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Interneurons Coactivating Hindleg Flexor and Extensor Motoneurons in the Locust

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Summary. 1. Using intracellular staining and recording techniques in the ventral nerve cord of locusts, we have identified a pair of large interneurons which monosynaptically excite hindleg flexor and extensor tibiae motoneurons. The somata and input processes of these interneurons are in the mesothoracic ganglion and each makes its connections to hindleg motoneurons via a large axon running in the meso-metathoracic connective contralateral to its soma.

2. We refer to these interneurons as C-neurons because single action potentials in one interneuron often caused the coactivation of the fast extensor tibiae motoneuron and a variable number of flexor tibiae motoneurons in one hindleg. The probability of activity in a C-neuron activating hindleg motoneurons depended on the position of the tibia, being highest when the tibia was flexed.

3. Coactivation of the fast extensor tibiae motoneuron and a number of flexor motoneurons could also be elicited by a variety of stimuli in intact animals when the tibiae were close to full flexion. This resulted in the tibiae being locked into full flexion and, very often, to the initiation of the co-contraction phase of the jump. We refer to this synchronous activation of hindleg flexor and extensor motoneurons in intact animals as the cocking response.

4. We propose that the C-neurons function to produce the cocking response. Consistent with this proposal is that the C-neurons receive input from all those sensory sources which evoke the cocking response.

5. A general feature of the interneuronal organization in insects derived from this and other investigations is the existence of individual interneurons for the production of single aspects of a behavior. By controlling transmission of sensory information to motoneurons via these unique interneurons, the same sensory stimulus can evoke a variety of motor response. This concept is discussed in relation to the jumping system of the locust.

Introduction

The application of intracellular recording and staining techniques in the central nervous system of insects has led to rapid progress in our knowledge of the neuronal events underlying relatively simple behaviors in these animals (Burrows 1977; Pearson 1980; Pearson et al. 1980). One of the best understood of these behaviors is the locust jump. This behavior consists of three phases: 1) a rapid movement of the hindleg tibia to full flexion, 2) a period of co-contraction in the hindleg flexor and extensor tibiae muscles, and 3) a sudden inhibition of flexor activity to allow the rapid shortening of the extensors and the release of energy stored in elastic elements (Heitler and Burrows 1977a). Recent studies have given insight into the cellular mechanisms responsible for the latter two phases (Heitler and Burrows 1977b; Pearson et al.1980) but not the first phase. Thus, the most outstanding problem is to determine the mechanism for the production of the initial rapid flexion of the hindleg tibia preceding the co-contraction phase.

During the course of our early studies on the nervous control of the locust jump, we noticed that the initial tibial flexion was often associated with an almost synchronous activation of hindleg flexor and extensor tibiae motoneurons (Pearson 1977). This preliminary observation led to the suspicion that single interneurons might exist for producing the simultaneous activation of the flexor and extensor motoneurons. Furthermore, if such interneurons did exist, then we anticipated considerable sensory convergence onto

Abbreviations: *DCMD* descending contralateral movement detector; *EMG* electromyogram; *FET* fast extensor tibiae

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them, since the coactivation and the corresponding rapid tibial flexion could be initiated by a variety of sensory stimuli. By chance, we recently discovered a pair of interneurons in the mesothoracic ganglion which coactivates hindleg flexor and extensor tibiae motoneurons and receives input from the visual, auditory and tactile sensory systems. In this paper, we describe the morphological and physiological properties of the interneurons, and discuss the proposal that they function to produce a rapid tibial flexion in preparation for a jump. We refer to these interneurons as C-neurons because of their property of coactivating hindleg flexor and extensor motoneurons.

Materials and Methods

Experiments were performed on male and female *Locusta migratoria* obtained from a long established colony at the University of Alberta. No consistent differences were observed related to sex. All experiments were performed at room temperature (22–24° C).

Intracellular Recording and Staining. Animals were mounted dorsal side up on a corkboard with the hindlegs positioned so that the tibia could move freely in an approximately horizontal plane. Pairs of 50 μm copper wire electrodes were implanted into the flexor and extensor tibiae muscles of both hindlegs. These electrodes were used either to record the electrical activity in the muscles or to stimulate antidromically the motor axons supplying the muscles.

The procedure for recording intracellularly from interneurons and motoneurons in the meso- and metathoracic ganglia has been described in detail elsewhere (Pearson et al. 1980). Briefly, the dorsal surfaces of the ganglia were exposed by removing the gut and overlying muscles. The ganglia were placed on a rigid stainless steel plate and constantly perfused with saline (NaCl – 147 mM, KCl – 10 mM, $CaCl_2$ – 4 mM, NaOH – 3 mM, Hepes – 10 mM). Extracellular recording electrodes (75 μm silver wires) were placed either on the pro-mesothoracic connectives to monitor the activity in the descending contralateral movement detector (DCMD) interneurons, or on the meso-metathoracic connectives to monitor activity in interneurons whose axons run in these connectives. The neuropile processes of motoneurons and interneurons were penetrated with microelectrodes which had a resistance of 40 to 60 megohms when filled with 1 M potassium acetate. Penetration of the fast extensor tibiae motoneuron was recognized by the 1:1 correspondence of intracellularly recorded spikes and rapid extension movements of the tibia, and by its antidromic activation with electrical stimulation of its terminals in the extensor tibiae muscle. Penetration of a flexor tibiae motoneuron was recognized by a large excitatory postsynaptic potential evoked by antidromic stimulation of FETi. These EPSPs followed the extracellular field potential from the central process of FETi by about 3 ms. Flexor motoneurons were also recognized by flexion movements of the tibia in response to evoked spike activity in the penetrated neuron. The classification of a flexor motoneuron as fast, intermediate or slow was judged by the movements of the tibia in response to a spike or to trains of spikes.

