Hearing and evasive behaviour in the greater wax moth, *Galleria mellonella* (Pyralidae)

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**Abstract.** Greater wax moths (*Galleria mellonella* L., Pyraloidea) use ultrasound sensitive ears to detect clicking conspecifics and echolocating bats. Pyralid ears have four sensory cells, A1–4. The audiogram of *G. mellonella* has best frequency at 60 kHz with a threshold around 47 dB sound pressure level. A1 and A2 have almost equal thresholds in contrast to noctuids and geometrids. A3 responds at +12 to +16 dB relative to the A1 threshold. The threshold data from the A-cells give no indication of frequency discrimination in greater wax moths.

Tethered greater wax moths respond to ultrasound with short-latency cessation of flight at +20 to +25 dB relative to the A1 threshold. The behavioural threshold curve parallels the audiogram, thus further corroborating the lack of frequency discrimination. Hence, the distinction between bats and conspecifics is probably based on temporal cues.

At a constant duty cycle (percentage of time where sound is on) the pulse repetition rate has no effect on the threshold for flight cessation, but stimulus duration affects both sensory and behavioural thresholds.

The maximum integration time is essentially the same: 45 ms for the A1-cell and 50–60 ms for the flight cessation response. However, the slopes of the time-intensity trade-off functions are very different: −2.1 dB per doubling of sound duration for the A1-cell threshold, and −7.2 dB per doubling of sound duration for the behavioural threshold.

The significance of the results for sexual acoustic communication as well as for bat defence is discussed.

**Key words.** Bat defence, evasive behaviour, moth hearing, neuroethology, Pyralidae, temporal integration, ultrasound hearing.

**Introduction**

Ears sensitive to ultrasound have evolved independently several times among nocturnal insects, presumably as a defence against bat predation (Hoy, 1992). The simplest ears are those of moths with only one to four sensory cells per ear depending on family (von Kennel & Eggers, 1933; Roeder, 1974b).

The best documented cases of detection and reaction to bat echolocation sounds involves noctuid moths thanks to the pioneering work of Roeder (see, e.g. Roeder, 1974b). Noctuid moths have two sensory cells in each ear, the A1 and A2 cells. The former is 20–30 dB more sensitive than the latter. This difference in threshold between the A-cells may explain why the evasive reactions of noctuids seem to depend on sound intensity. At low sound pressures, exciting only A1 cells, noctuids typically turn away from the sound source, whereas high sound pressures (a bat close by), recruiting also A2 cells, elicit unpredictable manoeuvres often including rapid dives to the ground (Roeder, 1974b).

Pyralidae (the single family of Pyraloidea) is characterized by a pair of tympanal organs with unique morphology. The ears are situated ventrally close together on the first abdominal segment, and contain a scolopale organ with four sensory cells besides a non-acoustic B-cell (von Kennel & Eggers, 1933; Mullen & Tsao, 1971). Although the Pyralidae is one of the most speciose families in the Lepidoptera (Scoble, 1992), hearing has been examined only in a few species: *Ostrinia nubilalis* (Hübner) (Belton, 1962; Agee, 1969), *Ephestia*
Several pyralid moths use sound presumably for social communication (see Conner, 1999, for a review). The greater wax moth, *G. mellonella*, combines acoustic and pheromone signalling in its sexual communication. Males use the tymbal organs on the tegulae of the forewings to produce short (100–500 μs), low intensity (81 dB sound pressure level at 1 cm) sound pulses with most energy around 75 kHz (Spangler, 1986). Spangler (1985) suggested that the pulses stimulate virgin females to fan their wings and produce substrate vibrations, in turn stimulating the males to emit pheromones that attract the females (Finn & Payne, 1977; Spangler, 1987). However, greater wax moths also use their ears for bat detection, and Spangler & Takessian (1983) and Spangler (1984) reported preliminary observations of rapid diving, dropping, spiralling and looping in response to echolocating insectivorous bats.

