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Tympanal mechanics in the parasitoid fly *Ormia ochracea*: intertympanal coupling during mechanical vibration

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Abstract The acoustic parasitoid fly *Ormia ochracea* locates its host, a singing field cricket, by means of a pair of small tympanal organs which are less than 2 mm in width. Nevertheless, laser vibrometric evidence shows that this tympanal system is directionally sensitive to sound through the action of a flexible intertympanal bridge that mechanically couples the tympana. Biomechanical data, a mechanical analogue and an analytical model lead to a testable prediction about the vibratory behavior of this tympanal system: if intertympanal coupling occurs, a force applied only unilaterally in non-acoustical conditions should be transmitted, at least to some degree, to the contralateral ear. This paper presents new experiments of direct mechanical stimulation that test the prediction of mechanical coupling. Stimulation on only one side of the intertympanal bridge elicited a contralateral mechanical response. Thus, coupling of the tympanal membranes through a flexible intertympanal bridge is demonstrated by mechanical as well as acoustical stimulation. These experiments also test for the possible presence of a pressure-difference system in *O. ochracea*. Intertympanal coupling is shown not to depend on the integrity of the air space backing the tympanal system, thus eliminating this possibility.

Key words Directional hearing · Tympanal ear
Laser vibrometry · Audition · Auditory mechanics

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Abbreviations IID Interaural intensity difference · ITD interaural time difference · mIID mechanical interaural intensity difference · mITD mechanical interaural time difference

Introduction

As parasitoids of singing Orthoptera, tachinid flies of the tribe Ormiini rely on their sense of hearing to locate their hosts (Cade 1975; Lakes-Harlan and Heller 1992; Robert et al. 1992). Several field and laboratory studies have highlighted the capabilities of ormiine flies to locate their singing hosts acoustically, and to approach and land on loudspeakers broadcasting their host's calling song (Cade 1975; Mangold 1978; Walker 1986; Robert et al. 1992). A remarkable feature of the tympanal ears of ormiine flies is their very small size. Located on the ventral prothorax, the ears of *Ormia ochracea* do not exceed 2 mm in width (average: 1.68 ± 0.19 (SD) mm; $n=16$; Robert et al. 1994), and thus are among the smallest described in the animal kingdom (Hoy and Robert 1996; Au 1997).

The auditory capabilities of *O. ochracea* were recently investigated at the biomechanical and neurophysiological levels (Miles et al. 1995; Oshinsky and Hoy 1995; Robert et al. 1996). Analysis of the mechanical response of the tympanal membranes and associated cuticular structures, along with deflection shape analysis and auditory nerve recordings, revealed that ormiine ears are sensitive to the direction of an incident sound wave at the carrier frequency of their host's calling song (4.6–5 kHz). Acoustical measurements made at the tympanal membranes with probe microphones revealed interaural time differences (ITDs) and interaural intensity differences (IIDs) in the sound pressure of $1.45 \mu\text{s}$ (SD = 0.49, $n=10$) and immeasurably below 1 dB (Robert et al. 1996), respectively. The *mechanical* interaural time difference (mITD) and a *mechanical* interaural intensity difference (mIID) between the mechanical response of the ipsilateral and contralateral tympanal ears were,

however, substantially larger than the ITD and IID from the sound field ($mITD = 48.3 \pm 11.2$ (SD) μs , $n = 8$; $mIID = 12.4 \pm 3.4$ dB, $n = 8$).

On the basis of this biomechanical evidence, the fly's directional sensitivity was proposed to rely on mechanical coupling between the two hemilateral eardrums (Miles et al. 1995; Robert et al. 1996). The anatomical basis for coupling was identified as a midline cuticular element (the presternum; Edgercomb et al. 1995) that mechanically links the two tympanal membranes; it was named the intertympanal bridge. To explain the mechanical response of the tympanal system, and the role of the intertympanal bridge in directional hearing, an analytical model and a mechanical analogue were developed (Miles et al. 1995). The mechanical analogue consists of two rigid beams, which represent the two distal projections of the bridge and are linked by a torsional spring of finite stiffness (Fig. 1A). The key to the directionality of the tympanal apparatus was shown to

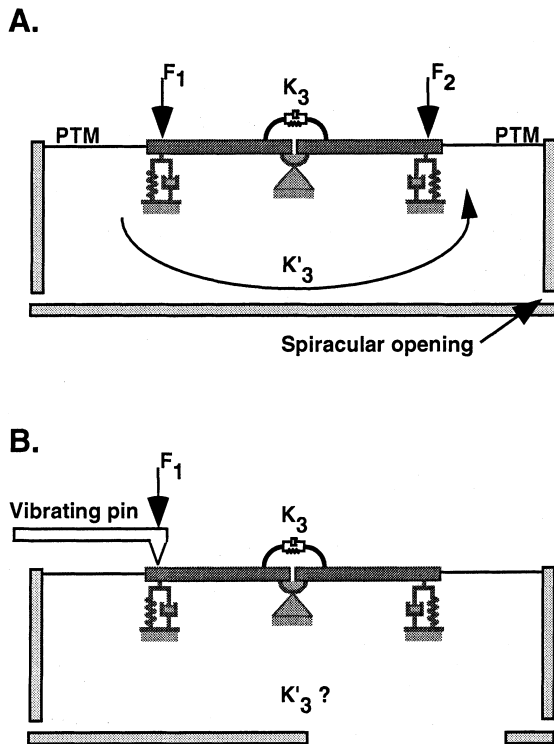


Fig. 1A, B Mechanical analogue of the hearing organs of the fly *Ormia ochracea*. **A** The two prosternal tympanal membranes (PTM) are connected by a cuticular intertympanal bridge represented by the two thick gray bars. The PTMs and the intertympanal bridge are backed by an undivided air space, the prosternal tracheal air sac. F_1 and F_2 represent the forces acting on the tympanal system during acoustic stimulation. The extremities of the intertympanal bridge are the tympanal pits (marked by the vertical arrows showing F_1 and F_2). K_3 is the bending stiffness assigned to the intertympanal bridge. K'_3 is the stiffness attributed to the air space of the tracheal sac. **B** During mechanical stimulation, the vibrating pin applies a force (F_1) on only one side of the intertympanal bridge. Removal of the prothoracic coxa creates a large opening of the air sac and causes a decrease of stiffness K'_3 . Comparison of the mechanical response in intact and open-ear conditions provides information on the relative contributions of K_3 and K'_3 to intertympanal coupling

reside in the flexibility of the bridge (Miles et al. 1995; Robert et al. 1996). When stimulated acoustically, both sides of the bridge are driven by two forces of equal amplitudes but with a slightly different phase (Fig. 1A; F_1 and F_2). Vibrometric measurements showed that, in response to an incident sound pressure, the intertympanal bridge undergoes an asymmetrical displacement about its center, in the manner of a floppy see-saw rocking about its pivot point.

