

## DIRECTIONAL HEARING IN GRASSHOPPERS: NEUROPHYSIOLOGICAL TESTING OF A BIOACOUSTIC MODEL

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*Accepted 29 October; published on WWW 14 December 1998*

### Summary

A recently proposed biophysical model for directional hearing in grasshoppers was tested using complex stimulus situations, with two loudspeakers, one on either side of the animal, synchronously emitting sinusoids with defined phase and amplitude relationships. Hearing responses were determined from whole nerve recordings and compared with the predictions of the model. In *Schistocerca gregaria*, there were only minor differences between the predictions of the model and measurements and, by reducing the value of the gain of the internal sound path measured previously, a close agreement was achieved between model and measured hearing responses. In *Chorthippus biguttulus*, larger discrepancies between model calculations using the values measured previously and neuronal response functions were found in both shape and amplitude. A better fit between measurements and model predictions was achieved by increasing the values of the internal delay over

those measured previously. The measurements presented here indicate high inter-individual variability of the parameters of the internal pathway, with a range of 60° for the internal phase delay. Calculating the directional characteristics using this range of values for the internal delay indicated that sufficient directional information was available down to 5 kHz. Increasing the value of the internal delay over that measured in an earlier study therefore provides an explanation for the discrepancy between the poor directional information attributed to *C. biguttulus* in that study and the excellent lateralization ability of males of this species at 5 kHz.

Key words: hearing, bioacoustic model, *Schistocerca gregaria*, *Chorthippus biguttulus*, directional hearing, sound localization, pressure difference receiver.

### Introduction

Directional hearing in small animals, with body sizes smaller than the wavelength of the sounds of interest, is achieved by ears that act as pressure difference (gradient) receivers (for a review, see Michelsen, 1994), with sound acting on both sides of a tympanum. The phase and amplitude of the sound pressures acting on the internal and external surfaces of the tympanum depend on the direction of sound incidence, resulting in directional responses of the ear. The hearing systems of acridids (grasshoppers and locusts) and gryllids have been particularly well investigated. In the cricket, hearing is tuned in both sensitivity and directionality to the frequency range of the pure tone calling song (Michelsen et al., 1994a; Michelsen and Løhe, 1995). In the grasshopper, however, the situation is more complex: here, the stridulatory songs are usually non-resonant and therefore cover a broad frequency range. An ear with a directionality tuned to one narrow frequency band is unlikely to be ideal for these insects.

The ears of grasshoppers and locusts are located on each side of the first abdominal segment. A sclerotized ring encircles the tympanal membrane, to which Müller's organ, containing 60–80 receptor cells, is attached. Between the ears are air-filled tracheal sacs, constituting an interaural sound pathway.

Various investigators, using biophysical and neurophysiological methods, have suggested that the locust ear acts as a pressure gradient receiver at low frequencies and as a pressure receiver at higher frequencies (Michelsen, 1971b; Römer, 1976; Miller, 1977). Recently, Michelsen and Rohrseitz (1995) investigated the physical mechanisms involved in directional hearing in *Schistocerca gregaria* and *Chorthippus biguttulus*. Using laser vibrometry, they determined the amplitude and phase relationships (their dependence on sound frequency and angle of incidence) for sound reaching the tympanum directly from the outside and through the interaural pathway. Using these data, they were able to calculate a model for the directional characteristic of the ear. For the larger insect (*S. gregaria*), this model was a good predictor of the directionality as measured by laser vibrometry and was in close agreement with the results of earlier investigations.

For the smaller insect (*C. biguttulus*), Michelsen and Rohrseitz (1995) found the measured phase delay at low frequencies too short to be physically meaningful. Using these measured values, the model of Michelsen and Rohrseitz (1995) predicted few if any directional cues at 5 kHz, which

contradicted direct measurements of directionality and also behavioural studies demonstrating that *C. biguttulus* males could reliably localize a song with this carrier frequency (Fig. 1). Predictions of model calculations based on longer, more realistic, phase delays were in good agreement with their measured directionality at this frequency. Whether the complications involved in calculating directional hearing in *C. biguttulus* were due to problems encountered in measuring the phase of the internally transmitted sound or instead to more general problems concerning the assumptions used in modelling the grasshopper hearing system was not clear.

In the present study, we challenge the model of Michelsen and Rohrseitz (1995) by comparing the predictions calculated according to their model, for complex stimulus situations, with electrophysiologically determined auditory responses. The stimulation followed the experimental arrangement used in behavioural experiments with *C. biguttulus* (von Helversen and Rheinlaender, 1988) with the insect placed between two loudspeakers that emitted sound synchronously. This experimental arrangement allows the amplitude and phase of the two signals to be manipulated independently and the dependence of the auditory responses on these parameters to be measured directly. The aim of the present study is to determine whether the model of Michelsen and Rohrseitz (1995) is able to explain directional hearing in acridids and whether modifications to the values of phase and amplitude of the sound transmitted through the internal pathway, similar to those suggested by Michelsen and Rohrseitz (1995), help to resolve the contradictions mentioned above.

## Materials and methods

### Animal preparations

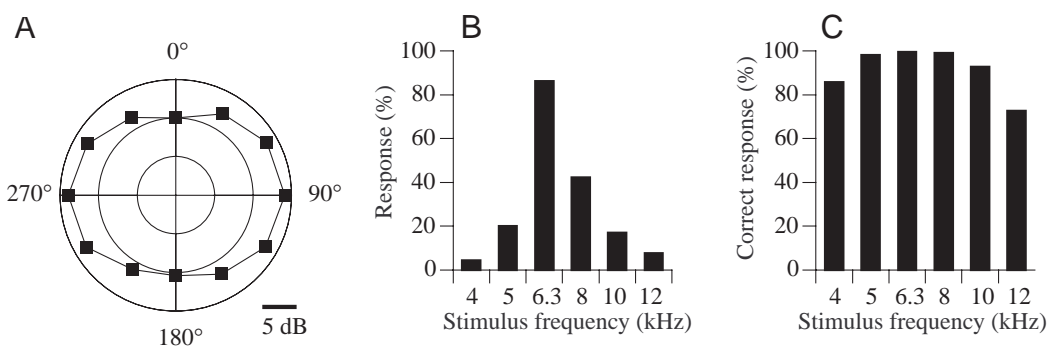
Adult male insects of *Schistocerca gregaria* and *Chorthippus biguttulus* were used in the experiments. *S. gregaria* came from a culture in the University of Konstanz, *C. biguttulus* were taken from wild populations in Bavaria. The animals were briefly anaesthetized with CO<sub>2</sub> and, after removal of all legs and wings, they were fixed ventral side up on a brass support (height 0.4 mm, length 60 mm, width 5 mm for *S.*

*gregaria*, 1.5 mm for *C. biguttulus*) with a wax/resin mixture. All wounds caused by the removal of the legs were sealed with the wax/resin mixture to prevent the insects from drying out. Whole nerve recordings of the tympanic nerve were used to determine the hearing responses. The preparations used in the two species were slightly different. In *S. gregaria*, an opening was cut into the metathoracic sternite, exposing both tympanic nerves close to their point of entry into the metathoracic ganglion. Both nerves were placed on electrolytically sharpened tungsten hook electrodes. The electrodes were attached to a brass rod (8 mm diameter) placed 10 cm behind the insect. The recording sites were sealed and insulated with petroleum jelly. In *C. biguttulus*, only one tympanal nerve was exposed by removing lateral parts of the mesothoracic sternite and apodeme. A thin steel wire (100 µm in diameter) was placed under the tympanic nerve and insulated and sealed with a silicone paste (Bayer Baysilone). The wire was glued to the animal holder; thus, no additional electrode holder was present in the sound field. A silver wire inserted into the abdomen served as an indifferent electrode in both preparations. The recorded signals were amplified using custom-made amplifiers (×400), bandpass-filtered (100–3000 Hz) and stored on digital audio tape (Sony PC 208A). A trigger signal was recorded on a separate track for off-line synchronization of stimulus and response.

### Stimulation

The stimuli were delivered *via* two loudspeakers (Technics 10TH400C), each positioned 45 cm from either side of the insect, perpendicular to its body axis (Fig. 2A). Signals were generated using computer-aided D/A converter systems (12- or 16-bit resolution, 200 or 250 kHz sampling rate). The signals were amplified and their amplitude was controlled with the aid of a computer. The stimuli were pure tone pulses of various frequencies (5–12 kHz) with a 10 ms plateau and 1 ms rise and fall time. Stimulus repetition rate was 1 Hz for all experiments. The entire apparatus was lined with sound-absorbing material. Because the loudspeakers faced each other, the front was also covered with sound-absorbing material, except for the sound-radiating slits. Nevertheless, slight echoes with maximum

Fig. 1. (A) Directional pattern for a right ear of *Chorthippus biguttulus* at 5 kHz calculated according to the model of Michelsen and Rohrseitz (1995). 0° corresponds to the front, 90° to the right-hand side of the insect. (B) Percentage of synthetic female songs evoking turning responses of male *C. biguttulus* at different stimulus centre frequencies. The songs consisted of bandpass-filtered noise (one-third octave bandwidth). (C) Percentage of correct turns towards the loudspeaker during the responses to the different stimulus centre frequencies. (B,C) Pooled data from five males, 145–424 stimulations per centre frequency. Stimulation from 90° lateral at 52–57 dB SPL (D. von Helversen, unpublished observations). At 5 kHz, stimuli were not attractive and evoked few responses (B), but all five males were able to localize these stimuli, since hardly any mistakes occurred in those cases where they responded (C).



amplitudes below  $-30$  dB were unavoidable (Fig. 2B). The sound field was tested for homogeneity using a probe microphone (Bruel & Kjaer 4182) and a sound level meter (B&K 2209). All sound amplitudes were calibrated using its 'peak hold' function, and are given as dB peak sound pressure level (SPL) (re.  $2 \times 10^{-5} \text{ N m}^{-2}$ ).