Based on behavioural observations, Spangler (1984) suggested that greater wax moths may distinguish between low and high ultrasound frequencies. However, this would require the ability to discriminate frequencies, a facility that has not yet been demonstrated in moths. Alternatively, greater wax moths could use temporal cues to distinguish between bats and conspecifics. The coding of temporal stimulus parameters, such as pulse repetition rate and pulse duration, has been studied in noctuid moth ears, where the acoustic sensory cells can follow repetition rates up to 200 Hz, corresponding to the highest repetition rates produced by bats (Surlykke, 1984). Furthermore, stimulus duration is traded for intensity, so that the threshold for short acoustic signals decreases when the stimulus duration increases to the integration time of around 10–30 ms (Surlykke et al., 1988; Tougaard, 1996) or as long as 69 ms (Waters & Jones, 1996). Temporal integration has been studied in most vertebrate groups, especially by psychophysical methods, but there are surprisingly few investigations of temporal integration in insects with rather simple ears.

The purpose of the present study was to investigate the effect of sound parameters such as frequency and temporal cues both on hearing and evasive behaviour of a pyralid moth with four sensory cells. When stimulated with ultrasonic pulses, greater wax moths in stationary flight respond consistently with short latency cessation of flight. This reliable behavioural response allowed us to study temporal integration both at the primary sensory level and at the behavioural (motor) level in the same insect species.

**Materials and Methods**

**Animals**

Wax moths, *Galleria mellonella*, were purchased as pupae from Saturnia, Copenhagen. After hatching, males and females were kept for a minimum of 24 h reversed phase LD 12: 12 h photocycle prior to the behavioural experiments to ensure that moths were in their active period while running the experiments in the daytime.

**Sound stimuli**

Ultrasonic sound pulses were generated using a Wavetek oscillator (model 186; San Diego, U.S.A.) controlled by either a pulse generator (Hewlett Packard 8011 A; Amsterdam, The Netherlands) or a computer (PC AT) and shaped to have linear rise- and fall times by a custom built trapeze modulator. The custom built electrostatic loudspeaker (6 cm diameter) was calibrated several times during the experimental period by means of a Brüel & Kjær Type 4135 1/4” microphone (Naerum, Denmark) (grid off), a Brüel & Kjær preamplifier Type 2619 and a measuring amplifier (Type 2607). The frequency response of the loudspeaker was flat (±3 dB) from 25 to 130 kHz. Sound pressure levels are given in dB relative to 20 μPa rms (dB sound pressure level).

Threshold curves were measured using single pulses of 50 ms duration and sound frequencies were tested in a random order. In other experiments we used pulse trains with different pulse durations and duty cycles (i.e. the percentage of time where the stimulus is on).

**Electrophysiology**

The animal was immobilized by cooling, and dissected ventrally using a modification of the technique described by Agee (1969). The tympanic nerve runs parallel to the abdominal connective from the metathoracic ganglion into the first abdominal segment where it was hooked onto a tungsten electrode. The nerve was covered by a 1 : 1 mixture of Vaseline and paraffin oil to avoid desiccation. In this way the preparation could last for up to 2 h. The preparation was placed ventral side up. The loudspeaker was mounted 30 cm above the moths such that both ears faced the loudspeaker. Cotton wool was placed beneath and behind the preparation to attenuate echoes.

The nerve signals were amplified and bandpass filtered (custom-build equipment) and recorded on a tape recorder (Hewlett Packard 3960) simultaneously with the DC-pulses controlling the sound stimuli. There were no obvious differences in spike characteristics, e.g. amplitude or shape, between the A-cells. Therefore, identification of spikes from the different cells had to be made by manual examination of every response train. We used the same method as Roeder (1974a) and Surlykke & Miller (1982), which is mostly based on firing frequency, i.e. when two spikes are spaced by less than 1 ms it is most unlikely that it is the same cell firing twice at a rate faster than 1000 Hz. Threshold was defined as the sound pressure level necessary to elicit at least two A1-spikes more than background firing in at least eight out of 10 stimulations. As a control the threshold for the first stimulus frequency was also determined at the end of each session. The individuals were discarded if the discrepancy was larger than ±2 dB from the control measurement.

