

Collision avoidance by running insects: antennal guidance in cockroaches

Yoshichika Baba^{1,*}, Akira Tsukada² and Christopher M. Comer^{1,†}

¹Laboratory of Integrative Neuroscience and Neurobiology Group, Department of Biological Sciences, University of Illinois at Chicago, IL 60607, USA and ²Department of Computer Engineering, Toyama National College of Technology, Toyama, 933-0293, Japan

*Author for correspondence at present address: Division of Biological Sciences, 32 Campus Drive, University of Montana, Missoula, MT 59812, USA (yoshi.baba@mso.umt.edu)

†Present address: Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

Accepted 11 March 2010

SUMMARY

Cockroaches were observed with videographic methods as escape running was initiated, but with obstacles in the path of their run. The goal was to determine the repertoire of possible responses to obstacles and the sensory cues used to trigger the responses. Intact cockroaches collided with obstacles on only about 10% of trials. The most common collision avoidance strategy was simply to stop running prior to impact. However, occasionally animals moved vertically and climbed over the barrier, or turned and navigated an edge of the obstacle, or completely reversed run direction. The avoidance strategies chosen depended on the size and configuration of the obstacle. Tests for the use of vision in detecting obstacles showed that its role, if any, is small. However, all manipulations that altered the antennal system changed behavior in a way consistent with the hypothesis that antennal mechanosensation plays a major role in collision avoidance. For example, reducing antennal length, or severing the main antennal nerve without altering the length produced significant increases in the frequency of collisions. Tests with tethered insects showed that (1) the antennae are preferentially directed forward as animals run, and (2) nearly simultaneous contact with both antennae is required to make the cockroach stop. Our data indicate that running cockroaches employ strategies that set their sensorimotor systems in a mode of readiness to deal with obstacles and they suggest that sensory information about the presence and configuration of obstacles is used to make choices, at very short latencies, about how to respond to obstructions.

Key words: cockroach, antenna, mechanosensory information, behavior, escape.

INTRODUCTION

The lifestyle of many advanced organisms requires significant processing of non-visual sensory information in order to navigate the immediate environment. For example, insects possess compound eyes, and often have additional visual receptors, but many also rely heavily on non-visual sensory guidance systems. Cockroaches (and other orthopteroid insects) provide a good model of such polysensory control. Cockroaches are nocturnal insects so they often explore and navigate the immediate environment under low light conditions. Thus they may depend on their long antennae for mechanosensory cues to guide behavior.

A good example is that cockroaches moving along a wall can use an antenna to measure the distance between the body and the wall, thus avoiding collision (Camhi and Johnson, 1999). Blind cockroaches walking toward an object use the antennae to determine object location (Okada and Toh, 2000). Cockroaches also can use the antennae to identify and react to a small predator (Comer et al., 1994; Ye and Comer, 1996; Ye et al., 2003; Comer et al., 2003) and to assist in interacting with surfaces and/or objects during climbing (Watson et al., 2002; Ritzmann et al., 2005; Harley et al., 2009). Even when visual cues are available, the antennae are important. So, for example, a reliance on antennal information is often a fundamental part of insect responses that are initially triggered by visual cues. Cockroaches (Baba et al., 2004), crickets (Honegger, 1981) and some other insects [e.g. honey bees (Erber et al., 1997)] exhibit visual guidance of the antennal motor system: they will orient the antennae toward novel objects, or track the movements of objects in the visual field with comparable antennal movements.

However, it remains an open question just how non-visual (antennal) information is integrated into ongoing behavior that is rapidly executed or that changes with a short latency and so requires quick integration of sensory feedback. Cockroach locomotion involves a range of speeds from walking (Delcomyn, 1971) to bursts of very high speed running (Full and Tu, 1991). Additionally, escape from predators routinely involves directional turning and running, and both are executed at high velocities (Ye et al., 2003; Gras et al., 2004). The present experiments were designed to test the hypothesis that antennal mechanosensory input can guide the trajectory of rapid running by cockroaches such as occurs during escape.

We challenged running cockroaches with barriers and assessed their collision avoidance behaviors. Using both intact and experimentally altered cockroaches, we showed that although both visual and antennal mechanosensory inputs might influence collision avoidance during running, antennal touch appears to be the major contributor. Furthermore, we show that the shape or dimensions of detected obstacles and apertures can influence the type of avoidance behaviors. Clearly, cockroaches have the ability to choose one of several collision avoidance strategies when encountering obstacles. They do this even when antennal information must be processed in time intervals of a few milliseconds. A preliminary description of this work was presented in abstract form (Baba et al., 2005).

MATERIALS AND METHODS

All cockroaches used were adult *Periplaneta americana* L. They were obtained from stocks maintained in our laboratory, occasionally supplemented with animals obtained from commercial suppliers.

Altering sensory feedback

Several approaches were taken to alter the sensory feedback available to experimental animals. In all cases, at least 1 day was allowed following any manipulation before behavioral tests were run.

Blocking vision was achieved by covering the compound eyes with opaque red paint (Testors, Rockford, IL, USA). Red was the preferred color because it contrasts well with the black surface of the compound eye and this made it easier to be sure that coverage was complete. The ocelli were not covered. Additionally, on some trials we further assessed the contribution of vision to collision avoidance by testing responses to obstacles that were translucent (the standard obstacle), or painted black (see below for description of object geometry).

In some animals the flagellum (the long, distal, multi-segmented portion) of each antenna was systematically shortened. The length of an intact adult antenna is about 4–5 cm. We trimmed antennae by removing the most distal flagellar segments bilaterally (and sometimes unilaterally) to produce one of three final lengths: 3–4 cm total length, 2–3 cm total length, or 1–2 cm total length. When transecting the antennal flagellum, care was taken to prevent excessive loss of hemolymph by temporarily blocking the exposed cut surface to allow for clotting of the hemolymph.

Finally, in one group of animals the flagellum was maintained at full length, but the afferent innervation was severed. This was done unilaterally so that comparisons could be made between an intact and denervated antenna for control of obstacle detection and avoidance. For this purpose a flap of cuticle was removed with a razor blade from the distal end of the pedicel of approximately the 10th flagellar segment, the nerve was severed with a fine insect pin, and the cuticular flap was replaced and glued in place with beeswax. As a control, some animals had the same flap cut in the antennal cuticle unilaterally and then it was replaced, but nerve inside the antenna were not cut. [This operation was performed where the nerve to flagellar receptors is distinct from the nerve supply to receptors associated with the scape and pedicel (see Baba and Comer, 2008).]

Observing behavior

Cockroaches were put in a circular arena (80 cm diameter with a wall 13 cm in height; see Fig. 1A), the wall and floor of which were painted white. The insects were allowed to acclimatize for at least 1 min before testing. During this period there were no obstacles in the arena. To elicit running, animals were touched abruptly on the

wings with a clear plastic probe. The cockroaches reliably responded to this tactile stimulus with escape that was almost always along the wall of the arena. All behavioral responses were recorded with a video camera (GR-DX97, JVC, Yokohama, Japan) mounted above the arena and the video data were converted to AVI format in the computer (Pinnacle Studio version 9, Pinnacle System Inc., Daly City, CA, USA). All experiments were done under room fluorescent illumination conditions.

