The Journal of Experimental Biology 205, 307–320 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3800

Implanted electrode recordings from a praying mantis auditory interneuron during flying bat attacks

Jeffrey D. Triblehorn* and David D. Yager
Department of Psychology, University of Maryland, College Park, MD 20742-4411, USA
*e-mail: jtriblehorn@psyc.umd.edu

Accepted 14 November 2001

Summary

Using an implanted electrode, we recorded the responses from the ultrasound-sensitive mantis interneuron 501-T3 during flying bat attacks in a large flight room where the mantis served as the target. 501-T3 responds to each vocalization emitted with multi-spike bursts when pulse repetition rates (PRRs) are below 55 pulses s^{-1}. As PRR increases and pulse durations fall below 3 ms, 501-T3 ceases burst activity. On average, spike bursts cease 272 ms before contact (when the bat is 73 cm away from the preparation). The timing of cessation of activity in 501-T3 is similar to the latency for the diving portion of the response of the mantid (242 ms). Experiments using vocalizing stationary bats confirm that 501-T3 responds more reliably to longer pulse durations (≥3 ms) when intensities are below 90 dB pe SPL. The cessation of 501-T3 activity is probably due both to the increasing PRR and to the decreasing pulse duration that occur in the terminal buzz phase of a bat attack. 501-T3 may be actively shut off at high PRRs and/or intensities to protect the interneuron from habituation while the mantis performs an escape response. The cessation of 501-T3 activity is consistent with the lack of a very late ultrasound-mediated evasive response by the mantis. However, cessation of 501-T3 activity may allow a true 'last-chance' response to be mediated by other neural systems.

Key words: audition, defence, behaviour, Dictyoptera, echolocation, insect, hearing, *Parasphendale affinis*, mantid, Mantodea, neuroethology, neurophysiology.

Introduction

Echolocating insectivorous bats pose a threat to insects flying at night. Many insects possess tympanate auditory systems sensitive to the ultrasonic frequencies used by these hunting bats (for reviews, see Hoy, 1998; Yager, 1999; Stumpner and von Helversen, 2001). Upon detection of ultrasonic bat vocalizations, these insects usually perform some evasive maneuver effective in eluding their bat predators. Evasive behaviors range from the simple (such as ceasing flight and dropping) to the complex (sharp turns and power dives). The timing of the response can be critical since beginning an escape response too early or too late could decrease its effectiveness. In addition, an insect species may exhibit multiple behaviors dependent on the intensity of the threat. For example, a moth will turn away from the direction of a bat that is far away but will fly erratically when the bat is close (Roeder, 1967). Some moths and tiger beetles even produce ultrasonic clicks when bats are close (Dunning, 1968; Acharya and Fenton, 1992; Yager and Spangler, 1997). Similarly, mantids will turn when a bat is far away but will enter a power dive when the bat is close (Yager et al., 1990). Therefore, it is important that insects use relevant cues produced by the bat to assess the level of danger and perform the appropriate response at the optimal time on the basis of this assessment.

Bat vocalizations contain several cues that insects could use to determine their distance from a bat. One cue is sound intensity, but this can be ambiguous. A bat may lower the intensity of its vocalization as it approaches a prey item (Surlykke and Moss, 2000). Therefore, a single intensity may represent a bat that is farther away and making loud vocalizations or a bat that is much closer but vocalizing at a lower intensity (Fullard, 1984). A bat also changes the emission rate of its vocalizations (referred to as pulse repetition rate or PRR) as it searches for and tries to capture an insect. These changes are divided into the search phase, the approach phase and the terminal buzz phase (comprising buzz I and buzz II) (Kalko, 1995; Surlykke and Moss, 2000). The vocalization pulses decrease in duration and increase in repetition rate in each successive phase. The increase in PRR provides more information to the bat about the location of the target and the decrease in pulse duration may prevent echo overlap (Schnitzler et al., 1980; Simmons and Grinnell, 1980; Kalko, 1995). Both PRR and pulse duration may indicate how close a bat is to capturing the insect. Finally, there are changes in the frequency-modulated (FM) sweeps that comprise the individual vocalizations of many bat species. As a bat approaches a target, there is a decrease in the beginning and ending frequencies of the FM sweeps as well as in the bandwidth.

Frequency changes are probably not very useful to most insects. Many ultrasound-sensitive interneurons are broadly tuned (Nolen and Hoy, 1987; Faure and Hoy, 2000a; Triblehorn and Yager, 2001) and may not be sensitive to the changes in frequency content in emitted pulses. Pulse duration may also not be a very useful cue to insects. Although bat pulses decrease predictably in duration as a bat approaches and attempts to capture a target, insect auditory systems are also less sensitive to these shorter pulses (Tougaard, 1999; Faure and Hoy, 2000a). As a result, these shorter bat pulses may not activate the insect auditory system strongly enough to elicit an evasive response. Although pulse duration may be important, PRR may be the most reliable cue for the insect to assess the level of danger posed by the bat.

In the arctiid moth *Cycnia tenera*, Fullard (1984) found that the lowest thresholds able to elicit ultrasonic clicking behavior occurred at 30–50 pulses s^{-1}, similar to PRRs in the beginning of the terminal buzz phase. Playback experiments using a recorded *Eptesicus fuscus* attack vocalization sequence (approach and terminal buzz phases) confirmed that *C. tenera* does not produce clicks until the terminal buzz phase, suggesting that PRR is a cue for the initiation of clicking behavior (Fullard et al., 1994). Fullard (1984) also found that the number of tympanal nerve spikes elicited per pulse decreased with increasing PRR.

Sound intensity and pulse duration can also interact to influence the auditory system's ability to follow PRRs. In katydids, the ultrasound-sensitive T-cell encodes at least 50 % of the pulses in a stimulus train using 40 kHz tones up to 70 pulses s^{-1} for loud tones (90 dB SPL), but performance decreases with decreasing intensity (42 pulses s^{-1} at 70 dB SPL, 24 pulses s^{-1} at 50 dB SPL) (Faure and Hoy, 2000a). The T-cell follows a pulse train mimicking a bat stimulus (10 ms pulse duration, 14.25 pulses s^{-1}) better than one mimicking a conspecific song (30 ms pulse duration, 14.25 pulses s^{-1}) (Faure and Hoy, 2000b).

The praying mantis possesses a single ear located on the ventral surface between the metathoracic legs (Yager and Hoy, 1987). Most species with auditory ability hear best within the range 30–50 kHz, but some species (termed broadly tuned species) are equally sensitive to higher frequencies (Triblehorn and Yager, 2001). A praying mantis typically responds to ultrasound during flight by dorsiflexing its abdomen, extending its forelegs, rolling its head and changing its wing-beat phase and frequency. In free flight, this produces evasive maneuvers ranging from random turns to power dives, depending on stimulus strength, that are effective in evading capture by bats (Yager et al., 1990).

Mantids have several mirror-image pairs of auditory interneurons; auditory interneuron 501-T3 has been most extensively characterized (Yager and Hoy, 1989). It is a broadly tuned interneuron sensitive to the ultrasonic frequencies used by echolocating bats. Its tuning is similar to the audiograms of other bat-avoiding insects. 501-T3 probably receives direct input from the auditory afferents, and it has a large axon (17 μm in diameter) with a short latency for action potentials to reach the prothorax (8–12 ms) and a high conduction velocity (4 m s^{-1}) (Yager and Hoy, 1989). These characteristics make it highly probable that 501-T3 has a role in initiating the evasive response of the mantis.

In the present experiment, we examined the neural responses of 501-T3 to echolocation vocalizations emitted by a free-flying bat in a large flight room. Other studies have examined the responses of physiological insect preparations to real bat vocalizations, but their focus was to determine either whether gleaning bats could be detected (Faure et al., 1993; Faure and Barclay, 1994) or to measure insect bat-detection distances (Fullard and Thomas, 1981; Schul et al., 2000). Many of these studies also focused on auditory responses to bat search calls not necessarily directed at the physiological preparation. Our study is unique since it focuses on the responses to bat approach and terminal buzz vocalizations during a capture attempt. A chronically implanted electrode allowed us to record the neural responses from a suspended mantis that served as the capture target for the free-flying bat. Since bat vocalizations emitted during attack sequences in the flight room are similar to those in the field and the preparation was the target of the attack (and thus the vocalizations), this paradigm provides the most accurate information to date to address how an insect auditory system responds to changes in bat vocalizations during capture attempts in the field.

Materials and methods

Animals

We tested male *Parasphendale affinis* (Beier, 1968) (Mantidae; Mantinae; Miomantini) 7–21 days after their molt to adulthood. The mantids were raised in our colony maintained at 25–30 °C and 30–50 % relative humidity with a 14 h day length. All mantids were housed individually as adults and fed flies twice a week.

We used three bats (*Eptesicus fuscus*) in these experiments (one male and one female for the bat attack experiments and a different male for the stationary bat vocalization experiments). The two males came from a cave in Ontario, Canada, captured in March 2000, and the female came from District Heights, MD, USA, captured in June 2000. The bats used for bat attack experiments were trained to capture tethered mealworms and were experienced in capturing free-flying mantids in the flight room (described below). The bat used for the stationary bat vocalization experiment was trained to echolocate while sitting on a platform for use in echolocation experiments.

Neurophysiology

Clip electrodes

We recorded neural responses using a single clip electrode similar to that described by Ye et al. (1995). The clip electrode consists of a 25 μm diameter formvar-insulated nichrome wire (A-M systems) embedded in 2-ton epoxy (Devcon).

