Wind generated by an attacking bat: anemometric measurements and detection by the praying mantis cercal system

Jeffrey D. Triblehorn* and David D. Yager

Department of Psychology, University of Maryland, College Park, MD 20742, USA

*Author for correspondence at present address: Department of Biology, Georgia State University, Atlanta, GA 30303, USA
(e-mail: jtriblehorn@hotmail.com)

Summary

The wind-sensitive cercal system, well-known for mediating terrestrial escape responses, may also mediate insect aerial bat-avoidance responses triggered by wind generated by the approaching bat. One crucial question is whether enough time exists between detection and capture for the insect to perform a successful evasive maneuver. A previous study estimated this time to be 16 ms, based on cockroach behavioral latencies and a prediction for the detection time derived from a simulated predator moving toward a simulated prey. However, the detection time may be underestimated since both the simulated predator and prey lacked certain characteristics present in the natural situation. In the present study, actual detection times are measured by recording from wind-sensitive interneurons of a tethered praying mantis that serves as the target for a flying, attacking bat. Furthermore, using hot-wire anemometry, we describe and quantify the wind generated by an attacking bat. Anemometer measurements revealed that the velocity of the bat-generated wind consistently peaks early with a high acceleration component (an important parameter for triggering wind-mediated terrestrial responses). The physiological recordings determined that the mantis cercal system detected an approaching bat 74 ms before contact, which would provide the insect with 36 ms to perform a maneuver before capture. This should be sufficient time for the mantis to respond. Although it probably would not have time for a full response that completely evades the bat, even a partial response might alter the mantid's trajectory enough to cause the bat to mishandle the insect, allowing it to escape.

Key words: mantis, bat, escape, wind, insect, cercal.

Introduction

The wind-sensitive cercal sensory system is an ancient structure possessed by many insects (Boudreaux, 1979; Hennig, 1981; Edwards and Reddy, 1986; Boyan and Ball, 1990). The cerci are two posterior appendages that contain many hair types, including filiform hairs that are sensitive to air currents. Afferents from these hairs project to the terminal ganglion where they synapse with ascending interneurons that carry the sensory information rostrally in addition to local interneurons (Burrows, 1996). In many species, the ascending interneurons have large axon diameters and are classified as giant interneurons (GIs). The cercal-GI system mediates terrestrial escape responses in several insects including cockroaches (Camhi, 1984; Ritzmann, 1984), crickets (Tauber and Camhi, 1995) and firebrats (Edwards and Reddy, 1986). However, other functions for the GIs have also been identified. These include providing sensory feedback during cricket song production (Kämper and Dambach, 1981; Kämper, 1984; Kämper and Dambach, 1985), serving as the detectors of wind-generated social signals in non-singing crickets (Kämper and

Dambach, 1979), and playing a role in flight initiation and maintenance (Fraser, 1977; Altman, 1983; Boyan et al., 1986; Libersat et al., 1987; Libersat and Camhi, 1988).

In flying insects possessing cercal systems, another hypothesized role for the GIs is mediating aerial evasive responses to avoid bat predation, triggered by detecting wind generated by an approaching bat (Ganihar et al., 1994). Although some insects possess auditory systems sensitive to the ultrasonic bat echolocation calls and perform evasive maneuvers effective in eluding these predators (Miller and Surlykke, 2001), most nocturnal flying insects lack these specialized active defenses. However, many of these insects do possess a cercal-GI system and could potentially benefit from engaging in evasive maneuvers triggered by the wind generated by an approaching bat.

Ganihar et al. (Ganihar et al., 1994) described cercal-mediated wind-evoked responses during tethered flight in the cockroach *Periplaneta americana*, an insect that lacks sensitivity to ultrasound. Side-directed wind initiated movements consistent with turns away from the source of the

stimulus in free flight. The investigators concluded this turning away behavior suggested an escape function whereas a turn toward the stimulus would suggest a flight correction function.

One of the issues with the bat-generated wind hypothesis is timing; does an insect have enough time between detecting the bat and being captured to perform an effective evasive maneuver? Based on the fastest behavioral component of the *P. americana* aerial response (wing phase change, 38 ms) and a detection time based on a moving predator simulation (54 ms), Ganihar predicted that a cockroach has 16 ms to evade the bat (Ganihar et al., 1994). Although some escape responses occur in this amount of time, i.e. crayfish tailflip <20 ms (Krasne and Wine, 1984) and fish Mauthner-mediated C-start 14 ms (Eaton et al., 1991), many responses have latencies of 40 ms or more, i.e. cricket terrestrial response 87 ms (Tauber and Camhi, 1995), locust jump up to 1100 ms (Heitler, 1974), insect flight responses 40–240 ms, depending on insect and response type (Nolen and Hoy, 1986; Yager et al., 1990). However, Ganihar et al. (Ganihar et al., 1994) noted that their simulated predator lacked certain characteristics of an actual attacking bat. For example, their simulated predator moved at a constant velocity throughout an attack while a bat decelerates before it captures a target (Schnitzler, 1987; Jones and Rayner, 1988). Also, a bat flapping its wings might generate more wind than their model. Both of these factors suggest that the amount of wind generated by an actual attacking bat and the amount of time between detection and capture were underestimated. Finally, instead of using the cockroach's cercal system to detect the wind generated by the simulated approaching predator, the investigators substituted smoke or *Lycopodium* spores. When the simulated predator's approach disturbed the continuous smoke/*Lycopodium* stream determined the detection time. However, the smoke/*Lycopodium* stream may not accurately reflect the actual physiological cercal response of the cockroach.

The present study addresses these issues using two methods. First, using hot-wire anemometry, we describe and quantify the wind generated by a flying bat attacking a target. Second, electrodes implanted in the abdomen recorded ascending wind-evoked neural activity from an insect that served as the target during the attack of a flying bat. Both methods provide actual measurements, rather than predictions, to evaluate the bat-generated wind hypothesis, *via* better understanding of both the wind stimulus generated by a bat when capturing a target and the reception of this wind by the cercal system.

This experiment used the praying mantis *Parasphendale agrionina* as a target. Mantids possess an ultrasound-sensitive auditory system (Yager and Hoy, 1989) and perform evasive maneuvers effective for eluding bats (Yager et al., 1990). However, they could also benefit from evasive responses triggered by bat-generated wind that are independent of the ultrasound-mediated responses. First, Triblehorn and Yager (Triblehorn and Yager, 2002) demonstrated that 501-T3, the ultrasound-sensitive interneuron likely involved in triggering mantis evasive responses, shuts down during the last 200–300 ms of a bat attack. Furthermore, activity from other

ultrasound-sensitive neurons was not observed during this period. These results indicate that ultrasound will not trigger any 'last-ditch' evasive responses in mantids. Second, as bats capture insects, their vocalization emission rate increases from a very low (10–15 pulses s⁻¹) to very high rates (over 100 pulses s⁻¹). Rapid transitions from low to high rates can potentially circumvent the mantis ultrasound defenses, either by not triggering an evasive response or by triggering the response too close to the capture point so that the mantis may not have enough time to perform its evasive response (Triblehorn and Yager, 2005). In both cases, mantids could potentially benefit from a 'last-ditch' response mediated by bat-generated wind cues.

Materials and methods
Animals
We tested male *Parasphendale agrionina* (Mantidae: Miomantinae: Miomantini; Ehrmann and Roy, 2002) raised in our colony maintained at 25–30°C and 30–50% relative humidity with a 13 h day length. Mantids were housed individually as adults and fed flies twice a week. Testing occurred 7–21 days after the final molt. All experiments used the big brown bat (*Eptesicus fuscus* L.) trained to capture tethered mealworms (*Tenebrio monitor* L.). Bats were collected locally in Washington, DC, USA and surrounding Maryland suburbs.

