Ultrasound Avoidance Behaviors in Two Species of Neoconocephalus (Orthoptera, Tettigoniidae)

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Source: Journal of Orthoptera Research, 19(1) : 81-88

Published By: Orthopterists' Society

URL: https://doi.org/10.1665/034.019.0114

Journal of Orthoptera Research July 28, 2010,19(1): 81-88

Ultrasound avoidance behaviors in two species of Neoconocephalus (Orthoptera, Tettigoniidae)

Submitted April 9, accepted June 7, 2010

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Abstract

The hearing system of the katydid genus *Neoconocephalus* functions in two contexts: intraspecific communication and predator (bat) avoidance. Male calls and bat echolocation signals differ spectrally and temporally. In order to respond appropriately, katydids must recognize and discriminate between signal types. We categorized bat avoidance behaviors and examined temporal recognition of bat cries behaviorally during tethered flight in *Neoconocephalus retusus* and *Neoconocephalus ensiger*.

We used simulated bat approaches to categorize bat avoidance behaviors in *Neoconocephalus*. Four distinct behaviors were observed: steering, wingbeat interruption, dives and leg kicks. The first three behaviors were amplitude dependent and could be elicited by a single pulse of ultrasound. The final behavior only occurred during the terminal phase and was found to be pulse-rate dependent.

Response probabilities of specific behaviors varied between species. A comparison of the two *Neoconocephalus* species showed that steering and leg kicks were performed consistently in both species. However, wingbeat interruptions and dives occurred more often in *N. retusus* than in *N. ensiger*.

We also tested how temporal properties of model bat cries are used to recognize a signal as aversive. Single-pulse experiments showed that katydids respond best to pulses with relatively short rise times and a minimum duration. Very long pulse durations did not negatively affect responsiveness if rise times were short enough. No differences were found in temporal recognition between the two species.

Key words

predator avoidance, auditory, bat/insect interaction, negative phonotaxis, hearing

Introduction

Diverse groups of nocturnally flying insects use their ears to monitor for echolocation cries of foraging bats taking evasive action when they detect such cries (Roeder 1967, Miller & Oleson 1979, Hoy *et al*. 1989, Yager *et al*. 2000, Miller & Surlykke 2001). In several of these groups (*e.g.*, Lepidoptera), hearing probably evolved for the purpose of bat detection (see Miller & Surlykke 2001). However, in Ensifera (Gryllidae, Tettigoniidae and allies) the ear evolved in the context of intraspecific communication, with bat avoidance likely being a secondary function of the hearing system (Stumpner & Helversen 2001).

Concomitantly with their separate evolutionary origins, positive phonotaxis to male calls and bat evasive responses are separate behaviors in ensiferans (Pollack & El-Feghaly 1993), controlled by independent neural circuits (Nolen & Hoy 1986, Schildberger & Hörner 1988). The need to attend to bats and males with different responses requires that the two signal classes are reliably discriminated from each other. In crickets, this discrimination is based on the carrier frequency of the signals with bat evasive responses largely limited to ultrasonic signals (Moiseff *et al*. 1978, Nolen & Hoy 1986). In katydids, however, discrimination based on carrier frequency appears less efficient, as male calls in many groups contain major ultrasonic components or are even limited to ultrasound (Heller 1988, Morris *et al*. 1994). Differences in temporal pattern likely contribute to the detection of bats within the background of male calls (Faure & Hoy 2000a, Schul & Sheridan 2006)

The need to reliably detect and avoid bats may impose constraints on the acoustic communication system of katydids. For example, the need to detect bats may lead to reduced female selectivity during flight, compared to selectivity in walking females (Schul & Schulze 2001). Also, it may limit the potential parameter space for the evolution of communication signals. Therefore, knowledge about bat avoidance behaviors is important to consider when studying the function and evolution of acoustic communication systems.

The katydid genus *Neoconocephalus* is a large group (25+ species) with a wide geographical range and high diversity in male calls (Greenfield 1990). Recently, this group has been extensively studied with regard to the co-evolution of male calls and female preferences (*e.g.*, Greenfield & Roizen 1993; Deily & Schul 2004, 2006; Beckers & Schul 2008; Bush *et al*. 2009) and the neuroethology of female phonotaxis and bat avoidance (Faure & Hoy 2000a, 2000b; Schul & Sheridan 2006; Höbel & Schul 2007; Triblehorn & Schul 2009). A robust reconstruction of the phylogenetic relationship in this genus is available (Snyder *et al*. 2009), allowing powerful comparative studies in this group.

Bat avoidance behaviors in this group have only been cursorily studied (Libersat & Hoy 1991, Faure & Hoy 2000c). Data for bat avoidance during flight is available for only one species (*N. ensiger*, Libersat & Hoy 1991), which differed significantly in its behavioral repertoire and sensitivity from another katydid species (Schulze & Schul 2001) and from crickets (Moiseff *et al*. 1978, Nolen & Hoy 1986). However, the methods used differed considerably among these studies, and thus it is unclear whether the differences among species are real or the result of methodological variation.

Here we study the bat avoidance behaviors of two *Neoconocephalus* species, *N. ensiger* and *N. retusus*, both of which are relatively small in size. Male calls of *N. retusus* have the fast pulse rate (approximately 150 pulses/s at 25°C) typical of this genus, while *N. ensiger* has a much slower pulse rate (12p/s at 25°C) (Greenfield 1990). The ranges of the two species overlap in the northeastern United States, with *N. ensiger* extending into Canada, while the range of *N. retusus* includes subtropical habitats in the southeastern United States (Walker 2008). The two species are at disparate positions in the phylogeny (Snyder *et al*. 2009).

