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REVIEW

Otoacoustic emissions from insect ears: evidence of active hearing?

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Abstract Sensitive hearing organs often employ nonlinear mechanical sound processing which generates distortion-product otoacoustic emissions (DPOAE). Such emissions are also recordable from tympanal organs of insects. In vertebrates (including humans), otoacoustic emissions are considered by-products of active sound amplification through specialized sensory receptor cells in the inner ear. Force generated by these cells primarily augments the displacement amplitude of the basilar membrane and thus increases auditory sensitivity. As in vertebrates, the emissions from insect ears are based on nonlinear mechanical properties of the sense organ. Apparently, to achieve maximum sensitivity, convergent evolutionary principles have been realized in the micromechanics of these hearing organs—although vertebrates and insects possess quite different types of receptor cells in their ears. Just as in vertebrates, otoacoustic emissions from insects ears are vulnerable and depend on an intact metabolism, but so far in tympanal organs, it is not clear if auditory nonlinearity is achieved by active motility of the sensory neurons or if passive cellular characteristics cause the nonlinear behavior. In the antennal ears of flies and mosquitoes, however, active vibrations of the flagellum have been demonstrated. Our review concentrates on experiments studying the tympanal organs of grasshoppers and moths; we show that their otoacoustic emissions are produced in a frequency-specific way and can be modified by electrical stimulation of the sensory cells. Even the simple ears of notodontid moths produce distinct emissions, although they have just one auditory neuron. At present it is still uncertain, both in vertebrates and in insects, if the nonlinear amplification so essential for sensitive sound processing is primarily due to motility of the somata of specialized sensory cells or to active movement of their (stereo-)cilia. We anticipate that further experiments with the relatively simple ears of insects will help answer these questions.

Keywords Hearing organs · Tympanal organ · Nonlinear sound processing · Electromotility

Abbreviations

OAE	Otoacoustic emission
DPOAE	Distortion-product otoacoustic emission
SOAE	Spontaneous otoacoustic emission
OHC	Outer hair cell
IHC	Inner hair cell
SPL	Sound pressure level
TRP	Transient receptor potential

Ears produce mechanical energy

Sense organs are comprised of sensory neurons and accessory cells that convert specific stimulus energy into changes in membrane potential. This conversion, termed sensory transduction, is based either on intracellular reception of the stimulus (as in photoreceptors) or on direct activation of membrane proteins, as is the case in mechanically sensitive systems. As a rule, such energy transduction operates only in one direction.

Hence it came as a great surprise, when in 1978 David Kemp recorded sound events in the human (outer) ear canal that were apparently produced by the inner ear (Kemp

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1978). He stimulated the ear with brief acoustic clicks and demonstrated that sound waves of a particular frequency appeared in the external ear meatus after a distinct delay of several milliseconds. Initially, these events were called "Kemp-echos". However, they do not originate from echo-like reflections at the ear-drum or the middle-ear but depend on an anatomically and physiologically intact cochlea. Subsequent studies in numerous mammalian species have shown that the outer hair cells (OHCs) in the cochlea are the elements responsible for the retrograde generation of sound (Kemp 2002). While the inner hair cells (IHCs) are extensively innervated by the dendrites of afferent auditory neurons, the OHCs possess only a few auditory nerve synapses but are directly contacted by *efferent* axons that provide centrifugal information from the brain stem to the inner ear. Zheng et al. (2000) discovered a protein, called prestin, in the cell membrane of OHCs which undergoes voltage-dependent changes in conformation, resulting in shortening or elongation of the hair cell soma upon depolarization or hyperpolarization of its membrane (Liberman et al. 2002; Dallos and Fakler 2002). Following an acoustic stimulus, OHCs are thereby capable of producing forces which can significantly augment the displacement amplitude of the basilar membrane in the inner ear. This voltage-dependent motility of the OHCs has been considered the basis of the so-called "cochlear amplifier", which provides an improvement of sensitivity by 40–60 dB in the mammalian ear (Geleoc and Holt 2003). It is up for debate, however, whether other amplification mechanisms might contribute to the cochlear amplifier in OHCs that are based on force production by the stereocilia. At present, the issue remains unsolved (see the discussion below).

Nonlinear mechanical amplification of sound energy is not restricted to the ears of mammals or of vertebrates as such, but seems to be a general principle in sensitive hearing organs. In the ears of insects, both nonlinear amplification and otoacoustic emissions have recently been demonstrated. The potential underlying mechanisms are currently being investigated; they are the subject of our present review.

Two types of otoacoustic emissions

Otoacoustic emissions (OAEs) are a by-product of the cochlear amplifier and have been used to study its function in detail. OAEs have also been recorded from the ears of non-mammalian vertebrates whose hair cells do not express electromotile prestin. Hence, other amplification processes must be responsible in these cases (Manley 2001, 2006; Manley et al. 2001). The most likely candidates are interactions of transduction proteins with actin and myosin in the stereociliary bundle of the hair cells (see our discussion below).

We distinguish two types of otoacoustic emissions: place-dependent OAEs and wave-dependent OAEs (see also Shera and Guinan 1999).

Place-dependent OAEs are comprised of distinct sound frequencies and hence can be attributed to a particular frequency position in the inner ear. Place-dependent OAEs include emissions induced by external sound stimulation of the inner ear (such as the click-evoked OAEs mentioned above) as well as spontaneous OAEs (SOAE) that are recorded in the absence of any external sound. SOAEs provide the most compelling indication of active sound generation in the inner ear (see Hudspeth 2000). In many subjects, SOAEs are measurable in the form of a narrow maximum of sound pressure at a distinct frequency (Fig. 1, left). Often several SOAEs appear in a given ear. In humans, they occur at frequencies that lie in the range of best hearing, i.e., between 0.5 and 11 kHz. Spontaneous motion of the OHCs has been considered as the cause of SOAEs (review Kemp 2002), but to explain the frequency-specificity of these emissions, additional mechanisms are required such as the emergence of standing waves at cochlear sites that are discontinuous (Russell and Kössl 1999).

Amphibians, reptiles, and birds display distinct SOAEs as well (Fig. 1, left). The main difference to mammals cases is their limitation to frequencies below ca. 10 kHz. SOAEs at this frequency have been measured in the barn owl (Taschenberger and Manley 1997). In the case of mammals, SOAE-frequencies up to 62 kHz have been recorded in bats (Kössl 1994; Fig. 1, left). This finding demonstrates that the mammalian ear can actively generate (i.e., without external sound stimulation) extremely rapid movements that could be employed for acoustic amplification, corresponding to the hearing range of many mammalian species that extends into extreme ultrasound. Electromotility of OHCs has in fact been found up to at least 79 kHz (Frank et al. 1999). Possibly, cochlear amplifiers that are based on prestin even operate up to the auditory frequency limits of mammals, which are beyond 200 kHz in certain species relying on echolocation.