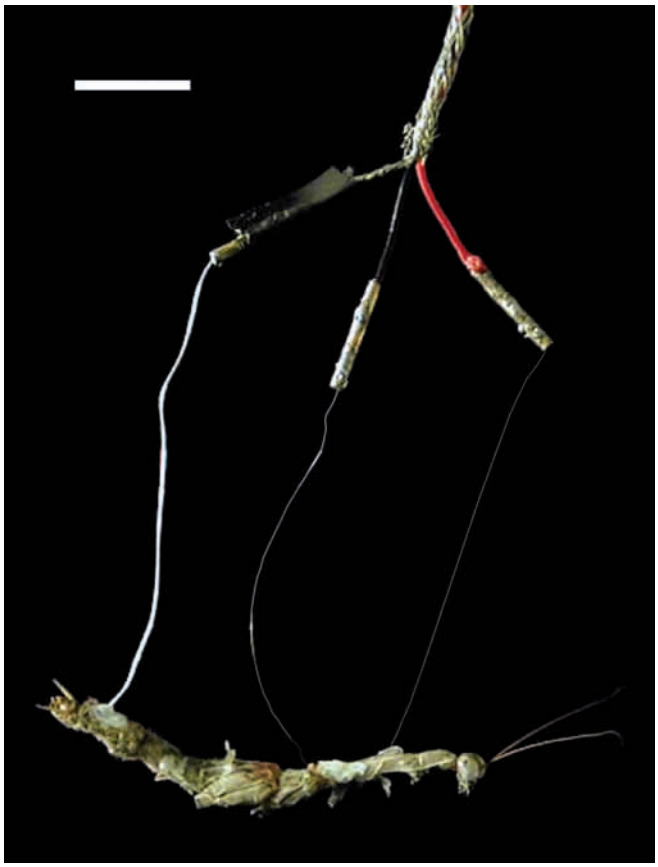

Fig. 1. The implanted electrode arrangement in the hanging tethered mantis preparation. Scale bar, 10 mm. The right-hand wire holds the clip electrode and the connective, the middle wire holds the indifferent lead and the left-hand wire stabilizes the mantis in a 'flight-like' posture.

Implantation

Each mantis was chilled slightly until immobile. The legs and wings of the mantis were removed before fixing it ventral-side up using wax. After removing a rectangular portion of the ventral cuticle between the prothoracic and metathoracic legs, the clip was inserted into the mantis body cavity and the connective was placed inside. Care was taken not to damage the tracheal system. We replaced the cuticle and applied agar (Fisher Science) to help keep the electrode in place. The connectives were cut just posterior to the prothoracic ganglion (to eliminate large-amplitude visual units from our recordings) and between ganglia A1 and A2 (to eliminate large-amplitude units from the wind-sensitive cercal system). A second single nichrome wire (not a clip electrode) was inserted dorsally into the prothoracic body cavity to serve as an indifferent electrode. A third, non-recording wire was also placed dorsally in the tip of the abdomen to provide additional support and relieve stress on the two recording wires. Agar was also applied at the insertion site of these two wires and at the dorsal and ventral sides of the abdomen. The three wires were soldered to gold connectors attached to 36-gauge braid-shielded stereo wire (Belden). The agar and the non-recording wire helped hold the mantis in a horizontal position during the experiment. Fig. 1 shows the tethered preparation.

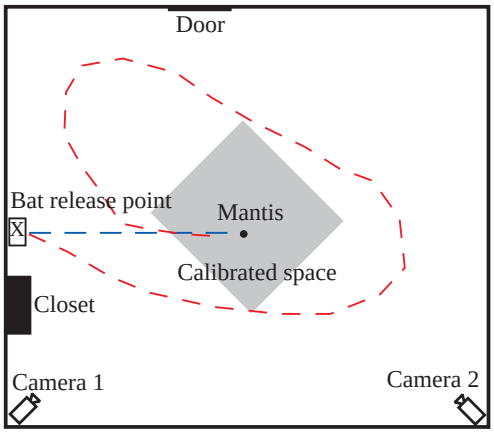

Fig. 2. Diagram of the flight room (6.4 m×7.3 m×2.5 m) and the experimental arrangement for the bat attack experiments. The gray box represents the calibration space for the high-speed video cameras (2.2 m×1.9 m×1.6 m). The dashed lines illustrate the two typical flight path types for the bat attacks (direct approach, blue line; indirect approach, red line).

Although the agar kept the electrodes in place, it was weak enough for the electrodes to slip out when the bat grabbed the mantis during an attack. In no case did the electrodes remain in the preparation as the bat grabbed the mantis and there was, therefore, no danger of the bat consuming the electrodes.

Flight room

All experiments were conducted in a carpeted flight room (6.4 m×7.3 m×2.5 m) at the University of Maryland, College Park, under low light levels. The ceilings and walls were lined with acoustic foam (Sonex I, Illbruck, USA). During bat attack experiments, we recorded the bats' flight and capture behavior on two synchronized high-speed video recorders (Kodak MotionCorders) at 240 frames s^{-1}. A 25-point calibration frame (2.2 m×1.9 m×1.6 m; Peak Performance Technologies) placed in the center of the room was filmed in both camera views. The three-dimensional positions of the bat and the mantis preparation (as well as the distance between them) were analyzed using these images and commercial motion-analysis software (Motus, Peak Performance Technologies). The preparation was placed within the calibration frame, 50 cm off-center towards the cameras. Fig. 2 shows a diagram of the flight room arrangement.

Bat attack experiments

The tethered mantis with electrode was hung 90–100 cm from the ceiling in the center of the flight room by alligator clips attached to shielded coaxial cable that carried neural signals to the amplifier (A-M Systems, model 1700). The bat was placed on a platform 1.5 m high, 3.37 m away from the hanging preparation. An Ultrasound Advice microphone positioned below the mantis preparation (30 cm above the floor, 175 cm below the mantis) recorded the bat vocalizations

for frequency and timing information (see *Sound recordings* section). A bat detector (Pettersson, model 100) was also placed at the site of the microphone. A four-channel DAT (Sony PCM-R500) stored the amplified neural signals, the bat detector output, the synchronization pulses from the video system and the trigger signal to stop the video and sound recording systems after digitization (BioLogic DRA-400) for off-line analysis. In some trials, the bat struck the tether, but the electrodes remained in the preparation. In these cases (four cases), another trial was acquired if 501-T3 still responded to ultrasound and the recording quality was still good.

Stationary bat vocalization experiments

To examine how the mantis auditory system responds to real bat vocalizations of different intensities and durations, chronic electrodes recorded auditory responses to a stationary bat vocalizing while on a platform at different distances from the mantis preparation. The electrode implantation procedure was the same for the static bat vocalization experiments except that we substituted superglue (CrazyGlue) for agar to keep the electrodes in place, but agar was still applied on the abdomen to help maintain a horizontal posture. The tethered mantis was placed in the corner of the flight room, attached to the ceiling in the same manner as for the bat attack experiments. A Brüel & Kjaer 2231 sound level meter with a 4135 6.25 mm microphone (protective grid off) mounted slightly below (microphone centered approximately 4 mm below) and behind (approximately 4–5 mm) the preparation's ear recorded bat vocalizations. This microphone is highly sensitive to direction and, because of its close proximity to the mantis ear, provided an accurate measurement of the vocalizations acting on the mantis ear. The bat sat on a movable platform placed, at 1 m increments, 1–6 m away from the preparation in random order. We recorded data in 4 s blocks and attempted to capture only the loudest calls at a given distance (since these calls indicated that the bat's head was aimed at the preparation). The same DAT recorder stored the neural responses and trigger signal used to stop the sound recording system.

Sound recordings

The output from the Ultrasound Advice or Brüel & Kjaer microphone was bandpass-filtered (10–100 kHz) and amplified using a Stewart Electronics (model VBF 44) filter (–110 dB at $1.5f_c$). An IoTech 512 Wavebook controlled by a Dell Inspiron laptop computer directly digitized the signals at 240 kHz during the bat attack experiments and at 250 kHz for the stationary bat vocalization experiments.

We used a MATLAB-based program developed by Aaron Schurger (in the laboratory of Dr Cynthia Moss) to analyze bat vocalizations from the bat attack experiments. This program provided the start and stop times and frequencies for the vocalizations as well as the peak frequency and relative amplitude. For the stationary bat vocalization experiments, we used Superscope II (GW instruments) on a Macintosh G4 computer to measure the amplitude and duration of the vocalizations. We converted amplitudes to peak equivalent dB SPL (dB pe SPL) (Stapells et al., 1982) by comparing the peak-to-peak amplitudes of the bat vocalizations with the amplitudes of 25 kHz pure tones of known sound pressure level (SPL) [based on maximum root mean square (RMS) in 1 s intervals using the Brüel & Kjaer 2231 sound level meter]. The calibration tones were 300 ms in duration with a 10 ms rise/fall time. We recorded calibration tones in the flight room using the same sound recording procedures as for the stationary vocalization experiments.

Data analysis

For both experiments, we analyzed neural signals stored on DAT using Superscope II after digitization (instruNet, model 100B) on a Macintosh G3 computer. Means and standard deviations are reported unless indicated otherwise. Statistical tests were conducted using InStat 2.02a (GraphPad Software, Inc). The Student's *t*-test was used in cases of equal variances; in other cases, we used the non-parametric Mann–Whitney *U*-test. The significance level for all statistical tests was 0.05.

