

Bat Avoidance in Non-Aerial Insects: The Silence Response of Signaling Males in an Acoustic Moth

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Abstract

While the evasive responses of many flying acoustic insects to aerial-hawking bats are duly recognized and studied, the responses of non-aerial insects to gleaning bats are generally overlooked. It has been assumed that acoustic insects are deaf to these predators because gleaning bat echolocation calls are typically low in amplitude, brief (1–3 ms) and very high in frequency (> 60 kHz). We tested this assumption in a series of playback experiments with a moth (*Achroia grisella*) that uses hearing in both predator evasion and mating. We report that ultrasound pulses ≥ 78 dB peSPL (peak equivalent sound pressure level) and ≥ 1 ms in duration inhibit stationary males from broadcasting their own ultrasonic advertisement calls, provided that the pulsed stimuli are delivered at a repetition rate ≤ 30 /s. Further analyses suggest that inhibition by pulsed ultrasound comprises two processes performed serially. First, a startle response with a latency < 50 ms is elicited by a single pulse ≥ 1 ms duration. Here, a male misses broadcasting several calls over a 50–100 ms interval. Secondly, the startle may be extended as a silence response lasting several to many seconds if subsequent pulses occur at a rate ≤ 30 /s. Call inhibition cannot represent a simple response to acoustic power because of the inverse interaction between pulse duration and rate. On the other hand, the temporal and energy characteristics of inhibitory stimuli match those of gleaning bat echolocation calls, and we infer that inhibition is a specialized defensive behavior by which calling males may avoid detection by eavesdropping bats.

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Introduction

The neurophysiologic and behavioral responses of nocturnal insects to bat echolocation calls are well known. Ultrasound-sensitive ears and evasive flight

maneuvers in response to pulsed ultrasound are highly developed in species distributed among five insect orders: Orthoptera, Mantodea, Coleoptera, Lepidoptera, and Neuroptera (Hoy 1992). Such hearing ability evolved independently in each of these orders; moreover, within the first four, multiple origins are inferred (Yager 1999). In the Lepidoptera, ultrasound-sensitive ears evolved at least six times on the various body segments and appendages (Yack & Fullard 2000), and they are widespread within three major superfamilies, Pyraloidea, Geometroidea, and Noctuoidea. Comparative analyses support the claim that ultrasonic hearing in the Lepidoptera has coevolved with insectivorous bats over the past 60 million years: Tympanal ears and ultrasound sensitivity are reduced or absent in noctuid moth species found in regions devoid of bats (Fullard 1994), as well as in species active at times of the day (Fullard et al. 1997) or season (Surlykke et al. 1998; but see Rydell et al. 1997 for lack of such reduction in geometrid moths) when bats are not present.

Aerial hawking is the most common foraging mode among insectivorous bats, and most studies of insect–bat coevolution have focused on these foragers and how flying insects avoid them via sudden changes in velocity, including diving (Fullard 1990). But, substrate gleaning occurs in approximately one-third of all insectivorous bat species (Arlettaz et al. 2001), and it is particularly common in various tropic regions. Members of this guild use echolocation calls only to avoid colliding with obstacles; they capture their prey by detecting and localizing sounds, incidental or otherwise, that insects on foliage and other substrates emit (Neuweiler 2000). And even among aerial-hawking bats, a variation of this method is found among some hipposiderid and rhinolophid species. Consequently, some acoustic insects may be expected to exhibit specialized responses to bat echolocation calls while they are running or perched, as well as in flight. These responses would include freezing all motion and silencing acoustic signaling (see Faure & Hoy 2000), which substrate-gleaning bats might eavesdrop on. To date, a limited number of studies (e.g. Werner 1981; Spangler 1984; Acharya & McNeil 1998; Bailey & Haythornthwaite 1998; Jones et al. 2002) have demonstrated inhibitory responses in non-aerial insects to pulsed ultrasound. Among these, the study by Bailey & Haythornthwaite (1998) is noteworthy in that it used live gleaning bats and showed that *Teleogryllus oceanicus*, a cricket with ultrasonic hearing, reduced the conspicuousness of its calls when in exposed locations subject to greater bat predation.

Findings from studies of lesser waxmoths (*Achroia grisella*; Pyralidae: Galleriinae), an unusual species that uses hearing in both bat evasion and pair formation, are consistent with the expectation of defensive responses in non-aerial insects. Like many moths, *A. grisella* in flight dive to the ground in response to ultrasound pulses that simulate the echolocation calls of aerial-hawking bats (Rodriguez 2002). But, male *A. grisella* also produce a pulsed ultrasonic signal attractive to females within 1–2 m (Spangler et al. 1984). Receptive females run toward signaling males or simulated signals broadcast from a loudspeaker, but they cease orientation and freeze movement if simultaneously presented with pulsed ultrasound resembling that typical of gleaning bat echolocation calls

(frequency = 83 kHz; pulse duration = 1–3 ms; pulse rate = 15/s; Greenfield & Weber 2000). On the other hand, continuous white noise that includes a broad range of ultrasonic frequencies elicits no behavioral response. Thus, it was inferred that freezing either represents the silencing of incidental sound or the suppressing of inappropriate responses to stimuli that are clearly not males. Although the latter benefit may be a less crucial one, expending available energy on mating activities economically should nonetheless be advantageous for an insect whose adult lifespan is normally 1 wk or shorter.

