

PROCESSING OF MECHANOSENSORY SIGNALS IN LOCAL REFLEX PATHWAYS OF THE LOCUST

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Summary

The processing of mechanosensory signals responsible for the reflex adjustment of the posture or movement of the legs of the locust is described in terms of the actions and connections of identified neurones. Signals can be followed from the major classes of exteroceptors of a leg, through their various integrative stages in the central nervous system to their emergence as specific patterns in known motor neurones. Particular emphasis is placed on the integrative roles of two classes of local interneurones. The spiking local interneurones map the leg as a series of overlapping receptive fields and reverse the sign of the afferent input. The nonspiking local interneurones control the output of the motor neurones by the graded release of chemical transmitter and can adjust the gain of a local reflex depending on the position and movements of the joints of that leg.

The reflex movements of one leg must not impair the stability of the animal and must therefore be influenced by events at the other legs. Populations of intersegmental interneurones convey sensory information from one segment to another to ensure such coordination. These interneurones do not produce stereotyped intersegmental reflexes but, instead, alter the performance of a local reflex in a distant leg by making synaptic connections with nonspiking local interneurones. These connections change the effectiveness of the outputs to the motor neurones and consequently the local reflex. The local interneurones therefore play a crucial role both in the production of local reflexes and in the integration of these actions with the movements of the other legs.

Introduction

For an animal to walk successfully on natural terrain, it is essential that the mechanisms in the central nervous system which generate the appropriate movements of the legs pay heed to the sensory signals generated by those movements and by the mechanical stimuli that might occur if the legs meet an external obstruction. This implies that the animal must be equipped with suitable proprioceptors to monitor the events at the joints of its limbs and exteroceptors to monitor possible contacts with external objects. These receptors must then make appropriate connections in the central nervous system so that adjustments can be made to a motor pattern. The neural mechanisms that underlie such reflexes are

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explored here for movements of the hind leg of the locust, because exteroceptors that respond to mechanical stimuli are sparingly distributed on the surface of the cuticle, and many are innervated by a single sensory neurone. Furthermore, the neurones in the central nervous system can be treated individually, so that the way that the surface of the leg is functionally mapped can be described in terms of the properties and connections of known neurones. These observations can then be directly related to the behaviour of the animal. In this way the neurobiology gives insights into the behaviour, and conversely the behaviour gives insights into many of the more puzzling properties of the neurones which would otherwise remain unexplained.

The integrative problem

The surface of a hind leg bears a variety of sensilla ranging from the singly innervated trichoid sensilla, that provide information about touch, to the multiply innervated basiconic sensilla, that may act as chemoreceptors and touch receptors. The trichoid sensilla (hairs) vary in stiffness and length, indicating a probable ability to detect different stimuli, and in the density of their distribution. Campaniform sensilla occur singly or in groups and give information about stresses in the cuticle. Internal receptors such as the chordotonal organs, multipolar joint receptors and strand receptors act as proprioceptors. This considerable battery of receptors give rise to some 10 000 sensory axons which converge onto the metathoracic ganglion, one of a chain of segmentally arranged ganglia consisting of the ganglion for the metathoracic segment fused with those of the first three abdominal segments.

The large number of sensory neurones contrasts with the small number of motor neurones that are responsible for controlling the movements of a hind leg. For example, the most distal three joints are controlled by no more than 25 motor neurones and the leg as a whole by less than 100. Most of these motor neurones can be treated as identified individuals whose shape and many of whose physiological characteristics are known. Nevertheless, the behaviour of locusts, which can balance on a twig, march as a band of hoppers, jump to escape predators or to launch into flight, and kick accurately to fend off an adversary, attests to the range, delicacy and precision of movements that are possible with this small complement of output elements.

Considerable convergence of sensory information from a leg and substantial integration within the central nervous system must therefore occur before a change in the output of the motor neurones is effected. Moreover, the spatial information provided by the receptors must be preserved because specific local reflexes of a leg can be evoked by stimulation of specific arrays of receptors. This is most obvious for the proprioceptors at a joint, where an imposed movement of that joint will readily call forth a reflex that opposes the imposed movement. It is less immediately obvious that the spatial information provided by the tactile hairs should be preserved. For example, touching hairs on the dorsal surface of the tibia

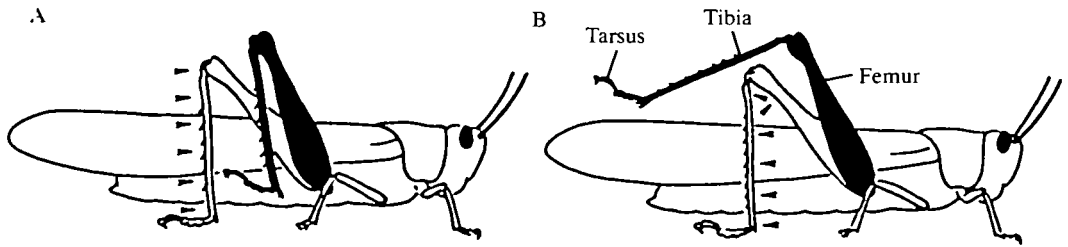


Fig. 1. Local reflexes of a hind leg elicited by touching specific arrays of mechanoreceptors (arrowheads) on that hind leg. (A) Touching hairs on the dorsal surface of the tibia causes the hind leg to move about three joints to the position indicated by the silhouette. (B) Touching hairs on the ventral surface leads to a different reflex movement. (Based on Siegler & Burrows, 1986.)

causes the trochanter to levate and move the femur forwards, the tibia to flex and the tarsus to levate (Fig. 1A). Touching hairs a short distance away on the ventral surface of the tibia causes the opposite sequence of movements (Fig. 1B). These are just two of many movements of a leg that occur depending on the location of the hairs that are touched (Siegler & Burrows, 1986). The result of these reflexes is that the leg moves away from the stimulus.

These movements illustrate the problems of sensory integration that must be solved in the central nervous system and the computations that must be performed for motor control. At present, these local reflexes are most conveniently studied in an animal that is not moving, but eventually it must be asked how they modify voluntary locomotory patterns.

