J Comp Physiol A (1985) 156:845–860

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Responses to model songs of auditory neurons in the thoracic ganglia and brain of the locust

Heiner Römer and Ulrich Seikowski
Lehrstuhl für Allgemeine Zoologie, Ruhr-Universität Bochum, D-4630 Bochum 1, Federal Republic of Germany

Accepted February 19, 1985

Summary. 1. Locust interneurons in the ventral nerve cord and the brain were tested with models of the stridulatory song. These models had the conspecific frequency composition (Fig. 1) but were varied with respect to the duration of the interchirp-interval.

2. In most ascending interneurons such model songs elicited complex responses comprising EPSP and IPSP of different thresholds and amplitude. As a consequence of EPSP and IPSP interaction neurons exhibit optimised intensity-response characteristics with each having a 'best intensity' at a different absolute intensity value (Figs. 2–4). Only a few neurons show sigmoid IR-characteristics; these are little inhibited or not at all.

3. The strength of inhibition seems to be correlated with the degree to which ascending interneurons habituate to a repeated stimulus (Fig. 5). When blocking the inhibitory synapses pharmacologically with picrotoxin, habituation is strongly reduced (Fig. 6). The suprathreshold responses of some neurons thus match the amplitude modulation of the conspecific song whereas others do not and give only on-responses. No tuning to the conspecific interchirp-interval was observed in ascending interneurons (Fig. 7).

4. In the brain (supraoesophageal ganglion) 5 out of 24 recorded interneurons responded selectively to the temporal configuration of the conspecific model song (Fig. 9). In contrast to ascending neurons these units failed to demonstrate a matching of their spike response with the chirp structure of the model song.

5. Such brain interneurons are not only tuned to the conspecific temporal configuration of the song, but also to a rather narrow range of sound intensity where the response is maximal (Fig. 10).

6. The results are discussed with respect to neural processing of auditory information at different levels of the afferent pathway and with respect to the selectivity of the recognition process as observed in the behaviour of other grasshoppers.

Introduction

Many orthopteran insects communicate by means of auditory signals which are essentially amplitude modulated and one of the problems they face is recognising the difference between the call of a conspecific from that of a congeneric. Indeed, the acoustic repertoire sometimes forms the only isolating barrier between two species (Perdeck 1957; Alexander 1960). Behavioural experiments with crickets, bushcrickets and grasshoppers have shown that they can recognize a song produced by a conspecific and that some temporal parameters, such as syllable or chirp rates and intervals, are more effective than others in activating the innate releasing mechanism for the phonoresponse and for phonotaxis (Weih 1951; Haskell 1958; Walker 1957; Dumortier 1963; Bailey and Robinson 1971; von Helversen 1972; Weber et al. 1981; Thorson et al. 1982; Popov and Shuvalov 1977).

A great many electrophysiological studies have been performed on the auditory pathway of various orthopterans in an attempt to elucidate the neuronal mechanisms underlying song recognition. In contrast to directional coding, which seems to take place at the level of the ventral nerve cord

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Abbreviations: SOG suboesophageal ganglion; *EPSP* excitatory postsynaptic potential; *IPSP* inhibitory postsynaptic potential; *AM* amplitude modulation; *SPL* sound pressure level; *IR-characteristic* intensity response characteristic; *AN* ascending neuron (for review see Rheinlaender 1984), song recognition appears to be centred at some higher level within the nervous system. It is true, however, that neurons in the VNC have been found in each of the three orthopteran groups whose activity quite accurately copies the amplitude modulation of the song being transmitted and the existence of such units must be regarded as a prerequisite for pattern recognition (Boyan 1984a). In addition, some interneurons respond most sensitively to the song of the conspecific because of their tuning properties although at higher sound intensities they will also respond to a congeneric's song (Rheinlaender et al. 1976). However, none of these neurons in the VNC respond specifically to those song parameters which elicit a phonoresponse or phonotaxis.

Although it is still possible that the neural elements underlying song recognition are located in the ventral nerve cord but have remained undetected, it is more probable that the neural circuitry envolved in species-specific recognition is located in the brain. Huber (1960) could elicit the walking movements associated with phonotaxis by electrical stimulation of the brain; furthermore, the animal could be forced to turn aside by appropriate stimulation. In addition certain lesions within the brain result in the loss of phonotaxis (in walking or flying animals; Huber, Pollack, personal communication) although locomotion was unaffected. And recently, Schildberger (1984) has demonstrated the existence of local interneurons in the cricket brain which possess the properties of a temporal filter that match the selectivity of song recognition demonstrated behaviouraly.

For grasshoppers, the interchirp-interval has been reported to be a key parameter for song recognition (von Helversen 1972). Thus, in the present study we examined the response of single auditory neurons in the CNS of the locust to model songs (Dörrscheidt and Rheinlaender 1980) in which the interchirp-interval had been varied systematically. Some synaptic mechanisms for ascending interneurons are proposed which may explain their different responses to the amplitude modulated song. Furthermore, brain elements were recorded that might act as part of the innate releasing mechanism in acridid grasshoppers.

Materials and methods

The locusts used in the experiments were *Locusta migratoria* from the institute culture. Generally females 1–3 weeks after the last moult were used. The animals were anaesthetised with CO_2 and the wings, legs and mouthparts removed and the gut freed at the mouth. The preparation was then waxed to a holder by its ventral surface, the pronotum opened dorsally and the gut removed. If recordings were to be made from the brain, the head capsule was opened posteriorly and fat and muscle were removed. For recordings from the metathoracic ganglion, all peripheral nerves and abdominal connectives were cut except the tympanal nerve.

The holder bearing the animal was mounted at the centre of a sound proof recording chamber. Sound stimuli were presented via a loudspeaker (PH 10, Visaton) at a distance of 50 cm from the preparation. The stimuli were either pure tone bursts (Stimulator II, Burchard) or models of the locust's stridulatory song, which is composed of syllables (terminology of Elsner 1974) generated by the up and down strokes of one hindleg against the wing (see Fig. 1). By repeating this action, chirps composed of pairs of syllables are produced which are repeated 5–10 times within the song. Chirps in the male's song are separated from each other by an interchirp interval of 47.5 ms (±12 ms) when the ambient temperature is 25 °C (Dörrscheidt and Rheinlaender 1980). In the same study, the sound energy of the song was found to be distributed over a frequency range of 2.5 kHz to about 15 kHz with a maximum at around 7 kHz. For this study the computer-based technique 'modulation analysis' (Dörrscheidt 1978; Dörrscheidt and Rheinlaender 1980) was used to construct model songs in which the frequency spectrum and temporal structure of the chirp were very similar to the normal song (see Fig. 1) but the interchirp-interval could be varied independently. In practice, songs with intervals of 35, 60 and 47 ms were used, the latter representing the species-specific form and the former the limits of variation of the interchirp-interval. In addition, songs with intervals of 20, 100, 180 and 280 ms were generated, consisting of 6 or 10 chirps (serving as stimuli for preparations of the metathoracic ganglion and brain respectively). Model songs were generated with the computer (PDP 12) and stored on magnetic tape (Nagra IV, Austerlitz electronic). They were played to the animal via an amplifier (Braun, A 301) and the visaton speaker. The direction of sound incidence was 90° re to the longitudinal axis of the animal. For statistical reasons, each model song was played to the animal 10 times at intervals of 2.5 s. After 5 s the next model was given at the same intensity. The sequence of the models was always 47, 60, 180, 35, 100, 20, 280 ms, as identified by the interchirp-interval of the respective song models. Sound intensity was varied between 45 and 90 dB SPL, attenuated in steps of 1 dB or 10 dB. Sound pressure level was measured using a 1/2″ condensor microphone (type 4133, Brüel and Kjaer) coupled to a measuring amplifier (type 2607, Brüel and Kjaer) and is given in dB re 2×10^{-5} N/m^2.

The neuronal responses were analysed and represented as peristimulus-time (PST) histograms. The strength of the response (spikes/song) was calculated for the 10 presentations of each model. As a reference (zero response) spontaneous activity was measured for 30 s without stimulation.

Recordings were made from neurons either in the brain or metathoracic ganglion using thin-walled glass capillary microelectrodes which had resistances of 50–100 MΩ when filled with an 5% aqueous solution of Lucifer Yellow (CH). Dye-filled electrodes were used for intracellular recordings from the metathoracic ganglion whereas recordings from the brain were made extracellularly using electrodes filled with 3 mol/l KCl. In both cases the passage of the electrodes into the tissue was facilitated by desheathing a small area of the ganglion. After intracellular recordings had been made the penetrated cells were filled with Lucifer Yellow and later observed in wholemounts in order to discriminate between local neurons and neurons ascending to the brain. Neuron types are named according to Römer and Marquart (1984).

