THE CONTROL OF WALKING IN ORTHOPTERA

II. MOTOR NEURONE ACTIVITY IN NORMAL FREE-WALKING ANIMALS

BY M. D. BURNS AND P. N. R. USHERWOOD*

Department of Zoology, University of Glasgow, Glasgow G12 8QQ

(Received 12 June 1978)

SUMMARY

A brief description is given of the anatomy, innervation and mechanical properties of the extensor tibiae muscles of the locust. Each is innervated by a 'fast' (FETi) and 'slow' (SETi) excitatory axon, one branch of a common inhibitor (CI) and a fourth small axon (DUMETi). The prothoracic and mesothoracic extensors contract more rapidly than the metathoracic muscle but exhibit a stronger 'catch', which can be relaxed by CI or FETi activity.

Records were made of electrical activity in the extensor motor nerves in all the legs of locusts and lubber grasshoppers. During standing only the SETi axons were active. During straight line walking in the locust all three motor axons were active, except in the metathoracic leg. A detailed description of the activity pattern of each axon is given. The activity in the grasshopper was similar, but rather more variable. Measurements were made of the mechanical responses of the extensor muscles to these patterns of activity. As walking speeds increased the response to SETi activity approached a constant tension or muscle length which could be strongly modulated by the phasic contractions due to FETi activity. It is suggested that the timing of SETi activity is only important at low walking speeds, and that at high speeds it simply provides a return force for the flexor muscle. The CI produces a slow effect and fires at the wrong time in the step to phasically relax the prothoracic or mesothoracic extensors, so it is suggested that its main role is in the relaxation of coxal muscles. The sources of motor neurone activity are discussed.

INTRODUCTION

Investigations of the neural mechanisms underlying the control and coordination of muscles in the legs of walking insects have used two main approaches. The first has been to work with fixed, dissected preparations in which leg reflexes (Wilson, 1965; Delcomyn, 1971) or the generation of motor patterns by the central nervous system (Pearson & Iles, 1970; Hoyle & Burrows, 1973; Burrows & Horridge, 1974) are studied. Such experiments are open to the criticism that the results obtained may not be relevant to the controlling mechanisms which operate during walking,

* Present address: Department of Zoology, University of Nottingham, Nottingham NG7 2RD.
since the conditions in the preparation are very different from those existing in the walking insect. This problem has been at least partially overcome in the second approach, which is to record electrical activity from restrained preparations walking on a 'free' surface (e.g. Wendler, 1966; Delcomyn, 1973) or from unrestrained walking insects (e.g. Usherwood, Runion & Campbell, 1968; Pearson, 1972). Since most of the restrained animals are clearly still subject to unusual conditions (e.g. a stick insect walking on a wheel cannot move sideways, a cockroach suspending a ball experiences reversed forces on its legs), it is desirable to use free-walking preparations.

The simplest way of obtaining information from a free animal is to record electromyograms (Pearson, 1972; Delcomyn & Usherwood, 1973), but only neurograms can supply accurate details of the activities of individual motor neurones. Neurograms can be obtained from peripheral nerves in the free-walking locust (Runion & Usherwood, 1966; Burns & Delcomyn, 1970) and here we examine the motor patterns involved in walking of the locust and grasshopper by recording from all the extensor tibiae motor neurones. These neurones were chosen because their axons are accessible, because there are only three of them for each muscle and because the physiology and control of the metathoracic extensor muscle have already received considerable attention.

MATERIALS AND METHODS

The animals used in this work were *Schistocerca gregaria* and *Romalea microptera*. Free-walking preparations

The recording technique and apparatus used have been described in a previous paper (Burns, 1973). In the experiments reported here each leg being monitored carried a second identical pair of recording wires. The cleaned ends of these wires were pushed into the femur of the leg through two small holes punched ventral to one of the insertions of the extensor tibiae muscle. Each pair of wires was adjusted so that the tips lay sufficiently near to the nerve to record neural activity clearly, and lay close enough together to prevent muscle potentials from swamping the nerve record (see Fig. 1B). Up to three legs could be monitored at one time. The records of nerve activity obtained by this method were useable for about 6 h, although after 2 h the noise level was usually significantly higher. All the records which were analysed were obtained within 2 h of electrode insertion.

Static preparations

In these experiments the locust was fixed on its back with the leg under investigation immersed in locust saline (Usherwood & Grundfest, 1965). For intracellular recording of muscle potentials the ventral cuticle of the femur, the flexor tibiae muscle and the retractor unguis muscle were successively removed to expose the extensor tibiae muscle. The femur–tibia joint was disarticulated and the apodeme of the extensor muscle was attached to a ‘Pixie’ silicon strain-guage transducer (compliance 0·05 mm/g), stretching the muscle by about half its maximum natural extension and enabling its tension to be monitored. Responses to spontaneous activity of motor neurones and to stimulation of nerves 3 and 5 in the thorax were recorded.
with glass microelectrodes filled with \(3 \text{ M-KCl}\). Some records of activity in the motor nerve to the extensor muscle were also obtained with plastic suction electrodes.

A similar procedure was used to record muscle tension alone but in this case the femur was not opened and wire electrodes were used to monitor the motor nerve potentials. A method similar to that described by Usherwood & Runion (1970) was used to record the mechanical responses of the extensor tibiae muscles to the motor neurone activity patterns used in walking. The times of occurrence of the spikes in each motor neurone were measured on recordings obtained from a walking locust and applied to a kymograph drum in the form of black marks on a reflective strip, one mark for each spike. These marks were then 'read' by two photosensors which triggered stimulators connected to electrodes arranged so that they activated the appropriate axons (Burns, 1974). Isometric tension developed by the muscle was monitored as before, while isotonic tibial movements were measured with a long glass whisker (compliance 4.5 mm/g) attached to the transducer.

### Analysis of records

Typical records of activity in the motor nerves to the extensor tibiae muscles of the legs of walking locust are shown in Fig. 3. Such records were not analysed unless the potentials from the muscles were small and spikes from three axons could be easily distinguished. Identifications of spikes were based on spike amplitude and the size and shape of the following muscle potential (non-existent for inhibitory spikes). The identifications were confirmed in some experiments by making intracellular recordings from the muscle fibres after recording nerve activity in the free-walking animal. All of the 'fast' spikes were probably recognized, while about 5% of the 'slow' and 10% of the inhibitory spikes were probably missed due to the high levels of activity in other axons. The activities of some of the flexor tibiae muscle units were also picked up and served as an indication of the level and timing of flexor activity. However, it is possible that some of these potentials may have originated in the retractor unguis muscle.

Measurements of the time of occurrence of each motor spike (±0.5 ms) were made from oscilloscope records and analysed with a digital computer. Each step made by a leg during walking was divided into protraction and retraction (defined in Burns, 1973). Each spike occurring in protraction was represented by a point whose abscissa was the phase of the spike in the protraction period and whose ordinate was the instantaneous frequency of the spike (e.g. Fig. 4). The spikes occurring in retraction were dealt with in a similar way and plotted separately. Retraction was shown following protraction, but the order is arbitrary and each plot should really be visualized as a cylinder on which the end of retraction and the start of protraction are continuous. Since retraction in the locust was on average twice as long as protraction (Burns, 1973) the horizontal scale used for the protraction part of each plot was half that used for the retraction part, so minimizing distortions of the temporal patterning of spikes.

