Independent Coding of Wind Direction in Cockroach Giant Interneurons

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Mizrahi, Adi and Frederic Libersat. Independent coding of wind direction in cockroach giant interneurons. *J. Neurophysiol.* 78: 2655–2661, 1997. In this study we examined the possible role of cell-to-cell interactions in the localization processing of a wind stimulus by the cockroach cercal system. Such sensory processing is performed primarily by pairs of giant interneurons (GIs), a group of highly directional cells. We have studied possible interactions among these GIs by comparing the wind sensitivity of a given GI before and after removing another GI with the use of photoablation. Testing various combinations of GI pairs did not reveal any suprathreshold interactions. This was true for all unilateral GI pairs on the left or right side as well as all the bilateral GI pairs (left and right homologues). Those experiments in which we were able to measure synaptic activity did not reveal subthreshold interactions between the GIs either. We conclude that the GIs code independently for a given wind direction without local GI–GI interactions. We discuss the possible implications of the absence of local interactions on information transfer in the first station of the escape circuit.

INTRODUCTION

One of the major tasks of sensory systems is to localize objects in space. In most sensory systems, this is achieved by comparing the input impinging upon two separate sense organs. For instance, in cockroaches, wind sensitive hairs located on a pair of appendages at the rear end of the animal, called the cerci, detect wind stimuli around the animal. Because cockroaches consistently turn away from a wind stimulus (Camhi 1984, 1988; Ganihar et al. 1994), it is clear that the animal's nervous system can determine the location of the wind stimulus in space.

Lateral inhibition is a widespread phenomena most often encountered in systems that encode location of a stimulus in the sensory space. In the *limulus* eye, the inhibition is mediated through small branches from the eccentric cells axons (Hartline and Ratliff 1957). The inhibition is reciprocal in that an axon that inhibits a neighboring axon is in turn inhibited by it. In cricket central neurons, an inhibitory relationship between acoustic local interneurons and an ascending interneuron may enhance directional sensitivity of this ascending interneuron and ultimately improve directional hearing (Selverston et al. 1985). Given the prevalence of lateral inhibition in localization processes, our goal in the present study was to examine the possibility of reciprocal interactions within a small and well-characterized neuronal assembly, the giant interneurons (GIs) of the cockroach escape network.

The cercal receptors provide sensory input to GIs whose soma are located in the most posterior ganglion of the nerve cord. The GIs are subdivided into two groups, the ventral GIs (vGIs) and the dorsal GIs (dGIs). Each group consists of three pairs of bilateral cells. The vGIs (GI_{1-3}) control the initiation of a highly directional behavior known as escape behavior of the cockroach on the ground (Camhi 1984, 1988; Liebenthal et al. 1994; Ritzmann 1993). Likewise, the dGIs (GIs_{5-7}) initiate directional evasive responses during flight (Libersat 1994a). Another GI, GI_4, shows morphological and physiological properties intermediate between the vGIs and the dGIs (Libersat 1994b) and was not included in the present study. Thus this small cell assembly consists of a total of 12 neurons.

All GIs display a peak wind sensitivity or best excitatory wind direction and both groups of GIs (the ventral and the dorsal) show similar organization of directional coding (Fig. 1*B*) (Kolton and Camhi 1995). For instance, vGI_2 in the ventral group and dGI_5 in the dorsal group respond best to wind from the rear ipsilateral quadrant. Moreover, vGI_3 and dGI_6 respond best to wind from the front ipsilateral quadrant and vGI_1 and dGI_7 to the ipsilateral front and rear quadrants. Finally, left and right homologues show mirror image receptive fields (Fig. 1*C*). Thus the combined activity of specific subpopulations of GIs code for the four quadrants of wind space in the longitudinal (front vs. back) and transverse (left vs. right) directions.

Each wind sensory neuron shows a broad but specific wind receptive field; these fields together code for all directions of the wind space (Westin et al. 1977). Although each GI shows a specific wind receptive field that resulted from excitatory connections of different synaptic strengths as well as inhibitory connections, for most GIs the shape of the wind receptive field cannot be extrapolated on the basis of the known pattern of sensory input (Daley and Camhi 1988; Hamon et al. 1994; Kolton and Camhi 1995). This suggests the possibility of synaptic local interactions among the GIs that could contribute to the shaping of their wind receptive fields. With this in mind, we wanted to determine to what extent the directionality of the GIs is derived from the pattern of sensory input and to what extent from local GI-to-GI interactions.