For pharmacological blocking of inhibitory synapses we

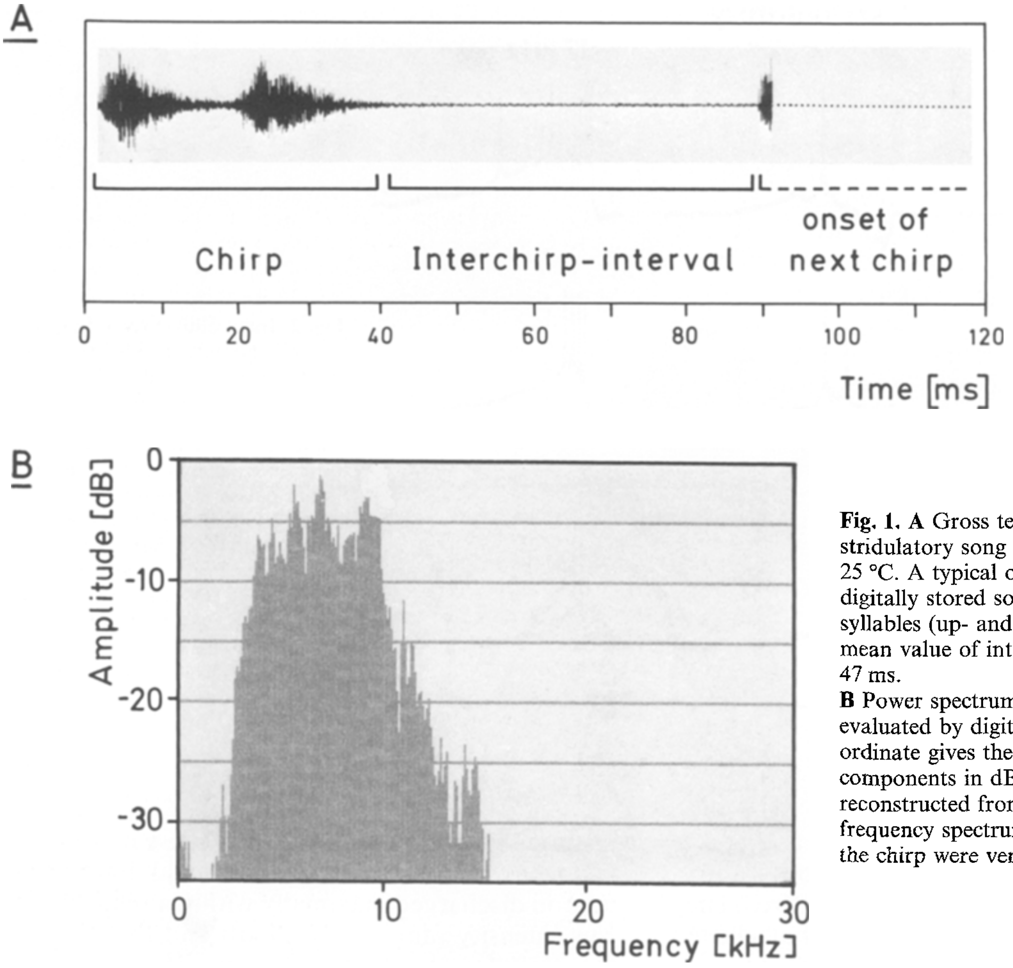


Fig. 1. A Gross temporal structure of the stridulatory song of *Locusta migratoria* at 25 °C. A typical oscillogram is plotted from digitally stored songs. The chirp consists of 2 syllables (up- and down stroke of the leg), mean value of interchirp interval is about 47 ms.
B Power spectrum of a chirp as in **A** evaluated by digital Fourier analysis. The ordinate gives the amplitude of the spectral components in dB. Model songs were reconstructed from these data in which frequency spectrum and temporal structure of the chirp were very similar to the normal song

used picrotoxin, which is a blocker of inhibitory synapses in general. There is conflicting evidence whether its effect may be mediated by the blocking of a Cl^- ionophore (Tackeuchi and Tackeuchi 1969; Marder and Paupardin-Tritsch 1978). In our experiments inhibitory potentials of all auditory interneurons recorded in the metathoracic ganglion were sensitive to low doses of picrotoxin (5×10^{-8} mol/l – 2.5×10^{-6} mol/l), applied to the whole metathoracic ganglion. In the same ganglion excitatory synapses seem not to be affected by similar doses of the drug. This was tested by recording the responses of SN1, a segmental auditory interneuron (Römer and Marquart 1984) which is not inhibited at all. The excitatory response of this neuron remained unaffected by the drug, even when the ganglion was bathed for more than 1 h in saline containing picrotoxin in a concentration of 2.5×10^{-6} mol/l. In no case a concentration of $> 5 \times 10^{-5}$ mol/l was used at which picrotoxin might begin to affect non-synaptic neuronal membrane (Freeman 1973).

Although the term 'habituation' derives from behaviour and means the decrement of a behavioural response that occurs when an initially novel stimulus is repeatedly presented it is also used to describe similar phenomena in single nerve cells. For auditory neurons of orthopterans, as in this study, the term is used equivalent to a gradual waning of the neural response, i.e., the amplitude of EPSP response or the spike number decrease with repeated presentations of the same stimulus (see Boyan 1984b).

Results

1. Ascending interneurons

Recordings were taken from a total of 47 ascending auditory interneurons either intracellulary, from their major processes in the metathoracic ganglion, or extracellularly at their terminal arborizations within the brain. Seven different types of neuron have been identified morphologically, most of them described by Rehbein et al. (1974) and Römer and Marquart (1984). These authors have shown that such neurons receive low and high frequency excitatory input and additional inhibitory inputs, elicited predominantly by low frequency sound. Thus the response to the conspecific song (which is a noisy signal, see Fig. 1) depends primarily on the degree to which each of these different inputs is activated. This is exemplified by the responses in Fig. 2 where it can be seen that the excitatory response to a pure tone of 4 kHz at threshold intensity is progressively suppressed by inhibitory inputs at higher intensities. In contrast,

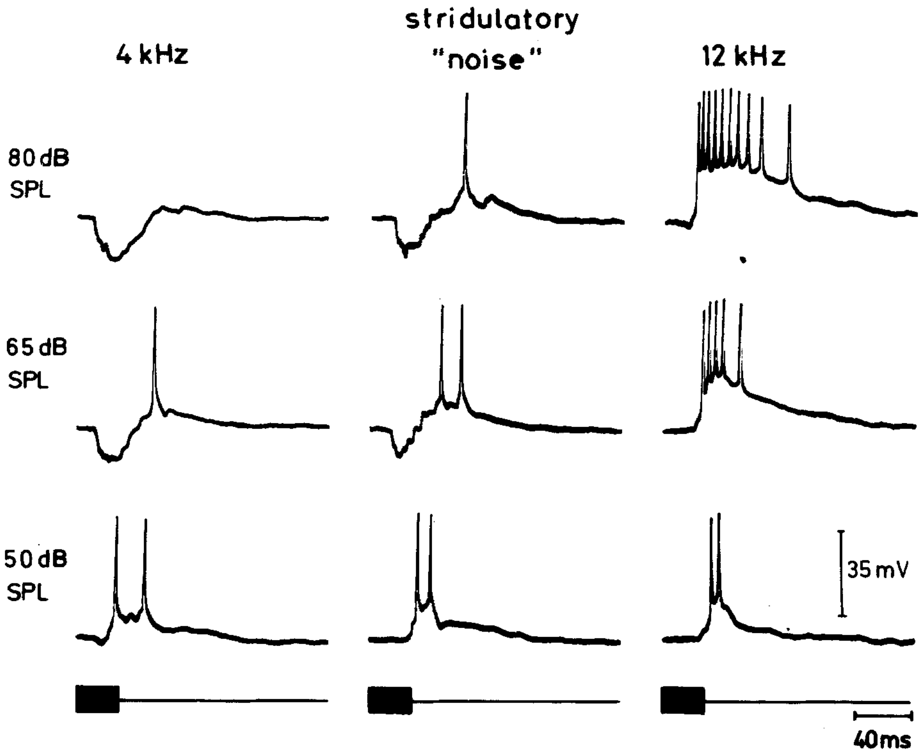


Fig. 2. Intracellular recording of synaptic activity of AN2 from its major processes in the metathoracic ganglion, in response to sound pulses of different frequency and intensity. Sound pulses were either pure tones (4 kHz and 12 kHz) or 'noisy' with a frequency spectrum very similar to the normal song (see also Fig. 1). Note that responses to the noisy sound pulse at 65 and 80 dB SPL consist of excitatory as well as inhibitory potentials

12 kHz stimulation elicits only EPSPs and spikes at all intensities. Thus the response to a 'noisy' sound stimulus containing low and high frequency components is intermediate between that elicited by the pure tones. At moderate and high intensities (65 and 80 dB SPL respectively) both EPSPs and IPSPs are elicited, although the earlier occurring IPSP inhibits most of the excitatory response (note the subthreshold EPSP activity at 65 and 80 db SPL). Although the interaction of excitation and inhibition and the relative threshold of the neuron to these inputs differ from neuron to neuron (Römer and Marquart 1984), the response of most ascending interneurons to the conspecific song strongly depends on the intensity of the stimulus (Fig. 3). This dependency is shown as an example for the underlying synaptic events in Fig. 3. The response of AN1 is resultant of two opposing effects, namely excitation at threshold and inhibition starting 5–10 dB above threshold. As a result of an intensity-dependent gradation of excitation and inhibition there is a rather narrow intensity range (10–15 dB) where the response of AN1 is maximal.

