

Mechanics of a ‘simple’ ear: tympanal vibrations in noctuid moths

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Summary

Anatomically, the ears of moths are considered to be among the simplest ears found in animals. Microscanning laser vibrometry was used to examine the surface vibrations of the entire tympanal region of the ears of the noctuid moths *Agrotis exclamationis*, *Noctua pronuba*, *Xestia c-nigrum* and *Xestia triangulum*. During stimulation with ultrasound at intensities known to activate receptor neurones, the tympanum vibrates with maximum deflection amplitudes at the location where the receptor cells attach. In the reportedly heterogeneous tympana of noctuid moths, this attachment site is an opaque zone that is surrounded by a transparent, thinner cuticular region. In response to sound pressure, this region moves relatively

little compared with the opaque zone. Thus, the deflections of the moth tympanic membrane are not those of a simple circular drum. The acoustic sensitivity of the ear of *N. pronuba*, as measured on the attachment site, is $100 \pm 14 \text{ nm Pa}^{-1}$ ($N=10$), corresponding to tympanal motion of a mere 200 pm at sound pressure levels near the neural threshold.

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Introduction

The ears of insects, and perhaps even their other sense organs, have generally been considered to be simple compared with those of vertebrates. Anatomically, they admittedly are. Yet, the mechanical responses and coding abilities of insect ears can be remarkably complex (for reviews, see Fullard and Yack, 1993; Hoy et al., 1998; Yager, 1999; Robert and Göpfert, 2002; Robert, 2004; Hedwig, 2006). Functionally, hearing in insects is used to find mates and hosts and to avoid predators (Hoy and Robert, 1996; Yager, 1999). The structure and neurobiology of insect ears have benefited from a long and fruitful research history [see a recent topical multi-author review by Robert and Göpfert (Robert and Göpfert, 2004)]. The mechanical aspects of auditory function have also received genuine attention. However, this has often been limited by technological, experimental and analytical difficulties. For example, only recently has it become possible to measure tympanal vibrations with sufficient magnitude resolution and spatial accuracy to characterise the nanoscale mechanics of external microscopic auditory structures (Robert and Lewin, 1998). In effect, as pointed out and calculated earlier by Autrum (Autrum, 1948) and Adams (Adams, 1972), the sensitivity of the insect chordotonal organs involved in hearing lies at the nanometre, if not tens of picometre, level.

Ideally, to understand hearing requires the full characterisation of the chain of mechanical events subtending the capture of acoustic energy; from impinging sound waves to the behaviour of assemblies of auditory neurones, including the mechanical

response of each physiologically uncompromised structural, histological and cellular constituent of the hearing organ. Of all insect ears, those of noctuid moths are considered to be one of the simplest, consisting of a nearly circular tympanum serviced by one (e.g. Notodontidae) or two (e.g. Noctuidae) auditory sensory cells contained within a chordotonal organ (Eggers, 1919; Ghiradella, 1971; Surlykke, 1984). Moths use their ears to detect the echolocation calls of hunting insectivorous bats (Roeder, 1967; Miller and Surlykke, 2001) and, in some cases, the mating signals of conspecifics (Spangler, 1988; Sanderford et al., 1998; Yack et al., 2000). The moth ear is also considered ‘simple’ because of the scant number of auditory neurones, a condition deemed to preclude the ability to discriminate between the frequencies of an incident sound wave (Suga, 1961; Roeder and Treat, 1961). Another reason to invoke simplicity is that moth ears appear to operate as pressure receivers without additional structural specialisations (but see Fullard, 1984) that condition the sound input to the structure effectively converting sound energy into mechanical energy (typically a tympanum). Such a structure, for instance, can be a specialised tracheal system that serves to amplify or phase delay sound waves, as shown, notably, for bushcrickets (Lewis, 1974; Michelsen et al., 1994; Hoffmann and Jatho, 1995). Compared with the early attention spent on the external morphology of the moth tympanic region (Richards, 1932; Treat, 1959; Minet and Surlykke, 2003) and the histology of the chordotonal sensory cells of the auditory organ (Eggers, 1919; Ghiradella, 1971; Yack, 2004), little is known about the biophysical characteristics of the tympanum

itself (but see Rodríguez et al., 2005). Swinton (Swinton, 1877) described the moth 'membranica tympanica' as 'extremely tense, beautifully iridescent and of great tenuity'. Roeder and Treat (Roeder and Treat, 1957) described the 'confusing and obscure' nature of the attachment of the auditory organ to the noctuid tympanum and reported the surprising observation that a tear across the tympanum did not appreciably change the responses of the auditory receptor cells. Ghiradella (Ghiradella, 1971) described the external attachment site (stigma) of the auditory sensilla in the noctuid *Feltia subgothica* as a dark spot surrounded by an 'opaque patch' composed of epithelial cells lining the internal surface of the tympanic membrane. The entire area comprising the stigma, opaque patch and surrounding transparent membrane extending to the cuticular tympanic frame is traditionally referred to as the tympanic (or tympanal) membrane [referred to as 'echtes Trommelfell' in Eggers (Eggers, 1919) and 'membrane tympanique' in Kiriakoff (Kiriakoff, 1956)] (see also Richards, 1932; Roeder and Treat, 1957; Ghiradella, 1971).

The minimal architecture of the noctuid tympanum has prompted some authors to use its anatomy as the main determinant of the ear's frequency tuning (Fullard et al., 1998) and sensitivity (Surlykke et al., 1999). Using laser vibrometry, Fullard et al. (Fullard et al., 1998) described non-linear intensity responses in the tympana of two species of notodontid moths to argue that mechanical factors were a probable cause of the non-monotonic firing pattern of the auditory sensilla reported by Coro and Pérez (Coro and Pérez, 1983). A recent study by Windmill et al. (Windmill et al., 2006) has shown that the mechanical response of the ear of the noctuid *Noctua pronuba* is variable, changing its resonant frequency as a function of the amplitude of the incident sound intensity.

Past studies have assumed that the entire moth tympanum vibrates in a unimodal, drum-like fashion when acoustically stimulated (Schiolten et al., 1981). However, a recent study by Rodríguez et al. (Rodríguez et al., 2005) on the pyralid moth (*Achroia grisella*) made a significant contribution by describing the predicted unimodal vibration using two transects each of three points (dorsal–ventral and anterior–posterior). An outline of tympanal deflection shapes was provided along two orthogonal axes intersecting at the centre of the membrane. Using high-resolution scanning laser Doppler vibrometry, another study revealed the detailed tympanal vibrations in other tympanate insect species; the desert locusts *Schistocerca gregaria* and *Locusta migratoria* (Orthoptera; Acrididae) (Windmill et al., 2005). In locusts, it was shown that the mechanical response of the eardrum to incident sound waves generates a series of travelling waves, which convey energy to the distinct attachment sites of the auditory sensory neurones. The geometry of these waves differs as a function of sound frequency, collecting and delivering sound energy – *via* the travelling wave – towards the attachment sites. The locust tympanum was thus shown not to undergo vibrations resulting from the oscillations of a circular drum. The question then arose as to what, in effect, happens mechanically to a 'simple' tympanal system, i.e. that of moths.