From this mechanical model, some predictions were made about the vibratory behavior of the tympanal complex. If, under acoustical stimulation, mechanical coupling between the ears is due to the action of the intertympanal bridge, a force applied only unilaterally in non-acoustical conditions should be transmitted to the other, contralateral ear. Additionally, if the bridge is flexible, both sides can be expected to oscillate at different amplitudes and with a phase difference other than 180 degrees (unlike a rigid see-saw). To test these predictions, one side of the intertympanal bridge was set into vibrations with a vibrating pin (Figs. 1B, 2A), and the mechanical displacement of the bridge was measured by laser vibrometry. These experiments show that the intertympanal bridge provides intertympanal mechanical coupling and is indeed flexible.

This paper also investigates an alternative explanation for directional sensitivity: that a pressure-difference receiver system is at work in *O. ochracea*. Indeed, the pressure-difference mechanism is not mutually exclusive with mechanical coupling and could contribute, at least in part, to the observed mechanical response. In such an alternative hypothesis, the air space backing the tympanal membranes – the ear cavity – is both small and stiff enough to act as a spring, and to contribute significantly to the observed directional response, at least for some sound frequencies. Since the fly's post-tympanal air space is unpartitioned, this system would be reminiscent of the cicada pressure-difference receivers (Fletcher 1992; Fonseca 1993). The air behind the tympana certainly experiences compression and rarefaction as sound pressure sets the tympanal system in vibration and sound is transmitted through it. It is therefore conceivable that this air space, with a stiffness K'_3 could play a role in intertympanal coupling (Fig. 1A).

To test this hypothesis, the fly's tympanal system was set in vibration without using acoustic energy by means of a vibrating pin, and the vibrational response was measured while the putative air stiffness K'_3 was altered (Fig. 1B). Then, the ear cavity was opened to the outside by removing the prothoracic coxa. This operation leaves the tympanal system intact while reducing the air stiffness of the ear cavity. Comparing the mechanical responses of the intertympanal bridge pre- and post-operatively indicates the degree to which coupling is influenced by the volume (stiffness) of the air behind the tympanal membranes. Mechanical coupling is shown to be independent of the integrity of the air space backing the tympanal membranes.

Materials and methods

Animals

The specimens used in this study were parasitoid tachinid flies *O. ochracea* from a laboratory colony in Ithaca, N.Y., USA. The colony was established with flies collected in the field near Gainesville, Fla., and at the Gulf Coast Research and Education Center at Bradenton, Fla. The methods of collection and culture of these parasitoid flies were adapted from those described by Walker (1986) and Wineriter and Walker (1990).

Laser vibrometry

The tethering system used for positioning the specimens is identical to that described in Robert et al. (1996). In this system, the orientation of the fly could be precisely adjusted with respect to both the laser beam and the mechanical stimulation probe.

The mechanical response of the fly's tympanal organs and the vibrations of the stimulation apparatus were measured with a Polytec laser vibrometer (Polytec, Waldbronn, Germany; model: OFV 2100 electronics unit and OFV 302 optical sensor head). Using this instrument, vibration velocities as low as $0.5 \mu\text{m/s}$ can be measured over a frequency range from 0.1 Hz to 500 kHz. The optical sensor head was mounted on a two-dimensional motorized micrometer stage (Oriel model 16647-16327, motor control unit 18000). The position of the laser beam could be adjusted in azimuth and elevation with a minimal displacement of about $1 \mu\text{m}$. Using a Nikkor macrolens (55 mm, 1:2.8, the laser beam could be focused down to a $5\text{-}\mu\text{m}$ -diameter spot. The position of the beam was visually monitored with a Wild-Leitz M3Z stereomicroscope equipped with a Wild DM-RB633 filter.

The optical sensitivity of the vibrometer was sufficient to obtain reproducible, highly coherent data, obviating the need for glass beads or other reflective particles used to enhance the reflectivity of membranous or other cuticular structures. Thus, tympanal vibrations were measured in an unloaded condition.

Mechanical stimulation

To set the fly's tympanal ears in vibration without using acoustic energy, a system of direct mechanical stimulation was developed.

