Measurement of sensitive distortion-product otoacoustic emissions in insect tympanal organs

In a recent paper in *The Journal of Experimental Biology*, Moir and colleagues did not find evidence for DPOAEs in the motion of the locust typanum (Moir et al., 2011).

Insects with tympanal organs produce sensitive DPOAEs (distortion-product otoacoustic emissions), as shown in locusts, several moth species and bushcrickets (for a review, see Kössl et al., 2008). Their characteristics are largely comparable to those reported from vertebrate ears. Insect DPOAEs are highly vulnerable to manipulations that affect the animal's physiology: hypoxia (Kössl and Boyan, 1998), ethyl ether (Kössl et al., 2007), electrical auditory nerve stimulation (Möckel et al., 2007), a neuroactive insecticide (Möckel et al., 2011) and acoustic suppression by third tones (Kössl and Coro, 2006). These findings strongly indicate a biological origin of acoustical two-tone distortions. Mechanical data from Michelsen (Michelsen, 1971) and Windmill et al. (Windmill et al., 2005) showed for locusts that the tympanum's thin part is maximally deflected by high frequency stimuli, and therefore scolopidia attached at the pyriform vesicle (PV) were most sensitive to that frequency range. Exclusion of these scolopidia *via* mechanical ablation specifically reduced the levels of high frequency DPOAEs evoked by stimuli above 15 kHz. Scolopidia were therefore suggested to be directly involved in frequency-specific DPOAE generation in insects (Möckel et al., 2007) [see also fig. 6 of Kössl et al. (Kössl et al., 2008)].

Moir et al. (Moir et al., 2011) aimed to record mechanical two-tone distortions in the movement of the tympanum and to relate these mechanical responses to the $2f1–f2$ DPOAE frequency defined by the used acoustical stimuli. Several times, the authors termed their measurements 'DPOAEs', which in association with their chosen method (laser Doppler vibrometry) is not quite correct. DPOAEs are defined as sounds that can be recorded in the outer ear canal, resulting from cochlear mechanical non-linearity and depending on physiological activity (for a review, see Kemp, 2008). Likewise, in the case of locusts, they are acoustically recorded close to the tympanum, to which the auditory receptors are directly attached. The authors' second aim was to visualize the effect that hypoxia has on tympanum vibration. Within the limits of their experimental setup, as stated in the last paragraph of Moir et al. (Moir et al., 2011), both parts of their work failed to show positive results. From our extensive experience with insect DPOAEs, we feel we should comment on the present study.

Possible reasons for difficulties in measuring mechanical two-tone distortions

One important factor for measuring sensitive DPOAEs is the acoustical coupler and its tip. The coupler consists of separate tubes for two speakers for stimulation and a microphone to record the emission. To record sensitive DPOAEs in locusts, it is crucial that the microphone is aimed perpendicular to the tympanal membrane, and is positioned as close to it as possible without making direct contact. Additionally, to measure sensitive DPOAEs especially in the low frequency range, it is essential to seal the ear with the coupler in position and hence produce a closed system (see also Kemp, 2008). This creates a situation where a large portion of the sound detected by the microphone comes from the tympanum. The study of Moir et al. (Moir et al., 2011), however, worked with an open system, as required for laser Doppler vibrometry. The authors used the stimulation parameters given in Kössl and Boyan (Kössl and Boyan, 1998), which in that study had produced an emission of 10 dB SPL in a closed system, but did not measure DPOAEs in their preparation. They also calculated the expected mechanical tympanum vibration associated with a 10 dB SPL tone based on a planar source model in an open system. However, the actual DPOAE recording situation (see above) and the complex mechanics of the locust tympanum might not be sufficiently reflected in this simple simulation. In this respect, it would be advisable, if possible, to record DPOAEs and mechanical tympanum vibration with the same closed coupler system, as Dalhoff et al. (Dalhoff et al., 2007) did for the human tympanum. This coupler included an optical fiber for the laser. Of course, the locust tympanum with its directly attached sensory cells and the strong membrane inhomogeneities cannot easily be compared with the human tympanum, and parallel acoustic and mechanical measurements in the locust are needed.

The second part of Moir et al.'s study involved the influence of hypoxia, which reduces DPOAE amplitude (Kössl and Boyan, 1998). As we interpret their data, the authors did not measure mechanical ($2f1–f2$) distortions or their modulation during hypoxia, but found that mechanical vibrations evoked by $f1$ or $f2$ were not affected by CO_2. Of course, during DPOAE recordings, we would not expect that hypoxia influences $f1$ or $f2$ responses but only the much smaller $2f1–f2$ response, whose mechanical analogue Moir et al. could not measure.

