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Intersegmental Interneurons Can Control the Gain of Reflexes in Adjacent Segments of the Locust by Their Action on Nonspiking Local Interneurons

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The gain of local reflexes of one leg of a locust can be altered by mechanosensory inputs generated by movements of or tactile inputs to an adjacent leg. Touching the mesothoracic tarsus, for example, increases the number of spikes that are produced by the metathoracic slow extensor tibiae motor neuron and enhances the depolarization of flexor tibiae motor neuron in response to imposed movements of the chordotonal organ in the ipsilateral hind femur. The sensory information from the middle leg is conveyed directly to nonspiking interneurons and motor neurons controlling the movements of the hindleg by a population of mesothoracic intersegmental interneurons (Laurent and Burrows, 1989). The metathoracic nonspiking interneurons receive direct inputs from receptors on a hindleg and are, therefore, a point of convergence for local and intersegmental inputs. We examine here the role of the connections between mesothoracic intersegmental interneurons and metathoracic nonspiking interneurons in controlling metathoracic local reflexes.

The amplitude of synaptic potentials evoked in leg motor neurons by the stimulation of local afferents can be modulated by altering the membrane potential of an interposed nonspiking interneuron with current injected through an intracellular electrode. These imposed voltage changes mimic a mesothoracic input and show that the state of a nonspiking local interneuron is a determining factor in the expression of a local reflex.

Inputs from mesothoracic intersegmental interneurons may cause large changes in the input conductance of nonspiking interneurons that can shunt a local afferent input. In some nonspiking interneurons, synaptic potentials caused by mesothoracic interneurons can be recorded, but no underlying conductance change can be detected at the recording site. Similarly, a particular nonspiking interneuron may receive synaptic inputs when two distinct regions of a middle leg are touched, but only one of these intersegmental inputs may be effective in reducing the amplitude of a synaptic potential caused by afferents from the hindleg. These results suggest that nonspiking local interneurons may be compartmentalized, with synaptic inputs and their associated conductance changes restricted to particular branches. In this way, an individual nonspiking neuron could contribute simultaneously to several local circuits. The inputs from different intersegmental interneurons could then modulate these pathways independently.

Nonspiking interneurons in the metathoracic ganglion of the locust receive direct inputs from mechanosensory afferents on one hindleg and are essential elements in local reflex movements of that leg (Laurent and Burrows, 1988; Burrows et al., 1988). They also receive direct inputs from intersegmental interneurons that process the mechanosensory inputs from an ipsilateral middle leg (Laurent and Burrows, 1989). They are, therefore, one of the sites at which inter- and intrasegmental signals converge and could provide the means by which local reflexes are adjusted to meet the constraints imposed by the movements of the other legs.

Evidence that the nonspiking interneurons are important in the execution of local reflexes comes from experiments in which the membrane potential of a single interneuron is altered by the intracellular injection of current. The result of such manipulations is that the pattern of motor spikes evoked by a particular sensory stimulus is changed (Siegler, 1981; Burrows et al., 1988). Moreover, imposing a sustained change in the membrane potential of a nonspiking interneuron may alter the movement of a leg about one joint and at the same time change the strength of reflexes elicited at other joints of the same leg (Siegler, 1981). Small voltage changes only are needed to effect these alterations in local reflexes. At certain membrane potentials, nonspiking interneurons may release transmitter tonically so that a sustained effect is observed in postsynaptic motor neurons (Burrows and Siegler, 1978) or other nonspiking interneurons (Burrows, 1979a). Synaptic potentials generated in nonspiking interneurons whose membrane potentials are at or close to these levels will, therefore, be able directly to increase or decrease the release of transmitter (Burrows, 1979b). The connections that the mesothoracic intersegmental interneurons make with the metathoracic nonspiking local interneurons (Laurent and Burrows, 1989) might, therefore, be able to alter the output of these interneurons and thereby influence the expression of local reflexes of the hindleg. A particular stimulus to a middle leg does not evoke a reliable intersegmental reflex of the ipsilateral hindleg but instead excites several intersegmental interneurons that make divergent connections with metathoracic neurons of the same or even antagonistic motor pools.

Here we show that changes in membrane potential or input

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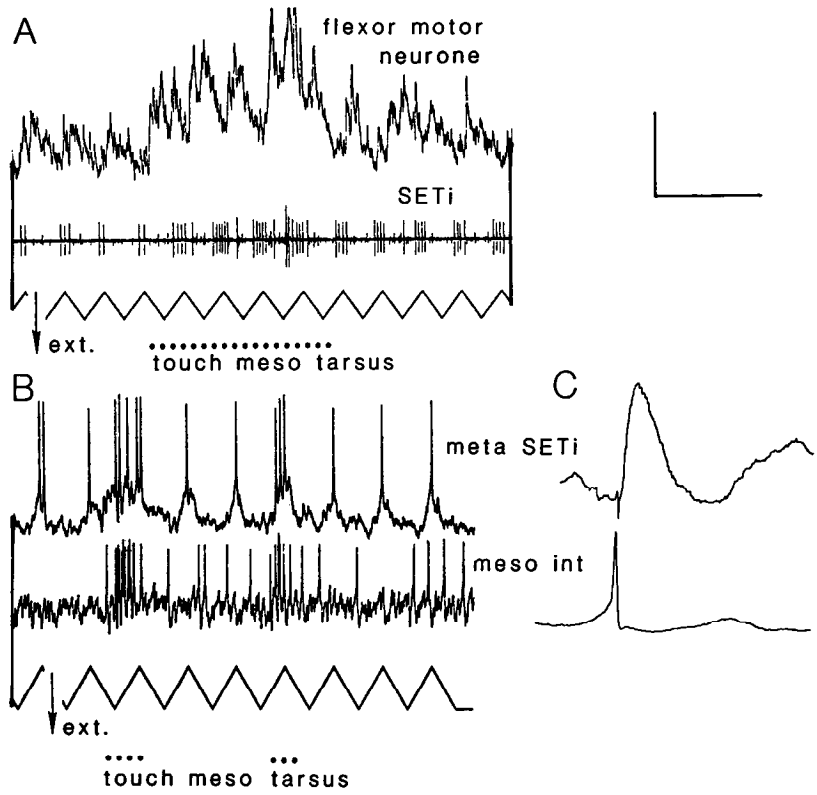

Figure 1. Mechanical stimulation of a middle leg produces changes in a local metathoracic reflex. *A*, The apodeme of the femoral chordotonal organ is moved rhythmically to simulate extension and flexion movements of the tibia of 40° amplitude. Each imposed extension causes a depolarization of a flexor tibiae motor neuron recorded intracellularly, and each flexion evokes spikes in the slow extensor tibiae motor neuron (*SETi*) recorded extracellularly by wires implanted in the extensor muscle. Touching the mesothoracic tarsus changes the response of both motor neurons to the metathoracic stimulus. *B*, A simultaneous recording from a mesothoracic intersegmental interneuron and SETi. Touching the tarsus of the middle leg evokes spikes in the interneuron and an increase in the number of SETi spikes on each metathoracic flexion. *C*, Signal averages (512 sweeps) show that an EPSP in SETi follows spikes in the interneuron, suggesting a direct connection (see Laurent and Burrows, 1989). Calibration: horizontal *A*, 1.25 sec, *B*, 0.5 sec, *C*, 42 msec; vertical, *A*, 5 mV, *B*, 10 mV.

conductance in metathoracic nonspiking interneurons, caused by the mesothoracic interneurons, produce alterations in local reflexes of a hindleg that would be of importance to the animal during locomotion. Furthermore, local increases in input conductance of an individual nonspiking interneuron are associated with changes in its integrative properties that can alter the release of transmitter onto postsynaptic neurons. This suggests that within a nonspiking interneuron certain actions may be compartmentalized, thus providing a refined control mechanism in which parts of the same interneuron participate simultaneously in different pathways.