These reflexes are organized locally by the metathoracic ganglion and still occur when it is isolated from other ganglia in the segmental chain. To produce a sensible movement, the local reflexes of one leg can, however, only take place if the movements of the other legs are appropriate. It is therefore necessary to consider two levels of organization. First, the organization within the metathoracic ganglion of the pathways responsible for the local movements and, second, the intersegmental pathways that place the local reflexes in the broader context of the movements of the other legs.

The integrative machinery

The sensory neurones from exteroceptors on the distal parts of a hind leg all end in the ipsilateral half of the metathoracic ganglion, but some more proximal receptors have axons that, after branching, project to the mesothoracic ganglion (Bräunig *et al.* 1981). All the motor neurones that innervate a hind leg originate in the ipsilateral half of the metathoracic ganglion. There is therefore the possibility that afferents from one leg may contact directly motor neurones of that leg, but interneurones must be involved in the transfer of information to the leg on the opposite side of the segment and, in general, to legs of an adjacent segment.

Each half of the ganglion contains some 1000 interneurones, many of which are

local interneurons with branches restricted entirely to this ganglion. These local interneurons form two classes; one whose members normally generate action potentials (spiking local interneurons) and a second whose members normally do not (nonspiking local interneurons). Additional interneurons have axons that project to other ganglia. The axons of these intersegmental interneurons either end in an adjacent ganglion or project to more distant ganglia, giving rise to axonal branches in each of the ganglia through which they pass. Some of these interneurons could thus provide information to the other legs and to the brain about the mechanical stimuli and movements of a hind leg, while others carry signals processed in the brain and elsewhere to the metathoracic ganglion so that the correct motor commands are issued to a hind leg.

Neurosecretory cells may modify the operation of these pathways both by adding a longer time scale to their processing and by allowing the same elements to participate in different pathways. Each ganglion contains a group of neurosecretory neurones and usually a neurohaemal organ associated with the median nerve. DUM (dorsal unpaired median) neurones either have axons that project to both legs (Plotnikova, 1969) or are local neurones with all their processes within the ganglion (Goodman *et al.* 1980). The neurones with peripheral axons alter both the contractions of the muscles and the release of transmitter from some of the motor neurones. They do not, however, appear to make recognizable output synapses in a ganglion (Watson, 1984) and thus are unlikely to affect the operation of the neuronal circuits. This may be the role of the local DUM neurones. Injection of one of the substances likely to be released by these neurones can bring about the expression of certain motor patterns (Sombati & Hoyle, 1984; Stevenson & Kutsch, 1988).

These basic sets of neurones provide the framework from which the motor pathways must be organized. To understand this organization, it is necessary to address the following four questions.

(1) How are the sensory signals processed so that spatial information is preserved?

(2) How are the small number of leg motor neurones controlled to provide movements that are coordinated and precise?

(3) How are the reflex movements of one leg adjusted so that they are appropriate for the wide variety of different circumstances that the locust must face?

(4) How are the local reflex pathways modified so that the movements of one leg are matched appropriately to those of the other legs?

Processing of afferent signals

The axonal branches of the sensory neurones synapse with several different classes of neurone in the central nervous system. Prominent amongst these are spiking local interneurons, of which three populations have so far been studied (Siegler & Burrows, 1984; Nagayama, 1989). These populations are defined

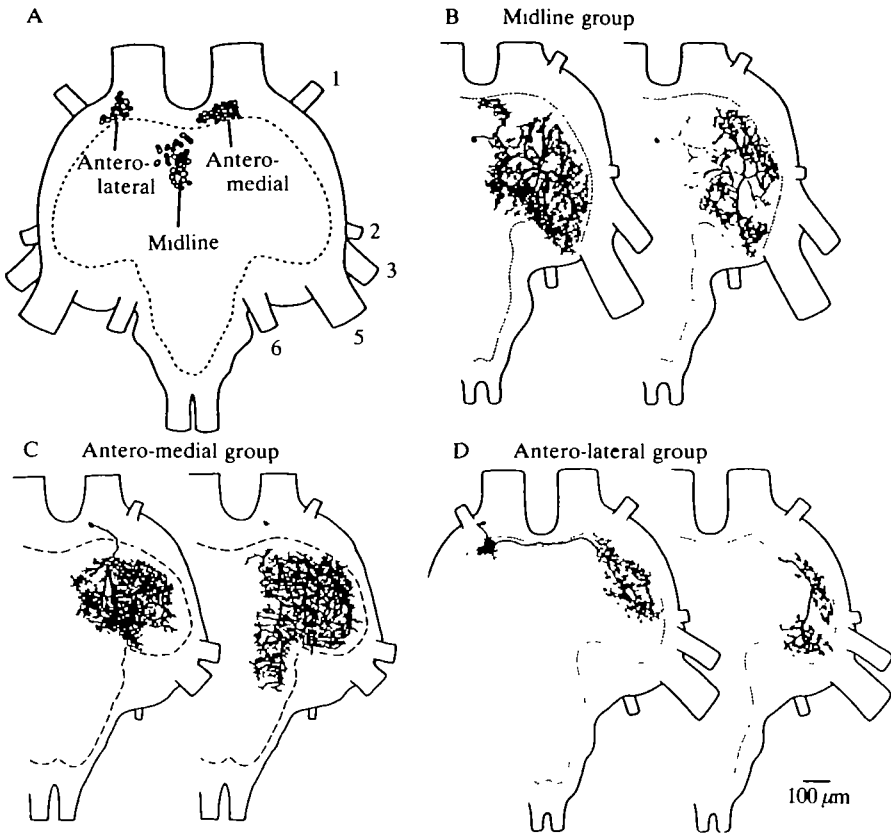


Fig. 2. The morphology of spiking local interneurons belonging to three populations in the metathoracic ganglion. (A) The groups of cell bodies belonging to members of the three populations. (B) An interneurone in the midline group. (C) An interneurone in the antero-medial group. (D) An interneurone in the antero-lateral group. Each neurone has two fields of branches; a ventral field shown on the left, and a dorsal field shown on the right. The interneurons were stained by the intracellular injection of cobalt (Brogan & Pitman, 1981) and its subsequent intensification with silver (Bacon & Altman, 1977). They were then drawn with the aid of a drawing tube attached to a compound microscope. The outline of half the metathoracic ganglion is shown with nerves 1–6 numbered. The dashed line indicates the edge of the neuropile.

