J. Exp. Biol. (1972), 57, 297–304
With 4 *text-figures*
Printed in Great Britain

MECHANICAL TUNING OF THE ACOUSTIC RECEPTOR OF *PRODENIA ERIDANIA* (CRAMER) (NOCTUIDAE)

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(*Received* 25 *January* 1972)

INTRODUCTION

The acoustic receptors of noctuid moths appear to serve a single purpose in nature —detection of the sonar signals of insectivorous bats (Roeder, 1966*b*). Accordingly, the receptors are most sensitive to acoustic stimuli that have frequencies between 20 and 100 kHz (Roeder, 1966*a*).

The tuning of the receptor may be separated into two components: coupling of impinging sound waves to movements of the eardrum, and transformation of eardrum movements into mechanical distortions of the transduction region. The first component depends on the acoustic properties of the system, in particular those of the tympanic membrane and the cavities that lie behind it. The second component results from the mechanical properties of the receptor organ and its mode of vibration.

The receptor organ, or scoloparium, is contained in the tympanic air sac, which arises as an enlargement of the tracheal system (Fig. 1). The scoloparium is attached directly to the tympanic membrane. At its proximal end the scoloparium is suspended by the beginning of the auditory nerve and a 'ligament'. Despite its suggestive name, the ligament does not appear to exert any tension on the scoloparium. In fact, the ligament is usually slack and kinked at its lower end.

In *Prodenia eridania* (Cramer), the moths used in these experiments, the width of the scoloparium varies between 3 and 25 μm while the total length is about 160 μm. The angle of attachment to the eardrum is small—30° or less. The dimensions and the shallow angle of attachment suggest that lateral flexure may form the principal mode of movement. If this is the case, one or more mechanical resonances should be expected.

The scoloparium contains two acoustic receptor cells of the scolopophorous type and several accessory cells (Eggers, 1919; Ghiradella, 1971). Probably the most special feature of a scolopophorous receptor is the cilium that arises from the end of the dendrite and extends to the distal end of the scolopod (Gray, 1960; Ghiradella, 1971). The cilia and scolopods of the two receptor cells lie near the middle of the scoloparium, between two large bulges. The receptor cell bodies occupy the proximal bulge, while the distal bulge is densely packed with microtubules (Ghiradella, 1971). Physiological studies have revealed similar response parameters for the two receptor cells, except for a difference of 20 dB or more in sensitivity (Roeder & Treat, 1957; Suga, 1961).

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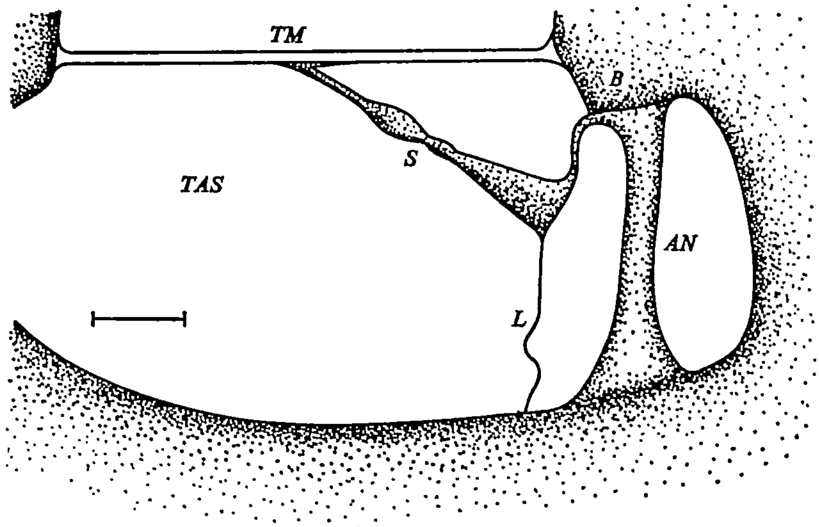

Fig. 1. Sagittal view of the noctuid ear, with special reference to *Prodenia eridania* (Cramer). The thickness of the tympanic membrane has been exaggerated. Note the small angle with which the receptor organ joins the tympanum and the kinked appearance of the 'ligament', implying that the ligament exerts little tension on the scholoparium. The dimensions were obtained from fresh preparations and from whole mounts in glycerol. *AN*, auditory nerve; *B*, Bügel; *L*, ligament; *S*, scoloparium; *TAS*, tympanic air sac; *TM*, tympanic membrane. Calibration bar, 50 μm.

