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Wind-sensitive interneurones in the terminal ganglion of praying mantids

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Summary. 1. Twelve types of wind-sensitive neurone have been identified in the terminal ganglion of the mantids, *Archimantis sobrina* and *A. latistylus* (Fig. 1). Nine have conspicuously larger axons than the others in the connective of the ventral nerve cord and are termed 'giant' interneurones (Figs. 1, 2, 3), although they are small by comparison with those of other orthopteroid insects (Fig. 15).

2. Transverse sections of connectives reveal nine large axon profiles whose spatial relationship changes along the ventral nerve cord (Fig. 4).

3. Transverse sections of ganglia containing stained giant cells show that major arborizations are found in neuropilar regions containing the terminals of cercal afferents (Fig. 5).

4. Recorded cells could be divided into two groups depending on whether their responses to wind stimuli were purely excitatory (Figs. 6, 7), or contained an inhibitory component (EI responses, Fig. 8). Electrical stimulation of cercal afferents confirmed the response patterns evoked by wind (Figs. 7, 8 and 9).

5. Dendritic arborizations are more strongly developed on the side of major synaptic input to each giant cell (Figs. 1, 2, 5) as established by electrical and wind stimulation of afferents in left and right cerci (Fig. 10).

6. Conduction velocities in the giant cells ranged from 2.5–3.1 m/s (Fig. 11, Table 1).These neurones are thus amongst the slowest conducting insect giant neurones, consistent with their small diameter relative to those of other insects (Fig. 15).

7. The short response latencies and the 1:1 nature of their response to high frequency (100 Hz) electrical stimulation of the cercal nerve indicated that at least neurone Types 5, 8 and 11 (Fig. 11, Table 1) probably have direct connections with cercal receptors.

8. Several cells had high rates of spontaneous activity and in one (Type 5, Fig. 12A), injection of hyperpolarizing current produced a rhythmical bursting at approximately half the spontaneous rate. The interburst interval could be altered by phasic stimulation of the cercal nerve (Fig. 12B).

9. Behavioural experiments with a tethered mantid showed responses in leg- and flight-motor pathways to wind stimulation of the cerci. In a dissected preparation, electrical stimulation of abdominal connectives and wind stimulation of the cerci both evoked responses in four neurones of the metathoracic ganglion: one spiking local interneurone and three motoneurones (Figs. 13, 14).

Introduction

The giant interneurones originating in the terminal ganglion of some orthopteroid insects have been extensively studied both to elucidate their functional significance (Roeder 1948; Ritzmann and Camhi 1978; Camhi 1980; Ritzmann et al. 1980, 1982; Camhi and Nolen 1981; Comer 1985; Boyan et al. 1986), and to study fundamental neuronal properties such as synaptic transmission (Harrow and Sattelle 1983; Miller and Jacobs 1984; Callec 1985); target cell recognition and neuronal plasticity (Edwards and Palka 1971; Palka and Edwards 1974; Murphey 1985; Murphey et al. 1981, 1985); and the effects of sensory deprivation (Matsumoto and Murphey 1977, 1978; Shankland and Goodman 1982; Shankland et al. 1982), and experience (Murphey and Matsumoto 1976), on identified interneurones during development.

It is now clear that giant interneurones with considerable morphological and functional similarities are present in crickets, cockroaches and lo-

custs. If it can be assumed that the orthopteroid insects had a common ancestor, then any similarities, or differences, between the neurones of any two orthopteroid species presumably reflect: (1) how closely related the two species are phylogenetically, and (2) the extent to which the processes of natural selection acted to alter the original pattern. The praying mantid is quite different in its way of life from either of the other two insects in which giant interneurones have been extensively studied – the cockroach and cricket. We therefore decided to investigate: (a) whether the mantid has giant interneurones extending up the ventral nerve cord from the terminal ganglion; and (b) if so, how these compare in structure and function with those of cockroaches and crickets.

The only previous detailed study of the terminal ganglion of the mantid is that of Cloarec (1968) who described its internal structure on the basis of wax sections, and summarized the pattern of nerve roots. Cloarec found it difficult to recognize homologies between *Mantis* and *Periplaneta* and did not encounter fibres which could be regarded as 'giants', although this term is clearly relative.

Since the majority of giant interneurones in the cricket (Tobias and Murphey 1979) and cockroach (Westin et al. 1977) are wind-sensitive, our initial approach was to record wind-sensitive cells in the terminal ganglion, and establish, by intracellular dye injection in conjunction with other histological techniques, those neurones that might be considered giant interneurones. We then examined the physiological response properties of these cells, such as conduction velocity, latency, side of major synaptic input and response to high frequency stimulation of cercal afferents, to determine their order in the synaptic pathway, and to allow a comparison with data from giant interneurones in other species. We also examined the outputs from the wind-sensitive cercal system to thoracic motor pathways, in both behaving and dissected preparations, as a clue to the role these neurones play in behaviour.

Materials and methods

Animals. All experiments were performed on adult female mantids (*Archimantis sobrina* Sauss. and *A. latistylus* Serv.). *A. sobrina* was previously misidentified as *A. brunneriana* Sauss. (Ball and Stone 1982; Ball et al. 1982). Mantids were collected in the wild near Canberra. No neurophysiological or neuroanatomical differences between the species were noted.

Dissection. Animals were generally pinned dorsal side up on a foam covered platform after the wings and legs had been removed. In addition, in order to obtain a more complete representation of wind-sensitive cells in the ganglion, some preparations were mounted ventral side up. The last abdominal segment bearing the cerci projected well beyond the end of the platform in all preparations. The cuticle covering the dorsal (or ventral) surface of the abdomen was removed, as were the gut, any eggs, and the internal reproductive organs, and the body cavity flooded with saline (mmol/l: NaCl 140, KCl 15, $CaCl_2$ 4, $MgCl_2$ 4, $NaHCO_3$ 10, Trehalose 35, pH 7.2). The large abdominal tracheae were disturbed as little as possible. Peripheral nerves of the abdominal ganglia immediately anterior to the terminal ganglion were severed, as were all but the cercal nerves of the terminal ganglion itself. A stainless steel spoon introduced posteriorly between the cercal nerves supported the terminal ganglion, and a silver wire inserted into the thorax served as the indifferent electrode.

Stimulation. The cerci were stimulated with wind puffs delivered by a Pasteur pipette with attached bulb, or, when quantified, by an 18 gauge syringe needle linked to a compressed air bottle. In the latter case, the stream of air was interrupted by a thin damped blade attached to a solenoid which was driven by a function generator via a transistorized switch. Wind velocities were calibrated using a hot wire anemometer and varied from 1.0–1.6 m/s when delivered at a distance of 9.5 cm from the nearest cercus.

Recording of neural potentials. Wind-related afferent activity from the cerci was monitored in every preparation by differential recording of the action potentials of receptors in each cercal nerve with bipolar 75 µm silver wire hook electrodes. The electrodes were placed as distally as possible on each cercal nerve (N10, Fig. 3) just as it entered the cercus. Another set of bipolar hook electrodes was placed on one or both connectives of the ventral nerve cord just posterior to the second abdominal ganglion to monitor activity in the ascending axons of cells being recorded intracellularly in the terminal ganglion. The signals from the hook electrodes were then amplified, and stored on magnetic tape along with the intracellular record. The various sets of hook electrodes were used both to record wind-sensitive activity, and also to electrically stimulate receptors and interneurones via a stimulus isolation unit. Appropriate voltages of stimulation produced a single afferent volley to the terminals (see also Pearson et al. 1985). All branches leading off the cercal nerve within the abdominal cavity were severed to minimize activation of non-cercal receptors during electrical stimulation.

Intracellular recordings were made from the neuropilar segments of wind-sensitive interneurones in the terminal ganglion using glass micropipettes that had resistances of 80–100 MΩ when filled with either a 200 m*M* cobalt nitrate/1 *M* potassium acetate mixture or a 5% Lucifer Yellow in 0.5 *M* LiCl solution. All electrodes were introduced into the ganglion through an untreated neural sheath. Intracellular penetrations were accompanied by a 40–60 mV drop in potential and the onset of synaptic activity. Neural potentials were amplified by a DC-amplifier with current-passing facility, displayed on an oscilloscope, and stored on magnetic tape.

Histology. At the completion of experiments either cobalt nitrate or Lucifer Yellow dye was injected into the recorded cell using the appropriate current polarity while continuously monitoring membrane potential. Immediately following dye injection the terminal ganglion was dissected from the preparation, and treated histologically as previously described by Bacon and Altman (1977) for cobalt and Stewart (1978) for Lucifer Yellow. Stained cells were photographed in wholemount and then drawn immediately using a camera lucida.

