J. exp. Biol. 158, 391–410 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

AUDITORY INTERNEURONES IN THE METATHORACIC GANGLION OF THE GRASSHOPPER *CHORTHIPPUS BIGUTTULUS*

I. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION

BY ANDREAS STUMPNER AND BERNHARD RONACHER
Institut für Zoologie II, Staudtstrasse 5, 8520 Erlangen, FRG

Accepted 25 March 1991

Summary

1. Auditory interneurones originating in the metathoracic ganglion of females of the grasshopper *Chorthippus biguttulus* can be classified as local (SN), bisegmental (BSN), T-shaped (TN) and ascending neurones (AN). A comparison of branching patterns and physiological properties indicates that auditory interneurones of *C. biguttulus* are homologous with those described for the locust.

2. Eighteen types of auditory neurones are morphologically characterized on the basis of Lucifer Yellow staining. All of them branch bilaterally in the metathoracic ganglion. Smooth dendrites, from which postsynaptic potentials (PSPs) can be recorded, predominate on the side ipsilateral to the soma. If 'beaded' branches exist, they predominate contralaterally. The ascending axon runs contralaterally to the soma, except in T-fibres.

3. Auditory receptors respond tonically. The dynamic range of their intensity–response curve covers 20–25 dB. Local, bisegmental and T-shaped neurones are most sensitive to stimulation ipsilateral to the soma. The responses of SN1 and TN1 to white-noise stimuli are similar to those of receptors, while phasic-tonic responses are found in SN4, SN5, SN7 and BSN1. The bisegmental neurones receive side-dependent inhibition that corresponds to a 20–30dB attenuation. One local element (SN6) is predominantly inhibited by acoustic stimuli.

4. Ascending neurones are more sensitive to contralateral stimulation (i.e. on their axon side). Only one of them (AN6) responds tonically to white-noise stimuli at all intensities; others exhibit a tonic discharge only at low or at high intensities. One neurone (AN12) responds with a phasic burst over a wide intensity range. The most directional neurones (AN1, AN2) are excited by contralateral stimuli and (predominantly) inhibited by ipsilateral stimuli. Three ascending neurones (AN13–AN15) are spontaneously active and are inhibited by acoustic stimuli.

5. All auditory interneurones, except SN5, are more sensitive to pure tones below 10 kHz than to ultrasound.

Introduction

Auditory interneurones of the locust (*Locusta migratoria*) have been thoroughly

Key words: grasshopper, auditory interneurones, metathoracic ganglion, *Chorthippus biguttulus*.

investigated (e.g. Adam, 1969; Kalmring, 1975*a*,*b*; Rehbein, 1976; Römer and Rheinlaender, 1983; Römer and Marquart, 1984; Boyan and Altman, 1985). However, no acoustic behaviour of the locust has been described that has any relevance for intraspecific communication. To compare neuronal and behavioural responses, therefore, it is more promising to investigate grasshoppers with an elaborate system of acoustic communication (see D. von Helversen, 1972; O. von Helversen, 1979; D. von Helversen and O. von Helversen, 1975*a*,*b*, 1983; Elsner, 1974, 1975; Hedwig, 1986, for a review; see also Elsner and Popov, 1978). For *Chorthippus biguttulus*, a strong correlation has been established between the response of an interneurone and certain properties of the female's song-recognizing system (Ronacher and Stumpner, 1988).

This study describes metathoracic interneurones of *C. biguttulus* that show distinct and reliable responses to acoustic stimuli (Boyan, 1984). Interneurones originating and branching in the metathoracic ganglion are of special interest, since lesion experiments with males of the same species indicated that the first important processing of auditory information takes place within this ganglion (Ronacher *et al.* 1986; see also Römer *et al.* 1981, 1988; Römer and Marquart, 1984). Furthermore, males and females with one tympanic nerve severed and one thoracic connective cut on the contralateral side between the mesothoracic and metathoracic ganglia were still able to recognize the species-specific song (Ronacher *et al.* 1986; B. Ronacher, unpublished results). In this experiment, the only functioning structures of the auditory receptors were in the metathoracic hemiganglion with the intact tympanic nerve. Since axons of auditory receptors only ascend ipsilaterally (Römer, 1985; Römer *et al.* 1988; Halex *et al.* 1988; Stumpner, 1988), the ascending collaterals of auditory receptors are not necessary for pattern recognition. Behavioural experiments with selective heating of ganglia in a related species (*C. parallelus*), however, suggest that the head ganglia make the final decision about whether a song is from a potential mate (Bauer and von Helversen, 1987). For song recognition, therefore, the filtering circuit in the brain must get its main input from auditory interneurones ascending from the thoracic ganglia.

This paper on the metathoracic auditory interneurones includes a morphological description and a physiological characterization. A second paper will describe the filtering characteristics of these interneurones for temporal parameters of the male's song (Stumpner *et al.* 1991).

Materials and methods

The animals used in the experiments were female *C. biguttulus* L., caught in the field in southern Germany. They were briefly anaesthesized with CO_2 and attached to a free-standing holder (thickness 4 mm) either by a wax–resin mixture or with minute insect pins. The head, legs, wings and gut were removed. The thorax was opened dorsally and the metathoracic ganglion was exposed. In some experiments the ganglion was partially desheathed; in most experiments it was

stabilized with a NiCr spoon. The whole torso was filled with locust Ringer (Pearson and Robertson, 1981).

The experiments were performed in an anechoic Faraday cage at room temperature (22–26°C). This must be kept in mind when comparing these results with behavioural ones, which are usually obtained at higher temperatures (30–35°C). The recorded signals were amplified with a List LM-1 electrode amplifier and stored on magnetic tape (with a Racal store 4DS or a Blaupunkt video recorder with Bio-Logic PCM-adapter). The stimuli were delivered *via* two Motorola speakers (PH10, 2.5–40 kHz) located 35 cm from the preparation on the left and right sides. The amplitudes of the white-noise (WN; Fa. Noizeg, 100 Hz–100 kHz) or sine-wave stimuli (5 or 20 kHz) were modulated by a computer (AIM 65, Rockwell). Sound intensities were adjusted with a Brüel & Kjäer condenser microphone (1/2 inch) located at the site of the preparation and with a Brüel & Kjäer measuring amplifier (type 2602), and are given in dB re 2×10^{-5} N m^{-2} SPL. The standard stimulation sequence consisted of WN stimuli, 100 ms in duration, 50–90 dB SPL in 10 dB increments on the left and right sides of the preparation (see Figs 1, 3, 5 and 7, left-hand column) and sine-wave stimuli, 23 ms in duration (1 ms rise and fall times), 50–90 dB SPL, usually tested on the side with the lower threshold (Figs 1, 3, 5 and 7, middle column). Each stimulus was repeated five times at a rate of 2 s^{-1}. The intensity range (50–90 dB) for WN stimuli was shifted to lower values (30–70 dB) when necessary. The sine-wave stimuli were, for technical reasons, usually not tested at lower intensities. Therefore, we present the neurones' responses to pure tones between 50 and 90 dB only. This is the intensity range where both low-frequency receptors and high-frequency receptors stimulate the interneurones (see Fig. 1). The low-frequency background noise (<1 kHz) was around 30–35 dB. The data were evaluated on a Data General Nova 4X with a 'spike-detector' interface (Zarnack and Möhl, 1977).

Intracellular or quasi-intracellular recordings were made with thin-walled borosilicate glass microelectrodes, whose tips were filled with a 3–5 % solution of Lucifer Yellow (Aldrich) in 0.5 mol l^{-1} LiCl or distilled water. After an experiment, the thoracic ganglia were fixed in 4 % paraformaldehyde, dehydrated, and cleared in methylsalicylate. The whole mount with the stained cell was viewed under a fluorescence microscope, photographed, and drawn *via* a drawing tube. The relative depth of the observed structure in the ganglion was monitored with a measuring device (1 μm resolution) and revealed the three-dimensional structure of the cells. For each neurone type described in this study, at least three specimens were recorded and stained. When the first stained cell in an experiment was clearly identified by its physiology, in several cases a second cell was recorded and stained in the same preparation.

More than 450 identified neurones were investigated in approximately 400 female *C. biguttulus*. Most of them had their soma located in the metathoracic ganglion and were classified into 27 distinct neurone types. For reasons discussed below, the nomenclature introduced by Römer and Marquart (1984) for *L.*

migratoria has been adopted for *C. biguttulus*. (SN, segmental neurone, only located in the metathoracic ganglion complex; BSN, bisegmental neurone, branching in the meta- and mesothoracic ganglia; TN, T-fibre with an ascending and descending axon; AN, neurone with an ascending axon). (For synonymity with other nomenclatural systems see Boyan, 1986; Stumpner, 1988; Robert, 1989.) Neurones that have not been described for *L. migratoria* are named according to the same system.

