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INTRACELLULAR RECORDINGS FROM INTERNEURONES AND MOTONEURONES DURING BILATERAL KICKS IN THE LOCUST: IMPLICATIONS FOR MECHANISMS CONTROLLING THE JUMP

BY I. C. GYNTHER AND K. G. PEARSON

Department of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7

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SUMMARY

Intracellular recordings were made from the neuropile processes of thoracic neurones of *Locusta migratoria* during bilateral kicks of the hindlegs. Electromyographic (EMG) recordings showed that the pattern of flexor and extensor tibiae muscle activity during kicks in this extensively dissected preparation was similar to that seen during a jump. Intracellular recordings from hindleg flexor and extensor motoneurones and from 13 identified interneurones revealed additional features of the motor programme for jumping and kicking and of the mechanism which triggers these events.

There was a discrete burst of activity in the fast extensor tibiae (FETi) motoneurone at the end of the co-contraction phase, generated by a system that appeared to be separate from that triggering the kick. The excitatory connection from FETi to flexors was not responsible for initiating flexor activity and was of little functional importance in maintaining this activity during the co-contraction phase. The initial flexor excitation came from another, unidentified, central source.

A pair of identified interneurones, the M-neurones, discharged with a high frequency burst just prior to the kick. Since these neurones inhibit hindleg flexor tibiae motoneurones, this observation provides further support for their proposed role as the neurones responsible for triggering kicks and jumps. Our data do not support the proposal that the activation of the M-neurones depends on them receiving progressively increasing proprioceptive input during the co-contraction phase. Throughout co-contraction, the M-neurones were hyperpolarized. Their activation was rapid and strong enough to cause them to discharge at rates as high as 400 spikes s^{-1}. We suggest that the pulse-like activation of the M-neurones is produced centrally by a higher order system of interneurones.

Another pair of previously identified interneurones, the C-neurones, were not necessary for the generation of the co-contraction phase of the motor programme. Their pattern of activity and their known connections indicated that they provide additional excitation to the flexors and extensors towards the end of co-contraction.

Many other interneurones discharged either during co-contraction or when a kick was triggered. We conclude that the system generating the motor programme for a kick (jump) is more complex than proposed in previous studies.

Key words: insect, locust, jump.

INTRODUCTION

The neuronal mechanisms controlling the locust jump have received considerable attention in past years. The activity of both motoneurones (Godden, 1975; Heitler & Burrows, 1977*a*) and interneurones (Pearson, Heitler & Steeves, 1980; Pearson & Robertson, 1981) during jumping has been examined, as has the involvement of sensory mechanisms in the jump (Heitler & Burrows, 1977*b*; Steeves & Pearson, 1982). The jump consists of several distinct phases: (1) an initial flexion of the hindleg tibiae to bring them into the position for jumping, (2) a co-contraction of the antagonist flexor and extensor tibiae muscles of the hindleg, during which time considerable force is generated by isometric contraction of the large extensor muscle, and (3) a sudden inhibition of the flexor activity allowing the tibiae to extend rapidly due to release of the energy stored in elastic elements of the femoral–tibial joint. These same phases are evident during kicking (Heitler & Burrows, 1977*a*).

Currently, the proposed neuronal circuitry controlling kicking and jumping (see Pearson, 1983) involves only two pairs of thoracic interneurones, the C- and M-neurones. The C-neurones are considered to cause the initial locking of the tibiae into full flexion by synchronously activating the flexor and extensor muscles at the onset of co-contraction (Pearson & Robertson, 1981). However, no recordings have been made from C-neurones during the rest of co-contraction leading to either a kick or a jump. The M-neurones are thought to be responsible for the sudden inhibition of flexor activity which triggers the jump (Pearson *et al.* 1980). The M-neurones make monosynaptic inhibitory connections with flexor motoneurones and receive depolarizing input resulting from visual, auditory and tactile stimuli, all of which are quite effective in eliciting jumps from intact animals. However, because the M-neurones have a characteristically high threshold for action potential generation, they are rarely induced to spike even upon simultaneous presentation of such stimuli. The M-neurones also receive depolarizing input from hindleg proprioceptors, and it has been proposed that the gradual increase in the strength of this input during co-contraction brings each M-neurone closer to its threshold for spiking, thus allowing it to discharge action potentials in response to other sensory stimuli (Steeves & Pearson, 1982). According to this idea, the proprioceptive information gates M's activity and ensures that each M-neurone will discharge (consequently inhibiting the flexors and triggering the jump) only when sufficient force has been generated within the appropriate femur.

The main purpose of the present study was to examine more closely the proposed roles of the C- and M-neurones by recording from the neuropile processes of these cells during the production of bilateral hindleg kicks. In addition, we wished to examine the pattern of synaptic input to hindleg motoneurones and to record from other interneurones within the thoracic ganglia to determine whether some of these may also play a part in producing the kick. We present further evidence which supports the idea that the M-neurones are the trigger neurones for the jump. However, their activity is not gated in a simple manner by proprioceptive feedback as proposed in earlier studies. This, together with the finding that other interneurones

are strongly activated during a kick, indicates that the circuitry controlling the jump is not as simple as has previously been proposed.

MATERIALS AND METHODS

All experiments were performed on adult male and female *Locusta migratoria* reared in a long-established colony at the University of Alberta. The sex of an animal had no influence on the results of this study. Experiments were carried out at room temperature (22–24°C).

EMG recording from intact animals

Details of the procedure used to record flexor and extensor tibiae muscle activity in intact locusts have been described previously (Pearson & Robertson, 1981). A pair of 50 μm copper wires, insulated except for their tips, was implanted into the extensor muscle of one hindleg. Flexor activity could still be recorded in this manner and was evident as the smaller amplitude activity in our recordings. The animal was then tethered to an overhead support by means of a cotton thread attached to the pronotum. This arrangement permitted the locust freedom of movement over an area of about 50 cm diameter. Jumps either occurred spontaneously or were elicited by making loud noises or by lightly touching the animal's body.

Preparation for intracellular recording

Animals were pinned dorsal side up on a cork board and their hindleg femora fixed firmly in place with Plasticene. The tibiae of these legs, cut so that only their proximal third remained intact, were usually allowed unrestricted movement. However, for some experiments a metal rod, mounted on a manipulator, was used to prevent full flexion of one of the tibiae. A combined myogram of the flexor and extensor tibiae muscle activity of each hindleg was obtained by implanting a pair of 200 μm copper wire electrodes into the extensor muscles. These electrodes could also be used to stimulate antidromically the fast extensor tibiae motoneurone. The thoracic ganglia were exposed by removing the gut, ventral diaphragm and overlying muscles (see Pearson *et al.* 1980 for details of the dissection). In the majority of experiments, nerves 3 and 4 of both the meso- and metathoracic ganglia were cut so as to denervate most of the thoracic flight musculature and thereby improve the stability of the preparation. These ganglia were then supported on a rigid stainless steel plate and kept covered with locust saline (in mmol l^{-1}: NaCl, 147; KCl, 10, $CaCl_2$, 4; NaOH, 3; HEPES, 10).