The freezing response of *A. grisella* females to simulated bat echolocation calls (Greenfield & Weber 2000) suggests that males too might exhibit specialized defensive behavior while on the ground. This prediction is reinforced by several additional points. First, males generate ultrasonic advertisement signals by fanning their wings, which allows an observer to recognize the activity visually as well as acoustically (with the aid of a bat detector), and males signaling from exposed perches in the field have been observed to stop when echolocating bats passed overhead or when presented with pulsed ultrasound (Spangler 1984). Secondly, hearing thresholds, determined from behavioral responses, are in the 45–50 dB SPL range for 100 kHz sounds (L. Brandt & B. Ludwar, unpubl. data), implying that the moths would readily hear the searching-phase echolocation calls of substrate-gleaning bats approaching to within several meters, before echolocation calls are shortened and weakened – and finally done away with – in the ultimate approach and capture phases (see Waters & Jones 1995). Thirdly, negative motor responses (freezing and silence) to substrate-gleaning bats at the close ranges where they would become clearly audible may afford terrestrial prey considerably more protection than aerial responses (evasive flight and diving) would afford a flying insect at comparable distances from an approaching bat.

Here, we report on the behavioral responses of signaling male *A. grisella* to experimental simulations of the pulsed ultrasounds of gleaning bats. By varying several temporal characters of the pulses, we determined that males are inhibited from signaling by the same stimuli that inhibit female movement. This silence response cannot be explained solely by stimulus power, and we suggest that males, and females, exhibit specialized inhibitory responses to these ultrasound pulses. Moreover, we infer that in the field such inhibitory responses afford a reduced risk of predation by substrate-gleaning bats.

Materials and Methods

General Methodology

Achroia grisella is a cosmopolitan symbiont of honeybees, *Apis mellifera*, in whose colonies the moth larvae feed on stored food, wax, and detritus (Künike 1930). Upon eclosion, the adult moths normally remain in the vicinity of the natal colony, and mating takes place there. Males advertise to females for 6–10 h per night while perched in, on, or near the colony (Greenfield & Coffelt 1983). Their wing-fanning advertisement, performed at approximately 50 wingstrokes per

second (at 25°C), causes a pair of tymbals at the forewing bases to vibrate briefly at the mid-point of each upstroke and downstroke of the wings. Tymbal vibrations yield highly-damped 100- μ s pulses of ultrasound, which are comparatively loud (95 dB SPL at 1 cm; 0 dB = 20 μ Pa), delivered rhythmically at a rate of approximately 100 pulse pairs per second ($2 \times$ wingstroke rate), and include frequencies ranging from 70–130 kHz (Jang & Greenfield 1996). Pulse pairs arise because stroking of the left and right wings is not perfectly synchronous, and onsets of the pulses generated by the left and right tymbals during a given upstroke or downstroke are normally separated by an ‘asynchrony interval’ approximately 250–500 μ s in duration. This interval is much shorter than that between upstrokes and downstrokes of the wings.

Both male and female *A. grisella* hear with a pair of abdominal tympanal organs (Knopik & von Hintze-Podufal 1986). These ears are broadly-tuned and sensitive to sounds ranging from 30–120 kHz (Spangler & Takessian 1983); playback experiments have shown that behavioral responses to pulsed stimuli are similar for sound frequencies ranging from 40–100 kHz (Spangler et al. 1984; Greenfield & Weber 2000). For loudspeaker broadcasts of the male advertisement call, whose peak frequency is approximately 100 kHz, receptive (virgin) females have a behavioral (phonotaxis) response threshold at 45–50 dB SPL (B. Ludwar, unpubl. data). Playback experiments have demonstrated that sound is the primary communication modality in pair formation, as loudspeaker broadcasts of male calls are as attractive to females as live males (Spangler et al. 1984). In addition to mate attraction, hearing functions in male–male competition and in avoidance of bat predation. Males temporarily elevate their pulse pair rhythm, which increases their attractiveness, when another male signals nearby (Jia et al. 2001). When flying, both males and females dive immediately to the ground if presented with long pulses (≥ 6 ms) of intense (\bar{x} threshold = 86 dB SPL) ultrasound (Rodriguez 2002).

We obtained our test insects from an *A. grisella* laboratory colony derived from several hundred insects collected in Lawrence, Kansas. Rearing and breeding procedures are described in Jang & Greenfield (1996). All test males were unmated adults between 1–3 d-old, except those used in the experiment testing the effects of age. We conducted a set of experiments designed to determine (i) whether pulsed ultrasound – as found in gleaning bat echolocation calls – influences signaling male *A. grisella* to become silent, (ii) whether the moth responses are age-dependent, as older individuals might be expected to be more risk-prone and fail to exhibit a silence response, and (iii) the threshold intensities above which the moths generally respond, from which we could infer the distance at which echolocating bats might influence moths to become silent in the field. Our basic method in each experiment consisted of presenting a signaling male, held in a small screen cage, with a 2-s playback of pulsed ultrasound and monitoring its calls prior to, during, and following the stimulus.

For playback stimuli, we created a 40-kHz continuous sine wave via a signal generator, edited the wave to one of three pulse durations (0.1, 1.0, 6.0 ms), and then repeated these pulses at one of four rates (10, 30, 60, 90 pulses per second).

These 12 stimuli were digitized (8-bit; sampling rate = 298 kHz) and saved to 12 separate computer files, each 220 ms in duration. For playback, a stimulus file was continuously looped, converted to analog via an 8-bit D:A converter in the computer's sound card (SoundFX, Engineering Version; SiliconSoft; San Jose, CA, USA), amplified (model S55, UltraSound Advice, London, UK), and broadcast through an ultrasound loudspeaker (UltraSound Advice, model S56; frequency response ± 6 dB from 10–200 000 Hz). To verify the quality of a stimulus, we monitored the frequency and pulse characteristics of the loudspeaker broadcast with a condenser microphone (model 7016, ACO Pacific, Belmont, CA, USA; frequency response ± 6 dB from 10–160 000 Hz) whose output was sent to an oscilloscope. We determined the sound pressure level associated with signal levels displayed on the oscilloscope by the method of peak equivalents (p.e.; see Jang & Greenfield 1996), wherein we directed the broadcasts an 8-kHz reference tone simultaneously toward a sound pressure level meter (model 1981, General Radio, Concord, MA, USA, with its band-pass filter centered at 8 kHz) and the condenser microphone. Thus, we adjusted the amplifier gain to broadcast a desired stimulus amplitude in each trial.