Most ascending neurons recorded responded like AN1 to variation of song intensity but the range of intensities over which the maximal response occurred was different from neuron to neuron, reflecting differences in the absolute threshold of excitatory and inhibitory inputs. Figure 4A illustrates such variation in the response of 4 identified neurons recorded from one animal. Each interneuron discharged maximally within a relative narrow intensity range of 10–20 dB and the 'best intensities' for the 4 neurons were 45, 55, 61 and 74 dB SPL, respectively. As the responses of all neurons were recorded in the same animal these differences in threshold and maximal response cannot be related to interindividual variation. The net effect of this is that variations in the stimulus intensity within a range of at least 30 dB are effectively encoded in the differential response of the ascending auditory interneurons by virtue of their optimized intensity characteristics.

The IR-characteristics of other auditory interneurons are different to those described above (Fig. 4B). In such cases the IR-characteristic is sigmoid and the response reaches a maximum at about 20–30 dB above threshold, although the absolute threshold values differ. This kind of intensity coding is very similar to that of tympanal receptor fibres (Römer 1976). Of the 47 ascending neurons investigated, the IR-characteristic of 38 exhibited a 'best intensity' (Fig. 4A) whereas only 9 exhibited a sigmoid relationship (Fig. 4B).

There is another consequence of EPSP/IPSP interplay for the neuron's response which becomes evident when the stimulus is not a singular sound pulse but a train of pulses at a pulse repetition

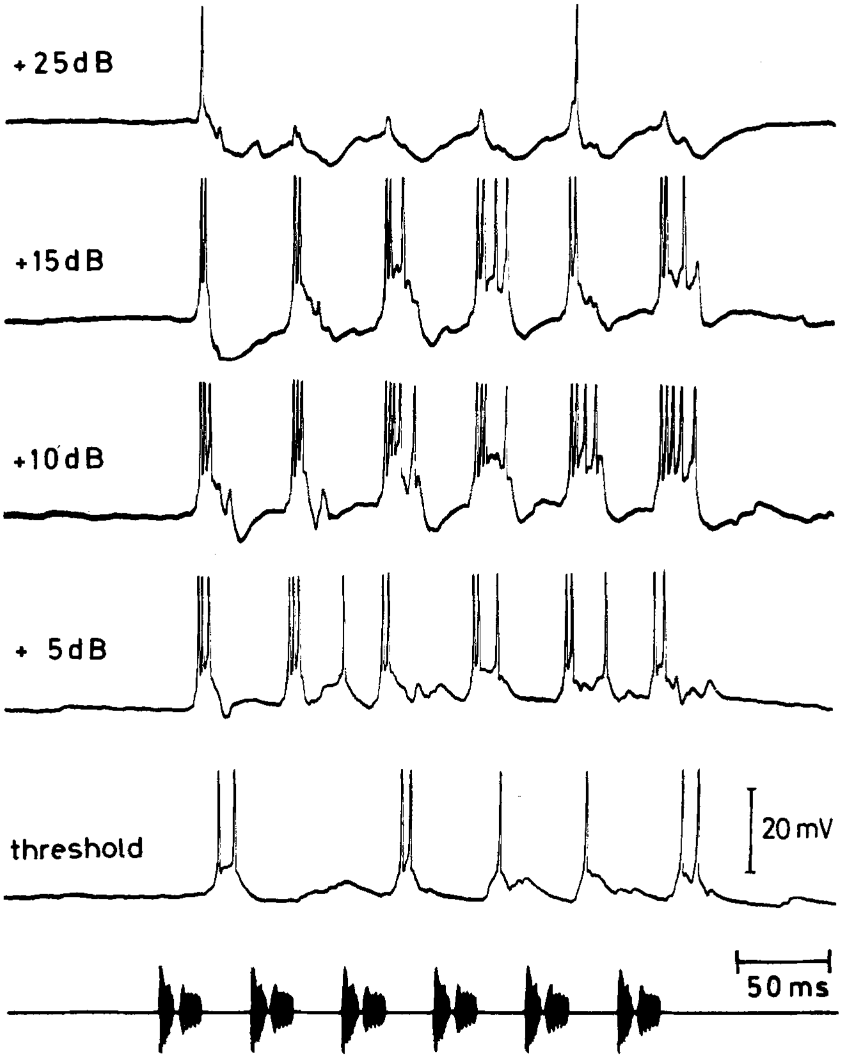


Fig. 3. Intracellular recording of synaptic activity of AN1 from its major processes in the metathoracic ganglion in response to model songs with the conspecific interchirp-interval, played at different intensities. At threshold (0 dB) there is only an excitatory response with little synchrony between the spikes and the chirps. At about 10 dB above threshold the response is maximal whereas inhibitory potentials suppress the EPSP at higher intensities to the extent that at 25 dB above threshold spikes are seldom elicited

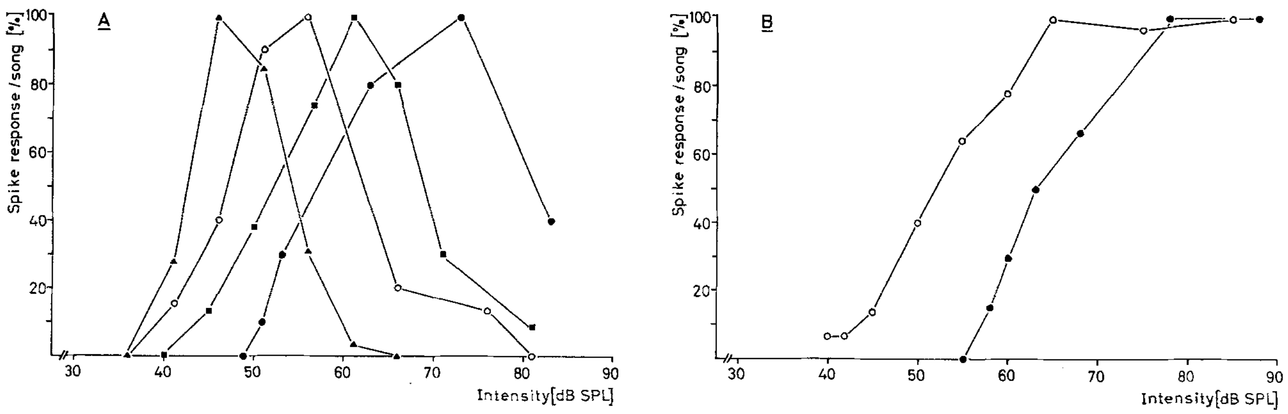


Fig. 4A, B. Intensity-response (*IR*) characteristics of 6 ascending interneurons to models of the conspecific song (interchirp-interval 47 ms). The response strength (as averaged no. of spikes per song) is plotted as a relative value, taking maximum response as 100% in each case. The responses of all neurons were recorded in the brain, their shortest latency was between 20 and 25 ms. **A** Response of 4 ascending interneurons recorded from one animal. Note that each neuron exhibits a 'best intensity' (range of maximum response) but that this range is different in each case. **B** Sigmoid IR-characteristics of two further ascending interneurons. Their slope is rather similar as is saturation at about 20 dB above threshold, which differs by 12 dB between the two neurons. Such IR-functions are similar to tympanic receptor fibres and local interneurons in the metathoracic ganglion