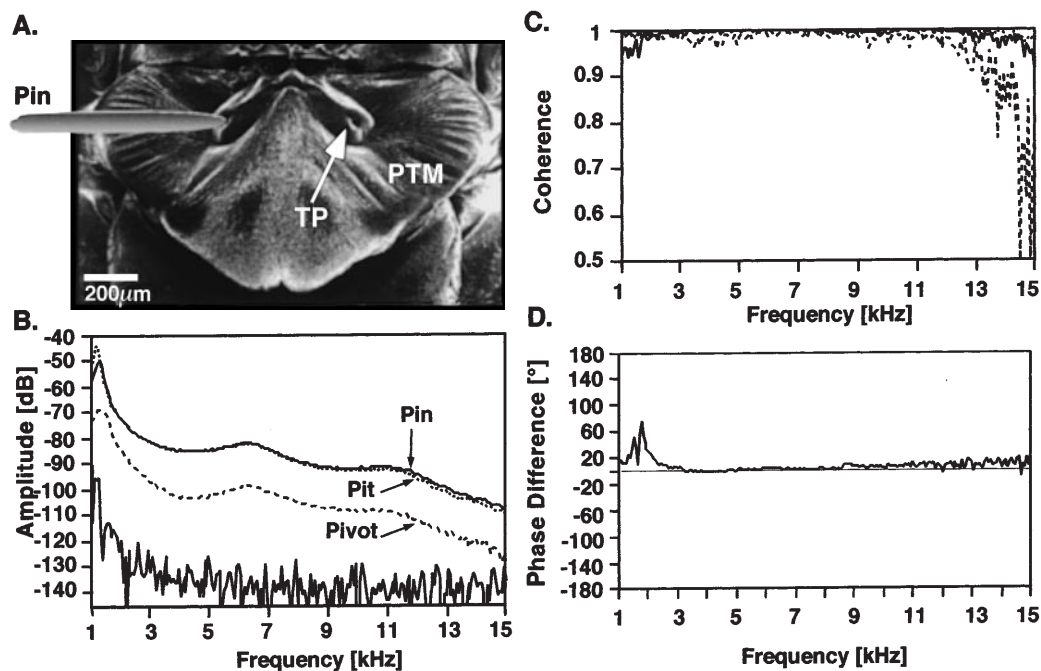
This actuating system consisted of a small probe – a steel entomological pin – fixed to the moving shaft of the electromagnetic actuator of an IBM hammer printer (model 3202 Impact Line). The entire actuating system was mounted on a micromanipulator that allowed three-dimensional positioning of the probe in front of the tympanal ears (Fig. 2A). The actuator was fed a random-noise electrical signal with a band width of 1–15 kHz. The resulting vibrations of the pin, as measured with the laser Doppler vibrometer, are shown in Fig. 2B. The damping between the electrodynamic actuator and the pin was adjusted with surgical wax and a visco-elastic damping adhesive to avoid sharp resonances in the range of frequencies of interest. Apart from a weak resonant peak at around 1 kHz, the frequency response of the actuator was reasonably flat (Fig. 2B). Several different combinations of pin length and damping were used during the experiments, accounting for some small variations in the shape of the frequency spectra. Such differences, however, do not affect the difference spectra shown here.

A series of important experimental controls were conducted to verify the legitimacy of this technique of direct mechanical stimulation. They are presented in the Results section.

Signal processing

Signals from the laser vibrometer and the electrical signal driving the electrodynamic actuator were digitized using an Analog Fast-

Fig. 2A–D Methods and experimental controls. **A** Electron scanning micrograph of the tympanal ears, showing the position of the vibrating pin on the ipsilateral tympanal pit. The positions of measurement by the laser vibrometer are on the tip of the vibrating pin, on the ipsilateral and contralateral pits, and on the pivot point (*PTM* prosternal tympanal membrane, *TP* tympanal pit). **B** Controls for mechanical contact between the pin and tympanal pit. Pin off (*Pin*): amplitude frequency spectrum of the mechanical displacement of the pin when the pin is not in contact with the tympanal system. Pin on (*Pit*): mechanical displacement of the pit when it is driven by the pin. *Pivot*: mechanical displacement of the pivot point. The *lower curve* shows the vibrations of the tympanal pit when it is not in contact with the vibrating pin. **C** Coherence functions of the “pin off”, “pin on” and “pivot” frequency spectra shown in **B**. Note that the scale shows the upper 50% coherence. **D** Frequency spectrum of the phase difference between the pin and the ipsilateral tympanal pit



16 A/D board (16 bit, 128 kHz/channel). A transient window was applied to the data in the time domain prior to analysis. Spectral analysis was performed using a Fast Fourier Transform to calculate the cross-power spectra, auto-power spectra, and transfer functions of the membrane velocity (or displacement) values relative to the electrical signal driving the electrodynamic actuator. The spectral analysis had a frequency resolution of 62.5 Hz which is sufficient given the absence of sharp resonances in the investigated system. The averaged responses of ten stimulus presentations were used to compute the transfer functions between the laser signal (output) and the driving signal (input). Transfer functions were computed as the cross-power spectra between the laser and electrical signals divided by the auto-power spectra of the electrical signal driving the pin.

To estimate the level of unrelated noise in the data, a coherence function was also computed for each stimulus presentation. Coherence is a frequency domain measure estimating to which degree the output of a system is linearly related to the input to the system (Kates 1992). The magnitude-squared coherence function was used (abbreviated "coherence" in the text) and is given by

$$\frac{|G_{xy}(f)|^2}{G_{xx}(f)G_{yy}(f)}$$

where $G_{xy}(f)$ is the cross-power spectrum between the laser signal $x(t)$ and the electrical input signal $y(t)$, and $G_{xx}(f)$ and $G_{yy}(f)$ are the auto-power spectra of the laser and electrical signal, respectively. The coherence function ranges from 0 to 1, where 0 indicates complete contamination of the signal by unrelated noise, and 1 indicates the absence of unrelated noise. Coherence values were typically above 0.90 over the frequency range 1–15 kHz (Fig. 2C).

Results

Mechanical stimulation

Mechanical coupling between the tympanic ears was tested by stimulating a single tympanum and measuring if and how well its vibrations were transmitted to the other tympanum. The series of experimental controls presented below were conducted to characterize the mechanical input to the system and to control for possible artifacts related to this technique. The interpretation of the subsequent experiments critically depends on the outcome of such controls.

Transmission of coplanar vibrations

The transmission of non-acoustic vibrational energy to the tympanic system was obtained by apposing the tip of the pin against one distal end of the intertympanic bridge (Fig. 2A). Care was taken that the movements of the probe applied to the tympanic pit were perpendicular to the longitudinal axis of the probe and perpendicular to the plane of the tympanic bridge. This careful alignment, made visually under a microscope, ensures an in-plane displacement of the tympanic system similar to that observed during sound stimulation. The displacement amplitude of the probe (measured with the vibrometer) was adjusted to be similar to a biologically relevant acoustic stimulus, causing displacements in the range of 10–50 nm (Robert et al. 1996).

