

Timing of praying mantis evasive responses during simulated bat attack sequences

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Summary

Praying mantids perform evasive maneuvers that vary with the level of danger posed by their bat predators. The vocalization pattern of attacking bats provides cues that mantids can potentially use to decide how and when to respond. Using pulse trains simulating bat attack echolocation sequences, this study determines when in the attack sequence the mantis power dive (its response to high-level threat) occurs and predicts the parameters within the echolocation sequence that are important for eliciting the response. For sequences with a rapid transition from low to high pulse repetition rates (PRRs), the evasive response occurred close to the point during the simulated sequence when the bat would have contacted the mantis. However, the evasive response occurred earlier if the transition was gradual. Regardless of the transition type, the prediction data show that sequences

trigger the response when PRRs reach 20–40 pulses s^{-1} . These results suggest that a bat gradually increasing its PRR could ‘tip off’ the mantis, enabling it to escape. Attack sequences contain gradual transitions when bats engage in strobing behavior, an echolocation phenomenon that may help the bat perceive the auditory scene. Conversely, bat attack sequences that contain rapid increases in PRR close to the point of capture could circumvent the mantid’s auditory defense. Based on these findings, mantids as well as other insects could benefit from having a back-up defense response to offset any advantage the bat gains by rapidly switching from low to high PRRs.

Key words: mantis, bat, escape, insect, hearing.

Introduction

Many animals exhibit rapid escape responses that protect them from attack by predators (Eaton, 1984). Though speed is a primary factor for successful escapes, the decision to initiate these behaviors can also be critical. This decision includes both whether to initiate a response and, if so, when the response should begin. The decision to initiate a response is especially crucial for cases where the prey detects the predator first. Escape responses performed before the predator poses a serious threat (i.e. the predator is far away) will squander energy and possibly hamper responses to future threats. False alarms could further cost the animal by unnecessarily interrupting ongoing behavior, such as tracking prey or a mate. Such improper responses can also make an undetected animal very conspicuous to nearby predators. The timing of an escape response can also be crucial for its success. An evasive response performed too early could allow the predator to adjust its own behavior and capture the prey, while a response occurring too late may not leave enough time to complete the maneuver and successfully escape.

Certain night-flying insects possess auditory systems sensitive to the echolocation calls of bats that prey upon them (Hoy, 1998; Yager, 1999). These insects perform evasive

maneuvers that are effective in eluding their bat predators. For moths (Roeder, 1967), lacewings (Miller and Olesen, 1979), and mantids (Yager et al., 1990), these maneuvers vary depending on whether the bat represents a low-level threat (i.e. gradual turns when the bat is far away) or a high-level threat (i.e. dramatic loops and dives when the bat is very close). The insect’s nervous system must detect the bat, determine the level of danger, and initiate the proper response at the appropriate time for the maneuver to be effective. Furthermore, these actions must occur on the scale of tens of milliseconds to succeed.

Bat vocalizations emitted during an attack sequence undergo a variety of stereotypic changes in pulse repetition rate (PRR), pulse duration (PD), pulse frequency and pulse intensity during attack sequences (Griffin, 1958; Simmons et al., 1979; Kick, 1982; Surlykke and Moss, 2000). These changes provide a variety of cues that insects could use to determine the level of danger posed by a bat and guide decisions such as whether to respond, the type of response, and when to initiate the response. This is the case for the triggering of ‘last chance’ responses in two different insects. High PRRs [>100 pulses s^{-1} (pps)] trigger ultrasonic ‘click’

production in dogbane tiger moths (Fullard et al., 1994). This finding supported the hypothesis that the ‘clicks’ may be attempts to startle (Möhl and Miller, 1976) or confuse (Fullard et al., 1979) the bat moments before capture. Similarly, green lacewings passively fall in response to bat echolocation calls and high PRRs further elicit ‘wing-flips’ in the passively falling lacewings. ‘Wing-flips’ alter the ballistic trajectory of the passive fall, causing the bats to miss the insect (Miller and Olesen, 1979).

Praying mantids possess an auditory system that is sensitive to bat vocalizations, with auditory tuning most sensitive between 20–60 kHz in most species (Yager and Hoy, 1989; Triplehorn and Yager, 2001). The single ear, located ventrally between the metathoracic legs, consists of two tympana within a midline chamber. Physiological and behavioral tests have demonstrated that the auditory system is nondirectional. Mantids respond to low-level threats by gradually turning and high-level threats by entering a power dive (Yager et al., 1990). These maneuvers are highly effective in preventing capture (Yager et al., 1990; Triplehorn, 2003).

Mantis auditory interneuron 501-T3 is a likely interneuron for triggering these responses since it is ultrasound-sensitive (best sensitivity between 25–45 kHz), responds with a short latency (8–12 ms minimum), and has a fast conduction velocity of 4 m s^{-1} (Yager and Hoy, 1989). Implanted electrodes recording 501-T3 activity during flying bat attacks (Triplehorn and Yager, 2002) demonstrated that this interneuron tracks bat vocalizations very well as the bat approaches the mantis and begins to increase its PRR during its attack (following PRRs up to 55 pps on average). However, 501-T3 ceased producing multi-spike bursts during the attack as the bat continued to increase its PRR before completely shutting down for the remainder of the attack. Although mantids have other auditory interneurons, 501-T3 responses were the only auditory interneuron activity observed during the bat attack. If 501-T3 is the trigger interneuron, the results from that study suggest that evasive responses must be triggered prior to 501-T3 shutdown.

This study uses a tethered flight paradigm to answer two pertinent questions: (1) when does the mantis initiate its power dive during simulated bat attack sequences; and (2) what parameters in the bat attack vocalization sequence are important for triggering the power dive response? The results demonstrate that bat emission patterns influence the triggering of the mantis evasive response in a way that potentially affects the mantis’ chance of escape.

Materials and methods

Animals

Subjects were 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 to adult.