Screen cages (1.5-cm diameter) holding the test males were placed on the periphery of a circular turntable kept in a darkened semi-anechoic room (see Jang & Greenfield 1996, 1998). We oriented the loudspeaker to face the turntable and positioned it at 15 cm distance from the peripheral circle along which the cages were arranged. For a given trial, we slowly rotated the table until the male to be tested was opposite the loudspeaker. Each cage was protected by a shield of acoustic insulation foam on all sides except that facing the loudspeaker. Thus, the male was exposed to stimuli only during its trial and was acoustically isolated from other calling males in the test room, a measure that reduced the opportunity for habituation. Previous studies in our laboratory showed that the screen of the holding cages did not interfere with ultrasound transmission, and we assumed that caged males were fully exposed to the loudspeaker broadcasts.

A given male was tested as many as 18 times in 1 d (see experiment 3 below), but a minimum of 15 min always elapsed between its successive trials. We used a randomized-block design to arrange the sequence with which the different stimuli were presented to the test males. To standardize conditions and improve the likelihood that a male was active and signaled consistently, we conducted the trials during the initial 5 h of the scotophase (night) under which the insects were reared. A 25-W incandescent red bulb above the turntable provided with dim illumination during the trials, and room temperature was maintained at 25°C.

Each trial lasted 10 s and included a 4-s pre-stimulus period, the 2-s stimulus, and a 4-s post-stimulus period. We monitored the male's signaling throughout the trial with the ultrasound microphone from a bat detector (UltraSound Advice, model S25) and sent the detector's output to one input channel of the stereo soundcard in a second (recording) computer (see Greenfield & Weber 2000). To compare the timing of the male's signals with the playback stimulus pulses, we split the stimulus at its output from the first (playback) computer and, via a patch cord, sent one branch to the second channel of the soundcard in the recording

computer. Thus, male and stimulus pulses in the sound files acquired by the recording computer were temporally aligned. From each stereo sound file, we measured (i) the latency of signaling interruption (interval from stimulus onset to the first missing pulse pair in the male's signal), (ii) the time when the male resumed signaling, and (iii) the male's pulse-pair rate prior to and during the stimulus (Fig. 1). We then used these data to calculate an inhibition index: the proportion of the combined stimulus and post-stimulus periods (6 s) during which the male did not signal.

Experiment 1

We determined the influence of two temporal characters, pulse duration and pulse rate, on the silence response by testing all 12 playback stimuli described above on 16 males. In every trial we held stimulus amplitude, as measured at the location of the test male, at 90 dB peSPL. This amplitude is equivalent to that measured in the searching phase echolocation calls of several species of European substrate-gleaning bats (*Myotis blythii*, *Myotis myotis*, *Myotis nattereri*, *Myotis septentrionalis*), whose signals have been thoroughly analyzed, at distances from 0.5–1.0 m (see Miller & Treat 1993; Waters & Jones 1995; Norman et al. 1999); however, for another thoroughly analyzed European substrate-gleaning species, *Plecotus auritus*, with weaker echolocation calls, our stimulus amplitude would only be equivalent to calls as measured at 0.25 m (see Waters & Jones 1995). The 40-kHz frequency in our stimuli is included in the downward frequency sweeps (120–30 kHz) of these bat echolocation calls, and it is also comparable with the

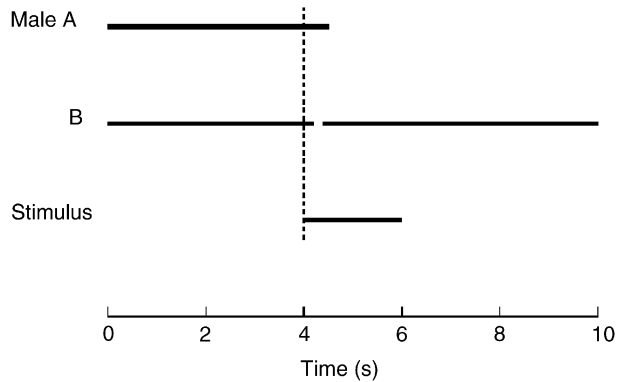


Fig. 1: Temporal relationships between stimulus presentation and male signaling in experiments 1, 2, and 3. Each playback trial included a 4-s pre- and post-stimulus period, a 2-s stimulus period. Durations of the stimulus and the signaling by two hypothetical males are depicted by thick horizontal lines. Male A: latency from stimulus onset to signaling interruption = 500 ms; inhibition index (proportion of stimulus + post-stimulus periods during which male is silent) = 0.9167. Male B: latency = 200 ms; inhibition index = 0.0333.

echolocation call frequency (approx. 50 kHz) bearing the most energy in several *Myotis* species.

Experiment 2

We determined the influence of age on the silence response by testing a subset of the 12 playback stimuli on 16 males when 1, 4, and 7 d old (post-eclosion). Again, stimulus amplitude was held at 90 dB SPL. Because we observed very few silence responses to 0.1-ms pulses and the responses to 60 and 90 pulse per second rates were similar to one another (experiment 1, see Results), we omitted 0.1-ms pulse durations and 60/s pulse rates from the stimulus array. From the data collected we determined whether males reduced their silence response as they aged.

Experiment 3

We evaluated the influence of amplitude on silence responses by testing on six males the same reduced (six-stimulus) playback array used in experiment 2. Each stimulus was tested at three different amplitudes, 66, 78 and 90 dB peSPL. Thus, each of the six males used in this experiment was tested in as many as 18 trials. From the data collected we determined threshold amplitude values for silence responses.

