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# Mechanics of the transduction of sound in the tympanal organ of adults and larvae of locusts

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**Summary.** The mechanical transmission of sound in the tympanal organ of adults and 5th instar larvae of *Locusta migratoria* and *Schistocerca gregaria* has been investigated by means of stroboscopic measurements within a frequency range from 1–20 kHz.

Frequency dependent spatial distributions of amplitudes and phases of oscillation on the tympanal membrane and the Müller's organ could be demonstrated. Cuticular structures on the membrane may act as a lever arm (e.g. elevated process) and cause a transformation of the (unidimensional) membrane motion into components of displacements in the Müller's organ perpendicular, as well as even parallel, to the membrane.

Sites of maximum relative displacements at distinct frequencies are found to be correlated to the course of the dendrites of the acoustic receptor cells. Differences in morphology of the tympanal organ between the two species as well as between adults and larvae always correspond to differences in the mechanical properties (resonances etc.). Consequently, differences or changes in the neurophysiological response characteristics of the different receptor cells have been found.

Based upon these findings a correlation between the anatomical and physiological classification of the receptor cell groups is presented.

## Introduction

With a relatively simple anatomy the caeliferan ear is able to fulfil the quite complex tasks of reception, transmission, transduction and coding of sound signals (review: Autrum 1963). Within the group of invertebrates it represents one of the few that can perform frequency discrimination with a fairly good resolution. The mechanisms underlying these operations were correlated to the resonance properties of the tympanal membrane (review: Michelsen 1979).

The anatomy of the tympanal organ (see Fig. 1) has been described in detail by several authors (e.g. Schwabe 1906; Gray 1960; Michelsen 1971a; Michel and Petersen 1982). According to anatomical criteria Gray (1960) distinguished between 4 groups of receptor cells (a-, b-, c- and d-cells).

The physiological characteristics of the tympanal receptor cells are also well known (Michelsen 1971a; Römer 1976; Petersen et al. 1982; Hill 1983a, b; Sippel and Breckow 1983, 1984). From their response properties (e.g. characteristic fre-

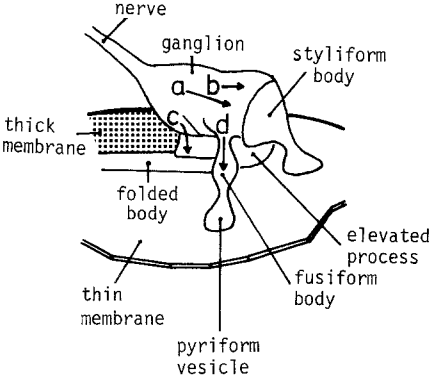


**Fig. 1.** Anatomy of the locust tympanal organ. Dorsal view of the inner surface of the right tympanal membrane with the Müller's organ. The location of the receptor cell bodies of the four different groups of receptor cells is given by the corresponding small letters. The course of the dendrites is indicated by arrows. Altered from Michelsen (1979) after Gray (1960)

*Abbreviations: T1, T2, T3, T6, T7* reference points on the tympanal membrane; *M1, M4* reference points on the ganglion of the Müller's organ; *K1, K2* reference points on the elevated process

quency and threshold) Römer (1976) was able to distinguish four distinct groups (type 1, 2, 3 and 4).

Michelsen (1971 b and c) described the very complex vibrations of the tympanal membrane and the resulting consequences for the transmission of signals onto the receptor cells. The pitch discrimination of the receptor cells which are anatomically identical is based upon spatially distributed resonances of the tympanum. Michelsen (1973) already supposed that the Müller's organ does not act only as a 'passive observer' of the oscillating membrane, but itself, as a mass loaded assembly of receptor cells is involved in the process of signal transmission. The investigations of Stephen and Bennet-Clark (1982) confirmed this supposition and showed, that the Müller's organ exhibits very complex properties in its oscillations and resonances. The motions show several degrees of freedom and there are even components of displacements parallel to the tympanum.

During larval development the tympanal organs of locusts show instar-specific characteristics in their membrane, their cuticular structures and the Müller's organ (Michel and Petersen 1982). All the receptor cells are already present in the 1st instar, whereas the tympanum and its cuticular structures differentiate step by step from one instar to the next. The electrophysiological properties of the receptor cells in larvae are described by Petersen et al. (1982).

If it is possible to demonstrate that the morphological and neurophysiological characteristics correspond to changes in the properties of vibration and resonance of the tympanum and Müller's organ, this would give a further clue to the hypothesis that the pitch discrimination in the receptor cells is due only to their site of attachment ('place principle', Michelsen 1971) and not to any 'intrinsic' feature of a cell. This hypothesis would be supported by finding a correlation between neurophysiological and mechanical differences. An exact correlation, however, of the morphological and physiological receptor classification is still missing. Therefore we investigated the mechanics of the tympanal organs of adults and larvae in two different, yet closely related species of locusts. In addition, some manipulations have been performed to intentionally alter the mechanics.

Many different methods have been used in the past to investigate the transmission properties of oscillating membranes. Von Békésy (1953) had already used stroboscopical methods to examine the organ of Corti, though with some restrictions in sensitivity and resolution. Michelsen (1971 b) used a special kind of laser-holography and the technique of capacitance electrodes. Laser vibrometry and other optical methods as well as the Mößbauer technique (Michelsen and Larsen 1978; Dragsten et al. 1974; Johnstone et al. 1970 a, b) have been applied to similar problems. Recently, very sensitive methods (amplitudes $\leq 10^{-11}$ m) have been developed (Drake and Leiner 1984). However, some of these techniques are restricted to investigations on even planes or vibrations in a particular direction, respectively. Frequently, there is a deficiency in the spatial resolution or the technique is very expensive. For those reasons, Stephen and Bennet-Clark (1982) returned to the stroboscopic method of von Békésy (1953). The investigations in the present paper also rely on that technique. The sensitivity is relatively poor ($\approx 1$ µm), but the spatial resolution is limited only by the optic device used in the experiment. Whereas Stephen and Bennet-Clark were mainly restricted to sound frequencies below 7 kHz, we are able to perform quantitative measurements of displacements and phases using frequencies up to 20 kHz.