primarily according to the position of their cell bodies (Fig. 2A). One group has cell bodies at the ventral midline, the second at the antero-lateral and the third at the antero-medial part of the ganglion. All have their neuropilar branches arranged in two fields (Fig. 2B–D). The ventral field consists of numerous, fine branches in the neuropile to which afferents from tactile hairs also project (Pflüger *et al.* 1981), and the dorsal field consists of fewer, more varicose branches in the neuropile to which motor neurones and other interneurons project (Watkins *et al.* 1985). Within a particular population, individual interneurons are con-

structed from the same basic ground plan (Fig. 2B–D). For example, interneurons of the midline group all have their primary neurites in ventral commissure II, ventral branches in the ventral association centre and a single branch that links the ventral and dorsal fields in the perpendicular tract (Siegler & Burrows, 1984). The different patterns of second- and higher-order branches and the different regions of neuropile in which the fine branches end give each neurone its characteristic and recognizable shape (Burrows & Siegler, 1984).

The two fields of branches of interneurons in the midline group differ in the distribution of their synapses as revealed with the electron microscope. Input synapses predominate on the ventral branches, while output synapses predominate on the dorsal branches (Watson & Burrows, 1985). This suggests a partition of function in these interneurons with the ventral branches processing the sensory input which is then delivered as a spike code to the dorsal branches through the single linking process. Nevertheless, output synapses do occur on the ventral branches and input synapses do occur on the dorsal branches, indicating that local processing may well modify either the sensory processing or the output pathways.

The afferents from a leg, like those from the cercus of a cricket (Murphey, 1981), the ear of tettigoniids (Oldfield, 1983; Römer, 1983; Römer *et al.* 1988) or the prolegs of larval tobacco hornworm moths (Peterson & Weeks, 1988), appear to project into the central nervous system in a precise pattern. Afferents from bristle hairs on a middle leg of a cricket, for example, have projections that are related to their position (Johnson & Murphey, 1985). Hairs that are dorsal project to neuropile that is more dorsal and medial than is the neuropile to which the ventral hairs project. Similarly, hairs that are progressively more distal project to progressively more lateral areas of neuropile. Finally, hairs that are on the anterior face of the leg project more anteriorly than do those on the posterior face. By contrast, hairs that are progressively more distal on a locust middle leg project to progressively more posterior regions of the metathoracic neuropile (Pflüger *et al.* 1981). More precise staining of individual hairs that can be recognised from locust to locust is needed to resolve this issue.

Physiological evidence shows that the afferents from tactile hairs on a hind leg make direct synaptic connections with these interneurons (Siegler & Burrows, 1983). All tactile hairs that have been tested make connections with at least one member of the midline population of interneurons. Each spike from a hair afferent evokes a depolarizing synaptic potential in a particular spiking local interneurone (Fig. 3A). The potential is altered by changing the membrane potential of the interneurone (Fig. 3B) and is caused by an apparent increase in the conductance of the membrane (Fig. 3C). These chemically mediated EPSPs, which often elicit spikes in the interneurone, summate but show no facilitation and consistently follow each afferent spike with a latency that indicates a monosynaptic connection.

The afferents also make direct synaptic connections with nonspiking interneurons (Laurent & Burrows, 1988*a*), with intersegmental interneurons whose axons project anteriorly (Laurent & Burrows, 1988*b*) and with some leg moto-

neurones (Laurent & Hustert, 1988). The connections are always excitatory. The divergent pattern of connections means that the sensory inflow will be processed by neurones with a range of different integrative properties and that parallel and