Simulated bat attack sequences

Experimental stimuli consisted of pulse trains simulating the PRRs and PDs of echolocation vocalizations emitted during the last 1200 ms of a successful bat attack sequence (i.e. capture). All pulses had a 0.1 ms rise/fall time. Each sequence (5 in total) was based on the temporal pattern (i.e. PRRs and PDs) of actual bat (*Eptesicus fuscus*) attack sequences recorded during a previous experiment conducted in a flight room designed for behavioral studies of bat echolocation behavior (Triplehorn and Yager, 2002). The temporal patterns of the attack sequences were typical of flight room echolocation behavior, including the absence of search phase vocalizations (Surlykke and Moss, 2000; Triplehorn and Yager, 2002). The temporal patterns of these sequences are similar to the most common type of echolocation pattern, those emitted by bats using frequency-modulated vocalizations (e.g. FM bats) as opposed to other echolocation strategies (e.g. constant frequency or gleaning bats; for a review, see Schnitzler and Kalko, 2001). During free flight encounters, mantids perform effective power dives in response to attacking, echolocating *Eptesicus fuscus* (Triplehorn, 2003).

Each sequence (Fig. 1) began with low, stable PRRs (around 12 pps, PDs=3–4 ms; traditionally called the ‘approach phase’) followed by a period of continuously increasing PRRs before reaching a consistent high PRR (130–150 pps, PDs <1 ms; called the ‘terminal buzz’) just before ‘contact.’ In this study, the period of continuously increasing PRRs (shaded regions in Fig. 1) is referred to as the transition period. The slope of the transition period (pps 10 ms^{-1}) provided a measure of how quickly the bat switched from low to high PRRs during an attack. Three of the sequences, termed rapid transition (RT) sequences, contained fast increases in PRR over a short transition period (slopes of 5.37, 8.25 and $14.01 \text{ pps ms}^{-1}$). The other two stimuli, termed gradual transition (GT) sequences, contained slower PRR increases over a longer transition period, indicated by lower slopes (1.78 and $3.45 \text{ pps } 10 \text{ ms}^{-1}$) in Fig. 1.

Sequences were presented using 35 kHz tones, the best frequency for 501-T3 in *P. agrionina*, at 85 dB sound pressure level (SPL) except where noted. At 35 kHz, *P. agrionina*’s behavioral threshold is about 70 dB SPL (Yager and May, 1990) and 501-T3’s threshold is 10 dB lower (Yager and Hoy, 1989). Stimuli 25 dB over threshold elicits maximum or near-maximum responses in 501-T3, (including spikes/stimulus and instantaneous firing frequency) at minimum firing latencies (Yager and Hoy, 1989) and evokes the full escape response (see below). Thus, bat attack sequences presented at this intensity should elicit the earliest mantis responses for each attack sequence and thus provide the ‘best case’ scenario for the mantis. Louder stimuli produce similar responses in 501-T3 (i.e. spikes/stimulus and instantaneous firing frequency do not decrease at higher intensities, up to 50 dB over threshold; Yager and Hoy, 1989), but higher intensities could habituate the behavioral response with repeated exposures.

Unlike the simulated trains in this study, bats vary the intensity of their emissions during attack sequences (Boonman

