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THE GLEANING ATTACKS OF THE NORTHERN LONG-EARED BAT, *MYOTIS SEPTENTRIONALIS*, ARE RELATIVELY INAUDIBLE TO MOTHS

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Summary

This study empirically tests the prediction that the echolocation calls of gleaning insectivorous bats (short duration, high frequency, low intensity) are acoustically mismatched to the ears of noctuid moths and are less detectable than those of aerially hawking bats. We recorded auditory receptor cell action potentials elicited in underwing moths (*Catocala* spp.) by echolocation calls emitted during gleaning attacks by *Myotis septentrionalis* (the northern long-eared bat) and during flights by the aerial hawker *Myotis lucifugus* (the little brown bat). The moth ear responds inconsistently and with fewer action potentials to the echolocation calls emitted by the gleaner, a situation that worsened when the moth's ear was covered by its wing (mimicking a moth resting on a surface). Calls emitted by the aerial-hawking bat elicited a significantly stronger spiking response from the moth ear. Moths with their ears covered by their wings maintained their relative hearing sensitivity at their best frequency range, the range used by most aerial insectivorous bats, but showed a pronounced deafness in the frequency range typically employed by gleaning bats. Our results (1) support the prediction that the echolocation calls of gleaners are acoustically inconspicuous to the ears of moths (and presumably other nocturnal tympanate insects), leaving the moths particularly vulnerable to predation, and (2) suggest that gleaners gain a foraging advantage against eared prey.

Introduction

A classic example of predator–prey sensory ecology is the response of tympanate moths to the ultrasonic calls of insectivorous bats. Moths from a variety of families have simple tympanal organs (ears) which detect the echolocation calls of sympatric, foraging bats (Roeder, 1970; Roeder and Treat, 1961; Miller, 1983; Fullard, 1987, 1990).

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Depending upon the intensity of the signal perceived by the moth, an indicator of the proximity of a hunting bat, moths initiate a series of defensive flight manoeuvres to avoid predation (Roeder, 1965; Agee, 1969). Faint echolocation calls are presumably translated by the moth's central nervous system (CNS) to mean that the bat is far away, causing a negative phonotaxic response (i.e. avoidance steering), whereas intense echolocation calls are perceived as coming from a nearby bat and cause the moth to initiate one or more escape (last-ditch) manoeuvres [e.g. looping flights, power dives or sound production (Arctiidae)] (Roeder, 1966; Roeder and Payne, 1966; Fullard, 1987). The selective advantage for moths performing these avoidance behaviours is not trivial since moths that try to evade aerially hunting bats better their chances for survival by up to 40% (Roeder, 1967).

The auditory characteristics of moth ears appear to have evolved to match the echolocation assemblages of sympatric bat communities exerting the heaviest selection (i.e. predation) pressure (Fullard, 1988). The ears of moths are most sensitive (i.e. have their best frequencies, BFs) in the frequency range from 20kHz to 50kHz. This bandwidth corresponds to the range of frequencies used in the intense echolocation calls emitted by aerial insectivorous bats (e.g. 110dB SPL at 10cm; Griffin, 1958). Bats, however, can reduce their acoustic conspicuousness to eared prey (Fullard, 1987; Surlykke, 1988; Faure *et al.* 1990). They can (1) emit calls of lower or higher frequency than the moth's BF range, (2) emit calls that are too short in duration to be reliably encoded by the moth's most sensitive auditory receptor (the A1 cell), (3) emit echolocation calls of low intensity, and thus approach closer without being detected, and (4) cease echolocating altogether and rely on passive sensory cues for prey detection.

Studies on the interactions between bats and moths have, traditionally, examined the relationship from the prey's perspective, focusing on the behavioural and auditory specializations that have evolved as adaptations to escape predation (e.g. Roeder, 1962; Surlykke, 1984; Morrill and Fullard, 1992). Previous studies have also limited their consideration to participants in flight since, until recently, aerial hawking has been the most investigated form of bat foraging behaviour (Fenton, 1990). Fewer studies have considered this interaction from the predator's perspective of trying to reduce the distance at which it first alerts its prey, and with insectivorous bats that employ alternative foraging behaviours (Fenton and Fullard, 1979; Fullard and Thomas, 1981; Faure *et al.* 1990).

Substratum gleaning is a comparatively uncommon style of foraging in which bats detect, locate and capture their prey on surfaces. Although it has been suggested that some gleaning bats use echolocation to detect insects on surfaces (e.g. Schumm *et al.* 1991), most rely on passive sensory cues for prey detection. Many gleaners listen for prey-generated sounds (Tuttle and Ryan, 1981; Fiedler, 1979; Faure and Barclay, 1992), while others use vision (Bell, 1985). The echolocation calls used by gleaning bats have certain features in common (Neuweiler, 1983; Fenton, 1990): (1) maximal spectral frequencies (i.e. peak frequencies) greater than 50kHz, (2) durations less than 2ms, and (3) intensities less than those of aerial-hawking bats (e.g. 'whispering' bats; Griffin, 1958). These acoustic characteristics imply that moths will find gleaning echolocation signals difficult to detect and will be particularly vulnerable to predation by bats that use this form of hunting.

In the present study, we test the prediction that underwing moths (Noctuidae: *Catocala* spp.) cannot adequately detect the gleaning echolocation calls of northern long-eared bats, *Myotis septentrionalis*, as well as the echolocation calls of the aerial-hawking little brown bat, *Myotis lucifugus*, and conclude that bats using a gleaning mode of foraging are able to exploit the sensory co-evolution of moth ears adapted to detect the echolocation signals uttered by aerial insectivorous bats.

