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A species-specific frequency filter through specific inhibition, not specific excitation

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Abstract Many bushcrickets produce specific song spectra for acoustic communication. Song detection and/ or recognition may make use of such specificity. Where in the nervous system are the filters for song frequency situated? A peripheral tuning for song frequency typically does not exist. Auditory receptor cells of bushcrickets connect to local and ascending neurons in the prothoracic ganglion. One of the ascending neurons (1) may function as a frequency filter in a group of four related bushcrickets (genera *Ancistrura*, *Barbitistes*). The frequency response of ascending neuron 1 is species-specific roughly corresponding to the frequency of the conspecific male song. The species-specific tuning of the neuron is not brought about by specific excitation, but by specific inhibition. By eliminating this frequency-dependent and species-specific inhibition the former filter neuron is transformed into an unspecific broad-band neuron in all four species. Its tuning then does not differ from omega neuron 1, a local neuron which is rather unspecific for frequency. Also, the supra-threshold responses of ascending neuron 1, which are different in intact animals, are similar to each other and similar to omega neuron 1 following elimination of inhibition. Only ascending neuron 1 of *Ancistrura* retains some species-specific features at low frequencies. In conclusion, evolution changed inhibition, not excitation of a species-specific neuron.

Keywords Hearing · Orthoptera · Phaneropteridae · Auditory interneuron · Frequency processing

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Abbreviations *AN1* ascending neuron 1 · *DUM* dorsal unpaired medium · *IPSP* inhibitory postsynaptic potential · *ON1* omega neuron 1 · *PTX* picrotoxin

Introduction

Animals with ears typically can hear a broad spectrum of frequencies including the behaviourally relevant frequencies of males, rivals, and predators. Accordingly, in many species the ear is not sharply tuned to a specific frequency, but either shows a more broad frequency tuning (e.g. bushcrickets, Kalmring et al. 1990; many vertebrates, e.g. Fay 1992) or a tuning with more than one minimum of sensitivity (e.g. crickets and grasshoppers, Pollack 1998; or certain bats, Neuweiler 1984). Most animals have sets of sensory cells tuned to different carrier frequencies. Tonotopic representation in the central nervous system, which is found in vertebrates (e.g. Suga 1989; Merzenich and Schreiner 1992; Kelly et al. 1998) and invertebrates (e.g. Oldfield 1988) may alleviate interneurons to pick up frequency-specific information. These interneurons constitute central filters for detection of specific signals (sexual partners, rivals, predators) and are found, for example, in the mammalian colliculus inferior (Cassedey and Covey 1996). Because of the tonotopic representation such filter neurons may easily be excited by a subset of afferent cells representing a specific portion of the whole hearing range (e.g. Römer et al. 1988; Evans 1992). Alternatively, central neurons may receive excitation from a larger set of sensory cells and response ranges then may be narrowed down by frequency-specific inhibition (in mammals: Evans 1992; Suga 1995; Wang et al. 2000; two-tone inhibition is also observed in insects, e.g. Boyan 1981; Moiseff and Hoy 1983; Boyd et al. 1984).

The bushcricket ear principally resembles the mammalian cochlea in that it contains an ordered series of tuned sensory cells projecting into restricted areas of the central nervous system in a tonotopic fashion (e.g. Oldfield 1988; Römer et al. 1988; Stölting and Stumpner 1998). Dendrites of local and intersegmental

interneurons overlap with these central projections (e.g. Römer 1985; Römer et al. 1988; Stumpner 1997, 1999a, 1999b). While some interneurons with restricted frequency responses seem to connect only to a limited number of sensory cells (e.g. Römer 1985; Römer et al. 1988), others receive frequency-specific inhibitions (Römer 1987; Schul 1997; Stumpner 1997). In higher brain centres of vertebrates, pharmacologically blocking of such inhibition has revealed a broader excitation underlying the neuronal responses (e.g. Suga 1995; Wang et al. 2000). A similar mechanism has recently been described in the bushcricket *Ancistrura nigrovittata* Stumpner 1998). The ascending neuron 1 (AN1) receives frequency-dependent inhibitions at low (<10 kHz) and high (>20 kHz) frequencies, which are seen as clear inhibitory postsynaptic potentials (IPSPs) (Stumpner 1997) and are eliminated following application of picrotoxin (PTX, Stumpner 1998), a competitive antagonist of chloride channels in invertebrates gated by GABA (γ-aminobutyric acid; Robbins et al. 1958), glutamate (e.g. Raymond et al. 2000) – and most likely glycine. At the same time this neuron of *A. nigrovittata* was turned from an element specifically tuned to the male song frequency into an unspecific broadband neuron.

This poses the question, whether also related species with other song frequencies have an AN1 tuned to relatively low frequencies, i.e. whether or not AN1 (a neuron on the first level of auditory processing) differs between species. If the latter is the case, one may ask, whether frequency specific inhibitions may be a mechanism to create species-specific neurons in this group of bushcrickets and whether elimination of such inhibitions has the same effect in different species with different tuning of AN1. Also, one may ask whether species-specific properties also extend to other prothoracic neurons. In the search for an answer I compared two neurons, AN1 and omega neuron 1 (ON1) of *A. nigrovittata* to those of three closely related species of the genus *Barbitistes*, most likely the next relatives of *Ancistrura*. The results may allow hypotheses about evolutionary processes in song processing of bushcrickets or even more general for sound processing in animals.

Material and methods

Animals

Some wild catches and mostly F1-reared males and females of *Ancistrura nigrovittata* (Brunner von Wattenwyl 1878) from northern Greece, *Barbitistes constrictus* (Brunner von Wattenwyl 1878) from Thüringen, Germany, *B. serricauda* (Fabricius 1798) from Lower Saxony, Germany, and *B. ocskayi* (Brunner von Wattenwyl 1878) from south-western Slovenia were used for the experiments. Altogether data of 45 individuals of either sex are presented (see also legend to Fig. 3).

Stimulation

Two broad-band speakers (Dynaudio DF 21, 2–50 kHz, placed on the left and right side at a distance of 37 cm) broadcasted the stimuli (50 ms, 1.5 ms or 2 ms rise and fall, 250-ms pause) which were synthesised using a custom-built DA board and amplifier (Lang et al. 1993). Each stimulus was repeated five times with intervals of 250 ms from 30 dB to 90 dB SPL (re. 2×10^{-5} Pa) in 10-dB steps. Calibration was done on a continuous sound wave using a Bruel and Kjaer amplifier (2610) and Bruel and Kjaer microphones (1/2" or 1/4"). All stimuli were applied from the soma-ipsilateral (ON1) or soma-contralateral (AN1) side, which is the side eliciting a stronger response.

