The neuronal control the mesothoracic flexor tibiae muscle of the locust.

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CONTENTS

SUMMARY

1. The anatomy, innervation and physiological properties of the mesothoracic flexor tibiae muscle were investigated. The muscle was found to have three functionally separate parts; the proximal, middle and distal flexors. Each part receives some axons which are common with the other parts and some which innervate exclusively this region of the muscle. By recording at the same time from the nerve branches and muscle fibres of the three parts of the flexor muscle the total number of axons was found to be 16 and their innervation pattern on the muscle was established. These axons can be distinguished as six fasts, three intermediate, three slows, two inhibitors and two DUM cells.

2. The tension/length curve for passive and active tension was plotted indicating a peak active tension increment at a femur-tibia angle of 90° to 100°. The response to prolonged high frequency stimulation was studied for the proximal part (proximal and middle flexors) and the distal flexor demonstrating clearly that the distal part can resist fatigue better than the proximal part.

3. The anatomy of the sensory nerve branches in the mesothoracic and prothoracic femur of locust is described. A single multipolar receptor cell on the cuticular end of the distal flexor tibiae muscle fibres is identified and examined. This cell is shown to be a tonic receptor for active and passive tension in the muscle fibres to which it is attached. It generally causes reflex excitation of flexor motoneurons and inhibition of the slow extensor neuron, although the sign of the reflexes can be reversed.

4. In order to study how the large number of flexor motoneurons was used, their responses to imposed tibial extension (resistance reflexes) were analyzed. Most of the fast and intermediate axons were activated by the movement of tibial extension while the slows fired continuously at a higher frequency during maintained extension. The inhibitors were excited only during maintained extension but their firing rate depended also on the angular velocity of the movement of tibial extension. The DUM cells were not found to be excited by any tibial movement.

5. The firing pattern of the flexor motoneurons was also studied by recording spontaneous activity from the flexor muscle of a tethered locust walking on a treadmill. First, the similarities in the firing motor pattern between a deafferented and a normal walking leg were established and then the activity of flexor motoneurons recorded from a deafferented leg was analyzed.

6. The effects of the femoral sensory inputs (Chordotonal Organ, CO) to the centrally produced walking motor pattern were studied. It was found that the negative feed back produced by the CO in a quiescent animal was effectively reversed when the animal started walking. Resistance reflexes were also found to be suppressed during flight.

7. The implication of the results obtained are discussed. The main conclusion is that in the mesothoracic flexor tibiae muscle different parts of the muscle with different mechanical properties are used for different purposes. For example the proximal and middle flexor produce fast tibial flexion while the distal flexor

is used to generate postural forces. Therefore the flexor muscle requires a large number of axons which can operate almost independently in the three different parts of the muscle.

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1. INTRODUCTION

In order to study the neuronal basis of behaviour in arthropods it has been found necessary to look at the motoneuronal activity generated within the ganglion. To obtain information on the spiking activity of the motoneurons (usually leg motoneurons), electromyograms (EMGs) were recorded peripherally from the muscle which they innervate. EMG recording proved a very useful technique since it permitted easy access to the muscular activity which generates behaviour, thereby allowing quantitative analysis of the timing and extent of activity of members of the excitatory motoneuron population innervating different behaviourally important muscles.

In the first electrophysiological experiments using EMGs Rijland (1932a, b) showed that the motor output to the leg muscles of the cockroach was changed by an imposed resistance to movement, one of the first examples of resistance reflexes in insects. Pringle, in an attempt to establish the input-output relationship in the cockroach leg, first studied the leg proprioceptors (Pringle, 1938) and then using myograms he established the double innervation of the coxal muscles (Pringle, 1939). He demonstrated a positive feed back reflex to the coxal depressor muscle (Pringle, 1940) and a weaker reflex to the muscle of the opposite leg. These reflexes led him to suggest (Pringle, 1961) that the whole leg movement cycle in walking is produced by a chain of reflexes. The importance of the reflexes in walking was also investigated by Wilson (1965) who demonstrated electrophysiologically that the leg reflexes of the cockroach were fast enough to be used in walking and also that reliable contralateral reflexes can be detected.

EMG's provided a convenient way of studying reflexes since they do not require very fine microdissections to expose muscles and nerves.

Dissections generally may damage sensory organs and the saline used may alter the properties of the sensory organs (Coillot and Boistel, 1969; Burns, 1974). Dissection also causes bleeding in some animals such as crustacea which may kill the preparation quickly. As a result reflexes in crustacean leg muscles were studied using EMG's (Bush, 1962, 1963, 1965) and have been extensively analyzed using the same technique (Evoy and Cohen, 1969; Spirito, 1970; Spirito et al., 1972; Ayers and Davis, 1977, 1978; Clarac et al., 1978).

However, behaviourally speaking, a stereotyped motor pattern such as a resistance reflex is less interesting than the motor control of walking. Walking is not only a rhythmic behavioural act, but one that exhibits considerable plasticity, for the animal has to adjust its leg movements to the terrain over which it is walking. The first attempt to study walking behaviour was made by observing free walking animals and information was obtained about the timing and the coordination of different legs during walking (Hughes, 1952, 1957). Wendler was able to show that although stick insects used feedback from coxal hair plates to regulate posture (Wendler, 1961) the timing of leg movements was determined by an independent central system of coupled oscillators (Wendler, 1966).

In an attempt to study with more accuracy the role of various elements in the organization of motor output in walking, particularly the influence of the sensory inputs, electromyograms were recorded from free walking insects (Hoyle, 1964; Ewing and Manning, 1966) and with more success in crustacea (Barnes et al., 1972; Evoy and Fourtner, 1973; Fourtner and Evoy, 1973; Barnes, 1977).

In insects the recording of myograms has been used further to study not only the walking motor pattern but also the interaction between

sensory inputs and centrally produced patterns (Pearson and Iles, 1970). Pearson (1972) recorded myograms from the coxal muscles of a free walking cockroach and compared this activity with the reciprocal pattern produced by the same animal when it was fixed and the ganglionic connectives were cut. Although he found differences in the motor patterns produced in the two preparations, he suggested that the similarities were sufficient to make it almost certain that the output resulted from a central neural programme. The fact that there was a difference indicated that there is normally some modulation of the motor activity by inputs from propriceptors and Pearson (1972) showed that in the free walking cockroach the activities in both levator and depressor muscles were strongly affected by changes in loading of the animal. He concluded that the effect was mediated by tonic and phasic inputs from the trochanteral companiform sensilla. The fact that leg sensory inputs affect the centrally generated walking pattern was also demonstrated using EMGs by Usherwood et al. (1968) who found that removal of either of the metathoracic chordotonal organs produced significant changes in walking and postural behaviour.

However the effects of the sensory inputs on centrally generated neuronal motor patterns were soon found to be capricious. Reflexes themselves were also found to be variable. In locust for example the stabilizing reflexes of the abdomen occurs only during flight (Camhi, 1970). In cockroaches, when they were engaged in righting behaviour, some of the reflexes were turned off, others were turned on and still others switched the sensory input to a different channel of motor output (Camhi, 1977). Resistance reflexes were also found not to be in evidence during simple walking (Barnes et al., 1972) but to occur only during unintended movements (Barnes, 1977). Finally, resistance

reflexes were found to be reversed in an aroused stick insect (Bässler, 1976) and resistance reflexes were turned into a positive feed-back reflex during activation of command fibres in crayfish (Evoy, 1977). It is not yet clear how the reflexes are linked to the motoneurons and how much they can influence the centrally programmed behaviour. Since the reflexes in a fixed preparation may not be typical of those operating in walking it is apparent that these questions can only be answered by obtaining information about the activity of individual motoneurons from walking preparations in which all the relevant reflexes are likely to be operating.

EMG's provide the easiest way to obtain information about the activation of the leg motoneurons during walking and can provide valuable information on the exact time of the activation of the muscles and the approximate frequency of the motoneurons but they are less accurate in the study of the activity of individual axons. The major drawback of the technique is that simultaneous activity of several motoneurons may make identification of individual axons difficult if not impossible. This difficulty was overcome by Runion and Usherwood (1966) who developed a method for recording from the motor nerves of free-walking locusts. Using this technique they were able to monitor the activity of the three axons(2 excitatory, 1 inhibitory) in the nerve to the metathoracic extensor tibiae muscle and they found that it was strongly influenced by the input from the tarsal sensory receptors during walking and standing (Runion and Usherwood, 1968). Using the same technique, Usherwood and Runion (1970) were also the first to show that the inhibitory axons, when active in the pattern used in normal walking, produced a significant relaxation in the muscle. In the smaller mesothoracic extensor tibiae muscle, Burns (1972, 1973) recording neurograms with very fine wires which did

not hamper the movement of the legs at all, was able to analyse in detail the activity of the extensor neurons during free walking. In such a preparation he was able to demonstrate that the removal of the mesothoracic chordotonal organ decreases the activity of the extensor SETi but does not much alter its firing pattern.

The difficulty in the identification of the activity of individual motoneurons was also overcome in insects and other arthropods by recording intracellularly from the motoneuron cell bodies (Kerkut, Pitman and Walker, 1969; Hoyle and Burrows, 1970). This technique produces valuable information not only on spiking activity of the motoneurons but also on the synaptic inputs which they receive. The functional and structural organization of the motoneurons and some of the interneurons related with them within an insect ganglion was investigated thoroughly (O'Shea et al., 1974; Pearson and Fourtner, 1975; Burrows and Siegler, 1977) since it was now possible to stain neurons through the microelectrode (Stretton and Kravitz, 1968; Remler et al., 1969). Soon maps of the topography of the somata of different leg motoneurons were produced for the locust ganglion (Burrows and Hoyle, 1973). Attempts have been made to combine this excellent technique with behavioural studies. Activity from the ventilatory motoneurons was recorded (Burrows, 1974) and the intracellular records were obtained from the motoneurons responsible for cricket singing (Bendley, 1969a). Behavioural motor patterns can be studied as far as they occur in a fixed and dissected animal, the necessary conditions for microelectrode recording. In an attempt to study walking motor patterns in such preparations Hoyle and Burrows (1973b) stimulated the connectives between the ganglia and produced a slow rhythmic movement of the metathoracic tibiae. Although they claim that this sequence of alternating flexion/extension movement

closely resembles those seen during locomotion in the freely-moving animal it is obvious that a fixed animal upside down is not the best way to study the walking motor pattern. Even if they were able to record from the cell bodies during walking it would be impossible to record the action of a large number of motoneurons simultaneously.

It seems that for the studies of motor patterns in a free animal the best technique to use is neurogram recording. This has allowed identification of the two excitors and one inhibitor axon in the mesothoracic and metathoracic extensor muscles of free locusts. However all muscles are not as simple as the extensor tibiae. Although no arthropod muscles receive as many axons as in vertebrates, there are muscles which receive far more than two excitors and one inhibitor. In the locust abdomen the median dorsal internal muscles are all innervated by eight axons in the dorsal nerve (Tyrer, 1971a, b). In the neck of the locust where one group of four muscles, consisting in total of less than 100 muscle fibres, receives more than 20 different motoneurons from three different ganglia (Shepheard, 1973). An extreme case is the dorsal longitudinal flight muscle in the flesh fly (Sarcophaga bullata) which has only six muscle fibres and receives five different motor axons (Ikeda, 1977). A similar compexity is seen in both groups of antagonistic abdominal muscles in the crayfish, the extensors (Parnas and Atwood, 1966) and the flexors (Kennedy and Takeda, 1965a, b). However not all antagonistic muscles in arthropods seem to be equally complicated. One clear example is the two antagonistic femoral muscles in the locust, the extensor tibiae with two excitors and the flexor tibiae with six (Hoyle and Burrows, 1973a, b) and maybe more excitors. The metathoracic extensor tibiae muscle is one of the most studied muscles in insects (Hoyle, 1955, 1978; Hoyle and O'Shea, 1974; Runion and Usherwood, 1966,

1968; Usherwood and Runion, 1970; Cochrane et al., 1972; and reviews, Hoyle, 1965; Usherwood, 1967, 1977). This is not surprising because as Hoyle (1955) admits "The Jumping muscle, the extensor tibialis of the metathoracic leg, was chosen primarily because it is large and therefore easy to study .....". However not all the femoral muscles are so large. In the smaller mesothoracic leg despite its size, the extensor muscle was also studied in detail by Burns (1972, 1973). Going through the literature it can be seen that, although there is a tremendous amount of information about the extensor tibiae muscle, there is a lack of knowledge about its antagonist, the flexor muscle. This is noticeable not only in the mesothoracic leg where the small size make it difficult to study, but also in the metathoracic leg where the flexor muscle is larger. It seems that reasons other than the importance of the flexor muscle in behaviour have led to this lack of information on the flexor muscle. This muscle is innervated by a large number of axons and this makes its study particularly difficult.

The purpose of the present work is to fill this gap and for this reason the mesothoracic flexor tibiae muscle was chosen. Although this muscle is small and it is difficult to obtain histological and electrophysiological data it was preferable to the larger metathoracic flexor tibiae for the following reasons. The mesothoracic flexor is larger than the extensor while the opposite is true of the metathoracic leg where the extensor is the most powerful. This suggests that the flexor muscle may have a more important function in the mesothoracic leg. Secondly, the mesothoracic leg seems to participate more in walking and posture while the metathoracic leg has been adapted for the jump and the defensive kick (Heitler and Burrows, 1977a, b; Heitler, 1977).

Since it is already known that the flexor tibiae muscle receives a large number of axons a number of significant questions can be asked. What is the exact number of axons which this muscle receives? How are these axons distributed on the muscle? Why does the flexor muscle require such a large number of axons? What is the significance of all these axons in behaviour? In an attempt to answer these questions anatomical, histological and electrophysiological methods were used to establish the anatomy and innervation of the muscle and some of the sensory receptors in the mesothoracic leg (Chapter 3 and 4). Electrophysiological techniques were also used to study the way in which the motoneurons are activated in various behaviour patterns (Chapter 5 and 6).

2. MATERIALS AND GENERAL METHODS

Most of the animals used in these studies were adult female locusts, Schistocerca gregaria Forskal (S.americana, Dirsch, 1974) kept in colonies at 32°C. Females were chosen because they have a thinner exoskeleton and they are larger in size; these features were advantageous for the dissections.

The size of the locust mesothoracic femur varies from 7.5 to 11mm (Burns, 1972). The average size of the femur in the animals used was 10 $\pm$ 0.2mm. To expose the flexor tibiae muscle and its sensory and motor nerves the femur (fig. 2.1A) was mounted ventral side down in Tackiwax. The dorsal cuticle of the femur, the extensor muscle and the retractor unguis muscle were removed and the final view under the dissecting microscope was the one shown in fig. 2.1B. The femur was immersed in 3ml of oxygenated saline (Usherwood and Grundfest, 1965) at room temperature 18° to 20°C.

Extracellular records from the nerve branches were made using special glass suction electrodes. These electrodes were made from glass micropipettes pulled from 0.7mm diameter glass, in such a way as to have a conical stem. The outside surfaces of these microelectrodes were coated with a layer of gold (1000Å) thick, by vacuum deposition. This external film was used as the reference electrode. The tip was broken giving an internal diameter similar to that of the nerve to be recorded from. This electrode had the following advantages.

1) The reference surface was very close to the recording tip giving a monophasic potential, good screening and a minimum of cross-talk.

2) When this suction electrode was used as stimulation electrode it produced a good local stimulation with very little spread of current to disturb other nerve axons.

Fig. 2.1

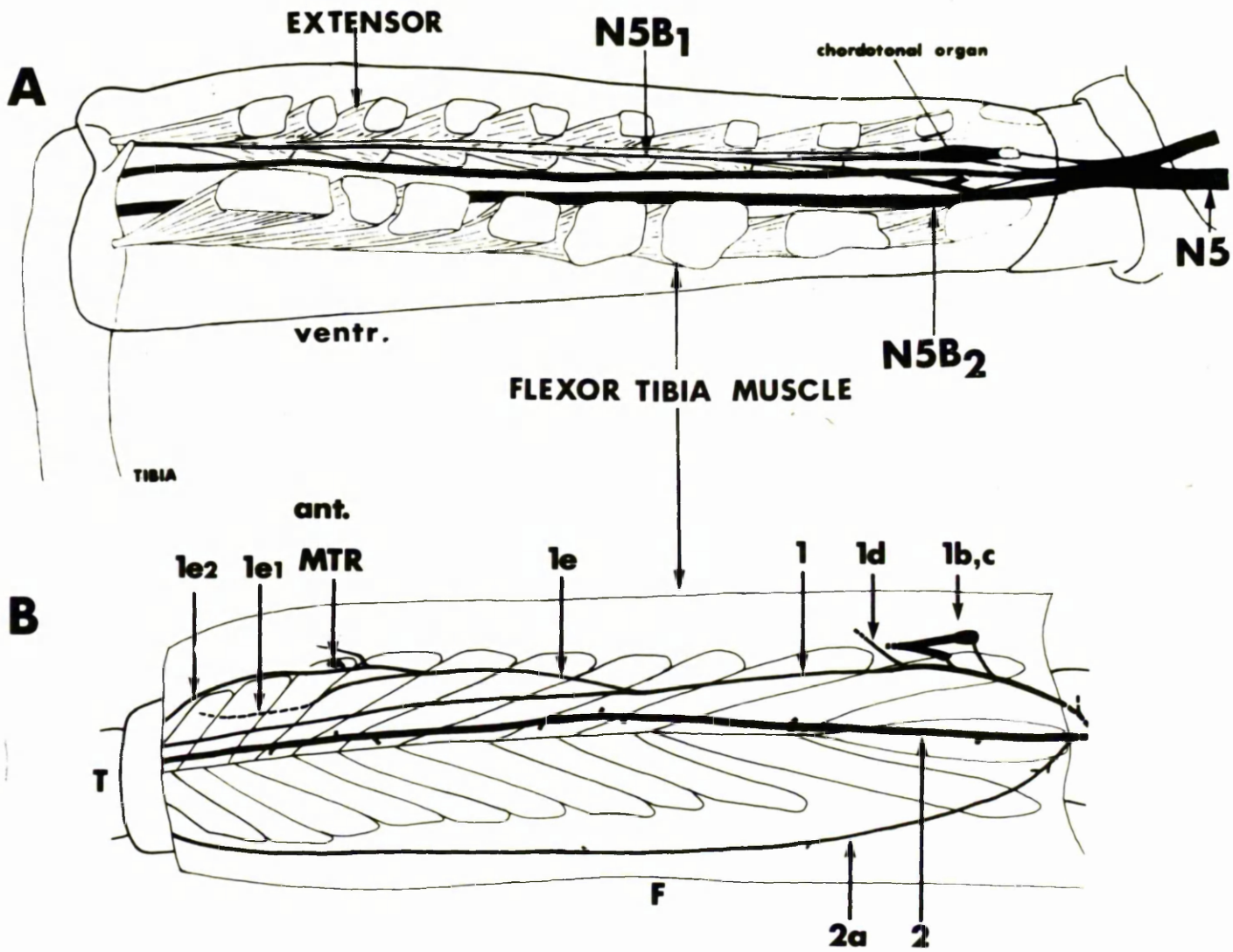
A.

A diagrammatic representation of the mesothoracic femur. The main muscles, nerves and the chordotonal organ are shown .
(Figure kindly supplied by Dr. Margaret Burns).

B.

Dorsal view of branches of N5 in the left femur. The basic numbering of the nerve branches follows Campbell (1961), but has been extended as shown. (see Chapter 4 for details). All nerves numbered are branches of nerve 5B.

F = femur T = tibia
MTR = muscle tension receptor


A
EXTENSOR
N5B1
chordotonal organ
N5
ventr.
N5B2
FLEXOR TIBIA MUSCLE
TIBIA
ant.
B
1e2
1e1
MTR
1e
1
1d
1b,c
T
F
2a
2

3) The layer of gold between the tip which was in the saline and the connection with the reference wire has a resistance of 15-20MΩ which improves the balance of the amplifier input.

4) The tip of the glass micropipette is very small and this allows records to be made from very fine nerves (diameter of 30-50μ). The very fine tip of the suction electrode also reduces the duration of each recorded spike, because of the proximity of the reference electrode. Records of nerve branches were made en passant also by holding them on a steel pin (diameter 0.0056mm) bent into a hook and raising the nerve into a plastic tube filled with a thick mixture of paraffin oil and vaseline. This hook electrode was modified from the design of Wilkens and Wolfe (1974). The signals from these electrodes were amplified by an Isleworth A101 preamplifier with capacitance input isolation.

Glass microelectrodes filled with 3M KCl having resistances of 5-20MΩ were used for intracellular recordings. The DC potentials were amplified by a WPI M701 amplifier.

Tension produced by the contraction of the flexor tibiae muscle was recorded under nearly isometric conditions with a semiconductor strain gauge (compliance 0.05mm/g). The changes of muscle tension were displayed usually by a Watanabe pen recorder.

A constant velocity movement was imposed on the tibia by a lever which had its one end attached to a pen motor and the other end to the tibia with a small drop of Cyanoacrylate adhesive (Avdel Bond No.3). The axis of the rotation of the lever was through the pivot of the femur-tibia joint and the rotation of the lever was monitored with a low friction potentiometer. Linear ramp functions were obtained by integrating a square wave by means of an integrated circuit operational amplifier, which in turn drove the pen motor. The ramp

generator also produced a trigger pulse before the beginning of each ramp.

All the records were displayed on a Tektronix 561 oscilloscope and stored with a Racal Store 4 FM tape recorder. For further analysis records were filmed with a camera (Nihon Kohden, PC-2) directly from the oscilloscope screen.

3. THE LOCUST MESOTHORACIC FLEXOR TIBIAE MUSCLE

A. Methods:

a) Physiological methods.

Physiology

When stimulation of the flexor motor axons was required (through N5) a locust mesothoracic leg was isolated by cutting the coxa-femur joint and dissected (fig. 2.1B) in a watch glass under oxygenated saline. The same dissection was used to record motoneuron activity from the femoral nerves, but in this case the leg under investigation was not removed from the body and was mounted (ventral side down) in a small bath made from plasticine. A similar preparation was also used to study the distribution of the Dorsal Unpaired Median cells (DUM cells, as defined by Hoyle et al., 1974) which give branches to both sides of the animal. In this case the contralateral leg was also fixed and dissected in the same way. To stimulate the somata of the flexor tibia motoneurons intracellularly, a wax covered platform was micromanipulated under the ganglion in the nearly intact insect, to provide a firm support for microelectrode penetration. The microelectrodes were driven with a Leitz micromanipulator and protease was used to make the sheath of the ganglion softer.

To record muscle tension, the tibia was cut transversely half way up and the femur-tibia joint was disarticulated. The tibial stump that remained was attached to a silicon strain gauge transducer thus stretching the muscle and allowing the tension generated to be monitored.

Glass microelectrodes were used to record potentials in the muscle fibres (EPSP's or IPSP's) in response to stimuli delivered to the proximal cut end of N5.

Records of spontaneous activity in the nerve branches on the flexor tibiae muscle were also made using hook electrodes.

Mechanical Properties

The animal was mounted ventral side up on a special curved piece of perspex and the leg under investigation was fixed with plasticine (fig. 3.1). A small window was opened in the ventral side of the mesothorax to expose the ganglion and a hook electrode was attached to N5 for stimulation. Passive and active forces were recorded from the end of the tibia with a strain gauge transducer. The tendon of the antagonist extensor was severed and the mesothoracic ganglion was destroyed to prevent any effects of the tension recorded from the flexor muscle. Forces recorded in such a way from the end of the tibia were converted to real muscle tension by multiplying this value by the factor of 12.1 obtained from the lever equation:

$$T.a = To.(a+b)$$

T is the real tension developed on the flexor apodeme

a (= 0.81mm) is the distance of the pivot to the flexor muscle tendon insertion on the tibia

To is the force recorded from the end of the tibia and

b (= 9mm) is the distance from the flexor tendon insertion to the end of the tibia.

Measurements made on the mesothoracic leg by Heitler (1977) gave very similar values for a and b.

To record changes in muscle tension during the passive extension, the tibia was extended to different angles with different angular velocities. This was accomplished by imposing a ramp movement on the transducer. To avoid inducing variation in the mechanical properties of the muscle no saline was used during these experiments.

Fig. 3.1.

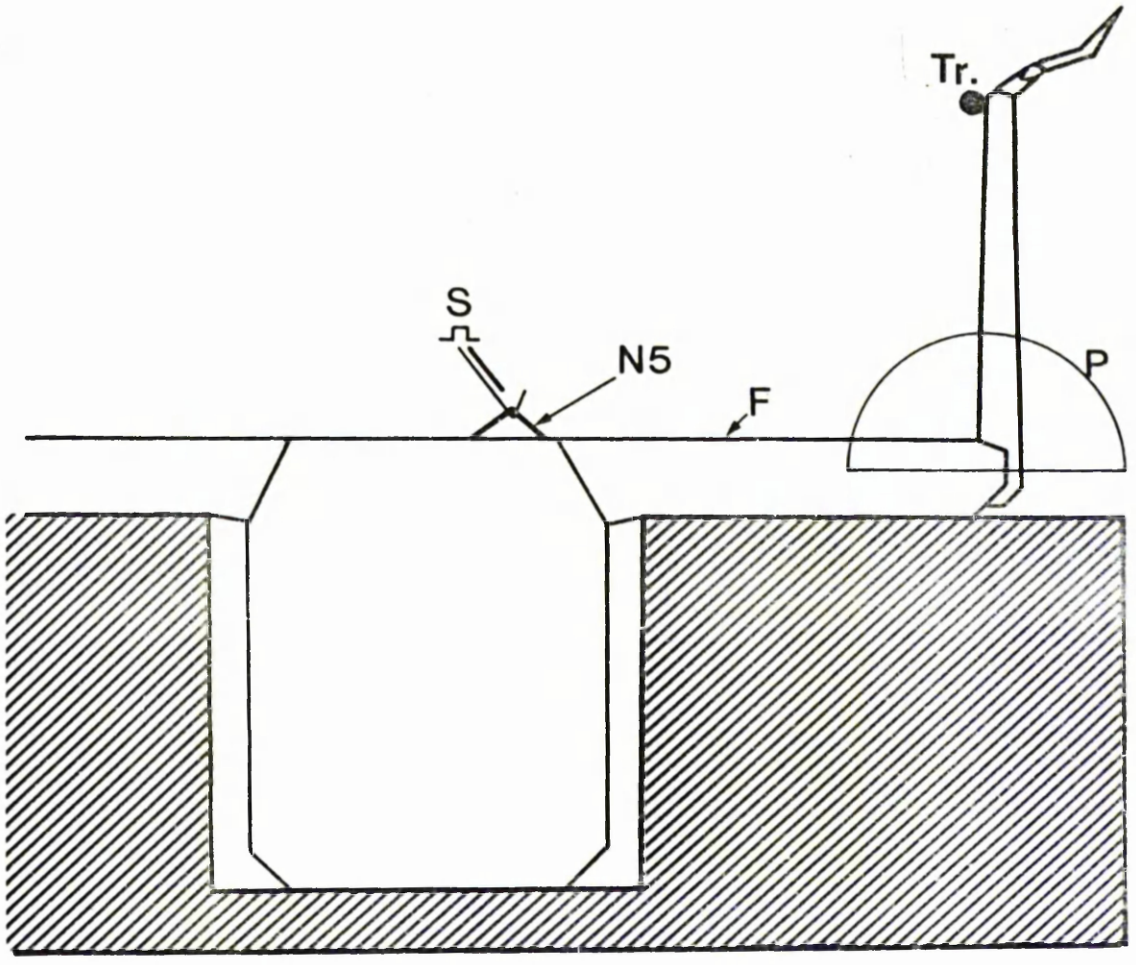
The apparatus used to record tension from the mesothoracic flexor tibiae muscle in its own haemolymph. The dissection to expose N5 was very small. No saline was used and care was taken to leave most of the tracheal system intact. To measure tension from either extensor or flexor tibiae muscles the apodeme of the antagonistic muscle was cut near its connection with the tibia.

S. Stimulation of N5 through a hook electrode. The exposed part of the nerve was covered by a mixture of paraffin and vaseline over the hook electrode.

Tr. = Transducer attached always at the end of the tibia

P = Miniature protractor

F = Femur


Tr.
S
N5
F
P

b) Anatomical methods.

1) Staining.

a) Staining with reduced methylene blue (Pantin, 1964).

A small quantity (less than 0.02ml) of a mixture of one part of reduced methylene blue and ten parts of locust saline, was injected into the mesothoracic femur. After 60 min. the leg was dissected in saline for further studies.

b) Back filling with Cobaltous Chloride ($CoCl_2$) (Pitman et al. 1972).

The cut end of the nerve was sucked firmly into the broken tip of a glass micropipette filled with saline. The saline in the micropipette was then replaced, using a long fine syringe needle, with distilled water for 3 min. to open the cut end of the axons. Finally the distilled water was replaced with 1M Cobaltous Chloride. In this case the interior of the microelectrode works as a pool isolated from the saline. Initially an electric current of 10^{-7} to 10^{-11} A was passed out of the electrode, but finally it was concluded that Cobaltous Chloride entered the nerve equally well without a current. After 6 to 24 hours the perfused Cobalt was precipitated as Cobaltous Sulphide with 10% ammonium sulphite. The preparation was fixed in 2% glutaraldehyde for 1 hour, dehydrated with series of ethanol (50-100%), cleared with methyl salicylate for 24 hours and mounted in Canada Balsam. The preparation was then photographed with a Leitz photomicroscope. Drawings were made using a camera lucida.

2) Electron microscopy.

The flexor muscle and its motor nerve were bathed in the saline for a period of 50-100 min. to equilibrate (Rees and Usherwood, 1972). Two fixatives were used and both gave satisfactory results.

a) The Formaldehyde - Glutaraldehyde fixative of high osmolarity (Karnovsky, 1965)

b) The glutaraldehyde fixative with similar osmolarity as that of locust haemolymph and saline (Rees and Usherwood, 1972).

For the final results the second fixative was used.

The muscle and its motor nerve $N5B_2$ were fixed at maximum body length as in fig.2.1B, in 2% glutaraldehyde fixative at $4^{\circ}C$ for 2 hours. Then the nerve branches from $N5B_2$ to the muscle were cut very close to the muscle and the whole nerve was removed and placed in 10ml bottles of buffered wash solution and washed for 18 to 24 hours at $4^{\circ}C$. It was then postfixed in 1% OsO_4 solution for 1 hour before being rinsed yet again in buffered wash solution for a similar length of time. Both the fixatives and the buffer wash solution were kept isosmotic to the saline using a small quantity of sucrose. The pH of the solution was maintained at 6.8 with sodium phosphate buffer. The tissue was quickly dehydrated and mounted in Spurr's low viscosity epoxy resin (Spurr, 1969). Thin transverse sections (70nm) and thick sections (1-5μm), from the flexor nerve branches and from $N5B_2$ at different levels were cut with an LKB ultratome. Thick sections for light microscopy were stained in methylene blue for 3 min. on a hot plate at $60^{\circ}C$ and rinsed in cold water. Light straw to silver grey sections were mounted on 100 mesh grids and double stained in lead citrate and uranyl acetate. All grids were examined in AEI EN801 Electron Miscroscope at 50KV.

3) Scanning Electron Microscopy.

The flexor muscle and its motor nerve 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.

B. Results:

1. Structure.

a) The muscle.

The mesothoracic flexor tibiae muscle lies in the ventral half of the femur (fig. 2.1A). In shape, it is a combination of fusiform and pinnate form and is composed of a number of muscle units (as defined by Hoyle, 1955). The muscle units will be called muscle bundles in this text. These muscle bundles form a row of more or less circular discrete anterior-dorsal insertions, a posterior row of elongated insertions very close to those of the extensor tibiae muscle and tending to merge into one another, and a single proximal dorsal insertion close to the trochanter. The structure of this muscle was partly described by Snodgrass (1929) who numbered this muscle at 107 and divided it into three major parts based on the anatomical characteristics of the muscle. The anatomy of this muscle is shown in fig. 3.2. and the three parts are as follows:

107b, described in this text as Proximal flexor
107a, " " " " " middle flexor
107c, " " " " " distal flexor

Proximal flexor (107b).

This is the only fusiform part of the flexor tibiae muscle and consists of a single muscle bundle (fig. 3.2). A transverse section through this bundle is shown in fig. 3.3A. This part of the muscle receives only one nerve branch which arises from the ventral side of $N5B_2$.

Middle flexor (107a).

This is the first pair of muscle bundles in the pinnate part of the flexor muscle (fig. 3.2). These bundles are attached ventrally to the proximal end of the femur and converge from either side of the

flexor apodeme. The angle between the muscle bundles and the apodeme, the pinnation angle, is about 9° (Table 3.1). Transverse sections through the middle part of the muscle are shown in fig. 3.3A and an analysis of the information obtained from similar sections is shown in Table 3.1.

Distal flexor (107c)

This forms the rest of the pinnate part of the flexor muscle and is attached to the walls of the distal two thirds of the femur (fig. 3.2). This part contains 8 to 10 pairs of muscle bundles with different pinnation angles (Table 3.1). A transverse section from the distal flexor is shown in fig. 3.3B. Due to the pinnate structure only four pairs of muscle bundles can be seen in this section which also shows that the muscle bundles of the anterior part of the muscle contain a larger number of muscle fibres than those of the posterior part (Table 3.1).

The anatomical features of the flexor muscle are summarized in Table 3.1 and will be discussed later.

b) The flexor nerve branches.

To establish the anatomy of the nerve branches on the flexor muscle bundles, reduced methylene blue was injected through the dorsal cuticle of the mesothoracic leg of an intact animal, or $CoCl_2$ was perfused into the fine nerve branches of $N5B_2$ (as described in Methods). The second technique was the most successful and some of the most densely filled nerve branches in the various parts of the flexor muscle are shown in fig. 3.4. The proximal flexor receives its motor nerves through a branch which arises from the ventral side of N5B2 and immediately after it reaches the muscle gives rise to two other branches. The middle flexor receives one pair of motor nerve branches. They

Fig. 3.2

Diagrammatic representation of the mesothoracic flexor tibiae muscle. The muscle bundles and the three different parts of the muscle are demonstrated. The exact dimension of the muscle bundles and their pinnation angles are not shown in this diagram. The nerve branches from the main nerve (N5B2) are the typical patterns from 25 left and 25 right legs, but there is no systematic difference between right and left legs.

AA and BB shows the approximate positions of the transverse sections in Fig. 3.3.

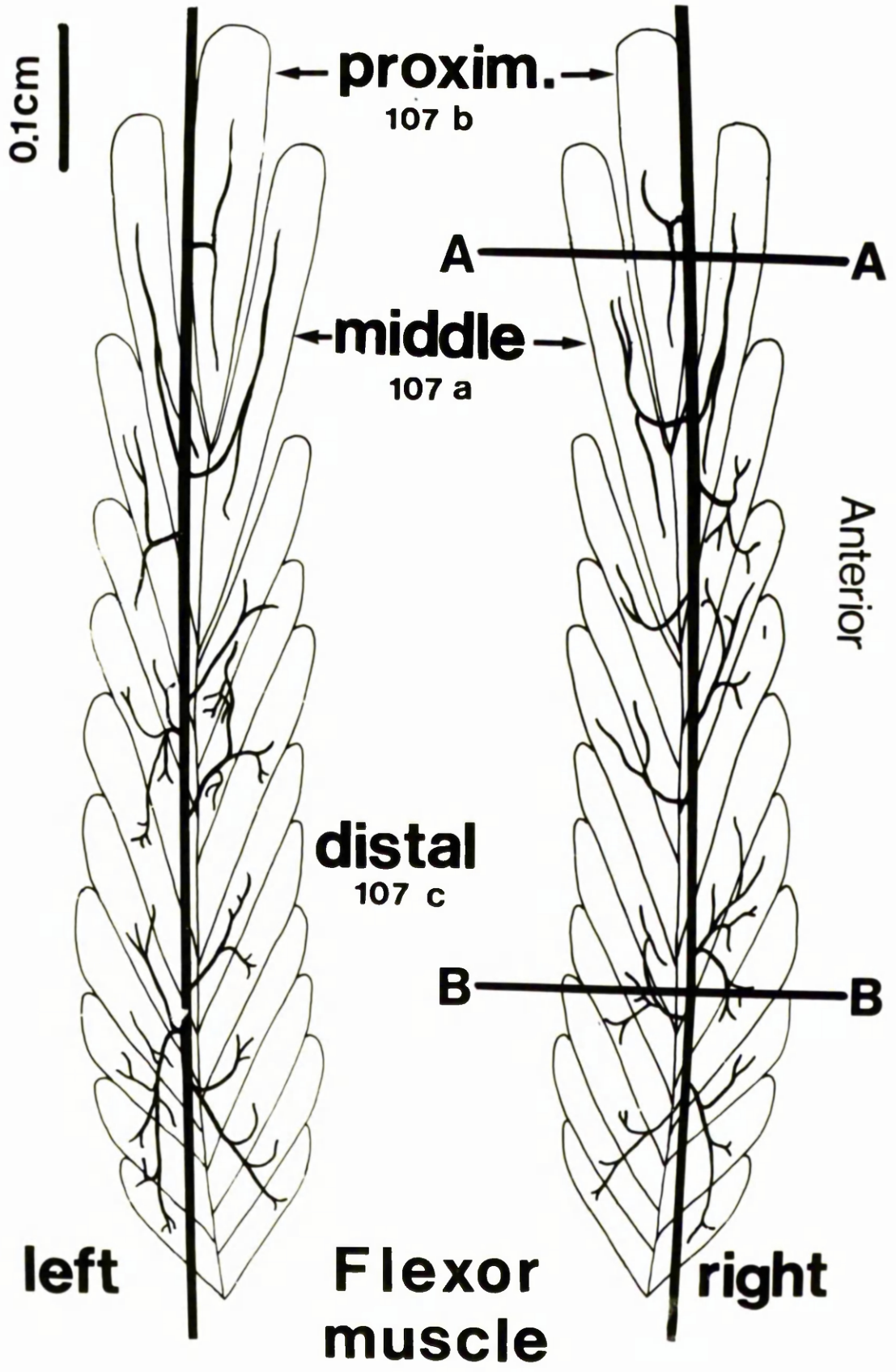

0.1cm
proxim.
107 b
A
A
middle
107 a
Anterior
distal
107 c
B
B
left
Flexor muscle
right

Fig. 3.3

Cross sections of the mesothoracic femur of the locust. The approximate locations of the sections on the muscle are shown in Fig. 3.2.

A = section AA (Fig. 3.2) through the proximal and middle flexors

B = section BB (Fig. 3.2) through the middle of the distal flexor.

C.O. - Chordotonal organ, R.U. muscle Retractor unguis muscle (only the apodemes are visible in B).

Scale bar 0.250mm

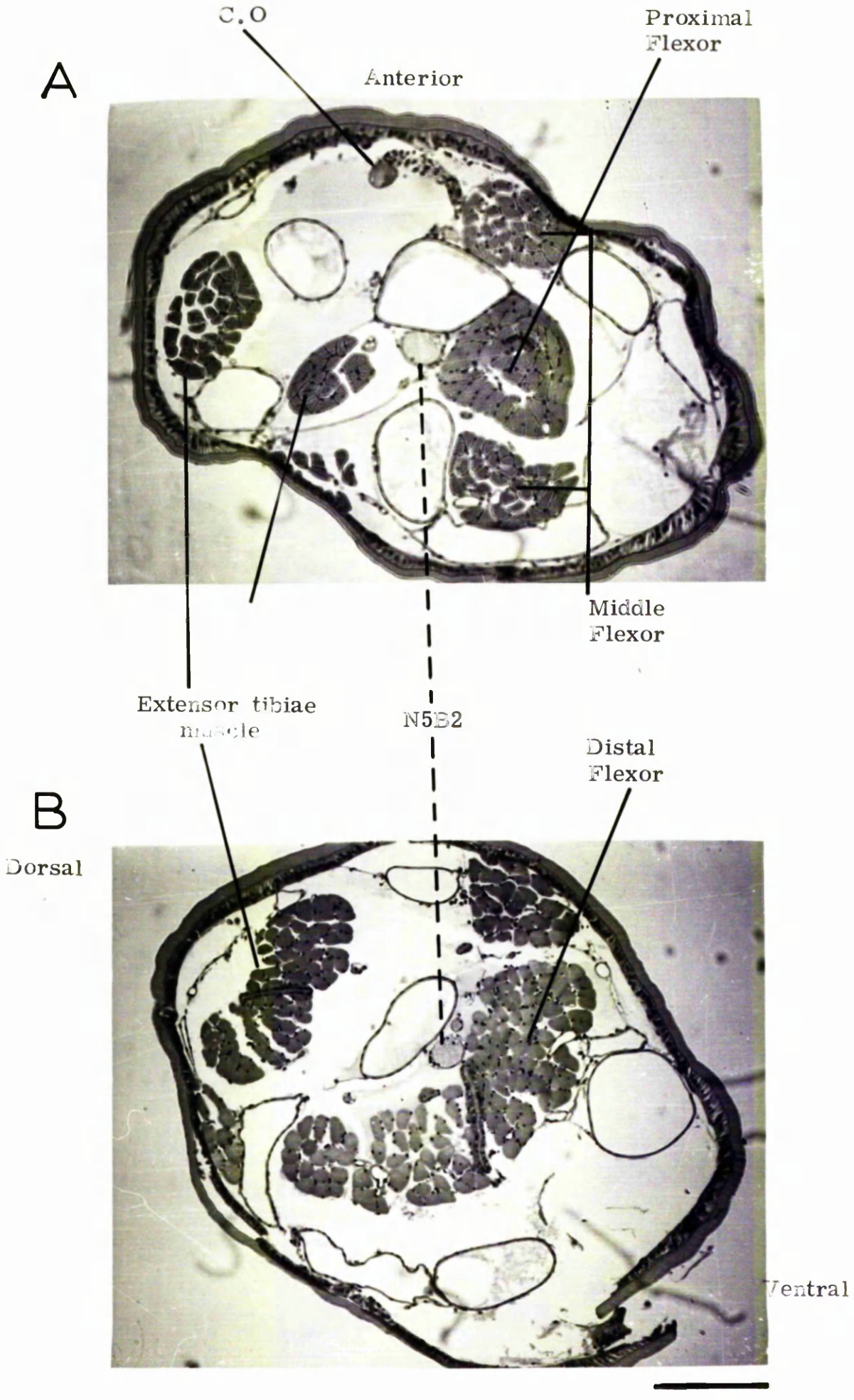

A
C.O
Anterior
Proximal
Flexor
Middle
Flexor
Extensor tibiae
muscle
N5B2
B
Distal
Flexor
Dorsal
Ventral

Table 3.1.

Dimensions and structural characteristics of the muscle bundles of the mesothoracic flexor tibiae muscle in female Schistocerca gregaria. Some data concerning the extensor and retractor unguis muscles are also given.

* Total : Indicates the number of muscle fibres which can be counted in the whole cross section of this part of the muscle

m.b. = muscle bundles

m.f. = muscle fibre

figures in brackets are standard deviations

Table 3.1.

	Region	Length of m.b. (mm)	Number of m.f. in each m.b.	Mean diameter of m.f. (μm)	Cross sectional area of m.b. (μ^2)	Pinnation angle of m.b. (degrees)
Prox. flexor		3.45(± 0.3)	31(± 3)	66(± 7.0)	102456	0
	Anterior	3.15(± 0.2)	33(± 2)	44.1(± 6.1)	42999	9.1(± 0.5)
Middle flexor	Posterior	3.80(± 0.3)	30(± 3)	47.9(± 5.3)	50411	
Distal flexor	Anterior					
	First m.b.	2.60(± 0.3)	28(± 4)			18.1(± 2.1)
	Last m.b.	4.90(± 0.2)	24(± 3)			27.4(± 2)
			* Total 80	42.7(± 3.1)	110948	
	Posterior					
	First m.b.	2.40(± 0.3)	19(± 3)			14.8(± 1.8)
	Last m.b.	1.70(± 0.1)	10(± 2)			22.1(± 1.4)
			Total 55	47.1(± 6.7)	50340	

continued next page

Table 3.1 (continued)

Mesothoracic Extensor tibiae muscle				
Proximal		32 (± 3)	41.15(± 5.2)	42770
	Anterior	Total(28± 3)	37.30(± 3.8)	30735
Distal	Posterior	Total(19± 4)	36.10(± 3)	17806
Retractor Unguis muscle				
		25(± 3)	32.3(± 5.5)	21974

Ratio of the effective diameter of proximal to distal muscle fibres

Flexor tibiae	1.40 (66 : 47.1)
Extensor tibiae	1.08 (41.15 : 37.90)

Ratio of total proximal and middle muscle fibre cross sectional area to total distal fibre cross sectional area.

Flexor tibiae muscle	1.21
Extensor tibiae muscle	0.88

Fig. 3.4

Nerve branches of the mesothoracic flexor tibiae muscle filled with $CoCl_2$.

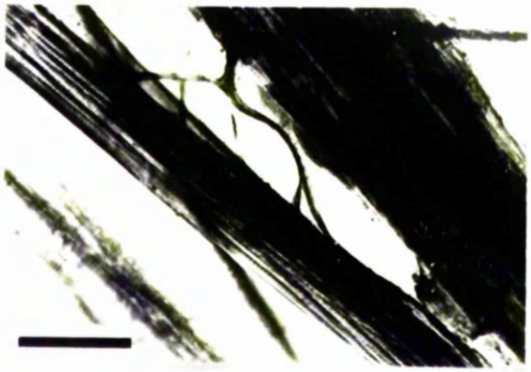
Proximal nerve (top left) This branch arises from the ventral side of N5B2 (see arrow) and directly innervates the proximal muscle fibres. Scale bar : 200 µm.

Middle nerve (top right) The only nerve which is symmetrical and innervates the middle flexor, which can be seen as the diagonal muscle bundle. Scale bar : 200 µm.

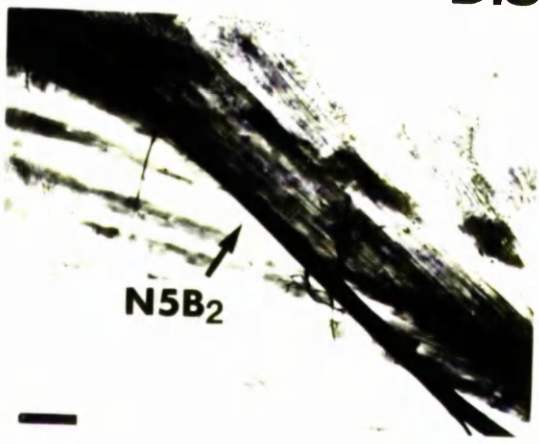
Distal nerve branches (central and bottom) Nerve branches arising from the sides of N5B2. Notice the nerve branch arrowed in the middle right picture. This is the last and the longest nerve branch of the distal flexor. Most of the extracellular records were obtained from this nerve branch using hook electrodes. The same branch is shown at a higher magnification in the right bottom figure. The middle left figure shows other branches of the distal flexor and the bottom left figure shows one of them at higher magnification. Scale bars : 100 µm.

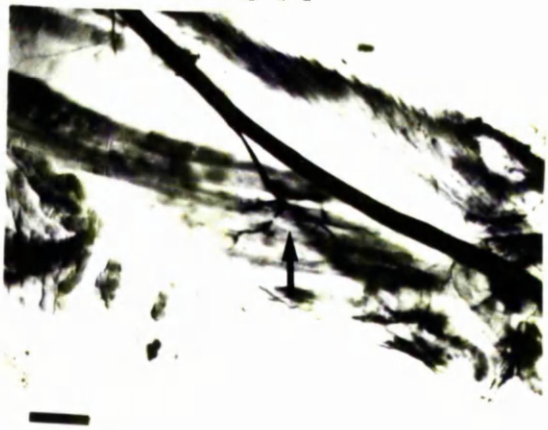
PROXIMAL

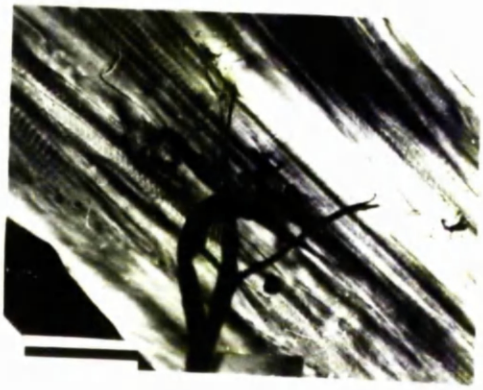
MIDDLE



DISTAL FLEXOR







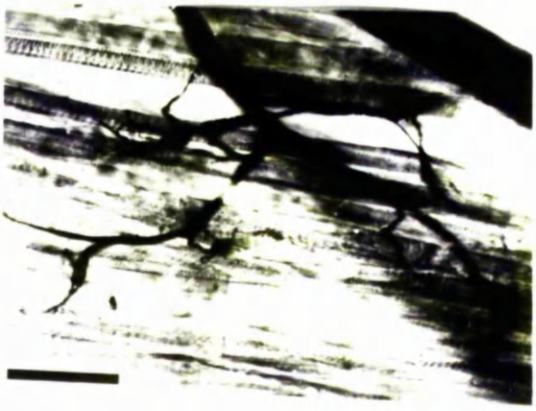
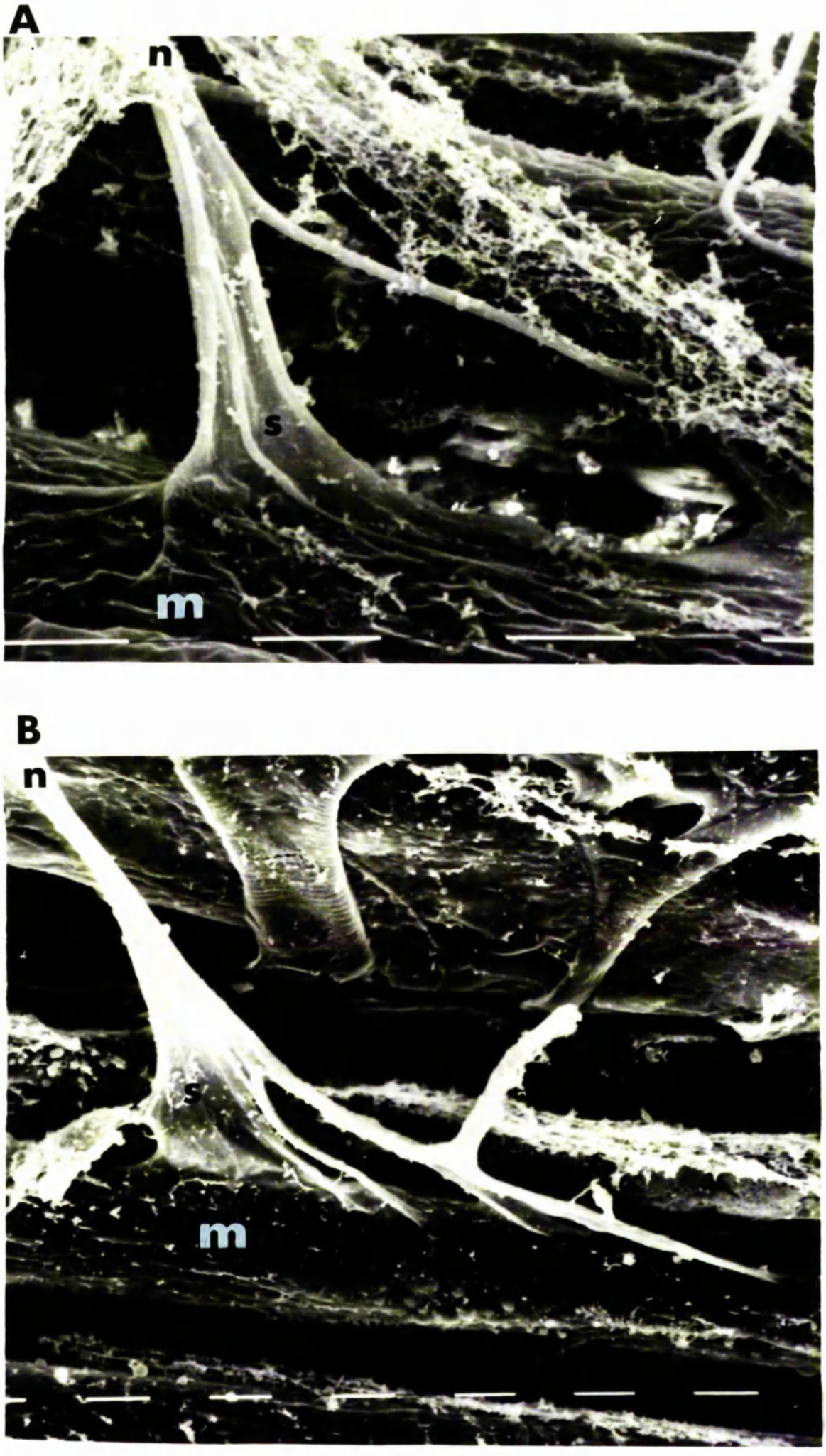


Fig. 3.5

A, B scanning electronmicrographs from the nerve branch terminals on the mesothoracic flexor muscle fibres.