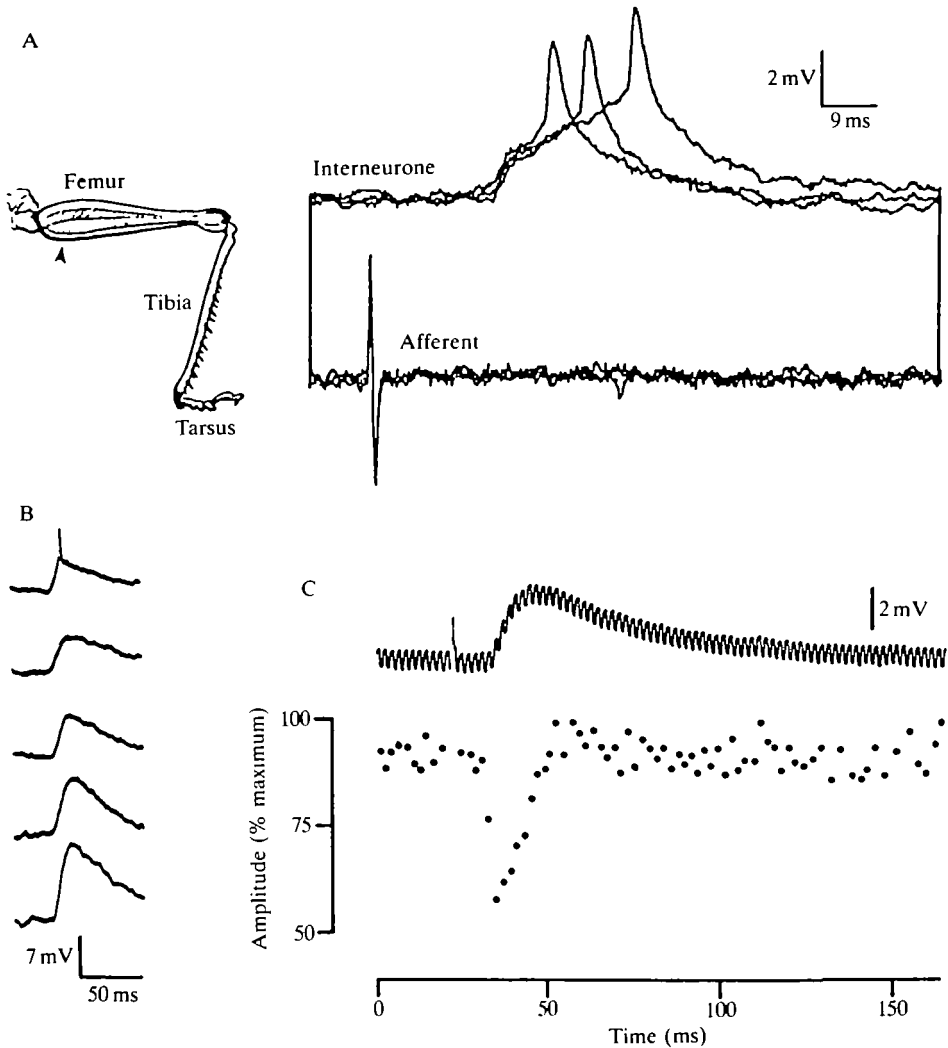


Fig. 3. Afferent connections with spiking local interneurons of the midline group. (A) Moving a single hair on the ventral surface of the proximal femur (arrowhead) evokes consistent EPSPs that elicit spikes in the interneurone. Intracellular recordings were made from the interneurone in the metathoracic ganglion. The spike of the hair afferent was recorded by placing a blunt microelectrode filled with saline over the cut end of the hair shaft. (B) The amplitude of the EPSP evoked by electrical stimulation of a single hair is progressively enlarged when the membrane of the interneurone is hyperpolarized with a steady current. (C) Constant current pulses are injected into the interneurone during an EPSP evoked by electrical stimulation. The graph shows that the voltage change caused by these pulses falls during the rising phase of the EPSP. (Fig. 3B,C is based on M. V. S. Siegler & M. Burrows, unpublished.)

distributed processing will dominate. It implies further that most of the sensory signals must pass through one or more layers of interneuronal processing before reaching the motor neurones. Direct connections with leg motor neurones are uncommon but may be more widespread than was initially thought, as they are present in larval stages of certain holometabolous insects (Weeks & Jacobs, 1987). They provide a means by which the local interneuronal processing can be circumvented, presumably so that rapid adjustments of the motor output can be made.

Receptive fields

Each afferent from a tactile hair synapses with more than one neurone in the central nervous system and, in turn, each neurone involved in the processing of afferent signals receives inputs from more than one receptor. The receptive field of a neurone can therefore be defined by the distribution of receptors which, when stimulated by natural mechanical stimuli, lead to an effect in the neurone under study (Fig. 4). For the majority of local interneurones, the excitatory regions of their receptive fields can be defined according to the pattern of monosynaptic connections made by the afferents themselves. This means that their fields are defined with great accuracy because intracellular recording allows access to an EPSP generated by any afferent, even though it may not evoke spikes. For the inhibitory regions, and for the greater part of the receptive fields of the other neurones, particularly the motor neurones, it is the connections they receive from the local interneurones that define their receptive fields. These connections are influenced by other pathways so that the extent of a field is not static and depends on the prevailing activity of the animal.

Each spiking local interneurone receives inputs from a particular array of receptors (Fig. 4A–E). An interneurone in the midline group is excited by receptors on one hind leg (Burrows & Siegler, 1985), but an interneurone in the antero-medial group has a receptive field that may include the other hind leg and one or both of the middle legs (Nagayama, 1989). These inputs must be provided by interneurones and not by direct afferent pathways. Many of the interneurones have inhibitory regions to their receptive fields which are often complementary to the excitatory regions; touching the posterior surface of the tibia may cause excitation of a particular interneurone, whereas touching the anterior surface causes inhibition. The juxtaposition of inhibitory and excitatory regions enhances the boundary. For many interneurones the two regions may not be contiguous. Such an organization may sharpen the effects of a stimulus moving across a leg, or exclude the action of the excitatory region when a certain movement is performed. For example, the ventral tarsus is often an inhibitory region and, as a consequence, the effectiveness of any excitatory region is diminished when the tarsus is placed on the ground during the stance phase of locomotion.

Deflection of an individual tactile hair evokes a high-frequency burst of afferent spikes but the transfer function to an interneurone has yet to be determined. For example, is the gain set so high that a single afferent spike will evoke a spike in an