To analyze the possible role of local interactions between the GIs, we used the photoablation technique developed by Miller and Selverston (1979). This technique, which has also been tested previously on cockroach interneurons (Libersat et al. 1989; Libersat and Mizrahi 1996), consists of filling a neuron with a fluorescent dye that, when excited

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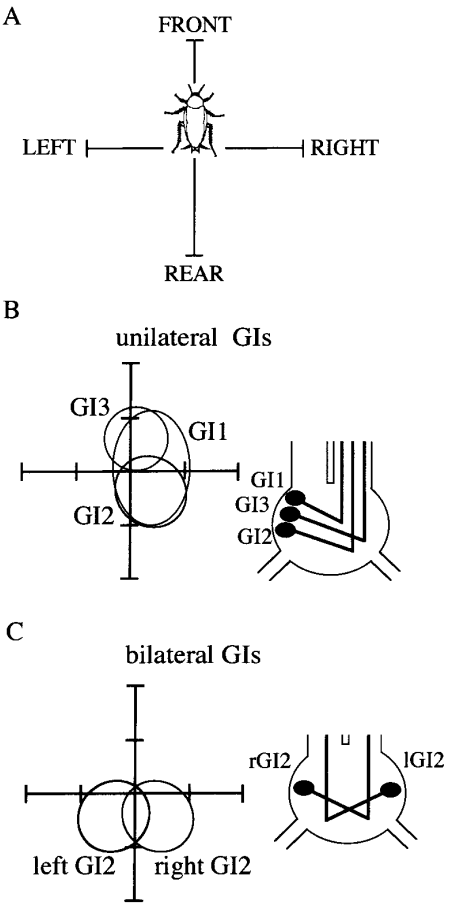

FIG. 1. Organization of giant interneuron (GI) wind receptive fields. *A*: representation of wind stimulus direction relative to the cockroach body. *B*: location of 3 right ventral GIs (vGIs) in the 6th abdominal ganglion (A_6) next to polar plots of GIs' wind directional sensitivity. Responses plotted as average spike count for each GI to a calibrated wind stimulus; tick marks represent 5 action potentials. All 3 right GIs show ipsilateral preference: GI_1 responds to wind from the front and back; GIs_{2-3} respond to wind from back or front, respectively. *C*: location of left and right GI_2s in A_6 next to the polar plots of the GIs' wind directional sensitivity. All bilateral homologue pairs of GIs show mirror image receptive fields. (Adapted with permission from Kolton and Camhi 1995.)

with the appropriate wavelength, causes neuronal death. We measured the wind sensitivity of a GI before, during, and after removing another GI.

A preliminary report of this data was published previously (Mizrahi and Libersat 1995).

METHODS

In all experiments we used adult male cockroaches raised in plastic barrels and kept at 27–32°C. They were provided with water and cat chow ad lib.

The animals were anesthetized with carbon dioxide and pinned dorsal side up on a recording platform; after removing the legs and wings, the nerve cord was exposed as described in Libersat (1992). A single GI was impaled with a glass microelectrode filled with 6% carboxyfluorescein in 0.44 M KCl. Antidromic spikes in the impaled GI were elicited with extracellular hook electrodes placed more anteriorly on the connectives between the fourth abdominal ganglion (A_4) and the third (A_3). The nerve cord was crushed between A_1 and A_2 to remove any descending modulatory input to the GIs from the thoracic or head ganglia. Carboxyfluorescein was injected by using 20 nA of steady hyperpolarizing current. Then, using the same procedure, we impaled another GI with a carboxyfluorescein filled electrode.

We tested the wind-evoked response of the second GI by delivering a wind stimulus produced by a custom-built wind generator (Weisel-Eichler and Libersat 1996). The wind stimulator delivered puffs of fixed velocity of 1.5 or 2 m/s at the cerci every 15 s to avoid habituation. This stimulus is known to activate suprathreshold responses in all the GIs (Westin et al. 1977; Libersat, unpublished observations). This wind stimulus was delivered in the head-to-tail direction relative to the animal's long axis (front wind) and in the tail-to-head direction (back wind; Fig. 1*A*). For the first ten trials the preparation was constantly illuminated with light filtered through a yellow barrier filter (515 nm). After the tenth trial, the first GI was photoablated while we continued to monitor the wind-evoked response of the impaled GI. For the photoablation procedure we removed the yellow barrier filter and inserted a blue excitation filter (470 nm) into the filter slot of a fiber optic system as described in Libersat and Mizrahi (1996). The dye-filled neuron was illuminated with the blue light emitted by the fiber optics, identified in situ, and photoablated. The filled neuron showed the characteristic signs of ablation within ~1–5 min depending on the intensity of the fill (Libersat and Mizrahi 1996). After photoablation we again tested the wind-evoked response of the second GI. At the end of the experiment, we also injected the second GI with carboxyfluorescein. Although we sampled and analyzed the wind-evoked responses of each GI to both front and back winds, we present only the response to wind direction that recruited a GI maximally (e.g., GI_2: back wind; GI_3: front wind; or GI_1: best of front or back wind). In each animal we tested only one pair of GIs.