S, the support structure between the nerve branch (n) and the muscle fibres (m). The nerve axons can also be seen.

This formation was found in some flexor nerve branches but not all. Scale marks 20 µm.


A
n
s
m
B
n
s
m

branch immediately they reach the muscle fibres. The rest of the muscle (the distal flexor) receives nerve branches from the sides of $N5B_2$. There are four pairs of nerve branches which usually branch again immediately after leaving the main nerve to innervate more than three muscle bundles.

More than 50 legs (25 right and 25 left) were examined and the nerve outlines were drawn with a camera lucida. Analysis of these drawings and photographs produced the diagram of the most common pattern of the flexor nerve branches and the organisation of the flexor muscle bundles that is shown in fig. 3.2. Although there is some variation between animals in the number of the nerve branches and in the way in which they approach the distal flexor, the innervation pattern of the proximal and middle flexors is always identical. No substantial differences in the anatomy were found between the prothoracic and mesothoracic muscles.

For further studies of the above flexor nerve branches, the surface of the flexor muscle was examined with a Scanning Electron Microscope. The terminal nerve branches on the muscle fibres are characteristic of the Orthopteron diffuse type (described by Hamory, 1961). Some of the nerve branches approach the muscle with a connective tissue link between them (fig. 3.5A, B). This may be a common membrane between nerve and muscle formed from the basement membrane of the lemnoblast and the muscle cell as found in EM sections by Edwards (1959) and Rees and Usherwood (1972) in the locust retractor unguis muscle. This link seems to "support" the neuromuscular junctions against any tension which is developed between nerve and muscle during passive movements or twitch contractions of the muscle.

c. The motor axons.

Transverse sections of the major flexor nerve branches were cut for transmission Electron Microscopy (EM) and they show a large number of axons in each nerve branch (fig. 3.6.). The number of the axons which can be seen in the flexor nerve branches varies a little between animals. Table 3.2. shows the number of axons which were counted in EM sections from three branches in five different animals. In order to count the axons to the distal flexor the last nerve branch from this part of the muscle was chosen. Comparison with a number of sections of other branches of the distal muscle showed no systematic differences in the number of the axons in each branch. The effective diameters of the motor axons in the flexor nerve branches calculated from the cross sectional areas are listed in Table 3.3. These measurements were taken from sections with numbers of axons very close to the average figures shown in Table 3.2. Some of the axons in the same nerve branch seem to have almost identical effective diameters (underlined values).

d. Nerve $5B_2$.

The ultrastructure of $N5B_2$ and of the nerve branches which enter the flexor tibiae muscle is similar to that of other peripheral nerves in insects (Edwards, Ruska and de Harven, 1958 ; Huddart, 1971; Elder and Moran, 1974; Lane and Treherne, 1973).

To study the location of the flexor motor axons in $N5B_2$, serial sections for light microscopy were taken from this nerve (fig. 3.7). The group of large axons in the ventral part of the section seems to contain most of the flexor motor axons. In the proximal part of the femur, $N5B_2$ (section A) has a diameter of 290 ± 20 µm while distally this diameter becomes 250 ± 20 µm (section C). There are two main

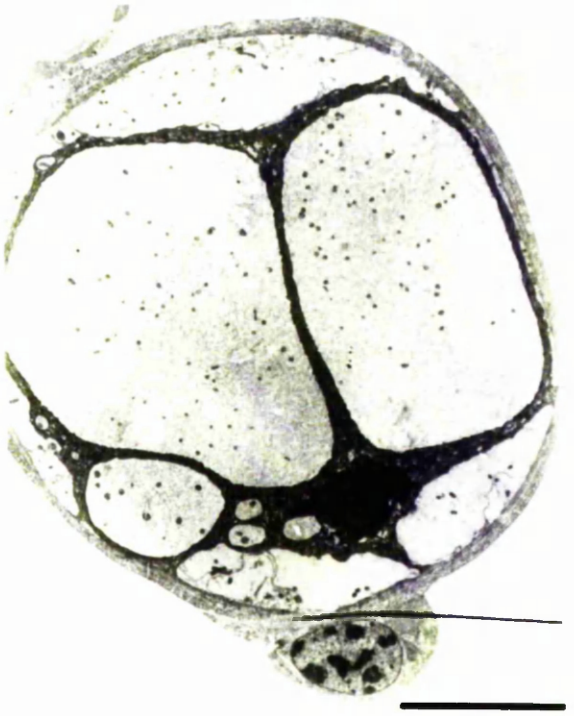
Fig. 3.6

Electron micrographs of transverse sections from the proximal nerve branch (section aa), middle branch (section bb) and distal branch (section cc). The exact locations of these sections are indicated in fig. 3.7. The shape of the nerve branches, from which these sections were taken, are shown in fig. 3.4.

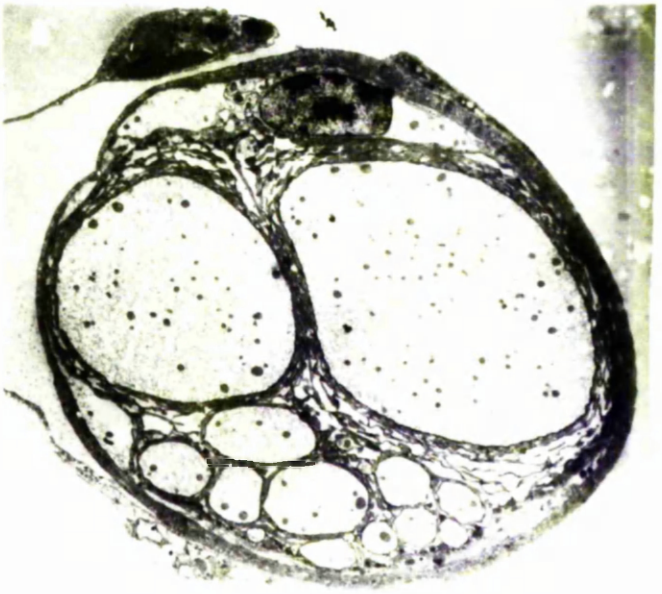
Scale bar : 10 μm.

PROXIMAL NERVE

BRANCH. Section aa



MIDDLE bb



DISTAL cc

10 µ

Table 3.2.

Numbers of axons in the nerve branches of the mesothoracic flexor tibiae muscle counted in electron micrographs from 5 different locusts.

Table 3.3.

Effective diameters of the axons in the mesothoracic flexor nerve branches. Each diameter was calculated from the cross sectional area. Measurements were taken from sections with approximately the mean number of axons shown in Table 3.2.

Table 3.2.

Animals	1	2	3	4	5	
	Number of axons					Mean
Proximal nerve branch (section aa)	9	10	9	11	9	9.6
Middle nerve branch (section bb)	14	15	17	16	15	15.4
Distal nerve branch (section cc)	18	19	17	20	18	18.3

Table 3.3

Effective diameter of axons (µm).

Proximal nerve branch (section aa)	25.50,	20.20,	8.80	
	2.20,	1.90,	1.60,	0.75,
	0.65,	0,40,	0.35	
Middle nerve branch (section bb)	23.50,	19.90,	7.50,	7.60
	6.80,	5.20,	4.90,	4.20,
	3.60,	3.70,	3.30,	2.90,
	2.30,	1.25,	1.20	
Distal nerve branch (section cc)	11.80,	10.10,	9.30,	9.10,
	8.35,	7.90,	6,40,	4.50,
	3.50,	3,40,	3.10,	
	2.80,	2.70,	1.60,	1.60,
	1.47,	1.46,		
	0.65,	0.35		

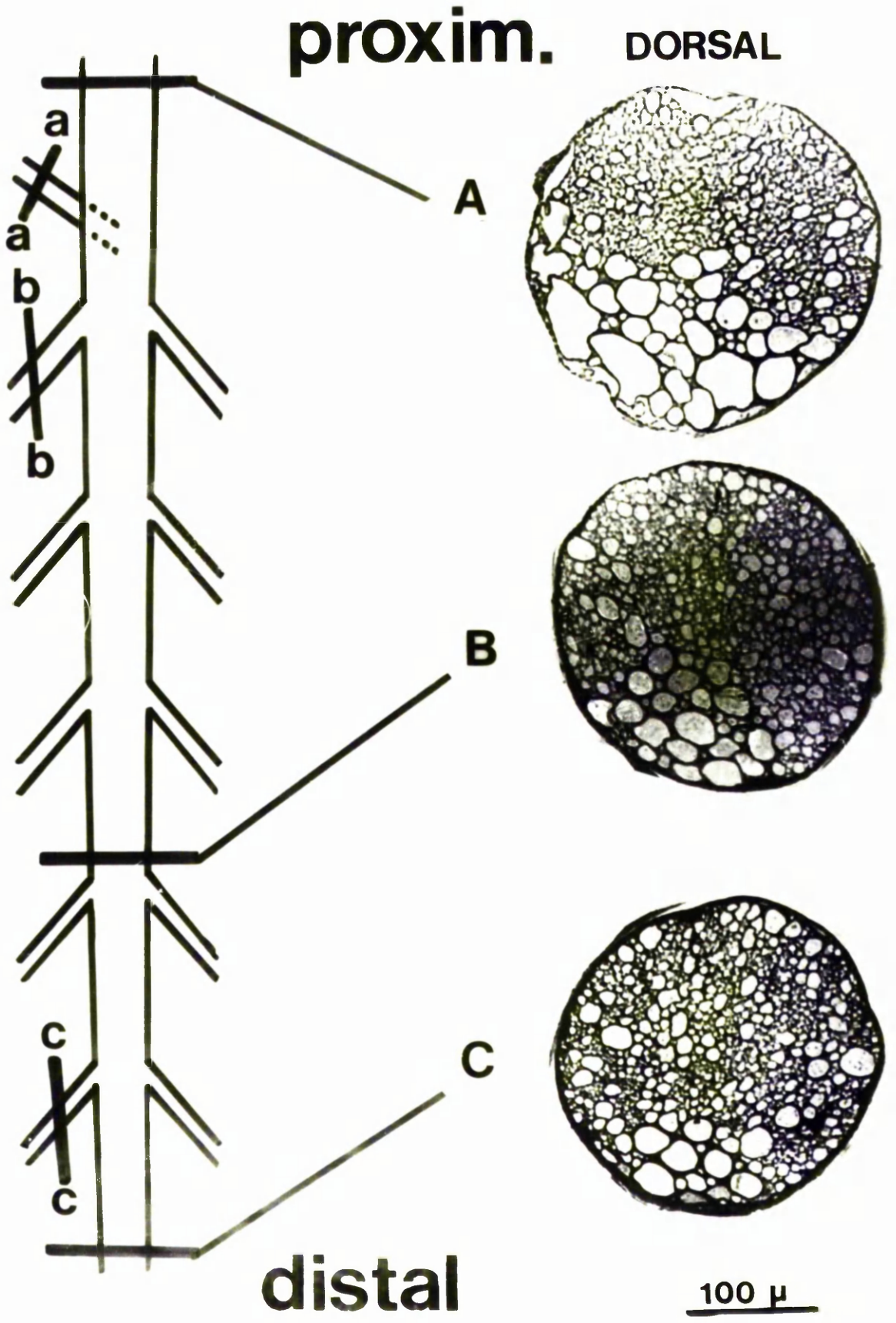
Fig. 3.7

Transverse sections for light microscopy taken from different levels of N5B2 as indicated in the diagram. This diagram also shows the flexor nerve branches from which thin sections for Transmission Electron Microscopy were taken.

Section (aa) The nerve branch to the proximal flexor

Section (bb) The nerve branch to the middle flexor

Section (cc) The main nerve branch of the distal flexor


proxim.
DORSAL
a
a
b
b
A
B
C
c
c
distal
100 µ

reasons for this reduction in diameter. 1) Some of the large axons (probably motor axons) innervating the proximal part of the muscle leave before the distal part and 2) there is a reduction in motor axon diameter when these axons reach the distal part of the nerve.

Nearly all the small axons are sensory and lie chiefly in the dorsal half of the proximal $N5B_2$ (section A). At the distal end of $N5B_2$ immediately after the last flexor nerve branch leaves, sections of the nerve reveal that sensory axons occupy most of the nerve area. Some of the large axons in this section could be the motor axons which innervate the tibial muscles (fig. 3.7, section C).

e. The flexor tibiae motoneurons (FlTiM)

To locate the cell bodies of the flexor motoneurons cobaltous chloride was perfused up $N5B_2$, to backfill these neurons. $N5B_2$ was cut 1mm distal to the middle nerve branch and the cut end was firmly sucked into the broken tip of a micropipette including the middle nerve branches (see Methods).

Most of the FlTiM (fig. 3.8) somata are very close together and they lie on the dorsal anterior side of the mesothoracic ganglion. It is very difficult to distinguish the members of clusters as described by Burrows and Hoyle (1973) in the metathoracic ganglion and Wilson (1977) in the mesothoracic ganglion. The number of motoneurons which can be stained using the above technique is in the range of 15-20 (10 different ganglia). This number includes also the tibial motoneurons. The two heavily stained cell bodies on the middle dorsal side of the ganglion are probably the two flexor inhibitors, as identified in the metathoracic ganglion by Burrows and Horridge (1974).

A disadvantage of this technique is that cobaltous chloride also fills the five tibial motoneurons and may possibly cross the axon membranes to fill other axons.

2. Innervation

The ideal technique for studying the innervation of the flexor tibiae muscle would be to identify the flexor tibiae motoneurons by passing current from an intracellular microelectrode into their somata and correlating the evoked somata spikes with extracellularly and intracellularly recorded events in the muscle. Using this technique Hoyle and Burrows (1973a, b) were able to identify six excitatory flexor tibiae motoneurons (FlTiM) in the large metathoracic ganglion. They found two fast motoneurons (FFlTiM), two intermediate (IMFlTi) and two slows (SFlTi) in an anterior and posterior cluster. The two inhibitory motoneurons were located by Burrows and Horridge (1974), the posterior inhibitor (PsInFlTiM) which has its soma between the midline and the root of N5, and the anterior inhibitor with its soma anterior to Common Inhibitor (CI). Wilson (1977) was able to fill with microelectrodes six excitatory flexor tibiae motoneuron somata in the locust mesothoracic ganglion but he did not investigate further the innervation of the flexor tibiae muscle.

The method described above was used to ensure that the stained motoneurons in fig. 3.8 were flexor tibiae motoneurons. Using the arrangement shown in fig. 3.9 recordings were made from the cell bodies of neurons which were found to innervate the flexor tibiae muscle. Although this technique produces much information about individual motoneurons, as a method for establishing the innervation of the flexor

Fig. 3.8

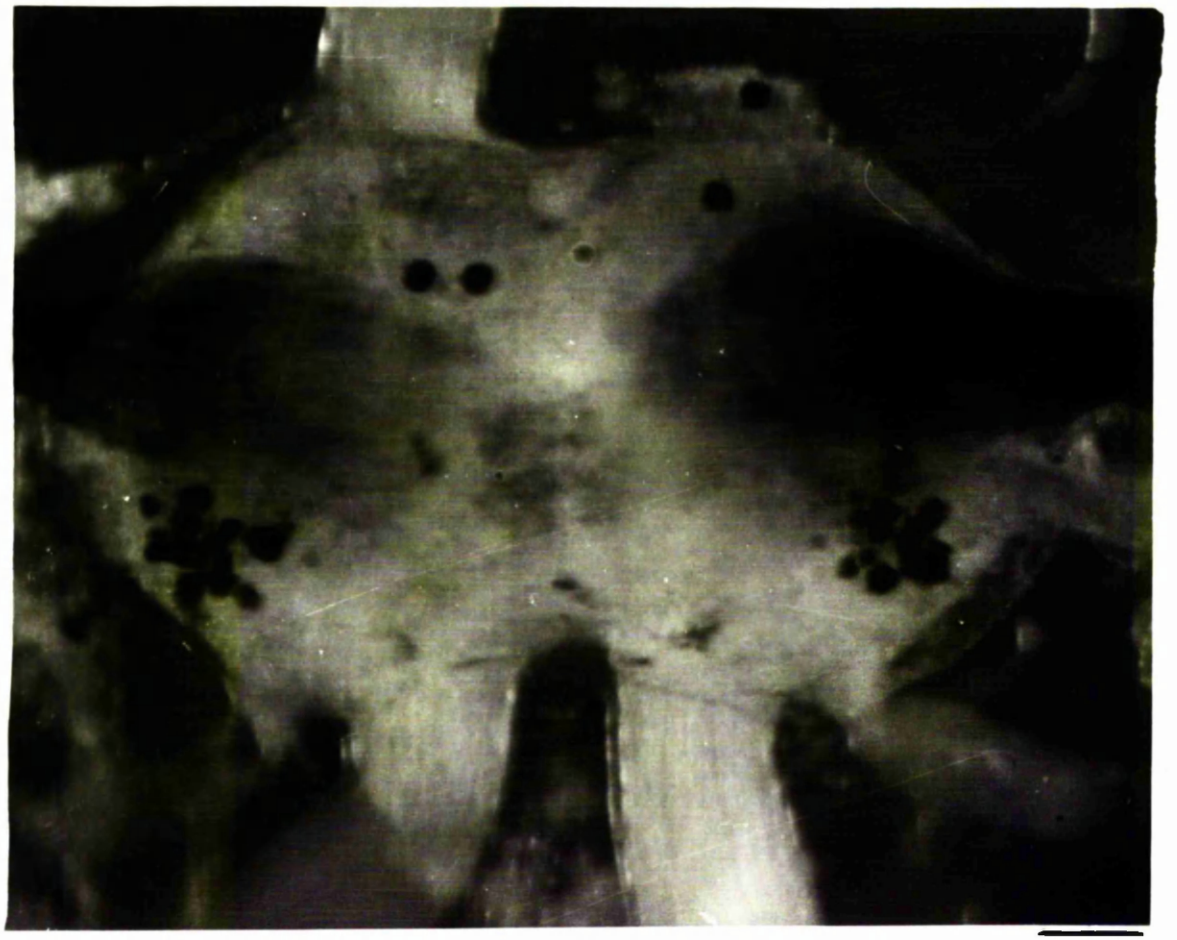
Photograph of a fixed, cleared, whole-mount preparation of the mesothoracic ganglion of Schistocerca gregaria viewed from the dorsal side. Most of the cell bodies of the flexor tibiae and tarsal motoneurons were back filled with $CoCl_2$ through N5B2. The positions of neurons are distorted owing to the pressures developed during fixation and mounting. This is especially true of the two inhibitors in the right side of the ganglion. The posterior part of the ganglion is at the top of the figure.

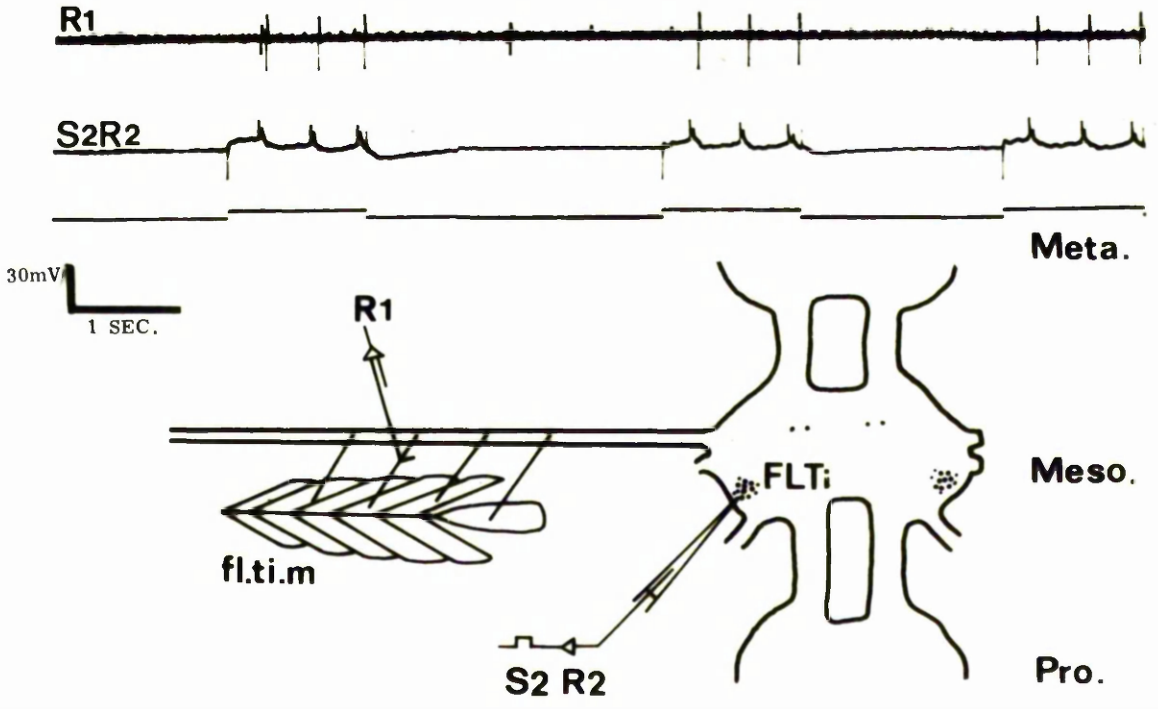
Fig. 3.9

Identification of a flexor motoneuron penetrated by a microelectrode and recording action potentials (R1) from the nerve branches of the flexor muscle (fl.ti.m). The motoneuron was activated by passing depolarising current through the microelectrode (S2 R2), monitored in the lowest trace.

FLT1 = flexor tibia motoneurons

R1 = extracellular records from the flexor nerve branches using a hook electrode.


200 µm


R1
S2R2
Meta.
30mV
1 SEC.
R1
FLTi
Meso.
fl.ti.m
S2 R2
Pro.

tibiae muscle, it suffers from the following disadvantages.

1) Most of the cell bodies lie very close together making it impossible to identify different flexor neurons from their position in the ganglion.
2) It is not possible to locate all the flexor motoneurons by probing with a microelectrode.
3) It is difficult to maintain stable records from the cell bodies for periods of time long enough to allow reliable investigation of the innervation of the muscle and for further behavioural studies.

As a result, this technique was not used.

Instead, the innervation pattern of the flexor motoneurons was accomplished using mainly two techniques.

1) In an isolated muscle, by stimulating peripherally the axon of the motoneurons and measuring the active tension increments generated in the distal flexor, while at the same time recording activity in the other two parts of the muscle with microelectrodes.
2) In an intact animal, by recording spontaneously occurring activity from the nerve branches which innervate the three different parts of this muscle.

In the first method, if a very sensitive tension transducer is used, this technique has the ability to detect most of the excitatory axons by the active tension increment which each of them produces in the muscle.

One of the problems with this method was the relaxation in the muscle fibres caused by the peripheral inhibitory axons. This phenomenon affects the tension records and makes the interpretation of these records difficult. This problem was solved by using one of the properties of the saline to diminish gradually the mechanical responses (Relaxation) to inhibitory stimulation of the muscle taken from starved

locusts. After 100 min in 10K saline, no relaxation can be recorded during inhibitory stimulation (Usherwood, 1968). This method, although it was not always sufficient, was preferable to eliminating relaxation with picrotoxin perfusion, since picrotoxin does not always perfuse properly between the muscle fibres and may affect the dondition of the muscle itself.

The equilibration time of 100 minutes was important not only for avoiding inhibitory effects but for the study of the excitatory axons. In the first 20 min after the immersion of the muscle in saline, the small axons appeared to have higher thresholds than the larger axons. Under these conditions tension produced by the larger excitatory axons masked the tension from the small axons and reduced the number of axons which could be identified to two or three. However, after 30 min twitch contractions caused by small axons could also be recorded and after 60 min records like those in fig. 3.11 could be obtained.

Generally the saline used reduced the peak active tension of the muscle by 3 to 5 times compared with that recorded under natural condition (see below). Occasionally when the muscle was bathed in saline no contractions could be recorded at all. In this case, the twitch contraction could be restored by adding more calcium chloride to the saline together with an appropriate reduction in the concentration of sodium chloride in order to maintain constant osmolarity. The effect of the Ca^{++} ion concentration on the size of the twitch contraction of the flexor tibiae muscle is illustrated in fig. 3.10. Similar effects were found by Aidley (1965) in the mesothoracic extensor muscle but in this case the maximum active tension appeared at 2 - 4mM Ca^{++}. At higher concentration than 2mM although the muscle produced higher peak contraction, the life time of the muscle in saline became shorter.

Fig. 3.10

The relationship between the maximum muscle twitch amplitude and the concentration of Ca^{++} in the saline. Tension was monitored from the distal flexor only.
Bars show ± 1 standard deviation. Data from 10 animals.

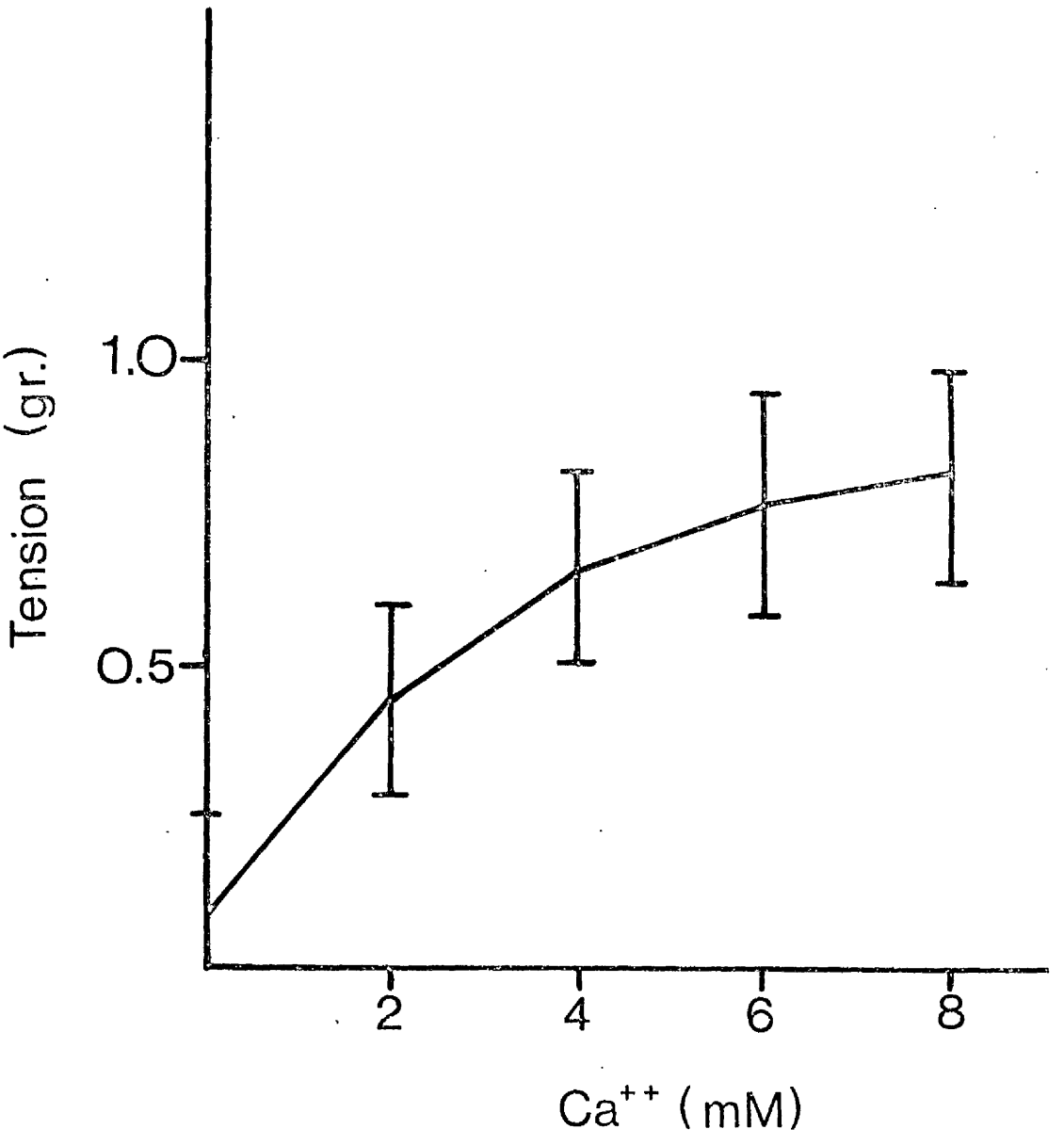

Tension (gr.)
1.0
0.5
2
4
6
8
Ca++ (mM)

Fig. 3.11

Twitch contractions separately recorded from the Proximal (P), the Middle (M) and the Distal (D) flexors. The flexor muscle was equilibrated with saline and the motor axons responsible were excited with single shocks to N5. To monitor tension only from one of the three parts of the muscle, the other two were completely denervated while tension was always recorded from the distal end of the flexor apodeme, as illustrated in the diagram (T. Tr).

Time and tension calibration for record D are the same as in record P.

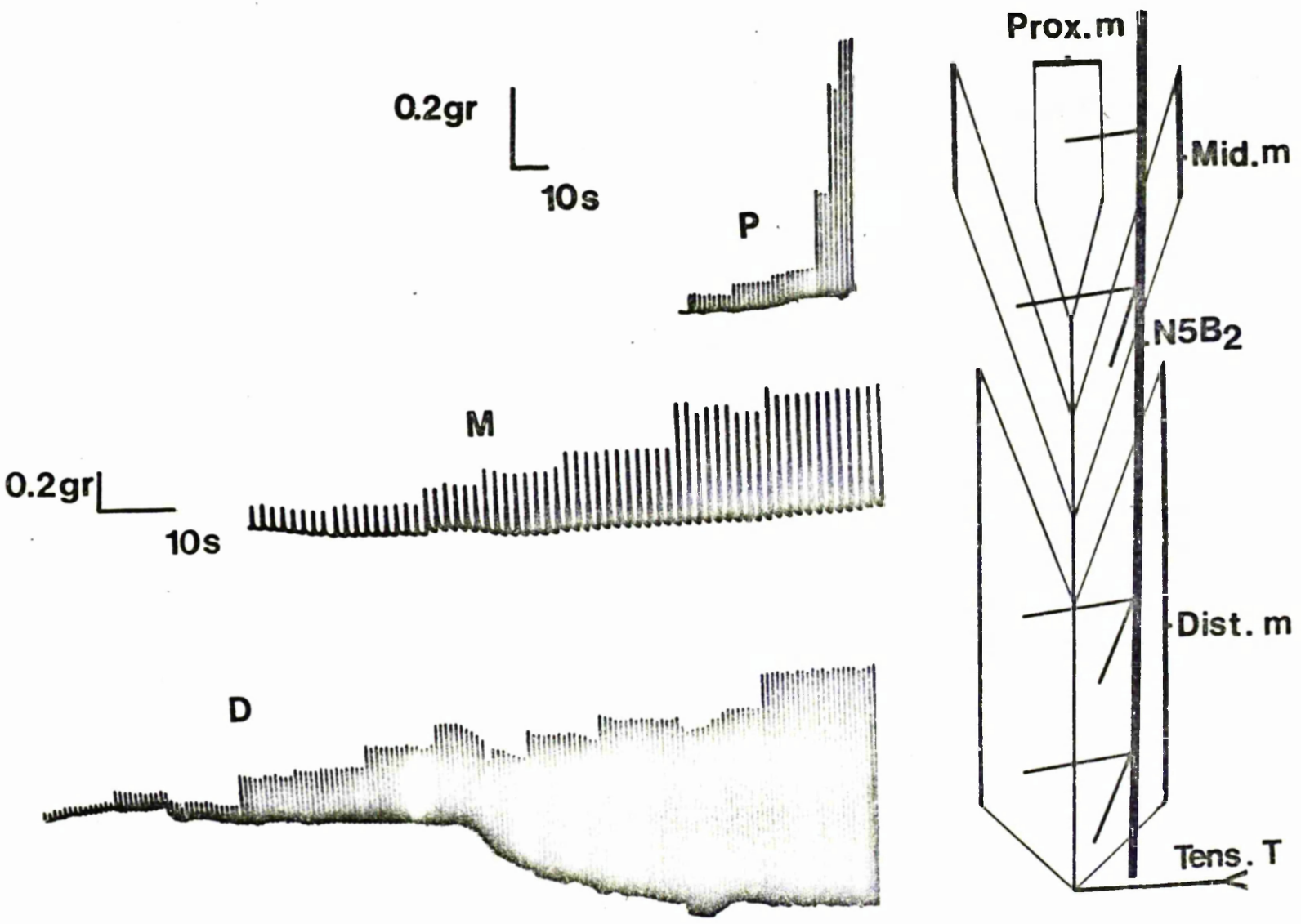

0.2gr
10s
P
M
0.2gr
10s
D
Prox.m
Mid.m
N5B2
Dist. m
Tens. T

Fig. 3.12

Diagrammatic representation of the distribution of the axons innervating the mesothoracic flexor tibiae muscle.

F = Fast axons

S = Slow axons

D = DUM axons

M = intermediate axons

I = inhibitory axons

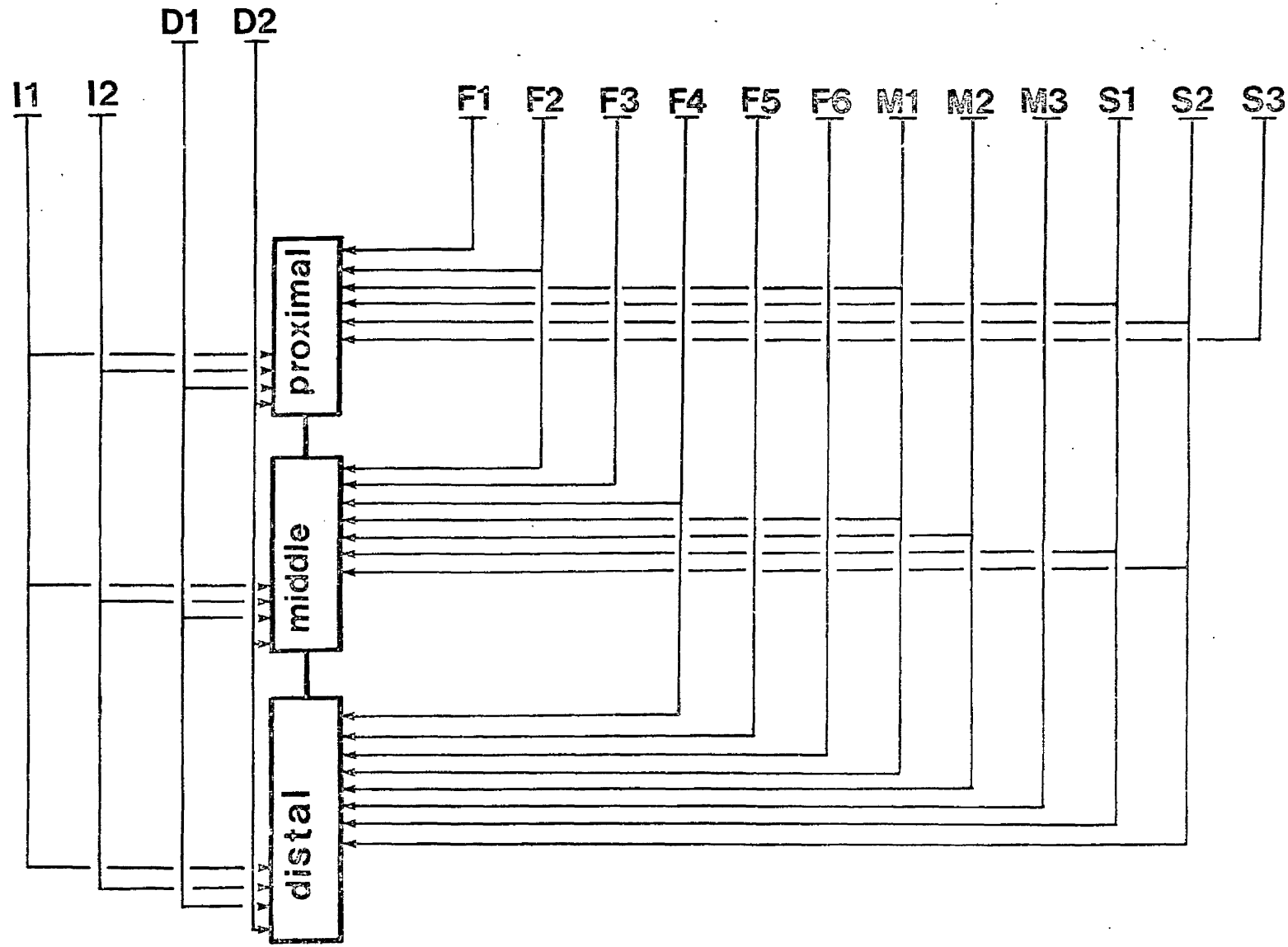

I1
I2
D1
D2
F1
F2
F3
F4
F5
F6
M1
M2
M3
S1
S2
S3
proximal
middle
distal

Table 3.4.

The number of excitatory axons to each part of the mesothoracic flexor tibiae muscle identified by the sizes of the twitch contractions they produced. Records taken from seven different animals.

Table 3.5.

Typical amplitudes of the postsynaptic potentials (PSP's) produced by the flexor axons.
Resting potential = 62 ± 10 mV.

Table 3.4.

	Number of Excitatory axons	Mode
Proximal flexor	6 7 5 6 6 5 7	6
Middle flexor	5 7 6 7 8 7 6	7
Distal flexor	9 8 8 7 8 6 8	8

Table 3.5

	PSP mV		PSP mV
F1	40 - 50	S1	2 - 4
F2	30 - 35 (5 - 10)	S2	3 - 5
F3	35 - 40	S3	5 - 10
F4	20 - 30 (10-15)	I1	- (1 - 2)
F5	20 - 25	I2	- (2 - 4)
F6	35 - 40		
M1	15 - 20 (5 -10)		
M2	10 - 15		
M3	10 - 15		

Having eliminated the effect of the inhibitory axons on the flexor muscle, the number only of the excitatory axons innervating the three parts of the muscle was established by graded stimulation of each part through N5 with single shocks (0.2ms long). The peak of the muscle active tension which was recorded through the tendon from each part of the muscle (fig. 3.11) increased in size in jumps which reflect the threshold of the axons which innervate the muscle. At the same time excitatory post synaptic potentials (EPSP's) were recorded from the fibres of the stimulated part of the muscle to establish that an active tension increment occurred for each EPSP recorded. This was necessary because of the possibility that some axons may cause tension increments too small to be detected. Using this technique of stimulation, only the excitatory axons can be demonstrated and the variation in the number of these axons counted in the three parts of the muscle was relatively small (Table 3.4). To investigate the possibility that some of the excitatory axons innervate only specific parts of the flexor muscle tension was recorded only from one part of the muscle (usually the distal) while microelectrodes penetrated fibres in other parts of the muscle, looking for EPSP, which were not correlated with the recorded active tension increments. The identification and the topography not only of the excitatory axons but also of the inhibitory was established by examining the size of spontaneously occurring action potentials in correlation with simultaneously recorded EPSP's or IPSP's (in haemolymph). The dorsal unpaired median (DUM) neurons were investigated by stimulating the contralateral nerve.

At that stage it was found necessary, for classification purposes, to separate the different axons according to the EPSP which they produce. Axons which innervate the metathoracic flexor tibiae

muscle were classified as Fast, Intermediate and Slow by Burrows and Hoyle (1973). Accordingly the excitatory axons which innervate the mesothoracic flexor muscle were defined as:

Fast (F): EPSP's which are within the range of 20 - 50 mV. They are usually suprathreshold and activate electrically excitable muscle membrane producing a fast rising potential. Similar fast responses have been recorded in many insect muscles (see reviews by Hoyle, 1965; Usherwood, 1967; also Hoyle, 1955 ; Usherwood, 1962).

Intermediate (M): EPSP's are depolarizations which are in the range of 10 - 20 mV.

Slow (S): Slow rising EPSP's within the range of 1 - 10 mV. They are produced by neurons which are spontaneously active.

Numbers were given to identify motor axons. This classification is not suitable for some flexor motor axons which taper (see axon F4). A similar phenomenon was described by Burns (1972) for the FETi in the mesothoracic extensor tibiae muscle and by Hoyle (1955) for the FETi in the metathoracic extensor.

Using all the above techniques and combining them, the final pattern of the innervation of the flexor muscle was established (fig. 3.12, Table 3.5). Detailed descriptions of the individual axons innervating each part of the flexor muscle are given below. These results were summarized from records obtained in 50 different animals and each axon was identified at least 5 times before being finally classified.

Fast 1 (F1).

This is the largest axon to the flexor muscle and innervates only the proximal flexor (fig. 3.12, Table 3.5). This was established

Fig. 3.13

a to c, Records from the mesothoracic flexor tibiae muscle of an isolated leg bathed in saline. The flexor axons were stimulated through the cut end of N5 in the coxa.

a and b. 1st trace: Intracellular records from the proximal flexor (see f for location).

2nd trace: Muscle tension recorded from only the middle and distal flexors. To avoid stimulating activity of the smaller axons these records were obtained in the first 30 min from the moment which the muscle was perfused with saline.

c, d. 1st trace: Intracellular records from the proximal flexor.

2nd trace: Tension record from the proximal flexor only Records were taken after the muscle was equilibrated with with the saline.

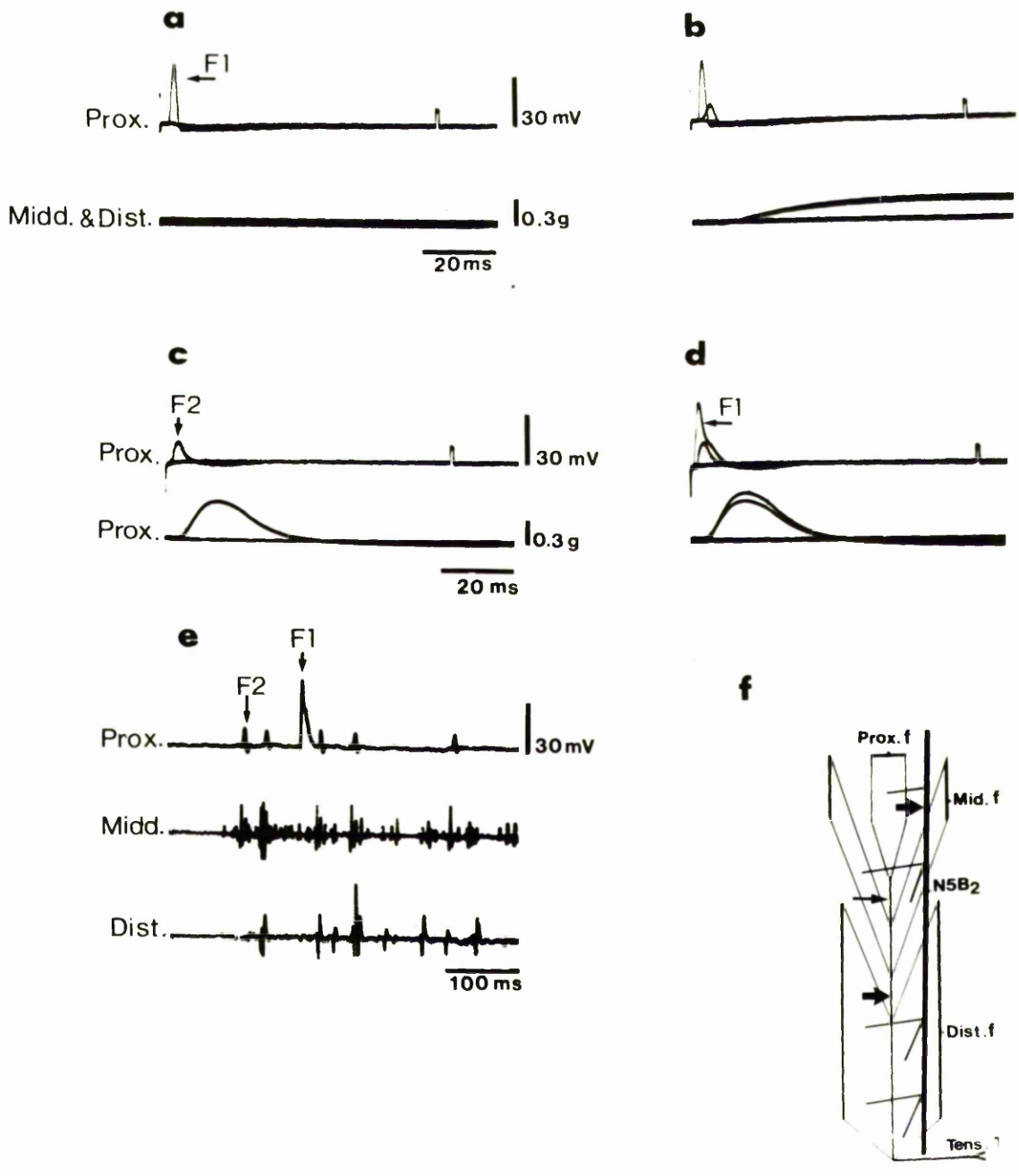
e. Records from the mesothoracic flexor tibiae muscle in a dissected femur of an immobilized locust. The femur was filled with haemolymph and a small amount of added saline. The flexor motoneurons were reflexly excited by tibial extension.

1st trace: Intracellular record from the proximal flexor

2nd trace: Extracellular record from the middle nerve branch

3rd trace: Extracellular record from the main distal nerve branch.

f. Diagrammatic representation of the proximal flexor (Prox. f), middle flexor (Mid. f) and distal flexor (Dist. f). Tens. T = the transducer attached to the end of the apodeme. Arrows indicate where the apodeme or N5B2 was cut as explained in the text.


a
F1
Prox.
30 mV
Midd. & Dist.
0.3g
20ms
b
c
F2
Prox.
30 mV
Prox.
0.3 g
20 ms
d
F1
e
F1
F2
Prox.
30mV
Midd.
Dist.
100 ms
f
Prox. f
Mid. f
N5B2
Dist. f
Tens.

by stimulating the axon through N5 and recording EPSP's from the proximal muscle fibres (fig. 3.13a, b). At the same time tension was monitored only from the middle and distal flexors by cutting the flexor apodeme between the middle and proximal flexors (see small arrow in fig. 3.13f). When the stimulus reaches the threshold of F1 a large EPSP is produced in the proximal muscle fibres but no tension movements are induced in the rest of the flexor muscle. However, when the stimulus reaches the threshold of a common axon a tension increment appears in the middle and distal muscle at the same time as an EPSP is produced in the proximal muscle fibres. The nature of the EPSP produced by axon F1 and the tension which this axon induces in the proximal flexor are shown in fig. 3.13c, d. In this case the tension produced only by the proximal flexor was monitored through the flexor apodeme by denervating the rest of the muscle. The twitch with the lower threshold than F1, was produced by axon F2 as will be explained below.

A series of penetrations of the proximal muscle fibres showed that F1 does not innervate more than 30% of the proximal flexor.

The fact that F1 innervates only the proximal flexor was confirmed by recording from the flexor muscle in a dissected femur of an immobilized locust (see Methods). When the animal was mechanically stimulated a large EPSP appeared in the proximal muscle fibres which did not correspond with any of the action potentials recorded in the middle and distal nerve branches (fig. 3.13e).

Fast 2 (F2).

This axon innervates only the proximal and middle flexors (fig. 3.12, Table 3.5). This was established by recording EPSP's in the proximal flexor and action potentials from the

middle and distal nerve branches of a fixed animal. Relatively large EPSP's appear in the proximal muscle fibres and correspond with some of the action potentials recorded in the middle nerve branch (fig. 3.14a). This suggests that axon F2 innervates both middle and proximal flexors. The absence of this axon in the distal flexor was established by simultaneously recording action potentials from the middle and distal nerve branches (fig. 3.14b). No action potentials were found in the distal branches corresponding with the EPSP's or action potentials produced by axon F2. EPSP's from axon F1 can also be seen in fig. 3.14a.

