

Ignoring the irrelevant: auditory tolerance of audible but innocuous sounds in the bat-detecting ears of moths

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Abstract Noctuid moths listen for the echolocation calls of hunting bats and respond to these predator cues with evasive flight. The African bollworm moth, *Helicoverpa armigera*, feeds at flowers near intensely singing cicadas, *Platypleura capensis*, yet does not avoid them. We determined that the moth can hear the cicada by observing that both of its auditory receptors (A1 and A2 cells) respond to the cicada's song. The firing response of the A1 cell rapidly adapts to the song and develops spike periods in less than a second that are in excess of those reported to elicit avoidance flight to bats in earlier studies. The possibility also exists that for at least part of the day, sensory input in the form of olfaction or vision overrides the moth's auditory responses. While auditory tolerance appears to allow *H. armigera* to exploit a food resource in close proximity to acoustic interference, it may render their hearing defence ineffective and make them vulnerable to predation by bats during the evening when cicadas continue

to sing. Our study describes the first field observation of an eared insect ignoring audible but innocuous sounds.

Keywords Moths · Auditory ecology · Bats · Cicadas · Discrimination

Introduction

Eared moths avoid insectivorous bats by listening for their echolocation calls and either flying away if the sounds are faint (i.e. a distant bat) or diving to the ground if they are intense (i.e. a near bat; Roeder 1962). While reacting to bats provides a significant survival benefit (Roeder and Treat 1962), unnecessary responses to non-bat sounds may present costs. Moths avoiding sounds not emitted by bats will waste flight otherwise spent searching for mates, food or egg-laying sites, while diving from the sky risks exposure to other predators (Guignion and Fullard 2004). It is therefore reasonable to presume that moths discriminate between bat and non-bat sounds. Although echolocation call design is remarkably diverse (Fenton 1986; Jones and Teeling 2006), most bats emit short (less than 10 ms) pulses of sound as they hunt (Schnitzler and Kalko 2001). Roeder (1964) observed that pulsed sounds were more effective than continuous (i.e. non-bat) sounds in eliciting evasive behaviours in free-flying noctuid moths. To provide a neural mechanism for this discrimination, he examined the spiking responses of the moths' two auditory receptors (A1 and A2 cells) and concluded that bursts (groups of spikes) with short A1 spike periods (the time between spikes) of 1.5–2.6 ms separated by inter-burst intervals of less than 100 to 200 ms cause moths to react to pulsed sounds. In contrast, continual tones produce adapted auditory receptor responses in noctuid moths with long

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spike periods and, by definition, no inter-burst intervals (Coro and Perez 1984). Discrimination in moths against non-bat sounds has been suggested (Paul 1974; Fullard 1984; Boyan and Fullard 1988), but to our knowledge, there has never been a documented example of a natural situation of a moth ignoring such sounds.

In South Africa, we observed African bollworm moths, *Helicoverpa armigera*, feeding on flowers near singing male cicadas, *Platypleura capensis*. The cicada's song was intense and continuous but had no apparent effect on the moths as they hovered as close as 10 cm from the calling cicadas. We hypothesized that this auditory tolerance is caused by one or more of the following: (1) The moths cannot hear the cicada, (2) the moths hear the cicada, but their auditory response adapts because of the song's continuous nature and (3) some another sensory modality (e.g. olfaction or vision) overrides the auditory response of the moths.

Materials and methods

Study site and organisms

This study was conducted in November 2006 at the De Hoop Nature Reserve (34°26'S, 20°25'E), 260 km east of Cape Town, South Africa. We observed swarms (>100 moths) of *H. armigera* as they fed from flowering *Agathosma riverdalensis* that surrounded the bush, *Carissa bispinosa*, that was simultaneously used by the cicada, *P. capensis* as a calling perch. Moths fed as close as 10 cm from calling cicadas, often flying directly towards flowers that were near cicadas and continued to feed on the flowers into the night (Jacobs et al., in preparation). Moths were collected during the day as they fed on flowers and at night from ultraviolet lights erected at a site 5 km away.

Auditory analyses

The action potentials of the auditory receptors in moth tympanic nerves (IIN1b: Nüesch 1957) were recorded with a stainless steel hook electrode referenced to another in the moth's abdomen. Neural responses were amplified (Grass Instruments P-15 Pre-amplifier, Astro-Med, West Warwick, USA) and observed either online or stored in a laptop PC using digital acquisition boards (ADC 212/3 [sampling rate=3 MHz]; Pico Technology, Cambridgeshire, UK, or UltraSoundGate 416-200 [sampling rate=250 kHz], Avisoft, Berlin, Germany) and oscilloscope-emulating software (PicoScope 5.10.7 or Recorder 2.9, respectively). Spike records were later analysed with a customized MATLAB (Version R2006b, The MathWorks, Natick, USA) application. In keeping with previous studies (Roeder 1964; Fullard

et al. 2003; Nabatiyan et al. 2003; Marsat and Pollack 2006), we report instantaneous rather than averaged spike periods as a direct measure of the auditory receptors' activity and their likelihood to activate post-synaptic neural components (Hedwig 2006).