In all experiments the size of the blue spot was ~1.5 cm and large enough to illuminate the cell body and dendrites in the sixth (last) abdominal ganglion (A_6) and a portion of the axon in the A_6-A_5 connective. Given that the ganglionic sheath is thicker than the connective sheath, we used the following procedure to remove any ambiguity as to whether we successfully ablated the entire cell. We filled a single GI with carboxyfluorescein and covered the connectives with a small piece of black cardboard to prevent the light from reaching the axonal portion of the cell, thereby illuminating only the ganglion. By using this procedure in three different preparations, we could verify that cell death occurred in A_6 on a similar timescale as when illuminating both A_6 and the A_{6-5} connectives.

After each recording the ganglion was removed and observed directly in a solution of 50% glycerol and 50% saline. Both GIs were identified based on their specific morphology within the last abdominal ganglion (Daley et al. 1981) with an Olympus epifluorescence microscope. Experiments in which we could not unequivocally identify both GIs were rejected from our sample.

Recordings of electrical activity were stored on video tape (Data Neurocorder) and digitized at 20 kHz with an A-D board (model No. NB.MIO.16; National Instruments). Data were acquired and analyzed with data acquisition software (Spike Studio, Eli Meir, Cornell University, Ithaca, NY). For the analysis of postsynaptic potentials (PSPs), the intracellular recording was filtered through a 2-kHz low-pass filter (Krohn-Hite 3550 filter). PSP occurrences were sampled using an amplitude window discriminator at 500-ms intervals; only PSPs >2 mV were included in the analysis.

Results are presented as means ± SD and significance tests are a mixed model, two-way analysis of variance (ANOVA) in which the animals are the random factor and the before-after comparison is the fixed factor (GB Stat, Dynamic Microsystems).

RESULTS

In the present report we have examined the connectivity in a population of GIs by testing 54 pairs of GIs. For the

sake of clarity, we use ''bilateral'' when referring to pairs of homologous GIs on opposite sides and ''unilateral'' for pairs of GIs on the same side. An example of one such experiment is shown in Fig. 2. Before the photoablation of left GI_1 (lGI_1), right GI_1 (rGI_1) gave a burst of action potentials in response to the wind stimulus delivered at the cerci (Fig. 2*A*). Then, within a few minutes of blue illumination, we photoablated lGI_1; its death is characterized by a dense, long-lasting burst monitored on the extracellular recording (Fig. 2*B*). It is known that this injury firing is followed by loss of the resting membrane potential and cell death (Libersat and Mizrahi 1996; Miller and Selverston 1979). Then we continued to measure the wind sensitivity of the rGI_1 to wind stimuli (Fig. 2*C*).

The response of a given GI to wind stimuli was rather stable from trial to trial in the course of an experiment (Fig. 3). Nevertheless, we sampled ≥10 responses before photoablating the first GI (Fig. 3*A*). In this example cell death started ~1 min after illumination and lasted for ~30 s, during which we applied five wind stimuli. The GI's responses during the period starting with the onset of blue illumination until the end of cell death were not included in our analysis. After cell death we sampled the response of the second GI_1 in 12 trials.

Most pairs of GIs were sampled in more than one preparation. For example, we studied the left-right GI_1 pair in six

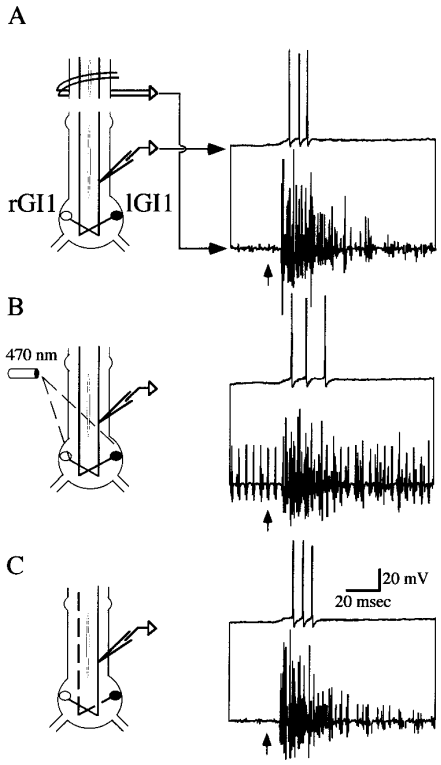

FIG. 2. Sample of recordings at 3 different stages of a typical experiment. *Left*: recording configuration. *Top trace*: intracellular recording from a rGI_1; *bottom trace*: extracellular recording of the GIs. Arrow, timing of the wind stimulus. *A*: after left GI_1 is filled with carboxyfluorescein (filled cell body) the right GI_1 is impaled and its response to a wind stimulus is measured. *B*: photoablation of left GI_1 (with a 470-nm light spot) is monitored on the extracellular recording electrodes as a tonic firing of spikes. *C*: after left GI_1 has been ablated (dashed axon) response of rGI_1 to a wind stimulus is measured again.