Intracellular recordings show that F1 and F2 are the only large axons innervating the proximal flexor. The fact that F2 has a large diameter in the proximal nerve branches (20.50, fig. 3.6aa) and the large action potential which this axon produces in the middle nerve branches (fig. 3.14a b) suggest that this axon must have a large diameter in the middle nerve too. In insects it was found by Pearson, Stein and Malhotra (1970) that the action potential recorded from an axon is related to the diameter of this axon ($d = 5.7\sqrt{V_3}$, d = diameter of axon, V_3 = peak to peak amplitude of a triphasic action potential). Transverse sections of the middle nerve branches reveal only two large axons with effective diameter of 23.50μm and 19.90μm. The most obvious candidate for F2 is the smaller axon due to the similarities in the effective diameter with the axon in the proximal nerve branch.

The innervation pattern of F2 on the flexor muscle can also be studied by recording EPSP's and active tension from various parts of an isolated flexor muscle in saline. The motor axons were electrically stimulated through N5. Three EPSP's were recorded in two different proximal muscle fibres (fig. 3.14c). The larger EPSP (40 mV) with the higher threshold is due to F1 and the other large EPSP (35 mV) due to F2.

Fig. 3.14

a and b. 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 added saline. The flexor motoneurons were reflexly excited by tibial extension.

1st trace: Intracellular record from the proximal flexor (see g for location)

2nd trace: Extracellular record from the middle nerve branch

3rd trace: Extracellular record from the main distal nerve branch. (b only)

c to f. Records from the mesothoracic flexor tibiae muscle bathed in saline. The flexor axons were stimulated through the cut end of N5 in the coxa.

c. 1st and 2nd trace: Intracellular records from two fibres of the proximal flexor.

3rd trace: Muscle tension recorded from only the distal flexor (see g for location)

d. 1st and 3rd trace: as in c.

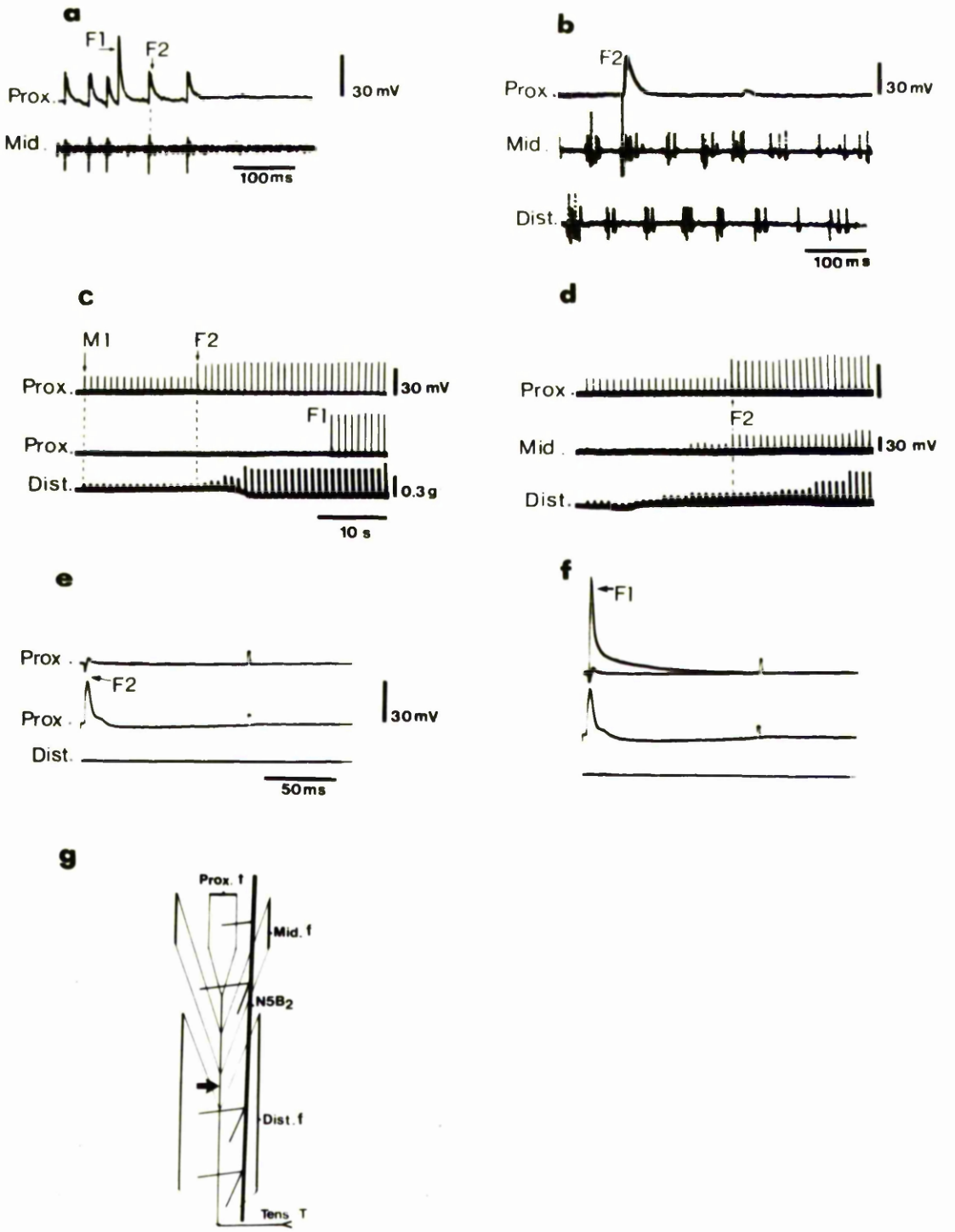
2nd trace: Intracellular record from the middle flexor

The muscle was left to equilibrate with the saline.

e, f. 1st and 2nd trace: Intracellular records from two fibres of the proximal flexor.

3rd trace: Muscle tension from the distal flexor. This experiment was undertaken immediately after the muscle was immersed in saline to eliminate activity from the smaller axons.

g. Diagrammatic representation of the mesothoracic flexor tibiae muscle. Arrow indicates the point were the flexor tendon was cut to allow tension records to be made from only the distal flexor.


a
F1
F2
Prox.
Mid.
30 mV
100 ms
b
F2
Prox.
Mid.
Dist.
30 mV
100 ms
c
M1
F2
Prox.
30 mV
F1
Prox.
Dist.
0.3 g
10 s
d
Prox.
F2
Mid.
30 mV
Dist.
e
Prox.
F2
Prox.
30 mV
Dist.
50 ms
f
F1
g
Prox. f
Mid. f
N5B2
Dist. f
Tens T

This identification is based on the fact that F1 and F2 do not innervate the distal flexor. No increase in active tension appears in the distal part of the muscle when either of these axons are stimulated. Tension from the distal muscle was monitored by cutting the apodeme between middle and distal flexors (fig. 3.14g). To show that F2 also innervates the middle flexor EPSP's were recorded from this part (fig. 3.14d). It is clear that two EPSP's with the same threshold (see dotted line) appeared in fibres of the proximal and middle muscles but the axon responsible for them does not produce any active tension in the distal flexor.

Axon F2 usually produces a large EPSP and does not innervate more than 70% of the proximal and middle flexors, as random penetration of these muscle fibres showed. Occasionally axon F2 innervates muscle fibres which also receive nerve endings from axon F1. In this case F2 behaves more like a slow axon producing a small EPSP. Figure 3.14e shows an extreme case where in two different muscle fibres axon F2 produces a fast and a slow EPSP at the same time. The fact that the muscle fibre in the first trace is also innervated by axon F1 is shown in fig. 3.14f. This phenomenon is also demonstrated in fig. 3.13c d where F2 produces a small EPSP (10 mV) but induces a large active tension increment. In this record spikes from F1 appear at a higher stimulus intensity.

Fast 3 (F3).

This is a large axon which innervates only the middle flexor (fig. 3.12, Table 3.5). This part of the flexor muscle usually receives its motor axons through a single pair of nerve branches. Transverse sections of these branches (fig. 3.6, sect. bb) show that in each nerve

there are two large axons and a few other axons of significantly smaller diameter. One of the large axons in the middle nerve branch has already been identified at F2. The other large axon is F3. To establish its innervation pattern, action potentials from the middle and distal nerve branches and EPSP's from the fibres of the middle muscle were recorded. In the middle nerve (fig. 3.15A) two large action potentials can be seen after strong mechanical stimulation of different parts of the animal. The action potential produced by the most active axon are from F2. The other action potential which usually appears once or twice is that of axon F3. This does not innervate the distal flexor because it does not produce any action potentials in the nerve to this part of the muscle (fig. 3.15A third trace). Random penetrations of the proximal muscle fibres also failed to show any EPSP's correlated with the action potentials of F3 recorded from the middle nerve branches.

It was found that the EPSP's of axon F2 are very small when they appear in the same muscle fibres as the large EPSP's from F3. This is similar to the situation in the proximal flexor when F2 innervates the same fibres as the large axon F1.

The fact that F3 innervates only the middle flexor can also be demonstrated by recording active tension only from the distal flexor and EPSP's from the proximal and middle parts of an isolated flexor muscle. Although when all the flexor motor axons are stimulated through N5 active tension increments can be seen in the distal flexor (fig. 3.15B third trace), when axons F2 and F3 are recruited no increase in tension occurs in the distal flexor. This shows that axons F2 and F3 do not innervate this part of the flexor muscle. Records taken simultaneously from the proximal muscle fibres (first trace fig. 3.15B) reveal that one of the two EPSP is common with one in the proximal flexor and this

Fig. 3.15

A. 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 added saline. The flexor motoneurons were reflexly excited by tibial extension.

1st trace: Intracellular record from the middle flexor (see E for locations)

2nd trace: Extracellular record from the middle nerve branche

3rd trace: Extracellular record from the main distal nerve branch

B to D Records from an isolated mesothoracic flexor tibiae muscle bathed in saline. The flexor axons were stimulated through the cut end of N5 in the coxa.

B 1st trace: Intracellular record from proximal flexor

2nd trace: Intracellular record from the middle flexor

3rd trace: Muscle tension from only the distal flexor (see E). The muscle was left to equilibrate in saline before any experiments were undertaken

C, D 1st and 2nd trace: Intracellular records from middle flexor.

3rd trace: Muscle tension from the distal flexor.

The records were taken before the muscle equilibrated with the saline to avoid twitches from smaller axons as described in the text.

E. Diagrammatic representation of the different parts of the flexor muscle and its nerve branches. Arrow shows the point where the flexor tendon was cut to allow the muscle tension records to be taken from the distal flexor alone.

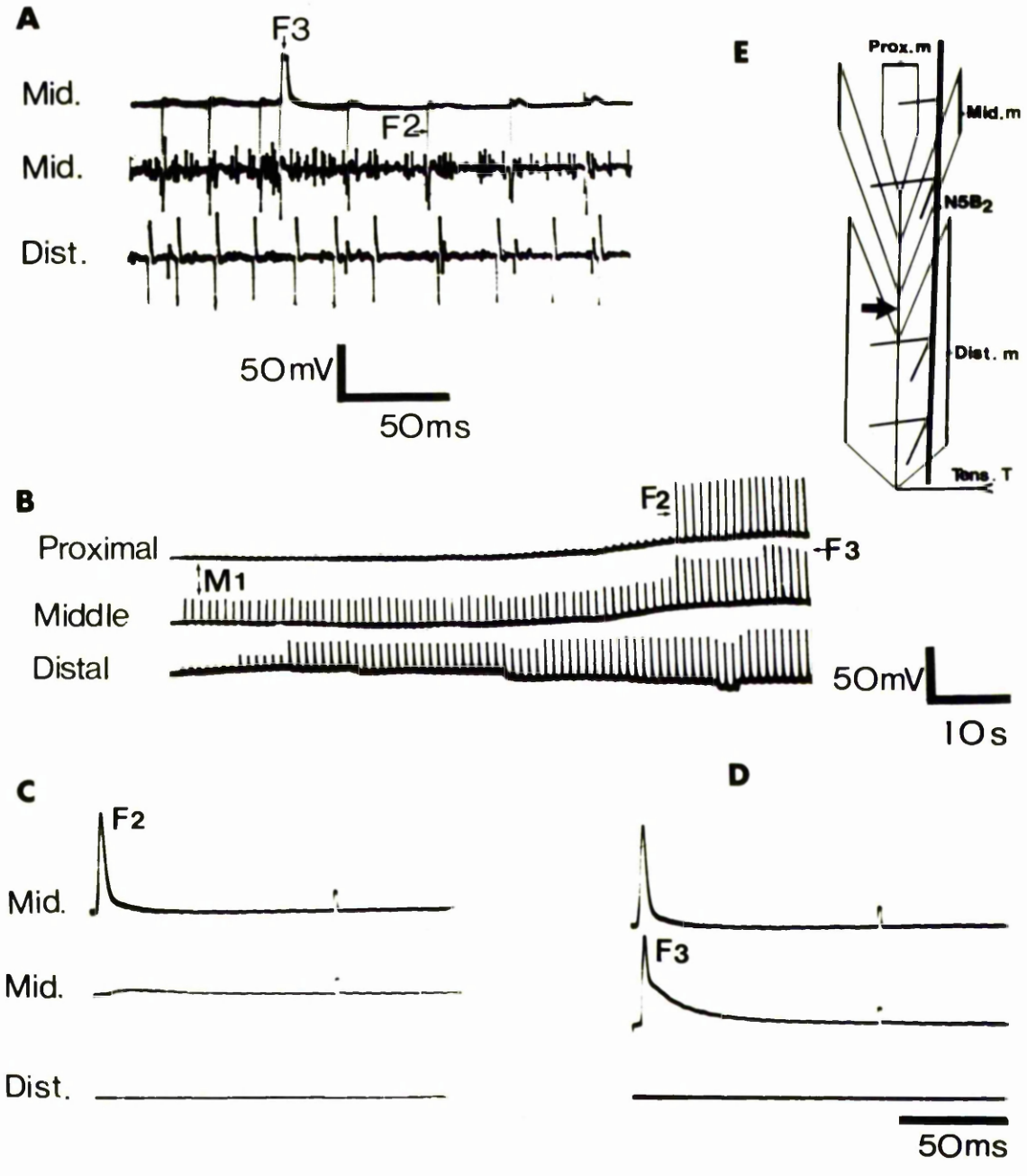

A
F3
Mid.
F2
Mid.
Dist.
50mV
50ms
E
Prox.m
Mid.m
N5B2
Dist. m
Tens. T
B
F2
Proximal
F3
M1
Middle
Distal
50mV
10s
C
F2
Mid.
Mid.
Dist.
D
F3
50ms

Fig. 3.16

Records from the mesothoracic flexor tibiae muscle in the dissected femur of an immobilzed locust. The femur was filled with haemolymph and a small amount of added saline. The flexor motoneurons were reflexly activated.

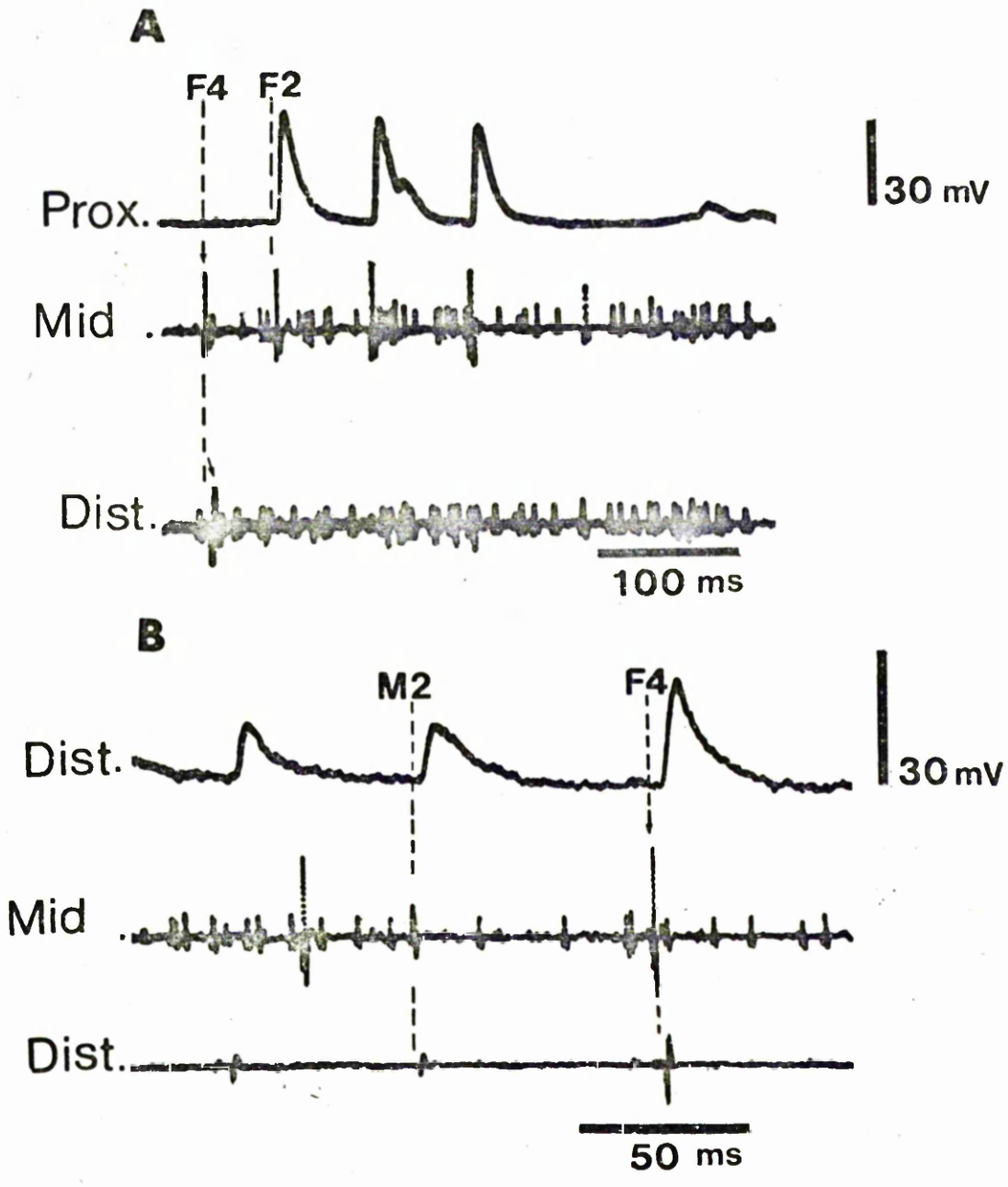
A. 1st trace: Intracellular records from a proximal muscle fibre.

2nd trace: Extracellular record from the middle nerve branch

3rd trace: Extracellular record from the distal nerve branches.

B. 1st trace: Intracellular record from the distal flexor

2nd and 3rd trace: as in A.


A
F4
F2
Prox.
Mid
Dist.
30 mV
100 ms
B
M2
F4
Dist.
Mid
Dist.
30 mV
50 ms

axon has been identified as F2. The EPSP with the higher threshold is produced by axon F3, since no EPSP's could be found in the proximal flexor occurring simultaneously with the middle muscle potentials.

The nature of the EPSP's F2 and F3 produce in the middle flexor fibres can be seen in fig. 3.15C, D. The first EPSP is due to axon F2 and the other with the higher threshold is produced by F3. These axons were identified by the fact that they do not produce any increase in active tension at the distal flexor.

Fast 4 (F4).

This axon innervates only the middle and distal flexors (fig. 3.12, Table 3.5). This was found by recording spontaneously occurring action potentials from the middle and distal nerve branches and EPSP's from the proximal muscle fibres. In the middle nerve branch (fig. 3.16A) axon F4 produces a relatively large action potential which can easily be distinguished from that of axon F2.(Axon F2 produces the fast EPSP's in the proximal muscle fibres). Axon F4 also produces a smaller action potential in the distal nerve branches suggesting that this axon tapers towards the distal part of the muscle. This has also been found in the FETi of the mesothoracic extensor muscle (Burns, 1972). EPSP's from axon F4 in the distal muscle fibres are shown in fig. 3.16B. This motoneuron is not very active and can be recruited only by strong stimulation of the body.

Fast 5 and 6 (F5, F6), Intermediate 3 (M3).

The identification of these three axons was based on the fact that they innervate only the distal flexor (fig. 3.12, Table 3.5). Records demonstrating this are shown in fig. 3.17A where no large action potentials common to middle and distal flexor nerve branches can be seen.

Three action potentials can be distinguished in the distal nerve branches. The largest action potential is produced by the axon called F6 (see arrows in fig. 3.17A) while the immediately smaller action potentials are from F5 and the third smaller action potentials are produced by axon M3. EPSP's recorded from these axons in the distal muscle fibres are shown in fig. 3.17B. Although there are similarities in size between the action potentials produced by F5 and M3, the M3 was classified as intermediate due to the fact that it produces a smaller EPSP.

Intracellular records taken by random penetration of the distal muscle fibres shows that axon F6 innervates approximately 30% of the distal flexor, axon F5 40-50% and axon M3 60-70%.

The fact that the distal flexor receives three axons which do not supply the middle muscle was also demonstrated by stimulating the flexor motor axons through N5 in an isolated leg. Activity of the excitatory motor axons to the middle flexor was displayed by recording EPSP's from two different middle muscle fibres. Activity of the distal excitatory motor axons was simultaneously demonstrated by recording the active tension increment produced when the threshold of each excitatory axon was reached during gradient stimulation (fig. 3.17C). The two large EPSP's in the middle muscle fibres are caused probably by axons F2 and F3. It has been shown that these axons do not innervate the distal flexor and this is obvious in the record because no EPSP's produced by F2 and F3 can be correlated with any distal active tension increments. The common axons between middle and distal flexor may be F4 and M2 but there is not enough information to identify these two common axons precisely. Finally in the same record it is clear that there are three large active tension increments in the distal flexor which do not correspond with any EPSP's recorded in the middle muscle. These results in combination with the

Fig. 3.17

A and B. Records from the mesothoracic tibiae muscle in a dissected femur of an immobilized locust. The femur was filled with haemolymph and a small amount of added saline. The flexor motoneurons were reflexly excited by tibial extension.

A. 1st trace: Intracellular record from the proximal muscle fibre (see E for locations).

2nd trace: Extracellular record from the middle nerve branch.

3rd trace: Extracellular record from the main distal nerve branch.

The dashed line indicates the tibial extension movement (angular velocity of $150^{o}/_{s}$).

B. 1st trace: Intracellular record from the distal flexor.

2nd trace: Extracellular record from the distal nerve branch.

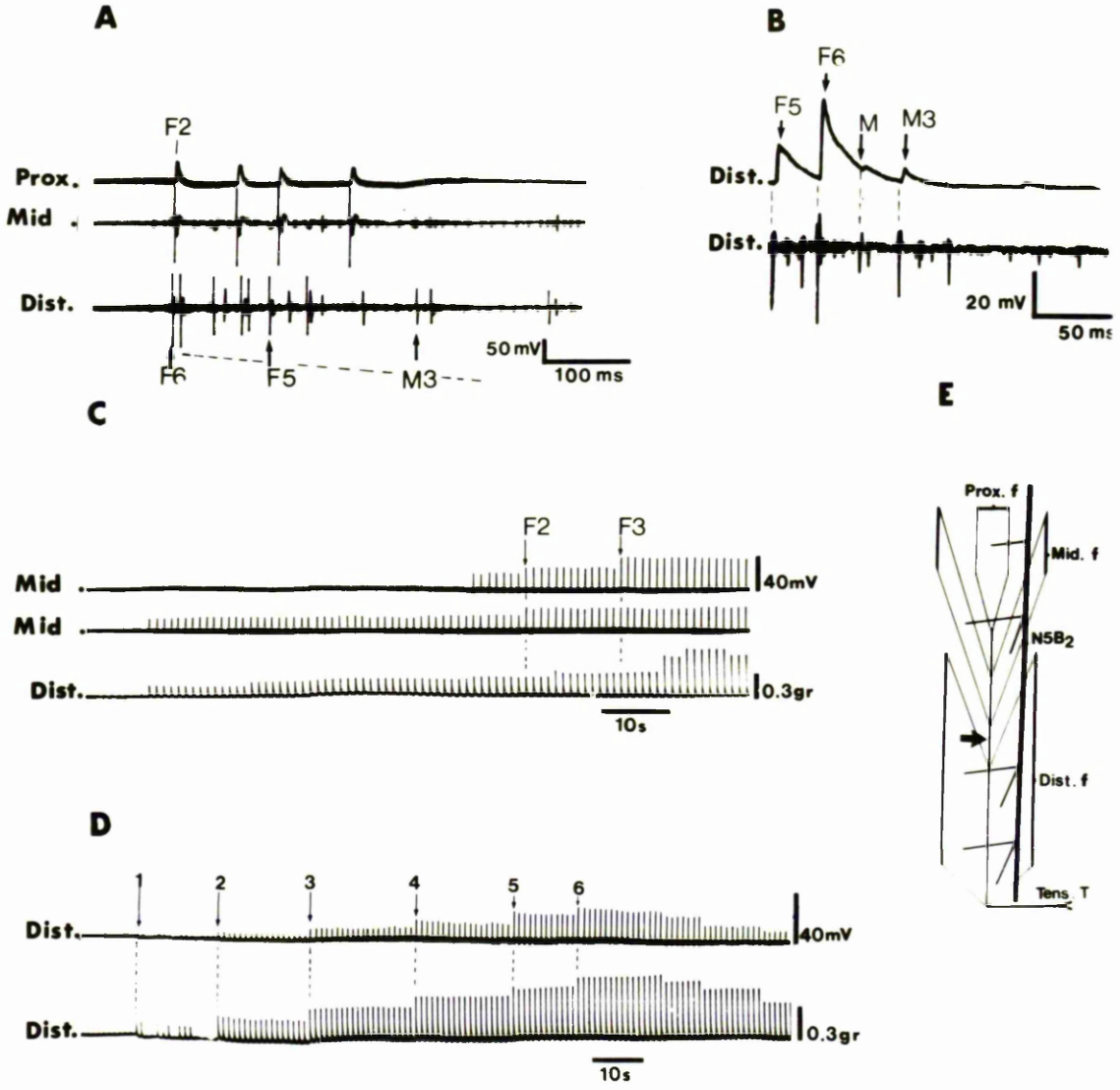
In most case records from the middle nerve branch, which are not shown here, were obtained to ensure that the recorded action potentials and EPSP's were not correlated with any activity in the middle muscle.

C and D. Records from an isolated mesothoracic flexor tibiae muscle equilibrated with the saline. The flexor axons were stimulated through the cut end of N5 in the coxa.

1st and 2nd trace: Intracellular records from two different muscle fibres in the middle flexor

3rd trace: Changes in muscle tension recorded from only the distal flexor by cutting the tendon between middle and distal flexor (see arrow in E).

E. Diagrammatic representation of the flexor muscle and its motor nerve.


A
F2
Prox.
Mid
Dist.
50 mV
100 ms
F6
F5
M3
B
F6
F5
M
M3
Dist.
Dist.
20 mV
50 ms
C
F2
F3
Mid
Mid
Dist.
40mV
0.3gr
10s
D
1
2
3
4
5
6
Dist.
Dist.
40mV
0.3gr
10s
E
Prox. f
Mid. f
N5B2
Dist. f
Tens. T

records in fig. 3.17A confirm that the distal flexor is exclusively innervated by three relatively large axons, F5, F6 and M3.

The ability of this technique to demonstrate the one to one relationship between a series of EPSP's and active tension increments is shown in fig. 3.17D. Active tension increments from the whole distal flexor are correlated with EPSP's produced by six different excitatory axons in one single distal muscle fibre. The unusual phenomenon in this case is that all six axons innervate the same muscle fibre, in contrast with the proximal and middle muscle fibres which were found to receive endings from a maximum of three excitatory axons.

Intermediate 1 (M1).

This is an axon which innervates mainly the proximal flexor but also gives smaller branches to the middle and distal flexors (fig. 3.12, Table 3.5).

In transverse sections of the proximal nerve branch there can be seen two large axons, F1 and F2 with diameters of 25.50μm and 20.20μm (fig. 3.6. and Table 3.3). There are also three very small axons, the largest having a diameter of 2.20μm and there is an axon which has an effective diameter 8.80μm which is intermediate between these two extremes. Tension recorded only from the proximal flexor showed six active tension increments when this part of the muscle was stimulated through N5. The two largest increments are due to axons F1 and F2, the small increments are probably produced by the three small axons. The twitch contraction intermediate between the large and the small active tension increments was caused by an axon called Intermediate 1 which is probably the 8.8μm diameter axon. Random penetration of the proximal muscle fibres shows that this intermediate twitch contraction is preceded by an EPSP with a maximum height of 15mV.

To establish the innervation pattern of this axon EPSP's and active tension were recorded in an isolated leg. A relatively small EPSP of 15 mV (fig. 3.14c) appears in the proximal muscle fibres, having the same threshold as a weak twitch contraction induced by the same axon in the distal flexor. The two large EPSP's in this figure have been already identified as from F1 and F2. Similar records are shown in fig. 3.15B where axon M1 produces a small EPSP in the proximal muscle fibres, an EPSP of 15 mV in the middle flexor and a small characteristic active tension increment in the distal flexor. This shows that M1 innervates most of the parts of the flexor muscle. The fact that axon M1 produces a small active tension increment in the distal flexor suggests that 1) M1 produces a very small EPSP in the distal flexor or 2) that M1 innervates very few of the muscle fibres in the distal flexor, or both. Due to the small size of this axon and the large number of axons in the distal branches it was very difficult to obtain evidence which could give a direct answer.

Due to the variation in the size of the EPSP's produced by M1, it was found necessary to confirm these results with records taken from the flexor nerve branches of a fixed locust during general stimulation of the animal. For this purpose a micrcelectrode was inserted in the proximal flexor while action potentials were recorded as usual from the middle and distal flexor nerve branches. EPSP's in the proximal muscle fibres (fig. 3.18a) can be correlated with small action potentials recorded in the distal nerve branches. Although M1 also innervates the middle flexor, no action potentials can be seen in the nerve branches to this part of the muscle (second trace). Often the branch of axon M1 which innervates the middle flexor does not run through the middle nerve branch but through other branches so that action potentials produced by

Fig. 3.18

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 added saline. The flexor motoneurons were reflexly excited by tibial extension.

a, b. 1st trace: Intracellular record from the proximal flexor (see d for locations).

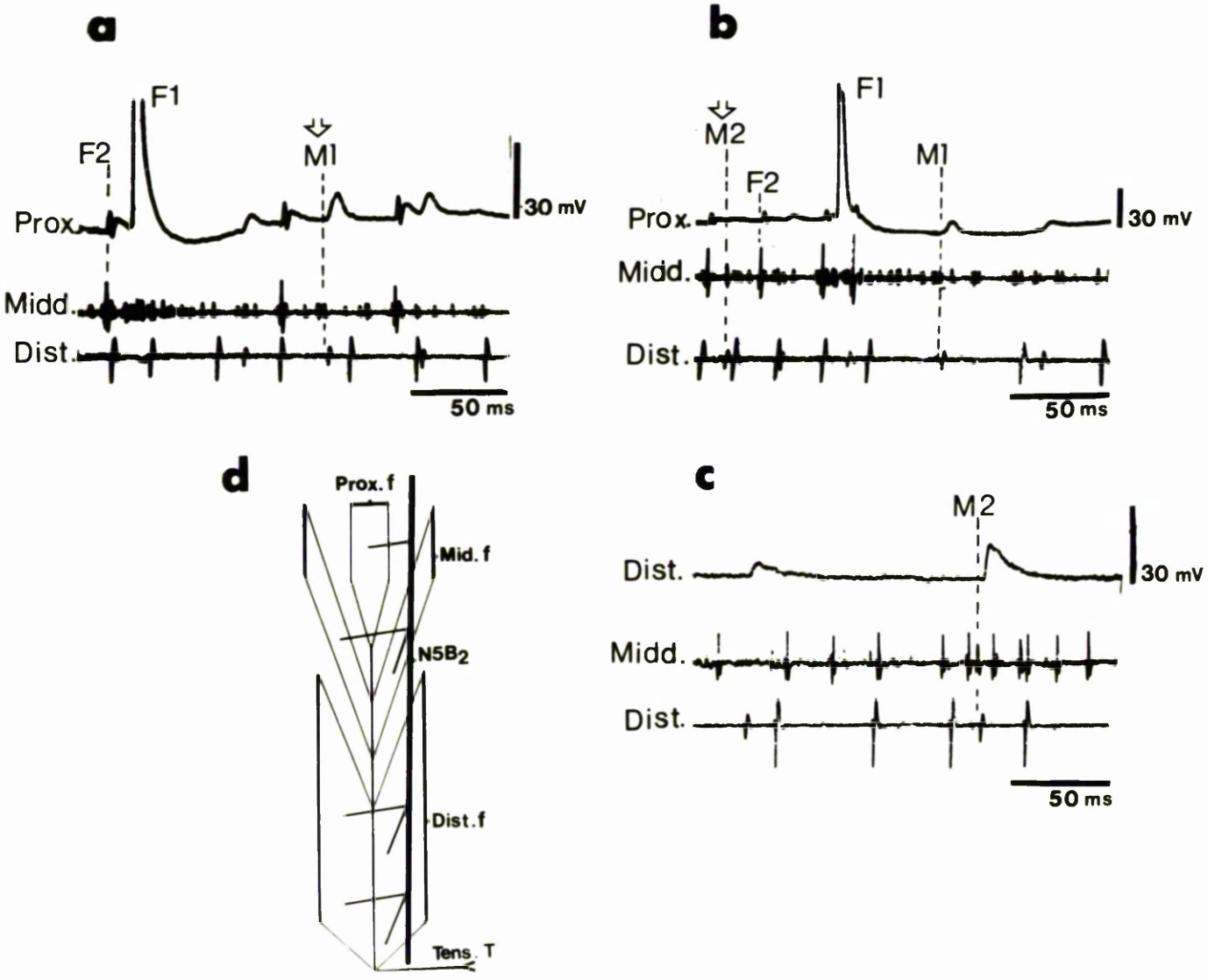
2nd trace: Extracellular record from the middle nerve branch.

3rd trace: Extracellular record from the distal nerve branch.

c. 1st trace: Intracellular record from the distal flexor.

2nd and 3rd trace: as in a and b.

d. Diagrammatic representation of the mesothoracic flexor tibiae muscle.


a
F1
F2
M1
Prox.
Midd.
Dist.
30 mV
50 ms
b
F1
M2
M1
F2
Prox.
Midd.
Dist.
30 mV
50 ms
d
Prox. f
Mid. f
N5B2
Dist. f
Tens. T
c
M2
Dist.
30 mV
Midd.
Dist.
50 ms

this axon cannot be recorded in the middle nerve branches. EPSP's from M1 have however been identified in the middle flexor. In some cases this unusual innervation pattern can be used to identify this axon.

Intermediate 2 (M2).

This axon innervates only the middle and distal flexors (fig. 3.12, Table 3.5). Two similar action potentials were recorded in the distal nerve branches (see arrows in fig. 3.18b). One of them is from axon M1 since it corresponds in time with the small EPSP's recorded in the proximal flexor. The other action potential is from axon M2 and does not correspond with any EPSP's in the proximal flexor but can be correlated with an action potential which can be seen in the middle flexor nerve branch. Random penetrations of more than thirty muscle fibres in three different animals failed to show any EPSP's in the proximal flexor when M2 was activated. An EPSP (15 mV) produced by axon M2 in the distal flexor is shown in fig. 3.18c. Axon M2 in this case is identified by the fact that it also produces action potentials in the middle and distal nerve branches. There is not enough evidence to identify the axon which produces the other small EPSP in this figure but it may be M1.(It does not innervate the middle nerve branch and it produces a small EPSP in the distal flexor).

Activity of axon M2 can also be seen in fig. 3.16B in comparison with F4. Axon M2 was found not to be very active when the animal was stimulated.

Slow 1 and 2 (S1 and S2).

These axons innervate the whole flexor tibiae muscle. Their effective diameters are very similar (1.0 - 1.2μm) when measured in the proximal nerve branch (fig. 3.6 section aa). The action potentials

which these axons produce are very small and are often masked by the noise of the recording electrodes. In some cases the noise level was low enough to allow recordings of spikes from these axons to be made. One of the characteristics of these slow motoneurons is that they fire spontaneously at a combined frequency of 5 to 10 Hz. EPSP's of 2 to 5 mV were recorded in the middle muscle fibres correlated with action potentials in the distal nerve branch (fig. 3.19A). In these records there is a variation in the size of the EPSP's which could be due to more than one axon in addition to the effects of noise and facilitation. Visual inspection of the records suggest that two different EPSP's are present. To confirm statistically the number of the neurons responsible for the activity the heights of the EPSP's were plotted in a histogram (fig. 3.19C). Two peaks appeared of 3 and 4.1 mV and this suggests that the recorded EPSP's were produced by two different neurons, S1 and S2. The mean sizes of the two EPSPs selected visually were shown to be significantly different with a t test ($P = 0.01$).

The potentials of S1 and S2 are about five times smaller than M1 in the distal nerve branches. Similar EPSP's recorded in the proximal flexor corresponded with the action potentials classified as S1 and S2 in the middle and distal nerve branches. Repeated penetrations of the flexor muscle fibres showed that these axons innervate approximately 60% of the distal and middle flexor but have fewer endings (30 to 40%) in the proximal flexor.

Slow 3 (S3).

This is an axon with a similar diameter to the other two slow axons but which was found only the proximal flexor. This axon also fires spontaneously at a lower frequency and produces larger EPSP's

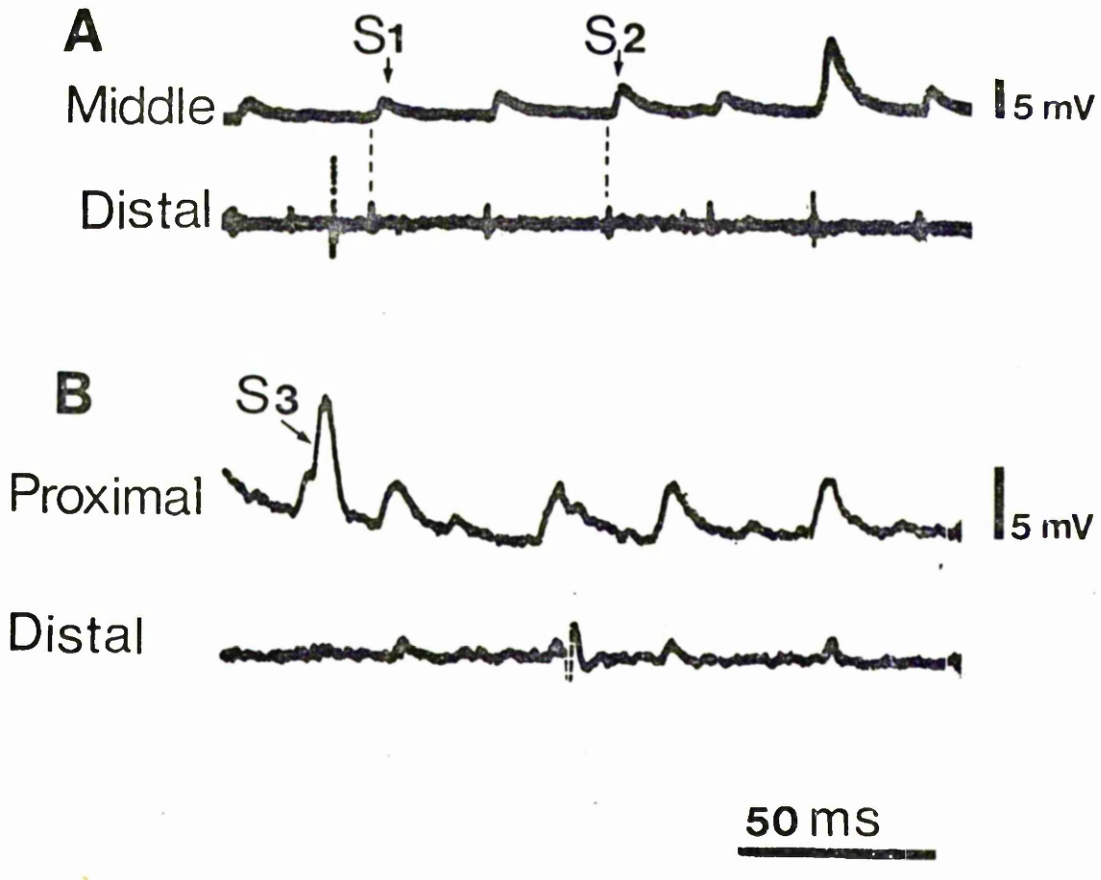
Fig. 3.19

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 added saline. The flexor motoneurons were reflexly excited by tibial extension.

A. 1st trace: Intracellular record from the middle flexor.
2nd trace: Extracellular record from a distal nerve branch.
The larger EPSP could be due to M1 or M2.

B. 1st trace: Intracellular record from the proximal muscle fibres.
2nd trace: Extracellular record from the main distal nerve branch.
The EPSP's smaller than that from S3 are probably due to S1 and S2 since they correspond with very small action potentials in the distal nerve branch.

C. Histogram of frequency against amplitude for slow EPSP's measured from the records shown in A. The two peaks indicate the presence of two axons labelled S1 and S2.


A
S1
S2
Middle
Distal
5 mV
B
S3
Proximal
Distal
5 mV
50 ms

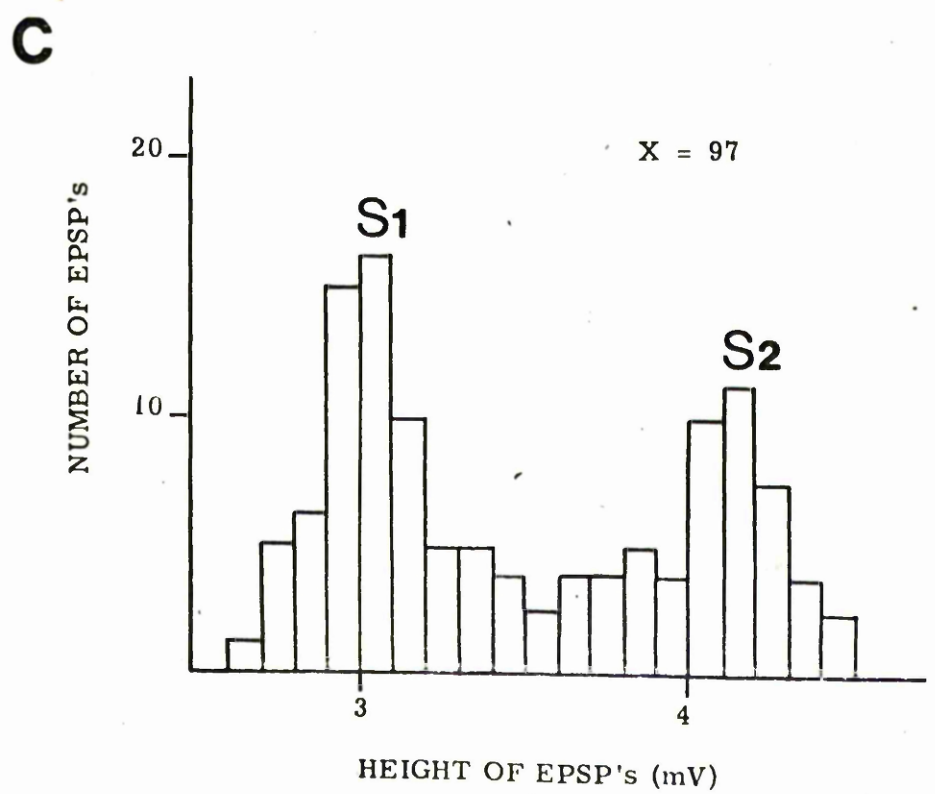
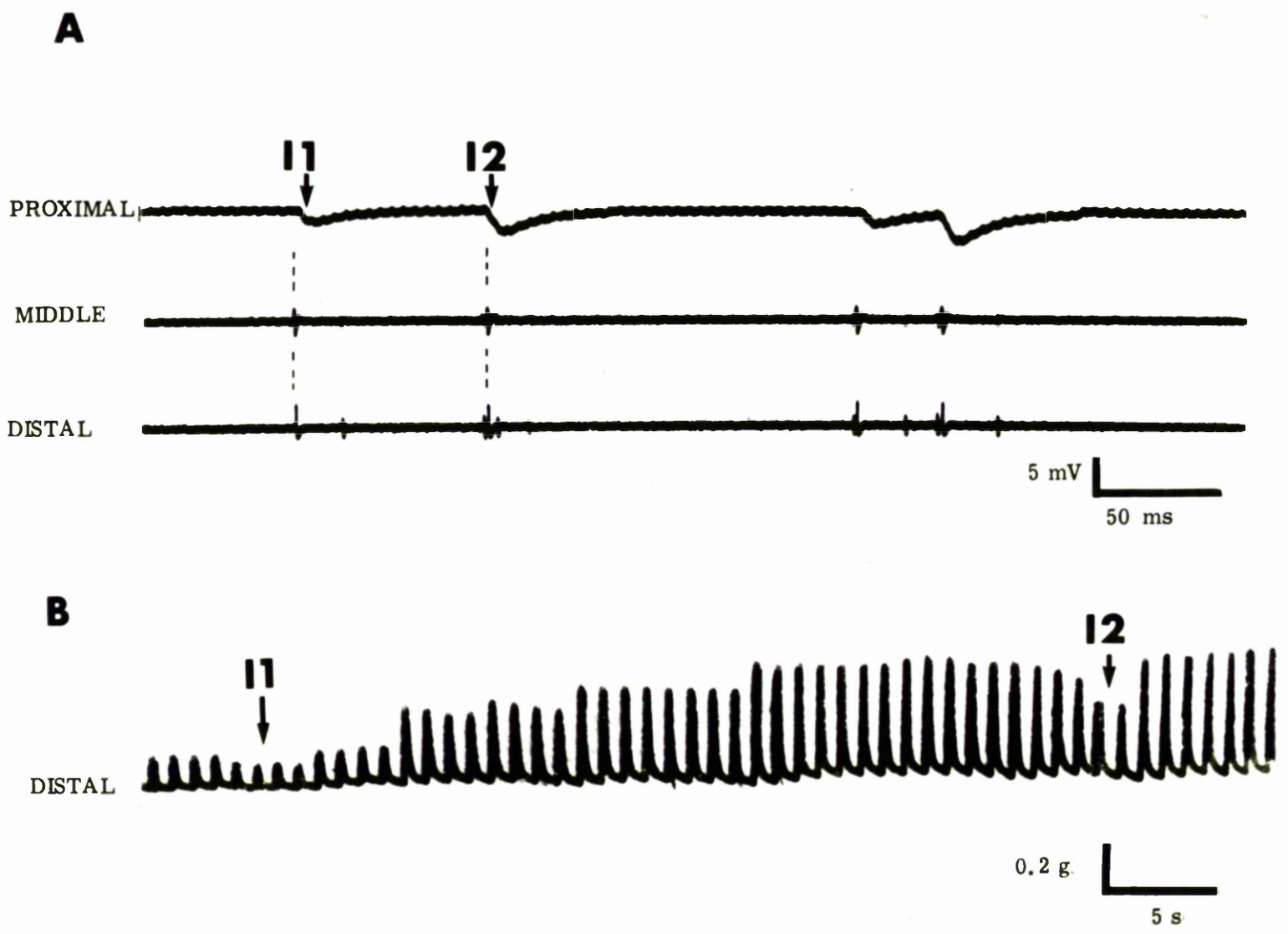

C
20
10
X = 97
S1
S2
NUMBER OF EPSP's
3
4
HEIGHT OF EPSP's (mV)

Fig. 3.20

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 added saline.

A. 1st trace: Intracellular record from the proximal flexor
2nd trace: Extracellular record from the middle nerve branch
3rd trace: Extracellular record from distal nerve branch
I1 = Inhibitor 1, I2 = Inhibitor 2.

B. Tension records from only the distal part of the flexor muscle. To stimulate the muscle a hook electrode was placed on the proximal part of N5B2 while the nerve branches to the proximal and middle flexor were cut to eliminate tension from these parts of the flexor muscle. The N5 was cut at the mesothoracic ganglion to eliminate spontaneous activity to the flexor.


A
I1
I2
PROXIMAL
MIDDLE
DISTAL
5 mV
50 ms
B
I1
I2
DISTAL
0.2 g
5 s

(7 - 10 mV) than the other two slows (fig. 3.19B). EPSP's from S3 cannot be correlated with any action potentials recorded in the middle or distal nerve branches even when the noise level is low.

Inhibitory axons (I1; I2).

These are two axons producing inhibitory post synaptic potentials (IPSP's) in the fibres of the whole flexor tibiae muscle (fig. 3.12, Table 3.5).

Stimulation of various parts of the animal excites these neurons which seems to have a low threshold. IPSP's were recorded from an intact animal where the flexor muscle was immersed in a mixture of haemolymph and saline. Under these conditions hyperpolarizing IPSP's could be seen for more than 30 min. Activity of these neurons recorded from proximal, middle and distal flexors is shown in fig. 3.20A. The action potentials recorded simultaneously in the middle and distal flexor can be identified as inhibitory since IPSP's were recorded at the same time from the proximal muscle fibres. Inhibitory axon number 2 (I2) was identified as the axon which produces the larger (3 - 4 mV) hyperpolarization and action potential. Axon I1 produces smaller IPSP's (1 to 2 mV).

To establish the distribution of the inhibitory nerve endings twenty distal, twenty middle and twenty proximal muscle fibres were penetrated in five different animals. The conclusion was that the two IPSP's always appeared together in the same muscle fibre and that the inhibitory axons innervate approximately 60-65% of the distal, 40-45% of the middle and less than 30% of the proximal flexor. Since the distal flexor receives a larger number of inhibitory endings, relaxation is very obvious in this part of the muscle (fig. 3.20B). Active tension increments produced by the slow axons were reduced in size when axon I1

was stimulated. When the threshold of I2 was reached the twitch contraction of the flexor muscle decreased by about 50% if the fast axons were not stimulated (see arrows).

Cobalt sulphide fills of the two inhibitory cell bodies are shown in fig. 3.8.

Dorsal Unpaired Medium neurons (DUM).

Two of the DUM neurons, with cell bodies on the dorsal side of the mesothoracic ganglion have axons which bifurcate and send branches into both right (R) and left (L) legs (fig. 3.21A). To study these cells, nerve impulses were initiated in the DUM axons innervating the flexor tibiae muscle by stimulating the contralateral flexor nerve branches. Since other neurons supplying the flexor muscle are unpaired, electric stimulation of the contralateral nerve does not lead to their excitation in the ipsilateral leg. This technique was used by Crossman et al. (1972) and Hoyle et al. (1974) to study the DUM neurons in the metathoracic leg of the cockroach and the locust.

