

## SHORT COMMUNICATION

### THE DORSAL GIANT INTERNEURONS MEDIATE EVASIVE BEHAVIOR IN FLYING COCKROACHES

FREDERIC LIBERSAT

*Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel*

*Accepted 22 July 1994*

Cockroaches escape from terrestrial predators by making an evasive turn and running away (Camhi, 1984). The air displacement produced by the predator's strike is sensed by wind-sensitive receptors on the cerci, two posterior antenna-like organs. These receptors excite two distinct populations of giant interneurons, the ventral giant interneurons (vGIs) and the dorsal giant interneurons (dGIs), in the last abdominal ganglion of the nerve cord (Camhi, 1984). The vGIs are known to control the direction of the escape turn when the animal is on the ground (Camhi, 1988; Ritzmann, 1993; Comer and Dowd, 1993; Liebenthal *et al.* 1994), while the dGIs are known to initiate and maintain flight (Ritzmann *et al.* 1982; Libersat *et al.* 1989; Libersat, 1992). Since cockroaches are not equipped with an ultrasonic hearing sense (Yager and Scaffidi, 1993) and consequently do not respond to ultrasound with escape maneuvers, as many other insects do (Hoy *et al.* 1989; Libersat and Hoy, 1991), it is reasonable to envisage that, while flying, cockroaches may recruit their wind escape circuit to evade aerial predators such as bats. Indeed, recently Ganihar *et al.* (1994) have shown that flying cockroaches produce various flight maneuvers that should cause an evasive turn away from a wind stimulus. Such flight maneuvers are not produced after cercal ablation. The most likely candidates to mediate these evasive flying maneuvers are the dGIs, because the wind sensitivity of the vGIs is greatly reduced during flight (Libersat *et al.* 1989; Libersat, 1992). In contrast, the dGIs retain their wind sensitivity during flight (Libersat, 1992) and also they respond in a directionally sensitive manner to a wind puff delivered from the side (Ganihar *et al.* 1994). In this study, the possibility that the dGIs mediate evasive flying maneuvers was investigated by stimulating individual identified dGIs during flight and measuring the asymmetrical responses in a pair of left and right flight depressor muscles, the subalaris of the metathoracic wings.

Adult cockroaches *Periplaneta americana* were anesthetized with carbon dioxide and pinned ventral side up on a recording platform after the legs and wings had been ablated. The ventral nerve cord was exposed and placed on a small platform coated with vinyl wax, for intracellular penetration, as described earlier (Libersat, 1992). Single dGIs were impaled in the abdominal nerve cord with glass microelectrodes filled with 6% carboxyfluorescein in  $0.44 \text{ mol l}^{-1}$  KOH and stimulated with trains of 15–30 depolarizing

current pulses at  $330\text{ s}^{-1}$ . The action potentials evoked by the intracellular stimulation were monitored by extracellular electrodes placed around the abdominal connectives in a more anterior position.

Since the subalar muscles contribute to wing twisting (supination) during the wing downstroke, and are thus involved in producing turns during flight, I recorded from the right and left subalar muscles of the metathoracic wings with pairs of  $50\text{ }\mu\text{m}$  copper wires. In this report, ipsilateral always refers to the side where the dGI axon is stimulated. Flight-like activity was initiated by a brief wind puff on the cerci and I measured the effects of this GI stimulation on two flight variables using these electromyogram recordings (EMGs). The first variable measured was the change in the number of EMG spikes per wingbeat in the ipsilateral and the contralateral subalar muscles. The second variable was the change in the relative delay between the spikes in the ipsi- and contralateral subalar muscles. The first spike in the ipsilateral subalar was taken as the reference point, and the delay was measured between this spike and the first spike in the contralateral subalar before and after the dGI stimulation (see Fig. 2B). After each recording, carboxyfluorescein was injected using  $20\text{ nA}$  of steady hyperpolarizing current, and the ganglion was removed and observed directly in a solution of 50% glycerol, 50% saline. Each dGI was identified on the basis of its morphology within the last abdominal ganglion (Daley *et al.* 1981) using an Olympus epifluorescence microscope. Recordings of electrical activity were stored on video tape (Data Neurocorder) and analyzed with built-in functions of a digital storage oscilloscope (Tectronix TDS 460).