Wave-dependent OAEs are evoked emissions that arise following auditory stimulation by several pure tones of different frequencies. Due to nonlinear sound processing in the inner ear, the amplitude of faint acoustic signals increases in comparison to louder signals and the overall signal wave form becomes distorted. Such distortions are most pronounced when several pure tone signals are applied simultaneously. The standard procedure to evoke such distortions is stimulation with two pure tone stimuli of different frequency (f1, f2). The wave form distortions are then measured in the frequency spectrum as additional frequency components, so-called distortion-products, at frequencies of nf1 − (n − 1)f2 and nf2 − (n − 1)f1. These components propagate back across the middle ear to the

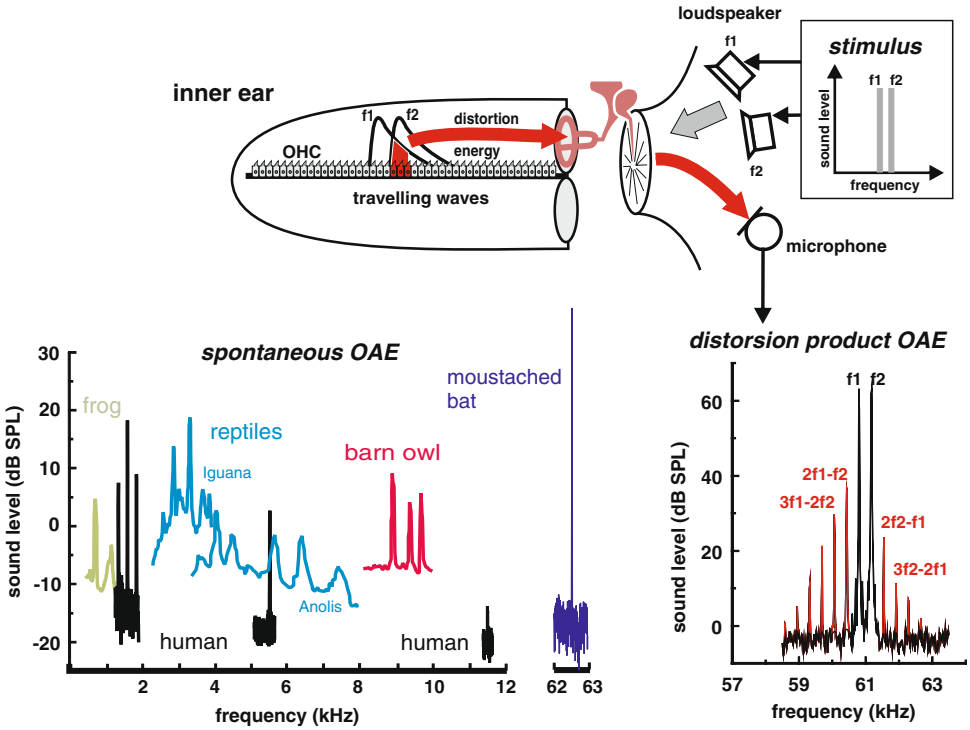


Fig. 1 Origin and characteristics of otoacoustic emissions (OAE) in various mammalian and non-mammalian vertebrates. *Lower left*: In the absence of acoustic stimuli, *spontaneous OAEs* are recordable at the tympanic membrane via a microphone; they appear as amplitude peaks at specific sound frequencies. *Lower right*: *Distortion-product OAEs* are evoked by simultaneous stimulation with two pure tones (f1, f2) and appear as additional peaks (*red*) at defined frequencies in the amplitude spectrum. They emerge at the basilar membrane in the region of overlap of the two travelling waves f1, f2 (*top*: *area in red*), due to nonlinear amplification by outer hair cells (OHC). Data sources: Long et al. 1996 (frog); Kössl, unpubl. (human); Manley 2006 (iguana and *Anolis*); Taschenberger and Manley 1997 (barn owl); Kössl 1994 (moustache bat, DPOAE on lower right)

tympanic membrane and are recorded as distortion-product OAE (DPOAE). In all hearing organs examined so far, the DPOAE at 2f1 − f2 is the loudest one (Fig. 1, right).

Distortion is the cost that has to be paid for highly sensitive sound amplification in biological systems. While many technical systems, such as hifi-setups, produce distortions when they become overloaded, auditory systems primarily generate distortions at low sound pressure when the cochlear amplifier is operating at maximal gain. Unlike place-dependent OAEs that are generally limited to one particular frequency, DPOAEs can be elicited across the entire hearing range of the subject and hence provide more detailed information on cochlear amplification. Increasingly, DPOAEs are used as diagnostic tools, for instance to examine newborn children for auditory impairment (see e.g., the review by Kemp 2002). It should be emphasized here that any nonlinear system can produce distortions, no matter whether the nonlinearity is due to active cellular processes or to passive properties of the system. Moreover, if a physical or biological system would produce linear amplification that does not change its gain with sound level, no two-tone distortions would be generated. In the case of the mammalian cochlea, however, numerous studies have shown that sensitive DPOAEs are due to active and nonlinear amplification that is dependent on metabolic processes in the OHCs (see e.g., Johnson and Canlon 1994; Frolenkov et al. 1998; Kössl and Vater 2000).

The auditory organs of insects and measuring OAEs

Many insects have excellent hearing and use it to detect food or predators and to communicate with conspecifics (see e.g., the reviews by Stumpner and von Helversen 2001; Gerhardt and Huber 2002). Unlike non-mammalian vertebrates, their hearing range is not limited to relatively low frequencies below 10 kHz but can extend far into the ultrasound range. This applies to many moth species and to crickets and katydids. For instance, certain oak bush-crickets (Meconematinae) produce sound at 129 kHz (Montealegre-Z et al. 2006); so far nothing is known of their auditory capabilities, but it seems likely that they can hear their own songs.

The anatomy of hearing organs in insects greatly differs from that of vertebrate ears. Tympanal organs, in which the primary auditory neurons lie next to a tympanic membrane,

are distinguished from antennal hearing organs, in which the sensory neurons are arranged ringlike at the base of each flagellar antenna (called "Johnston's organ"). Tympanal organs react to frequencies ranging far into ultrasound. Possessing a relatively stiff tympanic membrane, they can, in principle, generate sound in the form of otoacoustic emissions. Characteristically, tympanal organs are situated in the thorax, abdomen, or the legs of grasshoppers and moths (Hoy and Robert 1996). Antennal hearing organs, on the other hand, perceive air-particle movements in the acoustic near-field and are situated on the heads of insects such as flies and mosquitoes (Fig. 2c).

In the case of locusts, ca. 80 sensory neurons are gathered into a peripheral sensory ganglion called "Müller's organ" that lies beneath a relatively large tympanic membrane in the first abdominal segment (Fig. 2a). As in all insect auditory organs, both tympanal and antennal ones, the sensory neurons are scolopidial mechanoreceptors, each consisting of a bipolar sensory neuron with a ciliated dendrite and two types of accessory cells, the scolopale cell and the attachment cell (see Yack 2004 for a recent detailed review). The sensory cilium is anchored to the root-apparatus of the dendric process (Fig. 2d). Responsible for sound transduction is the distal region at the ciliary tip that is enclosed by an extracellular cap. The sensory cell somata are gathered in the ganglion of Müller's organ, and their dendrites reach out to various sclerotized attachment points on the tympanic membrane (Gray 1960). The tympanum is divided into relatively thin and thick regions. Upon acoustic stimulation, these respective regions are maximally deflected at high versus low frequencies. Thus, the sound frequency to which the sensory cells react best depends on the exact site of attachment on the tympanum. For instance, the sensory neurons whose dendrites attach via the "pyriform vesicle" in a region of thin membrane (Fig. 2a), react best to relatively high sound frequencies above 10 kHz.

