

Tympanal Membrane Motion is Necessary for Hearing in Crickets

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Summary. 1. Sound guided through the tracheal tube to the internal tracheal spaces in the region of the cricket ear is capable of eliciting auditory neural responses in the prothoracic ganglion if the tympanal membrane is allowed to vibrate freely. If the tympanal membrane motion is prevented mechanically neural responses are abolished (Fig. 3) whereas the sound pressure in the tracheal air spaces behind the tympanum is increased.

2. If the motion of the tympanum, as measured with laser vibrometry, is prevented by adjusting the internal and external sound pressure, then neural responses cease simultaneously (Fig. 5).

3. These findings demonstrate that motion of the large tympanum is a necessary requisite in the sound transduction process of the cricket ear.

(A) *Air Sac Pressure Hypothesis.* The tympanal membrane transmits the external sound into the air sac behind it. Here, the transmitted sound interferes with sound waves coming from other inputs. The resultant sound pressure is the essential stimulus for eliciting neural responses in the receptor cells. According to this hypothesis, the tympanal membrane merely acts as a sound-transmitting window for one of the sound pressure components in the air sac.

(B) *Tympanal Membrane Motion Hypothesis.* The auditory receptor cells respond to the motion of the tympanal membrane, which is proportional to the difference between the external and the internal sound pressure. That is, the tympanal membrane is the connecting link between all sound pressure components and those structures that mediate the resultant stimulus to the receptor cells.

The two hypotheses have very different implications for the mechanism of frequency tuning and directional sensitivity of the ear (Fletcher and Thwaites 1979). In this study, we present experiments with *Gryllus campestris* which prove unambiguously the validity of the second hypothesis.

Introduction

The auditory system of crickets (Gryllidae) has four major acoustic inputs: the large tympanal membrane (one in each foreleg) and the two prothoracic spiracles. The motion of the tympana has been studied under various input conditions using advanced experimental techniques such as Mössbauer spectroscopy (Johnstone et al. 1970) and laser vibrometry (Dragsten et al. 1974; Paton et al. 1977; Larsen and Michelsen 1978; Larsen 1981). However, the functional importance of tympanal membrane motion for the hearing process is still far from being understood. Two different hypotheses can be considered:

Materials and Methods

All experiments were performed using female crickets (*Gryllus campestris* L.) 2 to 3 weeks after adult moult. The animals were mounted ventral side up on a plexiglass stand. One front leg was placed with its ear in a closed sound cavity (Kleindienst et al. 1981). The nerve of this leg was cut close to the prothoracic ganglion to eliminate acoustic input to the CNS from this side. Both thoracic spiracles were covered with wax to ensure constant acoustic conditions. Sound pulses of 5 kHz were transmitted from the sound cavity to the contralateral ear (the active ear) via the tracheal tube inside the animal. Sound pressures at the plateau of the sound pulses are expressed in dB (root mean square) relative to 20 μ Pa. Excitation of the active ear

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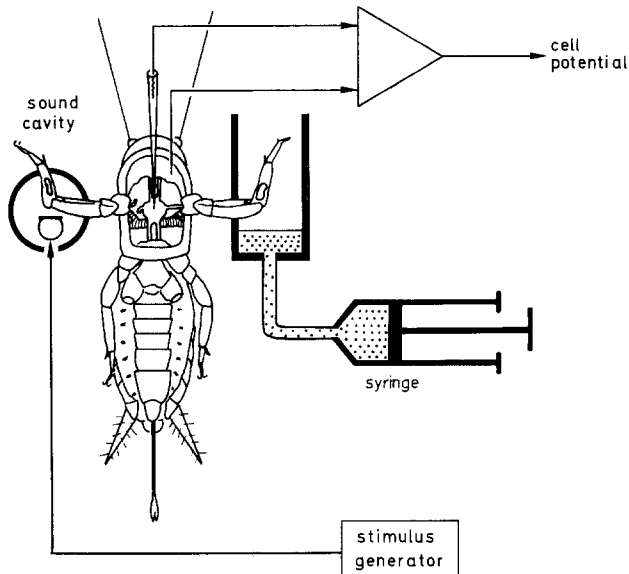