To quantify movement, changes in the position of the cockroach's head were measured frame-by-frame (some data were captured at 30 'frames' per second, but most were processed off-line to yield 60 images per second using customized computer software). The change in the position of the head coordinates between frames was defined as the movement distance per frame. Instantaneous velocity was calculated by the expression: movement distance per image (mm) \times imaging rate, and expressed as mm s^{-1} . Average velocity was defined as total distance moved divided by the total movement period.

Collision avoidance during running

On collision avoidance trials, cockroaches were stimulated to run as described above, but before the trial an obstacle was put against the wall. We used a standard obstacle of 24 mm height and 60 mm width (a microscope cover glass). The obstacle was not placed in the same spot in the arena for each trial. We did not try to keep the distance between the cockroach and the obstacle constant, but it was at least 65 cm (or 90 deg of travel around the perimeter of the arena; see Fig. 1B). The response to the obstacle was recorded videographically as described above and any collisions or collision-avoidance behaviors were analyzed frame by frame with the help of the computer.

In obstacle size experiments we changed some parameters of the standard obstacle (Fig. 1C). We tested responses to five different heights (5, 12, 20, 24 and 50 mm) while holding obstacle width constant at 60 mm. In other cases, we tested three different widths (15, 24, 60 and 80 mm) while holding height constant at 24 mm.

Additional experiments tested for an influence of specific geometric details of the obstacle on the success of collision avoidance. In a set of 'gate' experiments, we tested the ability of cockroaches to detect an aperture that might provide safe passage through (underneath) the obstacle. For these we used an obstacle of standard width (60 mm) and 29 mm in height, with a 'gate' or aperture along its lower edge (Fig. 1D). The width of the aperture was held at 50 mm, but aperture height was systematically changed

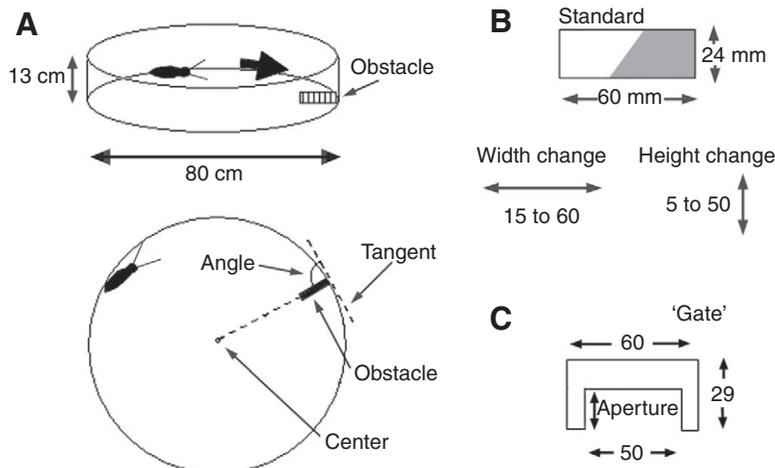


Fig. 1. Experimental arena and obstacles. (A) View of the arena and the general path of run toward an obstacle. (B) Standard obstacle. Height and width changes are shown just below. (C) 'Gate' obstacle. Other details of testing and obstacle characteristics are given in the text. All units in C and D are millimeters.

(2, 5 and 10 mm). In 'angle' experiments, the obstacle was varied from its standard orientation perpendicular to the path of the running cockroach (90 deg) to angles of 10 deg or 30 deg more acute to the run path, or 10 deg or 30 deg more obtuse to the run path.

Collision avoidance in stationary animals

To gain more control over the magnitude and timing of impact with each of the antennae, and to observe active antennal movements, we observed some animals as they were running but tethered and collisions were simulated. For this purpose, the flat head of a small nail was cemented to a cockroach's pronotum and its shaft (1 cm in length) was clamped in a holder affixed over a slippery surface (coated with a thin layer of petrolatum). This surface formed the floor of a small arena (20 cm diameter, 10 cm wall height; painted white). An obstacle to be tested was put in front of the cockroach at a distance of about 5 cm. The obstacles were clear and made of microscope slide glass (76 mm × 25 mm). The obstacle glass was moved toward the cockroach using a hydraulic (water filled) tube controlled from a syringe. The velocity varied slightly but averaged $2.5 \pm 0.8 \text{ cm s}^{-1}$ (mean \pm s.e.m., $n=10$ calculated from a random sample of videotaped trials). As tethered cockroaches walked or ran 'in place' the obstacle was moved toward the cockroach (with wind cues and the possibility of touch cues). We regarded pauses of leg movement, or abrupt shifts of leg positions, as 'responses' to the obstacle. Some trials included a pair of obstacles that could be brought into contact with the right or left antenna separately (see below).

RESULTS

General observations of running behavior of intact and modified cockroaches

Ablation of major sensory organs located on the head had noticeable effects on cockroach locomotion and its guidance. We first describe the ability to detect and avoid obstacles while animals ran with reduced visual input, shortened antennal flagella, or with disrupted flagellar sensory pathways. Removal of antennal receptors specifically located at the base (scape or pedicel) was quite variable and will be reported elsewhere (Y.B., in preparation).

Intact cockroaches, and those with altered flagellar input, ran along the wall of the circular test arena once running was stimulated. They touched the wall with the outside antenna (that closest to the wall) and 'probed the path' with the inside antenna as they ran. By using this phrase we mean that the antenna not in contact with the wall was moved both mediolaterally and vertically during the run. (We will examine one aspect of potential linkage between antennal movement and running behavior below.) The exact distance between a cockroach and the wall, which was usually less than 3 cm, has been reported to depend on escape speed (Camhi and Johnson, 1999) and it also depends on outside antennal length (see below).

No difference between intact and eye-covered cockroaches was observed in the general form of running, but average velocity was altered. The average running velocity of intact cockroaches was $430 \pm 17 \text{ mm s}^{-1}$ (mean \pm s.e.m., $N=5$ animals, $n=34$ trials). This was faster than for eye-covered insects ($380 \pm 23 \text{ mm s}^{-1}$, $N=5$, $n=28$; the difference is significant at $P < 0.01$, t -test). Average running velocities of cockroaches with shortened antennae tended to be slower than normal (velocity of insects with 1–2 cm antennae was $390 \pm 12 \text{ mm s}^{-1}$, $N=5$, $n=42$), but this difference is not quite significant.

One thing we noted occasionally in cockroaches with shortened antennae (1–2 cm length) was a tendency to run sufficiently close to the wall that they touched it with their body and this sometimes caused them to fall.

Definition of behaviors in response to obstacles

Several types of behavior were observed on trials with obstacles in a cockroach's path. Collision trials were straightforward – the head impacted the obstacle and immediately stopped (COLLIDE). (All cockroaches stopped after collision in the present experiment except in three cases, where they collided, stopped, turned, and escaped in the opposite direction (these were counted as collisions). Collision avoidance could be quite easily sorted into four basic types: stopping prior to impact (STOP), turning and reversing direction (REVERSE), changing path to clear the inner edge of the obstacle (REDIRECT), and climbing over (CLIMB) – see Fig. 2.

