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Escape responses following elimination of the giant interneuron pathway in the cockroach, *Periplaneta americana*

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The entire set of giant interneurons (GIs) in the nerve cord of the cockroach, *Periplaneta americana*, was ablated using either electrolytic or surgical techniques. All animals with these lesions were capable of turning and running away from standard wind puffs. However, all animals responded much less frequently to standard wind stimuli following lesion, and the latency of their responses was significantly increased. These results are discussed i* :erms of a GI role in extremely short latency escape responses, and the idea that non-GI pathways, perhaps associated with head sensory structures, need to be considered in the normal control of escape in the cockroach.

Many animals possess specialized neurons with large caliber axons whose function is related to the production of short latency startle or escape responses. Although the evidence for 'giant fiber' mediation of escape is quite compelling[2], it has become clear that there are 'non-giant' systems which may also initiate escape responses. These responses are typically initiated at a longer latency than escapes involving giant fiber systems. Wine and Krasne[22] described the organization of non-giant mediated tail flips in the crayfish and, based on evidence available at the time, speculated that non-giant systems would commonly be found in parallel with giant ones. Since then, similar responses also have been reported in teleost fish where 'non-Mauthner' pathways have been shown to be capable of sustaining the tail flip at the initiation of escape[11]. However, it is not yet clear just how widespread this phenomenon of parallel escape pathways may be.

The cockroach, *Periplaneta americana*, possesses a group of 7 bilaterally paired interneurons with large caliber axons (so-called 'giant interneurons' or GIs) traversing the ventral nerve cord. They receive wind-sensory input from receptors located on the cerci (at the rear of the abdomen — Fig. 1), and they can acti-

vate leg motor neurons in the thoracic ganglia (for recent reviews see refs. 4 and 15). In Roeder's classic work on this system[16,17], a role for the GIs in the mediation of escape was inferred from the good correlation between the properties of these cells (e.g. high conduction velocity) and the nature of escape behavior (response latencies reported in the range of 28 to 90 ms). Additional support for GI control of escape came from studies showing that the response is directional (beginning with a turn directed away from the source of wind), and that the GIs as a group encode directional information about wind stimuli[5,20].

Recent evidence has established more directly that the GIs play a role in escape: in both physiological[21] and behavioral[6] studies, discrete lesions of individual GIs have been shown to alter the escape motor output. This information, along with previous observations cited above, provides strong support for the hypothesis of GI control of escape. However, the evidence does not justify the conclusion that the GI pathway is the only interneuronal pathway capable of linking wind-sensory inputs with escape motor outputs.

If the cockroach, like the other animals mentioned above, has interneuronal systems which can operate

parallel to, or in place of, large caliber systems for the control of escape, then there are two general ways in which they might be organized. Cercal-derived, wind-sensory information might access turning motor circuitry in the thorax by way of ascending non-giant pathways. Alternatively or additionally, non-cercal pathways might access these motor circuits, perhaps descending to thoracic levels from rostral sensory structures. In order to make a direct test of this second possibility, we studied the behavior of a group of animals in which the nerve cord was completely transected or burned electrolytically at the abdominal level.

All animals which received lesions completely ablating the GI pathway in the abdominal nerve cord displayed a greatly lowered responsiveness to wind and an increased latency of response, but wind-triggered turning (and running) was never abolished. In this report we provide an initial characterization of wind-triggered turning in cockroaches lacking a functional GI pathway. The results establish that turning can be supported by a 'non-GI' pathway. However, they also suggest that the GI pathway may be essential for the generation of extremely short latency evasive turning responses. This work has appeared in abstract form[7].

Animals used in these studies were adult male *Periplaneta americana*. They were obtained from commercial suppliers or from our own laboratory stocks, and they were housed in plastic cages measuring 25 × 19 × 15 cm. Food and water were provided ad libitum.

Lesions were performed in a manner similar to previous studies in our laboratory[8]. Briefly, animals were first anesthetized with CO_2 and then restrained ventral side up on an ice-chilled platform. A flap of cuticle was opened along the midline of the abdomen to expose the nerve cord between either the 3rd and 4th or 5th and 6th (terminal) abdominal ganglia. No differences were noted in the behavior of animals as a function of which site was lesioned.