Acoustic stimulation

Auditory preparations were exposed to pulsed synthetic sounds generated by a customized MATLAB application running on a separate PC laptop, amplified (Avisoft) and broadcast from a speaker (Technics EAS-10TH400B, Panasonic, Mississauga, Canada) mounted 30 cm from the moths. Intensities were recorded as voltages delivered to the speaker and then converted to peak equivalent sound pressure levels (dB peSPL; rms re 20 μ Pa; Stapells et al. 1982) from equal-amplitude continual tones as measured with a Brüel and Kjær (B&K; Nærum, Denmark) 4135 6.35 mm microphone and 2610 measuring amplifier. The entire system was calibrated before and after the study with a B&K 4228 pistonphone. Auditory threshold curves (audiograms) were derived using 20-ms sound pulses, 0.1-ms rise/fall times from 5 to 100 kHz delivered 2 s^{-1} at randomly chosen 5-kHz increments with A1 cell threshold determined as the stimulus intensity that evoked two receptor spikes per stimulus pulse.

This setup was also used to playback a 20-s portion of a cicada calling song that was recorded syntemporally in the vicinity of the moths with an ultrasound microphone (UltraSoundGate CM16, Avisoft) and acquisition system (UltraSoundGate 416-200; Recorder 2.9, Avisoft). The frequency response of another CM16 microphone was subsequently calibrated using the B&K microphone and determined to be flat ± 2 dB from 5 to 100 kHz ensuring a faithful representation of the cicada's song. The natural intensity of the song was estimated by positioning an S200 Bat Detector (Ultra Sound Advice, London, UK) 30 cm from a single singing male and measuring the resultant voltage displacement as read by the PicoScope. The voltage was duplicated with the same microphone and power supply using a continual 8 kHz tone generated as described above. A test intensity of 85 dB at 30 cm was used in playbacks to the moth ears.

Results

The calling song of *Platypleura capensis*

The calling song of *P. capensis* has been previously described (Villet 1988), and we provide a brief summary here. The song (Fig. 1a, b) is a continuous trill of a constant amplitude motif alternating with an amplitude modulated

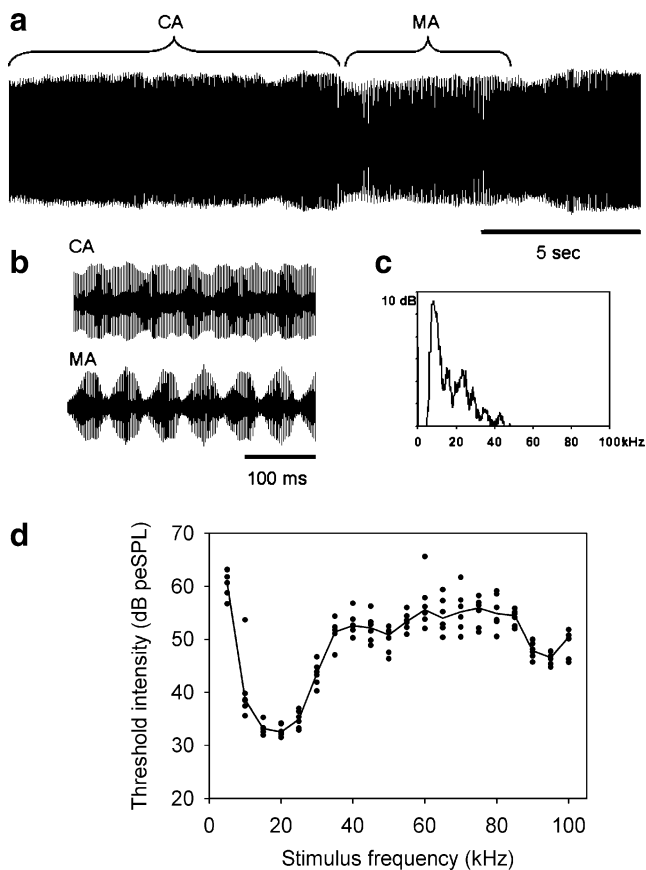


Fig. 1 **a** Oscillogram of a portion of single male cicada's calling song recorded in the field, *CA* constant amplitude motif, *MA* modulated amplitude motif. **b** Time expanded view of the two motifs. **c** Frequency spectrum (1,024 samples, Hanning-window) of 1 s of the *CA* motif (microphone sensitivity roll-off below 5 kHz has not been compensated). **d** Auditory sensitivity curve of six individual moths (median indicated by line)

