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THE INFLUENCE OF TRACHEAL PRESSURE CHANGES ON THE RESPONSES OF THE TYMPANAL MEMBRANE AND AUDITORY RECEPTORS IN THE LOCUST *LOCUSTA MIGRATORIA* L.

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Summary

In resting tethered locusts, the effect of slow changes in tracheal air pressure on peripheral auditory information processing was analysed. The tympanal membrane vibrations, the pressure inside the tracheal system and the summed activity of the auditory receptors were measured simultaneously.

With the membrane in the resting position, laser vibrometry and Fast Fourier Transformation analysis of sound-induced membrane vibrations demonstrated characteristic power spectra at the attachment sites of the high-frequency and low-frequency receptors. The spectra were different above 9 kHz, but very similar in the range 2–9 kHz.

During ventilation, tracheal pressure changed between −500 and 1500 Pa. This caused tympanal membrane peak-to-peak displacements in the range 70–90 μm outwards and 20–30 μm inwards, as measured by means of laser interferometry. For a quantitative analysis, sinusoidal tympanal membrane displacements with amplitudes such as those during natural ventilation could be induced by applying pressure to the tracheal system. There was a sigmoid relationship between the tracheal pressure and the corresponding membrane displacement.

Outward displacements of the tympanal membrane at the attachment site of the elevated process (a-cells) attenuated sound-induced membrane vibrations in the ranges 2–10 kHz and 14–22 kHz and increased them in the ranges 10–14 kHz and 22–25 kHz. At the pyriform vesicle (d-cells), the vibration sensitivity was reduced in the frequency range 2–14 kHz. Sensitivity was enhanced in the range 14–25 kHz.

As a consequence, the detection of acoustic signals was also influenced at the auditory receptor level. Tympanal membrane displacements during acoustic stimulation with 4 kHz sound pulses decreased the summed receptor response by approximately 15 dB. At 16 kHz, an increase of the response equivalent to 7 dB occurred. The effect on the response to white noise was intermediate.

Key words: ventilation, tympanal membrane, laser vibrometry, laser interferometry, auditory information processing, *Locusta migratoria*.

Introduction

The tympanal organs of locusts are situated in the first abdominal segment. The organ consists of a tympanal membrane suspended in a cuticular frame and about 80 highly sensitive scolopidial mechanoreceptors attached to the inside of the tympanum (Schwabe, 1906; Gray, 1960). The receptors respond to vibrations of the membrane and are classified according to their best frequency into one high-frequency group (d-cells) and three low-frequency groups (a-cells, b-cells, c-cells) (Michelsen, 1971*a*,*b*; Römer, 1976). Their axons project within the tympanal nerve (nerve 6) into the metathoracic ganglion complex.

In the body cavity behind the tympanal organ, there are a series of tracheal air sacs positioned between the ears. During ventilation, the air pressure in the tracheal system and the volume of the air sacs change. As a consequence, the tympanal membrane is displaced inwards and outwards in the rhythm of respiration (Schwabe, 1906; Michelsen, 1971*c*; Michelsen *et al.* 1990; Meyer and Elsner, 1995). The properties of the auditory organ and tympanal membrane of *Locusta migratoria* and *Schistocerca gregaria* have been described in experiments with isolated ears (Michelsen, 1971*a*,*b*; Stephen and Bennet-Clark, 1982; Breckow and Sippel, 1985). As a result of their biophysical and mechanical measurements, Michelsen (1971*c*) and Stephen and Bennet-Clark (1982) predicted that ventilatory pressure changes within the tracheal system should influence the transmission properties of the auditory organ. Ventilation has since been found to cause tympanal membrane displacements which modulate auditory information processing. The displacements influence the acoustically induced membrane oscillations, activate tympanal receptors and modulate their auditory responses (Hedwig, 1988; Michelsen *et al.* 1990; Meyer *et al.* 1992; Meyer and Elsner, 1995).

Locusta migratoria exhibits various types of respiration (Hustert, 1974, 1975). The ventilatory activity is irregular: it is often interrupted for minutes and the amplitude is also very variable. This prevents a quantitative evaluation of the impact of ventilation on the tympanal membrane and receptor activity. A device was therefore developed for precise pressure modulation of the tracheal system. This allowed a quantitative analysis of tympanal membrane displacements and their effect on tympanal membrane and auditory receptor responses in minimally dissected locusts.

Materials and methods

Animals

Adult male *Locusta migratoria* L. were obtained from a colony kept at the Zoological Institute of the University of Göttingen, Germany. After a number of preliminary experiments, the final data were obtained from 16 animals.

Preparation

The animals were fixed upside down on a platform covered with modelling clay (Fig. 1A). The hind leg, the middle leg and the forewing ipsilateral to the measured ear were removed. All other legs were fixed with wax. To provide access for the laser beam, the tympanal lid, which partly covers the tympanal membrane, was bent outwards and fixed to the thorax with a small drop of beeswax. To create stable conditions for the laser measurements, movements of the body wall at the side of the measured ear were prevented by attaching it to two insect pins stuck into the modelling clay. They were glued to the pleurae surrounding the tympanal membrane and served as stabilizing posts.

Laser measurements

A combined laser vibrometer and laser interferometer (Polytec OFV 2100 with sensor head OFV 300) was used to make simultaneous measurements of sound-induced high-frequency membrane oscillations and low-frequency membrane displacements due to pressure changes in the tracheal system (Fig. 1A). It allowed very sensitive measurements without any mechanical contact with the investigated surface. The vibrometer took advantage of the Doppler shift between the measuring beam and the light reflected from the illuminated object. In the frequency range from 0.1 Hz to 1 MHz, the system measured the velocity of object vibrations between 10^{-6} and 10 m s^{-1}. This corresponds to oscillation amplitudes from 1 nm up to 20 cm. The root mean square (RMS) value of the vibrometer signal was obtained on-line with an integrated circuit (Analog Devices type AD 637 JD). During acoustic stimulation with sinusoidal pulses, the vibrometer signal was bandpass-filtered around the selected frequencies (Krohn-Hite 3550).

The laser interferometer analysed the interference pattern of the emitted and reflected laser light and gave the amplitude of object displacement with a resolution of 8 nm in the frequency range 0.001 Hz to 10 kHz. Thus, the laser system allowed us to observe simultaneously the tympanal membrane displacements caused by ventilation and the acoustically induced membrane oscillations. To obtain a good signal-to-noise ratio for the laser measurements, the amount of laser light reflected from the tympanal membrane had to be enhanced. For this purpose, a