Here, we examine the tympanal response in four species of European noctuids: *Agrotis exclamationis* (Linnaeus 1758), *Noctua pronuba* (Linnaeus 1758), *Xestia triangulum* (Hufnagel

1766) and *Xestia c-nigrum* (Linnaeus 1758) (Lepidoptera; Noctuoidea; Noctuidae). Tympanal deflection shapes are analysed and shown here for the first three species. By analysing the mechanical deflections of the entire tympanum with high spatial resolution (Windmill et al., 2005), we establish that only the central, opaque zone of the tympanum vibrates in response to sound, while the translucent area surrounding this zone remains relatively still at biologically relevant sound frequencies and intensities. The mechanical sensitivity of moth ears is exquisite, with suprathreshold vibration amplitude below one nanometre. These findings challenge the conventional idea of a tympanum moving like the skin of a drum, also questioning the exact function of the moth tympanum. It is thus becoming apparent that moth ears are not simple auditory systems, even though they may well be one of the simplest found in the animal kingdom.

Materials and methods

Animals

Moths for this study were captured in a field outside the Leigh Woods nature reserve, near Bristol, UK, at location 51°27'20.88" N, 2°38'22.93" W. Moths were attracted after 22.00 h using white cotton sheet illuminated by ultraviolet fluorescent lamps. Moths were identified according to Koch (Koch, 1988) and Skinner (Skinner, 1998). Individuals were placed into cages and supplied with sugar water and used the next morning. No moths were tested more than 24 h following capture. Moths to be tested had their legs and wings removed and were then fastened upside down onto a drop of molten wax and mounted onto a platform that was positioned in line with the laser beam of the microscanning laser vibrometer. To allow for an unobstructed path for the laser beam, the abdominal hood and a portion of the cuticular cup that forms the tympanic recess were removed. No fluid escaped from the body during these operations. Control measurements on intact animals have shown that tympanal motion was not affected by this procedure.

Electrophysiological recordings

The action potentials of the auditory receptors in moth tympanal nerves (IIIN1b) (Nüesch, 1957) were recorded with a stainless-steel hook electrode referenced to another in the moth's abdomen. Neural responses were amplified (Grass Instruments P-15 Pre-amplifier; Astro-Med, West Warwick, RI, USA) and digitally acquired (sampling rate of 204.8 kHz) using one channel of the laser vibrometry data management system (PSV-300-F; Polytec, Waldbronn, Germany).

Mechanical analyses

Tympanal vibrations were examined in response to wideband (chirp) signals (1–30 kHz and 20–80 kHz). The acoustic signals were generated by a PCI data acquisition board (PCI-4451; National Instruments, Austin, TX, USA), amplified (Amplifier Model TAFE570; Sony, Tokyo, Japan) and passed to either of two loudspeakers (1–30 kHz, ESS AMT-1; ESS Laboratory Inc., Sacramento, CA, USA; 20–80 kHz, SS-TW100ED; Sony). Vibration velocities were measured using a microscanning laser Doppler vibrometer (PSV-300-F; Polytec) with an OFV-056 scanning head fitted with a close-up attachment. This allowed the laser spot (~1 µm diameter) to be positioned with an

accuracy of $\sim 5 \mu\text{m}$. Measurements across the entire tympana could be taken without readjusting the position of any component in the experiment. A single scan would consist of >500 measurement points in an array fitted to the shape of the specimen's membrane, or part thereof. The laser spot position was monitored *via* a live video feed to the vibrometer's controlling computer. The laser vibrometer thus allowed accurate measurement of the topography of tympanal motion in a contact-free way, without requiring the use of a reflective medium. Laser and microphone signals were sampled at rates of up to 204.8 kHz (rectangular window). Usually, 10–25 windows were averaged and subsequently transformed to the frequency domain by means of fast Fourier transforms (FFTs) (frequency resolution, 12.5 Hz).

The loudspeaker was positioned ~20 cm from the experimental animal. All moths were positioned ventral side up and had their left tympanum scanned. All experiments were carried out on a vibration isolation table (TMC 784-443-12R; Technical Manufacturing Corp., Peabody, MA, USA) at room temperature (24–26°C) and relative humidity of 40–62%. The vibration isolation table with the animal and the laser vibrometry measurement head were located in an acoustic isolation booth (IAC series 1204A; internal dimensions, length 4.50 m, width 2.25 m, height 1.98 m; Industrial Acoustics, Bronx, NY, USA). In line with the mechanical measurements, the sound pressure next to the animal's position was always recorded using a Brüel & Kjær Type 4138 pressure-field microphone and Brüel & Kjær 2633 preamplifier (Brüel & Kjær, Nærum, Denmark). The microphone's sensitivity was calibrated using a Brüel & Kjær sound level calibrator (4231, calibration at 1 kHz, 94 dB SPL). A calibrated stimulus sound level, at the tympanum, of 63 mPa (70 dB SPL) was used throughout these experiments, except for Fig. 2A, where a level of 20 mPa (60 dB SPL) was used. These levels of stimulus cause the insect's ear to mechanically tune to the higher frequency levels, as first reported by Windmill et al. (Windmill et al., 2006). Computer correction of the stimulus signals ensured that their amplitude was kept to a constant level across each range of frequencies. Frequency spectra of the laser signal were normalised to those of the microphone signal by the computation of transfer functions, calculated as the cross-power spectrum of the laser and the microphone signals divided by the auto-power spectrum of the latter. In addition, the amount of unrelated noise was estimated by calculating the magnitude-squared coherence (the ratio between the squared absolute value of the cross-power spectrum between the two signals divided by their auto-power spectra). Coherence values can range between zero and one, with a value of one indicating the absence of external, unrelated noise. Data were considered of sufficient quality when coherence exceeded 85%.

The mathematical calculations for the comparison of moth and microphone membrane tension were carried out using LabVIEW 8.2 (National Instruments).

Results

Tympanal morphology

The external tympanic region of one of the moths in our study (*N. pronuba*) is illustrated in Fig. 1. An opaque membrane, the conjunctivum (Cj) lies distal to the tympanic membrane (TM;

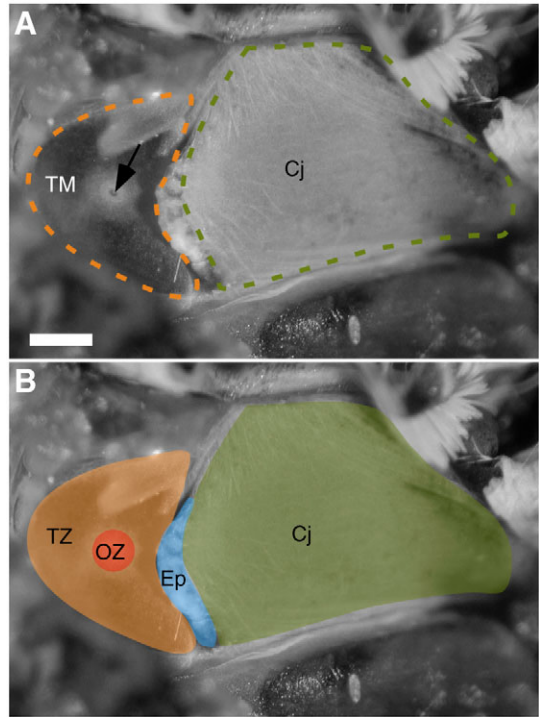


Fig. 1. (A) Close-up of the tympanum of the ear of *Noctua pronuba*. An opaque membrane, the conjunctivum (Cj), is situated distally from the tympanic membrane (TM), both of which lie above the tympanic cavity at the posterior lateral margin of the metathoracic segment. The stigma (arrow) marks the attachment site of the auditory receptor organ. Scale bar, 0.25 mm. (B) Morphological features of the tympanal system, highlighted as they pertain to the vibrational characteristics of the tympanum. The TM, *sensu stricto*, is bordered distally by a nodular sclerite, the epaulette (Ep), which separates it from the Cj. The attachment site of the auditory chordotonal organ (arrow in A) appears as a dark spot at the centre of the opaque zone (OZ), which is surrounded by the transparent zone (TZ).