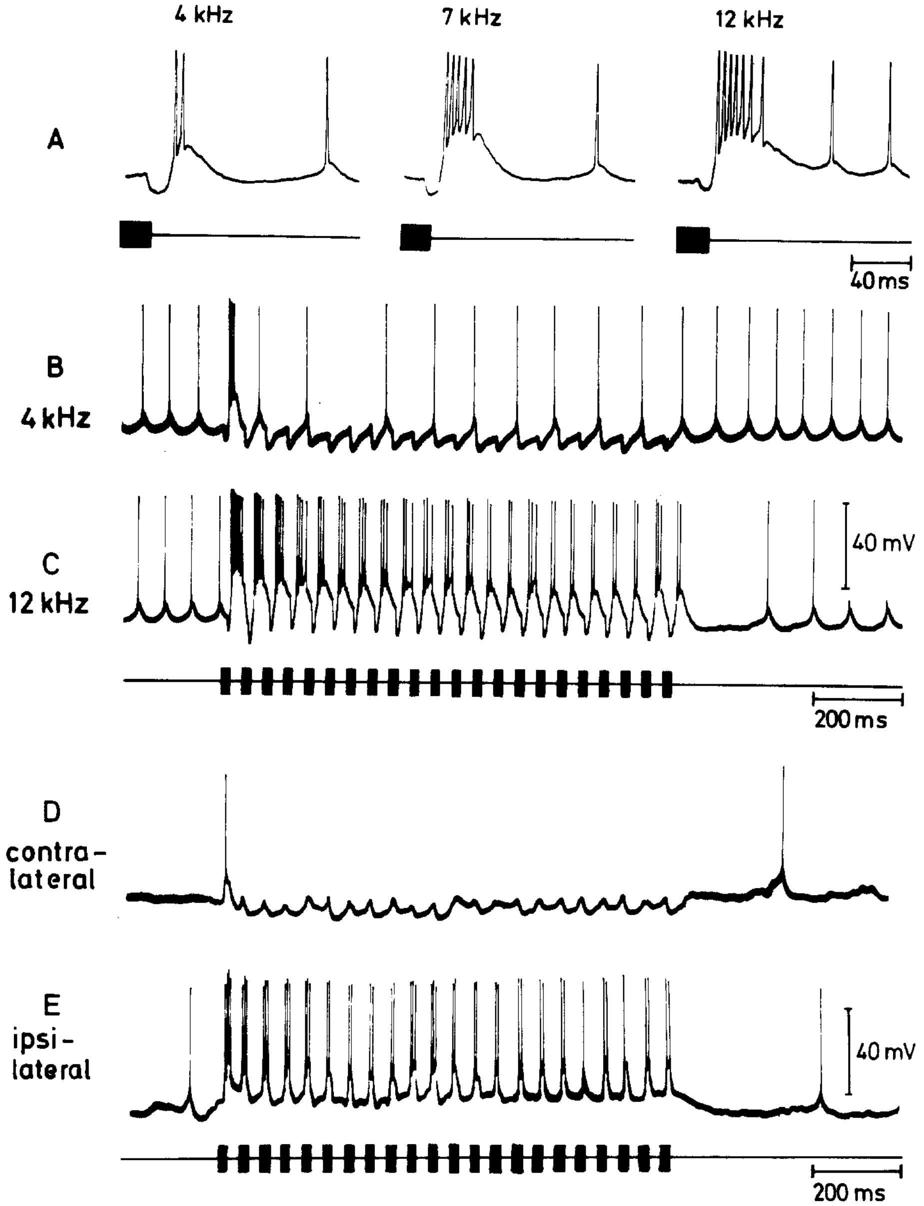


Fig. 5A–E. Intracellular recording of the synaptic activity of AN4 and AN1 from their dendrites in the metathoracic ganglion in response to single tone pulses (**A**) and pulse trains of different carrier frequency (**B** and **C**) and sound directions (**D** and **E**). Note that the stronger the inhibition is the stronger is the degree of habituation (**B** and **D**). Although there is still some habituation in the response in (**C**) even the last pulse within the pulse train gives rise to EPSP and spike response synchronized with the pulse. Stimulation 20 dB above threshold in each case, pulse repetition rate 1/s in **A**; 20/s in **B**–**E**. Carrier frequency of sound pulses in **D** and **E** 20 kHz

rate similar to that of the conspecific song. This is exemplified in Fig. 5 by the intracellular activity of AN4 to single sound pulses (Fig. 5A) compared to pulse trains (Fig. 5B, C). When the carrier frequency of the sound pulse is low (4 kHz) it elicits a stronger inhibitory than excitatory response and vice versa for 12 kHz. As a consequence the response to a pulse train habituates rapidly when the inhibition is stronger than excitation (Fig. 5B) and habituation is less (although still present) when excitation dominates (Fig. 5C). In the latter case each pulse within the train gives rise to suprathreshold responses. Thus the response of the neuron matches the temporal configuration of the stimulus quite well. Some ascending auditory interneurons of the locust receive strong side-dependent inhibition (Kalmring 1975; Römer and Dronse 1981; Römer and Marquart 1984) and so, for these neurons also, inhibition appears to be the main source of response decrement (Fig. 5D and E). When sound is applied to the tympanal organ on the axon side of AN1 the response is purely excitatory so that the EPSP activity and spikes copy the amplitude modulation of the stimulus quite well (Fig. 5E). In contrast, the same stimulus presented to the opposite ear elicits a weak on-response followed by IPSP activity for the whole sequence of sound pulses, demonstrating that response decrement can be extremely side-dependent. Similar to the two neurons shown in Fig. 5

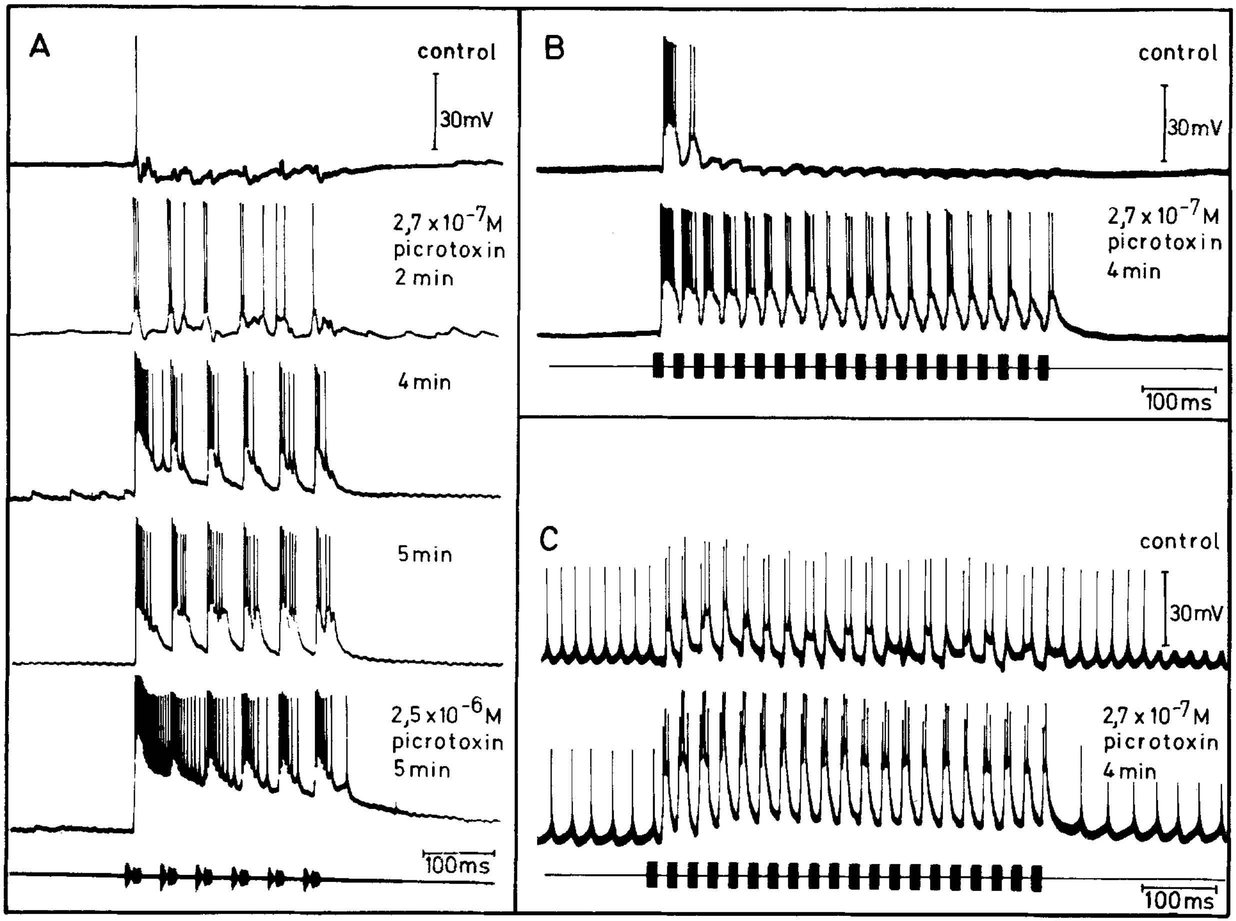
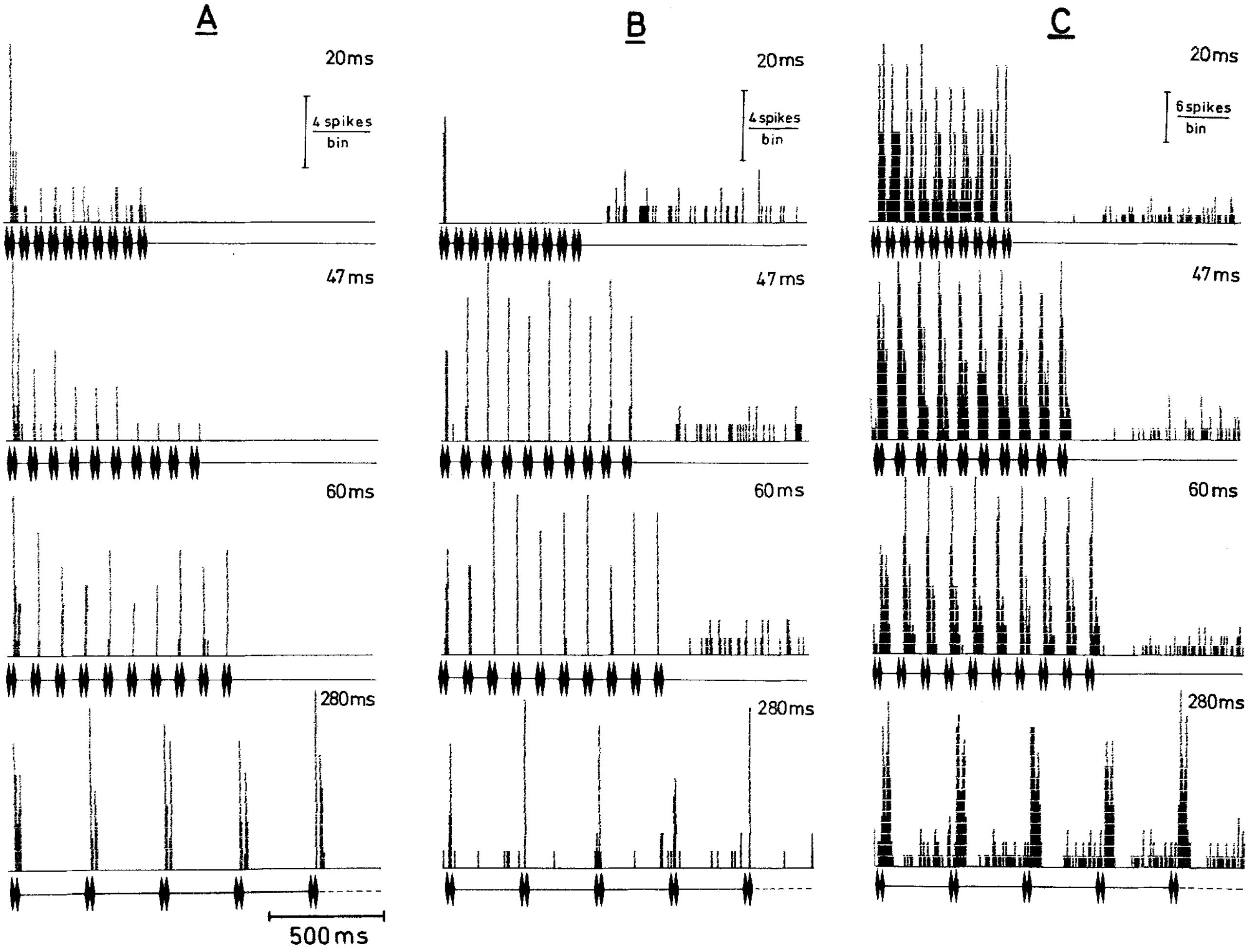