Data Analysis

Because the distributions of inhibition index and latency data could not be normalized by any standard transformation, these data were statistically analyzed with non-parametric tests. We used Mood's median test to evaluate the influences of playback stimulus features or male age on the inhibition index. Where a male's response to a given stimulus type was tested multiple times (e.g. 6-ms pulses tested at four different pulse rates), we calculated a mean value for each male and performed the median test on those values. A second evaluation of inhibition was obtained by determining the proportion of males who responded to a given stimulus type by becoming silent and remaining so for the remainder of the trial. We then used the binomial test (two-tailed), corrected for multiple tests via the Holm procedure, to compare this value with the proportion (0; we used 1/16 in order to perform the test) remaining silent in response to the 0.1-ms, 90 pulses per second playback, that which most closely resembled the *A. grisella* male advertisement call. Previous observations revealed that males do not cease calling in the presence of neighbors (Jia et al. 2001), and we take a high proportion of silence in response to a particular stimulus as indicating that for the moths it was outside the context of sexual behavior or competition. We used survival analysis (Kaplan–Meier estimates) to evaluate the influence of stimulus features or male age on signaling interruption. Survival curves showing the diminishing probability that a male had not yet interrupted its signaling at increased latencies, measured

from stimulus onset, were constructed for each playback or age category and then compared via a log-rank test. We used the two-sample Wilcoxon test for paired samples (two-tailed) to compare a male's pulse-pair rate prior to the stimulus with that occurring during the stimulus. Tests were performed separately for each stimulus, and the individual probabilities from each test were combined using Fisher's method.

Results

Experiment 1: How do Stimulus Characters Influence Startle and Silence Responses?

We found that the basic interruption of signaling, the missing of pulse pairs without regard to the duration of silence, was affected by pulse duration ($p < 0.01$, log-rank comparison of survival curves) but not by pulse rate ($p = 0.08$). In general, stimuli with 1-ms and 6-ms pulses elicited interruption whereas 0.1-ms pulses did not. When interruption occurred, its latency from stimulus onset was nearly always < 100 ms, and often < 25 ms (Fig. 2). But, in many cases, a male resumed signaling after a silent interval of 100 ms or less. We term such events startle responses. Extended silence, that is a high inhibition index (> 0.35), resulted when a male initiated a startle response and failed to resume signaling by the end of the stimulus or even the entire trial.

Unlike the startle response, a male's silence response was influenced by both the pulse rate and pulse duration of an ultrasonic stimulus. As with the startle response, we found that stimuli with 6-ms pulses elicited a high inhibition index, whereas 0.1 and 1.0-ms pulses did not ($p < 0.01$, Mood's median test; Fig. 3a).

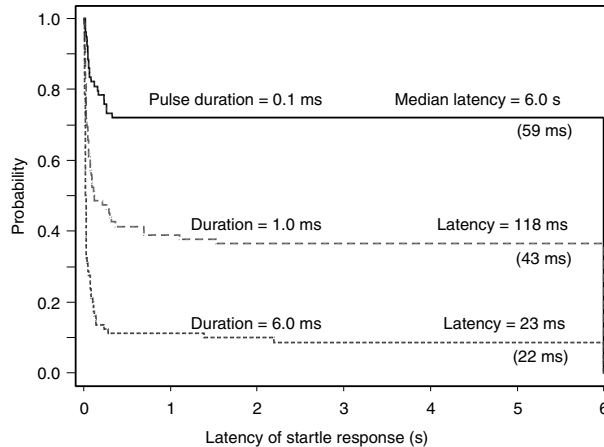


Fig. 2: Survival curves for uninterrupted signaling in response to stimuli of three different pulse durations. Each curve shows the diminishing probability that a male has not yet interrupted its signaling, (i.e. exhibited a startle response) at increased latencies, measured from stimulus onset. Parenthetic values are median latencies calculated only from males that responded to the stimulus.