Under our present recording condition, an effective 1/60 s imaging rate, there was some chance that COLLIDE would be judged as another response such as REVERSE. However, the probability seems low because head impacts during collisions made a sound and the obstacle could be seen on tape to move or vibrate slightly. We have not noticed any sound or recorded any obstacle movement for responses we judged to be REVERSE. We did not attempt to further delineate behaviors that occurred subsequent to a collision. On trials where there was an aperture in the obstacle, going through the 'gate' (UNDER) was sometimes observed.

Experiment 1: collision avoidance by intact cockroaches

In order to investigate the specific types of behavioral responses that constituted obstacle avoidance, we observed 250 trials by 25 intact cockroaches running toward the standard-sized, transparent obstacle. In just more than half of the insects (15/25), at least one collision was observed. The proportion of collisions ranged from 2 to 33% (individual data not shown). The average frequency of collisions among all intact cockroaches was 9% (Fig. 3A). Thus the majority of responses to barriers (91%) were collision avoidance behaviors.

The most frequently observed collision avoidance measure was a STOP (80.6% of responses overall). All cockroaches (25/25) chose

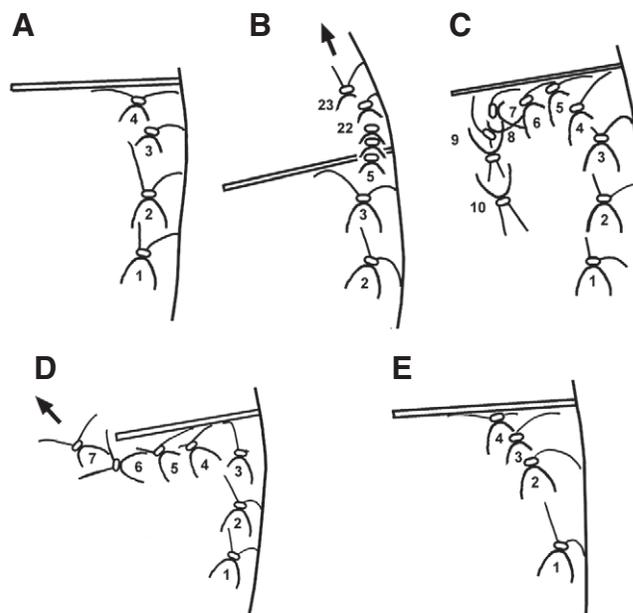


Fig. 2. Examples of the types of behavior observed at an obstacle. Each drawing is a reconstruction from frame by frame analysis of videotapes. Time between frames (numbered) was 60 ms. (A) STOP (B) CLIMB (C) REVERSE (D) REDIRECT (E) COLLIDE. Only the head and pronotum are drawn for each frame. For clarity, one antenna is occasionally omitted and the full length of the antennae is not always shown.

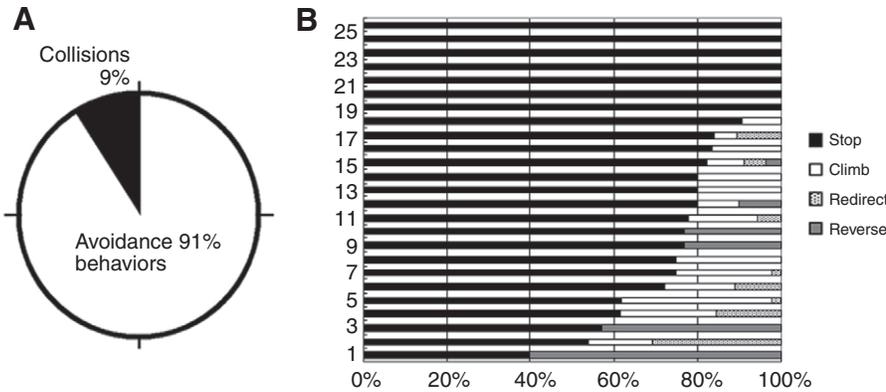


Fig. 3. Proportion of trials that were scored COLLIDE and the percentage of each type of avoidance behavior. Data are from 25 intact cockroaches. (A) summary of all responses. (B) Breakdown (horizontal bars) of trials that involved collision avoidance as defined in text (also see key). Examples of each type of behavior shown in Fig. 2 and the definition of each avoidance response is given in the text. Total data set represents 250 trials.

STOP at least once, and for seven individuals it was the only form of avoidance (Fig. 3B). CLIMB was the second most common avoidance response (10.8% overall, and at least once in 14/25 cockroaches). REDIRECT was next (4.6% overall, seen at least once in 8/25 animals) and REVERSE was least frequent (4% overall and observed at least once in 6/25 insects).

From the above data set, fifty-two trials with collision avoidance behaviors were reconstructed in detail from the video records and compared with collision trials. A key difference between trials that led to a collision *versus* avoidance was the velocity of the cockroach at the time an antenna first touched the obstacle. For example, in trials with collisions, average running velocity at first touch was $505 \pm 17 \text{ mm s}^{-1}$ ($n=26$; mean \pm s.e.m.), average running velocity on trials with a STOP was $410 \pm 16 \text{ mm s}^{-1}$ ($n=16$). This difference in running velocity in trials with a STOP *versus* COLLIDE is significant ($P < 0.01$, t -test). The average running velocity in trials with a CLIMB was $500 \pm 24 \text{ mm s}^{-1}$ ($n=10$), and this mean velocity is not significantly different from trials with collisions. We did not have a large sample of REVERSE responses to analyze, but what data we had suggested that these full turning responses might be associated with running at lower velocities (for five REVERSE trials the average was $430 \pm 25 \text{ mm s}^{-1}$).

A common characteristic of all trials in which a STOP was observed was that at least one antenna was visibly bent by contact with the obstacle, especially the antenna toward the center of the arena that was not in contact with the wall. From detailed analysis of the video records of these trials, we saw evidence that more than one type of contact with an obstacle can be effective. Sometimes the position of the insect at the time of stopping was close (less than 1 cm) and there appeared to be contact with the obstacle by, (1) the antenna held toward the center, which was bent along its length and/or (2) the prothoracic legs. In other cases, the STOP occurred about 2.5–1.0 cm before the obstacle and only the tip (most distal 25%) of the antennal flagellum touched the obstacle and was visibly bent.

It is also worth noting that on about 60% of trials with a COLLIDE cockroaches elevated the body after touching the obstacle with the ‘center-side’ antenna and also elevated the prothoracic legs against the obstacle. This behavior is similar to that observed during the initial phase of CLIMB responses (Watson et al., 2002). It is tempting to suggest that these collisions represent failures to fully initiate a CLIMB response despite the presence of the obstacle.

