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Otoacoustic Emissions from a Nonvertebrate Ear

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Otoacoustic emissions are produced by the inner ear of vertebrates and result from the active and nonlinear processing of input sound by sensory hair cells. We recorded pronounced distortion-product otoacoustic emissions from the ear of the grasshopper, and these emissions proved remarkably similar to those described for the mammalian ear. This is despite the fact that the grasshopper ear is structured very differently than that of the vertebrate in that it does not contain hair cells. Rather than being restricted to vertebrates, we suggest that nonlinear mechanical processing and associated otoacoustic emissions are a general property of sensitive hearing organs.

Otoacoustic emissions (OAEs) are emitted as a byproduct of active transduction in the inner ear and can be measured at the ear drum of vertebrates (for a review [29]). While spontaneous OAEs appear without sound stimulation, evoked OAEs require the presence of external sound. Among the various forms of evoked OAEs, the distortion-product OAEs (DPOAEs) are widely used to obtain insight into cochlear processing. In mammals the DPOAEs are a consequence of nonlinear mechanical amplification of low-level sound stimuli by active and motile outer hair cells [7]. They can be measured as distortion peaks during acoustic stimulation with two pure tones ($f1 < f2$), with the most pronounced DPOAE occurring at a frequency of $2f1–f2$. Mechanical correlates of the DPOAEs can be recorded in the motion of the basilar membrane [26] and during deflection of the sensory hair bundle of hair cells from the bullfrog sacculus [11]. If the outer hair cells in mammals are damaged due to ototoxic aminoglycosid antibiotics, the DPOAEs greatly deteriorate in amplitude [5], as also occurs during metabolic changes induced by hypoxia [25].

The presence of DPOAEs has, to our knowledge, not previously been examined in nonvertebrates. Our interest was to determine whether such emissions are also associated with ears representing a structural design very different from that of the vertebrate. If present, the form of the DPOAEs could provide insights into fundamental transduction mechanisms.

We selected the grasshopper *Locusta migratoria* for our experiments because it has a very well-developed and sensitive auditory system which has been extensively studied. The ear comprises a large tympanum, or eardrum, situated externally on each side of the first abdominal body segment, and behind the tympanum there is a receptor organ, the Müller's organ, which contains four groups of receptor cells (a–d) [9]. Each auditory receptor has a single modified cilium within its peripheral dendrite. The dendrites attach to the specialized cuticular thickenings, or sclerites, of Müller's organ [21, 28] which then contact the inner surface of the tympanum. The membrane of the tympanum is separated into a thin and a thick region (Fig. 1A) such that the a, b, and c cells are activated primarily by the thick membrane and the d cells by the thin membrane, as described in the closely related species *Schistocerca gregaria* [18–20]. Since the thin membrane reacts best to higher frequencies (>10 kHz) and the thick membrane to lower frequencies, a place-dependent mechanism for frequency analysis has been proposed at the level of the tympanum [18–20].

The grasshoppers (*Locusta migratoria*) used for these experiments were raised in crowded laboratory cultures at 30°C. The animals were prepared for experimentation by pinning them dorsal side up to a cork platform atop a thin metal post. Wings and legs were removed, but the animals not otherwise dissected for these experiments. The animals were alert for the entire duration of each experiment (up to 3 h). To test for DPOAEs we stimulated the ear with two pure tones of different frequency, f1 and f2, and the emitted acoustic energy was measured with a microphone placed close to the ear drum [13]. The recordings took place in a soundproof chamber heated to 28°C. An acoustic coupler consisting of two adjacent tubes for stimulation and recording, and with an overall tip diameter equal to the size of the tympanum of the insect, was positioned within a distance of about 0.3–1.0 mm from the tympanum. The experiments were performed in a completely closed acoustic system, and the connection between the body surface of the locust and the walls of the coupler tip was sealed thoroughly using toothpaste. The coupler was connected to Bruel & Kjaer 4133 microphone to measure responses up to 40 kHz or to a Bruel & Kjaer 4135 microphone for frequencies above 40 kHz. Two additional 4133 Bruel & Kjaer microphone capsules served as loudspeakers. The sound system was calibrated in situ using white noise, and sound pressure levels used in the experiments are expressed in dB SPL (dB re. $2 \cdot 10^{-5}$ Pa). DPOAEs were stimulated and recorded unilaterally. To test for any crosstalk between the ears via the tracheae we also applied white noise at the ipsilateral ear and recorded the response at the contralateral ear. In agreement with previous studies [22], we found considerable sound conduction through the body of