The dye Lucifer yellow (Stewart 1978) was used to stain interneurons intracellularly. Electrodes filled with 4% Lucifer yellow had a resistance well over 100 megohms. The dye was injected by the application of a constant hyperpolarizing current for 5 to 15 min and allowed to diffuse for 45 min following the injection. The ganglia were fixed in 4% paraformaldehyde (pH 7.2) for 1 h then dehydrated and cleared in methyl salicylate. Stained neurons were photographed and drawn as soon as they had cleared.

EMG Recording. Recordings were made from the flexor and extensor tibiae muscles in one hindleg in a situation where the animal was free to move in an area about 50 cm in diameter. The recording electrodes were pairs of 50 μm copper wires insulated except for the ends implanted into the muscles. To minimize interference in the flexor EMG from activity in the large extensor muscle, the flexor electrodes were inserted just under the surface of the ventral cuticle near the proximal end of the tibia (Fig. 11). The distance between the two electrodes of any one pair was about 1 mm. To elicit the first phase of the jump (tibial flexion), a hand was suddenly moved close to the animal. Since this response rapidly habituated with repeated stimuli, stimuli were presented at a rate not greater than one every 30 s. Dishabituation could sometimes be achieved by lifting the animal from the substrate to induce flight or by provoking a jump by stimulation of the abdomen.

Results

1. Structure of the C-Neurons

The structure of one C-neuron is shown in Fig. 1. Its soma was in the posterior-lateral region of the mesothoracic ganglion approximately midway between the dorsal and ventral surfaces of the ganglion. The neurite leaving the soma ran in an anterior-medial direction and eventually gave rise to a thick central process running directly across the midline. Two anteriorly directed processes arose from each side of this central process. The two anterior processes arborized extensively and extended to within 100 μm of the anterior margin of the ganglion. A few branches arising from these processes extended up towards the dorsal surface of the ganglion. Contralateral to the soma, the main central process narrowed and gave rise to an axon which initially ran laterally, then turned posteriorly, increased in diameter and left the ganglion in the meso-metathoracic connective. The axon lay close to the lateral edge of this connective and its diameter was approximately 15 μm. A very fine axon-like process arose from the main axon as it turned posteriorly in the mesothoracic ganglion. This process divided into two: one branch terminated in the lateral neuropile near nerve 5 and the other in the anterior neuropile. The depth of these two processes, as well as most of the more centrally located arborizations, was approximately 120 μm from the dorsal surface of the ganglion.

In the metathoracic ganglion, the main axon divided soon after entering the ganglion (about 200 μm from the anterior margin). One branch ran in a posterior-lateral direction and terminated with extensive arborizations in the lateral neuropile close to nerve 5. The other branch continued in a posterior direction, then turned laterally to end with numerous ar-

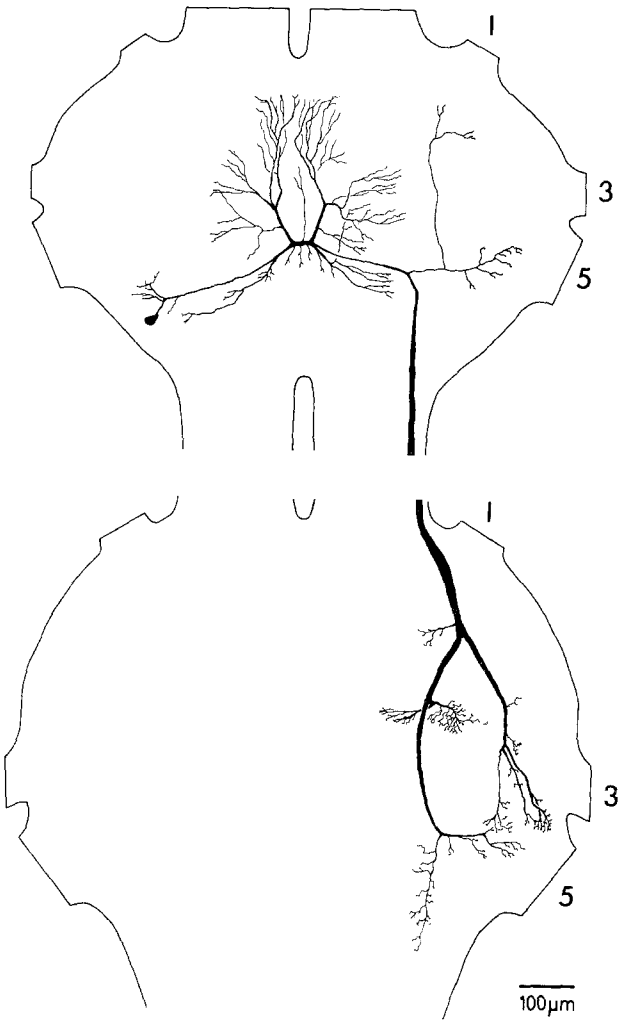

Fig. 1. Drawing of a C-neuron in the mesothoracic (top) and metathoracic (bottom) ganglia in *Locusta migratoria*. The soma is located approximately midway between the dorsal and ventral surfaces of the mesothoracic ganglion. The processes in the mesothoracic ganglion form an almost uniplanar sheet about 120 μm below the dorsal surface of the ganglion. An axon arises from the main central process and leaves the ganglion in the lateral part of the meso-metathoracic connective. Note the fine bifurcating branch leaving the main axon in the mesothoracic ganglion. In the metathoracic ganglion, the main axon divides and the two branches eventually terminate in the lateral neuropile close to the region through which the main processes of FETi and flexor motoneurons run

borizations located medial and slightly posterior to the terminal arborizations of the first branch. A prominent process arose from the second branch soon after the point of bifurcation. This process arborized immediately and the terminals extended to within 100 μm of the midline. Soon after the second branch turned laterally, a fine process arose which ran posteriorally to terminate close to the fused abdominal ganglia. The depth of the terminal arborizations within the metathoracic ganglion was from 100 to 130 μm from the dorsal surface.