**Behaviour**

All behavioural experiments were conducted in dim red light with the insect in stationary flight 30 cm from the loudspeaker. The thoracic movements of the moth were monitored using a gramophone pick-up as a transducer. We glued a 5-cm long syringe needle to the pick-up needle and mounted the animal to the end of the needle with wax (Cenco Softseal Tackiwax) dorsal side up, as in normal flight position. The transducer signal was amplified (custom-build amplifier) and recorded on tape (Hewlett Packard 3960) simultaneously with the sound stimuli (Fig. 1C). Using a stroboscopic light source we confirmed that the thoracic movements measured by the pick-up corresponded with the wingbeat frequency. One behavioural response, the cessation of flight, could be recorded on-line, whereas changes in wingbeat frequency were measured off-line from the tape recordings. The threshold curve was obtained in the following way: first the approximate threshold level was determined for each frequency; then the stimulus intensity was adjusted to 10–15 dB below this level and increased in 3 dB steps until a flight cessation response was elicited. A pause of 30–60 s between each stimulation was sufficient to avoid habituation. The threshold for flight cessation was defined as the sound pressure sufficient to elicit a flight cessation with short response latency (40–100 ms) in at least one out of two stimulations.

**Results**

**Hearing physiology**

The electrophysiological recordings showed spikes from at least three acoustic sensory cells and from a non-acoustic cell, the B-cell. Following Roeder (1974a), we named the auditory sensory cells A1–4 according to their sensitivity, with A1 being the most sensitive. A threshold curve was drawn only for the A1-cell (Fig. 1A). The spontaneous firing of the A-cell was always below 10 Hz. The audiogram obtained for nine females and six females showed best frequency at 60 ± 15 kHz.

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**Fig. 1.** (A) The upper curve is the behavioural threshold curve for the flight cessation response (n = 12). The lower curve is the sensory threshold for the A1-cell (n = 15). (Vertical bars represent ± SD). In some animals we also determined the A2-thresholds (○) and A3-thresholds (○). (B) Examples of neurophysiological recordings. A- and B-cell activity is marked according to our definitions. Trace a shows the response to a 5 ms pulse at threshold intensity. The two large spikes corresponds to the B-cell, and the medium sized spike in the middle of the trace is a summated response coming from simultaneous A1 + A2 firing. The two spikes labelled A could be either A1 or A2. Trace b shows the response to the same stimulus but 12 dB above threshold. Simultaneous A1 + A2 firing is seen at the beginning of the response. A3-cell activity is seen very close to the second A1 + A2 spike. The two spikes labelled A could be either A1, A2 or A3. (C) Traces a and b show the output from the gramophone pick-up, which corresponds to the wingbeat frequency (in this case 37 Hz). A flight cessation response is seen in both traces. In trace a the moth spontaneously resumes flight.
identify spikes from the A4 sensory cell, because its threshold either A1 or A2. cell. Synchronized A1 + A2 ®ring (spikes with double amplitude) was seen at intensities above the A2-threshold (Fig. 1B). It was not possible to identify spikes from the A4 sensory cell, because its threshold is probably about 20±30 dB above that of A1, where there is too much nerve activity.

The latency for the A1-spikes decreased from ±13 ms at threshold intensity (three individuals) to the minimum latency of 5 ms at 10 dB above threshold, beyond which further increase of intensity had no effect on latency. The time-intensity trade function for the A1-receptor in six individuals (Fig. 2, lower curve) showed that the threshold decreased by 2.1 dB per doubling of duration (dB/dd) for short pulse durations, but remained constant for longer durations. The sloping line for short durations and the horizontal line for long durations intersect at 45 ms (Fig. 2). Thus, the integration time for the A1-receptor is estimated at 45 ms.

Behavioural responses

The flight cessation response is easy to determine on-line due to its all-or-none character (Fig. 1B). We therefore used this response to determine the behavioural threshold for most stimulus paradigms. The threshold curve for cessation of flight (seven males and five females) parallels the curve for the A1-cell, but is shifted upwards by 20±25 dB (Fig. 1A). Thus, the best frequency for the behavioural curve was also at 60 ± 10 kHz (P<0.05, Mann–Whitney U-test), but with a lowest average threshold of 72 dB sound pressure level. We found no difference in hearing thresholds between the two sexes (paired t-test).

The latency for the flight cessation response, the time from stimulus onset to the end of the last wingbeat prior to cessation of flight averaged 72 ms (nine individuals) at threshold intensity and decreased with increasing sound pressure down to 55 ms on average at +15 dB above threshold and became constant above this intensity. The shortest latency measured for the behavioural response was 40 ms. The absolute difference between the latency for the A1-spikes and for the flight cessation response was around 50–60 ms through the whole intensity range tested, indicating consistent reaction time.