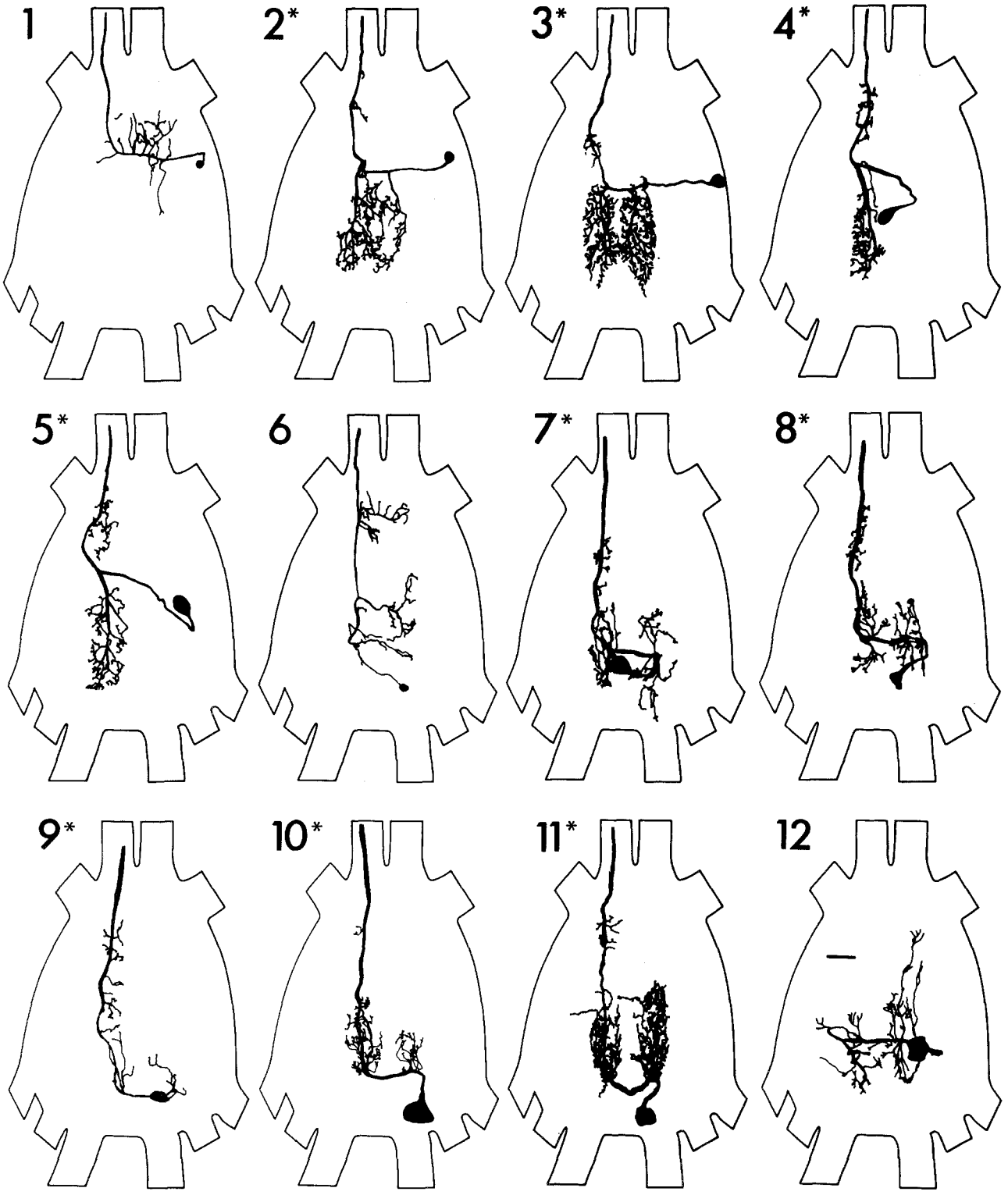


Fig. 1. Drawings of twelve types of wind-sensitive interneurone in the terminal ganglion of *Archimantis*. Neurones were stained intracellularly with Lucifer Yellow or cobalt (Types 3 and 11), and drawn from wholemount or reconstructed from photographs. (*) indicates neurones with a large ('giant') axon projecting anteriorly in the ventral nerve cord. Ganglia are viewed dorsally. Scale bar: 50 μm

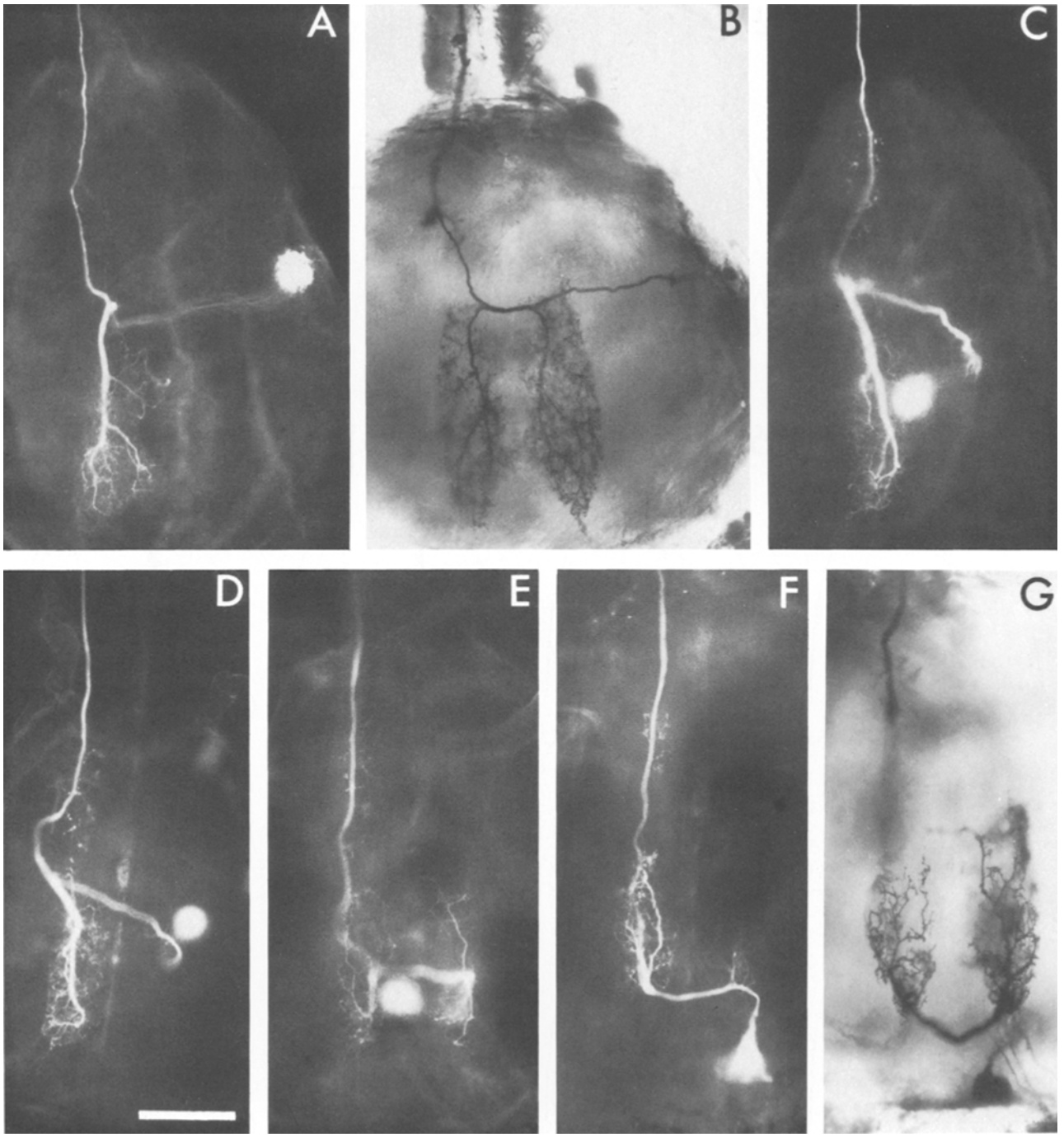


Fig. 2A–G. Photomicrographs of Lucifer Yellow or cobalt stained wind-sensitive neurones in the terminal ganglion in wholemount. **A** Type 2, **B** Type 3, **C** Type 4, **D** Type 5, **E** Type 7, **F** Type 10, **G** Type 11. Scale bar: 100 μm

Selected ganglia and connectives containing stained cells were embedded in soft araldite and sectioned at 25 or 50 μm in order to visualize the three-dimensional structure of filled cells.

The arrangement of unfilled giant fibres in the ventral nerve cord was studied in sections of material fixed and embedded for electron microscopy as described in Ball and Stone (1982). Cobalt chloride backfills were also used to determine giant interneurone projections and cell body positions in the terminal ganglion using the methodology previously described in Ball et al. (1982).

Nomenclature. Nerve roots were numbered according to previous studies by Viallanes (1891), Nesbitt (1941) and Cloarec (1968).

Behavioural experiments. Behavioural experiments were carried out on an intact adult female mantid, tethered by being waxed by the dorsal pronotum to a holder. The animal was held firstly in an inverted position, ventral side up, and horizontal to the ground, with the legs grasping a circular cardboard disc, and the wings free to move. Animals were often seen maintaining this body position in the wild. The room was dimly lit, but sufficient light was available for the animal to peer at objects. Puffs of air were delivered from a Pasteur pipette positioned behind the animal and just lateral to the longitudinal body axis. Wind strength was adjusted so that an air puff just deflected the cerci, and the diameter of the pipette's aperture ensured that only the cerci were being stimulated.

