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COMPARATIVE STUDIES ON THE AUDITORY SYSTEMS OF ENSIFERA.

Paul Jeremy Boyd B.Sc.

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the City of London Polytechnic, London (CNAA Board).

September, 1983.

CONTENTS

I hereby declare that this thesis is my own work, except where the contrary is specifically indicated. No other registration for an award of either the CNAA or any University occurred during the period of this research programme.

September, 1983.

P.J. Boyd.

Advanced studies undertaken in connection with this programme of research included attending the M.Sc. course "Neurophysiological Basis of Behaviour" at the City of London Polytechnic, and the attendance of several conferences and seminars.

Chapter 2 forms the basis of two papers which have been accepted for publication. In addition, investigations with other workers led to co-authorship of one paper on directional hearing in the Common Mole.

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ABSTRACT:

COMPARATIVE STUDIES ON THE AUDITORY SYSTEMS OF ENSIFERA

Paul Jeremy Boyd

The neural processing of acoustic information was investigated in examples of the Ensifera. Sound reception by these insects has two main functions: localization and recognition of the sound source. This study investigated aspects of each of these processes.

The degree of directional variation in the responses of the auditory organ was measured by recording responses of the primary acoustic fibres in the prothoracic leg nerve. In the bushcricket Tettigonia cantans and in the crickets Gryllus campestris and Teleogryllus oceanicus it was found that greatest directionality was achieved at frequencies close to their respective calling song frequencies. However, by comparing neural responses with measurements of sound diffraction around the insects, it was shown that directionality in T. cantans depends purely on sound diffraction by the body, whereas diffraction cannot account for directionality in the cricket auditory system. Experiments involving blockage of the sound input sites into the auditory systems of the two cricket species revealed details of a "pressure-gradient" mechanism, in which directionality is achieved by phase interactions.

The coding of auditory information by individual neurones in the ventral cord of G. campestris was investigated using glass microelectrodes. Cobalt-chloride electrolyte was used in order to simultaneously stain the recorded units. A number of neurones were characterized, both physiologically and morphologically. Some were most sensitive to the calling song frequency, while others responded best to the higher frequencies of the courtship song. Coding of song temporal patterning was also shown to be a characteristic of certain neurones, and a hypothesis that the neurones ascending from the prothoracic ganglion constitute a first stage in recognition is postulated. Additional experiments on processing of vibration stimuli by these and other units demonstrated that the degree of interaction between the sound and vibration pathways is not as extensive as has been shown in certain other orthopteran groups.

CHAPTER 1

GENERAL INTRODUCTION

1.1 ACOUSTIC BEHAVIOUR OF THE ENSIFERA

The well known singing of crickets and bushcrickets illustrates the importance of acoustic communication in these insects. Among the Orthoptera, most members of the sub-order Ensifera (Tettigoniidae and Gryllidae) use acoustic signals for intraspecific communication, although sound production is not quite so widespread among the Acrididae (Elsner 1983). Thus, in addition to the usual technical advantages of working with invertebrates for neurophysiological experiments, studies of auditory function in the Ensifera are made particularly worthwhile because the findings of such experiments can be relatively easily augmented by behavioural investigations carried out in the field and in the laboratory.

The most frequently used methods of sound production in the Orthoptera are wing and leg stridulation, and these have been described by several authors (e.g. Dumortier 1963; Haskell 1964; Michelsen & Nocke 1974; Sales & Pye 1974; Bennet-Clark 1975; Elsner & Popov 1978). The Ensifera employ an elytro-elytral mechanism, using the hardened fore-wings (tegmina), where contact is made between a denticulated vein (file) on the underside of one tegmen and the inside edge (plectrum) of the other. Sound is normally produced only during wing closure (Pierce 1948). In crickets, each tegmen bears a file and plectrum but the right tegmen usually overlaps the left so that only the left plectrum and right file are used (Huber 1963). A specialized area of the tegmen (the harp) functions as a

resonator and this emits the airborne sound. In bushcrickets, there is only one plectrum and one file (on the right and left tegmina respectively), and the sound is emitted by a stiffened region of the cuticle, the "mirror frame" (Bailey 1970) which largely surrounds a thin cuticular area of the wing, known as the mirror. The mirror itself appears to play only a minor role in the sound emission (Bailey 1970; Bailey & Broughton 1970).

A resonant or non-resonant song can be produced, depending on whether or not the tooth impact rate (i.e. the rate at which contact is made between the plectrum and the teeth of the file) corresponds to the resonant frequency of the emmitter. In all crickets and some bushcrickets the system is lightly damped (i.e. has a high Q-value) and sound emission is resonant, producing a narrow banded spectrum with a relatively low carrier frequency. In bushcricket species with a heavily damped (low Q-value) system, a low tooth impact rate produces transient pulses resulting in a broad-banded sound extending into high frequencies. In the latter case the fundamental frequency has been shown to depend mainly on the length of the mirror frame (Sales & Pye 1974).

The songs of the Ensifera can show complex temporal patterning. There is usually more than one song type; modification of a basic song pattern often provides for distinct calling (proclamation), aggression (rivalry) and courtship songs. This distinction is usually more highly developed in the gryllids than in the tettigoniids, the latter usually having only the calling song, although some

also appear to show a "disturbance call". The basic syllable rate (each syllable corresponds to one complete cycle of wing movement) is often divided into "chirps" or "trills". However, sound is usually produced only during wing closure. Each chirp may be further amplitude modulated and complex songs can be built up by combining chirps and trills of different durations. In both groups the male alone sings, and the number of songs produced, and their degree of complexity, varies greatly according to the species. In general, complicated songs occur in habitats where there are many species; when species have multiplied, sounds have become specialized, apparently to keep interspecific confusion minimal (Alexander 1962). Conversely, related species which do not live together often have nearly identical songs.

The functions of song are very similar in the crickets and the bushcrickets. They are used primarily in behavioural contexts related to courtship. When a male cricket starts to sing it first produces the calling song. This broadcasts its presence to conspecifics and serves to repel the males and attract the females. If two males become unacceptably close, fighting is likely to occur, interspersed with chirps of the aggression song. This behaviour presumably ensures even spacing of singing males throughout the biotope (such spacing has been demonstrated in bushcrickets by Latimer 1980, 1981). When a female approaches a male, in response to the calling song, the male cricket will usually change to a courtship song, used only at short distances and to release copulatory behaviour in

the female. This change in song type does not apparently occur in bushcrickets.

The phonotaxis of the female cricket towards a singing male was shown by the early experiments of Regen (1913) who first demonstrated that the only cue essential for female phonotaxis in Gryllus campestris was the calling song. As this behaviour is very repeatable and easily elicited in the laboratory it has since been used by numerous workers to study various aspects of audition in these insects. Another advantage in studying this behaviour is that the songs in the genus most generally used (Gryllus) are relatively simple and can therefore be synthesized electronically to determine the relative importance of the main song parameters, such as the temporal structure and the spectral components. Unfortunately, phonotaxis is much less easily elicited in bushcrickets, and as a result most of the information on acoustic behaviour of bushcrickets has been obtained by observations made in the field.

Before phonotaxis takes place, the female must perform two computations: the localization of the sound source, and the recognition of the caller as a conspecific male. The process of localization has been investigated behaviourally in crickets in various ways, often by altering the parameters of electronically generated songs (see Elsner & Popov 1978 for review). The main problem, however, has been in separating the processes of recognition and localization which must be performed by the CNS of the receiving animal. On the emitter side all the necessary parameters for these processes occur in the species song, and experimentally

manipulating one parameter may affect the efficacy of other information parameters. For example, if the carrier frequency of the song model is changed, in order to test any change in the accuracy of localization, the process of recognition may also be affected (the opposite is true when studying recognition). In other words, localization may be possible but phonotaxis (the measure used behaviourally) not probable when models differing in temporal pattern from the natural song are used.

Some of the methods most often used for studying accuracy of phonotaxis (which is a measure of the ability to localize) have included (i) analysis of turning angles in free-walking insects (Murphey & Zaretsky 1972; Bailey & Thomson 1977; Oldfield 1980), (ii) measuring side discrimination using a movable-axis Y-maze (Rheinlaender & Blatgen 1982) and (iii) analysis of turning angles in crickets walking on a "Kramer" treadmill (Wendler et al. 1980; Schmitz et al. 1982). Use of the Kramer treadmill, a locomotion compensator, involves studying the movements of a cricket walking on a polystyrene sphere. An electronic feedback mechanism uses motors to control the movement of the sphere such that the position of the insect remains constant. Analysis of walking movements is then made using a cine camera positioned directly above the insect. Despite the limitations imposed by the problem of separating recognition and localization, it now seems fairly clear that the process of localization depends on the frequency content, rather than the temporal structure of the song. Unfortunately, such experiments have not been possible on

bushcrickets, and hence little is known of their accuracy of localization.

There are two main parameters by which the songs of different species can be recognized: their temporal patterns and their frequency contents. The early view that insects are incapable of frequency discrimination (Pumphrey & Rawdon-Smith 1939) has not been confirmed since it has been clearly demonstrated neurally (e.g. Nocke 1972; Kalmring et al. 1978b) and behaviourally (e.g. Hill 1974; Popov et al. 1975) that crickets and bushcrickets are capable of at least frequency discrimination, if not complex frequency analysis. It is, therefore, at least theoretically possible that the songs of different species can be distinguished on the basis of their carrier frequencies or their overall spectra. However, while the songs of different cricket species generally have different carrier frequencies, they are mostly within the 3-6 kHz range, and therefore all fall within the hearing capabilities of each species. Many sympatric bushcricket species also have song spectra that overlap considerably, mainly because they often tend to be broad-banded. It now seems that recognition may be achieved predominantly on the basis of the amplitude modulation (temporal patterning) of the songs, rather than on the carrier frequency. Hill (1974), for example, showed that female Teleogryllus commodus moved towards an artificial conspecific song with the correct temporal pattern when the carrier frequency was between 2 kHz and 12 kHz. The natural carrier frequency in this species is around 3.8 kHz. The

early work of Walker (1957) showed that in crickets the syllable rate was the most important feature of the song for eliciting phonotaxis, and this has been supported by more recent work, such as that by Thorson *et al*. (1982). Syllable repetition rate has also been shown to be the most important parameter in trilling bushcrickets (Bailey & Robinson 1971).

It is clear from the investigations to date that experiments on recognition must be very carefully designed. It has been shown that female crickets will track artificial songs that have only a very limited resemblance to the natural conspecific song. Pollack & Hoy (1979) reported that female *Teleogryllus oceanicus* (a species with a complex song involving two phrases) recognized models of the song when the sequential order of the phrases was arranged randomly, but with the correct interpulse intervals. Thorson *et al*. (1982) showed that females of *Gryllus campestris* tracked songs even when they were played backwards. These experiments suggest that the main cue for recognition is the interpulse interval (time between each syllable) and that the recognition process is a rather crude one. However, it has also been shown in forced-choice Y-maze experiments that when a female is given a choice of two song models it always chooses the model that is closest to the natural song, both in temporal (Popov & Shuvalov 1977) and frequency (Rheinlaender & Blatgen 1982) content. This suggests much more accurate discrimination, making it difficult to understand why females have often been shown to track very poor copies of the male calling song. It is

possible that motivation plays an important role in this context. Thus the essential and motivational parameters work together to determine the specificity of the response. This type of preference was described by Popov & Shuvalov (1977) as working on a "probability principle", by which females always choose the signals that have the greatest probability of being the conspecific song. Certain "ideal" parameters of the song are presumed to be imprinted in the CNS in order to provide a reference. In some cases, where there is more than one conspecific song, the insect may also need to be able to distinguish different conspecific song types, such as calling, aggression and courtship. Motivation is particularly likely to be important in this process.

Apart from intraspecific communication, it is likely that hearing in insects is also very important for the detection and interpretation of signals produced by other animals, particularly predators. Crickets have been shown to respond to ultrasonic sound by negative phonotaxis (Popov et al. 1975; Moiseff et al. 1978) and this observation has suggested that bats may be the most common airborne predators. Rodents and various insectivores such as shrews are known to produce ultrasound (Sales & Pye 1974) and these could represent terrestrial predators. Once again, the data for the bushcrickets are lacking, but sensitivity to high frequencies shown neurally (Silver et al. 1980) has demonstrated that these insects can at least detect ultrasonic sounds.

There is much evidence that in bushcrickets the detection of substrate vibration is very important in addition to sensitivity to airborne sound. This may be of value both for intraspecific communication at short distances and for detection of terrestrial predators. However, little is known of the vibration sense in crickets, although they do possess well developed vibration-sensitive organs (see below). One of the most significant differences between the biology of crickets and bushcrickets, in relation to their acoustic behaviour, is that crickets usually live on more solid ground such as forest floors or fields; bushcrickets, as their name implies, tend to live in taller and more dense vegetation. This difference is of great importance when the various strategies involved in acoustic behaviour are considered, particularly regarding vibration reception; they are discussed fully in the forthcoming chapters.

1.2 ANATOMY OF THE ENSIFERAN AUDITORY SYSTEM

Within the Ensifera, detection of airborne sound is achieved by means of tympanal organs. In both Tettigoniidae and Gryllidae the tympanal organs are situated in the proximal tibiae of the prothoracic legs, and are closely associated with the tracheal system (see Hutchings & Lewis 1983a for review). The major organs concerned with the reception of substrate vibration are also situated in the proximal parts of the tibiae, and it has been shown that while the mesothoracic and metathoracic legs are atympanal they do

possess similar sensory structures to those of the prothoracic legs (Eibl 1978).

The tracheal apparatus of the forelegs and prothorax is modified, in both crickets and bushcrickets, in order to conduct sound towards the tympanal organs (Zeuner 1936; Lewis 1974). The proximal end of the main leg trachea ("acoustic trachea") opens at an "acoustic spiracle" on the prothorax, which allows access of sound to the rear surfaces of the tympana (Figs 1.1A, 1.2A). In crickets this spiracle is covered by cuticular folds by means of which the insect can, presumable, alter its patency, but in most bushcrickets it is large and permanently open. Near the spiracles, a branch of the trachea extends centrally. In most cricket species this meets the branch from the opposite side at the midline (Michel 1974; Young & Ball 1974; Zhantiev et al. 1975). The extent of this connection in bushcrickets is variable and is usually much less developed. The auditory systems of the two sides have been shown to be acoustically coupled in crickets, by means of this connection (Zhantiev et al. 1975; Hill & Boyan 1976, 1977), but no such coupling has been demonstrated in bushcrickets (Hill & Oldfield 1981).

The main leg trachea splits into anterior and posterior branches in the region of the tympana, and the two branches reunite distally. The tympana are formed from thin regions of the leg cuticle in contact with the walls of the tracheal branches, hence there are usually two tympana, one anterior and the other posterior (Figs 1.1B, 1.2B). The tympana and accessory structures differ between the crickets and

Fig. 1.1

The anatomy of the cricket auditory system (from Larsen & Michelsen 1978).

(A) shows the positions of the tracheal branches involved in sound conduction, in relation to the spiracle S and the tympanic membrane TM.

(B) is a cross-section through the tibia at the level marked CS in (A).

AM - anterior membrane

ATB - anterior tracheal branch

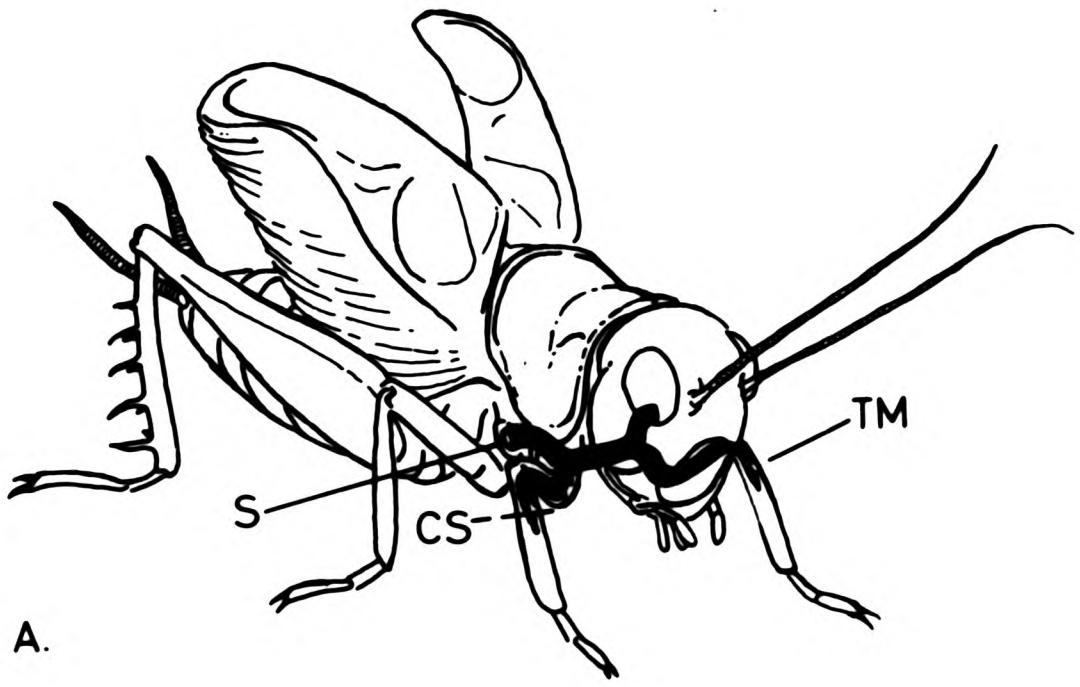
BC - blood canal

MC - muscle canal

PM - posterior membrane

PTB - posterior tracheal branch

SC - sensory cells



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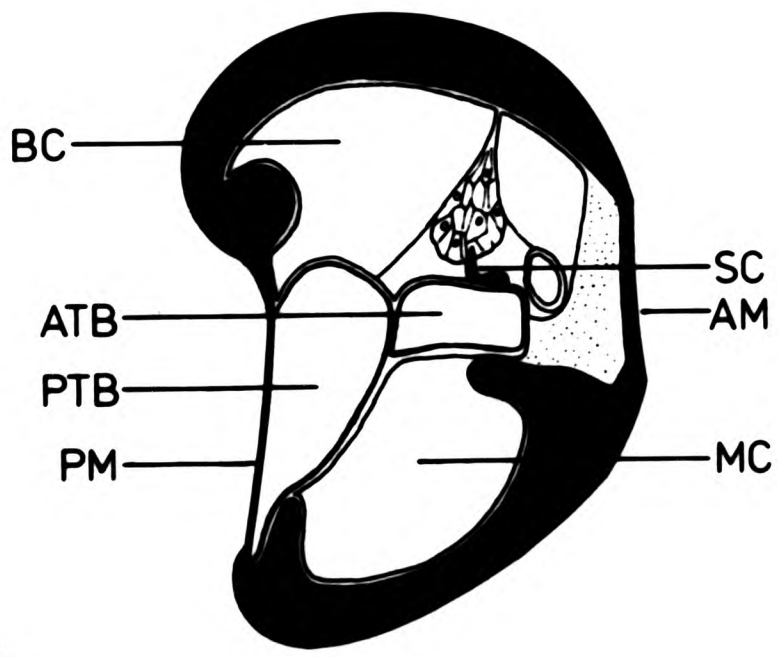

B.

Fig 1.2

The anatomy of the bushcricket auditory system.

(A) shows the tracheal branches at the level of the prothorax. Sound is conducted from the spiracle, down the trachea in the prothoracic leg, to the auditory organ in the tibia.

(B) shows a cross-section through the prothoracic leg at the level of the auditory organ. Note that the tympanic membranes are covered by large cuticular folds that leave only narrow tympanal slits communicating with the exterior.

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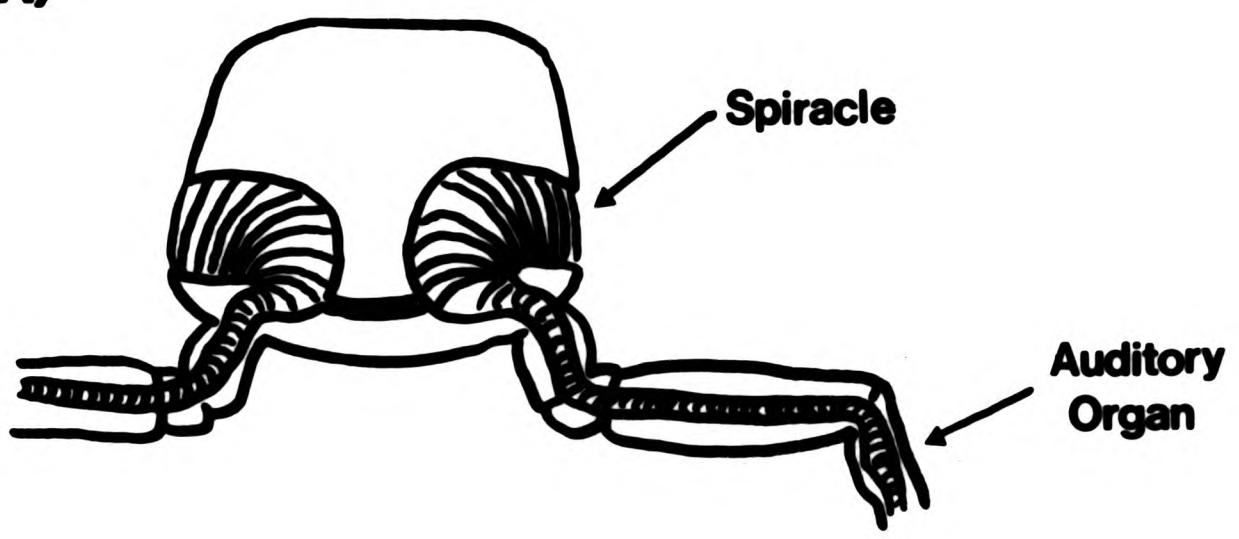

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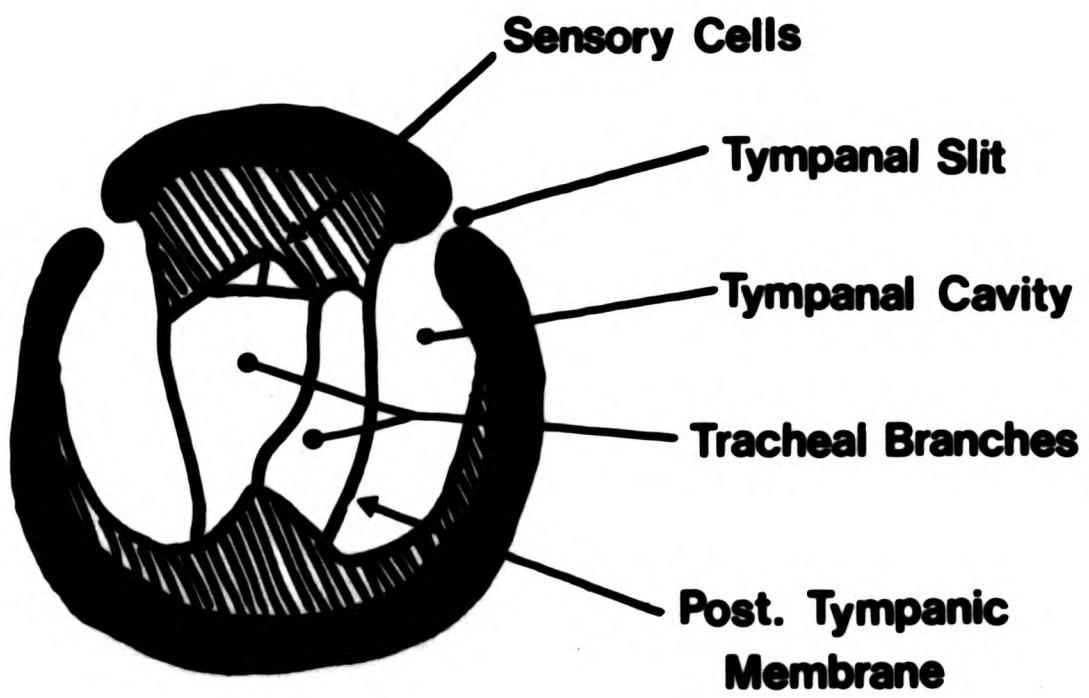


bushcrickets. In the gryllids the tympana are directly exposed (Fig. 1.1B) and the anterior tympanum is usually non-functional, being considerably smaller than the posterior and not directly apposed to the tracheal branch. The tympana of tettigoniids are usually more or less equally well developed, but are usually covered by cuticular folds which may leave only narrow "tympanal slits", often front-facing, communicating with the exterior (Fig. 1.2B).

The two branches of the acoustic trachea in the region of the tympana do not completely fill the lumen; a haemolymph canal occurs dorsally, and the ventral haemolymph space carries muscles and nerves (Autrum 1963). The sensory cells of the tympanal organs (scolopidia, Gray 1960), are grouped in the dorsal haemolymph canal, and insert on the dorsal wall of the anterior tracheal branch. In crickets the tympanal organ is divided into two parts: the proximal and distal cell groups. About 60-70 receptor cells are present (Michel 1974). Although the distal cells are smaller a tonotopic arrangement has not yet been clearly demonstrated. In the bushcrickets the more distal cells form the crista acustica, and are arranged in a row of 30-40 cells along the trachea, decreasing in size distally (Schumacher 1972). It has been shown that the larger, proximal receptor cells respond preferentially to low frequency sound, and cells towards the distal end to high-frequency sound (Zhantiev & Korsunovskaya 1978; Oldfield 1982). Also present in bushcrickets, between the crista acustica and the subgenual organ, is the intermediate organ. This structure is not present in the crickets,

although it may correspond to the proximal region of the tympanal organ, and its precise function in the bushcrickets is as yet unclear.

Apart from the tympanal organs, which respond to airborne sound, there are structures in the legs of Ensifera that respond to substrate vibration. The most highly developed of these are the subgenual organs which are situated proximal to the tympanal organ, and occur in all three pairs of legs. In the cricket these consist of 20-30 scolopidia in a fan-like arrangement (Michel 1974; Young & Ball 1974; Eibl 1978). Chordotonal organs and campaniform sensilla have also been shown to respond to substrate vibration (Kalmring et al. 1978).

The primary fibres from the tympanal organs and the vibration-sensitive organs run in the tympanal nerve and join the CNS at the prothoracic ganglion. In bushcrickets the tympanal nerve is discrete along its length from tibia to ganglion, but in crickets it becomes incorporated into the main leg nerve (Eibl & Huber 1979). The projections of the primary fibres all terminate within the "auditory neuropile", which is located near the centre of the ipsilateral side of the ganglion; they do not cross the midline, nor pass to other ganglia (Zaretsky & Eibl 1975; Eibl & Huber 1979; Esch et al. 1980).

1.3 AIMS OF THE STUDY

As was discussed in section 1.1, there are two distinct neural processes involved before phonotaxis can take place: localization and recognition. The present study investigated aspects of each of these processes.

The first part (chapter 2) investigates the peripheral mechanisms involved in sound localization. Localization of a sound is achieved by the central nervous system (CNS) on the basis of directional cues provided by the auditory organs. In order to provide directional cues the responses of the auditory organs must vary in relation to the angles of sound incidence. The extent to which this can be achieved defines the directionality of the auditory organs. The experiments described were carried out in an attempt to establish the degree and biophysical basis of this directionality in representative examples of crickets and bushcrickets.

The investigations into song recognition (chapter 3) were concerned with the coding of various sound parameters by some single auditory neurones ascending from the prothoracic ganglion, towards the brain, in the cricket G. campestris. They are all first- or higher-order interneurones and their response patterns therefore demonstrate the extent of their integration of frequency, intensity and temporal information, carried out within the prothoracic ganglion. Emphasis was placed on the investigation of mechanisms underlying the coding of the conspecific songs, and synthesized songs were generated

electronically in order to be able to independently control the various song parameters. The neurones were simultaneously stained by using cobalt-filled electrodes in an attempt to obtain morphological as well as physiological characterization.

CHAPTER 2

PERIPHERAL DIRECTIONALITY OF THE AUDITORY ORGANS IN BUSHCRICKETS AND CRICKETS

2.1 INTRODUCTION

As discussed in the previous chapter, localization of a sound by the CNS requires that the auditory organs vary in their responses to sound incident from different directions. Each organ must therefore be inherently directional when considered in isolation. Much work on mammals has shown that the directional variation in responses is achieved principally by diffraction of sound by the head and/or body (Lewis 1983). Thus at appropriate frequencies, a "sound shadow" is produced when a sound is contralateral to the ear in question, whereas a buildup of pressure occurs when the sound is ipsilateral (Shaw 1974). Other directional cues may be derived from comparing the time-of-arrival or the relative phase of sounds incident at the two ears. Differences in intensity can be established at two receivers if an object of a diameter greater than 1/10 of the wavelength of the sound is interposed between the receivers (Lewis 1983). Therefore smaller mammals tend to use higher frequencies for communication. Most of the Ensifera have body diameters in the range of about 3-7 mm, and so little diffraction would be expected to be produced by frequencies below 10 kHz. Directionality to low frequency sound is possible under certain conditions, if the two ears are acoustically coupled, so that sound may reach both the front and rear surfaces of the tympana. The ears then become pressure difference ("pressure-gradient") receivers, and directional sensitivity is achieved by interference at the tympanic membrane, which may be constructive or destructive,

depending on the relative path lengths of the two sound components (Beranek 1954). Such a system has been shown to be used by certain birds (Coles et al. 1980; Lewis & Coles 1981) and by moles (Coles et al. 1982).

The processes by which neural directional cues are derived may be investigated in two distinct ways: measurement of directional coding in primary or central neurones (measuring firing rates produced in response to sound incident from different angles), and measurement of directional sensitivity of the auditory organs. The latter determination is most often achieved by measuring the thresholds of neurones (which may be primary or central) to sound from different angles, although it is possible to measure the sensitivity by measuring suprathreshold responses. The present study examines directional sensitivity of the auditory organ of the bushcricket Tettigonia cantans and the crickets Gryllus campestris and Teleogryllus oceanicus, using a method described by Bailey & Stephen (1978). Suprathreshold responses of the primary auditory fibres, recorded as whole-nerve responses, were quantified in terms of directional sensitivity, using an intensity-response curve.

Little information concerning the acuity of sound localization in the bushcrickets is available from behavioural experiments, but several physiological studies have shown that the auditory organs are directionally sensitive. However, there has been much confusion as to the mechanism by which such directionality is achieved. Most of the species studied have rather broad-banded song spectra,

and sound diffraction by the body could account for the differences in pressure at the two ears for high frequencies. However, several workers have also reported directional sensitivity at low frequencies where negligible interaural intensity differences would be expected. Nocke (1975) found that the directional sensitivity in Acripeza was maximal around 8 kHz, the carrier frequency of the species song, but there have been no subsequent reports to support these findings. Lewis (1974) suggested that the ear of the bushcricket Ruspolia differens could act as a pressure-gradient receiver at low frequencies, a view supported by Seymour et al. (1978), whereas Hill & Boyan (1981) found that directionality was produced entirely by sound diffraction in another species Mygalopsis marki.

There has also been confusion as to whether the dominant site of sound access to the tympana is via the acoustic spiracles or through the tympanal slits, which has important implications regarding the origin of directionality. The model of Hill & Oldfield (1981), for the bushcricket M. marki, is based on sound diffraction by the body, by which the responses of the auditory organ vary in acccordance with the sound pressure at the spiracle. On the other hand, Bailey & Stephen (1978) presented a model in which directionally-sensitive responses in the same species were produced by sound entering via the tympanal slits. The present study uses the recording method employed by Bailey & Stephen (1978) to re-examine auditory directionality in the bushcricket T. cantans.

In contrast to most tettigoniids, the gryllids produce songs that are essentially narrow banded and of relatively low frequency (Nocke 1972; Hill 1974; Popov & Shuvalov 1974). Little sound diffraction can be produced around the body of the insects at these frequencies, yet behavioural investigations have shown that crickets are capable of very accurate sound localization (e.g. Murphey & Zaretsky 1972; Bailey & Thomson 1977; Wendler *et al*. 1980). These reports have suggested that several species are capable of distinguishing angles as small as 10-15° away from the anterior direction. A different mechanism from that used by animals relying on sound diffraction must therefore be present in the gryllid auditory system.

Directional sensitivity was first clearly demonstrated neurally by Zhantiev *et al*. (1975) in *G. bimaculatus*, and more detailed experiments by Hill & Boyan (1976,1977) showed that directionality was restricted to frequencies around the calling song carrier frequency in *Teleogryllus commodus*. On the basis of blockage experiments Hill & Boyan (1978) postulated a pressure-gradient mechanism by which sound incident on the front and rear surfaces of the posterior tympanum interacted to produce a very directional system. They suggested that the sound was transmitted to the rear surface of the tympanum of one ear from the other ear, by way of the tracheal connection in the prothorax (Fig. 1.1A). However, some of the details of this model have been questioned by the results obtained by other workers. In particular, the origin of the sound component incident at the rear of the tympanum has been disputed. Larsen &

Michelsen (1978), using laser vibrometry to measure the vibration of the tympanum, found that sound input via the spiracles provided a much greater proportion of the "back-pressure" produced at the tympanum than did the sound input from the contralateral tympanum. They therefore suggested that a pressure difference interaction occurred between sound incident on the front surface of the tympanum and sound reaching its rear surface via the spiracles. Kleindienst et al. (1981) showed that the attenuation of sound from one ear to the other was too great (11 dB) to permit the operation of the mechanism proposed by Hill & Boyan (1977). The present study uses the whole-nerve technique of Bailey & Stephen (1978) to critically examine the directionality of the auditory organ in two cricket species, G. campestris and T. oceanicus, the latter being morphologically very similar to T. commodus.

2.2 MATERIALS AND METHODS

2.2.1 EXPERIMENTAL ANIMALS

(A) Bushcrickets

The experiments were carried out on individuals of both sexes from a single group of about forty adult specimens of Tettigonia cantans, provided by the Zoology Department of the Philipps University, Marburg. These were maintained over a period of about 4 weeks in perspex tanks, in a separate room, at a temperature of 25 ±2°C and 30% relative humidity. They were fed on a mixture of fruit and vegetable matter, and were subjected to a 12-hour light/dark cycle. No attempt was made to breed these insects.

(B) Crickets

The crickets used were taken from a laboratory culture of Gryllus campestris. These were maintained under the same conditions as the bushcrickets, except that the staple diet was rat pellets (Dixons) for the adults and the cereal "Bemax" for the instars. Eggs were laid in large petri dishes filled with moist fine sand. These trays were removed from the adults' tanks every week and the first instars usually emerged after 14-20 days. Experiments were carried out on adults of both sexes, at least one week after their final moult. A small number of experiments were also carried out on individuals from a culture of Teleogryllus oceanicus, which was maintained under the same conditions as described for G. campestris. The culture of T. oceanicus has been maintained over 6 years, and that of

G. campestris over 4 years.

2.2.2 EQUIPMENT

(A) Stimulus Production

The circuitry involved in generation of the stimulus is given in Fig. 2.1. Sine-wave frequencies produced by a signal generator (Phillips GM2317) were led to a tone burst generator (TBG), the frequency being monitored by a digital frequency counter (Heathkit). The TBG was built in the laboratory from a design by Taylor (1978). Each time it was triggered by a stimulator (Grass S48) the TBG gated the sine-waves into a trapezoidal tone pulse with controlled duration and rise/fall times. The TBG was triggered using the "delay" output of the stimulator. Duration of the tone bursts could be set to any of several pre-set values between 25 and 500 ms, or set to produce a continuous tone. The onset and termination of the stimuli (rise/fall) were continuously variable and was usually set to 5 ms to avoid the production of transients by the loudspeakers. The tone bursts were passed to a power amplifier (Xelex DD8) via an attenuator (Hatfield 2123) which controlled the signal in 1 dB steps, and then to either of two loudspeakers situated within the anechoic chamber: an Audax HD17/HR37 (Frequency response 100 Hz - 10 kHz, ±8 dB) for frequencies up to 10 kHz, or an Audax TW8B (Frequency response 1-40 kHz, ±10 dB) for frequencies above 10 kHz. The stimuli were monitored on a digital storage oscilloscope (Gould OS4000), which was triggered by the "non-delay" output from

Fig. 2.1

Circuit diagram of equipment used for recording the response of the leg nerve in crickets and bushcrickets.

AC/DC .... AC/DC amplifier

Atten. ... attenuator

AUDIO .... audio amplifier

AV. ..... averager

CH. REC. . chart recorder

C.R.O. ... cathode ray oscilloscope

FILT. .... filter module

FREQ. .... frequency counter

H.S. ..... headstage

LS ..... loudspeaker

MON. ..... monitor loudspeaker

P.A. ..... power amplifier

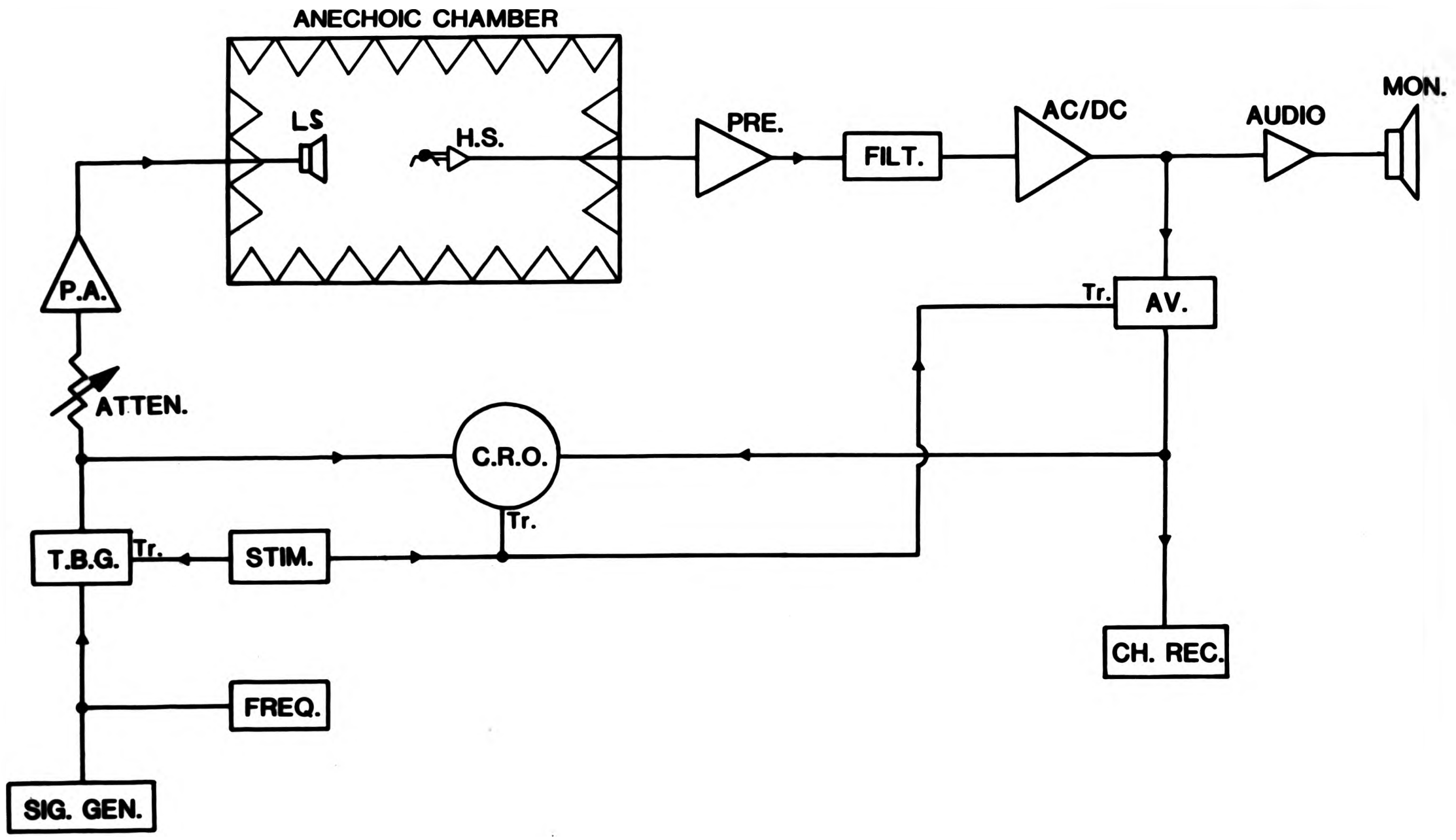
PRE. ..... preamplifier

SIG. GEN. signal generator

STIM. .... stimulator

T.B.G. ... tone-burst generator

Tr. ..... trigger input


ANECHOIC CHAMBER
LS
H.S.
PRE.
FILT.
AC/DC
AUDIO
MON.
P.A.
Tr.
AV.
ATTEN.
C.R.O.
Tr.
T.B.G.
Tr.
STIM.
CH. REC.
FREQ.
SIG. GEN.

loudspeaker positions in the horizontal plane, at several frequencies up to 40 kHz. The sound pressure level was found to be uniform within ± 1dB at 10 kHz, and within ± 2 dB at 40 kHz.

(C) Recording

The electrical signal picked up by the electrode was led to a headstage (Neurolog NL100) which was clamped to the wire preparation stand (described below). The signal was passed to an A.C. pre-amplifier (Neurolog NL103) located outside the anechoic chamber (Fig. 2.1), and was then filtered (Neurolog NL115) and amplified by an AC/DC amplifier (Neurolog NL106). The filters were set to pass frequencies between 10 Hz and 10 kHz. The signal was then led to an audio amplifier (Neurolog NL120) and subsequently to a small monitor loudspeaker. From the AC/DC amplifier the signal was also passed to an averager (Neurolog NL750) which stored the response , during averaging, until it was ready to be plotted on paper by a chart recorder (Mingograf 800). During averaging, the response was displayed, along with the stimulus, on the storage oscilloscope.

2.2.3 ANECHOIC CHAMBER

During an experiment an animal, waxed to the wire stand, was placed in the centre of an anechoic chamber having internal dimensions 2.5 x 2.5 x 2.2 m. All internal surfaces were lined with mineral wool (Rockwool - density 100 kg/m , 60 mm thick) cut into wedges having a lower cut-off frequency of 212 Hz. The two loudspeakers were mounted on a metal boom

(c)

.s.

Fig. 2.2

Anechoic chamber with boom control.

B. - boom

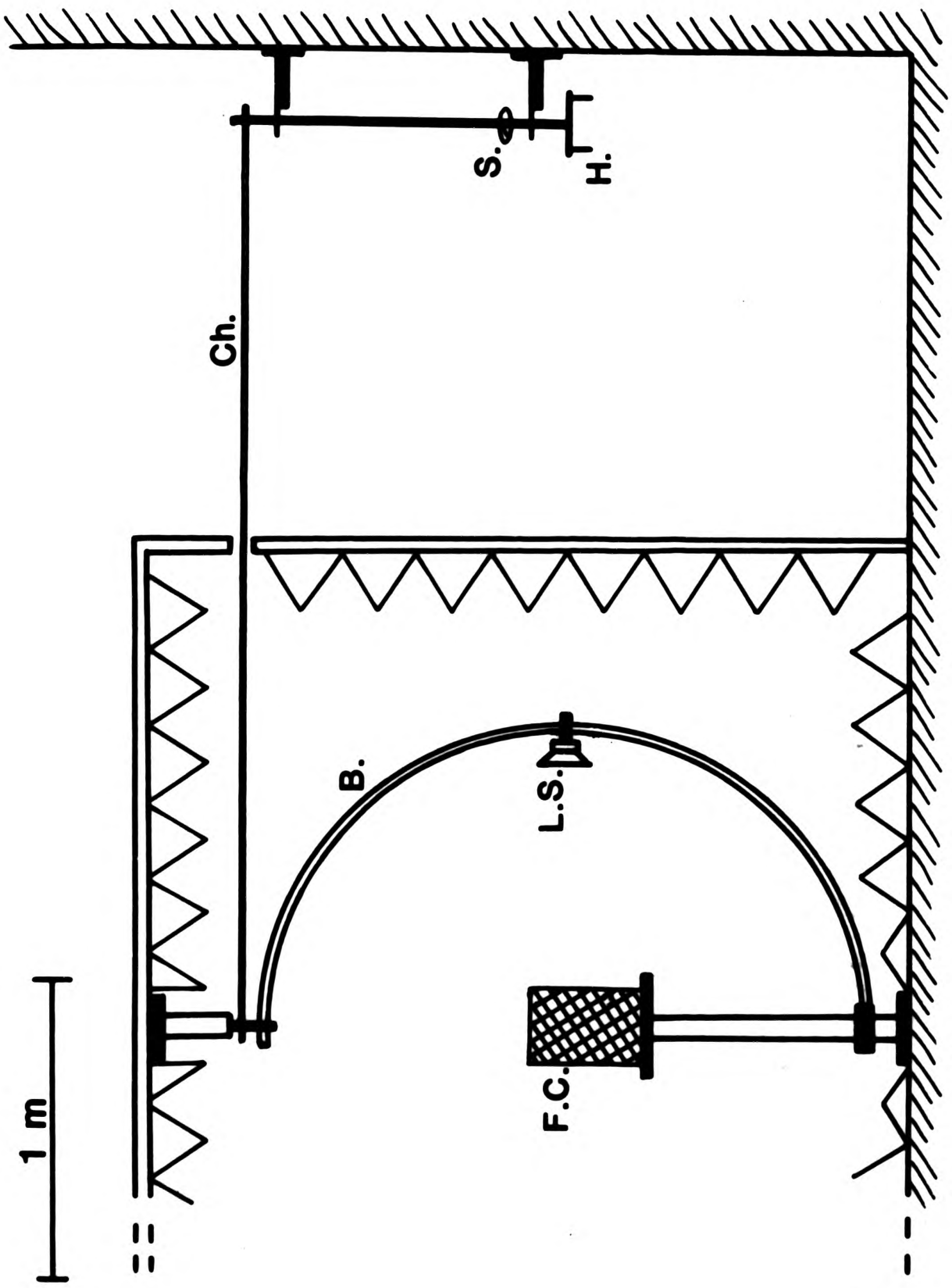
Ch. - chain

F.C. - Faraday cage

H. - handle

L.S. - loudspeaker

S. - protractor scale


S.
H.
Ch.
B.
L.S.
F.C.
1 m

that could be moved around the preparation in the horizontal plane, being controlled from outside the chamber (Fig. 2.2). The loudspeakers could also be moved up and down on the boom to allow for the study of elevation, if required. A circular clear plastic scale was attached to the boom control, outside the chamber, that showed the angle subtended by the loudspeaker to the longitudinal axis of the preparation. A vertical cylindrical stand, 90 cm high and with 7.5 cm diameter, supported a 42.5 x 38 x 0.6 cm metal plate in the centre of the chamber on which the wire stand, with preparation, was placed during experiments. A Faraday cage (30 x 30 x 40 cm), constructed of 5 mm wire mesh, was placed on the metal baseplate, over the preparation stand, to minimize pickup of electrical noise.

2.2.4 PREPARATION DISSECTION AND SET-UP

The insect was first immobilized by a short exposure to carbon dioxide. Wings and antennae were quickly removed and the animal was waxed (Cottrell Sticky Wax), in the normal standing position, onto a stand constructed of 0.8 mm wire (Fig. 2.3a). A length of 0.2 mm silver wire was inserted into the abdomen to serve as an indifferent electrode. In the case of the crickets, the main nerve of the recorded leg was sectioned proximal to the coxa. This was found to be necessary to reduce the background nervous activity picked up by the electrode, but was not necessary in the case of the bushcrickets.

Fig. 2.3

(A) Insect stand with preparation waxed in experimental position.

E - recording electrode

H.S. - headstage

I - indifferent electrode

(B) Prothoracic leg of G. campestris showing main leg nerve branches and position of electrode placement (redrawn from Esch et al. 1978).

A.T. - acoustic trachea

M.B. - motor branch

M.L.N. - main leg nerve

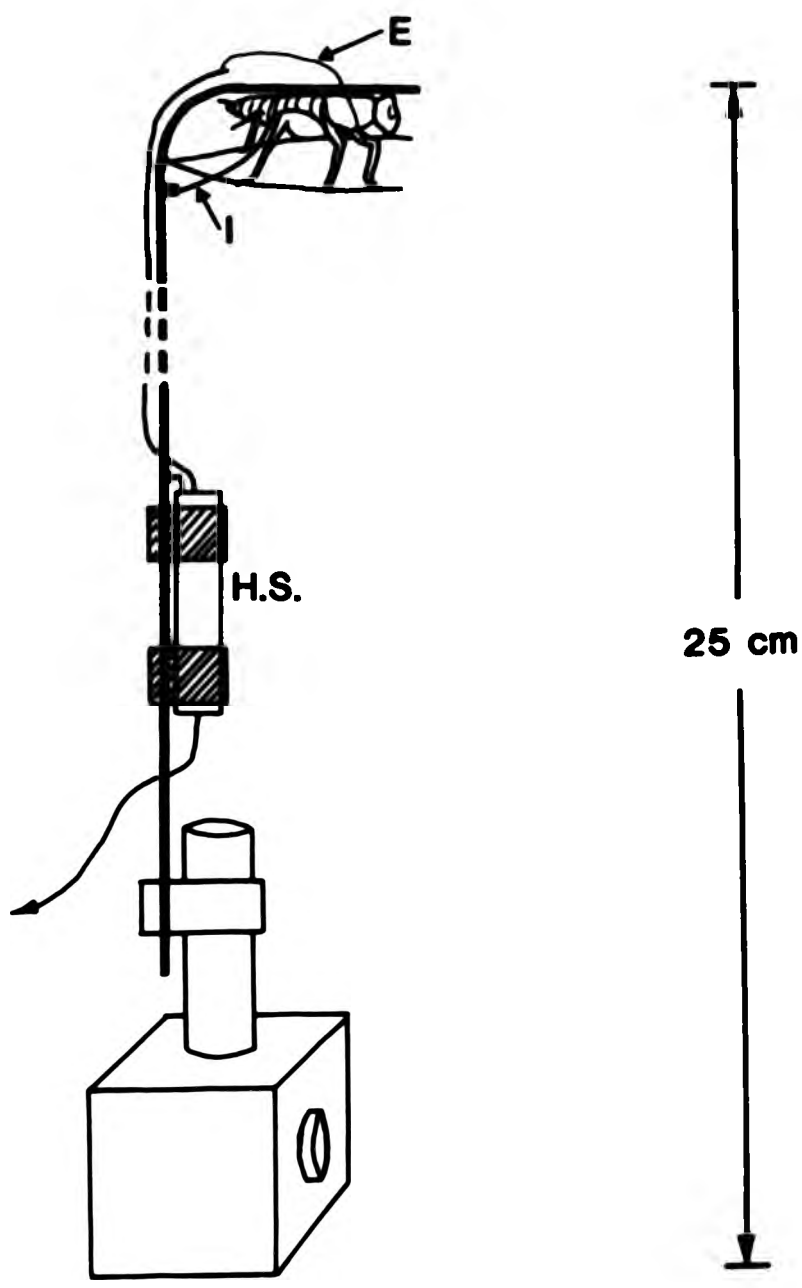
S.B. - sensory branch

S.O. - subgenual organ

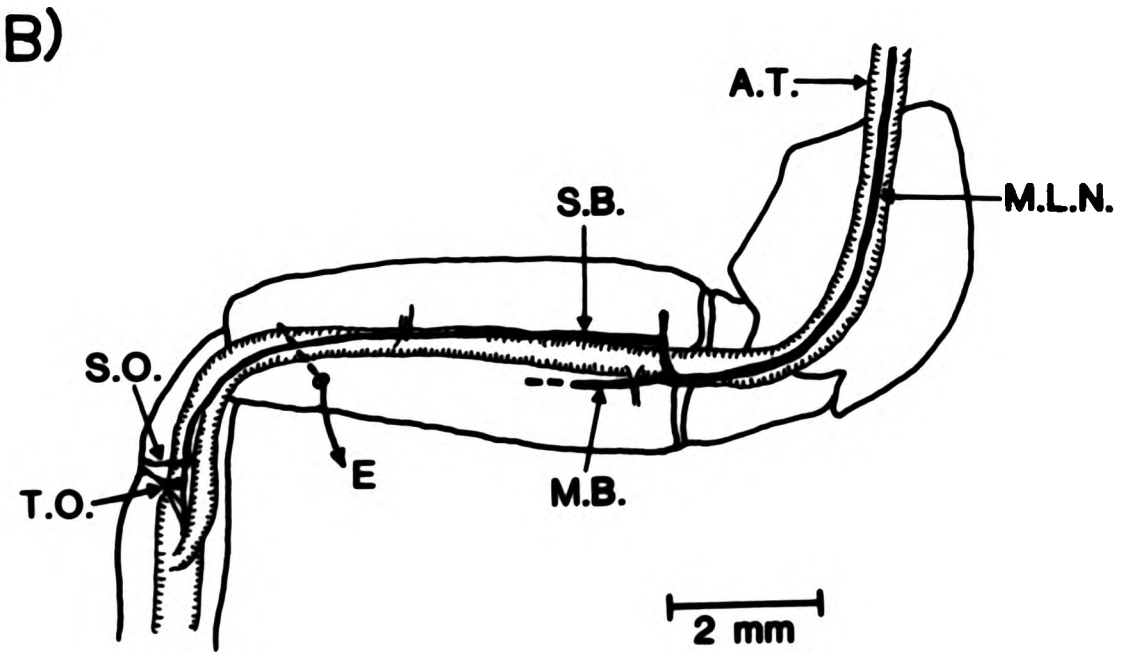
T.O. - tympanal organ

The electrode E was inserted through a hole in the cuticle to lie across the sensory branch of the leg nerve.

(A)



(B)



A small hole was then made in the femur of one of the prothoracic legs, using a 30-gauge syringe needle. An electrode consisting of a short length of 1/500" uncoated silver wire (Clark Electromedical Instruments) was then inserted through the hole to lie as close as possible to the sensory branch of the leg nerve. The other end of the elecrode wire was soldered to an insulated wire leading to the headstage (Fig. 2.3a).

In the cricket G. campestris the main leg nerve of the prothoracic ganglion splits into two main branches in the proximal femur, one branch being predominantly motor and the other sensory (Eibl & Huber, 1979). The latter runs along the dorsal surface of the trachea in the femur, and then passes anteriorly to the ventral surface of the leg proximal to the femoro-tibial (genual) joint (Fig. 2.3b). The bushcricket has a tympanal nerve that is discrete along its entire length from auditory organ to prothoracic ganglion (Schumacher 1973). This tympanal nerve, like the sensory branch of the leg nerve in the cricket, runs along the femur dorsal to the trachea. With knowledge of this anatomy in mind several positions of electrode placement were attempted in order to determine the optimum position for recording the nervous activity: this is shown in Fig. 2.3b.

2.2.5 EXPERIMENTAL PROTOCOL

(A) Analysis of Species Song

Recordings of the song produced by T. cantans males were made using a ¼" condenser microphone (B & K 4135) in

conjunction with a measuring amplifier (B & K 2107) and an instrumentation recorder (Racal Store 7). Several specimens were placed together in a cage 25 x 20 x 40 cm, constructed of muslin supported by thin wire over a 35 mm thick foam rubber base, and this was placed at the centre of the anechoic chamber. The microphone was hand-held, usually within 10 cm of a singing insect. The recordings were made at 15 i.p.s. in direct record (DR) mode (frequency response 250 Hz - 75 kHz, ±3 dB). The recording level was set to give as good a signal to noise ratio as possible, but avoiding any overloading which could introduce false harmonics into the recorded song spectrum.

The songs were later analysed in terms of spectral and temporal pattern using a high resolution signal analyser (B & K 2023). The spectra were plotted on paper using an X-Y plotter (Gould 3054) and photographs were made of the temporal patterns displayed on a storage oscilloscope (Tektronix S13).

The crickets G. campestris and T. oceanicus both have three distinct songs: calling, aggression and courtship. Analysis of these was carried out in the manner described for T. cantans.

(B) Biophysical Measurements

There are two sites of possible sound entry into the auditory system on each side of the bushcricket: the large acoustic spiracles on the prothorax and the tympanal slits on the tibiae of the forelegs (Zeuner 1936; Lewis 1974; Bailey & Stephen 1978). Measurements of the diffraction of

sound by the insect body were made for 5 specimens, waxed to the wire stand, after they had been used for physiological experiments. A 1 cm length of 2 mm diameter plastic tubing, acting as a probe, attached to a ⅛" condenser microphone (B & K 4138) was used (Fig. 2.4a), in conjunction with a signal analyser (B & K 2107). The probe was positioned vertically, with its opening next to the acoustic spiracle on the thorax. Polar plots of sound pressure were made (B & K Level Recorder 2305) at a number of pure-tone frequencies between 5 and 40 kHz, as the loudspeaker was rotated through 360°.

Sound pressure at the spiracle was also determined using an alternative method, in case the probe, which was positioned about 1 mm away from the spiracle, had recorded sound reflected from the body surface. A 1 mm metal probe (constructed in the laboratory), attached to a ¼" condenser microphone (B & K 4135), was inserted through a specimen from one side so that its opening was flush with the body surface at the opposite spiracle (Fig. 2.4b). Pressure levels were then measured at several frequencies, with the loudspeaker only in the ipsilateral position.

For each of these diffraction determinations the results were compared with records taken with the insect stand positioned normally, but without the insect present, so that any diffraction by the stand could be accounted for.

Sound diffraction by the body of the cricket G. campestris was determined as for the diffraction around T. cantans, using the first method described, except that only diffraction at 5 kHz, the carrier frequency of the

Fig. 2.4

Two methods for measurement of sound pressure levels at the spiracle of T. cantans.

In (A) a plastic probe was positioned vertically with its opening close to the acoustic spiracle.

In (B) a metal probe was passed through the thorax so that its opening was flush with that of the spiracle.

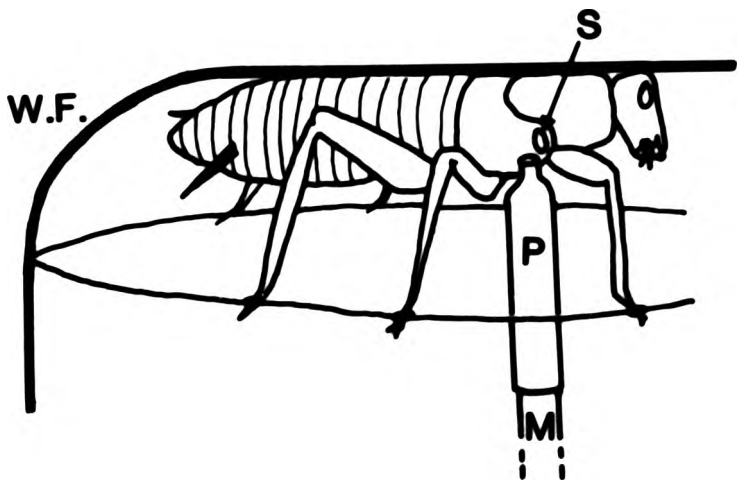
M - microphone

P - probe

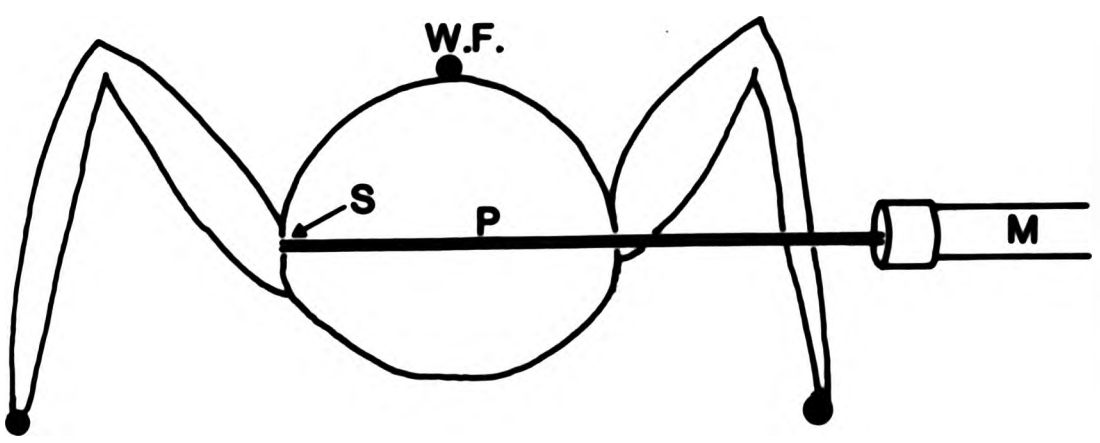
S - spiracle

W.F. - wire frame

(A)



(B)



calling song, was examined. The probe was positioned, in this case, near the posterior tympanum on one of the forelegs or near one of the acoustic spiracles.

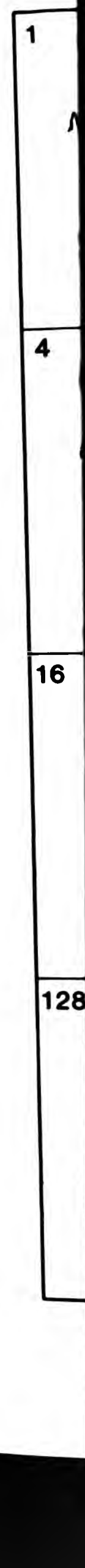
(C) Neural Responses

The neural activity recorded in response to a single tone burst was usually barely discernible above background activity, especially in the crickets, and it was therefore necessary to average the responses to many presentations of each stimulus type. Fig. 2.5 shows the response to a single stimulus and averaged responses of multiple (4-256) presentations in T. cantans. The clearest response outline is achieved with the maximum number of stimulus presentations, but time was limited as several procedures were usually to be tested. 128 presentations was the best compromise between time spent and clarity of the response. 25 ms stimuli were presented at a rate of 10 per second, which produced negligible adaptation. Fig. 2.6 shows the response, averaged over 128 presentations, to an identical stimulus presented at rates of 1, 3.5, and 10 per second, also for T. cantans. Very little difference can be seen between these responses.

Thresholds of nervous response were determined for sound frequencies over the range 1 - 40 kHz in both the bushcrickets and the crickets. The threshold at each frequency was taken as the intensity where the response was just discernible above noise level after averaging 128 presentations. It is appreciated, however, that there may have been nervous activity a few dB below the values

Fig. 2.5

Traces of responses to 5 kHz, 75 dB, recorded in G. campestris, averaged over the number of presentations given. The vertical scale was adjusted to show the waveforms to best advantage.



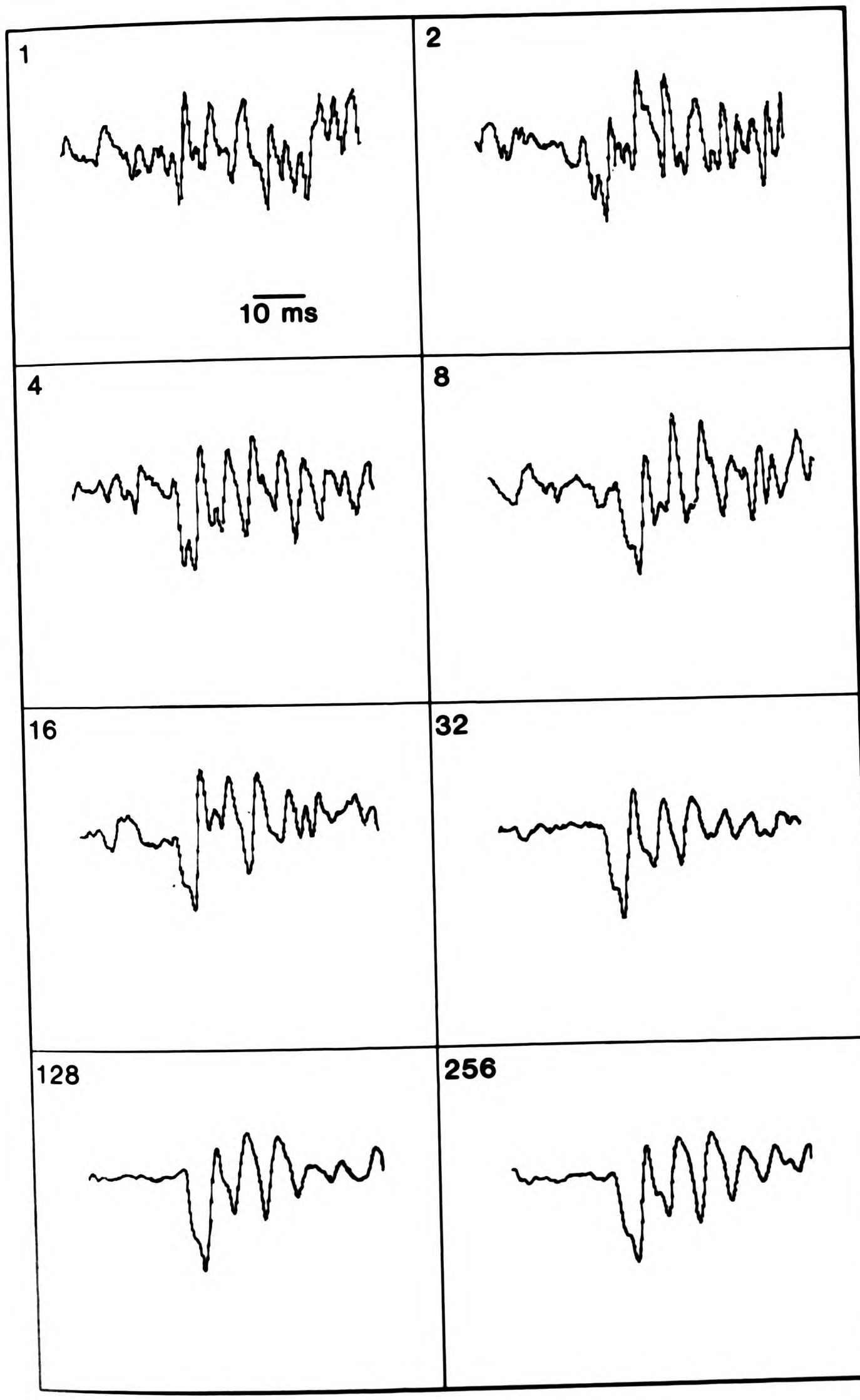
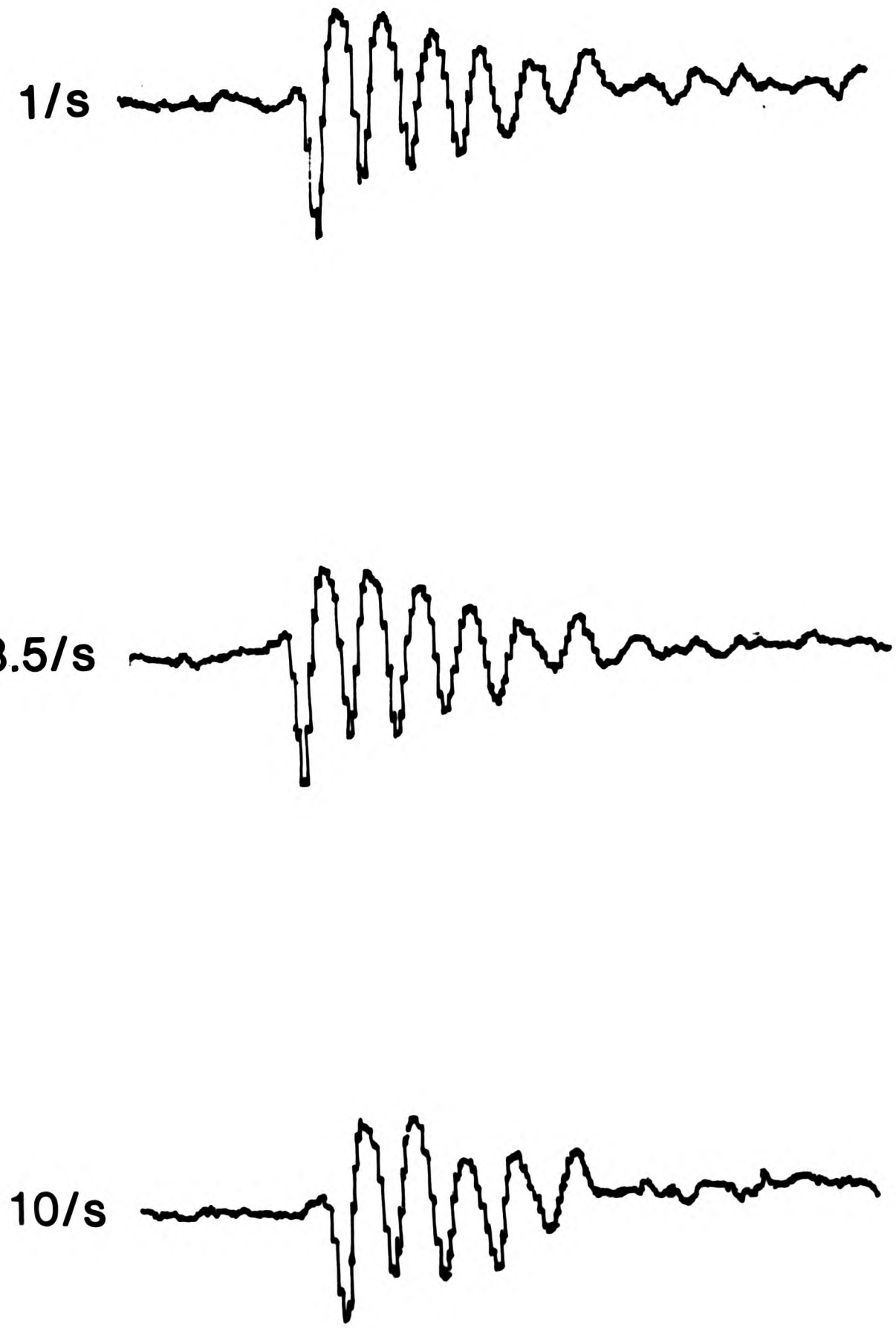

1
2
10 ms
4
8
16
32
128
256

Fig. 2.6

Traces of responses to 5 kHz 70 dB, recorded in G. campestris, averaged over 128 presentations. The 25 ms stimuli were presented at the rates given. Very little adaptation was evident at the highest presentation rate.


