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# Analyzing cockroach escape behavior with lesions of individual giant interneurons

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Individual giant interneurons (GIs) in the ventral nerve cord of the cockroach, *Periplaneta americana*, were lesioned by intracellular injection of proteolytic enzymes (pronase). This was accomplished with minimal dissection, so that the wind-evoked escape behavior of the animals could be studied following the lesion. Unilateral lesions of GI-2 had no obvious effect on escape behavior, but unilateral removal of GI-1, as well as combined unilateral lesions of GIs 1 and 2, influenced the direction of an animal's initial turning movement in response to a wind puff. These results support the hypothesis that GIs play a role in initiating and guiding the directional, wind-evoked escape response of the cockroach.

The ventral nerve cord of the cockroach contains a set of individually identifiable giant interneurons (GIs) which are generally believed to mediate wind-evoked escape behavior[2,11]. These GIs provide a rapid conduction pathway which links cercal wind-receptive afferents at the caudal end of the nerve cord with motor cells in the thoracic ganglia that generate leg movements[10,16]. Furthermore, the GIs, as a group, encode information about the direction of wind stimuli[15] which could presumably guide the initial movement of the escape response — a turn directed away from the wind source[3]. While this evidence is strong, it falls short of a direct demonstration of GI participation in cockroach escape behavior.

In order to specifically and directly test the role of the GIs in determining the direction of wind-evoked turning movements, I have adapted the technique of lesioning individual cells by intracellular injection of proteolytic enzymes[6] for use in behavioral studies of GI function. I have been able to produce lesions of individual GIs, either alone or in combinations, and have found that removal of certain GIs can produce specific changes in the directionality of wind-evoked turning movements. The results bear both on the hypothesis that the GIs mediate escape and on models of how particular GIs might be involved in specifying the direction of the escape turning movement.

Late instar cockroach nymphs (*Periplaneta americana*) obtained from commercial suppliers were used in all experiments. They were prepared for intracellular recording using a modification of Westin's procedures[15]. Animals were restrained ventral side up on a platform which was cooled by suspension in a salt-ice bath. Two segments of the abdominal cuticle were partially cut through near the midline and deflected to expose the underlying nerve cord. A portion of abdominal connective between the 3rd and 4th abdominal ganglia was freed of surrounding fat and lifted onto a small wax-covered platform which stabilized the cord for intracellular recording. Care was taken to keep the exposed portion of the cord moist during the entire operation. Glass microelectrodes filled with 0.5% pronase (Calbiochem-Behring) in 50 mM KCl were used to impale the axons of the GIs in the connective. After a cell was identified as a particular GI by testing its pattern of wind responsiveness[15], pronase solution was pressure injected into it. A small quantity of fast green dye in the pronase solution allowed the course of the injection to be monitored visually. In most cases, after several

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pulses of pressure (10–20 psi for 3 s) the dyed pronase solution could be seen in the cord confined within a longitudinal profile, which was presumed to be the axon of one GI. Occasionally injections were not confined to axon-like profiles. Instead, a diffuse halo of pronase solution appeared within the cord at the injection site. Such cases of gross leakage from an injected cell were discarded. In a few instances, however, pronase was intentionally injected extracellularly in the nerve cord to serve as a control for any inadvertent leakage in experimental animals. After completion of an injection the cord was returned to its normal position and a small quantity of PTU/penstrep (a mixture of phenylthiourea and penicillin-streptomycin)[13] was sprinkled over the area of incision. The flaps of cuticle were then replaced and sealed with several drops of dental wax. Animals were invariably up and walking within a few minutes after this surgery was completed.

Behavioral testing began at least 48 h after injection and followed previously described protocols[3]. Briefly, animals were placed in an arena where their behavior in response to standardized wind puffs was recorded on film with a Bolex 16 mm camera at 64 frames/s. Stop-frame analysis of all responses was carried out on a Vanguard motion analyzer to yield information on the angle of the animal with respect to the wind source at the time of stimulation, as well as the direction and angle of the initial turning response[4]. Tests were conducted for up to 7 days following injections, after which the nerve cord was extracted for histological study. The entire abdominal cord was fixed in Carnoy's solution, embedded in paraffin, cut in serial cross sections at 12 $\mu$m and then stained with Eosin. Sections were examined with a light microscope and photographed to verify the absence of injected axons.