Results

Anatomy

Twelve different types of wind-sensitive interneurones were identified on the basis of intracellular staining in the terminal ganglion of the mantid. Each of the neurones has its soma and major dendritic processes in the terminal ganglion. Eleven neurones have axons ascending to at least the metathoracic ganglion, while one is a local interneurone with all its processes restricted to the terminal ganglion. The gross morphology of each neurone type is shown in Fig. 1, in which the cells are ordered approximately from anterior to posterior according to cell body position. Representative stainings of recorded cells with either cobalt or Lucifer Yellow are shown in Fig. 2.

A series of cobalt backfills into the terminal ganglion from just posterior to the fourth abdominal ganglion (due to fusion of ganglia during ontogeny the terminal ganglion is the fifth abdominal ganglion), revealed a bilaterally symmetrical array of large cell bodies, along with a ladder-like arrangement of axons and major dendritic processes (Fig. 3A, B, C). In favourable preparations it was possible to recognize most of the cells previously stained intracellularly (Fig. 1).

In order to establish the size distribution and arrangement of axons in the ventral nerve cord, a series of transverse sections was made starting anterior to the fourth abdominal ganglion and continuing posteriorly up to the terminal ganglion. Although the decision as to what constitutes a 'giant' neurone is somewhat arbitrary, our sections showed nine axon profiles that were significantly larger than the rest (Fig. 4). This series of sections also revealed that the spatial relationship of any one of these profiles to its neighbours varied along the connective (Fig. 4A, B, C), thus making impossible an unambiguous identification of a cell from only a single filled profile. It was possible, how-

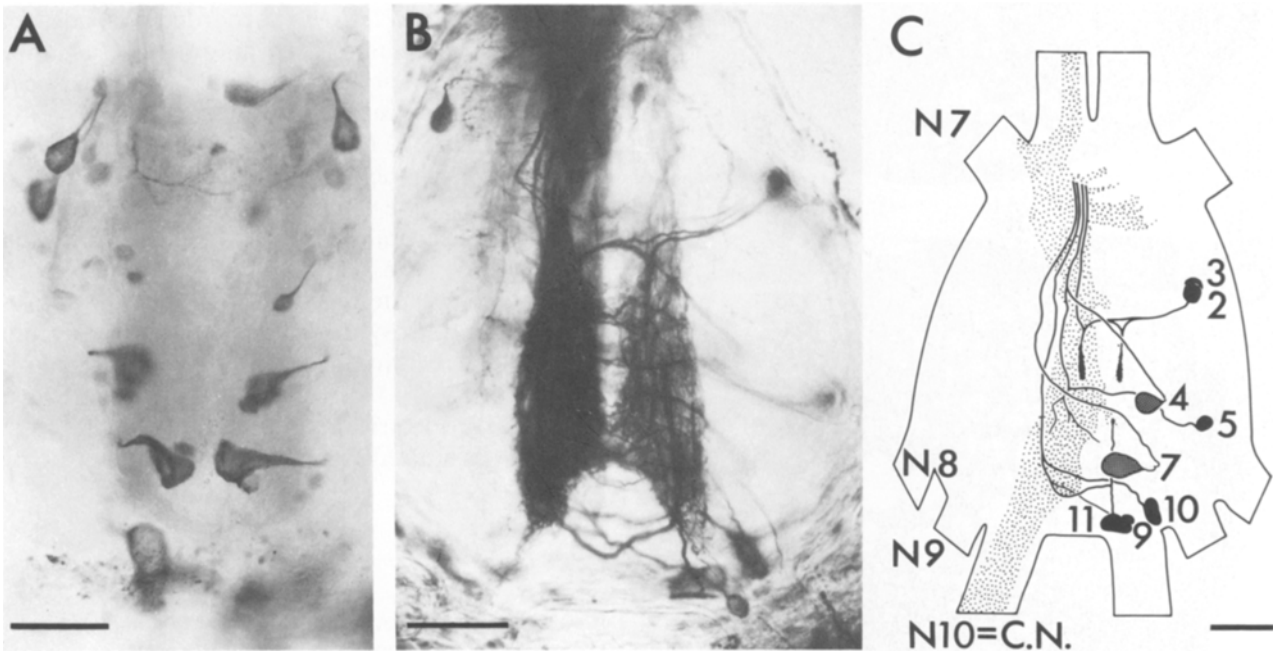
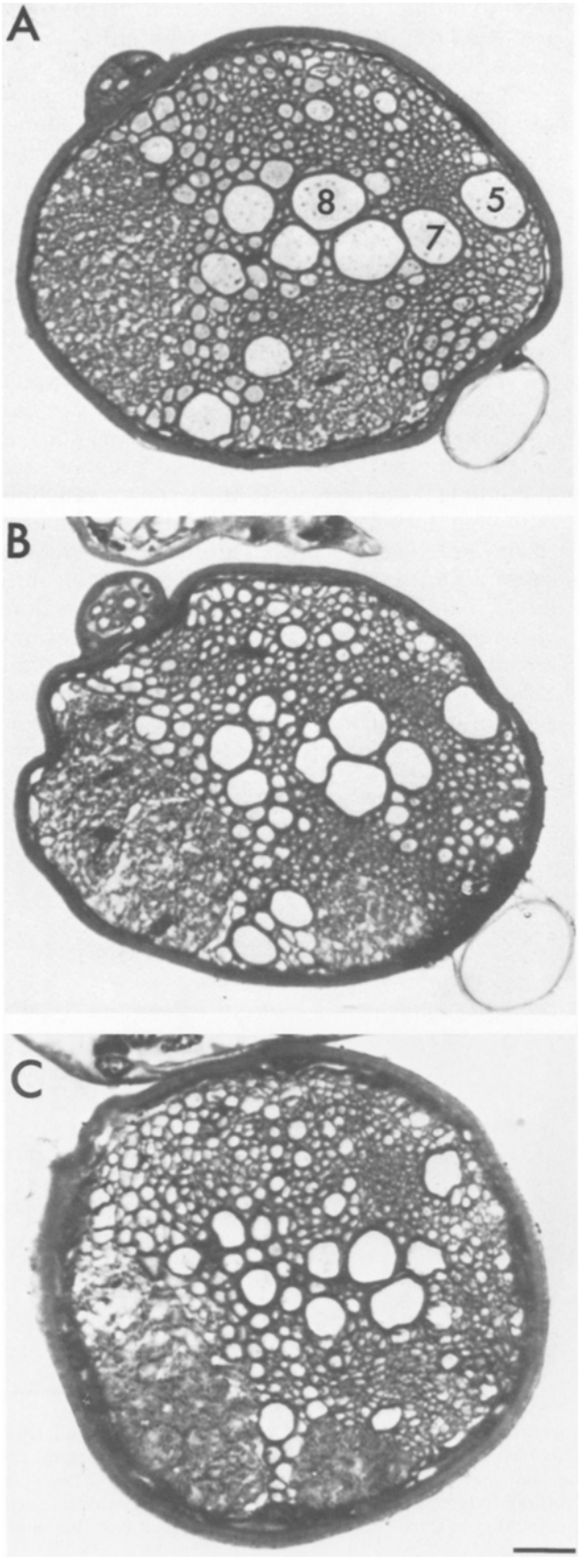


Fig. 3A–C. Wholemounts of cobalt backfills into the terminal ganglion showing the arrangement of the larger cells. **A** Bilateral fill from both connectives showing the large, bilaterally paired cells near the ventral surface of the ganglion. **B** Unilateral fill via the left connective showing the extent of dendritic arborization, and that most of the somata are contralateral. **C** Drawing of the stained cells shown in **B**. Identified somata are numbered according to type, and those with ventral cell bodies are indicated by cross-hatching. Nerve roots are numbered according to Cloarec (1968). The cercal nerve projection is stippled (see Ball et al. 1982). All scale bars: 100 μm



ever, on the basis of repeated recoveries of intracellular stainings with Lucifer Yellow, to identify three large profiles as belonging to previously identified cells (Fig. 4A), and these were cells already considered to be giants.

Several ganglia, each containing an intracellularly stained cell, were sectioned, in order to establish neurone morphology with respect to areas of cercal afferent endings (Fig. 5A). All of the neurone types which were reconstructed from sections (Types 5, 7, 8, 12) had a basically similar morphology, with a large ventral soma, a dorsally running neurite, dorsal crossing segment, and major dendritic processes located in one or both glomeruli, where the cercal afferents also terminate (Fig. 5B–D). Other neurone types which in wholemounts appeared to have arborizations in the glomeruli were Types 2, 3, 4 and 11.

Physiology

The physiological data presented below were gathered from over 50 animals. In most cases the data presented are from giant interneurones because these were encountered more often than the smaller wind-sensitive cells. Physiological data are only presented from cell types recorded a minimum of 3 times.

