

# Time-resolved tympanal mechanics of the locust

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A salient characteristic of most auditory systems is their capacity to analyse the frequency of sound. Little is known about how such analysis is performed across the diversity of auditory systems found in animals, and especially in insects. In locusts, frequency analysis is primarily mechanical, based on vibrational waves travelling across the tympanal membrane. Different acoustic frequencies generate travelling waves that direct vibrations to distinct tympanal locations, where distinct groups of correspondingly tuned mechanosensory neurons attach. Measuring the mechanical tympanal response, for the first time, to acoustic impulses in the time domain, nanometre-range vibrational waves are characterized with high spatial and temporal resolutions. Conventional Fourier analysis is also used to characterize the response in the frequency domain. Altogether these results show that travelling waves originate from a particular tympanal location and travel across the membrane to generate oscillations in the exact region where mechanosensory neurons attach. Notably, travelling waves are unidirectional; no strong back reflection or wave resonance could be observed across the membrane. These results constitute a key step in understanding tympanal mechanics in general, and in insects in particular, but also in our knowledge of the vibrational behaviour of anisotropic media.

**Keywords:** tympanal membrane; frequency; place principle; biomechanics; time-resolved laser vibrometry

## 1. INTRODUCTION

The ability to detect and process sound is a sense particularly important in many animals, including insects, playing a key role in predator, prey and mate detection. Acute hearing, both in the sense of extreme sensitivity to sound and sharp frequency selectivity, relies on the active participation of auditory mechanoreceptors (Hudspeth 1997; Hudspeth & Logothetis 2000; LeMasurier & Gillespie 2005). In the mammalian auditory system, the receptive structures are hair bundles located at the top of sensory hair cells. Remarkably, the hair cells of the inner ear make use of a mechanism of positive feedback, with which they increase the vibratory stimuli to which they respond in the first place (Hudspeth & Logothetis 2000). Possibly unique to the sense of hearing, this active amplification results in enhanced auditory sensitivity and sharpening of frequency discrimination (Robles & Ruggero 2001). Among insects, active hearing was first demonstrated for mosquitoes, fulfilling a function as in mammals by the action and reaction of mechanosensory neurons to sound-induced vibrations (Göpfert & Robert 2001).

In flies and mosquitoes, it was shown that the insect's auditory neurons can generate motions that mechanically drive the antenna (Göpfert & Robert 2003) and tune it to biologically relevant sounds (Jackson & Robert 2006). It was also shown that insectan mechanosensory neurons involved in hearing are capable of detecting exquisitely small mechanical displacements, down to some 100 pm (Windmill *et al.* 2007), close to the theoretical limits of sensitivity (Bialek 1987).

In addition to active cellular processes, hearing organs often also rely on complex passive mechanisms; the ear of the locust is a remarkable example of this. The ears of locusts (figure 1), and their function, have been known and studied for quite some time (Schwabe 1906; Autrum 1941; Gray 1960; Michelsen 1971; Stephen & Bennet-Clark 1982; Robert 1989; Meyer & Hedwig 1995; Jacobs *et al.* 1999). The two main parts of a locust ear are an external tympanal membrane (TM) and an internal mechanosensory organ, Müller's organ, containing four groups of mechanoreceptive units (Gray 1960), later categorized into three groups (Jacobs *et al.* 1999). Using electrophysiological techniques, it was shown that the ears of locusts could perform frequency discrimination (Horridge 1960). It was later discovered that the discrete groups of mechanosensory cells from Müller's organ, attached at different locations to the TM, transmit different frequency information (Michelsen 1968, 1971; Römer 1976; Miller 1977). The response of the locust auditory

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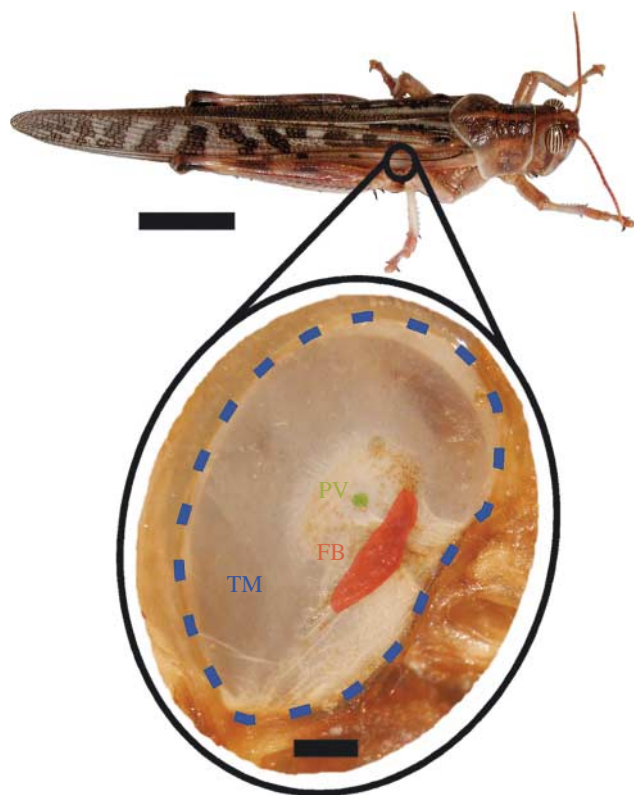


Figure 1. The tympanal membrane of the locust ear and its position on the animal's body. Body scale bar, 12 mm; membrane scale bar, 200  $\mu$ m. TM, tympanal membrane; PV, pyriform vesicle, location of the attachment sites of the mechanosensory neurons tuned above 10 kHz; FB, folded body, location of the attachment sites of the mechanosensory neurons tuned below 10 kHz.

system as respiratory ventilation varies the pressure in the tracheal system has also been investigated, showing a distinct modulation of neuronal signalling during the respiration cycle (Meyer & Hedwig 1995).

