

Acoustic feature recognition in the dogbane tiger moth, *Cycnia tenera*

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Summary

Certain tiger moths (Arctiidae) defend themselves against bats by phonoresponding to their echolocation calls with trains of ultrasonic clicks. The dogbane tiger moth, *Cycnia tenera*, preferentially phonoresponds to the calls produced by attacking *versus* searching bats, suggesting that it either recognizes some acoustic feature of this phase of the bat's echolocation calls or that it simply reacts to their increased power as the bat closes. Here, we used a habituation/generalization paradigm to demonstrate that *C. tenera* responds neither to the shift in echolocation call frequencies nor to the change in pulse duration that is exhibited during the bat's attack phase unless these changes are accompanied by either an increase in duty

cycle or a decrease in pulse period. To separate these features, we measured the moth's phonoresponse thresholds to pulsed stimuli with variable *versus* constant duty cycles and demonstrate that *C. tenera* is most sensitive to echolocation call periods expressed by an attacking bat. We suggest that, under natural conditions, *C. tenera* identifies an attacking bat by recognizing the pulse period of its echolocation calls but that this feature recognition is influenced by acoustic power and can be overridden by unnaturally intense sounds.

Key words: bat echolocation, tiger moth, phonoresponse, defensive behaviour, *Cycnia tenera*.

Introduction

Information is encoded in sounds *via* their frequency, duration and amplitude, and the auditory systems of vertebrates use multicellular neural systems to discriminate the salient from irrelevant components of those sounds (Alain and Arnott, 2000). Insects are also capable of similar discrimination abilities (Hoy, 1989; Wyttenbach and Farris, 2004; Schul and Sheridan, 2006) and perform them with far fewer neurons. Of all insects, the ears of moths represent the simplest auditory systems, possessing only one to four auditory receptor cells (Eggers, 1919; Yack, 2004). With some exceptions (Conner, 1999), moths use their ears solely to detect the echolocation calls of hunting bats and evoke escape behaviours appropriate to the threat presented by the bat. To determine what that threat is, the moth must acoustically estimate the proximity of the bat as gauged by its echolocation calls, since other cues (e.g. visual) are of presumably little or no use to these insects for bat detection when flying at night (Fullard and Napoleone, 2001; Soutar and Fullard, 2004).

Griffin et al. categorized the echolocation calls of aerial hawking bats into three phases based on changes in call duration, period (the time from the start of one call to the start of the next) and frequency (Griffin et al., 1960). Search-phase calls are emitted first and are defined as having periods of 50 ms or more. It is assumed that during this phase, bats have not yet detected a potential target. After a bat has detected a target (Kick and Simmons, 1984; Wilson and Moss, 2004) it emits approach-phase calls that are shorter in duration and period (10–50 ms)

and are marked by an increase in their lowest frequencies (Surlykke and Moss, 2000). Once the bat has decided to complete its attack, it emits terminal (buzz)-phase calls that are very short (~1–2 ms), have periods of less than 10 ms (which increases the duty cycle – the percentage of time that the bat is actively producing sound) and are of lower peak frequency than either search or approach calls. While this three-phase heuristic has proved a valuable tool for comparative analysis of bat species and hunting strategies (e.g. Kalko, 1995; Ratcliffe and Dawson, 2003), it can be simplified from the perspective of the insect into two phases: (1) search-phase calls that signify a bat before it has detected its target and (2) attack-phase calls (approach + terminal) that signify a bat after it has detected a target and has begun to actively pursue it. Insects react bimodally to these calls: to search-phase calls with primary defences intended to conceal them from the bat before it has detected them and to attack-phase calls with secondary defences designed to rapidly evade the now-aware bat.

Roeder proposed that the bimodal defence response of noctuid moths is based upon their perceived intensity of the bats' calls (Roeder, 1966; Roeder, 1974). According to this theory, moths react to distant bats (i.e. faint calls) with directional controlled flight away from the bat, a defensive behaviour evoked by the most sensitive auditory receptor, the A1 cell. When confronted by near bats (i.e. intense calls), moths switch to erratic flight or cease flying altogether, responses elicited by the less sensitive A2 receptor cell. Skals and Surlykke supported this hypothesis by concluding that flight

cessation in the moth *Galleria mellonella* was triggered by the rise in acoustic power (as perceived by the moth) caused by increased duty cycle of the attack-phase calls (hereafter the 'acoustic power hypothesis') (Skals and Surlykke, 2000). While changing call intensity will provide a measure of the relative distance of a searching distant bat (assuming it does not change its emitted level), this cue may become unreliable to an erratically moving moth for a bat beginning its attack. Temporal and spectral changes to the bat's calls, on the other hand, present acoustic cues that should provide less ambiguous information about the bat's switch from search to attack phase, and moths may possess the ability to recognize such cues (hereafter the 'acoustic recognition hypothesis').

When stimulated by the echolocation calls of an attacking bat, the dogbane tiger moth (*Cycnia tenera*) phonoresponds with trains of ultrasonic clicks generated by thoracic tymbals. These sounds warn the bat of its noxious qualities [aposematism (Dunning and Roeder, 1965; Hristov and Conner, 2005)] and/or interfere with the bat's echolocation [jamming (Fullard et al., 1979; Fullard et al., 1994; Miller 1991)]; aposematism and jamming may act synergistically because negative-cue/negative-consequence associations should be readily made (Ratcliffe and Fullard, 2005). The phonoresponse is a stereotyped behaviour that can be used to examine auditory perception in this moth and what cues it uses to evoke its acoustic defence (Fullard, 1979; Fullard, 1984; Fullard et al., 1994; Barber and Conner, 2006). Male, and rarely female, *Cycnia tenera* also emit these sounds during mating (Conner, 1987) but they are not elicited as a phonoresponse to conspecific clicks (Fullard and Fenton, 1977) and social functions should not influence whatever acoustic cues it uses to evoke this defensive behaviour. Fullard reported that *C. tenera* preferentially phonoresponds to stimulus pulse periods that resemble those of a bat's echolocation calls when it is in its attack phase and argued that recognizing the pulse period of the bat's echolocation attack sequence reduces the moth's chances of inappropriately clicking to sounds of pulse periods that are either too high (e.g. searching bats) or too low (e.g. chorusing insects) (Fullard, 1984).

