The Journal of Experimental Biology 214, 3165-3172

doi:10.1242/jeb.056465

RESEARCH ARTICLE

No evidence for DPOAEs in the mechanical motion of the locust tympanum

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Accepted 5 July 2011

SUMMARY

Distortion-product otoacoustic emissions (DPOAEs) are present in non-linear hearing organs, and for low-intensity sounds are a by-product of active processes. In vertebrate ears they are considered to be due to hair cell amplification of sound in the cochlea; however, certain animals lacking a cochlea and hair cells are also reported to be capable of DPOAEs. In the Insecta, DPOAEs have been recorded from the locust auditory organ. However, the site of generation of these DPOAEs and the physiological mechanisms causing their presence in the locust ear are not yet understood, despite there being a number of potential places in the tympanal organ that could be capable of generating DPOAEs. This study aimed to record locust tympanal membrane vibration using a laser Doppler vibrometer in order to identify a distinct place of DPOAE generation on the membrane. Two species of locust were investigated over a range of frequencies and levels of acoustic stimulus, mirroring earlier acoustic recording studies; however, the current experiments were carried out in an open acoustic system. The laser measurements did not find any evidence of mechanical motion on the tympanal membrane related to the expected DPOAE frequencies. The results of the current study therefore could not confirm the presence of DPOAEs in the locust ear through the mechanics of the tympanal membrane. Experiments were also carried out to test how membrane behaviour altered when the animals were in a state of hypoxia, as this was previously found to decrease DPOAE magnitude, suggesting a metabolic sensitivity. However, hypoxia did not have any significant effect on the membrane mechanics. The location of the mechanical generation of DPOAEs in the locust's ear, and therefore the basis for the related physiological mechanisms, thus remains unknown.

Key words: bioacoustics, hearing, tympanum, locust, distortion-product otoacoustic emissions, laser Doppler vibrometry.

INTRODUCTION

Insect hearing organs, despite their composition being relatively simple, have been found to function in a complex manner, and are highly sensitive and effective at detecting sound and transducing this energy into neural signals (Hoy and Robert, 1996; Yager, 1999). One of the main types of insect auditory organs is the tympanal system. This system consists of a membrane, with auditory neurones mechanically linked to it, which vibrates in response to sound (Yack and Fullard, 1993; Yager, 1999; Miller and Surlykke, 2001). Despite the large structural morphological differences in insect auditory organs they are all similar in that all contain chordotonal organs. These organs are mechanoreceptors and contain sensory neurons called scolopidia; the number of scolopidia varies between species and orders, and has been found to be correlated to the sensitivity and complexity of the auditory organ (Yack, 2004).

Within the class Insecta, the tympanal organs of locusts and grasshoppers are the most studied and one of the best understood (Yack, 2004). Locusts use their hearing sense for intra-specific communication in mating rituals and also in aggressive interactions (Hoy et al., 1989); it has also been suggested that they can detect ultrasound and therefore avoid predation by bats (Robert, 1989; Hoy et al., 1989; Miller and Surlykke, 2001). The structure of the hearing organ has been described in great detail by many authors (e.g. Gray, 1960; Michelsen, 1971a; Stephen and Bennet-Clark, 1982). Locust auditory organs are located on the first abdominal segment under the wing on either side of the insect and consist of a tympanal membrane (TM) and Müller's organ, the chordotonal organ in which 60–80 scolopidia attach to the membrane (Michelsen, 1971a; Gray, 1960). The scolopidia, originally classified into four groups (groups a–d), attach to the membrane at different points; each group is sensitive to a different range of frequencies (Gray, 1960). Previous studies using a laser Doppler vibrometer (LDV) found that the mechanics of the TM of locusts were surprisingly complex; it was observed that the received sound created a travelling wave across the membrane to direct the sound energy, depending on the frequency, to the specific point of attachment of the a–d cells on the membrane (Windmill et al., 2005), a form of tonotopy reminiscent of the mammalian cochlea.

Mammalian auditory organs amplify low-level sound through active mechanisms of the hair cells within the cochlea. This is an example of active hearing, where the sound is conditioned by metabolic processes in the ear. The cochlear amplification of sound in mammalian systems produces otoacoustic emissions (OAEs). These can be produced in mammalian auditory organs spontaneously with no external sound stimulus – spontaneous otoacoustic emissions (SOAEs) – or by electrically stimulating the auditory organ – electrically evoked otoacoustic emissions (EEOAEs) (Shera and Guinan, 1999; Kössl et al., 2008). Another example of evoked OAEs is distortion-product otoacoustic emissions (DPOAEs), first discovered in human ears (Kemp, 1978). These are acoustic emissions produced by non-linear hearing organs when two tones of distinct frequency, $f1$ and $f2$, are applied to the hearing organs of the animal under investigation. This effect is equivalent to the concept of intermodulation in the field of signal processing.

Expected DPOAEs are various arithmetic combinations of the applied tones, with the $2f1–f2$ products having the highest intensity and appearing first (Fig. 1). Active hearing processes, previously thought to be found only in mammalian ears, were also recorded acoustically in the locust ear using a closed acoustic system (Kössl and Boyan, 1998). The production of DPOAEs in the locust ear also appeared to be affected by CO_2, which implied that the presence of DPOAEs is metabolically sensitive (Kössl and Boyan, 1998). The site of origin and the cellular basis of the production of DPOAEs in invertebrates remain elusive (Kössl et al., 2008). The effect of ablation of the membrane, electrical stimulation and severing of the auditory nerve on the production of DPOAEs did not determine the location of their origin in the tympanal organ of *Locusta migratoria* (Möckel et al., 2007).