Since these activity plots were constructed to average activity over a number of steps, individual data points are not important. The features which are of interest are those concerned with the main concentrations of data points such as average height (frequency), horizontal position (phase), scatter, rate of rise of frequency and the phase of frequency maxima.
RESULTS

Anatomy and innervation of locust extensor tibiae muscles

The metathoracic extensor tibiae muscle of the locust and its innervation have received considerable attention (Hoyle, 1955a, b, 1978; Usherwood & Grundfest, 1965; Cochrane, Elder & Usherwood, 1972) and will not be further described here. However, the remaining extensor tibiae muscles have not been previously examined in sufficient detail to provide a basis for an analysis of the motor activity occurring in locomotion.

The femoral segments of the prothoracic and mesothoracic legs of *Schistocerca gregaria* are almost identical in structure. The mesothoracic leg is about 1.2 times the size of the prothoracic leg. The ratios of force at the tarsus to tension in the extensor tibiae apodeme (mechanical disadvantage) are 1/6 for the prothoracic and 1/15 for the mesothoracic legs when the femur-tibia angle is 90°.

The equivalent ratio for the metathoracic leg is 1/35 (Heitler, 1974). During the movements of the tibia which occur in walking (Burns, 1973) these ratios give normal movements of the extensor apodemes of 1.0, 0.7 and 0.4 mm for the prothoracic, mesothoracic and metathoracic legs, shortening the muscles by 13, 7 and 2% respectively.

The prothoracic and mesothoracic extensor tibiae muscles lie under the dorsal femoral cuticle, are pinnate in form and are composed of a number of muscle units (Hoyle, 1955a). These units are attached to the cuticle in an anterior–dorsal row of insertions, a posterior row of elongated insertions very close to those of the flexor
tibiae muscle Fig. 1A), and a single dorsal proximal insertion. The numbers and relative sizes of the insertions vary considerably between locusts.

Each extensor tibiae muscle is supplied by nerve 3Bc + 5Bd (Campbell, 1961), which runs across the ventral face of the muscle (Fig. 1) sending fine branches down between the muscle units. The nerve goes no further than the most distal extensor units except in the metathoracic leg where one branch continues to innervate the accessory extensor muscle. E.M. sections of the nerve in each leg show that it always contains four axons. The three largest have been identified physiologically as the 'fast' (FETi) and 'slow' (SETi) excitatory axons and the inhibitor (CI) in descending size order (nomenclature from Hoyle & Burrows, 1973a). The fourth axon produces a spike only 6% of the amplitude of the CI so its activity could not be recorded with implanted wire electrodes and no evidence was obtained about its function in walking. It has been identified as the axon of a dorsal unpaired median neurone (DUMETi) and neurosecretory and inhibitory functions have been suggested for it (Hoyle, Dagan & Colquhoun, 1974; Hoyle, 1974; Evans & O'Shea, 1977). All four axons taper progressively towards the distal end of the femur, but the FETi reduces in size more rapidly than the others so that it is proximally nearly twice the diameter of the SETi and at the distal end of the nerve it is about the same size as the SETi axon. The ratio of CI to SETi diameters is constant at 0.2 in the metathoracic and at 0.6 in the other legs.

Experiments in which the leg nerves were stimulated in the thorax confirm Hoyle's (1957) description of the route taken by the FETi and SETi axons in the proximal nerves. Thus in the prothoracic and mesothoracic legs the SETi runs in nerve 5 and the FETi in nerve 3B, the reverse of the metathoracic arrangement (Fig. 2). The inhibitory axon in the metathorax is known to be a common inhibitor with one branch in nerve 3C innervating the anterior coxal adductor muscle and branches in nerves 3A, 4, 5 and anterior ipsilateral connectives (Pearson & Bergman, 1969; Burrows, 1973). Stimulation experiments and peripheral filling with cobalt chloride have shown that the prothoracic and mesothoracic inhibitors have axonal branches in nerves 3A, 3C, 4 and 5 (Fig. 2).

Usherwood & Grundfest (1965) found that in the metathoracic extensor tibiae muscle of the locust the FETi innervates about 80% of the muscle fibres while the SETi innervates only 20–30% of the fibres, mostly at the proximal end of the muscle. Hoyle (1978) has now shown that the endings of the two axons are fairly uniformly distributed through the muscle. CI innervation is restricted to fibres receiving SETi endings. Hoyle (1957) states that in the other legs of the locust the FETi innervates the whole muscle, while the SETi supplies 40–50% of the more proximal fibres. However, intracellular recordings made from the prothoracic and mesothoracic extensor tibiae muscles indicate a different pattern. The muscles are arranged with most of the FETi endings at the proximal end, unlike the situation in the metathoracic leg. Thus about 80% of the muscle fibres examined at the proximal end of the muscle received FETi endings, reducing to about 30% at the distal end of the muscle. The extremes of the distribution were the dorsal–proximal muscle unit (P in Fig. 1B), which was the most phasic, with SETi endings only at its distal end, and the most distal units (D in Fig. 1B) which received large numbers of SETi and CI endings. The gradation of muscle fibre character was reflected by the FETi EPSP which often
had an amplitude and a time course similar to the SETi EPSP in the distal muscle fibres but which was larger and of shorter duration in the more proximal fibres. IPSPs were always hyperpolarizing potentials and in the distal muscle fibres they were often equal in amplitude to the SETi EPSPs. Similar findings have recently been reported by Wilson (1978b). A number of fibres were innervated by all three axons, but no fibres were encountered which received endings from the FETi and CI neurones only. Further support for this picture of the innervation pattern was provided by records of extracellular potentials and by examination of the extent of muscle degeneration following proximal nerve section.

The mesothoracic extensor tibiae muscle appeared to have a rather higher proportion of its fibres innervated by the SETi and the CI than did the prothoracic muscle, but was otherwise similar.

Mechanical properties of locust extensor tibiae muscles

Only a brief account of the mechanical properties of the prothoracic and mesothoracic extensor tibiae muscles will be given here. A more detailed report appears in another paper (Burns & Usherwood, 1978). In both muscles single stimuli to the FETi or the SETi gave rise to discrete tension twitches at the apodeme, but the SETi
induced twitch was too weak to overcome friction in the femur–tibia joint and produced no tibial movement. The rise times of the tension in both muscles were shorter than in their metathoracic counterpart (Cochrane et al. 1972; Hoyle, 1978) and the responses to FETi spikes were even more rapid than in the metathoracic retractor unguis muscle.

Stimulation of the FETi or the SETi at higher frequencies gave rise to sustained tetanic tension. The tetanic response to the FETi fatigued fairly rapidly (typically 5–10 s), but the response to the SETi showed no signs of fatigue until after 30 s of stimulation. The development of tension in response to high-frequency SETi activity was very slow with half rise and fall times of about 0.3 s at a stimulation frequency of 100 Hz. This is, of course, much longer than the step period of a rapidly walking locust, but might be shorter when the muscle is bathed in haemolymph rather than saline.

