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The Cockroach Escape Response

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1. Introduction

In the American cockroach *Periplaneta americana,* remarkably low velocity wind puffs evoke a running escape response oriented away form the source of the wind puff. This behavior and its underlying neural components provide considerable advantages for neurobiological studies. These include a quantifiably predictable behavior and numerous neural elements that are identifiable from animal to animal. The behavior requires that the cockroach not only must detect a wind stimulus, but also it must be able to determine the direction from which the wind originated. The information on wind direction is then used to establish a behavioral decision; that is, where and how to move. Far from being a simple movement, even the initial turn involves the coordination of six legs each of which possesses a complex musculature controlling five separate leg parts.

Several of the neurons and associated structures involved in this response have distinct advantages for experimental analysis. The sensory structures are hairs located in distinct columns on two abdominal appendages, the cerci (Nicklaus, 1965). Many of the motor neurons that control leg movements are identifiable both by intracellular and less technically demanding extracellular techniques (Ritzmann and Camhi, 1978). Finally, the information received by the sensory hairs is transported to the motor centers via two distinct populations of interneurons, the dorsal and ventral giant interneurons, and an as yet undetermined group of nongiant interneurons. The relatively large axonal diameter of the giant interneurons

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(GI) makes them particularly accessible to intracellular analysis. Moreover, in the abdominal ganglia, each GI assumes a reproducible position relative to the other GIs. Thus, using intracellular dye injection techniques, each GI can be unambiguously identified in any preparation.

1.1. Historical Background

Roeder (1948, 1967), in the first quantitative study of the escape system of the cockroach, calculated that the response from onset of wind stimulation to the initial leg movement takes 28–90 msec with a mean of. 54 msec. He suggested that the GIs described by Pumphrey and Rawdon-Smith (1936) probably carried the wind-activated stimulus signal to the leg motor neurons of the thoracic ganglia. Clearly, the GIs are excited by wind, and their large axonal diameter results in a rapid conduction velocity. Rapid conduction was believed to be an important factor for an escape system (Roeder, 1967).

The simple model in which sensory neurons from cercal hairs excite GIs that in turn excite leg motor neurons was accepted until Dagan and Parnas (1970) presented data that questioned the basic assumption that GIs excite leg motor neurons. They found that stimulation of the abdominal nerve cord at a current amplitude just suprathreshold for GI activation failed to evoke action potentials in metathoracic nerve 5 (N_5), which contains both the fast and slow depressor motor neurons of the coxa (D_f and D_s, respectively). Stimulus pulses of higher amplitude are required to elicit action potentials in these motor neurons. Moreover, in preparations missing GIs, action potentials in N_5 could still be evoked by stimulating the ventral nerve cord. These preparations were generated by severing the A5–A6 connective. Because the somata of GIs are located in the terminal ganglion (A6), this operation causes the axons of GIs to degenerate anterior to the cut. Finally, nicotine applied to the abdominal nerve cord reduced the effectiveness of cord stimulation in evoking a motor response. Nicotine blocks synaptic transmission in insects. Thus, this experiment suggests that cord stimulation evokes motor activity via a chain of neurons rather than by continuous interneurons such as the GIs.

Dagan and Parnas (1970) concluded from these data that GIs do not synapse directly with leg motor neurons. They suggested that a population of smaller interneurons excite the leg motor neurons. The GIs might prepare the animal for escape by evoking movements such as lowering of antennae and inhibiting all other on-going activities.

More recent data, which I will discuss in detail, demonstrate that several of the GIs can, in fact, excite leg motor neurons as suggested by

Roeder. However, as the observations of Dagan and Parnas (1970) indicate, the excitation does not occur over monosynaptic connections. Moreover, the GIs are not a homogeneous population of neurons. Rather, different GIs are excited by different wind directions and play distinct roles in evoking motor outputs. As a result, consistent with the observations of Dagan and Parnas (1970), a single action potential in a single GI is totally ineffective in evoking a motor response. However, gentle wind puffs actually cause high frequency trains of action potentials in multiple GIs and such activity in individual GIs can indeed evoke motor responses.

2. The Escape Behavior

Prior to discussing the neurophysiological data on escape, it is essential to understand, in detail, the animal's behavioral response to wind stimuli. A considerable amount of quantitative data is now available describing the wind-mediated escape response.

2.1. Directionality of Escape

Camhi and Tom (1978) employed high-speed cinematography and single frame analysis to quantify several aspects of wind-mediated escape. Free-ranging cockroaches were placed in an arena where they could be stimulated by a machine designed to deliver reproducible wind puffs from anywhere in the arena. Under these conditions, the initial response to a wind puff is highly directional (Figure 1). Cockroaches tend to turn away from the wind stimulus. This assures that the cockroach is at least initially directed away from an approaching predator. Thus, a wind originating from behind the cockroach causes a forward escape, and wind from the front causes a rearward escape. Likewise, wind from the right makes the animal turn left, and wind from the left elicits a right turn. After the initial turn, the animal's movements are considerably more variable.

2.2. Minimum Stimulus

The minimum velocity of wind needed to evoke escape movements is remarkably low. A velocity of 0.012 m/sec has been found sufficient to evoke a turn (Camhi and Nolen, 1981). With such a low minimum velocity, one might expect cockroaches to be constantly escaping. Certainly breezes in excess of 0.012 m/sec are quite often encountered in the animal's natural environment. In fact, they do not escape from these winds because acceleration is also a critical parameter for the wind stimulus.

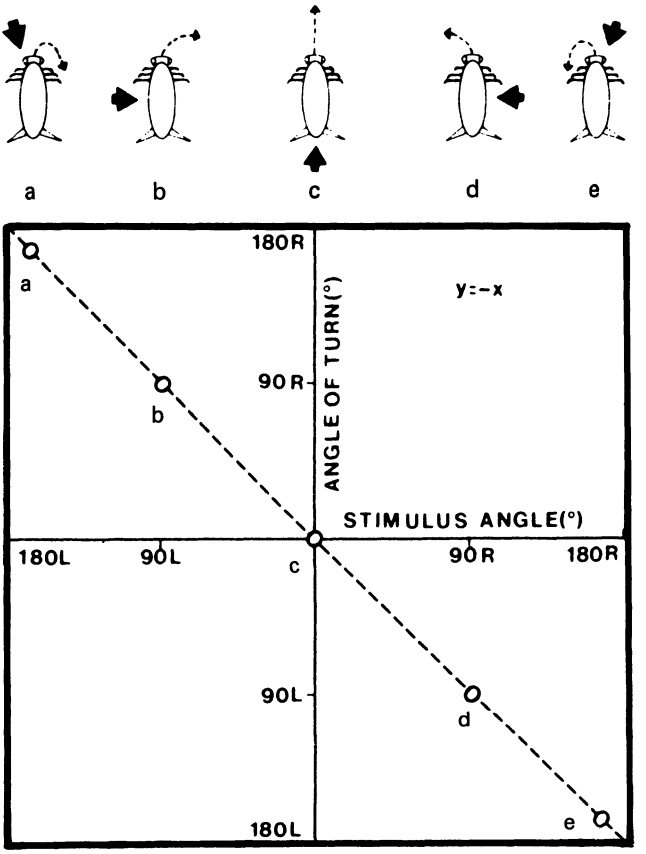
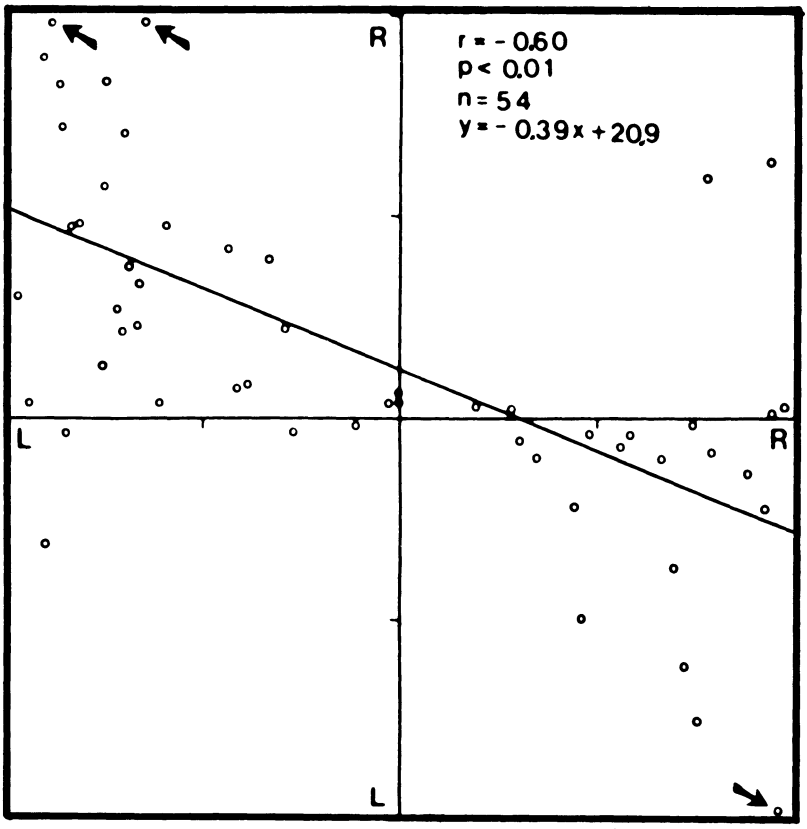


Figure 1. (Left) Conventions for plotting angle of turn vs. stimulus angle. For illustrative purposes, the cockroach is assumed to turn exactly away from the wind source. (a–e) Thick arrows represent stimulus angles, 170°L, 90L°, 0°, 90° R, and 170° R, respectively; thin arrows represent angle of turn, 170° R, 90° R, 0°, 90° L, and 170° L, respectively. The regression line for these idealized data points has the formula $y = x$. (Right) Turning

Plummer and Camhi (1981) developed a preparation for testing the importance of acceleration. The cockroach was held to a lightly greased plate by four insect pins inserted through the animal's cuticle and into a piece of wax. In this preparation the cockroach appears to walk in a very normal fashion. The plate was placed inside a movable box mounted on two tracks. Moving the box created a wind on the cockroach. By varying the acceleration of the box, the acceleration of the wind could be varied.



responses of ten free-ranging cockroaches. Angle of turn vs. stimulus angle plotted by conventions shown in top figure. Each point represents one turning response. Points indicated with an arrow represent turns larger than 180° R or 180° L (all 180°–245°). The regression line has a correlation coefficient (r), that is highly significant (from Camhi and Tom, 1978).

Acceleration of the wind stimulus is in fact positively correlated with success in generating escape responses. Stimuli accelerating at 0.6 m/sec^2 usually evoke running responses. Those accelerating between 0.3 and 0.6 m/sec^2 usually evoke a pause but no escape.

