

# The Physiology of the Locust Ear

## I. Frequency Sensitivity of Single Cells in the Isolated Ear

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*Summary.* The sensory responses of single receptor cells in the isolated ear of the locust *Schistocerca gregaria* were measured under controlled acoustical conditions. The four anatomical groups (Fig. 1) differ as to frequency sensitivity (Fig. 11). Although the isolated ear differs much from the intact ear, it may be concluded that fairly accurate information about sound frequency reaches the CNS. The responses of most units showed a maximum sensitivity at two (Figs. 4 and 9) or three (Fig. 8) different frequencies. But several units had only one maximum (Figs. 6 and 7, right).

### Introduction

Information about the frequency of a sound may be signalled by auditory receptors in two different ways. The anatomical groups of receptor cells may differ as to characteristic frequency (CF, i.e. the frequency of maximum sensitivity), thus providing the CNS with information about frequency (the place principle). At low frequencies the receptor cells may also send trains of nerve impulses to the CNS, each corresponding to a certain phase of the sound wave (the telephone principle). In the ear of vertebrates (see Whitfield, 1967), the place principle is the most likely explanation for frequency discrimination in the entire frequency range of the ear, and the telephone principle is normally thought to be of minor importance, and that only at frequencies below 2–4 kHz. So far, no examples of frequency discrimination based upon the place principle have been found outside the vertebrates.

In invertebrates the telephone principle is illustrated by the response of cercal hairs of insects to low pitched sounds (Pumphrey and Rawdon-Smith, 1936). It is doubtful, however, whether the insect CNS extracts the information about frequency carried by the cercal nerve.

Insects have generally been considered tone-deaf; the response of the insect ear was thought to be determined by changes in amplitude of the sound, and the frequency (pitch) was not considered important. In 1960,

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however, Horridge obtained evidence for some degree of pitch discrimination in the tympanal organ of locusts by recording from the tympanal nerve and central connectives. Popov (1965), by means of selective destruction, and Michelsen (1966), by recording from the individual receptor cells found evidence for two groups of receptor cells with widely different characteristic frequencies, and apparently with little overlap of the frequency responses at moderate intensities.

The present paper describes different frequency responses of the four anatomical groups of receptor cells in the *isolated* tympanal organ of the desert locust. The relationship between the results on the isolated ear and those obtained in the ear *in situ* will be dealt with in a separate publication (paper III). The amount of overlap of some of the frequency responses seems to allow a rather accurate determination of frequency in the CNS.

### Material and Methods

The locust has a tympanal organ on each side of the first abdominal segment. It consists of a sclerotized ring forming a recess in the abdomen and encircling a membrane, the tympanum. The tympanum is bean-shaped and about  $2.5 \times 1.5$  mm at its widest. The sensory units, a total of 60–80 chordotonal sensilla, are attached in four groups to four modified parts of the tympanum (Fig. 1). The attachment parts of the drum are thickened regions which form an *elevated process* (an invagination about  $100 \mu\text{m}$  deep), a *styliiform body*, a *folded body*, and a *pyriform vesicle*. The groups of sensory cells attached to these regions were named the a-, b-, c-, and d-groups respectively by Gray (1960), who described the fine structure of the ear, and this terminology will be used here. The receptor cells of the a-, c- and d-groups are orientated in three almost mutually perpendicular planes. The fourth group (b) is orientated in the same plane as the a-group. The a-group comprises about half of the total number of receptor cells, and the three other groups about 8–12 cells each.

The female locusts (*Schistocerca gregaria* Forskål, ph. gregaria) used were supplied by the Anti-Locust Research Centre, London, and were about 4 weeks after the final moult. The sclerotized ring encircling the tympanum and a small portion of the surrounding tissue were removed and mounted on a small platform of wax. When mounted the tympanum was about  $15^\circ$  askew relative to the vertical plane (see Michelsen, 1966).

The platform with the preparation was placed on the end of a vertical brass rod in an open box made of "soft masonite" and lined on the inside with 15 cm of mineral wool (Fig. 2). The box was situated in an isolation room, which had a special sound attenuating steel door.

