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ORIGINAL PAPER

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Neuronal basis of phonotactic behaviour in *Tettigonia viridissima*: processing of behaviourally relevant signals by auditory afferents and thoracic interneurons

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Abstract Information transmission in the auditory pathway of *Tettigonia viridissima* was investigated using song models and artificial stimuli. Receptor cells respond tonically to song models and copy the syllable pattern within a wide intensity range. The omega-neuron responds tonically to soma-ipsilateral stimuli. Contralateral stimuli elicit IPSPs both within dendritic (ipsilateral) and axonal (contralateral) branches, thereby emphasizing directionality. Both AN1 and AN2 respond with tonic, non-adapting responses, precisely copying the syllable pattern of the song. While AN1 is excited by sonic frequencies and inhibited by ultrasonic frequencies, AN2 responds predominantly to ultrasound. The TN1 only responds to the ultrasonic components of the song, with phasic responses, which adapt quickly. In the adapted state, it responds selectively to the time pattern of the conspecific song, but not to the song patterns of two syntopic *Tettigonia* species. TN2, which has not been described up until now, is tuned to ultrasonic frequencies. Its responses to song models vanish after a few syllables, because of quick adaptation. The morphology is unusual with the axon running contralateral to the input site. The behavioural relevance of auditory interneurons is discussed and compared with the auditory system of crickets.

Key words Auditory interneurons · Bushcricket · Song recognition · Phonotaxis · Neuronal information processing

Abbreviations *AN* ascending neuron · *cF* characteristic frequency · *EPSP* excitatory postsynaptic potential · *IPSP* inhibitory postsynaptic potential · *ON* omega neuron · *PSTH* peri stimulus time histogramm · *TN* T-shaped neuron

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Introduction

The auditory system of orthopterans is one of the best studied invertebrate sensory systems. The processing of sound signals and of communication signals especially has been intensely studied in grasshoppers and crickets (reviews: Boyan 1984; Schildberger 1988; Lewis 1992). In both groups it was possible to relate behavioural data on the recognition and localization of species-specific sound signals to the responses of identified neurons, e.g. grasshoppers, Römer and Seikowski (1985), Ronacher and Stumpner (1988), and Stumpner et al. (1991); crickets, Schildberger (1984), Nolen and Hoy (1984), Schildberger and Hörner (1988), Atkins et al. (1992); review: Huber et al (1989).

In the third large group of orthopterans, the Tettigoniids, some data about the connectivity and basic physiology of the auditory pathway are available (Römer 1985, 1987; Hardt 1988; Römer et al. 1988), but only some aspects of bushcricket acoustic behaviour have been investigated and discussed on the level of identified auditory neurons: song localization (Rheinlaender et al. 1986), male spacing behaviour (Römer and Bailey 1986; Römer 1987) and predator avoidance (Libersat and Hoy 1991). However, the neuronal processing of communication signals and the possible role of identified neurons in phonotactic behaviour has not been investigated in detail. Therefore, the neuronal structures and mechanisms of acoustic communication within the two main groups of Ensifera, Grylloidea and Tettigonioidea could not be compared satisfactorily. This comparison might yield further insights into the current debate on the evolution of stridulation and hearing within Ensifera. While current opinion tends towards a common origin of hearing and stridulation for Tettigonioidea and Grylloidea (Otte 1992, Michelsen 1992), Gwynne (1995) expressed a different view: he proposed independent evolution of Gryllid and

Tettigoniid stridulation and ears. His main point was that recent Raphidophorids, the basal family of the Tettigonioid clade, are both mute and deaf, which he assumes to be an ancestral trait.

In the bushcricket *Tettigonia viridissima,* males produce continuous disyllabic songs, which have to be discriminated by the females from the monosyllabic songs of the sympatric *T. cantans* and *T. caudata* [song patterns see Heller (1988)]. Phonotactic behaviour, and the important acoustic cues for song recognition have been studied by Jatho et al. (1994) and Jatho (1995). This paper describes the physiological properties of auditory receptor cells and thoracic auditory interneurons of the bushcricket *Tettigonia viridissima* and their responses to models of the conspecific song. It focusses on the processing of behaviourally relevant song features, including temporal, spectral and directional cues. The main purpose is to give a basis (1) for relating the responses of the thoracic auditory interneurons to acoustic behaviour, and (2) for comparison of the acoustic communication of Tettigoniids at the level of auditory interneurons with that of other orthopteran groups, especially Gryllids.

Materials and methods

Animals and preparation

Males and females of *Tettigonia viridissima* were collected as adults and nymphs from wild populations near Marburg and Erlangen. The animals were anesthesized by brief exposure to CO_2 prior to the removal of the wings and fixed on a metal holder with a wax-resin mixture. The forelegs were attached to a wire holder in a natural position. For intracellular recordings, the prothoracic ganglion was exposed and stabilized with a NiCr spoon, which also served as indifferent electrode. For the extracellular recordings, the cervical connectives were exposed and the gut and mouthparts were removed. A silver wire, inserted into the abdomen served as indifferent electrode. Exposed tissue was covered with saline (Fielden 1960).

Recording and staining

The experiments took place in anechoic Faraday cages at room temperature (20–25 °C). Intracellular or quasi-intracellular recordings were made with thick-walled borosilicate glass microelectrodes filled with a 3–5% solution of Lucifer Yellow in 0.5 $mol \cdot l^{-1}$ LiCl (resistance 40–100 MΩ). The recorded signals were amplified using a List LM-1 electrode amplifier and stored on magnetic tape (Racal 4DS or Sony PC208A). After an experiment, the prothoracic ganglion was fixed in 4% paraformaldehyde, dehydrated and cleared in methylsalycilate. Stained cells were photographed and drawn as whole mounts using a fluorescence microscope. The three dimensional structure was documented by a measuring device.

Extracellular recordings from the cervical connectives were obtained using either silver hook electrodes (100 μm diameter) or borosilicate glass electrodes filled with 3 $mol \cdot l^{-1}$ KCl (resistance 10–30 MΩ). The recorded signals were amplified using a custom made amplifier, band pass filtered (100–3000 Hz) and stored on magnetic tape (Racal 4 DS or Sharp RT 115). In all cases, a trigger signal was recorded on a separate track for off line synchronization of stimulus and response.

Acoustic stimulation

The stimuli were delivered via one or two loudspeakers (Technics 10TH400C) located 48 or 66 cm from the preparation, perpendicular to the body axis of the animal. The signals were generated using various computer and AD-converter systems. Signals had either an 8- or 12-bit resolution and sampling rate of 200 kHz (song models, white noise, sine waves 4–30 kHz) or 595 kHz (sine waves 4–60 kHz). The signals were amplified and their amplitude attenuated manually or computer aided. In some experiments sine waves of 40 and 60 kHz were generated with a Burchard ASII acoustic stimulator.