The purpose of this paper is to describe the mechanical tuning of the receptor organ as revealed by the frequency characteristic of the more sensitive receptor cell. Stimuli are delivered directly to the tympanic membrane with vibrating probes to eliminate the effects of acoustic tuning. The data are reported as the displacement required for criterion excitation of the receptor cell.

METHODS AND MATERIALS

Live material

Experiments were performed on approximately 75 moths. Preliminary investigations were carried out on various noctuid species caught at night during the summer months. Subsequently, specimens of *Prodenia eridania* (Cramer) were obtained as adults from the Niagara Chemical Division of FMC Corporation. The data in this report were obtained from these laboratory-reared moths.

Methods

The recording techniques have been described in full elsewhere (Adams, 1971*a*, 1971*b*). Briefly, a moth is mounted in paraffin and the legs, wings and abdomen are removed to expose the tympanic organ. Extracellular recordings from the auditory nerve are obtained with saline-filled pipettes that have heat-polished tips of 12–18 μm.

The tympanic membrane is exposed for stimulation by excision of the hood, an exoskeletal structure that forms the caudal wall of the tympanic recess (Eggers, 1919;

Richards, 1932; Roeder & Treat, 1957). The probe of a mechanical stimulator is set perpendicular to the plane of the tympanic membrane, centred over the scoloparium attachment, and lowered to make visual contact.

The stimuli consist of 50 millisecond (msec) sinusoidal tone bursts delivered once per second. The rise and fall times are 5 msec each. The number of spikes elicited from the receptor during the second half of the stimulus is used as a measure of the response magnitude. The responses to 50 identical stimuli are collected and averaged to derive an effective firing rate.

Mechanical stimulators

An electromagnetic stimulator, fashioned from a small permanent-magnet speaker, was used for low-frequency stimulation. Non-resonant displacements of 2·6 μm peak were obtained for frequencies up to 600 Hz. Usable displacements extended to 10 kHz. A prototype piezoelectric stimulator used in early experiments was calibrated to 40 kHz. Approximately 0·016 μm peak displacement was obtained with non-resonant drive. The primary resonance fell at about 3 kHz, with additional resonances at 14 and 35 kHz. A second piezoelectric stimulator was designed for extended frequency response. Non-resonant drive produced 0·025 μm peak displacement. The primary resonance fell at 28 kHz, with a second resonance at 70 kHz. Usable outputs were obtained for frequencies in excess of 100 kHz. Further information on the construction and calibration of the stimulators may be found elsewhere (Adams, 1971*a*, and in preparation).

RESULTS

The eardrum displacement that is required to elicit a criterion response provides a measure of the relative effectiveness of a stimulus. Determination of the criterion displacement was simplified by use of a standard intensity characteristic that had been derived in experiments with acoustic stimulation (Fig. 2; Adams, 1971*b*). The characteristic expresses the neural firing rate of the more sensitive receptor as a function of intensity above or below the intensity which would elicit a criterion response of 50 spikes per second. Intensity characteristics for individual receptors usually fall within a few dB of the standard characteristic. Thus, to determine the criterion intensity for a particular stimulus frequency, one need only stimulate with a supra-threshold stimulus, measure the response, and use the standard characteristic to extrapolate back to the criterion intensity. For example, a stimulus that elicits a firing rate of 150 spikes per second is found to be 8 dB more intense than a criterion stimulus of the same frequency.

The applicability of the standard intensity characteristic to the present experiments was tested by comparing the results that were obtained with mechanical and acoustic stimulation. Fig. 3 shows an intensity characteristic that was measured in an experiment employing mechanical stimulation. For comparison, it has been superimposed upon the standard intensity characteristic taken from Fig. 2. The agreement between the two curves indicates that the results obtained with acoustic stimulation may be applied to the mechanical stimulation experiments without significant error. This finding provides a substantial advantage, as experiments requiring mechanical stimuli present several problems. In particular, the

