J Comp Physiol (1982) 147:461–469

Journal of Comparative Physiology · A

Tonotopic Organisation of Auditory Receptors in Tettigoniidae (Orthoptera: Ensifera)

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Accepted May 4, 1982

Summary. 1. Removing the anterior tympanic membrane of the tettigoniid *Caedicia simplex* did not significantly alter either the tuning or the intensity-response characteristics of individual auditory fibers recorded in the leg nerve (Figs. 2 and 3). However, this operation did make the auditory sensilla in the crista acustica accessible for physiological recording.

2. The physiological responses of individual auditory receptors in the crista acustica have been recorded and the frequency-threshold characteristics of identified sensilla have been measured for sound frequencies between 1 kHz and 40 kHz.

3. In response to an acoustic stimulus a slow hyperpolarising potential (1–2 mV), lasting the duration of the tone and superimposed hyperpolarising spike potentials (2–4 mV) were recorded in the attachment cell of the sensillum (Fig. 4A). In two of the 28 sensilla from which recordings were obtained, depolarising spike potentials were recorded superimposed on the hyperpolarising potential (Fig. 4B). On rare occasions, depolarising spike potentials were recorded in the absence of any slow potential. Lucifer Yellow was successfully injected into the attachment cell of a sensillum only when the slow potential was recorded (Fig. 6).

4. Each sensillum has a typical V shaped tuning curve with the most sensitive sensilla tuned to sound frequencies between 7 kHz and 18 kHz (Figs. 7 and 8). The sensilla of the crista acustica are tonotopically organised with the more proximal receptors tuned to the lower sound frequencies (Figs. 6–8). The frequencies of optimal sensitivity of adjacent sensilla are separated by an interval of approximately 1 kHz (Fig. 8).

Introduction

The auditory receptors of tettigoniids (Orthoptera, Tettigoniidae) are located in the proximal portion of each prothoracic leg tibia. In this region there are three mechanosensory organs; the subgenual organ, the intermediate organ and the crista acustica. These organs were first described by Graber (1876) and later by Schwabe (1906). A more recent anatomical investigation (Schumacher 1979) has not significantly altered these original descriptions. Each organ contains chordotonal sensilla similar to those described by Gray (1960). The crista acustica however, has a highly organised arrangement of these sensory elements. In the crista the sensilla form a linear array in which the size of the attachment cells and the length of the sensory dendrites, distal to the soma, decrease from the proximal to the distal end of the array. The location of the crista acustica on the leg trachea between the two auditory tympana caused speculation that the sensilla of this organ may respond to air-borne sound (Schwabe 1906).

The pioneering work of Autrum (1940, 1941) demonstrated physiologically that the crista acustica of tettigoniids responds to sound frequencies higher than 1 kHz with greater sensitivity than either the subgenual or intermediate organs. Since the work of Autrum, there has been some progress toward defining the physiological characteristics of the auditory sensilla of tettigoniids. Zhantiev (1971), Rheinlaender (1975) and Kalmring et al. (1978) have measured the frequency and intensity characteristics of individual auditory fibers in the tympanal nerve. Their work, however, did not reveal the peripheral origin of the axons from which they were recording. They were unable, therefore, to associate physiological characteristics of specific axons with particular sensilla in the crista acustica. Rheinlaender (1975) and Kalmring et al. (1978) concluded that the more sharply tuned and sensitive auditory units recorded in the tympanal nerve could be the axonal projections of sensilla in the crista. The figure of Zhantiev and Korsunovskaya (1978, Fig. 1, p. 1013) implies that the tonotopic

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organization of the crista acustica has been demonstrated. The arrows in their figure, however, indicate the position in the tympanal nerve where single unit recordings were obtained. The positioning of the tuning curves along the receptor array was based on the assumption that a single unit record was obtained when the electrode tip was near the point of insertion of the receptor axon into the tympanal nerve. Single unit recordings of auditory receptor responses can be obtained at any point along the tympanal nerve and even in the prothoracic ganglion. Their work therefore, like that of Zhantiev (1971), Rheinlaender (1975) and Kalmring et al. (1978), did not demonstrate the peripheral origin of the axons from which they were recording. In order to attribute specific frequency and intensity characteristics to particular receptors in the crista acustica, it is necessary to record from identified sensilla. In this paper I describe the physiological response and frequency characteristics of identified sensilla in the crista acustica of a tettigoniid and demonstrate the tonotopic organisation of this organ.

Materials and Methods

Experiments were conducted on 66 wild adult male and female *Caedicia simplex* (Walker), formerly *C. longipennis* (Hill and Oldfield 1981), caught on vegetation in Canberra. Each preparation was mounted ventral side up with the forelegs supported so that the anterior tympanic membranes, which are fully exposed (e.g. Nocke 1975), were accessible for dissection. Following the removal of a small portion of the pronotal flap that partially covers the prothoracic spiracles, perspex emitter probes were manipulated next to each spiracle. The acoustic stimulus was presented to the preparation via an electromagnetic transducer fitted into each emitter probe. Each probe had an internal diameter of 1.5 mm at the tip and 15 mm at the transducer. The overall length of each probe was 60 mm with an approximately uniform wall thickness of 1 mm. The frequency response of each probe was measured with a $^1/_4''$ Brüel and Kjaer condenser microphone (type 4316), Brüel and Kjaer measuring amplifier (type 2607) and passband filter (type 1614). For a constant input voltage the output of each probe varied by 20 dB for sound frequencies between 1 kHz and 40 kHz. The attenuation levels required to flatten this frequency response were incorporated into the software routines used to control the intensity of the stimulus. The linearity of the probes was established by varying the level of the input voltage and monitoring the output of each probe. For sound frequencies between 1 kHz and 40 kHz the output varied by an amount that corresponded to the variation in the input voltage.