The two loudspeakers were situated at the hindmost wall of the box. A wall of mineral wool was placed about one meter from the opening of the box. Since this wall was somewhat askew relative to the box (see Fig. 2), very little echo reached the preparation. The micromanipulator was about 20 cm from the preparation and hidden behind 5 cm of mineral wool. The microscope could be swung away during the experiment. In this way a good approximation to an acoustic free field was obtained: the sound pressure was almost constant around the preparation, and the echo (measured with a microphone pointing towards the sound source) was at least 30 dB below the signal. In the later experiments the echo was

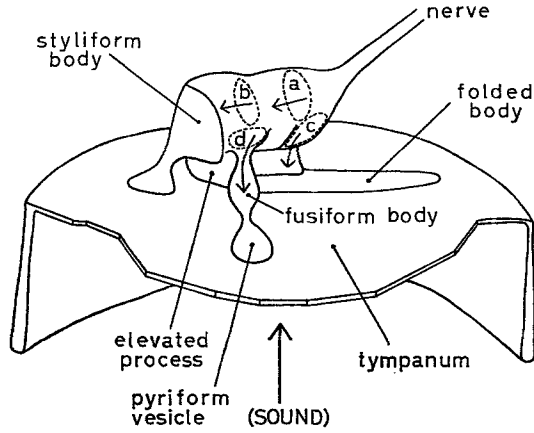


Fig. 1. The anatomy of the left tympanal organ (redrawn after Gray, 1960). The letters *a-d* indicate the position of the four groups of receptor cells. The arrows indicate the direction of the dendrites

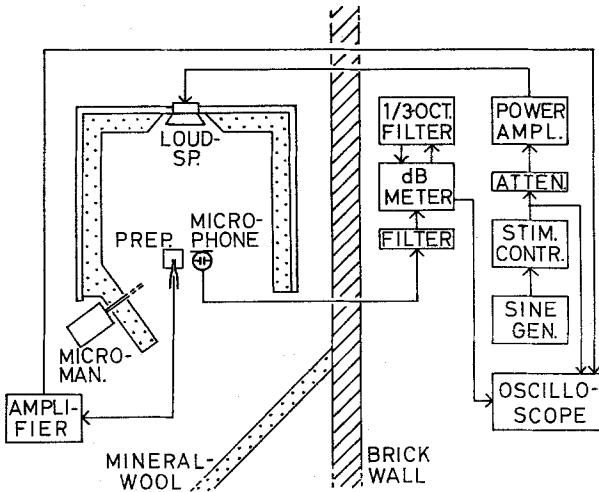


Fig. 2. The arrangement of the apparatus and sound absorbing material (cf. the text)

further reduced by means of cone-shaped Moltopren microwave absorbers (Grünzweig & Hartmann, type P100) placed behind the preparation.

The distance from the loudspeakers to the microphone and preparation was about 70 cm. At this distance and at the frequencies used here the sound reaching the ear and microphone could be regarded as plane waves. In a plane wave the pressure gradient (the sound parameter acting to move the *isolated* tympanum) can be estimated directly from the sound level measured by means of a condenser microphone.

The sound generating and sound measuring apparatus was essentially identical with that previously described (Michelsen, 1966), but with the sine wave generator, amplifiers, and loudspeakers replaced by selected low-distortion types. The sound levels are indicated in dB root mean square relative to  $2 \cdot 10^{-5}$  Newton/m<sup>2</sup> (about human threshold at 1 kHz). The background noise level at the preparation was below 25 dB root mean square in the frequency range 0.5–20 kHz. In the present experiments, sound pulses of 100 msec duration, with a rise and decay time of 2 msec were used. The sine wave started and ended at zero, and no “clicks” were produced at the beginning and end of the signals.

When the threshold is determined for units having a sharp low-frequency cut-off, the amount of harmonic distortion of the signal becomes critical at frequencies below the CF. The harmonics were measured by means of an  $1/3$  octave filter (Brüel & Kjær) and were at least 30 dB below a signal of 90 dB sound level. Although at most frequencies the harmonics were more than 40 dB down, they still presented a problem in some of the recordings.

Extracellular action potentials were recorded by means of glass capillary microelectrodes (tip diameter 0.2  $\mu$ m, impedance 20–40 Mohm, filled with 1.5 Molar NaCl). When the sensitivity of a cell to a reference sound pulse changed more than  $\pm 2$  dB during the course of the experiment, the cell was discarded.

In the absence of sound the receptor cells showed “spontaneous” activity, typically about 1–10 spikes/sec. Since spontaneously active cells do not have a well defined threshold, the “threshold” is defined here as the intensity necessary to give an average response of one spike more than the spontaneous activity to a sound pulse of 100 msec duration.