Neurophysiology

A CO_2-anaesthetised animal was waxed ventral side up on a holder. All legs and the head were fixed with a wax resin mixture. The prothoracic ganglion was exposed by removing the overlaying cuticle and was stabilised by a NiCr-spoon from below and a steel-ring from above. The dry sheath of the ganglion was treated with collagenase (Sigma) for 90–120 s to increase permeability of the neurolemm for PTX. The ganglion was then rinsed with saline (pH 6.8; Fielden 1960). Recordings were made with neurobiotin-filled (5% in 1 mol l^{-1} K-acetate; Vector) borosilicate capillaries (1.0/0.58 mm o.d./i.d.; 100–160 MΩ) and a custom-built intracellular amplifier. In the case of PTX application some of the saline was removed and replaced by 10–40 µl 10^{-3} mol l^{-1} PTX (Sigma) in saline (see Stumpner 1998). The effect of PTX became obvious between 2 min and 4 min following application. Staining of a cell was achieved by application of a 0.3- to 1.0-nA depolarising current for 1–10 min. Neurobiotin was visualised with the DAB method (DAKO StreptAB-complex/HRP; VECTOR Vectastain elite ABC kit and DAB substrate kit). Drawings of the cells were made using a Leitz microscope Dialux 20 and a drawing tube.

Data evaluation

Data were stored on a DAT recorder (Sony; 10 kHz sampling rate), digitised (DT 2128 F; Stemmer Turbolab 4.0 or 4.2) and analysed with the NEUROLAB program (Hedwig and Knepper 1992) and standard software. As threshold a response was defined which corresponds to one spike above spontaneous activity in three out of five stimuli. For AN1 only data are included from animals of successful PTX experiments.

Results

The songs

Since AN1 of *A. nigrovittata* responds best to frequencies contained in the male song (Stumpner 1997), it was compared to AN1 in three *Barbitistes* species to determine whether AN1 is similarly tuned in all species or whether it is also tuned to the conspecific male song frequency in *Barbitistes*. Therefore, song frequency in this group of bushcrickets has to be considered. Figure 1 presents data correlating average peaks of song frequency with body size in the four species treated in this article (see also Stumpner and Meyer 2001). The graph shows two general trends: song frequency decreases with increasing body size and males and females tend to have similar song frequency (which is also true for a larger sample of tropical Phaneropteridae (K.-G. Heller, personal communication). *A. nigrovittata* males are the only prominent exception, because they have a much lower frequency (around 15 kHz) than one would expect from their body size, while the song frequency of females is as expected from their size.

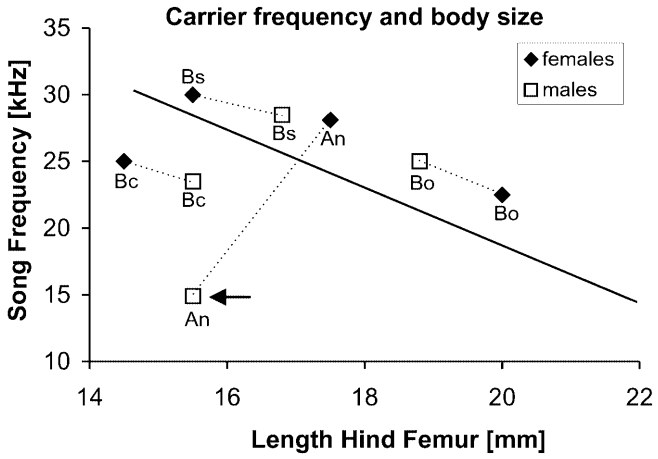

Fig. 1. Correlation between carrier frequency and body size (measured as length of hind femur) in four species of the Barbitistini (*B. serricauda* (Bs), *B. constrictus* (Bc), *B. ocskayi* (Bo), and *A. nigrovittata* (An). Males and females of each species are connected with *dotted lines*. For males a regression was calculated (*solid line*) including also the barbitistine species *B. yersini, Poecilimon affinis, P. ornatus, P. gracilis* and *P. laevissimus* but leaving out the value for *A. nigrovittata* males (*arrow*). The regression coefficient (r^2) is 0.78. The data for song frequencies are taken from Stumpner and Meyer (2001) for *Barbitistes*, from Dobler et al. (1994) for *Ancistrura* and from Heller (1988) for *Poecilimon*. The data for length of hind femur were measured from alcohol specimen in *Barbitistes* and *Ancistrura* and taken from Harz (1969) for *Poecilimon*

The neurons

I tested tuning of two auditory interneurons, one local and one ascending (Fig. 2). The local neuron, the well-known ON1 (Zhantiev and Korsunovskaya 1983; Römer 1985; Römer et al. 1988), was included in the study to see whether or not the results found for AN1 are neuron specific. Homology of neurons was determined on the basis of adult morphology and physiology. Identification of AN1 in the different species was slightly more demanding than that of ON1 (see Discussion). AN1 in all four species has a distinct morphology in the prothoracic ganglion with extensive dendrites originating solely on the soma-contralateral side (Fig. 2b). Very rarely single ipsilateral short dendrites occur. Additionally, brain projections of AN1 in all four species are quite similar in overall shape (Fig. 2c) and in the termination sites of the protocerebrum, which are found in ventral regions.

Responses of untreated AN1 and ON1

ON1 is similarly tuned in all four species with a minimum threshold around 20 kHz (Fig. 3a) and minor differences between species in absolute sensitivity. In contrast, when one compares frequency tuning of AN1 in the four species, it is evident that excitatory thresholds differ (Fig. 3b). At low frequencies (most clearly below 15 kHz), AN1 of all species receives inhibitions visible as

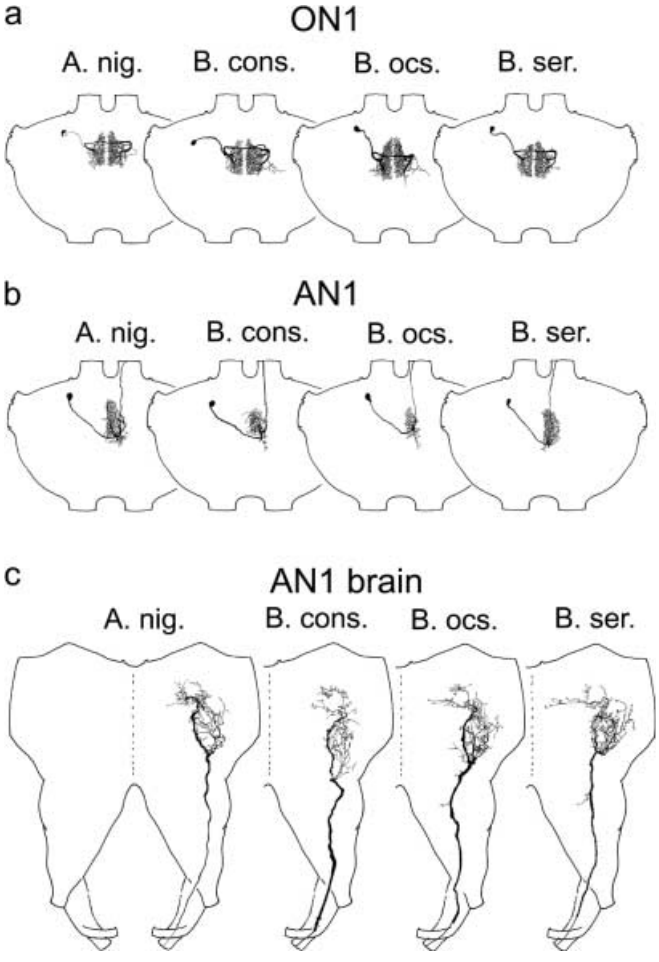

Fig. 2. Wholemount views of **a** the prothoracic ganglion with the omega neuron 1 (ON1) in (from left to right) *A. nigrovittata, B. constrictus, B. ocskayi* and *B. serricauda,* **b** the prothoracic ganglion with the ascending neuron 1 (AN1) of the four species, and **c** the brain projections of AN1 in the four species