Acoustic Stimulus. The acoustic stimulus, a 50 ms pure tone pulse with 10 ms rise and decay times presented at a rate of 1 Hz to 2 Hz, was produced by amplitude modulating (Schonemann 1172/04) the sine wave output of an audio oscillator (General Radio 1309). The temporal parameters of the pulse were chosen after recording (Nagra IV SJ recorder, $^1/_4''$ Brüel and Kjaer condenser microphone) and analysing (Nihon Kohden oscilloscope camera, Tektronix 5103N oscilloscope) the insect's call. The level of stimulation was adjusted in 1 dB steps (programmable attenuator, self constructed).

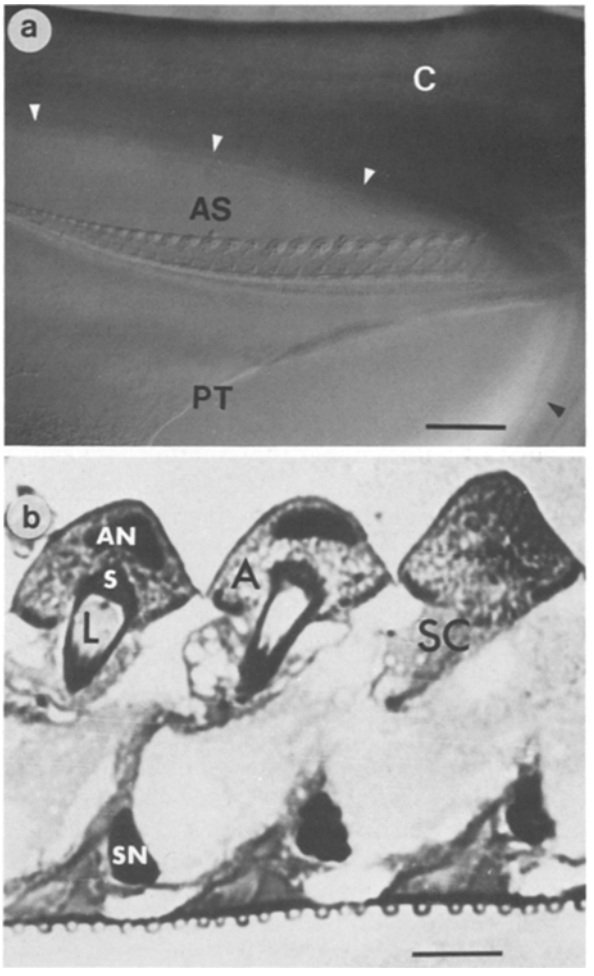

Fig. 1. Photomicrograph of the auditory sensilla in the crista acustica of the tettigoniid *C. simplex* **a** the portion of the crista that was visible through the opening in the leg (*arrowed*) left after the removal of the anterior tympanum. *Scale*: 50 μm. **b** Longitudinal section through three sensilla in the crista acustica. *Scale:* 12.5 μm. Note the location of the scolopale cell nucleus and the close anatomical association of the attachment cell and the cap cell into which the sensory cilium inserts. *A* attachment cell; *AN* attachment cell nucleus; *AS* auditory sensilla; *L* lumen; *C* leg cuticle; *PT* prothoracic leg trachea; *S* scolopale cap; *SC* scolopale cell; *SN* scolopale cell nucleus

Physiological Recording from Primary Auditory Axons. Prior to physiological recording, the insect's mandibles, the cuticle covering the prothoracic and oesophageal region and the major muscle groups of the prothorax were removed. A silver spoon, which acted as the indifferent electrode, was placed under the prothoracic ganglion and insect ringer (Fielden 1960, plus 3 g/l glucose) was applied. To reduce movements of the leg nerve, in which the auditory axons are located, it was sometimes necessary to remove the anterior portion of the insects' gut. When this procedure was adopted care was taken to prevent the gut contents from coming into contact with the nervous system.