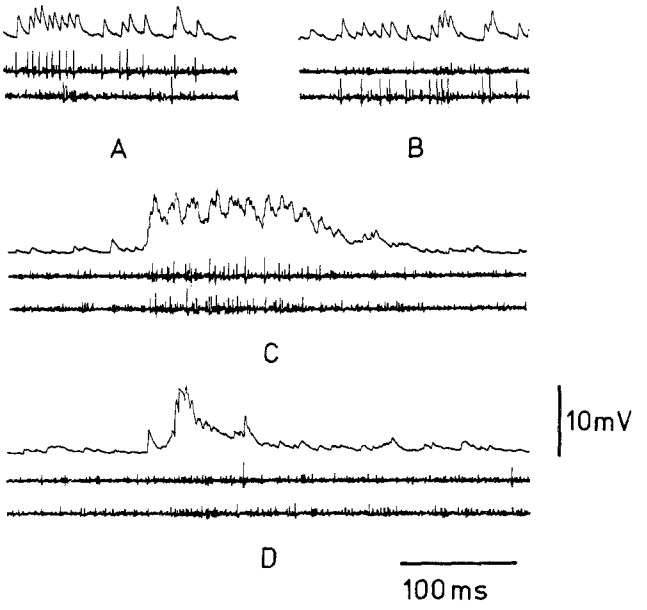

Fig. 2A–D. Inputs to a C-neuron from a variety of sensory sources. **A, B** Visual, large EPSPs (top) are evoked by activity in the left DCMD (large spike middle trace) and in the right DCMD (large spike bottom trace). **C** Auditory, depolarization produced by a brief hissing sound. **D** Tactile, depolarization produced by blowing air on the abdomen from a small pipette. Top traces: intracellular recording from the large midline process of a C-neuron in the mesothoracic ganglion. Middle and bottom traces: activity recorded extracellularly from the left and right pro-mesothoracic connectives respectively

The structure of a C-neuron was determined in the mesothoracic ganglion in seven animals. In two of these animals, sufficient dye was injected to reveal clearly the structure in the metathoracic ganglion. The basic structure was similar in all animals and there was no major structural variability between animals. The lack of structural variability together with the similarity of physiological properties from animal to animal (see below) indicates there are only two C-neurons (one for each side) in each animal. This conclusion is supported by our observations in three animals that multiple staining of neurons with the physiological properties used to identify the C-neurons only ever revealed one C-neuron, or one C-neuron and its mirror image homologue.

2. Identification of an Intracellular Penetration of a C-Neuron

A number of physiological criteria allowed the identification of an intracellular penetration of a process of a C-neuron within the mesothoracic ganglion. First, a C-neuron was encountered only after the electrode had progressed some distance into the posterior

midline region of the ganglion, and its penetration was usually not accompanied by the generation of spike activity (injury discharge). Second, the C-neuron received large EPSPs (from 3 to 6 mV in amplitude) from both descending contralateral movement detector (DCMD) interneurons, and was depolarized by a hissing sound and by tactile stimulation of the abdomen (Fig. 2). None of these inputs alone ever evoked spike activity in the C-neuron. Third, the current and voltage thresholds were high. The minimum constant current necessary to evoke action potentials was within the range of 12 to 15 nA and the voltage threshold was between 18 and 20 mV. Voltage threshold was measured by evoking just-threshold EPSPs by electrical stimulation of both pro-mesothoracic connectives. These three criteria were sufficient to identify reliably a penetration of a C-neuron. However, one further criterion was a 1:1 correspondence of evoked action potentials with a large spike ($\sim$60% of the amplitude of the DCMD spike) recorded extracellularly from the meso-metathoracic connective (Figs. 4 and 5).

A possible source of confusion in identifying a penetration of a C-neuron was that interneurons situated slightly more dorsally than the C-neurons receive similar input from the DCMDs and from auditory and tactile stimulation. However, these interneurons have a significantly lower current threshold (4 to 6 nA) than the C-neurons and their activity is not associated with large spikes in recordings from the meso-metathoracic connectives. They also receive large EPSPs (latency $\sim$25 ms) in response to just-threshold stimulation of either of the hindleg extensor tibiae muscles. These EPSPs were never seen in the C-neurons.

3. Coactivation of Flexor and Extensor Motoneurons by the C-Neurons

Action potentials in a C-neuron initiated by the intracellular application of depolarizing current pulses to the main central process in the mesothoracic ganglion often resulted in activation of the fast extensor tibiae motoneuron (FETi) and a variable number of flexor tibiae motoneurons of one of the hindlegs (Fig. 3). There were three important features of the C-neuron activation of FETi and the flexor motoneurons: 1) FETi and the flexor were activated almost simultaneously, 2) a burst of activity in the C-neuron (discharge rate greater than 0.5/s) never evoked more than one action potential in the motoneurons, and 3) activation of the motoneurons occurred more readily when the tibia was held close to full flexion.