Materials and Methods

The experiments reported in this paper were made using the same animals and same experimental procedures as in the preceding paper (Laurent and Burrows, 1989). The apodeme of the femoral chordotonal organ was grasped between the tips of fine forceps so that it could be moved in waveforms of controlled amplitude and frequency generated by a computer. Full details of this method are given in Burrows (1987).

Results

Changes in local reflexes of a hindleg

To test the possibility that a mechanical stimulus to a middle leg could alter a local reflex of the ipsilateral hindleg, the following experiment was performed. The apodeme of the chordotonal organ in the femur of a hindleg was moved rhythmically to mimic extension and flexion movements of the hind tibia (Fig. 1). The movements evoke a reliable resistance reflex in motor neurons innervating flexor and extensor tibiae muscles. A flexor motor neuron recorded intracellularly is excited during imposed extension, and the slow extensor tibiae (SETi) motor neuron spikes during flexion (Fig. 1*A*). Touching the ipsilateral middle does not evoke a reliable intersegmental reflex but instead alters the local metathoracic reflex. For example, when the mesothoracic tarsus is touched, the depolarization of the flexor motor neuron during extension of the metathoracic chordotonal organ is enhanced, and SETi produces more spikes on each flexion (Fig. 1*A*). Each cycle of the imposed movement now produces a large depolarization of the flexor, suggesting an alteration in the gain of the metathoracic reflex rather than a simple DC shift in the membrane potential of the metathoracic flexor motor neuron.

Each mesothoracic stimulus evokes spikes in several intersegmental interneurons. Touching hairs on the mesothoracic tarsus, for example, evokes spikes in one intersegmental interneuron and an increase in the number of spikes of SETi to the imposed metathoracic stimulus (Fig. 1*B*). The intersegmental interneuron makes a direct excitatory connection with SETi (Fig. 1*C*). The enhanced response of SETi could result from the summation between the intersegmental effects of this and possibly other interneurons and local sensory inputs at the level of the motor neuron itself. An additional enhancement could, however, result from the connections that the intersegmental interneurons make with premotor nonspiking interneurons in the metathoracic ganglion. It is the possible contribution of nonspiking interneurons to the alteration of local reflexes that will be further explored in this paper. Each mesothoracic stimulus excites several intersegmental interneurons in parallel. To assess the contributions of individual nonspiking interneurons, mesothoracic inputs were first simulated by injected current into one nonspiking interneuron.

The gain of a local reflex depends on the state of interposed nonspiking local interneurons

A reflex inhibition of a metathoracic flexor tibiae motor neuron occurs when cuticular strain is detected by receptors in the spines on the hind tibia (Fig. 2*A*). The afferents from these tibial re-

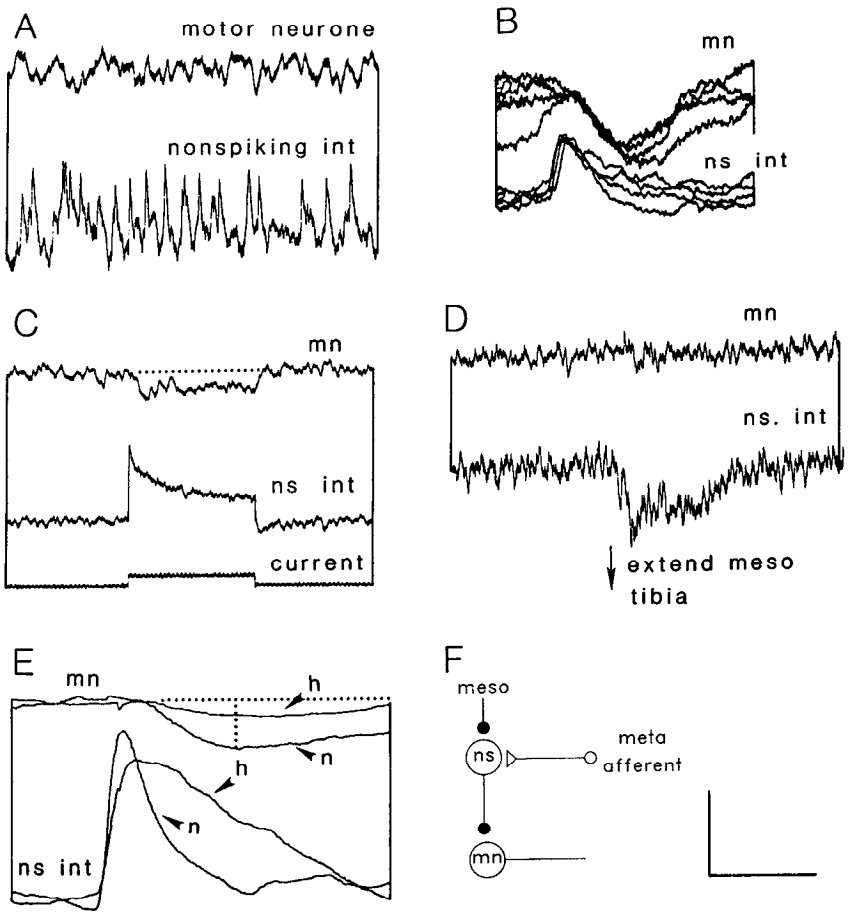

Figure 2. The gain of a local reflex depends on the "excitability" of a nonspiking interneuron. *A,* Afferent input from the spines on the metathoracic tibia evokes EPSPs in a nonspiking interneuron and IPSPs in a flexor tibiae motor neuron. *B,* Each EPSP in the nonspiking interneuron (*ns int*) is consistently followed by IPSPs, with a latency of less than 1 msec in the flexor motor neuron (*mn*). *C,* Depolarizing the nonspiking interneuron with a pulse of current produces a hyperpolarization of the flexor motor neuron. Dotted line indicates resting potential. *D,* Extension of the tibia of the ipsilateral middle leg produces a hyperpolarization of the interneuron but has no effect in the motor neuron. *E,* Two signal averaged sweeps, each representing the occurrence of 64 EPSPs, are superimposed. In one, the nonspiking interneuron is at its normal (*n*) membrane potential, and in the other, it is hyperpolarized (*h*) and displayed at half the gain. The IPSP evoked in the motor neuron is reduced by hyperpolarizing the nonspiking interneuron. The slower decay of the EPSP (*h*) in the hyperpolarized nonspiking interneuron probably indicates the existence of voltage-sensitive membrane currents. *F,* Diagram of the pathways revealed. Excitatory synapses are represented by *open triangles,* inhibitory synapses by *filled circles* in this and subsequent diagrams. Calibration: horizontal *A, D,* 250 msec, *B,* 20 msec, *C,* 500 msec, *E,* 14 msec; vertical, motor neuron *A, D,* 5 mV, *B,* 2.5 mV, *C,* 10 mV, interneuron *A, B, D,* 5 mV, *C,* 10 mV.

ceptors do not synapse directly on the motor neuron but instead evoke a barrage of EPSPs in a nonspiking interneuron (Fig. 2*A*). Each of these EPSPs in turn is followed by an IPSP in the motor neuron (Fig. 2*B*). Injecting a pulse of current into the nonspiking interneuron evokes a sustained hyperpolarization of the motor neuron (Fig. 2*C*). The change in postsynaptic voltage varies in a graded fashion with the applied presynaptic current and can be reversed in polarity by the injection of negative current into the motor neuron. The connection appears to be direct and synaptic transmission to be effected by the graded release of chemical transmitter. The EPSPs in the nonspiking local interneuron that occur when strain is imposed on spines on the tibia of the hindleg, therefore, transiently increase the release of transmitter and cause 1:1 IPSPs in the flexor motor neuron (Fig. 2*B*).