Auditory responses to the acoustic stimulus in primary auditory axons were recorded with glass microelectrodes filled

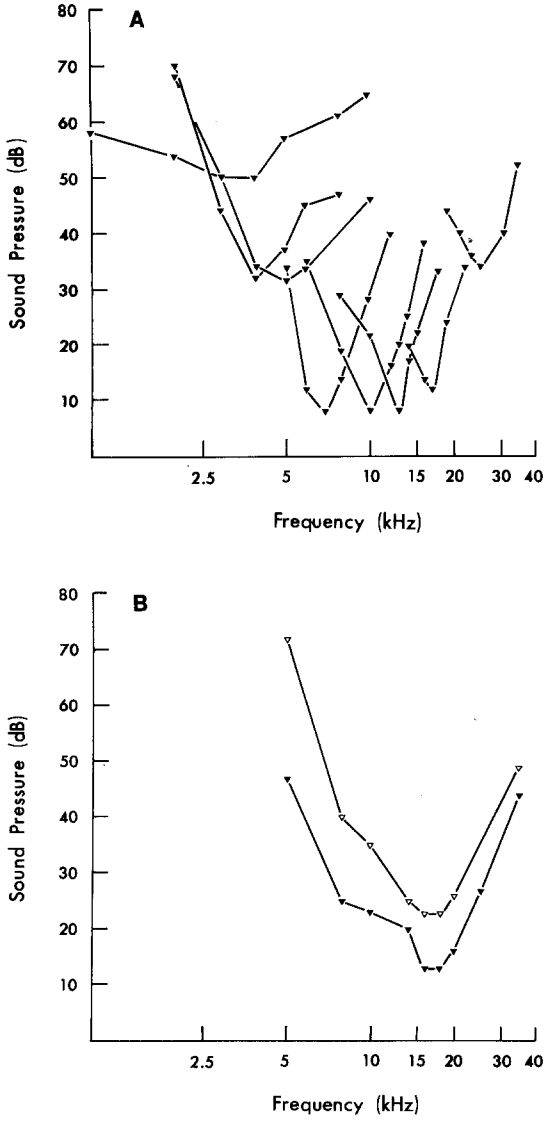

Fig. 2. A Frequency-threshold curves for several primary auditory neurons recorded in the leg nerve. Each point represents the sound pressure required to elicit 6–7 action potentials in the sensory axon. Note the lower thresholds of cells tuned to sound frequencies between 7 kHz and 18 kHz. **B** Frequency-threshold curves of a primary auditory neuron recorded in the leg nerve (▼) before and (▽) after the removal of the anterior tympanum. Note that the cell maintained maximum sensitivity to sound frequencies between 16 kHz and 18 kHz after the removal of the tympanum. **C** Frequency-threshold curves for several primary auditory neurons following the removal of the anterior tympanum. Note that all the cells maintained a tuned response and that the most sensitive cells were those tuned to sound frequencies between 7 kHz and 18 kHz

with 3 M potassium acetate (tip resistance 70–80 MΩ). The responses were amplified (Thomas 1977), monitored with headphones and an oscilloscope (Tektronix 5112) and recorded on magnetic tape (Hewlett Packard instrumentation recorder 3964 A). The frequency-threshold characteristic of each axon was quantified in terms of the sound pressure required to produce an average (n = 5) of 6–7 action potentials per stimulus. The intensity-response characteristic of each axon was quantified in terms of the average (n = 5) number of action potentials per stimulus for various sound pressure levels. In both cases the analysis was done on line by a microprocessor (Synertek 6502) that also controlled the amplitude and rate of stimulation.

Physiological Recording from the Crista Acustica. To record from the auditory sensilla of the crista acustica the darkly pigmented region of the anterior tympanum was removed. Insect Ringer solution was then applied and a silver wire, which acted as the indifferent electrode, inserted into the insects abdomen. The physiological responses of individual sensilla were recorded with glass microelectrodes, the tips of which were filled with a 5% aqueous solution of Lucifer Yellow CH (tip resistances 180–160 MΩ). The responses were amplified, monitored, recorded and the spike trains were analysed in the same way as recordings from the auditory axons. The slow hyperpolarising potentials (see Results) were analysed from chart recordings of the replayed responses (Watanabe V linear recorder).

To establish the position of the recording site in the crista acustica Lucifer Yellow was injected into the sensillum by applying 5 nA of negative current for 500 to 800 ms at a rate of 1 Hz for 1 to 2 min. The prothoracic leg was then removed from the insect and prepared as a whole mount by fixing for 30 min in 4% formaldehyde in 0.2 mol/l phosphate buffer at pH 7.2, dehydrated in an ethanol series and cleared in methyl salicylate. It was examined by fluorescence microscopy using a Zeiss photomicroscope. Several cristae were prepared for thin section microscopy. The prothoracic legs were removed from several insects and fixed for 2 h at 4 °C in 2.5% gluteraldehyde, 2% formaldehyde in 0.1 mol/l sucrose buffer (pH 7.2) containing 2 mmol/l CaCl. The material was then rinsed in buffer solution and post fixed in 1% osmium for 1 h, dehydrated in an acetone series and infiltrated with Spurr's resin. Longitudinal sections were then cut, 2 μm, stained with toluidine blue and examined with a Zeiss photomicroscope.

Results

Anatomy of the Crista Acustica

Figure 1a shows a photomicrograph of the crista acustica of *C. simplex*. The anatomical arrangement of the sensilla is similar to that described for other tettigoniid species (Graber 1876; Schumacher 1979). The crista acustica contains 35 sensilla with each sensillum composed of three cells, the attachment cell, the scolopale cell and the sensory neuron. The sensilla become smaller progressively from the proximal to the distal end of the array. This variation in size is predominantly due to a decrease in the volume of the attachment cell and the length of the distal portion of the sensory dendrite. The size of the scolopale cap, the length of the scolopale rods and the size of the lumen containing the sensory cilium (Fig. 1b) show less overall variation.

