Intersegmental interneurones are crucial for the appropriate coordination of the activity of local circuits located in different body segments. We have analysed the synaptic inputs to ascending intersegmental interneurones from a proprioceptor in the tailfan of the crayfish. Twenty identified interneurones responded during stimulation of the exopodite–endopodite chordotonal organ. Of these 20 interneurones, three were excited phaso-tonically, nine were excited phasically and eight were inhibited. All received convergent exteroceptive inputs from water-motion- or touch-sensitive hairs on the uropods. The effects of simultaneous exteroceptive and proprioceptive stimulation depended upon the identity of an interneurone. For interneurones that were inhibited by proprioceptive stimulation, suprathreshold exteroceptive responses were reduced to a subthreshold level by simultaneous proprioceptive stimulation. In contrast, for interneurones that were excited by proprioceptive stimulation, the simultaneous application of subthreshold proprioceptive and exteroceptive stimulation elicited action potentials.

Two of the interneurones that receive proprioceptive input (NE-1 and RC-8) are known to be presynaptic to giant interneurones that mediate and coordinate the tail-flip. Many of the other interneurones that receive proprioceptive inputs in the tailfan are known to excite abdominal extensor motor neurones. Thus, proprioceptive input to these intersegmental interneurones could serve two roles: first, to extend the abdomen during postural movements or prior to escape and, second, to drive the tail-flip escape response.

Key words: sensory, proprioception, chordotonal organ, interneurone, escape behaviour, crayfish, Procambarus clarkii.

Introduction

Coordinated behaviour requires that movements of one segment or limb fit into the context of what another may be doing. This means there must be bilateral and intersegmental coordination of the local circuits controlling the movement of each segment or limb. In insects, ascending and descending interneurones convey the signals necessary for the appropriate intersegmental coordination of the legs (Dean, 1989; Laurent, 1986; Laurent and Burrows, 1988; Newland, 1990). In locusts, for example, intersegmental interneurones with somata in the mesothoracic ganglion receive sensory input from receptors on the mesothoracic leg (Laurent, 1986) and convey those signals to the metathoracic ganglion where they can influence the gain of reflex movements of the metathoracic leg (Laurent and Burrows, 1989a,b).

In crayfish, each abdominal ganglion contains local circuits that control the movements of the abdominal appendages, the swimmerets (Murchison et al., 1993). These local circuits are linked by intersegmental interneurones that coordinate the rhythmic beating of these appendages (Paul and Mulloney, 1986). The local circuits controlling the movements of the terminal abdominal appendages, the uropods, are also linked by intersegmental interneurones to other abdominal ganglia (Sigvardt et al., 1982). Stimulation of water-motion- or touch-sensitive hairs on the uropods not only leads to local reflex movements (Nagayama et al., 1994) but also to forward walking or defensive postural movements depending upon the age of an animal (Nagayama et al., 1986). Ascending intersegmental interneurones must convey information from the terminal ganglion anteriorly to the segments that bear the legs where these behavioural responses are coordinated.

In the terminal ganglion of the crayfish, there are approximately 60 pairs of ascending intersegmental interneurones (Sigvardt et al., 1982; Kondoh and Hisada, 1986), of which approximately 30 have so far been identified as unique individuals (Nagayama et al., 1993, Sigvardt et al., 1982). These have been divided into different categories on the basis of their output effects on uropod closer and opener motor neurones (Nagayama et al., 1993). Many are involved in local...
reflex movements of the uropods in response to exteroceptive signals (Sigvardt et al., 1982), whereas others are involved in the ballistic escape reflex (Zucker, 1972).

Individual ascending intersegmental interneurones in the terminal abdominal ganglion do not act simply to coordinate different segments. Instead, they can also integrate and process sensory signals and can act as premotor controllers. The only inputs to have been examined in detail, however, are those that they receive from exteroceptors. We know that there are a number of proprioceptors in the tailfan (Barth, 1964; Field et al., 1990; Laverack, 1989; Maitland et al., 1982), but their inputs to only three intersegmental interneurones have so far been analysed (Newland and Nagayama, 1993). The aim of this study, therefore, was to analyse the responses of identified ascending interneurones in the terminal ganglion during proprioceptive stimulation.

