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Physical Basis for Auditory Frequency Analysis in Field Crickets (Gryllidae)

John A. Paton[1], Robert R. Capranica[1], Paul R. Dragsten[2], and Watt W. Webb[2]

School of Electrical Engineering and Section of Neurobiology and Behavior[1], and School of Applied and Engineering Physics[2], Cornell University, Ithaca, New York 14853, USA

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Summary. 1. The large posterior tympanic membrane in intact field crickets vibrates up to several hundred Ångströms in response to sounds of the same frequency and intensity as the cricket's calling song. The mechanical response is linear (Fig. 3), shows a peak near 5kHz (Figs. 2, 5), and the membrane vibrates in the same simple mode in response to tones from 4 to 20kHz (Fig. 4).

2. Reducing the movement of the large tympanic membrane by covering it with vaseline or by tearing holes in it (Fig. 7) causes a corresponding decrease in sensitivity of auditory interneurons (Figs. 6, 8).

3. The measured vibration of the small tympanic membrane is of the same order of magnitude as that of the cuticle adjacent to the membrane and it shows no selective tuning.

4. Introducing helium into the leg trachea changes the sensitivity of auditory interneurons. Thresholds near 5 kHz increase by up to 30 dB and thresholds near 18 kHz decrease by up to 13 dB (Fig. 9). This shift in tuning of the organ appears to be due to a change in the resonant frequency of the leg trachea.

5. Acoustical calculations are consistent with our conclusion that the dominant tuning of the large tympanic membrane and of the organ to 5 kHz is due to a tracheal resonance.

Introduction

Field crickets (Gryllidae) have been studied for over 50 years as a model for acoustical communication behavior. More recently, their auditory and sound producing systems have been investigated both as elements of communication behavior and as models of general neurophysiological principles such as command fibers (Bentley and Hoy, 1974), temporal pattern recognition (Stout and Huber, 1972), and central oscillators (Bentley, 1969). In this paper we will describe a further aspect of the cricket's communication system, namely the

physical basis for frequency selectivity in its auditory organ. This frequency selectivity (i.e., tuning) is behaviorally important in that it improves the isolation of species-specific calls from other sounds in the environment (Hill, 1974). It may also provide a means of discriminating acoustic communication signals within a species' repertoire (Nocke, 1972).

The most sensitive auditory organ of a field cricket is located in the proximal portion of the prothoracic tibia. The organ can be seen externally as two white tympanic membranes on opposite sides of the leg, and thus is referred to as the tympanal organ. Internally, two branches of the leg trachea are opposed to the two tympanic membranes. Cell bodies of the receptor cells are adjacent to the smaller anterior tracheal branch, and dendrites of the receptors run at right angles toward attachment cells connected to the leg cuticle. In all of the species of field crickets that have been studied, the tympanal nerve is most sensitive to tones near 5 kHz (Wever and Vernon, 1959; Loftus-Hills et al., 1971; Nocke, 1972), and at least in *Gryllus campestris* a second peak in sensitivity occurs near 14 kHz (Nocke, 1972). The 5 kHz sensitivity is closely matched to the carrier frequency in the calling songs of these same species.

In the tympanal organs of other species of insects, three mechanisms have been found which produce selective frequency sensitivity. In the locust a complex membrane resonance leads to different frequency responses for receptors at different locations on the tympanic membrane (Michelsen, 1971). In the tettigoniid ear, sound enters the leg trachea through a thoracic spiracle and the tuning of the organ is determined by acoustic properties of the trachea (Lewis, 1974; Nocke, 1974, 1975). And, in the noctuid moth, the column containing the receptor cells shows frequency tuning apart from that of the tympanal membrane and tympanal cavity (Adams, 1972). These examples suggest that the three elements of the cricket tympanal organ which may contribute to its frequency sensitivity are: (1) the tympanal membranes, (2) the tracheal system, and (3) the structures which couple energy directly to the receptor cells.

In this paper, based on mechanical measurements of sound induced vibration of the cricket tympanal organ, and from various mechanical manipulations which affect the frequency sensitivity of auditory interneurons, we present evidence that the 5 kHz tuning is due to a cavity resonance within the leg trachea. No evidence was found in the response properties of the tympanic membranes which would explain the 14 kHz sensitivity seen in some auditory nerve fibers.

Materials and Methods

Animals/Preparation

Three species of field crickets were studied: the house cricket (*Acheta domesticus*), the northern fall field cricket (*Gryllus pennsylvanicus*), and an Australian field cricket (*Teleogryllus oceanicus*). Adults of both sexes were used. The house crickets were obtained from a commercial supplier (Fluker's Cricket Farm in Baton Rouge, Louisiana). *Gryllus pennsylvanicus* were collected in the fall of 1973 in the Ithaca, N.Y. area. The Australian species were provided by R.R. Hoy from colonies in his laboratory at Cornell University.

Animals were anesthetized by lowering their temperature until they went into cold stupor. In all experiments the animals were restrained and allowed to recover before beginning any measurements. The position of the animal was the same for both neurophysiological and mechanical measurements. A groove the size of the body was formed in a block of "tackiwax" (Central Scientific Co.). The cricket was placed in the groove, ventral side up, and its body was held in place by straight pins pressed into the wax on either side of the neck, the mesothoracic legs, and the abdomen. All of the legs except the prothoracic were pinched off at the trochanter. This procedure ruled out possible input from receptors in these legs while recording from interneurons, and reduced body movements in the mechanical measurements. The prothoracic legs were fastened to the wax at 45° to the long axis of the body by U-shaped clips made from minuten pins. Each tibia was immobilized in a vertical position by waxing its distal portion to the pin stuck in the tackiwax.

For the mechanical measurements additional measures were taken to make the tibia as stationary as possible. The entire femur and the proximal portion of the tibia were fixed in place with "sticky wax" (Kerr Lab Products Division). In some cases the leg nerve was cut to reduce muscle movements in the leg. The tackiwax holder was embedded in a block of aluminum which was bolted in place.

In the neurophysiological experiments the prothoracic ganglion and leg nerve were approached from the ventral side. Once opened, the body cavity was kept moist with insect saline (Fielden, 1960).

In several experiments, various gases were infused into the leg trachea through the leg spiracle. The gases were bubbled slowly (2–4 ml/min) through water and then presented to the animal through a glass pipette whose tip fit loosely in the spiracle opening. In all cases, at least 20 min was allowed between gas changes for the trachea to be completely refilled.