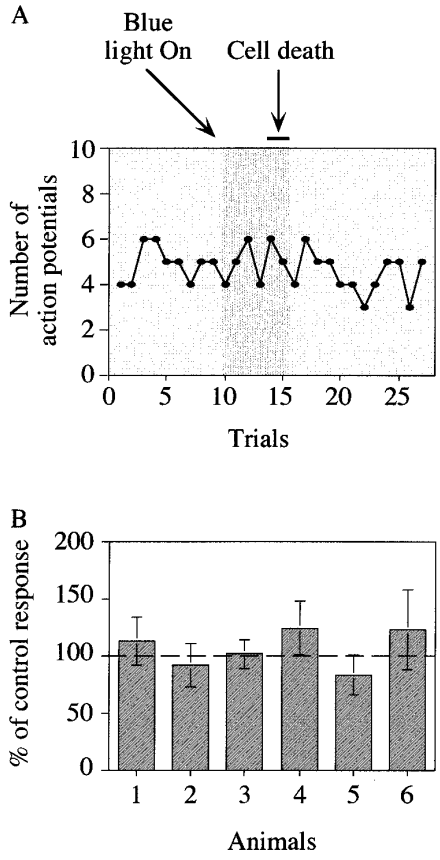

FIG. 3. Wind-elicited responses of GI_1 did not change significantly after removing its homologue. *A*: 1st, wind sensitivity of GI_1 is sampled by measuring the number of action potentials in response to wind stimulus every 15 s for 10 trials. Blue light is turned on while we continue to sample the GI's response every 15 s until cell death is completed. Afterward, GI_1's response is sampled again for 12 trials. Notice the stability of the response from trial to trial. *B*: histogram of change in GI_1's response after killing the contralateral homologue in 6 different preparations. Each data point is the percentage of the control response in a given pair (average of 10 responses after photoablation divided by average of 10 responses before photoablation). Response change showed some variability from preparation to preparation, albeit not significant in any of the preparations [$P > 0.05$, mixed model 2-way analysis of variance (ANOVA)].

different preparations. There was some variability in the change of the wind-evoked responses from preparation to preparation (Fig. 3*B*). Using this procedure, we tested 16 unilateral vGI pairs, 22 bilateral vGI pairs, 7 bilateral dGI pairs (Table 1), and 9 pairs of other combinations and looked for supra- and subthreshold interactions. For the sake

TABLE 1. *Different combinations of giant interneuron (GI) pairs tested*

	vGIs				dGIs		
	GI_1	GI_2	GI_3		GI_5	GI_6	GI_7
GI_1	6	3	3	GI_5	2	2*	2*
GI_2	4	12	2	GI_6	3*	3	1*
GI_3	2	2	4	GI_7	2*	3*	2

n, number of experiments for a given combination. The GIs on the vertical axis are those that were photoablated in a given pair. * Pairs presented in Libersat and Mizrahi (1996).

of clarity, when we present the results on a specific pair of GIs the first GI indicated in the pair is the one that was photoablated. The results on unilateral dGI pairs have already been reported (Libersat and Mizrahi 1996) and were not included in this paper (see Table 1).

Suprathreshold interactions

All GIs carry information about the direction of a wind stimulus to the thoracic centers. There are at least three parameters that could be used by the readout system in the thorax to extract information about the location of the wind stimulus in space (Liebenthal et al. 1994). These parameters are the difference in the following: *1*) the number of action potentials, *2*) the firing latency, and *3*) the temporal pattern of firing in the left- versus the right-biased GIs and front- versus back-biased GIs. We quantified the number of action potentials, the response latency, and, in some cases, the firing rate in the burst.

First, we present results on the bilateral pairs of homologous vGIs. When analyzing the bilateral pairs of homologous vGIs (lGI_1-rGI_1, $n = 6$; lGI_2-rGI_2, $n = 12$; and lGI_3-rGI_3, $n = 4$) we found no significant change (ANOVA, $P > 0.05$) in the number of wind-evoked action potentials or in the latency of responses after removing one GI of a given pair (Fig. 4).