The aim of our study was to investigate the vibration of the TM to try and establish the origin of DPOAEs in the locust ear. The locust ear was stimulated with two tones and the velocity of the membrane recorded with a LDV. The set-up of the experiment was altered to an open acoustic field in comparison to the closed system of previous acoustic experiments (Kössl and Boyan, 1998). The sound levels and the frequencies used were all within the acknowledged hearing range of the locusts used and the presence of a travelling wave in the tympanal membrane motion indicated normal auditory responses (Windmill et al., 2005; Windmill et al., 2008). The sound energy of the DPOAE would cause a vibration of the membrane (or *vice versa*) and should thus be detectable with the LDV – because of the large exposed TM of locusts the membrane mechanics are easily measurable. The site of the DPOAEs in locusts was previously speculated to be Müller's organ or more specifically the cilia of the scolopidia (Kössl and Boyan, 1998) and therefore a specific attachment point of a certain group of receptor cells might also be identifiable through the membrane deflections. In a recent study, the DPOAEs of bushcrickets were found to be dramatically affected by the application of an insecticide that is thought to specifically affect the scolopidia of insects; this suggested to the authors that the scolopidia are an integral part of DPOAE production in invertebrate hearing (Möckel et al., 2011). In the current experiments two species of locust, *Schistocerca gregaria* Forskål and *L. migratoria* L. were investigated. Specific points on the membrane where the receptor cells attach, including the pyriform vesicle (PV, where the high frequency receptor cells attach), along the folded body (FB), the styliform foot body, and the elevated process (where the lower frequency receptor cells attach), were selectively measured using the LDV. Furthermore, scans of the entire membrane were recorded. Scans and single-point recordings were also carried out when the animals were in a state of hypoxia induced with CO_2 application.

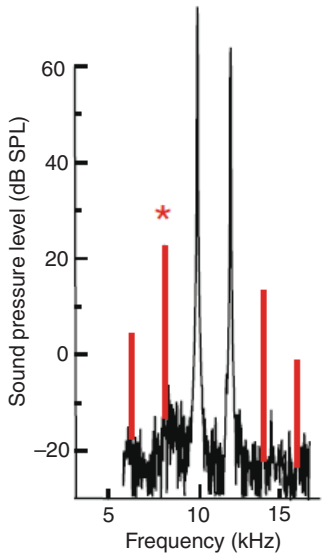

Fig. 1. Distortion-product otoacoustic emissions (DPOAEs; red) recorded acoustically from a locust tympanal organ when two tones ($f1$ and $f2$) are applied. The largest expected DPOAE ($2f1–f2$) is indicated with an asterix (modified from Kössl et al., 2008). The DPOAEs are arithmetically calculable from the two-tone input. The largest distortion product produced is always $2f1–f2$; it also the first to appear, with others appearing as the sound pressure level (SPL) is increased.

MATERIALS AND METHODS

Animals

Adult male and female *S. gregaria* and *L. migratoria* were obtained from Blades Biological Ltd (Cowden, UK). To prepare the locusts for experimentation, the wings were trimmed back to expose the tympanal organ; the locusts were otherwise unharmed and the alertness of the insect was confirmed during the experiment by checking for independent antennal movements. The locusts were placed dorsal side up whilst restrained using Blu-Tack (Bostik-Findley, Stafford, UK), ensuring that the tympanal organ was still exposed. The right tympanal organ was used in all experiments in order to keep the set-up consistent. The tympanal organs of *L. migratoria* have a piece of cuticle covering the membrane; to overcome this obstacle the animal was angled such that the laser beam could reach the PV and the majority of the membrane. This was done in keeping with the non-invasive nature of the experiments. The room where all of the experiments were carried out was kept at a constant temperature of 23°C. The animal was moved so that the video feedback on the LDV computer system was in focus and in line with the tympanal organ, allowing the laser beam to be positioned correctly on the membrane at all times. The loudspeaker was directed towards the tympanal organ and the stand was kept at the same height throughout the experiments. The microphone was placed in the holder and adjusted so that it was as close to the tympanal organ as possible.

Mechanical and acoustic measurements

The vibration and displacements of the TM were recorded using a scanning LDV (Polytec PSV-300-F; Waldbronn, Germany) with a close up head attached to the OFV-056 scanning head. The whole membrane was initially scanned using a wideband chirp acoustic stimulus that ranged from 1 to 30 kHz, which was generated using the PSV software-controlled digital-to-analog converter in the LDV. This initial scan was carried out to confirm that the membrane was functioning properly (i.e. was not damaged and the intrinsic travelling wave could be seen). If this test failed then the animal was not used. The test was repeated at the end of the experimental regime to ensure that the membrane was still functioning as expected. The two-tone stimulus was generated using a function generator (Tektronix, Dual Channel-AFG 3102; Bracknell, UK); the sound stimulus consisted of two distinct frequencies $f2$ and $f1$, where $f1<f2$ and the sound pressure of $f1$ was always 10 dB sound pressure level (SPL) higher than that of $f2$, as in previous studies this elicited the highest $2f1–f2$ distortion (Kössl and Boyan, 1998). The ratio of $f1/f2$ was previously found to be an important factor in DPOAE generation in both mammalian and invertebrate auditory organs (Kemp, 1986; Kössl and Boyan, 1998). The ratio of the frequency of $f1/f2$ was therefore altered, keeping $f2$ constant and altering $f1$

in 0.02 (ratio) increments. Frequencies between 2.5 kHz and 22.5 kHz were investigated as the locust organ has been found to be sensitive within this range (Michelsen, 1971a; Römer, 1976). The sound was amplified using an audio amplifier (Sony, TA-FE570; Tokyo, Japan) before being passed to a loudspeaker (4″ speakers, Riesen, Maplin; maplin.co.uk), which was directed towards the tympanal organ. The SPL of the two tones was measured using a calibrated microphone coupled to a preamplifier (Brüel & Kjær, microphone: 4138, preamplifier: Nexus 2690; Nærum, Denmark), placed as close as possible to the tympanal organ to ensure the correct SPL.