Static deflection

The apposition of the pin to the tympanic pit can provoke large static deflections of the tympanic system. A static deflection of a few micrometers is already at least 100 times larger than the acoustically induced displacements and could drastically alter the linear mechanical properties of the ears. To avoid such non-linearities, contact was monitored with the laser vibrometer aimed at the tympanic pit. While monitoring online the oscillographic trace of the laser signal, the pin was positioned to cause the smallest possible static deflection of the tympanic pit. Static contact between the pin and the pit was detected by the constant alteration of the noisy laser signal.

Continuity of the mechanical contact

However light the contact between the vibrating pin and the pit ought to be to avoid large static loading, this contact must still be continuous to ensure coherent transfer of mechanical energy to the tympanic system. This mechanical continuity was evaluated by measuring the vibration level of the pin as it is apposed on the pit, and comparing it to that of the tympanic pit. These two vibration levels (Pit amplitude spectrum subtracted from Pin amplitude spectrum) differed by 0.41 dB (SD 1.39) in the frequency range from 2 to 15 kHz (Fig. 2B, Pin and Pit spectra). This result indicates that the pin and the pit are moving with similar amplitudes in that frequency range. In another control, the vibrations of the pin, measured at the tip of the probe, did not differ whether the pin was in contact with the tympanic pit or not (not shown). Hence, the mechanical actuator is not subjected to any large mechanical impedance load from the tympanic system and can reliably transmit vibrational power to it within the energy range considered.

The continuity and linearity of the transmission of vibrational energy is further reflected by the high degree of coherence (Fig. 2C). Transfer functions of both ipsi- and contralateral pits have a coherence close to 1 over the frequency range 1–15 kHz. The phase response of the pin was also compared to that of the ipsilateral pit (Fig. 2D). At frequencies above 2 kHz, the phase difference between the pin and the pit is small (average 5.8°, SD 5.8°), indicating that they actually move together. Below 2 kHz, where imposed displacement is larger, there is a phase difference that can exceed 60°. Although coherence is still high at low frequencies (Fig. 2C), the pin and the pit do not strictly move together. This lack of synchronization may be related to a local deformation of the tympanic pit at the contact point of the pin. This phenomenon points to the possible problems that can arise at certain frequencies when using a point load (like a pin), as opposed to a distributed force field (like a sound field). The high degree of synchronization and high coherence at fre-

quencies above 2 kHz, however, show that the coupling between the pin and the tympanal pit is effectively linear.

Stimulus specificity

For this technique to be valid, it is also important for the mechanical probe to activate the tympanal system in conditions as close as possible to acoustic conditions. Since a point load can, at some frequencies at least, differ from a natural acoustic input, it is possible that other structures, or even the whole specimen, can be set into vibration. To test whether mechanical actuation can be representative of normal acoustic conditions, the vibratory behavior of the pivot point (the fulcrum of the intertympanal bridge) was also systematically measured. The vibration level of the pivot is significantly lower than that of the tympanal pit (Fig. 2B). Conditions of stimulation can also vary between preparations, since they depend on the fine positioning of the pin. When averaged for eight different animals, the difference between the vibration level of the pit and the pivot at 5 kHz amounts to 19.3 dB (SD 2.8) (Fig. 3). Across the frequency range 2–15 kHz, this difference is 17.6 dB (SD 2.9), showing that the mechanical displacement imposed upon one tympanal pit does not result in vibrations of the whole prosternal area, or the whole animal. As shown in Fig. 3, this experimental situation is reproducible. Coherence between the mechanical response of the pivot and the driving signal was reasonable across most of the frequency range tested (Fig. 2C, lower curve). Also, the difference in displacement amplitude between the ipsilateral pit and the thicker supporting cuticle of the probasisternum is 22.1 dB (SD 1.5). These results corroborate measurements made in acoustic conditions where the pivot point moved with an ampli-

tude 23.3 dB (SD 4.8) lower than the ipsilateral tympanal pit (Robert et al. 1996).

Controlling for non-acoustic conditions

Finally, it cannot be a priori excluded that the moving parts of the actuator produce an acoustic (near-) field that could elicit a mechanical response from the tympanal system. According to the high level of coherence obtained (Fig. 2C), inputs that are not related to the vibrations of the pin account for less than a few percent of the measured response. In addition, the possibility that the actuating system drives the tympanal system acoustically is refuted by the fact that the tympanal pit does not move above noise level (some 60 dB below signal) when the pin is vibrating a few micrometers in front of the tympanal system but without contacting it (Fig. 2B, lower trace).

Evidence for interaural mechanical coupling

The displacement of the contralateral tympanal pit was measured while the ipsilateral pit was driven by the actuating pin. The vibrations of the pivot were also systematically monitored during each of these experiments to ensure proper mechanical stimulation. The large difference in displacement amplitude (15–20 dB) observed between the mechanical response of the ipsilateral pit and the pivot guarantees, for each experiment, that the tympanal system is properly driven by the pin (Fig. 4, upper curves of left panels). The coherence function was computed for all flies tested but shown here for only three flies. The coherence of the amplitude spectra of the left and right pit was high, indicating that both ipsi- and contralateral responses were linearly related to the mechanical input to the system, and that little uncorrelated noise contributes to the measured mechanical response (Fig. 4, right panels).

The difference spectra between the displacement amplitudes of the ipsilateral and contralateral pits differ from zero by only a few decibels (Figs. 4, 5). In these conditions, the contralateral tympanal pit moves not as much as the ipsilateral pit, but clearly above noise level. As the driving force is applied on the ipsilateral pit only, this result provides good evidence for mechanical coupling between the two tympanal ears. When averaged for eight different animals, this difference is 4.8 dB (SD 2.4; $n = 8$ animals, $n = 225$ frequency point, Fig. 5).

