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Insect hearing in the field

I. The use of identified nerve cells as 'biological microphones'

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Summary. 1. For the bushcricket *Tettigonia viridissima* a portable recording unit was constructed which enabled long-term extracellular recordings of single and identified auditory nerve cells in the field. Thus, aspects of sound reception and of directional hearing could be studied in the animal's natural environment.

2. The applicability of the technique is demonstrated in two examples. Remarkable hearing distances of 40–50 m were found in a grassland habitat; in denser bushland, these distances are reduced by about 20 m (Fig. 2). Once the stimulus is suprathreshold, the gross temporal structure of the song is well encoded in the activity of the omega-neuron even within a reflecting and scattering vegetation.

3. By recording the activity of a pair of directionally-sensitive interneurons in the field we have direct experimental evidence that directional information is provided at remarkable communication distances and strongly depends on the spatial configuration between sender and receiver (Fig. 3).

Introduction

There is no paucity of reports of biophysical and neurophysiological studies on sound production and reception in orthopteran insects. However, as the great majority of these studies have been performed under laboratory conditions, they do not reveal complete information about an animal's hearing capacity outdoors. One fundamental reason is, that the habitats of most insects are in general quite complex, differing in terms of acoustic conditions far from those of a sound proof room.

In natural environments, sound propagation and attenuation between sender and receiver are complicated by many effects (for review see Wiley and Richards 1978; Michelsen and Larsen 1983), so that knowledge of the nature of such environments would necessitate the field measurement of all relevant parameters, requiring much technical effort. Attempts have been made to measure the degree to which sound is affected in the field by means of speakers and microphones (Griffin 1971; Popov et al. 1974; Michelsen and Larsen 1983; Keuper and Kühne 1983), but these data are of limited value, as the properties of physical emitters and receivers normally differ widely from those of insect stridulatory and auditory organs. For example, the frequency range of a bushcricket ear extends from about 5 up to 100 kHz with an absolute sensitivity of about 20–30 dB SPL (Autrum 1960; Rheinlaender 1975; Kalmring et al. 1978); such a combination of both features can barely be mimicked by technical receivers. In particular, many insect ears are known to act – at least in certain frequency ranges – as pressure gradient receivers (Michelsen and Nocke 1974; Hill and Boyan 1977; Michelsen 1979), so that they have species-specific directional characteristics. Again, this feature differs from that of pressure sensitive condenser microphone being normally used.

We have tried to bypass some of these problems by using a portable recording unit which has enabled us to record the activity of single (and identified) auditory neurons in the field, whose characteristics are known in great detail from laboratory experiments. We thus are able to study the biologically relevant information by using the CNS of the insect itself as the receiver. By recording from identified interneurons in the field a first insight into the hearing capacity of an insect in its natural environment was obtained.

