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Identification of thoracic interneurons that mediate giant interneuron-to-motor pathways in the cockroach

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Summary. 1. Paired intracellular recordings were made to identify thoracic interneurons that receive stable short latency excitation from giant interneurons (GIs).

2. Eight metathoracic interneurons were identified in which EPSPs were correlated with GI activity which was evoked either by wind or intracellular electrical stimulation or occurred spontaneously. In all cases EPSPs in the thoracic interneurons followed GI action potentials faithfully at short latencies.

3. EPSPs associated with GI action potentials consistently represented the upper range of amplitudes of a large sample of EPSPs recorded in the thoracic interneurons.

4. Seven of the interneurons were correlated with activity in ventral GIs but were not correlated with activity in dorsal GIs. Four of these interneurons were part of a discrete population of interneurons whose somata are located in the dorsal posterior region of the ganglion. The eighth interneuron (designated the T cell) was positively correlated with activity in dorsal GIs.

5. The four dorsal posterior group interneurons and the T cell were depolarized intracellularly to establish their potential for generating motor activity. In all cases evoked activity was stronger in leg motor neurons (primarily D_s and the common inhibitor) located on the side contralateral to the interneuron's soma.

6. The results indicate that significant polysynaptic pathways exist by which GI activity can evoke motor activity. The implications of this conclusion to investigations on the cockroach escape system are discussed.

Introduction

The giant interneuron (GI) system in the cockroach *Periplaneta americana* consists of 7 bilateral pairs of identifiable interneurons that span the length of the ventral nerve cord (Fig. 1). They can be divided into two groups, 4 ventral and 3 dorsal giants (vGIs 1–4 and dGIs 5–7 respectively). Because of the accessibility of the GIs, this system has been a popular preparation for studying neural organization (Ritzmann 1984). Several studies have demonstrated that each GI is selectively excited by wind from a characteristic set of directions (Westin et al. 1977) and that this information on wind direction is used to direct escape away from the wind source (Camhi and Tom 1978; Camhi et al. 1978). However, the pathway between GIs and motor neurons, once thought to be a simple monosynaptic connection, has proved to be extremely difficult to demonstrate.

Roeder (1967) proposed that the GIs provide a rapid pathway from wind receptive hairs on the cerci (sensory structures on the posterior abdominal segments) to the motor neurons that control leg movements in thoracic segments. Such a rapid pathway would be appropriate for a wind mediated escape behavior. Although this organization seemed logical, several subsequent findings were inconsistent with a monosynaptic pathway from GIs to motor neurons. Dagan and Parnas (1970) reported that the threshold value for extracellular stimulation of the abdominal cord which is needed to generate action potentials in leg motor neurons is higher than that which produces action potentials in GIs. Moreover, intracellular stimulation of unidentified GIs also failed to evoke activity in motor axons of the metathoracic legs.

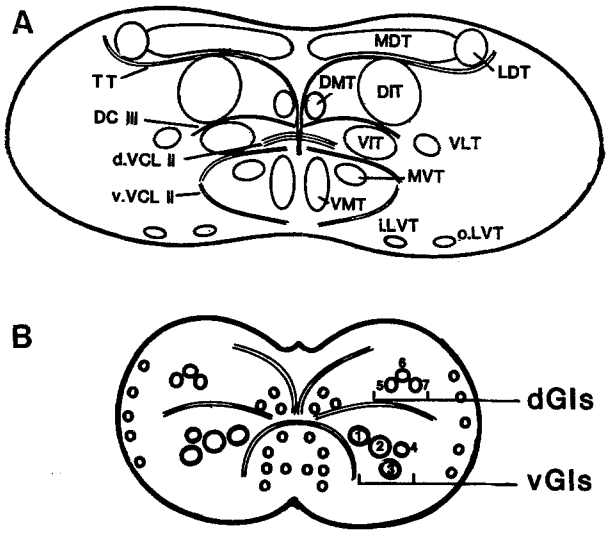

Fig. 1 A, B. Landmarks used in cross-sections to aid in identifying thoracic interneurons and GIs. **A** Diagrammatic representation of a cross-section of T_3 near the level of N_3, indicating the principle tracts and commissures (after Gregory 1974). *MDT* median dorsal tract; *LDT* lateral dorsal tract; *DMT* dorsal median tract; *DIT* dorsal intermediate tract; *VIT* ventral intermediate tract; *VLT* ventral lateral tract; *MVT* median ventral tract; *VMT* ventral median tract; *i.LVT* inner lateral ventral tract; *o.LVT* outer lateral ventral tract; *TT* T-shaped tract; *DC III* dorsal commissure III; *d.VCL II* dorsal part of ventral commissural loop II; *v.VCL II* ventral part of ventral commissural loop II. **B** Diagram of a cross-section of an abdominal ganglion. The positions of vGIs (1–4) and dGIs (5–7) on one side are indicated

In contrast to this finding, Ritzmann and Camhi (1978) were able to generate outputs in leg motor neurons by stimulating specific GIs intracellularly. They used trains of current pulses reaching frequencies that had been recorded for GI action potentials in response to gentle wind puffs (Westin et al. 1977). In addition, each stimulated GI was marked with intracellular dye for subsequent identification. All the dGIs, plus vGI1 evoked reproducible motor responses. For example, dGI5 always excited primarily depressor motor neurons, whereas dGI6 excited primarily levators. However, the responses had relatively long latencies and were weaker than expected for a vigorous escape circuit. This was attributed to two factors. First, the intracellular technique made it necessary to stimulate a limited number of GIs at any given time. The activity in a single GI represents only a small fraction of the neural activity generated by a wind stimulus (Westin et al. 1977). Paired GI stimulation produced stronger responses with shorter latencies, but still not to the degree that might be expected for a vigorous escape response (Ritzmann 1981; Ritzmann and Pollack 1981). Second, the dissection and restraint required for intracellular techniques may have severely reduced the efficacy of the system. Nevertheless, these results along with those of Dagan and Parnas (1970) again suggest that although a GI-to-motor pathway exists, it is probably not a significant monosynaptic one.

Additional support for a polysynaptic pathway comes from intracellular studies of motor neurons that function in both walking and flying movements. Subthreshold responses evoked by stimulation of identified dGIs had latencies in excess of 20 ms; i.e. much longer than that expected for a monosynaptic pathway (Ritzmann et al. 1982).

In addition to the suggestion of a polysynaptic organization, other studies indicated that the dorsal and ventral GIs are distinctly different populations of interneurons. The dGIs are excited during walking while the vGIs are inhibited (Daley and Delcomyn 1980). Moreover, in paired stimulation experiments, vGIs do not augment motor outputs evoked from dGIs but if anything decrease their effectiveness (Ritzmann 1981). Finally, dGI activity can initiate either flying or running depending on afferent activity from the legs (Ritzmann et al. 1980). This type of bifunctional activity has not been demonstrated for vGIs.

Thus, the picture that is developing is far more complex than that originally envisioned by Roeder (1967). The data suggest a neural organization involving at least one interneuron interposed between the GIs and the leg motor neurons, and, given the differences between dGIs and vGIs, several interneurons may be involved. However, the evidence for a polysynaptic pathway remains circumstantial. A direct demonstration of a polysynaptic pathway requires the identification of interneurons that both receive inputs from GIs and are capable of evoking activity in leg motor neurons. To date no such interneurons have been identified. In this paper we describe a study in which numerous interneurons in the metathoracic ganglion were tested for both inputs from GIs and outputs to leg motor neurons. We found 5 thoracic interneurons that meet both of these criteria, and an additional 3 interneurons that receive inputs from vGIs but have as yet not been tested for motor outputs.

Methods

All experiments were performed on adult male *Periplaneta americana*. The colony was housed in large plastic garbage cans at 27–30 °C. Food (Purina Lab Chow Pellets) and water were available ad lib.

Animals were pinned dorsal side up to a cork. The dorsal cuticle from A_5 to T_2 was removed to expose the CNS. Care was taken to limit the disruption to the musculature and the tracheal system. The animal was continuously superfused with cockroach saline. Originally, standard Callec and Sattelle (1973) saline was used. However, in later preparations we found that substituting MOPS for the $NaHCO_3$ buffer provided a more active preparation (Carr and Fourtner 1980).

Stainless steel platforms were inserted under the A_3–A_4 connective for impaling GIs and under T_3 for impaling thoracic interneurons. The sheath surrounding T_3 was softened with 1 mg/ml protease in saline (Sigma type XIV) applied for 20 min.

Silver bipolar hook electrodes were placed under the abdominal cord at the A_1–A_2 connective to record from or stimulate the whole cord. Extracellular recordings were also made from leg nerves 6Br4 and 5 or nerve branch 5rl using single ended silver hook electrodes or from leg muscles via small copper wire EMG electrodes. Hook electrodes were always insulated with a mixture of petroleum jelly and mineral oil. Nerve 5 was crushed distal to the recording electrode to eliminate sensory activity.

Intracellular microelectrodes were made from single filament glass tubing. The tips were filled with 4% Lucifer Yellow CH (LY) (Stewart 1978), and the remainder of the electrode was filled with 0.1% lithium chloride. The tips were beveled to 60–80 MΩ with a jet stream beveler (Ogden et al. 1978). In experiments involving intracellular stimulation of interneurons, we had more success with unbeveled electrodes whose tips were filled with LY that had been dissolved in 0.5 M lithium acetate.