The insect, attached to a free-standing holder (brass, diameter 5 mm), was placed midway between the loudspeakers, 3–4 cm above the bottom lining (Fig. 2A). The exact centre of the apparatus was determined acoustically: during simultaneous playback of two sine waves of identical

frequency, the sound pressure in the centre depends on the phase relationship between the two sine waves. For  $0^\circ$  phase difference, the amplitude in the middle should be twice the amplitude of one sine wave alone; with a phase shift of  $180^\circ$ , the two signals should cancel each other, and the recorded amplitude should be at a minimum. Using a small microphone (probe B&K 4182 or 1/8 inch B&K 4138), the position of minimum amplitude was determined for a phase difference of  $180^\circ$ , and the midline of the insect was placed below the microphone (Fig. 2B). The homogeneity of the sound field was verified prior to each experiment by checking the centre position using at least two frequencies (5 kHz and 8 or 12 kHz). All phase and amplitude relationships for simultaneously presented stimuli refer to the central position between the loudspeakers.

In *S. gregaria*, the responses of the two tympanic nerves recorded in the same preparation showed a high degree of similarity during both experimental situations (see below), which also demonstrates the symmetry of the sound field. In *C. biguttulus*, where only one nerve of each preparation was used, there were no qualitative differences in response between preparations in which recordings were made from the left or the right tympanic nerve.

#### Stimulus protocol

An amplitude/response function (reference function) was constructed with ipsilateral stimulation for each frequency used, over a range of 45 dB in steps of 4.5 dB. This function was used to convert the recorded responses to sound pressure levels during two-loudspeaker stimulation (see below). Two sets of experiments were conducted with each species.

#### Phase experiment

In this experiment, the phase relationship between the two simultaneously presented stimuli was varied. In practice, the phase of one loudspeaker (the reference speaker) was held constant, while the phase of the other loudspeaker was varied. Signals of 5 kHz were used for the phase experiment in both species. For *C. biguttulus*, the amplitudes of the two signals were identical during the phase experiment; for *S. gregaria*, the amplitude of the loudspeaker with the modified phase was lower by 5 dB relative to the reference loudspeaker.

#### Amplitude experiment

In this experiment, the simultaneously replayed signals had no phase shift, but were varied in amplitude: the amplitude of one loudspeaker was held constant (and set to 0 dB), while the amplitude of the other was reduced stepwise from 0 dB to  $-15$  dB (*S. gregaria*) or  $-21$  dB (*C. biguttulus*). The frequencies used here were 5 kHz for *S. gregaria* and 5 kHz, 8 kHz and 12 kHz for *C. biguttulus*.

During both phase and amplitude experiments, all stimulus combinations were presented twice, with loudspeaker positions reversed. The amplitude of the loudspeaker held constant was 71 dB SPL in both types of experiments using *C. biguttulus*, and at 59 dB SPL or 71 dB SPL, depending on the dynamic

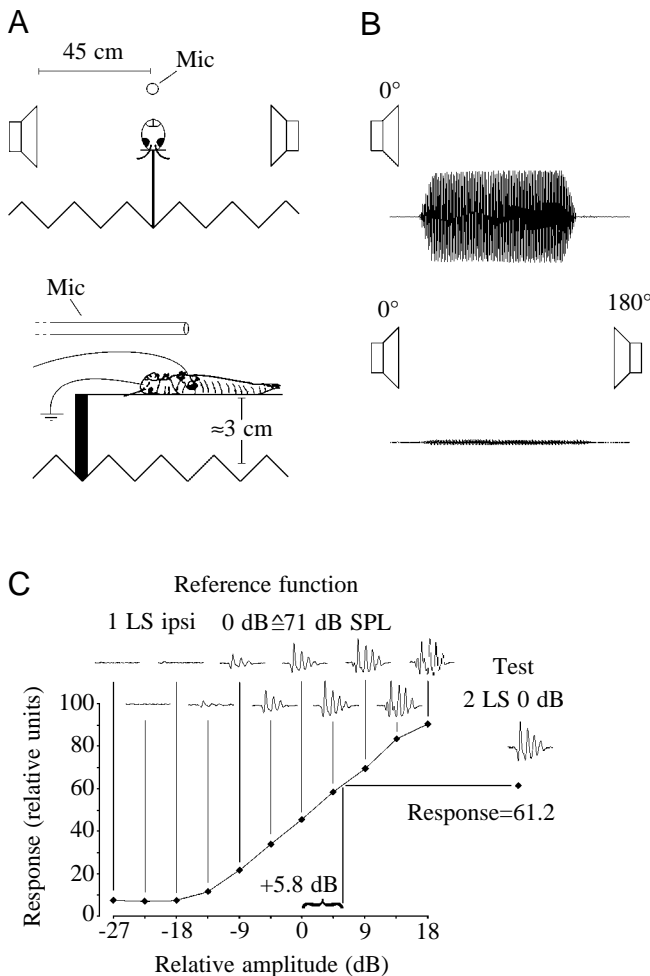


Fig. 2. (A) Schematic drawing (not to scale) of the experimental arrangement (Mic, microphone). (B) Oscillograms of an 8 kHz stimulus recorded at the position of the insect. Stimulation with one loudspeaker (LS) (top) or two loudspeakers with a phase relationship of  $180^\circ$  (bottom). (C) The dependence of the neuronal response on sound pressure level at one ear. The trace length of the summed action potential of the tympanic nerve was taken as the magnitude of the response. To convert the responses during a test situation with two loudspeakers into sound pressure, we used the trace length of the response (in this example 61.2) to interpolate the corresponding sound pressure level resulting in the same response magnitude (in this case +5.8 dB). ipsi, ipsilateral. For further explanation, see the text.

range of the reference function, for experiments with *S. gregaria*.

All stimulus combinations were presented 50 times. For *S. gregaria*, the 50 repetitions of each stimulus were presented consecutively. At the beginning and end of each experiment, the reference function was tested to check for the effects of changes in recording quality. In *C. biguttulus*, a different protocol was used: all stimulus combinations including the reference function were presented once, and the whole sequence was repeated 50 times. This procedure guaranteed almost simultaneous measurement of all stimulus combinations, thus excluding effects due to changes in recording quality.

#### Data evaluation

The whole-nerve recordings were digitized (12-bit A/D converter, 10 kHz sampling rate), and the 50 responses to each stimulus were averaged. Averaged responses well above threshold resembled damped oscillations (Fig. 2C). The length of the trace during the 30 ms following stimulus onset was taken as a measure of response magnitude. This measure provided a larger dynamic range for the amplitude/response function than the peak-to-peak amplitude (see Löhe and Kleindienst, 1994). For each test situation (with two-loudspeaker stimulation), the response magnitude was used to read out the corresponding sound pressure level required in the one-loudspeaker situation. In practice, interpolation was carried out if the response fell within the dynamic range of the reference function (Fig. 2C). In both phase and amplitude experiments, the amplitude of the signal that was held constant and presented alone from the ipsilateral loudspeaker was used as the reference amplitude (i.e. 0 dB). The data were evaluated separately for the ear ipsilateral to the loudspeaker held constant and for the ear ipsilateral to the loudspeaker varied in phase or amplitude. To calculate the interaural response difference, the animal was assumed to be perfectly symmetrical, and differences between responses of one ear to reciprocal stimulus situations were computed.

