

The next step in cicada audition: measuring pico-mechanics in the cicada's ear

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SUMMARY

Female cicadas use sound when they select a mate from a chorus of singing males. The cicada has a tympanal ear; and the tympanal membrane, and constituent tympanal ridge, act as both acousto-mechanical transducers and frequency filters. The tympanal ridge is physically connected to a large number of mechanoreceptor neurons via a cuticular extension known as the tympanal apodeme. Using microscanning laser Doppler vibrometry, we measured for the first time the *in vivo* vibrations of the apodeme of female *Cicadatra atra* in response to the motion of the tympanum driven by sound. These measurements reveal that the nanoscale motion of the tympanal membrane is over a magnitude greater than that of the apodeme. Furthermore, the apodeme acts as an additional mechanical frequency filter, enhancing that of the tympanal ridge, narrowing the frequency band of vibration at the mechanoreceptor neurons to that of the male calling song. This study enhances our understanding of the mechanical link between the external ear of the cicada and its sensory cells.

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INTRODUCTION

Many species of insect have developed methods to produce and receive sound. Auditory capabilities play a central role in individual reproductive success as they ensure species recognition and sexual selection. Auditory pair-formation process is amply displayed in the cicada, one of the loudest animals in the world (Bennet-Clark and Young, 1992). Although only males possess organs for calling song production, the abdominal tymbals, both males and females possess fully functioning auditory systems, in the form of tympanal ears (Dugès, 1838; Powell, 1873; Vogel, 1921; Pringle, 1954; Bennet-Clark and Young, 1992; Young and Bennet-Clark, 1995; Sueur et al., 2006).

A cicada's ear consists of two main components: a tympanum and a chordotonal (sensory) organ containing multicellular auditory receptors (scolopidia; type I monodynamal receptors). The tympanum is a thin tympanal membrane (TM) partly crossed by a dark spear-like structure called the tympanal ridge (TR), as shown in Fig. 1A. When driven by sound, the membrane and the ridge show complex oscillatory modes (Fonseca and Hennig, 2004), including travelling waves (Sueur et al., 2006) or standing waves (Sueur et al., 2008). Unlike standing waves, such as the vibrations of a guitar string, the travelling waves found on the TM and TR of *Cicadatra atra* (both male and female) do not remain constant in position but move with time, and so the vibration amplitude varies with both distance and time. The important point to note regarding the formation of these travelling waves is that their displacement shows increasing phase lag along the TR, such that at a given position the motion of the membrane increasingly lags the motion of its point of origin. This is indicative of a travelling wave response (Windmill et al., 2005; Sueur et al., 2006).

Male tympana are larger with a smaller ridge than those of the female, and are coupled to an air-filled space in the abdomen. This morphological dimorphism has been associated with the mechanism of sound radiation (Young, 1990; Bennet-Clark and Young, 1992; Fonseca and Popov, 1994) and audition (Sueur et al., 2006; Sueur et al., 2008) revealing the complex dual purpose of this structure. The ridge is extended by the tympanal apodeme (TA), a cuticular extension inside the sensory capsule of the organ where the mechanosensory auditory receptors (scolopidia) are attached (Fig. 1B) (Michel, 1975). The tympanal ear sensory organ of the cicadas contains large numbers of mechanosensory receptors, from 600 up to 2100 in each ear, equalling or even surpassing the number found in most lower vertebrates (Fonseca et al., 2000).

The motion of the TM of two species of cicada, *C. atra* and *C. orni*, was found to be different in previous studies using the microscanning laser vibrometry technique (Sueur et al., 2006; Sueur et al., 2008). However, in both cases it was clearly seen that the TR acts to transfer the motion of the TM, as driven by the incident sound. Previous studies have measured the frequency tuning of cicada species at the auditory nerve level (Popov et al., 1992; Hennig et al., 1994; Fonseca et al., 2000), and interneuron level (Huber et al., 1980; Fonseca et al., 2000; Fonseca and Correia, 2007). However, this still leaves open the question of how the mechanical energy focussed down the ridge is converted to motion at the mechanosensory neurons in the sensory organ. In this study we have investigated the mechanical link between the TM and the sensory organ, namely the TA, in the female cicada *C. atra*. Tackling, for the first time, the mechanics of the internal structure of the cicada ear, this work reveals the sub-nanometre motion of a small hidden structure – the tympanal apodeme – essential to the audition of these insects but never studied before.