We also investigated unilateral interactions among the three vGIs on a given side. For example, right GI_2 responds best to wind from the right rear ipsilateral quadrant whereas right GI_3 responds best to wind from the right front ipsilateral quadrant (Fig. 1). Thus, for this pair, inhibitory interactions would enhance directionality. Conversely, right GI_3 and right GI_1 wind receptive fields greatly overlap in the front right quadrant of wind space (Fig. 1). Here we could expect excitatory interactions in response to a front wind stimulus.

We analyzed six pairs of vGIs on the same side (GI_1-GI_2, $n = 3$; GI_2-GI_1, $n = 4$; GI_1-GI_3, $n = 3$; GI_3-GI_1, $n = 2$; GI_2-GI_3, $n = 2$; and GI_3-GI_2, $n = 2$). For some of these pairings, the GIs show opposite front versus back best wind directions (Fig. 1*B*). Therefore we analyzed the change in a GI's response to both wind directions. For these unilateral pairs we found no significant change (ANOVA, $P > 0.05$) in either the number of wind-evoked action potentials or the latency of responses after removing one GI of a given pair for either wind direction. Figure 4 summarizes the results for best wind direction only. We carried out the same analysis on the three bilateral pairs of dorsal GIs (GI_5-GI_5, $n = 2$; GI_6-GI_6, $n = 3$; and GI_7-GI_7, $n = 2$). There also we found no significant change (ANOVA, $P > 0.05$) in the number of wind-evoked action potentials or the latency of responses after removing one GI of a given pair (Fig. 4).

Finally, we looked at the firing rate changes in several pairs of GIs (unilateral vGI pairs: GI_1-GI_2, $n = 3$; GI_1-GI_3, $n = 2$; GI_2-GI_3, and $n = 1$; bilateral vGI pairs: lGI_1-rGI_1, $n = 3$; lGI_2-rGI_2, $n = 5$; and lGI_3-rGI_3, $n = 3$; unilateral dGI pairs: lGI_5-rGI_6, $n = 1$; lGI_5-rGI_7, $n = 1$; and lGI_6-rGI_7, $n = 4$; and bilateral dGI pairs: lGI_6- rGI_6, $n = 2$ and lGI_7-rGI_7, $n = 2$). Here as well we found no significant change in the firing rate after removing one of the GIs (ANOVA, $P > 0.05$).

Although we tried to limit our investigation to the testing of unilateral pairs of the same functional group (dGIs or vGIs) or bilateral homologue pairs (dGIs or vGIs), we also sampled unilateral ventral-dorsal combinations (GI_5-GI_1, GI_1-GI_6, GI_6-GI_2, GI_1-GI_7, and GI_7-GI_1; $n = 1$) and a few bilateral combinations (lGI_5-rGI_6, rGI_6-lGI_1, rGI_5-lGI_1, and lGI_7-rGI_6; $n = 1$). Testing these combinations of GI pairs did not reveal any changes in latency or number of action potentials after ablating one of the pair (Student's *t*-test, $P > 0.05$).