Materials and methods

Study area and species

The study was conducted at the Queen's University Biological Station (QUBS), Chaffeys Locks, Ontario, Canada (44° 34′N, 76° 19′W), between June and August 1991. Bats were caught in Tuttle traps (Tuttle, 1974) near QUBS. The species used in our study were the northern long-eared bat (*Myotis septentrionalis*) and the little brown bat (*Myotis lucifugus*). *Myotis septentrionalis* were housed in an outdoor holding cage (3.7 m×3.7 m×2 m) in which food (moths, dragonflies and caddisflies collected from ultraviolet lights at QUBS) and water were available *ad libitum*. In this way, the bats maintained themselves for the duration of the study with minimal disturbance. *Myotis septentrionalis* quickly adapted to captivity and began foraging (using substratum gleaning and aerial hawking) within an hour of their release into the enclosure. The fact that wild *M. septentrionalis* gleaned so readily in captivity demonstrates that gleaning is part of this species' natural foraging behaviour. Individuals of the aerial-hawking species, *M. lucifugus*, were caught as needed. Although the area near QUBS contains other aerial-hawking species (e.g. *M. leibii*, *Eptesicus fuscus*, *Lasiurus cinereus*, *L. borealis*, *Pipistrellus subflavus*), we chose *M. lucifugus* because it was similar in body size to *M. septentrionalis* and had little difficulty manoeuvring in our flight cage.

We chose underwing moths (*Catocala* spp.) to conduct our auditory studies and predator–prey exposure trials. These moths are abundant in the deciduous woodlands near QUBS and their ears are among the most sensitive of moths in this nearctic region (J. H. Fullard, unpublished data). The sugaring technique of Holland (1968) was used to collect the following species: *C. neogama*, *C. subnata*, *C. cerogama* and *C. retecta* (identified using Covell, 1984; Ward *et al.* 1974).

Moth auditory analysis

Moths were fastened dorsum up to a block of modelling clay and extracellular recordings were made of the moth's tympanic nerve (IIIN1b; Nüesch, 1957) with a stainless-steel monopolar hook electrode referenced to a ground electrode in the abdomen (see Fullard, 1984, for complete details). Responses of the most sensitive auditory receptor, the A1 cell, were observed on-line. Auditory thresholds were defined as a stable response of three action potentials per stimulus pulse for four consecutive pulses. This response criterion is more conservative than that used by other authors (e.g. Madsen and Miller, 1987) but resulted in a consistent estimate of threshold (cf. Surlykke, 1984). Replicated audiograms performed to verify the stability of the acoustic test conditions revealed maximum threshold differences of less than 3dB at only a few frequencies.

Thresholds were measured for moths in two situations: (1) with the ipsilateral ear covered by the wing (mimics a resting moth and is relevant to predation by gleaning bats since the ear of a moth is located beneath its wings and would be covered when the moth is at rest), and (2) with the wings outstretched and ears exposed (this mimics a flying moth and is relevant for predation by aerial-hawking bats).

Pulses were produced by feeding a continuous tone from a Wavetek model 23 synthesized function generator into a Coulbourn S84-04 envelope shaper and broadcast through a Technics EAS-10TH400B leaf tweeter. Each moth was tested at stimulus durations of 1, 5 and 10ms (rise/fall=0.2ms, hence total pulse durations were 1.2, 5.2 and 10.2ms, respectively), and with the ears exposed or covered by the wings. Pulses were delivered at a rate of $1\,s^{-1}$. Auditory threshold curves (audiograms) were derived from these responses for stimulus frequencies from 5 to 125kHz. Threshold intensities were determined by converting millivoltages into decibels sound pressure level (dB SPL rms re $20\,\mu Pa$) relative to a continuous tone using a Brüel and Kjær (B&K) type 4138 1/8inch condenser microphone (without protecting grid) coupled to a B&K type 2606 measuring amplifier and a Krohn-Hite model 3500 bandpass filter. Since the rise/fall times for the 1 ms stimulus pulses resulted in a power drop relative to a pulse with no rise/fall time, a correction factor of -1.39dB was applied to thresholds calculated for this duration. Although power losses due to the rise/fall times for the 5 and 10ms stimulus pulses were considerably smaller, correction factors of -0.29 and -0.15dB, respectively, were applied to thresholds obtained at these pulse durations. The entire system, including calibration equations for converting voltages into decibels, was controlled by a customized MS-DOS programme written by J.W.D.

Echolocation recordings and predator–prey exposure trials

Echolocation recordings and moth exposure trials were conducted in an indoor flight cage ($2.2\,m\times2.2\,m\times1.9$m) kept at ambient light conditions. At night, the cage was dimly lit with a CGE 25W Colaramic red bulb to permit observation. Bats were transferred from the holding cage to the flight cage the evening prior to an experiment. The cage walls were lined with plastic, except for one corner where the bats roosted, so that bats started from the same position for each experiment.

A moth auditory preparation was used to compare the auditory responses of *Catocala* spp. to the echolocation calls of gleaning *versus* aerial bats. The dissection was performed outside the cage and, once the tympanic nerve had been hooked by the recording electrode, the preparation was moved into the cage and mounted on a tripod behind a bark-covered vertical trellis. The bark trellis simulated a natural substratum on which to present moths to gleaning *M. septentrionalis*. The tripod allowed us to position the moth neural preparation so that one of its ears faced towards the bats, allowing it to detect echolocation calls.

Myotis lucifugus is an aerial-hawking species and does not glean (except for insects taken off the surface of water, Fenton and Bell, 1979) and emits echolocation calls typical of aerial insectivorous bats. Moth auditory responses to its echolocation calls were recorded as bats flew around the cage (*M. lucifugus* never responded to moth fluttering sounds and never gleaned insects from the bark trellis). *Myotis septentrionalis*, however,

readily took moths from the walls, the ground and our fingers and, by pinning a live, fluttering moth to the surface of the bark beside the moth mounted on the tripod, we could lure bats to hover in front of, and attack moths beside, our auditory preparation. This technique enabled us to record the action potentials evoked by echolocation calls emitted during actual gleaning attacks.