The intensities of the signals were calibrated with a condenser microphone (Bruel and Kjaer 4135 or 4138) and a B&K measuring amplifier (2209) using its "peak hold" function. To ensure comparability, all signal intensities are given in dB peak SPL (re. 2×10^{-5} Pa), which is for sine waves 3 dB above the respective RMS value. Sound measurements were obtained on the preparation site, with no animal present.

For further analyses the sound signals were recorded on magnetic tape (Racal 4DS) and later replayed, digitized (sampling frequency 200 or 400 kHz) and analyzed by custom made computer programs. The computer synthesized sine waves had a signal-to-noise ratio of at least 40 dB. At the recording site (especially in the intracellular setup) at frequencies below 10 kHz, slight echo influences were unavoidable. They did not alter the intensity or the envelope of the signals by more than 2 dB.

For characterization of the tested neurones white noise (4–50 kHz) of 20 ms or 100 ms duration (+ 1 ms rise/fall time respectively) and pure tones (4, 7, 12, 20, 30, 40, 60 kHz) of 20 ms duration (+ 1 ms rise/fall time) were used.

The song models were generated by inverse Fourier transformation of an averaged spectrum of the songs of 15 *T. viridissima* males. The resulting impulse was chained to an almost natural series and modulated with a typical envelope. The resulting song parts had a duration of about 400 ms. At the time of the experiments, they were repeated without additional gaps to mimic the continuous song of *T. viridissima*. The duration of the song models used ranged from 7 to 20 s. In behavioural experiments, the song models with both, 8- bit and 12-bit resolution, evoked phonotaxis in more than 80% of the tested females.

To test the influence of the various frequency components of the song, a model with the complete spectrum (model CS) was split by digital filtering into a sonic song model (low-pass filtered at 16 kHz, model LP) and an ultrasonic one (high-pass filtered at 16 kHz, model HP). The digital filtering changed neither the gross impulse structure nor the envelope of the syllables (Fig. 1). The filtered song models were delivered with the same intensity as the respective frequency components occur in the unfiltered song. Due to differences between the various loudspeakers the absolute intensities of the song components differed by ± 2 dB. The actual values are given in the figures. Unless otherwise stated, the text and the figures refer to the song model with the complete spectral components.

Actual sound pressure level at the acoustic spiracles ipsi- and contralateral to the loudspeaker position was measured with a probe microphone (B&K 4182) and the measuring equipment described above.

Data evaluation

For analysis, the neuronal responses were replayed and digitized (10 kHz sampling frequency, 8- or 12-bit resolution). Response magnitudes were calculated as spikes per s or spikes per double syllable for song models or as spikes per stimulus for other stimuli. Additionally, the responses to song models are presented as peristimulus-time-histograms with a bin width of 2 ms. All analyses were carried out with individually designed computer programs for DOS computers.

Results

Receptor cells

The auditory receptor cells of *Tettigonia,* show a distinct frequency tuning with highest sensitivity for a charac-

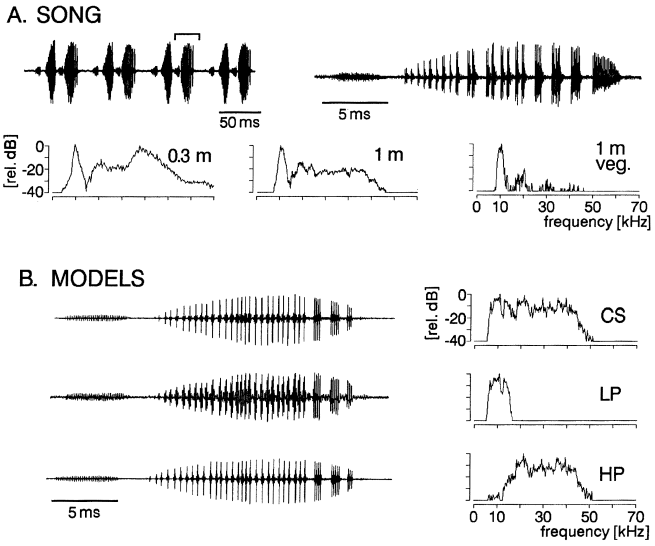

Fig. 1A, B Oscillograms and power spectra of natural songs of *T. viridissima* and the song models used: **A** *upper trace*: oscillogram of a song of *T. viridissima*, with one syllable shown on a larger time-scale (*right*). The song comprises double syllables, which are chained to continuous songs, often lasting minutes without interruption. The double syllables are produced during two opening and closing movements of the elytra. *Lower trace*: three examples of power spectra calculated from songs recorded in the biotope. The recording distances are indicated. The left and middle spectra were calculated from songs recorded in open air, the one on the right from a song recorded through vegetation; **B** time amplitude pattern of one syllable (*left*) and the corresponding power spectrum (*right*) of the song models used (top *CS*, middle *LP*, bottom *HP*). The models were recorded at the position of the preparation of the neurophysiological experiments

teristic frequency (ranging from about 3 to above 50 kHz) and a threshold roll off in the order of 20 dB per octave on both sides of this frequency (Kalmring et al. 1978; Römer 1983). As the spectral sensitivity of the tympanal organ includes the complete frequency content of the songs (Fig. 1), interest was focussed on the temporal response characteristics. Independent from their spectral tuning, all receptor cells of *T. viridissima* showed tonic responses, i.e. they followed the time pattern of the song models tested. No deterioration of the response magnitude during the stimulus was observed, except during the first two or three syllables of a song, which elicited a stronger response than those following. After a few syllables, a stable response magnitude was reached, which stayed constant for the remainder of the song. This emphasis of neuronal response at the very beginning of a song was also found in all auditory interneurons.

Receptors with characteristic frequencies (cf, the frequency of the lowest threshold for pure tone stimulation) between 12 and 30 kHz were the most sensitive ones for model song stimulation, with thresholds of approximately 40 dB. Receptor cells with characteristic frequencies outside the spectral range of the songs were less sensitive for song models (thresholds above 80 dB for cells with cF of 4 kHz). At low intensities, only the song parts with high amplitudes (i.e. the closing hemisyllables) evoked responses and with increasing intensity the receptors responded additionally to song parts of lower amplitude, especially in the opening hemisyllables (Fig. 2A). The dynamic range of the intensity-response function for the model songs was much greater than 24 dB (Fig. 2B), compared to approximately 15 dB as described for stimulation with pure tones (Kalmring et al. 1978, 1993).

The shift of the receptor's response threshold by contralateral stimulation depended on the characteristic frequency of the receptor. For cells tuned to 12 kHz the threshold increased by approximately 10 dB, whereas the threshold difference increased to about 18 dB for a cF of 20 kHz (Fig. 2B) and above 25 dB for receptors with cFs above 25 kHz. This reduction matched the intensity difference between ipsilateral and contralateral stimulation measured with a probe microphone in front of the acoustic spiracle at the characteristic frequency of the respective receptor, which was 9–11 dB at 12 kHz and 18–20 dB at 20 kHz. Due to the wide dynamic range of the receptor's responses, directionality was maintained over a wide range of song intensity.