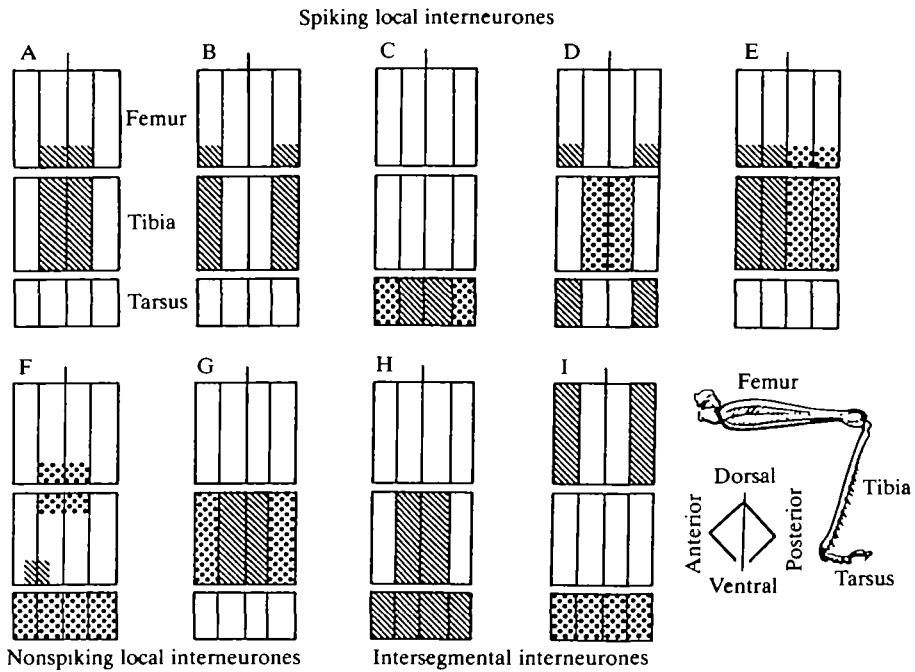


Fig. 4. Receptive fields of interneurons that process exteroceptive signals from a hind leg. (A–E) Five spiking local interneurons in the midline group. (F,G) Two nonspiking local interneurons. (H,I) Two intersegmental interneurons with axons that project anteriorly. The surface of the leg is displayed as if the leg were opened by an incision along the ventral midline and then laid flat. Each part of the leg is divided into four quadrants as shown in the diagram at the bottom right. Hatched areas represent excitatory regions of the receptive fields formed by direct connections of the afferents, and stippled areas represent inhibitory regions formed by interactions in the central nervous system.

interneurone, or is it set much lower so that inputs from many hairs must be summed before an interneurone will spike? It is also pertinent to ask whether all receptors within a field connect with an interneurone and whether those that do connect all have the same effect on the interneurone. Hairs of a particular type that respond to one feature of a stimulus may connect with an interneurone, whereas those coding different features may not, as in the connections of cricket cercal hairs with giant interneurons (Shepherd *et al.* 1988). Hairs at the centre of a field might have more powerful inputs than those towards the periphery, as in the connections of cat hair afferents with spinocervical tract neurones (Brown *et al.* 1987), or strategically placed hairs near a joint might be more effective than those further away. Clues that different weighting may be given to the various inputs comes from receptors that monitor the movements of the spurs at the distal end of the tibia (Burrows & Pflüger, 1986). The two spurs on one side of the tibia evoke EPSPs of different amplitudes in a particular spiking local interneurone. The amplitudes of these EPSPs may reflect a difference in their ability to evoke spikes

in the interneurone, or simply the location of their synapses relative to the site of the recording electrode.

Two important features of the receptive fields of the spiking local interneurones emerge from these studies. First, one region of a hind leg will be represented by several interneurones. Parallel and distributed processing therefore occurs within this class of interneurone. Second, these connections ensure that the spatial information provided by the receptors is preserved. For example, information from receptors on the dorsal tibia needed for the correct execution of the reflex in Fig. 1A is preserved by the interneurone in Fig. 4A and information from the receptors on the ventral tibia needed for the reflex in Fig. 1B is preserved by the interneurone in Fig. 4B.

The receptive fields of nonspiking interneurones (Fig. 4F,G), intersegmental interneurones (Fig. 4H,I) and hence of motor neurones, on which all these interneurones converge, show many of the same characteristics. For example, the nonspiking interneurones have excitatory regions of their receptive fields derived from the direct connections of afferents (Laurent & Burrows, 1988a), and inhibitory regions that are often the opposite of the excitatory regions of specific spiking local interneurones. This suggests that there may be inhibitory interconnections between the two classes of local interneurone.

Controlling the motor output

If the afferent signals diverge to many interneurones of different types, then how does the processed sensory information converge onto the motor neurones? To answer this question it is necessary to determine the output connections of the local interneurones. This has been achieved by simultaneous intracellular recordings from a local interneurone and a postsynaptic neurone.

The spiking local interneurones of the midline group make inhibitory output connections. A few of these connections are made directly with motor neurones (Fig. 5A,B) (Burrows & Siegler, 1982), but more common connections are made with nonspiking local interneurones (Fig. 5C,D) (Burrows, 1987a) and with intersegmental interneurones (Laurent, 1987a, 1988). Immunocytochemistry shows that some midline spiking local interneurones, first characterized physiologically and then labelled with Lucifer Yellow, stain with a polyclonal antibody raised against γ -aminobutyric acid (GABA) (Watson & Burrows, 1987). Moreover, the output connections of one of these interneurones can be blocked with picrotoxin (Watson & Burrows, 1987).

A mechanical stimulus to a particular array of tactile hairs will therefore evoke spikes in a given spiking local interneurone that are each followed after a short and constant latency by IPSPs in particular nonspiking local interneurones and intersegmental interneurones (Fig. 5C,D). The connections appear to be monosynaptic and to depend on the occurrence of a spike in the presynaptic interneurone. No evidence for graded synaptic transmission from these spiking local interneurones has yet been uncovered. Injecting current into these interneurones to