The stimuli used by Fullard (Fullard, 1984) did not closely resemble bat calls in that they were of a constant frequency and duration while real bats manipulate these acoustic characteristics as they change from search to attack phase. Fullard et al. (Fullard et al., 1994) provided a more realistic stimulus to *C. tenera* by using the calls recorded in a laboratory from a free-flying bat (*Eptesicus fuscus*) as it attacked a target and again demonstrated that *C. tenera* times its phonoresponse to attack calls. These results were confirmed in flight-cage experiments with wild-caught, free-flying *Myotis septentrionalis* bats (Ratcliffe and Fullard, 2005). Barber and Conner (Barber and Conner, 2006) also reported the attack-stage phonoresponse of *C. tenera* and demonstrated that other (but not all) tiger moths phonorespond when the bat is less than a second from contact. While these studies confirm that *C. tenera* is most sensitive to the calls of an attacking bat, the question remains, does this insect recognize some specific signature (i.e. frequency, duration, period) of these calls, as suggested by Fullard (Fullard, 1984), or does it simply respond to its perceived increase in the calls' acoustic power as the bat

closes (Skals and Surlykke, 2000)? In the current paper, we use the phonoresponse of *C. tenera* and two auditory psychophysical methods [thresholds and habituation/generalization (H/G)] to test for acoustic feature recognition in this insect and interpret these results in the context of this animal's defence against naturally hunting bats.

Materials and methods

Animals

Cycnia tenera Hübner were reared in the field from eggs collected from wild specimens captured at the Queen's University Biological Station in Chaffeys Lock, Ontario, Canada, raised to pupae on local plants (*Apocynum androsaemifolium* and *A. cannabinum*) and stored in constant-temperature rooms at 4°C with a 12 h:12 h light:dark photoperiod for 5 months. Pupae were transferred to 16 h:8 h light:dark rooms at 25°C, and adults emerged 2–3 weeks later. Adults were allowed to mature for 24–48 h and were then tested during the nocturnal part of their diel cycle.

Acoustic stimulation

Individual moths were fastened by their descaled mesothoracic terga to the head of a dissecting pin with a drop of molten Cenco Softseal Tackiwax (Cenco Scientific, Chicago, IL, USA) and suspended 20 cm above a Technics EAS10TH400B (Panasonic, Matsushita Electric Industrial Co. Ltd, Kadoma City, Japan) speaker in a chamber lined with sound-attenuating foam. Moths were positioned under red light and were then left in complete darkness for 20 min before playbacks began. Continual tones produced by either a Wavetek (model 23) (Willtek Communications, Ismaning, Germany) or Hewlett-Packard (Hewlett-Packard, Palo Alto, CA, USA) signal generator (model 3311A) were shaped with a 0.5 ms rise/fall time to various durations and periods (Coulbourn S84-04; Coulbourn Instruments, Allentown, PA, USA), amplified (National Semiconductor LM1875T; National Semiconductor Corp., Santa Clara, CA, USA) and broadcast from the Technics speaker. Certain of the stimulus trains were stored on a Racal Store 4D tape recorder (Racal Acoustics Ltd, Harrow, UK) running at $\sim 76 \text{ cm s}^{-1}$ (as internally calibrated), while others were recorded as .wav files onto a PC laptop using a 500 kHz sampling rate PCMCIA card (DAQ Card-6062E; National Instruments, Austin, TX, USA) controlled by the programme BatSound Pro v.3.30 (Pettersson Elektronik AB, Uppsala, Sweden). Stimulus trains were either played back using the Racal tape recorder or the playback feature of the BatSound Pro programme and DAQ Card. Playback intensities were recorded as mV peak-to-peak and were later converted to peak equivalent dB sound pressure level (peSPL) (re 20 μPa rms) from equal-amplitude continual tones using a Brüel and Kjær (B&K) (Nærum, Denmark) type 4135 6.35 mm microphone and type 2610 B&K measuring amplifier. The system was regularly calibrated with a B&K type 4228 pistonphone. Stimuli were presented to the moth as trains of pulses of different durations, frequencies and periods depending upon the experiment.

Phonoresponse

The phonoresponse in *C. tenera* (Fig. 1A) was generated by exposing moths to acoustic stimuli as generated by the

mentioned methods. Tymbal sounds are generated as trains of clicks [modulation cycles (MC) (Blest et al., 1963)] that result from the in and out buckling of the striated tymbal surface (Fullard and Fenton, 1977) and were recorded with the B&K microphone and measuring amplifier onto the Racal tape recorder. Phonoresponse recordings were played at $\frac{1}{4}$ real-time tape speed into a data acquisition board (TL-2; Axon Instruments, Molecular Devices Corporation, Sunnyvale, CA, USA) at a 20 kHz sampling rate and stored on a PC, and files were subsequently analysed using the programme AxoScope 8.1 (Axon Instruments). Certain trials were recorded using the DAQ Card and stored and analysed as .wav files using BatSound Pro.