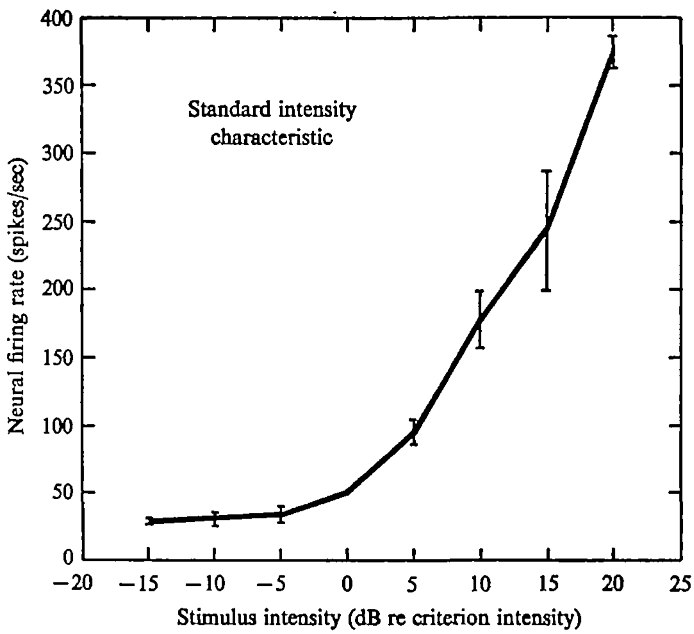

Fig. 2. Standard intensity characteristic. The effective neural firing rate is calculated from the responses occurring during the second half of a 50 msec stimulus. The response for a single preparation is measured twice during the experiment, averaging the response to 50 stimuli for each determination. The abscissa gives the stimulus intensity referred to the intensity that elicits the criterion response of 50 spikes per second. The curve represents average values obtained from six preparations. Vertical bars: ± 1 sample standard deviation.

additional surgical procedures and the placement of the stimulator probe are likely to result in damage to the thin and fragile tympanic membrane. The equivalence of acoustic and mechanical stimulation allows the criterion displacement to be obtained from a single measurement of a supra-threshold response.

The frequency characteristic was derived from the results of several experiments. For a particular measurement, the frequency was fixed and the intensity was adjusted to produce a stimulus-related response of approximately 100 spikes per second. Fifty stimuli were presented to obtain an accurate measure of the response. The standard intensity characteristic was then used to extrapolate to the criterion intensity.

The extrapolation yielded a measure of the eardrum displacement required to produce a criterion response at the selected stimulus frequency. By repeating the procedure at several frequencies a segment of the frequency characteristic was obtained. Usually the entire frequency range could not be investigated in any one experiment. The stability of the preparation limited the number of stimulus runs to between four and sixteen, with an average of eight or ten data points being obtained in a single experiment. In addition, different stimulators were required for different segments of the frequency range. For low frequencies, i.e. below c. 2 kHz, large displacements were needed and the electromagnetic stimulator was employed. However, the useful range of this stimulator extended only to 10 kHz, and above that frequency the piezoelectric stimulators were used.

To compensate for the limited number of data points that were obtained from

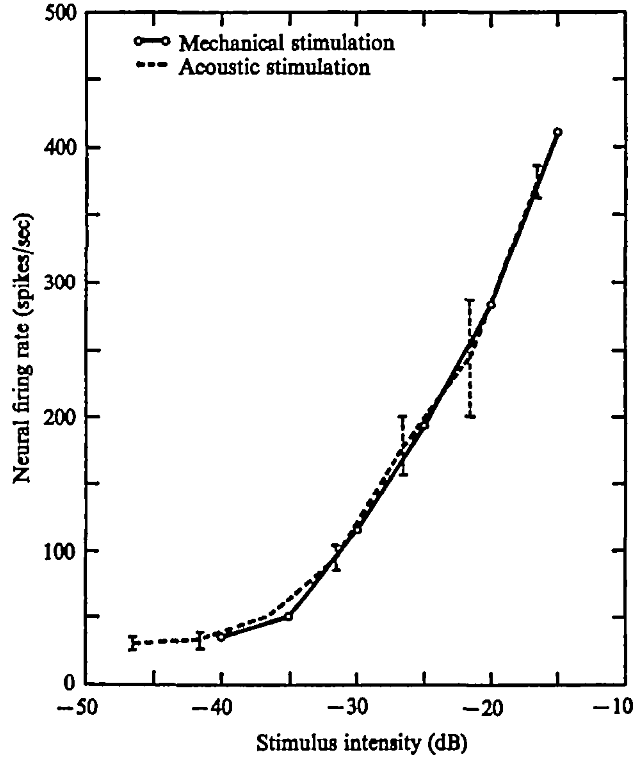

Fig. 3. Intensity characteristics for mechanical and acoustic stimulation. An intensity characteristic determined with mechanical stimulation from a single preparation is superimposed on the average standard intensity characteristic that was derived from acoustic stimulation experiments (Fig. 2). The reference point for stimulus intensity was chosen to show the overlap of the two curves.

individual preparations, the results of several experiments are combined in Fig. 4. The upper graph is a composite of measurements from seven preparations. In the lower graph the average values of these measurements are plotted to simplify reading. In both cases, the vertical axis gives the stimulus intensity—in dB referred to 1 μm peak displacement—necessary to elicit the criterion response.