The cockroach's behavior at the time of wind stimulation has important consequences for the escape response (Camhi and Nolen, 1981). If the cockroach is walking slowly (4 steps/sec), the mean latency from

stimulus onset to the initial escape movements is 14 msec compared with 54–58 msec for standing cockroaches. However, the latency increases as the speed of walking increases. The threshold wind velocity is also reduced during slow walking (Camhi and Nolen, 1981).

2.3. Sensory Structures That Evoke Escape

Is wind and indeed wind impinging on the cerci the critical stimulus for initiating escape? The wind machine used in the experiments described above is quite massive and would provide visual and auditory cues that could initiate or direct the escape rather than the actual wind it produces. To control for this possibility, Camhi and Tom (1978) placed a smaller wind tube in the arena along with the wind machine. The shutter valve of the wind machine was activated in the same way as in previous experiments. However, the fans were turned off so that no wind was released. In all cases the escape was directed away from the wind emanating from the smaller tube rather than from the windless wind machine. This established that wind is the critical stimulus.

Many structures in addition to the cercal hairs are wind sensitive (e.g., antennae). To establish that the cerci provide the principle sensory cue for escape, the cercal hairs were covered with glue. This prevented initiation of the escape response. The same amount of glue applied to the dorsal surface of the cerci, which is devoid of hairs, had no effect on escape.

Another experiment established that inputs from cerci not only initiate the escape but also direct the initial turn (Camhi and Tom, 1978). The cerci were rotated in their sockets 60–70° to the left. The turning angles in these animals was shifted by approximately 65° left. In addition, in manipulated animals, wind from 60° right to 180° would be interpreted as wind from the animal's right side by any structure other than the cerci and therefore would evoke a left turn. However, the receptors on the rotated cerci would interpret these stimuli as coming from the left side and evoke right turns. In fact the mean angle for turns to stimuli in this range was 28° right, thus demonstrating that the important structures for directing turns are the cercal hairs.

2.4. Predator–Prey Encounters

Does the wind-mediated escape response really increase the cockroach's ability to survive predator–prey encounters? To answer this question, Camhi *et al.* (1978) studied encounters between *P. americana* and a natural predator, the toad *Bufo marinus*. Intact cockroaches successfully

escape attacks from toads 50% of the time; that is, about half of the strikes by toads fail to catch a cockroach. Moreover, 48% of the cockroaches tested managed to escape at least once. The success rate decreased significantly in cockroaches that had their cerci covered with wax. With these cockroaches, 92% of the toad strikes were successful and only 5% of the tested cockroaches escaped at least once. A control group had an equivalent amount of wax applied to abdominal sternites. This had no significant effect on the success of toad strikes.

Cinematic analysis revealed that the escape is initiated as the toad lunges prior to any movement of its tongue. This part of the toad's attack generates a wind of approximately 0.02 m/sec at the cockroach, well above the threshold for initiation of escape.

Thus, the wind-mediated escape system does impart a significant benefit to the cockroach. Moreover, the response threshold appears to be tuned to the cues generated by the attacker.

3. Neural Elements of the Escape System

With the knowledge of the precise behavioral repertoire of escape in the cockroach, we can begin to determine what neural elements control this response and how that control is effected.

3.1. Sensory Structures

Two types of hairs are located on the ventral surfaces of the cerci, thread hairs and bristle hairs (see Gnatzy, 1976). The approximately 200 thread hairs are freely deflected in response to wind (Nicklaus, 1965; Gnatzy, 1976). Deflection excites the single bipolar neuron associated with each hair. The thread hairs and their associated sockets are located in 14 distinct columns (Figure 2A) (Nicklaus, 1965). Their sockets are asymmetric, causing the hair to bend preferentially in one plane. All hairs in any single column have the same preferential planes of movement (Nicklaus, 1965).

The asymmetry of movement in the hairs confers a directionality in the response properties of their associated sensory neurons. Extracellular records from single hairs show a best response in one direction of the preferred plane and an inhibition in the opposite direction (Nicklaus, 1965). Intracellular recordings from sensory axons in the cercal nerve confirm the monopolar response characteristics of the hairs (Westin, 1979). Each axon is excited by wind from one set of directions and inhibited by wind in the opposite directions. Winds from directions not in the preferred

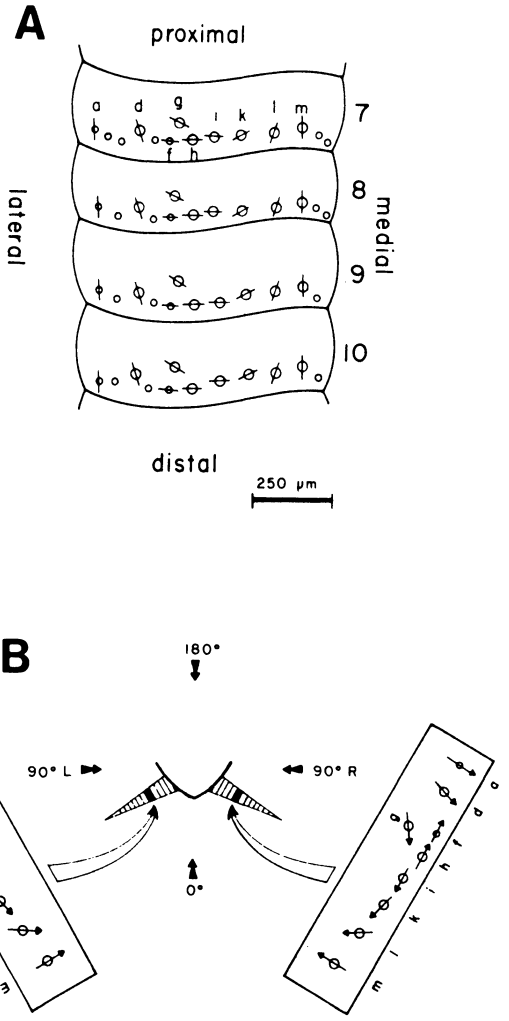


Figure 2. (A) Distribution of filiform hairs on the ventral segments 7–10 in an adult *Periplaneta americana*. Each circle shows the position of one hair. Larger circles indicate larger hairs. A line through a circle indicates the two opposite wind directions that are most effective in deflecting the hair. Letter designations are given for those columns of hairs studied by Nicklaus (1965) and Dagan and Camhi (1979). Modified from Nicklaus (1965) as shown in Dagan and Camhi (1979). (B) Best excitatory wind directions (thin arrows) for hairs of the nine most prominent columns on the left and right cercus as determined by cercogram recordings. Schematic representation of one cercal segment on each side, dorsal view, drawn at normal orientation to the body. Larger circles represent larger hairs. Letter designation for each hair corresponds to that in (A) (from Dagan and Camhi, 1979).

planes did have an effect, but in these cases the hair still moved in its preferred plane (Nicklaus, 1965). Polar plots of the responses to wind of individual sensory axons revealed seven to nine distinct groups of curves having different mean best angles (Figure 3) (Westin, 1979).

Dagan and Camhi (1979) determined the response for 9 of the 14 columns of hairs by recording extracellularly from the cercal nerve (cercogram) after immobilizing all but one column. When stimulated by air movements generated by a speaker, each column responds best to movement in the preferred plane and not at all to wind orthogonal to that plane. In the preferred plane, one direction causes an initial depolarization and the other direction causes an initial hyperpolarization. The absolute nature of the cercogram records suggests that the directional response properties of all hairs in any column are in fact the same.

As in the intracellular study, the cercograms revealed nine different preferred wind directions, one for each column studied. The best direction for each of the nine columns is consistent from animal to animal (Figure 2B).

Taken together, the best directions for all nine columns cover all four quadrants surrounding the animal (Figure 4A). Some quadrants are represented by only one or two columns. However, because each hair responds to a broad range of angles (Westin, 1979), all angles of wind should generate activity in the cercal nerve. Indeed, cercograms from an uncoated left cercus reveal a continuous curve, with smaller responses from approximately 30° right to 120° right (Figure 4B). A minimum response occurs at 90° right.

As a result of the directional properties of the thread hairs located on the cerci, the information on wind stimuli entering the CNS encodes the directional origin of wind and not just the time of onset. This information is then available to the CNS for directing the escape movements away from a potential predator.

3.2. Sensory Structures of First Instar Nymphs

In contrast to adults that possess approximately 200 thread hairs on each cercus, first instar nymphs have only four hairs, two on each cercus (Dagan and Volman, 1982). Nevertheless, these nymphs turn away from wind as well as adults do. In test cases, 84.0% of the first instar nymphs make correct turns. This compares with 83.7% for adults. Moreover, the regression line depicting angle of turn vs. angle of wind presentation for first instar nymphs is not significantly different from that calculated for adults.

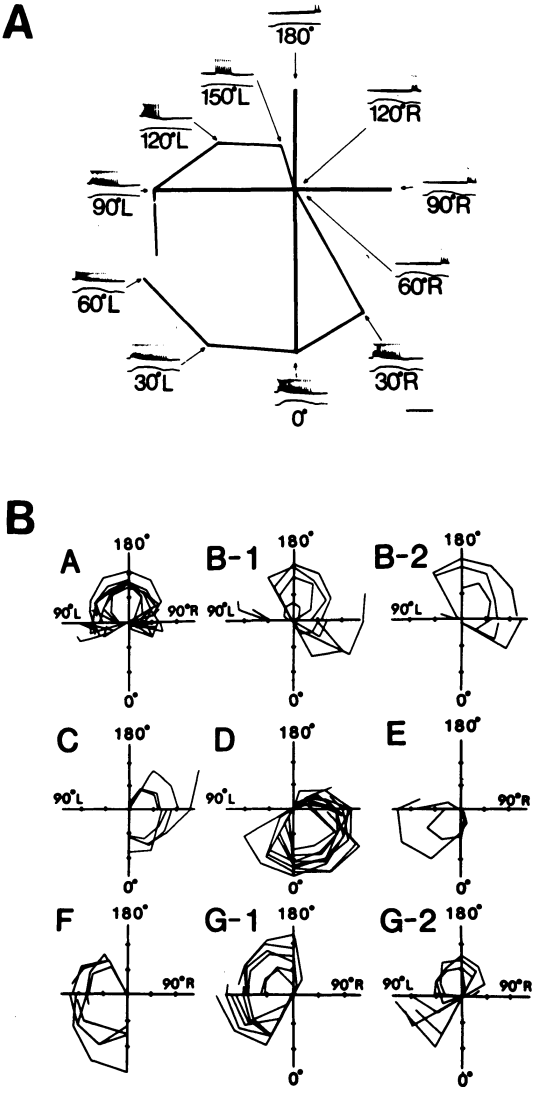


Figure 3. (A) Responses from a single sensory cell to different angles of wind. Polar plot of number of action potentials vs. wind angle. 0° represents wind from directly behind animal, 90°L represents wind flowing from animal's left to right, 180° represents wind from in front, and 90°R represents wind from right to left. Bars mark units of ten action potentials. Each point represents mean of three trails. A sample record for most wind positions is shown. Top trace shows action potentials recorded intracellularly from sensory axon. Bottom trace shows wind speed. For 60°L, 30°L, 0°L, 30°R, 60°R, 90°R, and 120°R peak speed (at the cerci) = 2.6 m/sec, acceleration = 130 m/sec^2 (outward puffs); and for 90°L, 120°L, 150°L, and 180°L peak speed (at the cerci) = 0.6 m/sec, acceleration = 25 m/sec^2 (inward puffs). Time bar = 50 msec. (B) Sensory response types. Polar plots of numbers of action potentials vs. wind angle, constructed as in part (A). Responses from several different animals are plotted on each set of axes (from Westin, 1979).