Results

Receptor fibres

There are no obvious differences between the morphology of tympanic receptor fibres of *C. biguttulus* and of *L. migratoria* as far as the rough branching patterns in the metathoracic ganglion are concerned (see Römer, 1985; Halex *et al.* 1988). Fig. 1 shows the physiological characteristics of a low-frequency receptor and a

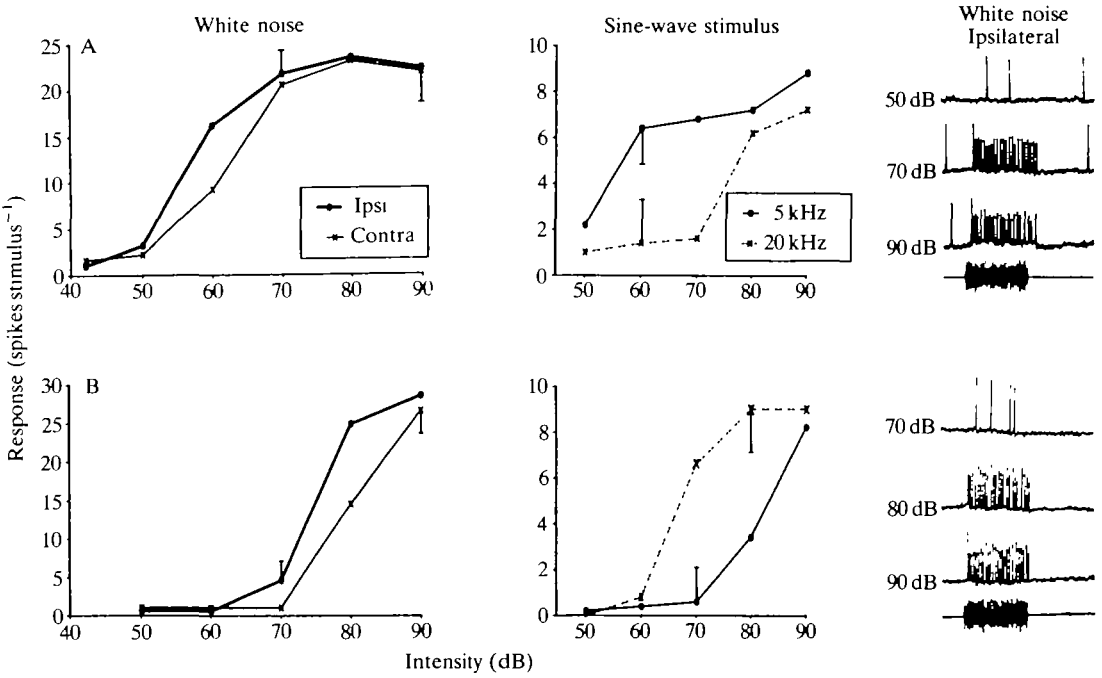

Fig. 1. Intensity–response functions for white noise and pure tones of a low-frequency receptor (A) and a high-frequency receptor (B) from the same preparation. In this figure and Figs 3, 5 and 7 ipsi and contra refer to the soma position; the left-hand diagrams give the response functions for white-noise stimuli of 100 ms (WN), the middle diagrams for pure-tone stimuli of 23 ms, delivered ipsilaterally. For each curve the largest standard deviations are shown (vertical bars). On the right are sample recordings (corresponding to the diagrams on the left) obtained with 100 ms WN stimuli at the indicated intensities. Lowest trace, stimulus trace; stimulus duration, 100 ms.

high-frequency receptor. The intensity–response functions have a dynamic range of 20–25 dB. In the neurophysiological preparation, contralateral stimulation reduced the receptor's sensitivity for WN by about 6 dB (range 3–8 dB) compared with ipsilateral stimulation. In behavioural tests a sensitivity difference of 8–9 dB was measured between the two ears (von Helversen, 1984); a 2 dB difference evoked 100 % correct turns in the males (see D. von Helversen and O. von Helversen, 1983).

Local and bisegmental neurones

Morphology

The local auditory neurones SN1, SN4, SN5 and SN6 and BSN1 (which ascends to the mesothoracic ganglion) have several morphological features in common (Fig. 2). The somata of these cells lie in the frontal part of the metathoracic ganglion, in a lateral or ventral location. Dense dendritic processes with smooth endings predominate in the frontal auditory neuropile (fNP) ipsilateral to the soma. Branches in the contralateral fNP are less dense, have beaded endings, and are often restricted to the anterior part of the neuropile. The segment connecting these two branching areas differs among the neurone types: the crossing segment of SN1 and BSN1 is located near the anterior border of the fNP, the crossing segment of SN4 and SN5 runs through the caudal half of the fNP, and SN6 has a deep, ventral crossing segment. SN4, SN5, SN6 and BSN1 have descending branches on the contralateral side, which reach the region of the caudal neuropile (SN4, SN5) or the second or third abdominal neuromere (SN6, BSN1). Medially directed processes of these branches have beaded endings. The axon of BSN1 ascends to the mesothoracic ganglion, where it ends in two or three medially directed collaterals with a beaded appearance (in one out of 36 stainings, the axon ascended further than the mesothoracic ganglion).

The morphology of SN7 corresponds to that of DUM-type neurones (dorsal unpaired median, Evans and O'Shea, 1977), with a dorsomedially located soma and a symmetrical branching pattern in both frontal auditory neuropiles (Fig. 2). One auditory, non-spiking DUM neurone with very similar morphology has been described in the locust (SN3, Marquart, 1985*a*); SN7, however, is a spiking neurone.

Several local and bisegmental neurones exist as 'twins' (Römer and Marquart, 1984; Stumpner, 1989), i.e. two (or more) neurones of very similar morphology are found on each side of the ganglion. SN1 twins and BSN1 twins were demonstrated in double stainings.

Physiology

SN1, SN4, SN5, SN6 and BSN1 show some similarities in their physiological properties. These neurones are more sensitive to ipsilateral stimulation (the terms ipsilateral and contralateral are used with respect to the soma of the neurones). Ipsilateral recordings reveal postsynaptic potentials (PSPs), whereas contralateral