In 12 different experiments, intracellular stimulation of a single dGI produced alterations in the flight EMGs. These motor alterations consisted of a change in the delay

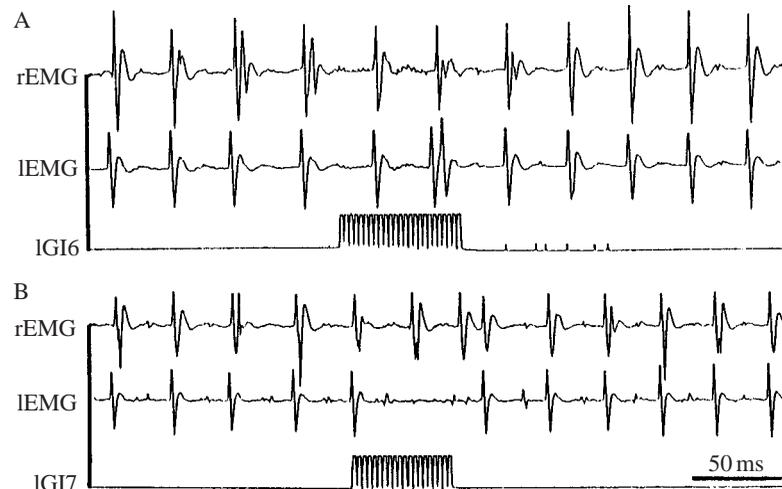


Fig. 1. Effect of stimulation of a dorsal giant interneuron (dGI) on the metathoracic subalar muscles. (A) The left GI6 was stimulated ( $25\text{ spikes at }330\text{ s}^{-1}$ ) during flight-like activity. This produced two effects: (1) the ipsilateral subalar (IEMG) fired a spike doublet, and (2) the delay between the ipsilateral and contralateral subalar muscles increased. (B) The right GI7 was stimulated with 20 spikes in a different preparation. This produced similar motor alterations to those in A but also a spike failure in the contralateral homolog (IEMG).

between the spikes in the homologous pair of metathoracic subalars and a change in spike number in these two muscles. In some trials, the number of subalar spikes increased in the ipsilateral subalar while this number was unchanged in the contralateral homologue (Fig. 1A); in other trials, spike failures lasting for 1–3 wingbeat cycles occurred in the contralateral homologue (Fig. 1B, Fig. 2B). However, in most trials, an increase in the number of spikes in the ipsilateral subalar and spike failures in the contralateral subalar occurred. In addition, there was also an increase in the delay in spiking between left and right subalar muscles of the metathoracic wings (Figs 1, 2). This shift in the delay between the homologous subalars following the dGI stimulation increased significantly by 2–13 ms relative to that prior to the stimulation ( $P < 0.001$ ;  $\chi^2$ -test;  $N = 20$ ). The mean latency from the onset of the dGI stimulation to the first change in left–right subalar firing latency or number of spikes was  $42.5 \pm 14.5$  ms (11 flight sequences in 11 preparations; range 15–65 ms). When analysing all dGI stimulation experiments, no clear correlation was found between a given motor response and a given dGI.

There also appeared to be a correlation between the number of dGI spikes presented during flight and the motor responses. A brief intracellular stimulation of left GI7 (15 spikes) produced both asymmetrical changes in the number of spikes and a latency shift between the right and left subalars (Fig. 2A). This asymmetrical motor response lasted for three wingbeat cycles. In the same animal but in a different trial, a longer stimulation delivered in GI7 (20 spikes) produced similar motor alterations, but these lasted for 13 wingbeat cycles (Fig. 2B). An increase in the number of dGI spikes was always accompanied by a consistent increase in the duration of the motor response. This was observed in two different preparations on seven successive trials (three trials in one animal, four trials in the other; dGI stimulation ranging from 10 to 35 action potentials produced motor responses ranging from 60 to 520 ms).

The present study shows that the dGIs of the cockroach are very good candidates for mediating evasive maneuvers during flight in response to wind stimuli delivered from the side. The motor alterations are asymmetrical and include changes in the relative delay and number of spikes in the subalar flight muscles (Figs 1, 2). These consisted of (1) an increase in the subalar activity in the metathoracic wing ipsilateral to the dGI stimulation, and a decrease in activity on the contralateral side, and (2) an increase in the delay between the ipsilateral and the contralateral subalar activity (Figs 1, 2). Although these motor output alterations were variable in different preparations, in that changes in spike number in the subalars did not always occur on both sides simultaneously, they were always consistent with an attempt to turn away from the stimulated side. An increase in the number of spikes per wingbeat cycle in a subalar muscle of the ipsilateral wing helps to produce increased supination of that wing (Dugard, 1967). Conversely, a decrease in the number of spikes per wingbeat cycle in a subalar muscle of the contralateral wing results in more pronation (less supination) of that wing. In addition, a change in the delay between homologous muscles, including the subalar muscles, accompanies changes in supination (Baker, 1979; Thüring, 1986). An increase in this delay between the ipsi- and contralateral subalars would produce an earlier supination of the ipsilateral wing. Together, these motor alterations, which were all observed during the course of these experiments, should produce a banked turn away from the stimulated side. However, one