Fig. 6A–C. Effect of blocking inhibitory synapses on the responses of three interneurons in the metathoracic ganglion. **A** Responses of AN1 at various times after application of 2.7×10^{-7} mol/l picrotoxin. Note, that a first effect is visible as a reduction of IPSP amplitude and that at 5 min there is no response decrement in the response of AN1. **B** and **C** Responses of AN4 and AN3 before and 4 min after application of picrotoxin to the metathoracic ganglion. AN4 was hyperpolarized by a constant negative current of 0.5 nA to prevent spontaneous firing of the cell

most ascending interneurons with considerable IPSP activity exhibit habituation with repetitive stimulation, irrespective of whether inhibition is elicited either by stimulus frequency, intensity or stimulus direction.

If postsynaptic inhibition was indeed the major source of response decrement in these interneurons a blocking of inhibitory synapses should affect habituation remarkably. Cutting the contralateral tympanic nerve alone would not result in a total blocking of inhibition, as some interneurons receive as well ipsilateral inhibitory input (Kalmring 1975; Römer and Dronse 1981; Römer and Rheinlaender 1983). Thus we dissected the inhibitory input pharmacologically with the drug picrotoxin, which is a blocker of inhibitory synapses in general. The effect of such blocking on the responses of 3 ascending interneurons (AN1, AN3 and AN4) is shown in Fig. 6. In each of the 3 experiments recordings were made intracellularly for 2 min in normal saline (see control in Fig. 6A, B and C), then the Ringer's solution was changed to saline containing 2.7×10^{-7} mol/l picrotoxin, in which the metathoracic ganglion was kept under a constant flow of 5 ml/min. In Fig. 6A recordings of responses of AN1 to a locust model song are shown at different time after the drug application. The control response shows a compound response of EPSP and IPSP with the result of extreme response decrement due to membrane hyperpolarization (see Fig. 6A, control). Starting at about 2 min after drug application the IPSP amplitude decreased more and more, the EPSP amplitude reached spiking threshold and the cell started firing to each of the six syllables of the model song. After 5 min the response was purely excitatory and no response decrement was observed. Irregular and abnormal long lasting firing of cells only occurred



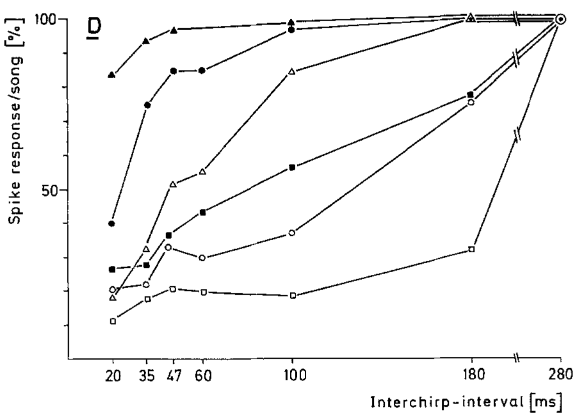


Fig. 7A–D. PST-histograms of the spike responses of 3 ascending interneurons (**A–C**) to song models with interchirp-intervals of 20, 47, 60 and 280 ms as indicated (only the first 5 chirp responses are shown for the last). The response of the neuron in **C** was recorded intracellularly in the metathoracic ganglion and identified as AN9 (Marquart, pers. communication) by staining with Lucifer Yellow, whereas those in **A** and **B** were recorded extracellularly in the brain. Their shortest latencies of 23 ms (**A**) and 21 ms (**B**) strongly indicate, that these neurons are also thoracic ascenders. Temporal configuration of the stimulus is shown below each histogram. Note that the response in **B** and **C** gives a clear copy of the stimulus when the interchirp-interval is that of the conspecific song whereas that in **A** does not because of habituation. Similar data taken from another 6 ascending interneurons are plotted in **D** with each point giving average response to 10 presentations of a song. Note that there is a gradation in the degree to which the different neurons become habituated to the stimulus. Stimulus intensity for all neurons was 20 dB above threshold. Bin width of histograms 6.2 ms

when the picrotoxin concentration was increased 10 times (see Fig. 6A, 2.5×10^{-6} mol/l picrotoxin). Two further interneurons (AN4 and AN3, Fig. 6B and C) were affected by picrotoxin similarly. The responses of both neurons to a repetitive pulse train showed remarkable habituation in the control response, whereas after only 4 min of drug application each sound pulse within the pulse train gave a clear EPSP and spike response, although there was still some inhibitory influence left in the response of AN4.

In order to test the responsiveness of the different ascending neurons to repetitive stimuli and to search for any specific responses to the temporal pattern of the conspecific song, animals were tested with model songs which had different interchirp-intervals. The degree to which the response of a cell matches the amplitude modulation of the song model is illustrated by PST-histograms from two ascending interneurons (Fig. 7A, B). The response of the neuron in Fig. 7A habituated the more, the shorter the interchirp-intervals are. As a consequence the response of the cell did not match the song except at very long intervals (280 ms). This contrasts to the neuron in Fig. 7B where each chirp within the model elicited a spike response of similar strength at all but the shortest interchirp-interval (20 ms). Finally, Fig. 7C gives the response of an ascending interneuron (AN9) the response of which matches the amplitude modulation of any model song presented. It shows some spontaneous activity and tonic spike discharge in response to each chirp in the sequence of the song. This neuron is not inhibited at all by any sound parameter. The responses of 6 ascending interneurons to the variation of interchirp-intervals are summarized quantitatively in Fig. 7D. Evidently, all units discharge maximally at the largest interchirp-interval; none of the neurons is tuned to the conspecific interval with respect to spike count. As a result of the different strength of habituation the responses to song models with conspecific interchirp-intervals may differ considerably, ranging from more than 90% down to 20% of the maximal response.

2. *Responses of auditory interneurons in the brain*

Extracellular recordings have been made from a total of 56 neurons in the brain (supraoesophageal ganglion). In each case the recording site was an area including the lower lateral lobe of the protocerebrum, the medio-dorsal protocerebrum and an adjacent part of the deutocerebrum. The former may represent an auditory neuropil in the locust as it is here that thoracic ascending interneurons and those from the suboesophageal ganglion terminate (Eichendorff and Kalmring 1980; Boyan 1983; Boyan and Altman 1985). Local auditory interneurons also have branches in this area (Boyan, personal communication).