Here, we have identified 20 interneurones receiving input from a small chordotonal organ that monitors the movements of the exopodite of the uropods (Field et al., 1990). We show that most receive phasic proprioceptive input during opening of the joint. Only three are phasotonically excited and, of these, two are known to be involved in escape circuitry through their excitation of the lateral giant interneurones (Zucker, 1972).

Materials and methods

Adult male and female crayfish, Procambarus clarkii (Girard), 7–12 cm in body length, were purchased from a local supplier, maintained in running freshwater tanks and fed weekly on a diet of chopped potato and liver.

The abdomen was isolated from the thorax and pinned ventral-side-uppermost in a small chamber containing 8 ml of cooled physiological saline (van Harreveld, 1936) that immersed the tailfan completely. The chamber was constantly perfused with fresh saline using an Eyela MP-3 microtube pump (Tokyorikakiki). The bathing solution could be changed at a rate of 4 ml min\(^{-1}\) so that different drugs could be bath-applied. The swimmerets were removed, and the terminal (sixth) abdominal ganglion was exposed by removing the overlying fifth ventral sternite and surrounding soft cuticle. The ganglion was supported on a silver platform and treated directly with protease (granular form; Sigma type XIV) for 20–30 s to aid penetration of intracellular electrodes through the surrounding sheath.

The exopodite–endopodite chordotonal organ (exo-end CO) was exposed by fixing the exopodite at an angle of 60° to the long axis of the body. A small window was cut in the hard cuticle in a medial region of the ventral protopodite to expose nerve 3 from the terminal ganglion and extended distally through the soft cuticle to the endopodite (for details, see Field et al. 1990). The hypodermis and connective tissue were removed to expose the chordotonal organ spanning the joint between the endopodite and exopodite (Fig. 1A). The sensory neurones with somata in this chordotonal organ have their axons in nerve 3 of the terminal ganglion (Fig. 1B), and their activity was monitored by placing an oil hook electrode on this nerve between the protopodite and ganglion.

The chordotonal organ was mechanically stimulated using methods described previously (Nagayama and Newland, 1993). Briefly, the receptor strand was displaced by a fine pin attached to a vibrator (Ling Dynamic Systems) and moved through distances equivalent to 10° of exopodite movement. The vibrator was driven by ramp waveforms generated using a Shoshin OI-8 computer-controlled stimulator at velocities within the range of normal uropod movement (Cooke and MacMillan, 1985). Our stimulus method produced controlled displacements only in the stretch direction since the chordotonal organ was free to return to its resting position at rates depending on its own elasticity (and therefore at uncontrolled rates). While the exo-end CO is known to encode movements in two directions (Field et al., 1990) and the results of experiments described here show specific interneurones responding to stretch and relaxation, our descriptions are restricted to the stretch phases of stimulation only. This method of CO stimulation did not activate the sensory neurones of exteroceptive hairs that respond to water movements (Newland et al., 1996).

Intracellular recordings from neuropilar processes of interneurones were performed using microelectrodes filled with 3% Lucifer Yellow CH (Stewart, 1978) dissolved in 0.1 mol l\(^{-1}\) lithium chloride. Electrodes had resistances of...
Proprioceptive processing by intersegmental interneurones

We have analysed the input properties of 20 of these (Table 1) that receive input from sensory neurones innervating the exopodite-endopodite chordotonal organ (exo-endo CO), which monitors movements of the exopodite of the uropod relative to the endopodite (Nagayama and Newland, 1993). Twelve interneurones were excited during stretches of the chordotonal organ strand that were equivalent to opening movements of the exopodite (Field et al., 1990). Three of these were excited phasotonically, whereas the other nine were excited phasically during CO stimulation. A further eight different interneurones received inhibitory inputs during stretches of the exo-endo CO.