In the mesothoracic leg when the left flexor nerve branches (LF, fig. 3.21A) were stimulated nerve impulses of about the same size were recorded in the right flexor (RF) and the right extensor (RE) as fig. 3.21B shows. These action potentials were almost certainly recorded from the branches of the same axon since they drop out at a single threshold stimulus amplitude. When the intensity of the stimulus was increased another action potential, smaller than the first one, was recorded in the contralateral flexor nerve branch (RF) but not in those of the extensor. The difference in absolute latency between action potentials recorded from the points RE (proximal to the femur) and RF distal, is caused mostly by the difference in distance (approximately 1 cm) between the recording electrodes, although there may be also differences in conduction velocity.

The records in fig. 3.21B suggest that the flexor muscle receives axons from two DUM neurons, called D1 and D2. The axon coming from D1 also gives a branch to the antagonist extensor muscle, in contrast with the metathoracic leg where it was found that the homologous DUM neuron gives a branch only into the extensor muscle (Hoyle et al. 1974 - named DUMETi).

In the experiments described above, when the frequency of the stimulation was increased (4 - 20 Hz) the action potentials recorded contralaterally were unable to follow the stimulus pulses 1:1. Prolonged stimulation of the axons can cause failure due to a fatigue phenomenon. To eliminate fatigue effects in these experiments, the records were taken in the first 0.5 sec. of stimulation and the failure frequency was considered to be the frequency at which the first action potential failed to show a 1:1 response to stimuli. Stimulation of the extensor nerve on one side of the animal (LE, fig. 3.21A) and recordings from the contralateral extensor (RE) show that the nerve impulses follow the stimulation 1:1 up to 4 to 8 Hz. When the frequency was increased the spikes began to fail (fig. 3.22A). Some of the impulses persist at higher frequencies of stimulation but their number falls dramatically. When the same experiment was repeated for conduction from the flexor nerve branch LF to RE , nerve impulses do not fail until the frequency exceeds about 16 Hz (fig. 3.22A last trace). The fact that nerve impulses failed at a higher frequency when they were propagated from LF to RE than when they run from LE to RE is also shown in fig. 3.22B which demonstrates the percentage of spike failures in the first 5s of the stimulus. To investigate this failure phenomenon further nerve impulses initiated at point LF were recorded in branch LE and RE. Figure 3.22C shows that nerve impulses in both extensor nerve branches follow stimulation frequencies higher than 14 Hz. When the frequency

Fig. 3.2 1.

A. The experimental arrangements for the study of the flexor DUM cells. Both mesothoracic femurs of an immobilized locust were dissected to expose the nerve branches of the right (R) and left (L) flexor (fl.t.m) and extensor (ex. t.m) muscles.

LF = Left flexor. LE = Left extensor,
RF = Right flexor RE = Right extensor.

B. Top trace simultaneous record from RF while a stimulus was applied to LF. This stimulation and recording situation is indicated by the label LF⌐¬RE. All traces are identified by the same convection (including Fig. 3.2 2.). A comparison can be made in RF of the action potentials produced by axons D1 and D2 with those of CI and SETi.

Fig. 3.2 2.

A. 1st, 2nd and 3rd traces show the failure of action potentials in the branches of D1 in LE during stimulation of increasing frequency. In the same animal no failure occurred at the frequency of 16 Hz when the stimulus was applied to LF (4th trace).

B. The relationship between the percentage of spike failure in the first 5s of applied stimulation and the frequency of stimulus, plotted for two different routes LE⌐¬RE and LF⌐¬RE in the same locust. The standard deviations are from 15 repetitions.

C. Simultaneous records from RE and LE while the stimulus was applied to LF at two different frequencies. Notice in lower pair of records that the action potentials of D1 in LE have disappeared.

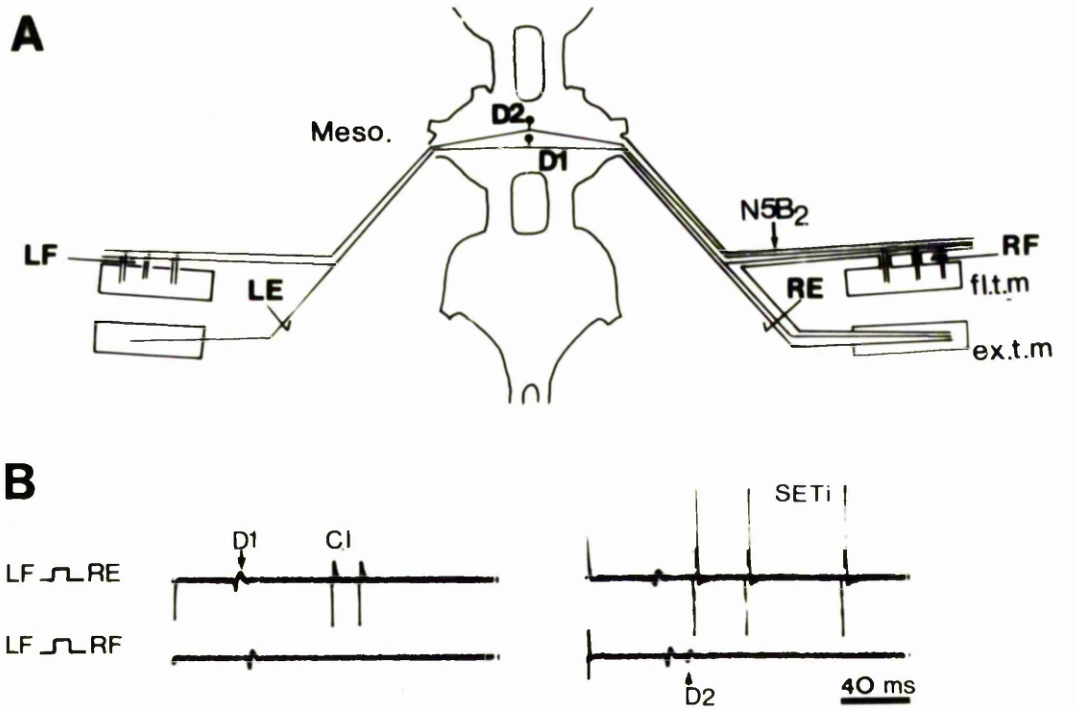


Fig. 3.21

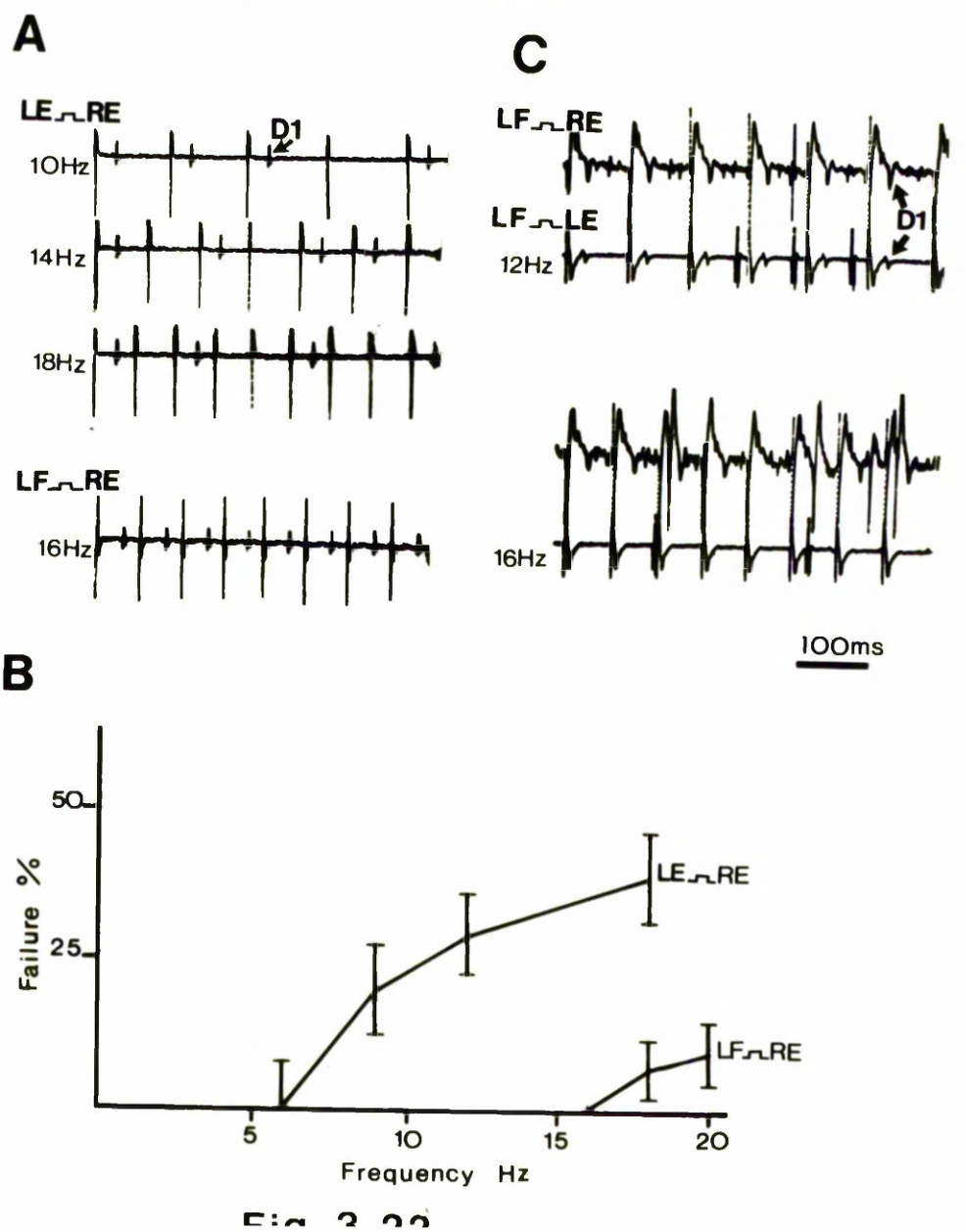


Fig. 3.22

rises to 16 Hz nerve impulses fail in the ipsilateral extensor (IE) but persist in the contralateral extensor nerve (RE). This suggests that the low frequency failure point is in the extensor nerve branches of axon D1 and the high frequency failure point is proximal to the flexor nerve branches of the same neuron.

Similar properties were described by Parnas (1972) for a differential block of high frequency in the branches of a single axon innervating two crustacean muscles. Although there are significant functional and anatomical differences between the insect DUM neurons and the excitatory motoneurons in crustacea it seems that the properties of the bifurcation points of these axons are similar.

Cobalt chloride perfused through $N5B_2$ failed to stain the cell bodies of D1 and D2. Similar difficulties were found by Crossman et al. (1972) who failed to back fill the DUM neurons in cockroach. One of the contributary factors could have been the small diameter of the peripheral axons of these neurons. However Hoyle et al. (1974) derived the complete morphology of DUMETi by combining pictures of several neurons which were partially stained by intracellular injection of cobalt.

3. Mechanical properties

Most of the previous work on the mechanical properties of locust muscles has been done on muscles bathed in various physiological salines. However salines may affect the mechanical properties of the muscle fibres. For example, Burns and Usherwood (1978) found that tetanic tension of the mesothoracic extensor tibiae muscle is about 5.5gr while this tension can rise up to 15 to 20gr under in vivo conditions as described below (and also see Aidley, 1975). Hoyle (1978) shows in his records that the isometric force developed by the fan of

the metathoracic extensor muscle when the SETi is stimulated in locust saline changes dramatically after equilibration. Changes in concentration of Ca^{++} ions can also affect the tension produced by the flexor tibiae muscle (see previous pages) or the extension tibiae muscle (Aidley, 1965). In this work the mechanical responses of the mesothoracic flexor tibiae muscle were investigated under "in vivo" conditions to avoid any such misleading effects. To achieve these conditions the mesothoracic leg was mounted (as in fig. 3.1) and the flexor muscle was stimulated electrically or mechanically stretched as described in Methods. No saline was used during cutting of the nerves or the tendon, which were done by local microoperations. The animal was left long enough to recover before any experiments were undertaken. The blood circulation was not disturbed and none of the trachael systems were damaged. The fact that there were no alterations in the recorded twitch contractions after 48h shows that the muscle was kept in good condition.

In behavioural terms, passive extension or tetanic contraction of the flexor tibiae muscle occurs very often, for instance in walking during retraction of the leg a tetanic contraction of the extensor muscle produces a force which passively stretches the antagonistic flexor muscle. During protraction, the opposite occurs and the flexor muscle flexes the tibia by producing a tetanic contraction.

Passive stretch of the flexor muscle

In the mesothoracic leg, an imposed extension of the tibia causes an increase in the length of the flexor muscle. The increase of muscle length in relation to the femur tibia angle (FTA) is almost linear (fig. 3.23A). To obtain this data the tibia was extended slowly to different angles and the increase in muscle length was measured with a pointer mounted on a micromanipulator.

Fig. 3.23

A. The relationship between flexor muscle length and femur tibia angle (FTA). Data from 5 repetitions in 5 animals. Bars show ± 1 standard dev.

B. Changes in flexor muscle tension during passive tibial movement at different angular velocities, as indicated at the top left of each record.

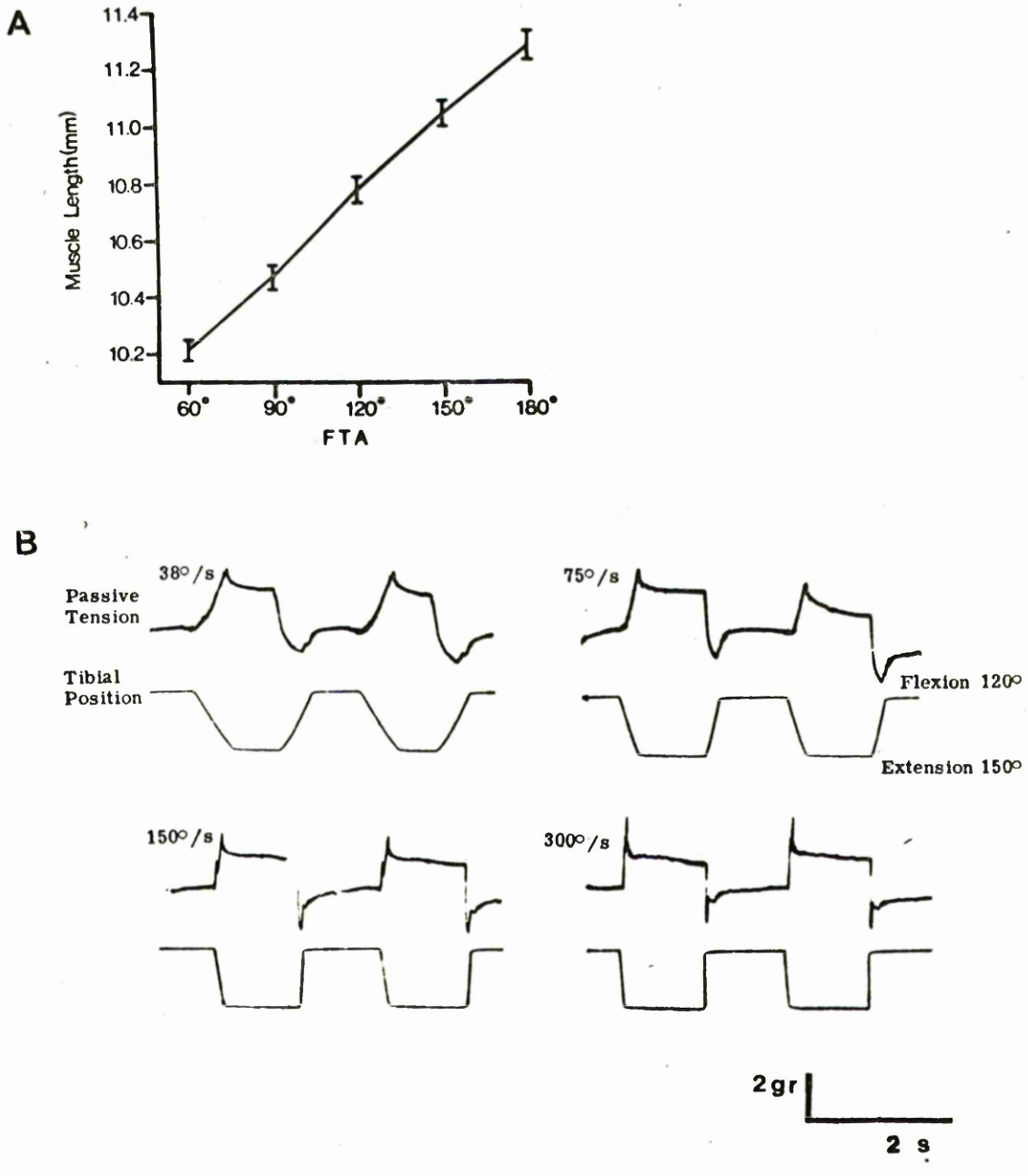

A
11.4
11.2
11.0
10.8
10.6
10.4
10.2
Muscle Length(mm)
60°
90°
120°
150°
180°
FTA
B
38°/s
Passive Tension
Tibial Position
75°/s
Flexion 120°
Extension 150°
150°/s
300°/s
2gr
2 s

Fig. 3.24

A. Tension from the mesothoracic flexor tibiae muscle recorded at the distal end of the tibia (see Methods and Fig. 3.1).

Scale bars = 2g muscle tension. Data from 15 repetitions in 3 animals. Bars = ± 1 standard dev.

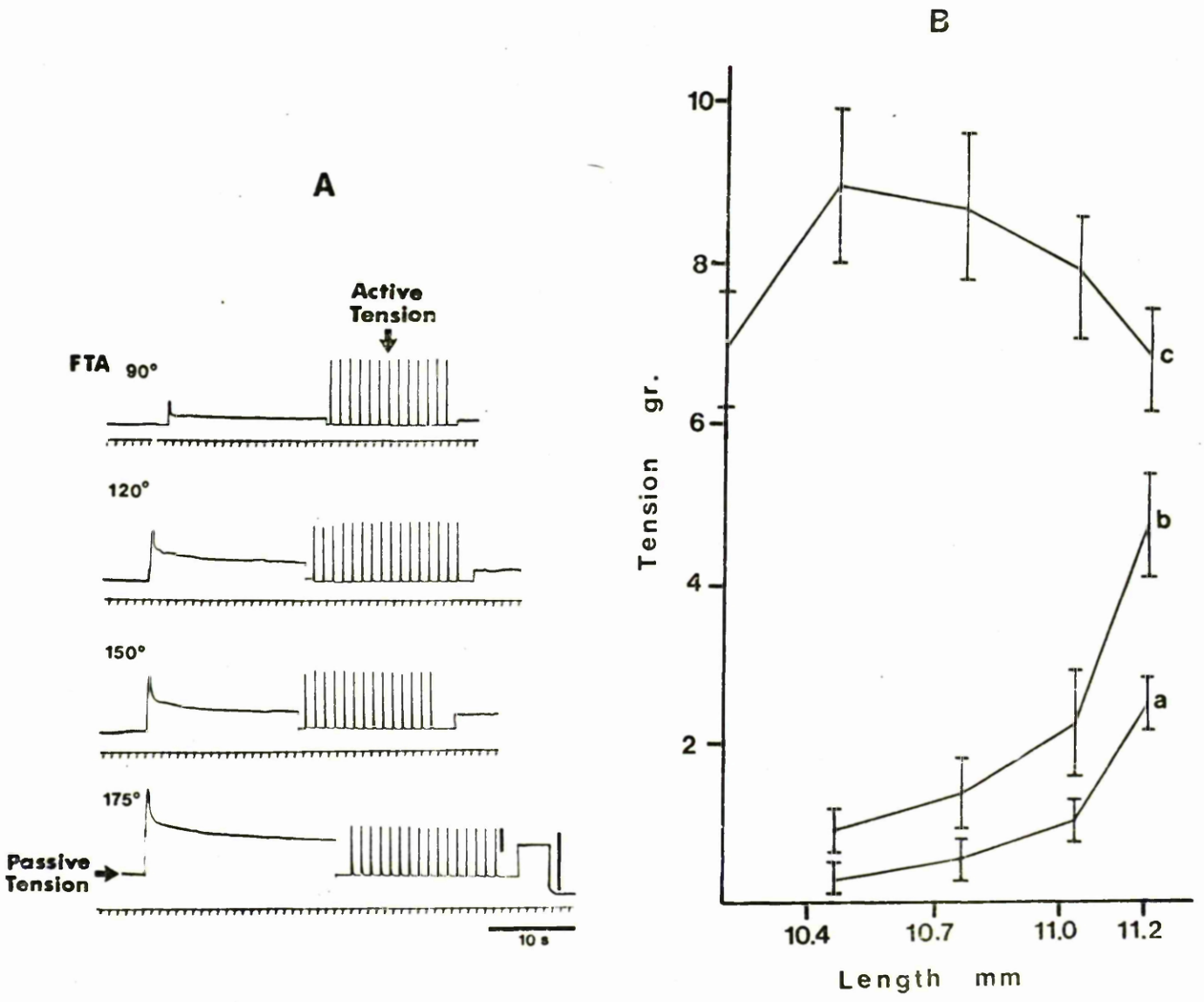
Passive tension: Changes in muscle tension caused by passive tibial extension from 90° to 175° in steps with an angular velocity of 150 °/s.

Active tension: Twitch contraction of the flexor muscle induced 20 or 30s after the passive extension. The sensitivity of the pen-recorder was reduced while twitch contractions were recorded.

B. The tension recorded in A plotted against flexor muscle length.

b - peak passive tension, a - plateau tension,

c - amplitude of twitch. The data were obtained from 15 experiments on each of three different locusts. The muscle length in mm was calculated from Fig. 3.23A since the FTA was known.


A
Active Tension
FTA 90°
120°
150°
175°
Passive Tension
10 s
B
Tension gr.
10
8
6
4
2
c
b
a
10.4
10.7
11.0
11.2
Length mm

In a free walking animal the tibia is extended rapidly from 70° to 130°, with an average angular velocity of 150°/s in an animal walking at a speed of 2 steps/second (st./s), with an average angular velocity of 240°/s when it walks at a speed of 3 st/s and 430°/s when it walks at 4 st/s (Burns, 1973). To simulate these movements, a constant velocity was imposed on the tibia of an immobilized animal by a lever. Muscle tension was monitored by the probe of a tension transducer attached between the lever and the end of the tibia as described in Methods. Fig. 3.23B shows the changes in muscle tension when the tibia was extended at different angular velocities. The overshooting during lengthening or shortening of the flexor muscle depends on the velocity of the stretch or release. Similar properties were described by Gaisser and Hill (1924) in a frog sartorious muscle, who found that these responses also persist in a tetanized muscle. In their simple viscous elastic model they attributed the overshooting to the viscous components. During extension of the flexor muscle in normal walking, the muscle cannot be considered completely relaxed, although most of the flexor motoneurons are silent, because there is probably a residual tension in the tonic fibres of the muscle. Similar effects in the mesothoracic extensor muscle were described by Burns (1972).

To investigate changes in muscle tension during the stretch of the muscle, the isometric length tension curve was plotted, by extending the tibia from 90° to 175°, in steps with a constant velocity of 150°/S. Although the mesothoracic tibia operates from 30° to 180°, the angle of 180° was avoided because at that angle (maximum FTA) forces developed by cuticular formations may affect the estimates of the muscle tension, since these were measured from the tibia. Typical records from the above

procedure are shown in fig. 3.24A and the results of an analysis of fifteen experiments on three different animals are shown in fig. 3.24B (curves a b).

Active tension produced by the flexor muscle

Active tension increments were induced in the flexor muscle by stimulating N5 (see methods) while the length of the muscle was increased by extending the tibia with an angular velocity of $150^{\circ}/S$ (fig. 3.24A). The twitch contraction reduced in strength when the muscle was stretched. The peak of the active tension (fig. 3.24B, curve c) occurred at the normal length of the muscle for an FTA of 90° to 100°. Similar curve was plotted by Aidley (1975) for the locust mesothoracic extensor tibiae muscle.

In walking, tetanic forces due to high frequency activity in the flexor muscle, cause a fast flexion of the tibia in protraction. Strong flexion of the tibia is vital also in other behaviour patterns such as climbing or grasping. To simulate these conditions the flexor muscle was stimulated through N5 (see Methods) at different frequencies for 20 sec. and tetanic forces were measured at the end of the tibia which was kept at a FTA of 90°. The stimulus voltage used was high (15 - 20 V) to ensure that all the flexor motor axons were excited. This meant that the inhibitory axons were also excited, but relaxation of the flexor muscle is not significant. Usually the inhibitors have little inhibitory effects when muscles are contracting under the influence of activity in the fast fibres (Usherwood and Grundfest, 1965).

The tetanic tension produced by the flexor tibiae muscle rises quickly (see Table 3.6) and produces a plateau for 5 - 8s for frequencies of stimulation higher than 30 Hz and then slowly fatigues to about 50%

of the peak tension in a further 12 - 15s. To investigate the components of this tetanic tension the proximal part of the muscle (proximal and middle flexors) were stimulated separately from the distal part (distal flexor). These two parts were chosen because they receive many different axons (fig. 3.12). Tension from the proximal part (fig. 3.25B) was recorded by cutting $N5B_2$ immediately distal to the middle nerve branch (denervation of the distal flexor). During high frequency stimulation (50 Hz) the tetanic tension rises very rapidly and in 20 sec. fatigues by about 60% of the peak tension. To record active tension only from the distal part of the muscle the apodeme between middle and distal flexors was severed to eliminate tension produced by the rest of this muscle. During stimulation at a high frequency (fig. 3.25C), the distal flexor produces also a fast rising peak which fatigues by about 35 - 40% of the peak tension in 20 sec.

The half rise times, half decay times and the twitch/tetanus ratios at a frequency of stimulation of 50 Hz for the distal part, the rest and the whole flexor tibiae muscle are shown in Table 3.6. The tetanic tension in the proximal and middle flexors rises and decays faster than that in the distal flexor. This suggests that the proximal muscle fibres are more phasic than the distal fibres and this is also supported by the fact that the proximal flexor has larger diameter muscle fibres (Table 3.1) and also receives larger motor axons than the rest of the muscle (Table 3.3). Similar anatomical and physiological characteristics can be seen in the antagonist extensor tibiae muscle (Burns and Usherwood, 1979) where the distal part of the muscle is less phasic than the proximal part.

The study of the contraction which each individual motor axon causes in the fibres of the flexor muscle would require excitation of

Fig. 3.25

Muscle tension recorded from different parts of the mesothoracic flexor tibiae muscle while they were stimulated for 20s (for the duration of the horizontal line) at frequencies of 20, 30 and 50 Hz. The muscle was bathed in its own haemolymph (Fig. 3.1).

A. From the whole flexor muscle. The tension transducer was attached to the distal end of the tibia.

B. From the middle and proximal flexor. The distal part was denervated by cutting N5B2 immediately distal to the middle nerve branch.

C. From the distal flexor. The flexor apodeme was cut between middle and distal flexors to eliminate tension developed in the two proximal parts.

Vertical calibration = 12.1g muscle tension.

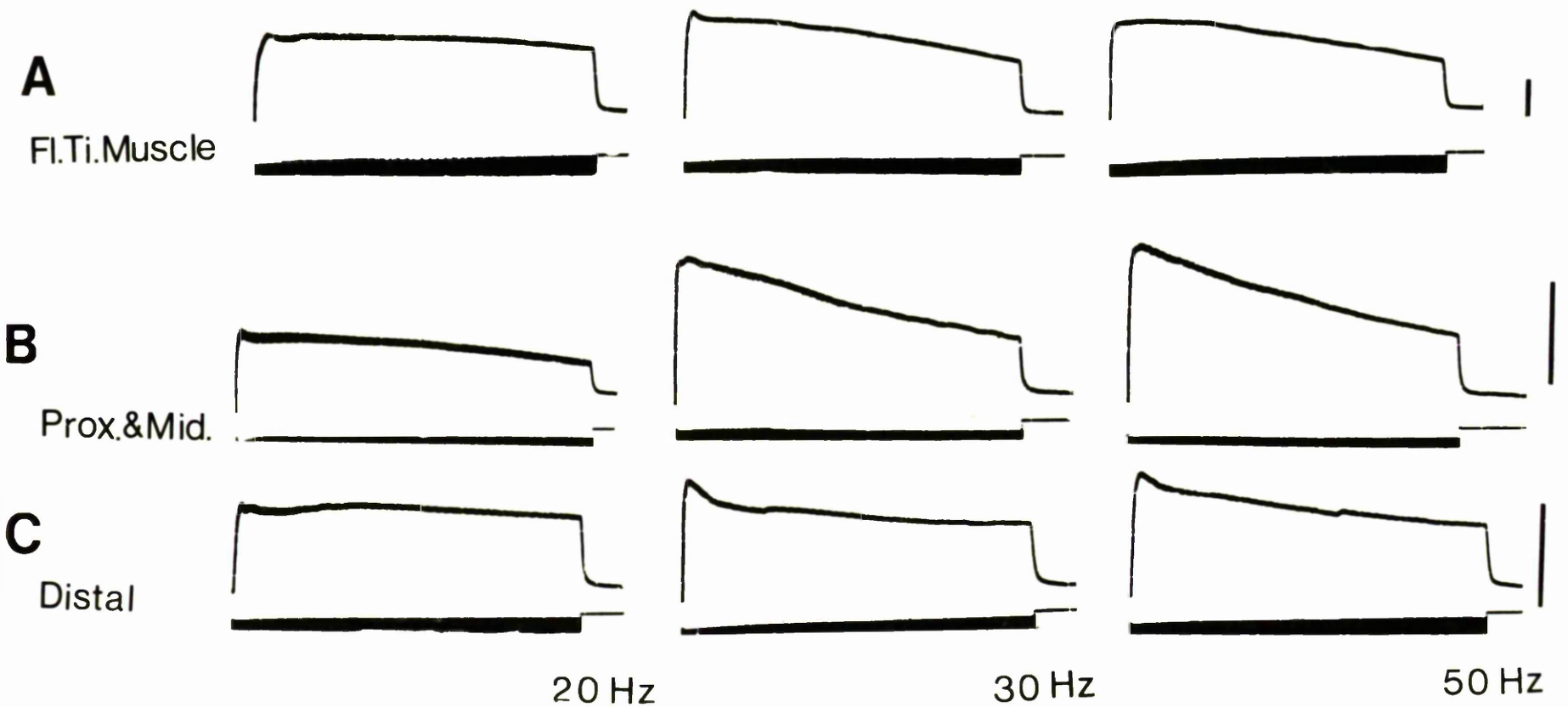

A
Fl.Ti.Muscle
B
Prox.&Mid.
C
Distal
20 Hz
30 Hz
50 Hz

Table 3.6.

Mechanical characteristics of active contractions of the mesothoracic flexor tibiae muscle. The muscle was in its own haemolymph and excited through N5. Recording conditions were almost isometric (transducer compliance = 0.05 mm/g). No significant changes of the rise and decay times were observed at higher stimulus frequencies (120 Hz).

Table 3.6.

	Whole Flexor Muscle	Prox. & middle flexor	Distal flexor
Twitch/Tetanus ratio	0.225	0.172	0.142
Half rise time(50Hz) (ms)	80	60	125
Half decay time(50Hz) (ms)	100	50	90

The above numbers are the average values of records taken from three different animals.

these axons from their motoneuron cell bodies. This technique which has been described above (fig. 3.9) is suitable if there is only small number of motoneurons, but in this case the total number of excitatory and inhibitory axons detected is 14, a fact which makes the use of this technique almost impossible.

CHAPTER 4

A Muscle Tension Receptor in the Locust Leg.

by

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1. Introduction

In vertebrate limbs the Golgi tendon organs are well known as receptors for active muscle tension (e.g. Houk & Henneman, 1967). Tension receptors have also been found on the apodemes of leg muscles in Cancer (MacMillan & Dando, 1972) and Limulus (Eagles, 1978). However, no receptors directly sensitive to muscle tension have so far been identified in the legs of insects, although there is physiological evidence for their existence in the locust (Burrows & Horridge, 1974) and in the stick insect (Bässler, 1977). In addition to receptors for joint position and movement (Burns, 1974; Coillot & Boistel, 1963) the insect leg is well supplied with campaniform sensilla which detect cuticular stress (Pringle, 1938) and it has been suggested that the latter receptors also function indirectly as muscle tension receptors.

In this paper we describe the anatomy of sensory nerve branches in the femur and report a single receptor cell attached to the flexor tibiae muscle of the prothoracic and mesothoracic legs of the locust, which responds to passive and active tension in the muscle, and which is capable of mediating reflexes in the femoral muscles.

2. Materials and Methods

The present work was performed on the mesothoracic leg of the adult female locust, Schistocerca americana gregaria. The leg was removed from the animal and mounted ventral side down on Tackiwax. In order to measure isometric tension in the flexor tibiae muscle the tibiae was disarticulated, cut to a 1mm stump and attached to a silicon strain gauge transducer (compliance 0.05mm/g) which could be moved to stretch the muscle. In some experiments the tibia was left intact to ensure

that the muscle length remained within its natural range and the femur-tibia angle was measured visually against a protractor scale. The dorsal cuticle of the femur and the extensor tibiae muscle were removed and the whole leg was immersed in circulated, oxygenated saline (Usherwood & Grundfest, 1965) at room temperature. Records of activity from the sensory nerves in the femur were made with a glass suction electrode, gold plated to increase the signal to noise ratio. Passive tension was produced in the flexor tibiae muscle by extending the tibia or by stretching the muscle directly with a lever on its apodeme which was attached to the armature of a small relay. Active tension in the flexor muscle was induced by stimulating the motor nerve (N 5B2). Neural activity was recorded in magnetic tape and either photographed from the oscilloscope screen or photographed from an instantaneous frequency display.

In order to study reflexes mediated by the tension receptor the leg was fixed and dissected in the same way, but was left attached to the locust. Nerve 5B2 was cut at the proximal end of the femur (see Fig.4.1A) and motoneuron activity was recorded from the proximal side of the cut and from the extensor tibiae motor nerve. Tension was developed in the flexor tibiae muscle by stimulating N 5B2 distal to the cut. All branches of n5 were cut except the one from the tension receptor which was monitored to check receptor function. In some experiments the femur-tibia joint and nerves 5B1b and 5B1c were left intact. The tibia was then moved with a galvanometer motor driven by an electronic ramp generator so that the flexor muscle was both passively stretched and reflexly excited via the chordotonal organ.

The anatomy of the sensory nerve branches in the leg was determined by perfusing cobalt chloride peripherally down the nerves

Fig. 4:1

Anatomy of the sensory nerve branches of the mesothoracic leg of the locust. A - dorsal view of branches of nerve 5B in the left femur, B - anterior view of branches of nerve 5B1 in the right proximal tibia, C - posterior view of branches of nerve 5B2 in the right proximal tibia. All nerves numbered are branches of nerve 5B. MTR = muscle tension receptor, CS = campaniform sensilla, S = subgenual organ, C = coxa, F = femur, T = tibia.

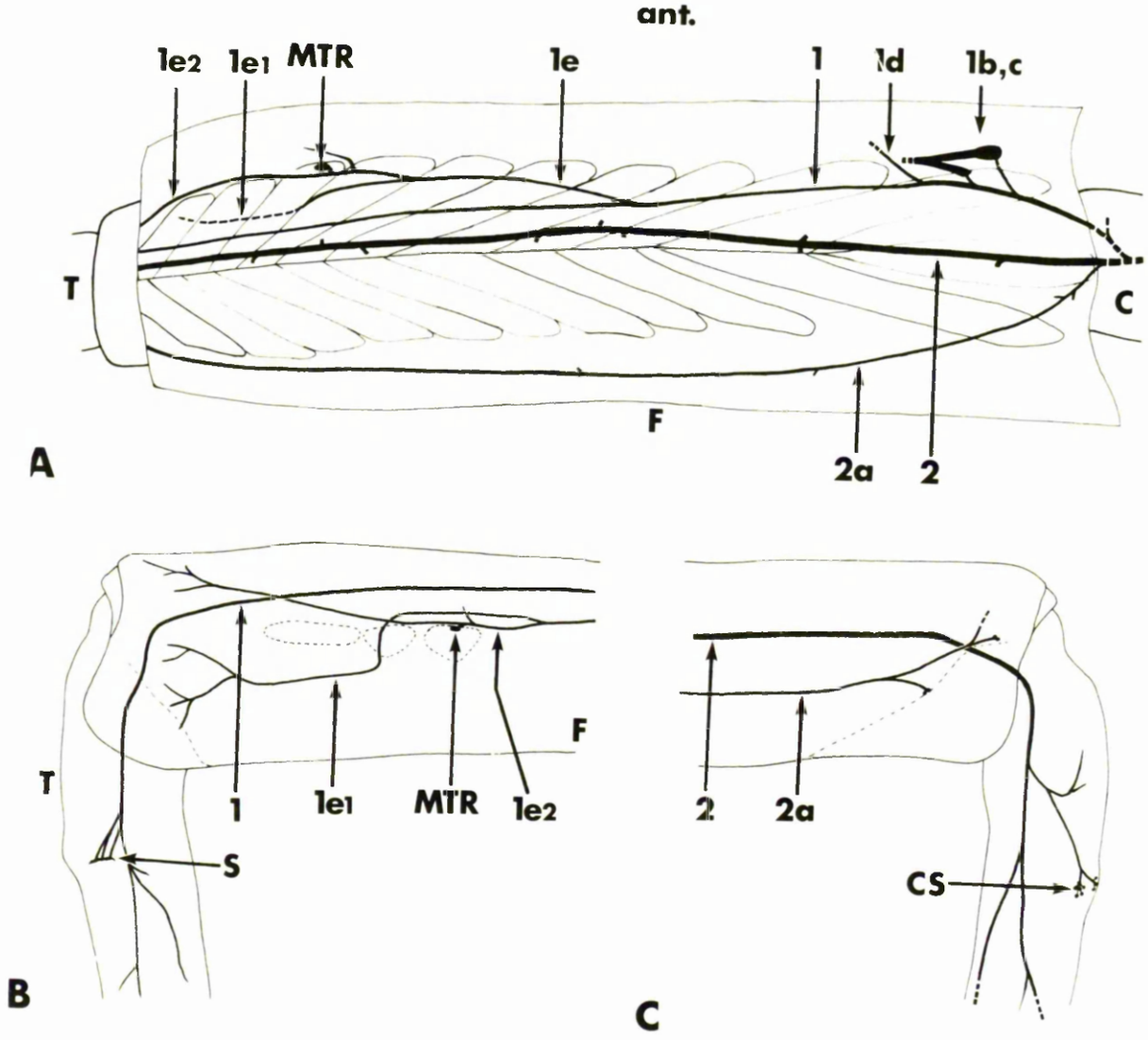

ant.
1e2
1e1
MTR
1e
1
1d
1b,c
T
C
F
2a
2
A
T
1
1e1
MTR
1e2
F
S
B
2
2a
CS
C

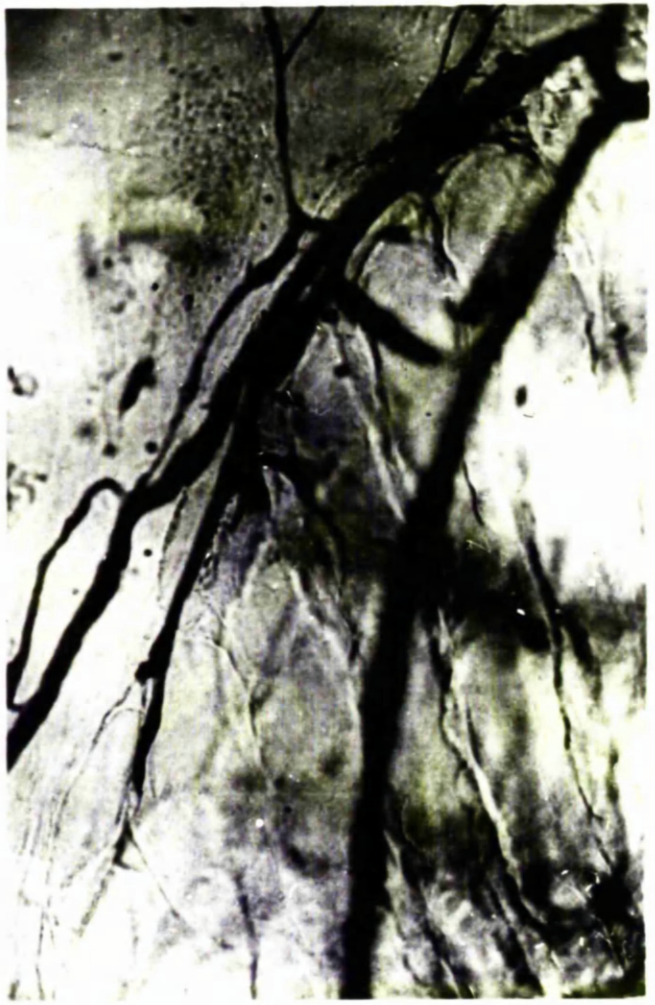
Fig. 4:2.

Morphology of the flexor muscle tension receptor (MTR).

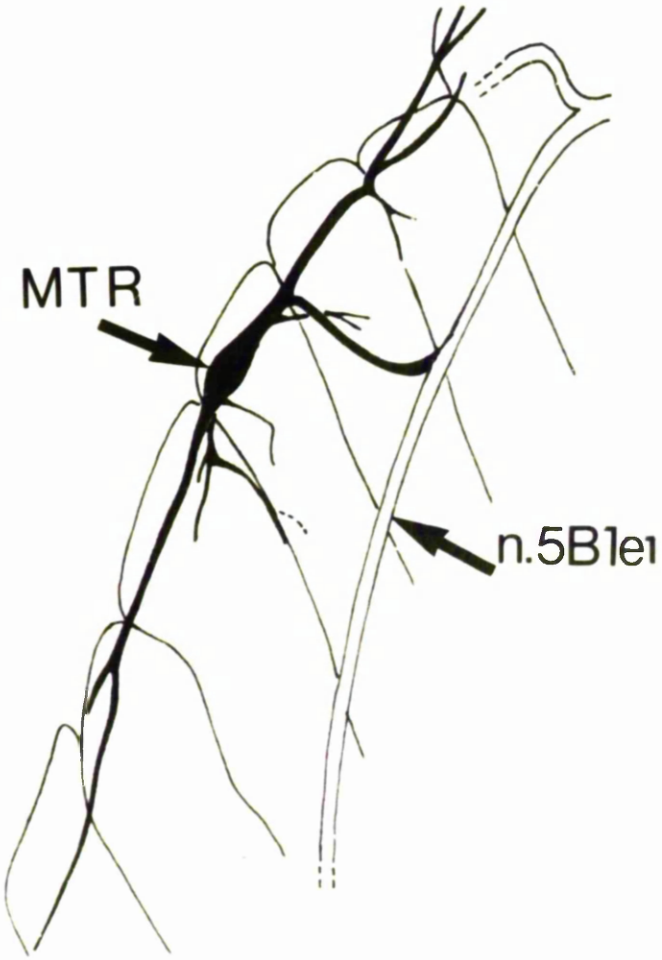
A - branches of nerve 5Ble filled with cobalt sulphide,

B - diagram of the same preparation. Scale bar = 100μm.

A



B



and precipitating it as cobalt sulphide. This was done by sucking the cut end of the nerve into a glass suction electrode filled with 0.5 M cobalt chloride.

3. Results

Anatomy.

In the femur of the prothoracic and mesothoracic legs of the locust the extensor tibiae muscle lies under the dorsal cuticle while the larger flexor tibiae muscle lies in the lower half of the femur. In structure both muscles are a combination of pinnate and fusiform type and they are composed of a number of discrete muscle bundles or units (defined by Hoyle, 1955) attached to a common apodeme. The flexor tibiae muscle consists of 10-12 anterior muscle bundles attached to the cuticle in a row of approximately circular discrete insertions, the same number of posterior bundles with elongated insertions tending to merge into one another, and a single proximal bundle with a ventral insertion very close to the trochanter. There is no accessory flexor corresponding to that in the metathoracic leg.

A single large nerve trunk enters the femur from the coxa. It is formed by the fusion in the coxa of nerves 3B2 and 5B1 (Campbell, 1961) and divides into two major branches in the femur, n.5B1 and n.5B2 as shown in Fig. 4.1A.

Nerve 5B1: This nerve innervates the chordotonal organ (Burns, 1974) and then gives off two more branches in the femur before passing into the tibia where it innervates sensory hairs near the joint, the subgenual organ and cuticular receptors a little further down the tibia (Fig. 1B). The first of the two major branches (n.5B1d) carries the only motor axons

in the nerve and supplies the extensor muscle. The second branch (n.5B1e) separates from the main nerve half way along the femur and runs along the anterior dorsal surface of the tibiae muscle for a short distance before dividing into two smaller nerves. The first of these (n.5B1e1) continues along the surface of the muscle and then dives down between two flexor muscle bundles to innervate hairs on the anterior face of the distal femur. The close association between the nerve and the muscle at this point suggested that there might be sensory receptors involved, but none could be identified. The second half of the nerve (n.5B1e2) gives rise to a small branch connected to the flexor tension receptor (Fig. 2) and one innervating a small field to cuticular hairs before passing along the anterior cuticle to innervate dorsal mechanosensory hairs including the very large ones immediately dorsal to the femur-tibia joint.

Nerve 5B2: This nerve contains the motor axons of the flexor tibiae, the retractor unguis and the tibial muscles. It gives rise to one sensory branch in the femur, the lateral nerve (n.5B2a) which innervates the multipolar joint receptors (Williamson & Burns, 1978) and sensory hairs in the posterior face of the femur. All the remaining sensory fibres in the nerve originate in tibial or tarsal receptors. Upon entering the tibia n.5B2 divides into three branches (Fig. 4.1C) the first of which supplies three groups of five campaniform sensilla and some sensory hairs. The remaining branches continue to the tarsus, forming the dorsal and ventral nerves (Kendall, 1970).

Flexor tension receptor: This receptor is a single large multipolar cell located at the base of the second or third most distal anterior bundle of the flexor tibiae muscle. It is about 60µm long and 20µm in diameter (Fig. 4.2) and has four major dendrites which branch profusely to connect

with more than seven muscle fibres over a distance of about 600μm. Some of the fine processes of the dendrites attach to the surface of the outermost flexor muscle fibres within 20μm of their attachment to the cuticle, while most of them pass between the fibres at the same level so that their sites of attachment are within the muscle. No direct connections between the cell and the cuticle were found. From its shape the receptor cell can be classified as a type II mechanosensory neuron (Zarwarzin, 1912) or a multiterminal cell (Finlayson, 1963). It is similar in shape to those found in the abdomen of Orthoptera (Slifer & Finlayson, 1956) but unlike them it is not attached to a single specialised muscle fibre.

The anatomical features described above are almost identical in the prothoracic and mesothoracic legs of the locust, but are different from those of the metathoracic leg. In the latter, the chordotonal organ is distal in position, Brunner's organ is present, the proximal tibia has a specialised buckling region and fewer campaniform sensilla (Heitler & Burrows, 1977) and nerve branch 5B1e is absent. No structure corresponding to the mesothoracic flexor tension receptor could be found.

Physiology

In order to evaluate the sensory contributions of nerve branches described above, their activity was monitored in the femur of an isolated leg while the tibia was passively flexed and extended over the 90°-120° range used in walking (Burns, 1973). Recordings from the distal parts of nerve 5B1 (Fig. 4.3.E) and 5B2 (Fig. 4.3D) show activity from tibial receptors which may be responding to vibration (subgenual organ) and cuticular stress (campaniform sensilla). Activity in n.5B2a, the only sensory branch of n.5B2, comes from the multipolar joint receptors

Fig. 4:3.

Records made from sensory nerve branches in the isolated mesothoracic femur of the locust while the tibia was moved passively. Records are from: A - n.5B1 proximal (chordotonal organ), B - n.5B2a (multipolar joint receptors), C - n.5B1e (flexor tension receptor), D - nerve 5B2 distal (tibial receptors), E - nerve 5B1 distal (tibial receptors). F shows the movements of the tibia between a femur-tibia angle of 90° (trace up) and 120° (trace down). The gain was different for each trace.

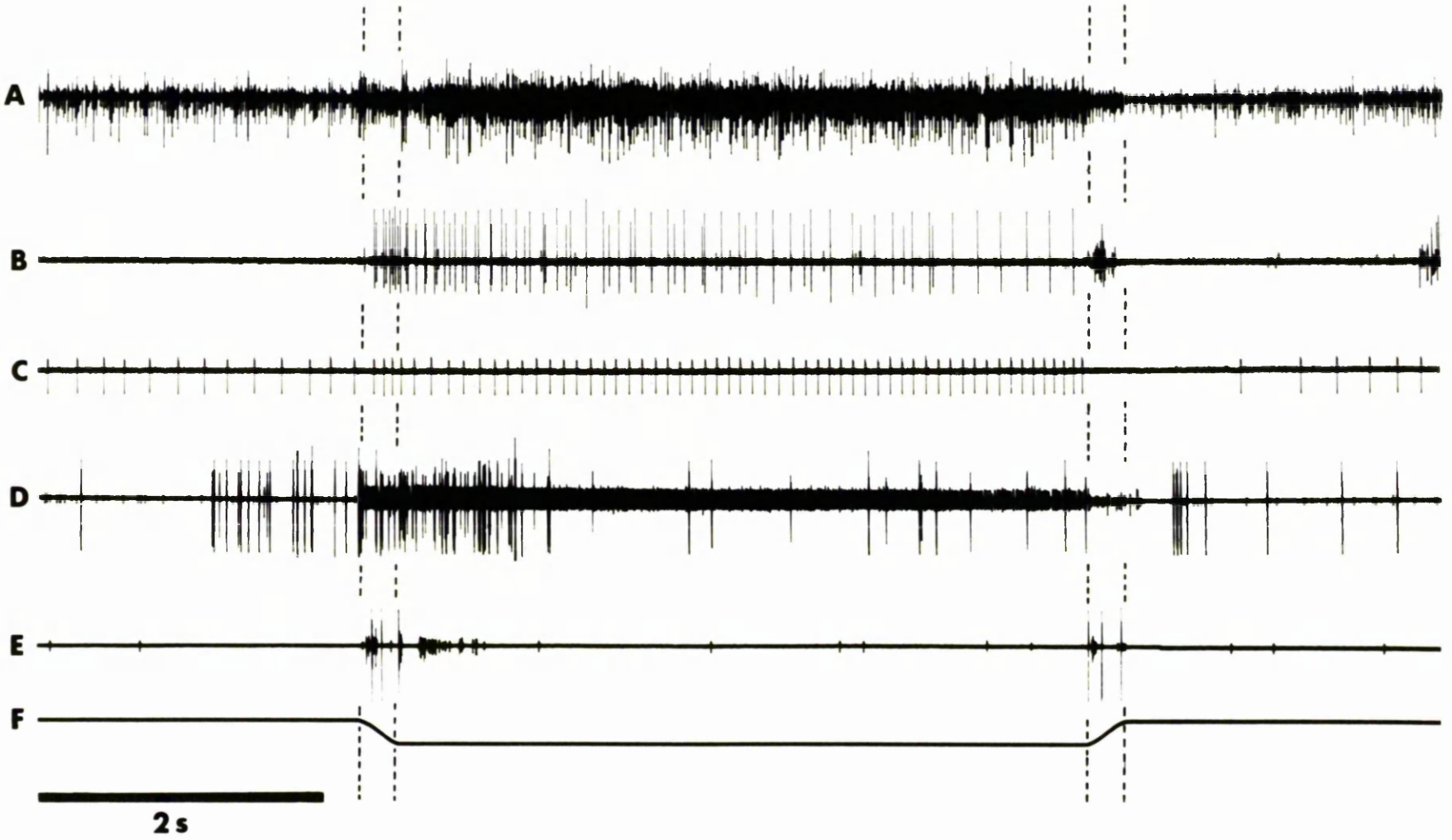

A
B
C
D
E
F
2 s

Fig. 4:4.