A detailed analysis of the mechanics of oscillation of the tympanal membrane with its cuticular structures and the Müller's organ should give an answer to the following questions:

- How does the 'place principle' work?
- How can differences in the response characteristics of the receptor cells be related to changes in the properties of resonance and oscillation of the involved structures?
- Is it possible to predict certain response characteristics of receptor cells when the mechanics of the tympanal organ in question are known?

## Material and methods

*Animals and preparation.* Data have been obtained from 68 adults and 32 larvae (fifth instar) of both sexes of *Locusta migratoria* and from 40 adults and 32 larvae (fifth instar) of *Schistocerca gregaria*. The age of the larvae (in days after the 4th moult) was taken into consideration in all measurements.

The tympanal organ with its surrounding chitinal structures was removed from the anesthetized animal and waxed to the bevelled end of a small tube. The tympanal membrane was parallel to the acoustic and optic axis and managed such that the loudspeaker output acted on the external face of the membrane. The internal face with the Müller's organ was viewed from above with the microscope. All the tissue covering the tympanal organ from the inside of the animal was removed carefully so that the whole organ was freely visible. The interesting structures were dusted with small corundum granules ($Al_2O_3$. $\varnothing \approx 1$ µm) to provide land marks for the measurements. As a rule, the whole preparation takes less than 15 min, much below the critical time of drying of the preparation.

*Stroboscopy.* The motion of quickly oscillating objects can be observed when they are illuminated with phase-coupled flashes. When the flashes are given phase-locked to the sound signal

the image of the object appears to be stationary. When the phase-delay of the flash is shifted slowly each phase of the objects' motion can be observed, particularly maxima and minima or zero-crossings of the displacements.

The arrangement for stroboscopical illumination of oscillating parts of the tympanal organ is essentially the same as described by Stephen and Bennet-Clark (1982) with some modifications. Our stroboscopic device is manually adjustable to provide constant phase-delays from 1 up to 360° (stationary image) as well as an automatic scanning mode where the phase-delay is shifted in one degree steps with an adjustable velocity of 0.5 to 4 periods/s (slow motion). The phase scan could be stopped at each desired point of displacement. This was very usefull for measuring the relative amplitudes and phases at maximum and minimum or zero displacement of marked points on the tympanal organ in a particular direction of the plane of observation. This procedure provides the advantage of a short duration of the measurements. For measuring arbitrary movements of a defined point in the plane of observation fixed phase-delays were used to produce stationary images.

*Opto-acoustic arrangement.* For observation and optical measurements of the vibrating objects a microscope with a ×20 objective (Leitz) providing a large working distance (6 mm) and a ×10 curtain measuring eyepiece (Malies Instruments) was used. With 200-times magnification we got an accuracy of about 1 µm measuring (optically) stationary objects.

To obtain adequate sound-pressure levels we produced a closed sound field inside a small plastic tube. The sound source was located at one end and the tympanal organ at the other end of the tube. Working under closed field conditions one must ensure that the dimensions of the acoustic apparatus allow the approximation of plane waves. Likewise, reflections which would cause standing waves must be avoided. In the range of 2–7 kHz the wavelength is at least ten times greater than the diameter of the tube (5.5 mm), so that the propagation of plane sound waves can be assumed. In order to damp the reflections from the walls of the tube and the covering of the loudspeaker we used some fleece paper. In the frequency range from 2–20 kHz we obtained fluctuations in the sound pressure of less than ±3 dB. Measurements of the phase differences between sound emitter and receiver show a proportionality to the frequency of the sound signal which means that our closed sound field is sufficiently free of reflections. Even measurements inside the tube did not reveal any local fluctuations in the sound pressure. All acoustic measurements were done with a sound-level meter (Brüel & Kjaer 2209) and a $^1/_4$ inch microphone (Brüel & Kjaer 4135).

The actual sound pressure at the site of the tympanum cannot be measured directly because of different acoustic impedance of the microphone and the tympanum, but the values measured inside the tube are probably the best approximation because the attenuation of the amplitudes of the plane wave on its way to the end of the tube is negligible. Nevertheless, distortions of the sound field cannot be excluded completely. Therefore, instead of the parameters of the sound field, defined points on the tympanum or the Müller's organ were used as a reference for the measurements (e.g. the phase difference $\Delta\varphi$ is measured relative to the phase of the point T2 on the tympanum instead of to the phase of the sound wave).

Generally, sound pressure levels of 103 dB SPL were used for frequencies $f \leq 5$ kHz and 113 dB SPL for frequencies $f >$ 5 kHz.

*Linearity of the system.* Because the stroboscobic method used here is relatively insensitive, displacements less than 1 µm cannot be measured quantitatively. In the near suprathreshold range (according to the reactions of the receptor cells) displacements occur in the range of a few nm. To be able to make statements which are valid for the threshold range and the total dynamic and saturating range the system must show a linear transmision characteristic. We found by measurements of defined points on the tympanal membrane and the Müller's organ a good linearity over a range from about 90 to 110 dB SPL (see Fig. 2). Below this level we may also assume linearity because with smaller displacements the displacement of an oscillatory system should be proportional to the driving force (Hooke's law). Schiolten et al. (1981) have already shown that the d-cell attachment area of the locust tympanum works as a linear transducer up to at least 100 dB. Equivalent results have been found for comparable systems in gryllids and bush-crickets (Paton et al. 1977; Seymour et al. 1978).