Acoustic Stimulation

A number of different acoustic waveforms were used to stimulate the tympanal organ. A diagram of the sound generating system is shown in Figure 1a. Pure tones were obtained from two Hewlett-Packard 200 CD oscillators and could be combined in a passive adder. The tones were either presented continuously, as in the mechanical measurements, or were turned on and off by an electronic switch (Grason-Stadler model 1287) as in the neurophysiological measurements. Tone bursts had durations of 30, 50, or 100 ms and rise times of 2.5 or 5 ms.

Acoustic stimuli were amplified by one channel of a Dynaco stereo 80 amplifier. The output level was controlled by several attenuators (Hewlett-Packard 350D) and a 30 dB voltage divider between the amplifier and the loudspeaker. The 30 dB attenuator was used at low SPL's to minimize the effect of amplifier noise.

The mechanical measurements were made in a relatively noisy open lab area; the neurophysiological recordings were conducted in an IAC audiometric room (model 1203A). Acoustic stimuli were radiated by either a Philips dome tweeter, type AD0160T8 ($\pm$3 dB from 2 to 30 kHz), or the speaker from a KLH model 10 audio system. For the mechanical measurement, a tube damped with glass wool was mounted on the Philips tweeter to direct the sound toward the animal. To reduce direct vibration of the animal, the sound source was vibration isolated. For the neurophysiology experiments the speaker was placed on a foam rubber pad at a distance of 30 to 50 cm from the preparation. In the mechanical measurements involving scattered laser light from the tympanal membranes, the speaker was 15 to 20 cm away and was suspended from a floor stand which was mechanically independent of the optical bench. To reduce standing waves in the local sound field, the surfaces around the animal were covered with 2 to 3 cm of sound absorbing glass wool, and the area was kept as clear of sound reflecting objects as possible. The microscope used to position the laser beam on the membrane was swung out of the way during all measurements.

Sound pressure level (SPL) was monitored by a free-field condenser microphone (Bruel and Kjaer 4133) and measured on an impulse sound level meter (B & K 1613). The microphone and its preamplifier were connected to the meter by a 3 m cable. In most of the experiments, a 2 or 4 mm probe tube was attached to the microphone and the end of the probe tube was placed within several mm of the tympanal organ. The tube diameters were both less than the wavelength of sound at 20 kHz and therefore had little effect on the sound field at frequencies of interest. The probe tubes were calibrated outdoors in free-field conditions. One of the possible sources of error in measuring SPL was spatial variation of the sound level due to reflections and standing waves from surrounding

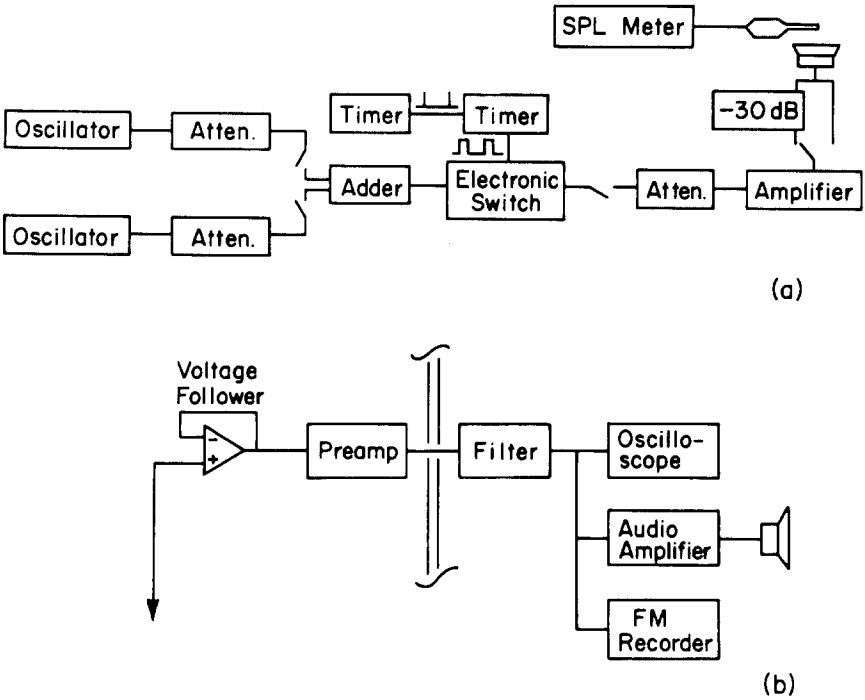


Fig. 1. **a** Sound stimulus equipment. **b** Neurophysiological recording electronics. The voltage follower and the preamplifier were within the soundproof room

apparatus. To test for this, the probe tube was moved around the leg and, for the normal range of probe tube placement, the SPL varied less than 1 dB up to 12 kHz and less than 3 dB up to 20 kHz.

Neurophysiological Recordings

In most of the single-unit experiments the prothoracic ganglia were supported from beneath by an insect pin running from one side of the wax through to the other side. In some cases, the ganglia were further stabilized by covering them with gelatin dissolved in insect saline.

Two different types of microelectrodes were used. In a few cases indium-filled pipettes (Gesteland et al., 1959) were advanced into the ganglion through holes torn in the connective tissue sheath. In most of the experiments glass-insulated tungsten electrodes were used (Merrill and Ainsworth, 1972). The electrodes had uninsulated tip sizes of 2 to 10 μm and DC resistances of 0.5 to 5 megohms. To minimize deterioration of the ganglion caused by contact with hemolymph, the same hole was used for more than one electrode pass by repositioning the electrode without exiting from the ganglion (Roeder, 1966).

The position of the electrode entrance was noted, using as landmarks the connectives, leg nerves, and ganglionic trachea. The electrode was advanced by a hydraulic microdrive system (David Kopf Industries) until activity was isolated in response to a search stimulus with energy at both 5 and 16 kHz. Relative electrode depth was noted; however the entire ganglion moved somewhat as the electrode was advanced so that exact depth measurements were not reliable.

The electrode voltage, with respect to an indifferent electrode in the abdomen, was sensed by a high input-impedance (10^{12} ohms) voltage follower built from an FET operational amplifier (Teledyne Philbrick model 1420). The signal was preamplified by two stages of AC-coupled, noninverting amplifiers and then bandpass filtered by a Krohnhite model 3550 filter. The passband

of the filter was set at 200–10,000 Hz during the search for a unit, and was then reset to improve the isolation of the desired unit. The filter output was monitored on a Tektronix storage oscilloscope and an audio monitor and simultaneously recorded on magnetic tape. A summary of the signal processing electronics is shown in Figure 1b.

Auditory thresholds were determined differently for units with and without spontaneous activity. Threshold for normally silent units was defined as the SPL necessary to elicit responses in at least 3 out of 5 consecutive trials. In spontaneously active units, threshold was defined as the SPL which first caused the action potentials to become time-locked to the stimulus.