The metathoracic nonspiking interneuron is hyperpolarized by inputs from a group of mesothoracic intersegmental interneurons that are excited by an imposed extension of the ipsilateral middle leg (Fig. 2, *D, F*). These intersegmental interneurons have no direct effect on the metathoracic flexor tibiae motor neuron, so if the mesothoracic inputs are to modify the metathoracic reflex, they can act only through the nonspiking interneuron. To test the effect of combining meso- and metathoracic stimuli, the normal metathoracic input was provided while the mesothoracic input was mimicked by altering the membrane potential of the nonspiking local interneuron with injected current. Hyperpolarizing the nonspiking local interneuron with a DC current of 1 nA reduces by as much as 70% the amplitude of the IPSP evoked in the motor neuron by the metathoracic stimulus (Fig. 2*E*). The local reflex inhibition of the motor neuron has, therefore, been reduced by changing the effectiveness of the EPSPs generated by metathoracic afferents in releasing transmitter from the nonspiking interneuron. The reduction cannot be explained by changes at the motor neuron because the steady hyperpolarization that is applied to the interneuron is expected to diminish any direct tonic synaptic effect that it might exert. This should result in a depolarization of the motor neuron and an increase in its membrane resistance, both of which would increase the amplitude of an IPSP.

In a second metathoracic local reflex, an imposed extension of the tibia of a hindleg to its most extreme position evokes a sequence of EPSPs in the SETi motor neuron (Fig. 3*A*). At the same time, a nonspiking interneuron also is depolarized by a parallel barrage of EPSPs (Fig. 3, *A, B*). Signal averages triggered from an EPSP in either the nonspiking interneuron (Fig. 3*E*) or the motor neuron reveal a simultaneous EPSP in the other neuron, suggesting that both are caused by a common presynaptic neuron. This neuron is probably an afferent from a femoral receptor excited tonically by tibial extension (Fig. 3 inset) (Burrows, 1987; Laurent and Hustert, 1988; Laurent and Burrows, 1988; Burrows et al., 1988), but the identity of the common presynaptic neuron is not crucial to the following argument. Depolarizing the nonspiking interneuron with a pulse of current

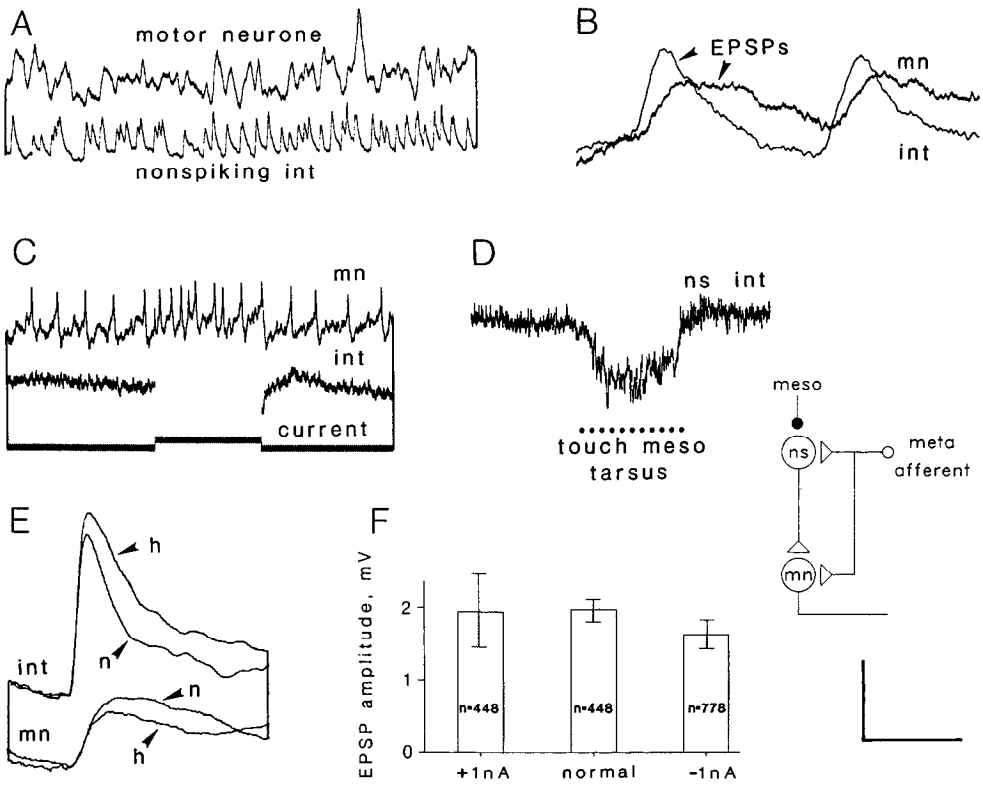

Figure 3. Gain control in parallel pathways. *A*, A metathoracic nonspiking local interneuron and the slow extensor tibiae motor neuron both receive a barrage of EPSPs when the hind tibia is forcibly extended from an angle of 160° to 175°. *B*, Most of the individual EPSPs can be matched in both neurons. *C*, Depolarizing the nonspiking interneuron with a pulse of current (bridge circuit unbalanced) produces an increase in the frequency of spikes in the extensor motor neuron. *D*, The nonspiking interneuron is hyperpolarized when the mesothoracic tarsus is touched. *E*, The amplitude of the EPSP in the motor neuron evoked by the metathoracic stimulus is reduced by about 20% if the interneuron is held hyperpolarized (*h*) by 1 nA DC current. Two signal averaged sweeps (each 64 sweeps) triggered by the EPSP in the interneuron are superimposed. In one, the interneuron is at its normal (*n*) membrane potential, and in the other, it is hyperpolarized (*h*). *F*, Histograms of the amplitude of the EPSP in the motor neuron while the interneuron is held depolarized, at its normal membrane potential, and hyperpolarized. Calibration: horizontal *A*, 250 msec, *B*, 12.5 msec, *C*, *D*, 500 msec, *E*, 29 msec; vertical, interneuron *A*, 10 mV, *B*, 3 mV, *C*, 8 mV, *D*, 20 mV, motor neuron *A*, 4 mV, *B*, 2.6 mV, *C*, 8 mV.

produces a depolarization of SETi and an increase in the frequency of its spikes (Fig. 3*C*). The effect is caused by the graded release of chemical transmitter from the interneuron. In the local reflex, SETi is, therefore, excited by a, presumably direct, connection from the afferent and, in parallel, by an indirect connection through a nonspiking interneuron (Fig. 3 inset).