Flight room
All experiments were conducted in a carpeted, acoustically lined (Sonex I, Illbruck, Minneapolis, MN, USA) flight room (6.4 m×7.3 m×2.5 m) at the University of Maryland, College Park, USA, under low-light level conditions (Fig. 1A). Two synchronized high-speed video recorders (Kodak MotionCorders) recorded the bat flight and capture behavior at 240 frames s⁻¹. A 25-point calibration frame (2.2 m×1.9 m×1.6 m; Peak Performance Technologies, Centennial, CO, USA) placed in the center of the room was filmed in both camera views. Both the three-dimensional position of the bat and the target (either the mantis for physiology experiments or the anemometer probe) as well as the distance between them were analyzed using these images and commercial motion analysis software (Motus, Peak Performance Technologies). The mantis was placed near the edge of the calibrated space farthest from the release site (indicated by a filled circle on Fig. 1A). This positioning provided the bat with the greatest amount of time and distance to orient itself after leaving the perch and accelerate to attack velocity before the capture attempt.

Mantis cercal wind reception during bat attacks
Bat-detection times and distances for the mantis cercal system were determined by using the mantis as a 'biological anemometer'. Two hook electrodes (Teflon-coated silver wires, 200 μm when bare; A-M Systems, Carlsborg, WA, USA) were glued together (SuperGlue) and implanted in the mantis abdomen. The electrodes recorded ascending wind-
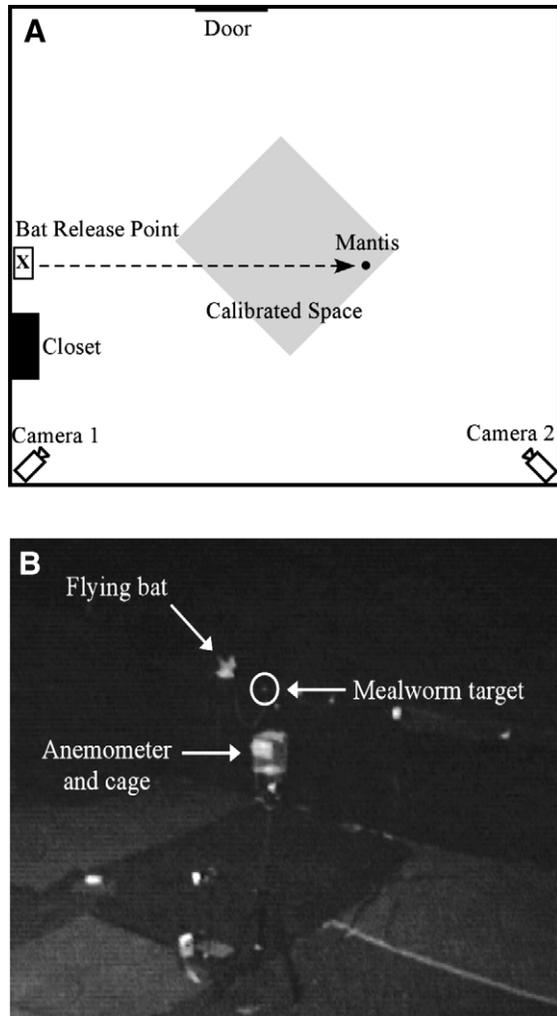
Fig. 1. (A) Schematic of the flight room setup for the physiological experiments, viewed from above. The gray region represents the high-speed video-system's calibrated area for distance measurements. The dark circle marks the location of the mantis during the experiments. (B) Photo of a trial during the anemometer measurements of bat-generated wind. The anemometer probe sits inside the protective cage except for the sensor region positioned about 3 cm outside the top of the cage. In the photo, the sensor sits 24 cm below the mealworm target (inside circle) as the flying bat approaches the target just before capturing it. The photo is from Camera 2 (A) of the high-speed video system.

evoked activity during bat attacks, where the mantis served as the target.

Mantis implanted electrode procedure

Each mantis was slightly chilled until immobile. After removing the legs and wings, the dorsal abdominal cuticle was removed, exposing the gut. The caudal and rostral ends of the gut were tied off and the gut removed to expose the ventral nerve cord. The abdominal connectives were cut between A1–T3 to record only ascending activity. Recording from the connectives between A1–A2, the left connective was placed in one hook while the right connective was placed in the other. A mineral oil–Vaseline mixture isolated the recording from the body cavity while preventing the connectives from drying out. After replacing the dorsal cuticle, an application of agar (Fisher Science, Hampton, NH, USA) held the cuticle in place and prevented the electrodes from slipping out. Placement of the indifferent wire in the prothoracic cavity held the mantis in a 'flight-like' posture. The prothoracic connectives were removed beforehand to prevent the indifferent electrode from recording the electrical activity from large units (such as mantis auditory interneuron 501–T3) that would mask activity from wind-sensitive units in the abdomen.

Recording procedure

The hook electrodes and indifferent wire were connected to a 32-gauge braid-shielded stereo cable (Belden, Richmond, IN, USA) that served as the tether. In the flight room, the cable tether connected to shielded coaxial cable that carried the neural signals to the amplifier (A-M systems model 1700). Once in place, the mantis was 90–100 cm from the ceiling of the flight room. The bat's perch was 1.5 m high and 3.37 m away from the hanging mantis. The mantis cerci were pointed directly at the bat's perch since bats approached and captured mantids from behind during free-flight encounters conducted in the same flight room (J. D. Triblehorn, personal observations).

After hanging and positioning the mantis in the flight room, but prior to releasing the bat, neural responses to a gentle wind stimulus (the experimenter blowing) were recorded. These wind-evoked responses were compared to neural responses elicited during the bat attack to verify that the activity came from wind-sensitive interneurons (based primarily on spike height). Fig. 2 shows an example of neural activity evoked by blowing on the cerci before the trial began with activity recorded from the same mantis just before capture by the bat. The similarity between some of the individual units indicates that both stimuli evoked responses from wind-sensitive interneurons. Signal-to-noise ratios for the largest units were 5 or better in 85% of the trials (71% of these were over 10) and their appearance indicated the beginning of a response. A bat detector (Pettersson model 100, Uppsala, Sweden) placed on the floor below the mantis recorded timing information of the bat vocalizations. Neural data were stored on DAT (Sony PCM-R500) after digitization (BioLogic DRA-400) and analyzed offline using Superscope II (GW Instruments, Somerville, MA, USA) after digitization (instruNet, Model 100B, Somerville, MA, USA) on a Macintosh G3 computer.

Bat training

Since the bat's approach was critical for a successful trial, a single bat was trained to fly consistently from a perch directly to the mantis with little deviation. This training lasted 6 weeks, but increased the probability that the bat would attack the mantis target (instead of the thick recording wire) from the appropriate direction. Collection of physiological data did not occur until the bat performed the task correctly on almost every

trial. The bat typically captured and ate the mantis off the tether. The electrodes posed no harm to the bat since they always slipped out of the mantis and remained attached to the wire tether.