1/s
3.5/s
10/s

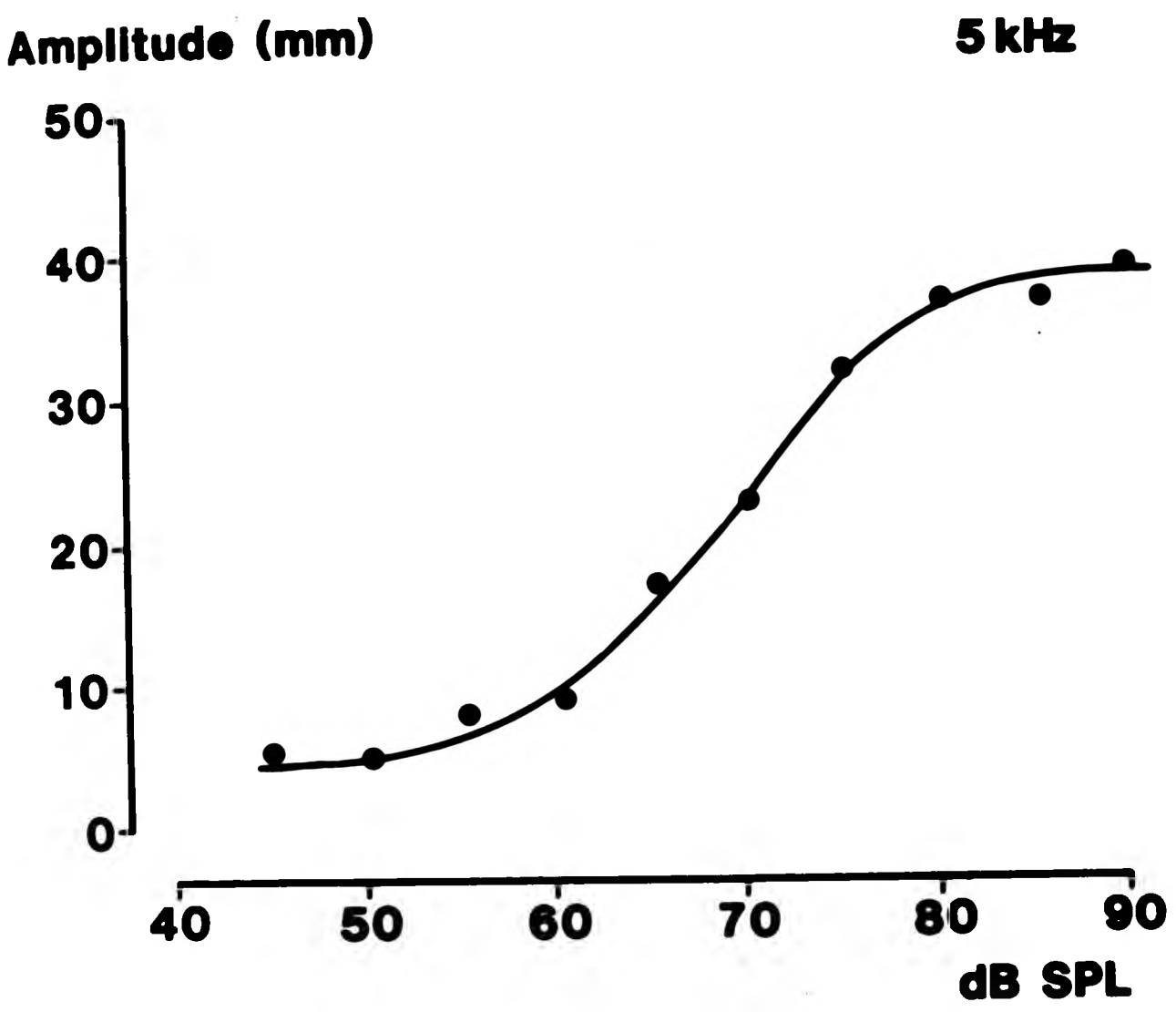
recorded as thresholds.

Directionality of the auditory organ was determined over a range of frequencies from 5 to 40 kHz in T. cantans by comparing the responses in the auditory nerve to selected pure-tone frequencies incident from several angles around the specimen in the horizontal plane. Investigations into the directionality of the cricket auditory organs were confined to frequencies around the carrier frequency of the species calling songs (around 5 kHz for G. campestris). At each frequency the response to the stimulus was first measured at a variety of intensities, with the loudspeaker ipsilateral to the recorded ear. The maximum peak-to-peak vertical amplitudes of the responses printed out by the chart recorder were measured for each intensity and plotted against the free field sound intensity to produce an intensity-response curve for that frequency (Fig. 2.7). The amplification of the signal was arbitrarily set on the AC/DC amplifier, at the beginning of each experiment, to provide an easily measurable response on the chart recorder paper. This setting was then maintained for the total duration of the experiment.

After taking measurements for an intensity response curve at a given frequency an intensity was chosen of some 20-30 dB above ipsilateral threshold and the response to this intensity was measured at several loudspeaker positions around the preparation - usually every 20° or 30°. The maximum peak-to-peak amplitude of each of these responses was then converted to effective dB using the intensity response curve and these values were later plotted on polar

Fig. 2.7

Intensity-response curve produced at 5 kHz in G. campestris with the loudspeaker ipsilateral to the recorded ear. Amplitudes measured were maximum peak-to-peak values of the chart recorder traces.


Amplitude (mm)
5 kHz
50
40
30
20
10
0
40
50
60
70
80
90
dB SPL

graph paper as dB relative to the anterior (0°) position.

Quantification of responses recorded in this way (which are compound action potentials) has been carried out in a number of ways. Bailey & Stephen (1978) measured the total length of averaged response outlines, claiming this measurement to be linearly proportional to the Fourier analysis of the power of the response. Lewis et al. (1975) summated the amplitudes of constituent peaks of responses to single stimuli. Both of these methods take far longer than measuring the maximum peak-to-peak amplitude of the whole response. Comparison between the methods was made by selecting 20 typical response plots of a range of sizes and plotting total response outline against peak-to-peak amplitude and against the summed amplitudes of the constituent peaks (Fig. 2.8). A straight line relationship was found in both cases, validating the use of maximum peak-to-peak amplitude.

Subsequent to establishing the directionality of the auditory organ in intact specimens an attempt was made, in T. cantans, to determine whether the acoustic spiracles or the tympanal slits (or both) was the principal site of sound entry into the auditory system, and which was the more important in providing directionality. Thresholds and directionality plots were produced for each insect over the frequency range 1 - 40 kHz (1) in the intact state, and either (2) after placing a sleeve of plastic tubing, sealed at both ends with wax, around the tibia of the recorded ear (to block sound entry to the tympanal slits), or (3) after blocking the ipsilateral acoustic spiracle with wax.

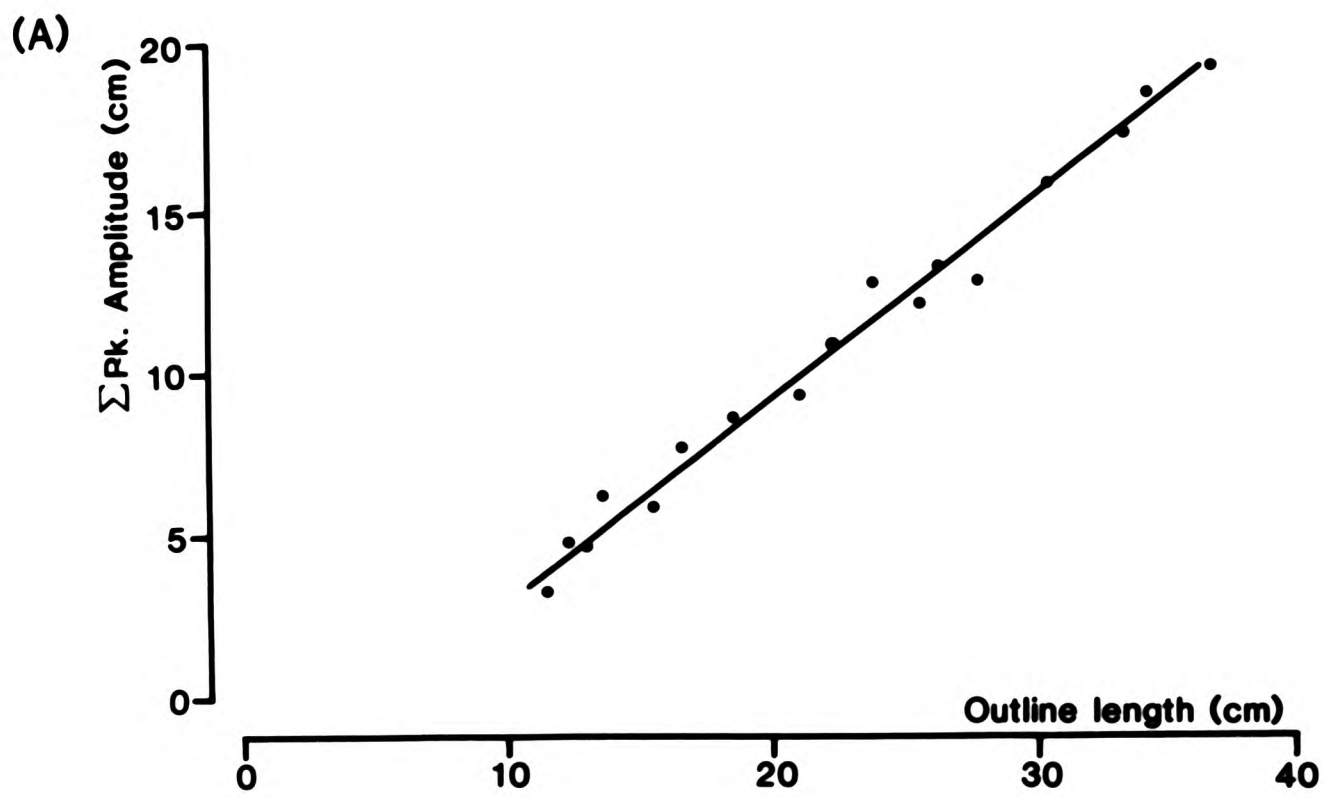
Fig. 2.8

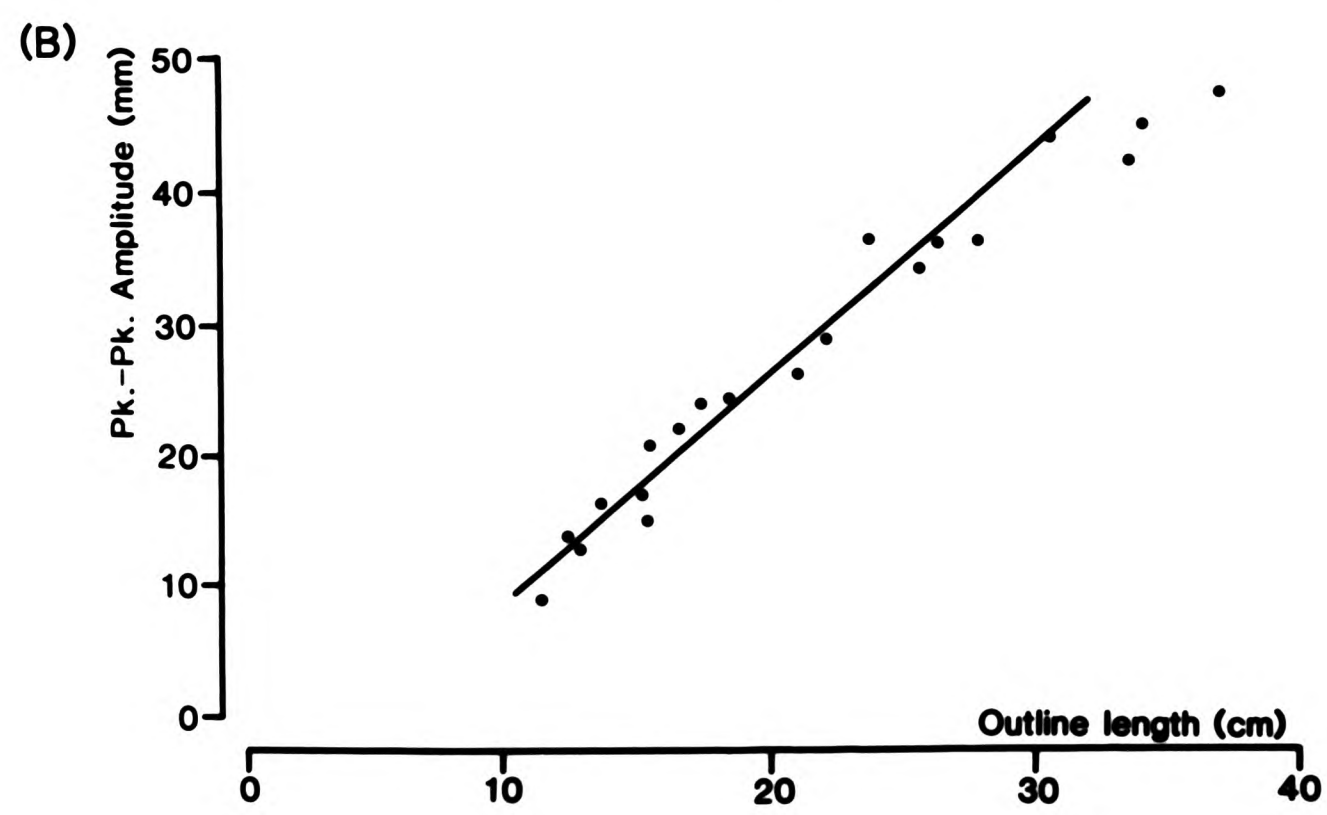
Comparison of outline lengths of 20 response traces of different sizes with (A) the summed amplitudes of the constituent peaks, and (B) the maximum peak-to-peak amplitudes of the whole response traces.

(A)

(B)

rent


(A)
ΣPk. Amplitude (cm)
0
5
10
15
20
Outline length (cm)
0
10
20
30
40


(B)
Pk.-Pk. Amplitude (mm)
0
10
20
30
40
50
Outline length (cm)
0
10
20
30
40

For the crickets, a similar attempt was made to find the main site(s) of sound entry into the auditory system. Unlike the bushcrickets, however, the tympana of crickets are exposed directly to the air. The left and right auditory systems are acoustically coupled by a trachea traversing the body in the prothorax , and so contralateral as well as ipsilateral inputs were investigated. Manipulations involved blocking tympana and spiracles with wax and determining resultant changes in directionality or overall sensitivity. Additional details of procedure are given with the results.

2.3 RESULTS

2.3.1 BUSHCRICKETS

(A) Song Analysis

Fig. 2.9 shows the temporal pattern and spectrum of the calling song of T. cantans. The insect produces a chirp of about 1-4 seconds duration which consists of syllables of some 12 ms repeated at a rate of about 40/s. Two syllables are shown, each composed of two hemisyllables; the major ones are produced by wing closure and the minor ones by wing opening. The spectral analysis shows the song to be broad-banded, between 8 and about 60 kHz, and with peaks at 8-12 kHz, 20 kHz and 30-40 kHz. The traces below 8 kHz and above 60 kHz are at background noise level. There is also a low frequency, low amplitude, component around 4 kHz, corresponding to the tooth impact rate, but this is not clear in the spectrum shown.

(B) Biophysical Measurements

The diffraction of sound by the body of the insects was investigated in 5 specimens. A series of polar plots showing the pattern of sound diffraction around a representative specimen of T. cantans is shown in Fig. 2.10, for a range of frequencies from 5 to 40 kHz. At 5 kHz there is practically no diffraction, but at 10 kHz a small ipsilateral augmentation in the sound pressure is evident, maximal at 90° (about 4 dB relative to the free field), and there is a slight decrease in intensity of 2-3 dB at some

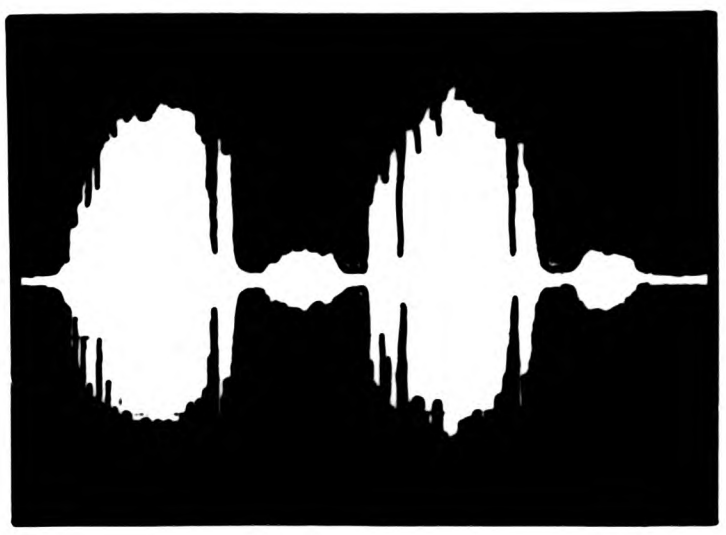
Fig. 2.9

Analysis of T.cantans song.

(A) Oscillogram showing the temporal structure of the calling song. Two syllables are shown, each composed of two hemisyllables. The larger were produced during wing closure and the smaller during wing opening.

(B) Spectral pattern of the song, over the range 1 - 80 kHz.

(A)



20 ms

(B)

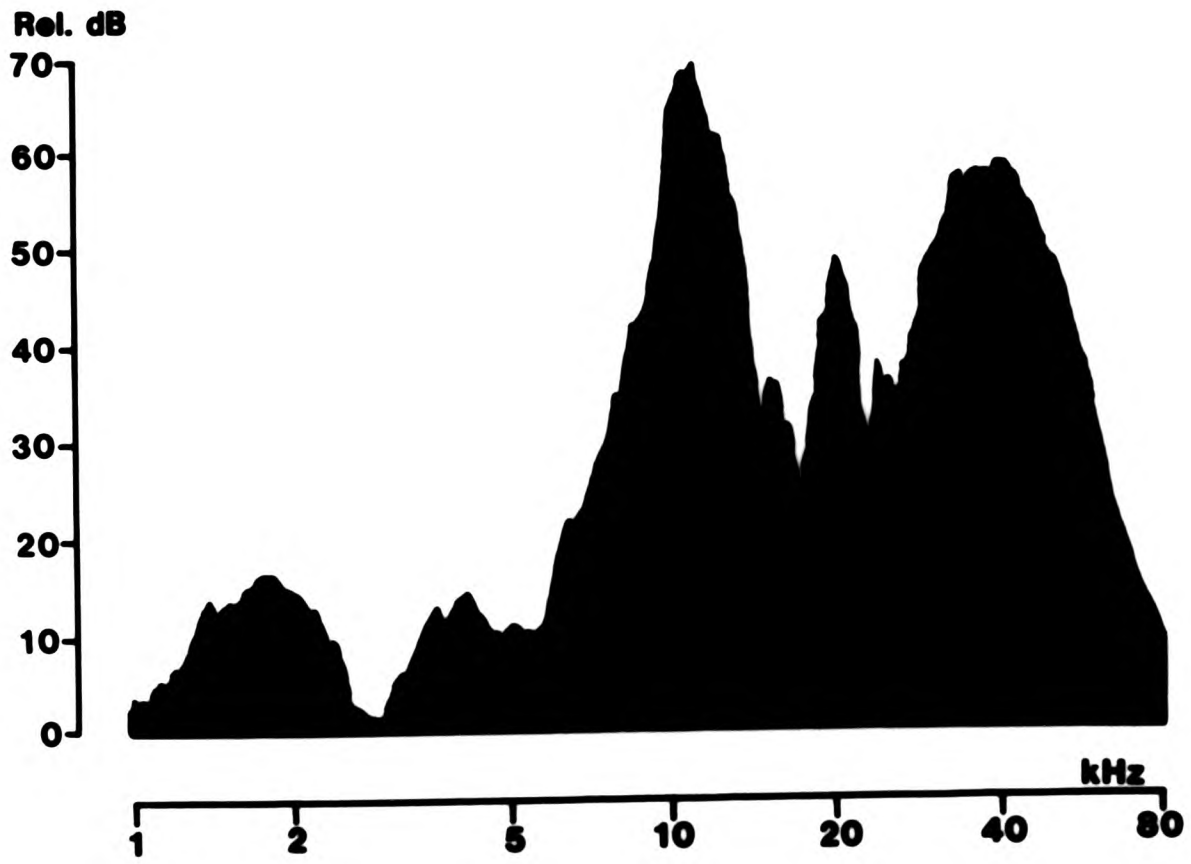
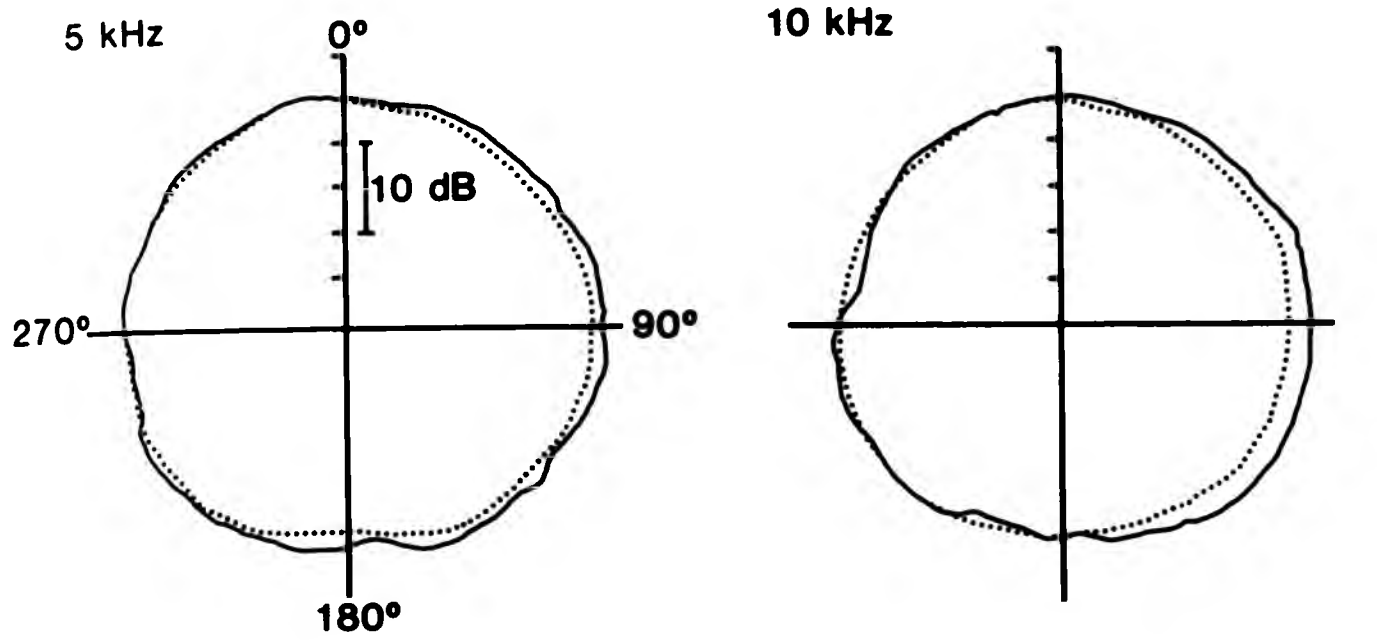
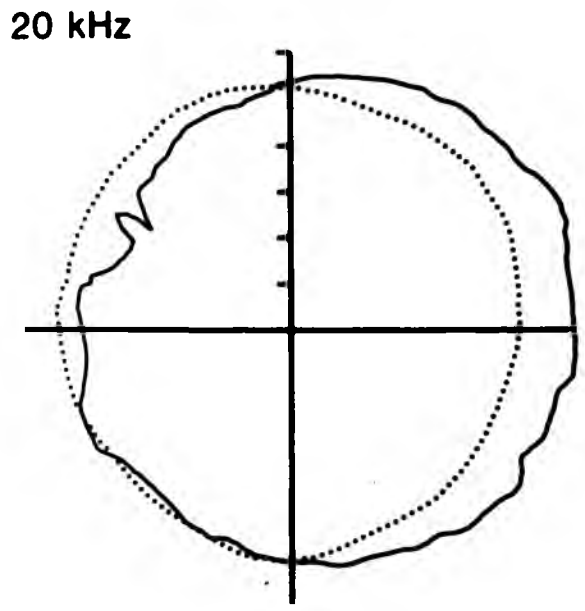


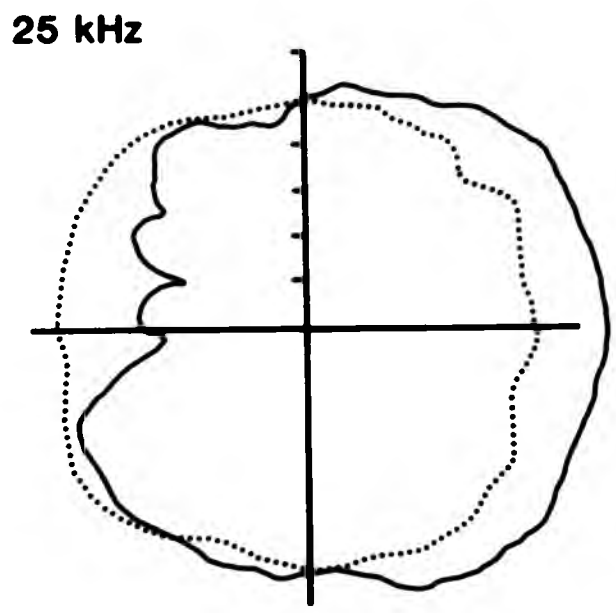
Fig. 2.10

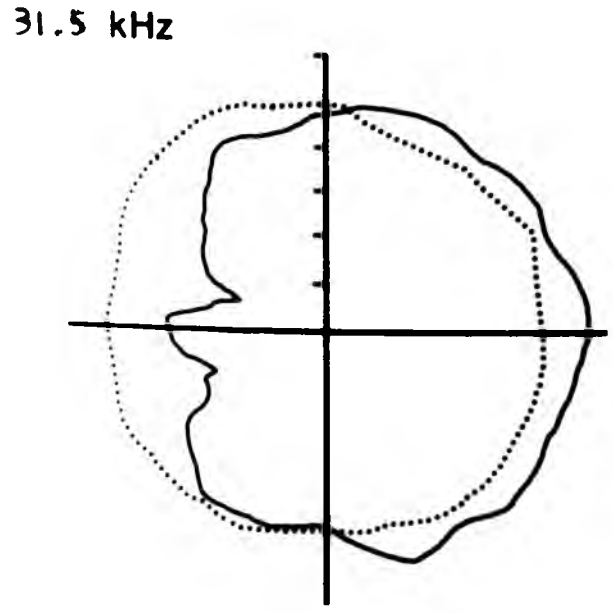
Diffraction of sound around the body of T. cantans, at the frequencies given. Solid lines = sound pressure measured at the spiracle as the sound source was moved in the horizontal plane. The dotted lines show the variation in sound pressure in the presence of the stand alone. In each case 0° represents anterior and 90° the position ipsilateral to the spiracle.

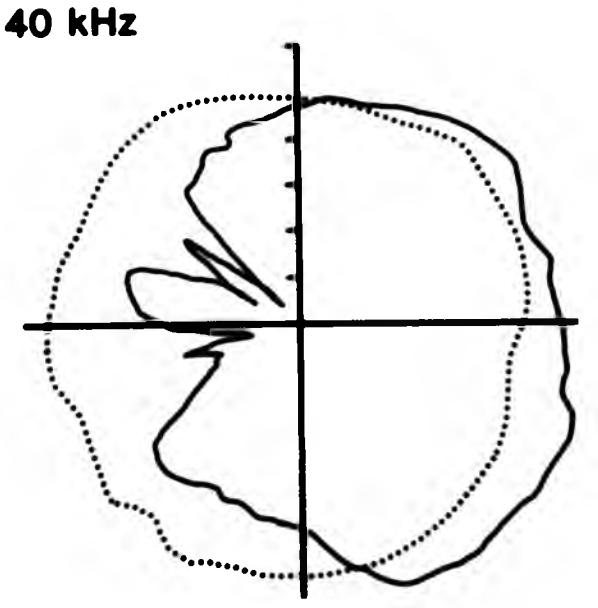

5 kHz
0°
10 dB
270°
90°
180°

10 kHz


20 kHz


25 kHz


31.5 kHz


40 kHz

contralateral positions. At 20 kHz the ipsilateral augmentation has increased to about 7 dB and the maximum contralateral "sound shadow" is about 5 dB. Ipsilateral augmentation reaches a maximum at 25 kHz (10 dB at 90°), and at this frequency sharp dips or "nulls" of some 15-20 dB start to appear on the contralateral side. At 31.5 and 40 kHz the ipsilateral augmentation decreases slightly while the contralateral dips increase greatly becoming maximal at 40 kHz (over the range investigated). All these trends were consistent in all specimens tested.

The dotted lines in Fig. 2.10 represent the sound pressure levels measured without the insect present, but with the microphone and probe tubing in the same position relative to the wire stand. Slight irregularities of up to 2-3 dB occurred at some positions, particularly at the higher frequencies. These may be interpreted as diffraction by the wire stand or irregularities of the anechoic chamber, but may also have been due to peculiarities in sound admission of the tubing attached to the microphone. These irregularities were very slightly greater than those produced by the anechoic chamber, but their contributions towards the diffraction patterns shown will be minimal.

It was considered a possibility that the pronounced ipsilateral augmentation of sound pressure, observed around 25 kHz, may have been caused by measuring sound reflected by the body, as the sound pressure was actually measured about 1 mm away from the spiracle. Therefore a second method of measuring the diffraction was used to check for this possibility. The sound pressure at the spiracle, relative

Fig. 2.11

Comparison of the two methods of measuring diffraction around T. cantans (cf Fig. 2.4). Each point represents the difference between the sound pressure at the spiracle and that of the free field, over the 5 - 40 kHz frequency range, measured with the loudspeaker ipsilateral to the spiracle. O——O = measurements made using a vertically positioned probe (mean of 5 preparations); ●····● = measurements made using a probe passed through the thorax to emerge flush with the spiracle (n = 1).

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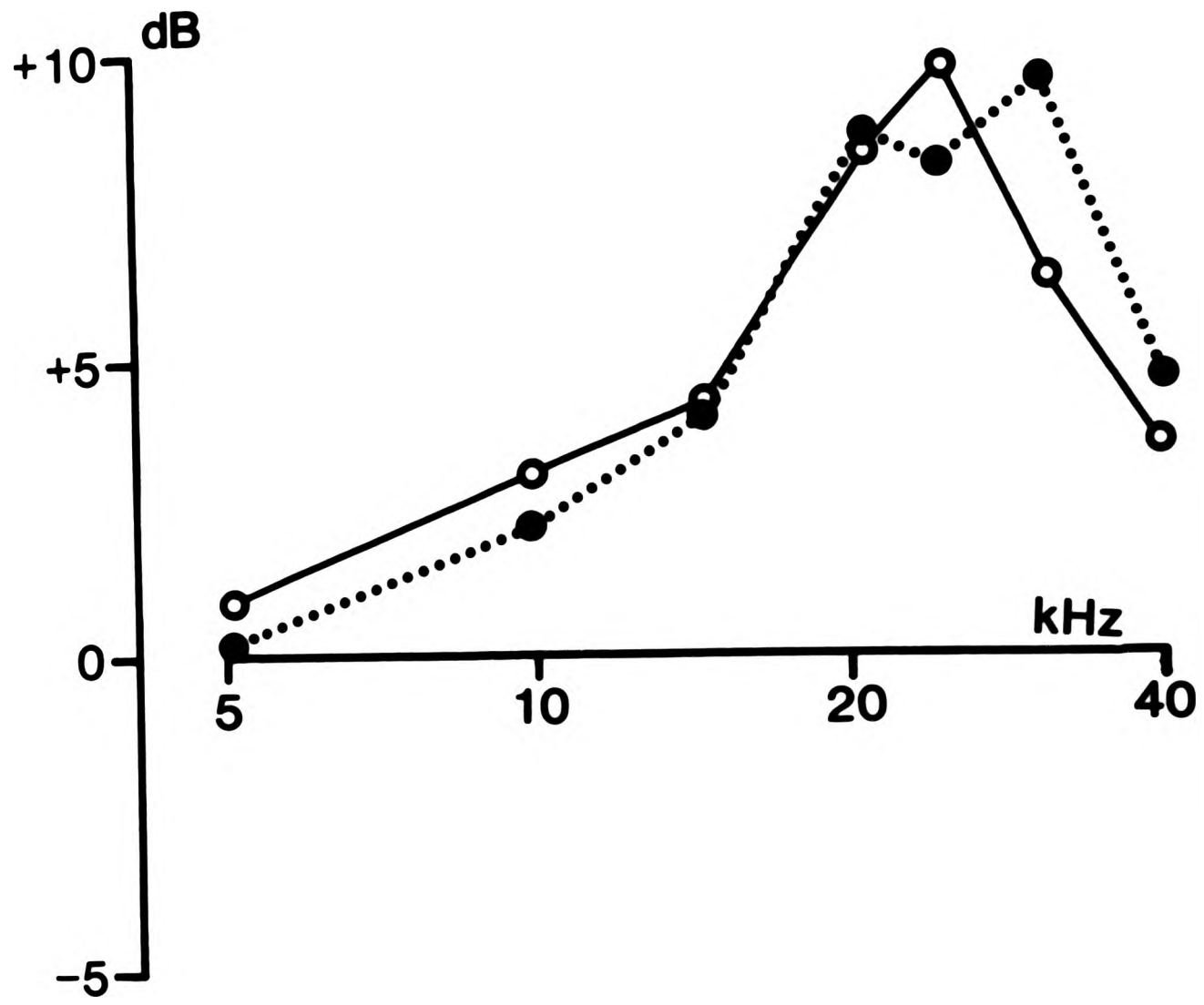


to the free field, was measured at frequencies from 5 to 40 kHz, using a metal probe inserted through the body to emerge at the opposite spiracle flush with the body surface (Fig. 2.4b). Sound pressures were measured for sound presented ipsilaterally only. The results are shown in Fig. 2.11. Augmentation of the sound levels relative to the free field were found, as by the original method, which was maximal around 25 kHz. The results are compared with measurements of the 90° position taken from the polar plots constructed in experiments on 5 specimens, and were very similar. This was considered to validate the results for the polar plot constructions.

(C) Neural Responses

(i) Intact State Thresholds

A typical threshold curve of the total auditory response in T. cantans is shown in Fig. 2.12. Measurements from all the insects tested showed that the auditory organ is most sensitive to 20-25 kHz sound. The threshold sound pressure at the most sensitive frequencies was usually around 20-25 dB SPL.

(ii) The Effects of Tympanal Slit and Spiracle Blockage on Thresholds

The effects of preventing sound entry through the ipsilateral tympanal slits or through the ipsilateral acoustic spiracle are shown in Fig. 2.12. Sound entry via the tympanal slits was prevented by fitting a plastic sleeve around the tibia and spiracular sound entry was prevented by

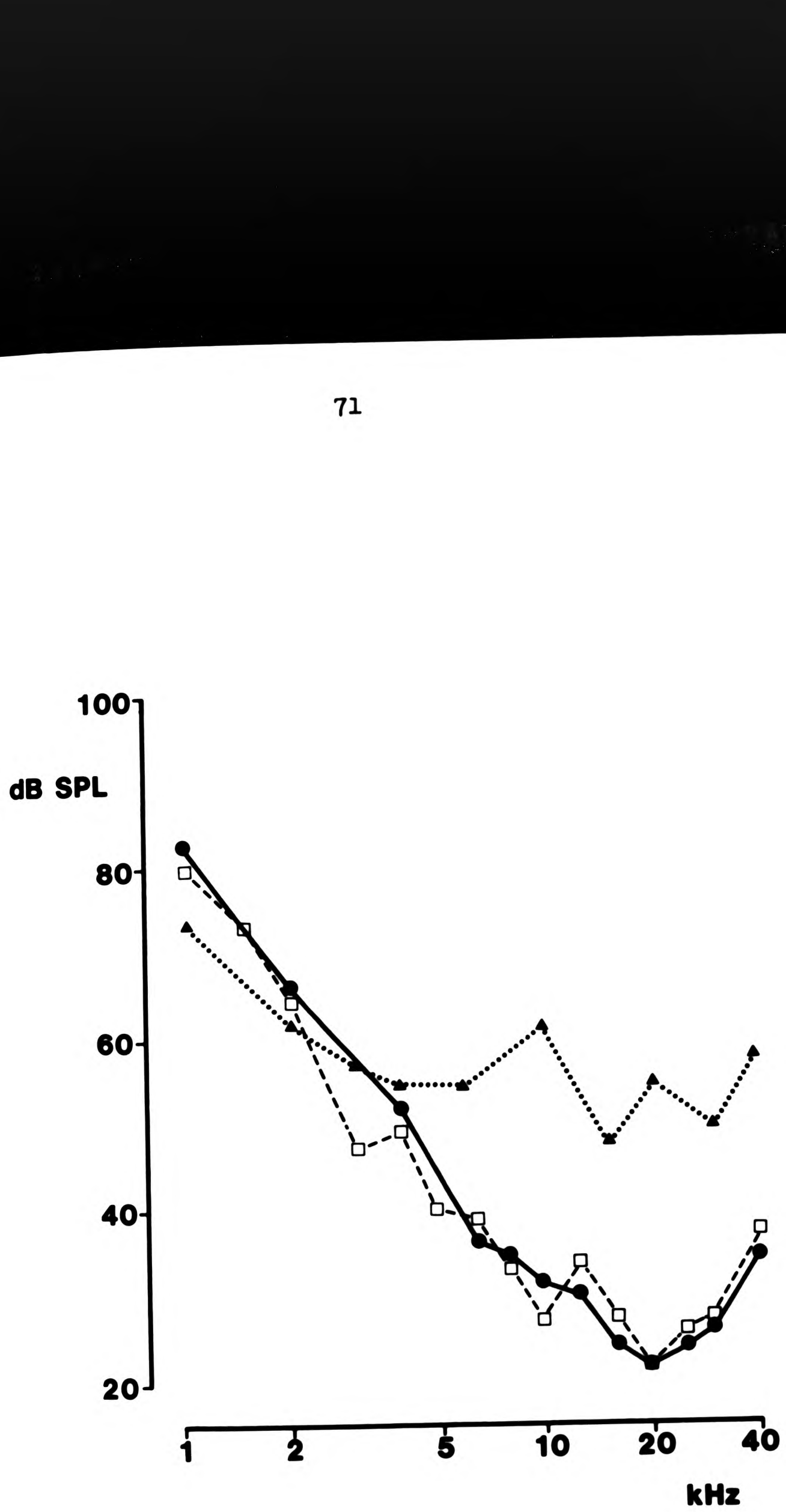
Fig. 2.12

Thresholds, to ipsilaterally presented sound, measured from the tympanal nerve of one specimen of T. cantans.

●——● = intact.

□- - □ = sleeve fitted around tibia.

▲····▲ = acoustic spiracle blocked.


100
dB SPL
80
60
40
20
1
2
5
10
20
40
kHz

blocking the spiracle with wax. Application of the tibial sleeve did not appreciably affect the thresholds. This test was carried out on 4 specimens. The ipsilateral spiracle was blocked in 1 specimen and decreased the sensitivity by up to 35 dB, the maximum decrease being around 20 kHz.

(iii) Directional Responses in the Intact State

Fig. 2.13 shows a typical series of directional responses to frequencies from 5 to 40 kHz. Responses are shown as dB relative to 0° (anterior). This is because it is assumed that the two auditory organs have mirror-image response patterns overlapping anteriorly (and posteriorly). 90° represents the ipsilateral position. At 5 kHz and 10 kHz the maximum left-right (L-R) diffference was always around 5 dB. Above 10 kHz L-R difference gradually increases both as a result of ipsilateral increase and contralateral decrease of the response. Maximum L-R difference may be used as a rough quantitative measure of directionality, and is used here as the difference, in dB, between the maximum response on the ipsilateral side and the minimum response on the contralateral side (not necessarily at exactly 90° and 270°). Ipsilateral response augmentation develops to a maximum of about 8 dB around 25 kHz, above which it gradually decreases. The contralateral decrease develops smoothly up to 20-25 kHz, above which sharp dips in the response patterns begin to occur.

Fig. 2.13

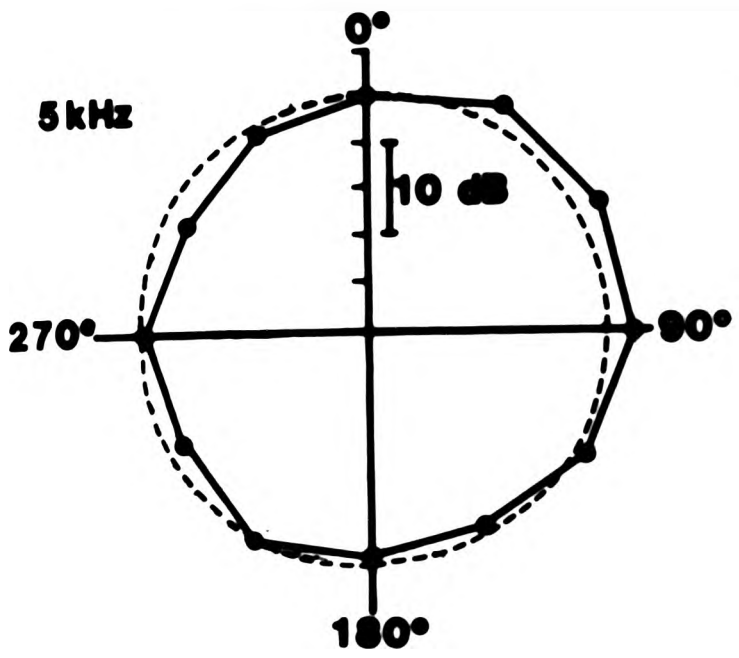
Directional variation in responses in T. cantans at the frequencies given. Responses are plotted as dB relative to the response at 0° (dashed line). In each case 0° represents anterior and 90° the position ipsilateral to the recorded ear.

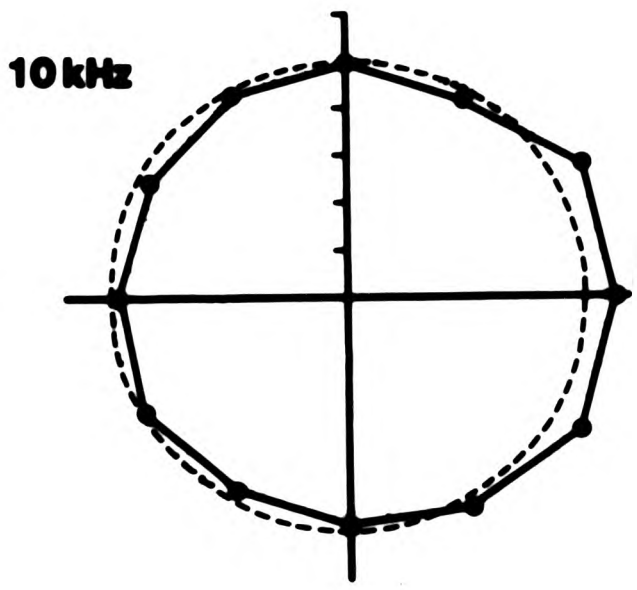
5k

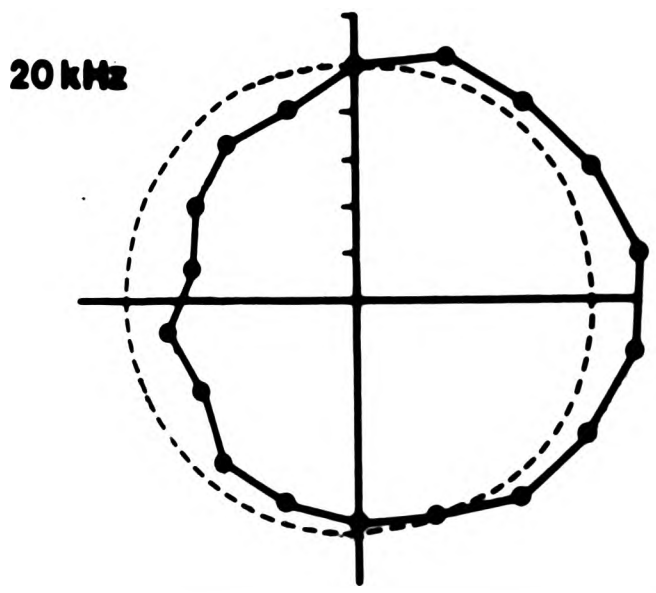
270°

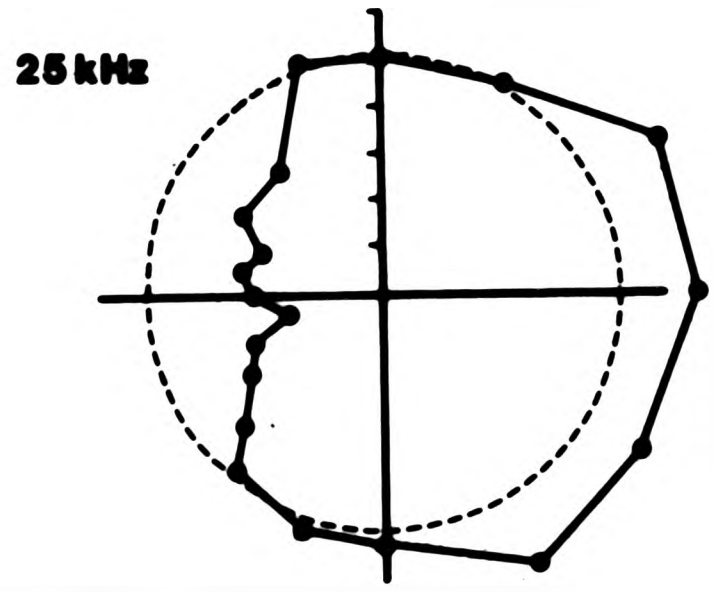
20k

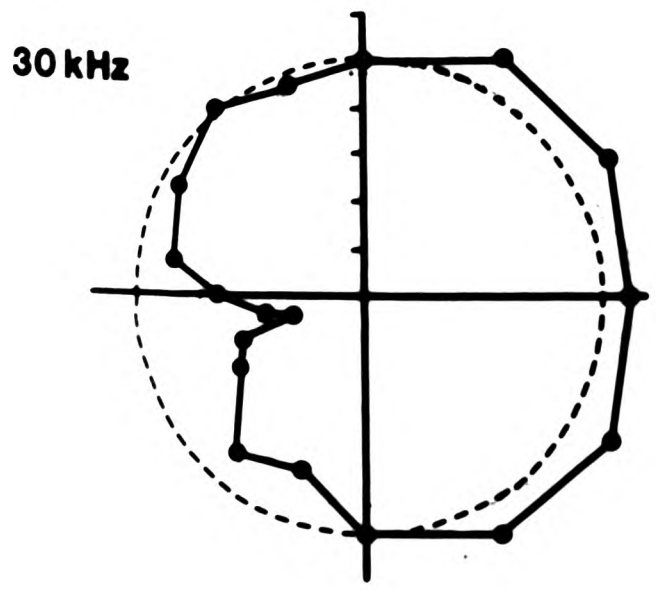
30k

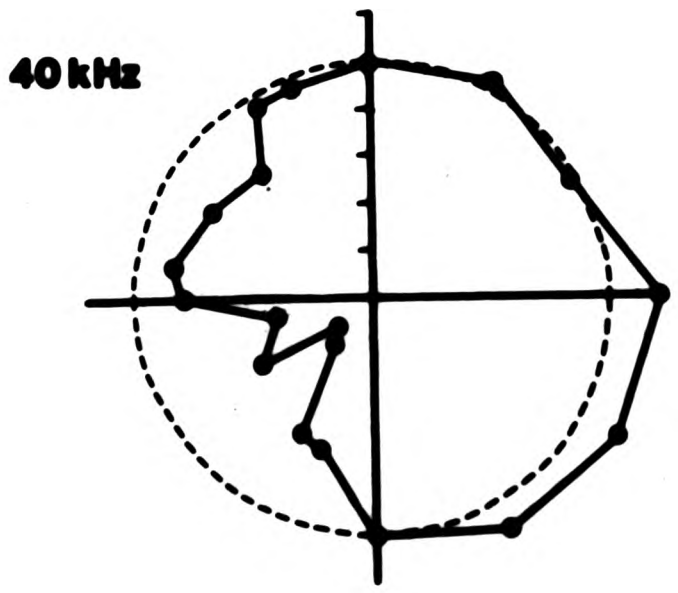

0°
5 kHz
10 dB
270°
90°
180°


10 kHz


20 kHz


25 kHz


30 kHz


40 kHz

(iv) The Effects of Tympanal Slit and Spiracle Blockage on Directionality

The effect of the tibial sleeve application on directionality is shown in Fig. 2.14. In no case was directionality appreciably changed after blockage of the sound input at the tympanal slits by this method (tested in 5 specimens). Conversely, blockage of the acoustic spiracle (1 specimen) caused a very large reduction in directionality, as shown in Fig. 2.14. Maximum L-R difference at 25 kHz was originally about 20 dB. This decreased to 7 dB when the spiracle was blocked, and increased again to 17 dB after clearance of the spiracle.

A few representative plots of sound diffraction patterns are compared with neural response plots in Fig. 2.15 for 10, 20, and 30 kHz. The similarities are clear, although the details of the contralateral minima in the neural responses may not exactly correlate with those in the diffraction patterns as they were not derived from the same specimen. Precise details of minima varied between individual specimens.

2.3.2 CRICKETS

(A) Song Analysis

Fig. 2.16 shows oscillograms of the temporal patterns of the calling, aggression and courtship songs of G. campestris. The calling song consists of repeated chirps of usually between 3 and 6 syllables, each of about 20 ms duration which, together with a similar inter-syllable interval,

Fig. 2.14

Upper: the effect, on directionality at 30 kHz, of blocking sound entry to the tibia of the recorded ear.

Lower: the effect, on directionality at 25 kHz, of blocking the ipsilateral acoustic spiracle with wax.

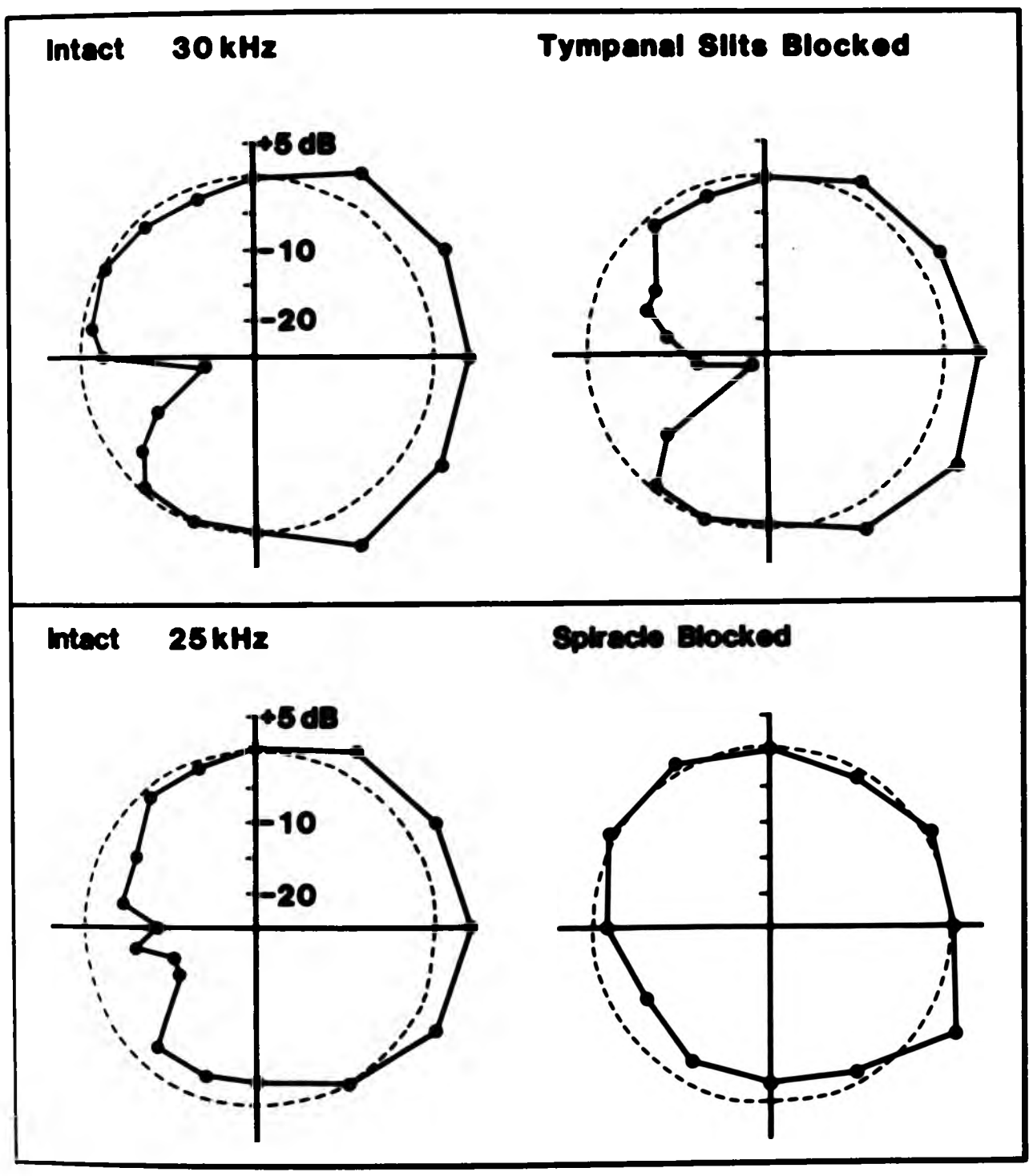
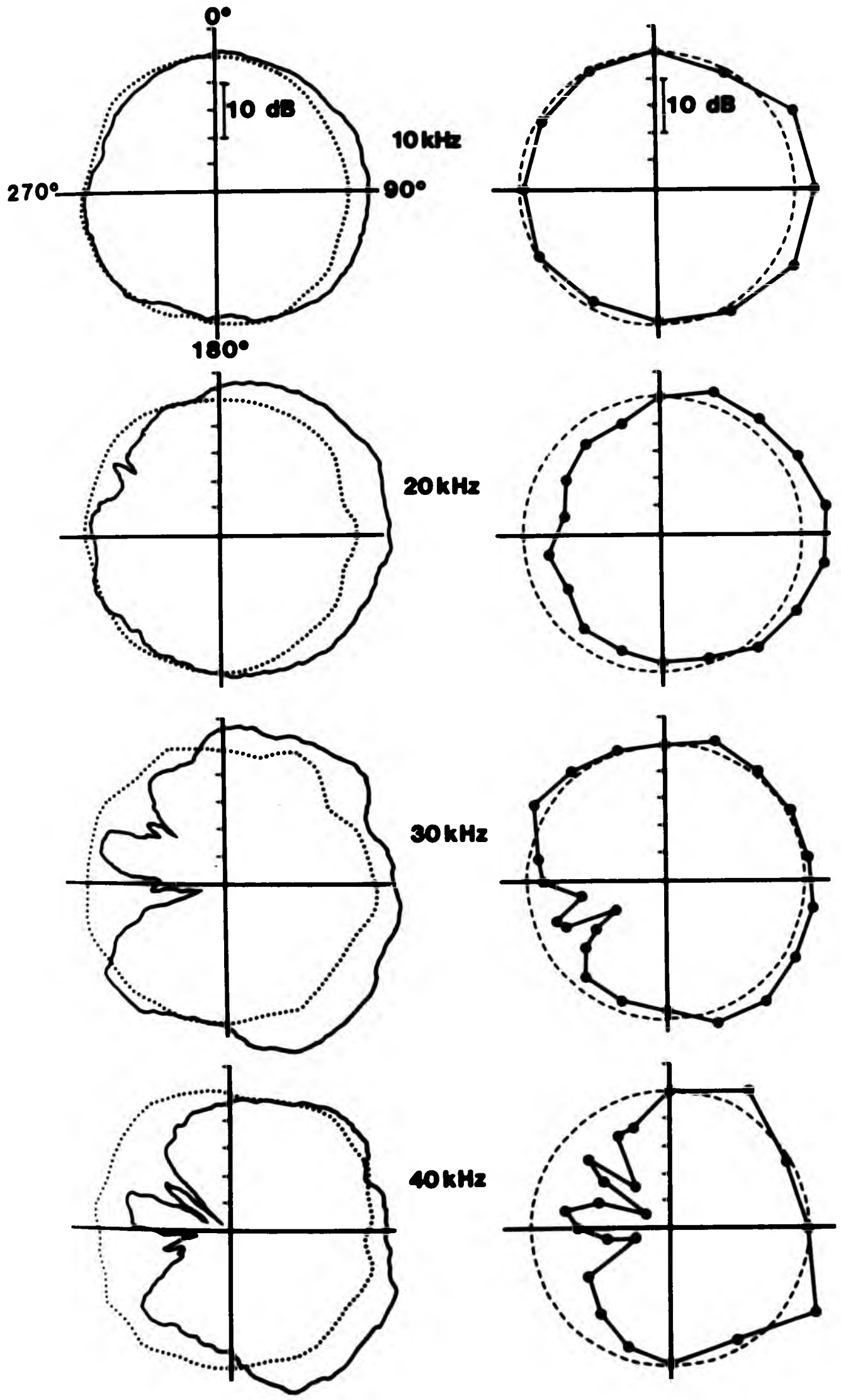

Intact 30 kHz
Tympanal Slits Blocked
+5 dB
-10
-20
Intact 25 kHz
Spiracle Blocked
+5 dB
-10
-20

Fig. 2.15

Comparison of diffraction patterns measured at the acoustic spiracle (left) and neural response patterns (right) in T. cantans, at the frequencies given. The diffraction series were taken from a different specimen from the neural response series. Dotted lines (left) = sound diffraction produced by the stand alone. Dotted lines (right) = response to sound incident from 0°. In both cases 0° represents anterior and 90° the position ipsilateral to the recorded ear.


0°
10 dB
10 kHz
270°
90°
180°
20 kHz
30 kHz
40 kHz

gives a total chirp duration of some 100-200 ms. The chirp rate is 2-3/s. Each syllable is produced by one wing closure. The first few syllables are always of increasing amplitude, later syllables being of roughly constant amplitude. Aggression is identical to the calling song except that the chirps are usually longer (up to 1 or 2 seconds), and they are not repeated regularly, but may be discrete. The courtship song consists of single syllables of about 12 ms, shorter than those of calling or aggression, repeated at a rate of 3-4/s.

The spectral patterns of these songs are shown in Fig. 2.17. The calling and aggression songs clearly have very similar spectra. The sound energy is concentrated, with a calculated Q value of 10.9, around the carrier frequency of 4.9 kHz, with lower intensity peaks at higher harmonic frequencies. Courtship has a very different spectral pattern, the main energy peak being centred at 13 kHz, with secondary peaks near 29 and 40 kHz. The main peak at 13 kHz is not as narrow-banded as that of the calling and aggression songs; the calculated Q was 3.7. All the spectra shown were measured at points near the middle of the relevant syllables. Spectra at other points on the song temporal pattern always have peaks at the same frequencies, but may show slight variations in their relative amplitudes.

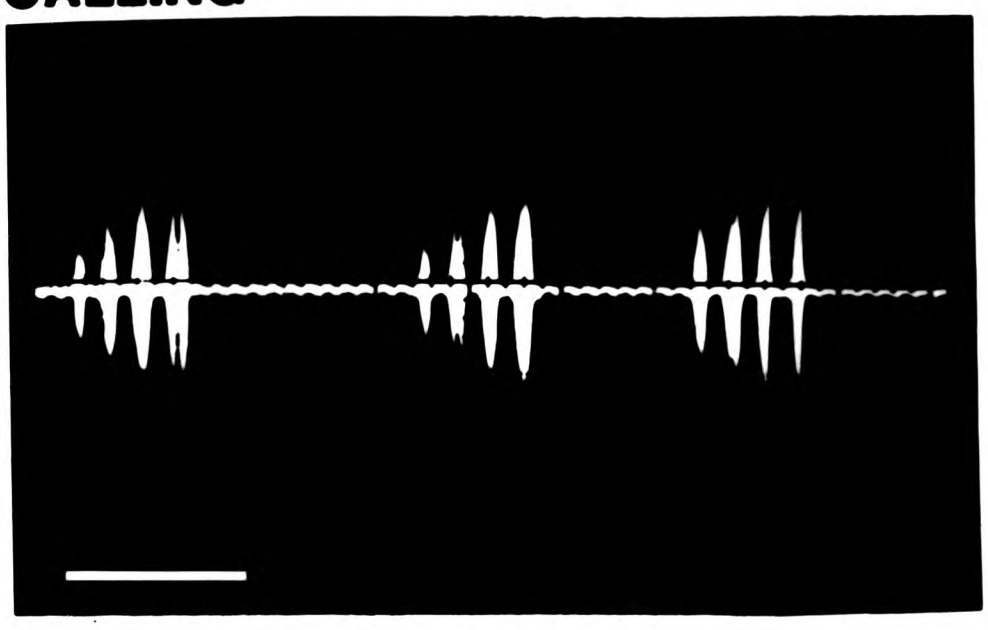
The temporal patterns of the songs of T. oceanicus, given in Fig. 2.18, are rather more complex than those of G. campestris. The calling song consists of two "phrases"; a short chirp of about 6-8 syllables followed by a series of

Fig. 2.16

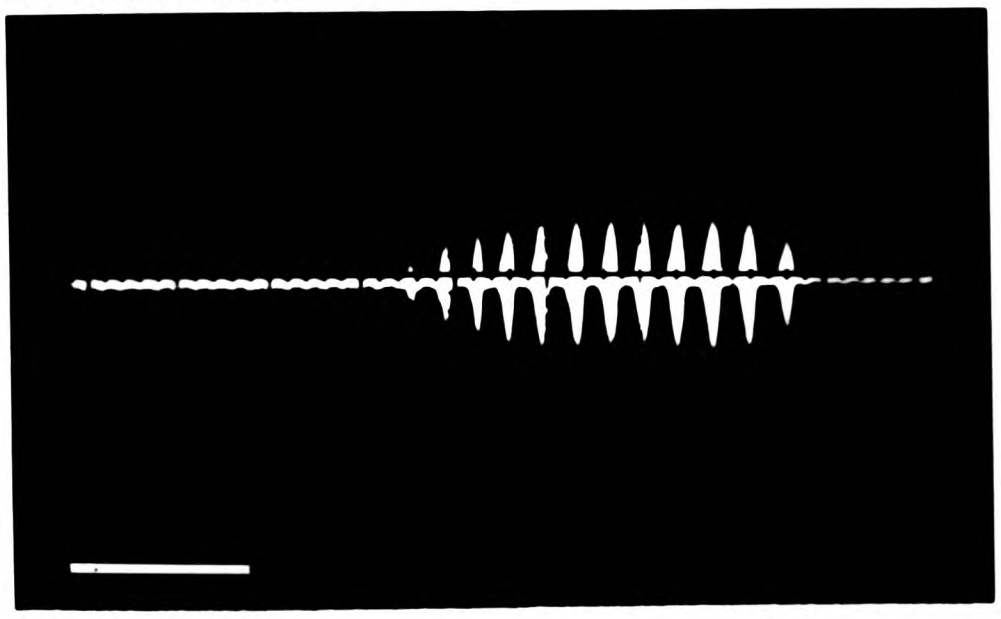
Oscillograms showing the temporal patterns of the calling, aggression and courtship songs of G. campestris.

Scale bars = 200 ms.