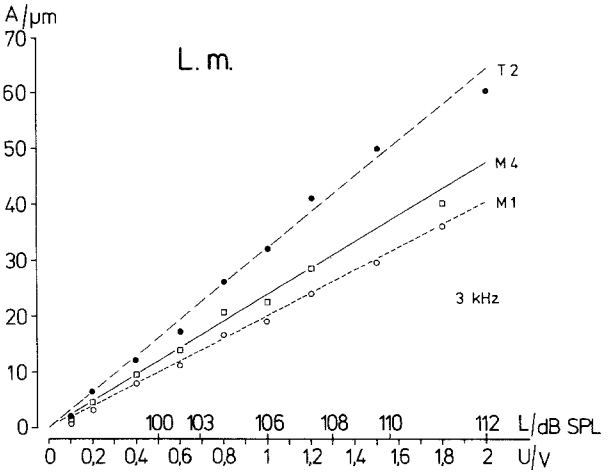


**Fig. 2.** Oscillation amplitudes A of different points on the tympanal membrane (T2) and the Müller's organ (M1, M4) in relation to the output voltage U of the amplifier, driving the loudspeaker, and the sound pressure level L, respectively, at a stimulus frequency of 3 kHz

## Results

### *1. Oscillation properties of the tympanal membrane*

As already known from Michelsen (1971b) and Stephen and Bennet-Clark (1982) from investigations on adults of *Schistocerca gregaria* the tympanal membrane produces resonances with different modes of vibration. The thin part of the membrane oscillates mainly independently from the entire tympanum. Because of the good spatial resolution of the stroboscopic technique we are able to present an improved spatial attachment of resonances, nodal lines and phase differences which are spatially distributed. In addition, we performed comparative measurements on adults of *Locusta migratoria* and *Schistocerca gregaria* and on 5th instar larvae.

In this study each spatial minimum displacement is termed a 'node' in the sense of the theory

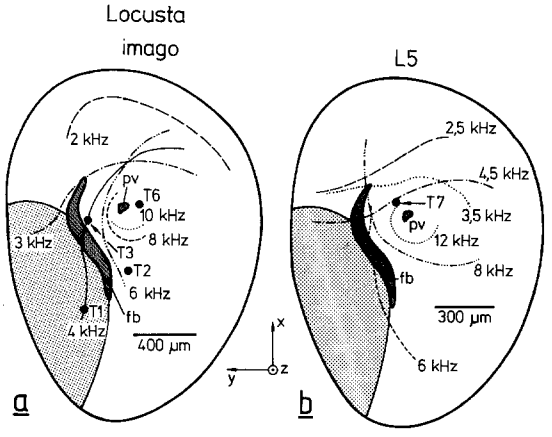


**Fig. 3a, b.** Nodal lines (minimum displacements) on the tympanal membrane of *Locusta migratoria* at several frequencies. Cuticular structures are hatched, the thick part of the membrane is dotted. Reference points of the mesurements are indicated; *fb* folded body; *pv* pyriform vesicle. **a** imago, **b** 5th instar larvae

of oscillating membranes (Skudrzyk 1971). Two points which are separated by an 'ideal' node are oscillating 'antiphasically', that means with a relative phase of $\Delta\varphi = 180°$. The spatial distribution of nodes depends on the frequency of the stimulus signal. Increasing the excitatory frequency, an (ideal) membrane starts to vibrate in higher modes and the minima of oscillation (nodal lines) shift concentrically towards the center of the membrane. The vibration amplitude of the center reflects the resonances corresponding to the different modes of vibration, but the center is not affected by the nodal lines (cf. Morse 1948).

Figure 3a shows the spatial distribution of nodal lines upon the tympanal membrane of adults of *Locusta migratoria*. With low frequencies the lines appear close to the border of the tympanum and the maximum of vibration is located at the folded body (fb) near the point marked T1. The entire membrane is oscillating with uniform phase in its fundamental mode. With increasing frequency the course of the nodal lines becomes less concentric and the thick and thin part of the membrane are getting out of phase. With high frequencies ($f \geq 8$ kHz) the maximum is found near the pyriform vesicle (pv, T6) with the nodal lines circling around it. Other parts of the membrane only vibrate with very poor amplitudes.

The elevated process represents the site of attachment of the b-cells. It is a finger-shaped cuticular invagination of the membrane which is firmly attached and may act as a lever arm with the center of rotation at its base ($\approx$T3, cf. Fig. 5). Upon stimulating the tympanum at 4 kHz, it vibrates with a nodal line going through the point T3 which means minimum displacement at this point and antiphasic motion on the right and left. This results in a motion of the lever arm which may cause a transformation of the direction of the power vector and oscillatory movements with a maximum component parallel to the plane of the tympanum. Altering the stimulus frequency causes the nodal line to move away from T3 and results in a diminishment in the component of displacement parallel to the membrane (cf. Sect. 2, Fig. 5).

The distribution of nodal lines upon the tympanum of the 5th instar of *Locusta migratoria* about 3 days after moulting is shown in Fig. 3b. It is obvious that the larval tympanum too, exhibits spatial and frequency dependent distributions of the amplitudes of vibration and resonances. Apart from a shift of about 2 kHz to higher frequencies in the nodal lines at the same site on the membrane, we find almost the same characteristics of oscillation as with adults, i.e., nodal lines near the border of the membrane for low frequencies, and nodal lines circling around the pyriform vesicle for high frequencies.

The frequency dependence of the vibration amplitudes of the tympanum as well as their spatial distributions measured in our experiments are almost the same as described by Stephen and Bennet-Clark (1982). There are only slight differences in some values of the resonant frequencies and phase relations between *Locusta migratoria* and *Schistocerca gregaria*. Apart from the above mentioned points for larvae, the differences in the properties of oscillation of the tympanum between adults and larvae of the 5th instar are small (cf. Sect. 4).