Anemometer wind measurements

Wind generated by a flying, attacking bat was characterized using a single axis hot-wire anemometer (Model 1700 constant temperature anemometer, Model 1210-60 hot-film probe; TSI, Inc., Shoreview, MN, USA). For one trial, the sensor was placed 2 cm below the target to record bat-generated wind as the bat successfully captured the mealworm target. Although the bat only tapped the sensor when capturing the target, this contact damaged the anemometer probe. To obtain a larger set of bat-generated wind velocity measurements, the anemometer was placed at a distance from the target. A small cage (17 cm×13 cm×13 cm) protected the sensor by dissuading the bat from attacking the probe instead of the mealworm target. The cage was constructed from wire mesh (wire thickness=1 mm; mesh holes=1 cm×2 cm). The sensor extended 5 cm beyond the top of the cage. This protective cage, however, limited how close the sensor could be to the target and still have the bat capture the mealworm (23 cm below the

target was the closest distance the bat would readily approach). Fig. 1B shows a photo from the high-speed video camera of the experimental setup during one of these trials.

Results
Recordings of wind-sensitive neuron activity during flying bat attacks

Data of implanted electrode wind-sensitive activity elicited during bat attacks were recorded from 18 different mantids. Two of the mantids had data collected from two trials and the results from the two trials were averaged for each individual. Fig. 3 contains the neural activity in the connective during the last 280 ms preceding capture recorded for five of these trials. Each trace comes from a different mantis and the traces span the range of detection times for all trials. We are confident that the neural activity immediately preceding capture is related to wind-detection because: (1) these responses resembled the
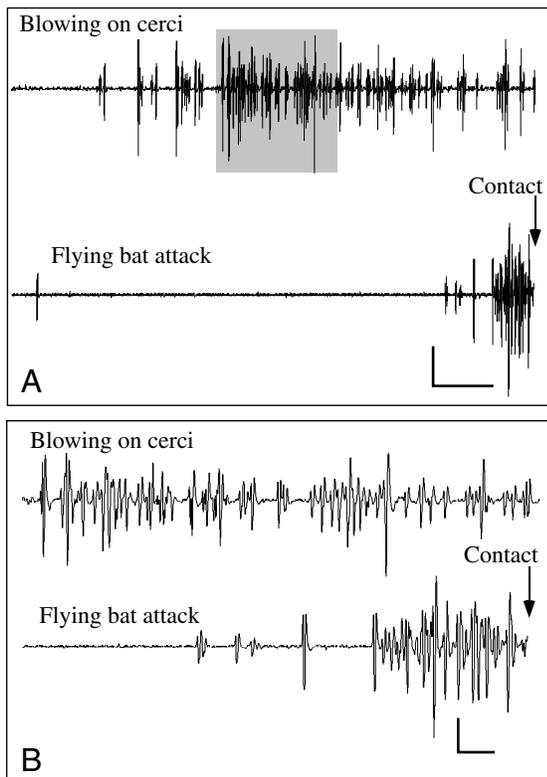

Fig. 2. Implanted electrode recordings of wind-sensitive interneuron activity in the abdominal connective of a mantis. (A) Neural activity from the mantis recorded while blowing on the cerci (top) and just before the bat captured the mantis (bottom). The similarity between the two responses indicates that both traces result from wind-sensitive interneuron activity. Scale bars: 200 mV, 50 ms. (B) Same trial (shaded area in A) viewed on an expanded time scale. Scale bars: 200 mV, 20 ms.
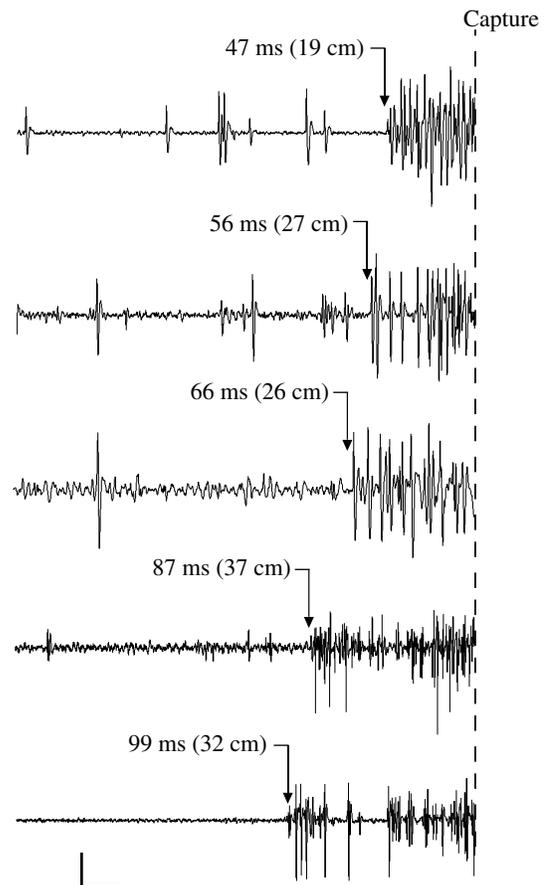

Fig. 3. Five examples of abdominal connective activity recorded by an implanted electrode during the last 280 ms before a flying, attacking bat captured the mantis. Arrows mark the beginning of the response in each trial and the numbers state the time (distance) that the response began before capture. The examples illustrate that wind-related activity evoked by the bat's approach was characterized by a sudden increase in neural activity that was sustained until the bat captured the mantis. Scale bars: 100 mV, 25 ms.

activity elicited by gently blowing on the cerci before releasing the bat (see Materials and methods), and (2) only ascending activity was recorded since the VNC between A1-T3 was cut (i.e. the activity did not originate from other sensory systems rostral to the lesion, such as visual or auditory responses). The cercal system is by far the most likely origin for ascending activity in an isolated mantis abdomen that would generate the responses observed just before capture illustrated in Fig. 3.

The wind-sensitive interneurons generally have a low level of spontaneous activity in the absence of a stimulus (indicated by the activity to the left of the arrow in each trace). Therefore, the point at which the wind-sensitive interneurons began responding to bat-generated wind (the detection time, marked by the arrow) was clear.

The neural responses across trials all consisted of a multi-unit response that included both large and small amplitude units. Each response continued from the point of detection until the bat contacted and consumed the mantis (broken line). However, detection times varied across trials as did the spike train patterns.

On average, the mantis cercal system detected bat-generated wind during an attack 73.9±18.8 ms (median: 77.5, range: 38–109 ms) before contact when the bat was 27.5±7.69 cm (median: 27.5 cm, range: 14–40 cm) away. Fig. 4 shows the distribution of detection times (Fig. 4A) and distances (Fig. 4B) for the 18 trials. The bat's behavior could influence when the cercal system detects the bat's approach during an attack. One potential factor is the bat's flight velocity as it approaches the target. In these trials, the bat's velocity in the last 200 ms before the response ranged from 292–363 cm s^{-1}. Within this range, there was no relationship between flight speed and detection time or distance (velocity vs time: $r=-0.04$, $t(16)=-0.1763$, n.s.; velocity vs distance: $r=+0.36$, $t(16)=1.5523$, n.s.; data not shown). Another potential factor is the changes in flight posture a bat performs to reduce its velocity, which could potentially increase wind production suddenly, marking the point when the mantid's cercal system detects the bat's approach. For individuals where data from only one trial was collected, the neural responses began within 70 ms of the time that the bat decelerated in 94% of the trials (15 out of 16). However, the response began before the bat decelerated in 50% of the trials (8 out of 16) and after the response began in 44% of the trials (7 out of 16). In one trial, the response coincided with the bat's deceleration. For the two mantids where data from two trials were collected, responses began with 70 ms of the time the bat decelerated in all four trials. However, for both mantids, responses occurred before deceleration in one trial and after deceleration in the other trial.