Bilateral kicks of the hindlegs were evoked by gently stroking or pinching the animal's abdomen, mouthparts or antennae with a pair of forceps. We were able to evoke kicks from approximately 80% of these fully dissected animals. A kick could easily be distinguished from rapid tibial extension because continued simultaneous activity of flexor and extensor muscles (co-contraction) did not occur during the latter, and because kicks produced an audible 'click'. Instability of intracellular recordings during kicking was generally not a problem. It was only rarely that the

violent movement of the tibiae during kicking dislodged a microelectrode from within a cell. All electrical recordings were stored on magnetic tape and later displayed on a Gould ES 1000 chart recorder.

Intracellular recording and staining

All recordings from motoneurones and interneurones were made by penetrating their major neuropile processes. Glass microelectrodes were filled with either $1\,mol\,l^{-1}$ potassium acetate or a 5% aqueous solution of the dye Lucifer Yellow. Lucifer staining enabled the morphology of a penetrated neurone to be determined following recording of its physiology. After staining, the ganglia were fixed for 30 min in a 4% solution of paraformaldehyde, dehydrated in alcohol and then cleared in methyl salicylate for 15 min before being viewed as whole mounts.

The criteria for recognizing penetrations of fast extensor motoneurones (FETi), flexor motoneurones, and M- and C-neurones have been described previously (Pearson *et al.* 1980; Pearson & Robertson, 1981). The cells could be recognized so reliably that dye-filling was not necessary for their identification. All other inter- and motoneurones, however, were always stained following recording.

RESULTS

Motor patterns during jumping and bilateral kicking

Although jumping and defensive kicking in locusts are related behaviours, there are distinct differences between them. One of the more obvious is that jumping involves both metathoracic legs whereas kicking usually involves just one. Another is that, before jumping, a freely moving locust assumes a crouched position with both hind coxae depressed, such that the femora lie parallel to the substrate. When the tibiae extend during the jump, force is directed downwards and backwards (Godden, 1975). Defensive kicks, however, can be accurately directed towards a source of irritation by rotation of the leg about the thoraco–coxal joint. Here the force of the extending tibia is often directed upwards and backwards. There has been no comprehensive EMG study of all muscles involved in jumping and kicking, and consequently a detailed account of the differences between the motor programmes for these two behaviours is not available. However, in as far as the activity of the metathoracic flexor and extensor tibiae muscles is concerned, there is good evidence to suggest that the motor programmes for jumping and kicking in the locust are very similar (Godden, 1975; Heitler & Burrows, 1977*a*; Pflüger & Burrows, 1978). From here on in this paper we use the term 'motor programme for a jump or kick' to mean only this pattern of flexor and extensor activity.

Our preparation for intracellular recording in the locust involved such an extensive dissection that we had to question whether the behaviour elicited from these dissected animals resembled anything seen in intact, freely moving locusts. A comparison of the myograms recorded during jumps by intact animals and restrained kicks by dissected animals showed that the main features of the motor patterns for these behaviours were very similar (Fig. 1A) and closely resembled those described

in detail by Heitler & Burrows (1977*a*). Typically, a period of flexor activity of about 50–500 ms duration was followed by co-contraction of the flexor and extensor muscles, as indicated by a period in which spikes occurred in both muscles. Spikes in the extensor muscle EMG were due to the activity of the single fast extensor motoneurone innervating that leg. Co-contraction lasted for a period of some 300–800 ms (mean for a jump = 340 ms, mean for a kick = 450 ms) and ended with a sudden cessation of flexor activity. Usually, extensor activity continued for a short period (about 20 ms) after the termination of activity in the flexors.

Although the femora of the hindlegs were held fixed so that they could be neither depressed nor rotated upwards, as would normally occur during a jump or kick, the great majority of kicks elicited from our dissected animals involved the synchronous extension of both hind tibiae (Fig. 1B) rather than just one as is usual for a defensive kick. This, together with the basic similarity between the patterns of motor activity seen in intact and dissected animals, led us to believe that the behaviour we observed in dissected locusts resembled jumping more than it did defensive kicking. For this reason it may be more correct to refer to this behaviour as fictive jumping. However, in order to retain consistency with the terminology of earlier studies (e.g. Heitler & Burrows, 1977*a*,*b*) we refer to this fictive jumping as kicking throughout the paper. It should be borne in mind, then, that the results of this study are likely to have more direct relevance to the jump system than to the system underlying defensive kicking.

The total duration of the programme for an intact animal's jump was usually shorter than that for kicking in a dissected animal. This was due to shorter periods of both initial flexion and co-contraction in the intact animal. In some highly aroused locusts, co-contraction began not with an initial activation of flexor motoneurones alone but with synchronous activation of FETi and flexor motoneurones (see Pearson & Robertson, 1981). This was never observed in dissected animals.

A major difference between the motor programmes we recorded and those reported by others was that the extensor muscle commonly displayed two phases of activity. Throughout most of co-contraction this muscle spiked at a more or less constant frequency of 10–40 Hz and on a few occasions almost 60 Hz (Fig. 2D). Sometimes the muscle spikes occurred in discrete groups of two or three, but with their overall frequency still in the 10–40 Hz range. The final phase of co-contraction, however, usually involved a higher frequency of activity in the extensor muscle just prior to the jump or kick (Fig. 1A,B). The timing of this final burst was variable. Usually, it commenced before, and extended just beyond, the termination of flexor activity. Occasionally it began after flexor activity had ceased. The number of spikes during the final extensor burst was typically 4–7 and they occurred with a frequency of 70–130 Hz. A noticeable feature was that the burst often began synchronously in both legs (Fig. 1B).

Recordings from motoneurones during bilateral kicking

The motoneurones driving the power-producing extensor and power-controlling flexor muscles of the hindleg constitute the output stage of the neuronal circuitry

controlling the jump. As such, a careful analysis of the synaptic input that these motoneurones receive during bilateral kicking can reveal much about the underlying mechanism of the jump. We present here recordings, made during kicking, from the neuropile processes of the fast extensor tibiae motoneurone and some of the nine excitatory flexor motoneurones. All previous recordings (Heitler & Burrows, 1977*a*,*b*) have been made from neuronal somata and consequently provide an attenuated view of synaptic events.

