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'Absolute steepness' of ramps as an essential cue for auditory pattern recognition by a grasshopper (Orthoptera; Acrididae; *Chorthippus biguttulus L.*)

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Abstract. Male grasshoppers of the species *Chorthippus biguttulus* react to female songs with a characteristic turn towards the female. The probability of turning towards female song models was used to evaluate those parameters which are essential for a signal to be interpreted as female song.

1. The shape of sound pulses turned out to be most decisive; pulses with ramps rising gradually over 3 and more ms were efficient (Figs. 2, 3), whereas rectangularly modulated pulses evoked only weak responses and only when pulse intervals were between 3 and 5 ms (Fig. 2). The decline of a pulse did not influence its efficiency (Fig. 3). In particular, pulses with sudden onsets and gradual declines were as weakly effective as rectangularly modulated ones and thus remarkably less effective than pulses with ramp-like onsets (Fig. 4).

2. Intensity tuning curves suggest, that the absolute steepness of ramps (expressed as µbar/ms) is detected by the grasshopper nervous system (Figs. 6, 7), possibly by processing the delay in excitation onset of at least two receptor types differing in threshold sensitivity.

3. The sawtooth shape of pulses in female signals is suggested to be adaptive with respect to directional hearing.

Key words: Acoustic pattern recognition – Envelope of sound pulses – *Chorthippus biguttulus* – Grasshopper

Introduction

For all orthopteran species investigated so far, a most decisive feature for recognition of species specific song is amplitude modulation, i.e. a specific temporal pattern of sound pulses and intervals. This is evidenced by the enormous variety of species specific song patterns in various orthopteran groups, and has been confirmed experimentally whenever the efficiency of artificially varied song pattern was tested (for reviews see Dumortier 1963; Elsner and Popov 1978; v. Helversen and v. Helversen 1987; Ewing 1989). Short rise times, often referred to as 'transients', were found to essentially influence the effectiveness of acoustic patterns (Busnel and Loher 1961; Busnel 1963). Indeed, stimuli with syllables the onsets of which had been accentuated by an additional short pulse at the beginning, were preferred to rectangularly modulated ones by females of *Ch. biguttulus* (v. Helversen and v. Helversen 1983). But apart from these studies the general importance of such rapid rise times never has been investigated.

However, the shape of pulses might be an essential cue for discrimination of male and female song in the grasshopper *Chorthippus biguttulus*. In this species the pattern of stridulatory movements is principally the same in both sexes; as a rule, 3 down- and upward movements of the hindlegs rubbing against the elythra generate 6 short sound pulses, which are grouped to a syllable by insertion of a short stop before the next downstroke (Elsner 1974). The shape of such pulses, however, is distinctly different in the signals of both sexes, male pulses having nearly rectangular envelopes, whereas female pulses are characterized by a rise time in the order of some milliseconds (Fig. 1 and v. Helversen and v. Helversen 1983). This led to the hypothesis, that slowly rising ramps might be important for recognition of female song.

This can be tested using a peculiar behaviour of *Ch. biguttulus;* a male hearing a female song in reply to its own stridulation is immediately alerted and abruptly turns toward the sound source, moves forward a short distance, then sings again and waits for the next answer. This characteristic turn occurs exclusively in response to a female signal, never in response to the song of another male. Therefore, when a male is tested with artificial sound models, its turning probability will indicate to what degree a given sound pattern is interpreted as female song.

In a first series of experiments, rise time of pulses will be shown to be a decisive feature in recognition of female song, then it will be asked whether rising and declining ramps are of equal importance, and finally intensity