A - passive tension in the flexor tibiae muscle when the muscle was stretched from 10.5 to 10.8 mm length for the duration of the bar, B - peak (a) and plateau (b) passive tension in the muscle when stretched to different lengths at 1.4 cm/s, C - the relationship between muscle length and femur-tibia angle (FTA), D - instantaneous frequency display of the tension receptor response when the muscle was stretched from 10 to 11.5 mm length for the duration of the bar, E - spike frequency in the receptor axon in the first 10s after extending the tibia to the angle shown (a) and in the subsequent 100s. All experiments were done on legs of the same size. Bars show ± 1 standard deviation.
B: Data from 15 experiments from 3 animals
E: Data from 10 experiments from 5 animals.

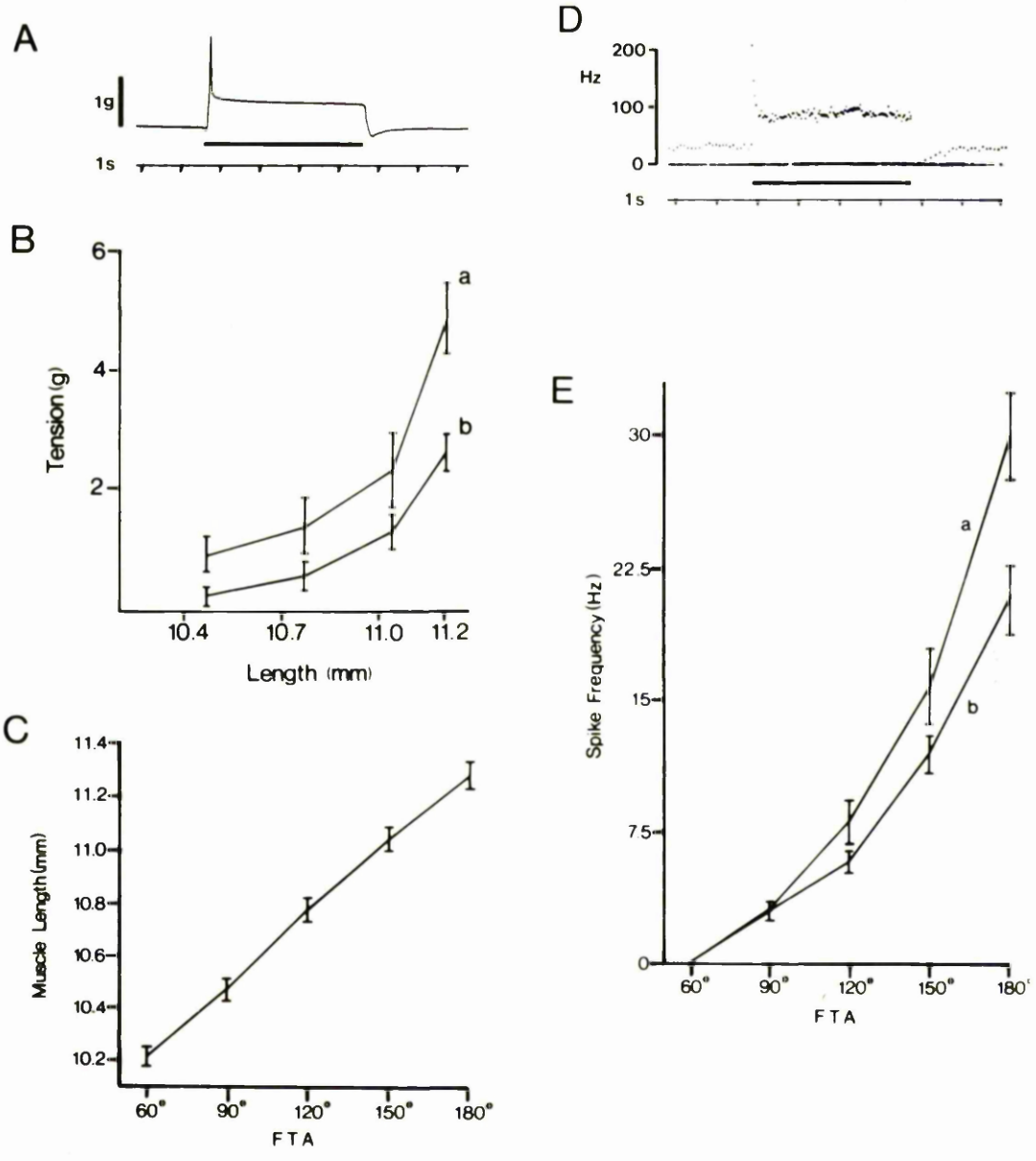
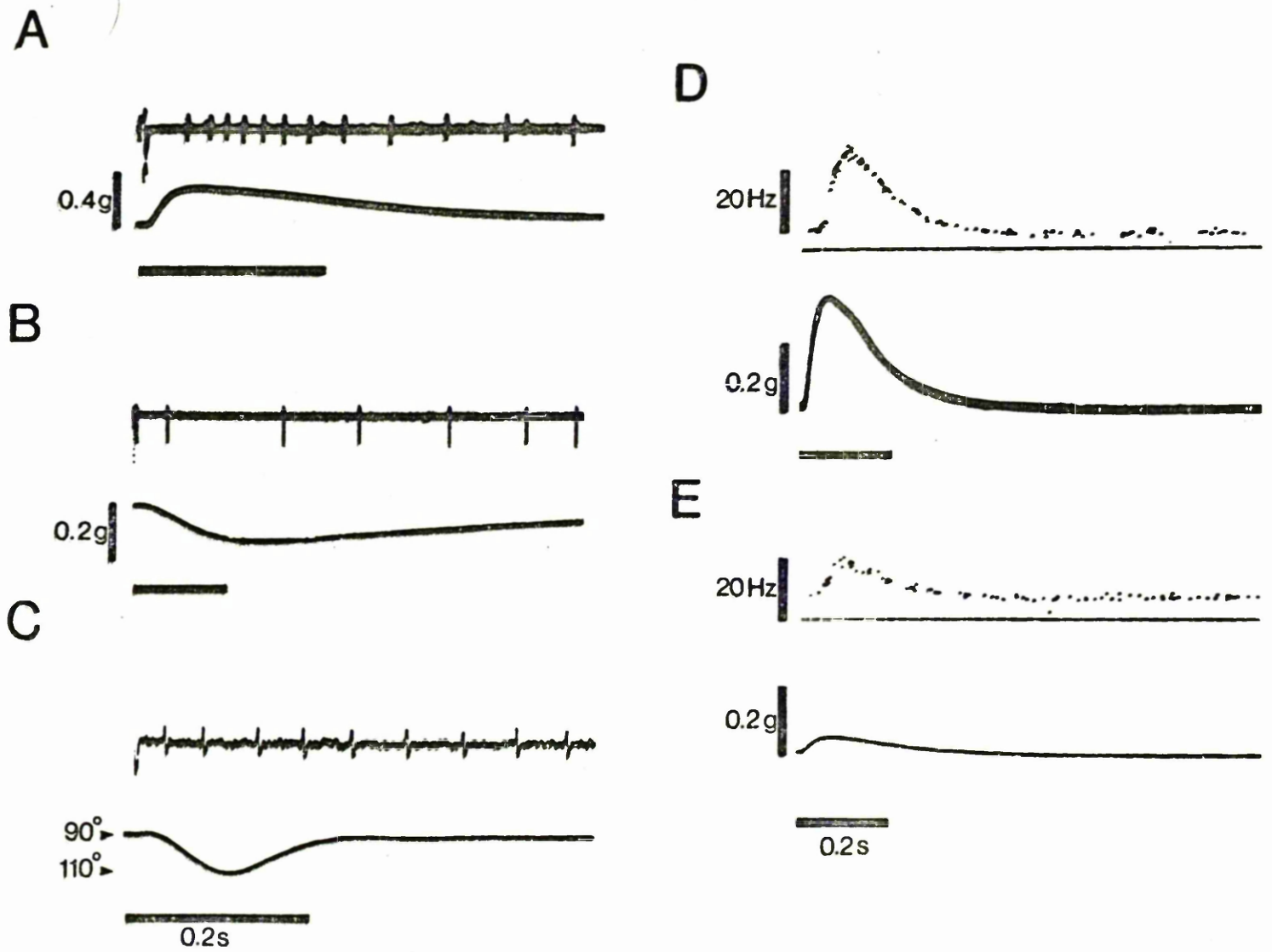

A
1g
1s
D
200
Hz
100
0
1s
B
6
4
2
Tension (g)
a
b
10.4
10.7
11.0
11.2
Length (mm)
C
11.4
11.2
11.0
10.8
10.6
10.4
10.2
Muscle Length (mm)
60°
90°
120°
150°
180°
FTA
E
30
22.5
15
7.5
0
Spike Frequency (Hz)
a
b
60°
90°
120°
150°
180°
FTA

Fig. 4:5

Responses of the tension receptor to twitch contractions of the flexor tibiae muscle. A - maximal isometric twitch of the whole muscle, B - isometric relaxation due to single stimulus to the inhibitory axons, C - near isotonic twitch of the muscle with the femur-tibia angle recorded, D and E - instantaneous frequency plots of responses to isometric twitches of two different strengths in the part of the muscle to which the receptor is attached. The calibrations are the same for all records.


A
0.4g
B
0.2g
C
90°
110°
0.2s
D
20Hz
0.2g
E
20Hz
0.2g
0.2s

responding directly to tibial position (Williamson & Burns, 1973). Nerve 5B1 shows the highest level of activity (Fig.4.3A), most of which comes from the chordotonal organ in response to tibial position (Burns, 1974). However, after cutting nerves 5B1b, 5B1c and 5B1 to the tibia, some sensory activity can be seen coming from n.5B1e (Fig. 4.3C). This activity comes from a single small axon with a spike amplitude only 3-5 times the noise level and some 30 times smaller than the spike from the large sensory hair afferents running in n.5B1e. It persists when all the branches of n.5B1e are cut distal to the multipolar cell but ceases when the nerve is cut proximal to the cell. It is also greatly increased by any mechanical disturbance to the flexor muscle near to the cell. Thus the axon almost certainly originates in the multipolar cell.

The receptor axon is continuously active at 5-10 spikes per second when the flexor tibiae muscle is fully relaxed, but when the muscle is stretched by rapidly extending the tibia the firing frequency increases in a response which has both phasic and tonic components (Fig. 4.4D). The phasic response is confined to the first 0.5s after the muscle is stretched and also appears as a transient reduction in firing frequency below the rest level when the muscle is relaxed. If the muscle is stretched to different lengths within its physiological range by increasing the femur-tibia angle (FTA), both the tonic activity in the receptor axon and the activity in the first 10 seconds after the movement increase nonlinearly (Fig. 4.4E). The relationship between muscle length and FTA is almost linear (Fig. 4.4C) showing that the non-linearity in the response must reside in the muscle fibres or the receptor. It also suggests that the apodeme of the flexor muscle is fairly stiff where it attaches to the tibia since a completely flexible apodeme would result in a sinusoidal relationship between length and FTA. From the responses to passive extension of the muscle it is not

possible to show that the effective stimulus is muscle tension rather than length, although the firing frequency in the receptor axon (Fig. 4.4E) appears to be very closely related to the passive tension in the muscle (Fig. 4.4B). The close similarity between the instantaneous frequency in the receptor axon (Fig. 4.4D) and the passive muscle tension during a quick stretch (Fig. 4.4A) also suggests that the phasic component of the response may be entirely due to the mechanical properties of the muscle.

The responses of the receptor to active contractions of the flexor muscle do show that it is primarily sensitive to muscle tension. If the muscle is made to twitch isometrically by stimulating the excitatory axons in n.5B2 after cutting the nerve branches to the rest of the muscle, the receptor activity increases (Fig. 4.5A), but if the tibia is free to move so that conditions are isotonic, the receptor does not respond (Fig. 4.5C). When the region of the muscle containing the receptor twitches under isometric conditions the frequency in the receptor nerve accurately follows the tension, with a lag of about 40 ms (Fig. 4.5D, E). The response frequency increases with increasing rest tension in the muscle (Fig. 4.6) when the muscle is passively stretched, although this reduces the amplitude of the muscle fibre movement. The receptor also responds to the reduction in tension induced by stimulating the inhibitory fibres in the flexor motor nerve (Fig. 4.5B).

Like the chordotonal organ (Burns, 1974) the responsiveness of the tension receptor depends on its environment during the experiment. It is most responsive in haemolymph with the tracheal system functioning and its sensitivity is similar in circulated, oxygenated saline. However, if the saline is still, or is not oxygenated the responses of

the receptor to isometric twitches disappear, leaving only the responses to passive tension. This may be the result of a loss of phasic responsiveness.

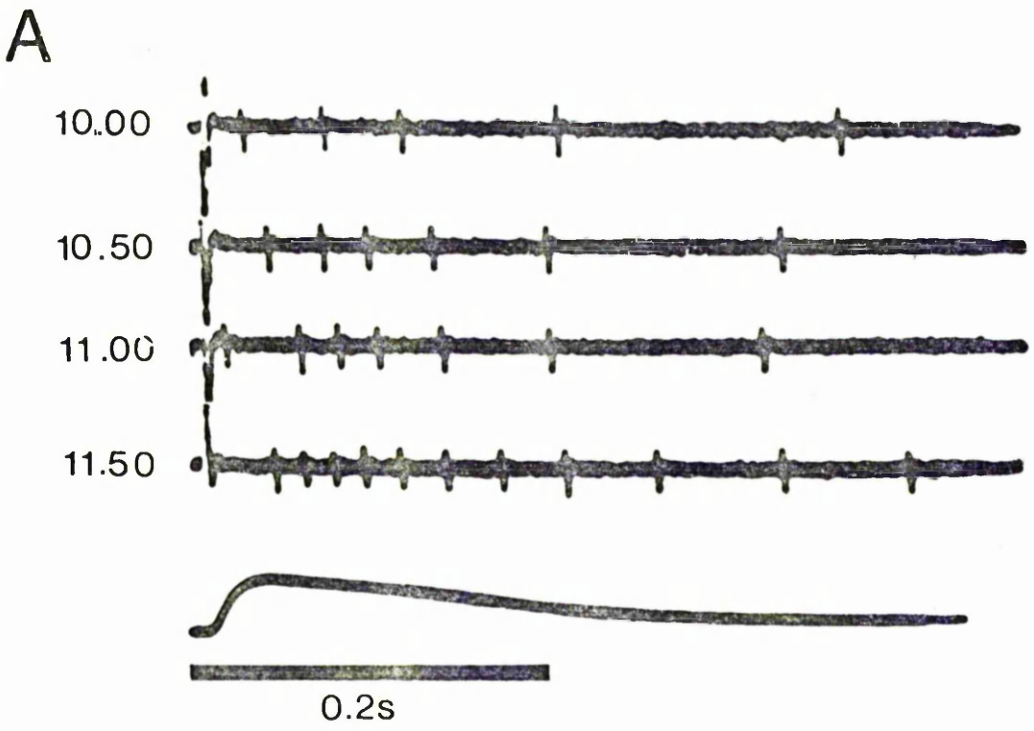
Reflexes mediated by the tension receptor.

If the tension receptor normally participates in the coordination of leg muscles it should be possible to demonstrate that its activity has an influence on motoneuron firing patterns. This was attempted by developing tension in the part of the muscle connected to the receptor and looking for reflex responses in the flexor and extensor motoneurons. Typical results are shown in Fig. 4.7. This demonstrates firstly that the receptor responds well to maintained contraction of the muscle (Fig. 4.7A), which is more like the natural behaviour of the muscle in the intact locust, and secondly, that this pattern of receptor activity causes a reflex activation of a number of slow flexor motoneurons (Fig. 4.7B) and a transient inhibition of the slow extensor motoneuron (SETi) (Fig. 4.7C). These positive feedback reflexes onto the flexor neurons were found in 18 out of 20 cases, while the inhibition of the SETi occurred in 6 out of 7 cases. In the remaining animals the signs were reversed, so that the flexor reflex became negative and the SETi was excited by the receptor.

The reflex onto the SETi is less clear than the flexor reflex and can be demonstrated more convincingly by looking at its effect on the normal resistance reflex of the intact leg. With all the sensory systems in the leg intact the SETi is usually activated when the tibia is passively flexed and its firing frequency depends on the velocity of the movement (Fig. 4.8A). When the flexor tension receptor axon is cut the resistance reflex is considerably enhanced (Fig. 4.8B), suggesting

Fig. 4:6.

Responses of the tension receptor to isometric twitch contractions of the flexor tibiae muscle at different rest lengths. A - typical records at the rest lengths marked, B - increase in receptor firing frequency above resting rate. The dotted line shows the maximum natural length of the muscle. Bars show ± 1 standard deviation. Data from 15 experiments on 3 animals.


A
10.00
10.50
11.00
11.50
0.2s

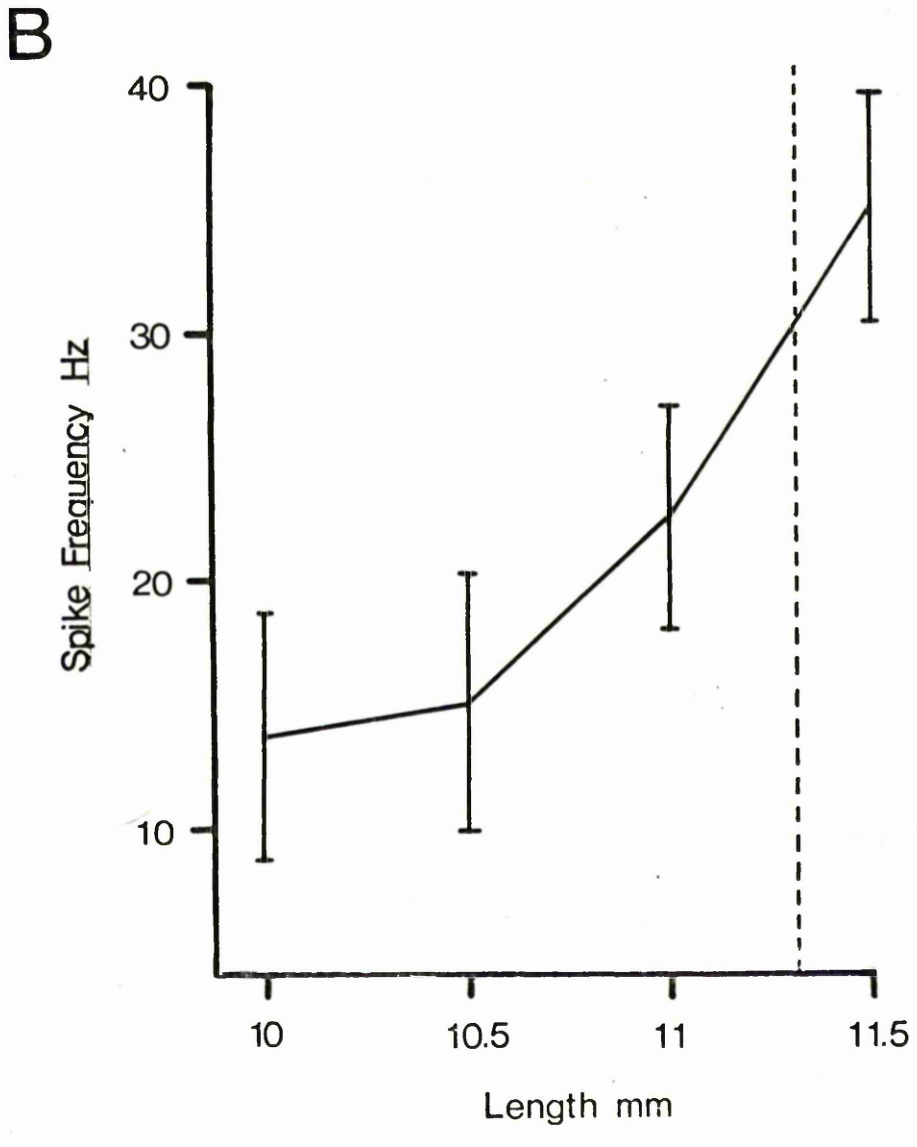

B
40
30
20
10
Spike Frequency Hz
10
10.5
11
11.5
Length mm

Fig. 4:7.

A - activity in the tension receptor axon during a maintained flexor muscle contraction, B and C - reflexes evoked by the receptor in the flexor motor nerve (B) and the slow extensor tibiae (SETi) motoneuron (C) in response to active tension in the distal part of the flexor muscle, as shown in the lower traces. The flexor motor nerve was cut half way along the femur and stimulated distal to the cut to induce a contraction in the part of the muscle containing the receptor. The cut prevented the flexor reflex from affecting the receptor. Calibrations are the same for all records.

Fig. 4:8.

Influence of the flexor tension receptor on the resistance reflex in the slow extensor tibiae (SETi) motoneuron. A - resistance reflex in intact leg, B - resistance reflex with the tension receptor axon cut. Lower traces show imposed movement of the tibia.

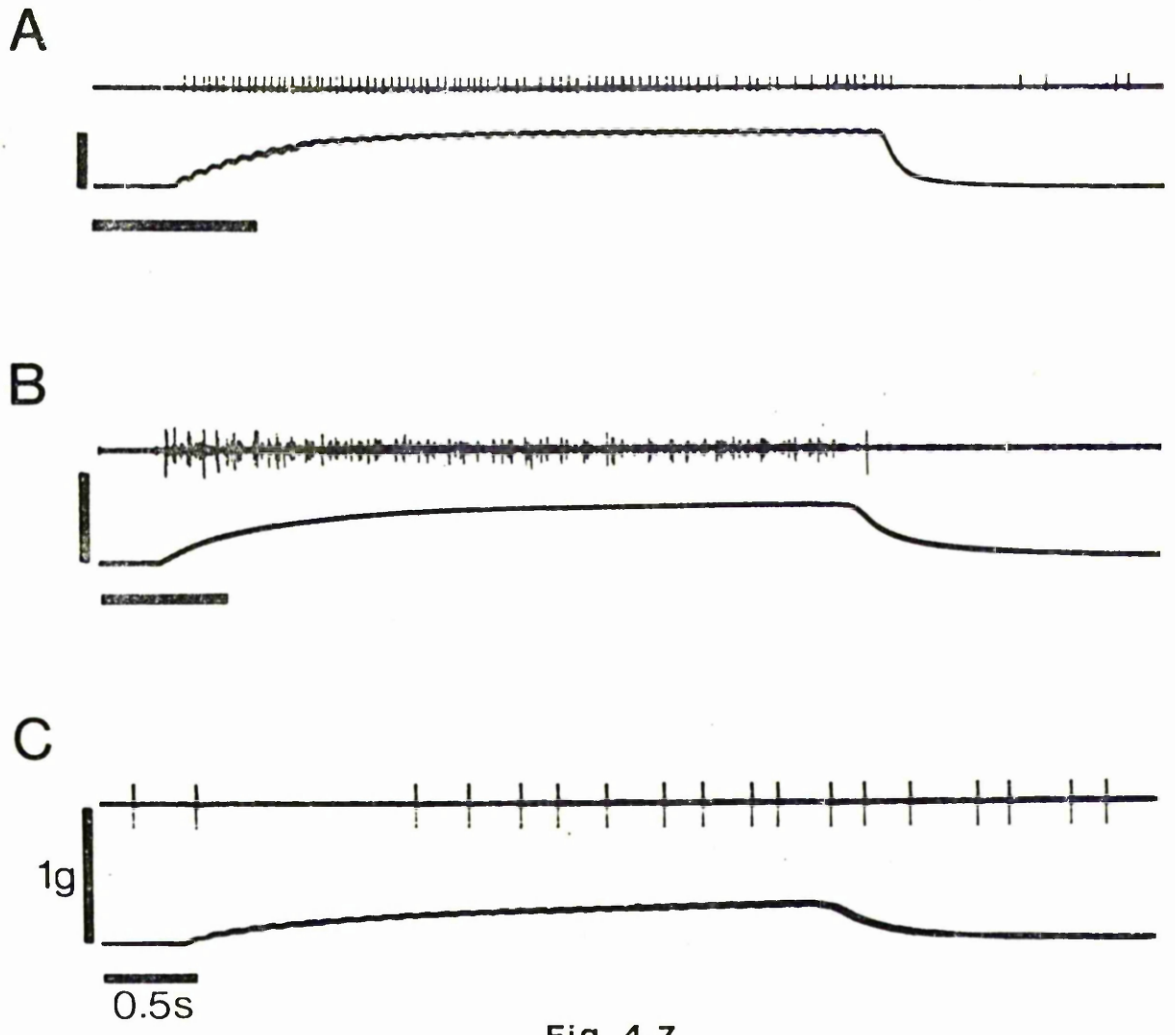


Fig. 4.7

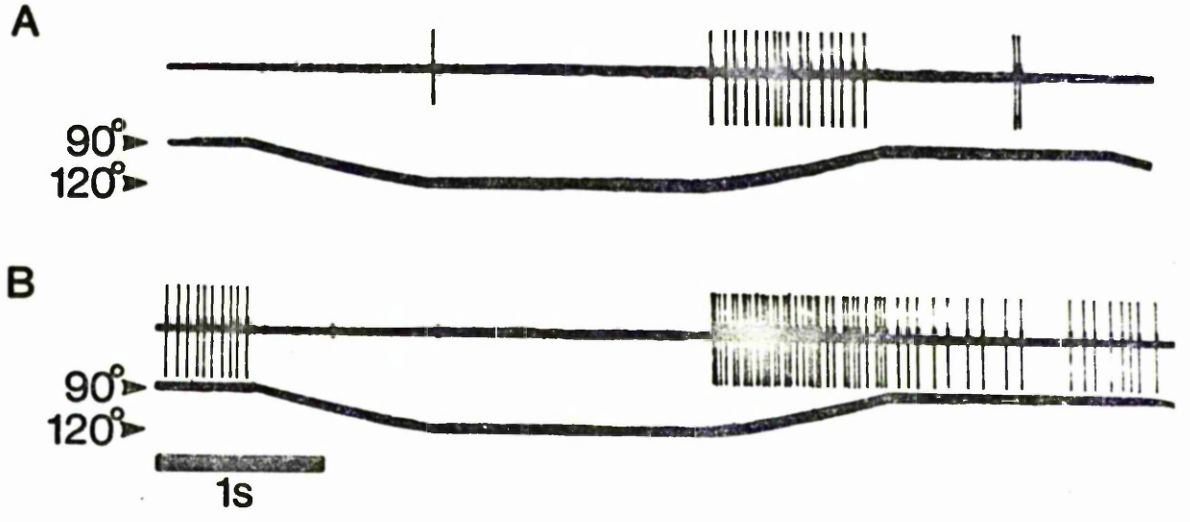


Fig. 4.8

the removal of an inhibitory input. When the chordotonal organ is also removed, the resistance reflex disappears altogether.

4. Discussion

The results show conclusively that the prothoracic and mesothoracic femurs of the locust contain a single neuron whose axon runs in nerve 5Ble2 and which is a tonic receptor for tension in one of the distal bundles of the flexor tibiae muscle. There seems little doubt that this muscle tension receptor (MTR) is the large multipolar neuron located at the base of one of these bundles. The properties of the receptor are very similar to those of the single unit investigated by Bässler (1977) in the stick insect. Bässler was unable to identify the neuron responsible, but comparison with the locust leg suggests that the large nucleus he found on stick insect nerve F121 is the correct choice. The locust MTR may be analogous to the large multipolar neurons found at both ends of the tibial flexor muscle of Limulus polyphemus (Eagles, 1978; Eagles & Gregg, 1978). However, in this animal the cells at the insertion of the muscle are reported to be receptors for muscle length rather than tension.

The MTR is morphologically very similar to the multiterminal stretch receptors attached to abdominal muscle fibres in many species of insect, in centipedes and in scorpions (Finlayson, 1976). In Rhodnius (Hemiptera) the abdominal receptors resemble the locust MTR in that their dendrites are attached to a number of muscle fibres (Anwyl, 1972) but in most insects so far examined each receptor is connected to only one muscle fibre. If the muscle fibres concerned are part of the main segmental muscle the receptors could function similarly to the femoral MTR and monitor tension, but if they are innervated separately the

receptors may function as segment length detectors.

The locust MTR differs from tension receptors on the leg muscles of crustaceans and vertebrates in that it is a single cell located in a contractile region near the fixed end of the muscle rather than a multicellular organ located in the tendon. Although this means that it can only monitor tension in a small region of the muscle this is also true of a single neuron in the vertebrate Golgi tendon organ which usually monitors the tension in a restricted number of muscle fibres (Barker, 1967) all of which may be separately innervated (Reinking et al., 1975). In both receptors the sensitivity to active tension is greater than to passive force applied to the tendon because much of the passive tension is developed in connective tissue or muscle fibres not connected to the receptors (Houk, 1967). There is currently no evidence that the neurons on the crustacean tendon organ are differentially sensitive to different parts of the muscle although the fact that the neurons are distributed along the length of the apodeme in a pinnate muscle (MacMillan & Dando, 1972) would seem to place them in a good position to do so.

Since the locust flexor tibiae muscle is also pinnate in form it is not clear why the MTR is located on the muscle fibres rather than the tendon. It may be that this position confers a special sensitivity to local contraction of the associated fibres, although there was no indication that these fibres are innervated differently from others in the same region. This region of the muscle is more tonic than the more proximal parts so that sensory feedback of flexor muscle activity may be divided between the MTR responding to tension in the tonic distal fibres, the cuticular campaniform sensilla responding to stress from the more powerful phasic fibres and the chordotonal organ monitoring

movements of the phasic proximal part of the flexor (Burns, 1974).

In spite of the fact that the MTR is only a single cell it mediates a strong positive excitatory reflex onto the flexor motoneurons and an inhibition of the slow extensor motoneuron powerful enough to interfere with the reflex from the much larger chordotonal organ. Similar positive reflexes have also been found in the abdomen of the caterpillar (Weevers, 1955) where the single celled MRO receptor excites parallel muscles. The locust MTR reflexes are opposite in sign to the equivalent reflexes in the stick insect (Bässler, 1977), the crab (Clarac & Dando, 1973) and in mammals (Granit & Ström, 1951). However, the sign of such reflexes may change with the behavioural state of the animal. Thus Macmillan (1976) reports that the crab tension receptor reflex is sometimes reversed when the animal is active and chordotonal reflexes in the stick insect femur change sign with changes in the state of arousal on the insect (Bässler, 1976). In a few locusts negative feedback reflexes from the MTR were found.

The positive feedback may be part of a load compensation reflex similar to that mediated by campaniform sensilla in the cockroach (Pearson, 1972). A similar load sensitive reflex has been reported in the metathoracic leg of the locust (Burrows & Horridge, 1974) and was ascribed to muscle tension receptors which were not identified. In the mesothoracic leg it is possible that the MTR excitation of the flexor motoneurons is also a mechanism for distributing loads over the whole muscle. Unlike the extensor motoneurons, most of the flexor neurons innervate only restricted areas of the muscle (Theophilidis & Burns, in preparation). The muscle bundle containing the MTR does not appear to have its own unique motor supply, but it lies in the distal part of the muscle which shares few of its motor axons with the more phasic proximal

parts. Thus tension developed in the distal muscle fibres may cause the MTR to reflexly excite motoneurons supplying other parts of the muscle, producing a contraction which will reduce the load on the distal fibres. In this connection it is interesting that it is the distal part of the flexor muscle which is most used in posture and in walking (Theophilidis & Burns, in preparation), so the MTR reflex may be able to provide additional tension when it is needed.

Acknowledgements

We thank Drs. D.M. Neil and W.J.P. Barnes for comments on the manuscript, and Mr. D.S. Little and K. Ritchie for assistance with the diagrams.

5. RESISTANCE REFLEXES

A. Methods

The locust was fixed ventral side down, in the middle of a platform made from plasticine (4 x 4 x 2cm). The femur of the mesothoracic leg was mounted horizontally on the same platform in such a way as to allow free movement of the tibia in a vertical plane (fig. 5.1.). The rest of the legs were also mounted on the platform. The animal was left in this position for five or six hours to settle before any experiments were undertaken. Then the femur of the leg under investigation was dissected, as described in fig. 2.1B. Care was taken not to destroy any of the sensory nerves from the main femoral mechanoreceptors. A constant velocity movement, between 90^{o} and 120^{o} was imposed by a lever on the tibia at three different angular velocities (see Chapter 2). The movement of the tibia produced resistance reflexes in the femoral muscles which were recorded from the nerve branches of the flexor and extensor muscles using hook or suction electrodes. Cross reflexes and reflex activity from the DUM cells were recorded from the contralateral leg.

B. Results

Reflex excitation of the flexor tibiae motoneurons (FlTiM's) is a variable phenomenon which depends on the strength of the sensory inputs and the excitability of the animal. Most of the reflexes studied in this section are resistance reflexes which tend to resist passively imposed femur-tibia joint movements by excitation of the motoneurons innervating the femoral muscles. Sensory activity responsible for these reflexes is shown in fig. 4.3. where it can be seen that most of the proprioreceptors detecting femur-tibia movement and position are

Fig. 5.1

The arrangement used to record femoral reflexes produced by tibial movement.

T = tibia

F = femur

P = protractor

M = shaft of the pen motor connected to the tibia via the lever (L). The axis of the rotation of the shaft and lever were in line with the tibial articulation. To record activity from the DUM cells, or cross reflexes the contralateral leg was also dissected.

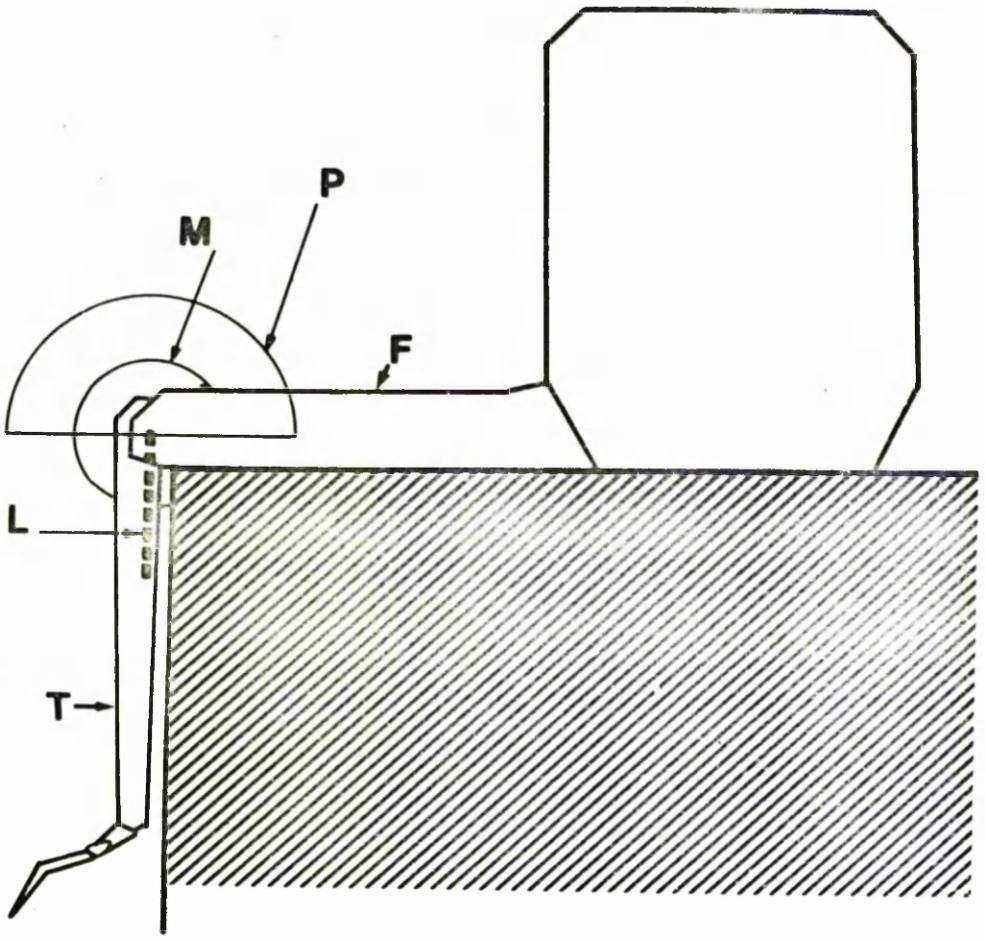

P
M
F
L
T

Fig. 5.2

Records from the mesothoracic flexor tibia muscle in the dissected femur of an immobilized locust. The femur was filled with haemolymph and a small amount of saline was added and replaced with fresh oxygenated saline every 3 to 5 min. All leg sensory inputs were removed except from the chordotonal organ. Tibial movement was imposed with the arrangement described in Methods.

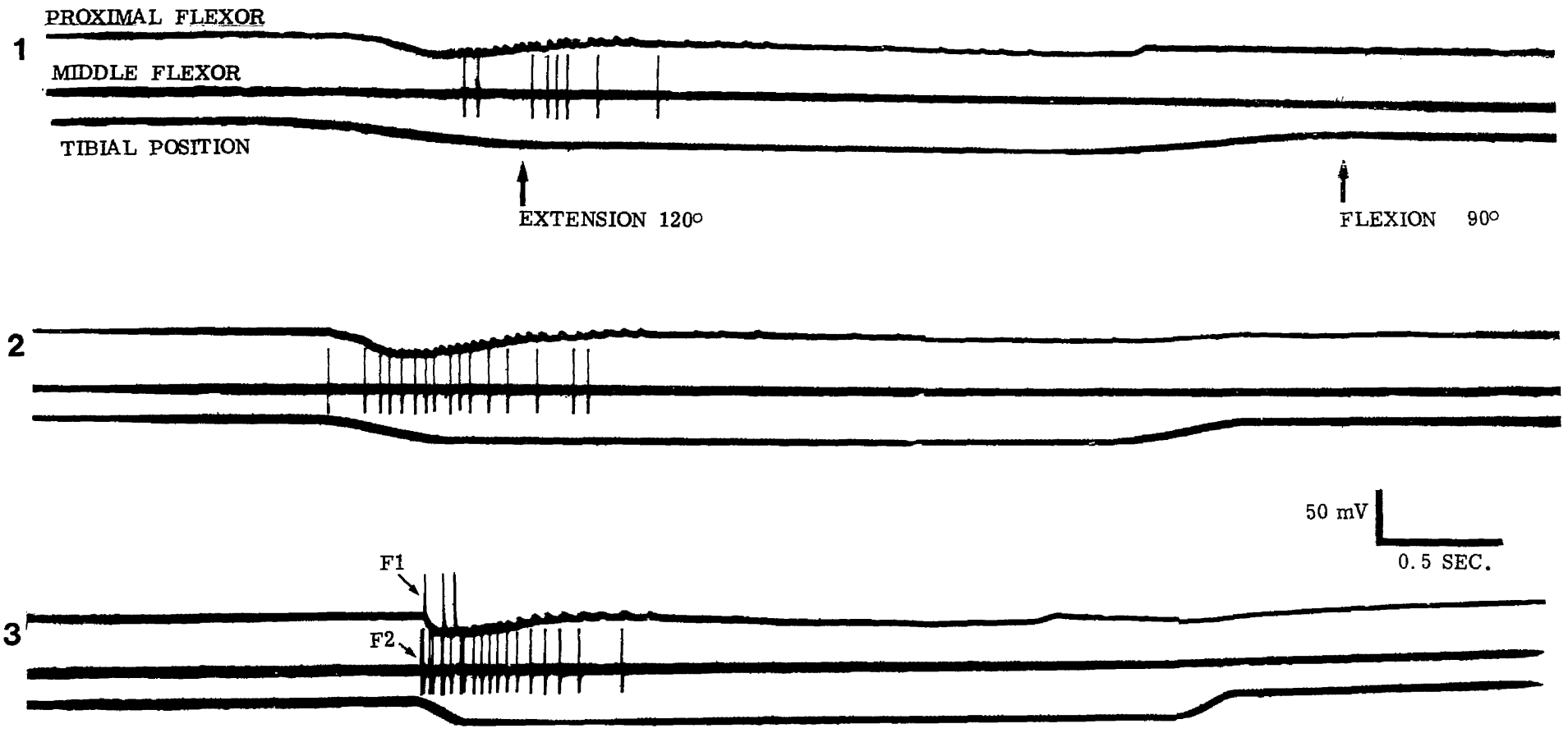
Top trace: Intracellular record from the proximal flexor muscle fibre .

Middle trace: Extracellular record from the middle flexor nerve branch.

Bottom trace: Tibial position.

Angular velocities were: 1: 38°/s, 2: 75°/s, 3: 150°/s.

At the higher velocity (3), the three large EPSP's can be identified as from F1 since they are not correlated with any of the action potentials in the middle flexor nerve branch. Small EPSP's in the proximal flexor could be due to M1.


1
PROXIMAL FLEXOR
MIDDLE FLEXOR
TIBIAL POSITION
EXTENSION 120°
FLEXION 90°
2
50 mV
0.5 SEC.
3
F1
F2

active during imposed extension of the tibia and, except for the chordotonal organ (CO), they are not very active during flexion. The CO is the most important sensory input in the generation of resistance reflexes involving femoral motoneurons. This is demonstrated by the fact that removal of this mechanoreceptor abolishes most of the resistance reflexes.

Reflexes are not only dependent on the strength of the sensory inputs to the central nervous system but also on the excitability of the animal. To avoid variation in the responses caused by differences in the behavioural state of different animals the locusts were fixed (as in fig. 5.1) and experiments undertaken after six hours. This was an attempt to bring the excitatory state of each animal to about the same level.

Motoneuronal activity was recorded from the three parts of the flexor tibiae muscle to allow identification of the different flexor motoneurons. The tibia was extended from 90 to 120° at constant angular velocities of 38°/s, 75°/s and 150°/s which correspond to the average velocities at which a locust extend its tibia when walking at speed of 0.5, 1 and 2 steps/s (calculated from Burns, 1973).

Excitatory flexor motoneurons.

The flexor motoneurons are excited during passive extension of the tibia indicating a negative feed back from the leg sensory receptors. The fast flexor motoneurons fired continuously only when the tibia was extending at frequencies which depended on the velocity of the extension. The intermediate motoneurons responded to the extension of the tibia in the same way as the fast axons but often their activity was prolonged after the end of the extension. The slow motoneurons were spontaneously

active and increased their firing frequency when the tibia was extended.

Typical records demonstrating resistance reflexes are shown in fig. 5.2 where a full cycle of the imposed tibia movement can be seen. This figure also demonstrates reflex excitation of motoneuron F1, which has a large axon innervating only the 30% of the proximal flexor (fig. 3.12). F1 is a high threshold motoneuron which is excited only by fast tibial movement and produces a maximum of three spikes. The relation between firing rate of F1 and the angular velocity of tibial extension is given in fig. 5.3A.

The large action potentials, in fig. 5.2 (second trace), are responses of motoneuron F2 to the imposed extension of the tibia. Responses of motoneuron F2 also can be identified, in fig. 5.4A which shows intracellular records from the proximal muscle fibres and nerve records from the middle and distal nerve branches. Axon F2 has been identified as the motoneuron whose branches innervate only the proximal and middle flexors (fig. 3.12). This motoneuron seems to reach threshold when the tibia is extended at velocities greater than $38^{\circ}/s$ (fig. 5.3A). Below this value F2 is usually silent but in some animals the threshold was lower.

A similar pattern of reflexes as that of F2 is shown by motoneuron F4 (fig. 5.4B). Axonal branches from this motoneuron have been identified only in the middle and distal flexor (fig. 3.12). This motoneuron was sometimes completely inactive but when it was active it responded to tibial extension of all the velocities as can be seen in fig. 5.3B.

Figures 5.4A and B demonstrates the two most common patterns of resistance reflexes recorded from the flexor tibia motoneurons. The main difference between these two patterns is that in the first case,

Fig. 5.3

Typical responses of the mesothoracic flexor tibiae axons in relation to the angular velocity of tibial extension. The axons are usually activated in groups where some of them are fully active and others suppressed. These graphs were plotted from animals in which each particular axon was fully active. For each axon the above curves represent records taken from at least two different animals from 10 different imposed extension movements. Bars show ±1 standard deviation.

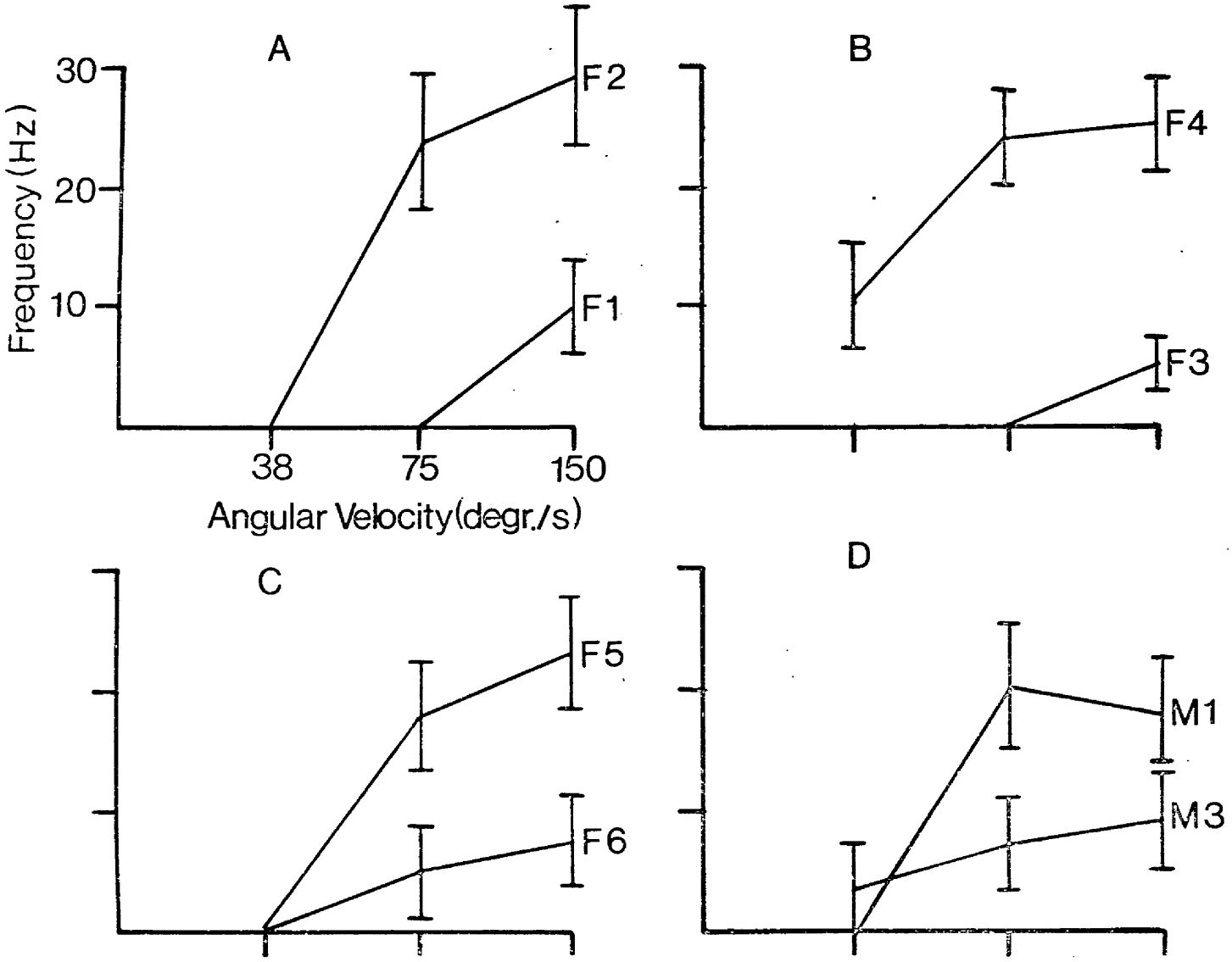

A
B
C
D
30
20
10
Frequency (Hz)
F2
F1
F4
F3
F5
F6
M1
M3
38
75
150
Angular Velocity (degr./s)

Fig. 5.4

Typical records used to identify most of the mesothoracic flexor tibiae motoneurons in resistance reflexes produced by extending the tibia of three different velocities, 1: $38^{\circ}/s$, 2: $75^{\circ}/s$, 3: $150^{\circ}/s$.

A. The most common flexor motor pattern produced by tibial extension.

1st trace: Intracellular record from a proximal muscle fibre.

2nd trace: Extracellular record from the middle flexor nerve branch.

3rd trace: Extracellular record from the main distal flexor nerve branch.