Vibration Measurements

Vibrations of the tympanic membranes were measured by laser heterodyne spectroscopy (Dragsten et al., 1974; Dragsten et al., 1976). In this technique a laser beam is focused on the vibrating surface; the light scattered from the surface is then mixed with a reference light beam to obtain information on the motion of the vibrating surface. Several controls were routinely carried out to avoid possible artifacts in the mechanical data. First, the vibration of the leg cuticle adjacent to the membrane was measured to verify that neither the leg nor the optical apparatus was vibrating at the sound frequency. Second, we confirmed that there was no signal when either the probe or reference light beam was blocked. This ruled out the possibility of intensity modulation of the light beams. Third, we checked for the presence of light scattered from points not on the surface of the animal. Finally, after each set of measurements, a second set was taken to determine reproducibility. On live animals reproducibility was typically better than 5 dB for all measurements and better than 3 dB for the majority. For mechanical scatterers such as an electrically-driven piezoelectric mirror mount the reproducibility was better than 1 dB.

Results

Two different approaches were used in studying the mechanics of the tympanal organ. First, we measured the vibration of various elements of the organ directly. Second, we measured changes in the neural frequency sensitivity of the organ when the ear was mechanically disturbed in various ways. In the latter experiments, isolated interneurons in the prothoracic ganglion were used as monitors of the organ's frequency sensitivity. The interneurons were most sensitive near 5 kHz (e.g., Figure 8b) and their responses were purely excitatory and tonic (see Paton, 1975 for more details).

Large Tympanic Membrane

The large posterior tympanic membrane normally vibrates up to several hundred Ångströms in response to sounds of the same frequency and intensity as a cricket's calling song. As will be shown, the mechanical response is linear and frequency sensitive, and the membrane vibrates as a whole in response to tones of up to at least 20 kHz.

The mechanical sensitivity of the tympanic membrane was measured as a function of frequency at a constant intensity of 90 dB SPL for 15 animals of two species of field crickets. The peak displacement near the center of the membrane is plotted versus frequency for a typical animal in Figure 2. The mechanical frequency response of this animal's tympanic membrane is representative in

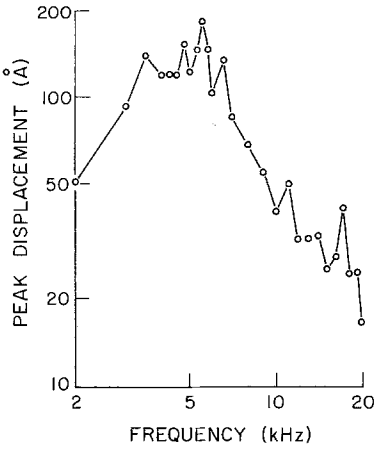


Fig. 2. Typical frequency response of the large tympanic membrane. Results are displayed as the peak displacement as a function of frequency for a constant 90 dB SPL stimulus. These data are from a *Gryllus pennsylvanicus*, 23 August 73

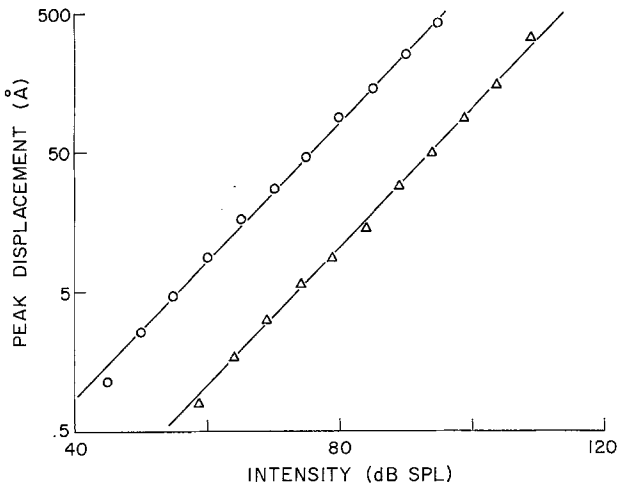


Fig. 3. Linearity of the large tympanic membrane vibration. Data from *Gryllus pennsylvanicus*, 6 August 73. The circles are displacements in response to a 5 kHz tone; the triangles are displacements in response to an 18 kHz tone

having the following features: (1) a maximum displacement near 5 kHz, (2) a response which decreases at roughly 25 dB per decade above 5 kHz, and (3) a decrease in sensitivity below 5 kHz.

As might be expected in a mechanical system whose vibrational amplitudes are small compared with its dimensions, the displacement is a linear function of stimulus intensity. The sample intensity functions in Figure 3 were taken on the same membrane at two different frequencies (5 and 18 kHz). In addition to indicating linearity, the lack of scatter is a measure of the precision of the measuring technique. Even at displacements of less than one Ångström, the measured values differ by less than 3 dB from the expected linearity. For comparison, the intensity of the calling song in the field might be as great as

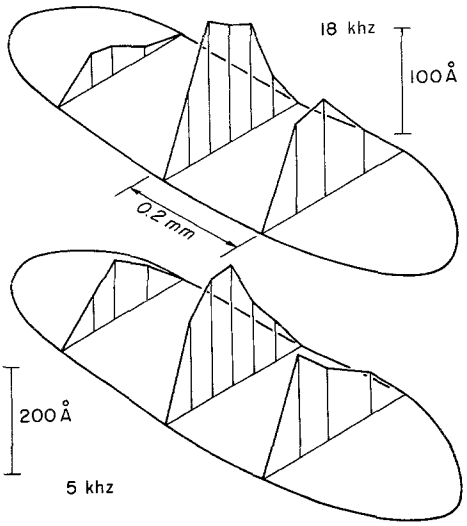


Fig. 4. Spatial pattern of displacement of the large tympanic membrane to tones at 90 dB SPL. Top, 18 kHz; bottom, 5 kHz. (*Gryllus pennsylvanicus*)

100 dB SPL (Popov and Shuvalov, 1974) and the neural threshold of the most sensitive prothoracic interneurons can be as low as 30 or 40 dB SPL. Thus the mechanical response is linear over the entire intensity range of biological interest.

The spatial pattern of vibration in response to tones was measured in 6 animals by scanning the large membrane with the laser beam. Figure 4 shows the peak displacements at a number of locations on the oval membrane. For both 5 kHz and 18 kHz, the vibrational phase relative to the sound phase was constant over the entire membrane. Therefore the membrane vibrates in its simplest (fundamental) mode in response to tones at both frequencies, and measurements at the center of the membrane are indeed a valid measure of the acoustic frequency response.