Both prothoracic and mesothoracic muscles displayed a 'catch effect' in response to high-frequency SETi activity similar to that seen in Crustacea (Blaschko, Cattel & Kahn, 1931). This effect appeared when a brief high-frequency burst of stimuli to the SETi was added to low-frequency stimulation and took the form of a slowly decaying tension following the burst which was much greater than could be developed in response to the low frequency activity alone. The muscle relaxed as soon as the low-frequency stimulation ceased and could also be considerably relaxed by activity in the CI or in the FETi (Burns & Usherwood, 1978). The phasic contraction resulting from a burst of FETi spikes produced a tension increase, but it was followed by a relaxation in the SETi-induced tension. This relaxing effect of the FETi may not be so marked under isotonic conditions, but it means that tensions produced by a combination of FETi and SETi activity depend in a complex way on the relative frequencies and time courses of the activities in the two axons and cannot easily be predicted from records of neuronal activity.

Activity in the CI had three effects on tension due to the SETi; a reduction in the rate of rise of tension, a rather slow fall of tetanic tension (half-time of relaxation typically 0.5 s) and a marked acceleration of relaxation when SETi stimulation ceased. The effectiveness of the inhibition increased with the frequency of firing in the CI and decreased with the frequency in the SETi. CI activity had no effect on the 'fast' twitch.

Motor-neurone activity in the standing locust

The activities of the metathoracic extensor tibiae motor neurones in a stationary locust have been described by Runion & Usherwood (1968). They found that both the SETi and the CI were continuously active at a fluctuating level which appeared to be correlated with the frequency of afferent impulses from the tarsus.

In the present work the postural activities of the prothoracic and mesothoracic motor neurones were briefly examined. In both legs the CI and the FETi appeared to be silent when the locust was not moving, but the SETi was continuously active at a low frequency (average 5–30 impulses/s (ips)) which fluctuated with the respiratory movements of the abdomen. The average firing frequency of any of the SETi neurons appeared to depend on three factors: (a) the position of the leg, (b) the stance of the animal (height and angle at which the body was held), and (c) the rigidity of the
Fig. 3. Activity recorded from the femoral nerves in the prothoracic, mesothoracic and metathoracic legs of different walking locusts. Typical spikes from each of the three axons in the extensor tibiae motor nerve are marked in extensor nerve records (N). In the step traces (S) the heavy bars represent protractions (foot off the ground). Also shown are: activity from the tarsal sensory nerve (T) and the frame times of the cine camera filming the locust (F).

Leg (level of simultaneous activity in antagonistic muscles). Loading of the animal was not an important factor since placing weights (up to 2 g) on the back of a locust produced no discernible increase in SETi activity. If the surface on which the locust was standing was inclined steeply at right angles to the body axis, the activities of the flexor tibiae muscles were affected but those of the SETi axons were not.

If the locust made peering movements (Wallace, 1959) so that its thorax swayed from side to side, the activities in the flexor tibiae muscles and the SETi neurones oscillated up and down in antiphase. Thus a movement to the right was caused by a rise in the firing frequency of the left SETi neurones and the right flexors with a concomitant fall in the activity of their antagonists. The CIs were also occasionally active during this activity, but the frequency of firing was the same on both sides of the insect.

The CI neurones could be activated in the standing locust by lightly brushing the tarsi or by strong vibrations in the surface on which the locust was standing. Thus the CI probably receives inputs from both tarsal receptors and the subgenual organ. The SETi responded similarly but has a higher threshold. The FETi was active only when the locust began to walk.

**Motor-neurone activity in the walking locust**

Typical records obtained from the extensor motor nerve in one femur of each of the three pairs of legs of a walking locust are shown in Fig. 3. These records show clearly the patterns of motor-neurone activity during a single step of each leg. How-
ever, to present the standard motor-neurone behaviour during walking, the activity

data from about ten steps have been measured from filmed oscilloscope records and

averaged by plotting as described above. For each leg analyses of walking records

from about ten locusts have been made and any differences found between animals

are described.

Prothoracic leg. At the end of retraction the prothoracic femur is almost perpen-
dicular to the body. As it swings forwards in protraction the tibia is extended so

that the tarsus is put down well in front of the head (Burns, 1973). Thus one might

expect that the extensor tibiae muscle would be active during protraction and the

flexor tibiae muscle active in retraction. This was found, but the activity patterns

showed some unexpected features. For example, the flexor muscle activity (Fig. 4D)

began before the foot was placed on the ground and instead of continuing right through

retraction it ceased at a phase of about 0-3 (i.e. after three-tenths of the retraction
time).

The extensor motor neurones were most active during protraction but were not

silent during retraction. The FETi produced only a few potentials during retraction

but produced a large burst of spikes during protraction with a clearly defined biphasic

pattern (Fig. 4A). The highest frequency occurred in the first period of FETi activity

(up to a protraction phase of 0-3); this was followed by a slight reduction in frequency

and the firing frequency then rose to a second peak (protraction phase 0-5–0-7).

The movements of the tibia often reflected the biphasic FETi activity pattern by

resuming extension as the tarsus was being lowered to the surface.

Unlike the FETi, the SETi was almost equally active during protraction and

retraction (Fig. 4B). The distribution of SETi potentials during protraction was very

similar to that of the FETi but, at least at high walking speeds, the firing frequency

of the SETi was always lower and more constant. During retraction the firing pattern

of the SETi became more variable (a greater scatter of points in Fig. 4B) with about

the same maximum frequency as in protraction, but a lower minimum frequency.

The SETi activity usually started as soon as the foot was placed on the substrate and

ceased at a retraction phase of about 0-6. Thus the SETi was firing at a high frequency

while the flexor tibiae muscle was active (Fig. 4D). The tibia was being flexed through-

out retraction, suggesting that the flexor muscle, being larger, could overcome the

tension in the tonic fibres of the extensor muscle. The function of the extensor

muscle at this time may only be to assist in maintaining posture by increasing the

rigidity of the leg.

The few FETi potentials which occurred during retraction appeared to be used to

reinforce excitation due to the SETi and increased in number at high walking speeds.

It was expected that the CI neurone would become active just before or during

the flexor muscle activity, but this was not found to be the case. The CI fired at a low

frequency through each retraction (Fig. 4C) and its firing frequency rose consider-

ably after a retraction phase of about 0-5, as the activity of the SETi declined. The

high level of CI activity at this time in the step suggests that it is designed to accelerate

the relaxation of the tonic extensor tibiae muscle fibres, but it is not clear why this

should be necessary when the same fibres are reactivated almost immediately at the

start of the next protraction.

Mesothoracic leg. The protraction movements of this leg never bring it further
Fig. 4. Locust prothoracic leg. Typical patterns of spike activity in the three extensor tibiae motor neurones (A–C) and in the flexor tibiae muscle (D) during 10 steps of straight-line walking at 4.7 steps/s. (A–C) Plots of instantaneous frequency against phase constructed as described in the text. Numbers on the plots indicate the number of superimposed data points. Most of the CI potentials occurring in protraction in C were not recorded. (D) Histogram of the average number of cross-talk potentials from the flexor muscle in different parts of the step. In some locusts the biphasic nature of the activity during protraction was even more obvious.
Forward than a position perpendicular to the body (Burns, 1973) and so it can only contribute propulsive force by swinging the coxa backwards and by extending the tibia towards the end of retraction. The tibia thus flexes in protraction and extends during retraction, the reverse of the sequence in the prothoracic leg. The flexor tibiae muscle therefore might be expected to act during protraction and the extensor tibiae
during retraction, where it would assist in propulsion. In fact the flexor muscle did not become active until about half-way through protraction (at a phase of about 0.4) and its activity continued into retraction (Fig. 5D). This timing means that all the tibial flexion occurred in a short period just before the foot was put down. Since the tarsal claws could be seen to flex when the tarsus touched the surface, some of the muscle potentials recorded during retraction may have originated in the retractor unguis muscle. The remainder probably represented the contribution of the flexor tibiae muscle to postural support.