Interneurones that receive phasotonic excitatory input from chordotonal afferents

Three ascending interneurones (NE-1, RC-8 and CI-3/RO-1/RO-3) were excited phasotonically during stretches of the chordotonal organ strand. Interneurones CI-3, RO-1 and RO-3 cannot be distinguished from each other on the basis of shape and input properties alone and have therefore been grouped in this study. Interneurones RC-8 and NE-1, also known as interneurones C and A (Zucker, 1972), respectively, which form part of a disynaptic pathway that excites the lateral giant (LG) interneurones via electrical synapses, were strongly depolarised during stretch movements of the exo-endo CO strand. For example, interneurone NE-1 received a barrage of excitatory postsynaptic potentials (EPSPs) during stretch

Table 1. Summary of responses of identified ascending interneurones to chordotonal organ stimulation

<table>
<thead>
<tr>
<th>Neurone type</th>
<th>Phasotonic excitatory input</th>
<th>Phasic excitatory input</th>
<th>Phasic inhibitory input</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-3/RO-1/RO-3* (6B2)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RC-8 (interneurone C/6C1)</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>NE-1 (interneurone A/6B1)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>CA-1 (6D1)</td>
<td>2‡</td>
<td>2‡</td>
<td>2‡</td>
</tr>
<tr>
<td>CI-1 (6B6)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CI-2 (6A7)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CI-4 (not previously described)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RC-2 (6B6)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RC-3 (not previously described)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>RC-6 (6P1)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>RO-2 (6B3)</td>
<td>3‡</td>
<td>3‡</td>
<td>3‡</td>
</tr>
<tr>
<td>NE-5 (not previously described)</td>
<td>4‡</td>
<td>4‡</td>
<td>4‡</td>
</tr>
<tr>
<td>RC-4 (6A5)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RC-7 (6E3)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>RO-4 (6A4)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>RO-5 (6A2)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>RO-6 (not previously described)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VE-1 (6D2)</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>NE-3 (not previously described)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>NE-4 (6A1/CPR)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The numbers of each type of neurone recorded and the type of input received during ramp stimulation of the chordotonal organ strand are given.

*These neurones cannot be distinguished on the basis of anatomical structure alone.
‡Subthreshold depolarising responses. No spikes were evoked in these interneurones.
Details in parentheses correspond to alternative names for these neurones based on anatomical similarities with previous descriptions (Sigvardt et al., 1982; Wilkens and Larimer, 1972; Wine, 1984; Zucker, 1972).
movements of the chordotonal organ strand at a velocity of 4°s⁻¹ (Fig. 2A). The amplitude of the compound EPSP increased with increasing stimulus velocity up to 40°s⁻¹ (Fig. 2B) as a result of both an increase in spike frequency and the recruitment of more sensory spikes. Action potentials were evoked in NE-1 when the stimulus velocity was increased to 400°s⁻¹ (Fig. 2C). Individual chordotonal sensory spikes, with particular amplitudes, were consistently followed at short latency by EPSPs in NE-1 during a maintained stretch of the chordotonal organ (Fig. 2D). The amplitude of the compound EPSP mediated by stretches of the chordotonal organ at 1600°s⁻¹ increased during injection of a 1 nA hyperpolarising current (Fig. 2E). Taken together, these results, in addition to previous tests (Newland and Nagayama, 1993), are indicative of a chemically mediated monosynaptic connection.

Interneurones that receive phasic excitatory input from chordotonal afferents

Nine ascending interneurones (CA-1, CI-1, CI-2, CI-4, RC-2, RC-3, RC-6, RO-2 and NE-5) received phasic excitatory inputs during stretch movements of the chordotonal organ strand. Three of these interneurones (CA-1, RO-2 and NE-5) only received subthreshold inputs even with ramp displacement at 1600°s⁻¹ (Table 1).