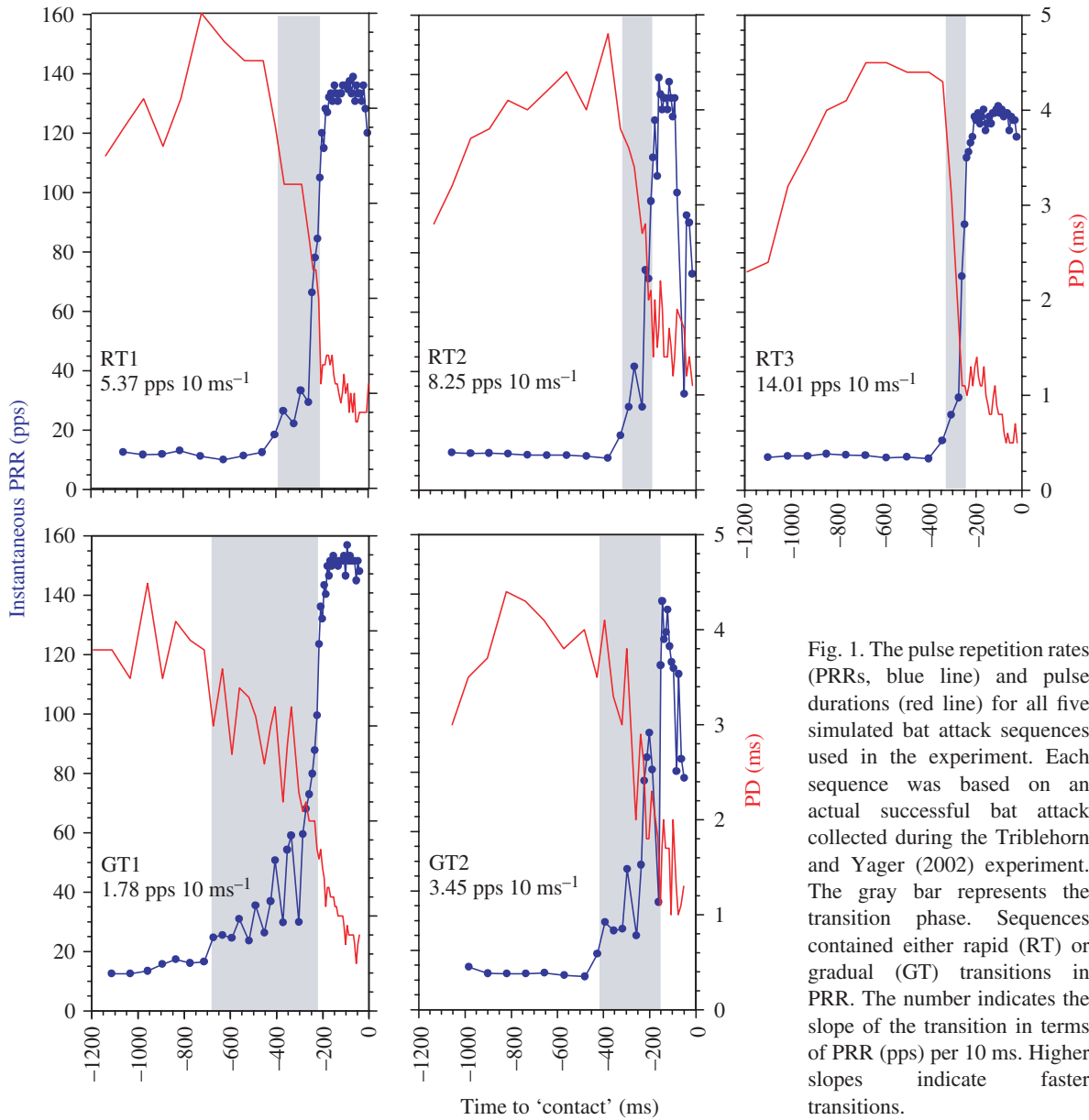


Fig. 1. The pulse repetition rates (PRRs, blue line) and pulse durations (red line) for all five simulated bat attack sequences used in the experiment. Each sequence was based on an actual successful bat attack collected during the Triplehorn and Yager (2002) experiment. The gray bar represents the transition phase. Sequences contained either rapid (RT) or gradual (GT) transitions in PRR. The number indicates the slope of the transition in terms of PRR (pps) per 10 ms. Higher slopes indicate faster transitions.

and Jones, 2002). To ensure the simulated sequences produced similar 501-T3 activity as flying bat attack sequences, 501-T3 responses to the simulated trains (fixed intensity) were compared to the responses recorded during the corresponding flying bat attacks (changing intensity; Triplehorn and Yager, 2002). For all five sequences, 501-T3 responses during the first 400 ms of the sequence were greater (i.e. more spikes/stimulus) for the simulated sequences. In four of the five sequences, 501-T3 activity was similar (i.e. similar spikes/stimulus) during the last 700–800 ms of the attack sequence. The fifth simulated sequence (RT1) elicited more 501-T3 spikes/stimulus than during the corresponding bat attack sequence until the last 200 ms, when 501-T3 responses shut down in response to both simulated and flying bat sequences. Since 501-T3 responses were similar during the flying bat (changing intensity) and simulated sequences (fixed

intensity) during the last 700–800 ms (containing the transition period and post-transition period leading up to capture), the simulated sequences should elicit behavioral responses similar to those that the flying attacks would have elicited.

Experimental setup

All experiments were conducted in a chamber (2.0 m×0.8 m×0.8 m) lined with acoustic foam and a speaker (EAS10TH400B leaf tweeter, Technics, Secaucus, NJ, USA) at one end. SuperScope I with a MACADIOS board generated the train stimuli by gating a sine wave generator (Model DS345, Stanford Research Systems, Sunnyvale, CA, USA). Custom-built electronics shaped the sine wave pulses. A Hewlett-Packard 350D attenuator (Palo Alto, CA, USA) controlled intensity and a Harmon Kardon HK-655 power

amplifier amplified the signal to the speakers. Stimulus calibration followed the procedure described in Triplehorn and Yager (2001), in which the stimuli were 300 ms pulses (0.1 ms rise/fall). The calibration was referenced to a Brüel & Kjaer 4135 6.36 mm microphone (Nærum, Denmark).

Mantids were first pre-tethered with wax (Kerr brand sticky wax, Emeryville, CA, USA) on the dorsal surface of the pronotum as far caudal as possible (toward the mantid's center of mass). Thread melted in the wax and a touch of Superglue held the pre-tether in place. The tether (a thin wire tipped with sticky wax) attached to the pre-tether by melting the wax together. The mantis was positioned 0.7 m from the speaker, 11 cm from the floor of the chamber. A fan provided a gentle headwind that induced flight initiation as well as promoted longer bouts of stable flight. Stable flight was defined as the mantis assuming standard flight posture (with all three pairs of legs tucked in and still; Yager and May, 1990) and both pairs of wings beating continuously (i.e. without brief or long pauses). Both conditions had to be present for several seconds before stimulus presentation.

Sequences were presented in blocks of five trials, with each sequence presented once per block. Interstimulus intervals of 1 min separated each sequence presentation. Each mantis was

exposed to 3–5 trial blocks, with 5 min separating each block. Thus, each mantis was exposed to each attack sequence 3–5 times. Attack sequences were presented in a different order in each block, but the order was consistent across mantids. In between trials, the mantis rested on a platform without headwind stimulation. During trials, stimulus presentation occurred only after the mantis achieved a stable flight pattern. For an individual mantis in a single day, testing ended if the mantis appeared fatigued (demonstrated by short flight bouts and/or unstable flight patterns). If this occurred in the middle of a block, the data from that entire block was discarded. In many cases, testing resumed on another day. For each mantis, a control sequence representing the vocalization pattern preceding the transition phase (PRR=12 pps, PD 4 ms for 1200–2000 ms, 85, 90, and 95 dB SPL) was presented.

Behavioral responses and predicted trigger points

Pulse trains of ultrasound elicit the mantis evasive response while in tethered flight. The response is a multi-component behavior consisting of: (1) foreleg extension, (2) abdominal dorsiflexion, (3) wing-beat phase and frequency changes and (4) a head roll (Yager and May, 1990). To determine when the behavioral response occurred during a simulated bat attack sequence, a laser directed at a

photocell was positioned in front of the mantis so the forelegs would break the beam when extended during evasive response production (Fig. 2A). Although the beginning of foreleg extension is the fastest component of the evasive response (66 ms; Yager and May, 1990), the positioning of the laser measured nearly full foreleg extension that provided latencies (typically between 130–210 ms) that were closer to the latencies for changes in flight paths during free flight (125–230 ms; Yager et al., 1990).

