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STRUCTURE AND PHYSIOLOGY OF A CHORDOTONAL ORGAN IN THE LOCUST LEG

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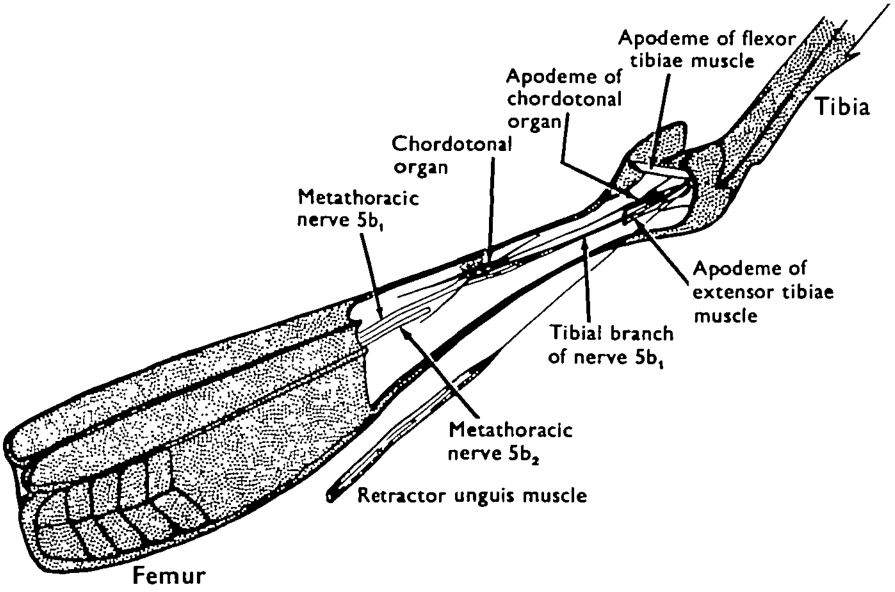
INTRODUCTION

Mechanoreceptors or proprioceptive organs associated with the wings, legs, antennae, palps and abdominal and thoracic cavities are thought to be involved in the co-ordination of insect body movements (e.g. Pringle, 1938*a–c*; Hughes, 1952, 1957; Wilson, 1965, 1966). Dethier (1963) suggests that mechanoreception or proprioception is accomplished in the insect by five different sensory structures; hair plates, companiform sensilla, stretch receptors, chordotonal organs and statocyst-like organs.

Of the various known types of insect mechanoreceptors the chordotonal organ is perhaps anatomically and electrophysiologically the most complex. There are four main types of chordotonal organs: (1) simple chordotonal organs associated with position detection in the legs, wings and antennae; (2) subgenual organs concerned with the perception of vibrations of the substratum (Autrum, 1940); (3) complex auditory organs, i.e. the tympanal organs or insect 'ears'; and (4) Johnson's organs which are located in the second segment of the antenna of some insects and act as receptors for movement of the antenna, as gravity detectors and as modified hearing organs with directional sensitivities for sound localization (Bullock & Horridge, 1965). In this paper the structural and electrophysiological properties of a so-called simple chordotonal organ are described. The organ is found in the femoral segments of the pro- meso- and metathoracic legs of the locusts *Schistocerca gregaria* and *Locusta migratoria* and the American grasshopper *Romalea microptera*, and is thought to be involved in co-ordinating the activities of the metathoracic femoral muscles (Runion & Usherwood, 1966*a*). Only the metathoracic chordotonal organ has been studied so far in any detail. The occurrence of chordotonal organs in the legs of insects has, of course, been known for some time (e.g. Eggers, 1928), but little information on the electrophysiological properties of the mechanoreceptors has been published, apart from some preliminary studies by Becht (1958) on the femoral chordotonal organ of the cockroach, and Hubbard (1959) on the chordotonal organ found in the metathoracic leg of *Locusta* and *Schistocerca*. The present studies are part of a much broader investigation of the mechanisms underlying postural and walking behaviour in locusts and grasshoppers.

The neural mechanisms controlling insect walking are not well understood. One reason for this is that it has not been possible, so far, to differentiate clearly between peripheral and central influences. A major advance in the study of flight-control mechanisms in locusts was made by Wilson (1961) and Gettrup (1962) when they partly deafferented the flight apparatus. By this means they were able to show that control of the flight muscles is mainly central and that sensory input from proprioceptors associated with the flight system only modifies the frequency of the output signals to the flight muscles, i.e. the proprioceptive information does not determine the patterning of the output. Unfortunately the proprioceptors associated with the legs are less accessible than those associated with the wings and cannot usually be removed without damaging the motor system. This apparent inability to deafferent the walking apparatus satisfactorily has, in the past, been something of a stumbling block to further progress in studies of insect walking. In the present investigations we have successfully removed part of the sensory system, i.e. the metathoracic femoral chordotonal organs in the leg of the locust and grasshopper, without damaging either the femoral muscles or the leg nerves. This is admittedly only a small step towards the desired goal of complete deafferentation of the walking apparatus, but even so it has provided some useful information on the mechanisms controlling and co-ordinating the contractions of the leg muscles.



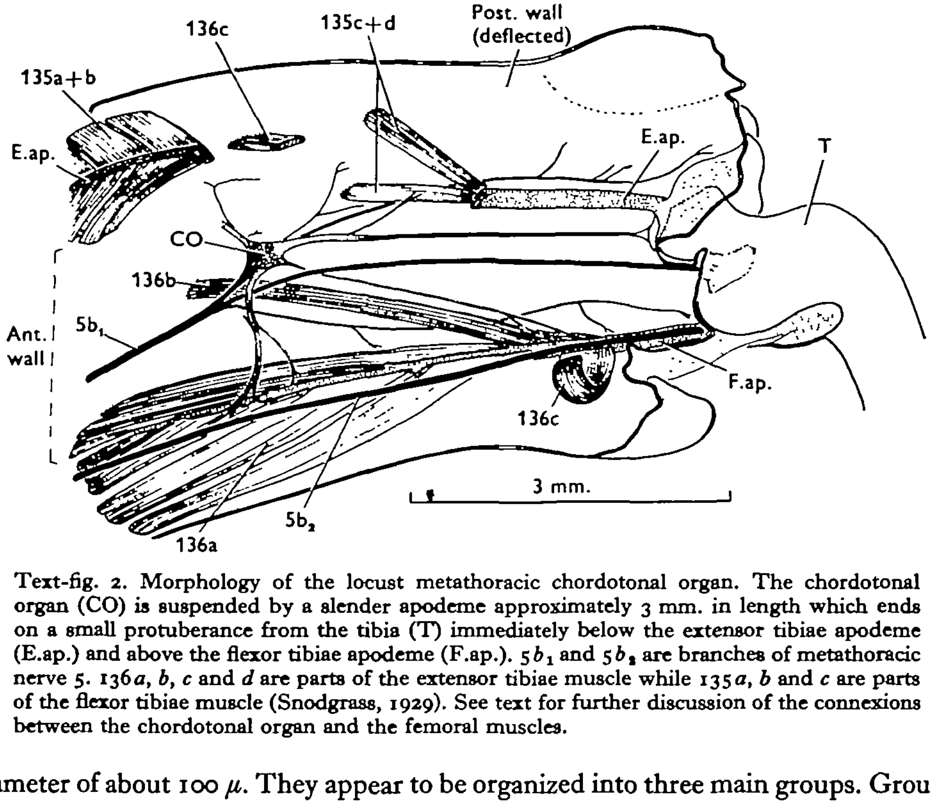
Text-fig. 1. Diagrammatic representation of the metathoracic femur of the locust or grasshopper showing position of chordotonal organ.