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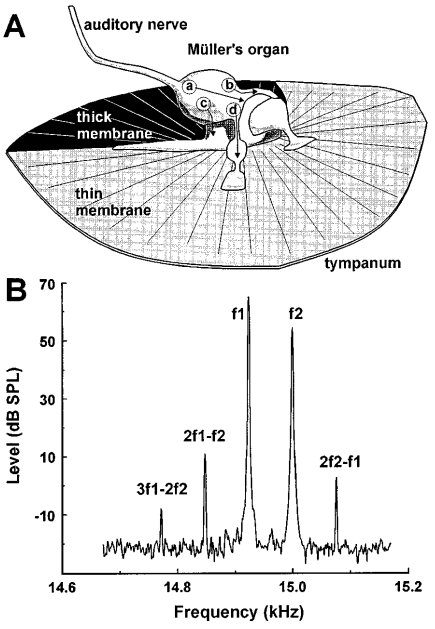

Fig. 1. A) The tympanum and Müller's organ in *Locusta migratoria* as seen from the interior. The dendrites of groups of receptor cells (a–d) contact sclerites attached to either the thick or the thin region of the tympanum (adapted from [9, 18–20]). B) A typical spectrum of DPOAEs measured at the tympanum. The level of the f1 stimulus was 10 dB above that of f2. An acoustic coupler designed for measurements in mammals was used (see text)

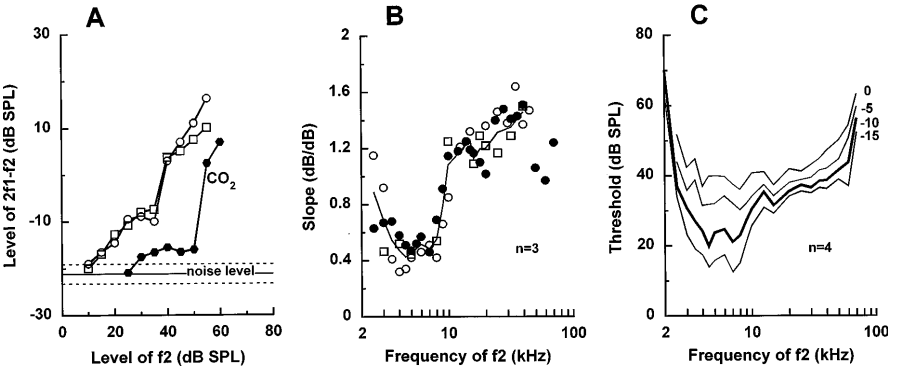

Fig. 2. A) Growth functions of the 2f1–f2 DPOAE (f1: 7.2 kHz; f2: 8 kHz; the level of f1 was always 10 dB above that of f2) before (*open circles*), during (*solid hexagons*), and after (*open squares*) ventilation with CO_2. *Horizontal lines*, the noise level (with SD) of the stimulus setup. B) Initial slope of 2f1–f2 growth functions for different f2 frequencies in three animals (*different symbols*; *solid line*, average values). To yield maximum level of the distortion growth functions for each f2 frequency the corresponding f1 frequency was chosen according the optimum ratio (between 1.006 and 1.18 in the displayed data). The slopes were calculated from the linear regression function for stimulus levels between the first appearance of a distortion above noise level and that 15 dB higher. C) Distortion threshold curves, averaged from four animals. Shown is the f2 level that was sufficient to elicit a 2f1–f2 distortion of –15, –10, –5, and 0 dB SPL for different f2 frequencies. The level of f1 was always 10 dB above that of f2. The frequency ratio f2/f1 lay between 1.006 and 1.18. As with mammalian thresholds, the data are expressed with respect to f2

the insect up to frequencies of about 20–30 kHz. To exclude that the ipsilaterally recorded DPOAEs are influenced by the contralateral ear we destroyed the contralateral tympanum and either closed the ear with resin or left it open. In both cases the ipsilaterally recorded DPOAEs did not change within the accuracy of the measurements (±2 dB).