Time parameters such as pulse duration, interpulse length and duty cycle, i.e. the percentage of time in a stimulus train where the sound is on (duty cycle = pulse length/flush length + interpulse length) × 100% affected the flight cessation thresholds. For example, we never observed a moth (n > 30) that would stop flying in response to a single stimulus shorter than 6 ms, even at an intensity of 100 dB sound pressure level. However, when in response to pulse trains of high pulse repetition rate (pulses/s) moths showed flight cessation response to pulses as short as 1.5 ms. The threshold decreased by 15 dB, when the pulse repetition rate increased from 50 pulses/s to 333 pulses/s using 1.5 ms pulses in pulse trains which varied in length from 500 ms to 75 ms (Fig. 3). Over this range the duty cycle increased from 7.5% to 50% in the pulse train, while the total number of pulses and thus the total energy per stimulus train was kept constant. This means that the decreased threshold could reflect either the increase in
pulse repetition rate, or the increase in duty cycle (and thus in the energy per unit time). To assess this (Fig. 4), we increased the pulse repetition rate and kept the duty cycle and the energy per unit time constant. This was done by varying pulse repetition rate, pulse duration and the number of pulses in every stimulus train. The duty cycle was 35% and each stimulus train was 60–70 ms long, thus a little longer than the integration time (see below). Under these conditions we found no significant difference ($t$-test: d.f. = 6, $t = -1.05$, $P = 0.34$) in flight cessation threshold between the fastest and slowest pulse repetition rate (Fig. 4), nor between the other values.

Single pulses longer than 6 ms decreased the flight cessation threshold in the same range as for the sensory cell, but with a much steeper slope of the curve for the time-intensity trade function (Fig. 2, three individuals). The slope of the steep part of the curve was determined by linear regression to $-7.2$ dB/dd corresponding to 24 dB per decade. At long durations the threshold intensity was constant irrespective of duration. We chose the first value on the horizontal curve, i.e. 60 ms, as an estimate of the behavioural integration time for the flight cessation response.

We also determined the integration time for the behaviour using double-pulses, where each pulse alone was too short to elicit a flight cessation, and measured the flight cessation as a function of the interpulse interval (Fig. 5). First, we determined the threshold intensity for flight cessation (two reactions out of two stimulations) for an 8 ms pulse. Then, we stimulated with two 4 ms pulses of this intensity and systematically decreased the interpulse interval until a flight cessation response was observed (Fig. 5A). We tested 10 different animals once at every interpulse duration. The proportion of responding animals increased gradually from interpulse intervals of $\geq 60$ ms, where none responded, to pulse intervals of $\leq 35$ ms, where the response rate was close to 100% (Fig. 5B). The integration time was defined as the interpulse length where 50% of the animals responded, i.e. 50 ms. By choosing the 50% response level the threshold definition was identical to the one used in the time-intensity trade-off experiment.

Greater wax moths in stationary flight show other behavioural responses to ultrasound, such as changes in wingbeat.

Fig. 4. Flight-stop threshold as a function of pulse repetition rate when the duty cycle, as represented by the dotted line, is constant (35%), the pulse train duration is 60–70 ms and the total energy in each train is constant. 0 dB corresponds to the flight cessation threshold for a 50 ms single pulse ($n = 5$, vertical bars represent $\pm$ SD).

Fig. 5. Integration time measured with double pulses. Ten wax moths were tested with double pulse stimuli consisting of two 4 ms pulses with different interpulse intervals ($\Delta t$). The sound pressure of the two 4 ms pulses was set to the threshold level for a single 8 ms pulse. (A) shows the principle for the experiment. Trace a shows the response when $\Delta t$ is too long (65 ms) to elicit a reaction. Trace b shows a flight cessation response when the $\Delta t$ is decreased to 45 ms. (B) The bars show that the number of moths reacting to a double pulse increased from 0 at $\Delta t \geq 60$ ms to 9–10 at $\Delta t \leq 40$ ms.
frequency, abdominal steering and movements of the antenna. We measured the wingbeat frequencies from the tape recordings. The wingbeat frequency for G. mellonella in stationary flight varied between individuals from 27 to 50 Hz (72 individuals). The changes in wing beat frequency were measured in moths that flew with an almost constant wingbeat frequency. Stimuli with intensities below the threshold for cessation of flight often led to a change in the wingbeat frequency, but the response was not an unambiguous decrease or increase in wingbeat frequency. Figure 6 shows examples from two animals. Animal A increased its wingbeat frequency by more than 10% relative to the pre-stimulus frequency, whereas animal B decreased its wingbeat frequency by more than 20%. Furthermore, the same individual could respond by increasing the wingbeat frequency to the first stimulus, and decrease the wingbeat frequency to a later stimulus. The threshold for changes in wingbeat frequency ranged from 2 to 12 dB below the threshold for the cessation of flight.