The laser point from the vibrometer system can be moved to specific positions on the membrane to test for the presence of DPOAEs, for example at the points where the scolopidia attach. The vibration of each point of interest on the membrane was recorded using the LDV's single-point acquisition setting whilst the tympanal organ was acoustically stimulated by two tones. The attachment points of the groups of receptor cells were initially investigated in relation to the two-tone stimulus frequency applied to the membrane. Following this the PV was chosen as the site from which recordings were made for the remaining experiments as it was easily distinguishable on the membrane of every animal and could be returned to with the laser point if the animal moved.

Software was written in LabVIEW (National Instruments, version 8.5.1; Austin, TX, USA) to control the function generator using a laptop computer, whilst simultaneously recording the LDV velocity signal for the membrane using a data acquisition card (National Instruments, BNC-2110 and USB-6251). This software automatically ran through all the $f1$ and $f2$ frequencies, ranging from 2.5 kHz to 22.5 kHz [corresponding to the ratios previously published by Kössl and Boyan for each $f1$ (Kössl and Boyan, 1998)]. The velocity of the membrane and the signal from the microphone were averaged 10 times; the time trace for both parameters was also recorded. This was repeated for 15 individuals of each species of locust.

Simulation of sound production from a planar source

To confirm our experimental set-up was sufficient to measure mechanically the DPOAEs observed acoustically in previous studies (Kössl and Boyan, 1998), the locust tympanum was modelled as an acoustic radiator, moving as a piston (guaranteeing maximum power output for a given surface displacement). The acoustic field and intensities were calculated using the Rayleigh integral, which states that the pressure p (ignoring frequency-dependent attenuation) at a point R away from a surface S=$S(R_0)$ moving with a velocity v, in a medium with density ρ and speed of sound c, obeys the equation (Benny et al., 2000):

$$p(R,t) = \frac{\rho}{2\pi}\frac{\partial}{\partial t}\int_S \frac{v(R_0, t - R/c)}{R}\,\mathrm{d}S\ . \qquad (1)$$

For a membrane oscillating at a single frequency f, the velocity can be described as:

$$v = v(R_0)\,\exp\,[i2\pi f(t - R/c)]\ . \qquad (2)$$

The pressure can therefore be made independent of time, and discretised for numerical calculation, such that the absolute pressure P obeys:

$$P(R) = \frac{\rho A f}{N}\sum_i^N \frac{d(R_0)\exp[-2\pi R/c]}{R}\ , \qquad (3)$$

where A is the area of the surface, N is the number of discretisation points and d is the surface displacement.

At frequencies dealt with in this paper (<22.5 kHz) there exists no geometric near field for an emitter the same size as the locust tympanum – the field pattern is simply a main lobe radiating directly from the surface. Simulations show that for a surface 2.2×1.5 mm (from Stephen and Bennet-Clark, 1982) radiating as a piston, surface displacements equivalent to the experimental noise floor of ~3 pm (velocity: 0.2 μm s^{-1}) would result in a sound intensity at 10 kHz of –2 dB SPL at the surface and –6 dB SPL at 1 mm. To generate ~10 dB SPL at such close proximity would require a surface displacement 4 times the noise floor (~12 pm). Our experimental set-up is able to detect DPOAEs greater than 0 dB. Note that the assumption of a radiating flat piston is an idealised description of the tympanal membrane. More realistic representations of the surface displacement (i.e with fixed boundaries) would need to vibrate with an even larger displacement to produce the same sound as the ideal piston case, increasing our ability to measure these vibrations.

In Kössl and Boyan's previous experiments it was found that DPOAEs could be recorded that were over 10 dB SPL in emission (Kössl and Boyan, 1998). The parameters used previously of frequency and SPL were therefore repeated in the current study to see whether these large emissions could be detected on the membrane using the LDV. The simulations show that a DPOAE of 10 dB SPL would be caused by a membrane to be displaced by 12 pm (velocity: 0.8 μm s). The same set-up was used as before, recording the FFT for the velocity of the PV using the PSV software on the single-shot setting. Scans were also carried out initially. The data averaging was increased to 500 times using the single shot setting focused on the PV; this was done to reduce the noise floor. The values of SPL and the ratio of $f1/f2$ were used from previous values (Kössl and Boyan, 1998). The effect of CO_2 hypoxia at these levels on the velocity of the membrane was also investigated by applying CO_2 and recording the PV velocity during hypoxia; repeated for 10 insects of each species.