Fig. 1. Experimental set-up used in the flooding experiments. A condenser microphone is used as a sound source in a closed cavity containing a cricket foreleg. The nerve of this leg is cut to eliminate neural auditory input to the prothoracic ganglion from this side. Sound is passed through the tracheal tube to the inner parts of the opposite ear, the tympanum of which can reversibly be loaded by water from a syringe. The neural response to the internal sound stimulus is recorded from an acoustic interneuron in the prothoracic ganglion

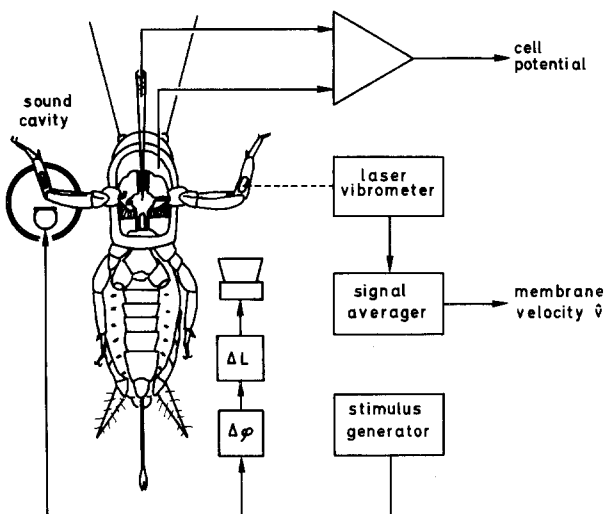


Fig. 2. Diagram of the experimental set-up in the interference experiment. Sound is guided through the tracheal tube and elicits tympanal membrane vibration in the opposite ear. This vibration can be cancelled by an external sound pressure. The phase and amplitude of the external sound are adjusted by means of a phase shifter $\Delta\phi$ and an attenuator ΔL until the tympanal vibration disappears. The motion of the tympanum is measured with a laser vibrometer. Neural responses are recorded simultaneously from an acoustic interneuron in the prothoracic ganglion

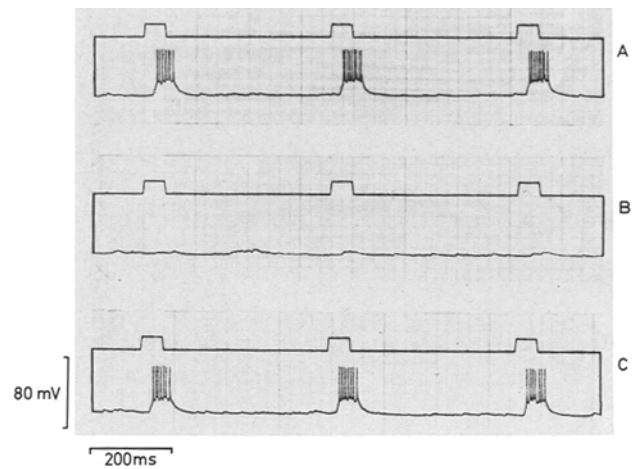


Fig. 3A–C. Response of an acoustic interneuron in the prothoracic ganglion to internal sound pulses during the flooding experiment. *Upper traces*: time pattern of the 5 kHz sound stimulus. *Lower traces*: neural response before the flooding (A), after the tympanum is covered with water (B), and after the water has been removed (C)