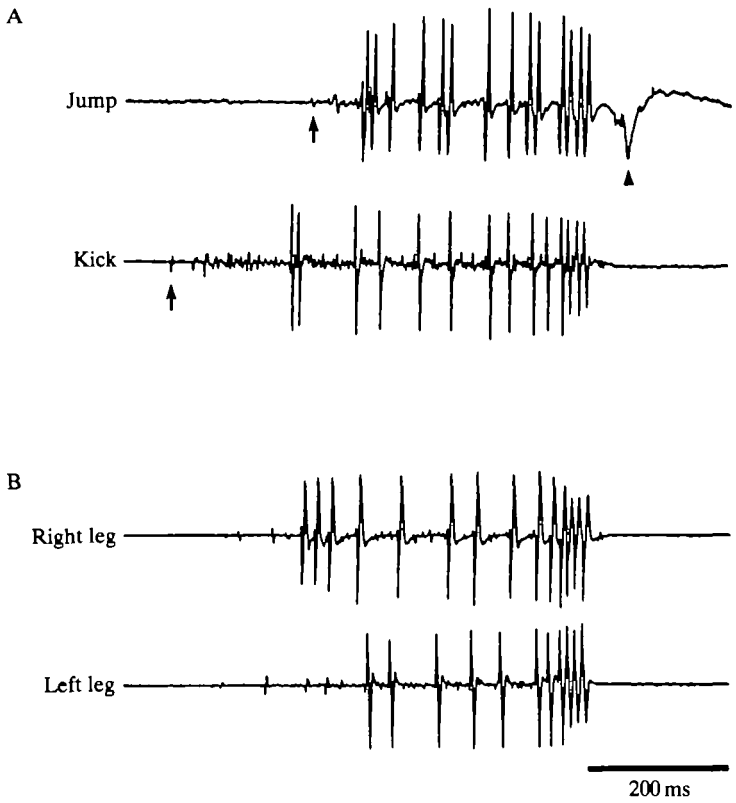

Fig. 1. Electromyographic (EMG) recordings from hindleg flexor and extensor tibiae muscles of locusts during bilateral kicking and jumping. (A) Top trace, EMG from an intact locust during an unrestrained jump; bottom trace, EMG recorded from one leg during a bilateral kick by a locust dissected for intracellular recording. The large spikes in both traces were due to activity in the single fast extensor tibiae motoneurone. The small amplitude spikes were caused by activity in the flexor tibiae muscle. Except for the shorter duration of the sequence during jumping, the motor programmes for kicking in the dissected animal and jumping in the intact animal were similar. Each began with a period of flexion (commencing at arrows) followed by a co-contraction of both the flexor and extensor muscles. As illustrated here, the motor programme often ended with a rapid burst of 4–7 spikes in the extensor muscle. The movement artifact in the top trace (arrowhead) was caused by the animal jumping. (B) EMG recordings from a dissected locust during a bilateral kick of the hindlegs. All the features of the motor programme are as described in A. Note the simultaneous burst in the extensor muscle of each leg which terminated the kick sequence. Note also that the kicks were triggered simultaneously in the two legs as indicated by the synchronous cessation of muscle activity.

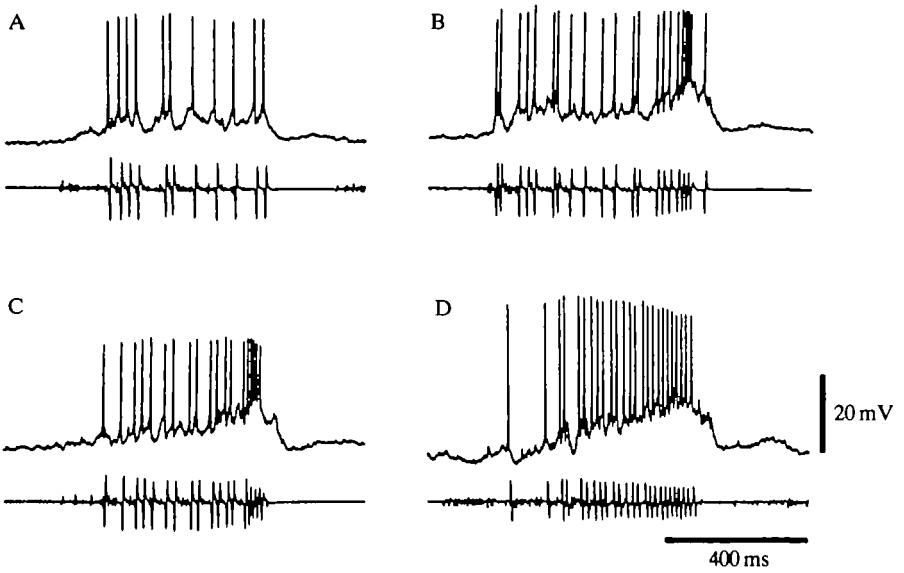

Fig. 2. Intracellular recordings from the fast extensor tibiae motoneurone during kicking. Top traces, intracellular recordings; bottom traces, electromyograms from flexor and extensor tibiae muscles of the innervated legs. (A) A weak kick in which no final burst occurred in the motoneurone. (B),(C) Examples in which FETi's activity ended with a marked depolarization and rapid burst of spikes. (D) A sequence in which FETi received a ramp depolarization throughout co-contraction and fired at higher than normal frequencies. The patterns of activity shown in B,C were those most commonly observed.

Fast extensor tibiae motoneurones

The pattern of activity seen in FETi during kicking was variable. During weak kicks, FETi displayed a slight plateau of depolarization interrupted by partial membrane repolarizations (Fig. 2A). No final, higher frequency burst occurred during these kicks. More commonly, FETi displayed the same biphasic pattern that we often observed in the EMG recordings of extensor muscle activity, with a period of relatively low frequency spiking followed by a rapid burst of action potentials in the cell. During the first phase of co-contraction, action potentials could occur at either a fairly constant frequency or in groups of 2–3, with the groups separated by roughly constant intervals. Two such biphasic patterns were evident. In one, FETi showed a plateau depolarization throughout most of co-contraction before receiving a marked, often pulse-like, depolarization accompanied by a rapid burst of 4–7 spikes just preceding the kick (Fig. 2B). In the other, it received a weak ramp-like depolarization throughout co-contraction leading to the distinct final burst (Fig. 2C). In both cases, the duration of the higher frequency burst of spikes was in the range of 30–100 ms. Another pattern of FETi activity seen in one animal during co-contraction consisted of a much stronger ramp depolarization which did not end with a rapid burst of action potentials (Fig. 2D). In these cases, the spike frequency was usually higher than normal throughout co-contraction. At times the ramp could be interrupted by marked membrane repolarizations. Except for this last example, each of the described patterns of activity could be observed in a single preparation, but the

patterns shown in Fig. 2B,C were the most common. The boundaries between the various patterns were not so clear cut as to suggest functionally separate motor programmes.

The recordings do suggest, however, that FETi could receive two distinct inputs: (1) a tonic or ramp-like excitation throughout most of co-contraction and (2) a final phasic excitation just preceding the kick. This conclusion was supported by observations of the pattern of synaptic input to FETi when kicks were elicited from the contralateral leg but prevented in the ipsilateral leg. (It was possible to prevent one hindleg from kicking by placing an obstruction between the tibia and femur so that the leg was unable to flex fully.) Throughout most of co-contraction of the opposite leg, FETi innervating the obstructed leg was often quiescent. On no occasion was it continuously depolarized and active during co-contraction of the opposite leg as it normally would have been. This suggested that FETi's normal pattern of activity during the first part of co-contraction was not due to a bilaterally symmetrical central excitation but more probably to a sensory source, as proposed by Heitler & Burrows (1977*b*). However, the ipsilateral FETi was consistently depolarized above threshold at, or near, the time of the final burst of spikes in the opposite leg. This depolarization was usually weak with only one or two spikes resulting (see Fig. 5), but sometimes as many as six occurred. As mentioned, the exact timing of this input was variable. In most cases it coincided with the burst in the opposite FETi, but in others it occurred just before this burst started or just after it had finished.

Flexor tibiae motoneurones

Each flexor tibiae muscle of the locust is innervated by nine excitatory motoneurones (Phillips, 1980), which can be classified physiologically as fast, intermediate or slow flexor motoneurones. Although we did not attempt to record from all nine flexors in this study we have made numerous recordings during kicking from neurones in each group. There is a basic similarity between the groups in their patterns of activity and we have therefore not made a complete catalogue of flexor motoneurone responses.