motif that produces a composite frequency spectrum peaked at 8–8.5 kHz with reduced energy at the second and third harmonics of approximately 16 and 24 kHz (Fig. 1c). The intensity of the song as measured at 30 cm from a single male was 89 dB peSPL, which extrapolates to a source level intensity (i.e. at 10 cm) of 98 dB, which would be the highest intensity encountered by feeding moths that we observed. Cicadas sang throughout the day ceasing only during the highest temperatures in the mid-afternoon after which they resumed singing until after dusk. A casual survey of singing males determined that singing usually ended at 21:00, earlier for single males than for those singing within groups. The latest singing male was heard at 21:45, approximately 90 min after the Cape horseshoe bat, *Rhinolophus capensis*, a sympatric, insectivorous species whose echolocation call is audible to *H. armigera* (Jacobs et al., unpublished data) left its day roost in a cave approximately 150 m from the site where moths were observed feeding.

Auditory analyses of *Helicoverpa armigera*

Figure 1d describes the auditory sensitivity of six *H. armigera* collected in the same vicinity as the calling cicadas. The moth has an auditory best frequency at 15–30 kHz with high sensitivity at secondary frequencies above 40 kHz, an adaptation seen in other African moths exposed to diverse echolocation assemblages (Fullard 1982). At the peak frequency emitted by the cicada's calling song, the median frequency threshold of this moth would be approximately 45 dB, which would make it sufficiently sensitive to hear the cicada at the distances it was commonly seen foraging (i.e. <50 cm).

Figure 2a shows a typical *H. armigera* auditory response to a cicada's song. This record reveals that at a playback intensity of 85 dB, which approximates a distance of 48 cm from the cicada, both A1 and A2 auditory receptors

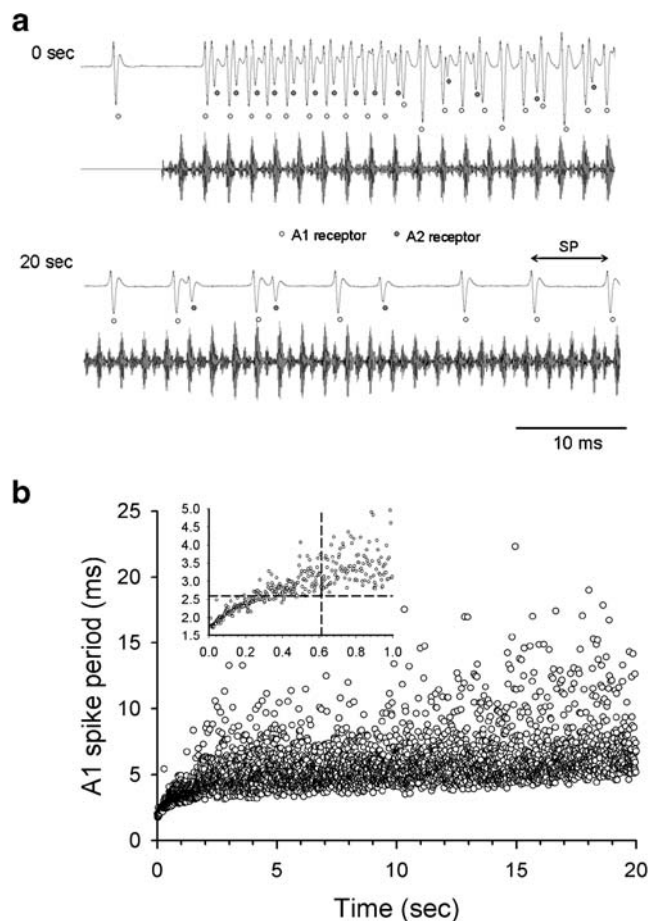


Fig. 2 **a** Representative traces of the response of *H. armigera*'s auditory receptors to the calling song of *P. capensis*. *Top trace*, the first 100 ms of the song, *bottom trace*, 20 s later (*SP* spike period). **b** spike periods of the A1 receptor in a representative preparation (actual $n=8$) as a function of the time exposed to a calling song. *Inset*, time-expanded view of whole song response; *horizontal dashed line* indicates spike period of 2.6 ms (refer to text for explanation); *vertical dashed line* corresponds to the time required to reach this period

vigorously respond throughout the entire playback. Of the eight moths exposed to the cicada song, all responded with A1 firing that persisted until the end of the playback, and seven of the eight exhibited continuous A2 firing. Figure 2b illustrates that the A1 response rapidly adapts with increasing spike periods throughout the duration of the playback. We used a spike period of 2.6 ms as a conservative estimate of Roeder's (1964) range of A1 spike periods as the cue for activating diving responses and determined by eye the time from stimulus onset at which A1 spike periods rose above 2.6 ms. We determined that the average (± 1 standard deviation) duration from the start of the playback until the point that A1 spike periods exceeded 2.6 ms was 0.75 ± 0.41 s ($n=8$).