Experiment 2: testing a visual contribution to obstacle avoidance

One group of six animals was first tested for the possibility that vision could be a sensory channel alerting running cockroaches to

an obstacle. We predicted that if vision was important in collision avoidance, (1) the frequency of collisions should be increased by covering the eyes to block vision, and (2) the frequency of collisions should be reduced by giving the obstacle high visual contrast with the white arena (by painting the obstacle black).

When these animals were intact, the frequency of trials that were scored COLLIDE was 10% ($\pm 2.9\%$) with the standard obstacle, and 18% ($\pm 3.7\%$) with the eyes covered. When the obstacle was painted black to increase visual contrast, collision rate was measured at 8% ($\pm 2.9\%$). An analysis of variance showed that only one individual comparison just reached significance (that between the eye covered and intact animals with the black object; F -test; $P < 0.05$; see Fig. 4).

Experiment 3: cockroaches with altered flagellar length or innervation

In cockroaches with normal vision but shortened antennae, the collision rate depended on antennal length (Fig. 5). The collision rate of the animals prior to cutting their antennae was 10% (six animals, 60 trials). When the antennae were shortened to 3–4 cm, COLLIDE occurred in 47% of trials. With yet shorter antennae, the frequency of collision increased even further: to 70% with antennae of 2–3 cm, and to 97% with antennae of 1–2 cm (in all groups: $n=30$ trials; Fig. 5A). ANOVA indicated that antennal length significantly affected collision rate; $P < 0.01$.

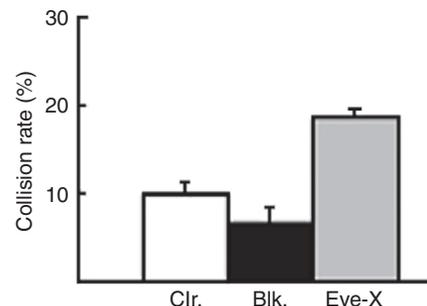


Fig. 4. Visual information had little influence on the collision rate. The percentage of trials that were scored COLLIDE for animals in each condition. Bars show the standard error of the mean, $N=6$ animals and 60 trials for each group. White bar, clear obstacle (Clr.) and intact cockroaches. Black bar, black painted obstacle (Blk.) and intact cockroaches. Gray bar, clear obstacle and cockroaches with both eyes covered (Eye-X). The obstacle was standard size in all of these experiments. The percentage collisions with eyes covered is just significantly different from the percentage for intact animals interacting with the black obstacle (F -test, $P < 0.05$). No other differences are statistically significant.

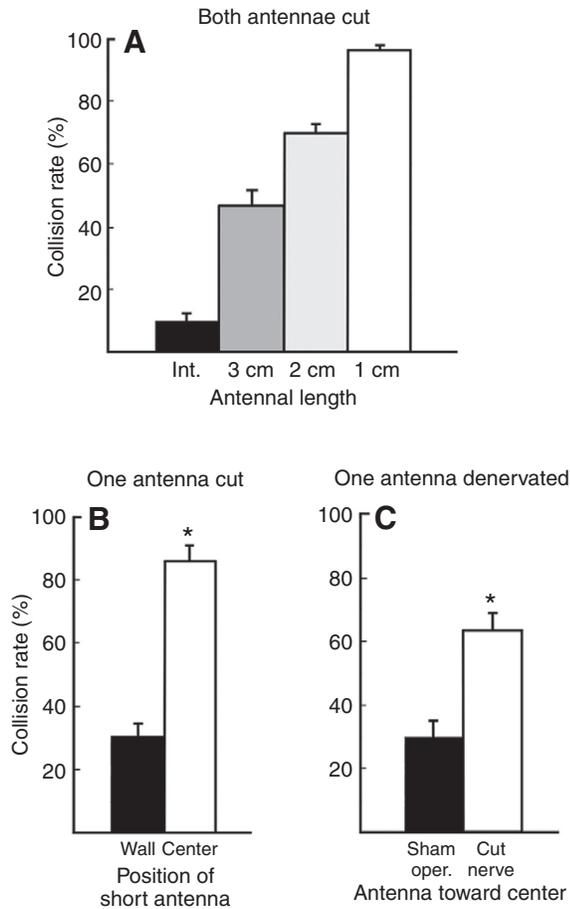


Fig. 5. Collision rate of cockroaches with altered antennae. Height of each bar gives the percentage of all trials scored as COLLIDE, with error bars indicating the standard error for collision rates averaged across five animals. (A) Animals with various lengths of antennae interacting with the standard obstacle. Int., intact antenna; lengths of antennae in other groups were from 1–3 cm as shown. (B) Animals with one shortened antenna (1 cm length) and one intact antenna. Black bar: intact antenna toward center of arena. White bar: shortened antenna toward center of arena. (C) Animals with one antennal nerve cut, or control animals (Sham oper.) that were operated on, but the nerve was not cut. All data in this histogram come from trials where the denervated antenna, or sham-operated antenna, was toward the center of the arena. Asterisks indicate statistically significant differences as described in the text (the trend of differences in panel A is also significant).

When one of the two antennae was shortened, the importance of the center-side antenna (away from the arena wall) became obvious. When the center-side antenna was short, the percentage of COLLIDE responses was 86% (four animals, 40 trials; Fig. 5B, white bar). By contrast, when the center-side antenna was the longer intact one, it was only 30% (four animals, 40 trials; Fig. 5B, black bar). This difference is significant at $P < 0.001$ (χ^2 -test).

In cockroaches with normal length antennae, but with one antennal nerve transected, the frequency of COLLIDE responses was 63% (when the denervated antenna was toward the center of the arena; Fig. 5C, white bar). However, for the controls, with the cuticle opened but the nerve uncut, the frequency of COLLIDE responses was 30% (when the antenna serving as surgical control was toward the center of the arena; Fig. 5C, black bar). The difference between these two groups is also significant at $P < 0.001$ (χ^2 -test).

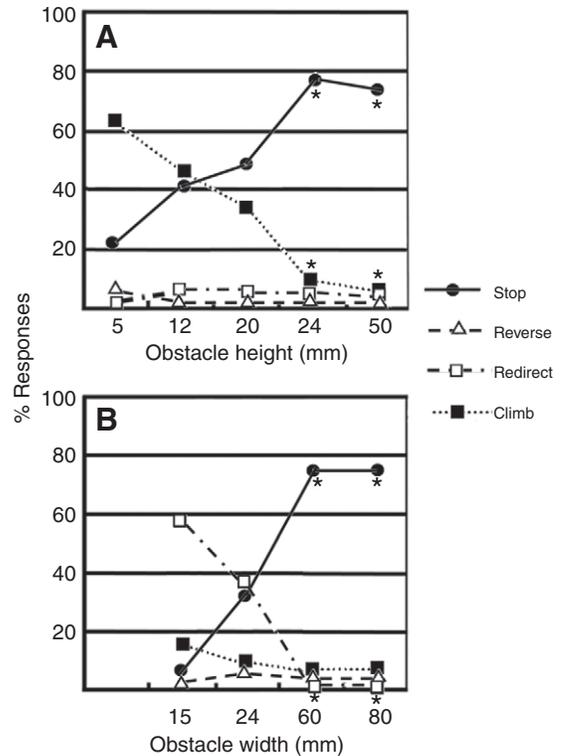


Fig. 6. Relationship between obstacle size and shape, and type of avoidance behavior. (A) Avoidance behaviors with obstacles of different heights. (B) Avoidance behaviors with changes in obstacle width. % response is the percentage of all trials that resulted in each type of response, as indicated by the key. Asterisks indicate statistically significant difference in the indicated parameter from the value at minimal height or width.