IPSPs in many recordings (see, for example, 8 kHz in Fig. 4), while only AN1 of *A. nigrovittata* has high thresholds also at high frequencies, where it receives strong inhibitions at low intensities (see Stumpner 1997). The suprathreshold responses of AN1 in the three *Barbitistes* species are also different. When one compares sample traces (Fig. 4) and intensity functions (Fig. 5) at 8 kHz, 16 kHz and 28 kHz of AN1 and ON1 of all four species, it is quite obvious that ON1 responses are rather similar with only some differences of the absolute spike number (Fig. 5a, b, c). Also, there is little difference in the responses at different frequencies. This is very different in AN1 (Fig. 5d, e, f). Neither are the suprathreshold-responses similar in the four species, nor are the responses at 8 kHz, 16 kHz and 28 kHz similar within any species. A common property of AN1 of the *Barbitistes* species seems to be that the higher the frequency (8 kHz, 16 kHz, 28 kHz) the stronger are the responses. In AN1 of *A. nigrovittata* intermediate frequencies are most effective. Sample traces in Fig. 4 underline the species differences, which are largest at

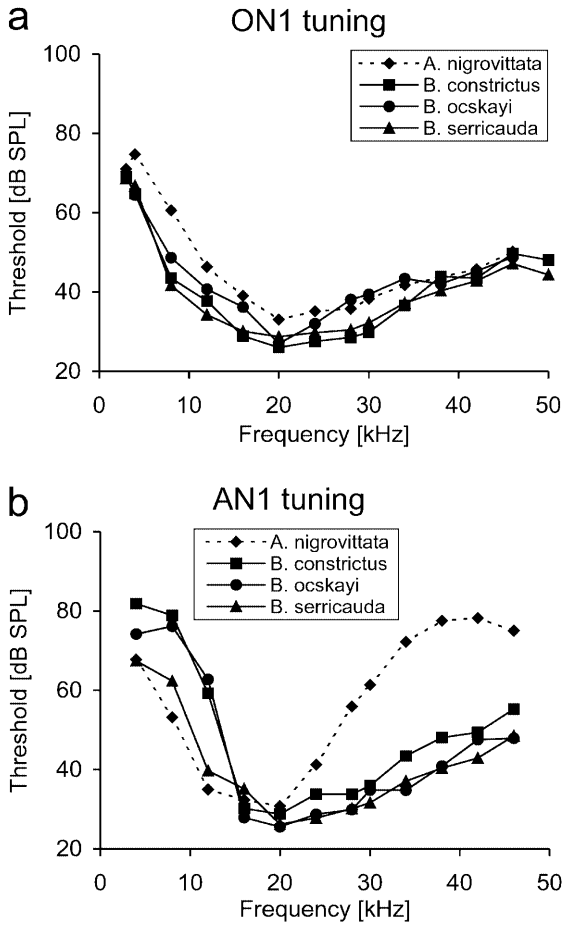

Fig. 3. a Mean excitatory threshold of ON1 in the four species (both sexes). **b** Mean excitatory threshold of AN1 in the four species (both sexes). Sample sizes (*m* males, *f* females): *A. nigrovittata* ON1: 8 m, 6 f; AN1 1 m, 2 f; *B. constrictus* ON1: 4 m, 1 f; AN1 3 m, 1 f; *B. ocskayi* ON1: 2 m, 3 f; AN1: 1 m, 3 f; *B. serricauda* ON1: 6 m, 1 f; AN1: 1 m, 2 f

28 kHz. Responses of ON1 to the different stimuli, on the other hand, are similar between species. The main difference in the traces in Fig. 4 come from different recording sites within the large soma-ipsilateral branch and the correspondingly different relative sizes of EPSPs and action potentials. This means that most clearly at high frequencies or high intensities there are different and therefore species-specific response properties of an auditory interneuron, namely AN1, already on the first level of auditory processing in these closely related bushcrickets, while responses of another neuron (ON1) are similar in all species.

Responses of AN1 after treatment with picrotoxin

For *A. nigrovittata* it has been demonstrated that excitatory tuning of AN1 broadens considerably following bath application of picrotoxin (Stumpner 1998). In AN1 of *Barbitistes*, as in *Ancistrura*, PTX eliminates frequency-dependent inhibitions and thereby changes the threshold of these neurons (Fig. 6) as well as their suprathreshold responses (Fig. 7). Following PTX application, the tuning of AN1 is very similar in all four species (Fig. 6a). This is especially obvious, when thresholds of AN1 are compared to those of ON1: in untreated animals (Fig. 6b) they differ within each species by up to 36 dB at certain frequencies – mostly below 20 kHz in *Barbitistes* and above 20 kHz in *Ancistrura*. After PTX treatment (Fig. 6c) the differences are mainly lost and only occasionally reach 10 dB and thereby are very similar to ON1 threshold differences between the different individuals (compare Fig. 3a to Fig. 6a). The same is largely true for the intensity response functions (Fig. 7). They are nearly identical in the three *Barbitistes* species following PTX-application and look quite similar to those of untreated ON1 (Fig. 5), although show-

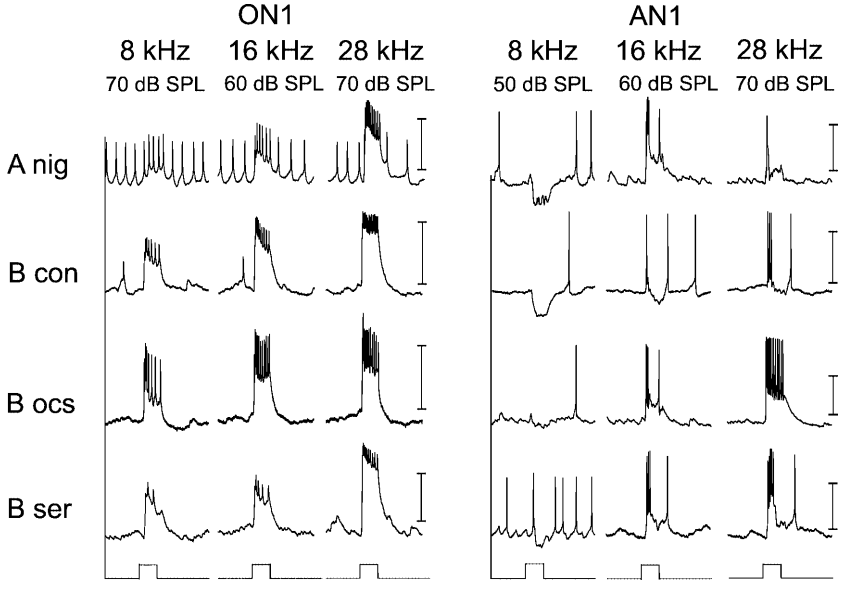

Fig. 4. Sample traces of an individual ON1 of the four species recorded in the soma-ipsilateral dendrites (*left*) and of an individual AN1 recorded in the soma-contralateral dendrites (*right*) of the four species at the frequencies and intensities given above the traces. The *bar to the right* of each neuron corresponds to 25 mV. A stimulus is exactly 50 ms