In some experiments in which thoracic interneurons were stimulated intracellularly to study motor outputs, the abdominal cord was severed between A_5 and A_6 either immediately before the experiment or several days prior to using an animal. This was done to eliminate vGI inputs to the thoracic interneurons which we thought might fatigue the escape circuitry during the set-up procedure. We noted no systematic differences between animals that had had their abdominal cords lesioned and those with intact cords.

Thoracic interneurons were impaled primarily at or near the midline of T_3 in the posterior region of the ganglion. Good penetrations showed considerable spontaneous synaptic activity. GIs were initially recognized by stimulating the abdominal nerve cord and noting a very short latency between stimulus artifact and the intracellularly recorded action potential. Also action potentials from the presumptive GI could usually be found in extracellular records of the abdominal nerve cord. These action potentials, which were correlated with the intracellularly recorded action potentials, were typically considerably larger than the rest of the action potentials recorded in the abdominal cord. Electrical records were stored on magnetic tape and analyzed with the aid of a Nicolet 4064 digital oscilloscope.

Subsequent to electrical recording, both cells were filled by iontophoretic injection with hyperpolarizing 1 s, 5 nA current pulses every 2 s for a period of 5–20 min. The nerve cord from A_5 to T_2 was removed and fixed for 30 min in 10% formalin in saline. It was dehydrated in an alcohol series, cleared in methyl salicylate and viewed in wholemount under a fluorescence microscope where the cells of interest were photographed and drawn with the aid of a 10×10 grid located in one eyepiece or with a drawing tube.

The tissue was then embedded in wax directly from methyl salicylate or indirectly through a xylene series (the former conserved the dye and tissue as well as the latter and was more rapid). Sections were made at 10 μm and again viewed under the fluorescence microscope. In cross-sections the GI could be uniquely identified by its position in abdominal ganglia (Fig. 1B) (Westin et al. 1977). The thoracic tract containing the main axon of the thoracic interneuron could also be located in cross-section (Fig. 1A) (Gregory 1974). This greatly facilitated the identification of these interneurons (Ritzmann and Pollack 1983). Electrophysiological records were analyzed only from preparations in which the neuron or neurons that were studied intracellularly were positively identified on morphological grounds.

Results

A total of 63 thoracic interneurons were recorded intracellularly in conjunction with GIs where both cells were positively identified by dye injection. Of these, 24 pairs, made up of 8 distinctly different thoracic interneurons, showed evidence of connection. Two criteria were used to establish that a connection existed. In all positive cases, we found 1 for 1 correlations with consistent short latencies between intracellularly recorded GI action potentials and postsynaptic potentials recorded in the thoracic interneuron. This is an often used criterion for connectivity (Bacon and Tyrer 1979; Robertson and Pearson 1983; Pearson et al. 1980). In addition, in most preparations we verified conclusions that were based on correlation by stimulating the GI intracellularly. No cases were found in which the two procedures yielded contradictory results.

Lambda cell

We designated the most prominent interneuron we identified as Lambda cell due to its characteristic morphology in wholemount. Unlike most neurons in the cockroach nervous system, the soma of this cell is located on the dorsal surface (Fig. 2A). It is part of a group of about 20 interneurons whose somata are located in the posterior region of T_3. At least three other interneurons that receive inputs from GIs (see below) also have somata in this region. We have designated the entire population as the Dorsal Posterior Group (DPG). The main neurite of Lambda proceeds ventrally from the soma, bends around the lateral dorsal tract (LDT) then continues ventrally to a cross-commissure just dorsal to the ventral intermediate tract (VIT) which contains the vGIs (Fig. $2A_{1+2}$). In this region four large symmetrical dendrites are given off around the midline of the ganglion. In cross-section these filled processes approach vGI axons. After passing the contralateral VIT the main fiber proceeds dorsally again and exits T_3 anteriorly in the dorsal intermediate tract (DIT). The primary axon is 25 μm in diameter in the T_2–

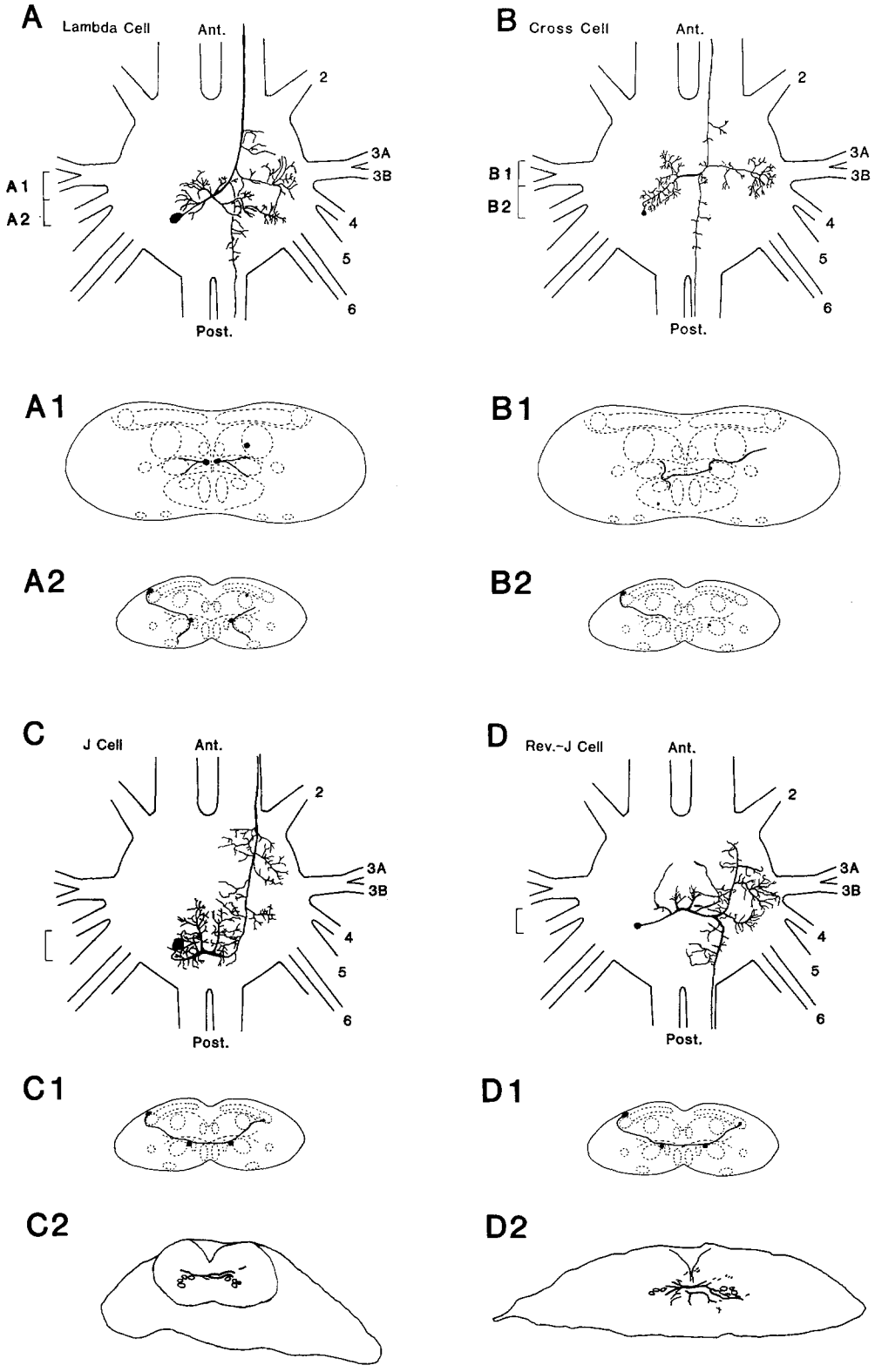

Fig. 2A–D. Morphology of four DPG interneurons in T_3. Each cell is represented in wholemount and in one or more composite drawings taken from a series of cross-sections. Cross-sectional drawings were made relative to standard location of tracts (as in Fig. 1A) on a series of templates. Drawings were made from cross-sections in the regions indicated by brackets on the wholemounts. **A** Lambda cell. The primary axon in DIT is the large anteriorly directed axon. **B** Cross cell. **C** J cell. **D** Reverse-J cell. C_2 and D_2 are camera lucida drawings of cross-sections of J cell and Reverse-J cell showing the proximity of branches of these cells to vGI axons (seen as circles in both Figs.). Only the DPG interneurons were filled with LY