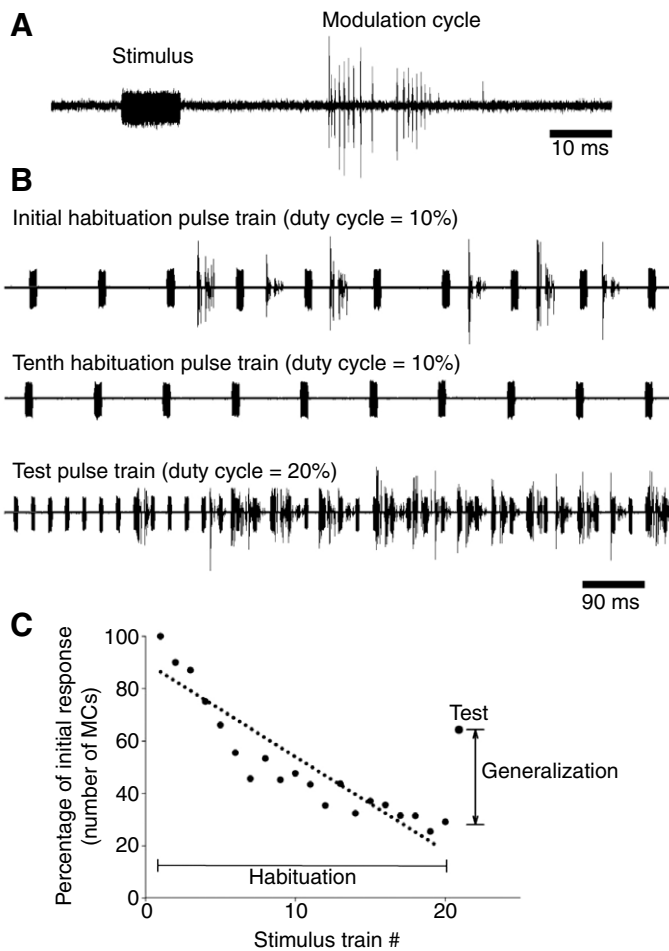


Fig. 1. The tymbal phonoresponse of *Cynia tenera*. (A) Oscillogram of one stimulus pulse in a pulse train and the tymbal sounds as a series of clicks within a single modulation cycle (Blest et al., 1963). (B) The habituation/generalization (H/G) paradigm. (Top) Initial stimulus pulse train and moth phonoresponse. (Middle) Tenth pulse train where the moth has completely habituated. (Bottom) Test pulse train stimulus showing lack of generalization in the moth's phonoresponse. (C) Measurements taken from one H/G trial. Responses were the number of tymbal modulation cycles per stimulus pulse train (normalized to the percentage change from initial response). Habituation was determined if the slope of a linear regression applied to the response decrease was significantly less than 0; lack of generalization (i.e. expressed stimuli discrimination) was determined from pooled habituated animals if the test stimulus response was significantly greater than the average of the last habituated responses.

Threshold trials

For neural examinations, we exposed the auditory nerves (IIN1b) (Nüesch, 1957) of male and female *C. tenera* and recorded auditory receptor cell action potentials with a stainless steel hook electrode referenced to another electrode placed in the moth's abdomen (Fullard et al., 1998). Responses were amplified with a Grass Instruments P-15 pre-amplifier (Quincy, MA, USA). Auditory threshold curves (audiograms) were derived for each moth using trains of 20 ms acoustic pulses (produced as described above) with 500 ms periods at 5 kHz frequency increments randomly chosen from 5 to 100 kHz. A 1 cell threshold was determined as the stimulus intensity that evoked two receptor spikes per stimulus pulse.

For behavioural threshold trials, we positioned individual *C. tenera* above the speaker as described above and exposed them to trains of pulses of various durations, frequencies and periods. The intensity of the pulse trains was raised from zero to the point when the moth just began to phonorespond.

Habituation/generalization trials

We used a habituation/generalization (H/G) paradigm (Thompson and Spencer, 1966) to test whether *C. tenera* discriminated changes to various parameters in the acoustic stimulation applied to them. Fig. 1 describes the stimulation regime applied to each moth; in all cases, response was measured as the number of tymbal modulation cycles that the moth produced during the stimulus trains (counting MCs was facilitated by treating the files to a 40 kHz high-pass filter that eliminates the stimulus pulses while preserving the tymbal clicks). The first part of the trial began with the habituating stimulus (Fig. 1B, top and middle) consisting of a 95 dB peSPL [20 cm, equal to approximately 101 dB source level (10 cm)] train of pulses of a particular frequency, duration and period. This stimulus train was one second in duration and was repeated 20 times. Each trial was separated from the next by one second of silence. This was followed one second later by the test stimulus, consisting of a single train of pulses that differed from the habituating stimulus in a single acoustic parameter (Fig. 1B, bottom). Habituation was determined for each individual moth by applying a linear regression to the raw response data and then testing for a significantly negative departure from a slope of zero (*F*-test) (Fig. 1C). Only moths that habituated were used in subsequent analyses. To control for inter-individual responsiveness, modulation cycle numbers were normalized as the percentage of the response to the first pulse train. We tested for stimulus generalization by comparing test stimulus responses (trial 21) to responses to the last pre-test stimulus train (trial 20) by using paired-sample *t*-tests (Zar, 1999).

Results

Stimulus frequency

Threshold trials

The individual and median neural audiograms for five male and five female *C. tenera* are shown in Fig. 2A and reflect the broad ultrasound sensitivity reported for this moth both neurally (Fullard and Dawson, 1999) and behaviourally (Fullard, 1984). The moth reveals a uniform sensitivity for frequencies between 30 and 50 kHz. This bandwidth was used in habituation/test of

stimulus generalization trials to determine if the moth would discriminate between pulse trains of different frequencies.

H/G trials

Sixteen individual *C. tenera* were habituated using trains of 5 ms, 50 kHz pulses at 95 dB peSPL (20 cm) and independently tested with pulse trains of 42, 44, 46, 48 and 50 kHz with equal duty cycles of 10%. Fig. 2B shows that no significant ($P>0.05$, paired-sample *t*-tests) differences exist between habituated 50 kHz responses and those to any of the test frequencies.

Stimulus duration

H/G trials

We first ran a series of 5 ms/10 ms H/G trials in which we compared habituated and test stimuli responses to pulse trains of equal (10%/10%) and unequal (10%/20%) duty cycles. The results are illustrated in Fig. 3 and indicate that *C. tenera* generalizes (i.e. does not differentiate) between a 5 ms increase or decrease in pulse duration when duty cycles are maintained at 10%. When pulse train duty cycles were doubled, moths generalized to pulse durations that were twice the duration but if this increase in duty cycle was accompanied by a halving of pulse period, moths exhibited a vigorous re-initiation of clicking to shortened pulses.

To test for the effects of pulse train duty cycle, we ran a set of H/G trials in which we exposed moths to habituation pulse trains of 5 ms in duration with a period of 50 ms (i.e. duty cycle of 10%) and then tested for generalization to pulse train pulses

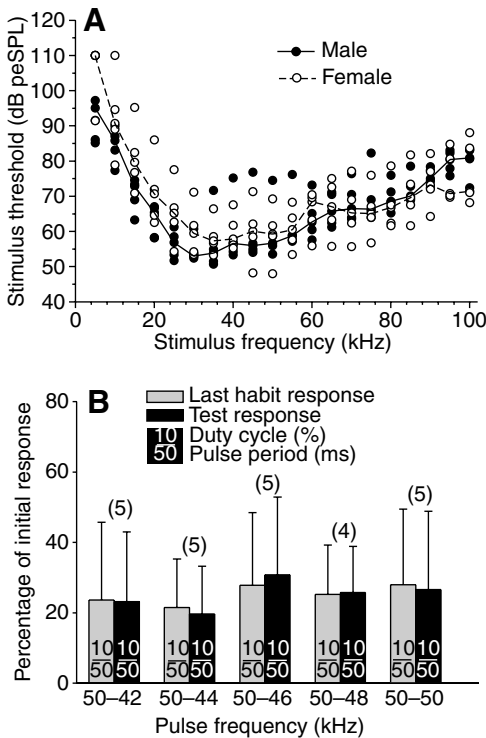


Fig. 2. (A) Audiograms of *C. tenera* ($N=5$ males, 5 females), individuals as circles, gender medians as bold lines. (B) Habituation/generalization (H/G) trials of individual *C. tenera* ($N=20$). Values are means + 1 s.d., sample sizes in parentheses; no significant differences ($P>0.05$, paired-sample *t*-tests) exist in any of the trials.

