

## SHORT COMMUNICATION

### MOTION ANALYSIS OF ESCAPE MOVEMENTS EVOKED BY TACTILE STIMULATION IN THE COCKROACH *PERIPLANETA AMERICANA*

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The escape behavior of the American cockroach, *Periplaneta americana*, has provided a large amount of neuroethological data on the control of orientation movements. In the typical behavior, the animal responds to gentle wind puffs by turning away from the wind source and running (Camhi and Tom, 1978). However, cockroaches also escape in a directional manner in response to tactile stimuli (Comer *et al.* 1989, 1993). This additional category of escape response raises two important questions. Do the neural circuits for wind- and tactile-evoked escape responses converge, and if so where? At one extreme, the two behaviors may be under the control of completely separate circuits. Alternatively, the two sensory pathways could converge on a single set of controlling interneurons.

To address this question, circuit analysis studies must be complemented with behavioral observations. Shared neurons could certainly be demonstrated by analyzing the neural pathways from sensory structures to motor output. However, during the actual behavioral response, tactile information could still bypass common elements and utilize a separate pathway from that used during the wind-evoked response. If the escape turns resulting from tactile stimulation include totally different leg movements from those generated by wind, it would be difficult to rationalize common control elements. However, a finding that the leg movements that generate turns are essentially the same regardless of the sensory modality that triggered them would support the notion of common control circuits. A detailed circuit analysis study would then be in order. In this paper, we report behavioral observations of the initial turns of the tactile-evoked escape behavior in both freely moving and tethered animals. We then compare these leg movements with those that have been documented for wind-evoked escape turns. In the interest of space and clarity, we will continue to refer to these behaviors as the tactile- and wind-evoked escape responses. However, we should point out that our analysis is directed specifically at the initial turn of the escape response not the subsequent running movements.

The leg movements that produce wind-mediated turns have been documented in detail

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(Camhi and Levy, 1988; Nye and Ritzmann, 1992). For any wind angle, the prothoracic and mesothoracic legs determine the direction of the turn by flexing the femur–tibia (FT) joint on the leg that is contralateral to the stimulus while extending the FT joint on the ipsilateral leg. These joints move the leg laterally. The ipsilateral FT extension pushes the tarsus of that leg laterally towards the stimulus source, while the contralateral FT flexion pulls the tarsus of that leg towards the animal's midline. As a result, both legs create forces directed at the stimulus, thereby turning the animal in the opposite direction. The actions of the coxa–femur (CF) joint move the leg in an anterior–posterior direction. Wind-evoked turns can be divided into three categories based upon the actions of various CF joints (Nye and Ritzmann, 1992). Type 1 turns are characterized by an extension of the CF joints on each leg, resulting in a backward thrust of the metathoracic legs, which drives the animal forward while the FT movements turn it away from the stimulus. In type 2 turns, the metathoracic legs provide additional directionality. The contralateral leg moves forward by flexing its CF joint while the ipsilateral leg extends its CF joint and pushes backwards. The tarsi of both legs are in contact with the ground during this maneuver, so that they provide power in opposite directions. The result is similar to turning a rowboat, where the oarsman pulls on one oar while pushing on the other. It results in a sharper turn angle than the type 1 turn and no forward movement of the body. Type 3 turns are characterized by flexion of CF joints on both metathoracic legs. This pulls both legs forwards, causing the animal to move backwards, away from the stimulus. The incidence of the different types of turns is correlated with the anterior–posterior orientation of the stimulus source. Both type 2 and 3 turns are predominantly associated with wind from the front of the animal, while winds from the rear of the animal evoke almost exclusively type 1 turns.