could argue that asymmetrical responses in the subalar muscle pair of the metathoracic wings represent only one indicator of a steering attempt. However, such an asymmetrical response has also been described in detail in tethered, but intact, cockroaches flying in front of a wind tunnel, upon receiving a wind stimulus from the side (Ganihar *et al.* 1994) and, in this same study, the authors have also shown that the direction of the delay changes were opposite in the forewings and the hindwings, just as was found in locusts carrying out steering maneuvers (Thüring, 1986).

It is worth noting that such effects on the flight activity could be obtained with stimulation as brief as 15 spikes elicited in a single dGI (Fig. 2A). This number

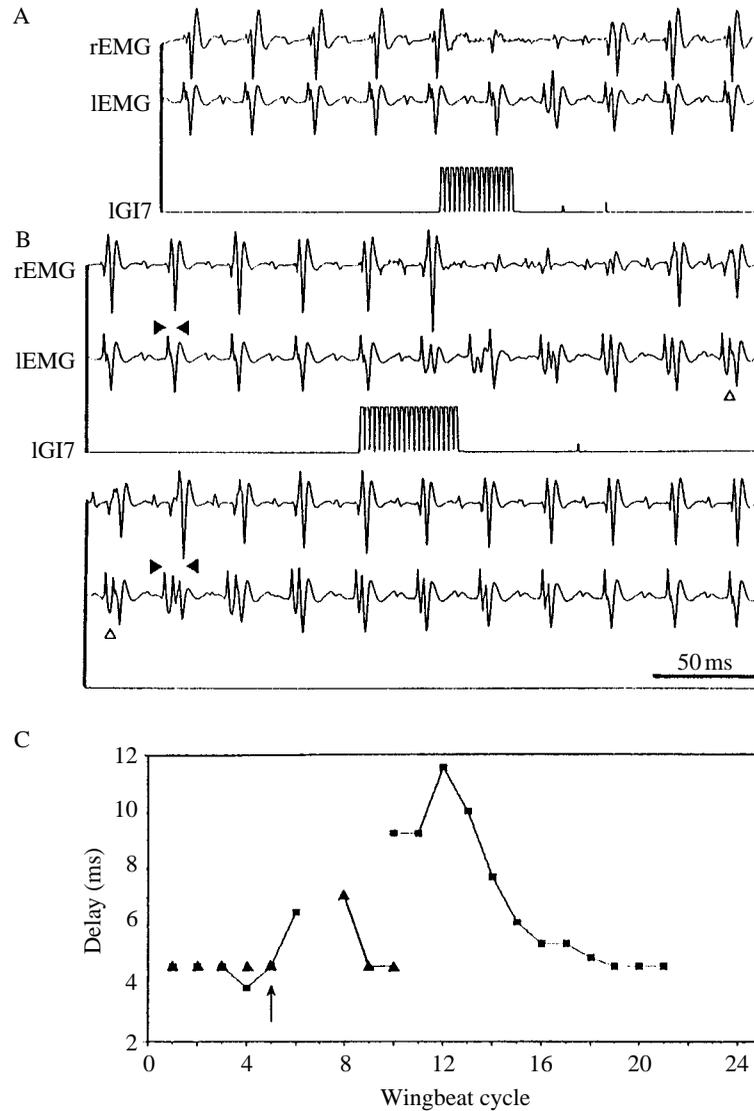


Fig. 2

corresponds rather well with the difference in the number of spikes of  $16 \pm 10$  (Ganihar *et al.* 1994) recorded from the ipsilateral and the contralateral dGIs in response to a wind puff from the side.