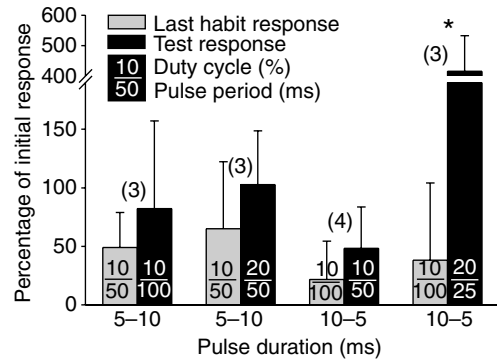


Fig. 3. Habituation/generalization (H/G) trials of *C. tenera* ($N=3-6$) stimulus duration discrimination. 5-10, habituation pulse trains of pulse durations of 5 ms, test pulse train of pulse duration of 10 ms, equal and unequal duty cycles; 10-5, habituation pulse trains of pulse durations of 10 ms, test pulse train of pulse duration of 5 ms, equal and unequal duty cycles. Values are means + 1 s.d., sample sizes in parentheses; asterisks mark significant differences ($P<0.05$).

of 15, 10, 8, 6 and 5 ms of equal periods, which resulted in decreasing duty cycles. Fig. 4 reveals that, for moths to cease generalizing, the test pulse duty cycle needed to be 60% or higher than the habituating stimuli.

Stimulus period

Threshold trials

When stimulus pulses were delivered using variable duty cycles (Fig. 5A, top), moths exhibited a maximum sensitivity to a pulse train period of 20 ms (i.e. 50 pulses s^{-1}) with increased thresholds to shorter and longer pulse periods (i.e. the response was tuned). The duty cycle at 20 ms for a 2 ms pulse duration was 10% so we ran another series of threshold trials using duty cycles below (6.7%) and above (20%) this value to determine if acoustic power would account for the tuning. To hold the duty cycles constant we had to change the pulse durations for each period used. Fig. 5A (middle) illustrates the pulse period tuning curve for a duty cycle of 6.7% and shows that the moths exhibited no particular tuning. However, when the duty cycle

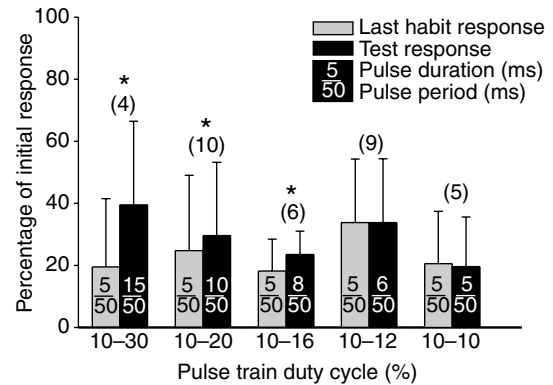


Fig. 4. Habituation/generalization (H/G) trials of *C. tenera* ($N=18$) to test responses after habituation to pulse trains of 5 ms pulses to different test duty cycles (increasing pulse duration, periods equal). Values are means + 1 s.d., sample sizes in parentheses; asterisks mark significant differences ($P<0.05$).

was increased to 20%, the moth's response exhibited less sensitivity displayed a high-pass filter at pulse periods of 20–40 ms (Fig. 5A, bottom).

H/G trials

To separate the effect of pulse period from that of duty cycle in *C. tenera*'s phonoresponse tuning we ran a series of H/G trials using pulse trains that simulated a typical bat's search

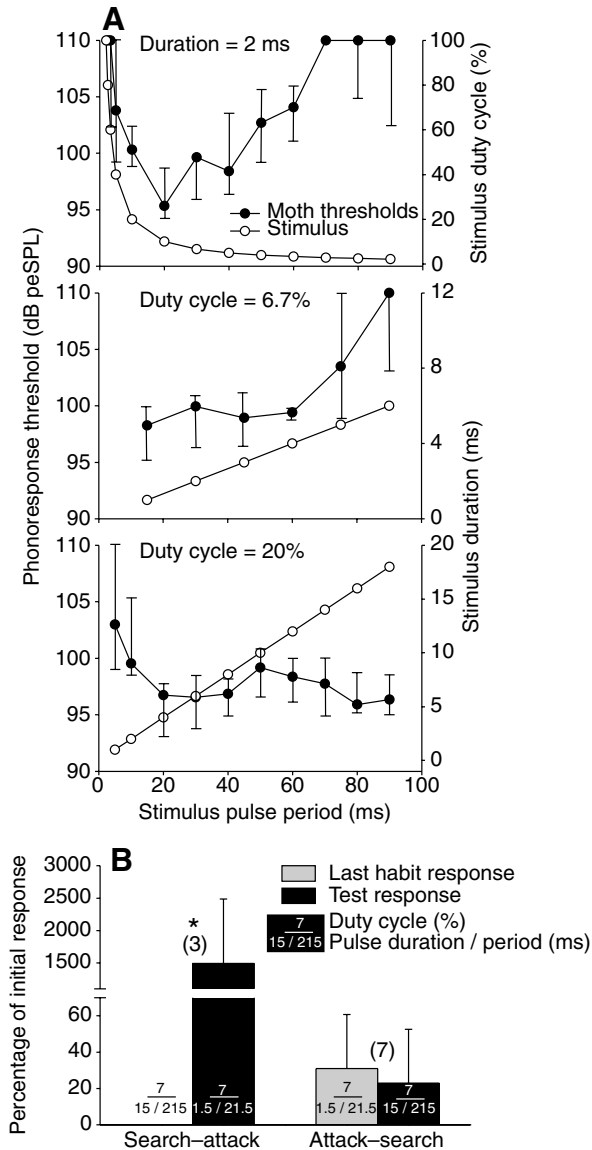


Fig. 5. (A) Threshold phonoresponse curves of *C. tenera* ($N=9$, for all graphs) to stimulus pulses of constant and variable duty cycles as a function of stimulus pulse periods. (Top) Pulse duration of 2 ms and variable duty cycles; (middle) constant duty cycle of 7% and variable pulse durations; (bottom) constant duty cycle of 20% and variable pulse durations. (B) Habituation/generalization (H/G) trials using equal duty cycles (7%) and search versus attack (terminal phase) echolocation pulse durations (15 and 1.5 ms, respectively) and periods (215 and 21.5 ms, respectively) played in normal and reversed sequence [simulations modelled after field recordings of wild *Eptesicus fuscus* hunting in an open area (Surlykke and Moss, 2000)]. Values are means \pm 1 s.d., sample sizes in parentheses.