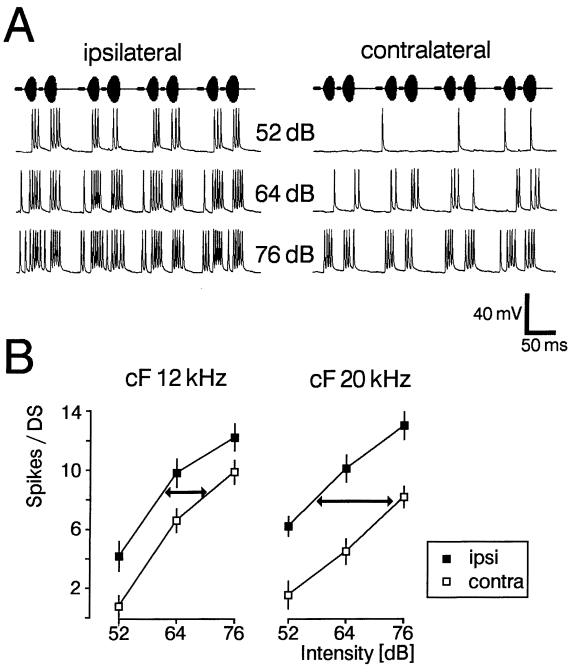

Fig. 2 **A** Responses of an auditory receptor cell of *T. viridissima* to song models during ipsi- (*left*) and contralateral (*right*) stimulation. The receptor had a characteristic frequency (i.e. the frequency of the lowest threshold) of 20 kHz. **B** Response-intensity functions for two receptor cells for stimulation with song models. The response magnitudes are given in spike/double syllable (DS), mean ± standard deviation (n = 63 per data point). The right diagram corresponds to the responses shown in **A**. The dynamic range covers the complete intensity range tested. For contralateral stimulation the functions are shifted by about 10 dB (cF 12 kHz) or about 18 dB (cF 20 kHz)

Omega neuron

The ON is a segmental neuron connecting the auditory neuropiles of both hemiganglia (Römer et al. 1988). In 2 out of 18 cases a thin ascending axon was found on the soma-contralateral side, coursing into the cervical connectives. With electrodes positioned in the soma-ipsilateral, dendritic arborizations, the ON was spontaneously active, while in recordings from the soma-contralateral side no or only weak spontaneous activity was recorded.

Ipsilateral stimulation with white noise or pure tones evoked tonic responses at all frequencies tested (4–60 kHz, Fig. 3A). Only in some preparations was a frequency-dependent inhibition at 4 kHz at low intensities visible (data not shown). With stimuli of longer duration (100 ms) the EPSPs diminished and the spike rate decreased.

Figure 3B shows the ON responses to contralateral stimulation. In dendritic recordings an inhibition was observed as IPSPs for low stimulus intensities or as a reduction of the EPSPs for louder signals. This inhibition lasted approximately as long as the stimulus. Spiking responses were observed only at the beginning of the stimuli.

In recordings from the soma-contralateral branches during contralateral stimulation, IPSPs were visible concomitant with the spikes. This inhibition had a long time-course, by far exceeding the end of the stimulus. Thus, it is likely that the IPSPs recorded in the axon had a different source than those seen in dendritic recordings.

The responses of the ON to soma-ipsilateral stimulation with song models were tonic, with thresholds of approximately 40 dB. Both the EPSPs and the spike pattern followed the syllable structure of the song (Fig. 4). No deterioration of the responses was noticeable during the duration of the song models, except for the first few syllables of a song that elicited a stronger response than those following (Fig. 4). The responses with song models delivered from soma-contralateral were evidently weaker than with the respective ipsilateral stimulation (Fig. 4), but varied in strength between the different preparations. While in one animal no spikes occurred during contralateral presentation of the song model, in others, a spike count of up to 55% of the response during ipsilateral stimulation was found (for the intensity range tested from 52 to 76 dB).

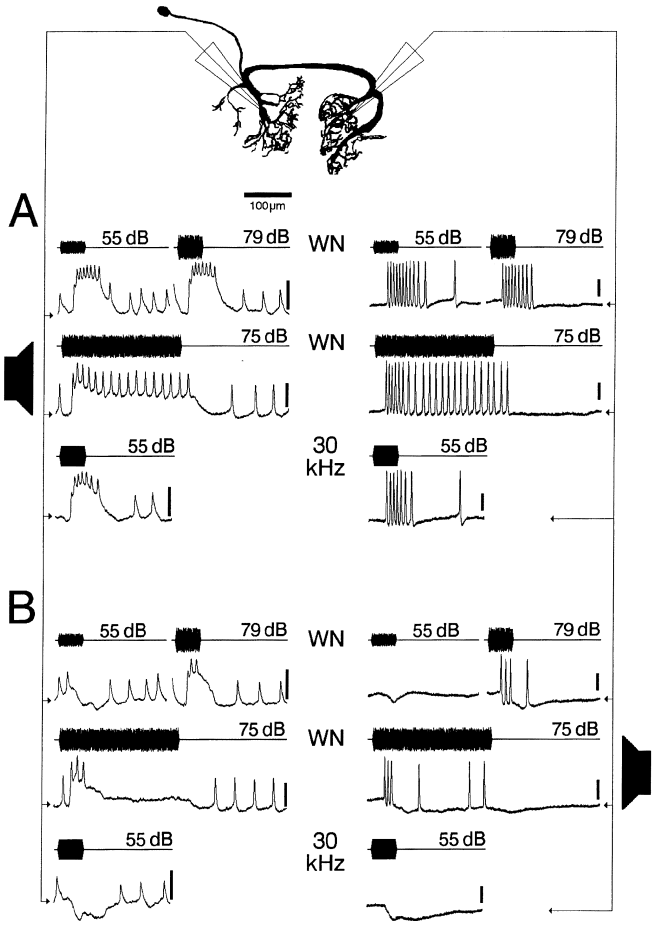

Fig. 3A, B Responses of the *T. viridissima* ON recorded from the soma-ipsilateral branches (*left half* of the figure) and the soma-contralateral branches (*right half* of the figure). ON morphology and recording positions are indicated at the top. Stimuli: white noise (*WN*), 20 or 100 ms duration, 30 kHz, 20 ms duration, stimulus intensities are indicated. Vertical scale bars: 10 mV: **A** stimulation from the soma-ipsilateral side. The soma-ipsilateral recordings reveal EPSPs and spikes, in the soma-contralateral recordings only spikes; **B** stimulation from the soma-contralateral side. IPSPs were visible both in dendritic and axonal recordings. The dendritic recordings in **A** and **B** are from two different preparations

Ascending neurons: AN1 and AN2

Intracellular recordings and stainings revealed the morphologies of two ascending neurons which both show tonic response characteristics. Their gross morphology is quite similar (Fig. 5A). Römer et al. (1988) demonstrated that these cells most probably receive monosynaptic inputs from soma-contralateral receptor cells. In the following, all stimuli were delivered from this side. Both AN1 and AN2 responded tonically to the song models without adaptation (Fig. 5B), at least for the stimulus periods tested here. Only the first few syllables of a song elicited a stronger response than those following, similar to the responses of the ON (data not shown, cf. Fig. 4).