We also recorded the echolocation sounds reaching the moth's ear (for both bat species) by placing a B&K type 4138 microphone beside the auditory preparation. The microphone (with protecting grid) was coupled to a B&K type 2606 measuring amplifier and Krohn-Hite model 3500 bandpass filter. The echolocation calls and tympanic nerve action potentials were recorded on separate channels of a RACAL 4DS instrumentation tape recorder operating at $152\,\mathrm{cm\,s^{-1}}$ (entire system response ±2dB, 0.02–140kHz). Chart recorder print-outs of these exposure trials were used to count the number of action potentials (spikes) evoked per echolocation pulse.

Echolocation calls were analyzed with a customized Fast Fourier Transform (FFT) programme written by J.W.D. using a Cooley–Tukey algorithm (Press *et al.* 1986). The minimum time and frequency resolution of the system were $10\,\mu s$ and 0.8kHz, respectively. Call durations were measured from the oscillograms whereas highest, lowest and peak frequencies were obtained from the FFT spectra. The highest frequency and lowest frequency on the power spectra were arbitrarily defined as the −18dB point from the peak frequency (0dB).

Echolocation intensities were estimated for *M. septentrionalis* during gleaning attacks and from *M. lucifugus* during calling flights (fly-bys) in the cage. Calls were monitored with a B&K type 4138 microphone. The amplitudes of the pulses were measured as voltages (Tektronix 564 storage oscilloscope), while the bat-to-microphone distance was estimated by an observer in the cage. Continuous pure tones generated with a Wavetek model 23 synthesized function generator centred near the PF for each bat and broadcast through a Technics EAS-10TH400B leaf tweeter were used to estimate dB SPLs. The peak-to-peak intensity of the generated signal was adjusted until the output peak-to-peak voltage equalled that emitted by the bat. The SPLs at the recorded distance and from 10cm (i.e. the emitted intensity) were then read from the measuring amplifier.

Statistical analysis

Data are expressed as either median ± 1quartile or mean ± 1standard deviation. Audiograms were analyzed using Friedman's nonparametric randomized block analysis of variance (ANOVA) with a Tukey-type *a posteriori* multiple-comparison test. For purposes of ranking, non-obtainable auditory thresholds (i.e. thresholds that could not be determined within the dynamic range of our system) were arbitrarily assigned the maximum value of 100dB SPL. Spike count and echolocation characteristic data were analyzed using a Mann–Whitney *U*-test (Zar, 1984). To correct for differences in the number of calls emitted per sequence per bat, data were averaged within and then across sequences, and the grand means for each bat were used in the rankings. Owing to non-normality and heteroscedasticity, decibel values were first converted to absolute sound pressure levels before calculating means ± S.D. (reconverting the data to decibels results in asymmetrical S.D. values). All tests employed a rejection criterion of $P \leqslant 0.05$.

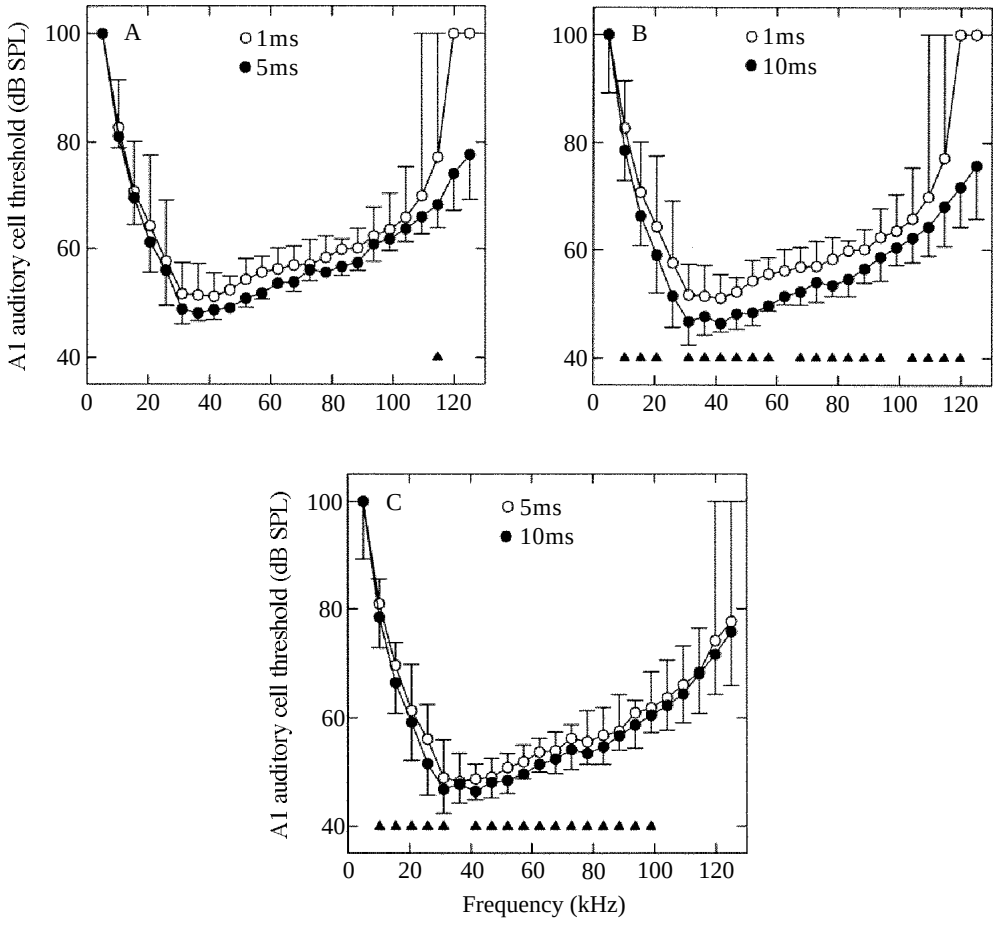

Fig. 1. The effect of pulse duration on hearing sensitivity of *Catocala* moths (N=31). Frequency sensitivity curves (audiograms) at each pulse duration are shown. (A) Comparison of the audiograms obtained with the ears exposed using stimulus durations of 1 and 5ms, (B) 1 and 10ms, and (C) 5 and 10ms. Data shown are median +75% and −25% quartiles. Filled triangles indicate significant differences (P<0.05) at the frequency tested.