CALLING



AGGRESSION



COURTSHIP

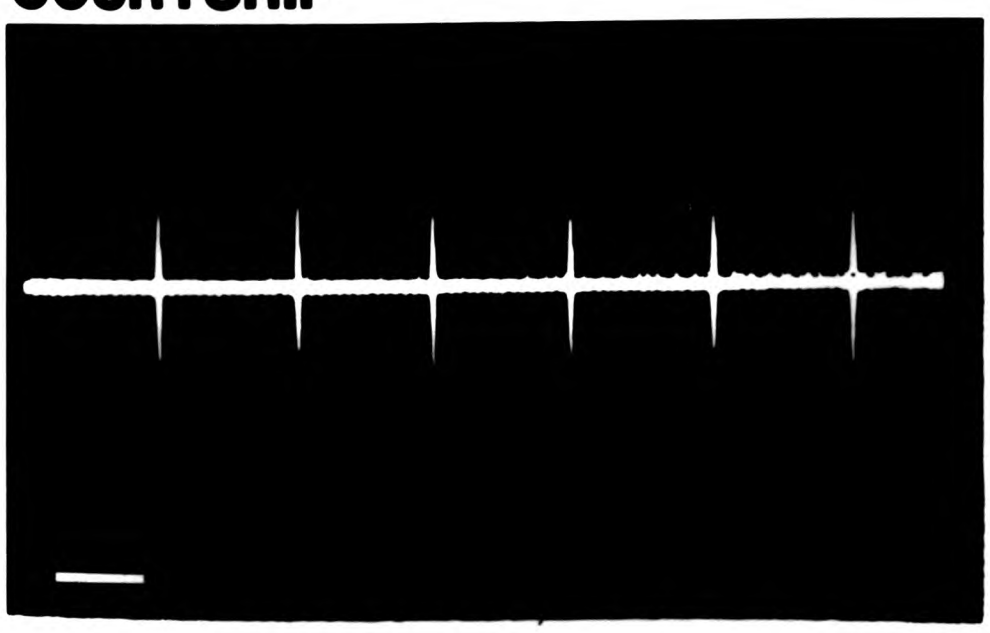
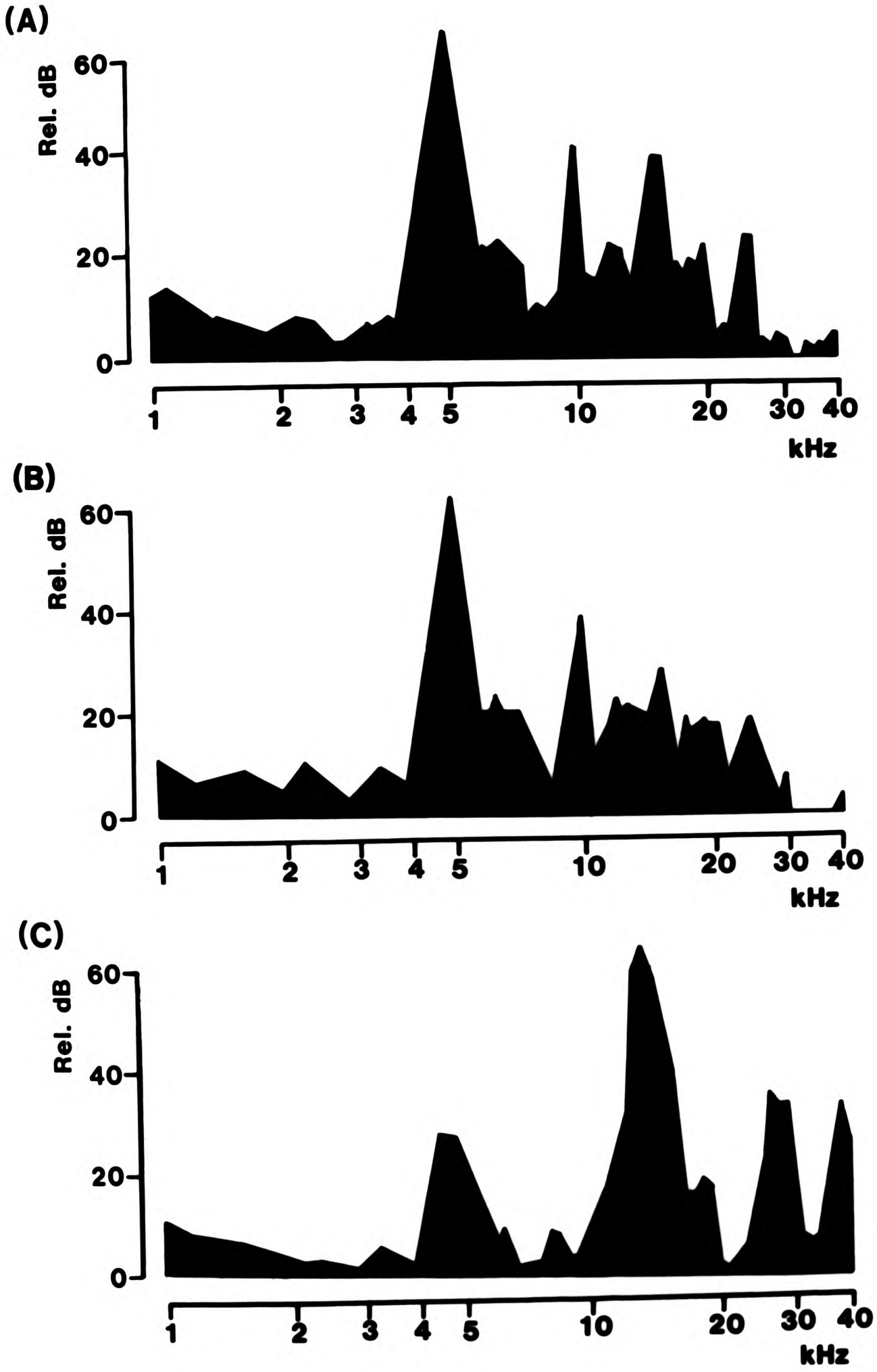


Fig. 2.17

Spectrograms of the calling (A), aggression (B) and courtship (C) songs of G. campestris, over the frequency range 1 - 40 kHz. Spectra were measured near the midpoint of a syllable of each song. Note differences in relative harmonics in (A) and (C).


(A)
Rel. dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz
(B)
Rel. dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz
(C)
Rel. dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz

syllable "doublets". The chirp phrase is of about 300 ms duration, with a syllable repetition rate of 15/s, while the duration of the doublet phrase is more variable, usually some 1-1.5 s, syllable rate being 18/s and doublet rate 5/s. This sequence is then repeated at a rate of about one every 2 seconds. The courtship song also consists of two phrases; an initial chirp, similar to that of the calling song, followed by a long "trill" of smaller amplitude syllables repeated at about 40/s for a duration of about 1 s. This sequence is repeated as for the calling song. Aggression in T. oceanicus is similar to the first phrase of the calling and courtship songs. It consists of a single prolonged chirp with a syllable repetition rate of 20-25/s.

The spectral patterns of all three songs of T. oceanicus are very similar and are shown in Fig. 2.19. The main energy peak is always around 4.7 kHz (Q of 11.5), and smaller peaks occur at harmonics of this frequency, mostly of progressively lower intensity.

(B) Biophysical Measurements

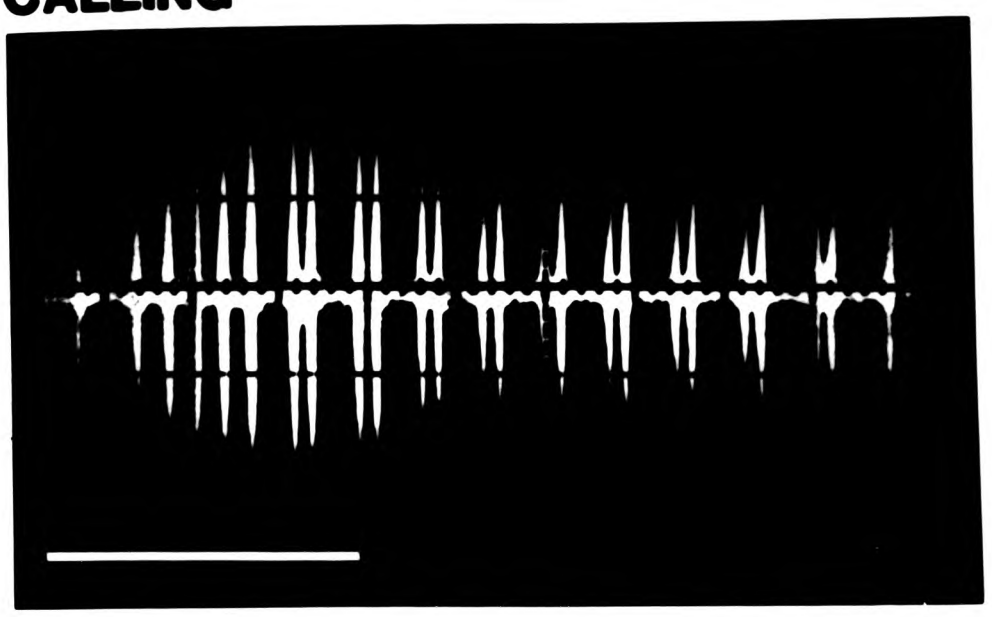
Fig. 2.20(a) and (b) show the plots of the sound pressure recorded near the posterior tympanum and acoustic spiracle respectively as a loudspeaker, producing a 5 kHz pure-tone, was rotated around a specimen of G. campestris in the horizontal plane. The variation was within ±2 dB in both cases, demonstrating that the sound diffraction by the cricket body is negligible at the carrier frequency of the calling and aggression songs of G. campestris, and of all three songs of T. oceanicus. Diffraction was not tested at

Fig. 2.18

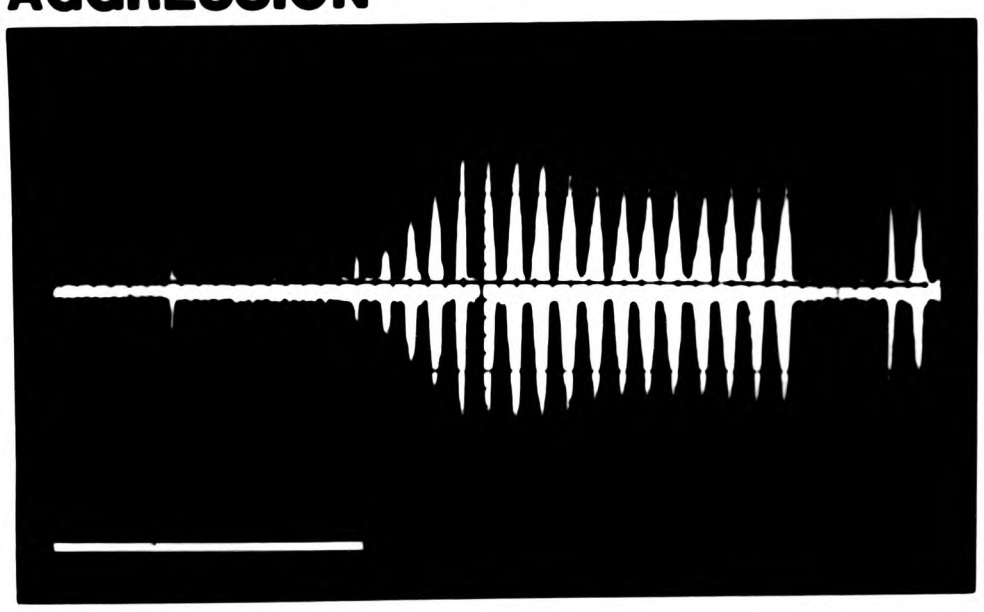
Oscillograms showing the temporal patterns of the calling, aggression and courtship songs of T. oceanicus.

Scale bars = 500 ms.

CALLING



AGGRESSION



COURTSHIP

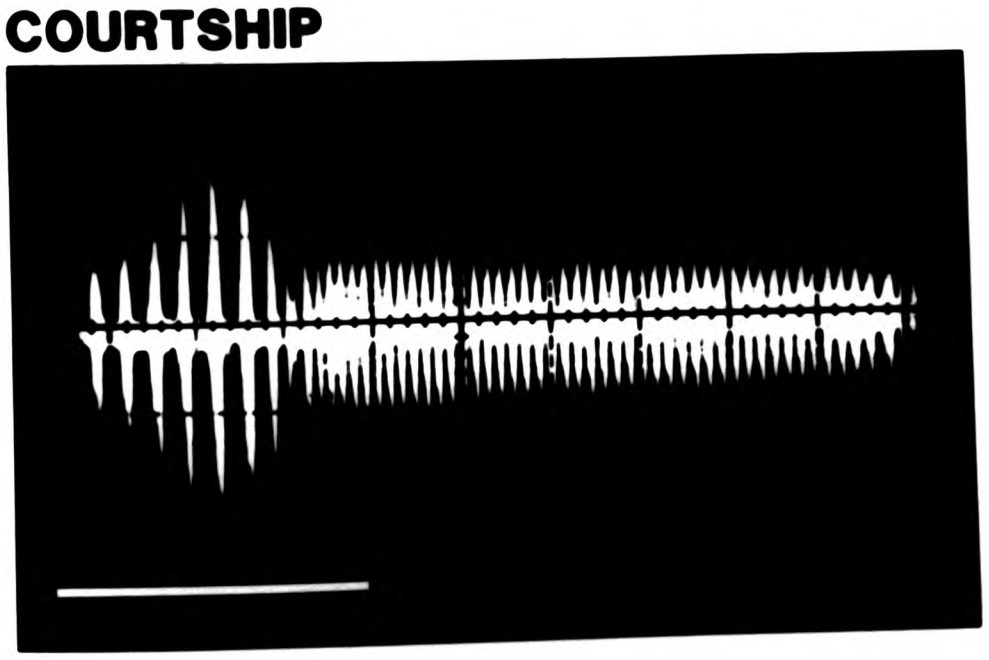
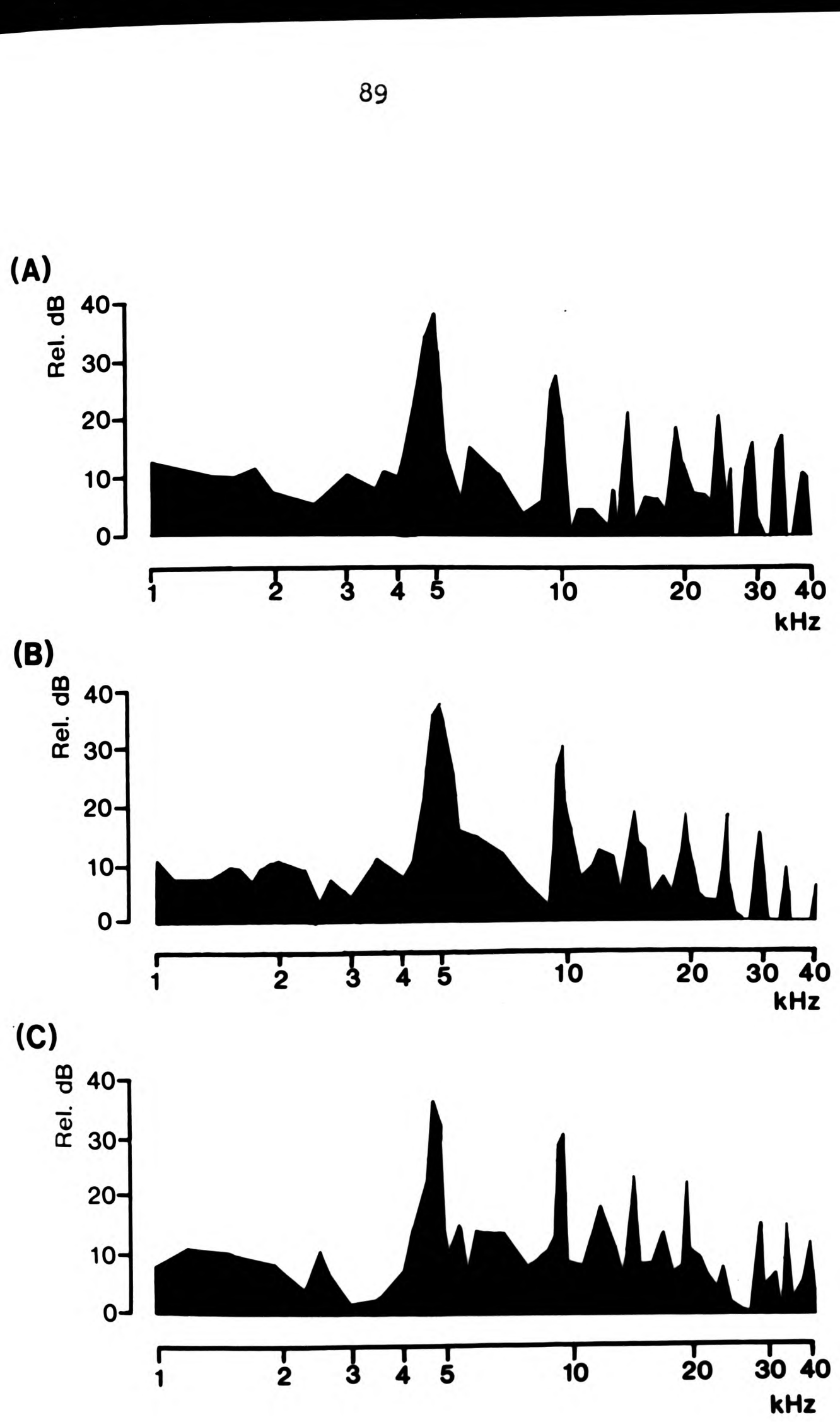


Fig. 2.19

Spectrograms of the calling (A), aggression (B) and courtship (C) songs of T. oceanicus, over the frequency range 1 - 40 kHz. Note the similarity of the relative amplitudes of the harmonics in all three songs.


(A)
Rel. dB
40
30
20
10
0
1
2
3
4
5
10
20
30
40
kHz
(B)
Rel. dB
40
30
20
10
0
1
2
3
4
5
10
20
30
40
kHz
(C)
Rel. dB
40
30
20
10
0
1
2
3
4
5
10
20
30
40
kHz

90

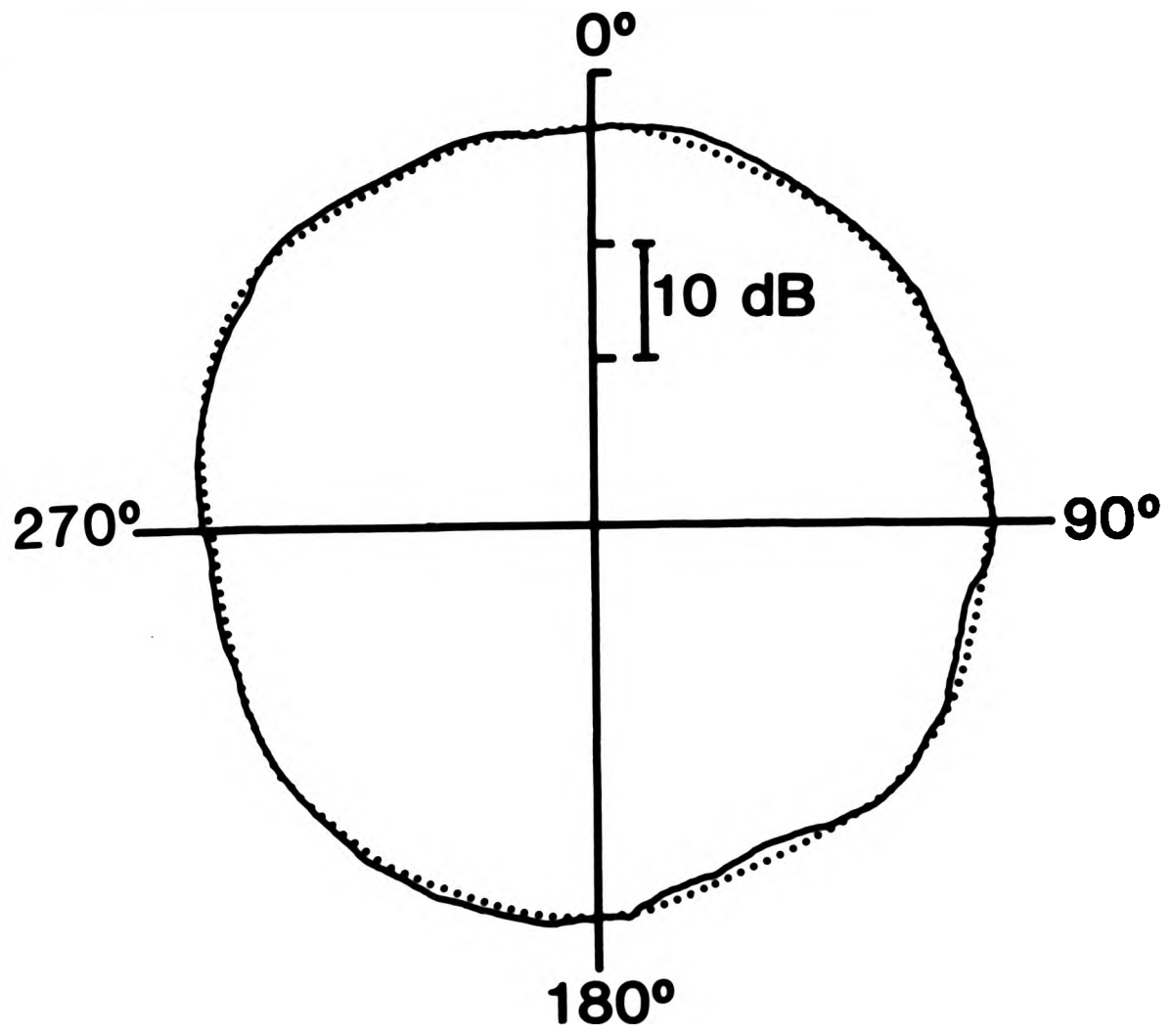
(A)

(B)

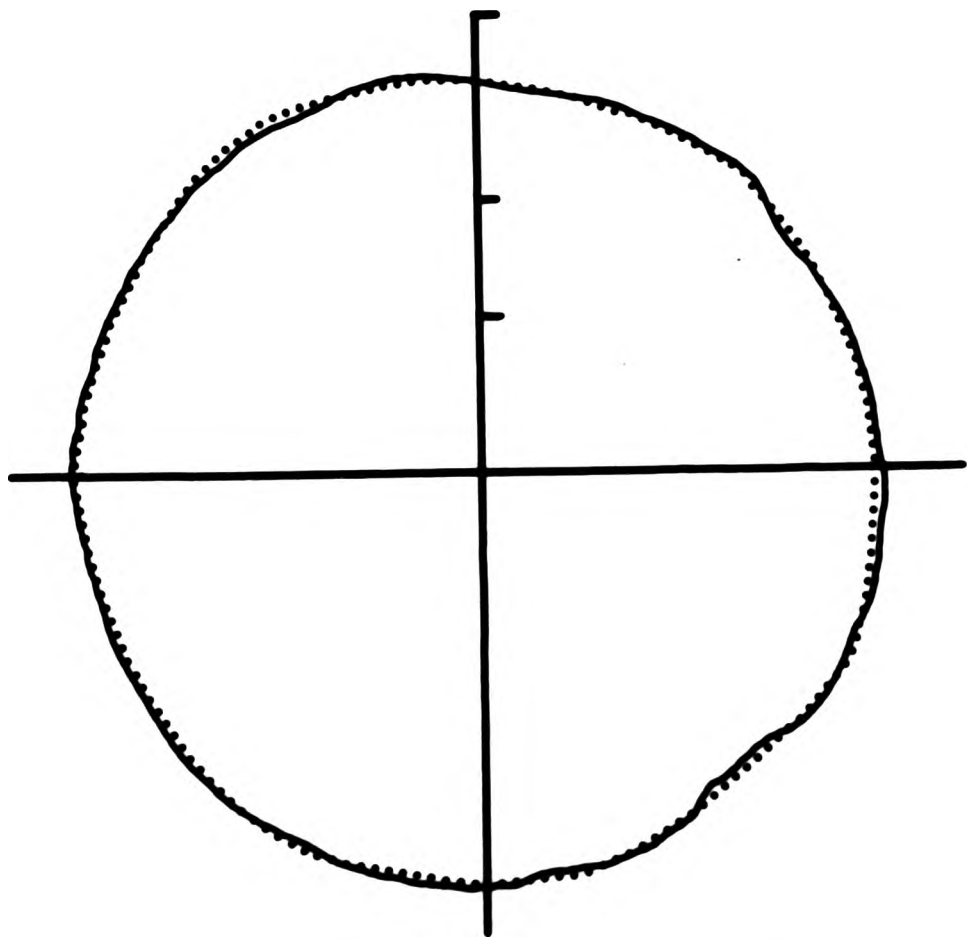
Fig. 2.20

Diffraction patterns around G. campestris at 5 kHz. Solid lines = directional variation in the sound pressure at the acoustic spiracle (A) and near the posterior tympanum (B), relative to the free field (dotted lines). In each case 0° represents anterior and 90° ipsilateral.

(A)



(B)



other frequencies, nor for T. oceanicus. The latter is slightly smaller than G. campestris, and will therefore produce slightly less diffraction.

(C) Neural Responses

(i) Threshold Values

The loudspeakers used allowed the testing of auditory thresholds at frequencies up to 40 kHz. Thresholds were tested in 3 specimens of G. campestris and 1 specimen of T. oceanicus, and examples are given in Fig. 2.21. The technique employed is not ideal for threshold determinations as the response near threshold becomes lost in background noise. Thresholds were taken as the lowest sound pressure for which the response was visible above background activity after 128 presentations, but the real thresholds of the auditory organ may be a few dB below the values shown. Nevertheless, relative sensitivities are clear; although the organ can be seen to be responsive to sound frequencies of up to 40 kHz in both species, peaks of sensitivity are evident at 4.8 kHz in G. campestris and 4.5 kHz in T. oceanicus, both with lesser sensitivity peaks around 15 kHz.

(ii) Directional Responses in G. campestris

Directionality of the Intact System

Fig. 2.22 shows a typical series of plots showing directional variation in the responses at several

Fig. 2.21

Audiograms of (A) G. campestris and (B) T. oceanicus, measured at the level of the leg nerve. Sound was presented ipsilaterally to the recorded ear. Threshold values were obtained from 128 averaged responses for each of the frequencies shown.

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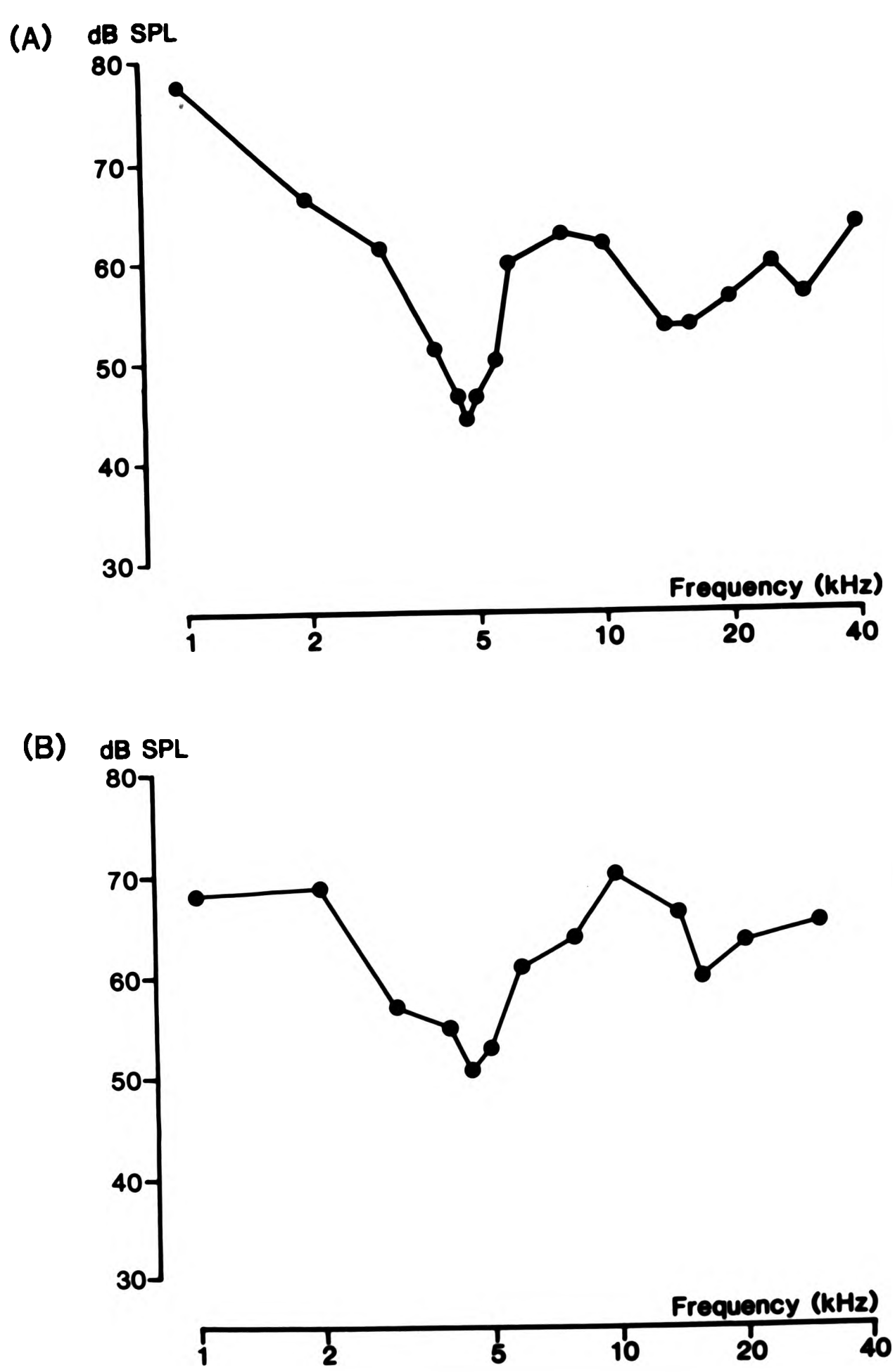

(A)
dB SPL
80
70
60
50
40
30
Frequency (kHz)
1
2
5
10
20
40
(B)
dB SPL
80
70
60
50
40
30
Frequency (kHz)
1
2
5
10
20
40

frequencies around the calling song carrier frequency. From 4.5 kHz directionality increases with frequency, to a maximum at 4.8 kHz, where the response pattern is "cardioid", with a "null" at 260°. Above this "best frequency" the directionality declines, in terms of L-R difference, and the pattern becomes almost omnidirectional at 5.2 kHz. By using a monitor loudspeaker to listen to the response it was possible to locate the null, or position of minimum response, precisely in both azimuth and frequency. Plots at the best frequency were constructed for several specimens. The mean value of the best frequency was 4.92 kHz (range 4.4-5.3 kHz, n=23), the angle of the null was 268.7° (range 240-300°, n=23), and the maximum L-R difference was 27.9 dB (range 18-35 dB, n=21).

Although it was possible to accurately locate the point of minimum response there was some variation in its depth between specimens, as is illustrated by the range in the maximum L-R differences. In a small number of specimens (5) a "supercardioid" pattern was observed at the best frequency. Whenever this occurred all other features of the directionality, such as its development around the calling song carrier frequency, were identical to those observed in specimens showing cardioid response patterns. Fig. 2.23 shows the development of directionality in a specimen showing supercardioid response patterns. It can be seen that while the depth of the nulls varies for different frequencies, the spatial positions of the nulls is fairly constant.

Fig. 2.22

Directional variation in the responses of the auditory organ in G. campestris, at frequencies around the best frequency (4.8 kHz). Points are plotted as dB relative to 0° anterior (dashed lines).

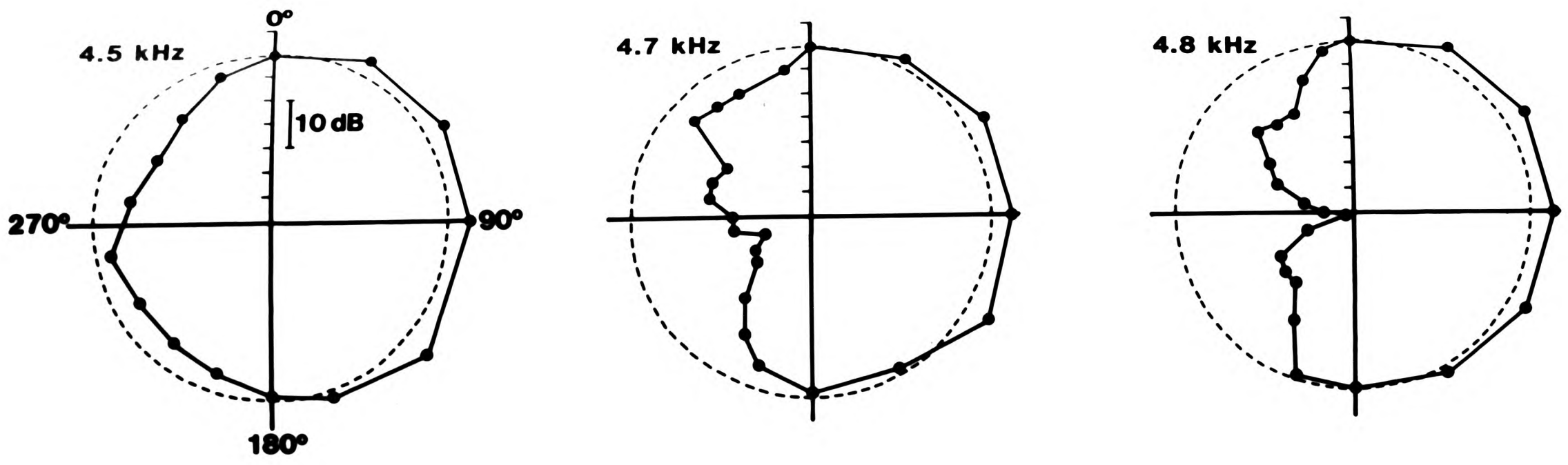
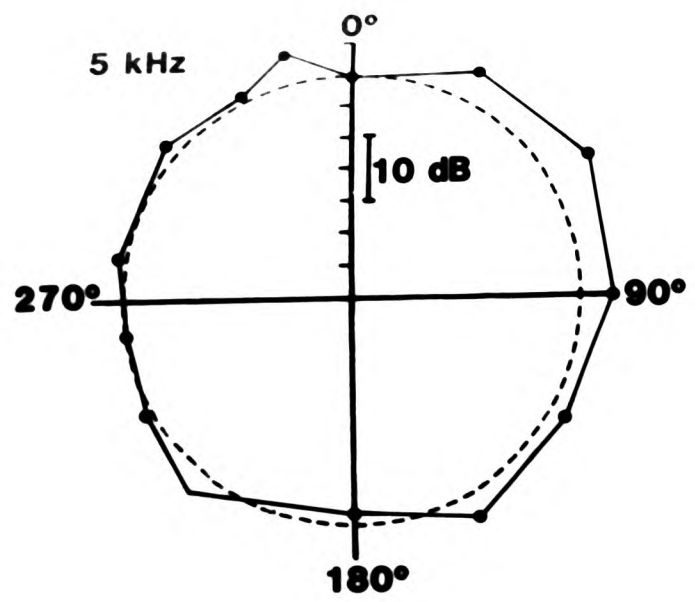
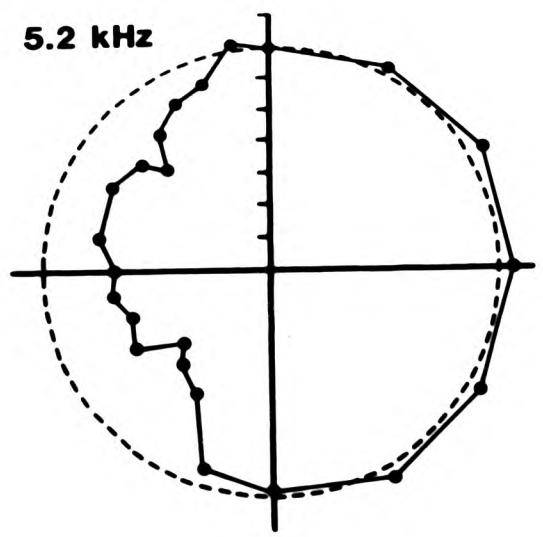
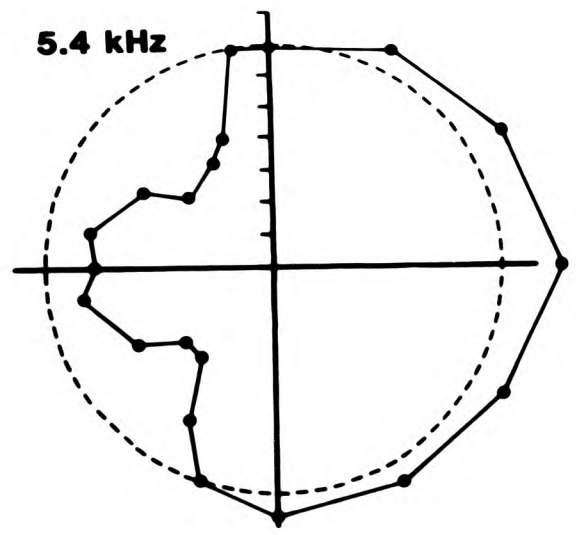

4.5 kHz
0°
10 dB
270°
90°
180°
4.7 kHz
4.8 kHz
5.0 kHz
5.2 kHz

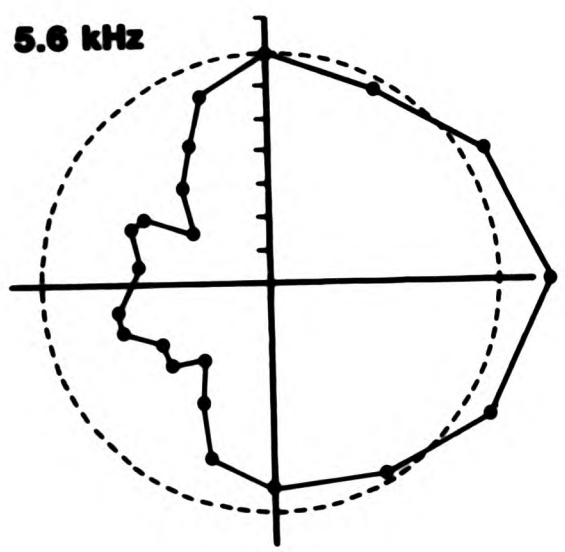
Fig. 2.23

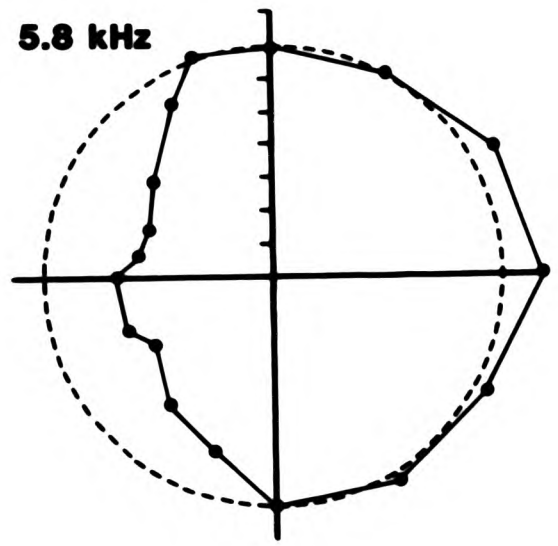
Development of peripheral directionality of the auditory organ of G. campestris at frequencies around the best frequency (5.6 kHz) in a specimen producing supercardioid patterns. Responses are plotted relative to 0° (dashed lines).

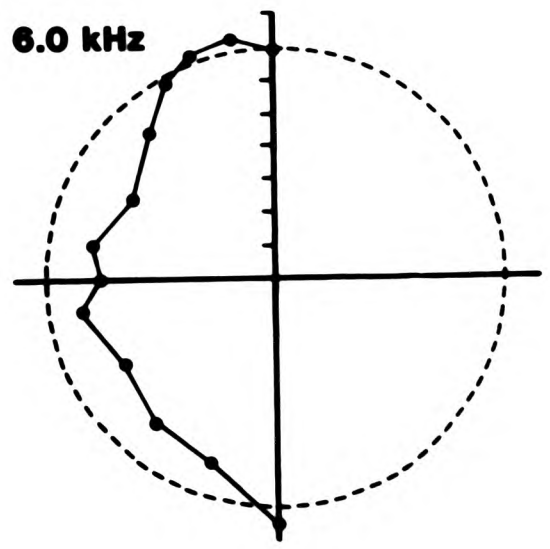

5 kHz
0°
10 dB
270°
90°
180°


5.2 kHz


5.4 kHz


5.6 kHz


5.8 kHz


6.0 kHz

The Effects of Contralateral Tympanum Blockage

The effects, on directionality, of preventing sound entry into the auditory system via the contralateral tympanum was tested by blocking it with wax in 12 specimens, an example being given in Fig. 2.24a. The anterior tympanum was assumed to be non-functional (Nocke 1972; Larsen & Michelsen 1978) and so its effect was not tested. Blockage of sound entry via the contralateral tympanum in this way did not affect the magnitude or shape of the directionality. In a few experiments the best frequency was shifted to a slightly lower point during tympanal blockage. This was measured precisely in 5 specimens; the mean best frequency for these specimens in the intact state was 4.65 kHz, and the best frequency with the contralateral tympanum blocked was 4.49 kHz. This difference was not statistically significant ($p=0.23$, paired t-test).

The Effects of Bilateral Spiracular Blockage

Blockage of both acoustic spiracles always eliminated almost all directionality. This test was performed on 7 specimens, and a typical result is shown in Fig. 2.24b. At no frequency could any greater L-R difference be found during spiracular blockage, average maximum L-R difference being 5 dB (n=7). It was difficult to clear the spiracles of wax without damage, but whenever this was achieved the response pattern always returned to its original form (Fig. 2.24b).

The Effects of Unilateral Spiracular Blockage

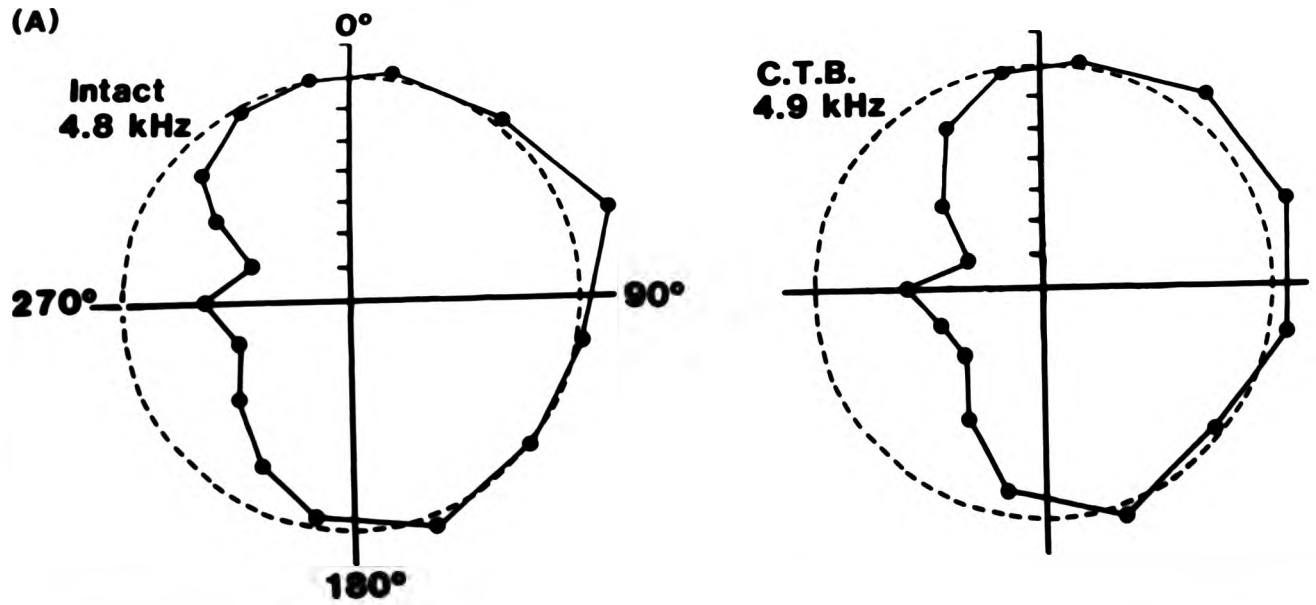
It was considered a possibility that during experiments involving unilateral spiracular blockage the insect may

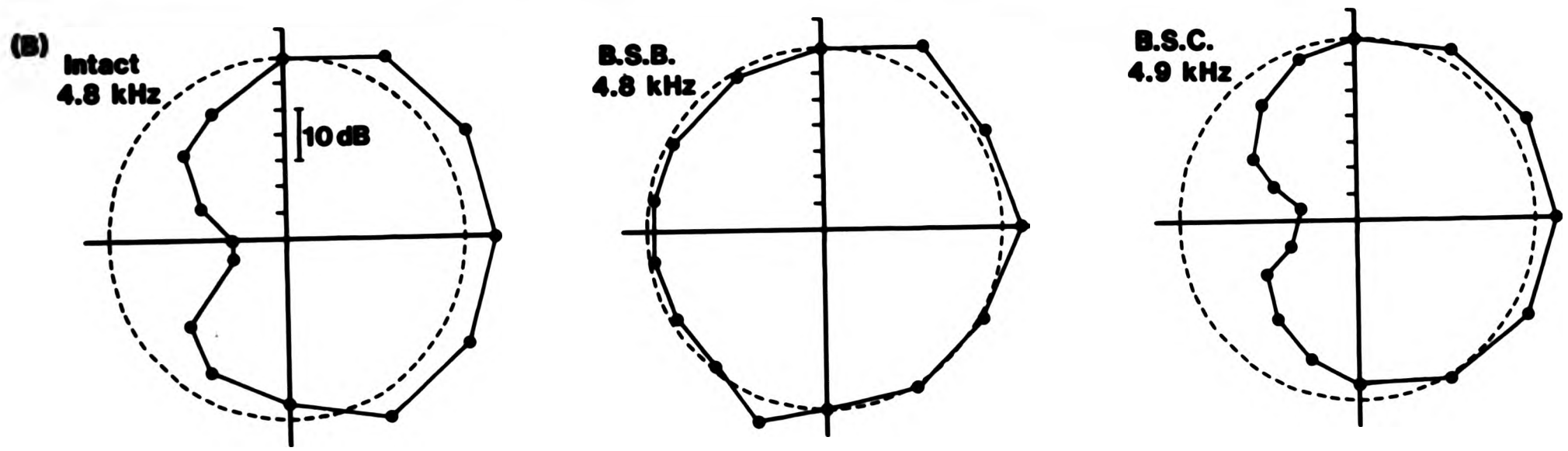
Fig. 2.24

The effect of blockage of the contralateral tympanum and of the spiracles on directionality patterns in *G. campestris*.

(A) Plots at the best frequency in the intact state and with the contralateral tympanum blocked (CTB).

(B) Plots at the best frequency in the intact state, with both spiracles blocked (BSB) and with both spiracles cleared (BSC).


(A)
0°
Intact
4.8 kHz
270°
90°
180°
C.T.B.
4.9 kHz


(B)
Intact
4.8 kHz
10 dB
B.S.B.
4.8 kHz
B.S.C.
4.9 kHz

respond to wax blockage of one spiracle by closing its other spiracle. Therefore, these experiments were conducted with the relevant spiracle waxed slightly open to prevent this possibility. Subjectively, it appeared that as long as a spiracle was not completely blocked, the extent of its patency had very little effect. Blockage of the contralateral spiracle alone, carried out on 9 animals, typically produced a response pattern as in Fig. 2.25a, where most of the original L-R difference has been lost. It was not possible to improve the directionality by changing the frequency of the stimulus. The mean maximum L-R difference under these conditions was 8.25 dB (n=9). Clearance of the spiracle returned the response pattern to its original form.

Most directionality was also lost, at the best frequency, following blockage of the ipsilateral spiracle, but there was usually some suggestion of a supercardioid pattern. The contralateral response diminutions relative to 0° were 5-10 dB in most preparations. It was found that paired nulls occurred that were consistently tuned to a lower frequency than the cardioid null of the intact state. In Fig. 2.25b the normal cardioid pattern occurred at 5.3 kHz. After blockage of the ipsilateral spiracle most directionality was lost, but changing the frequency to 4.6 kHz produced a supercardioid response pattern with nulls at 195° and 330°. Each of the paired nulls were always tuned to approximately the same frequency, and this frequency was a mean of 650 Hz lower than the respective intact state best frequencies (this result was highly

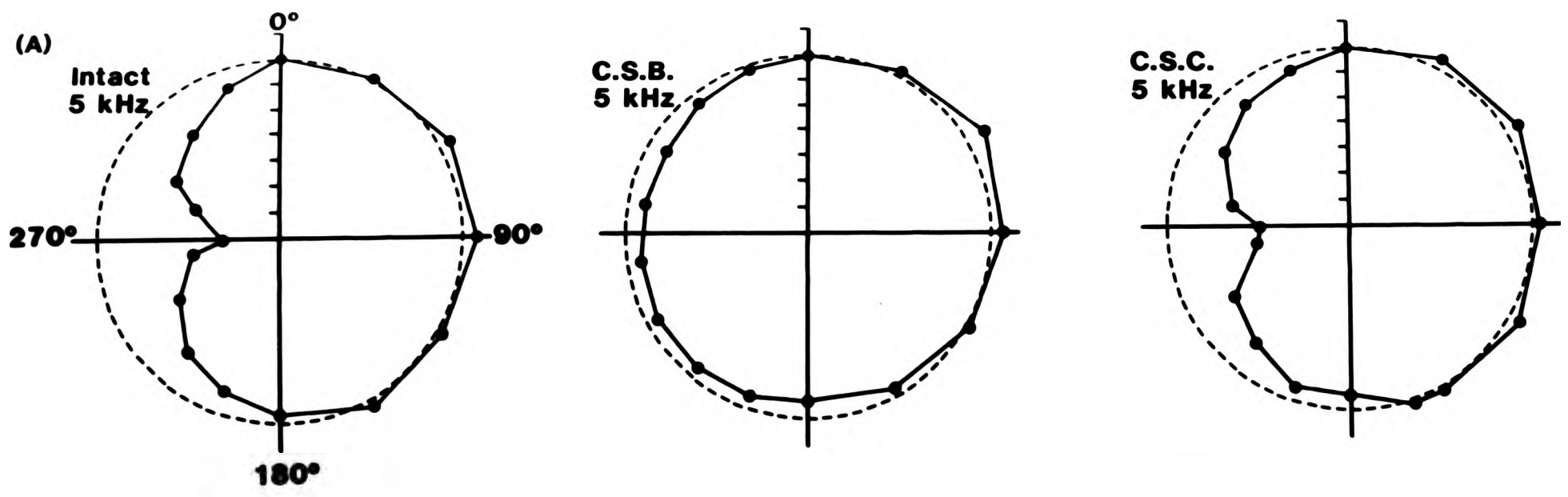
Fig. 2.25

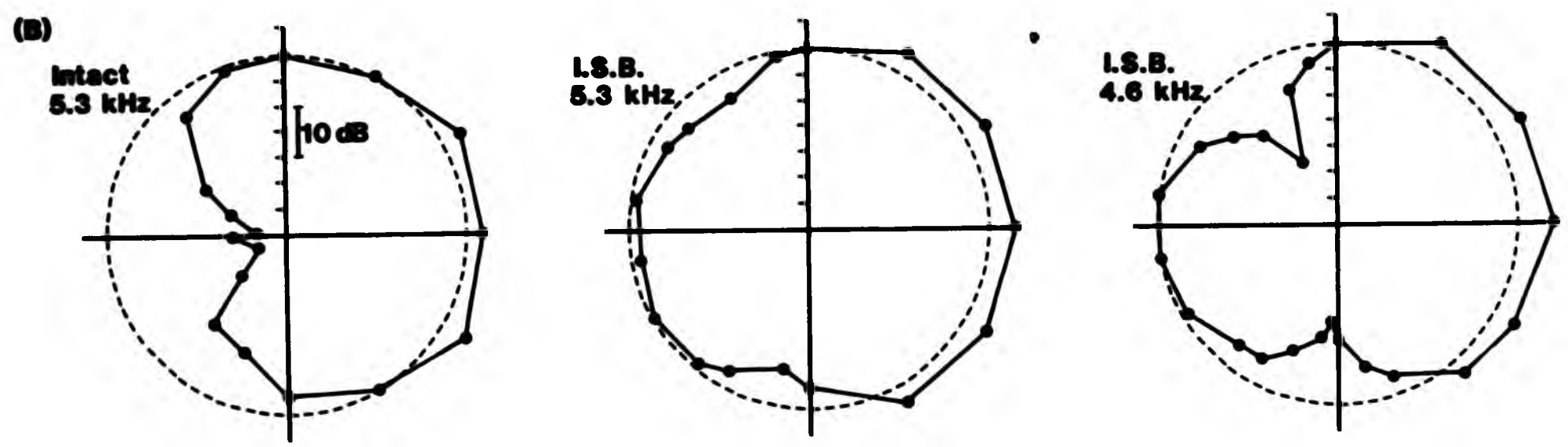
The effects of unilateral spiracular blockage on directionality patterns in G. campestris.

(A) Plots at the best frequency in the intact state, with the contralateral spiracle blocked (CSB) and with the contralateral spiracle cleared (CSC).

(B) Plots at the best frequency (originally 5.3 kHz) in the intact state, with the ipsilateral spiracle blocked (ISB centre), and after also changing the sound stimulus frequency to 4.6 kHz (ISB right).

Data in series (A) from a different specimen to series (B).


(A)
Intact
5 kHz
0°
90°
180°
270°
C.S.B.
5 kHz
C.S.C.
5 kHz


(B)
Intact
5.3 kHz
10 dB
I.S.B.
5.3 kHz
I.S.B.
4.6 kHz

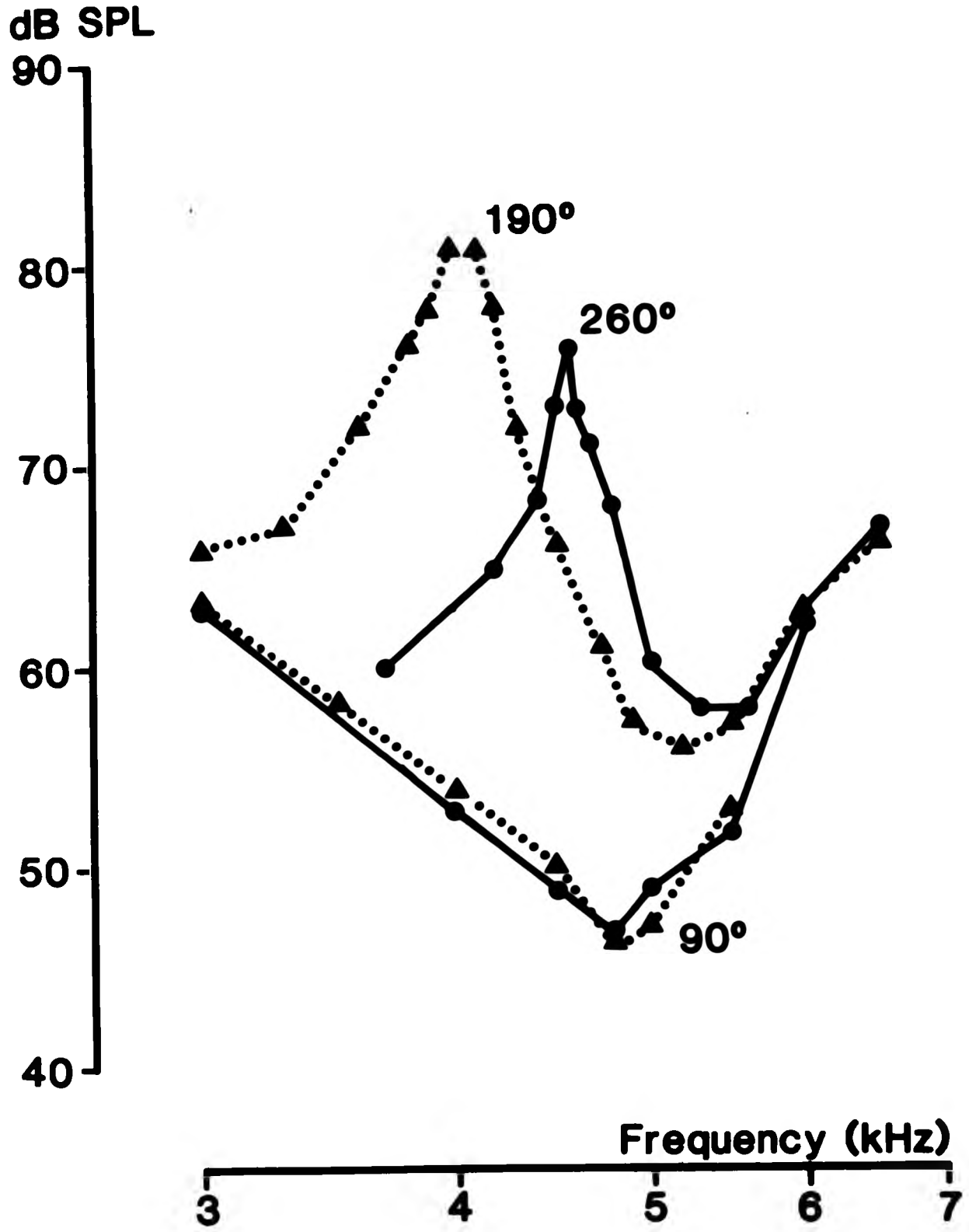
significant: $p<0.001$, paired t-test). The supercardioid nulls had a mean separation of 119°, centred about the 262° position (n=12). For these specimens the mean position of the intact state cardioid null was 271°, but this was not statistically different from the position at the centre of the supercardioid nulls (262°) when the ipsilateral spiracle was blocked ($p=0.245$).

There was no evidence that changing the stimulus frequency altered the position of the nulls; only their depth was affected. When this test was performed on a specimen that showed a supercardioid pattern in the intact state, ipsilateral spiracular blockage produced a pattern with nulls that were more widely spaced and tuned to a lower frequency than in the intact state.

Thresholds to frequencies around the best frequency of the null were determined in the intact state for 3 specimens with the loudspeaker (a) at the 90° ipsilateral position and (b) at the position of the null. A typical result is given in Fig. 2.26a. The difference between the thresholds at the ipsilateral and null positions effectively shows the tuning of the intact state directionality. This was repeated with the ipsilateral spiracle blocked; blocking the spiracle did not affect the thresholds at the 90° position but the null can be seen to be tuned to a lower frequency. Thresholds shown are for only one of the nulls occurring with the spiracle blocked, as both showed the same threshold curve.

Fig. 2.26

Thresholds of the auditory response measured at 90° (ipsilateral) and at the position of the null in the intact state ●——● (260°) and after blockage of the ipsilateral spiracle ▲···▲ (190°).


dB SPL
90
80
70
60
50
40
190°
260°
90°
Frequency (kHz)
3
4
5
6
7

The Tuning of the Nulls

The change in the suprathreshold response with frequency was tested at the position of the null(s) in the intact state and during various test situations in 7 specimens. In each case the null was first located spatially, and then the responses to various constant intensity frequencies around the null frequency were measured. In the example shown (Fig. 2.27) the tuning of the null is shown in the intact state, after blockage and after clearance of the contralateral tympanum, and after blockage and after clearance of the ipsilateral spiracle. Responses were converted to dB from an intensity-response curve constructed at the null frequency. The null in the intact (cardioid) state was tuned to 4.85 kHz. After contralateral tympanum blockage there was a slight decrease in the null frequency to 4.8 kHz, and this increased to 4.9 kHz after clearance.

Blockage of the ipsilateral spiracle produced a supercardioid response pattern, with nulls at 185° and 315°. These nulls can be seen to be tuned to 4.3 kHz and 4.2 kHz respectively. Clearance of the spiracle returned the directionality to cardioid form with a null tuned to 4.9 kHz. It should be noted that as the points for each curve were converted to effective dB from an intensity-response curve produced at the null frequency there may be small errors in the points recorded off the null frequency as the intensity-response curves are not identical at all frequencies.

Fig. 2.27

Variation in null tuning curves at the positions given, under several conditions in one representative specimen of G. campestris. Intact = intact state; CTB = with the contralateral tympanum blocked; CTC = with the contralateral tympanum cleared; ISB = with the ipsilateral spiracle blocked; ISC = with the ipsilateral spiracle cleared. Responses for each plot were converted to dB from the intensity-response curve of the respective null frequency. The ordinate is non-continuous and the curves are vertically displaced for clarity.

Ipsilateral = 90°.

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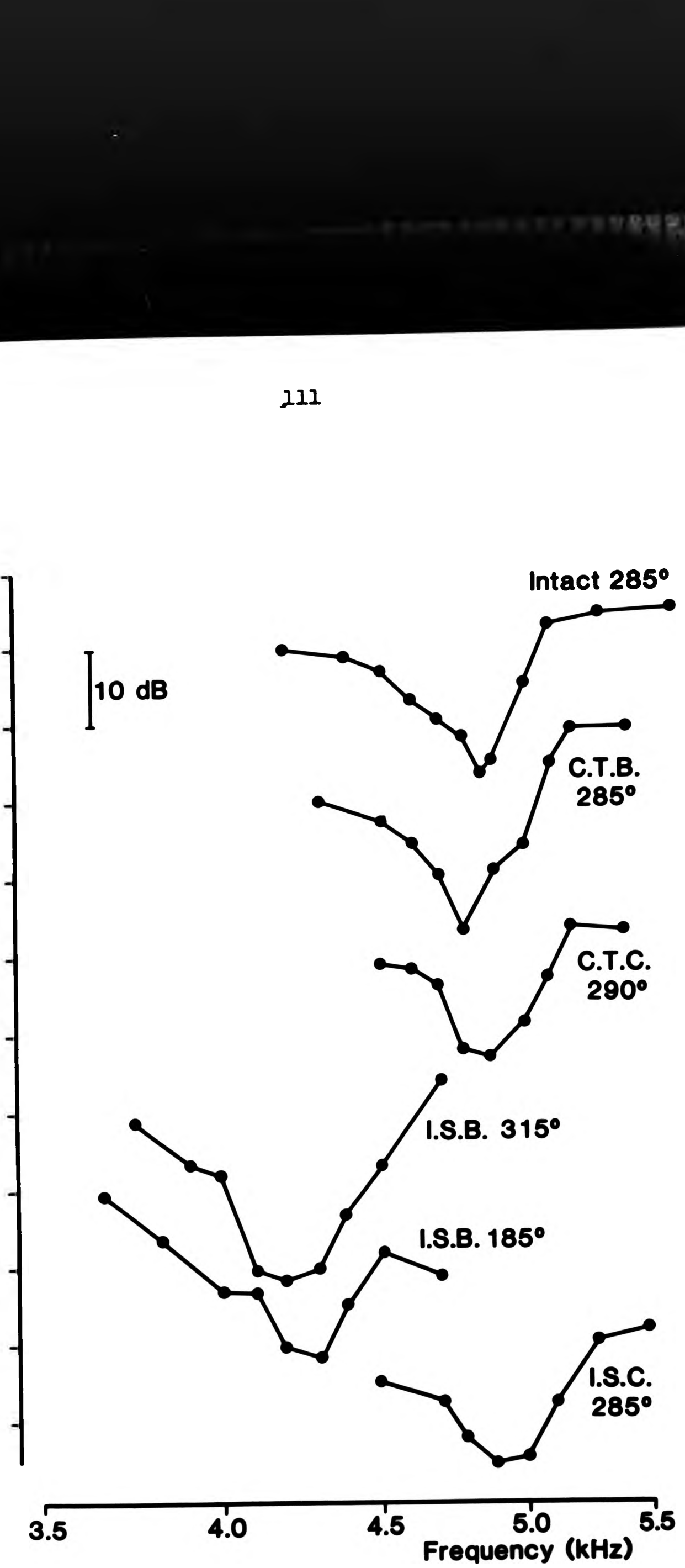

Intact 285°
10 dB
C.T.B. 285°
C.T.C. 290°
I.S.B. 315°
I.S.B. 185°
I.S.C. 285°
3.5
4.0
4.5
5.0
5.5
Frequency (kHz)

(iii) Directional Responses in T. oceanicus

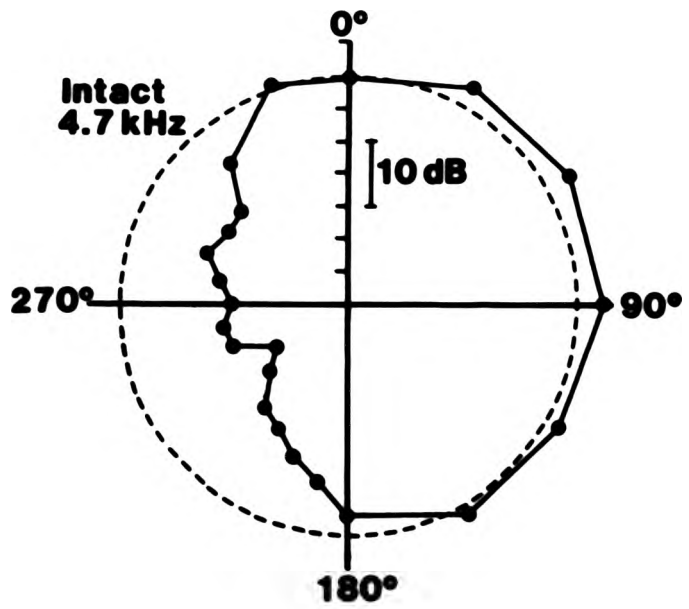
Most of the experiments described for G. campestris were repeated, for comparative purposes, on T. oceanicus. The results were essentially identical and a summary is provided by Fig. 2.28. The mean value of the best frequency in the intact state was 4.74 kHz (n=4). Fig. 2.28 shows the effect, on directionality, of blocking the contralateral tympanum and of blocking both spiracles. The results are very similar to those given in Fig. 2.24 for G. campestris; blocking the contralateral tympanum had no appreciable effect, whereas bilateral spiracular blockage reduced the maximum L-R difference from 25 dB to 7 dB. In another specimen (not shown) blockage of the contralateral spiracle reduced the L-R difference from 28 dB to 8 dB, whereas blockage of the ipsilateral spiracle produced a supercardioid directionality pattern with nulls tuned 350 Hz lower than the intact state cardioid null.

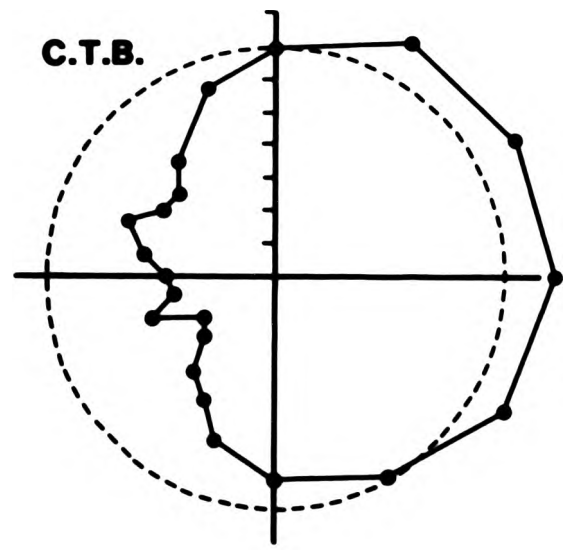
(iv) Transmission Through the Interaural Pathway

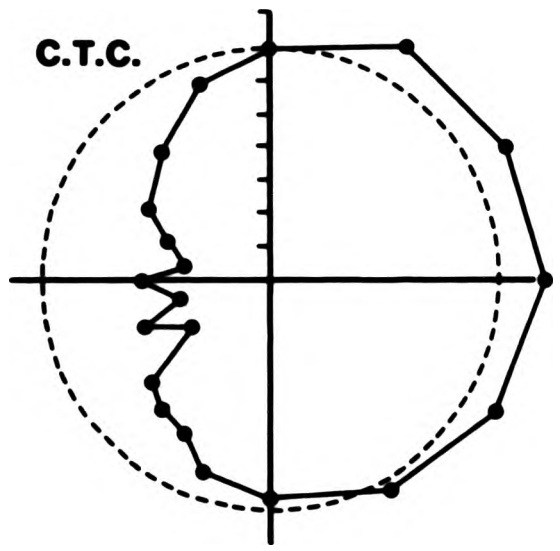
Transmission of sound to the rear surface of the posterior tympanum was investigated by waxing a sleeve of plastic tubing around the ipsilateral tibia, to prevent sound access to the front surface, and comparing the sensitivity of the auditory organ to sound entering the auditory system via the two acoustic spiracles or the contralateral tympanum (hence incident on the rear surface of the tympanum). As the experimental method was not ideal for measuring thresholds, intensity-response curves were constructed under each of several conditions, to compare sensitivities. Fig. 2.29a

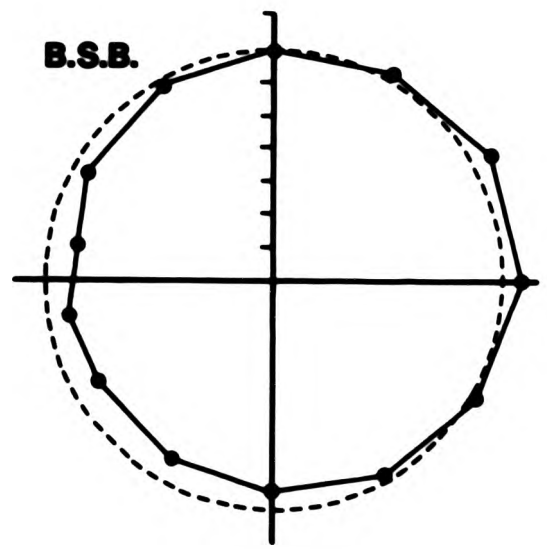
Fig. 2.28

Directionality patterns in T. oceanicus at 4.7 kHz. Plots are given for the best frequency in the intact state, with the contralateral tympanum blocked (CTB), with the contralateral tympanum cleared (CTC) and with both spiracles blocked (BSB). All data from one specimen.