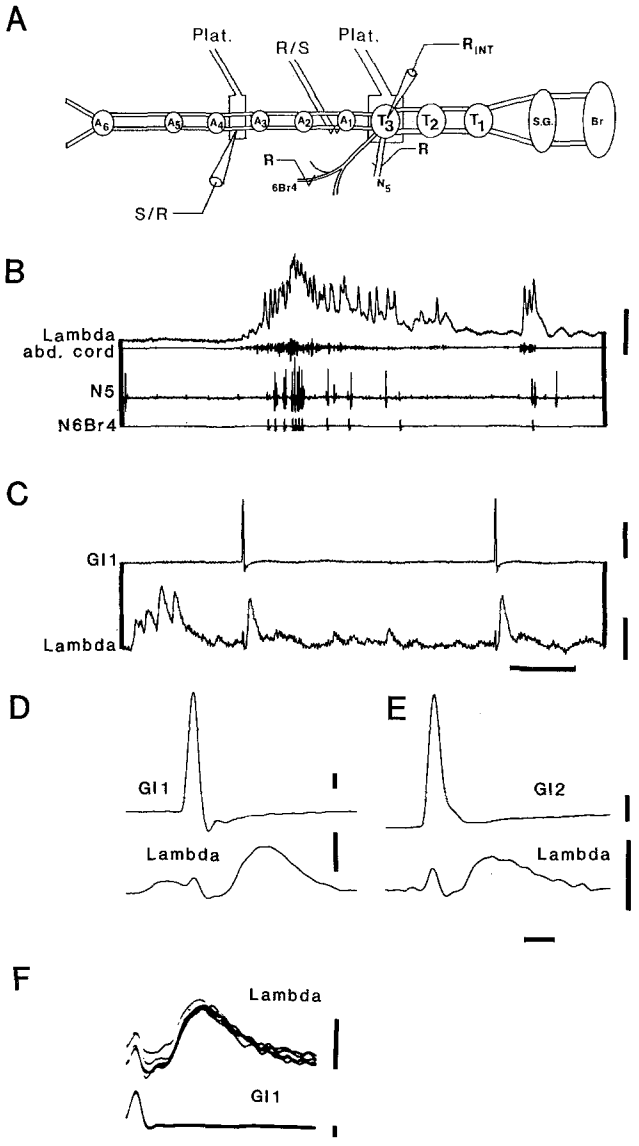

Fig. 3A–F. Evidence that vGIs excite Lambda cells. **A** Diagram of cockroach central nervous system indicating position of all recording and stimulating electrodes for dual intracellular experiments. *Br* brain; *S.G.* suboesophageal ganglion; T_1–T_3 thoracic ganglia 1–3; A_1–A_6 abdominal ganglia 1–6; *Plat* support platforms (the platform under the abdominal connectives was in some preparations placed under the A_2–A_3 connective); *R/S* hook electrodes on abdominal cord that are used for stimulation while impaling GIs and for recording during tests for connectivity; *R* single ended hook electrodes on nerves 5 and 6Br4; *S/R* microelectrode for recording or stimulating GIs, R_{INT} microelectrode for recording from thoracic interneuron. **B** Typical response of a Lambda cell (top trace) in response to a wind puff. Note that the cell depolarizes along with onset of activity in the abdominal cord (middle trace) and prior to arrival of action potentials in N5 and N6Br4. **C** In another preparation EPSPs from a Lambda cell (bottom trace) are correlated with action potentials recorded intracellularly from GI1 contralateral to the Lambda cell soma (top trace). Some crosstalk between the top and middle traces results in a small artifact of the GI1 action potential in the Lambda cell record. **D** An averaged record of 8 sweeps triggered off of the GI1 action potential (pretrigger allows visualization of record prior to trigger event). An averaged EPSP from the Lambda cell is clearly evident. **E** From another preparation, 7 averaged sweeps triggered off of an intracellularly recorded GI2 action potential. Again an EPSP in the Lambda cell is clearly evident. **F** Five superimposed EPSPs from the Lambda cell in **D** (top trace) triggered off of GI1 action potentials (bottom trace) to show consistency of latency measures. Amplitude calibrations: 10 mV for all Lambda records except **E** where it is 5 mV; 100mV for GI record in **C**, 20 mV for GI records in **D** and **E**, 50 mV for GI record in **F**. Time calibration: Bar in **C** represents 50 ms in **B**; 20 ms in **C**. Bar in **E** 1 ms in **D**, **E** and **F**

T_3 connective leading some investigators to refer to the cell as the thoracic giant (Delcomyn and Pitman, personal communication). A smaller axon is given off posteriorly, enters the abdominal nerve cord and is located in the DIT in a bundle which also contains the dGIs (Fig. $2A_2$).

As is the case for the GIs, the Lambda cell is depolarized by wind directed at the cerci (Fig. 3B). All four of the DPG interneurons on which we report had thresholds for action potentials well over 10 mV above resting potential. In some cases this threshold was greater than 20 mV.

We have recorded from Lambda cells in conjunction with vGIs in 4 different pairs, including contralateral GI1 and 2 and ipsilateral GI3 (ipsilateral and contralateral in pair data refers to the location of the GI axon relative to the soma of the thoracic interneuron). All of these pairs showed evidence of connections. In each case, EPSPs often reaching 8–14 mV were recorded in Lambda whenever an action potential was recorded in the vGI (Fig. 3C–F). In a sample of 105 PSPs taken from one Lambda cell, those PSPs associated with vGI action potentials were among the largest recorded (Fig. 4A). The latencies from the vGI action potential recorded in A_2–A_3 to the EPSP recorded in the Lambda cell ranged from 0.7 to 1.17 ms. The maximum conduction velocity for vGI action potentials reported in the literature (Spira et al. 1969) and in our recordings is 7 m/s. Thus, even for the longest delays from A_2–A_3, only 0.4 ms would remain for conduction within T_3 and actual transmission between the vGI and Lambda.

In two preparations, connections were verified by stimulating vGIs intracellularly while monitoring the Lambda cell intracellularly (Fig. 5). Each suprathreshold pulse generated an EPSP in Lambda. When the vGI was stimulated with trains of current pulses at just threshold for vGI action potentials, the stimulus occasionally failed to produce an action potential. These instances were always correlated with failures of the EPSP in the Lambda cell (Fig. 5B). In suprathreshold trains the

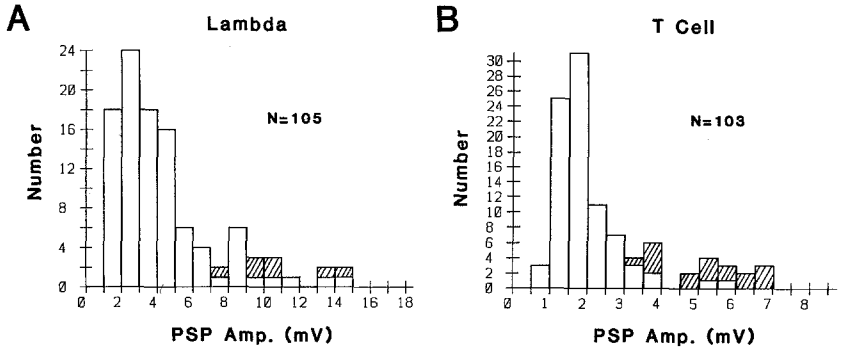

Fig. 4. Histograms showing amplitudes of 105 PSPs sampled from a Lambda interneuron (**A**) and 103 PSPs sampled from a T cell (**B**). Hatched bars: PSPs associated with GI1 (**A**) and GI7 (**B**)

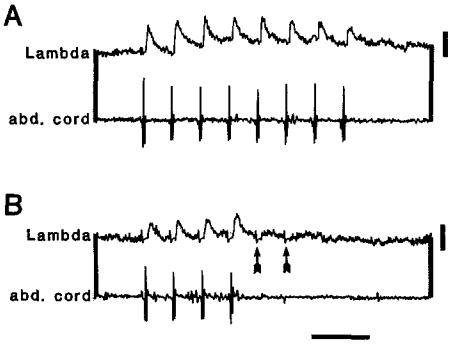

Fig. 5A, B. Intracellular stimulation of vGIs evokes EPSPs in Lambda (top traces). GI2 is stimulated intracellularly with trains of current pulses at a frequency of 100 Hz. Suprathreshold stimuli are indicated by large action potentials in the abdominal cord extracellular record (bottom traces). In **A** each stimulus pulse evokes a GI spike and an associated PSP in Lambda. In **B** the last two stimuli (arrows) failed to evoke action potentials. These are the only stimuli that failed to depolarize the Lambda cell. In these and subsequent records involving intracellular stimulation of GIs, artifacts in the interneuron records were electronically reduced. Calibrations 3 mV and 20 ms

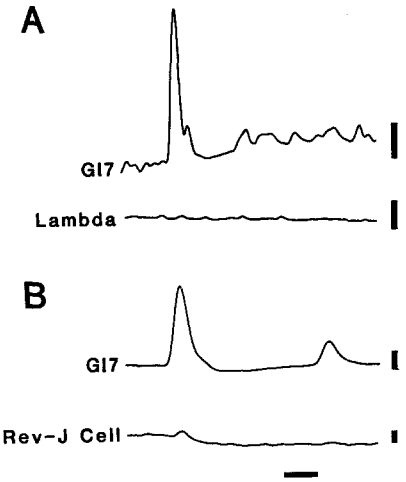

Fig. 6A, B. DPG PSPs are not associated with dGI action potentials. Averaged intracellular records from Lambda and Reverse-J cells triggered off of intracellularly recorded action potentials in dGI7. **A** Is an average of 16 sweeps, **B** is an average of 6 sweeps. Neither shows significant PSPs associated with the dGI action potentials. Amplitude calibrations: GI7 (both records) 10 mV; Lambda 1 mV; Reverse-J cell 4 mV. Time calibration: **A** 2 ms; **B** 1 ms

EPSPs followed vGI action potentials at 100 Hz without failure. In one of these preparations the vGI was subsequently identified as contralateral GI2. In the other preparation two vGIs were stimulated (not concurrently) and filled. They were subsequently identified as contralateral GI2 and ipsilateral GI3. Both excited the Lambda cell with similar latencies.