For example, the amplitude of depolarising potentials evoked in interneurone NE-5 increased with increasing stimulus velocity from 40 to 1600°s⁻¹, although chordotonal inputs alone never elicited spikes in the interneurone (Fig. 3A–C). The amplitude of the depolarising potential mediated by stretches of the chordotonal organ decreased during injection of a +1 nA depolarising current and increased during injection of a −1 nA hyperpolarising current (Fig. 3D). Interneurone NE-5 also received exteroceptive inputs during stimulation of water-motion-sensitive hairs on the exopodite (Fig. 3E). Directing jets of saline onto the mechanosensory hairs on the exopodite evoked EPSPs and spikes in the interneurone. Interneurone RC-3, like most of the interneurones that received phasic inputs studied here, spiked during stretch movements of the chordotonal organ strand...
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Low-velocity stimulation at 200° s⁻¹ evoked a compound EPSP in interneurone RC-3 (Fig. 4A). Stimulation at 400° s⁻¹ also elicited spikes in RC-3 (Fig. 4B), and further increases of stimulus velocity produced a burst of spikes in the interneurone (Fig. 4C,D). Jets of saline directed onto the mechanosensory hairs also elicited bursts of spikes in interneurone RC-3 (Fig. 4E).

Interneurones that receive phasic inhibitory input from chordotonal afferents

Eight ascending interneurones (RC-4, RC-7, RO-4, RO-5, RO-6, VE-1, NE-3 and NE-4) received inhibitory inputs during chordotonal organ stimulation. For example, interneurone NE-3 was hyperpolarised during stretches of the strand of the exo-endo CO equivalent to opening movements of the exopodite (Fig. 5). Increasing the velocity of a stretch movement of the strand from 40 to 1600° s⁻¹ resulted in a summation of the inhibitory potentials (Fig. 5A–C). The hyperpolarisation of NE-3 increased in amplitude during a +1 nA depolarising current injection and decreased and reversed during injection of hyperpolarising currents (Fig. 5D). In contrast to the proprioceptive inhibition, interneurone NE-3 was excited by jets of saline directed onto the water-motion-sensitive hairs on the tailfan (Fig. 5E), producing bursts of action potentials.

Bath application of the Cl⁻ channel blocker picrotoxin (PTX) decreased the amplitude of the hyperpolarisation in NE-3 evoked by chordotonal organ stimulation (Fig. 6). In normal crayfish saline, a stretch of the chordotonal organ strand evoked hyperpolarising potentials in NE-3. After 15 min bath application of 50 μmol l⁻¹ PTX, the amplitude of the hyperpolarisation decreased to approximately 25 % of the initial amplitude. After a 10 min wash with normal saline, the amplitude of the hyperpolarising potential recovered to approximately 75 % of the initial amplitude.

Convergence of exteroceptive and proprioceptive inputs

Many ascending interneurones receive inputs during both exteroceptive and proprioceptive stimulation. The inhibitory inputs recorded in ascending interneurones during chordotonal organ stimulation reduced the efficacy of excitatory inputs from simultaneously occurring exteroceptive afferents (Fig. 7). Interneurone VE-1, for example, received inhibitory inputs during stretch movements of the chordotonal organ but was excited by stimulation of mechanosensory hair afferents. Stretch of the chordotonal organ strand with a ramp velocity of 1600° s⁻¹ evoked a long-duration inhibitory potential in the interneurone (Fig. 7A). In contrast, electrical stimulation of nerve 2, which contains exteroceptive afferents innervating
Fig. 4. Response of interneurone RC-3 to proprioceptive inputs. (A–D) Stretching the strand of the chordotonal organ, equivalent to an opening movement of the exopodite relative to the endopodite, with a ramp velocity of $200^\circ\text{s}^{-1}$ evoked a depolarisation in the interneurone. Lower velocities of stimulation failed to evoke a response in the interneurone (not shown). (B) When the stimulus velocity was increased to $400^\circ\text{s}^{-1}$, the chordotonal inputs evoked an action potential (*) in the interneurone. (C,D) Increasing the stimulus velocity still further to 800 and $1600^\circ\text{s}^{-1}$, respectively, resulted in a summation of potentials and more spikes (*) in the interneurone. Jets of saline directed towards exteroceptive hairs on the surface of uropods (arrows) again evoked bursts of spikes in the interneurone. CO, chordotonal organ.