Both SETi and the nonspiking interneuron are affected by stimulation of particular parts of the ipsilateral middle leg. Touching exteroceptors on the dorsal tarsus of this leg produces a hyperpolarization of the nonspiking interneuron (Fig. 3*D*) and a small depolarization of SETi. These effects are caused by the parallel activation of at least two intersegmental interneurons, one with inhibitory connections with the nonspiking interneuron, the other with excitatory connections with SETi. To test the effect of combining meso- and metathoracic stimuli, the normal metathoracic input was provided while the membrane potential of the nonspiking interneuron was altered by the intracellular injection of current. Hyperpolarizing the nonspiking interneuron with a DC current of 1 nA reduces by about 20% the amplitude of the EPSP in the motor neuron that results from the combined afferent and interneuronal input (Fig. 3*E*). By contrast, the EPSP is unaffected if the interneuron is held depolarized with 1 nA of DC current. The applied hyperpolarization presumably cancels the contribution of the interneuron to the afferent excitation of the motor neuron but has no effect on the direct afferent connection with the motor neuron. In this example and in these experimental conditions, therefore, the mesothoracic input appears able to produce a limited reduction of the local reflex pathway because it only has access to one of the afferent pathways to the motor neuron.

Gating of a metathoracic reflex by mesothoracic inputs

In a third metathoracic local reflex, the slow extensor tibiae motor neuron is inhibited by exteroceptive input from the distal tibia of the hindleg (Fig. 4*A*). This inhibition can be overridden by touching exteroceptors on the ipsilateral mesothoracic tarsus (Fig. 4*A*). The excitation from the middle leg is caused in part by a direct excitatory input to the metathoracic SETi from a mesothoracic intersegmental interneuron (see Fig. 1, *B*, *C*). This is manifest as a small depolarization and sometimes an increase in the frequency of spikes of SETi if the mesothoracic tarsus alone is touched (Fig. 4*B*). At the same time, however, other mesothoracic intersegmental interneurons with tarsal inputs evoke a barrage of IPSPs in a metathoracic nonspiking interneuron presynaptic to SETi (Fig. 4*B*). Depolarizing this nonspiking interneuron with a pulse of current evokes a depolarization of SETi and an increase in the frequency of its spikes (Fig. 4*C*). Hyperpolarizing the interneuron causes a hyperpolarization of SETi, indicating that at its normal membrane potential, the interneuron is releasing transmitter tonically. An applied depolarization increases the release, thereby depolarizing the motor neuron, whereas a hyperpolarization reduces release and allows the membrane of the motor neuron to repolarize.

To test the contribution of the nonspiking interneuron to both inter- and intrasegmental effects, its membrane potential was altered while the same metathoracic input as in Figure 4*A* was presented, and the inhibition of SETi was monitored. At the normal membrane potential of the nonspiking interneuron, equivalent to the situation where no mesothoracic input is pro-

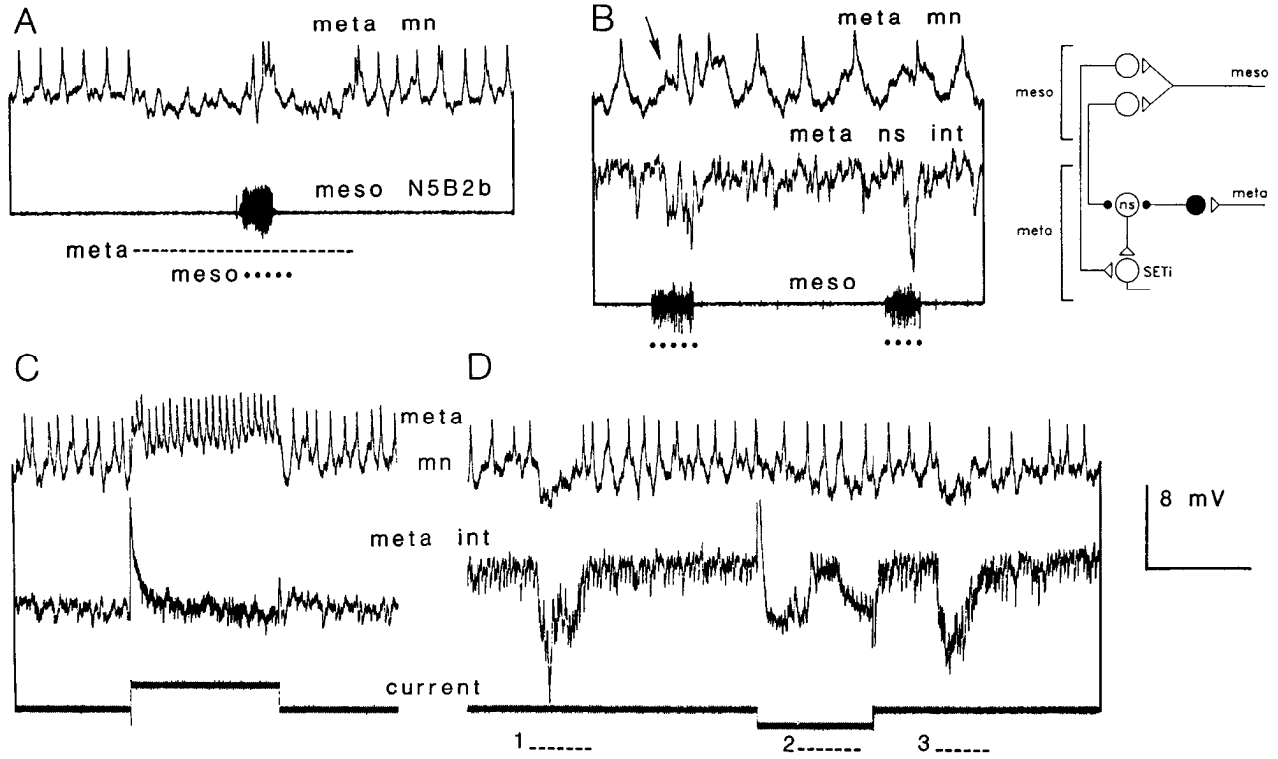

Figure 4. Gating of a metathoracic local reflex by mesothoracic inputs. *A*, Touching the spur region on the hindleg tibia (*dashed line*) inhibits the spikes in the metathoracic slow extensor tibiae motor neuron (*SETi*). The inhibition is overridden if the mesothoracic tarsus is brushed (burst of sensory spikes in mesothoracic tibial nerve 5B2b). *B*, Touching the mesothoracic tarsus (*dots*) in the absence of the metathoracic stimulus leads to a small depolarization of metathoracic SETi (*arrow*) and a hyperpolarization of a metathoracic nonspiking interneuron. *C*, Depolarizing this nonspiking interneuron with a pulse of current depolarizes and increases the frequency of spikes in the extensor motor neuron. *D*, Touching the metathoracic tibia (*dashed lines 1, 3*) while the nonspiking interneuron is held depolarized leads to the inhibition of both SETi and the nonspiking interneuron. Providing the same metathoracic stimulus while the nonspiking is hyperpolarized with a pulse of current (*dashed line 2*) evokes depolarizing IPSPs in the interneuron but fails to inhibit SETi. The diagram shows the pathways involved. Calibration: horizontal *A*, *B*, 0.5 sec, *C*, *D*, 1 sec.

