Picrotoxin Eliminates Frequency Selectivity of an Auditory Interneuron in a Bushcricket

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Stumpner, Andreas. Picrotoxin eliminates frequency selectivity of an auditory interneuron in a bushcricket. *J. Neurophysiol.* 79:2408–2415, 1998. AN1, an auditory interneuron in the bushcricket *Ancistrura nigrovittata*, is narrowly tuned to the male song frequency (~15 kHz). It receives pronounced inhibitory input at frequencies below and, more prominently, above this fundamental frequency. It is also subject to side-dependent inhibition producing asymmetric response functions for left- and right-side stimulation. In addition, intensity-response functions of AN1 peak as stimulus intensities increase. Application of the GABA A channel-blocker picrotoxin eliminates all subthreshold inhibitory postsynaptic potentials, revealing underlying excitation that is particularly obvious in the high-frequency range. Excitatory thresholds close to the song frequency remain unchanged by picrotoxin. Thus a specifically tuned neuron is shown to become broadly tuned after elimination of frequency-dependent inhibition. Although average maximum response strength is increased by 150% after picrotoxin application, at male song frequencies a slight reduction of the responses is still present at high intensities. Side-dependent inhibition remains largely unaffected by picrotoxin, suggesting that side- and frequency-dependent inhibitions are caused by different transmitters from different neurons.

INTRODUCTION

Inhibition is an extremely important factor in shaping neuronal response properties. Among many other functions, inhibitions may sharpen neuronal frequency tuning (vertebrates: Evans 1992; Fuzessery and Feng 1982; Suga 1989; invertebrates: Marquart 1985; Moiseff and Hoy 1983; Popov and Markovich 1982; Schildberger 1984; Stumpner et al. 1995). A broadening of excitatory tuning of neurons after removal of inhibition has been demonstrated in some vertebrate (but not yet invertebrate) species (e.g., Evans 1992; Fuzessery and Hall 1996; Suga et al. 1997; Sun et al. 1996; Yang et al. 1992). This effect can be tested, in principal, by application of selective blockers for inhibitory channels. Bicuculline, a frequently used blocker of inhibition in vertebrate studies (Macdonald and Olsen 1994) has proven ineffective in most insects (Benson 1993; Sattelle et al. 1991). The most frequently used blocker of inhibition in invertebrate studies has been picrotoxin, a known γ-aminobutrylic acid-A (GABA A) channel-blocker (Maynard and Walton 1975; Robbins and van der Kloot 1958).

GABA has been shown to be abundant in invertebrate nervous systems. There are ~10% (between 250 and 400 cells) of GABA immunoreactive neurons in the prothoracic ganglion of grasshoppers (Watson 1986) and crickets (Spörhase-Eichmann et al. 1989). In the metathoracic ganglion of grasshoppers, GABA immunoreactive neurons were identified that respond to acoustic stimuli (Sokoliuk et al. 1989; Thompson and Siegler 1991). One of these (TN1) is likely responsible for an intensity-dependent inhibition of ascending interneurons at low frequencies (Römer et al. 1981; Sokoliuk et al. 1989). Immunohistochemical and electron-microscopic studies using *Grillus bimaculatus* have revealed that a considerable proportion of identified synapses at AN1, an identified auditory interneuron, are GABA immunoreactive (Hardt and Watson 1994). However, another inhibitory interneuron (ON1) (Selverston et al. 1985) is known to be non-GABAergic, possibly using serotonin as a transmitter (Hardt and Agricola 1991; Spörhase-Eichmann et al. 1989). The degree to which excitatory input is actually overridden by inhibition is not known for crickets or any other invertebrates. Because picrotoxin has proven effective in the auditory system of a grasshopper (Römer and Seikowski 1985) it was deemed a suitable tool to investigate the extent to which responses of auditory neurons are shaped by patterns of excitatory and inhibitory interaction in other insects.