Discussion

Our study describes the first field observation of an eared insect ignoring audible but innocuous sounds. *H. armigera* responds to the calling song of *P. capensis* with its complete two-celled auditory repertoire, and we therefore reject the first of the three explanations for this auditory tolerance that *H. armigera* cannot hear the cicada's song. The second possibility that the continuous nature of the cicada's song results in an adapted receptor response is borne out by our results. In less than 1 s of exposure to the cicada's song, A1 spike periods increase above those observed by Roeder (1964) to coincide with evasive flight responses in noctuid moths, and the adapted spike periods never return to these levels. In addition, the cicada's continuous song never produces A1 inter-burst intervals that Roeder (1964) suggested were necessary for evoking flight responses. We suggest that *H. armigera*'s A1 cell adaptation constitutes a sensory level temporal filter that allows this moth to ignore continuous, audible sounds. Evidence from thoracic inter-neuron recordings (Roeder 1966; Paul 1974; Boyan and Fullard 1988) further suggests that temporal filters exist within the central nervous system. Phasically firing inter-neurons ("pulse markers"; Roeder 1966) respond to pulsed sounds, and the firing of such cells may be necessary to activate anti-bat evasive flight responses perhaps via oscillatory neural templates (Bush and Schul 2006) that mimic the pulse period patterns of echolocating bats. We did not measure the response characteristics of the less sensitive auditory receptor, the A2 cell, but this cell appears to adapt even more quickly than that of the A1 cell (as previously reported for noctuids from Cuba; Pérez and Coro 1985), and there is debate over its actual involvement in the avoidance behaviour of noctuid moths (Surlykke 1984; Fullard et al. 2003). It is

interesting to note that the time at which A1 intervals completely exceed 2.6 ms (0.75 s) in *H. armigera* is qualitatively similar to values given in Roeder (1964; 0.5–0.7 s) for a North American noctuid, *Amanthes bicarnea*, to continuous constant frequency tones, so tolerance of continual sounds may be a common auditory adaptation in nocturnal insects dealing with acoustic interference.

Our results do not exclude the third possibility that the daytime foraging activity of these moths provides non-auditory sensory stimuli (e.g. olfaction, vision) that override the acoustic input of the cicada. Under certain conditions, flying moths (*Agrotis segetum* and *Plodia interpunctella*) ignore bat-like sounds when tracking pheromones (Svensson et al. 2004), but other species (*Autographa gamma*) do not when tracking plant odours (Skals et al. 2003). This suggests that olfactory override may be context specific (Rodríguez and Greenfield 2004) and is determined by the selective benefits of the behaviour the moth is expressing (e.g. mating vs feeding). For vision, flying during the day suggests that the simplest explanation for why bollworm moths ignore cicada songs is that bats are not diurnal. The moth, *Catachysta lemnata*, shows different responses to ultrasound when tested in the day vs the night suggesting that vision plays a role in determining behavioural responses to ultrasound (Svensson et al. 2003). Visual override, however, is unlikely after the sun has set when the moths continue to feed alongside singing cicadas as bats begin to hunt (Jacobs et al., in preparation). It is also unlikely that the feeding motivation in moths that have spent the day foraging would be high enough at night to suppress auditory responses invoked by bat predation. These predictions could be tested by observing ultrasound responses in blinded and/or de-antennated moths.

Lastly, the evening overlap between feeding moths, singing cicadas and foraging bats presents the possibility that auditory tolerance may be a fatal liability to *H. armigera*. Bats that hunt in the presence of calling cicadas may be better able to attack sensory-adapted moths feeding at flowers, particularly gleaners whose calls are typically of lower intensity than the calling songs of cicadas (Schnitzler and Kalko 2001). Cape horseshoe bats (*R. capensis*) are sympatric, forage close to vegetation and have diets containing a large percentage of moths (Jacobs et al. 2007). Whether these diets include *H. armigera* and what effect bat predation has on this moth, a major African agricultural pest (Abate and Ampofo 1996), remains to be seen.

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