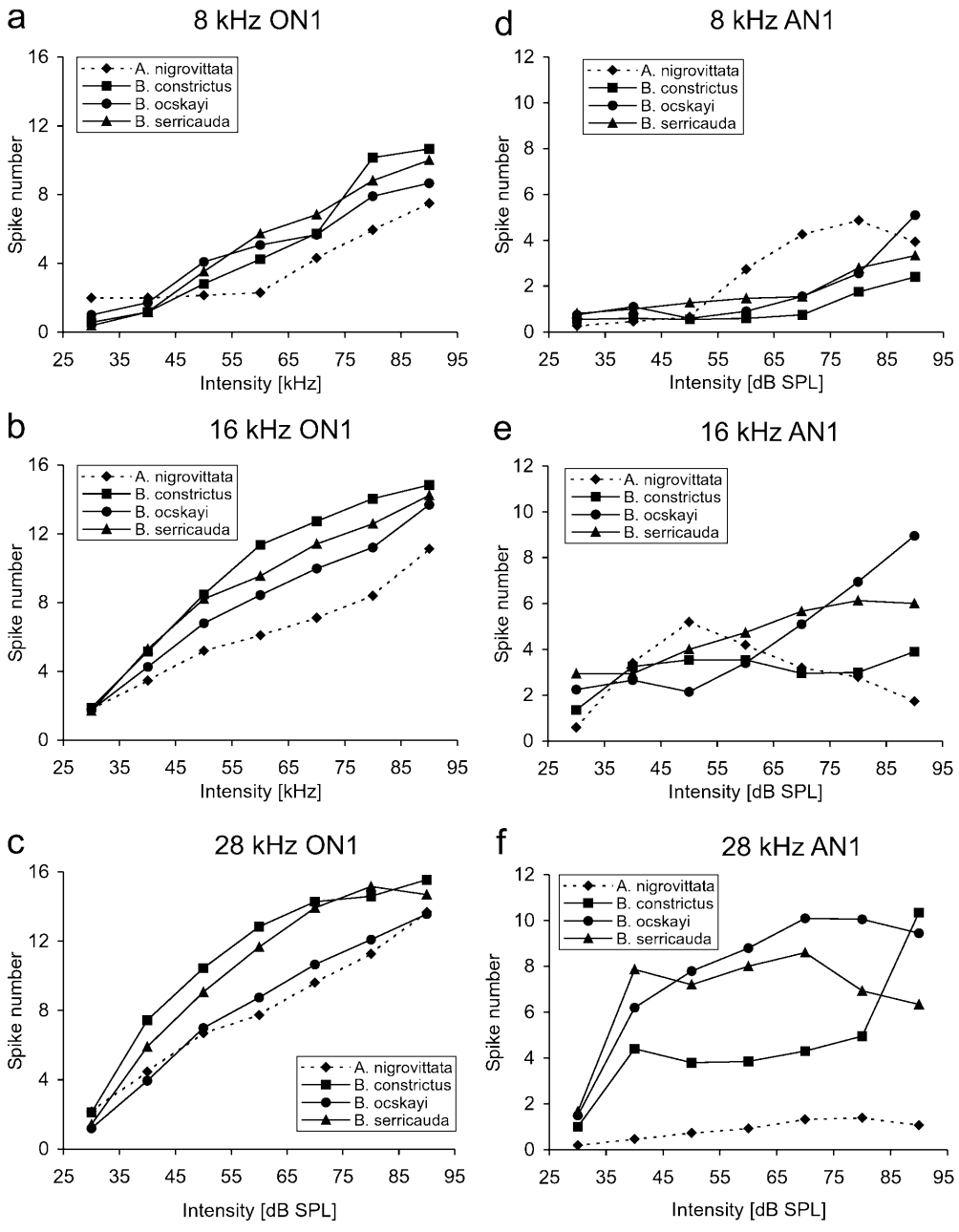

Fig. 5. Intensity response functions of ON1 (**a**, **b**, **c**) and AN1 (**d**, **e**, **f**) for 8 kHz, 16 kHz and 28 kHz in the four species. Sample sizes as in Fig. 3

ing a somehow stronger tendency of saturation than ON1. AN1 of *A. nigrovittata* seems to be an exception (most clearly at 16 kHz), since it retains some reduction of its responses at high intensities (Fig. 7a). The sample traces in Fig. 8 in comparison to those of Fig. 4 underline the dramatic change of AN1 characteristics: Now the AN1 of all four species shows rather similar spiking with tonic responses at all frequencies. Differences mainly result from different levels of spontaneous activity, which often is elicited as longer lasting post-stimulatory activity at higher intensities. Both, spontaneous activity and post-stimulatory activity, have a tendency to increase with increasing duration of PTX-presence. All in all, it seems justified to conclude that PTX application made the response properties of AN1 similar to those of ON1, and therefore species-specificity of AN1 responses is largely lost.

Discussion

Homology of neurons

Two neurons, ON1 and AN1, originating in the prothoracic ganglion, were compared between four barbitistine bushcrickets. While homology of ON1 is undisputed, since no other cell similar to ON1 has been described so far in any bushcricket, identification of AN1 needs more detailed arguments. There are three ascending neurons with the soma in the same anterior