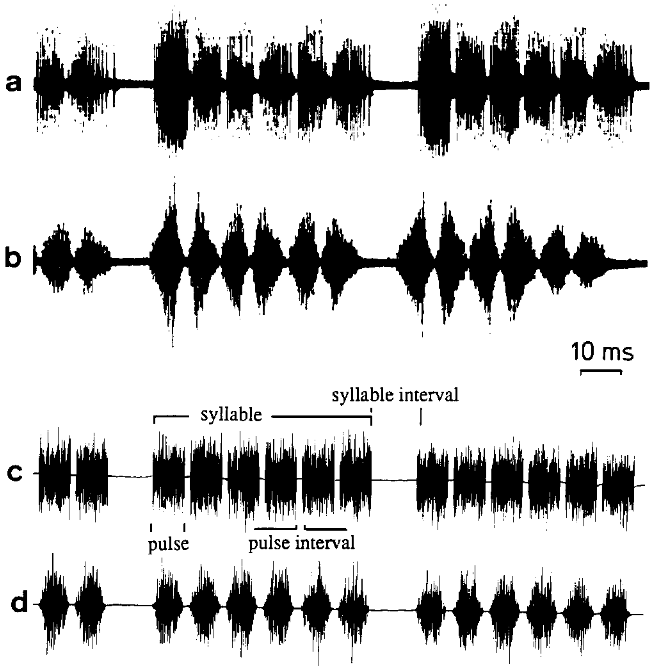

Fig. 1. Parts of male (**a**) and female (**b**) song pattern in *Chorthippus biguttulus*, demonstrating the different pulse shape in the sounds of the two sexes. The characteristic syllable structure of the song was present in all song models, represented by two examples with ramp times of 0 ms (**c**) and with a rising and declining ramp of 3 ms (**d**)

tuning curves will be measured to reveal whether 'steepness' or 'duration of rise time' is the parameter detected by the nervous system of the animal.

Methods

Chorthippus biguttulus males were taken from the field, kept in cages of 40 × 40 × 40 cm, and fed with grass *(Dactylis glomerata)*, oat flakes, and fish food.

The experiments were performed in a reflection-attenuating room at temperatures between 28° and 30 °C and under dimmed illumination, in order not to disturb the animals by movements of the experimenter. During tests the male moved freely on a cloth covered platform. Whenever the male began to sing spontaneously, its song triggered an artificial female song, broadcast via a small loudspeaker (AKG-acoustics, type DKC 29/51) from 20 cm laterally. An efficient model caused the male to turn immediately towards the loudspeaker. This was recorded as a positive reaction. Before the male started the next song, the movable loudspeaker was rearranged, and while doing so, the stimulated side of the animal was changed frequently. The duet was continued until the male stopped turning and singing after about 3 up to 30 responses depending on the efficiency of the stimulus. A motivated male resumed singing within about 1 min.

One type of sound model was offered for at least 20 times, then the next type was given. Sequence of stimuli was out of order, efficient stimuli followed unefficient and reverse.

The artificial song models had been stored on a tape recorder (Racal 4 Ds). They passed an attenuator, which allowed the intensity to be varied in steps of 0.375 dB. Intensities are given in dB SPL. For this purpose, the intensity of a continuous noise the amplitude of which referred to the maximal amplitude of a test stimulus was measured (Bruel & Kjaer microphone 4133 and amplifier 2231). The frequency spectrum was between 2 and 12 kHz with a main band between 4–8 kHz, which is typical for the sound spectrum of the *Chorthippus biguttulus* female.

Because in *Chorthippus biguttulus* the characteristic pattern of syllables and intervals turned out to be most important for recognition of species specific song when investigated in females (v. Helversen 1972), the characteristic species-specific song pattern was maintained in all artificial sound stimuli presented here. Syllables were between 70 and 80 ms with intervals of 12 to 15 ms inbetween, which is in the optimal range of efficiency.

Results

(i) Efficiency of ramps

It is impossible to vary the shape of pulses without simultaneously affecting the ratio of pulses and intervals in a song model. With ramps becoming less steep, effective pulse intervals necessarily increase because threshold intensity of auditory receptors will be attained later in each pulse. Therefore, to determine whether ramps of pulses are decisive for a sound pattern to be recognized as female song, a set of response curves had to be measured, in which the pulse interval was varied for each given ramp time. Pulses were composed of rising and falling ramps between which the amplitude was kept constant. Total duration from the beginning of the rising ramp to the end of the falling ramp was 8 ms. In Fig. 2 the probability of a turning reaction is plotted for 4 different ramp times of pulses. Stimuli with rectangularly modulated pulses elicited turning responses infrequently, with maximum response rates of 20% for pulse intervals between 3 and 5 ms. However, efficiency of stimuli was raised drastically, when rise time of pulses was length-