RESULTS

Laser vibrometry scans

Accurate membrane mechanics could be recorded using the LDV whilst the membrane was stimulated with two tones, in an open set-up, non-invasively. It was expected that the initial scans of the membrane would detect a distinct area of deflection, such as one of the attachment points of a group of receptor cells. However, the scans of the membrane at the expected $2f1–f2$ DPOAE frequency did not find any coherent displacement of the membrane. This was the case at all of the frequencies investigated. Altering the SPL of the applied two tones did not alter the membrane's behaviour at the DPOAE $2f1–f2$ frequency. At the frequencies of $f1$ and $f2$ the intrinsic travelling wave was observed, funnelling the sound to the relevant receptor cells, indicating that the membrane was functioning correctly (Fig. 2).

Single shot experiments

The animal was stimulated with two tones at a range of frequencies at different SPL while the laser point was moved across the membrane. The velocity at the point was measured continuously to enable detection of any significant deflection at the $2f1–f2$ frequency. This was initially carried out on a small number of locusts and there did not appear to be any significant points on expected parts of the membrane; namely, the receptor–cell attachment sites on the FB and PV. This concurs with the scan data (data not shown). The single-shot recordings of the velocity of just the PV, whilst stimulated with various $f1/f2$ frequencies, were carried out for 15 locusts of each species. The PV was chosen

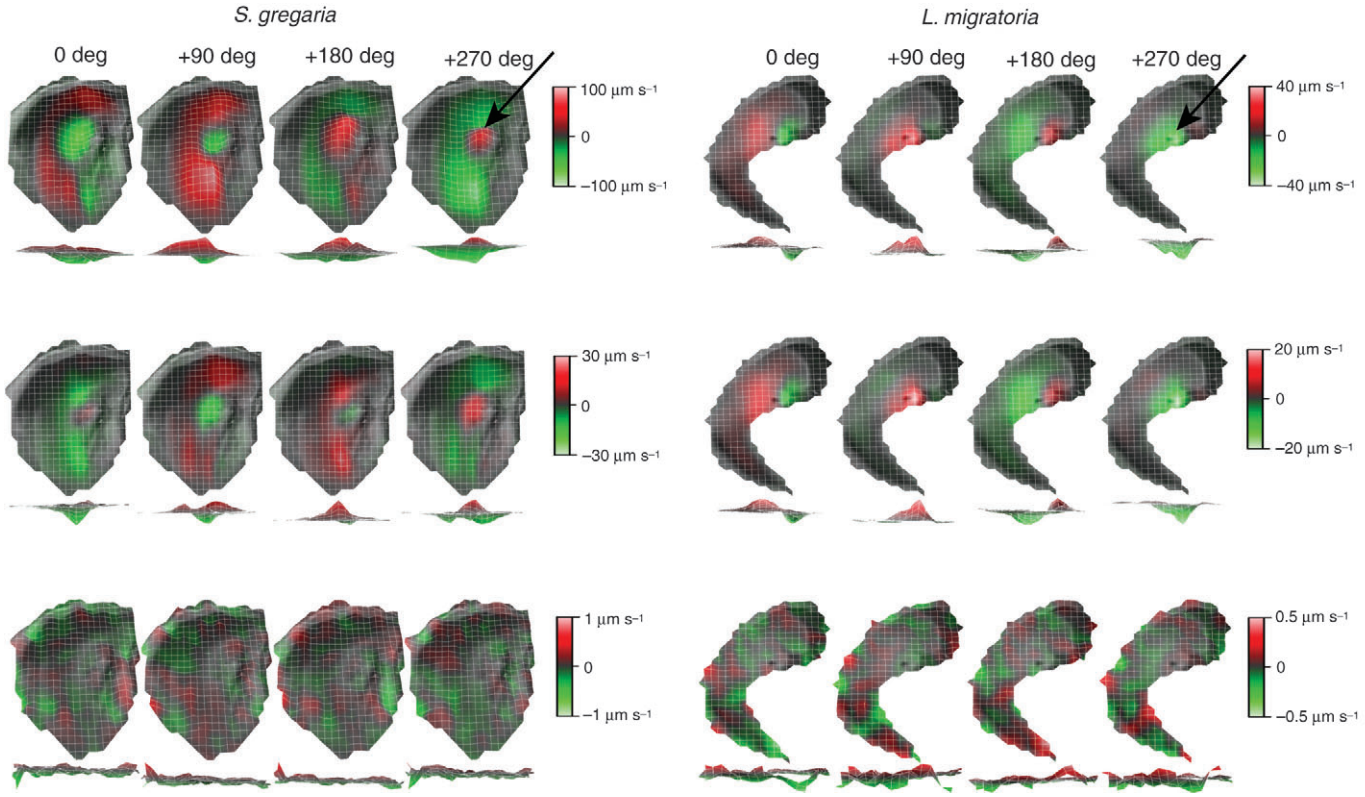

Fig. 2. Laser Doppler vibrometer (LDV) scans of the tympanal membrane of each locust species: *Schistocerca gregaria* and *Locusta migratoria*. The acoustic stimulus was two tones $f1$=12.25 kHz and $f2$=12.5 kHz at sound levels of 45 and 55 dB SPL for $f2$ and $f1$, respectively. The travelling wave can be seen on the membrane for $f1$ and $f2$ but at the expected DPOAE of $2f1–f2$=12 kHz there are no coherent points of deflection on the membrane. The velocity of the membrane is shown for four phases of the oscillation cycle; the side view of the tympanum is also shown. The scale bars represent the inward (green) and outward (red) velocity for each frequency; arrows show the pyriform vesicle (PV) location on the membrane.

as no DPOAEs were detected on any part of the membrane, the PV is easily locatable on the membrane so that the recordings would be consistent, and the PV is known to be mechanically sensitive to a wide range of frequencies. The results of the single-point experiments did not show any significant difference between the velocity of the PV at $2f1–f2$ and the background noise floor of the velocity recordings (paired t-test $P\leq0.1$, N=15 for both *S. gregaria* and *L. migratoria*); the velocity of the PV for different $f2$ and increasing ratios of $f1$ are shown for both species (Fig. 3). The background noise floor was calculated as the mean velocity over the recorded spectrum of 1–30 kHz with $f1$, $f2$ and $2f1–f2$ values removed for each ratio of $f1/f2$ and then averaged over the 15 recordings for each species.