Results

The effect of pulse duration and wing placement on hearing sensitivity

Auditory thresholds curves for underwing moths (N=31) at each pulse duration and frequency for the ear exposed are shown in Fig. 1. These thresholds are similar to those reported for other nearctic and palearctic moths (Fullard and Barclay, 1980; Surlykke, 1984) and are higher than those of some other insects (e.g. tachinid flies; Robert *et al.* 1992) and of tropical moths from areas possessing more diverse bat communities (Fenton and Fullard, 1979; Fullard and Thomas, 1981; Fullard, 1988). Thresholds for the 5ms pulses were significantly higher than those for 10ms pulses from 10 to 100kHz (Fig. 1C). Significant losses in sensitivity occurred across a broader bandwidth when thresholds obtained with 10ms and 1ms pulses were compared (Fig. 1B). Although auditory

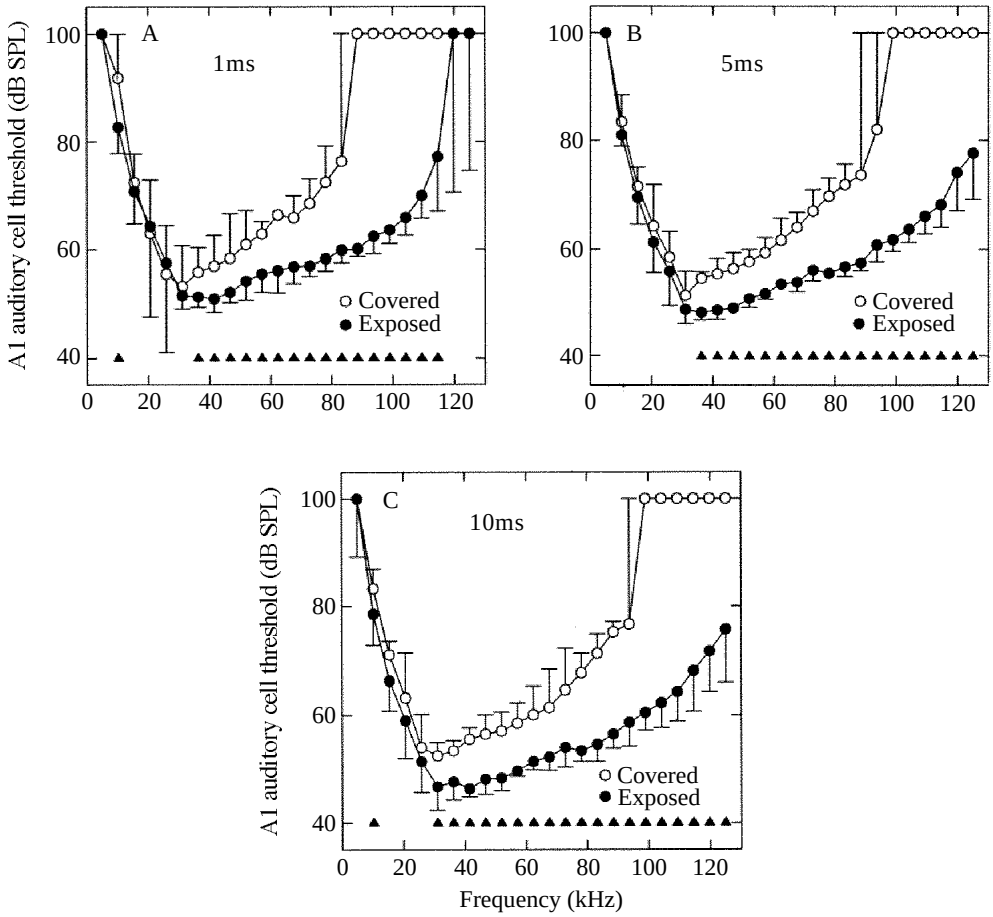

Fig. 2. The effect of wing placement on hearing sensitivity of *Catocala* moths. (A) Comparison of hearing thresholds obtained with the moth's ears exposed (N=31) and with the ipsilateral ear covered by the wings (N=15) using stimulus durations of 1ms, (B) 5ms and (C) 10ms. Data show median +75% and −25% quartiles. Filled triangles indicate significant differences at the frequency tested.

thresholds obtained with 1ms pulses were usually higher than those obtained with 5ms pulses (Fig. 1A), these differences were not statistically significant across any particular bandwidth. We stress that whether the differences at each duration are significant or not depends only upon the ranks of the thresholds. If thresholds at particular durations are consistently higher or lower than those at other durations, significant differences can exist even though these differences may be small (e.g. Fig. 1C).

Fig. 2 illustrates the influence of wing position on hearing sensitivity. When the ear is covered by the wing, significant losses occur at all durations. Although the loss in sensitivity is statistically significant at approximately 35kHz, it is particularly evident at frequencies above 50kHz. Moths with their ears covered maintained hearing sensitivity at their BF range (20–50kHz), even when tested with short stimulus pulses (Fig. 2A).