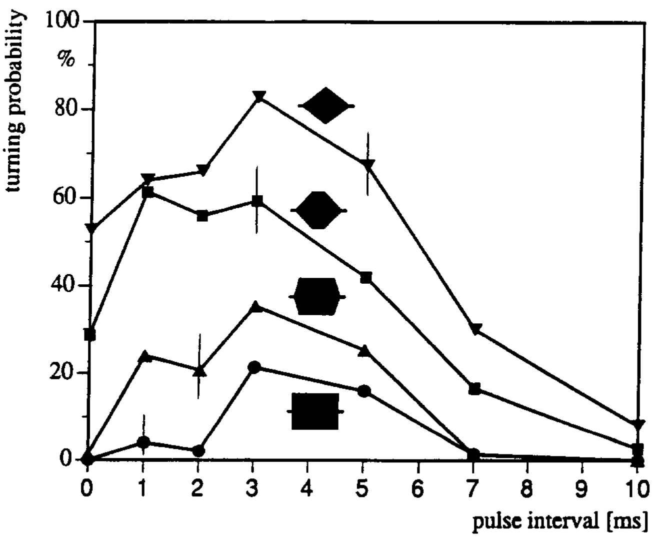

Fig. 2. Turning probability of *Ch. biguttulus* to artificial song models (means of 6 males). Ramps of pulses (0, 1.2, 3, and 4 ms) were constant for each curve, duration of pulses was 8 ms, intensity was 58 dB SPL at the maximum amplitude. Pulses were grouped to syllables of 70 to 80 ms duration, separated by pauses of 15 ms, pulse interval was varied. (*n*/data point: 91–153, 123–158, 105–184, 129–206 for 0, 1.2, 3, 4 ms ramp time). Bars indicate 95% confidence intervals

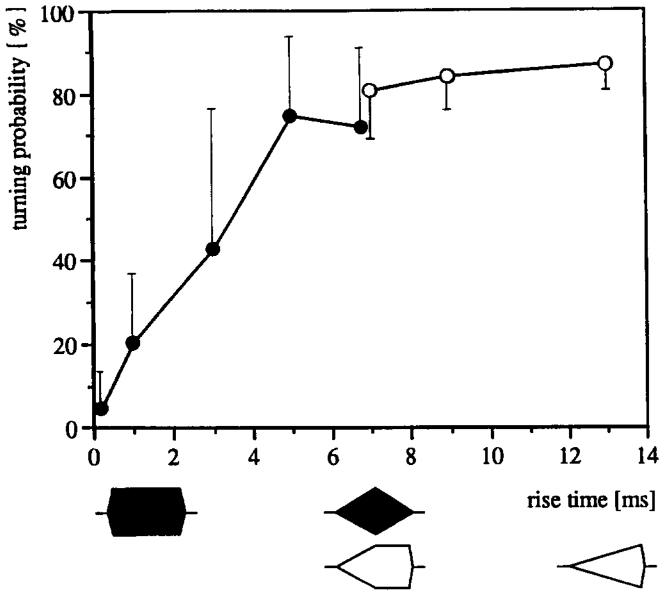

Fig. 3. Turning probability to increasing ramp time of pulses (means and standard deviations of 6 males; *n*/data point: 198–273). Two types of pulses were used, one with symmetrically rising and declining ramps, which allowed ramp times up to 7 ms at a constant pulse duration of 14 ms. The second type allowed rise times up to 13 ms, as the decline was within 1 ms. Pulse interval was 2 ms, intensity was 55 ± 3 dB at the maximum amplitude

ened to 3 and 4 ms; the animals then continued the dialogue over 10 to 30 replies. With such ramp times pulse intervals of even 0 and 1 ms became effective, which indicates, that slower rise times created sufficiently long intervals between pulses. From this set of response curves it can be concluded, that the shape of pulses is decisive for recognition of female song.