Physiological Characteristics of Primary Afferent Fibres

In order to record from the sensilla of the crista acustica (see below) it was necessary to remove the anterior tympanum. To characterise the physiological response of the auditory receptors in the intact preparation and verify that this operation did not alter these characteristics, recordings were obtained from several auditory neurons in the leg nerve before and after the removal of the tympanum.

The frequency-threshold curves of several primary auditory neurons in intact preparations are shown in Fig. 2A. The most sensitive neurons are those tuned to sound frequencies between 7 kHz and 18 kHz. Neurons tuned to sound frequencies outside of this range have higher absolute thresholds. Neurons tuned to sound frequencies above 7 kHz have symmetrical frequency-threshold curves with roll-offs in sensitivity of 20 dB to 30 dB/octave. Neurons with optimal sensitivity to sound frequencies below 7 kHz have asymmetrical frequency-threshold curves with low and high frequency roll-offs of 40 dB and 20 dB or less/octave, respectively.

Figure 2B shows the frequency-threshold curves for a primary auditory neuron before and after the removal of the anterior tympanum. The cell showed a decrease in sensitivity of 10 dB for sound frequencies near 16 kHz and 20 dB for sound frequencies one octave or more below 16 kHz. The cell remained, however, tuned to sound frequencies between 16 kHz and 18 kHz after the removal of the anterior tympanum. Similar results were obtained from 5 preparations.

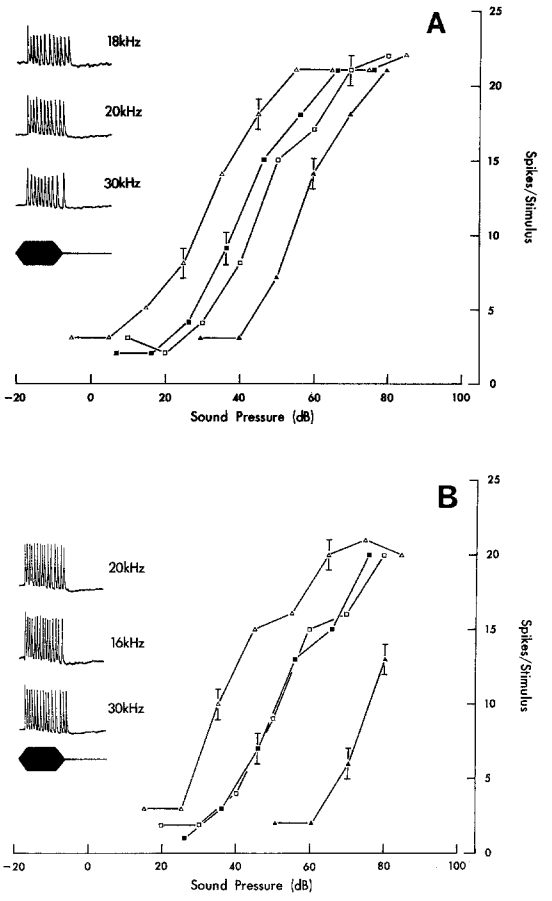

Fig. 3. A Intensity-response curves of a primary auditory neuron recorded in the leg nerve of an intact preparation. Note that the slope and maximum number of action potentials for each sound frequency is similar. *Insert* shows the response of a cell in an intact preparation to 18 kHz (33 dB), 20 kHz (40 dB), 30 kHz (64 dB). **B** Intensity-response curves from the same primary auditory neuron after the removal of the anterior tympanum. Note that the slope and maximum number of action potentials produced are similar for each sound frequency and similar to that of the intact preparation. *Insert* shows the response of the cell to 20 kHz (43 dB), 16 kHz (56 dB), 30 kHz (61 dB). (▲) 5 kHz, (■) 8 kHz, (△) 20 kHz, (□) 30 kHz

Figure 2C shows the frequency-threshold curves of several primary auditory neurons after the anterior tympanum had been removed. The most sensitive cells were, as in the intact preparation, those tuned to sound frequencies between 7 kHz and 18 kHz. For several cells that were most sensitive to sound frequencies below 7 kHz the asymmetry in the frequency-threshold characteristics, noted previously, disappears following the removal of the anterior tympanum.