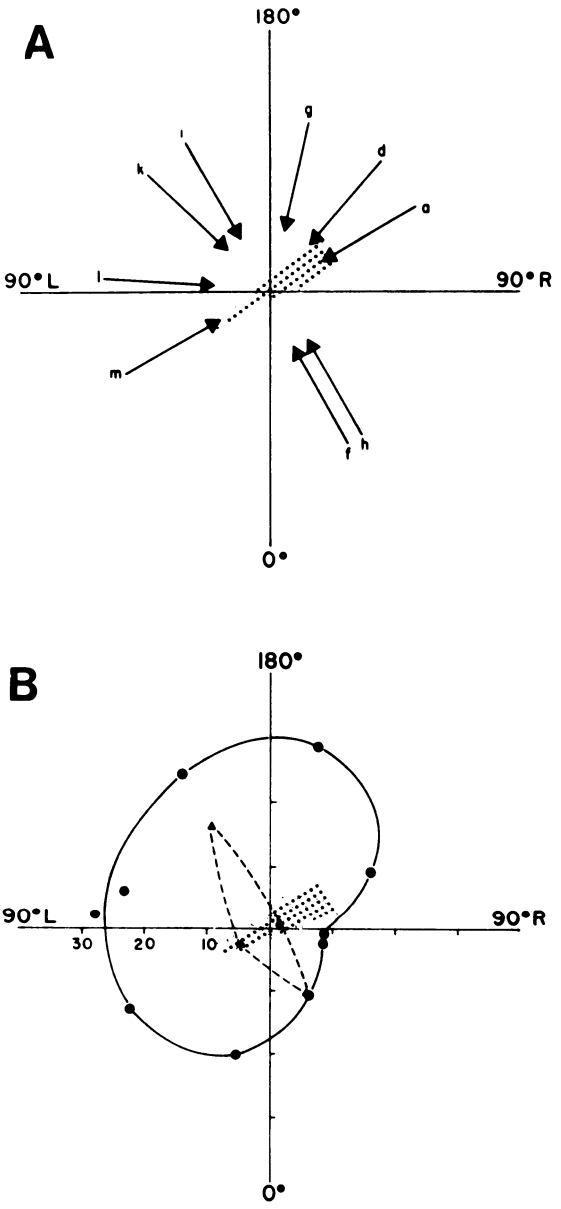


Figure 4. (A) Best excitatory directions relative to the body's axes of all columns of filiform hairs on the left cercus only. For wind stimulus from 60°L–0°–120°R, one finds the best excitatory direction of only two columns (f and h). Stippling represents orientation of left cercus. (B) Polar plot of filtered, summated cercograms obtained from an uncoated, intact left cersus (— and •) and repeated after covering all columns except f, g, h, i, and as well as all the most proximal and distal cercal segments (--- and ▲). Numbers on abscissa indicate relative size of peak positive response. The smallest response of the uncoated cercus occurs for wind from the range of directions represented by the fewest columns of hairs. Stippling represents orientation of left cercus (from Dagan and Camhi, 1979).

As in adults, each hair is depolarized in one direction and hyperpolarized in the opposite direction (Dagan and Volman, 1982). On both cerci the more lateral hair responds primarily to wind from the ipsilateral front and the medial hair responds primarily to wind from the ipsilateral rear. As a result, although there is some overlap, the four hairs do cover all four quadrants surrounding the animal. The morphology of the ventral giant interneurons in first instar nymphs (Blagburn and Beadle, 1982) is similar to that found in adults (Daley *et al.*, 1981).

The success of this system raises the as yet unanswered question of why an adult cockroach requires so many hairs to accomplish the same task that a first instar nymph does with four hairs.

3.3. The Giant Interneurons

Information on wind stimuli is conducted from the terminal ganglion (A_6) anteriorly to motor centers in the thoracic ganglia via 14 giant interneurons (seven bilateral pairs) (Figure 5A). These are found in two morphologically distinct groups, the ventral (vGIs) and dorsal (dGIs) giant interneurons (Figure 5B). A considerable amount of data (to be presented later) indicate that these are functionally as well as morphologically distinct groups.

Each GI has a single cell body that is located in A_6 (Figure 6). In all cases a single neurite exits the soma, crosses the midline, then turns anteriorly (Daley *et al.*, 1981). The axon exits A_6 in the connective contralateral*. The vGIs generally have most of their dendritic branches located at the point where the neurite turns anteriorly. The side branches of the dGIs tend to be more evenly distributed along the fiber leading out of A_6 as well as on the side of the ganglion in which the soma is located. The soma position and the general distribution of dendritic branches in A_6 is sufficient for positive identification of each GI (Daley *et al.*, 1981).

Positive identification can also be made in cross sections of abdominal ganglia (Figure 5B). (Camhi, 1976; Westin *et al.*, 1977). In abdominal connectives, the axons of GIs often change position. However, on entering an abdominal ganglion, each GI consistently assumes a typical position (Camhi, 1976). In this array, GIs 1–4 form a close-fitting ventral group and GIs 5–7 form a more dorsal triangular-shaped group. Occasionally, a fourth axon is seen between GIs 5 and 7 ventral to GI-6. However, no physiological characteristics have been ascribed to this neuron.

*The convention in this system is that the words "ipsilateral" and "contralateral" are used with reference to the position of the axon of the giant fiber, not the soma. Thus, a stimulus to the contralateral cercus would be delivered to the side of the animal opposite the axon.

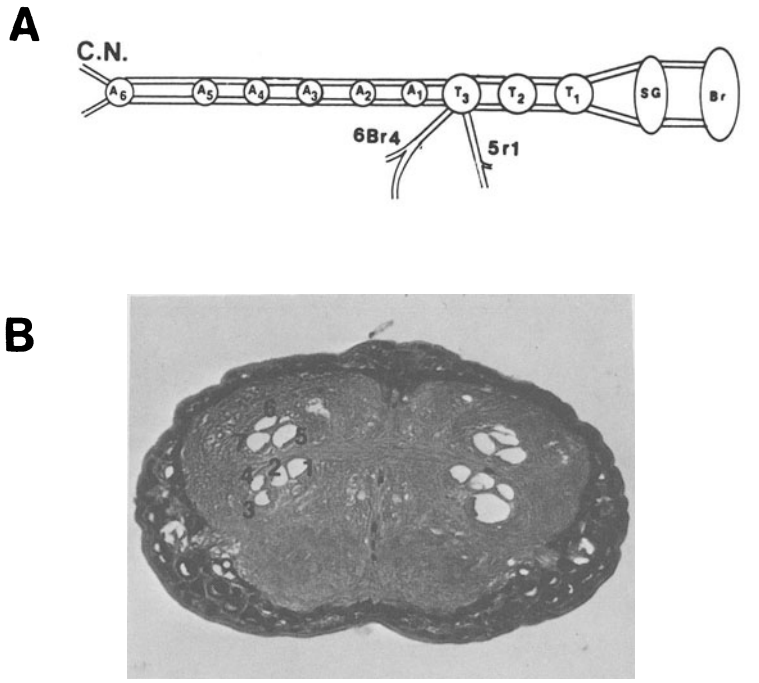


Figure 5. (A) Diagramatic representative of the cockroach CNS. Br, brain; SG, subesophageal ganglion; T_1–T_3, thoracic ganglia 1–3; A_1–A_6, abdominal ganglia; CN, cercal nerves; 6Br4, nerve in metathoracic leg containing levator axons, and 5rl, nerve in metathoracic leg containing depressor axons. (B) Cross section of an abdominal ganglion stained with hematoxylin and eosin. Numbers that have been assigned to each giant interneuron are indicated on the left side. The same numbers apply to the mirror image GIs on the right (from Ritzmann and Camhi, 1978).

The axons of vGIs, with the exception of GI-4, are larger in diameter than those of dGIs. In the abdominal connectives, GIs 1–3 have diameters of approximately 60 μm. Axons of dGIs are 25–30 μm in diameter (Spira *et al.*, 1969*a*). Giant interneuron 4 is approximately the same size or slightly smaller than the dGIs. Conduction velocities are predictably faster for the large vGIs (6–7 m/sec) than for the dGIs (4–5 m/sec). All of the GIs taper to a narrower diameter in ganglia and are smaller in thoracic segments than in abdominal connectives (Parnas and Dagan, 1971). The vGIs have been followed uninterrupted to the supraesophageal ganglion (Farley and Milburn, 1969; Spira *et al.*, 1969*b*). However, dGIs have not been detected anterior to T_2 (Farley and Milburn, 1969).

A considerable amount of information on the biophysics of GIs has

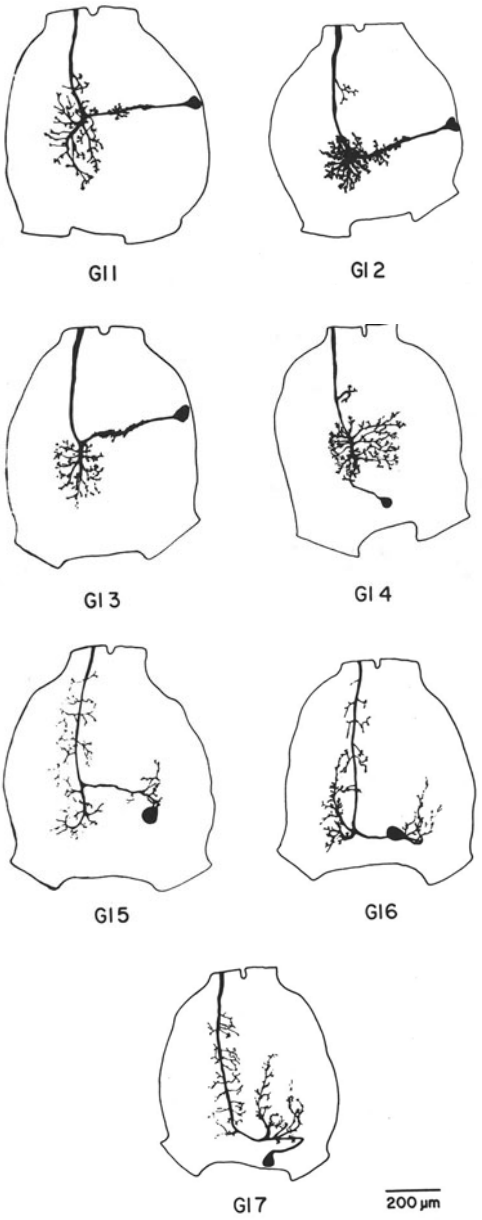


Figure 6. Morphology of each GI in the terminal ganglion (A_6) from camera lucida drawings of interneurons filled intracellularly with cobalt chloride or nitrate (from Daley *et al.*, 1981).

been documented. However, these data are beyond the scope of this review. The reader is referred to the works of Parnas, Spira, and their colleagues for this information (Parnas and Dagan, 1971; Dagan and Parnas, 1974; Spira *et al.*, 1976; Yarom and Spira, 1982).