Anemometric measures of bat-generated wind velocity

The detection times provided by the physiological recordings are crucial for determining how much time the mantis has to perform an evasive maneuver between detecting the bat and capture. However, it is also of interest to quantitatively and qualitatively characterize the wind generated by a bat as it captures a target.
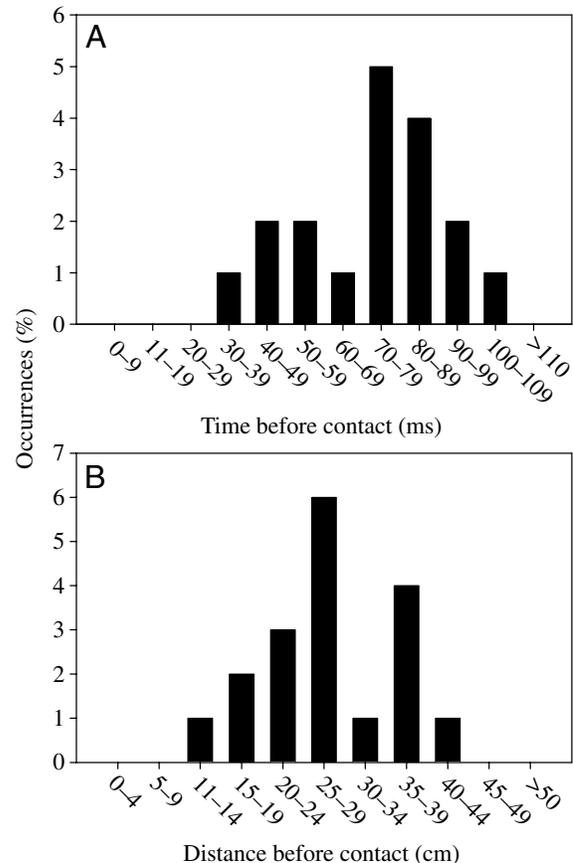

Fig. 4. Histograms of times (A) and distances (B) before capture when wind-sensitive interneuron activity began during flying bat attacks.

The sacrifice of one anemometer probe allowed the collection of a velocity–time waveform and peak velocity measurement at the point of the attack. Fig. 5 shows the anemometer voltage output leading up to the point when the bat contacted the probe. The bat-generated wind had a high acceleration (800 cm s^{-2}) and reached a peak velocity of 175 cm s^{-1}. The fact that the wind velocity continued increasing until the point of contact suggests that the wind may not have reached its peak before the bat arrived at the target. The anemometer probe detected the bat-generated wind 75 ms before contact when the bat was 18 cm away. The rest of the anemometric data were collected with the anemometer protected and placed near (but not at) the target (see Materials and methods).

Anemometer voltage waveforms of bat-generated wind

Bat-generated wind may vary during different phases of an attack. Normal flight generates a certain amount of wind from the flapping wings and movement displaces air particles in front of the bat (bow wave). Bat-generated wind likely changes as the bat decelerates by spreading its wings and tail membrane during a capture attempt. After capturing the target, wind follows the bat as it leaves the capture area (slipstream).

The anemometer waveforms in Fig. 6 illustrate these changes in the bat-generated wind during attacks. All of these
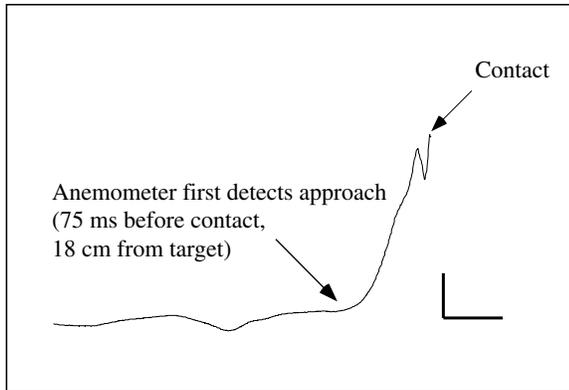
Fig. 5. Anemometer output from a single trial where the probe was placed 2 cm from the mealworm target. Contact occurred as the wind velocity continued to increase, but possibly before the wind velocity reached its peak. Scale bars: 50 cm s^{-1}, 50 ms.

examples come from trials where the anemometer was 20–30 cm directly below the target. Since the anemometer was at a distance from the target, the capture time cannot be determined exactly from the anemometer. However, the experiment above demonstrated that the bat's approach was characterized by a sudden voltage increase in the anemometer trace prior to contact. Therefore, a sudden voltage increase served as a marker to indicate the bat's approach (indicated by the arrow for traces with peak velocities of 56 cm s^{-1} or greater) for the anemometer waveforms collected when the anemometer was near the capture point.
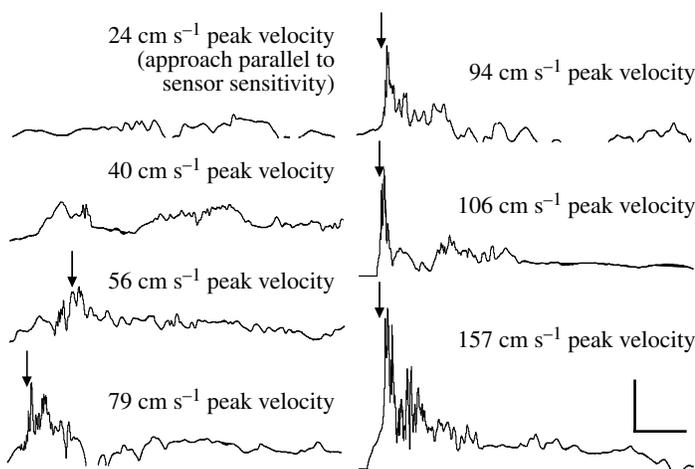

Fig. 6. Anemometer traces of wind generated by an attacking bat as it approaches a mealworm target, captures it, and flies away. Since the anemometer was not directly at the target's location, contact times (arrows) for each trace were predicted based on the one trial when the anemometer was located at the target (see Fig. 7). The examples illustrate the major changes in the anemometer traces as peak velocities increase; see text for details. The anemometer traces indicate that the bat generates a short but strong stimulus as it approaches and captures the target and a longer but weaker stimulus follows as the bat flies away. Scale bars: 50 cm s^{-1}, 500 ms.

The anemometer waveforms collected during these measurements illustrate several points. First, the duration of the bat-generated wind was long. Even for the lowest wind peak velocity measurements (24 and 40 cm s^{-1} peak velocity traces), the anemometer detected bat-generated wind for at least 1 s. Second, during this period, the magnitude of the bat-generated wind fluctuated. Weakly generated winds (24 and 40 cm s^{-1} peak velocity) contained only slow fluctuations in velocity and lacked a distinct peak. A distinct peak in the velocity appeared in traces with 50–60 cm s^{-1} peak velocities, but was not prominent until around 80 cm s^{-1} peak velocity. Waveforms with peak velocities greater than or equal to 80 cm s^{-1} contained both rapid fluctuations in velocity and the slower fluctuations observed in weaker wind profiles. Third, for stronger wind measurements, there was a rapid transition from a 'no wind' condition (zero velocity) to a strong wind condition. In fact, the early portion of the profile usually contained the strongest component for trials measuring peak wind velocities over 80 cm s^{-1}. In summary, these anemometer measurements indicated that attacking bats generated wind detectable at a single point near the target over a long period and that the strongest portion of the stimulus (both in terms of velocity and acceleration) occurred at the beginning of the generated wind stimulus.