Two examples of flexor activity during kicking are shown in Fig. 3. A striking feature, seen in all flexor motoneurones, was the large plateau depolarization they received throughout co-contraction. The initial depolarization leading to this plateau could occur gradually, in a ramp-like manner (Fig. 3A), or more quickly, following several summating excitatory postsynaptic potentials (EPSPs) (Fig. 3B). In some motoneurones, the rise time of this plateau was so rapid that it became difficult to distinguish the individual EPSPs. It is important to note that the flexors received this initial depolarization independently of activity in FETi, as indicated by the absence of extensor spikes in the myogram at the time the flexors became active. Consequently the central excitatory connection that exists between FETi and the flexor motoneurones (Hoyle & Burrows, 1973) could not have been responsible for initiating flexor activity. This connection probably does contribute some excitation to the flexors during co-contraction, however, and may account for the oscillations in membrane potential sometimes seen superimposed on the plateau depolarization in

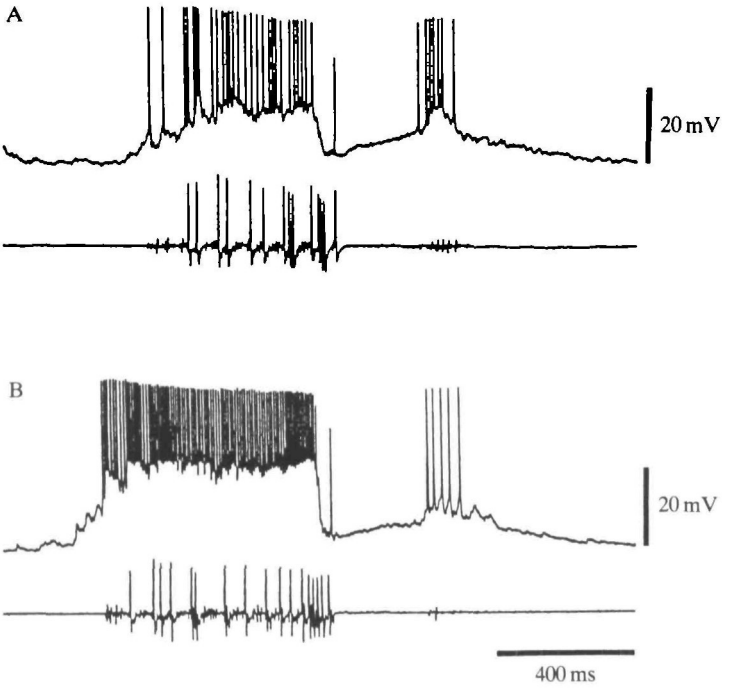

Fig. 3. Intracellular recordings from flexor motoneurones during kicking. Top traces, intracellular recordings; bottom traces, electromyograms from flexor and extensor tibiae muscles of the innervated legs. (A) Intermediate flexor motoneurone. (B) Slow flexor motoneurone. Note the inhibition of the flexors (particularly marked in B) which just preceded the termination of extensor activity and triggered the kick. The second burst of spikes in each flexor motoneurone, 200–300 ms after the kick, caused the tibia to re-flex.

our records of flexors. These oscillations corresponded on a 1:1 basis with FETi spikes seen in the accompanying myogram. This correspondence declined, however, as the co-contraction sequence continued, most probably because of a rapid decrease in the strength of the central FETi–flexor interaction which occurs with repeated spike activity in the fast extensor tibiae motoneurone (Heitler & Burrows, 1977*b*).

The frequency at which flexors spiked during the plateau depolarization was related to their physiological category, as has been reported by Heitler & Burrows (1977*a*). Fast and intermediate flexor motoneurones commonly spiked at 70–90 Hz during co-contraction (Fig. 3A), whereas slow flexors reached spike frequencies of 200 Hz or more (Fig. 3B).

The most important feature observed in all flexors during kicking was their rapid inhibition which terminated the co-contraction and triggered the kick. Its timing relative to the end of extensor activity in the ipsilateral leg was variable. In our records, it occurred over a range of between 4 and 67 ms prior to the last spike in the EMG. So, too, the timing of flexor inhibition relative to the start of the final high frequency burst in the ipsilateral FETi was variable. We have recorded examples in which it occurred as much as 20 ms before the start of the final burst in FETi.

However, it was more usual for the inhibition to occur after this burst had commenced and while it was still in progress (mean = 15·7 ms following the first FETi spike of the burst). This degree of variability in timing of the two phenomena – the flexor inhibition and the final FETi excitation – suggested that these events did not have a common underlying origin.

Recordings from interneurones during bilateral kicking

To gain more insight into the cellular mechanisms for patterning motor activity for the jump, we recorded the activity of numerous identified interneurones in the meso- and metathoracic ganglia during bilateral kicking. We concentrated initially on the C- and M-neurones because they are known to be intimately involved in the mechanisms by which the jump motor programme is initiated and by which the jump is triggered, respectively (Pearson & Robertson, 1981; Pearson *et al.* 1980). No attempt was made to elucidate the connections between these and other interneurones.

The M-neurones

Our recordings in the M-neurones revealed two previously unreported features: (1) throughout the period of initial flexion and the subsequent co-contraction the M-neurones were hyperpolarized by several millivolts; (2) at the end of co-contraction they received a strong, pulse-like depolarization causing them to spike at extremely high frequencies.

It has been reported that the M-neurones (Fig. 4A) are gradually depolarized during co-contraction and then, due to the occurrence of visual and auditory stimuli, are caused to discharge in a burst (Steeves & Pearson, 1982). In contrast, we never observed any slow depolarization in an M-neurone prior to a kick, but consistently noted a hyperpolarization of up to 6 mV (Fig. 4B,C). The magnitude of this hyperpolarization depended on both the site and quality of the intracellular penetration. In recordings from the central processes of the cell (Fig. 4B,C) the hyperpolarization was very obvious. In recordings from the transverse process, proximal to the point where it bifurcates into the ascending axon and the large lateral branch, the hyperpolarization was less marked (Fig. 4D), as was also true in cases where the cell had been injured on penetration. It was possible that this pattern of activity in the M-neurone may not have been the same as that during kicks in the intact animal, because of the absence of proprioceptive information from afferent fibres projecting from the hindleg femur and tibia to the central nervous system (CNS) *via* nerve 3 (in most experiments this nerve was cut). However, these same features of M's activity were evident even when all peripheral nerves were left intact. For this reason we consider the recordings presented here to be typical of the M-neurone's normal activity.

The initial flexion movements of the two hind tibiae did not always occur synchronously. Flexion in one leg could sometimes precede the other leg by as much as 500 ms. The onset of the hyperpolarization in an M-neurone appeared to coincide with the beginning of flexor activity in whichever leg was first to flex. Thus, it was