Experiment 4: the effect of obstacle geometry

From the group of 25 intact animals we used to characterize the various types of responses to obstacles, we randomly selected 10 cockroaches and conducted tests of responses to obstacles of different dimensions and orientations.

4A: basic obstacle dimensions

Height

The frequency of trials scored as COLLIDE was not significantly altered by any of the height changes we made, ranging from only 10% to 13% in all types of trials (data not shown). The frequencies of REVERSE and REDIRECT responses also were low across all heights tested (Fig. 6A). However, the height of an obstacle affected the frequency of STOP and CLIMB responses. The frequency of CLIMB responses decreased and the frequency of STOP responses increased as obstacle height increased (Fig. 6A). The frequency of STOP responses went from 23% at 5 mm to 78% at 24 mm obstacle height. Correspondingly, the frequency of CLIMB responses went from 63% at 5 mm to 11% at 24 mm obstacle height. Analysis of variance revealed that there was a significant impact of height on both CLIMB and STOP responses, but not of other categories ($P < 0.001$, ANOVA).

The importance of antennal mechanosensation could be directly observed in this experiment. In the situation where STOP responses were infrequent – when encountering the lowest obstacle (5 mm) – all trials that resulted in a STOP response (7/7) involved bending

of the center-directed antenna (that away from the wall) against the obstacle.

Width

The frequency of trials scored as COLLIDE was not significantly altered by any of the width changes we made, ranging from 10% to 20% on all types of trials (data not shown). As obstacle width went from narrow to wide, the frequency of REDIRECTs (turning to skirt the inner edge of the obstacle) was reduced. It was at its highest level (58%) with a narrow obstacle and dropped to near zero (2%) with the two widest obstacles. Correspondingly, the frequency of STOP trials increased as width increased from a low of 8% to a high of 76% (Fig. 6B). Analysis of variance revealed that the changes in these responses with width were significant ($P < 0.001$, ANOVA), but that the frequency of the other types of response did not vary significantly over this range of obstacle widths.

4B: obstacle orientation and surface features

Angle of encounter

The frequency of trials scored as COLLIDE was not significantly altered by changes in the angle of encounter, ranging from 6% to 12% on all types of trials (data not shown). As the angle between the run path and the obstacle went from its typical orthogonal orientation to either an acute angle or an obtuse angle, avoidance behavior changed. First, the frequency of STOP responses was highest (84%) when the obstacle was directly orthogonal to the run path. Second, the frequency of CLIMB behavior was highest at acute angles and lowest at obtuse angles. The frequency of REDIRECT responses increased from near zero at acute angles to more than 40% at obtuse angles. Analysis of variance revealed that both CLIMB and REDIRECT categories were significantly influenced by barrier angles ($P < 0.01$, ANOVA). The other types of response were consistently below 20% and did not change significantly as a function of angle of encounter.

Aperture

Cockroaches are widely known to move through narrow spaces. Therefore, another aspect of the response to geometry that we checked was the ability to interact with apertures while running. For this we used a 'gate obstacle' (Fig. 1D). When aperture height was greater than 2 mm from the substrate, the rate of entering UNDER the aperture increased from near zero to a high of 43% (Fig. 7B). There was an opposite pattern in the frequency of trials with a STOP response, which decreased as the aperture size increased. Both of these changes with aperture height were statistically significant ($P < 0.01$, ANOVA). Interestingly, there was an increase in the frequency of COLLIDE responses whenever the aperture was present. Without the aperture, this group of insects collided in 10% of trials, but this number increased to between 40 and 60% when any aperture was present. This observation suggests that animals may be influenced (fooled?) by having an antenna pass into an aperture on some trials. We could not clearly see the antennae on all COLLIDE trials, but on those where we could, at least one antenna did pass into the aperture. This is at least consistent with a model where collisions can become more frequent at some apertures if the aperture decreases the probability of antennal contact during obstacle approach.

Experiment 5: antennal movement during running by tethered and free-moving cockroaches

The range of antennal movements we observed in a group of ten tethered, but running, insects depended on leg movement cycles.

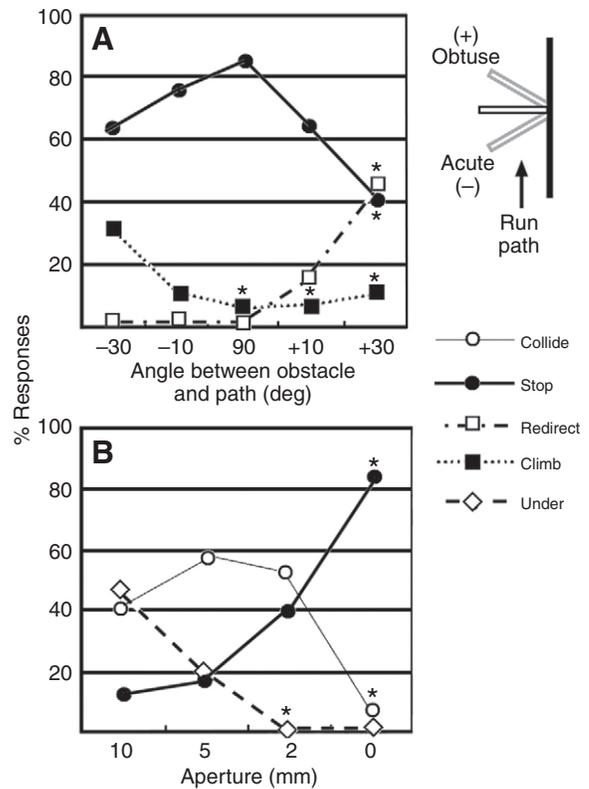


Fig. 7. Relationship of specific obstacle geometry to frequency of collisions or type of avoidance behavior. (A) Avoidance behaviors of obstacles at different angles. Horizontal axis is obstacle orientation with respect to run path. Vertical axis is the percentage of all trials that resulted in each type of response. Scheme for obstacle orientation with respect to run path is shown on the right. (B) Collision and avoidance behavior in response to 'gate' obstacles with different sized apertures. Horizontal axis is height of the 'gate' or aperture, vertical axis is the same as in A. Asterisks indicate statistically significant difference in the indicated parameter from the value at the opposite end of the geometric range.