While specific lesions of GIs have not been made before, earlier experiments in which the wind sensory inputs to the cockroach nerve cord were manipulated provide some background for interpreting lesions of GIs. When the responsiveness of wind-sensory cells on one side of the nerve cord was reduced by gluing down the cercal filiform hairs on that side, animals frequently responded inappropriately to wind puffs directed at them from the side of the 'silenced' cells, turning toward rather than away from the wind source. However, they continued to turn away from winds directed at them from the 'intact' side[3]. Thus the predominant response of experimental animals was to turn away from the side of the nerve cord with the more active wind-sensory cells, suggesting that a comparison of wind-evoked activity on the two sides of the nerve cord could explain an animal's ability to turn appropriately to the right or left depending upon the laterality of a wind source. Such a comparison could be performed by the GIs, but a contribution by non-giant wind interneurons of the ventral nerve cord[15] cannot be excluded on the basis of this experiment.

All of the results reported here concern GIs No. 1 and 2. As seen in Fig. 1, these two GIs lie adjacent to each other in the abdominal nerve cord, and they have different directional selectivities for wind. Their close anatomical association makes them ideal for testing the specificity of the pronase lesioning technique in the cockroach. Furthermore, their differing directional selectivities suggest that different behav-

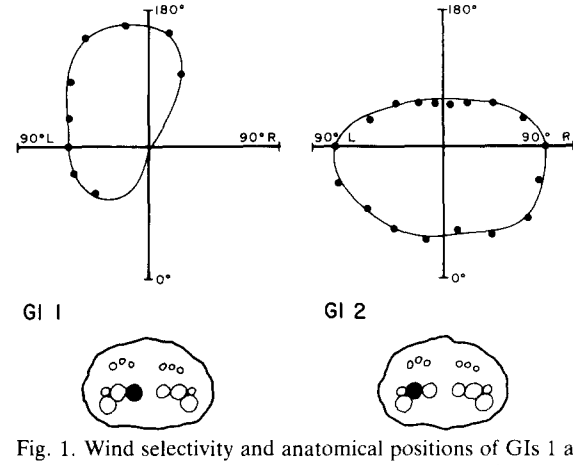


Fig. 1. Wind selectivity and anatomical positions of GIs 1 and 2. Semi-schematic polar plots at the top are derived from physiological experiments in which GI axons were impaled in the abdominal connectives and standardized wind puffs were directed at the animal from various angles in the horizontal plane (e.g. ref. 16). To understand the plots, visualize the animal positioned at the center of the coordinate system; 0° is directly behind the animal, 180° is directly in front, 90° L (left) and R (right) are self-explanatory. Each dot represents the mean number of action potentials evoked by wind puffs from that direction (the range of the mean values in this experiment was from 2 to 5 action potentials, n = 3 trials per point). The closed figures then are 'directional selectivity curves' for each of these two GIs. (The curve for GI-1 Right would be a mirror image to the curve for GI-1 Left which is shown here.) Schematic cross-sections of the ventral nerve cord are shown below. On each side there is a subgroup of 3 dorsal GIs and another group of 4 larger ventral GIs. GIs 1 and 2 on the left side have been darkened to show their positions. The other GIs, Nos. 3–7, are not individually identified in this drawing (see Westin et al.[15]).

ioral outcomes would be expected following unilateral lesions of one or the other of these cells. GI-1 responds differentially to winds depending on whether they are to the right or left of the animal's midline. A comparison of its wind-evoked activity with that of the contralateral GI-1, or perhaps other GIs, could provide an indication of the appropriate direction for an escape turn. GI-2 is basically omnidirectional and thus should be of little use in determining the laterality of a wind stimulus. (Of the large, ventrally situated GIs, only GI-1 responds differentially to winds based upon their laterality — see discussion below.) Therefore only GI-1 lesions might be expected to have a significant influence on the direction of escape turning.

Fig. 2 summarizes the anatomical effects of injecting GIs 1 and/or 2 with pronase. In all cases, the axon of the injected cell was absent from the nerve cord when it was examined 7 days after the injection. This was true not only at the level where the injection was made but throughout the entire length of the abdominal nerve cord. Occasionally, the degenerating axon of GIs 1 or 2 could be seen at one of the extreme ends of the abdominal cord. While no attempt has yet been made to determine if the cell bodies of injected GIs (in the terminal abdominal ganglion) or their rostral extensions in the thoracic cord are also destroyed, clearly the elimination of ascending wind-sensory information in treated GIs was achieved. When one axon was injected, only that specific cell was killed (Fig. 2A, B). By impaling and injecting two or more axons, multiple cell kills were accomplished (Fig. 2C). Nerve cords were in some cases examined histologically as early as 48 h after injection, with results comparable to those seen at 7 days. Behavioral testing was in all cases begun at least 48 h after injection.