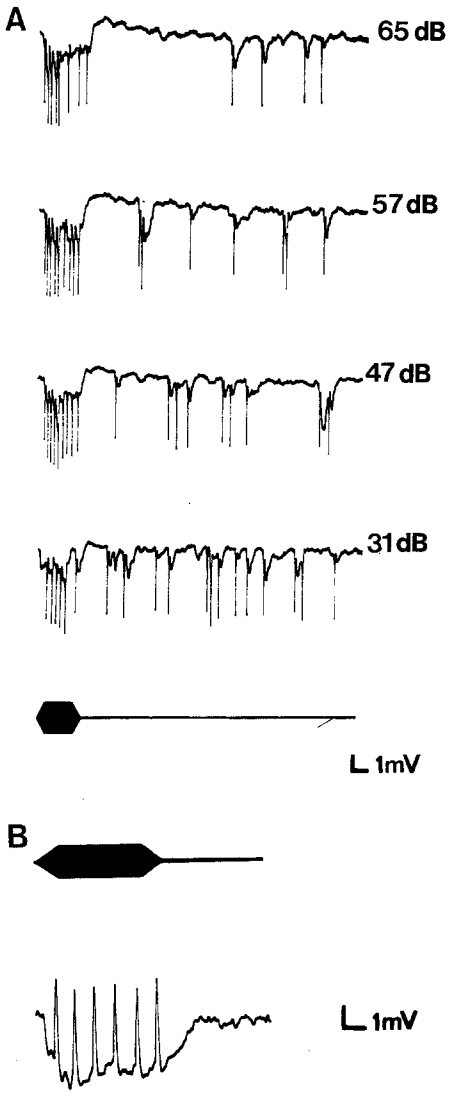

Fig. 4A, B. Physiological recordings from an auditory sensillum. **A** Typical response (as recorded from 26 sensilla) showing a slow hyperpolarising potential and hyperpolarising spike potentials. **B** Atypical response (as recorded from 2 sensilla) showing depolarising spike potentials. The sound pulse duration in both diagrams is 50 ms

Figure 3A shows the intensity-response curves of a primary auditory neuron for several sound frequencies in the intact preparation. Figure 3B shows corresponding curves for the same cell following the removal of the anterior tympanum. Both sets of intensity-response curves have the typical sigmoidal shape. The maximum number of action potentials in the response and the slope of the curves were similar in both the intact and operated preparation. The intensity-response curves for an individual cell were similar for each sound frequency.

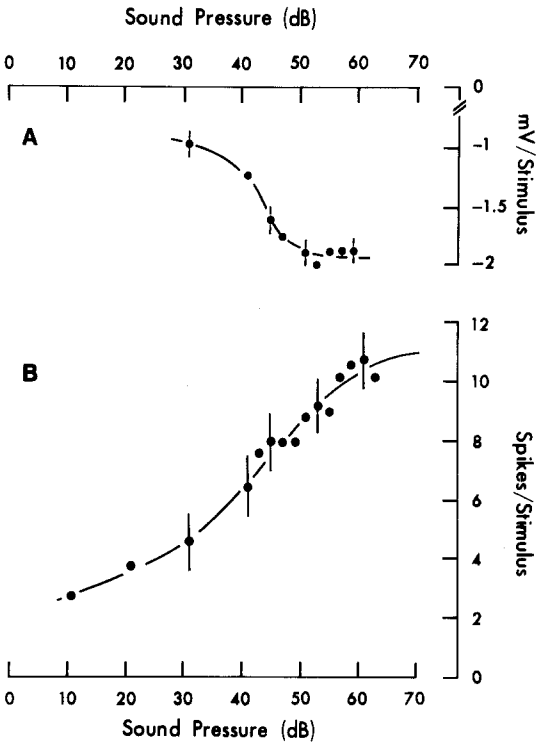

Fig. 5. Intensity-response characteristic of an auditory sensillum quantified in terms of **A** the magnitude of the slow potential; **B** the number of hyperpolarizing spike potentials

Physiological Responses of Identified Sensilla in the Crista Acustica

After removing the anterior tympanum, the auditory sensilla of the crista acustica and in particular their attachment cells can be seen through a dissecting microscope. Recordings were obtained by advancing the electrode tip toward the attachment cell of a specific sensillum while gently tapping the micromanipulator. No auditory responses were recorded until the electrode tip was in contact with or had penetrated the sensillum. Figure 4A shows the typical response obtained from an auditory sensillum in the crista acustica of *C. simplex*. To a low, but suprathreshold sound pressure, a 1 mV hyperpolarising slow potential that lasts the duration of the stimulus and, superimposed on this potential, 2 to 4 mV rapid hyperpolarising potentials of 1–2 ms duration (spike potentials) were recorded. As the sound intensity was increased both the magnitude of the slow potential and the number of spike potentials increased until the response became saturated (Fig. 5). Following each sound pulse the attachment cell briefly depolarised by 1 to 2 mV before regaining its resting membrane potential (-30 to -50 mV). The duration of the