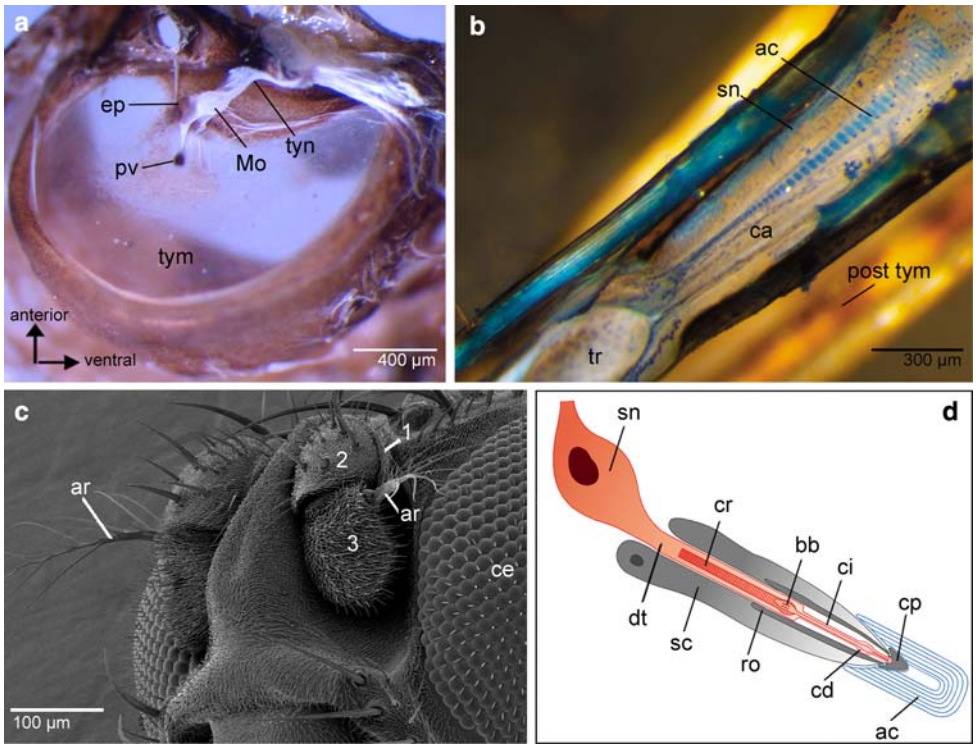


Fig. 2 Important anatomical features of auditory organs in insects. **a** Tympanal organ in the first abdominal segment of the locust (*Locusta migratoria*), inside view. The relatively large tympanum (tym) spans across a cuticular ring. The peripheral sensory ganglion (Müller's organ, MO) lies near the anterior edge, from where sensory dendrites reach out to sclerotized attachment points on the tympanum. The dendrites in the pyriform vesicle (pv) insert in a relatively thin membrane region, those of the elevated process (ep) in a thicker region; *tyn* tympanal nerve. **b** Tympanal organ of tropical bushcricket (*Mecopoda elongata*) in the opened foreleg tibia and after impregnation with methylene blue. The characteristic crista acustica (ca) extends along a branch of the acoustic trachea (tr). It comprises ca. 40 scolopidial sensory neurons and is part of a larger mechanosensory organ complex in the insect leg; *sn* sensory neurons; *ac* attachment cells; *post tym* posterior tympanum. **c** Scanning electronmicrograph of *Drosophila* head showing the two antennal hearing organs next to the compound eyes (ce). The branched arista (ar) is firmly attached to the third antennal segment (3); both oscillate in the acoustic near-field (see also Fig. 7); *1*, *2*, *3* segments of left antenna. **d** Schematic representation of a single scolopidium with bipolar sensory neuron and accessory cells. The dendrite (dt) of the sensory neuron (sn) runs out into a ciliated terminal (ci); *cd* ciliary dilatation; *ac* attachment cell; *sc* scolopale cell; *cr* ciliary root; *bb* basal body; *ro* rod; *cp* scolopidial cap

Other dendrites terminate via sclerites in thicker membrane regions (such as the "folded body") and react to lower frequencies. Hence the tympanal organ of locusts is characterized by mechanical frequency filtering and reaction to distinct sound frequencies just like the vertebrate ear (Michelsen 1971; Römer 1976; Breckow and Sippel 1985; Jacobs et al. 1999; Windmill et al. 2005).

Distinct tonotopic representation of sound frequencies is found in the tympanal organ of bushcrickets (katydids). Here the auditory receptors form a linear array of cells inside the tibia of each foreleg called "crista acustica" (Fig. 2b). The anatomical arrangement resembles the situation in the cochlea of the vertebrate ear. The ears of bushcrickets are primarily stimulated by sound entering the foreleg via a spiracle in the prothorax, from where it is transmitted to the crista acustica by an acoustic trachea (Lakes and Schikorski 1990). The spiracular tracheal system acts primarily as a high-frequency gain filter, amplifying sound in the high audio-frequency range or ultrasonics. Tympanic membranes exist near the sense organ in the tibia, but these are not directly contacted by the sensory dendrites; rather they seem to aid in impedance matching (Bangert et al. 1998). The scolopidial sensory neurons (with their dendritic caps) are oriented perpendicular to the long axis of the crista acustica. The linear anatomical organization is the basis for a linear tonotopic coding of the receptor neurons, that is, sensory cells in the proximal region of the crista code for low sound frequencies and those in the distal part for high frequencies, with a continuous gradient of frequency coding along the sense organ (Oldfield 1982, 1988; Stumpner 1996). Microscopic inspection of the organ shows that the size of the dendritic caps gradually decreases from proximal to distal (Fig. 2b), suggesting that the mechanical features of these structures might contribute to the tonotopic coding characteristics. Of course this does not rule out that other, tonotopically changing properties of the sensory cells might exist as well (see also the discussion by Pollack and Imaizumi 1999).