In the experiments so far, pulse duration was kept constant at 8 ms, which allowed rise and decay times up to 4 ms only. To investigate the efficiency of longer ramp times, a pulse duration of 14 ms (still accepted by males; v. Helversen, unpubl.) was chosen, allowing symmetrical pulses up to 7 ms ramp time (filled circles in Fig. 3) Further lengthening of ramps could be achieved, when the decline was reduced in favour of rise time (open circles in Fig. 3). With increasing ramp time mean turning probabilities increased, too, reaching a saturation level at about 5 to 7 ms, regardless of whether the pulse ended abruptly or not.

(ii) Efficiency of declining ramps

As shown in Fig. 3, the 7 ms rise time pulses were equally effective irrespective of ending abruptly or declining gradually suggesting, that only rise time is important. This was tested directly in the following experiment, the results of which are given in Fig. 4. Three types of stimuli were presented; syllables, the pulses of which had slowly rising ramps (10 ms) and an abrupt end, and the mirror imaged ones, starting with a sudden onset and declining towards the end of the pulse. The third type of pulse was

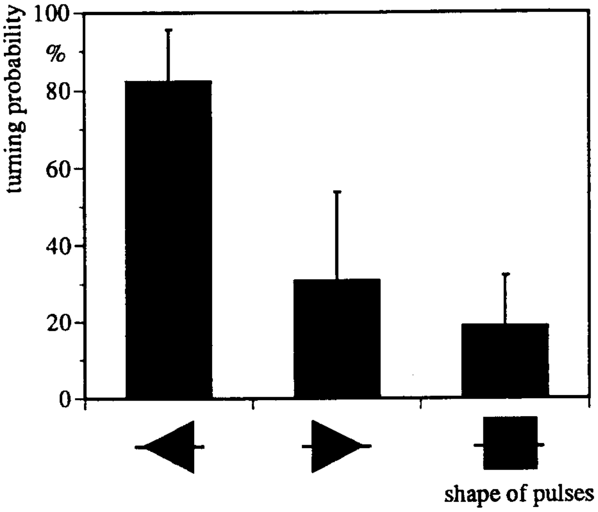

Fig. 4. Efficiency of pulses with rising or declining ramps (means and standard deviations of 13 males) and rectangularly modulated pulses (mean and standard deviation of 8 males). Duration of pulses was 10 ms, intervals between pulses 2 ms