Although all of the large tympanic membranes tested were most sensitive to frequencies near 5 kHz, the frequency response curves varied in detail from animal to animal and from species to species. To illustrate the range of responses found, the tympanic tuning curves for two *Gryllus pennsylvanicus* are shown in Figure 5a and for two *Acheta domesticus* in Figure 5b. In interpreting these figures it should be remembered that the reproducibility of individual data points was within 3 dB and the accuracy of measuring the sound field was within 1 dB up to 12 kHz and 2 dB up to 20 kHz.

To help compare measurements in the two species, several quantitative measures have been abstracted from the frequency response data and are presented in Table 1. In the first two columns is information on the peak sensitivity at 90 dB SPL. The data for *Gryllus pennsylvanicus* and *Acheta domesticus* are quite similar, with an average most-sensitive-frequency of just above 5 kHz and an average peak displacement of 100 to 200 Ångströms.

In the next two columns of the Table are measures of the sharpness of mechanical tuning. The slope of the line fitted by eye to the data points from the

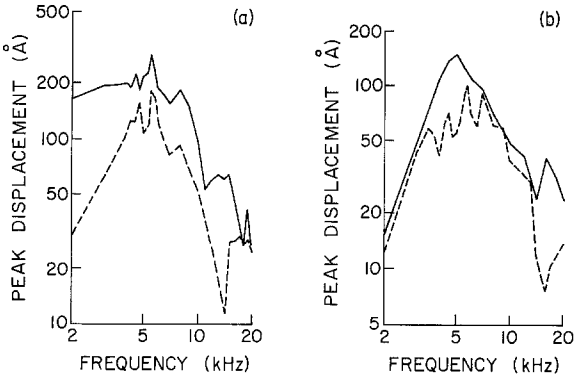


Fig. 5a and b. Frequency response of the large tympanic membrane at a constant intensity of 90 dB SPL. **a** *Gryllus pennsylvanicus:* (i) 8 June 73 ———; (ii) 6 August 73 -------. **b** *Acheta domesticus:* (i) 7 December 73 ———; (ii) 23 February 74 -------

Table 1. Summary of large tympanic membrane measurements

Animal	Frequency for maximum displacement (kHz)	Maximum displacement at 90 dB SPL (Å)	High frequency slope (dB/decade)	Maximum displacement Displacement at 18 kHz (dB)
G. pennsylvanicus				
7 June 73L[a]	5.5	32	12	6
R	5.5	130	24	13
3 July 73	5.25	260	25	14
18 July 73	5.5	310	15	9
23 July 73	5.5	185	32	18
6 August 73L	5.25	330	21	12
R	5.5	295	32	20
Range	5.25–5.5	32–330	12–32	6–20
A. domesticus				
7 December 73	5.75	99	34	26
10 December 73	4.5	16.4	26	18
30 January 74	4.5	375	38	22
2 February 74	5.5	134	varied	7
14 February 74	5.5	57	22	7.5
21 February 74	4.5	40.7	18	10
23 February 74	5	150	26	13.5
28 February 74	5.5	58	18	9
6 March 74	5.5	167	32	19
8 March 74	5.5	155	20	13
Range	4.5–5.75	16.4–375	18–38	7–26

[a] Animals are identified by the date on which measurements were taken. When both ears were used, the ears are further denoted as left (L) or right (R)

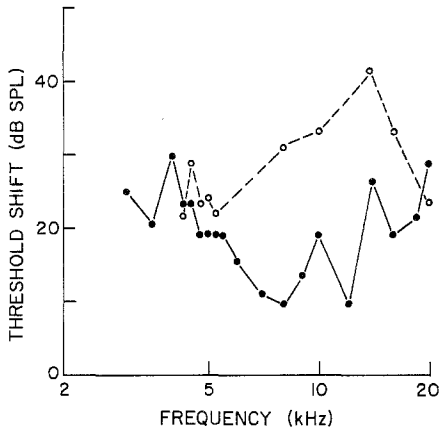
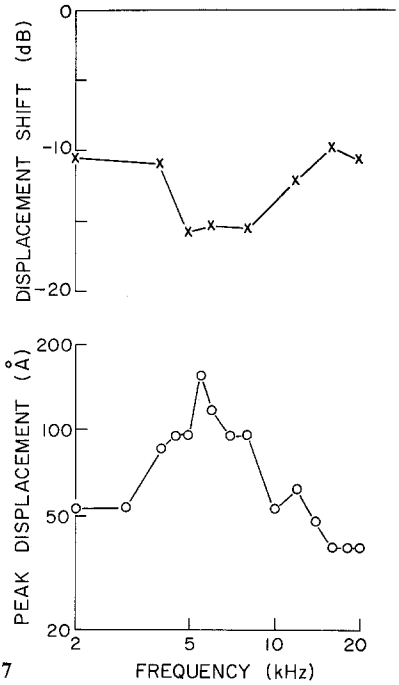


Fig. 6. Change in the threshold of two interneurons due to vaseline over the large tympanic membrane. Closed circles, unit 8 May 74-1-2; open circles, unit 4 May 74-1-2 (both from *T. oceanicus*)

most sensitive frequency to 30 kHz varied from 12 to 38 dB/decade in the entire population of mechanical tuning curves. Unfortunately it is difficult to interpret this variability since the value of the slope depended on a subjective estimate of the best fitting line. Without a model for the relation between sensitivity and frequency and without knowledge of the distribution of error, it did not seem worthwhile to explore more quantitative curve fitting schemes. The second measure used was the ratio between maximum displacement and the displacement at 18 kHz. This more clearly defined measure ranged from 6 to 26 dB, which again reflects considerable variability between animals. In general the bandwidth of Δf of the frequency response (measured at 6 dB above peak sensitivity) is about 5 kHz, corresponding to a quality factor "Q"$=\Delta f/f \simeq 1$ for this response peak. We conclude that because of variability no clear species difference can be seen between the mechanical responses of tympanal membranes in *G. pennsylvanicus* and *A. domesticus.*

When the amplitude of the tympanal membrane vibration was reduced, the auditory activity of the interneurons was also reduced. In four animals vaseline was placed over the entire large tympanic membrane of one leg. Presumably this greatly reduced the membrane vibration. In every case the thresholds of the interneurons increased for *all* frequencies. The results of two experiments are shown in Figure 6. Despite these changes in threshold sensitivity the maximum remained near 5 kHz. This result is not consistent with a tympanic membrane resonance as the determinant of the peak frequency sensitivity of the organ since in this case the increase in membrane mass due to vaseline application should lower resonant frequencies. Instead, the large membrane may simply serve as an acoustic window into the trachea which transmits less energy when coated with vaseline.