The bushcricket *Ancistrura nigrovittata* possesses an auditory interneuron (AN1) tuned to the male song frequency and receiving strong inhibition at lower and higher frequencies (Figs. 1 and 3) (Stumpner 1997). Dendrites of the neuron branch into the auditory neuropile of the prothoracic ganglion, overlapping largely with termination of primary afferents. However, afferents tuned to the same frequencies that excite AN1 only project on a restricted area of AN1’s dendritic arbor (Stumpner 1996, 1997). This result indicates that excitatory input into AN1 may be more broadly tuned than what appears when this neuron is receiving both excitation and inhibition. To demonstrate this directly, excitatory and inhibitory tuning [spike and inhibitory postsynaptic potential (IPSP) thresholds, respectively] in AN1 was measured before and after application of picrotoxin. Because Stumpner (1997) demonstrated that AN1 receives frequency-dependent excitation and inhibition from the soma-contralateral ear and direction-dependent inhibition from the soma-ipsilateral ear, experiments were performed to examine how the inputs of both ears shape AN1’s responses under influence of picrotoxin. This examination was done by the following techniques. 1) Registering the directional responses of AN1 before and after picrotoxin application to show whether picrotoxin affected directional responses of AN1 in the same way it affected frequency tuning. 2) Registering the directional responses of AN1 after picrotoxin application before and after cutting the soma-ipsilateral input to reveal whether side-dependent inhibition continued in the presence of picrotoxin. 3) Registering the excitatory and inhibitory tuning of AN1 with the soma-ipsilateral input sev-
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FIG. 1. Sample traces of AN1 responses of Ancistrura nigrovittata to soma-contralateral stimuli of selected frequencies and intensities before (top traces) and after application of picrotoxin (middle traces; bottom trace shows the stimulus). A: low and high frequency both evoking subthreshold inhibitory postsynaptic potentials (IPSPs) before application and medium frequency evoking no IPSPs; all elicit excitatory responses after application. B: 3 responses to same stimulus superimposed demonstrate reliability of responses before and after application.

METHODS

Animals

Both sexes of the bushcricket Ancistrura nigrovittata (von Wat tenwyl 1878) were used in this investigation. Experimental animals were laboratory-reared F1 and F2 generations of individuals captured wild in Greece.

Preparation and picrotoxin application

Electrophysiological methods used were described in detail by Stumpner (1997). In short, the procedure was as follows. An experimental animal was briefly anesthetized with CO₂ and fixed ventral side up to a plastic holder with a wax-resin mixture. The forelegs were fixed in a normal (inverse) standing position. The prothoracic ganglion was exposed and stabilized by a Ni-Cr spoon from below and a steel ring from above. Before starting the experiment, a tiny crumb of collagenase (Sigma) was laid on the air-exposed ganglion for 90–120 s followed by several washes with saline. This procedure was needed to facilitate picrotoxin penetration of the ganglionic sheath. Three experiments were conducted in which collagenase was not used. One served as a control for nonspecific effects of picrotoxin outside the ganglion housing AN1; in each of the other two the sheath was punctured with a broken electrode tip. Exposed tissue was bathed with Fielden’s saline (Fielden 1960). Thick-walled borosilicate glass capillaries filled with Neurobiotin (Vector, 5% in 1M K-acetate) were used for intracellular recording. Electrode resistances ranged between 80 and 160 MΩ. Recordings were amplified by using a DC amplifier, continuously displayed on screen and stored on a digital recorder (Sony). After physiological characterization a portion of the saline and hemolymph mixture covering the ganglion was carefully removed and 10–40 μL of 10⁻³ M picrotoxin (Sigma) dissolved in saline was added to the preparation. Then the neuron was again physiologically characterized. Picrotoxin was then either removed followed by three washes with saline (2 experiments) and/or the cell was stained ionophoretically (see Stumpner 1996 for developing procedure of the neurobiotin stain). AN1, which can be identified by its physiology, was continuously recorded from during and after picrotoxin application (12 cases) and stained at the end (9 of these cases); cells lost during picrotoxin application were repenetrated shortly after and identified morphologically after staining (2 cases). In two instances the soma-ipsilateral leg was cut before the picrotoxin experiment. In two other cases the soma-ipsilateral leg was cut after registering picrotoxin effects to reveal the independent influence of ipsilateral inhibition under picrotoxin (see RESULTS). The results presented in this paper are based on 11 AN1s recorded from females and 3 AN1s recorded from males. One of these experiments was a control animal in which (in addition to 5 more experiments without using collagenase) picrotoxin application had no effect on the neuron’s physiology (for possible explanations see DISCUSSION).