1. Response characteristics. Recorded neurones fell into two broad classes according to whether their physiological responses to wind, and electrical, stimulation of cercal afferents were purely excitatory (Types 2, 3, 4, 6, 7, 11, 12), or contained an inhibitory component (Types 5, 8, 10).

Representative excitatory responses to wind stimuli from several neurones are shown in Fig. 6. The responses are very similar, having an initial large depolarization supporting one or more spikes, followed by a more or less rapid decline of the EPSP amplitude to result in a lower spiking rate (Types 2, 7, 11), or to a subthreshold level (Types 3, 4). Electrical stimulation of cercal receptor axons produced excitatory responses in the in-

Fig. 4A–C. Transverse sections of one ventral nerve cord cut at varying intervals between the last two abdominal ganglia. The sections are arranged from anterior **A** to posterior **C**. Several of the larger fibres can be followed from section to section, but few can be unequivocally identified from a single section due to changes in their relative positions. Profiles identified on the basis of several fills and/or a series of sections containing a filled profile are numbered according to neurone type in **(A)**. Scale bar: 10 μm

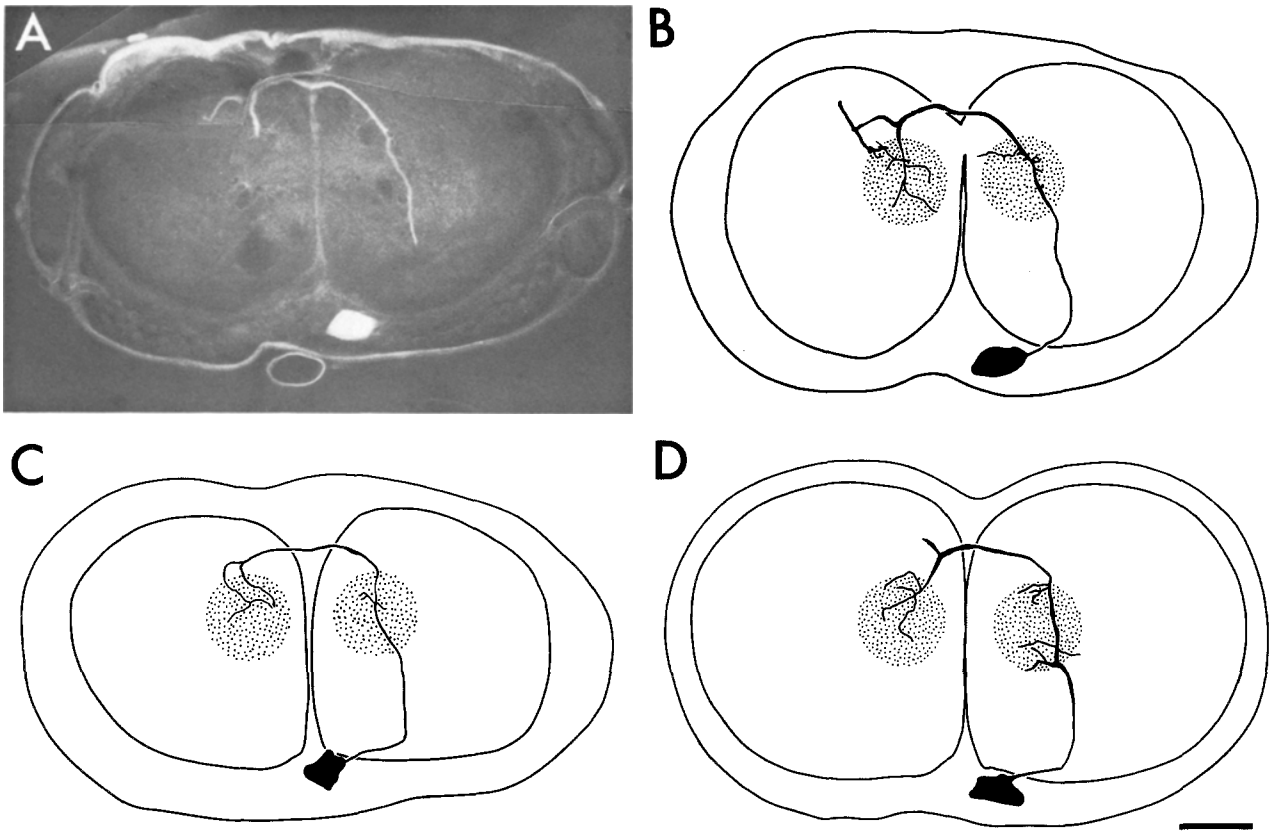


Fig. 5A–D. Transverse sections of terminal ganglia containing wind-sensitive interneurones stained with Lucifer Yellow. **A** Photomicrograph of a Type 7 neurone, and **B** a reconstruction of the same neurone. **C** Reconstruction of a Type 8 neurone, **D** reconstruction of a Type 12 local neurone. The approximate maximum extent of the glomeruli is shown by stippling in the reconstructions; note that most branching occurs within the glomeruli. Scale bar: 100 μm

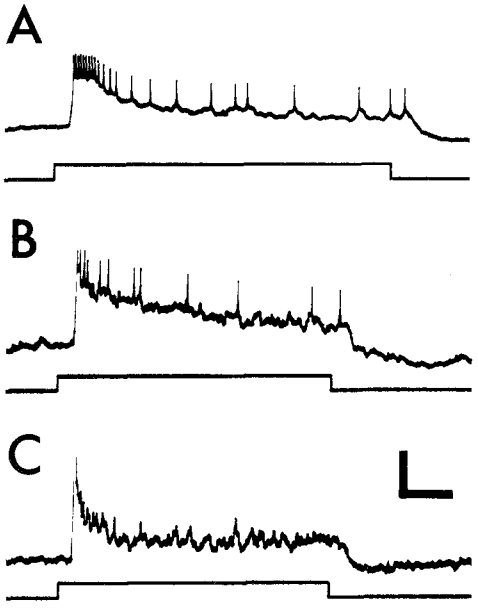


Fig. 6A–C. Responses from three neurone types excited by wind stimulation of the cerci. **A** Type 2, **B** Type 11, **C** Type 3. Lower traces show the duration over which wind was presented. Scale bars: vertical **(A)** 30 mV, **(B, C)** 15 mV; horizontal 100 ms

terneurones similar to those produced by wind (Fig. 7A, B).

Neurone Types 5, 8 and 10 responded with varying degrees of excitation and inhibition (EI) to wind stimulation of the cerci (Fig. 8A–C). There also appeared to be some variation in the degree of inhibition exhibited by the same neurone type in different preparations. Again, the neuronal response evoked by electrical stimulation of cercal receptors mimicked that to wind (Fig. 9A), and on occasion also revealed the presence of discrete IPSPs otherwise masked in the response to wind (Fig. 9B).

2. Input side. The effects of electrical stimulation of cercal afferents closely matched those evoked by wind stimuli, so this method was used to characterize the side of major synaptic input, and test the directness of connection of interneurones with receptors by measurement of response latency and ability to follow high frequency (100 Hz) stimulation 1:1.

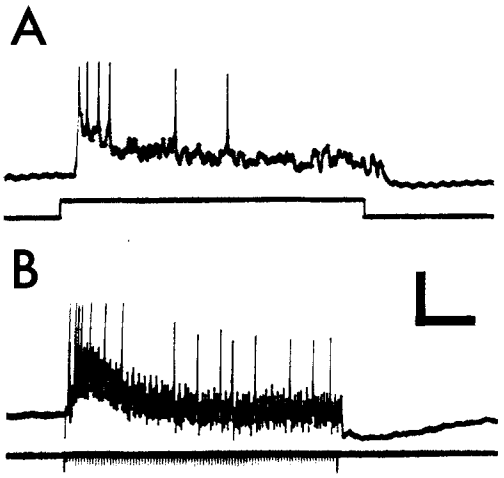


Fig. 7A, B. Excitatory response recorded in neurone Type 7 on wind stimulation of the cerci **A**, can be mimicked by high frequency (200 Hz) electrical stimulation of the cercal nerve **B**. Larger deflections in the lower trace in **B** are artefacts which indicate the beginning and end of electrical stimulation. Record in **B** is AC-coupled. Scale bars: vertical **(A)** 15 mV, **B** 9 mV; horizontal 100 ms

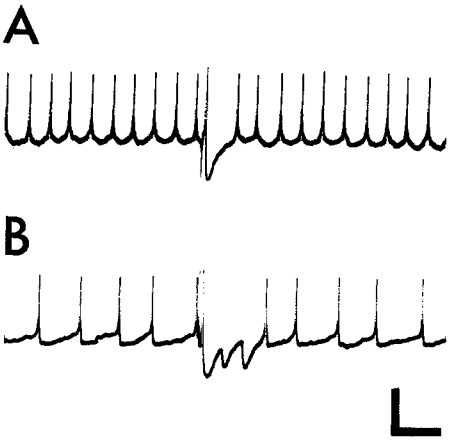


Fig. 9A, B. Results from two neurone types suggest that the afterhyperpolarization evoked by electrical stimulation of the cercal nerve has a postsynaptic origin: **A** the hyperpolarization evoked by an electrical stimulus is not present after spontaneous spikes (Type 8); **B** the afterhyperpolarization often reveals discrete inhibitory events, probably IPSPs (Type 5). Stimulus artefacts are present in both records. Scale bars: vertical **A** 15 mV, **B** 6 mV; horizontal 50 ms