Several risks are incurred in averaging data obtained from mechanical systems. If the systems are not tuned to exactly the same frequencies, the peaks in the average frequency response are broader than in the individual response curves. Furthermore, since each preparation covers only a segment of the frequency range, individual differences in overall sensitivity can produce spurious maxima or minima in the average curve. Fortunately, the data obtained from different preparations are fairly consistent. The critical frequencies, i.e. the frequencies at which local maxima and minima of sensitivity were found, were nearly identical in all the experiments. At frequencies where two or more experiments overlapped, the agreement is generally satisfactory. To be sure, there is some scatter in the data. Nevertheless, the average curve resembles the individual curves in all respects.

Three regions of high sensitivity were found. The first of these regions is quite broad, and centred at about 2 kHz. For stimulus frequencies below 2 kHz the

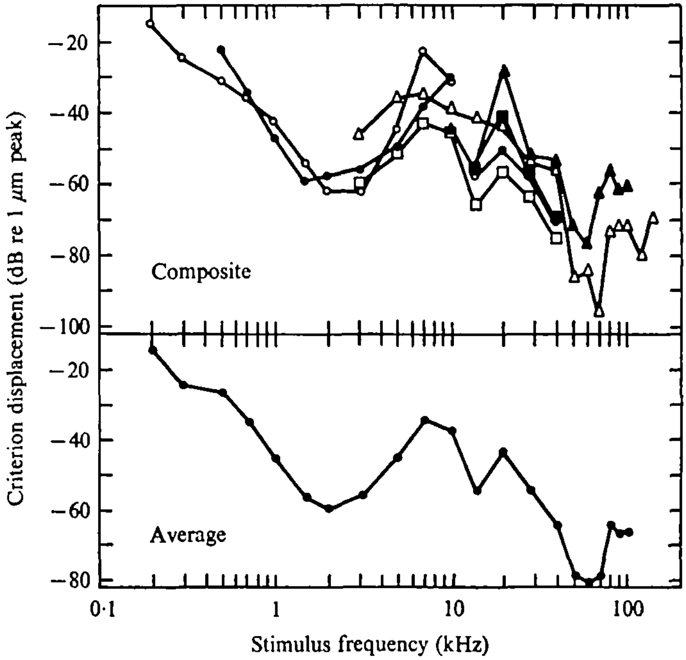

Fig. 4. Frequency characteristic determined with mechanical stimulation of the tympanic membrane. The upper graph is a composite of the results from seven experiments. The arithmetic averages of these data are shown in the lower graph. In both cases the ordinate gives the eardrum displacement, in dB referred to 1 μm peak, that would elicit the criterion response of 50 spikes per second. The electro-magnetic stimulator was used in the two experiments that include the lowest frequencies, the second piezoelectric stimulator in the two experiments extending to the highest frequencies, and the original piezoelectric stimulator for the remainder of the experiments.

displacement required for criterion excitation appears to continue rising indefinitely as the frequency is lowered. Above 2 kHz the necessary displacement again increases, reaching a maximum at approximately 8 kHz.

The next region is sharply delimited around a centre frequency of approximately 14 kHz. Near the centre frequency the tuning is probably sharper than is indicated by the graphs, since no effort was made to locate the precise frequency of maximum sensitivity. Instead, a standard set of frequencies (8·00, 10·00, 13·80, and 19·50 kHz) was used in all experiments to facilitate comparison of the data from different preparations.

The third low-threshold region is broad again. It covers the frequency range from 20 to 100 kHz, with maximum sensitivity at 60 or 70 kHz. In one preparation the threshold displacement reached a minimum at 70 kHz of nearly −100 dB or $\frac{1}{10}$ Ångstrom (Å). In other words, the displacements necessary to elicit a criterion response at this frequency were of subatomic size.