Stimulation

The mounted bushcricket was placed in an anechoic chamber with dynamic speakers (Dynaudio DF 21) on both the left and right at a distance of 37 cm from the animal. Acoustic stimuli were produced with a computer-controlled stimulator (Lang et al. 1993). In a standard test series, five 50-ms stimuli (1.5-ms rising and falling ramp) were presented at 250-ms intervals with frequencies ranging from 3 to 46 kHz and intensities from 30 to 90 dB SPL from the more sensitive (soma-contralateral) side. For directional tests, 100-ms stimuli (16 kHz, 2-ms ramps) were presented at 250-ms intervals at intensities ranging from 30–90 dB SPL with five repetitions from the left side followed by the same number from the right side at any given intensity. Calibration was done using a Bruel & Kjaer amplifier (2610) and Bruel & Kjaer microphones (‘4 or ‘5’). Repeated measurements gave an accuracy of ±2 dB.

Data evaluation

Digitized data were evaluated using the NEUROLAB program (Hedwig and Knepper 1992). Threshold values for excitatory tuning curves were defined as the intensity of sound of the respective frequency eliciting one spike above spontaneous activity in three of five stimuli. Thresholds of IPSPs occurring below the threshold
RESULTS

Principal effects of picrotoxin

AN1 is an auditory interneuron of male and female A. nigrovittata with its soma and dendrites in the prothoracic ganglion and an ascending axon with terminations in the protocerebrum (Stumpner 1997). Dendritic recordings of AN1 revealed strong subthreshold IPSPs at moderate intensities of both low frequencies (e.g., 8 kHz, 50 dB SPL) and ultrasonic frequencies (e.g., 38 kHz, 50 dB SPL; see Fig. 1A) (see also Stumpner 1997). At frequencies close to the peak of the male song spectrum (~15 kHz) (see Dobler et al. 1994) IPSPs were not elicited (Fig. 1A). Two to three minutes after application of 10–40 μl of 10−3 M picrotoxin to the hemolymph, IPSPs began to disappear. Stimuli previously evoking inhibition now evoked a spiking response in AN1 (Fig. 1), whereas stimuli previously excitatory at low intensities (12 and 16 kHz) remained excitatory after picrotoxin application (Fig. 1A). Spiking responses after picrotoxin application were elicited as reliably as IPSPs had been elicited before picrotoxin application (Fig. 1B). When hemolymph containing picrotoxin was removed and replaced with normal saline, the responses close to threshold remained purely excitatory for many minutes, frequently longer than the recording could be held. There were two cases, however, in which slight IPSPs became visible 12 and 19 min, respectively, after washing (in the latter case, this computed to 31-min post-application of picrotoxin). In another experiment in which the ganglionic sheath was not treated with collagenase, picrotoxin application produced no obvious effects on auditory processing of AN1. This result indicates picrotoxin did not enter the ganglion and further suggests that nonspecific actions of picrotoxin capable of changing AN1’s responses do not exist outside the prothoracic ganglion. A repeated frequency scan (70 dB SPL; Fig. 2B) using the same preparation underscored that suggestion. Although some variability in responses between 12 and 20 kHz did occur, inhibition at both low frequencies (5 kHz) and high frequencies (~34 kHz) remained intact over a time period of 12 min after picrotoxin application.