Table 1. *Summary of bat vocalization parameters for the three phases of the bat attack (18 trials, 14 mantis preparations)*

	Approach	Buzz I	Buzz II
Phase begins (ms before contact)			
Mean	1162.5	458.3	219.4
S.D.	216.3	104.9	30
Minimum	867	305	185
Maximum	1820	672	283
Phase begins (cm before contact)			
Mean	–	128.3	61.8
S.D.	–	37.6	10.5
Minimum	–	81	47
Maximum	–	204	85
Phase duration (ms)			
Mean	704.2	229.8	167
S.D.	223.6	93.7	42.4
Minimum	366	66	93
Maximum	1258	355	228
Peak frequency of vocalizations (kHz)			
Mean	38.2	30.1	24.9
S.D.	5.3	1	1.1
Pulse repetition rate (pulses s^{-1})			
Mean	12.9	46.7	130.1
S.D.	1.3	10	14.7
Minimum	10.4	29.2	100
Maximum	15.5	66.8	147.4
Pulse duration (ms)			
Mean	3.8	2.7	1.3
S.D.	0.3	0.37	0.3
Sweep rate (kHz ms^{-1})			
Mean	12.4	17.9	24.7
S.D.	1	1.5	2.7

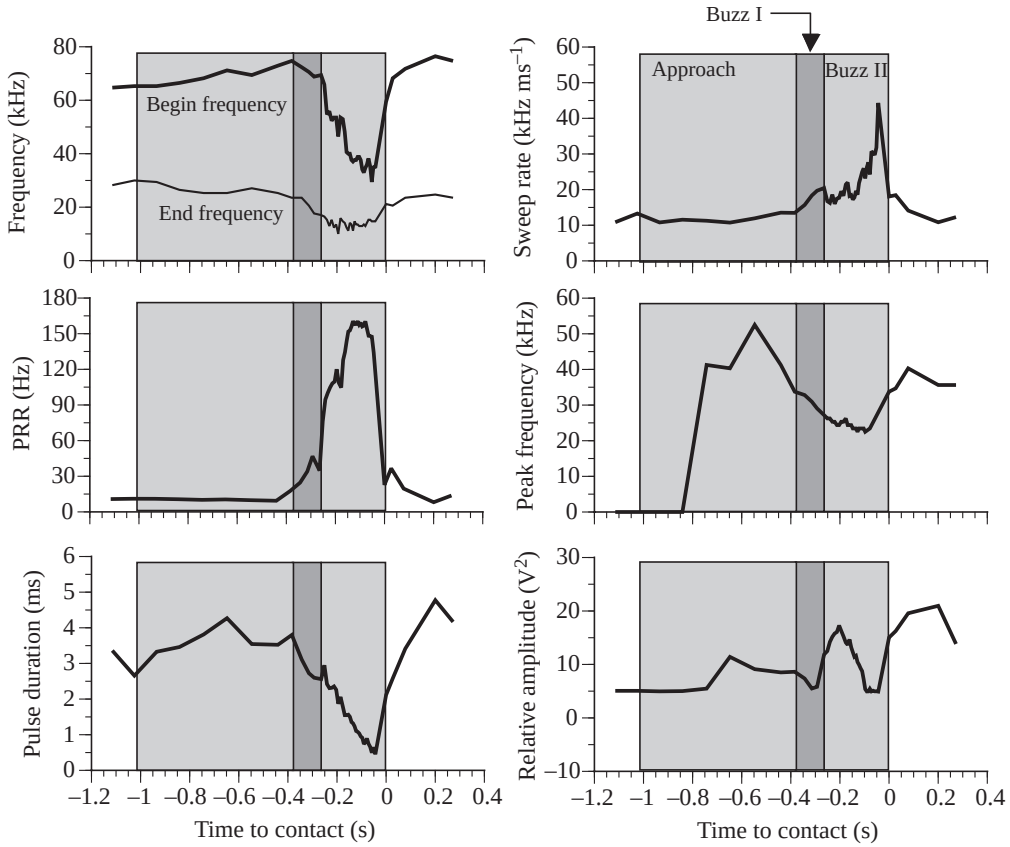

Fig. 3. Example of the changes in different bat vocalization parameters during an attack sequence from one trial. The shaded areas mark the beginning and end of the approach, buzz I and buzz II phases. In the approach phase, pulse repetition rate (PRR) is consistent, pulse durations are over 3 ms and the bandwidth of the echolocation vocalizations is broad (based on the beginning and end frequencies). In the buzz I phase, PRR increases and pulse duration decreases. In the buzz II phase, PRR is over 100 pulses s^{-1}, pulse duration continues to decrease, the sweep rate increases and the bandwidth of the echolocation vocalizations narrow. In the beginning of the buzz II phase, the relative amplitude of the echolocation vocalizations increases (probably as a result of the bat approaching the microphone located near the mantis target), but the relative amplitude drops in the second half of the buzz II phase (approximately 100 ms before contact).

Results

The bat attack results presented here come from 18 trials (14 different mantis preparations) in which the bat made contact with the mantis or the tether just above the mantis at the end of the attack sequence.

Bat vocalization behavior

The bats vary their vocalizations as they navigate around the room and as they approach and attempt to capture the mantis neural preparation (Fig. 3). PRR increases, while pulse duration decreases. The bandwidth of the vocalizations decreases (based on the beginning and ending frequencies of the vocalizations) and the sweep rate increases. The peak frequencies and relative amplitude also change.

In the present experiment, the attack sequence is divided into three phases: stable approach, buzz I and buzz II (Fig. 3). Bats do not produce search-type vocalizations within the flight room (Surlykke and Moss, 2000). We defined the beginning of the stable approach phase as the time when the bat reached a stable vocalization PRR after leaving the platform. We took the start of the buzz I phase as the time when the bat increased its PRR above 20 pulses s^{-1} as it neared the target and the start of the buzz II phase as the time when the PRR reached 100 pulses s^{-1} and once again stabilized at this high PRR. PRR changed the most during the buzz I phase.

Fig. 2 illustrates the flight room arrangement and the two types of path that the bat used when attacking the mantis. The majority of the trials (lasting between 1200 and 1600 ms) consisted of the bat leaving the platform and flying directly towards the target. For longer trials (in which the bat flew around the room), we reduced the analysis to the point in the trial when the bat began its attack on the target (on the basis of the stable approach phase definition). Table 1 provides a summary of the bat vocalization parameters (see also *Bat vocalization behavior* section in the Discussion).

Clip electrode recordings of 501-T3

Previous extracellular recordings using a suction electrode placed over the caudal cut end of the prothoracic connective from 47 mantis species reveal three spike types in response to ultrasound (Yager, 1999). Large action potentials (signal-to-noise ratio typically >5:1) with a latency of 8–12 ms firing in a phasic-tonic pattern are always present. Occasionally, a very low amplitude tonic unit is visible above the background activity, and a very high amplitude phasic unit appears with a long latency at high sound pressure levels. Simultaneous extracellular and intracellular recordings reveal that the large phasic-tonic unit is activity in 501-T3, first described in *Mantis religiosa* (Yager and Hoy, 1989). 501-T3 exhibits several characteristics that distinguish it from other units in extracellular recordings: (i) it responds to ultrasound, (ii) it is not spontaneously active, (iii) action potentials reach the prothorax with a short latency (8–12 ms) and (iv) its action potentials are of large amplitude because of its large axon diameter.

The ultrasonic bat vocalizations in this experiment evoked neural activity in the prothoracic connective, as measured by

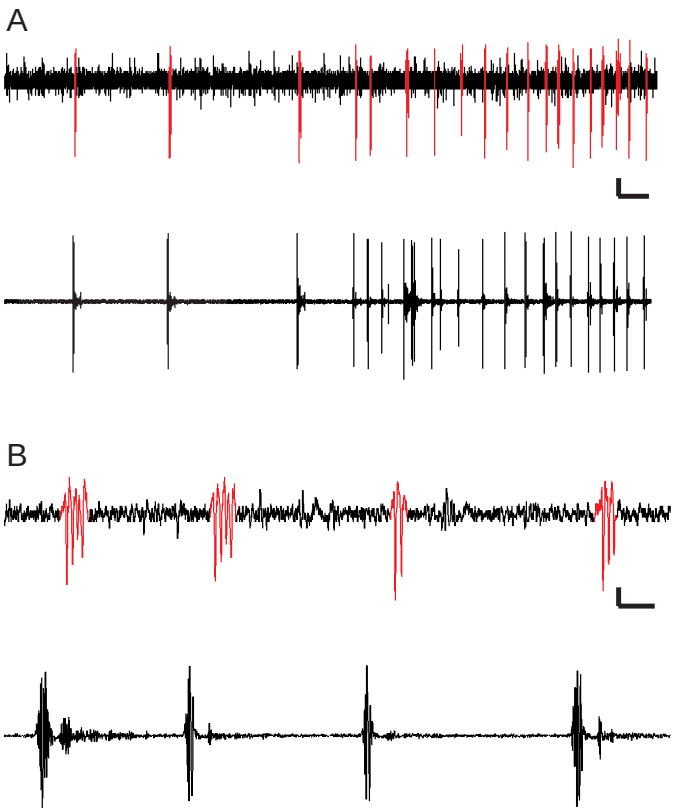

Fig. 4. (A) Example of an electrode implant recording (upper) with the corresponding bat vocalizations taken from the bat detector (below). The responses of 501-T3 are in red. 501-T3 responses occur only after a bat vocalization, and each vocalization elicits a 501-T3 response, indicating that the interneuron can encode the bat's pulse repetition rate. Time scale, 50 ms; voltage scale, 20 mV. (B) Same recording with the time scale expanded to illustrate that the responses of 501-T3 (in red) to a single bat vocalization contain multiple spikes. The first spike in each burst of 501-T3 activity has a larger amplitude than the following spikes because of its very high phasic firing rate. Time scale, 10 ms; voltage scale, 20 mV.

the clip electrode. Fig. 4 shows an example of a typical extracellular recording of auditory responses (in red in Fig. 4A) to bat vocalizations using the clip electrode technique. 501-T3 burst rate (top trace) increases as the repetition rate of the bat vocalizations (bottom trace) increases. Each vocalization can elicit a burst of spikes from 501-T3, evident when the time scale is expanded (Fig. 4B). The amplitude of auditory-evoked spikes was large and easily distinguishable even in recordings exhibiting high levels of background neural activity. Neural responses elicited by bat vocalizations did not resemble in amplitude and/or shape other responses occurring spontaneously in the absence of a bat vocalization (thus indicating a non-spontaneously active unit). The latency of vocalization-elicited responses was 10–14 ms (corrected for distance differences between the mantis and microphone locations). On the basis of these parameters, we are confident that the recorded auditory-evoked neural responses came from 501-T3. In the present study, 501-T3 responds only in a phasic manner, with no tonic component. This is probably because of the short-duration bat vocalizations. Neither the low-amplitude unit nor the very large amplitude, long-latency component appeared in the neural recordings during any of the trials.

