The Journal of Experimental Biology **199**, 847–856 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0035

THE PERIPHERAL AUDITORY CHARACTERISTICS OF NOCTUID MOTHS: RESPONSES TO THE SEARCH-PHASE ECHOLOCATION CALLS OF BATS

DEAN A. WATERS* AND GARETH JONES
School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

Accepted 5 January 1996

Summary

The noctuid moths *Agrotis segetum* and *Noctua pronuba* show peak auditory sensitivity between 15 and 25 kHz, and a maximum sensitivity of 35 dB SPL. *A. segetum* shows a temporal integration time of 69 ms. It is predicted that bats using high-frequency and short-duration calls will be acoustically less apparent to these moths. Short-duration frequency-modulated (FM) calls of *Plecotus auritus* are not significantly less acoustically apparent than those of other FM bats with slightly longer call durations, based on their combined frequency and temporal structure alone. Long-duration, high-frequency, constant-frequency (CF) calls of *Rhinolophus hipposideros* at 113 kHz are significantly less apparent than those of the FM bats tested. The predicted low call apparency of the 83 kHz CF calls of *R. ferrumequinum* appears to be counteracted by their long duration. It is proposed that two separate mechanisms are exploited by bats to reduce their call apparency, low intensity in FM bats and high frequency in CF bats. Within the FM bats tested, shorter-duration calls do not significantly reduce the apparency of the call at the peripheral level, though they may limit the amount of information available to the central nervous system.

Key words: noctuid moths, audition, call apparency, bats, auditory sensitivity, *Agrotis segetum*, *Noctua pronuba*, echolocation, sonar.

Introduction

Moths from the families Noctuidae, Notodontidae, Geometridae, Pyralidae and some Sphingidae have auditory systems sensitive to ultrasound (Roeder *et al.* 1968; Roeder, 1974; Spangler and Takessian, 1983; Surlykke, 1984). It is proposed that these auditory systems have evolved in relation to predation by echolocating bats which forage using ultrasonic sonar (Roeder, 1967*a*; Miller, 1984; Fullard, 1987; Surlykke, 1988). Stimulation of the auditory system triggers a variety of escape responses from negative phonotaxis at low sound intensities (Roeder, 1967*b*) to complex loops, spirals and dives at high sound intensities (Roeder, 1962, 1975). The auditory system is simple, consisting of only two cells per ear in the Noctuidae, the A1 cell and the less-sensitive A2 cell. The characteristics of the peripheral system appear to be matched to the call characteristics of the sympatric bat community (Fullard, 1984, 1987, 1988; Surlykke, 1988). It has been proposed that bats using calls mismatched to the moths' auditory system could gain a foraging advantage (Fenton and Fullard, 1979, 1981; Fenton, 1980). The ultimate currency by which the acoustic apparency of the call can be measured is the distance from the moth at which the bat is initially detected. A number of studies have used echolocating bats in free flight as stimuli to tympanic preparations to determine the distance at which the A1 cell detects the bat (Roeder, 1966; Fenton and Fullard, 1979; Faure *et al.* 1990, 1993). While this reveals the overall apparency of the call, it does not reveal the mechanism by which this level of apparency is produced; the frequency structure, the temporal structure, the emitted intensity, or a combination of these factors. It is necessary to eliminate the factor of emitted intensity to separate the mechanisms governing call apparency into those dependent on time and frequency structure and those dependent on emitted intensity and foraging strategy. Even once this is done, the way in which apparency is defined can depend on how the signal level necessary to stimulate the auditory system is measured and how this interacts with the physiology of the auditory system itself. This study generates hypotheses as to the types of call structure which would render the bat acoustically less conspicuous to the moth. We test the hypothesis that bats which have diets containing a high proportion of Lepidoptera have search-phase calls that have frequency and temporal structures which are relatively inaudible to moths. We then assess the contribution of call intensity to the overall level of apparency and examine how call apparency can be reduced.

Materials and methods

Two European noctuid moths were selected for the experiments. *Agrotis segetum* (Denis & Schiffermuller) was

*Present address: Department of Biology, University of Leeds, Leeds LS2 9JT, UK.

supplied from an existing culture and *Noctua pronuba* (Linnaeus) was captured from the wild using a mercury vapour light trap. All experiments were performed in a 4 m×4 m×2.6 m room lined with 1 cm thick foam. Tympanic preparations were made, derived from the methods of Roeder (1966) and Agee (1967). Briefly, the moth was decapitated and the legs removed. The thorax and abdomen were fastened dorsal side up in a shaped polystyrene block with the wings clamped spread open. A groove in the block allowed sound to reach the tympanic organ situated at the base of the hindwing. The notum was brushed free of scales and a circular incision made around it. On removal of the notum, the mesophragma was gripped with a pair of forceps and pulled free, the longitudinal flight muscles being severed at the anterior end. The resulting cavity was flooded with saline (Fielden, 1960). A bipolar silver hook electrode was scanned over the surface of the exposed dorsoventral muscle until the tympanic nerve IIIN1 was encountered, characterised by the regular firings of the non-auditory B cell. The nerve was then hooked over the tip of the electrode. Output was preamplified (CEP 8120) and bandpassed between 50 Hz and 10 kHz with a gain of 1000×. Output was passed to an audio amplifier and Tektronix 5113 dual-beam storage oscilloscope. Recordings were made with the nerve still attached to the metathoracic ganglion. The nerve was left in the saline solution except when threshold determinations were made, when it was pulled gently out of the solution. Between these determinations, it was returned to the saline bath. The movement of the nerve in and out of the saline was not sufficient to increase the discharge rate of the B cell, which appears to encode stress in the tympanal region. Once established, preparations remained stable for between 2 and 6 h, with no sign of lowered auditory sensitivity. All experiments were completed within 1 h of establishing the preparation.

Ultrasonic stimuli were generated by a custom-made sine-wave generator and pulse shaper, amplified and broadcast through an Ultra Sound Advice amplifier and matched capacitance loudspeaker (frequency response ±4 dB 10–120 kHz, total harmonic distortion <1 %). Where required, oscilloscope voltages were converted to sound pressure levels immediately after the experiments by a Brüel and Kjær 2204 sound pressure meter equipped with a 6.35 mm type 4135 microphone (grid off) with a frequency response of ±2 dB 0.01–120 kHz. In order to take account of the local sound field at the preparation, the moth was removed and the microphone placed in the slot in the polystyrene block at the position of the moth's ear.

Frequency sensitivity

Audiograms were constructed by playing sine-wave carrier pulses of 10 ms duration and 1.5 ms rise/fall time at the preparation. Pulses were manually triggered at a rate of between 0.2 and 1 Hz. The threshold criterion was 1–2 action potentials in four out of five stimulus presentations, the threshold being measured as a speaker voltage. The carrier frequency of the pulse was incrementally increased in 5 kHz stages over the range 10–115 kHz. At the completion of the first data set, the threshold values were remeasured at 20 kHz intervals and any preparation which deviated by more than 2 dB was discarded. Repeated measurements were usually within 1 dB. The sensitivity of the A2 cell was measured in the same way. At threshold values of the A2 cell, A2 cell action potentials could be differentiated within the A1 cell spike trains by their short interspike intervals, which were too short to be consecutive A1 cell action potentials (Fig. 1). Owing to fatty deposits around the tympanic nerve of *N. pronuba* resulting in a poor signal-to-noise ratio, only representative measurements of A2 threshold values were established as A2 cell spikes could not be reliably differentiated from A1 cell spikes over the full frequency range.