## Results

A series of responses to sound stimuli were recorded from 28 individual a-cells, 20 b-cells, 16 c-cells, and 18 d-cells. In about half of the recordings the cell met the stability criteria mentioned above, thus permitting a determination of the threshold curve. In a few cases the frequency response of two receptor cells was determined from the same preparation, but most of the recordings were from different preparations. The properties of the receptor cells varied considerably within each of the four anatomical groups. However, the difference in frequency response between the groups appeared to be highly significant.

In a large number of cells there was a tendency for a second (and sometimes a third) maximum sensitivity at a higher frequency than the CF. These “resonances” in the response cannot be explained as artefacts due to errors in the calibration of the sound sources, since in several cells the “resonance” was very small or entirely lacking. In a few cells, on the other hand, the response at the second maximum was as large or even larger than the response to the “CF” itself (see below).

### *The a- and b-Cells*

The anatomical position and frequency response of the b-cells are so close to those of the a-cells that for some time the recordings from the

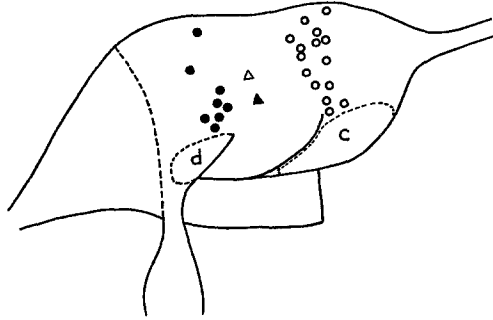


Fig. 3. The anatomical position of the recordings from a- and b-cells (○ a-cell, ● b-cell, △ cell with a-cell properties, ▲ cell with b-cell properties)

Table. Data for 15 a-cells and 8 b-cells. Further explanation in the text

	a		b		P
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	
Characteristic frequency (Hz)	3747	64	3463	98	0.02
Threshold at CF (dB)	46	0.9	51	1.3	<0.01
SDB (dB/kHz)	7.8	0.6	16.1	1.8	<0.001
SDA (dB/kHz)	7.6	0.6	13.4	1.5	<0.001
Intensity-response slope (spikes/10 dB)	12.1	0.6	15.0	0.7	<0.01

two groups were not considered different. There are, however, a number of statistically significant differences between the properties of the two groups. In Fig. 3 the anatomical position of the recordings from 15 a-cells and 8 b-cells are shown, and some of their properties are compared in the table. The Table indicates the mean values ( $\bar{x}$ ), the standard error of the mean ( $S\bar{x}$ ), and the probability ( $P$ ) that the groups are identical ( $t$ -test).

The average threshold at CF was calculated for the most sensitive  $\frac{2}{3}$  of the cells in each group (10 a-cells and 6 b-cells). The sensitivity decrease for frequencies below (SDB) or above (SDA) the CF are given in dB per kHz. These two values taken together indicate the degree of tuning to the CF, but as seen on Fig. 5 they are not always of the same magnitude. The intensity-response curve is sigmoid, and therefore the "intensity-response slope" is calculated from the increase in sound level needed at CF to obtain an increase from 5 to 15 in the number of spikes per 100 msec. Smaller values for the intensity-response slope were found on both sides of the CF.

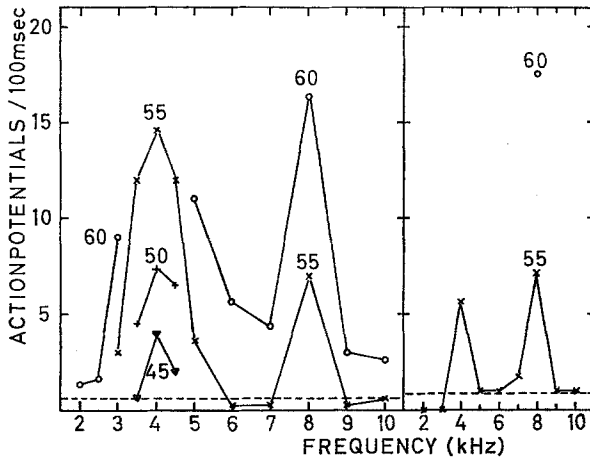


Fig. 4. The responses of two a-cells to sound pulses of 100 msec duration at varying frequencies. Numbers by each curve indicate the sound level in dB (rel.  $2 \cdot 10^{-5}$  N/m<sup>2</sup>). Broken lines show the average level of spontaneous activity. Note the second sensitivity maximum at 8 kHz