We test the responses during tethered flight to stimulation with models of echolocation cries during the different phases of an attack by an aerially hunting bat. We quantify the temporal parameters of the signals that elicit bat (ultrasound) avoidance behaviors. The results were similar between the two species and largely agree with those described for other katydids.

Materials and Methods

We collected adult males and females of *N. retusus* (Scudder) and *N. ensiger* (Harris) from wild populations near Columbia, Missouri. The insects were kept in the laboratory under a photoperiod of 14h:10h L:D cycle at 20-25°C. Prairie fescue, apples and water were provided *ad libitum*.

Experiments were performed at 25°C in a temperature-controlled chamber (2m × 3m × 5m) lined with 10-cm anechoic foam. Insects were tethered at the pronotum and placed dorsal side up 20 cm in front of a fan producing a wind speed of ~2.5 m/s. Under these conditions, the animal assumed a flight posture (Fig. 1) similar to that described by Libersat and Hoy (1991) and Schulze and Schul (2001). Stimuli were only presented if the insect was in stable flight position with all wings beating for at least 15s before stimulation. We monitored insect activity through an infrared-sensitive video camera placed underneath the insect and a Sony GV-D1000 digital video recorder. The experiments took place in total darkness with the exception of an infrared light source for the video camera.

Acoustic stimulation.—Acoustic stimuli were delivered through one of two Technics EAS 10TH400C speakers placed 50 cm from the animal and perpendicular to its longitudinal axis on either side of the insect. We generated ultrasonic stimuli using a custom developed DA-converter/amplifier system with 16-bit resolution and a 250 kHz sampling rate. The amplitude of the signals was controlled manually using an attenuator with a resolution of 0.375 dB. The amplitude of the signals was calibrated using a ¼" condenser microphone (G.R.A.S. 40BF) and a Bruel and Kjaer sound level meter (B & K 2231), using its "peak amplitude" function. Sound measurements were obtained at the level of the animal without the animal present. Sound levels are given as dB SPL (re 20 µPa).

Simultaneously with the playback of a bat-call model, an infrared LED flashed to mark the stimulus timing on the video recording. This LED was invisible to the insect.

We generated a model of a bat echolocation call of the frequency-modulated (FM) type, resembling search-phase calls of aerially hunting bats occurring in the habitats of *Neoconocephalus* katydids (*e.g., Eptesicus fuscus*). The carrier frequency was linearly modulated from 80 to 45 kHz during the first 50% of the pulse duration, and from 45 kHz down to 35 kHz during the reminder of the pulse. The main energy of these pulses was focused around 40 kHz. Duration of the model bat pulses was 10 ms if not stated otherwise, and had rise and fall times of 0.5 ms. In the experimental series where pulse duration and pulse rise time were manipulated (see below), the pulses had a constant carrier frequency of 40 kHz. The model bat pulses were presented in various combinations during the different experimental series of this study.

We first simulated the approach of an echolocating bat flying at a speed of 6.3 m s^{-1}, assuming a call amplitude of 125 dB SPL at a distance of 10 cm (Jensen & Miller 1999, Holderied & von Helversen 2003), and a spreading loss of 6 dB per doubled distance and atmospheric attenuation of 1 dB m^{-1} (Sivian 1947, Lawrence & Simmons 1982). The search and approach phase of the simulated bat approach had a pulse rate of approximately 7 Hz (period = 140 ms) and 4 s duration. The amplitude of the bat pulses increased from 43 dB SPL (approx. 31-m distance) to 83 dB SPL (6.3-m distance). The final second of the simulated bat approach mimicked the terminal phase (Moss *et al.* 2006). It consisted of three sections with increasing pulse rate (18, 45, 111 Hz) and decreasing pulse durations (4, 3, 2 ms, respectively). Pulse amplitude was held constant at 84 dB SPL during this phase. We presented the approach stimulus four times to each animal, twice from each side. We determined the response probability of the different behaviors from 23 individuals in *N. retusus* and 21 individuals in *N. ensiger*. From a subset (*N. retusus* N=9, *N. ensiger* N=11), we measured the timing of the behaviors (see below).

We measured amplitude thresholds using single-model bat pulses, or series of 7-model bat pulses of constant amplitude, presented with a pulse period of 140 ms. This experiment was conducted only with *N. retusus* (N=8).

To measure the importance of pulse duration and pulse rise time duration, we used sound pulses with a constant carrier frequency of 40 kHz, instead of the frequency modulated carrier described above. This was necessary, as changes in pulse durations would have changed the modulation rate in the FM stimuli and would have introduced an additional independent variable. In the pulse durations series, rise and fall time were kept constant at 0.5 ms. The sample size of this experiment was N=10 in *N. retusus* and N=11 in *N. ensiger*.

The influence of pulse rise time was tested in two series. In the first series, the plateau time of the pulses was kept constant at 9 ms, while rise and fall time were varied between 0.5 to 1000 ms; *i.e.*, total pulse duration varied between 10 and 2009 ms. In the second series, the total pulse duration including rise and fall times was kept constant at 2000 ms and rise and fall time varied between 0.5 and 1000 ms. The amplitude during the rise time experiments was set at 12 dB above the threshold for the stimulus, with 0.5 ms rise time, which was determined for each individual prior to the experiment (see below). We collected data for N=8 individuals in both *N. retusus* and *N. ensiger*.