In AN1, only the low-frequency components of the song evoked a strong response, while only weak responses were recorded during stimulation with the ultrasonic song component alone (Fig. 5C). In AN2, the responses to the different spectral components were strikingly different from those of AN1, in that AN2 responded strongly to the ultrasonic components alone, whereas the responses to the low-frequency components were weaker and the fidelity of copying the stimulus pattern decreased (Fig. 5C).

As both cells are rather small, penetration often led to increased spontaneous activity and the responses changed or vanished during recording. Using glass-micro-

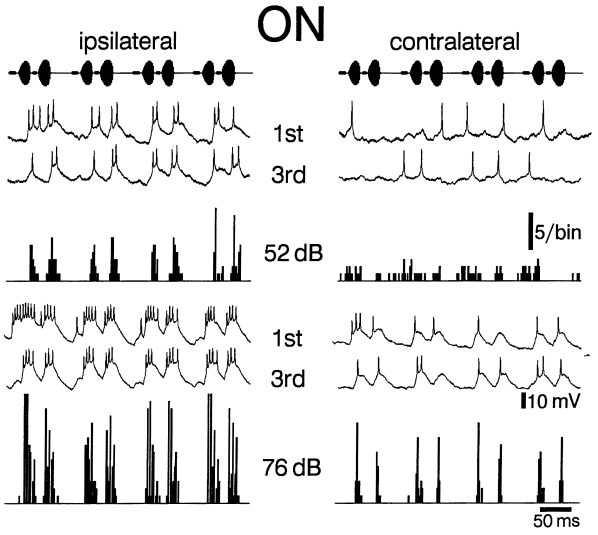

Fig. 4 Dendritic recordings from ON and PSTHs to stimulation with song models (CS), delivered from the soma-ipsilateral (*left*) and soma-contralateral side (*right*). Stimulus intensities are indicated. *Top traces* correspond to the first stimulus sweep, *bottom traces* to the third one, demonstrating the pronounced responses to the first syllables of a song. PSTH: 15 repetitions, bin width 2 ms

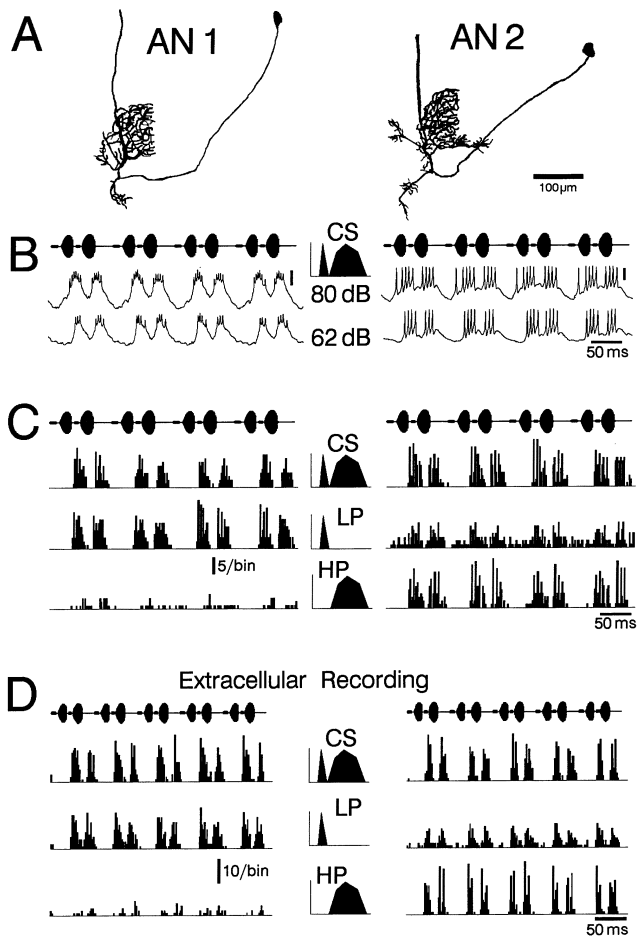

Fig. 5 **A** Reconstructions of AN1 and AN2 morphology in the prothoracic ganglion of *T. viridissima*. **B** Dendritic recordings of the responses of AN1 (*left*) and AN2 (*right*) during stimulation with the song model *CS* (complete spectrum). Stimulus intensities are indicated. Scale bars: 10 mV. **C** PSTHs of AN1 (*left*) and AN2 (*right*) responses to the intact song model (*CS*), the low frequency components (*LP*) and the ultrasonic components of the song (*HP*) (bin-width 2 ms, 15 repetitions; stimulus intensities: CS 62 dB, LP 57 dB, HP 60 dB.). **D** PSTHs calculated from extracellular recordings in the cervical connectives to song models comprising the same spectral components as the stimuli of **C**. The time pattern of the song models used here is faster than that used in **C**, matching a 2–3° higher temperature. (Bin-width 2 ms, 25 repetitions; stimulus intensities: CS 72 dB, LP 70 dB, HP 66 dB.) The stimulation was delivered from the axonal (i.e. soma-contralateral) side

electrodes for extracellular recordings from the cervical connectives, two cells with the same response characteristics to either AN1 or AN2 were encountered. Both showed tonic, non-adapting responses, with the one cell responding only to the low frequency components of the song, and the other with the best responses to the ultrasonic components (Fig. 5D). None of the morphologically identified cells ascending to the brain apart from AN1 and AN2, showed tonic responses (both T-shaped cells have phasic responses, see below). Thus, there is no doubt, that the extracellular recordings belong to the cells identified as AN1 and AN2. As the extracellular recordings were often stable for several minutes, it seemed appropriate to use these extracellular data for a quantitative analysis of AN1 and AN2 responses.

AN1 responded to pure tone stimulation from 7 to 20 kHz with thresholds below 40 dB and the strongest responses at 12 kHz. Between 20 and 30 kHz there was a steep rise in the threshold. In contrast to AN1, AN2 was broadly tuned from 7 to 60 kHz with the lowest threshold and the strongest response at 20 and 30 kHz (Fig. 6A). The intensity-response functions for white noise stimulation were different in both cells. For AN1, this function had optimum characteristics with a maximum 20–25 dB above threshold, while for AN2 the responses saturated 30–40 dB above threshold (Fig. 6B). The latencies for spiking responses in the cervical connectives (white noise, 70 dB) were in the range 22–28 ms in AN1, and 16–21 ms in AN2 (Fig. 6C).