A number of the higher SPL two-tone experiments that elicited DPOAEs above 10 dB in previously published work did reveal DPOAEs of small magnitude on the membrane at a few $f1/f2$ values tested using the single-shot setting. However, these were also present on the sound recording, indicating that it was in fact the loudspeaker producing the distortion (Fig. 4). This was confirmed by recording the sound stimulus in the absence of an animal. The DPOAEs were still present on the animal-absent sound recording eliminating the possibility that the locust tympanal organ was producing the DPOAEs. The sound level required to cause the distortion of the loudspeaker is comparatively not very high; the levels in the current experiments never went above 70 dB SPL. The scans also confirmed the lack of any distinct emission from the membrane at the higher SPL levels (Fig. 5).

It was previously found that applying CO_2 to locusts reduced the growth rate of the $2f1–f2$ DPOAE [see fig. 10 of Kössl and Boyan (Kössl and Boyan, 1998)]; this was suggested to be due to the fact that the process was physiologically sensitive. The results of the current experiments did not show any drastic or uniform alteration in the membrane behaviour when CO_2 was applied. Membrane velocity was also measured before and during CO_2 application to investigate whether the induced hypoxia affected the velocity of the membrane at the $f1$ and $f2$ tones and also the $2f1–f2$ frequency; the velocity at these frequencies was found not to significantly change in any of the recordings in either of the species, as shown in Fig. 6 (two-sample Kolmogorov–Smirnov test $P\leq0.1$, N=10 for both *S. gregaria* and *L. migratoria*).

DISCUSSION

In previous experiments the acoustic presence of DPOAEs recorded from the locust ear suggested that the organ possesses non-linear hearing derived from active and mechanical non-linearities (Kössl and Boyan, 1998; Möckel et al., 2007). Complex sound processing in the locust ear has been known for some time; it is one of the few insect auditory organs known to be capable of frequency discrimination and having specific groups of cells for particular frequency ranges (Gray, 1960; Michelsen, 1971a). Regardless of this, the organ was previously considered to be a linear system (Breckow and Sippel, 1985; Michelsen, 1971b; Michelsen, 1971c). It was thought that all insect tympanal organs functioned passively through the mechanical motion of the membrane as no non-linear

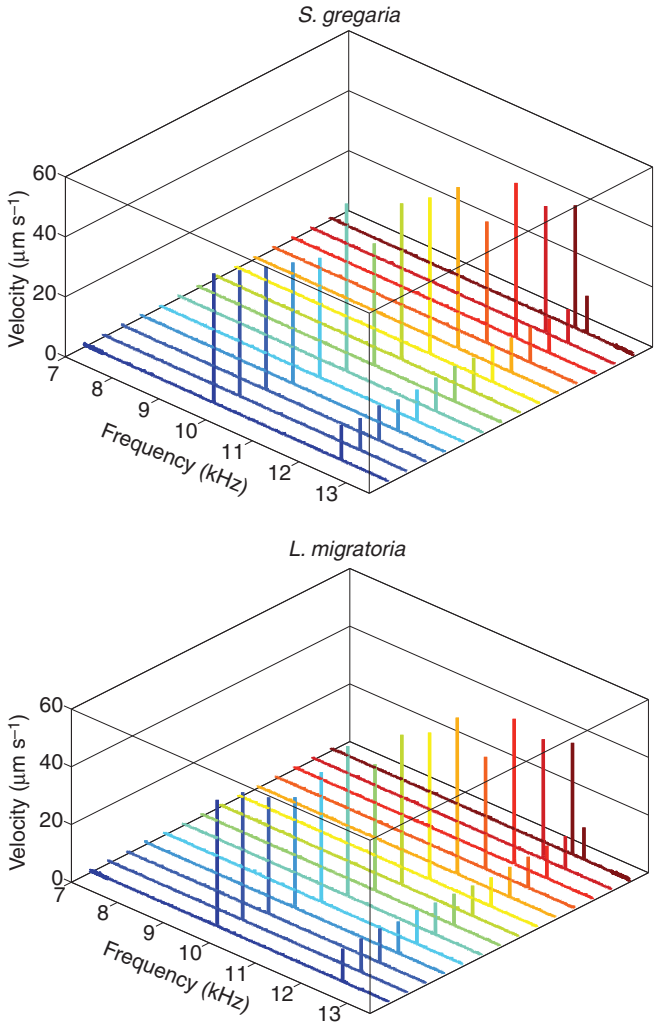

Fig. 3. Waterfall plots of the velocity of the PV of an individual locust of each species. The stimulus frequency was $f2$=12.5 kHz, where $f1$ was increased in a ratio of 0.02 from 1.02 up to 1.28. The sound levels were $f1$=55 dB SPL and $f2$=45 dB SPL. Fast Fourier transform (FFT) graphs show no DPOAEs from the recorded membrane velocity at the PV. There is not a significant change in velocity at the expected $2f1-f2$ frequency in comparison to the noise floor, for the individual locusts (*S. gregaria*, paired *t*-test, P=0.473, t=0.74; *L. migratoria*, P=0.605, t=0.53).