In the final experiment, we tested the importance of the pulse rate during the terminal phase of the simulated bat approach (see above). We kept the search/approach part of this stimulus as described above (*i.e.*, 28 model bat pulses of increasing amplitude at 7 Hz), and held the pulse rate constant during the terminal phase. We used pulse rates between 7 Hz and 100 Hz. Pulse duration and amplitude of the terminal phase were held constant at 4 ms and 84 dB SPL. The sample size in this experiment was N=11 in *N. retusus* and N=9 in *N. ensiger*.

Experimental protocol.—The sequence of the stimuli presented during each experimental series was pseudorandomly varied among the individuals tested in each series. We kept a silent period of at least 2 min between successive stimulus presentations. We could not detect changes of behavioral sensitivity in the course of our experiments. Individuals were tested as long as they maintained consistent flight, for up to 60 min.

If not stated otherwise, each experimental series was tested in a repeated measures approach, *i.e.*, each individual contributed a data point for all values of the independent variable tested. In the experimental series during which we scored response probability, each stimulus was presented four times, two times from each side. Here, data are given as 'grand-mean', *i.e.*, as the mean of the mean values of all individuals tested.

To determine the amplitude dependence of three of the ultrasound avoidance behaviors, we tested each individual with all

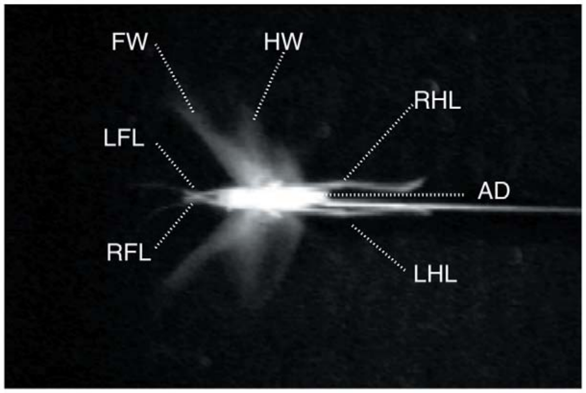

Fig. 1.Ventral view of a male *N. ensiger* during tethered flight. On the right, the tether extends behind the insect to the edge of the frame. The points used in the position analysis (see Fig. 2) are indicated (RFL, LFL right/left forewing; RHL, LHL right/left hindleg; AD abdomen; FW, HW fore/hindwing).

stimulus amplitudes (40 – 70 dB SPL) in 3-dB steps for series of 7 model bat pulses and for single bat pulses. Each amplitude was presented twice. We defined the threshold for each behavior as the lowest stimulus amplitude at which the insect consistently responded to both stimulus presentations.

To determine thresholds as functions of pulse duration, we used a 1/2 up, 0/2 down paradigm (Levitt 1971). In short, if at least one response occurred to two presentations of a given amplitude, the amplitude was lowered. If no response occurred, then the amplitude was increased. At each reversal of direction the step size was halved. We started each experiment at 84 dB SPL, with a step size of 12 dB. Threshold determination was stopped after a final step size of 1.5 dB. The lowest stimulus amplitude that elicited a response was defined as threshold.

Threshold data are presented as medians. Error bars give the range excluding the extreme values (*i.e.*, from second highest to second lowest threshold).

Analysis.—Response probabilities were determined offline from video recordings, using single-frame analysis. We used the criteria described in the results to decide when the behaviors occurred. We confirmed the reliability of these decisions by blind re-scoring a subset of the data through an independent observer.

For a subset of the experiments, the video recordings were digitized and position traces for select body parts generated using motion analysis software (MaxTRAQ Lite v 2.20, Innovision Systems). This analysis was used to determine the nature and timing of behavioral responses.

Results

During tethered flight, both *N. retusus* and *N. ensiger* assumed a flight posture with the antennae and forelegs pointing forward. Mid- and hindlegs were extended straight back, parallel to the abdomen (Fig. 1). The tarsi of the forelegs were often crossed. Both fore and hind wings were fully opened and beating, although the forewings had a much smaller range of movement than the hindwings.

In response to the simulated bat approach, the insects displayed four distinct behaviors. The first behavior, 'steering', consisted of swinging the hind leg contralateral to the sound source, away from the abdomen (Fig. 2). Occasionally, the other legs moved in the same direction. However, we could not detect directional movement of the abdomen during steering. This behavior was clearly observable in the position traces of the hind legs (Fig. 2, label S). The second behavior, 'wingbeat interruption', consisted of closing the hindwings (*i.e.*, aligning with the abdomen) while the legs remained in flight posture (Fig. 2, label W). Forewings either remained open, or were partially or totally closed during this behavior. The third behavior, 'dive', consisted of folding all four wings and rapidly aligning the forelegs with the abdomen (Fig. 2, label D). The position traces of the forelegs show a characteristic movement away from, and back to the body axis, as the forelegs were swung backward. The fourth behavior 'leg kick', was a rapid swinging of all 6 legs laterally away from the insect's midline (Fig. 2, label K). In many cases, a short burst of wingbeats occurred in conjunction with the leg kick. We discuss the function and the reason for the assigned names below.