Figure 6D quantifies the responses to the different spectral song components which correspond to the characteristics described above. In all AN1 the responses to the low-frequency components were strongest, while the presence of ultrasonic frequencies in the complete model reduced activity. The response to the ultrasonic components alone were significantly weaker than to the complete song model ($P < 0.05$, *u*-test). For AN2, the responses to the ultrasonic model were almost as strong as those to the complete model, while the responses to the low-pass filtered model were significantly weaker than to model CS ($P < 0.05$, *u*-test).

The response characteristics of AN1 can be explained by its inputs (Fig. 7). With increasing intensity of white noise, a short latency IPSP overrode the excitatory inputs thus reducing and delaying the spiking response of AN1. This inhibitory input is tuned to ultrasonic fre-

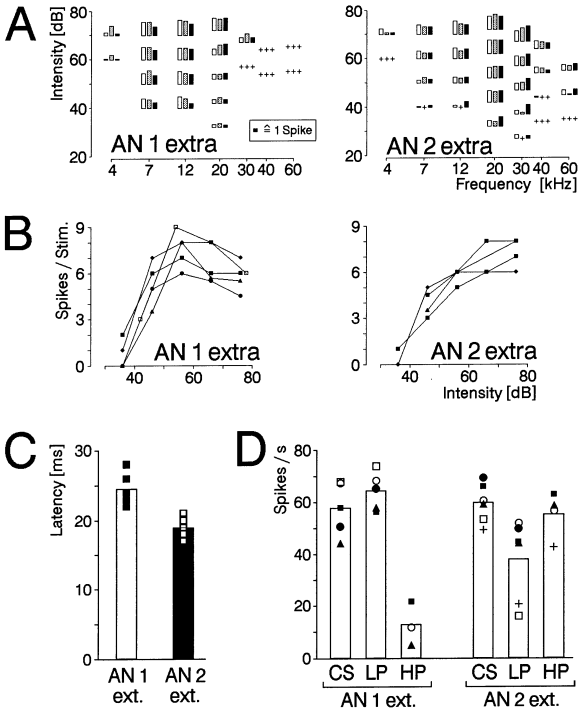

Fig. 6 A–D Quantitative analyses of AN1 and AN2 responses from extracellular recordings with stimulation from the axonal side: **A** typical "response maps" of three individual AN1 (*left*) and AN2 (*right*) for stimulation with pure tones. The height of a *bar* indicates the response magnitude (mean of three sweeps) at the given frequency and intensity, *crosses* indicate no spikes. The neurons were not tested below 30 dB from 7 to 20 kHz; **B** response intensity functions of single units of AN1 and AN2 for stimulation with white noise (20 ms duration). Each point represents a mean of two to four responses; **C** mean latency ($n = 5$) of AN1 and AN2 recorded in the neck connectives for stimulation with white noise 70 dB (100 ms duration), which is more than 30 dB above the threshold for both cells (symbols indicate the individual values); **D** mean responses of AN1 and AN2 during stimulation with the song models *CS* (complete spectrum), *LP* (sonic components only) and *HP* (ultrasonic components only). Each symbol represents the data of one cell. (Stimulus intensities: CS 72 dB, LP 70 dB, HP 66 dB.)

quencies. While stimulation with 7 and 12 kHz elicited pure excitation, frequencies above 30 kHz elicited pure inhibition. Intermediate stimuli resulted in mixed responses similar to those shown for white noise [similar data is shown by Römer (1987), Fig. 7]. In about half of the AN1 tested an IPSP occurred at 4 kHz (Fig. 7), while in the others, the same stimulus was purely excitatory.

T-shaped Neuron, TN1

The TN1 (Fig. 8A) has the largest axonal diameter of all auditory neurons identified in *T. viridissima* so far (up to 10 µm within the cervical connectives). Like AN1 and AN2, it most probably receives monosynaptic excitatory input from soma-contralateral receptor cells (Römer et al. 1988). Stimulation with pure tones elicited responses in a wide frequency range from 4 to 60 kHz,

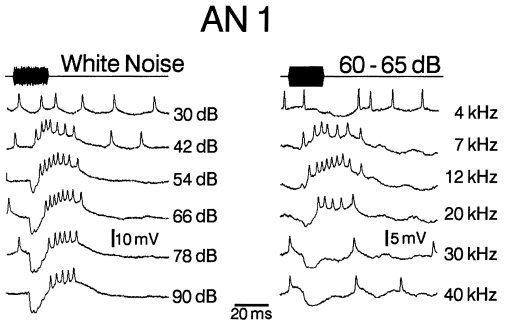

Fig. 7 Dendritic recordings of AN1 with white noise (*left*) and pure tones of 60–65 dB (*right*), delivered from the soma-contralateral side

with the lowest thresholds in the range from 12 to 30 kHz. The spiking responses of TN1 were phasic and adapting, while the graduated depolarization lasted as long as the stimulus (Fig. 8B). The responses were variable, according to the preceeding stimulus conditions. The responses often differed significantly between different presentations of the same stimulus (Fig. 8B).

The responses to soma contralateral stimulation with song models are shown in Fig. 8C. The phasic responses

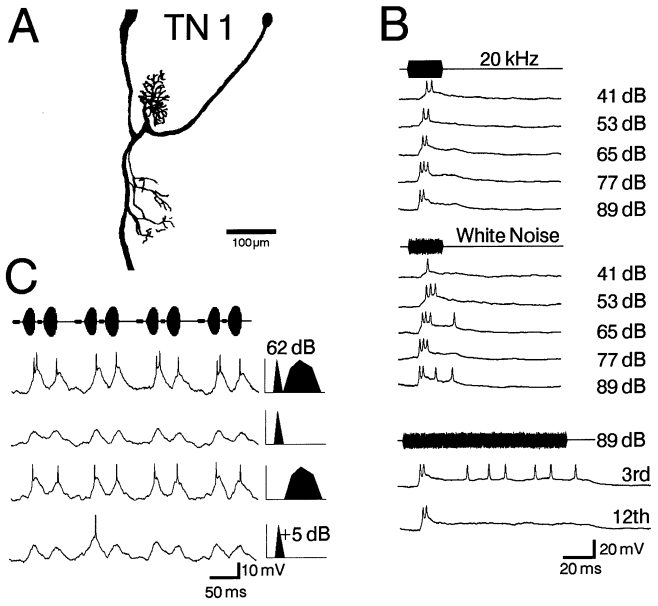

Fig. 8 A Reconstruction of TN1 morphology in the prothoracic ganglion of *T. viridissima*. **B** Dendritic recordings of TN1 for stimulation with pure tones (20 kHz, *top*), white noise, 20 ms (*middle*) and white noise, 100 ms (*bottom*). The stimuli were delivered from the soma-contralateral side; repetition rate 2 per s. The responses to white noise 100 ms corresponded to the 3rd and 12th stimulus presentation. The phasic-tonic responses in the early sweeps vanished in later ones. **C** Dendritic recordings of TN1 responses for stimulation with different song models presented from the soma-contralateral side. From top to bottom: model with the complete spectrum (CS) 62 dB; low frequency components alone (LP 57 dB, corresponding to CS 62 dB); ultrasonic components alone (HP 60 dB, corresponding to CS 62 dB); model LP 62 dB. LP 62 dB demonstrates that the missing responses to model LP were not due to low intensity