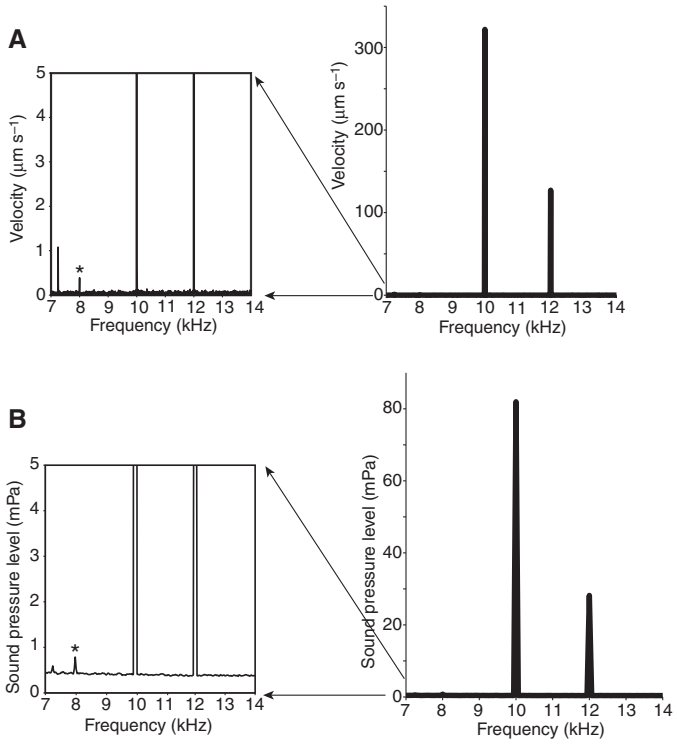

Fig. 4. (A) The recording of the velocity of the PV of an individual *S. gregaria* using the single-shot setting of the LDV. The two tones applied are $f1$=10 kHz and $f2$=12 kHz. The $2f1-f2$ expected DPOAE at 8 kHz is present on the membrane at very low velocity (0.5 μm s^{-1}). (B) Microphone recording of the SPL; the DPOAE at 8 kHz is also visible. The SPL of the two tones is $f1$=28 mPa, $f2$=88 mPa. The presence of the DPOAE on the microphone recording indicates that it is not the hearing organ that is producing the DPOAE (marked with an asterisk). The velocity of the membrane is only 0.5 μm s^{-1}, the same as that of the scans at the $2f1-f2$ frequency (Fig. 2). The graphs on the left side of A and B show the data on an expanded axis.

behaviour was found in the mechanical motion of the membrane of any species (Yager, 1999). Despite this, the travelling wave on the locust TM shows some similarity to the vertebrate travelling wave auditory membranes in terms of, for example, wavelength and wave velocity. However, the tympanal mechanics were not found to change in dead locusts, suggesting that the frequency discrimination is a passive system and does not require extra metabolic processing (i.e. activity) (Windmill et al., 2005). Another insect auditory organ, in the cicada *Cicadatra atra*, was also found to possess a travelling wave that is reminiscent of the mechanics of the locust membrane. The TM of *C. atra* was also found to behave linearly with respect to sound pressure; these auditory organs are therefore also believed to be passive despite the presence of the travelling wave, again with the same membrane behaviour observed post-mortem (Sueur et al., 2006).

Mammalian DPOAEs are thought to be created by the active amplification of sound due to motile hair cells in the cochlea (Frank and Kössl, 1996; Ruggero, 1992), whereby force generated by the hair cells introduces non-linearity. However, a study of non-mammalian hearing organs that do not possess hair-cell somatic motility found that both spontaneous and evoked OAEs were present (Bergevin et al., 2008), suggesting that other generation mechanisms of OAEs may also be a possibility. In non-mammalian hearing organs the presence of SOAEs has been described as giving the clearest evidence that a hearing organ is capable of active processes (Manley, 2001). SOAEs have so far never been recorded in any invertebrate tympanal organ investigated (Kössl et al., 2008). However, another characteristic of OAE-capable non-mammalian hearing organs is a travelling wave on the basilar membrane (Bergevin et al., 2008). Does this indicate that the hearing organs with membranes possessing travelling waves in both vertebrates and invertebrates are capable of producing DPOAEs? In LDV-recorded basilar membrane displacements of mammals it was found that there was a linear response to increasing SPL at frequencies far removed from the best frequency, but extremely non-linear behaviour at the characteristic frequency, suggested to be due to the outer hair cells (Ruggero, 1992). This non-linearity of the membrane mechanics was not seen in the TM of locusts or cicadas, with the TM reacting linearly at all frequencies.