#### Model calculations

The model of Michelsen and Rohrseitz (1995) is based on the assumption that the tympanum is driven by two components: one component ( $\mathbf{p_r}$ ) is the sound wave acting on the external surface, the other ( $\mathbf{p_b}$ ) is the sound wave acting from the inside (Fig. 3A), with  $\mathbf{p_b}$  originating only from sound entering through the contralateral tympanum and not through other possible sound inputs. These two sound pressures can be conveniently represented as vectors, thus allowing the total sound pressure ( $\mathbf{P}$ ) driving the tympanum to be calculated by summing these two sound pressures (Fig. 3A). The amplitudes and phases of the sound pressures acting on the outside of the tympana were measured by Michelsen and Rohrseitz (1995; Figs 4, 7) using a probe microphone. The transmission gain ( $b$ ) and the phase shift ( $\beta$ ) of the internal pathway were calculated from measurements of the tympanal

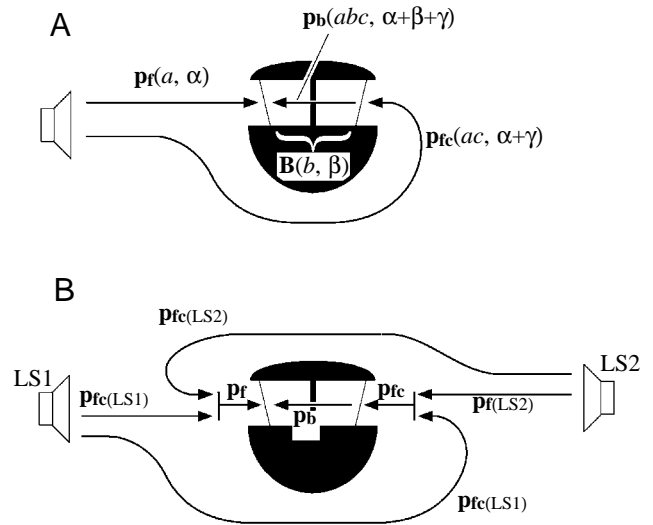


Fig. 3. Schematic drawing of the sound paths in the grasshopper auditory system. This model (adapted from Michelsen and Rohrseitz, 1995) was used to predict the excitation of the ear of the grasshopper. Sound paths for stimulation by one loudspeaker (LS) (A) and by two loudspeakers (B). (A) Two forces act on the tympanum:  $\mathbf{p_r}$  on the external surface, and  $\mathbf{p_b}$  on the internal surface.  $\mathbf{p_b}$  is determined by the force  $\mathbf{p_{rc}}$  acting on the external surface of the contralateral tympanum and the transfer function of the internal sound pathway B. (B) For the two-loudspeaker situation, the forces acting on the external surface of both tympana ( $\mathbf{p_r}$  and  $\mathbf{p_{rc}}$ ) are the vector sum of the signals from the two loudspeakers ( $\mathbf{p_{r(1S1)}}$  and  $\mathbf{p_{r(2S2)}}$  for  $\mathbf{p_r}$ , and  $\mathbf{p_{rc(1S1)}}$  and  $\mathbf{p_{rc(2S2)}}$  for  $\mathbf{p_{rc}}$ ). For further explanation, see the text.  $a$ , stimulus amplitude;  $b$ , internal gain;  $c$ , external gain;  $\alpha$ , stimulus phase;  $\beta$ , internal delay;  $\gamma$ , external delay.

vibrations and are given in Figs 5 and 8 in Michelsen and Rohrseitz (1995).

For our model calculations, we used the stimulus parameters to describe  $\mathbf{p_r}$ , the vector acting on the outside of the tympanum: for the stimulus held constant in our experiments, we set the amplitude ( $a$ ) to 1 and the phase ( $\alpha$ ) to  $0^\circ$ ; for the manipulated stimulus, the amplitude and phase were set relative to the parameters of the constant stimulus. The sound pressure acting on the outside of the contralateral tympanum is determined by the parameters of the stimulus ( $a, \alpha$ ) and the transmission of the sound around the insect, with an amplitude (=gain,  $c$ ) and a phase (=delay,  $\gamma$ ) component. The value of  $\mathbf{p_{rc}}$  was calculated by multiplying the amplitudes ( $ac$ ) and summing the phase components ( $\alpha + \gamma$ ). The values of  $c$  and  $\gamma$  were taken from Figs 4 and 7 in Michelsen and Rohrseitz (1995) as the difference between the values given for the right (ipsilateral) ear and the left (contralateral) ear for  $90^\circ$  sound incidence (in their notation, the right side). Values needed for our model calculations that were not measured by Michelsen and Rohrseitz (1995) (8 kHz and 12 kHz stimuli for *C. biguttulus*) were interpolated from their measurements.

The sound pressure acting on the inner surface of the

tympanum  $\mathbf{p_b}$  is the sound pressure acting on the outside of the contralateral tympanum  $\mathbf{p_{fc}}$  modified by the internal sound pathway ( $\mathbf{B}$ ), which is again described by an amplitude (=gain,  $b$ ) and phase (=delay,  $\beta$ ) component (Figs 5 and 8 in Michelsen and Rohrseitz, 1995). To calculate  $\mathbf{p_b}$ , the amplitudes of  $\mathbf{p_{fc}}$  (=ac) and  $\mathbf{B}$  (=b) were multiplied, and their phases ( $\alpha+\gamma$ ;  $\beta$ , respectively) were summed (Fig. 3A).

Finally, we determined the driving force acting on the tympanum by vector summation of  $\mathbf{p_r}$  and  $\mathbf{p_b}$ . To account for the different signs of  $\mathbf{p_r}$  and  $\mathbf{p_b}$  (which act on opposing sides of the tympanum),  $180^\circ$  was added to the phase angle of the internal pathway. The total sound pressure  $\mathbf{P}$  acting at the contralateral tympanum was determined in an analogous manner.

Each phase shift ( $\gamma$ ,  $\beta$ ) can also be expressed as a delay. For the external phase shift ( $\gamma$ ),  $0^\circ$  means that the sound reaches the ipsilateral and the contralateral tympana at the same time; for the internal phase shift ( $\beta$ ),  $180^\circ$  means that sound propagates from one ear to the other instantaneously. Smaller or negative phase shifts mean that sound propagation takes a certain time.

For our purposes, we introduced a second loudspeaker (LS) to the model. Therefore, the forces acting on the external surfaces of the tympana ( $\mathbf{p_r}$  and  $\mathbf{p_{fc}}$ ) had to be adapted (Fig. 3B). They were calculated by vector summation of the vectors representing the sound pressures from the two loudspeakers reaching one ear, i.e.  $\mathbf{p_r}$  was the sum of  $\mathbf{p_{r(1S1)}}$  and  $\mathbf{p_{r(1S2)}}$  (Fig. 3B). The individual vectors were calculated according to the scheme given above. After calculation of  $\mathbf{p_r}$  and  $\mathbf{p_{fc}}$ , the procedure to determine the driving force acting at one tympanum ( $\mathbf{P}$ ) was identical to the one-loudspeaker situation.

We normalized the model calculations for the two-loudspeaker situations, in that we defined 0 dB total sound pressure acting on the tympanum as the sound pressure calculated for ipsilateral presentation alone of the signal held constant in the different experiments. This procedure was equivalent to the normalization applied to the neurophysiological results, where the responses measured for the ipsilateral presentation of the signal held constant in the different experiments was set to 0 dB (see above).

The model responses are therefore determined by four parameters describing the biophysical properties of the grasshopper: the gain and delay of the external sound pathway ( $c$ ,  $\gamma$ ) and the gain and delay of the internal transmission ( $b$ ,  $\beta$ ). We calculated model predictions for all our experimental situations using the parameters determined by Michelsen and Rohrseitz (1995). Additionally, we made calculations for models using modified parameter values in order to achieve better congruence between experimental and theoretical results.

The model suggested by Michelsen and Rohrseitz (1995) is approximative in nature, since it does not consider other possible factors that might influence the driving force on the tympana. Such a factor could be sound being reflected on the internal surfaces of the tympana. These factors are likely to be

small (e.g. because of impedance mismatches), so that it is reasonable not to include them into the model.

#### Calculation of directionality

Using the model described above, we calculated the directionality for one tympanal organ of *C. biguttulus*. The sound pressures acting on the two tympana ( $\mathbf{p_r}$  and  $\mathbf{p_{fc}}$ ) for the different directions of sound incidence were taken from Fig. 7 in Michelsen and Rohrseitz (1995). The calculation of the driving forces on one tympanum was performed according to the scheme given above. Interaural sensitivity differences were determined as the difference between the directionality of one ear and its mirror image. Directionalities were calculated using the model parameters determined by Michelsen and Rohrseitz (1995) and also using modified parameters for the internal pathway.

### Results

#### Schistocerca gregaria

##### Phase experiment

In this experiment, the phase and amplitude of the signal from one of the loudspeakers (taken as reference) were kept constant, while the phase of the signal from the contralateral speaker was varied and its amplitude set at a value 5 dB lower than that of the reference. Fig. 4A shows the responses of the ear ipsilateral to the reference loudspeaker for different phase relationships of the two signals. The response function was rather flat, with maximum responses appearing between  $60^\circ$  and  $120^\circ$  phase shift, and minimum responses at  $240^\circ$ . The peak-to-peak amplitude was approximately 5–8 dB for the individual preparations. The responses of the ear contralateral to the reference had a distinctly larger range of 15–20 dB (Fig. 4B). Minimum responses were found for a phase shift of  $120^\circ$ , and the maximum appeared at approximately  $300^\circ$ . The interaural response difference, i.e. the difference between the responses shown in Fig. 4A and Fig. 4B, is given in Fig. 4C. Here, the peak-to-peak amplitude ranges for the individual curves fell between 10 and 25 dB, with a difference of 15 dB between the peaks of the averaged responses.