Artefacts

In their Results section, Moir and colleagues report the presence of setup artefacts at the DPOAE frequency of about 32 dB SPL (0.8 mPa). The acoustical stimuli used may amount to ~72 dB SPL for $f1$ (82 mPa) and to ~63 dB SPL for $f2$ (28 mPa) and not the other way round as stated in their fig. 4 legend (which is obviously a typing error). This very high distortion level within their sound-producing system might have been caused by the use of a single loudspeaker to deliver both pure-tones. Using two loudspeakers would cause much lower setup distortion during DPOAE measurements and also could be beneficial for investigating their mechanical analogue on the tympanal membrane. It is a common praxis in our lab to use two loudspeakers for delivering the $f1$ and the $f2$ stimulus and also to keep the stimulation and DPOAE recording channels separated as far as the tip of the acoustical coupler, positioned close to the tympanum. In the original report on locust DPOAEs, setup-produced distortions appeared just above the background noise level (–15 to –25 dB SPL) at high stimulation levels of 83 to 91 dB SPL (Kössl and Boyan, 1998). Sensitive locust preparations allow DPOAE measurements at stimulation levels as low as 10–20 dB SPL, giving a safe distance of more than 60 dB to any setup-generated distortion. DPOAEs from tympanal organs reach their highest threshold sensitivity at frequencies defined by the hearing range of the investigated species (cf. Kössl and Boyan, 1998; Coro and Kössl, 1998; Kössl et al., 2008). The pronounced differences in DPOAE threshold characteristics between diverse animal groups (moths, locusts, bushcrickets and several mammalian species) rules out setup distortions whose characteristics would depend on the recording system and not the animal species.

10.1242/jeb.067306

References

Coro, F. and Kössl, M. (1998). Distortion-product otoacoustic emissions from the tympanic organ in two noctuoid moths. *J. Comp. Physiol. A* **183**, 525-531.

Dalhoff, E., Turcanu, D., Zenner, H. P. and Gummer, A. W. (2007). Distortion product otoacoustic emissions measured as vibration on the eardrum of human subjects. *Proc. Natl. Acad. Sci. USA* **104**, 1546-1551.

Kemp, D. T. (2008). Otoacoustic emissions: concepts and origins. In *Active Processes and Otoacoustic Emissions* (ed. G. A. Manley, R. R. Fay and A. N. Popper), pp. 1-38. Springer, New York: Springer Handbook of Auditory Research.

Kössl, M. and Boyan, G. S. (1998). Acoustic distortion-products from the ear of a grasshopper. *J. Acoust. Soc. Am.* **104**, 326-335.

Kössl, M. and Coro, F. (2006). L1,L2 maps of distortion-product otoacoustic emissions from a moth ear with only two auditory receptor neurons. *J. Acoust. Soc. Am.* **120**, 3822-3831.

Kössl, M., Coro, F., Seyfarth, E.-A. and Nässig, W. A. (2007). Otoacoustic emissions from insect ears having just one auditory neuron. *J. Comp. Physiol. A* **193**, 909-915.
Kössl, M., Möckel, D., Weber, M. and Seyfarth, E.-A. (2008). Otoacoustic emissions from insect ears: evidence of active hearing? *J. Comp. Physiol. A* **194**, 597-609.
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, III. Acoustical properties of the intact ear. *Z. Vergl. Physiol.* **71**, 49-128.
Möckel, D., Seyfarth, E.-A. and Kössl, M. (2007). The generation of DPOAEs in the locust ear is contingent upon the sensory neurons. *J. Comp. Physiol. A* **193**, 871-879.
Möckel, D., Seyfarth, E.-A. and Kössl, M. (2011). Otoacoustic emissions in bushcricket ears: general characteristics and the influence of the neuroactive insecticide pymetrozine. *J. Comp. Physiol. A* **197**, 193-202.
Moir, H. M., Jackson, J. C. and Windmill, J. F. C. (2011). No evidence for DPOAEs in the mechanical motion of the locust tympanum. *J. Exp. Biol.* **214**, 3165-3172.
Windmill, J. F. C., Göpfert, M. C. and Robert, D. (2005). Tympanal travelling waves in migratory locusts. *J. Exp. Biol.* **208**, 157-168.