Integration time

Constant-frequency pulses with the carrier frequency set to 50 kHz were generated and broadcast at preparations of *A. segetum*. Pulse duration was varied between 1.5 ms and 1000 ms with the rise/fall time set to 10 % of the plateau duration. This constant proportion of ramp time ensures that a constant percentage of the energy transmitted in the pulse is within the ramp (Faure *et al.* 1993). Threshold values were obtained as 1–2 action potentials in four out of five stimulus presentations. For stimuli over 250 ms long, spontaneously generated action potentials occurring at rates of approximately 7 Hz made threshold determination difficult. In these instances, the first detectable rise in action potential number was taken as threshold. Stimuli were presented in halved durations from 1000 ms to 1.5 ms, and then in reverse. The means of the two threshold values at each stimulus duration were used and usually fell within 2 dB of each other. For all stimulus durations, peak pressure from the speakers was found to be proportional to speaker voltage.

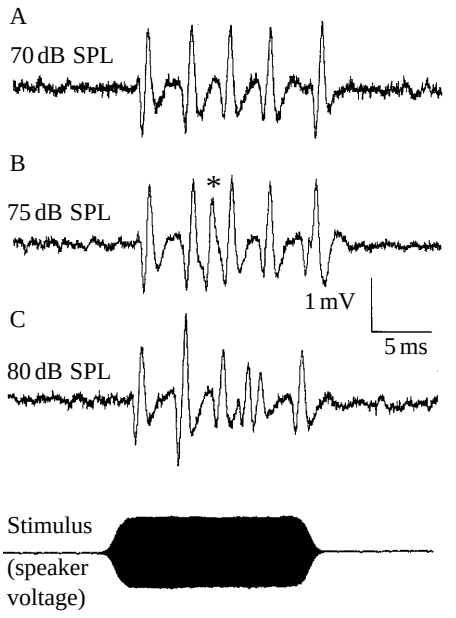

Fig. 1. (A) A1 cell spike train in response to a 10 ms 50 kHz pulse at 70 dB SPL. (B) A1 cell spike train and A2 cell action potential (marked with an asterisk) using the same stimulus at 75 dB SPL. (C) Superimposed A1 and A2 action potentials with the same stimulus at 80 dB SPL. At this stimulus level, it is not possible to differentiate separate A1 and A2 cell action potentials since they coincide.

Bat echolocation call playbacks

Search-phase calls from the bats *Pipistrellus pipistrellus*, *Myotis brandtii*, *M. nattereri*, *Plecotus auritus*, *Rhinolophus hipposideros* and *R. ferrumequinum* were used to broadcast at tympanic preparations of *A. segetum* and *N. pronuba*. Bats were recorded flying in a 4 m×4 m×2.6 m room lined with sound-attenuating foam using a Brüel and Kjær type 4135 microphone (grid off) attached to a 2204 sound pressure meter recording onto a Racal Store 4DS recorder at 76 cm s^{-1} onto a DR channel (total system linear ±3 dB 200 Hz to 120 kHz). The trajectory of the bats flying towards the microphone was recorded using multiflash stereophotogrammetry, and sequences of calls were selected only if the bat was flying directly towards the microphone (Waters and Jones, 1995). These calls were characterised by high bandwidth and good signal-to-noise ratio (>40 dB). These calls were sampled from the tape by an Ultra Sound Advice S-350 memory bat recorder at a sampling rate of 400 kHz and played into a Kay DSP 5500 Sonagraph at 10× time expansion. Calls from *R. ferrumequinum* were recorded from the wild in a stone mine using an Ultra Sound Advice S-25 bat detector, sampled by the S-350 memory bat recorder and downloaded at 10× time expansion onto metal tape using a Sony WM-DC6 Professional Walkman. Single calls were edited and compiled with 11 s of silence on either side to create a 22 s sequence. This was downloaded onto a Sony TCD-D3 digital audio tape (DAT) recorder. Three calls from one individual of each of the six species were selected and spaced evenly throughout the tape. An identical sequence was created using a different individual bat from each species to control for intraspecific differences in call structure between bats. To broadcast call sequences, they were replayed into the S-350 at a sampling rate of 40 kHz and recompressed back to real time. The signals were high-pass-filtered at 18 kHz prior to the input to the Ultra Sound Advice amplifier. This process did not significantly affect the structure of any of the calls (Waters and Jones, 1995). While call structures obtained in the laboratory may not mimic those from free-flying bats in the field, the calls we recorded were similar to search-phase calls obtained from the same species in the wild (Ahlén, 1981; Jones and Rayner, 1989), and laboratory recordings under controlled conditions probably better represent the actual call structure than do calls collected in the field using non-linear apparatus at unknown distances or angles to the emitted call (Pye, 1993).

Each call was replayed individually at a rate of 0.5 Hz to the preparations. Threshold was taken as 1–2 A1 action potentials in four out of five presentations, peak-to-peak speaker voltage levels being converted to absolute dB peSPL (peak-equivalent SPL, after Stapells *et al.* 1982) after the experiment. The root mean square (rms) pressure levels over the duration of the call were calculated following the method of Waters and Jones (1995) and Prestwich *et al.* (1989) using the analogue impulse response of a sound pressure meter and compensating for the call duration. The rms value provides a measure of the mean sound pressure level over the duration of the call and is a more accurate representation of the energy content of the call and of the mean rate of transmitted energy, since it makes no assumptions about the shape of the pressure–amplitude envelope. Both sets of recordings (i.e. each set of six species) were replayed to individuals of *A. segetum*; however, owing to seasonal availability of *N. pronuba*, only the first set was used for this species. Three types of control sounds were used randomly interspersed within the stimulus set to test for apparatus or tape noise triggering the A1 cell. These consisted of a 5 ms and 50 ms sequence of tape noise from the Racal and a 50 ms sequence of tape noise from the Sony Walkman. Thresholds to these noises were determined as above.