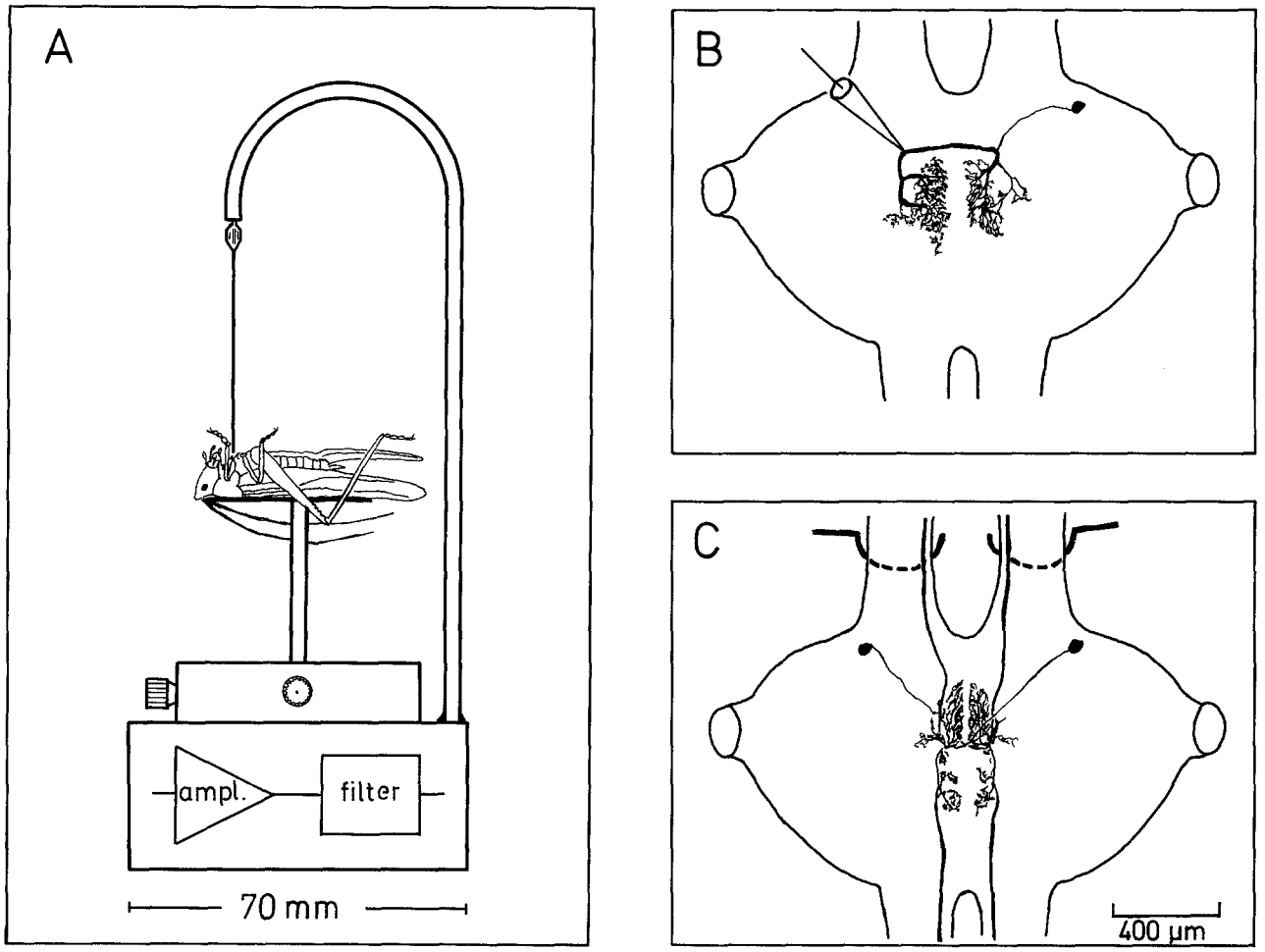

Fig. 1. **A** Portable unit for field recording of the omega-cell activity. The unit could be placed within the habitat at any distance, height and angular orientation relative to a sound source. **B, C** Anatomical reconstruction after intracellular Lucifer Yellow injection of the omega-neuron (**B**) and a pair of T-fibres (**C**) in the prothoracic ganglion

Material and methods

We constructed two slightly different portable recording units (one addressing the problem of sound reception in general, the other that of directional hearing), which enabled long-term extracellular recordings of single auditory nerve cells in the prothoracic ganglion of the bushcricket, *Tettigonia viridissima* (Fig. 1). The animal was mounted ventral side up with the forelegs (bearing the hearing organs) fixed in a natural position. To study sound reception in the field, the activity of the omega-cell (Römer 1985; Fig. 1 B) was recorded with a glass-insulated tungsten electrode (resistance 1–3 MΩ) which was inserted into the anterior part of the prothoracic ganglion with a small microdrive under optic and acoustic control (monitored by headphones). After positioning the electrode tip in close proximity to the cell's crossing segment (Fig. 1 B), the opening in the cuticle was sealed with vaseline, thus preventing dessication. The mechanical stability of the preparation provided reliable single cell recordings lasting at least several hours (for the typical recording quality see Fig. 2 B).