Two different methods were used to produce lesions of the GI pathway. In 7 animals the nerve cord was completely transected surgically. In these instances a small length of both interganglionic connectives was removed with iridectomy scissors. In one additional animal, the cord was burned with a microcautery probe. The probe was fabricated in our laboratory: it had a 5 mm length of bare platinum wire exposed at its tip. Current was passed through the tip by connecting contacts on the probe to the output of a variable AC transformer. Both the left and right interganglionic connectives were lifted just enough to allow the tip of the microcautery probe to be placed under them, with the platinum filament against the dorsal surface of the cord. The lesion was produced by passing sufficient current for several seconds to heat the filament just below the point at which it would glow. This did not sever the cord. Following either type of lesion the cord was returned to its normal position, and the cuticle was resealed with a few drops of warmed dental wax. Animals were usually active 10–15 min after the completion of surgery. Wind-elicited responses were studied from 5 h to 2 weeks postlesion and compared with behavioral responses observed prior to lesion.

High-speed videography was used to record responses of the cockroaches to standardized puffs of wind. Our system for generating wind puffs and videotaping behavioral responses has been described in detail elsewhere[8], so only a few salient features will be mentioned here. Cockroaches were studied individually in an arena with a video camera mounted above. When an animal was standing still, a wind puff with a peak velocity of approximately 1.8 m/s was delivered through a tube projecting into the arena. The puff was directed toward the animal's cerci, but the aperture of the wind tube was large enough (4 cm) that the wind stream enveloped the entire body. When a wind puff was triggered, a red LED visible to the camera (but out of the animal's view) was simultaneously activated. The number of 'frames' between LED onset and the animal's first movement was used as a measure of latency. The imaging rate of our video system during these studies was set at 60 pictures/s. From frame-by-frame playback of the video tapes, we reconstructed the angle of the wind stimulus with respect to the animal, the angle of his initial turning movement, and the latency of the response in frames.

At the completion of all postlesion testing, animals were sacrificed, and the abdomen was dissected to establish the condition of the nerve cord. In some cases histological sections of the cord were examined by light microscopy and photographed.

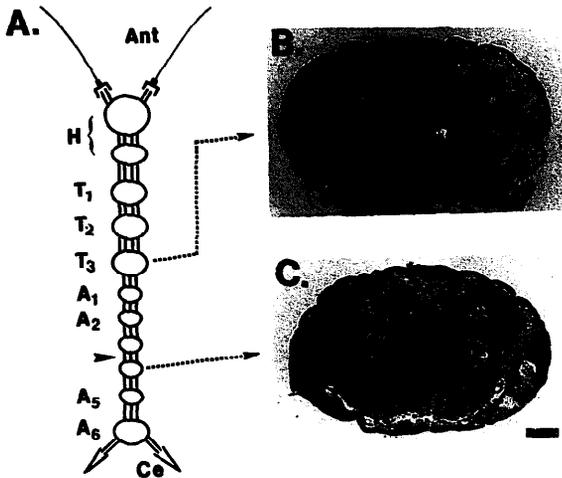All 8 cockroaches with abdominal cord lesions

Fig. 1. The CNS of the cockroach and an example of a complete lesion of the GI pathway. A: schematic drawing of the CNS as seen from the dorsal view. Rostral is upward on the page. A_n, abdominal ganglia; Ant, antennae; Ce, cerci; H, head ganglia; T_n, thoracic ganglia. Lesions were usually made in the A_3–A_4 interganglionic connective (arrow), although a few were made between A_5 and A_6 (see text). B,C: histological cross-sections (stained with eosin) from an animal receiving an electrocautery lesion. The sections are through the specific ganglia indicated. Arrows in B give positions in T_3 where the axons of GIs in the dorsal and ventral subgroups would normally be located. Bar = 100 μm.