Determining the trigger point is more difficult since there is only one known variable (the behavioral response) and two unknowns (when the attack sequence triggers the response and the latency between the trigger point and the behavioral response). To provide an estimate of the latency between the trigger point and the behavior, the following procedure preceded simulated bat attack sequence presentations for each mantis (illustrated in Fig. 2B,C). After tethering the mantis and positioning the laser (Fig. 2B), a standard 300 ms train (PRR=50 pps, PD=10 ms, 35 kHz,

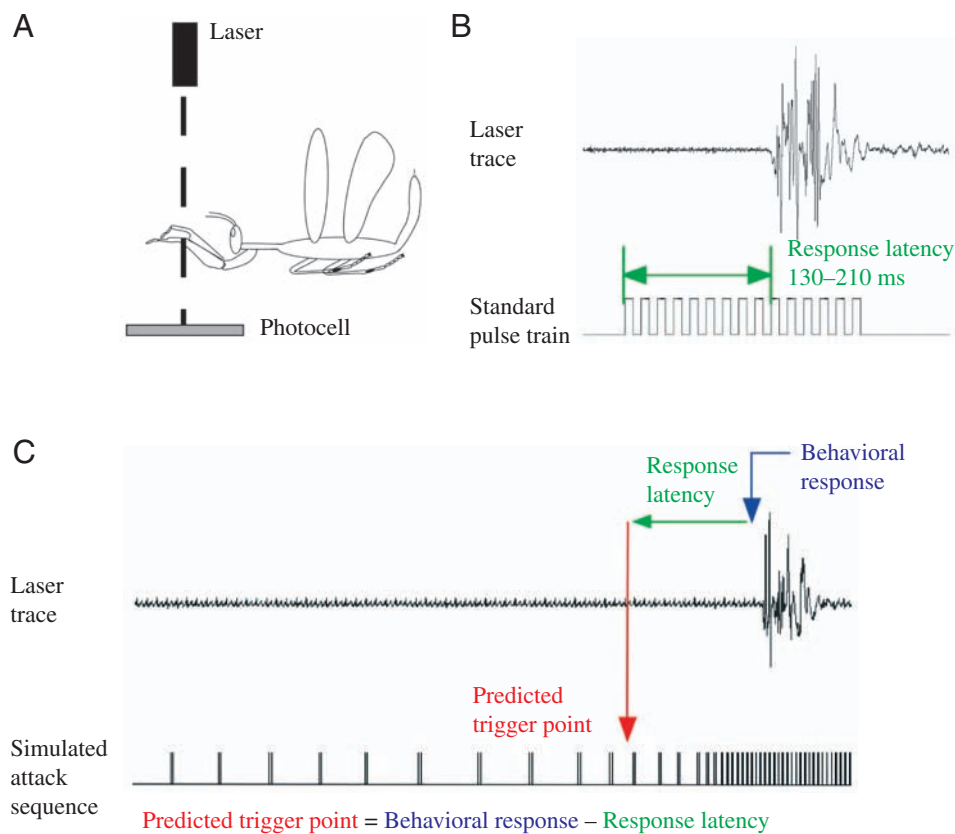


Fig. 2. (A) Schematic of tethered flight behavior paradigm and the method for marking when the behavioral response occurs during the simulated attack sequences using a laser and photocell. (B) Example of laser trace as mantis forelegs break the laser during a standard pulse trains to obtain an estimate of the response latency (see text for details). (C) Example of laser trace as mantis forelegs break the laser during a simulated attack sequence and the calculation of the predicted trigger point (see text for details).

85 dB SPL) presentation evoked an evasive response in the flying mantis. This is the typical pulse train stimulus used by our laboratory for eliciting consistent, strong evasive responses during tethered flight across mantis species (see Triplehorn and Yager, 2001). Since the standard train had consistent parameters with a known onset, the time between the beginning of standard train and the behavioral response provided the response latency (Fig. 2B). The average results from five standard stimulus presentations provided an average response latency. Trigger point predictions were calculated by subtracting the average response latency from the time of the behavioral response to obtain the predicted trigger point (Fig. 2C). These individually calculated predicted trigger points were averaged to obtain the average predicted trigger point for each individual mantis. If testing occurred over different days, a new response latency estimate was determined for that day's data.

The effects of stimulus intensity on the timing of behavioral responses were determined by testing two sequences (RT1 and GT1) presented at 75–95 dB SPL in 5 dB intervals in increasing order. A 1 min interstimulus interval separated each trial within a block and a 5 min period separated each block. Six mantids were tested with each sequence. Three mantids were tested using both sequences while six other mantids were tested with only one of the sequences (data from nine mantids total). For mantids exposed to both sequences, responses were collected on separate days. For both sequences, some of the mantids (one for GT1 and two for RT1) were tested only between 75–90 dB SPL.

Results

Behavioral responses to simulated bat attack sequences

The behavioral experiments addressed two questions regarding the mantis evasive response during bat attack sequences. (1) When does the mantis evasive response occur during a bat attack sequence (behavioral response)? (2) When does the bat attack sequence trigger the mantis evasive response (i.e. when does the mantis nervous system recognize that the bat represents a large enough threat to initiate an evasive response – trigger point)? Although the two questions are related to one another, there is also an important distinction between them. There is some delay between when the response triggers and when the overt behavior occurs due to multiple factors, primarily biomechanical, but also neural conduction time, synaptic delays and neural processing. It is important to consider both events, for two reasons. First, there must be enough time between the mantis detecting the bat as a threat and performing the evasive response before being captured to survive a bat attack. Second, the trigger point, not the behavioral response, reveals the salient parameters of the bat attack sequence for recognizing the bat as a high-level threat requiring a power dive response.

Fig. 3 illustrates the results for the behavioral responses to the three RT attack sequences. Each symbol (diamonds for behavioral responses, triangles for predicted trigger points)