### *2. Oscillation characteristics of the Müller's organ*

Stephen and Bennet-Clark (1982) demonstrated with *Schistocerca gregaria* that the Müller's organ does not act only as a passive observer of the vibrating tympanum, but due to its own mass and compliance it exhibits its own properties of oscillation and is actively involved into the process of transmission of signals. Although the mass of Müller's organ is about $^1/_3$ of the membrane's mass it mainly affects the membrane as a damping or resistive load. Furthermore, the authors describe the transformation of the unidimensional movement of the tympanum into two orthogonal components of displacement which result in two-dimensional trajectories of motion (loci, orbits) of certain points of the Müller's organ. These facts and their underlying mechanisms are now described in more detail using adults and 5th instar

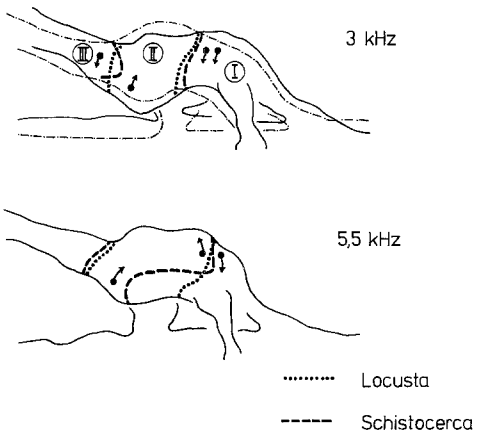


**Fig. 4.** Areas of antiphasic relative motion of the Müller's organ of *Locusta* and *Schistocerca* at two stimulus frequencies. The direction of motion of some points at the same phase is indicated by arrows. At 3 kHz the contours of the Müller's organ are shown at minimum (broken line) and maximum displacement (solid line) of the tympanal membrane

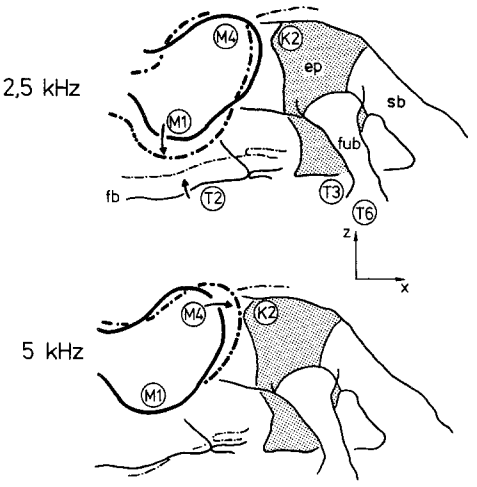


**Fig. 5.** Semischematic drawing of the motion of the Müller's organ at two sound frequencies (2.5 and 5 kHz). The direction of motion at the same point in time is indicated by arrows. Further explanations in the text. *ep* elevated process; *fb* folded body; *fub* fusiform body; *sb* styliform body

larvae of *Locusta* and *Schistocerca* testing an expanded frequency range from 1–20 kHz.

According to the frequency-dependent phases of motion of distinct points on the Müller's organ relative to the membrane it is possible to distinguish between certain 'phase areas' of the Müller's organ. Figure 4 presents areas (I, II and III) of 'antiphasic' relative movements on the Müller's organ in *Schistocerca gregaria* and *Locusta migratoria* for two different stimulus frequencies. The arrows indicate the direction of motion at a certain phase or time, respectively. At a given frequency (i.e. 5.5 kHz), two points belonging to *different* phase-areas are oscillating antiphasically, that means with large relative amplitude. If the borderline moves by altering the stimulus frequency it may happen that both points belong to the *same* phase area (i.e. area I at 3 kHz). Both points are now oscillating almost in phase, which results in a reduction of the relative amplitude even if the absolute amplitudes remain constant. Increasing the frequency, the orientation of the right borderline to the surface of the membrane in *Schistocerca gregaria* turns from initially perpendicular ($f=$ 3 kHz) to approximately parallel ($f > 5$ kHz) to the membrane. This effect is nearly absent in *Locusta migratoria.* At 1.5 kHz the Müller's organ of *Locusta migratoria* is divided into only two areas of antiphasic motion with a borderline almost perpendicular to the membrane's plane. In *Schistocerca gregaria,* however, at 1.5 kHz the entire Müller's organ oscillates with equal phase (not shown).

Semischematically, Fig. 5 shows the motion of the Müller's organ of *Locusta migratoria* for 2.5 kHz and 5 kHz. With 2.5 kHz we find relative displacements mainly between M1 and T2, that means along the dendrites of the c-cells which are attached to the folded body near T2 (Michel and Petersen 1982). M4 nearly reaches a deadlock and acts as an axis of rotation of the left area (surrounded by a thick line) at this frequency. This is near the resonant frequency of M1 ($f_0=$ 1.7 kHz), but far below that of M4 ($f_0=4.3$ kHz). Furthermore T2 ($f_0=3.3$ kHz) nearly reaches maximum displacement at this frequency. With higher frequencies (5 kHz) this axis turns to M1, while M4 is getting components of displacement parallel to the x-axis (towards K2). The stimulus frequency in this case is near the resonant frequency of M4 according to the x-axis, but far above that of M1. The junction line between M4 and K2 corresponds to the course of the dendrites of the b-cells. At 5 kHz the nodal line appears near T2 which means minimum displacement. Over the whole frequency range the main direction of motion of M1 remains in the z-axis, whereas with higher frequencies M4 is getting components of motion parallel to the x-axis. This description of the oscillations of the Müller's organ is rather crude because it does not oscillate like a stiff solid. It becomes obvious, however, that the frequency dependent amplitudes of oscillation are spatially distributed, due to the different resonant and damping properties of the Müller's organ as well as to the spatial distribution of resonances upon the membrane.