3.3.1. Inputs to GIs

On entering A_6, the sensory neurons of the cerci make connections with the 14 GIs. In some cases the connections between sensory neurons and GIs has been shown to be monosynaptic (Callec *et al.*, 1971). The GIs tested in these experiments were not positively identified but were probably vGIs. Wind-mediated activity in dGIs tends to have a longer latency than that of vGIs (Westin *et al.*, 1977). Because only part of this discrepancy could be due to differences in conduction velocity, the sensory-to-dGI pathway may be polysynaptic.

The pharmacology of the monosynaptic connection between cercal afferents and vGIs has been studied extensively. The data indicate that these are cholinergic synapses (for a review, see Callec, 1974). The GIs are also cholinergic. This has been demonstrated by the finding that choline acetyltransferase (CAT), which is the rate-limiting enzyme in the synthesis of ACh, is significantly reduced following specific degeneration of GIs (Dagan and Sarne, 1978).

3.3.2. GI Response to Wind

All GIs respond positively to gentle wind puffs, but they do not all respond to wind from the same directions. Each GI has a specific set of wind directions to which it will respond (Westin *et al.*, 1977). The directional nature of each GI was determined by impaling individual GIs with microelectrodes filled with Procion yellow M4RS and recording the number of action potentials in response to wind stimuli from various directions. Other parameters including burst duration, average frequency, and reciprocal latency had directional properties similar to number of action potentials.

The stimulus was delivered by a device capable of repeatedly generating wind puffs of the same amplitude, duration, and acceleration. The delivery tube was mounted on a track that could be rotated around the animal. Thus, the wind stimulus could be presented from almost every angle around the animal. The presence of micromanipulators prevented the tube from rotating into the front quadrants. To stimulate from the front of the animal, wind was drawn into the delivery tube. This resulted in a similar, but lower velocity, wind stimulus. With this device it was possible to map the response to wind from all directions for a single GI.

Procion yellow could then be injected into the GI for subsequent identification in cross sections of abdominal ganglia. Response curves for all 14 GIs were generated, and they were consistent for each GI from animal to animal (Westin *et al.*, 1977).

As shown in Figure 7, only GIs 2 and 4 respond equally well to wind from any direction. Giant interneuron 1 responds to wind from all directions, but wind ipsilateral to its axon evokes a stronger response than wind from the contralateral side. Giant interneuron 7 also responds best to ipsilateral wind, but in addition it responds to wind from some contralateral front angles. It is insensitive to wind only from the contralateral rear. Giant interneurons 3, 5, and 6 are the most directional, and GIs 3

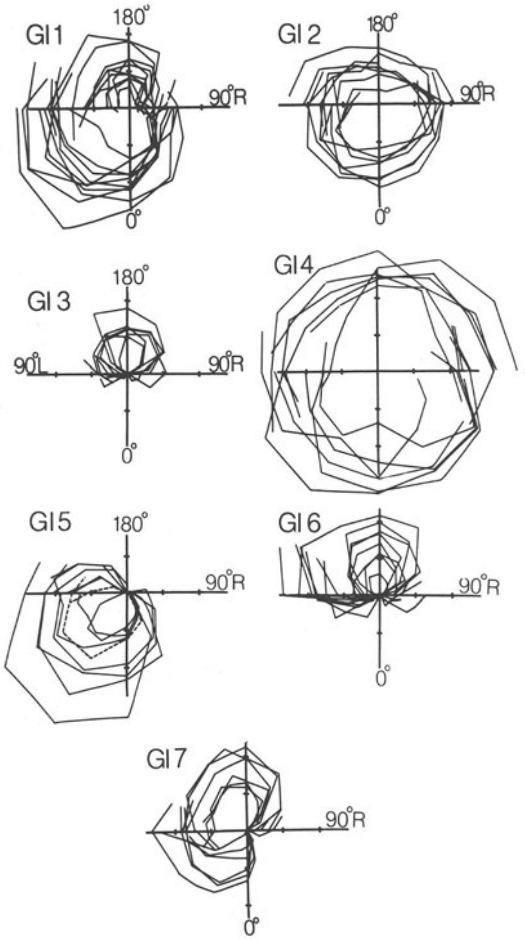


Figure 7. Directional selectivity curves for all seven histologically identified GIs. Polar plots of number of action potentials vs. wind angle constructed as in Figure 3. For each GI, curves from several different animals are plotted as if recorded from the left connective. Each point represents the mean of five trials, all σ's < 1.5 (from Westin *et al.*, 1977).

and 6 respond to wind only from the front. Each of the two GI-5s respond primarily to winds from their contralateral rear quadrant.

The mean frequency of action potentials in response to winds of 2.6 m/sec ranged from 207 ± 61/sec for GI-6 to 354 ± 79/sec for GI-2. The first two action potentials of GI-2 reach an instantaneous frequency of 900/sec. All of the GIs respond to winds of 0.1 m/sec (the lowest wind velocity tested in these experiments). With GI-1 and all of the dGIs, the number of action potentials in a response increases as the wind velocity is increased between 0.1 m/sec and 2.6 m/sec. However, GIs 2, 3, and 4 (all vGIs) reach a plateau at 0.5 m/sec. Camhi and Nolen (1981) have subsequently demonstrated that the threshold wind velocity for the GIs is much lower than 0.1 m/sec. Indeed, puffs of 0.012 m/sec (the behavioral threshold for escape) are suprathreshold for all of the vGIs and for GI-5 of the dGIs.

The frequency of action potentials for the dGIs remains relatively constant throughout the duration of wind stimulation. In contrast, the instantaneous frequency for the vGIs declines throughout the response burst. With GIs 1 and 4 the decline is gradual. However, GIs 2 and 3 are quite phasic.

To demonstrate that the responses recorded in GIs were totally due to cercal inputs, experiments similar to those described in the behavioral section were performed (Westin *et al.*, 1977). Covering the two cerci with petroleum jelly completely abolished the wind response in the GIs. Covering the animal's entire cuticle except the cerci had no influence on the response.

If only one cercus was covered or removed, the response of each GI was reduced. Indeed, the responses of GIs 1, 2, 3, and 6 ipsilateral to the ablated cercus were virtually eliminated, whereas the responses in these GIs contralateral to the ablated cercus were not appreciably affected. These GIs must receive little if any input from the contralateral cercus. In contrast, GIs 4, 5, and 7 are still excited, albeit much less, in the absence of their ipsilateral cercus. Responses for these GIs are also reduced following removal of the contralateral cercus. Therefore, they must receive inputs from both cerci.

The manner in which the sensory neurons confer directionality on the GIs is not totally understood. Certainly some differential connection of various columns of hairs must exist. However, the difference between the wind response curves of GIs 1 and 2 appear to result from differential strength of sensory inputs (Daley, 1982). Both GIs 1 and 2 receive inputs from all nine major columns of hairs. The ipsilateral bias of GI-1 arises directly from the wind input represented in cercograms of one intact cercus (cf. Figures 4B and 7). In contrast, hairs in columns *a* and *h* have

proportionally stronger inputs to GI-2 than to GI-1. This increases the responses of GI-2 to wind from the contralateral front and rear quadrants, resulting in a symmetrically omnidirectional wind response curve.

The information on wind direction encoded in the relative activity of the various GIs is rapidly conducted to the thoracic ganglia. By comparing the activity of all members of either the dGI or the vGI populations, the animal should be capable of determining the direction from which the wind originated. This can then be used to direct leg motor neurons in the ultimate behavioral response.

3.3.3. Cricket GI System

Very similar and probably homologous GI systems exist in crickets. As in *P. americana,* the cricket *Acheta domesticus* has two populations of GIs (Mendenhall and Murphey, 1974). In cross sections of connectives, only three vGIs are found, one of which is small (as in GI-4 in *P. americana*). The two large vGIs are referred to as the medial and lateral giant interneurons (MGIs and LGIs, respectively) and are probably homologous to two of the large vGIs in *P. americana.* There are four dorsal interneurons Although most cross sections of *P. americana* connectives show three obvious dGIs, occasionally a fourth one is also seen. The morphology of the cricket GIs in the terminal ganglion (Mendenhall and Murphey, 1974) is also similar to that of cockroach GIs. A single soma is located contralateral to the axon, and in the vGIs the major dendritic branches are also contralateral to the soma.

There are only two sets of hairs in the cricket. L hairs bend longitudinally with respect to the long axis of the cercus. T hairs bend transversely across the long axis (Palka *et al.,* 1977). As in the cockroach, these respond to low frequency sound (Palka *et al.,* 1977) and to wind (Tobias and Murphey, 1979). The sensory neurons and GIs have been studied in more detail in cricket (Palka and Olberg, 1977; Matsumoto and Murphey, 1977*a*) than in cockroach, and this system has been used extensively in developmental experiments (Palka and Edwards, 1974; Matsumoto and Murphey, 1977*b*; Murphey and Levine, 1980; Levine and Murphey, 1980). However, less is known about the motor outputs and the behavioral role of these systems in the cricket than in the cockroach.

3.3.4. Motor Outputs from GI Activation

The primary problem with models that have implicated GIs in escape has been the difficulty in eliciting motor responses by stimulating GIs. Dagan and Parnas (1971) demonstrated that in a dissected preparation a

single stimulus delivered extracellularly to the abdominal cord at threshold for activating GIs fails to evoke a motor response. This was later confirmed by Iles (1972), who further could not detect synaptic potentials in D_f (the fast depressor of the coxa) associated with GI action potentials. Intracellular stimulation of individually identified GIs also consistently failed to evoke motor responses when single stimulus pulses were used (Ritzmann and Camhi, 1978). However, the responses of GIs to wind stimulation, as determined by Westin *et al.*, (1977), indicate that a single action potential in a single GI is in fact a very weak stimulus. Wind puffs of 2.8 m/sec generate trains of action potentials with frequencies as high as 300/sec. Moreover, this occurs in 8–12 GIs depending on the direction of the wind stimulus. When high-frequency stimulus trains were tested even in individual GIs, several of them consistently evoked motor responses (Ritzmann and Camhi, 1978). Moreover, the motor neurons that were excited by each GI would produce movements consistent with the behavioral outputs observed in response to wind directions to which that GI responded.