Fig. 1A; orange surface area in Fig. 1B), both of which reside within the tympanic cavity at the posterior lateral margin of the metathoracic (MT) segment. The tympanic membrane consists of two distinct anatomical zones, a transparent zone (TZ) surrounding a central opaque zone (OZ) that, in turn, encloses the stigma (Fig. 1A, arrow), the external manifestation of the attachment site of the auditory receptor organ (Ghiradella, 1971). The epaulette (Ep) is a nodular sclerite that separates the conjunctivum from the tympanic membrane partially overhanging the transparent zone along its distal margin. The epaulette is believed to be a protective device against parasitic mites, and its morphology is quite variable amongst noctuids (Treat, 1975).

Mechanical analyses

The entire tympanic region of each moth is accessible to optical vibration analysis using a microscanning laser Doppler vibrometer. This allows hundreds of measurement points to be selected and repeatedly measured during acoustic stimulation. The mechanical response of the entire sound-receiving structure can thus be established, for instance, in the

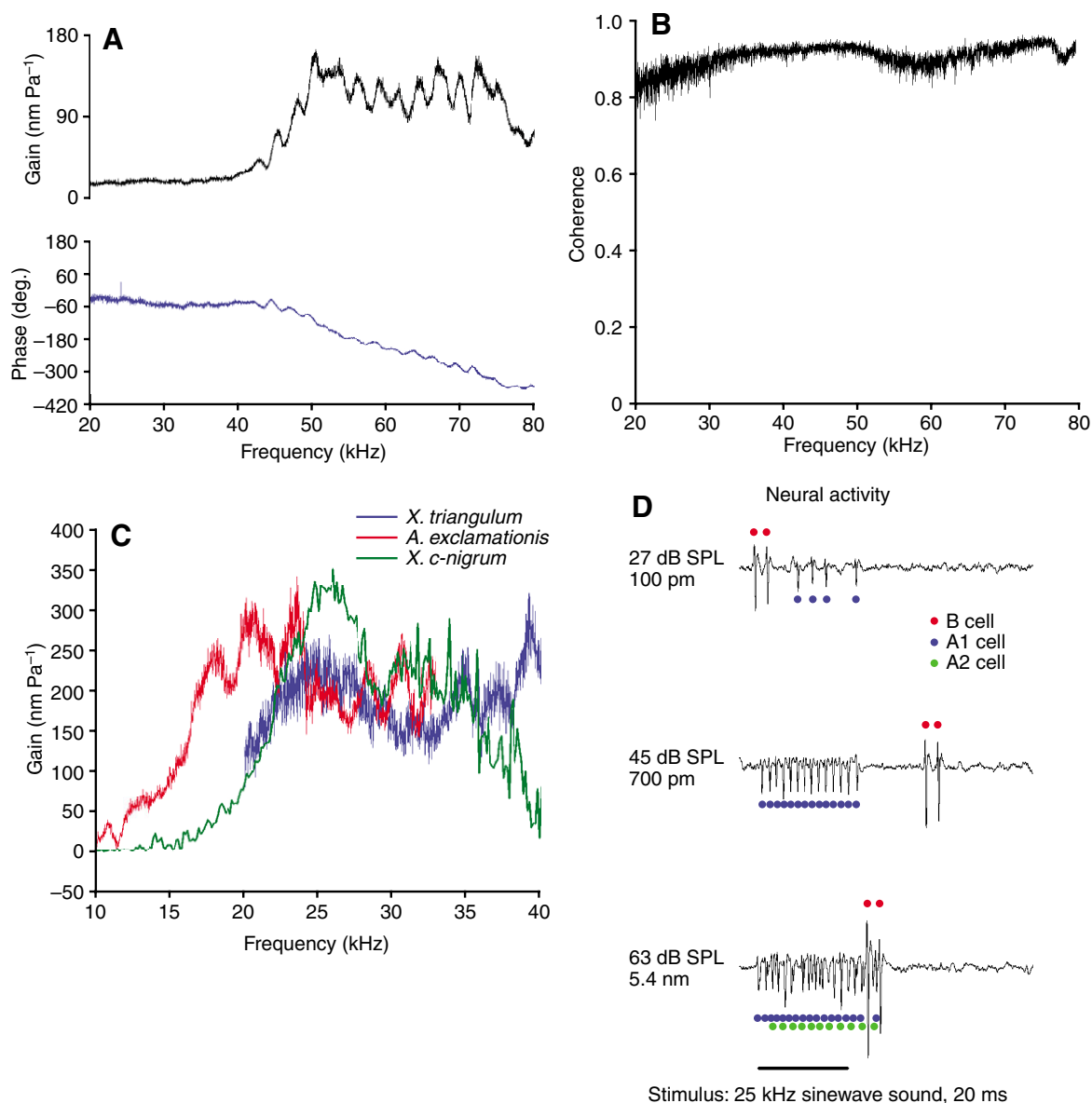


Fig. 2. Response of the moth tympanic membrane to sound. (A) The amplitude-phase gain response of the transparent zone (TZ) of *N. pronuba*. (B) The coherence signal for the measurement in A. (C) The amplitude gain response of the TZ of *A. exclamatoris*, *X. c-nigrum* and *X. triangulum*. (D) Measurement of the auditory neural activity of *X. c-nigrum* combined with the laser vibrometric measurement of TZ displacement (A1 and A2: auditory mechanosensory neurones).

frequency domain, as a gain (or magnitude) and phase response (Fig. 2A). The quality of each point of measurement is then evaluated by estimating the magnitude-squared coherence across the frequency range in use, as shown in Fig. 2B. Each measurement point thus provides coherent (reliable) amplitude and phase data on the mechanical response of the membrane at that spatial position. The mechanical response of the attachment site of the auditory receptor organ to the TM (centre of OZ in Fig. 1B) for *N. pronuba* was measured in response to broadband (20–80 kHz) acoustic stimulation and evaluated as an amplitude-phase frequency spectrum (Fig. 2A). The amplitude response, displayed as the response gain (nm Pa⁻¹), shows a flat, very low response from 20 to 40 kHz, then a relatively sudden

increase to a regime indicating much larger response fluctuations from 45 to 80 kHz. The resonant frequency (phase lag of -90° in a displacement measurement) in this insect is at approximately 50 kHz. A gain of 100 nm Pa⁻¹, as seen in Fig. 2A, at a steady-state sound level of 60 dB SPL (20 mPa), equates to a vibration amplitude of 2 nm. Thus, by linear extrapolation, at a sound level of 40 dB SPL, at which the sensitivity threshold of the A1 cell of *N. pronuba* was recorded (Waters and Jones, 1996), the tympanic vibration amplitude amounts to a mere 200 pm. The mechanical response of the neural attachment point was evaluated for different species of noctuids (Fig. 2C). The response spectra highlight some minor but notable variations, such as the higher response to lower frequencies (down to 15 kHz) in *A.*