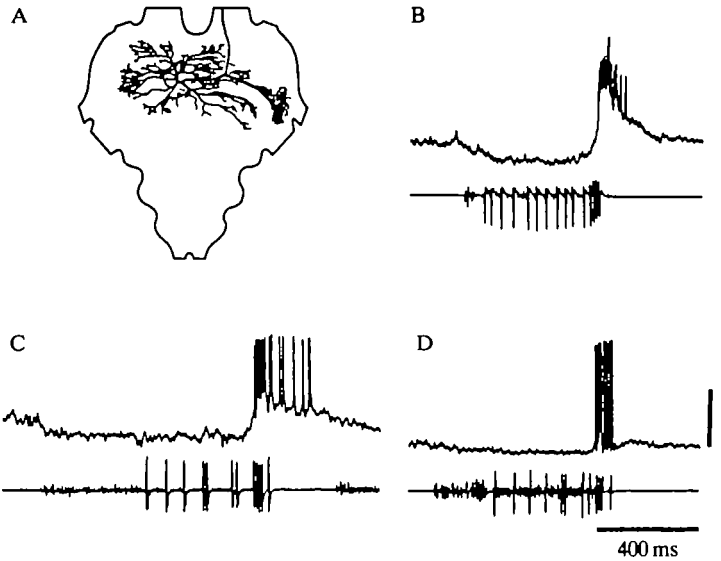

Fig. 4. Intracellular recordings from the M-neurone during kicking. Top traces, intracellular recordings; bottom traces, electromyograms from hindleg flexor and extensor muscles on the side to which the M-neurone sent its major branch. (A) Diagram of M's structure within the metathoracic ganglion. (B),(C) Recordings from central processes during kicking. The exact sites of penetration were not determined but judging from spike amplitudes, the recording in B was from a finer central process than was the recording shown in C. (D) Recording from the transverse process of M, proximal to the point where it bifurcates into the ascending axon and the large lateral branch. The recording site was quite close to the spike-initiating zone. The M-neurone fired at spike frequencies of 250–400 s^{-1} during its burst (the individual spikes cannot be discerned here). Note the hyperpolarization (evident in B,C, but less so in D) which commenced at or near the time of tibial flexion. Scale bar, 10 mV in B,D, 20 mV in C.

not always coincident with flexion on the side to which the M-neurone sent its major branch (which here we term the ipsilateral side though it is actually the side contralateral to M's soma). The duration of the hyperpolarization in M was variable, being dependent on the length of the initial flexion and co-contraction periods combined.

The depolarization that each M-neurone received just prior to a kick could occur even in the complete absence of visual, auditory and tactile stimuli. It was triphasic in form, with a rapid ramp depolarization leading to a pronounced pulse-like excitation, followed by a slow ramp repolarization (Fig. 4B,C). The pulse phase usually lasted 40–90 ms during which time the cell discharged at spike frequencies of 250–400 Hz. It was common for an M-neurone to continue spiking as its membrane gradually repolarized.

As was suggested by the variability in timing of the flexor motoneurone inhibition relative to the end of extensor activity, the timing of the M-neurone's burst relative to the last FETi spike was also variable. In an analysis of 42 kicks by five animals the first spike in M preceded the last spike in the ipsilateral extensor EMG by an average of 23·4 ms (S.D. = 15·7 ms). The greatest separation observed was 60·4 ms. In two

kicks M's discharge followed the last extensor spike by periods of 4·0 and 6·4 ms, respectively. These were the only two cases out of all of our recordings in which M became active after extensor activity had ceased. In these cases, flexor EMG activity was also observed following the last FETi spike. During kicks in which FETi's activity ended with a rapid burst, the ipsilateral M was observed to begin firing before, during, or even after this burst. This variability in the timing of the M-neurone's activity was present even during repeated kicks by the same animal. Again it indicated that the M-neurone was not responsible for the final burst in FETi. However, the correlative evidence of the range of times of flexor shutdown relative to the end of extensor activity as compared with the timing of the burst in each M-neurone was entirely consistent with the idea that the M-neurones provided the trigger inhibition to the flexor motoneurones at the end of co-contraction.

In an attempt to determine whether the pattern of input each M-neurone received during kicking was of a central, bilateral origin or whether it depended on incoming sensory information from the ipsilateral leg, we recorded from an M-neurone whilst preventing the ipsilateral leg from kicking. A kick was then elicited from the opposite leg. In this situation, the M-neurone displayed the same pattern of activity – initial hyperpolarization followed by a triphasic depolarization – that it would have if the locust had been able to kick its ipsilateral leg (Fig. 5). This result indicated that the input to the M-neurones during kicking was bilateral and that each

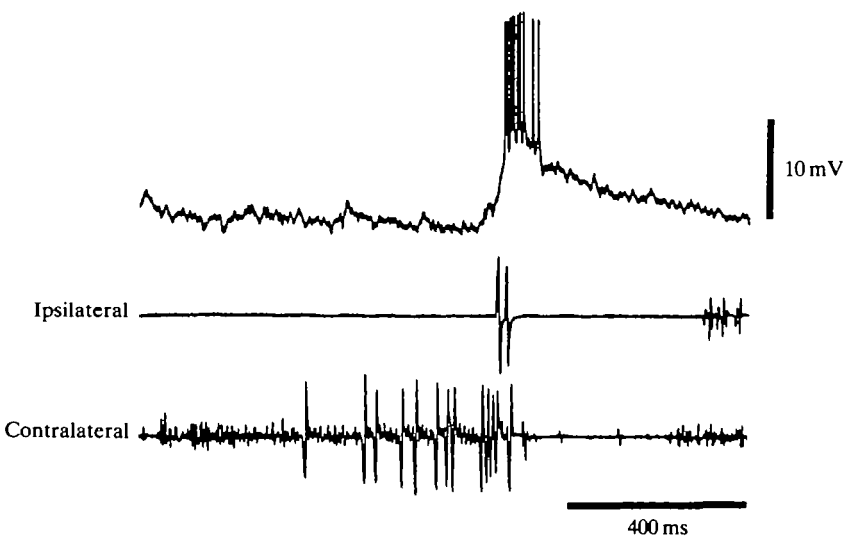

Fig. 5. Intracellular recording from an M-neurone made while its ipsilateral leg (i.e. on the side to which the M-neurone sent its major branch) was prevented from fully flexing and therefore could not kick. A kick was elicited from the contralateral leg. Top trace, intracellular recording; middle trace, electromyogram (EMG) from the ipsilateral leg; bottom trace, EMG from the contralateral leg. Note that M's pattern of activity was unaltered by obstructing its ipsilateral leg. Note also the two spikes in the extensor muscle of the ipsilateral leg (caused by activity in FETi) which occurred close to the time of the extensor burst in the unobstructed leg.

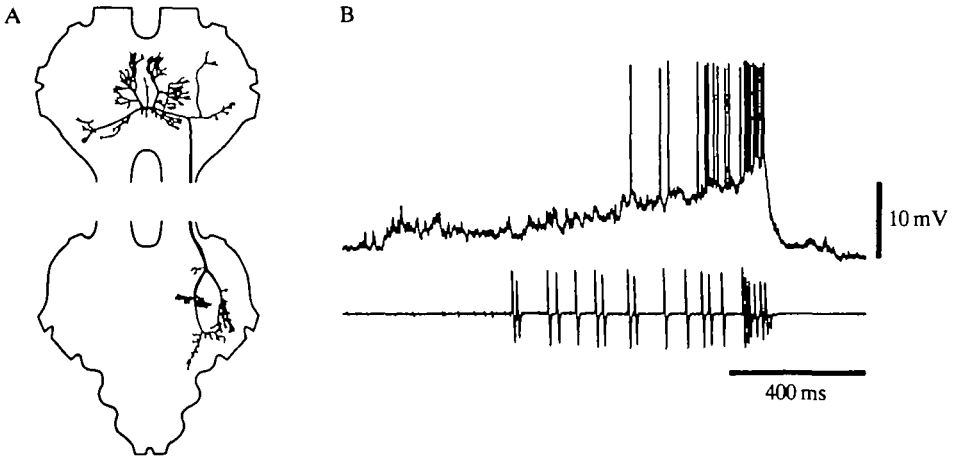

Fig. 6. Intracellular recording from the C-neurone during kicking. (A) Diagram of C's morphology in the meso- and metathoracic ganglia. (B) Recording during kicking. Top trace, intracellular recording; bottom trace, electromyogram from the flexor and extensor tibiae muscles on the side to which the C-neurone projected. The recording was made from the large transverse process in the midline of the mesothoracic ganglion.