The ability to process the frequency composition of sound is an important attribute for many auditory systems. In the mammalian ear, this ability relies on the particular physical properties of the basilar membrane in the cochlea (von Békésy 1960). This method of frequency analysis is based on the spatial mapping of sound frequencies along the basilar membrane. Sound causes mechanical displacements of the basilar membrane, taking the shape of slow travelling waves (Peterson & Bogert 1950; Olson 2001; Robles & Ruggero 2001). As a wave propagates along the cochlea, the speed of its propagation gradually diminishes while its amplitude increases. At a certain location, the wave's amplitude reaches a maximum, and then rapidly attenuates. The exact location at which this takes place is frequency specific, due to the interaction of the fluids in the cochlear duct and the anisotropic nature of the basilar membrane (Olson & Mountain 1991; Olson 1999; Robles & Ruggero 2001). This is the physical basis for the spatial representation of sound in the mammalian ear, referred to as tonotopy, or the place principle, first established by von Békésy (1960).

Given that the locust ear has different groups of mechanosensory cells, attached at different positions to the TM, the question was whether the attachment sites

of these cell groups were related to the vibrational patterns of the TM. A correlation was established between the frequency response of the four groups of mechanoreceptors and the mechanical response of the TM (Michelsen 1971). The locust TM was described as undergoing different 'drum-like' resonant modes at different frequencies. The locations of maximal membrane displacements, the antinodes, were seen to vary as a function of the driving frequency, and then also to correspond to the attachment points of mechanoreceptor groups. This was the mammalian place principle applied to the locust ear.

Recent work has shown that the 'drum-mode' theory of how the locust's TM functions is incorrect (Windmill *et al.* 2005). The recent study has shown that the spatial decomposition of incident sound into discrete frequency components on the locust TM involves a tympanal travelling wave, funnelling mechanical energy to specific tympanal locations, correctly mapping incident sound frequency to the attachment of the groups of frequency-sensitive mechanosensory cells. These nano-metre-scale travelling waves show a close analogue in both operation and function to those of the basilar membrane. However, whereas the cochlea wave is born from interactions between the anisotropic basilar membrane and the surrounding fluids, the locust's travelling wave rides on an anisotropic membrane suspended in air (Stephen & Bennet-Clark 1982). Recent studies have also reported travelling waves on mammalian TMs, providing an explanation of the mechanical delay in the conduction of vibration in the middle ear (Decraemer *et al.* 1999; Fay *et al.* 2005). However, the locust's ear combines in one structure the functions of both sound reception and frequency decomposition (Windmill *et al.* 2005).

The study by Windmill *et al.* (2005) describing the locust's TM travelling wave relied solely on frequency-domain fast Fourier transform (FFT) reconstructions from 'continuous wideband' chirps. We now present a further analysis of the membrane's motion in the time domain. This allows, for the first time, the examination of the TM's response to an impulse stimulus in time with a temporal resolution not obtainable by conventional Fourier transform frequency analysis.

## 2. METHODS

### 2.1. Animals

Experiments were performed using adult male and female *Schistocerca gregaria* Forskål, with a total of 15 animals used. The animals were supplied by Blades Biological (Cowden, UK) and kept in standard conditions (temperature 24–26°C, RH 60–70%). For the laser vibrometric mechanical measurements, the animal's wings were cut back to allow direct optical access to the TM. No other surgery was required and all insects remained alive throughout the experiment. While measured, the animals were firmly attached, ventrum down, to a horizontal brass bar (5 mm wide, 1 mm thick and 60 mm long) using BLU-TACK (Bostik-Findley, Stafford, UK). The brass bar was connected to a metal rod (150 mm long, 8 mm in

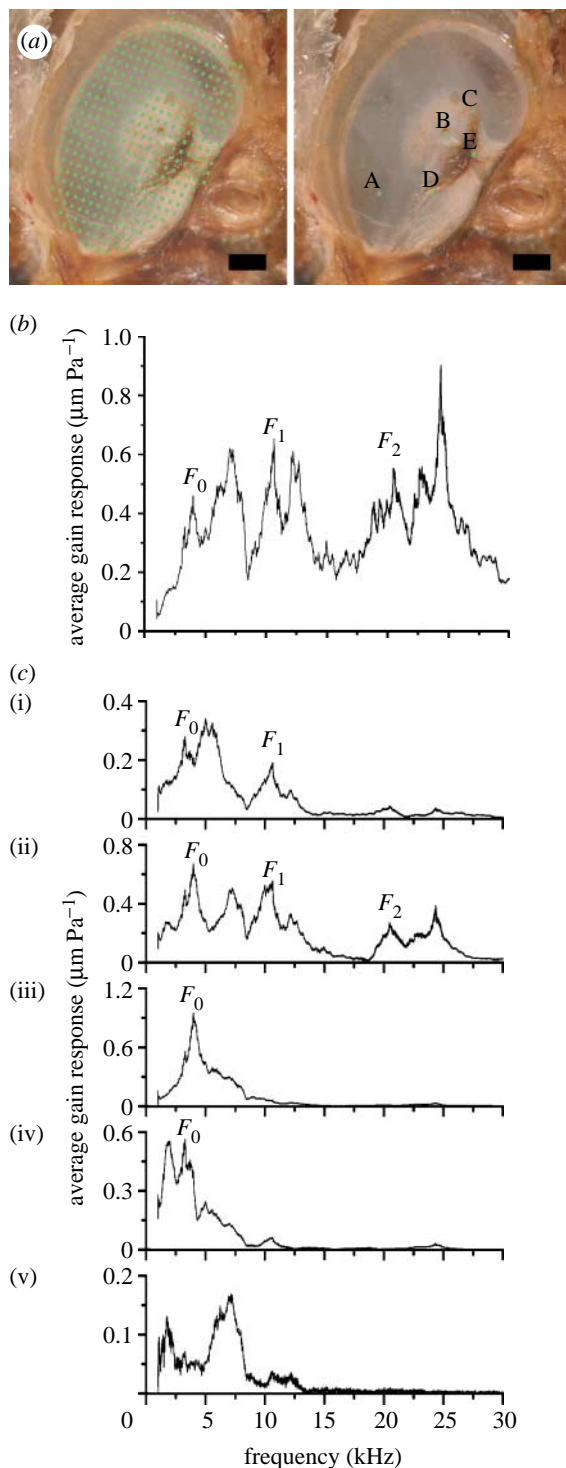


Figure 2. Frequency point spectrum of the vibration response of the TM. (a) Micrograph of the TM indicating the positions of the point spectrum response measurement, A–E (green dots). Scale bar, 200  $\mu\text{m}$ . (b) Average gain response spectrum for 380 points measured across the entire TM (acoustic stimulation with wideband (chirp) signals of range 1–30 kHz). Three frequencies of interest are identified:  $F_0$ , 4.4 kHz;  $F_1$ , 10.76 kHz;  $F_2$ , 19.85 kHz. (c) Gain response spectrum at the five points, (i) A, (ii) B, (iii) C, (iv) D and (v) E, identified in (a), and correlated with the frequencies of interest,  $F_0$ – $F_2$ , of (b).