The basic experimental paradigm for the motor response experiments of Ritzmann and Camhi (1978) was as follows. The animal was pinned to a cork dorsal-side-up, and a window was opened in the dorsal cuticle. After removing the gut, fat, and extraneous muscle tissue, the ventral nerve cord was raised onto a wax-covered stainless steel platform. This supported the cord so that a GI could be impaled with a microelectrode. A pair of hook electrodes were placed under the A_3–A_4 connective. These could be used to stimulate the cord while GIs were being penetrated. They could also monitor cord activity as the impaled GI was stimulated through the microelectrodes, thus assuring that each stimulus pulse excited one and only one GI.

Leg motor neurons were monitored by recording with extracellular suction electrodes on nerve branches 5r1 and 6Br4. Activity from depressor motor neurons D_f and D_s could be readily detected in 5r1 records and levator activity could be identified in 6Br4. After recording the motor response to GI stimulation, Procion yellow M4RS or Lucifer yellow CH was injected into the GI iontophoretically so that it could be identified in cross sections of abdominal ganglia.

3.3.4a. Dorsal Giant Interneurons. In terms of selectivity to wind inputs, GI-5 is the most directional GI. It responds exclusively to wind from the ipsilateral rear quadrant. Stimulation of GI-5 consistently excites the ipsilateral slow depressor (D_s) motor neuron (Figure 8A). Levator motor neurons may also be excited but only after D_s. The D_s motor neuron of the contralateral metathoracic leg is also excited by GI-5, but it is much

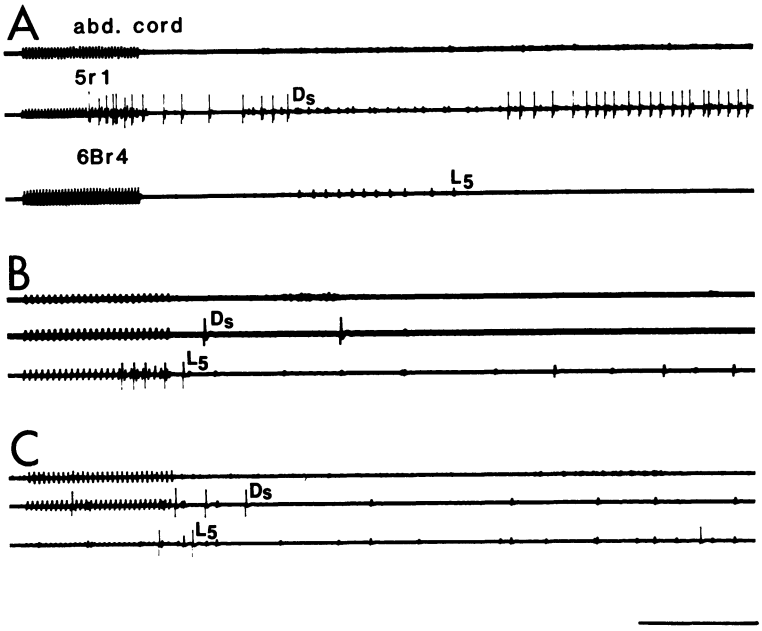


Figure 8. Motor responses recorded extracellularly in metathoracic leg nerves 5rl and 6Br4 in response to trains of current pulses delivered intracellularly to GIs 5, 6, and 7 (A, B, and C, respectively). (A–C) Top trace represents abdominal nerve cord; middle trace represents nerve branch 5rl, which innervates depressor muscle; and bottom trace represents nerve branch 6Br4, which innervates levator muscle. Regular pulses in the abdominal cord are GI action potentials. Pulses associated with these in 5rl and 6Br4 are stimulus artifacts. Action potentials from the slow depressor (D_s) and a levator motor neuron (L_5) are labeled. Calibration represents 75 msec for (A) and 60 msec for (B and C).

weaker and the latency is consistently longer than the activity generated in ipsilateral D_s. The bias toward ipsilateral D_s activation would, in a free-ranging animal, result in a turn away from the ipsilateral rear quadrant, the origin of wind stimuli that excite GI-5.

It should be noted that even in the ipsilateral D_s response to GI-5 activation, the latency from the first GI action potential to the first motor action potential is always quite long (mean of 33.7 ± 13.4 msec reported in Ritzmann and Camhi, 1978). This is also true for all other GI-activated motor outputs and suggests that the GI-to-motor neuron pathway is not monosynaptic. There is a possibility that this time is taken by subthreshold events summing to reach threshold for action potentials. However, motor neurons involved in flight that are also excited by dGIs have been recorded intracellularly in conjunction with dGI stimulation (Ritzmann *et al.*, 1982*b*).

In these motor neurons the initial depolarization is detected only after latencies of 24–34 msec. Similar results have been found in motor neurons with axons in nerve 5 that presumably control leg movements (Ritzmann and Pollack, unpublished data).

Giant interneuron 6 (Figure 8B) is opposite to GI-5 with regard to both sensory input and motor output. It responds only to wind from the front and excites initially ipsilateral levator motor neurons (Ritzmann and Camhi, 1978). The leg movements in response to wind from the front are not as consistent as those in response to wind from the rear. Nevertheless, the most typical initial movement of the ipsilateral rear leg in response to wind from the front is levation. Presumably turns of greater than 90° require more than one leg movement. The turn may be started by another set of legs (perhaps those of the prothorax) while the ipsilateral metathoracic leg is levated and protracted for a subsequent depression that completes the turn.

Giant interneuron 7 combines the inputs of GIs 5 and 6 in that it is excited by wind from the front and ipsilateral rear. The motor output reflects this combination (Figure 8C). Both levator and depressor motor neurons are excited by GI-7 with no consistent bias towards one or the other.

The unbiased input and output of GI-7 makes it an excellent candidate for a general activation neuron that would augment the more biased outputs of GIs 5 or 6. Paired intracellular stimulation of GIs indicates that GI-7 can indeed play this role (Ritzmann and Pollack, 1981). When paired with either GIs 5 or 6, GI-7 increases the motor output and decreases the latency to that output while maintaining the bias towards depressor or levator activity of the other dGI. Thus, paired stimulation of GIs 5 and 7 evokes significantly more D_s action potentials at a shorter latency than either GIs 5 or 7 individually. Similarly, stimulation of GIs 6 and 7 evokes an augmented levator response. GIs 5 and 6 are coactivated only by winds perpendicular to the animal's long axis. Nevertheless, stimulating GIs 5 and 6 together also evokes a stronger response. However, although the levator/depressor bias is consistent in trials of a single preparation, it varies from preparation to preparation.

3.3.4b. Lesioning Single GIs. Since any wind excites 8–12 GIs and summation among GIs occurs, is any single GI essential to the motor output? To answer this question, Westin and Ritzmann (1982) determined the motor response to wind stimulation before and after lesioning a single GI.

Wind stimuli were presented from various different directions while recording extracellularly from metathoracic leg motor neurons. In this

preparation, activity in either D_s motor neuron is stronger in response to wind from its ipsilateral rear quadrant than from its contralateral rear quadrant (Figure 9). Levator activity was less consistent.

The stronger response from the ipsilateral rear quadrant could be due to activation of the ipsilateral GI-5. To test this hypothesis, GI-5 was impaled with a microelectrode filled with $CoCl_2$. In addition to its utility in intracellular dye marking, $CoCl_2$ is a strong neurotoxin (Dagan and Sarne, 1979). Injection of $CoCl_2$ into GI-5 created a block for anterior propagation of action potentials, thus selectively eliminating it from the wind-to-motor pathway. As a result of lesioning GI-5, the strong D_s response from the ipsilateral rear was drastically reduced or eliminated (Figure 9). Thus, at least GI-5 plays a critical role in the biased initiation of D_s activity.

3.3.4c. Ventral Giant Interneurons. Giant interneurons 5–7, which consistently evoke motor outputs, are all dGIs. Of the vGIs, only GI-1 has been found to be effective in evoking motor outputs. Stimulating GIs 2 or 3 does not elicit a motor response. Giant interneuron 4 has also been ineffective, but because of its small diameter relative to GIs 2 and 3, which are located on either side of it, GI-4 has been tested in only a few preparations.

In most cases, stimulation of GI-1 evokes action potentials in the widespread common inhibitor (CI) and one to two action potentials to D_s (Ritzmann and Camhi, 1978). Fourtner and Drewes (1977) have reported that tactile stimulation of cerci or electrical stimulation of cercal nerves

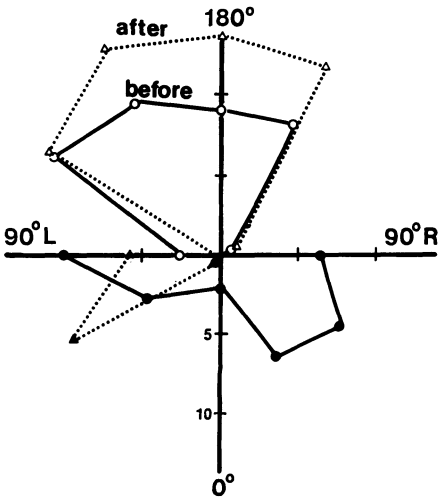


Figure 9. The effect on D_s output of blocking conduction in GI-5. Responses to wind stimulation of the right D_s motor neuron are shown in an intact animal (——) and in the same animal after conduction in the right GI-5 had been blocked (– – –) by injection of cobalt ions into its axon. The D_s response to wind from the ipsilateral rear was abolished by blocking GI-5, while responses to other wind angles remained. Responses to wind from the front of the animal actually increased slightly following blockage of GI-5, but this was not seen in other animals (from Westin and Ritzmann, 1982).

produces initially a burst of action potential in CI. This is also true for most wind-activated responses (Westin and Ritzmann, 1982) and could be due to activation of GI-1.

Although the D_s response is typically very weak, occasionally the initial response is quite strong (Ritzmann, 1981). However, even in these cases the response wanes quickly in subsequent trials (Figure 10). This suggests that the excitatory pathway between GI-1 and depressor motor neurons contains one or more extremely labile connections. In many preparations, the process of setting up the animal and impaling GIs probably renders this pathway refractory.