Changes of tuning after picrotoxin application

AN1 is tuned to frequencies of the male song (peaking at ~15 kHz), when the threshold for eliciting spikes is measured. Lower intensity stimuli <12 and >20 kHz (Fig. 3, ■ and ×, respectively) evoked IPSPs. IPSPs were more clearly visible in females than in males at low frequencies (<12 kHz). Application of picrotoxin changed the tuning: all IPSPs disappeared and thresholds for excitation dropped at those frequencies that previously elicited subthreshold inhibition (Fig. 3). At ultrasonic frequencies, thresholds of excitation after picrotoxin application and thresholds of inhibition before application matched nearly perfectly, in males as well as in females (Fig. 3, A and B). Excitatory thresholds after application were significantly lower than before application at frequencies >24 kHz (t-test, P < 0.05 or P < 0.01), whereas inhibitory thresholds before application and excitatory thresholds after application did not differ significantly. As a consequence, in the complete frequency-intensity field of stimuli >20 kHz eliciting IPSPs in an untreated animal, excitation occurred after picrotoxin application. Although picrotoxin application seemed to lower thresholds for excitation at 6 and 8 kHz (both sexes), average threshold of inhibition before application was even lower (Fig. 3A). Because differences between inhibitory and excitatory thresholds at low frequencies were ≤10 dB before treatment, the effect of picrotoxin on thresholds at low frequencies was hard to detect. At these frequencies excitatory thresholds were not significantly different before and after application (t-test, P > 0.05). In conclusion, all IPSPs disappeared after picrotoxin application and excitatory tuning of AN1 became considerably broader, especially in the high-frequency range. However, thresholds were still ~10 dB higher than the hearing threshold of
the whole ear as calculated from summed recordings of the tympanic nerve (see Dobler et al. 1994), except for the range between 16 and 20 kHz.

Included in Fig. 3A are data from two females in which the soma-ipsilateral leg was cut before the experiment. This experiment was done to test whether soma-ipsilateral inputs that were demonstrated to be of no relevance for normal excitatory and inhibitory tuning using stimuli from the more sensitive (soma-contralateral) side (Stumpner 1997) respond similarly after picrotoxin application. In fact, after picrotoxin application the frequency-dependent responses of AN1 with the soma-ipsilateral input cut did not differ from those of intact animals; data of lesioned and intact animals are thus shown together in Fig. 3A.

Latencies of pretreatment IPSPs and posttreatment excitatory postsynaptic potentials (EPSPs) using the same stimulus parameters were on average quite similar (IPSPs: 21.8 ± 5.7 ms; EPSPs: 18.5 ± 4.6 ms; n = 44). In the range between 24 and 30 kHz, most EPSP latencies were shorter than IPSP latencies, on average by 7.0 ± 4.9 ms (n = 16).

At those frequencies it was not unusual to see compound potentials instead of pure IPSPs before application. In some of the compound potentials, EPSP onset was clear enough for latency to be measured. Those EPSP-latencies were nearly identical before and after treatment (difference of 0.4 ± 1.4 ms, n = 5).

Changes of intensity-dependent responses after application of picrotoxin

Responses of AN1 in females without picrotoxin application depend on intensity, and this dependence varies with frequency (Fig. 4C) (Stumpner 1997). At most frequencies a clear peaked intensity-response function is found with a strongly reduced response at high intensities. After picrotoxin application suprathreshold responses became clearly more tonic than before application. The compound EPSPs and IPSPs typically underlying action potentials of AN1 in response to high-intensity stimulation were not observed after treatment (Fig. 4A, A and B). Maximum response strength increased on average by 150%. Intensity-response functions at some frequencies now resembled saturating functions (e.g., 28 kHz; Fig. 4C), whereas at other frequencies a decline at high intensities was still observed (e.g., 16 kHz; Fig. 4C). However, this decline was much weaker than in untreated neurons (e.g., 30% of the maximum response at 16 kHz compared with 70% before). After picrotoxin application the maximal increase in spike number per standard stimulus varied from 7 to 9 spikes at all frequencies, irrespective of the number of spikes that frequency elicited before treatment (Fig. 4D). This maximal increase was observed at moderate intensities by using 16-kHz stimulation, whereas at 8 and 28 kHz the largest increase was observed at the highest intensity tested (Fig. 4D). A different picture is seen, however, when relative response changes are calculated. Frequencies exhibiting subthreshold inhibition before treatment (e.g., 8 and 28 kHz) showed the largest relative changes close to IPSP thresholds (Fig. 4E). At male song frequencies (e.g., 16 kHz), on the other hand, the greatest relative changes were seen at high intensities (intensities at which response reduction was strongest before treatment; Fig. 4E).