M-neurone was able to produce its trigger discharge without the usual proprioceptive information from the ipsilateral leg. However, although the overall pattern of activity was normal, it appeared that the spike frequency attained during M's discharge was not as high as when both legs were able to kick (compare Figs 5 and 4C). We have insufficient data to quantify this, but it may be that the ipsilateral proprioceptive input directly or indirectly affects the intensity of the M-neurone's burst.

The C-neurones

In intact animals the C-neurones are thought to initiate the motor programme for a jump by synchronously activating FETi and flexor motoneurones at the very start of co-contraction. This brings the hindlegs into the correct position for jumping (Pearson & Robertson, 1981). Co-contraction is a separate phase of the motor programme which then follows. Because of the lower levels of arousal in dissected animals, this synchronous activation of FETi and flexors at the start of co-contraction never occurs. Instead, flexor activity may precede the start of extensor activity by as much as 500 ms. This proposed role of the C-neurones therefore cannot be established using intracellular recording techniques. Rather, we studied their activity during the remainder of the kick motor programme (i.e. the rest of co-contraction and the trigger activity) to determine whether they might contribute to other aspects of the jump.

Commencing some time during the initial flexion of the leg or co-contraction itself, the C-neurones (Fig. 6A) received a slow ramp depolarization. The spike frequency usually increased during this depolarization, reaching 100–200 Hz just before the cell was rapidly inhibited (Fig. 6B). From the EMG records alone it was not possible to determine whether this inhibition was coincident with that which occurred in the flexor motoneurones.

Because the burst in C corresponded to the burst in FETi and because C makes a strong excitatory connection to FETi (Pearson & Robertson, 1981), the C-neurones may have been responsible for generating the final, rapid burst in FETi just prior to the kick. However, several observations did not support this. During some kicks C did not have such a well-defined burst of spikes but instead discharged at a more or less constant frequency throughout the ramp, even though the accompanying EMG record still showed the rapid FETi burst concluding the co-contraction. During one kick (not shown), in which the C-neurone only fired spikes when at its maximum depolarization, this discharge preceded the rapid FETi burst by 90 ms. Moreover, C was then inhibited more than 10 ms before the last spike occurred in the FETi motoneurone and so could not be solely responsible for the continuing extensor burst. The most convincing observation, however, was that locusts were still able to kick when the meso/metathoracic connectives had been cut (see also Godden, 1975). The motor programme for kicking was not altered significantly by severing these connectives or the abdominal connectives, posterior to the metathoracic ganglionic mass. Thus, even without the descending input from the C-neurones, the rapid burst often occurred in FETi. This observation that kicking can still occur after cutting the meso/metathoracic connectives also clearly demonstrates that the C-neurones are not necessary for the generation of co-contraction as a whole. This finding was suggested too by the fact that C usually begins to spike only late in the co-contraction phase (Fig. 6B).

It would not be inconsistent to suggest that the M-neurones were responsible for the rapid inhibition of the C-neurones, based purely on the timing of this event. Clearly, investigation of this proposal must await simultaneous recordings from the C- and M-neurones. From anatomical evidence alone, however, we feel it is most unlikely that M would inhibit C because the M-neurones do not possess output branches in those regions of the mesothoracic ganglion where C is thought to receive its input.

Other interneurones

Our recordings from the M-neurones suggested that some interneuronal trigger system may account for the pattern of activity seen in M during kicking. With this in mind, we searched the meso- and metathoracic ganglia for cells which might be members of this trigger system. To date this has revealed 11 interneurones which discharged either during co-contraction or at the time the kick was triggered. Here we describe the characteristics of some of these interneurones. We were also interested in finding cells which may provide excitatory input to the C-neurones.

The responses of two mesothoracic interneurones during kicking are illustrated in Fig. 7. The cell in Fig. 7A displayed a characteristically high level of synaptic activity and responded to input from several sensory modalities. It received small EPSPs from the descending contralateral movement detector (DCMD) and was strongly depolarized by high frequency sounds and by tactile stimulation of the abdomen. During co-contraction it was depolarized in a ramp fashion and reached high spike frequencies (around 200 Hz) before being inhibited prior to the kick

(Fig. 7B). The M-neurones were not responsible for the inhibition of this neurone since flexor activity continued beyond the last spike in the cell. Also, the intensity of its inhibition was not of the same degree as that seen in flexors. This is the only interneurone we have found that consistently discharges strongly throughout co-contraction.

Another interneurone that received input from DCMD and auditory and tactile stimuli is shown in Fig. 7C. The similarity of its inputs, discharge pattern and some features of its anatomy (such as soma position within the ganglion, looped neurite leading from the soma, general position of its central branches, variability in structure of the large central processes and possession of a distinct lateral branch) with those of M suggests that it may possibly be the mesothoracic homologue of the M-neurone. (The presence of a descending axon in a mesothoracic cell and the absence of one in a metathoracic cell does not indicate a lack of homology between the two neurones – see Pearson, Boyan, Bastiani & Goodman, 1985.) This cell received a very strong depolarizing input during the final phase of co-contraction and at about the time M would be expected to discharge (Fig. 7D). High spike frequencies (also near 200 Hz) occurred during this depolarization.

At present we have identified seven interneurones within the metathoracic ganglion which also discharged in bursts prior to a kick (see Fig. 8A, C). These cells displayed similar patterns of activity during kicks, with their peak depolarizations

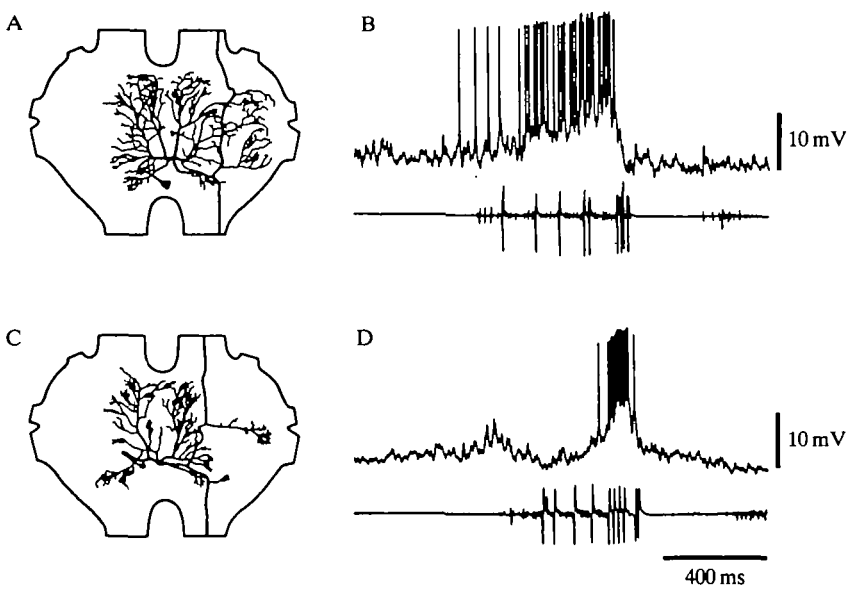

Fig. 7. Intracellular recordings from two mesothoracic interneurones during kicking. Top traces, intracellular recordings; bottom traces, electromyograms from the hindleg flexor and extensor tibiae muscles on the side to which the interneurones projected. (A),(B) Diagram of one interneurone's structure and its pattern of activity during kicking. (C),(D) Diagram of another interneurone and its discharge pattern during a kick. This cell fired at frequencies of about 200 spikes s^{-1} during its burst.

occurring 10–30 ms before the last extensor spike in the accompanying EMG (Fig. 8B,D). The cell in Fig. 8A also received strong DCMD and auditory input.