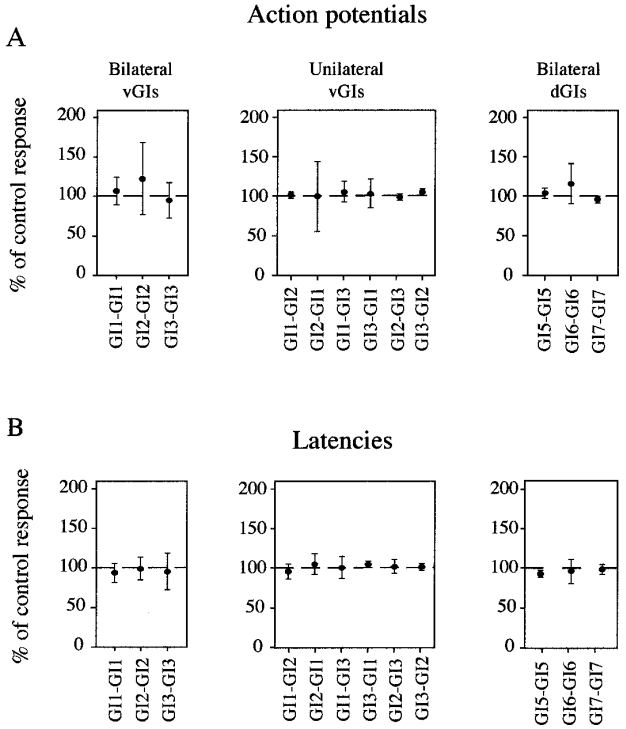

FIG. 4. Number of action potentials and latency of 1st action potential elicited in response to wind stimulus in a given GI before and after photoablating another GI. Response average from 10 trials before photoablation was compared with the response average from ~10 trials after photoablation in each pair tested. Changes are expressed as percentage of the control response. Results from 3 groups of pairs: *1*) bilateral vGI pairs ($n = 22$), *2*) unilateral vGI pairs ($n = 16$), and *3*) bilateral dGI pairs ($n = 7$). Each data point represents mean ± SD (each point is calculated from several animals). *A*: GIs responded with a similar number of action potentials to wind stimulus before and after ablation of another GI ($P > 0.05$, mixed model 2-way ANOVA). *B*: no significant change in latency of response (measured from onset of wind stimulus to 1st action potential in the burst) before and after ablation ($P > 0.05$, mixed model 2-way ANOVA).

Subthreshold interactions

In a number of experiments ($n = 12$) we impaled the GI axon very near to A_6. In this recording configuration we were able to monitor synaptic activity and look for subthreshold interactions.

We wanted to evaluate if a specific GI contributed to the wind-evoked synaptic activity in another GI. To test this, we measured the amplitude of the wind-evoked compound PSP in a given GI before and after photoablating the other GI of a given pair. Although we did not exhaust all possible combinations, we accumulated at least one example in most pairs. Figure 5*A* shows an example of a wind-evoked compound PSP in a lGI_2 before and after ablation of rGI_2. In

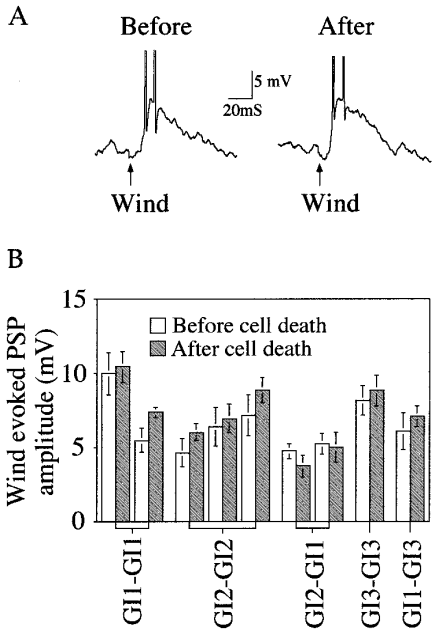

FIG. 5. Amplitude of wind-evoked PSPs in a given GI before and after photoablating another GI. *A*: intracellular trace shows a wind-evoked PSP recorded in lGI_2 before and after ablating rGI_2. *B*: histogram of average amplitude of wind-evoked compound PSPs for different GI combinations before (□) and after (■) photoablation of 1 of a pair. Each pair of bars is data from 1 GI in 1 animal. Data are presented as means ± SD calculated from 10 consecutive wind stimuli. No significant change was detected (ANOVA or Student's *t*-test, $P > 0.05$).

nine vGI pairs (lGI_1-rGI_1, $n = 2$; lGI_2-rGI_2, $n = 3$; lGI_3-rGI_3, $n = 1$; GI_2-GI_1, $n = 2$; and GI_1-GI_3, $n = 1$) we found no change in the amplitude of the compound PSP after photoablation (Fig. 5*B*; ANOVA or Student's *t*-test, $P > 0.05$).

In the absence of any sensory stimuli (''at rest''), the intracellular trace often showed spontaneous PSPs (Fig. 6*A*). We compared the occurrences of the spontaneous PSPs during a 500-ms time window to the occurrences of PSPs recorded during the high firing rate of the dying GI in an equivalent time window (Fig. 6*B*) in three vGI combinations (lGI_1-rGI_1, $n = 2$; lGI_2-rGI_2, $n = 6$; and GI_2-GI_1, $n = 2$). No differences in PSP occurrences were observed in any couple when comparing during firing and at rest (Fig. 6*C*; ANOVA, $P > 0.05$).

DISCUSSION

In the present study we investigated specific aspects of the connectivity in a small assembly of interneurons, the giant interneurons of the cockroach, which are involved in the coding of directional information. To understand the physiological basis for the directional properties of the GIs, it is critical to determine whether these properties are strictly caused by the specific pattern of synaptic inputs or also specific interactions among the GIs. With this in mind we have searched for lateral inhibition and/or excitation among the GIs. To examine the connectivity within the assembly of GI we have measured the wind sensitivity of each GI before and after removing another GI. Our expectations were that *1*) for those GIs that had a large degree of overlap in their receptive fields, such as GI_1 and GI_3, we would observe excitatory interactions and *2*) for those GIs that have antagonist receptive fields in the longitudinal (front vs. back) or transverse (left vs. right) direction, such as left GI_2 versus left GI_3 or left GI_2 versus right GI_2, respectively (Fig. 1) (Kolton and Camhi 1995), we would observe inhibitory interactions. However, our results demonstrated a distinct lack of inhibitory or excitatory connections either between unilateral GI pairs or between bilateral homologous GI pairs (Figs. 3–6).