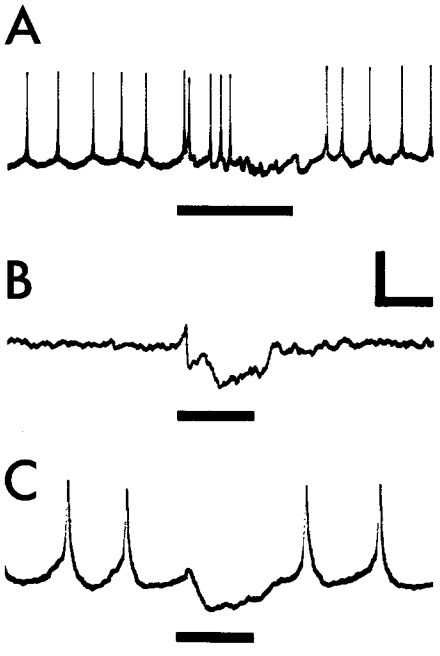


Fig. 8A–C. Responses of three neurone types displaying varying degrees of excitation and inhibition (EI) on wind stimulation of the cerci. **A** Type 5, **B** Type 8, **C** Type 10. Stimulus bars in each case indicate the onset and duration of wind-related excitatory activity in the cercal nerve. Scale bars: vertical **A, C** 15 mV, **B** 6 mV; horizontal 50 ms

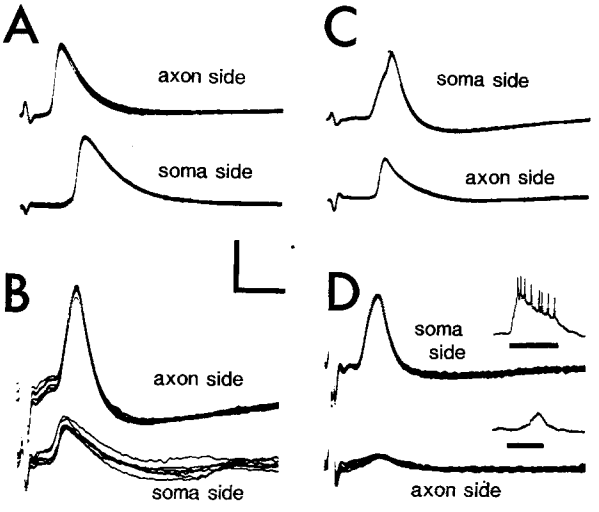


Fig. 10A–D. The side of major synaptic input to four neurone types as revealed by responses evoked by electrical stimulation of left and right cercal nerves. **A** Type 11, **B** Type 5, **C** Type 7, **D** Type 12. Insets to **D** show that responses evoked by wind stimulation of the cerci were consistent with the directionality of the input recorded on electrical stimulation. Initial deflections in each record are stimulus artefacts, and each trace consists of multiple sweeps of the oscilloscope. Scale bars: vertical **A, C** 20 mV, **B, D** 10 mV, inset to **D** 25 mV; horizontal 4 ms, inset to **D** 85 ms

Recorded neurones fell into two groups depending on whether the major input to the cell (excitatory or inhibitory) was from the cercus on the neurone's axon side (Types 5, 6, 8, 10, 11), or soma side (Types 7, 12) (Fig. 10). There was a further distinction within the former group with respect to the dominance of the axonal over the somatic input. In neurones 8, 10 and 11 the input from the soma side sometimes almost equalled that generated contralaterally, as seen by the amplitude

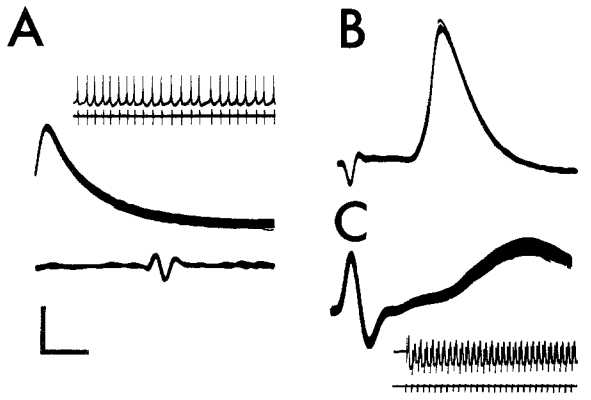


Fig. 11. **A** (Inset) Records from cell Type 10 showing 1:1 relationship of spikes recorded intracellularly in the terminal ganglion (upper trace) and extracellularly in a connective (lower trace). Triggering the oscilloscope from the intracellular spike reveals a constant latency between spikes, which allowed measurements of conduction velocity to be made. **B** Latency of response in cell Type 7 evoked by electrical stimulation of a cercal nerve. The trace shows superimposed responses to 10 successive stimuli. **C** The response of neurone Type 8 follows high frequency (100 Hz) electrical stimulation of the cercal nerve 1:1 (inset), and at a short and consistent latency (top trace), indicating a direct connection with cercal afferents. The initial deflection in **B, C** is the stimulus artefact (as is the bottom trace in inset to **C**) and multiple sweeps are shown in each case. Traces in **C** are AC-coupled. Scale bars: vertical (**A, B, C** 10 mV; insets, upper traces only, 28 mV); horizontal (**A, B, C** 2 ms; insets 85 ms)

Table 1. Table showing conduction velocity, response latency, and capacity to follow 100 Hz electrical stimulation of the cercal nerve 1:1, in seven identified wind-sensitive neurones of the terminal ganglion. (*) excludes one measurement of 3.4 m/s; ($n=$) number of preparations, minimum of five repetitions for each; n.t. not tested; n.a. not applicable, local interneurone

Cell type	Physiological measurement		
	Conduction velocity (m/s)	Latency (ms)	Follows 100 Hz stimulation 1:1
5	2.6 ± 0.08 ($n=4$)	1.84 ± 0.26 ($n=5$)	yes
6	n.t.	2.34 ($n=1$)	no
7	2.5 ± 0.09 ($n=6$) (*)	3.15 ± 0.37 ($n=8$)	no
8	3.1 ± 0.03 ($n=2$)	2.05 ± 0.05 ($n=2$)	yes
10	3.1 ± 0.02 ($n=2$)	2.32 ± 0.35 ($n=3$)	n.t.
11	3.0 ± 0.07 ($n=2$)	1.69 ($n=1$)	yes
12	n.a.	2.02 ($n=1$)	n.t.

of the EPSP recorded in the crossing segment, but the response latency to axonal input was always considerably shorter (Fig. 10A). In neurones 5 and 6 there was a considerable difference between the strength of input from axon and soma sides (Fig. 10B), and this difference was just as strong, but reversed, in neurones 7 and 12 (Fig. 10C, D). Wind stimuli to the cerci in each case were consistent with the directionality of the input recorded on electrical stimulation (e.g. cell 12, Fig. 10D).

3. Conduction velocities. One physiological indicator of axon diameter in unmyelinated fibres is the velocity with which the action potential is conducted in the axon. This was measured in five cell types by impaling the cell intracellularly in the terminal ganglion and simultaneously recording the spike with hook electrodes located on the connectives ascending to the thorax. A 1:1 correspondence between intracellularly and extracellularly recorded spikes, at a constant latency, confirmed the same cell was being recorded at each site (Fig. 11A), and allowed measurements of conduction velocity to be made, since electrode separation could also be measured (Table 1). The data show that the five cell types from which measurements were obtained had conduction velocities ranging from 2.5–3.1 m/s.

4. Response latency. Response latency is one physiological characteristic that can be used to place a neurone in the synaptic hierarchy from receptors to motor output. Latencies were obtained from seven neurone types by electrically exciting cercal afferents and measuring the delay to the commencement of the evoked response (Fig. 11B, Table 1). Delays ranged from 1.69 ms for cell Type 11 to 3.15 ms for cell Type 7, and the data show that giant interneurones did not necessarily have shorter latencies than non-giants (e.g. cell Types 6, 12). Considering the time from electrical stimulation to the arrival of afferent information at the receptor terminal in the ganglion (ca. 1 ms), and the delay associated with one synapse, a response latency of 2 ms or less would probably represent a direct connection between an interneurone and receptors. Neurone Types 5, 8, 11 and 12 are therefore the most likely to make direct connections with receptors.

5. High frequency stimulation. Another indicator of whether a direct connection exists between two cells is whether the synapse continues to transmit information 1:1 at high stimulus frequencies. The cercal afferents were therefore stimulated at 100 Hz and the evoked activity in five interneurone types was checked for a corresponding 1:1 following of the stimulus (Fig. 11C, Table 1). Only three of the interneurones (Types 5, 8, 11) followed the stimulus 1:1, and these were also the neurones with the shortest response latencies (Table 1).