In one animal *(A. domesticus)* the membrane vibration was measured before and after tearing a hole of about 75 μm diameter in the large tympanic membrane. The results are shown in Figure 7. The mechanical sensitivity decreased 10 dB or more at all frequencies from 2 to 20 kHz. In three other animals (1 *Gryllus pennsylvanicus* and 2 *Acheta domesticus*) similar small holes



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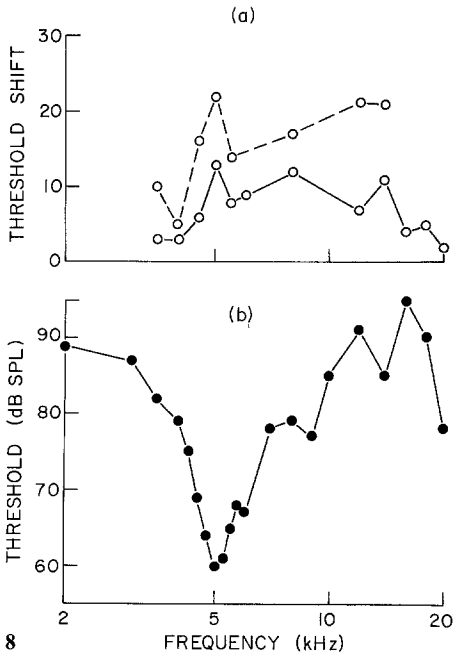

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Fig. 7. Mechanical frequency response before and after tearing a hole in the large tympanic membrane. Circles, membrane intact; crosses, after hole torn in membrane. *Acheta domesticus*, 8 March 74

Fig. 8. a The change in threshold in decibels of interneuron 27 June 74-2-1 due to a hole in the large tympanic membrane. The change for a small hole is shown by the solid line and the change after the hole was enlarged is shown by the dashed line. **b** Threshold curve of the same unit with the membrane intact

were torn in the large tympanic membrane. In these preparations the thresholds of units innervating the damaged ear increased, and the threshold shift was greatest at the peak sensitivity of the unit (see Fig. 8). Note that both neural threshold curves and mechanical tuning curves are presented in their conventional formats, such that a minimum neural threshold corresponds to a maximum displacement. Similarly, increased thresholds are equivalent to decreased movements. As the hole was enlarged, the thresholds increased further until the peak in the tuning curve became deemphasized. Thus the integrity of the membrane is important to, although not directly responsible for, the tuning of the organ.

Small Tympanic Membrane

The vibration of the small anterior tympanic membrane was measured in 6 animals, and in every case the displacement of the membrane was of the same order of magnitude as that of the cuticle adjacent to the membrane and showed no selective tuning. Furthermore, covering the small membrane had no effect

either on the large membrane vibration or on the sensitivity of the interneurons. In two animals the small membrane was covered with tackiwax and no significant change in peak displacement of the large membrane was seen for frequencies from 2 to 20 kHz. In seven animals (1 *A. domesticus* and 6 *T. oceanicus*) vaseline was placed over the entire small tympanic membrane of one leg. No significant change occurred between 2 and 20 kHz in the thresholds of interneurons innervated by this leg. Hence vibration of the small tympanic membrane appears relatively unimportant for normal acoustic transduction.

Effect of Trachea

Another possible source of mechanical tuning is the tracheal cavities behind the tympanic membranes. The acoustic resonant frequency of a cavity depends on its size and shape and is directly proportional to the speed of sound within the cavity. In four animals *(Teleogryllus oceanicus)* the air in the leg trachea was replaced by either helium or oxygen. Of these gases, oxygen and air have a velocity of sound near 330 m/s while the velocity of sound in helium is 965 m/s. This means that any cavity resonant frequencies which might contribute to the tuning of the organ near 5 kHz would be increased by a factor of about 3 when helium is introduced.

In two animals the sequence of oxygen, then helium, and then oxygen as a control were infused into the leg trachea. Tuning curves for a single unit in the prothoracic ganglion were recorded during each of the three infusions. The tuning curves for oxygen before and after helium were within 3 dB of each other and were averaged in plotting the data. Results for two animals are shown in Figure 9. The tuning curve of the unit with oxygen in the trachea is shown below the graph of the change in threshold due to helium. Near 5 kHz the threshold increases up to 20 dB, while near 18 kHz the threshold decreases by as much as 13 dB. These two changes are consistent with a shift in cavity resonance by a factor of about 3, namely from 5 kHz to near 18 kHz. Note that the *increased* sensitivity at higher frequencies indicates that the *decreased* sensitivity near 5 kHz was not due to anoxia.

An obvious question is "what portions of the trachea determine the tuning?" The tracheal branches near the membranes are certainly of a different shape and size than the rest of the leg trachea and might conceivably be responsible for all of the mechanical tuning. To test this possibility, in five animals *(Teleogryllus oceanicus)* thresholds for an interneuron were recorded before, during, and after pinching the trachea in the middle of the femur with a glass probe mounted in a micromanipulator. The change in threshold for one animal is graphed in Figure 10. In all of these animals the greatest reduction in sensitivity occurred near 5 kHz, with little or no change for other frequencies. This frequency dependence is evidence against uniform changes in neural threshold as might be produced by pinching the nerve. Another alternative is that by immobilizing the trachea in the femur, we are directly damping the trachea of the auditory organ within the tibia. We cannot rule this out; however, both the distance between the probe and sensory transduction ($\sim$0.5 cm) and the abruptness of the change in threshold as the sides of the trachea meet argue against the possibility of direct

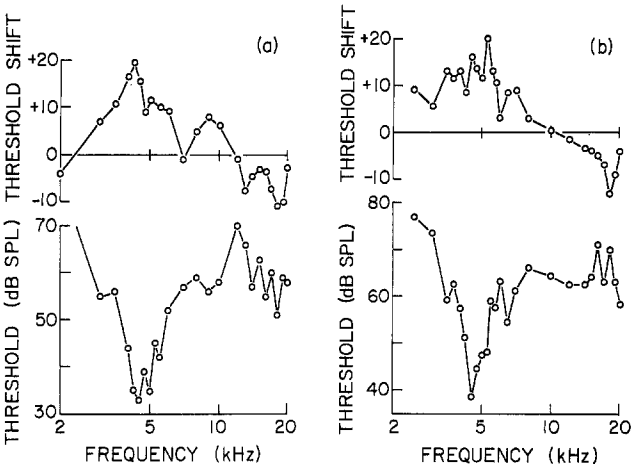

Fig. 9. a Change in threshold in decibels of interneuron 11 October 74-1-1 *(T. oceanicus)* as a result of replacing the oxygen in the leg trachea with helium. Below is the threshold curve of the unit with oxygen in the trachea. **b** Change in threshold in decibels of unit 27 June 74-2-1 *(T. oceanicus)* as a result of replacing oxygen in the trachea with helium. Below is the threshold curve of the unit with oxygen in the trachea