The neural traces shown in Fig. 4 (and subsequently in Figs 5 and 7) seem to show that the amplitude of the auditory spike is not constant, suggesting that the spike bursts may contain action potentials from auditory units other than 501-T3. Several observations argue against this. Because of the very high initial firing rate of 501-T3, spike heights decrease progressively. This decrease is evident in both intracellular and extracellular recordings from 501-T3 (Yager and Hoy, 1989) and is also exhibited in the trace shown in Fig. 4B. Another source of variation in spike amplitude comes from the background neural activity and noise. Although the auditory-evoked spikes are easily distinguishable within this background activity, it can alter the spike amplitude. A third source of variation occurs in the long neural traces showing responses during the entire attack sequence (Figs 4A, 5, 7). The lower resolution of the Superscope II data analysis program for viewing long traces of data alters the spike amplitude. These differences disappear, however, when the time scale is expanded (thus increasing the resolution).

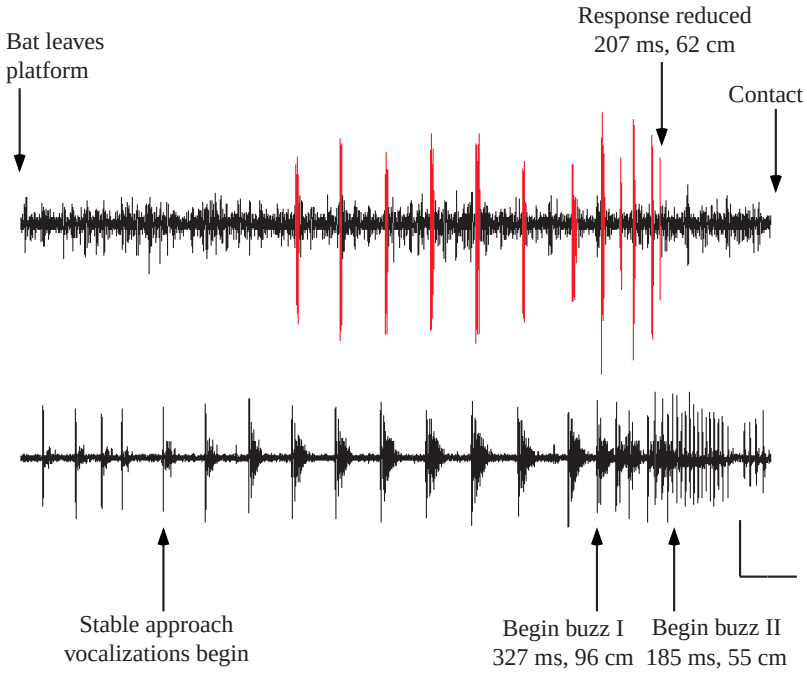

Fig. 5. Example of a B1 flying bat attack trial. The upper trace is the neural recording and the bottom trace the corresponding bat vocalizations taken from the bat detector. The responses of 501-T3 are in red. The different phases of the bat attack are indicated. Time scale, 50 ms; voltage scale, 10 mV.

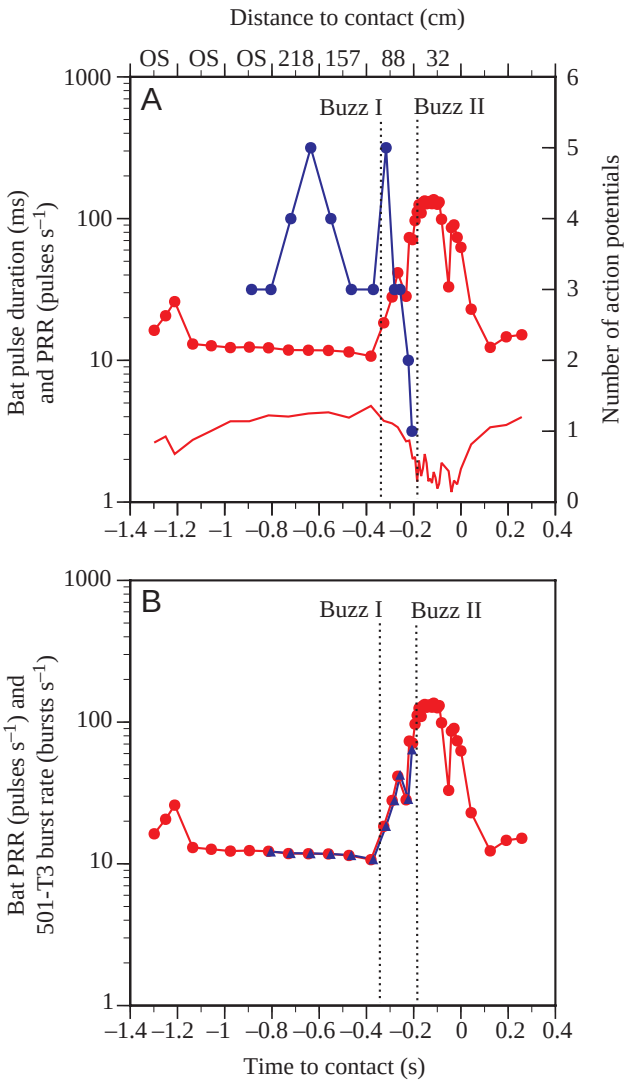

Fig. 6. (A) The bat pulse duration (red line, no symbols, left axis in ms), pulse repetition rate (PRR; red line/circles, left axis in pulses s^{-1}) and 501-T3 spikes per burst (blue line/circles, right axis) for the corresponding B1 trial in Fig. 5. The top axis is distance to contact (cm). OS, out of the calibrated space. 501-T3 burst responses cease before the buzz II phase begins (indicated by the right-hand dotted line). (B) A comparison of the bat PRR (red line/circles, in pulses s^{-1}) with the burst rate of 501-T3 (blue line/triangles, in bursts s^{-1}) to illustrate accurate following of bat vocalization emissions by 501-T3 during the stable approach phase and into the buzz I phase until responses cease entirely.

Responses of 501-T3 to flying bat attacks

Fig. 5 shows the neural trace and corresponding bat vocalizations for a 1400 ms trial. Fig. 6A shows, for the same trial, the bat PRR (red line, circles), the pulse duration (red line, no symbols) and the number of spikes elicited in each 501-T3 response burst (blue line, circles). Fig. 6B compares the bat's PRR (red line, circles) with the burst rate of 501-T3 (blue line, triangles).

When the bat first leaves the platform, its flight can be somewhat unstable. This is reflected in its vocalizations: the first vocalizations in Figs 5 and 6 have shorter durations and the PRR is not consistent. The later constant PRRs (approximately 13 pulses s^{-1}) and corresponding pulse durations (>3 ms) are indicative of a stable flight pattern with goal-directed echolocation (the goal being the mantis target).

In this trial, the bat achieves stable approach behavior 1150 ms before contact. The stable approach vocalizations are emitted at 12 pulses s^{-1} and are 3–4 ms in duration. 501-T3 begins responding 900 ms before contact, and each vocalization elicits a burst response of 3–5 spikes (shown clearly in Fig. 5 and Fig. 6A). The accurate coding of bat PRR (Fig. 6B) continues into the buzz I phase, when PRRs increase. However, in the middle of buzz I, the interneuron stops responding completely, and no more responses are elicited even though the bat continues to vocalize. The number of spikes elicited in each burst declines earlier than the accuracy of coding of bat PRR, changing from 3–5 spikes per burst during the approach and the beginning of the buzz I phases to 1–2 spikes per burst as PRR increases (Fig. 6A). However, it is important to note that the decline in the number of spikes elicited per burst also correlates with the decrease in pulse duration (Fig. 6A).

To summarize this trial, 501-T3 follows bat PRRs up to 64 pulses s^{-1}. The number of spikes elicited per burst declines as PRR increases and pulse duration falls below 3 ms. Pulse duration continues to decrease until contact. The last response from 501-T3 (a single spike) occurs 207 ms before contact when the bat is 62 cm from the target. This trial is categorized as a B1 trial. B1 trials include cases in which the output of 501-T3 changes from spike bursts to either complete cessation of neural activity or one (and only one) single-spike between burst cessation and complete cessation of neural activity. B1 cases accounted for 55 % of all trials. In B1 trials, the last spike burst occurred 266.6±53.6 ms before contact (75.4±9.8 cm away from the preparation, N=10). In cases where a single spike occurred after burst cessation (half of B1 trials), the single spike occurred 221.8±49.1 ms before contact (66.8±8.7 cm away from the preparation, N=5).

Fig. 7 shows the neural responses of 501-T3 and the corresponding bat vocalizations for a different 1400 ms trial. Fig. 8 is comparable with Fig. 6. This trial is similar to the previous trial in many ways. 501-T3 responses begin once the bat achieves a stable approach PRR and pulse duration. Each vocalization elicits a burst (3–5 spikes each) through the approach phase and into the beginning of the buzz I phase. As in the previous trial, burst responses cease during the buzz I phase as PRR increases. This is also the point at which accurate coding of the bat PRR by 501-T3 begins to fail (60 pulses s^{-1}) and the vocalization pulse duration falls below 3 ms. Burst responses cease 211 ms before contact, when the bat is 59 cm from the target. However, this trial differs from the B1 trials because, after burst cessation, single-spike responses continue to be produced into the buzz II phase. In the buzz II phase, 501-T3 completely ceases all activity. In this trial, single-spike responses end 127 ms before contact

when the bat is 42 cm away. We refer to trials in which 501-T3 burst responses and accurate PRR coding end during the buzz I phase but several single-spike responses continue to be produced into the buzz II phase as B2 trials. B2 trials accounted for 45 % of the trials. In these trials, the last spike burst occurred 278.9±87.8 ms before contact (69.8±15.4 cm away from the preparation, N=8). The last single spike response occurred 96±54.8 ms (34.4±14.3 cm away from the preparation, N=8).