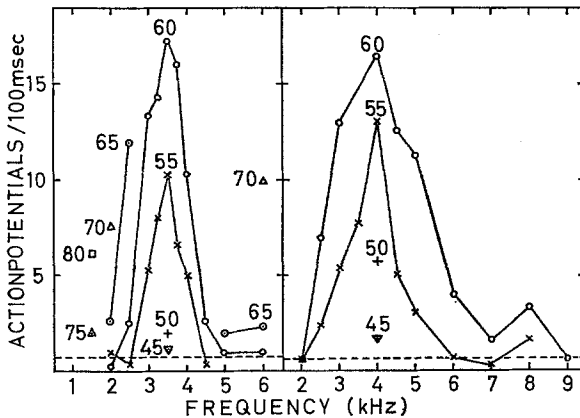


Fig. 5. The responses of two a-cells (for explanation see Fig. 4). Note the small second maximum in the right graph

For all parameters except the characteristic frequency, the difference between the two groups is highly significant. Thus, the b-cells are about 5 dB less sensitive than the a-cells, and they are more sharply tuned to their CF, which may be about 0.3 kHz lower than that of the a-cells.

In about half of the a-cells investigated a second response maximum was found around 8 kHz (Fig. 4). In a few cells this second maximum

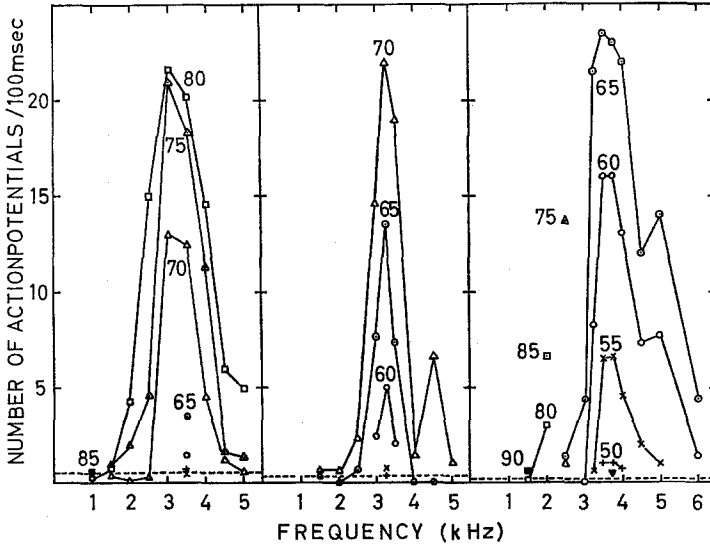


Fig. 6. The responses of three b-cells. Explanation see Fig. 4. Note the very sharp tuning of the cell in the middle graph and a second sensitivity maximum at 5 kHz in the right graph

was found at 6 or 7 kHz. The sensitivity at 8 kHz was on the average 4 dB lower than that at the CF (3.5–4 kHz), but two cells responded more vigorously at 8 kHz than at their “CF” (Fig. 4). In about one third of the a-cells, however, a second response maximum was reduced or absent (Fig. 5).

In the b-cells (Fig. 6) about half of the units had a small second maximum (5–10 dB down) at about 5 kHz.

The patterns of adaptation of the two groups are very similar to each other. The spike frequency is a maximum during the first 50 msec after the onset of sound and decreases during the next 200–300 msec to reach a slowly decreasing plateau (see Michelsen, 1966).

#### *The c-Cells*

The frequency responses obtained from the individual c-cells may be divided into two groups. In some c-cells the sensitivity was rather uniform within the frequency range 2–3.5 kHz, and outside this range the sensitivity decreased markedly (Fig. 7, right). In most c-cells, however, the sensitivity at 1.5 kHz was as high or even higher than that at 2.5–3 kHz (Fig. 7, left). In such cells the sensitivity to 2 kHz