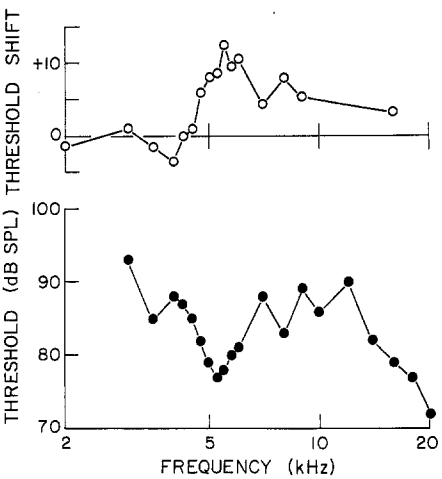

Fig. 10. Change in threshold in decibels of unit 27 June 74-2-1 (*T. oceanicus*) as a result of pinching the leg trachea near the middle of the femur. Below is shown the threshold curve of the unit with the trachea unobstructed

mechanical interference. We suggest instead that pinching the trachea shortens the acoustic cavity responsible for auditory tuning, and therefore that the entire leg trachea rather than just the tracheal branches may be involved in tuning.

Discussion

In this discussion we analyze our experimental results in terms of simple acoustic models based on the anatomy of the tympanal organ. We consider the various mechanical elements of the cricket ear which might be responsible for the observed frequency selectivity of auditory nerve fibers.

Acoustic Model of the Tracheal Organ

Our problem involves (1) the two tympanic membranes, (2) the spiracle opening at the base of the leg trachea, (3) the leg trachea, and (4) the attachments between the trachea and the receptor cells. The mechanical properties of these elements will be discussed individually below. We fully realize the difficulties in applying acoustic theory to a biological system with complex geometry and nonideal materials, so the following calculations are intended as first approximations to be compared with our experimental results. We hope the model will offer a qualitative feeling for physical processes involved in frequency sensitivity within the cricket's ear; a more quantitative model will require further study of the precise geometry involved and the properties of the mechanical elements within the tracheal organ.

The Leg Spiracle

The spiracle has been implicated as the major input channel to the tympanal organ of long-horned grasshoppers (Tettigoniidae) and by analogy might also be an input in crickets. Lewis (1974) found that blocking the spiracle in tettigoniids decreased the sensitivity of the leg nerve by 30 dB, whereas blocking either tympanal membrane had less than a 10 dB effect. Nocke (1974, 1975) also found that blocking the spiracle destroyed the directionality of the auditory organ. From these and other experiments, both investigators concluded that the open spiracle was the major acoustic input to the grasshopper's ear.

In the cricket the role of the spiracle is less certain. Although the cricket ear is very similar to the tettigoniid ear internally, there are two important external differences. First, the tympanic membranes of the grasshopper are partially enclosed by cuticular folds so that the only acoustic pathway to the membranes is through a narrow slit. Second, tettigoniids lack the flap-like covering over the spiracle which is found in crickets. As in most Orthoptera the spiracles of field crickets actively open and close during ventilation. The primary auditory fibers and interneurons show a cyclic variation in sensitivity which is also correlated with the ventilatory rhythm (Nocke, 1972; Stout and Huber, 1972). These changes in auditory sensitivity may in fact be due to changes in spiracle position, but direct evidence is lacking.

If the spiracles are normally open for 5–20% of the ventilatory cycle (as in other Orthoptera; Miller, 1960) then all of our measurements represent the closed spiracle situation because the mechanical measurements were averaged over many cycles and are therefore weighted in favor of the closed spiracle values. Similarly by sampling the neural thresholds during five 50 ms periods, the closed spiracle case is also favored. For this reason we will only discuss the acoustics of the ear in the closed spiracle condition.

Small Tympanic Membrane

Our measurements indicate that the small tympanic membrane does not vibrate any more than the surrounding leg cuticle, and has no reproducible frequency

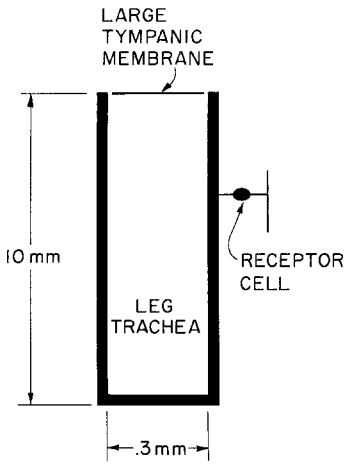

Fig. 11. Schematic model of the mechanical system of the cricket tympanal organ. The tympanic membrane is the only acoustic opening to the tracheal cavity; the receptor cells run from the tracheal wall to fixed sites

tuning. In addition, waxing over the small membrane had no effect either on vibrations of the large membrane or the neural thresholds of auditory interneurons most sensitive near 5 kHz. These results contrast with the findings of Johnstone et al. (1970), namely that "the small membrane tended to mirror the large membrane but was about 20 dB less sensitive." In their experiments sound was presented in a closed system, which might account for some of the difference. Also they do not report the vibration amplitude of the leg cuticle adjacent to the membrane. Therefore, because all three types of our small membrane experiments are consistent, and because there are at least two possible sources of difference between Johnstone's experiments and our own, we believe that the small tympanic membrane has no effect on the tympanal organ at low frequencies.

The lack of tuning to higher frequencies in our study suggests that the small membrane is also unimportant for the reception of frequencies above 10 kHz. On the other hand, Nocke (1972) found that if the small tympanic membrane is detached at its margins from the leg cuticle, the high frequency units in the tympanal nerve stop responding whereas 5 kHz units are unaffected. Unfortunately the receptor cells are very close to the small membrane and might be selectively damaged by such an operation. It would be useful to know whether the same high frequency units can be silenced by immobilizing the small membrane with wax. It would also be interesting to study the frequency sensitivity in species such as ground crickets (Nemobinae) which do not have a small tympanic membrane. Until further evidence such as this is available, the role of the small tympanic membrane is uncertain. To simplify our acoustic model the small tympanic membrane will be ignored, at least in discussing low frequency stimuli. A schematic of our resultant model is shown in Figure 11.