diameter) via a thumbscrew, allowing the animal to be rotated and tilted into the required position. Only one ear was examined per animal. The animal was oriented

such that the measuring laser Doppler vibrometer could scan the entire TM and that the tympanum was perpendicular to the direction of sound wave propagation. 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 a RH 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 and height 1.98 m; Industrial Acoustics, Bronx, NY, USA).

## 2.2. Mechanical and acoustic measurements

Tympanal vibrations were examined in the frequency domain in response to acoustic stimulation with wideband (chirp) signals of range 1–30 kHz. In the time domain, the response of the tympanum to acoustic impulses (clicks) was measured. The vibrations were analysed by the simultaneous recording of the vibration velocity of the TM, and the sound pressure level (SPL) adjacent to the tympanum. The velocity was measured using a microscanning laser Doppler vibrometer (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head fitted with a close-up attachment. This allowed the laser spot (approx. 5  $\mu\text{m}$  in diameter) to be positioned with an accuracy of approximately 1  $\mu\text{m}$ .

Measurements at any position on the entire TM could be taken without readjusting the position of any component in the experiment. 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 the amplitude, time and frequency domains, in a contact-free way and without requiring the use of a reflective medium.

The acoustic signals were generated by the PSV 300 internal data acquisition board (National Instruments PCI-4451; Austin, TX, USA), amplified (Sony amplifier model TAFE570; Tokyo, Japan) and passed to a loudspeaker (ESS AMT-1; ESS Laboratory, Inc., Sacramento, CA, USA) positioned 300 mm from the animal. Thus, for the relevant frequency range (3–30 kHz), the animal was in the far-field of the sound source. SPL was measured using a 1/8 in. (3.2 mm) precision pressure microphone (Bruel & Kjaer, 4138; Nærum, Denmark) and preamplifier (Bruel & Kjaer, 2633). The microphone had a linear response in the measured frequency range. The microphone's sensitivity was calibrated using a Bruel & Kjaer sound level calibrator (4231, calibration at 1 kHz, 94 dB SPL). The microphone was positioned 10 mm from the tympanum, with its diaphragm parallel to the sound direction, thus maximizing the response.

The broadband stimulus was corrected in the frequency domain to ensure that the sound amplitude was kept to a constant level (60 dB SPL) across the complete range of frequencies (1–30 kHz). The incident sound spectrum, as measured by the reference microphone, was measured in signal voltage and inverted with respect to amplitude. The resulting voltage–frequency function was fed back to the waveform generator to create another, corrected, arbitrary



wave form presenting a power spectrum with a flat frequency distribution. This procedure allows for the accurate control of the stimulus in the frequency domain at the position of the reference microphone. Across the frequencies used, the amplitude did not vary by more than 3 dB.

In order to generate a single acoustic impulse for the time-domain analysis, a short voltage signal from the generator to the amplifier and loudspeaker would cause the loudspeaker to resonate, outlasting the single acoustic impulse required. To improve on this limitation, the initial single voltage pulse played through the loudspeaker was recorded, using the reference microphone, with a high temporal resolution (sample rate 2.56 MHz). In order to remove the unwanted resonance due to loudspeaker mechanics, compensatory sinusoid-like oscillations were introduced into the original recorded impulse stimulus. Notably, these compensatory oscillations had to be introduced before and after the initial impulse. This process was carried out iteratively in order to produce the best possible, compensated acoustic pulse. This compensated impulse was carried out for each individual animal tested and every time the geometry of the experimental set-up was changed. A pulse duration of approximately 15  $\mu$ s was achieved through this method (figure 4b).

### 2.3. Evaluation of data

The analysis of the membrane vibration and the SPL was carried out by the vibrometer's control PC. For frequency-domain measurements, the signals were simultaneously sampled at 102.4 kHz. Sets of 25 data windows of 80 ms duration were acquired and averaged for each point across the TM. Using an FFT with a rectangular window, a frequency spectrum was produced for each signal with a resolution of 12.5 Hz. The laser and microphone signals were then used to calculate different quantities, such as gain and phase responses. By combining the results from all the points recorded across the TM, oscillation profiles and animations of tympanal deflections were generated for specific frequencies. Here, we calculated the transfer function ( $H_1$ , the cross-spectrum of the displacement signal (nm) and reference signal (Pa) divided by the auto-spectrum of the reference signal), to produce the amplitude gain and the phase response of the system at different frequencies. The magnitude-squared coherence between the vibrometer and microphone signals was also computed for each data point to assess data quality for the entire dataset. The data were considered of sufficient quality when coherence exceeded 85%.

In the time domain, the velocity signals of the vibrometer and the SPL were simultaneously sampled at 2.56 MHz for a measurement duration of 1.6 ms. A set of 100 measurements were averaged for each point on the membrane. A custom-written program, using the LABVIEW v. 8.0 environment (National Instruments) was used to reconstruct the averaged point time data into spatial vibration profiles.

## 3. RESULTS

### 3.1. Membrane frequency spectra at different positions

The laser vibrometer system was used to measure the frequency spectrum of the vibration of the locust's TM with a high spatial resolution, at many different points in response to sound (figure 2). The average response across all the points measured was then calculated, as shown in figure 2b. This gives a relative measurement of all the frequencies present in the vibration response of the TM. It clearly shows that certain frequency bands are responding mechanically more than others, permitting the identification of three specific frequencies for further examination:  $F_0$ , 4.4 kHz;  $F_1$ , 10.76 kHz;  $F_2$ , 19.85 kHz.