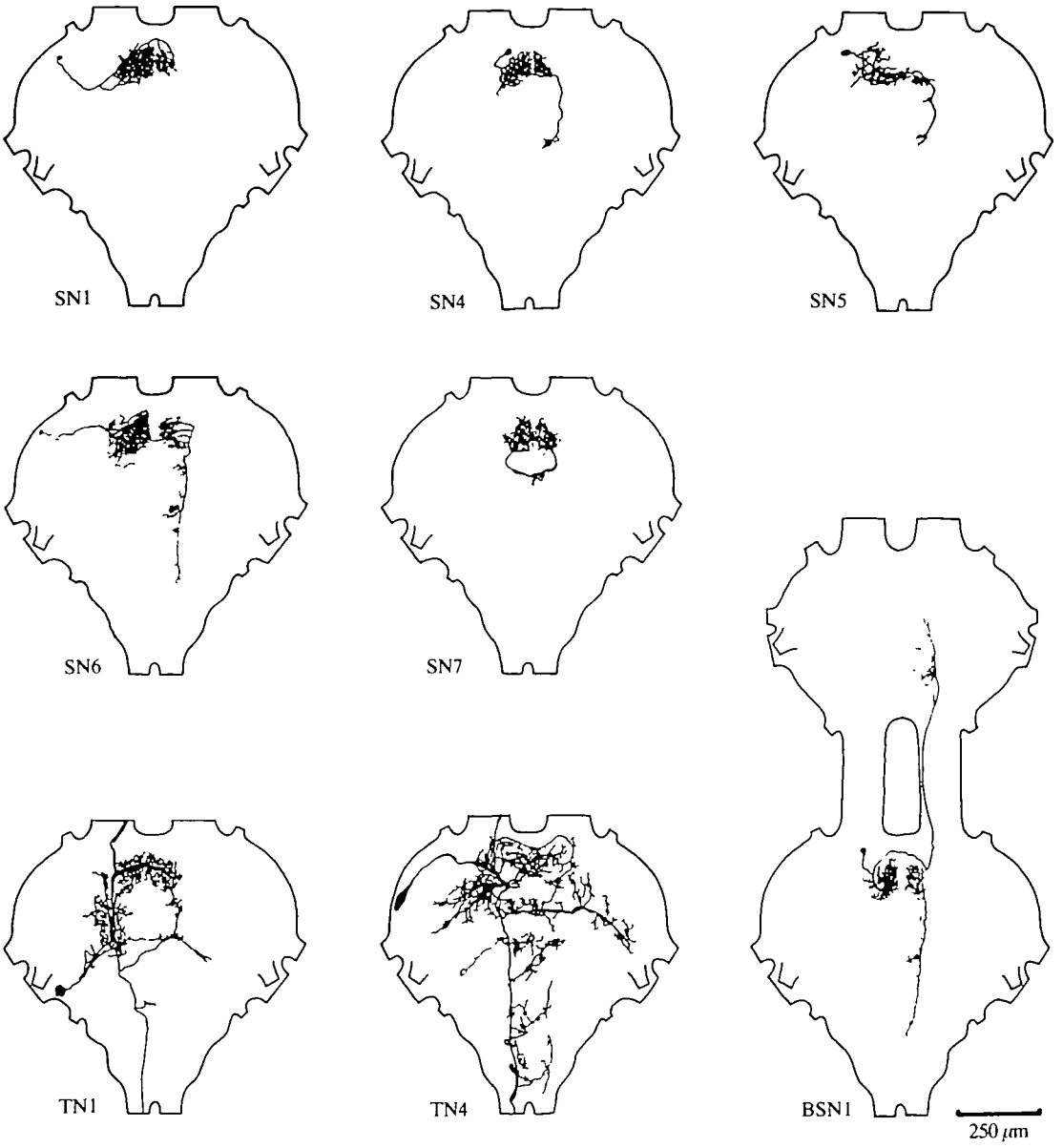

Fig. 2. Morphology of local (SN), bisegmental (BSN) and T-shaped (TN) neurones in the metathoracic ganglion. Additionally, the ramifications of BSN1 in the mesothoracic ganglion are presented.

recordings appear to be similar to axonal penetrations. For SN7, the terms 'ipsilateral' and 'contralateral' cannot be defined relative to the soma position; this neurone shows identical reponses to stimulation from the left and from the right (Fig. 3D).

SN1 responds tonically and its intensity–response curve (Fig. 3A) is similar to

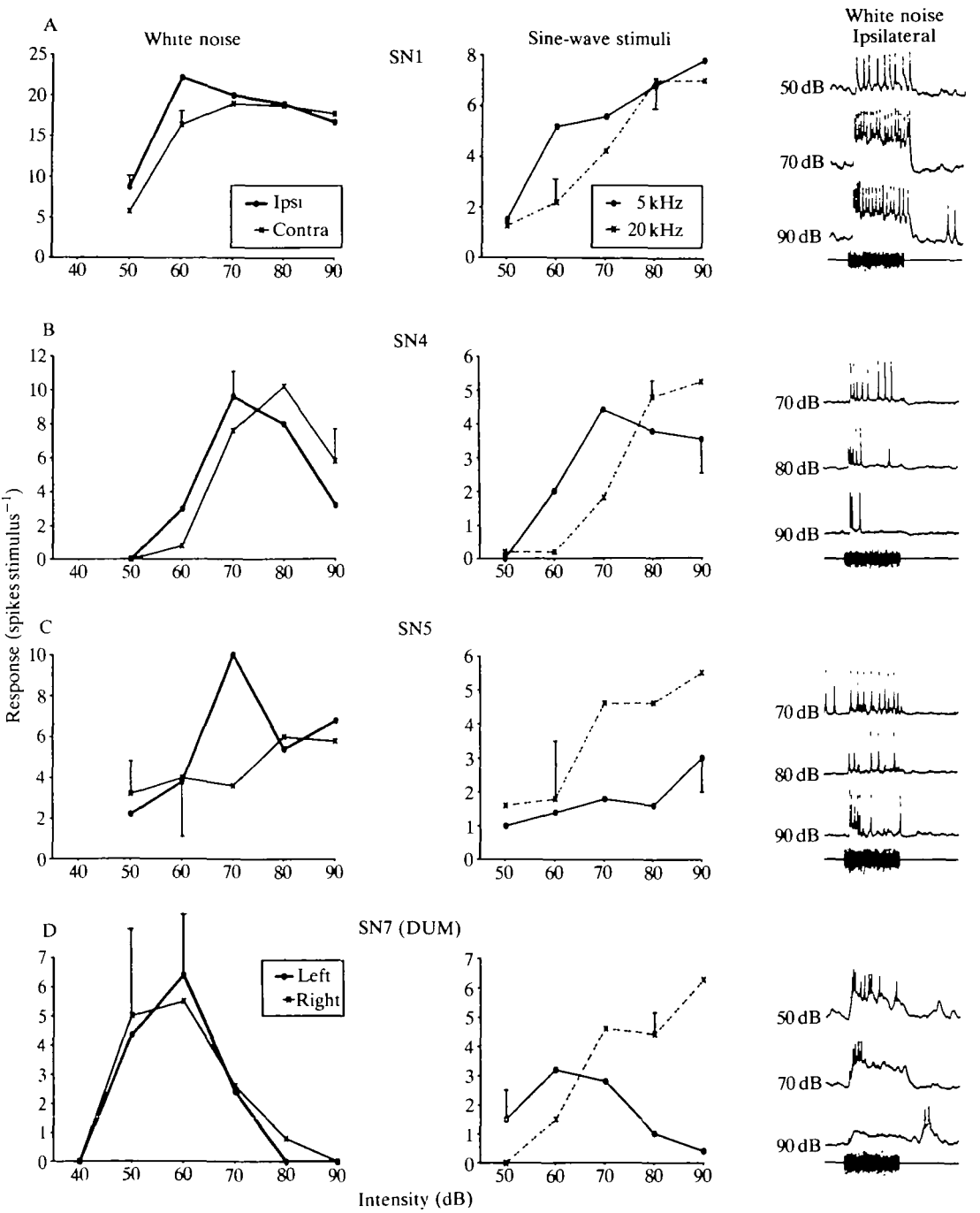

Fig. 3. Intensity–response functions of local neurones for white noise (100 ms) and pure tones (23 ms, delivered ipsilaterally). Further details are given in Fig. 1. For SN7, a DUM-type neurone, ipsilateral and contralateral are not defined.

that of receptors, though its spiking rate is typically lower (about 200 Hz at maximum), and a slight reduction in spike numbers may occur at high intensities. SN4, SN5, SN7 (Fig. 3B–D) and BSN1 (see Fig. 5A,B), in contrast, are inhibited to a certain degree at intensities more than 20 dB above threshold. This results in a phasic response or even total suppression of spikes (SN7, some examples of BSN1). The responses of different BSN1 neurones, however, depend quite differently on stimulus intensity: whereas some BSN1 cells can be called tonically responding neurones with a (sometimes only slight) reduction of spike numbers in response to louder stimuli (see Fig. 5A), others have a phasic-tonic to phasic spiking pattern, especially at higher stimulus intensities (see Fig. 5B). Differences were found not only in different preparations but also between the twins in one preparation (Stumpner, 1989).

The directionality of SN1 and SN4 is comparable to that of auditory receptors. Most BSN1 cells, however, exhibited reduced responses to contralateral stimulation, corresponding to an attenuation of 20–30 dB (see Fig. 5A,B).

The lowest thresholds of SN1, SN4, SN7 and BSN1 lie in the low-frequency range (below 10 kHz), while SN5 neurones are most sensitive to stimuli above 20 kHz (compare middle diagrams in Fig. 3). Intensity–response curves for different frequencies shift along the x-axis for SN1 and for some SN4 cells. Other SN4 cells as well as SN7 and BSN1 respond with a phasic pattern to low-frequency stimuli at any intensity, while high-frequency stimuli elicit tonic responses.

SN6 is usually spontaneously active; this activity is suppressed by auditory stimuli. A tonic hyperpolarization can be seen in ipsilateral recordings; often, this inhibition is interrupted by a single action potential a few milliseconds after the onset of the hyperpolarization (arrows in Fig. 4). With high-frequency stimulation, some SN6 cells exhibit a pure excitation near threshold, and, at high intensities, a postinhibitory rebound (Fig. 4).