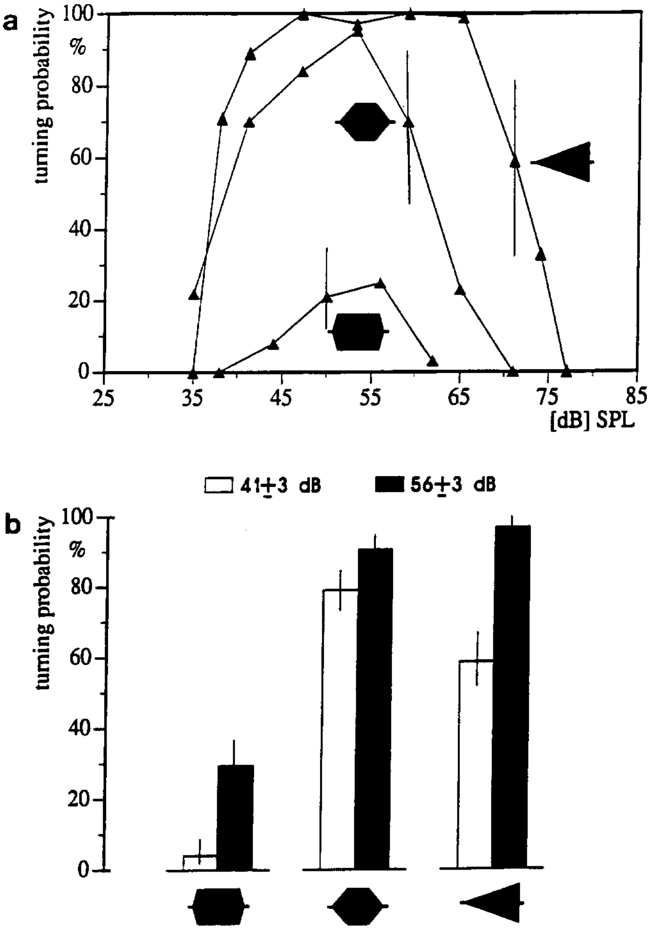

Fig. 5a, b. Turning probability to female model songs as function of intensity at 3 different rise times of pulses (1, 3 and 10 ms). **a** Reaction curves of an individual male (*n*/reaction curve: 215, 120 and 182), **b** mean reactions of 6–8 males to the same stimuli at two different intensities. Note, that the animals discriminate between flat and steep ramps already at threshold intensities (*n*/configuration of rise time and intensity: 161–237). Bars indicate 95% confidence intervals