MATERIALS AND METHODS

Female *Cicadatra atra* (Olivier) were caught during July 2007 in Cuges-les-Pins (Bouches-du-Rhône, France, N43°16'18"–E5°41'24"). The ambient temperature was 29–32°C. Animals were cooled down to 8–10°C and were immediately transferred to Bristol (UK) in an ice-box. Before measurements, animals were placed at 25–26°C in a plastic cage (0.53 m×0.22 m×0.22 m) and allowed to return to ambient temperature. The wings and the legs were cut back before mechanical measurements. The operculum and the meracanthus that ventrally cover the tympanal organs, but are not mechanically linked to them, were also removed. Then a dissection was made to partially open the outer cuticle of the auditory capsule, exposing the TA without damaging the sensory neurons attached to it. This delicate procedure was only possible in the female of the species, because although the ear of the male is larger, access to the TA was not possible without damaging the mechanical integrity of the tympanum. Measurements of the TM and TR were carried out before and after the dissection procedure to ensure that the tympanal mechanical response did not change, and was similar to that in a previous study (Sueur et al., 2006). The vibration amplitude and frequency response, and the presence of travelling waves, on the TM and TR were measured and found to remain the same pre- and post-dissection and TA measurement. For example, the gain response at 10 kHz on the TR was reported as $244 \pm 168 \text{ nm Pa}^{-1}$ ($N=7$) in Sueur et al. (Sueur et al., 2006), and in this study the TR gain response at 10 kHz was $241 \pm 22 \text{ nm Pa}^{-1}$ ($N=8$, post-dissection).

Animals were not anaesthetised during measurements, but were firmly attached to a horizontal brass bar (6 mm wide, 1 mm thick and 16 mm long) using Blu-Tack (Bostik-Findley, Stafford, UK). Only one ear was examined per animal, with a total of eight animals studied. Vibrations were measured with a microscanning laser Doppler vibrometer (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head. The animal was orientated such that the measuring Doppler vibrometer could scan the entire TM, TR and TA, and that the tympanal system 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, 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 a dedicated acoustic isolation booth (Industrial Acoustics Company, Winchester, Hampshire, UK; IAC series 1204A, 20 m³).

The vibrations of the TM, TR and the TA were studied following the same general procedure used in a previous study on the same species (Sueur et al., 2006). The vibrations of the whole TM, and of a focused area along the TR and TA, were examined in response to a frequency modulated signal (duration, 80 ms) sweeping at similar intensity all frequencies from 1 kHz to 30 kHz. All acoustic stimuli were amplified with a Sony Amplifier Model TAFE570 (Tokyo, Japan) and broadcast with a loudspeaker ESS AMT-1 (ESS Laboratory, Sacramento, CA, USA) positioned at 0.25 m from the cicada. Thus, for the relevant frequency range (1–30 kHz), the animal was in the far-field of the sound source.

As described in detail by Windmill et al. (Windmill et al., 2005), the TM, TR and TA vibrations were analysed by simultaneous recording of the vibration velocity of the tympanum and the sound pressure level (SPL) adjacent to the tympanum. The laser vibrometer allowed accurate measurement (laser positioning $\sim 1 \mu\text{m}$) 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. SPL was measured using a 1/8 inch (3.2 mm)