Results

Frequency sensitivity

Both species of moth show a marked sensitivity to lower-frequency ultrasound, and mean audiograms of both species are presented in Fig. 2. The best frequency for the A1 cell in

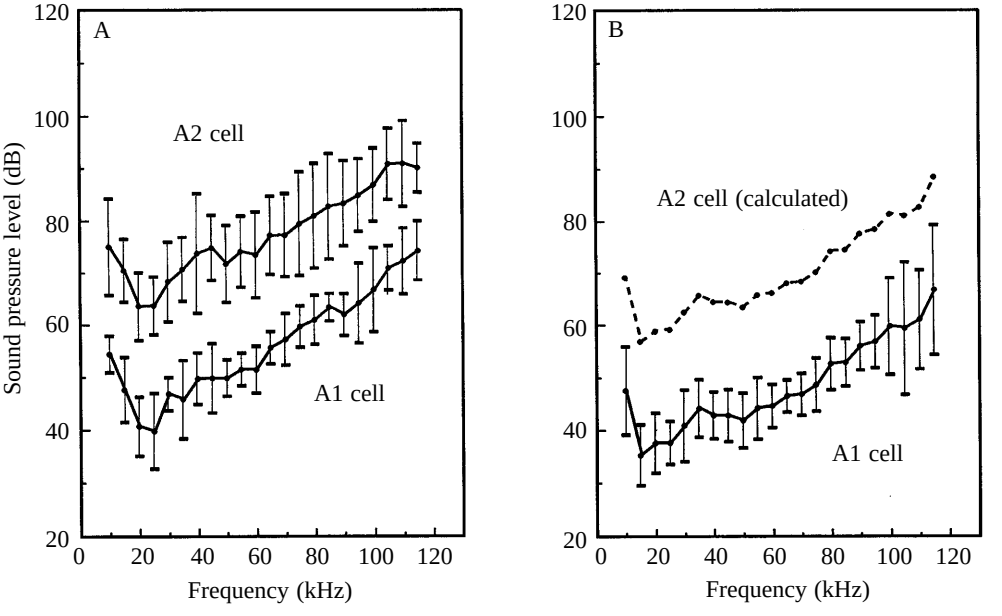

Fig. 2. Audiograms for (A) *A. segetum* (*N*=9) and (B) *N. pronuba* (*N*=8). Data are means ± S.D. A2 cell values for *N. pronuba* are derived from 28 paired A1 and A2 thresholds from the eight individuals.

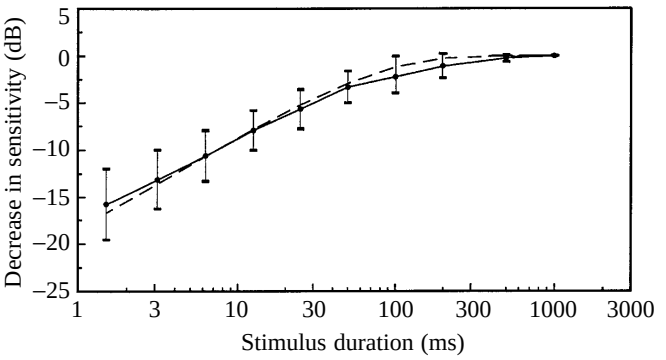

Fig. 3. Reduction in preparation sensitivity with reduced stimulus duration (N=6). The model (dotted line) is plotted with a value for the integrating time constant τ of 69 ms (see text). Data are means ± S.D.

A. segetum is 25 kHz at 40±7.0 dB SPL and 15 kHz at 35.4±5.7 dB SPL in *N. pronuba*. In *A. segetum*, the difference in sensitivity between the A1 and A2 cells is 21.8±18.9 dB, and in *N. pronuba* it is 22.1±14.0 dB (28 determinations of paired A1 and A2 sensitivities from eight individuals).

Integration time

The sensitivity of preparations of *A. segetum* to a stimulus as the stimulus is reduced in duration is shown in Fig. 3. Sensitivity is reduced as the stimulus duration falls below approximately 500 ms, i.e. the stimulus must be increased in amplitude as it decreases in duration to maintain the same threshold level. A model is fitted to the data as proposed by Plomp and Bouman (1959) defined as:

$$S = 1 - e^{-t/\tau},$$

where S is the sensitivity (dB), t is the stimulus duration (ms) and τ is an integrating time constant.

The line was fitted manually to the data and τ (ms) was altered to minimise the overall mean square value. The best estimate for τ was 69 ms. The linear part of the line followed a slope of 2.5 dB loss in sensitivity per halved stimulus duration.

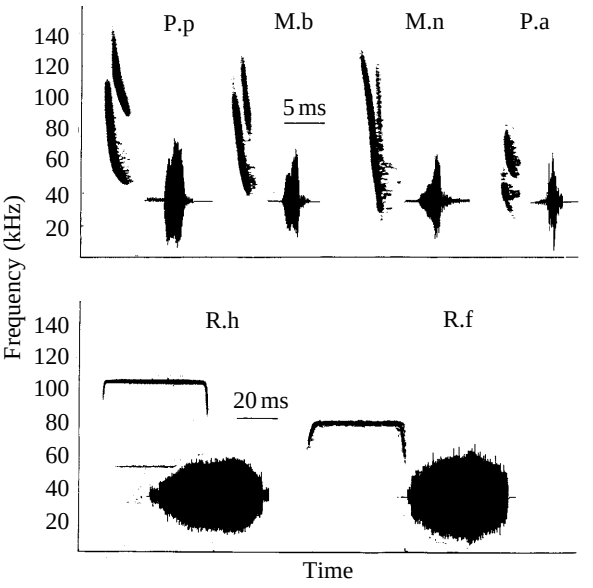

Fig. 4. Representative sonograms and waveforms of bat calls used during playback experiments. P.p, *P. pipistrellus*; M.b, *M. brandtii*; M.n, *M. nattereri*; P.a, *P. auritus*; R.h, *R. hipposideros*; R.f, *R. ferrumequinum.*

Response to echolocation call playbacks

Structures and sonograms of the rebroadcast calls are given in Table 1 and Fig. 4 as recorded at the preparation by a Brüel and Kjær microphone type 4135 (grid off) attached to a 2204 sound pressure meter and recording onto a Racal Store 4DS recorder at 76 cm s^{-1}. The calls of *P. pipistrellus*, *M. brandtii*, *M. nattereri* and *P. auritus* are frequency-modulated (FM) calls. The calls of *R. hipposideros* and *R. ferrumequinum* are constant-frequency (CF) calls with initial and terminal FM sweeps. The structures of the rebroadcast calls are similar to those exhibited by these species in the wild (Ahlén, 1981; Jones and Rayner, 1989).