and terminal echolocation phases. For pulse periods, we used the call parameters as described by Surlykke and Moss' analyses of wild *Eptesicus fuscus* foraging in an open area (Surlykke and Moss, 2000) and picked search- and attack-phase examples that shared the same duty cycle (Fig. 6). This bat represents a common (Fullard et al., 1983; Brooks and Ford, 2005; Kurta and Baker, 1990) sympatric, moth-feeding species that should form a significant part of this moth's natural predation potential. The pulse train sequences that matched these criteria had pulse durations of 15 ms (search) versus 1.5 ms (terminal) and equal duty cycles of 7% (pulse periods of 215 and 21.5 ms, respectively). The results from these trials indicate that when *C. tenera* is first habituated to search-phase calls it vigorously responds to terminal calls but does not respond when exposed to the opposite sequence of terminal to search calls (Fig. 5B).

Discussion

Cycnia tenera times its phonoresponse to the calls of bats after they have switched from search to attack phase so it is not surprising that the sounds most likely to evoke the phonoresponse are those mimicking the bat at this point. The question is which of the spectral, temporal and intensity cues available to the moth at this critical part of the encounter does it use to evoke its defensive behaviour?

Frequency

Surlykke and Moss observed that searching *Eptesicus fuscus* raise the minimum frequencies of their calls by 3–4 kHz as they begin their approach phase (Surlykke and Moss, 2000) and we suggest that this shift would be a reliable spectral cue to insects capable of frequency discrimination. Based on physiological evidence, authors have predicted that moths cannot frequency discriminate (Suga, 1961; Roeder, 1966) and our study behaviourally supports this conclusion (but see Spangler, 1984). While moths may be tone-deaf, the Pacific field cricket, *Teleogryllus oceanicus*, categorically perceives sound frequencies representing diametrically opposed signals (mating versus bat sounds) (Wytenbach et al., 1996) and could use spectral cues to identify an attacking bat. Crickets discriminate frequencies by using a range-fractionated auditory receptor array (Imaizumi and Pollack, 2005) that reserves approximately 25% of its cells for the ultrasonic calls of bats. The frequency non-fractionating, two-celled receptor organ of the noctuid moth ear appears to preclude a similar ability for these insects. Tone-deafness appears to extend to more complex moth ears, as demonstrated in the four auditory-celled pyralid *Galleria mellonella* (Skals and Surlykke, 2000).

Duration

Once a searching bat enters its attack phase, it shortens the duration of its echolocation calls, and if *C. tenera* recognizes such a temporal change it could use this as a trigger to elicit its phonoresponse. Our H/G trials demonstrate that *C. tenera* does not respond differentially to a 5 ms increase or decrease in stimulus pulse duration when duty cycles are held constant (Fig. 3), suggesting that changes to duration alone will not trigger a phonoresponse. However, a real attacking bat differentially decreases its pulse period as well as its duration,

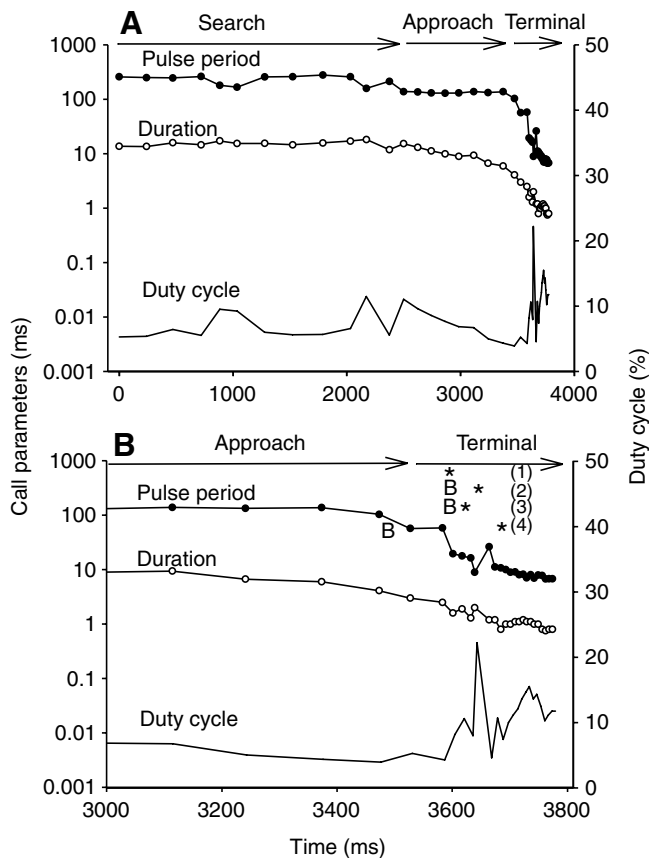


Fig. 6. (A) Call parameters of a wild *Eptesicus fuscus* foraging in an open area as it attacks a flying insect [duration and instantaneous pulse period data computed from original data of Surlykke and Moss (Surlykke and Moss, 2000) with their definitions of echolocation phase]. (B) Expansion of the approach and terminal phases shown in A; points are marked where *C. tenera*'s phonoresponse begins (B) and/or where it is most pronounced (*), as extrapolated from stimulus pulse periods described in the following studies: 1 (Fullard, 1984); 2 (Fullard et al., 1994); 3 (Ratcliffe and Fullard, 2005); 4 (Barber and Conner, 2006). For both graphs, duty cycles were computed from the original data.

resulting in an increase in duty cycle (Kalko, 1995). Using the data of Surlykke and Moss (Surlykke and Moss, 2000) (Fig. 6), the echolocation duty cycle increases from an average of 7% during *E. fuscus*' search phase to an average of 11% during its attack phase (i.e. an increase of 57%). This value is similar to the 60% increase that appears to be the minimum required to re-elicite *C. tenera*'s phonoresponse (Fig. 4), suggesting that increasing duty cycle could serve as a natural cue in telling the moth that the bat has entered its attack phase, as predicted by an acoustic power hypothesis (Skals and Surlykke, 2000). This hypothesis would predict, however, that given the same increase in duty cycle, the moth would show an equal degree of differential responsiveness whether the pulse duration was increased or decreased. This, however, was not the case, with the moth exhibiting a more than sevenfold increase in responsiveness when the pulse duration was decreased by 5 ms compared with when it was increased by the same amount (Fig. 3). To obtain an equal doubling of duty cycles with shorter pulse durations, we were required to change the pulse train pulse

periods. Whereas the pulse period was the same (50 and 50 ms) in the equal duty cycle trials, it had to be reduced to 25% (100 and 25 ms) for the unequal duty cycle trials. The dramatic increase in phonoresponse suggests the primary role that pulse period plays in eliciting *C. tenera*'s behaviour.