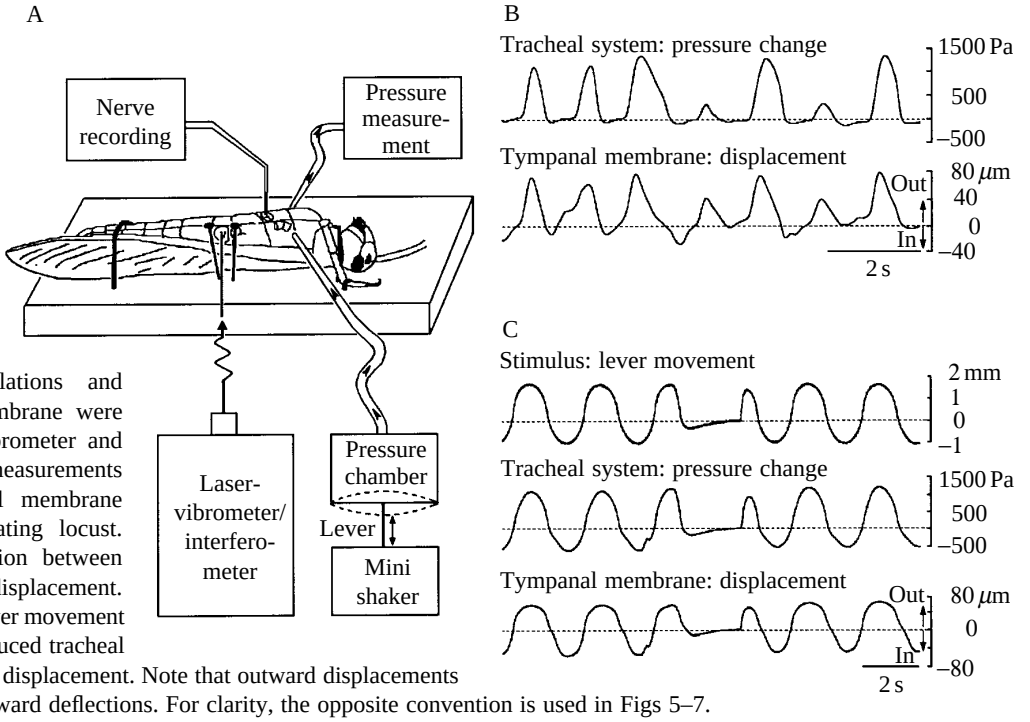

Fig. 1. (A) Preparation used for the experiments. A manometer was used to measure pressure at the contralateral mesothoracic spiracle. The activity of the auditory nerve was recorded using a hook electrode. The hole in the cuticle necessary for the recording was tightly sealed with wax. A mini-shaker connected to a pressure chamber was used to apply pressure to the ipsilateral mesothoracic spiracle. The oscillations and displacements of the tympanal membrane were recorded with a combined laser vibrometer and interferometer. (B) Simultaneous measurements of tracheal pressure and tympanal membrane displacement in a normally ventilating locust. The signals show a close correlation between the pressure and the membrane displacement. (C) Simultaneous recordings of the lever movement driving the pressure chamber, the induced tracheal pressure and the tympanal membrane displacement. Note that outward displacements of the membrane are presented as upward deflections. For clarity, the opposite convention is used in Figs 5–7.

small glass sphere (diameter 70 μm, mass 0.2 μg) was positioned on the outer surface of the tympanal membrane at the point of measurement. Experiments with and without the sphere demonstrated that the influence of the load on the oscillations of the tympanal membrane was below the resolution of the measuring system (Völker, 1991). All experiments were carried out in an anechoic room with a background noise level of 25 dB SPL at the investigated frequencies.

Neurophysiological recordings

A small opening was cut into the metathoracic sternites above the tympanal nerve (nerve 6). Special care was taken not to damage the main tracheae and air sacs. The indifferent electrode was in contact with the haemolymph. The tympanal nerve was placed on a hook electrode (125 μm diameter silver wire) and was insulated with Vaseline. This recorded the activity of all axons within the nerve. Of course, under these conditions, the activity of all receptors interferes in the extracellular recording. After a stable nerve recording had been obtained, the opening in the cuticle was completely covered and tightly sealed with wax. Thus, the pressure in the animal's body was not influenced by the preparation.

Tracheal pressure recordings

Changes of the air pressure in the tracheal system were recorded with a digital manometer with an upper limiting frequency of 200 Hz (Furness Controls Limited FC016). For this purpose, the valves of the mesothoracic spiracle contralateral to the laser recording were immobilized with wax in their open position. A glass tube (inner diameter 2.5 mm) was then put over the spiracle and the attachment site between the tube and the animal tightly sealed with wax. The tube was connected to the manometer and the variation of tracheal pressure relative to the momentary ambient air pressure could be recorded (Fig. 1A).

As the experiments demonstrated, ventilatory activity caused both pressure changes and movement of the tympanal membrane. These membrane displacements are caused by changes in the tracheal pressure and were measured with the laser interferometer.

Experimental modulation of the tracheal pressure

It was the aim of the experiments to analyse quantitatively the influence of tracheal pressure on tympanal membrane displacements, acoustically induced membrane oscillations and the activity of the tympanal nerve. To induce repetitive systematic tympanal membrane displacements, the tracheal air pressure was experimentally modulated in a defined way. For this purpose, a second glass tube was put over the opened mesothoracic spiracle ipsilateral to the measured ear. The tube was connected to a pressure chamber (Fig. 1A). The elastic membrane of the chamber was moved sinusoidally by a mini-shaker (Brüel & Kjaer type 4810) driven by a function generator (Philips PM 5134). The movements of the chamber membrane caused changes in air pressure which were transmitted *via* the tube into the tracheal system. With this system, tracheal pressure changes greater than the physiological range could be induced. However, pressure was set to amplitudes corresponding to normal ventilation. In these experiments, only the mesothoracic spiracles were used to apply or to measure the pressure. The ten others pairs of spiracles were not affected by the preparation and worked in their natural way.

Additionally, control experiments with isolated ears were performed. For this purpose, a glass tube (inner diameter 3.5 mm) was put over the tympanal membrane from the outside. The connection between tube and animal was again sealed with wax. Thereafter, the animal was decapitated and the whole body bisected. The gut, the muscles and surrounding tissue were removed. Finally, the air sac covering the tympanal membrane and Müller's organ was opened. Pressure was applied to the outside of the tympanal membrane and laser measurements of the membrane displacement were made from inside the organ.

Acoustic stimulation

One sound source used was a programmable digital-to-analogue converter (DAC) which was an integral part of an Fast Fourier Transform (FFT) analyzer (Hewlett-Packard 3567A). With this system, an analysis of the power spectra of membrane vibrations was performed with frequency-modulated sound pulses of 16 ms duration (75 dB SPL). These sound pulses included frequencies from 1 to 25 kHz with approximately the same intensity.

The other stimulus generator, used in combination with tympanal nerve recordings, was designed at the First Department of Zoology in Göttingen. White noise (frequency components 1–25 kHz) and pure tones at 4 or 16 kHz (20 ms duration, 1 ms rise time, 10 Hz repetition rate) could be produced. The amplitude of all sound pulses could be adjusted between 30 and 100 dB SPL. The acoustic stimuli were transmitted by a broad-band speaker (Dynaudio D-21AF), with a flat frequency range from 2 to 40 kHz, which was checked by the FFT analyzer. The speaker was positioned 33 cm away from the investigated tympanal membrane and perpendicular to its surface.

Sound recordings

The sound pressure level of the acoustic stimuli was determined with a Brüel & Kjaer microphone (type 4133) connected to a Brüel & Kjaer amplifier (type 2608). The microphone was positioned 5 cm above the animal.

Data evaluation

Power spectra of tympanal membrane vibrations within the range 2 to 25 kHz were obtained on-line with an FFT analyzer (Hewlett-Packard 3567A). The FFT analyzer sampled the laser signal at 130 kHz. All spectra presented are averages of 50 successively measured spectra and were obtained using the system's software. The power spectra obtained during membrane displacements were divided by the power spectra

for the resting position to calculate the relative alterations of membrane oscillations.

The stimulus marker, the membrane displacement, the RMS of the membrane velocity and the tympanal nerve activity were stored on magnetic tape (Racal store 7DS). A high-speed A/D board (Data Translation DT2821-F-8DI) was used to digitize the analogue recordings off-line and to transfer them to the hard disk of an IBM-compatible PC. Data were sampled at 10 kHz per channel using Turbolab software, (Stemmer, Puchheim). This sampling rate was sufficient for these low-frequency signals. Evaluation of the binary data files was carried out with the program NEUROLAB (Hedwig and Knepper, 1992). For every acoustic stimulus, the corresponding displacement of the tympanal membrane, the velocity of membrane vibrations (RMS) and the tympanal nerve activity could be determined. The peak-to-peak amplitude of the summed nerve recording is not constant under identical stimulus conditions. Therefore, as a measure of the tympanal nerve response, the sum of the absolute voltage changes of the amplified summed nerve recording ($\Sigma|dV|/dt$) was calculated in a time window of 40 ms after the stimulus. This variable showed only a small variation. The software package Quattro Pro (Borland) was used to calculate dot plots for the resulting data points. These were displayed with a Gaussian weighted gliding average function.

Results

Normal ventilation

Ventilation in locusts consists of an active expiration and an almost passive inspiration (Hustert, 1974, 1975). Gas exchange occurs *via* the spiracles. During expiration, the abdomen is constricted, the pressure in the tracheal system rises and air is pressed outwards through the spiracles. During inspiration, the abdomen expands almost passively to its normal size, pressure decreases and fresh air enters the tracheae *via* the spiracles. Thus, expiration is associated with high air pressure in the tracheal system and inspiration with values below atmospheric pressure.