For the experiments involving ventilation with CO_2 a grasshopper was mounted on its side to the cork platform described above. The temperature of the CO_2 delivered to the preparation was maintained at 28°C and the air humidity kept constant. CO_2 was then applied for 10 min or until the antennae of the animals assumed the depressed attitude typical for hypoxia. Recovery of the grasshopper from hypoxia was signaled by the antennae, again assuming their normal elevated position and beginning to move freely.

The recorded frequency spectra show pronounced two-tone distortions, and as in vertebrates the 2f1–f2 distortion-product had the largest amplitude (Fig. 1B). Such DPOAEs were measured in nine individuals of *Locusta migratoria*, both male and female. To study DPOAE generation over the whole hearing range of this species the frequency of the f2 stimulus was first adjusted to a value between 2 and 70 kHz, and both the frequency and the level of the f1 stimulus were then varied. This was repeated for each f2 stimulus frequency tested. The amplitude of the 2f1–f2 distortion was maximal when the level of the f1 stimulus was between 5 and 15 dB above that of the f2 stimulus – a situation comparable to that in mammals [3]. In contrast to vertebrates, for a given f2 frequency a wider range of f1 frequencies and hence of f2/f1 frequency ratios induced large 2f1–f2 distortions. The optimum ratios at which the 2f1–f2 level was maximal lay between 1.006 and 1.3, but the corresponding maxima were clearly less distinct than in vertebrates, and the general frequency dependence of the distortions displayed a high pass characteristic rather than the bandpass characteristic of mammals. This bandpass has been suggested as being the product of a secondary cochlear filter element, namely the tectorial membrane [1, 6].

The growth functions associated with the 2f1–f2 distortion-product were then measured during a progressive increase in stimulus levels over the entire range of f2 frequencies investigated (Fig. 2A). For higher stimulus levels (f2 level of 35–65 dB SPL) the growth functions often displayed an increase in slope. The initial slope of the growth functions for low stimulus levels differed for different frequency ranges. At f2 frequencies below 10 kHz the average initial slope was 0.57 dB/dB. This increased to 1.29 dB/dB above 10 kHz (Fig. 2B). A comparable change in slope is observed between the intensity response functions for the b and d receptor cells in the ear [27]. We interpret these data to mean that the low-frequency distortions below about 10 kHz originate from the thick region of the tympanum, while the high-frequency distortions originate from the thin region. This interpretation is consistent with direct mechanical measurements of tympanal vibration [2, 17–20].

Isodistortion threshold curves were then calculated from the 2f1–f2 growth functions (Fig. 2C). In vertebrates such threshold curves are known to run parallel to neuronal threshold data and to provide a noninvasive means to measure hearing sensitivity [8, 12, 15, 16]. In the grasshopper the distortion thresholds showed a pronounced minimum for frequencies between 3 and 8 kHz. This coincides exactly with most sensitive neuronal thresholds [27], with the main energy in the grasshopper's communication signals, and with maximum motion of the thick membrane [17].

To determine whether the OAEs depend on the physiological state of the animal we induced hypoxia by ventilating the grasshopper with CO_2. The DPOAE levels for f2 frequencies below about 10 kHz were seen to decrease reversibly during CO_2 exposure (Fig. 2A). The distortion thresholds increased correspondingly by 4–28 dB in the sensitive low-frequency part of the audiogram; for frequencies above 10 kHz there was no significant effect of CO_2.