In addition, we have found local spiking interneurones within the metathoracic ganglion which discharged strongly at the end of co-contraction. The cell illustrated here (Fig. 9A) received a ramp-like depolarization and fired a high-frequency burst of spikes (about 200 Hz) just preceding and during the final burst in FETi (Fig. 9B).

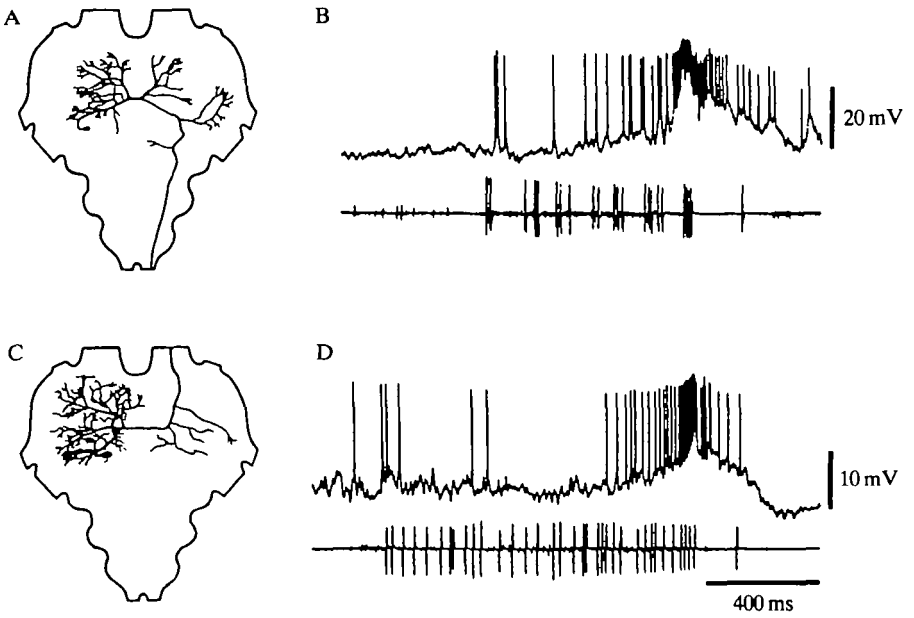

Fig. 8. Intracellular recordings from two metathoracic interneurones during kicking. Top traces, intracellular recordings; bottom traces, electromyogram recordings from the flexor and extensor tibiae muscles on the side on which the cells had their predominant output. (A),(B) Diagram of the anatomy of one interneurone and its activity pattern during kicking. (C),(D) Structure of another interneurone and its response during a kick.

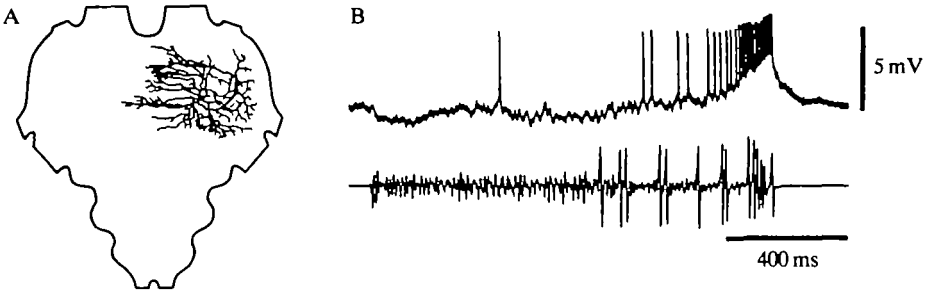

Fig. 9. Intracellular recording during kicking from a local interneurone within the metathoracic ganglion. (A) Diagram of the structure of the interneurone. (B) Recording of the cell's discharge pattern during a kick. Top trace, intracellular recording; bottom trace, electromyogram from the flexor and extensor tibiae muscles of the leg ipsilateral to the cell.

It was then rapidly inhibited. It was also noted that the injury discharge that accompanied the initial penetration of this neurone caused rapid and maintained extension of the ipsilateral hind tibia. Consequently this local interneurone may play a role in generating the rapid burst in FETi.

DISCUSSION

The major finding of this investigation is that the pattern of synaptic input to the M-neurones (the jump trigger neurones) is not consistent with earlier models of the jump circuitry, and instead suggests that there is a higher order trigger system which is activated just prior to a kick or jump. Support for this proposal comes from our identification of numerous interneurones which discharge either during co-contraction or at the time a kick is triggered. In this section we discuss the implications this new evidence has on our understanding of the triggering of jumping in locusts. First, however, we discuss the motor programme for jumping and kicking, and consider some additional features of the programme not described in previous studies.

The motor programme

The major features of the motor programmes for kicking in the dissected locust and jumping in intact locusts are similar. In each the programme commences with a period of flexor activity, 50–500 ms in duration, followed by a longer (300–800 ms) period of co-contraction of the flexor and extensor muscles (Fig. 1A). Flexor activity then ceases due to a rapid inhibition and the kick or jump follows. Godden (1975) has described these same features in jumping locusts, as have Heitler & Burrows (1977*a*) for kicks in animals less radically dissected than our preparation.

The programme for jumping does differ to a minor degree from kicking in that the periods of flexion and co-contraction, and consequently the duration of the entire programme, tend to be shorter. This was also noted by Pflüger & Burrows (1978). Another difference is that in intact animals the motor programme is often initiated by synchronous activation of the flexor and extensor motoneurones (Pearson & Robertson, 1981). In contrast, this never occurs in dissected locusts, presumably because of the animals' low levels of arousal. We do not feel that these differences are sufficient to suggest that kicking in our dissected preparation and jumping have separate underlying motor programmes. Indeed in view of the fact that dissected locusts most commonly kick with both legs simultaneously, as if performing a jump (Fig. 1B), we are satisfied that the motor programme we studied in our dissected preparations does represent the programme for jumping in the intact animal.

One aspect of the motor programme which has not been reported previously is that FETi often has two distinct phases of activity during co-contraction. This is evident in EMG records (Fig. 1) but is especially clear in intracellular recordings from FETi (Fig. 2B,C). The first phase is a weak tonic excitation which lasts throughout most of co-contraction, and during which FETi spikes at a quite constant frequency of 10–40 Hz. The second and concluding phase of FETi's activity is marked by a

stronger pulse-like excitation causing the motoneurone to fire a burst of spikes at a frequency of 70–130 Hz.