Period

If *C. tenera* recognizes an attacking bat's echolocation call period we should expect to see its phonoresponse tuned to a specific period, with reduced sensitivity to lower and higher values. By contrast, if the moth responds to an increase in acoustic power arising from changing duty cycle (Skals and Surlykke, 2005), the phonoresponse threshold will decrease regardless of period. Our threshold trials using increasing duty cycles (Fig. 5A, top) show that *C. tenera* exhibits maximum sensitivity to pulse periods of 20 ms (Fullard, 1984). These pulse periods simulate the echolocation calls of *E. fuscus* after it has detected its intended prey and is approaching for an attack (Kick and Simmons, 1984; Surlykke and Moss, 2000) and are similar to values that initiate the phonoresponse in laboratory experiments (Fullard et al., 1994; Barber and Conner, 2006). Our results also demonstrate that *C. tenera*'s phonoresponse thresholds are not linearly related to the duty cycles of the pulse trains but instead exhibit a tuned response to the preferred period value of 20 ms. When pulse train duty cycles were maintained at 6.7%, a value representing natural search calls (Fig. 5A, middle), intensity thresholds decreased as pulse periods decreased, which would be predicted by an acoustic recognition hypothesis. As shown by its response to pulse train duty cycles of 20%, those in excess of a natural terminal phase (Fig. 5A, bottom), *C. tenera* discriminates against pulse periods below 20 ms, suggesting an adaptive function for its period tuning. Fullard observed that auditory receptor response in *C. tenera* decreases with pulse period until continual firing occurs to periods of less than approximately 10 ms (Fullard, 1984), implying that the moth treats these sounds as continuous and non-threatening (Fullard et al., 2003). Similar neural responses have been reported by Waters (Waters, 1996) and Coro et al. (Coro et al., 1998) in sound-producing arctiids and silent noctuids. Roeder reported that continuous sounds were less effective than pulsed sounds in evoking evasive manoeuvres in flying noctuid moths (Roeder, 1964). That pulsing is an essential element in evoking the defensive behaviour of flying noctuid moths is not surprising since these are the types of sounds that hunting bats emit, but these results suggest that continual tones are actively ignored, perhaps since they represent non-dangerous stimuli (e.g. chorusing insects).

Skals and Surlykke concluded that echolocation pulse period is not used by *G. mellonella* to evoke its defence against near bats and suggested instead that the increased acoustic power from an attacking bat's echolocation calls triggers the moth's defences (Skals and Surlykke, 2000). The duty cycle of the pulse trains used in their experiments (35%) is approximately seven times higher than values reported for typical vespertilionid bats (Kalko, 1995; Surlykke and Moss, 2000; Ratcliffe and Fullard, 2005), and we suggest that the high stimulus power delivered by Skals and Surlykke (Skals and Surlykke, 2000) to the *G. mellonella* masked the effect of pulse period in triggering this particular moth's defensive behaviour.

Furthermore, Skals and Surlykke's acoustic power hypothesis (Skals and Surlykke, 2000) depends upon a reliable increase in the received intensity of sound pulses, something not likely experienced in nature by erratically flying moths whose wings would already be attenuating the intensities of the bat calls by frequently obscuring their ears (Payne et al., 1966).

Our H/G trials using simulated natural search and attack echolocation pulse trains (Fig. 5B) of equal duty cycles indicate that the sequence of calls as encoded by pulse period is important for evoking *C. tenera*'s natural phonoresponse. The first set of pulse trains (search – attack) simulates the situation where an insect would naturally hear the approach of a bat that has detected its target and is attacking, while the second set (attack – search) represents a bat that has missed its intended target and has reverted to searching. It is expected that *C. tenera* would not generalize the first pair of pulse train stimuli since waiting until the bat is within a metre or so represents the optimal time for the moth's clicks to have their deterrent effect (Ratcliffe and Fullard, 2005). On the other hand, phonoresponding to a bat that is departing would not serve the moth any useful purpose since the bat had already passed and might, in fact, draw that bat's attention or that of eavesdropping bats (Balcombe and Fenton, 1987). Since duty cycles and intensities were held constant in these trials, the only cue available to *C. tenera* for its response would be the pulse period of the calls.

Intensity

While *C. tenera* exhibits preferences for particular pulse trains, it readily phonoresponds to unnatural stimuli if the intensity is great enough [e.g. jingling keys (Fullard and Fenton, 1977)]. This suggests that, if an acoustic recognition mechanism exists in *C. tenera*, it can be overridden by stimulus intensities and cautions an appreciation of the natural relevance of the sounds used as stimuli in these experiments. To evoke phonoresponses in *C. tenera*, Fullard et al. (Fullard et al., 1994) used pre-recorded echolocation calls with source level intensities (i.e. dB @ 10 cm) that were matched to those produced by the bat, which resulted in an increase in the intensity at the moth's ear as the bat began its terminal phase. An increase in attack call intensity (as received by the target) was also reported for the bat *Myotis daubentonii* (Boonman and Jones, 2002) from approximately 80 to 95 dB. By contrast, Holderied et al. (Holderied et al., 2005) report approach/terminal echolocation call source intensities in *Eptesicus bottae* of 105–115 dB, equating to much lower target received intensities of 75–85 dB [assuming a distance from the bat of 3 m when it begins its approach (Kick and Simmons, 1984) and not accounting for excess atmospheric attenuation]. The range of reported approach/terminal call intensities serves as a further warning that unnaturally high stimulus intensities may result in artifactual responses. In our present study, habituating and test pulse trains were delivered at a constant received sound level intensity of 95 dB, resulting in unnaturally intense search sequences, which may explain *C. tenera*'s phonoresponse to these otherwise sub-threshold pulse train periods (Fig. 5A, top). The observed maximum phonoresponse sensitivity to terminal pulse periods of 20 ms at 95 dB suggests that this represents the closest simulation of the combination of temporal and intensity variables that naturally evokes *C. tenera*'s defensive behaviour.