We first wanted to observe tactile-evoked escape behavior in its most natural state. To do this, we observed the responses of animals that were free to move in a 23 cm by 31 cm plastic container. Twenty animals were tested and each was placed in the cage 10 min prior to testing. Stimuli were only delivered to animals that were standing still. Tactile stimuli were delivered with a small hand-held solenoid that moved a 15 cm glass rod with a bent insect pin glued to its tip. Activation of the solenoid caused the head of the pin (1 mm in diameter) to contact the cuticle with a single pulse of 0.18 N as measured by testing the stimulator on a strain gauge. Although we attempted to hold the stimulus probe at the same height each time, the hand-held nature of the stimulus undoubtedly introduced some variability. In the free-ranging tests, we had to use a hand-held stimulator in order to get near freely moving animals. We videotaped the escape movements with a NAC HSV400 high-speed video recorder, operating at 200 frames s<sup>-1</sup>. As indicated in Fig. 1, we tested seven areas on the animal's body. The order of presentation was determined from a random number generator. The animals were tested once at each site with 5 min between trials. We are confident that this stimulation paradigm did not activate antennal or cercal afferents, because similar paradigms used in electrophysiological studies failed to activate giant interneuron activity in the ventral nerve cord and evoked activity in thoracic interneurons that was qualitatively distinct from the responses associated with tactile stimulation of cerci or antennae (Pollack and Ritzmann, 1993).

Out of the 140 trials, 120 (85.7 %) resulted in vigorous escape movements. Most of the failures occurred with head stimulation. The animal always turned away from stimuli applied to lateral areas of the body. We attempted to place the responses into categories similar to those found for wind-evoked behavior. With free-ranging animals, the legs were partially obscured from view, making a definitive classification of turns difficult. Nevertheless, we could categorize the responses based upon body movement. Type 1 responses tend to be small-angle turns associated with forward movement of the animal's body. Type 2 and 3 responses turn the animal through greater angles and generate rearward body movements (type 3) or turns that pivot the animal in place (type 2) (Nye and Ritzmann, 1992).

By either of these criteria, tactile stimuli to the head and thoracic regions produced predominantly type 2 and 3 turns, while stimuli to the abdominal region produced almost exclusively type 1 turns. We divided the responses into large-angle turns (greater than  $60^\circ$ ) reminiscent of types 2 or 3 and small-angle turns (less than or equal to  $60^\circ$ ) reminiscent of type 1. Escape responses evoked by tactile stimulation of the abdomen (areas 4–7) evoked exclusively small-angle turns (Fig. 1). The mean angle of all responses in this region was  $12.3 \pm 9.9^\circ$  (S.D.). Stimulation of the head and pronotum (the cuticular plate above the anterior thoracic segments) resulted in predominantly large-angle turns (mean  $91.8 \pm 44.9^\circ$ ). The differences in turning angle between the abdominal regions and the head–thorax regions were highly significant ( $P < 0.001$ , Wilcoxon–Mann–Whitney  $U$ -test).

The criteria of body displacement produced the same conclusion as did turning angle. Stimuli to the head and thorax generated 89.4 % of turns in which the animal pivoted in

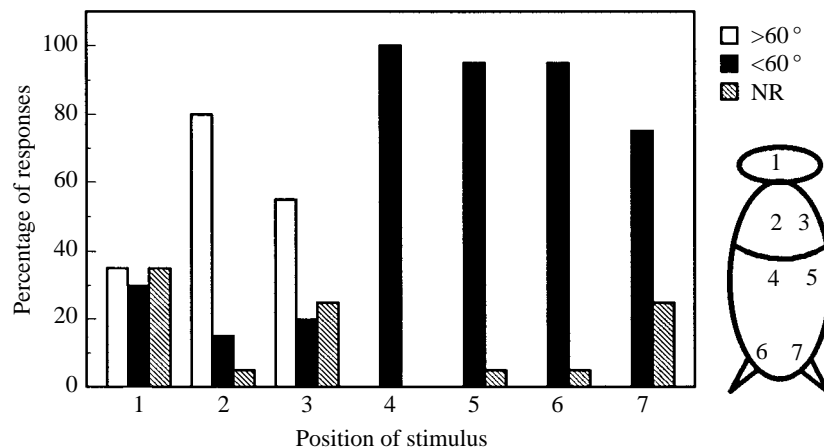


Fig. 1. Escape responses to tactile stimulation. Intact free-ranging animals were stimulated on the areas indicated on the inset. The results are divided into three groups. Turns of  $60^\circ$  or less (filled bars) were similar to type 1 turns seen in wind-evoked escape. Large-angle turns (open bars) were similar to type 2 or 3 turns in wind-evoked escape. Trials in which the animal made no turning movements are indicated as no response (NR, hatched bar). Note that large-angle turns predominated when the head or pronotum was stimulated. Abdominal stimulation evoked exclusively small-angle turns.

place (type 2) and 4.2 % with rearward movement (type 3). Only 6.4 % of these trials produced forward movement (type 1). In contrast, all but one of the escape responses evoked by abdominal stimulation produced forward movement of the animal's body (type 1).