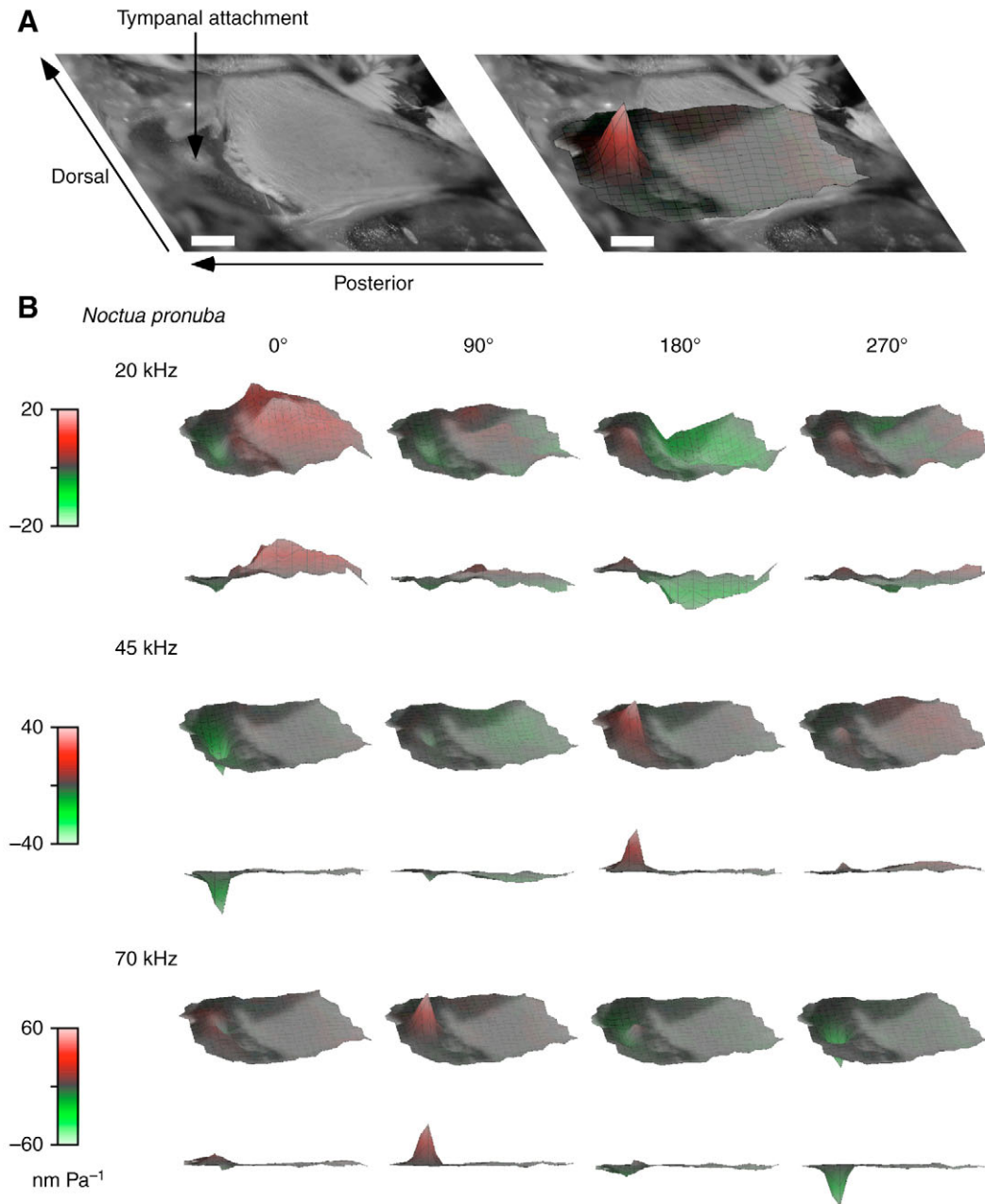


Fig. 3. Area scan and deflection shapes of the tympanic membrane (TM) in *N. pronuba*. (A) Orientation image relating tympanal topography (left image) to the position of the scanning lattice (right image). (B) Area scans of tympanal deflections for three different frequencies. The deflections are shown each time for four different phases along the oscillation cycle (see also Movies 1–3 in supplementary material). Deflections are additionally shown as profiles, looking at the tympanum from its side. Red indicates positive displacement (or outward tympanal deflections), and green indicates negative displacement (or inward tympanal deflections).

exclamationis, and the distinct resonant frequencies. These results were used to focus the analysis of tympanal motion on the relevant frequencies.

The broadband gain amplitude, and phase response, of the attachment site were measured for 10 *N. pronuba* individuals. At a frequency of 20 kHz, the mean amplitude gain was 6.37 ± 0.777 nm Pa⁻¹ (\pm s.d.), at 45 kHz the gain was 28.83 ± 5.23 nm Pa⁻¹, at 70 kHz it was 53.65 ± 9.885 nm Pa⁻¹ and at the mean resonant frequency it was 105.8 ± 14.336 nm Pa⁻¹. The mean resonant frequency for the 10 individuals was found

to be 61.21 ± 3.08 kHz. The same set of measurements was also taken on the centre of the conjunctivum on eight individuals. The mean resonant frequency was found to be 20.39 ± 0.31 kHz with an amplitude gain of 36.375 ± 7.78 nm Pa⁻¹.

In order to document the amplitude range of tympanal deflection that is relevant to the process of hearing, neural recordings were taken from the tympanal nerve as described above, using a hook stainless-steel electrode. In effect, the high sensitivity of the laser vibrometry technique reports vibration in the picometre range of some tympanal elements, yet their