Usually, the position of the antennae in the horizontal plane during locomotion was from 0 deg to 70 deg (antennal position of 0 deg being straight ahead or parallel to body axis, 90 deg being directly lateral), and from -20 deg from 40 deg in the vertical plane (antennal position of 0 deg in this plane being parallel to the long axis of the body and the ground, angles above are positive and below are negative). During relatively slow running (leg cycles 2–4 Hz), the average antennal position in the horizontal plane was 43.0 ± 5.4 deg (mean \pm s.e.m.; $n=40$). In the vertical plane, the average position was 18.4 ± 7.2 deg ($n=40$). As the frequency of the leg movement increased, the mean antennal position became more centered and range of movement decreased in both horizontal and vertical dimensions (Fig. 8). The net effect was that although the antennae remained in motion during running, they were pointing anterior to the head and near the mid-horizontal plane a greater portion of the time.

Horizontal antennal movement during escape in freely moving cockroaches was so fast, and the antennae so slender, that we could not generally record it well on video. We also could not detect ventral movement because of the angle of the camera. However, we found four trials where the video records were particularly good so we checked to see if they suggested congruence with our results in tethered preparations. The average horizontal antennal position on

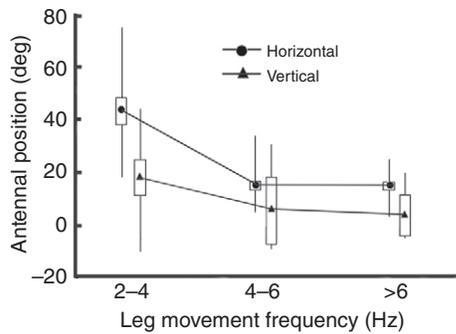


Fig. 8. Relationship of leg movement rate to typical antennal position and movement range during running. Horizontal axis is leg movement frequency expressed as cycles per second (Hz). Vertical axis is the average antennal position in the horizontal plane (circles) and the vertical plane (triangles). The orientation of these planes is described in the text. Boxes around each mean indicate the standard error of the mean; vertical lines show the angular range of all observed movements.

these trials was 29.0 ± 1.6 deg ($n=40$). These data are well within the range of mean positions we saw in tethered preps and would be consistent with a rapid rate of locomotion – which was typical of free-ranging trials. We suggest that antennal movement in freely running animals is restricted to more forward angles in ‘obstacle detection mode’ as estimated from measurements in tethered animals. Further quantification of antennal movement during fast runs will require higher speed recording methods.

Experiment 6: the antennal logic underlying the STOP response

When obstacles were moved toward stationary cockroaches that were already moving their legs, STOP responses were induced. If, because of antennal movements, one antenna was put under or over the approaching obstacle while the other antenna made contact, cockroaches kept moving the legs until the obstacle reached the head (COLLIDE). This suggested the importance of near simultaneous contact with both antennae (under conditions where the animal is not running with one antenna perhaps already in contact with a wall). STOPS that occurred were typically recorded when obstacles were about 1 cm in front of the tethered cockroach. With each flagellum typically several centimeters in length, substantial bending of a flagellum was easy to observe in these tethered experiments. Slight bending of the most distal segments at the tip of the antennal flagellum did not trigger STOP responses.

In the baseline conditions for these tests, an armature was moved toward a tethered cockroach that had one ‘obstacle’ (really two separate obstacles, but at the same distance with respect to the insect, see Fig. 9A). Because the ‘obstacles’ could be independently positioned, some trials also were conducted in which the two ‘obstacles’ were at different distances from the insect. Thus contact with the left and right antenna was largely independent and bending of the flagellum on one side would occur without bending the other. We thus obtained a sample of cases where approach of an ‘obstacle’ occurred with corresponding contact of both left and right flagella, and cases where an ‘obstacle’ also approached but only one flagellum was contacted (sometimes the left, others the right). These data were collected from a group of 10 animals. On trials with dual antennal contact, STOP responses were frequent (collision rate=32%, $n=40$ trials; Fig. 9B). However, on trials where only one

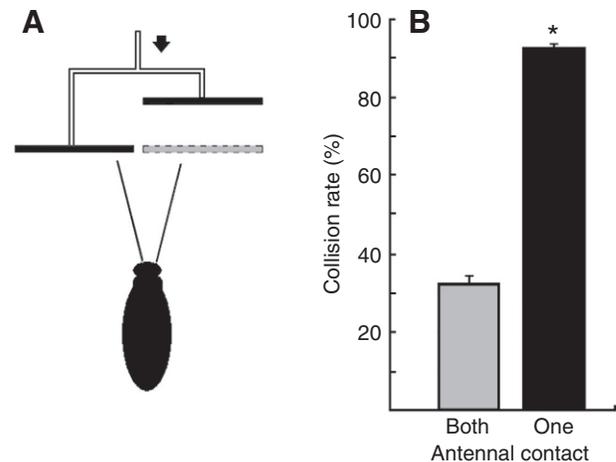


Fig. 9. The antennal logic of the STOP signal. (A) The set up for the test. (B) The collision rate when one antenna contacted the obstacle (black bar) and when two antennae contacted the obstacle (gray bar).

antennal flagellum made contact and was bent, STOP responses were much less frequent (collision rate=93%, $n=40$ trials; Fig. 9B). The difference in frequency of collision under the two conditions is significant at the level of $P < 0.05$ (χ^2 -test).

DISCUSSION

There is a substantial literature on the neurobiology and biomechanics of running by insects in general, and cockroaches in particular (e.g. Delcomyn, 1985; Full and Tu, 1990). In addition, it has long been known that cockroach escape consists of rapid running, often preceded by an initial turn away from a threatening stimulus (e.g. Camhi, 1984; Ritzmann et al., 1991; Comer and Dowd, 1993; Ye et al., 2003). However, much less is known about the mechanisms by which rapid locomotion is guided by systems that can react quickly to perturbations. Our data revealed that collisions are avoided on most occasions (about 90% of trials; Fig. 3A) so there must be fairly reliable sensorimotor mechanisms for obstacle detection and avoidance. Two sensory systems would seem suited to warning a running cockroach of an obstacle: vision and mechanosensation delivered through one or both of the antennae.

Obstacle avoidance as a decision

The initial finding that cockroaches have a small repertoire of behavioral possibilities when encountering an obstacle during a run indicates that there is more to collision avoidance than simply throwing an ‘off’ switch. At least four different types of responses to obstacles were observed (Fig. 2) with differing frequencies: STOP>CLIMB>REDIRECT>REVERSE. It is also notable that animals differed individually in their tendencies. Some only displayed one type of response (STOP), but others displayed two, three or four of the possible responses (Fig. 3). Many of our experiments were aimed at determining the factors that cause animals to make one response *versus* another.

Clearly, variations in obstacle size (experiment 2) and geometry and orientation (experiment 4) resulted in different patterns of response (Figs 6 and 7). The nature of the ‘choices’, e.g. tending to climb in response to shorter obstacles, or tending to redirect locomotion around the edge of obstacles oriented at oblique angles with respect to run direction, make sense in terms of insuring progress of the run despite the presence of an obstacle. In addition

the timing of these responses is of note. Any processing of sensory input that underlies these decisions must be rapid because a response has to be initiated within a time window in the order of 50–100 ms if a collision is to be avoided (see below). This also means that collisions may sometimes occur when the physical limitations of stopping in time were exceeded, and need not always indicate that the obstacle went undetected.