0°
Intact
4.7 kHz
10 dB
270°
90°
180°


C.T.B.


C.T.C.

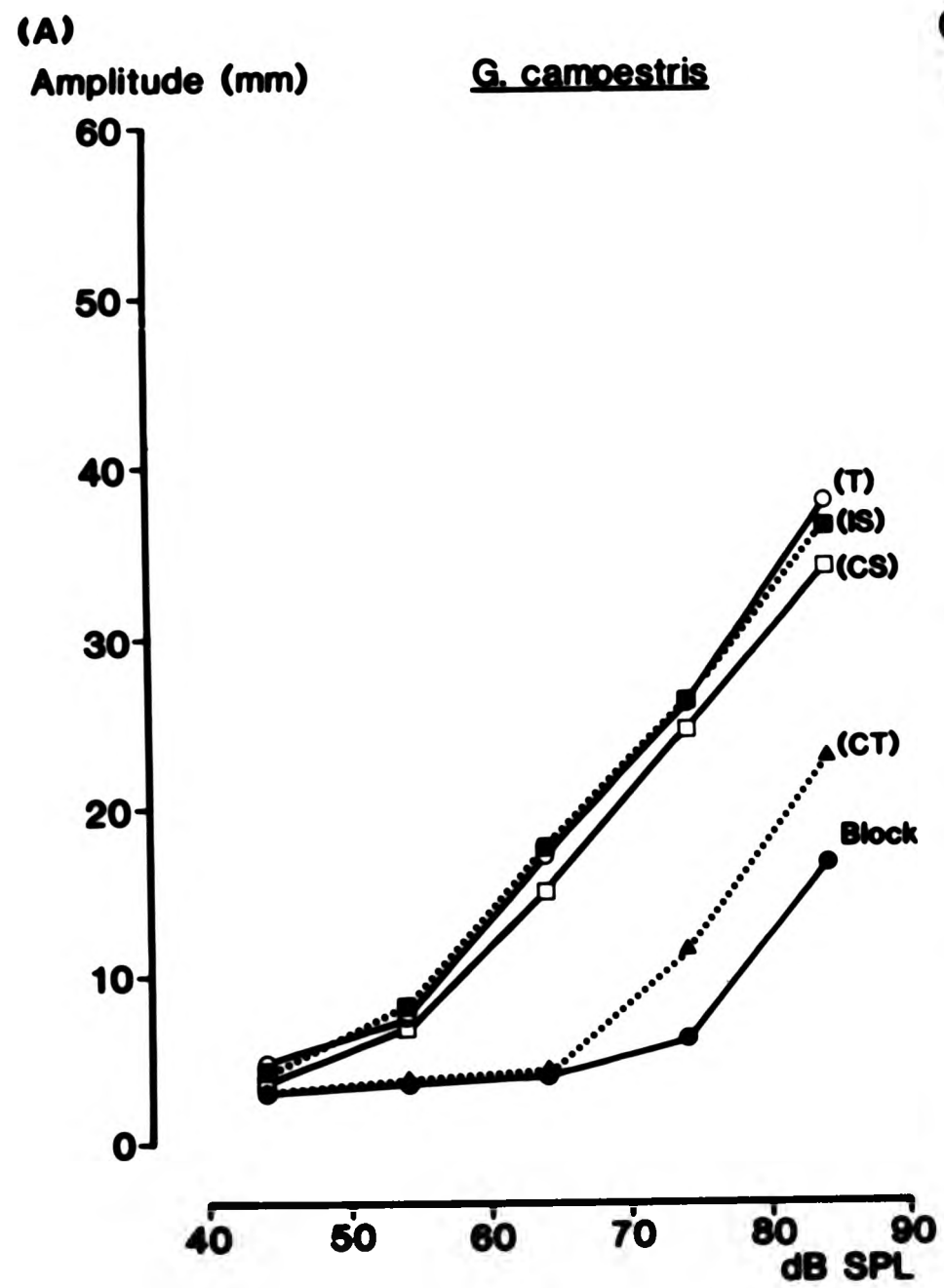

B.S.B.

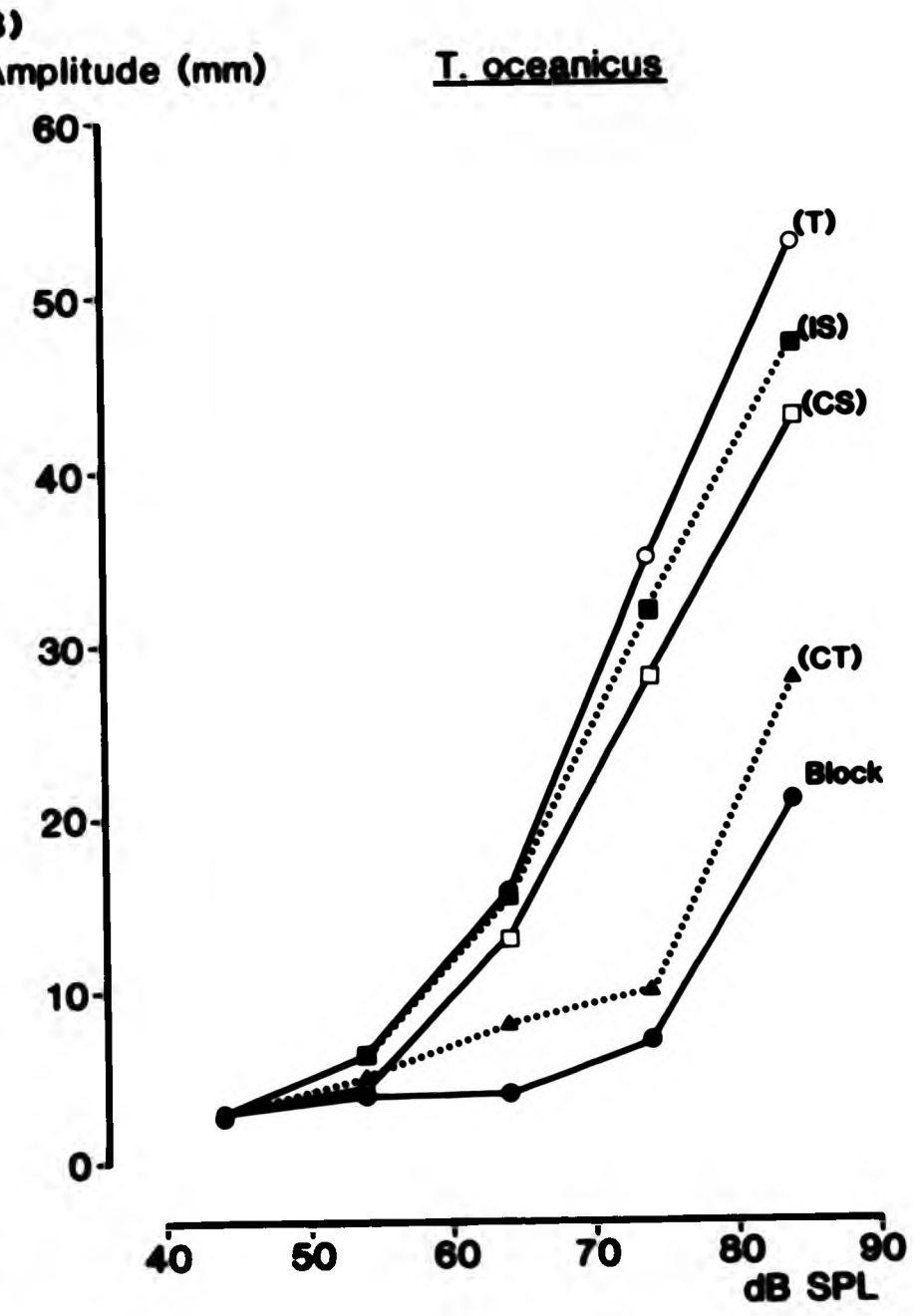
shows mean values for 6 specimens of G. campestris, for ipsilaterally presented sound. (T) is the intensity-response curve produced with all three inputs open (the ipsilateral and contralateral spiracles and the contralateral tympanum). (IS), (CS) and (CT) are the curves produced with the ipsilateral spiracle, contralateral spiracle and contralateral tympanum exposed individually, the other two inputs being blocked with wax in each case. The "block" curve was produced with all inputs blocked. The further to the left a curve is, the more sensitive the organ is to that input. Fig. 2.29b gives the results of a single experiment on T. oceanicus; these were very similar to those for G. campestris. It is evident, for both species, that with either spiracle open substantial "back-pressure" is produced which is almost as great as the back-pressure produced with all three inputs exposed. The input from the ipsilateral spiracle produced a slightly larger response than that from the contralateral spiracle. The contribution of the contralateral tympanum was considerably smaller, however, producing a response only slightly greater than that produced in the totally blocked condition. The response in the latter case may have been due to leakage of the blockages, but was more likely to have been caused by resonance of the leg by the wire frame as it was very consistent in all specimens and did not appear above threshold levels until about 70 dB SPL.

When sound was presented contralaterally the sensitivity of the auditory organ to sound entering via the single inputs changed in accordance with expectations based

Fig. 2.29

Intensity-response curves for ipsilaterally presented sound with a plastic tube around the ipsilateral tibia for 6 specimens of G. campestris (left), and one specimen of T. oceanicus (right). (T) = curve constructed with all other sound input sites open;
(IS) = with the ipsilateral spiracle alone open;
(CS) = with the contralateral spiracle alone open;
(CT) = with the contralateral tympanum alone open;
Block = with all input sites blocked.
Stimulus frequency = 5 kHz.


(A)
Amplitude (mm)
G. campestris
60
50
40
30
20
10
0
(T)
(IS)
(CS)
(CT)
Block
40
50
60
70
80
90
dB SPL


(B)
Amplitude (mm)
T. oceanicus
60
50
40
30
20
10
0
(T)
(IS)
(CS)
(CT)
Block
40
50
60
70
80
90
dB SPL

on sound diffraction, i.e. the sensitivity to the contralateral inputs increased by about 2 dB while the sensitivity to the ipsilateral input decreased by a similar ammount. The ipsilateral spiracle still produced a slightly larger response than the contralateral spiracle. However, the sensitivity to sound entering via all three inputs combined (T) was less by about 10 dB, in G. campestris, indicating some phase interactions between the three inputs. The response for the totally blocked condition remained the same as for ipsilaterally presented sound.

2.4 DISCUSSION

The experimental technique employed in this study has two distinct advantages over other methods of physiologically determining peripheral directionality. The first is that investigations are carried out on almost intact insects in the natural standing position. Most of the studies on directionality performed by other workers have involved considerable dissection, especially when recording from units in the CNS (e.g. Hill & Boyan 1977; Rheinlaender & Romer 1980). Their methods have therefore neccesitated recording from incomplete specimens. Secondly, the recorded responses remain reasonably stable over a long period of time, thereby enabling many successive tests to be made on one specimen. A disadvantage of the technique is that the responses cannot be quantified directly, but must be quantified by converting their amplitudes (or outline lengths, or areas) to effective dB using an intensity-response curve. The responses are thereby used to predict the changes in the effective pressure at the tympanic membrane, and so directional plots show the directionality available to the receptor (i.e. the peripheral directionality), as may be obtained by determining the thresholds of any auditory neurone at different angles of sound incidence.

The information that is utilized by the insect from the available peripheral directionality will depend on the absolute thresholds and dynamic ranges of single units, and the degree of intensity range fractionation in the auditory

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The information that is utilized by the insect from the available peripheral directionality will depend on the absolute thresholds and dynamic ranges of single units, and the degree of intensity range fractionation in the auditory

pathways. From the intensity-response curves produced in this study (e.g. Fig. 2.7) it is clear that the receptors may code the intensity of sound at frequencies relevant to the calling songs over a range of about 60 dB above their respective thresholds. Single-unit studies, in both crickets and bushcrickets, have demonstrated primary and central auditory neurones with dynamic ranges of 25-35 dB but with range fractionation sufficient to code intensity over at least 50 dB (Boyan 1979; Esch et al. 1980; Hutchings & Lewis 1981). There is also evidence that peripheral directionality may be coded in terms of response latency (Nocke 1972; Morchen et al. 1978; Boyan 1979). This process is not based on the difference between the time of arrival of a sound at the two ears, but depends on the fact that the responses of the receptors to high intensity stimuli have considerably shorter latencies than those to low intensity stimuli. The difference in the latencies can be up to about 50 ms (Nocke 1972; Boyan 1979). This directional cue would obviously only be produced at the beginning of a sound pulse, and so insects with an amplitude modulated song would be more likely to use it than those with a continuous song.

Directionality of the Bushcricket Auditory Organ

The results of the investigations on T. cantans show the auditory organ in this bushcricket to be most sensitive to sound of 15-30 kHz, within the frequency range tested (Fig. 2.12). At 40 kHz, however, the threshold was still

less than 40 dB SPL. It is therefore unfortunate that the loudspeakers used did not allow the testing of frequencies above 40 kHz. This is particularly true as the spectrum of the calling song extends to above 50 kHz (Fig. 2.9B). However, this missing frequency band amounts to less than half an octave, and the sensitivity of the auditory organ appears to be tailing off at 40 kHz. Silver et al. (1980) recorded single central units in T. cantans that responded to frequencies up to at least 100 kHz, but also found that the sensitivity of the units was greatest around 25 kHz. It seems likely that the high frequency sensitivity is necessary for predator avoidance, rather than conspecific communication (Silver et al. 1980).

The directionality plots (Fig. 2.13) indicate that substantial L-R differences are achieved only at frequencies above 10 kHz. Although the directionality increased with frequency over the entire range tested, many sharp dips in the response patterns occurred above 30 kHz. The directional cues, therefore, may become rather ambiguous at these high frequencies, and so it is likely that most reliable directional cues may be achieved at around 20-30 kHz. This possibility is supported by the fact that the auditory organ is most sensitive to this frequency band.

The effects of blockage of the acoustic spiracle and of the tympanal slits on the thresholds (Fig. 2.12) suggest that the sensitivity of the ear (at least for 4-40 kHz) is due primarily to sound entering the auditory system via the spiracles. As the directionality increases gradually with frequency it might be reasonable to suppose that the L-R

response differences are due to changes in the sound diffraction around the insect body at different angles of sound incidence (this would increase with sound frequency - Shaw (1974)). The results of the diffraction measurements, made at the acoustic spiracle, confirm this hypothesis; in Fig. 2.15 there is a close correlation between the trends of the neural response and diffraction patterns at each frequency. Particularly of interest is the fact that ipsilateral augmentation provides a major contribution to the total L-R difference, and this augmentation, both in the neural responses and the sound diffraction, is maximal near 25 kHz, which is also the frequency optimum of the auditory organ (Figs 2.15,2.12).

The results presented here agree, in most respects, with the findings of several other authors who have investigated the mechanisms of auditory directionality in bushcricket species. Most particularly, these results agree with those of Hill & Oldfield (1981) who investigated the directionality in another bushcricket species, Mygalopsis marki, by recording from auditory units in the cervical connectives. Certain aspects of some reports, however, are not consistent with the data presented here, or by Hill & Oldfield (1981), and these require some comment.

Nocke (1975), recording from the tympanal nerve of Acripeza reticulata, using hook electrodes, found that the magnitude of the L-R response difference increased with frequency up to a maximum of about 15 dB at 8 kHz, then decreased to 5 dB at 16 kHz, and then increased slightly at higher frequencies. He suggested, on the basis of

biophysical measurements of sound diffraction around a plasticine model of the insect, that the directionality was due to diffraction of sound by the insect body. However, it is difficult to understand how 8 kHz could produce such pressure differences around a body of this size (A. reticulata is very slightly larger than T. cantans). In the present study, diffraction produced L-R differences of less than 5 dB at frequencies below 10 kHz (Fig. 2.10). The diffraction measurements of Seymour et al. (1978), for a cylinder of similar dimensions, also do not agree with Nocke's measurements.

Some of the features of the directionality reported by Nocke (1975) for A. reticulata are characteristic of a pressure-gradient system. The directionality is tuned to a particular frequency that could not produce sufficient diffraction to account for the directionality. The auditory organ was also shown to be sharply tuned to this frequency. Nocke found that blocking the sound access to the front surfaces of the tympana, using a tibial sleeve, did not affect the directionality, but it is possible that pressure-gradient interaction could occur between sound components entering the auditory system via the contralateral as well as the ipsilateral acoustic spiracle. The tracheae of the two sides are much more closely apposed in A. reticulata than in certain other tettigoniids, such as M. marki (Hill & Oldfield 1981). The possibility of the bushcricket ear operating as a pressure-gradient receiver at low frequencies has been postulated by several authors (Autrum 1942; Lewis 1974; Seymour et al. 1978) but they

have envisaged interaction of sound components entering via the ipsilateral spiracle and the ipsilateral tympanal slits. The most puzzling feature of the results of Nocke (1975) is the shape of the reported threshold curves for A. reticulata. He showed the auditory organ to be very sharply tuned to 8 kHz (Q_{10} of about 1.6), whereas Hill & Oldfield (1981) showed the organ, in the same species, to be broadly tuned to 7-14 kHz (Q_{10} of about 0.5). This discrepancy needs to be explained before the directionality reported by Nocke can be understood.

While most studies have agreed that the spiracle is the dominant site of sound entry into the auditory system (Lewis 1974; Nocke 1975; Seymour et al. 1978; Hill & Oldfield 1981; Larsen 1981; this study) an alternative mechanism for producing directionality has been proposed by Bailey & Stephen (1978). They reported the occurrence of paired ipsilateral augmentations in the directional response patterns of M. marki, using the technique employed by the present study. These "lobes" occurred in positions that suggested that they were due to sound entry at the tympanal slits. However, the biological significance of these lobes, if they do exist, is doubtful for several reasons. They were reported to occur only over a very narrow frequency band of a few hundred Hz around 11 kHz, whereas the auditory organ is most sensitive to sound of 20-25 kHz (Hill & Oldfield 1981). The song is very broad-banded, although it does have its major peak at about 13 kHz. Furthermore, the lobes amount' to about 6 dB in intact specimens (Stephen & Bailey 1982) which could not be expected to provide

particularly useful directional cues in any case, especially as they are paired. The existence of these lobes was not confirmed by the present study, although it is possible that they may be more important in certain tettigoniid species than others, as the morphology of the tympanal slits varies greatly between species (W. Bailey, personal communication).

Although the directionality patterns at the frequencies tested (Fig. 2.13) show a reasonable L-R difference at higher frequencies, their shape is rather inconsistent. The positions of the contralateral minima also change to some extent with frequency. It is possible, therefore, that directional variation in responses to the natural song, which is very broad-banded (Fig. 2.9), will be such that only a gross L-R discrimination, rather than a fine angular discrimination, is possible.

The bushcricket T. cantans lives in tall dense foliage and the path of a female approaching a singing male will be very complex. Climbing up and down stems will be necessary as there will usually be no direct route (this phonotaxis is not achieved by flight). A precise localization is therefore not essential, at least at long distances. As the female gets closer to the male the spectrum of the song perceived will change (Keuper & Kuhne 1983; Michelsen 1983), high frequencies gradually becoming proportionally more audible as the distance decreases. This phenomenon, coupled with a gross L-R discrimination, may be sufficient to lead the female into the vicinity of the male. At short distances, the role of vibration reception becomes very

important, when the broadcaster and receiver are on the same plant. The coding of the species song by ventral cord auditory units has been shown to be enhanced when presented together with vibration in several bushcrickets (Kalmring & Kuhne 1980; Kuhne et al. 1980). This may be important for the female to unequivocally recognize the singer as a conspecific male. It is possible that the directional cues necessary for the female to approach the male are provided mainly by vibration at these short distances. Localization using vibrational cues is likely to prove very important for tettigoniid phonotaxis. Investigations in this direction have recently commenced (e.g. Latimer & Schatral 1983).

Directionality of the Cricket Auditory Organ

The development of cardioid directionality around the carrier frequency of the calling song (Fig. 2.22) is similar to that reported by Hill & Boyan (1976, 1977) for Teleogryllus commodus. In G. campestris an appreciable L-R difference was usually evident between about 4 kHz and 6 kHz, but differences of more than 10 dB were restricted to frequencies within a band of some 300 Hz either side of the best frequency.

The directionality patterns of most specimens were cardioid, but there was variation in some of the characteristics of these patterns at their respective best frequencies. The main parameters that showed variation were the spatial position of the null, the "best frequency" of the directionality, and the maximum L-R difference at the best frequency.

The position of the null, while near 270° on average, varied from about 240° to 300°. Since the alignment of the preparations, including their leg positions, varied by less than 5° this variability may correspond to the left-right assymetry measured physiologically by Boyan (1979) and behaviourally by Hoy & Paul (1973). Boyan recorded, bilaterally, the responses of single homologous central units in T. commodus, at different angles of sound incidence, and found that the spatial position of equal response in both neurones was rarely directly anterior. Most specimens exhibited a degree of "auditory handedness", by which the position of equal response was consistently to one side of the anterior position. The magnitude and direction of this bias varied between preparations. Hoy & Paul (1973) found that phonotactically orientating females often consistently tracked an emitted calling song erroneously, either to the left or to the right.

The frequency that produced the greatest L-R difference ("best frequency") was at a mean of 4.92 kHz, in G. campestris, with a range from 4.4 to 5.3 kHz. Kleindienst et al. (1981), using a closed-field sound stimulation system, and recording from the "omega neurone" (Wohlers & Huber 1978) in the prothoracic ganglion, showed transmission of sound to the rear surface of the posterior tympanum from the contralateral ear to be best at a mean of 5 kHz, but with a range extending from 3.15 to 5.6 kHz. This variation could explain the range in best frequencies found in the present study, although the range found here is not as great as that reported by Kleindienst.

In a few specimens, supercardioid directionality patterns were found in the intact state. In order to achieve a perfect null by the pressure-gradient system the phase and pressure of the sound incident on the front and rear surfaces of the tympanum must be equal (Hill & Boyan 1977; Larsen & Michelsen 1978). If the phase angles of the sounds are equal, but the sound pressures are not, an imperfect null will be produced, although the directionality pattern could still be cardioid. Furthermore, in the ideal situation, the sound components acting on the two surfaces of the tympanum should change from being out of phase (ideally 180° relative phase) to being in phase (0° relative phase) when the sound source is moved from the ipsilateral to the contralateral position. This perfect situation would produce a 6 dB augmentation ipsilaterally and an ∞ dB diminution at the contralateral position. If the relative phase at the contralateral position does not reach 0° a cardioid pattern with an imperfect null will result. If the relative phase reaches 0° before the contralateral position a supercardioid pattern with perfect nulls will result (assuming that the sound pressures are equal). Thus the variation in the cardioid patterns observed, and the occurrence of supercardioid patterns, may be explained on the basis of imperfect matching of pressure and/or phase of the sound components interacting at the tympanic membrane. One possible reason for this mismatch could be that since the position of the null was rarely exactly on the interaural axis it may also have been rarely located exactly on the horizontal plane. No investigations into the effects

of elevation were made. It may be that the closely cultured experimental animals used in this study were imperfect, due to the age of the culture, and so did not show the normal response patterns. Alternatively, it may be that a perfect cardioid pattern is not essential, and a mere gross left-right difference is all that is needed. This possibility is discussed below.

The directionality patterns constructed at the best frequencies represent the maximum degree of directionality possible in the horizontal plane. It is clear from Figs 2.22,2.26 that this may only be realized at one frequency; even sound 100 Hz away from the best frequency will produce a maximum L-R difference that is reduced by several dB. The main energy peak in the calling song of G. campestris extends over about 500 Hz, between 4.5 and 5 kHz (Fig. 2.17), so that the directional variation in the responses to the species song will probably be less than to the best pure-tone frequency.

The results of investigations involving manipulations of the auditory system by blockages need to be interpreted with caution as blockage of one input may change the acoustic properties of the system for sound entering via other input sites (Larsen & Michelsen 1978). In the present study this is particularly true for blockage of the ipsilateral spiracle; its blockage may cause a change in the impedance of the acoustic trachea. Nevertheless, it is clear from Figs 2.24,2.28 that little directionality is achieved when both the spiracles are occluded, whereas blockage of the contralateral tympanum has very little

effect. These findings are contrary to those of Hill & Boyan (1976,1977), who found, in T. commodus, that either the spiracular inputs or the contralateral tympanum input alone provided a back pressure at the tympanum sufficient to provide considerable directionality, although all the inputs were required for a normal response pattern. They found that blocking the contralateral tympanum alone, or blocking both spiracles, decreased the L-R difference only slightly, but blockage of the contralateral tympanum and the spiracles together abolished almost all the L-R difference.

The effects of unilateral spiracular blockage show that both inputs are necessary to produce a normal directionality pattern (Fig. 2.25). Sound access to the rear surface of the tympanum via the ipsilateral spiracle alone would not be expected to produce much directionality by pressure-gradient interaction at the tympanum because the difference in the path lengths of the sound components reaching the tympanum can never be very long (although it is possible that additional phase changes could be produced by the morphology of the trachea). The mean value of 8 dB, for the maximum L-R difference with the contralateral spiracle blocked, is close to the figure of 9 dB calculated theoretically for this condition by Thorson et al. (1982).

Sound input via the contralateral spiracle alone (ignoring the contribution of the contralateral tympanum) produced a supercardioid directionality pattern that was strongest at a frequency lower than the normal best frequency (Figs 2.25,2.27). It is possible that blockage of

the ipsilateral spiracle changes the best frequency of transmission across the body, thereby changing the tuning of the directionality. In any case, the system does not appear to act in the same way as the 2-input pressure-gradient system that has been described for various vertebrates (e.g. Coles et al. 1980; Feng & Shofner 1981) because the separation of the paired nulls of the supercardioid patterns do not change with frequency. The same is true for the supercardioid patterns observed in intact preparations. The 2-input system functions in a manner similar to a pressure-gradient microphone (Beranek 1954).

The comparison of the thresholds at the 90° position and at the null (Fig. 2.26) indicates the variation in L-R difference with frequency, and shows that the intact state null is tuned to approximately the same frequency as that to which the auditory organ is most sensitive. The tuning of the auditory organ to the calling song carrier frequency has been shown to be primarily due to factors other than the characteristics of the acoustic tracheae (Ball & Hill, 1978; Larsen & Michelsen, 1978), although these characteristics may enhance the tuning. Blockage of the ipsilateral spiracle produced supercardioid directionality with nulls tuned at a lower frequency than in the intact state (Fig. 2.25), but it is clear from Fig. 2.26 that such blockage does not affect the tuning of the auditory organ, as measured by ipsilateral thresholds. In the example shown the organ was tuned to 4.9 kHz in both the intact state and when the ipsilateral spiracle was blocked.

The transmission experiments described were performed as a further test for any species differences between G. campestris and T. oceanicus. They confirm that the magnitude of the back pressure provided by the spiracular inputs is considerably greater than that from the contralateral tympanum in both species. While some of the results of Hill & Boyan (1977) do not agree with those of the present study, the results of their transmission experiments are mostly in agreement with those described here (see their Fig. 2). They found that blocking the contralateral tympanum had no effect on the thresholds of a ventral cord unit when the ipsilateral tibia was surrounded by a metal tube, but that subsequently blocking the spiracles greatly increased the thresholds. G. campestris is morphologically very similar to Gryllus bimaculatus, but Larsen (1981), measuring transmission of impulse sounds in the tracheal tubes, found that the acoustic trachea produces a gain of 3.5 in G. bimaculatus and T. commodus, and a gain of 1.5 in G. campestris and Acheta domesticus. These impulse sounds had a very broad spectrum, strongest between 18 and 42 kHz. There have been no reports, however, of differences in the auditory processing of frequencies around 5 kHz between these cricket species.

The findings of the present study also suggest that the ipsilateral spiracle allows more sound input than the contralateral spiracle. However, the difference between the two inputs was not found to be as great as has been suggested by Larsen & Michelsen (1978) or by Larsen (1981). In any case, this does not necessarily mean that the

ipsilateral spiracular input is the more important in providing directionality, since phase changes are at least as important as pressure levels. The total back-pressure, i.e. the sound reaching the rear surface of the tympanum via all three sites together, varied considerably with the direction of the sound source. This indicates that the magnitude of the back-pressure, as well as its phase, changes with sound direction, and is another observation demonstrating that the pressure-gradient function in these insects is considerably more complex than the 2-input pressure-gradient system of other animal groups.

Clearly, any model based on the dimensions of the auditory system of these crickets (e.g. Hill & Boyan 1977) must be at best incomplete, as no correlation between insect size and calling song carrier frequency has been demonstrated. It would be expected that smaller crickets would have to use sound of shorter wavelength for intraspecific communication, yet this does not seem to be the case. For example, G. campestris is slightly larger than T. oceanicus, yet its calling song has its carrier frequency at a slightly higher frequency (hence the sound is of shorter wavelength); T. oceanicus and T. commodus are the same size but T. commodus produces a calling song of about 3.7 kHz (Loftus-Hills et al., 1971), compared to the 4.5-5 kHz of T. oceanicus (Hutchings & Lewis 1983b). Factors which might be involved in the mechanism, not so far investigated in detail, include the role of the thin septum dividing the two auditory systems, and also the degree of patency of the acoustic spiracles.

Although results involving blockages must be interpreted with caution, it is of interest to consider such results in the context of behavioural experiments conducted under the same conditions. If one spiracle of a free walking cricket is blocked, the ear ipsilateral to the blockage will have a supercardioid directionality pattern while the other will show almost omnidirectional responses (Fig. 2.25). Assuming that the insects use the point of maximum response difference between the two ears for localization, it might be expected that such an insect would show errant phonotaxis towards the intact side, as no nulls can occur on the blocked side. There may also be some front/rear error, as there will be two points of maximum L-R difference. Wendler et al. (1980) showed behaviourally that G. campestris females with one blocked spiracle tracked a target along a path that was at an angle to the correct course, the error being always towards the intact side. They did not, however, report any front/rear confusion.

The variation in the shape of the directionality patterns constructed in this study suggest that their exact shape is not critical and that a gross L-R discrimination may be all that is necessary. The zig-zag path of a female approaching a conspecific singing male has been described by several workers (Murphey & Zaretsky 1972; Bailey & Thomson 1977; Schmitz et al. 1982) and this behaviour suggests that angular discrimination is not very precise. However, investigations designed to measure the minimum audible angle for side discrimination ("lateralization") by walking crickets have demonstrated side discrimination of 12-14° in

T. oceanicus (Oldfield 1980), 10° in Scapsipedus marginatus (Bailey & Thomson 1977) and 15° in G. bimaculatus (Rheinlaender & Blatgen 1982). Furthermore, it has been shown that orientating females grade the magnitude of their turns, to some extent, according to the angle of the sound source (Bailey & Thomson 1977; Oldfield 1980). These findings suggest that angular discrimination is possible.

Female crickets can approach singing males not only by walking but also by flying (Ulgaraj & Walker 1973; Moiseff et al. 1978). Experiments on tethered flying crickets by Pollack & Plourde (1982) have shown that flying T. oceanicus can discriminate angles only 5-10° from the anterior position. During tethered flight no leg or body scanning movements are possible, so these experiments also imply very accurate side discrimination. A particularly interesting observation of Pollack & Plourde (1982) was that the magnitude of the turning response was approximately equal when the sound source was placed anteriorly or posteriorly, i.e. 170° produced the same response as 10°, and 130° produced the same response as 50°. This front/rear error is exactly what could be predicted when no references are provided by body and/or leg movements, given the directional cues that have been shown to be produced by the auditory organs in this study.

The observations of this study illustrate two distinct strategies by which the auditory organs of insects may provide directional cues for sound localization. The ear of the bushcricket T. cantans functions as a pressure receiver

for localizing broad-banded, high frequency sounds, while the ear of the crickets G. campestris and T. oceanicus functions as a pressure-gradient receiver, providing cues for localization of narrow-banded, low frequency sounds. A pressure receiver depends on sound diffraction to produce directional variation in the pressure detected, whereas pressure-gradient receivers depend on access of sound to both surfaces of the tympanum so that phase interactions produce directional variation in the net pressure detected.

In the species studied here, there is a clear distinction between the mechanisms of the two groups; one is a fairly clear-cut pressure system and the other a pressure-gradient system. However, it may be that some insects utilize a system that lies between these two extremes. Miller (1977) showed that although the locust ear functions as a pressure system at high frequencies (above 10 kHz), it acts as a mixed pressure and pressure-gradient receiver at frequencies of 2-8 kHz. The tympana are situated on the abdomen in locusts (Acrididae), and there is no tracheal connection between the auditory systems of the two sides, but at these low frequencies sound seems able to pass across the body with little attenuation. Several other workers have suggested that the ears of certain bushcricket species may function as pressure-gradient receivers at low frequencies and pressure receivers at high frequencies (e.g. Seymour et al. 1978). Thus it is likely that there is a broad spectrum of mechanisms, and the systems employed by different insects may lie anywhere between pure pressure systems and pure pressure-gradient systems.

CHAPTER 3

CODING OF AUDITORY INFORMATION IN IDENTIFIED VENTRAL-CORD NEURONES IN THE CRICKET *GRYLLUS CAMPESTRIS*

3.1 INTRODUCTION

Most of the neural investigations into auditory reception in the Gryllidae have been carried out fairly recently compared to studies on other orthopteran groups, such as the locusts and bushcrickets. The earliest reports were of recordings from the afferents in the tympanal nerve and from the cervical connectives ascending from the prothoracic ganglion. Comparison of the responses at these two levels shows the degree of integration at the prothoracic ganglion. These early studies employed whole-nerve recording techniques, usually using silver or tungsten hook electrodes (Nocke 1972) or glass or PVC suction electrodes (Hill 1974). Mass responses so recorded could show the preferred frequency and/or intensity ranges of the ear or the CNS as a whole, but although "single units" were often distinguishable in the recordings, these were inevitably the largest fibres in the connectives.

Since the adoption, in the mid-seventies, of glass micropipettes as the standard electrodes progress has been very rapid. They have enabled recordings from unequivocally single units, presumably of all but the smallest size. They have also permitted intracellular recordings to be made within the prothoracic ganglion itself (Popov et al. 1978; Wohlers & Huber 1978) and, recently, in the brain (Boyan 1981). Recordings from axons in the cervical connectives, however, are still all extracellular (Hutchings & Lewis 1983b).

Coupled with the use of glass microelectrodes for recording has been the use of stains to mark the recorded units. Initially cobalt electrolytes were used in the microelectrodes, and the resulting cobalt release stained the recorded units. These cobalt stains were often subsequently silver intensified as described by Bacon & Altman (1977). This method is suitable for intracellular and extracellular recordings. More recently, the use of fluorescent Lucifer yellow as the intracellular marker has become popular (Wohlers & Huber 1982). Unfortunately, with either technique neurones are rarely completely filled from the prothoracic ganglion to the higher centres; most recordings made in the prothoracic ganglion or the cervical connectives reveal only the prothoracic ganglion anatomy.

Since the earliest investigations, the intention has been to correlate neurophysiological and behavioural data, much of the latter being already available when physiological recordings were first made. Zaretsky (1971) claimed to demonstrate the first neurone in the CNS (the ♀-neurone) that responded specifically to the conspecific calling song, in a species of Liogryllus. Stout & Huber (1972) described two units in the cervical connectives of Gryllus campestris that responded to specific parameters of the calling song; the "pulse-coder" copied the syllable structure well while the "chirp-coder" coded the duration of each chirp as a whole, but did not code each of the syllables within each chirp. Since these studies, much effort has been concentrated on determining how such coding is achieved.

Cricket songs may be readily synthesized electronically, and the responses of individual auditory neurones to the different song parameters have been investigated, particularly in the frequency domain. It is hoped that such data will establish the relative importance of the different song parameters for the processes involved in recognition. Neurally, species specific song recognition would be best demonstrated experimentally by recording a unit that responded only to the complete conspecific song. No such neurone has yet been found, but at the lower levels of the CNS some degree of recognition can be assumed if a unit responds preferentially to one or more parameters of the conspecific songs, such as their spectral or temporal patterns.

Until recently, neurophysiological studies have generally used pure-tone stimuli, presented as single tone bursts or amplitude modulated into the species song patterns. This has been largely due to the fact that most gryllid songs are essentially narrow-banded, although lower intensity harmonics also occur (Hutchings & Lewis 1983b). However, it is now becoming evident that the overall spectral content of the songs may be important in the recognition process, at least in some species (Hutchings & Lewis 1983b). Wohlers & Huber (1982), recording intracellularly from prothoracic ganglion neurones in G. campestris, reported that certain frequencies elicited IPSPs in some of the recorded units. This suggests that the responses of the interneurones ascending from the prothoracic ganglion result from integration of excitatory

and inhibitory inputs from the primary fibres and/or lower-level interneurones. Wohlers & Huber (1982) characterized the physiological and morphological characteristics of 3 units, in G. campestris, that send axons anteriorly from the prothoracic ganglion. Two of these had ascending axons only; one was sharply tuned to the carrier frequency of the species calling song, AN1 (ascending neurone 1), and the other was broadly tuned to high-frequency sound - AN2. A "through-unit" (TN1) had a descending as well as an ascending axon, and responded weakly to mid/high frequency sound. The cell bodies of all 3 were located within the prothoracic ganglion. The present study carried out further investigations into the integration of the primary responses to different frequencies by these and other units ascending from the prothoracic ganglion.

By simultaneous presentation of two frequencies, inhibitory as well as excitatory effects may be demonstrated, although without intracellular recordings it is still difficult to establish whether or not integration occurs primarily in the recorded unit. A similar technique was employed by Boyan (1981), who investigated an auditory neurone in the brain of Gryllus bimaculatus that responded positively to high frequencies but was inhibited by frequencies around the carrier frequency of the calling song. Hutchings & Lewis (1983b) also used this method to study responses of prothoracic ganglion neurones in Teleogryllus oceanicus.

As well as studying the integration of the responses produced to different sound frequencies, studies were also performed on the processing of vibratory information at the level of the prothoracic ganglion. Although much work has been carried out on the processing of vibration in other orthopteran groups, notably locusts and bushcrickets (e.g. Dambach 1972; Kalmring et al. 1978a), very little information is available on such processing in crickets. In the present study vibratory stimuli were applied to all six legs, both alone and together with sound stimuli. Information was thereby gained on interaction of sound and vibration inputs as well as information on responses to vibration alone.

3.2 MATERIALS AND METHODS

3.2.1 EXPERIMENTAL ANIMALS

The crickets used in the study were adults of both sexes of G. campestris. They were maintained as a laboratory culture as described in Section 2.2.

3.2.2 STIMULUS GENERATION DURING SINGLE-UNIT RECORDING

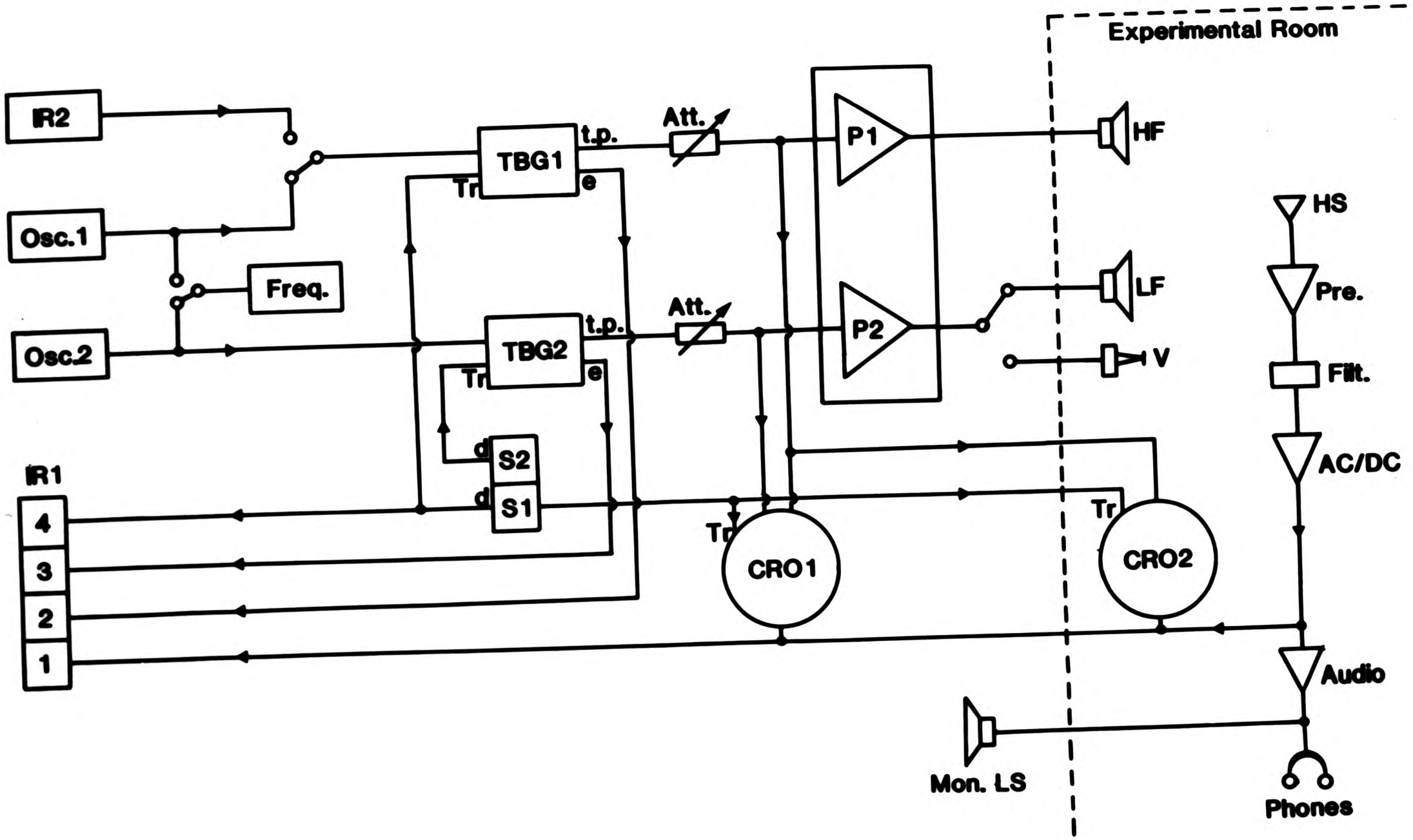
(A) Single Pure-tone Pulses

The circuit for the production of the stimuli is given in Fig. 3.1. Sine-waves were produced by two function generators, Osc.1 and Osc.2 (Farnell FG2 and FG3 respectively). The frequency of their outputs was monitored by a digital frequency counter (Heathkit) and the output of each was passed to a tone burst generator (TBG1, TBG2 in Fig. 3.1). These were identical to the TBG described in section 2.2. A two-channel stimulator (Grass S88) was used, each channel (S1, S2 in Fig. 3.1) triggering one TBG using the "delay" output. The tone pulses were then passed to a 2-channel (P1,P2) power amplifier (Quad 405) via attenuators (Hatfield) that could alter the signal voltage in 1 dB steps. Channel 1 was used for sound stimuli of 5 kHz and upwards; channel 2 was used for sound below 5 kHz, or for the vibration stimulus. The output of P1 was therefore connected to a high-frequency loudspeaker (Audax TW8B, specified frequency range 1-40 kHz ± 10 dB) and the output of P2 could be switched to either a low-frequency loudspeaker (Audax HD17/HR37, frequency range 100 Hz

Fig. 3.1

Experimental circuitry employed during recording of the responses of single units in the cervical connectives.

AC/DC - AC/DC amplifier
Att. - attenuator
Audio - audio amplifier
CRO1,2 - cathode ray oscilloscope 1, 2
d - delayed trigger output
e - envelope output of TBG
Filt. - filter module
Freq. - frequency counter
HF - high-frequency loudspeaker
HS - headstage
IR1, 2 - instrumentation recorder 1, 2
LF - low-frequency loudspeaker
Mon. LS - monitor loudspeaker
Osc.1,2 - oscillator 1, 2
P1, P2 - 2-channel power amplifier
Phones - headphones
Pre. - preamplifier
S1, S2 - 2-channel stimulator
TBG1,2 - tone-burst generator 1, 2
t.p. - tone pulse output of TBG
Tr - trigger input
V - minishaker


Experimental Room
IR2
Osc.1
Freq.
Osc.2
TBG1
TBG2
t.p.
Att.
P1
P2
HF
LF
V
HS
Pre.
Filt.
AC/DC
Audio
Phones
IR1
4
3
2
1
S2
S1
CRO1
CRO2
Mon. LS

- 10 kHz, ± 8 dB) or a minishaker (Bruel & Kjaer 4810, frequency range DC to about 11 kHz with the T-piece attached) which produced the vibration stimuli. Together with the tone-pulses, the TBG also produced a rectified trapezoidal DC outline ("envelope") of the pulses and these were led to channels 2 and 3 of a four-channel instrumentation recorder, IR1 (Phillips Ana-Log 7). During the production of stimuli the main monitor oscilloscope CR01 (Tektronix D13) was triggered by the "non-delay" output of S1 and the stimuli were displayed from the inputs of P1 and P2. The same information was also displayed on CR02, within the experimental room.

(B) Two-tone Pulses

Both of the TBGs could be triggered together by the two channels of the stimulator. Thus the preparation could be presented with sound of two frequencies simultaneously. Alternatively, channel 2 could be used to provide vibration at the same time as sound was generated by channel 1. Using the delay controls on the stimulator it was also possible to move the onset of the two stimuli, relative to one another, in order to determine temporal features of two-tone interactions.

(C) Pulse Trains

The stimulator could be set to produce trains of triggers, thus generating trains of tone pulses from either TBG. The duration and frequency of the trains, the frequency of the triggers within each train, and the duration and rise/fall of the pulses thus produced were adjusted to achieve trains

of pulses that approximated to the temporal pattern of the species calling song. This "synthesized natural song" (SNS) could then be filled with whatever carrier frequency was required. Both channels of the TBG could be set to produce these trains simultaneously, with different carrier frequencies, or it was possible to produce a synthesized chirp in one channel and a continuous tone with the duration of the synthesized chirp in the other channel. This was often carried out when testing the effect of vibration on responses to SNS. In this case, channel 1 produced acoustic SNS while channel 2 produced continuous vibration during each chirp.

(D) Pre-recorded NS and SNS

The three previous stimulus types were all generated during each experiment. In addition to these, a pre-recorded tape was produced containing short sections of the three natural songs, together with sections of SNS filled with selected frequencies. This tape could then be replayed during an experiment whenever required.

Recordings of the three natural songs were made onto IR1 as described in section 2.2. Short sections of each were then dubbed onto IR2 (Racal Store 4), at 15 i.p.s. in DR (direct record) mode (frequency response 100 Hz - 75 kHz ±3 dB), together with relevant announcements on the voice channel. The sections of SNS were amplitude modulations of the three song types, with appropriate temporal characteristics, but filled with either 5 kHz, or 16 kHz, or a combination of both. These were produced as described for

the pulse trains, except that only the channel 1 TBG was used. The parameters of S1 and TBG1 were set to produce aggression and courtship SNS as well as calling SNS.

The spectra of the SNS were analysed as described in section 2.2. These are shown in Fig. 3.2. The peak at the fundamental frequency in each case had a Q of about 8.0. Secondary peaks occurred at harmonic frequencies, but these were more than 40 dB less intense.

3.2.3 CALIBRATION OF THE LOUDSPEAKERS AND THE MINISHAKER

The output of the loudspeakers had to be periodically calibrated in a similar manner to the calibrations described in Section 2.2. It was also important to check that the sound field was homogeneous around the preparation as there were several objects in the vicinity, such as the minishaker and the preparation stand, which could cause sound diffraction. The output of both loudspeakers was calibrated by measuring the sound pressures produced at different frequencies with a given attenuation. The low-frequency loudspeaker was calibrated from 1 to 10 kHz in steps of 1 kHz; the high frequency loudspeaker was calibrated from 5 to 40 kHz in steps of 1 kHz up to 20 kHz, and in larger selected steps above 20 kHz.

A ¼" condenser microphone (B & K 4135) was positioned horizontally above the preparation plate, with the grid at the position normally occupied by the ipsilateral foreleg of the preparation, and with the diaphragm facing the sound source. For each frequency tested a pure-tone was produced

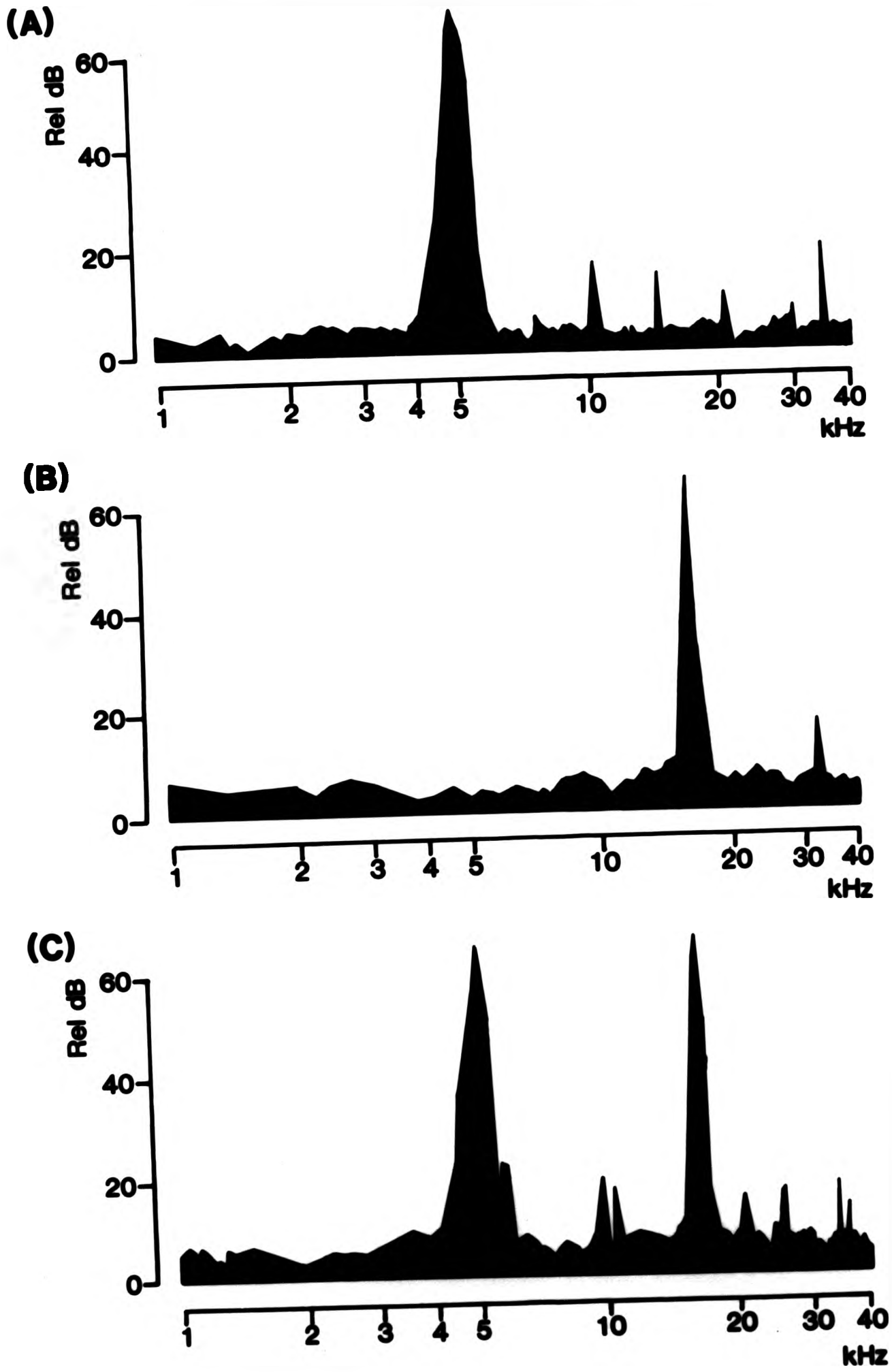
Fig. 3.2

Spectral analysis of synthesized natural song (calling song temporal pattern).

(A) 5 kHz

(B) 16 kHz

(C) 5 kHz + 16 kHz combined


(A)
Rel dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz
(B)
Rel dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz
(C)
Rel dB
60
40
20
0
1
2
3
4
5
10
20
30
40
kHz

in the same way as the tone bursts, but with the TBG set to provide a continuous duration. Sound pressures were recorded using the microphone in conjunction with a frequency analyser (B & K 2107) as dB relative to 2×10^{-5} Pa. A table was thereby constructed of the sound pressures produced at any given frequency and attenuation setting.

The variation in the sound pressure around the preparation was checked by moving the microphone around in the region of the preparation stand. The sound field was found to be uniform to within ± 2 dB up to 40 kHz at a distance of 5 cm around the preparation.

The output of the minishaker was calibrated from 30 Hz to 10 kHz using an accelerometer (B & K 4369) in conjunction with the frequency analyser. The accelerometer produced a voltage output proportional to the acceleration produced by the minishaker for a constant voltage input. The minishaker also produced a small degree of airborne sound at high input levels, particularly at high frequencies. The level of this output was measured using the condenser microphone as for the loudspeaker calibrations, with the minishaker in its normal position.

3.2.4 THE EXPERIMENTAL ROOM

A plan of the room in which the preparation was set up during recording is shown in Fig. 3.3A. The preparation stand, together with the electrode mounting, was located on a metal baseplate (100 x 60 cm) just outside the opening of

Fig. 3.3

Organization of the experimental room.

(A) Plan of the room showing position of the sound tunnel and Faraday cage enclosing the preparation.

B - beam support

ER - equipment rack

FC - Faraday cage

LS - loudspeakers

S - stool

ST - sound tunnel

(B) View inside the Faraday cage.

eh - electrode holder

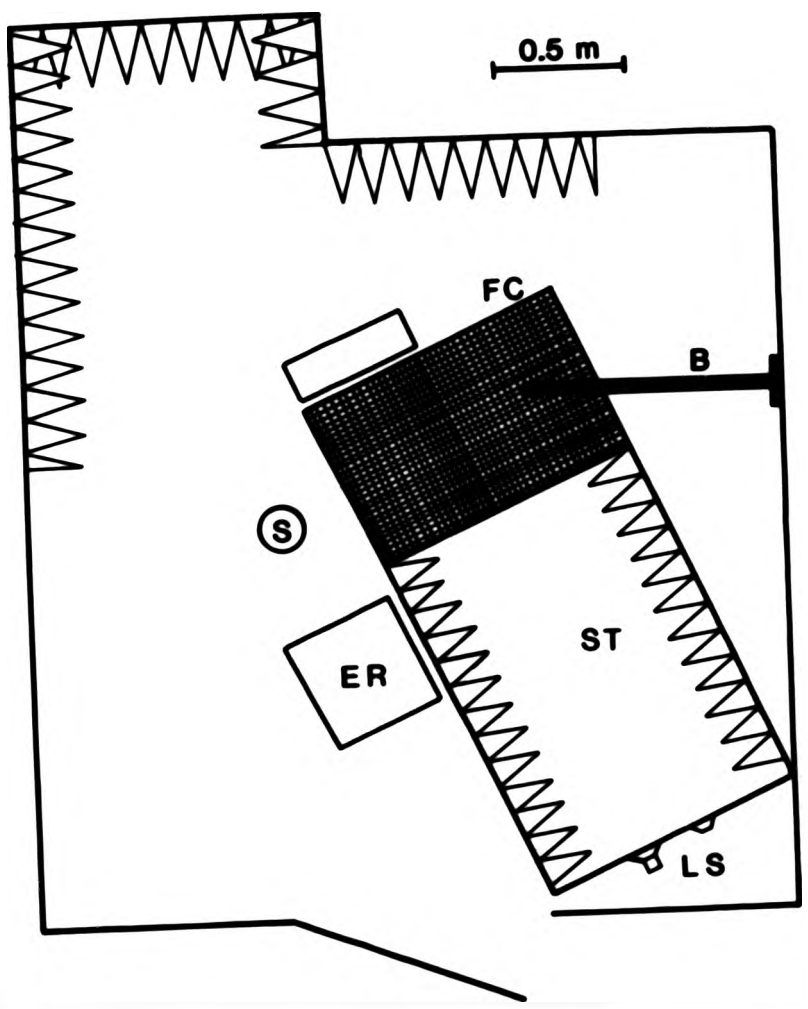
hs - headstage

lg - light guide

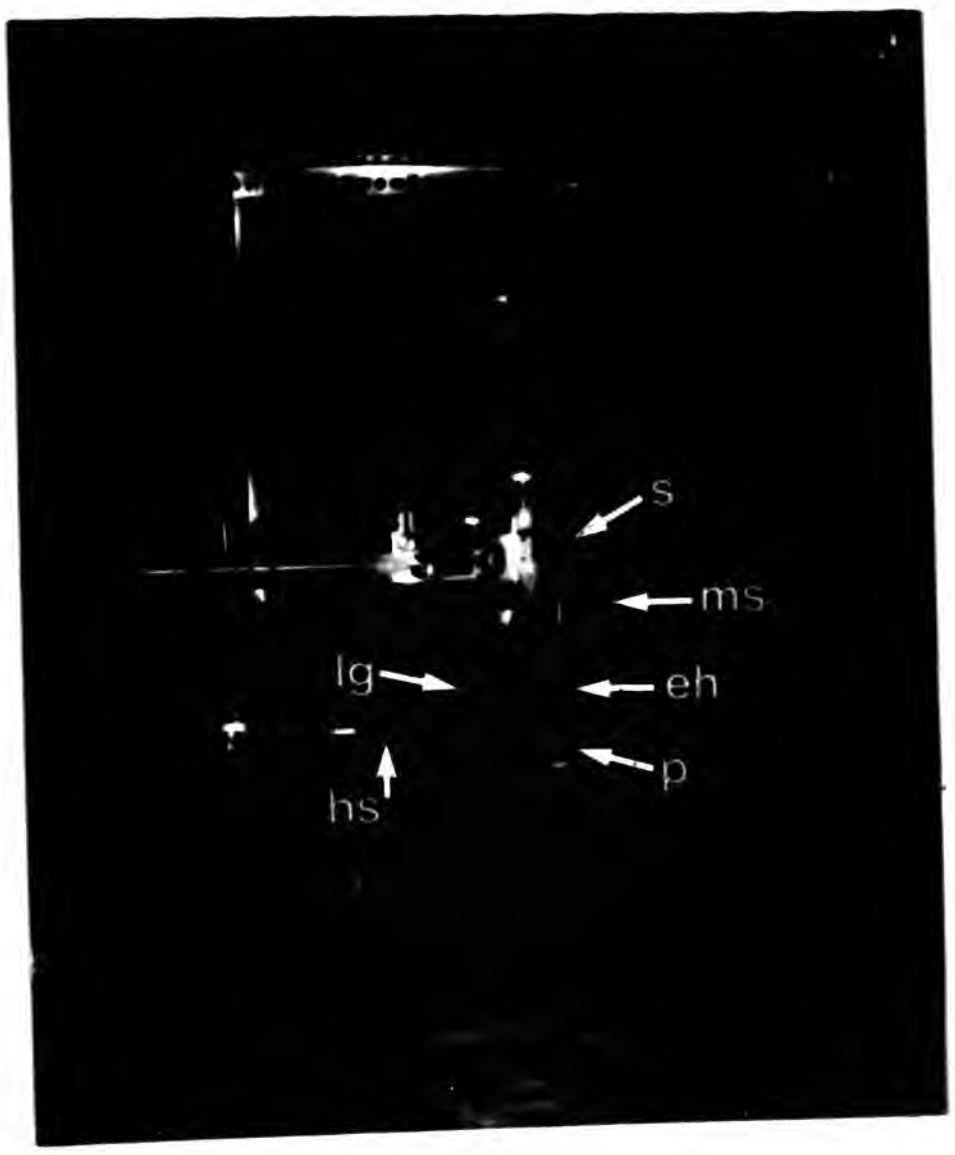
ms - minishaker

p - preparation

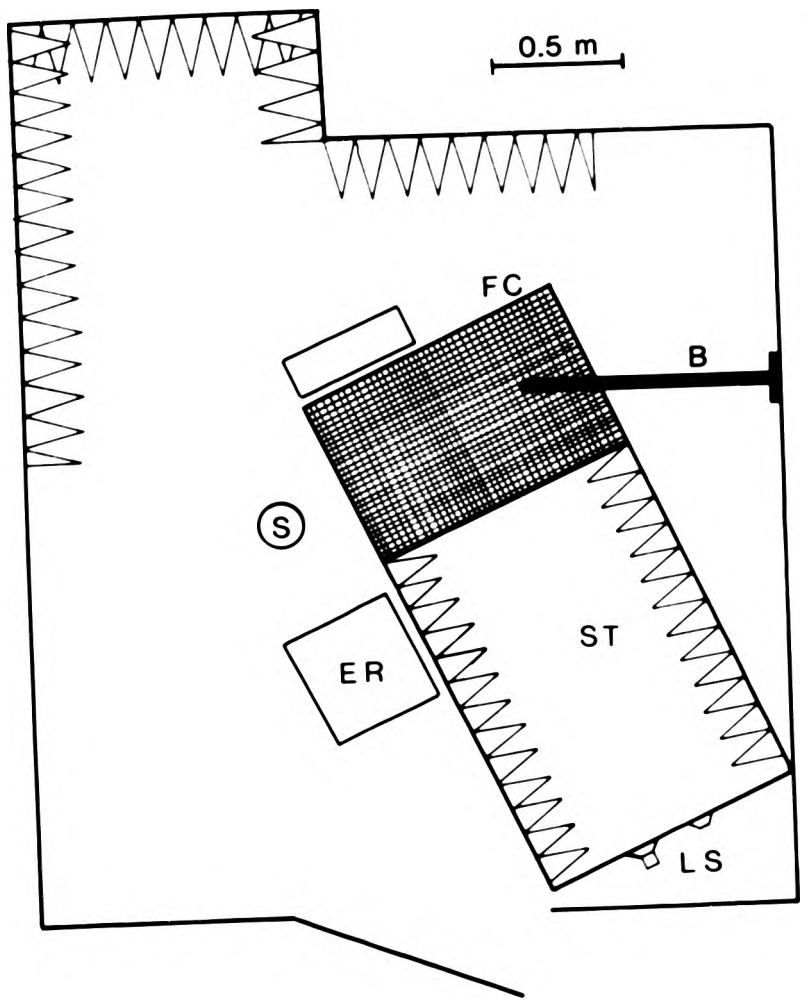
(A)



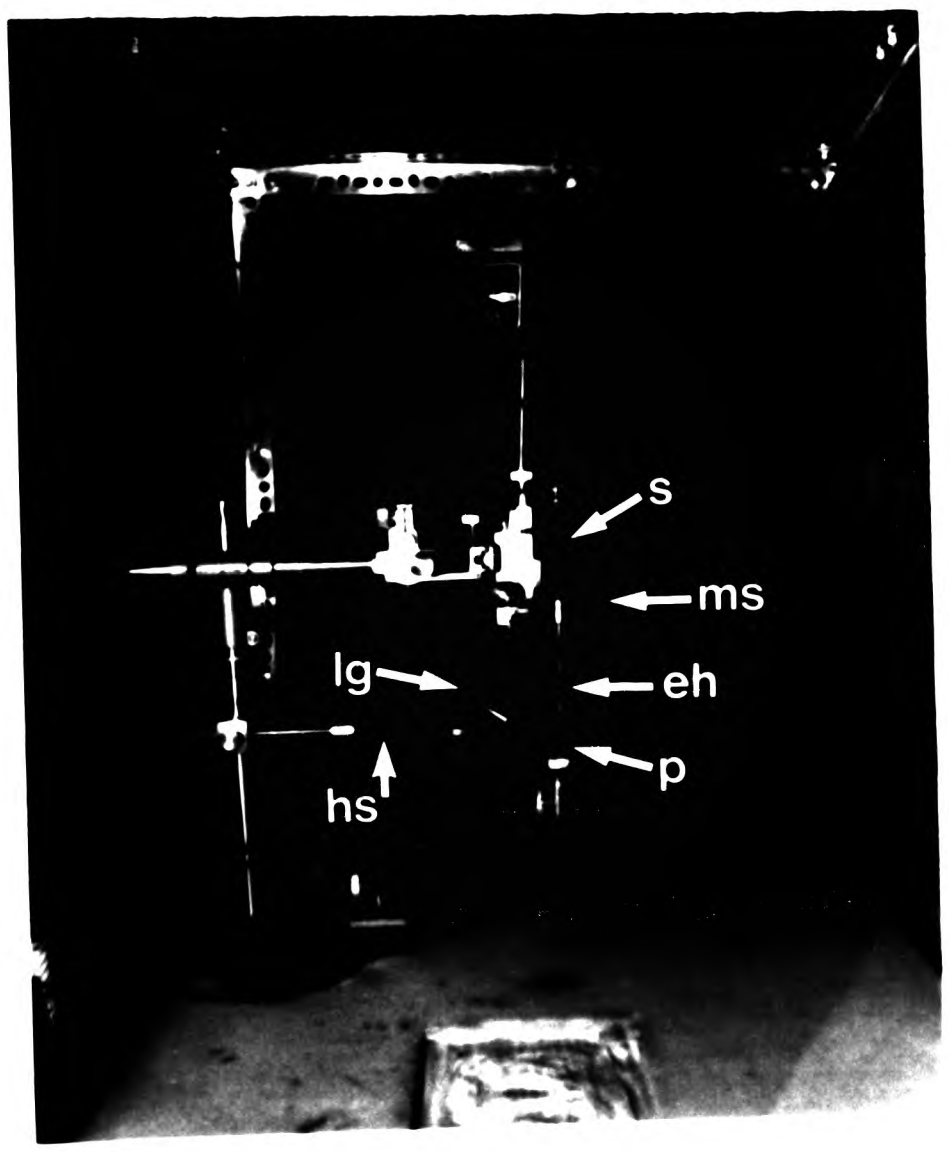
(B)



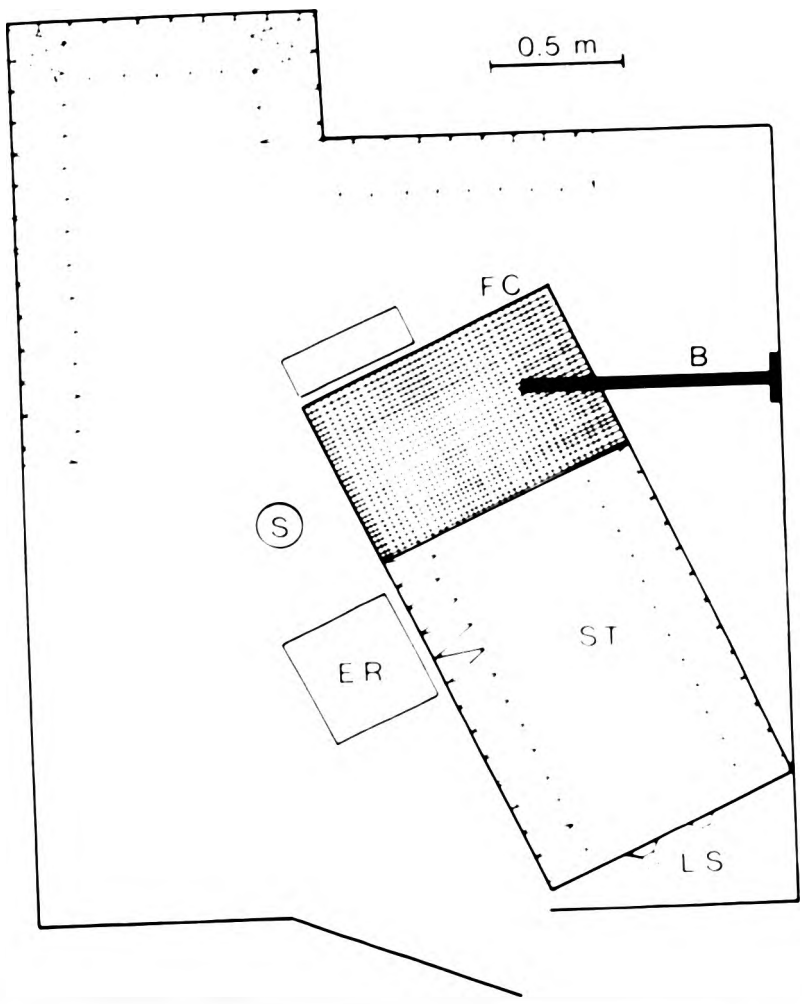
(A)



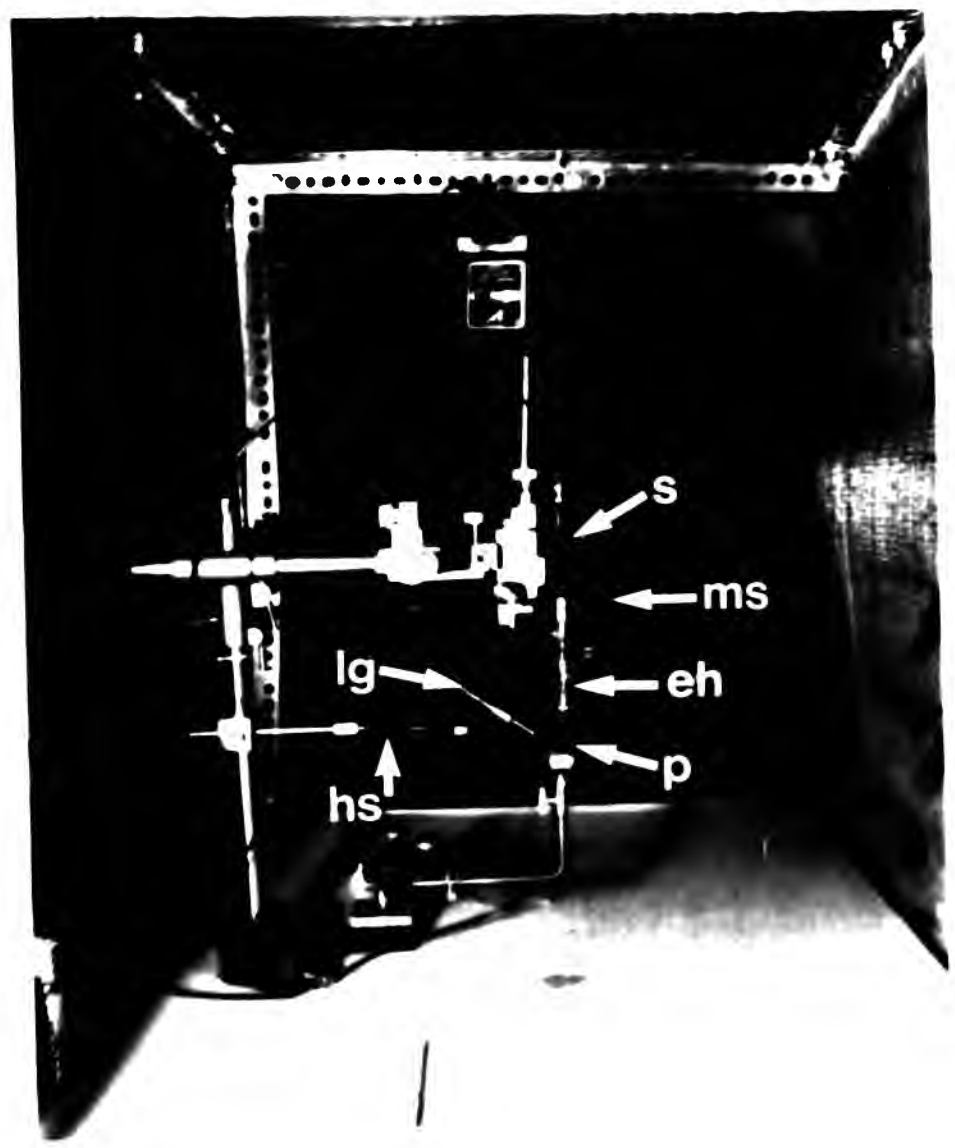
(B)



(A)



(B)



a sound tunnel. The baseplate was covered with 3 cm foam rubber. The sound tunnel (1.0 x 1.0 x 1.3 m) was constructed of a 2 cm chipboard shell lined with mineral wool (Rockwool, density 100 kg/m , 60 mm thick) cut into wedges having a calculated lower cut-off frequency of 212 Hz. The loudspeakers were fixed into the closed end of the tunnel which served to produce a plane sound wave at the preparation. The metal baseplate and the base of the tunnel were positioned 95 and 75 cm above the floor respectively, but with the top of the wedges on the base of the tunnel above the level of the baseplate.