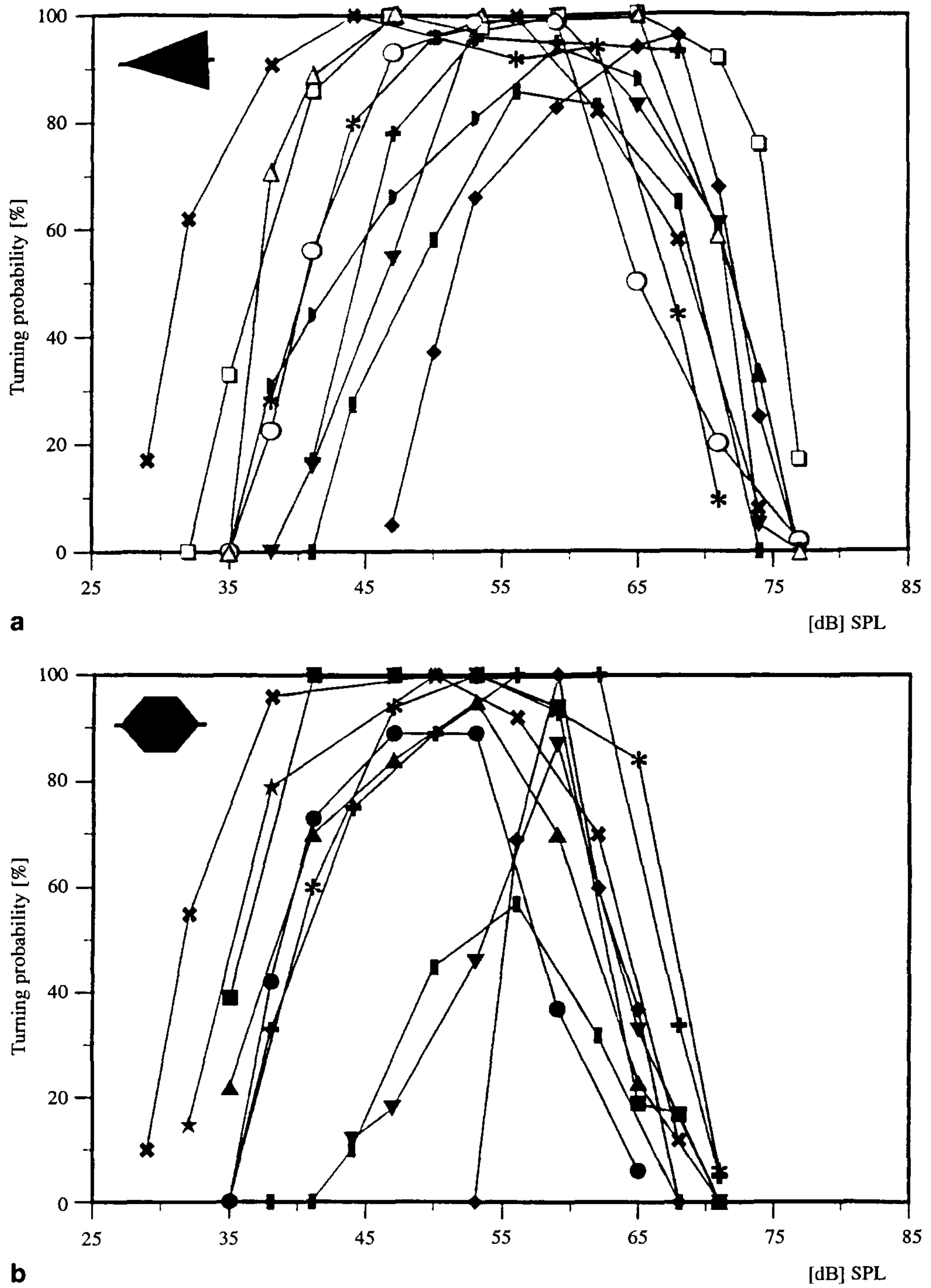

Fig. 6. Intensity tuning curves of 10 males for model songs with pulses of 10 ms (**a**) and 3 ms (**b**) ramps. Same symbols in **a** and **b** refer to the same individual (*n*/reaction curve: 91–369)

modulated rectangularly at beginning and end, and thus was of higher energy than the first two, which were of equal energy. Stimuli with rising ramps were significantly ($P<0.001$; *t*-test) more efficient than stimuli the pulses of which had declining ramps. The efficiency of such pulses, however, when compared with purely rectangularly modulated ones were not significantly different ($0.1<P<0.2$; *t*-test). Thus, declining ramps do not substantially influence efficiency. A comparison, however, is problematic insofar, as the pulses to compare differ with respect to intensity and gap width between pulses.

(iii) Intensity dependence

Pulses with different rise times – though equal with respect to their maximum amplitude (= equal intensity, as defined here) – differ with respect to sound energy. Therefore, to find out whether the effectiveness, especially the low effectiveness of steep ramps, might be due to differences in sound energy of stimuli, intensity tuning curves were measured. In Fig. 5a a typical example of three response curves with rise times of 1, 3 and 10 ms is given for one individual male. Response curves for 3

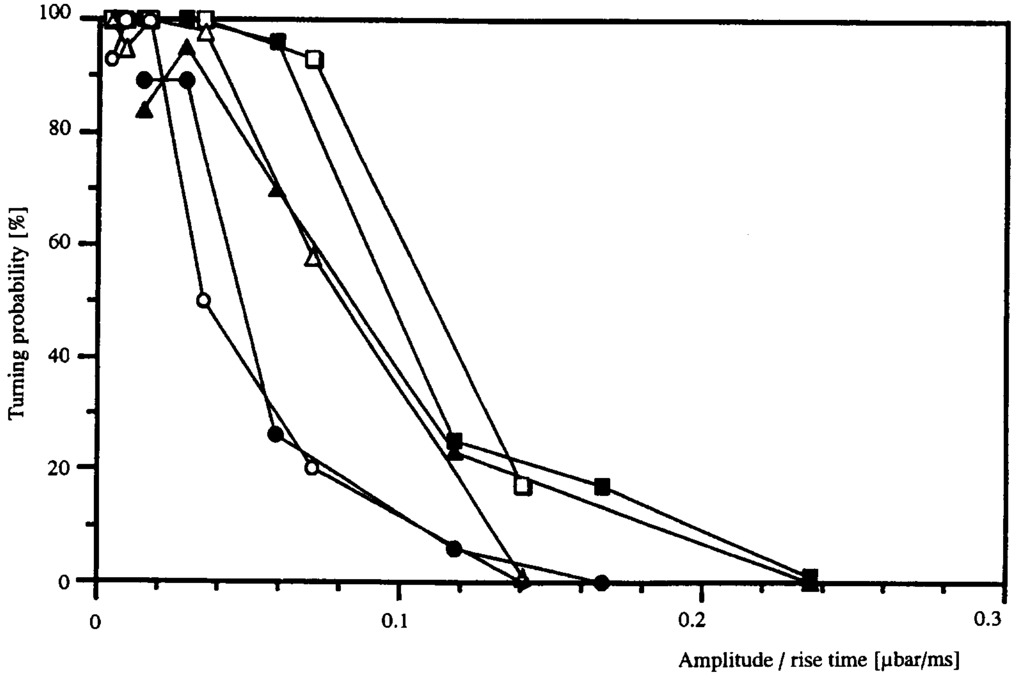

Fig. 7. Turning probability plotted as function of steepness of pulses in female model songs. The turning probabilities refer to the data of 3 individual males (open symbols in Fig. 6a). For each male two reaction curves are plotted, one derived from 10 ms ramp time stimuli *(open symbols)* and one derived from 3 ms ramp time stimuli *(filled symbols)*. Note that every two curves of a male have the same decline