Changes of directional responses

AN1 of A. nigrovittata might contribute to song recognition and song localization in females because it receives frequency-dependent excitation and inhibition from the soma-contralateral side and inhibition from the soma-ipsilateral side (Stumpner 1997). Low-intensity stimulation from the soma-ipsilateral side with 16 kHz renders higher-threshold responses with a lower maximum than soma-contralateral stimuli, whereas at high intensities stimuli from both sides evoke similar responses (Fig. 5A, solid curves) (see also Stumpner 1997). Lesion experiments and comparison to receptor responses had shown that the left to right difference at high intensities is due to ipsilateral inhibition (Stumpner 1997). After picrotoxin treatment the response strength for stimulation from both sides was increased (Fig. 5A, dotted curves) as described previously (Fig. 4). The threshold difference between the responses to ipsi- and contralateral stimulation was not affected and the response to high-intensity stimuli (≥70 dB SPL) from both sides still elicited identical responses as before application (Fig. 5A). To directly demonstrate that soma-ipsilateral inhibition remains in effect after picrotoxin application, additional experiments were conducted in which the soma-ipsilateral leg was cut after the response changes through picrotoxin were
FIG. 4. Changes of intensity-dependent responses in AN1 of females. A and B: responses at 16 and 28 kHz before and after picrotoxin application. Intensities are indicated below stimulus traces. C: intensity-response functions at 8, 16, and 28 kHz before and after picrotoxin application (n = 6). D: change in spike number caused by picrotoxin application (same data set as in C). E: relative change in response strength induced by picrotoxin. The peak at 8 and 28 kHz demarks intensities with subthreshold IPSPs before picrotoxin application. Error bars in C and D represent SE of the means.

documented; directional responses were then measured again (Fig. 5B). At high intensities this produced responses that were more effective than soma-contralateral responses. The shift induced by removing the ipsilateral inhibition is indicated by the shaded area in Fig. 5B. The responses to both sides are not completely symmetrical, which might be an effect of the intensity steps used, but probably was also influenced by biophysical differences of sound waves reaching a given ear from different sides of the animal.

DISCUSSION

Did picrotoxin specifically block inhibitory inputs?

In this study picrotoxin remained ineffective unless the sheath was punctured by a broken electrode or treated with collagenase, indicating the sheath serves as an efficient barrier against picrotoxin (see control animals). In all experiments with collagenase pretreatment, an effect of picrotoxin on responses of AN1 was observed.

Freeman (1973) described nonsynaptic effects of picrotoxin on axonal membranes, when high doses of picrotoxin (starting at $5 \times 10^{-3}$ to $1 \times 10^{-4}$ mol/l) were continuously superfused over the preparation. These effects included considerable broadening of action potentials and increased excitability to electrical stimulation. In this study $10-40 \mu l 10^{-3}$ M picrotoxin in saline were applied to the preparation. Several observations indicate that changes in the responses of AN1 were not caused by nonsynaptic effects of picrotoxin. First, it must be considered that a singular application of picrotoxin will be considerably diluted in the hemolymph and saline mixture surrounding the ganglion (roughly estimated to be $<10^{-2}$ mol/l). Second, the greater portion of the sheath covering the prothoracic ganglion remained untreated with collagenase and therefore continued to serve as a barrier against picrotoxin throughout the experiment. As a consequence, the actual concentration of picrotoxin within the neuronal tissue housing the neurons under investigation was almost certainly $<10^{-5}$ mol/l and therefore below the threshold for nonsynaptic effects on superfused axons (Freeman 1973).