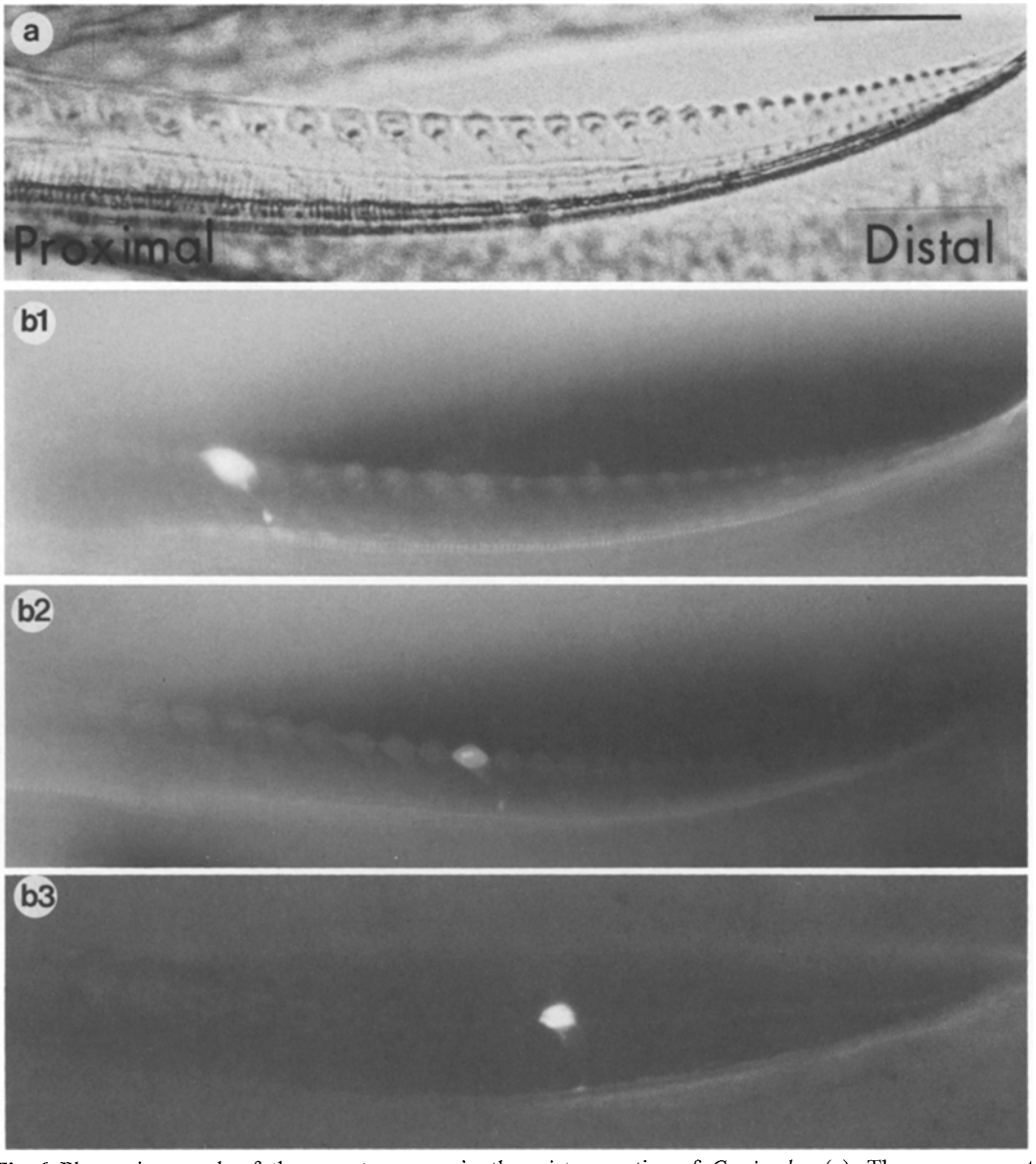

Fig. 6. Photomicrograph of the receptor array in the crista acustica of *C. simplex* (**a**). The arrangement and relative location of auditory sensilla visible through the dissecting microscope (**b1**–3). Corresponding portions of the crista from three different insects photographed under ultraviolet light showing the relative location of three auditory receptors stained with Lucifer Yellow. Note that the dye is located in the attachment cell and the scolopale cell nucleus at the base of the sensillum and that, even though a considerable amount of dye has been injected, no sensory axons were stained. *Scale:* 50 μm

recovery period was dependent on the level of stimulation. During the recovery period, the number of spontaneous spike potentials decreased.

In two of the 28 sensilla from which recordings were obtained (Fig. 4B), depolarising spike potentials were recorded superimposed on a hyperpolarising slow potential. On the rare occasions when spike potentials were recorded in the absence of the slow potential, dye injection failed to stain the sensillum. Sensilla were stained only when the slow potential was recorded and then only one sensillum in the crista was marked.

Figure 6a shows a photomicrograph of the portion of the crista acustica of *C. simplex* that was visible through the dissecting microscope. Figure 6b shows three cristae from different preparations each containing a cell that was stained with Lucifer Yellow. Typically, the dye was concentrated in the attachment cell and the scolopale cell nucleus at the base of the sensillum. On the occasions when

dye was injected for a period of less than 1 min, due to the loss of the recording during dye injection, only the attachment cell was stained. The dye marking confirms that the attachment cell was the site of the physiological recordings reported in this paper.

Figure 7 shows the tuning curves obtained from the three auditory sensilla shown in Fig. 6b. All three receptors had typical V shaped tuning curves with cells 1 and 2 having approximately 20 dB to 30 dB/octave rolloffs in sensitivity above and below their optimal sound frequency (i.e. characteristic frequency or CF). The CFs of the three cells were 9.5 kHz (1), 13.5 kHz (2) and 16 kHz (3), with the most proximal sensillum having the lowest and the most distal the highest CF. Threshold curves obtained from 11 other marked auditory sensilla, four of which having the same optimal frequency and the same position in the crista, confirmed this relationship between the position of the sensillum in the receptor array and the sound frequency of maximum sensitivity. Figure 8 shows tuning curves from identified auditory sensilla in the crista acus-