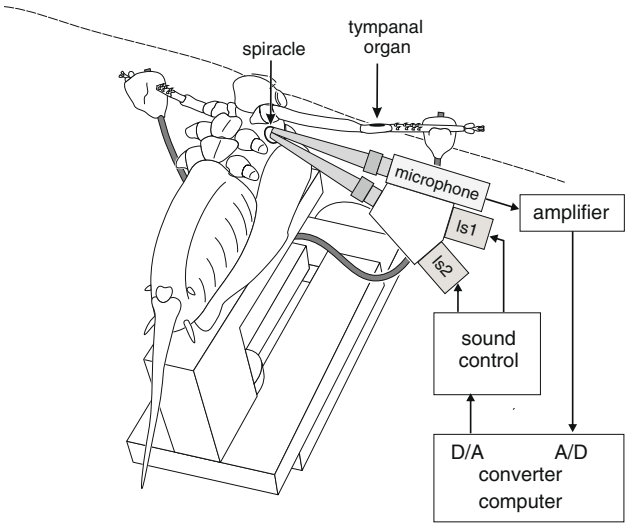


Fig. 3 Recording DPOAEs in the bushcricket *Mecopoda elongata*, experimental setup. The insect is secured to a metal holder and the forelegs fixed to two supports with beeswax. For acoustic stimulation, two pure-tone signals are generated via D/A-conversion and fed into two loudspeakers (ls1, ls2) following calibration and power amplification. Separation of the two stimulus channels is essential to preclude distortions in the technical sound-producing system. The resulting sound signal, which consists of the two stimuli as well as the DPOAE, is recorded via a microphone, then amplified and fed into a A/D converter. Sound-producing and sound-recording channels are gathered into a coupling device, whose tip is adapted to the diameter of the opening of the acoustic trachea (spiracle) in the prothorax of *Mecopoda*. One advantage of this arrangement is that the stimulating/recording device is applied to the prothoracic spiracle while the tympanal organ with its crista acustica remains freely accessible for experimental manipulations

Acoustic distortions in tympanal organs

Upon sound stimulation with pure tones, tympanal organs generate distinct DPOAEs (Fig. 4, 5) that resemble those measured in vertebrate ears in many respects (Kössl and Boyan 1998a, b; Coro and Kössl 1998, 2001; Kössl and Coro 2006; Möckel et al. 2007; Kössl et al. 2007). The emissions are recordable via a probe that is adapted to the diameter of the tympanum (in the case of locusts and moths) or the spiracle (of bushcrickets) and that comprises a stimulation channel with two loudspeakers and a recording channel with a microphone (for technical details see Fig. 3). In locusts and moths, DPOAEs can be induced even with sound stimuli that lie near the auditory threshold; the 2f1 − f2 emission is always the loudest (see the example in the power spectrum of Fig. 4a). This indicates that the motion of the tympanum/sense organ is nonlinear close to the auditory threshold. By determining the stimulus level (at various sound frequencies) that suffices to evoke a DPOAE of a certain amplitude (e.g., −10 dB SPL), it is possible to calculate DPOAE-threshold curves. Such calculations predict the frequency-specific sensitivity of the nonlinear mechanics of the tympanal organ. In fact, they resemble the neuronal threshold of the locust ear, exemplified by two neuronal tuning curves (Fig. 4b). Hence, noninvasive DPOAE-measurements can be used to determine objective auditory thresholds in insects, comparable to the procedure applied in mammals, particularly in humans.

Insect DPOAEs are vulnerable to manipulations that affect the physiological state of the animal, such as the application of certain anesthetics or hypoxic substances (Fig. 4c). Similar treatment clearly reduces DPOAE-levels also in mammals, due to apparent blockade of the cochlear amplifier. In the case of insects, it is still unclear if it is actually the sensory cells and potential amplification mechanisms that are affected by

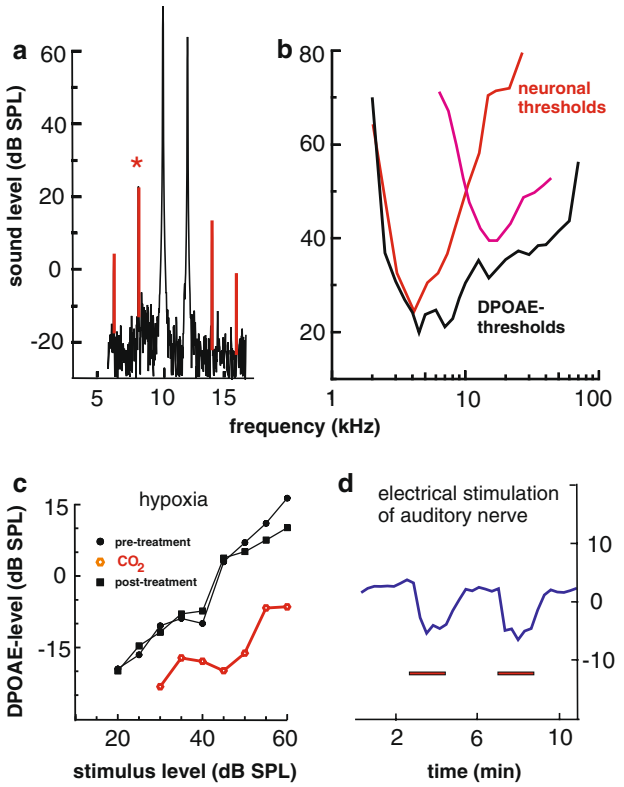


Fig. 4 DPOAE-measurements in the tympanal organ of the locust. **a** DPOAE-spectrum following two-tone stimulation with 10 and 12 kHz; distortion products (*peaks in red*) reach sound intensities that are clearly distinguishable from background noise. Asterisk denotes 2f1 − f2 distortion product. **b** Auditory threshold curves determined electrophysiologically for two groups of neurons (*in red*; adapted from Römer 1976) and expressed as sound intensity sufficient to elicit DPOAEs (*in black*; adapted from Kössl and Boyan 1998b) within the normal hearing range of locusts. **c** Reduction of DPOAE-levels following CO_2-treatment demonstrates the importance of active metabolic processes for generation of otoacoustic emissions (adapted from Kössl and Boyan 1998a, b). **d** Brief electrical stimulation of the auditory nerve (*horizontal bars*) causes transient reduction of emission levels (acoustic stimuli: 10.5/12 kHz with 60/50 dB SPL; electrical nerve stimulation: bipolar pulses of 10 μA, pulse frequency 200 Hz, pulse duration 2 ms; modified from Möckel et al. 2007)

such manipulations. Scolopidial sensory neurons have (afferent) axons that connect directly to the central nervous system via the auditory nerve. This fortuitous anatomical situation offers an opportunity to stimulate auditory afferents electrically at some distance away from the sense organ so that its mechanical structures remain unharmed by surgical procedures. This way we were able to test if induced changes of the membrane potential affect the generation of otoacoustic emissions. In fact, electrical stimulation causes reversible changes in DPOAE-amplitude (Fig. 4d; Möckel et al. 2007). The result suggests that events intrinsic to the sensory neurons themselves (and possibly the cap cells) contribute to the production of emissions.

The number of sensory cells in tympanal organs varies from species to species. Notodontid moths like the maple-prominent, *Ptilodon cucullina,* or the buff-tip, *Phalera* sp., seem to have the most simple organs. Here the tympanum is stretched tightly between two elastic exoskeletal elements, and only a single scolopidium is positioned between a cuticular anchor and the tympanum (Fig. 5a,b; Eggers 1919; Surlykke 1984). In this strategic position, the auditory neuron will react very sensitively to deflections of the tympanum. Should the scolopidial sensory cell possess active components of amplification, it should also be capable to influence the mechanics of the tympanic membrane directly. It turns out that even the simple metathoracic ear of notodontids emits prominent DPOAEs, whose growth function is comparable to that of more complex tympanal organs and that of vertebrate ears (Fig. 5c). Following treatment with ether (as an anesthetic), the DPOAE-amplitudes decrease considerably, which suggests that metabolic processes are actively involved in the generation of emissions (Kössl et al. 2007). In notodontids, DPOAEs can be elicited at frequencies near 100 kHz which is in accordance with a sensitive high-frequency hearing range in these moths (Surlykke 1984).

