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Physiology and mechanics of insect hearing organs

Abstract

Tympanal hearing organs of insects emit distortion-product otoacoustic emissions (DPOAEs) which are indicative of nonlinear mechanical sound processing. General characteristics of insect DPOAEs are comparable to those measured in vertebrates, despite distinct differences in ear anatomy. DPOAEs appear during simultaneous stimulation with two pure tones (f1<f2) as additional spectral peaks at frequencies of nf1-(n-1)f2 and nf2-(n-1)f1, with the 2f1-f2 emission being the most prominent one. Insect DPOAEs are highly vulnerable to manipulations that interfere with the animal's physiological state and disappear after death. First evidence from locusts suggested that scolopidial mechanoreceptors might play a role in frequency-specific DPOAE generation (Möckel et al. 2007). The overall aim of this thesis was to determine the source of sensitive, nonlinear hearing at high frequencies and of DPOAE generation in tympanal organs of insects.

The first project of the present thesis involved general characteristics of DPOAE generation in the bushcricket *Mecopoda elongata* and the selective exclusion of the scolopidial mechanoreceptors using the neuroactive insectizide pymetrozine (Möckel et al. 2011). Pymetrozin appears to act highly effective and selectively on chordotonal organs, without affecting other sensory organs that lack scolopidial receptors. Pymetrozine solutions were applied as closely as possible to the scolopidia via a cuticle opening in the tibia, distally to the organ. Applications at concentrations between 10^{-3} and 10^{-7} M led to a pronounced and irreversible decrease of DPOAE amplitudes. Both this study on bushcrickets (Möckel et al. 2011) and an earlier one on locusts (Möckel et al. 2007) hence indicate the involvement of scolopidia in DPOAE generation in insects, by using complementary methods (pharmacological versus mechanical manipulation) and different animal models.

The second project of the present thesis investigated the temperature-dependence of DPOAEs in the locust *Locusta migratoria* (Möckel et al. 2012). The suggested biological origin of acoustic two-tone distortions in insects should involve metabolic processes, whose temperature-dependence would directly affect the DPOAE generation. Body temperature shifts resulted in reversible, level- and frequency-dependent effects on the 2f1–f2 emission. Using low f2 frequencies of up to 10 kHz, a body temperature increase (median +8–9°C) led to an upward shift of DPOAE amplitudes of approximately +10 dB, whereas a temperature decrease (median –7°C) was followed by a reduction of DPOAE amplitudes by 3 to 5 dB. Both effects were only present in the range of the low-level component of DPOAE growth functions below f2 stimulus levels of approximately 30-40 dB SPL. Emissions induced by higher stimulus levels and frequencies (e.g. 12 and 18 kHz) remained unaffected by any temperature shifts. The Arrhenius activation energy of the underlying cellular component amounted to 34 and 41 $kJmol^{-1}$ (for growth functions measured with 8 and 10 kHz as f2, respectively). Such activation energy values provide a hint that an intact dynein-tubulin system within the scolopidial receptors could play an essential part in the DPOAE generation in tympanal organs.

The third project of this thesis demonstrated mechanical DPOAE analogs in the tympanum's vibration pattern during two-tone stimulation in the locust *Schistocerca gregaria*, using laser Doppler vibrometry (Möckel et al. 2014). DPOAE generation crucially relies on the integrity of the scolopidial mechanoreceptors (Möckel et al. 2007, 2011), which in locusts, directly attach to the tympanal membrane. During two-tone stimulation, DPOAEs were shown to mechanically emerge at the tympanum region where the auditory mechanoreceptors are attached. Those emission-coupled vibrations differed remarkably from tympanum waves evoked by external pure tones of the same frequency, in terms of wave propagation, energy distribution, and location of amplitude maxima. In contrast to traveling wave-like characteristics of externally evoked vibrations, intrinsically generated waves were locally restricted to the region around the high frequency receptors' attachment position. The mechanical gradient of the tympanal membrane that leads to direction-dependent properties probably avoids the spreading of these locally evoked waves, which are then reflected and occur only in restricted areas as standing waves. Selective inactivation of mechanoreceptors by mechanical lesions did not affect the tympanum's response to external pure tones, but abolished the emission's displacement amplitude peak. These findings provide evidence that tympanal auditory receptors, comparable to the situation in mammals, comprise the required nonlinear response characteristics, which during two-tone stimulation lead to additional, highly localized deflections of the tympanum.