Examples of active hearing have been found in insect auditory organs of an entirely different structure to the tympanal organ. The

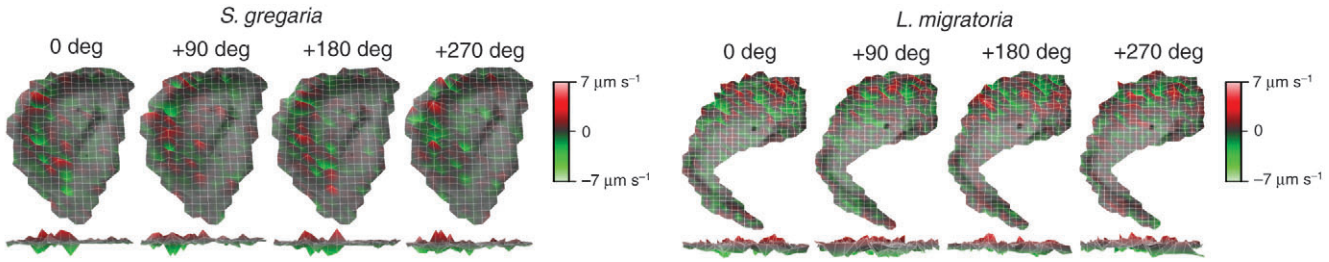

Fig. 5. Membrane scans of each species when the membrane is stimulated with two tones, previously found to produce a 2*f*1−*f*2 DPOAE of 10 dB, where *f*1=10 kHz and *f*2=12 kHz and the sound level was 60/70 dB SPL for *f*2/*f*1. There are no coherent points of oscillation on the membrane at the expected DPOAE of 2*f*1−*f*2=8 kHz. The velocity of the membrane is shown for four phases of the oscillation cycle and the side view of the membrane is also shown. The scale bar represents the inward (green) and outward (red) velocity.

antennal displacements of mosquitoes and fruit flies have both been shown to amplify received sound through mechanical and active non-linearities (Göpfert and Robert, 2001; Jackson and Robert, 2006; Jackson et al., 2009). Sound is received by antennal structures on the head with a chordotonal organ, the Johnston's organ, at the base. The non-linearity is produced by mechanical amplification of the oscillations of the antennae, generated through the mechanosensory cells within the Johnston's organ (Göpfert and Robert, 2001; Jackson and Robert, 2006; Albert et al., 2007; Nadrowski et al., 2008; Jackson et al., 2009). As Müller's organ is composed of scolopidia, in common with Johnston's organ in Diptera, it would not be surprising if Muller's organ was capable of active amplification.

There remains a great deal to be understood regarding the processing of sound by the locust tympanal organ. This is further complicated by the lack of knowledge regarding the functioning and reaction of Müller's organ to sound and also an incomplete understanding of the reasons for the travelling wave on the membrane. Breckow and Sippel previously investigated the oscillation of Müller's organ when isolated from the body of the