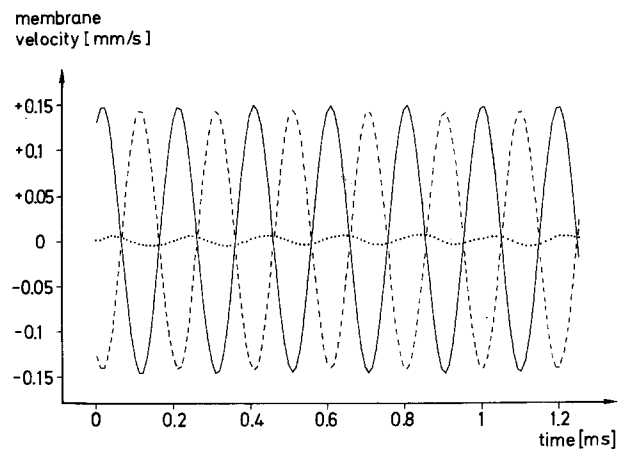


Fig. 4. Vibrational response of the large tympanum at 5 kHz in the interference experiment. The response to internal sound (*dashed line*) is 180° out of phase relative to the response to external sound (*solid line*). The response is reduced by a factor of 25 if both stimuli are presented simultaneously (*dotted line*)

was studied by recording intracellularly from acoustic interneurons in the prothoracic ganglion using electrodes filled with 2 mol/l potassium acetate and with resistances of 50–70 MOhm. Two types of experiments were carried out:

Flooding Experiments (Fig. 1). The leg containing the active ear was placed in a short tube (internal diameter 3 mm) which could be filled with water. Flooding the leg greatly reduces the motion of the tympanum. 4 animals were used to investigate the change of nervous response after flooding, and for 8 animals the change of sound pressure in the tracheal air sac was measured. In these animals, the leg was cut distal to the large tympanum and a probe microphone (orifice diameter 20–40 μm) was inserted into the leg trachea. The tracheal opening (diameter 200–250 μm) was then closed with wax. The probe was made

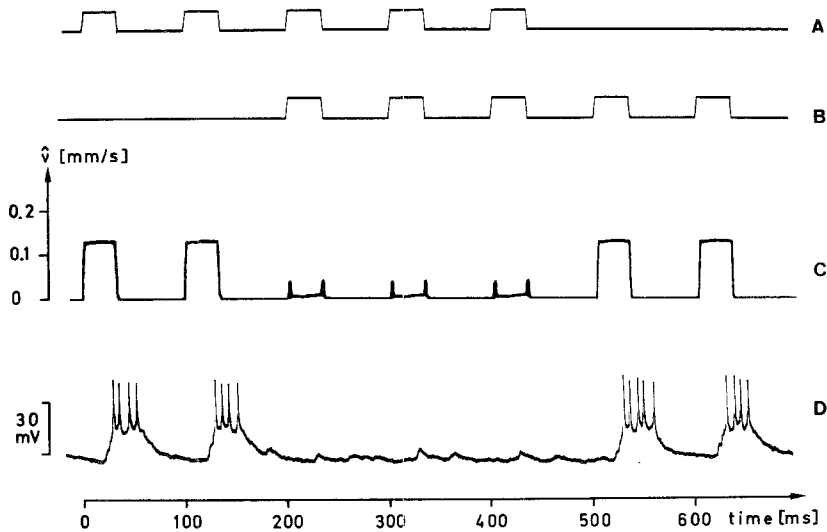


Fig. 5A–D. An interference experiment with neural recording and simultaneous measurement of tympanal membrane motion.

A, B Time pattern of external (A) and internal (B) 5 kHz sound pulses.

C Velocity amplitude \hat{v} of tympanal membrane vibration.

D Response of an acoustic interneuron in the prothoracic ganglion

from a glass microelectrode. It was coupled to a 1/2" condenser microphone (Brüel and Kjaer 4133, adaptor kit UA 0040). The microphone signal was filtered in a 1/3 octave filter (Brüel and Kjaer 1614) and averaged (Nicolet type 1074, sampling rate 0.1 MHz, 1024 repetitions). The duration of sound pulses used in the flooding experiments was 54 ms with 2 ms rise and decay times. The pulse repetition rate was 2/s.