In order to make precise observations of individual leg joints, we observed 35 animals that were tethered over a lightly oiled plate (see Nye and Ritzmann, 1992, for details). We used a preparation that was essentially the same as that used previously to determine leg movements in response to wind stimuli (Nye and Ritzmann, 1992). Under conditions where tethered animals were suspended over a lightly oiled glass plate, normal leg movements could be made without actual displacement of the body. The camera viewed the leg segments from the ventral surface through the glass plate, giving a clear image of all joint angles. The thin oil layer did not distort the video image.

We had to make one change in the attachment for the tether. In the previous study, the animal was attached by inserting pins through the lateral margin of the pronotum into a cork pad that was glued to the tether. Since we wanted to reach the pronotum with our tactile stimulator, we had to redesign the attachment. We did this by replacing the cork on the tether with a small magnet. A thin piece of steel was glued to the middle of the pronotum (3M Vetbond glue). The animal could then be secured to the magnetic tether. With tactile stimulation, it was also necessary to prevent all rotational movements prior to stimulation, so that the stimulus probe could be accurately positioned. This was accomplished by adding a second magnet and another metal strip on the dorsal surface of the animal's abdomen. Thumbscrews provided adjustments for the height of the front attachment magnet and the distance between the two magnets, so that the animal rested in a normal posture.

In the tethered preparations, a reproducible stimulus was generated with a Pasco model SF-9324 mechanical wave driver. A lever ending in an L-shaped metal rod (approximately 1 mm in diameter) was used to amplify the motion of the wave driver. The tip of the rod contacted the animal with a reproducible force of 0.18 N, the same as that used in the free-ranging experiments.

In all cases, the animal was allowed to acclimate to the tether for 30 min after attachment. Each animal was stimulated five times or until two successive trials failed to produce a response. Each area was stimulated at least 10 times during the study. There were 171 trials. This paradigm was different from that used in the free-ranging experiments because the tethered animals often failed to respond after several stimuli. In order to ensure vigorous responses on all test trials, we chose to restrict our tests to five trials per animal rather than to attempt a complete set on each subject. A minimum 5 min interval was maintained between trials. As with the free-ranging paradigm, animals were only stimulated when they were standing still. Occasionally, we had to wait longer than 5 min if the animal was walking. The order of stimulus position was chosen from a random number generator.

The areas that were stimulated are indicated in Fig. 2. In general, tactile stimulation of the abdominal areas was more reliable than on the pronotum (Fig. 2A). This may reflect the fact that the tether covered a larger proportion of the pronotum than of the abdomen.