When Lambda cells were recorded in conjunction with dGIs, no connection was revealed (Fig. 6A). Although both cells were excited by wind in a similar manner, no close correlation was found between dGI action potentials and individual Lambda cell PSPs. However, a correlation was noted in these preparations with large action potentials in the extracellular record of the abdominal nerve cord. The amplitudes of these extracellular action potentials suggest that they came from vGIs.

J cell and Reverse-J cell

Like the Lambda cell, the J and Reverse-J cells also have somata in the dorsal posterior group of T_3 (Fig. 2C and D). Also like Lambda the main neurite of the J and Reverse-J cells wrap around the LDT before descending ventrally to the VITs (Fig. $2C_1$ and D_1). At the VITs two prominent dendrites project anteriorly on either side of the midline close to vGI axons. Branches from these processes approach the vGI axons providing possible sites for contact with side branches from these axons (Fig. $2C_2$ and D_2). The main axon then proceeds dorsally, but unlike Lambda cell, enters and continues in the LDT rather than the DIT. The Reverse-J cell differs from the J cell in that it exits T_3 posteriorly into the abdominal nerve cord, whereas the J cell exits anteriorly into the T_2–T_3 connective. Also, the Reverse-J cell soma and crossing neurite are consistently more anterior than that of the J cell. Nevertheless, the basic shapes of the two interneurons are very similar in cross-section.

As with the Lambda cell, the J and Reverse-J cells are excited by wind directed at the cerci and by vGIs (Figs. 7 and 8). Action potentials in both ipsilateral and contralateral GI2 were recorded 1 for 1 with EPSPs in J cells with latencies ranging from 0.8–1.17 ms. Similar observations were made in preparations in which ipsilateral GI1 and contralateral GI2 were recorded in conjunction with a Reverse-J cell. Here latencies ranged from 0.83–1.5 ms (Fig. 8). In the preparation involving the Reverse-J cell and GI2, the GI was also excited intracellularly. This consistently evoked short latency EPSPs in the Reverse-J cell. Again, dGI action potentials were not closely correlated to PSPs in these cells (Fig. 6B).

Cross cell

The Cross cell has its primary axon in the ventral intermediate tract (VIT) making it the only DPG interneuron we have filled which has an axon in a ventral tract. It is also more asymmetrical than the other DPG interneurons we described (Fig. 2B). Branches that would provide overlap with vGIs are only found on the side ipsilateral to the soma.

The Cross cell was recorded in conjunction with GIs in two preparations. However, three GIs were tested in one of those animals. Consistent with Cross cell's asymmetrical morphology, positive results were only found with vGIs on the side ipsilateral to the cell's soma (vGI 1 and 2) (Fig. 9). Both contralateral vGI1 and ipsilateral dGI5 failed to show evidence of connection (data not shown).

Correlation with extracellular records from the abdominal cord

In addition to the paired intracellular records described above, we also found 12 preparations in which synaptic activity in thoracic interneurons could be correlated with action potentials in extracellular records from the abdominal nerve cord. In this analysis taped records were played onto a digital oscilloscope triggered by action potentials in the abdominal cord records. The trigger was set at the narrowest possible sensitivity, and initially corresponded to the largest action potentials in the cord record. The records were then replayed at lower and lower trigger levels until only the smallest action potentials were sampled. The results of this procedure were analyzed both as individual trials and as averaged records taken at three distinctly different trigger levels.

The averaged records consistently showed PSPs

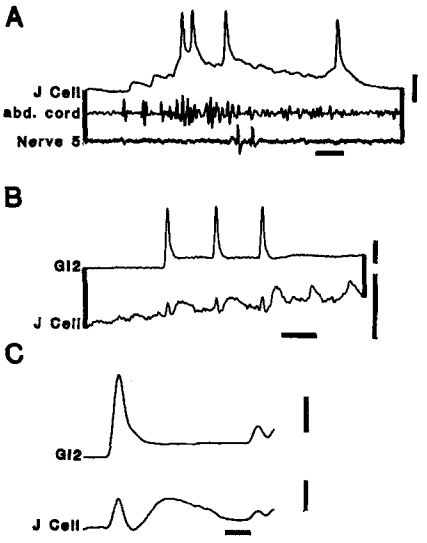

Fig. 7A–C. J cell is excited by vGI2. **A** Response of J cell (top trace) to a gentle wind puff. Wind puff is indicated by activity in abdominal cord record (middle trace). Note EPSPs in J cell associated with first 2 action potentials in abdominal cord. These large action potentials are probably from vGIs. J cell is depolarized to threshold prior to the onset of motor activity as recorded in nerve 5 (bottom trace). **B** In another preparation J cell (bottom trace) is recorded intracellularly in association with intracellular record from GI2 (top trace). Each action potential in GI2 is associated with an EPSP in the J cell. **C** Average of 5 sweeps triggered off of the action potential in GI2. An EPSP is clearly seen in the J cell record. In **B** and **C** the sharp pulses preceding the PSPs in the J cell record are artifacts resulting from cross-talk with the GI action potentials. Amplitude calibrations: **A** 20 mV; **B** 20 mV (top trace), 10 mV (bottom trace); **C** 20 mV (top trace), 2 mV (bottom trace). Time calibrations: **A** and **B** 5 ms; **C** 1 ms

correlated with the largest and second largest spike amplitudes (Fig. 10). The largest action potentials are presumably from the largest axons in the abdominal nerve cord; i.e. the vGIs. In all preparations, the largest averaged PSPs were correlated with the largest extracellular action potentials. The smallest action potentials were always associated with the smallest averaged PSP or had no discernable PSP associated with them.

Since the averaged records are triggered off of action potentials from several different axons in the abdominal cord, individual trials were also analyzed. In Fig. 11A amplitudes of action potentials in the cord are plotted against the amplitudes of their associated PSPs. As in the averaged records these are positively correlated, suggesting that the axons with the largest spikes are associated with the largest PSPs. Since amplitude of extracellular action potentials can be influenced by recording properties such as distance of the axon from the recording electrodes, we also plotted PSP amplitude against the latency from the action potential

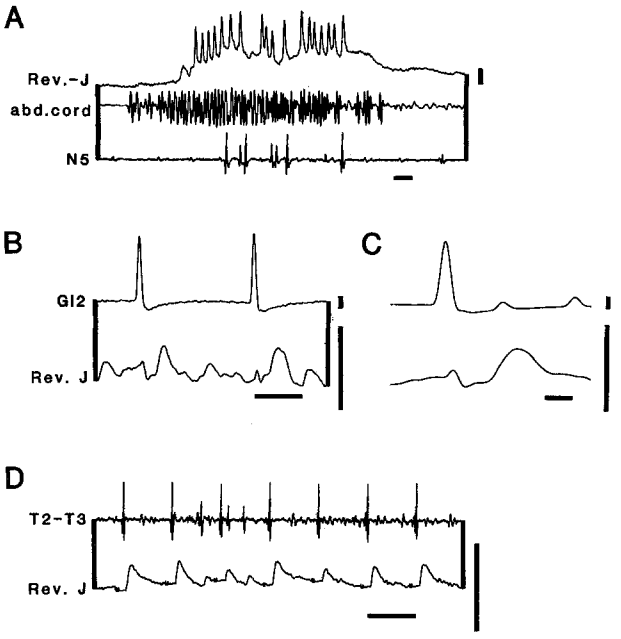

Fig. 8A–D. Reverse-J cell receives input from vGI2. **A** Wind puff indicated by activity in the abdominal cord record (middle trace) generates suprathreshold depolarization in the Reverse-J cell (top trace) and, subsequent to the initiation of action potentials in the Reverse-J cell, motor activity in nerve 5 (bottom trace). **B** In another preparation a Reverse-J cell is recorded in conjunction with GI2. EPSPs in the Reverse-J cell (bottom trace) are associated with each GI2 action potential (top trace). **C** Average of 7 sweeps triggered off of the GI2 action potential. **D** Intracellular stimulation of GI2 generates action potentials in GI2 monitored in the T2–T3 connective (top trace) which in turn evoke EPSPs in the Reverse-J cell (bottom trace). All amplitude calibrations represent 10 mV. Time calibrations: **A** and **D** 10 ms; **B** 5 ms; **C** 1 ms