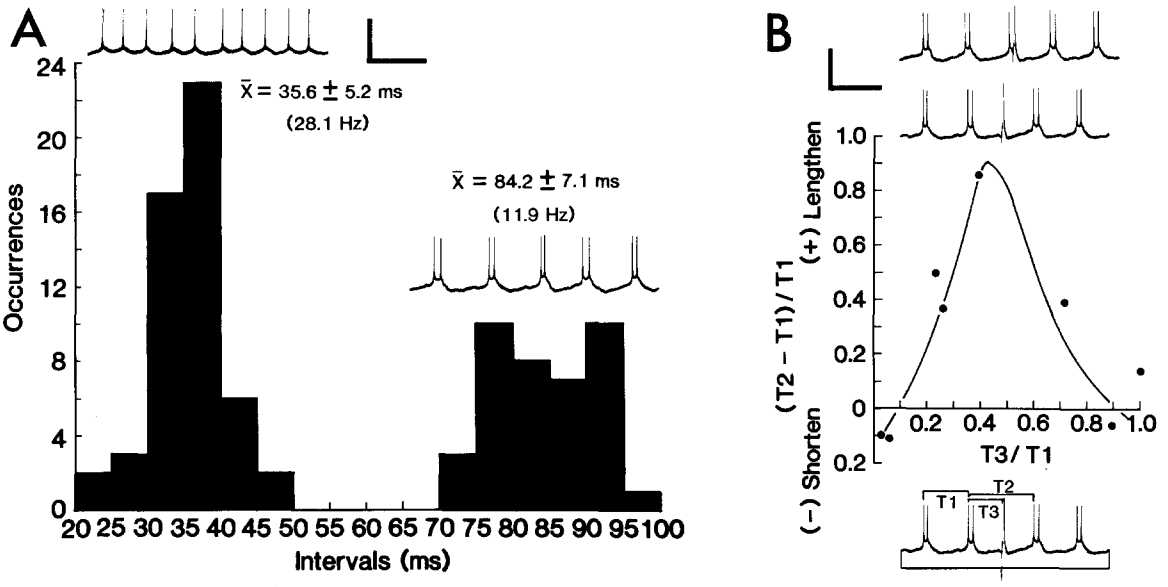


Fig. 12. **A** Histogram showing interspike intervals during spontaneous activity in neurone Type 5 normally (left), and intervals between the first spikes of doublets (right) after injection of 2 nA of hyperpolarizing current has converted the cell into a burster (right). Scale bars: vertical 25 mV; horizontal 100 ms. **B** Relationship between the change in cycle time of intervals between the first spikes of successive bursts (T2-T1)/T1, and the time of electrical stimulation of the cercal nerve (T3/T1). Both variables were normalized by dividing by the mean duration of the three cycles (shown in the inset at bottom as the one cycle T1) immediately preceding the cycle in which the stimulus was presented (T2). Time of stimulation (T3) was measured from the first spike of a preceding burst. Scale bars: vertical 25 mV; horizontal 100 ms

6. Descending effects on responsiveness. The results of Roeder et al. (1960) demonstrated that some neuronal activity in the terminal ganglion of the mantid was under the influence of descending input from the brain. To test whether the responsiveness of the wind-sensitive interneurones we were studying was influenced by the head ganglia, we recorded the response of giant neurone Type 3 (Fig. 1) to a constant wind stimulus before and after sectioning the VNC between the suboesophageal and prothoracic ganglia. In a preparation stimulated well above threshold, the response of neurone 3 under control conditions was 20.5 ± 0.58 spikes per stimulus, and after severing the VNC 20.0 ± 1.15 spikes per stimulus. In another preparation the same neurone type was stimulated at just above threshold intensity and responded with 2.71 ± 0.95 spikes per stimulus normally, and with 2.75 ± 1.26 spikes after severing the VNC. If neurone Type 3 is representative of the wind-sensitive cells in the terminal ganglion, then removal of the head ganglia does not affect responsiveness to wind.

7. Burst-like activity in the terminal ganglion. Most wind-sensitive cells recorded in the terminal ganglion displayed no, or little, activity in the absence of a stimulus. In some cases, however, penetration of a cell was accompanied by a maintained discharge which did not alter with time (e.g. cell Types 5, 8, 10, Figs. 8, 9). The fact that the discharge was at a constant rate, and was repeatable for cell Type 5 in different preparations, suggested that the activity might be physiological, and either a property of the cell itself or the result of presynaptic input to it. In the case of neurone Type 5 (Fig. 1), the cell recorded in Fig. 12A was spontaneously active at 28.1 Hz, but tonic hyperpolarization (2 nA) transformed this to a rhythmically oscillating depolarization, bearing only spike doublets, at a rate of 11.9 Hz (Fig. 12A). When the cercal afferents were then electrically stimulated (Fig. 12B), there was a strong phase dependent effect on the oscillatory rhythm. A stimulus occurring early in an oscillatory period shortened that period (i.e. sped up the rate), whilst a stimulus occurring in the middle of a period almost doubled the length of that period.

8. Responses to sound. A number of studies have demonstrated that giant interneurones of the terminal ganglion in crickets (Counter 1976; Belosky and Delcomyn 1977; Murphey et al. 1977; Kämper 1984), bushcrickets (Shen 1983), cockroaches (Guthrie 1966; Schwab and Josephson 1977), and locusts (Rozhkova et al. 1984) are sensitive to low frequency sound. We placed hook electrodes on the ventral nerve cord anterior to the

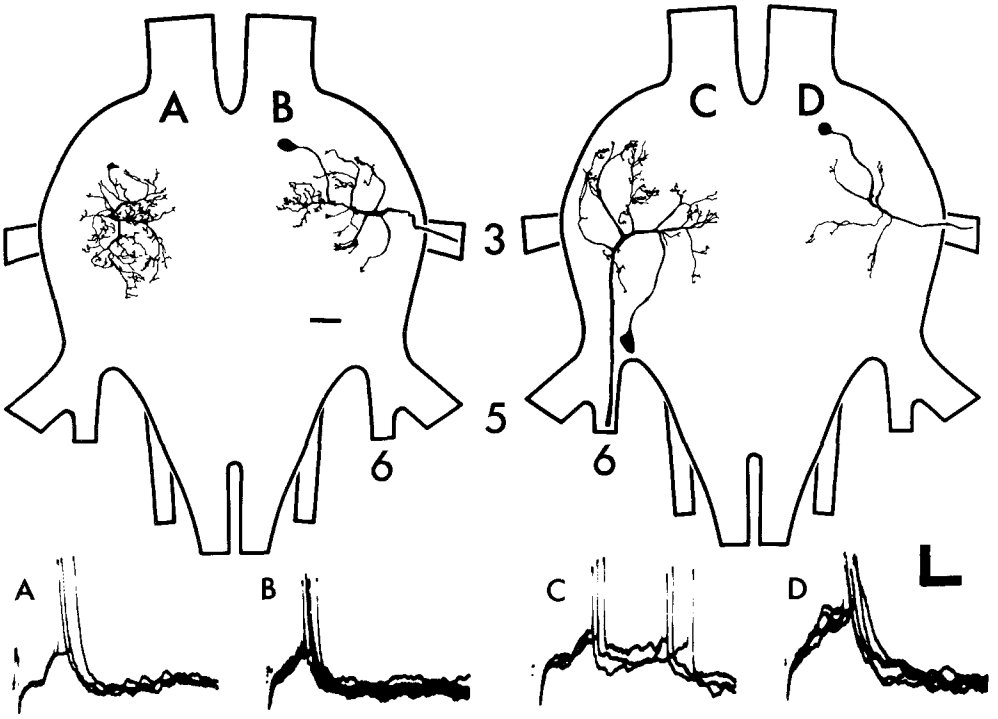


Fig. 13. Neurones in the metathoracic ganglion and the responses evoked in them by electrical stimulation of abdominal connectives. Numbers refer to nerve roots. Initial deflections are the stimulus artefact and multiple sweeps are shown in each case. All traces AC-coupled. Scale bars: vertical 6 mV; horizontal **(A)** 10 ms, **(B, C)** 20 ms, **(D)** 40 ms

terminal ganglion in five mantids, located in anechoic conditions (Nocke 1975), and found that several unidentified giant interneurones responded to low frequency sound. All responded best in the 30–40 Hz frequency range, where there was a phase-locked following of the sinusoidal stimulus.

9. Cercal inputs to thoracic motor pathways. It has been established in both cockroaches (Ritzmann and Camhi 1978; Ritzmann et al. 1980, 1982) and locusts (Boyan et al. 1986) that wind-sensitive giant interneurones exert powerful effects on motor pathways associated with walking and flight. Our interest in the comparative, as well as functional, aspects of giant interneurones in arthropods, therefore led us to examine the inputs from such neurones to these same motor pathways in the mantid.