The walls opposite the open end of the tunnel were also covered with mineral wool wedges to prevent echoes reaching the preparation. The minishaker was suspended from a bracket bolted to the wall to one side of the sound tunnel, and on the opposite side, out of the sound path, was a rack housing the initial amplification stages and a monitor oscilloscope, CRO2. The apparatus on the baseplate was surrounded by a Faraday cage constructed of 5 mm wire mesh, on a Dexion frame, that fitted over the baseplate. The cage was open only on the side facing the experimenter during preparation set-up.

A view of the inside of the Faraday cage is given in Fig. 3.3B. The preparation plate was attached to the support plate (10 x 18 x 38 mm) fixed to a brass rod. This was attached to a micromanipulator (Prior), allowing precise positioning of the preparation. A steel pole (20 mm diameter) mounted on a second magnetic base (Eclipse 901) supported a second micromanipulator allowing coarse

positioning of the electrode. This micromanipulator held the slave cylinder of a hydraulic microdrive (Clark HMD-1M), the control of which was placed just outside the Faraday cage and below the level of the baseplate. The electrode holder was attached to the slave cylinder by a small brass fitting. This received the signal from the electrode holder which was passed to the headstage by a short lead. A second lead connected the indifferent electrode to the headstage. The T-piece of the minishaker was positioned just above the preparation so that the three pairs of legs of the preparation could be waxed to it when required.

3.2.5 MICROELECTRODES

The electrodes used were glass micropipettes filled with a 3-Molar cobalt-chloride electrolyte. These allowed good physiological recordings with simultaneous staining of the recorded units. Most of the units were recorded extracellularly and under these conditions the use of the cobalt electrolyte rather than a light metal electrolyte such as potassium does not seem to impair the responses of these units during recording to any great extent (Hutchings, personal communication).

It was found that the performance of the electrodes was more reliable when they were pulled immediately prior to an experiment. Less reliable results after long periods may have been due to collection of dust particles, although the electrodes were kept in a closed petri dish after pulling. Glass tubing (1.5 mm) was used (Clark Electromedical

Instruments, GC150F) which had a fine glass capillary within the lumen, facilitating quick filling. The electrodes were pulled on a vertical action electrode puller (SRI) and were filled with electrolyte using a syringe with 37-gauge needle. Filling was carried out immediately prior to the use of each electrode as the tip tended to become blocked with crystals if left for any length of time in air. The electrolyte was made up freshly and thoroughly filtered every 3-4 weeks to avoid excessive crystalization.

The controls of the electrode puller were adjusted to obtain the tip characteristics required. A fairly short shank was needed with high impedance. The impedance was measured using a DC amplifier (Neurolog NL102) and was usually 15-35 MΩ when filled with 3M cobalt chloride. These measurements were repeated every few weeks to check for drift.

3.2.6 EXPERIMENTAL PROTOCOL

(A) Preparation Dissection and Set-up

Each specimen was initially carefully inspected to ensure that it was morphologically normal, particularly at the tympana. The specimen was immobilized by a short exposure to carbon-dioxide and the wings and antennae removed. It was then waxed (Cottrel Sticky Wax), ventral side upward, to a thin perspex plate (40 x 10 x 1 mm). A short silver wire, soldered to a 1 mm Cambion plug fixed into the perspex plate, was inserted into the abdomen to serve as an indifferent electrode. When the preparation was to be

tested for responses to vibration as well as sound all three pairs of legs were left intact but immobilized with plasticine during the rest of the dissection. When the preparation was to be tested for responses to sound alone the meso and metathoracic legs were removed and the forelegs lightly waxed to fine tungsten wires on the plate, in a position 90° to the longitudinal body axis, and as near as possible to the normal standing posture.

The mouthparts were removed and the tissue around the pharynx cut to free it. The fore and midgut were then removed via an incision made in the abdomen. The resultant space in the abdomen was filled with tissue soaked in "Clarke's Ringer". A rectangular window was cut in the thin ventral cuticle of the neck to expose the cervical connectives. As little of the surrounding musculature as possible was disturbed, but it was usually found beneficial to insert a small piece of Ringer-soaked tissue through the mouth cavity and under the connectives to provide support during penetration by electrodes.

The preparation, mounted on the perspex plate, was attached to the support plate in front of the opening to the sound tunnel (Fig. 3.3B) and a lead was attached to the Cambion plug to connect the indifferent electrode to the headstage. The preparation was aligned with the body axis normal to the direction of the sound source so that one foreleg pointed towards the sound source (this side is hereafter termed ipsilateral). When vibration responses were being investigated the T-piece of the minishaker was positioned about 5 mm above the centre of the preparation,

at right-angles to the body axis. The tips of the legs were then lightly waxed to the T-piece. A small drop of distilled water was placed on the connectives to facilitate penetration of the connective sheath.

(B) Recording Procedure and Circuitry

A microelectrode was filled with electrolyte and fixed into the electrode holder. Using the micromanipulator, the electrode was positioned just above the ipsilateral connective, under visual guidance from a binocular microscope (Bausch & Lomb). Illumination was provided, via a light guide, by a cold light source (Schott KL150B). When the electrode was in position the microscope was removed from the vicinity of the preparation and the light source switched off. The electrode was then advanced into the connective using the hydraulic microdrive.

During penetration of the connective an acoustic and/or vibratory search stimulus was applied, and this stimulus, together with the neural activity, was displayed on the monitor oscilloscopes CRO2, CRO1 inside and outside the experimental room. The acoustic search stimulus was usually alternated between 5 kHz and 16 kHz, at least one of which was found to excite most of the acoustic neurones encountered. It was found that few units responded well to white noise, and so this was not used as a search stimulus. When specific neurones were being searched for the stimulus was set to a relevant frequency for that neurone. The vibratory search stimulus was usually of 200 or 500 Hz, to which most vibration-sensitive units responded. When a unit

was located and the electrode position optimized all further tests were carried out from outside the experimental room.

The signals picked up by the electrode were passed to a headstage (Neurolog NL100) and then to an AC preamplifier (Neurolog NL103), a filter module (Neurolog NL125), and an AC/DC amplifier (Neurolog NL106) situated within the experimental room (Fig. 3.1). The filters were set to pass frequencies above 100 Hz and below 1.5 kHz. The neural activity was monitored on headphones (Danasound) within the experimental room, and on a loudspeaker (Radiospares) outside, after passing through an audio amplifier (Neurolog NL120). It was also monitored on the oscilloscopes inside and outside the experimental room (CRO2, CRO1 respectively). The neural activity was passed to channel 1 of the instrumentation recorder IR1 and, during testing of a unit, was recorded at 15 i.p.s. in FM mode (frequency response DC to 5 kHz, ±3 dB). The "delay" trigger from S1 was recorded on channel 4 in DR mode (frequency response 250 Hz to 50 kHz, ± 3 dB) and the stimulus envelopes from TBG1 and TBG2 were recorded on channels 2 and 3 in FM mode. When the NS/SNS tape was used as the stimulus (through TBG1) it was recorded (channel 2) in DR mode.

As soon as a unit was located, certain tests were given priority in order to characterize it, in case the unit was lost after a short time. The thresholds of the unit over its frequency range were measured, and intensity-response determinations were made at its best frequencies. Each stimulus type was presented 8 times to show variability in the responses. The NS/SNS tape was run as early as

possible, and preliminary two-tone tests were made. If time permitted, additional tests were carried out to fill in details of the response characteristics. In some instances, particularly when recording from vibration sensitive units, legs were then selectively sectioned to ascertain their relative inputs.

3.2.7 REPLAY OF PHYSIOLOGICAL DATA FOR ANALYSIS

The circuitry employed during analysis of physiological data is given in Fig. 3.4. Most analysis was carried out by displaying the neural responses in various forms on the oscilloscope CRO1. Analogues of responses could be displayed by passing them directly from channel 1 of IR1 to CRO1. The triggers stored on channel 4 during recording were amplified (laboratory-built amplifier) and used to trigger S1. The non-delay output of S1 was then used to trigger CRO1. During replay of responses to the NS/SNS tape (on which there were no triggers) CRO1 was triggered internally on "free-run". The information thus displayed was photographed using a Canon FP camera, on 35 mm film (Kodak Recording Film 2475).

Most analysis, however, was carried out on responses converted to digital form using a spike trigger as a discriminator. This simplified the analysis of multiple presentations and statistical tests. Responses from the tape were passed to an AC/DC amplifier (Neurolog NL106), and then to a spike trigger (Neurolog NL200). This was set, for each unit, to produce a TTL pulse to each action potential.

Fig. 3.4

Circuitry employed during data analysis.

Amp. - amplifier

Ch. Rec. - chart recorder

CRO1 - cathode ray oscilloscope 1

d - delayed stimulator output

D.C. - D.C. bias

DW - delay width module

IR1 - instrumentation recorder 1

Pre. - preamplifier

PST - post-stimulus time module

S1, S2 - 2-channel stimulator

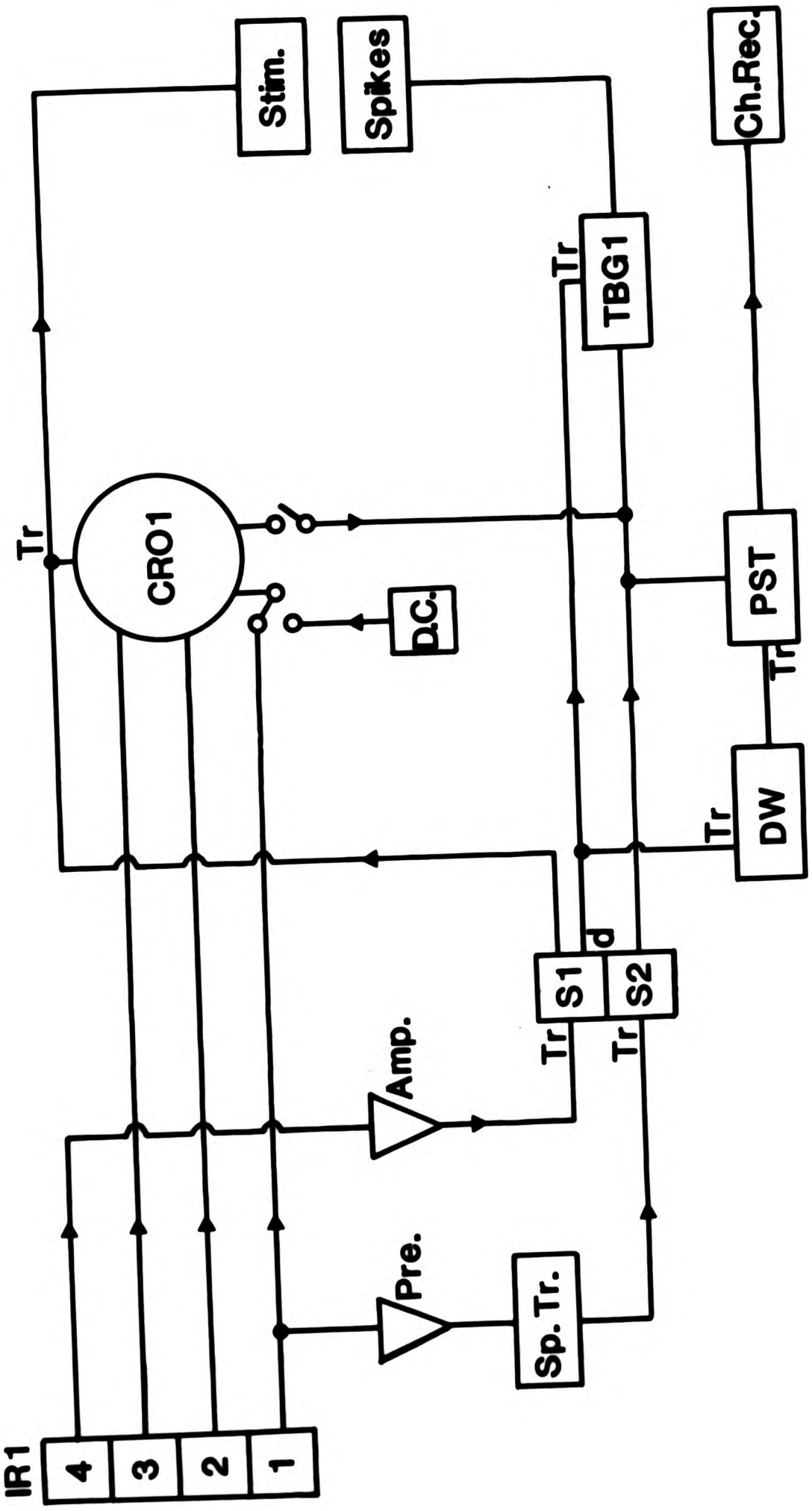
Spikes - spike counter

Sp. Tr. - spike trigger

Stim. - stimulus counter

TBG1 - tone-burst generator 1

Tr - trigger input


Stim.
Spikes
Ch.Rec.
Tr
TBG1
Tr
CRO1
D.C.
PST
Tr
DW
Tr
d
S1
S2
Tr
Tr
Amp.
Pre.
Sp. Tr.
IR1
4
3
2
1

These TTL pulses triggered the S2 channel of the stimulator to produce controllable output pulses. The neural responses, in this form, could then be analysed in several different ways.

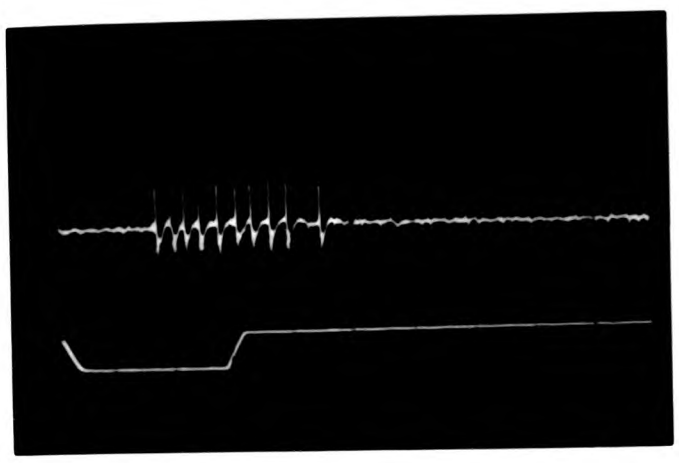
The number of spikes produced in response to each stimulus presentation could be counted by passing the output of S2 to a spike counter (Radiospares 258-798). In order to count only spikes that were stimulus locked, they were gated by TBG1 before reaching the spike counter in the following way. The trigger from the tape was used (via S1) to trigger the TBG for each stimulus presentation. The TBG then passed all the spikes that occured within the gate or "window", whose onset and duration was set to include only stimulus locked activity. The onset of the window relative to the stimulus could be controlled by using the delay control of S1, so that the entire stimulus-locked response could be encompassed by the window whatever its latency or duration. The rise/fall time was set to zero (transient) to prevent amplitude modulation of the discriminated spikes. The windowed spikes were then passed to the spike counter which showed the cumulative count for successive stimuli. The number of stimuli were monitored by a second counter which recieved triggers from S1.

A "raster display" of the responses to all 8 presentations of a stimulus could be produced on the screen as follows (Fig. 3.5). A DC resistor-division network was used to apply a DC bias to the input of the response channel of CRO1. This could be varied in a stepwise manner to move the beam up or down the screen. The output of S2

Fig. 3.5

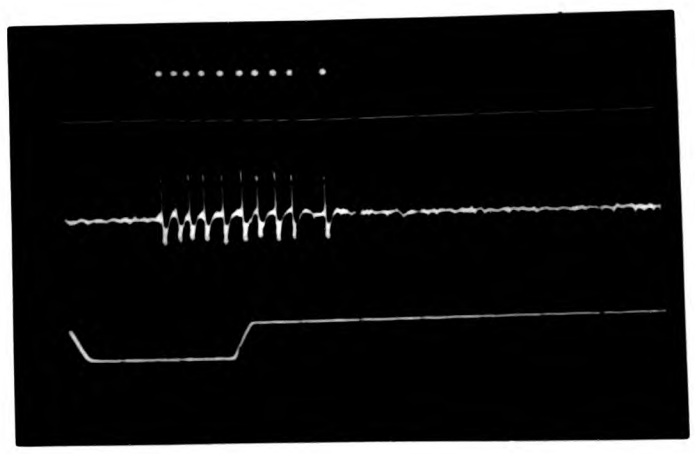
Production of raster dispays.

For explanation see text.

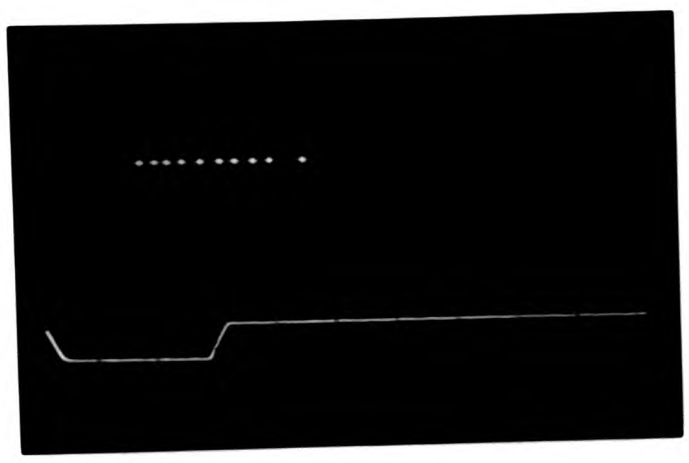


Response analogue

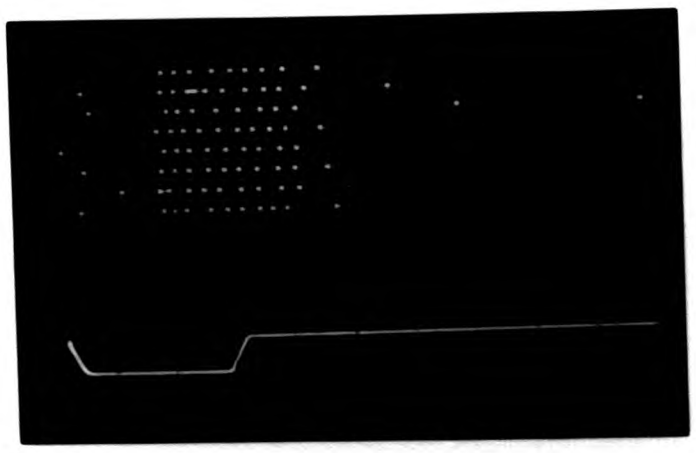
Stimulus



Discriminated spikes



Z-modulated
Response Channel



Raster Display of 8
successive stimulus
presentations

(discriminated spikes) was passed to the Z-modulation input of the response channel, thus brightening the trace at the positions of the discriminated spikes (Fig. 3.5). The brightness control for this channel could then be decreased to leave only the bright dots. For each successive stimulus presentation the response, displayed as a row of dots, was moved down the screen by the DC shift network. A summary was thereby obtained of the responses to 8 sequential identical stimuli. These displays demonstrated adaptation and could also be used for measuring response latencies.

Post-stimulus time (PST) histograms were constructed using the discriminated spikes. These showed the total response to the 8 presentations of each stimulus over a given time. Histograms were produced by an averager (Neurolog NL750) in "PST" mode. It was necessary that the averager was triggered by a TTL pulse and so the output of S1 (non-TTL) was passed through a delay-width module (Neurolog NL403) set to zero delay. This produced a TTL output that was used to trigger the averager. Discriminated spikes were led to the averager signal input directly from S2. Each PST histogram was then plotted on paper using a chart recorder (Mingograf 800).

3.2.8 PROCESSING OF STAINED UNITS

The recorded units, cobalt-filled by diffusion, were silver-intensified using a modification of Timm's method (1958), as described by Tyrer & Bell (1974) and improved by Bacon & Altman (1977). During recording, the units had to

be held for as long as possible to optimize filling, and with as good a signal to noise ratio as possible to avoid multiple unit fills. Recordings from the posterior region of the cervical connectives usually filled processes well in the prothoracic ganglion, but only rarely in the suboesophageal and mesothoracic ganglia.

At the termination of the experiment the ganglion chain was dissected out from the suboesophageal to the mesothoracic ganglia, and was placed in a few mls of Ringer in a welled glass block. A few drops of ammonium sulphide were added and left for 5 minutes to precipitate the cobalt as cobalt sulphide. The ganglion chain was rinsed in Ringer and fixed in alcoholic Bouin's for at least an hour. It could then be dehydrated and cleared for viewing, but in order to show the fine details of the fill the cobalt stain was usually silver-intensified (after Bacon & Altman 1977).

From the Bouin's fixative the ganglion chain was hydrated to distilled water (steps of 70%, 50%, and 30% ethanol, 10 minutes in each) and then placed in the developer stock solution (Appendix 1) at 60°C, for a one hour pre-soak. Active developer was prepared by combining the stock solution with 1% silver nitrate in a 9:1 ratio, and the ganglion chain was placed in a few mls of this. It was essential that the action of the developer was carried out in the dark, and at 60°C, so the chain was placed in a 60°C oven and viewed every few minutes under red light. When the chain had turned a pale yellow colour (usually after 20-30 minutes) the process was terminated by transferring it to distilled water for 10 minutes (several

changes). Dehydration was then carried out in steps of 30%, 50%, 70%, 90% and 100% ethanol. The steps up to 50% were carried out in the dark. The chain was passed through acetone (10 minutes) and cleared in methyl salicylate. Long term storage was also in methyl salicylate. Viewing was carried out using a Zeiss Standard microscope and camera lucida drawings were made using a drawing tube attachment.

If fills were insufficiently intensified it was possible to re-intensify them by rehydrating to water, repeating the intensification procedure (the development was then only about 5 minutes) and dehydrating and clearing as before. Alternatively, in some instances the preparation became overstained, usually with non-specific deposition of silver on the ganglion surfaces. This was often the case in preparations that had been re-intensified. It could be rectified to some extent using a destaining technique described by Pitman (1979). The ganglion chain was rehydrated down to 50% ethanol and placed in a solution of 1.25% sodium thiosulphate and 1% potassium ferricyanide in 50% ethanol. The chain could be observed under a binocular microscope (Olympus) as it became gradually lighter. This process took 2-5 minutes and was terminated by placing the chain in 50% ethanol. The chain was then dehydrated and cleared as described above.

3.3 RESULTS

3.3.1 CHARACTERIZATION OF THE UNITS BY THEIR THRESHOLD CURVES

(A) Responses to Acoustic Stimuli

A preliminary classification of the units encountered was possible on the basis of their threshold curves. These showed the absolute sensitivity of each unit, and also their relative sensitivities to different frequencies. Taken together, they demonstrate the frequency and intensity ranges over which these central units respond, and show the variation between the frequency and intensity sensitivities of the individual units.

Most units were tuned to one or more particular frequency. This "characteristic frequency" was most often around 5 kHz or around 14-16 kHz. These frequencies correspond to the carrier frequencies of the calling and courtship songs respectively. Fig. 3.6 shows a selection of the threshold curves of several units (a) tuned near 5 kHz, (b) tuned near 14-16 kHz, and (c) tuned to more than one frequency or broad banded. Frequency discrimination is thereby demonstrated by these groups of neurones. In each group, differences between the absolute thresholds of units were often found (range fractionation), even when their relative sensitivities to different frequencies were similar.

(B) Responses to Vibratory Stimuli

The range of vibration frequencies to which the recorded units responded was from about 50 Hz to 2 kHz. Although

(B)

Fig. 3.6

Threshold curves showing representative examples of acoustic neurones recorded in the cervical connectives and tuned to

(A) frequencies around 5 kHz,

(B) frequencies around 10-20 kHz,

(C) more than one frequency or broad-banded.

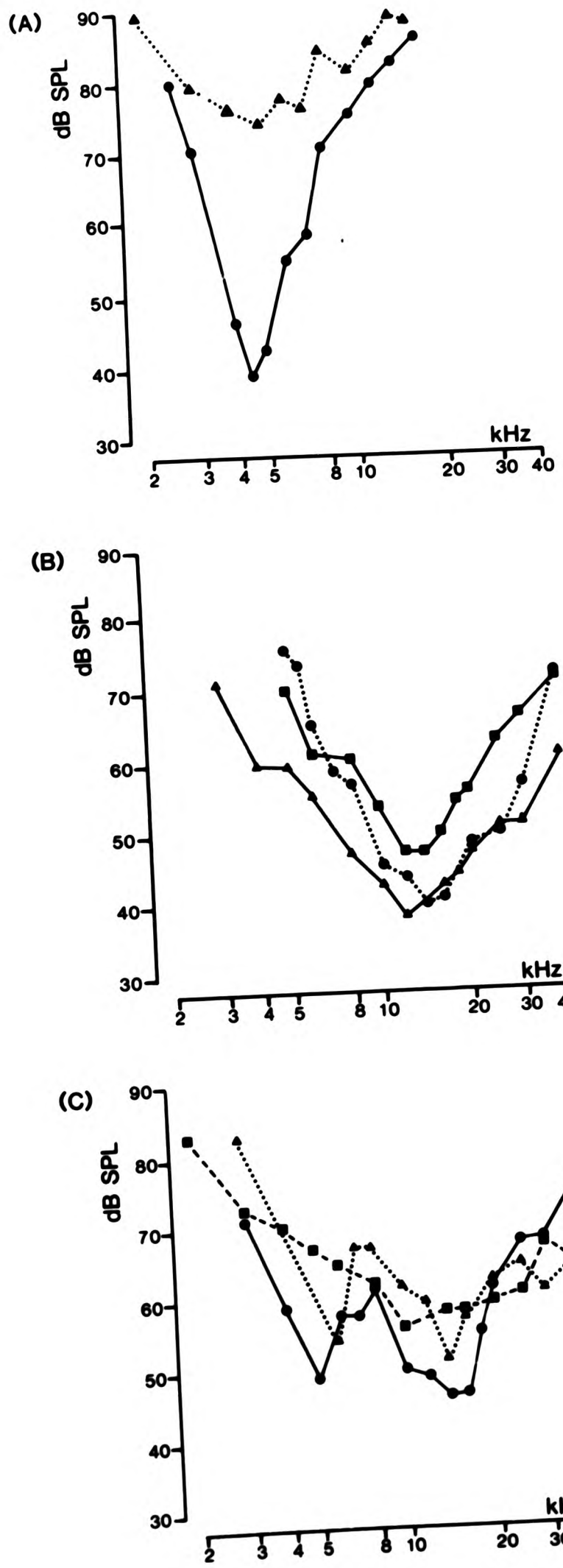

(A)
dB SPL
90
80
70
60
50
40
30
kHz
2
3
4
5
8
10
20
30
40
(B)
dB SPL
90
80
70
60
50
40
30
kHz
2
3
4
5
8
10
20
30
40
(C)
dB SPL
90
80
70
60
50
40
30
kHz
2
3
4
5
8
10
20
30
40

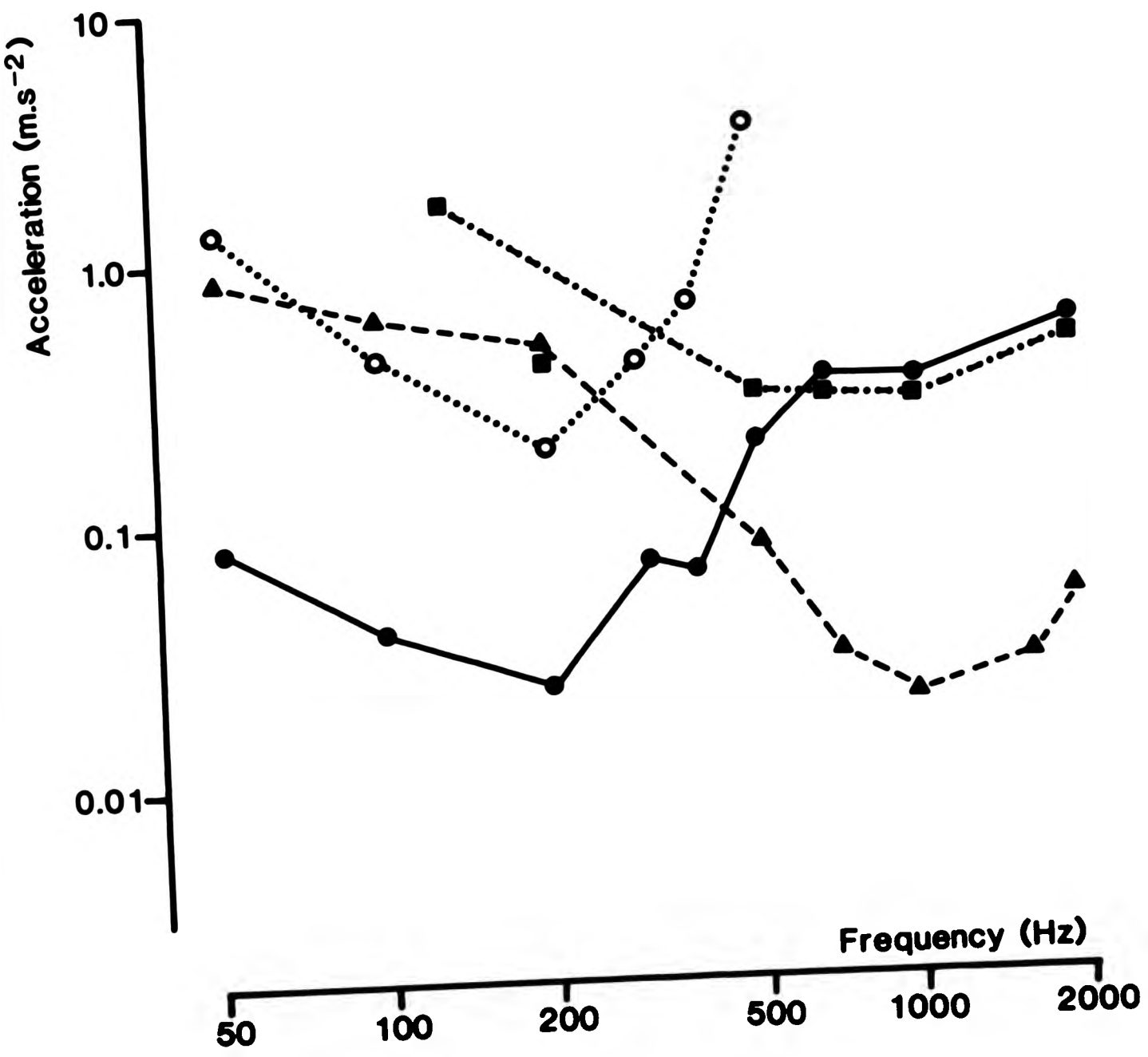
Fig. 3.7

Threshold curves showing representative examples of vibration-sensitive neurones recorded in the cervical connectives. Threshold values are peak-to-peak accelerations.

f

cal

celer-


Acceleration (m.s^{-2})
10
1.0
0.1
0.01
Frequency (Hz)
50
100
200
500
1000
2000

many of the units responded best to one frequency or frequency band, the degree of this tuning was not as marked as it was for the thresholds to acoustic stimuli. Furthermore, no units had more than one characteristic frequency, in contrast to many of the acoustic neurones. A cross-section of the types of threshold curve encountered is provided by Fig. 3.7. It is evident that units have characteristic frequencies covering the range from about 200 Hz to 1 kHz, in addition to units that are relatively broad-banded.

Most neurones preferentially sensitive to either sound or vibration were influenced, to some degree, by the other modality, and a few of these had measurable thresholds for sound as well as vibration stimuli. The shapes of these threshold curves followed the same trends as those described for neurones preferentially sensitive to either sound or vibration, and are discussed in detail in section 3.3.2.

3.3.2 RESPONSE CHARACTERISTICS OF MORPHOLOGICALLY AND PHYSIOLOGICALLY IDENTIFIED NEURONES

(A) Neurones Sensitive to Low Frequency Sound

AN3

The physiological characteristics of this neurone were clearly distinct from the AN1 (low-frequency) and AN2 (high frequency) units described by Wohlers & Huber (1982). The cobalt staining showed it to be an "ascending" unit, hence it is labelled here as AN3.

Anatomy

Although this unit was recorded in 13 preparations, staining was successful in only one instance. Fig. 3.8 shows a camera lucida drawing of the neurone in the prothoracic ganglion. The stain was not complete and most of the fine dendrites have not been filled. However, the major dendritic branches are clear, and they show the neurone to be similar to the ascending unit AN2 described below.

The axon runs half-way between the midline of the cervical connective and its medial side, and joins with the main lateral process near the centre of the ganglion. This lateral process extends towards the leg nerve of the axon-ipsilateral side, terminating in many fine dendritic branches close to the origin of the leg nerve. In the angle between the lateral process and the ascending axon is a large dendritic branch, but further arborization is not visible. Leading away from the axon, in an opposite direction to the lateral process, is a fine neurite which runs to the soma which is situated in the anterior quadrant of the ganglion, contralateral to the axon.

Physiological Responses

The thresholds measured for this unit were remarkably consistent between the examples recorded. The maximum variation is shown by the threshold curves from three preparations, given in Fig. 3.9. Each can be seen to be sharply tuned to around 4.5-5 kHz, with a roll-off of about 45 dB/octave on the low frequency side and about 27 dB/octave on the high frequency side. The threshold at

Fig. 3.8

Camera lucida drawing of the anatomy of AN3 in the prothoracic ganglion. Arrow indicates anterior.

pro-

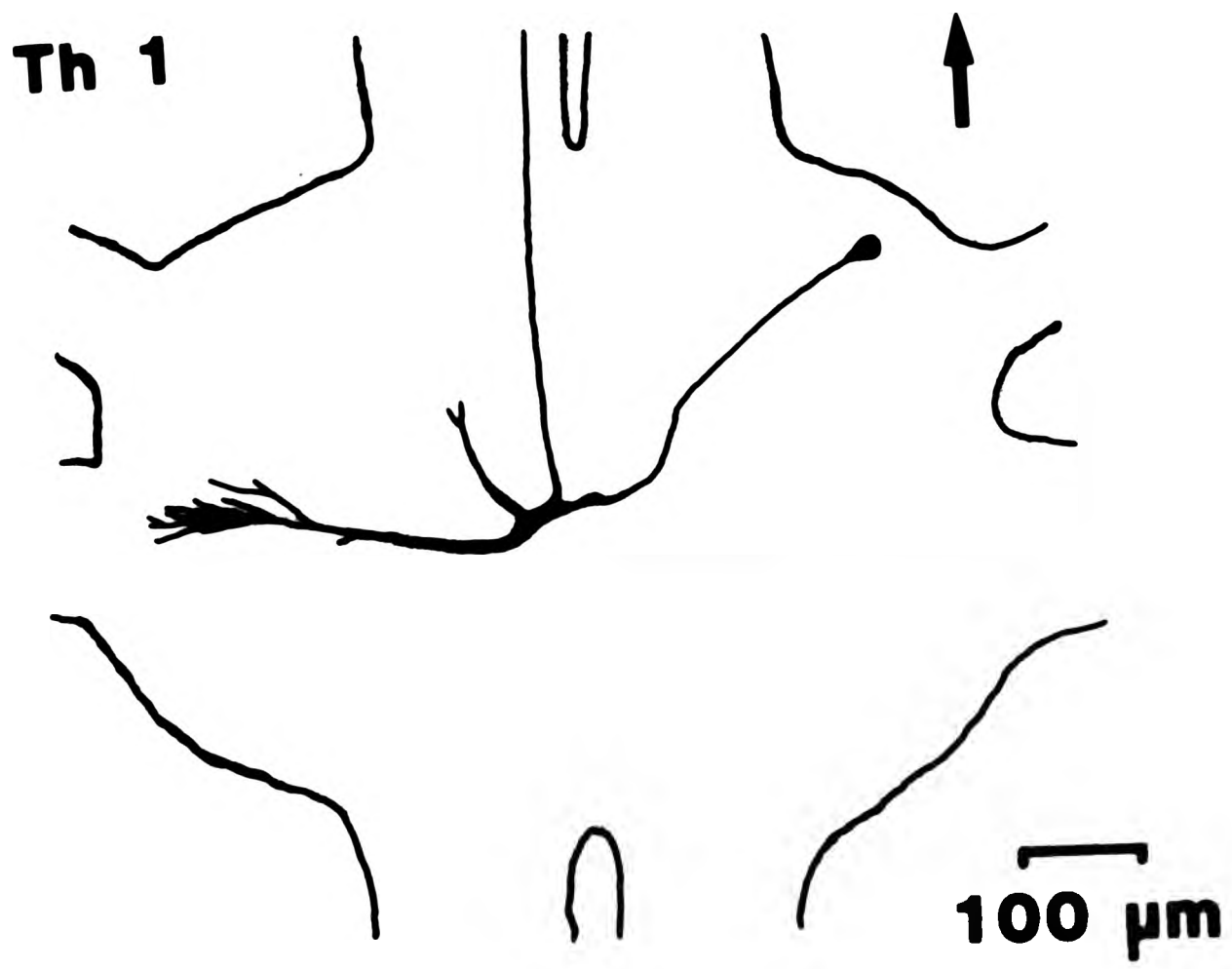
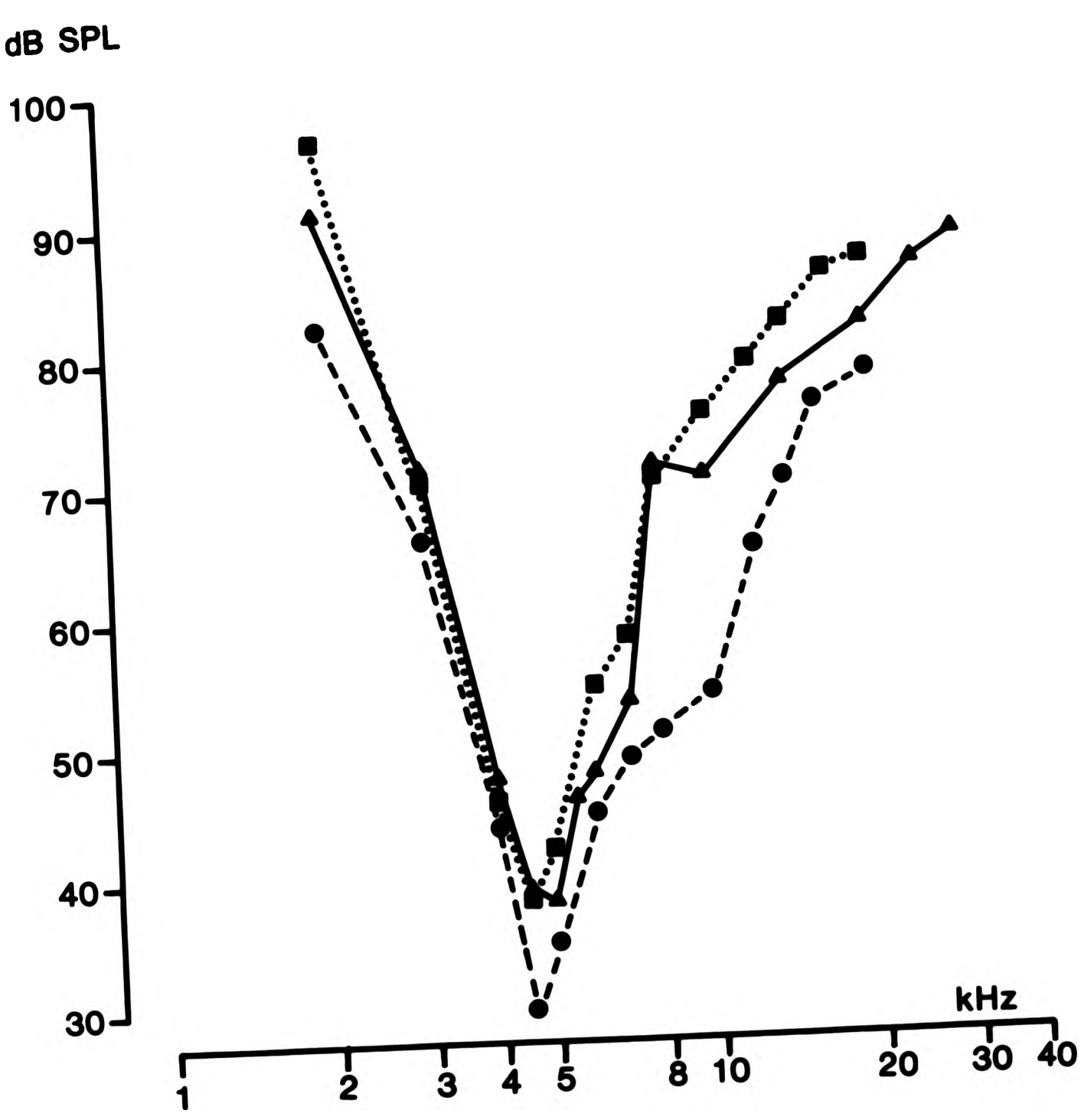


Fig. 3.9

Threshold curves of three examples of AN3. Note greatest variability in thresholds at frequencies above 5 kHz.

greatest

Hz.



the characteristic frequency was usually 30-35 dB SPL. Threshold points below this characteristic frequency were notably more consistent than those above it. None of the units recorded showed secondary peaks of sensitivity at other frequencies.

The suprathreshold response patterns at 5 kHz were also very consistent between preparations, in most respects. Fig. 3.10A shows a selection of intensity-response curves at 5 kHz for 3 different units, together with curves for 4 and 6 kHz from one of these units. Fig. 3.10B shows raster displays of the responses to 50 ms, 5 kHz stimuli at several intensities. At 41 dB a weak tonic response is elicited with a latency of about 25 ms. As the intensity of the stimulus is increased, the firing rate increases and the latency decreases. The latency also becomes more consistent to successive stimulus presentations at higher intensities, and reaches a minimum of about 11 ms (taking into account the 4 ms delay for sound to pass from the loudspeaker to the preparation). At intensities of 80 or 90 dB the firing rate can reach at least 500/s. Very little adaptation is evident during the duration of any given stimulus, or between successive stimulus presentations. There is no after-discharge (although the termination of the response is more variable than its onset) and the spike bursts have almost exactly the same duration as the stimulus.

The unit always showed some level of spontaneous activity. The rate of this was rather variable, however, limits of 10/s and 60/s being encountered in the units recorded. It was suppressed following the response of the

Fig. 3.10

Suprathreshold response characteristics of AN3.

(A) Intensity-response curves of 3 examples of AN3 at 5 kHz, together with the curves for 4 kHz and 6 kHz for one of these units. Spike counts are means of 8 presentations (standard deviations shown by vertical bars when permitted by clarity).

Spon = mean spontaneous activity.

(B) Raster displays of the responses of a representaive example of AN3 to 5 kHz, at the intensities given. Responses to 8 presentations are shown; the bottom trace shows the 50 ms stimulus.

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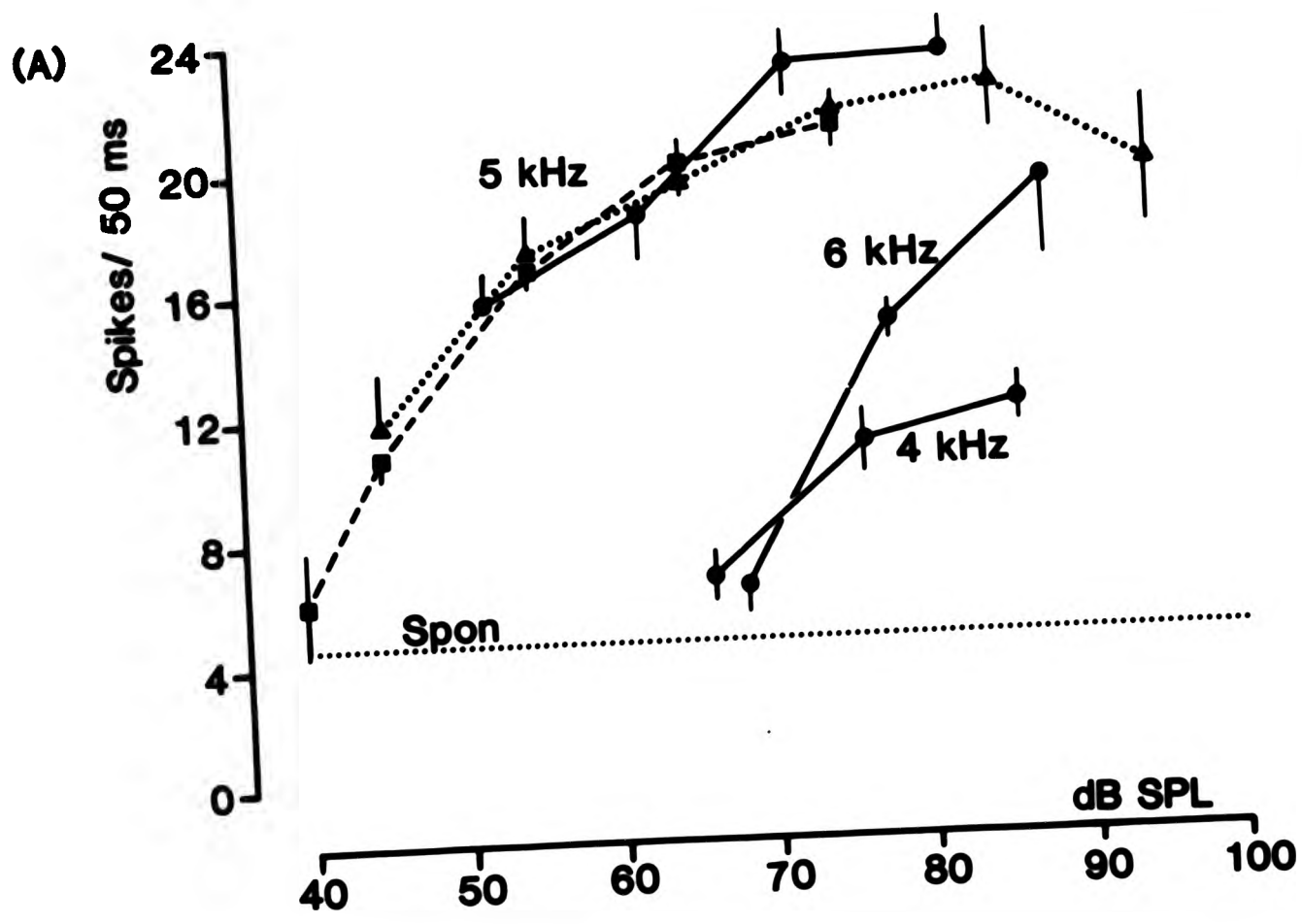

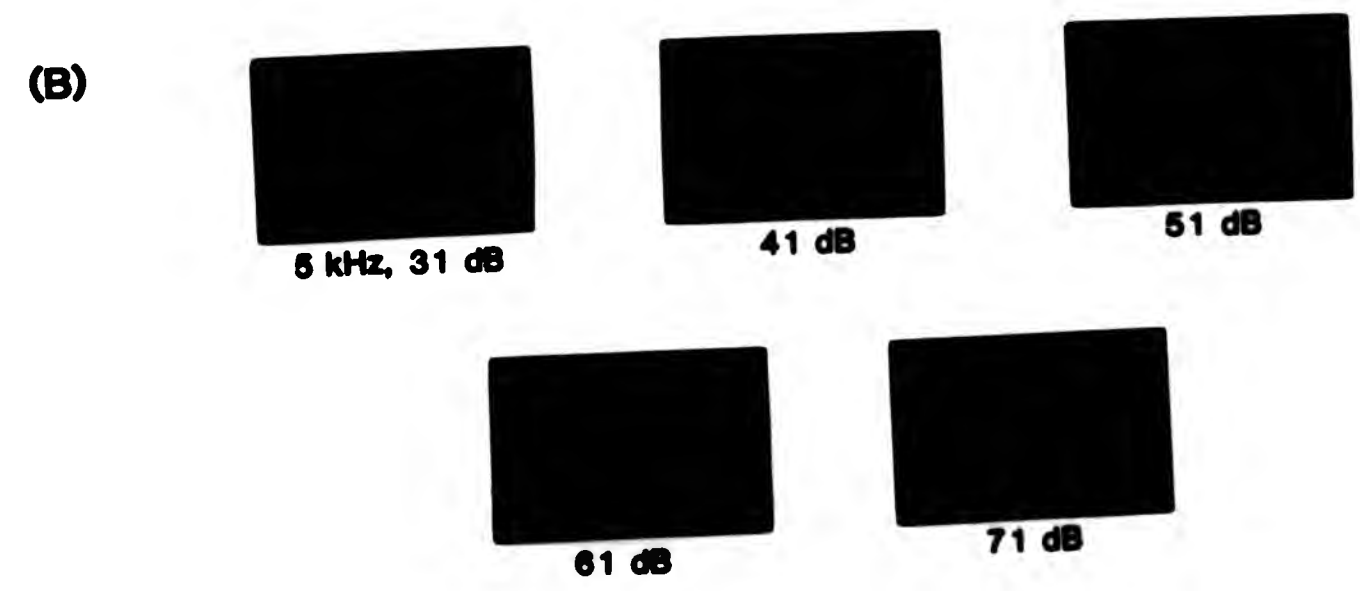

unit to an acoustic stimulus, the duration of the suppression being greater following strong responses, up to a maximum of about 300 ms.

The response of the unit to 5 kHz was found to be strongly suppressed by simultaneous presentation of sound of both higher and lower frequencies. Fig. 3.11A shows a typical normal threshold curve, together with thresholds of inhibition derived from 5 preparations. Each point represents the minimum test-tone (TT) intensity (measured for several TT frequencies) that caused a reduction in the response to a 5 kHz control tone (CT) of 52 dB SPL, when the TT and the CT were presented simultaneously. Two inhibitory fields are evident, centred around 3-4 kHz and around 16 kHz. Rasters of responses to a 5 kHz CT, presented alone and with 3 kHz and 16 kHz TTs, are given in Fig. 3.11B. Both 3 kHz and 16 kHz produce strong, intensity-dependent inhibition, but there were differences between the characteristics of the inhibition by the two frequencies. The major difference is that 3 kHz appears to have very little effect on the spontaneous activity of the unit, unlike 16 kHz; when the response to 5 kHz is reduced to almost zero by the simultaneous presentation of 3 kHz the spontaneous activity remains, whereas when the suppression is by 16 kHz the spontaneous activity does not return for at least 300 ms.

It is evident, from the raster displays, that the inhibition of the response to 5 kHz by 3 kHz or 16 kHz has a latency that is not greater than that of the 5 kHz

Fig. 3.11

Two-tone interactions in AN3.

(A) Normal threshold curve of AN3 (solid lines) together with thresholds of inhibition, produced by simultaneous presentation of other frequencies (TTs), of the response to a 5 kHz 52 dB CT (dotted lines).

(B) Raster displays of 8 stimulus presentations, showing the response of AN3 to 5 kHz at 51 dB when presented alone and together with 16 kHz, 75 dB or 3 kHz, 76 dB.
Bottom trace = 5 kHz control tone.
Middle trace = test tone.
50 ms stimuli.

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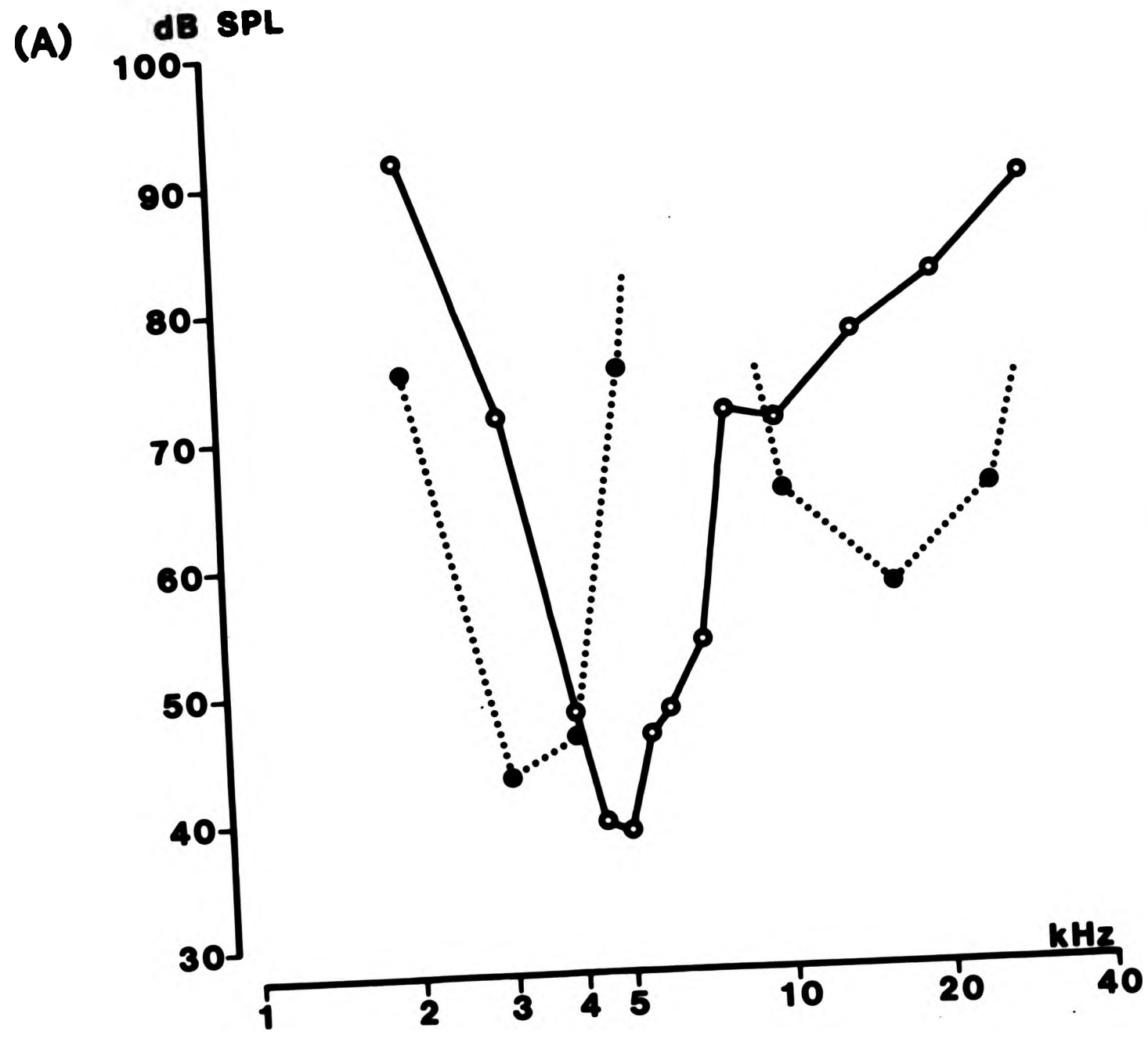

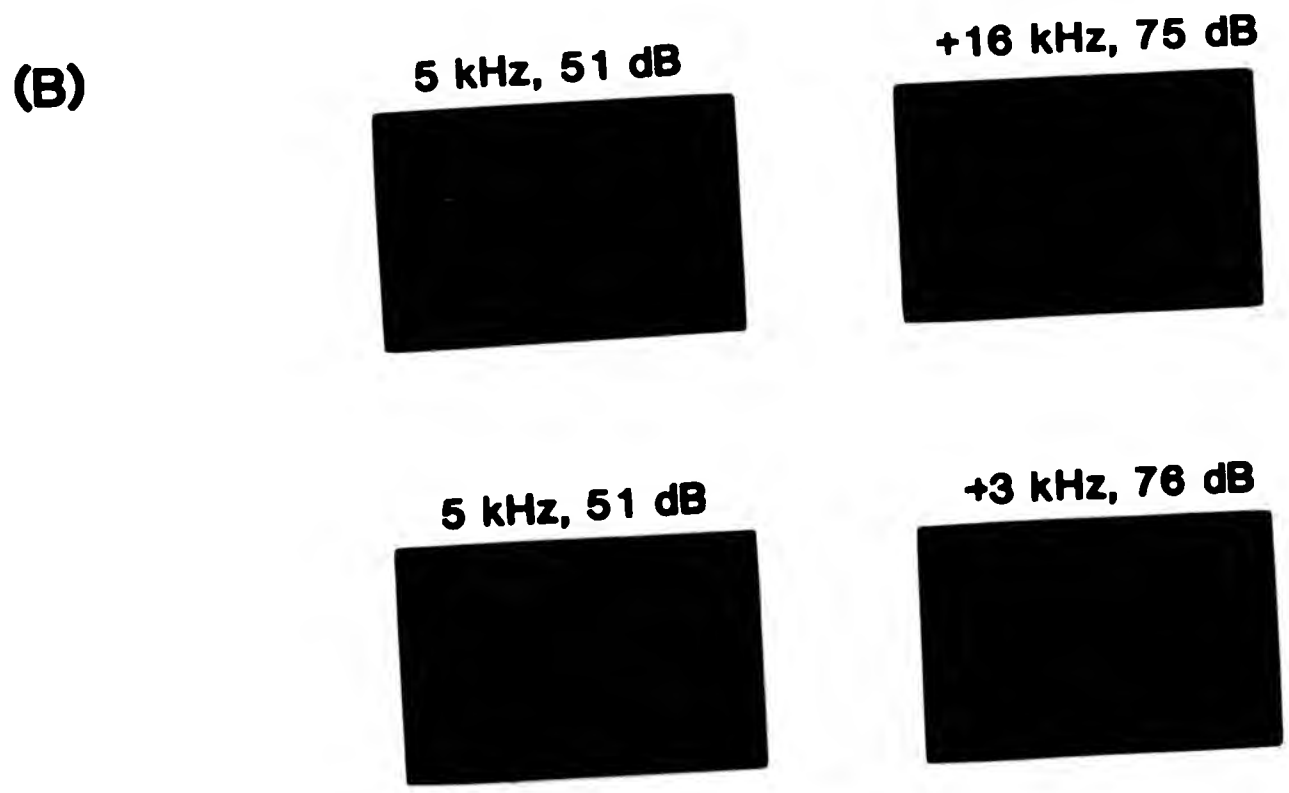

excitation. Experiments involving changing the relative onset and duration of the CT and TT were not carried out and it is therefore not possible to determine the duration of the inhibition (other than it is as least as long as the excitation) as any level of positive response suppressed the spontaneous activity for a considerable time after the response.

Fig. 3.12 provides a comparison between the intensity response characteristics and the inhibition by 16 kHz in a single preparation. Fig. 3.12A shows the intensity response curves for 5 kHz and 16 kHz. The 5 kHz curve is similar to those in Fig. 3.10A, but the 16 kHz curve is far to the right; positive responses are only elicited by intensities of above 75 dB (c.f. 16 kHz threshold in Fig. 3.11A), whereas by intensities of between about 55 and 75 dB there is suppression of the spontaneous activity. The effect of presenting the 16 kHz TT at several intensities, simultaneously with a 5 kHz CT of 52 dB, is shown in Fig. 3.12B. Strong suppression of the CT response is produced at TT intensities from about 50 to 95 dB, above which the CT response is augmented. The limited effects of 12 kHz and 20 kHz TTs show the inhibition to be greatest near 16 kHz (c.f. Fig. 3.11A). It is clear that the intensity range over which there was suppression of the CT response, in this example, was greater than that over which there was suppression of the spontaneous activity in Fig. 3.12A.

Fig. 3.12

(A) Intensity-response curves of AN3 at 5 kHz and 16 kHz. Points show means and standard deviations of responses to eight 50 ms stimulus presentations.
Spon = mean spontaneous activity.
Note suppression of spontaneous activity by 16 kHz at intermediate intensities.

(B) The effect of presenting test-tones of 12 kHz, 16 kHz and 20 kHz, at several intensities, together with a 5 kHz, 51 dB control tone. The dotted line gives the mean response to the control tone presented alone.

Data in (A) and (B) from the same neurone.