More detailed information on the location of DPOAE-generation can be obtained from the ear of locusts after setting fine surgical lesions at the attachment points of sensory dendrites at the tympanum (Fig. 6c). Here the vibrations of the tympanic membrane are frequency-specific in the form of travelling waves (Fig. 6a,b; Windmill et al. 2005). At the location of the "pyriform vesicle" (PV in Fig. 6c), maximal deflections are achieved at frequencies > ca. 12 kHz (26 kHz in the example of Fig. 6b). At the "folded body" (FB), that is, another site of dendritic attachment, maximal deflection can only be induced at much lower frequencies (3.3 kHz in the example of Fig. 6b). After the connection between pyriform vesicle and the peripheral sensory ganglion has been severed, high-frequency-DPOAEs are drastically reduced (Fig. 6d, top). Additional ablation of the folded body together with the whole sensory ganglion results in a reduction of DPOAE-levels across the entire frequency range (5–30 kHz; Fig. 6d, bottom). This finding underpins the notion that the mechanosensory cells themselves are contributing to DPOAE-generation in a frequency-specific way.

Vibrating antennal hearing organs

Although the results discussed in the previous chapter provide circumstantial evidence, final proof is still lacking in the case of tympanal organs demonstrating that an active amplification process does in fact exist within the sensory cells and how such a putative mechanism is constituted. In the case of the antennal ears of *Drosophila* and of male mosquitoes, however, Göpfert and Robert (2001, 2003) and Jackson and Robert (2006) have demonstrated with laser-

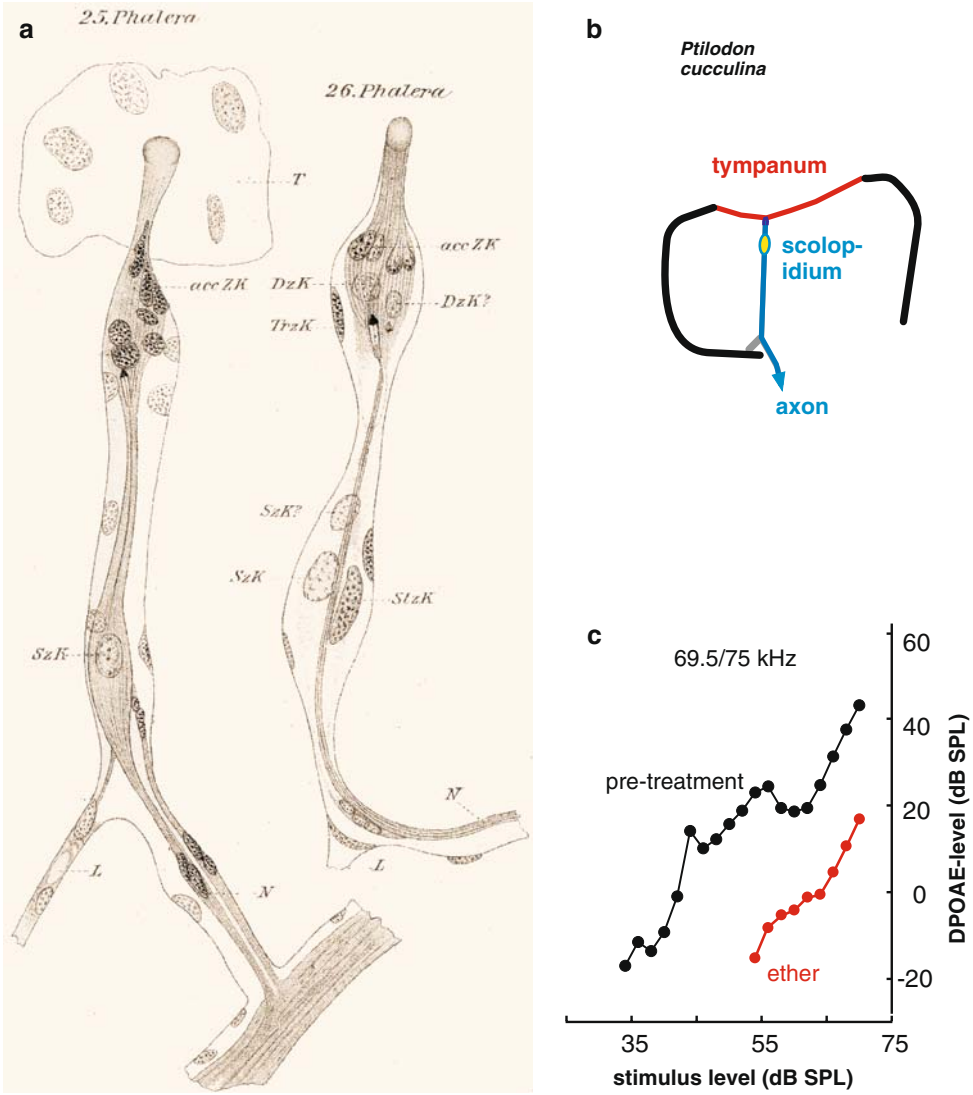


Fig. 5 The metathoracic tympanal organ of notodontid moths has only one auditory neuron; nonetheless, the ear produces distinct DPOAEs in the high frequency range. **a** Detailed drawings of Eggers (1919; plate 23)demonstrate that the tympanal scolopidium of the buff-tip (*Phalera bucephala*) comprises only one sensory neuron (*T* tympanum; *N* tympanal nerve; *L* ligament; remaining labels denote putative cell nuclei). **b** Schematized representation of the tympanal organ of notodontids; the scolopidial dendrite is attached to the tympanum. **c** DPOAE growth-functions in tympanal organ of another notodontid, the maple-prominent (*Ptilodon cucculina*), before and after treatment with ether acting as an anesthetic; the two sound stimuli were at 69.5 kHz (f1) and 75 kHz (f2) (modified from Kössl et al. 2007)

vibrometric measurements that mechanical energy is in fact generated in these organs (see the examples in Fig. 7). Compelling evidence of such active force generation are the spontaneous vibrations of the antennae at frequencies below ca. 1 kHz. They can be induced by pharmacological and genetic manipulations (Fig. 7b,c). Such vibrations do not generate sound pressures that are loud enough to be recordable as OAEs (because the organs lack a tympanic membrane), but the underlying cellular mechanisms may resemble those in tympanal organs. The quest for the molecular basis of force generation focuses on proteins associated with the transduction apparatus at the cilium. Although it is still inconclusive in the case of vertebrate hair cells which protein constitutes the transduction channel (see the review by Corey 2006), with some circumstantial evidence that a member of the TRP-group (TRP Transient Receptor Potential) may be the most promising candidate (Cuajungco et al. 2007), in *Drosophila* at least, there is good evidence that a TRP-protein is indeed responsible for sensory transduction (Kernan 2007). Antennal vibrations are significantly altered after deleting the genes that either code for a putative TRP transduction channel or for associated proteins (Göpfert and Robert 2003; Göpfert et al. 2005, 2006). These results indicate that the transduction apparatus with its associated proteins is critically involved in force production, even though the detailed molecular interactions are still unclear (Göpfert et al. 2006). Interestingly, the scolopidial sensory neurons of *Drosophila* also possess a prestin-homologous protein (Weber et al. 2003), but so far it appears unlikely that it is relevant here for the production of mechanical forces.