Anatomy and histology

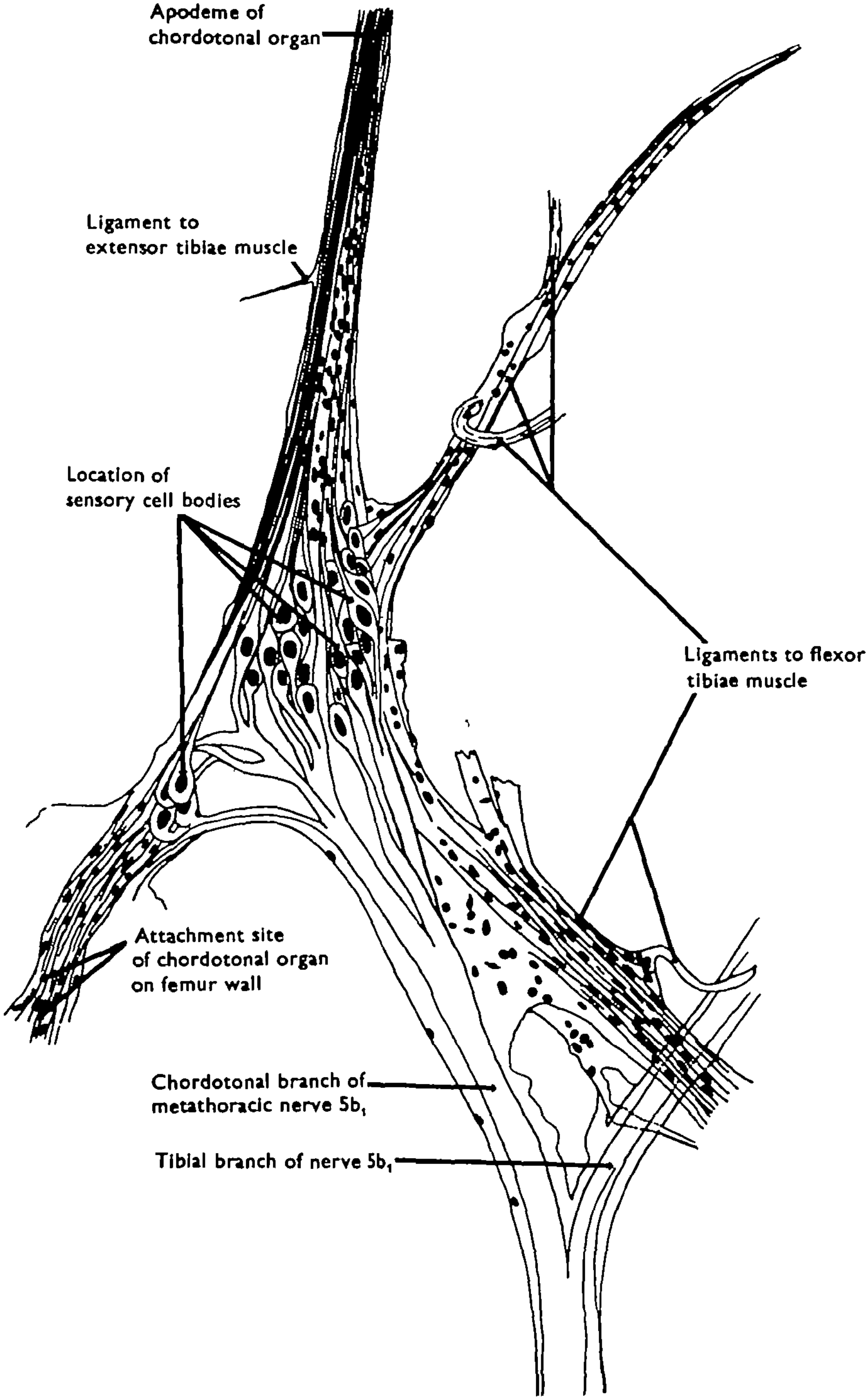
The chordotonal organ (CO) is located in the distal dorsal region of the femur and is firmly attached to the posterior wall of the femur by connective ligaments (Text-fig. 1). A multicellular filamentous apodeme about 3 mm. in length runs from the distal end of the CO body to a protrusion on the tibia near the tibia-femur articulation. Numerous connective ligaments arise from the body of the CO and from the apodeme and these attach to the apodemes of the extensor tibiae muscle and the flexor tibiae muscle (Text-fig. 2). Axons from the CO are contained in a branch of metathoracic nerve 5 ($N5b_1$, Campbell, 1961). $N5b_1$ divides into three main trunks approximately

1 mm. proximal to the CO body. One trunk of $N5b_1$, which will be called the chordotonal nerve, enters the CO while another passes to the extensor tibiae muscle and to receptors in the femoral cuticle. A small branch from the third nerve trunk goes to the flexor tibiae muscle and ends in cuticular receptors, while the major portion of this nerve trunk continues on to the tibial segment.

The CO contains approximately twenty-four sensory cells arranged as shown in Fig. 3 and Pl. 1. The sensory cells are oblate spheroid bodies with a maximum diameter of about $100\ \mu$. They appear to be organized into three main groups. Group 1 consists of four cell bodies, and processes from these are associated with the connective ligaments which link the CO with the posterior wall of the femur. Group 2, consisting of ten cell bodies, is located in the middle of the CO and appears to be associated with chordotonal apodeme. Group 3, also consisting of approximately ten cell bodies, is associated with the chordotonal apodeme and with the ligaments which connect the CO with various parts of the flexor tibiae muscle (Text-fig. 3). The connective ligaments appear to contain dendrites from the cell bodies. The body of the CO is surrounded by a tough connective tissue sheath consisting of a single layer of cells (Plate 1). This sheath also covers the CO apodeme and parts of the connective ligaments. A more detailed study of the histological and ultrastructural features of the CO is currently in progress, and will be reported elsewhere.



Text-fig. 2. Morphology of the locust metathoracic chordotonal organ. The chordotonal organ (CO) is suspended by a slender apodeme approximately 3 mm. in length which ends on a small protuberance from the tibia (T) immediately below the extensor tibiae apodeme (E.ap.) and above the flexor tibiae apodeme (F.ap.). $5b_1$ and $5b_2$ are branches of metathoracic nerve 5. 136*a*, *b*, *c* and *d* are parts of the extensor tibiae muscle while 135*a*, *b* and *c* are parts of the flexor tibiae muscle (Snodgrass, 1929). See text for further discussion of the connexions between the chordotonal organ and the femoral muscles.



Text-fig. 3. Detailed histology of the locust metathoracic chordotonal organ. The diagram is principally derived from the phase contrast micrographs shown in Plate 1.

MATERIAL AND METHODS

COs found in the metathoracic femoral segments of *Schistocerca gregaria*, *Locusta migratoria* and *Romalea microptera* were examined. Studies were made primarily on female *Schistocerca* supplied by the Anti-Locust Research Centre, London. Three different types of preparation were used to investigate the properties of the CO and the role that this organ plays in controlling and co-ordinating the activities of the leg muscles.

(1) *Isolated leg preparations.* These were placed in a moist chamber so that extra-cellular recordings could be made from the CO nerve over extended periods (24–36 hr.) in a saline-free environment. Locust saline (Hoyle, 1953) was used during the preliminary stages of these investigations, but was considered unsatisfactory since it often markedly modified the responsiveness of the CO. P. N. R. Usherwood (to be published) has recently found that this saline is in fact hypertonic to the haemolymph of locusts and grasshoppers. Petroleum jelly and paraffin oil were also found to be unsatisfactory, since they altered the responsiveness of the CO.

The femoral segment of a metathoracic leg was fixed in low-temperature sealing wax with the ventral surface uppermost. The ventral cuticle was removed immediately anterior (1 mm.) to the tibia-femur articulation for a distance of 15–20 mm. The flexor tibiae muscle was then removed, leaving the extensor tibiae muscle, branches of N5 and the retractor unguis muscle. The CO was exposed by removing the membraneous sheath which separates the flexor and extensor tibiae muscles. Extra-cellular recording electrodes, consisting of two 40 μ insulated copper wires, were placed on the CO nerve (Text-fig. 3) using a micromanipulator. The electrodes were initially shaped into a U with the insulation removed from the inner margins of the U. Once in position, the U was closed around the nerve thus ensuring maximum contact between electrodes and nerve. Impulses recorded from the chordotonal nerve were amplified using an a.c. differential amplifier, displayed on a Tektronix 565A oscilloscope and photographed with a Grass C-4 camera. The impulses were counted by connecting the output of the a.c. differential amplifier through a bio-potential analyser (Runion, 1964) to a Hewlett Packard 522B frequency rate counter.