The portable recording unit consisted of a brass socket (including two 9 V-batteries) with a set of OP-amplifiers (providing a gain of 100 and a 24 dB/octave bandpass filter from 0.5–5.0 kHz) and a miniature microdrive to move the preparation in three dimensions relative to the fixed tungsten electrode from above (see Fig. 1 A).

For the study of directional hearing the nervous activity of a pair of directionally sensitive auditory T-fibres (Fig. 1 C) was used. Their large axonal diameter of 10–15 µm provided reliable single cell spike recordings by means of hook-electrodes applied to the connectives between prothoracic and suboesophageal ganglion (Suga and Katsuki 1961; Rheinlaender and Römer 1980). For this bilateral recording approach a two-channel setup was used, similar to the one shown in Fig. 1 A. The preparation could be moved in the horizontal plane relative to a sound source in calibrated angular steps.

The portable recording unit was connected via a 20 m extension cord to a storage oscilloscope to display spike recordings in the field. Simultaneously, they were stored on tape for later conventional spike analysis in the laboratory.

Conventional sound stimulation techniques were used to produce pure tone sound pulses. A portable computer system (Duet 16, 8 bit A/D-D/A-converter) generated song models being identical in temporal structure and frequency content (upper range 45 kHz, limited by the sampling rate of the converter) to the song of a male bushcricket *Tettigonia viridissima*. Both types of stimuli were broadcast via a broadband speaker (Matsushita technics, EAS-10TH800B, frequency range 3–100 kHz