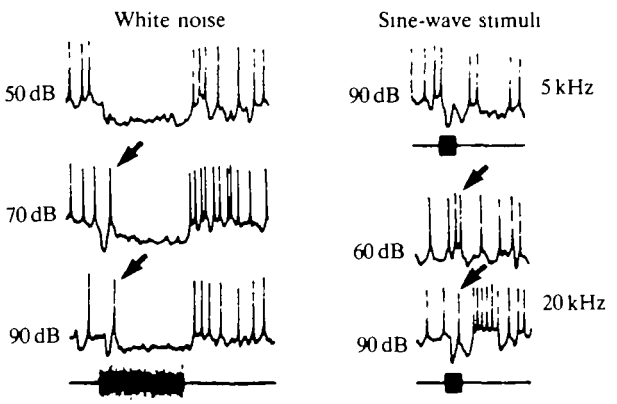

Fig. 4. Sample recordings of the response of SN6 to ipsilateral stimuli of white noise (100 ms, on the left) and pure tones (23 ms, on the right). Arrows denote suprathreshold excitation. With high-frequency tones of low intensity the excitation occurs without a preceding IPSP (see 20 kHz, 60 dB).

T-fibres

Morphology

Two metathoracic T-fibres, TN1 and TN4, have their soma in a dorsolateral location of the first abdominal neuromere (TN1) or the metathoracic neuromere (TN4). The ascending and descending axons run ipsilateral to the soma (Fig. 2), unlike those of the ascending neurones (see Fig. 6). Both neurones show extensive branching: branches of TN4 cover nearly all the metathoracic ganglion complex, while the most prominent processes of TN1 are positioned in both frontal auditory neuropiles with a very thick, ventrally situated segment crossing the midline. This branch of TN1 seems to be mainly presynaptic, because recordings in this position never showed PSPs (see Peters *et al.* 1986; see also Römer and Marquart, 1984, who reported a slight hyperpolarization induced by high-frequency tones in TN1 in *L. migratoria*). PSPs were visible in recordings of the more posterior, ipsilateral structures of TN1. One of many smaller dendrites in this posterior half crosses the midline and branches in the region of the caudal neuropile. The ascending axon was not stained beyond the prothoracic ganglion; in one TN1 cell the axon clearly ended in the mesothoracic ganglion.

Physiology

TN1 and TN4 respond tonically. TN1 cells spike (Fig. 5C) in a similar way to receptors: the tonic discharge shows a dynamic range from 50 dB SPL (threshold) to 70 or 80 dB SPL. The threshold for contralateral stimulation is 5–9 dB higher than for ipsilateral stimulation.

TN4 (Fig. 5D) is usually less sensitive than TN1 and its response is more variable; most TN4 cells show irregular spontaneous activity. In addition to acoustic stimuli, vibrations and air currents elicit suprathreshold responses in TN4 and some TN1 neurones.

The threshold of both neurones lies below 5 kHz and is thus lower than in other interneurones. 20 kHz stimuli elicit spikes only at intensities of 80 dB SPL or more.

Ascending neurones

Neurones with an ascending axon form the most prominent group of metathoracic auditory interneurones; at least 17 different types have been identified so far. The physiology of 10 neurones will be described in some detail. The remaining neurones (AN7, AN10, AN16–20) showed either a weak response to acoustic stimuli or were recorded only once or twice. The responses of AN1 (=B-neurone) have been described for *C. biguttulus* by Wolf (1986) in an extracellular preparation, and will be mentioned here only briefly.

Morphology

The soma of ascending neurones can be found in a frontal lateral location (AN2, AN11, AN12, AN13), in a dorsolateral location near to the entrance of the tympanic nerve (AN3), in a more dorsal location (AN1, AN15) or in a ventral

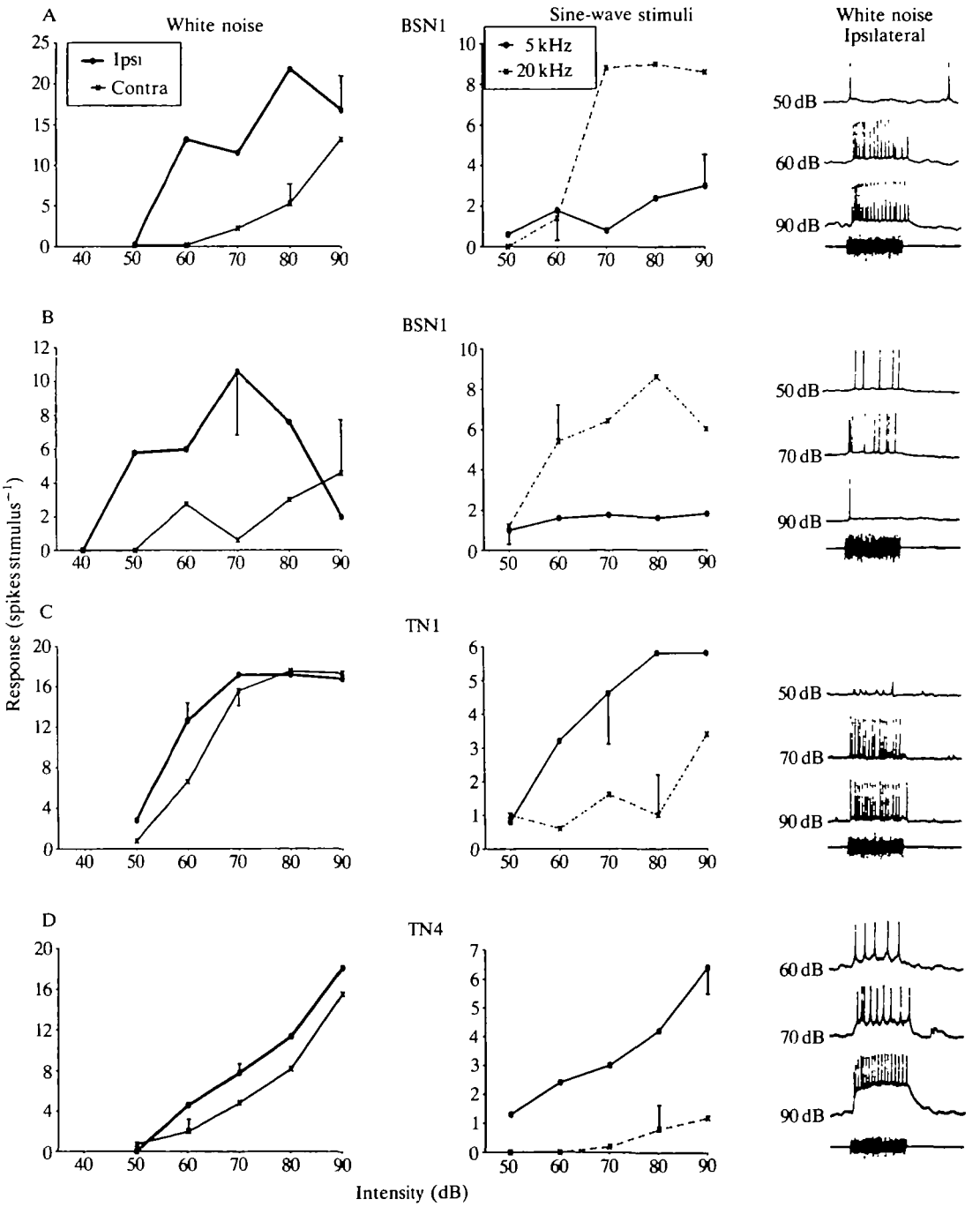

Fig. 5. Intensity–response functions of bisegmental neurones and T-fibres for white noise (100 ms) and pure tones (23 ms, delivered ipsilaterally). For BSN1, examples of two different response types are shown. Further details are given in Fig. 1.

location near the midline (AN4, AN6, AN14) (Fig. 6). The axon ascends on the contralateral side to the brain; this has been proved for AN1, AN3, AN4, AN11 and AN12, and probably also holds for the other ascending neurones (see Hedwig, 1985, for *Omocestus viridulus*, and Eichendorf and Kalmring, 1980, for *L. migratoria*). The smooth dense dendrites in the frontal auditory neuropile originate ipsilaterally, except in AN4 where they originate contralaterally, and may cross the midline (AN3, AN4); AN11–AN15 have additional contralateral dendrites of the same structure. Recordings from regions with smooth dendrites show clear PSPs. Where beaded branches exist, they lie contralaterally in the metathoracic ganglion (AN1, AN6, in the region of the fNP; AN2, in the frontal dorsal one-third of the ganglion; AN3, only sparse). Single beaded branches can regularly be found in anterior thoracic ganglia.

Physiology

Spiking pattern and intensity characteristics. Only one ascending neurone, AN6, responds tonically to WN stimuli at all intensities (Fig. 7F). This response does not saturate at the highest intensities tested. The highest spiking rates only exceptionally exceed 200 Hz. It is typical of this neurone that the intensity–response curves for ipsilateral and contralateral stimulation cross at approximately 60 dB.