In order to find where in the CNS the evasive reactions are controlled, we conducted behavioural experiments on brainless animals. Six animals were decapitated and four others had either the neck connective cut or the brain damaged by a thin needle. The treated animals maintained stationary flight after the surgery and most showed stable flight. One of the headless animals flew with exactly the same wingbeat frequency as before decapitation – even 2 days after decapitation. Three animals had a reduced wingbeat frequency after decapitation, but none reacted behaviourally when stimulated with ultrasonic pulses – even at the maximum output intensity of the system (104 dB sound pressure level, corresponding to 30 dB above the threshold for cessation of flight).

Discussion

Sensory and behavioural threshold curves

Wax moth ears are broadly tuned with best frequency from 40 to 80 kHz, corresponding fairly well to the peak frequency of 75 kHz for the male clicks (Spangler, 1986). The reason why the hearing is not perfectly tuned to their own sounds is probably that they also need to listen for bat calls at lower frequencies. Thus, their hearing may represent a compromise between listening for mating calls and listening for sounds from a predator. The threshold for the flight cessation was +20 to +25 dB relative to the A1 threshold at all frequencies and thus comparable with behavioural thresholds of noctuids (Roeder, 1967; Madsen & Miller, 1987). This means that other initial evasive behaviours occur at lower stimulus intensities, when the insect has detected the bat, but not stopped flying. Changes in flight patterns, for example turning, might be an initial reaction in wax moths because the thresholds for changes of wing beat frequency were 2–12 dB below the threshold for cessation of flight.

The sensory threshold agrees well with those reported for other pyralids (Agee, 1969; Perez & Zhantiev, 1976; Heller & Krahe, 1994). Only Spangler & Takessian (1983) and Spangler (1984) found much lower thresholds by monitoring the tympanic tilting in both lesser and greater wax moths, Achroia grisella and G. mellonella, respectively. In G. mellonella they found a best frequency around 30 kHz with a threshold of only 18 dB sound pressure level, which is 40 dB below the sensory threshold we found. Furthermore, the best frequency of 30 kHz found in their study differs from the 60 kHz found in this study. We have no explanations for these discrepancies.

In noctuids the difference in threshold between the A1- and A2-cell is 20–30 dB (Roeder, 1974a; Surlkyke & Miller, 1982; Madsen & Miller, 1987). This is approximately equal to the dynamic range of the single cells, hence maximizing the total dynamic range of this two-celled ear. In Geometrids the individual thresholds of the four sensory cells are closer than in noctuids (10–15 dB apart), but they are equally spaced (Surlkyke & Filskov, 1997). In contrast, the two most sensitive cells in greater wax moths have almost equal thresholds. Thus, A1 and A2 may not be the same cells in different animals because the definition is based only on threshold. A similar small threshold difference between A1 and A2 was indicated for another pyralid species, E. kuehniella (Perez & Zhantiev, 1976), which also produces sound (Trematerra & Pavon, 1995). We suggest that greater wax moths may have a more precise coding of the intensity and time structure of the sound at sound levels just above threshold, and this could be correlated with the use of low intensity ultrasonic signals in intraspecific communication. If the small threshold difference between A1 and A2 also exists in the lesser wax moth, Achroia grisella, this could help to explain that these moths can distinguish signal events as brief as 150 μs (Jang & Greenfield, 1996).