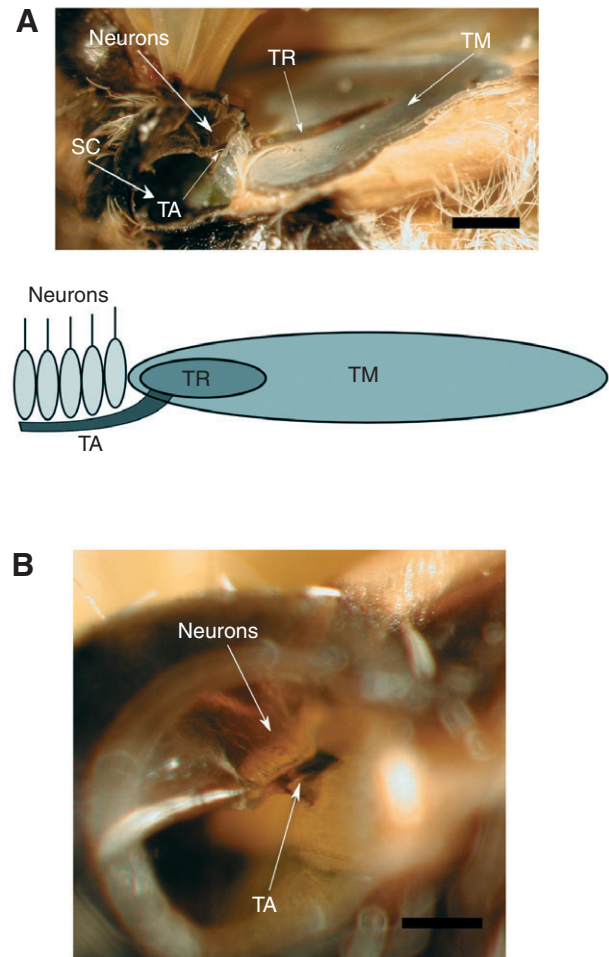


Fig. 1. External views of the right tympanal membrane (TM), and inside the sensory organ capsule (SC), of a female *C. atra*. (A) The SC is lateral to the TM, with the TM in plane with the dorsoventral plane. The dark tympanal ridge (TR) lies across the TM. The TR extends out under the TM, becoming the tympanal apodeme (TA) inside the SC, the site of attachment of the mechanosensory neurons. For clarity a diagram of the sensory arrangement is also depicted. Scale bar, 0.5 mm. (B) View inside the sensory capsule. The TA protrudes into the SC. The mechanosensory neurons are attached along the length of the TA. Scale bar, 0.25 mm.

precision pressure microphone (Bruel & Kjaer, 4138; Nærum, Denmark) and preamplifier (Bruel & Kjaer, 2633). The microphone has a linear response in the measured frequency range. The sensitivity of the microphone 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 TM, with its diaphragm parallel to the sound direction, thus maximising the response. The stimulus acoustic signals were computer corrected to ensure that their amplitude at the reference microphone was kept to a constant level (66 dB SPL) across the complete range of frequencies (1–30 kHz).

The analysis of the tympanal system velocity and SPL was carried out by the control PC of the vibrometer. The laser signals resulting from the FM sweep and natural sound stimuli 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 membrane. Using an FFT (Fast Fourier Transform) with a rectangular window, which weights all sample points equally, a frequency spectrum was

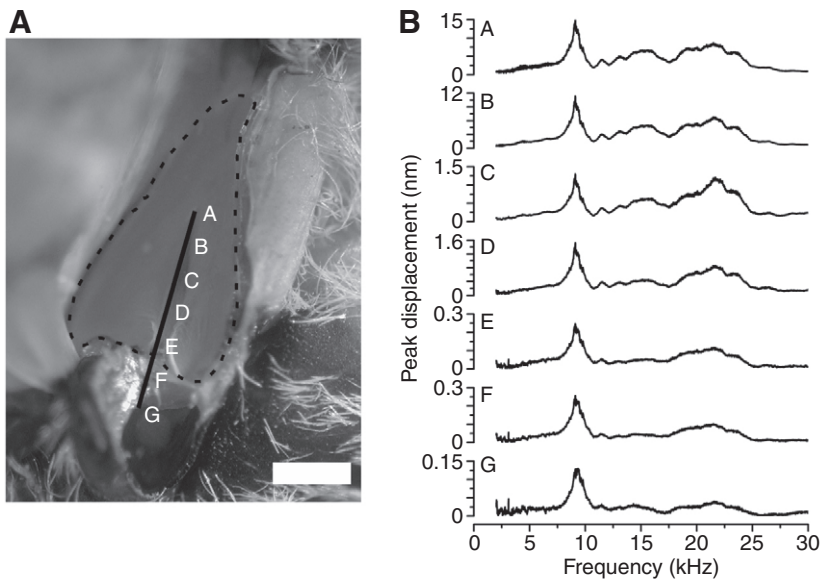


Fig. 2. The frequency response of the tympanal ridge and apodeme of a female *C. atra*. (A) External view of the experimental preparation showing the tympanal membrane (dotted line), and a line of points of measurement for the scanning laser vibrometer, from A at the apex of the tympanal ridge, to G at the end of the tympanal apodeme. (B) The frequency response of the points A–G, measured in peak displacement.