The activity in the SETi also differed from the expected pattern in that the SETi often started to fire before the end of protraction (at a phase of about 0.9) and reached a maximum frequency just before the foot was placed on the substrate (Fig. 5B). This initial activity was relatively stereotyped and constant and merged into a period of more variable, lower-frequency activity during retraction (retraction phase of 0.2-0.8). In a few animals the SETi was also active at a low frequency throughout protraction. The activity of the SETi rarely continued until the end of protraction and so it seems unlikely that it contributed to propulsion.

Most of the activity in the mesothoracic FETi was restricted to the end of retraction (Fig. 5A) when the frequency of firing in the SETi was declining. Maximum firing frequency in the FETi occurred at a retraction phase of 0.8-0.9 depending on walking speed. In some animals the FETi was also recruited to assist the SETi at the start of retraction and, although it usually produced only a single spike at this time, this was sufficient to generate considerable tension (see page 88). The timing of this activity was unusual since it coincided with the end of the activity in the flexor tibiae muscle, but it may function to brake over-rapid flexion movements.

The timing of CI activity in the mesothoracic leg was what one would expect if the role of the axon is to relax the tonic extensor tibiae muscle fibres at the end of each step. During protraction and throughout most of retraction the CI fired at a low frequency (Fig. 5C). At the end of retraction the frequency rose, probably relaxing the extensor muscle fibres prior to the next tibial flexion. This burst of CI potentials was almost as well defined as that of the FETi, with its maximum frequency occurring a little earlier at a retraction phase of 0.7 to 0.8. However, the maximum firing frequency was never as high as that in either excitatory axon. The abrupt termination of the CI activity just before the FETi burst may have been an artifact resulting from the swamping of CI spikes by high frequency FETi activity.

**Metathoracic leg.** Since the metathoracic leg is held almost parallel to the body in walking, flexion and extension of the tibia correspond to protraction and retraction respectively and the extensor tibiae muscle must provide considerable propulsive force. Activity in the flexor tibiae muscle was confined almost completely to protraction, but started a little before the end of retraction (Fig. 6C). The early start to flexor muscle activity is a result of the way in which protraction was defined. Since protraction was judged to begin when the tibia stopped extending (when the tarsus started to move relative to the surface), the flexor muscle activity must have already commenced in order to arrest tibial extension.

Of the two metathoracic excitatory extensor motor neurones, only the SETi is used in walking. It usually fired 3-10 times between a protraction phase of 0 (just before the foot was put down) and a retraction phase of about 0.6 (Fig. 6A).
Like the burst of SETi activity during protraction in the prothoracic leg, the metathoracic SETi activity appeared to be biphasic, starting abruptly at a fairly high frequency and continuing beyond a retraction phase of about 0.2 with a lower frequency, more variable pattern. The large size of the metathoracic extensor muscle and its slow tension development (Usherwood & Runion, 1970; Cochrane et al. 1972) mean that relatively few potentials early in retraction were able to produce a force sufficient for propulsion. Some animals produced only 2–3 spikes per step whereas others showed a great deal more activity spread out over a longer period of retraction (e.g. Fig. 6).

Runion & Usherwood (1968) reported that the metathoracic CI produced 3–4 spikes per step just prior to tibial flexion, but the present studies indicated a higher level of inhibitory activity. Fig. 6B shows the pattern in an animal whose CI started fire at a retraction phase of 0.3, reached maximum frequency between 0.6 and 0.8 and then continued at a low frequency through protraction. This is the pattern one
might expect if the function of the CI was to accelerate the relaxation of the tonic extensor muscle fibres following SETi activity and then to assist flexion by reducing the remaining extensor tension. The discrepancies between these results and those reported by Runion & Usherwood (1968) may have arisen because they recorded neural activity in the thorax (Runion & Usherwood, 1966) and so may have missed some of the smaller potentials and because of their method of analysis which did not involve multi-step averaging.

**Motor-neurone activity in the grasshopper**

Activity patterns of the extensor tibiae motor neurones of the prothoracic and mesothoracic legs of the grasshopper were analysed for comparison with the locust. Only three animals were investigated and it was not found to be possible to monitor the activities of the CI neurones.

The motor-neurone activity patterns during walking are shown in Fig. 7. They are very similar to those of the locust except that the SETi is more active than the FETi, probably because the greater weight of the grasshopper necessitates a larger contribution to postural support. The lower SETi activity in the mesothoracic than the prothoracic leg may be due to the gait used by the grasshopper (Burns, 1973) which does not rely as heavily on support from the mesothoracic leg as does the alternating tripod gait of the locust.

The activity of the SETi in posture in the prothoracic and mesothoracic legs of the standing grasshopper appeared to be similar to that of the locust and was not examined in detail. However, *Romalea* does display one behaviour pattern besides walking which is of interest and which I shall call ‘jerking’. This is equivalent to the defensive kick of the locust (Runion, 1968) and occurs in an unreceptive female when a male attempts to mount her or is placed on top of her. ‘Jerking’ takes the form of a series of simultaneous depressions of all the legs causing the insect to move up and down 2–3 times/s. This continues until a short time after the male grasshopper loses contact with the female. The ‘jerking’ behaviour is of interest because it involves almost simultaneous bursts of potentials in the FETi and SETi axons in all the legs, a situation which otherwise never occurs. The bursts of potentials in the different legs occur up to 10 ms apart with no fixed sequence, suggesting that the connections between the neurones in different segments are polysynaptic.

Examination of the bursts of motor-neurone activity showed that each contained 3–10 FETi spikes with an average frequency of about 300 i.p.s. During each burst the frequency of firing in the SETi rose above its ‘postural’ level. The rise in the frequency of SETi activity during each ‘jerk’ remained as the intensity of the behaviour declined, but the FETi spikes disappeared, suggesting that the FETi was recruited to assist the SETi neurone. Since no rise in SETi spike frequency could be seen in each burst before the first FETi potential occurred, the burst of activity must have been initiated by a very rapid rise in central excitation.