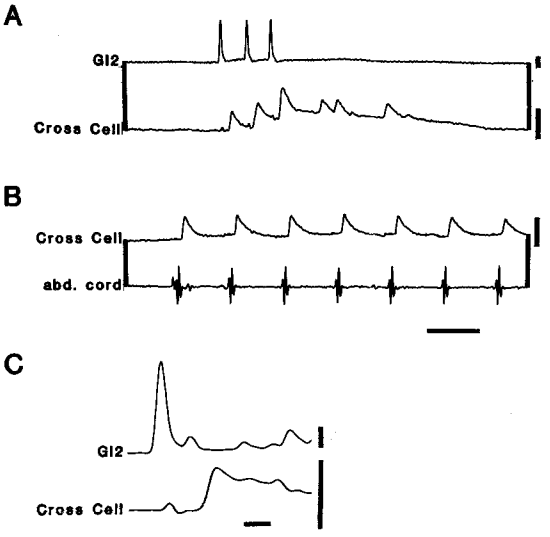

Fig. 9A–C. Cross cell receives inputs from vGI2. **A** Gentle wind puff generates three action potentials in GI2 (top trace) each associated with a large EPSP in the Cross cell. Note three smaller EPSPs are not associated with activity in GI2. **B** Intracellular stimulation of GI2 with a 100 Hz train of suprathreshold current pulses generates EPSPs in Cross cell (top trace) associated with the GI2 action potentials recorded in the abdominal nerve cord (bottom trace). **C** Average of 16 sweeps triggered off of the GI2 action potential (top trace) clearly showing the associated EPSP in the Cross cell record (bottom trace). All amplitude calibrations 10 mV. Time calibrations: **A** and **B** 10 ms (mark in **B**); **C** 1 ms

in the abdominal cord to the PSP in the thoracic interneuron (Fig. 11B). The principle variable in latency (assuming a direct pathway) should be conduction velocity, which is directly proportional to axon size. As expected, this relationship showed an even closer correlation than did action potential amplitude.

The conclusion from these analyses is that, at the site of microelectrode penetration (i.e. near the midline), the largest PSPs in these interneurons are associated with the largest axons of the abdominal nerve cord; i.e. the vGIs. This, therefore, supports the conclusion from the paired intracellular recordings that vGIs provide a major input to these interneurons. In addition, our samples included several action potentials that were not associated with PSPs. These failures are indicated as 0 amplitude in Fig. 11A and tend to be clustered in the intermediate to upper intermediate range of action potentials. These would be the sizes associated with dGI axons. Thus, this observation is consistent with the failure to detect connections between dGIs and DPG interneurons in paired recordings (Fig. 6).

Motor outputs from DPG interneurons

The motor outputs resulting from excitation of the four DPG interneurons were tested by stimulating them intracellularly while recording extracellularly from leg nerves 5r1 and 6Br4 on both sides of the ganglion. Extracellular recording was employed to maximize our chances of locating motor effects. Nerves 5r1 and 6Br4 were chosen because they contain the principle depressor and levator axons used in walking (Pearson and Iles 1970). The source of activity for individual axons in these nerves can be identified based on well-established properties seen in extracellular records (Pearson and Bergman 1969; Pearson and Iles 1970, 1971; Pearson et al. 1970). Nerve 5r1 contains the slow and fast depressor motor neurons (D_s and D_f respectively). Nerve 6Br4 contains three readily excited levators L_4–L_6. In addition the wide-spread common inhibitor (c.i.) has axons in both of these nerves and can, therefore, be readily identified by the presence of 1 for 1 action potentials in the two records.

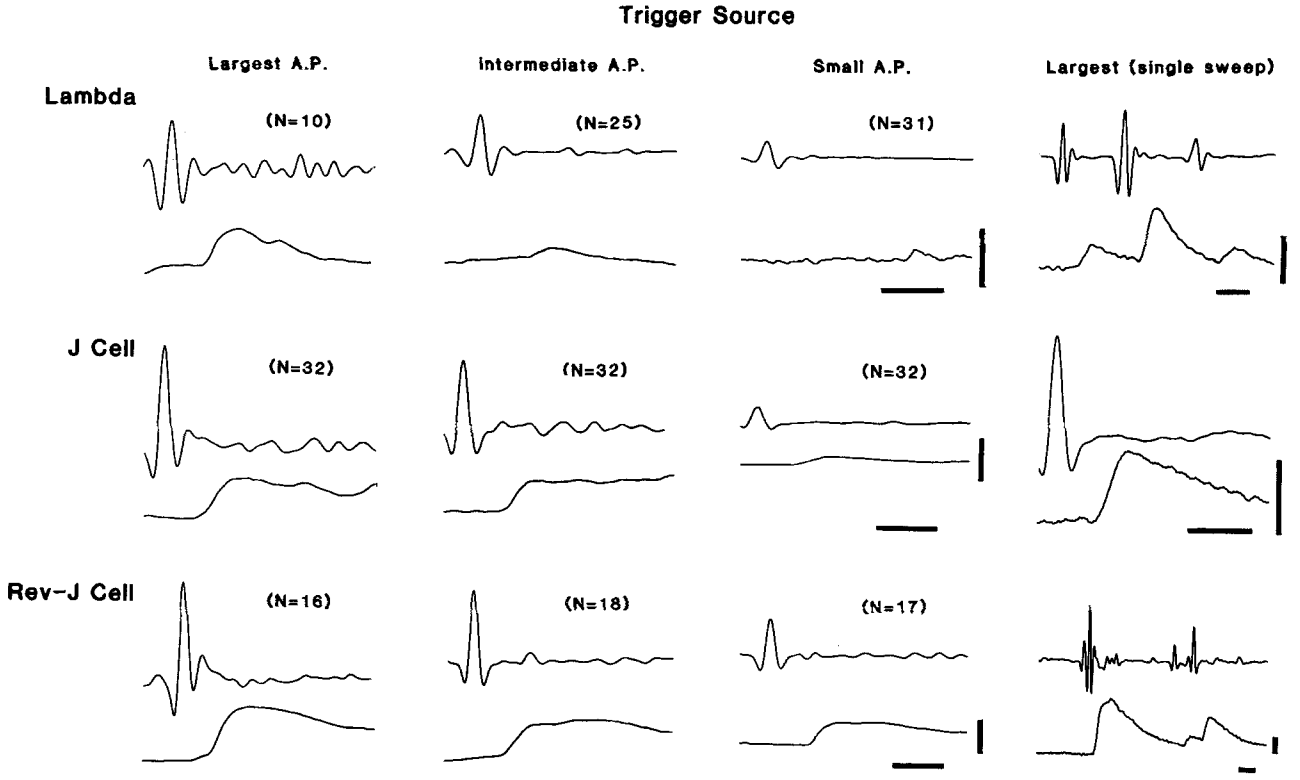

Fig. 10. Averaged records for Lambda, J cell and Reverse-J cell (bottom traces) triggered off of action potentials recorded extracellularly from the abdominal nerve cord (top traces). For each interneuron the trigger level was sequentially set to trigger off of three different amplitudes of action potentials. In each record N=number of sweeps that were averaged. Note that for all three interneurons the largest average PSP is associated with the largest action potentials in the abdominal cord record. Next largest PSP is associated with the intermediate size action potential and the smallest action potential is associated with the smallest PSP or, for Lambda, no PSP is discernible. For each interneuron a sample of a single sweep is shown in the last column. These were triggered off of the largest spike amplitude, but the examples for Lambda and Reverse-J cell were fortuitous in that they showed all three spike sizes. The different size PSPs are clearly evident in these records. Calibrations for all averaged sweeps for a given cell are seen in the small A.P. column. All calibrations 5 mV and 2 ms

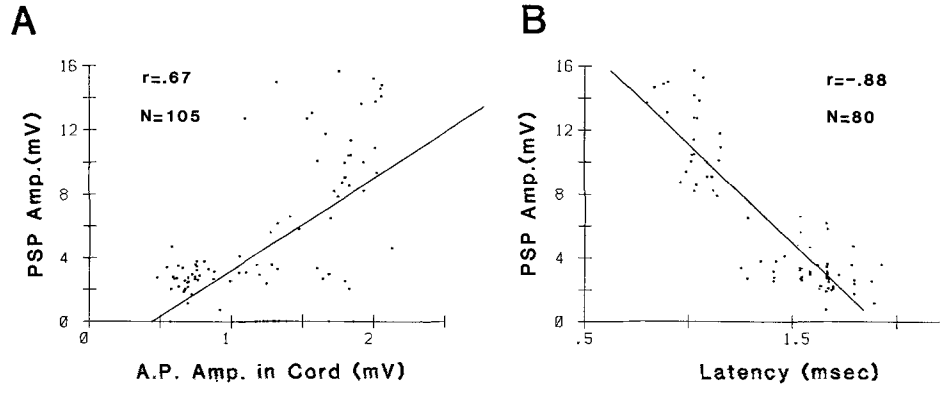

Fig. 11. PSP amplitude, recorded in a Reverse-J cell, plotted against the size of associated extracellularly recorded action potentials in the abdominal nerve cord (**A**) and latency from the action potential to the PSP (**B**). Both are proportional to axon diameter, but amplitude of the action potential has the extra variable of distance from the axon to the recording electrode. Thus, the correlation seen for latency is, as expected, closer. Both graphs indicate a close correlation between axon diameter and size of associated PSP in the Reverse-J cell. Regression lines for both graphs are indicated along with r values. Note in **A** several points clustered between 1.0 and 1.5 mV were associated with no PSP in the Reverse-J interneuron (0 mV). Only three failures were seen outside this range of amplitudes. $P<0.001$ for both graphs