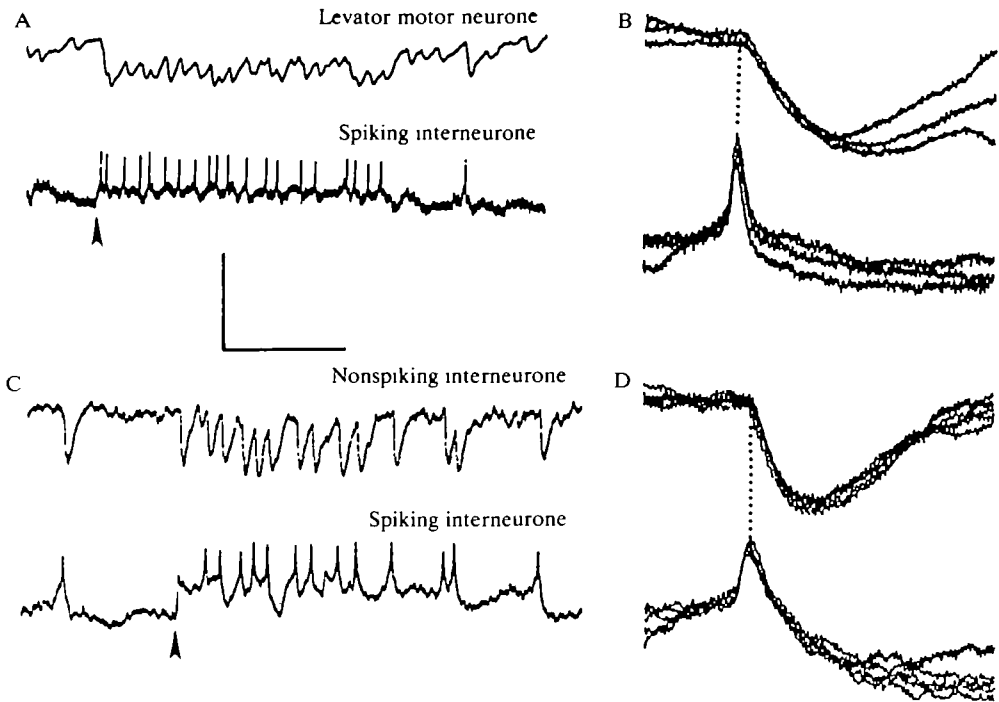


Fig. 5. Output connections of spiking local interneurons in the midline group. (A) Touching hairs on the ventral surface of the distal tibia evokes spikes in an interneurone and a hyperpolarization of the levator tarsi motor neurone. (B) Each spike in the interneurone is followed at a constant latency by an IPSP in the motor neurone, as shown in the superimposed records. (C) Touching hairs on the dorsal surface of the distal femur evokes spikes in a different interneurone and IPSPs in a nonspiking local interneurone. (D) Superimposed records show that the connection is direct. Calibration: horizontal, A 500 ms, B 6 ms, C 250 ms, D 10 ms; vertical, A int 5 mV, mn 25 mV, B int 1.6 mV, mn 6 mV, C nonspiking int 10 mV, spiking int 4 mV, D nonspiking int 4 mV, spiking int 2 mV.

produce spikes does not generate a readily detectable movement of the leg. This is in sharp contrast to the effects of injecting a similar pulse of current into a nonspiking local interneurone. The current does not evoke spikes in the interneurone and yet alters the output of several motor neurones that cause a readily observed movement (Burrows & Siegler, 1978; Burrows, 1980).

The nonspiking local interneurons make direct excitatory and inhibitory connections with motor neurones (Fig. 6). Injecting more current into a nonspiking local interneurone evokes a progressively greater change in the amplitude of the membrane potential of the postsynaptic neurone (Fig. 6A). Small changes in the voltage of the presynaptic nonspiking interneurone are sufficient to effect these changes and presynaptic spikes are not required. The changes in the postsynaptic neurone are sustained for as long as the depolarization is maintained in the nonspiking interneurone. The effects are caused by the sustained release of

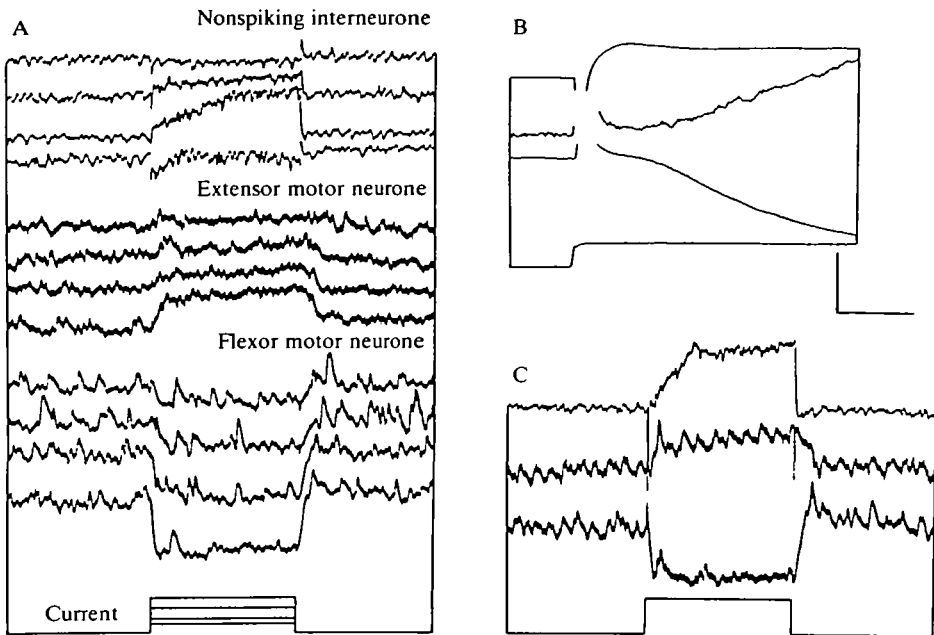


Fig. 6. Graded synaptic transmission from a nonspiking interneurone to two motor neurones. (A) Depolarizing the interneurone with four successively larger pulses of current does not evoke spikes but causes a graded depolarization in the slow extensor tibiae motor neurone and a parallel but graded hyperpolarization in a flexor tibiae motor neurone. (B) The onset of the responses of the two postsynaptic neurones appears to be the same. (C) Common depolarizing potentials occur in the motor neurones. The amplitude of these potentials in the flexor is greatly reduced during the evoked hyperpolarization caused by the nonspiking local interneurone, but in the extensor they are unaffected. Calibration: vertical, interneurone 10 mV, motor neurones 2 mV, current 16 nA; horizontal, A, C 250 ms, B 6.4 ms.

a chemical transmitter from the nonspiking local interneurone (Burrows & Siegler, 1978). The net result of this method of intercellular signalling is that sustained shifts of the presynaptic membrane potential will result in tonic release and that single synaptic potentials of either polarity produced by inputs to the nonspiking local interneurone are potentially capable of altering release.