The anatomic relationships among the GIs in A_6 is consistent with the possibility for such interactions and motivated this investigation. The morphology of each GI in the whole-mount of the ganglion shows that dendritic arborizations appear to overlap considerably for GIs on the same side and, to a lesser extent, for GIs on opposite sides (Daley et al. 1981; unpublished observations). Thus interactions could be mediated via dendro-dendritic synapses.

Alternatively, a local interneuron interposed between two GIs could also provide a communication pathway without the necessity of dendritic overlap. Indeed, in cockroaches and crickets there are quite a few wind-sensitive local interneurons that process the sensory input from the cerci; some of these interneurons are highly directional (Bodnar 1993; Kondoh et al. 1993; Okuma and Kondoh 1996). Possible interactions among local wind sensory interneurons and one ascending GI were investigated in the cricket cercal system, which is in many ways comparable to the cockroach cercal system (Bodnar 1993; Miller et al. 1991). Although photoablating any of three wind-sensitive local interneurons did

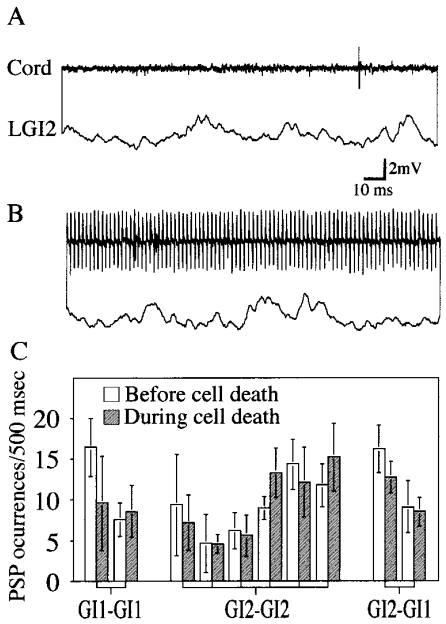

FIG. 6. Number of spontaneous PSPs in a given GI before and during another GI's death. *A, top trace*: extracellular recording of nerve cord showing the spontaneous activities of GIs. *Bottom trace*: intracellular recording showing synaptic activity in a single GI (lGI_2) in the absence of wind stimuli. *B*: same traces as *A*. *Bottom trace*: synaptic activity of lGI_2 during tonic burst of photoablated rGI_2 (recorded on the extracellular trace). *C*: occurrences of PSPs during 500-ms intervals are plotted for different vGI pairs before cell death (□) and during cell death (■). Each pair of bars is data from 1 GI in 1 animal. Data are presented as means ± SD for 5 samples. No significant change was detected (ANOVA, $P > 0.05$).

not change the overall shape of the wind receptive field of this GI, it resulted in a decrease in its wind-evoked response (Bodnar 1993). However, it is not known whether these local interneurons receive input from the GIs in addition to the input they receive from the cercal receptors.

We used the photoablation protocol, although the most popular method to investigate the connectivity in a circuit of neurons is simultaneous penetration of two cells and a stimulation paradigm. One paradigm consists of injecting current pulses in a given cell and looking for synaptic potentials in the other. However, showing that the GIs are synaptically connected by using pair recording does not necessarily prove that this connection is used in the coding of the wind direction. An alternative approach consists of presenting a stimulus that recruits a given cell and measuring changes in its response during hyperpolarization of a second cell. However, while hyperpolarizing the second cell it is difficult to ensure that the hyperpolarization is effective in both preventing the firing of action potentials and/or preventing synaptic activity at electrotonically distant sites on the dendritic arborization.

In the locust, dual penetrations of pairs of GIs were used to search for synaptic connections between them. Testing for connections between bilateral homologues failed to reveal any supra- or subthreshold connections between two cells (Boyan and Ball 1989). Excitatory connections were found in a few cases between ipsilateral GIs but these resulted in only subthreshold postsynaptic responses. These connections were from neurons with cell bodies in the anterior portion of the last abdominal ganglion to neurons with cell bodies located posteriorly. The former correspond to the cockroach's vGIs and the latter to the cockroach's dGIs. We did not look extensively for such connections in the cockroach cercal system because the vGIs and the dGIs represent two different functional groups and our rationale was to look for connections within the same functional assembly. However, in the pairs that we did test ($n = 5$), we could not reveal any suprathreshold connections.