Peak velocity and peak acceleration measurements of bat-generated wind

A large sample of peak velocity measurements of bat-generated wind were collected with the anemometer probe placed close to the target (but not directly at the target). Fig. 7A shows the peak velocities of bat-generated wind for 94 samples as a function of the anemometer distance. The highest wind velocity recorded was 326 cm s^{-1} (28 cm from the target) while the lowest was 6 cm s^{-1} (27 cm from target). The distance between the anemometer and the target accounts for some of the variation seen in the data. However, even when the anemometer was between 18–30 cm from the target (representing 77% of the trials; shaded area in Fig. 7A), bat-generated wind velocity measurements still exhibited substantial variation unrelated to distance. In 38.5% of the trials, the measured peak wind velocities were below 50 cm s^{-1}. In 31% of the trials, bat-generated wind contained peak velocities between 50–100 cm s^{-1} and peak velocities exceeded 100 cm s^{-1} in the remaining 30.5% of the trials.

In the other 23% of the trials, the probe was 30–50 cm away from the target. In these cases, the anemometer probe was below the target, but off to the side and not directly below. This setup simulated situations where a mantis evades capture by performing a power dive and determined how much wind would reach the mantis as the bat flies by. Since the mantis auditory system lacks the ability to localize sound (Yager and Hoy, 1989; Yager et al., 1990), bat-generated wind could indicate the location of the bat to the mantis. Mantids could then

potentially incorporate this new sensory information into the power dive response to direct the mantis further from the bat's location and increase its chances of survival. At these distances, the anemometer probe still detected bat-generated wind, but the peak velocities were lower, ranging between 20–60 cm s^{-1} peak velocity (Fig. 7A).

The bat-generated wind had a high acceleration component, shown in Fig. 7B. Although the measured peak accelerations spanned a wide range, measurements exceeding 200 cm s^{-2}

account for over one-third of the data. Peak acceleration was significantly correlated with peak velocity (r=+0.92, t(64)=18.7794, P<0.001), increasing exponentially with peak velocity (Fig. 7C).

Discussion

The possibility that insects possessing cercal systems may detect wind generated by an attacking bat and use this cue to mediate flight evasive responses is intriguing for both deaf and hearing insects. For deaf insects, in the absence of visual input, this cue may be the only indication that an attack is imminent. For insects sensitive to bat echolocation calls, bat-generated wind detection may act either in conjunction with the auditory system or as a backup response in case the auditory defense fails. The usefulness of this cue for insects depends on how much wind a bat generates as it approaches a target and whether the insect detects the wind early enough to perform an effective evasive response.

Physiological recordings of wind-sensitive interneuron activity during bat attacks provide good measurements of detection times and possess three distinct advantages over the hot-wire anemometric measurements: (1) they provide the most accurate information about bat-generated wind (although uncalibrated regarding the wind's magnitude) since the 'sensor' serves as the target, (2) they provide the most relevant information for evaluating the possible success of wind-evoked responses (by providing detection time and distances) since the natural receiver detects the stimulus from the natural producer, and (3) they allow the collection of multiple trials without risking the bat or expensive equipment.

Hot-wire anemometry provided calibrated measurements of bat-generated wind not available in the physiological recordings. The measurements include the magnitude of the wind generated as well as how the generated wind pattern changes at a single point over a period of time.

The nature of wind generated by an attacking bat and its measurement

The wind stimulus created by an approaching bat should be very complex and will vary with the angle of approach, initial bat flight speed, wing stroke angle and frequency, rate and
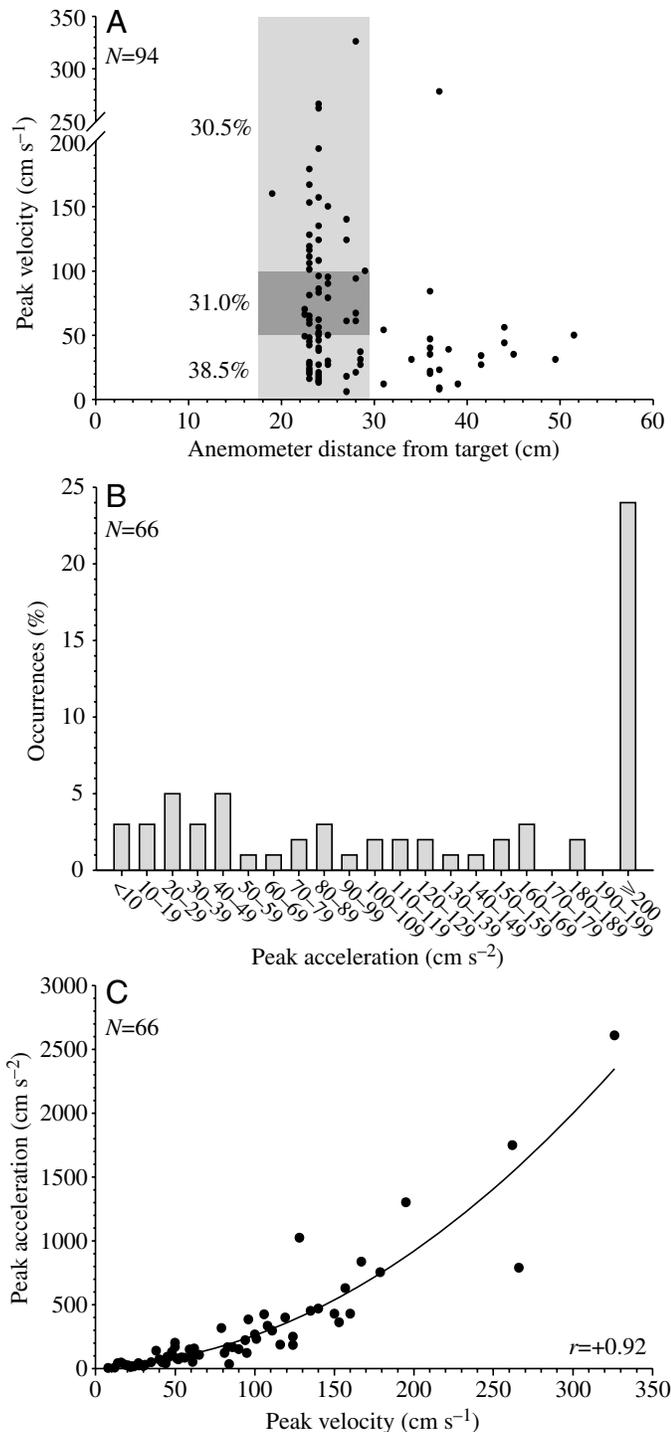

Fig. 7. (A) Peak wind velocities measured by the anemometer at distances 20–60 cm from the target (N=94). The anemometer was within 18–30 cm of the target in 77% of the trials. The shaded boxes indicate the percentage of measured peak wind velocities that fell between 0–49, 50–99 and >100 cm s^{-1} for the data collected with the anemometer within 20–30 cm of the target only. (B) Distribution of peak accelerations for 66 of the trials in A. Peak acceleration was calculated using the onset time of the anemometer detecting the bat-generated wind to the time of the peak velocity. (C) Comparison of peak velocity and peak acceleration for each trial in B. For bat-generated wind, peak velocity and peak acceleration were closely related, with peak acceleration increasing exponentially with increasing peak velocity. The equation for the exponential best fit line is $f(x)$=4.980398×10^{-1}*exp(4.814839×10^{-2}*x).

timing of deceleration, and the effective area of the bat facing the anemometer probe. The air motion will be turbulent, hence varying unpredictably in velocity and direction. The complexity and variability of the wind produced by an attacking bat makes the measurements of the bat-generated wind using a single-axis hot-wire anemometer probe with a 2 mm sensor surface difficult. In addition to the factors mentioned above, the probe only samples a small region of the entire wind stimulus. However, these are the same difficulties faced by an insect's cercal system when detecting the wind from an approaching bat. *P. agrionina*'s cerci occupy as much space as the anemometer probe and, therefore, sample only a small portion of the bat-generated wind. Furthermore, cercal systems are directionally sensitive (Palka et al., 1977; Dagan and Camhi, 1979; Westin, 1979), similar to the anemometer probe. Therefore, certain characteristics of the data collected using the anemometer, such as the large variation in the data when the probe was within 20–30 cm of the target, fully represent and describe the situation involving the cercal system.