The tibia was cut leaving a stump of about 4–5 mm. The tissues inside the tibial stump were destroyed by macerating them with a blunt glass probe. Since the apodeme of the CO is attached to the tibial segment (Text-figs. 1, 2), the CO can be excited by moving the tibial stump. Movement of the tibia is restricted to an arc of 170° which corresponds to a 1·9 mm. movement of the chordotonal apodeme. The dynamic or phasic properties of the CO were examined by moving the tibial stump either sinusoidally ($>$ 0·03 Hz.) or in a ramp function. Movements were controlled by an electro-magnetic driver, which was itself driven by a sine-wave and ramp-function generator. The output of the driver was in phase with the output of the generator. A photo-conductive cell with good low-frequency and high-frequency response characteristics registered the direction, velocity and amplitude of the tibial movements. The static or tonic properties of the CO were investigated by recording the CO discharges when the tibia was set at different angles to the femur. The moisture chamber was fitted with an angle indicator enabling the femur-tibia angle

(FTA) to be measured directly, i.e. 0° = tibia flexion, 170° = maximum tibia extension.

(2) *Restrained insect preparations.* The relationship between afferent information from the metathoracic CO and activity of the metathoracic extensor tibia muscle was examined using restrained intact insect preparations. The insect was held down on its back and the femur of one of the metathoracic legs (ipsilateral) was enclosed in a high-humidity chamber similar in design to that used for isolated leg preparations. Low-temperature wax was used to immobilize the coxal and femoral segments of the ipsilateral and contralateral metathoracic legs. The flexor tibiae muscle of the ipsilateral leg was removed and recording electrodes were placed on the CO nerve. Recording electrodes were also placed in the metathorax on ipsilateral metathoracic nerves 5 (N5) and 3b (N3b). Metathoracic N5 contains the afferent tracts from the CO as well as a 'fast' motor axon to the metathoracic extensor tibiae muscle, while N3b contains a 'slow' motor axon and an inhibitory axon to the metathoracic extensor tibiae muscle (Hoyle, 1955; Usherwood & Grundfest, 1964, 1965). Recordings from metathoracic N5 and N3b provide a clear picture of the activity of the muscles and sense organs of the metathoracic leg (Runion & Usherwood, 1966*a*).

In some experiments the ipsilateral leg was left intact, i.e. the CO was not exposed and the relationship between the CO discharge and activity of the leg muscles was studied by recording from metathoracic nerves N5 and N3b in the metathorax. The CO was stimulated as before, either sinusoidally or in a ramp function.

(3) *Free-walking preparations.* The role of the femoral chordotonal organ during postural and walking activity was studied using two types of free-walking intact locust preparation (Runion & Usherwood, 1966b). In some preparations the afferent discharges of the metathoracic femoral CO were recorded from metathoracic N5, while activity of the 'slow' excitatory axon to the extensor tibiae muscle was recorded from N3b. Photographic records of the insect were taken concurrently with the neurographic records (Galloway, Runion & Usherwood, 1966) so that the relationships between limb movements and limb position and afferent and efferent activity levels could be determined.

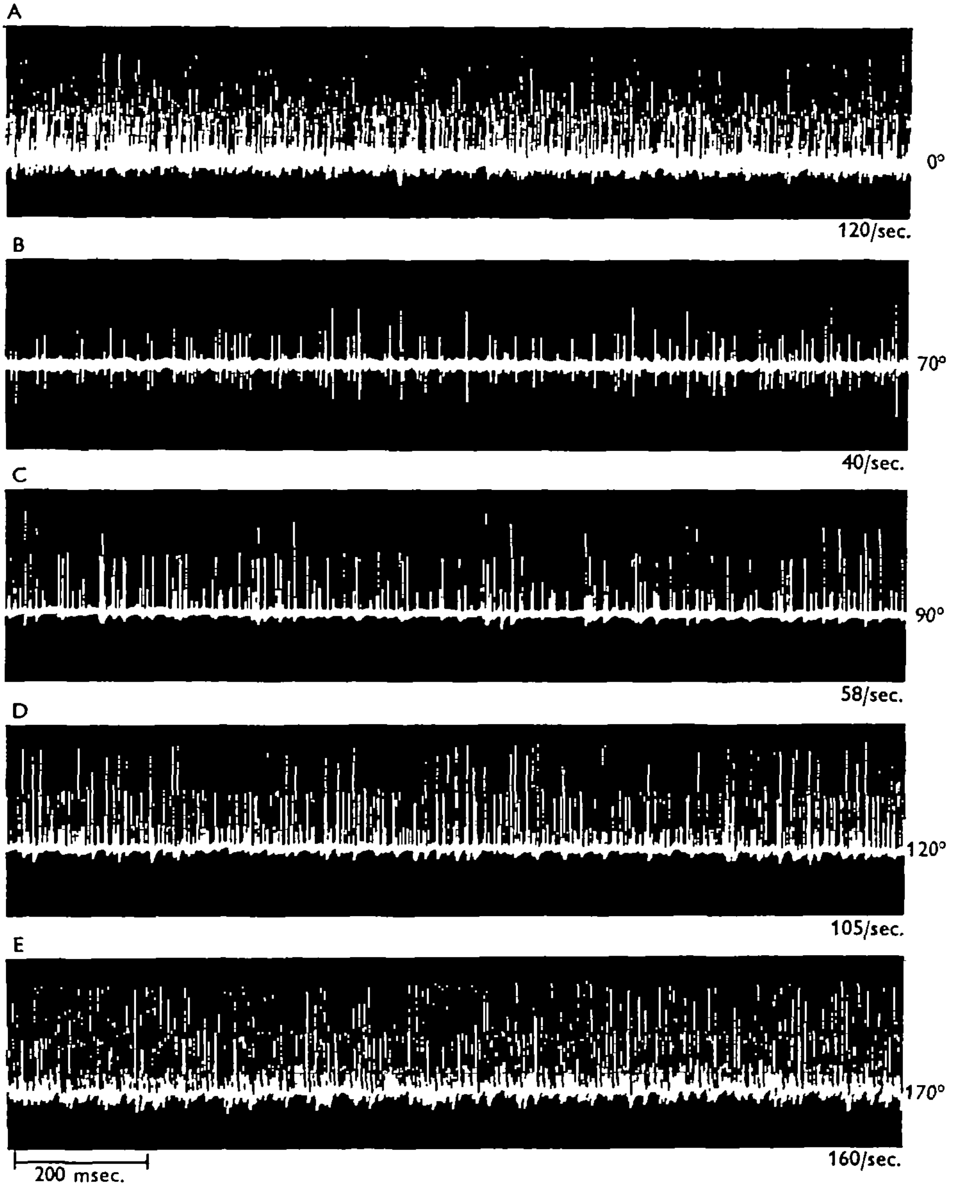
RESULTS

The femoral CO in locusts and grasshopper is a position detector and movement indicator responding to displacement of the tibia and also probably to contractions of the femoral muscles which do not result in movements of the tibial segment.

Tonic or static properties of the CO. The position of the tibial segment is indicated by the activity of tonic units which fire at different frequencies for different fixed positions of the tibia or femur-tibia angles (FTAs), (Runion & Usherwood, 1966b). Many sensory units in the CO are apparently involved in signalling the position of the tibia. It was not possible to determine with any degree of certainty whether the same tonic units fire at all FTAs, i.e. a change in tibia position being signalled by an alteration of the firing frequency of these units, or whether different combinations of units signal the different tibia positions. The latter seems more likely. It appears from records of the type illustrated in Text-fig. 4 that an increase in tonic frequency is caused by recruitment of extra units, although an increase in the firing frequency of the units already active presumably also occurs. Recordings from the chordotonal nerve merely gave an indication of over-all level of activity of the CO, and provided little information on the activities of individual sensory cells comprising the CO. Unfortunately it has not been possible to date to record from single tonic units.