Interference Experiment (Fig. 2). In addition to the internal sound stimulus, the active ear was exposed to an external sound produced by a piezo horn tweeter (VISATON PH8) located 15 cm away. This external sound was phase-shifted ($\Delta\phi$) and attenuated (ΔL) relative to the sound pressure in the closed sound cavity. $\Delta\phi$ and ΔL were adjusted until the vibrational response of the tympanum disappeared. The motion of the tympanal membrane was measured by a DISA laser vibrometer (Larsen and Michelsen 1978). The output signal of the vibrometer was averaged to improve the signal-to-noise ratio (Nicolet type 1170 A, sampling rate 0.1 MHz, 128 repetitions). In the interference experiments, sound pulses had a duration of 34 ms. The pulse repetition rate was 10/s.

Results

Flooding Experiments

Figure 3 shows the neural responses to 5 kHz sound pulses (cavity sound pressure 70 dB). The sound pressure reaching the active ear via the internal tracheal tube was 15 dB above threshold and elicited a neural response of 7–9 spikes per sound pulse in the auditory interneuron (Fig. 3A). This response disappeared completely when the tympanal membrane was covered with water (Fig. 3B) and was entirely restored after the water was removed (Fig. 3C). As soon as the water covered the tympanum, the sound pressure in the tracheal air sac increased by 2.5 to 5 dB at 5 kHz.

From the results of the flooding experiments, it is obvious that the auditory receptor cells of

Gryllus campestris do not respond to sound pressure in the tympanal air sac as postulated in the air sac pressure hypothesis.

Interference Experiments

For the example discussed here, the external sound pressure was set to 80.5 dB at the exposed ear and the cavity sound pressure to 95 dB. These settings resulted in almost identical vibrational responses of the tympanum (Fig. 4). The phase angle between the cavity sound and the external sound was adjusted such that the responses to either sound signal were 180° out of phase. When both sound signals were presented simultaneously, the resultant vibration of the tympanal membrane was very weak (dotted line in Fig. 4). Figure 5 demonstrates the correlation between tympanal membrane motion and central auditory neuron activity at 5 kHz. As long as only one sound source was active, each sound pulse elicited both a tympanal vibration and a neural response. With the two sound sources activated simultaneously, *both* the spike responses *and* the tympanal membrane motion were almost cancelled. Since both sound signals differed in their time of arrival at the active ear, the cancelling effect was not complete and short-lasting oscillations of the tympanum could be seen at the beginning and end of each sound pulse. The neuron exhibited e.p.s.p. activity that was correlated to these oscillations. The result of the interference experiment indicates that the amplitude of the tympanal membrane motion and the strength of the neural response are highly correlated.

Discussion

Changes in the neural response caused by experimental operations on the large posterior tympanum have been reported by several authors. In *Gryllus campestris*, Nocke (1972) detached the tympanum at its margins from the leg cuticle and obtained a threshold increase of 30 dB at 4 kHz. A similar loss of auditory sensitivity was found in *Teleogryllus commodus* by Hill (1974) and in *Teleogryllus oceanicus* by Paton et al. (1977) when the tympanum was covered with vaseline. Hearing thresholds also increased after tearing a hole in the tympanal membrane (Paton et al. 1977). These experiments, carried out in a free sound field, demonstrate that an intact large posterior tympanum is important for hearing. However, they do not show whether the tympanal membrane only acts as a sound window to the tracheal air sac or whether its vibration is intimately related to the transduction process. Our results confirm that the tympanal membrane responds to the (vectorial) difference between external and internal sound pressures (Larsen and Michelsen 1978). In addition, they demonstrate that tympanal membrane motion is a necessary part of the transduction process. From the anatomy of the cricket hearing organ (Michel 1974; Young and Ball 1974), it is apparent that the tympanal membrane is probably only the first of several elements in the transduction pathway to the receptor cells.

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