Simultaneous intracellular recordings from a C-neuron and FETi, or a C-neuron and a flexor motoneuron, demonstrated that activity in the C-neuron produced short constant latency EPSPs in both FETi and the flexor motoneurons (Figs. 4 and 5). The short latency ($\sim$1.4 ms) from the spike in the axon of the C-neuron to the EPSPs indicates that the connections from the C-neuron to FETi and flexors are monosynaptic. The EPSPs in flexors occurred in the absence of spikes in FETi and therefore could not have been produced via the excitatory pathway from FETi to flexors. Our conclusion that the C-neuron connections to FETi and flexors is monosynaptic is consistent with the anatomy since the locations of the lateral terminations of a C-neuron in the metathoracic ganglion correspond very closely to the locations of the main processes of FETi and the flexor motoneurons (Wilson 1979; Pearson et al. 1980). The amplitude of the EPSPs in FETi when the C-neuron was discharging at about 5 impulses/s was in the range of 4 to 6 mV, while the EPSP amplitude in flexors varied from 2 to 6 mV depending on the type of flexor motoneuron. The amplitude was large in fast flexors and small in the slow and intermediate flexors.

A characteristic of the EPSPs in FETi and flexor

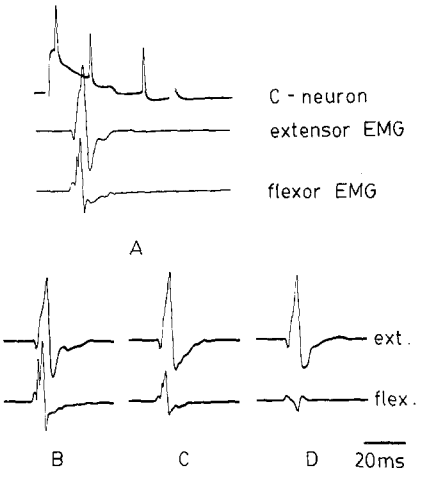

Fig. 3A–D. Coactivation of FETi and flexor tibiae motoneurons by action potentials in a C-neuron. **A** Action potentials evoked in a C-neuron (top trace) by a 12 nA depolarizing current pulse caused excitation of FETi (middle trace) and a number of flexor tibiae motoneurons (bottom trace). Activity in FETi and flexor tibiae motoneurons was monitored by extracellular recordings (EMGs) from the extensor and flexor tibiae muscles. Note that a single action potential in the C-neuron produced the coactivation of the motoneurons. **B, C** Other examples of activation of FETi (top) and flexor tibiae motoneurons (bottom). The activation of the C-neuron is not shown in these records. The variation in the response in the recording from the flexor muscles (see also **A**) indicates that a varying number of flexor motoneurons can be activated by the C-neuron. **D** In this example, no flexors were activated and the response in the flexor record (bottom trace) is cross-talk from the activity in the large extensor muscle

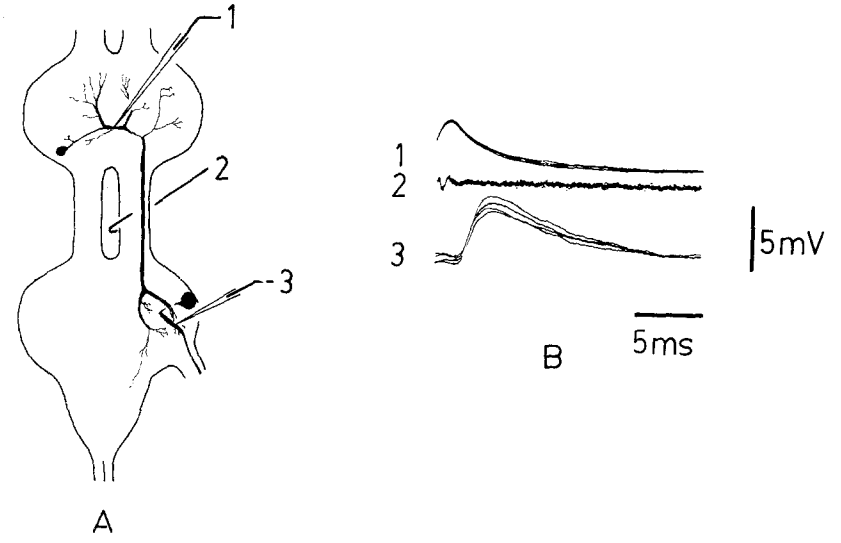

Fig. 4A, B. Excitatory connection of a C-neuron to FETi.
A Diagram of experimental arrangement. One C-neuron and FETi were penetrated simultaneously and activity in the C-neuron recorded extracellularly from its axon in the meso-metathoracic connective.
B 1:1 correspondence of spikes in the C-neuron (top trace) and large EPSPs in FETi (bottom trace). Note the short latency of the EPSPs and the lack of variability in the time of their occurrence. These records were obtained when the C-neuron discharges at about 5 impulses/s in response to the application of a constant depolarizing current