Using our protocol, we looked for both suprathreshold (action potentials) and subthreshold (synaptic potentials) interactions and observed neither. We cannot definitively rule out the possibility of subthreshold interactions as these may occur at some site electrotonically distant from our recording electrode. In addition, subthreshold interactions provided by more than one GI could be relevant to the processing of wind information if they were to summate and generate suprathreshold responses. We have not tested the effect of removing two GIs on the remaining GI. However, the neural code carried by the GIs is brought to a population of thoracic interneurons (TIs) in the thorax (Ritzmann and Pollack 1986), and these TIs almost certainly perform the computation for determining wind direction (Camhi 1988). Given that the TIs are recruited only by action potentials carried by the GIs, subthreshold GI-to-GI interactions that are not translated into a change in either the action potential threshold or in the number of action potentials evoked in a GI cannot participate in the coding for wind direction. We did not notice any subthreshold interactions when either looking for changes in wind-evoked compound PSPs after removing one GI or in synaptic activity in one GI during the intense tonic firing occurring in another GI during photoablation (Figs. 5 and 6). Thus, because of the lack of evidence for subthreshold interactions, summation of PSPs is unlikely to participate in the coding of wind direction.

In our experiments we did not measure a GI's response to different wind directions. However, if there were any excitatory or inhibitory connections in a given pair, our experimental procedure would have revealed them, providing that the particular wind stimulus simultaneously activated both GIs. Because of this we used only straight front and rear winds, each of which are known to activate strongly 8 of the 12 GIs and weakly activate the other four (Kolton and Camhi 1995; Westin et al. 1977). In addition, straight front or rear winds would be most ambiguous in left-right discrimination and if any ipsicontralateral interactions existed, front and rear winds would be most appropriate to reveal them.

Given that the initial escape turn is highly directional, is there any evidence of contralateral interactions occurring at other stations in the escape circuitry? Indeed, it has been shown that there are interactions between neurons whose large axons run through T_3 (Spira et al. 1976; Yarom and Spira 1982). However, such interactions were subthreshold and were revealed with DC injection rather than with natural wind stimuli. Unfortunately, the impaled neurons were, in most cases, not identified; thus the recorded axons could belong to neurons whose cell bodies are located anterior to T_3 with axons descending through the nerve cord. Because the vGIs feed information about the direction of the wind stimulus to the TIs, the next station in the escape circuit, directionality could be enhanced if the activities of left- and right-biased TIs were mutually inhibitory. So far, for those pairs of TIs that have been tested, there is no evidence for mutual inhibition (Ritzmann and Pollack 1986). Whether such interactions occur at other points in the circuit (e.g., at the level of motor outputs) remains to be determined.

The question also arises as to whether there is a functional advantage to enhancing the acuity of the escape network through, for instance, lateral inhibition at the level of the GIs. Providing that one function of sensory processing is to maximize or ''sharpen'' the information transfer to the thoracic centers, lateral inhibition could serve as a significant filter (van Hateren 1992). For example, in the vertebrate olfactory bulb lateral inhibition via reciprocal dendro-dendritic interaction among mitral cells plays an important role in sharpening the tuning specificity and refining the information pathway (Nakanishi 1995). Thus, because lateral inhibition was shown to participate in sensory processing in visual and olfactory systems, one could speculate as to why it is absent in the early processing of wind by the GIs. All sensory systems share the function of encoding and transferring sensory information to higher centers. Yet several differences exist between vision and olfaction where lateral inhibition is a dominant principle and the escape sensory system. The escape behavior is essential for survival and requires fast sensory processing. The neurons involved in this processing are mostly, if not only, dedicated to one behavior (i.e., escape) and encode a relatively simple stimulus. In contrast, visual and olfactory primary sensory interneurons encode complex stimulus configurations and are certainly not dedicated to one behavior. Neural networks in which lateral inhibition is implemented are assumed to have some iteration

process (Majernik and Kral 1993) and thus require a longer processing time, a ''luxury'' the escape sensory system cannot afford. In conclusion, it appears that lateral inhibition may well be an adaptive feature of sensory systems that are mostly not behavior dedicated and for which time is not a critical constraint.

To conclude, information transfer from the sensory neurons to the interneurons about the location of a wind stimulus appears not to involve local interactions among the interneurons themselves but, presumably, requires complex synaptic relationships among the sensory neurons, local interneurons, and the plurisegmental giant interneurons. Thus, at the first station of sensory processing, the information about the direction of the wind stimulus is transmitted to the higher thoracic center without cross talk among the first-order sensory interneurons.

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