Considerable behavioral variability existed during stimulation with the simulated bat approaches. Not every individual showed all four behaviors (*e.g.*, Fig. 2A); for example, the 'wingbeat interruption' was often skipped, especially in *N. ensiger*. Also, responses of one individual could vary both in which behaviors occurred (Fig. 2B) and in the timing of the behaviors during the approach. Table 1 gives the response probabilities for each behavior.

The timing during the approaches differed distinctly for the four behaviors (Fig. 3). Steering occurred typically during the first second of the stimulus in both species. Wingbeat interrupts and dives occurred later during the stimuli, typically during the search/approach phase with 7/s pulse rate. We never observed leg kicks during the first 4 s of the stimulus, but only during the terminal phase with pulse rates > = 18/s (Fig. 3).

In the previous experiment the first three behaviors occurred during the part of the approach with increasing amplitude of the bat-cry models. To quantify the importance of the stimulus amplitude for these behaviors, we measured their behavioral thresholds with constant amplitude stimuli. In response to a series of 7 bat pulses (at 7/s), median thresholds for leg steering were 49 dB SPL (n=8 for all measurements); median thresholds for wingbeat interruption were at 53 dB SPL and the diving response occurred at 63 dB SPL (Fig. 4). When we used a single bat pulse as stimulus, median response thresholds were two to five dB higher (54, 55, 66 dB SPL, respectively; Fig. 4). This indicates that single pulses are sufficient to trigger these behaviors and that specific pulse rates were not necessary.

In the next sets of experiments, we quantified the importance of the temporal parameters of a single pulse for the bat avoidance. Because of the response variability of the first three behaviors, we scored both wingbeat interrupt and dive as a single response (see methods). In the first series, we determined relative behavioral thresholds as a function of pulse duration (Fig. 5). In *N. retusus*, thresholds decreased by approximately 6 dB for each doubling of the pulse duration. In *N. ensiger*, the function was steeper for pulses shorter than 10 ms (8 dB/doubling duration), while from 10 to 20 ms, thresholds decreased by only 4.5 dB. Thus, thresholds in both species changed significantly more than the -3 dB/double duration (Fig. 5, dashed line) predicted by an energy integrator (Surlykke 1988, Tougard 1998).

We next tested the importance of the pulse rise time in two experimental series, one keeping plateau duration constant, and the

Table 1. Response probabilities of bat avoidance behaviors during stimulation with simulated bat approach (2-4 trials/insect).

	Steering	Wingbeat interrupt	Dive	Leg Kick
N. retusus (n=23)	51%	44%	80%	75%
N. ensiger (n=21)	64%	28%	43%	73%