Despite the predicted and observed variation in the wind stimulus, the anemometer data consistently show an initial peak with a high acceleration and the maximum velocity for the trial. The early appearance of these components represents the insect's first indication of a bat's approach based solely on wind. Furthermore, the anemometer data unquestionably underestimate the maximum velocity and probably the acceleration, since the probe was not directly at the target for the majority of the measurements. Variation in maximum velocity may be relatively unimportant to the insect, because even our underestimates are orders of magnitude above detection threshold for the cercal system (Triblehorn, 1997). The physiological recordings confirm that such a bat-generated wind stimulus sufficiently excites several wind-sensitive interneurons.

Wind stimulus acceleration, not peak velocity, may be the more important parameter for triggering an evasive response. This is the case in cercal-mediated terrestrial responses and is important for the animal distinguishing between wind generated from a predator (with high accelerations) that trigger an escape response *vs* changes in ambient wind conditions (with low accelerations though potentially high velocities) that do not trigger escape responses (Plummer and Camhi, 1981). For cercal-mediated aerial responses, the acceleration component may be even more important since flight provides continuous wind stimulation on the cerci with relatively high velocities (around 180 cm s^{-1} in mantids and cockroaches) but with low accelerations during stable flight.

In cockroaches, wind stimuli with high accelerations (60 cm s^{-2} or higher) evoke escape running, while stimuli with accelerations around 30 cm s^{-2} evoke a pause in walking (Camhi and Nolen, 1981). The peak acceleration of the bat-generated wind often exceeded 200 cm s^{-2}, more than three times the acceleration necessary to evoke escape running in cockroaches. The peak accelerations measured in our experiments using stationary targets (both the anemometer and

mantis physiological preparation) may be overestimates compared to the natural situation where the mantis would be flying. As mentioned previously, however, the anemometer probe was not located directly at the target and, therefore, likely underestimates the actual peak acceleration. Even so, the peak accelerations measured were very high (a large proportion were >200 cm s^{-2}). Furthermore, the peak acceleration was 800 cm s^{-2} for the one trial where the anemometer was located at the target.

Evaluating the bat-generated wind hypothesis: is there enough time to escape?

The physiology results showed that the mantis cercal system detected the bat an average of 74 ms before contact, when the bat was 27.5 cm away. Since the electrodes were implanted to record from the connectives between A1–A2, these detection times actually incorporate the detection of the bat-generated wind by cercal hairs, afferent neural conduction, processing and information transfer to ascending interneurons in the terminal ganglion, and conduction time to reach the electrodes. These results correlate well with the detection time measured by the anemometer placed within 2 cm of the target (75 ms), but not as well with the detection distance measurement (18 cm). Still, 18 cm is within the range of detection distances from the physiological measurements. It is interesting that this detection time matches the cockroach cercal system's detection time of the wind produced by the tongue strike of an attacking toad, a natural predator that evokes the terrestrial wind-evoked response (Camhi et al., 1978). Based on the wind cue alone, cockroaches escaped 55% of the time (47% advantage over cockroaches with cerci covered and unable to detect wind). Therefore, it is not unreasonable that using the wind cue may improve the mantid's odds at escaping when the auditory defense fails.

Comparisons between the bat's flight velocity at detection indicated that this parameter was not correlated to either an earlier detection time or distance. However, the tail-flip used by a bat to capture an insect and transfer it to its mouth begins about 65±15 ms before capture (*N*=10; K. Ghose, personal communication). This value is close to the 74 ms average detection time, suggesting that the tail-flip may be a major contributor to the bat-generated wind that the cercal system detects.

Based on their predator simulations, Ganihar et al. (Ganihar et al., 1994) estimated that an insect could detect an approaching bat predator 54 ms before contact (40–68 ms range). The current assessment of average detection time (74 ms) based on physiological recordings allows the insect 20 ms more to escape. The new estimate allows the cockroach a total of 36 ms to evade the bat (based on the cockroach's 38 ms behavioral response latency; Ganihar et al., 1994). Even with the increase in the detection time from Ganihar's estimate, it still seems unlikely that enough time exists for a cockroach (or any insect) to detect the bat and perform a response that will cause a bat to completely miss it. However, there may be enough time for the insect to alter its position just before

capture. Although researchers suggest that echolocating bats update the target location on an echo-by-echo basis (Masters et al., 1985; Masters, 1988), others suggest that bats plan their capture attempt early by predicting the location of the insect at the point of capture (Wilson and Moss, 2002; Ghose et al., in press). If bats do plan their capture attempt in advance, any wind-evoked behavior altering the insect's flight would result in a difference between the bat's prediction and the insect's actual location. This deviation could cause the bat to mishandle the insect (i.e. drop it) since the bat would not be able to adjust to the unpredicted change. Earlier wind detection would give the insect more time to respond, resulting in a larger discrepancy between the bat's prediction and the mantid's actual location. Therefore, successful wind-evoked insect evasive maneuvers would likely not cause the bat to completely miss the insect, but cause the bat to mishandle the target during capture by tipping the insect with its wing or tail membrane and give it one final chance to avoid the bat. Such late changes in the insect's trajectory could also pose problems if bats do update a target's location on an echo-by-echo basis. Although the bat may detect the change, it may not be able to act on this information and adjust its capture attempt to compensate for the alteration of the insect's location in such a short time.

In staged free-flight encounters between mantids and bats, we have observed bats making contact with deafened mantids (thus eliminating the ultrasound-triggered evasive response) and dropping them (Triblehorn, 2003). These mantids were not bitten, so they were not dropped after the bat transferred the mantis to its mouth and began eating. Although no behavioral responses from the mantids were observed, such responses would occur very close to the time of capture (within 100 ms) and would not likely be seen by the observers (though the sound of the bat making contact with the mantis was evident). Although such observations could be coincidental mistakes in the bat handling the mantis, it is also possible that these dropped, deafened mantids successfully detected and responded to wind from the approaching bat, enabling them to survive the attack.

The fragility of the implanted mantis preparation and the nature of the experiment as a whole prevented establishing, without a doubt, that the activity recorded during the bat attacks are from ascending wind-sensitive interneurons. However, in addition to the data presented here (see Fig. 2 and Results), data from other studies conducted in our laboratory indicate that this is most likely the case. First, mantids do possess filiform hairs that are sensitive to wind stimulation, and removing and/or shortening these hairs increases wind detection thresholds measured by recording from the cercal nerve (Triblehorn, 1997). Second, extracellular recordings from abdominal connectives in *P. agrionina* reveal that wind puffs of varying velocities elicit responses from multiple ascending interneurons (Triblehorn, 2003). These interneurons vary in spike height and firing properties (i.e. find both phasic and tonic units). Furthermore, covering the cerci with Vaseline eliminated these wind-elicited responses (J. D. Triblehorn,

personal observation). Finally, in the current study, with the exception of testing the mantis preparation by blowing on the cerci, neural responses resembling those elicited by the approach of the bat did not occur, either spontaneously or as a result of the experimenter's actions while preparing for the trial.