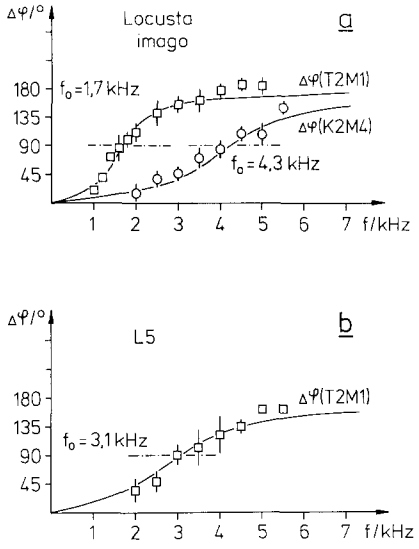


**Fig. 6a, b.** Phase spectra of the motion of points T2 relative to M1 (parallel to the z-axis, squares) and K2 relative to M4 (parallel to the x-axis, circles) on the Müller's organ of *Locusta migratoria*. **a** imago, **b** 5th instar larvae (for better clarity $\Delta\varphi$(K2 M4) is not shown, $f_0 = 3.5$ kHz)

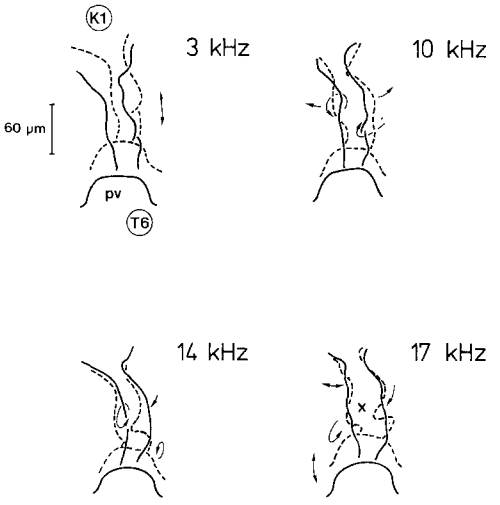


**Fig. 7.** Motions of the fusiform body and the pyriform vesicle (*pv*) of *Locusta migratoria* at different stimulus frequencies. The contours are drawn at minimum (solid line) and maximum displacement (broken line) of the pv. The estimated 'center of gravity' of fub is indicated by the cross at 17 kHz

More quantitatively, Fig. 6a shows the phase differences $\Delta\varphi$ of M1 relative to T2 and of M4 relative to K2 in adults of *Locusta*. In both cases the oscillatory characteristics can be described with very good accuracy by the estimated functions of a linear harmonic oscillator, plotted as solid lines. The resonant frequencies ($\Delta\varphi = 90°$) are found to be 1.7 kHz for M1 and 4.3 kHz for M4. In *Schistocerca gregaria* in contrast, we find resonant frequencies of 2.6 kHz and 5.1 kHz, respectively (not shown). However, in larvae (Fig. 6b) the conditions are rather different. In this case the resonant frequency of M1 (3.1 kHz) is identical to that of M4 and the course of $\Delta\varphi$(K2 M4) is nearly the same as $\Delta\varphi$(T2 M1). For better clarity the values of $\Delta\varphi$(K2 M4) are not shown. In larvae (independent from age) the area of points M and K of the Müller's organ oscillates as a uniform system, and we did not find any spatial distribution of resonances. Therefore the capability of frequency discrimination is very much reduced, although the same mechanical transformation of motion as we found in adults is working even in larvae.

The relative displacements A(T2 M1) and A(K2 M4) which correspond to the tension on the dendrites can be described by an unidimensional coupling of the oscillators T2 and M1 or K2 and M4, respectively. For *Locusta* we find a broadened resonant curve because of the different resonant frequencies of T2 (3.3 kHz) and M1 (1.7 kHz) with an absolute maximum at 3.3 kHz. In *Schistocerca* on the other hand, we find a relatively sharp tuning curve with a clearly higher maximum at 2.9 kHz ($f_0$(T2) = 2.9 kHz, $f_0$(M1) = 2.6 kHz). For $f_0$(K2 M4) we find 4.0 kHz in *Locusta* and 4.7 kHz in *Schistocerca*. Not all oscillations of other points on the Müller's organ can be described in terms of linear harmonic oscillators and normally the relations appear to be more complex.

### *3. Pyriform vesicle and fusiform body*

The fusiform body is formed by the dendrites of the d-cells (cf. Fig. 1). It represents an independent unit separated from the main mass of the Müller's organ and extends to the pyriform vesicle, a small cuticular structure at the thin part of the membrane to which the dendrites are attached. In adults of *Locusta migratoria* we find the maximum oscillation of the entire tympanum at the pyriform vesicle for frequencies above 7 kHz. In this frequency range the nodal lines concentrate more and more concentrically around the pyriform vesicle (Fig. 3a).

Figure 7 shows some details of the very complex stress patterns of the fusiform body depending on the stimulus frequency. We see the contours of fusiform body and pyriform vesicle at maximum and minimum displacement of the pyriform vesicle, respectively. The course of motion of certain parts of the fusiform body is indicated by arrows. The sound-pressure levels for the different frequencies are not identical. At 3 kHz the whole system follows the motion of the pyriform vesicle and K1

which oscillate with the same phase. Therefore, the relative motion is nearly zero. At 10 kHz K1 is stationary and the fusiform body is strained and jolted. Certain parts of the fusiform body vibrate with different phases and show relative motions (indicated by arrows). This effect becomes magnified at 14 kHz and forces of flexion and torsion affect the fusiform body. At 17 kHz about $^2/_3$ of the fusiform body are resting. Only that part close to the pyriform vesicle is jolted and very complex patterns of motion appear. If we define a 'center of gravity' of the fusiform body (marked with a cross at 17 kHz, Fig. 7) we are able to measure the phases relative to the motion of the pyriform vesicle which transmits the driving force onto the fusiform body. This leads to a resonant frequency of the fusiform body of $f_0 = 13$ kHz ($\Delta\varphi = 90°$).