Threshold values of each call are presented for both sets in Fig. 5. Threshold levels in response to the control noises were at 45.1±2.22 dB peSPL, less than 20 dB below the maximum threshold value to any call. Since the measured signal-to-noise

Table 1. *Characteristics of the bat calls used in the playback experiments*

	Call type	Maximum frequency (kHz)	Minimum frequency (kHz)	Peak frequency (kHz)	Duration (ms)
P. pipistrellus	FM	129±4.5	43±1.4	49±1.9	3.1±0.48
M. brandtii	FM	126±2.5	33±2.1	50±5.1	2.8±0.58
M. nattereri	FM	132±3.4	24±1.3	73±19.4	2.8±0.36
P. auritus	FM	84±2.4	26±2.2	49±12.1	2.1±0.35
R. hipposideros	CF	113±2.5	84±2.7	110±1.2	50.5±1.61
R. ferrumequinum	CF	86±1.5	64±3.4	83±0.3	51.5±4.77

Data are means ± S.D., N=6.
FM, frequency-modulated; CF, constant-frequency.

ratios of the call sequences were greater than 40 dB, noise levels would have to have been over 20 dB greater to interfere with the response to broadcast calls. A three-factor analysis of variance (ANOVA) was performed on each data set with the three calls of each bat nested within that of the individual bat. The model was run on SPSS using the error term of the moth×bat species interaction as the denominator in the *F* tests. Prior to analysis, data on threshold levels were tested for normality (Shapiro–Wilks test) and heterogeneity of variances (F_{max} test) to satisfy the underlying assumptions of ANOVA (Zar, 1984). *A posteriori* multiple comparisons are based on the SPSS Contrasts test allowing the pairwise comparisons of 'bat species' as a factor. The significance level was adjusted for multiple comparisons following the Bonnferoni method (Altman, 1991). Results from pairwise comparisons are presented in Table 2.

For the threshold values using the dB peSPL values, the calls of *R. hipposideros* in presentation set 1 are significantly acoustically less apparent to *A. segetum* and *N. pronuba* than those of all other species with the exception of *M. nattereri* (Table 2A,B). This pattern is repeated in set 2, with the exception that only the calls of *R. hipposideros* are significantly acoustically less apparent than those of all other bat species (Table 2C). The calls of *R. ferrumequinum* are more acoustically apparent than all other calls for *N. pronuba* in presentation set 1 and for *A. segetum* in presentation set 2. The data are clearer for the measurements using rms pressure values. In both sets, the calls of *R. hipposideros* are significantly less acoustically apparent than the calls of all other bats. In set 1, the calls of *M. nattereri* are also less acoustically apparent than the calls of *P. pipistrellus*, *M. brandtii* and *P. auritus* (and *R. ferrumequinum* for *N. pronuba*).

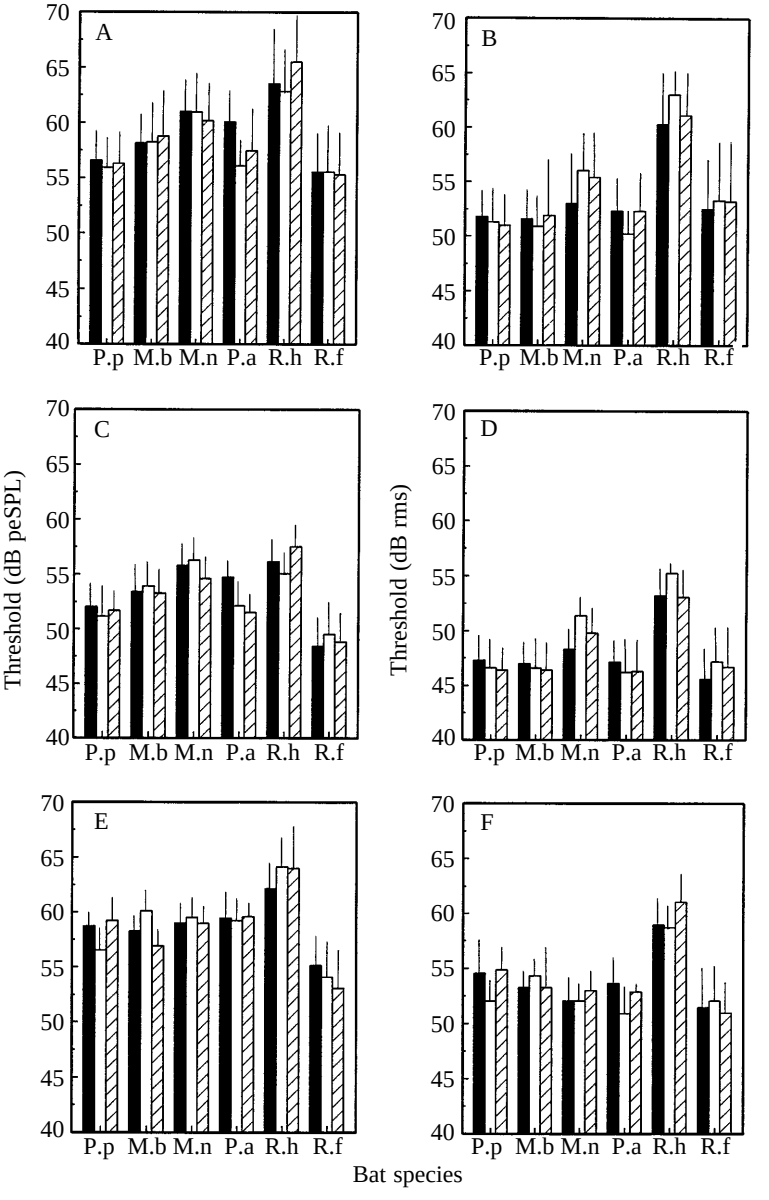

Fig. 5. Threshold values of broadcast calls. Presentation set 1 broadcast to *A. segetum* and *N. pronuba*, set 2 broadcast to *A. segetum* only. Set 1 and set 2 differ only in that the calls were recorded from different individuals bats. Within each set and species, results from three individual calls are presented. Both measures, dB peSPL and dB rms, are presented, data are means + S.D., *N*=6. (A,B) Set 1 to *A. segetum.* (C,D) Set 1 to *N. pronuba.* (E,F) Set 2 to *A. segetum.* P.p, *P. pipistrellus;* M.b, *M. brandtii;* M.n, *M. nattereri*; P.a, *P. auritus*; R.h, *R. hipposideros*; R.f, *R. ferrumequinum*.

Table 2. *Contrast pairwise comparisons for the factor 'bat' in the playback presentations of the echolocation calls*

		M. brandtii	*M. nattereri*	*P. auritus*	*R. hipposideros*	*R. ferrumequinum*
A	*P. pipistrellus*	NS	*P*<0.001	NS	*P*<0.001	NS
		NS	*P*<0.001	NS	*P*<0.001	NS
	M. brandtii		NS	NS	*P*<0.001	NS
			P<0.0033	NS	*P*<0.001	NS
	M. nattereri			NS	NS	p<0.001
				P<0.0033	*P*<0.001	NS
	P. auritus				*P*<0.001	NS
					P<0.001	NS
	R. hipposideros					*P*<0.001
						P<0.001
B	*P. pipistrellus*	NS	*P*<0.001	NS	*P*<0.001	*P*<0.001
		NS	*P*<0.001	NS	*P*<0.001	NS
	M. brandtii		NS	NS	*P*<0.001	*P*<0.001
			P<0.001	NS	*P*<0.001	NS
	M. nattereri			*P*<0.001	NS	*P*<0.001
				P<0.001	*P*<0.001	*P*<0.001
	P. auritus				*P*<0.001	*P*<0.001
					P<0.001	NS
	R. hipposideros					*P*<0.001
						P<0.001
C	*P. pipistrellus*	NS	NS	NS	*P*<0.001	*P*<0.001
		NS	NS	NS	*P*<0.001	NS
	M. brandtii		NS	NS	*P*<0.001	*P*<0.001
			NS	NS	*P*<0.001	NS
	M. nattereri			*P*<0.001	*P*<0.001	*P*<0.001
				P<0.001	*P*<0.001	NS
	P. auritus				*P*<0.001	*P*<0.001
					P<0.001	NS
	R. hipposideros					*P*<0.001
						P<0.001

In each cell of the table, the top value corresponds to the dB peSPL measure and the bottom one corresponds to the rms measure.
A, call set 1 presented to *A. segetum*; B, call set 1 presented to *N. pronuba*; C, call set 2 presented to *A. segetum.*
NS, not significant.