Behaviour

For behavioural experiments the animal was mounted as described in the Methods. Once the animal had adapted to the conditions, wind puffs were delivered to the cerci at approximately 10 s intervals, and the animal's response was firstly to move its abdomen away from the stimulus source, and then maintain the new position until again stimulated. The abdomen did not return to its original position in line with the body axis, but could be brought back by stimulating from the other side. On removal of the cardboard disc the animal maintained its legs in the normal stance position, forelegs cocked for a strike, although the tarsi of the middle- and hindlegs were not in contact with any substrate. Each puff of air delivered to the cerci under these circumstances was observed to reliably evoke two distinct, but possibly linked, behaviours. The first behaviour consisted of all the legs moving in unison, the forelegs forward and outward, and the middle- and hindlegs, dorsally and forward. The second behaviour consisted of the forewings being opened and extended, the hindwings only partially so. At no time did the legs contact the wings and so influence the response.

To test if this latter behaviour represented a part of the flight response, the holder bearing the animal was placed so that the animal was dorsal side up, horizontal to the ground, and with its head about 12 cm from a tube through which a continuous warm air stream could be delivered. When placed in the air stream, and without tarsal contact, the mantid opened and extended both sets of wings; the middle- and hindlegs were extended and moved dorsally so that the femora were

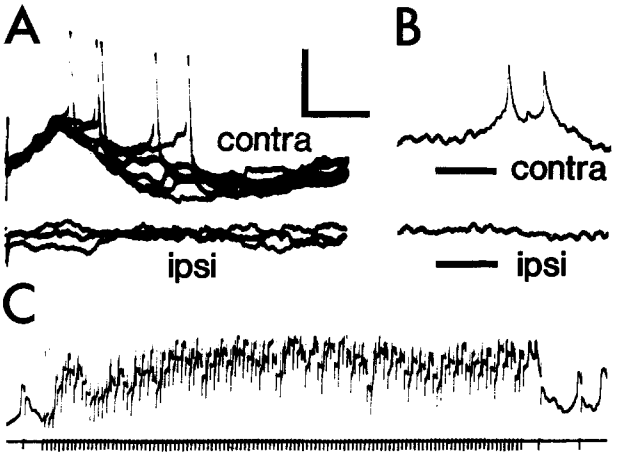


Fig. 14. **A** Electrical stimulation of each abdominal connective shows that the subalar motoneurone only receives synaptic input from the side contralateral to its soma. Initial deflections are stimulus artefacts and multiple sweeps are shown. Traces were AC-coupled. **B** Responses evoked by wind stimulation of the cerci are consistent with the directionality established by electrical stimulation in **A** above. **C**. Responses of the subalar motoneurone summate on high frequency (200 Hz) electrical stimulation of abdominal connectives. Lower trace shows stimulus artefacts. Scale bars: vertical **A** 10 mV, **B** 15 mV, **C** 25 mV; horizontal **A** 40 ms, **B, C** 85 ms

aligned at right angles to, and in the median plane of, the longitudinal body axis. The forelegs were moved firstly forward and outward, and then together so that they closely apposed the head.

The behavioural observations above suggested that both leg and flight motor pathways received input from wind-sensitive neurones of the cercal system. We tested this proposition by firstly recording extracellularly with hook electrodes from a connective posterior to the metathoracic ganglion, and simultaneously from the same connective between meso- and metathoracic ganglia. We observed that a number of wind-sensitive units appeared in both recordings and so projected to at least the mesothoracic ganglion. We then placed recording/stimulating electrodes on each connective between the first and second abdominal ganglia (see Methods), and looked for evoked activity in intracellularly recorded neurones of the metathoracic ganglion in response to electrical stimulation. Wind puffs were also directed at the cerci to ensure that recorded cells were in fact receiving wind input from the cerci.

We found that electrical stimulation of the abdominal connectives, and wind stimulation of the cerci, both evoked activity in a spiking local interneurone and three motoneurones in the metathoracic ganglion (Fig. 13). One motoneurone projected out peripheral nerve root 6 and probably innervated the subalar muscle, which in the locust is a bifunctional walking (coxal remotor) and flight (depressor, wing opener) muscle, but whose role in the mantid is unknown. The other two motoneurones both projected out nerve root 3, and although not individually identifiable, probably innervated walking muscles of the leg (Ball, in preparation).

The responses evoked by electrical stimulation of abdominal connectives and wind stimulation of the cerci were excitatory and suprathreshold in all the thoracic neurones recorded (Figs. 13C, 14A, B). Since the stimulating electrodes were always placed on the connectives in the same positions along the ventral nerve cord, it was possible to compare the relative latencies of evoked responses in the four metathoracic neurones, although not the absolute response latency with respect to cercal stimulation. The local interneurone received the shortest latency EPSP from the ventral nerve cord, followed by the two anterior (N3) motoneurones, and finally the subalar motoneurone (Fig. 13C).

Where tested, motoneurone responses were directional; the major input side for the local interneurone was ipsilateral to the soma, but contralateral for the subalar motoneurone (Fig. 14A, B). The responses of the subalar motoneurone also summated on high frequency stimulation of presynaptic cells (Fig. 14C).

Discussion

The present study shows that praying mantids of the genus *Archimantis* possess a group of large wind-sensitive interneurones in the terminal ganglion (Fig. 1) which are activated by cercal hair sensilla (Ball et al. 1982). These cells are bilaterally paired (Fig. 3) and send large-diameter axons up the ventral nerve cord to at least the meso- and metathoracic ganglia, where they excite neurones in both walking and flight motor pathways (Figs. 13, 14).

Anatomy of the cercal receptor/giant interneurone system in an evolutionary context

It is not known whether the ancestral orthopteroid insect had giant fibres, but it seems likely that it did have cerci (Kukalova-Peck 1985). Edwards and Reddy (1986) have recently summarized our knowledge of the evolution of cercal receptor/giant interneurone systems. In the absence of fossil nervous systems the evolution of giant fibres must remain speculative, but there are clearly enormous differences in the extent of development of the

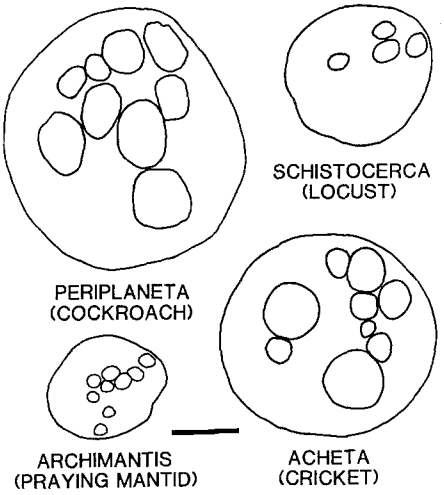


Fig. 15. Tracings from published photomicrographs of transverse sections of the ventral nerve cords of some orthopteroid insects to show the relative development of the giant fibre system between the last two abdominal ganglia. *Periplaneta americana* from Farley and Milburn (1969), *Schistocerca gregaria* from Seabrook (1970), *Acheta domestica* from Edwards and Palka (1974), *Archimantis sobrina*, this study, Scale bar: 50 μm

giant fibre system among present orthopteroid insects. The 'giant' interneurones of mantids are small compared to those of other orthopteroid insects both in absolute terms, and relative to the size of the other interneurones in the ventral cord (Fig. 15). To quantify these differences we have taken the cross-sectional areas both of the largest axon, and of the nine largest axons (except for *Schistocerca* which only has four giants), as a percentage of the cross-sectional area of the connective (Fig. 15). These are, respectively, for *Periplaneta americana* 5.7% and 30.9%, for *Acheta domestica* 8.2% and 28.8%, for *Schistocerca gregaria* 2.9% and 8.9%, and for *Archimantis latistylus* 2.0% and 12.3%. These figures are not exactly comparable due to differences in fixation technique and position of the section in the ventral nerve cord, but the overall picture which emerges is almost certainly correct. It is plausible that the relatively large size of the giant fibres in cricket and cockroach reflects the importance of the cercal sensilla in predator detection and of the giant fibres in a fast escape, while for other orthopteroid insects such as locusts (Cook 1951; Seabrook 1970, 1971; Boyan et al. 1986; and in prep.), grylloblattids (Edwards and Mann 1981), earwigs (Edwards and Ball 1980) and mantids (this paper) selective pressures on conduction velocity have been less.

The pattern and extent of embryonic fusion of the abdominal ganglia in mantids remains unresolved, probably due to differing views on the status of the eleventh abdominal segment (Viallanes 1891; Nesbitt 1941; Roeder et al. 1960; Cloarec 1968). However, regardless of whether it comprises four or five neuromeres, the terminal ganglion is a fused structure and our cobalt backfills reveal putative serially homologous cells (e.g. Types 4 and 7 of Figs. 1, 3).