In tethered locusts, the tracheal pressure and tympanal membrane displacements were measured simultaneously during ventilation. The tympanal membrane moves in and out in the rhythm of respiration; each pressure change in the tracheal system producing a corresponding membrane movement (Fig. 1B).

During expiration, the tracheal pressure reached approximately 1500 Pa. As a consequence, the tympanal membrane was simultaneously displaced outwards by 70–90 μm. During deep breathing cycles, the pressure reached 2500 Pa and the displacement up to 130 μm. Inspiration caused only slight negative pressures of -200 Pa in the tracheal system. Therefore, inward displacements of the tympanal membrane were only in the range 20–30 μm and only occasionally reached 50 μm. The signal of the laser interferometer revealed small membrane displacements not visible in the pressure signal. This may be due to the limited upper frequency of the manometer and the low-pass filter characteristics of the connected tube. The maximum amplitude of membrane displacement varied only slightly in different animals.

Experimental modulation of tracheal pressure

The pressure chamber was used to induce repetitive tympanal membrane movements with adjustable amplitudes (Fig. 1A,C). Three cycles of a sine wave (0.5 Hz) separated by an interval of 2 s were constantly repeated for several minutes. To test the effectiveness of the system, the movement of the mini-shaker, the tracheal pressure and the tympanal membrane movement were measured simultaneously. The laser interferometer was positioned to monitor the attachment site of the pyriform vesicle (d-receptors). The amplitude of the pressure system was adjusted to induce maximum tympanal membrane outward displacements of about 75 μm.

The output of the pressure system elicited pressure changes in the tracheal system and tympanal membrane displacements with almost identical waveforms (Fig. 1C). At the end of the intervals between the sinusoidal movements, no pressure was applied and the membrane returned to its resting position (dotted baseline in Fig. 1C). Changes in the tracheal pressure and membrane displacement also occurred as a result of additional spontaneous ventilatory activity. This, however, was rare and occurred only at the beginning of the experiments.

The amplitudes of displacement and pressure were simultaneously sampled at intervals of 100 ms. The membrane displacement was plotted as a function of pressure and gave a sigmoid relationship (Fig. 2A). At around zero pressure there was, at best, a small range of about ±10–20 μm within which the displacement was almost a linear function of the tracheal pressure. A pressure of approximately 130 Pa was needed to displace the membrane from 0 to 20 μm. At membrane displacements above 20 μm the curve flattened out. Thus, with increasing displacement, the force acting on the membrane had to be larger to move it an equivalent distance. Between 40 and 60 μm, a pressure of 340 Pa was needed to displace the membrane by 20 μm. That is, the compliance (the ratio of displacement to force) decreased rapidly with increasing displacement. As a consequence, it may be expected that even small displacements of the tympanal membrane would modulate its oscillations and influence auditory information processing. There was a small hysteresis for inward and outward movements (Fig. 2A), but we ignored this in our subsequent considerations.

Because the measuring device allowed no direct access to the pressure directly behind the tympanum, we compared the pressure–displacement relationship with control measurements on isolated ears (Fig. 2B). Pressure was applied to the outside of the membrane and the displacement was measured at the inner side of the isolated ear. These experiments gave a very similar sigmoid relationship, with identical amplitudes, between the membrane displacement and the pressure, but without hysteresis (Fig. 2B).

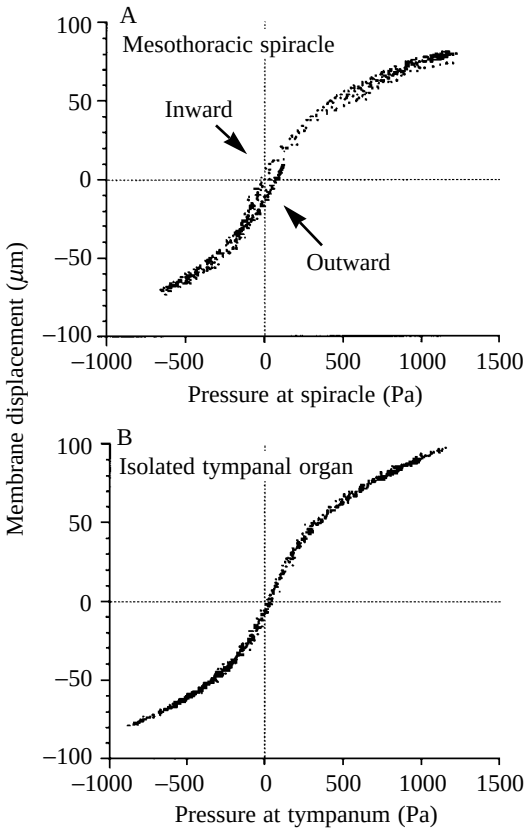

Fig. 2. Dot plots showing tympanal membrane displacement as a function of pressure. (A) The tracheal pressure was measured at the mesothoracic spiracle in the intact animal. (B) In the isolated ear of the same animal, the applied pressure was measured directly at the tympanum. A linear relationship between pressure and displacement is present at best only for ±10–20 μm. Note the small hysteresis for inward and outward displacements in A. Data for pressure and displacement were sampled at 100 ms intervals for a total of 617 measurements.

Oscillation properties of the tympanal membrane

The attachment sites of the different receptor groups can easily be identified by examining the outer surface of the tympanal membrane. During acoustic stimulation, the tympanal membrane shows resonances with different modes of vibration at the various receptor attachment sites. These vibrations and those of Müller's organ are thought to cause the specific frequency-sensitivity of the receptor groups (Michelsen, 1971*b*; Stephen and Bennet-Clark, 1982).

To analyse the vibrational sensitivity of the tympanal membrane at the different attachment sites of the receptors, the membrane was stimulated with frequency-modulated sound pulses (see Materials and methods) and the resulting membrane oscillations were FFT-analysed to obtain the spectral composition of the vibrations. The shapes of the spectra were very similar for all animals investigated; a representative example is given in Fig. 3. We found that at the elevated process (a-cell group) the membrane vibrated at maximum velocity between 3.5 and 10 kHz (Fig. 3B). The sensitivity was maximal at 8 kHz, with a second sensitivity peak at 5 kHz. At lower and higher frequencies, the amplitude decreased rapidly. At the pyriform vesicle (high-frequency receptor group, d-cells), the membrane vibrations were in the range from 3.5 kHz to at least 23 kHz (Fig. 3C). The maximum of the membrane sensitivity was in the range 13–18 kHz.

The power spectra indicated clear differences above 9 kHz in the vibrational sensitivity at the low- and high-frequency receptor attachment sites. Surprisingly, however, in the low-frequency range between 2 and 9 kHz, the tympanal membrane exhibited a very similar sensitivity at the two attachment sites. In this range, the velocity of acoustically induced membrane vibrations in some animals was larger at the attachment site of the high-frequency d-cells than at the attachment site of the a-cells: at 5 kHz, the relative oscillation velocity of the membrane at the latter site was −37 dB, corresponding to 1270 μm s^{-1}, whereas at the pyriform vesicle (d-cells) it was −33 dB, corresponding to 1790 μm s^{-1}. At higher frequencies, the difference between the membrane velocities at the two locations was much larger. At 16 kHz, for example, there was a difference of 26 dB between the two membrane locations: at the pyriform vesicle, the membrane oscillated at 4500 μm s^{-1}, whereas at the elevated process it vibrated at only 230 μm s^{-1} at the same frequency (Fig. 3B,C).

Influence of displacement on membrane vibrations

The influence of membrane displacement on the sound-induced membrane oscillations was analysed during experimentally modulated membrane movements. For this analysis, the spectrum of the membrane oscillations in the resting position was measured first as a reference for the membrane tuning at the attachment sites of the a- and d-cells. The power spectra of tympanal membrane vibrations during specific displacements were then analysed. These were divided by the reference value to obtain the relative change of vibrational sensitivity in the total spectrum. Correspondingly, in the resting position, the curve indicated no relative change in the spectrum over the whole frequency range (details in Michelsen *et al.* 1990).