Table 1. *Echolocation call characteristics of* M. septentrionalis *and* M. lucifugus *in the flight cage*

	Myotis septentrionalis			*Myotis lucifugus*			
Echolocation call variable	Bats tested	Mean	S.D.	Bats tested	Mean	S.D.	*P*
Duration (ms)	6	1.01	0.20	3	1.71	0.58	<0.05
Lowest frequency (kHz)	6	60.69	5.60	3	39.82	4.16	<0.05
Peak frequency (kHz)	6	97.41	7.79	3	56.55	8.52	<0.05
Highest frequency (kHz)	6	126.21	7.56	3	81.78	12.62	<0.05
Bandwidth (−18dB from peak)	6	65.52	6.02	3	41.97	13.40	<0.05
Intensity (dB at 10cm)	6	78.0	+1.9/−2.3	1	95.3	+1.4/−1.6	−

Except for intensity data, all values are averages of 25 pulses per bat (*M. septentrionalis*) or 40 pulses per bat (*M. lucifugus*).

These data demonstrate that bats using pulse durations of 1ms or less will be less detectable than bats employing longer pulse durations (10ms). Additionally, moths with their ears covered by their wings maintain their relative hearing sensitivity at their BF range, but experience profound losses in sensitivity to frequencies greater than 50kHz, when all durations are considered. These data suggest that moths are less sensitive to bat echolocation pulses with peak energies of more than 50kHz, and that this deficiency is most severe when the duration of the pulse is short and the ear is covered by the wing.

Echolocation calls of gleaning versus *aerial insectivorous bats*

Table 1 describes the acoustic characteristics of the echolocation calls emitted by *M. septentrionalis* during substratum-gleaning foraging sequences and by *M. lucifugus* during approaches and calling flights in the cage. Both species emitted short-duration, broad-band, FM signals. However, the pulses employed by *M. septentrionalis* had significantly larger bandwidths (U=18, d.f.=3 and 6) and contained more energy in their upper frequencies than those of *M. lucifugus* (Table 1, Fig. 3). The high-frequency content of *M. septentrionalis*' calls may be even greater than we report since the microphone's protecting grid was left on, out of necessity, during these recordings. The calls of *M. septentrionalis* were significantly shorter in duration (U=18, d.f.=3 and 6), higher in highest frequency (U=18, d.f.=3 and 6), higher in peak frequency (U=18, d.f.=3 and 6) and higher in lowest frequency (U=18, d.f.=3 and 6) than those of *M. lucifugus*. Additionally, the echolocation calls emitted by *M. septentrionalis* were, on average, 17dB less intense than those of *M. lucifugus*. *Myotis septentrionalis* emitted relatively few pulses during gleaning attacks and, on average, bats ceased calling 140.58±58.65ms (N=4 bats, 20 sequences) before attacking the fluttering moth on the bark trellis. Terminal feeding buzzes, the rapid increase in pulse repetition rate associated with aerial prey captures (Griffin, 1958; Simmons *et al.* 1979), were never recorded during gleaning attacks by *M. septentrionalis.* Although *M. lucifugus* never gleaned insects from the bark, they frequently produced a rapid series of pulses (buzzes) when they approached the trellis.

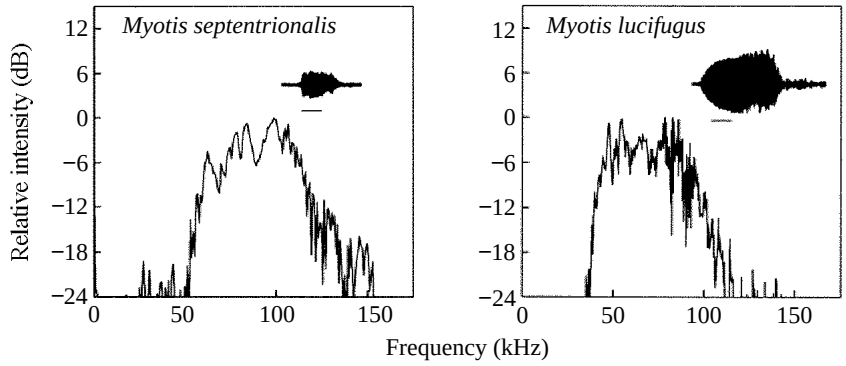

Fig. 3. Fast Fourier Transform spectra of the echolocation calls used by bats in the flight cage. (A) An attack pulse from *M. septentrionalis* during a substratum-gleaning foraging sequence. (B) A call from *M. lucifugus* flying in the cage. Insets: oscillograms of the same calls. Note the difference in amplitude. Scale bars, 500 μs.

Tympanic nerve responses to the echolocation calls of gleaning versus *aerial insectivorous bats*

Fig. 4 displays typical A1 cell responses of a *Catocala* moth to the echolocation calls of *M. septentrionalis* approaching and attacking (Fig. 4A,C) and of *M. lucifugus* as it flew in the cage (Fig. 4B,D). The ear's response to the echolocation calls of *M. septentrionalis* and *M. lucifugus* originated solely from the A1 cell. The least-sensitive cell, A2, did not respond. The calls emitted by *M. septentrionalis* during gleaning attacks were poorly detected by the moth's ear, even when bats hovered less than 50cm away from the front of the neural preparation. Although *M. lucifugus* rarely approached closer than 50cm to the neural preparation, its echolocation calls consistently produced a stronger spiking response from the A1 cell. Even when the pulses emitted by *M. septentrionalis* were larger in amplitude than those of *M. lucifugus*, the calls of *M. lucifugus* usually produced more action potentials. Sometimes the pulses emitted by *M. lucifugus* produced substantial echoes within the flight cage, but these echoes appeared to have little influence on the response of A1 because similar responses were obtained in pulse–nerve interactions of equal amplitude but with little or no echo.