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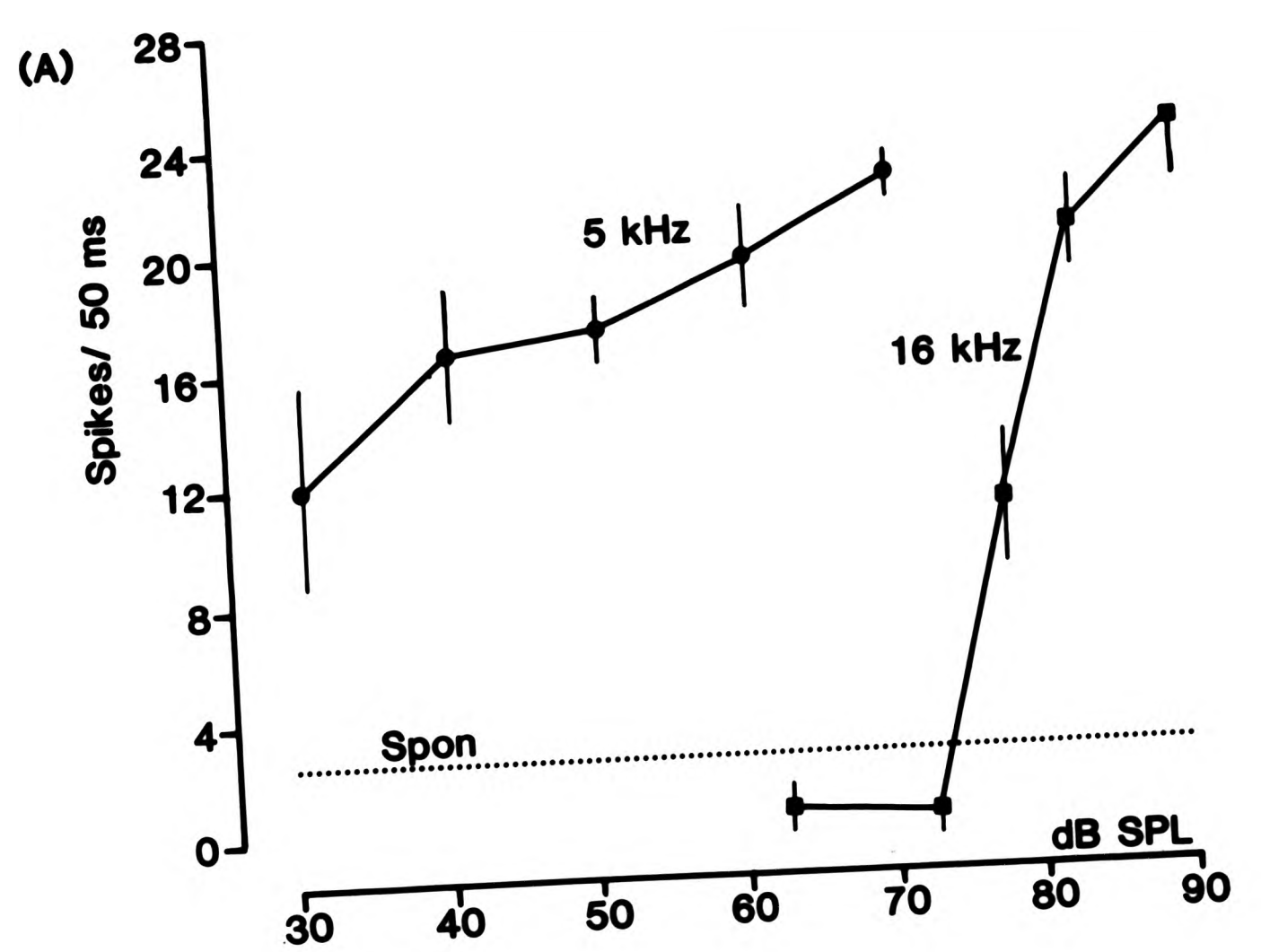

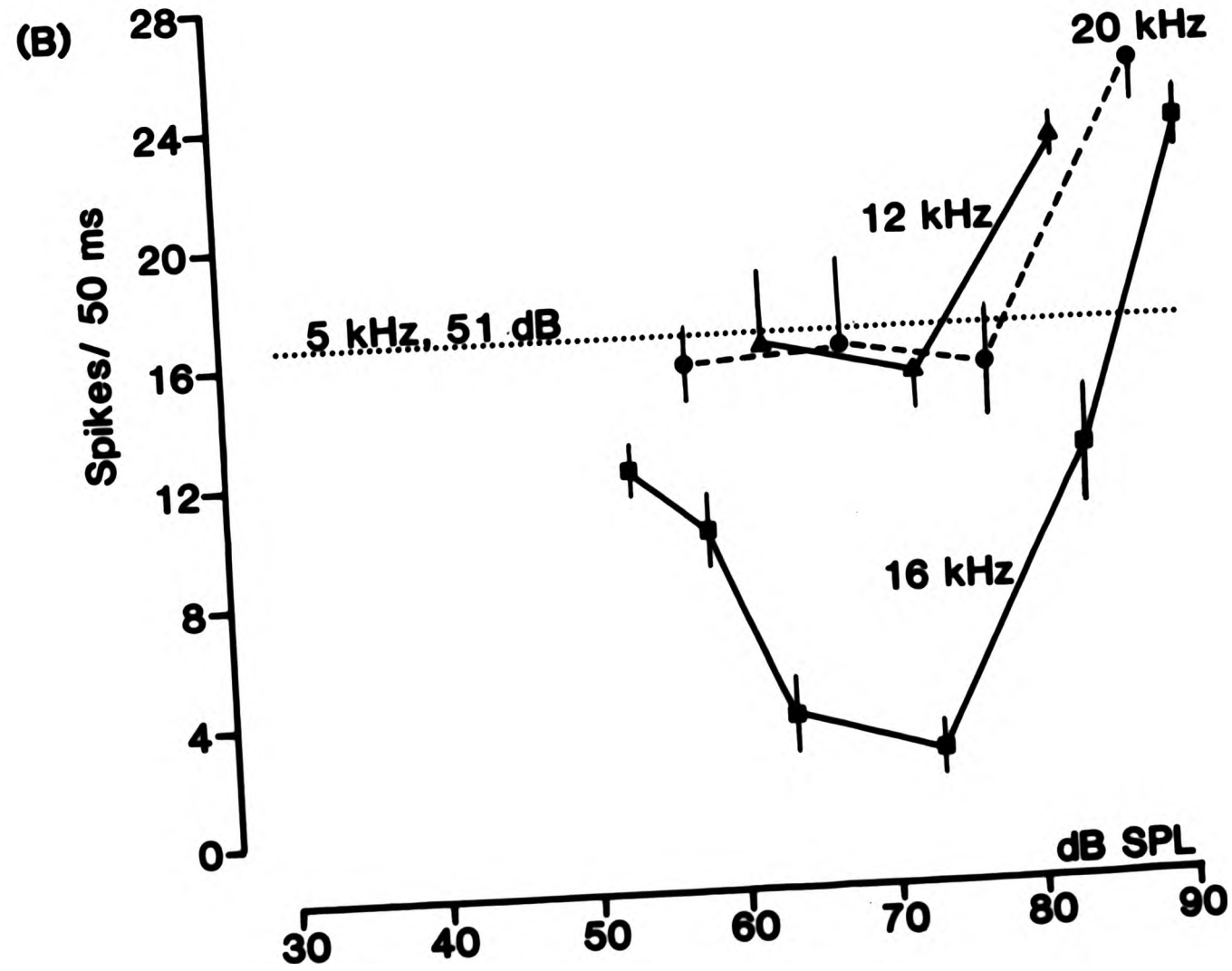

Leg sectioning experiments revealed that the 16 kHz inhibition is mediated primarily contralaterally and the 3 kHz inhibition ipsilaterally. Fig. 3.13A shows the effects of adding 3 kHz or 16 kHz TTs, at several intensities, on the magnitude of the response to a 5 kHz CT of 52 dB. 5 kHz alone produced a response of 16 spikes/50 ms stimulus. The addition of either 3 kHz or 16 kHz decreased the spike number when presented at intensities above about 50 dB. In each case, as the intensity of the TT was increased, the suppression was greater, although for 16 kHz the response started to increase for intensities above about 75 dB (c.f. Fig. 3.12B). Raster displays are shown of the responses to 5 kHz alone, and together with 3 kHz and 16 kHz at the intensities shown.

After sectioning the contralateral foreleg the response to 5 kHz alone was very slightly lower (15 spikes/50 ms stimulus). Addition of the 3 kHz TT suppressed the response to a similar extent as in the intact state, whereas the suppression by 16 kHz was almost totally absent (Fig. 3.13B). Very small reductions in spike number were produced by low 16 kHz TT intensities, and the response was then augmented by intensities above about 75 dB. Raster displays are provided of the responses to the CT with and without the TTs after leg sectioning.

Fig. 3.14 shows analogues of the responses of AN3 to natural song and synthesized song (NS and SNS). Responses to NS are given in Fig. 3.14A. The unit can be seen to

Fig. 3.13

Two-tone suppression in an AN3 neurone in the intact state (A) and after cutting the contralateral foreleg (B). Left: reduction of the response to a 5 kHz, 52 dB control tone (dashed line) by the simultaneous presentation of a 3 kHz and a 16 kHz test tone of increasing intensity. Right: dot rasters of eight successive responses to the control tone presented alone, and together with 3 kHz or 16 kHz.

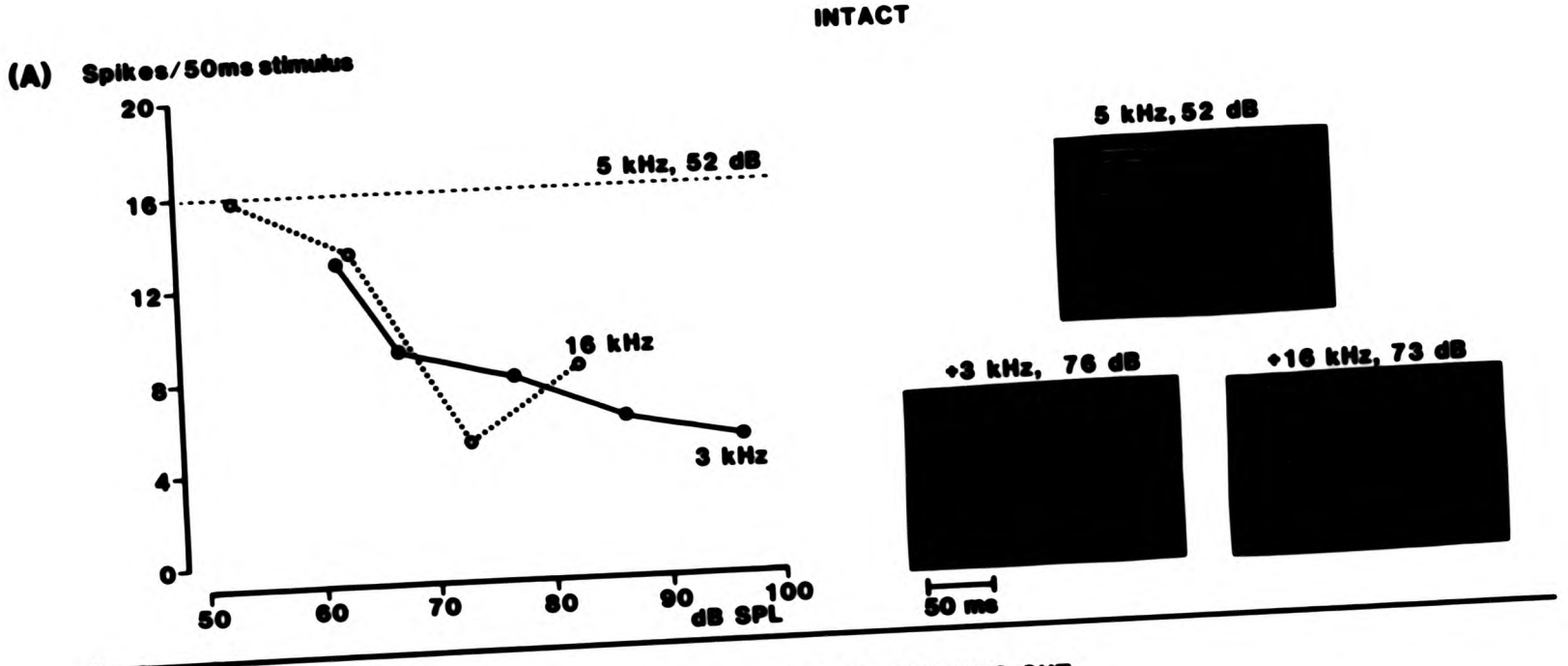
INTACT
(A) Spikes/50ms stimulus
20
16
12
8
4
0
5 kHz, 52 dB
16 kHz
3 kHz
50
60
70
80
90
100
dB SPL
5 kHz, 52 dB
+3 kHz, 76 dB
+16 kHz, 73 dB
50 ms
CONTRA. FORELEG CUT
(B) Spikes/50ms stimulus
20
16
12
8
4
0
16 kHz
5 kHz, 52 dB
3 kHz
50
60
70
80
90
100
dB SPL
5 kHz, 52 dB
+3 kHz, 76 dB
+16 kHz, 73 dB

Fig. 3.14

Responses of AN3 to natural and synthesized songs.

(A) Responses to the natural calling, aggression and courtship songs.

Time scale = 100 ms.

Peak intensities: calling - 55 dB
aggression - 54 dB
courtship - 33 dB

(B) Responses to the 3 types of synthesized songs with carrier frequencies as given. Note suppression of spontaneous activity during and after strong responses.

Time scale = 100 ms.

Peak intensities: 5 kHz - 50 dB
16 kHz - 45 dB
5+16 kHz - 50+46 dB

Data in (A) and (B) from different specimens.

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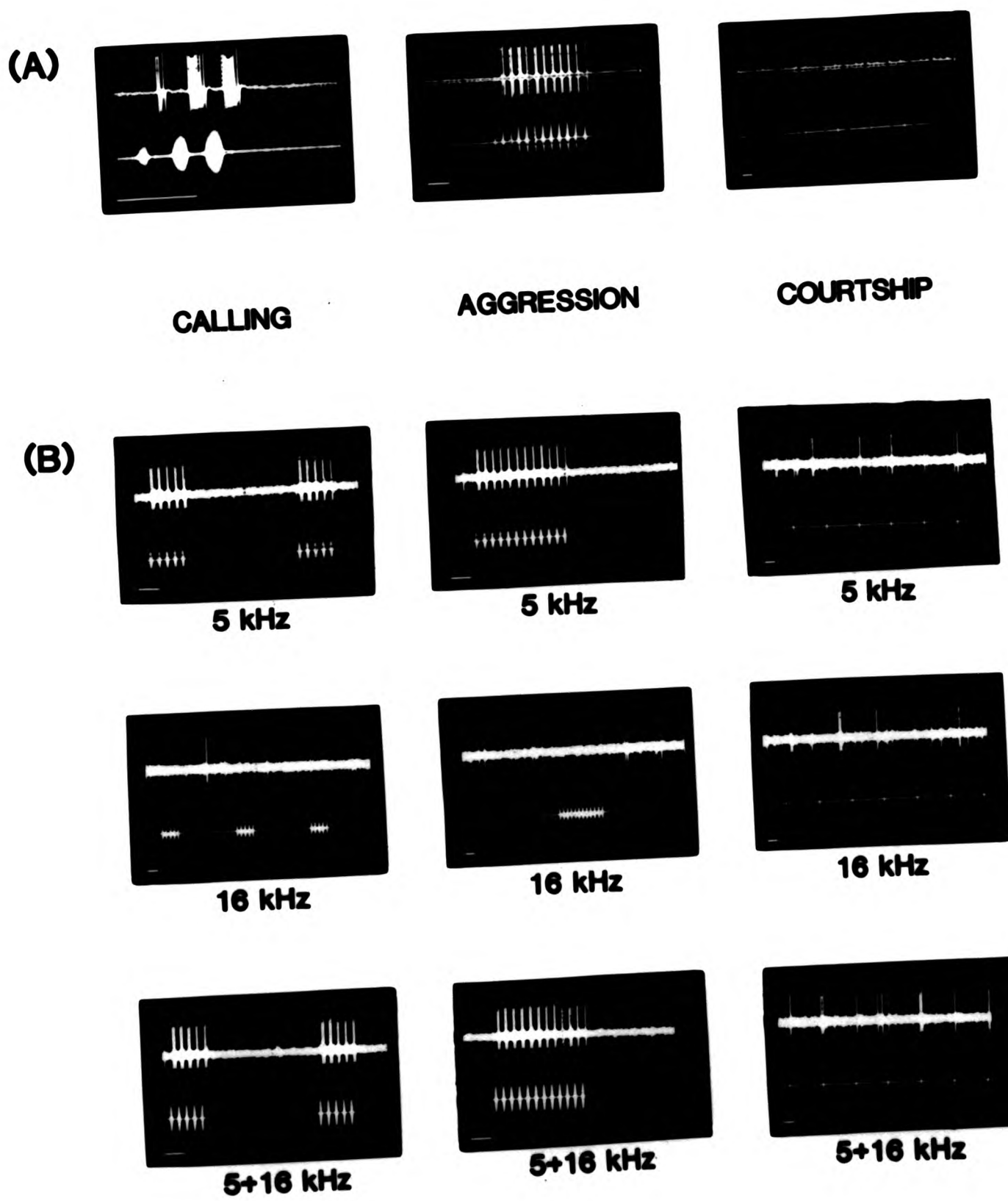

produce strong responses to the calling and aggression songs, but not to the courtship song (the activity shown during the courtship song stimulus is spontaneous, at a rate of about 10 spikes per second). The temporal coding of the calling and aggression songs is very accurate; in both cases a strong burst of spikes is elicited by each syllable, and very little spiking occurs between the responses to separate syllables.

Responses to SNS are shown in Fig. 3.14B (taken from a different preparation from the responses to NS). The unit is clearly preferentially sensitive to 5 kHz sound, whether presented alone or together with 16 kHz. Very little two-tone interaction is evident, as the 5 kHz and 16 kHz components were of the same intensity. Syllable coding of the calling and aggression songs is equally good for 5 kHz alone and for 5 kHz presented together with 16 kHz. When presented alone, the 16 kHz SNS suppressed the background spontaneous activity - shown most clearly here for aggression. The responses to courtship SNS were largely obscured as the responses were not strong enough to suppress the spontaneous activity (about 30 spikes per second in this recording).

(b) Neurones Sensitive to High-Frequency Sound

(i) AN2

The morphological and most of the physiological characteristics of this neurone correspond closely to the AN2 neurone described by Wohlers & Huber (1982). Their

terminology is therefore retained here. Units with similar physiology but different anatomy were recorded in some instances, and so physiological responses are described only for those units that stained unequivocally as AN2 (see discussion).

Anatomy

The unit was recorded and successfully stained in 14 preparations. Drawings of 3 examples are shown in Fig. 3.15. These show some of the extremes of the morphological variation, although the basic anatomy was very consistent. AN2 is an ascending neurone whose cell body is located in the anterior quadrant of the prothoracic ganglion, contralateral to the ascending axon. Viewed laterally, the cell body is situated anteriorly in the middle of the ganglion and is about 30 μm in diameter. A thin neurite runs from the soma towards the midline of the ganglion, and joins the axon near a point where the latter turns sharply laterally, forming the "main lateral branch", which extends towards the leg nerve. Numerous dendritic branches originate from this, particularly near the the distal end and in the angle between the lateral branch and the ascending axon.

It is in the detail of this dendritic branching that most of the variation between examples is to be found. Many of the fills showed a distinct bifurcation in the main lateral process, and this occurred more distally in some examples than in others. Another important variable feature was the angle between the main lateral process and the

Fig. 3.15

Camera lucida drawings showing the anatomy of AN2 in the prothoracic ganglion. Ventral views of three examples are shown (A,B,C) and (D) gives the lateral view of the unit in (C). Arrows indicate anterior and ventral directions.

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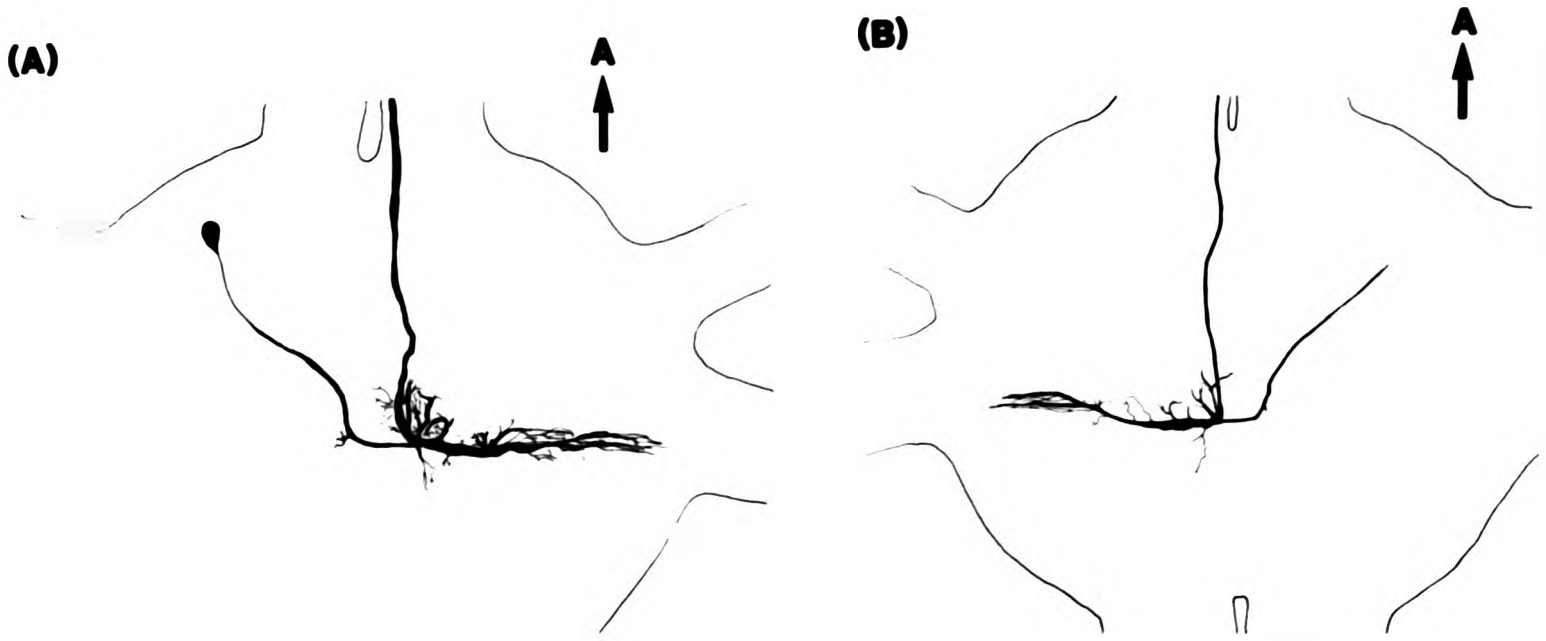

ascending axon; in some cases this was a simple 90° angle, whilst in others (e.g. Fig. 3.15C) the lateral process showed a distinct dip posteriorly near its proximal end. The axon is known to enter the suboesophageal ganglion but has not been filled far enough to determine any further dendritic branching.

Physiological Responses

This neurone was always most sensitive to sound frequencies of between 10 and 20 kHz. Its relative sensitivity to other frequencies, however, was more variable, particularly around 5 kHz. Some examples showed a distinct secondary peak of sensitivity at around 5 kHz, while in others this was totally absent. Fig. 3.16 shows the threshold curve for AN2, measured in 3 preparations. Whilst all can be seen to be tuned to around 14-16 kHz, there is considerable variation in their sensitivities around 5 kHz. The threshold at the "characteristic frequency" (i.e. 14-16 kHz) was 40-50 dB in all examples recorded.

The relative sensitivities of AN2 to high and low frequency sound are also very clearly illustrated by its suprathreshold intensity-response characteristics. Intensity-response curves for 3 preparations are given in Fig. 3.17, for (A) 5 kHz and (B) 14 kHz. These show the extremes of variability in this unit. While the 14 kHz curves are rather similar, there is considerable variation in the 5 kHz curves. Unit 86 responded equally well to 5 kHz and 14 kHz; unit 31 showed a weaker response to 5 kHz, which appeared to be suppressed at higher

Fig. 3.16

Threshold curves of 3 examples of AN2. Note variability in thresholds to 5 kHz.

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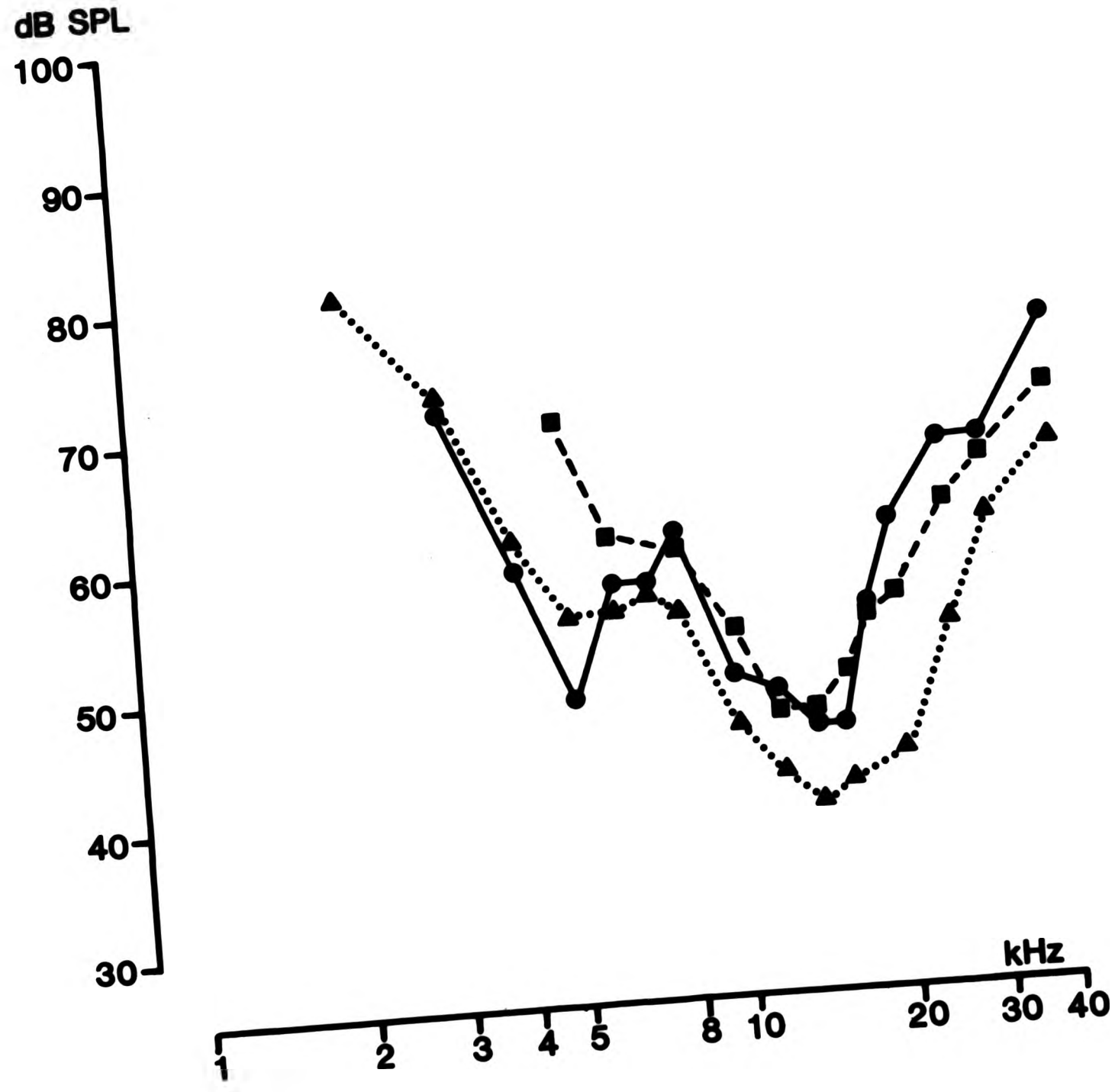

Fig. 3.17

Intensity-response curves for three AN2 units at (A) 5 kHz, (B) 14 kHz. Points show means and standard deviations of the responses to eight 50 ms stimulus presentations.

■ - - - ■ - Gc86

●——● - Gc31

▲·····▲ - Gc38

(C) shows raster displays of another AN2 unit to 5 kHz and 16 kHz at the intensities given.

Sound stimulus = 50 ms.

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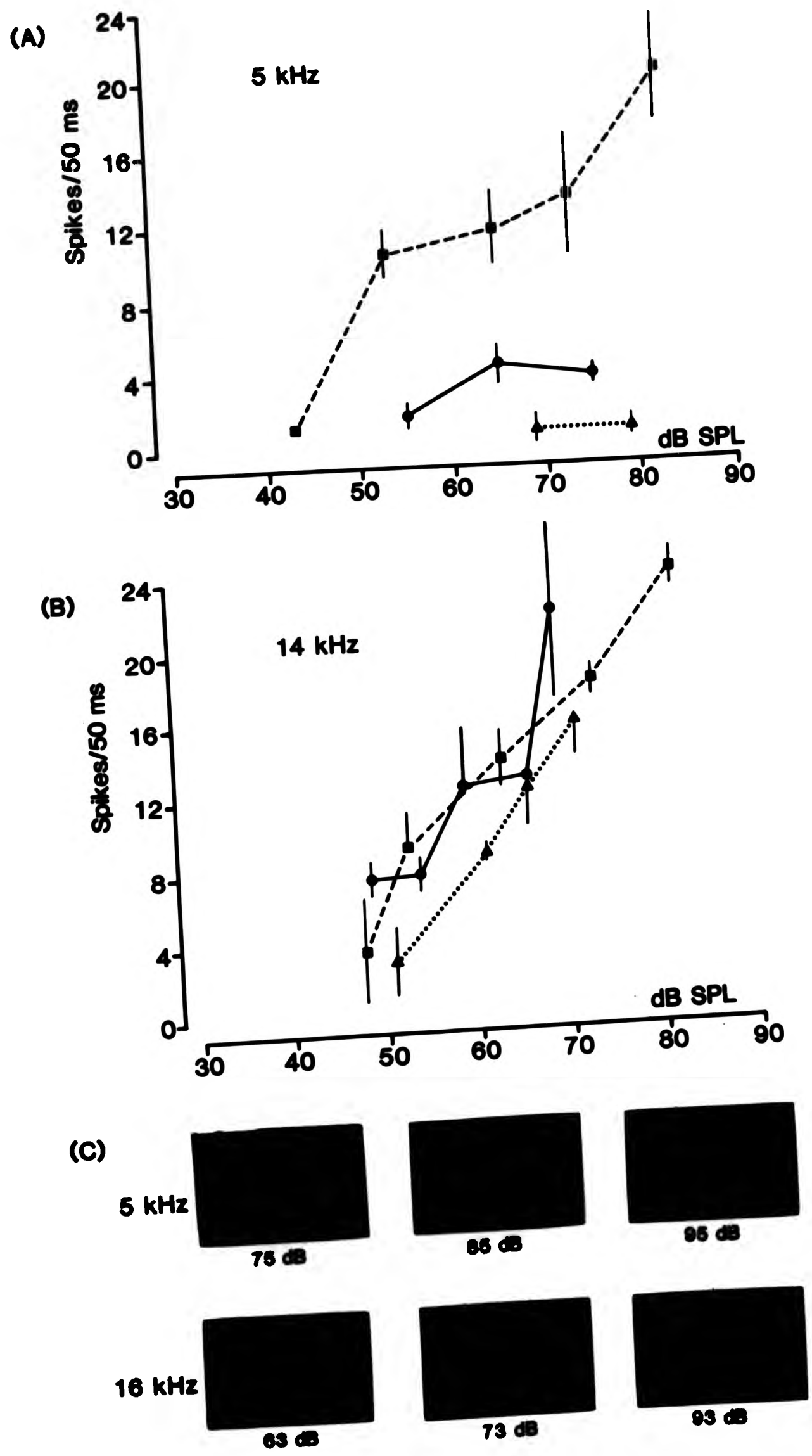
(A)
5 kHz
Spikes/50 ms
24
20
16
12
8
4
0
dB SPL
30
40
50
60
70
80
90
(B)
14 kHz
Spikes/50 ms
24
20
16
12
8
4
0
dB SPL
30
40
50
60
70
80
90
(C)
5 kHz
75 dB
85 dB
95 dB
16 kHz
63 dB
73 dB
93 dB

intensities; almost no response could be elicited by 5 kHz in unit 38.

Raster displays of responses to 5 kHz and 16 kHz at several intensities are shown in Fig. 3.17C. In this example the unit responded well to both frequencies. The responses to both frequencies are tonic, with a small degree of after-discharge at high intensities. There is a slight degree of adaptation over the first few stimulus presentations at high intensities (evident in Fig. 3.17C for 16 kHz at 73 dB), but apart from this very little adaptation occurred. The firing rate reached a maximum of 600-700 spikes per second at high intensities. At higher intensities the latency became shorter and more regular, reaching a minimum of about 6-7 ms.

The response of AN2 to high frequencies was found to be selectively suppressed by simultaneous presentation of sound around 5 kHz. The degree of this inhibition, however, was extremely variable; almost total suppression of the response occurred in some examples, whilst in others the effect was almost totally absent. Generally, those units showing poor positive responses to 5 kHz presented alone (e.g. unit 38 in Fig. 3.17) showed greater suppression of the responses to high frequencies by simultaneous presentation of low-frequency sound. The low frequency suppression was found to be always tuned to 4-5 kHz. Fig. 3.18A demonstrates the tuning of this inhibition in one specimen. Each curve gives the intensity necessary, at several frequencies, to suppress the response to a 16 kHz, 56 dB CT by the % stated, in terms of spike number. The

Fig. 3.18

Two-tone inhibition in AN2.

(A) Suppression of the response to 16 kHz at 56 dB, by simultaneous presentation of test-tones around 5 kHz. Each point gives the intensity required to decrease the response by the % stated, in terms of spike number.

(B) Raster displays of the responses of Gc48 (the same unit as in (A)) and Gc45 to high-frequency control tones presented alone and together with low-frequency test-tones.
Middle trace = control tone (50 ms)
Bottom trace = test tone
Note much greater suppression effects in Gc45.

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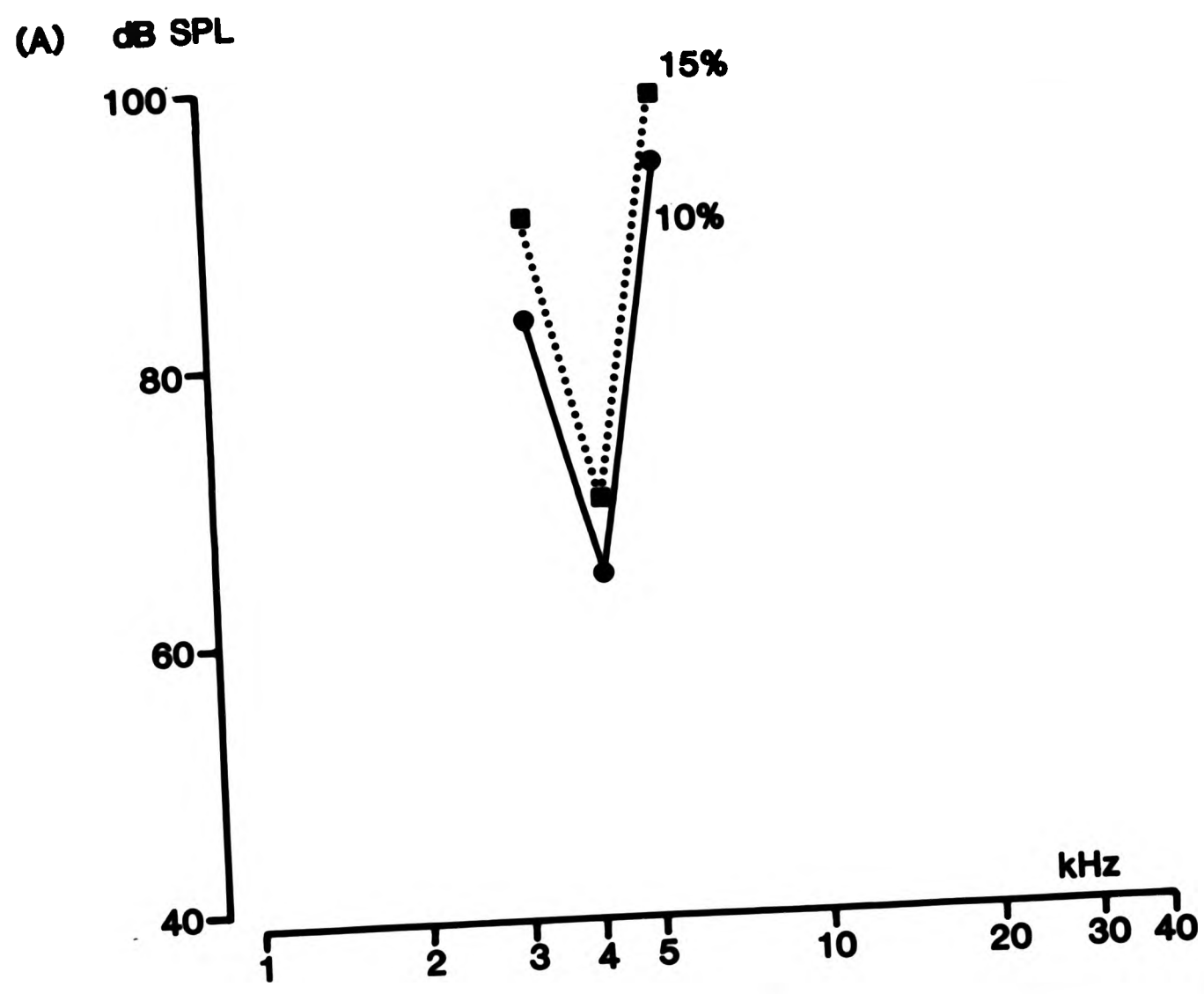
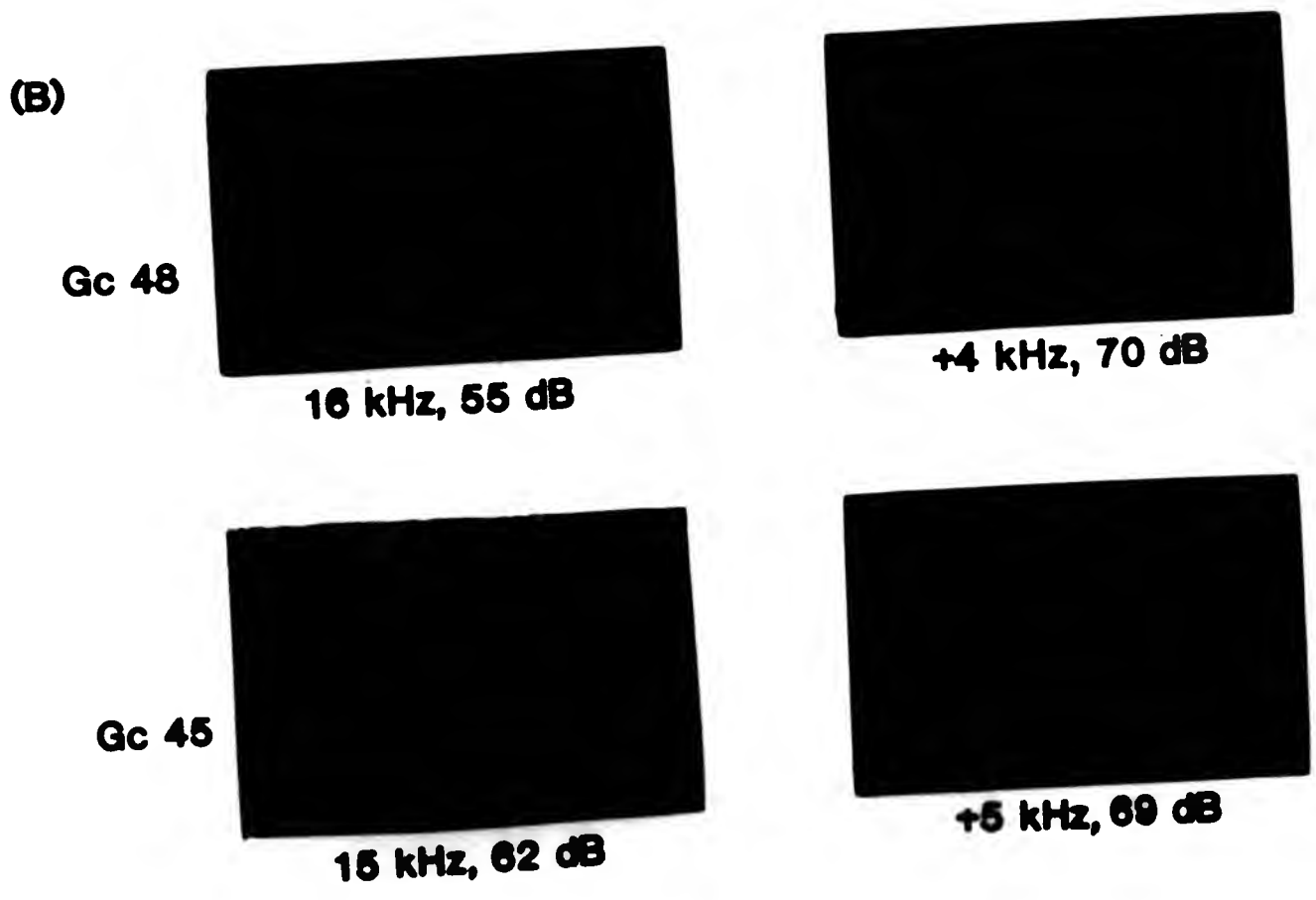




spike number is reduced by 10%, by 4 kHz at 66 dB, whereas 3 kHz had to be presented at 84 dB to produce the same reduction. The inhibition in this unit was evidently sharply tuned near 4 kHz. Fig. 3.18B gives raster displays of 16 kHz presented alone and together with 4 kHz in the same unit as in Fig. 3.18A, and in another unit showing considerably greater inhibition.

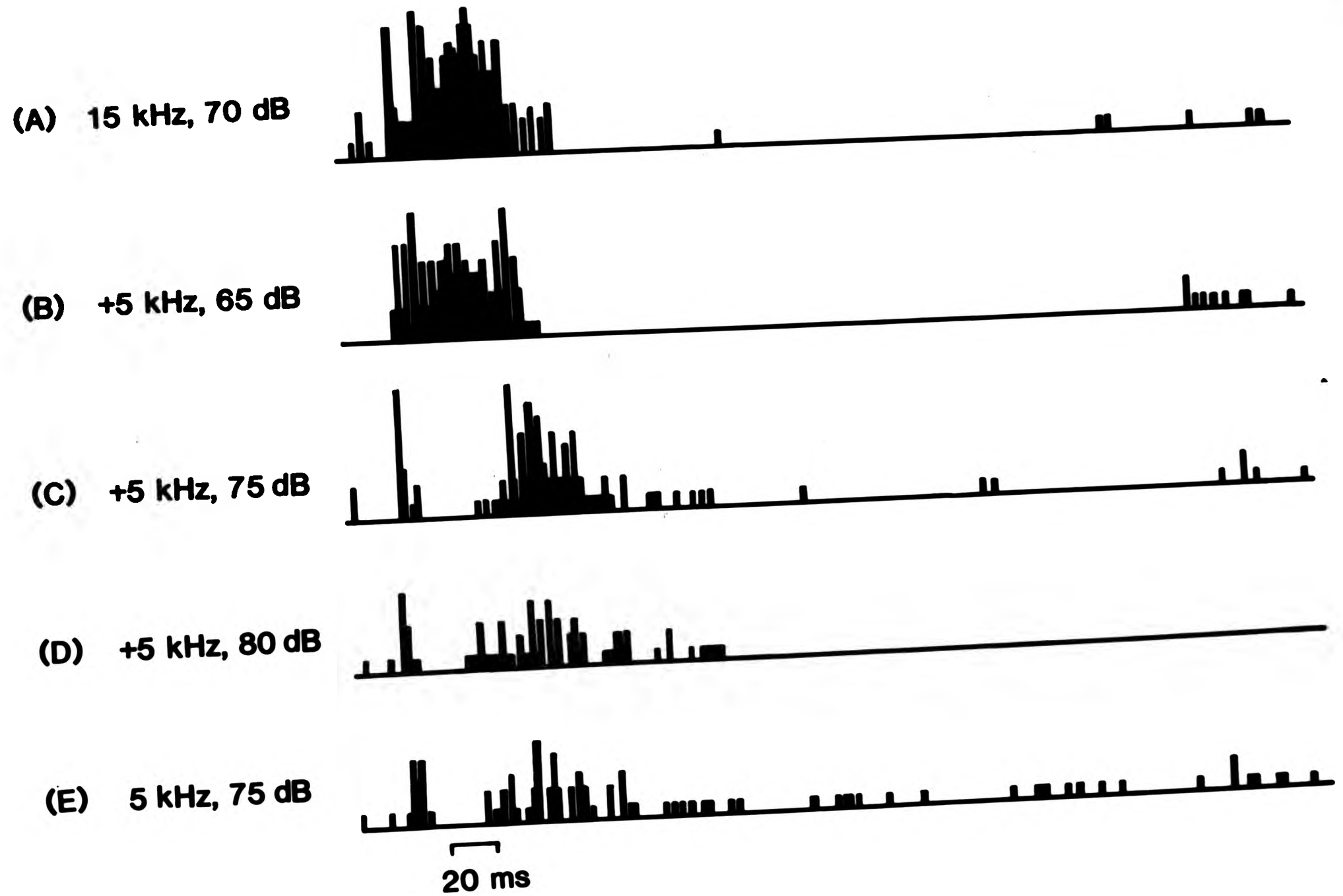
Post-stimulus-time (PST) histograms are shown, in Fig. 3.19, of the responses of AN2 to 15 kHz presented alone, and together with 5 kHz at several intensities. Presented alone (A), 15 kHz at 70 dB produced a strong response for about 60 ms. Simultaneous presentation of 5 kHz at 65 dB (B) had very little effect on the magnitude of the response, but at higher intensities (C and D) considerable suppression of the response occurred. When the response was strongly inhibited the response pattern showed an initial short excitation followed by a silent period of about 15-20 ms, and then a longer period of excitation (60-70 ms). This pattern was also produced when 5 kHz was presented alone (E), except that in this case there was a long lasting after-discharge.

It was found that the response of AN2 to 5 kHz sound was inhibited by simultaneous presentation of vibration. The inhibition was tested at vibration frequencies from 200 Hz to 1 kHz, and did not appear to be tuned to any particular frequency. The response of the unit to high frequency sound, however, was not affected by vibration. In Fig. 3.20A rasters of the responses to calling song SNS are

Fig. 3.19

PST histograms of the responses of AN2 to a 15 kHz, 50 ms stimulus presented alone (A), and together with 5 kHz at the intensities given (B,C,D). (E) shows the response to 5 kHz presented alone.

Bin width = 2 ms.


(A) 15 kHz, 70 dB
(B) +5 kHz, 65 dB
(C) +5 kHz, 75 dB
(D) +5 kHz, 80 dB
(E) 5 kHz, 75 dB
20 ms

given for 5 kHz and for 14 kHz sound presented alone, and together with 200 Hz vibration, continuous for the duration of the SNS chirp. Vibration at 200 Hz clearly inhibits the response to 5 kHz, but has very little effect on the response to 14 kHz.

Fig. 3.20B shows the effects of 500 Hz at different accelerations on the response to 5 kHz in another recording. The response to 5 kHz SNS alone is given, each syllable producing 2-3 spikes. Addition of 500 Hz vibration at 0.18 $m.s^{-2}$ produces a very slight inhibition, and possibly improves the temporal coding slightly. With stronger vibration stimulation the response is further suppressed, and at 1.8 $m.s^{-2}$ vibration each syllable produces only one spike. The temporal coding also becomes less reliable.

In Fig. 3.20C the temporal features of the vibration inhibition are investigated using 50 ms sound and vibration pulses. In each case a 500 Hz, 0.56 $m.s^{-2}$ tone is presented with a 5 kHz, 50 dB tone, and the relative onset of the two tones is varied. Delays are given for the onset of the sound stimulus relative to the vibration stimulus. With decreasing delays of the sound stimulus, the response always commences at a minimum of 20-30 ms after the end of the vibration stimulus, which suggests that the inhibition lasts 20-30 ms longer than the duration of the inhibitory stimulus. When there is zero delay between the two stimuli, no response occurred over the duration of the stimuli, demonstrating that the latency of the inhibition was no greater than that of the excitation by sound.

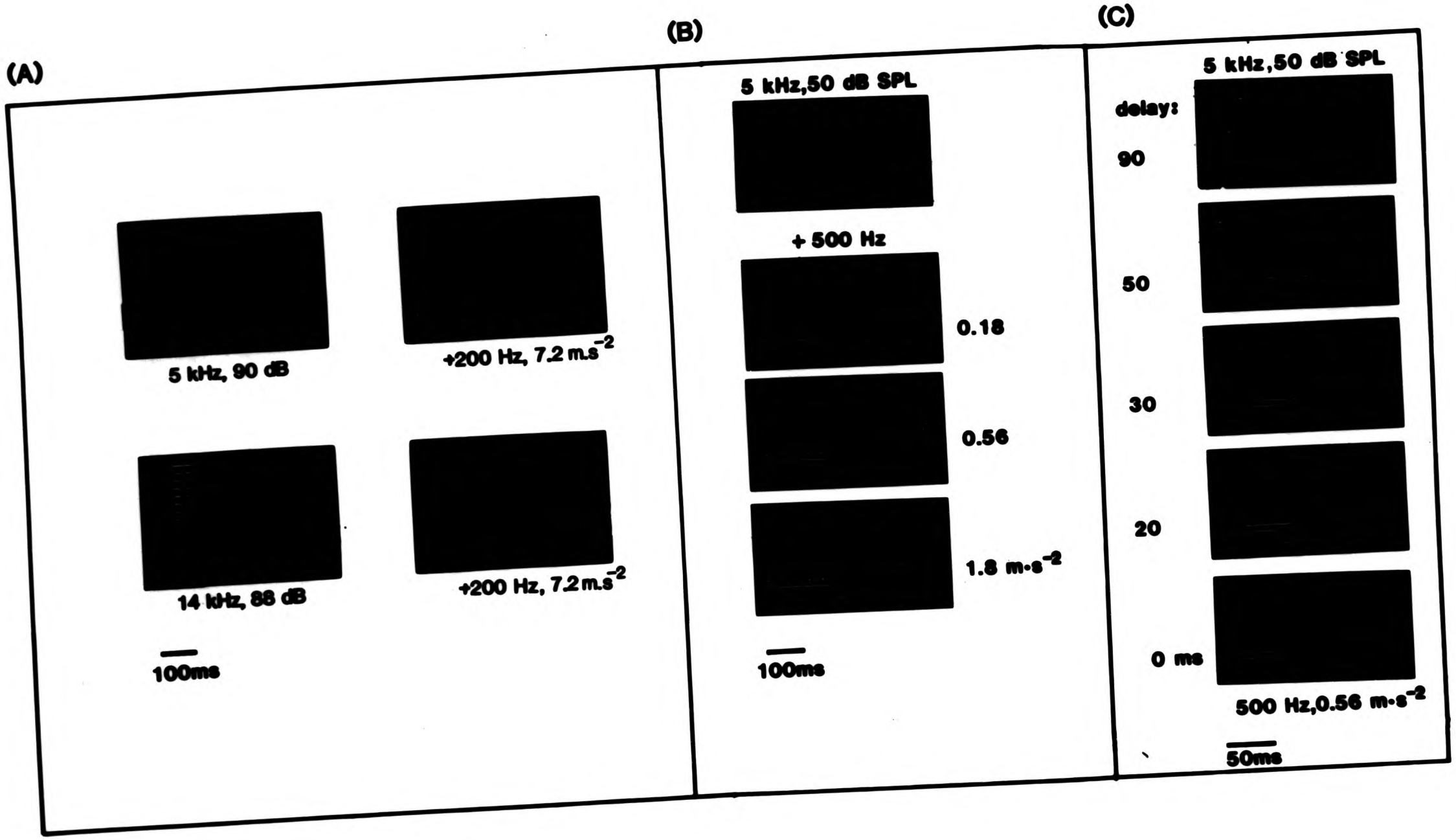
Fig. 3.20

Raster displays of responses showing the integration of sound and vibration inputs by AN2.

(A) Responses to 5kHz and 14 kHz sound stimuli having the calling song temporal pattern (calling SNS), presented alone and together with 200 Hz vibration continuous for the duration of each SNS chirp.
Middle trace = sound stimulus.
Lower trace = vibration stimulus.

(B) Response to 5 kHz calling SNS presented alone (middle trace) and together with 500 Hz continuous vibration (lower trace) at the acceleration values given.

(C) Response to 5 kHz sound (middle trace) presented together with 500 Hz vibration (lower trace). The onset of the sound stimulus was delayed relative to that of the vibration stimulus by the amounts given.


(A)
5 kHz, 90 dB
+200 Hz, 7.2 m.s-2
14 kHz, 88 dB
+200 Hz, 7.2 m.s-2
100ms
(B)
5 kHz,50 dB SPL
+ 500 Hz
0.18
0.56
1.8 m·s-2
100ms
(C)
5 kHz,50 dB SPL
delay:
90
50
30
20
0 ms
500 Hz,0.56 m·s-2
50ms

Responses to stimuli from the NS/SNS tape were similar, in most respects, to the responses described for single tone presentations. Fig. 3.21A shows the responses of one unit to the three NS types. In this unit courtship produced 2-3 spikes in response to each syllable, whereas the calling and aggression songs elicited almost no response (this unit was very insensitive to 5 kHz sound). The responses of the same unit to calling, aggression and courtship SNS are given in Fig. 3.21B. In each case the unit responded well to 16 kHz and to 5+16 kHz but very little to 5 kHz alone. For the calling and aggression songs there is evidence of slight suppression of the 16 kHz response when presented together with 5 kHz.

In many cases, strong responses of AN2 were followed by a period of rebound excitation, which could last up to several seconds. This occurred particularly after responses to prolonged stimuli. Fig. 3.21C shows the responses of a unit to certain NS and SNS, on a slow time base (this unit was relatively sensitive to 5 kHz). The repeated NS calling song chirps produced strong rebound excitation. This activity is regarded as rebound excitation, rather than after-discharge, as there was always a short silent period after the main response and before the rebound activity. The responses of this unit to calling SNS suggest that the rebound activity is strongest following low-frequency stimulation; it is clear, in Fig. 3.21C, that it was considerably stronger during 5 kHz than during 16 kHz SNS, even though the positive response to 16 kHz was as least as strong as it was to 5 kHz.

Fig. 3.21

Analogue responses of AN2 to natural and synthesized songs.

Lower trace = stimulus.

Upper trace = neural response.

(A) Responses to calling, aggression and courtship natural songs.

Peak intensities: Calling - 75 dB
Aggression - 74 dB
Courtship - 53 dB

(B) Responses of the same unit as in (A) to SNS containing the frequencies given.

Peak intensities: 5 kHz - 70 dB
16 kHz - 65 dB
5+16 kHz - 70+66 dB

(C) Responses of a different unit to calling SNS at 16 kHz and 5 kHz, and to the natural calling song (NS).

Peak intensities: 5 kHz - 60 dB
16 kHz - 55 dB
NS - 65 dB

Time scale in all traces = 400 ms.

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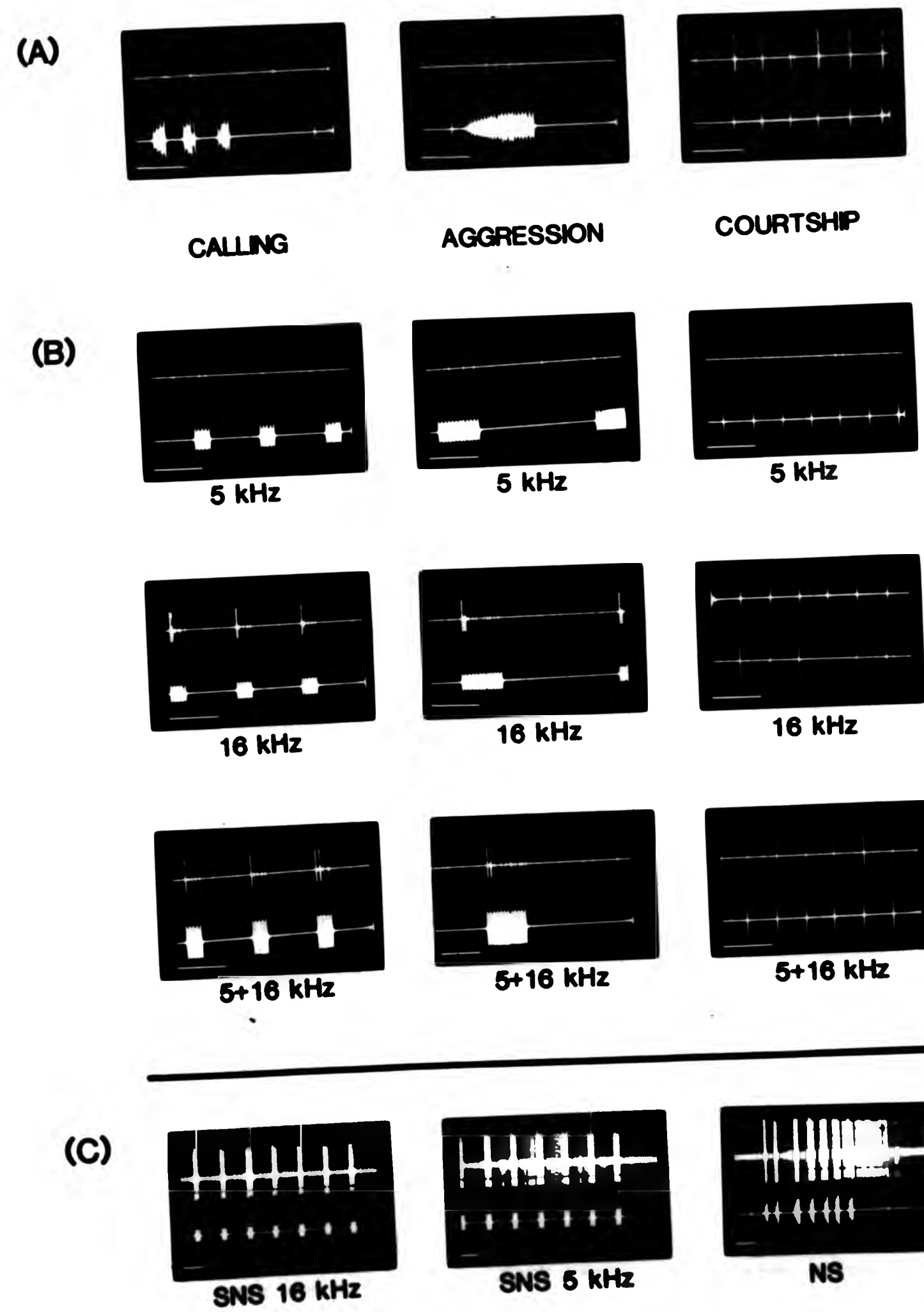
g (NS).


(A)
CALLING
AGGRESSION
COURTSHIP
(B)
5 kHz
5 kHz
5 kHz
16 kHz
16 kHz
16 kHz
5+16 kHz
5+16 kHz
5+16 kHz
(C)
SNS 16 kHz
SNS 5 kHz
NS

(ii) TN2

This neurone was recorded and successfully stained as a single fill in only one instance, but subsequent recordings in this laboratory have shown the data reported here to be characteristic of this unit (Kuhne & Silver, personal communication). The neurone is a "through-unit", i.e. it communicates directly with both posterior and anterior ganglia. As it is clearly distinct, morphologically and physiologically, from the through-unit (TN1) described by Wohlers & Huber (1982) it is termed here as TN2.

Anatomy

Fig. 3.22 shows the morphology of TN2 in the prothoracic ganglion, viewed ventrally (A) and laterally (B). A single axon passes through the prothoracic ganglion, in which it appears thinner than in the connectives. It runs close to the midline in the ganglion, and near the medial side of the ascending and descending connectives. The neurone has not been filled as far as the mesothoracic or suboesophageal ganglia. Within the prothoracic ganglion several dendrites branch from the axon along its length. These extend mostly laterally and dorsally, but do not appear to be concentrated in any particular region of the ganglion. The cell body is not located in the prothoracic ganglion.

Physiological Responses

Limited tests were carried out on TN2, but the recordings that were made show it to produce very similar responses to AN2. The only threshold curve measured for this unit is

Fig. 3.22

Camera lucida drawing showing the anatomy of TN2 in the prothoracic ganglion, viewed (A) ventrally, and (B) laterally. Arrows indicate anterior and ventral directions.

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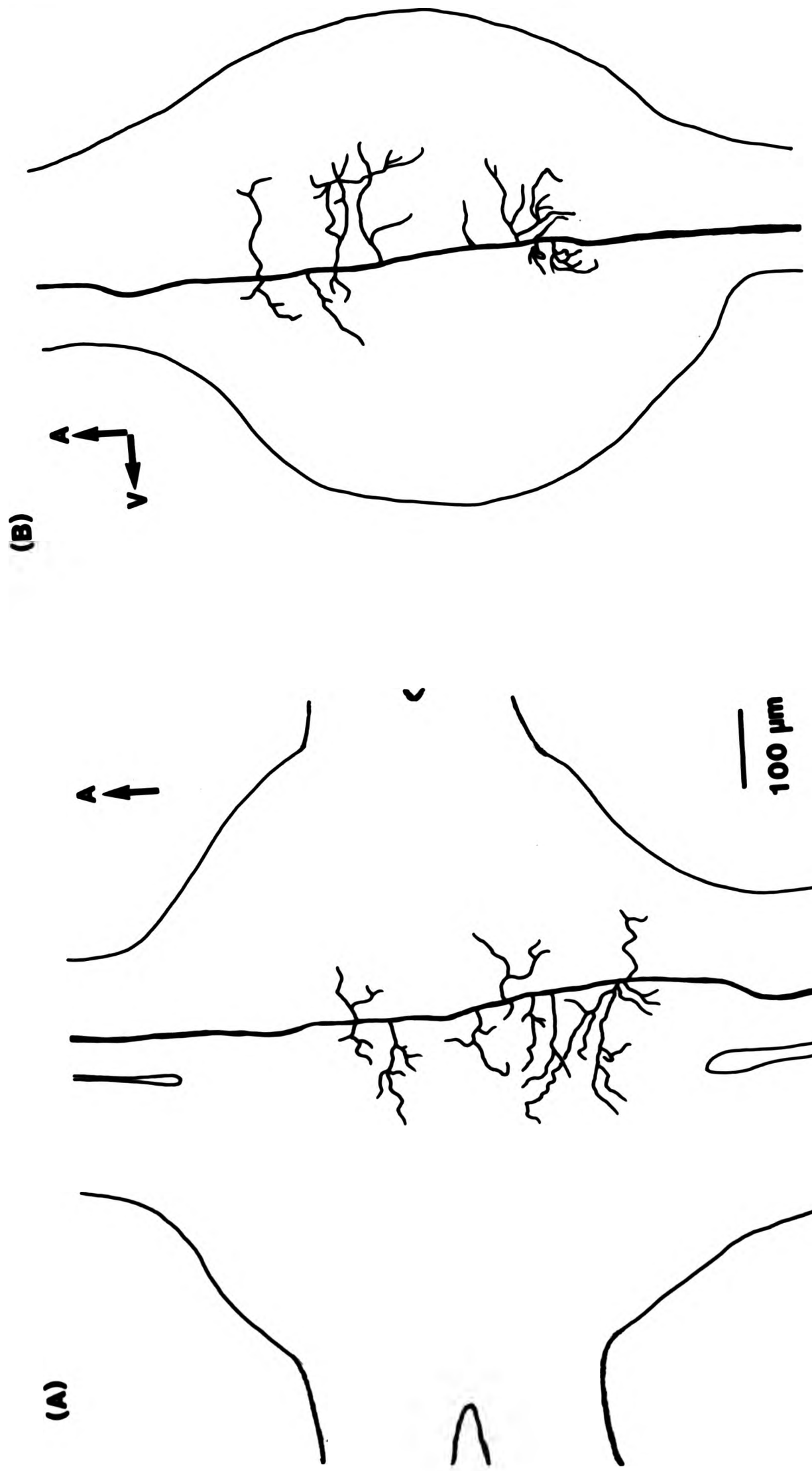


given in Fig. 3.23A, and shows it to be most sensitive to sound frequencies of 10-20 kHz, where the threshold is about 40 dB. There is also an indication of a small secondary peak of sensitivity at 5 kHz.

Fig. 3.23B shows raster displays of the responses of TN2 to 14 kHz sound at several intensities. The responses can be seen to be tonic, showing very little adaptation, and with a small ammount of after-discharge. At very high intensities the maximum firing rate of about 600 spikes per second is reached, and the latency is 7-8 ms. Responses to NS or SNS were not tested in this preparation.

(iii) Unstained Units

Recordings were made, from 17 preparations, of units that were not successfully stained, but were preferentially sensitive to high frequency sound. Most of these showed similar threshold curves and/or suprathreshold responses to those of AN2 and TN2. It is quite likely that many of them were these units, but as the characteristics of AN2 and TN2 were similar, and those of the former very variable, no attempt was made to classify these unstained neurones.

(C) Neurones Sensitive to Low-Frequency Vibration

A homogeneous group of 8 units (Gc 39,52,54,61,65,67,71,78) was found to be tuned to vibration frequencies of 100-200 Hz. Suprathreshold response patterns were consistent with the exception of 2 units (Gc 61 and Gc 78) which are discussed separately below. Unfortunately very

Fig. 3.23

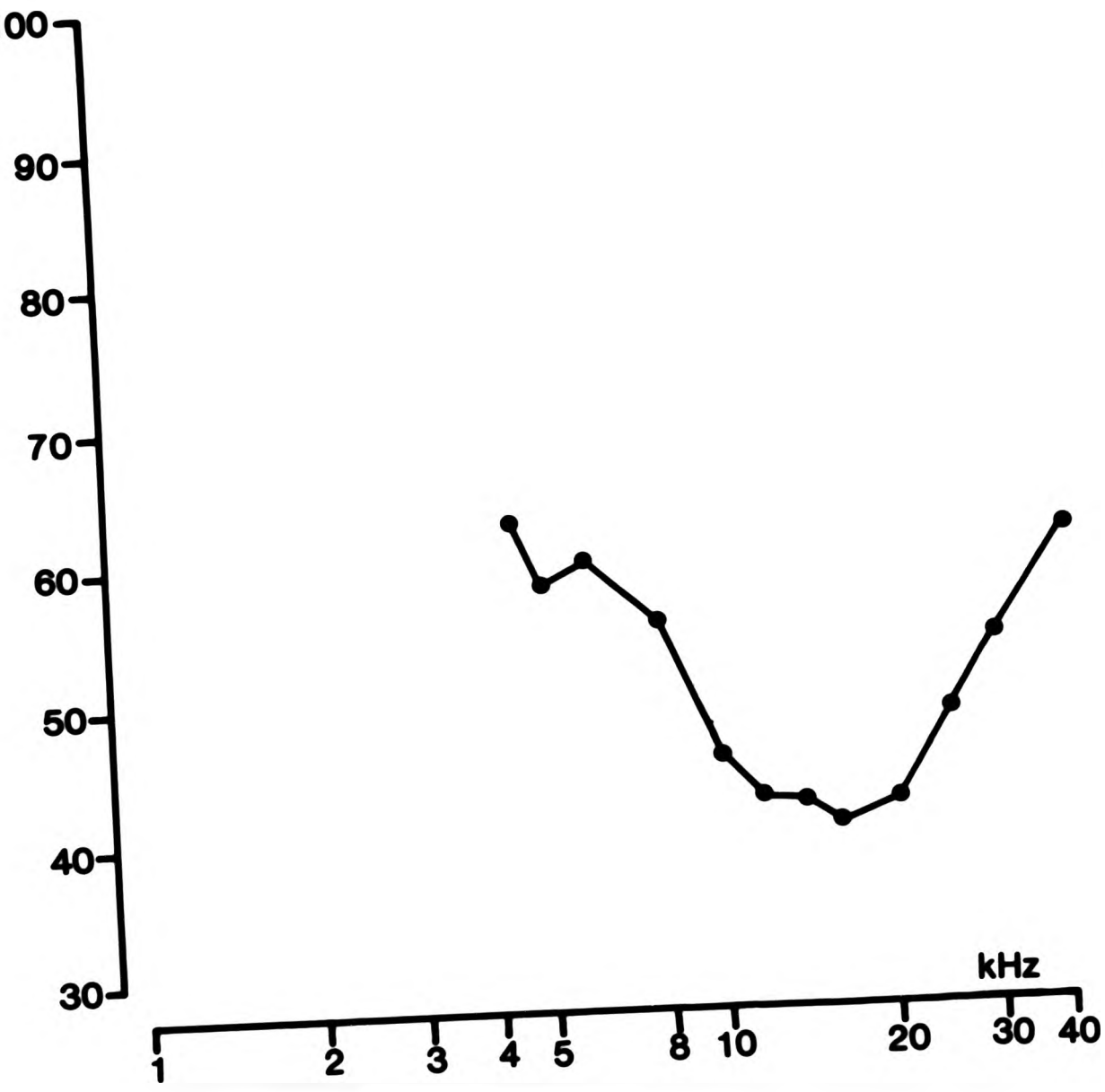
(A) Threshold curve of TN2.