We found that the thresholds for both A2- and A1-cells and for the behavioural response paralleled the audiogram (the A1

Fig. 6. Typical examples of changes in the wingbeat frequency following ultrasonic stimulation shown for two individuals. The arrow shows the start of the pulse (50 ms single pulse: 60 kHz, 4 dB below threshold for cessation of flight). The y-axis gives the change in percentage relative to a running average of 10 wing beats before the stimulus. The x-axis gives the wingbeat number, respectively, before and after stimulation. In both examples the changes in wingbeat frequency are significant (paired t-test: Moth A: d.f. = 9, t = 4.5, P < 0.002; Moth B: d.f. = 9, t = -6.5, P < 0.0001).
threshold) in the whole frequency range tested. Our results, therefore, give no indication of frequency discrimination in greater wax moths as suggested by Spangler (1984, 1988). Furthermore, the anatomical data contradict frequency discrimination, as pyralids have all four sensory cells attached to the same area on the tympanic membrane (von Kennel & Eggers, 1933; Mullen & Tsao, 1971). Therefore, we find it more likely that greater wax moths make this discrimination using temporal cues. Recently, Greenfield & Weber (2000) showed that the lesser wax moth, *A. grisella*, discriminates between bat sounds and conspecific sounds on the basis of temporal cues.

**Temporal integration**

The flight cessation threshold for greater wax moths decreased with increasing pulse repetition rate, corresponding with results obtained for crickets (Nolen & Hoy, 1986) and noctuid moths (Zhantiev, 1988). However, none of these experiments controlled for duty cycle and thus for energy content in the stimulus. Our results demonstrated that at constant duty cycle there was no effect of pulse repetition rate, suggesting that greater wax moths and probably other insects respond to the power of the signal, not to the pulse repetition rate per se. Experiments where the duty cycle was increased in parallel with the pulse repetition rate suggest that moths use the pulse repetition rate to judge the distance to an approaching bat (Fullard, 1984; Surlykke, 1984). Our results do not support this interpretation. In fact, many bats in the field tend to keep the duty cycle constant while increasing the pulse repetition rate throughout the pursuit sequence until the terminal phase (Schnitzler et al., 1987; Surlykke et al., 1993). Duty cycle rather than the pulse repetition rate could explain the fact that some arctiids do not click until the terminal phase in a bat’s hunting signal (Fullard et al., 1994). In Fullard et al.’s experiment the moth’s clicks began at a moment in the stimulus sequence where the duty cycle increased in parallel with the pulse repetition rate.

Temporal integration experiments reveal that time is traded for intensity at stimulus durations up to the maximum integration time. Temporal integration has been studied at the receptor level (Surlykke et al., 1988; Tougaard, 1996), but not at the behavioural level in moths. Behavioural integration time, however, is presumably the more relevant integration time for discussions of bat–moth interactions.

The maximum integration time for the A1 receptor was 45 ms. Similar experiments for noctuid moths revealed integration times of 25 ms (Surlykke et al., 1988) and 69 ms (Waters & Jones, 1996). Tougaard (1996) used double clicks, fitted his data to an exponential model and found a characteristic time (τ) of 5 ms for the noctuid receptor, suggesting (Tougaard, 1998) that the differences in threshold definitions may explain the differences in integration times measured by traditional trade-off experiments.

The behaviourally determined integration time of 60 ms for the flight cessation response was confirmed by the double-pulse experiment (50 ms). Nolen & Hoy (1986) measured the threshold for negative phonotactic steering in crickets as a function of stimulus duration and the data in their Fig. 5 suggest a maximum integration time of about 15 ms.

Although the behavioural integration time approximates the integration time for the sensory cell, the slope of the curve for the behavioural response is much steeper, ~7.5 dB per doubling of duration. This is much more than the ~3 dB per doubling of duration expected for a perfect energy detector (Au, 1988) and has been termed ‘over-integration’ (Schmidt & Thaller, 1994). For technical reasons it was not possible to determine the integration time for the other sensory cells, but we would expect their slopes to be less steep than ~3 dB per doubling of duration. However, several factors are relevant in the comparison of integration at the behavioural level and temporal integration at the receptor level. First, the behaviour is elicited at 20–25 dB above the sensory threshold, i.e. at a level so high that most, if not all, sensory cells are excited and the most sensitive cells are close to saturation. Rate-level curves for sensory cells become more horizontal at stimulus intensities just below saturation, perhaps explaining the steeper time-intensity trade function, because at such high stimulus levels a further increase of intensity will not produce more spikes, whereas an increase in duration could increase the total number of spikes elicited by the pulse. Secondly, the behavioural response involves activity at higher neural levels, including both interneurones and motorneurones. Thus, at high sound intensities other forms of integration (facilitation, spatial summation, coincidence, etc.) could contribute to the discrepancy between the slopes of the curves.