The comparison between the phase response of the ipsi- and contralateral tympanal pits also provides valuable information on the nature of coupling. Phase data for each pit were first unwrapped, subtracted from each other, and then averaged. For convenience, the unwrapped phase values were converted into time delays (Fig. 6A). At 5 kHz, the time delay between the ipsilateral and contralateral pits reaches 117 μ s (SD 13.7, $n = 7$ animals). In comparison, for the same frequency,

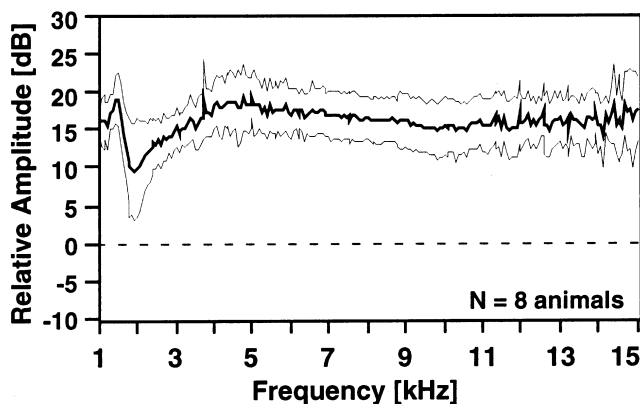


Fig. 3 Difference in the displacement amplitude between the ipsilateral pit and the pivot of the intertympanal bridge. The amplitude frequency spectrum (*thick line*) is the result of the subtraction of the displacement amplitude spectra of the ipsilateral pit and the pivot. The *thin lines* show the 1-SD interval. On average, the amplitude of displacement of the ipsilateral pit is 17.6 dB higher than the pivot ($n = 8$ animals)

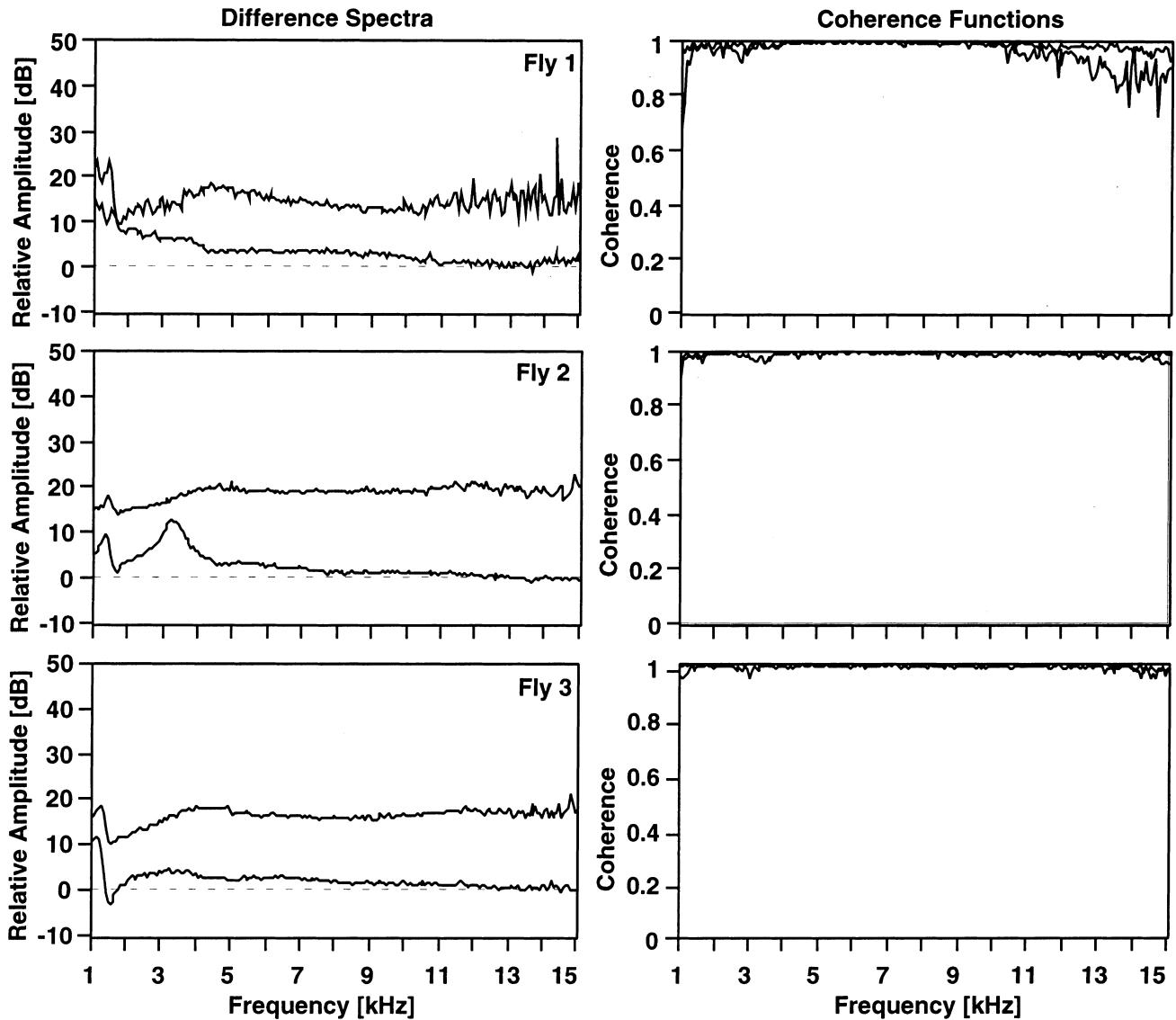


Fig. 4 Difference spectra in the displacement amplitudes of the tympanal pits and the pivot, and coherence functions. For three examples (*Fly 1–3*), the frequency spectra are calculated as the difference between the displacement amplitude spectrum of the pit and that of the pivot (*upper curves*), or the difference between the response of the ipsilateral pit and the contralateral pit (*lower curves*). The coherence functions of the mechanical responses of the ipsi and contra pits indicate the low noise level and the linearity of the mechanical response

the delay in the mechanical response of a putative rigid intertympanal bridge is $-100 \mu\text{s}$ (half a period at that frequency; Fig. 6A). At first sight, the predicted delay of a putative rigid bridge and the measured data seem to coincide. However, a closer look at the difference in delay between the predicted and measured curves indicates that the measured response lags the prediction (larger delays) for frequencies below 9 kHz (Fig. 6B). Interestingly, at 9.0 kHz, this difference in delay is nil, indicating the absence of bending of the intertympanal bridge at this particular frequency.