No significant differences exist between the bat vocalization parameters occurring in B1 and B2 trials. Table 2 shows these comparisons.

Fig. 9 summarizes our data on the cessation of 501-T3 activity (for both multi-spike bursts and single-spike responses) for B1 and B2 trials (in terms of time to contact and distance to contact). This figure also illustrates the difference between B1 and B2 trials. The last burst (diamonds) and last spike (crosses) data points for B1 (red symbols) are clustered, while those for B2 (blue symbols) have a greater separation. There are no significant differences between the times and distances when spike bursts end in B1 and B2 trials [Student's t-test; time, $t(16)=0.3664$, not significant; distance, $t(16)=0.9506$, not significant]. However, there are differences between the times and distances when single-spike responses end [Student's t-test; time, $t(10)=4.0840$, $P<0.01$; distance, $t(10)=4.4600$, $P<0.01$]. Spike burst responses are more likely to be involved in eliciting evasive behavior (see Discussion). Therefore, if we combine the burst cessation data for B1 and B2 (which are not significantly different), the mean time before contact at which activity ceases is 272.06±68.8 ms (72.9±12.5 cm away from the preparation, N=18). The mean bat PRR that 501-T3 can follow is 55.3±21.1 pulses s^{-1} (N=18) (minimum, 25.7 pulses s^{-1}; maximum, 100.5 pulses s^{-1}).

Fig. 10 presents a timeline to summarize the significant events in both the bat attack sequence (right, red) and the mantis 501-T3 responses (left, dark blue). The timing of these events is based on the mean values obtained from our trials presented in the tables and the text.

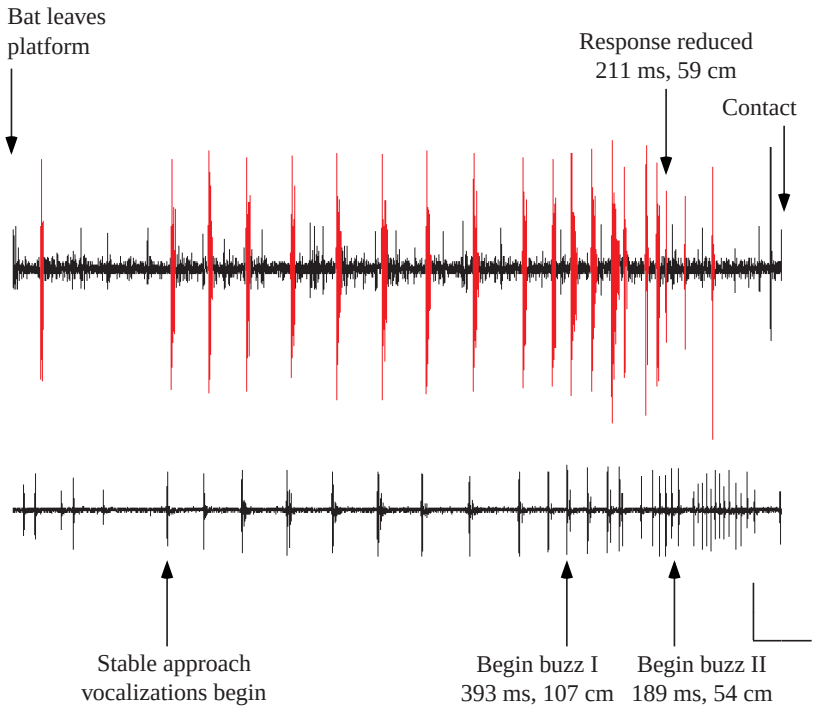

Fig. 7. Example of a B2 flying bat attack trial. The upper trace is the neural recording and the bottom trace the corresponding bat vocalizations taken from the bat detector. The different phases of the bat attack are indicated. Time scale, 50 ms; voltage scale, 10 mV.

Table 2. *Statistical test results comparing bat vocalization parameters in B1 and B2 trials for the three different stages of a bat attack sequence*

	Approach phase	Buzz I phase	Buzz II phase
Begin time	t=0.4057	t=0.6796	t=1.9334
Phase duration	t=0.2086	t=0.1381	t=0.2534
Begin distance		t=0.4379	t=0.9243
Mean peak frequency	t=0.2421	t=0.4500	U=32.00
Mean PRR	t=0.9464	t=0.5925	t=1.0751
Mean pulse duration	t=0.5200	U=35.5	t=0.6191
Mean sweep rate	t=0.7512	U=27.00	t=1.7430
Buzz duration			t=0.2228

PPR, pulse repetition rate.

The Student's t-test was used in cases of equal variances; in other cases, we used the non-parametric Mann–Whitney U-test. The significance level for all statistical tests was 0.05.

There were 10 B1 trials and 8 B2 trials. Bat vocalization patterns cannot account for the response differences in B1 and B2 trials, none of the comparisons were statistically significant.

Responses of 501-T3 to vocalizations from stationary bats

We also looked at the responses of 501-T3 to actual bat vocalizations of different intensities and durations, allowing us to assess the dynamic range of 501-T3. The bat produced single vocalizations similar in frequency and structure to those used when echolocating. Fig. 11 illustrates the range of intensities emitted at each distance from the preparation. There is a decrease in intensity with distance, partly due to spherical spreading. The large variation at a given distance and the large overlap in intensities across distances is probably due to the aim of the bat's head when vocalizing. Fig. 12 shows the responses from two animals (PAR-17-16, top; PAR-16-36, bottom) to static bat vocalizations. Vocalizations can elicit as many as 5–6 spikes per burst. Although there is a trend for louder vocalizations to elicit neural bursts containing more spikes, a large amount of overlap exists. The data are also divided into responses to pulses of 3 ms or longer (purple

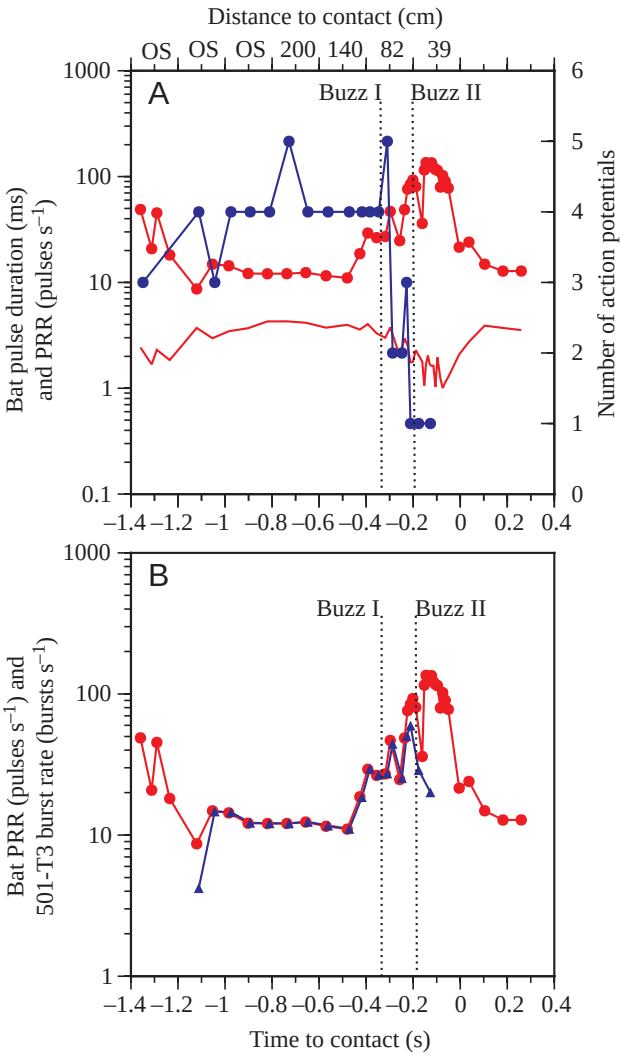

Fig. 8. (A) The bat pulse duration (red line, no symbols, left axis in ms), pulse repetition rate (PRR; red line/circles, left axis in pulses s^{-1}) and 501-T3 spikes per burst (blue line/circles, right axis) for the corresponding B2 trial in Fig. 6. The top axis is distance to contact (cm). OS, out of the calibrated space. 501-T3 burst responses cease before the buzz II phase begins (indicated by the right-hand dotted line), but single-spike responses continue into the buzz II phase; these cease before contact. (B) A comparison of the bat PRR (red line/circles, in pulses s^{-1}) with the burst rate of 501-T3 (blue line/triangles, in bursts s^{-1}) to illustrate accurate following of bat vocalization emissions by 501-T3 during the stable approach phase and into the buzz I phase. Although 501-T3 continues to respond to bat vocalizations into the buzz II phase, accurate encoding of bat PRR breaks down before the transition from the buzz I phase to the buzz II phase (indicated by the decrease in 501-T3 burst rate before the buzz II phase).