The amplitude spectra of T6 and K1 are shown quantitatively in Fig. 8a. For T6 we find two maxima. The second maximum at 12 kHz is about 15 dB below the first at 3.3 kHz. This second maximum does not occur for any other point of the tympanum and not even for K1. The solid line in Fig. 8 represents the simple difference A(T6 K1) between both amplitude spectra. This corresponds (as a primary approach) to the relative displacement of the dendrites of the d-cells. Almost only the second maximum in the amplitude spectrum A(T6) of the pyriform vesicle determines the course of A(T6 K1). This leads to a relatively flat spectrum with a single maximum at 14 kHz. Phase effects in this case can be neglected because for low frequencies ($f < 5$ kHz) T6 and K1 are almost in phase and for higher frequencies the amplitude of K1 almost vanishes. For adults of *Schistocerca gregaria* we obtained very similar results.

Although the fusiform body is completely developed by the 5th instar (Michel and Petersen 1982) it is not able to act as an independent oscillator with its own resonant patterns as in adults. The site of attachment of the fusiform body at the Müller's organ is essentially the same as in adults. The columnar fusiform body in adults has no additional contact to the Müller's organ or to the tympanum (see Fig. 1 or Fig. 5). In larvae, however, the entire length lies closely against the elevated process and the membrane and attaches near T7 (between elevated process and pyriform vesicle, Fig. 3b). This contact is enhanced by the developing epidermal layer (cf. Sect. 4) which presses the fusiform body onto the Müller's organ and the tympanum, resulting in an increase in damping of the vibrations of the fusiform body. For that reason it is difficult to describe the relative displacements concerning the dendrites of the d-

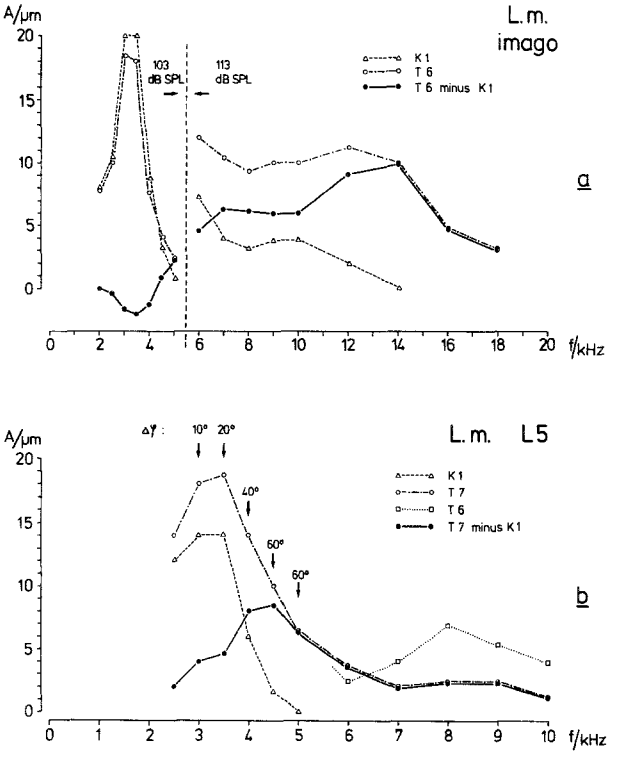


**Fig. 8a, b.** Amplitude spectra of certain points of the tympanum and the Müller's organ of *Locusta migratoria*. **a** imago, the solid line represents the difference A(T6)–A(K1), $L = 103$ dB SPL for $f \leq 5$ kHz, $L = 113$ dB SPL for $f > 5$ kHz. **b** 5th instar larvae, the solid line represents the difference A(T7)–A(K1). $L = 113$ dB SPL, phase differences $\Delta\varphi$ of the point K2 relative to T7 are given at different frequencies

cells. As a primary approach we can refer to the amplitude spectra of K1 and T7 plotted in Fig. 8b. Beyond its maximum at 3.5 kHz the amplitude of K1 rapidly decreases and nearly vanishes at 5 kHz. Altogether, the amplitudes of T7 are distinctly larger. The decrease is much slower and amplitudes could be measured even with higher frequencies. The solid line corresponds to the simple difference of the absolute values, A(T7 K1) := A(T7) – A(K1) and forms a maximum around 4.5 kHz. For $f = 3.5$ kHz we find phase differences of $\Delta\varphi < 20°$ and A(T7 K1) is almost equal to the absolute value of the complex difference. For $f > 5$ kHz A(T7 K1) is determined only by A(T7). In the frequency range between 3.5 kHz $\leq f \leq$ 5 kHz we find phase differences from 40°–60°, which means that the actual relative displacement (proportional to the absolute value of the complex difference) should be even stronger than determined by A(T7 K1). Additionally, Fig. 8b shows the amplitude spectrum of T6 in the frequency range from 6–10 kHz. Below 6 kHz the course of A(T6) is almost identical to that of A(T7), but at 8 kHz it exhibits a secondary maximum.

Phases and amplitudes in this area exhibit relatively large interindividual fluctuations, mainly caused by the developing epidermal layer. We found maximum displacements of A(T7 K1) even at 8 kHz as well as infinitely small relative motions.

### *4. Age dependent mechanical properties in larvae*

Whereas we did not find any significant age dependent variations in the oscillatory properties of the tympanal organ of adults, such variations are evident in larvae. Structural developments take place even in the period between moulting and influence the mechanics and the function of the tympanal organ. For this reason we measured the most important features in 5th instar larvae of *Locusta* and *Schistocerca* depending on their age – in days after the 4th moult. The final moult follows after $7.2 \pm 1$ days in *Locusta* whereas only after $10 \pm 2$ days in *Schistocerca*. The growth rate is somewhat higher in *Schistocerca*.

Although the average duration of the 5th instar of *Schistocerca* is around 10 days it is not possible to observe the tympanal structures for more than 6 or 7 days. A new epidermis develops (predeposition of the tympanum of the adult animal) which slowly covers the entire inside of the tympanal organ. At the beginning this cellular layer is very thin and transparent, but with increasing age it becomes more and more thick and heavy and finally presses upon the Müller's organ and the tympanum so that relative motions are suppressed. In larvae of *Locusta* this phenomenon is much less extensive and measurements could be performed throughout the whole duration of the 5th instar.