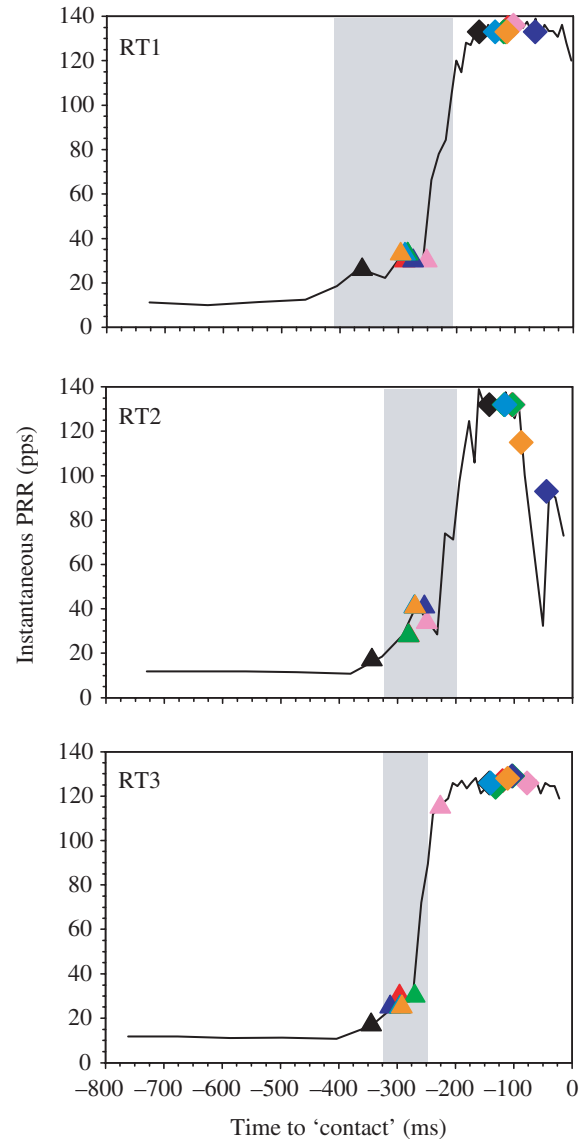


Fig. 3. The average behavioral responses (diamonds) and predicted trigger times (triangles) relative to time to 'contact' for eight mantids (represented by color) to the three rapid transition simulated attack sequences. The black line represents the PRR. The gray bar is the same as in Fig. 1. Placement of each symbol along the y-axis is based on the PRR of the sequence at that time relative to 'contact.' The spread of the trigger point and behavior data differ due to individual differences in the response latency measured for predicting the trigger points (see Materials and methods). Responses occur after the transition period, while the predicted trigger times mostly occur during the transition phase.

represents the results from a single mantis ($N=8$, each individual represented by a color) and is the average from 3–5 trials. The deviation between the behavioral response and trigger point data differ because the 'standard' latency used in predicting the trigger points differed across individuals. For all mantids, behavioral responses occurred very close to the 'contact' time, i.e. the time the bat reached and captured the target during the actual attack sequence. Behavioral responses

occurred between 50–150 ms (mean=114.0 ms, s.d.=42.4 ms) before ‘contact,’ when the ‘bat’ reached its maximum PRR after the transition period (PRR >120 pps in the three sequences). Behavioral responses did not systematically decline during the course of the experiment, indicating that the presentation paradigm effectively prevented habituation.

Trigger point predictions fell between 250–370 ms (mean=287.4 ms, s.d.=47.2 ms) before ‘contact.’ In the majority of the cases, this corresponded to the transition period of the bat attack, shortly after PRR began to increase. For another individual (purple triangle, RT3), the prediction fell just after the transition period, when the PRR reached 120 pps. In the majority of individuals, however, the trigger point predictions indicated that only a moderate increase in PRR early in the transition phase was necessary for triggering the mantis power dive. From a stable PRR of 12 pps prior to the transition phase, 73.92% of the trials predicted that PRRs between 15–40 pps triggered the responses.

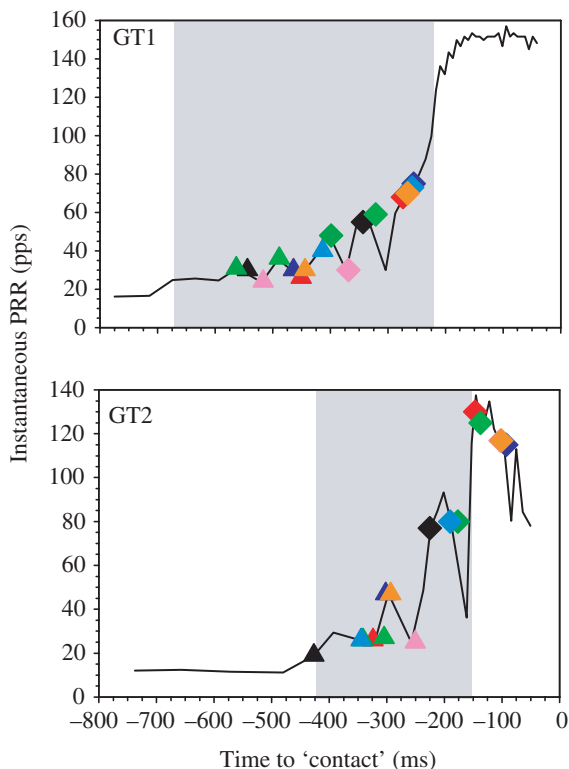


Fig. 4. The average behavioral responses (diamonds) and predicted trigger times (triangles) relative to time to ‘contact’ for eight mantids (represented by color) to the two gradual transition simulated attack sequences. Colors represent the same individuals as in Fig. 3. The black line represents the PRR. The gray bar is the same as in Fig. 1. Placement of each symbol along the y-axis is based on the PRR of the sequence at that time relative to ‘contact.’ The spread of the trigger point and behavior data differ due to individual differences in the response latency measured for predicting the trigger points (see Materials and methods). Both predicted trigger times and behavioral responses occur during the transition period for GT1. The same is true for half of the individuals for GT2, although half of the behavioral response occur just after the transition period.

Bat attack sequences containing gradual transitions (GT bat attack sequences) elicited a very different pattern in the timing of the behavioral response (shown in Fig. 4). GT1 elicited behavioral responses 260–400 ms (mean=308.2 ms, s.d.=99.3 ms) before ‘contact,’ earlier than responses elicited by the RT sequences. This period corresponded to the transition phase of the attack sequence. GT2 contained a shallower overall transition than any of the RT sequences, but was steeper than GT1. All mantids responded to the GT2 sequence 100–225 ms (mean=154 ms, s.d.=60 ms) before ‘contact.’ Half of the mantids responded after the transition period (as seen in the RT sequences) while the rest responded during the transition period (similar to the responses to the GT1 sequence, though not as early). Mantis behavioral responses also exhibited greater individual variation to GT sequences than to RT sequences (standard deviations for behavioral responses were 44.8, 45.3 and 36.2 for RT1–3 and 99.3 and 60 for GT1–2).