DISCUSSION

The frequency characteristic of the receptor cell clearly reveals the consequences of mechanical tuning by the receptor organ. In particular, the enhanced sensitivity at 2, 14, and 60 kHz suggests that the scoloparium is set into resonant vibration at these frequencies. Unfortunately, the vibration amplitudes appear to be too small to allow direct confirmation of the resonant behaviour. Indirect evidence is available, however, from preliminary studies of the vibration patterns of a mechanical model of the scoloparium (Adams, 1971*a*). These studies support the hypothesis that lateral flexure forms the principle mode of vibration and, furthermore, indicate that the enhanced sensitivity may be accounted for by the first, third and fifth order mechanical resonances of the scoloparium. The receptor is not excited by the even-order resonances because their vibration peaks fall outside the postulated transduction region. Further work is in progress to describe more precisely the form of the mechanical distortions at the site of the transducer elements in preparation for a closer study of the transduction process.

The role of acoustic tuning by the accessory structures may be evaluated by comparing the frequency characteristic obtained here with results obtained with acoustic stimulation. Roeder (1966*a*) has found in similar moths that excitation with a 5 msec stimulus requires a minimum acoustic power of about 40 dB SPL, i.e. 40 dB referred to 0·0002 dynes/cm^2. When a 50 msec stimulus is substituted, a criterion response is obtained with a stimulus 15 dB less intense (Adams, 1971*a*). Hence, an acoustic stimulus similar to the mechanical stimuli used here should require an intensity of about 25 dB SPL to elicit a criterion response at the best frequency. The peak particle displacement in a 60 kHz sound wave at 25 dB SPL amounts to 0·03 Å. In comparison, the criterion eardrum displacement as measured with mechanical stimulation was between 0·1 and 1 Å (Fig. 4). This result suggests that a high-frequency acoustic resonance is produced by the accessory structures or, allowing for experimental error and interspecies differences, that the movements of the eardrum are at least closely coupled to the impinging sound wave.

Quite different results are obtained at low frequencies. Although mechanical stimulation is effective below 10 kHz, acoustic stimulation is not. Roeder has found that the acoustic threshold at 2 kHz is greater than 85 dB SPL, corresponding to a particle displacement amplitude of 1100 Å. Since the threshold displacement of the tympanic membrane is only 10 Å (Fig. 4), the tympanic membrane must be poorly coupled to the incident sound wave at low frequencies.

The acoustic tuning may arise from several accessory structures, including the tympanic membrane, the internal cavities, and the external tympanic recess. However, the tympanic membrane appears to make the most important contribution. Based on his findings for the locust ear (Michelsen, 1971), Michelsen has suggested (personal communication) that the primary resonance of the noctuid tympanic membrane should fall at 60 or 70 kHz. Although the eardrum resonance has been eliminated in these experiments by the use of mechanical stimulation, the resonance would take on major importance in the detection of acoustic stimuli.

The overall frequency response for acoustic stimulation thus appears to depend on both acoustic and mechanical tuning. The mechanical properties of the scoloparium provide high sensitivity for several discrete frequency ranges, while the low-frequency ranges are filtered out by the acoustic system.

SUMMARY

1. Mechanical stimulation of the tympanic membrane with vibrating probes eliminates the acoustic tuning of the receptor and allows the mechanical tuning to be determined.

2. Using neural firing rates as a measure of the response magnitude, three separate regions of high sensitivity are found. They are centred at approximately 2, 14 and 60 kHz. Displacements of 0·1 to 1·0 Å suffice for excitation at 60 kHz.

3. The regions of high sensitivity appear to correspond to the first, third and fifth order mechanical resonances of the scoloparium.

4. Comparison with the results of acoustic stimulation experiments indicates that low-frequency acoustic stimuli are filtered out by the acoustic system of the receptor.

My thanks go to Drs Robert B. Barlow, Jr. and Jozef J. Zwislocki for their counsel, support and assistance throughout these studies, and to Drs Kenneth D. Roeder and Asher E. Treat for continuing encouragement. Interpretation of the data was aided by access to results of electronmicrographic studies by Dr Helen Ghiradella in advance of their publication. The moths used in these experiments were kindly supplied by Mr Dominic Albanese and Dr Kenneth Ensing of the Niagara Chemical Division of FMC Corporation.

This work is based in part on a dissertation submitted to the faculty of Syracuse University in partial fulfilment of the requirements for the Ph.D.

Research support was provided by Grant No. NS-03950 from the U.S. Department of Health, Education, and Welfare, National Institute of Neurological Diseases and Stroke. Manuscript preparation was supported by Grant No. 5-S04 RR 06013 from the Division of Research Resources, National Institutes of Health.

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