This pattern is not repeated for the *M. nattereri* calls in set 2, which are no less acoustically apparent than the calls of the other FM bats and *R. ferrumequinum*.

Discussion

The frequency sensitivity exhibited by *A. segetum* at a best frequency of 25 kHz is broadly consistent with that found in other species of noctuids, which are generally between 20 and 40 kHz (Fenton and Fullard, 1979; Fullard and Barclay, 1980; Fullard and Thomas, 1981; Surlykke and Miller, 1982; Fullard *et al.* 1983; Surlykke, 1986; Faure *et al.* 1990, 1993). The 15 kHz best frequency exhibited by *N. pronuba* is, however, lower than any previously encountered with the exception of those of female gypsy moths *Lymantria dispar* (Cardone and Fullard, 1988) and the Hawaiian noctuid moth *Elydna nonagrica* (Fullard, 1984). The low-frequency sensitivity of the former is argued to be due to the flightless females having ears in a state of evolutionary degradation. The sensitivity of the latter appears to be adapted to the low-frequency social calls of the only bat species present on the island of Hawaii. Individuals of *N. pronuba* are large moths with typical wingspans of 50–60 mm and are probably only taken by larger bats. In the United Kingdom, species which are known to take *N. pronuba* include *P. auritus* (Robinson, 1990) and *R. ferrumequinum* (Jones, 1990). Neither of these bats uses calls as low as 15 kHz, so low-frequency sensitivity would be of little benefit in detecting these bats. Two species of bats with low-frequency calls occurring in the United Kingdom are *Nyctalus noctula*, with a call frequency in the range 16–22 kHz (Ahlén, 1981), and *N. leisleri*, with calls with peak energy between 24 and 28 kHz (Waters *et al.*1995). It is possible that this low-frequency sensitivity may have evolved specifically in response to calls from these large species of bat.

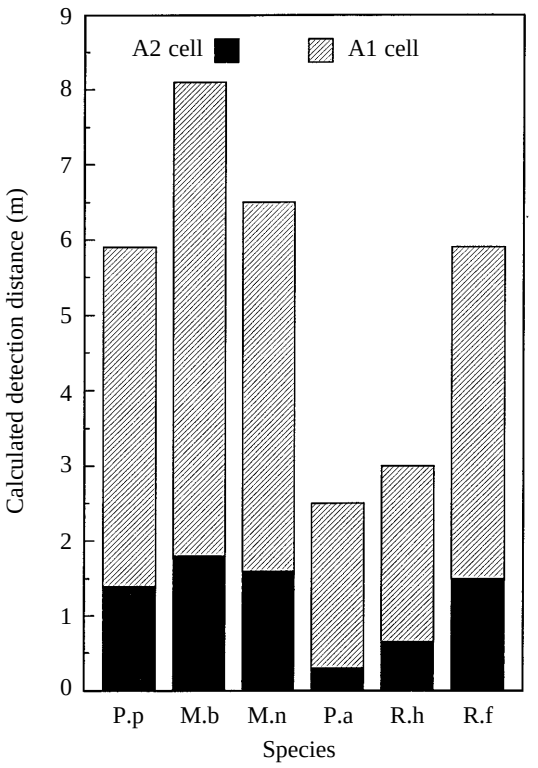

Fig. 6. Calculated distances at which the A1 and A2 cells of *A. segetum* are predicted to detect the search-phase echolocation calls of the bats tested. Call intensity data are from Waters and Jones (1995) and excess atmospheric data are from Bazley (1976). P.p, *P. pipistrellus*; M.b, *M. brandtii*; M.n, *M. nattereri*; P.a, *P. auritus*; R.h, *R. hipposideros*; R.f, *R. ferrumequinum*.

Schiolten *et al.* (1981) have measured the resonant frequency of the tympanum of *A. segetum* to be 25±5 kHz, in good agreement with the best frequency of 25 kHz from this study. Surlykke and Miller (1982) have also produced an audiogram for *A. segetum* showing a best frequency of 30 kHz at 51 dB SPL, some 11 dB less sensitive than that measured in this study. The difference may have arisen as a result of the different stimulus protocol used (5 ms pulses delivered at 10 Hz, as opposed to 10 ms pulses delivered at >1 Hz, this study) or of time spent in culture, as this is known to affect audiogram variability (Cardone and Fullard, 1988) and auditory interneurone morphology (Pallas and Hoy, 1986).

The data on frequency sensitivity support the hypothesis that bats using echolocation calls with a high peak frequency should be less acoustically apparent than calls with a low peak frequency.

A reduction in the stimulus duration results in a reduced sensitivity of the tympanic preparation. This reduction closely fits the function proposed by Plomp and Bouman (1959), which has been fitted to data from a variety of vertebrates (Dooling 1980). In the parakeet *Melopsittacus undulatus*, the value of the integrating time constant is approximately 200 ms (Dooling, 1980) and this value appears to be universally accepted for vertebrates (Green, 1985). The flat part of the curve in Fig. 3 represents the region where only the amplitude of the stimulus has an effect on the auditory threshold. Below this, the function is dependent on the integral of stimulus power over stimulus duration (Adams, 1971). This predicts a line of constant energy of a 3 dB increase in stimulus amplitude with halved duration. This is close to the 2.5 dB found in the present study and correlates with the 2.5 dB determined for *A. segetum* by Surlykke *et al.* (1988), though the integrating time constant of 25 ms from that study is considerably less than that of 69 ms reported here. The discrepancy between the measured integration times may have arisen from the different rise/fall times used by Surlykke *et al.* (1988) of 0.05 ms and the constant 10 % of plateau duration used in this study. The problem with using a constant-duration short time course rise/fall time is that of sidebands which produce frequencies outside that of the carrier (Pye, 1983). These sidebands may stimulate a more sensitive area of the frequency response outside that of the carrier and so produce an erroneous threshold value. The use of slower rise/fall times means that sidebands are reduced, but that stimulus duration is less well defined. Since we have plotted plateau duration against threshold SPL, and used a longer rise/fall time to minimise sidebands, more energy is contained within the pulse than is represented by the duration, and this may lead to a different (and higher) integrating time constant from that obtained by Surlykke *et al.* (1988). The value of 69 ms for the integrating time constant is at odds with the requirements of the moth to detect most FM bats. Many species of bats using FM calls have call durations shorter than 10 ms (data from Obrist *et al.* 1993). A reduction in integrating time constant would allow detection of FM bats at greater range without affecting the ability to detect the generally long-duration calls of CF bats. This level of time integration may be physiologically limiting in that a finite amount of energy may be required to stimulate firing of the A1 cell within the sensillum attached to the tympanic membrane. It may also be that this time constant allows the filtering out of transient noise events caused by the flexion of the tympanal frame during flight or of the noises produced from the wing-beat cycle, which can raise the A1 cell discharge rate (Waters and Jones, 1994). The overall effect of this value of the integrating time constant is to render short-duration calls acoustically less apparent than long-duration calls of the same amplitude (Surlykke *et al.* 1988). It is predicted that bats using short-duration FM calls are acoustically less apparent than those using long-duration CF calls to these moths and that, within FM bats, shorter-duration FM calls also confer an advantage.