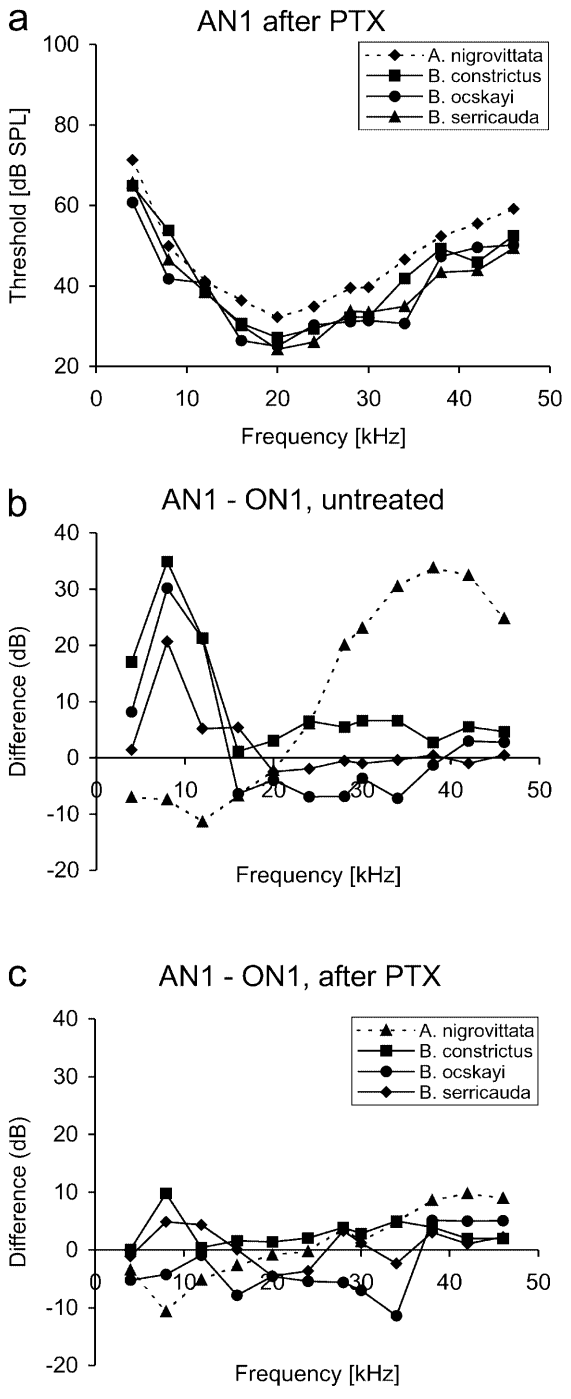

Fig. 6. a Excitatory thresholds of AN1 following application of picrotoxin (PTX). **b** Differences between mean tuning of AN1 and mean tuning of ON1 in each species in untreated animals [significant differences, *t*-test, 2-tailed ($P \leq$)]: *A. nigrovittata*: 4 kHz (0.05), 16 kHz (0.05), 28–46 kHz (0.0001); *B. constrictus*: 4 kHz (0.05), 8 kHz (0.0001); *B.ocskayi*: 8 and 12 kHz (0.001); *B. serricauda*: 8 kHz (0.01). **c** Differences between mean tuning of AN1 following PTX application and mean tuning of ON1 in each species [significant differences, *t*-test, 2-tailed ($P \leq$)]: *A. nigrovittata*: 8, 42 and 46 kHz (0.05); *B. constrictus*: 24 and 28 kHz (0.05), 34 kHz (0.01); *B. ocskayi*: no significant differences; *B. serricauda*: no significant differences

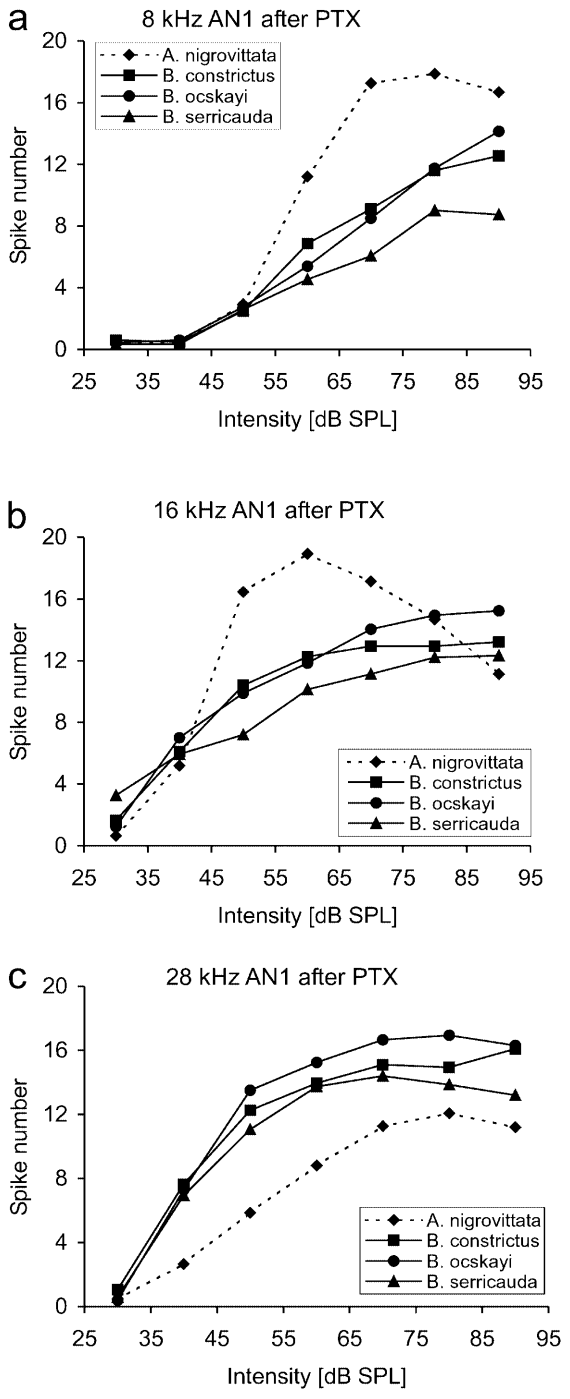

Fig. 7. Intensity response functions of AN1 for 8 kHz (**a**), 16 kHz (**b**) and 28 kHz (**c**) following PTX application in the four species. Sample sizes as in Fig. 3

dorsolateral cluster and with brain projections in the same protocerebral area in the Barbitistini. Only AN1 of *A. nigrovittata* has been described in detail so far (Stumpner 1997). Homology of AN1 to other ascending cells published for crickets and bushcrickets was discussed in detail in Stumpner (1997). In all four species treated here, AN1 can be unequivocally distinguished from the other two by its physiology (temporal response pattern, intensity dependence and responses to different

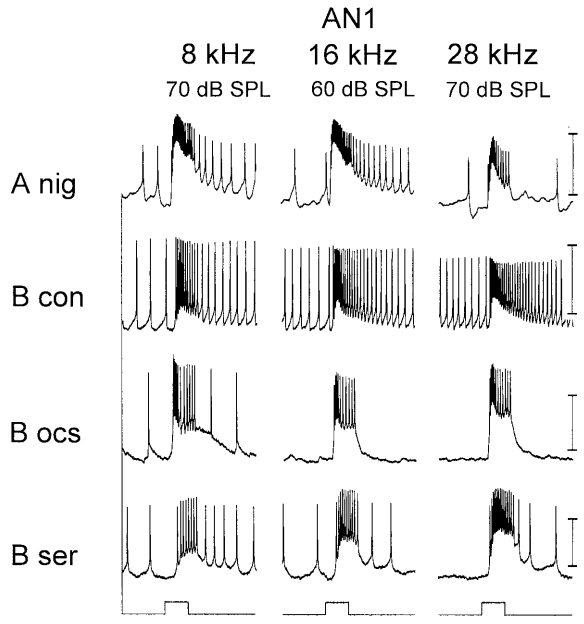

Fig. 8. Sample traces of an individual AN1 recorded in the soma-contralateral dendrites at the frequencies and intensities given above the traces following PTX application. Same individuals as in Fig. 4. The *bars to the right* correspond to 25 mV. A stimulus is exactly 50 ms

pulse rates; A. Stumpner, unpublished observations) and by its distinct morphology in the prothoracic ganglion: the other two ascending cells have extensive dendrites originating soma-ipsilaterally, while AN1 does not. There are also clear differences in the brain projections of the three cells in *A. nigrovittata*, but these have not been verified in all *Barbitistes* species. The similarity of brain projections of AN1 in the four species, however, make it likely that also the other ascending cells have similar brain projections in the four species.