Some other ascending neurones also show tonic discharges in response to WN stimuli. However, these responses occur in the range near threshold (AN1, AN3, AN11: Fig. 7A,C,D,G) or at high intensities (AN2, AN3, AN4; Fig. 7B,D,E). At intermediate intensities these neurones respond phasic-tonically (e.g. Fig. 7D) or with scattered spikes (Fig. 7B). The spiking pattern of most ascending neurones adapts strongly. Furthermore, in some neurones, especially in AN2, the response is highly variable: the spiking pattern may be tonic, phasic or irregular for the same stimulus in one individual.

AN12 is an ascending neurone with a predominantly phasic response. From 5 to 10 dB above threshold (45–50 dB SPL) to 90 dB SPL, a phasic burst of 3–6 spikes is produced at the onset of a stimulus (Fig. 7H) with very short interspike intervals (sometimes less than 2 ms). At high intensities, this phasic burst is followed by additional spikes.

A characteristic of several neurones, an initial inhibitory postsynaptic potential (IPSP) before the excitatory response, can best be seen in dendritic recordings of AN4, AN3 and, to a lesser extent, AN12 and some AN6 cells (Fig. 7C,E,H). This IPSP is most clearly triggered by the onset of white-noise stimuli (see Ronacher and Stumpner, 1988) and low-frequency stimuli (less than 10 kHz), but also occurs in response to high-frequency tones.

Three ascending neurones (AN13–AN15) were inhibited by acoustic stimuli. As an example, the intensity–response functions of an AN13 are shown in Fig. 8A. All inhibited cells were spontaneously active (Fig. 8B), though some of them had a rather low spiking rate (less than 5 Hz). The inhibition was detectable around 40–50 dB SPL; contralateral stimuli were slightly more effective than ipsilateral ones. The duration of the inhibition in all cases was diminished by adaptation.

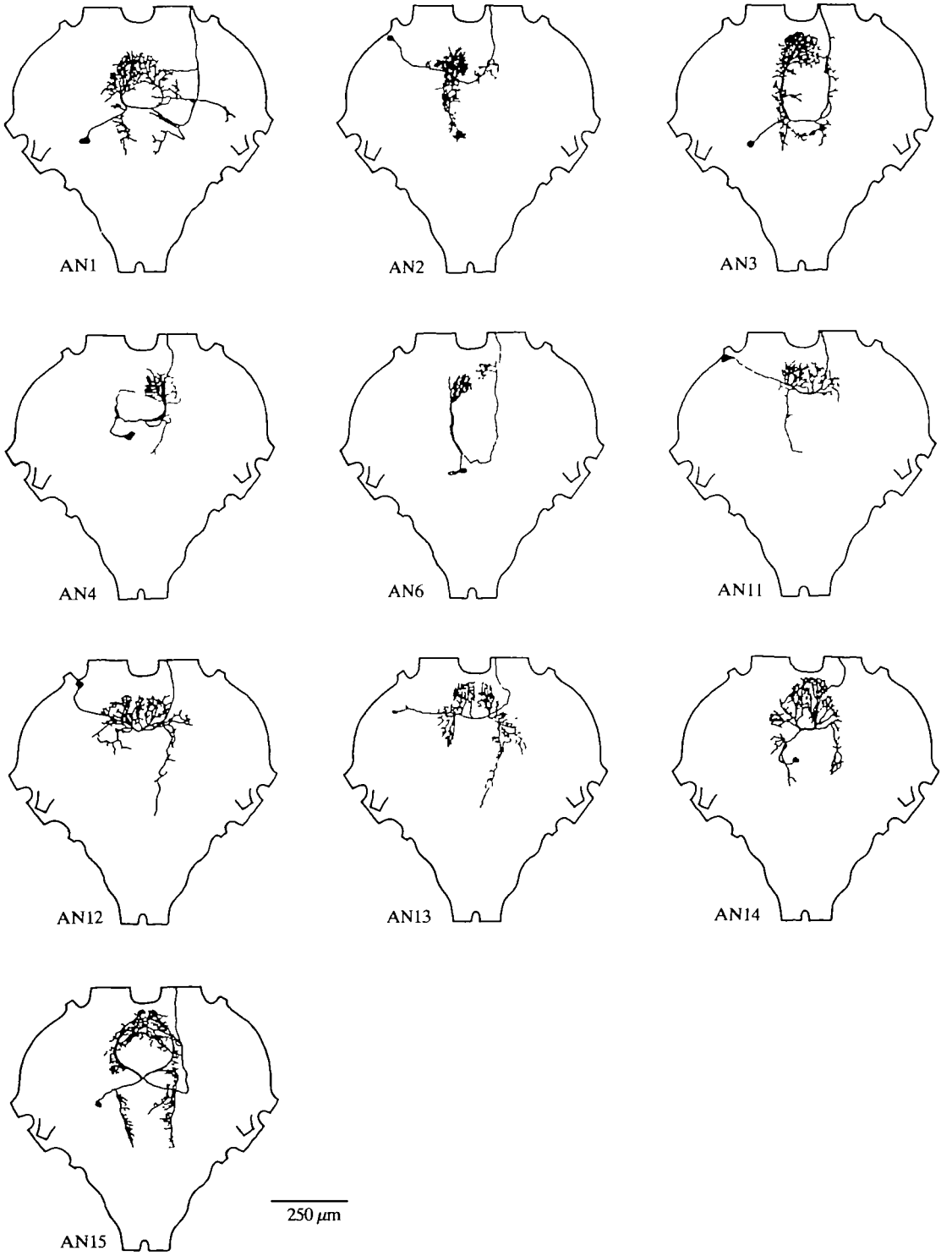

Fig. 6. Morphology of 10 ascending neurones in the metathoracic ganglion.

Directionally. The influence of stimulus direction on the spiking response differs substantially between ascending neurones. Nevertheless, all those described here exhibit the lowest threshold with contralateral stimulation, and the majority show a reduced response to ipsilateral stimuli. Note, however, that most of these neurones are also influenced by intensity, and this influence usually exceeds the dependence on stimulus direction (see Fig. 7C,G). AN2, however, is completely suppressed by ipsilateral stimuli up to 80 dB SPL (Fig. 7B); only the loudest ipsilateral stimuli evoke suprathreshold activity. AN1 shows a clear directionality, too, but is usually slightly excited by ipsilateral stimuli up to about 10–20 dB above threshold (Fig. 7A, see also Wolf, 1986).

Tuning. Ascending neurones of *C. biguttulus* are most sensitive below 10 kHz. The responses to low-frequency stimuli are similar to the responses to white noise. High-frequency sounds elicit more uniform responses: the discharges are tonic over a broad intensity range. Only at the highest intensities can a decrease in spike number per stimulus be seen (Fig. 7A,C,E). Adaptation is less conspicuous with high frequencies than with low frequencies or white noise.

Discussion

In grasshoppers, the metathoracic ganglion accommodates the first important level of auditory processing (see Introduction). The head ganglia must perform further filtering steps on the basis of information ascending from the thoracic ganglia. The diversity of information is obviously delimited by the set size and by the properties of ascending auditory neurones. Thus, it is important to have as complete a survey as possible of the thoracic auditory pathway. Several identified neurones (Stumpner, 1988) are not included in this study because of low excitability to acoustic stimuli or fragmentary physiological data.

The main objective of this study is to further the understanding of the auditory pathway of a grasshopper with elaborate acoustic communication. For a comparison of the neuronal responses with behavioural data one has to know that model songs composed of several WN syllables (as used here) are as effective as natural songs, provided that the temporal pattern is correct. The intensity of the male's song reaches 76 dB at 10 cm distance. In most behavioural tests with females, intensities between 64 and 76 dB were used (O. von Helversen, 1979). The behaviourally effective intensities of model songs (WN) ranged from approximately 45–50 to 80 dB SPL (D. von Helversen, 1984, and personal communication). Stimuli lacking the high-frequency component are less effective. In both respects (intensity and spectrum) the females exhibit large interindividual variability.