were found to be capable of turning and running in response to standard wind stimuli following the lesion, although they responded much less frequently than before lesion. Each of the 7 animals in which cord transection was attempted had a complete severance of the nerve cord (with no evidence of reconnection) when examined after the completion of behavioral testing. Results of the electrocautery lesion of the nerve cord are shown in Fig. 1. As the histological sections reveal, rostral to the site of lesion the axons of the GIs were not discernible in the cord, but there was a dark staining material characteristic of GI degeneration[10,18] (Fig. 1B). Caudal to the site of lesion, however, the GI axons were still present (Fig. 1C). (These are the segments of the axons proximal to the GI cell bodies in the 6th abdominal ganglion.)

Fig. 2 summarizes the effect of transection of the abdominal cord on wind-elicited turning behavior. All 7 animals in this group were similar to each other in terms of responsiveness to wind and the directionality of their turning (see below) and so their data have been pooled. The histograms at the bottom of Fig. 2 indicate that before lesion the animals were
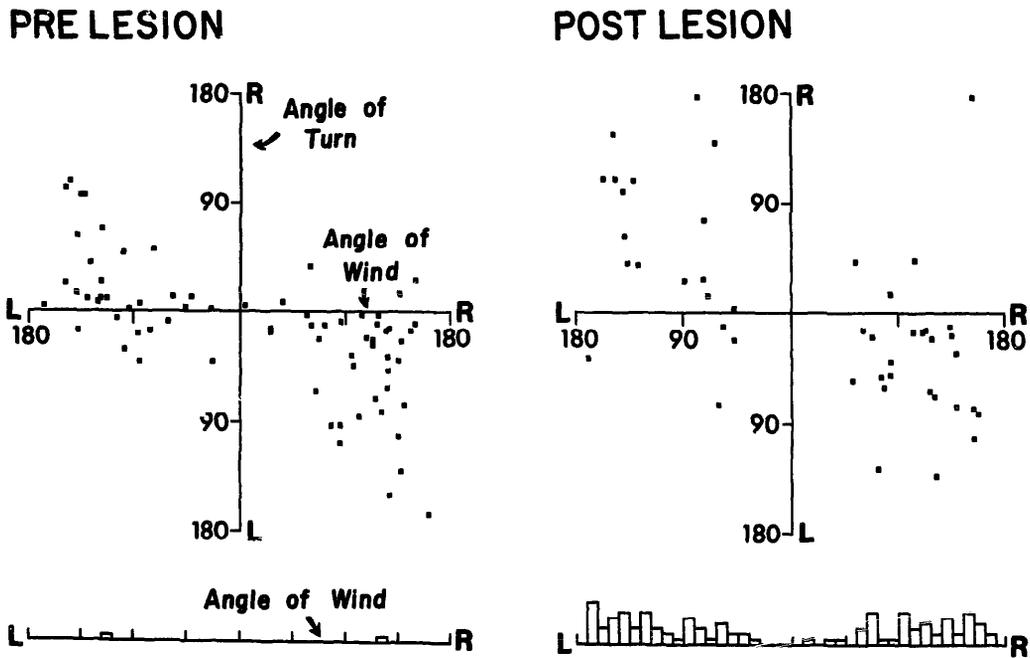
PRE LESION

POST LESION

Fig. 2. Wind-elicited turning responses of cockroaches both before (left) and after (right) completely severing the abdominal nerve cord. Each plot represents the pooled responses of 7 animals. Angle of turn is plotted versus angle of wind stimulus as indicated on the left. Wind from the front = 180°, wind from the rear = 0°. L and R, wind puffs from the left and right side of the animal (abscissa), or turns to the left and right (ordinate). Histogram at bottom shows the distribution of trials on which no response to wind was observed.

highly responsive to wind (97.3% response rate, $n =$ 74 trials), but after lesion their responsiveness was greatly reduced (30.4% response rate, $n = 148$ trials). (The responsiveness of the animal with the electrocautery lesion was also reduced; it responded on 44% of all trials ($n = 34$).) In previous work involving gross lesions of the ventral nerve cord[8], animals receiving sham lesions did not display a lowered responsiveness to wind, or alterations in response latency (see below).