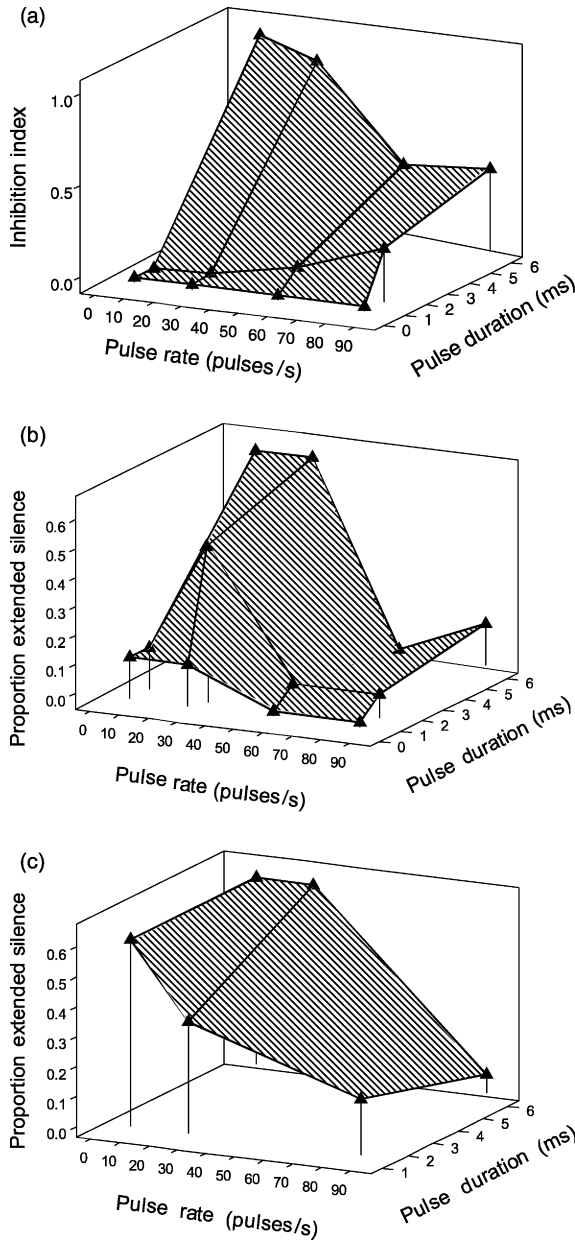


Fig. 3: Silence response to stimuli of three different pulse durations and four different pulse rates. (a) Silence response measured by the inhibition index, the proportion of stimulus + post-stimulus periods during which a male is silent; data taken from experiment 1. (b) Silence response measured by the proportion of males who, after interruption of signaling during the stimulus, did not resume before the end of the trial; data taken from experiment 1. (c) Silence response measured as above; data taken from 1- and 4-d-old males in experiment 2. Solid triangles indicate median values of inhibition index or of proportion extending silence until the trial end.

But, the high level of silence in response to 6-ms pulses only occurred when the pulses were delivered at the two slower pulse rates, 10 and 30/s ($p < 0.01$; Fig. 3a). We found similar results by calculating the proportion of males that ceased signaling during the stimulus and remained silent for the remainder of the 10-s trial, save that here a high proportion of extended silence occurred for 1-ms pulses as well as for 6-ms ones delivered at 30 pulses per second ($p < 0.01$, binomial test; Fig. 3b). Extended silence responses to both 1- and 6-ms pulses, delivered at either 10 or 30/s, were also found in experiment 2 ($p < 0.01$, Fig. 3c).

When males continued to signal during a stimulus, they usually increased their rate of delivery of pulse pairs ($p = 0.05$, Fisher's combined probability test). The average increment was 3 pulse pairs per second, a 4% increase, and was not influenced by pulse duration or rate of the stimulus.

Experiment 2: Are Startle and Silence Responses Age-dependent?

A male's inhibition index did not change as he aged. This invariance held whether we analyzed the entire data set from this second experiment ($p = 0.742$, Mood's median test) or only responses from trials using 6-ms pulses delivered at either 10 or 30/s ($p = 0.086$), the stimuli eliciting the highest inhibition indices in experiment 1. The latency to signaling interruption, however, increased with age ($p = 0.0156$, Fig. 4). Median latency to the first missing pulse pairs was 29 ms in 1-d-old males, less than 40% of the values observed in 4- and 7-d-old males.

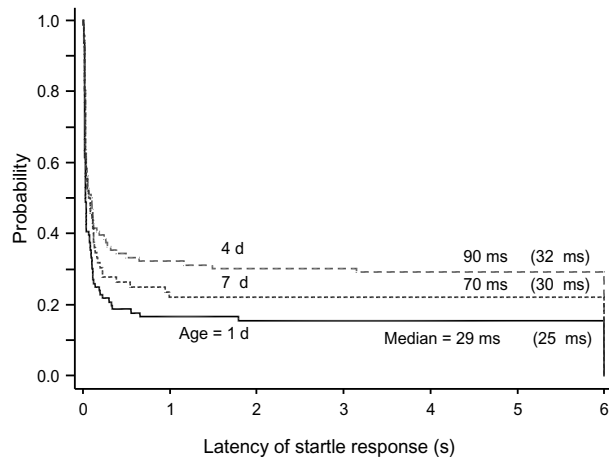


Fig. 4: Survival curves for uninterrupted signaling in males aged 1, 4, and 7 d (post-eclosion). Each curve shows the diminishing probability that a male has not yet interrupted its signaling, (i.e. exhibited a startle response) at increased latencies, measured from stimulus onset; data taken from experiment 2. Parenthetic values are median latencies calculated only from males that responded to the stimulus. See Fig. 2 for explanation.

Experiment 3: Threshold Amplitudes for Startle and Silence Responses.

Stimulus amplitude had a strong influence on the silence response. Inhibition indices were significantly higher for 90 and 78 dB than for 66 dB, and this result held whether we analyzed the entire data set from this experiment ($p < 0.01$, Mood's median test; Fig. 5a) or restricted analysis to responses to 10 and 30 pulse per second playback stimuli ($p < 0.01$); these slower pulse rates elicited significantly greater inhibition in Experiments 1 and 2. Latencies to signal interruption were also significantly shorter in response to 78 and 90 dB than to 66 dB stimuli. Again, this result held for the entire data set ($p < 0.01$, log-rank