Since GT sequences generally elicited behavioral responses earlier than RT sequences, the predicted trigger times naturally occurred earlier relative to ‘contact’ time. Despite these earlier predictions, GT attack sequences triggered mantis responses at similar PRRs to the RT attack sequences (77.02% of trials predicted PRRs between 15–40 pps triggered the behavioral response). The trigger point predictions exhibited less individual variation than the behavioral response times.

It is important to note that mantids never responded prior to the transition period (i.e. the ‘approach phase’) in any of the five simulated bat attack sequences, when the PRR was low and stable (PRR=12 pps). To ensure that such vocalization sequences fail to elicit the mantis power dive, a static pulse sequence (PRR=12 pps, PD=4 ms) was presented for 2000 ms at 90 or 95 dB SPL. These presentations never elicited a power dive response in tethered flying mantids.

Effects of attack sequence intensity on mantis behavioral responses

Previous studies using static pulse trains demonstrated that louder stimuli elicited a stronger behavioral response (greater yaw: Yager and May, 1990; higher percentage of power dives: Yager et al., 1990) and decreased the latency of the response (Yager and May, 1990). To determine whether intensity affects when simulated bat attack sequences elicit responses, two different sequences (GT1 and RT1) were presented at five different intensities (75, 80, 85, 90 and 95 dB SPL) to tethered flying mantids.

Fig. 5 shows the average behavioral response times (relative to the response time elicited at 85 dB SPL) for all six mantids for both sequences. Comparing the average responses elicited by 75 and 95 dB SPL (75 and 90 dB SPL for gray and black data for RT1 and blue data for GT1), increasing intensity had a tendency to evoke behavioral responses earlier in the sequence for most of the mantids (the exception is green data for RT1). Across individuals, the difference in the average response times at 75 vs 95 dB SPL (or 75 vs 90 dB SPL) ranged from 29–218 ms (median=129.5 ms, omitting the green data) for

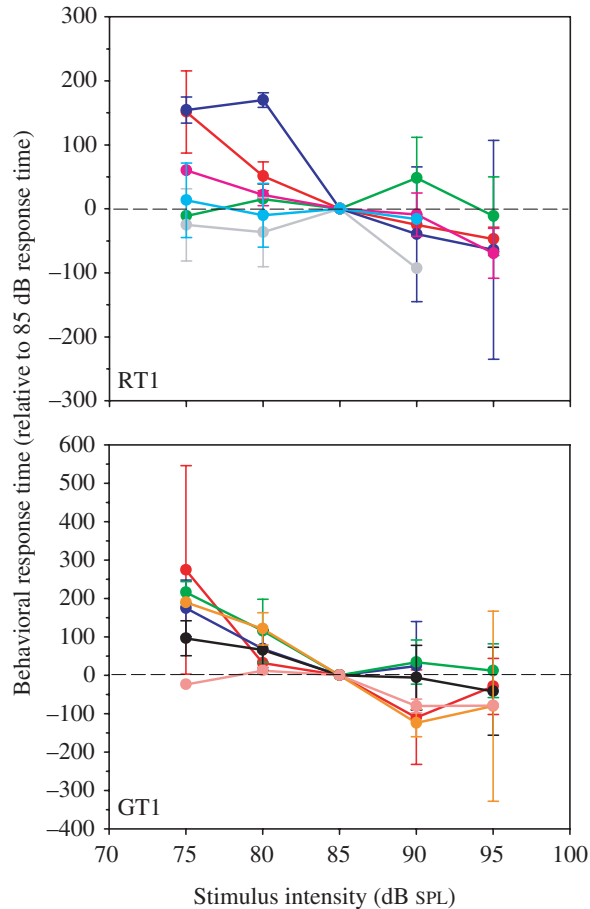


Fig. 5. Effects of intensity on behavioral response times during two simulated attack sequences. Behavioral response times are relative to response times elicited by 85 dB SPL. Positive values indicate longer latencies while negative values indicate shorter latencies. Each color represents the means \pm s.d. for individual mantids (1–3 observations per intensity per mantis). The same color in both graphs represents the same mantis. Since all data is relative to 85 dB SPL, data at this intensity lacks error bars.

RT1. For GT1, this difference ranged from 55–303 ms (median=188 ms).

The effect of intensity on behavioral response times was not equivalent across the range of intensities tested. For mantids tested on the full range of intensities (75–95 dB SPL) during GT1 presentations, the difference in the response times elicited by 75 vs 85 dB SPL (100–300 ms differences) were greater than the differences in the response times elicited by 85 vs 95 dB (11–81 ms) for four of the five mantids (80%; pink data the exception). For RT1, this was true in two of the four mantids (50%, red and blue data, 150 ms vs 48 and 64 ms, respectively) and a third mantis showed no difference (green data).

Discussion

While searching, tracking, and eventually capturing their insect prey, bats increase the rate of vocalizations from a consistently low PRR to a stable high PRR in a non-linear

fashion. The results of the present study suggest that this transition period in the bat attack sequence is important for triggering the mantis power dive. Trigger point predictions indicated that PRRs between 20–40 pps initiated the power dive in tethered flying mantids. This range of PRRs is below the average maximum PRR rate of 55 pps that 501-T3 could follow during flying bat attacks (Triblehorn and Yager, 2002).

The results of the present study further suggest that the characteristics of this transition period could be an important factor influencing the outcome of a bat–mantis interaction. Longer transition periods (such as in GT1 and GT2) can ‘tip off’ the mantis, evoking a power dive before the bat is very close, allowing the mantis to escape. On the other hand, very short transition periods could circumvent the mantis auditory defense, allowing the bat to capture the mantis. Although not specifically analyzed in previous studies, examples of echolocation sequences show that transition periods do vary (e.g. Kalko and Schnitzler, 1989; Kalko, 1995). The transition period may also affect other insect ultrasound-mediated evasive responses as well. If so, not only mantids, but also other insects, could benefit from having a back-up system for situations when the auditory defense fails.