**Analysis of locust motor-neurone activity parameters**

The method used in this paper for plotting the activity patterns of motor neurones tends to conceal the effects of factors which are not phasically related to the two part.
Control of walking in Orthoptera

Fig. 7. Grasshopper. Typical patterns of spike activity in two of the extensor tibiae motor neurones in the prothoracic (A, B) and mesothoracic (C, D) legs during 6 steps of straight-line walking at 1-3 steps/s. Details as for Fig. 4. The horizontal lines in A and C show the approximate timing of flexor tibiae muscle activity. For comparison with the locust plots the number of points should be multiplied by about 2.

of each step. The most important of these factors is likely to be the speed of walking of the locust, which is a major factor in determining the tension produced by the extensor tibiae muscles (see next section). Large fluctuations in speed are common during even short periods of walking and may well account for some of the apparent differences in motor-neurone behaviour between animals. However, other factors may also have strong influences on motor-neurone activity; two obvious candidates
Fig. 8. Two examples of locust prothoracic motor neurone activity plotted against step duration. The regression coefficients (r) and Student’s t (t) are shown. (A) Number of FETi spikes per protraction against protraction duration; 19 points, not significant. (B) Mean firing frequency of the SETi in retraction against retraction duration; 19 points, significant.

being the relative timing of the movements of other legs and the activity of other motor-neurones of the same leg.

Some of the effects of changing walking speed can be shown by comparing activity plots for different walking speeds. However, the effects are more easily detected graphically and statistically by determining the strength of the relationship between motor neurone spike parameters (e.g. mean frequency, mean phase, number/step) and the durations of protraction or retraction, a method similar to that used by Davis (1969) for the burst characteristics of lobster swimmeret motor neurones. Each analysis was carried out on data from a single animal, since combination of data from different animals led to a masking of any relationship by the variation between animals. The statistical results are given in Table 1 while two typical graphs are shown in Fig. 8.

Prothoracic motor neurones

There is only a very weak correlation between walking speed and burst length or mean phase of the FETi and SETi activity in protraction. This implies that the pro-
Control of walking in Orthoptera

Table 1. Coefficients of correlation between motor neurone spike parameters and the parameters listed on the right

<table>
<thead>
<tr>
<th>Axon</th>
<th>Protraction or retraction</th>
<th>No. of spikes</th>
<th>Mean frequency</th>
<th>Mean phase</th>
<th>Burst length</th>
<th>Against:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothoracic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protraction or retraction duration</td>
</tr>
<tr>
<td>FETi</td>
<td>P</td>
<td>0.22</td>
<td>-0.93</td>
<td>0.27</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>-0.36</td>
<td>-0.62</td>
<td>0.30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SETi</td>
<td>P</td>
<td>-0.80</td>
<td>-0.79</td>
<td>-0.48</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>-0.43</td>
<td>-0.81</td>
<td>0.60</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>R</td>
<td>0.62</td>
<td>-0.53</td>
<td>0.55</td>
<td>-</td>
<td>SETi mean freq in retraction</td>
</tr>
<tr>
<td>CI</td>
<td>R</td>
<td>0.23</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FETi</td>
<td>R</td>
<td>0.72</td>
<td>0.75</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

| Mesothoracic|                           |               |                |            |             | Retraction duration                        |
| FETi       | R                         | 0.31          | -0.73          | 0.53       | 0.68        |                                            |
| SETi       | R                         | 0.87          | -0.25          | 0.61       | -           |                                            |
| CI         | R                         | 0.34          | -0.75          | 0.23       | -           |                                            |
| CI         | R                         | -             | 0.72           |            | -           | FETi mean freq in retraction               |
| FETi       | R                         | -             | 0.21           |            | -           | SETi mean freq in retraction               |
| Metathoracic|                           |               |                |            |             |                                            |
| SETi       | R                         | 0.80          | -0.72          | 0.72       | -           | Protraction or retraction duration         |
| CI         | R                         | -0.28         | 0.33           | -0.33      | -           |                                            |
| CI         | R                         | 0.36          | -0.62          | 0.39       | -           | SETi mean freq in retraction               |
|            |                           | 0.58          |                |            | -           |                                            |

Trtraction burst is fairly constant and that it is not initiated a fixed time after the foot is lifted but after a time which is related to protraction duration. During retraction both the mean frequency in the FETi and the number of spikes were more strongly correlated with SETi firing frequency than with retraction duration, supporting the suggestion that the FETi is recruited to assist the SETi rather than being independently driven.

The effects of variation in the relative times of the movements of other legs were also investigated. However, most of the correlations found were probably due to the effects of changes in the duration of prothoracic protraction or retraction, since these are also correlated with inter-leg phases (Burns, 1973).

Mesothoracic motor neurones

The mean firing frequency in the mesothoracic FETi increased with walking speed, but the frequency in the SETi did not. Thus the SETi appears to be driven separately from the FETi with a pattern of excitation which is probably related to the supporting role of the mesothoracic leg rather than its stepping movements and which may therefore be controlled almost entirely by proprioceptive inputs.

The CI firing frequency seemed to be independent of SETi frequency and showed correlations similar to those of the FETi, suggesting that the FETi and CI may share common source of excitation.
Fig. 9. Locust mesothoracic leg. Records of isometric tension developed by the extensor tibiae muscle in response to stimulation of the motor axons with patterns derived from a walking locust. Stimulus patterns are shown as dots for each axon and are those shown in Fig. 5 (3.2 steps/s). Protractions are marked as heavy bars in the steps line. SETi stimuli to the right of the last retraction in these plots are artificial and were added to maintain tension until the stimulation restarted. Each curve is marked with the stimulation programme used. Rest tension = 1.16 g.

Metathoracic motor neurones

The relationships between walking speed and the activity in the metathoracic motor neurones were generally similar to those in the other legs. However, the mean frequency of spikes in the CI was much more strongly correlated with the SETi frequency than in the other legs, suggesting some common sources of excitation.

Mechanical responses of the extensor tibiae muscles in the walking locust

Much of the interpretation of motor-neurone activity patterns in locust walking depends on a knowledge of the mechanical responses of the muscles to those patterns. Some predictions can be made on the basis of mechanical responses to regular, artificial stimuli (Burns & Usherwood, 1978) but these cannot take account of the complex interactions between the activities of the motor neurones. In the present study tension in the extensor tibiae muscle was measured in a static dissected leg while the motor
axons were stimulated with patterns derived from a walking locust (Usherwood & Runion, 1970). Although this gave a much closer approximation to the situation in walking, muscle conditions were still quite artificial since tension was recorded under nearly isometric conditions (corresponding to a femur–tibia angle of about 110°).

**Mesothoracic leg**

In the mesothoracic leg isometric records provide a moderately good simulation of the tensions developed by the extensor tibiae muscle in walking since the tarsus will always be on the ground when the extensor muscle is active and there will therefore be considerable resistance to tibial extension.

When the SETi axon was stimulated with the pattern of potentials used in walking the very slow response of the extensor tibiae tonic fibres meant that the tension peaks caused by the bursts of activity during retraction were not very pronounced and occurred considerably later than the neural activity. When the SETi stimulation programme corresponded to a low walking speed, separate contractions were visible for each step (Fig. 9 A) with the maximum tension appearing in late retraction, some time after the peak axon activity. When a higher speed SETi programme was used the separate steps were only just distinguishable in the tension record (Fig. 10 A). Thus, if only the SETi were active and the locust was walking faster than about 3.5 steps/s, the tonic fibres of the extensor tibiae muscle would probably produce an
almost constant tension. The very slow development of tension in the tonic fibres also means that maximum muscle forces would not be developed until several steps after the locust first started to walk.