The shapes of the curves for the averaged responses exhibit high congruence with those of the model calculations based on the values determined by Michelsen and Rohrseitz (1995). However, although the maxima and minima of both curves appeared at approximately the same values of phase shift, a distinct difference in amplitude occurred, with the model predicting much smaller values than those found in our measurements; for example, in the difference curve, a measured mean amplitude of 15 dB contrasts with a predicted amplitude of 9 dB (Fig. 4C).

The influence of the four model parameters on the predictions for interaural response difference is shown in Fig. 5. We varied one parameter at a time, while holding the other three at the original values. Changes in both external and internal delay ( $\gamma$  and  $\beta$  in Fig. 3A) resulted in a shift of the peak values of phase shift, but no clear changes in amplitude

occurred (Fig. 5B,D). Changing the external and internal gain ( $c$  and  $b$  in Fig. 3A) resulted in changes in the response amplitude only (Fig. 5A,C). While the external gain had only moderate effects on the amplitude (Fig. 5A), a decrease in the internal gain led to a large increase in amplitude (Fig. 5C). Adjusting the internal gain from the Michelsen and Rohrseitz (1995) value of 0.45 down to 0.25 predicts an amplitude of approximately 20 dB. The curves predicted by the model using this modified value of 0.25 for the internal gain show an almost perfect match with our measured response curves (Fig. 6).

#### Amplitude experiment

In this experiment, the phase relationship of the two signals remained constant at  $0^\circ$ , while the amplitude of one signal was attenuated from 0 dB down to  $-15$  dB. The interaural response difference, i.e. the response of the ear ipsilateral to the attenuated signal subtracted from the response of the ear ipsilateral to the constant signal, is shown in Fig. 7. Up to amplitude differences of 10 dB, the mean response difference is slightly greater than half the magnitude of the amplitude difference between the speakers. For greater amplitude

#### *Schistocerca gregaria*

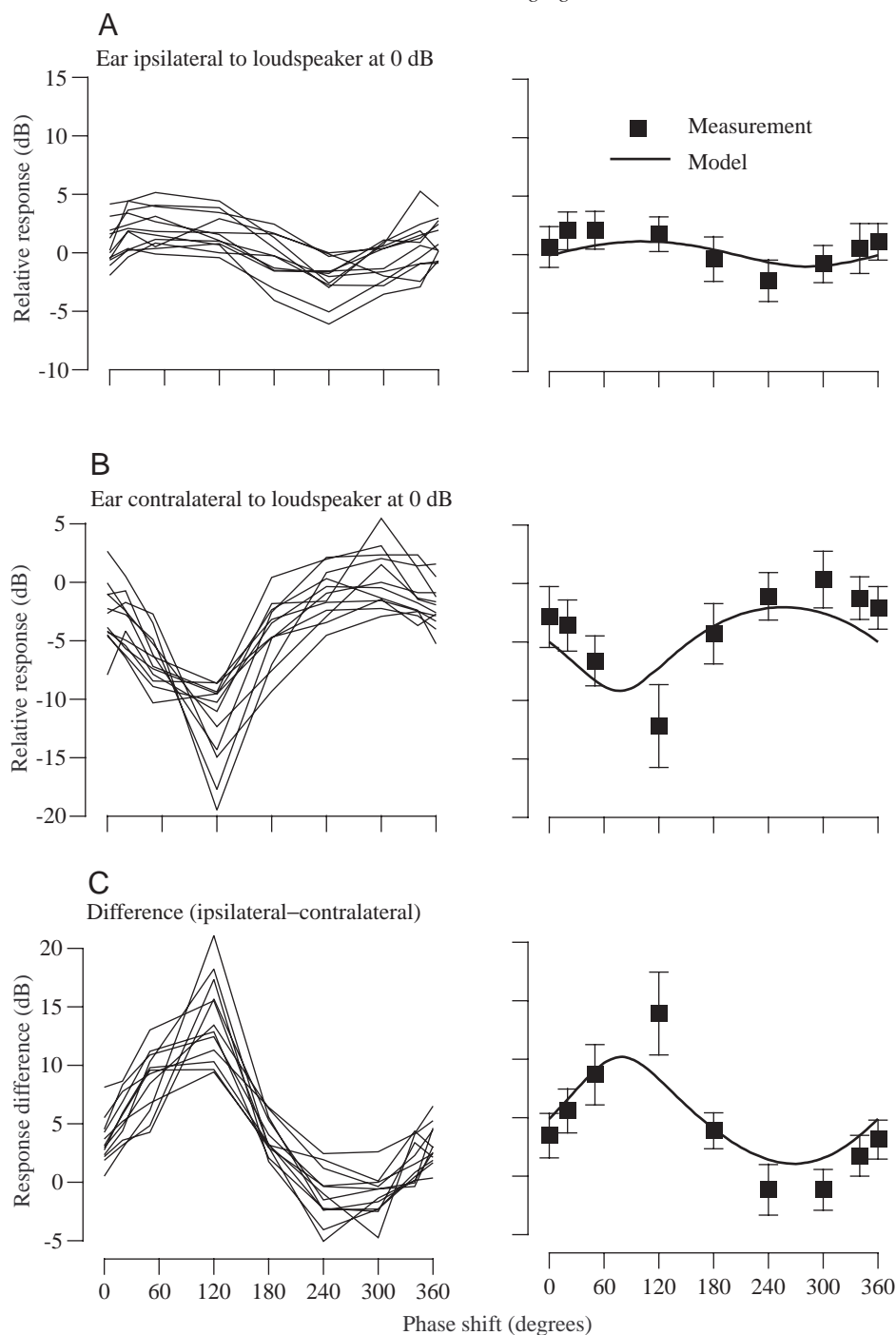


Fig. 4. Tympanic responses of *Schistocerca gregaria* to two synchronous 5 kHz tones differing in phase (phase experiment) measured and calculated according to Michelsen and Rohrseitz (1995). The neuronal responses were converted to sound pressure levels using the calibration procedure shown in Fig. 2C. The phase angle of one signal (with 0 dB amplitude) was held constant, while the phase angle of the other (with  $-5$  dB amplitude) was varied. (A) Response of the ear ipsilateral to the signal at 0 dB. (B) Response of the ear contralateral to the signal at 0 dB. (C) Response difference between the ears ipsilateral and contralateral to the signal at 0 dB. Results for individual preparations are shown on the left, averaged response and predictions according to the model are shown on the right. The error bars indicate standard deviations ( $N=12$ ). The model predictions are based upon the parameter values determined by Michelsen and Rohrseitz (1995).



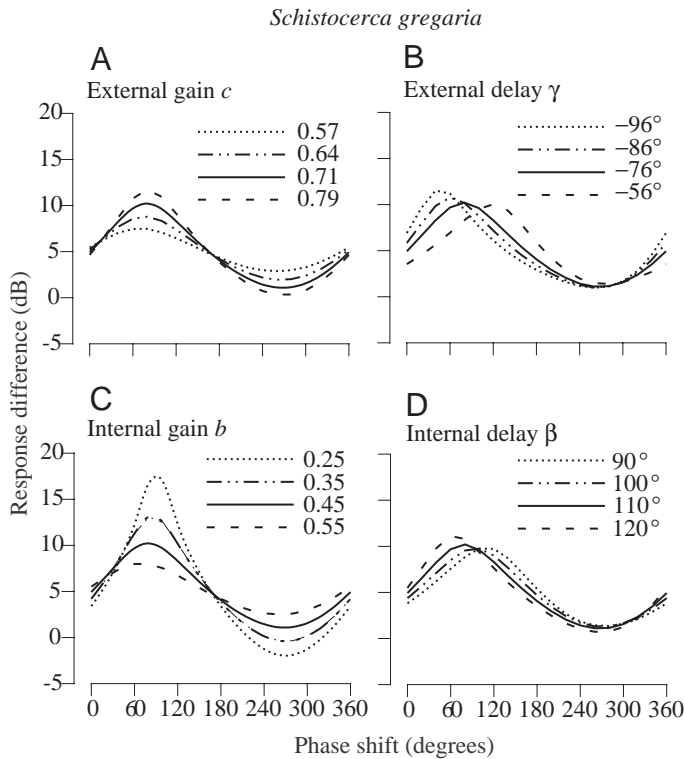


Fig. 5. Influence of the four model parameters describing the external and internal sound path on the model predictions for the phase experiment in *Schistocerca gregaria* for a frequency of 5 kHz. One parameter was changed at a time, while the three others were kept at the original values determined by Michelsen and Rohrseitz (1995) (M&R). Solid line: original parameters ( $c_{M\&R}=0.71$ ,  $\gamma_{M\&R}=-76^\circ$ ,  $b_{M\&R}=0.45$ ,  $\beta_{M\&R}=110^\circ$ ). Modification of (A) the external gain ( $c$  in Fig. 3), (B) the external delay ( $\gamma$ ), (C) the internal gain ( $b$ ) and (D) the internal delay ( $\beta$ ). See the text for further details.

differences, the steepness of the response difference function decreases, reaching a response difference of 7 dB at an amplitude difference of 15 dB between the stimuli. The curve predicted by the model using the original value of internal gain,

Fig. 7. The dependence of the tympanic responses of *Schistocerca gregaria* on the amplitude relationships of two synchronous 5 kHz tones (amplitude experiment). The amplitude of one signal was held constant (at 0 dB), while the amplitude of the other was varied; the phase relationship of the two tones was  $0^\circ$ . The response difference between the ear ipsilateral and the ear contralateral to the signal at 0 dB is shown. Results for individual preparations are shown on the left, and averaged response and model predictions are shown on the right. The error bars indicate standard deviations ( $N=16$ ). The predictions are calculated using the original parameter values (dashed line) and using an internal gain  $b$  of 0.25 (solid line) instead of  $b_{M\&R}=0.45$ , the original value determined by Michelsen and Rohrseitz (1995).