Using bat-generated wind during escapes

Anemometer measurements show that bats can generate wind detectable at least 50 cm away from the target. Mantids are unable to localize sound and cannot determine the approaching direction of the attacking bat (Yager and Hoy, 1989; Yager and May, 1990). When the bat is close, the mantis executes a power dive to evade the bat and does not necessarily need to know the bat's approach direction. However, the wind created as the bat passes the diving mantis could serve as a cue to the bat's location. The mantis could incorporate this new sensory information into its power dive, giving the dive a directional component allowing the mantis to not only escape from the bat's initial attack, but also enable it to avoid a second attack.

The use of bat-generated wind in these scenarios only seems reasonable if the mantis is within 30 cm of the bat (see Fig. 7). Within this distance, peak velocities exceed $100\ \mathrm{cm\ s^{-1}}$ in 30.5% of the trials. However, beyond this range, peak velocities rarely exceeded $60\ \mathrm{cm\ s^{-1}}$. Wind puffs at $60\ \mathrm{cm\ s^{-1}}$ failed to elicit strong behavioral responses in flying cockroaches (Ganihar et al., 1994) or significant changes in *P. agrionina* wind-sensitive interneuron activity in the presence of a flight-simulated headwind (Triblehorn, 2003). In the latter experiments, the flight-simulated headwind was set to $180\ \mathrm{cm\ s^{-1}}$ (equal to the mantis stable flight velocity). When executing a power dive, mantids double their flight velocity, which would potentially make it more difficult for the cercal system to detect the bat-generated wind. However, this increase is not instantaneous and mantids should be too far from the bat by the time they reach this velocity for the bat-generated wind to be a factor. However, even when flying at $180\ \mathrm{cm\ s^{-1}}$ at the initiation of a power dive, it seems unlikely that mantids should rely on bat-generated wind for determining the direction of the attack and incorporate this information into their response.

Candidates for putative wind-mediated evasive responses

Insects that would benefit from wind-mediated evasive response to avoid bat predation are those that possess cerci and fly nocturnally. Despite the prevalence of either of the characteristics across insect species, those that possess both are in the minority. The majority of insects that possess cerci are wingless and do not fly. On the other hand, all of the holometabolous (complete metamorphosis) insects (such as Diptera, e.g. flies; Lepidotera, e.g. moths and butterflies; Homoptera, e.g. cicadas and aphids; Hymenoptera, e.g. bees, ants and wasps; and Coleoptera, e.g. beetles) as well as Hemiptera (e.g. the true bugs) and Neuroptera (e.g. lacewings and caddis flies) have lost their cerci but have the ability to fly.

The main candidates for putative wind-mediated evasive responses are members of Dictyoptera (e.g. mantids and cockroaches) and Orthoptera (e.g. crickets, locusts, grasshoppers, katydids).

Insect 'last chance' bat avoidance responses

Late 'last-chance' behaviors, mediated by ultrasound-sensitive auditory systems, have been described in several insects. Green lacewings passively fall in response to bat vocalizations emitted at low rates, but as the bat closes in on the capture, the higher emission rates trigger 'wing-flips' in the lacewing that abruptly slow its descent (Miller and Olesen, 1979). 'Wing-flips' occur about 50–100 ms before capture and this unexpected alteration in the lacewing's descent occurring late in the bat's capture attempt causes the bat to miss the insect 70% of the time. Arctiid moths produce ultrasonic 'clicks' that deter bats from capturing the insects as an acoustic aposematic signal (Dunning, 1968; Dunning et al., 1992; Hristov and Conner, 2005), by startling the bat (Möhl and Miller, 1976) or 'jamming' its ability to echolocate (Fullard et al., 1979). High bat emission rates trigger arctiid moth 'clicks' during the late stage of the bat attack (Fullard, 1984; Fullard et al., 1994) and begin between 142–270 ms before capture, depending on stimulus level. Acharya and Fenton (Acharya and Fenton, 1992) showed that bats rarely contact 'clicking' moths even though the arctiids show little, if any, evasive behaviors during the attack.

These 'last-chance' maneuvers do not require significant alterations in flight trajectories to be effective, which likely contributes to their high success rate. The lacewing passive fall relies on gravity and the 'wing-flip' simply alters the velocity of this fall, not its trajectory. Arctiid moth 'clicks' effectively protect the insect without any alteration in its flight path. However, for the putative wind response, success potentially relies on how much the insect can alter its trajectory from the bat's prediction during its capture attempt. This requires time to initiate the response internally (neuromuscular response) and for the response to take effect (resulting in a change in flight path). Given the short amount of time for this to occur, the success rate will likely be much lower than the other 'last chance' maneuvers. Still, very low survival advantages can be very important evolutionarily (Endler, 1986). Since the response makes use of the neural, muscular and behavioral elements already present and in use during normal flight, the cost to the insect is minimal while providing a slim chance to survive the encounter, which is better than no chance.

References

Acharya, L. and Fenton, M. B. (1992). Echolocation behavior of vespertilionid bats (*Lasiurus cinereus* and *Lasiurus borealis*) attacking airborne targets including arctiid moths. *Can. J. Zool.* **70**, 1292-1298.

Altman, J. (1983). Sensory inputs and the generation of the locust flight motor pattern: from the past to the future. In *Biona Report 2* (ed. W. Nachtigal), pp. 127-136. Stuttgart: Gustave Fischer.

Boudreaux, H. B. (1979). *Arthropod Phylogeny with Special Reference to Insects*. New York: John Wiley & Sons.

Boyan, G. S. and Ball, E. E. (1990). Neuronal organization and information processing in the wind-sensitive cercal receptor/giant interneurone system of the locust and other orthopteroid insects. *Prog. Neurobiol.* **35**, 217-243.

Boyan, G. S., Ashman, S. and Ball, E. E. (1986). Initiation and modulation of flight by a single giant interneuron in the cercal system of the locust. *Naturwissenschaften* **73**, 272-274.

Burrows, M. (1996). *The Neurobiology of an Insect Brain*. Oxford: Oxford University Press.

Camhi, J. M. (1984). *Neuroethology: Nerve Cells and the Natural Behavior of Animals*. Sunderland, MA: Sinauer Associates.

Camhi, J. M. and Nolen, T. G. (1981). Properties of the escape system of cockroaches during walking. *J. Comp. Physiol. A* **142**, 339-346.

Camhi, J. M., Tom, W. and Volman, S. (1978). The escape behavior of the cockroach *Periplaneta americana*. II. Detection of natural predators by air displacement. *J. Comp. Physiol. A* **128**, 203-212.

Dagan, D. and Camhi, J. M. (1979). Responses to wind recorded from the cercal nerve of the cockroach, *Periplaneta americana*. II. Directional selectivity of the sensory neurons innervating single columns of filiform hairs. *J. Comp. Physiol. A* **133**, 103-110.

Dunning, D. C. (1968). Warning sounds of moths. *Z. Tierpsychol.* **25**, 129-138.

Dunning, D. C., Acharya, L., Merriman, C. B. and Dal Ferro, L. (1992). Interactions between bats and arctiid moths. *Can. J. Zool.* **70**, 2218-2223.

Eaton, R. C., DiDominico, R. and Nissanov, J. (1991). The role of the Mauthner cell in sensorimotor integration by the brainstem escape network. *Brain Behav. Evol.* **37**, 272-285.