Fig. 5. Ascending interneurones that receive inhibitory inputs. Response of interneurone NE-3 to proprioceptive and exteroceptive inputs. (A) Interneurone NE-3 received phasic inhibitory inputs during a ramp stimulus of $40^\circ\text{s}^{-1}$. Increasing the stimulus velocity to $400^\circ\text{s}^{-1}$ (B) and $1600^\circ\text{s}^{-1}$ (C) resulted in summation of inhibitory postsynaptic potentials and a greater hyperpolarisation of the interneurone. (D) Averaged sweeps of the oscilloscope triggered from the ramp stimulus. Each ramp stimulus evokes potentials of similar latency in the interneurone. Each trace consists of eight averaged sweeps. Constant depolarising and hyperpolarising current changed the amplitude of the hyperpolarising potentials in the interneurone. (E) Directing a long jet of saline onto hairs on the surface of uropods (arrow) evoked a depolarisation and spikes in the interneurone. CO, chordotonal organ.
Fig. 6. Effects of bath application of picrotoxin (PTX) on the inhibitory response of interneurone NE-3. The relative change in inhibitory postsynaptic potential (IPSP) amplitude during PTX application is plotted against time. The amplitude of IPSPs is expressed as a percentage of the initial IPSP level just before application of PTX (0 min). All values are the mean of four trials. After application of 50 μmol l⁻¹ PTX, the amplitude of the summed IPSPs decreased to approximately 25% of the initial amplitude. After a 10 min wash with normal saline, the amplitude of IPSPs recovered to approximately 75% of the initial amplitude. Insets show typical responses at the times shown. CO, chordotonal organ.

Fig. 7. Interaction between exteroceptive and proprioceptive inputs in interneurone VE-1. (A) Stretches of the chordotonal organ strand with a ramp velocity of 1600 ° s⁻¹ evoked a compound inhibitory postsynaptic potential in interneurone VE-1. (B) Electrical stimulation of nerve 2 evoked a compound excitatory postsynaptic potential (EPSP) in the same interneurone. Superimposed sweeps show that increasing the intensity of electrical stimulation gave rise to a spike in the interneurone. Responses to three different intensities of stimulation are superimposed. (Ci) Simultaneous stimulation of nerve 2 and the chordotonal organ (CO) strand. Suprathreshold electrical stimulation of nerve 2 alone evoked a spike in the interneurone (first arrow), whereas a simultaneous stimulation of nerve 2 and the chordotonal organ produced only an EPSP (second arrow). (Cii) Increasing the intensity of electrical stimulation of nerve 2 resulted in an increased amplitude of the EPSP. (Ciii) Further increasing the intensity of electrical stimulation elicited a spike (second arrow) in the interneurone even though the interneurone was receiving simultaneous inhibitory input from chordotonal afferents. Two superimposed sweeps are shown.
hairs on the exopodite, evoked a compound EPSP in VE-1 (Fig. 7B, middle trace). Increasing the stimulus amplitude resulted in a spike in the interneurone (Fig. 7B, upper trace). During chordotonal stimulation, the spike in VE-1 mediated by suprathreshold electrical stimulation of nerve 2 was blocked by the inhibitory chordotonal organ input and, under these conditions, the electrical stimulus resulted in a small compound EPSP (Fig. 7Ci, second arrow). Increasing the amplitude of electrical stimulation still further produced an increase in the amplitude of the EPSP (Fig. 7Cii) until it eventually gave rise to a spike in the interneurone, even though the interneurone was receiving a strong proprioceptive inhibitory drive from the exo-endo CO (Fig. 7Ciii).

Excitatory inputs elicited by stretch of the chordotonal organ also had an effect on the efficacy of exteroceptive inputs (Fig. 8). For example, interneurone RC-3 received depolarising inputs during both proprioceptive and exteroceptive stimulation. Stretch of the chordotonal organ strand with a ramp velocity of 1600 ° s⁻¹ evoked a compound EPSP in RC-3 (Fig. 8A) and a subthreshold electrical stimulation of nerve 2 also evoked a compound EPSP in the same interneurone (Fig. 8B). When these inputs occurred simultaneously, the subthreshold depolarisations summed to evoke spikes in interneurone RC-3 (Fig. 8Ci,ii).