In mammals DPOAEs reflect cochlear sensitivity, the action of the cochlear amplifier [23], and cochlear tuning [4]. There has therefore been considerable interest in developing this measurement technique into a powerful noninvasive tool both for animal experimentation and for clinical diagnosis of hearing deficits (see [29]). By contrast, the grasshopper ear uses different structures than that of the vertebrate. A major difference is that the grasshopper ear does not contain hair cells. Despite such profound differences we recorded pronounced OAEs from the ear of the grasshopper, and these emissions proved remarkably similar to those described for the mammalian ear.

What then is responsible for the nonlinear mechanical characteristics and the associated OAEs in the grasshopper ear? Previous mechanical measurements at higher sound pressure levels led to the supposition that the motion of the tympanum is linear [2]. However, a nonlinearity sufficient to produce the OAEs must be present at low stimulus levels close to the hearing threshold. We suspect the involvement either of an interaction between different modes of tympanal vibration [18–20] or of the complex mechanical characteristic of the sclerites through which the receptor cells attach to the tympanum [2, 28]. The sclerites exhibit frequency-dependent rotational movements and strains which must be important for adequate stimulation of the sensory dendrites [28]. On the other hand, the properties of the receptor cells themselves, and in particular their cilia, may also contribute to the ear's nonlinear mechanical characteristics, as is the case for the hair bundles of vertebrate hair cells [10]. Indeed in birds and reptiles the hair bundle may be the main source for OAEs since there is no apparent hair cell motility [14]. In the insect cilia may play an active role in sensory transduction [24]; however, their mechanical involvement in OAE generation awaits investigation. It remains to be seen whether the susceptibility of the DPOAEs to CO_2 is induced by changes within the transduction apparatus at the ciliated dendrites of the receptor cells. Whatever the mechanism for their generation is, we suggest that rather than being restricted to vertebrates nonlinear mechanical processing and associated OAEs is a general property of sensitive hearing organs.

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Solar UV Monitor with Yeast and the Possibility of Estimating Ozone-Layer Thickness

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The possibility of using cell inactivation rates as a monitor of the biologically effective dose of solar UV rays was investigated. The cell survival of a radiation-sensitive strain exposed to sunlight was measured for several years. It was confirmed that a sufficient inactivation rate of the cells was obtainable to determine the biological effectiveness of solar UV rays relative to the effect of a germicidal lamp at 253.7 nm. To validate the data we calculated the term corresponding to the ozone thickness by the dose ratio of two factors in different UV absorption conditions obtained with a quartz and a glass cuvette. The results indicate that the data of biologically determined thickness were in accordance with those observed by the optical method at the nearest observatory.

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Introduction

UV exposure of the human body has become an increasing concern because of an anticipated increase in skin cancer incidence, which may be caused by a decrease in the thickness of the ozone layer [10, 11]. Monitoring of the biologically harmful component of UV rays is required in addition to physical dosimetry. Physical methods are generally accurate but are expensive cost and difficult to perform technically. The use of biological dosimetries is convenient for expressing the anticipated degree of hazard caused by solar UV, which is often indicated as an equivalent to those with germicidal lamp (253.7 nm). The survival curve of cells exposed to sunlight is related to the effective amount of UV rays reaching the ground. Here we report the possibility of determining the thickness of the ozone layer by comparing two sets of survival data with that obtained under different conditions in UV penetration. A quartz and a glass cuvette were used for this purpose. The ratios of the biologically effective UV doses are directly combined to the ozone thickness by canceling ambiguous factors independent of wavelength. The sun's spectrum [2] at the surface of the earth and the action spectrum of cell inactivation [6] are used to calculate the effectiveness of sun light as a function of the thickness of ozone layer.

Biological dosimeters of *Bacillus subtilus* spores [4, 7, 12, 13], yeast cells [3], DNA molecules [8], and phage T7 [9] have been suggested to possess radiometric properties that allow evaluating the effects of solar UV radiation on the human body. The most extensive studies have been carried out with UV-sensitive spore of *Bacillus subtilus*. Practical uses have been reported with the daily accumulation of UV doses in Tokyo [4] and in Antarctica [7].

The present study used a repair-deficient mutant of yeast as a eukaryotic biological monitor. The survival measurements accompanied by the estimation of ozone thickness were carried out with this strain for recent several years.