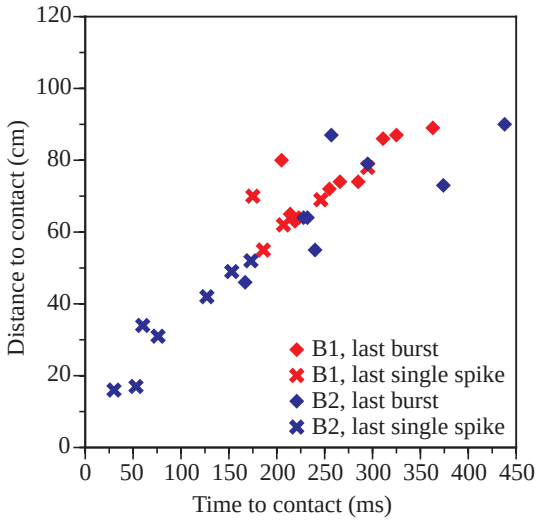

Fig. 9. Summary of the time and distance to contact for the last burst response (diamonds) and the last single-spike response (crosses) for both B1 (red symbols) and B2 (blue symbols) trials. The last burst responses for B1 and B2 trials form a cluster. The last single-spike response for B1 trials also falls within this cluster. However, the last single-spike response for B2 forms a separate cluster.

symbols) and of less than 3 ms (green symbols). Pulse duration does not seem to greatly affect the number of spikes within a burst, but can affect the chance of eliciting a response. PAR-17-16 responded to 47 % of the vocalizations of 3 ms or longer compared with 4 % of the vocalizations of less than 3 ms. The results were slightly more evenly distributed for PAR-17-16 (57 % response to vocalizations of 3 ms or longer, 17 % response to vocalizations of less than 3 ms).

Fig. 13 shows the percentage of vocalizations that elicited at least one spike on the basis of duration and intensity, combining all the data from five mantids and 1130 bat vocalizations. For pulse durations greater than or equal to 3 ms (purple line), there is a sigmoidal response *versus* intensity function. Intensities over 70 dB pe SPL reliably elicit at least one spike, resulting in response rates of 70 % for intensities between 70 and 79 dB pe SPL, 93 % for intensities between 80 and 89 dB pe SPL and 100 % for intensities over 90 dB pe SPL. Below 70 dB pe SPL, response reliability drops to 15 %. For pulse durations shorter than 3 ms (green line), the curve is quite different. There was only one case in which a pulse duration of less than 3 ms and intensity greater than 90 dB pe SPL elicited a response. When pulse durations less than 3 ms in duration were between 80 and 89 dB pe SPL, response reliability was 48 %; response reliability decreased with decreasing intensity.

Fig. 14 describes whether 501-T3 responds to a bat vocalization with a multi-spike burst (squares) or a single spike (circles) for pulse durations of 3 ms or longer (purple lines) and for pulse durations less than 3 ms (green lines). For pulse durations of 3 ms or longer, the percentage of responses that are spike bursts decreases with decreasing intensity. However, more than 60 % of the positive responses are spike bursts for intensities over 60 dB pe SPL. For pulse durations shorter than 3 ms, single-spike responses make up more of the positive responses compared with pulse durations of 3 ms or longer. However, for intensities over 60 dB pe SPL, spike bursts still occur more often than single-spike responses (57–58 % for 60–79 dB pe SPL, 75 % for 80–89 dB pe SPL and 100 % over 90 dB pe SPL).

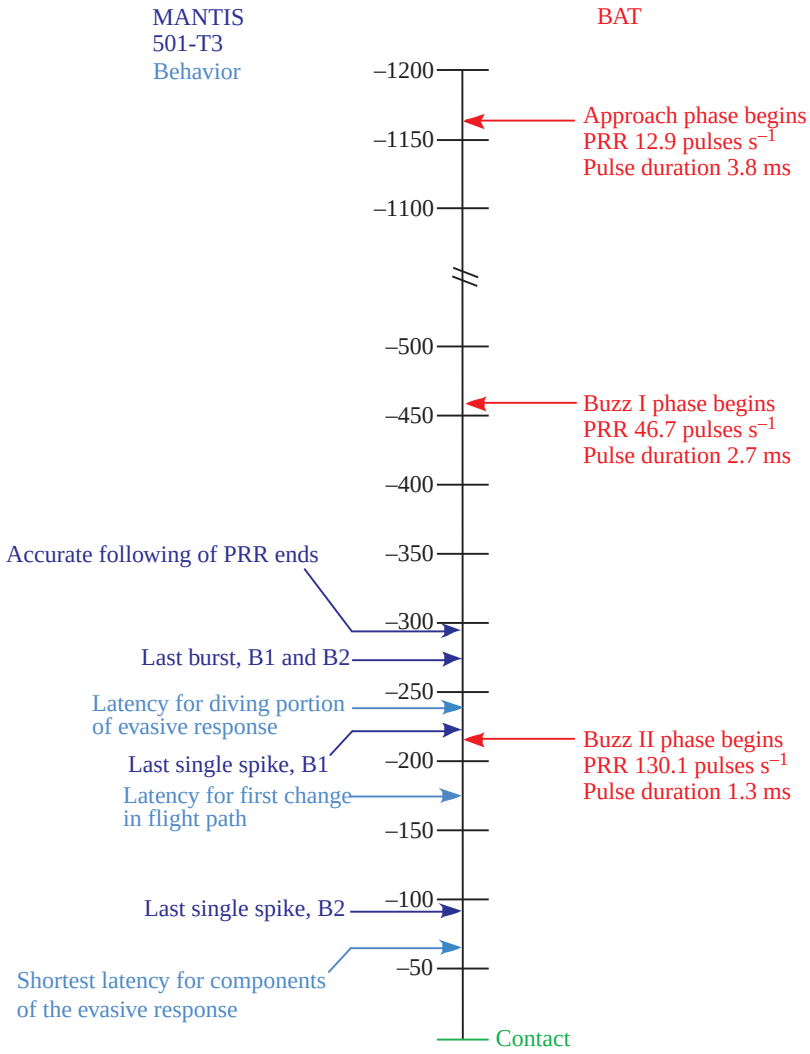

Fig. 10. Summary of the pertinent events in the bat attack vocalization sequence (red, right) and the corresponding 501-T3 neural events (dark blue, left) and mantis evasive behavior latencies (light blue, left). 501-T3 ceases producing burst responses and accurately following bat pulse repetition rate (PRR) during the buzz I phase, and this correlates well with the latency for the diving portion of the mantis evasive response.

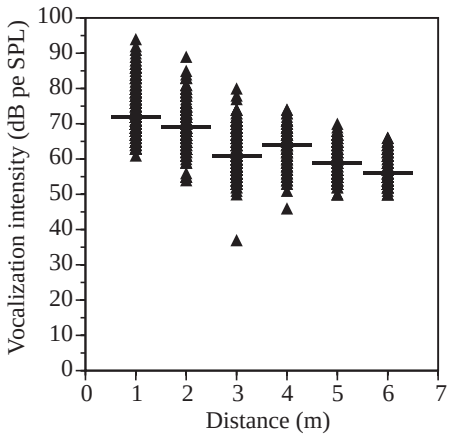

Fig. 11. Range of intensities at a range of distances from the 501-T3 preparation for all vocalizations for the stationary bat. The bars indicate the median intensity at each distance. Although there is considerable overlap, there is a general decrease in vocalization intensity as distance increases.

Discussion

In this study, we recorded the responses of mantis auditory interneuron 501-T3 during the last 1500 ms before capture by a flying bat. 501-T3 responds reliably to bat vocalizations at low PRRs (emitted during the approach phase) with multi-spike bursts, but ceases to produce bursts as PRR increases and pulse duration decreases during the buzz I phase. Neural activity ceased during the buzz I phase in 55 % of the trials, while neural bursts ended during the buzz I phase but single-spike responses continued into the buzz II phase in the remaining trials. We also recorded the responses of 501-T3 to single bat vocalizations emitted by a stationary bat. 501-T3 response reliability decreases when sound duration falls below 3 ms and the intensity is under 90 dB pe SPL. These short-duration sounds also decrease the chance of eliciting a neural burst response in 501-T3 at higher intensities (≥70 dB pe SPL). This is consistent with results from the flying bat attack experiments suggesting that high PRRs and shorter pulse durations may interact to decrease the number of spikes in a burst and also with unpublished data from our laboratory demonstrating that 501-T3 thresholds increase as pulse durations shorten and that 501-T3 responds poorly to pulse trains mimicking the terminal buzz stage of a bat attack. The cessation of activity in this auditory interneuron may possibly be related to the mantid's evasive behavior.

Bat vocalization behavior

Since we are interested in how 501-T3 responds to vocalizations produced by attacking bats in the natural situation, our experiment is a reasonable approximation only if the echolocation behavior in the laboratory closely mimics that observed in the field. Surlykke and Moss (2000) published a detailed comparison of the echolocation vocalizations of *E. fuscus* in the field and in the laboratory (using the same flight room described in the present study). They found that long-duration, low-PRR search-type calls emitted in the field are not produced in laboratory. The PRRs of approach calls in the laboratory match those in the field vocalizations, but the pulse durations are not as long as some approach call durations in the field (2–4 ms in the laboratory, up to 12 ms duration in the field). The laboratory approach pulse durations, however, do mimic field vocalizations in the late approach phase, just before the transition to the terminal buzz. The duration of the terminal buzz (buzz I + buzz II) observed in the laboratory was longer (range 230–1005 ms) than that of terminal buzzes in the field (range 170–660 ms). In the laboratory, the decrease in