and 10 ms rise time are clearly bell-shaped, increasing at about the same threshold, but declining at different intensity levels. The efficiency of 1 ms rise time stimuli remained below 20% turning probability throughout the whole range of intensities. Figure 5b summarizes this result for several males; when the efficiency of pulses with abrupt onsets (0 or 1 ms ramp time) was compared to such with slowly rising ramps (3 and 10 ms) at a low intensity of 41 ± 3 dB and at the most effective of 56 ± 3 dB, it turned out, that discrimination between steep and slow ramps is present already at threshold intensities.

(iiii) What is decisive – 'rise time' or 'absolute steepness'?

At a given maximum intensity the steepness of a ramp is entirely defined by the duration of increasing amplitude, but, of course, when the intensity level is changed, the absolute steepness of a ramp (measured as amplitude/time) will change also. Therefore, a comparison of intensity tuning curves with different ramp times should reveal, whether the 'duration of rise time' is measured or whether an 'absolute steepness' is detected by the auditory system.

In Fig. 6 the turning probabilities of 10 males are plotted in dependence of intensity for two different rise times, 10 ms (a) and 3 ms (b). Whereas response thresholds were scattered over a broad range of intensities (though being similar for both response curves in individual animals), the declines of reaction curves differed much less and were shifted about 10 dB in mean towards higher intensities for 10 ms ramp time stimuli. Such declines at different levels of intensities should be expected when 'absolute steepness' of ramps is detected by the animal. Stimuli beyond a critical value become inefficient as they are classified as too steep to be accepted as female specific. Of course, for short rise times this critical value will be reached at lower intensities than for longer rise times, which causes the two curves to decline at different intensity levels.

In Fig. 6 both curves (3 and 10 ms ramp time) had been measured for 7 animals. When for each male the shift between the two curves was calculated at a turning probability of 50%, a mean shift of 10.1 ± 1.4 dB was found. This value is quite near to 10.46 dB which is the expected shift between the declines of two response curves with ramps of 3 and 10 ms when steepness is detected.

Therefore, when plotted against steepness, the declining parts of the two intensity tuning curves with 10 and 3 ms ramps should fall together. This is shown for the reaction curves of 3 individual males (open symbols in Fig. 6a). In Fig. 7, for each male the turning probability is different at a given steepness, but the declines for every two curves of a male are the same regardless whether derived from 3 or 10 ms rise time stimuli (filled and open symbols, respectively). When, similar to the calculation of the mean dB-shift, the steepness of every two intensity tuning curves is compared at the interpolated turning probability of 50% a mean difference of only 3 ± 15 nbar/ms ($n = 7$ males) results between both reaction curves.

Discussion

The experiments reported here reveal sawtooth shaped pulses to be a decisive feature for recognition of female song in the grasshopper species *Chorthippus biguttulus*. Sound patterns with ramps beyond a critical steepness are classified as female sound. Moreover, only increasing ramps are decisive. Declining ramps seem neither to improve nor reduce effectiveness of a signal, as shown in Figs. 3 and 4.

For perception of a specific temporal pattern of sound and intervals, as it is so essential for all the species investigated so far, sawtooth pulses are surely not particularly suited, as for the receiver, the pattern changes with increasing distance. Pulses are perceived to be shorter (reduced by that amount remaining below threshold) while the intervals appear enlarged. Therefore, the Innate Releasing Mechanism of a male for female song should accept sound patterns within a broad range of pulses and intervals.

Not only the temporal pattern, but also steepness as such is perceived differently by a receiver nearby or far away. However, steepness *below* a critical value is important for female recognition. Therefore, with increasing distance a pattern will appear even more effective, as ramps will be perceived more flat. Thus, there is no need for the grasshopper to evaluate a 'relative steepness', which would require some sort of gain control somewhere in the neuronal network.