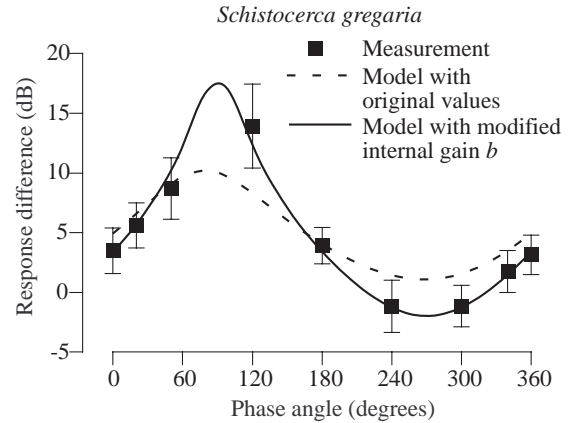
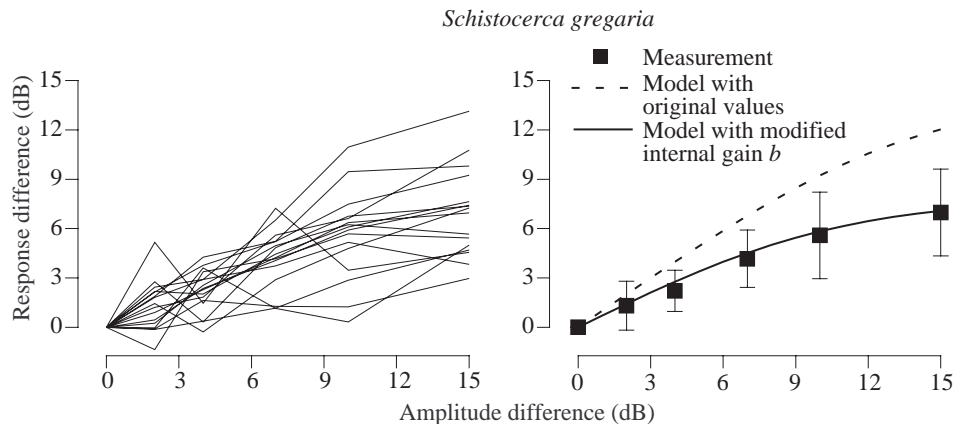


Fig. 6. Averaged interaural response difference during the phase experiment in *Schistocerca gregaria* compared with the prediction of a model using the original parameters of Michelsen and Rohrseitz (1995; dashed line) and a model using an internal gain of 0.25 instead of 0.45 (solid line). These are the same measurements as those presented in Fig. 4C. Measured values are means  $\pm$  S.D.,  $N=12$ .

0.45, proposed by Michelsen and Rohrseitz (1995), had a similar shape but, as in the phase experiment, was quite different in amplitude. In this case, the predicted amplitudes were approximately twice as large as those measured. Applying the same modifications to the model calculation (a reduction of the internal gain from 0.45 to 0.25) resulted in an almost perfect match between measurements and model predictions.

Thus, the neurophysiological responses of the ear of *S. gregaria* in both the phase and amplitude experiments are in close agreement with the model of Michelsen and Rohrseitz (1995) when the gain of the interaural pathway for 5 kHz is reduced from 0.45 to 0.25.

#### *Chorthippus biguttulus*

##### Phase experiment

The phase experiment with *C. biguttulus* was conducted with the signals from both the loudspeakers presented at the

same amplitude, resulting in mirror-imaged functions for the ears ipsilateral and contralateral to the signal kept at  $0^\circ$ . We therefore present only the measurements for the ipsilateral ear. The responses of the different preparations showed high variability (Fig. 8A). The phase shift resulting in minimum responses varied from  $180^\circ$  to  $300^\circ$ , while that resulting in maximum responses varied from  $330^\circ$  to  $180^\circ$ . The peak-to-peak amplitude ranged from 3 dB to 14 dB ( $9.8 \pm 3.0$  dB, mean  $\pm$  s.d.;  $N=13$ ) for different preparations. Owing to shifts in the

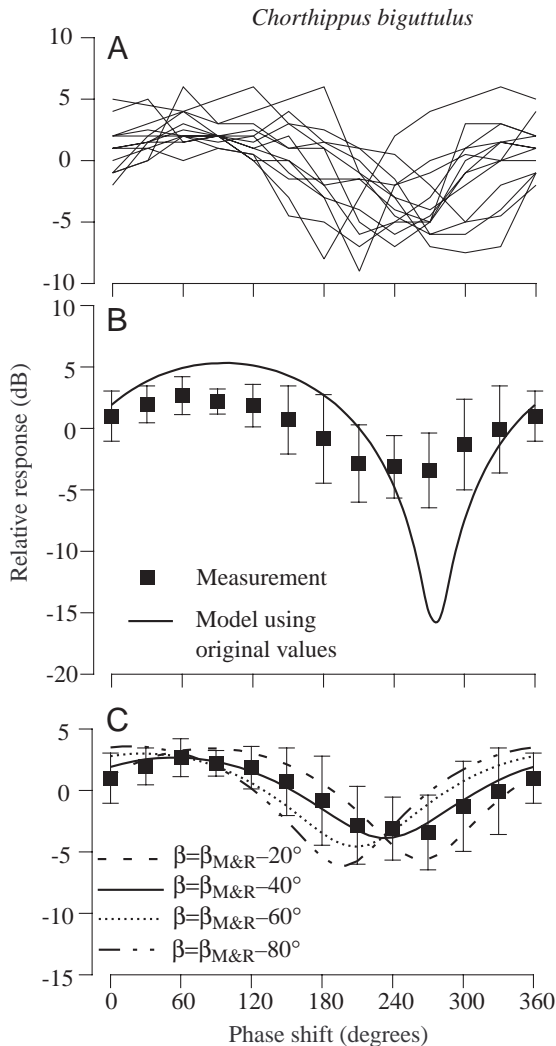


Fig. 8. Tympanic responses of *Chorthippus biguttulus* to two synchronous 5 kHz stimuli differing with respect to their phase relationship (phase experiment). The phase angle of one signal was held constant, while the phase angle of the other was varied; both signals had an identical amplitude. (A) Responses of individual preparations ipsilateral to the signal with  $0^\circ$  phase angle. (B) Mean ( $\pm$  s.d.,  $N=13$ ) values of the responses shown in A and model predictions based upon the original values measured by Michelsen and Rohrseitz (1995). (C) Mean ( $\pm$  s.d.,  $N=13$ ) values of the responses shown in A and predictions of the model with varied internal delay  $\beta$ . The original phase angle ( $\beta_{M\&R}=180^\circ$ ) measured by Michelsen and Rohrseitz (1995) was reduced by  $20$ – $80^\circ$ , i.e. the delay was increased.

minima of individual curves, the averaged response (Fig. 8B) had a peak-to-peak amplitude of only 6.1 dB.

Calculations using the parameter values given by Michelsen and Rohrseitz (1995) predicted that *C. biguttulus* would exhibit a minimum response at a phase shift of  $270^\circ$  at 5 kHz, with a difference between the maximum and minimum responses of approximately 21 dB (Fig. 8B). This predicted curve differs markedly from both our individual and averaged measurements in both position and amplitude. The influence of the four model parameters measured by Michelsen and Rohrseitz (1995) on the predictions of the model is shown in Fig. 9. Modifications of the values of the external delay and the internal gain shift the position of the minimum, but have only a small effect on the peak-to-peak amplitude (Fig. 9B,C). Changes in the external gain cause a decrease in amplitude, but have no effect on the position of the minimum (Fig. 9A). Both parameters of the predicted response were influenced by increasing the internal delay (Fig. 9D): increasing the delay by  $20^\circ$  (to  $160^\circ$  instead of the value of  $180^\circ$  measured by Michelsen and Rohrseitz, 1995) reduces the peak-to-peak amplitude to 9 dB. When the delay is further increased to  $80^\circ$ , additional changes in amplitude occur, and the position of the minimum moves from  $280^\circ$  towards  $180^\circ$ . The predicted responses of models with the internal delay increased by