P. auritus, *R. hipposideros* and *R. ferrumequinum* have diets which contain a high proportion of Lepidoptera, including noctuid moths (Swift and Racey, 1983; McAney and Fairley, 1989; Jones, 1990). *M. nattereri* and *P. pipistrellus* have diets which contain only a small proportion of moths (Shiel *et al.* 1990; Swift *et al.* 1985). Only the calls of *R. hipposideros* can be considered to be less acoustically apparent on the basis of the combined effects of the frequency and temporal structure. The high-frequency calls of this species at approximately

113 kHz probably render the bat less acoustically conspicuous to the moths since both *A. segetum* and *N. pronuba* have sensitivities at this frequency 32 dB below that at their most sensitive frequency.

The calls of *R. ferrumequinum* and *P. auritus* would be predicted to be acoustically less apparent since these species prey heavily on moths; however, this is not the case considering the combined frequency and temporal data alone. It appears that any advantage of using high-frequency CF calls at 83 kHz in *R. ferrumequinum* is counteracted by the disadvantage of the longer duration of 50 ms. In addition, the calls of *R. ferrumequinum* are moderately intense at approximately 110 dB peSPL at 10 cm (Schnitzler, 1968; Schnitzler and Grinnell, 1977; Konstantinov *et al.* 1973). The calls of *P. auritus* are, however, of comparatively low intensity, between 87 and 95 dB peSPL at 10 cm, compared with the calls of the other FM bats tested, which are generally 103–109 dB peSPL at 10 cm (Waters and Jones, 1995). The lower intensity of the search-phase calls of *P. auritus* probably allows the bat to approach more closely to a noctuid moth before being initially detected, despite the actual call structure being no less acoustically apparent than those of the other FM bats tested (using the threshold criteria we have defined). The net effect of reduced call intensity would thus confer a foraging advantage during aerial hawking. The benefit of low-intensity calls to avoid detection by the moths when this species is gleaning is less easy to predict since tympanate moths exhibit reduced auditory sensitivity at rest when the folded wings cover the tympanic recess (Faure *et al.* 1993). When gleaning, *P. auritus* also often ceases echolocation prior to prey capture and occasionally does so during aerial attacks (Anderson and Racey, 1991). While ceasing echolocation prior to prey capture may prevent the initiation of a last-minute escape response, the low search-phase echolocation call intensity of this species will avoid alerting the moth while the bat is too far from the prey to detect it by passive sounds.

We can estimate the distances at which the A1 and A2 cells would detect the search-phase echolocation calls of the bats tested since the threshold values of the calls are known (this study) and the echolocation call intensities are also available (Waters and Jones, 1995). Using typical excess atmospheric data for the field in the United Kingdom of 12 °C and 80 % relative humidity (Bazley, 1976) and applying it to the peak frequency of the calls recorded, the distance at which the arbitrary threshold we have defined for the A1 cell is elicited can be calculated (the threshold for the A2 cell to the calls is assumed to be 22 dB above that of the A1 cell). These data are presented in Fig. 6. As can be seen, the calls of *R. hipposideros* and *P. auritus* would allow these species to approach more closely to *A. segetum* before being detected. For *R. hipposideros*, this is a consequence of the high frequency of the call at 113 kHz being outside the moths' zone of best hearing, and also the high excess attenuation at this frequency (approximately 3.5 dB m^{-1}), lowering the received intensity at the moth. These factors combine to reduce the overall apparency. For *P. auritus*, the actual call structure is no more or less apparent than those of the other FM bats tested, but it is predicted to allow the bat to approach a moth more closely by virtue of the low emitted intensity.

The ability of *R. ferrumequinum* to capture noctuid moths remains problematic, since Lepidoptera account for almost 100 % of faecal volume in early summer (Jones, 1990). Airapet'yants and Konstantinov (1973) have reported that individuals of this species may switch off their echolocation in the final approach to a flying insect. This would allow the bat to pursue its prey using passive acoustic cues, which may explain the marked sensitivity to lower ultrasound frequencies outside its normal echolocation frequencies (Neuweiler, 1970). This startling observation has never been repeated however. Ceasing echolocation prior to prey capture is documented in bats which hunt for prey on the ground or foliage (Bell, 1982; Neuweiler, 1990), but rarely occurs during active aerial hawking (Anderson and Racey, 1991).

This study cannot address the problems of call plasticity dependent on foraging situation or phase of prey capture. Gleaning bats, such as *P. auritus* and *M. nattereri*, are expected to reduce call duration considerably prior to prey capture from a surface (Faure *et al.* 1990, 1993; Faure and Barclay, 1994), as well as reducing call intensity (Neuweiler, 1990). While this will reduce the overall apparency of the call, it may have an additional effect in that it is unclear which level of activity from the A1 cell is necessary to trigger the negative phonotactic escape mechanism, although indirect evidence suggests it is close to the threshold level of the A1 cell (Roeder, 1967*b*). Since shorter-duration stimuli produce fewer A1 action potentials (Faure *et al.* 1993), this may reduce the apparency of the call still further since the A1 cell response to the call may be lost in the noise from the cell's spontaneous activity.

The data presented here suggest that the bats studied use two mechanisms to reduce their call apparency. *R. hipposideros* uses high-frequency calls which render them less acoustically conspicuous to noctuid moths with their lower-frequency biased auditory systems. In addition, the high frequency of the CF call means that excess atmospheric attenuation reduces the received intensity at the moth. These factors combine to render the call less apparent, and would allow the bat to approach the moth more closely before being detected. *P. auritus* uses a call structure which, at least in the search phase, is no more or less apparent than those of other FM bats, but which is much lower in amplitude. The lowered amplitude would allow this species to approach noctuid moths more closely before being detected.