Figure 9a shows the values of the resonant frequency $f_o$(T2) and the maximum amplitudes $A_{max}$ at T2 depending on the time after the 4th moult. With increasing age $f_o$(T2) decreases by about 10% and $A_{max}$ (T2) by about 35%. The same effect was found in *Locusta*. The reduction in amplitude results mainly from the increase in damping induced by the growing epidermal layer, because the resonant frequency, which is virtually independent from damping, is reduced only a little, according to the increase of mass of the tympanum.

In *Schistocerca*, the resonant frequencies of the Müller's organ are strongly age-dependent. During the whole observable period after the 4th moult the Müller's organ vibrates as a homogeneous unit without internal relative displacements. Figure 9b shows the course of the resonant frequency $f_o$(M1) which is approximately identical for all other points of the Müller's organ. From an initial 4.5 kHz $f_o$ decreases to 3 kHz at the 3rd and 4th

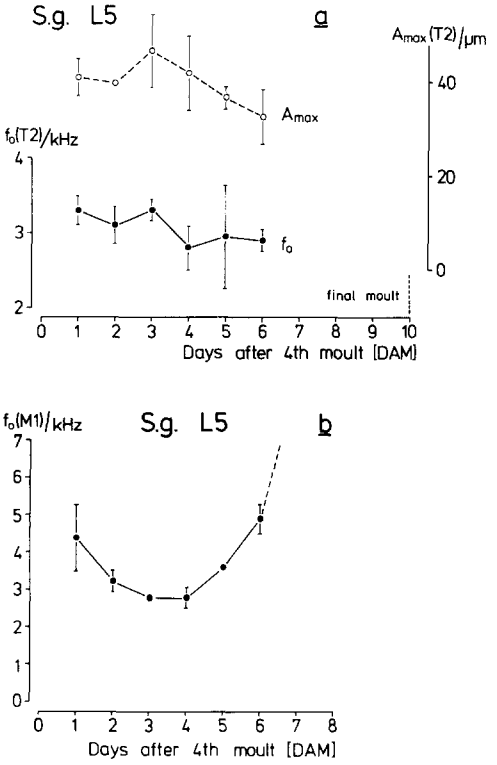


**Fig. 9a, b.** Age dependent properties in 5th instar larvae of *Schistocerca gregaria* in relation to the age in days after the 4th moult. **a** Resonant frequency $f_o$(T2) (left ordinate) and maximum amplitude $A_{max}$ (T2) (right ordinate), **b** $f_o$(M1)

day and afterwards increases very rapidly. The increase of mass of the Müller's organ results in a reduction of $f_o$. The growth diminishes somewhat after the 4th day, but now the influence of the relatively thick epidermal layer becomes more and more apparent. Because it covers the entire Müller's organ and the tympanum as well, the elastic connection between the ventral part of the Müller's organ and the folded body (dendrites of the c-cells) is 'short-circuited'. This results in an increase of the resonant frequency and a decrease of the relative displacements between Müller's organ and the tympanum ($\Delta\varphi \approx 0°$). After carefully removing this epidermal layer we find almost the same mechanical characteristics as in young larvae. With 5th instar larvae of *Locusta* we could not ascertain any significant age dependence of the resonant frequencies of the Müller's organ.

## Discussion

### *Stroboscopy and linearity of the tympanal organ*

The stroboscopic method used in the present paper provided a sensitivity of about 1 µm which is about 10 to 100 times below that of some other tech-

niques, but we obtained a spatial resolution of about 1 μm. Because of the relatively poor sensitivity it was necessary to use high sound pressure levels to obtain measurable effects. However, this implies no essential restriction in using this method because the tympanal system is proved to be linear and any displacement caused by smaller sound-pressure levels can be extrapolated. The nonlinear filter element assumed by Sippel and Breckow (1984) to describe the non-monotonic response intensity characteristics of the tympanal receptor cells can't be reduced to any mechanical process of signal transmission within the considered range of sound intensity.

*Closed-field conditions*

The exact measurements of the actual parameters of the sound field at the site of the tympanum appear to be somewhat problematical. Due to the damping we obtained a linear relationship between phase and frequency of the signal relative to the sound source and an almost flat power spectrum. Defined points on the tympanum or the Müller's organ were normally used as a reference for the measurements instead of the parameters of the sound field. Therefore, distortions of the sound field by some reflections that can't be excluded completely should be not critical in this context.

*The isolated tympanal organ*

All investigations described in the present paper have been performed on isolated tympanal organs. Therefore, all acoustic effects due to the animal's body are not considered. Resulting restrictions have been investigated in detail by Michelsen (1971c). These effects essentially influence the directional sensitivity of the tympanal organ and the effective sound pressure. The statements on the mechanisms of mechanical transmission of signals are not affected.

*Basic mechanisms of mechanical pitch discrimination*

The pitch discrimination of the tympanal organ of locusts is based upon the interactions between the tympanal membrane (performing spatial and frequency dependent modes of vibration) and the Müller's organ (exhibiting its own independent resonant properties). The importance of spatial and frequency dependent resonances in the tympanum is already emphasized by Michelsen (1971b). But his description of the way the receptor cells perceive the oscillatory patterns is yet incomplete, e.g. nodes have been described as sites of diminishing responses of the corresponding receptor cells. But as demonstrated (Fig. 5) the very nodes and the corresponding cuticular structures are responsable for transforming the membrane motions into components parallel to the plane of the tympanum and thus lead to spatial dependent resonances within the Müller's organ as an *active observer* of the membrane.

The basic mechanism of frequency discrimination can be described in a very simplified way by spatially separated complex oscillators with differing resonant frequencies. The importance of the phase relations between points of the tympanum and Müller's organ as well as the efficiency of cuticular lever arms is pointed out (Fig. 5). The main directions of maximum relative displacements (at distinct $f_0$, the 'characteristic frequencies') correspond to the courses of the dendrites of the different groups of receptor cells.