Furthermore, several physiological arguments support the assertion that picrotoxin exerted specific synaptic effects only. A broadening of action potentials was never observed (see Figs. 1 and 4, A and B). At frequencies at which no IPSPs were seen before treatment (e.g., 12 and 16 kHz), responses close to threshold remained unaffected by picrotoxin (Figs. 1, 3, and 4A). Also, number of spikes per standard stimulus observed in AN1 after picrotoxin application were comparable with those of other broadly tuned auditory neurons exhibiting tonic responses in the absence of picrotoxin (10–20 spikes/stimulus in ON1 and AN3, a sister cell of AN1; 11–19 spikes/stimulus in treated AN1). Finally, in AN3, which does not receive obvious inhibitory inputs, application of picrotoxin was without effect (unpublished observation).
due to an inhibition that is not eliminated completely after is the local interneuron ON1. First, ON1 of crickets failed ipsilateral acoustic input had been cut excluding any directionality not mediated by a GABAergic neuron. A probable candidate to encounter some reduction of response strength of auditory Agricola 1991; SpoÈrhase-Eichmann et al. 1989; Watson 1994) . The roll-off at about excitatory input at different frequencies. In Fig. 4 the high-frequency flank of AN1’s excitatory response singing (Dobler et al. 1994; Stumpner 1997), whereas after treatment AN1 was nearly as unselective for the male song frequency as is true for other auditory interneurons (e.g., ON1 and AN3) (unpublished observations). One can thus conclude that frequency-dependent inhibition in AN1 covers a broad frequency range of excitatory synaptic input that is overridden by the inhibition under normal conditions. Available evidence suggests that AN1, like its homologous cell in crickets (Hennig 1988), receives excitation directly from auditory afferents (Römer et al. 1988), whereas inhibition is believed to be evoked by interneurons, some of which are GABA immunoreactive (e.g., Hardt and Watson 1994).

Even with the broad tuning under picrotoxin, AN1 was less sensitive than the whole hearing organ except at frequencies exhibiting equal sensitivities for both before application (14–20 kHz). Before treatment excitatory tuning relatively closely resembled the behavioral threshold of female response singing (Dobler et al. 1994; Stumpner 1997), whereas after treatment AN1 was nearly as unselective for the male song frequency as is true for other auditory interneurons (e.g., ON1 and AN3) (unpublished observations). One can thus conclude that frequency-dependent inhibition in AN1 covers a broad frequency range of excitatory synaptic input that is overridden by the inhibition under normal conditions. Available evidence suggests that AN1, like its homologous cell in crickets (Hennig 1988), receives excitation directly from auditory afferents (Römer et al. 1988), whereas inhibition is believed to be evoked by interneurons, some of which are GABA immunoreactive (e.g., Hardt and Watson 1994).

In all experiments using picrotoxin, some reduction in spike number to high-intensity (12 or 16 kHz) stimuli was seen and resulted in peaked intensity-response functions (Fig. 4C). Relative reductions were much smaller, however, than before treatment. This was true also when the soma-ipsilateral acoustic input had been cut excluding any directional effects (see next section). This reduction might be due to an inhibition that is not eliminated completely after picrotoxin application. On the other hand, it is not unusual to encounter some reduction of response strength of auditory receptors ≥40 dB above threshold (unpublished observations). This effect might at least contribute to the peaked intensity-response functions of AN1 after picrotoxin application.

Changes in intensity-response functions seen before and after picrotoxin application might also provide information about excitatory input at different frequencies. In Fig. 4D it is obvious that the increase in absolute spike number after picrotoxin application has a steeper slope at 16 kHz than at 8 or 28 kHz. The largest increase at 16 kHz occurs 20 dB above threshold, whereas it was found 40–50 dB above threshold for 8 and 28 kHz. This difference might indicate that AN1 receives its strongest excitation from auditory receptors tuned to the male song frequency and therefore its input at this frequency is nearly saturated 20 dB above threshold, a typical response for the individual receptor to the type of stimuli used here (Stumpner 1997; unpublished observations). Other receptors (tuned to frequencies lower or higher than the male song) might excite AN1 with a lower efficacy. See Pollack (1994) for an example of frequency-dependent efficacy of excitatory input to an auditory interneuron. At higher intensities receptors tuned to the male song are excited by these other frequencies as well and could still evoke an increase in AN1’s responses.