Conclusion and a comparison of active ear mechanics in vertebrates and insects

In reptiles and birds (sauropsids) active and nonlinear mechanical amplification in the inner ear is not based on

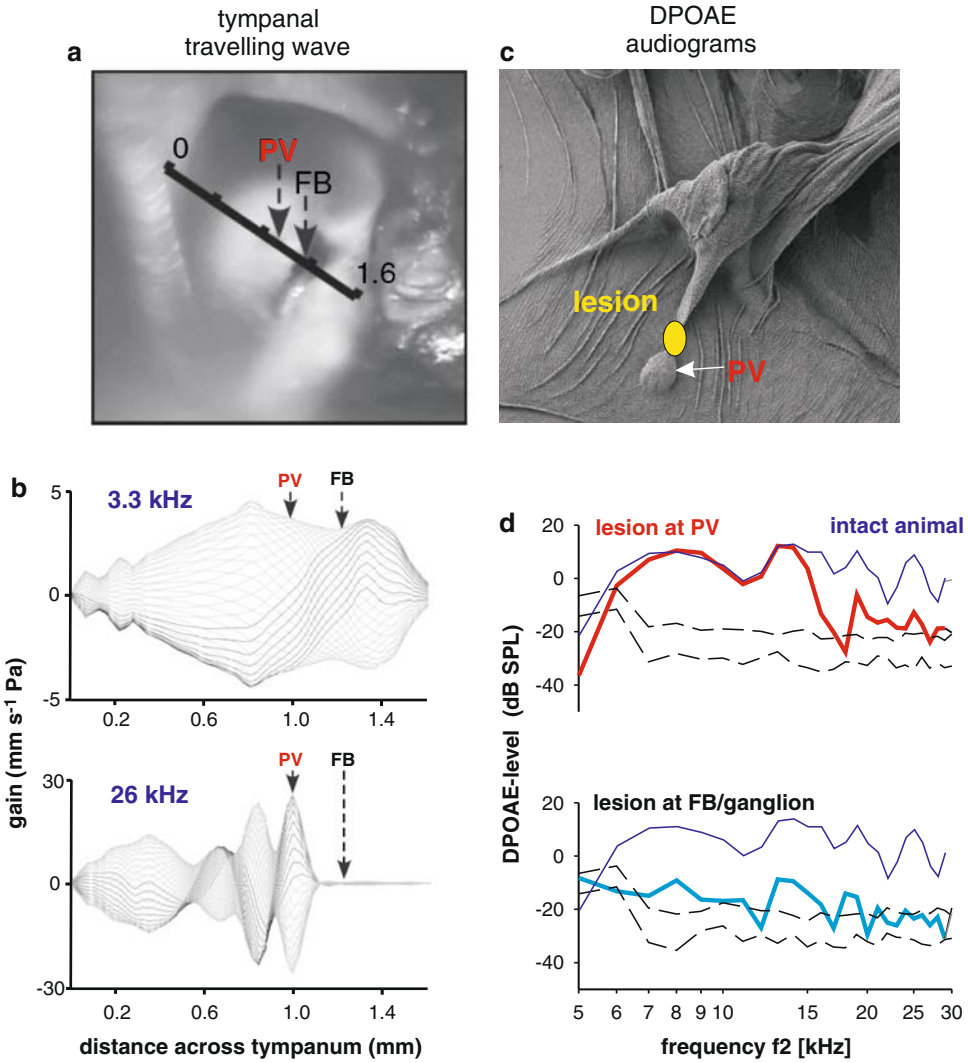


Fig. 6 Müller's organ of *Locusta migratoria*; travelling waves and DPOAEs before and after lesions. **a** External view of tympanum; *diagonal line* indicates measuring points (in mm) for laser-vibrometric recordings of tympanic vibrations. *Arrows* mark position of pyriform vesicle (PV) and of folded body (FB). **b** Stimulation with pure tones generates vibration patterns that resemble travelling waves across measuring points. Low frequency stimulation (at 3.3 kHz) causes displacements of PV and FB, while high-frequency stimulation (at 26 kHz) generates amplitude maxima at the PV without displacing FB. **c** Internal view of locust tympanum showing Müller's organ and PV (scanning electronmicrograph). The PV was severed from Müller's organ by setting a lesion through the dendritic attachments (*yellow spot*). **d** DPOAE-levels at sound stimuli of constant intensity and varying frequency (f2), i.e., so-called DP-gram (f2/f1 ratio maintained at 1.08; L1/L2 of 60/50 dB SPL). *Upper:* following a lesion at PV, DPOAE-levels above ca. 15 kHz (*red curve*) drop nearly to noise level (*dashed black lines* noise level expressed as the average (±1SD). *Lower:* subsequent lesion of FB and of Müller's organ itself (in the same preparation) cause a marked reduction of DPOAE-levels across the entire frequency range. The results indicate that high-frequency DPOAEs are only generated as long as the connection between sensory neurons and PV remains intact (**a**,**b** modified from Windmill et al. 2005; **c**,**d** modified from Möckel et al. 2007)

motility of the sensory cell soma, but on motility of the stereovilli of the hair cells. Correspondingly, the stereovilli are responsible for the generation of spontaneous OAEs (Manley et al. 2001; Manley 2001). In mammals, there is ample evidence that prestin-based somatic motility of outer hair cells is an important component of the cochlear amplifier (reviewed by Ashmore 2008). Recent evidence, however, has shown that there is also active force production in the stereocilia of inner and outer hair cells. It is employed for adaptation during auditory transduction and could amplify sound waves on a cycle-by-cycle basis (reviewed by Fettiplace and Hackney 2006). Hence at present, the question remains unresolved which amplifying mechanism is the relevant one in mammals. Techniques are required here that can dissect the somatic versus the stereocilia mechanisms in vivo. As to somatic motility of outer hair cells, it is known that changes in the membrane potential cause prestin to change its conformation which instantly shortens or