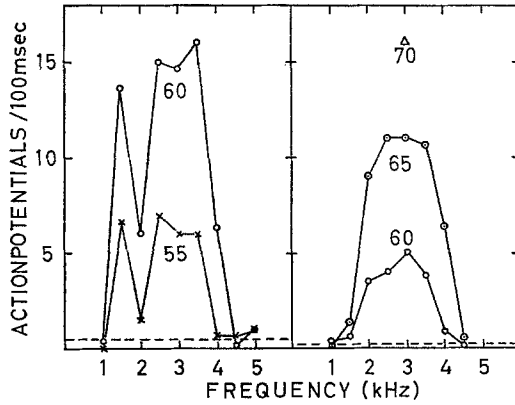


Fig. 7. Two response types in c-cells (for explanation see Fig. 4)

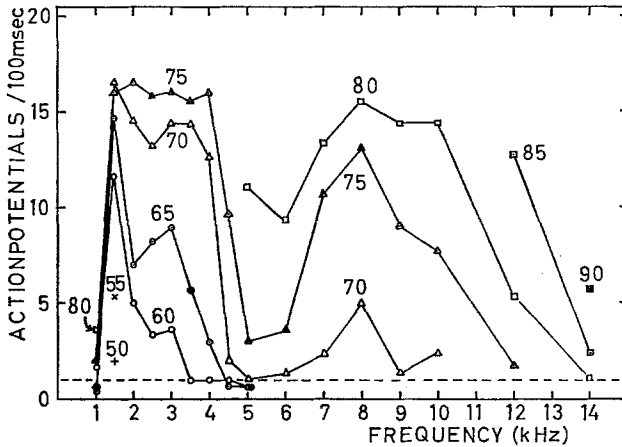


Fig. 8. The responses of a c-cell having three sensitivity maxima (for explanation see Fig. 4). Note the 28 dB difference in sensitivity to 1 kHz and to 1.5 kHz

was about 5 dB below that at 1.5 kHz. In some units a small third maximum of sensitivity was found at 8 kHz (Fig. 8).

The pattern of adaptation of the c-cells is rather different from that of the other groups. The spike frequency increases more slowly, to reach a maximum 100–400 msec after the onset of sound. After the initial rise the spike frequency decreases slowly in a phasic manner. The slow response of the c-cells, in contrast to the fast response of the other groups, is also evident by their failure to respond to single “clicks” at high repetition frequencies (Michelsen, 1966).



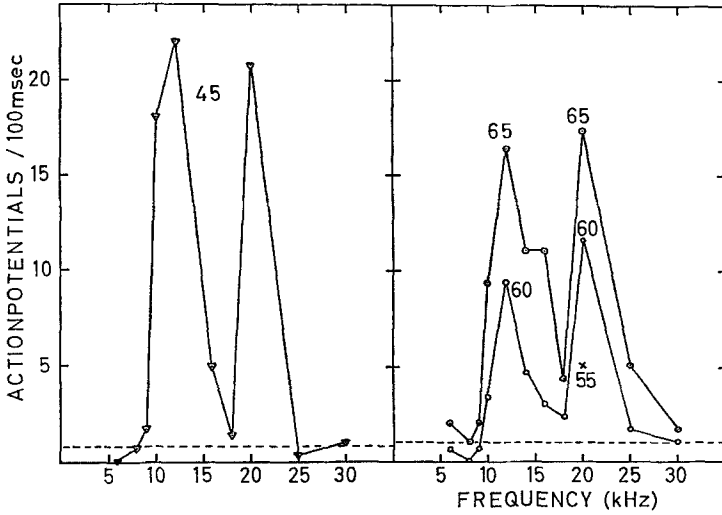


Fig. 9. The responses of d-cells having two sensitivity maxima (for explanation see Fig. 4)

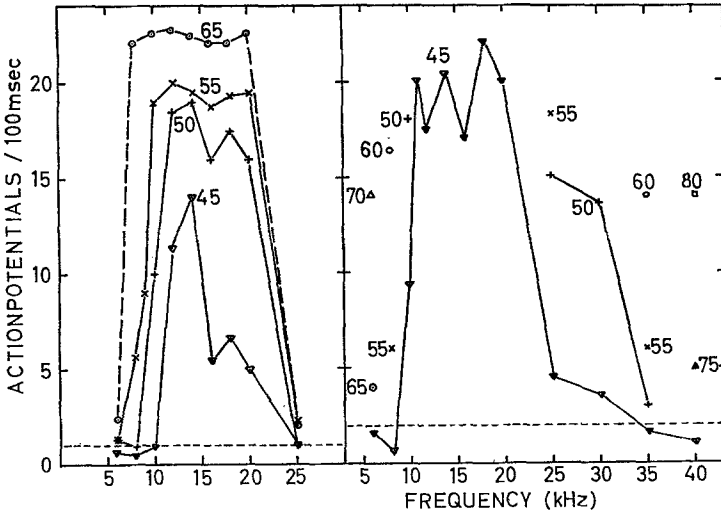


Fig. 10. The responses of d-cells (for explanation see Fig. 4). Left: a cell with a small second sensitivity maximum (unbroken line), and the response of another cell (long, broken lines). Note the large decrease in sensitivity towards low frequencies in both graphs