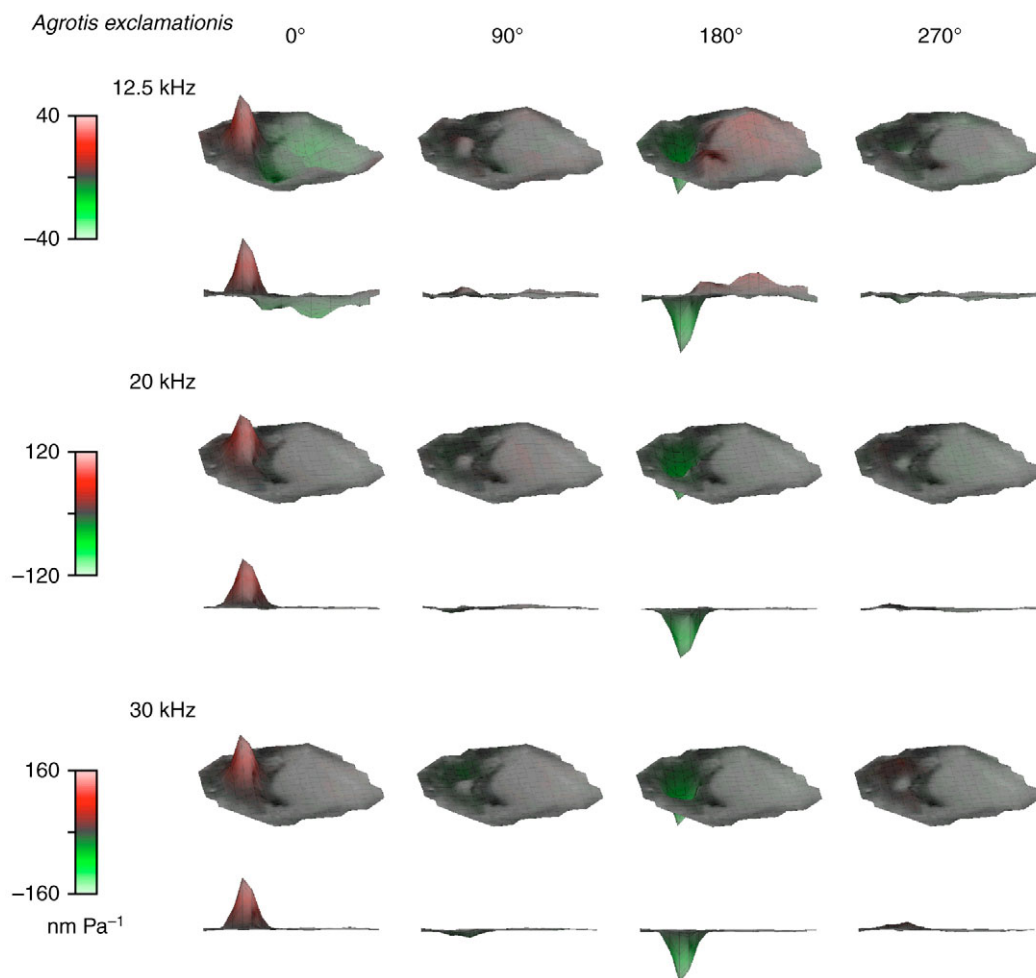


Fig. 4. Area scan and deflection shapes of the tympanic membrane (TM) in *A. exclamationis*. Area scans of tympanal deflections for three different frequencies. The deflections are shown each time for four different phases along the oscillation cycle. Deflections are additionally shown as profiles, as if looking at the tympanum from its side. Red indicates positive displacement (or outward tympanal deflections), and green indicates negative displacements (or inward tympanal deflections).

relevance to the process of hearing requires a direct evaluation. In Fig. 2D, neural activity is represented by the A1 receptor cell at tympanal vibrations as low as 100 pm and is then augmented by firings of the A2 cell at amplitudes above 700 pm.

The entire tympanic region was scanned on three different species of moth, with examples shown in the corresponding figures: *N. pronuba* (Fig. 3), *A. exclamationis* (Fig. 4) and *X. triangulum* (Fig. 5). The response gain of the membrane was evaluated for three different frequencies for each moth and is shown for each frequency, at four different phase angles, 90° apart, in the cycle of that frequency. The scan area data for each frequency is shown in both relief and profile views. Different frequencies were used for each species, as each moth species – and individual – exhibited a slightly different response spectrum.

The high-resolution three-dimensional reconstruction of the laser Doppler data reveals the patterns of motion of the noctuid tympanal system. For *N. pronuba*, the data show both the tympanal membrane (TM) and the conjunctivum (Cj) vibrating at the lower frequency of 20 kHz, but in anti-phase (see Fig. 3).

At the higher frequency of 45 kHz the motion on the Cj is much reduced, and at 70 kHz there is no discernible Cj motion. Further, the recorded peak gain of the motion of the TM increases threefold from that measured at 20 kHz. These results clearly show that the vibration of the TM is not that of a simple 'drum' in its fundamental mode. Rather surprisingly, only the area around the point of sensory attachment (OZ; Fig. 1B) appears to undergo motion.

Tympanal deflections in *A. exclamationis* (Fig. 4) show a much lower frequency response range than that of *N. pronuba*. Here also, the motion of the Cj is only discernible at the lower frequency and is in anti-phase with the motion of OZ. Further, the gain increases with increasing frequency. For higher frequencies (≥ 20 kHz), only OZ displays significant motion, but, notably, the rest of the TM does not appear to move either. Similar data were also gathered for *X. triangulum* (Fig. 5). Motion of Cj decreases as the frequency increases and is in anti-phase with OZ, as only the central portion of the TM displays any motion. Hence, this pattern of mechanical response is consistent across the noctuid species investigated in this study.

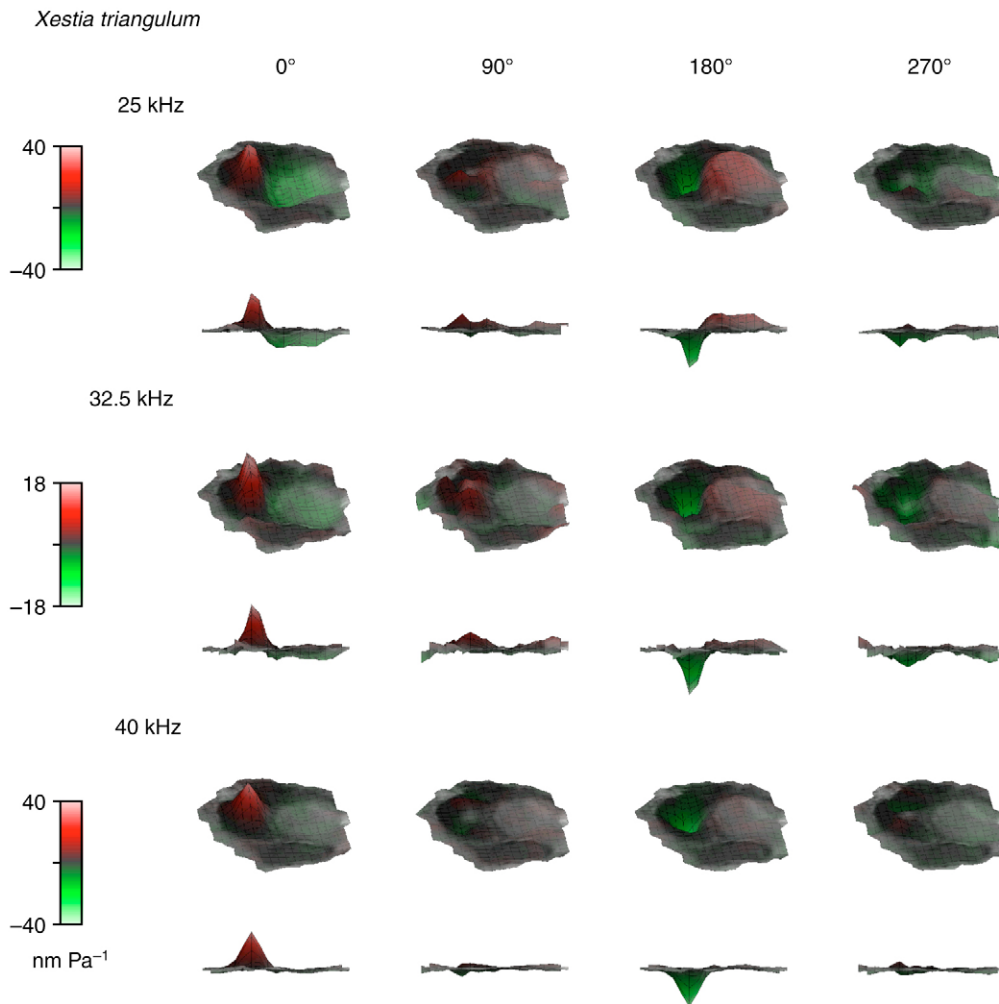


Fig. 5. Area scan and deflection shapes of the tympanic membrane (TM) in *X. triangulum*. Area scans of tympanal deflections for three different frequencies. The deflections are shown each time for four different phases along the oscillation cycle. Deflections are additionally shown as profiles, as if looking at the tympanum from its side. Red indicates positive displacement (or outward tympanal deflections), and green indicates negative displacement (or inward tympanal deflections).