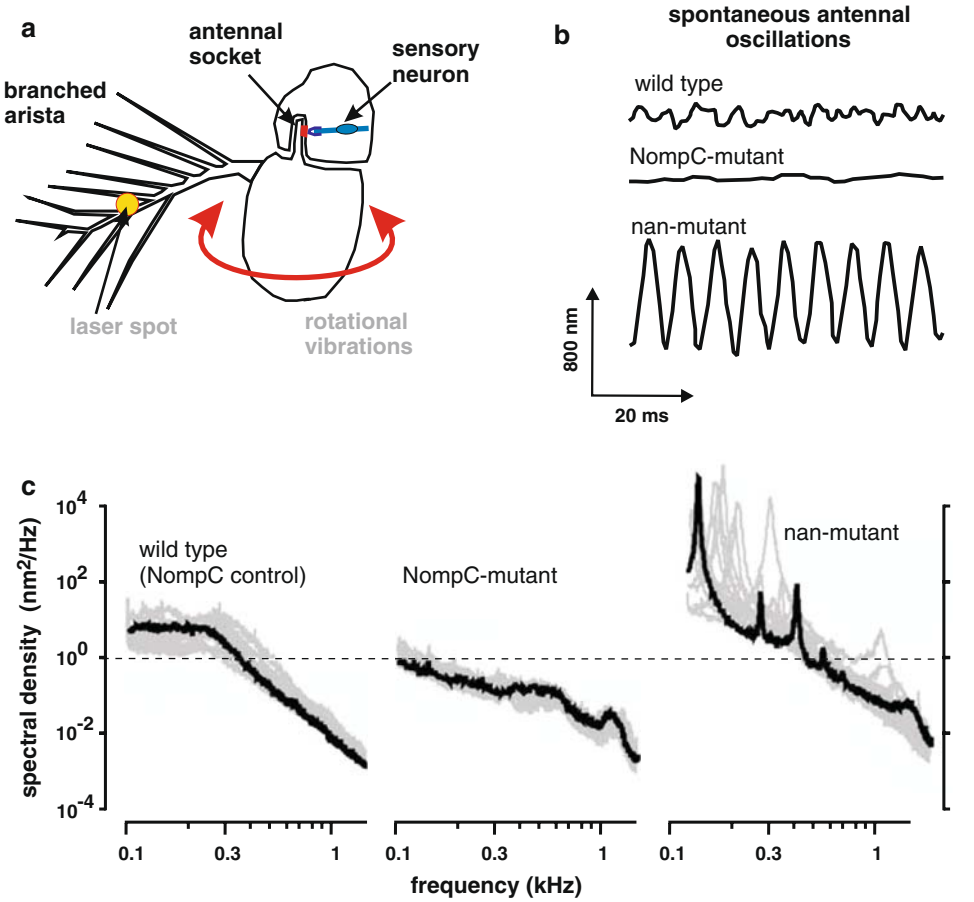
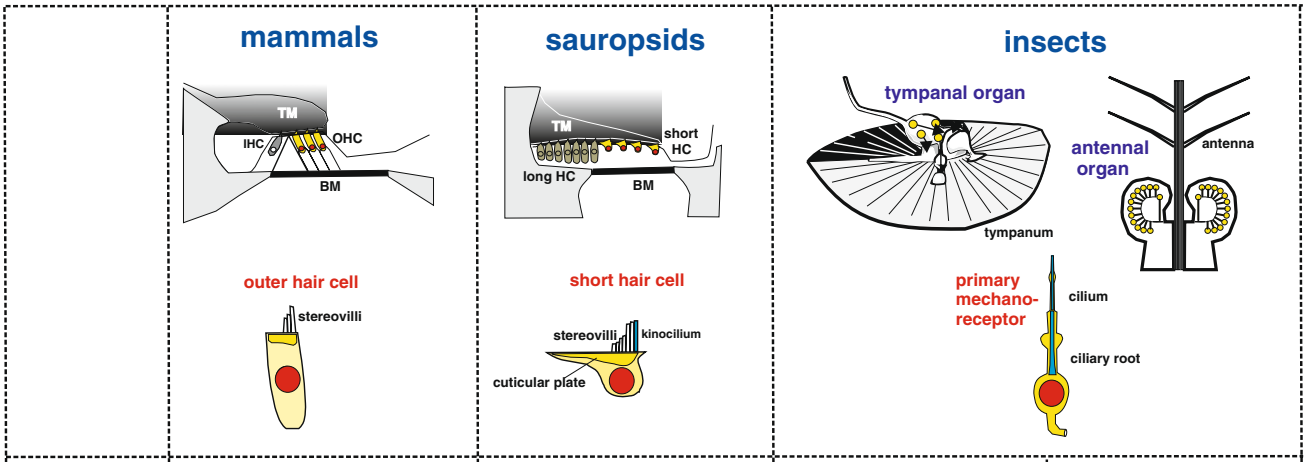


Fig. 7 Vibrating antennal ear of *Drosophila*. **a** Schematic representation of the two distal antennal segments with branched arista and arrangement of laser-vibrometric measurements (adapted from Göpfert et al. 2005). Near-field sound vibrations of the arista rotate the third antennal segment relative to the second. **b** Time course of spontaneous ("self-sustained") antennal oscillations in nan-mutant (having deficient TRPV channels); by comparison, in animals with deficient NompC TRP-channels spontaneous activity is reduced below that found in wild type flies. **c** Power spectra of such oscillations in the same *Drosophila* strains. *Black traces*, spectrum obtained for one arista; *gray*, spectra from additional specimens of the same strain (**b**, **c** adapted from Göpfert et al. 2006)

elongates the cell soma. This process is rapid and can follow very high sound frequencies as indicated by fast motile responses induced electrically in isolated outer hair cells up to at least 79 kHz (Frank et al. 1999). However, since the receptor potential of these cells in vivo decreases with frequency due to membrane capacitance, it is not clear if it is sufficient to drive somatic motility at very high frequencies. Several possibilities have been discussed as to how the required driving voltage could be provided at very high frequencies, including extracellular potentials (Liao et al. 2007; Ashmore 2008).

Amplification in stereovilli, which relies on interactions between the transduction molecules and actin/myosin-like proteins, seems to operate at a much slower rate than somatic motility. So far, all available evidence gathered in non-mammalian vertebrates demonstrates that active stereovilli movements, adaptation mechanisms, and even electrically induced OAEs are restricted to frequencies below ca. 3 kHz (Manley et al. 2001; Fettiplace et al. 2001). Incidentally in mammals, potential active stereovilli processes are limited to a similar, low frequency range (Ricci et al. 2002; LeMasurier and Gillespie 2005). Correspondingly, also the hearing range of non-mammalian vertebrates is limited to relatively low frequencies (Fig. 8). Barn-owls appear to be the front-runners among non-mammalian vertebrates as far as high-frequency auditory processing is concerned; their hearing range extends to ca. 11 kHz. Accordingly in these owls, spontaneous OAEs are recordable at ca. 9–11 kHz (Fig. 1). If an adaptation time constant of 120 µs in mammalian outer hair cells can serve as an indicator of the frequency limits of hair bundle mechanisms (Kennedy et al. 2003), then the calculated frequency limit would be close to 8 kHz. To summarize, two potential modes of cochlear amplification exist in the case of vertebrates: (1) a stereovilli-based process that covers low frequencies, and (2) a somatic mechanism that amplifies both low and very high frequency signals.