As the bat closes on its target, the combination of changing pulse intensities and duty cycles, counteracted by decreasing pulse durations [and their concurrent effects due to the temporal integration of the moth's ear (Tougaard, 1998)], results in a complex transformation of the acoustic energy received by *C. tenera*. The neural simplicity of the moth ear provides the unique opportunity to empirically examine how these acoustic changes are encoded. Fullard et al. (Fullard et al., 2003) examined the responses of *C. tenera*'s two auditory receptor neurons to the same echolocation sequence they had used previously (Fullard et al., 1994) and reported that, at intensities evoking phonoresponse, both of *C. tenera*'s auditory receptor neurons *reduce* their firing as the bat enters its terminal phase. This indicates that the total acoustic power received by *C. tenera*'s ear *decreases* as the bat nears its target, further reducing its natural role as a cue activating its phonoresponse.

The precise point at which *C. tenera* phonoresponds to an attacking bat may be a result of the species of bat it is facing. While *C. tenera* begins its phonoresponse to the terminal calls of a lab-reared *Eptesicus fuscus* (Fullard et al., 1994), experiments with free-flying wild *Myotis septentrionalis* indicate that *C. tenera* phonoresponds earlier, during this bat's approach-phase calls (Ratcliffe and Fullard, 2005). If wild-flying bats emit louder echolocation calls than lab-confined animals (Surlykke and Moss, 2000), these behavioural variations may be related to the different intensities of the two bats. Fullard et al. showed that increasing the intensity of the attack sequence of *E. fuscus* results in *C. tenera* beginning its phonoresponse earlier in the bat's attack sequence (Fullard et al., 1994). Praying mantids also exhibit an advance in their anti-bat flight responses when confronted by higher intensities (Triblehorn and Yager, 2005).

Conclusion

Our original belief was that the situation-specific behavioural response of *C. tenera* to attacking bats would support either an acoustic power hypothesis (Skals and Surlykke, 2000) or an acoustic recognition hypothesis (Fullard, 1984), but our results suggest that both mechanisms play a role in the moth's natural behaviour. The ability of *C. tenera* to discriminate searching from attacking bats may exist by means of a pulse period identification mechanism (e.g. a central nervous system template) but this template is influenced and can be overridden by the acoustic power of the stimuli reaching it. Recent work on pulse period recognition in singing insects (reviewed by Hedwig, 2006) suggests that such a template could exist as pattern-specific (oscillatory) neurons (Bush and Schul, 2006) matched to the same period as the calls of an attacking bat. This template (or others running with different periods) could also account for the flight patterns exhibited by other noctuid moths when exposed to similar rates (Roeder, 1964). Decreasing pulse periods also trigger flight reaction in lacewings (Miller and Olesen, 1979) and praying mantids (Triblehorn and Yager, 2005) and we suggest that pulse period recognition is an auditory feature shared by many flying, nocturnal insects that have to avoid hungry bats.