**Acoustic communication**

The 75 kHz signals produced by the male’s tymbals are short (100–500 μs) and only 81 dB peak sound intensity 1 cm from the animal (Spangler, 1986). As the minimum threshold for detection of a 0.5 ms pulse in the A1-cell is 62 dB sound pressure level (Fig. 2, lower curve), the acoustical communication distances must be shorter than 20 cm, even considering that the threshold for multiple pulses may be lower than for a single pulse. Therefore, we propose that the sound signals produced by greater wax moth males function only in close range communication. *Galleria mellonella* males are observed to leave bee hives shortly after eclosion and fly to nearby trees or bushes (Nielsen & Brister, 1977). A high density of males and a huge amount of pheromones produced from individual males might attract females from a longer distance. When the females have arrived in the vicinity of the males, then they might rely on the acoustic cues to direct them towards individual males, as sound signals have a strong directional component compared with chemical cues. Other pyralids, however, emit more intense signals, enabling acoustic communication over longer distances (Heller & Krahe, 1994), perhaps substituting pheromones for long-range communication in this species.

In addition to intraspecific communication, wax moths use their ears for bat defence, and temporal parameters combined...
with intensity cues are important for both types of signals. The relative broad hearing spectrum in greater wax moth makes them sensitive to a large range of bat calling frequencies, which is an advantage for their cosmopolitan occurrence. For example, a typical sympatric bat as *Pipistrellus pipistrellus* emitting search calls with main energy around 57 kHz and a source level of 115 dB sound pressure level at 10 cm (data kindly provided by Marianne Egebjerg Jensen) will be detected by the A1-cell at approximately 12 m using an atmospheric attenuation of 2 dB/m. However, reaction distances cannot be extrapolated from sensory cell activity (Waters, 1996). Instead, we use the data from the behavioural experiments. The threshold for flight cessation will be surpassed when the bat is less than 5 m away. This leaves the wax moth around 2.4 s from detection to collision at a typical flight speed of 5 m/s for the bat and negligible speed for the moth. There is around 1 s available for the flight cessation reaction. The situation is different for bigger bats flying faster and emitting lower frequencies. For example, the serotine bat, *Eptesicus serotinus*, calls at 27 kHz (source level: 115 dB sound pressure level at 10 cm; Jensen & Miller, 1999) and flies at 8 m/s. The wax moth’s detection distance is around 16 m (atmospheric attenuation 0.7 dB/m) and flight cessation reaction distance is about 3 m, leaving the moth 2 s and 0.4 s for detection and reaction, respectively. This should still give the moth enough time to escape, because the latency for cessation of flight is only 70 ms. The threshold for changes in wing beat frequencies lies between the detection threshold and the flight cessation threshold, indicating that flight pattern changes are elicited at distances between the detection and reaction distances calculated above. Hence, the evasive behaviours of greater wax moths seem to depend on intensity and thus distance to the bat, corresponding to what has been demonstrated for noctuids (Roeder, 1974b).

In noctuids the current hypothesis is that the A1-cell is responsible for the negative phonotactic response, whereas the non-directional and unpredictable responses (diving, looping, etc.) are close-range behaviours elicited at high sound intensities (+20 dB above the A1-threshold), where the A2-cell fires (Roeder, 1974b). In greater wax moths the changes in wing beat frequency and perhaps turning behaviour are evoked at intensities corresponding with the threshold of A2-cells, whereas flight cessation is elicited 2–12 dB higher probably around the threshold of A4. The A3 and A4 may be functionally analogous to the noctuid A1 and A2, whereas the A1 and A2 in the wax moth may be associated mostly with intraspecific communication. However, the behavioural thresholds of tethered greater wax moths might not be the same as those of freely flying moths.

It has been suggested that last chance evasive manoeuvres of noctuids are elicited by A2-cell activity directly through the meso- and metathoracic ganglion (Fullard, 1982; Boyan & Fullard, 1986; Agee & Orona, 1988). The situation appears to be different in greater wax moths. Behavioural reactions can be elicited in headless noctuid moths (Treat, 1955), but not in greater wax moths, suggesting that all evasive responses in wax moths are controlled from the brain.

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References


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