In order to further characterise the different deflection shapes described, high-resolution transect line scans were taken across both the TM and Cj of *N. pronuba*. Examples of the results of this experiment are shown in Fig. 6. The envelope of the motion across a transect becomes apparent when the instantaneous response is displayed for every 10° of phase through a full cycle of oscillation. A transect line was taken across the TM and Cj together for three different frequencies (see blue line in Fig. 6A). At 20 kHz (Fig. 6B), Cj shows a greater deflection than OZ and is moving as a large 'drum-like' membrane, exactly out of phase with OZ. At 45 kHz (Fig. 6C), OZ shows much greater motion than Cj. The Cj motion appears to show a nodal line. The Cj membrane could be moving in a higher mode, given that the driving frequency is much higher than the mean Cj resonance of 20.39 kHz. At 70 kHz (Fig. 6D), the motion of the transect is clearly dominated by the sharp peak of the OZ area. With 8 pm peak to peak, the motion of the Cj at this frequency is negligible. In order to describe the motion of the TM and OZ area in greater detail, a second transect (labelled A–B in Fig. 6A) was taken across only the TM, aligned so as to exactly

cross the centre of OZ. This measurement was done at the same three frequencies as the first transect line and, in addition, a fourth measurement at the resonant frequency was determined for each individual animal. The motions of TM and OZ do not resemble the deflection of a simple circular membrane in its first mode of oscillation. Unexpectedly, the motion of OZ dominates the motion of the entire tympanal system. This is most salient for higher frequencies: at 45 kHz (Fig. 6F), 70 kHz (Fig. 6G) and 58.76 kHz (resonance; Fig. 6H). At the lower frequency of 20 kHz (Fig. 6E), the envelope of TM deflection is apparently asymmetric. As frequency increases this asymmetry gradually vanishes.

Finally, to provide material for direct comparison, the deflections of the circular membrane of a 1/8" Brüel & Kjaer (4138) pressure-field microphone were characterised. The resulting mechanical deflections for driving frequencies of 25 and 70 kHz, shown with transect lines and 3-dimensional scans, are provided in Fig. 7. These results clearly show the microphone membrane moving as it would be expected from a 'drum-like' circular membrane in its (0,1) mode. It is worth

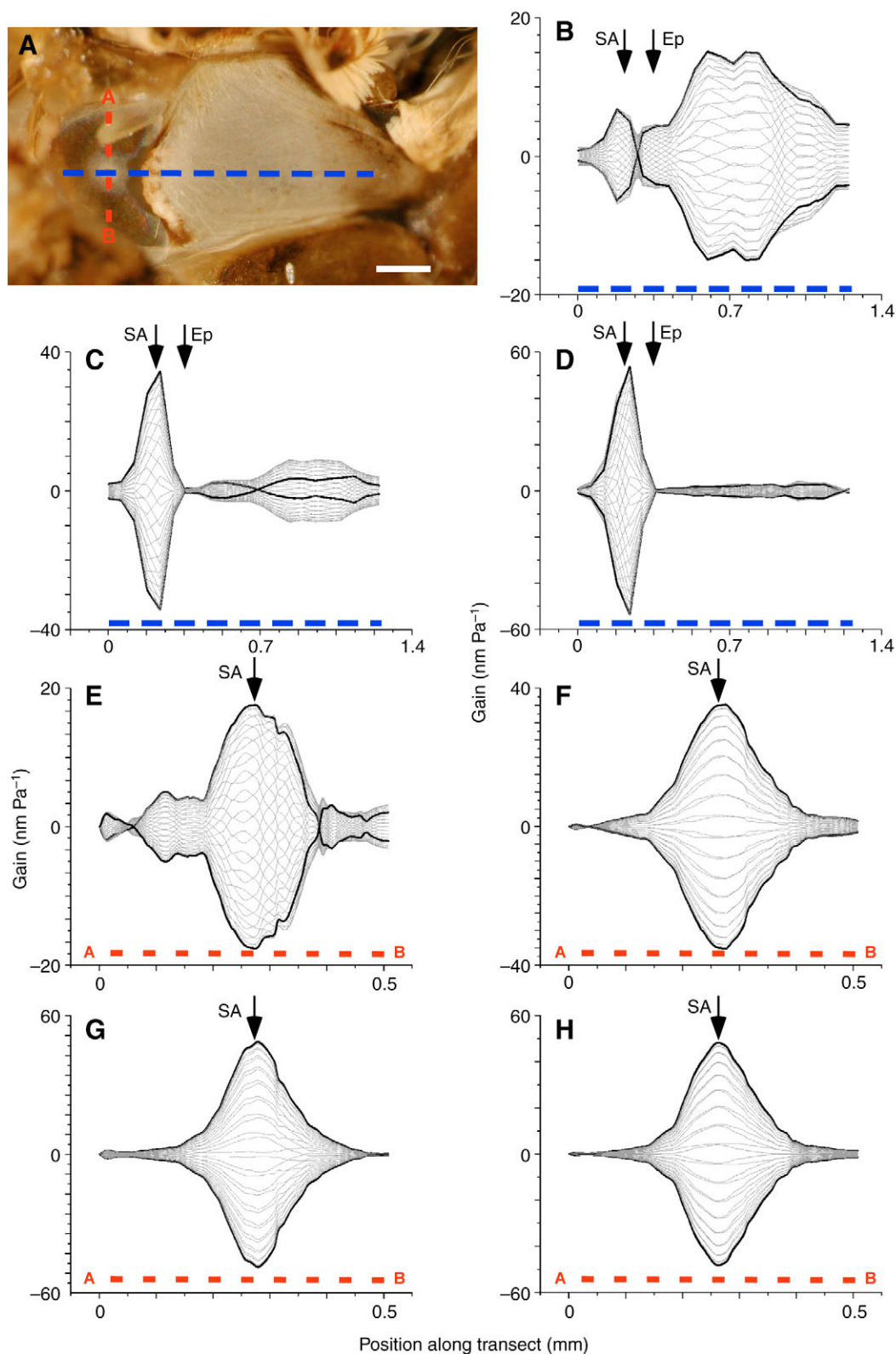


Fig. 6. Envelopes of mechanical deflections (nm Pa^{-1}) across the tympanic membrane (TM) of *N. pronuba* along transect lines for different driving frequencies. (A) The position along the transect lines is given with a calibrated scale. This scale constitutes the x -axis in B–F. Scale bar, 0.25 mm. (B–F) The deflection envelopes are constructed by displaying the instantaneous deflection velocities along the transect for a series of phases (in 90° increments) along the full oscillation cycle. Driving frequencies: (B) 20 kHz; (C) 45 kHz; (D) 70 kHz; (E) 20 kHz; (F) 45 kHz; (G) 70 kHz; (H) 58.76 kHz (resonance). SA, sensory attachment; Ep, epaulette.