**Discussion**

We show that many identified ascending interneurones in the terminal ganglion of the crayfish are excited or inhibited by stretches of a chordotonal organ that monitors movements of the exopodite relative to the endopodite of the tailfan (Field et al., 1990). The exo-endo CO contains approximately 12 sensory neurones that between them encode position and velocity and have distinct velocity thresholds (Field et al., 1990; Nagayama and Newland, 1993). The ascending interneurones encountered in this study also showed distinct response properties, some receiving phaso-tonic inputs during an imposed stretch movement, while others received phasic inputs. Of the three interneurones that receive phaso-tonic inputs, two (NE-1 and RC-8) are known to be part of the circuitry mediating the characteristic tail-flip escape response of crayfish. These two interneurones form part of a disynaptic pathway from exteroceptive afferents on the tailfan and abdomen exciting the pair of lateral giant (LG) interneurones that mediate and coordinate the tail-flip (Zucker et al., 1971). They also receive synaptic input from the exo-endo CO (Nagayama et al., 1997) and, in turn, make monosynaptic connections with LG interneurones (Zucker, 1972). Tonic sensory input from the exo-endo CO also produces a sustained subthreshold activation of LG interneurones (Newland et al., 1997). This input to the LG interneurones and ascending interneurones NE-1 and RC-8 was never found to lead to spikes in the LG interneurones, and it is therefore unlikely that CO input alone could evoke a tail-flip. Chordotonal organ input could therefore sum with other inputs, probably exteroceptive input from water-motion-sensitive hairs, to evoke a response in the LG interneurones.

Many of the interneurones received a phasic excitation during proprioceptive stimulation. The tail-flip response occurs with very high velocities (Cooke and MacMillan, 1985), so that feedback relating to the positions of the endopodite and exopodites will occur at high frequency. Many of the ascending interneurones responded to movements of the chordotonal organ at very high velocities (up to 1600 ° s⁻¹), which would mean they could respond to variations in joint angle during the actual tail-flip itself whether mediated through giant or non-giant pathways.

**Intersegmental reflex effects**

Whilst exteroceptive inputs to ascending intersegmental interneurones have been analysed in detail in the crayfish, their

![Fig. 8. Interaction of exteroceptive inputs and proprioceptive inputs in interneurone RC-3. (A) Stretches of the chordotonal organ (CO) with a ramp stimulus velocity of 1600 ° s⁻¹ evoked a compound excitatory postsynaptic potential (EPSP) in the interneurone. (B) Subthreshold electrical stimulation of nerve 2 (N2) also evoked a compound EPSP in the same interneurone. Three responses to electrical stimulation are superimposed. The amplitude of EPSPs was constant. (Ci,ii) Two examples showing that simultaneous subthreshold stimulation of nerve 2 and the exopodite-endopodite CO evoked a spike in the interneurone (N2+CO).](image-url)
output effects are far less well documented. Nevertheless, Aonuma et al. (1994) described the influence of 16 identified ascending interneurones on the spike frequency of abdominal flexor and extensor motor neurones in each abdominal segment, in addition to their output effects on the exopodite opener and closer motor neurones in the terminal ganglion. Of the 12 interneurones receiving excitatory input from the exo-endo CO found in our study, we know the output effects of seven (Aonuma et al., 1994); of these, six (CA-1, CI-1, RC-2, RC-6, RC-8 and NE-1) have an excitatory effect on abdominal extensor motor neurones, thereby increasing their spike frequency. The other interneurone (RO-2) has an inhibitory effect on the abdominal extensor motor neurones. The input that RO-2 received from the chordotonal organ, however, was always subthreshold so that the summed activity of all these interneurones during an opening movement of the exopodite could increase extensor motor neurone activity in all abdominal segments, which may lead to abdominal extension. In terms of escape behaviour, such a movement is again essential in preparation for a tail-flip, in particular for the longer-latency non-giant tail-flips, since the escape movement is driven by a rapid abdominal flexion from an extended position. Clearly, the input to these two motor systems underlies conflicting motor patterns. The LG interneurones are activated at short latency through mono- and polysynaptic pathways, yet at the same time the abdominal postural extensor motor neurones will also be activated. The most likely explanation of how this conflict is resolved must lie in the fact that, if the LG interneurones reach threshold and spike, then the giant neurones themselves mediate an inhibition of both the tonic and phasic extensor motor neurones (Wine, 1977, 1984) during the tail-flip. A similar inhibitory effect must also occur when many of the same ascending interneurones are excited directly by water-motion-sensitive afferents (Nagayama and Sato, 1993) in parallel to the LG interneurones (Miller et al., 1992; Yeh et al., 1993).