When the tympanal membrane was displaced outwards by 75 μm, a frequency-dependent change in vibration sensitivity occurred at the attachment sites of low- and high-frequency receptors (Fig. 4). At the elevated process (a-cells) in the frequency ranges 2–10 kHz and 14–22 kHz the membrane vibrations were damped. The attenuation was greatest (−13 dB) between 3 and 6 kHz. Vibrations in the ranges 10–14 kHz and 22–25 kHz were enhanced by approximately 4 dB. At the pyriform vesicle (d-cells), the sensitivity was reduced in the frequency range 2–14 kHz. The maximum attenuation, −13 dB, occurred at 3–6 kHz. Vibrations in the range 14–25 kHz were enhanced. The maximum enhancement, at 17 kHz, was approximately 6 dB. When the membrane was displaced inwards, the change in sensitivity was similar but generally smaller. Apart from some individual variations, these response curves were obtained in all individuals studied and correspond to the results of Michelsen *et al.* (1990) and Völker (1991).

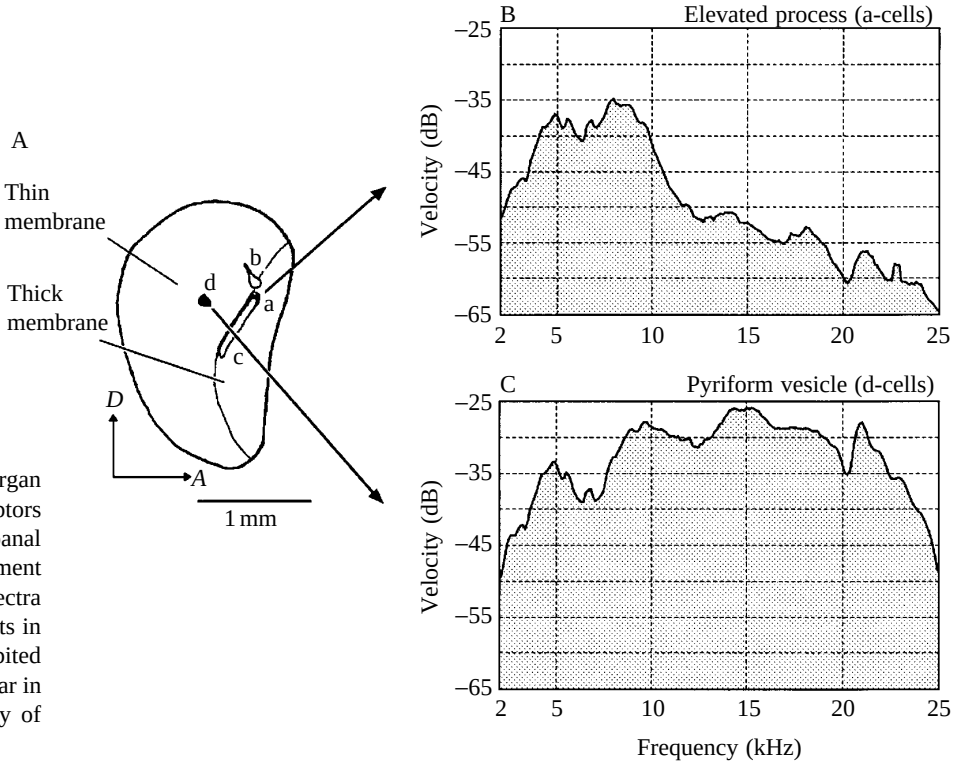

Fig. 3. (A) Outside view of the tympanal organ with the attachment sites of the different receptors indicated. (B) Power spectra of the tympanal membrane vibrations obtained at the attachment site of the a-cells and (C) the d-cells. The spectra represent averages of 50 single measurements in one locust. The two attachment sites exhibited different spectra above 9 kHz, but were similar in the range 2–9 kHz. Note: dB=20log[velocity of tympanal membrane)/90(mm s^{-1})].

Biophysical and neurophysiological recordings

To determine the degree to which modulations of tympanal membrane vibrations were reflected in the tympanal receptor activity, stimuli were chosen according to the most significant changes in the spectra and the sensitivity of the auditory receptors: 4 kHz, 16 kHz or white noise sound pulses (20 ms duration, repetition rate 10 Hz, 40–90 dB SPL). The tympanal membrane was displaced sinusoidally by altering the tracheal pressure, and the summed tympanal nerve activity was recorded. For the 4 kHz stimuli, the velocity of the tympanal membrane vibrations was determined at the attachment site of the a-cells; for 16 kHz and white noise pulses, velocity was measured at the attachment site of the d-cells.

For every acoustic stimulus, the tympanal membrane displacement, the corresponding velocity of the membrane vibration and the amplitude of the nerve activity were measured. Thereafter, data points were plotted as a function of the membrane displacement, and a gliding average function was fitted.

During stimulation with 4 kHz pulses, the velocity of membrane vibrations was reduced whenever the membrane was displaced (Fig. 5A,B). At the same time, the activity of the tympanal nerve distinctly decreased (Fig. 5A,C). The effect was more pronounced for outward movements, since these were of larger amplitude. With 4 kHz, 60 dB SPL stimulation

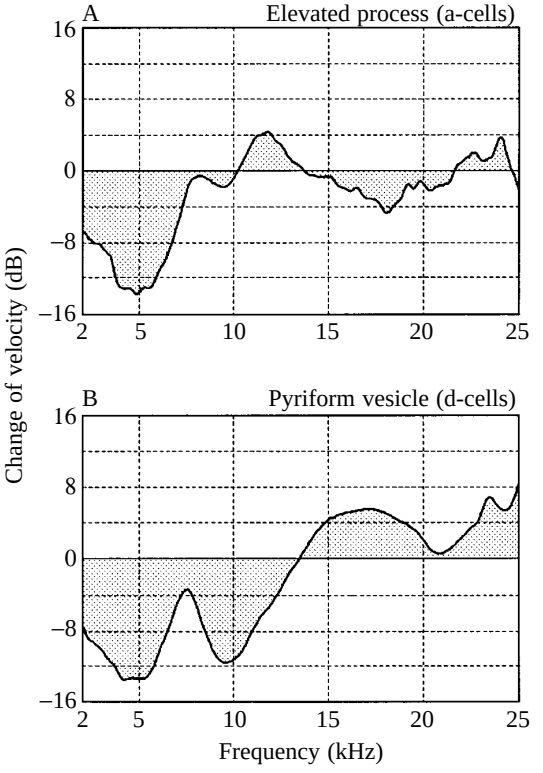

Fig. 4. Relative changes in the power spectra of tympanal membrane vibration obtained with a 75 μm membrane outward displacement. (A) At the elevated process (a-cells), membrane vibrations decreased in the ranges 2–10 kHz and 14–22 kHz. Vibrations increased in the ranges 10–14 kHz and 22–25 kHz. (B) At the pyriform vesicle (d-cells), membrane vibrations decreased between 2 and 14 kHz and increased above 14 kHz. The spectra represent averages of 50 single measurements in one locust.