### *The d-Cells*

The d-cells have their maximum sensitivity at about 12 kHz (range 10–14 kHz), and most cells also have a second sensitivity maximum around 19 kHz (Fig. 9). The sensitivities at these two maxima were almost the same. In some cells, however, the second maximum was much less conspicuous (Fig. 10). At 6 and 40 kHz (i.e. one octave below and above the two sensitivity maxima) the sensitivity is about 35 dB less. A remarkable property of many d-cells is their very limited “measuring range” (i.e. the intensity range from threshold to saturation, see Fig. 10). The pattern of adaptation is equivalent to that of the a- and b-cells.

### *Low-frequency Units*

During the investigation of d- and b-cells, action potentials from a fifth kind of unit were occasionally recorded. The spikes, as judged from their small amplitude and negative polarity, were probably generated by axons of small diameter. These units responded to sound of high intensity (90 dB) and low frequency (some hundred Hz). In two cases it was possible to record similar responses from units in the fusiform body (see Fig. 1). Probably, these responses originate from mechanoreceptors situated upon the tympanal membrane near to the pyriform vesicle.

## **Discussion**

### *Frequency Discrimination and the Intact Ear*

These studies show that the maximum sensitivity of each group of receptor cells is limited to a small number of discrete frequency bands. For most cells the frequencies of maximum sensitivity were around 3.7 kHz for the a-cells, 3.5 kHz for the b-cells, and 12 kHz for the d-cells. In some cells a second sensitivity maximum was observed around 8 kHz (a), 5 kHz (b), and 19 kHz (d). The response of the c-cells is more complex, but the preferred frequencies are 1.5 kHz, 2–3 kHz (all cells), and 8 kHz. In the following publication (paper II) the physical properties of the isolated ear will be considered with special reference to the problem of frequency discrimination. It will be shown that most of the frequencies mentioned above correspond to the expected and observed resonance frequencies of the tympanum.

In the present experiments the sensory units attached to different anatomical parts of the tympanum showed different frequency sensitivities. In these experiments the ear was completely *isolated*, i.e. the preparation merely consisted of the sclerotized ring, tympanum, and receptor cells. Although the isolated ear, considered as a mechanical

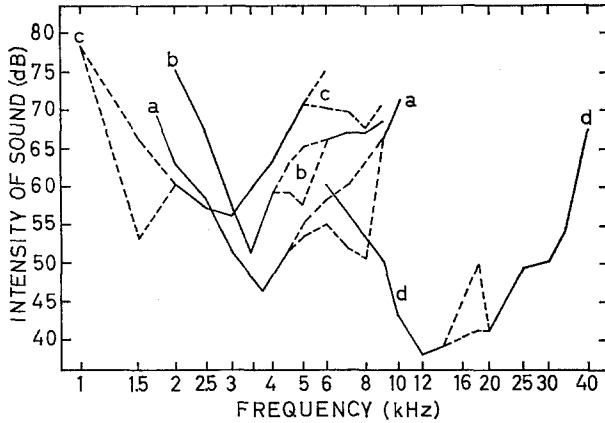


Fig. 11. The threshold curves for the four groups of receptor cells in the isolated locust ear. Broken lines indicate variations in threshold curves for different cells within each group

system, is far from simple, the acoustic properties of the *intact* ear are much more complicated. The intact pair of ears are connected by a series of air sacs formed by tracheal membranes. In a following publication (paper III) it will be shown, how the presence of these air sacs may affect the sensory response of the ear to sound stimuli.