Responses to standard stimuli

Local elements and T-fibres

The short response latencies and the location of the input regions (where PSPs can be recorded) on the side of greater sensitivity suggest that the local neurones

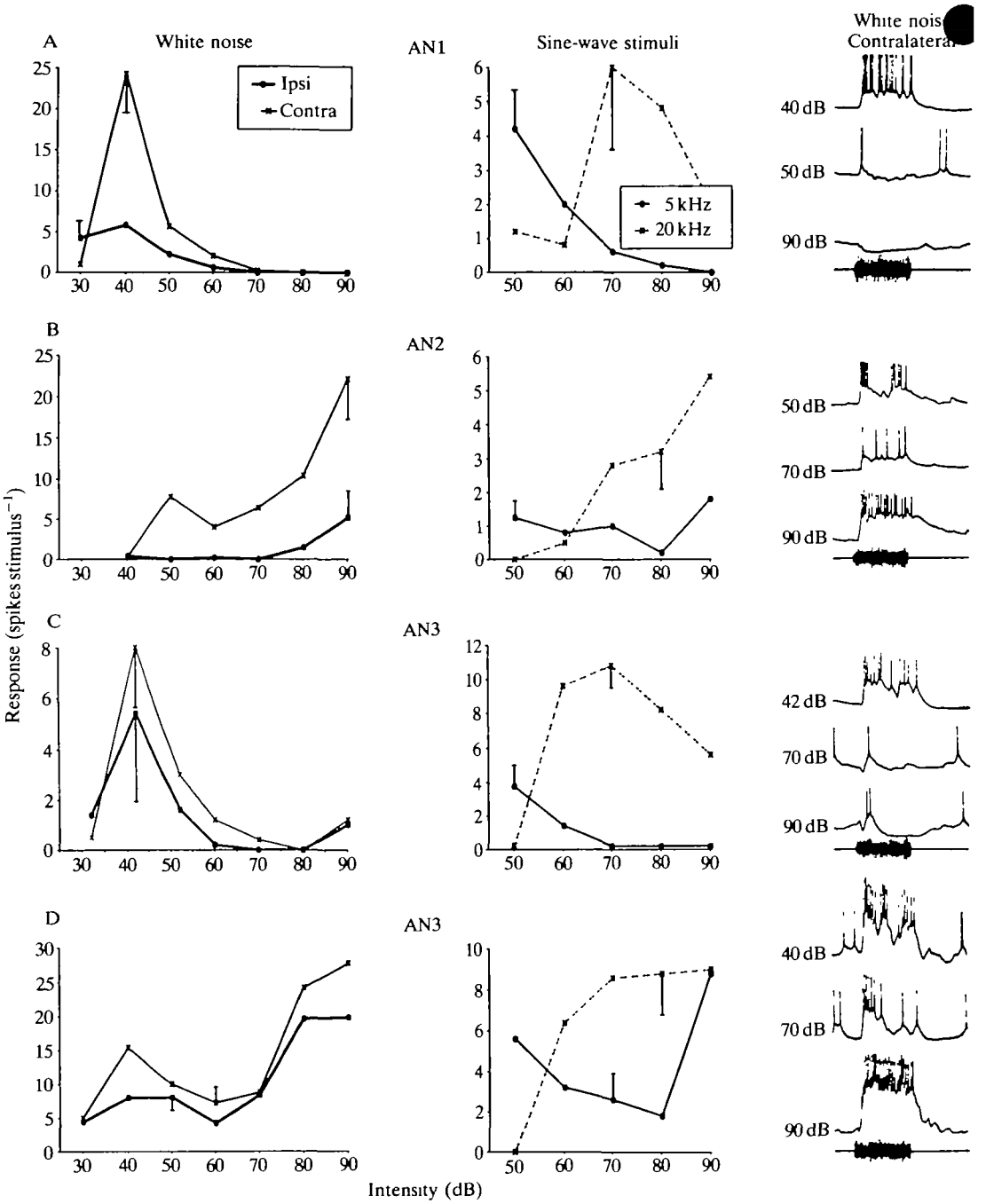

Fig. 7. Intensity–response functions of ascending neurones for white noise (100 ms) and pure tones (23 ms, delivered contralaterally). In B, D, E and G the two diagrams are not from the same cell but from two cells with similar responses. The sample recordings on the right (100 ms WN stimuli) represent single data points from the WN intensity curves on the left. Further details are given in Fig. 1.

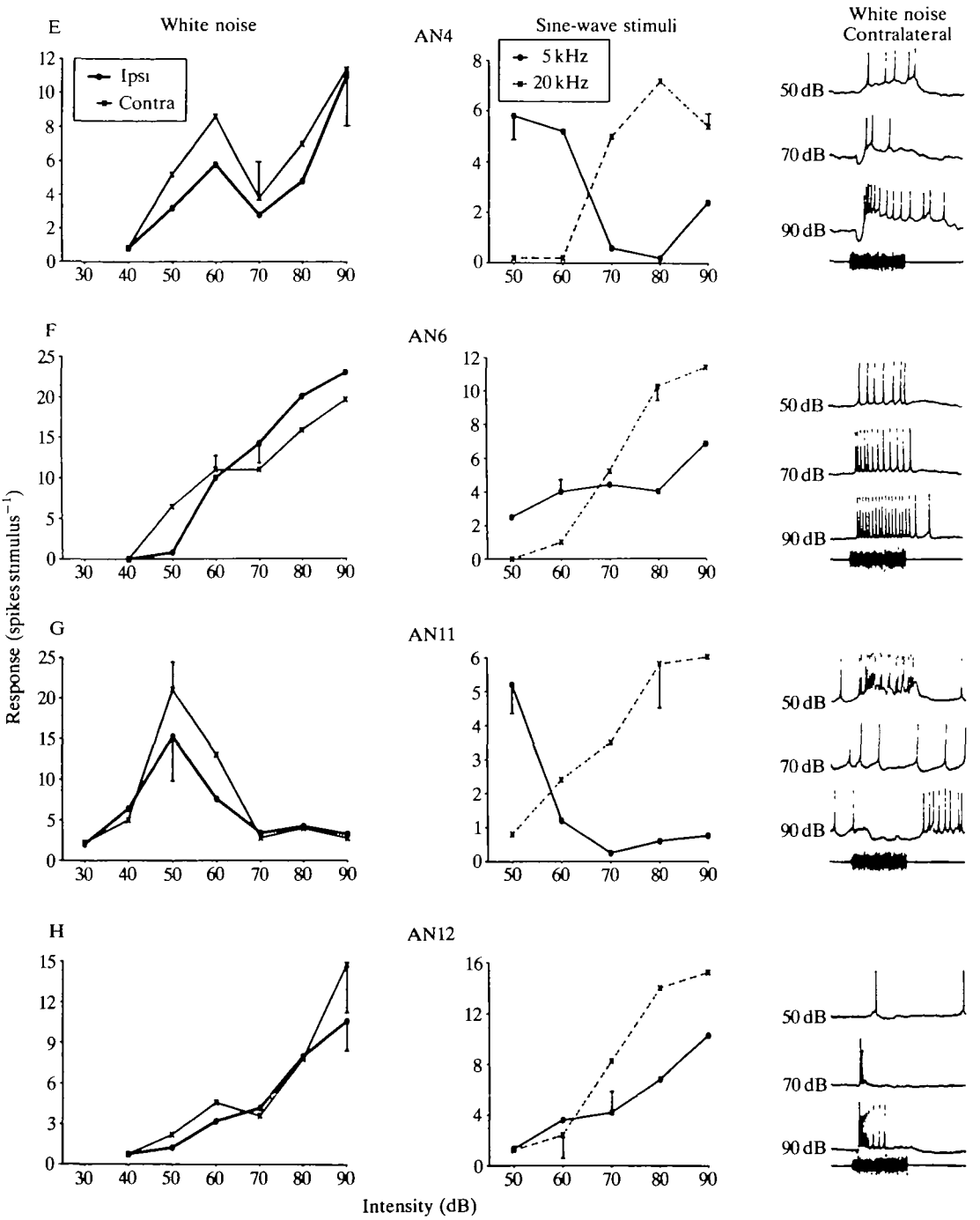

Fig. 7 (continued)

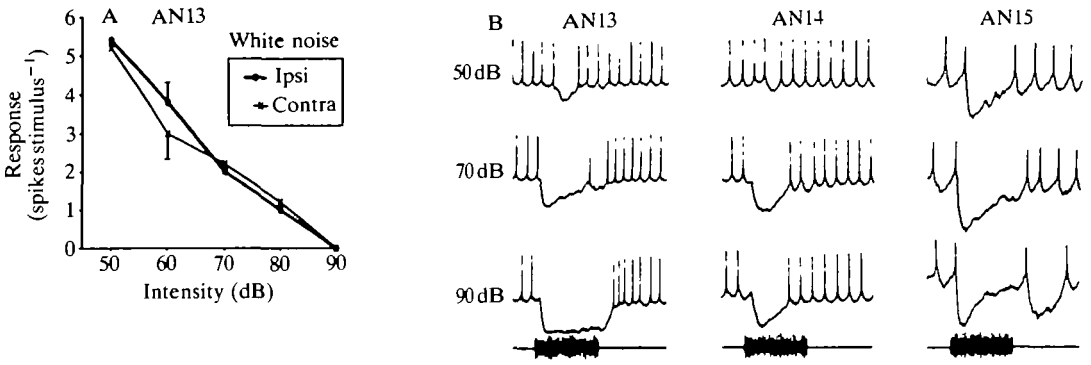

Fig. 8. Responses of ascending neurones that are inhibited by all acoustic stimuli. (A) Intensity–response function of AN13. (B) Sample recordings (100 ms WN stimuli, delivered contralaterally) of AN13, AN14 and AN15. The recording of AN13 in B is from the same neurone as the intensity curves in A.