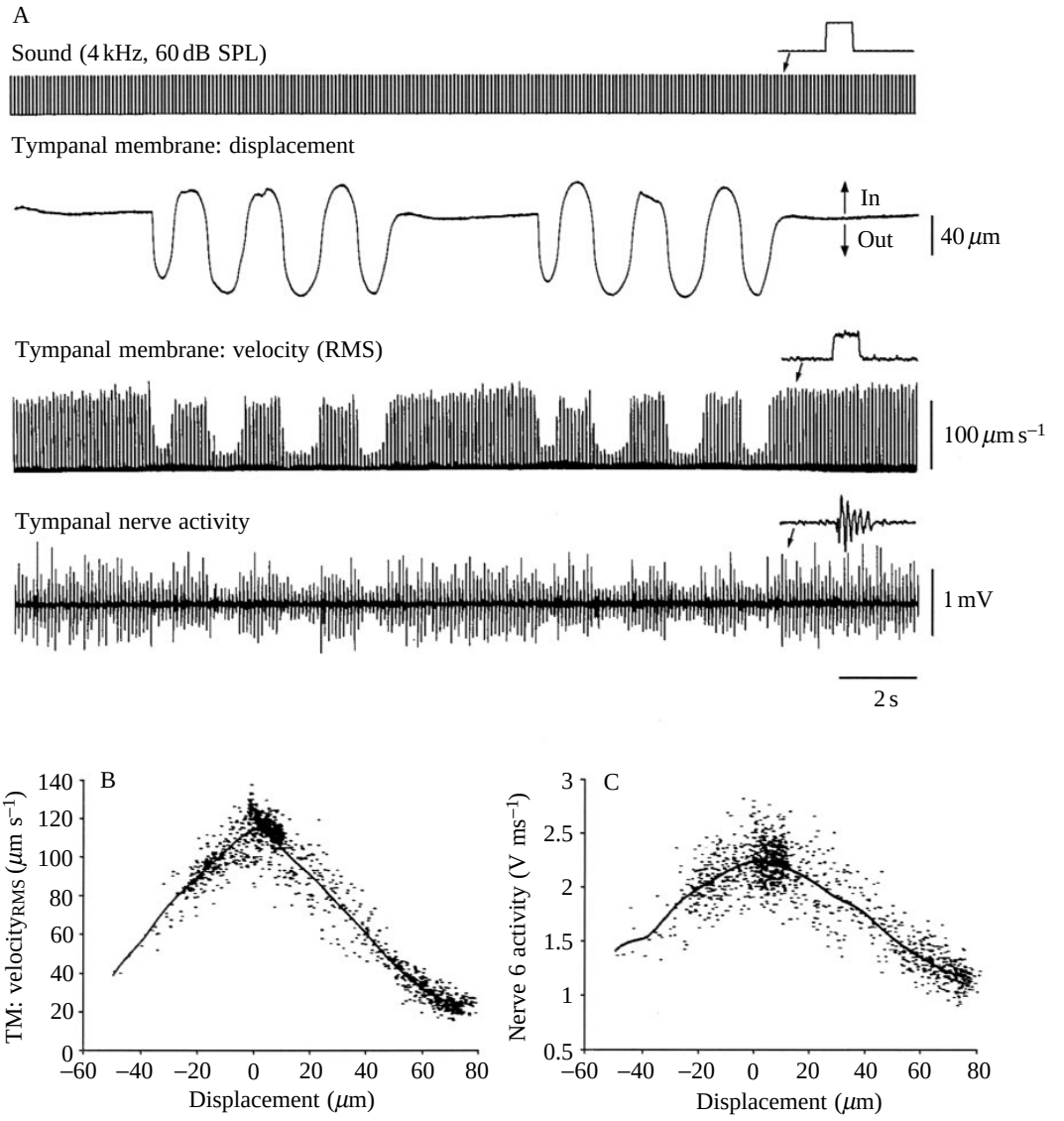

Fig. 5. Influence of tympanal membrane displacements on responses to 4 kHz sound pulses. (A) Simultaneous recording of the sound pulse marker, the tympanal membrane displacement, the RMS of the tympanal membrane velocity at the elevated process and the summed activity of the tympanal nerve. The insets demonstrate a single acoustic marker, RMS measurement and summed nerve response on a faster time scale. Acoustic stimulus: 4 kHz, 60 dB SPL, 20 ms duration, 100 ms interval. Outward displacements of the membrane are presented as downward deflections here and in Figs 6 and 7. (B) Dot plot and gliding average function representing the effect of tympanal membrane (TM) displacement on membrane vibration. (C) Effect of tympanal membrane displacement on tympanal nerve activity. Negative displacements correspond to inward membrane movements. Note the similarity in the shape of membrane and nerve modulation. Changes in the tympanal membrane response and nerve activity were evoked by even small membrane displacements.

and zero displacement, the tympanal membrane vibrated at 110 $\mu m\,s^{-1}_{RMS}$. Inward and outward displacements of up to 50 μm caused a symmetrical linear reduction of the velocity to 40 $\mu m\,s^{-1}$. The velocity signal decreased almost to 20 $\mu m\,s^{-1}_{RMS}$ at a 75 μm outward displacement, corresponding to a reduction of 14.8 dB. These data are in agreement with the change of velocity at 4 kHz of the power spectrum previously presented (cf. the 4 kHz value in Fig. 4A). Like the velocity, the nerve activity also decreased with increasing membrane displacement. The nerve response had a maximum of 2.2 $V\,ms^{-1}$ at the resting membrane position. Membrane displacements of up to ±40 μm caused an almost linear reduction by approximately 37 % to 1.4 $V\,ms^{-1}$. However, with a 75 μm outward displacement, the response was reduced by 45 %, to 1.2 $V\,ms^{-1}$ (Fig. 5C). Thus, the summed tympanal nerve activity clearly reflected the change in vibrational sensitivity. In addition, even small displacements of the tympanal membrane, of less than 10 μm, produced a significant reduction in the membrane velocity and receptor activity, although, in this range particularly, there was an almost linear relationship between pressure and displacement.

The power spectra (Fig. 4B) indicated an increase of

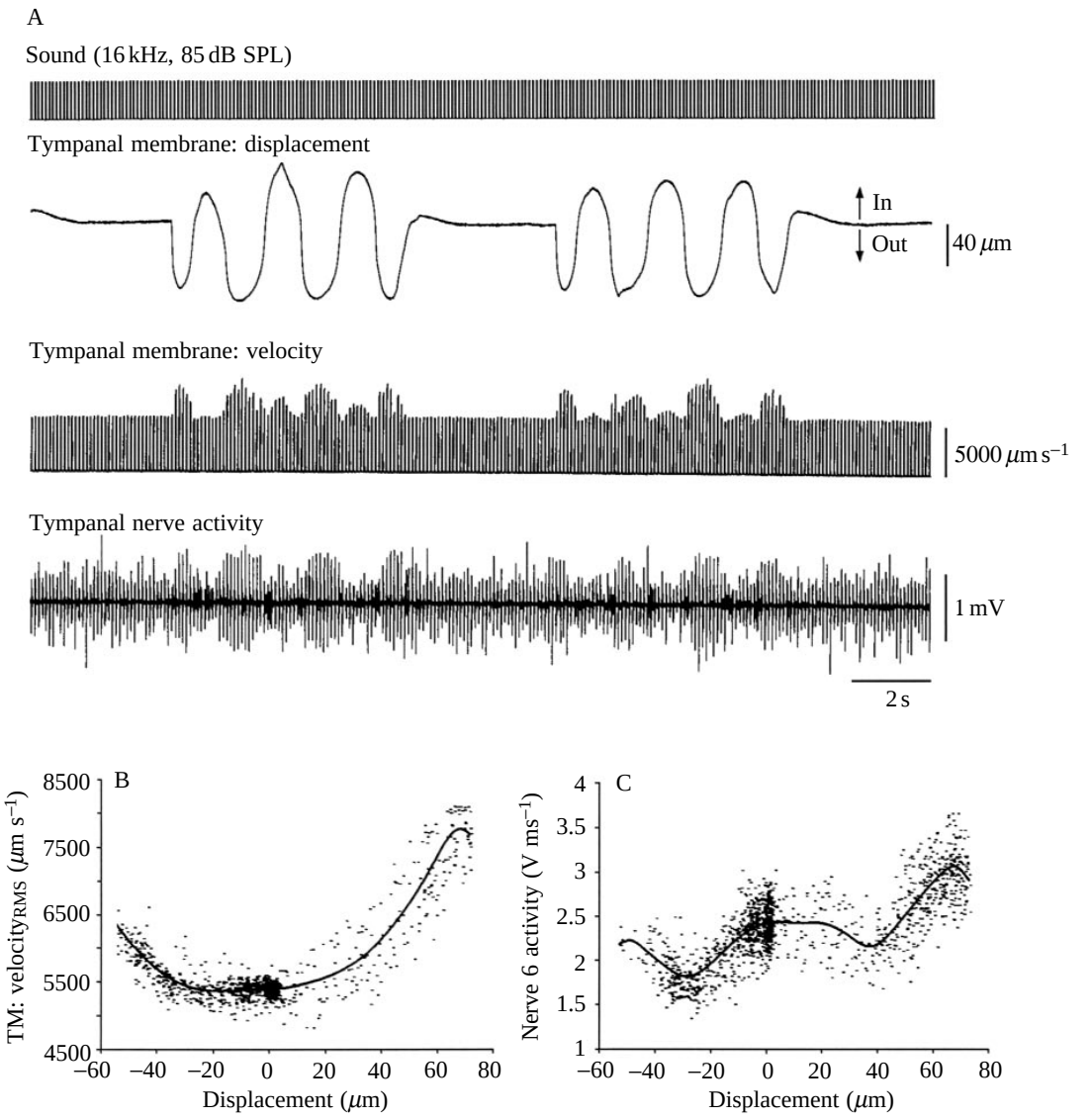

Fig. 6. Influence of tympanal membrane (TM) displacements on responses to 16 kHz sound pulses. (A) Simultaneous recording of the sound pulse marker, the tympanal membrane displacement, the RMS of the tympanal membrane velocity at the pyriform vesicle and the summed activity of the tympanal nerve. Acoustic stimulus: 16 kHz, 85 dB SPL, 20 ms duration, 100 ms interval. (B) Dot plot and gliding average function representing the effect of tympanal membrane displacement on membrane vibration. (C) Effect of tympanal membrane displacement on tympanal nerve activity. Note that the effect on tympanal nerve response changes with displacement amplitude.