vided, the metathoracic stimulus leads to a hyperpolarization of both SETi and the nonspiking local interneuron (bars *1* and *3*, Fig. 4*D*). The metathoracic inhibitory reflex is, therefore, expressed as in Figure 4*A*. If the nonspiking interneuron is held hyperpolarized below reversal potential for the IPSPs by a pulse of current, equivalent to the situation where an input from the mesothoracic tarsus occurs, the metathoracic stimulus now evokes depolarizing IPSPs in the nonspiking interneuron and only a small hyperpolarization of SETi (bar *2*, Fig. 4*D*). The frequency of SETi spikes is not altered, so that the metathoracic reflex is, therefore, no longer expressed as an inhibition of SETi (Fig. 4*D*). The pulse of hyperpolarizing current applied to the nonspiking interneuron should prevent tonic release of transmitter and allow the motor neuron to repolarize. Repolarization of the motor neuron during the hyperpolarizing pulse can sometimes be seen, but other pathways linking the nonspiking interneurons with the motor neuron must be involved. Hyperpolarizing the nonspiking interneuron, therefore, mimics part of the effect of a mesothoracic tactile stimulus and gates the metathoracic reflex. In this experiment, the gating effect was reliable, providing that the nonspiking interneuron was at a membrane potential at which it could release transmitter tonically. If the membrane potential was already held at a hyperpolarized level, further hyperpolarization, from either an applied pulse of current or a mesothoracic stimulus, had no effect in gating the metathoracic reflex. The ability of the nonspiking local interneuron to gate this metathoracic local reflex is hence itself dependent on other local synaptic inputs to the nonspiking interneuron.

Local changes in input conductance can modulate local integration

In about 70% of examples where a connection was found between a mesothoracic intersegmental interneuron and a metathoracic nonspiking interneuron (see Table 1 in Laurent and Burrows, 1989), the evoked depolarization or hyperpolarization in the nonspiking interneuron recorded from neuropilar branches was transient and of small amplitude (Fig. 5*A*). Nevertheless, the application of constant hyperpolarizing current pulses through the intracellular electrode shows that detectable changes in input resistance as large as 60% may commonly underlie these small voltage changes (Fig. 5*B*). These resistance changes are not explained by voltage-dependent rectifying currents, since they can occur in the absence of a detectable depolarization but are a reflection of the chemically mediated synaptic inputs from the mesothoracic interneurons. In most instances, however, a particular stimulus to the mesothoracic leg would evoke barrages of synaptic potentials in a metathoracic nonspiking interneuron, but no underlying conductance change could be detected with the applied pulses of current (Fig. 5*C*). This suggests that any conductance changes associated with the synaptic potentials are restricted to a part of the interneuron not accessible to the re-

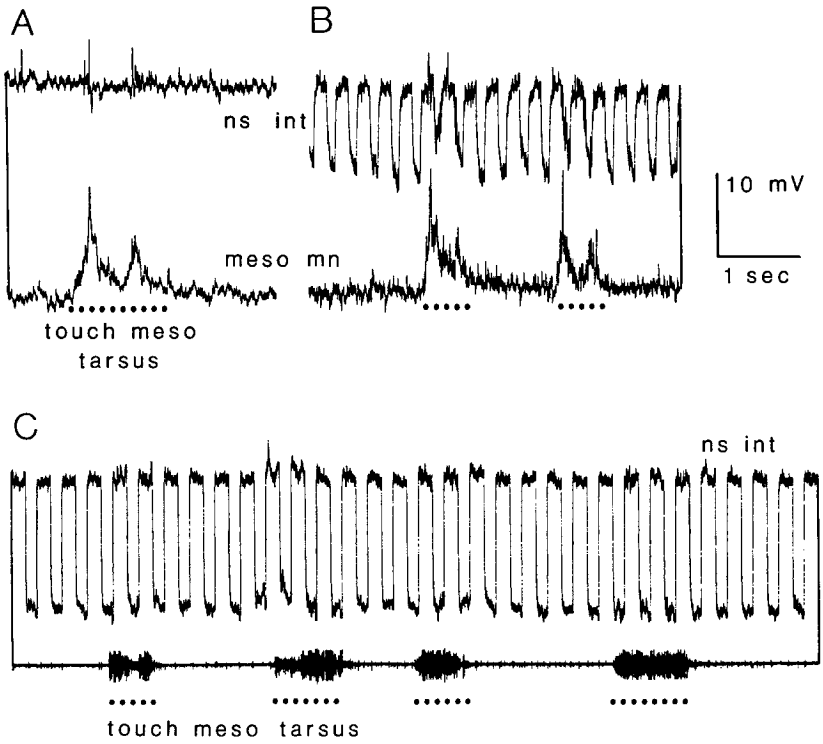

Figure 5. Conductance changes in metathoracic nonspiking local interneurons produced by mesothoracic inputs. *A,* Touching the mesothoracic tarsus (*dots*) evokes EPSPs in a metathoracic nonspiking interneuron and a depolarization of a mesothoracic tarsal depressor motor neuron. *B,* Pulses of constant hyperpolarizing current (0.2 nA) injected into the nonspiking interneuron reveal a change in its input resistance during the mesothoracic stimuli. *C,* Mesothoracic inputs to a different metathoracic nonspiking interneuron produce PSPs, but pulses of constant current reveal no detectable change in input resistance. The mesothoracic stimulus is marked by dots and by bursts of afferent spikes in a mesothoracic tibial nerve.

cording electrode. We thus tested whether changes in input conductance caused by intersegmental interneurons could modify the ability of metathoracic nonspiking interneurons to process metathoracic afferent inputs.

A metathoracic nonspiking interneuron is hyperpolarized by tactile stimulation of the distal hind tibia and slightly depolarized by touching the ventral tarsus of the ipsilateral middle leg (Fig. 6, *Ai, ii*). Electrical stimulation of the nerve containing these metathoracic afferents reliably evokes large IPSPs in this interneuron, probably caused by an interposed spiking local interneuron (pulses *1* and *2,* Fig. 6*Aii*). If the same electrical stimuli are now delivered to the metathoracic nerve while touching the ventral surface of the mesothoracic tarsus, the evoked IPSPs are reduced in amplitude by more than 30% (pulses *3* and *4,* Fig. 6*Aii*; signal averages in Fig. 6*Aiii*). During this mesothoracic stimulus, whose time course is seen clearly by its effect on a mesothoracic motor neuron, the membrane potential of the nonspiking interneuron is slightly depolarized, a voltage change that would in itself be expected to increase the amplitude of the evoked IPSPs. In fact, spontaneous IPSPs that are not associated with the metathoracic stimulus are enhanced during the mesothoracic stimulus (open triangles, Fig. 6*Aii*). As the effect of the mesothoracic stimulus declines, the IPSPs evoked by metathoracic stimulation resume their original amplitude (pulses *5–7,* Fig. 6*Aii*).

In other nonspiking interneurons, EPSPs evoked by metathoracic stimuli also can be reduced by mesothoracic inputs. This reduction is of functional significance because the transient change in transmitter release from the nonspiking interneuron as a direct result of the PSPs will be reduced, and the gain of a reflex pathway will thereby be changed. If these conductance changes in a nonspiking interneuron are localized, as is suggested by the result in Figure 5, the spatial arrangement of synapses assumes importance in the modulation of inter- and intrasegmental effects. Indeed, not all inputs from mesothoracic interneurons can alter the amplitude of a PSP in a nonspiking interneuron caused by a metathoracic stimulus. The metathoracic nonspiking interneuron in Figure 6*B* is excited by exteroceptive inputs from the metathoracic tarsus (Fig. 6*Bi*). The metathoracic nerve containing these afferents was stimulated to evoke a reliable and consistent EPSP whose size was compared in the presence and absence of mesothoracic inputs. A mesothoracic tactile stimulus evokes a barrage of EPSPs in the metathoracic nonspiking interneuron but no sustained depolarization (Fig. 6*Bii*). EPSPs evoked by electrical stimulation of the metathoracic nerve during this mesothoracic input (pulses *3* and *4,* Fig. 6*Bii*) are of the same amplitude as those that occur before or after it (Fig. 6*Biii*).