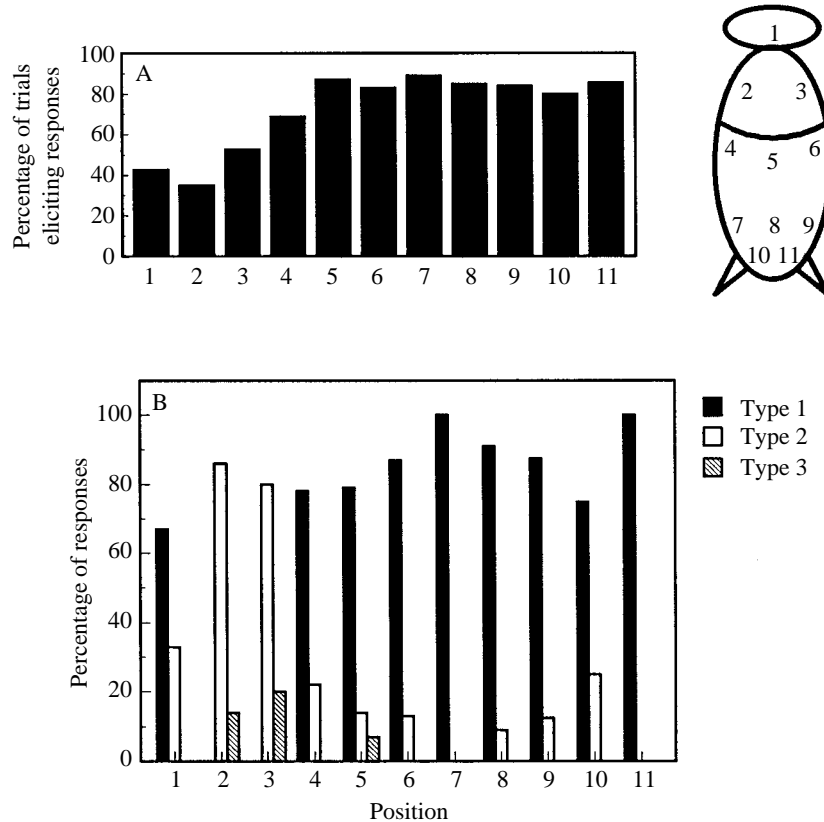


Fig. 2. Leg movements recorded from tethered animals. (A) The percentage of trials resulting in vigorous escape responses is indicated for each area stimulated. Most of the areas on the abdomen resulted in approximately 80% success. The lower success rate for the pronotum areas could reflect the location of the tether. (B) The responses were categorized according to type of turn, based upon the movements of CF joints. See text for details. They were then arranged by stimulus area (see inset). The results of stimulating areas on the abdomen are clearly dominated by type 1 turns that would generate forward movements in free-ranging animals. Responses from the pronotum areas are dominated by type 2 and 3 turns which, in free-ranging animals, would generate escape responses with greater turn angles, moving the animal in a more posterior direction. Stimulation of the head region resulted in a mixture of type 1 and 2 turns, reflecting a more complex sensory architecture.

Nevertheless, enough responses were recorded to provide an indication of the leg movements resulting from tactile stimulation of the pronotum.

The basic leg movements associated with tactile stimulation were indistinguishable from those observed in response to wind. The FT and CF joints of each leg made movements typical of those observed in wind-evoked behaviors. In all responses to stimuli on lateral body regions, the FT joints on the mesothoracic legs extended on the ipsilateral side and flexed on the contralateral side. In a free-ranging animal, FT movements of this kind would generate a turn away from the stimulus. The three turn

types that were associated with CF movements in wind trials were also found in tactile responses. Moreover, the correlation between the type of turn and the anterior–posterior location of the stimulus that was documented for wind stimuli was also found in tactile trials. The incidence of type 2 and 3 turns was greater in trials in which the pronotum (anterior region) was stimulated than in trials in which the abdomen was stimulated (Fig. 2B). This relationship was significant ( $P < 0.001$ ,  $\chi^2$ -square test) and consistent with the distribution of responses seen in the free-ranging trials. Type 2 turns dominated in areas 2 and 3 (pronotum) and were rare in the abdominal areas. Type 3 turns were rare in all areas but, with one exception, all resulted from stimulation of the pronotum. The responses to touching the abdominal regions (areas 4–11) were almost exclusively type 1. The only exception to the anterior–posterior correlation with type of turn was in the head region. Tactile stimulation of the top of the head capsule between the compound eyes resulted in a greater number of type 1 turns than type 2 turns. No type 3 turns were recorded in response to tactile stimulation of the head. This may reflect the very complex sensory situation associated with the head region relative to the escape circuitry (R. E. Ritzmann and A. J. Pollack, in preparation).

We noted only one difference between wind-mediated responses and tactile responses. In tactile trials, the latency from the onset of stimulation to the first leg movements was  $17.5 \pm 10.6$  ms (s.d.). This is actually shorter than the latency observed for wind-mediated escape ( $55.2 \pm 16.4$  ms) timed from the arrival of the wind stimulus at the cerci (Nye and Ritzmann, 1992), but this may reflect difficulties in determining exactly when the wind stimulus reaches the cerci. This problem does not arise with the tactile stimulator. Here latency was determined directly from the videotape records as the time between contact of the stimulus probe with the cuticle and the first sign of leg movement. Since the video system was operating at a frame rate of one frame per 5 ms, the accuracy of each measurement could be up to 5 ms longer (if the probe contacted the cuticle early in the inter-frame interval) or 5 ms shorter (if the legs moved early in the inter-frame interval). In either case, the actual latency is considerably shorter than that reported for wind-evoked responses.