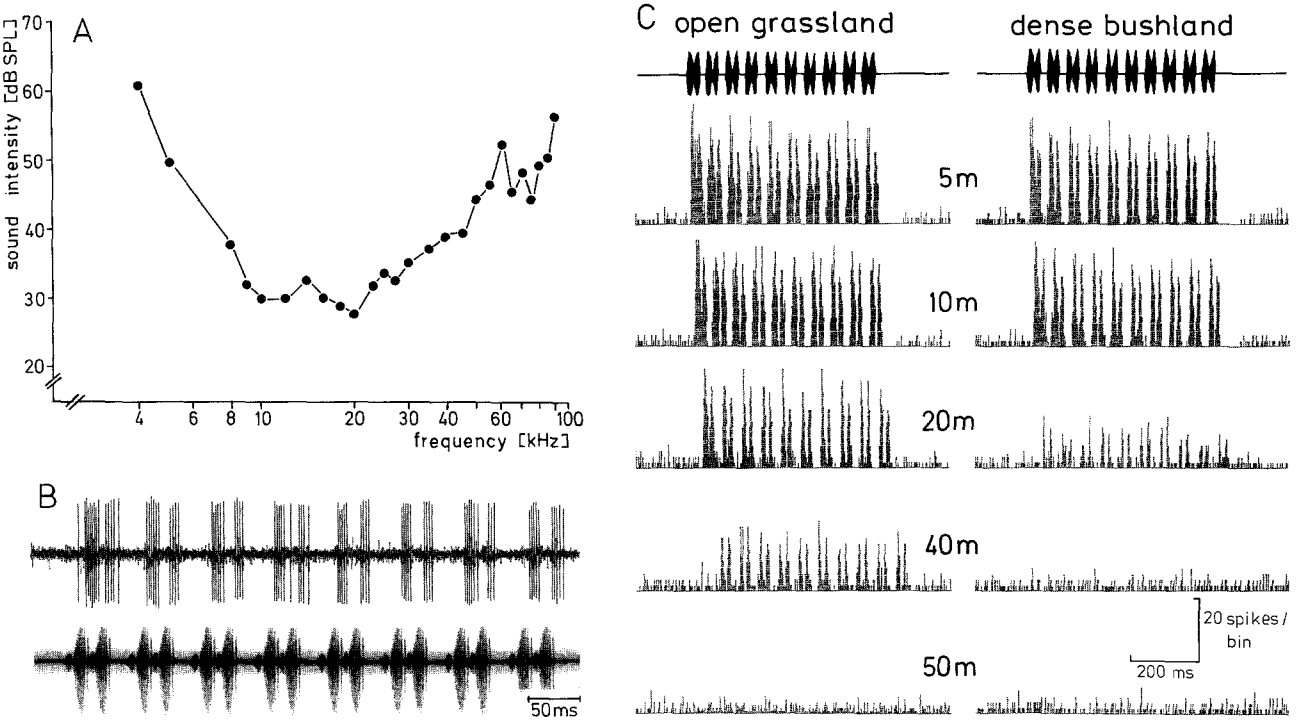

Fig. 2. **A** Tuning curve of the omega-cell, recorded under controlled acoustic conditions in the laboratory (for methods see Rheinlaender and Römer 1980). **B** Response of the cell (upper trace) as monitored with the portable recording unit in the field at a distance of 2 m to the speaker. The sound stimulus was the conspecific male stridulatory signal. **C** Post-stimulus-time histograms of the omega-cell responses in two different habitats at various distances (speaker and preparation 1.5 m above ground). Sound stimulus was the same as in (**B**) repeated ten times to obtain each histogram; bin width 4 ms. Note the difference in maximum hearing distance in the two habitats (differing by about 20 m) and the relative increase in response latency, which is mainly due to increased travelling time of sound and less to a reduction of stimulus intensity. Air temperature 24–25 °C, wind speed 0–1 m/s, measured with a mechanical anemometer; wind direction was transverse to the broadcasting direction. The mean height of vegetation in the open grassland was about 0.3 m and in the dense bushland about 1.5 m

at 3 dB points) at various distances to the preparation. Thus the overall frequency range of the entire sound stimulation set-up was 3–45 kHz.

Results

The applicability of the portable omega-cell preparation is demonstrated in Fig. 2. The cell is sensitive over a broad range of frequencies between 5 and 90 kHz (Fig. 2A). Also, its threshold sensitivity is similar to that of the whole auditory organ except in the frequency range below 8 kHz (Römer 1985); thus monitoring the omega-response is almost equivalent of monitoring the sensitivity of the whole ear. Furthermore, the neuron copies the syllable pattern of the conspecific song (Fig. 2B) and does not habituate even with faster rates of sound modulation. Thus the spike pattern of the omega-neuron, recorded in the field, might prove as an excellent indicator for the hearing capacity of the insect under natural conditions. Fig. 2C shows the result of an experiment with the omega preparation positioned in two different environments, in open grassland and dense bushland, with a sound signal being identical to the conspecific song in syllable structure, frequency, and intensity. The speaker was placed at a height of 1.5 m above ground (which was the top level of the vegetation), thus copying the broadcasting position of a conspecific singing male. It can be seen that threshold for the detection of the conspecific song is close to a distance of 40 m in the open grassland. In the dense bushland, however, a similar spike pattern can be recorded starting at a maximum distance of only 20 m. Thus the two experiments demonstrate maximum hearing distances in both habitats, where the differences must be due to stronger sound attenuation in the denser bushland.

Furthermore it is notable, that for all suprathreshold stimulations the temporal structure of the song is well reflected in the spike pattern of the omega-neuron. This is especially surprising for the recordings in the dense bushland, where one might have expected the time structure of the stimulus to be blurred due to, e.g., multiple scattering of the sound in the vegetation.