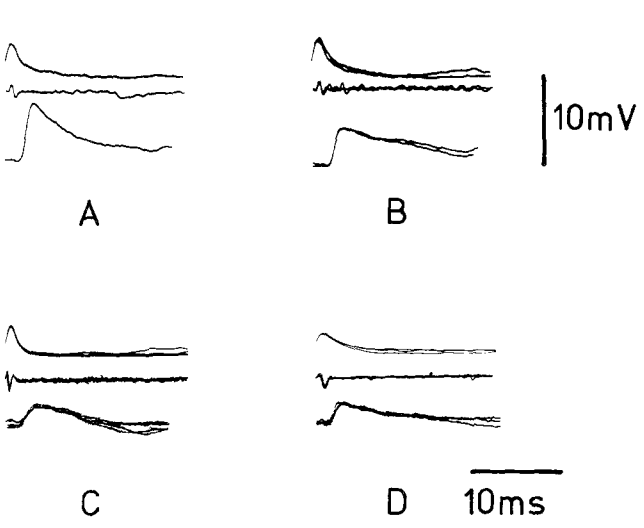

Fig. 5A–D. Excitatory connection of C-neurons to flexor motoneurons. The experimental arrangement was the same as shown in Fig. 4 except a flexor motoneuron rather than FETi was penetrated with electrode #3. Spikes in the C-neuron (top traces) evoked EPSPs (bottom traces) in a fast flexor (**A** and **B**), an intermediate flexor (**C**) and in a slow flexor (**D**). The middle traces show the action potential in the C-neuron recorded extracellularly from the meso-metathoracic connective. **A** EPSP evoked in a fast flexor by a single impulse in a C-neuron. **B** EPSPs evoked in the same fast flexor when the C-neuron was discharging at about 2 impulses/s. In comparing **A** and **B**, note the significant depression of the EPSP amplitude with repetitive activity. The latency of the EPSPs in all flexor motoneurons was constant and very similar to the latency of the EPSPs evoked in FETi by the C-neurons (compare with Fig. 4)

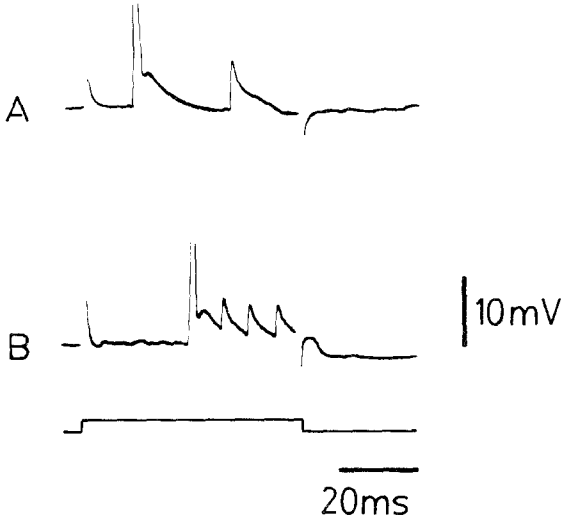

Fig. 6A, B. Depression of EPSPs in FETi with repetitive activity in a C-neuron. One C-neuron and the FETi excited by the C-neuron were penetrated simultaneously (see Fig. 4A) and a 60 ms depolarizing current pulse (bottom trace) was injected into the C-neuron every 5 s. The current pulse (varying in amplitude from 12 to 15 nA) evoked varying numbers of action potentials in the C-neuron from trial to trial, as indicated by the varying number of EPSPs recorded in FETi. Traces **A** and **B** show the intracellularly recorded activity in FETi for two separate trials. In all trials, the first EPSP was sufficiently large to evoke an action potential in FETi. Subsequent EPSPs in any one trial never evoked further action potentials. Comparison of **A** and **B** shows the depression of EPSP amplitude with increased discharge rate of the C-neuron

motoneurons was that they depressed with repetitive activation of the C-neuron. This depression prevented bursts of activity in a C-neuron from initiating more than one action potential in the motoneurons. In Fig. 6, a 60 ms current pulse to a C-neuron evoked a number of EPSPs in FETi, the first of which initiated an action potential. Subsequent EPSPs were too small to evoke further action potentials. Since the voltage threshold of FETi was approximately 10 mV, it follows that the amplitude of the first EPSP must

have been larger than this. In the example shown in Fig. 6, the amplitude of the second EPSP was 7 mV when occurring 25 ms after the first, and 4.5 mV when occurring 8 ms after the first.

Because simultaneous penetrations of a C-neuron and FETi, or a C-neuron and a flexor motoneuron, were not easily achieved and rarely persisted for long periods of time, we extracellularly stimulated the main axon of the C-neuron in the meso-metathoracic connective to investigate in more detail the time course of depression and also the effects of tibial position on transmission from the C-neurons to FETi. The extracellular stimulating electrode consisted of a glass-insulated silver wire approximately 0.05 mm at the tip. The electrode tip was positioned on the lateral edge of one of the meso-metathoracic connectives. Three quite distinct EPSPs were recruited in FETi with increasing stimulus strength to the ipsilateral meso-metathoracic connective. The first was small (1–2 mV) with a short constant latency, the second was approximately the same amplitude as the first but had a long variable latency, and the third was large (6–8 mV) with a short constant latency. We believe the third EPSP results from the stimulation of the axon of the C-neuron because its amplitude is similar to the directly evoked EPSPs and its occurrence usually corresponded to the excitation of flexor motoneurons. Flexor motoneurons were never activated by the stimulation of the connective in the absence of the occurrence of the large EPSP in FETi.

Repetitive stimulation of the connective caused a depression in the amplitude of all three components of the compound EPSP. Figure 7 shows the time course of depression for the short latency component of the compound EPSP (see Fig. 8). Immediately following one EPSP, the amplitude of a second EPSP was reduced to almost 50%. Subsequently, there was a slow recovery from depression, with full recovery taking approximately 5 s. Stimulation of the meso-metathoracic connective evoked compound EPSPs in flexor motoneurons, and these EPSPs also depressed with repetitive stimulation of the connective. The time course of this depression was comparable to that for depression in FETi.