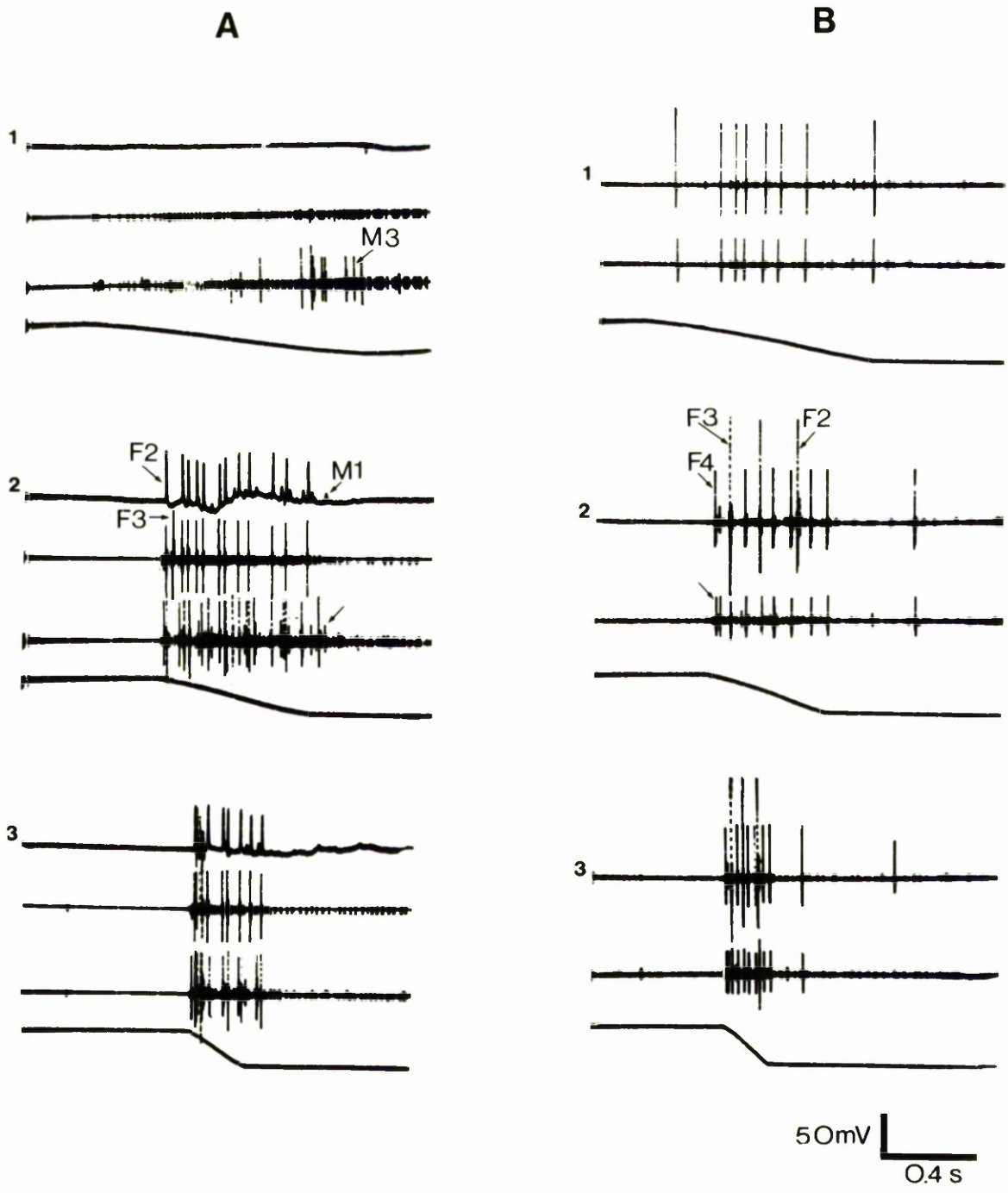
4th trace: Tibial position, downward movement = extension from 90° to 120°. Action potentials of the inhibitors cannot be confused here since they usually activated immediately after the end of the extension movement (Fig. 5.5B). The identification criteria for the flexor motoneurons as described in Chapter 3.

B. An extreme case where although most of the flexor axons were silent F4, F3 and F2 were active.

1st trace: Extracellular record from the middle flexor nerve branch

2nd trace: Extracellular record from the main distal flexor nerve branch

3rd trace: Tibial position.


A
B
1
M3
2
F2
M1
F3
3
1
F3
F2
F4
2
3
50mV
0.4 s

F2 is the dominant active motoneuron while F4 is silent (8 locusts) and in the second case (fig. 5.4B) their roles are reversed (3 locusts). In both cases motoneuron F3 is activated by fast tibial movements, producing one or two spikes (fig. 5.4A, B and fig. 5.3B).

Among the motor axons which innervate the distal flexor only M3 is activated during slow tibial movements (fig. 5.4A), F5 and F6 are activated by the faster movements. The frequency-angular velocity curves from these motoneurons are shown in fig. 5.3C, D. Motoneuron M1 is activated usually by the fast tibia movement and when the angular velocity increases, paradoxically the activity of this motoneuron decreases (fig. 5.4A and fig. 5.3D).

Motoneurons S1 and S2 which innervate the whole flexor muscle (fig. 3.12) fire continuously with frequencies of 5 to 10 Hz. When the tibia is passively extended to 120° the firing frequency of these motoneurons becomes 15 - 20 Hz and remains at this value without any adaptation, as long as the extension is maintained (fig. 5.5A). This firing frequency is almost independent of the velocity at which the tibia is moved. Motoneuron S3 in the proximal flexor responds in a similar way.

Reflexes in the fast flexor tibiae motoneurons often habituate if the mechanical stimulation of the femur tibia proprioreceptors is applied for prolonged periods. This phenomenon is demonstrated in fig. 5.6. Habituation could cause variation in the results described above and for this reason most of the records were taken in the first five cycles of stimulation.

Flexor inhibitory neurons.

The I1 and I2 motoneurons innervate the whole flexor muscle (fig. 3.12). These neurons have a very low threshold and can be easily activated by touching different parts of the animal. They are silent in a quiescent animal at a femur-tibia angle of 90°, however they are excited by imposed tibial extension (fig. 5.5B). Neuron I1 and I2 remain silent during the extension of the tibia from 90° to 120° but they are activated immediately after the end of this movement. Their firing frequency depends on the velocity of the extension and it declines rapidly from a peak frequency immediately after the tibia stops moving. When the tibia is flexed these neurons are silent. In the six animals examined, two failed to show any responses of the inhibitory neurons to the extension of the tibia.

D.U.M. neurons.

It is a difficult task to monitor activity from the flexor DUM cells (D1 and D2) during movement of the tibia, because most of the D.U.M. action potentials are masked by the activity from the larger flexor tibiae motor axons. It has been shown (section 3) that the D1 neuron (DUMETi) innervates the flexor and extenson tibiae muscles on both sides of the animal. Using this property the above difficulties were eliminated by recording activity from D1 in the extensor nerve from the contralateral side to the reflexively excited flexor motoneurons. The D1 spike was identified from an action potential produced every second by stimulating the extensor nerve from the side on which the tibial movement was imposed (fig. 5.7A). The D1 neuron is not excited by flexion or by extension of the tibia at any of the angular velocities. Occasionally it fires at about one spike every two or three seconds. D1 can fire with a burst of increased frequency up to 5 or 8 Hz whenever

Fig. 5.5

Records from the mesothoracic flexor tibiae muscle in the dissected femur of an immobilized locust (conditions as in Fig. 5.2).

A. 1st trace: Intracellular record from a distal muscle fibre.

2nd trace: Extracellular record from the middle flexor nerve branch.

3rd trace: Tibial position.

Notice the size of the action potentials produced by axons S1 and S2 (2nd trace). They are very small and very difficult to distinguish from the noise level of the record, especially when other larger axons are activated.

B. 1st trace: Intracellular record from the distal muscle fibre.

2nd trace: Tibial movement.

Angular velocity: 1: 18°/s; 2: 38°/s; 3: 78°/s.

I1 = inhibitor 1, I2 = inhibitor 2.

Axons I1 and I2 were identified also in the other parts of the muscle using similar method to that described in Fig. 3.20.

In this preparation the thresholds of the inhibitory axons were very low and the activity of the excitatory motoneurons was correspondingly low (see small group of EPSP's).

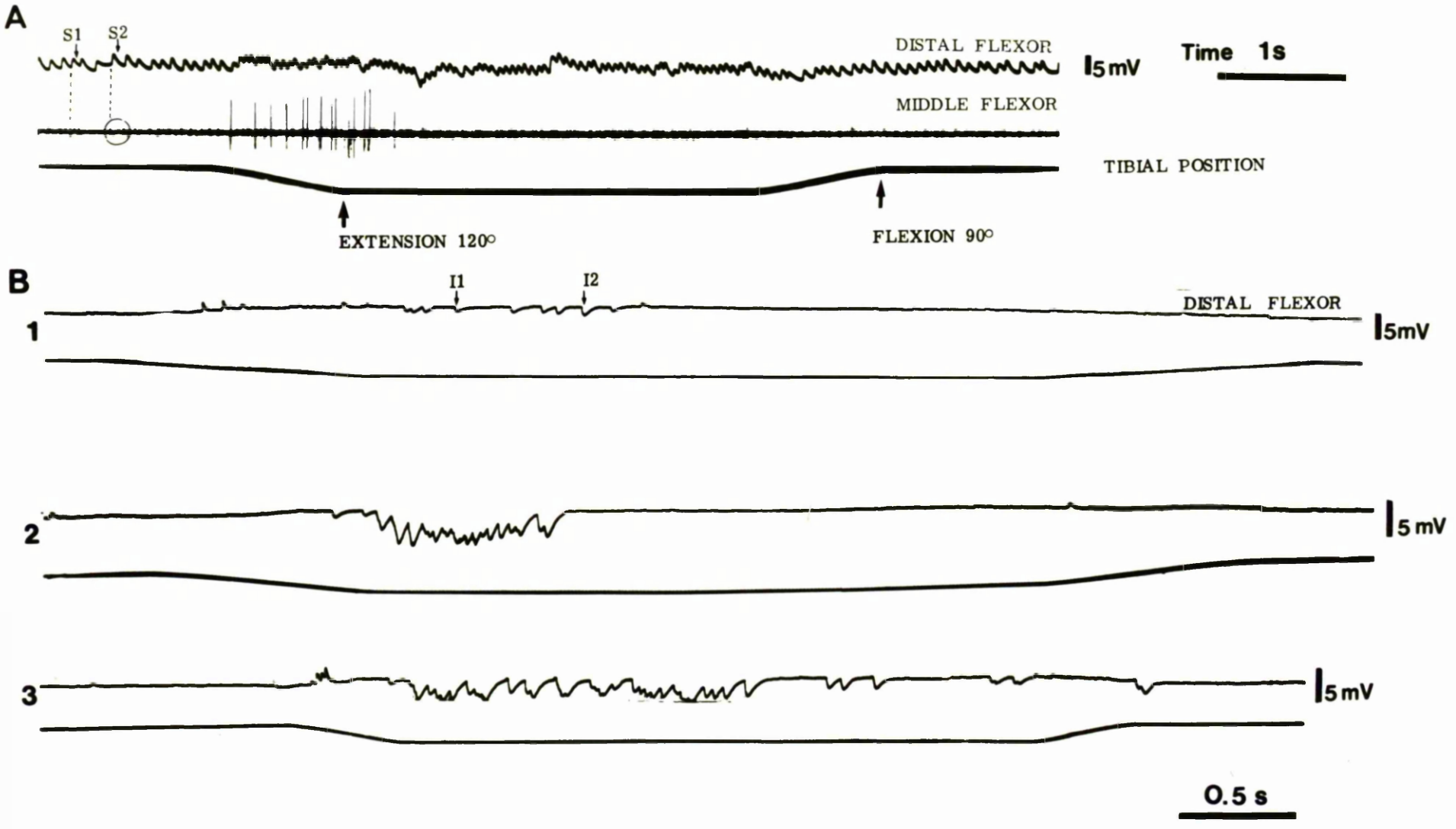

A
S1
S2
DISTAL FLEXOR
5 mV
Time 1s
MIDDLE FLEXOR
TIBIAL POSITION
EXTENSION 120°
FLEXION 90°
B
I1
I2
DISTAL FLEXOR
1
5mV
2
5 mV
3
5 mV
0.5 s

Fig. 5.6

Records from the mesothoracic flexor tibiae muscle in a dissected femur of an immobilized locust (conditions as in Fig. 5.2).

1st trace: Intracellular record from the proximal flexor.

2nd trace: Extracellular record from the middle nerve branch.

3rd trace: Extracellular record from the distal nerve branch

4th trace: Tibial position. Downward movement of the trace = tibial extension. Angular velocity of movement $78^{\circ}/s$.

Each record was taken after the number of cycles of tibial movement shown at the left side of the figure.

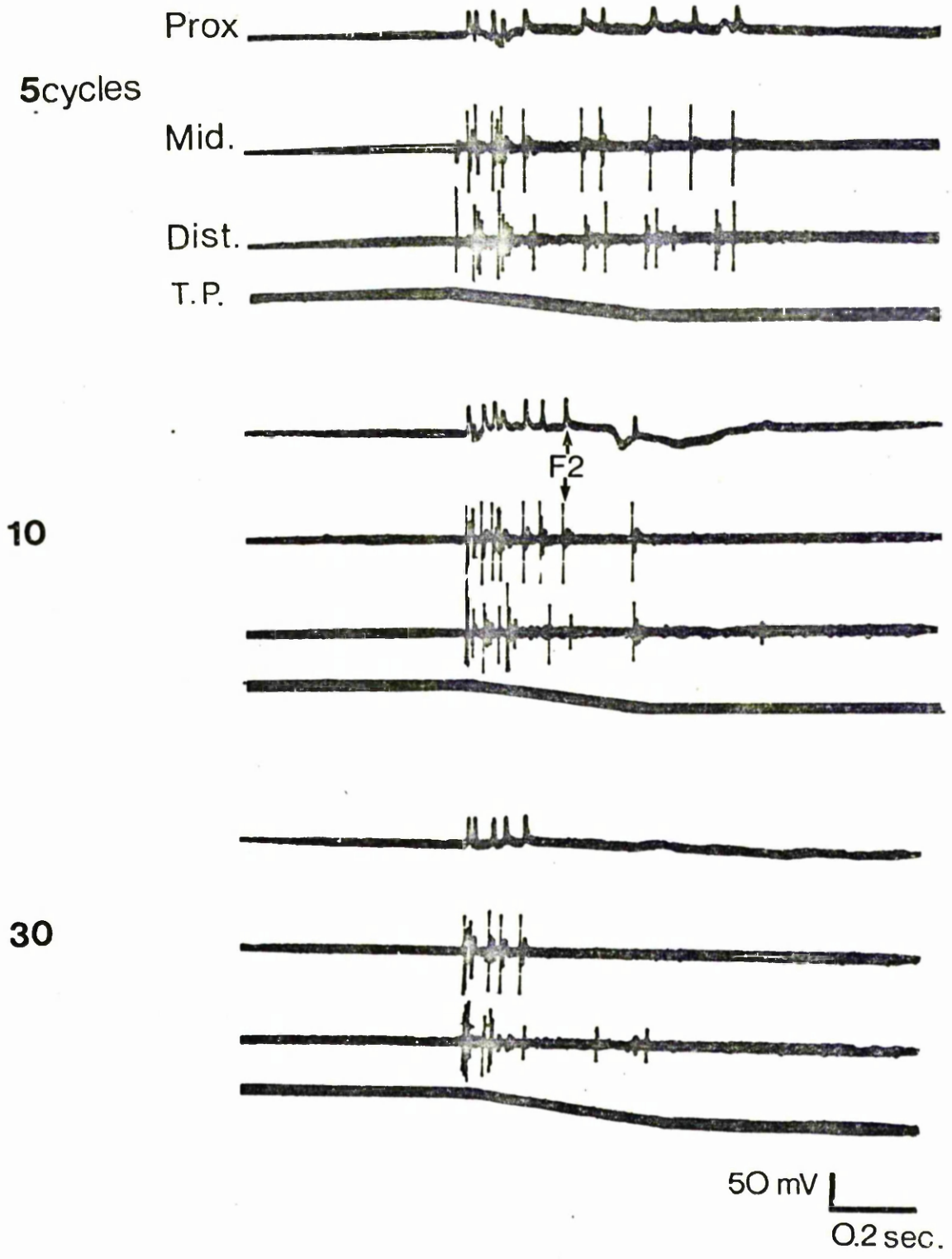

Prox
5cycles
Mid.
Dist.
T.P.
F2
10
30
50 mV
0.2 sec.

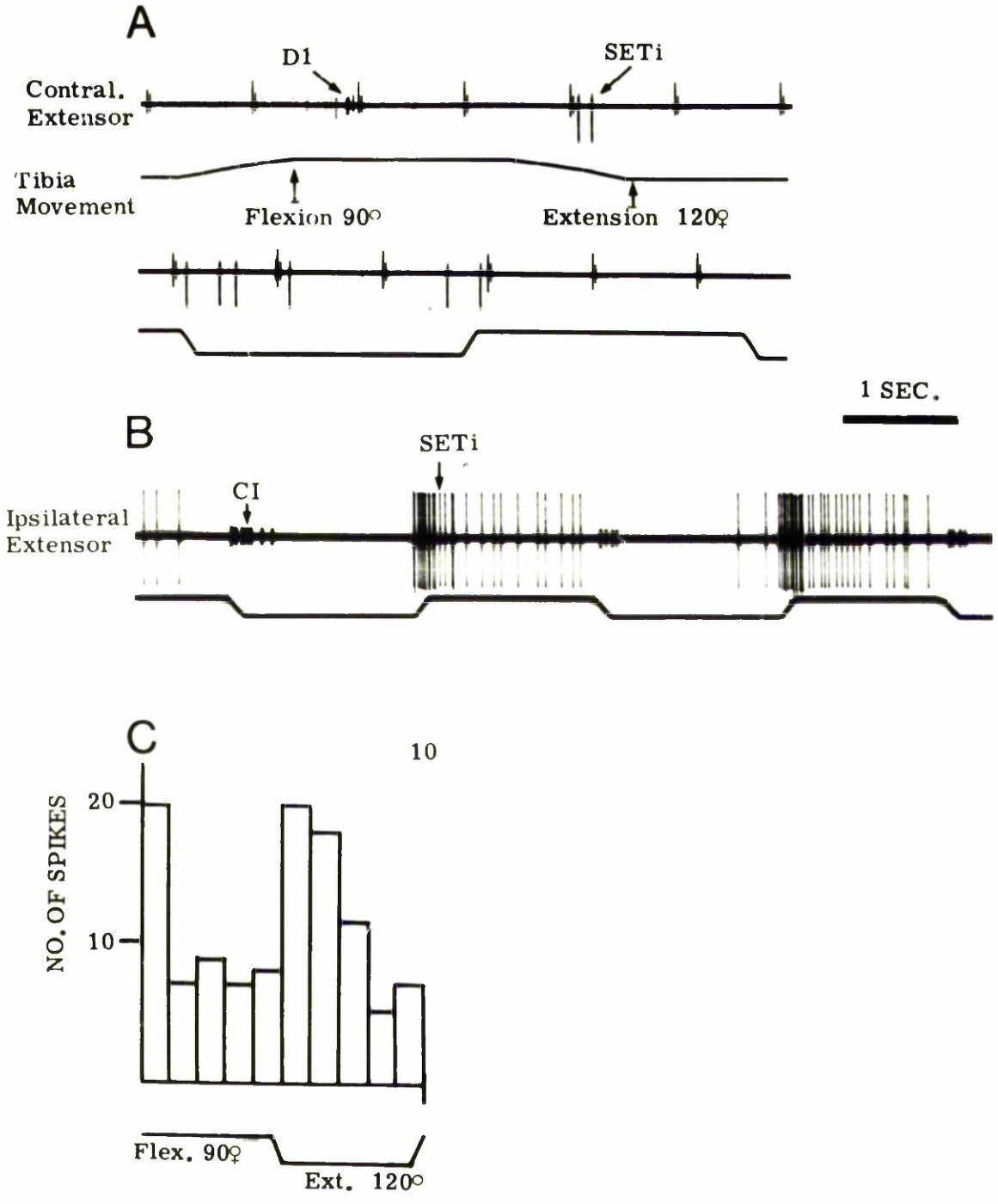
Fig. 5.7

A and B. Records from the extensor nerves in both dissected mesothoracic femurs of an immobilized locust (conditions as in 5.2).

A. Responses of the DUM cell (D1) and the extensor SETi from one side of the animal (1st trace), to the movement of the opposite mesothoracic tibia (2nd trace) imposed at two different angular velocities (38°/s first record, 300°/s second record). The large "action potential" repeated every second is an artefact due to a stimulus applied to the ipsilateral extensor nerve in order to identify axon D1 which runs in both mesothoracic extensor nerves.

B. Reflexes produced in the extensor tibiae neurons (CI, SETi, 1st trace) by extension or flexion of the tibia of the same leg as indicated in the 2nd trace.

C. Peristimulus time histogram plotted for the SETi from similar records to those in A. Tibial movement of the opposite leg as indicated below the histogram.
Duration of plot = 3s.


A
D1
SETi
Contral.
Extensor
Tibia
Movement
Flexion 90°
Extension 120°
1 SEC.
B
SETi
CI
Ipsilateral
Extensor
C
10
20
10
NO. OF SPIKES
Flex. 90°
Ext. 120°

the insect is aroused (fig, 5.7A, see arrow). Similar behaviour is shown by the DUMETi neuron in the metathoracic leg (Hoyle et al., 1974). Activity from neuron D2 was not recorded due to the fact that its action potential is very small and is hidden by the activity of the other axons.

Extensor motoneurons.

Resistance reflexes in the mesothoracic extensor tibia motoneurons were reported by Burns (1974) and in the metathoracic by Usherwood et al. (1968) who found that extension of the chordotonal apodeme invariably activated the SETi and the resulting frequency of firing and duration of activity increased with the rate of stretch of the chordotonal organ. This is also shown in fig. 5.7B where it can be seen that extension of the tibia also excites the common inhibitory neuron (CI). The CI, which innervates the extensor but not the flexor tibiae muscle, responds only when the tibia is extending, in contrast with the flexor inhibitors (fig. 5.5B) which are inactive during this tibial movement. Reflexes from the SETi and CI motoneurons are very reliable and they persist for more than 50 cycles.

Cross reflexes.

The slow and intermediate axons to the mesothoracic flexor tibiae muscle of the ipsilateral leg are excited during constant velocity movement of the contralateral mesothoracic tibia. However, these cross reflexes only occur in animals with a relatively high central excitatory state and the phase relationships between motor output and contralateral input are not very precisely defined.

Studies of the extensor motoneurons show that the SETi is also excited during the movement of the contralateral mesothoracic tibia producing more reliable cross reflexes (fig. 5.7A), a fact which makes

this motoneuron a better subject than the flexor motoneurons for studying contralateral reflexes. Fast movements of the contralateral tibia ($300^{o}/S$) produce a weak reflex activation of the SETi. This neuron responds to both extension and flexion of the contralateral tibia but it seems from fig. 5.7C that its response is stronger during extension which is opposite in signe to the ipsilater reflex.

6. MOTONEURONAL ACTIVITY DURING WALKING ON A TREADMILL

A. Methods

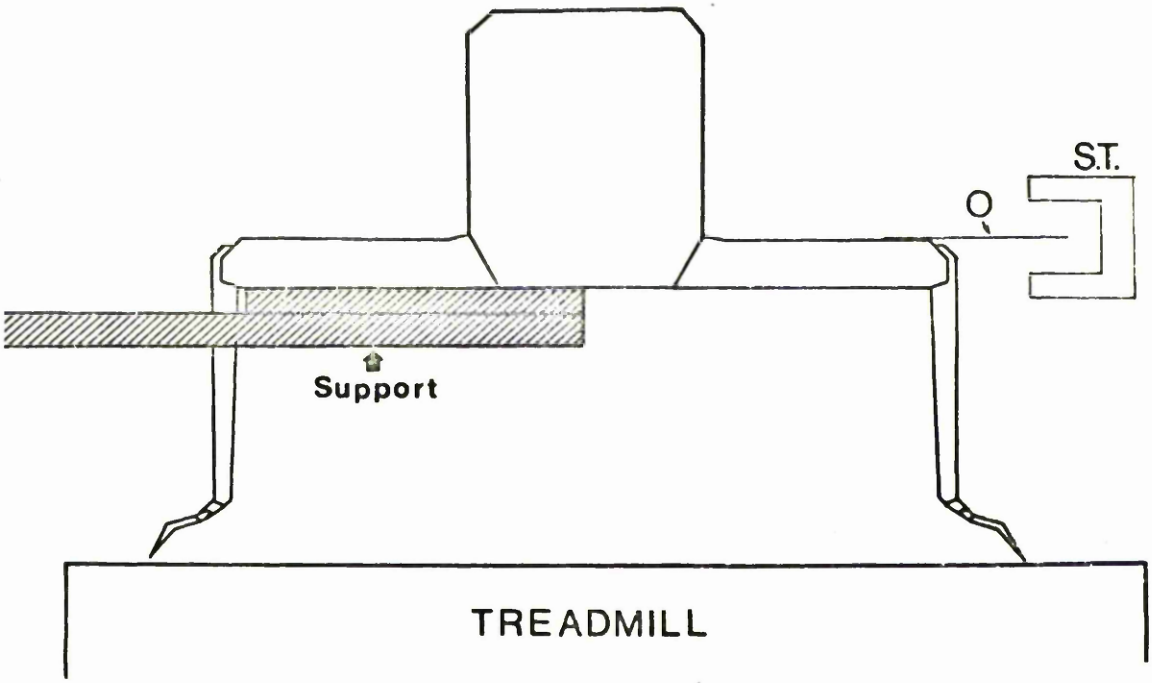
The thorax of the locust was fixed ventrally on a small platform (2 x 12 x 2mm) made from balsa wood. This platform was firmly attached to the end of horizontal mounting rod which was held horizontally in a magnetic stand (fig. 6.1). A treadmill was put under the animal so that the locust was able to walk on it. The treadmill was made from light foam perspex (Rohacell), was free to rotate under the animal and was 20cm in diameter and 8cm wide. The weight of the wheel was counterbalanced with a lever so that the upthrust on the legs of the locust was approximately equal to the animal's weight. Light card was glued to the outer rim of the treadmill to provide a non-slip surface for the tarsi of the locust. The legs were free to move except for one mesothoracic leg, the femur of which was mounted on the balsa platform (fig. 6.1) and dissected as in fig. 2.1B. This leg was deafferented by cutting the sensory nerves always at the same point.

When they were required, care was taken to leave all the main sensory inputs intact, while a constant velocity movement (see chapter 5) was imposed on the mesothoracic tibia to excite femoral reflexes. In this case the ramp generator produced a trigger pulse before the beginning of each ramp to start analysis by a microcomputer (Burns, 1977) which generated a peristimulus histogram. Neuronal activity was recorded extracellularly with hook electrodes and postsynaptic potentials in the muscle were recorded with glass micropipettes filled with 3MKCl. As an indication of the walking activities of the animal, the movement of the contralateral femur (protraction, retraction) were monitored with a capacitive movement detector (Sandeman, 1967).

The treadmill was also used to study an intact unrestrained

Fig. 6.1

The arrangement used to record from the mesothoracic extensor and flexor tibiae muscles and nerves in the dissected femur of a tethered locust walking on a treadmill. The transmitting wire (O) of the Sandeman Transducer (S.T.) was fixed to the contralateral femur to monitor protraction and retraction of this leg. (Horizontal movement of the femur).


S.T.
Q
Support
TREADMILL

leg during walking. In this case, the animal was fixed with its pronotum glued dorsally to a metal saddle at the end of a vertical holding rod. All the legs were free to walk on the treadmill placed under the animal. Pairs of 40μm copper wires were implanted into the main femoral muscles to record muscle activity during walking. The movements of the femur of the leg under investigation or of the contralateral leg were also monitored. Electrophysiological data was stored on tape and was analysed from film using a semi-automatic analyser (Burns and Delcomyn, 1976) in conjunction with a general purpose digital computer. Histograms were plotted on a graphical output device.

B. Results

In the study of the use of the flexor tibiae motoneurons during walking, it is important to identify the activity of each individual motoneuron. This requires separate records from the three different parts of the flexor muscle. In order to obtain such records from a walking animal, it was found necessary to immobilize the mesothoracic leg and to allow the locust to walk with the remaining legs on a treadmill (fig. 6.1). The femur of the fixed leg could then be dissected to expose the sensory and motor nerves (see Methods). Under these conditions "walking" was defined as the occurrence of regular stepping movements in the free legs and of alternating bursts of activity in the antagonistic muscles of the clamped femur. Walking on the treadmill was stimulated by touching the abdomen of the animal with a fine brush. In a clamped leg it is possible for abnormal forces to be developed which may cause high frequency firing from the femoral and tibial proprioceptors. For this reason the main sensory nerves were

cut, so eliminating reflex effects on the motoneuron activity. Since the deafferented flexor motoneurons of a locust walking on the treadmill are activated reciprocally with the extensor motoneurons it will be interesting to know if this pattern is related with the alternating bursts produced by the antagonistic femoral muscles in a free walking leg. If there are any similarities between the activity of a deafferented and a normal leg these studies will also provide valuable information about the activity of the flexor motoneurons in a normal walking animal. In order to establish this, the activity of the femoral motoneurons in a deafferented and a normal leg were compared.

Activity in a deafferented leg.

Typical records taken from the nerves of femoral muscles in a deafferented leg (fig. 6.2A) show that the extensor and flexor motoneurons fired in alternating bursts. In order to determine the time relationship between the activity of these motoneurons phase histograms were plotted for 13 steps from an animal walking at speed of 1 step/s. In the case of the extensor muscle the compound activity of the SETi and FETi was plotted, while for the flexor muscle the combined activity of all the axons which innervate the distal flexor was plotted (fig. 6.3A). These histograms show that in a deafferented femur, although the extensor and flexor motoneurons produce a reciprocal firing pattern there is an overlap between their activities. Usually the end of the flexor burst overlaps with the beginning of the slow extensor burst. Fig. 6.2A shows also that there is a variation in this overlap and sometimes the activity of the small distal flexor motor axons can be prolonged even in the burst of the antagonistic FETi. In an animal walking slowly (fig. 6.2C) the interval frequency of the flexor burst was low enough to allow identification of axons in the distal nerve

Fig. 6.2.

Records from the mesothoracic extensor and flexor tibiae muscles and nerves of a tethered locust walking on the treadmill.

A. Records from a deafferented dissected femur.
   1st trace: Extracellular record from the extensor nerve
   2nd trace: Extracellular record from the main distal flexor nerve branch.
   The distal axons cannot be individually identified since they fire at a high frequency.
   3rd trace: Horizontal movement of the contralateral femur. Upward movement of the trace = protraction; downward movement = retraction.

B. Electromyograms (EMG's) recorded from the femur of a free leg (see Methods).
   1st trace: EMG's from the distal flexor
   2nd trace: EMG's from the proximal part of the extensor muscle. In this case the wires were very close to the proximal flexor and the activity from the flexor F1 was recorded as cross talk.
   3rd trace: The movement of the contralateral femur.

C. As in A.
   1st trace: Extracellular record from the middle flexor nerve branch.
   2nd trace: Extracellular record from the main distal flexor nerve branch.
   3rd trace: movement of the contralateral leg.

In both A and C small action potentials in the 2nd trace occurring between the flexor bursts could be due to the flexor inhibitors. From A and B it is obvious that the SETi always fires at the beginning of the retraction of the contralateral leg in both deafferented and normal legs.

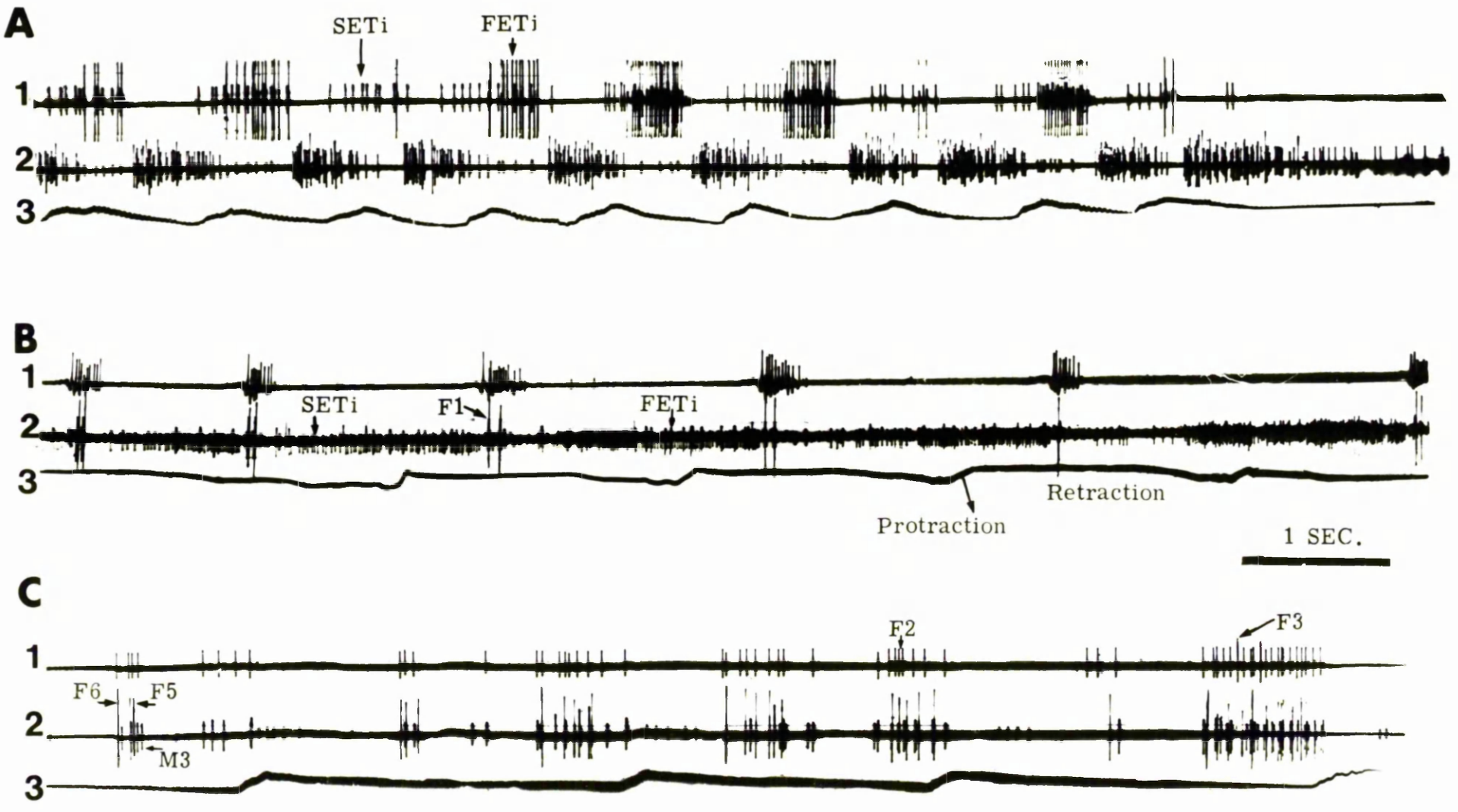
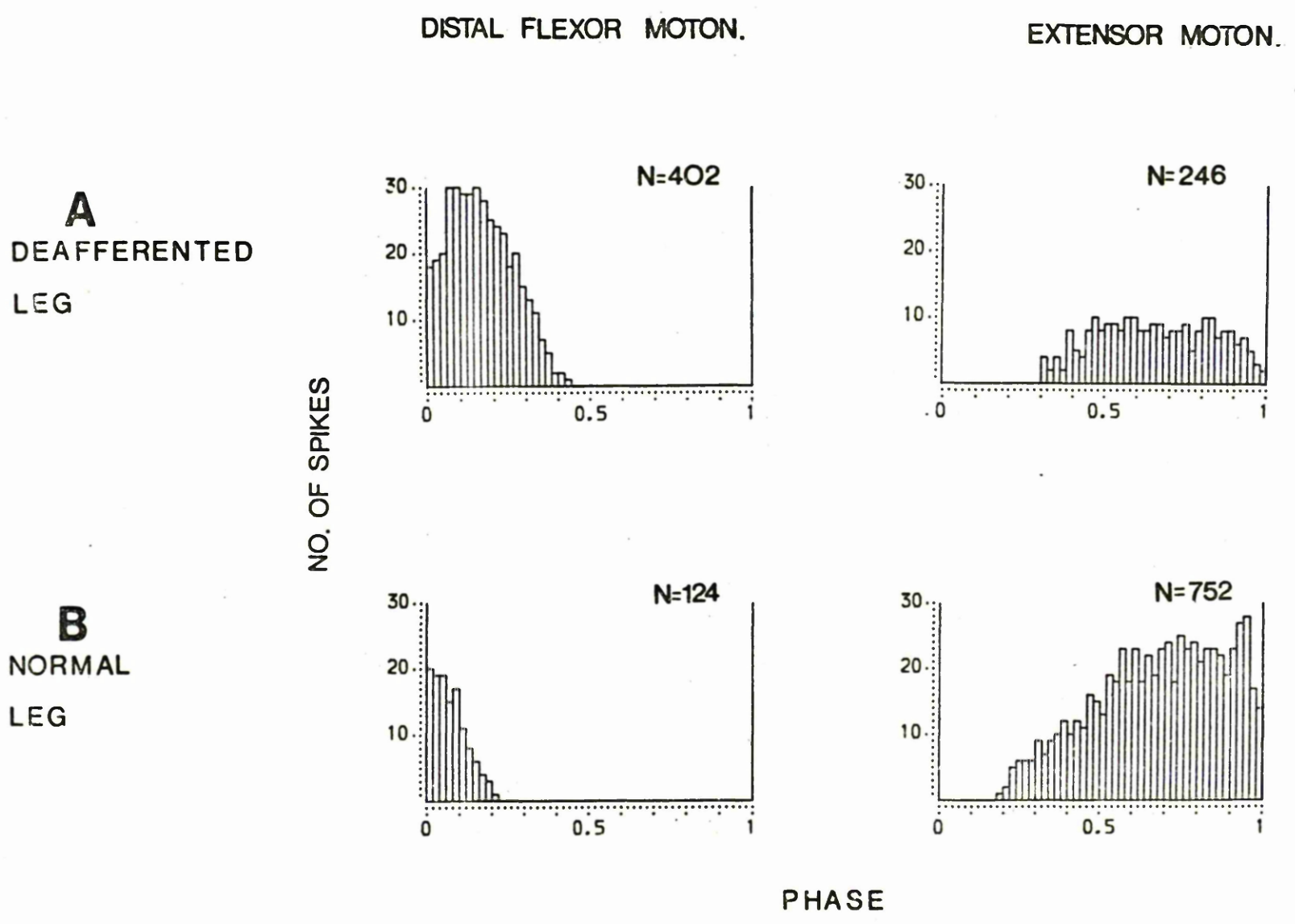

A
SETi
FETi
1
2
3
B
1
2
3
SETi
F1
FETi
Retraction
Protraction
1 SEC.
C
1
2
3
F2
F3
F6
F5
M3

Fig. 6.3

Phase histograms plotted for 12 different steps in a deafferented leg (A) and a normal leg (B) of a tethered locust walking on the treadmill.

A phase of zero was defined as the first spike of the burst of activity in the distal flexor motoneurons. Each histogram includes all the active excitatory motor axons.


DISTAL FLEXOR MOTON.
EXTENSOR MOTON.
A
DEAFFERENTED
LEG
B
NORMAL
LEG
N=402
N=246
N=124
N=752
30
20
10
0
0.5
1
NO. OF SPIKES
PHASE

branch. In this case the flexor burst started with a spike from F6 which immediately after was followed by F5 and M3. The activity of M3 is usually prolonged after F5 stopped. Notice also in fig. 6.2A and 6.2C (third trace) that although no movement could occur in the clamped mesothoracic leg, the alternating bursts recorded in the deafferented femur correspond in time with the stepping movement of the contralateral femur. (in antiphase).

To check that the recorded activity in the extensor nerve is really a walking pattern rather than unrelated firing of the femoral motoneurons, the records obtained from the mesothoracic extensor motoneurons of deafferented preparation (fig. 6.2A) were compared with the neurograms described by Burns (1972) for the extensor nerve of a normal free walking locust. He reported that the SETi fires continuously during early retraction and the FETi is activated at the end of this period as also can be seen in fig. 6.2B (second trace). This firing pattern is very similar to the activity recorded from the extensor nerve in a deafferented leg of a locust walking on a treadmill. However in the deafferented extensor motoneurons there are differences in the frequency within bursts and a variation in burst length which probably are caused by the fact that not only femoral and tibial proprioceptors are absent but also the coxal receptor can no longer detect movement since the femur is immobilised.

Activity from an intact mesothoracic leg walking on the treadmill.

In order to obtain an indication of how much the pattern produced by the flexor and extensor motoneurons in a deafferented animal walking on the treadmill differs from the pattern in an almost free animal, a comparison was made with the activity from an intact free leg.

For this purpose myograms and neurograms were recorded from the femoral muscles of a tethered locust walking on the same treadmill (see Methods). Recordings were made with copper wires implanted in the distal flexor and the proximal part of the extensor muscle (fig. 6.2B). This technique for recording muscle potentials is not accurate as far as identification of individual motor axons is concerned. However this disadvantage of myograms was reduced since the number of the motoneurons which innervate the distal flexor is already known (Chapter 3). Activity such as that in fig. 6.2B was recorded from six different animals and filmed at high speed. These records show that the flexor burst in the distal part of this muscle consists of a large action potential which appears at the beginning of the burst and is followed by another two action potentials of different sizes. The similarities between this firing pattern and the pattern produced in the distal nerve of a deafferented animal walking at the same speed on a treadmill (fig. 6.2C) suggest that there is a close relationship between the bursting pattern of the distal flexor motoneurons in a deafferented and a normal walking leg.

Activity histograms for the antagonistic femoral muscles plotted by a computer are shown in fig. 6.3B. These show that in a normal walking leg, immediately after the end of the extensor burst, a short flexor burst appears. The bursts of the distal flexor and extensor muscle form a perfect reciprocal pattern. The bursts of the extensor motoneurons in a normal leg are longer than in a deafferented, with significantly larger number of spikes. The burst of the distal flexor motoneurons are shorter with fewer spikes in a normal leg than in a deafferented. The conclusion here is that the difference in firing pattern between a deafferented and a normal leg concerns mainly changes

in length and internal frequency of the alternating bursts of the extensor and flexor muscles while the sequence in which the motoneurons are activated in both muscles seems to be independent of the peripheral sensory inputs. Therefore the activity from the flexor motoneurons in a deafferented preparation can reasonably be considered as normal for further investigation bearing in mind that this activity is slightly higher and more prolonged than the normal.

All the above evidence suggests the existence of an internal programme which drives the femoral motoneurons during walking on the treadmill. One could argue here that the sensory inputs from the other legs could cause intersegmental reflexes which may generate these alternating bursts in the deafferented femur. In order to eliminate this possibility the same experiments were repeated on animals where the prothoracic and metathoracic legs were amputated at the coxal joint. Under these conditions the remaining contralateral mesothoracic leg was able to step on the treadmill while the flexor and extensor tibiae motoneurons of the fixed deafferented leg produced a reciprocal pattern similar to that shown in fig. 6.2A.

Activity of individual flexor motoneurons during walking on the treadmill.

The composition of the flexor burst in a deafferented leg can now be analyzed in more detail. The identification of the individual flexor motoneurons was based on records taken from the three different parts of the flexor muscle in a locust walking on the treadmill. Neuron F2 fires continuously throughout the flexor burst as can be seen in fig. 6.2C which shows action potentials recorded from the middle and distal nerve branches in an animal walking on the treadmill. The spike frequency with the F2 burst is approximately 20 - 25 Hz. The

relationship between the activity of F2 and that of the axons which innervate the distal flexor is also shown in this figure. It is worth notice that the burst of F2 in the middle flexor corresponds in time and length with the compound burst of F6, F5 and M3 in the distal part of the muscle. The activity of axon F3 was recorded in the middle nerve branches (fig. 6.2C). This neuron which innervates only the middle flexor is inactive during walking on the treadmill and fires two or three spikes when the animal is aroused. During walking neuron F1 which innervates only the proximal flexor produces two or three spikes in the first half of the period in which the other flexor motoneurons are active (fig. 6.4A). Muscle activity recorded from this axon in a free walking leg (fig. 6.2B) shows that neuron F1 produces a similar firing pattern in a normal walking leg.

Motoneurons F5, F6 and M3 which innervate only the distal flexor are also active during walking on the treadmill, as fig. 6.2C shows. Here axon F6 fires a single spike at the beginning of the flexor burst and it is followed by a compound burst from F5 and M3. Muscle activity produced by these motoneurons in the distal flexor of a normal free walking leg is shown in fig. 6.2B. In this case it is clear that the duration of their burst is shorter. Axon M1 is not active in a stationary animal but fires with a similar pattern to that of F2 when the animal is walking (Fig. 6.4B). The slow flexors are the only motoneurons which are active when the animal is not walking. S1 and S2 fire continuously at a frequenxy of 5 -10 Hz and during walking this frequency rises approximately by two or three times. Motoneuron S3 shows a similar pattern but fires at lower frequencies. Fig. 6.4B demonstrates activity from neuron S3 which produces a relatively large EPSP in the proximal flexor and most of the time masks the activity of S1 and S2 in these records.

Fig. 6.4

Records from the mesothoracic flexor and extensor muscle and nerves of a dissected and deafferented femur of a tethered locust.

A and B, the locust was walking on the treadmill.

1st trace: Intracellular record from a proximal flexor muscle fibre.

2nd trace: Extracellular record from the main distal nerve branch

3rd trace: Horizontal movement of the contralateral femur as indicated in B.

Vertical scale bars = A : 50 mV, B : 25 mV.

The flexor motor axons were identified as described in Chapter 3.

C and D, the locust was beating its wings continuously as a result of wind stimulation of the head hairs.

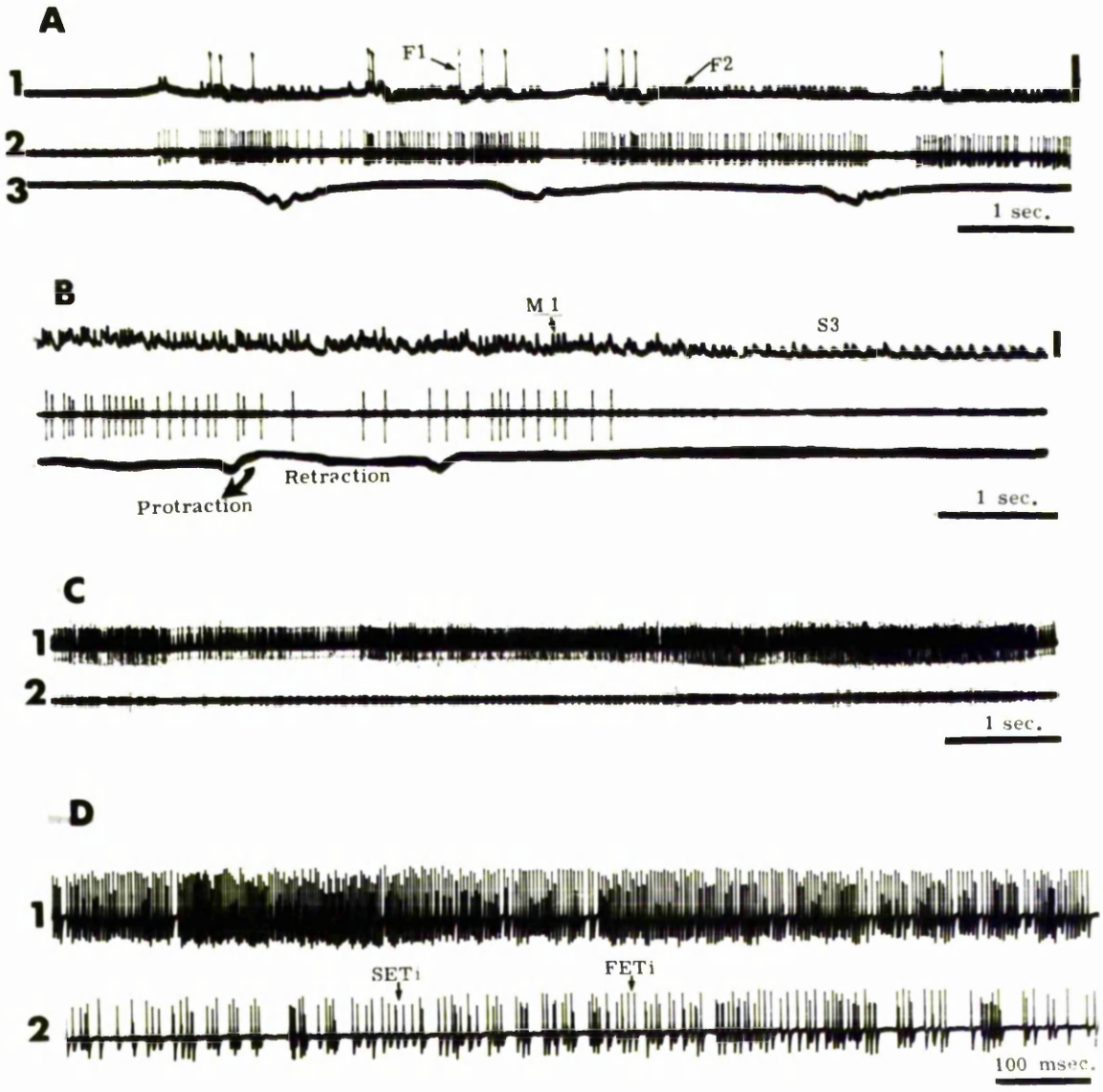
C. (1) Extracellular record from the extensor nerve

(2) Extracellular record from the main distal nerve branch.

D. Activity from the extensor nerve.

(1) Immediately after the beginning of flight

(2) 8 - 10s later.


A
F1
F2
1
2
3
1 sec.
B
M 1
S3
Retraction
Protraction
1 sec.
C
1
2
1 sec.
D
1
SETi
FETi
2
100 msec.

During walking, it was difficult to record from the inhibitors I1 and I2 due to the high level of activity from the flexor excitors. However IPSP's were recorded immediately after the animal stopped walking and most of the flexor excitors became inactive. The DUM cells seem not to be active during walking. Activity like that in fig. 6.2A from the extensor nerve was replayed at high amplification to reveal any smaller action potentials, but none were present. Of course these observations concern only the period when the FETi was not active. During FETi burst it was not possible to distinguish any other activity. It will be interesting to investigate the activity of D1 during the FETi bursts since this large axon is anatomically related with DUMETi in the metathoracic leg (Hoyle, 1978).