Manfred Kössl and Doreen Möckel
Institut für Zellbiologie und Neurowissenschaft, J. W. Goethe-Universität, Max-von-Laue-Straße 13, D-60438 Frankfurt am Main, Germany
koessl@bio.uni-frankfurt.de

Response to ‘Measurement of sensitive distortion-product otoacoustic emissions in insect tympanal organs’

We thank Kössl and Möckel for their correspondence regarding our article (Moir et al., 2011) and the Editor for the opportunity to reply.

In general, the occurrence of distortion products (also called ‘intermodulation’ or ‘intermodulation distortion’) indicates that the system in question is non-linear. This non-linearity can really be anything: from active feedback to passive structural non-linearities. A single-frequency input into a non-linear system will result in a distorted signal that, spectrally, will contain a series of harmonics of the fundamental. Similarly, if two frequencies are input, then intermodulation will occur and sidebands at various arithmetic combinations of the two input frequencies will exist in the frequency spectrum.

A distortion-product otoacoustic emission (DPOAE) is thus a result of such an intermodulation, recorded as a sound emission from an ear. For sound to be emitted from any system, a structure within that system must be vibrating (as in a loudspeaker). In the case of locust ears, the DPOAE sounds are understood to originate from the tympanal organ. As a result, there should be a mechanical signature on the motion of the tympanal organ – something we aimed to measure. Over a large number of animals, at both ethologically relevant sound pressures and louder, we were unable to measure the largest distortion product (at $2f1–f2$) in the mechanical motion of the tympanum using laser Doppler vibrometry. As pointed out by Kössl and Möckel, sensitivity of this effect to the physiology of the insect indicates a biological origin, and the mechanosensitive neurones (scolopidia), which attach to the tympanum, were the most likely candidate for the production of DPOAEs in insects (Kössl et al., 2008).

If the locust ear produces DPOAEs as part of its everyday sound transduction behaviour, then we should be able to measure the structure vibrating to generate them (within the limits of our measurement system), regardless of whether the system is closed or not. Therefore, if the tympanal membrane is that structure, and is receiving the correct two-tone sounds [as is clearly evident from measurements showing that the membrane vibrates at those tone frequencies; see fig. 2, Moir et al. (Moir et al., 2011)], such that DPOAEs are being produced by the same membrane (or the scolopidia attached to the membrane) then that tympanal membrane must also be vibrating at the $2f1–f2$ distortion-product frequency. Such vibrations were not recorded (down to the laser vibrometer’s 3 pm noise floor).

Kössl and Möckel suggest that the use of an acoustic coupler and its tip are important for measuring DPOAEs. The use of an acoustic coupler is simply a method by which faint sounds from a small source can be recorded by a microphone. While this is clearly beneficial for measuring very quiet sounds, it is irrelevant for our report. The existence of a DPOAE at a known frequency and sound pressure must correlate with the motion of a structure emitting the sound. Our investigation could not find evidence of the tympanal membrane vibrating in relation to known DPOAEs.

The reasonable concern of artefacts in the current experiments was also raised. When studying an effect that is derived from any non-linearity, artefacts must be carefully noted and avoided. However, we were very careful not to overdrive our single loudspeaker so as not to generate artefactual two-tone distortion; this was monitored using the microphone. It was only possible to produce distortion-products by overdriving the loudspeaker and not the animal, and we took this into account throughout our experiments [see fig. 4, Moir et al. (Moir et al., 2011)]. In any case, the presence or not of some acoustic artefacts in the setup used does not in any way relate to the absence of any evidence for measured vibrations at any distortion-product frequencies.

Finally, we are not questioning the existence of DPOAEs in locusts, or their physiological dependence, the evidence for which is strong. However, it is concerning that we were not able to measure a mechanical two-tone vibration on the locust tympanum with an experimental setup that is capable of doing so. Rather than put these results in the ‘file drawer’, we believe it is better that the scientific community is made aware of these results so that the question of where DPOAEs are generated can be resolved more quickly. We very sincerely hope that our work will be followed by that of others in order to continue to examine, and seek to explain, all the intricate workings of the locust (and other) insect ears.

10.1242/jeb.068098

References

Kössl, M., Möckel, D., Weber, M. and Seyfarth, E.-A. (2008). Otoacoustic emissions from insect ears: evidence of active hearing? *J. Comp. Physiol. A* **194**, 597-609.
Moir, H. M., Jackson, J. C. and Windmill, J. F. C. (2011). No evidence for DPOAEs in the mechanical motion of the locust tympanum. *J. Exp. Biol.* **214**, 3165-3172.

Hannah M. Moir, Joseph C. Jackson and James F. C. Windmill
University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, UK
hannah.moir@eee.strath.ac.uk