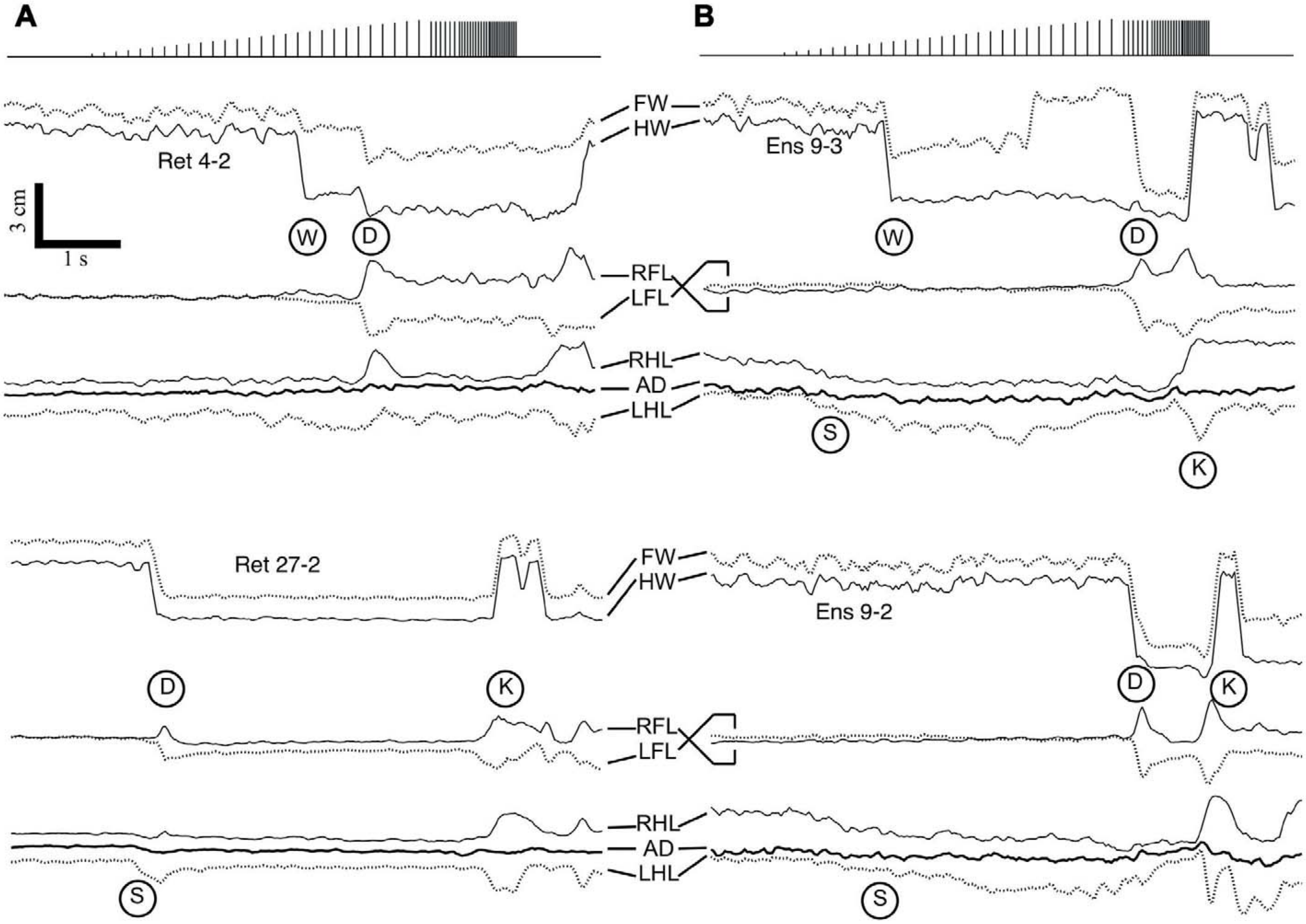

Fig. 2. Relative position traces of various body parts of *N. retusus* (left) and *N. ensiger* (right) in response to the simulated bat approach during tethered flight. Top trace indicates the timing of the stimulus; amplitudes not shown to scale. The stimulus presentation was from the right (from the top in the figure). Two examples are shown for each species. For *N. retusus* (left) the two examples are from two different individuals; for *N. ensiger* (right) the two examples are from the same individual. For clarity, traces of wings, forelegs, and hindlegs have been shifted relative to each other. Positions are shown only for the right fore and hindwing (FW, HW). RFL, LFL right/left foreleg, RHL, LHL right/left hindleg, AD abdomen tip. The measuring points are indicted in Fig. 1. The circled labels indicate the timing of behavioral responses; S steering, W wingbeat interruption, D dive, K leg kick. Note that in the examples of *N. ensiger*, the forelegs were crossed at the beginning of the trial.

other pulse duration constant. Insects responded best to short pulse rise times, with response probabilities dropping sharply as rise time increased beyond 40 ms. Response probabilities were low for rise times of 250 ms and longer (Fig. 6). In both species, response probabilities were somewhat higher for the constant-plateau stimuli.

Leg kicks were never observed in response to single pulses or slow pulse rates (7 pulses/s), even at amplitudes exceeding the maximum amplitude of the simulated approaches. This suggests that a higher pulse repetition rate may be necessary to trigger this behavior. Correspondingly, we observed leg kicks only during the terminal phase of the simulated approaches, *i.e.*, after pulse rates increased above 7 Hz (Fig. 3). To test the threshold pulse rate required to trigger leg-kick behavior, we presented the insects with approach stimuli with the terminal phase held at a constant pulse rate between 7 and 100 Hz.

In *N. retusus*, response probabilities were low for pulse rates up to 25 Hz and increased sharply towards higher pulse rates (Fig. 7). In *N. ensiger*, response probabilities increased sharply between 50 and 75 Hz, while no responses occurred up to 25 Hz.

Discussion

We studied behavioral responses to ultrasonic stimulation during tethered flight. Three behaviors were elicited by single sound pulses and occurred during the model search/approach phase. A fourth behavior was triggered only by stimulation with fast pulse rates and consequently was only seen in response to a terminal phase mimic. We could not detect significant differences between the species for the stimuli tested here.

At low pulse amplitudes the hindleg contralateral to the sound source swung away from the body, where it impaired the hind wingbeat, which should cause turning away from the sound source during free flight (May & Hoy 1990). Accordingly, we refer to this behavior as steering. At higher pulse amplitudes, the insect stopped wingbeat but remained in flight posture, which should result in a sudden drop of altitude. During experiments with single pulses or short series (Fig. 4) the insects resumed normal wingbeat within one second, thus we refer to this behavior as wingbeat interruption.

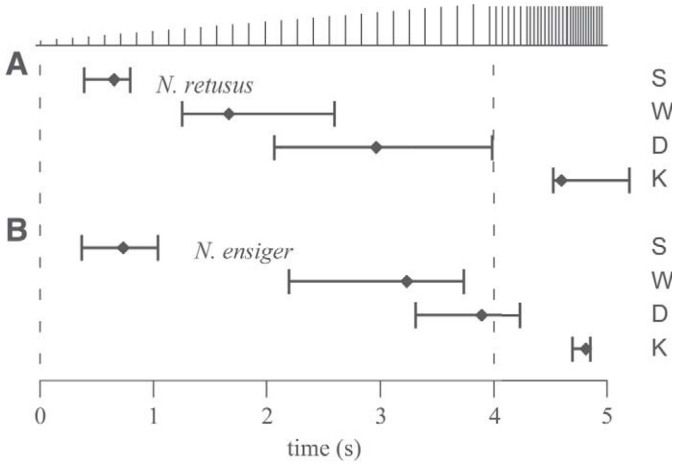

Fig. 3. Timing of the four bat avoidance behaviors during the simulated bat approach in *N. retusus* (A) and *N. ensiger* (B). Top trace indicates the timing of the stimulus; amplitudes not shown to scale. Dashed lines mark the beginning of the stimulus and beginning of terminal phase. Median response times shown; error bars indicate range of data, excluding the highest and lowest value. N=9 (*N. retusus*) and 11 (*N. ensiger*). S steering, W wingbeat interruption, D dive, K leg kick.