auditory sensitivity in the high-frequency range during membrane displacement. A corresponding change in sensitivity had been predicted by Michelsen (1971*c*) and Stephen and Bennet-Clark (1982). Owing to the relatively small number of high-frequency receptors and their higher threshold, at 16 kHz 60 dB SPL, the summed nerve response was relatively low. However, a clear extracellular nerve response was obtained at 85 dB SPL, which was therefore used for the experiments documented in Fig. 6. With high-frequency acoustic stimulation, the vibration velocity was hardly influenced between +20 μm and −20 μm displacement, remaining at 5400 $\mu m\,s^{-1}_{RMS}$. At +40 μm and −40 μm it increased slightly, to 5600 $\mu m\,s^{-1}_{RMS}$ during inward and to 6100 $\mu m\,s^{-1}_{RMS}$ during outward deflection. The maximum increase occurred at 70 μm outward displacement, the vibration velocity reaching 7800 $\mu m\,s^{-1}$, approximately 44 % higher than at the resting position (Fig. 6B). Thus, these data also correspond to the data from the spectral analysis. The tympanal nerve activity exhibited the same tendency, but with some differences in detail. For displacements up to approximately ±40 μm, the nerve response decreased slightly. The reduction was higher during inward movements than during outward movements. With greater displacements, however, the nerve response increased again, and at 75 μm outward displacement the response was approximately 29 % larger than at the resting position. Thus, in parts of the displacement range, the nerve activity and tympanal membrane vibrations changed in opposite senses.

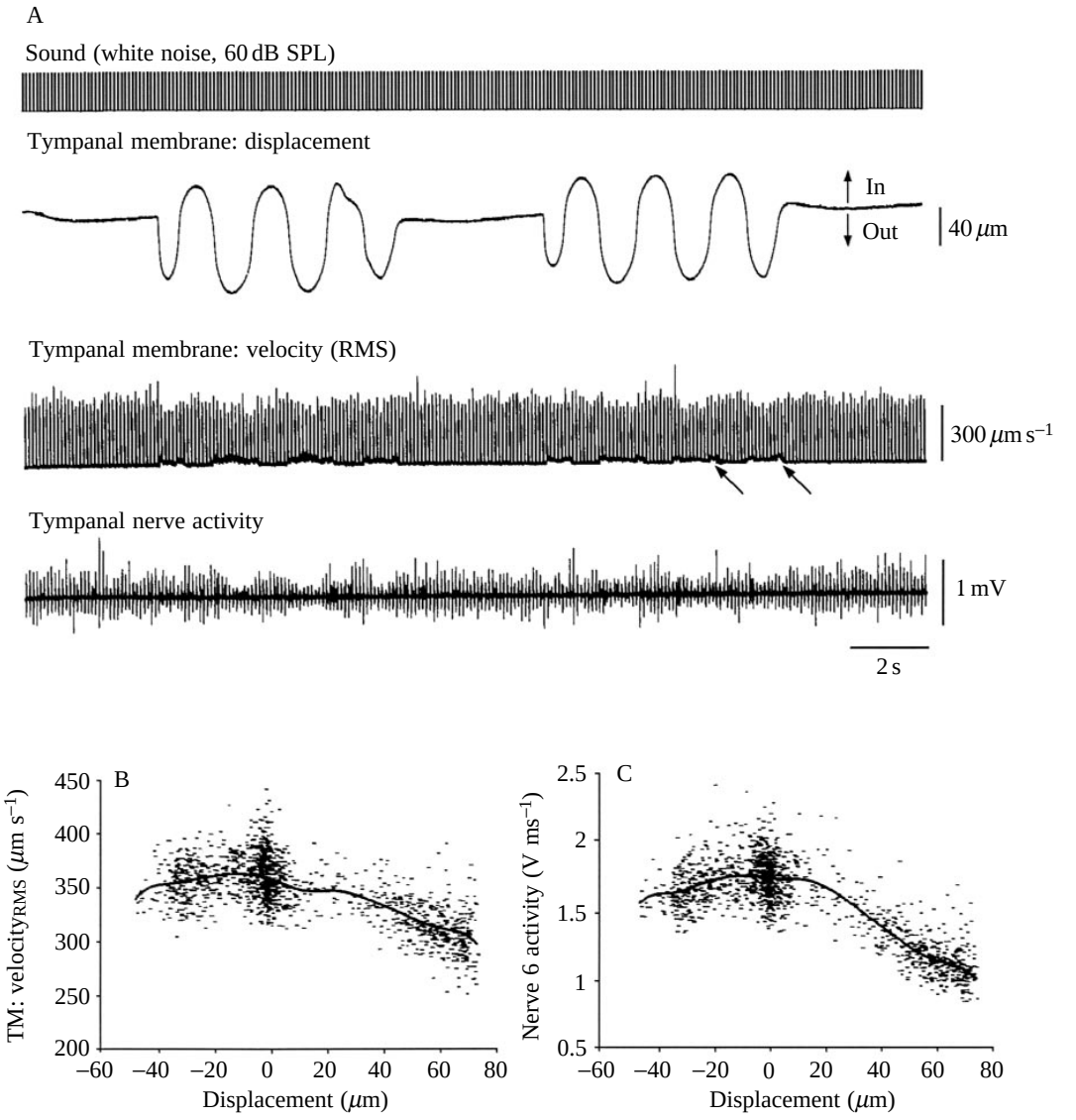

Fig. 7. Influence of tympanal membrane (TM) displacements on responses to white noise sound pulses. (A) Simultaneous recording of the sound pulse marker, the tympanal membrane displacement, the RMS of the tympanal membrane velocity at the pyriform vesicle and the summed activity of the tympanal nerve. Acoustic stimulus: white noise, 60 dB SPL, 20 ms duration, 100 ms interval. (B) Dot plot and gliding average function representing the effect of tympanal membrane displacement on membrane vibration. (C) Effect of tympanal membrane displacement on tympanal nerve activity. Note the similarity in the shape of membrane and nerve modulation. Arrows show examples of small offsets in the velocity signal whenever the membrane passed the resting point.

During stimulation with white noise, there was a small offset in the velocity signal whenever the membrane passed the resting point (Fig. 7A, arrows). At this moment, the velocity of the membrane due to pressure application was at a maximum and produced a velocity component which interfered with the acoustically induced velocity component. These vibrations also caused some tympanal receptor activity. For stimuli with pure tones, this effect did not occur, because the velocity signal was bandpass-filtered for the tone frequency. During stimulation with white noise pulses, only a small reduction of the membrane and receptor response occurred during inward displacements. However, the velocity of the membrane vibration (Fig. 7A,B) and the nerve response (Fig. 7A,C) were markedly reduced with increasing outward displacement. In the resting position, the membrane vibrated at approximately 350 μm s^{-1} and the tympanal nerve response was 1.7 V ms^{-1}. At 75 μm displacement, the oscillation velocity was reduced by 14 %, to 300 μm s^{-1}, and the nerve response by 41 %, to 1.0 V ms^{-1}. In total, the quality of the response modulation was very similar for the tympanal membrane and the nerve.