The important question arising from these studies of the motor programmes for jumping and kicking is how the patterns of activity seen in the flexor and extensor tibiae motoneurones are generated. The two phases of FETi's activity during co-contraction probably have separate origins: the tonic excitation stemming from peripheral reflex mechanisms (Heitler & Burrows, 1977*b*) and the final phasic excitation arising from a central neuronal system with bilateral outputs. The latter is suggested by the fact that the final burst in FETi very often commences simultaneously in both legs (Fig. 1B), and because the phasic excitatory input can still be seen in a leg which is prevented from kicking and cannot therefore be sending relevant proprioceptive information to the CNS (Fig. 5). The exact origin of this central input to FETi is unknown. Because the rapid FETi burst persists after the meso/metathoracic connectives have been cut and, in separate experiments, after the abdominal connectives have been severed, the neurones necessary for generating it must be restricted to the metathoracic ganglionic mass. This also excludes the C-neurones as the main source of the phasic input. Furthermore, the M-neurones, or whatever produces their depolarization, cannot be implicated because they sometimes discharge after the rapid burst in FETi has occurred (Fig. 4B). The interneurones described in Fig. 8 might contribute to the depolarization of FETi. They discharge at an appropriate phase and also possess branches which terminate in the lateral neuropile in the region where branches of FETi occur. It is probable that the local neurone shown in Fig. 9 is also involved. Spikes in this cell are known to excite FETi and during a kick it also discharges at an appropriate phase. It is possible that the proprioceptive input which builds up during the initial stages of the motor programme excites this hypothetical central system of interneurones, which in turn delivers a phasic excitation to both FETi motoneurones. Because successful kicks do occur in which the rapid FETi burst is lacking, the neuronal system responsible for the final extensor excitation cannot be an integral part of the kick trigger system itself, as the latter must still be producing its trigger pulse. Obviously, the FETi burst is not necessary for kicking or jumping to occur. When it does occur it probably ensures the maximum development of tension in the hind femora immediately prior to a jump and consequently increased force output during take-off.

The excitation which depolarizes the flexor motoneurones and initiates the entire motor sequence for kicking comes from an as yet unknown central source. Although the role of abdominal (and other) receptors excited by tactile stimuli in producing the flexor depolarization cannot be ruled out for every case, we have clear examples in which this depolarization occurred many seconds after the tactile stimulation of the body had ceased, i.e. there was no strict temporal association between the time of touching the insect's body and the onset of the flexor excitation. There were also times when a locust kicked several times in response to the one tactile stimulus and here the flexor motoneurone displayed a strong excitation during each kick. Once co-contraction is under way, however, sensory feedback from the hindlegs probably

maintains the depolarization of the flexors (Heitler & Burrows, 1977*b*). We have unpublished results which confirm this conclusion. When the tibia of one hindleg is held in the fully-flexed position (as it would be during co-contraction) the flexor motoneurones on that side receive a larger and longer reflex excitation following an extensor muscle spike than they do when the tibia is allowed to move freely. Presumably, during the train of FETi spikes in a co-contraction sequence this greater peripheral feedback would summate to produce the characteristic plateau depolarization seen in flexor motoneurones.

The importance of the central FETi–flexor connection in generating the pattern of activity in flexors is probably minor. As can be seen in Fig. 3, the membrane potential of each flexor motoneurone is already close to its maximal depolarization before FETi even becomes active. This central connection adds a little extra excitation to the flexors early in co-contraction, but as the motor programme continues its strength and importance rapidly diminish.

In all our recordings from flexor motoneurones the timing of the trigger inhibition which terminates flexor activity corresponds to the time at which the M-neurones become active. Although we have not yet recorded simultaneously from an M-neurone and a flexor during kicking, all the available evidence continues to support the proposal that the M-neurones act as trigger interneurones for kicking and jumping. This is not to say, however, that the M-neurones are the only source of the inhibitory input to flexor motoneurones. For example, the interneurone shown in Fig. 7C appears to be the mesothoracic homologue of the M-neurones and has the appropriate discharge pattern to produce flexor inhibition.

One aspect of the jump motor programme not observed in our preparation was the C-neurone's proposed synchronous activation of FETi and the flexor motoneurones at the beginning of co-contraction to lock the hind tibiae into full flexion (Pearson & Robertson, 1981). Unfortunately, this response never occurs in dissected animals and so we have not been able to test this proposal during the present intracellular study. The C-neurones, however, play no part in maintaining the simultaneous activity of the flexor and extensor muscles during the first phase of co-contraction (Fig. 6). Instead, they probably provide a general excitation to these muscles towards the end of co-contraction and so generate further tension in the hindlegs.

Triggering of the jump

It has been proposed that in order for the M-neurones to become activated and trigger the jump they must receive feedback from leg proprioceptors during the co-contraction phase (Steeves & Pearson, 1982). This input is postulated to bring the membrane potential of the M-neurones closer to threshold, thereby activating them directly or enabling them to discharge in response to auditory or visual stimuli. If this proposal were true, we would expect to see a gradual depolarization of the M-neurones during co-contraction leading to their rapid discharge. Yet we do not. Instead we see a maintained hyperpolarization terminated by a very sudden and strong depolarization (Fig. 4B–D).

This argues against the proposal that proprioceptive signals increase the excitability of the M-neurones during co-contraction and instead suggests that the excitatory input to each M-neurone arises from the sudden activation of a system of interneurones within the CNS. We refer to this system as the trigger system. The notion of a trigger system exciting the M-neurones is supported to some extent by our discovery of other interneurones which discharge near the end of the co-contraction phase (Figs 7–9), although their activity may serve a different function such as generating the final phase of FETi's excitation (Fig. 9). The trigger system, and other neurones necessary to generate the motor programme, must be located within the metathoracic ganglionic mass because the main features of the motor programme can be produced after severing the meso/metathoracic connectives or the abdominal connectives. The neurones in the mesothoracic ganglion, whose activity suggested they were involved in generating the kick, must either play a minor, supporting role in producing the motor programme or play another role, such as providing input to the flight system before a jump or generating appropriate movements in the pro- and mesothoracic legs prior to take-off.

That the timing of the M-neurone discharge falls under central rather than peripheral control would allow more precise control of motor events. The maintained hyperpolarization of the M-neurones throughout co-contraction ensures these cells remain inactive and thus prevents them from inhibiting the flexor motoneurones until sufficient tension has developed in the legs. Furthermore the rapid, strong depolarization of both M-neurones would ensure the synchronous inhibition of the flexor motoneurones of both hindlegs and thus the synchronous timing of leg extension during jumping. This synchrony would be more difficult to achieve if M-neurone activation depended on a gradual depolarization by proprioceptive input during the co-contraction phase. This is because synchronous activation would require the membrane potential of each M-neurone to be in an identical state with respect to spike threshold and each to receive identical synaptic input.

Although the M-neurones, based on their known connections and discharge pattern, definitely inhibit the flexors and so contribute to triggering the kick or jump, we have not yet been able to establish whether they are necessary for triggering. Attempts to hyperpolarize an M-neurone, thereby preventing its burst of activity during kicking, have failed because we have not been able to deliver enough negative current to the cell to silence its discharge. Since there are a number of other interneurones which burst at the time the M-neurones do (Figs 7C, 8A,C) it is possible that M acts in concert with some of these to produce the trigger inhibition in the flexors.

In summary, our data indicate that an important event in triggering the jump is the strong, pulse-like depolarization of the M-neurones at the end of co-contraction. We propose that an interneuronal system (the trigger system) is responsible for the generation of this input. The future direction of studies on the neuronal mechanisms for jumping must now be to determine the cellular connections between neurones of this trigger system.

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