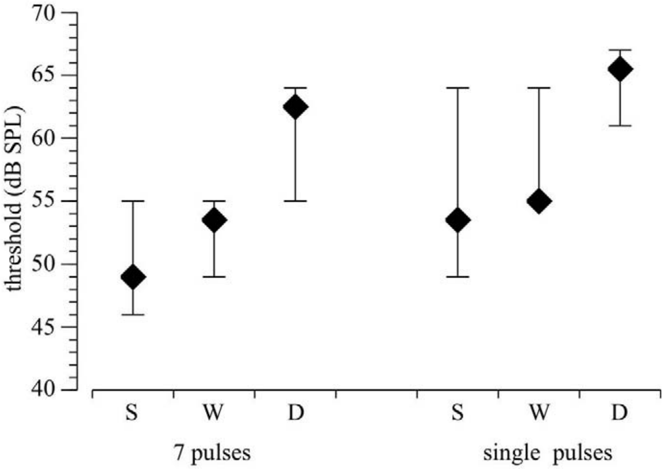

Fig. 4. Behavioral thresholds of the three early warning behaviors in *N. retusus* in response to a series of 7 model bat cries presented at a rate of 7/s (left) and a single model bat cry (right). Median thresholds (n=8) are shown with error bars showing the range of data, excluding highest and lowest value. S steer, W wingbeat interruption, D dive.

At even higher pulse amplitudes, all wings were folded parallel to the body and the animal moved out of flight position ('dive'). During stimulation with single pulses and series of seven pulses (Fig. 4), flight did not resume for several seconds after the dive, so that a free flying insect would likely fall to the ground. Note that during the stimulation with the approach stimuli (Fig. 2) the leg kick behavior often coincided with a short burst of wingbeats. All of these behaviors increase the likelihood that the insect will move away from the echolocation cone (Hartley & Suthers 1989) of the bat, thus we interpret them as bat-avoidance behaviors.

Our approach stimulus assumed a constant bat flight speed of 6.3 m/s (see methods). In both species tested, steering occurred at amplitudes equivalent to a distance of ~26 m between bat and insect (Fig. 3). In *N. retusus*, wingbeat interruption and dive occurred at distances of approximately 20 and 12 m (*i.e.*, stimulus amplitudes of 56 and 69 dB SPL; Fig. 3). Responses occurred at similar amplitudes during the threshold measurements (Fig. 4). In *N. ensiger*, the latter two responses occurred later during the simulated approach, equivalent to distances of 10 and 7 m between the bat and the insect (Fig. 3).

Estimates for the detection ranges of bats for medium to large insects range between 5 m (Kick 1982) and 10-15 m (Holderied & von Helversen 2003, Surlykke & Kalko 2008). Thus, these three avoidance behaviors would occur either before or close to the time when the bat would detect the insect. Therefore, these three behaviors are early warning behaviors, which reduce the likelihood of being detected by the bat (Miller & Oleson, 1979). However, early warning behaviors also have potential costs for the insect: any deviation from the original flight path should cause a loss in time or energy. A dive would also increase the chance that the insect is exposed to terrestrial predators (*e.g.*, spiders, mantises). Seemingly, as the risk of bat predation increases (decreasing distance) the insect employs more costly behaviors (Fig. 3).

The consistency with which the leg kick occurs (Table 1), and the low variability in time of occurrence (Fig. 3) support its function as bat avoidance behavior. The leg kick never occurred to single pulses, or stimuli with slow pulse rates. It was triggered only by fast pulse rates (Fig. 7), as they occur during the terminal phase of a bat approach ("feeding buzz", Griffin *et al.* 1960, Schnitzler & Kalko 2001). We therefore consider the leg kick a 'last chance behavior' (Miller & Oleson 1979). Last chance behaviors triggered by fast pulse rates have been described in several other insects including green lacewings (Miller 1975), dogbane tiger moths (Fullard *et al.* 1994) and praying mantids (Triblehorn & Yager 2005).

The effect of this behavior during free flight is not obvious. Potentially, the leg kicks could cause a sudden change in the flight path of the insect. The burst of wingbeats, that often occur in conjunction with this behavior (Fig. 2), supports this interpretation.

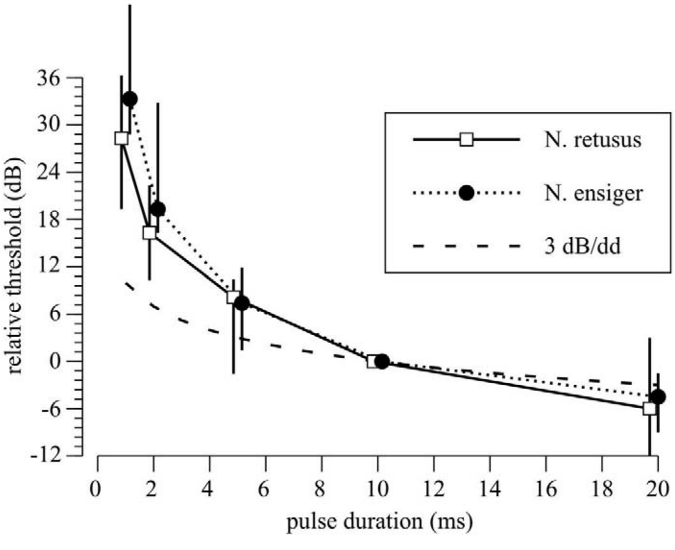

Fig. 5. Relative behavioral threshold for wingbeat interruption in response to single sound pulses of varying duration. Thresholds are given relative to the threshold for 10 ms pulse duration. Symbols indicate median thresholds (open squares *N. ensiger*, n=11; filled circles *N. retusus*, n=10); error bars represent range, excluding highest and lowest values. Dashed line indicates the relative thresholds expected for an energy integrator with a slope of -3dB per doubling duration. Stimuli had a constant carrier frequency of 40 kHz.