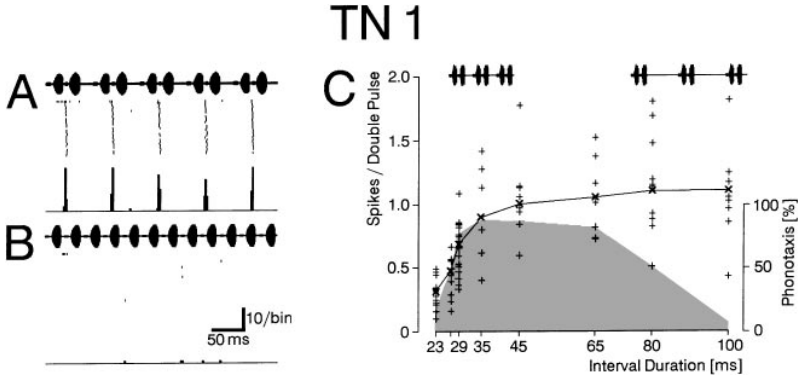

Fig. 9A–C Responses of TN1 recorded with hook electrodes from the neck connectives of *T. viridissima*: **A, B** PSTHs of responses to a disyllabic (conspecific) song model (**A**) and a monosyllabic one (similar to the song of *T. cantans*, **B**). Above each PSTH the occurence of spikes in 26 stimulus sweeps is given. The PSTHs include the 2nd to 26th sweep; bin width 2 ms. The TN1 responded to the disyllabic song model with an ongoing response of 1 spike/double syllable **A**, whereas the monosyllabic song evoked only a few spikes **B**. For the stimulus protocol of the hook recordings see text; **C** mean responses of 16 TN1 to song models with varied interval duration between the double syllables . The graph shows mean responses in spikes/double syllables, the values of the individual cells are indicated. The individual cells were not tested with every stimulus (stimulus duration 10 s, intensity 72 or 82 dB). The shaded area gives the phonotactic response of females to the respective stimuli. The females were tested in an arena, and the percentage of females that responded phonotactically is given (stimulus intensity ranging in the arena form 77–88 dB). For a detailed description of the experiments see Jatho (1995)

only occurred in models containing the ultrasonic components, while the sonic components alone did not evoke regular spiking.

The responses to song models varied between different TN1 and even within single preparations, affected strongly by the preceeding stimulus conditions. To guarantee a comparable stimulus situation in all preparations, hook-recordings from the neck connectives were used to monitor TN1 activity. Due to the large axonal diameter, this setup gave reliable single cell activity from TN1 [for identification criteria see Rheinlaender (1984) and Rheinlaender and Römer (1980)]. This approach allowed a stimulus protocol comprised of 5 min of silence followed by an "adapting stimulus" (a song model of 20 s duration, 75 dB) before the actual testing of a cell started. This procedure ensured comparable states of adaptation in the different preparations. A typical response to soma-contralateral stimulation obtained in such experiments is shown in Fig. 9A. The song model with the conspecific time pattern elicited a stable response of one spike per double syllable. When using a song model without the typical double syllable pattern (i.e. mimicking a song of the sympatric *Tettigonia cantans* or *T. caudata*) no regular responses occurred in TN1 (Fig. 9B). The preference for the conspecific time pattern in TN1 was found at intensities from at least 60–80 dB. Song models delivered from the soma-ipsilateral side (at 60–80 dB) evoked no or weak responses, evidently weaker (< 30%) than those of soma-contralateral stimulation (data not shown).

As the double syllable pattern is crucial for phonotaxis in *T. viridissima* (Jatho et al. 1994) the influence of the duration of the interval between the double syllables on TN1 responses was tested. In these experiments, the double syllable was left constant, but the intervals were varied between 23 and 100 ms (the interval matching the syllable durations used was 30 ms). The averaged response of the TN1 cells was around one spike per double syllable at interval durations of 35 ms and longer (Fig. 9C). At shorter intervals (below 30 ms), the response dropped sharply (29 ms: 0.7 spikes/double syllable, 27 ms: 0.45 s/ds; 23 ms: 0.3 s/ds). This drop of TN1 responses followed female behaviour under the same stimulus conditions, where intervals shorter than 27 ms were not sufficient to evoke phonotactic responses (Fig. 9C; for a detailed description of the behavioural experiments cf. Jatho 1995).

T-shaped Neuron, TN 2

In *T. viridissima*, an auditory neuron so far undescribed with the soma in the prothoracic ganglion and with both an ascending and an descending axon, could be identified. It was named TN2. The morphology (Fig. 10A) was quite different from that of other intersegmental auditory cells. The soma position was found close to the midline of the ganglion, anterior to the arborizations, in contrast to the fronto-lateral soma position of ON, AN1, AN2 and TN1. Dense arborizations within the auditory neuropile, like in the other auditory interneurones, were not found for TN2, but four branches on the soma-ipsilateral side and one lateral branch on the soma-contralateral side arborize in wide areas of the prothoracic ganglion with the main dendritic regions to be found on the soma-ipsilateral side. The TN2 responses were strongly phasic, marking only the beginning of a stimulus (Fig. 10B) and tuned to the ultrasonic range, with thresholds below 40 dB for 30 kHz (Fig. 10C). TN2 was most sensitive to stimuli delivered from the soma-ipsilateral side, with sensitivity for soma-contralateral stimulation reduced by about 25 dB (at 30 kHz, Fig. 10C). The TN2 latencies were in the same