The activity of I1 and I2 and also D1 and D2 requires further investigation. Improvement of the recording methods could also supply more detailed information on axons F4 and M2 which have not been identified in a walking preparation.

Interaction between motor patterns and resistance reflexes.

The above information suggests that the pattern of activity produced by the motoneurons of the femoral muscles during walking is a product of an internal programme which functions from the moment that the animal starts to walk. Removal of the leg proprioceptors does affect this pattern but has been shown that it does not alter the sequence in which extension and flexor motoneurons fire. To investigate the effects of the sensory inputs on this internal programme the locust was fixed (as in fig. 6.1) and the immobilized leg was dissected, leaving the chordotonal organ (the main femur-tibia mechanoreceptor) intact. The tibia was cut transversely half way up and a constant velocity movement was imposed on the remaining part of the tibia to excite femoral

reflexes (see Methods). The activity of the SETi was recorded. This motoneuron was chosen for these experiments because identification of its activity is very easy. This makes it possible to plot activity histograms with an on line microcomputer which required input impulses from a window discriminator.

Fig. 6.5A shows an analysis of reflexes produced in the SETi during imposed movement of the tibia in a stationary animal on the treadmill. The flexion of the tibia produces a strong response in this motoneuron, its activity adapts to a prolonged flexion and is inhibited when the tibia is extended (see also fig. 5.7B). When the animal was left to walk on the treadmill while at the same time a ramp movement was imposed on the tibia to stimulate reflexes, the SETi neuron produced the bursting pattern typical of walking, but during imposed flexion of the tibia there was a decrease in its firing rate (fig. 6.5C). This suggests that the SETi motoneuron is inhibited during flexion of the tibia, which is the reverse of a resistance reflex. A control histogram of the SETi activity during walking on the treadmill was plotted while the tibiae was immobilized at a femur-tibia angle of 90° (fig. 6.5B). These experiments were done on four animals and the most obvious reflex was that which is demonstrated in fig. 5.5D. In two animals, when the locust was stationary after a long run on the treadmill, the SETi of the deafferented leg failed to show any reflex activity during imposed movement of the tibia. Sometimes in the same preparations SETi motoneuron fires rythmically every 400 - 500 ms in bursts of five or six spikes (fig. 6.6A). This activity can be prolonged for 3 to 5 min and this is very similar to the pattern with which the SETi fires when the locust walks on a treadmill at a speed of 2 steps/s (fig. 6.2A). In this case imposed flexion of the tibia slightly reduces the number of spikes in each burst of the SETi

Fig. 6.5

Peristimulus histograms plotted from records obtained from the mesothoracic extensor tibiae nerve in the dissected femur of a tethered locust on the treadmill. The femur was filled with haemolymph and a small amount of saline was added and replaced with fresh oxygenated saline every 3 to 5 min. All sensory input was removed except from the chordotonal organ.

A. Activity of the extensor SETi during the tibial movement indicated below the histogram (upward movement of the trace = tibial flexion) in a quiescent animal. This shows typical resistance reflexes (negative feedback).

B. Activity of the extensor SETi from a walking animal in the treadmill with the tibia fixed at an FTA of 90^{o}.

C and D. Repeats of A in two different locusts walking on the treadmill. The sign and phase of the feedback are different from those in A.

The right top number in each histogram indicates the number of sweeps making up the plots.

Vertical axis = number of spikes.

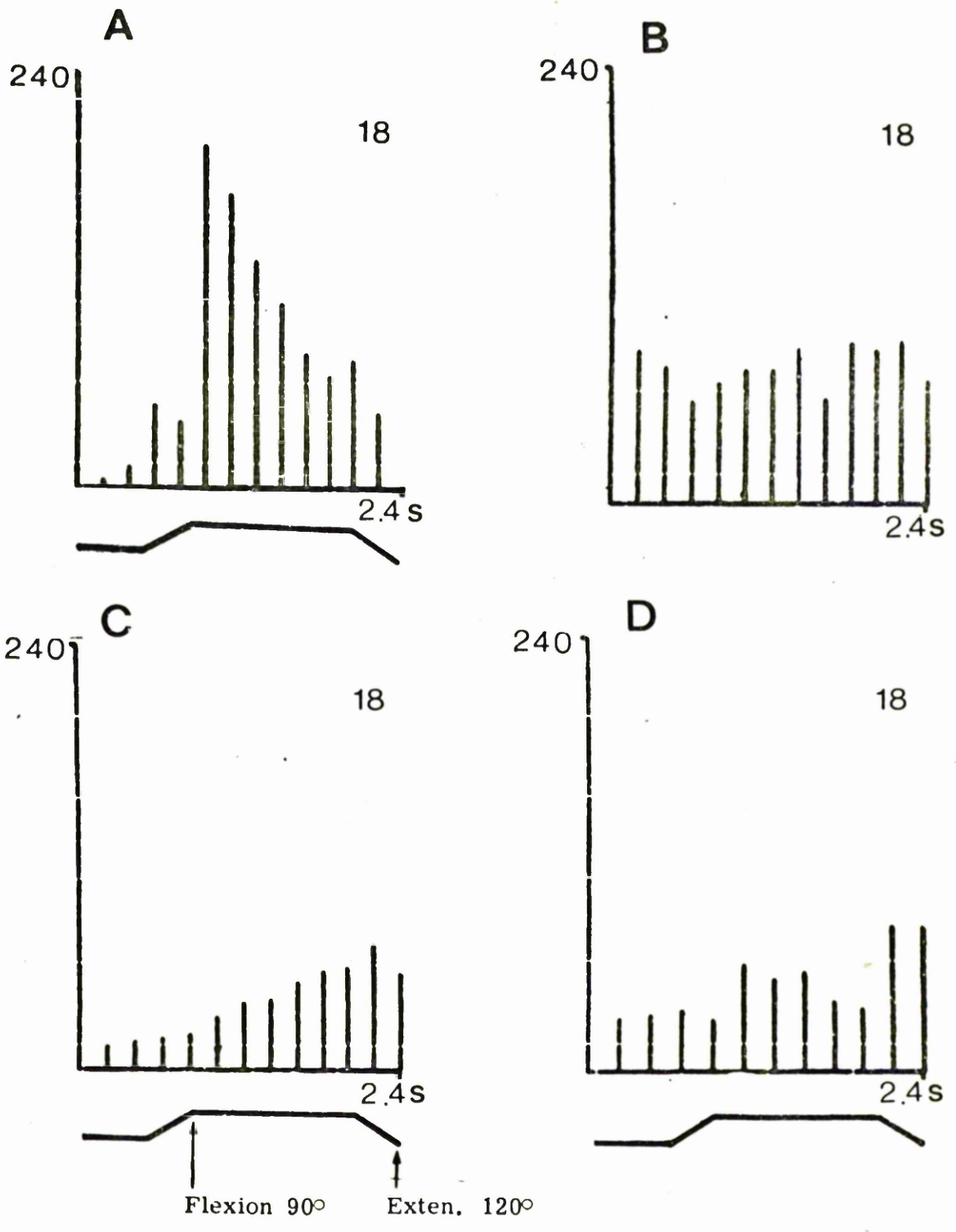
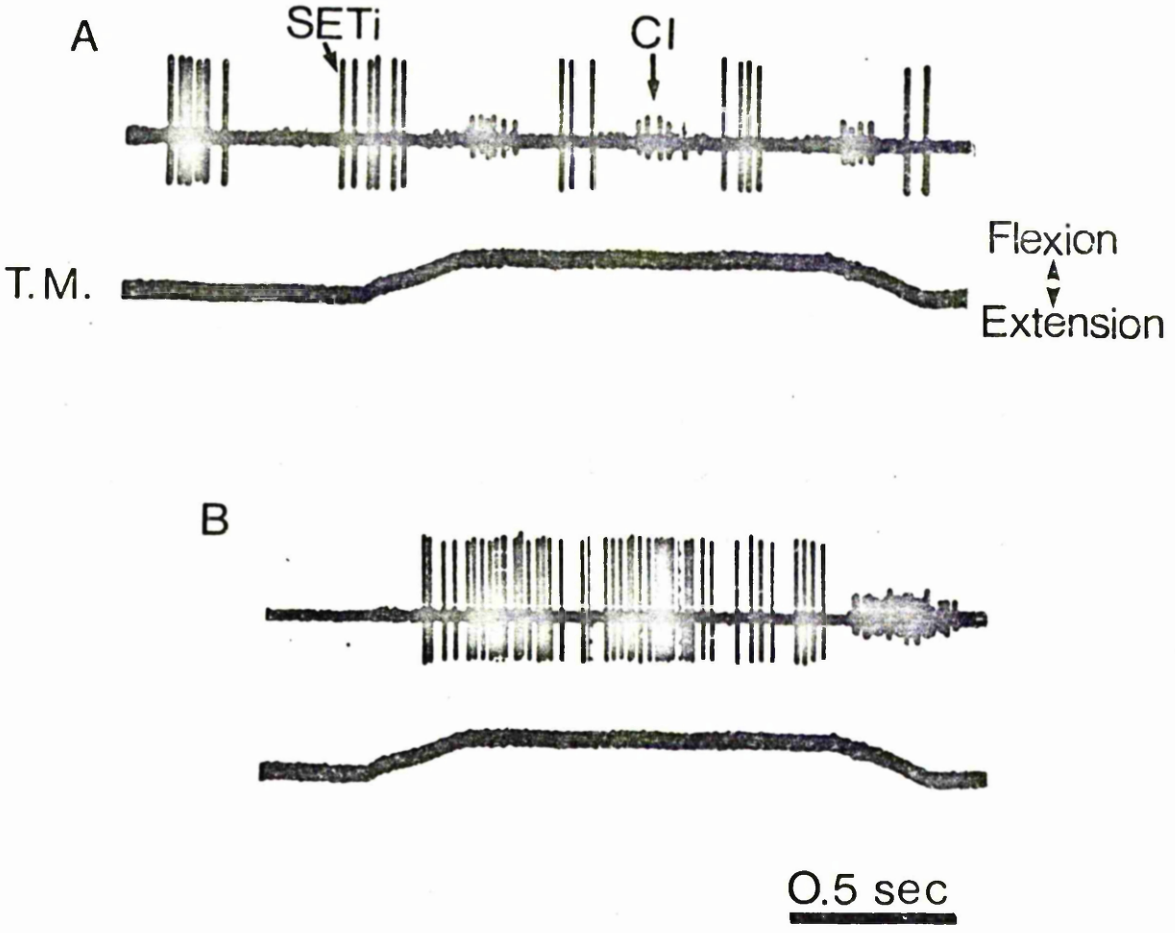

A
240
18
2.4 s
B
240
18
2.4s
C
240
18
2.4s
Flexion 90°
Exten. 120°
D
240
18
2.4s

Fig. 6.6

Records from the mesothoracic extensor nerve in the dissected femur of a tethered locust on the treadmill. The chordotonal organ was intact.

A. A self generated bursting pattern which continued for more than 5 min, (1st trace).
Imposed flexion of the tibia (2nd trace) reduced the number of spikes in the extensor burst (SETi) but also produced excitation of the Common Inhibitor (CI) (positive feedback). This record represents a full cycle and this phenomenon occurred in more than 20 cycles.

B. A repeat of A. with the self generated activity dismissed by strong mechanical stimulation of the animal. Flexion of the tibia produced normal resistance reflexes (negative feedback, see also Fig. 5.7B).


A
SETi
CI
T.M.
Flexion
Extension
B
0.5 sec

(fig. 6.6A), but flexion seems to have stronger effects on the Common Inhibitor which fires in bursts when the SETi is silent, for as long as the flexion lasts. When this rhythmic firing of the SETi stops, usually as a result of a strong stimulus, the responses of the motoneuron to the tibial movement were reversed (fig. 6.6B).

The above experiments show the effects of the femoral proprioceptors on the reciprocal walking pattern produced by the extensor motoneurons. To find out whether motor patterns of behaviour other than walking can affect the resistance reflexes, experiments were undertaken on a 'flying' animal. 'Flying' in this case is defined as the behaviour of an animal which, although it was firmly fixed, was continuously beating its wings. Flying activity was stimulated by a stream of air on the front of the animal. The legs were free except for one mesothoracic leg which was fixed (fig. 6.1) and dissected. The treadmill under the animal was removed. Movement of the tibia was imposed as described before and reflex activity from the extensor motoneurons was analyzed with an activity peristimulus histogram which was initiated in the middle of the imposed tibial flexion. Reflex activation of the SETi neuron, produced by flexion of the tibia in a non-flying animal is shown in fig. 6.7A. The FETi neuron is silent during this flexion. When the locust was stimulated to fly, with the leg fixed at a femur-tibia angle of 90°, the FETi and the SETi fired continuously at a high frequency. Some of this activity was also filmed at a different film speed to allow measurements of firing frequency to be made (fig. 6.4D). At the beginning of flight the FETi is activated at a very high frequency (fig. 6.4D1) which can reach the level of 300 - 350 Hz. Activity of the SETi is usually masked by the high firing frequency of the FETi so there are not any indications about the activity of this motoneuron.

Fig. 6.7

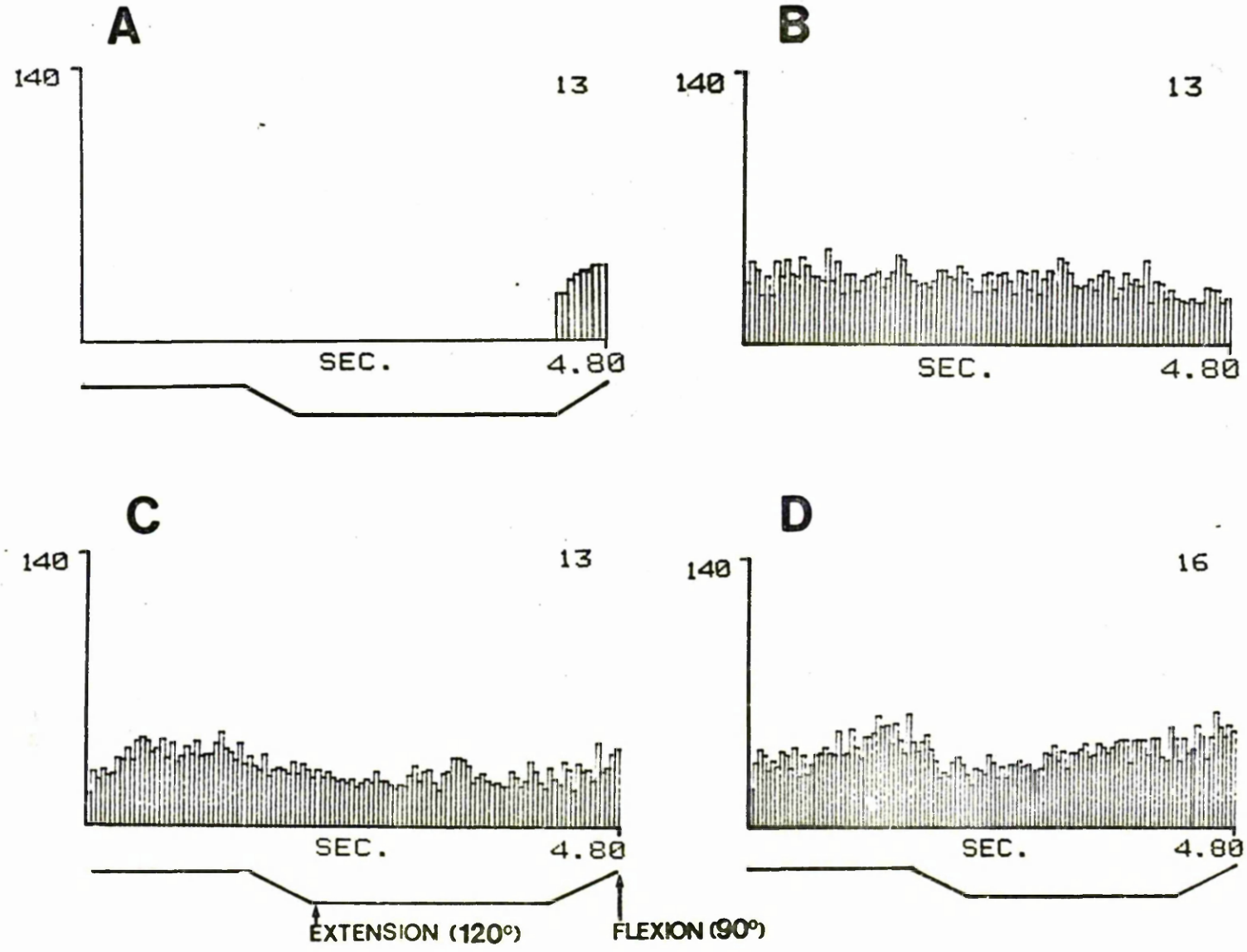
Peristimulus time histograms of the extensor tibiae motoneurons in a tethered locust. Conditions as for Fig. 6.5 but the locust was stimulated to produce flight activity.

A. Reflex responses of the SETi, in a quiescent animal, the imposed tibial movement indicated below the histogram.

B. Summed activity of the SETi and FETi during flight of a tethered locust. The tibia was fixed at a FTA of 90°.

C and D. Repeats of A in two different animals during flight activity.

The top right number on each plot indicates the number of sweeps making up the histogram. The vertical axis represents the number of spikes.


A
140
13
SEC.
4.80
B
140
13
SEC.
4.80
C
140
13
SEC.
4.80
EXTENSION (120°)
FLEXION (90°)
D
140
16
SEC.
4.80

This high firing frequency drops gradually in 8 to 10 sec to a relatively low frequency of 50 to 80 Hz and the animal flies normally (fig. 6.4D2). Similar firing pattern was found for the ventilatory motoneuron during flight by Hinkle and Camhi (1972). Continuous firing of the FETi at such a high frequency is unusual and similar activity has not been observed in other behavioural patterns. The activity of the extensor motoneurons seems to be modulated, during flying, with small regular changes in firing frequency in cycles of 0.3 to 0.5 sec period. This modulation is also visible in peristimulus histograms plotted every 4.8 sec (fig. 6.7B) for the activity of FETi. Records taken from the distal flexor nerve branches (fig. 6.4C second trace) show that most of the distal flexor motoneurons are silent during flight.

In flight, movements of the tibia from 90 to 120° seem to have only a small effect on the firing pattern of the extensor motoneurons (fig. 6.7C, D). Extension of the tibia produces a slight inhibition of the firing rate of FETi and when the tibia was flexed this inhibition was cancelled. These effects can be seen better in comparison with activity plotted from a locust with the tibia fixed at femur-tibia angle of 90° (fig. 6.7B). There is no strong excitation of the extensor motoneurons during flexion as it appears in a non-flying animal (fig. 6.7A). Three of the five animals examined showed these effects while in the other two cases no reflex responses occurred during the flight, although strong reflexes were visible when flight ceased. This indicates that although sensory inputs from the leg proprioceptors can affect the activity of the extensor motoneurons during flight, these effects are sometimes completely suppressed. Similar records were obtained from the prothoracic extensor motoneurons.

One or two seconds before the animal stops flying or, in cases where the animal is not flying properly, the flexor motoneurons were activated, firing in prolonged burst alternating with those of the extensor motoneurons. In one animal it was found that for a few seconds the extensor and flexor bursts were alternating at the wing beat frequency.

7. DISCUSSION

It is well known from physiological evidence that many insect muscles receive a fast, a slow and, in some cases, an inhibitory motor axon (see reviews by Hoyle, 1965; Bullock and Horridge, 1965; Aidley, 1967; Usherwood, 1967). The locust extensor tibiae muscle is a typical example which shows characteristically this pattern (Hoyle, 1955, 1978; Burns and Usherwood, 1979). However, not all insect muscles are as simple as this. Some cockroach muscles have been described with as many as seven or eight axons (Dresden and Nijenhuis, 1958). Many of the neck muscles of the locust receive innervation from six or more different axons and some from as many as 13 - 15 (Shepheard, 1973). Tyrer (1971a, b) demonstrated also that some locust abdominal muscles which might be presumed to be simple, are innervated by eight axons. In the femur of the locust the flexor tibiae muscle, the antagonist of the extensor described above, had been reported to be innervated by a considerably larger number of axons (Hoyle, 1955). Hoyle and Burrows (1973a, b) and Wilson (1977) were able to identify six excitatory motoneurons to the metathoracic flexor muscle but they left open the possibility that there could be more. Preliminary anatomical and electrophysiological studies showed that the number of axons in the flexor nerve branches is much larger than the number of flexor motoneurons previously reported. This makes the flexor muscle very interesting for further studies since it is so much more complex than the antagonistic extensor. It also produces problems concerning the study of the distribution of these axons on the muscle. There are three possible innervation patterns which might be expected; a) the flexor axons innervate all the parts of the muscle as happens in the extensor tibiae muscle, b) each flexor axon innervates only a specific muscle bundle or c) a compromise between (a) and (b). Hoyle (1955) suggested that

(b) was the correct pattern for the metathoracic flexor tibiae muscle.

The purpose of this work was to investigate:

1) The exact number of axons which innervate the mesothoracic flexor tibiae muscle.
2) To produce as far as possible an accurate description of the innervation pattern for the flexor motor axons, and
3) To attempt to explain why the flexor muscle receives such a large number of motor axons and how the locust uses these axons to control the position of the tibia during various behavioural patterns.

In order to understand the function of the flexor muscle it is necessary first to study its structure and mechanical properties and then to investigate the neuronal control of the muscle.

The flexor muscle

The two antagonistic femoral muscles of the mesothoracic leg have very similar morphological features. However, Snodgrass (1929) gave the extensor tibiae muscle a single number (106) while he subdivided the flexor into three parts, 107 a, b, c, based on the morphological differences between these parts. Muscle 107a is a long pinnate muscle arising ventrally in the proximal part of the femur, 107b arising in the base of trochanter, 107c arises anteriorly and posteriorly in the distal two thirds of the femur (fig. 3.2). Initially when the innervation of the muscle was studied electrophysiologically no subdivision of the flexor was made but it was soon found that the innervation pattern suggested a division of the muscle into three parts identical to those of Snodgrass and which are here named proximal flexor, middle flexor and distal flexor. The distal flexor which is the largest part might possibly be divided further but no physiological evidence was found to suggest a further

subdivision. This division of the muscle solved many problems especially these related with the identification of individual flexor motor axons. Such problems do not exist in the extensor muscle which receives fewer axons with a homogeneous innervation. The mesothoracic extensor was studied as a single muscle by Burns (1972).

A large part of both femoral muscles has a pinnate bundle arrangement. This means that the fibres are shorter than they would be in a simple fusiform muscle. It also means that they have a greater fibre cross sectional area per unit volume and hence a pinnate muscle is able to produce more force per unit volume than a fusiform muscle. When it contracts its tendon moves through a shorter distance but exerts more force. Making a long muscle such as a femoral muscle pinnate is equivalent to increasing the mechanical advantage of its fibres and it is not surprising that the flexor and extensor tibiae muscles are the only pinnate muscles in the locust. As the direction of pull of the muscle fibres is not directly in line with the tendon, they do in fact exert slightly less force per unit of fibres cross sectional area than the fusiform fibres. In a relaxed flexor muscle (FTA of 90^{o}) the pinnation angle is not uniform and increases from the proximal to the distal end of the muscle. The anterior part has a larger pinnation angle than the posterior (Table 3.1). How this variation in pinnation angle affects the mechanical properties of the muscle is a question which requires further investigation.

The total cross sectional area per flexor muscle bundle is 2.5 to 3 times larger than in the equivalent part of the extensor muscle (Fig. 3.3). In the distal flexor and extensor muscle bundles this is mainly a result of the greater number of muscle fibres, although there is also a very small difference between the diameters of the flexor and extensor muscle

fibres. In the proximal flexor and extensor the difference in cross sectional area is mainly due to the diameter of the muscle fibres since both muscles have almost the same number of muscle fibres in this region.

Within the flexor muscle there is a significant difference between muscle fibres in different parts of the muscle. The ratio of the effective diameter of the proximal muscle fibres to the distal flexor muscle fibres is 1.40 while the same ratio for the extensor tibiae is 1.08 indicating only a small difference between the distal and proximal (Table 3.1) extensor muscle fibres. The anterior half of the flexor muscle has a greater cross sectional area than the posterior part (almost double, Table 3.1). This suggests that there could be a significant assymetry in the distribution of the forces developed by the contraction of the muscle. This assymetry is also due to the difference in pinnation angle. 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 is smaller than the equivalent and opposite force developed by the anterior part. This difference in forces developed on the tendon by the anterior and posterior part of the flexor muscle 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. This is a very odd structural organization which needs further investigation. A possible purpose for this powerful movement of the whole flexor inside the femur is that it would help the flow of the haemolymph as Usherwood (1974) suggested for the intrinsic rhythm of the metathoracic extensor muscle.

Tetanic contraction of the mesothoracic flexor tibia muscle can develop a force of about 35g and closure of the tibiae from 180° to 30° producing a sudden reduction of muscle length of about 1.2mm (12% of

the whole muscle length). This, in combination with the fact that the short nerve branches which innervate the muscle arise along the stiff N5B2 suggest that high tension could be developed on the neuromuscular junctions. Overstretching of the neuromuscular junctions may be prevented by a protective link between the nerve branches and the surface of the muscle fibres (fig. 3.5). This connecting tissue is shorter than the attached axons and may absorb any tension between muscle and nerve, protecting the neuromuscular junctions. On the surface of the flexor muscle such "support mechanism" appeared in a few of the visible nerve branches while axons could be seen attached to the muscle fibres without any support. This suggests that only a few nerve branches have this "support mechanism" which produce enough resistance to equalize the developed tension by the contraction of the whole muscle. Another suggestion could be that only phasic muscle fibres have this link since they produce fast and powerful contraction, but this hypothesis requires further investigation. Similar formations were reported by Rees and Usherwood (1972) from electron micrographs of the retractor unguis muscle which has similar structure as the proximal flexor and receives a very short nerve branch from N5B2. A comparison with extensor nerve is inevitable since this muscle receives a single long nerve which rises from the proximal part of N5B2 and bifurcates further along the muscle. Such a pattern provides the neuromuscular junction with a very good suspending system which absorbes better the imposed tension. This tension developed during contraction or stretch of the extensor muscle is lower than in the flexor, since the extensor muscle has a mechanical disadvantage 2:1 in comparison with the flexor (Heitler, 1977). Although the extensor muscle has been thoroughly studied such protective mechanisms have not been reported and it would be interesting to look further for them.

The structural and anatomical differences between the flexor and extensor tibiae muscles also affect the mechanical properties of these muscles. For instance the peak active tension of the mesothoracic extensor tibiae muscle appears when the muscle is almost fully extended (FTA of 150° - 180°) (typical of a pinnate muscle; Aidley, 1975), while the peak active tension increment for the flexor appears in the middle of the normal muscle length range (FTA of 90°-100°,fig. 3.24). This feature of the flexor muscle is typical of fusiform muscle (Weis-Fogh, 1956). The conclusion here is that the flexor muscle behaves more like a fusiform than a pinnate muscle. This is mainly caused by the large fusiform proximal flexor; the effective diameter of the proximal muscle fibres being about 40% larger than those of the distal muscle fibres in the flexor muscle while the difference is only 8% in the extensor. This also results from the presence of the middle flexor bundles which, although they have pinnation arrangement, they have a very small pinnation angle (9°, Table 3.1), so they can be considered as fusiform muscle bundles.

The marked structural differences between the proximal part (proximal and distal flexors) and the distal part of the flexor muscle also results in a difference in the tension developed by these parts. During prolonged high frequency stimulation (30-50 Hz, fig. 3.25) the proximal part not only has a faster tension rise time but also fatigues faster than the distal part which at stimulation of 30 Hz can maintain tetonic tension for prolonged periods. This is a very good indication that the proximal part of the flexor is more phasic than the distal part although the difference is not so marked as the difference between the retractor unguis muscle and the tonic fibres of the extensor tibiae muscle (Cochrane et al., 1972).

The proximal and distal regions of the flexor muscle are not only histologically different but also receive different sized motor axons. For example, the muscle fibres of the proximal flexor in addition to having diameters about 40% greater than the rest of the muscle also receive the largest motor axon (F1, fig. 3.12). Although the distal flexor receives fast axons (F5, F6, fig. 3.12) the diameter of these axons is significantly smaller (about 50%) than the proximal motor axons. This "matching" between the size of the muscle fibres and the size of the motor axons which innervate them may be important for the function of the muscles. In some arthropods muscles this matching can be achieved by separating the large muscle fibres from the smaller as happens in the locust mesothoracic flexor tibiae muscle (Table 3.1) or in the abdominal adductor muscle in crayfish (Susuki , 1977) and in some other cases. While in other arthropods muscles both muscle fibres and axons develop a gradient of properties along the muscle (tapering) as was found for the locust extensor tibiae muscle (Hoyle, 1955, 1978; Wilson, 1977; Burns, 1972) and in the superficial abdominal muscle in crayfish (the tonic part of the abdominal flexor) as was demonstrated by Velez and Wyman (1978). Matching of motor axons and muscle fibres was also demonstrated in the dorsal superficial extensor muscle (tonic part of the abdominal extensor) by Parnas and Atwood (1966). They showed that this part of the muscle not only receives the smaller axons but that there is a homogeneous population of slow fibres with no indication of medial or lateral regions with different contractile properties. Of course there are some exceptions where although strongly differentiated slow and fast muscle fibres are known, as in the accessory flexor muscle of the crab (Cohen, 1963), this muscle receives only a single motor axon (Doray-Raj, 1964).

The flexor muscle is a typical example in which the different sized muscle fibres are not randomly mixed, in the different regions of the muscle. This differs from the vertebrate muscles where mixture of different sized muscle fibres occurs (Henneman and Olson, 1965). This matching between size of muscle fibres and axons has two possible explanations.

a) The motoneurons selectively innervate only those muscle fibres which are of the proper type, Frank (1973) ascribes matching to the postsynaptic elements; synapses are modified according to instructions from the muscle fibre, or b) the motoneurons innervate muscle fibres before their type is determined and they cause the fibres to differentiate into the proper type by their activity or some sort of trophic effects (Atwood, 1973; Barony and Close, 1971; Close, 1972).

Velez and Wyman (1978) working on the crayfish slow abdominal flexor muscle suggested that axons act trophically, not through a specific trophic substance, but by imposing a pattern of activity and metabolic demands on the muscle fibres. The mesothoracic flexor tibiae muscle offers also a good system on which this theory can be tested by cutting N5 in early embryonic stage (1 or 2 instar). If the difference in the diameter between proximal and distal flexor remains (40%) when the animal becomes adult, the trophic role of motor axons is not important in the development and growth of the muscle or vice versa.

To study electrophysiologically the innervation of the mesothoracic flexor tibia muscle the axons were separated into types, fast, intermediate and slow, according to the aplitute of the EPSP which they produce. Since the relationship of the axon diameter and size of EPSP has been well established by recording from single muscle fibres (Atwood, 1963, 1967; Doray Raj, 1964) and tapering of the flexor axons

occurs only in one or two cases because they innervate specific regions of the muscle, this classification was useful.

The mesothoracic flexor tibiae motor axons can also be divided into three categories according to the way their endings are distributed on the muscle.

1) Axons which innervate only specific areas of the muscle. In the proximal flexor, axons S3 and F1, in the middle flexor axon F3 and in the distal flexor axons F6, F5 and M3.

2) Axons which innervate the whole flexor muscle. Such axons are the spontaneously active S1 and S2 which mainly innervate the distal flexor with a progressive reduction of the number of their nerve endings to the middle and proximal flexors. Since the proximal flexor has the largest muscle fibres and is innervated by the largest motor axons, it was not surprising that they do not receive many endings from the two smaller axons. It does however receive an extra tonic input from S3. Axon M1 also innervates most of the flexor muscle but gives very few endings in the distal flexor and can be considered that innervates mainly the proximal and middle flexor.

Other axons innervating the whole flexor muscle are the inhibitors I1 and I2. They innervate most of the distal flexor with fewer endings in the middle and proximal flexors. The inhibitors usually occur with slow axons (Usherwood and Grundfest, 1965; Usherwood, 1967; Atwood, 1967). Both flexor inhibitors were found to innervate the same muscle fibre. Double inhibition of muscle fibres may provide a more powerful relaxation of the muscle. Triple inhibitory innervation was found in the coxal depressor muscles of cockroaches by Iles and Pearson (1969). An interesting point here is that the three coxal inhibitors of the cockroach were found to be common with other muscles, in contrast

with the flexor tibiae muscle in the locust which is innervated by two specific inhibitors. The flexor muscle does not receive endings from the Common Inhibitor (CI) although Burrows (1973) found that a branch of this neuron runs in N5.

Other axons found to innervate most of the flexor muscle are the axons from the DUM cell, D1 and D2. Since axons of DUM cell in the mesothorax were found to run in the peripheral nerves in parallel with the Common Inhibitor (Crossman et al., 1972) it is not surprising the fact that D1 and D2 run in most of the flexor nerve branches following probably the inhibitory branches of the two flexor inhibitors. The interesting points here are that the DUM neuron which innervates both the mesothoracic flexor and extensor tibiae muscle was found to innervate only the extensor tibiae in the metathoracic leg (DUMETi, Hoyle et al, 1974).

3) Axons with an innervation pattern which overlaps two parts of the flexor muscle. For example, axon F2 which innervates the proximal and middle flexor and axon F4 and M2 which can be found only in the middle and distal flexors.

The way which the motoneurons innervate the flexor muscle also match the way in which they are activated. The slow axons with low thresholds innervate the whole muscle. The intermediate axons with higher thresholds innervate only combinations of the three parts and most of the fast axons innervate exclusively specific regions of the muscle. The fast axons with higher thresholds are probably used separately. This kind of innervation of the flexor muscle increases its functional abilities. There are behavioural patterns which require activation of the different parts of the flexor separately as will be discussed below. However in some cases it is important to use the whole power of the flexor muscle.

It is also rather interesting that although each proximal muscle fibre receives the maximum of two or three axons, the muscle fibres in the distal region receive a maximum of six excitatory axons (fig. 3.17D). This suggests that the distal flexor may be able to develop a finely controlled tension. Thus it is not surprising that the tension receptor (Theophilidis and Burns, 1979) is attached almost at the end of the distal flexor. The close relationship between the muscle properties and the type of proprioceptors attached to the muscle is also shown by the chordotonal organ. This receptor monitors muscle movement (Burns, 1974) with its distal scolopidium attached to the middle flexor. The advantage of this part of the flexor is that it acts very like a fusiform muscle since it has a pinnation angle of only 9^{o} and therefore produces larger tendon movement when it contracts. Preliminary studies with reduced methylene blue, additional to those of Burns (1974) showed that the CO is also connected distally with the flexor muscle by a fine tendon-like branch arising from the distal scolopidium. All the above evidence suggests that the flexor muscle is well coupled with some of the main femoral proprioceptors. In contrast there are no receptors exclusively related with the extensor muscle. Since the flexor muscle is the largest muscle in the mesothoracic femur and innervated also by a large number of axons providing a fine control, it may be expected to have a large number of proprioceptors associated with its activity. The importance of the leg proprioceptor in the control of the flexor muscle will be discussed in the following pages.

The control of the flexor muscle

In order to understand the control of a muscle it is necessary to study how the animal activates the motoneurons innervating the muscle in various behavioural patterns such as posture, walking, flying, etc. The control of the mesothoracic extensor tibiae muscle during walking

has been studied thoroughly (Burns, 1972, 1973), but no information has been published about the control of its antagonist flexor. This is not surprising since this muscle is innervated by a larger number of axons (12 excitors) and it is difficult to study the control of the muscle without knowing its innervation pattern. Having established the innervation pattern of the flexor muscle and developed a technique which is able to identify the flexor motoneurons in a tethered animal, the further investigation of the control of the flexor motoneurons was possible. Motoneuronal activity was monitored from the flexor nerve branches and muscle fibres in such a manner as to allow identification of the individual motor axons. For this kind of recording it was necessary to use a dissected femur which may have altered the activity of the flexor motoneurons. This was a compromise which was made in order to achieve accurate electrophysiological records. In most cases it was found that disturbances due to dissection did not very much alter the reflex responses of the flexor motoneurons. This contrasts with disturbances produced centrally by cutting the connectives, which usually produce a disinhibition of reflexes (Rowell, 1969) and generally increase the efferent activity (Weiant, 1958). The problems due to the dissection could have been avoided by recording electromyograms (EMG's), but EMG analysis has proved inaccurate in the study of individual motoneurons (Runion and Usherwood, 1966). Bowerman (1977) describes as the major disadvantage of this technique the fact that simultaneous activity of several motoneurons may make identification of the activity of individual axons difficult, if not impossible and additionally, activity of inhibitory fibres may be completely overlooked as a result of the small size or absence of inhibitory post synaptic potentials.

Reflex activiation of motoneurons

The simplest way to activate the flexor motoneurons was through reflexes. Strong mechanical stimulation of almost every part of the locust's body produced reflex excitation of the flexor motoneurons but the most powerful and reproducible responses were resistance reflexes produced by extending the tibia. To achieve systematic activation of the mesothoracic flexor tibiae motoneurons within the normal physiological range, the tibia was extended at a speed matching the mean angular velocity of tibial flexion during walking. It is important to emphasize here that care was taken to activate only the proprioceptors which were normally excited by the tibial movement. Careless imposition of tibial movement in the small mesothoracic leg can create extra strain in the coxal or tibial receptors which may alter the original pattern of the femoral resistance reflexes. The most reliable reflexes were produced by sensory inputs such as the chordotonal organ (CO), the flexor muscle tension receptor (MTR) and the multipolar femoral tibial joint receptors (fig. 4.2). The MTR reflexively inhibits the extensor tibiae motoneurons and produces a reflex activation of the flexor tibiae motoneuron. The other properties of this receptor have been discussed in Chapter 4 (Theophilidis and Burns, 1979). The femur-tibiae multiterminal joint receptors also affect the activity of the femoral motoneurons (Williamson and Burns, 1978). The Chordotonal organ of the mesothoracic leg seems to produce the largest sensory activity and this is not surprising since this proprioceptor consists of about 200 small and 50 larger cells (Burns, 1974). Sensory activity, mainly from the CO, during extension of the tibia produces an excitation of the flexor motoneurons producing contraction of the flexor muscle opposing the movement. These are typical resistance reflexes as named by Bush (1965) and removal of this receptor inactivates most of these reflexes. The CO mediates strong reflexes in the metathoracic extensor tibiae muscle

(Usherwood et al., 1968) and in the mesothoracic extensor (Burns, 1974). Strong reflexes from similar receptors were described in the stick insect by Bässler (1972) and Wilson (1965) also studied leg reflexes in cockroaches but there is no report on the activation of the flexor tibiae motoneurons.

The activity of the mesothoracic flexor tibiae motoneurons during passive extension of the tibia is summarised in fig. 7.1A. In the proximal flexor axon F2 is very active and produces a discharge which lasts as long as the imposed tibial extension. The burst in F2 sometimes can be prolonged further in animals with a high excitability. The burst produced by F2 produces a powerful co-contraction of the proximal and middle flexor and as a consequence a large movement of both parts which is detected by the distal scolopidium of CO. Axon F2 seems to be an unorthodox axon because although the difference in diameter(Table 3.3 between F2 and F1 or F3 is not more than about 10%, there is a significant difference in their thresholds. Axons F1 and F3 are activated only by a very fast tibial extension producing a small number of spikes but sometimes they are silent. Axon M1 has a lower threshold and is also activated by tibial extension, producing a similar firing pattern to that of axon F2. Activity from M2 does not appear very often in resistance reflexes. This axon seems to have a high threshold and is excited together with the large motor axons which probably mask its activity in the extracellular records required for the identification of this axon. Axons S1, S2 and S3 fire spontaneously at a low frequency in a quiescent animal and tibial extension produces a strong increase in their activity.

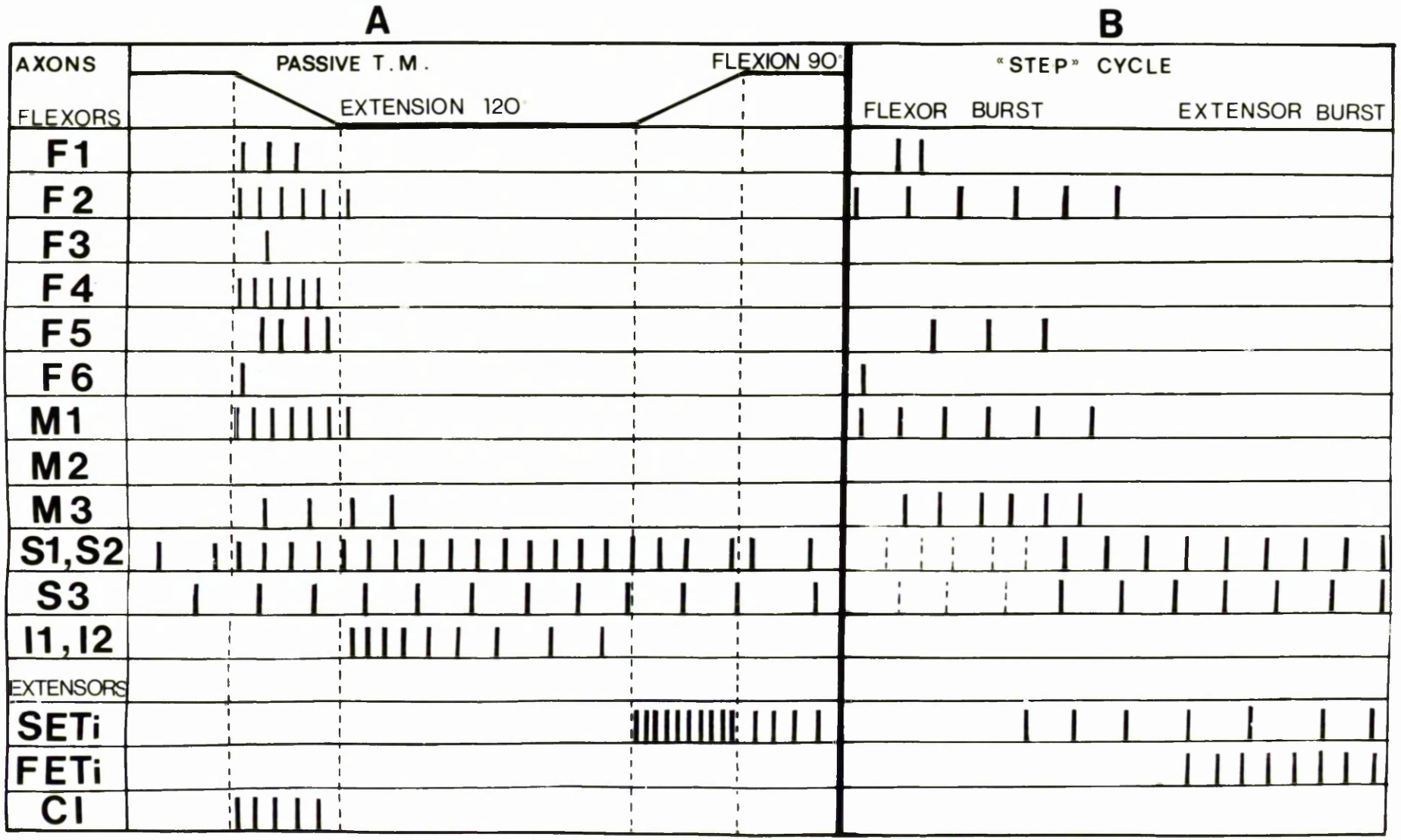
Other low threshold flexor motoneurons are the inhibitors I1 and I2, which respond to any mechanical stimulation of the locust's body.

Fig. 7.1

Summarized activity of the mesothoracic flexor and extensor tibiae motoneurons.

A. Resistance reflexes produced by tibial imposed extension and flexion at a constant angular velocity of 150°/s. The duration of the cycle is 2.4s.

B. Motor pattern of the flexor and extensor motoneurons recorded from a deafferented leg of a tethered locust walking on the treadmill with a speed of 1st/s.


A
B
AXONS
FLEXORS
PASSIVE T.M.
EXTENSION 120°
FLEXION 90°
"STEP" CYCLE
FLEXOR BURST
EXTENSOR BURST
F1
F2
F3
F4
F5
F6
M1
M2
M3
S1,S2
S3
I1,I2
EXTENSORS
SETi
FETi
CI

Since the inhibitors act on the flexor muscle they should be excited by the movement of tibial flexion, as the extensor CI is excited by tibial extension (fig. 5.7). However they remain silent during either tibial flexion or extension and are excited only during maintained extension (fig. 5.5B). They usually fire in bursts in which the length and the internal frequency depends on the angular velocity of the previous extension. This indicates that although the inhibitors seem to be excited by the position detectors of the CO there is an effect from the velocity detectors of the same proprioceptor which occurs with a certain delay. Other workers have also found that the inhibitory neurons always fire in alternation with motoneurons that innervate the same muscle (Kennedy and Takeda, 1965a, b; Hoyle and Burrows, 1973a). Activation of the flexor inhibitors immediately after the burst of the flexor excitors may accelerate relaxation of the flexor muscle, as suggested for the metathoracic extensor tibiae muscle by Runion and Usherwood (1968). It is worth notice here that the extensor inhibitor is also activated during flexor burst produced reflexly by tibial extension. A similar firing pattern was found in crayfish where motor axons of the claw closer muscle fire at the same time as the opener inhibitor and activity in the closer inhibitor (Weins and Gerstein, 1975).

Although there are differences in the way which the resistance reflexes are organised in the flexor and extensor tibiae muscles the intensity of reflexes discharge in both muscles is in most cases directly related to the angular velocity (fig. 5.3), suggesting that the evoked reflexes were driven from the velocity sensitive cells of the CO, which has been identified as the main cause of these reflexes. It appears that angular velocity is the most important input variable for resistance reflexes as was found by Ayers and Davis (1977) and Bush (1965). The relationship between angular velocity of the movement and the instantaneous

frequency of motoneurons activity is linear (Evoy, 1977). The slope of the line can be taken to represent the gain of the sensory to motoneuron pathway (Average discharge rate / unit change in velocity).

Neither extension nor flexion of any angular velocity were able to activate axon D1 (the homologous of the metathoracic DUMETi). It is not known yet what synaptic input excites these neurons. Hoyle and Dagan (1978) found sensory inputs to the DUM cells, but claimed that all natural pathways that excites these cells are extremely labile. It appears that the synaptic inputs which drive DUMETi are several levels of interneurons removed from primary sensory inputs (Heitler and Goodman, 1978). However, the DUM cells are active at low frequencies which sometimes rise to 5 - 7 Hz (fig. 5.7A and Hoyle et al., 1974). In the case of D1 which bifurcates to innervate both extensor and flexor tibiae muscles, it seems strange that one neuron innervates two antagonistic muscles. However it was found that nerve impulses transmitted in the extensor branch of D1 failed at a lower frequency than those in the flexor branch. Thus it is possible that this is a mechanism which at least prevents height frequency discharge of axon D1 ending in both antagonistic muscles.

The reflexes described above do not always appear with the same strength. In some preparations although the responses of the flexor motoneurons always remain phasic to the imposed tibial extension, the number of spikes in the flexor discharges decrease during prolonged stimulation. This phenomenon, called here habituation, may be due to the fatigue of the synaptic interconnections between interneurons and the

flexor motoneurons since the sensory inputs, the discharge from the CO, is unaltered. The same explanation was given by Usherwood et al. (1968) for a similar phenomenon in the SETi. They suggested that this waning represented central adaptation. Another interesting point is that reflexes from the flexor motoneurons sometimes almost disappeared and reappeared within seconds leaving the same strength as before. Extensive modifications of reflexes were also found during behavioural motor patterns such as posture, walking or flight.

Spontaneous activity of motoneurons

Another way of studying how the locust uses the large number of flexor motoneurons was to record motoneuronal activity from the nervous system of a tethered locust on a treadmill (fig. 6.1). From the details of this method which have been described, it is obvious that the animal was under rather abnormal conditions and it would be unrealistic to claim that a natural walking pattern was recorded. However, the locust in this situation was able to walk on the treadmill. There are of course differences from free walking and this can be seen in the length and the interval frequency of the alternating bursts of the mesothoracic extensor and flexor muscles the sequence in which the femoral motoneurons were activated with the stepping pattern in both cases was very similar. The activity of the individual flexor tibiae motoneurons of an animal walking on the treadmill is demonstrated in fig. 7.1B. A comparison in fig. 7.1 between A and B shows that the reflex burst of activity in the flexor axons, produced by forcibly extending the tibiae, is similar to the burst produced by some axons in a deafferented animal walking on a treadmill. However, there are differences, for example axon F3 which is regularly activated in reflexes was not very active during the flexor burst in walking and was excited only when the locust was

aroused. Axons M1 and F2 fired at a slightly higher frequency during walking than in the reflex. Axon F4 which was identified in reflexes in a few preparations was not recorded in walking. What caused this difference is not known and requires further investigation. The three distal axons were activated in the same way in both cases. Their burst of activity usually started with some delay and usually the large F6 fires first. Spikes from axon M2 appeared very rarely and did not produce any regular firing pattern. The slow axons which were continuously active, increased their frequency from the moment the animal started to walk. However it was not possible to see if the frequency of the slows was modulated during the flexor burst since the small EPSP's from the slows were masked by the activity of the larger axons. Although sensory inputs are cut off, it is interesting that the timing of the bursts of the different flexor axons is very accurate. Most of them end at the same time, except M3 whose activity is sometimes prolonged. Although the inhibitors were not positively identified, small action potentials occurred regularly between the flexor bursts where usually the antagonistic FETi was expected to fire. (fig. 6.2A, C).

In both reflexes and walking the large axons seem to have a high threshold and produce a small number of spikes. Axons M1, M3 and F5 which have a smaller diameter are more excitable while the small slow axons have a very low threshold and are continuously active. This relationship between axon diameter and motoneuron excitability seems to be common in muscles which are innervated by a large number of motor axons. In the locust's abdominal muscles which receive six large and six small axons, Hinkle and Camhi (1972) demonstrated that the firing sequence of these motoneurons was correlated with axon diameter. In other invertebrates such as the crab, the relationship of axon diameter and motoneuron threshold was also described (Davis, 1971; Wiens, 1976).