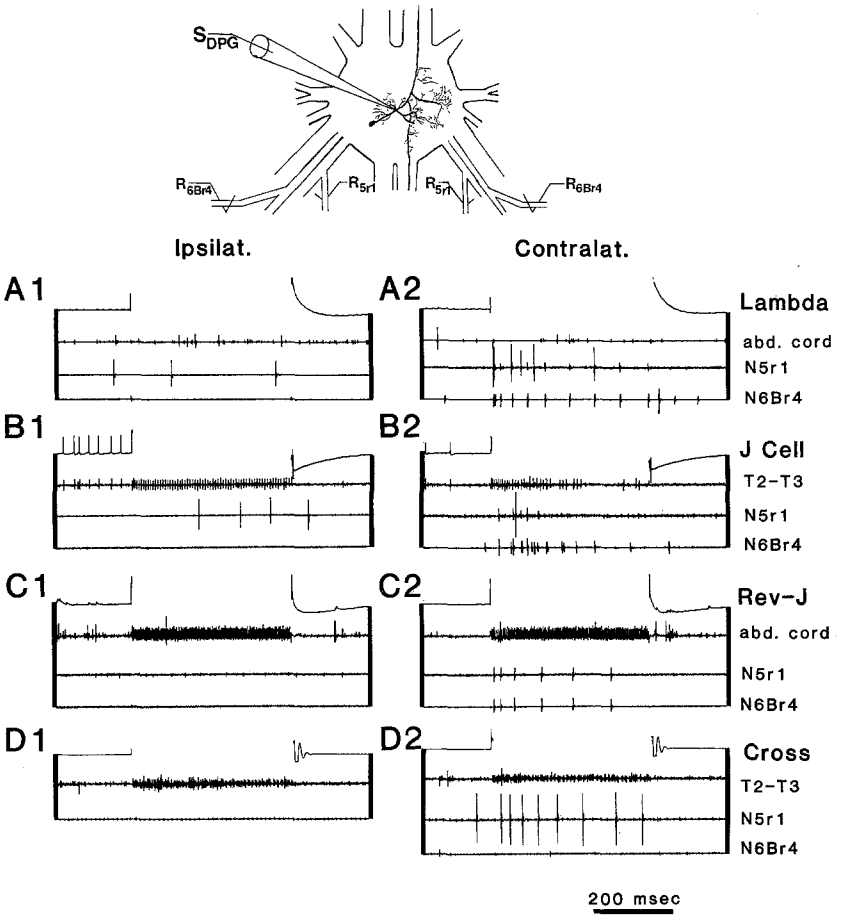

Fig. 12A–D. Intracellular stimulation of DPG interneurons evokes responses in motor nerve roots. Diagram indicates recording and stimulation set-up. In each case the interneuron was stimulated with a depolarizing current pulse lasting 400 ms which caused the bridge to go out of balance. This is provided as a stimulus monitor (top trace). Extracellular recording amplifiers could be switched between hook electrodes on the side ipsilateral to the interneuron's soma and another set of electrodes on the side contralateral to the soma. Nerve branches recorded (traces 2–4 in each record) are indicated to the right of the traces. Either the abdominal cord or the T_2–T_3 connective was monitored in addition to nerve 5r1 (containing depressor axons from Ds and Df) and nerve 6Br4 (containing levator axons). **A** Lambda evoked little if any activity in Ds on the ipsilateral side, but evoked several action potentials in Ds and c.i. (indicated by 1 for 1 action potentials in both nerve records) contralateral to the interneuron's soma. **B** J cell evoked a short latency response contralaterally in Ds, c.i. and a small axon in 6Br4 (probably a small levator). On the ipsilateral side Ds was excited but with a much longer latency. The axon of J cell was monitored in the T_2–T_3 connective. Note that although fewer Ds action potentials were evoked contralaterally, the J cell was excited only over half of the stimulus pulse (as indicated by activity in the T_2–T_3 record). The stimulus became ineffective soon after this record was taken. **C** Reverse-J cell excited c.i. contralateral to its soma. No response was ever seen on the ipsilateral side. Reverse-J cell has a large axon in the abdominal cord and action potentials from that axon can be seen in the cord records during stimulation. **D** Cross cell excites Ds on the contralateral side. The first Ds action potential was spontaneous (i.e. it preceded the stimulus). However, the excitation during stimulation was consistent in all trials. Ipsilaterally no activity is seen in nerve 5r1. Nerve 6Br4 was not monitored. Calibration 200 ms in all records

The motor responses resulting from electrical stimulation of the Lambda cell are consistent with motor responses resulting from wind puffs (Westin and Ritzmann 1982) and with those resulting from intracellular stimulation of GI1 (Ritzmann and Camhi 1978). Depolarizing Lambda evoked a response in D_s and c.i. contralateral to the Lambda soma (Fig. 12a). The D_s response began 5 ms after the onset of the depolarization to Lambda. On the ipsilateral side little if any motor output was recorded in association with Lambda stimulation. These response patterns have been recorded in 4 separate preparations involving stimulation of an identified Lambda cell. C.i. responses have also been recorded during filling procedures as Lambda rebounded from large hyperpolarizing potentials.

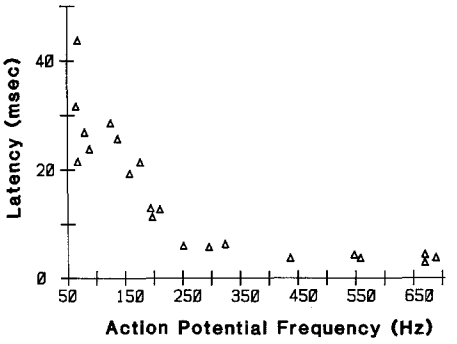

Fig. 13. Latency to motor response is influenced by frequency of interneuron activity. Reverse-J cell shown in Fig. 12C was stimulated at several different current levels. This resulted in varying frequencies of Reverse-J action potentials recorded in the abdominal cord. In this graph the latency from the first Reverse-J action potential is plotted against the frequency of Reverse-J action potentials preceding the first contralateral c.i. action potential. Latency clearly declines with increasing Reverse-J frequency until it levels out near 3 ms

In these cases the c.i. action potentials occurred with delays of 5 ms or less from the first recorded Lambda action potential.

Stimulation of J cell (2 preparations) excited D_s, c.i. and another cell with a small axon in 6Br4 contralaterally (Fig. 12B). The first action potential was from c.i. with a latency of 19 ms. D_s action potentials were also recorded on the ipsilateral side but with a latency of 166 ms versus 62 ms for the contralateral D_s motor neuron.

The Reverse-J cell was successfully stimulated in 4 preparations. It consistently evoked activity in contralateral c.i. (Fig. 12C). The latency from the first Reverse-J cell action potential to the first c.i. action potential ranged from 3–44 ms. The large variation in latency is correlated with the frequency of Reverse-J action potentials evoked by the intracellular stimulation (Fig. 13). In one preparation stimulation of the Reverse-J cell also excited the contralateral D_s motor neuron. No activity was ever observed ipsilaterally in response to Reverse-J cell stimulation.

Depolarization of Cross cell (one preparation) evoked a strong response in D_s contralateral to the interneuron soma (Fig. 12D). No response was recorded in ipsilateral N5r1. However, N6Br4 (containing levator motor axons) was not monitored in this preparation.

T cell

Of all the thoracic interneurons sampled only one cell type was found to be directly correlated with dGI activity. We refer to this cell as the T cell. In contrast to the interneurons described above, the T cell has a ventral soma (Fig. 14A and B). Its main neurite crosses the midline at a more anterior position than do those of the DPG interneurons. It then divides to send branches anteriorly and posteriorly. The axon exits T_3 anteriorly in the DIT.

The T cell also responds to wind directed at the cerci (Fig. 15A). However, unlike the other interneurons we describe, PSPs in the T cell are correlated with action potentials in dGIs (Fig. 15B–E). In two separate preparations 1 for 1 correlations were found between dGI action potentials (GIs 5 and 7 both ipsilateral to the T cell soma) and EPSPs in T cells. Amplitudes of the EPSPs associated with GI7 ranged from 3–7 mV. These are among the largest PSPs found in a random sample of 103 PSPs measured in the T cell (Fig. 4B). In another preparation a correlation was seen with a non-giant interneuron that was located in the dGI bundle. Several axons are located in the dGI bundle that are not identifiable from animal to animal and have, therefore, not been numbered as specific dGIs. However, these are approximately the same diameter as the dGIs, are wind activated (Westin et al. 1977) and excite motor neurons in the same way as dGIs (Ritzmann and Pollack 1981). Thus, they may be part of the same population of cells as the dGIs. In one preparation a T cell was recorded in conjunction with a vGI. No evidence of connection was detected.

Although the dGIs were not successfully stimulated intracellularly during T cell recordings, extracellular stimulation of the nerve cord revealed a very close correlation between the thresholds for dGI action potentials and that for large EPSPs in the T cell (Fig. 15E).