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 scanned, oscillation profiles and animations of deflections can be generated for specific frequencies.

RESULTS

The motion of the TR and TA was measured at points along a transect (Fig. 2A). The displacement responses in the frequency domain of seven points (A–G) along this transect, starting over the TR, through to the TA, show that every point has a maximum response peak within the male calling frequency range (Fig. 2B). However, there is also clearly motion occurring at higher frequencies, over the range 12 to 20 kHz. The displacement measured at the TA is over an order of magnitude less than that at the TR, such that where the TR is moving 15 nm (peak), the TA is moving less than 150 pm (peak). The frequency responses of the measurements across the transect were also normalised (Fig. 3). In this case the displacement response is first normalised with respect to SPL (the gain function), then normalised relative to the height of the amplitude peak, showing that the frequency response of the measured vibration changes along the transect. Fig. 4 shows the two normalised frequency spectra for points A and G, clearly demonstrating a decrease in the response at higher frequencies. The normalised response measured between 12 and 20 kHz is seen to decrease *ca.* 50–75%, as the measurement moves from the TR to the TA.

The phase response changes along the TR from apex to base as a function of both frequency and spatial location [see figure 8 in Sueur et al. (Sueur et al., 2006)]. In the female *C. atra* the phase at the calling song centre frequency (~10 kHz) changes from a ~60 deg. lead at the apex to lag of ~80 deg. at the base, a change of ~140 deg. At higher frequencies the lag increases, up to ~300 deg. at 20 kHz, with a corresponding lag of ~90 deg. at the apex, resulting in a change of ~210 deg. This phase response represents the travelling wave observed on the TR. The TA does not display such a phase response (Fig. 3), with very little change in phase recorded along the length of the TA (8.75±2.9 deg. at 10 kHz and 24±9.1 deg. at 20 kHz; $N=8$). This small phase change along the ridge results in the apodeme moving in an 'up-and-down' motion, whereas the TR (and TM) are crossed by travelling waves.

The motion of the points along the TR and TA transect were also measured at single frequencies (Fig. 5A). From this the displacement of the transect can be displayed, clearly demonstrating how the large

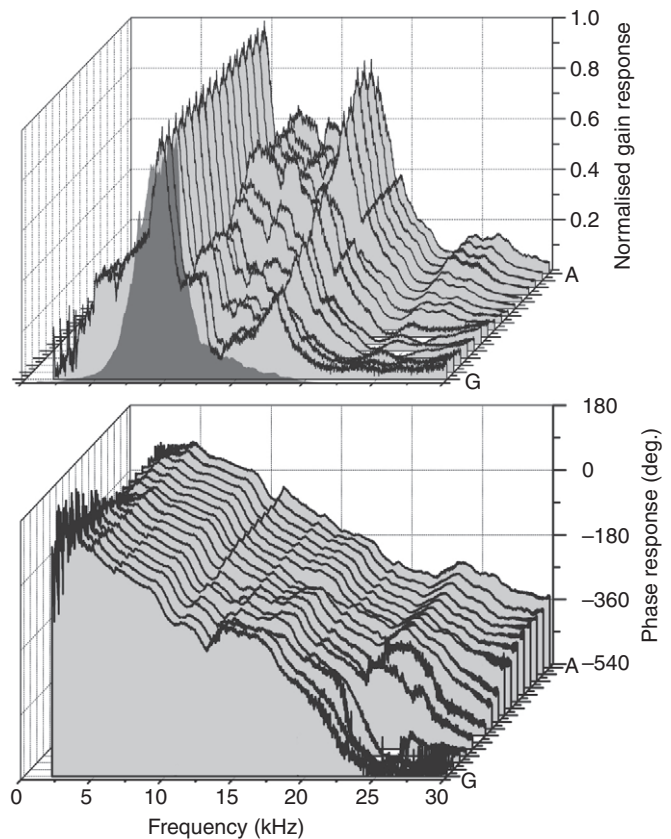


Fig. 3. The normalised gain response of the tympanal ridge and apodeme of a female *C. atra*. The normalised gain response (upper panel) in frequency of many points from within the span of points A–G of Fig. 2. The average calling song frequency spectra of the male cicada is shown as the shaded graph. The phase response (lower panel) represents the travelling wave observed on the tympanal ridge. The tympanal apodeme does not display such a phase response, with very little change in phase recorded along the length of the TA.