In spite of its inability to elicit motor responses on its own, GI-2 activity can enhance the motor output of GI-1. In several preparations, paired stimulation of GIs 1 and 2 evoked significantly more D_s action potentials than did GI-1 alone (Figure 11). An interesting aspect of these pairs is that beyond some threshold, the duration of the GI-2 train has no bearing on the extent of the increase in motor output. This is unlike the case for dGIs and correlates with the phasic nature of the responses to wind stimulation recorded in GIs 2 and 3. Pairing GI-3 and GI-1 has not been effective in altering motor responses. However, its role may be more

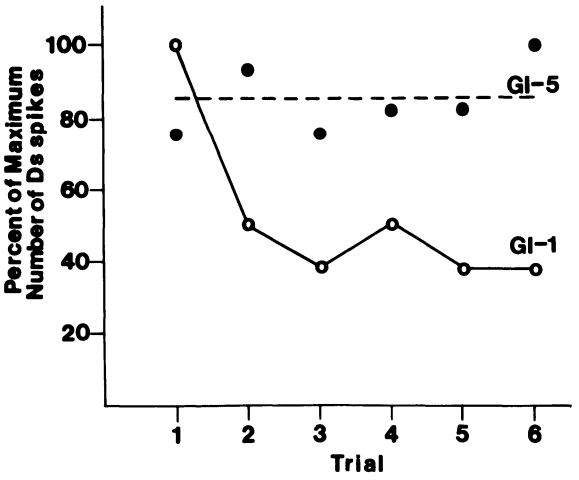


Figure 10. A comparison of the motor response of the first six trials of a preparation in which a ventral GI (○) was stimulated and the first six trials of another preparation in which a dorsal GI (●) was stimulated. The strongest response was taken as 100%. Note that in the ventral GI trials, the response declined sharply after the first trial. However, in the dorsal GI the response stayed at about the same level throughout the six trials. Lines were fitted by eye (from Ritzmann, 1981).

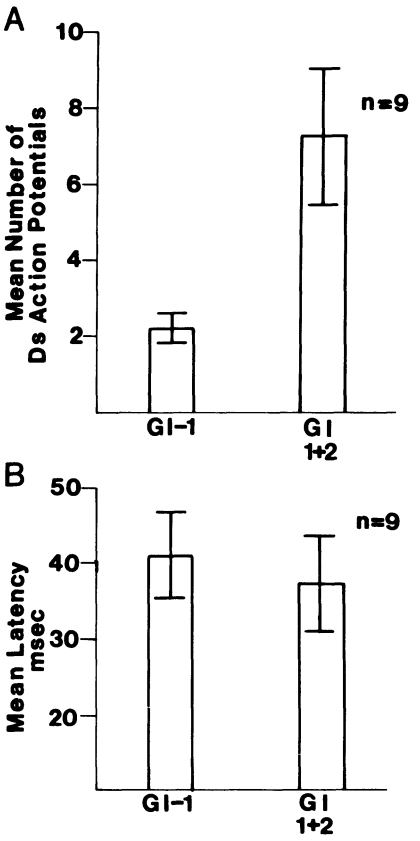


Figure 11. Bar graphs showing motor response magnitudes and latencies for a preparation in which GIs 1 and 2 were stimulated alone and together. (A) Mean number of D_s spikes resulting from stimulation of GI-1 alone and with GI-2. GI-2 alone evoked no response. Note the larger D_s response in the paired trials. (B) Latency from stimulus onset to the first D_s spike was not significantly different in paired and GI-1 trials. Error bars in this figure and in Figure 12 indicate ± 2 SE (from Ritzmann, 1981).

complex. Perhaps GI-3 combines with GIs 1 and 2 and reverses the bias from depression to levation. If so, this effect might only be detected if all three GIs were stimulated simultaneously, a technically demanding experiment.

It should be noted that all of the motor outputs reported for dGIs or vGIs are considerably weaker than that expected in an escape response. They are all limited to slow motor neurons and all have fairly long latencies. Escape, in a relatively freely moving animal, involves much stronger responses including activation of fast motor neurons. Stronger responses with shorter latencies were found in paired experiments. This could be further improved if the full complement of GIs activated by wind could be stimulated simultaneously. Indeed, it is possible that the relatively high threshold for fast motor neurons (Pearson and Iles, 1970) could be surpassed in this manner.

An additional and potentially more important factor is that the restraint and dissection required in intracellular experiments greatly reduces the responsiveness of the system. Restraint has been shown to inhibit the escape system of crayfish (Krasne and Wine, 1975), and Camhi (unpublished data) has noted a similar effect in cockroach; Eaton and Hackett describe a similar effect in goldfish (Chapter 8, this volume). Indeed in an animal that is freely walking on a greased plate, holding one leg can totally eliminate vigorous escape responses (Ritzmann, unpublished data).

3.3.4d. Pairs Involving dGIs and vGIs. Another possibility is that strong motor outputs require activity in both dGIs and vGIs. The motor responses from excitation of dGIs would provide a base of activity on which vGI activation could sum to yield the strong responses observed in escape. Without the dGI-mediated activity, vGIs would be incapable of reaching threshold for any but the weakest motor responses.

As attractive as this hypothesis is, it is not supported by the results of paired experiments involving one dGI and one vGI. Rather than revealing positive summation, the only effect found in dGI/vGI pairs was a decrease in the motor output below that found for the dGI alone. This was true even in pairs including GIs 1 and 5 (Figure 12). Since both of these normally excite the same motor neuron (D_s) this would be the optimal pair for testing the hypothesis.

3.3.5. Functional Separation of dGIs and vGIs

The experiments pairing a dGI and a vGI suggest that these are not only morphologically distinct, but also functionally separate groups of interneurons. This conclusion is supported by several other lines of evidence. In addition to the various physiological differences between dGIs and vGIs that have been noted above, the effects during walking observed in these two groups are totally different. In many systems sensory inputs that are used to evoke locomotor activity are suppressed during locomotion, thus preventing positive feedback loops (Kennedy *et al.*, 1974; Russell, 1976). This is also true for the large GI system of cricket (Murphey and Palka, 1974). In the cockroach, vGI activity is reduced during walking, but dGI activity is actually enhanced (Figure 13) (Delcomyn and Daley, 1979; Daley and Delcomyn, 1980*a*,*b*). Both of these effects appear to arise in large part by central connections. However, the reduction of vGI activity during walking does have a peripheral component, perhaps due to the physical deflection of hairs caused by wind generated by walking movements (Orida and Josephson, 1978; Daley and Delcomyn 1980*b*).

Beyond the physiological and morphological characteristics of dGIs

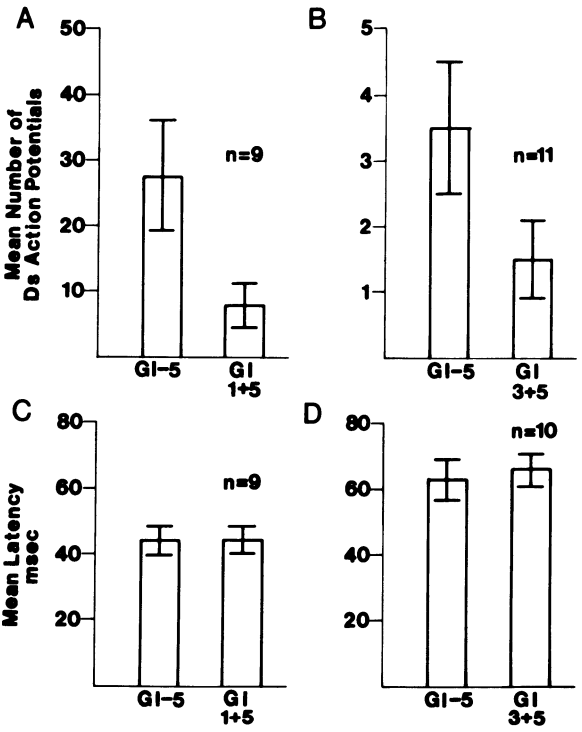


Figure 12. Bar graphs showing the mean responses in paired and unpaired trials involving one dorsal and one ventral GI. Data from two preparations are shown; one (A, C) involving GIs 1 and 5, and one (B, D) involving GIs 3 and 5. In both preparations, the number of D_s spikes in the paired trails was less than that in the GI-5 trials (A, B). However, the mean latency to the first D_s spike was not changed (C, D) (from Ritzmann, 1981).

and vGIs, Westin *et al.* (1977) pointed out that summation between dGIs and vGIs is simply not necessary. Either group could detect wind direction perfectly well independent of the other group. In this discussion, GI-4 (a small omnidirectional vGI) was included with the dGIs due to its similar latency. But it is not required in either group in order to determine wind direction. In the ventral system, wind from the right rear would be detected by activity in right GI-1 coupled with a lack of activity in either GI-3. Wind from the left rear quadrant would be signaled by activity in left GI-1 and again silence in GI-3s. Wind in the front quadrants would be indicated by the same relative activities in GI-1s plus activity in the two GI-3s. Giant interneuron 2 would be excited in all cases. This would enhance the signal and assure against the generation of escape responses due to spurious activity in GI-1 alone.

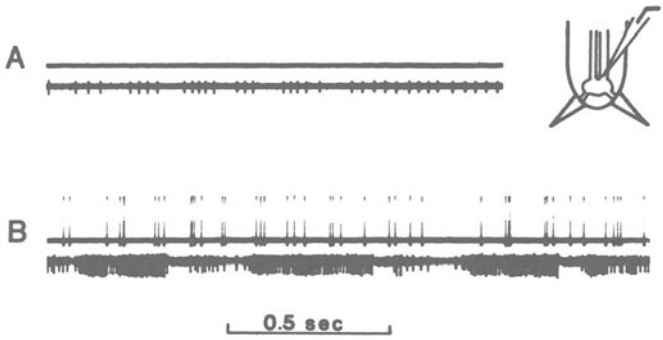


Figure 13. Intracellularly recorded activity from the axon of a dorsal GI-7 during (A) a period of rest and (B) slow walking. Inset represents intracellular recording arrangement. Upper traces are the intracellular record and the lower traces are electrical activity in extensor muscle 177 of the right rear leg (from Dale and Delcomyn, 1980).

In the dorsal system the two GI-7s serve as general excitors. Activity in either right or left GI-5 clearly denotes wind from right or left rear quadrants respectively. Wind from the front excites the two GI-6s. Right or left front is more difficult to surmise with the dorsal system. However, both GIs 6 and 7 respond more to ipsilateral wind. This could provide the left–right bias. In fact, in experiments where the dorsal system is probably employed, motor responses to wind from the front are not as predictable as those from the rear (Westin and Ritzmann, 1982).