If the CI axon was also driven with the pattern of activity used in walking, the extensor tension fell to well below the level produced by SETi activity alone (Figs. 9A, 10A). Since the CI activity accelerated relaxation of the muscle and always occurred after the SETi potentials in each step, it reduced the tension slightly more in protraction than in retraction, especially at the lower walking speed. In all other respects the effects of CI activity seemed to be independent of the time of its occurrence and of the speed of walking so that it would only produce a useful step-to-step modulation of tension at very low walking speeds (< 1 step/s).

With the experimental arrangement used it was not possible to stimulate all three extensor motor axons at the same time and so the combined effects of activity in the SETi and FETi were examined using a second stimulation programme. When the FETi axon alone was stimulated a tension record resulted which was composed of brief contractions corresponding to the burst of FETi activity during the retraction periods (Figs. 9B, 10B). These contractions were larger and more sharply defined at the higher walking speed. Maximum tension was always reached after the end of the FETi burst and often occurred during the following protraction, although it is possible that in a walking animal lifting the foot from the ground might reduce the tension developed in protraction. Since the analysis in the previous section showed that the FETi burst occurred earlier in retraction as speed increased, the activity is probably timed so that maximum tension is always developed at about the time the foot is lifted, regardless of walking speed. This arrangement would maximise the propulsive thrust produced by the mesothoracic leg.

When both the FETi and SETi axons were stimulated and the resulting tensions compared with those resulting from the SETi pattern alone, the most obvious difference was the relaxation induced by the FETi in protraction and early retraction (Fig. 9B). The relaxation was more marked at the higher walking speed (Fig. 10B) because the phasic contractions were faster and stronger and because the tension due to the SETi alone was more nearly constant. Thus at high walking speeds FETi bursts are powerful agents for producing within-step modulations of tonic tension in addition to causing large phasic contractions. The possibility that the relaxation was wholly due to simultaneous activation of the CI axon was eliminated by stimulating the CI alone with the FETi pattern. The resulting reductions in tension (not shown) were considerably smaller than those in Figs. 9B, 10B.

In the walking locust a few FETi potentials may occur early in retraction, producing rapid twitches in the muscle (Fig. 9B). At the low frequency involved their relaxing effect on SETi induced tension is very small so they accelerated the development of tension at the start of retraction. The role of these FETi potentials in walking is not certain but they may provide a rapid boost in tension for postural regulation and to brake tibial flexion, particularly at low walking speeds.

The above results do not allow an exact determination of the tensions which would result from the combined activity of all three extensor motor neurones, but they suggest that the addition of CI activity to the excitatory stimulation programmes would slightly reduce the maximum tensions developed and cause a significantly faster relaxation during each protraction.
Prothoracic leg

Both the FETi and the SETi are active in this leg for most of each step, leaving only a short period when they are both silent (see Fig. 4). As a result the muscle tension developed in response to the SETi stimulation pattern showed little variation from step to step even in slow walking (Fig. 11 A), while in fast walking it was virtually constant (Fig. 12 A).

The addition of the CI programme in the slower walking situation caused an overall relaxation below the tension due to the SETi activity alone. This was a little smaller than that in the mesothoracic muscle since the prothoracic extensor muscle receives fewer inhibitory endings (Burns & Usherwood, 1978). At the higher walking speed the CI stimulation programme had only a small effect on the tonic muscle tension, reducing it by 5–10% to a lower constant level. This reduction in effectiveness with
increasing walking speed was probably due to the reduction in the effectiveness of the CI as the frequency of the SETi stimuli increased and to the fact that the number of CI spikes in each step decreased with increasing walking speed (see previous section).

When the FETi axon was stimulated with a slow speed programme the tension peaks occurring during protraction (Fig. 11B) reflected the biphasic nature of the FETi burst (see Fig. 4A). The recruitment of additional FETi potentials during retraction at higher walking speeds maintained a fairly high tension between protraction bursts and so the extensor tibiae muscle never fully relaxed (Fig. 12A). At the higher walking speed the biphasic shape of the FETi protraction spike pattern was no longer visible in the tension curves, presumably because of the limited speed of response of the muscle.

At the lower walking speed used, combination of the FETi and SETi stimulation programmes produced an effect (Fig. 11B) similar to that found in the mesothoracic leg in which each burst of FETi activity was followed by a reduction of the tonic tension. The continuation of SETi activity through retraction meant that relaxation was never as complete as in the mesothoracic muscle and the occurrence of a few FETi potentials during retraction reduced it still further. When the effective walking speed was increased to 4·6 steps/s the mean spike frequency in the SETi rose high
Control of walking in Orthoptera

enough to cause the tonic tension developed to exceed the peaks produced in response to the bursts of FETi activity. As a result, when both axons were stimulated with their respective patterns (Fig. 12A) there was very little relaxation below the tension due to the SETi pattern alone. The small size of the relaxation was also partly due to the high level of FETi activity during retraction which left only a short period in each step when the FETi was silent. These factors may also be responsible for the fact that the relaxing effect of FETi activity took up to 10 s to reach maximum after the FETi stimulation was initiated, which was much longer than in the mesothoracic leg and indicates a cumulative process. In the equilibrium which was ultimately reached the tension at the prothoracic extensor tibiae apodeme did not vary by more than 20% through all four steps of the stimulation.

The relative constancy of the tension in the extensor muscle at high walking speeds does not seem to be compatible with the movements actually executed by the tibia in walking. There are two possible explanations for this. One is that the extensor tibiae muscle tension is nearly constant during high speed walking and the tibial movements observed are brought about by the flexor tibiae muscle, which may contain more phasic fibres. The alternative explanation is that the isometric recording conditions under which the above results were obtained were too far removed from the situation in the walking insect, since in the prothoracic leg the maximum extensor activity occurs in protraction when the foot is off the ground and the only forces opposing extension of the tibia are friction in the tibial articulation and residual flexor tension. For the remainder of the step the isometric simulation may be adequate.

In order to check the second explanation the experiment involving stimulation of the FETi and SETi axons was repeated under nearly isotonic conditions, recording the movements of the tibia against a very weak restoring spring. Under these conditions the responses of the muscle were markedly different. When the SETi programme was used alone the contraction and relaxation times appeared to depend more on the degree of contraction (length of the muscle), probably because of the addition of viscosity effects, and the effects of changing walking speed were much reduced. Thus at the lower walking speed used (Fig. 11C) the shape of the SETi tension curve was similar to that of its isometric equivalent with slightly less relaxation between steps, whereas at the higher speed (Fig. 12B) there was more relaxation than under isometric conditions.

The effects of activating the FETi axon under these conditions were dependent on the speed of walking. When the lower speed FETi programme was used alone (Fig. 11C) the tibial movement only just exceeded the extension due to the SETi and twitches due to individual FETi spikes could be clearly seen, causing the extension movements to be rather irregular. If this activity was added to the SETi programme there was very little relaxation. At the higher walking speed (Fig. 12B) the FETi-induced extension in protraction was smoother than at the lower walking speed because of the higher stimulation frequency. It was now smaller than the extension resulting from SETi activity and there was even less relaxation during the retraction periods than under isometric conditions. However, when the FETi programme was added to the SETi stimulation, where the isometric records had shown almost no relaxation below the tonic tension level, the isotonic records showed large tibial movements resulting from FETi induced relaxations. The movements were very
similar to those occurring in the walking locust but their amplitudes were reduced by the absence of flexor muscle effects.