Fig. 5 summarizes the results of the spike count data from 71 bat exposure trials. We used 6 *M. septentrionalis* in 35 trials with the ear exposed (N=646 pulse–nerve interactions) and in 17 trials with the ear covered (N=313 pulse–nerve interactions), and 3 *M. lucifugus* in 17 trials with the ear exposed (N=230 pulse–nerve interactions) and in 2 trials with the ear covered (N=33 pulse–nerve interactions, data not shown in the figure).

Whether one compares the median number of A1 cell spikes per echolocation pulse (Fig. 5A), the median maximum number of A1 spikes elicited during an exposure trial (Fig. 5B) or the median percentage of echolocation calls that elicited less than or equal to 1 spike per pulse (Fig. 5C), the results demonstrate that the echolocation calls of *M. septentrionalis* are significantly less detectable than those of *M. lucifugus.* We suggest that the criterion of no more than 1 spike per pulse can be considered as a non-response,

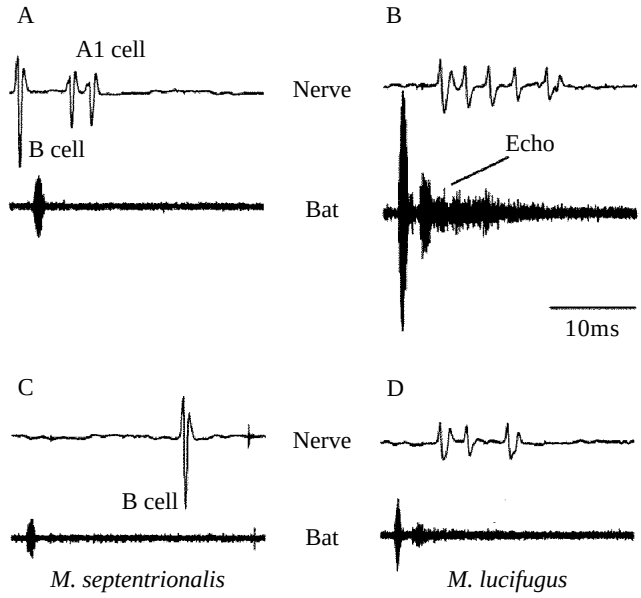

Fig. 4. Representative traces from the bat–moth exposure trials. Each trace displays typical A1 cell responses of *Catocala* moths to the echolocation calls emitted by *M. septentrionalis* (A,C) during gleaning attacks and by *M. lucifugus* (B,D) during calling flights within the cage. This is a qualitative representation of typical A1 responses (for quantitative data and analyses, see Fig. 5) and is not a quantified measure of bat echolocation pulse SPLs (see Table 1). Although gleaning *M. septentrionalis* were almost always closer to the recording microphone (and thus the moth's ear) than aerial-hawking *M. lucifugus*, their echolocation calls were fainter. The larger-amplitude B cell is non-auditory. Scale bar, 10ms.

since the A1 cell regularly exhibits spontaneous firing activity in the absence of acoustic stimuli.

The calls of flying *M. lucifugus* elicited a significantly greater number of spikes per pulse (Fig. 5A) and caused, on average, a significantly larger maximum spiking response than the calls uttered by *M. septentrionalis* during gleaning attacks (Fig. 5B). However, even when the ear was fully exposed, over 50% of the pulses emitted by *M. septentrionalis* during gleaning attacks elicited no more than 1 spike, whereas only 9% of the calls emitted by *M. lucifugus* resulted in a similar response (Fig. 5C). This difference in A1 spiking response was statistically significant.

The gleaning attacks of *M. septentrionalis* elicited significantly fewer A1 spikes when the ipsilateral ear was covered than when it was exposed by the wing (Fig. 5D–E). The average number of spikes per pulse and the maximum number of spikes per pulse were both significantly less for moths with their ears covered by their wings (Fig. 5D,E) and, in both cases, the observed spiking rates were close to (or less than) spontaneous firing rates. Almost 100% of the echolocation calls emitted by *M. septentrionalis* elicited no more than 1 spike when the moths' ears were covered (Fig. 5F), indicating that moths sitting or walking on surfaces with their ears covered by their wings are unable to detect (or detect only poorly) the echolocation calls of gleaning *M. septentrionalis* as they approach.

We also flew one *M. lucifugus* in the flight cage with a moth that had its ears covered by its wings (2 trials). The mean number of spikes per pulse was 4.9, the maximum number of spikes per pulses was 7.0, and the percentage of pulses that elicited 1 spike or fewer was zero.