CLIMB was the second most frequently chosen response to the normal obstacle in the present study. Climbing has been well studied in another cockroach, *Blaberus discoidalis* (Watson et al., 2002; Harley et al., 2009). Climbing behavior in *Blaberus* is influenced by antennal contact with the top surface of an obstacle (Harley et al., 2009). The present study seems generally consistent because we found that the frequency of climbing increased with low obstacle height (Fig. 6A). However, it should be noted that our obstacles were thin and presented little in the way of an upper surface as would be provided by a ledge or an obstacle with depth.

Ultimately, the question of the extent to which cockroaches can actually make choices using antennal information at short latencies, must be answered neurophysiologically. It will be necessary to identify the specific sensory pathways involved and demonstrate that they can activate appropriate motor pathways at short latency during bouts of locomotion. We believe a preparation with a tethered animal receiving highly controlled antennal stimulation (such as in experiment 6) will be useful in such experiments.

Does vision contribute to obstacle avoidance?

The fact that animals with their eyes covered ran at lower average velocities than intact animals (experiment 1) is consistent with a previous report on the effect of vision on the run phase of escape (Ye et al., 2003). It might have been expected in the present study that the lower running velocity would help reduce the tendency of blind cockroaches to collide with obstacles, but it may also help cockroaches with shortened antennae in the same way. Although we did not collect data in a way that could be used to directly test these ideas, there are hints that running speed makes a difference. *Post-hoc* analysis of trials with collisions by intact animals showed that they involved the high end of running speeds (about 500 mm s⁻¹), whereas those where collision was avoided were clearly at lower speeds (closer to 400 mm s⁻¹).

The data from experiment 2 provide perhaps weak evidence for visual control of obstacle avoidance. We predicted that making the obstacle more salient for visual detection would decrease collisions, and that blocking vision would increase collisions. The data showed only small effects in these directions, but the overall trend was just statistically significant (Fig. 4). So this would support the idea that vision might play a small role in detection of obstacles during running. Given the form of the data, however, we would not be surprised if more extensive tests revealed a larger impact for visual cues, under some conditions. Indeed, in *Blaberus* it has been found that visual context (detected by the ocelli) may influence what sort of response an animal has to some obstacles (Harley et al., 2009). It should be emphasized that our animals with eyes covered did not have their ocelli covered. The idea that light input may modify interactions with obstacles is intriguing and should be a fertile area of future investigation in *Periplaneta* as well as *Blaberus*.

It is worth noting that the apparent collision rate in our animals with eyes completely covered, just below 20%, is much less than the clear-cut elevations in collision rate seen after various alterations of the antennae. This implies that any role of vision in obstacle detection or avoidance under the conditions we tested is, at best, secondary to the role of mechanosensory cues of antennal origin.

Centrality of the antennal flagella to avoidance

Active exploration of the environment with the antennae may guide orientation toward objects of interest. This sort of orientation is believed to depend on antennal mechanosensory information derived from hair plates at the base of the antennae (Okada and Toh, 2000; Okada and Toh, 2001). The behavior we were testing here is different from exploratory searching and orientation mostly in the time domain: the insects in our study were running at high velocity and challenged to react to obstacles rapidly. Given that a cockroach antenna is a bit longer than one body length (typically about 5 cm), animals running at the velocities observed here (approximately 400 mm s⁻¹) would have to react within times as short as 50–100 ms to avoid a collision if using the antennae (latency depending to some extent on the orientation of an antenna on the head). Given that flagellar receptors are believed to help cockroaches space themselves with respect to a wall – and this spacing behavior can be adjusted multiple times per second (see Camhi and Johnson, 1999) – then flagellar receptors are reasonable candidates for obstacle detection and avoidance.

Consistent with this idea, we observed that flagellar length and innervation was a critical factor in collision-avoidance behavior. Trimming one flagellum (or both) increased collisions in a way that was scaled to the length of the flagellum that remained (Fig. 5A,B). Cutting the main sensory nerve in the proximal flagellum also increased collisions to a significant extent (Fig. 5C). With the nerve cut, collision frequency was about 60%, and with the antennae at 1–2 cm length it exceeded 80%. Although this indicates involvement of flagellar receptors, it leaves at least some room for involvement of other, basal receptors that may be activated by mechanical transmission of impact towards the base *via* the cuticle, where basal receptors could then be activated (see Baba and Comer, 2007). One aspect of our data consistent with this suggestion is that the control animals in experiment 3, in which only the cuticle was cut, did not display the expected background rate of collisions seen intact animals (10%), but displayed an increased collision rate of 30% (Fig. 5C). This may have been because excising the cuticle near the base of the flagellum reduces mechanical transmission from the distal to the proximal antenna. This point is currently under investigation.

How is the sensorimotor organization of running adapted for collision avoidance?

Throughout the course of this study, numerous observations were made that suggested that there is a cluster of sensorimotor synergies that represent ways that running is automatically made flexible in an unpredictable running environment. First, animals were somewhat less likely to collide with an obstacle if they were running at a lower velocity, and when vision was compromised running velocity was reduced (experiment 1). This makes sense if one assumes that vision plays at least a small role in obstacle detection and we believe that this is likely.

Second, active antennal movements are related to running behavior in a useful way. As is true for other insects (e.g. Dürr et al., 2001) antennal movements are related to the cycle period of leg movements. In particular, antennal movements became more restricted spatially as locomotion speed increased and their orientation was then typically directed more forward (experiment 5; see Fig. 6). Apparent differences between tethered and free-ranging animals need to be confirmed with more precise methods, and such methods might reveal any quantitative dependency of avoidance choices on antennal angle on a trial-by-trial basis. Additionally, it will be of interest to map the trajectory of each

antenna (that toward the wall, and that away) at high resolution, as escape and obstacle interactions proceed. Nonetheless, we suggest a shifting of the function of the antennae during locomotion from a general exploratory mode to an obstacle-detection mode.

What neural mechanisms might underlie obstacle detection?

Bending of the distal flagellum on at least one antenna was observed to be associated with avoidance behavior in experiment 4 and in observations on tethered animals. Additionally, in the aperture experiment, failure of an antenna to impact the obstacle – by moving through the aperture – resulted in collisions. Because running animals typically had one antenna in contact with the wall to their side, the logic of a signal to adjust the run may be the abrupt stimulation of the second antenna. This was tested in additional studies of tethered cockroaches (experiment 6). The data suggest that impact of one antenna with a surface or an object is not sufficient to reliably trigger a STOP or other avoidance measures, but that near simultaneous impact of both antennae with objects and/or surfaces is highly efficacious in triggering collision-avoidance responses. This makes sense for an animal that often runs with one antenna opposed to a surface.

Our data suggest that the disposition of each antenna is important as an obstacle is approached. In the present experiments, the time resolution of our video system was not fast enough to map the trajectory of each mobile antenna throughout a behavioral response. It will be important in future studies to precisely describe the dynamics of the antennae to fully define the logic of antennal control of collision avoidance and to rule out simple physical and biomechanical factors in producing changes in running trajectory *versus* active involvement of antennal mechanosensation. Parallel neurophysiological studies also will be required.