When two postsynaptic motor neurones are recorded together, one may be depolarized and the other hyperpolarized by the action of a single nonspiking local interneurone, particularly if they are motor neurones that act antagonistically during locomotion (Fig. 6). The two postsynaptic neurones show the same parallel and graded effects as the current is increased in the presynaptic interneurone, thereby posing the question as to whether the nonspiking interneurone makes monosynaptic connections of opposite sign with each. The latencies to the depolarization of one neurone and to the hyperpolarization of the other appear to be the same, but the graded nature of the signals makes the onset of change difficult to measure accurately (Fig. 6B). A measurement of conductance changes

in the postsynaptic neurones, however, suggests that in the example shown only the inhibitory effect is caused by a direct action of the nonspiking interneurone (Fig. 6C). Any conductance change caused directly by the nonspiking interneurone can be gauged by its effect on the common depolarizing synaptic potentials that occur in both the postsynaptic neurones. During the evoked hyperpolarization of one postsynaptic neurone, but not during the evoked depolarization of the other, the amplitude of these potentials is markedly reduced (Fig. 6C). This is interpreted to indicate that the inhibition results from the release of transmitter directly onto just one postsynaptic neurone and that the depolarization of the other neurone results from disinhibition in a more complex pathway. For example, the latter postsynaptic neurone could be subjected to tonic inhibition from another nonspiking interneurone that would be shut off by the inhibitory action of the impaled nonspiking interneurone. This explanation implies that a nonspiking local interneurone makes connections with other nonspiking local interneurones in addition to those it makes with the motor neurones. Simultaneous recordings from pairs of nonspiking local interneurones show that such connections do exist and that they are always inhibitory (Burrows, 1979).

Each nonspiking local interneurone connects with several motor neurones and, in turn, each motor neurone receives inputs from several nonspiking local interneurones (Burrows, 1980). The connections seem to be designed to call forth movements of the leg that are part of normal locomotory patterns. These interneurones thus organize the motor neurones into sets appropriate for particular movements. The lateral inhibitory connections with other nonspiking local interneurones ensure that the unwanted outputs of these neurones are prevented. The sets of motor neurones overlap and each movement results from the concerted output of many nonspiking local interneurones and their postsynaptic motor neurones. For example, the slow motor neurone to the extensor tibiae muscle receives inputs from at least 12 nonspiking local interneurones, and one nonspiking local interneurone may alter the output of motor neurones at three joints of the leg (Burrows, 1980).

Design of the local reflex pathways

From these patterns of connections, it is possible to trace the route by which a particular stimulus to a mechanoreceptor on the leg results in the execution of a specific local reflex movement (Fig. 7). Five main features of the pathways can be discerned.

First, the afferent signals diverge to several classes of interneurone with which they make exclusively excitatory synapses. The divergence is such that the input from one afferent will be processed by several interneurones of one class and by interneurones of different classes with differing integrative properties. The sensory stimulus delivered to the motor neurones is therefore derived from the parallel and distributed processing by many interneurones whose receptive fields overlap and whose outputs converge on the same motor neurones. The connec-

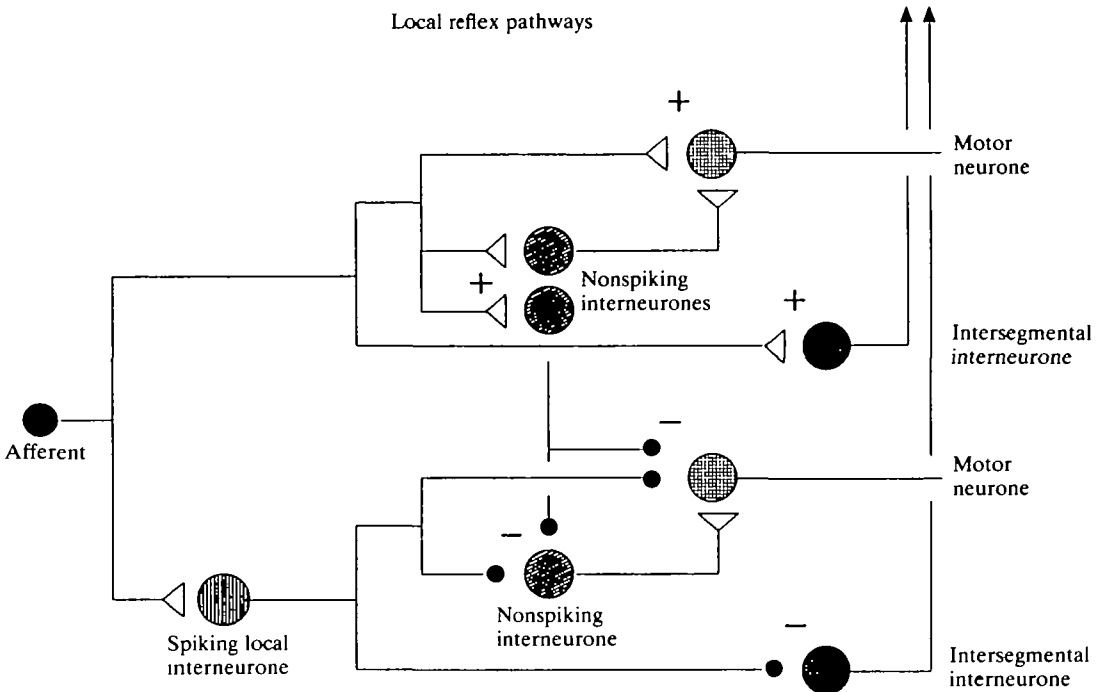


Fig. 7. Diagram of the pathways that underlie a local reflex adjustment of a hind leg initiated by mechanical stimulation of exteroceptors on that hind leg. (From Burrows & Laurent, 1989.)

tions are, however, specifically ordered so that an array of receptors connects only with a specific set of interneurons. In this way a stimulus can be spatially localized.

Second, spiking local interneurons in the midline group make further divergent sets of connections that are exclusively inhibitory. Additions will have to be made to these pathways as more is learned about the output connections of the other two populations of spiking local interneurons. Perhaps these neurons provide an excitatory drive to the motor neurons and other interneurons for, at present, inhibition and disinhibition do seem to be the dominant features of the networks.

Third, the local interneurons make lateral inhibitory connections with other local interneurons that are again exclusively inhibitory. Although the networks appear to be complex as a result of these interactions, the principles are basically simple. There is a directional flow of information, so that each interneuron, or class of interneurons, has a clearly defined place in the pathways. For example, no connections have been found from nonspiking interneurons to spiking local interneurons, although the converse interactions are common.