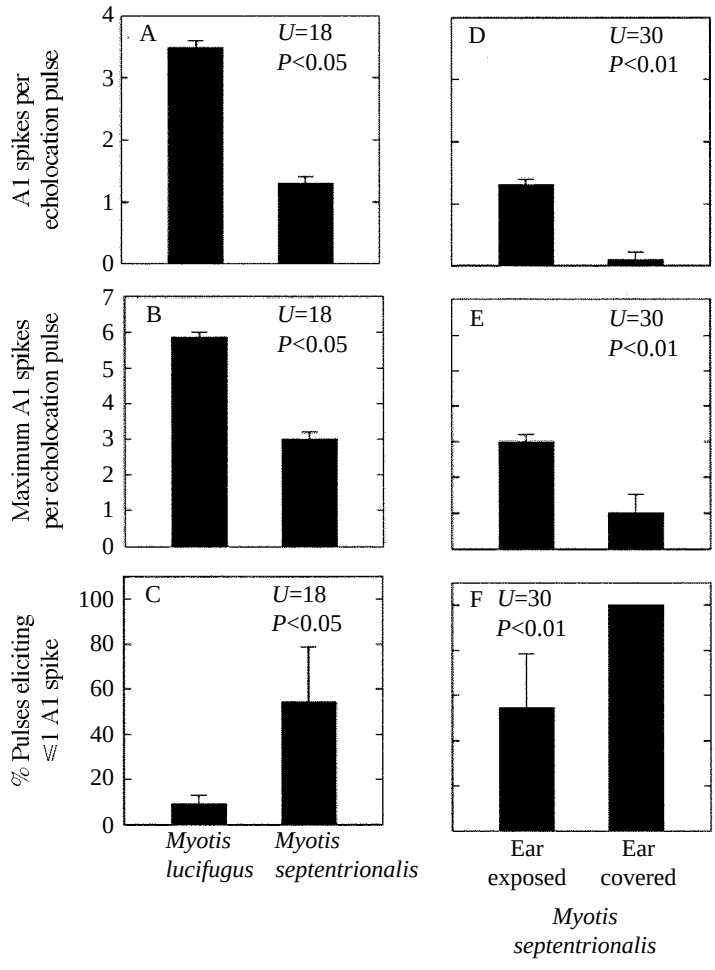

Fig. 5. Summary of the auditory nerve spike count data from the bat–moth exposure trials. (A–C) Comparison of A1 spiking response to the echolocation calls used by *M. septentrionalis* during gleaning attacks (*N*=6) and by *M. lucifugus* while flying in the cage (*N*=3). (D–F). The effect of moth wing placement on A1 spiking response to the echolocation calls of *M. septentrionalis* during gleaning attacks. Left: ears exposed (*N*=6); right: ears covered by the wings (*N*=5). (A, D) The average number of spikes per bat pulse. (B, E). The maximum number of spikes per bat pulse. (C, F) The percentage of bat pulses that elicited 1 spike or fewer. In all cases, data were averaged within an exposure trial and, where multiple exposures per bat occurred, the mean of the averages from each exposure was used as the final value for that bat. The medians of the grand means for all bats are shown. Error bars: ±quartiles for *M. septentrionalis* and ±range for *M. lucifugus.*

Discussion

Our audiogram data predicted that *Catocala* moths would not be able to detect the faint, short-duration, high-frequency echolocation calls of gleaning *M. septentrionalis* as well as the calls of *M. lucifugus*. The data also predicted that moths with their ears covered by their wings (i.e. in a substratum-gleaning situation) would be at a further disadvantage in detecting the calls of gleaning bats as they approached. These predictions were empirically tested in exposure trials with echolocating bats, and the results confirm that the calls used by *M. septentrionalis* are acoustically mismatched to the ears of moths.

Bats using pulse durations of 1ms or less will be harder to detect than bats employing longer pulse durations (e.g. >10ms). However, short pulse durations alone are unlikely to render gleaning bats inaudible since our audiograms, although revealing significant differences between 1 and 10ms stimulus pulses, do not reveal particularly large decibel spreads. Bats should also use echolocation calls outside the BF range of the moth (20–50kHz). This is supported by the observation that moths with their ears covered by their wings maintained their relative hearing sensitivities at their BF range, but experienced significant (and substantial) losses in sensitivity to frequencies greater than 50kHz (i.e. those used by *M. septentrionalis*). This implies that, if gleaners were to emit calls with frequencies used by aerial-hawking bats, they would be more easily detected by the moths. Echolocating *M. lucifugus* continued to elicit a strong A1 spiking response even though the ears of the moth were covered by its wings. However, because our sample size for these trials was small, our conclusions remain tentative.

The echolocation calls uttered by *M. septentrionalis* were shorter in duration, lower in intensity, higher in frequency and broader in bandwidth than those of *M. lucifugus*, and were typical of bats using a gleaning mode of foraging (Neuweiler, 1983, 1990; Fenton, 1990). The functions of these types of signals are multifold. If bats are relying on passive audition for prey detection, these calls may be important for accurate monitoring of the substratum on which the prey is sitting (Faure and Barclay, 1992) since flying and landing in thorny vegetation can be dangerous (Davis, 1968). Alternatively, for bats relying on echolocation for prey detection, high-frequency broad-band calls are ideal for acoustically imaging targets on surfaces (Schmidt, 1988). The use of short-duration signals reduces the problems associated with pulse–echo and echo–echo overlap when approaching close targets (Neuweiler, 1990). Finally, because high frequencies attenuate in the environment more rapidly than low frequencies (Griffin, 1971; Lawrence and Simmons, 1982), the bats' use of broad-band, high-frequency calls in acoustically cluttered environments may be an adaptation to avoid swamping their auditory systems with irrelevant echoes from the distant background (Kober and Schnitzler, 1990).

The frequency, duration and intensity characteristics of the echolocation calls emitted by *M. lucifugus* in our study were typical of aerial-hawking species (Neuweiler, 1983, 1990) and, except for the peak frequency, the frequency variables measured for *M. lucifugus* in our flight cage were similar to values reported for *M. lucifugus* flying freely in the field (Fenton and Bell, 1981). The higher peak frequency recorded for *M. lucifugus* from in the cage in our study compared with that in the field probably resulted from the close proximity of our recording microphone. This would cause an

increase in recording of higher harmonics. No published field data exist on the echolocation characteristics of eastern populations of *M. septentrionalis* for comparison with our cage data, but analyses of echolocation calls recorded from western Canadian populations (Fenton *et al.* 1983) agree with our observations of high frequency. Our values are also very similar to those obtained from field recordings of wild *M. septentrionalis* in Massachusetts (Miller and Treat, 1993).