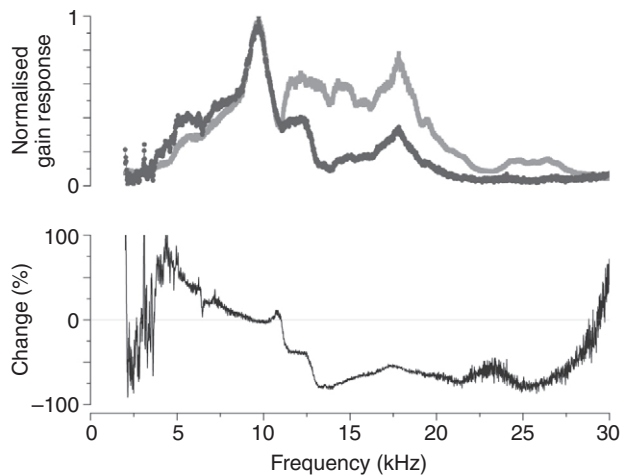


Fig. 4. The change in the frequency response from the tympanal ridge to the apodeme of a female *C. atra*. The normalised gain response in frequency of points A (light grey line and squares) and G (dark grey line and circles) of Fig. 2 is shown. The percentage change between the two spectra is also shown (lower trace).

motion of the TR relates to the much smaller motion of the TA. In this individual the maximal motion of the transect occurs at 9.4 kHz, within the male calling song frequency band. The movement of the TA at frequencies outside the male calling range is reduced by a much greater factor than for the calling song. The reduction factor across all specimens measured was 117.5 ± 14.3 ($N=8$) at 15.9 kHz and 27.4 ± 11.1 ($N=8$) at 9.4 kHz. The data presented in Fig. 5A represent a snapshot of the motion of the end of the TR and the TA at two opposite points in the cycle of the sinusoidal sound stimulation (supplementary material Movies 1 and 2).

DISCUSSION

As in many other cicada species, *C. atra* gather and form chorus centres. Within these aggregations, females have to select and localise a single male for mating. This task might be complicated by the presence of other species calling in the same area. This is particularly true for *C. atra*, which is often found with two other species, *Cicada orni* and *Lyrystes plebejus*. The tympanum of *C. atra* is mechanically tuned to the male's calling song ensuring a certain level of selective attention (Sueur et al., 2006). However, when compared with the acoustic environment the insect may inhabit, this tuning is relatively broad, such that the tympanal membrane would not filter out all of the background noise. In these new experiments it is seen that the apodeme, the internal cuticular connection between the external tympanal system and the mechanosensory neurons, acts a further bandpass filter. It reduces higher frequency components found in the motion of the external tympanal membrane and ridge. This means that at the attachment site of the mechanosensory neurons in the sensory organ the motion of the apodeme is tuned very closely to the male's calling song. This ensures an efficient selective auditory response to conspecific males in the multi-species choruses.

Intracellular recordings from interneurons of different cicadas, although not *C. atra*, have shown distinct frequency tuning (Huber et al., 1980; Huber et al., 1990; Fonseca et al., 2000; Fonseca and Correia, 2007). Studies of other insect ears, such as tettigoniids, have also demonstrated the ability of individual sensory neurons and interneurons to encode frequencies (Oldfield and Hill, 1983; Stumpner, 1996; Hennig et al., 2004). Thus, it is possible that the