Large Tympanic Membrane

The large tympanic membrane clearly vibrates most readily at frequencies near 5 kHz. Johnstone (1970) suggested that the sensitivity of the membrane was due

to its resonant properties. We will compare the theoretical properties of ideal membranes and plates with experimental observations to see whether a membrane resonance could plausibly explain the tuning of the tympanic membrane. The driving force can be assumed to be a spatially uniform sound pressure which varies sinusoidally with time since the tympanic membrane is much smaller than the shortest wavelength of sound used (16 mm at 20 kHz). To simplify the discussion, the membrane will be assumed circular even though it is approximately elliptical (0.3×0.7 mm) since predictions for the modes of vibration are qualitatively the same for the two ideal shapes. Furthermore the *ratios* of resonant frequencies should be relatively independent of the details of membrane shape. This last assumption seems reasonable since the *ratios* of resonant harmonic frequencies for a similar problem, namely a rectangular membrane, are independent of the membrane's length and width (Kinsler and Frey, 1962).[1]

The mechanical model for the tympanic membrane depends on the restoring forces. If the membrane is sufficiently thin and anchored at its edges under tension, then it can be treated as a true thin membrane in the mechanical sense. If however it is sufficiently thick that it resists bending forces, then it must be treated as a plate. Suppose we consider both limits.

The displacement of a driven, circular membrane of radius a is well known. In complex notation the displacement y as a function of distance r from the center as a function of time t is for the undamped case

$$y = \frac{P}{k^2 T}\left[\frac{J_0(\omega r/c)}{J_0(\omega a/c)} - 1\right] e^{j\omega t} \tag{1}$$

where ω is the angular frequency, c is the velocity of sound, $k = \omega/c$ is the wavelength constant, $J_0(\,)$ is the zero-order Bessel function, T is the tension, and P is the magnitude of the sinusoidal driving pressure (Kinsler and Frey, 1962, p. 94). At the center of the membrane, namely for $r = 0$, $J_0(0) = 1$ and the displacement as a function of frequency (without damping) is

$$|y| = \frac{c^2 P}{\omega^2 T}\left[\frac{1}{J_0(\omega a/c)} - 1\right]. \tag{2}$$

The displacement at the center is maximum for resonant frequencies such that $J_0(\omega a/c) = 0$. The first three resonant frequencies are

$$f_0 = 0.3828\, c/a, \quad f_1 = 2.295 f_0, \quad \text{and} \quad f_2 = 3.598 f_0.$$

Thus, if $f_0 = 5$ kHz, the next two resonances should occur near 11.5 kHz and 18 kHz.

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[1] The exact solution for the vibrational pattern of an elliptical membrane involves Mathieu functions and will not be developed in this discussion. For references, see Whittaker and Watson, 1927, pp. 404–428

If the tympanic membrane behaves as a circular elastic plate, its displacement as a function of position and frequency is

$$|y| = \frac{P}{2h\rho\omega^2} \cdot \frac{I_1(\gamma a)[J_0(\gamma r) - J_0(\gamma a)] + J_1(\gamma a)[I_0(\gamma r) - I_0(\gamma a)]}{I_1(\gamma a)\, J_0(\gamma a) + J_1(\gamma a)\, I_0(\gamma a)} \tag{3}$$

where h = plate thickness, ρ = density of plate, $J_1(\,)$ is the first order Bessel function, $I_0(\,)$ is the zero order hyperbolic Bessel function, $I_1(\,)$ is the first order hyperbolic Bessel function, and γ is a variable which is proportional to $\sqrt{\omega}$ (Morse and Ingard, 1970, p. 217). The plate resonates at those frequencies at which the denominator of Equation (3) vanishes; that is, f_0, $3.91 f_0$, $8.73 f_0$, etc. These resonant frequencies are more widely spaced than for the circular membrane but even so the second harmonic does appear by 18 kHz.

Although our experiments covered this entire frequency range, only the fundamental mode at $f \simeq 5$ kHz was seen (Figs. 2 and 5). Thus, the predicted higher order plate or membrane resonances are not observed in the mechanical response.

The strongest evidence that only the fundamental mode of vibration of the tympanic membrane is excitable is our observation of the spatial distribution of the vibration amplitude of the membrane. It always increased smoothly with frequency from the periphery to the center with no nodes at either 5 or 18 kHz. The phase of the vibration was constant across the membrane to within the accuracy of our measurement at both frequencies. In the case of a resonant membrane or plate model, higher modes should be excited as the driving frequency approaches the second and third harmonics. A node, i.e. region of zero displacement, should appear near the edge of the membrane, and as ω increases, the node should move toward the center of the membrane and a new nodal circle should appear for every resonance. Furthermore, corresponding points on opposite sides of the nodes should vibrate with opposite phases.

Our measurements of the frequency and spatial response of the membrane vibration amplitude clearly show that only the fundamental mode of vibration is excited with maximum response around 5 kHz. Although the expected harmonics fall within our range of measurement, they are not excited. The response peak associated with the fundamental is very broad, about 5 kHz half maximum width, and damping alone may be strong enough to exclude vibration in higher modes. However their absence leads us to consider another possible tuned element – the trachea.

The Leg Trachea

When the membrane is intact and the spiracle closed, the tracheal cavity presents an additional mechanical load on the tympanic membrane. This load can either interfere with or aid membrane vibration depending on the acoustic properties of the trachea.

The trachea can be treated in two different ways depending on the dimensions of the cavity. If all of the dimensions are less than one fourth of the wavelength of sound, then the cavity is a Helmholtz resonator in which the air

volume determines the first resonant frequency. In the cricket leg, the trachea has a diameter of about 0.3 mm and a length of approximately 10 mm which should produce a Helmholtz resonance of 34.2 kHz. Therefore the trachea does not act as a simple Helmholtz resonator within the frequency range of our measurements. Considering the trachea instead as a tube with diameter much less than the wavelength of sound, i.e. as an acoustic waveguide directly analogous to electrical waveguides, then the length determines the resonant frequencies. The resonant frequencies for an acoustic waveguide terminated by a rigid ending are

$$f_{t,n}=\frac{2n-1}{4}\cdot\frac{c}{l} \tag{4}$$

where c is the speed of sound in air, l is the length of the pipe, and n is any positive integer (Kinsler and Frey, 1962, p. 202). Thus the fundamental undamped geometrical resonance of the trachea would appear at $f_t=c/4l=(3.30\times10^{-1})/(4\times1)\simeq8.25$ kHz and at all odd harmonics of this fundamental frequency.