It may be the case that rate of PRR change (the ‘slope’ of the transition period) and not absolute PRR (or both together) may be an important parameter for triggering evasive responses in mantids and other insects. Most studies only consider physiological and behavioral responses to static PRR trains (Fullard, 1984; Moiseff and Hoy, 1983; Faure and Hoy, 2000a,b) or to a single attack sequence containing changes in PRR (Fullard et al., 1994, 2003). The results of the current study identify: (1) the importance of the transition phase and pulse train stimuli that include this phase, which more closely resemble natural bat echolocation sequences, and (2) the importance of testing multiple attack sequences. Both should be considered in future studies of insect physiological and behavioral auditory responses.

Risk assessment and the triggering of the power dive

The nature of echolocation requires bats to perceive the world in a strobe-like manner, as bats must produce a vocalization and wait for the returning echo to obtain information about the environment. When tracking and hunting an insect, a bat must continuously update its information concerning the insect’s location and these updates need to occur more often as the bat comes closer to capturing the insect. Thus, PRRs increase closer to capture and PDs decrease to prevent overlap between the next vocalization and the returning echo from the previous emission (Schnitzler et al., 1980; Simmons and Grinnell, 1980; Kalko, 1995).

Assuming 501-T3 is the interneuron primarily responsible for triggering the mantis evasive response, the decision to dive will be based on two factors: how often 501-T3 fires and how many spikes comprise each response. 501-T3 is not spontaneously active and only produces spikes in response to ultrasound (Yager and Hoy, 1989). Triblehorn and Yager (2002) confirmed this was true during flying bat attacks and

demonstrated that 501-T3 burst rates followed the bat's PRR reliably into the transition phase. Therefore, how often 501-T3 fires (501-T3 burst rate) determines the rate of information provided to the mantis, dictated by the bat's PRR. The number of spikes comprising each 501-T3 response is a function of the 'effective strength' of each pulse in eliciting behavioral responses. A variety of parameters, including vocalization duration and intensity, influences 501-T3's response. Two examples illustrate that both factors of 501-T3's response are important for triggering the mantis evasive response.

First, pulse trains simulating bat vocalization patterns prior to the transition phase (low PRR around 12 pps with PDs around 4 ms presented at 85 dB SPL or higher) failed to elicit evasive responses. However, physiological recordings in non-flying mantids reveal that 501-T3 responses to such pulse trains typically contain 5–6 spikes (J. D. Triplehorn, unpublished data), but this activity fails to trigger an evasive response. The low 501-T3 burst rate likely causes this failure. However, this is beneficial as it limits false alarms and prevents the evasive response from occurring too early, possibly allowing the bat to adjust to the mantid's maneuver. Second, pulse trains simulating bat vocalization patterns after the transition period (very high PRRs >100 pps with PDs <1 ms) also fail to elicit power dives (J. D. Triplehorn, personal observation). In this case, the high PRR potentially provides a large amount of input, but 501-T3 responds either weakly (single spikes) or not at all to each vocalization (Yager and Hoy, 1989; Yager et al., 2001). As the average mantis power dive latency is 242 ms, responses initiated after the transition period would generally be ineffective anyway as the bat would capture the mantis before the power dive is fully developed.

The transition period of the bat attack sequence provides the best indication to the mantis that it is in danger. Although a bat can break off an attack during this period, it does represent a level of commitment by the bat that is absent earlier in the attack sequence (when the bat vocalizes using low PRRs). Although it may lead to some false alarm responses in the mantis, waiting for an indication of the late stage of the bat attack (i.e. PRRs >100 pps) would not leave the mantis enough time to escape. Fullard (1984) used similar reasoning to explain why PRRs between 30–50 pps optimally elicit clicking behavior in the arctiid moth *Cygnia tenera*. Low pulse rates indicate a distant bat that may trigger early defenses, but not close range defenses while very high pulse rates are similar to non-predator sounds such as singing insects.

The transition period and 501-T3's responses may not only affect the initiation of the power dive response, but also the magnitude of the response. This would not be unusual. In crickets, the magnitude of Int-1's response directly correlates with the strength of the response in the dorsal longitudinal muscles, and thus the magnitude of the evasive turn (Nolen and Hoy, 1984). In mantids, the magnitude of the power dive may, at least in part, be attributed to how long 501-T3 continues responding to bat vocalizations after initiating a response. If so, then attack sequences with rapid transitions would not only

evoke later power dives, but also weaker ones, while gradual transitions would evoke strong power dive responses.

The implications of transition rates on the outcomes of mantis-bat encounters

The proposed design for initiating the mantis power dive based on the current results suggests how the bat vocalization pattern (and, more specifically, the transition period) can significantly influence the chance of a mantis successfully escaping an attacking bat. Attack sequences containing longer transition periods (such as GT1 and GT2) trigger the response before the bat gets too close, thus preventing any chance of the bat catching the mantis.

As bats increase their PRR, they may exhibit a phenomenon known as strobing, where the bat emits several (2–4) vocalizations with short (15–20 ms) interpulse intervals separated by a single pulse with a longer interpulse interval (30–40 ms; Moss and Surlykke, 2001). These vocalization clusters, known as strobe groups, may facilitate the bat's perception of the auditory scene through perceptual grouping of auditory events, especially when tracking and capturing insects (Moss and Surlykke, 2001). Such grouping of perceptual stimuli is common to both visual and auditory systems in many animals, including humans (Bregman, 1990). Moss and Surlykke (2001) demonstrated that *Eptesicus fuscus* exhibit strobing behavior, typically during the transition phase of the attack sequence, when the bat begins pursuing an insect but before fully committing to the attack (represented by PRRs over 100 pps). Strobing bat attack sequences have transition slopes between 0.94 and 1.45 pps 10 ms⁻¹ (based on examples in Moss and Surlykke, 2001). Attack sequences containing strobe groups provide the best examples of gradual transitions in natural attack sequences. Although our study did not include attack sequences containing strobing behavior, GT1, with its long transition phase (slope=1.78 pps 10 ms⁻¹), best represents such a sequence and GT1 clearly triggered the mantis evasive response the earliest. Though strobing behavior could potentially reduce a bat's hunting efficiency, this would only be true against insects that have the ability to detect the ultrasonic emissions. Since a large number of night-flying insects lack ultrasound-sensitivity, the potential benefits of strobing may, in the long run, be outweighed by the drawbacks of early warning to the ultrasound-sensitive insects.