Both the cercal receptors of the mantid (Ball and Stone 1982; Ball et al. 1982), and the interneurones to which they are presynaptic (Fig. 1), reflect insect phylogeny in being more similar to those of the cockroach than the cricket (Blackith and Blackith 1968; Sharov 1971; Kamp 1973; Mendenhall and Murphey 1974; Kristensen 1975, 1981; Kevan 1977; Daley et al. 1981; Hue 1983). However, a search for homology between the giant interneurones of the terminal ganglion of different orthopteroid insects based only on adult anatomy and physiology is subject to several potential errors. Firstly, in the embryo of the cricket the lateral wind-sensitive giants originate from clusters of cells which are morphologically similar (Jacobs and Murphey 1981), and it is therefore possible for different cells in the clusters to have become giants in different species (G. Jacobs, personal communication). Secondly, Wilson (1979) described morphological variability amongst locust leg motoneurones with somata in the anterior-central part of the thoracic ganglia that was as great, or greater, within a class of cells from different individuals as it was between classes. Thirdly, Pearson et al. (1985) have established that locust interneurones which are quite dissimilar in both morphology and physiology are in fact true serial homologs. Wilson and Hoyle (1978) even describe a functional switch between serially homologous motoneurones in locust thoracic ganglia. In the mantid we found variability in some wind-sensitive cells of the terminal ganglion similar in extent to that described by Wilson (1979), so that we have had to use physiological as well as morphological criteria to determine both the number of anterolateral (Types 2 and 3) and posterior ventral midline (Types 7, 8 and 9) cells, and the differences between them. Given these provisos, there are still striking morphological and physiological similarities between the giant interneurones of several orthopteroid species. For example, mantid lateral cell Types 2 and 3 may be comparable to cricket 8-1 (MGI) and 9-1 (LGI) (Murphey et al. 1976, 1977), and to cockroach GI2 and GI3 (Daley et al. 1981; Hue 1983). Of the more posterior cells, there is a marked similarity between cricket cell 10-2 (Mendenhall and Murphey 1974 – note that this appears to differ from cell 10-2 of Levine and Murphey 1980), cockroach GI6 (Daley et al. 1981; Hue

1983), and our cell type 7 (Fig. 1); between cricket cell 10-2 (Levine and Murphey 1980) and our cell Type 11 (Fig. 1); and between cockroach GI5 (Daley et al. 1981; Hue 1983) and our cell Type 5 (Fig. 1). We feel that these similarities probably reflect homology: if not, they represent a remarkable example of convergent evolution.

Physiology

The physiological responses of several of the twelve wind-sensitive interneurones in the terminal ganglion (Fig. 1) are consistent with the interspecific anatomical similarities pointed out above.

With few exceptions, the neurone types we recorded as being purely excited by wind stimuli tended to have the more anterior, and those displaying inhibition the more posterior, cell body positions in the ganglion (Figs. 1, 7, 8). In this respect their responses appear to follow the pattern in the cockroach, where inhibition from cercal hair sensilla does not play any role in the formation of the receptive fields of anterior GIs 1 and 2 (Daley 1982), and in the cricket, where both pre- and postsynaptic inhibition are seen in posterior giants 10-2 and 10-3 (Levine and Murphey 1980).

The afterhyperpolarization we recorded from several neurones on electrical stimulation of a cercal nerve (Fig. 9) is similar to that reported for the cockroach (Narahashi 1960; Callec and Boistel 1966; Dagan and Parnas 1974; Bernard et al. 1983). Dagan and Parnas (1974) have reported hyperexcitability as a result of the negative afterpotential following a spike, an effect which might be attributable to non-synaptic events such as the accumulation of K^+ around the membrane (Narahashi 1960). However, in the mantid, the hyperpolarization also occurred in response to low intensity wind stimulation of cercal sensilla (Fig. 8), was not present after spontaneous spikes, and could often be resolved into discrete subunits (Fig. 9). These data suggest that the afterhyperpolarization was probably a postsynaptic effect similar to that described by Levine and Murphey (1980) in the cricket. Indeed Jacobs et al. (1985) have identified possibly GABAergic neurones as the origin of such polysynaptic inhibitory inputs to a number of wind-sensitive neurones in the cricket, whilst Bernard et al. (1983) have recently demonstrated an inhibitory input to giant interneurones of the cockroach from a chordotonal organ at the base of the cercus. The chordotonal receptors project into the ganglion via the cercal nerve, so if such an organ also exists in the mantid, electrical stimulation of the entire cercal nerve would excite the chordotonal as well as the hair cell afferents, and might account for some of the inhibitory responses seen.

The evoked responses in giant interneurones to electrical stimulation of left and right cercal nerves correlated in all cases with the major input side as established on the basis of wind stimulation (Fig. 10). The major input side (as determined by EPSP amplitude or latency) was generally axonal, and in all cases clearly reflected the relative extent of branching in the glomeruli on each side of the ganglion (compare Figs. 1, 10).

Without dual recordings from both afferent cell and postsynaptic interneurone, we relied on two indirect measures – response latency and a 1:1 following of a high frequency stimulus – to ascertain whether a particular interneurone made a direct connection with cercal receptors. The sensory axons in the cercal nerve are up to 5 μm in diameter (Ball et al. 1982), and, given a 2 m/s conduction velocity (from Pearson et al. 1970), and 2 mm distance from receptor to CNS, the afferent information will arrive in the CNS with approximately a 1 ms delay. If we then allow up to a further 1 ms for synaptic delay, this means that interneurone response latencies should be 2 ms or less for a direct connection. Our measurements of response latency for seven neurone types (Table 1) show that only neurones 5, 8, 11 and 12 meet this criterion.

Our second method of testing for direct connections was to electrically stimulate the cercal nerve at a high frequency (100 Hz) (see also Horridge and Burrows 1974) and note whether interneurone EPSPs could follow the stimulus 1:1 (Fig. 11C, Table 1). Of the neurones tested only Types 5, 8 and 11 could do so, and all of these also meet the response latency criterion for a direct connection.

Consistent with the axon diameters presented in Fig. 15, and discussed above, the conduction velocities of 2.5–3.1 m/s which we measured for five mantid neurone types, including the largest 'giants' (Table 1), are amongst the slowest recorded for 'giant' interneurones in an invertebrate (c.f. *Locusta* 1.5–4.5 m/s, Seabrook 1971; *Periplaneta* 4–6 m/s, Farley and Milburn 1969, Dagan and Parnas 1970; *Acheta* 2.5–5.5 m/s, Counter 1976; *Anax* 3.5–4.5 m/s, Fielden 1960), but accord well with the model of Pearson et al. (1970) and reflect the small axon diameters of 'giants' in the mantid.

A number of giant interneurones in the cercal systems of the cricket (Edwards and Palka 1974; Belosky and Delcomyn 1977; Levine and Murphey 1980) and mantid (cell Types 5, 8, 10, Figs. 8, 9, 12), were found to have high, maintained, rates

of spontaneous activity. In both insects these neurones also displayed an inhibitory component in their electrical- and wind-evoked responses (e.g. Figs. 8, 9). In the mantid, however, tonic hyperpolarizing current injected into one of these cells (cell Type 5, Fig. 12) resulted in bursting activity (see Pinsker and Ayers (1983) for similar effects in other systems) whose timing could be altered in a phase-dependent manner by cercal input. Resetting of rhythmical activity by sensory input is a feature of several motor pathways in vertebrates and invertebrates (Farley and Case 1968; Andersson et al. 1981; Fitch and Kammer 1982; Grillner and Wallen 1984; Pearson et al. 1983) but rhythmical activity is not common in purely sensory systems. The oscillatory activity in cell Type 5 may result from some intrinsic endogenous mechanism within the cell, in which case the phase-dependent effect of cercal input may be explained by the recovery course of membrane potential after a spike (Katayama 1971). Alternatively, the oscillations may be the result of synaptic input from an underlying endogenous oscillatory circuit, as is the case with the CV1 cells in the feeding motor pathway of the snail *Lymnaea* (McCrohan 1984). One circuit in the terminal ganglion of the mantid that might produce the oscillatory synaptic drive to neurone 5 is the copulatory motor program which can be readily released by decapitation (Roeder et al. 1960). If this is the case, then cercal giant 5 may provide sensory information to this motor pathway in the form of a variable shift in the phase of its oscillatory activity.

Behaviour

Much of the interest in cercal receptor/giant fibre systems has related to their role in escape behaviour. However, it is becoming increasingly apparent that they also play an important role in other behaviours as well. Behavioural experiments have demonstrated a powerful effect of the cerci on flight in the cockroach (Fraser 1977), and giant interneurones have been identified that activate motor pathways involved in the initiation of flight (Ritzmann et al. 1980, 1982). Similarly, but for a greatly reduced cercal system comprising only four giant interneurones (Cook 1951; Seabrook 1970, 1971; Potente 1975), wind stimulation of the cerci of the locust can initiate flight, and a single giant interneurone has been shown to both initiate and modulate flight (Boyan et al. 1986).

Our behavioural experiments with a tethered mantid showed that wind stimulation of the cerci can initiate leg and wing movements consistent with an initiation of flight (see Results). The flight response itself closely resembled that of the locust, in that the forelegs were closely apposed to the head, and tarsal contact exerted a powerful inhibitory effect on the release of the behaviour.

We then showed in a dissected preparation, that wind-sensitive circuits in the terminal ganglion of the mantid activated several thoracic motoneurones which may be active during walking and flight (Figs. 13, 14). Motoneurones similar to these, and receiving cercal input, have previously been shown to be involved in walking in the cockroach (Iles 1972; Ritzmann and Camhi 1978; Ritzmann 1981; Ritzmann and Pollack 1981; Tobias and Ritzmann 1984), and both walking and flight in the locust (Wilson 1962).

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