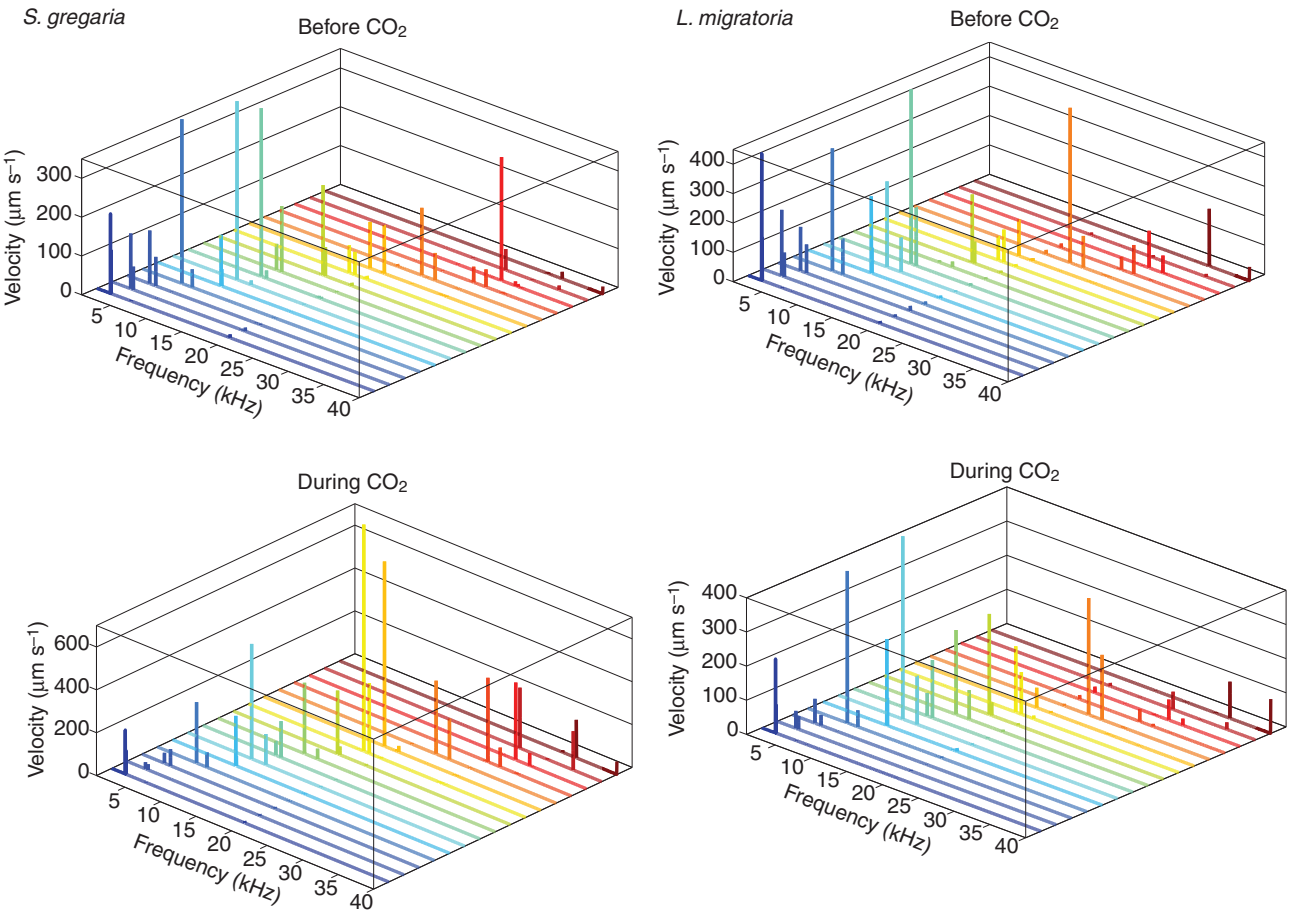

Fig. 6. LDV single-shot results of the velocity of the PV before and during CO_2 hypoxia for individuals of both species. The velocity of the membrane was found not to react in a specific manner. The velocity at the *f*1, *f*2 and 2*f*1−*f*2 frequencies did not significantly change as a result of hypoxia for any of the animals tested of either species (two-sample Kolmogorov–Smirnov test $P \geq 0.1$).

locust, showing that the oscillations of Müller's organ were very complex (Breckow and Sippel, 1985). Oscillations were recorded by a stroboscopic method using stimulus frequencies up to 20 kHz; the technical recording of the mechanical displacement was not of high accuracy, however, and the SPL required to produce recordable oscillations was extremely high and unnatural (103–113 dB SPL). The membrane and Müller's organ vibrations were linear when stimulated with high SPLs (Breckow and Sippel, 1985). Another study found that Müller's organ creates a load on the membrane and when removed the amplitude of vibration increased dramatically; this suggests that Müller's organ has a damping effect on the membrane oscillations (Stephen and Bennet-Clark, 1982).

The mechanical deflection of the tympanal membrane at the expected $2f1–f2$ frequencies relating to DPOAEs should be detectable using the laser vibrometry system in our experiments. The LDV recordings did not show any vibration at the expected frequencies on any point on the membrane. In contrast, for human and other vertebrates, experiments with LDV techniques found that the DPOAE-induced vibration could be measured on the eardrum (Dalhoff et al., 2007). Recordable human eardrum displacements due to the $2f1–f2$ DPOAE were 1.3 and 5.6 pm when the two tones applied were 25 and 65 dB SPL for $f1$ and $f2$, respectively. It should also be noted that other tested vertebrate hearing organs, in guinea pigs, produced even larger displacements of the basilar membrane (Dalhoff et al., 2007).

In previous studies, the reduction of the DPOAE magnitude in SPL when CO_2 was applied was previously seen as an indication that the DPOAEs being produced were due to metabolic processes (Kössl and Boyan, 1998; Möckel et al., 2007). Our inability to measure mechanically any DPOAEs render it difficult to comment upon this. Meyer and Hedwig previously showed that altering the pressure in the tracheal sacs backing the tympanum causes changes in the TM displacement (Meyer and Hedwig, 1995). It was noted in the current study that the insects' respiratory cycle was altered as a result of the application of CO_2; the respiratory movement of the abdomen ceased and the spiracle adjacent to the tympanal organ that opens and closes during normal breathing was closed throughout hypoxia. The results of the LDV recordings, however, show that the velocity of the membrane did not significantly differ as a result of CO_2 application (Fig. 6). More research needs to be carried out to understand the complex effect that respiration has on the tympanal mechanics and in turn the response of the receptors and how this affects sound processing.

The measurements in this study did not reveal any DPOAEs on the membrane vibration at any point. The origin of the previous DPOAEs acoustically recorded in the locust tympanal organ therefore remains elusive. In previous work, the site was put forward as being the individual groups of receptor cells, emitting frequency-specific DPOAEs (Möckel et al., 2007). It was also suggested that the membrane could be producing the DPOAEs; the lower frequencies being produced by the thick membrane and/or the interaction between the thick and thin membrane, and the higher frequencies being produced by the thin membrane (Kössl and Boyan, 1998). However, this does not appear to be the case from this study's LDV recordings of the membrane; within the limits of our experimental set-up there do not appear to be any DPOAEs emitted. More research needs to be carried out to solve this mystery. One of the main areas where knowledge of sound processing in the locust ear is lacking is in the role of Müller's organ, which may be responsible for the production of DPOAEs in the locust ear. Further work needs to be done to understand the sophisticated functioning and processing of sound by the locust hearing organ.

LIST OF ABBREVIATIONS

DPOAE	distortion-product otoacoustic emission
FB	folded body
FFT	fast Fourier transform
LDV	laser Doppler vibrometer
OAE	otoacoustic emission
PV	pyriform vesicle
SOAE	spontaneous otoacoustic emission
SPL	sound pressure level
TM	tympanal membrane

ACKNOWLEDGEMENTS

This work was financially supported by funding from the BBSRC (J.F.C.W., grant no. BBH0046371), EPSRC (H.M.M., grant no. EP/F01371X/1 and J.C.J., grant no. EP/H02848X/1) and the Royal Society (J.F.C.W., grant no. RG080039). We thank Dr Jerome Sueur for his helpful comments on the development of the manuscript, Dr Shira Gordon for careful reading of the manuscript and two anonymous reviewers for their constructive feedback.

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