3.3.6. Relative Roles of dGIs and vGIs

Why does the cockroach have two separate systems for detecting wind stimuli? At this time we can propose only hypotheses and several are possible. First of all, we must realize that only a small part of the motor consequences of stimulating either system is presently known. The GIs have branches in all abdominal and thoracic ganglia, not just in T_3 (Farley and Milburn, 1969; Harris and Smyth, 1971). Indeed, the vGIs proceed uninterrupted to the supraesophageal ganglion (Spira *et al.*, 1969*b*; Farley and Milburn, 1969). Even in T_3 the motor neurons that have been monitored are those only of the coxal levator and depressor motor neurons. All segments of all six legs are involved in the escape movements. If the entire constellation of motor outputs was known for each system, we might find that the dGIs and vGIs are not at all redundant.

Nevertheless, given that our present knowledge suggests that the two systems are similar, at least two explanations for their existence are pos-

sible: (1) the dGIs may serve as a slower, less labile backup system for the vGIs, and (2) the two systems may function sequentially; the vGIs initiating the escape response, and the dGIs completing it.

In several systems employing giant axons for escape, a smaller fiber system is also present in parallel with the giant axons (Krasne, 1965; Schrameck, 1970; Wine and Krasne, 1972; Eaton *et al.*, 1982). The GIs provide more rapid responses to novel threats, but tend to habituate rapidly and perhaps as a result may be actively inhibited if the animal is restrained and escape is hopeless (Krasne and Wine, 1975). The smaller fiber system provides a slower but safer backup system to generate escape movements.

In the cockroach, the vGIs may represent the "true" GI system, whereas the dGIs along with other non-GIs (Westin *et al.*, 1977, Ritzmann and Pollack, 1981) perhaps provide a smaller backup system. The vGI system is significantly faster than the dGIs. The latency for wind-evoked activity reaching the thoracic ganglia is approximately 5 msec less for the vGIs than for the dGIs (Westin *et al.*, 1977). The vGI system is also much more labile than the dGI system (Figure 10B). This could explain the difference in the changes during walking in the two systems. The more labile vGI system must be inhibited to protect the vGI-to-motor pathway for habituation. The dGI system does not readily habituate and can therefore be potentiated by the increase in ongoing activity. The negative influence of vGI activity on motor outputs from dGI stimulation could establish a hierarchy. In a novel situation, vGI activity would not only provide strong motor outputs, but would also effectively prevent the expression of motor outputs from the dGIs. As the motor response habituates, the repression of dGI activity would also habituate, allowing the dGI pathway to function.

An alternate hypothesis has been put forward by Camhi and Nolen (1981). They reason that, at least during slow walking, the vGIs are solely responsible for initiating escape, and the dGIs are responsible for subsequent turning and running movements (Figure 14). During slow walking the mean latency for escape is 14 msec and can be as low as 11 msec (Camhi and Nolen, 1981). According to their calculations, if the vGI pathway is used, at least 8 msec are needed for neural information to flow through the entire escape system. In the shortest latency (11 msec), this would only allow 3 msec more for conduction through GIs. Even the mean latency of 14 msec would allow only 6 msec of additional time. Wind-evoked activity in the dGIs reaches the thoracic ganglia about 5 msec after activity in the vGIs. Thus, under these conditions, the dGIs would probably not be fast enough to evoke the escape movements. During the initiation phase, the vGIs would suppress the dGI system. How-

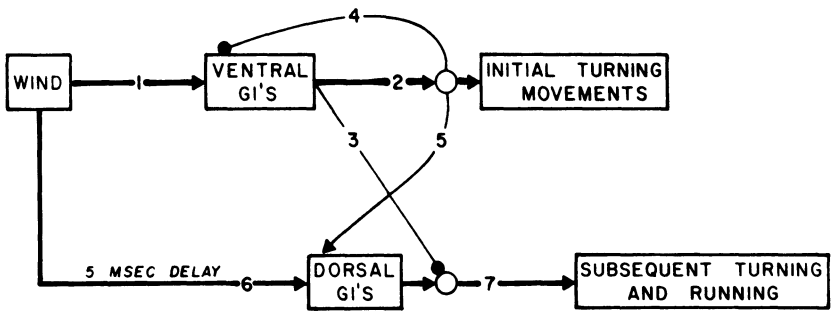


Figure 14. Tentative flow diagram of the activation of escape behavior by the dorsal and ventral GIs. The two circles, one following the ventral GIs (on path 2) and the other following the dorsal GIs (on path 7) represent thoracic GI-to-motor centers. All interactions shown hav been demonstrated. The model reveals that two sets of GIs may work sequentially; first the ventrals would initiate the oriented escape response, then the dorsals would continue to drive the turn during running (from Camhi and Nolen, 1981).

ever, once the cockroach started running, the vGIs would themselves be suppressed, releasing the dGIs to continue the escape movements.

The finding that vGIs are inhibited during walking would seem to argue against this model. However, the extent to which the vGIs are inhibited is correlated with walking speed. Under slow walking the inhibition of vGIs is minimal (Daley and Delcomyn, 1980*a*). With increasing walking speeds, vGI inhibition increases, but so does the latency to escape (Camhi and Nolen, 1981).

Support for this, or any model proposing that vGIs provide the primary input for escape, will require more information on the motor outputs generated by vGI activity. Although the dGI-mediated motor outputs are consistent with behavioral data, the only motor response to vGI stimulation is activation of D_s. How does wind from the front quadrants evoke rearward turns via vGIs? The lability of the vGI system makes this a difficult problem to resolve. Until recently all behavioral analyses have been performed on free-ranging or minimally restrained animals, whereas neurophysiological experiments have been performed on highly dissected and restrained preparations. As is evident from the relatively weak motor outputs recorded in these preparations, one cannot assume that the escape circuitry performs in the same manner under these two conditions. Attempts are presently being made to perform neurophysiological experiments on preparations that are closer to those used in behavioral experiments.

One approach being used by C. M. Comer and J. M. Camhi (personal communication) is to impale a vGI through a small window in the cuticle,

inject a toxin into the vGI to kill it, and then allow the wound in one cuticle to heal. Once the cockroach recovers, it can then be tested as a free-ranging animal. A postmortem reveals which GI was lesioned. Preliminary experiments have been encouraging, but more are required. Until more information is available on how the vGIs function, we will not be able to establish what the relative roles of the vGIs and dGIs are.

4. Alterations of the Escape Response

Rapid escape responses are often thought to arise from stable neural circuits. A system that is designed to simply remove the animal from a threatening situation as fast as possible needs little modification. Nevertheless, the circuitry involved in the cockroach escape system does undergo modulation. Two examples have been documented. One is a long-term form of recovery in which activity in the GIs is modified over a period of several days in response to cercal ablation. The other example is a more transient switch in the motor response to dGI activation caused by changes in the momentary conditions the animal encounters.

4.1. Recovery from Cercal Ablation

Quite often individual cockroaches are found with one cercus missing. A missing cercus alters the response properties of GIs to wind stimuli and thus should create serious problems for the directionality of the escape system. Recent experiments by Vardi and Camhi (1982 *a,b*) indicate that the cockroach can in fact adjust to such losses.

Behavioral tests on adult animals 1–5 days after their left cercus was removed confirm that wrong turns are made (Vardi and Camhi, 1982*a*). These animals incorrectly turn left into winds from the left. Wind from the right still evokes correct left turns away from the wind source.

Over a period of 30 days these animals gradually adjust to the absence of a cercus. After 30 days, winds from the left once again evoked right turns, although the mean angle was still less than normal. During this period the cercus was not allowed to regenerate.

Last instar nymphs were also tested. They corrected even better than the adults, indicating a greater potential for plasticity in younger animals.

The mechanism for recovery involves the return of activity patterns in GIs closer to that of a normal animal (Vardi and Camhi, 1982*b*). One day after ablation of the left cercus, left GIs 1, 2, 3, and 6 show little if any response to wind stimuli. These GIs do not normally receive significant inputs from the contralateral cercus (Westin *et al.*, 1977). In contrast,

the directionality curves of these GIs 30 days after cercal ablation approach the normal condition. Giant interneurons 4, 5, and 7, which do receive contralateral inputs, have reduced and altered wind response curves immediately after ipsilateral cercal ablation. Their curves are also considerably restored after 30 days.

The source of this correction appears to come from inputs originating in the intact right cercus. However, the cellular mechanism is not as yet understood.

4.2. dGIs as Bifunctional Interneurons

Leg movements associated with running occur only when the cockroach has one or more legs in contact with some surface. In the absence of leg contact, the same wind puffs evoke a totally different behavior, flying. Even though many of the same motor neurons are used in running and flying (Fourtner and Randall, 1982; Ritzmann *et al.*, 1983), the movements are quite distinct. In flight the wings unfold and beat at approximately 42 Hz, legs are held up against the animal's abdomen, and the leg levator motor neurons in nerve 6Br4 burst in phase with both the elevator and depressor cycles on the windbeat (Figure 15A, B).

The switch between running and flying could represent an inhibition of the GI systems allowing a different set of wind-activated interneurons to evoke flight. Alternatively, the GIs could remain active, but have their activity rerouted to the circuitry controlling flight. This latter possibility is in fact what happens (Ritzmann *et al.*, 1980).

Flight can be initiated in a restrained preparation by simply removing all of the legs distal to the coxa-trochantor joint. Under these conditions flight can be evoked with either a wind puff or a train of current pulses in any of the 6 dGIs (Figure 15B, C). The stimulus trains are identical to those used to generate running responses in animals with intact legs. None of the vGIs have been shown to be capable of initiating flight.