Motor output of T cell

T cells have been stimulated intracellularly in two preparations. In both cases stimulation consistently excited contralateral D_s and contralateral c.i. Latency from the first T cell action potential to the first D_s action potential was 35 ms (Fig. 16B). The latency to the first c.i. action potential was 50 ms. Occasionally a large action potential, possibly from D_f, was also seen in the N5r1 response to T cell stimulation. D_f is never spontaneously active in these preparations. Levator activity in N6Br4 was also evoked contralaterally in a burst that started 80–120 ms after the first T cell action potential. On the side ipsilateral to the T cell soma, the only consistently evoked activity was in c.i. with a 10 ms latency.

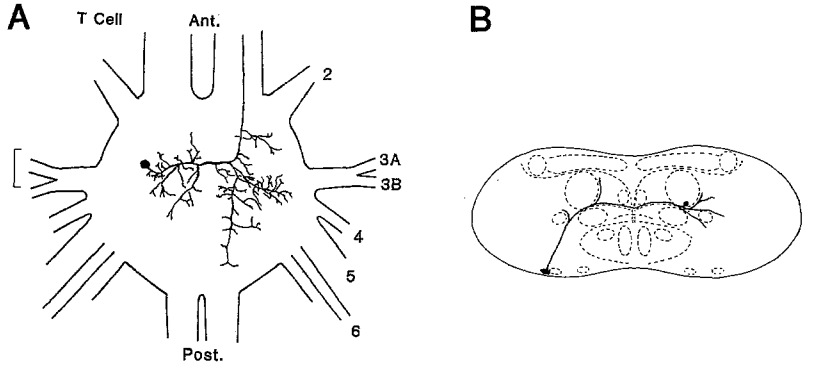

Fig. 14. Morphology of the T cell that was recorded in Fig. 15 is shown both (**A**) in wholemount and (**B**) in a composite drawing of cross-sections taken from the region indicated by a bracket in **A**

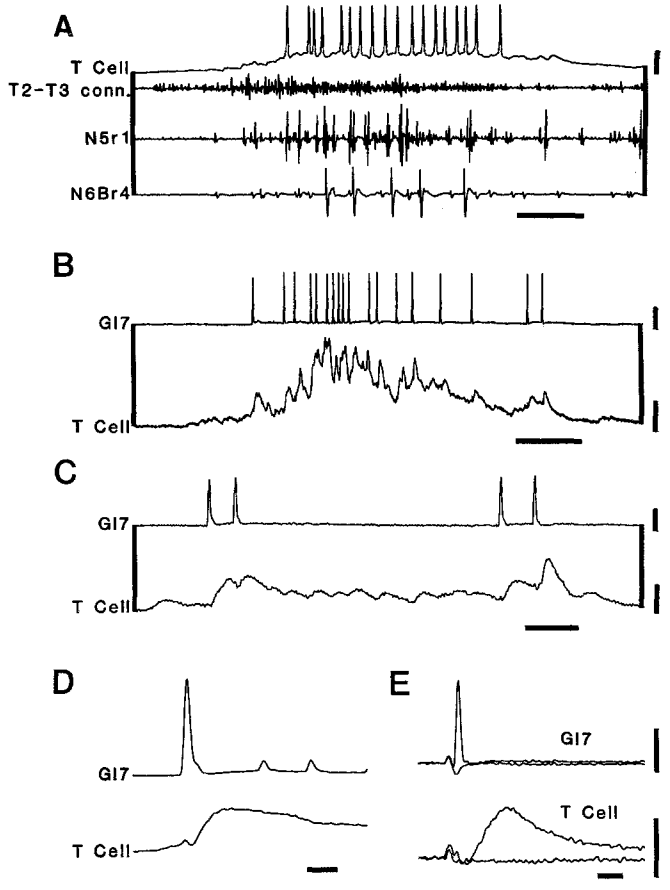

Fig. 15A–E. T cells receive inputs from dGIs. **A** Wind puff indicated by increase in activity in the T_2–T_3 connective. Wind causes a suprathreshold depolarization in the T cell. Motor activity is associated with the wind puff, but precedes the activity in the T cell. Thus, this response is probably evoked by a faster pathway, possibly the vGI-DPG pathway. **B** EPSPs in the T cell (bottom trace) are associated with action potentials recorded in GI7. This is especially evident in the early and late PSPs where summation is limited. Note that in the preparation shown in **B–E** the GI7 axon was impaled in the T_2–T_3 connective. Thus, the action potential has already passed through the metathoracic ganglion, and the delay between GI7 action potentials and T cell PSPs is not as short as it appears to be in these records. **C** Correlation between GI7 action potentials and T cell EPSPs is more evident in weak responses and when records are more spread out. **D** Average of 9 sweeps triggered off of GI7 action potentials. **E** Stimulating the abdominal cord at threshold for the GI7 action potential occasionally fails to generate action potentials. These also failed to generate EPSPs in the T cell. Here two sequential trials are superimposed. One generated a GI7 action potential and an associated EPSP in the T cell; the other failed to evoke either an action potential or an EPSP. Amplitude calibrations: **A** 20 mV; **B–E**. GI7 25 mV; T cell 10 mV. Time calibrations: **A** 25 ms; **B** 50 ms; **C** 10 ms; **D** and **E** 2 ms

Additional interneurons with GI input

In addition to the interneurons described above, one other interganglionic interneuron and two intraganglionic interneurons in T_3 were also found to receive inputs from GIs. In all three cases connections were confirmed with dual recording and intracellular stimulation of vGIs (Fig. 17). None of these have been tested for motor outputs. They are presented here simply to complete our survey of thoracic interneurons excited by GIs.

One intraganglionic cell had a ventral soma and a single fiber that crossed over perpendicular to the long axis of the nerve cord before sending out several short branches (Fig. 17A). This cell received inputs from GI2 contralateral to the interneuron's soma.

The other example of intraganglionic interneuronal profiles showed two small dorsal somata near the midline. Two fairly symmetrical branches emanate from the somata and loop around to form a horseshoe shape. Although distinctions were difficult, it appeared that two complete, very similar cells were filled (Fig. 17B). These cells have been found in three separate preparations. Each time similar multiple fills were found even though dye was injected after only one recording. The dorsal midline position of the somata would suggest that they are small DUM cells (Goodmann et al. 1980). If so, they are the only DUM cells which we have recorded that are directly excited by GIs.

The remaining interneuron (Fig. 17C) was an

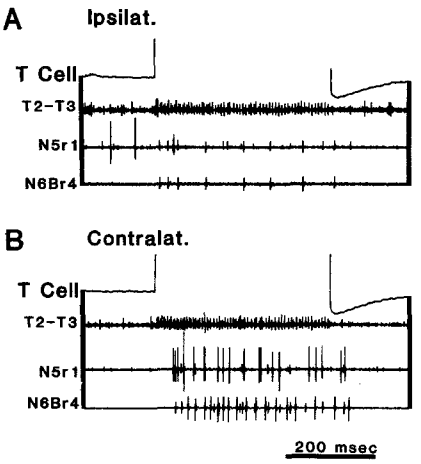

Fig. 16A, B. Stimulation of T cell evokes motor output. Traces as in Fig. 12. Depolarization evokes activity in c.i. and another small inhibitor in nerve 5r1 on the side ipsilateral to the soma (**A**). On the contralateral side (**B**) depolarization evokes activity in Ds, c.i., a levator in nerve 6Br4 and one spike in Df (the one spike that is larger than the Ds action potentials). Calibration applies to both records

interganglionic interneuron with a ventral soma located near the midline. It was impaled in conjunction with simultaneous penetrations of contralateral GI2 and an ipsilateral vGI whose specific identity could not be determined. Both showed positive connections.

Discussion

We have described 8 interneurons that are excited by GIs via stable, short latency pathways. The PSPs associated with GIs are among the largest recorded in the DPG and T cell interneurons. The connections are summarized in Table 1. If any cell is interposed between the GIs and these interneurons, it would have to be either nonspiking or, if spiking, be driven 1 for 1 via a very short latency pathway (Roberts et al. 1982). With the four DPG interneurons and the T cells, information from the GIs is subsequently routed to motor neurons of the legs. Since the motor neurons excited by these interneurons are the same as those that are normally excited by GIs, these observations provide a possible route for GI excitation of motor neurons (Ritzmann and Camhi 1978; Ritzmann 1981; Ritzmann and Pollack 1981).

As was the case with the studies involving GI stimulation, stimulation of these thoracic interneurons activated primarily slow motor neurons and the common inhibitors. In the escape behavior, one would expect to record vigorous activity from fast motor neurons. However, that is true only in an animal that has been minimally dissected and is not restrained to any great degree. Neither of these conditions is possible in experiments involving intracellular penetration in the neuropil. Moreover, in stimulating single thoracic interneurons, we are probably only exciting a portion of the interneurons that are normally activated in response to wind. All of these factors, plus inputs to the DPG interneurons from proprioceptive structures in the legs (M. Murrain, personal communication), would serve to potentiate the system and probably would result in excitation of the higher threshold fast motor neurons.