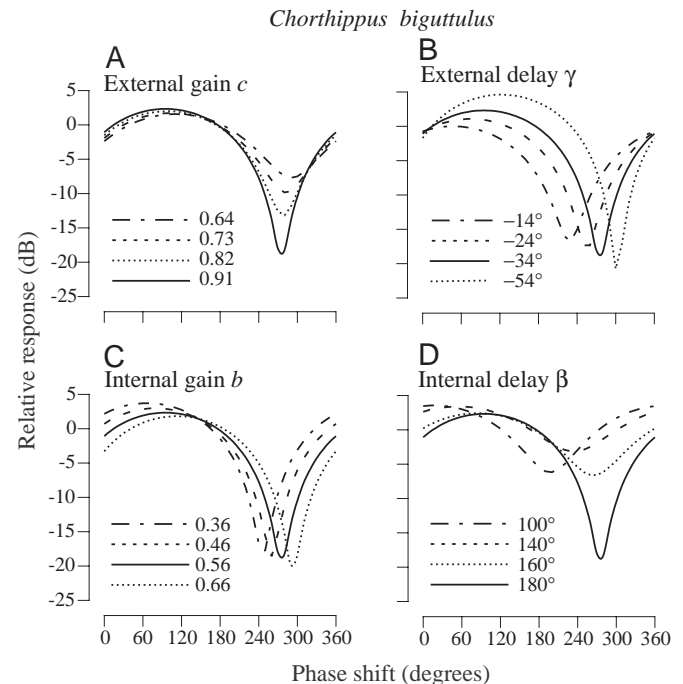


Fig. 9. The influence of the four model parameters describing the external and internal sound path on the model predictions for the phase experiment in *Chorthippus biguttulus* at a sound frequency of 5 kHz. One parameter was changed at a time, while the other three were kept at the original values determined by Michelsen and Rohrseitz (1995). Solid line: original parameters ( $c_{M\&R}=0.91$ ,  $\gamma_{M\&R}=-34^\circ$ ,  $b_{M\&R}=0.56$ ,  $\beta_{M\&R}=180^\circ$ ). Modification of (A) the external gain ( $c$  in Fig. 3) (B) the external delay ( $\gamma$ ), (C) the internal gain ( $b$ ) and (D) the internal delay ( $\beta$ ).



20–80° span a large part of the range of variability of the individual responses (Fig. 8A,C). The mean response fitted well with the model assuming a 40° increase in the delay (Fig. 8C). In another approach, we determined the internal delays that corresponded best to each individual measurement. We approximated the best fits by minimizing the sum of squares of the deviations between modified model and measurement. The mean internal phase shift determined in this way was 138.5°. This value corresponds to an increase in the delay of 41.5° over the value measured by Michelsen and Rohrseitz (1995) and is within the range they assumed to be realistic.

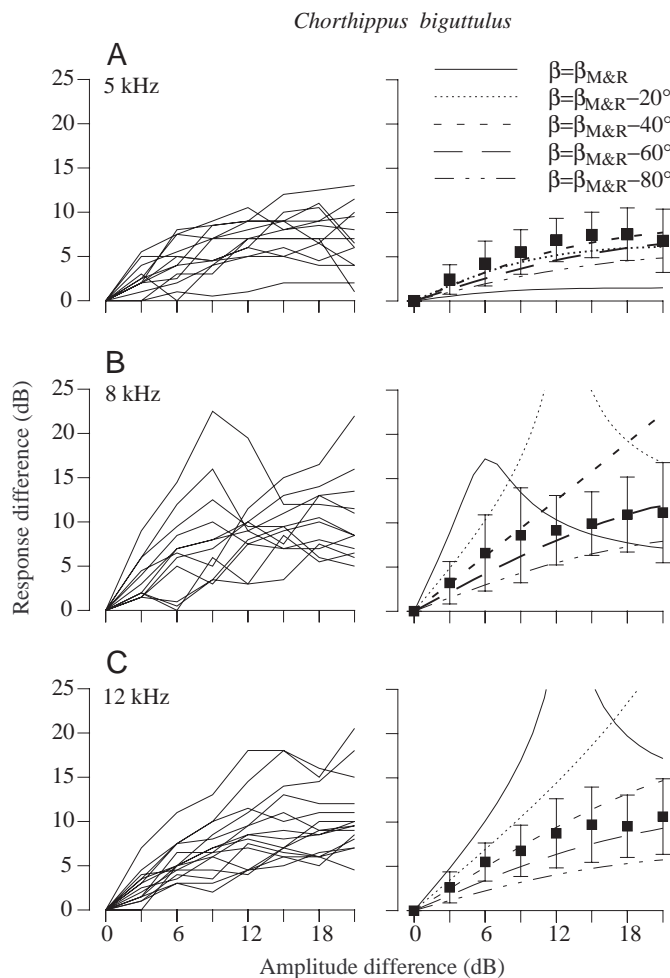


Fig. 10. Tympanic responses of *Chorthippus biguttulus* for different amplitude relationships of two synchronous tones of 5 kHz (A), 8 kHz (B) and 12 kHz (C) (amplitude experiment). One signal was held constant in amplitude (at 0 dB), while the amplitude of the other was varied; the phase relationship of the two sine waves was 0°. The response difference between the ear ipsilateral and the ear contralateral to the signal at 0 dB is shown. Results for individual preparations are shown on the left; and averaged response ( $\pm$  S.D.,  $N=12-15$ ) and model predictions are shown on the right. The predictions are calculated using the original parameter values (solid line) and using modified internal delays  $\beta$  (dashed lines).  $\beta_{M\&R}$  is the original internal delay measured by Michelsen and Rohrseitz (1995).

#### Amplitude experiment

We conducted the amplitude experiment in *C. biguttulus* for three frequencies (5, 8 and 12 kHz). As in the phase experiment, the results were highly variable between individual preparations. At 5 kHz (Fig. 10A), the response difference between the ear ipsilateral to the loudspeaker held at 0 dB and the ear ipsilateral to the attenuated loudspeaker increased with increasing amplitude difference, with the mean value reaching saturation at a stimulus difference of 12–15 dB (for 15 dB,  $7.4 \pm 2.5$  dB, mean  $\pm$  S.D.;  $N=13$ ). Calculations using the parameter values given in Michelsen and Rohrseitz (1995) predicted a response difference not exceeding 1.5 dB throughout the experiment, reflecting the poor directionality they found at this frequency. Model calculations with internal delay values increased by 20–80° resulted in predicted response differences of 6–8 dB (Fig. 10A), in close agreement with the mean response measured.

At 8 kHz, the variability of the measurements was even more pronounced (Fig. 10B). In some animals, the response differences had optimum characteristics, reaching a maximum at a stimulus difference of approximately 12 dB. In others, however, the response differences increased over the complete range tested, and in some they saturated in a pattern similar to the response differences found at 5 kHz. The original model for 8 kHz predicted an optimum function with a maximum at 6 dB stimulus difference, once again outside the range of our measurements (Fig. 10B). Applying the same changes to the model as for 5 kHz transformed its responses towards the measured values: with an increase in the internal delay of 20°, the maximum of the response function increased and moved to a stimulus difference of 14 dB. A further increase in the internal delay transformed the predicted response function into a linear rise throughout the range of interest (at 40° increase) and finally to a saturating curve (at 60–80° increase).

At 12 kHz, the situation was similar to that described at 8 kHz. Response differences with optimum characteristics were found, together with linear and saturating characteristics (Fig. 10C). Increasing the internal delay by between 20° and 80° predicted curves similar to the neurophysiologically measured curves. A model calculation using the original values of Michelsen and Rohrseitz (1995) produced a curve outside the measured range of variability.

In *C. biguttulus*, the results of both the phase and amplitude experiments fit the predictions of the model suggested by Michelsen and Rohrseitz (1995) quite well if the delay of the internal pathway is assumed to be 20–80° larger than that measured by them. The responses of two individual insects to all four experiments are shown in Fig. 11. In each of the two preparations shown, the internal delay had to be increased by the same phase shift for all three frequencies to achieve a good correlation between model predictions and measurements. In the first insect (Fig. 11A–D), a phase increase of 40° resulted in good agreement between measurements and predictions; for the second insect (Fig. 11E–G), a phase shift of 80° resulted in a better fit. For five out of ten preparations in which all four

experiments were successfully carried out, a good fit for all three frequencies could be obtained using phase shifts differing by less than  $20^\circ$  from each other (see Discussion). Nevertheless, there were also a few individuals for which no model fitted convincingly when only the internal phase was modified.