Opening the air cavity

As presented in the Introduction, a direct way to test whether a pressure-difference receiver system is at work in the orniine ear is to modify the stiffness of the air volume backing up the tympanal system (Fig. 1). Such modification can be achieved by removing the prothoracic coxa while leaving the tympanal system mechanically intact. The effect of altering air stiffness can be seen in the difference in the mechanical response of the tympanal pits before and after the operation (Figs. 7, 8). As seen previously, it is important for the mechanical input to the tympanal system to be characterized for each animal. And since the specimen is taken out of the vibrometry setup for removal of the coxa, it is equally important that the mechanical stimulus to the ipsilateral pit remains comparable, if not identical, before and after the operation. The difference in the mechanical displacement of the ipsilateral pit before and after the operation indicates very little amplitude difference (Fig. 7,

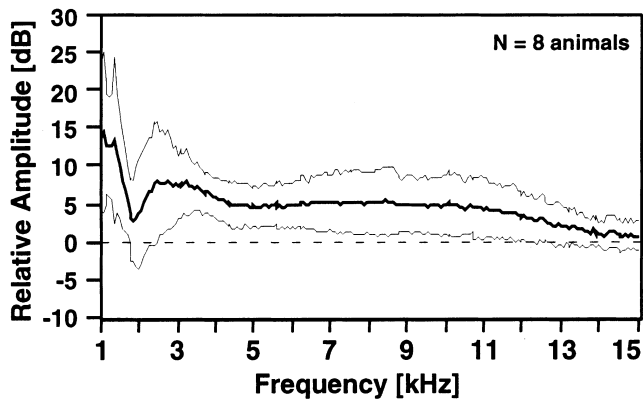


Fig. 5 Difference spectrum in the displacement amplitudes of the ipsilateral and contralateral tympanal pits (*thick line* average of eight animals, *thin lines* 1-SD interval). Positive values indicate a larger displacement of the ipsilateral pit. Average difference: +4.8 dB (SD 2.4; $n = 225$ frequency points)

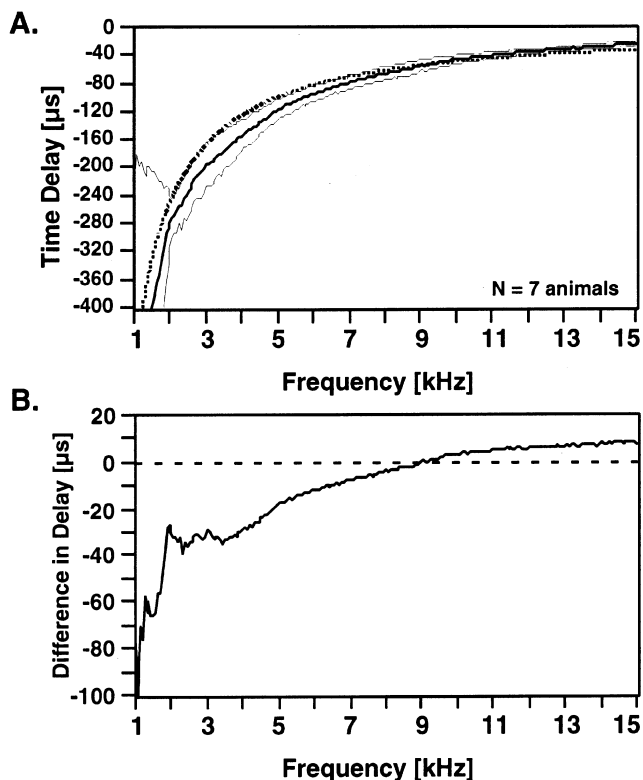


Fig. 6 A Time delay, as function of frequency, between the mechanical displacement of the ipsi- and contralateral pits as the ipsilateral pit is driven by the vibrating pin (*thick solid curve* average time delay, *thin curves*: 1-SD interval, *dotted curve* predicted time delay for a putative rigid-bridge model). **B** Difference between the measured time delay (*solid curve*) and the time delay predicted for a rigid intertympanal bridge (*dotted curve*). Note: negative time values indicate a time lag of the contralateral pit

left panels). When averaged for eight animals, this difference is -1.38 dB (SD 0.65; $n=8$ animals, $n = 225$ frequency point; Fig. 8A). These measurements guar-

antee that after the operation, the specimen can be positioned again properly and that its ipsilateral tympanal pit can be mechanically actuated in near-to-identical, thus comparable, conditions.

The displacement of the contralateral pit (as a response to the mechanical actuation of the ipsilateral pit) undergoes little change when the prothoracic coxa is removed (Fig. 7, right panels). The difference in displacement amplitude amounts to 1.75 dB (SD 1.64; $n=8$ animals, $n=225$ frequency points) over the frequency range 1–15 kHz. For the intact tympanal system, the ipsi- and contralateral difference in response amplitude is 4.8 dB (SD 2.4; $n=8$; see Fig. 5). When the tympanal system is open to the outside, this difference is 5.4 dB (SD 2.2; $n=8$). Hence, opening up the tympanal system does not significantly alter the mechanical characteristics of the intertympanal bridge.