Thus the records of tibial movement under nearly isotonic conditions show that when the tibia is free to move, as it is during protraction in the free-walking locust, the extensions produced by the extensor tibiae muscle are fast enough and of large enough amplitude to account for most of the tibial movements actually observed in walking. Relaxation of the tonic fibres by FETi activity is very effective, especially at high walking speeds (> 3 steps/s). However, it is difficult to visualize the result of combining nearly isotonic conditions during protraction in the walking animal with the more nearly isometric conditions of retraction. The most likely possibility seems to be that there is an isotonic type of tibial extension in each protraction due mostly to FETi activity, followed by a tibial flexion in which the flexor muscle is assisted by FETi induced relaxation of the extensor, and then a slowly falling extensor tension through each retraction. The absolute level of extensor tension during retraction could only be ascertained by imposing complicated mechanical load changes.

**DISCUSSION**

The first two pairs of legs in the Orthoptera, although less obviously specialized than the metathoracic legs, also have their special functions. The mesothoracic legs are probably the most important for postural support while the prothoracic legs seem to have an exploratory role (Burns, 1973). In the metathoracic legs the femoral muscles are probably as important as the coxal muscles in providing propulsive force and postural support, but in the other legs it is likely that these forces are largely generated at the body-coxa joint with a small contribution from the muscles moving the tibia. In the prothoracic and mesothoracic legs the flexor tibiae muscles are larger than the extensors and may be more important. This is the reverse of the metathoracic arrangement. The functional differences between legs are reflected in the innervation and properties of their extensor tibiae muscles, the patterns of motor-neurone activity driving them, and the responses of the muscles themselves.

**Innervation and properties of the extensor tibiae muscles**

The differences in innervation pattern, muscle fibre distribution and axon paths between the metathoracic leg and the other legs probably result from the adaptation of the locust for jumping. The distribution of SETi innervated muscle fibres and the paths of the motor axons in the leg nerves suggest that in the metathorax the SETi and FETi neurones have exchanged their functions. The axons of these cells are fairly similar in diameter and are named ‘fast’ and ‘slow’ only as a result of the relative speed of the muscle contractions they induce (Hoyle, 1955a) and so the exchange implies only a change in the effect of each axon on the muscle fibres. Since the endings of the CI are associated with those of the SETi in all the legs, it must be supposed that its endings were redistributed on the metathoracic extensor muscle. Further evidence for the exchange of neurones is provided by back-filling the cells with cobalt sulphide. This shows that the positions of the somata of the FETi and SETi in the mesothoracic ganglion are reversed compared to the positions given by Burrows & Hoyle (1973) for the metathoracic cells (see Fig. 2). This is in disagree-
Control of walking in Orthoptera

ment with the diagram produced by Hoyle (1975). Wilson (1978a) has independently arrived at a similar conclusion on the basis of intracellular recording from the neurones and examining their morphology.

The reason for the evolutionary exchange of neurone function in the metathorax may have been that it was a simpler way of increasing the proportion of phasic fibres in the extensor tibiae muscle than by redistributing the nerve endings on the muscle. This idea is supported by the fact that the few tonic fibres in the metathoracic muscle are mostly proximal, while the much larger number of tonic fibres in the other legs are at the distal ends of their muscles. These changes may have entailed a reduction in contraction speed of the metathoracic muscle but this is probably unimportant since the FETi is not used in walking and the jump depends largely on stored energy (Heitler, 1974; Bennet-Clark, 1975). Response time is probably more important in the prothoracic and mesothoracic extensor tibiae muscles, particularly in view of the greater muscle movement in walking necessitated by their lower lever factors. Thus it is interesting that these muscles show a phasic twitch which is faster than that measured for other locust leg muscles (Cochrane et al. 1972; Hoyle, 1966), but slower than those of the faster running cockroach (Usherwood, 1962).

The high contracture tension developed by the mesothoracic extensor tibiae muscle when immersed in high potassium saline (Aidley, 1965) and the long time courses of contraction and relaxation under SETi stimulation in both prothoracic and mesothoracic muscles suggest that they contain a high proportion of tonic fibres whose properties are similar to those of the metathoracic leg (Usherwood & Grundfest, 1965; Hoyle, 1978). These fibres may well be responsible for the ‘catch’ effect, which is considerably stronger than that shown by the metathoracic extensor muscle (Wilson & Larimer, 1968). The ‘catch’ property of these muscles may provide a way in which postural forces can be maintained without continuous high frequency SETi activity. However, both the slow muscle response and the ‘catch’ effect would be an embarrassment to a rapidly walking locust unless there were efficient mechanisms for inducing relaxation. In fact, the CI was found to accelerate muscle relaxation following SETi activity almost as efficiently as in the coxal depressor muscles of the cockroach (Iles & Pearson, 1971), but it caused only a slow relaxation of ‘catch’ tension. Large contractions in the phasic muscle fibres provide a faster way of releasing ‘catch’ tension which they may do by momentarily removing the load from the parallel tonic fibres of the same muscle (Burns & Usherwood, 1978).

Functions and effects of motor-neurone activity patterns

The closest one can presently get to measuring the tension developed by the extensor muscles of a walking locust is to record the mechanical responses of the muscles to artificial stimuli mimicking the naturally occurring nerve activity. The artificial conditions of such an experiment mean that the results will not accurately represent the free-walking situation. However, useful comparisons can be made between the motor neurone activity patterns and the tensions they induce, and these allow some deductions to be made about the functions of the motor neurones in walking.

Muscle tension and length both follow FETi activity fairly closely, although there is a delay in the development of the mechanical response. However, the mechanical properties of the tonic fibres mean that, over much of the walking speed range,
neither tension nor length appear to follow SETi activity at all. The amplitudes of step to step tension changes reduce with increasing walking speed and are greater in the mesothoracic than in the prothoracic leg.

What then is the function of the SETi in walking? At very low speeds (<0.5 steps/s for the prothoracic leg, <1 step/s for the mesothoracic leg) when FETi activity is low, the step to step modulation of SETi activity will produce a useful variation in muscle tension which will suffice for most leg movements. Analysis of the motor neurone activity suggests that the SETi pattern changes very little with increasing walking speed, and so there is little compensation for the slow muscle responses. Thus at higher speeds the timing of SETi activity becomes largely irrelevant, and it results in continuous development of tension by the tonic muscle fibres. The function of this tension may be to maintain postural stiffness and to provide a return force to extend the tibia after flexor muscle contraction. However, continuous tension in the extensor tonic fibres would result in considerable energy being wasted by the flexor muscle in stretching its antagonist, so it is necessary to have a means of phasically relaxing the tonic fibres. At higher walking speeds this is accomplished by phasic contractions in response to bursts of FETi potentials. This mechanism may be particularly effective in the prothoracic leg just before tibial flexion, when the tibia is off the ground and the condition of the extensor muscle is therefore nearly isotonic. The relaxing function of phasic contractions may be important in many insect muscles which are composed of phasic fibres in parallel with tonic fibres.

In the front four legs the FETi is also recruited during retraction to induce additional tension in support of SETi activity. Since the relaxing effect of phasic activity depends on a high frequency of firing in the FETi, the low frequencies produced at this time in the step do not cause a significant relaxation.