Five distinct measurement points were selected at different positions on the membrane (figure 2c). From these individual point vibration spectra, it can be seen that the TM responds spatially to different frequencies. At A, a point on the membrane away from the attachments to Müller's organ, the frequencies  $F_0$  and  $F_1$  are clearly represented, as well as a response in the range 5–6 kHz. At B, the point on the membrane at which the group of mechanosensory neurons that are tuned above 10 kHz attach,  $F_0$ ,  $F_1$  and  $F_2$  are all present. This demonstrates the correspondence between the vibration of the TM at a particular frequency and the tuning of the attached neurons, in this case responsive above 10 kHz. However, these data also show B moving in response to frequencies below the responsive frequency range of the neurons attached at that position. This simple measurement immediately suggests a more complex TM vibration response than that of a resonant drum skin. The points C and D have no response above 10 kHz. Their response corresponds to the frequency selectivity of the groups of mechanosensory neurons attached in proximity to those positions on the TM. Finally, figure 2c(v) shows a response around 7 kHz, with both the lower and higher frequency ranges present, but with a lower amplitude.

### 3.2. Mechanical response of the TM across a transect

Using the laser vibrometer to measure the response of many points to a repeated stimulus, it is possible to reconstruct the vibration pattern of a transect across the TM from the frequency-domain data (figure 3). This can only be carried out given that the stimulus is known and recorded with the vibration response, such that the phase change between the two signals is accurately measured. Thus, for a chosen frequency, the response amplitude of all the measured locations is displayed at a chosen time in the phase cycle of that frequency. Displaying these data through the entire phase cycle reveals the vibrational pattern of the TM at that frequency.

In figure 3b, waterfall plots for the reconstructed data for  $F_0$ ,  $F_1$  and  $F_2$  are presented. At  $F_0$ , the entire membrane transect is vibrating. The wave peak shows movement from the left (near X) of the transect (where there are no neural attachments) to the right (near Y) where the neurons attach. Thus, the acoustic energy at

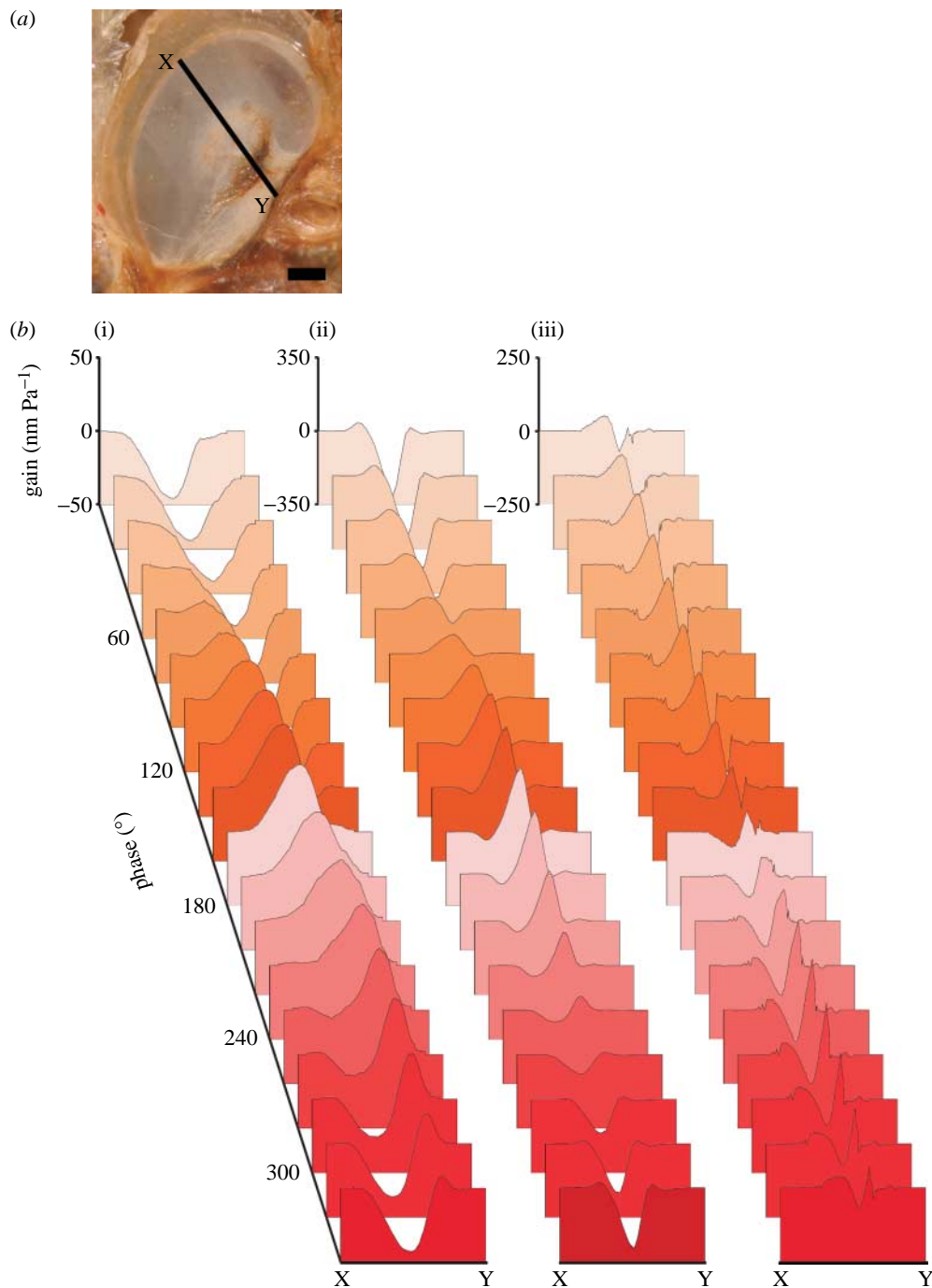


Figure 3. FFT reconstruction of the gain response as a deflection profile across a transect XY of the TM. (a) Micrograph of the TM indicating the transect XY of the FFT reconstruction. Scale bar, 200  $\mu\text{m}$ . (b) Waterfall plots showing the changing gain response across the XY transect for one cycle of each of the three frequencies ((i) 4.4 kHz, (ii) 10.76 kHz and (iii) 19.85 kHz) taken from figure 2b.