Our data do not establish that the connection between the interneurons and the leg motor neurons are monosynaptic. The answer to this question will require dual intracellular penetrations, which were beyond the scope of this study. Nevertheless, in experiments involving intracellular stimulation of DPG interneurons, latency measure-

Table 1. Summary of inputs from specific GIs and outputs to specific motor neurons

Thoracic interneuron			GI inputs							Motor outputs	
	n		Ventrals			Dorsals			Non-GI[1]	ipsi.	Contra.
	(vGI)	(dGI)	1	2	3	5	6	7			
Lambda	(4)	(2)	c	c	i	0		0		Ds[3]	Ds, c.i.
J Cell	(2)	(1)		i/c		0				Ds[3]	Ds, c.i., L
Rev-J	(2)	(2)	i	i		0		0		0	c.i., Ds[4]
Cross	(3)	(1)	i/O_c^2	i		0				0	Ds
T Cell	(1)	(3)	0			i		i	i	c.i.	Ds, Df, c.i., L

[1] Non-GI refers to axons other than GIs 5–7 located in the dorsal GI bundle
[2] Cross cells had positive connections with ipsilateral GI 1 but no correlation with contralateral GI 1
[3] Weak or long latency response
[4] Only seen in 1 out of 4 preparations

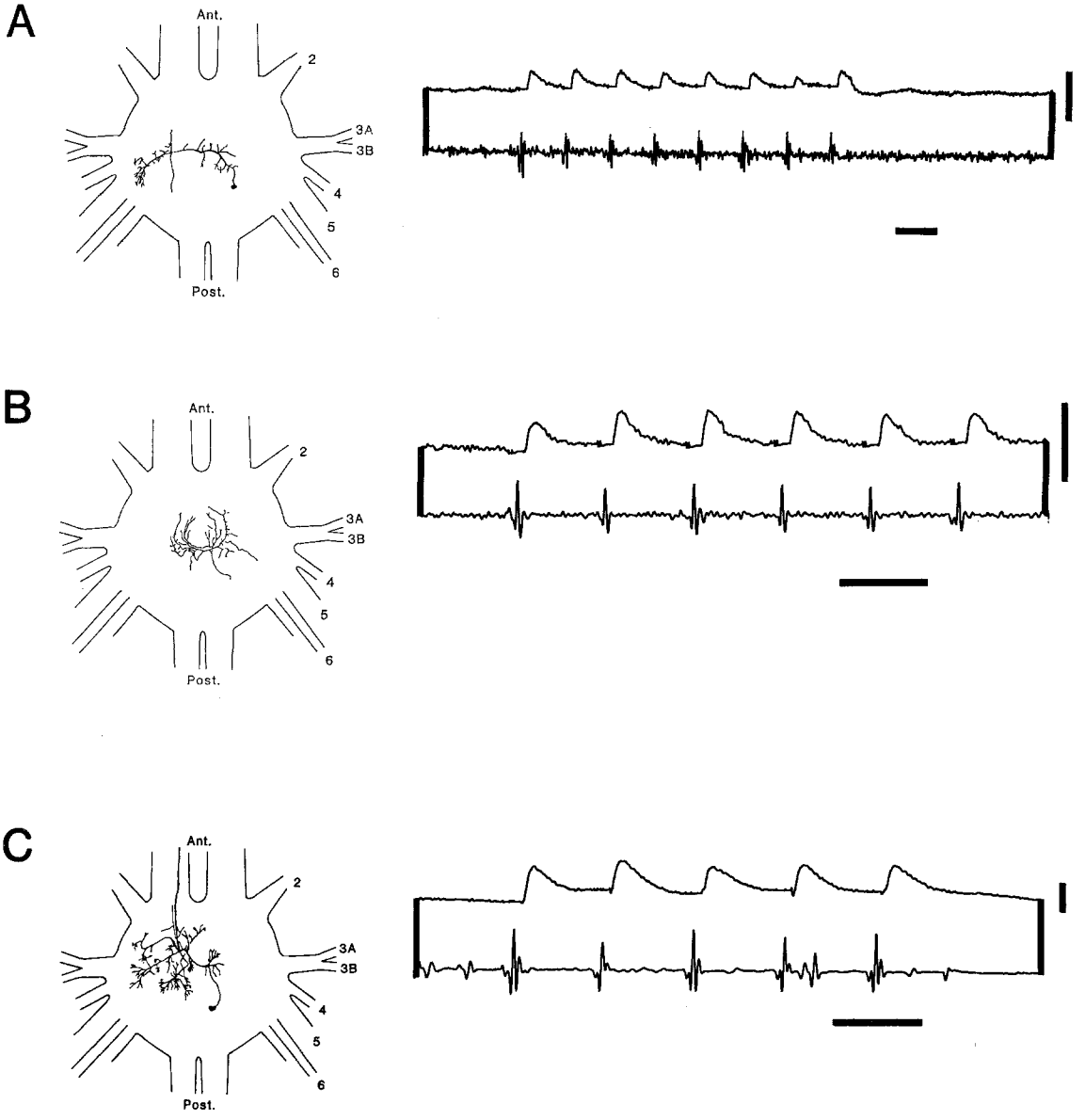

Fig. 17A–C. Three cells that are not part of the DPG or T cell populations but receive inputs from vGIs. **A** and **C** have ventral somata. **B** Has two dorsal somata. Traces associated with wholemounts show PSPs (top traces) resulting from stimulating vGIs (bottom traces). In **A** and **C** GI2s were stimulated. In **B** GI1 was stimulated. In all three cases the GI was contralateral to the somata. All calibrations: 10 mV; 10 ms

ments from the first evoked action potential to the first motor spike were often as short as 3–5 ms. The motor spike usually arrived after only 2–3 action potentials in the interneuron. This would suggest a direct pathway. In contrast the latency from evoked T cell action potentials to the first motor spike was on the order of 10 ms. This certainly allows time for more interneurons interposed in the pathway. In either case, the interneurons described in this paper represent the first sets of identified thoracic interneurons that have been positively linked to the GI-to-motor pathway.

Inputs from vGIs and dGIs

Previous data suggest that the dGIs and vGIs represent two functionally separate pathways that do not normally operate in conjunction and may indeed have mechanisms built in to prevent simultaneous activation. Aside from being physically distinct in the nerve cord, the latency from wind onset to the appearance of action potentials in the A_4–A_5 connective is 4–5 ms longer for dGIs than for vGIs (Westin et al. 1977). This is too long to be explained solely on the basis of conduction velocity,

suggesting differences in input circuitry. Also, the vGIs are inhibited during walking while the dGIs are excited (Daley and Delcomyn 1980). Finally, in experiments involving paired stimulation, there is no evidence for summation between ventral and dorsal GIs. Even GIs that would be excited by the same wind puffs and excite the same motor neuron population, e.g. GIs 1 and 5, do not tend to interact positively. In fact, quite often vGI stimulation reduced the effectiveness of the dGI (Ritzmann 1981).

The pattern of connections which has been described between GIs and interneurons in T_3 is consistent with the evidence for functionally distinct vGI and dGI pathways. As of this time, no interneuron has been found that was excited by both sets of GIs. Each DPG interneuron that was recorded in conjunction with a vGI showed a 1 for 1 correlation between vGI action potentials and DPG EPSPs. However, when tested with dGIs no such correlation was found. Only the T cells showed consistent correlation with dGI activity, and in at least one preparation involving simultaneous penetration of a T cell and a vGI no evidence of connection was indicated. If this pattern holds true, it would mean that the separation between dGI and vGI systems extends to the level of thoracic interneurons.

The reason for having two functionally separate systems remains to be seen. Several other giant fiber systems have small fiber pathways in parallel (Krasne 1965; Reichert and Wine 1983; Eaton et al. 1982). However, in cockroach the GIs may be more analogous to sensory interneurons than to premoter interneurons, and, therefore, such analogies may not be useful. In any case, conclusions regarding the relative roles of the two GI systems in cockroach must await more complete information on their ultimate motor outputs and their activity in behaving animals.

The need for a polysynaptic pathway

Monosynaptic connections between GIs and leg motor neurons were originally suggested because of the time constraints of an escape system. It was reasoned that the large diameter of the GIs provides a rapid path by which information on wind inputs could reach the thoracic ganglia. To then pass this information through an interneuron would seem to reverse that advantage. Nevertheless, data described in the introduction suggest that direct connections from GIs to motor neurons either do not exist, or represent a very weak input to the motor neurons. Conversely, the data presented here confirms that a strong polysynaptic pathway does exist between the GIs and the motor neurons which control leg movements. Does utilization of a polysynaptic pathway make sense for a rapid escape system? If the interposed interneurons represent the minimum circuit necessary for interpreting the information being brought to the thoracic ganglion and then activating, in a proper sequence, all of the necessary motor neurons involved, then it does make sense.

One must keep in mind that the escape turn of the cockroach is a complex directional movement involving a large number of muscles located in 3 pairs of limbs. A simple group of monosynaptic connections would almost certainly be insufficient to perform this task. Indeed even in the crayfish escape system, where monosynaptic connections have been demonstrated, a parallel polysynaptic pathway is the primary route for flexor activation (Roberts et al. 1982).

Given that the interposed circuit is necessary, it would still be advantageous to make the pathway as fast as possible. Thus, the GIs will get the information to the thoracic ganglia rapidly where communication with the postsynaptic interneurons will occur via short latency pathways. The postsynaptic interganglionic interneurons also have very large axons (especially in the vGI pathway) for further rapid conduction. Thus, the pathways which include the interneurons under investigation would appear to provide a rapid circuit for processing the information transmitted by GIs and controlling the motor response.

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