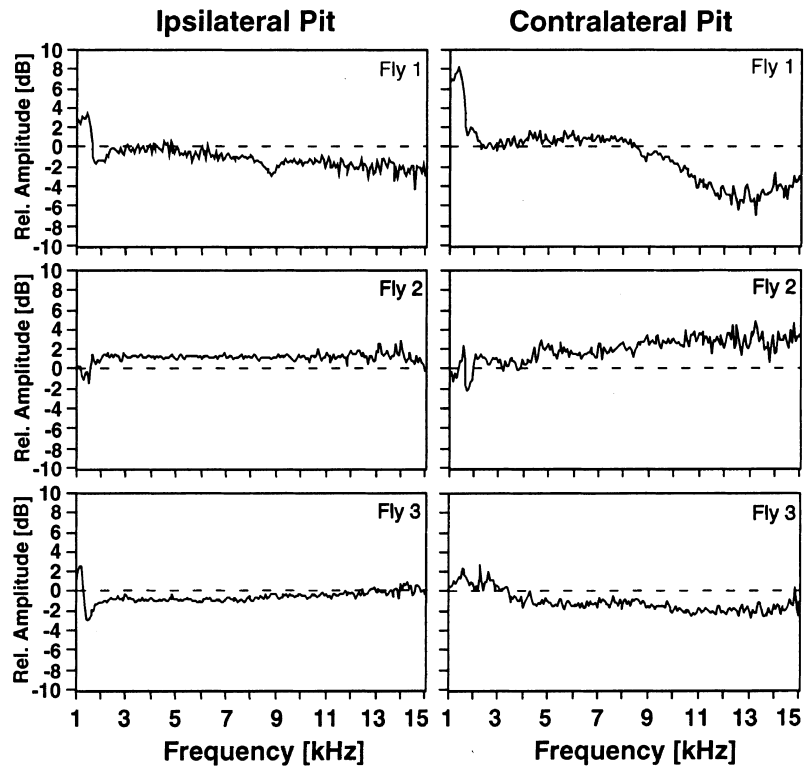
Discussion

The tympanal ears are mechanically coupled by a flexible bridge

The mechanism proposed to explain directional hearing in the fly *O. ochracea* relies on the mechanical coupling between the two hemilateral tympanal systems (Miles et al. 1995; Robert et al. 1996). This proposition forcefully predicts that mechanical coupling should also occur in the absence of acoustic stimulus. In essence, the mechanical displacement of one tympanal membrane – regardless of the nature of the force that causes it – should elicit a displacement of the contralateral tympanal membrane. Alternatively, if no coupling occurs, when one side is mechanically actuated, the contralateral side is expected to vibrate with an amplitude close to noise level (e.g., 60 dB below signal; Fig. 2B). The results show that the contralateral tympanal pit undergoes displacement with amplitudes similar, but not identical, to the side driven by the pin (see difference spectra in Figs. 4, 5). In fact, the average difference in displacement amplitude between the ipsi- and contralateral sides of the intertympanal bridge amounts to 4.8 dB (SD 2.4). In addition, it was shown that the central point of the intertympanal bridge vibrates at much lower levels than the two tympanal pits (17.6 dB, SD 2.8; $n = 8$; Fig. 3). This corroborates previous measurements made in acoustic conditions (23.3 dB, SD 4.8; Miles et al. 1995; Robert et al. 1996), and demonstrates that the bridge is rocking about its pivot point. The difference in the vibration amplitude of the pits also confirms the flexible nature of the bridge.

Another way to illustrate this flexibility is to consider the phase difference, or time difference, in the mechanical response of the pits (Fig. 6A). It is true that a rigid lever oscillating about its fulcrum elicits a phase lag of 180° between each of its extremities. At a frequency of oscillation of 5 kHz, this lag corresponds to a time difference of half a period, or 100 μ s. For the same

Fig. 7 Difference spectra in the mechanical response of each pit before and after the opening of the ear cavity. In both panels, the spectrum measured before the operation is subtracted from that measured after the operation. Examples are given for three different flies



frequency, the intertympanal bridge, however, shows a time lag of $117 \mu\text{s}$ (SD 13.7, $n=8$) between its two tympanal pits. This means that the contralateral side is slightly delayed when compared to an ideal rigid lever. When the comparison is made for a broad range of frequencies (1–15 kHz), the delay of the measured data differs from the delay predicted for a rigid lever (Fig. 6A). An important difference lies in the fact that the contralateral side consistently lags for frequencies below 9.0 kHz (Fig. 6B), which are the frequencies of salient behavioral significance for the fly (Robert et al. 1992). Since the pivot point vibrates much less, such a response is only observed if some bending occurs. This observation corroborates the previous findings made in acoustic conditions, in which two fundamental modes – a rocking and a bending mode – were identified (Miles et al. 1995; Robert et al. 1996). Interestingly, at 9 kHz, the delay measured corresponds to that predicted for a rigid bridge that would rock about its fulcrum (Fig. 6B). The results of Fig. 5 however show an amplitude difference of about 4 dB, indicating that some additional translational displacement occurs at the level of the pivot point.

The flexibility of the intertympanal bridge plays a key role in the asymmetrical mechanical response of the tympanal system. This response gives rise to the two cues essential to directional hearing: a mechanical interaural time difference, and a mechanical amplitude difference (Miles et al. 1995; Robert et al. 1996). The experiments of direct mechanical stimulation presented here first demonstrate that the intertympanal bridge mechanically connects the two hemilateral systems but also show that,

because of its flexibility, it generates mechanical interaural delays and amplitude differences.

Testing for the presence of a pressure-difference receiver system

The other mechanism known to generate a directional response in small hearing organs is the pressure-difference receiver system (Autrum 1940; Fletcher 1992; Michelsen 1994; Michelsen et al. 1994). In principle, it is not formally excluded that intertympanal coupling in *O. ochracea* is caused, even partially, by the action of a pressure-difference system. It is therefore important to formulate and test an alternative hypothesis that permits one to experimentally distinguish between the two fundamental but non-exclusive mechanisms constituted by the pressure-difference receivers and mechanically coupled receivers.

An essential feature of any pressure-difference system is that sound pressure must reach the back face of the tympana via an air column, as well as the external front face. The sound transmission characteristics of the tracheal tubes connecting the inside of the ears to the outside condition the relative amplitude and phase of the internal sound pressure. In such a system, the interaction of the external and internal sound pressures at the tympanum constitute the driving force to the tympanum (Fletcher 1992; Michelsen et al. 1994). In *Ormia*, the tympanal membranes are backed by an unpartitioned air space that is very small ($< 1 \text{ mm}^3$) and that could rapidly propagate sound pressures from one eardrum to the

other. A priori it cannot be excluded, therefore, that this small air column could generate acoustic coupling and contribute to the observed asymmetrical response. In such a situation, the stiffness of the air column ought to be high enough to provide acoustic coupling, like an air spring. One way to test the role of this is to open the internal cavity to the outside, thus increasing both the actual air volume and surface area of the opening, and dramatically decreasing the stiffness of the air spring.