A stimulus to one region of a middle leg may evoke synaptic potentials in a particular metathoracic nonspiking interneuron that do not alter its response to a metathoracic stimulus, whereas a stimulus to another region of the middle leg may be effective. One metathoracic nonspiking interneuron is excited by touching ventral exteroceptors on the hind tarsus, and when the nerve containing these afferents is stimulated electrically, a consistent EPSP is evoked (Fig. 7*A*). It is also depolarized by a series of EPSPs when the tarsus or tibia of the ipsilateral middle leg is touched (Fig. 7, *A, B*). When the meso- and metathoracic stimuli are combined, the EPSP from the metathoracic stimulus is unchanged by input from the mesothoracic tarsus (Fig. 7*A*) but is greatly reduced by input from the mesothoracic tibia (Fig. 7*B*). This result suggests that the two mesothoracic stimuli excite different sets of intersegmental interneurons whose output synapses have a different spatial arrangement on the nonspiking interneuron. Only the synapses of one set can interact with those of the metathoracic afferents.

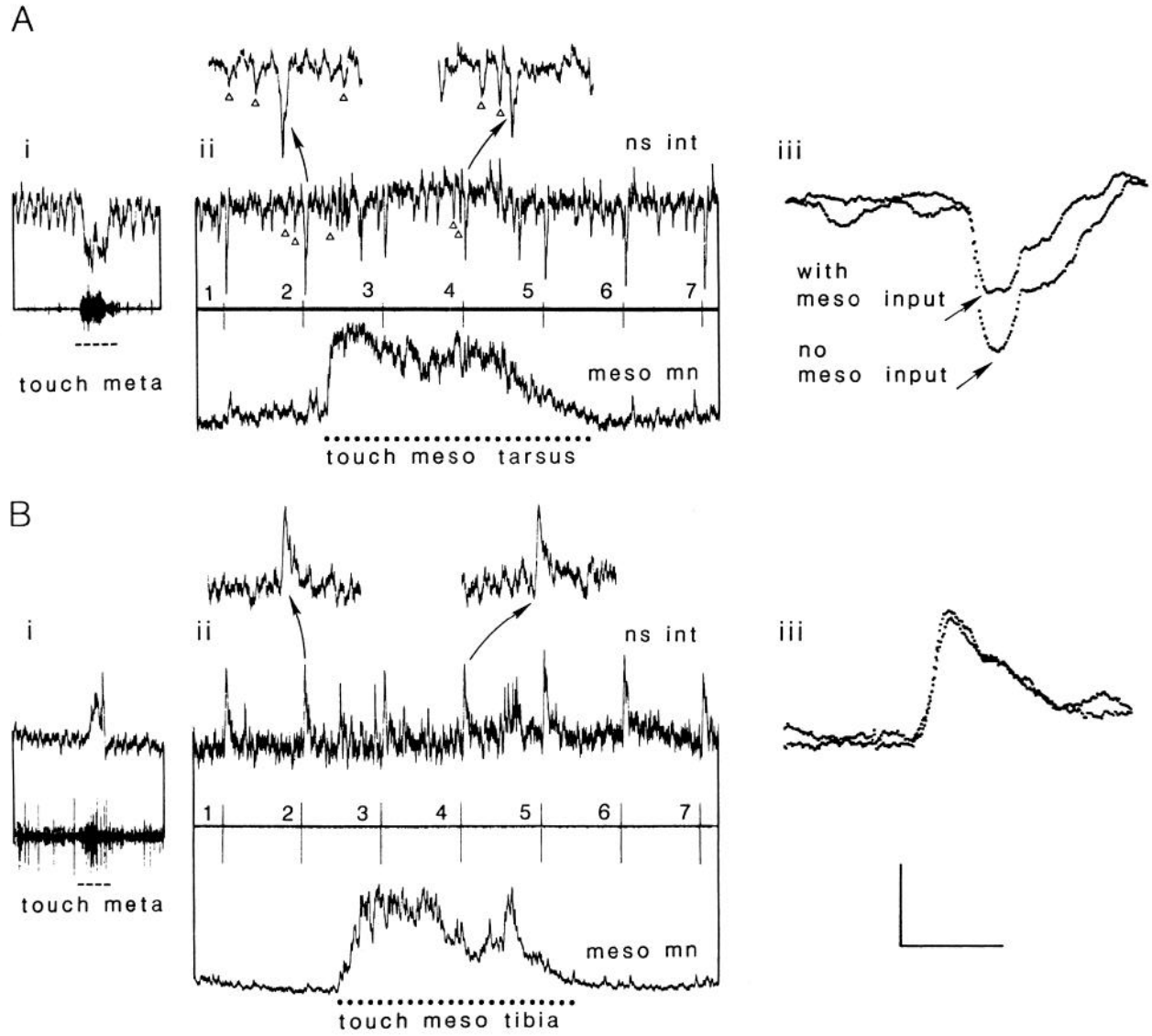

Figure 6. Effects of mesothoracic inputs on the responses of metathoracic nonspiking interneurons to metathoracic inputs. *Ai,* A metathoracic nonspiking interneuron receives inhibitory inputs from the hind tibia. The afferent spikes are recorded extracellularly from a tibial nerve (*lower trace*). *Aii,* Repetitive electrical stimulation of this nerve evokes a large IPSP in the nonspiking interneuron (stimuli 1 and 2, *middle trace*). These IPSPs are reduced in amplitude (stimuli 3 and 4) when the ipsilateral mesothoracic tarsus is touched (*dots*) but assume their original amplitude (stimuli 5–7) as the mesothoracic stimulus declines. The duration of the stimulus is seen clearly by its effect on a mesothoracic depressor tarsi motor neuron (*bottom trace*). IPSPs not associated with the metathoracic stimulus (*triangles*) are increased in amplitude during the mesothoracic stimulus. *Aiii,* Superimposed signal averages (5 sweeps each) of IPSPs evoked in the presence or absence of mesothoracic input. *Bi,* A different metathoracic nonspiking interneuron receives excitatory inputs from the hind tarsus. The afferent spikes are recorded extracellularly from N5B2 in the tibia. *Bii,* Repetitive electrical stimulation of this nerve evokes EPSPs whose amplitude is not changed during a simultaneous mesothoracic stimulus (*dots*). The duration of the mesothoracic stimulus is seen clearly by its effect on a mesothoracic motor neuron. *Biii,* Superimposed signal averages (5 sweeps) of EPSPs evoked in the presence or absence of the mesothoracic stimulus. Calibration: horizontal *Ai, Bi,* 1 sec, *Aii, Bii,* 1.25 sec (*insets* 0.5 sec), *Aiii, Biii,* 14 msec; vertical, interneuron *Ai, ii, Bi,* 4 mV, *Aiii, Bii,* 2 mV, *Biii,* 1.25 mV, motor neuron *Aii,* 4 mV, *Bii,* 10 mV.