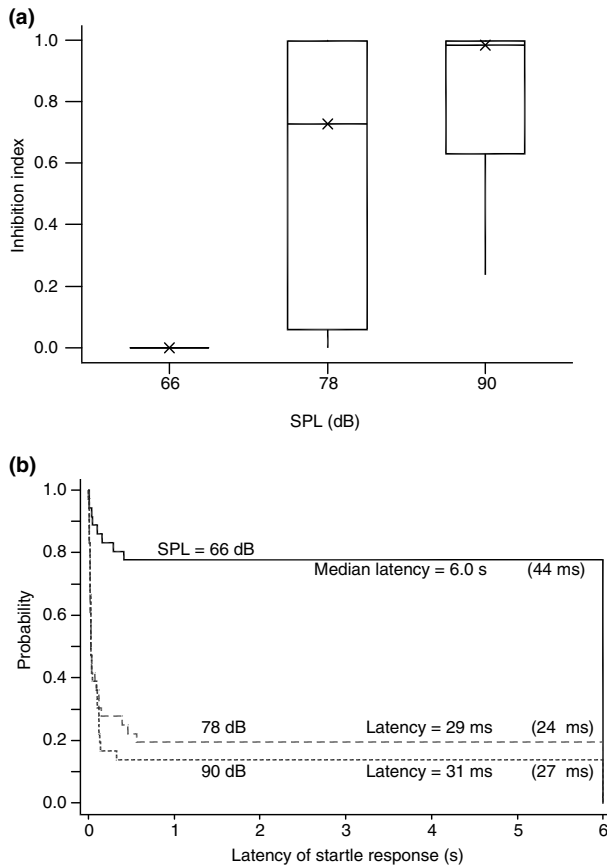


Fig. 5: Inhibition of signaling as a function of stimulus amplitude. (a) Silence response measured by the inhibition index, the proportion of stimulus + post-stimulus periods during which a male is silent; data taken from experiment 3, with median values (\bar{x}), first and third quartiles, and ranges shown for each of three SPLs. (b) Survival curves for uninterrupted signaling in response to stimuli of three different SPLs. Each curve shows the diminishing probability that a male has not yet interrupted its signaling (i.e. exhibited a startle response) at increased latencies, measured from stimulus onset; data taken from experiment 3. Parenthetic values are median latencies calculated only from males that responded to the stimulus. See Fig. 2 for explanation.

comparison of survival curves; Fig. 5b) and for the reduced set restricted to 10 and 30 pulse/s stimuli ($p < 0.01$). For 66-dB stimuli, most males failed to interrupt signaling at any time during the trial.

Discussion

Our playback experiments clearly demonstrated that *A. grisella* males interrupt their sexual advertisement calls when presented with lengthy ultrasound pulses delivered at slow rates. Males showed extended silence in response to 1-ms and 6-ms pulses presented at 10 or 30 pulses/s. Silence was elicited by pulse amplitudes ≥ 78 dB SPL, and the response level did not decline with age. The 78-dB stimulus amplitude is equivalent to that of an *A. grisella* male signal at approximately 8 cm, whereas the 90-dB stimuli used in many trials is equivalent to a male signal at approximately 2 cm. Thus, our playback stimuli were not supernormal. The initiation of silence could therefore be viewed as a natural startle response with a brief latency (< 50 ms) measured from the onset of the stimulus pulses (cf. Faure & Hoy 2000). This latency was markedly shorter in response to longer (1-ms and 6-ms) pulses, insensitive to pulse rate, and lengthened in older (≥ 4 d) males. Startle responses were seldom elicited by 0.1-ms pulses or by stimuli of any type presented at 66 dB SPL.

Because extended silence was strongest in response to pulses ≥ 1 ms in duration but delivered at a rate ≤ 30 /s, the behavior cannot be interpreted as a straightforward reaction to acoustic power (pulse duration \times pulse rate); that is, an intense conspecific stimulus. It is also unlikely to represent a simple terrestrial expression of the diving response in flying moths, as the latter is readily elicited by pulse rates as high as 100/s. Rather, the silence response is best reconciled with a specialized behavior of non-flying moths. In the field, this behavior may afford protection from predatory bats, specifically those species that detect and glean terrestrial prey perched or running on foliage and other substrates. While in the searching phase, these bats typically emit brief (1–3 ms) ultrasound pulses repeated at slow rates (10–20/s); their ultrasound frequencies are either very high (≥ 60 kHz) or sweep rapidly downward through a broad range (e.g. 120–30 kHz) (Neuweiler 2000), and pulse amplitudes are approximately 90 dB peSPL at 1 m (Miller & Treat 1993; Waters & Jones 1995). Such characteristics match those stimulus features that elicit high levels of silence in signaling *A. grisella* males (and of freezing in running *A. grisella* females). Thus, *A. grisella* males signaling in the vicinity of a honeybee colony might be expected to exhibit the silence response when exposed to the searching-phase echolocation calls of nearby bats (see Spangler 1984). Importantly, bats that may elicit silence responses would include substrate-gleaning species, as described in experiment 1 in the Materials and Methods. Here, the origin of *A. grisella* in the Old World alongside honeybees (*A. mellifera*) is relevant: these moths have experienced a long evolutionary history of exposure to bats such as the European *Myotis* spp., and it is for this reason that we used their signals as representative of ecologically relevant echolocation calls.

Based on reported echolocation call amplitudes in *Myotis* and *Plecotus* spp. bats (77–90 dB at 1 m; Miller & Treat 1993; Waters & Jones 1995; Norman et al. 1999) and the behavioral thresholds we found in experiment 2 (between 66 and 78 dB), *A. grisella* may be expected to initiate silence responses if substrate-gleaning bats approach to within 1–2 m. This distance is considerably closer than that reported for evasive maneuvers by flying noctuid moths responding to the echolocation calls of aerial-hawking bats (Roeder 1967; cf. Waters & Jones 1996 and Surlykke et al. 1999 for data on hearing sensitivity in noctuid moths; cf. Schulze & Schul 2001 for data on evasion distances in Tettigoniidae). However, the final approach and capture phases of substrate-gleaning species are not nearly as condensed as in aerial-hawking ones, where they can encompass a mere fraction of a second. Consequently, a silent, stationary moth the size of *A. grisella* and 1–2 m from a gleaning bat may not be a readily localized target (see Arlettaz et al. 2001), and silence responses as observed in our experiments are expected to afford a large measure of protection against predation. At the same time, the threshold for the silence response – approximately 30 dB above that for female phonotaxis to male calls – is sufficiently high that calling would not be interrupted by distant bats, who would be unable to hear the moth's signals.