The frequency of impulses recorded from the chordotonal nerve is within certain limits a linear function of the FTA (Text-fig. 5). The linear relationship of discharge frequency (y) to FTA (x) may be expressed according to the equation:

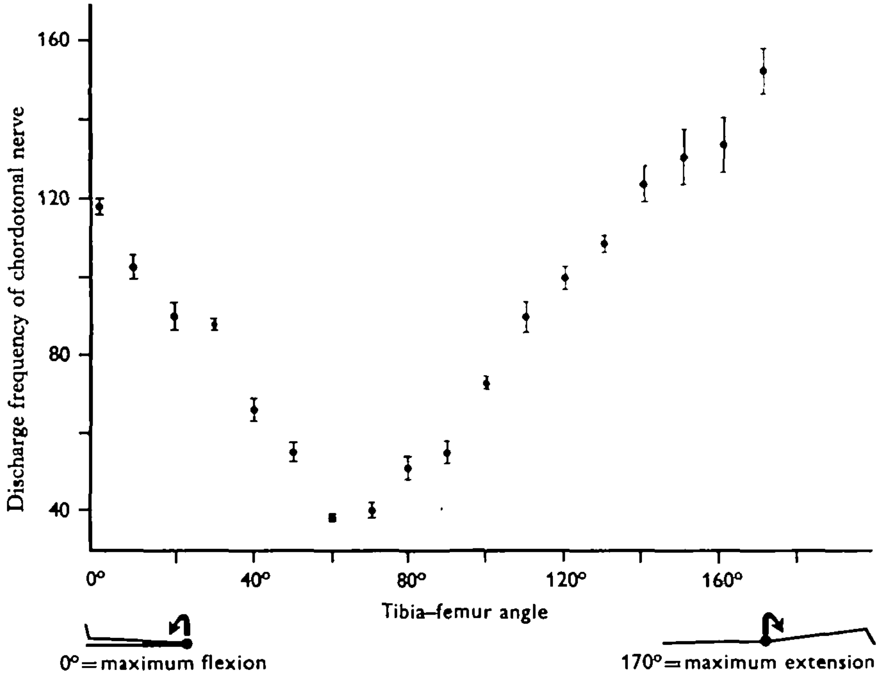
$$Y = M(X-A)+B,$$



Text-fig. 4. Tonic discharges of the locust chordotonal organ. The tibial segment was firmly fixed in different positions with reference to the femur and the recordings illustrated (A–E) were obtained 5 min. later. The femur-tibia angles were: (A), 0°; (B), 70°; (C), 90°; (D), 120°; (E), 170°. The discharge frequencies are shown below each record.

where A is the femur-tibia angle at the minimum resting discharge frequency (B), i.e. 60°. $M = -m_1$ when $0 \leqslant x \leqslant A$; $M = +m_2$ when $A \leqslant x \leqslant 170°$; $-m_1$ and $+m_2$ are the slopes of the graph (Text-fig. 6) (Runion & Usherwood, 1966*a*). The tonic properties of the femoral COs of *Romalea* and *Locusta* are almost identical with those of *Schistocerca*.

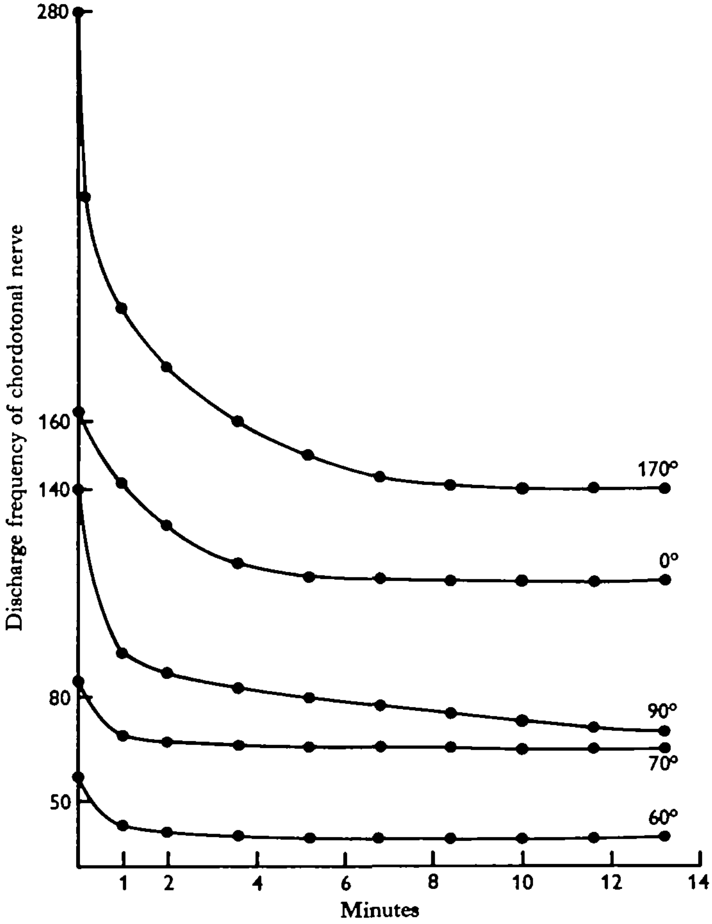
The tonic units which signal the position of the tibia do adapt to some extent (Text-fig. 6). Adaptation was always almost 90% complete 1 min. after moving the tibial segment to a new position, i.e. a different FTA. Thereafter the frequency of tonic impulses stayed relatively constant although random variations of ± 10 impulses per sec. were recorded in the isolated leg preparations. The tonic discharge frequency was more variable in intact preparations. Changes of the order of ± 30 impulses per sec. were recorded over long periods from some preparations but these fluctuations in tonic activity were not random. In fact, they appear to be directly related to the respiratory activity of the insect. It is perhaps significant that the rate and magnitude of the initial fall in discharge frequency when the tibia is set in a new position is not the same for all FTAs. For example, at an FTA of 90° the rate and magnitude of the initial drop in discharge frequency was much greater than at an FTA of 0°, although the steady-state firing frequency is greater at 0° than at 90° (Text-fig. 6). There is no obvious explanation for this. Possibly the units active at an FTA of 90° adapt quickly



Text-fig. 5. Variation of the tonic or static discharge frequency (±s.d. of mean) of a locust metathoracic chordotonal organ with femur-tibia angle. The tibia was maximally flexed at 0° and maximally extended at 170°. The activity was counted during the second minute after positioning of the tibia, i.e. after complete adaptation of phasically responsive units in the chordotonal organ. Adaptation of the tonic units to a lower steady firing frequency was also almost complete by this time. (After Runion & Usherwood, 1966*b*.)

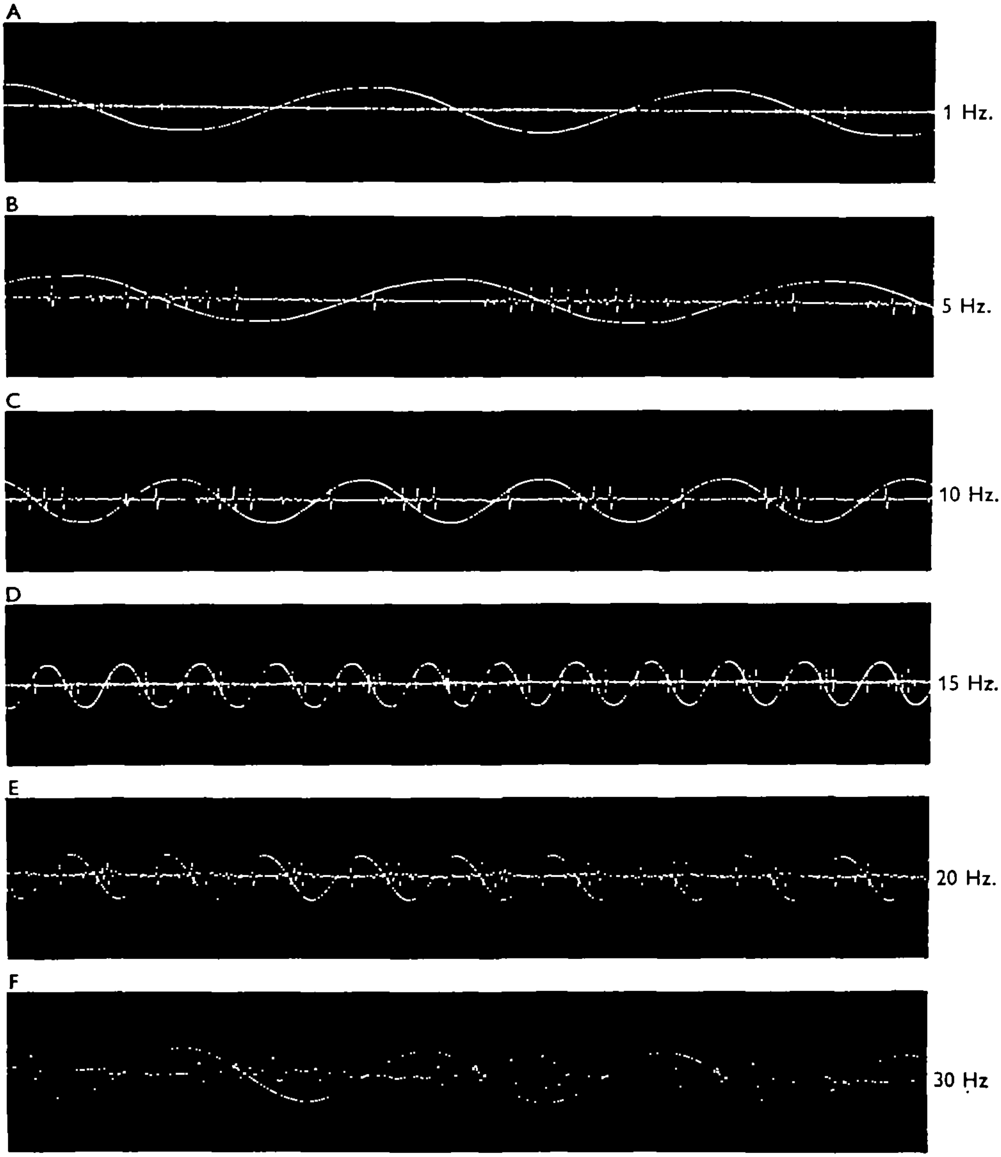
and to a greater extent than those active at an FTA of 0°. Another possibility is that an FTA of 90° is the optimum for mechanical accommodation of the over-all receptor structure and the femoral muscles.