These mechanisms of pitch discrimination are basically different to those of Ensifera whose tympana vibrate homogeneously in the fundamental mode without spatially dependent phase delays. The pitch discrimination in this case cannot be reduced to spatially dependent resonances of the tympanum but to associated acoustic elements (trachea, air sacs etc.). The tympanum itself only acts as a transformer of acoustic impedance for the sound perceiving neuronal structures which are not connected directly to the membrane (Lewis 1974; Fletcher and Thwaites 1979).

*Comparison with neurophysiological results*

Single cell recordings from tympanal receptor cells in locusts have been performed e.g. by Michelsen (1971a), Römer (1976) and Petersen et al. (1982). For comparison with the presented results we mainly refer to Petersen et al. (1982) who investigated larvae and adults of *Locusta migratoria*.

The most sensitive receptor cells are those of type 2 with a characteristic frequency at about 3 kHz and a threshold intensity of about 30 dB SPL. At the 5th instar (all receptor cells are already present) the type 2 cells are about 8 dB less sensitive and with frequencies below 3 kHz the decrease in sensitivity becomes ca. 12 dB. The same is true for the amplitudes of oscillation we measured in the attachment area of the c-cell dendrites. Here, by far, we find the largest relative displacement A(T2 M1) tuned to 3 kHz which diminishes by about 8 dB in larvae. At lower frequencies the relative amplitudes are even more reduced. A compari-

son of the threshold values of the type 2 receptor group of adults and 5th instar larvae (from Petersen et al. 1982) and the corresponding amplitudes of displacement A(T2 M1) reveals a very good conformity. Therefore, it is evident that the c-cells and the type 2 receptor cells are identical.

Type 3 receptor cells show characteristic frequencies at 6 kHz and threshold values about 5 dB above that of type 2 (Römer 1976) and exhibit a relatively broad response spectrum in the low frequency range. The highest frequencies of maximum displacements within the low frequency range are found for the area of attachment of the b-cells (K2 M4, Fig. 5). These resonant frequencies with values of 4–5 kHz are below that of the threshold curves but the amplitude spectrum is also broad band and yet the maximum is about 5 dB below the c-cells. With 5th instar larvae we find a shift of the characteristic frequency to 3 kHz (Petersen 1982). The same shift becomes apparent for the displacements in the area K2 M4. This suggests a correspondence of the b-cells and the type 3 receptor cells.

Type 1 is the third group of low frequency receptors. The characteristic frequency is about 3.5 kHz and the threshold is about 15–20 dB higher than that of type 2 receptors. This corresponds to a 6–10 times reduction of displacement. Those small amplitudes cannot be measured quantitatively using the stroboscopic method. However, the following consideration seems to be plausible: the separately differentiated area of attachment of the a-cells reaches from the dorsal part of the Müller's organ to the basal part of the elevated process. This area shows maximum displacements at frequencies of 3–4 kHz. Proper resonances do not occur and we always find phase differences of $\Delta\varphi < 90°$. This means a reduction of the relative amplitudes in the area of the dendrites of the a-cells and one would conclude a rather poor sensitivity (to sound stimuli). The a-cells therefore appear to correlate with the type 1 receptors.

In the high frequency range (10–20 kHz) the correspondence between morphologically and neurophysiologically identified types of receptors is very clear. Only the motion of pyriform vesicle and fusiform body can determine the response characteristics of the d-cells, because the remaining parts of the Müller's organ and of the tympanum are stationary in this frequency range. The type 4 receptor cells respond in a broad frequency band around their characteristic frequency of 12–16 kHz. This agrees with the resonant frequency of the fusiform body at about 13 kHz. The strong shift of the characteristic frequency in 5th instar larvae towards 4 kHz (Petersen et al. 1982) is striking. Also mechanically this frequency shift can be ascertained in the pattern of oscillation of the tympanal organ (Fig. 8). This is due to the changes in the oscillatory properties of the involved structures (mainly the fusiform body) as well as to a shift of the site of attachment of the d-cells. With high reliability, the d-cells are identical to the type 4 group of receptor cells.

Comparable neurophysiological data of adults and larvae of *Schistocerca gregaria* are not yet available. Based upon the results presented in this paper we are able to predict some response characteristics of the receptor cells of *Schistocerca*:
– the type 2 cells should be somewhat more sensitive and tuned more sharply to the resonant frequency of T2 (2.9 kHz) than in *Locusta,*
– in comparison with *Locusta,* the characteristic frequency of type 3 cells should be shifted to higher frequencies and the response characteristics of type 2 and type 3 should be better differentiated and more discernable. The performance of pitch discrimination by the low frequency receptor system (types 1, 2 and 3) should be better developed,
– concerning the response characteristics of type 4 receptors we don't expect any differences,
– for type 1 cells predictions cannot be made,
– the response characteristics of all receptor groups in 5th instar larvae should depend much more on the age than in *Locusta.*

The statement that adequate stimuli of the acoustic receptor cells are evoked by tensions in their dendrites seems to be justified and leads to an agreement with neurophysiological results. Michelsen (1971 b) already assumed that the receptor cells themselves are nonspecific and their ability for pitch discrimination is based only on their site of attachment at the membrane. This is supported by the fact that morphological changes during the larval development are always correlated with changes in the physical properties of the oscillating structures. Therefore changes in the response characteristics of the receptor cells can be reduced to changes in the mechanics. The 'place principle', that means the spatial separation of receptors with different characteristic frequencies and thresholds originates in the arrangement of the receptor cells in the Müller's organ. It is continued by the course of the axons in the tympanal nerve (Rehbein 1976) and in their areas of projection in the acoustic neuropils of the thoracic ganglia where a tonotopical organisation was found (Breckow et al. 1982; Keuper 1984). The spatial and time dependent separation of frequency components is also present in the further transmission of information in the

ventral cord (Kalmring 1975) and seems to be a basic principle of processing of acoustic signals within the CNS of insects.

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