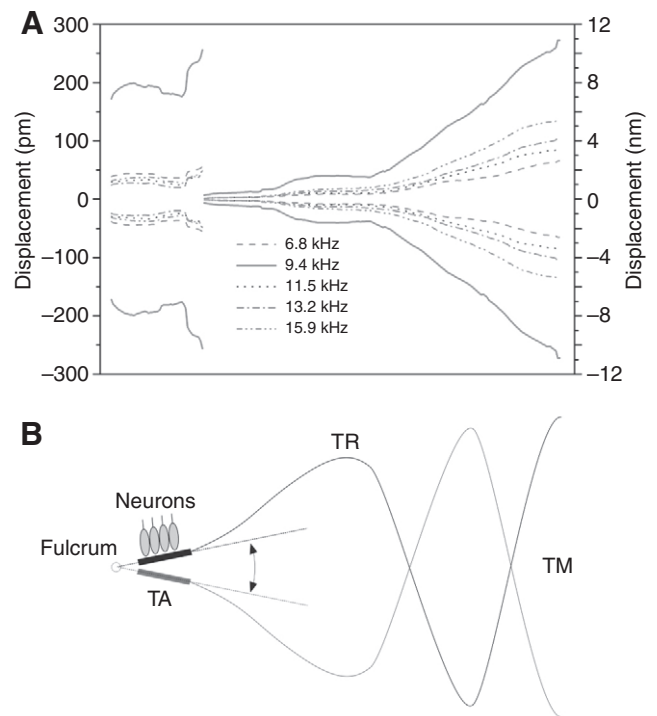


Fig. 5. The displacement of the tympanal ridge (TR) and apodeme (TA) of a female *C. atra*. (A) Motion of the transect from Fig. 2A, shown at the maximal positive and negative values for the end point of the transect on the TR, for five different frequencies. At 15.9 kHz the TR maximal motion is ± 5 nm, reducing to ± 40 pm at the TA, a reduction factor of 125, whereas at 9.4 kHz the reduction factor is 50 (TR: ± 10 nm, TA: ± 200 pm). (B) Schema of the 'lever' motion of the TA, with an imaginary fulcrum past the TA, generated by the travelling wave through the tympanal membrane (TM) and TR.

neurons within the sensory organ also contribute to frequency filtering, following from the mechanical filtering of the tympanal membrane, ridge and apodeme, although no neurological studies have been carried out on the *C. atra*.

These new experiments have also directly measured the displacement undergone by the apodeme in response to biologically relevant sound levels. These measurements, carried out *in vivo*, show for the first time how small the motion of the apodeme is. Typically the apodeme moved only a few tenths of a nanometre. Although, unfortunately, it was not possible in these experiments to carry out electrophysiological recordings simultaneously with the laser recordings, previous work on an insect tympanal system demonstrated that insect mechanosensory neurons can be sensitive to such small motions (Windmill et al., 2007).

Surprisingly, the travelling wave observed along the tympanal ridge disappears at the apodeme level. The apodeme moves in a simple up-and-down motion behaving somewhat like a lever, displaying very little phase change along its length, as shown in Fig. 5A and B. The transduction of mechanical oscillations between the ridge and the apodeme is probably constrained by an impedance mismatch between the two structures. The apodeme is not only smaller than the ridge but also appears thicker, and so stronger, suggesting a higher stiffness. This might explain the change of oscillatory mode and the drastic amplitude reduction. The absence of a travelling wave along the apodeme rejects the hypothesis that this system could work as the basilar membrane of the mammal inner ear (Békésy von, 1960). The apodeme selects a range of frequencies but does not seem to be able

to decompose sound frequencies as previously expected (Sueur et al., 2006). It could be that the apodeme, made of stiff cuticule, is mechanically tuned to the main frequency of the calling song, with the mechanosensory neurons, made of soft tissue, measuring the motion of the apodeme. Or, the combined system of the apodeme and mechanosensory neurons, coupled mechanically, provides the observed signal filtering.

So the outstanding problem is to understand how the apodeme and mechanosensory cells interact at a mechanical level. In part, an examination of the material properties of the system is required, to help analyse the mechanical transduction process. Furthermore, there is not yet an explanation of why the system requires so many mechanosensory neurons. Although this paper describes the missing step in the acousto-mechanical transduction in the cicada ear, it is clear there is still much work to be done to understand how a cicada hears.

LIST OF ABBREVIATIONS

SC	sensory organ capsule
SPL	sound pressure level
TA	tympanal apodeme
TM	tympanal membrane
TR	tympanal ridge

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