Phasic properties of the CO. Investigations of the phasic responses of the CO were made on isolated leg preparations and restrained intact preparations using sinusoidal or ramp function movements of the tibia. Phasic discharges could be elicited from the CO when the tibial segment was displaced by as little as ±3′. When the tibia was moved, for example in an arc between FTA 120° and FTA 135°, sinusoidal extension and flexion of the tibia at frequencies of 0·1–170 Hz. evoked a constant phasic response (Text-fig. 7), but with frequencies greater than 170 Hz. the response of the CO no longer coincided exactly with the movements of the tibia. The phasic units in the CO adapt very rapidly. They also presumably recover equally rapidly since prolonged sinusoidal stimulation of the CO at frequencies less than 170 Hz.



Text-fig. 6. Time course of adaptation of the tonic units in the locust chordotonal organ for different femur-tibia angles (shown above each curve). The chordotonal discharges were counted starting 10 sec after the tibia was repositioned, i.e. after complete cessation of phasic firing.

does not result in the disappearance of the phasic response. In fact, the tibial segment could be moved sinusoidally for periods of up to 2 hr. without any detectable change in the response pattern. The recovery time would presumably be critical only with high-frequency sinusoidal stimulation, which could account for the failure of the CO to follow high-frequency movement of the tibia.



Text-fig. 7. Phasic responses of a locust metathoracic femoral chordotonal organ to sinusoidal movement of the tibia (±7° 30′), at different frequencies: (A), 1 Hz.; (B), 5 Hz; (C), 10 Hz.; (D), 15 Hz.; (E), 20 Hz.; (F), 30 Hz. Note increase in frequency but decrease in duration of phasic discharge with increasing input frequencies. Note also smaller and briefer response during flexion of tibia (up on transducer trace) than during extension (down on transducer trace). A photoconductive cell monitored the movements of the tibia and the output of this cell is displayed superimposed on the records from the chordotonal nerve.

The CO responded phasically during both extension and flexion of the tibia, although the discharge during extension was always much greater than during flexion (Text-fig. 7). There was little evidence for any change in the phase relationship between input and output as the input frequency was increased, although the duration of the CO discharge declined while the discharge frequency increased with higher input frequencies. The phasic responses of the CO during flexion and extension of the tibia seemingly involve different sensory units (Text-fig. 7).

Text-fig. 8. Response of locust chordotonal organ to ramp movement of the tibia. The tibia was alternately extended (upward deflexion of transducer trace) and flexed through an angle of 6° 30′ at different velocities. Each complete cycle of extension followed by flexion was repeated: (A) once per second; (B) 15 times per second and (C), twice per second. Note during extension increased frequency of discharge with increased tibial velocity and differences between discharges during flexion and extension of tibia.

The relationship between velocity of displacement of the tibia and activity of the phasic or dynamic units was further studied by using a linear ramp function to control movements of the tibia. The tibial segment was moved, at different velocities, through an angle of 6° 30′ (Text-fig. 8). Discharges of the CO were quantified by integrating the impulses recorded from the chordotonal nerve. It can be seen from Text-fig. 8 that a clear relationship exists between tibia velocity and phasic firing of the CO, the output frequency from the CO increasing with increasing velocity of tibial displacement.

Studies on intact insects. Results similar to those obtained from isolated leg preparations were also obtained from intact, restrained preparations and free-walking preparations. Phasic responses were recorded from the chordotonal nerve in the intact, restrained preparation during sinusoidal displacement of the tibial segment and the behaviour of the tonic or static units of the CO was more or less the same as in the isolated leg preparations. In addition to studying the properties of the CO it was possible, with the intact preparation, to study also the relationship between the CO discharge and activity of the femoral muscles (Text-fig. 9). This was achieved either by recording from metathoracic N5 and N3b in the metathoracic cavity or by placing electrodes in the extensor tibiae muscle (Runion & Usherwood, 1966b). Metathoracic N5 contains most of the sensory fibres from the CO as well as the 'fast' motor axon to the metathoracic extensor tibiae muscle and a number of 'fast' axons to the flexor tibiae and retractor unguis muscles, while N3b contains a 'slow' excitatory axon and an 'inhibitory' axon which innervate the extensor tibiae muscle. During sinusoidal displacement of the tibia at frequencies between 1 and 10 Hz., spike potentials were recorded extracellularly from the chordotonal afferent axons in N5 and the phasic firing of the CO was accompanied by activity of the 'slow' excitatory axon in N3b. Firing of the 'slow' axon to the extensor tibiae muscle usually coincided with imposed flexion of the tibia (Text-fig. 9), although this was not always the case.

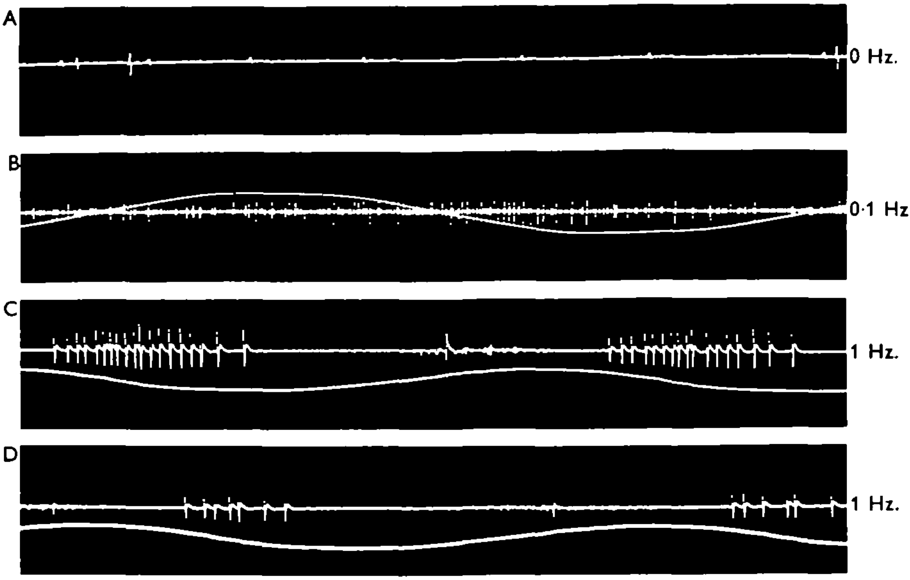
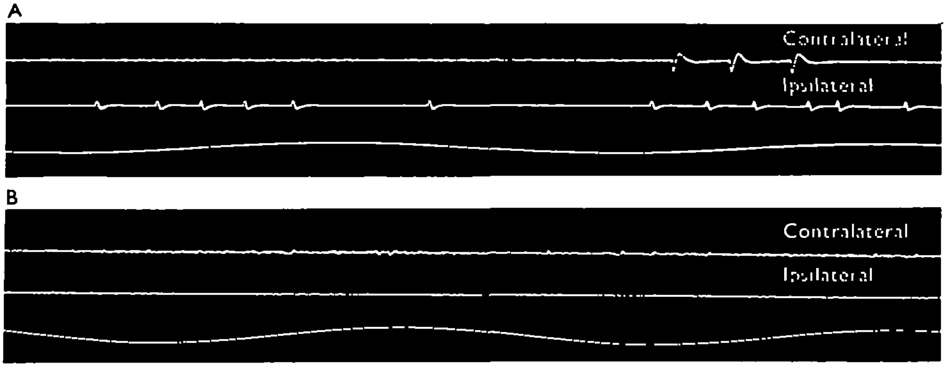