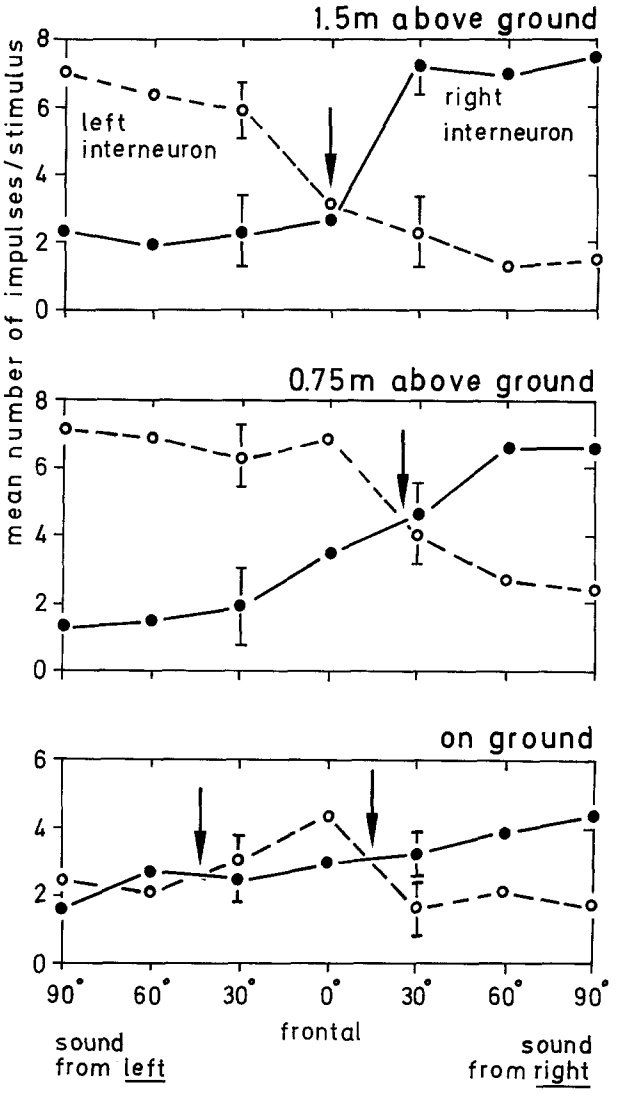

Fig. 3. Directional characteristics of a pair of auditory T-fibres recorded in dense bushland at a distance of 10 m from the sound source (stimulus frequency 20 kHz, duration 30 ms, sound intensity 90 dB SPL at a distance of 1 m). Note that the greater the height of the preparation from the ground, the greater is the directional information encoded by the two fibres

In addition to signal detection, the animals also have to face the problem of localizing a sound source, which might prove rather difficult in such complex environments. However, by recording the activity of directionally sensitive nerve cells, we can obtain direct and undistorted information of relevance for the animal in its natural environment. As an example Fig. 3 shows the directional characteristics of a pair of auditory interneurons in dense bushland, at three different heights of the preparation from the ground and at a distance of 10 m to the speaker. The discharges of right and left auditory interneurons are plotted for sound from different angles of incidence. With the preparation on the ground, there is no directional information as there is no systematic tendency in the neuronal discharges to different angular stimulations. But it should be noted that each sound pulse elicited several spikes/stimulus in this configuration. From this result we can conclude, that in such a receiving position the animal was able to receive the signal, but unable to localize the sound source.

This discrepancy changed, however, with raising the preparation 0.75 m above ground level (although still positioned within the vegetation), as the right-left differences in spike number now showed a clear preference for the neuron of the stimulated side. Finally, at a height of 1.5 m (top of vegetation) the crossing point of the curves (arrow in Fig. 3) coincided exactly with frontal sound stimulation. Similar results could be found at greater distances and extended in the open grassland up to 40 m. Thus we have direct experimental evidence that directional sensitivity strongly depends on the spatial configuration between sender and receiver, and it is not only an inherent property of the insect's auditory system, as seen in laboratory experiments.

Discussion

Our results show, that such 'biological microphones' can be put to use in a wide range of biologically relevant applications, such as determination of hearing distances for conspecific and heterospecific sound communication, for pattern recognition and directional hearing in any habitat. Furthermore, the filtering properties of an environment can be evaluated with the use of, for example, pure tones as stimulus and the response of the cell as an indirect measure of sound energy at the receiving site. Of course, the presented data give only a first insight into the hearing capacity of the animal under study, concerning both sound reception and directional hearing. And there is no doubt, that these methods cannot replace microphone measurements, which give an insight into the physical mechanisms of environmental sound propagation.

In conclusion, in addition to conventional sound measurements and laboratory methods, such outdoor neurophysiology will reveal indispensable information about evolutionary adaptations of the sensory system to the animal's environment.

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