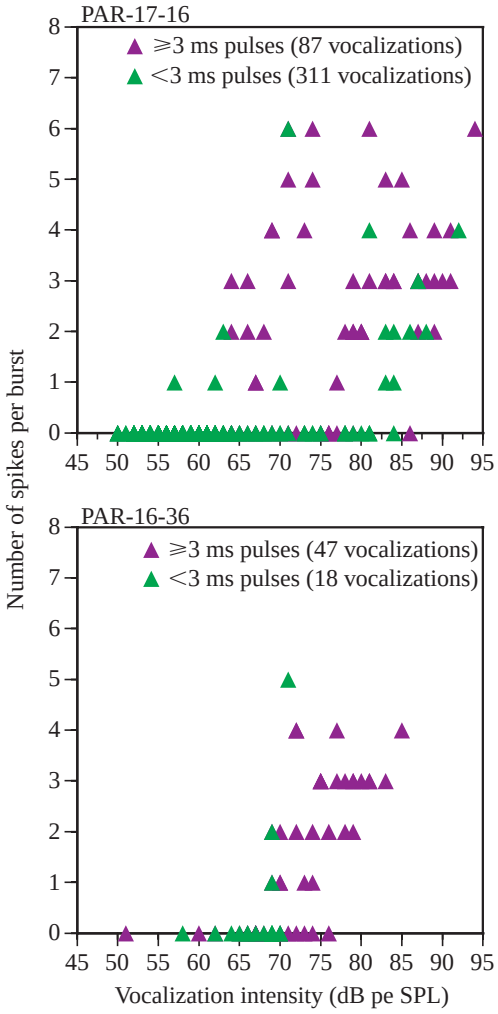

Fig. 12. Number of 501-T3 spikes per burst elicited by bat vocalizations at different intensities for two mantids in the stationary bat vocalization experiment. Responses are separated into vocalizations 3 ms and longer (purple triangles) and those less than 3 ms (green triangles). For PAR-17-16, 269 of the 311 vocalizations shorter than 3 ms and 26 of the 87 vocalizations of 3 ms or longer between 50 and 65 dB pe SPL did not elicit a response, and their data points overlap. For PAR-16-36, the only large overlaps are at 69 dB pe SPL (no response) for vocalizations of less than 3 ms (five cases) and at 57 dB pe SPL (no response) for vocalizations of 3 ms or longer (six cases).

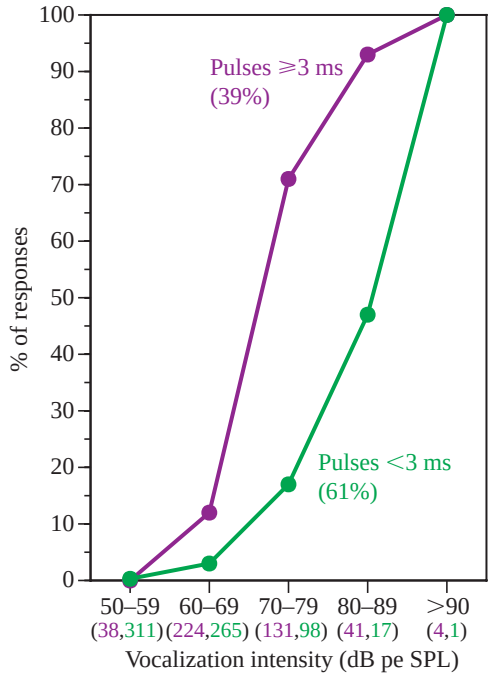

Fig. 13. Percentage of 501-T3 responses (either single-spike or multi-spike bursts) to vocalizations of different intensity ranges for pulses 3 ms and longer (purple circles) and pulses less than 3 ms (green circles). The numbers in parentheses indicate the number of vocalizations at each intensity (purple for pulses 3 ms or longer, green for pulses less than 3 ms). 501-T3 responds more reliably to longer bat vocalizations (durations of 3 ms or longer) at all intensities except those that are very loud (>90 dB pe SPL) or very quiet (<60 dB pe SPL).

vocalization duration and increase in PRR characteristic of the terminal buzz resembled those of field recordings. Laboratory PRRs during the buzz II phase reached 167 pulses s^{-1}.

In our study, we also did not detect search vocalizations, but this does not concern us since we are interested in the vocalizations emitted in the later stages of a bat attack. Average maximum PRRs in our study (133 pulses s^{-1}) were lower than those recorded both in the field and in the laboratory by Surlykke and Moss (167 pulses s^{-1}). However, this difference is not important because 501-T3 shuts down before the bat reaches these PRRs. In our study, the buzz durations (mean 430.2±112.2 ms; range 235–686 ms, N=18) were shorter than those measured by Surlyyke and Moss (2000), but were actually within the range of buzz durations recorded in the field. Therefore, the terminal buzzes in our study provide a good match to the vocalization stimuli acting on the mantis auditory system in the natural situation.

Why does 501-T3 cease activity during bat attacks?

The most intriguing result in our study is the finding that 501-T3 stopped producing action potentials before the bat (which continued to vocalize) made contact with the mantis neural preparation. Since PRR, pulse duration and vocalization intensity all covary during a bat attack, it is difficult to determine which factor is most important in causing 501-T3 to cease responding in the later stages of a bat attack. It is probably a combination of these three parameters, each contributing to the eventual cessation of this auditory interneuron's activity. The cessation of 501-T3 activity during the last moments of a bat attack is somewhat puzzling since the closer a bat is to the mantis, the greater danger it poses. So why does 501-T3 cease responding at the apparent time when it is needed the most? The answer may lie in the evasive behavior of the mantis.

Fig. 10 includes the latencies for different events in producing the mantis evasive behavior (left, light blue) in the

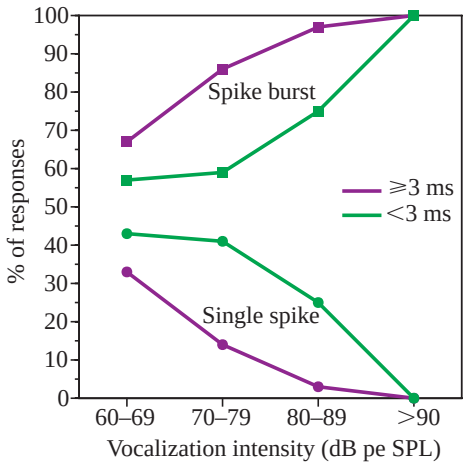

Fig. 14. Diagram showing whether a response of 501-T3 to a bat vocalization was a multi-spike burst (squares) or a single spike (circles) for pulse durations of 3 ms or longer (purple lines) and pulse durations of less than 3 ms (green lines).

context of the neural activity of 501-T3 (left, dark blue) and the bat vocalizations (right, red). Components of the mantis evasive behavior begin as early as 66 ms after stimulus onset (Yager and May, 1990). Changes in the flight path of the mantis occur 172.5±38.9 ms (range 125–230 ms, *N*=10) after stimulus onset, and the power dive portion of its response is fully developed at 242±47.2 ms (range 140–290 ms, *N*=16) (Yager et al., 1990) after stimulus onset. High-frequency neural bursts from 501-T3 (and possibly other auditory interneurons) may be necessary to initiate these behaviors, i.e. single spikes are ineffective (see below). In the present study, 501-T3 stops producing neural bursts 272 ms (range 167–438 ms) before contact, which correlates well with the power dive latency (242 ms).

The correlation between the cessation of 501-T3 activity and power dive latency could have several functional implications. The diving portion of the response should begin at least 242 ms (latency for the response) before contact to have a good chance of reaching completion. Once the dive has started, new auditory information could be irrelevant to the mantis and there may be no reason for 501-T3 to continue responding to ultrasound. Although the auditory information may not benefit the mantis once the dive has begun, it could have a harmful effect. Very high PRRs and/or high-intensity vocalizations could over-stimulate 501-T3, causing habituation. Intracellular recordings from 501-T3 show that the interneuron does habituate (Yager and Hoy, 1989). If this habituation were to last minutes or even seconds, 501-T3 (and the mantis) might not respond to ultrasound once the mantis had finished its initial dive. Experiments involving encounters between free-flying bats and mantids (in the flight room used in these experiments) demonstrate that bats enter the terminal buzz phase even when a mantis produces a power dive (J. D. Triblehorn, K. Bohn, C. F. Moss and D. D. Yager, unpublished data). Therefore, the mantis potentially experiences very high PRRs when escaping from an attack. If habituation occurred, it could leave the mantis vulnerable to multiple attacks by the same bat or by other bats hunting in the same area. The cessation of 501-T3 activity may prevent this habituation from occurring.

Possible mechanisms for cessation of 501-T3 activity

There are three possible mechanisms that could account for the cessation of 501-T3 activity. The first possible mechanism involves the possibility that auditory afferents cannot follow the high PRRs emitted during the buzz I and buzz II phases of a bat attack. However, extracellular recordings from many insects demonstrate that auditory afferents can respond to these high PRRs. Auditory afferents in moths and green lacewings can respond to individual pulses for PRRs over 100 pulses s^{-1} (Fullard, 1984; Miller, 1971). Mantis tympanal recordings from Yager and Hoy (1989) showed that afferents can follow short (5 ms) pulses presented at repetition rates up to 80–100 pulses s^{-1}, and more recent results illustrate that the afferents can follow pulse durations of 1 ms or less up to 300 pulses s^{-1} (D. D. Yager, unpublished data).

Despite the ability of the auditory afferents to follow high PRRs, a second possible mechanism may involve either the synapse between the afferents and 501-T3 or the intrinsic properties of 501-T3. The afferents may not be able to drive auditory interneurons at the same repetition rate, causing PRR coding to break down. For example, 501-T3 activation may require synchronous afferent input. As PRR increases, afferent activity could become asynchronous and unable to activate 501-T3. Alternatively, some intrinsic property of 501-T3 (such as a long time constant) may prevent faithful reproduction of high PRRs.

A third possible mechanism involves active inhibition of 501-T3. Some evidence supports the possibility that another auditory-sensitive neuron (or neurons) may actively turn off 501-T3. Intracellular recordings of 501-T3 revealed that a delayed inhibitory effect does act on 501-T3 beginning at intensities 15 dB over threshold and increasing in strength with higher intensities (Yager and Hoy, 1989). Results from pulse pair experiments show that this inhibition begins 4–7 ms after 501-T3 responds and is completed by 20 ms, with maximal effects occurring between 8 and 12 ms (Yager and Hoy, 1989). As a bat closes in on the mantis, both vocalization intensity and increasing PRR may strongly activate this inhibition, shutting off 501-T3 responses. The static vocalization experiments also show that short-duration calls activate 501-T3 less strongly, making it easier to inhibit 501-T3 totally.