(B) Raster displays showing the response of TN2 to 14 kHz at the intensities given. 50 ms stimulus.

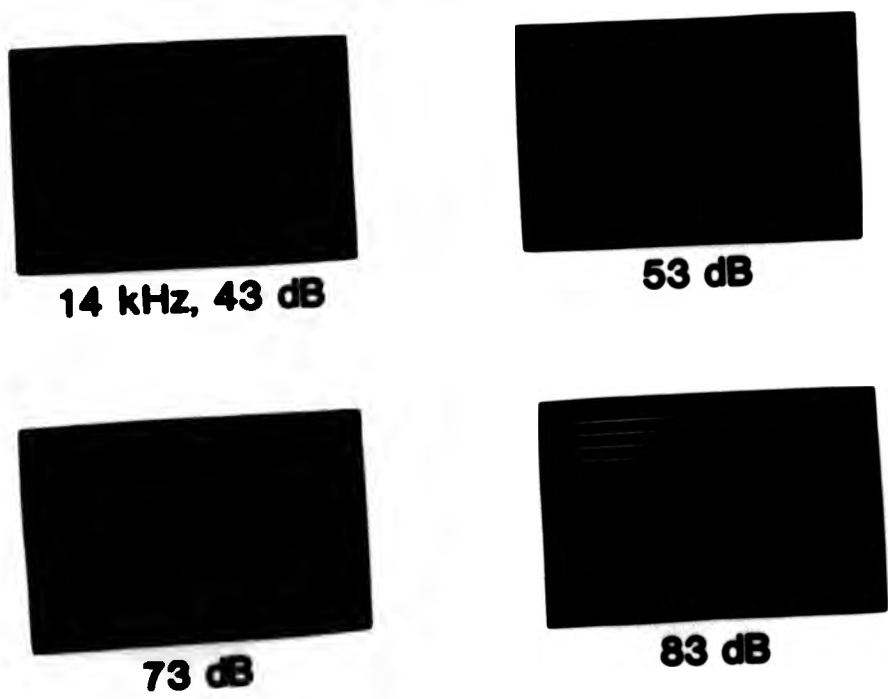
Only four responses to 83 dB are shown.

4 kHz

(A) dB SPL



(B)



little success was achieved in staining these units. The only example stained showed a single axon passing through the prothoracic ganglion, with no dendritic branches, and was not filled as far as the suboesophageal or mesothoracic ganglia. The characterization of these units, therefore, is based entirely on their physiological properties.

Fig. 3.24A gives the threshold curves of 3 of these units to demonstrate the range encountered within this group. They are clearly tuned, to varying degrees, around 200 Hz, and have thresholds in the physiological range from about 50 Hz to 1000 Hz. Raster displays in Fig. 3.24B show the typical response patterns near the characteristic frequency of one unit (Gc 52). In response to a 50 ms stimulus the unit typically produced a weak tonic discharge with a latency of about 15 ms and a duration approximately equal to the stimulus duration. Increasing the stimulus duration shows that the response adapts over durations longer than 100-200 ms; the response to 500 ms stimulus lasted about 300 ms. In all units recorded spontaneous activity was either very low or totally absent. None were responsive to sound stimulation.

The intensity-response characteristics of this group are shown in Fig. 3.25. Fig. 3.25A gives the intensity-response curves at 200 Hz and 500 Hz for Gc65. At both frequencies the spike number increases gradually with increasing stimulus acceleration over the range tested, but 200 Hz clearly produces a stronger response than 500 Hz (the whole dynamic range of this unit was not tested). Fig. 3.25B gives raster displays of the responses of this

Fig. 3.24

(A) Thresholds of 3 units sensitive to low-frequency vibration, over the frequency range 50-2000 Hz. Threshold values are peak-to-peak accelerations.

(B) Responses of Gc 52 to 200 Hz, 1.0 $m.s^{-2}$ vibration stimuli, of the durations given.
Lower trace = vibration stimulus.

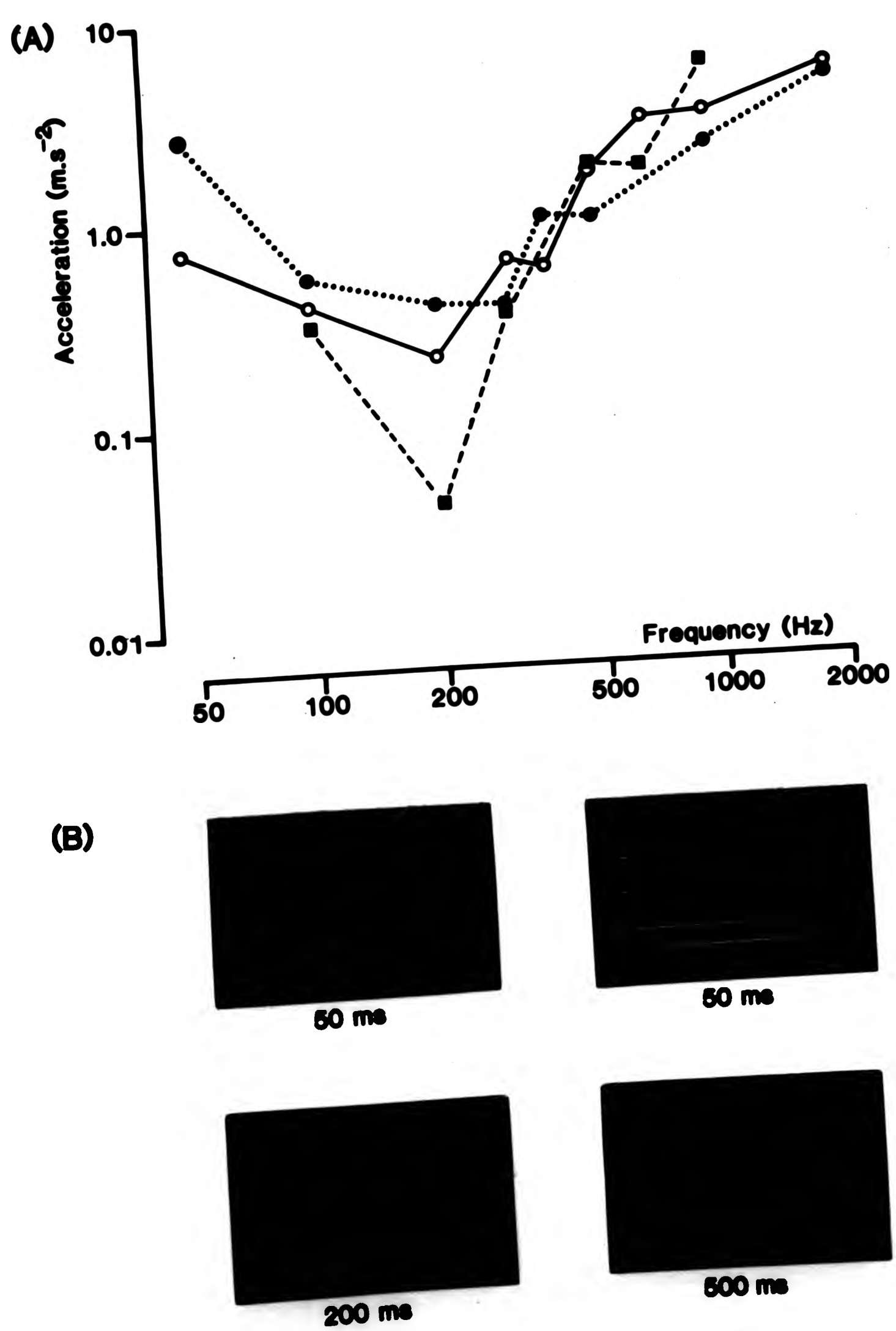

(A)
10
1.0
0.1
0.01
Acceleration (m.s-2)
Frequency (Hz)
50
100
200
500
1000
2000
(B)
50 ms
50 ms
200 ms
500 ms

Fig. 3.25

(A) Intensity-response curves showing responses of Gc 65 to 200 Hz and 500 Hz vibration. Points show means and standard deviations for eight presentations of the 50 ms stimulus.

(B) Raster displays showing responses of the same unit to 200 Hz vibration at the acceleration values given. Bottom trace = vibration stimulus.

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kit
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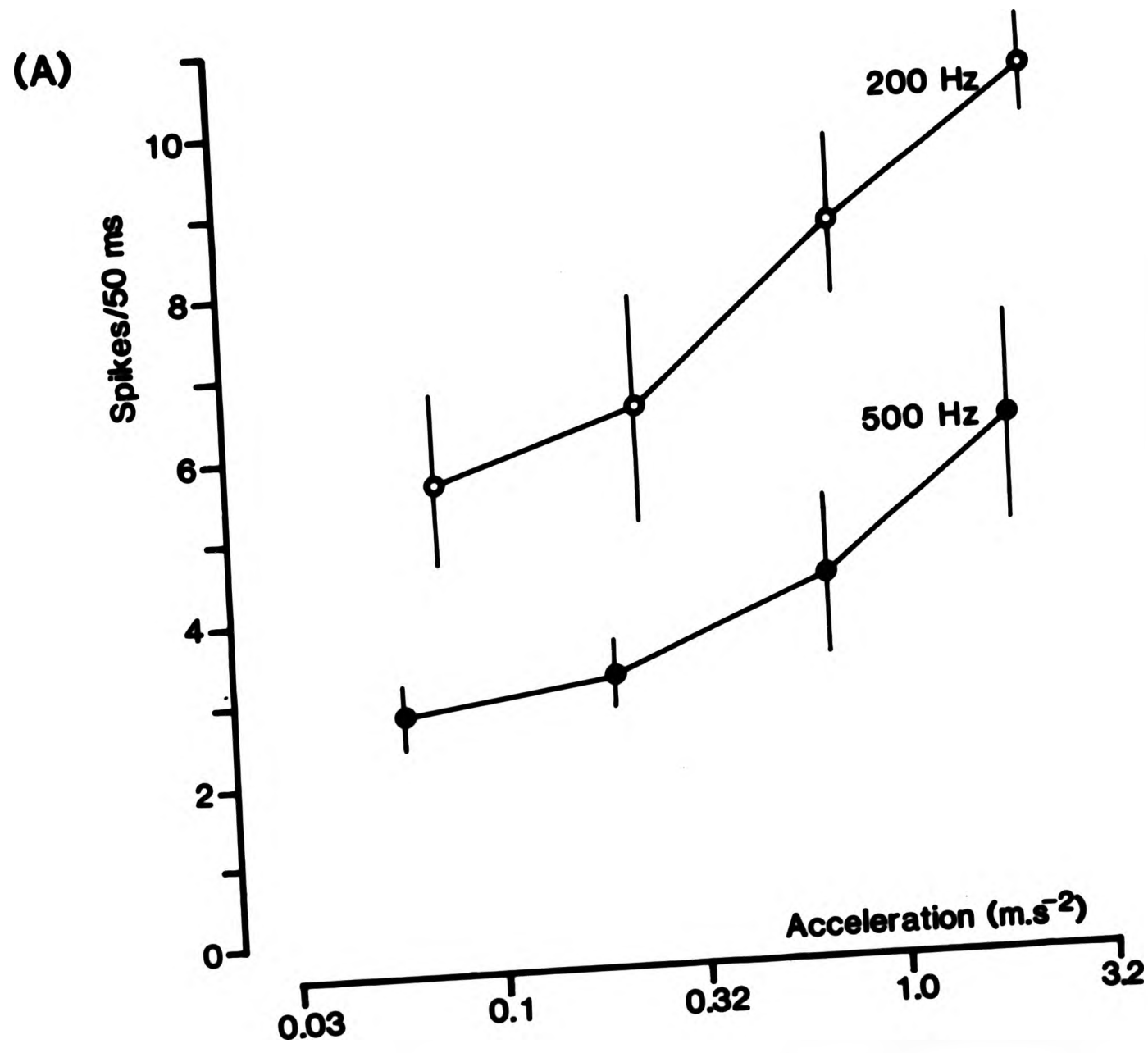

unit to 200 Hz. An interesting feature is that the onset of the response becomes more synchronized at higher acceleration values, up to 0.72 $m.s^{-2}$, above which the onset again becomes more variable. This trend was evident in several of the units recorded.

In one preparation (Gc 67) the contribution of the inputs from the different legs was investigated by sectioning experiments. Fig. 3.26 shows the threshold curve for the unit recorded in the intact state, after sectioning all the contralateral legs, and after sectioning the ipsilateral hind leg. The unit in the intact state was most sensitive to 200 Hz. Sectioning all the contralateral legs reduced the sensitivity of the unit at most frequencies, except that the sensitivity to 200 Hz was retained. After sectioning the ipsilateral hind leg this peak of sensitivity was lost, although the unit still responded with moderate sensitivity to vibration from 50 Hz to 2000 Hz.

Unit 61 was most sensitive to vibration of 100 Hz, but its threshold curve was more broad-banded than the other units sensitive to low-frequency vibration, and it is therefore likely that this unit is distinct from the group described above. Fig. 3.27 shows raster displays of the responses of the unit to 200 Hz at several acceleration values (A) in the intact state, (B) after removal of the contralateral hind leg, and (C) after removal of the ipsilateral hind leg. The response in the intact state is clearly phasic, of 1-2 spikes per stimulus at intermediate accelerations, and 2-3 spikes at high accelerations.

Fig. 3.26

Threshold curves to vibration, over the frequency range 50-2000 Hz, for unit Gc 67 in the intact state (all 6 legs present), after cutting the contralateral legs (all ipsilateral legs present), and after also cutting the ipsilateral foreleg (ipsilateral fore and mid-legs present).

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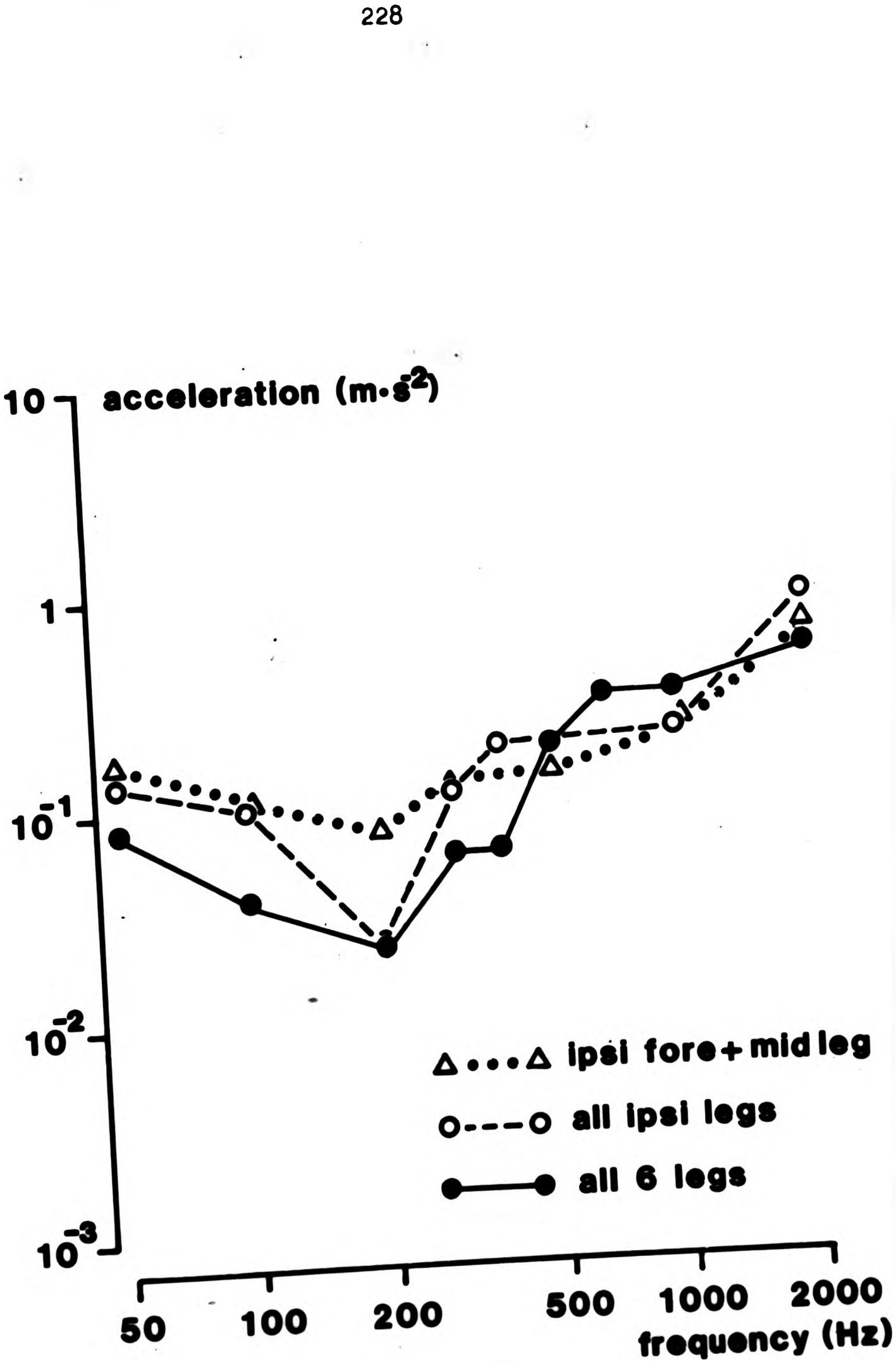


Fig. 3.27

Raster displays showing the responses of unit Gc 61 to 200 Hz vibration at the acceleration values given

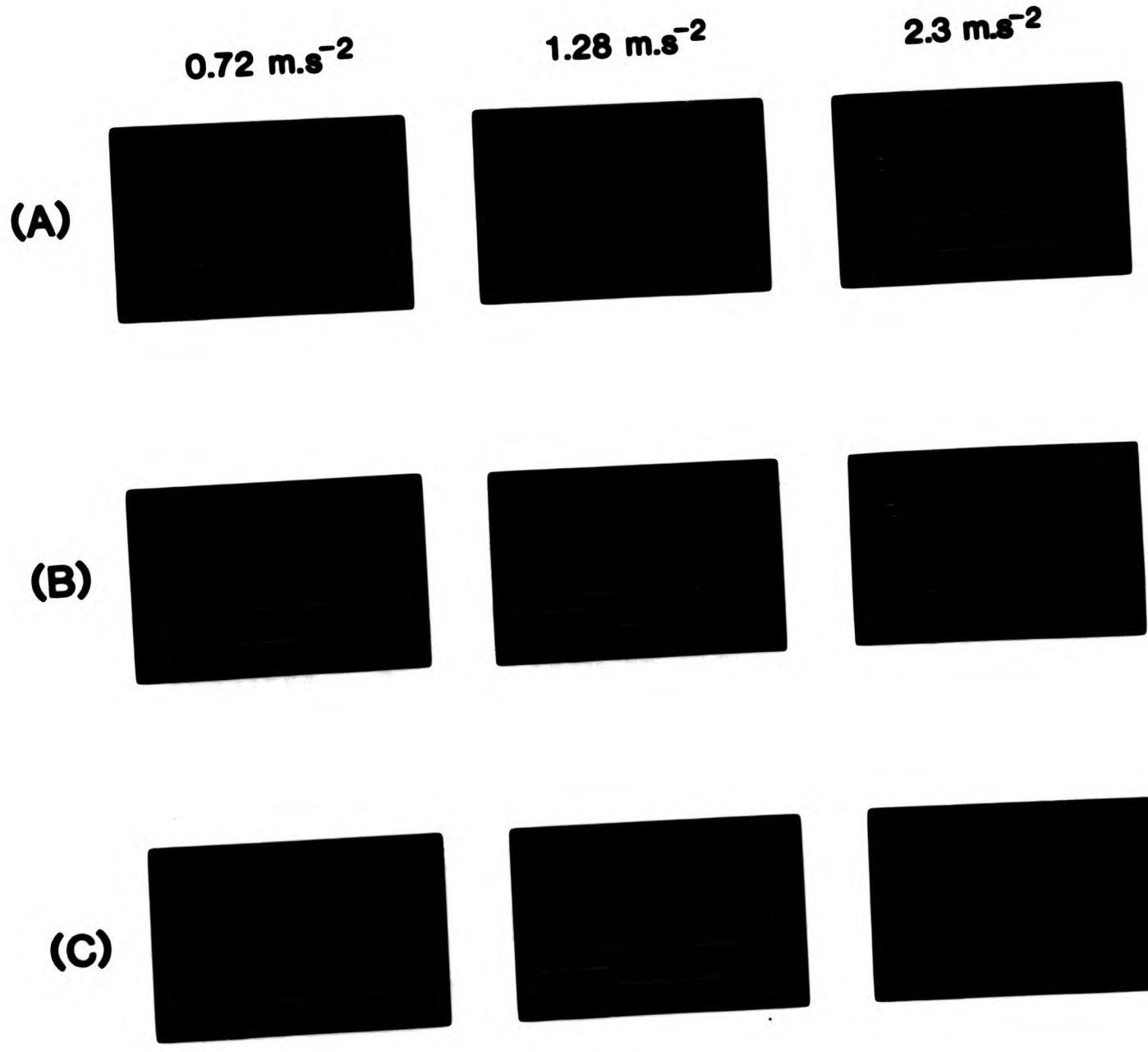
(A) in the intact state,

(B) after removal of the contralateral hind leg, and

(C) after removal of the ipsilateral hind leg.

50 ms stimuli.

Bottom trace = vibration stimulus.


0.72 m.s^{-2}
1.28 m.s^{-2}
2.3 m.s^{-2}
(A)
(B)
(C)

Sectioning the contralateral hind leg increased the response considerably, and it became slightly more tonic. Spike numbers of 5-6 were produced in response to high intensity stimuli. Sectioning of the ipsilateral hind leg had little further effect on the responses. This unit was not held sufficiently long to carry out further leg sectioning experiments.

Gc 78 was a vibration-sensitive unit, tuned around 200 Hz, which produced responses considerably stronger than those of the other low-frequency units described. Fig. 3.28A shows the intensity-response characteristics of the unit at 200, 500, and 1000 Hz. The unit responded preferentially to 200 Hz at all acceleration values. There is also a suggestion of high-intensity inhibition in the 200 Hz curve. The raster displays of these responses are given in Fig. 3.28B. They show the unit to produce a strong tonic response, particularly to higher acceleration values of 200 Hz vibration. A small degree of adaptation is evident.

(D) Neurones Sensitive to Mid/High Frequency Vibration

A population of units was found to be preferentially sensitive to vibration frequencies of 500-1000 Hz. The tuning of none of these units was very sharp, and many of them were more broad-banded than tuned to a particular frequency. It was considered expedient to divide these units into two groups: one in which the units did not respond to sound, and another in which low-frequency sound

Fig. 3.28

Suprathreshold response characteristics of unit Gc 78.

(A) Intensity-response curves for 200 Hz, 500 Hz and 1000 Hz. Means and standard deviations of the spike counts to 50 ms stimuli are shown.

(B) Raster responses to 200 Hz, 500 Hz and 1000 Hz, at the acceleration values shown.
50 ms stimulus (lower trace).

78.
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spike
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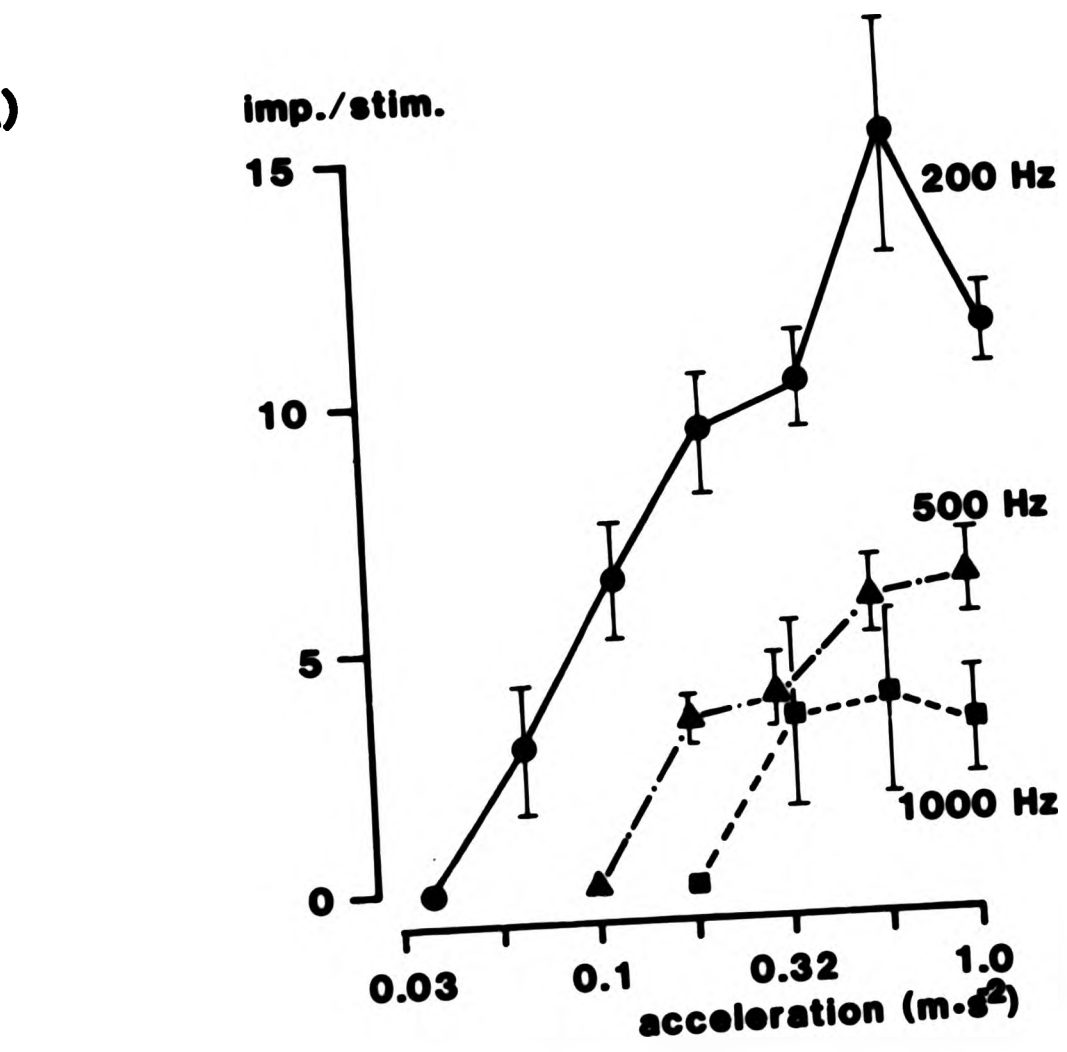

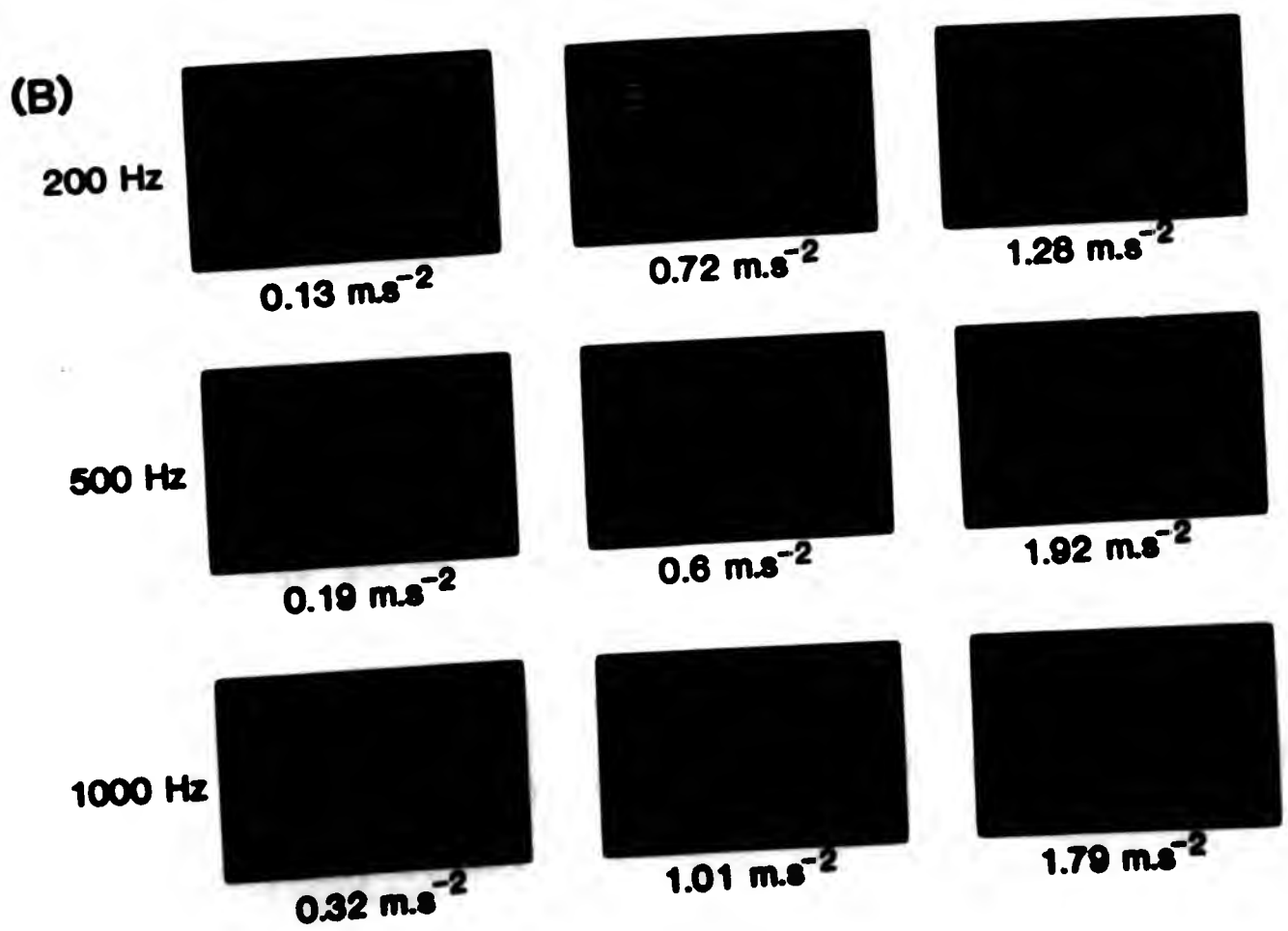

produced an augmentation in the responses to vibration.

The units unresponsive to sound formed a very homogeneous group (preparations 42,62,80,81,83). They all showed spontaneous activity of 30-50 spikes per second and produced weak tonic discharges in response to vibration of 200-1000 Hz. Fig. 3.29 shows the threshold curves of three of these units. They are all rather broad-banded, but preferentially sensitive to frequencies between 500 Hz and 1 kHz. The threshold acceleration was around 0.05-0.1 $m.s^{-2}$ at the characteristic frequency in all 5 specimens.

The suprathreshold characteristics are shown by intensity-response curves of 200 Hz, 500 Hz and 1 kHz in Fig. 3.30A, for specimen Gc81. The strongest response is produced by 500 Hz at all acceleration values; 200 Hz produces a stronger response than 1 kHz, although the thresholds for these two frequencies are the same. The raster displays in Fig. 3.30B demonstrate the response patterns for these frequencies. The relative sensitivities shown in the intensity-response curves are evident in the responses, and they also show the influence of the stimuli on the background spontaneous activity. Generally, at high acceleration values the spontaneous firing is suppressed to some extent for about 50 ms following the response of the unit. The firing rate reached a maximum of about 200 spikes/s, and no adaptation was evident in any of the responses. The latency of the response was notably long, the minimum being 25 ms.

Fig. 3.29

Threshold curves of three units sensitive to mid/high frequency vibration, and not sensitive to sound. Thresholds to vibration are given, over the frequency range 50-2000 Hz.

...reshold
...2000 Hz.

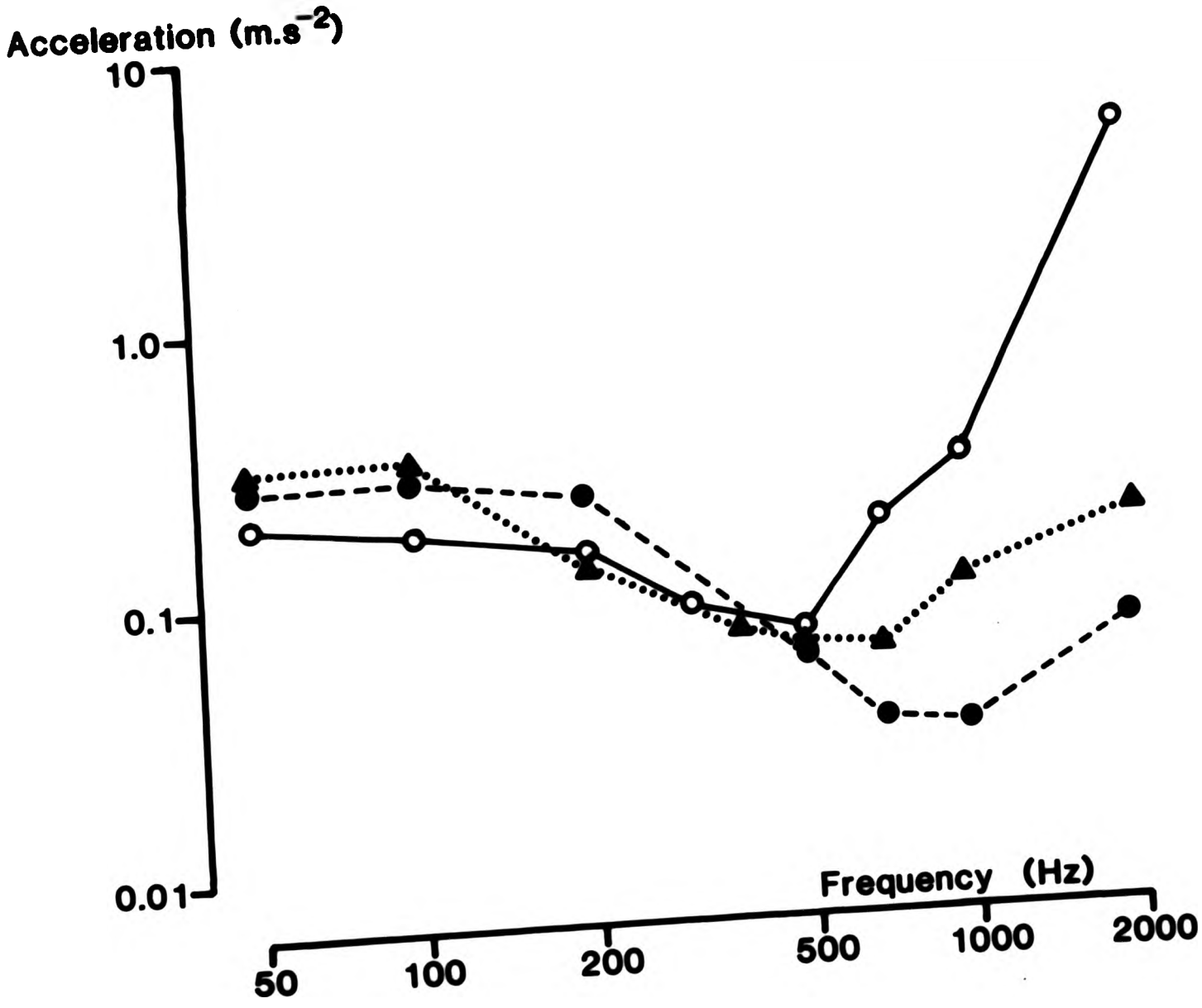

Fig. 3.30

Suprathreshold response characteristics of unit Gc 81.

(A) Intensity-response curves for 200 Hz, 500 Hz and 1000 Hz. Means and standard deviations of the spike counts to 50 ms stimuli are given.

(B) Raster responses to 200 Hz, 500 Hz and 1000 Hz, at the acceleration values shown.
50 ms stimulus (lower trace).

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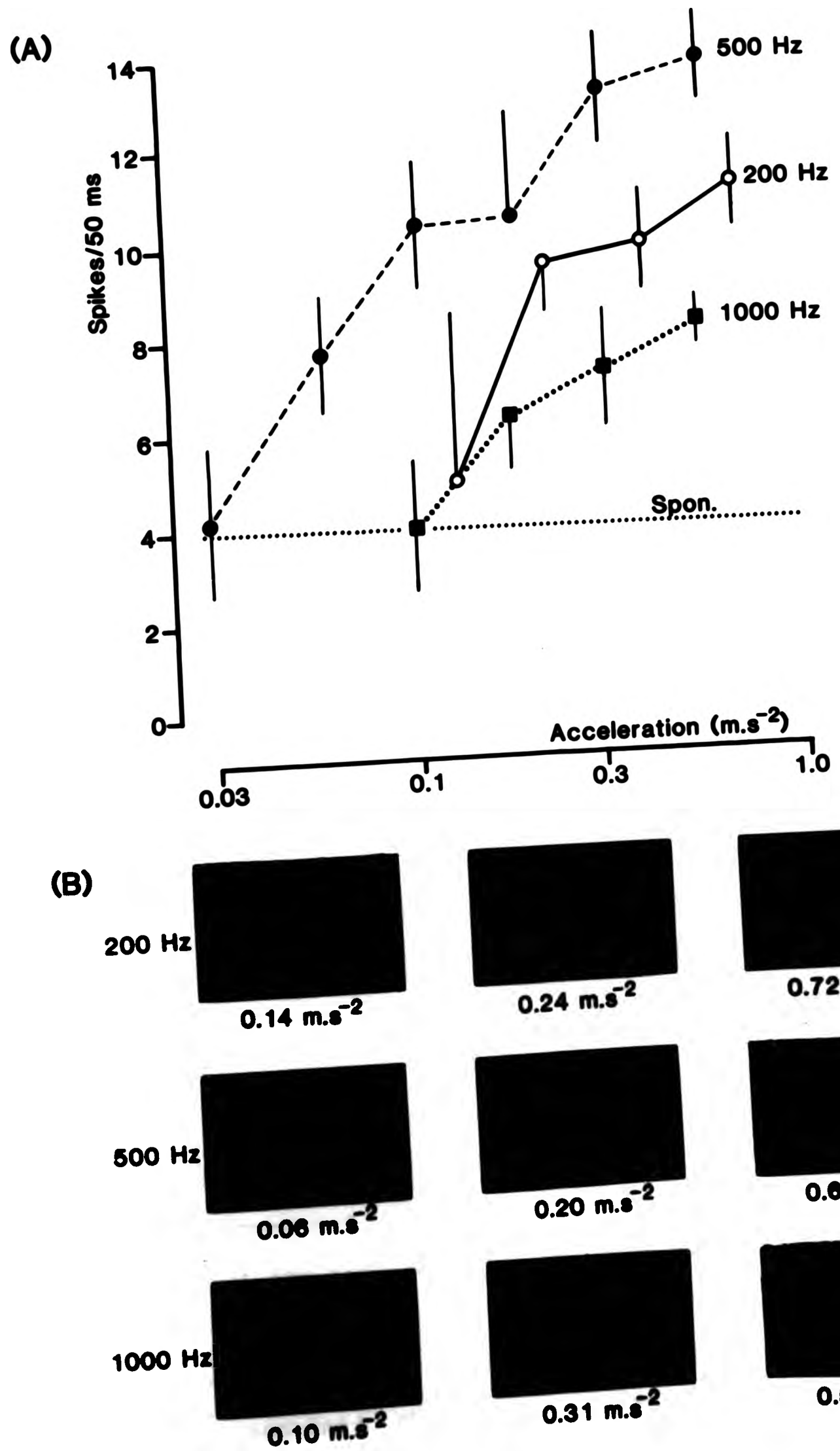
(A)
Spikes/50 ms
0
2
4
6
8
10
12
14
500 Hz
200 Hz
1000 Hz
Spon.
Acceleration (m.s^{-2})
0.03
0.1
0.3
1.0
(B)
200 Hz
0.14 m.s^{-2}
0.24 m.s^{-2}
0.72 m.s^{-2}
500 Hz
0.06 m.s^{-2}
0.20 m.s^{-2}
0.60 m.s^{-2}
1000 Hz
0.10 m.s^{-2}
0.31 m.s^{-2}
0.56 m.s^{-2}

The threshold curves of preparations 37,46,65 & 91 were similar to those of the previous group, but their responses were augmented by sound, particularly of around 5 kHz. However, this group was not very homogeneous, and differences in several of the response characteristics gave the impression that the group comprises several different neurones. Only one (Gc65) was spontaneously active.

The influence of sound on the responses of these units to vibration is given in Fig. 3.31, for two examples. In Fig. 3.31A the response of Gc91 to 500 Hz vibration is shown when presented alone, and together with 5 kHz sound. In each case the response is enhanced by the sound stimulus, particularly for low intensity vibration. However, the response of the unit to 5 kHz sound alone can be seen to be minimal. In Fig. 3.31B the response of Gc46 (a more phasic unit) can be seen to be enhanced by sound of 5 kHz and 12 kHz. The minimum latency in all these units was 10-15 ms. In Fig. 3.32 the effects of leg sectioning on the thresholds of Gc37 to vibration (A) and sound (B) are given. In the intact state the unit was most sensitive to vibration of 500-1000 Hz, and to sound of 3-5 kHz. After sectioning both hind legs the thresholds to vibration increased (the unit became less sensitive) at all frequencies except 700 Hz (sound thresholds not measured). The thresholds to vibration increased further, at all frequencies, following sectioning of the middle legs. The thresholds to sound were considerably higher after sectioning the hind and middle legs, as compared to the intact state, although the shape of the curve remained the same. This unit produced fairly

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Fig. 3-31

Raster responses of vibration-sensitive units whose responses were augmented by sound stimulation.

(A) Responses of Gc91 to 5 kHz sound alone, 500 Hz vibration alone, and the two presented together, at several intensities.

(B) Response of Gc46 to 500 Hz vibration presented alone, and together with 5 kHz and 12 kHz sound.

50 ms stimuli in both (A) and (B).

Middle trace = sound stimulus

Lower trace = vibration stimulus

responses

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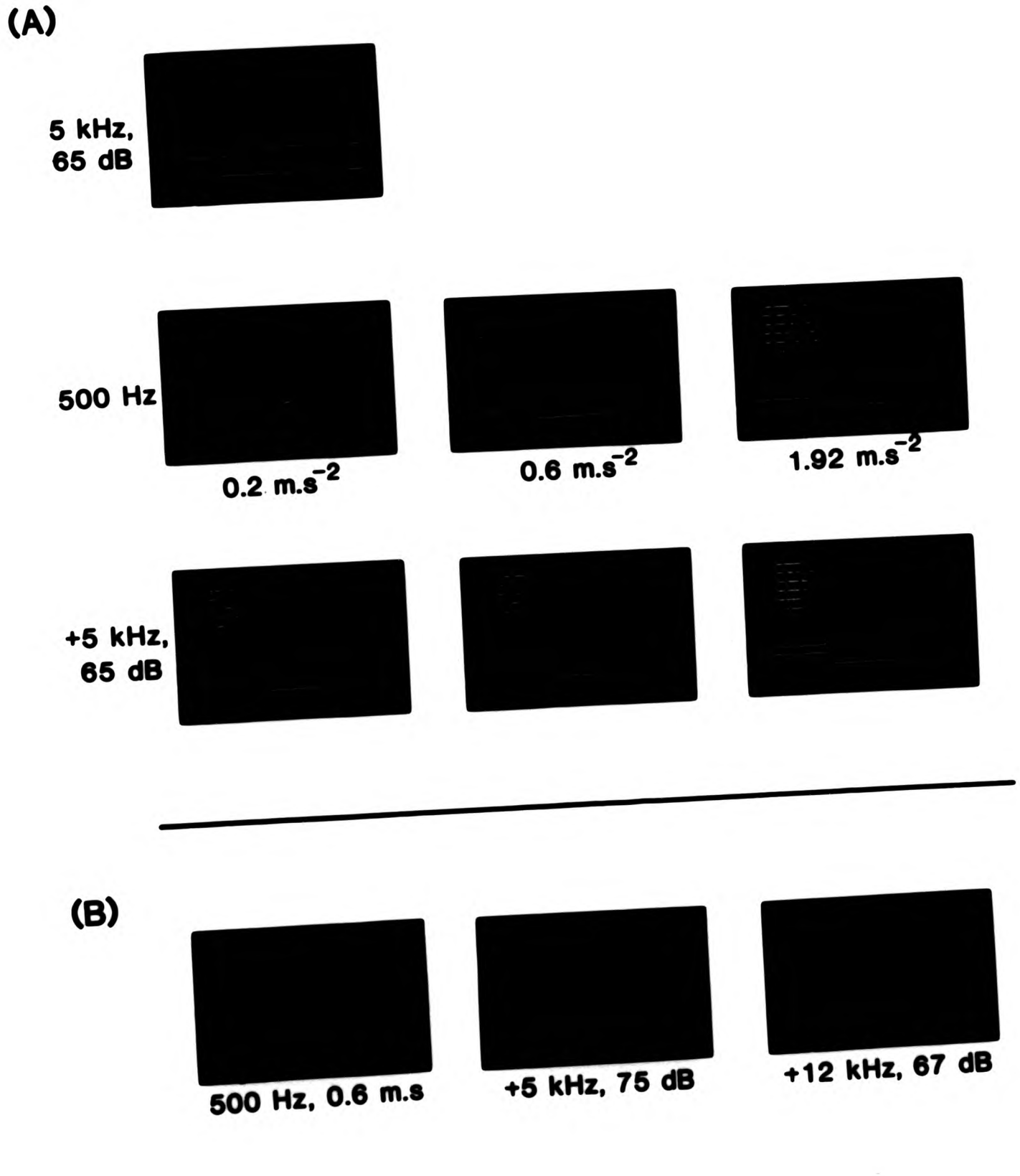

Fig. 3.32

Effects of leg sectioning on thresholds to sound and vibration of unit Gc37.

(A) Thresholds to vibration, over the frequency range 50-2000 Hz, in the intact state (all 6 legs present), after removal of the hind legs (fore and mid-legs present), and after removal of the mid legs (fore legs only present). Insert shows analogue of response to 500 Hz at 0.6 $m.s^{-2}$ (50 ms), in intact state.

(B) Thresholds to sound, over the frequency range 1-20 kHz, in the intact state (all 6 legs present), and after removal of the hind and mid-legs (fore-legs only present).

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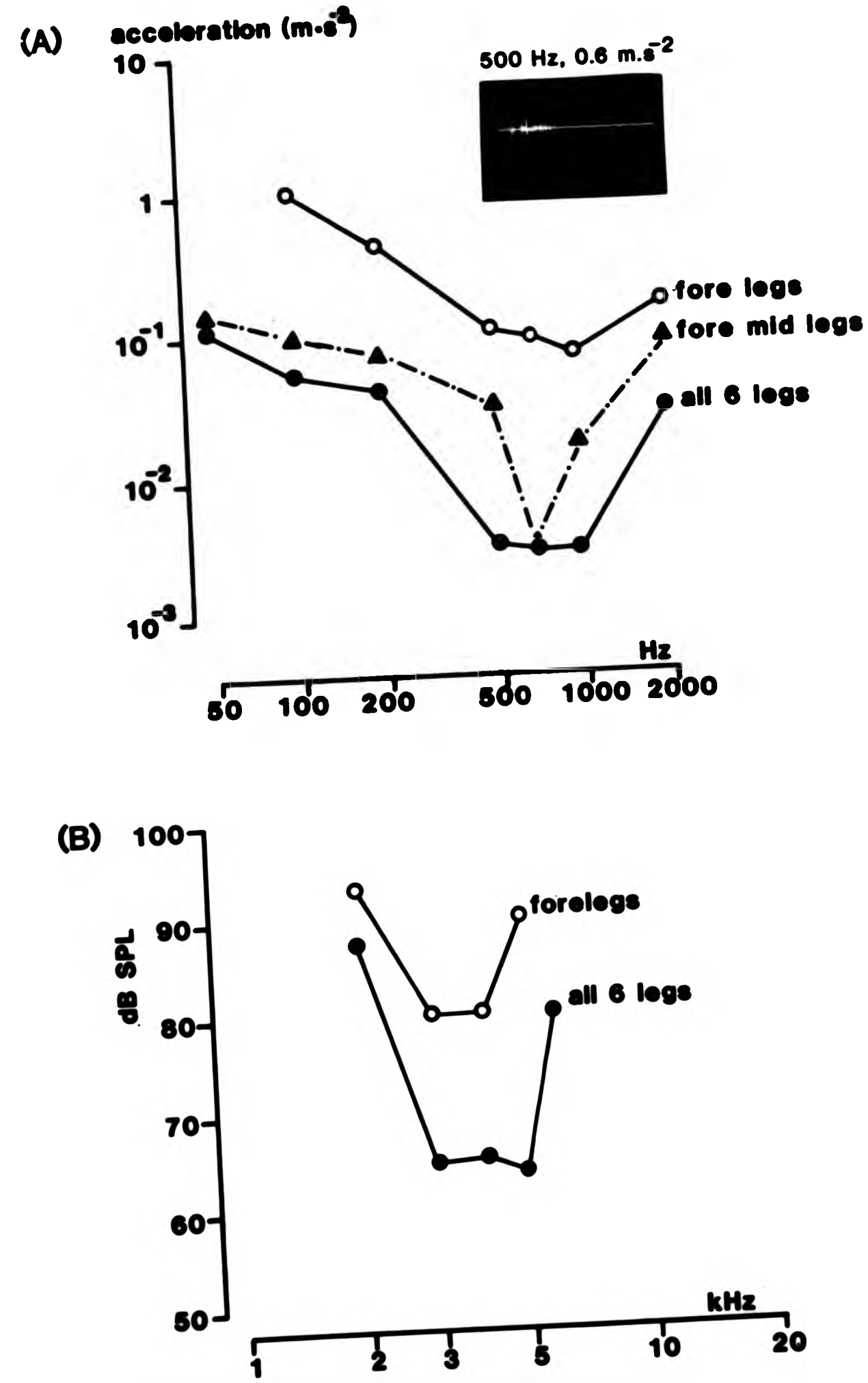

strong tonic responses (Fig. 3.32A, inset).

(E) Neurones Sensitive to Sound and Vibration

TN1

The anatomy of this unit was described by Wohlers & Huber (1981), with its responses to sound, and their terminology is retained here. It was the only unit recorded that responded well to both sound and vibration, as opposed to being preferentially sensitive to one modality and influenced by the other.

Anatomy

This neurone was successfully stained in 2 preparations and these fills were very similar. A drawing of the unit from Gc44 is given in Fig. 3.33. TN1 is a neurone whose cell body lies contralateral to the axon, in a position close to the cell bodies of AN2 and AN3. The ascending and descending axons run between the middle and the medial side of the connectives and meet at a point slightly lateral to the centre of the ganglion. Three main dendritic branches extend from this central region. One branch extends laterally, towards the axon-ipsilateral leg nerve; one extends contralaterally, with a large degree of branching, and includes the neurite which connects to the cell body; the third also extends into the contralateral side, but more posteriorly, and with fewer branches than the other two main processes.

Fig. 3.33

Camera lucida drawing showing the anatomy of TN1 in the prothoracic ganglion. Arrow indicates anterior.

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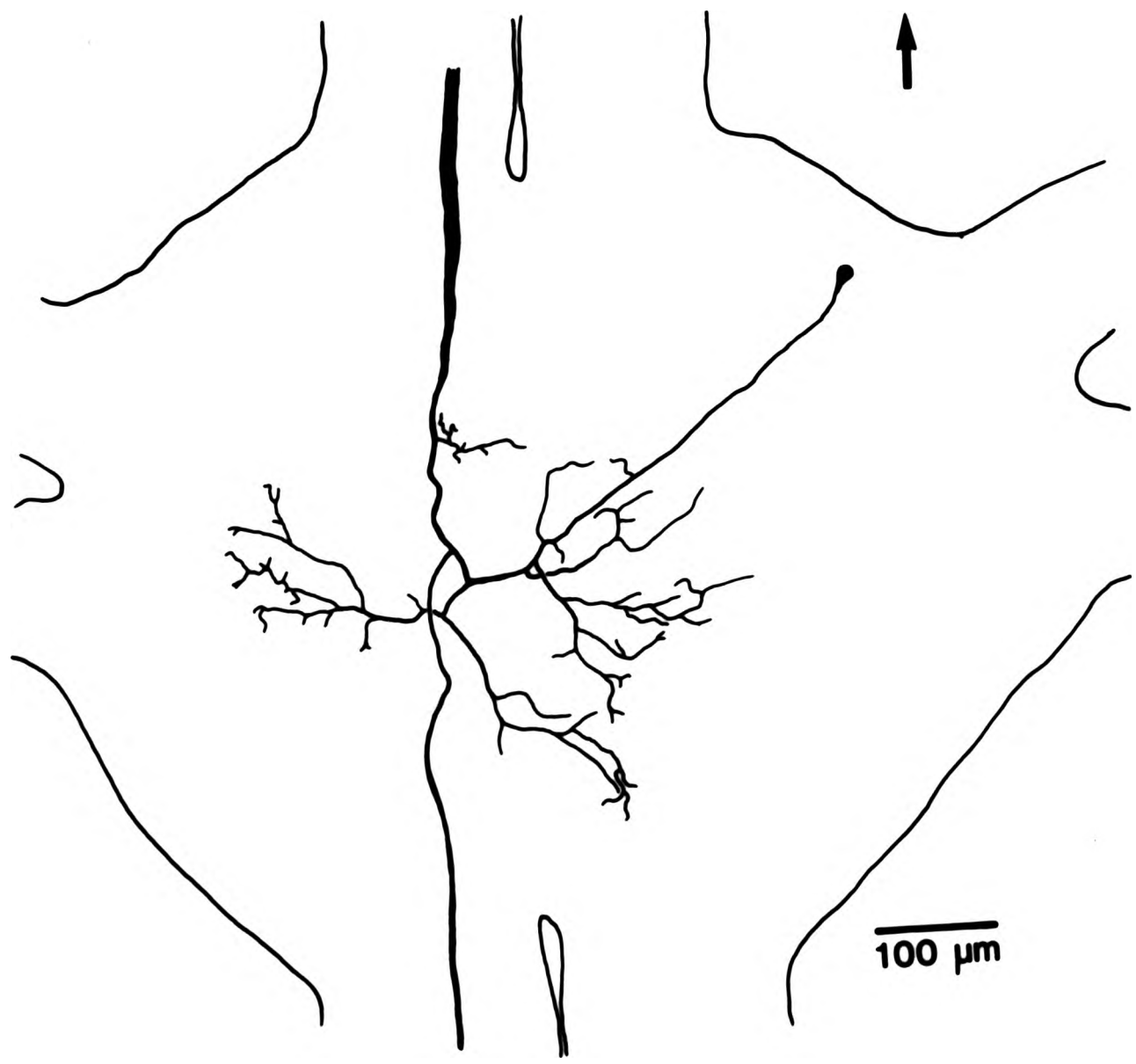

Physiological Responses

The thresholds of TN1 were easily measurable for sound and for vibration, and are given in Fig. 3.34(A) and (B) respectively. The thresholds for sound were fairly broad-banded, but lowest between 10 and 20 kHz (about 50 dB SPL), and the thresholds for vibration were lowest around 1 kHz (about 0.02 $m.s^{-2}$)

The responses of the unit to 50 ms tone pulses were phasic-tonic, and more phasic to sound than to vibration. In Fig. 3.35A raster displays of the responses to 5 kHz sound, 500 Hz vibration, and the two combined, demonstrate the integration of acoustic and vibratory inputs by this neurone. Pronounced adaptation of the responses to successive presentations of 5 kHz sound alone occurred, but this was not evident when the sound was presented together with 500 Hz vibration. However, the responses to 500 Hz + 5 kHz still showed adaptation within each individual response. The spike counts of these responses are shown in Fig. 3.35B, and show more clearly the summation of the influences of sound and vibration stimuli.

The responses of this unit to NS and SNS were not tested.

Fig. 3.34

Threshold curves for TN1 to sound (A) and vibration (B).

(B).

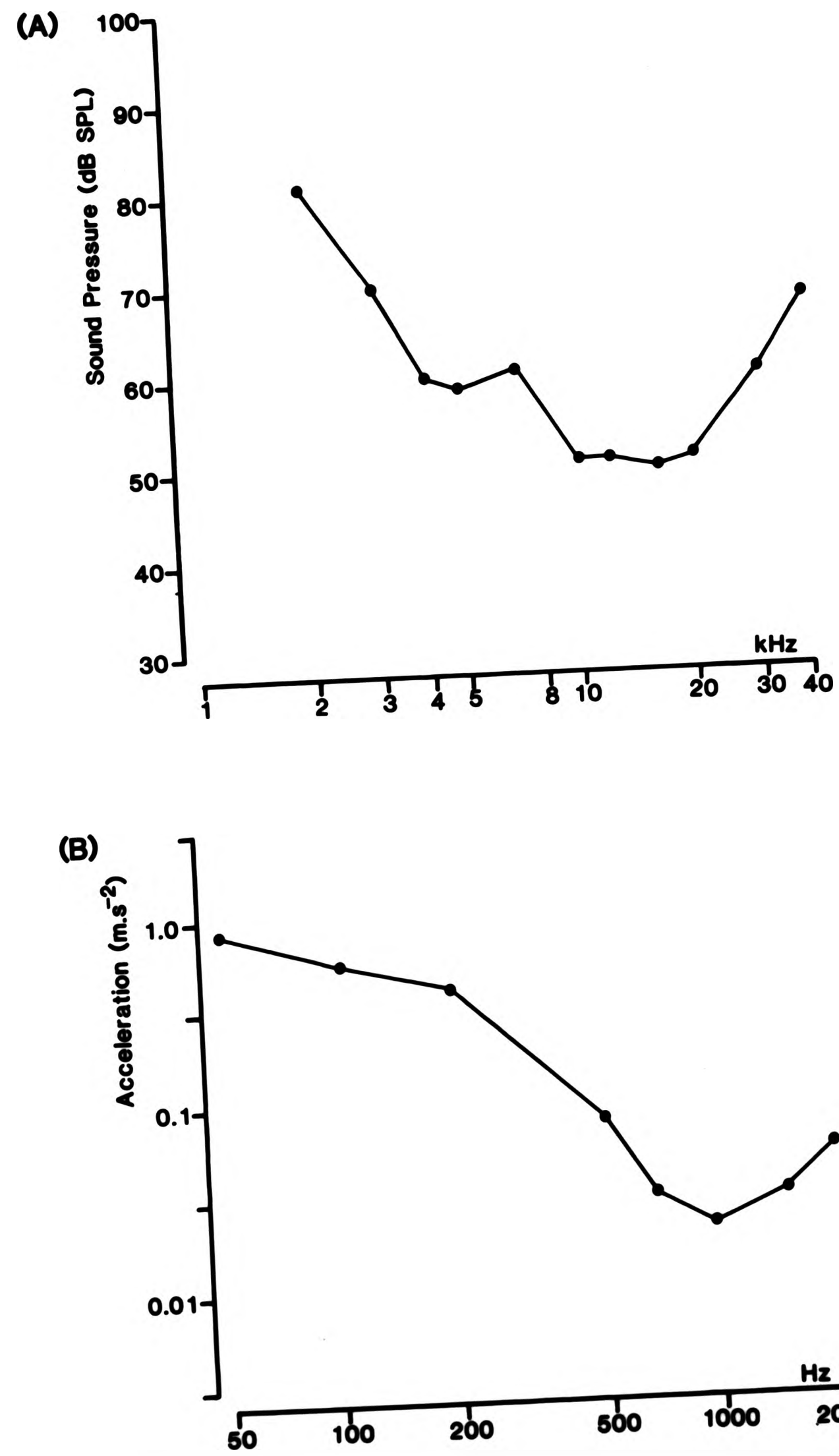
(A)
Sound Pressure (dB SPL)
100
90
80
70
60
50
40
30
kHz
1
2
3
4
5
8
10
20
30
40
(B)
Acceleration (m.s^{-2})
1.0
0.1
0.01
Hz
50
100
200
500
1000
2000

Fig. 3.35

Suprathreshold response characteristics of TN1.

(A) Raster responses to 5 kHz sound alone, 500 Hz vibration alone, and the two presented simultaneously (50 ms stimuli). Note strong adaptation in the response to sound alone.

(B) Responses as in (A) illustrated graphically to demonstrate summation of responses to sound and vibration. The shaded area represents the augmentation of the response to vibration by simultaneous presentation of sound.

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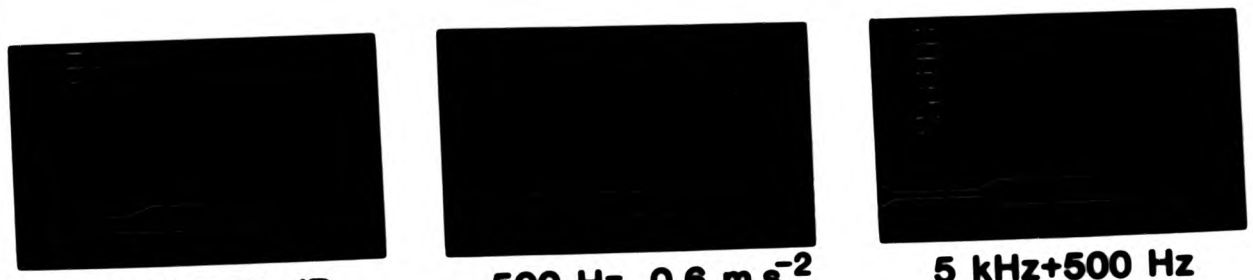
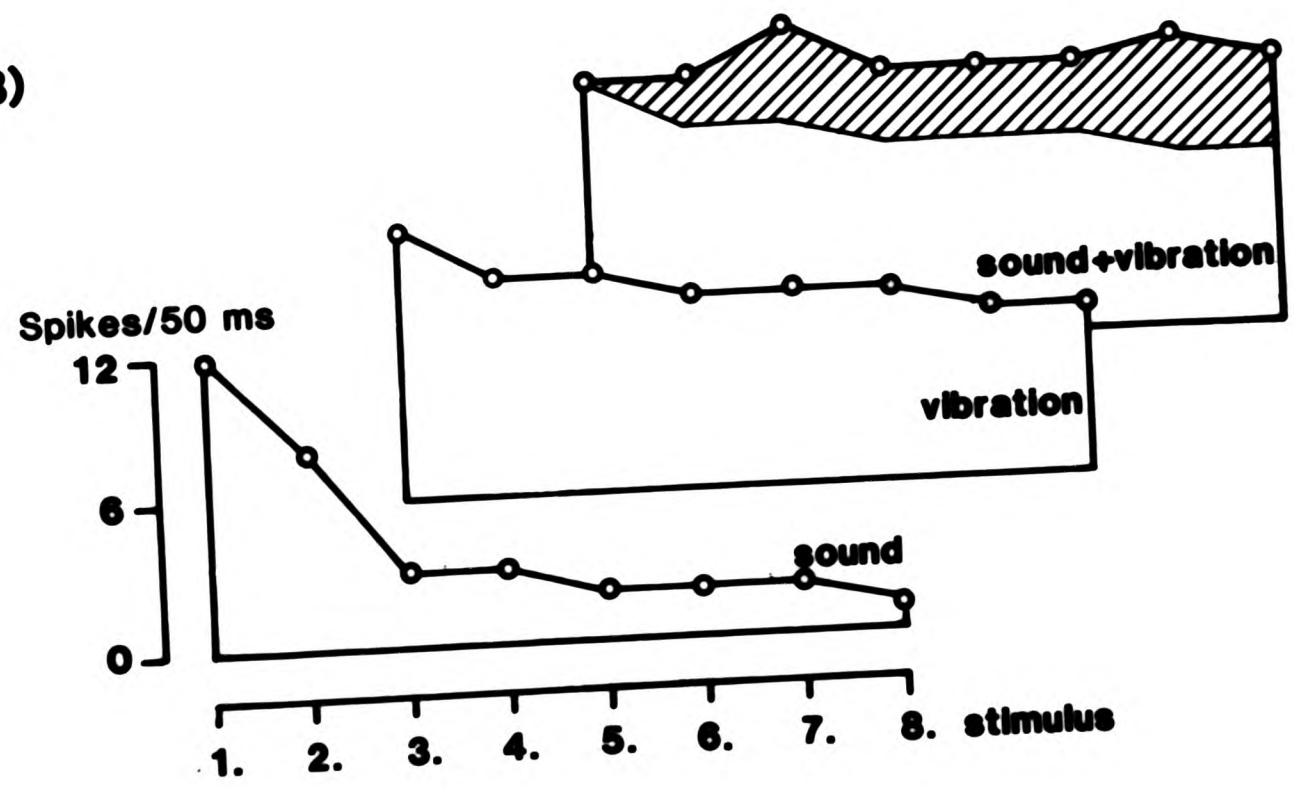
(A)



(B)



3.4 DISCUSSION

It has been shown, by previous workers, that cricket auditory receptors may be physiologically distinguished into at least two groups: the first with maximum sensitivity around 5 kHz and the second with maximum sensitivity above 10 kHz (Popov 1971; Nocke 1972). It has been assumed that these responses are from the proximal and distal cells, respectively, of the tympanal organ (Zhantiev & Tshukanov 1972). One of the most obvious observations that may be made concerning the data presented in this study is that the sound-sensitive units are most often tuned around either 5 kHz or 14-16 kHz. Indeed, within the frequency band tested, no exclusively sound-sensitive units were recorded with characteristic frequencies at any other frequencies. Peripheral "clustering" is therefore retained at this level of the CNS. This distribution is not too surprising when one considers that, at least in G. campestris, these are the only frequencies of obvious behavioural significance; the carrier frequency of the calling song is about 5 kHz and that of the courtship song about 13-16 kHz (see Chapter 2, Fig. 2.17). However, hearing is likely to be very important in predator avoidance, as well as for intraspecific communication. This function is discussed in greater detail below.

Numerous workers have described the physiological characteristics of auditory neurones recorded, at the level of the tympanal nerve or the cervical connectives, in several cricket species. The neurones have been

characterized primarily on the basis of their characteristic frequencies, and therefore often given names such as "the low-frequency neurone" or "the high-frequency neurone". One of the main problems has been the drawing of homologies between similar unit types reported by different workers. Without knowledge of anatomy it has been impossible to be sure how many neurones are involved, particularly as most of the early experiments were carried out using whole-nerve recording techniques. More recently, however, methods for intracellular (Pitman et al. 1972) and extracellular (Rehbein 1974) staining have been developed, and it has often been possible to identify homologous units recorded in different species as well as by different workers. The present nomenclature of the auditory units in the ventral cord takes into account both anatomical and physiological characteristics. This has not solved all the problems of neurone homologies, but as a result there now appear to be rather fewer neurones ascending from the prothoracic ganglion than was previously envisaged. Estimates give numbers of around 10 each side of the ventral nerve cord (Elsner & Popov 1978), but one must bear in mind that it is very difficult to record from very small axons with present techniques, and this may cause an underestimation in the number of units actually involved.