Varying the angle of delivery of the wind stimulus around the animal reveals the directional sensitivity of each of the three dGIs (Westin *et al.* 1977; Kolton and Camhi, 1993). GI5 responds best to wind stimuli from the rear ipsilateral quadrant, GI6 to the front ipsilateral quadrant and GI7 to the front and rear ipsilateral quadrants. Given this fact, a wind stimulus reaching the animal from a given angle would activate the dGIs differently. Yet, in these experiments, no clear correlation between the motor response and a given dGI was found. However, this observation is based on recordings from the subalar flight muscles only. There is, presumably, an endless combination of patterns of muscle activation for producing different amounts of steering. Linkage between the amount of turn during flight and a specific combination of dGIs firing could result from specific changes in, among other factors, (1) the latency shift between several ipsilateral muscles and/or ipsi- and contralateral muscles and/or (2) an increase in the duration of the asymmetrical response in the ipsilateral and contralateral flight muscles. To test these possibilities, one would like to record simultaneously from several ipsilateral flight muscles, or from ipsi- and contralateral muscles other than the subalars. These possibilities are worth testing since recent investigations have shown that the amount of escape turn on the ground is correlated with the relative number of spikes given by each vGI on the ipsilateral side (Levy and Camhi, 1994). Whether this is also true for the dGI-evoked evasive behavior patterns during flying remains to be demonstrated.

In only a few instances has it been possible to determine a clear causal relationship between flight maneuvers and the activation of specific interneurons by intracellular stimulation. In locusts, stimulation of various visual and wind-sensitive descending neurons (the DN<sub>s</sub>) located in the brain produces motor alterations consistent with corrective steering maneuvers (Möhl and Bacon, 1983; Hensler, 1989; Reichert and Rowell, 1986). However, in contrast to these brain interneurons, which mediate yaw-

---

Fig. 2. Effect of increasing the duration of the dGI stimulation on the subalar depressor muscles. (A) The left GI7 was stimulated briefly (15 spikes at  $330 \text{ s}^{-1}$ ) during flight-like activity. This resulted in an increase and decrease in the number of EMG spikes in the ipsilateral and contralateral flight muscles, respectively, as well as an increase in the delay between the ipsilateral and the contralateral muscle. These effects lasted for three wingbeat cycles. (B) The duration of the dGI spike train was increased to 20 spikes. Similar motor alterations to those in A occurred, but these lasted for 13 wingbeat cycles. The open triangle under the middle EMG recording (IEMG) labels the same EMG spike in both top and bottom panels, indicating the continuity of the recordings. Filled triangles indicate how the delay between the left and right subalars was determined. (C) Delay between the ipsi- and the contralateral subalar muscles for the trials shown in A and B. The arrow indicates the onset of the dGI stimulation. The missing data points after the arrow correspond to those wingbeat cycles where the spikes in the contralateral subalar are missing (see A and B) and, thus, for which the delay could not be evaluated. For a brief dGI stimulation, the delay increased from 4.6 ms to 7 ms for only one wingbeat cycle (triangles). For a longer dGI stimulation (20 spikes), this delay increased from 4.6 ms to roughly 10 ms for four consecutive cycles and decreased thereafter to the baseline, or 4.6 ms, during the next five wingbeat cycles (squares).

corrective steering during flight, the dGIs mediate yaw-enhancing steering (Ganihar *et al.* 1994). In this respect, the dGIs are functionally comparable to interneuron 1, the bat-detector acoustic interneuron that initiates yaw-enhancing steering in crickets (Hoy *et al.* 1989). Since, in cockroaches, it appears that ultrasonic avoidance behavior does not occur (D. D. Yager, personal communication; D. Ganihar and F. Libersat, unpublished observations), cockroaches would be defenseless during bat encounters if they were not equipped with an alternative escape behavior. Such an escape behavior appears to be mediated by the wind-sensitive dGIs. Interestingly, the motor alterations are graded with the duration of the dGI spike train (Fig. 2), just as, in crickets, the ultrasonic evasion is graded with stimulus intensity (Hoy *et al.* 1989). Although there is a clear relationship between ultrasound intensity and the proximity of the predator, it is not yet clear how wind velocity correlates with predator distance. Nevertheless, such a graded motor response indicates that the wind cercal system might be tracking the approach of the hunting bat.

This work was supported by grant I-151-012.1/90 from the German–Israel Foundation for Scientific Research and Development and the Raschi Foundation (Guastela Fellowship to F.L.). I thank J. M. Camhi and A. Weisel Eichler for critically reading this manuscript.