A comparison of B1 and B2 trials

In the cricket *Teleogryllus oceanicus*, the Int-1 auditory interneuron produces ultrasound-triggered evasive behavior only if neural bursts exceed 220 spikes s^{-1} (Nolen and Hoy, 1984). If a similar relationship exists between 501-T3 and the initiation of the mantis evasive response, does it matter that single spikes occur after burst cessation in some trials but not

in others (the only distinction between B1 and B2 trials)? The difference in the pattern of neural activity exhibited in B1 and B2 trials may not affect the initial escape response. However, this difference may be important for understanding how multi-spike bursts cease during an attack. In the active inhibition model proposed above, the lack of difference in 501-T3 burst cessation between B1 and B2 trials suggests that active inhibition occurs at approximately the same time in both trial types and may be linked to some reliable stimulus parameter (such as PRR). However, maintaining the inhibition may be difficult as the bat gets close to capturing the mantis. In some cases (B1 trials), the inhibition works to eliminate 501-T3 responses. In other cases (B2 trials), the inhibition starts to break down, allowing single spikes to occur. These single spikes may serve to reinforce the inhibition so that 501-T3 does not begin producing spike bursts until after the initial attack. Differences between B1 and B2 trials may be due to the one parameter that we could not measure reliably: intensity. Compared with buzz II bat vocalization intensities in B1 trials, those in B2 trials could be louder (strong stimulation of 501-T3 overcomes the inhibition, allowing the production of single spikes but still preventing bursts) or softer (moderately stimulating 501-T3 while invoking less inhibition, allowing single spikes through).

A second reason for the distinction is that there was a nearly equal distribution of B1 and B2 trials in our experiments. Therefore, we cannot and should not make any assumptions about which situation is more likely to occur in an encounter between a bat and a mantis in the field.

Risk assessment and insect evasive responses

In the optimal case, insects must not only detect an approaching bat but also assess the level of danger posed by the bat and execute an appropriate evasive maneuver. An inappropriate evasive response can be almost as detrimental as not evading the bat at all. A bat closer to the insect poses a greater threat and elicits a more vigorous evasive response. In addition to early and late responses, green lacewings and some moths (such as arctiids) have a 'last-chance' defense as well, occurring in the last hundreds of milliseconds before capture. For arctiid moths, this is ultrasonic click production. For green lacewings, it is a 'wing-flip' that alters the ballistic trajectory of their passive nosedive, causing bats to miss a seemingly easy target 70% of the time (Miller and Olesen, 1979). These behavioral results suggest that insects exhibiting 'last-chance' maneuvers (green lacewings and arctiid moths) possess auditory systems that are apparently active throughout a bat attack.

The relatively predictable changes in the echolocation calls as bats detect and pursue insect prey provide the best indication of the level of danger posed by the bat. Bat vocalizations provide many possible cues (PRR, intensity, pulse duration and frequency) that the insect could use in assessing this danger. Some cues are very helpful (such as PRR), while others can be ambiguous (intensity) or detrimental (duration). Since each of these parameters covaries during a bat attack, it is unlikely that the insect monitors only one. Instead, interactions between these cues probably affect the insect auditory system and probably improve the insect's ability to assess the level of risk. For example, intensity may be less ambiguous when combined with PRR. Also, increases in intensity may compensate for decreases in duration. It is important to note, however, that none of these parameters would aid insects to avoid gleaning bats using echolocation vocalizations containing low frequencies outside the region of greatest sensitivity of most tympanate insects.

Mantids have both an early-warning response (a gradual turn that is not dependent on the direction of a bat) and a late response (power dives). In this study, we show how the activity of 501-T3 changes during a bat attack, reliably encoding bat vocalizations in the approach phase into the buzz I phase before ceasing its activity. This indicates that information is continuously available to the mantis for risk assessment. On the basis of its sensitivity, short latency and high conduction velocity, it is very likely that 501-T3 is involved in initiating evasive behavior in the mantis, allowing it to avoid bat predators when flying, but this does not imply that other mantis auditory interneurons do not have a role as well. However, we have little information about many of these other neurons and were unable to monitor their activity with the clip electrode.

Experiments have revealed no ultrasound-triggered 'very late' response in *P. affinis*. The cessation of 501-T3 activity in the last 200–300 ms before contact is consistent with this finding. A neural system other than 501-T3 (such as other auditory interneurons, wind-sensitive interneurons or visual interneurons) may become active during the last 200 ms before contact to provide a last chance of escape, and cessation of 501-T3 activity may 'clear the way' for a true 'last-chance' response.

We would like to thank Dr Cynthia F. Moss for allowing us to use the flight room, the video/sound recording equipment and the video/sound data-analysis hardware and software. We would also like to thank Kari Bohn and Erin Gillam for their help with the static bat vocalization experiments and Sachin Vadiya for his excellent care of the mantids. Finally, we would like to thank two anonymous reviewers for their helpful comments. This research was supported by NSF Grant No. IBN9808859 (D.D.Y.) and NIMH (NRSA) Grant No. F31MH12025 (J.D.T.).

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.

Beier, M. (1968). Mantodea Fangheuschrecken. In *Handbuch der Zoologie IV*, part 2/12 (ed. J.-G. Helmeke, D. Starck and H. Wermuth), pp. 1–47. Berlin: De Gruyter.

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

Faure, P. A. and Barclay, R. M. (1994). Substrate gleaning vs. aerial hawking: plasticity in the foraging and echolocation behavior of the long-eared bat, *Myotis evotis*. *J. Comp. Physiol. A* **174**, 651–660.

Faure, P. A., Fullard, J. H. and Dawson, J. W. (1993). The gleaning attacks of the northern long-eared bat, *Myotis septentrionalis*, are relatively inaudible to moths. *J. Exp. Biol.* **178**, 173–189.

Faure, P. A. and Hoy, R. R. (2000a). Neuroethology of the katydid T-cell. I. Tuning and responses to pure tones II. *J. Exp. Biol.* **203**, 3225–3242.

Faure, P. A. and Hoy, R. R. (2000b). Neuroethology of the katydid T-cell. II. Responses to acoustic playback of conspecific and predatory signals. *J. Exp. Biol*. **203**, 3243–3254.

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., 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.

Fullard, J. H. and Thomas, D. W. (1981). Detection of certain African, insectivorous bats by sympatric tympanate moths. *J. Comp. Physiol. A* **143**, 363–368.

Hoy, R. R. (1998). Acute as a bug's ear: An informal discussion of hearing in insects. In *Comparative Hearing: Insects* (ed. R. R. Hoy, A. N. Popper and R. R. Fay), pp. 1–17. New York: Springer.

Kalko, E. K. V. (1995). Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera). *Anim. Behav.* **50**, 861–880.

Miller, L. A. (1971). Physiological responses of green lacewings (Chrysopa, Neuroptera) to ultrasound. *J. Insect Physiol.* **17**, 491–506.

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.

Nolen, T. G. and Hoy, R. R. (1984). Initiation of behavior by single neurons: The role of behavioral context. *Science* **226**, 992–994.

Nolen, T. G. and Hoy, R. R. (1987). Postsynaptic inhibition mediates high-frequency selectivity in the cricket *Teleogryllus oceanicus*: implications for flight phonotaxis behavior. *J. Neurosci.* **7**, 2081–2096.

Roeder, K. D. (1967). *Nerve Cells and Insect Behavior*. Cambridge, MA: Harvard University Press.

Schnitzler, H.-U., Kalko, E., Miller, L. and Surlykke, A. (1980). How the bat, *Pipistrellus kuhli*, hunts for insects. In *Animal Sonar: Processes and Performance* (ed. P. E. Nachtigal and P. W. B. Moore), pp. 619–623. New York: Plenum Press.

Schul, J., Matt, F. and von Helversen, O. (2000). Listening for bats: the hearing range of the bushcricket *Phaneroptera falcata* for bat echolocation calls measured in the field. *Proc. R. Soc. Lond. B* **267**, 1711–1715.

Simmons, J. A. and Grinnell, A. S. (1980). The performance of echolocation: Acoustic images perceived by echolocating bats. In *Animal Sonar: Processes and Performance* (ed. P. E. Nachtigal and P. W. B. Moore), pp. 353–385. New York: Plenum Press.

Stapells, D. R., Picton, T. W. and Smith, A. D. (1982). Normal hearing thresholds for clicks. *J. Acoust. Soc. Am.* **72**, 74–79.

Stumpner, A. and von Helversen, D. (2001). Evolution and function of auditory systems in insects. *Naturwissenschaften* **88**, 159–170.

Surlykke, A. and Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *J. Acoust. Soc. Am.* **108**, 2419–2429.

Tougaard, J. (1999). Receiver operating characteristics and temporal integration in an insect auditory receptor cell. *J. Acoust. Soc. Am.* **106**, 1711–1718.

Triblehorn, J. D. and Yager, D. D. (2001). Broad vs. narrow auditory tuning and corresponding bat-evasive behaviour in praying mantids. *J. Zool., Lond.* **254**, 27–40.

Yager, D. D. (1999). Structure, development and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380–400.

Yager, D. D. and Hoy, R. R. (1987). The midline metathoracic ear of the praying mantis, *Mantis religiosa*. *Cell Tissue Res.* **250**, 531–541.

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.

Yager, D. D. and Spangler, H. G. (1997). Behavioral response to ultrasound by the tiger beetle *Cincidela marutha* Dow combines aerodynamic changes and sound production. *J. Exp. Biol.* **200**, 649–659.

Ye, S., Dowd, J. P. and Comer, C. M. (1995). A motion tracking system for simultaneous recording of rapid locomotion and neural activity from an insect. *J. Neurosci. Meth.* **60**, 199–210.