#### Calculation of directionality in *Chorthippus biguttulus*

The values for the biophysical parameters measured by Michelsen and Rohrseitz (1995) in *C. biguttulus* predicted almost no directionality at 5 kHz but, during behavioural tests, the insects were easily able to detect the direction of a song with this carrier frequency (see Fig. 1). According to the

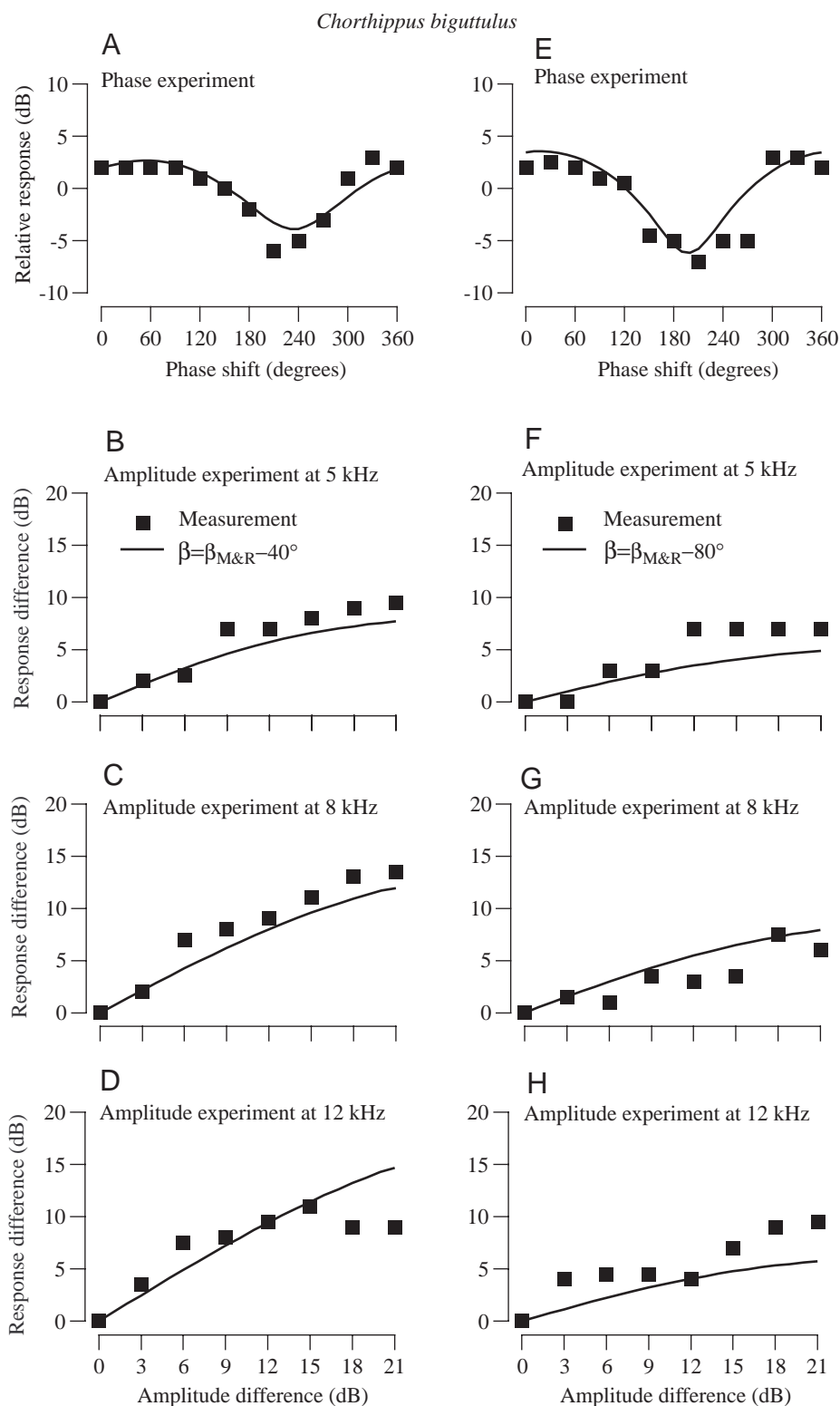


Fig. 11. Responses of two preparations of *Chorthippus biguttulus* from all experiments conducted and comparisons with predictions of models with modified internal delays. In one insect (left-hand column), decreasing the internal phase shift by  $40^\circ$  resulted in a good agreement with the results of the phase experiment (A) and the amplitude experiments at 5 kHz, 8 kHz and 12 kHz (B–D). The same was true for the second insect (right-hand column, E–H, respectively) when the internal phase shift was decreased by  $80^\circ$ .

measurements presented here, it should be assumed that the internal delay is 20–80° greater than that found by Michelsen and Rohrseitz (1995). We calculated the directionality of the ear over a range of values for the internal delay (Fig. 12A). No directionality was apparent for the original value (delay 180°). Increasing the internal delay increased directionality mainly by decreasing the total sound pressure at the ear for sounds from the contralateral hemisphere.

The directional information available to the animal is the interaural sensitivity difference, i.e. the difference between the directional characteristic and its mirror image. The interaural sensitivity difference was close to zero for the unmodified model, but reached maximum values when the internal delay was increased by 40–60° (Fig. 12B). Nevertheless, an interaural amplitude difference of at least 4 dB was found with a delay of 20°. This value of 4 dB occurred at an angle of only 30° from the longitudinal axis of the insect. Michelsen and Rohrseitz (1995) measured similar values by determining the directionality of tympanal vibrations directly and showed that model calculations using an increased internal delay predict a directionality of comparable magnitude (Figs 9 and 11 in Michelsen and Rohrseitz, 1995).

### Discussion

Our results from neurophysiological recordings using a more complex acoustic stimulus situation in principle support the model of the biophysical mechanisms involved in directional hearing in grasshoppers proposed by Michelsen and Rohrseitz (1995). However, a comparison of our results with the model predictions based on the parameter values measured by Michelsen and Rohrseitz (1995) revealed remarkable discrepancies both in *Schistocerca gregaria* and in *Chorthippus biguttulus*. In both species, modification of the value of a single model parameter led to a better fit: in *S. gregaria* the transmission gain, and in *C. biguttulus* the delay of the internal pathway, had to be modified. For *C. biguttulus*, our results support the assumption of Michelsen and Rohrseitz (1995) that the internal delay had to be increased over their measured value.

For *S. gregaria*, our measurements were quite consistent, with only moderate inter-individual variation. The results of Michelsen and Rohrseitz (1995) for this species were also unambiguous. The directional characteristic they obtained by direct measurement of tympanal vibrations was in good agreement with that predicted by their model. However, there were differences between the measured and modelled response functions, mainly in amplitude. The differences in the value of the internal gain found by Michelsen and Rohrseitz (1995) and in the present study may be due more to differences between the animals used than to differences between the experimental arrangements.

The most marked difference between our experimental procedure and that of Michelsen and Rohrseitz (1995) was that we fixed the insects ventral side up on a free-standing holder, while Michelsen and Rohrseitz (1995) fixed the insect ventral side down on a cork platform, which could result in different

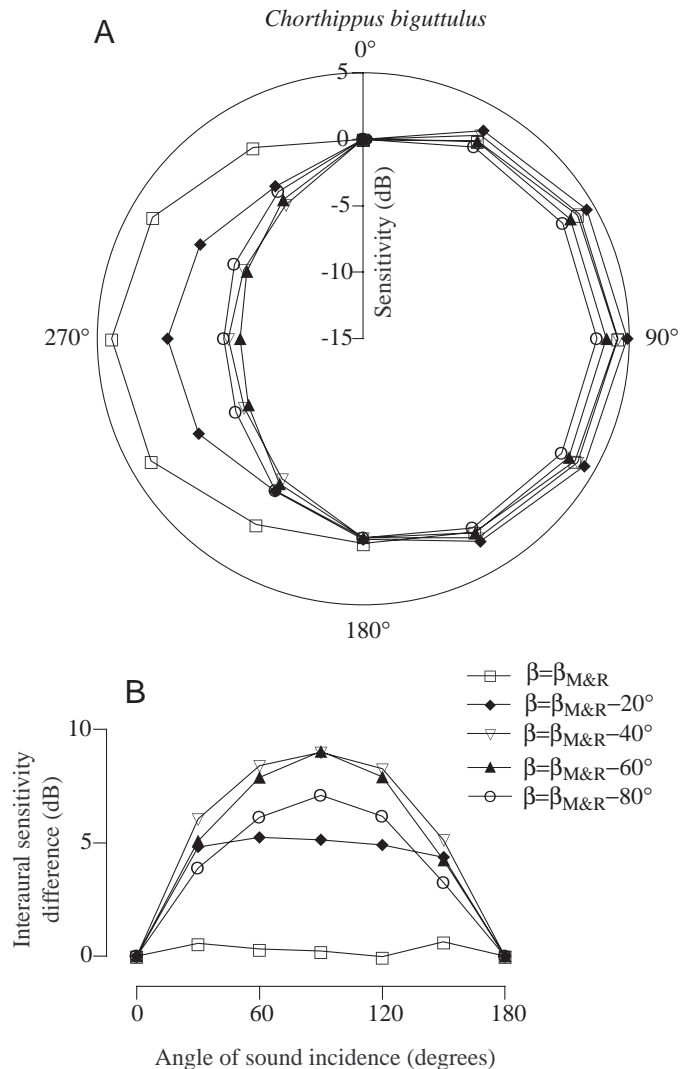


Fig. 12. (A) Directional pattern at 5 kHz in *Chorthippus biguttulus* calculated from models with different internal delays  $\beta$ . The model using the original value ( $\beta_{M\&R}=180^\circ$ ) of Michelsen and Rohrseitz (1995) shows almost no directionality (open squares), while directionality is more pronounced for increased values of delay. (B) Interaural sensitivity differences for the directional patterns in A, calculated as the difference between each directional pattern and its mirror image.

gain and phase values for the external sound pathway. Because of the small body sizes, however, the diffraction of the sound was rather low at 5 kHz in both the species used and for higher frequencies in *C. biguttulus*. The phase shifts of the external pathway were mainly due to the different time of arrival of the sound at the ear (Michelsen and Rohrseitz, 1995), which should not be influenced by the posture of the insect. Therefore, it seems unlikely that large differences between these two studies were caused by the different postures of the insects, although this possibility cannot be excluded. In addition, our model calculations (Fig. 5A,B) indicate that changes to the external pathway should have no great effect on the responses in our stimulus situations.