this frequency is captured and translated through the TM system to the location of the mechanosensory neurons. At  $F_1$ , the wave travels in the same direction, but is sharper (shorter wavelength), and does not activate the entire TM transect. In this case, the wave peak stops at the B position (figure 2a), where the mechanosensory neurons tuned to this frequency attach. These data clearly show that the TM is carrying out the function of a spatial frequency filter, using a travelling wave to convey mechanical energy to different spatial locations. Finally, at the yet higher frequency of  $F_2$ , a response is seen similar to that of  $F_1$ .

In this case, the wave peak is sharper, and here more than one wave cycle can clearly be seen in the travelling wave response across the TM transect (figure 3b). Again, the wave peak halts at the position of the mechanosensory neuron attachment, correlating with that higher frequency range.

### 3.3. Time-resolved mechanical response at different locations

The time response of the TM to an acoustic impulse was measured at several positions along the same XY transect

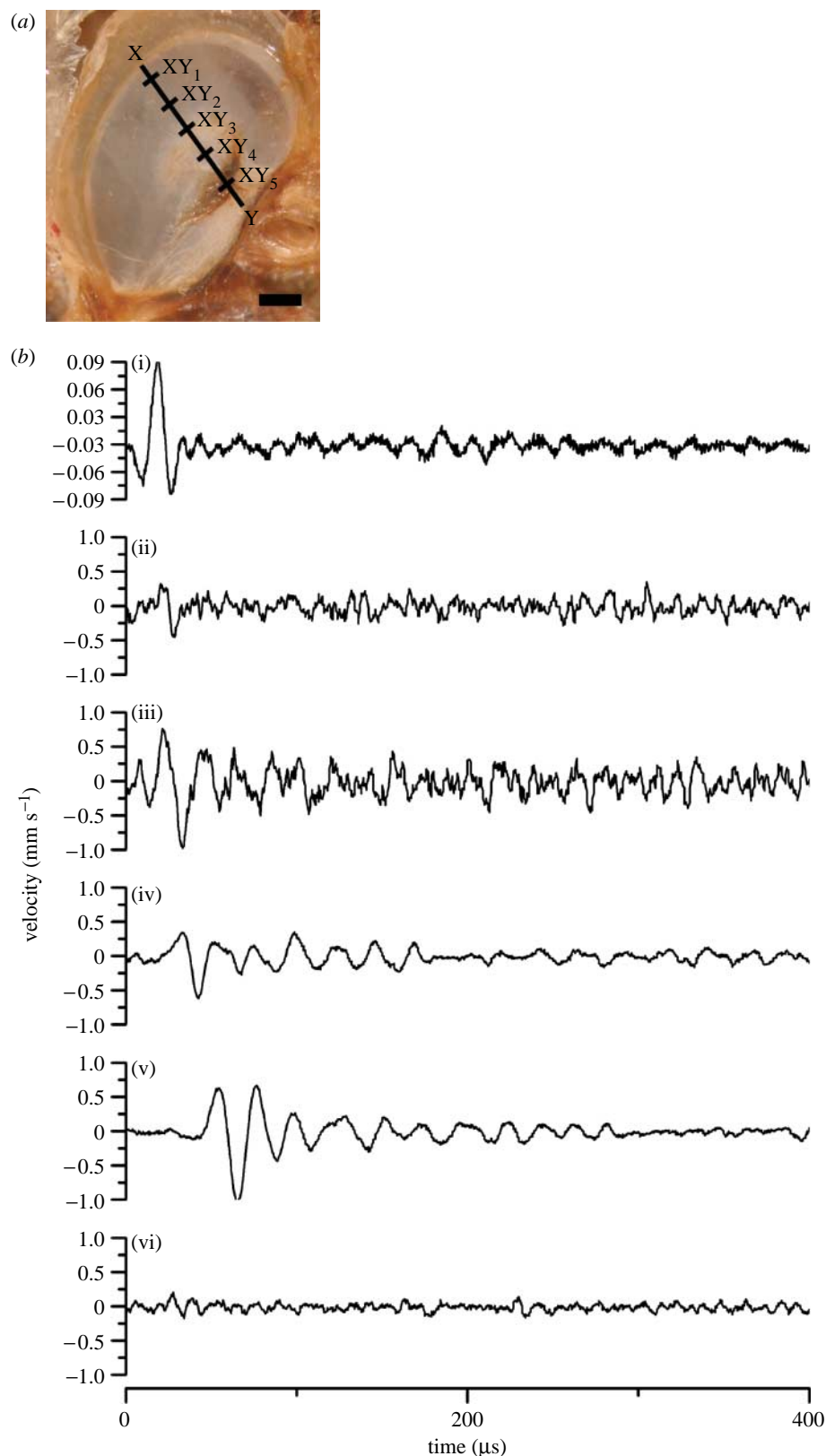


Figure 4. Time-resolved mechanical response of the membrane at different points. (a) Micrograph of the TM showing the positions of the vibration response measured, along the same XY transect of figure 3, from XY<sub>1</sub> to XY<sub>5</sub>. Scale bar, 200  $\mu\text{m}$ . (b) Graphs showing the acoustic impulse as recorded by the (i) reference microphone (in Pa) and the resultant vibration responses (velocity) at each of the XY points ((ii) XY<sub>1</sub>, (iii) XY<sub>2</sub>, (iv) XY<sub>3</sub>, (v) XY<sub>4</sub> and (vi) XY<sub>5</sub>) from (a). Each time trace is sampled at 2.56 MHz and contains 1024 samples.