Species specificity of AN1

ON1 tuning is similar in all four species, while peak frequencies of male songs are much less similar (see Fig. 1 and Stumpner and Meyer 2001). Actually, *A. nigrovittata* males have the lowest song frequency (around 15 kHz) of the four species while at the same time, between 5 kHz and 20 kHz, ON1 of *Ancistrura* is the least sensitive. All in all, frequency tuning of ON1 is broad and, consequently, tuning of the whole auditory system to the conspecific song frequency does not exist.

In *A. nigrovittata* AN1 has been described as a "low-frequency filter" (Stumpner 1997). While it is strongest excited at 12–16 kHz (the peak of the male song), it receives prominent inhibitions seen as IPSPs at lower and higher frequencies. Therefore, it might constitute the relevant filter for male song frequency in this species (*A. nigrovittata*) which is concluded from a close, though not perfect, correspondence of neuronal tuning and the threshold of female duetting behaviour (Dobler et al. 1994; Stumpner 1997). In *A. nigrovittata*, male song frequency is unusually low compared to the typical relation between size and song frequency in several barbitistine species (Fig. 1) and therefore probably has shifted during evolution to lower frequencies. Female song frequency in this species is in the typical range around 28–30 kHz and a separate filter neurone for detecting female songs may exist (Stumpner 1999a). There are two alternative explanations for the correspondence of tuning of AN1 and male song frequency in *A. nigrovittata*: either the males have shifted their song frequency to exploit an existing "low-frequency filter neuron" in the auditory system of females (sensory exploitation, see Ryan and Rand 1990). In this case, AN1 probably would have similar properties in closely related species. Alternatively, there has been a co-evolution between song and AN1 tuning, and AN1would be expected to be species-specific, most obviously to be seen in *A. nigrovittata* due to its unusually low male song frequency. The crucial test for this is a comparison of related species.

The data presented here give a definite answer: the *Barbitistes* species investigated have higher male song frequencies than *Ancistrura* and have an AN1 tuned to higher frequencies than in *Ancistrura*. Therefore, the sensory exploitation hypothesis can be discarded as an explanation for the correspondence of male song frequency and tuning of AN1 in *A. nigrovittata*. Not only frequency tuning, but also intensity dependence of AN1 responses in the different species is quite different. This is not an effect of differences of the peripheral system, since thresholds and supra-threshold responses of the omega neuron are very similar in all species. The ON1 neuron has a broad tuning and is most likely involved in lateral inhibition (Schul 1997; Römer und Krusch 2000) and certainly not in frequency discrimination. In conclusion, these bushcrickets have a species-specific frequency filter, the AN1 neuron, already in the prothoracic ganglion, which constitutes the first level of auditory processing. The fit between AN1 responses and male song frequency does not seem to be perfect, though, since AN1 of *B. ocskayi* shows the strongest responses at ultrasonic frequencies at higher intensities, while *B. serricauda* males produce the highest song frequency of all species. However, also the fit of AN1 and male song in *A. nigrovittata* is not perfect: while the male song peaks between 14 kHz and 16 kHz, AN1 threshold is lowest at 20 kHz – even though supra-threshold responses are stronger at 12 kHz and 16 kHz than at 20 kHz (see Stumpner 1997).

It seems that AN1 is a special case, since also the other two ascending cells (AN2 and AN3; A. Stumpner, unpublished observations) and TN1 (Stumpner 1999b; A. Stumpner, unpublished observations) are similar in tuning and in supra-threshold responses in the four species. This may be true only for the closer kin or for the Phaneropteridae. The rather patchy knowledge from auditory neurons in other bushcricket species indicates a close similarity between ON1 of many species (Römer

et al. 1988, 1989), while even homology of ascending cells is disputed, since in tettigoniids (especially *Tettigonia viridissima* and *T. cantans*; Hardt 1988; Römer et al. 1988; Schul 1997) only two ascending neurons have been described – just like in crickets (e.g. Hennig 1988). Römer (1987), however, concerning ascending neurons in the copiphorine species *Mygalopsis marki*, states that physiological data indicate there might be more than two ascending cells.

Species specificity through specific inhibition

Bushcricket ears have a series of sensory cells tuned to different frequencies (e.g. Römer 1987; Oldfield 1988; Kalmring et al. 1990; Stölting and Stumpner 1998). On the level of bushcricket interneurons, there is a remarkable convergence of sensory inputs. This even holds true for interneurons like AN1, which seem to act as species-specific frequency filters with a narrow excitatory response range compared to broadband neurons. This response range is similar to that of single sensory neurons (A. Stumpner, unpublished observations). What might the convergence of sensory inputs be good for? Mainly, it increases reliability of neuronal responses: it improves the signal to noise ratio (e.g. Spiridon et al. 2000) and enlarges the dynamic range for coding intensities (e.g. Pollack 1994; Römer et al. 1998). Moreover, it most likely allows the detection of spike synchronisation in sets of sensory cells processing acoustic information in parallel (Ronacher and Römer 1985; Engel et al. 1997); however, this would be at the cost of narrow-frequency tuning. Therefore, sharpened species-specific spectral response ranges are achieved by means of frequency-dependent inhibitions. Such inhibitions, in turn, may limit the dynamic range of responses, since at higher intensities a larger frequency range is affected by the inhibition (e.g. Suga 1995; Stumpner 1997).