get a least part of their input directly from ipsilateral receptors. In addition to low-frequency excitation, most local interneurones receive an inhibitory input at higher intensities (some of them perhaps from TN1, see Sokoliuk *et al.* 1989). BSN1 seems to be the only one of these thoracic interneurones that receives contralateral inhibition (Fig. 5A,B). Furthermore, BSN1 is obviously excited not only by the most sensitive low-frequency receptors but also by high-frequency receptors. The same might be true for SN7, while SN5 is exclusively excited by high-frequency input. The spiking patterns of local and bisegmental neurones in response to WN stimuli range from tonic respones (SN1), phasic-tonic responses (most BSN1), phasic responses (though usually only at higher intensities) to predominant inhibition at all intensities (SN6).

Ascending neurones

Ascending neurones, too, exhibit the whole range of responses from tonic (AN6) to distinct phasic activity (AN12). Obviously, most ascending neurones are excited by sensitive low-frequency receptors – probably *via* the local interneurones mentioned above. The most sensitive neurones show a response peak approximately 10–20 dB above threshold. WN stimuli above 50–60 dB SPL evoke complex patterns of excitation and inhibition, resulting in reduced spiking activity (Fig. 7). This inhibition usually begins at intensities at which the high-frequency receptors are not yet excited by the WN stimuli. Therefore, this inhibition must be caused by low-frequency receptors, probably mediated by interneurones like TN1 (see Römer *et al.* 1981; Sokoliuk *et al.* 1989). In some neurones (AN2, AN3, AN4) a further excitation can be seen at intensities above 70 dB SPL. The intensity–response functions at 5 and 20 kHz in Fig. 7B,D,E suggest that this response can be interpreted as high-frequency excitation which overcomes the intensity-dependent low-frequency inhibition.

Lateralization and binaural summation

BSN1, AN1 and AN2 are the neurones whose responses are most influenced by the direction of the sound (for AN1 see Wolf, 1986; for the locust see also Rheinlander and Mörchen, 1979). The activity of these neurones is rather effectively suppressed by contralateral (BSN1) or ipsilateral (AN1, AN2) stimuli. This would be in accordance with the BSN1 neurones being presynaptic to AN1 and AN2. In simultaneous recordings in *L. migratoria*, BSN1 has been demonstrated to excite AN1 (Marquart, 1985*b*). The response difference for ipsilateral and contralateral stimuli becomes smaller at middle intensities owing to the intensity-dependent inhibition mentioned above. Other auditory interneurones receive nearly equal excitation from both ears, as is most obvious for AN6 and AN12. A neuronal summation of auditory inputs from both ears has also been postulated from behavioural results (D. von Helversen, 1984; D. von Helversen and O. von Helversen, 1990).

AN3 and AN4 show a conspicuous IPSP which precedes the excitation at most intensities (see above); similar IPSPs can be seen also in AN12 and in some AN6 cells. What could be the function of an initial IPSP? In the first place, its effect is to delay the first action potential, which might be important in coding directional information (Römer *et al.* 1981; Rheinlaender, 1984). In AN3 and AN4 the latency is usually shorter for a contralateral stimulus than for the same stimulus delivered ipsilaterally. Another effect might be to trigger the first action potential more precisely; AN12, for example, shows a very constant latency (with less than 0.5 ms standard deviation in most cases).

Comparison of auditory neurones of C. biguttulus *with those of other grasshoppers*

There is extensive congruence of the morphological characteristics of thoracic auditory neurones in *L. migratoria* and in *C. biguttulus*. Only 5 out of 22 neurones described for the locust (Römer and Marquart, 1984; Marquart, 1985*a*) have not yet been found in *C. biguttulus* (SN3, TN2, AN5, AN8, AN9). 10 'new' neurones have been identified in *C. biguttulus* (SN6, SN7, AN13-AN20, Stumpner, 1988); 6 of these could be stained in the locust (SN6, SN7, AN15, AN16, AN17, AN20). The morphological similarity extends to other acridid species, especially *Omocestus viridulus* (Hedwig, 1985, 1986; see also Römer *et al.* 1988). The responses of the neurones to the stimuli used here are also very similar in both species. Interspecific differences exist, of course, in the characteristic frequencies (higher in the respective neurones of *C. biguttulus*) and in the sensitivity, with the locust being about 10–15 dB more sensitive to white-noise stimuli at the same temperature. At least for one auditory interneurone (TN1), the interspecific similarity also extends to the putative transmitter; in both *L. migratoria* and *C. biguttulus* the TN1 neurone shows GABA-like immunoreactivity (Sokoliuk *et al.* 1989; see also Robertson and Wisniowski, 1988).

Uncertainty about homology exists only with the sister cells of the AN11/AN12

group and with SN5, which, in *C. biguttulus*, possesses a contralaterally descending branch like SN4; this branch, however, is missing in the locust (Marquart, 1985*a*).

The information flow in the auditory pathway of *C. biguttulus* seems to be the same as in the locust (see Römer and Marquart, 1984; Römer *et al.* 1988). Local interneurones probably receive direct input from auditory receptors (see above). All local neurones (except the DUM-type SN7) are assumed to have their main input region (smooth, dense dendrites) on the side ipsilateral to the soma, and their main output region (less-dense, beaded dendrites) on the side contralateral to the soma. In ascending neurones, too, the input regions are largely located on the soma side. The lower thresholds, however, are found on the axon side. As far as is known from *L. migratoria*, ascending neurones do not get direct input from auditory receptors (except AN10, see Pearson *et al.* 1985). Thus, local and bisegmental neurones seem to be interposed between receptors and ascending neurones. Of course, other interactions are to be expected in addition to this basic connectivity scheme.

In conclusion, there is little doubt that corresponding neurones of *C. biguttulus* and *L. migratoria* are homologous (criteria *der Lage* and *der spezifischen Qualität*, Remane, 1952; see also Rowell, 1989). However, stridulation has obviously been developed independently in the subfamilies Gomphocerinae (*Chorthippus*) and Oedipodinae (*Locusta*), since the stridulatory pegs are on the hind femur in *Chorthippus* but on the forewing in *Locusta*. Therefore, we conclude that the ancestral common set of auditory interneurones has been modified only slightly during the radiation of these grasshoppers and can be interpreted as a preadaptation for the evolution of acoustic communication. Consequently, we expect that in different species the mechanisms for recognizing conspecific songs (innate releasing mechanisms) will reflect in many details the properties of these common local and ascending neurones of the auditory pathway.

We want to thank Alfred Schmiedl and Erwin Schreier for many years of excellent technical assistance; Otto von Helversen and two anonymous referees gave valuable comments on the manuscript.

References

ADAM, L. J. (1969). Neurophysiologie des Hörens und Bioakustik einer Feldheuschrecke (*Locusta migratoria*). *Z. vergl. Physiol.* **63**, 227–289.

BAUER, M. AND VON HELVERSEN, O. (1987). Separate localization of sound recognizing and sound producing neural mechanisms in a grasshopper. *J. comp. Physiol.* A **161**, 95–101.

BOYAN, G. S. (1984). What is an 'auditory' neurone? *Naturwissenschaften* **71**, 482–484.

BOYAN, G. S. (1986). Modulation of auditory responsiveness in the locust. *J. comp. Physiol.* A **158**, 813–825.

BOYAN, G. S. AND ALTMAN, J. S. (1985). The suboesophageal ganglion: a 'missing link' in the auditory pathway of the locust. *J. comp. Physiol.* A **156**, 413–428.

EICHENDORF, A. AND KALMRING, K. (1980). Projections of auditory ventral cord neurons in the supraoesophageal ganglion of *Locusta migratoria*. *Zoomorphologie* **94**, 133–149.

ELSNER, N. (1974). Neuroethology of sound production in gomphocerine grasshoppers. I. Song patterns and stridulatory movements. *J. comp. Physiol.* **88**, 67–102.