Discussion

Gain control mechanisms

A particular mechanosensory stimulus to a middle leg is coded by a group of mesothoracic intersegmental interneurons with overlapping receptive fields of varying complexity (Laurent, 1988). The outputs of these intersegmental interneurons both diverge and converge onto metathoracic nonspiking local interneurons (Laurent and Burrows, 1989) so that an "image" of the mechanosensory inputs to a leg is conveyed to them. During locomotion, therefore, the mesothoracic intersegmental interneurons will provide the metathoracic local circuits with information about the movements of the ipsilateral middle leg. These inputs provide a mechanism by which the gain of metathoracic local reflexes could be adapted to the movements of the adjacent middle leg. What advantages are to be gained from such intersegmental modulatory pathways?

The reflex effects of tactile or proprioceptive inputs to the body or limbs of many vertebrates depends on the time at which they occur in the cycle of locomotory movements (Forssberg, 1979; Wallen, 1980; Lennard, 1985; Dietz et al., 1986; Capaday and Stein, 1986; Sillar and Roberts, 1988). Similarly, in the

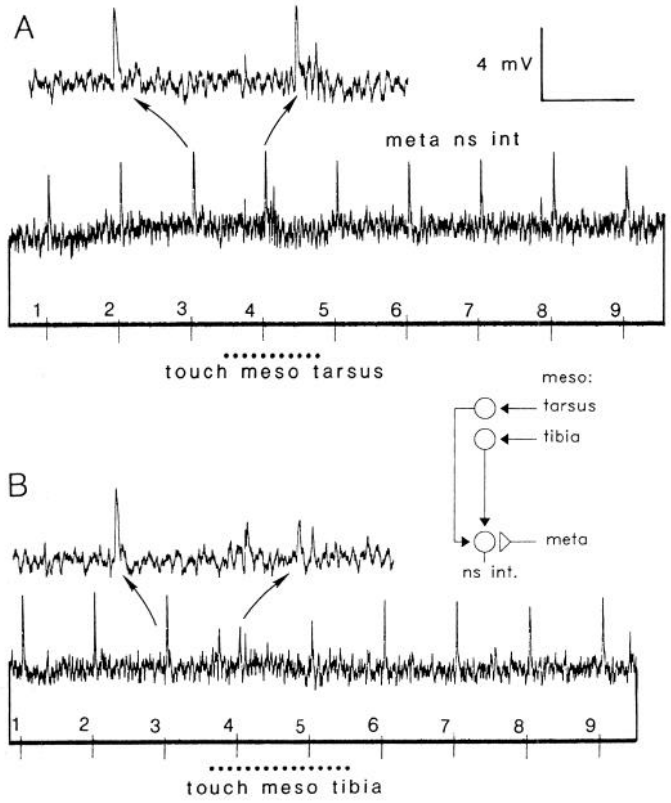

Figure 7. Different effects of 2 mesothoracic inputs on the integrative properties of a metathoracic nonspiking interneuron. *A,* The nonspiking interneuron is depolarized by touching exteroceptors on the ventral tarsus of the hindleg. Electrically stimulating the nerve containing these afferents evokes EPSPs (stimuli 1–3, 5–9) whose amplitude is not changed if the tarsus of the middle leg is touched (stimulus 4). *B,* If, however, the tibia of the middle leg is touched, the evoked EPSPs are reduced in amplitude (stimuli 4, 5). Calibration: horizontal 1.25 sec, inset 0.5 sec.

locomotion of invertebrates, the effects of proprioceptive feedback are also phase-dependent (Reichert and Rowell, 1985; Sillar and Skorupski, 1986). Although the neural mechanisms underlying this phase-dependent control are partially understood in only a few animals (Reichert and Rowell, 1985; Sillar and Skorupski, 1986; Sillar and Roberts, 1988), a general conclusion is that they are the result of central rather than peripheral mechanisms. In both the cat and the crayfish, for example, central rhythmic inputs occur on the afferent terminals (Bayev and Kostyuk, 1982; Sillar and Skorupski, 1986), and in *Xenopus* embryo, central gating occurs at the level of a particular class of sensory interneuron (Dale, 1985; Sillar and Roberts, 1988).

In these examples, the central origin of the phase-dependent modulation is explained by two facts. First, the reflex modulation is linked to the phase of the movement of a particular segment rather than to that of a neighboring segment. The central pattern generator output is thus a reliable indicator of the phase. Second, in flying locusts and swimming fish or amphibian embryos, the coupling between adjacent segments is rigid enough to allow the phase of one segment to be predicted from that of another. A central signal is thus presumably sufficient to permit correctly adjusted phase-dependent modulation.

By contrast, in a walking insect, the central coupling between the rhythmical motor output to each leg is less rigid (Graham and Bässler, 1981; Delcomyn, 1985), and the temporal and spatial coordination between the legs relies on sensory cues from each of the legs (vow Buddenbrock, 1921; Dean and Wendler, 1983; Bässler, 1983). It follows, therefore, that corrective reactions of one leg should depend not only on the state of that leg but also on the state of the adjacent legs. For example, a particular tactile stimulus to the hindleg of a locust should lead to a specific avoidance reflex of this leg (Siegler and Burrows, 1986) only if stability is ensured by the position of the adjacent legs.

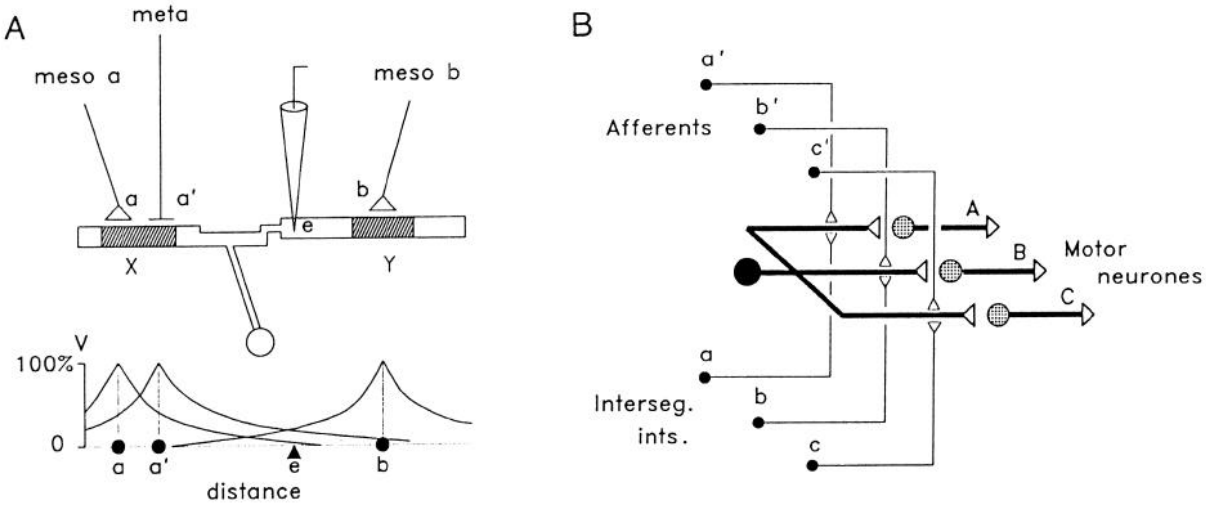

Figure 8. Compartmentalization of function in a nonspiking interneuron. *A,* Model of a nonspiking interneuron, to explain the experimental findings in Figure 7. Inputs from mesothoracic interneuron a (*meso a*) cause local conductance changes in branch X, reducing the effectiveness of the metathoracic afferent input *a'*. A second mesothoracic interneuron b (*meso b*) makes synapses on branch Y that are electronically distant from *a'* so that the 2 inputs do not interact. The position of the recording microelectrode (*e*) is shown. The graph indicates the attenuation of the various signals with distance. V, voltage. *B,* Model of a nonspiking interneuron that receives inputs from 3 local afferents (*a', b', c'*) and from 3 intersegmental interneurons (*a, b, c*). It makes output connections with three motor neurons (*A, B, C*). In this model, the 3 local circuits (*a', a, A*), (*b', b, B*), and (*c', c, C*) can be modulated separately.