In view of the interactions between the effects of the SETi and FETi, what is the function of the common inhibitor? Its effects on the tensions in the extensor muscles are small at high levels of SETi activity and, at least in the prothoracic leg, its activity appears to occur at the wrong time in the step. The situation is complicated by the fact that the neurone innervates a number of different muscles (Pearson & Bergman, 1969; Burrows, 1973). Usherwood & Runion (1970) suggested that the metathoracic CI is used to regulate postural tension in the extensor, but very little CI activity occurs in the other legs unless the locust is actually walking. These authors and Iles & Pearson (1971) considered that the CI was used to accelerate relaxation after ‘slow’ axon activity, but this interpretation seems doubtful since in cockroaches the CI innervates both members of an antagonistic pair of muscles.

The present work has shown that the CI accelerates relaxation in all the extensor tibiae muscles of the locust, and that its effect is very long-term. Thus, at least at high walking speeds the acceleration of relaxation does not depend on the time of firing of the CI. The average firing frequency is the most important parameter and this rises with increasing walking speed. Since the maximum activity in the CI in all the legs occurs at the end of retraction and the CI appears to be closely linked to the ‘central oscillator’ (M. D. Burns, in preparation) is seems likely that its primary role is in the direct relaxation of the propulsive coxal muscles (such as the coxal remotors) where it may act more rapidly. The secondary innervation of other leg muscles may have evolved by branching to those muscles which would benefit,
Providing decreasing relaxation times as walking speed increases, a general relaxation at the end of each step at very low walking speeds and possibly some assistance in postural adjustments. Thus it may be a general rule in insects that the CI is an effective phasic inhibitor of primary propulsive muscles (e.g. cockroach, Iles & Pearson, 1971) but that it has a slower, reduced effect on other leg muscles.

This study has shown that at different walking speeds the relative usefulness of the extensor tibiae motor neurones is very different. At very low speeds the SETi and CI may adequately control the muscles, but as the speed increases SETi induced tension becomes increasingly modulated by FETi activity leaving the CI only to provide a general acceleration of relaxation. There are some central adjustments to the activities of the motor neurones corresponding to their change of role with speed such as the advance in phase of the mesothoracic extensor activity and the recruitment of FETi potentials, but the patterns of activity seem to be basically designed for low walking speeds.

No information was obtained on the function of the DUMETi neurone in walking. Hoyle et al. (1974) report that on the metathoracic extensor muscle the endings of this neurone parallel those of the FETi and suggest that it has a trophic role on the phasic muscle fibres. If this is the case it would be interesting to know whether its endings also follow those of the FETi in the other legs where the distribution of phasic fibres is different.

**Generation of motor neurone activity patterns**

The detailed information contained in the activity patterns of the extensor motor neurones in the walking insect (Figs. 4–7) and in the statistical analysis of these patterns enable some suggestions to be made about the nature of the central mechanisms involved in exciting the motor neurones. A more complete picture of the functional connexions responsible can be constructed only if it is known what use the central nervous system makes of sensory information (M. D. Burns, in preparation).

**Prothoracic motor neurones**

The protraction activity of the FETi is biphasic, the two frequency peaks corresponding to the times during which the femur is being lifted and then depressed. The duration and phase of the activity were not related to walking speed so the burst is probably initiated centrally at the same time as the coxal levator muscles are activated and is controlled, in parallel with the coxal depressors and levators, by reference to sensory information about the position of the leg (M. D. Burns, in preparation). After the protraction burst is terminated, the FETi remains available for recruitment by the system which controls the SETi. The threshold for FETi activation is high since the neurone is only recruited when a number of SETi potentials is produced at a high average frequency, and it presumably rises still further towards the end of retraction. This phenomenon represents the simplest case of the 'size principle' demonstrated in lobster swimmeret motor neurones (Davis, 1971) and agrees with the threshold difference observed by Burrows & Horridge (1974) between the metathoracic FETi and SETi. It cannot alone account for the FETi activity in protraction since the FETi is then clearly independently activated.
At the beginning of the SETi activity in protraction the spikes are frequently paired with those of the FETi, suggesting a common source of excitation. There is no evidence of the SETi inhibition following FETi spikes observed by Hoyle & Burrows (1973) in the metathorax. For the rest of each step the variability of the SETi firing pattern suggests that the SETi may be controlled by sensory feedback. It is inhibited while the flexor tibiae muscle is active, either through the reciprocal synaptic inputs found by Burrows & Horridge (1974) or through chordotonal organ responses to flexor muscle shortening (Burns, 1974).

**Mesothoracic motor neurones**

The movements of the mesothoracic leg are simpler and more stereotyped than those of the prothoracic leg and almost no extension occurs during protraction (Burns, 1973), so the activity patterns of the extensor motor neurones are less complex. The fact that the firing frequency of the SETi is independent of walking speed and the high variability it displays suggest that in this leg too the SETi is largely controlled by reference to sensory feedback. The initial period of high-frequency activity at the start of retraction which is visible in some animals may result from a rebound from the inhibition which operates while the flexor neurones are active during protraction. In the early part of retraction the mesothoracic FETi is recruited to assist the SETi, but the following propulsive burst of FETi potentials occurs after the SETi activity has ceased and must be independently driven.

**Metathoracic motor neurones**

The activities of the extensor motor neurones have been less thoroughly examined here than their counterparts in other legs since they have since been the subject of previous work (Runion & Usherwood, 1968; Usherwood & Runion, 1970; Usherwood *et al.* 1968; Burrows & Horridge, 1974). However, the results obtained here show that the SETi produces a burst of activity in early retraction, at least partly for propulsion, in which the mean frequency rises with walking speed and, unlike the situation in the other legs, the SETi firing frequency is correlated with that of the CI. Thus both the SETi and the CI are probably fairly tightly coupled to the ‘central oscillator’ responsible for timing the activities of the primary propulsive muscles. It is conceivable that both neurones may be driven by the same non-spiking interneurones (Pearson & Fourtner, 1975; Burrows & Siegler, 1976) and that these may also drive the coxal motor neurones. The fact that the timing of the CI activity in all the legs is similar in spite of the differences between other motor neurones and that the correlations with SETi activities in the front four legs are poor suggests that all the CI neurones are controlled in the same way.

Comparison of the grasshopper data with that for the locust suggests that the central connexions to the excitatory motor neurones are very similar. The greater weight and lower walking speed of the grasshopper is reflected in a higher SETi firing frequency throughout each step and a greater variability suggestive of a high degree of sensory modulation, but in most other respects the activities in all the axons were very similar to the locust patterns. Since the grasshopper employs a different gait to the locust (Burns, 1973), the similarity of the motor neurone behaviour in
Two species implies that the motor neurones are not strongly influenced by the phasic inputs from other legs. This is also demonstrated by the poor correlations between motor neurone activities and the movements of other legs and implies that the weak cross-segmental reflexes observed by Burrows & Horridge (1974) are no stronger during walking.

The ‘jerking’ behaviour of the grasshopper shows that a common excitatory connexion between motor neurones in different ganglia exists. If this is mediated by an interneurone it cannot synapse directly with the motor neurones since the relative timing of activities in different segments is variable. A similar behaviour has been observed in stick insects after cutting the circumoesophageal connectives (D. Graham, personal communication).

REFERENCES