By comparison, insects possess an auditory amplifier in the lower frequency range (up to ca. 1 kHz) that seems to be based on a ciliary mechanism. This has clearly been demonstrated in the antennal organs of *Drosophila* (Göpfert and Robert 2001, 2003) and of male mosquitoes (Jackson and Robert 2006); it is analogous to the situation in non-mammalian vertebrates (see Albert et al. 2007). So far in the case of insect tympanal organs, which can process very high frequencies above 100 kHz like the ear of mammals, a mechanical amplifier based on transduction proteins of the cilium has not been demonstrated (Fig. 8). If force is generated here similar to the mechanism in antennal organs, the putative amplification process would be much more

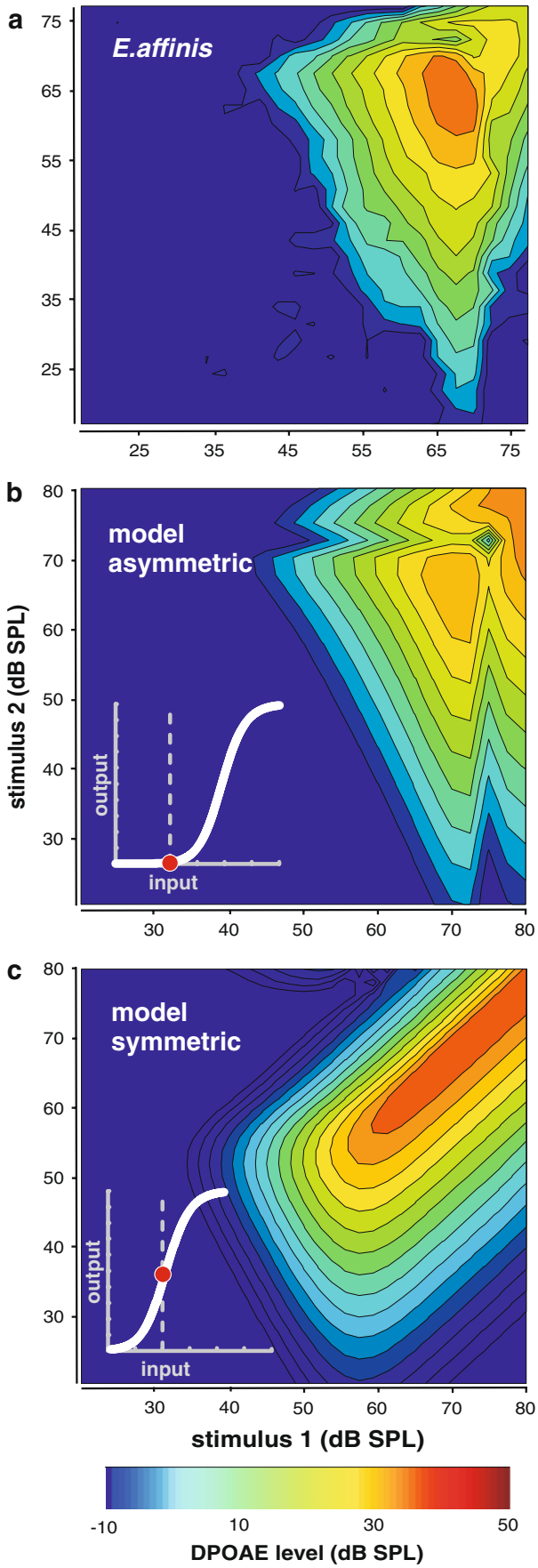


	mammals	sauropsids	insects	
hearing range	10 Hz - >200 kHz	80 Hz - 11 kHz	1 kHz - 100 kHz	100 - 1000 Hz
spontaneous OAE	some species 0.5 - 62 kHz up to 40 dB SPL	some species 0.8 - 11 kHz up to 25 dB SPL	?	spontaneous vibrations 100 - 1000 Hz
DPOAE-sensitivity	ca. -3 dB SPL	ca. 10 dB SPL	ca. 12 dB SPL	?
cell soma motility	70 kHz	negative	?	?
motile cilia/ stereovilli	≤ 3 kHz (force generation)	bis ~3 kHz (electr. evoked OAE)	?	?

Fig. 8 Comparing the ears of mammals, sauropsids, and insects: fundamental anatomical and bio-acoustical parameters. Hearing ranges are indicated without considering infra-sound perception. DPOAE-sensitivities are listed for minimal stimulus-levels that are just sufficient to evoke DPOAEs of −10 dB SPL. *IHC* inner hair cell; *OHC* outer hair cell; *TM* tectorial membrane; *BM* basilar membrane. Data sources: Frank et al. 1999 (motility of OHC-somata); Ricci et al. 2002 (motile stereovilli in mammalian cochlea); Manley et al. 2001 (electrically evoked OAEs in reptiles); Manley et al. 1993 (DPOAEs in lizards). See text for detailed discussion

rapid than is currently known to exist in cilia or stereovilli. Unlike the finding in antennal organs, so far no spontaneous vibrations or SOAEs have been observed in tympanal organs. Nonetheless, DPOAE-recordings from tympanal organs show that the sensory cells are capable of processing faint sound in a sensitive, nonlinear fashion and that this process retroacts on the vibration properties of the tympanic membrane.

Even if a potential amplifier does not directly act on the transduction apparatus but in the cell soma (like prestin in mammals), it will still crucially depend on the transduction apparatus and the resulting receptor (generator) potential. Voltage-dependent mechanical deformation of the cell that contributes to raising the receptor potential (which drives the process), is a typical positive feedback system. If the amplification factor is close to 1, the system is prone to spontaneous oscillations. The generation of DPOAEs in mammals can easily be simulated, using the characteristic behavior of the transduction currents in hair cells as the nonlinear element. Such S-shaped transduction characteristics are generally approximated by Boltzmann-functions; they reflect the level-dependent properties of DPOAEs quite well. A single Boltzmann-function suffices to simulate the DPOAEs in mammals realistically (see e.g., Frank and Kössl 1996; Lukashkin and Russell 1999; Lukashkin et al. 2002). Using such simulations, and comparing how the DPOAE-levels depend on the respective stimulus levels in tympanal organs and in the mammalian cochlea, reveals clear differences between the two types of auditory organ. In the case of the tympanal organs of moths (for example in *Empyreuma affinis*, Kössl and Coro 2006), varying the two stimulus levels results in a triangular DPOAE-activation range (Fig. 9a), which can be simulated by a Boltzmann-function (Fig. 9b), but only as long as the zero-point or operating point of the function lies asymmetrically. Using symmetrical Boltzmann-functions, the DPOAE-activation range has a different shape (Fig. 9c) and resembles the one found in the ear of mammals (Kummer et al. 2000) or frogs (Meenderink and Van Dijk 2005). Interestingly, receptor potentials measured directly in tympanal cells of locusts are restricted to depolarizations and do not show hyperpolarizations upon acoustical stimulation (Hill 1983a, b). This



◀ **Fig. 9** DPOAE-generation in the (metathoracic) tympanal organ of the moth *Empyreuma affinis* (Arctiidae) (**a**), and in an approximation model that is based on a nonlinear Boltzmann-function (**b**, **c**). Such functions are used to describe the transduction behavior of hair cells in the vertebrate cochlea. Here, the DPOAE-amplitude (color-coded) is represented as a function of the amplitude of the two pure-tone stimuli. The insect data are well represented by the approximation in (**b**) as long as the operating point (or zero-point) of the function lies asymmetrically. Using a symmetrical model (**c**), the DPOAE-activation range resembles the one characteristically found in vertebrate ears (see text for a detailed discussion)

finding can be explained by strongly asymmetrical transfer functions. Hence, the single cilium of scolopidial sensory neurons seems to create somewhat different constraints than the hair cells in the mammalian cochlea. Nevertheless, similar proteins appear to be involved in both types of cells (scolopidial and hair cells), and mechanical feedback appears to be an integral part of sensitive hearing.

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