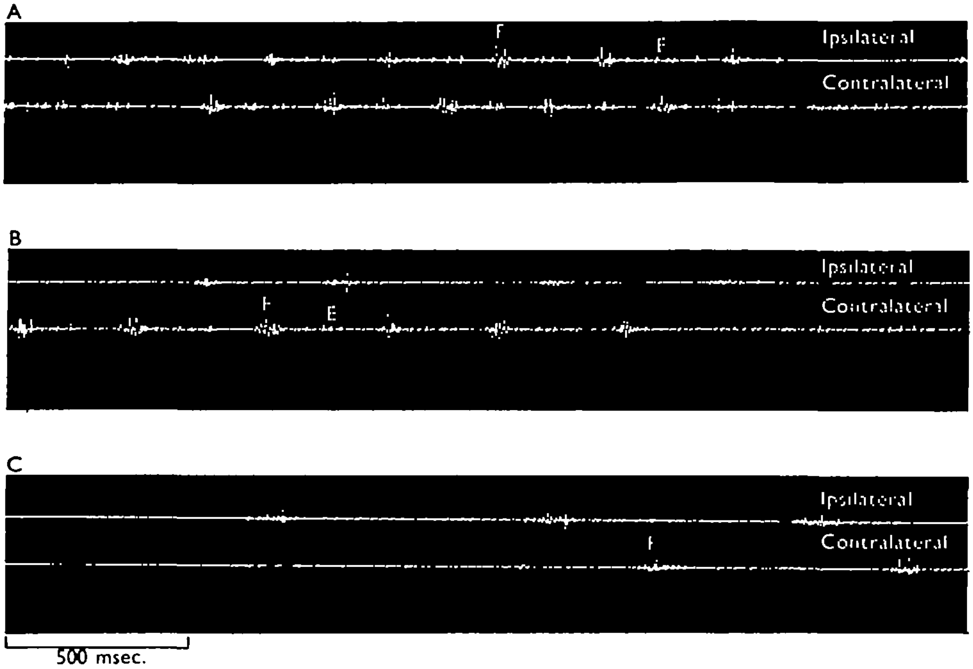
Fig. 9. Discharge of a locust chordotonal organ (A), with the tibia in a fixed position (FTA = 70°) and (B), during sinusoidal movement of the tibia at 0·1 Hz. Note in (B) different responses during flexion (up on transducer trace) and extension (down on transducer trace) of tibia. (C–D) 'slow' excitatory potentials recorded from the metathoracic extensor tibiae muscles during sinusoidal movement of the tibia (extension, up on transducer trace) at 1 Hz. The myographic electrode in the extensor muscle also recorded some activity from the flexor tibiae muscle especially during extension of the tibia. Record (D) taken about 5 min. after record (C) demonstrates waning of the flexor muscle responses with repeated stimulation of the chordotonal organ. This waning presumably represents central adaptation since the discharge of the chordotonal organ is unaltered.

The myographic electrodes in the extensor tibiae muscle also recorded some of the activity of the flexor tibiae muscle although the flexor potentials were usually much smaller than those recorded from the extensor tibiae muscle. Significantly, the flexor tibiae muscle was usually excited during extension of the tibiae. In some preparations the responses of the femoral flexor and extensor muscles were initially phasically related to the sinusoidal movement of the tibia, but then they became irregular, slowly waned and eventually disappeared. In other preparations sinusoidal movements of the tibia failed to evoke any response from the femoral muscles unless additional afferent pathways involving other receptors were excited at the same time. For example if the abdomen was touched a large burst of activity was recorded from the femoral muscles, and this activity could be modulated (frequency) by sinusoidal movements of the tibial segment, with the activity of the flexor tibiae muscle increasing during extension and the activity of the extensor tibiae muscle increasing during flexion of the tibia. In some preparations the 'slow' neurone to the extensor tibiae muscle was for some unknown reason 'spontaneously' active, and it was with these preparations that the phase relationships between CO input and 'slow' axon activity were best illustrated. The discharges of the CO never evoked any 'fast' responses from the extensor muscle unless other receptors were vigorously stimulated at the same time. When 'fast' activity did occur it could be modulated (frequency) by sinusoidal movement of the tibia. When the chordotonal nerve was transected or the CO body crushed, the 'slow' excitatory axon was not excited during sinusoidal displacement of the tibia (Text-fig. 10B). It appears therefore that, during movement of the tibia, activity of the extensor tibiae muscle is causally related to the output from the CO. There are, of course, other reflex pathways involving the extensor tibiae muscle and sense organs in the leg which remain unimpaired following transection of the chordotonal nerve or selective destruction of the CO body. For example, touching the tarsus pads of the metathoracic leg evokes firing of the 'inhibitory' and 'slow' excitatory axons and, in some cases, the 'fast' excitatory axon to the extensor tibiae muscle.



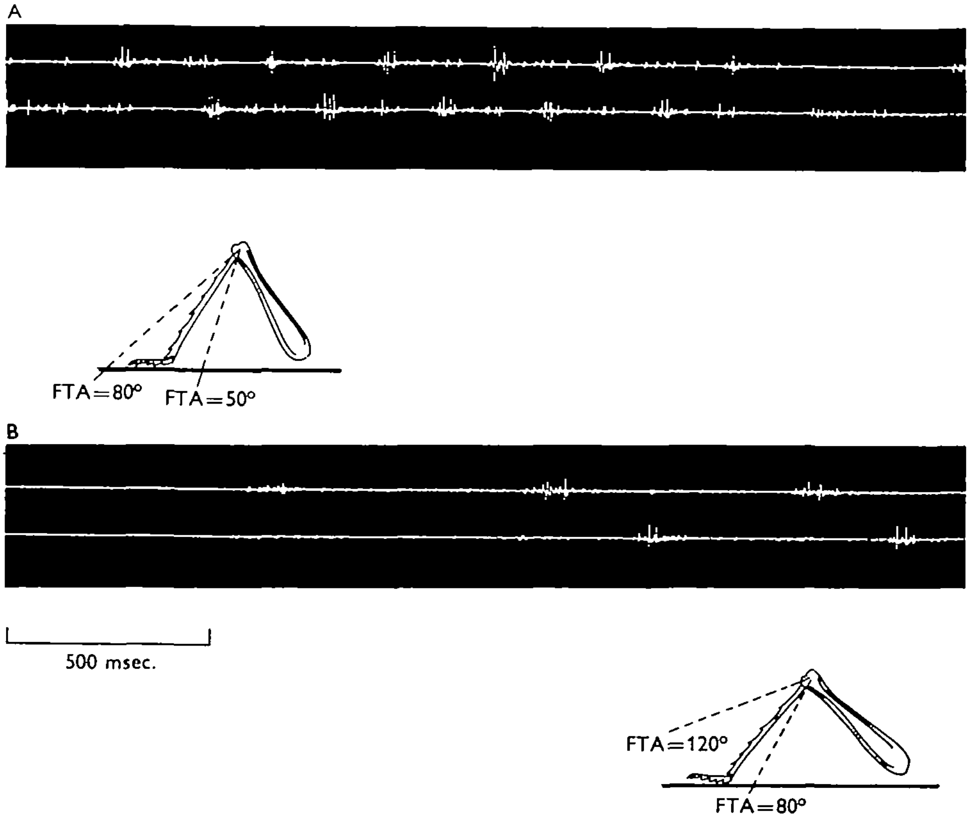
Text-fig. 10. Effects of removal of ipsilateral metathoracic chordotonal organ on the responses of ipsilateral and contralateral metathoracic extensor tibiae muscles. (A) Both ipsilateral and contralateral chordotonal organs were intact and when the ipsilateral tibia was moved sinusoidally (monitored in bottom trace) at 1 Hz. 'slow' responses were recorded from the ipsilateral extensor tibiae muscle (centre trace) during flexion (up on transducer trace). 'Slow' responses were also recorded from the contralateral extensor tibiae muscle (upper trace). However, they only occurred after some delay and they were never phasically related to the movements of the ipsilateral tibia. (B) After removal of the ipsilateral chordotonal organ sinusoidal movement of the ipsilateral tibia fails to evoke 'slow' activity in either the contralateral or ipsilateral extensor tibiae muscles although phasically related responses of the contralateral extensor tibiae muscle and phasically unrelated responses of the ipsilateral extensor muscle could still be evoked by moving the contralateral tibia.