Intrasegmental reflex effects

The local output effects on opener and closer motor neurones of the terminal ganglion are known for six of the ascending interneurones we describe in this study. Four of these interneurones are known to excite the closer motor neurones, while the other two interneurones inhibit them. Thus, the net effect of an opening of the exopodite (if only proprioceptors were activated) would be a local reflexive closing movement about the joint. Unlike the metathoracic femoral chordotonal organ of locusts (Burrows, 1987) or the coxo-basal chordotonal organ of the walking legs of crayfish (El Manira et al., 1991), Newland and Nagayama (1993) found no monosynaptic input from proprioceptive afferents of the exo-endo CO onto exopodite motor neurones. This lack of direct drive means the precise reflex response may depend on the behavioural state of the animal when the exopodite is displaced. Thus, if chordotonal organ stimulation were insufficient to lead to an escape response, it could induce a bilateral closing of both exopodites, an extension of the abdomen and possibly forward walking in a posture typical of the ‘dart response’ described by Nagayama et al. (1986).

Convergence of extero- and proprioceptive inputs

All the ascending interneurones we describe received convergent inputs via sensory pathways from water-motion-sensitive hairs on the tailfan and from the exo-endo CO. The input from these two sources was not always the same. For example, some interneurones were depolarised and spiked in response to stimulation of both types of mechanoreceptor. Other interneurones, however, were inhibited by proprioceptive stimulation but depolarised in response to exteroceptive stimulation. Since it is unlikely that the exopodite would be moved without simultaneous displacement of the water-motion-sensitive hairs, it is very probable that there will be substantial interactions between the two types of input that will either enhance or reduce a particular sensory drive. At the stimulus strengths used in this study, proprio- and exteroceptive inputs to interneurone RC-3 were both subthreshold. When both qualities of input were applied simultaneously, however, the inputs summed to produce spikes in the interneurone.

However, interneurone VE-1, for example, received excitatory input from exteroceptive afferents (see also Nagayama et al., 1993). Stimulation of the exo-endo CO was inhibitory. Simultaneous stimulation led to a reduction in excitability so that normally suprathreshold exteroceptive inputs became subthreshold. This implies that the output effects of a given interneurone could be abolished when inputs to those interneurones are considered in a more behaviourally relevant manner.

To conclude, we have shown that many identified ascending interneurones receive proprioceptive inputs during stimulation of a receptor in the tailfan. These inputs interact with those from exteroceptive hairs to change the level of excitability of the interneurones and their potential role in behaviour. The next step in our analysis is to analyse the output effects of ascending interneurones on the phasic extensor motor neurones, since we know that they receive three sources of input during the tail-flip. The most important of these is derived from activation of the sensory receptors during the power stroke of the tail-flip (Reichert et al., 1981; Wine, 1977). An opening of the uropods during the power stroke could evoke a reflexive closing movement (a prerequisite of the return stroke) and an activation of the ascending interneurones that activate the tonic extensor motor neurones. A better understanding of the role of the exo-endo CO during normal behaviour would then help us to understand more fully the contribution played by these ascending interneurones in controlling and coordinating behaviour.

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References