Edwards, J. S. and Reddy, R. (1986). Mechanosensory appendages and giant interneurons in the firebrat (*Thermobia domestica*, Thysanura): a prototype system for predator evasion. *J. Comp. Neurol.* **243**, 535-546.

Ehrmann, R. and Roy, R. (2002). Systematische aufstellung der gattungen. In *Mantodea: Gottesanbiterinnen der Welt* (ed. R. Ehrmann), pp. 374-378. Munich: Natur und Teil-Verlag.

Endler, J. A. (1986). *Natural Selection in the Wild*. Princeton, NJ: Princeton University Press.

Fraser, P. (1977). Cercal ablation modifies tethered flight behaviour of cockroach. *Nature* **268**, 523-524.

Fullard, J. H. (1984). Listening for bats: pulse repetition rate as a cue for a defensive behavior in *Cycnia tenera* (Lepidoptera: Arctiidae). *J. Comp. Physiol. A* **154**, 249-252.

Fullard, J. H., Fenton, M. B. and Simmons, J. A. (1979). Jamming bat echolocation: the clicks of arctiid moths. *Can. J. Zool.* **57**, 647-649.

Fullard, J. H., Simmons, J. A. and Saillant, P. A. (1994). Jamming bat echolocation: the dogbane tiger moth *Cycnia tenera* times its clicks to the terminal attack calls of the big brown bat *Eptesicus fuscus*. *J. Exp. Biol.* **194**, 285-298.

Ganihar, D., Libersat, F., Wendler, G. and Camhi, J. M. (1994). Wind-evoked evasive responses in flying cockroaches. *J. Comp. Physiol. A* **175**, 49-65.

Ghose, K., Horiuchi, T. K., Krishnaprasad, P. S. and Moss, C. F. (in press). Echolocating bats use a nearly time-optimal strategy to intercept prey. *PLoS Biol.*

Heitler, W. J. (1974). The locust jump. Specializations of the metathoracic femoral-tibial joint. *J. Comp. Physiol.* **89**, 93-104.

Hennig, W. (1981). *Insect Phylogeny*. New York: John Wiley & Sons.

Hristov, N. I. and Conner, W. E. (2005). Sound strategy: acoustic aposematism in the bat-tiger moth arms race. *Naturwissenschaften* **92**, 164-169.

Jones, G. and Rayner, J. M. V. (1988). Flight performance, foraging tactics, and echolocation in free-living Daubentons bats *Myotis daubentoni* (Chiroptera, Verspertilionidae). *J. Zool. Lond.* **215**, 113-132.

Kämper, G. (1984). Abdominal ascending interneurons in crickets: responses to sound at the 30-Hz calling-song frequency. *J. Comp. Physiol. A* **155**, 507-520.

Kämper, G. and Dambach, M. (1979). Communication by infrasound in a non-stridulating cricket. *Naturwissenschaften* **66**, 530.

Kämper, G. and Dambach, M. (1981). Response of the cercus-to-giant

interneuron system in crickets to species-specific song. *J. Comp. Physiol. A* **141**, 311-317.

Kämper, G. and Dambach, M. (1985). Low-frequency airborne vibrations generated by crickets during singing and aggression. *J. Insect Physiol.* **12**, 925-929.

Krasne, F. B. and Wine, J. J. (1984). The production of crayfish tailflip escape responses. In *Neural Mechanisms of Startle Behavior* (ed. R. C. Eaton), pp. 179-211. New York: Plenum Press.

Libersat, F. and Camhi, J. M. (1988). Control of sensory feedback by movement during flight in the cockroach. *J. Exp. Biol.* **136**, 483-488.

Libersat, F., Goldstein, R. S. and Camhi, J. M. (1987). Nonsynaptic regulation of sensory activity during movement in cockroaches. *Proc. Natl. Acad. Sci. USA* **84**, 8150-8154.

Masters, W. M. (1988). Prey interception: predictive and nonpredictive strategies. In *Animal Sonar: Processes and Performance* (ed. P. E. Nachtigal and P. W. B. Moore), pp. 467-470. New York: Plenum Press.

Masters, W. M., Moffat, A. J. M. and Simmons, J. A. (1985). Sonar tracking of horizontally moving targets by the big brown bat *Eptesicus fuscus*. *Science* **228**, 1331-1333.

Miller, L. A. and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.* **131***, 113-120.

Miller, L. A. and Surlykke, A. (2001). How some insects detect and avoid being eaten by bats: tactics and countertactics of prey and predator. *Bioscience* **51**, 570-581.

Möhl, B. and Miller, L. A. (1976). Ultrasonic clicks produced by the peacock butterfly: a possible bat-repellent mechanism. *J. Exp. Biol.* **64***, 639-644.

Nolen, T. G. and Hoy, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. *J. Comp. Physiol. A* **159**, 423-439.

Palka, J., Levine, R. and Schubiger, M. (1977). The cercal-to-giant interneuron system of crickets. I. Some attributes of the sensory cells. *J. Comp. Physiol. A* **119**, 267-283.

Plummer, M. R. and Camhi, J. M. (1981). Discrimination of sensory signals from noise in the escape system of the cockroach: The role of wind acceleration. *J. Comp. Physiol. A* **142**, 347-357.

Ritzmann, R. E. (1984). The cockroach escape response. In *Neural Mechanisms of Startle Behavior* (ed. R. C. Eaton), pp. 93-131. New York: Plenum Press.

Schnitzler, H.-U. (1987). Echoes of fluttering insects: information for echolocating bats. In *Recent Advances in the Study of Bats* (ed. M. B. Fenton, P. A. Racey, and J. M. V. Rayner), pp. 226-243. Cambridge: Cambridge University Press.

Tauber, E. and Camhi, J. M. (1995). The wind-evoked escape behavior of the cricket *Gryllus bimaculatus*: integration of behavioral elements. *J. Exp. Biol.* **198**, 1895-1907.

Triblehorn, J. D. (1997). Near-field sound reception in the praying mantis *Sphodromantis aurea*. Masters thesis, University of Maryland, College Park, MD, USA.

Triblehorn, J. D. (2003). Multisensory integration in the ultrasound-triggered escape response of the praying mantis, *Parasphendale agrionina*, PhD thesis, University of Maryland, College Park, MD, USA.

Triblehorn, J. D. and Yager, D. D. (2002). Implanted electrode recordings from a praying mantis auditory interneuron during flying bat attacks. *J. Exp. Biol.* **205**, 307-320.

Triblehorn, J. D. and Yager, D. D. (2005). Timing of praying mantis evasive responses during simulated bat attack sequences. *J. Exp. Biol.* **208**, 1867-1876.

Westin, J. (1979). Responses to wind recorded from the cercal nerve of the cockroach *Periplaneta americana*. I. Response properties of single sensory neurons. *J. Comp. Physiol. A* **133**, 97-102.

Wilson, W. W. and Moss, C. F. (2002). Sensory-motor behavior of free-flying FM bats during target capture. In *Echolocation in Bats and Dolphins* (ed. J. A. Thomas, C. F. Moss and M. Vater), pp. 22-27. Chicago: University of Chicago Press.

Yager, D. D. and Hoy, R. R. (1989). Audition in the praying mantis, *Mantis religiosa* L.: identification of an interneuron mediating ultrasonic hearing. *J. Comp. Physiol. A* **165**, 471-493.

Yager, D. D. and May, M. L. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. II. Tethered flight. *J. Exp. Biol.* **152**, 41-58.

Yager, D. D., May, M. L. and Fenton, M. B. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight. *J. Exp. Biol.* **152**, 17-39.