used in the frequency-domain experiment (figure 4). The short impulse caused the membrane to vibrate at each position along the transect (apart from XY<sub>5</sub>). As the measurement is repeated at each position from X towards

Y, a time delay between the stimulus impulse and the initial vibration of the TM appears. This delay increases along the transect, with a total time delay of approximately 45  $\mu\text{s}$  from XY<sub>1</sub> to XY<sub>4</sub>, where XY<sub>4</sub> is the

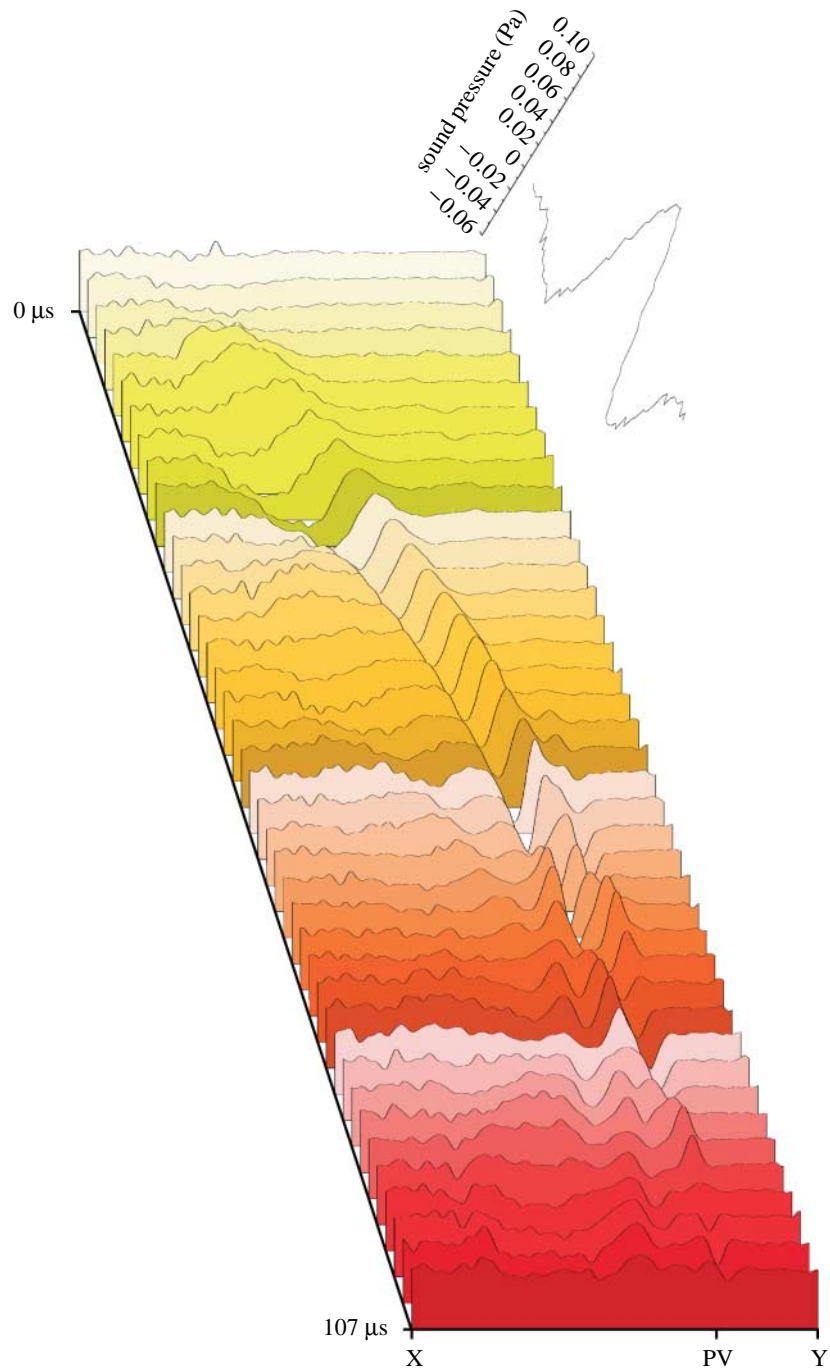


Figure 5. Waterfall plot of the vibration response of the TM to an acoustic impulse. The data taken from 190 points along the same transect as that of figure 4 are used to spatially reconstruct membrane deflections in response to an impulse stimulus of 15  $\mu\text{s}$  duration. Temporal resolution of the deflection shapes in this plot is approximately 2.73  $\mu\text{s}$ .

position at which the high-frequency tuned mechanosensory neurons attach to the TM. Although stimulated with a short impulse (duration 15  $\mu\text{s}$ ), the TM oscillates for several cycles, outlasting the stimulus duration by a factor of 10. At position  $XY_5$ , the membrane did not react to the stimulus impulse.

### 3.4. Time-resolved mechanical response across a transect

Using the impulse time response data taken for each point along the XY transect, it is also possible to reconstruct the vibration pattern of the TM across the entire transect (figure 5; electronic supplementary

material). This is accomplished by transforming a matrix of all the data points measured (each to the same stimulus). In this experiment, the actual time resolution required was 390.625 ns; however, for clarity of presentation, the resultant waterfall plot shown in figure 5 only shows every seventh time step. This result clearly shows the left part of the membrane being first stimulated by the acoustic impulse, while the right side remains inactive. As the impulse stimulus ends, the vibration pattern on the TM transect forms into a travelling wave. The leading peak of this travelling wave then moves across the transect line. The wave peak then stops travelling at the pyriform vesicle (PV, the point on the membrane where the high-frequency



neurons attach to the membrane; figure 5). At this location on the membrane, wave amplitude is at its largest, some 42  $\mu\text{s}$  after the end of the impulse stimulus. Finally, it takes several more stimulus cycles (approx. 40  $\mu\text{s}$ ) for the free oscillations to completely decay, returning the TM to its quiescent state. There is clearly no strong reflection of the travelling peak back across the membrane transect. This result reveals the temporal development of the locust's tympanal travelling wave, whereby it captures sound energy at one place on the membrane and transforms it into a wave travelling across the membrane, to dissipate the mechanical energy at a specific membrane location, imparting energy and therefore information into the mechanosensory neurons. Thus, this is a clear demonstration of the locust's place principle spatial frequency analysis, as it occurs in time.