The 'slow' axon to the metathoracic extensor tibiae muscle of the ipsilateral leg is also excited during sinusoidal movement of the contralateral metathoracic tibia (Text-fig. 10A). However, these cross-reflexes only occur in animals with a relatively high central excitatory state, and the phase relationships between ipsilateral output and contralateral input are not very clearly defined. The pattern of 'slow' axon impulses could be altered in some preparations by varying the frequency and the amplitude of the sinusoidal movement of the contralateral leg. However, the response pattern was not always predictable. When the ipsilateral tibia is moved sinusoidally



Text-fig. 11. Changes in the responses of the ipsilateral (upper traces) and contralateral (lower traces) femoral muscles following removal of the metathoracic femoral chordotonal organs. Recordings were obtained using myographic electrodes. These electrodes were implanted in the extensor muscles, but also monitored some of the flexor responses. In fact, in these records the 'fast' flexor potentials (F) were considerably greater in amplitude than the 'slow' extensor potentials (E). Normally 'slow' contractions of the ipsilateral and contralateral extensor tibiae muscles alternate with the 'fast' contractions of the flexor tibiae muscles although the responses of the ipsilateral and contralateral flexor and extensor tibiae muscles are opposite in phase (A). The very small potentials were probably from the retractor unguis muscle or trochanteral muscles. When the ipsilateral chordotonal organ was removed (B) the responses of the contralateral femoral muscles were not significantly altered during walking, but in the ipsilateral femur no extensor activity and only weak flexor activity was recorded. Photographic records of the walking insect show that stepping by the ipsilateral leg was abnormal at this time. When both ipsilateral and contralateral chordotonal organs were removed (C) the stepping frequency of the metathoracic legs fell to about one-third of the normal stepping frequency (4 steps/second) and was highly irregular at times. It is significant that little or no activity was recorded from either metathoracic extensor tibiae muscle following removal of both metathoracic chordotonal organs, and activity of the flexor tibiae muscles was weak and at times very irregular. Presumably the flexor motoneurones are still being driven, albeit irregularly, from sources other than afferents from the metathoracic femoral chordotonal organs. In fact, removal of the tarsi from the metathoracic legs makes the flexor discharges even more irregular, which suggests that afferents from the tarsi are of some importance in regulating the motoneurones to the flexor muscles.

the number of 'slow' responses of the ipsilateral extensor tibiae muscle is to some extent related to the magnitude and frequency of the tibial displacement. The extensor tibiae muscle contains both tonic and phasic muscle fibres (Usherwood, 1967). Myographic recordings from the extensor tibiae muscles of free-walking locusts and grasshoppers indicate that the muscle is under a constant but varying barrage of 'slow' motor axon discharges. The frequency of the 'slow' axon activity varies from 0 impulses/sec. to well over 150 impulses/sec. and this often occurs without any visual movement of the tibia. Concurrent photographic and neurographic-myographic recordings of the free-walking intact insect preparation suggest that the variation in 'slow' motor axon discharges could be linked with the femoral CO input to the metathoracic ganglion. Small postural adjustments of the contralateral metathoracic leg or the prothoracic and mesothoracic legs are readily detected by the ipsilateral metathoracic CO and are immediately followed by changes in the firing



Text-fig. 12. Influence of the femur-tibia angle (FTA) on walking in the partly deafferented locust. (A) Normal flexor tibiae activity (large potentials) and extensor tibiae activity (small potentials) of ipsilateral (top trace) and contralateral metathoracic legs in an intact locust. Myographic electrodes were placed in the extensor tibiae muscles. Note regular alternation of flexor (F) and extensor (E) responses. Inset: arc described by tibia during walking. N. Tibia shown in rest position (approx. 70°). (B) Ipsilateral and contralateral metathoracic femoral chordotonal organs removed. Inset: arc described by tibia during walking. Tibia shown in rest position (approx. 90°). When femur-tibia angle is less than 80° activity of the flexor muscles becomes less frequent and very irregular (see Text-fig. 11 C).

frequency of the 'slow' excitatory axon to the ipsilateral metathoracic extensor tibiae muscle (Usherwood & Runion, to be published).

The walking behaviour of the locust is significantly altered when the femoral CO is removed from one or both of the metathoracic femoral segments (Text-figs. 11, 12). It is possible to remove the CO thereby partly deafferenting the metathoracic leg without damaging the femoral muscles or their innervation and without affecting other afferent pathways in the leg. Removal of the metathoracic femoral CO alters the behaviour of the insect in several distinct ways. (1) Following the destruction of the metathoracic femoral CO, the femoral segment assumes a new angular relationship with the thorax. (2) When the leg is making contact with the substratum the FTA is about 80° compared with 60°–70° in the intact insect. Furthermore, during walking the leg moves in an arc of 80°–120° instead of 50°–80° (Text-fig. 12). (3) The frequency of stepping by the metathoracic legs is reduced from an average of 4 steps per sec. to a maximum rate of 2 steps per sec. and an average rate of 0·8 steps per sec. (4) The phase relationship between stepping of the ipsilateral and contralateral metathoracic legs is frequently irregular. (5) There is great reduction in tonic activity of the ipsilateral extensor tibiae muscle which is also reflected in reduced flexor tibiae activity in this leg during flexion. In fact, in many preparations the extensor muscle became completely inactive during walking. No stepping by the leg from which the CO has been removed occurs unless the FTA is greater than 80°. If, for example, the tibia is flexed stepping does not occur until the tibia returns to the required position, and for this to occur the tibia must be dragged back passively as the insect moves forwards.

DISCUSSION

Lissmann (1950) suggested that an insect proprioceptor is an organ which is capable of responding continuously to deformations, and provides some information concerning the relationships between parts of the body. The metathoracic femoral CO of locusts and grasshoppers has been shown to provide information on the position, velocity and direction of movement of the tibia. The CO certainly appears to be involved in the over-all control of the tonic activity of the metathoracic extensor tibiae muscle. Furthermore, phasic activity of the flexor and extensor tibiae muscles during sinusoidal movement of the tibia supports the contention that there is a relationship between CO activity and phasic activity of these muscles. The CO contains approximately twenty-four sense cells and under certain conditions many of these cells fire simultaneously although apparently they are never all active at the same time. Flexion of the tibia appears to excite a large number of these units or cells, and it is during flexion that slow responses occur in the extensor tibiae muscle. During extension, when the chordotonal nerve discharge is often smaller, there is no consistent activity of the 'slow' excitatory axon to the extensor tibiae muscle, although the flexor tibiae muscle is very active at this time. Presumably the afferent fibres from the CO which fire during flexion of the tibia are different from those that fire during extension of the tibia. The former are probably 'connected' reflexly with the motoneurones which innervate the metathoracic extensor tibiae muscle, while the latter are 'connected' reflexly with the motoneurones to the flexor tibiae muscle. There is also some evidence for cross-reflex pathways, although the detailed relationships between the ipsilateral and contralateral COs and femoral muscles have not been established so far.

Jumping behaviour in the locust involves initially activity of both metathoracic flexor tibiae and metathoracic extensor tibiae muscles. The flexor muscle is apparently used to brake the shortening of the extensor muscle until the muscle has developed sufficient tension to ensure a successful jump (Brown, 1967). It is perhaps significant that when one or both of the metathoracic COs are removed the locust can no longer jump. Presumably the CO provides the animal with information on the tonic state of the femoral muscles as well as information on the FTA, and this information is of importance in determining the magnitude and phasing of the outputs to the femoral muscles during jumping. This suggestion is fully supported by the fact that the CO has many connexions with the flexor tibiae and extensor tibiae muscles as well as a main connexion with the tibia. There is little doubt that in the absence of the femoral CO the outputs to the femoral muscles are markedly different from normal. It seems reasonable to assume, therefore, that the tonic input from the CO is of some considerable importance in setting the outputs to the femoral muscles of the ipsilateral leg and of somewhat less importance for controlling the muscles of the contralateral leg. So far there is no evidence for any intersegmental influences of the COs although these sensory structures are found in the pro- and mesothoracic femurs as well as in the metathoracic femur.