One interesting characteristic of the coactivation of FETi and the flexor motoneurons by intracellular activation of a C-neuron was that it usually occurred only when the tibia was close to full flexion. Correspondingly, intracellular recordings from FETi and the flexor motoneurons showed that the amplitude of the evoked EPSPs with meso-metathoracic connective stimulation was strongly dependent on the femoral-tibial angle (Figs. 8 and 9). As the tibia was extended, the EPSP amplitude was reduced. The sharpest decline occurred just as the tibia was moved away from full flexion. Beyond an angle of about 20°, the decline with further extension was less marked. We did not investigate in detail the mechanism for the dependence of EPSP amplitude on tibial position, but two observations indicate the involvement of a postsynaptic mechanism. The first was that, in a few animals, tibial flexion produced a slight depolarization of FETi. The second was an increase in the current threshold for spike initiation with tibial extension. Extension resulted in an almost parallel shift upwards in the strength duration curve (Fig. 10), and this shift was most marked just as the tibia was moved away from full flexion. Although these data indicate that postsynaptic mechanisms are involved in producing the decline in EPSP amplitude with tibial extension, they do not exclude the involvement of a presynaptic inhibitory component.

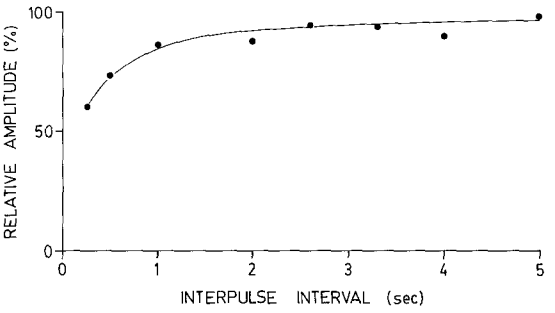

Fig. 7. Time course of recovery from depression of compound EPSPs evoked in FETi by electrial stimulation of the ipsilateral meso-metathoracic connective. The connective was stimulated by a pair of pulses and the relative amplitude of the EPSP evoked by the second pulse is plotted against the interpulse interval. Each data point represents the average of at least 5 measurements. The evoked EPSPs were similar to those shown in Fig. 8. The amplitude measurement was for the first large component of the compound EPSP

4. The Cocking Response

The initial phase of the locust jump is a rapid movement of the hindleg tibia to full flexion followed by a period of co-contraction of the flexor and extensor tibiae muscles (Heitler and Burrows 1977a). EMG recordings from the extensor and flexor tibiae muscles of one hindleg showed that there were two motor patterns associated with the initial tibial flexion. The first consisted of an almost simultaneous activation of the fast extensor tibiae motoneuron and a variable number of flexor tibiae motoneurons followed by asynchronous activity in flexor motoneurons (Fig. 11). This response was elicited only when both tibiae were initially at, or close to, full flexion. Despite activation of the fast extensor tibiae motoneuron, the

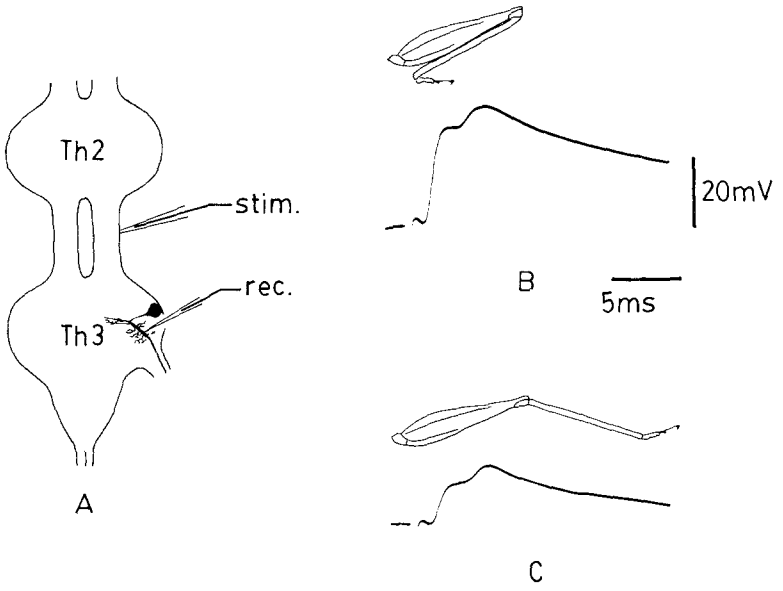

Fig. 8A–C. Decrease in amplitude of electrically evoked EPSPs in FETi with extension of the tibia. **A** Experimental arrangement – one meso-metathoracic connective was stimulated electrically while recording intracellularly from the ipsilateral FETi.
B, C Compound EPSPs recorded in FETi when the tibia was fully flexed (**B**) and fully extended (**C**). Note the large reduction in the amplitude of the first component of the compound EPSP. Stimulus strength was the same in **B** and **C**. To prevent spiking in FETi, a constant hyperpolarizing current of 5 nA was applied throughout the experiment

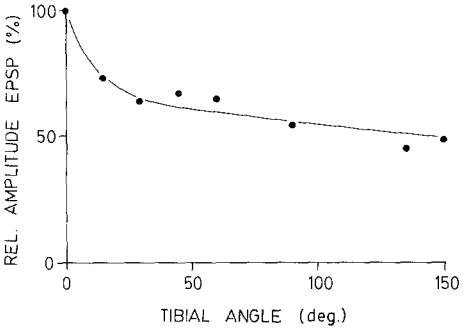

Fig. 9. Relationship between the relative amplitude of the first component of the electrically evoked EPSP in FETi (see Fig. 8) and the position of the tibia. Full flexion corresponds to a tibial angle of 0°

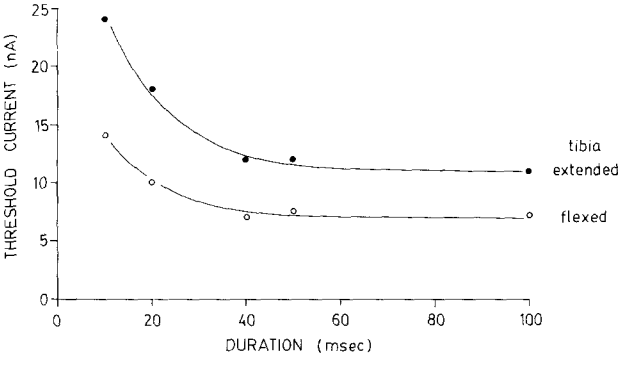

Fig. 10. Increase in current threshold for spike initiation in FETi with tibial extension. The strength-duration curve was shifted upwards when the tibia was moved from fully flexed (open circles) to fully extended (closed circles)