Directional responses of AN1

AN1 receives a clear soma-ipsilateral (side-dependent) inhibition. Removal of this input by cutting the leg containing the tympanic nerve nearly eliminates the difference in shape of response functions of AN1 to stimuli from the left and right side at 16 kHz (whereas the threshold difference remains unaffected because it is caused by peripheral directionality) (Stumpner 1997). Picrotoxin application, however, did not have the same effect. Although the difference between maximal responses to stimuli from both sides decreased with picrotoxin, the curves still differed in shape. The increase of AN1’s responses to soma-ipsilateral stimuli after the additional removal of ipsilateral input demonstrated that at least one important fraction of soma-ipsilateral inhibition is not sensitive to picrotoxin and, therefore, probably not mediated by a GABAergic neuron. A probable candidate for this side-dependent, but picrotoxin-insensitive inhibition, is the local interneuron ON1. First, ON1 of crickets failed to exhibit GABA immunoreactivity (Gryllus: Hardt and Agricola 1991; Spöhrase-Eichmann et al. 1989; Acheta: F. J520-7/ 9k28$$my04 04-13-98 07:17:55 neupa LP-Neurophys

FIG. 5. A: directional responses in AN1 of females (n = 5) before and after picrotoxin application. Ipsi and contra refer to soma position. Note that response functions for ipsi and contra retain different shapes at intensities >70 dB SPL even after picrotoxin application. B: change of response functions by removal of soma-ipsilateral input after picrotoxin had been applied before. Shaded area, difference between ip PTX and ip PTX + cut, which compares to the amount of soma-ipsilateral inhibition insensitive for picrotoxin. Response functions were normalized to maximum response before and after the cut (n = 2).
Lang and A. Stumpner, unpublished observations). Second, AN1 in crickets (the homologous neuron to AN1 of *A. nigrovittata*) receives a side-dependent inhibition by the local neuron ON1 (Horseman and Huber 1994; Stumpner et al. 1995).

**Organization of auditory system in ensifera**

The results presented in this paper allow some general hypotheses about the organization of the auditory pathways in *A. nigrovittata* and possibly in ensifera in general. The majority of auditory neurons encountered in the prothoracic ganglion, the first center of auditory processing, exhibit relatively broad frequency tuning compared with auditory receptors (e.g., Rheinlaender 1975) (for the only exception described thus far see Oldfield and Hill 1984). These interneurons are then excited by more than one of the many receptors with differing best frequencies (e.g., Lin et al. 1993; Oldfield 1988). What, then, is the basis for the known differences in frequency selectivity between interneurons? The results of this study indicate it may be due to additional inhibitory influence overriding excitation in certain frequency-intensity ranges and not because of restricted receptor input. Although examples of restricted tuning without noticeable inhibition exist as well (e.g., for descending neurons in crickets and bushcrickets see Atkins and Pollack 1987; Wohlers and Huber 1982; unpublished observations), these cells seem to be relatively similar in different species, whereas there are distinct differences between species regarding the frequency range of the highest sensitivity of neurons like AN1 (Schul 1997; Stout et al. 1988; Stumpner 1997; Wohlers and Huber 1982). It is conceivable that evolutionary change of acoustic communication in bushcrickets with respect to frequency tuning involves changes of interneuronal (e.g., inhibitory) pathways more than changes of the connections of primary afferents to these neurons. To put these observations on a broader experimental basis, comparative studies on the effects of blocking frequency-dependent inhibitory inputs to auditory interneurons should be performed now with bushcricket species that use other frequency ranges for their songs and with crickets.

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