ELSNER, N. (1975). Neuroethology of sound production in gomphocerine grasshoppers. II. Neuromuscular activity underlying stridulation. *J. comp. Physiol.* **97**, 291–322.

ELSNER, N. AND POPOV, A. V. (1978). Neuroethology of acoustic communication. *Adv. Insect Physiol.* **13**, 229–355.

EVANS, P. D. AND O'SHEA, M. (1977). The identification of an octopaminergic neurone which modulates neuromuscular transmission in the locust. *Nature* **270**, 257–259.

HALEX, H., KAISER, W. AND KALMRING, K. (1988). Projection areas and branching patterns of the tympanal receptor cells in migratory locusts, *Locusta migratoria* and *Schistocerca gregaria*. *Cell Tissue Res.* **253**, 517–528.

HEDWIG, B. (1985). Untersuchungen zur Kontrolle des Feldheuschreckengesanges durch intersegmentale Interneurone. Thesis, University of Göttingen.

HEDWIG, B. (1986). On the role in stridulation of plurisegmental interneurones of the acridid grasshopper *Omocestus viridulus* L. II. Anatomy and physiology of ascending and T-shaped interneurones. *J. comp. Physiol.* A **158**, 429–444.

KALMRING, K. (1975*a*). The afferent auditory pathway in the ventral cord of *Locusta migratoria* (Acrididae). I. Synaptic connectivity and information processing among the auditory neurons of the ventral cord. *J. comp. Physiol.* **104**, 103–141.

KALMRING, K. (1975*b*). The afferent auditory pathway in the ventral cord of *Locusta migratoria* (Acrididae). II. Responses of the auditory ventral cord neurons to natural sounds. *J. comp. Physiol.* **104**, 143–159.

MARQUART, V. (1985*a*). Auditorische Interneurone im thorakalen Nervensystem von Heuschrecken. Morphologie, Physiologie und synaptische Verbindungen. Thesis, Universität Bochum.

MARQUART, V. (1985*b*). Local interneurons mediating excitation and inhibition onto ascending neurons in the auditory pathway of grasshoppers. *Naturwissenschaften* **72**, 42–44.

PEARSON, K. G., BOYAN, G. S., BASTIANI, M. AND GOODMAN, C. S. (1985). Heterogeneous properties of homologous interneurons in the ventral nerve cord of locusts. *J. comp. Neurol.* **233**, 133–145.

PEARSON, K. G. AND ROBERTSON, R. M. (1981). Interneurones coactivating hindleg flexor and extensor motoneurones in the locust. *J. comp. Physiol.* **144**, 391–400.

PETERS, B. H., RÖMER, H. AND MARQUART, V. (1986). Spatial segregation of synaptic inputs and outputs in a locust auditory interneurone. *J. comp. Neurol.* **254**, 34–50.

REHBEIN, H. (1976). Auditory neurons in the ventral cord of the locust: morphological and functional properties. *J. comp. Physiol.* **110**, 233–250.

REMANE, A. (1952). Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik. Theoretische Morphologie und Systematik. Leipzig: Akadem. Verlagsges.

RHEINLAENDER, J. (1984). Das akustische Orientierungsverhalten von Heuschrecken, Grillen und Fröschen: Eine vergleichende neuro- und verhaltensphysiologische Untersuchung. Habilitationsschrift, Universität Bochum.

RHEINLAENDER, J. AND MÖRCHEN, A. (1979). 'Time–intensity trading' in locust auditory interneurones. *Nature* **281**, 672–674.

ROBERT, D. (1989). The auditory behaviour of flying locusts. *J. exp. Biol.* **147**, 279–301.

ROBERTSON, R. M. AND WISNIOWSKI, L. (1988). GABA-like immunoreactivity of identified interneurones in the flight system of the locust, *Locusta migratoria*. *Cell Tissue Res.* **254**, 331–340.

RÖMER, H. (1985). Anatomical representation of frequency and intensity in the auditory system of Orthoptera. In *Acoustic and Vibrational Communication in Insects* (ed. K. Kalmring and N. Elsner), pp. 25–32. Berlin, Hamburg: Parey.

RÖMER, H. AND MARQUART, V. (1984). Morphology and physiology of auditory interneurons in the metathoracic ganglion of the locust. *J. comp. Physiol.* A **155**, 249–262.

RÖMER, H., MARQUART, V. AND HARDT, M. (1988). The organization of a sensory neuropile in the auditory pathway of two groups of Orthoptera. *J. comp. Neurol.* **275**, 201–215.

RÖMER, R. AND RHEINLAENDER, J. (1983). Electrical stimulation of the tympanal nerve as a tool

for analyzing the response of auditory interneurones in the locust. *J. comp. Physiol.* **152**, 289–296.

RÖMER, H., RHEINLAENDER, J. AND DRONSE, R. (1981). Intracellular studies on auditory processing in the metathoracic ganglion of the locust. *J. comp. Physiol.* **144**, 305–312.

RONACHER, B. AND STUMPNER, A. (1988). Filtering of behavioural relevant temporal parameters of a grasshopper's song by an auditory interneurone. *J. comp. Physiol.* A **163**, 517–523.

RONACHER, B., VON HELVERSEN, D. AND VON HELVERSEN, O. (1986). Routes and stations in the processing of auditory directional information in the CNS of a grasshopper, as revealed by surgical experiments. *J. comp. Physiol.* A **158**, 363–374.

ROWELL, C. H. F. (1989). The taxonomy of invertebrate neurons, a plea for a new field. *Trends Neurosci.* **12**, 169–174.

SOKOLIUK, T., STUMPNER, A. AND RONACHER, B. (1989). GABA-like immunoreactivity suggests an inhibitory function of the thoracic low-frequency neuron (TN1) in acridid grasshoppers. *Naturwissenschaften* **76**, 223–225.

STUMPNER, A. (1988). Auditorische thorakale Interneurone von *Chorthippus biguttulus* L.: Morphologische und physiologische Charakterisierung und Darstellung ihrer Filtereigenschaften für verhaltensrelevante Lautattrappen. Thesis, Universität Erlangen.

STUMPNER, A. (1989). Physiological variability of auditory neurones in a grasshopper. Comparison of twin cells and mirror-image cells. *Naturwissenschaften* **76**, 427–429.

STUMPNER, A., RONACHER, B. AND VON HELVERSEN, O. (1991). Auditory interneurones in the metathoracic ganglion of the grasshopper *Chorthippus biguttulus*. II. Processing of the temporal patterns of the song of the male. *J. exp. Biol.* **158**, 411–430.

VON HELVERSEN, D. (1972). Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* L. *J. comp. Physiol.* **81**, 381–422.

VON HELVERSEN, D. (1984). Parallel processing in auditory pattern recognition and directional analysis by the grasshoppper *Chorthippus biguttulus*. *J. comp. Physiol.* A **154**, 837–846.

VON HELVERSEN, D. AND VON HELVERSEN, O. (1975*a*). Verhaltensgenetische Untersuchungen am akustischen Kommunikationssystem der Feldheuschrecken. I. Der Gesang von Artbastarden zwischen *Chorthippus biguttulus* und *Ch. mollis*. *J. comp. Physiol.* **104**, 273–299.

VON HELVERSEN, D. AND VON HELVERSEN, O. (1975*b*). Verhaltensgenetische Untersuchungen am akustischen Kommunikationssystem der Feldheuschrecken. II. Das Lautschema der Artbastarde. *J. comp. Physiol.* **104**, 301–323.

VON HELVERSEN, D. AND VON HELVERSEN, O. (1983). Species recognition and acoustic localization in acridid grasshoppers: a behavioral approach. In *Neuroethology and Behavioral Physiology* (ed. F. Huber and H. Markl), pp. 95–107. Berlin: Springer.

VON HELVERSEN, D. AND VON HELVERSEN, O. (1990). Pattern recognition and directional analysis: routes and stations of information flow in the CNS of a grasshopper. In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. V. Popov), pp. 209–216. Basel: Birkhäuser.

VON HELVERSEN, O. (1979). Angeborenes Erkennen akustischer Schlüsselreize. *Verh. dt. Zool. Ges.* **72**, 42–59.

WOLF, H. (1986). Response patterns of two auditory interneurons in a freely moving grasshopper (*Chorthippus biguttulus* L.). I. Response properties in the intact animal. *J. comp. Physiol.* A **158**, 689–696.

ZARNACK, W. AND MÖHL, B. (1977). A data acquisition processor with data reduction for electrophysiological experiments. *Fortschr. Zool.* **24**, 321–326.