The use of an acoustic stimulus in open-ear conditions is not favorable since it actually increases the number of unknown parameters in the system. Opening the tympanal system allows sound waves to reach the back of the tympana, in effect making the system under test a pressure-difference receiver. Any change in the vibratory behavior of the intertympanal bridge could then not be assigned to changes in air stiffness alone since newly created and unquantified sound pressures act from inside on the tympana and the intertympanal bridge. In contrast, direct mechanical stimulation to one ear applies only one quantifiable, non-acoustic input and can be useful for testing the presence of a pressure-difference system.

Opening of the ear cavity

As shown in Fig. 7 and 8, opening the ear cavity does not alter the vibratory behavior of the intertympanal bridge. The level of vibration of the contralateral tympanal pit does not differ much when the ear is surgically opened (average difference 1.75 dB, SD 1.64; $n=8$ animals). Hence, the difference in displacement amplitude between the ipsi- and contralateral pits is not greatly affected by opening the air cavity (Fig. 8). From these results, one can deduce that the volume of air backing the tympanal membranes does not contribute significantly to intertympanal coupling. A word of caution is however necessary at this point. Since this evidence relies on the absence of a change, the effect of opening the ear cavity deserves critical assessment and comment.

The removal of one prothoracic coxa substantially decreases the effective stiffness of the air in the tracheal sac behind the tympana. The opening resulting from the removal of one coxa causes the cavity to resemble a small Helmholtz resonator with a very short neck. At frequencies sufficiently below the resonance frequency (which we estimate to be ca. 40 kHz), when the ipsilateral tympanum is displaced inward, the opening acts to relieve the internal pressure and air will flow readily through the opening while the contralateral tympanum sees a diminished response. Because stiffness K'_3 cannot be measured directly, it remains questionable whether the opening is large enough to have the desired effect. In fact, the actual diameter of the opening due to coxal removal is that of the proximal coxal joint. For the fly specimens used in this study, the surface area of the coxal opening was measured as 0.242 mm^2 (SD 0.042;

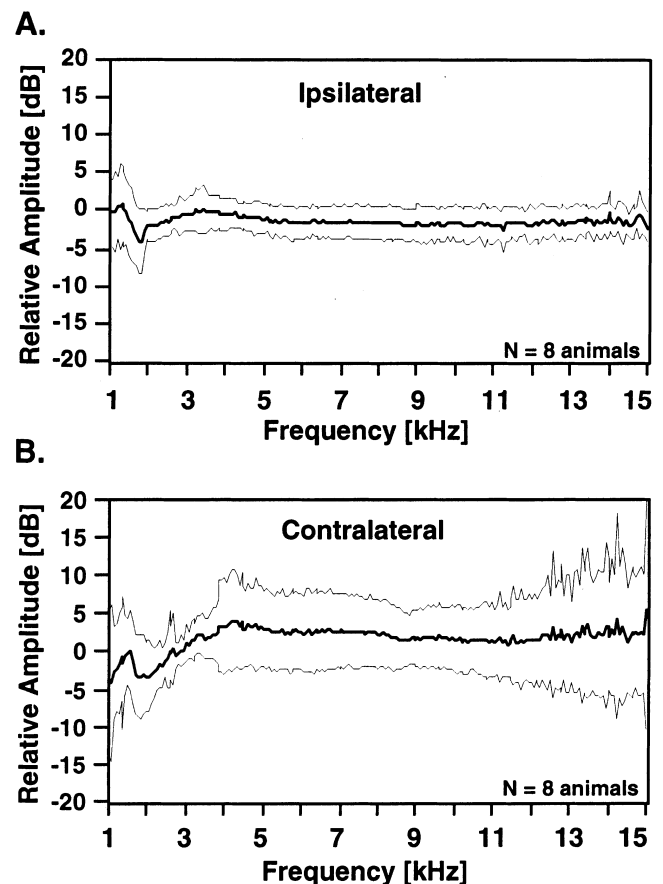


Fig. 8A, B Mechanical response of the tympanal pits after opening the ear cavity minus that obtained before opening the cavity. **A** Difference spectrum of the ipsilateral pit. Average difference: -1.38 dB (SD 0.65; $n=8$ animals, $n=225$ frequency points). **B** Difference spectrum of the contralateral pit. Average difference: 1.75 dB (SD 1.64; $n=8$ animals, $n=225$ frequency points) (thin curves ± 1 -SD interval)

$n=8$). By comparison, the surface area of one tympanal membrane is about 0.288 mm^2 (SD 0.056; $n=5$; Robert et al. 1994). It is very likely that an opening about the size of one tympanal membrane, and adjacent to it, has a significant effect on the acoustical characteristics of the tracheal sac. In fact, in conditions of acoustic stimulation, the response amplitude of the tympanal system decreases significantly as the prothoracic coxa is removed (data not shown). Such a decrease is due to sound acting on the back of the tympanal system. This shows that the hole resulting from coxal removal is sufficiently large, or acoustically transparent, for sound of travel in the ear cavity and alter internal acoustical conditions. It seems therefore reasonable to surmise that, in the absence of sound, this opening increases the actual volume of the air cavity so as to decrease its stiffness significantly.

In conclusion, it has been shown that the application of the method of direct mechanical actuation constitutes a powerful technique to investigate the mechanics of a tympanal system. The results presented show that the vibrations of one side of the intertympanal bridge are

accompanied by vibrations of the other side. Thus, the two hemilateral tympanal systems are mechanically coupled by the intertympanal bridge. This corroborates the findings made earlier in acoustical conditions (Miles et al. 1995; Robert et al. 1996). In addition, coupling could not be shown to depend on the volume of the air cavity backing the tympanal membranes. It is concluded that the directionality of the mechanical response of the ears of *O. ochracea* occurs through the sole action of intertympanal mechanical coupling and does not rely on a pressure-difference receiver mechanism.

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