The long duration of CF calls is a consequence of the need to detect frequency and amplitude glints from flying prey (Schnitzler *et al.* 1983). This increased duration, however, imposes a cost to the bat in that these calls are also more apparent to tympanate moths. The evolution of higher-frequency CF calls may thus have been driven by the need to counteract this increased apparency. A positive correlation has been found between call frequency and the proportion of Lepidoptera in the diet for CF bats from the families Rhinolophidae and Hipposideridae (Jones, 1992). The option

of raising the call frequency may not be available to FM bats since the amount of echo energy returning to each frequency bin in the cochlea will be severely reduced by atmospheric attenuation. Since virtually all the energy of the call of a CF bat returns to the same frequency bin, CF bats may be able to exploit a greater detection range (Neuweiler, 1983). Reducing call duration over the range 4–2 ms does not have a significant effect on the apparency of FM calls at the peripheral level, though the encoding of information in A cell action potentials may be limited at very short durations. In these instances, reducing emitted call intensity appears to be the only available option to gain a foraging advantage over tympanate moths.

D.A.W. was supported by a Science and Engineering Research Council studentship. G.J. was supported by a Royal Society University Research Fellowship. Thanks to Drs Innes Cuthill and Henry O. Null for statistical advice. We thank English Nature for providing the licences to undertake this work. Comments from two anonymous referees are gratefully acknowledged. Moths were kindly supplied by DowElanco Europe.

References

ADAMS, W. B. (1971). Intensity characteristics of the noctuid acoustic receptor. *J. gen. Physiol.* **58**, 562–579.

AGEE, H. R. (1967). Response of the acoustic sense cell of the bollworm and tobacco budworm to ultrasound. *J. econ. Ent.* **60**, 366–369.

AHLÉN, I. (1981). *Identification of Scandinavian Bats by their Sounds.* Report no. 6. Uppsala, Sweden: The Swedish University of Agricultural Sciences Department of Wildlife Ecology.

AIRAPET'YANTS, E. S. AND KONSTANTINOV, A. I. (1973). *Echolocation in Animals*. Jerusalem: Israel Program of Scientific Translations.

ALTMAN, D. G. (1991). *Practical Satistics for Medical Research.* London: Chapman & Hall.

ANDERSON, M. E. AND RACEY, P. A. (1991). Feeding behaviour of captive brown long eared bats *Plecotus auritus. Anim. Behav.* **42**, 489–493.

BAZLEY, E. N. (1976). Sound absorption in air at frequencies up to 113 kHz. *National Physics Acoustics Laboratory Acoustics Report* no. Ac 74.

BELL, G. P. (1982). Behavioural and ecological aspects of gleaning by a desert insectivorous bat, *Antrozous pallidus* (Chiroptera: Vespertillionidae). *Behav. Ecol. Sociobiol*. **10**, 217–223.

CARDONE, B. AND FULLARD, J. H. (1988). Auditory characteristics and sexual dimorphism in the Gypsy moth. *Physiol. Ent.* **13**, 9–14.

DOOLING, R. (1980). Behaviour and psychophysics of hearing in birds. In *Comparative Studies of Hearing in Vertebrates* (ed. A. Popper and R. Fay), pp. 261–288. Berlin: Springer.

FAURE, P. A. AND BARCLAY, R. M. R. (1994). Substrate-gleaning *versus* aerial-hawking: plasticity in the foraging and echolocation behaviour of the long-eared bat, *Myotis evotis*. *J. comp. Physiol.* A **174**, 651–660.

FAURE, P. A., FULLARD, J. H. AND BARCLAY, R. M. R. (1990). The response of tympanate moths to the echolocation calls of a substrate gleaning bat, *Myotis evotis. J. comp. Physiol.* A **166**, 843–849.

FAURE, P. A., FULLARD, J. H. AND DAWSON, J. W. (1993). The gleaning attacks of the northern long-eared bat, *Myotis septentrionalis*, are relatively inaudible to moths. *J. exp. Biol.* **178**, 173–189.

FENTON, M. B. (1980). Adaptiveness and ecology of echolocation in terrestrial (aerial) systems. In *Animal Sonar Systems* (ed. R. G. Busnel and J. F. Fish), pp. 427–446. New York: Plenum.

FENTON, M. B. AND FULLARD, J. H. (1979). The influence of moth hearing on bat echolocation strategies. *J. comp. Physiol*. **132**, 77–86.

FENTON, M. B. AND FULLARD, J. H. (1981). Moth hearing and the feeding strategies of bats. *Am. Sci.* **69**, 266–275.

FIELDEN, A. (1960). Transmission through the last abdominal ganglion of the dragonfly nymph *Anax imperator*. *J. exp. Biol*. **37**, 832–844.

FULLARD, J. H. (1984). Acoustic relationships between tympanate moths and the Hawaiian hoary bat. *J. comp. Physiol.* A **155**, 795–801.

FULLARD, J. H. (1987). Sensory ecology and neuroethology of moths and bats: interactions in a global perspective. In *Recent Advances in the Study of Bats* (ed. M. B. Fenton, P. A. Racey and J. M. V. Rayner), pp. 244–272. Cambridge: Cambridge University Press.

FULLARD, J. H. (1988). The tuning of moth ears. *Experientia* **44**, 423–428.

FULLARD, J. H. AND BARCLAY, R. M. R. (1980). Audition in spring species of arctiid moths as a possible response to differential levels of insectivorous bat predation. *Can. J. Zool.* **58**, 1745–1750.

FULLARD, J. H., FENTON, M. B. AND FURLONGER, C. L. (1983). Sensory relationships of moths and bats sampled form two nearctic sites. *Can. J. Zool*. **61**, 1752–1757.

FULLARD, J. H. AND THOMAS, D. W. (1981). Detection of certain African, insectivorous bats, by sympatric, tympanate moths. *J. comp. Physiol.* **143**, 363–368.

GREEN, D. (1985). Temporal factors in psychoacoustics. In *Time Resolution in Auditory Systems* (ed. A. Michelsen), pp. 122–140. Berlin: Springer.

JONES, G. (1990). Prey selection by the greater Horseshoe Bat (*Rhinolophus ferrumequinum*): optimal foraging by echolocation? *J. Anim. Ecol*. **59**, 587–602.

JONES, G. (1992). Bats vs moths: studies on the diets of rhinolophid and hipposiderid bats support the allotonic frequency hypothesis. In *Prague Studies in Mammalogy* (ed. I. Horácek and V. Vohrálik), pp. 87–92. Praha: Charles University Press.

JONES, G. AND RAYNER, J. M. V. (1989). Foraging behaviour and echolocation of wild horseshoe bats *Rhinolophus ferrumequinum* and *R. hipposideros* (Chiroptera, Rhinolophidae). *Behav. Ecol. Sociobiol.* **25**, 183–191.

KONSTANTINOV, A. I., MOVTCHAN, V. N. AND MAKAROV, A. K. (1973). Influence of band limited noise on the efficiency of echolocation detection of targets by *Rhinolophus ferrumequinum. Period. Biol.* **75**, 7–11.

MCANEY, C. M. AND FAIRLEY, J. S. (1989). Analysis of the diet of the lesser horseshoe bat *Rhinolophus hipposideros* in the West of Ireland. *J. Zool., Lond.* **217**, 491–498.