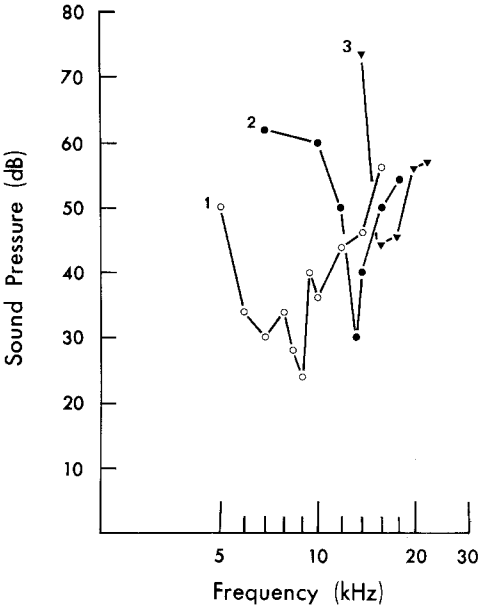

Fig. 7. Frequency-threshold curves for three auditory sensilla shown in Fig. 6. Note, the numbers shown on each curve corresponded to the numbers used to identify the marked cells in Fig. 6b (1–3)

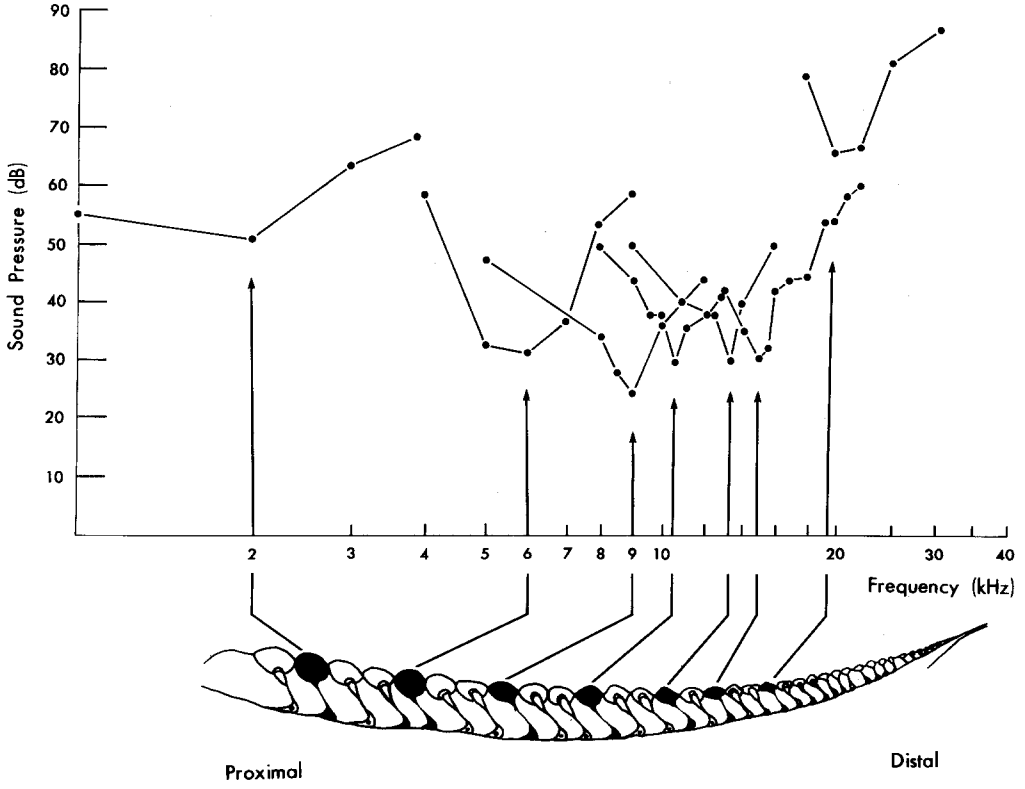

Fig. 8. Frequency-threshold curves for 7 identified auditory sensilla in the crista acustica of *C. simplex* showing the relationship between the optimal sound frequency (CF) and the position of the receptor in the array

tica of *C. simplex* and their position in the receptor array.

Discussion

This study demonstrates that the sensilla in the crista acustica of tettigoniids are tonotopically organised. For *C. simplex* the sensilla in the crista are tuned to sound frequencies between 1 kHz and 35 kHz with an interval of approximately 1 kHz between the CFs of adjacent sensilla (Figs. 6–8).

The frequency-threshold characteristics of the primary auditory axons of *C. simplex* (Fig. 2A) are consistent with the single unit responses of the group-a type-R units described by Rheinlaender (1975) and the pure auditory units described by Kalmring et al. (1978). These auditory units, like those reported here, were sensitive to sound frequencies between 3 kHz and 40 kHz, with the most sensitive units tuned to sound frequencies greater than 7 kHz. Similarly, the intensity-response curves reported here (Fig. 3A) have similar slopes to some of the intensity functions of auditory fibers reported by Rheinlaender (1975) and Kalmring et al. (1978). The recordings I have presented from the auditory sensilla confirm that at least some of the primary auditory units recorded by Rheinlaender (1975) and Kalmring et al. (1978) were the axonal projections from receptors in the crista acustica.