Elsner & Popov (1978) classify the ventral cord auditory neurones into five separate types according to their connections to other parts of the central nervous system. These are "(i) segmental auditory neurones which distribute auditory information within one ganglion; (ii) ascending auditory neurones which send axons to the anterior

ganglia; in most cases up to the brain; (iii) descending auditory neurones sending axons to the posterior ganglia; (iv) T-shaped neurones having descending and ascending axons and thus supplying both thoracic ganglia and the brain with auditory information; (v) 'through-passing' neurones, coming from the brain or ascending from the abdominal cord, which receive additional inputs from the auditory neuropiles through lateral branches of their axons." In the present study, recording from the cervical connectives, it was clearly only possible to expect to record from units in groups (ii), (iv) and (v).

Neurones Tuned to the Calling Song Carrier Frequency

Low-frequency sound-sensitive units have been described by many workers in several gryllid species, and they have shown certain consistent characteristics that suggest they are homologous. These include the "low-frequency neurone" (Popov et al. 1974, 1975) and the LF1 unit (Rheinlaender et al. 1976), both in Gryllus bimaculatus, the "pulse coder" in G. campestris (Stout & Huber 1972), and the "small tonic unit" (STU) in Teleogryllus commodus (Hill 1974; Ball & Hill 1978). These have all been shown to be very sensitive, to be sharply tuned to the respective calling song carrier frequency, to be spontaneously active, and to accurately code the temporal pattern of the calling song. Reports have indicated that at the single unit level the low frequency neurone(s) is difficult to record, and even more difficult to stain (Rheinlaender et al. 1976; J. Stout, personal communication). So far, there has been

only one report of the morphology of a low-frequency unit in the brain of G. bimaculatus (Boyan & Williams 1982), and one of the prothoracic ganglion morphology of a similar unit in G. campestris (Wohlers & Huber 1982). Wohlers & Huber termed this unit "AN1" (ascending neurone 1) on the basis of its anatomy.

Although the recent anatomical data have revealed only one low-frequency neurone, there have been other reports, based on physiological data, suggesting that more than one may be involved in the ascending pathway from the prothoracic ganglion. Rheinlaender et al. (1976) described their LF1 unit in detail, but also showed the threshold curve of an LF2 unit that was less sensitive and not so sharply tuned, but which was otherwise very similar to LF1. The limited staining of the highly-tuned low-frequency unit in this study (AN3) has shown it to be morphologically distinct from the less sensitive AN1 of Wohlers & Huber (1982). Subsequent investigations in this laboratory have established that the AN1 unit is distinct in its morphology and its response characteristics from the AN3 described in the present study (Kuhne & Silver, personal communication). This is therefore the first report describing the AN3 unit both physiologically and morphologically.

The sharp tuning, high maximum firing rate, and low adaptation and after-discharge, shown in responses to 50 ms tone pulses (Figs 3.9,3.10), suggest that this unit should accurately code the temporal pattern of the calling and aggression songs. The responses to NS and SNS confirm this (Fig. 3.14). In response to calling NS and 5 kHz SNS each

syllable produces a strong discrete burst of spikes. The very sharp tuning of AN3 is clearly achieved, at least in part, by inhibition from frequencies on either side of the characteristic frequency. Inhibition was also suggested by Rheinlaender *et al*. (1976) as a possible feature contributing towards the sharp tuning of LF1, particularly on the low-frequency side.

It is of interest to consider the modes of action of the low and high frequency inhibition, as they appear to be rather different (Figs 3.11, 3.13). The most striking difference is that the low-frequency inhibition appears to be mediated ipsilaterally and the high-frequency inhibition contralaterally (Fig. 3.13). It is well known that the primary fibres do not cross the midline (Esch *et al*. 1980), and so the high-frequency inhibition (contralateral input) must be mediated via an interneurone. The only segmental auditory neurones so far identified in the prothoracic ganglion have been the two "omega neurones" (Popov *et al*. 1978; Wohlers & Huber 1982), but neither of these is tuned to around 16 kHz.

The spontaneous activity of the unit is suppressed for a time following a positive response to 5 kHz. When the response was inhibited by simultaneous presentation of 3 kHz the spontaneous activity remained, but when it was inhibited by 16 kHz it did not (Fig. 3.11). This suggests that the low-frequency inhibition may be mediated via an interneuronal stage and that the high-frequency inhibition is postsynaptic (as the latter suppresses all the neural activity). The latency of the response to 5 kHz was about

10 ms, suggesting AN3 to be a second order neurone (first order interneurone). However, the inhibition by 3 kHz and 16 kHz (which, as discussed above, must both involve an interneurone stage) was not delayed relative to the positive response. With the data available it is not possible to account for this discrepancy. Intracellular recordings are now necessary to enable examination of the EPSPs and IPSPs produced.

Neurones Tuned to High-frequency Sound

The most readily recorded neurone encountered, particularly sensitive to high-frequency sound in the frequency range 10-20 kHz, was the AN2 unit. This unit was stained comparatively easily and many single fills were obtained. The anatomy is similar to that described for high-frequency neurones, in related cricket species, by several other workers. The first report of a neurone showing this anatomy was that of "Interneurone-1" by Casaday & Hoy (1977) in Teleogryllus oceanicus. This unit has also been recorded and marked by Hutchings & Lewis (1983b) who termed it ANA. Wohlers & Huber (1978) described their AIAA (auditory interneurone with ascending axon) in G. bimaculatus, which is probably homologous with the HF1AN (high-frequency-1, ascending neurone) reported by Popov & Markovich (1982), in the same species. The first report of this unit in G. campestris was by Wohlers & Huber (1982) who termed it AN2 (ascending neurone 2) and their terminology is retained in this study.

The characteristics of AN2, both at threshold and suprathreshold levels (Figs 3.16, 3.17) show the unit to respond best to frequencies of 10-20 kHz. Above 20 kHz the sensitivity decreases, although the unit still responds at 40 kHz, with a threshold of 60-70 dB SPL. Popov & Markovich (1982) show that this unit (in T. oceanicus) will respond up to 80-100 kHz at high intensities. The sensitivity also decreases below 10 kHz, but there may be a secondary peak of sensitivity at or near 5 kHz. The unit typically produced a tonic discharge to 50 ms stimuli of 14-16 kHz (Fig. 3.17C) and showed a small degree of adaptation and after-discharge. This adaptation was more evident during responses to the repeated syllables of the SNS (Fig. 3.21B). There was little or no spontaneous activity. Almost all the variation between examples of AN2 was concerned with their relative sensitivity to 5 kHz. As is shown by the 5 kHz intensity-response curves (Fig. 3.17A), some examples responded well to 5 kHz while others were almost completely unresponsive.

The response characteristics described above for AN2 correspond quite well to the reports by other workers of "homologous" neurones in the same and in different species. Popov & Markovich (1982) stress the adaptation and after-discharge as a characteristic feature of this unit. Wohlers & Huber (1982) report wide variation in the responses of AN2 (in G. campestris) to low-frequency sound stimuli. Similar variation was described by Popov & Markovich (1982), and they suggested that "the fact that functional variability at the low-frequency range is much

stronger than that in the high-frequency range can be the result of stronger processes of natural selection for stability in the high-frequency range covering the spectrum of sound of predators and conspecific courtship song". They also reported a great variation in the morphology of the unit, similar to that found in the present study. Although attempts were made to correlate variations in physiology with variations in morphology there was no strong evidence suggesting that more than one similar neurone is present. The morphological variation observed could be natural variation of the anatomy of one neurone or a small population of similar neurones. Popov & Markovich (1982) report that cobalt back-filling of units in the whole circumoesophageal connectives, down to the prothoracic ganglion, consistently filled only one AN-type neurone. However, it may be that other AN2-type units ascend only as far as the suboesophageal ganglion. Hutchings & Lewis (1983b) for example, report two high-frequency AN-type units with distinct physiological characteristics in the prothoracic ganglion of T. oceanicus.

The experiments using two-tone stimulation demonstrated inhibitory influences produced by sound of around 4-5 kHz. When presented together with a high-frequency sound stimulus the response to the latter was reduced, but by a variable amount in different preparations (Fig. 3.18). These findings correspond to the observations of Wohlers & Huber (1982) who reported that the responses by AN2 to 5 kHz ranged from weak IPSPs to strong spiking responses.

Boyan (1981) described the physiological characteristics of a morphologically identified unit in the brain of G. bimaculatus, the "Plurisegmental auditory brain neurone 2" (PABN2). The data were derived largely from two-tone experiments. The unit had a characteristic frequency of around 13 kHz and its responses were selectively suppressed by sound of 4-6 kHz. The intracellular recordings showed no IPSPs. Boyan therefore suggested that the suppression was presynaptic. However, he did not consider that the suppression could occur at a lower level in the CNS, although he did suggest that this neurone may receive input from the AIAA (=AN2) neurone described by Wohlers & Huber (1978). This possibility is supported by the fact that both AN2 and PABN2 respond almost exclusively to ipsilateral stimulation. Boyan & Williams (1982) described a high-frequency unit, stained in the brain of G. bimaculatus, which appears to be physiologically homologous to AN2. Its arborizations were shown to overlap those of PABN2, and were all ipsilateral. They did not, however, test two-tone interactions in this unit.

The different responses of AN2 to low and high-frequency sound suggests that the cell receives input from at least two airborne sound receptor groups: one tuned to the calling song carrier frequency and the other sensitive to high frequencies. This is substantiated by the fact that simultaneous presentation of vibration suppressed the response to 5 kHz, but not to 14 kHz (Fig. 3.20). The origin of the inhibition in terms of which leg inputs were effective was not analysed, but it was not tuned to any

particular vibration frequency. Wiese (1981) found that input from the foreleg vibration receptors in G. campestris suppressed the activity of the omega neurone ON1, which is tuned around 5 kHz. However, it is unlikely that the 5 kHz input to AN2 is mediated via ON1, since the output of ON1 is contralateral to its input, whereas the excitatory input to AN2 has been shown to be principally ipsilateral (Wohlers & Huber 1982).

The suppression of the 5 kHz response by vibration was rather unspecific. It did not appear to significantly improve the temporal coding of the conspecific calling song, a phenomenon that has been shown to be produced by interaction of sound and vibratory inputs in ventral cord neurones of certain bushcrickets (Kuhne et al. 1980) and locusts (Kalmring et al. 1978a). However, amplitude modulation of the vibration stimulus to the calling song temporal pattern was not carried out; this may have produced a more reliable temporal coding of the song.

The anatomy described for TN2 is the first report of a through-unit in G. campestris that does not have its cell body within the prothoracic ganglion, although several units with rather similar morphology were described in T. oceanicus by Hutchings & Lewis (1983b). The only other through-unit described morphologically in gryllids has been that of TN1 (Wohlers & Huber 1982; this study) which has its cell body in the prothoracic ganglion, close to that of AN2.

The responses of TN2 were remarkably similar to those of AN2, but insufficient numbers of recordings were made to be able to make any statement as to the variability of these responses. Given the anatomy of TN2, the strong responses to acoustic stimuli are rather surprising. The limited arborization in the region of the primary acoustic neuropile would suggest only modulation of the activity of the unit by sound. Moreover, the latencies seem too short to postulate that the major acoustic input occurs in another ganglion, via an intersegmental interneurone. However, similar strong responses were reported in the through-units described by Hutchings & Lewis (1983b).

Vibration-sensitive Neurones

As for the sound-sensitive units, the neurones sensitive to vibration tended to respond preferentially to one of two frequency ranges: either around 1-200 Hz or around 500-1000 Hz. A homogeneous group of units was recorded that was tuned to each of these frequency ranges. The behavioural relevance of these frequencies is not immediately obvious, but this point is discussed further below.

With the exception of the units recorded in Gc61 and Gc78, the units responsive to low-frequency vibration formed a homogeneous group with very consistent response characteristics. They were not spontaneously active and produced tonic responses showing some adaptation to stimuli of long duration (Fig. 3.24B). Their responses were not affected by sound stimulation. Without anatomical data it

is not possible to say whether this type is a single or a group of several neurones. Leg sectioning experiments (e.g. Fig. 3.26) demonstrate that the units must receive input from all three pairs of legs, but insufficient tests were carried out to be able to clearly show the relative contribution of each leg.

The other homogeneous group (which also may consist of only one unit) was that of the units which responded best to vibration of 500-1000 Hz and were not affected by sound stimuli. These were all spontaneously active and produced good tonic responses to preferred frequencies. The spontaneous activity was suppressed for a time following positive responses (Fig. 3.30). The long latencies of around 25 ms indicate that these are not first order interneurones.

The high-frequency vibration units whose responses were augmented by simultaneous sound presentation (Figs 3.31, 3.32) were mostly recorded only once; they did not form a distinct group. In the main, they responded only very weakly to sound presented alone; in Fig. 3.32B the thresholds to sound for Gc37 are given, but it is rather insensitive, and cutting the hind and mid-legs reduced the sensitivity, suggesting that the responses may be largely due to sound-induced vibration of the legs. The absence of anatomical data and the limited amount of physiological data obtained on each of these units makes it very difficult to speculate on the inputs to these neurones.

There is virtually no published literature on the responses of gryllid ventral cord auditory neurones to vibration. However, several studies have been carried out on acridids and tettigoniids, and the units responsive to vibration have been classified into 5 types on the basis of their physiological characteristics (Kalmring et al. 1978; Kalmring & Kuhne 1980; Kuhne et al. 1980; Kuhne 1982b). The groups of vibratory units described here, in G. campestris, do not appear to fit well into this classification, with the possible exception of the low-frequency vibration units. The "V1" unit, in Locusta and Decticus, shows similar thresholds to those given here, responding from about 30 Hz to 500 Hz (Kuhne 1982b). The present study did not demonstrate any units similar to the V4 and V5 units in Tettigonia cantans, which respond in a phase-locked manner up to about 200 Hz (Kuhne 1982b). These are assumed to receive input directly from the campaniform sensilla, whereas most of the other vibration-sensitive units are believed to receive input mainly from the subgenual organ (Dambach 1972; Kuhne 1982a,b).

Integration of Sound and Vibration Inputs

The responses of the units discussed so far have shown a certain degree of integration between the effects of sound and vibration stimuli. Both excitatory and inhibitory influences have been demonstrated. The response of AN2 to 5 kHz was seen to be inhibited by vibration stimulation (Fig. 3.20), while the responses of certain vibration-sensitive units were enhanced by simultaneous

sound stimulation (e.g. Fig. 3.31). In each case, however, the unit in question responded with stimulus-locked activity only to the preferred stimulus type, whether sound or vibration, when presented alone; its response was then modified (enhanced or inhibited) by the other modality. The only unit recorded that could be described as truly bimodal was TN1 which responded fairly well to sound and vibration in an excitatory fashion (Fig. 3.35).

Assuming that recordings were made from a representative cross-section of the unit types present in the ventral cord, the limited extent of sound/vibration integration shown is in contrast to that found in the other orthopteran groups. Among the Tettigoniidae, it has been shown, in D. verrucivorus and T. cantans, that all acoustic units respond to both sound and vibration (Kalmring & Kuhne 1978; Kuhne et al. 1980). However, on the basis of their preferred stimuli, these authors classified them as vibration (V), vibration and sound (VS) and sound (S) neurones. Similar integration has been reported in the acridid L. migratoria (Cokl et al 1977; Kalmring et al. 1978a; Kuhne 1982b). The functional significance of this interaction has been shown to be that coding of temporal song patterns is enhanced when the sound stimulus is presented together with vibration. This enhancement appears to occur even when the vibration stimulus is not amplitude modulated, but is continuous for the duration of each chirp (shown in T. cantans by Kalmring & Kuhne 1980). For the units recorded in the present study there was little evidence of improved temporal coding when vibration was

presented together with calling SNS (e.g. AN2 in Fig. 3.20). Possible behavioural explanations for this are discussed below.

All those units that were responsive to vibration and which were enhanced by sound stimuli were most sensitive to high-frequency vibration. This was also the case for TN1 (Fig. 3.34); its characteristic frequencies for sound and vibration were 10-20 kHz and 1000 Hz respectively. This unit was first described by Wohlers & Huber (1982), but they tested only responses to sound stimuli. They found it to respond rather weakly to sound, best between 5 and 15 kHz (at suprathreshold levels) and suggested on the basis of its widely distributed dendritic arborization that it might be multimodal. They also showed that it received some level of excitatory input from the contralateral ear. The results presented here show that it is indeed at least bimodal, responding quite strongly to combined sound and vibration stimuli. Inputs from the contralateral side, however, were not tested in this series of experiments.

Behavioural Significance of Single-unit Responses

In order to best understand the responses of the single units that have been found at the various neuronal levels it is essential to consider them in a behavioural context. We need to be aware what the insect is capable of hearing and what sounds it will respond to. Fortunately, considerable work has been carried out on certain aspects of acoustic behaviour in crickets and other orthopteran species, and these observations have led to considerable speculation as

to the function of individual auditory neurones. Although it may be attractive to label neurones as discrete functional units it is important that these speculations are not taken too far, as it is likely that certain units play different roles in different behavioural contexts. Nevertheless, it is possible to be fairly clear about some of the roles of certain of the ventral cord neurones. There can be little doubt, for example, that the low frequency sound channel (receptors and central units), tuned to the calling song carrier frequency, has a definite function in mediating the parameters of the conspecific calling song. The AN3 unit has been termed the "pulse coder" by Stout & Huber (1972) which is perhaps a fair label because of its accurate coding of both the syllable duration and the syllable rate. The high degree of tuning and the sensitivity of the low-frequency (=AN3) unit ensures that the calling song is coded over distances of tens of metres and with little interference from other sounds, although it is capable of coding other biologically important sounds, such as other cricket species, with less accuracy (Rheinlaender et al. 1976). Behaviourally, within this narrow frequency range, the cricket is only likely to be interested in conspecific communication.

Sensitivity to high-frequency sound may be important in several behavioural situations. In G. campestris it is natural to assume that the high sensitivity to sound around 15 kHz corresponds to the carrier frequency of the courtship song. However, peaks of sensitivity between 10 and 20 kHz have also been shown in other cricket species which do not

have a high-frequency song (e.g. T. oceanicus, Hutchings & Lewis 1983b). High-frequency sensitivity is also likely to be important for predator avoidance, and the peaks of sensitivity may represent a compromise between sensitivity to the conspecific songs (including the harmonics of the calling song) and to predators producing ultrasound. There may, of course, be other peaks in sensitivity above 40 kHz that were not revealed by the present study.

There is behavioural evidence, shown by several workers, that links high-frequency sensitivity with predator avoidance in several insect groups. Silver et al. (1980) found single units that responded up to 100 kHz in locusts and bushcrickets. Since the spectrum of the locust song does not extend into ultrasonic frequencies they concluded that sensitivity to ultrasound was likely to be important for predator avoidance. Miller & Olesen (1979) demonstrated complex evasive behaviour in green lacewings, triggered by ultrasound. Negative phonotaxis has also been shown in flying G. bimaculatus in response to ultrasound (Popov et al. 1975; Popov & Shuvalov 1977; Moiseff et al. 1978). The predators most often suggested are bats, and Popov & Markovich (1982) showed that the HF1AN unit in T. oceanicus was sensitive to echolocation sound of 3 sympatric bat species, especially to those of Nyctalus noctula, the spectrum of which is entirely within the range of AN2 (Miller & Degn 1981).

Although there is an apparent ambiguity between AN2 responding to courtship song (positive phonotaxis) on the one hand and predators (negative phonotaxis) on the other,

it must be remembered that arousal plays a very important role during courtship. Many other cues, such as visual and olfactory, are present. The effects of AN2 on higher centres will therefore depend on the inputs from these other modalities. Kuhne et al. (1980) suggested that units showing adaptation might also be involved in arousal. Initially, such units are in an unhabituated state, and would therefore respond well to the first few chirps of a calling conspecific. Following this arousal the unit may continue to monitor chirp rate by means of a more phasic response. On the other hand, responses to predators would also be strong for unhabituated neurones.

As a female approaches a singing male, the song spectrum received changes. In general, all frequencies will appear more intense and high frequencies, which are particularly heavily attenuated at long distances, will be more likely to be at suprathreshold levels. Over short distances, the highly sensitive low-frequency units may be saturated. Hutchings & Lewis (1983b) showed, in T. oceanicus, that ANA (=AN2) can accurately code the species song under these conditions due to the fact that the low frequencies exert two-tone suppression on the high-frequency responses, thereby reducing saturation.

In addition to the increase in the intensity of the high-frequency components at short distances, vibration is likely to play an important role. Vibratory inputs could cause inhibition of the sound sensitive units, thereby reducing saturation and after-discharge, so maintaining syllable coding. This phenomenon has been shown to be the

case in certain units recorded in bushcrickets (Kalmring & Kuhne 1980) but there was little evidence of it in the present study on G. campestris. This may be because crickets live on more solid ground than bushcrickets, and so are less likely to detect vibration produced during singing. On the other hand, vibration could enhance the responses of adapted units such as TN1 (Fig. 3.35). Vibration sensitivity may also be important for avoidance of terrestrial predators producing substrate vibration. Such predators might include rodents and other small mammals. Toads have also been reported to prey on crickets (Cade & Rice 1980).

Thus the ventral cord units certainly channel specific kinds of information in the frequency and temporal domains, but whether individual units can be ascribed specific "functions" is not so clear. As was discussed above, the term "pulse-coder" applied to AN3 may be a reasonable one. However, certain other units have been termed "chirp coders" in crickets (Stout & Huber 1972) and bushcrickets (Kuhne et al. 1980) merely because they discharge during each chirp but do not accurately copy syllables. Any neurone responding to sound in a tonic manner will code the chirp duration, but this does not necessarily mean that this is its sole function. In any case, the "pulse-coder" can code chirp duration more accurately than the "chirp-coder" as it shows negligible after-discharge. The intensity of the stimuli used probably has great importance in this context. Other units have been shown to follow syllable repetition rates, but not duration, by responding phasically to each

syllable (Kalmring & Kuhne 1980), but this is unlikely to be the only "function" of these units, as such coding occurs only over a narrow range of stimulus parameters.

Therefore, although certain types of information appear to be channelled in specific neurones ascending from the prothoracic ganglion, this may be too low a level at which to ascribe individual units specific functions. Without a doubt, further processing of acoustic information, in conjunction with inputs from other modalities, will occur at higher levels of the CNS, and it may be that precise song "templates" are located at these higher levels. Indeed, specific acoustic, vibrational, and other sensory inputs may interact directly with descending information at the level of the final motor pathways. As yet, studies are only at an early stage.

CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS

It is clear both from the results on peripheral directionality and on the neural basis of song coding (chapters 2 and 3 respectively) that the auditory systems of the Ensifera are highly specialized towards reception of the conspecific songs. The thresholds of the auditory organs of bushcrickets (Fig. 2.12) and particularly of crickets (Fig. 2.21) show greatest sensitivity at the dominant frequencies of the calling songs. At the single unit level, in the cricket G. campestris, individual neurones may also be found with characteristic frequencies at the carrier frequencies of the calling and/or courtship songs (Fig. 3.6). In addition, sound localization is clearly tuned to the frequencies of the calling song, both in bushcrickets and crickets (chapter 2). Thus the two processes of sound production and reception complement each other, although this is clearest in the frequency domain. The tuning of the auditory system is much sharper in the crickets than in the bushcrickets, at least for the species used in the present study, presumably corresponding to the relative bandwidths of the calling songs.

Tuning of the auditory system to the temporal patterns of the songs was not so clearly demonstrated. The unit AN3 responded very well to the syllable structure of the calling song of G. campestris, but different syllable repetition rates were not tested, which may have revealed worthwhile information. Rheinlaender et al. (1976), however, showed that the LP1 unit, in G. bimaculatus (probably AN3 in this study), did show species specificity; while it responded to the calling songs of other cricket species, these were coded

with considerably less accuracy than the conspecific song. Behaviourally, Thorson et al. (1982) showed that G. campestris females tracked synthesized male calling songs most easily when their syllable rate was equal to that of the natural song. It may be that specificity towards temporal patterning occurs at higher levels of the CNS than were investigated in the present study.

The recordings of single-unit responses to vibration carried out by this study, in G. campestris, provide a useful basis for further investigations. Many of the recorded units showed very low thresholds to vibration, indicating that the vibration sense is important to crickets. However, integration of responses to vibration and sound was not as extensive as reports have indicated it to be in bushcrickets and locusts. There is much scope for further work, not least concerning the anatomy of the vibration-sensitive units. Behavioural data are particularly lacking on the vibration sense of both bushcrickets and crickets. Also of interest would be information, physiological or behavioural, on directional cues achieved through vibration.

Considerable work has been carried out on the peripheral mechanisms of the directionality of the ensiferan ear, but very little is known of coding of directional information at higher levels in the CNS. Directional responses of units ascending towards the brain (e.g. Boyan 1979; Rheinlaender & Romer 1980) show no integration between the two sides; the responses simply reflect the information received from each of the auditory organs. At

some level of the CNS the responses from the two sides must be compared before localization can be acheived, but as yet no such site has been found.

Since most of the information on song temporal patterning and on sound direction is carried in the ventral cord by neurones tuned to the calling song carrier frequency, and very few of these have been definitely identified, it seems that the two types of information are carried in the same neurones ascending from the prothoracic ganglion. Centres for localization and song recognition are therefore probably located within the brain. However, it might be more worthwhile to obtain as full an understanding of the integration processes occuring at the prothoracic and suboesophageal ganglia before the pathways in the brain are investigated in detail. Obviously, much work has yet to be carried out at these lower levels.

APPENDIX

Developer Stock Solution for Silver-Intensification of Cobalt-Stained Wholemount Ganglia

3.0 g. Gum Arabic.

0.8 g. Citric acid.

0.17 g. Hydroquinone.

10.0 g. Sucrose.

100 ml. Distilled water.

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ACOUSTIC TRANSMISSION THROUGH THE HEAD OF THE COMMON MOLE, *TALPA EUROPAEA*

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Cues for directional hearing in mammals are traditionally based on inter-aural intensity differences (IIDs) established as a result of the diffraction of a progressive sound wave by the head and pinna, or on inter-aural time differences (ITDs) established because of the separation of the two ears in space. This 'dual hypothesis' has been extended to other animal groups despite the fact that the majority of sub-mammalian animals have head dimensions that are small relative to the wavelengths of the sounds used in communication. Under these conditions, little if any diffraction can occur and time differences become very small. As an alternative to the pressure-receiver principle of the mammalian ear, crickets (Michelsen, 1979) and the quail (Coles *et al.* 1980) use the pressure-difference principle for directional hearing. In a pressure-difference receiver, sound has access to both surfaces of the tympanic membrane (TM) because of an air-filled cavity between the ears which allows sound transmission through the head or body. When the sound pressure level (SPL) acting on the inner surface of the TM is equivalent to that acting on the external surface, the response of the TM (and, therefore, the cochlea) will be determined by the phase difference between the external and internal components. At any one frequency this phase difference will, in turn, vary with the angle of incidence of the sound.

Moles (Talpidae) have no pinna and are regarded as low-frequency hearers. Unfortunately, no audiogram has been reported for the European mole, but positive behavioural reactions have been reported to frequencies between 250 Hz and 3·5 kHz (Kriszat, 1940). They also possess an unusual middle ear structure for mammals showing extensive trabeculation of the caudal ventral skull between the ears. However, the significance of this cavity for directional hearing has not been considered previously. We report the results of experiments to determine the extent to which sound is transmitted across this cavity from one ear to the other.

The plane of the tympana is almost horizontal, the manubrial tips being separated by about 8 mm. The distance between the closest edge of each membrane is 4 mm. A Bruel and Kjaer $\frac{1}{8}$ in microphone was sealed into position so that its membrane

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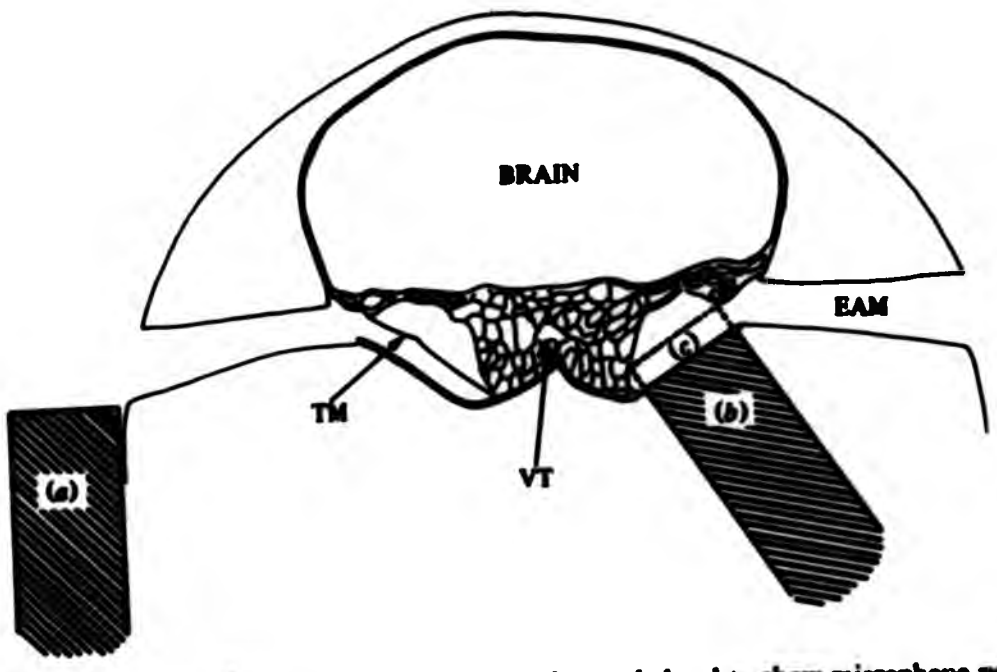
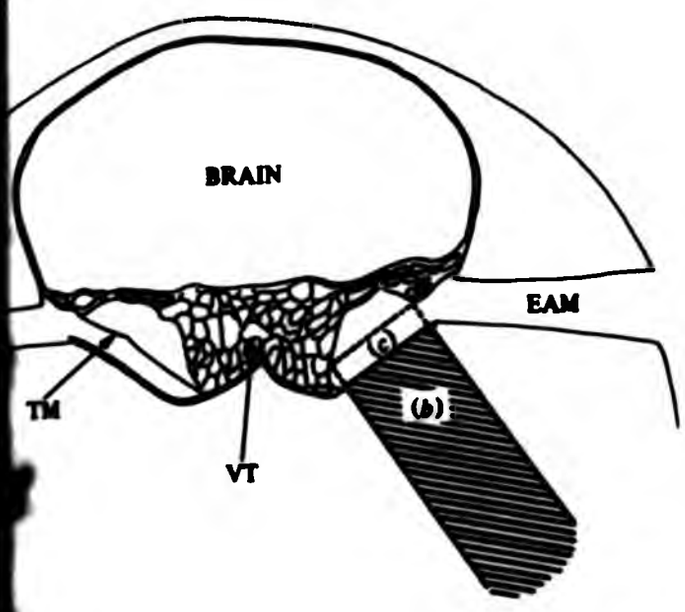


Fig. 1. Diagrammatic transverse section through a mole head to show microphone positions (a), (b) and (c). EAM, external auditory meatus; TM, tympanic membrane; VT, ventral trabeculation.

occupied the position of the removed TM (Fig. 1, position (c)). A second microphone was placed external to the opposite ear (Fig. 1, position (a)). Sound transmission through the interaural cavity was measured in seven preparations in the free field in an anechoic chamber. A representative example of the results is given in Fig. 2A, where the output of microphone (c) is plotted relative to the output of microphone (a) for a number of different frequencies when the loudspeaker was ipsilateral to microphone (a). From 500 Hz to 6 kHz, the internal SPL values are within 6 dB of the SPL values measured externally. Above 6 kHz there is increasing attenuation of the internal sound level with frequency. These experiments were repeated with the meatus proximal to microphone (a) blocked with tissue glue and petroleum jelly. This resulted in severe attenuation of the internal sound at all frequencies. Acoustic transmission across the head was then comparable to that found in similar experiments in the rat (Fig. 2B).

The effects of changing the angle of azimuth of the sound source were also determined for a number of frequencies. Fig. 3A shows that the internal SPL at 1600 Hz (Fig. 1, position (c)) was within 6 dB of the external SPL (Fig. 1 position (a)) for all angles through 360°. Phase measurements were taken internal (Fig. 1 position (c)) and external (Fig. 1 position (b)) to the TM as the sound source was rotated through 360°. When the speaker is contralateral to the recorded ear (Fig. 3B), the phases on each side of the membrane are the same; with the speaker ipsilateral, a phase difference of 180° was recorded.

These results indicate that there is good acoustic transmission through the head of the European mole for a range of low frequencies. Acoustic interaction is therefore to be expected at each tympanic membrane. The fact that the phases are matched



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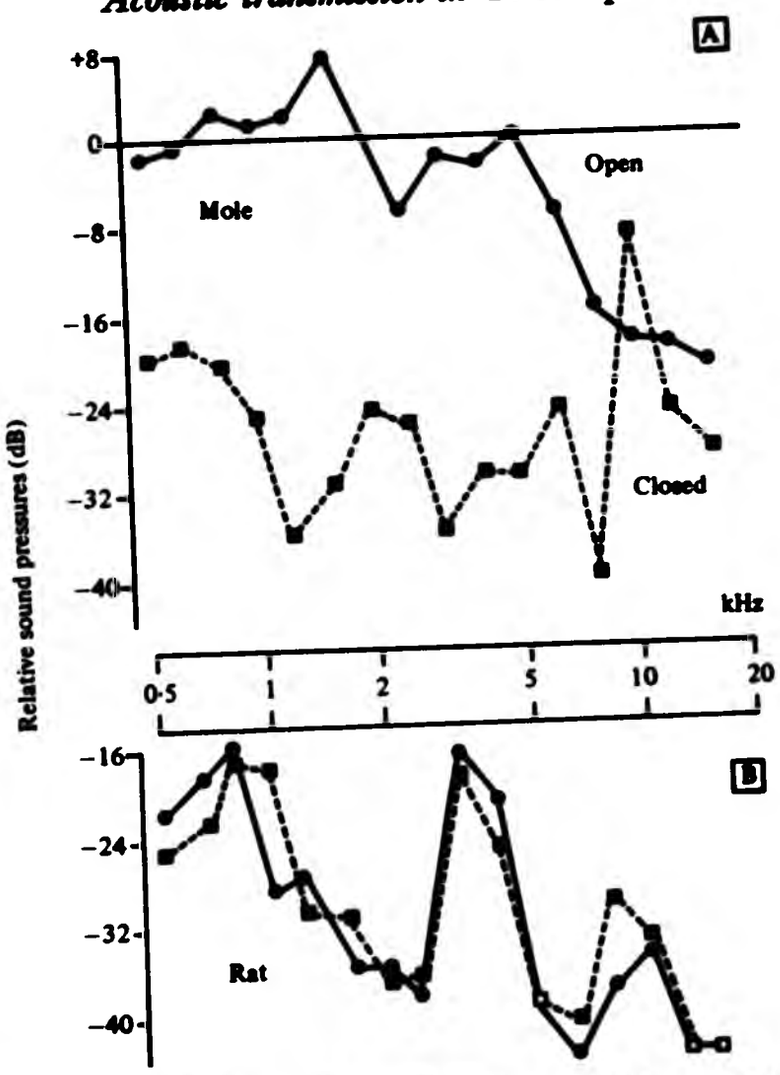


Fig. 2. Measurements of the inter-aural acoustic transmission in the mole (A) and the rat (B). Sound pressure levels were measured at the position of the tympanic membrane [(c), Fig. 1] and are given relative to the sound pressure levels measured at the opening of the meatus, [(a), Fig. 1], ●——●, with contra-meatus open; ■——■, with contra-meatus blocked.

when the speaker is contralateral to the recorded ear also suggests that the response of the TM will be 'nulled' when the sound source occupies this position. At the same time, for the ipsilateral ear the response may be enhanced by up to 6 dB because of the 180° phase difference. Very large IIDs may therefore be created by this means, even at low frequencies.

These biophysical data suggest that the ears of European moles may act as balanced, pressure-difference receivers. This is the first time that such a system has been suggested for a mammal, although many reptiles and amphibia (Henson, 1974), as well as birds and crickets, have been shown to have a direct air pathway between the tympana. Thus, pressure-difference receivers may be common to all animals, regardless of their evolutionary position, that lack pinnae and are preferentially sensitive to low frequencies of sound.

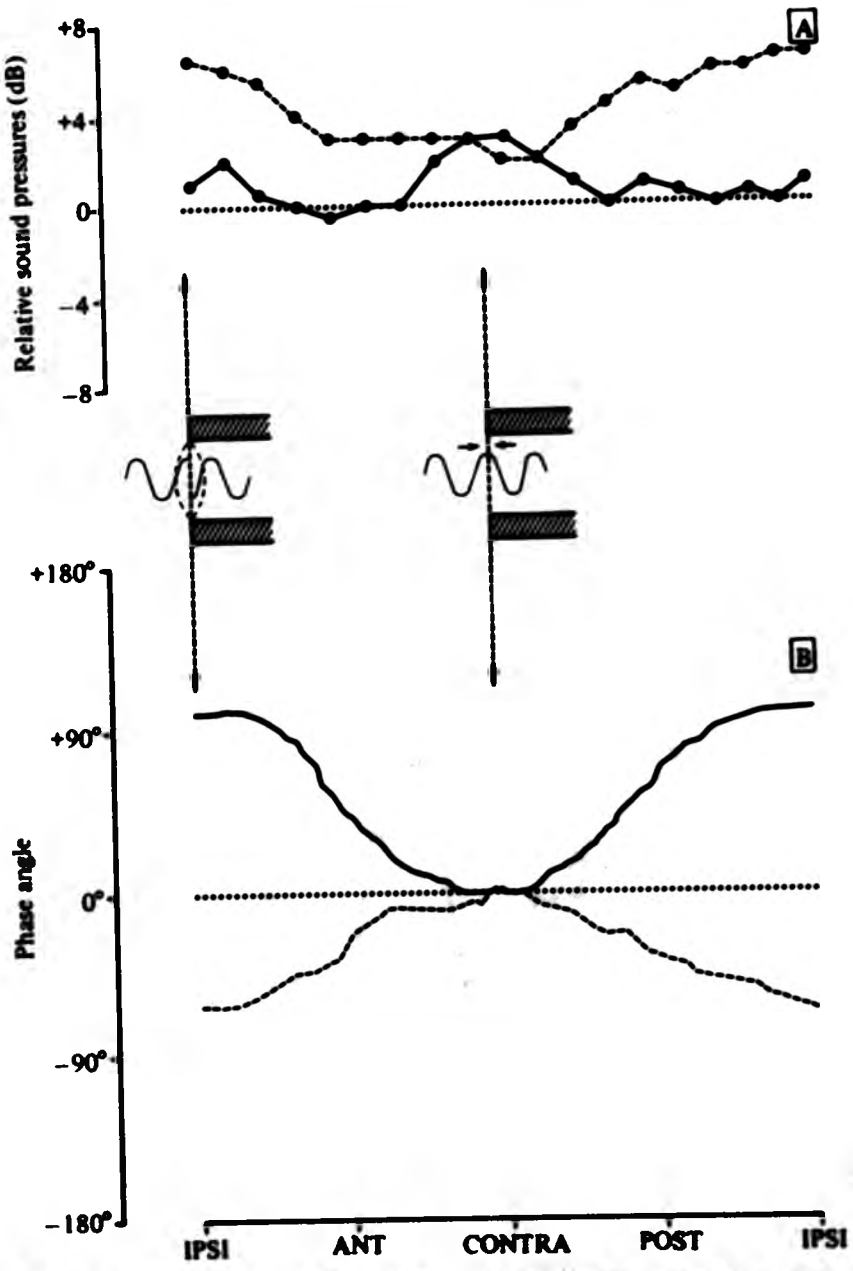
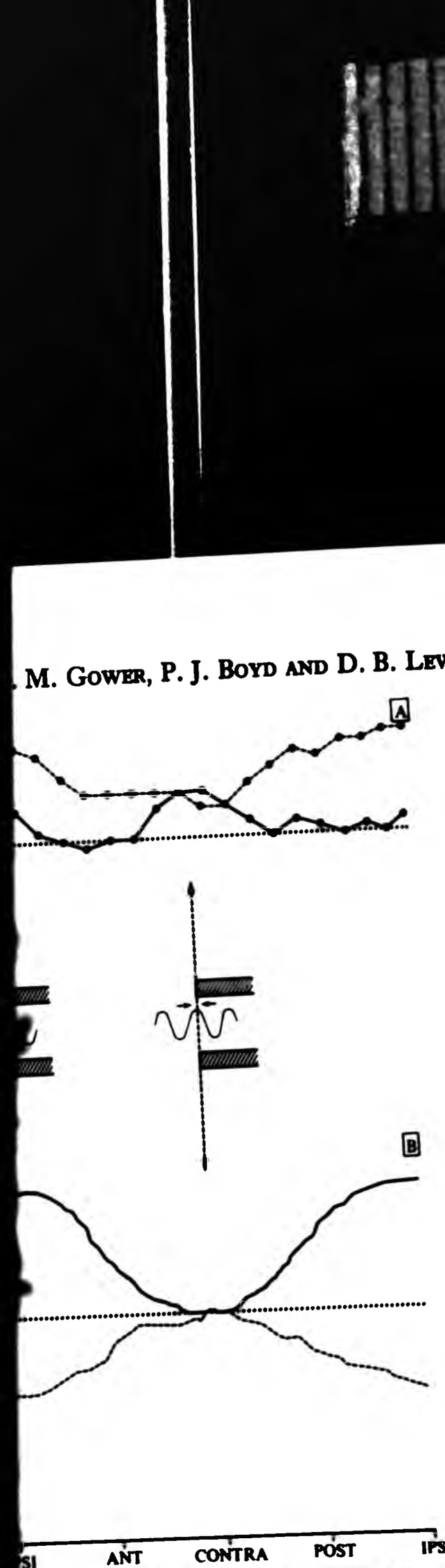


Fig. 3. Relative sound pressure levels (A), and phases (B), recorded internal (——) and external (– – –) to the tympanic membrane at a frequency of 1·6 kHz as the sound source was rotated through 360° azimuth. The sound pressures are plotted relative to the free field and are within 5 dB of each other at all angles; the phase differences change predictably from 180°, when the sound is presented ipsilaterally to the recorded ear, to 0° when the sound is contralateral. These conditions fulfil the requirements for a pressure-difference receiver.

This work was supported by a Royal Society Scientific Investigations Grant to D. B. Lewis and a Sir John Cass Research Grant to R. B. Coles. We would also like to thank Gill Sales for the loan of a ½ in B. & K. microphone and Axel Michelsen and Lindsay Aitken for critizing an early version of the manuscript.



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Auditory Localisation in the Bushcricket *Tettigonia Cantans* (Orthoptera, Tettigoniidae)

P.J. Boyd and D.B. Lewis

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AUDITORY LOCALISATION IN THE BUSHCRICKET *TETTIGONIA CANTANS* (ORTHOPTERA, TETTIGONIIDAE).

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Auditory localisation is of primary importance to an animal, both for intraspecific communication and for the localisation of predators. The *localisation* of a sound by an animal is achieved by the Central Nervous System (CNS) on the basis of *directional cues* provided by the auditory organs. In order to provide directional cues the responses of the auditory organs must vary with the angle of incidence of the sound. The extent to which this can be achieved defines the *directionality* of the auditory organs. The aim of the present study was to establish the extent of this directionality, at primary neurone level, in the bushcricket *Tettigonia cantans*, and to determine its basis.

The auditory organs of bushcrickets are located in the tibiae of the forelegs. The sensory cells of each organ are stimulated via a pair of tympana which are formed from thin regions of the leg cuticle in contact with the walls of the modified leg trachea (Zeuner 1936; Lewis 1974). The proximal end of this trachea opens at a large 'acoustic spiracle' on the prothorax which allows access of sound to the rear surfaces of the tympana. Access of sound to the front of the tympana is largely restricted by cuticular folds that leave only narrow 'tympanal slits' communicating with the exterior.

Hill and Oldfield (1981) showed directional response patterns in the bushcricket *Mygalopsis marki*, at 25 kHz (its most sensitive frequency), by recording the responses of a single auditory unit in the ventral nerve cord. They suggested that sound entry was mainly via the spiracle and that directionality was entirely due to sound diffraction by the body. However, neural interactions that alter intensity coding may occur centrally (Rheinlander 1975), and so it is important to establish first the details of directionality at primary level in order to be able to evaluate fully results obtained in the CNS. Bailey and Stephen (1978) studied the directional responses of primary neurones in *M. marki* and proposed a model of directionality which was based on sound entry at the tympanal slits, in contrast to the results of Hill and Oldfield (1981).

Physiological and biophysical investigations on the directionality of the auditory organ were carried out on a group of 10 adult *T. cantans* of both sexes. For the physiological studies the insects were waxed, in the natural standing position, to a stand made of 0.8mm wire, and placed at the centre of an anechoic chamber having internal dimensions 2.5 x 2.5 x 2.2m. A calibrated loudspeaker mounted on a metal boom could be rotated in the horizontal plane from outside the chamber. The sound field at the centre of the chamber was uniform to within ± 2dB over the range 1-40kHz. Recordings were made using a method employed by Bailey and Stephen (1978): Whole-nerve recordings of the auditory responses were obtained from tympanal nerve just above the genual joint, using an electrode consisting of a short length of 0.002in. silver wire. Evoked responses to sound stimuli of 20ms duration and 3ms rise/fall time were averaged (Neurolog NL750) over 128 presentations and plotted for later analysis (e.g. Fig 1a). Audiograms and polar plots of directional responses were produced for each insect over the frequency range 1-40kHz (a) in the normal state, and either (b) after fitting a sleeve of plastic tubing, sealed at both ends with wax, around the tibia of the recorded ear, or (c) after blocking the acoustic spiracle ipsilateral to the recorded ear with wax.

At each frequency tested, an intensity-response curve was first derived by measuring the maximum peak-to-peak amplitude of the averaged responses obtained, over a range of intensities, with the loudspeaker ipsilateral to the recorded ear (e.g. Fig 1b). Directionality was then determined by recording the responses to stimuli of 20-30 dB above ipsilateral threshold at several angles of azimuth around the insect. The amplitudes of these responses were converted to dB using the intensity-response curve for the relevant frequency.

Measurements of the diffraction of sound by the insect body were made for 5 specimens waxed to the wire stand after they had been used for physiological experiments. A 1cm length of 2mm plastic tubing, acting as a probe, attached to a ⅛in. condenser microphone (Bruel & Kjaer 4138) was used, and the opening of the probe was placed at the

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position of the acoustic spiracle on the thorax. Polar plots of sound pressure were made (B&K Level Recorder 2305) at a number of pure-tone frequencies between 5 and 40kHz, as the loudspeaker was rotated through 360°.

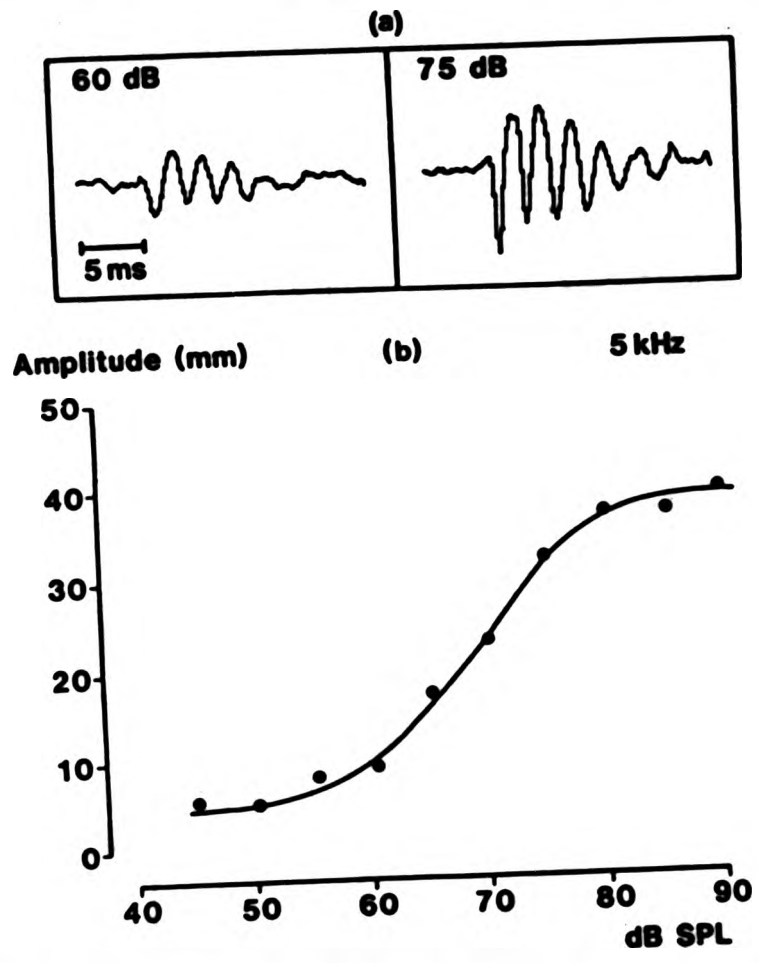

Figure 1. a. Examples of averaged responses to 20ms duration sound stimuli of 5kHz at 60dB and 75dB SPL (Threshold +10 and +25dB respectively).
b. Intensity-response curve constructed at 5kHz with loudspeaker ipsilateral to the recorded ear.

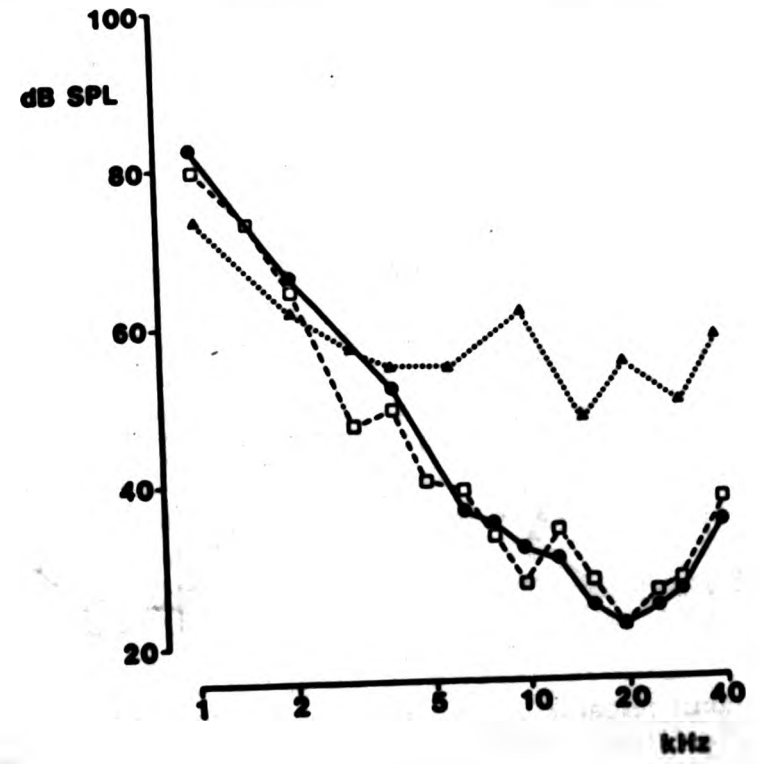

Figure 2. Audiograms constructed from thresholds to ipsilaterally presented sound; ●—● Intact; □----□ After blockage of ipsilateral tympanic slits; ▲····▲ After blockage of ipsilateral acoustic spiracle.

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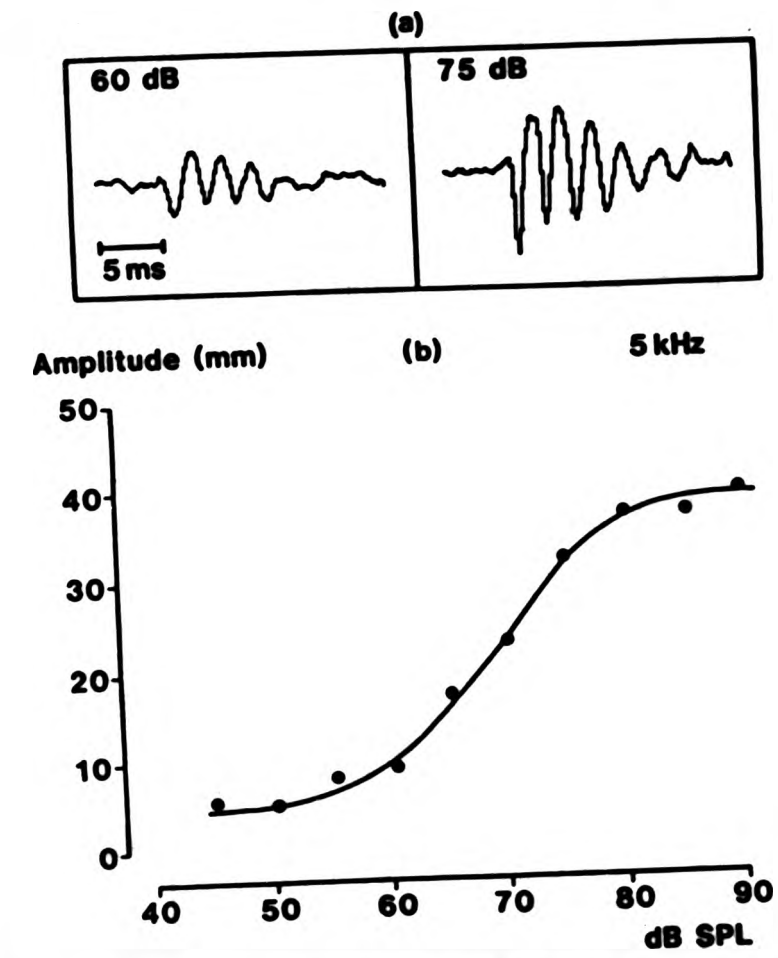

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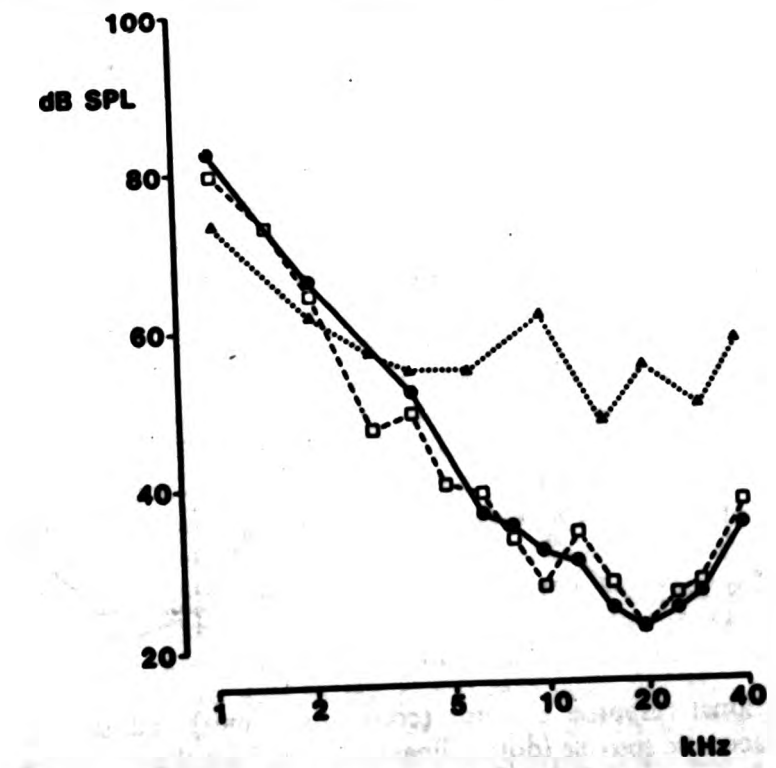

Figure 2. Audiograms constructed from thresholds to ipsilaterally presented sound; ●—● Intact; □—□ After blockage of ipsilateral tympanic slits; ▲···▲ After blockage of ipsilateral acoustic spiracle.

A typical audiogram for the whole auditory nerve is given in Fig 2. The audiograms of all the animals tested were similar, and showed that the auditory organ is most sensitive to sound of 20-25 kHz, the threshold at the most sensitive frequency being about 25 dB SPL (re 20μPa). Blockage of sound entry at the tympanal slits caused no appreciable change in thresholds, whereas blockage of sound entry through the acoustic spiracle caused a reduction in sensitivity, above 5 kHz, of up to 30dB. The maximum reduction occurred between 10 and 30kHz.

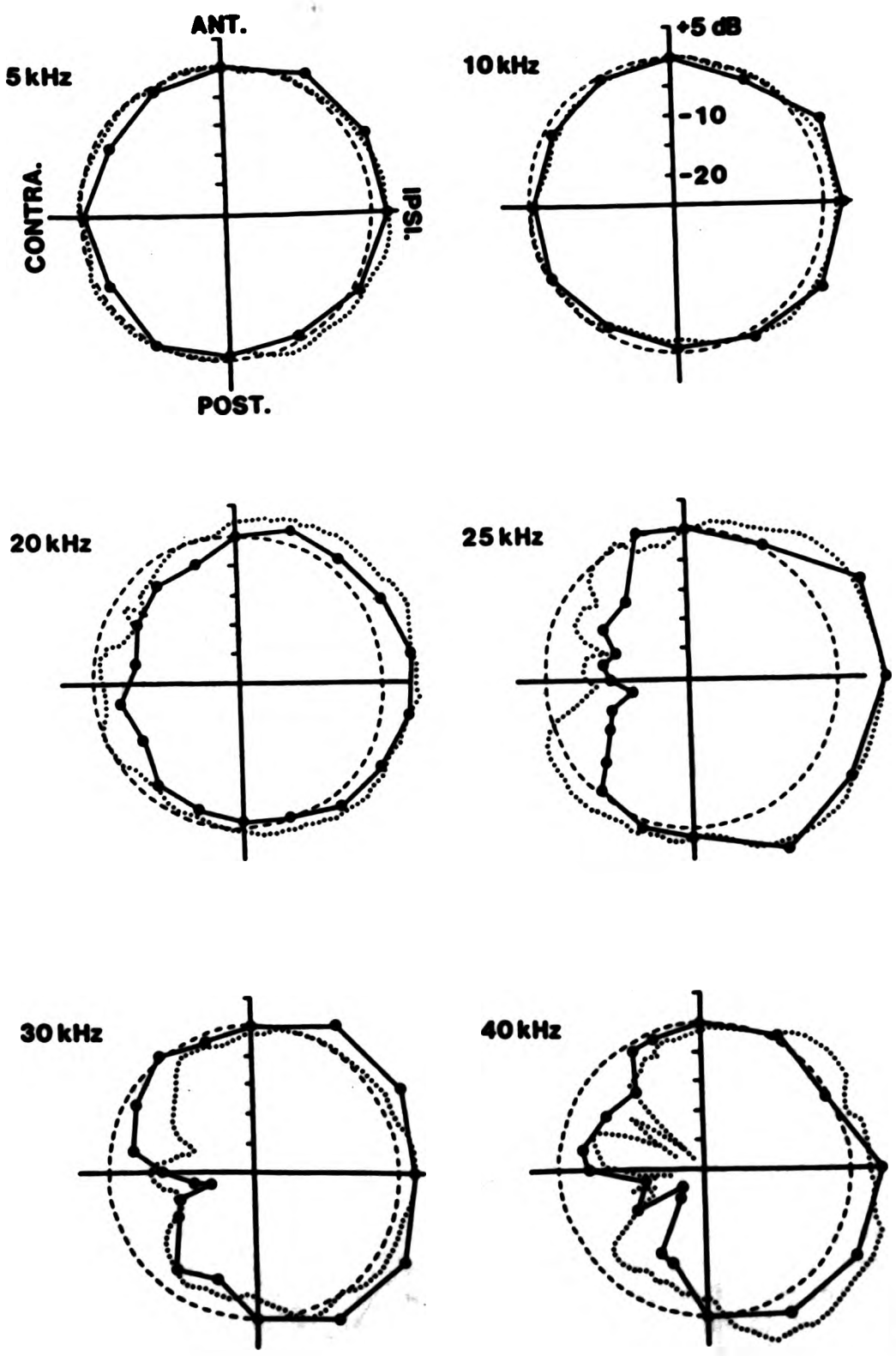

Figure 3. Neural directional response patterns (continuous lines) and sound pressure levels at the acoustic spiracle (dotted lines) measured for the sound frequencies given. Neural responses are plotted as dB relative to the anterior response; sound pressure levels are plotted as dB relative to the free field. The dashed line represents 0dB in both cases.

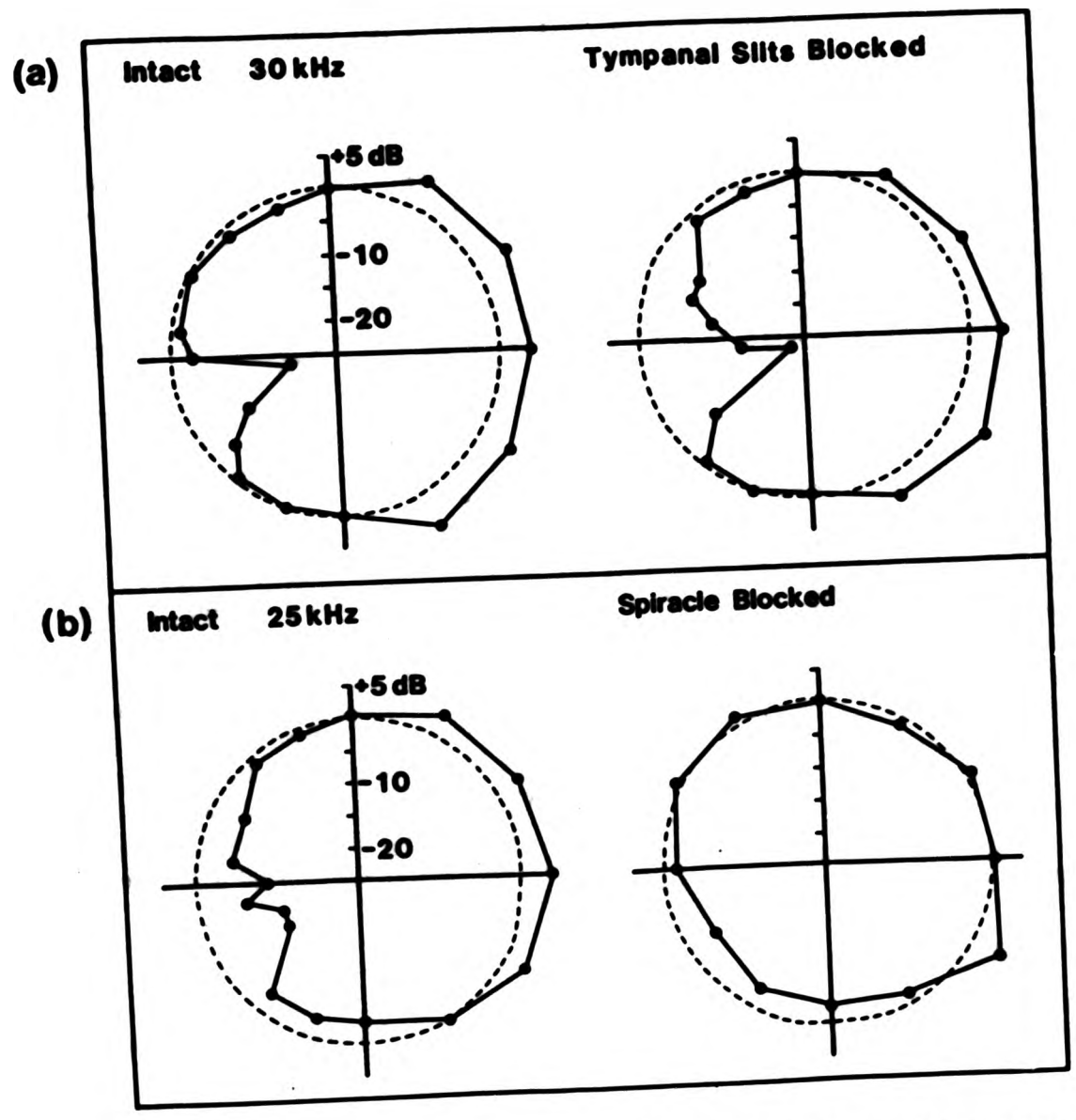

Figure 4. a. Effect of blocking sound entry at the tympanal slits on the directional responses to a 20kHz sound stimulus.
b. Effect of blocking the acoustic spiracle on the directional responses to a 25kHz sound stimulus.

A representative series of neural directional response patterns is given in Fig 3, together with the sound diffraction patterns measured at the acoustic spiracle. The neural responses are plotted as dB relative to the anterior position, as the two auditory organs should have almost mirror-image response patterns that overlap anteriorly (and posteriorly); the sound pressure levels are plotted as dB relative to the free-field sound levels. At frequencies up to 10kHz the maximum left-right (L-R) difference in the neural response was about 5dB. Above 10kHz the L-R difference can be seen to increase as a result of both ipsilateral augmentation and contralateral diminution of the response. Ipsilateral augmentation develops to a maximum of about 8dB around 25kHz, above which it gradually decreases. Contralateral diminution develops smoothly up to 20-25kHz, above which sharp dips in the response begin to appear. These trends were evident in all the animals tested.

The sound diffraction patterns show marked similarity to the neural responses; it is clear that the same trends of ipsilateral augmentation and contralateral diminution occur. These are consistent with the physical theory governing the diffraction of sound about a body of dimensions similar to those of the insect (Beranek 1954; Shaw 1974). The precise details of the sharp dips in the neural responses and the sound pressure levels shown may not correspond to each other exactly because they were not derived from the same specimen. The sound field around the insect stand alone was uniform within ± 2dB at all frequencies.

The effect of tibial sleeve application on the directionality is shown in Fig 4a. In no case was directionality appreciably changed following blockage of sound input at the

tympanal slits by this method. Conversely, blockage of the ipsilateral acoustic spiracle always caused a very large reduction in directionality, as measured by maximum L-R difference. In the example shown (Fig 4b) this was originally about 20dB at 25kHz and decreased to 7dB when the spiracle was blocked.

These results indicate that in the bushcricket *T. cantans*, appreciable directionality is achieved only for sound frequencies above 10kHz, A comparison of neural response patterns and sound diffraction patterns indicates that the neural response varies in direct accordance with the sound pressure at the spiracle, which is in agreement with the central recordings of Hill and Oldfield (1981). The effects of blocking sound input via the acoustic spiracle on both the audiogram and the directional responses confirm that the spiracle is the major site of sound entry to the auditory system (as suggested by Lewis 1974; Seymour *et al* 1978; Hill and Oldfield 1981). Bailey and Stephen (1978) reported the occurrence of paired augmentation lobes in the directional responses of *M. marki* in positions that suggest that they were due to sound entry through the tympanal slits. The present study did not confirm the existence of these lobes.

Thus the bushcricket (like most mammals) uses a 'pressure-system' to achieve directionality. This type of system requires interaural distances that are large compared to the wavelengths of the sound received. *T. cantans* has a high frequency species song with a bandwidth of about 9-55kHz (λ = 36-6mm), while the interaural distance is about 7mm. Substantial sound diffraction should therefore be expected at least at the higher frequencies within this range. However, although the maximum L-R difference was seen to continue to increase above 30kHz, it may be that directional cues become more ambiguous at these high frequencies, because many sharp dips appear in the response patterns. It is likely, therefore, that most useful directional cues are achieved around 25-30kHz, a view that is supported by the fact that the auditory organ is most sensitive to this frequency range. However, behavioural tests and recordings from central units that integrate left and right peripheral inputs are needed to confirm this hypothesis.

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II