Wind puffs directed at normal animals from frontal locations usually cause large obvious turns to the right or the left. The direction of the turn depends upon the side of the wind source[3]. For frontal winds, defined here as any puffs originating from in front of an animal and within 45 degrees of its midline, normal animals (n = 6) usually turned *away* from the wind source. Pooling their responses to both left and right frontal winds produced a level of only 9.1% incorrect turns (turns *toward* the wind source — dotted line in Fig. 3).

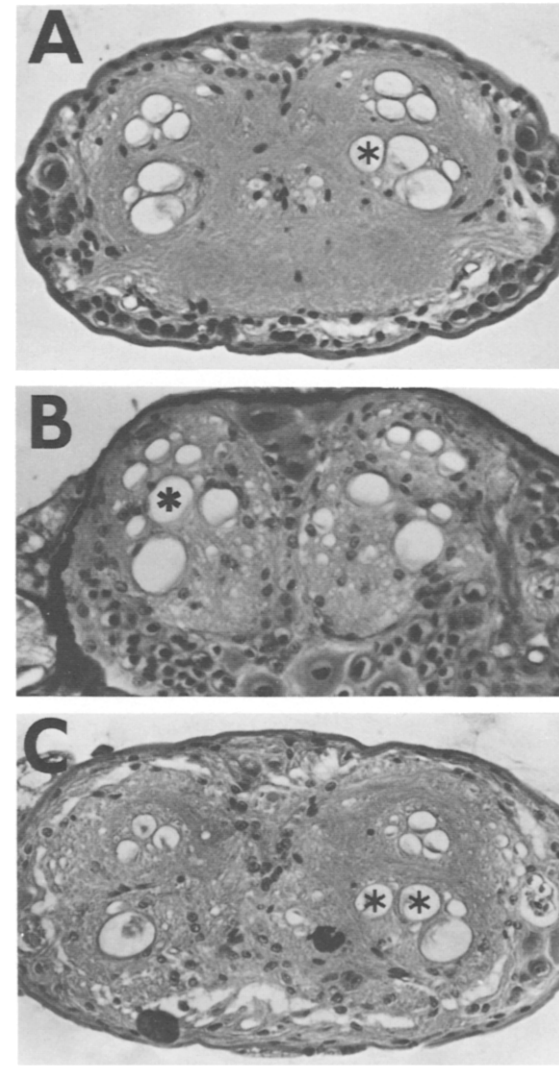


Fig. 2. Histological confirmation of GI lesions in 3 representative animals. In each case the GI which was injected has been marked with a star on the intact side, so that its absence can be seen clearly on the experimental side. Tissue was extracted and fixed 7 days after injection as described in the text. A: a cross-section through the middle of the 5th abdominal ganglion after injection of GI-1 (left) with pronase. B: a cross-section through the caudal end of the 4th abdominal ganglion following injection of GI-2 (right). C: a section through the middle of the 3rd abdominal ganglion following injection of GIs 1 and 2 (left). In all animals the site of injection was the connective between the 3rd and 4th abdominal ganglia.

When GI-2 was removed unilaterally (3 animals) there was no significant elevation in the percentage of incorrect turns compared with normal animals nor any significant difference in this percentage on the side of the lesion versus the intact side. However, when GI-1 was removed unilaterally (5 animals),