It is important to note that the leg movements we observed are not the only possible movements that could generate a rapid escape turn. Indeed, the typical running pattern is the tripod gait, which is distinctly different from any of the escape turns that have been observed (Camhi and Levy, 1988). It is certainly possible that the animal could escape from a tactile stimulus by simply generating a rapid tripod gait movement. A turn could be generated by making larger or more forceful movements on one side of the body, while still maintaining the alternating tripod pattern. However, we did not observe tripod leg movements in the initial turning phase of any vigorous escape responses.

Our results are consistent with the hypothesis that tactile and wind stimuli evoke escape movements through common neural control elements. The leg movements resulting from tactile stimuli were indistinguishable from those generated by wind stimulation. In addition, the proportion of type 1, 2 and 3 turns was correlated with the anterior–posterior orientation of the stimulus in the same way in both cases. This was true for both free-ranging and tethered animals. For both behaviors, the control circuit must be able to detect both left–right and anterior–posterior orientations of the stimulus. The

circuit responds to stimuli from the left or right side by generating appropriate asymmetrical movements of the mesothoracic FT joints that will turn the animal away from the stimulus. It also responds to the anterior–posterior orientation of the stimulus by influencing the actions of the CF joints, particularly in the metathoracic legs. Both tactile and wind stimuli from the rear result in extension of both CF joints. More anterior orientation must be detected by the control circuit and read out as a reversal of the contralateral CF joint movement, causing it to flex and pull the animal backwards. This movement results in the larger-angle turn that is required to move the animal away from a threat coming from the front. On some occasions, stimuli from the front will induce both CF joints to reverse direction and flex, causing the animal to back away from the stimulus. These type 3 turns are relatively rare in both tactile- and wind-evoked escape movements. Given that the animal's response to stimuli of various orientations is the same for both tactile- and wind-evoked behaviors, the most parsimonious conclusion is that the same control circuit is operating regardless of the sensory modality to which it is responding.

What neural components could logically be expected to be shared between tactile- and wind-mediated escape? Certainly the leg motor neurons are by necessity shared and many of the local circuits that coordinate multi-joint movement (Burrows, 1980) are probably also the same. However, on the sensory side, it is unlikely that tactile stimuli evoke escape behavior *via* the giant interneurons (GIs). There is no evidence that the GIs respond to tactile stimulation anywhere other than on the cerci. Moreover, tactile stimuli can evoke escape movements after specific lesion of all ventral GIs (Stierle *et al.* 1993), the subgroup of GIs that is largely involved in generating wind-mediated responses.

The remaining interneurons in the wind-mediated escape pathway are the type A thoracic interneurons (TI<sub>As</sub>). These are approximately 100 interneurons that receive direct excitation from ventral GIs (Casagrand and Ritzmann, 1991; Ritzmann and Pollack, 1988) and activate or inhibit motor neurons either directly or *via* local interneurons (Ritzmann and Pollack, 1990). The TI<sub>A</sub> population appears to control the relative strength of activity among leg motor neurons based upon the directionality of the input signal (Ritzmann, 1993). They do have the capacity to act in both wind-mediated and tactile-mediated escape. Individual TI<sub>As</sub> are excited by many types of sensory input in addition to wind-related activity carried by the GIs (Ritzmann *et al.* 1991). These additional modalities include tactile stimulation. In addition, recent observations indicate that the location of the tactile stimulus can be encoded in the response properties of the TI<sub>A</sub> population (Pollack and Ritzmann, 1993). These direct observations from the TI<sub>As</sub>, taken together with the behavioral data presented here, suggest that the TI<sub>As</sub> represent a common locus for the control of escape movements originating from either wind stimulation or tactile stimulation.

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