The moths used in our exposure trials were significantly less sensitive to the calls of gleaning *M. septentrionalis* than to the aerial calls of *M. lucifugus*. In addition, *M. septentrionalis* often ceased echolocating prior to attacking, presumably relying on passive auditory cues (moth fluttering sounds) for prey detection, thereby denying moths the acoustic cues necessary for predator detection. The calls of *M. septentrionalis* rarely elicited more than three auditory spikes per sound pulse, similar to levels of firing reported for congeneric moths exposed to hunting bats (probably *Myotis* and *Eptesicus* spp.) 30–40m away (Roeder, 1962). The moths in our flight cage were never more than 2 m from a bat, and most of the action potentials elicited in response to calling *M. septentrionalis* were from bats that were hovering less than 50cm away or attacking. Even when the ear was fully exposed, the average A1 receptor firing rate was approximately 1 spike per echolocation pulse. These spike rates are no greater than spontaneous firing levels for A1, and should be rejected as background noise by interneurones within the moth's CNS (e.g. Boyan and Fullard, 1988).

The most sensitive cell in the moths' ear, the A1 cell, presumably functions by affecting the moth's flight in such a way as to cause it to move away from a distant, calling bat. At this distance, the bat is probably unaware of the moth because the echo containing this information will be severely attenuated. The effects of the A2 cell, the least sensitive cell, are to disrupt and/or terminate the moth's flight pattern, presumably as a last-ditch evasive response to an attacking bat (Roeder, 1965; Roeder and Payne, 1966). The auditory nerve response elicited by the calls of *M. septentrionalis*, originate solely from A1, so it should activate only the moth's initial avoidance flight circuitry (negative phonotaxis) and it should not terminate the wing or body movements that generate the sounds the bat uses to locate its prey. Furthermore, because moth ears are tone-deaf (Roeder, 1974), they would be unable to differentiate between the faint echolocation calls of an attacking *M. septentrionalis* and those from a more distant *M. lucifugus*, resulting in inappropriate behaviour.

If a moth were sufficiently alerted to the approach of a gleaning bat it might cease walking or fluttering and reduce its body profile so as to deny the bat the acoustic cues (both passive and active) required for prey detection. However, such an adaptive response would require a total cessation of wing and body movements to ensure an adequate defence, since slight movements or sounds are readily detectable by gleaning bats that have momentarily lost sensory contact with their prey (e.g. Faure and Barclay, 1992). Adopting this behavioural tactic does not guarantee the moth's safety, since some gleaners use vision for prey detection when adequate light is available (Bell, 1985), whereas others may use echolocation to detect motionless targets on surfaces (Schumm *et al.* 1991). An alternative strategy for the moth might be to leave the substratum and either fall to the ground or fly away. Resting *Catocala* do adopt this strategy, at least when

attempting to escape from humans trying to capture them, but this defence probably evolved as a generalized response to diurnal predators since *Catocala* are among the most acceptable lepidopteran prey to common woodland birds (MacLean *et al.* 1989).

Insects on surfaces do not appear to take immediate defensive actions as echolocating bats approach (e.g. Belwood, 1990; Faure *et al.* 1990; Yager *et al.* 1990). It appears, therefore, that the neural circuitry controlling the ultrasound avoidance and startle responses of insects is not adapted to the predation threat imposed by gleaning bats. For example, interneurone-1, which is responsible for initiating negative phonotaxis in crickets, does not initiate this behaviour (or initiates it poorly), no matter how strongly it is stimulated, unless the insect is in flight (Nolen and Hoy, 1984). The ultrasound-sensitive T-neurone in katydids (bush crickets) may function in a similar manner (Libersat and Hoy, 1991). Hence, even if a standing or walking insect hears a gleaning bat approaching, the specific behavioural context (i.e. standing or walking *versus* flight) may preclude its neuronal circuitry from producing an effective response. Insects under threat from gleaning bats use passive defences and these have been shaped by natural selection to be minimally acoustically conspicuous. For example, some tropical katydids have adopted intermittent singing, apparently to reduce their detectability to gleaning *Micronycteris* and *Tonatia* spp. (Belwood and Morris, 1987). Moths may have been selected by the predation pressure of gleaners to reduce their incidental noises by remaining still or concealing themselves beneath vegetation (cf. the reduced flight of earless moths, Morrill and Fullard, 1992). In any case, the echolocation calls employed by gleaning bats appear to have imparted little selection pressure on the auditory characteristics of sympatric moths.

Although the echolocation call characteristics of gleaning bats may originally have evolved as adaptations for hunting in cluttered environments (Neuweiler, 1990; Simmons *et al.* 1988), they are, by their acoustic nature, less audible to tympanate prey. Why do all bats not glean? Gleaning requires that bats have short, broad wings with low wing loading and aspect ratios. This allows them to fly slowly, to have high manoeuvrability and good lifting capacity and to be able to hover and carry prey from surfaces (Norberg and Rayner, 1987; Norberg and Fenton, 1988). Hovering flight is metabolically expensive (U. M. Norberg, personal communication) and, when compared with aerial hawking and considering the relatively high abundance of aerial insects, substratum gleaning may be more energetically costly. If this were true, one would expect the total proportion of gleaners in any bat community to be relatively small. Evidence from a number of studies on bat community structure supports this notion (Willig, 1986; McKenzie and Rolfe, 1986; Crome and Richards, 1988).

Why have insects not evolved more sensitive ears to cope with gleaning bats? Natural selection has produced in moths ears tuned to the echolocation call characteristics of the predominant predation potential, that is, aerial-hawking bats. We suggest that the relative scarcity of gleaners (or bats using gleaning behaviour) imparts a selection pressure that is too low to have resulted in auditory adaptations in sympatric moths (and presumably other nocturnal insects) and, in this respect, gleaners may be viewed as predatory ‘cheaters’ in the natural selection mosaic that acts upon the defensive behaviour of eared insects.

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