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References

- Alain, C. and Arnott, S. R. (2000). Selectively attending to auditory objects. *Front. Biosci.* **5**, 202-212.
- Balcombe, J. and Fenton, M. B. (1987). Eavesdropping by bats: the influence of echolocation call design and foraging strategy. *Ethology* **79**, 158-166.
- Barber, J. R. and Conner, W. E. (2006). Tiger moth responses to a simulated bat attack: timing and duty cycle. *J. Exp. Biol.* **209**, 2637-2650.
- Blest, A. D., Collett, T. S. and Pye, J. D. (1963). The generation of ultrasonic sounds by a New World arctiid moth. *Proc. R. Soc. Lond. B Biol. Sci.* **158**, 196-207.
- Boonman, A. and Jones, G. (2002). Intensity control during target approach in echolocating bats: stereotypical sensorimotor behaviour in Daubenton's bats, *Myotis daubentonii*. *J. Exp. Biol.* **205**, 2865-2874.
- Brooks, R. T. and Ford, W. M. (2005). Bat activity in a forest landscape of central Massachusetts. *Northeast. Natur.* **12**, 447-462.
- Bush, S. L. and Schul, J. (2006). Pulse-rate recognition in an insect: evidence of a role for oscillatory neurons. *J. Comp. Physiol. A* **192**, 113-121.
- Conner, W. E. (1987). Ultrasound: its role in the courtship of the arctiid moth, *Cynia tenera*. *Experientia* **43**, 1029-1031.
- Conner, W. E. (1999). 'Un chant d'apell amoureux': acoustic communication in moths. *J. Exp. Biol.* **202**, 1711-1723.
- Coro, F., Pérez, M., Mora, E., Boada, D., Conner, W. E., Sanderford, M. V. and Avila, H. (1998). Receptor cell habituation in the A1 auditory receptor of four noctuid moths. *J. Exp. Biol.* **201**, 2879-2890.
- Dunning, D. C. and Roeder, K. D. (1965). Moth sounds and the insect-catching behavior of bats. *Science* **147**, 173-174.
- Eggers, F. (1919). Das thoracale bitympanale Organ einer Gruppe der Lepidoptera Heterocera. *Zool. Jahrb. Anat.* **41**, 273-376.
- Fullard, J. H. (1979). Behavioural analyses of auditory sensitivity in *Cynia tenera* Hübner (Lepidoptera: Arctiidae). *J. Comp. Physiol.* **129**, 79-83.
- Fullard, J. H. (1984). Listening for bats: pulse repetition rate as a cue for defensive behavior in *Cynia tenera* (Lepidoptera: Arctiidae). *J. Comp. Physiol. A* **154**, 249-252.
- Fullard, J. H. and Dawson, J. W. (1999). Why do diurnal moths have ears? *Naturwissenschaften* **86**, 276-279.
- Fullard, J. H. and Fenton, M. B. (1977). Acoustic and behavioural analyses of the sounds produced by some species of Nearctic Arctiidae (Lepidoptera). *Can. J. Zool.* **55**, 1213-1224.
- Fullard, J. H. and Napoleone, N. (2001). Diel flight periodicity and the evolution of auditory defences in the Macrolepidoptera. *Anim. Behav.* **62**, 349-368.
- Fullard, J. H., Fenton, M. B. and Simmons, J. A. (1979). Jamming bat echolocation: the clicks of arctiid moths. *Can. J. Zool.* **57**, 647-649.
- Fullard, J. H., Fenton, M. B. and Furlonger, C. L. (1983). Sensory relationships of moths and bats sampled from two Nearctic sites. *Can. J. Zool.* **61**, 1752-1757.
- Fullard, J. H., Simmons, J. A. and Saillant, P. A. (1994). Jamming bat echolocation: the dogbane tiger moth *Cynia tenera* times its clicks to the terminal attack calls of the big brown bat *Eptesicus fuscus*. *J. Exp. Biol.* **194**, 285-298.
- Fullard, J. H., Forrest, E. and Surlykke, A. (1998). Intensity responses of the single auditory receptor of notodontid moths as a test of the peripheral interaction hypothesis in multi-celled moth ears. *J. Exp. Biol.* **201**, 3419-3424.
- Fullard, J. H., Dawson, J. W. and Jacobs, D. S. (2003). Auditory encoding during the last moment of a moth's life. *J. Exp. Biol.* **206**, 281-294.
- Griffin, D., Webster, F. and Micheal, C. (1960). The echolocation of flying insects by bats. *Anim. Behav.* **8**, 141-154.
- Hedwig, B. (2006). Pulses, patterns and paths: neurobiology of acoustic behaviour in crickets. *J. Comp. Physiol. A* **192**, 677-689.
- Holderied, M. W., Korine, C., Fenton, M. B., Parsons, S., Robson, S. and Jones, G. (2005). Echolocation call intensity in the aerial hawking bat *Eptesicus bottae* (Vespertilionidae) studied using stereo videogrammetry. *J. Exp. Biol.* **208**, 1321-1327.
- Hoy, R. R. (1989). Startle, categorical response and attention in acoustic behavior in insects. *Annu. Rev. Neurosci.* **12**, 355-375.
- Hristov, N. I. and Conner, W. E. (2005). Sound strategy: acoustic aposematism in the bat-moth arms race. *Naturwissenschaften* **92**, 164-169.
- Imaizumi, K. and Pollack, G. S. (2005). Central projections of auditory receptor neurons of crickets. *J. Comp. Neurol.* **493**, 439-447.
- Kalko, K. V. (1995). Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera). *Anim. Behav.* **50**, 861-880.
- Kurta, A. and Baker, R. H. (1990). *Eptesicus fuscus*. *Mamm. Spec.* **356**, 1-10.
- Kick, S. A. and Simmons, J. A. (1984). Automatic gain-control in the bat's sonar receiver and the neuroethology of echolocation. *J. Neurosci.* **4**, 2725-2737.
- Miller, L. A. (1991). Arctiid moth clicks can degrade the accuracy of range difference discrimination in echolocating big brown bats. *J. Comp. Physiol. A* **168**, 571-579.
- Miller, L. A. and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.* **131**, 113-120.
- Nüesch, H. (1957). Die Morphologie des Thorax von *Telea polyphemus* Cr (Lepid). I. Nervensystem. *Zool. Jahrb.* **75**, 615-642.
- Payne, R. S., Roeder, K. D. and Wallman, J. (1966). Directional sensitivity of the ears of noctuid moths. *J. Exp. Biol.* **44**, 17-31.
- Ratcliffe, J. M. and Dawson, J. W. (2003). Behavioural flexibility: the little brown bat, *Myotis lucifugus*, and the northern long-eared bat, *M. septentrionalis*, both glean and hawk prey. *Anim. Behav.* **66**, 847-856.
- Ratcliffe, J. M. and Fullard, J. H. (2005). The adaptive function of tiger moth clicks against echolocating bats: an experimental and synthetic approach. *J. Exp. Biol.* **208**, 4689-4698.
- Roeder, K. D. (1964). Aspects of the noctuid tympanic nerve response having significance in the avoidance of bats. *J. Insect Physiol.* **10**, 529-546.
- Roeder, K. D. (1966). Auditory system of noctuid moths. *Science* **154**, 1515-1521.
- Roeder, K. D. (1974). Acoustic sensory responses and possible bat-evasion tactics of certain moths. In *Proceedings of the Canadian Society of Zoologists Annual Meeting* (ed. M. D. B. Burt), pp. 71-78. Fredericton, NB: University of New Brunswick Press.
- Schul, J. and Sheridan, R. A. (2006). Auditory stream segregation in an insect. *Neuroscience* **138**, 1-4.
- Skals, N. and Surlykke, A. (2000). Hearing and evasive behaviour in the greater wax moth, *Galleria mellonella* (Pyralidae). *Physiol. Entomol.* **25**, 354-362.
- Soutar, A. and Fullard, J. H. (2004). Nocturnal anti-predator adaptations in eared and earless Nearctic Lepidoptera. *Behav. Ecol.* **15**, 1016-1022.
- Spangler, H. G. (1984). Responses of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) to continuous high-frequency sound. *J. Kansas Entomol. Soc.* **57**, 44-49.
- Suga, N. (1961). Functional organization of two tympanic neurons in noctuid moths. *Jpn. J. Physiol.* **11**, 666-677.
- Surlykke, A. and Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *J. Acoust. Soc. Am.* **108**, 2419-2429.
- Thompson, R. F. and Spencer, W. A. (1966). Habituation: a model phenomenon for the neuronal studies of behavior. *Psychol. Rev.* **173**, 16-43.
- Tougaard, J. (1998). Detection of short pure-tone stimuli in the noctuid ear: what are temporal integration and integration time all about? *J. Comp. Physiol. A* **183**, 563-572.
- Tribblehorn, J. D. and Yager, D. D. (2005). Timing of praying mantis evasive responses during simulated bat attack sequences. *J. Exp. Biol.* **208**, 1867-1876.
- Waters, D. A. (1996). The peripheral auditory characteristics of noctuid moths: information encoding and endogenous noise. *J. Exp. Biol.* **199**, 857-868.
- Wilson, W. W. and Moss, C. F. (2004). Sensory-motor behavior of free-flying FM bats during target capture. In *Advances in the Study of Echolocation in Bats and Dolphins* (ed. J. Thomas, C. F. Moss and M. Vater) pp. 22-27. Chicago: University of Chicago Press.
- Wytenbach, R. A. and Farris, H. E. (2004). Psychophysics in insect hearing. *Microsc. Res. Tech.* **63**, 375-387.
- Wytenbach, R. A., May, M. L. and Hoy, R. R. (1996). Categorical perception of sound frequency by crickets. *Science* **273**, 1542-1544.
- Yack, J. E. (2004). The structure and function of auditory chordotonal organs in insects. *Microsc. Res. Tech.* **63**, 315-337.
- Zar, J. H. (1999). *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice-Hall.