Finally, we are unsure if there is a smooth transition from running to avoidance that is mediated by antennal signals in a simple way. Given the rate at which we sampled behavior, it is always possible that there are changes in velocity or even short pauses occurring that we did not detect. Higher sampling rates for behavior should be able to dissect the run and interaction with various obstacle at a millisecond-by-millisecond level.

Obstacle avoidance in relation to other behaviors

One behavior that has some similarities with the obstacle avoidance studied here is wall following. This is an antennal-mediated behavior and appears to depend on bending of the distal flagellum (Camhi and Johnson, 1999; Cowen et al., 2006). Indeed, because cockroaches make small turns to follow contours of a vertical surface, wall following may overlap with at least one of the obstacle-avoidance tests used here – the response to an oblique obstacle. The tendency of cockroaches to successfully run around the edge of a barrier, and especially when it was at an obtuse angle with respect to run direction (see Fig. 7) suggests that there is an area where obstacle avoidance is not very different from wall following. This is an example of a situation in which mapping antennal movements with precision and being able to visualize antennal impacts may prove helpful. It could allow us to determine where steering along an uneven wall ends (perhaps relying mostly on the outer antenna), and where obstacle avoidance begins (perhaps requiring stimulation of the inner antenna).

The most noteworthy general point from these experiments is the complexity of the behavioral constellation of obstacle avoidance responses that are integrated with running. There are multiple types

of avoidance responses, their control is determined at short latencies, and a host of sensory and motor details seem designed to work together to make running sensitive to obstacles that may unpredictably appear during evasion or other high-speed runs. Understanding the evolution of evasive running will need to consider design features of multiple sensory and motor circuits.

ACKNOWLEDGEMENTS

This research was supported by grant no. 04-22883 from the National Science Foundation. We thank Yoshiya Baba for invaluable assistance with the collection of data. Two anonymous reviewers provided comments that have substantially improved the manuscript.

REFERENCES

- Baba, Y. and Comer, C. M. (2007). Characterization of sensory neurons and interneurons receiving information from chordotonal organs in cockroaches. *Soc. Neurosci. Abstr.* 211.23.
- Baba, Y. and Comer, C. M. (2008). The antennal motor system of cockroaches. *Cell Tissue Res.* 331, 751-762.
- Baba, Y., Tsukada, A., Hansen, A. N., Baba, Y. J. and Comer, C. M. (2004). Control of cockroach antennal movements in relation to visual attention. *Soc. Neurosci. Abstr.* 555, 2.
- Baba, Y., Tsukada, A., Beyerlein, A., Baba, Y. J. and Comer, C. M. (2005). Antennal input works for cockroaches' avoidance and gap crossing behavior. *Soc. Neurosci. Abstr.* 176, 16.
- Camhi, J. M. (1984). *Neuroethology*, pp. 79-104. Sunderland, MA: Sinauer Associates.
- Camhi, J. M. and Johnson, E. N. (1999). High-frequency steering maneuvers mediated by tactile cues: antennal wall-following in the cockroach. *J. Exp. Biol.* 202, 631-643.
- 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. 88-112. New York: Academic Press.
- Comer, C. M., Mara, E., Murphy, K. A., Getman, M. E. and Mungy, M. C. (1994). Multisensor control of escape in the cockroach *Periplaneta americana*. II. Patterns of touch-evoked behavior. *J. Comp. Physiol. A* 174, 13-26.
- Comer, C. M., Parks, L., Halvorsen, M. B. and Breese-Tertling, A. (2003). The antennal system and cockroach evasive behavior II. Stimulus identification and localization and separable antennal functions. *J. Comp. Physiol. A* 189, 97-103.
- Cowan, N. J., Lee, J. and Full, R. J. (2006). Task-level control of rapid wall following in the American cockroach. *J. Exp. Biol.* 209, 1617-1629.
- Delcomyn, F. (1971). The locomotion of the cockroach *Periplaneta americana*. *J. Exp. Biol.* 54, 443-452.
- Delcomyn, F. (1985). Factors regulating insect walking. *Annu. Rev. Entomol.* 30, 239-256.
- Dürr, V., König, Y. and Kittmann, R. (2001). The antennal motor system of the stick insect *Carausius morosus*, anatomy and antennal movement pattern during walking. *J. Comp. Physiol. A* 187, 131-144.
- Erber, J., Pribbenow, B., Grandy, K. and Kierzek, S. (1997). Tactile motor learning in the antennal system of the honeybee (*Apis mellifera* L.). *J. Comp. Physiol. A* 181, 355-365.
- Full, R. J. and Tu, M. (1990). Mechanics of six-legged runners. *J. Exp. Biol.* 148, 129-146.
- Full, R. J. and Tu, M. (1991). Mechanics of a rapid running insect. Two-, four-, and six-legged locomotion. *J. Exp. Biol.* 156, 215-231.
- Gras, H., Hörner, M. and Schürmann, F.-W. (2004). A comparison of spontaneous and wind-evoked running modes in crickets and cockroaches. *J. Insect Physiol.* 40, 373-384.
- Harley, C. M., English, B. A. and Ritzmann, R. E. (2009). Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*. *J. Exp. Biol.* 212, 1463-1476.
- Honegger, H.-W. (1981). A primary note on a new optomotor response in cricket, antennal tracking of moving targets. *J. Comp. Physiol.* 142, 419-421.
- Okada, J. and Toh, Y. (2000). The role of antennal hair plates in object-guided tactile orientation of the cockroach (*Periplaneta americana*). *J. Comp. Physiol. A* 186, 849-857.
- Okada, J. and Toh, Y. (2001). Peripheral representation of antennal orientation by the scapal hairplate of the cockroach. *J. Exp. Biol.* 204, 4301-4309.
- Ritzmann, R. E., Pollack, A. J., Hudson, S. E. and Hyvonen, A. (1991). Convergence of multimodal sensory signals at thoracic interneurons of the escape system of the cockroach, *Periplaneta americana*. *Brain Res.* 563, 175-183.
- Ritzmann, R. E., Pollack, A. J., Archinal, J., Ridgel, A. L. and Quinn, R. D. (2005). Descending control of body attitude in the cockroach *Blaberus discoidalis* and its role in incline climbing. *J. Comp. Physiol. A* 191, 253-264.
- Watson, J. T., Ritzmann, R. E., Zill, S. Z. and Pollack, A. J. (2002). Control of obstacle climbing in the cockroach, *Blaberus discoidalis*. I Kinematics. *J. Comp. Physiol. A* 188, 39-53.
- Ye, S. and Comer, C. M. (1996). Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, *Periplaneta americana*. *J. Neurosci.* 16, 5844-5853.
- Ye, S., Leung, V., Khan, A., Baba, Y. and Comer, C. M. (2003). The antennal system and cockroach evasive behavior. I. Role for visual and mechanosensory cues in the response. *J. Comp. Physiol. A* 189, 89-96.