Fourth, the outputs of the sensory neurons and local interneurons converge on the motor neurons. Most of the afferent signals are processed through the

local circuitry, although a few of the exteroceptive signals and most of the proprioceptive signals (Burrows, 1987*b*) are also delivered directly to the motor neurones. The reasons for the existence in parallel of the direct and of the more complex pathways are not clear. The direct pathway does not imply that a stereotyped motor response will always occur if this route prevails, because the terminals of many insect sensory neurones receive input synapses (Altman *et al.* 1980; Watson & Pflüger, 1984) that, by analogy with other animals (Sillar & Skorupski, 1986), can be expected to modify the effectiveness of the sensory signals.

Fifth and finally, the raw sensory input and the sensory input as processed by the local circuitry are conveyed to an adjacent leg by intersegmental interneurones.

Properties and actions of the local interneurones

Within these pathways, the actions of the two different classes of local interneurones can be defined. The spiking local interneurones collate afferent information from arrays of receptors and preserve spatial information. They then reverse the sign of the afferent signals from excitatory to inhibitory. Their output connections delimit the receptive fields of nonspiking and intersegmental interneurones, and either exclude the action of nonspiking interneurones whose motor effects would be inappropriate, or disinhibit those whose action would be appropriate.

The nonspiking interneurones, by virtue of their graded release of transmitter, exert a fine control over the output of motor neurones so that the frequency of their spikes and hence the resulting contractions of the muscles can be delicately adjusted. Furthermore, inhibitory connections with other nonspiking interneurones exclude the contribution of certain motor neurones to a particular movement while allowing others to participate through the disinhibition of any tonic inhibitory input to them.

Adjustment of local reflexes

Stimulation of the same set of receptors does not lead to an invariant motor response. Instead the reflexes are adaptive and depend upon the circumstances in which they are evoked. This implies that within the pathways are elements whose outputs can be altered by inputs from other neurones. Nonspiking interneurones could provide ideal elements for such adjustments because they receive inputs from afferents and from spiking local interneurones, make connections with other nonspiking local interneurones and control the output of pools of motor neurones. This idea can be tested directly by placing an intracellular electrode in one of these interneurones and manipulating its membrane potential, while an array of receptors is stimulated to evoke a local reflex. The nonspiking interneurone must receive direct inputs from the afferents that are stimulated and connect with some of the relevant motor neurones. Hyperpolarizing such an interneurone with a steady current to reduce the release of its transmitter reduces the effectiveness of

the reflex response as measured by the frequency of spikes in the participating motor neurones. Likewise, a steady depolarizing current increases the effectiveness of the reflex. All these effects are graded and depend upon the current that is injected into the interneurone.

These results indicate that the nonspiking interneurone is directly involved in the reflex and that, despite the parallel processing, a single interneurone can produce a measurable change in a reflex pathway. Any synaptic input to such a nonspiking interneurone could, under appropriate circumstances, alter the graded release of transmitter and change the gain in the reflex pathway. This provides a plausible mechanism by which local reflexes are adapted to the behaviour of the animal.

Intersegmental influences on local reflexes

Does this intrasegmental mechanism of reflex adjustment give any clues as to how the movements of one leg take heed of the movements of other legs? Consider how the movements of a middle leg might influence the movements of a hind leg on the same side of the body. Interneurones in one population in the mesothoracic ganglion receive inputs from a middle leg and have an axon that projects ipsilaterally to the metathoracic ganglion (Laurent, 1987*b*, 1988). The receptive fields of these interneurones are shaped by the same series of connections that have been described for the neurones controlling a hind leg; direct excitation from afferents, inhibition by spiking local interneurones. These interneurones make direct excitatory or inhibitory inputs to nonspiking interneurones in the metathoracic ganglion (Laurent & Burrows, 1989*a*). The connections are specific and are related to the receptive fields of the intersegmental interneurones and the output connections of the nonspiking interneurones. As with the afferent pathways, some connections are also made directly with the motor neurones of a hind leg. The connections do not seem designed to produce stereotyped intersegmental reflexes, but to act on the nonspiking interneurones and modify the local reflex movements of a hind leg (Laurent & Burrows, 1989*b*).

Conclusions

The types of pathways used for local reflexes in a locust have been defined in sufficient detail to allow the flow of sensory signals to be followed through its various integrative stages to the final emergence of an adaptive movement. Moreover, it has been possible to pin-point some of the crucial elements in these pathways where modification occurs. For example, the nonspiking interneurones adjust the gain of local reflexes and act as the summing points for both intra- and intersegmental effects. The intention has been to extract the general principles that govern the processing in these pathways as a basis for a future understanding of how the animal may use them during voluntary movements. Inevitably, describing the pathways in these terms gives a rather static impression of the

integrative processes, which are impressive for their dynamic features. Two important considerations have deliberately been ignored.

First, no consideration has been given to the action of neuromodulators, although they exist and are known to exert profound effects on the patterns that can be generated by networks of neurones (Flamm & Harris-Warrick, 1986; Marder & Hooper, 1985).

Second, simply looking at the patterns of connections ignores a further layer of complexity provided by the processing within a single neurone. For example, examination with the electron microscope of the distribution of synapses on a nonspiking interneurone shows that input and output synapses are closely opposed (Watson & Burrows, 1988). The intermingling of inputs and outputs on the same small branches means that compartmentalization of action may occur, with the consequence that a single neurone could perform several computations simultaneously. Estimates of the attenuation of synaptic potentials in these interneurones (Rall, 1981) suggest that such compartments may exist. Furthermore, the effects of certain intersegmental interneurones on a nonspiking local interneurone can be explained by such mechanisms (Laurent & Burrows, 1989b).

The task that must now be undertaken is to weave these further complexities into our understanding of the processing of local reflexes and then to place these observations in the context of their role in influencing voluntary movements.

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