#### 4. DISCUSSION

The locust TM uses a travelling wave as a method for spatial frequency analysis. The membrane is shown to react differently to particular frequency ranges through the frequency-domain analysis. These results were then reconstructed to show how the membrane vibrates across a transect to illustrate the membrane's capacity for frequency analysis. The travelling waves generated by different sound frequencies exhibit specific vibrational patterns on the membrane, leading to energy transduction to specific sites on the membrane at which cognately tuned mechanosensory neurons attach. This study also demonstrates that the travelling wave can be instigated by a controlled impulse stimulus, and then accurately measured in the time domain.

These results uncover some important and previously unknown aspects of tympanal dynamics. Previous results had described the travelling wave's presence, yet solely through FFT analysis resulting from a constant and sustained stimulus. Several questions were extant from this frequency-domain analysis. What is the physical mechanism underlying this particular mechanical response? How, where and when is the travelling wave generated on the TM? Although accurate in the frequency domain, previous data precluded exact analysis in the time domain and the characterization of key quantities, such as the instant of sound incidence on the membrane, the temporal development of the travelling wave, its location of initiation, its travel time, velocity and decay. Alternative hypotheses, such as the simultaneous build-up of the wave across the entire membrane, can also be tested using the impulse response.

Unlike the response from a conventional drum skin, such as the eardrum of a moth or a precision condenser microphone (Windmill *et al.* 2007), the locust membrane starts oscillating at one distinct location only. This behaviour gives rise to a wave that then travels to another position. This result shows that the locust membrane's behaviour is similar to that of the mammalian cochlea's anisotropic basilar membrane. The underlying mechanism might be similar as well, whereby a stiffness gradient, as previously described for

the basilar membrane, causes the generation and propagation of travelling waves. The impulse response also shows that once the travelling wave is initiated on the membrane, it continues its propagation, outlasting the initial stimulus. This fact can only be interpreted as the conversion of incident acoustic energy into a mechanical travelling wave propagating across the membrane.

The time response also reveals the fate of the travelling wave once it reaches the mechanosensory neuron attachment site. Notably, in the impulse response experiment, the membrane is driven by sound pressure for only approximately 15  $\mu\text{s}$ , implying that, at the moment the wave reaches the PV (at a time 35  $\mu\text{s}$  post-stimulus offset), the membrane undergoes free, unforced oscillations. This situation provides a clear opportunity to observe where and when mechanical energy is dissipated from the membrane. The pattern of free oscillations, the deflection shapes, observed clearly indicates that vibrations dissipate where the wave stops (at the PV), also highlighting that there is minimal wave reflection back across the membrane. This result could not have been gained from a more conventional frequency-domain analysis. This corroborates the notion that nearly all of the wave's energy is dissipated by the membrane structure and/or the mechanosensory cells attaching to that location. The evidence for this claim resides in the observation that the peak of the wave stops travelling, generating a stationary oscillation, a local resonance dissipating at the attachment site.

The locust's ear combines in one structure the functions of both sound reception and frequency analysis. The TM structure performs three mechanical functions: it captures sound energy, it then transfers this mechanical signal across the membrane using a travelling wave, before finally dissipating the signal's energy at a certain membrane location. This mechanical distribution of sound energy into the mechanosensory organ is frequency specific, and carried out without a strong wave 'echo' reverberating back into the structure, indicating that the process is reasonably efficient in its energy transfer.

This study prompts further research into the signal-processing properties of TMs in general. Along the years, it has become apparent that the TMs of auditory animals are not, in structure and function, simple eardrum structures (Van Staaden & Römer 1998; Decraemer *et al.* 1999; Fay *et al.* 2005, 2006; Windmill *et al.* 2005, 2006, 2007; Sœur *et al.* 2006). First and foremost, it appears that the construction and the material of TMs, in general, are often anisotropic, opening the possibility of complex mechanical responses (Funnell & Laszlo 1982; Fay *et al.* 2006). One possible significant aspect currently under investigation is the relative role of both anisotropic stiffness and heterogeneous material tension, an arrangement that could generate the observed travelling waves. One enticing outcome of this study is the possibility now offered to study how multi-frequency sound waves can be mechanically processed by anisotropic thin, pressure-sensitive membranes (Windmill *et al.* 2008). Possible developments are miniature embedded



sensors that report on frequency composition and direction of air and substrate-borne wave propagation.