Our results suggest that interruption of signaling and extended silence are two separate processes performed serially (see von Helversen & von Helversen 1995; Pollack 1998). Interruption is elicited by ultrasound pulses 1 ms or longer, whereas extended silence requires a low pulse rate in addition to lengthy pulses. Thus, males may interrupt signaling – the startle response – upon perceiving a single long pulse (see latency data in Fig. 2), but they only remain quiet – the silence response – if subsequent pulses recur at a low rate. A high pulse rate ($\geq 60/s$) would likely represent a neighboring male or, possibly, the capture-phase echolocation calls of an aerial-hawking bat foraging nearby (see Neuweiler 2000), neither of which would pose a threat. However, the latter event would represent a clear risk for an aerial insect, and *A. grisella* in tethered flight exhibit immediate defensive behavior – diving – in response to lengthy (≥ 6 ms) ultrasound pulses delivered at any rate ≥ 10 pulses per second (Rodriguez 2002). Serial processing as proposed above may be adaptive for terrestrial moths, because the startle response would afford a male the opportunity to assess the rate of ultrasonic pulses unmasked by its own signals (cf. Faure & Hoy 2000). Studies of various acoustic insects indicate that a signaler's hearing is greatly enhanced when silent (Greenfield 2002).

When males do not exhibit the silence response and continue to signal during the stimulus, they typically elevate the rate at which they generate pulse pairs. The rate increments we observed suggest that when males do continue signaling they are interpreting the stimulus as another male because males also elevate their pulse pair rate when signaling neighbors are close (Jia et al. 2001). Acoustic perception of pulsed ultrasound in *A. grisella* may thus be categorical (sensu Green & Marler 1979): The insects interpret the graded stimuli as discrete categories representing either conspecifics or natural enemies, a simplifying neural process that could accelerate evaluation. Categorical perception has been reported

in other acoustic insects (Wytenbach et al. 1996), and its general occurrence in situations demanding rapid responses needs further consideration.

Previous reports in the literature (e.g. Faure et al. 1990, 1993) have suggested that acoustic insects are relatively defenseless against substrate-gleaning bats and that the echolocation call features of these bat species – very high ultrasound frequencies, extremely brief pulses, and low amplitudes, which have caused them to be known as ‘whispering bats’ – are adaptations for hunting while undetected by their prey. Importantly, our findings show that some acoustic insects have the perceptual ability to detect these echolocation calls, and that they may take effective defensive measures upon hearing them. We do not claim that the sensitivity of *A. grisella* to extremely brief pulses of very high (≥ 60 kHz) ultrasound per se is a specific adaptation to evade substrate-gleaning bats. Some of their auditory abilities may simply reflect their small size (1-cm body length) and the high resonant frequencies expected, from mechanical limitations (Fletcher 1992), in their minute tympana. But, regardless of the origin of their sensitivity, *A. grisella* apparently use it in a specialized manner which, in the field, may lower the risk of predation from gleaning bats. We therefore call for further studies on the defenses of non-aerial acoustic insects and a more thorough examination of their natural interactions with a diverse range of bat predators in the field.

Acknowledgements

We are grateful to LaRoy Brandt and Rafa Rodriguez for technical assistance with playback experiments; to Eric Siegfried for rearing the moths; and to Ken Ratzlaff, Tom Peters, and Ric Roggero (University of Kansas Instrumentation Design Laboratory) for developing the software used in ultrasound signal processing. Critical reading by Rafa Rodriguez, Johannes Schul, and two anonymous referees greatly improved earlier versions of the manuscript. The project was supported financially by NSF grants IBN-9807915 and DBI-0097223.

References

- Acharya, L. & McNeil, J. N. 1998: Predation risk and mating behavior: the responses of moths to bat-like ultrasound. *Behav. Ecol.* **9**, 552–558.
- Arlettaz, R., Jones, G. & Racey, P. A. 2001: Effect of acoustic clutter on prey detection by bats. *Nature* **414**, 742–745.
- Bailey, W. J. & Haythornthwaite, S. 1998: Risks of calling by the field cricket *Teleogryllus oceanicus*: potential predation by Australian long-eared bats. *J. Zool., Lond.* **244**, 505–513.
- Faure, P. A., Fullard, J. H. & Barclay, R. M. R. 1990: The response of tympanate moths to the echolocation calls of a substrate gleaning bat, *Myotis evotis*. *J. Comp. Physiol. A* **166**, 843–849.
- Faure, P. A., Fullard, J. H. & 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. & Hoy R. R. 2000: The sounds of silence: cessation of singing and song pausing are ultrasound-induced acoustic startle behaviors in the katydid *Neoconocephalus ensiger* (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **186**, 129–142.
- Fletcher, N. H. 1992: *Acoustic Systems in Biology*. Oxford Univ. Press, Oxford.
- Fullard, J. H. 1990: The sensory ecology of moths and bats: global lessons in staying alive. In: *Insect Defenses* (Evans, D. L. & Schmidt, J. O., eds). SUNY Press, New York, pp. 203–272.