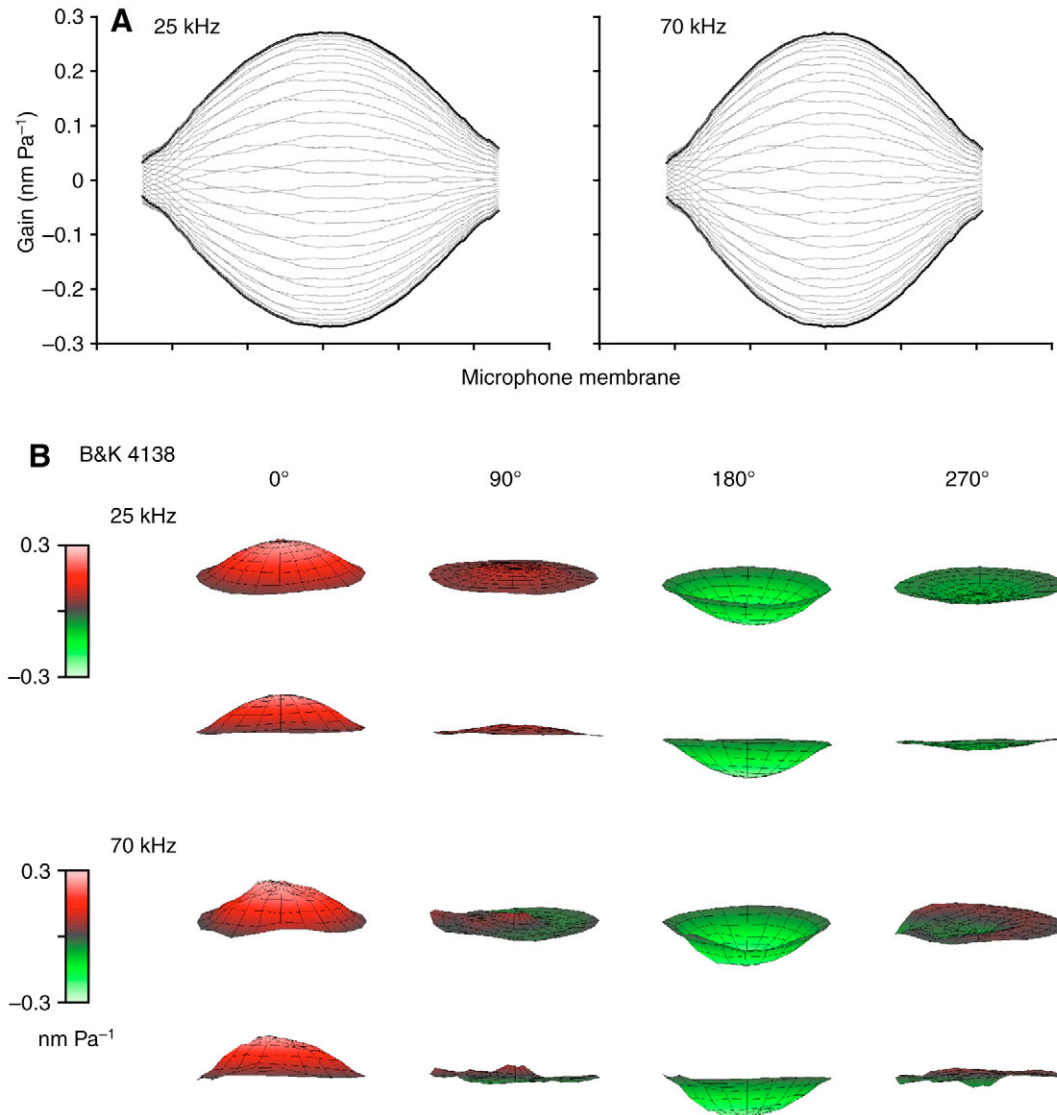


Fig. 7. Mechanical deflection across the membrane of a Brüel & Kjaer type 4138 pressure-field microphone. (A) Envelopes of deflection (gain) for driving frequencies of 25 and 70 kHz. (B) Area scans of microphone membrane deflections for driving frequencies of 25 and 70 kHz. The deflections are shown each time for four different phases along the oscillation cycle. Deflections are additionally shown as profiles, looking at the membrane from its side. Red indicates positive gain (or outward membrane deflections), and green indicates negative gain (or inward membrane deflections).

noting here that the measured gain of the microphone membrane – e.g. its acoustic sensitivity – is a factor of 100 below that of the moth. This illustrates a fundamental difference between the freely oscillating membrane of the microphone and the tympanic system of the moth's ear.

Discussion

Our results demonstrate that, contrary to expectations, or the previous report by Rodríguez et al. (Rodríguez et al., 2005), only the central, opaque zone of the moth tympanum vibrates in response to sound while the surrounding transparent zone, the traditionally labelled tympanum, shows little sign of motion even at sound intensities 30 dB above the reported neural threshold (Waters and Jones, 1996). Adams (Adams, 1972) reported the neural response of the ear of *Prodenia eridania*

(Noctuidae) at 2, 14 and 60 kHz. The ear was mechanically stimulated with a small probe driving the OZ directly whilst extracellular recordings of the auditory nerve were carried out. These results of Adams are corroborated by our study; for *N. pronuba*, sound-induced vibrations of the OZ alone in that frequency range are sufficient to stimulate the mechanosensory neurones.

Our results show that the Cj moves in anti-phase to the TM, corroborating the results of Rodríguez et al. (Rodríguez et al., 2005). However, in the species investigated here, we find that the Cj has a far lower resonant frequency than that of the OZ on the TM. The function of this secondary membrane is uncertain although Richards (Richards, 1932) and Yack et al. (Yack et al., 1999) have argued that it is the remnant of the original wing-hinge membrane from which the TM evolved.

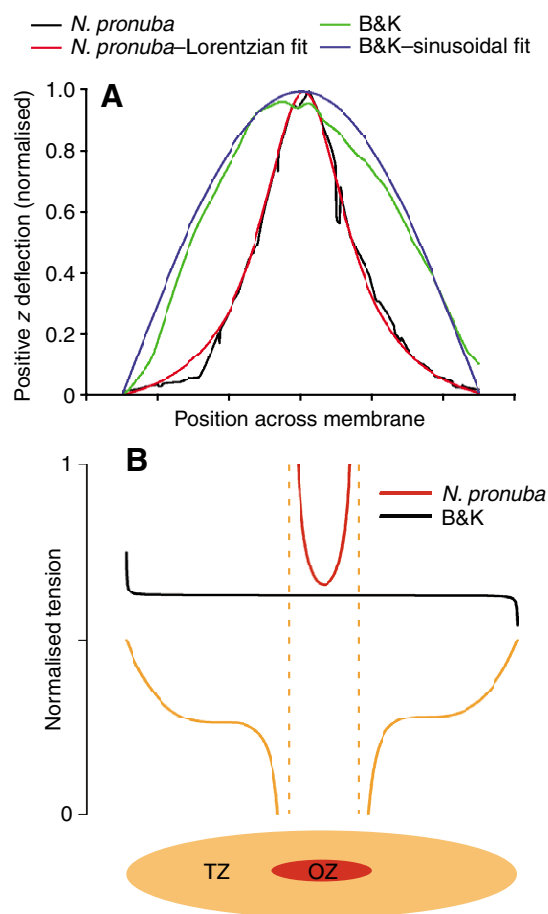


Fig. 8. Comparison between moth and microphone membrane tension. (A) Normalised maximum positive deflection (displacement) of the *N. pronuba* tympanic membrane (TM) and the B&K 4138 microphone membrane at 70 kHz. Each displacement is shown with its corresponding 'fit', a Lorentzian for the moth and a sinusoid for the microphone. (B) Tension across a B&K microphone, fitted to a sinusoidal model, and across the *N. pronuba* TM, and fitted to a Lorentzian, when both are driven at 70 kHz.