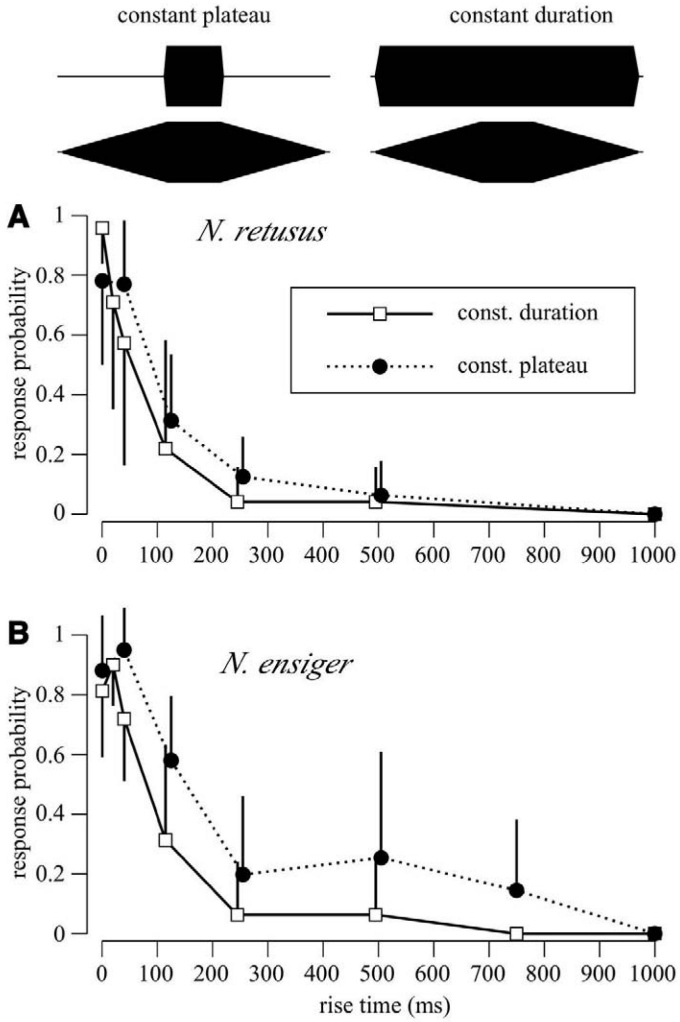

Fig. 6. Mean response probabilities of *N. retusus* (A, n=8) and *N. ensiger* (B, n=8) for wingbeat interruption in response to pure tone (40 kHz) pulses with varying rise times. Top: sketches of the stimuli, not drawn to scale: in one experimental series (dotted lines) the plateau duration was held constant at 10 ms; in the other (solid line) the pulse duration (including rise and fall time) was held constant at 2010 ms. Grand means (3-4 trials/individual) ± standard deviation are shown.

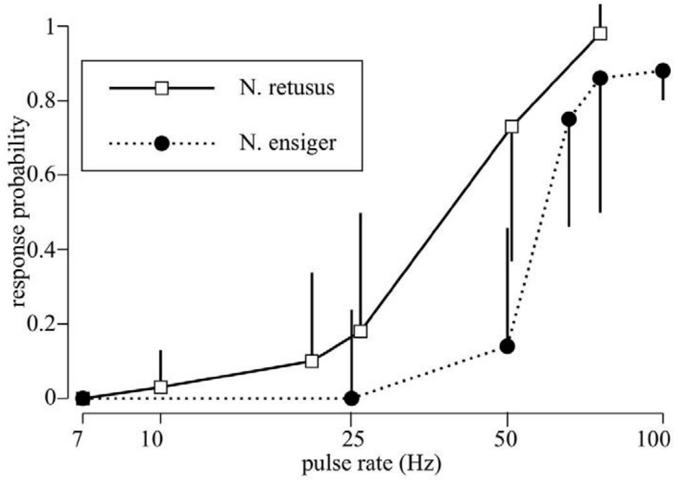

Fig. 7. Response probabilities of *N. ensiger* (closed circles, n=9) and *N. retusus* (open squares, n=11) for leg kicks in response to approaches with varying pulse rate in the terminal phase. Grand mean (3-4 trials/individual) ± standard deviation are shown.

Alternatively, the leg kick might make the insect more difficult for the bat to capture. The species studied here (body length 37-55 cm) are probably at the upper end of the prey size range captured by larger bats (*e.g.*, *Eptesicus fuscus*, Agosta *et al.* 2003). Thus, the leg kick might be a successful strategy used to avoid capture. A third alternative is that the leg-kick behavior could change the echo signature received by the bat, potentially confusing or startling it.