- Fullard, J. H. 1994: Auditory changes in noctuid moths endemic to a bat-free habitat. *J. Evol. Biol.* **7**, 435—445.
- Fullard, J. H., Dawson, J. W., Otero, L. D. & Surlykke, A. 1997: Bat-deafness in dayflying moths (Lepidoptera: Notodontidae: Dioptrinae). *J. Comp. Physiol. A* **181**, 477—483.
- Green, S. & Marler, P. M. 1979: The analysis of animal communication. In: *Handbook of Behavioral Neurobiology*, Vol. 3. Social Behavior and Communication (Marler, P. & Vandebergh, J. G., eds). Plenum Press, New York, pp. 73—158.
- Greenfield, M. D. 2002: *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford Univ. Press, Oxford.
- Greenfield, M. D. & Coffelt, J. A. 1983: Reproductive behavior of the lesser waxmoth, *Achroia grisella* (Pylalidae: Galleriinae): signaling, pair formation, male interactions, and mate guarding. *Behaviour* **84**, 287—315.
- Greenfield, M. D. & Weber, T. 2000: Evolution of ultrasonic signalling in wax moths: discrimination of ultrasonic mating calls from bat echolocation signals and the exploitation of an anti-predator receiver bias by sexual advertisement. *Ethol. Ecol. Evol.* **12**, 259—279.
- von Helversen, D. & von Helversen, O. 1995: Acoustic pattern recognition and orientation in orthopteran insects: parallel or serial processing? *J. Comp. Physiol. A* **177**, 767—774.
- Hoy, R. R. 1992: The evolution of hearing in insects as an adaptation to predation from bats. In: *The Evolutionary Biology of Hearing* (Webster, D. B., Fay, R. R. & Popper, A. N., eds). Springer-Verlag, New York, pp. 115—129.
- Jang, Y. & Greenfield, M. D. 1996: Ultrasonic communication and sexual selection in wax moths: female choice based on energy and asynchrony of male signals. *Anim. Behav.* **51**, 1095—1106.
- Jang, Y. & Greenfield, M. D. 1998: Absolute versus relative measurements of sexual selection: assessing the contributions of ultrasonic signal characters to mate attraction in lesser wax moths, *Achroia grisella* (Lepidoptera: Pylalidae). *Evolution* **52**, 1383—1393.
- Jia, F. -Y., Greenfield, M. D. & Collins, R. D. 2001: Ultrasonic signal competition among wax moths. *J. Insect Behav.* **14**, 19—34.
- Jones, G., Barabas, A., Elliott, W. & Parsons, S. 2002: Female greater wax moths reduce exual display behavior in relation to the potential risk of predation by echolocating bats. *Behav. Ecol.* **13**, 375—380.
- Künike G. 1930: Zur biologie der kleinen wachsmotte, *Achroia grisella* Fabr. *Z. Angew. Entomol.* **16**, 304—356.
- Knopek, L. & von Hintze-Podufal, C. 1986: On the morphology of the abdominal tympanic organ of the little wax moth *Achroia grisella* (Fbr.). *Zool. Jahr. Abt. Anat. Ontog. Tiere* **114**, 83—93.
- Miller, L. A. & Treat, A. E. 1993: Field recordings of echolocation and social signals from the gleaning bat *Myotis septentrionalis*. *Bioacoustics* **5**, 67—87.
- Neuweiler, G. 2000: *The Biology of Bats*. Oxford Univ. Press, Oxford.
- Norman, A. P., Jones, G. & Arlettaz, R. 1999: Noctuid moths show neural and behavioural responses to sounds made by some bat-marking rings. *Anim. Behav.* **57**, 829—835.
- Pollack, G. S. 1998: Neural processing of acoustic signals. In: *Handbook of Auditory Research*, Vol. 10, Comparative Hearing: Insects (Hoy, R. R., Popper, A. N. & Fay, R. R., eds). Springer-Verlag, New York, pp. 139—196.
- Rodriguez, S. R. 2002: Functional design in the communication of an ultrasonic moth. PhD Thesis. Univ. of Kansas, Lawrence, Kansas.
- Roeder, K. D. 1967: *Nerve Cells and Insect Behavior*. Harvard Univ. Press, Cambridge.
- Rydell, J., Skals, N., Surlykke, A. & Svensson, M. 1997: Hearing and bat defence in geometrid winter moths. *Proc. R. Soc. Lond.* **B 264**, 83—88.
- Schulze, W. & Schul, J. 2001: Ultrasound avoidance behaviour in the bushcricket *Tettigonia viridissima* (Orthoptera: Tettigoniidae). *J. Exp. Biol.* **204**, 733—740.
- Spangler, H. G., Greenfield, M. D. & Takessian, A. 1984: Ultrasonic mate calling in the lesser wax moth. *Physiol. Entomol.* **9**, 87—95.
- Spangler, H. G. & Takessian, A. 1983: Sound perception by two species of wax moths (Lepidoptera: Pylalidae). *Ann. Entomol. Soc. Am.* **76**, 94—97.
- Spangler, H. G. 1984: Silence as a defence against predatory bats in two species of calling insect. *Southwest. Nat.* **29**, 481—488.

- Surlykke, A., Skals, N., Rydell, J. & Svensson, M. 1998: Sonic hearing in a diurnal geometrid moth, *Archicaris parthenias*, temporally isolated from bats. *Naturwissenschaften* **85**, 36–37.
- Surlykke, A., Filskov, M., Fullard, J. H. & Forrest, E. 1999: Auditory relationships to size in noctuid moths: bigger is better. *Naturwissenschaften* **86**, 238–241.
- Waters, D. A. & Jones, G. 1995: Echolocation call structure and intensity in five species of insectivorous bats. *J. Exp. Biol.* **198**, 475–489.
- Waters, D. A. & Jones, G. 1996: The peripheral auditory characteristics of noctuid moths: responses to the search-phase echolocation calls of bats. *J. Exp. Biol.* **199**, 847–856.
- Werner, T. K. 1981: Responses of nonflying moths to ultrasound: the threat of gleaning bats. *Can. J. Zool.* **59**, 525–529.
- Wytenbach, R. A., May, M. L. & Hoy, R. R. 1996: Categorical perception of sound frequency by crickets. *Science* **273**, 1542–1544.
- Yack, J. E. & Fullard, J. H. 2000: Ultrasonic hearing in nocturnal butterflies. *Nature* **403**, 265–266.
- Yager, D. D. 1999: Structure, development, and evolution of insect auditory systems. *Microscop. Res. Tech.* **47**, 380–400.

Received: May 22, 2002

Initial acceptance: December 22, 2002

Final acceptance: January 30, 2003 (J.-G. Godin)