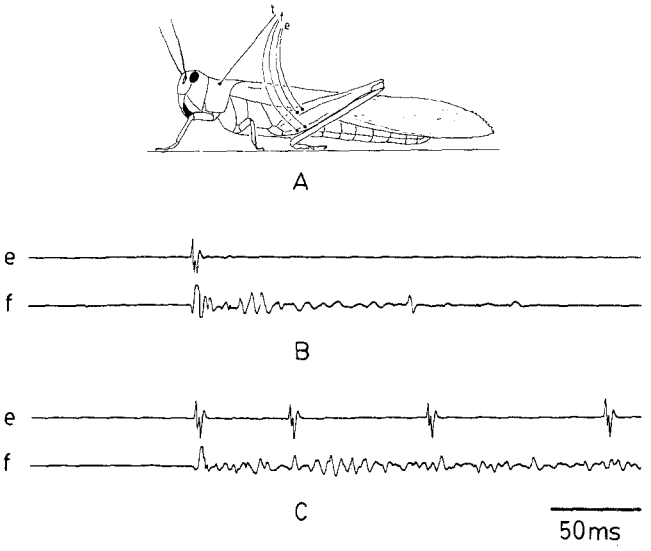

Fig. 11A–C. Coactivation of FETi and flexor tibiae motoneurons in a tethered animal. **A** Diagram showing the position of the tethering lead (t) and the recording electrodes in the flexor (f) and extensor (e) muscles. **B** Movement in the animal's visual field evoked a single spike in FETi (top trace, e) and an almost simultaneous activation of flexor motoneurons (bottom trace, f). Activity continued in flexor motoneurons for almost 200 ms following the coactivation. **C** Repetitive activity in FETi following the initial coactivation of FETi and flexor by movement in the animal's visual field. Note that there was no obvious cross-talk in the flexor EMG from activity in FETi. The variation in the form of the initial flexor response in **B** and **C** indicates that a variable number of flexor motoneurons were coactivated with FETi. The coactivation of FETi and flexors occurred when the tibia was within about 20° of full flexion, and its occurrence resulted in a rapid movement of the tibia to full flexion

tibia moved to full flexion, or was held at full flexion if that was the initial position. This is because the flexors enjoy a considerable mechanical advantage when the tibia is close to full flexion (Heitler 1974). The second motor pattern consisted of a burst of activity in flexor motoneurons and a delayed excitation of FETi which was not associated with a synchronous activation of flexors. This response could be elicited with the tibia initially at any angle. An important difference between the two types of motor pattern was that in the first a number of flexor motoneurons were simultaneously activated at the beginning of the response, whereas in the second the initial activation of the different flexors was asynchronous.

We refer to the first of these two motor patterns, i.e. the simultaneous activation of FETi and flexor motoneurons, as the cocking response. Provided the tibia were at or close to full flexion, this response could be elicited by a variety of stimuli such as a slight movement in the animal's visual field, a loud sound (e.g. a hissing sound or a hand clap), or by a puff of air on the animal's body. The cocking response was initiated only in aroused animals, arousal being judged by the frequency of occurrence of spontaneous jumping and walking, and it habituated rapidly with repeated presentations of the same stimulus.

Discussion

The main result of this study was the identification of a pair of large interneurons, the C-neurons, which monosynaptically excited hindleg flexor and extensor tibiae motoneurons of the locust. In this discussion, we first consider the function of the C-neurons, and then discuss some of the features of these interneurons in comparison with those of other identified interneurons in the locust thoracic ganglia.

1. Function of the C-Neurons

We propose that the C-neurons function to coactivate the fast extensor tibiae motoneuron and the flexor tibiae motoneurons of the hindlegs in response to a variety of stimuli such as movements in the visual field, a loud sound, and wind on the body (Fig. 11). We have termed this synchronous activation of the hindleg flexor and extensor tibiae motoneurons the cocking response because it results in a rapid movement of the tibia to full flexion and, in many instances, it is the first event in the co-contraction phase of the jump. In this section, we consider the evidence to support the proposal that the C-neurons mediate the cocking response, as well as discussing problems which must be resolved before this proposal can be fully accepted.

There are a number of characteristics of the C-neurons which are consistent with them evoking the cocking response. First, they make strong monosynaptic excitatory connections to both FETi and the fast flexor tibiae motoneurons (Fig. 4 and 5). These connections are sufficiently strong that a *single* action potential in a C-neuron can simultaneously activate FETi and some of the flexors (Fig. 3). In the cocking response, FETi and a variable number of flexors are also activated simultaneously (Fig. 11). Second, intracellular activation of the C-neurons more readily elicits action potentials in FETi and flexor motoneurons when the tibiae are close to full flexion. Correspondingly, the cocking response occurs only when the tibiae are at or close to full flexion. Third, the C-neurons receive input from a variety of sensory sources (Fig. 2), all of which will produce the cocking response in aroused animals. Fourth, the input to the C-neurons is bilaterally symmetrical and the magnitudes of the inputs from different sources are similar in both C-neurons. We presume, therefore, that both C-neurons are normally activated together and participate in a bilaterally symmetrical behavior. Provided both hindleg tibia are at or close to full flexion, the cocking response occurs almost at the same time in both legs and the overall behavior is bilaterally symmetrical. One further reason, albeit negative, for considering that the C-neurons mediate the cocking response is that we know of no behavior other than the rapid tibial flexion associated with the cocking response which involves synchronous activation of FETi and flexor tibiae motoneurons. It is conceivable that the C-neurons function to coactivate FETi and flexor motoneurons throughout the co-contraction phase of the jump, but the fact that spikes in FETi and flexors do not occur synchronously during this phase makes this function unlikely.