Physiological criteria were used to distinguish between neurons ascending from the ventral nerve cord and those neurons located in the brain. The most reliable criterion was the latency of the response: more specifically, the shortest latency measured from responses to a number of different stimuli (recorded at an ambient temperature of 23 °C). Graphic presentation of the latencies of all neurons recorded in the brain (Fig. 8) shows that they fall into two groups. Those with latencies up to 35 ms respond to stimulation in the same way as the previously identified and described ascending neurons, which they are taken to be. Other neurons, with latencies of between 40 to 100 ms or more, very probably represent local neurons or possibly descending neurons, since such latencies were never found in thoracic ascending neurons. Thus, in general, cerebral neurons could be distinguished from ascending interneurons with some confidence.

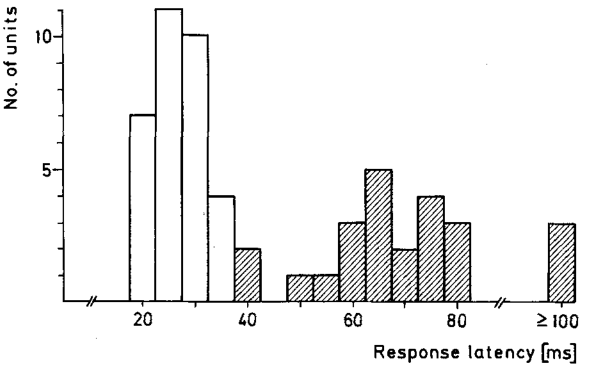


Fig. 8. Histogram of the response latency of 56 auditory interneurons recorded from the brain (see text). Two classes can be distinguished which can be identified as corresponding to interneurons ascending from the VNC (white columns) and brain neurons (hatched columns). The response of the two neurons with an intermediate latency of about 40 ms was characteristic of cerebral neurons as their spike discharge did not follow the chirp structure of the stimulus (see also Fig. 9). They are therefore taken as such though their latency is somewhat in between that of ascending and brain neurons

Compared with the relatively uniform response of ascending neurons to sound models, the responses of brain interneurons differed significantly. Some cells show a strong preference for the song with the conspecific interval (Fig. 9), others were inhibited by every model song (Fig. 11A) and a third group did not respond at all to model songs of any interval, but rather to pure, high frequency tones (Fig. 11B).

The responses of two brain interneurons are shown in Fig. 9A and B; both are excited maximally when a song model with the conspecific temporal configuration is presented. Such cells did not copy the temporal form of the stimulus in their spike discharges as well as did most ascending neurons (compare with Fig. 5), rather there was an overall excitation during the presentation of the song model. Quantitative analysis of these responses in Fig. 9C shows that the four neurons

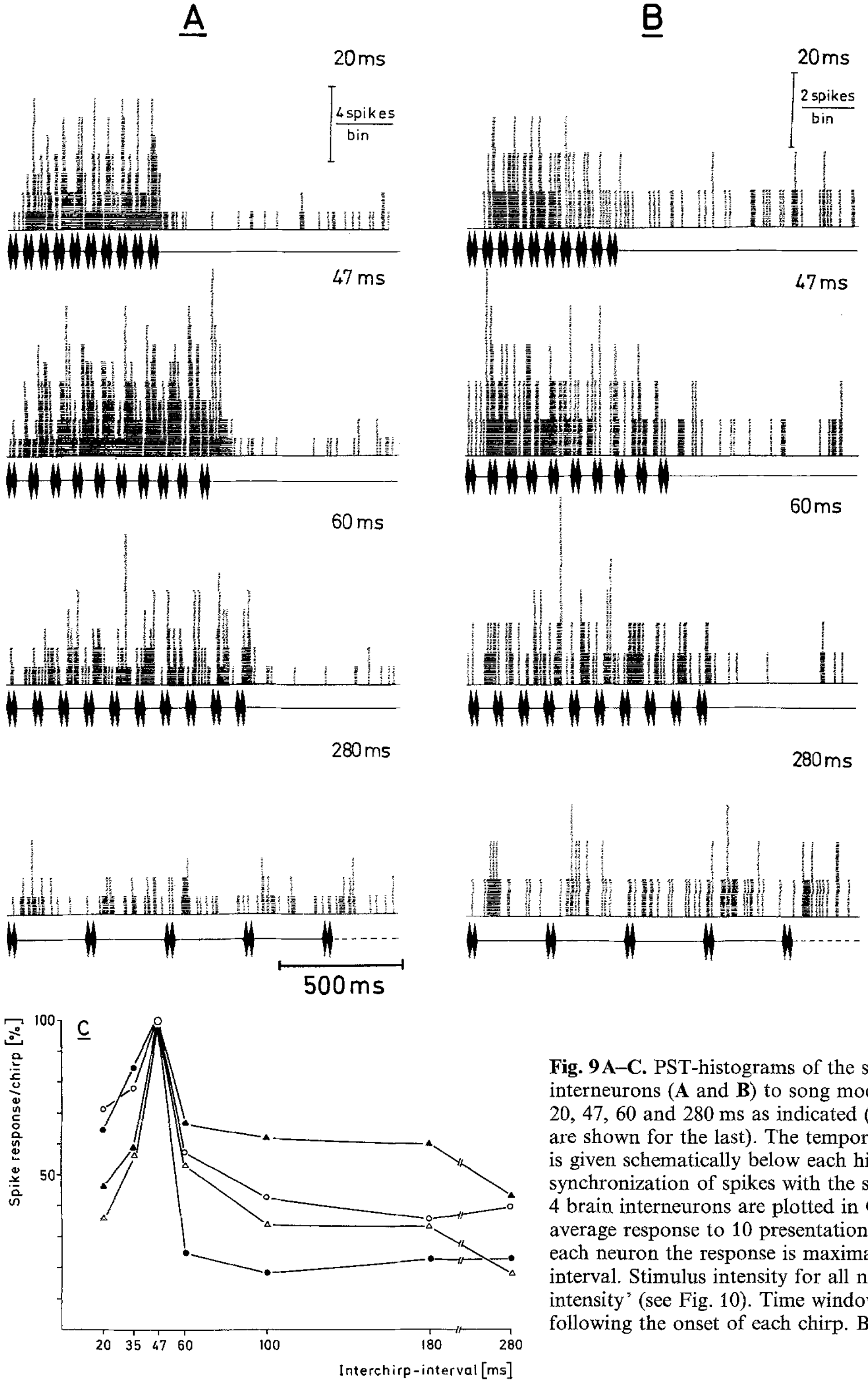


Fig. 9A–C. PST-histograms of the spike responses of two brain interneurons (**A** and **B**) to song models with interchirp-intervals of 20, 47, 60 and 280 ms as indicated (only the first five chirp responses are shown for the last). The temporal configuration of each stimulus is given schematically below each histogram. Note little synchronization of spikes with the stimulus. Similar data taken from 4 brain interneurons are plotted in **C** with each point giving the average response to 10 presentations of a model song. Note that for each neuron the response is maximal at the conspecific interchirp-interval. Stimulus intensity for all neurons was at their 'best intensity' (see Fig. 10). Time window for spike counting 100 ms following the onset of each chirp. Bin width of histograms 6.2 ms

are sharply tuned to the conspecific interchirp-interval with pronounced cut-off at both sides of the maximum. Only 5 of the 24 brain interneurons tested responded in this way. They had latencies of between 60 and 81 ms and exhibited low levels of irregular spontaneous activity.

Such cerebral interneurons responded to songs of different intensity in a way similar to most ascending interneurons, i.e. they exhibited a 'best intensity' (Fig. 10), although the cerebral neuron was more sharply tuned to this intensity than was the ascending neuron (Fig. 10C). Consequently, the