Compartmental structure of nonspiking interneurons

Changes in the gain of metathoracic local reflexes can be produced by synaptic inputs from intersegmental interneurons to specific nonspiking local interneurons. These inputs cause a change in membrane potential or a change in input conductance of the nonspiking interneuron, both of which can alter the efficacy of synaptic transmission to postsynaptic neurons. A synaptic input from a mesothoracic interneuron may, however, sometimes fail to produce any reflex modulation (Fig. 6*B*). Indeed, in the same nonspiking interneuron, one mesothoracic stimulus may reduce the synaptic potential caused by a metathoracic stimulus, but another may have no effect. Two possible explanations can be proffered. First, more powerful parallel effects of the mesothoracic interneurons may dominate. For example, the mesothoracic interneurons may synapse on the terminals of metathoracic afferents and reduce the effectiveness of their connections with nonspiking interneurons by presynaptic inhibition. Contacts between their axonal terminals are possible because stains of the intersegmental interneurons (Laurent, 1987) and the afferents (Pflüger et al., 1981) show that some project to the same regions of neuropil. Reflex modulation would thus depend on a precise pattern of connections between intersegmental interneurons and metathoracic mechanosensory afferents. Preliminary experiments, however, have so far failed to indicate any such connections.

Second, a nonspiking interneuron could consist of several independent "compartments," so that a conductance change in one branch does not necessarily affect other, electrotonically distant branches. Such a proposed action stems directly from the experimental observations in Figures 6 and 7. The nonspiking interneuron shown diagrammatically in Figure 8*A* receives input synapses from metathoracic afferents (a′) that are restricted to branch X and inputs from two mesothoracic interneurons, one of whose synapses (a) occur on branch X close to a′, the other (b) on branch Y.

Consider two combinations of inputs. First, if inputs a and a′ occur simultaneously, the local conductance change in branch X (hatched area in Fig. 8*A*) caused by the synapses of mesothoracic interneuron a could swamp the metathoracic afferent input a′. As a result, the metathoracic local reflex will be altered. Second, if inputs b and a′ occur simultaneously, the conductance change caused by the synapses of the mesothoracic interneuron b will be restricted to branch Y and will have no effect on the metathoracic afferent input a′ (see Fig. 6*B*). As a result, the metathoracic local reflex will be unchanged.

It may be more useful, therefore, to consider nonspiking local interneurons, not as "integrative units," but as several compartments, each capable of potentially independent processing of information. A nonspiking interneuron has already been shown to:

1. Release chemical transmitter in a graded fashion according to changes in its membrane potential (Burrows and Siegler, 1978). This means that at certain membrane potentials it will release transmitter tonically, and inputs of either polarity will then be able to alter release.

2. Generate EPSPs that may effect the release of transmitter so that the pattern of outputs is a reflection of the pattern of inputs (Fig. 2; Burrows, 1979b).

3. Synapse with several members of a motor pool and with members of different pools (Burrows, 1980).

4. Receive convergent input from various classes of afferents from the leg whose motor neurons it controls (Laurent and Burrows, 1988; Burrows et al., 1988; Laurent and Hustert, 1988).

5. Receive convergent inputs from several intersegmental interneurons (Laurent and Burrows, 1989).

6. Show interactions between intersegmental and local inputs (this paper).

7. Have closely apposed input and output synapses on the same fine branches (Watson and Burrows, 1988).

A simple model of a nonspiking local interneuron that incorporates these properties is shown in Figure 8*B*. Three local circuits involving different sets of local afferents (a′, b′, c′), intersegmental interneurons (a, b, c), and with outputs to different motor neurons (A, B, C) coexist within the same interneuron. Intersegmental interneuron a, for example, can alter the gain of the metathoracic reflex mediated by the action of afferent a′ on motor neuron A but has no effect on the other metathoracic afferent pathways (Fig. 8*B*).

To support this hypothesis, it will be necessary to determine the attenuation of electrical signals between distant branches of the same nonspiking local interneuron. The terminals of some nonspiking interneurons can be 1.5 mm away from other terminals (Burrows and Siegler, 1978), and potentials recorded by two electrodes at least 200 μm apart in the same interneuron can be attenuated by 60–70% (Burrows and Siegler, 1978). Moreover, models based on the morphology of these nonspiking neurons (Rall, 1981), suggest that passive voltage attenuation between small diameter processes (<0.25 μm), where many input and output synapses occur (Watson and Burrows, 1988), that are linked by a primary neurite of 5 μm or more in diameter may be sufficient to make these small processes electrically autonomous (Rall, 1981; Siegler, 1984). Furthermore, any isolation would be enhanced by increases in the local conductance shunting the spread of potentials.

It also will be necessary to determine the spatial relationship of the synapses from both the local afferents and the intersegmental interneurons on the nonspiking interneurons. We need to know whether the contacts made by one group of afferents on a nonspiking interneuron are restricted to one region (neurite) of the interneuron or spread over its whole arbor. We have no information on this, since it would require double and triple labeling and EM serial reconstruction of the branches. We know, however, that the projections of the intersegmental interneurons in the metathoracic ganglion are sparse (Laurent, 1987), whereas the branches of the afferents are more numerous.

The existence of independent local circuits and nonlinear interactions in neurons has often been hypothesized (e.g., horizontal cells in the retina, Nelson et al., 1975; amacrine cells, Miller and Bloomfield, 1983; Masland et al., 1984; ganglion cells, Koch et al., 1983), although not easily demonstrated (Douglas et al., 1988). A convincing example of possible independent functioning of branches in the same neuron is in a nonspiking interneuron (I cell) of the visual system of the barnacle (Oland et al., 1983). Two dendritic arbors of this neuron are separated by about 200 μm, and each receive signals from one eye only. Hyperpolarizing inputs to one arbor can, presumably through local conductance changes, shunt the invasion of this arbor by depolarizing inputs from the arbor. As a consequence, the effect of these depolarizing inputs can be expressed only through one of the two arbors. It is, therefore, assumed that local interactions occur independently in the two dendritic fields of this interneuron (Oland et al., 1987).

Directions for further analysis

Our results indicate that nonspiking local interneurons are a point of convergence of intersegmental inputs and of local sensory inputs and thereby play an important role in shaping the strength of local reflexes. Any input to the nonspiking interneurons, whether of peripheral or central origin, should be able to modulate a reflex pathway. It is, therefore, imperative to consider the action of nonspiking interneurons during voluntary movements and then assess their role in controlling the gain of reflexes. The richness of the integrative capabilities of nonspiking interneurons may, however, make further considerations essential. A single nonspiking local interneuron can give rise to a coordinated movement of the leg by virtue of its divergent connections with several motor neurons (Burrows, 1980). The precise shaping of the different movements it causes about the various joints could, however, be further modulated as a result of its compartmentalized integrative properties.

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