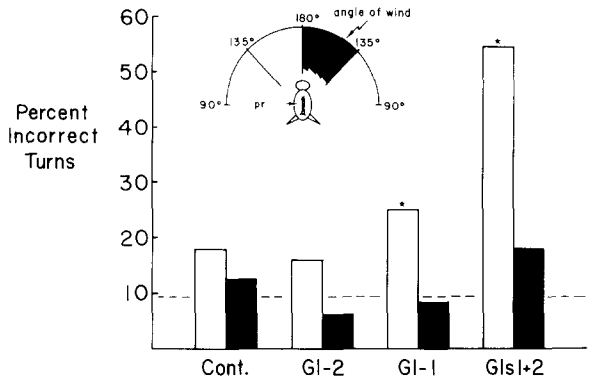


Fig. 3. Summary of the influence of the GI lesions on turning behavior in response to frontal winds. Inset at top shows that only responses to wind puffs from the frontal 45° on each side are being considered. In each experimental group most of the pronase injections (pr.) were made on the left side of the nerve cord. In histograms below responses elicited by winds from the side ipsilateral to the injection are represented on the left (open bars), while responses elicited by contralateral winds are represented on the right side (dark bars). Experimental groups and their percentages of incorrect turns (ITs) are as follows: Cont. = 3 control animals; 18% ITs on the left, 13% ITs on the right (total n = 69). GI-2 = 3 animals; 16% ITs on the left, 6% ITs on the right (total n = 43). GI-1 = 5 animals; 25% ITs on the left, 8% ITs on the right (total n = 92). GIs 1 and 2 = 3 animals; 52% ITs on the left, 19% ITs on the right (total n = 48). Dotted line = overall level of ITs seen in 6 normal animals in response to frontal winds (9.1%). Stars indicate a statistically significant elevation of percent incorrect turns over that seen in normal animals, as well as a significant difference in ITs on the sides ipsilateral and contralateral to the lesions as described in the text.

there was a significant increase in the percentage of incorrect turns only in response to wind puffs from the side of the lesion ($P < 0.05$, $2 \times 2$ $\chi^2$ test). Interestingly, when GIs 1 and 2 were both removed on the same side (3 animals), there was a much larger increase in the percentage of incorrect turns on the side of the lesion (significant at the $P < 0.01$ level, $\chi^2$). In both groups which received lesions involving GI-1, the level of incorrect turns elicited by wind stimuli on the side opposite the lesions was not significantly higher than that seen in normal animals.

In two cases pronase was injected extracellularly on one side of the nerve cord and in one case a non-giant wind interneuron was impaled and injected on one side. The results from these 3 animals are pooled in Fig. 3 as 'controls'. There was no significant difference in the percentage of incorrect turns on the side of the injection versus the intact side in this group.

The lesioning technique used in this experiment appears to be anatomically specific since one GI axon from a set of closely situated GIs was effectively deleted without obviously damaging the other axons. Single cell selectivity of pronase killing comparable to that seen here has also been achieved in leech[1,5,14] and lobster[7,8]. The general approach of lesioning one cell and then monitoring the behavior of the whole animal has recently been used in the analysis of feeding behavior in the marine mollusc *Aplysia*[12]. The success of pronase lesioning in the cockroach, and the fact that it can be carried out so that the behavior of the whole animal can subsequently be examined, should make this technique powerful for analyzing the behavioral functions of neuronal systems in insects and perhaps some other arthropods.

The present results show that lesions which are restricted to the GIs can produce changes in the direction of wind-evoked escape turning in the cockroach. They thus provide direct evidence linking GI function with the control of this well-characterized behavior. Lesion effects were specific in all animals in that large elevations in the percentage of incorrect turns were seen only in response to wind puffs delivered from the side ipsilateral to the lesioned GIs.

The present results also bear on models of how the GIs may control the orientation of wind-evoked escape turns in *P. americana*. The observation that lesions of GI-1 influenced an animal's choice of turn direction for frontal winds, whereas lesions of GI-2 did not, can be understood in terms of the different degree of selectivity these cells show for the direction of wind stimuli. GI-1 is the only member of the subgroup of large, ventrally located GIs (Fig. 1, and see Westin et al.[15]) which responds differentially to winds based upon their laterality. A comparison of activity in GI-1 Left versus GI-1 Right could be the basis for determining the initial direction of turning movements. However, the observation that removal of GI-2 in combination with GI-1 produces a much greater lesion effect than removal of GI-1 alone, raises the possibility that GI-2 might play some role in specifying the laterality of wind stimuli. For example, a comparison of activity in GI-2 with either the ipsilateral or contralateral GI-1 could yield information on the location of a wind stimulus. Clearly, there are a variety of different GI–GI comparisons that might be used to determine the location of a stimulus. In fact, if wind location were specified not by one particular comparison, but by a set of GI–GI compari-

sons, this could provide an explanation for the apparent synergy between the effects of GI-1 and GI-2 lesions. It is interesting to note that in physiological experiments where GIs are stimulated electrically to drive leg motor neurons some evidence of a synergism between GIs 1 and 2 has been described[9]. GI-1 is the only ventral GI that strongly activates the leg motor neurons in these experiments. While GI-2 does not drive leg motor neurons by itself, concomitant stimulation of GI-2 enhances the ability of GI-1 to activate leg motor neurons. In order to determine if there is a specific synergy between GIs 1 and 2 in specifying the direction of escape turns, it will be necessary to examine the behavior of animals with lesions of other GIs, and especially animals with other combinations of GIs killed.

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