As can be seen from the scatter plots in Fig. 2, turns were usually directed away from the wind source following lesion, just as they had been prior to lesion. The overall percentage of turns which were contraversive, i.e. directed away from the side of the wind, was 81.9% before lesion and 82.2% after lesion. Both of these figures compare quite closely to the percentages reported for normal animals in previous studies[8].

This similarity in the percent of contraversive turns is not sufficient, however, to argue that the directionality of lesioned animals is really comparable to intact animals. As has been noted in previous work[5], and recently examined in some detail[8], the size of a turn is normally graded as a function of the angle of the wind stimulus. Winds from the rear cause cockroaches to make small angle turns, whereas winds directed at them from increasingly frontal angles lead to increasingly large angles of turn. This gradation in the size of turns can easily be seen by inspecting the plot of prelesion behavior in Fig. 2. The postlesion behavior displayed in the same figure shows much less evidence of a systematic grading of turn amplitude as a function of wind angle. Thus the directionality of turning triggered by non-GI pathways may be different in this regard from GI-mediated turns. This point will need to be investigated with a larger sample of turning responses.

An additional difference between pre- and postlesion behavior which was particularly striking was response latency. The average latency in frames before lesion was 3.2 which, given our video sampling rate, produces an estimate of actual behavioral latency of 53 ms. This figure is in close accord with previously published estimates of latency for wind-triggered escape in the cockroach[5,16]. Following lesion, the latency of the animals was 8.7 frames on average, for an estimate of actual behavioral latency equalling 144
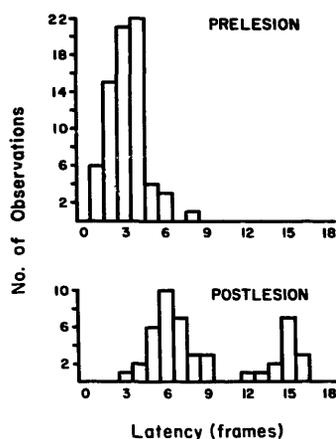

Fig. 3. The distribution of response latencies both before (top) and after (bottom) completely severing the abdominal cord. For each histogram, the responses of 7 animals were pooled. Latency is measured as the number of video frames between opening of the shutter on the wind machine and the first detectable movement made by the animal. The time between frames was 16.6 ms.

ms. This difference between pre- and postlesion latencies is highly significant ($P < 0.001$, Mann–Whitney U-test).

Histograms displaying the observed latencies both before and after lesion are presented in Fig. 3. As will be noted, the distribution of latencies following lesion was distinctly bimodal. The mean of the 'early' group (from 3–10 frames) was 6.3 (= 104 ms), while the mean for the 'late' group (12–16 frames) was 14.7 (= 244 ms). Since there was considerable overlap between prelesion latencies and the 'early' postlesion latency group, the difference between these was tested separately. With all very long latency responses (>9 frames) deleted, there was still a significant difference between the latency of responses observed before and after lesion ($P < 0.001$). The fact that the latency of postlesion responses was so clearly bimodal suggests that there may be two very different pathways controlling the turning behavior in lesioned animals. A similar idea, that there may be multiple circuits controlling the latency of cockroach escape, has recently been noted in studies where GI function was apparently altered by drugs that interfere with cholinergic neurotransmission[12].

These results indicate that *P. americana* is capable of generating wind-triggered evasive turns following complete elimination of the GI pathway. While re-

sponses in our lesioned animals were clearly initiated at a longer average latency, it should be noted that the turning movements themselves were not obviously deficient. Frame-by-frame analysis of turns before and after lesion revealed no indication of decreased motor performance. Indeed, inspection of Fig. 2 will reveal that following lesion, animals generated many turns of considerable angular amplitude.