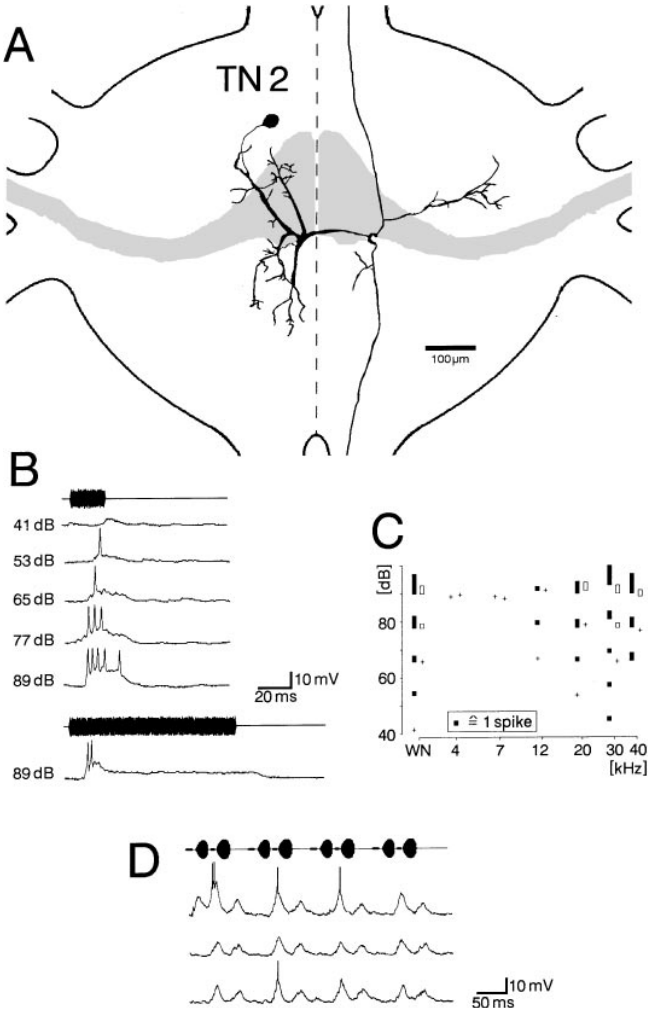

Fig. 10 **A** Whole mount drawing of TN2 morphology within the prothoracic ganglion of *T. viridissima*. The shaded areas schematically indicate the projections of the auditory receptor cells. **B** Dendritic recordings of TN2 responses for stimulation with white noise, demonstrating the phasic response characteristics. The stimuli were delivered from the soma-contralateral side. **C** Typical "response maps" of a TN2 for stimulation from the soma-ipsilateral (*filled bars*) and soma-contralateral (*open bars*) side. The height of a *bar* indicates the response magnitude (mean of 2 sweeps) at the given frequency and intensity, *crosses* indicate no response. The neuron was not tested below 40 dB. (Stimulus duration 20 ms; *WN* white noise). The stimuli from ipsi and contralateral were not presented at the same level. **D** Dendritic recordings of TN2 during stimulation with the song model, presented from the soma-ipsilateral side. The responses of the first three sweeps are shown. During the remainder of the song only a few occasional spikes occurred

range as those recorded for ON and TN1 (10–12 ms), which is 1–2 ms after the first receptor spikes arrived in the neuropile. The responses to song models adapted quickly. Figure 10D shows the responses to the beginning of a song. Only the first few syllables evoked spikes; in the remainder of the song only a few occasional spikes occurred, while EPSPs could be recorded throughout the stimulus without an apparent deterioration in amplitude.

Discussion

Intracellular versus extracellular recordings

In this study the responses of three cell types were recorded both extra- and intracellularly to combine the advantages of both methods: morphological identification and the recording of dendritic responses by the intracellular approach, and the quantitative analysis of spiking responses for much longer periods without penetrating artifacts by the extracellular method. Penetration with an electrode often injures even large neurons, leading to changed physiological properties. For example, Stumpner et al. (1995) found higher spontaneous activity during dendritic recordings of ON1 in *Acheta domesticus,* when compared to extracellular or axonal recordings, and the same was found here for the ON of *T. viridissima*. In smaller cells, important characteristics may even be masked by penetration artifacts during intracellular recordings [e.g. cricket AN1, see discussion in Horseman and Huber (1994)].

Of course, extracellular recordings are useful only when neuronal characteristics allow unequivocal identification of the neuron: AN1 and AN2 are the only neurons found so far in the *T. viridissima* neck connective with tonic, non-adapting discharges, and their different frequency responses and latencies allow discrimination between them. Rheinlaender (1984) used *Platycleis affinis* for simultaneous extra- and intracellular recordings and demonstrated that the large spikes recorded from the neck connective belong to TN1. Additionally, TN1 is the only auditory cell found in *T. viridissima* with phasic responses ascending in the connective ipsilateral to the main input side. For TN2, no convenient way for making extracellular recordings was found because of the small axonal diameter.

Song recognition and localization

Female phonotaxis requires the recognition of the conspecific signal and the localization of a male. As the brain most probably plays a decisive role in these processes, the sensory information necessary has to be present within the neurons that ascend from the prothoracic ganglion. These cells can serve this function in two ways, they can either copy the information and thereby transmit it unchanged to higher centres, or they can weigh or extract specific parameters from the signal and route this "preprocessed" information to the brain. Of course, both possibilities are not mutually exclusive, as one cell could encode one song quality (e.g. the time pattern) and weigh another one (e.g. the spectrum or direction).

The songs of *T. viridissima* males have a disyllabic rhythm that distinguish them from the monosyllabic songs of *T. cantans* and *T. caudata* males (Heller 1988), two species which occur syntopically with *T. viridissima*

in parts of their distribution area. Behavioural tests demonstrated two important features for female phonotaxis in *T. viridissima*: (i) in choice situations females prefer disyllabic song models over monosyllabic (Jatho et al. 1994) and (ii) song models must comprise both sonic and ultrasonic components to elicit phonotaxis [Jatho (1995) for *T. viridissima*; Hardt (1988) for *T. cantans*].

The spectral information provided by the receptor cells is split at the level of the ascending pathway into a low-frequency channel (AN1) and a high-frequency channel (AN2 and TN1). Despite the spectral resolution of the tympanic organ and the tonotopic frequency representation in the auditory neuropile, more detailed frequency information has not been found at the level of interneurons (Rheinlaender 1975; Silver et al 1980; Hardt 1988). For the exceptional finding of a sharply tuned interneuron see Oldfield and Hill (1983). The behavioural results suggest that the activity of both channels is necessary for phonotactic response. Thus, it may be concluded, that the responses of AN1 and either AN2 or TN1 (or both) are necessary to evoke phonotaxis in *T. viridissima*.

The syllable pattern of songs is transmitted reliably by AN1 and AN2 to the brain, providing sufficient information for filter networks recognizing the conspecific syllable pattern. On the other hand, the selective response of TN1 to the conspecific time pattern, which is similar to the course of female selectivity in phonotactic experiments (Fig. 9), may suggest the participation of TN1 in temporal song discrimination. The disyllabic song of *T. viridissima* was most probably evolutionarily derived from a monosyllabic one, similar to that of *T. cantans* [similar examples of disyllabic songs between more primitive monosyllabic ones are found in other bushcricket genera, e.g., *Platycleis, Metrioptera, Antaxius* (Heller 1988), *Neoconocephalus* (Walker 1975)]. This rhythmic change must result in the appearance of a second, slower syllable rate that alternates with the original, faster one.