Convergence of sensory inputs and shaping of response properties of interneurones through inhibition are not specific features of auditory interneurones, but have also been described to great detail in the cercal receptor/giant interneurone system of various insects (Orthoptera, Blattodea, Mantodea; for extensive reviews see Boyan and Ball 1990; Comer and Robertson 2001). In this system we find topographical maps of sensory projections and a corresponding anatomical basis for specific directional properties similar to the hearing system of bushcrickets (e.g. Jacobs and Theunissen 2000). There are not only parallel pathways of excitatory input of interneurones (direct and via local elements; Boyan and Ball 1989), but also pre- and postsynaptic inhibition (e.g. Blagburn and Sattelle 1987; Boyan 1988) of (mainly) giant cercal interneurones which are involved in determining whether and in what direction a wind-induced escape response should occur. Patterns of overlap with afferents alone cannot sufficiently explain interneuronal behaviour (e.g. Paydar et al. 1999). All such factors may also contribute to response properties of auditory neurones in Ensifera (parallel pathways: Pollack 1994; presynaptic inhibition: Hardt and Watson 1999; postsynaptic inhibition: many papers, including the present study). However, the driving forces are probably quite different in the two systems. The one is mainly an escape system with the need for fast decision making, probably tolerating false alarms. Two or more interneurones receive similar inputs and may serve a similar function. Differences between closely related species are not expected, although differences between taxa were found (see Boyan and Ball 1990). The auditory system, on the other hand, serves pair forming with less need of speed but probably greater need of reliability. Responding to the wrong partner (species) should not occur. Differences between closely related species are expected, though not necessarily on a low level of information processing. When studying the cercal system, one big advantage exists which finds no parallel in the auditory system: it is possible to examine early instars with very few or single filiform hairs on the cercus and thereby study the function of the system in a very reduced situation (e.g. Blagburn et al. 1986, 1991). The bushcricket larva, on hatching, has its complete set of sensory cells in the crista acustica but nevertheless is probably nearly deaf, partly due to lack of tympanic membranes on the legs (Rössler 1992).

Evolutionary aspects

Why, during evolution, have inhibitory connections been changed instead of excitatory connections? Excitation in AN1 probably comes directly from afferents, while inhibition almost certainly is evoked by local neurons, e.g. dorsal unpaired medium (DUM) cells (Bate 1976), some of which have shown to be GABAergic in grasshoppers (Thompson and Siegler 1991). So far, DUM cells of bushcrickets are the only known local cells which show frequency-specific responses in *Ancistrura* and *Barbitistes* (A. Stumpner, unpublished observations). Such DUM cells can be assumed to receive direct excitation from receptor cells: they show extensive arborisations in the auditory neuropile overlapping with receptor terminals (see also Thompson and Siegler 1991) and they have shortest latencies for onset of EPSPs between 11.5 ms and 15 ms, which is exactly the same range as in ascending cells like AN1 which are thought to be directly connected to afferents (A. Stumpner, unpublished observations; Stumpner 1997). Presumably di- or polysynaptic IPSPs in DUM cells and in ascending cells have longer latencies (not shorter than 15 ms, see also Stumpner 1998). A set of DUM cells exists in all orthopteran species (at least three auditory DUM cells in *A. nigrovittata*) and evolution may just have changed the strength of their inhibitory influence onto ascending neurons. For the latter we find some supporting evidence in the intensity response functions of AN1 of the three *Barbitistes* species. While in untreated animals, AN1 responds very differently in the four species despite similar

tuning, these differences are nearly eliminated following PTX application. Therefore, the species-specific response strength of untreated AN1 may reflect different degrees of inhibitory influence (lowest *in B. ocskayi*, highest in *A. nigrovittata*). Another evolutionary advantage of such a system, namely speed of evolutionary change, is also feasible. If the strength of inhibition of one (or few) interneurons has to be changed compared to the connection of many receptors to an interneuron, the former may simply be faster or easier to achieve.

For the future, it would be promising on the one hand to extend such studies to more distantly related groups of bushcrickets which use other frequencies in male calling and to directly test the influences of single DUM cells on AN1, e.g. by cell killing, to find the network properties evoking the divergent tuning of AN1 in *Ancistrura* and *Barbitistes*. Comparison with detailed phylogenetic data in this group, which hopefully will be available in the close future, may also help to demonstrate a correlation between physiological and genetic distances. Such studies may be easier to follow up in groups of bushcrickets than in vertebrates, where similar mechanisms of specific sharpening of tuning curves (e.g. in mammals) have been found.

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References

Bate CM (1976) Embryogenesis of an insect nervous system. I. A map of the thoracic and abdominal neuroblasts in *Locusta migratoria*. J Embryol Exp Morphol 35:107–123

Blagburn JM, Satelle DB (1987) Presynaptic depolarization mediates presynaptic inhibition at a synapse between an identified mechanosensory neurone and giant interneurone 3 in the first instar cockroach, *Periplaneta americana*. J Exp Biol 127:135–157

Blagburn JM, Beadle DJ, Satelle DB (1986) Differential synaptic input of filiform hair sensory neurones on to giant interneurones in the first instar cockroach. J Insect Physiol 32:591–595

Blagburn JM, Blanco RE, Thompson KSJ, Bacon JP (1991) Positional information determines the anatomy and synaptic specificity of cockroach filiform hair afferents using independent mechanisms. J Comp Physiol A 169:607–614

Boyan GS (1981) Two-tone suppression of an identified auditory neuron in the brain of the cricket *Gryllus bimaculatus* (De Geer). J Comp Physiol 144:117–125

Boyan GS (1988) Presynaptic inhibition of identified wind-sensitive afferents in the cercal system of the locust. J Neurosci 8:2748–2757

Boyan GS, Ball EE (1989) Parallel inputs shape the response of a giant interneurone in the cercal system of the locust. J Insect Physiol 35:305–312

Boyan GS, Ball EE (1990) Neuronal organization and information processing in the wind-sensitive cercal receptor/giant interneurone system of the locust and other orthopteroid insects. Prog Neurobiol 35:217–243

Boyd P, Kühne R, Silver S, Lewis B (1984) Two-tone suppression and song coding by ascending neurones in the cricket *Gryllus campestris* L. J Comp Physiol A 154:423–430

Cassedey JH, Covey E (1996) A neuroethological theory of the operation of the inferior colliculus. Brain Behav Evol 47:311–336

Comer CM, Robertson RM (2001) Identified nerve cells and insect behavior. Prog Neurobiol 63:409–439

Dobler S, Stumpner A, Heller K-G (1994) Sex-specific spectral tuning for the partner's song in the duetting bushcricket *Ancistrura nigrovittata* (Orthoptera: Phaneropteridae). J Comp Physiol A 175:303–310

Engel AK, Roelfsema PR, Fries P, Brecht M, Singer W (1997) Binding and response selection in the temporal domain: a new paradigm for neurobiological research. Theor Biosci 116:241–266

Evans EF (1992) Auditory processing of complex sounds: an overview. Philos Trans R Soc Lond B 336:295–306

Fay RR (1992) Structure and function in sound discrimination among vertebrates. In: Webster DB, Fay RR, Popper AN (eds) The evolutionary biology of hearing. Springer, Berlin Heidelberg New York, pp 229–263

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

Hardt M (1988) Zur Phonotaxis von Laubheuschrecken: Eine vergleichend verhaltensphysiologisch/ neuroanatomische Untersuchung. Dissertation, Universität Bochum

Hardt M, Watson AH (1999) Distribution of input and output synapses on the central branches of bushcricket and cricket auditory afferent neurones: immunocytochemical evidence for GABA and glutamate in different populations of presynaptic buttons. J Comp Neurol 403:281–294