The preceding calculation for simple closed pipes must be corrected for several secondary factors for application to the trachea. First the diameter of the trachea is small enough to produce an appreciable attenuation and a decreased velocity due to viscous aerodynamic drag on the walls. The wave velocity in tubes is

$$v=c\left[1-\frac{1}{2a}\sqrt{\frac{2\nu}{\omega}}\right] \tag{5}$$

where c is the velocity in air, a is the radius of the tube, and ν is the effective kinematic coefficient of viscosity (Kinsler and Frey, 1962, pp. 240–241). To give an idea of the magnitude of the change in velocity, for a leg trachea of average radius 0.15 mm the wave velocity at 7 kHz is approximately $v=0.9\,c$. Because the acoustic wave propagates at a slower velocity in the trachea than in open space, the resonance is shifted to lower frequencies than calculated from Equation (4). Thus a more accurate value of f_t can be found by replacing c in Equation (4) with the expression for v from Equation (5), giving a tracheal resonance around 7.5 kHz which is somewhat damped.

A second effect, yielding of the tracheal walls, would reduce the resonant frequency even further. However (Morse, 1948, pp. 305–308), at reasonable vibration amplitudes this effect seems negligible.

With aerodynamic damping reducing the speed of sound in the trachea, the estimated tube resonance should be near 7.5 kHz. Therefore, allowing for imprecision of the model and uncertainties of anatomy, the fundamental resonance of the main leg trachea might plausibly provide a mechanical resonance that couples to the tympanic membrane to develop the membrane displacement maximum observed around 5 kHz.

Direct proof of the involvement of a tracheal resonance is provided by our experiments with helium infusion and pinching the trachea. Both shifted the peak frequency of interneuron sensitivity. Increasing the speed of sound in the

trachea from 330 m/s to 965 m/s by introducing helium should shift the tracheal resonance to a frequency 2.92 times higher [see Eq. (4)]. Experimentally we found that the frequency of maximum sensitivity of interneurons shifted upward by factors of 3 and just above 3 in two different animals on helium infusion. Pinching the trachea also abolished the tracheal resonance at 5 kHz. Therefore we suppose that the entire tibial portion of the leg trachea in the cricket is involved in the primary tuning near 5 kHz of the auditory organ but it remains to be verified whether the relevant resonance mode is indeed a fundamental pipe resonance. For example, the leg trachea narrows distal to the tympanic membrane and the effect of such a termination is uncertain. At the other end of the leg, the leg trachea opens into the much larger prothoracic trachea. The prothoracic trachea runs between the prothoracic spiracles, and is separated into two compartments by a septum at the midline. In effect then, the proximal end of the leg trachea enters the prothoracic trachea to form a *T*-shaped termination. Thus the prothoracic trachea might possibly contribute to the overall tracheal resonance. In fact, Hill and Boyan (1976) have recently concluded that sound propagates from one leg trachea to the other by way of the prothoracic trachea. If this is the case, then acoustic resonance responsible for auditory tuning would also be affected by the contralateral trachea. The involvement of the prothoracic trachea therefore might account for the discrepancy between the theoretical frequency response in our simple model and the observed results.

The leg trachea "determines" the pressure on the inner side of the tympanic membrane while the incident sound determines the pressure on the outer side. The difference between these two pressures drives the membrane; however the mode of membrane vibration should not be affected by coupling to the tracheal resonance. Therefore it is quite possible that the fundamental natural resonance of the isolated tympanic membrane, whether effectively a plate or a thin stretched membrane, occurs at a frequency above $\simeq 10$ kHz. Only if damping suppressed excitation of higher harmonics could the fundamental frequency be as low as 5 kHz. It is of course not possible to calculate the fundamental resonant frequency of the tympanic membrane from the anatomical data available to us.

Receptor Cell Attachments

Very little evidence other than morphology (Young and Ball, 1974) is available on the function of the attachments between the trachea and the receptor cells. Anatomically the axons of the receptor cells follow beside the trachea and then turn at an abrupt angle toward the leg cuticle. No direct mechanical connection to the trachea has been found. The receptor cell bodies are arranged along the length of the small tracheal branch, and the solitary dendrite of each cell points toward a common locus on the surface of the tibia. The dendrites contact attachment cells of different shapes and sizes. The more proximal receptors are connected to small attachment cells, whereas the distal receptors are connected to long, narrow attachment cells extending from the nerve cell to an attachment site near the cuticle. Finally, the fine structure of the sensory cells, especially the

dendrite shapes, varies systematically within the organ. To summarize, axonal paths, dendritic structure, and attachment cell morphology are all factors which vary from one receptor to another and might contribute to their individual frequency sensitivity.

Neural Sensitivity of the Tracheal Organ

The frequency sensitivity of the auditory organ is generally presumed to be determined by the mechanical structures which transfer energy from the environment to the receptor cells. As we now understand the system, sound impinges on the large tympanic membrane, whose movement drives the air within the trachea, and the resulting coupling to the tracheal resonance modifies the resultant membrane response. The small tracheal branch is set into vibration and this vibration couples to the receptor cells to excite them.

The neural frequency sensitivity of cricket ears has been determined in a number of species. In all of the species studied, the tympanal nerve is most sensitive to frequencies near 5 kHz but is more sharply tuned than the vibrations of the tympanic membrane. For example, the maximum slope of the mechanical tympanic tuning curve above 5 kHz in *T. oceanicus* is about 10 dB/octave, while the slope of the neural curve in the same species is about 25 dB/octave (Loftus-Hills et al., 1971). This difference suggests that the mechanical tuning manifested in the tympanic membrane response is not entirely responsible for tuning of neural sensitivity.

Single receptor cells or interneurons in the prothoracic ganglion are in turn more narrowly tuned than the whole tympanal nerve (Loftus-Hills et al., 1971; Paton and Capranica, unpublished). This means that energy at different frequencies is differentially distributed to the sensory cells in the tympanal organ; therefore further tuning must occur near or at the receptor cells, possibly in coupling to the trachea or attachment cells or in receptor cell morphology. The most striking example of this selective tuning is found in *Gryllus campestris* where each sensory cell is tuned to either 5 kHz or 14 kHz, but not both (Nocke, 1972). One possibility which we have considered is that these two sets of receptors may be driven by different modes of tracheal resonance, that is, the fundamental and third harmonic. However, harmonic peaks were not found in the mechanical tuning curves of tympanic membranes.

The tympanic membrane does not seem to play an important role in the frequency selectivity of the tympanal organ. Thus we are left with the question of the immediate coupling of energy to the receptor cells via the trachèa. Measurements of sound pressure within the trachea, tracheal vibrations, and the deformations of supporting structures connected to the receptor cells may help clarify this question.

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