Influence of membrane displacement on membrane vibration and nerve activity at different sound intensities

For all types of stimuli tested (4 kHz, 16 kHz, white noise), the amplitude of the sound pulses was increased in 5 dB steps

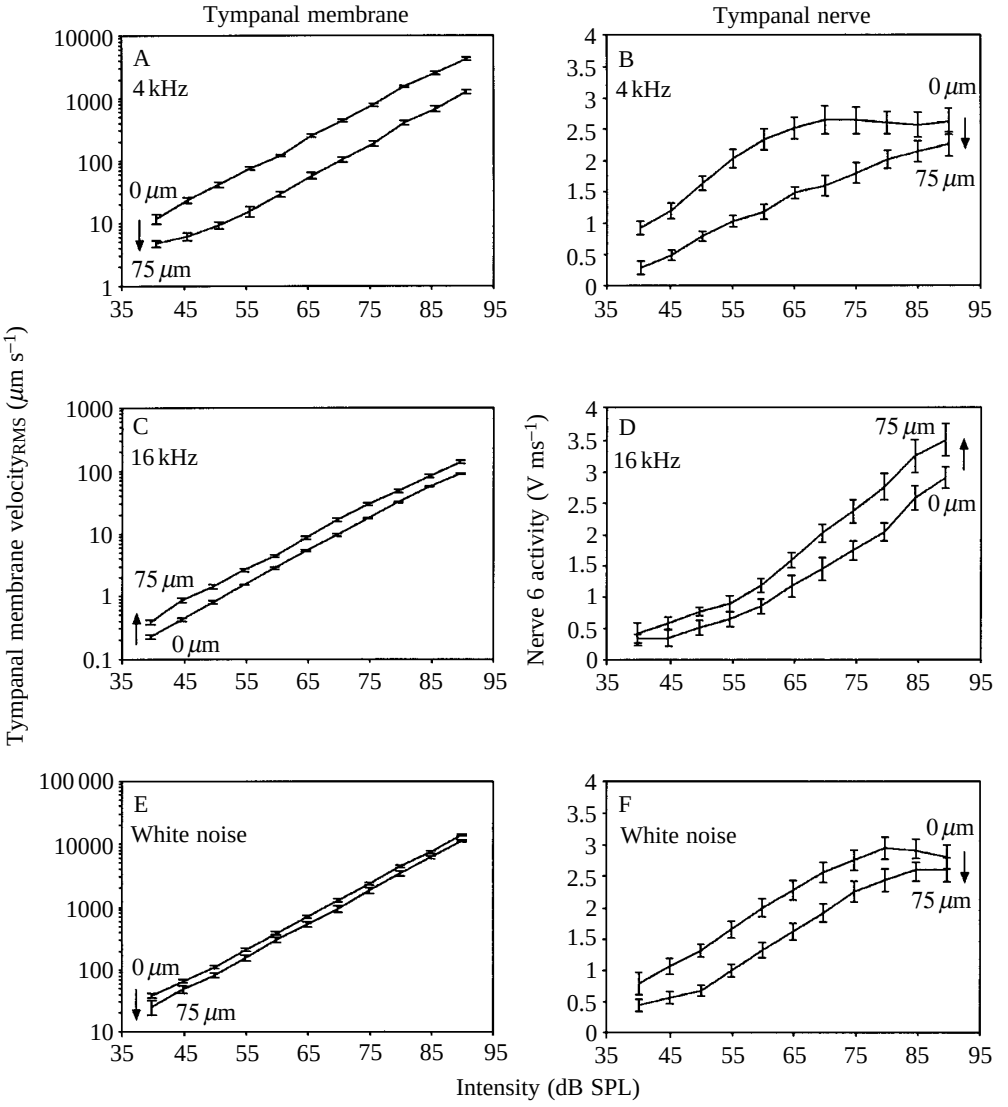

Fig. 8. Comparison of the intensity functions of tympanal membrane vibrations (A,C,E) and tympanal nerve activity (B,D,F). Two functions were obtained at 0 and 75 μm membrane displacement. Arrows indicate the shift in the functions due to membrane displacement. Sound intensity was tested from 40 to 90 dB SPL in steps of 5 dB. (A,B) Stimulus frequency, 4 kHz. Tympanal membrane vibrations and nerve activity were reduced by approximately 15 dB. (C,D) Stimulus frequency, 16 kHz. An increase up to 7 dB occurred in the membrane and nerve responses. (E,F) Stimulus white noise. Note that the membrane vibrations were reduced by approximately 4 dB, whereas the nerve response was reduced by approximately 12 dB. All values are means ± S.D. (N=80–120).

from 40 to 90 dB SPL and was combined with experimentally modulated tympanal membrane displacements. The average RMS of the tympanal membrane velocity and the average auditory receptor response were determined at zero and at approximately 75 μm membrane outward displacement, thus permitting a quantitative description of the influence of membrane displacement on tympanal membrane vibrations and receptor activity. Each point of the curves (Fig. 8) is based on 80–120 measurements. All standard deviations (with two exceptions) are significantly separated. For all three stimulus types, the velocity of the membrane vibration increased logarithmically with increasing sound pressure level (note that SPL is a logarithmic scale). The nerve activity showed an almost linear response up to approximately 70 dB SPL. With 4 kHz and white noise stimuli the response decreased slightly at higher intensities, since the receptors were saturated and began to adapt. With 16 kHz stimulation, there was no saturation above 70 dB SPL. This is because the high sound pressures probably additionally activated the low-frequency receptors.

In the resting position, 4 kHz pulses elicited a logarithmically increasing membrane vibration with increasing sound pressure level (Fig. 8A). When the membrane was displaced by about 75 μm a similar response was obtained, but the membrane vibration was reduced by approximately 15 dB. That is, the vibration velocity at 50 dB SPL and 0 μm displacement was the same as the velocity elicited by 65 dB SPL pulses at 75 μm displacement. The tympanal nerve

response increased with increasing sound pressure level almost linearly up to 70 dB SPL. It decreased above 70 dB SPL, probably because the receptors adaptated to the high sound intensity. The receptor responses for 0 and 75 μm membrane displacement were almost parallel between 40 and 70 dB SPL. However, because of the membrane displacement, the receptor activity was also reduced by approximately 12–15 dB (Fig. 8B). As a consequence, at 75 μm displacement, the nerve response was not saturated above 70 dB SPL but showed a continuous increase up to 90 dB SPL.

With 16 kHz stimulation, the tympanal membrane vibrations were enhanced during the 75 μm displacement (Fig. 8C). The velocity response of the displaced membrane corresponded to that obtained in the resting position with a stimulus approximately 7 dB SPL louder. The nerve activity was also enhanced by the same magnitude. The effect was even more pronounced at higher sound intensities. However, it is possible that a simultaneous reduction of low-frequency receptor activity could also contribute to this effect, since the summed nerve activity was evaluated.

The reduction of the responses to the 4 kHz stimulation and the enhancement at 16 kHz is in agreement with the relative changes of the vibration spectra obtained by FFT analysis (Fig. 4). We also tested the effect of membrane displacements on the response to white noise, which contained frequency components from 1 to 25 kHz (Fig. 8E,F). Because low and high frequencies occurred simultaneously in the noise pulses, we expected the positive and negative effects to compensate for each other. Displacement of the membrane caused a decrease in membrane vibration equivalent to 4 dB SPL of stimulus intensity. Thus, this shift seemed to be the sum of the response reduction at 2–14 kHz and the response enhancement at 14–25 kHz. The nerve response was also reduced, by an amount corresponding to about 10 dB SPL at 60 dB SPL stimulus intensity. Owing to the small number of high-frequency receptors (12) in comparison with the low-frequency receptors (approximately 70), the attenuation of the activity of a-, b- and c-cells will cause a larger change in the summed nerve activity than the amplification of the d-cell activity. This may explain why the change in the nerve activity was larger than the measured change in the membrane vibrations.

These data demonstrate that tympanal membrane displacements not only influenced the acoustically induced tympanal membrane vibrations but also had a corresponding effect on the tympanal receptor activity. Thus, tracheal pressure changes caused marked spectral distortions in signal detection with corresponding changes in receptor activity.

Discussion

Methodological considerations

The aim of these experiments was to analyse the influence of ventilation on the processing of acoustic stimuli at the level of the tympanal membrane and auditory receptors in nearly intact locusts. Changes of tracheal pressure during normal ventilation were analysed and an air application device was used to induce experimental tracheal pressure changes that gave rise to membrane displacement amplitudes like those during normal ventilation.