In vertebrate muscle, Somjen, Carpenter and Henneman (1964) show that the relative excitability of the motoneuron is determined by their size or by the diameter of the axons and finally, Henneman, Somjen and Carpenter (1965) and Henneman and Olson (1965) demonstrated that in the median gastrocnemius and soleus muscle the size of the motoneuron dictates its excitability, its excitability determines the degree of the use of the motor unit and its "usage" in turn specifies or influences the type of muscle fibre required.

However, in locust not all the flexor motoneurons follow this "size principle". For example F2, which is only 10% smaller than the high threshold axons F1 and F3, has the same low threshold as axon M1 which is about three times smaller. Axon F6, although it is impossible to measure its exact diameter, belongs to the group of distal axons where the maximum diameter is no more than 11μm (fig. 3.6, Table 3.3) and seems to have a similar threshold to F1 (25.50 μm). Thus the excitability of the flexor motoneurons depends on a priority factor and this seems to be independent of the axon diameter, and may depend on the significance of the motoneurons in behaviour such as posture and walking. Therefore an examination is required firstly of the function of the flexor neurons in posture and secondly of the activity of these neurons in generating the tibial movements of walking.

a) Posture

In a standing locust, most of the time the femur-tibia angle is about 90° to 100°, while the angle between the tibia and the horizontal plane is usually 110 - 120°. The contribution of the femoral muscles to postural forces requires further investigation using tension recording techniques similar to those used in stick insects by Cruse (1976). However in a standing locust on a horizontal plane the

mesothoracic extensor SETi fires at a low frequency (Burns, 1972), but no information has been published for the slow flexor motoneurons. The study of these neurons in a deafferented preparation, and in a preparation with most leg proprioceptors intact (see reflexes) shows that the slow flexors are continuously active while the extensor SETi has a reduced firing rate. The question then arises how is this spontaneous slow activity used?

Since S1 and S2 were found to innervate mainly the distal flexor, which is the largest part of the flexor muscle, it seems that this part is more used in the maintainance of posture than is the proximal part although the proximal flexor does receive a tonic input. For this purpose the distal flexor has the following advantages:

1) The distal flexor has smaller diameter muscle fibres than the proximal flexor. This means that they can contract for prolonged periods without fatigue (see fig. 3.25C) since it has been shown that small diameter muscle fibres contain larger numbers of mitochondria and therefore are more resistant to fatigue (Henneman and Olson, 1965). In the retractor unguis muscle and the metathoracic extensor, it was found that fast muscle fibres (white) contain fewer and smaller mitochondria than the slow (red) and fatigue more quickly under sustained neural stimulation (Usherwood, 1967; Hoyle, 1978). This property is very important in maintaining at FTA of 90° by producing a constant isometric tonic force.

This is also helped by the fact that an FTA of 90° to 100° the flexor muscle produces its peak active tension increment. Since this isometric tension may be important for the balance of the animal it is not surprising that the tension receptor (Chapter 4) is located at the end of the distal flexor.

2) The distal flexor is a pinnate muscle with graded pinnation angles (Table3.1). Such a muscle can exert powerful force with small tendon movement which is important for finely controlled movements such as postural readjustments. This fine control depends also on the fact that changes in body position are detected by the C.O. which in a quiescent animal reflexly affects the tonic activity of the three slow flexor motoneurons and the slow extensor SETi.

The functional importance of the flexor tibia muscle in posture depends also on the tonic activity and function of the antagonistic extensor. However the flexor muscle seems to dominate because it is larger than the extensor, not only having larger diameter muscle fibres but also having about three times more muscle fibres (Table 3.1). The flexor muscle also has a mechanical advantage of 2:1 (Heitler, 1977) over the extensor. It seems to be generally true of the construction of arthropods leg that the tibial flexor muscles have more tonic axons. This can be seen in the rock lobster where the flexor muscle (equivalent to the flexor tibiae muscle) receives three tonic axons while its antagonistic receives only one (Ayers and Clarac, 1978).

b) Walking

In walking the main function of the mesothoracic flexor muscle is to flex the tibia during protraction from an FTA of 130° to 70°. The protraction (flexor burst) varies from 50 to 110 ms in duration while the retraction (extensor burst) is from 120 to 400 ms long in an animal walking at a speed of 2 to 5 steps per second (Burns, 1973). This shows that the duration of the flexor burst must be much shorter and more constant than that of the extensor burst. This rapid flexion of the tibia may create problems in the accurate firing time of the flexor motoneurons and the speed of muscle contraction.

The flexor motoneuron activity during attempted tibial flexion in a deafferented leg of an animal walking on a treadmill is shown in fig. 7.1B. The main components of the flexor activity consist of axons F2 and M1 which are firing continuously during flexion. F6 usually fires immediately after the first action potential of the burst of F2 and it is followed by F5 and M3. Thus the large motoneuron innervating of the proximal and middle flexor are used as much as the smaller neurons innervating the distal part. This is odd since according to the "size principle", as hasbeen discussed before, one might expect that the small axons having lower threshold would be used more than the larger motor axons innervating the proximal and middle flexors. What is the function of the two first parts of the flexor muscle when such large motoneurons as F1, F2 and M1 are active at an almost constant frequency during the flexion burst?

During protraction the flexor burst causes a shortening in muscle length of about 7 - 9% in a time of approximately 50 ms in a locust walking at a speed of 5 st/s. This fast muscle contraction can only be produced by the proximal part (middle and proximal flexors) for the following reasons:

a) The proximal flexor contains exclusively the largest muscle fibres of the flexor muscle (Table 3.1) arranged in parallel with the flexor tendon so they can produce maximum movement of the tendon during fast muscle contraction and driven mainly by F1 and F2 can produce a considerable acceleration of the tibia. The middle flexor has a similar function since its pinnation angle is only 9^{o} and it is thus nearly parallel to the tendon. In contrast, the distal flexor with its greater pinnation angle is able to produce less movement and more force.

b) The proximal and middle flexors have faster rise and relaxation

times and the twitch/tetanus ratio is larger than in the distal flexor (Table 3.6). Fast relaxation is also important in the short flexor burst to eliminate the residual tension which could cause strong opposition to the following extensor contraction. All the above evidence suggests that the proximal flexor activated by F1, F2 and M1 is ideal to produce the fast flexion of the tibia during walking.

However fig. 7. B shows that the distal flexors are also active. The fact that there is a delay before their activation suggests that the distal flexor may be used to reinforce the proximal part of the muscle during flexion. Reinforcement of the proximal flexor at the end of the flexor burst may be important because : 1) The high firing frequency in F2 may produce fatigue effects in the large, fast contracting muscle fibres of the proximal flexor, especially in slow walking locusts where the burst of F2 is prolonged and 2) A high level of tension in the flexor muscle at the end of the retraction may be required when the leg touches the ground and rigidity may be important as will be discussed below. It must also be borne in mind that the flexor tibiae muscle is used in entirely different ways in prothoracic leg. Here the flexor muscle is active during retraction and flexion of the tibia produces propulsive force. The requirement for tension from the distal flexor is thus much greater. Of course, locusts do not spend their lives walking on flat, horizontal surfaces. They usually walk on uneven surfaces and they climb. Under these variable conditions an increase in tibial flexor force is essential and maybe caused by extending the activity of the motoneurons innervating the distal flexor.

There is no information in the locust about the motoneuronal patterns during other walking situations. Pearson (1972) demonstrated in the cockroach that an effect of increasing the resistance to

retraction, making the animal drag a weight, was an increase in the average discharge rate of the slow axon innervating the coxal depressor muscle. Cruse (1976) found in the stick insect that the function of the different groups of muscles and the function of the whole leg can vary considerably depending on the type of walking situation. His conclusion was that the neuronal programme itself is changed, when the walking situation changes. The fact that the locust mesothoracic tibiae muscle has such a large number of motoneurons and is subdivided into parts with specialized mechanical and physiological properties increases the possibilities for changes in the neuronal programme required by various behavioural patterns.

Fig. 7.1B shows that all the flexor motoneurons except the slow flexors are silent during attempted retraction. The activity of these motoneurons increases when the animal starts to walk and continues right through the periods between the flexor bursts. In contrast, in the extensor nerve the SETi is activated only for a short period before the FETi is active. Thus the tonic tension of the flexor muscle opposes tension produced by the SETi in the extensor. The fact that in a normal walking animal the mesothoracic tibia keeps extending from the beginning of the retraction means that the extensor muscle must dominate. At the moment where both extensor and flexor tibiae slow neurons fire together in the first half of retraction the flexor muscle acts against the extensor and must produce a very rigid femur-tibia joint. Rigidity of the femur-tibia pivot is probably very important at that particular moment to support the body weight and ensure stability. One result of the approximately alternating tripod gait used by the locust is that for at least half of the retraction the animal is supported by only three legs and as a result the mesothoracic member of the three must carry more than one-third of the body weight (Burns, 1973).

All the above evidence suggests that the flexor motoneurons have more complicated functions than those of the antagonistic extensor. It can be seen that different motoneurons are used in a standing animal from those which cause the fast tibial flexion which occurs during walking. However the most interesting point is that some of the flexor motoneurons active at the same time have different functions. This kind of neuronal control where synergistic motoneurons have independent functions can also be seen in the locust neck muscles nos. 57, 58, 59 and 60 (Shepheard, 1973). When they act as a single muscle unit in response to the activity of the common excitors during the fast phase of head movement, their function clearly differs to the functions carried under control of motoneurons providing specific innervation to different muscles within the group (Shepheard, 1974). Similar functional separation also occurs in crabs where the eye muscles produce three types of movement; optokinetic, compensatory and protective withdrawal (Burrows and Horridge, 1968a, b). The first two involve the same motoneurons which are excited in different proportions for the different directions of movement while the third involves additional motoneurons although the same muscle participates in all movements.

Muscles with complicated innervation receiving a large number of motor axons and subdivided into synergistic parts are not common in arthropods. Homologous muscles in other insects are, of course, similar. For instance, the flexor tibiae muscle in the cockroach was also found to be divided into three parts (143 a, b, c) by Carbonell (1947). Dresden and Nijenhuis (1958) showed that this muscle is innervated by 11 axons. Muscle 143a receives only two axons. Muscle 143b and 143c receive 9 axons and they have some 4 or 5 axons in common whereas two axons are confined to 143b and two or three to 143c. A few other arthropods muscles with similarly complicated innervation have

been described. For example, the coxal depressor muscle of cockroaches (Pearson and Iles, 1971), the group of protergal muscles of the cervical sclerites in the neck of the locust (Shepheard, 1973) and some of the abdominal muscles which innervated by eight axons (Tyrer, 1971a, b). In the crustacea the complexity of the abdominal flexor and extensor muscles is also high (Kennedy and Takeda, 1965a, b; Parnas and Atwood, 1966). Multifunctional muscles also exist in vertebrates. Henneman and Olson (1965) claimed that since both the M.G. and the soleous muscle cause extension their contribution might be expected to differ with respect to maximum tension, speed of contraction, amount of shortening, economy of action and degree of usage. The same authors finally speculate that "nature discovered long ago that two heads are better than one".

Interaction between proprioceptive reflexes and motor patterns

Most of the discussion above was based on results taken from deafferented preparations. However the sensory information from the leg proprioceptors during tibial movements was found to be repeatable over prolonged periods. The main effect of these sensory inputs on the femoral motoneurons of an immobilized animal was in the form of negative feedback (resistance) reflexes. Resistance reflexes, apart from the fact that they sometimes habituate (fig. 5.6) appear to be very regular in the flexor and extensor tibiae motoneurons (see also Wilson, 1965). However it appears that during centrally driven movement, the whole reflex organization is arranged to facilitate it, and no opposing neuronal activity is generated (Barnes et al., 1972; Barnes, 1977; Ayers and Davis, 1977). This does not mean that sensory inputs are not important in the control of movement. In some cases the centrally produced rhythms can be modulated by sensory feedback (Burrows, 1975; Wendler, 1974; Wong and Pearson, 1976) and by orienting cues (Camhi, 1970). The

forms of modulation of the walking rhythm found in cockroaches include variations in the frequency of cycling and in the proportion of the cycle occupied by the propulsive and returnstroke movement of an appendage (Pearson and Iles, 1970; Pearson, 1972; Delcomyn, 1973). The way in which the resistance reflexes were modulated in a centrally driven motoneuron was studies here in a tethered locust (fig. 6.1) with only the C.O. intact in an otherwise deafferented, fixed leg. A centrally programmed motor activity of the SETi was produced during walking on the treadmill and it interacted with sensory information produced by tibial movement (stretch or release of the C.O.). Fig. 6.5 shows that resistance reflexes of the SETi were not only cancelled but that the sensory input produced a positive feedback during tibial flexion inhibiting the SETi, functioning to enhance tibial flexion during walking. Such reversal of reflexes were also described by Bässler (1976) in a decerebrated stick insect fixed on cork plate. He demonstrated that ramp-wise stretching of the femoral chordotonal organ excites the slow extensor tibia in an "inactive" animal (an animal which only moves the stimulated leg) while in "active" animals (animals which move also the other legs) the same stimulus decreased the firing rate of this motoneuron. It is worth noticing the similarities of Bässler's "active" stick insect and the tethered locust where five of the legs were free to move, walking on the treadmill.

In an inverted dissected animal Hoyle and Burrows (1973b) were able to produce a sequence of alternating flexion and extension movements of the tibia closely resembling those seen during locomotion, by stimulating the connectives between the ganglia. During this particular action they found that although the fast extensor to the tibia is not used in walking, its membrane potential is driven more negative by IPSP's during the flexion part of the cycle. However Burrows (1973)

demonstrated that during tibial flexion in a quiescent locust the FETi received EPSP's. This shows that for the metathoracic extensor FETi at least, the reflexes reversed during this self generated tibial movement. Similar rhythmic activity can also be recorded from the nerve of the mesothoracic SETi of a tethered locust immediately after a long run on the treadmill. The rhythmic bursts which occur last for a prolonged period and can be stopped only by a strong stimulation of the animal. Is this self generated pattern related to the walking central programme or is it a rhythmic activity of the motoneurons caused by the excited state of the animal? Imposed tibial flexion during this period not only reduces the number of spikes in the SETi but also excites the Common Inhibitor (fig. 6.6A). When the bursting pattern ceases resistance reflexes reappear. This reversal of reflexes supports the idea that the spontaneous rhythmic bursting is caused by a mechanism related to the walking programme although it is not known if the flexors were activated. Fig. 6A and B also confirm the fact that resistance reflexes were reversed during the centrally generated walking motor pattern. Of course in the extensor bursts the FETi is not active but it may be that the threshold of this neuron is higher than in walking or the strength of the central input is not enough to activate this neuron when the animal is stopped. Pearson (1972) also found that the large motor axons to the levator muscle of cockroach are active during walking but when alternating bursts between levator and depressor occurs in an immobilized animal the large axons were inactive.

The interaction between the femoral sensory input and another centrally generated firing pattern, flight was also studied in the extensor motoneurons. At the beginning of flight the excitatory extensor motoneurons fire at an unusually high frequency which gradually drops to a level of about 50 - 80 Hz (fig. 6.4D). This is an unusually

high firing frequency for the mesothoracic extensor motoneurons, especially the FETi which generally acts as a high threshold motoneuron in other behavioural motor patterns (walking - Burns, 1972). Why and how the locust can achieve this prolonged high firing activity of extensor excitors is not known but it seems that in flight this firing pattern would ensure that the mesothoracic leg is kept extended. The flexor motoneurons are practically silent. A similarly high activity level also appears in the abdominal muscles and falls after 10 sec. (Hinkel and Camhi, 1972). It seems that the centrally produced flight pattern modulates the activity of femoral and abdominal motoneurons and probably many others essential for the balance and orientation of the animal. An umusually high excitation of extensor tibiae motoneurons and an inhibition of the antagonistic flexors, controlled by a central programme was also found by Godden (1972) in a completely different behaviour in the stick insect, Thanatosis.

During flight, imposed tibial flexion or extension (stretch or release of the C.O.) generally has no effect on the excited extensor motoneurons. However, in some cases tibial extension causes an inhibition and flexion a small excitation of the extensor motoneurons (fig. 6.7). This shows that sensory inputs produce weak resistance reflexes indicating that during flight, reflexes on the extensor motoneurons were dramatically reduced but not reversed. During flight, resistance reflexes have no functional meaning since the leg is kept continuously extended and imposed tibial flexion is unlikely. What causes this suppression or cancellation of reflexes requires further investigation. Kennedy et al. (1974) found in crayfish that sensory inputs to intraganglionic interneurons were suppressed when the interneurons were excited by stimulating command fibres. They suggested that this was due to a presynaptic inhibition. Presynaptic inhibition was also suggested for

locust motoneurons by Burrows and Horridge (1974). They found that when motoneurons have inputs in common, indicating driving from a common interneuron, the post synaptic potentials to one but to others can be dropped out, as if gated presynaptically.

The study of the function and the innervation of the mesothoracic flexor motoneurons was achieved by recording motoneuronal events peripherally from the flexor nerve branches and muscle fibres. Although valuable information was obtained, it is not possible with this technique to study the intraganglionic connections of the flexor motoneurons themselves. For this purpose further investigation is required, primarily by intracellular recordings from the cell bodies of the flexor tibiae motoneurons and their associated premotor interneurons (Hoyle and Burrow, 1973a, b; Burrows and Hoyle, 1973; Burrows 1973; Burrows and Horridge, 1974).

In the investigation of the neuronal control of the movement, modelling of the neuronal connections responsible for the specific behaviour patterns is very popular (Pearson, 1972; Pearson and Iles, 1973; Burns, 1972; Burrows and Horridge, 1974 etc.). It was not thought that the information on the drive to the flexor motoneurons was sufficient to justify the construction of a model. However, any attempt at modelling the input connections of the femoral motoneurons must be based on the following information.

1) There is not enough evidence about the nature of the synaptic coupling between the femoral and tibial sensory inputs and the flexor motoneurons. The minimum latency for proprioceptive resistance reflexes in the mesothoracic leg was found to be 20 to 25 ms for the flexor axon F2. This suggests that at least one interneuron was interposed as was also found by Burrows and Horridge (1974) who showed in the metathoracic leg that reflex pathways and patterned central commands act on

interneurons and not directly on the motoneurons. It is not yet known certainly if there are monosynaptic connections between femoral motoneurons and leg sensory inputs. Wilson (1965) found a latency of less than 10 ms in the proprioceptive leg reflexes of the cockroach and he suspected monosynaptic connection with many parallel input fibres converging on a few motoneurons. Such monosynaptic coupling with a ganglionic delay of approximately 10 msec. has been reported in locust only between wing stretch receptors and flight motoneurons (Burrows, 1975). In the cockroach, Wong and Pearson (1976) have also discovered a monosynaptic reflex between the trochanteral pair-plate afferent and the slow depressor neuron.

2) There is no direct synaptic coupling between mesothoracic flexor motoneurons (Wilson, 1977). No evidence was found by Hoyle and Burrows (1973a) for either directly or indirectly mediated cross excitation or inhibition between any of the different types of flexor neurons in the metathoracic ganglion in spite of their strong synergistic behavioural action. They suggested that the synergistic action of many flexor motoneurons is not achieved by close coupling of the motoneuronal level but by inputs from higher interneurons which are either common or are themselves closely coupled. This is in contrast to the motoneurons of other multiple innervated insect muscles, for example those involved in flight, in which electrotonic coupling between the motoneurons themselves is implicated (Kendig, 1968; Bentley, 1969b). Monosynaptic interconnections were found between motoneurons of the crayfish claw muscles. Three neurons whose activity contributes to closing the claw - the Fast (FCE) and the Slow (SCE) Closer Excitors and the Opener Inhibitors (OI) - are linked by mutual excitatory synapses (Wiens and Atwood, 1978).

3) There is no information about the synaptic interconnections between motoneurons of antagonistic femoral muscles in the mesothoracic ganglion. However the associations between the extensor and different classes of flexor motoneurons were described by Hoyle and Burrows (1973a, b) in the metathoracic ganglion. They found a positive feedback between these neurons with a latency of 20 - 25 ms which suggests that there are one or more interneurons interposed between the extensor and flexor motoneurons.

4) The excitatory flexor motoneurons can be separated into four groups according to the way in which they function in reflexes and walking (fig. 7.1A, B).

A) Axons F1, F2 and M1 which activate mainly the proximal and middle flexors. M1 and F2 are active in long lasting bursts.

B) Axons F6, F5 and M3 which activate exclusively the distal flexor usually do so with short bursts.

C) Axons S1, S2 and S3 are responsible for the tonic activity of the whole flexor.

D) Axons F3, F4 and M2 have high thresholds and are probably used only in extreme cases.

The functional separation between flexor motoneurons would suggest that there are four different interneurons (or groups of interneurons) which drive the motoneurons in each group. The only way to obtain more accurate information about this kind of organization is to record intracellularly from the cell bodies within these groups. This grouping of inputs will be confirmed if common inputs are found exclusively between the motoneurons in each group. In the metathoracic leg Burrows and Horridge (1974) found by recording simultaneously from the slow extensor (SETi) and the Posterior Intermediate (PIF1Ti), the Posterior Fast (PFF1Ti) or Lateral Fast (LFF1Ti) flexor motoneurons

that all three motoneurons, but certainly not all flexors, receive IPSP's when there are EPSP's to the SETi, and vice versa, so that one record resembles a mirror image of the other. The three motoneurons described above (two Fast and one Intermediate) could be homologous to either group A or group B. Hoyle and Burrows (1973b) described four different functional interneurons connected with the metathoracic flexors. However further investigation is required to reveal more about the interneurons which drive the mesothoracic flexor tibiae neurons.

The conclusion that the slow motoneurons are driven separately, which was obtained by recording peripherally, was confirmed by Burrows and Horridge (1974) in the metathoracic ganglion recording intracellularly from the cell bodies of the slow motoneurons. They found that the fast and slow motoneurons must always be excited by separate interneurons which derive their excitation from phasic and tonic receptors.

5) The flexor motoneurons are active in resistance reflexes when the ipsilateral leg is stimulated but the contralateral reflexes of these neurons seem to be very weak. Therefore it seems that the regular alternation of the two sides in walking is centrally coordinated. However since it has been shown that ipsilateral reflexes are cancelled or reversed during some centrally produced behavioural motor patterns and switching of reflexes has also been found, it is premature to claim that cross reflexes are not important in walking. Whether the cross reflexes are as weak in a walking animal as they are in a quiescent one is a subject for further investigation. A locust walking on a treadmill is a good preparation in which to study further the function of reflexes during walking. For instance cross reflexes can be investigated by imposing a movement on the immobilized contralateral tibia and recording the effects of this movement on the motor pattern produced in the ipsilateral leg while the remaining four legs can walk on the treadmill.

Resistance reflexes and the motor pattern produced by a tethered locust walking on the treadmill were mainly used to activate the flexor neurons in order to study the way which this large number of axons is used (fig. 7.1A and B). However since there is a large number of neurons there is still the possibility that some of them may operate in a different manner during other behavioural motor patterns. To complete this study it would be necessary to investigate these possibilities further. For example, how do leg sensory inputs other than the CO, such as the coxal proprioceptors, influence the activity of flexor motoneurons. This would be answered by imposing a levation-depression or protraction-retraction movement on the femur of a fixed locust, or by immobilizing the femur and moving the rest of the body through the appropriate angles, since the method used here for identifying the flexor motoneurons requires a fixed femur. The activity of the flexor motoneurons could be further investigated on the treadmill and it would be interesting to find out which flexor motoneurons are mainly used for postural readjustments. This could be achieved in a tethered locust by tilting the treadmill or moving it sideways or up and down. Since the locations of the axons on the flexor muscle are known it would also be possible to study, using myograms or neurograms, the way which the flexor axons are activated in a free locust walking or climbing, this is not only for the mesothoracic but also for the prothoracic flexor muscle. Since there are strong similarities between the way in which the flexor motoneurons respond in reflexes and the way in which they are activated during walking, it would be interesting to find out if the interneurons which activate the separate groups of flexor motoneurons are always active in the same manner, whether they are excited by the central programme or by the peripheral sensory inputs.

REFERENCES

Aidley, D.J. (1965). The effects of calcium ions on potassium contracture in a locust leg. J. Physiol., Lond. 177, 94-102.

Aidley, D.J. (1967). The excitation of insect skeletal muscles. Adv. Insect Physiol. 4, 1-31.

Aidley, D.J. (1975). Excitation - Contraction coupling and mechanical properties. In : Insect muscle (ed. P.N.R. Usherwood) London, New York, San Francisco, Academic Press.

Anwyl, R. (1972). The structure and properties of an abdominal stretch receptor in Rhodnius prolixus. J. Insect Physiol. 18, 2143-2154.

Atwood, H.L. (1963). Differences in muscle fibre properties as a factor in "fast" and "slow" contraction in Carcinus. Comp. Biochem. Physiol. 10, 17-31.

Atwood, H.L. (1967). Crustacean neuromuscular mechanisms. Am. Zoologist 7, 527-551.

Atwood, H.L. (1973). An attempt to account for the diversity of crustacean muscles. Amer. Zool. 13, 357-378.

Ayers, J. and F. Clarac (1978). Neuromuscular strategies underlying different behavioural acts in a multifunctional crustacean leg joint. J. Comp. Physiol. 128, 81-94.

Ayers, J. and W.S. Davis (1977). Neuronal control of locomotion in the lobster Homorus americanus. II. Types of walking leg reflex. J. Comp. Physiol. 115, 29-46.

Ayers, J. and W.J. Davis (1978). Neuronal control of locomotion in the lobster Homorus americanus III. Dynamic organization of walking leg reflexes. J. Comp. Physiol. 123, 289-298.

Barany, M. and Close, R.I. (1971). The transformation of myosin in crossinnervated muscles. J. Physiol., London 213 455-474.

Barker, D. (1967). The innervation of mammalian skeletal muscle. In: Myotatic Kinesthetic and Vestibular Mechanisms (ed. A.V.S. de Reuk), pp 3-15. Boston : Little, Brown and Co.

Barnes, W.J.P. (1977). Proprioceptive influence on motor output during walking in the crayfish. J. Physiol., Paris 73 543-563.

Barnes, W.J.P., Spirito, C.P. and Evoy, W.H. (1972). Nervous control of walking in the crab, Cardisoma guanhuni. II. Role of resistance reflexes in walking. Z. vergl. Physiol. 76 16-32.

Bässler, U. (1972). Der "Kniesehmenreflex" bei Carausius morosus : Ubergangsfunktion und frequenzgang. Kybernetik 11, 32-50.

Bässler, U. (1976). Reversal of reflexes to a single motoneuron in the stick insect, Carausius morosus. Biol. Cybernetics, 24, 47-49.

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.

Bentley, D.R. (1969a). Intracellular activity in cricket neurons during generating of song patterns. Z. vergl. Physiologie, 62, 267-283.

Bentley, D.R. (1969b). Intracellular activity in cricket neurons during generation of behaviour patterns. J. Insect Physiol. 15, 677-699.

Bowerman, R.F. (1977). The control of arthropod walking. Comp. Biochem. Physiol. 56A, 231-247.

Bullock, T.H. and Horridge, G.A. (1965). Structure and function in the nervous system of invertebrates. San Francisco and London. W.H. Freeman.

Burns, M.D. (1972). The nervous control of walking in Orthoptera. Ph.D. Thesis, University of Glasgow.

Burns, M.D. (1973). The control of walking in orthoptera. I. Leg movements in normal walking. J. Exp. Biol. 58 45-58.

Burns, M.D. (1974). Structure and physiology of the locust femoral chordotonal organ. J. Insect Physiol. 202 1319-1339.

Burns, M.D. (1977). Microprocessor analyser for neurophysiological data. J. Physiology 273 D13.

Burns, M.D. and Delcomyn, F. (1970). Motor neuron activity during walking in insects. J. Physiol., Lond., 211 1-2.

Burns, M.D. and Delcomyn, F. (1976). Semiautomatic processing of neurophysiological data. Part I. A device for measuring potential intervals for film. Int. J. Bio-medical Computing, 7 307-317.

Burns, M.D. and Usherwood, P.N.R. (1978). The mechanical properties of locust extensor tibiae muscles. Comp. Biochem. Physiol. 61A 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 animal. J. exp. Biol. (in press).

Burrows, M. (1973). Physiological and morphological properties of the metathoracic common inhibitory neuron of the locust. J. Comp. Physiol. 82 59-78.

Burrows, M. (1974). Modes of activation of motor neurons controlling ventilatory movements of the locust abdomen. Phil. Trans. R. Soc. Lond. Ser. B. 269 29-48.

Burrows, M. (1975). Monosynaptic connexions between wing stretch receptors and flight motoneurons of the locust. J. Exp. Biol. 62 189-219.

Burrows, M. and Horridge, G.A. (1968a). Motoneuron discharges to the eyecup muscles of the crab Carcinus. J. exp. Biol. 49 251-267.

Burrows, M. and Horridge, G.A. (1968b). Eyecup withdrawal in the crab, Carcinus, and its interaction with the optokinetic response. J. exp. Biol. 49 285-297.

Burrows, M. and Horridge, G.A. (1974). The organization of inputs to motoneurons of the locust metathoracic leg. Phil. Trans. R. Soc. Lond. B 269 49-94.

Burrows, M. and Hoyle, G. (1973). Neural mechanisms underlying behaviour in the locust Schistocerca gregaria. III. Topography of limp motoneurons in the metathoracic ganglion. J. Neurobiol. Vol. 4, No.2, 167-186.

Burrows, M. and Siegler, M.V.S. (1976). Transmission without spikes between locust interneurons and motoneurons. Nature (London) 262 222-224.

Bush, M.B.H. (1962). Proprioceptive reflexes in the legs of Carcinus maenas (L). J. exp. Biol. 39 89-105.

Bush, B.M.H. (1963). A comparative study of certain limp reflexes in decapod crustaceans. Comp. Biochem. Physiol. 10 273-290.

Bush, B.M.H. (1965). Leg reflexes from chordotonal organs in the crab, Carcinus maenas. Comp. Biochem. Physiol. 15 567-587.

Cambell, J.I. (1961). The anatomy of the nervous system of the mesothorax of Locusta migratoria migratoroides R. and F. Proc. Zool. Soc. Lond. 137 403-432.

Camhi, J.M. (1970). Sensory control of abdomen posture in flying locusts. J. exp. Biol. 52 533-537.

Camhi, J.M. (1977). Behavioural switching in cockroaches : Transformation of tactile reflexes during righting behaviour. J. Comp. Physiol. 113 283-301.

Carbonell, C.S. (1947). The thoracic muscles of the cockroach, Periplaneta americana. Smisth. Misc. Coll. 107 No.2.

Clarac, F. and Dando, M.R. (1973). Tension receptor reflexes in the walking legs of the crab, Cancer pagurus. Nature, London 243 94-95.

Clarac, J., Vedel, J.P. and Bush, B.M.H. (1978). Intersegmental reflex coordination by a single joint receptor organ (CB) in rock lobster walking legs. J. exp. Biol. 73 29-46.

Close, R.I. (1972). Dynamic properties of mammalian skeletal muscles. Physiol. Rev. 52 129-197.

Cochrane, D.G., Elder, H.Y. and Usherwood, P.N.R. (1972). Physiology and ultrastructure of phasic and tonic skeletal muscle fibres in the locust Schistocerca gregaria. J. Cell Sci. 10 419-441.

Cohen, M.J.(1963). Muscle fibres and efferent nerves in crustacean receptor muscle. Quart. J. micr. Sci. 104 551-559.

Coillot, J.P. and Boistel, J. (1968). Localisation et description des recepteurs a l'etirement au niveau de l'articulation tibio-femorale de la patte sauteuse du criquet, Schistocerca gregaria. J. Insect Physiol. 14 1661-1667.

Crosman, A.R., Kerkut, G.A. and Walker, R.J. (1972). Electrophysiological studies on the axon pathways at specified nerve cells in the central ganglia of two insect species, Periplaneta americana and Schistocerca gregaria. Comp. Biochem. Physiol. 43A 393-415.

Cruce, H. (1976). The function of the legs in a free walking stick insect, Carausius morosus. J. comp. Physiol. 112 235-262.

Davis, W.J. (1971). Functional significance of motoneuronal size and soma position in swimmeret system of the lobster. J. Neurophysiology 34 274-288.

Delcomyn, F. (1973). Motor activity during walking in the cockroach, Periplaneta americana. II. Tethered walking. J. exp. Biol. 59 643-654.

Dirsh, V.M. (1974). Genus Schistocerca (Acridomorpha, Insecta). The Hague : Dr. W. Junk, B.V.

Dorai-Raj, B.S. (1964). Diversity of crab muscle fibres innervated by a single motor axon. J. cell comp. Physiol. 64 41-54.

Dresden, D. and Nijenhuis, E.D. (1958). Fibre analysis of the nerves of the second thoracic leg in Periplaneta americana. Proc. K. ned. Akad. Wet. C61 213-223.

Eagles, D.A. (1978). Tension receptors associated with muscles in the walking legs of the horseshoe crab, Limulus polyphemus. Mar. Behav. Physiol. 5 215-230.

Eagles, D.A. and Gregg, R.A. (1979) Receptors sensitive to muscle length in the horseshoe crab. Mar. Behav. Physiol. (In press).

Edwards, G. (1959). The fine structure of a multiterminal innervation of an insect muscle. J. Biophysic. and Biochem. Cytol. Vol. 5 No.2 241-244.

Edwards, G.A., Ruska, H. and de Harven, E. (1958). Electron microscopy of peripheral nerves and neuromuscular junctions in the wasp leg. J. biophys. biochem. Cytol. 4 107-114.

Eldred, W.D. and Moran, D.T. (1974). Fibre analysis of an insect peripheral nerve : Light vs. Electron microscopy. Tissue and Cell 6 (4) 751-756.

Evoy, W.H. (1977). Crustacea motor neurons. In: Identified neurons and behaviour of arthropods. (ed. G. Hoyle). Plenum Press, New York.

Evoy, W.H. and Cohen, M.J. (1969). Sensory and motor interactions in the locomotor reflexes of crabs. J. exp. Biol. 51 151-169.

Evoy, W.H. and Fourtner, C.R. (1973). Nervous control of walking in the crab, Cardisoma guanhumi. III. Proprioceptive influence on intra- and intersegmental coordination. J. comp. Physiol. 83 303-318.

Ewing, A.W. and Manning, A. (1966). Some aspects of the efferent control of walking in three cockroach species. J. Insect Physiol. 12 1115-8.

Frank, E. (1973). Matching of facilitation of the neuromuscular junction of the lobster: A possible case for influence of muscle on nerve. J. Physiol. (London) 233 635-658.

Finlayson, L.H. (1968). Proprioception in the invertebrates. Symp. Zool. Soc. London. 23 217-249.

Finlayson, L.H. (1976). Abdominal and thoracic receptors in insects, centipedes and scorpions. In: Structure and Function of Proprioceptors in the Invertebrates (ed. P.S. Mill), pp. 153-212. London: Chapman and Hall.

Fourtner, C.R. and Evoy, W.H. (1973). Nervous control of walking in the crab, Cardisoma guanhumi. IV. Effects of myochordotonal organ ablation. J. comp. Physiol. 83 319-329.

Gaisser, H.S. and Hill, A.V. (1924). Dynamics and muscular contraction. Proc. R. Soc. Lond. B 96 398-437.

Godden, D. (1972). The motor innervation of the leg musculature and motor output during thanotosis in the stick insect, Carausius morosus. J. comp. Physiol. 80 201-255.

Granit, R. and Ström, G. (1951). Autogenic modulation of excitability of single ventral horn cells. J. Neurophysiol. 14 113-132.

Hamory, J. (1961). Innervation of insect leg muscle. Acta. Biol. Hung. 12 219-230.

Heitler, W.J. (1977). The locust jump. III. Structural specializations of the metathoracic tibiae. J. exp. Biol. 67 29-36.

Heitler, W.J. and Burrows, M. (1977a). The locust jump. I. The motor programme. J. exp. Biol. 66 203-219.

Heitler, W.J. and Burrows, M. (1977b). The locust jump. II. The neural circuits of the motor programme. J. exp. Biol. 66 221-241.

Heitler, W.J. and Goodman, C.S. (1978). Multiple sites of spike initiation in a bifurcating locust neurone. J. exp. Biol. 76 63-84.

Henneman, E. and Olson, C.B. (1965). Relations between structure and function in the design of skeletal muscles. J. Neurophysiol. 28 581-598.

Henneman, E., Somjen, G. and Carpenter, D. (1965). Functional significance of cell size in spinal motoneurons. J. Neurophysiol. 98 560-580.

Hinkle, M., Camhi, J. (1972). Locust motoneurons bursting activity correlated with axon diameter. Science 175 553-556.

Houk, J. (1967). A visco-elastic interaction which produces one component of adaptation in responses of Golgi tendon organs. J. Neurophysiol. 30 1482-1493.

Houk, J. and Henneman, E. (1967). Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. J. Neurophysiol. 30 466-481.

Hoyle, G. (1955). The anatomy and innervation of a locust skeletal muscle. Proc. Roy. Soc. 143 281-292.

Hoyle, G. (1964). Exploration of neuronal mechanisms underlying behaviour in insects. In: Neural Theory and Modelling (R.F. Reiss, ed.), . Statford Univ. Press pp. 346-76.

Hoyle, G. (1965). Neural control of skeletal muscle. In: The Physiology of Insecta. Vol. II (ed. M. Rockstein) New York and London : Academic Press.

Hoyle, G. (1974). A function for neurons (DUM) neurosecretory on skeletal muscles of insects. J. Exp. Zool. 189 401-406 .

Hoyle, G. (1978). Distribution of nerve and muscle fibre types in locust jumping muscle. J. exp. Biol. 73 205-233.

Hoyle, G. and Burrows, M. (1970). Intracellular studies on identified neurons of insects. Fed. Proc. 129 : 1922.

Hoyle, G. and Burrows, M. (1973a). Neural mechanisms underlying behaviour in the locust, Schistocerca gregaria. I. Physiology of identified motoneurons in the metathoracic ganglion. J. Neurobiology 4 3-41.

Hoyle, G. and Burrows, M. (1973b). Neural mechanisms underlying behaviour in the locust, Schistocerca gregaria. II. Integrative activity in metathoracic neurons. J. Neurobiology, 4 No.1 43-67.

Hoyle, G., Dagan, D., Moberly, B. and Colquhoun, W. (1974). Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscles. J. Exp. Zool. 187 159-165.

Hoyle, G. and Dagan, D. (1978). Physiological characteristics and reflex activation of DUM (Octopaminergic) neurons of locust metathoracic ganglia. J. Neurobiol. 9 No.1 59-79.

Hoyle, G. and O'Shea, M. (1974). Intrinsic rhythm contractions in insects skeletal muscle. J. exp. Zool. 189 407-417.

Huddart, H. (1971). Ultrastructure of a peripheral nerve of Locusta migratoria migratoroides R. and F. (Orthoptera : Locustidae). Int. J. Insect Morph. Embryol. 1 29-41.

Hughes, G.M. (1952). The coordination of insect movements. I. The walking movements of insects. J. exp. Biol. 29 267-84.

Hughes, G.M. (1957). The coordination of insect movements. II. The effect of limb amputation and cutting of commissures in the cockroach (Blatta orientalis). J. exp. Biol. 34 306-33.

Ikeda, K. (1977). Flight motor innervation of Flesh Fly. In: Identified Neurons and Behavior of Arthropods (ed. Hoyle). Plenum Press, N.Y.

Iles, J.F. and Pearson, K.G. (1969). Triple inhibitory innervation of insect muscle. J. Physiol., Lond. 204 124pp.

Karnovsky, J.M. (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. cell Biol. 27 137 Abstract.

Kendall, M.D. (1970). The anatomy of the tarsi of Schistocerca gregaria Forskal. Z. Zellforsch. Microsk. Anat. 109 112-137.

Kending, J. (1968). Motoneurons coupling in locust flight. J. exp. Biol. 48 389-404.

Kennedy, D. and Takeda, K. (1965a). Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. J. exp. Biol. 43 211-227.

Kennedy, D. and Takeda, K. (1965b). Reflex control of the abdominal flexor muscles in the crayfish. II The tonic system. J. exp. Biol. 43 229-246.

Kennedy, D., Calabrese, R.L., Wine, J. (1974). Presynaptic inhibition: primary afferent depolarization in crayfish neurons. Science 186 451-454.

Kerkut, G.A., Pitman, R.M. and Walker, R.J. (1969). Iontophoretic application of acetylocholine and GABA onto insect central neurons. Comp. Biochem. Physiol. 31 611-633.

Lane, N.J. and Trehern, J.E. (1973). The ultrastructural organization at peripheral nerves in two insect species (Periplaneta americana and Schistocerca gregaria). Tissue and Cell 5 (4) 703-714.

MacMillan, D.L. (1976). Arthropod apodeme tension receptors. In: Structure and Function of proprioceptors in the Invertebrate. (ed. P.J. Mill) pp 427-442. London : Chapman and Hall.

Macmillan, D.L. and Dando, M.R. (1972). Tension receptors on the apodemes of muscles in the walking legs of the crab, Cancer magister. Mar. Behav. Physiol. 1 185-208.

O'Shea, M., Rowell, C.H.F. and Williams, J.L.D. (1974). The anatomy of a locust visual interneuron : The descending contralateral movement detector. J. exp. Biol. 60 1-12.

Pantin, C.F.A. (1964). Note on microscopical techniques for Zoologists. Cambridge University Press.

Parnas, I. (1972). Differential block at high frequency of branches of a single axon innervating two muscles. J. Neurophysiol. 35 903-914.

Parnas, I. and Atwood, H.L. (1966). Phasic and tonic neuromuscular systems in the abdominal extensor muscles of the crayfish and rock lobster. Comp. Biochem. Physiol. 18 701-723.

Pearson, K.G. (1972). Central programming and reflex control of walking in the cockroach. J. exp. Biol. 56 173-93.

Pearson, K.G. and Fourtner, C.R. (1975). Nonspiking interneurons in walking system of the cockroach. J. Neurophysiol. 38 33-52.

Pearson, K.G. and Iles, J.F. (1970). Discharge pattern of coxal levator and depressor motoneurons of the cockroach, Periplaneta americana. J. exp. Biol. 52 139-65.

Pearson, K.G. and Iles, J.F. (1971). Innervation of coxal depressor muscles in the cockroach, Periplaneta americana. J. exp. Biol. 54 215-232.

Pearson, K.G. and Iles, J.F. (1973). Nervous mechanism underlying intersegmental coordination of leg movements during walking in the cockroach. J. exp. Biol. 58 725-744.

Pearson, K.G., Stein, R.B. and Mulhotra, S.K. (1970). Properties of action potentials from insect motor nerve fibres. J. exp. Biol. 53 290-316.

Pitman, R.M., Tweedie, C.D. and Cohen, M.J. (1972). Branching of Central neurons : Intracellular Cobalt Injection for light and electron microscopy. Science Vol. 176 pp 412-414.

Pringle, J.W.S. (1938). Proprioception in insects. II The action of the companiform sensilla on the legs. J. exp. Biol. 15 114-31.

Pringle, J.W.S. (1939). The motor mechanism of insect leg. J. exp. Biol. 16 220-31.

Pringle J.W.S. (1940). The reflex mechanisms of insect leg. J. exp. Biol. 17 8-17.

Pringle, J.W.S. (1961). Proprioception in arthropods. In: The Cell and the Organism (J.A. Ramsay and V.B. Wigglesworth, eds.) Cambridge Univ. Pres. pp 256-82.

Rees, D. and Usherwood, P.N.R. (1972). Fine structure of normal and degenerating motor axons and nerve muscle synapses in the locust Schistocerca gregaria. Comp. Biochem. Physiol. 43A 83-101.

Reinking, R.M., Stephens, J.A. and Stuart, D.G. (1975). The tendon organs of cat medial gastrocuemius : significance of motor unit type and size for the activation of Ib afferents. J. Physiol. 250 491-512.

Remler, M., Selvestron, A. and Kennedy, D. (1968). Lateral giant fibres of crayfish : location of somata by dye injection. Science 162 281-283.

Rijland, P. (1932a). Le manifestations electriques du touus et des contractions voluntaires et reflexe chez les arthropodes C.r. Seanc. Soc. Biol. III 631-5.

Rijland, P. (1932b). Introduction a l'etude du functionnement des centres nerveux. C.r. Seanc. Soc. Biol. III 636-9.

Rowell, P.C.H. (1969). Central control of an insect segmental reflex. II. Analysis of the inhibitory input from the metathoracic ganglion. J. exp. Biol. 50 191-201.

Runion, H.I. and Usherwood, P.N.R. (1966). A new approach to neuromuscular analysis in the intact free walking insect preparation. J. Insect Physiol. 12 1255-1263.

Runion, H.I. and Usherwood, P.N.R. (1968). Tarsal receptors and leg reflexes in the locust and grasshopper. J. exp. Biol. 49 421-436.

Sandeman, D.C. (1968). A sensitive position measuring device for biological systems. Comp. Biochem. Physiol. 24 635-638.

Shepheard, P. (1973). Musculature and innervation of the neck of the desert locust, Schistocerca gregaria (Forskal). The Journal of Morphology 139 No.4 439-464.

Shepheard, P. (1974). Control of the head movements in the locust, Schistocerca gregaria. J. exp. Biol. 60 735-767.

Slifer, E.H. and Finlayson, L.H. (1956). Muscle tension receptor organs in grasshoppers and locusts (Orthoptera, Acrididae). Q.J. Microsc. Sci. 97 617-620.

Snodgrass, R.E. (1929). The thoracic mechanism of a grasshopper and and its antecents. Smithsom misc. Coll. 82 No. 2.

Somsen, G., Carpenter, D. and Henneman, E. (1964). A precise correlation between the sizes of motor neurons and their reflex excitabilities. Fed. Proc. 23 11.

Spirito, C.P. (1970). Reflex control of the opener and stretcher muscles in the cheliped of the fiddler crab, Uca pugnax. Z. vergl. Physiol. 68 211-228.

Spirito, P., Evoy, H. and Barnes, W.J.P. (1972). Nervous control of walking in the crab, Cardisoma guanhumi. I. Characteristics of resistance reflexes. Z. vergl. Physiol. 76 1-15.

Spurr, A.R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure research 26 31-43.

Stretton, A.O.W. and Kravitz, E.A. (1968). Neuronal geometry determination with technique of intracellular dye injection. Science, N.Y. 162 132-134.

Suzuki, Y. (1978). Abdominal abductor muscle in crayfish. Physiological properties and neural control. I. Twitch system. Comp. Biochem. Physiol 59A 249-258.

Tyrer, N.M. (1971a). Innervation of the abdominal intersegmental muscles in the grasshopper. I. Axon counts using unconventional techniques for electron microscopy. J. exp. Biol. 55 305-314.

Tyrer, N.M. (1971b). Innervation of the abdominal intersegmental muscles in the grasshopper. II Physiological analysis. J. exp. Biol. 55 315-24.

Theophilidis, G. and Burns, M.D. (1979). A muscle tension receptor in the locust leg. J. comp. Physiol. (in press).

Usherwood, P.N.R. (1962). The nature of "slow" and "fast" contractions in the coxal muscles of the cockroach. J. Insect Physiol. 8 31-52.

Usherwood, P.N.R. (1967). Insect neuromuscular mechanisms. A. Zool. 7 553-82.

Usherwood, P.N.R. (1968). A critical study of the evidence for peripheral inhibitory axons in insects. J. exp. Biol. 49 201-222.

Usherwood, P.N.R. (1974). Nerve-muscle transmission. In: Insect Neurobiology (J.E. Treherne) 245-305. North Holland, Amsterdam.

Usherwood, P.N.R. (1977). In : Identified neurons and Behaviour of Arthropods. (ed. G. Hoyle). Plenum Press. New York and London.

Usherwood, P.N.R. and Grundfest (1965). Peripheral inhibition in skeletal muscle of insects. J. Neurophysiol. 28 497-518.

Usherwood, P.N.R. and Runion, H.I. (1970). Analysis of the mechanical responses of metathoracic extensor tibiae muscle of the free walking locust. J. exp. Biol. 52 39-58.

Usherwood, P.N.R., Runion, H.I. and Campbell (1968). Structure and Physiology of a chordotonal organ in the locust leg. J. exp. Biol. 48 305-323.

Velez, S.J. and Wyman, R.I. (1978). Synaptic connectivity in a crayfish neuromuscular system. II Nerve muscle matching and nerve branching patterns. J. Neurophysiol. 41 No. 1 85-96.

Weevers, R. de G. (1966). The physiology of the lepidopteran muscle receptor. III Stretch reflex. J. exp. Biol. 45 229-249.

Weiant, E.A. (1958). Control of spontaneous efferent activity in certain efferent nerve fibres from the metathoracic ganglion of the cockroach. Proc. 10th Int. Congr. Ent. 2 81-82.

Wendler, G. (1961). Die regelung der korperhaltung dei stubheuschrecken (Carausius morosus). Naturwissenschaften 48 676-7.

Wendler, G. (1966). The coordination of walking movements in arthropods. Symp. Soc. exp. Biol. 20 229-49.

Wendler, G. (1974). The influence of proprioceptive feedback on locust flight co-ordination. J. comp. Physiol. 88 173-200.

Weis-Fog, T. (1956). Tetanic force and shortening in locust flight muscle. J. exp. Biol. 33 668-684.

Wiens, T.J. (1976). Electrical and structural properties of crayfish claw motoneurons in an isolated claw ganglion preparation. J. comp. Physiol. 112 213-233.

Wiens, T.J. and Atwood, H.L. (1978). Motoneuron interaction in crayfish claw control. Evidence from intracellular recordings. J. comp. Physiol 124 237-247.

Wiens, T.J. and Gerstein, G.L. (1976). Reflex pathways of the crayfish claw. J. comp. Physiol. 107 309-326.

Wilkens, L.A. and Wolfe, G.E. (1974). A new electrode design for en passant recording, stimulation and intracellular dye infusion. Comp. Biochem. Physiol. 48A 217-220.

Williamson, R. and Burns, M.D. (1978). Multiterminal receptors in the locust mesothoracic leg. J. Insect Physiol. 24 661-666.

Wilson, D.M. (1965). Proprioceptive leg reflexes in cockroaches. J. exp. Biol. 43 397-409.

Wilson, J.A. (1977). The structure and function of serially homologous leg motoneurons in the locust. Ph.D. thesis, University of Oregon.

Wong, R.K.S. and Pearson, K.G. (1976). Properties of the trochanteral hair plate and its function in the control of walking in the cockroach. J. exp. Biol. 64 233-249.

Zarwarzin, A.R. (1912). Histologische studien über insecten. II. Das sensible nervesystem der Aeschnalarven. Z. wiss. Zool. 100 245-246.