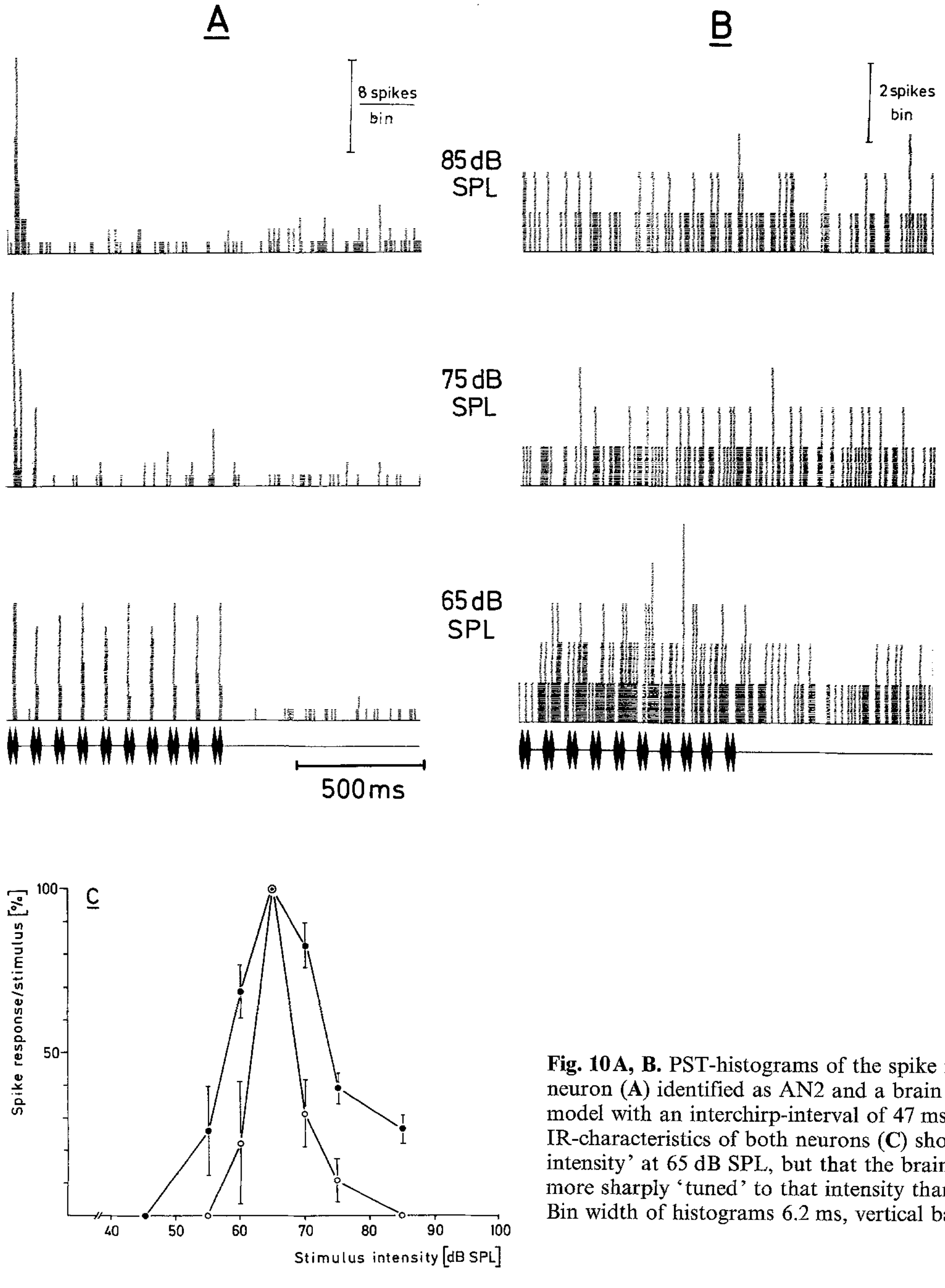


Fig. 10A, B. PST-histograms of the spike responses of an ascending neuron (**A**) identified as AN2 and a brain interneuron (**B**) to a song model with an interchirp-interval of 47 ms at three different intensities. IR-characteristics of both neurons (**C**) shows that they have a 'best intensity' at 65 dB SPL, but that the brain neuron (open circles) is even more sharply 'tuned' to that intensity than is the ascending interneuron. Bin width of histograms 6.2 ms, vertical bars in **C**: standard error

cerebral neurons are tuned both to the interchirp-interval of the conspecific song and to a rather narrow range of intensity.

Responses different from those described above were recorded in the brain with one group of 5 neurons being almost completely inhibited by song models with an interchirp-interval of up to 47 ms (Fig. 11 A). There was some excitatory off-response at the end of the song presentation. The inhibitory effect elicited by each chirp evidently lasted for about 80 ms (judging from the neuron's response to chirps 280 ms apart; Fig. 11 A), which is consistent with the total inhibition of spontaneous activity in response to songs with a shorter interchirp-interval. Such neurons showed no preference for the conspecific song model in either their inhibitory responses or excitatory off-responses both of which remained constant over the suprathreshold intensity range tested.

A third kind of interneuron (a total of 3 neurons, Fig. 11 B) gave no reaction – neither excitatory nor inhibitory – to any of the song models over

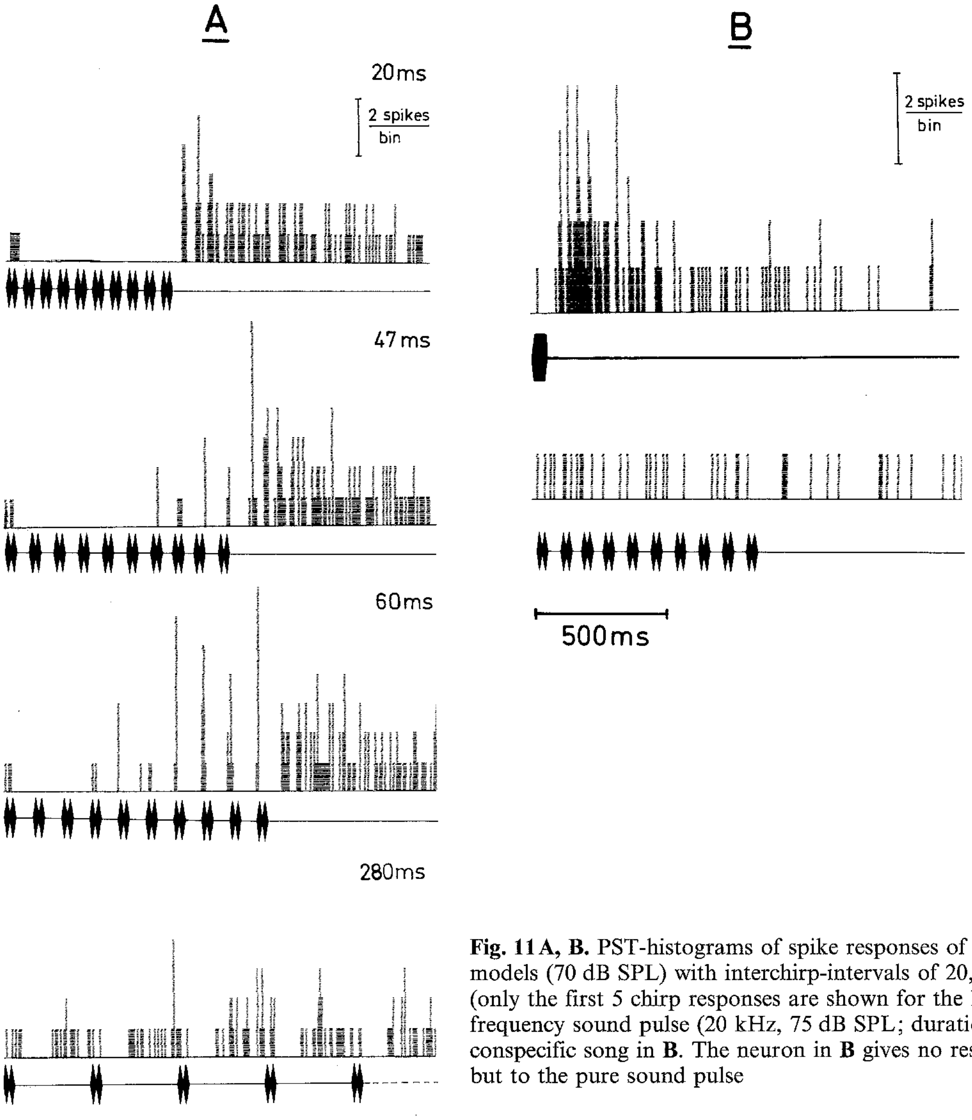


Fig. 11 A, B. PST-histograms of spike responses of two brain interneurons to song models (70 dB SPL) with interchirp-intervals of 20, 47, 60 and 280 ms as indicated (only the first 5 chirp responses are shown for the last) in **A** or to a high frequency sound pulse (20 kHz, 75 dB SPL; duration 50 ms) compared to the conspecific song in **B**. The neuron in **B** gives no response to the conspecific song, but to the pure sound pulse

the full range of intensities tested. Only when using pure tones of high frequency (12–40 kHz) and rather high intensities (about 65 dB SPL or more) did the neurons respond with a long lasting excitation. The response latency was between 42 and 60 ms. It seems probable that the energy of the high frequency components within the stridulatory song of the locust is too low to excite these neurons.

Discussion

Although there is a large body of literature dealing with the physiology and morphology of auditory interneurons at various levels of the CNS in the locust, the neuronal site of the putative conspecific signal analyser is still unknown. This is because in many studies either artificial stimuli were used or tape recordings of the conspecific song which could not be modified with respect to those parameters which are essential for species-specific recognition (Kalmring 1975a, b). This problem was overcome in the present study using a computer-aided technique of modulation analysis (Dörrscheidt 1978) by means of which the interchirp-interval of the songs was varied independently of either chirp substructure or the spectral composition of the song.