Systems with different sensitivity were used to measure the tracheal pressure and tympanal membrane displacements. The manometer has a frequency range from d.c. to 200 Hz. However, the laser interferometer is much faster and detects displacements from d.c. to 10 kHz. Thus, during rapid pressure changes and membrane displacements, the signals of the two systems may be out of phase. A comparison between the pressure measured at the mesothoracic spiracle (Fig. 2A) and the pressure measured at the isolated ear (Fig. 2B) showed no distinct difference except that the small hysteresis in the measurement made at the spiracle disappeared when pressure was measured directly at the tympanum. This indicates that the hysteresis may be due to pressure spreading within the tracheal system and not to any special properties of the membrane or the measuring system. The small hysteresis will increase the scatter of the data points in Figs 5–7, but it will not invalidate the measurement. Therefore, measurements at the mesothoracic spiracle reliably indicate the pressure in the tracheal system of the intact animal.

It was essential that the measurement procedure should not affect the tracheal system and the pressure stability within the thorax. This ruled out intracellular recordings from the receptor axons, since then the recording site could not be tightly sealed. At the least, there would be a distinct loss of pressure, so that the membrane displacements would be unlike those during natural ventilation. During extracellular summed recordings of the tympanal nerve activity, the recording site could be completely sealed but, of course, the activity of many low- and high-frequency receptors is measured simultaneously, and the receptor types cannot easily be distinguished. By stimulating with appropriate frequencies, however, the different receptor groups can be activated preferentially.

In addition, we tried to extract significant changes in receptor responses by means of suitable data evaluation algorithms and the analysis of a large number of nerve responses. The close correlation between the membrane velocity and the summed receptor response (Figs 5, 7) supports the use of this approach.

Oscillation properties of the tympanal membrane

During acoustic stimulation, there are different modes of vibration at the different attachment sites of the receptors (place principle: Michelsen, 1971*b*). Our laser vibrometic measurements confirm that the vibrational response of the tympanal membrane is not identical at all receptor attachment sites. At the site of low-frequency receptors (a-cells), the membrane oscillated maximally in the frequency range 3.5–10 kHz, with peaks at 5 and 8 kHz (Fig. 3). The frequencies of maximum oscillation velocity coincide with the tuning curves of the auditory receptor cells. The best frequencies for the low-frequency group are between 3.5 and 6 kHz, and in some receptors there is a second maximum of sensitivity at 8 kHz (Michelsen, 1971*a*; Römer, 1976). At the attachment site

of the high-frequency receptors (d-cells), the membrane vibrates maximally in the frequency range 3.5–23 kHz with a peak in the range 13–18 kHz. This again correlates with the tuning curves of the receptors described by Michelsen (1971*a*) and Römer (1976). Additionally, however, the membrane at this position is also sensitive to low-frequency sound in the range 2–9 kHz. These data are in agreement with the results of Völker (1991) and Völker *et al.* (1991), but may not be expected by a strict place principle. A corresponding situation occurs in the grasshopper *Chorthippus biguttulus* (Meyer and Elsner, 1995). Here, the membrane vibration is also greatest in the range 6–8 kHz at the attachment sites of the low-frequency receptors and from 6 to 20 kHz at the attachment site of the high-frequency receptors. In both species, the low-frequency response is sometimes even larger at the d-cell attachment site than at that of the a-cells. Why then are the d-cells less sensitive in the lower frequency range than the a-, b- or c-cells? Frequency discrimination depend not only on the modes of tympanal membrane vibrations but may also depend on the movements and oscillations of Müller's organ (Michelsen, 1973; Stephen and Bennet-Clark, 1982; Breckow and Sippel, 1985). The laser vibrometer measured only membrane vibrations perpendicular to the membrane, but vibrations in its plane could also be important. That is, the difference in membrane sensitivity and d-receptor tuning may be due to the complex response of the whole tympanal organ. In any case, the sensitivity of the pyriform vesicle to low frequencies remains an interesting result in the context of frequency analysis at the level of the tympanal membrane and auditory receptors.

Modulation of auditory information processing by tympanal membrane displacements

The use of a laser interferometer allowed exact measurements of the membrane displacements caused by tracheal pressure changes. The normal outward displacement amplitude was between 70 and 90 μm and the maximum was between 130 and 150 μm. Inward displacements were distinctly smaller as a result of the smaller negative pressure, which corresponds to data from *Chorthippus biguttulus* (Meyer and Elsner, 1995).

Measurements of the compliance of the isolated ear during local loading indicated that there is a linear relationship between the membrane displacement and the applied force and that, for tympanal membrane displacements of 50–100 μm, Hooke's law is obeyed (Michelsen, 1971*b*,*c*; Stephen and Bennet-Clark, 1982). However, compliance measurements with pressure gradients showed a sigmoid relationship and indicated a compliance only about one-tenth as great as when loaded locally (Stephen and Bennet-Clark, 1982). Our data based on pressure modulation and membrane displacement in intact animals also demonstrate a sigmoid relationship between pressure and membrane displacement, with a linear segment over no more than ±10 to ±20 μm. In the intact animal, therefore, in agreement with Stephen and Bennet-Clark (1982), Hooke's law is obeyed only in a very narrow range. Moreover, our measurements show distinct modulation of tympanal membrane vibrations and of the auditory nerve activity even at membrane displacements smaller than ±10 μm (Figs 5–7). Therefore, even minute pressure changes within the tracheal system will affect the detection of acoustic stimuli. Since the resonant frequency of a membrane depends in part on its tension (Michelsen, 1971*b*; Stephen and Bennet-Clark, 1982), even minute changes in the tension would be expected to have an impact on auditory sensitivity. This is what is actually found in the auditory system of locusts.

Ventilation was expected to influence the responses of the tympanal membrane and auditory receptors to acoustic stimuli. In particular, it should cause a shift in resonance responses of the tympanal membrane to higher frequencies (Michelsen, 1971*c*; Stephen and Bennet-Clark, 1982). The laser vibrometric measurements showed that displacement of the tympanal membrane increases its sensitivity in the high-frequency range. These data are in good agreement with the previous assumptions. A second effect, however, was that acoustically induced oscillations at lower frequencies decreased (Michelsen *et al.* 1990). Thus, even at the level of tympanal membrane vibrations, the spectral composition of a sound pattern was modulated by tracheal pressure changes. Ventilation will generally reduce low-frequency components by about 15 dB SPL and enhance the high frequencies of the sound pattern by about 7 dB SPL.

The altered tympanal membrane sensitivity was also reflected in the activation of the tympanal receptor cells. It is surprising how closely the modulation of tympanal nerve activity follows the course of tympanal membrane vibrations (Figs 5, 7). The curves for membrane velocity and nerve response differed only for high-amplitude stimulation at 16 kHz (Fig. 6B,C). The reason for this deviation may be due to the small number (about 12) of high-frequency receptors. Their activity may have been superimposed on, and masked by, simultaneous activation of the low-frequency receptors, of which there are about 70. At 16 kHz and 85 dB SPL, even low-frequency receptors will respond to the sound pulses (Michelsen, 1971*a*; Römer, 1976).

At sound pressure levels of 50 or 55 dB SPL, the auditory nerve response to 4 kHz or white noise pulses was almost completely extinguished by the membrane displacement, whereas the response of the high-frequency receptors was enhanced. The shift of the receptor response occurred not only near threshold, however; even with much louder sound pulses (80 dB SPL), membrane displacements modulated the nerve activity. Not only deep ventilatory cycles but also small tracheal pressure changes caused these modulations, although to a smaller degree.

The close mechanical coupling of the tracheal system and the auditory organ has a significant impact on auditory information processing in the locust and in the acridid grasshopper *Chorthippus biguttulus* (Meyer and Elsner, 1995). Whenever the tracheal pressure changes, auditory sensitivity and information about the spectral composition of a sound pattern are modulated at the level of tympanal membrane vibrations and auditory receptor activity. As a consequence, the

animals cannot rely on a frequency analysis within their auditory pathway. This may be relevant to the acridid grasshoppers, which use relatively broad-band signals for acoustic communication.

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