### References

- BAKER, P. S. (1979). The wing movements of flying locusts during steering behavior. *J. comp. Physiol.* **131**, 49–58.
- CAMHI, J. M. (1984). *Neuroethology: Nerve Cells and Natural Behavior of Animals*. Sunderland, MA: Sinauer Associates Inc. Chapter 4. A case study in neuroethology: The escape system of the cockroach, pp. 79–105.
- CAMHI, J. M. (1988). Escape behavior in the cockroach: Distributed neural processing. *Experientia* **44**, 361–462.
- COMER, C. M. AND DOWD, J. P. (1993). Multisensory processing for movement: Antennal and cercal mediation of escape turning in the cockroach. In *Biological Neural Networks in Invertebrate Neuroethology and Robotics* (ed. R. D. Beer, R. E. Ritzmann and T. McKenna), pp. 89–112. New York: Academic Press.
- DALEY, D. L., VARDI, N., APPIGNANI, B. AND CAMHI, J. M. (1981). Morphology of the giant interneurons and cercal nerve projections of the American cockroach. *J. comp. Neurol.* **196**, 41–52.
- DUGARD, J. J. (1967). Directional change in flying locusts. *J. Insect Physiol.* **13**, 1055–1063.
- GANI HAR, D., LIBERSAT, F., WENDLER, G. AND CAMHI, J. M. (1994). Wind-evoked evasive response in flying cockroaches. *J. comp. Physiol.* **175**, 49–65.
- HENSLER, K. (1989). Corrective flight steering in locusts: convergence of extero- and proprioceptive inputs in descending deviations detectors. In *Neurobiology of Sensory Systems* (ed. R. N. Singh and N. J. Strausfeld), pp. 531–554. New York, London: Plenum Press.
- HOY, R. R., NOLEN, T. AND BRODFUEHRER, P. (1989). The neuroethology of acoustic startle and escape in flying insects. *J. exp. Biol.* **146**, 287–306.
- KOLTON, L. AND CAMHI, J. M. (1993). Re-evaluating the directional sensitivity of identified giant interneurons of the cockroach. *Soc. Neurosci. Abstr.* **19**, 702.
- LEVY, R. AND CAMHI, J. M. (1994). Testing for a population vector code for wind direction in the cockroach giant interneurons. *Soc. Neurosci. Abstr.* (in press).
- LIBERSAT, F. (1992). Modulation of flight by the giant interneurons in the cockroach: *Periplaneta americana*. *J. comp. Physiol.* **170**, 379–392.

- LIBERSAT, F. AND HOY, R. R. (1991). Ultrasonic startle behavior in bushcrickets. (Orthoptera; Tettigonidae). *J. comp. Physiol.* **169**, 507–514.
- LIBERSAT, F., LEVY, A. AND CAMHI, J. M. (1989). Multiple feedback loops in the flying cockroach: Excitation of the dorsal and inhibition of the ventral giant interneurons. *J. comp. Physiol.* **165**, 651–668.
- LIEBENTHAL, E., UHLMAN, O. AND CAMHI, J. M. (1994). Critical parameters of the spike trains in a cell assembly: coding of turn direction by the giant interneurons of the cockroach. *J. comp. Physiol.* **174**, 281–296.
- MÖHL, B. AND BACON, J. (1983). The tritocerebral commissure giant (TCG) wind-sensitive interneurone in the locust. II. Directional sensitivity and role during flight stabilization. *J. comp. Physiol.* **150**, 453–465.
- REICHERT, H. AND ROWELL, C. H. R. (1986). Neuronal circuits controlling flight in the locust: how information is processed for motor control. *Trends Neurosci.* **9**, 281–283.
- RITZMANN, R. E. (1993). The neural organization of cockroach escape and its role in context dependent orientation. In *Biological Neural Networks in Invertebrate Neuroethology and Robotics* (ed. R. D. Beer, R. E. Ritzmann and T. McKenna), pp. 113–137. New York: Academic Press.
- RITZMANN, R. E., POLLACK, A. J. AND TOBIAS, M. L. (1982). Flight activity mediated by intracellular stimulation of dorsal giant interneurons of the cockroach *Periplaneta americana*. *J. comp. Physiol.* **147**, 313–322.
- THÜRING, D. A. (1986). Variability of motor output during flight steering in locusts. *J. comp. Physiol.* **158**, 653–664.
- WESTIN, J., LANGBERG, J. J. AND CAMHI, J. M. (1977). Responses of giant interneurons of the cockroach to wind puffs of different directions and velocities. *J. comp. Physiol. A* **121**, 307–324.
- YAGER, D. D. AND SCAFFIDI, D. J. (1993). Cockroach homolog of the mantis tympanal nerve. *Soc. Neurosci. Abstr.* **19**, 142.12.