Wilson (1965) found that sinusoidal movement of the entire leg of *Periplaneta* evokes reflex firing of the leg muscles, usually the extensor tibiae muscle, and that the response is usually phasically related to the input, although not always repeated at every cycle. It is difficult to make satisfactory comparisons of his results with those presented in this paper since he reflexly activated the leg muscles by moving the entire leg and presumably excited more receptors than just the femoral CO. Nevertheless, one significant difference between the locust and cockroach systems is that cross-reflexes appear to be much stronger in the cockroach. Here sinusoidal movement of the leg produces reflex activation of the contralateral extensor tibiae muscle opposite in phase to that found for the ipsilateral extensor tibiae muscle. In the locust the activity of the contralateral metathoracic extensor tibiae muscle bears little or no phase relationship to the input to the ipsilateral CO. However, perhaps movement of the entire locust leg instead of just the tibia would evoke phasically related responses of the contralateral muscles similar to those seen in the cockroach.

It is perhaps significant that removal of both metathoracic femoral COs completely uncouples movement of the metathoracic legs on the one hand and the mesothoracic and prothoracic legs on the other. The metathoracic legs step at a lower frequency, and the movements of these appendages bear no apparent phase relationships with those of the anterior legs. However, the metathoracic legs are driven to some extent by commands from more anterior segments since section of the longitudinal connectives between the mesothoracic and metathoracic ganglion completely abolishes stepping of the metathoracic legs. It would be unwise at this stage to speculate on the relative merits of peripheral and central control of insect walking merely on the basis of the small amount of data presented herein, but it does appear at least superficially that peripheral mechanisms are more important for control of walking in locusts than they appear to be for control of locust flight (Wilson, 1966). One way of assessing the importance of the CO in controlling and co-ordinating the movements of the femoral muscles would be to determine the effects on the output to these muscles of electrical stimulation of the chordotonal nerve after removal of the CO.

The fact that in many preparations sinusoidal movement of the tibia failed to excite the motoneurones to the femoral muscles unless other sensory pathways were activated at the same time is of some interest. Presumably reflex activation of the femoral muscles, through CO stimulation will only occur provided the excitatory states of either the femoral motoneurones or the interneurones which possibly couple the afferents from the CO with the femoral motoneurones are above a certain minimum level. During walking the excitatory state of motoneurones can be expected to be very high and any movements of the tibia can be expected to be followed by contractions of the femoral muscles: the extensor tibiae muscle contracting following flexion and the flexor tibiae muscle contracting following extension of the tibia. The phasic coupling between the CO input and the output to the femoral muscles is most 'efficient' when the tibia is moved sinusoidally at between 2 and 4 Hz., i.e. the normal frequency observed during walking. It seems reasonable, therefore, to assume that the CO plays a very important part in controlling and co-ordinating the activities of the femoral muscles during walking. Information from the CO may only reinforce centrally determined impulse sequences to the femoral muscles during walking, although there is a distinct possibility that the CO plays a more significant role than this.

SUMMARY

1. The structural and physiological properties of a chordotonal organ found in the metathoracic femoral segments of the locust and grasshopper are described.

2. Recordings from the afferent fibres of this mechanoreceptor reveal two patterns of activity: a static or tonic discharge and a dynamic or phasic, rapidly adapting discharge. The frequency of the tonic discharge varies with the femur-tibia angle, while the phasic discharge occurs in response to angular movements of the tibia of $> 3'$. The frequency of the phasic response is related to the velocity of tibial displacement. The responses recorded during flexion of the tibia are different from those recorded during extension.

3. Removal of either of the metathoracic chordotonal organs produces significant changes in walking and postural behaviour.

4. Leg reflexes involving the femoral chordotonal organ and femoral muscles appear to play a very significant role during postural and walking activity.

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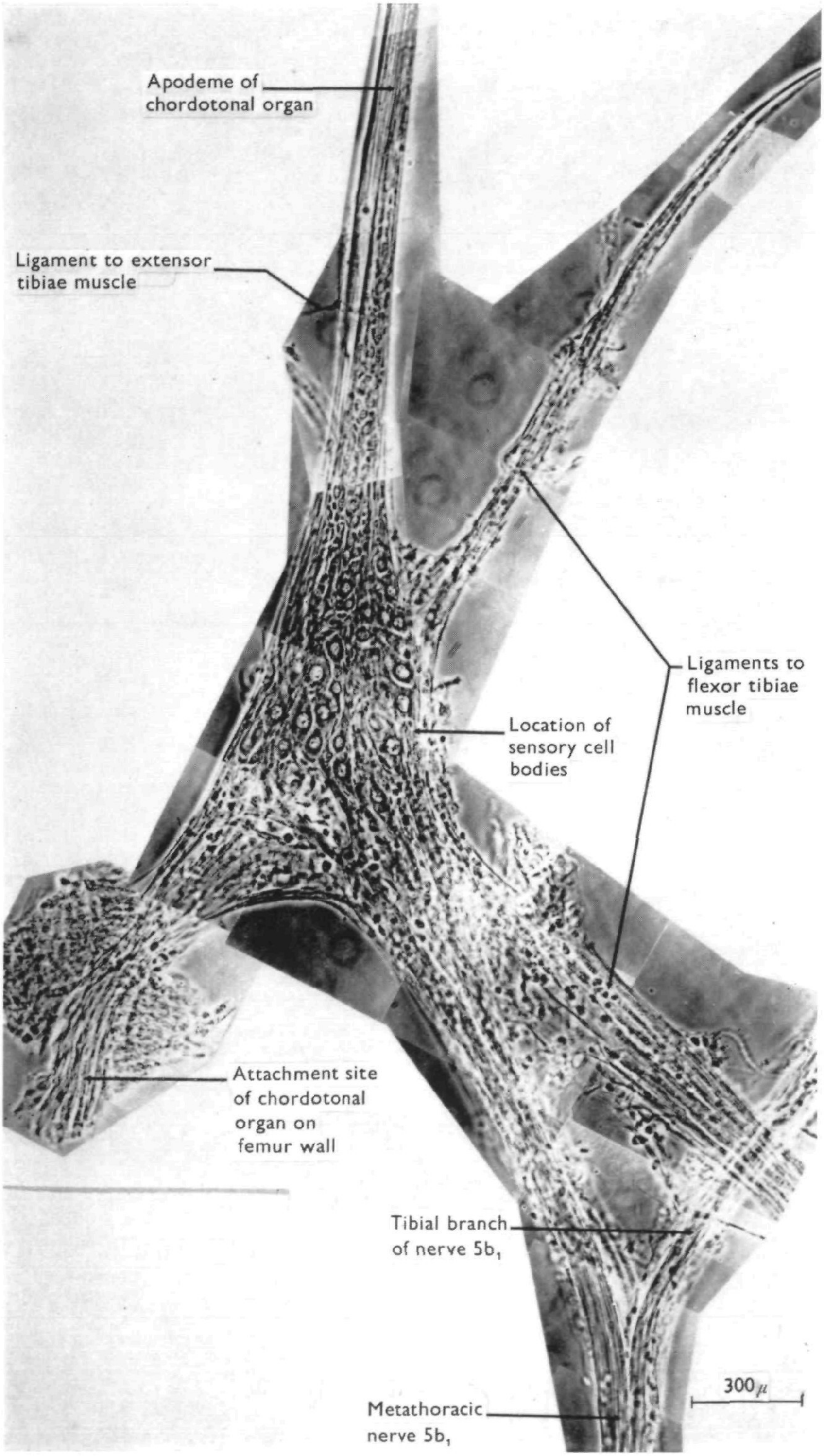

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EXPLANATION OF PLATE

This photograph of the almost complete organ shown in Text-fig. 3 was reconstructed from a large number of phase contrast micrographs of various parts of a whole mount of a locust chordotonal organ. See text for further explanation.