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## REFERENCES

- Autrum, H. 1941 Ueber Gehör und Erschütterungssinn bei Locustiden. *Z. Vgl. Physiol.* **28**, 580–637. (doi:10.1007/BF00297960)
- Bialek, W. 1987 Physical limits to sensation and perception. *Annu. Rev. Biophys. Biophys. Chem.* **16**, 455–478. (doi:10.1146/annurev.bb.16.060187.002323)
- Decraemer, W. F., Khanna, S. M. & Funnell, W. R. J. 1999 Vibrations at a fine grid of points on the cat tympanic membrane measured with a heterodyne interferometer. In *EOS/SPIE Int. Symp. on Industrial Lasers and Inspection, Conference on Biomedical Laser and Metrology and Applications, München, Germany*.
- Fay, J., Puria, S., Decraemer, W. F. & Steele, C. 2005 Three approaches for estimating the elastic modulus of the tympanic membrane. *J. Biomech.* **38**, 1807–1815. (doi:10.1016/j.jbiomech.2004.08.022)
- Fay, J. P., Puria, S. & Steele, C. R. 2006 The discordant eardrum. *Proc. Natl Acad. Sci. USA* **103**, 19 743–19 748. (doi:10.1073/pnas.0603898104)
- Funnell, W. R. & Laszlo, C. A. 1982 A critical review of experimental observations on ear-drum structure and function. *J. Otorhinolaryngol. Relat. Spec.* **44**, 181–205.
- Göpfert, M. C. & Robert, D. 2001 Active auditory mechanics in mosquitoes. *Proc. R. Soc. B* **268**, 333–339. (doi:10.1098/rspb.2000.1376)
- Göpfert, M. C. & Robert, D. 2003 Motion generation by Drosophila mechanosensory neurons. *Proc. Natl Acad. Sci. USA* **100**, 5514–5519. (doi:10.1073/pnas.0737564100)
- Gray, E. G. 1960 The fine structure of the insect ear. *Phil. Trans. R. Soc. B* **243**, 75–94. (doi:10.1098/rstb.1960.0005)
- Horridge, G. A. 1960 Pitch discrimination in Orthoptera (Insecta) demonstrated by responses of central auditory neurones. *Nature* **185**, 623–624. (doi:10.1038/185623a0)
- Hudspeth, A. J. 1997 Mechanical amplification of stimuli by hair cells. *Curr. Opin. Neurobiol.* **7**, 480–486. (doi:10.1016/S0959-4388(97)80026-8)
- Hudspeth, A. J. & Logothetis, N. K. 2000 Sensory systems. *Curr. Opin. Neurobiol.* **10**, 631–641. (doi:10.1016/S0959-4388(00)00133-1)
- Jackson, J. C. & Robert, D. 2006 Nonlinear auditory mechanism enhances female sounds for male mosquitoes. *Proc. Natl Acad. Sci. USA* **103**, 16 734–16 739. (doi:10.1073/pnas.0606319103)
- Jacobs, K., Otte, B. & Lakes-Harlan, R. 1999 Tympanal receptor cells of *Schistocerca gregaria*: correlation of soma positions and dendrite attachment sites, central projections and physiologies. *J. Exp. Zool.* **283**, 270–285. (doi:10.1002/(SICI)1097-010X(19990215)283:3<270::AID-JEZ5>3.0.CO;2-C)
- LeMasurier, M. & Gillespie, P. G. 2005 Hair-cell mechanotransduction and cochlear amplification. *Neuron* **48**, 403–415. (doi:10.1016/j.neuron.2005.10.017)
- Meyer, J. & Hedwig, B. 1995 The influence of tracheal pressure changes on the responses of the tympanal membrane and auditory receptors in the locust *Locusta migratoria* L. *J. Exp. Biol.* **198**, 1327–1339.
- Michelsen, A. 1968 Frequency discrimination in the locust ear by means of four groups of receptor cells. *Nature* **220**, 585–586. (doi:10.1038/220585a0)
- Michelsen, A. 1971 The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear, II. Frequency discrimination based upon resonances in the tympanum, and III. Acoustical properties of the intact ear. *Z. Vgl. Physiol.* **71**, 49–128. (doi:10.1007/BF01245154)
- Miller, L. A. 1977 Directional hearing in locust *Schistocerca gregaria* Forskal (Acrididae, Orthoptera). *J. Comp. Physiol.* **119**, 85–98. (doi:10.1007/BF00655874)
- Olson, E. S. 1999 Direct measurement of intra-cochlear pressure waves. *Nature* **402**, 526–529. (doi:10.1038/990092)
- Olson, E. S. 2001 Intracochlear pressure measurements related to cochlear tuning. *J. Acoust. Soc. Am.* **110**, 349–367. (doi:10.1121/1.369098)
- Olson, E. S. & Mountain, D. C. 1991 *In vivo* measurement of basilar-membrane stiffness. *J. Acoust. Soc. Am.* **89**, 1262–1275. (doi:10.1121/1.400535)
- Peterson, L. C. & Bogert, B. P. 1950 A dynamical theory of the cochlea. *J. Acoust. Soc. Am.* **22**, 369–381. (doi:10.1121/1.1906615)
- Robert, D. 1989 The auditory-behavior of flying locusts. *J. Exp. Biol.* **147**, 279–301.
- Robles, L. & Ruggero, M. A. 2001 Mechanics of the mammalian cochlea. *Phys. Rev.* **81**, 1305–1352.
- Römer, H. 1976 Processing of information by tympanal receptors of *Locusta migratoria* (Acrididae, Orthoptera). *J. Comp. Physiol.* **109**, 101–122. (doi:10.1007/BF00663438)
- Schwabe, J. 1906 Beiträge zur Morphologie und Histologie der tympanalen Sinnesapparate der Orthopteren. *Zoologica* **20**, 1–154.
- Stephen, R. O. & Bennet-Clark, H. C. 1982 The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. *J. Exp. Biol.* **99**, 279–314.
- Sueur, J., Windmill, J. F. C. & Robert, D. 2006 Tuning the drum: the mechanical basis for frequency discrimination in a Mediterranean cicada. *J. Exp. Biol.* **209**, 4115–4128. (doi:10.1242/jeb.02460)
- Van Staaden, M. J. & Römer, H. 1998 Evolutionary transition from stretch to hearing organs in ancient grasshoppers. *Nature* **394**, 773–776. (doi:10.1038/29517)
- von Békésy, G. 1960 *Experiments in hearing*. New York, NY: McGraw-Hill.
- Windmill, J. F. C., Göpfert, M. C. & Robert, D. 2005 Tympanal travelling waves in migratory locusts. *J. Exp. Biol.* **208**, 157–168. (doi:10.1242/jeb.01332)
- Windmill, J. F. C., Jackson, J. C., Tuck, E. J. & Robert, D. 2006 Keeping up with bats: dynamic auditory tuning in a moth. *Curr. Biol.* **16**, 2418–2423. (doi:10.1016/j.cub.2006.09.066)
- Windmill, J. F. C., Fullard, J. H. & Robert, D. 2007 Mechanics of a ‘simple’ ear: tympanal vibrations in noctuid moths. *J. Exp. Biol.* **210**, 2637–2648. (doi:10.1242/jeb.005025)
- Windmill, J. F. C., Zorab, A., Bedwell, D. J. & Robert, D. 2008 Nanomechanical and electrical characterization of a new cellular electret sensor-actuator. *Nanotechnology* **19**, 035 506. (doi:10.1088/0957-4484/19/03/035506)