The leg kick bears a striking resemblance to a terrestrial startle behavior described in other Copiphorinae. In response to terrestrial predators, *Mygalopsis* spec. spread fore legs, hind legs, and mandibles rapidly, while keeping the mid legs on the substrate (Sandow & Bailey 1978). In Saginae, such startle behavior was accompanied by a flaring of the wings (Kaltenbach 1990).

The similarities of these terrestrial startle behaviors to the in-flight leg kick leads us to hypothesize that the leg kick is an aerial manifestation of the terrestrial startle response. The change in behavioral context (sitting *vs* flying insect) is accompanied by a change in sensory modality (optical to acoustic stimulation). A similar behavioral transfer has been reported in praying mantids (*Parasphendale agrionina)*, which have incorporated an optically/tactilely-induced, terrestrial startle response into their flight repertoire (Yager & May 1990). When performed in flight, the behavior can only be elicited by acoustic stimulation (Yager & May 1990).

Previous bat-avoidance studies with flying *Neoconcephalus* reported only one behavior with very high thresholds (*N. ensiger*, Libersat & Hoy 1991). However, these authors used very different experimental methods than our own, as well as insects from geographically different populations. Either of these factors might account for the differences in observed behavioral repertoires.

The behavioral repertoire and sensitivities that we found in *Neoconocephalus* were similar to those described for another katydid, *Tettigonia viridissima* (Schulze & Schul 2001), tested with methods similar to our own. Only minor differences were detected between the behavioral repertoires of *T. viridissima* and *Neoconocephalus*, namely a lack of abdominal steering in *Neoconocephalus*. The consistency in behavioral repertoires and behavioral sensitivities in these two distant genera of Tettigoniidae suggest that bat-avoidance strategies are relatively well conserved within the family. While the leg kick has not been described in previous studies of in-flight, bat-avoidance behaviors (Libersat & Hoy 1991, Schulze & Schul 2001), the stimulation used by these authors would not have revealed this behavior, as no fast pulse rates were used.

The three early warning behaviors were all elicited by a single pulse of ultrasound, indicating that pulse repetition rate played no role in the recognition of the bat pulses. Therefore, some feature of a single pulse must be used to recognize the signal as a bat cry. In order to narrow down the necessary parameters, we tested two fine-scale temporal parameters, pulse rise time and duration.

Both *N. retusus* and *N. ensiger* had the highest response probabilities to pulses with short rise time durations, regardless of the overall duration of the plateau or the total pulse duration. Total length of the stimuli, which reached nearly 2 s in the constant length stimuli, did not negatively affect response probabilities, indicating that maximum pulse duration is probably not a critical value. We confirmed this by measuring behavioral thresholds for pulses of varying duration. Although long duration pulses do not negatively affect thresholds, decreasing the duration did have an effect.

We measured shifts in the slope of behavioral thresholds at 6-8 dB per double duration (dB/dd), much higher than expected from the usage of an energy detector alone (3dB/dd, Au 1988). This difference indicates that some parameter other than signal energy determines behavioral thresholds for signals of varying duration. A similar conclusion was reached for the greater wax moth (*Galleria mellonella*), which had a slope in behavioral threshold shift of 7.2 dB/dd (Skals & Surlykke 2000).

The effect of variations in rise time and the relative unimportance of lengthening pulse duration indicate that the signal onset is critical for recognition. In order to be recognized a signal must have a minimum duration (>2 ms) and have a short rise time. We hypothesize that very short duration signals are not recognized as aversive because of their similarity to sounds produced by other katydids (*e.g.*, Phaneropterinae: Heller 1988, Walker 2008).

The increase in behavioral thresholds as a function of decreasing pulse duration suggests a potentially useful strategy for bats. If a bat could decrease the duration of its pulses (and thus the total energy), its prey detection range would inevitably decrease. However, the detection range of the katydid for bat echolocation would decrease by a greater amount, because of the greater effect of pulse duration on behavioral thresholds in katydids. Thus, decreasing the duration of echolocation cries might be a successful hunting strategy for bats.

One additional problem that katydids face, beyond simple recognition of signals, is that bats must be detected in the midst of the background noise produced by other insects, including conspecific calls. One solution proposed for *Neoconocephalus* katydids with 'typical' calls (fast pulse rate), such as *N. retusus*, is that signals are discriminated based on differences in pulse rate, via the TN-1 neuron. TN-1 responds to stimuli with slow pulse rates (<20 Hz), while no responses occur to faster pulse rates. TN-1 detects slow pulse rates even in the presence of fast rates, if the two signals differ sufficiently in carrier frequency (Schul & Sheridan 2006). In *N. retusus*, this interneuron is broadband, responding to frequencies associated with both bats and male calls. This solution would not work for *N. ensiger*, which has a call with a slow pulse rate. *N. ensiger* may have solved this problem by shifting the tuning of TN-1 to higher frequencies (Faure & Hoy 2000b). We hypothesize that *N. ensiger*, unable to discriminate signals based on pulse rate, instead relies on spectral differences between signals for discrimination.

Acknowledgements

We would like to thank S.L. Bush, H.C. Gerhardt, R.-M. Muzika, J.D. Triblehorn, and K.H. Frederick Hudson for support and critical comments. MKK was supported by a Life Sciences fellowship and BBB by an LSUROP fellowship of the University of Missouri. This work was supported by NSF grants IOB-0445286 and IOS-0722109 to JS.

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