The proposed design of the mantis evasive response trigger circuit also means that very short transition periods (as in RT1, RT2 and RT3) may circumvent the mantis auditory defense, allowing the bat to capture a mantis even though the mantis can hear the bat. This circumvention could occur in several ways. A short transition period occurring close to the point of capture is one way a bat attack sequence could thwart the mantis auditory defense. A late transition would allow the bat to close with the mantis without triggering the power dive since vocalization patterns preceding the transition do not trigger mantis power dives. A rapid, late transition to high PRRs when the bat is close to the mantis would allow the bat to capture the mantis even after triggering a power dive during the

transition period due to the long latency of the mantis power dive response. A rapid transition occurring farther away could also render the mantis auditory defense ineffective. Vocalizations emitted when the bat is farther away would reduce the sound intensity reaching the mantis and, consequently, 501-T3's response. Even though the transition period normally provides a favorable rate of 501-T3 bursts, the weaker 501-T3 responses might fail to initiate a response or only elicit a weak behavioral response. A rapid transition occurring far away would require the bat to continue pursuing the mantis using very high PRRs. If the bat is capable of echolocating at very high PRRs for long durations, these vocalizations will fail to elicit a mantis response and negate the mantid's auditory defense.

Unlike gradual transitions, it is not clear under what circumstances bats would use rapid transitions. A bat that makes a late decision to attack an insect could potentially employ a rapid transition as it tries to capture the insect. It may also be possible that bats continuously encountering evading insects with auditory capabilities could potentially learn that attack sequences with rapid transitions increase their capture success. This would be another strategy bats could employ to circumvent insect auditory responses (reviewed in Schnitzler and Kalko, 2001). Field research of bat–insect encounters would provide the best method for identifying the type of transitions bats use while capturing insects and under what conditions bats employ both gradual and rapid transitions.

The effects of intensity on power dive behavioral responses

The results in the present study are based on simulated sequences presented at a constant intensity of 85 dB SPL, for reasons previously stated. However, during actual attack sequences, bats systematically change the intensity of their vocalizations during attacks (Griffin, 1958; Simmons et al., 1979; Kick, 1982; Surlykke and Moss, 2000; Boonman and Jones, 2002) and the intensity of the vocalizations may exceed 85 dB SPL even during the intensity changes (Jensen and Miller, 1999; Boonman and Jones, 2002; Holderied and von Helversen, 2003). 501-T3 does produce more spikes/stimulus in response to louder single bat vocalizations (Triblehorn and Yager, 2002) and to louder, short, pure tone pulses (Yager et al., 2001), especially when the sounds are <3 ms in duration, which occurs during the transition phase (see Fig. 1). However, our simulated sequences (fixed intensity) did produce similar 501-T3 responses to those of actual bat attack sequences (changing intensity) and the mantis behaviors elicited by the simulated sequences should reflect the behavior that would be evoked by the actual sequences. Still, it is important to consider how bat vocalization intensity might influence mantis evasive responses.

The production of mantis power dives involves both triggering and executing the response. Triggering the power dive will be tightly linked to 501-T3 activity (and potentially other auditory interneurons), while execution involves the activity of descending neurons in the cephalic ganglia and motor neurons (as well as any interposed interneurons), and muscle activation. Our data indicate that triggering is linked to PRR and intensities

over 85 dB SPL have little effect on triggering the response. This is evident from the findings that the trigger point already appears to be set to a low PRR (15–40 pps) and that control sequences with a fixed PRR and pulse duration (12 pps, 4 ms) do not trigger the power dive response, even when presented at 95 dB SPL. Since the trigger point is already set to a low PRR, any further reduction could increase the number of false alarms, causing the mantis to perform a power dive before the bat truly poses a threat. The slope of the transition phase also does not alter the triggering point.

Intensity appears to have greater influence in the execution of the response, affecting both the latency and magnitude of the response. Yager and May (1990) demonstrated that higher intensity pulse trains reduce the latency for yaw production in the mantis evasive response and increase yaw magnitude. Yager et al. (1990) also demonstrated that evasive responses in free-flying mantids become more dramatic as pulse train intensity increases (from gradual turns to shallow dives to steep power dives). The results from our intensity experiment indicate that louder sequences can emit earlier responses and give the mantids more time to escape. However, this effect varied across mantids (average behavioral responses in some mantids were unaffected by stimulus intensity) and sequences (increasing intensity seemed to evoke earlier responses in mantids when exposed to GT1 compared to RT1). Intensities below 85 dB SPL also appear to affect when behavioral responses occurred during simulated attack sequences more than intensities over 85 dB SPL. As with the Yager and May (1990) study, our data showed a high degree of variation, which may add an element of unpredictability in the response that could increase its effectiveness.

Our study did not examine how the transition phase affects response magnitude. However, to address this issue, an ideal experimental paradigm would to record 501-T3 activity in tethered flying mantids while quantifying the magnitude of elicited evasive responses. It is important to know the 501-T3 response pattern that elicits evasive responses, in order to account for the variability that exists in behavioral data alone. These recordings could detect the descending motor command, which would provide more accurate information regarding the trigger point since the biomechanical factors and some of the neural delays that affect the behavioral latency would be eliminated.

Back-up systems and 'last-ditch' evasive responses

The results of this study reveal the effectiveness of the mantis evasive response, but also reveal some vulnerabilities that bats could exploit (either as a strategy or serendipitously). Such vulnerability could explain, at least to some extent, why insect auditory defenses are not 100% effective. It also indicates that mantids could benefit from possessing back-up systems for initiating 'last-ditch' responses. Insects such as arctiid moths and lacewings, have auditory systems that do mediate 'last-ditch' responses, triggered by the high PRRs emitted by bats late in the attack. However, *P. agrionina* does not have an obvious sound-mediated last-chance behavior like

these. Such ‘last-ditch’ responses could be mediated by other sensory systems such as the mantid’s wind-sensitive cercal system. Bats do produce a substantial amount of air currents before capturing their target that is detectable by the mantis, and cercal-directed wind puffs elicit brief wing-beat changes in tethered flying mantids that could alter the flight path sufficiently in free flight to make it more difficult for the bat to capture the mantis (Triplehorn, 2003).

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