The variation in the absolute thresholds of auditory receptors (Fig. 2A) indicates that the acoustic transmission properties of the prothoracic trachea (Hill and Oldfield 1981, Fig. 10, p. 176) determine the overall auditory sensitivity of the crista acustica. Receptors tuned to sound frequencies below the cut-off frequency of the trachea (about 6 kHz for *C. simplex*) are 20 dB to 30 dB less sensitive at their CF than those tuned to sound frequencies between 7 kHz and 18 kHz (Fig. 2). The reduced sensitivity of receptors tuned to sound frequencies above 18 kHz (Fig. 2A) is consistent with the reduced transmission of sound in the prothoracic trachea at high sound frequencies. Individual receptors are obviously more sharply tuned (Figs. 2, 7 and 8) than the broad band frequency characteristic of the trachea (Hill and Oldfield 1981). This enhanced frequency selectivity must be due either to properties intrinsic to the individual receptor or to an as yet undescribed mechanical frequency analyser. It is clear from the anatomical data presented here (Fig. 1 and 6) and elsewhere (Graber 1876; Schwabe 1906; Schumacher 1979) that the auditory sensilla in the crista acustica are coupled by a supporting membrane. The frequency-threshold characteristics of individual auditory sensilla may result, therefore, from the characteristic vibration patterns of this membrane in a manner analogous to the mammalian basilar membrane. This issue will remain unresolved until the effective stimulus at the auditory sensillum is identified.

Because the sensory dendrite within the sensillum is continuous with the auditory axon in the leg nerve (Schumacher 1979) and the axon produces depolarising action potentials (Fig. 3A, B), the sensory dendrite must depolarise in response to a sound stimulus. The slow hyperpolarising potential recorded in the attachment cell (Fig. 4), therefore, most probably is an extracellular recording of the receptor potential. The ability to record this slow potential and the spike potentials extracellularly is most probably a consequence of the tight physical coupling between the attachment cell and the sensory dendrite (Fig. 1). Similar potentials have been recorded by Suga (1960, Fig. 4D, p. 538) in Müllers organ of the locust *Locusta migratoria danica*. He reports that this type of recording was rare and difficult to maintain since slight movements of the electrode resulted in the loss of the recording. The "negative monophasic slow potential" reported by Suga (1960) may have been obtained from the attachment cell of a sensillum in Müllers organ.

Acknowledgements. I thank Dr. W. Stewart for providing the Lucifer Yellow CH, Dr. G.S. Boyan for instruction in its use, R. Yanka for technical assistance and Dr. K.G. Hill for providing a scientific standard that I hope to one day achieve.

References

Autrum H (1940) Über Lautäußerungen und Schallwahrnehmung bei Arthropoden II. Das Richtungshören von *Locusta* und Versuch einer Hörtheorie für Tympanalorgane vom Locustidentyp. Z Vergl Physiol 28:326–352

Autrum H (1941) Über Gehör und Erschütterungssinn bei Locustiden. Z Vergl Physiol 28:580–637

Fielden A (1960) Transmission through the last abdominal ganglion of the dragonfly nymph, *Anax imperator*. J Exp Biol 37(4):832–844

Graber V (1876) Die tympanalen Sinnesapparate der Orthopteren. Denkschr Akad (Wien) Math Naturwiss Kl (Abt II) 36:1–140

Gray EG (1960) The fine structure of the insect ear. Philos Trans R Soc Lond [Biol] 243:75–94

Hill KG, Oldfield BP (1981) Auditory function in tettigoniidae (Orthoptera: Ensifera). J Comp Physiol 142:169–180

Kalmring K, Lewis B, Eichendorf A (1978) The physiological

characteristics of the primary sensory neurons of the complex tibial organ of *Decticus verrucivorus* L. (Orthoptera: Tettigoniidae). J Comp Physiol 127:109–121

Nocke H (1975) Physical and physiological properties of the tettigoniid ("grasshopper") ear. J Comp Physiol 100:25–57

Rheinlaender J (1975) Transmission of acoustic information at three neuronal levels in the auditory system of *Decticus verrucivorus* (Tettigoniidae: Orthoptera). J Comp Physiol 97:1–53

Schumacher R (1979) Zur funktionellen Morphologie des auditiven Systems der Laubheuschrecken (Orthoptera: Tettigoniidae). Entomol Gen 5:321–356

Schwabe J (1906) Beiträge zur Morphologie und Histologie der tympanalen Sinnesapparate der Orthoptera. Zoologica (Stuttgart) 50:1–154

Suga N (1960) Peripheral mechanism of hearing in locust. Jpn J Physiol 10:533–546

Thomas MV (1977) Microelectrode amplifier with improved method of input-capacitance neutralization. Med Biol Eng Comput 15:450–454

Zhantiev RD (1971) Frequency characteristics of tympanal organs in grasshoppers (Orthoptera: Tettigoniidae). Zool Zh 50:507–514

Zhantiev RD, Korsunovskaya OS (1978) Morphofunctional organization of tympanal organs in *Tettigonia cantans* (Orthoptera: Tettigoniidae). Zool Zh 57:1012–1016