Given the locus of our lesions, it is clear that the source of sensory input controlling the responses observed here is not the cerci. In one previous study on this system, it has been noted that some locomotor responses to wind survive complete removal of the cerci in nymphal cockroaches[19]. An explicit test for the possibility of non-GI mediated evasive turning in the cockroach has not been attempted before. Our observations establish that wind-triggered turning and running, comparable in form to that by which the cockroach escapes from natural predators[4], persists following complete lesion of the cercal-to-GI pathway at the abdominal level.

We suspected that the postlesion behavior of animals in the present study was due to a descending sensory pathway originating from rostral sensory structures. Since the antennae have been implicated in the control of other types of wind-mediated behavior in P. americana such as anemotactic orientation[1], 3 of the transected cockroaches subsequently had their antennae removed bilaterally. This was done by cutting them off at the level of the pedicel, thereby removing all of the flagellar (distal) segments and leaving only the scape (articulating segment) intact. The responsiveness to wind in this group was further reduced: they responded on only 4.2% of all trials (n = 48). This reduction did not reflect either an inability to generate turning and running responses, or a generalized lowering of responsiveness to sensory input, since all 3 readily responded to tactile stimulation of the pronotum (a gentle tap with an applicator stick) by turning contraversively and running in a manner similar to wind-triggered escape.

The results of antennal removal suggest that receptors on, or associated with, the antennae may be one source of afferent input supporting wind responsiveness in animals without a functional GI pathway. While it is possible that such pathways normally are not present or organized so as to generate turning movements, but rather depend upon a lesion-in-

duced reorganization of the CNS, we feel that this is unlikely for two reasons. First, wind-evoked turning and running was observed at the earliest postlesion times tested in our experimental animals (5 h). Second, electrophysiological studies support the existence of pathways originating in head structures that descend to thoracic levels and can be activated by wind and tactile stimuli[3]. Finally, it is important to note that in our lesioned animals wind-sensory information is not carried to motor cells by any surviving GI axon segments distal to the site of lesion, as these segments were no longer present (Fig. 1B).

Given the longer than normal average latency of the turning responses in lesioned animals and the greatly reduced response rate, it is important to ask if a non-GI pathway such as demonstrated here could contribute to escape in intact animals. That is, there is clearly a substrate for wind-triggered behavior which can parallel the function of the GI pathway, but does it actually operate in parallel with the GIs in an intact animal? It is worth noting that the lesions made here were not selective for the GI pathway. Any generalized effects on the CNS of cutting the abdominal cord might contribute to reducing responsiveness and lengthening the latency of behavioral responses. Thus the level of response and the latency of non-GI turning observed here may underestimate the responsiveness and overestimate the true latency of these pathways. Furthermore, the earliest latencies of turning observed in our lesioned animals are well within the range seen in these animals before lesion. Therefore a non-GI contribution to escape in intact cockroaches cannot be completely excluded on the basis of time considerations. To understand the importance of non-GI pathways to escape it will be necessary to identify the relevant interneurons and assess their relationship to the GIs and to motor cells in intact animals. For example, non-GI pathways normally might be inhibited by the GIs or they may exert less direct control over motor cells than the GIs, perhaps acting primarily as modulatory pathways for escape behavior.

The existence of non-GI evasive turning clearly has an operational significance for future research on this system. A contribution of non-GI pathways to escape could make it difficult to detect the effects of partial lesions of the GI system, and this may be especially true in the case of discrete single-cell lesions[6].

Thus it will be important to identify sensory receptors for non-GI pathways, so that their activity can be controlled during experiments involving the GIs.

In other systems where 'non-giant' escape behavior has been studied the general picture seems to be that giants are essential for production of the very shortest latency responses[13]. Alternative pathways can 'functionally substitute' for the giants and produce similar responses, but at somewhat longer latencies. There seem, additionally, to be differences between the two types of interneuronal pathways in terms of the types of sensory stimuli to which they are maximally sensitive or the performance characteristics of the motor outputs that they generate (for the

crayfish see ref. 14, for teleost fish see refs. 9 and 11). These initial studies suggest that the cockroach escape system also may conform to this general scheme. The exact source of descending sensory information relating to escape behavior, and its relationship to the GI pathway under normal circumstances are currently under investigation in our laboratory.

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