To expose the tympanic nerves in *S. gregaria*, only small incisions in the cuticle were necessary, and no muscles or apodemes had to be removed. Furthermore, the incisions were filled and sealed with petroleum jelly. Nevertheless, the preparation could possibly influence the internal pathway. The most likely explanation, however, is that the differences between the internal gain measured by Michelsen and Rohrseitz (1995) and the value fitting our measurements are due to the quantity of fat in the insect. Michelsen (1971b) found large variations in hearing sensitivity depending on the amount of fat within the locust, and Miller (1977) demonstrated that this factor influences the gain of the internal pathway. Thus, different rearing conditions alone may be sufficient to explain the differences found for *S. gregaria* between the two studies. The model suggested by Michelsen and Rohrseitz (1995) seems, therefore, to be a good approximation of directional hearing in *S. gregaria* at 5 kHz.

Our measurements in *C. biguttulus* showed a much greater range of variation than in *S. gregaria*, particularly in the phase experiment (compare Figs 4, 8). This could be due to the fact that the preparation of *C. biguttulus* was more invasive than that of *S. gregaria*, owing to the smaller size of the insect. Part of the metathoracic apodeme and the attached muscles had to be removed to expose the tympanic nerve, and this procedure may have affected the mechanical properties of the cuticle more than in *S. gregaria*. We attempted to prepare the different insects in an identical manner, and no systematic differences were found between preparations of the right and left ear (which differed slightly as a result of the handedness of the experimenter). Since the measurements of the transmission gain of the interaural pathway in *C. biguttulus* also showed considerable variation (Fig. 8 in Michelsen and Rohrseitz, 1995), we are confident that the variation in the responses of *C. biguttulus* in our study was not due to experimental artefacts but reflects differences between individual animals (see below).

The experimental results of Michelsen and Rohrseitz (1995) for *C. biguttulus* were not as clear as for *S. gregaria*. There were considerable discrepancies between the directional patterns measured directly by means of laser vibrometry and the calculations of directional patterns based on their measurements of the different sound pathways. These discrepancies were particularly pronounced at lower frequencies (5–10 kHz), but even at higher frequencies calculated and measured characteristics differed by up to 6 dB (at 17 kHz) and 9 dB (at 12 kHz) for some angles of sound incidence. In addition, the conclusion of Michelsen and Rohrseitz (1995) that the ear of *C. biguttulus* provides poor directional information at 5 kHz contradicts the behaviour of the insect: behavioural results demonstrate that the males are able to lateralize a 5 kHz signal with almost no errors (Fig. 1C), whereas frequencies above 8 kHz which, according to Michelsen and Rohrseitz (1995), are suited for song localization, are much less effective in eliciting male responses (Fig. 1B; von Helversen and von Helversen, 1997).

In their experiments on *C. biguttulus*, Michelsen and

Rohrseitz (1995) measured, for frequencies below 7 kHz, phase shifts of the internal pathway greater than 180°, which would correspond to a negative travel time of sound. They recognized and considered this problem for their model calculations, but did not discuss possible sources of this error. Michelsen and Rohrseitz (1995) used a wall of beeswax placed on the dorsal side of the animal to reduce the transmission of sound from the local sound source to the opposite tympanum. This wall may have influenced the mechanical properties of the cuticle, resulting in alterations to the properties of the internal sound pathway. Furthermore, Michelsen (1971b) demonstrated that a considerable amount of sound energy can be transmitted through the cuticle outside the tympanum. This sound pathway was probably also altered by the wall of beeswax placed on the insect. This effect would probably be greater in *C. biguttulus* than in *S. gregaria* because of its smaller body size.

A major problem in determining the driving forces on the tympanum using laser vibrometry may involve the different vibrational modes of the tympanum: Michelsen (1971a) demonstrated that, with variation of the stimulus frequency, different vibrational modes with different centres of vibration appear on the tympanum. Nodal lines, i.e. lines with no displacement, were found at the tympanum, with their positions changing at different stimulus frequencies (Breckow and Sippel, 1985). Laser vibrometry picks up only the vibrational amplitudes in the direction of the laser beam (i.e. in this case perpendicular to the tympanum). The receptor cells of Müller's organ are most probably stimulated by displacement of their cell bodies relative to their dendritic attachment sites. These relative movements are not necessarily linked to large amplitudes of movement of the tympanum, which would be picked up by laser vibrometry (Breckow and Sippel, 1985). This mechanism of signal transduction may interfere with the prerequisite of using this method, which is that the tympanal vibrations as picked up by the laser faithfully reflect the driving force acting on the tympanum. Unfortunately, Michelsen and Rohrseitz (1995) did not present data proving that this prerequisite was always met. This problem could be the reason for the difficulties encountered in applying this method, which has been used successfully in other insects (e.g. Michelsen et al., 1994a,b) and frogs (e.g. Jørgensen et al., 1991), to grasshoppers.

Whether the dubious phase measurements of Michelsen and Rohrseitz (1995) were caused by methodological problems or by the inadequacies of the two-input model for the grasshopper ear (e.g. the presence of additional sites of sound input besides the two tympana) cannot be determined from the data so far available. Because measurements of the sound pressures acting on the outer surface of the two tympana are not affected in either case, it is justifiable to modify the parameters of the internal sound pathway to achieve a better fit between the measurements and the model calculations. The good correlation between our measurements and the predictions calculated using an internal delay modified in the direction as suggested by Michelsen and Rohrseitz (1995) supports the two-input model of the grasshopper ear and the calculation

procedures used by Michelsen and Rohrseitz (1995) and in our study.

For *C. biguttulus*, we achieved the best match between the model and our measurements by assuming a mean phase angle of the internal pathway of  $138.5^\circ$  at 5 kHz, i.e. the delay had to be increased by approximately  $40^\circ$  over the value given by Michelsen and Rohrseitz (1995). The calculation of the directional pattern using this value resulted in an interaural sensitivity difference of 9 dB (Fig. 12), which is consistent with the interaural response difference for large-amplitude differences found here (Fig. 10A) and also with the directional pattern for 5 kHz measured by Michelsen and Rohrseitz (1995, their Fig. 9). The high variability in our neurophysiological measurements suggests that there is a high inter-individual variability in the phase shift of the internal sound pathway in *C. biguttulus*. The range of responses indicates a variability between individuals of the internal delay of approximately  $60^\circ$  (Fig. 8). The directional patterns calculated using delay values throughout this range all result in reasonable directionalities with interaural sensitivity differences of at least 4–5 dB (Fig. 12).

In *C. biguttulus*, pair formation is achieved by duetting, in which the song of the male elicits an acoustic reply from the receptive female, to which the male then reacts phonotactically. The orientation turn is followed by a short forward movement in the new direction, after which the male initiates the next phrase of the song. The turns towards the sound source during the phonotactic approach of the male are based on simple right–left decisions. As amplitude differences of 1–2 dB are sufficient for these decisions in an undisturbed sound field (von Helversen and Rheinlaender, 1988), the interaural amplitude differences calculated in Fig. 12 should be sufficient to ensure lateralization under natural conditions. Therefore, the variations in the internal phase delay probably do not have important effects on the ability of the insect to locate a responding female. In crickets, a similar variability in the transmission gains of the contralateral sound inputs was described by Michelsen et al. (1994b). To obtain a good fit, a change in the internal delay in *C. biguttulus* was necessary not only in the low-frequency range, as Michelsen and Rohrseitz (1995) suggested, but over at least the complete frequency range used in this study (5–12 kHz); for approximately half the preparations, we achieved a good fit with the same or similar phase angles added at all three frequencies tested.

Our findings generally support the two-input model for directional hearing in grasshoppers and demonstrate that the model suggested by Michelsen and Rohrseitz (1995) can be used to predict hearing responses even in complex stimulus situations. While the data presented by Michelsen and Rohrseitz (1995) for the sound diffraction due to the body of the insect, i.e. the sound pressures acting on the external surfaces of the tympana, appear to be a good basis for quantitative calculations, there is strong evidence to question the data they presented on the internal sound transmission. Our

measurements suggest a considerably larger delay for the internal pathway in *C. biguttulus*, resulting in good directional hearing at low frequencies. In the light of the behavioural performance of these insects, this situation seems to be biologically meaningful.

We wish to thank A. Michelsen and K. Rohrseitz for their intensive discussion of our results. Our thanks are also due to H.-U. Kleindienst for commenting on the manuscript and to S. L. Bush and R. Balakrishnan for linguistic help.

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