The recognition of the ancestral (monosyllabic) song could be performed by a bandpass filter network, as proposed by Schildberger (1984) for the cricket brain. The introduction of a second (slower) syllable rate into the song would require a second, slower temporal filter for recognition. The low-pass filter properties of TN1 for double syllable rates might serve this function in *T. viridissima*.

Strong directionality should not eliminate the possibility that TN1 is involved in pattern recognition. Behavioural experiments with *Gryllus bimaculatus* indicated that song recognition and localization are processed serially, involving common neuronal elements (Wendler 1989; Stabel et al. 1989). No data are yet available that bear on this question in bushcrickets. Processing of song patterns and direction could also be parallel as is the case in grasshoppers (v. Helversen and v. Helversen 1995).

Behavioural data (Jatho 1995; J. Schul, unpubl. obs.) indicate that sonic song components play only a minor role in song localization. Therefore, AN1 seems to be involved only in song recognition, while the high-frequency channel (AN2, TN1) probably serves for both functions, i.e. recognition and localization.

Detection of predators

Flying bushcrickets stop their wingbeat in the presence of ultrasonic signals that mimic echolocation calls of bats [Libersat and Hoy (1991) for *Neoconocephalus ensiger*; J. Schul, personal observations for *T. viridissima*]. Correlations between this bat avoiding behaviour and TN1 responses have been made (Libersat and Hoy 1991). In crickets, one auditory interneuron (AN2 = INT1) has been proven responsible for eliciting bat-evasive behaviour during flight (Nolen and Hoy 1984). This same neuron participates in positive phonotaxis during walking and is involved in song localization (Schildberger und Hörner 1988).

A similar gating by the behavioural context is possible in the TN1 of *T. viridissima*: predator detection during flight and song localization during walking phonotaxis. On the other hand, if the selective TN1 responses to the conspecific song of *T. viridissima* are not only mere coincidence, but really used for song recognition, TN1 should be involved both during walking and flying phonotaxis. What could produce difficulties is the confusion between a mate and a bat. In this case another element would be necessary for bat detection. A possibility is TN2 which has ultrasonic tuning and strong adaptation properties.

The TN2-morphology is different from that of both ANs and TN1, in that information ascends to the brain (and descends towards the abdomen) contralateral to the input-site. This unusual morphology might be understood in terms of bat evasive responses, a manoeuver that should lead the insect away from the sound source. The interpretation that TN2 functions in intraspecific communication seems unlikely because the cell responds only to the first few syllables of a song (Fig. 10), while the insects often stridulate for minutes without interruption.

Comparison to crickets

The properties of the auditory pathway in *T. viridissima* described in this paper allow a comparison with the well known cricket system, in regard to both physiological properties and presumed functions in behaviour.

The *T. viridissima* ON and cricket ON1 have similar connectivity and physiology. Both receive excitatory input of soma-ipsilateral receptor cells and lateral inhibition from the soma-contralateral side, probably via their mirror image cell. Both have tonic responses, with high copying fidelity for the syllable pattern (Wohlers and Huber 1982; Horseman and Huber 1994; Stumpner

et al. 1995), while the inhibition recorded in the soma-contralateral branches of *T. viridissima* ON has not yet been described for the cricket ON1. However, the similarities strongly suggest homology of *Tettigonia* ON and cricket ON1.

Both AN1 and AN2 in *T. viridissima* and crickets have some similar physiological properties, too. In both groups, the AN1 is tuned to low frequencies and receives inhibitory inputs during ultrasonic stimulation. The AN2 of both groups receive excitation predominantly from ultrasonic signals and have no prominent frequency depending inhibition [review for crickets in Horseman and Huber (1994) and Schildberger (1994)]. A difference between *Gryllus* and *Tettigonia* exists in the copying abilities of AN2: while in *T. viridissima* AN2 is a "syllable coder", AN2 in crickets is mostly described as "chirp coder", since it does not usually resolve the syllable pattern (review: Hennig 1988). This difference may be due to different functions (see below). For the similarities in physiology and connectivity, homology of AN1 and AN2 in crickets and *Tettigonia* can be assumed.

There are some similarities between cricket TN (Wohlers and Huber 1982) and *T. viridissima* TN1 in gross morphology (frontolateral soma position, axon running in the medial third of the connectives) but also striking differences: cricket TN has few arborizations within the auditory neuropile but many in other regions of the prothoracic ganglion, while *Tettigonia* TN1 has dense arborizations within the auditory neuropile. Additionally, the physiological properties are quite different: *Gryllus* TN is rather insensitive and tuned to low frequencies, while *T. viridissima* TN1 is quite sensitive to ultrasonic frequencies. Homology of both cells cannot be ruled out for the data available, but seems to be unlikely. Homology of *Gryllus* TN and the TN2 of *T. viridissima* can be excluded due to the medial soma position of TN2. A cricket cell corresponding to TN2 may not yet have been found in crickets.

A comparison of the complete ascending auditory system between crickets and *T. viridissima* indicates some differences in neuronal functions. The cricket AN1 has been demonstrated to be necessary for both song recognition and localization (Schildberger and Hörner 1988; Atkins et al. 1992), while *T. viridissima* AN1 is probably necessary for song recognition but probably plays only a minor role in song localization.

The high-frequency channel (comprising AN2) is not necessary for positive phonotactic behaviour, but sharpens song localization at higher intensities in crickets (Schildberger and Hörner 1988). In contrast, the high frequency channel of *T. viridissima* (comprising AN2 and TN1) is necessary for eliciting positive phonotaxis, as the females respond only to songs containing the ultrasonic band (Hardt 1988; Jatho 1995). The large axonal diameters of cricket AN2 and *T. viridissima* TN1 suggest alarm or arousal function for these neurons. In some respects (ultrasound avoidance), cricket AN2 and Tettigonia TN1 appear to have convergent functions.

The comparison of the auditory system of Tettigoniids and Gryllids at the level of thoracic interneurons shows a high degree of similarity, especially when compared to the completely different auditory system of the Acrididae (Römer and Marquardt 1984; Stumpner and Ronacher 1991). A common, ancestral auditory system for Gryllids and Tettigoniids seems quite probable, with the differences being due to the long period of independent evolution after the Tettigonioid and the Grylloid clades separated. Of course, at the moment the hypothesis of independent evolution of the auditory systems in Grylloid and Tettigonioids cannot be rejected, as both groups might have incorporated the same, predisposed neuronal elements into the new sensory system. Data on the thoracic interneurons of Rhaphodophoridea and Gryllacrididea, two basal Tettigonioid clades, could give further evidence for either hypothesis.

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