Because of the physical difference between the isolated and intact ears, the present experimental results do not tell us how much information about frequency is actually sent to the CNS by the receptor cells in the intact ear. In paper III it will be shown that some of the main preferred response frequencies of the isolated ear are also found in recordings from intact ears. Therefore on the basis of the threshold curves of the isolated ear, one may speculate about the ability of the intact insect to carry out a pitch discrimination. According to Haskell (1957) the flight noises of locusts are in the intensity range 50 to 65 dB and around 4 kHz. It has been suggested (Michelsen, 1968) that in this range locusts might be able to perform an approximate determination of frequency by means of very few central neurons: a shift of frequency at an appropriate intensity causes different groups of receptor cells to respond in turn (Fig. 11). At low frequencies the c-cells alone respond, and at higher frequencies the combinations are: ac, abc, ab, a, ad, and d. If this primitive type of frequency discrimination is used, the functional significance of the b-cells becomes comprehensible. Assuming this kind of central mechanism exists, the response of the b-cells would be useful, even if the CF's of the a- and b-cells were identical. If a larger number

of central neurons were used, the pitch discrimination (together with the intensity discrimination) might improve considerably.

It is known that the information about frequency signalled from the intact ears is transmitted to the brain. In *Locusta migratoria*, Popov (1967) found two ascending neurons on each side of the third thoracic ganglion. The responses of these neurons are very complex, and information about frequency seems to be signalled by means of different types of adaptation; low (3–8 kHz) and high (12–20 kHz) frequencies evoke a phasic and tonic response, respectively. According to Adam and Schwartzkopff (1967) the same information is found in the response of neuron groups in the protocerebrum of *Locusta*. They found three types of neurons, sensitive to 4–8, 12–30, and 4–30 kHz, respectively. Thus, it is known that at least some information about frequency reaches the brain. The results of Yanagisawa, Hashimoto and Katsuki (1967) suggest, however, that the response patterns of higher neurons may be markedly influenced by inhibitory interactions between various auditory inputs. The studies of Adam (1969) on the behaviour of auditory neurons in the brain likewise suggest that the processing of auditory information in the CNS is extremely complex. Unfortunately, most authors do not indicate the exact acoustical condition of their experiments, and therefore their results are difficult to compare.

#### *Sources of Error*

In the present experiments the microelectrode was inserted into the Müller organ. Although the electrodes used were long and thin, their presence in the organ may have influenced the vibration of the membrane and the sensory response to sound. In a few cases it was possible to record from single fibers in the acoustic nerve, and these recordings did not differ from those obtained from the Müller organ. Such recordings were seldom successful, because the sheet covering the nerve hindered the penetration of the microelectrode. Recordings from entire nerves at some mm distance from semi-isolated ears gave threshold curves very similar to those expected from the present results.

#### *Anomalous Results*

The results presented above have been selected from the results of all the experiments on the basis of two criteria: First, the cell should meet the criterion for stability mentioned above, and secondly the sensitivity and frequency response should be reasonable "normal". For example, the threshold at CF for the most sensitive  $\frac{2}{3}$  of the a-cells was about 46 dB (range 42–50 dB). In some cases and previously (Michelsen, 1966), thresholds of about 60 dB and frequency ranges of 2–10 kHz have been found for a-cells, and these less sensitive cells also tend to have a higher CF. Typically, a-cells with a threshold of 60–70 dB may have a CF of 5–8 kHz, as compared with an average CF of about 3.7 kHz for the most sensitive a-cells. Similarly, d-cells with a CF of about 25 kHz have occasionally been observed. It will be shown in the following publication that this shift in CF was probably due to a change in the membrane compliance caused by the drying of the preparation.

*Recordings from Damaged c-Cells*

The c-cells seem to be rather sensitive to disturbances during the recording of single unit activity. I have previously described (1966) the inhibition of spontaneous activity in c-cells by stimulation. I also described the response of an "e-unit". These types of response were probably recorded from damaged c-cells:

In one experiment spikes from two c-cells were recorded simultaneously. Initially, one of the cells was excited by low intensity sound (60–70 dB, 3 kHz) and inhibited by intense sound (80–90 dB), whereas the other cell was excited by all the intensities mentioned. Later, the first cell was inhibited at all sound levels above 60 dB, whereas the behaviour of the other cell remained constant.

In another experiment it was possible to follow the gradual change of the response pattern of a cell from the "e-unit" type to that of a typical c-cell. The small spikes, which often initiate the spikes in "e-units" (Michelsen, 1966, Fig. 11), have now been observed in all four groups of receptor cells. They seem most likely to occur in recordings near to the dendrite of the receptor cell; their nature is unknown.

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