Harz K (1969) Orthoptera of Europe I. Junk, The Hague, pp 1–434

Hedwig B, Knepper M (1992) NEUROLAB, a comprehensive program for the analysis of neurophysiological and behavioural data. J Neurosci Methods 45:135–148

Heller K-G (1988) Bioakustik der europäischen Laubheuschrecken. Ökologie in Forschung und Anwendung Bd 1. Markgraf, Weikersheim

Hennig RM (1988) Ascending auditory interneurons in the cricket *Teleogryllus commodus* (Walker): comparative physiology and direct connections with afferents. J Comp Physiol A 163:135–143

Jacobs GA, Theunissen FE (2000) Extraction of sensory parameters from a neural map by primary sensory interneurons. J Neurosci 20:2934–2943

Kalmring K, Keuper A, Kaiser W (1990) Aspects of acoustic and vibratory communication in seven European bushcrickets. In: Bailey WJ, Rentz DCF (eds) The Tettigoniidae: biology, systematics, and evolution. Crawford House Press, Bathurst, pp 191–216

Kelly JB, Liscum A, Adel B van, Ito M (1998) Projections from the superior olive and lateral lemniscus to tonotopic regions of the rat's inferior colliculus. Hear Res 116:43–54

Lang F, Brandt G, Glahe M (1993) A versatile multichannel acoustic stimulator controlled by a personal computer. In: Elsner N, Heisenberg M (eds) Gene-Brain-Behaviour. Thieme, Stuttgart, p A892

Merzenich MM, Schreiner CE (1992) Mammalian auditory cortex – some comparative observations. In: Webster DB, Fay RR, Popper AN (eds) The evolutionary biology of hearing. Springer, Berlin Heidelberg New York, pp 673–689

Moiseff A, Hoy R (1983) Sensitivity to ultrasound in an identified auditory interneuron in the cricket – a possible neuronal link to phonotactic behavior. J Comp Physiol A 152:155–167

Neuweiler G (1984) Foraging, echolocation and audition in bats. Naturwissenschaften 71:446–455

Oldfield BP (1988) Tonotopic organization of the insect auditory pathway. Trends Neurosci 11:267–269

Paydar S, Doan CA, Jacobs GA (1999) Neural mapping of direction and frequency in the cricket cercal sensory system. J Neurosci 19:1771–1781

Pollack GS (1994) Synaptic inputs to the omega neuron of the cricket *Teleogryllus oceanicus*: differences in EPSP waveforms evoked by low and high sound frequencies. J Comp Physiol A 174:83–89

Pollack GS (1998) Neural processing of acoustic signals. In: Hoy RR, Popper AN, Fay RR (eds) Comparative hearing: insects. Springer, Berlin Heidelberg New York, pp 139–196

Raymond V, Sattelle DB, Lapied B (2000) Co-existence in DUM neurones of two GluCl channels that differ in their picrotoxin sensitivity. Neuroreport 11:2695–2701

Robbins J, Kloot WG van der (1958) The effect of picrotoxin on peripheral inhibition in the crayfish. J Physiol (Lond) 143:541–552

Römer H (1985) Anatomical representation of frequency and intensity in the auditory system of Orthoptera. In: Kalmring K, Elsner N (eds) Acoustic and vibrational communication in insects. Parey, Hamburg, pp 25–32

Römer H (1987) Representation of auditory distance within a central neuropile of the bushcricket *Mygalopsis marki*. J Comp Physiol A 161:33–42

Römer H, Krusch M (2000) A gain-control mechanism for processing of chorus sounds in the afferent auditory pathway of the bushcricket *Tettigonia viridissima* (Orthoptera; Tettigoniidae). J Comp Physiol A 186:181–191

Römer H, Marquart V, 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 H, Bailey W, Dadour I (1989) Insect hearing in the field. III. Masking by noise. J Comp Physiol A 164:609–620

Römer H, Spickermann M, Bailey W (1998) Sensory basis for sound intensity discrimination in the bushcricket *Requena verticalis* (Tettigoniidae, Orthoptera). J Comp Physiol A 182:595–607

Ronacher B, Römer H (1985) Spike synchronisation of tympanic receptor fibres in a grasshopper (*Chorthippus biguttulus* L, Acrididae) J Comp Physiol A 157:631–642

Rössler W (1992) Postembryonic development of the complex tibial organ in the foreleg of the bushcricket *Ephippiger ephippiger* (Orthoptera: Tettigoniidae). Cell Tissue Res 269:505–514

Ryan MJ, Rand AS (1990) The sensory basis of sexual selection for complex calls in the tungara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). Evolution 44:305–314

Schul J (1997) Neuronal basis of phonotactic behaviour in *Tettigonia viridissima*: processing of behavioural relevant signals by auditory afferents and thoracic interneurons. J Comp Physiol A 180:573–583

Spiridon M, Chow CC, Gerstner W (2000) Effects of correlations on signal transmission in a population of spiking neurons. Neurocomputing 32:529–535

Stölting H, Stumpner A (1998) Tonotopic organization of auditory receptor cells in the bushcricket *Pholidoptera griseoaptera* (Tettigoniidae, Decticini). Cell Tissue Res 294:377–386

Stumpner A (1997) An auditory interneuron tuned to the male song frequency in the duetting bushcricket *Ancistrura nigrovittata* (Orthoptera, Phaneropteridae). J Exp Biol 200:1089–1101

Stumpner A (1998) Picrotoxin eliminates frequency selectivity of an auditory interneuron in a bushcricket. J Neurophysiol 79:2408–2415

Stumpner A (1999a) An interneuron of unusual morphology is tuned to the female song frequency in the bushcricket *Ancistrura nigrovittata* (Orthoptera, Phaneropteridae). J Exp Biol 202:2071–2081

Stumpner A (1999b) Comparison of morphology and physiology of two plurisegmental sound-activated interneurones in a bushcricket. J Comp Physiol A 185:199–205

Stumpner A, Meyer S (2001) Songs and the function of song-elements in four duetting bushcricket species (Ensifera, Phaneropteridae, *Barbitistes*). J Insect Behav 4:511–534

Suga N (1989) Principles of auditory information processing derived from neuroethology. J Exp Biol 146:277–286

Suga N (1995) Sharpening of frequency tuning by inhibition in the central auditory system: tribute to Yasuji Katsuki. Neurosci Res 21:287–299

Thompson KJ, Siegler MVS (1991) Anatomy and physiology of spiking local and intersegmental interneurons in the median neuroblast lineage of the grasshopper. J Comp Neurol 305:659–675

Wang J, Caspary D, Salvi RJ (2000) GABA-A antagonist causes dramatic expansion of tuning in primary auditory cortex. Neuroreport 11:1137–1140

Zhantiev RD, Korsunovskaya OS (1983) Structure and functions of two auditory neurons in the bush cricket *Tettigonia cantans* Fuess (Orthoptera, Tettigoniidae). Rev Entomol URSS 62:462–469