Despite these reasons supporting the proposal that the C-neurons produce the cocking response, there are a number of features of these neurons which are seemingly inconsistent with this proposal. One problem is that in dissected preparations, auditory, visual and tactile stimuli never evoked spike activity in the C-neurons, yet all these stimuli can give the cocking response in normal aroused animals. This problem would be resolved if dissection leads to an increase in the voltage threshold of the C-neurons or to a decrease in the amplitude of the synaptic inputs to these neurons. Consistent with this suggestion is that the cocking response (i.e. synchronous activation of FETi and flexor motoneurons) was never observed in dissected preparations in response to sensory stimuli (unpublished observations).

Two further problems arise because the C-neurons and the jump trigger neurons, the M-neurons (Pear-

son et al. 1980), receive similar visual, auditory and tactile inputs, and the C-neurons appear to have a higher voltage threshold than the M-neurons for spike initiation. The first of these is that an external stimulus capable of activating the C-neurons to give a cocking response would be expected to also activate the M-neurons. If this occurred, then the inhibition of the flexor motoneurons by the M-neurons would oppose the excitation of the flexors by the C-neurons. There are a number of possible resolutions to this problem. Firstly, there is presynaptic inhibition of some inputs to the M-neurons which may function to prevent these inputs alone initiating spike activity in the M-neuron (Pearson and Goodman, 1981). Secondly, the voltage threshold at the spike initiating site may actually be lower in the C-neurons than in the M-neurons and our measurement of a relatively higher threshold is due to our recording site in the C-neurons being relatively closer to the site of synaptic input. Thirdly, in normal behaving animals, the excitability of the C-neurons may be much higher than that measured in dissected preparations and our measurements of voltage threshold may be meaningless in a behavioral context. The second problem is that an external stimulus capable of activating the C-neurons to give a cocking response would be expected to activate the C-neurons if used to trigger a jump. If the latter occurred, then the excitatory input of the C-neuron to flexor motoneurons would antagonize the inhibitory input to these motoneurons from the M-neurons at the time of triggering. This problem would be resolved if the activity of the C-neurons, or the synaptic transmission from the C-neurons, was suppressed just prior to triggering the jump. One possibility is that this suppression could be produced by proprioceptive feedback during the co-contraction phase of the jump, but there is presently no evidence for this.

In summary, then, there are compelling reasons to support the proposal that the C-neurons produce the cocking response, and none of the difficulties raised in the foregoing discussion forces us to reject this proposal. Clearly, it would be useful to obtain further data, particularly on the activity of the C-neurons immediately preceding a cocking response and when the jump is triggered by external stimuli. This may be possible by recording extracellularly from the axons of the C-neurons in the meso-metathoracic connectives provided the spikes from the large axons can be unambiguously identified.

2. Functional Specificity of Interneurons in the Locust Jump System

The question we wish to consider in this section is the extent to which identified interneurons in the jumping system of the locust have unique behavioral roles. From our data so far, the C-neurons and the M-neurons each appear to have only one function: the C-neurons causing a rapid flexion movement of the tibia in preparation for a jump, and the M-neurons triggering the jump. These two interneurons share a number of features. Both have high thresholds, receive inputs from a variety of sensory sources and make strong synaptic connections to motoneurons. By contrast, the descending contralateral movement detectors (DCMDs) receive input predominantly from a single sensory modality, are very easily activated, make only weak monosynaptic connections onto motoneurons and appear to have multiple functions. The DCMDs connect monosynaptically to the C-neurons, to the M-neurons and to flight motoneurons. Therefore, they have the potential to produce a rapid tibial flexion preceding a jump, to trigger a jump, and to modify activity in flight motoneurons following a jump. The important question now arises of how transmission of information from multifunctional DCMDs is controlled to allow these interneurons to produce different behaviors. We have found that this control depends to a large extent on sensory input from leg receptors. First, the production of the rapid tibial flexion by movements in the visual field requires the tibia to be at, or close to, full flexion. Tibial flexion progressively increases the amplitude of the EPSPs from the C-neurons to flexor and extensor motoneurons (Fig. 9) and thereby increases the probability that C-neuron activity (which we presume can be initiated by DCMD bursts in intact animals) will evoke action potentials in the motoneurons. Following the initiation of the co-contraction phase of the jump, proprioceptive feedback increases the excitability of the M-neurons to allow their activation by the DCMDs (Steeves and Pearson, in preparation). We must assume that during co-contraction, either the C-neuron excitability is reduced to prevent activation by DCMD or that there is suppression of synaptic transmission from the C-neurons to the motoneurons (see Sec. 1 above). Finally, flight activity is suppressed by tarsal contact with the ground. Therefore, the DCMDs cannot have any influence on flight until the animal has initiated a jump and inhibitory influences from leg receptors to the flight system have been removed.

From these studies of the locust jump, we are beginning to get a glimpse of some organizational principles of interneurons controlling simple behaviors in insects. The most important of these is that visual, auditory and tactile sensory systems initiate behavior by acting via interneurons specialized for producing single aspects of behavior rather than acting directly onto motoneurons. Another is that inter-

neurons associated with sensory systems can produce a variety of behaviors depending on the animal's state at any particular time.

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