Thoracic ascending neurons

All the known responses of ascending auditory interneurons derive from elements identified as AN1–9 (Römer and Marquart 1984; Marquart, pers. communication) and the G-Neuron (Rehbein et al. 1974) which have their somata and postsynaptic branches in the meta- and mesothoracic ganglia, respectively. All these neurons do not respond specifically to model songs having the conspecific temporal configuration. Rather, their responses differ in the degree to which they habituate to a repeatedly presented stimulus, like the chirp sequence in the song (Fig. 7). Habituation to the conspecific song has also been reported by Kalmring (1975b), who speculated that the excitatory synapses underwent decrement without the participation of inhibition. The intracellular approach used in the present study indicates that the main cause of habituation at this level of the auditory pathway is a direct, postsynaptic inhibition, independently of whether such inhibition is elicited by stimulus frequency or by the stimulation of the contralateral tympanal organ (Fig. 5). Ascending neurons receiving no inhibitory input (Fig. 7C) and local interneurons in the metathoracic ganglion exhibit little or no habituation which is consistent with the finding that neurons in close connection with receptor fibres do not exhibit habituation (Horn 1967). That postsynaptic inhibition is the major cause of habituation in ascending interneurons is also indicated by experiments in which, after blocking the inhibitory synapses pharmacologically, little or no habituation occurred and all neurons responded like auditory receptor fibres and local interneurons (Fig. 6). Thus, response decrement of these metathoracic interneurons seems to be based on another mechanism compared to that described by Hawkins et al. (1983) and Walters and Byrne (1983) for *Aplysia*.

A further consequence of postsynaptic inhibition is that all neurons receiving inhibitory input exhibit optimum IR-characteristics which result simply from the opposing effects of excitation and inhibition in each neuron. A gradation of best intensities exists between different ascending neurons due to their different thresholds of excitatory and inhibitory inputs (see also Römer and Marquart 1984). As stimulus intensity is mainly a function of distance between the sender (male) and the receiver (female), a set of neurons each having a narrow range of best intensity may well be used to encode the distance to the sound source.

It is interesting to note that frequency dependent excitatory and inhibitory effects have been reported for many auditory interneurons (Wohlers and Huber 1978; Boyan 1981), although the site of the inhibitory input often remained unclear because extracellular recordings from axons were made (Moiseff and Hoy 1983; Boyd et al. 1984; Hutchings and Lewis 1984). There are, however, different interpretations about the functional significance of such a phenomenon, depending on the kind of signal in different species and the auditory behaviour these signals will elicit. For example, in the cricket *Gryllus bimaculatus*, which has a calling song at 5 kHz and a courtship song at 15 kHz, Boyan (1981) reported responses of an auditory neuron to a 15 kHz tone being suppressed by the frequency of the calling song. This author proposed a model with high and low frequency networks, each being activated and resulting in the appropriate behaviour when the corresponding fundamental frequencies would be present in the stimulus.

In *Teleogryllus oceanicus* however, the calling and courtship songs have similar carrier frequencies but different temporal patterns (Hill et al. 1972; Hutchings and Lewis 1984) and in this case two-tone suppression effects were also reported and advanced as representing the basic mechanism for the accurate encoding of the temporal characteristics of the songs, especially at high intensities (Hutchings and Lewis 1984). In the same animal, frequency dependent suppression plays a role in a neuronal system which might be involved in avoidance or escape behaviour (Moiseff and Hoy 1983). Finally, ascending thoracic neurons in bushcrickets also exhibit inhibitory sidebands which result in their IR-characteristics having optima (Rheinlaender and Kalmring 1973; Rheinlaender 1975). Thus it seems likely that for insects using broadband signals, such as the locust and many bushcrickets the frequency dependent excitation and inhibition of auditory interneurons represents a mechanism for the encoding of signal intensity (and thus distance to the sender) which is 'read' by a set of 'intensity detectors' that each responds maximally to a different level of sound intensity.

Brain neurons

Recordings were made from two areas of the brain, the lower lateral lobe of the protocerebrum and the medio-dorsal protocerebrum. The former has been regarded as an auditory neuropil within the brain as it is here that the terminal branching areas of ascending auditory interneurons (Eichendorff and Kalmring 1980; Boyan 1983) and branches of local interneurons are located (Boyan, personal communication). Boyan and Altman (1985) describe auditory interneurons in the SOG which send projections to the brain that terminate either in the lateral lobe of the protocerebrum or the medio-dorsal protocerebrum. The shortest spike latencies of most of these neurons (37–80 ms) are considerably longer than those of the ascending interneurons described here, and coincide closely with latencies of those neurons interpreted here – on the basis of physiological properties only – to be brain neurons (Fig. 7). Stainings of such cells in the future will reveal which of these long latency responses are from brain neurons or from ascending axons of SOG-neurons.

The responses of neurons recorded from both protocerebral areas were remarkably different to those of the thoracic ascending neurons and some of them (5 out of 24) gave a maximal response only when the conspecific song was presented. Their tuning to the conspecific interchirp-interval was rather sharp with respect to spike count/song whereas the responses of these neurons did not copy the amplitude modulation of the stimulus. The shape of the tuning curve (Fig. 8) resembles that measured behaviourally in another acridid grasshopper, *Chorthippus biguttulus* (von Helversen 1972). Surprisingly similar response characteristics were reported for neurons in the brain of the cricket (Schildberger 1984) and in the torus semicircularis of the toad (Rose and Capranica 1984). Those in the cricket's brain have been identified as local or possibly descending interneurons. In both the cricket and the locust such selective elements represent only a small percentage of the population of cells studied and the response of these neurons is not matched in time to the amplitude modulation of the song, as it is with many thoracic ascending neurons. This suggests that in both orthopteran families the specificity of the behavioural (primarily phonotactic) response is not based on a matching between the discharge of brain neurons and the AM pattern of the stimulus.

The tuning of these selective elements to the AM of the conspecific song resembles that of a bandpass filter and one may ask whether or not this can be explained by the nature of their input. This input may come from at least two sources: firstly, information ascends from auditory interneurons in the thoracic ganglia, and secondly, as a separate loop in the auditory pathway, information comes from neurons in the SOG, at least 3 of which have axons ascending to the brain (Boyan and Altman 1985). On the basis of their anatomical projections in the brain and their response latencies, the information from the SOG neurons reaching the brain is separated temporally, and to some extent spatially, from the direct input carried by thoracic ascending neurons (Boyan and Altman 1985). This configuration is the basis of the model presented in Fig. 12 to explain the selectivity of the brain neurons. The spike discharge of both presynaptic neurons (one ascending from the thorax and one from the SOG) is separated temporally because of their different latencies and this results in the distinct EPSP activity in the postsynaptic brain neuron. With long interchirp-intervals (Fig. 12A) the postsynaptic potentials do not coincide and the result is a subthreshold or weak suprathreshold response. However, with the shorter interchirp-interval of the conspecific model song, both postsynaptic events coincide and summate so that a maximal suprathreshold response is elicited in the postsynaptic neuron, although the presynaptic input habituates to some extent (Fig. 12B). With intervals shorter than that of the conspecific song both presynaptic inputs fully habituate, consequently resulting in a decrease of the postsynaptic response, which accounts for the sharp cut-off of the tuning curves at short interchirp-intervals (see Figs. 6 and 8). This simple model of temporal filtering, comprising only two different input neurons, from the thorax and SOG, respectively, and employing the common phenomenon of summa-

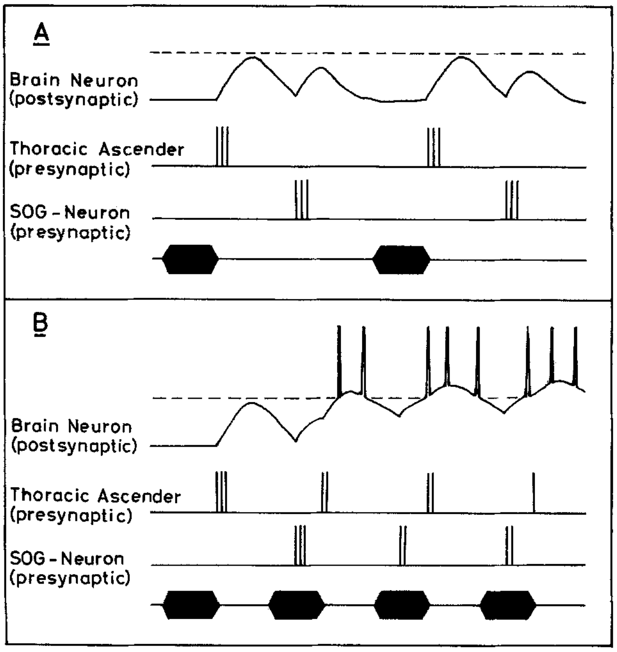


Fig. 12A, B. Neuronal model to explain the temporal selectivity of a brain neuron comprising only two presynaptic ascending auditory neurons. The SOG-neuron has a latency more than twice that of the thoracic ascender. Because of some spatial separation of synapses at the dendrites of the brain neuron, the EPSP elicited by each presynaptic unit may differ in amplitude and size. With long interchirp-intervals (A) both presynaptic units discharge synchronously with the chirp and elicit separate EPSP activity in the brain neuron. This contrasts to the conspecific temporal configuration of the stimulus where EPSP activity summates to that extent that a series of irregular suprathreshold spike activity is elicited. For further explanation see text tion fully accounts for the selectivity of the responses described here.

Acknowledgements. This work was supported by Sonderforschungsbereich 'Bionach SFB 114'; we especially thank Drs. J. Rheinlaender and G.S. Boyan for support and helpful discussion throughout the study and Dr. G.J. Dörrscheidt for providing us with the computer-generated model songs. We also thank Dr. Whittle for revising the manuskript.

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