The switch between running and flying is provided by campaniform sensilla on the legs (Ritzmann *et al.*, 1980). These have been previously implicated in tarsal contact inhibition of flight (Krämer and Markl, 1978). Axons of the companiform sensilla are located in nerve N_5 of each leg. If only the prothoracic and mesothoracic legs are removed, dGI stimulation evokes a typical running response with no flight-related activity (Figure 16A). Severing N_5 in one of the remaining metathoracic legs may release some flight tendencies, but usually does not. However, when the remaining N_5 is severed, stimulation of a single dGI will evoke flight (Figure 16B). The converse experiment can also be performed. All legs are removed, and dGI stimulation elicits flight activity. However, if stim-

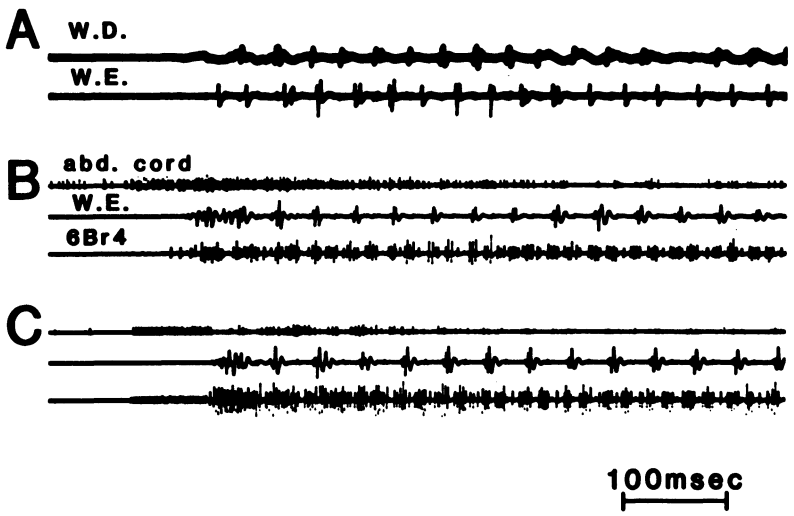


Figure 15. Demonstration of flight in legless restrained animals. (A) For comparison, EMG records from wing depressor (top trace) and elevator (bottom trace) muscles of the metathoracic segment taken during flight in an animal that has been tethered off of the ground. Note the regularly occurring muscle potentials that alternate between elevation and depression. (B) Recording of a flight sequence in an animal that was pinned to a cork but had all legs removed except the right metathoracic leg. In that leg, nerve 5 was severed. Flight was initiated by a brief wind puff directed at the cerci. In (B, C) top trace is the extracellular recording from the abdominal nerve cord, middle trace is an EMG from wing elevator muscles, and bottom trace is from leg nerve 6Br4 (contains levator motor axons of the leg). Note the EMG in the wing elevator muscle is essentialy the same as that in (A). Axons in 6Br4 burst approximately in time with elevator and depressor phases of flight. (C) GI-6 is stimulated intracellularly, in the same preparation as in (B). A train of 73 msec with 2.5 msec interpulse intervals (indicated by dGI spikes in top trace) initiates a flight sequence that is essentially the same as that in (B). Calibration applies to all records (from Ritzmann *et al.*, 1982).

ulation of N_5 in any leg is paired with dGI stimulation, flight is prevented (Figure 16C).

4.2.1. Analysis of Flight Initiation

In dissected and restrained preparations, flight behavior is in fact easier to initiate than a complete running response. This may be because the wings although cut back to stubs, are not hindered from free movement. In any event, dGI activation of flight may be instructive in understanding how dGIs evoke running.

In flight the motor neurons that control wing movements are activated in two parts (Ritzmann *et al.*, 1982). An initial depolarization is followed by a series of rhythmic depolarizations that are in phase with either el-

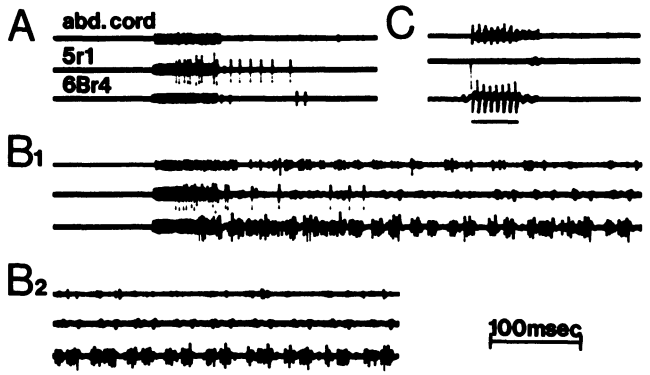


Figure 16. The effect of nerve 5 activity on flight initiation. The order of traces in (A, B): top trace is abdominal cord recording, middle trace is nerve branch 5rl containing leg depressor motor neurons, and bottom trace is nerve branch 6Br4. The prothoracic and mesothoracic legs were removed but both metathoracic nerve 5s were still intact. Under these conditions stimulation of GI-5 (73 msec duration, 2.0 msec interpulse interval) evoked activity in the slow depressor motor neuron D_s but did not evoke flight related activity. Severing the left metathoracic nerve 5 had no effect on the motor output. (B) After the right nerve 5 was also severed, so that no nerve 5 remained intact in any leg, the same stimulus to GI-5 initiated strong flight activity as indicated by the bursts in 6Br4. B_1 and B_2 are continuous records. (C) In the same preparation as in Figure 15, the same stimulus was presented to GI-6 as in Figure 15C. However, at the same time nerve 5 was stimulated with 8 pulses at a 7 msec interval. This was sufficient to prevent flight activity from occurring. A bar under the bottom trace indicates the time of nerve 5 stimulation. The calibration applies to all records (from Ritzmann *et al.*, 1980, copyright 1980 by the American Association for the Advancement of Science).

evation or depression of the wing (Figure 17). In wind-evoked flight, dGI activity is seen at or before the initial depolarization (Figure 17B, C). The activity recorded in dGIs outlasts vGI activity, but does not continue throughout the flight sequence. Thus, dGI activity is sufficient for flight initiation, but not necessary for maintaining the flight sequence.

Nevertheless, the number of dGI action potentials in the input train can influence the duration of the flight sequence. The number of bursts in the flight sequence increases linearly as more dGI action potentials are added to the input (Ritzmann *et al.*, 1982). Flight duration can also be increased by pairing two dGIs. Any two dGIs stimulated together evoke more flight bursts and have a lower threshold duration for the input train than does either dGI alone (Ritzmann *et al.*, 1982). In either case, the input signal eventually gets strong enough that the flight becomes self-sustaining. At that point, flight sequences can last well over several seconds.

All of this suggests that dGIs initiate flight by depolarizing some elements in the flight circuitry, and that the amplitude of the depolarization

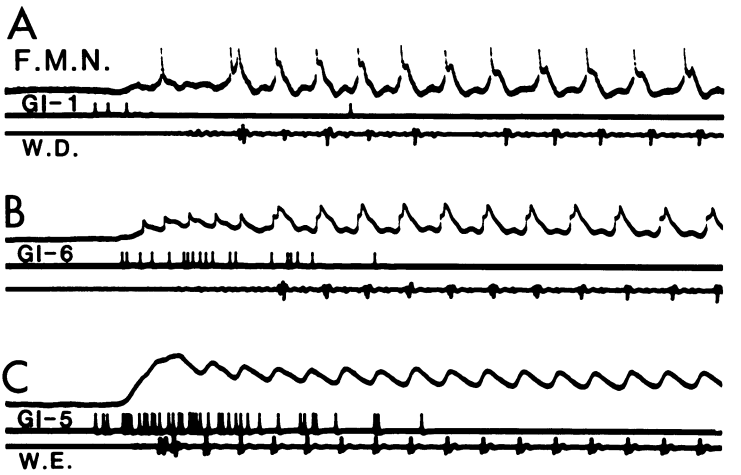


Figure 17. Records showing the relationship between GI action potentials and flight initiation in wind-mediated flight. A flight motor neuron (FMN) and a GI were impaled simultaneously and flight was evoked by a brief wind puff. GI action potentials are recorded before the initial depolarization of the FMN in all cases except (B). In (B), dGI activity occurs slightly after the initial depolarization. Presumably in this case another GI caused the initial depolarization. Even in (B), dGI activity occurs well before the onset of rhythmic activity. In each record the top trace is an intracellular record from the FMN, the middle trace is an intracellular record from a GI, and the bottom trace is an EMG from either a wing depressor (WD) or a wing elevator (WE) muscle. (A, B) From records of the same FMN (a wing depressor motor neuron) but in association with a vGI (A) and dGI (B). (C) From a different preparation in which GI-5 (a dGI) and a wing elevator motor neuron were impaled. The vertical calibration represents 160 mV in all GI traces. In the FMN traces the vertical calibration represents (A) 10 mV, (B, C) 20 mV. The horizontal calibration represents 50 msec in all records.

determines the duration of the flight sequence. This would be similar to the ramp depolarization recorded in dorsal swim interneurons of *Tritonia* (Getting *et al.*, 1980). In *Tritonia* the amplitude of the ramp depolarization affects not only the duration of the swim but also the frequency of motor bursts (Lennard *et al.*, 1980). In cockroach flight the frequency of bursts remains relatively constant throughout the flight sequence (Ritzmann *et al.*, 1982).

5. Summary

Our present evidence points to the dGIs and vGIs as playing important but separate roles in initiating locomotory movements. The larger vGIs may provide the primary initiation signal for most escape responses, especially those that occur while the animal is walking slowly. Information

reaches the thoracic ganglia more rapidly via vGIs. However, the vGI system becomes ineffective after a few trials, and the dGIs do not.

The dGIs provide a bifunctional pathway by which wind stimuli can initiate one of two forms of locomotion. In the presence of leg contact, dGIs evoke a running response biased in the proper direction for the escape response. When leg contact is removed, a "switch" directs dGI activity toward the circuitry controlling flight. Thus, the dGIs are capable of initiating at least two totally different behaviors. The result is a very efficient use of a population of interneurons. One would expect to find such multifunctional interneurons in other systems also. Indeed, different kinds of multifunctional interneurons have been located in the cricket (Bentley, 1977) and crayfish (Kramer *et al.*, 1981).

Although the existence of excitatory pathways between GIs and motor neurons has been demonstrated, they appear to be polysynaptic. Does it make sense to use large-diameter axons for rapid escape only to have them connect to motor neurons via one or more interneurons? Perhaps the answer to this lies in the complexity of the cockroach locomotor systems. Unlike systems that do employ monosynaptic connections between GIs and motor neurons (e.g., crayfish and earthworm), the cockroach must coordinate six legs, each of which is composed of five segments. Moreover, the legs of each thoracic segment make movements that are different from the others. A multiplicity of monosynaptic connections between the various GIs and the constellation of motor neurons required for a turning movement may produce an insurmountable problem in coordination. One or more interneurons might be required to collect information from the GIs and direct the motor response. Given this minimal circuit, conduction of the necessary sensory information from the cerci to the motor centers of the thorax as rapidly as possible would still be advantageous. Indeed, even in the crayfish system where giants synapse directly onto fast flexor motor neurons, a parallel polysynaptic pathway now appears to provide most of the excitation (Roberts *et al.*, 1982).

Of course the large axons of the GIs also provide advantages to neurobiologists. The number of large identifiable axons in these two populations is unique. This has allowed detailed experiments to be performed on a variety of neural problems that have been reviewed in this chapter. These include processing of information on wind direction both into and from the various GIs, initiation of motor outputs, switching between different patterned outputs, recovery from sensory ablation, and the relationship of all these factors to a quantified behavior.

Note Added in Proof. Ritzmann and Pollack (in preparation) have recently identified 6 interneurons in the metathoracic ganglion that are monosynaptically excited by GIs. At least one of these can excite leg motor

neurons that are excited by GI stimulation. The identification of these interneurons verifies the existence of a polysynaptic pathway between the GIs and the leg motor neurons. Moreover, they are likely to be involved in deciphering the directional information encoded in the GIs and in controlling motor responses.

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