MILLER, L. A. (1984). Hearing in green lacewings and their responses to the cries of bats. In *Biology of Chrysopidae* (ed. M. Canard, Y. Séméria and T. R. New), pp. 134–149. The Hague: D. R. W. Junk Publishers.

NEUWEILER, G. (1970). Neurophysiologische Unterschungen zum Echoortungssystem der Großen Hufeisennase *Rhinolophus ferrumequinum. Z. vergl. Physiol*. **67**, 273–306.

NEUWEILER, G. (1983). Echolocation and adaptivity to ecological

constraints. In *Neuroethology and Behavioural Physiology* (ed. F. Huber and H. Markle), pp. 280–302. Berlin: Springer-Verlag.

NEUWEILER, G. (1990). Auditory adaptations for prey capture in echolocating bats. *Physiol. Rev.* **70**, 615–641.

OBRIST, M. K., FENTON, M. B., EGER, J. L. AND SCHLEGEL, P. A. (1993). What ears do for bats: a comparative study of pinna sound pressure transformation in Chiroptera. *J. exp. Biol*. **180**, 119–152.

PALLAS, S. L. AND HOY, R. R. (1986). Regeneration of normal afferent input does not eliminate aberrant synaptic connections of an identified auditory interneurones in the cricket *Teleogryllus oceanicus. J. comp. Neurol.* **248**, 348–359.

PLOMP, R. AND BOUMAN, M. A. (1959). Relation between hearing thresholds and duration for tone pulses. *J. acoust. Soc. Am.* **31**, 749–758.

PRESTWICH, K. N., BRUGGER, K. E. AND TOPPING, M. (1989). Energy and communication in three species of hylid frogs: power input, power output and efficiency. *J. exp. Biol.* **144**, 53–80.

PYE, J. D. (1983). Techniques for studying ultrasound. In *Bioacoustics, a Comparative Approach* (ed. B. Lewis), pp. 39–65. London: Academic Press.

PYE, J. D. (1993). Is fidelity futile? The 'true' signal is illusory, especially with ultrasound. *Bioacoustics* **4**, 271–286.

ROBINSON, M. F. (1990). Prey selection by the brown long-eared bat. *Myotis* **28**, 5–18.

ROEDER, K. D. (1962). The behaviour of free flying moths in the presence of artificial ultrasonic pulses. *Anim. Behav.* **10**, 300–304.

ROEDER, K. D. (1966). Acoustic sensitivity of the noctuid tympanic organ and its range for the cries of bats. *J. Insect Physiol.* **12**, 843–859.

ROEDER, K. D. (1967*a*). *Nerve Cells and Insect Behaviour*. Cambridge, MA: Harvard University Press.

ROEDER, K. D. (1967*b*). Turning tendency of moths exposed to ultrasound while in stationary flight. *J. Insect Physiol.* **13**, 873–888.

ROEDER, K. D. (1974). Responses of the less sensitive acoustic sense cells in the tympanic organs of some noctuid and geometrid moths. *J. Insect Physiol.* **20**, 55–66.

ROEDER, K. D. (1975). Neural factors and evitability in insect behaviours. *J. exp. Zool*. **194**, 75–88.

ROEDER, K. D., TREAT, A. E. AND VANDEBERG, J. S. (1968). Auditory sense in certain sphingid moths. *Science* **159**, 331–333.

SCHIOLTEN, P., LARSEN, O. N. AND MICHELSEN, A. (1981). Mechanical time resolution in some insect ears. I. Impulse responses and time constants. *J. comp. Physiol*. **143**, 289–295.

SCHNITZLER, H.-U. (1968). Die Ultraschall-Ortungslante der Hufeisen-Fledermause (Chiroptera: Rhinolophidae) in verscheiden Orientierungssituationen. *Z. vergl. Physiol*. **57**, 376–408.

SCHNITZLER, H.-U. AND GRINNELL, A. D. (1977). Directional sensitivity of echolocation in the horseshoe bat *Rhinolophus ferrumequinum.* I. Direction of sound emission. *J. comp. Physiol.* **116**, 51–61.

SCHNITZLER, H. U., MENNE, D., KOBER, R. AND HEBLICH, K. (1983). The acoustical image of fluttering insects in echolocating bats. In *Neurophysiology and Behavioural Physiology* (ed. F. Huber and H. Markle), pp. 235–250. Berlin: Springer.

SHEIL, C. B., MCANEY, C. M. AND FAIRLEY, J. S. (1990). Analysis of the diet of natterer's bat *Myotis nattereri* and the common long-eared bat *Plecotus auritus* in the West of Ireland. *J. Zool,. Lond.* **223**, 299–305.

SPANGLER, H. G. AND TAKESSIAN, A. (1983). Sound perception by two species of wax moths (Lepidoptera: Pyralidae). *Ann. ent. Soc. Am*. **76**, 94–97.

STAPELLS, D. R., PICTON, T. W. AND SMITH, A. D. (1982). Normal hearing threshold for clicks. *J. acoust. Soc. Am.* **72**, 74–79.

SURLYKKE, A. (1984). Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *J. exp. Biol.* **113**, 323–335.

SURLYKKE, A. (1986). Moth hearing on the Faeroe islands, an area without bats. *Physiol. Ent.* **11**, 221–225.

SURLYKKE, A. (1988). Interaction between echolocating bats and their prey. In *Animal Sonar: Processes and Performance* (ed. P. E. Nachtigall and P. W. B. Moore), pp. 551–566. NATO ASI Series A, Life Sciences, vol. 156. New York: Plenum.

SURLYKKE, A., LARSEN, O. N. AND MICHELSEN, A. (1988). Temporal coding in the auditory receptor of the moth ear. *J. comp. Physiol.* A **162**, 367–374.

SURLYKKE, A. AND MILLER, L. A. (1982). Central branchings of three sensory axons from a moth ear (*Agrotis segetum*). *J. Insect Physiol*. **28**, 357–364.

SWIFT, S. M. AND RACEY, P. A. (1983). Resource partitioning in two species of vespertilionid bats occupying the same roost. *J. Zool,. Lond.* **200**, 249–259.

SWIFT, S. M., RACEY, P. A. AND AVERY, M. I. (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during lactation. I. Diet. *J. Anim. Ecol.* **54**, 217–225.

WATERS, D. A. AND JONES, G. (1994). Wingbeat generated ultrasound in noctuid moths increases the discharge rate of the bat-detecting A1 cell. *Proc. R. Soc. Lond. B* **258**, 41–46.

WATERS, D. A. AND JONES, G. (1995). Echolocation call structure and intensity in five species of insectivorous bats. *J. exp. Biol.* **198**, 475–489.

WATERS, D. A., RYDELL, J. AND JONES, G. (1995). Echolocation call design and limits on prey size: a case study using the aerial-hawking bat *Nyctalus leisleri. Behav. Ecol. Sociobiol*. **37**, 321–328.

ZAR, G. (1984). *Biostatistical Analysis* (2nd edition). New Jersey: Prentice Hall International Inc.