Hypothetically, it could play a role in changing, as a function of frequency, the acoustic impedance of the entire tympanal system and its series of acoustic chambers and have a function relating to the frequency adaptivity recently shown in the ear of *N. pronuba* (Windmill et al., 2006).

Our measurements also confirm that the surrounding cuticular support does not vibrate in response to impinging acoustic energy, suggesting a particular role of the OZ as the main mobile structure of the tympanum of the moth ear. This finding explains the surprising observation of Roeder and Treat that tearing the noctuid tympanum did not appreciably change the neural responses to sound (Roeder and Treat, 1957). Similar operations on the tympanum of the field cricket result in severe changes in tympanal movements and neural responses (Paton et al., 1977), implying that the motion of the entire TM in this insect is required for sound reception. Roeder and Treat stated that only interfering with the sensilla attachment site or preventing the tympanum from moving (and hence presumably the OZ as well) diminished the neural response (Roeder and

Treat, 1957). Our findings suggest that puncturing anywhere within the OZ should affect the receptor responsiveness by interfering with the mechanical response of the functional tympanum.

It may now become apparent that most insect tympana are neither simple circular membranes nor move like simple circular membranes when acoustically stimulated. By examining the mechanical response of the locust tympanum in both frequency and time domains, Windmill et al. (Windmill et al., 2005) concluded that its pattern of motion relies on travelling waves rather than drum-like motions. Functionally, the travelling waves are used to channel mechanical energy to discrete locations on the membrane and thus endow the locust ear with the capacity for frequency discrimination. Another example of complex mechanical behaviour is the tympanum of the bushcricket, which has been reported to move as a hinged flap (Bangert et al., 1998) in response to sound waves delivered by tracheal conduction. In a recent study, the tympanic ridge, a salient anatomical feature of the tympanum of cicadas, has been shown to undergo the motion of a travelling wave and has been suggested to support peripheral frequency analysis (Sueur et al., 2006). Our tympanal deflection measurements show that the noctuid moth tympanum behaves in yet another fashion. The acoustically driven deflections of the central circular area (the opaque zone) suggest that this tympanum deflects more like the membrane of an air balloon poked by a blunt needle than the canonical membrane of a drum. This can be clearly illustrated by comparing the deflections of the moth's ear with that of a microphone membrane, as shown experimentally in this study.

At this time, it is not clear why the extremely thin (0.4 μm) (Ghiradella, 1971) TZ vibrates relatively little compared with the receptor attachment site, nor what its function is. This measurement alone prompts several questions about the auditory function of the tympanum. Why, in the process of reception of acoustic waves at the adequate and relevant amplitude and frequency, is the TZ (the so-called tympanum) moving so little? Is the moth tympanum in effect the actual site of conversion of acoustic energy into mechanical energy? Could it be, as originally suggested by Adams (Adams, 1972), that other membranes or suitably compliant structures inside the auditory system vibrate in response to sound and generate the mechanical energy to drive the scolopidial auditory sensilla? Such an indirect relationship between the tympanum and the auditory sensilla has been reported for other insects, notably for the ultrasound-sensitive ears of tettigoniid Orthoptera for which the activation of the auditory sensilla is achieved by the mechanical actuation of accessory trachea (Bangert et al., 1998).

A further analytical step can be taken, which is briefly developed and discussed here, shedding some light on the mechanical behaviour of the OZ part of the moth tympanum. In keeping with the transect data gathered, the motion of the TM can be considered in two dimensions using the linear wave equation for a string:

$$T \frac{d^2 z}{dx^2} - \rho \frac{d^2 z}{dt^2} = 0, \quad (1)$$

where T is tension, z is displacement normal to the membrane, x is the distance across the membrane, t is time and ρ is density.

However, this form of the linear wave equation assumes that the tension is constant. Therefore, to consider the potential change in tension, we need to introduce a small perturbation $\theta(x)$ such that $T \rightarrow T + \theta(x)$. If ρ is uniform across a membrane, and so normalised to 1, then Eqn 1 can then be rearranged for the tension, $T + \theta(x)$, as:

$$T + \theta(x) = \frac{(d^2z/dt^2)}{(d^2z/dx^2)}. \quad (2)$$

It is then possible to use Eqn 2 to formulate a representation of the tension across any membrane, given that we can measure z (see Fig. 8A), and so can calculate the second differential d^2z/dx^2 , and can also measure acceleration d^2z/dt^2 . From this, the tension across the membrane of the moth can be qualitatively compared with that across the isotropic membrane of the Brüel & Kjaer 4138 microphone. Unfortunately, it was found that performing a second differential directly on the measured data of either membrane injected such a large amount of noise that the result was completely indecipherable. This is because the sharp changes in gradient from any noise on the displacement measurement are greatly accentuated by carrying out two differentials on the signal. To answer this we carried out the calculation of Eqn 2 on functions fitted to the z and d^2z/dt^2 measurements of the moth ear and B&K microphone membranes (as shown in Fig. 8A). In the case of the microphone membrane, a simple sinusoidal function could be fitted, and for the moth TM a Lorentzian function yielded the best fit. These results were then normalised to allow qualitative comparison, as shown in Fig. 8B.

The tension distribution across the moth TM is different to that of the microphone. Tension across the microphone membrane is constant, as expected for an isotropic membrane (Berg and Stork, 1995). For the moth, membrane tension greatly varies across the transect. TZ experiences lesser tension than OZ. Notably, at points immediately adjacent to OZ, tension goes through a discontinuity (broken line on Fig. 8B); these points relate to the points of inflection on the deflection shape data. This analysis highlights the possible effect of the anisotropy of the moth tympanum. The heterogeneous tension distribution indicates higher tension at the centre of the membrane, at the insertion point of the mechanosensory neurones. This result suggests that differences in material properties (thickness, density, compliance) between the membrane's TZ and OZ may contribute to localisation, or focussing, of mechanical energy at the mechanosensory insertion point. Further experiments are clearly needed to quantify the local (as opposed to bulk) material properties of the tympanic membrane. This will provide the data necessary to construct further models, establish predictions of mechanical behaviour as a function of measured membrane heterogeneity and aim at a better understanding of the exact relationship between structure and function in a tympanum. In particular, the mechanisms subtending the conversion of acoustic energy into mechanical energy by tympanal and ancillary structures remains elusive. More generally, the acousto-mechanical processing capacity of eardrums, those of other insects and vertebrates, may be worth further consideration. It is becoming increasingly apparent that, as the first stage in hearing, eardrums process incident sound pressure

in a way more complex than previously surmised, and thus deserve further attention.

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