Recognition of steepness may not be restricted to *Ch. biguttulus.* Flat ramps were also decisive for female recognition in *Ch. brunneus,* which is closely related to *Ch. biguttulus,* but this may be of significance in many other insect species, too, producing songs with characteristic rise times of pulses or syllables. The discrimination ability between 'steep' and 'flat' is in the order of 1 to 2 ms rise time at medium intensities, and must be based on a mechanism different of rise time recognition as e.g. in anuran auditory sytems, in which far longer rise times (ca. 10 to 20 ms), are discriminated neuronally (Hall and Feng 1988).

Possible neuronal mechanism of steepness detection

On the neuronal level the information on steepness can hardly be transmitted by a single receptor element. Spikes on a low frequency receptor fibre follow each other at about 5–10 ms (22 °C, Ronacher and Römer 1985). Such intervals and even spike intervals of 3 ms, as recorded in *Ch. biguttulus* at high intensities (Stumpner, pers. com.) would hardly suffice to reliably transmit information on rise times of the same order. Additionally, short pulses or pulses of low intensity would elicit only a single spike per pulse in most cases, and thus information about rise time would be lost.

Therefore, more than one receptor type will have to cooperate for coding rise time. Information on rapid and slow rise time could be coded by a mechanism similar to that proposed for gap detection by Ronacher and Römer (1985), in which the degree of synchronization in a group of receptor elements could be used to neuronally distinguish between rapid and slow rise times. However, it is difficult to imagine, how the difference between rapid and slow rise times could be coded at low intensities, at which – according to Fig. 5a, b – the animal is able to discriminate between rapidly and slowly rising ramps.

In principle, two types of receptors with *different* thresholds would be well-suited to translate steepness into a latency difference. Rectangularly modulated pulses then would excite both types of receptors simultaneously, whereas slow rise times would bring about a delayed onset of activation of the less sensitive receptor type. This time delay in excitation could be processed by postsynaptic interneurons, functioning either as detector elements for steep ramps (excitation only when both receptor types are active simultaneously) or smooth ramps (inhibitory influence of the less sensitive receptor type would lead to excitation only when delayed).

Anatomical and physiological data are consistent with this hypothesis of steepness detection. In the low frequency range three receptor types, mainly characterized by their different thresholds, have been described (Römer 1976, for *Locusta* and Stumpner, Ronacher unpubl. for *Ch. biguttulus*). These elements may not only function in intensity partitioning (Römer 1976), but permit rise time recognition as well.

On the level of interneurons, too, many examples have been described, demonstrating the complex interactions of inhibitory and excitatory inputs on interneurons (Römer et al. 1981; Römer and Marquart 1984; Marquart 1985) for *Locusta;* and Stumpner and Ronacher 1991; Stumpner et al. 1991 for *Ch. biguttulus*). Further electrophysiological studies should reveal, whether such units could work as detector for smooth ramps.

It seems worthwhile to mention that males were able to discriminate between slow and steep ramps even at very low intensities (Fig. 5). If the proposed mechanism is working, one asks for the threshold of the sensitive receptor type. We observed in a highly motivated male, that the threshold for a turning response was 3 dB above the threshold for a stridulatory answer. Possibly, turning needed excitation of receptor elements with different thresholds. A sensitivity difference of 3 dB between two receptors seems to be a very low value, but – see v. Helversen and Rheinlaender (1988) – it might be sufficient for evaluation of a detectable time delay.

Consequences of sawtooth shaped pulses for directional hearing

At first sight, clicks or pulses with steep ramps seem to be best suited for directional analysis of sound, and that is actually true for vertebrates, which evaluate the time lag between the arrival of sound at their two ears. Grasshoppers also could evaluate time delays (Mörchen et al. 1978; Rheinlaender and Mörchen 1979) but these delays are created internally by the intensity dependent latency of the tympanal elements. Such binaural time differences between excitation on both sides would be further enlarged by ramped pulses, as threshold will be reached later on the side away from sound. This was already pointed out by Adam (1977) who showed that the intensity dependence of latency becomes less steep with rising ramps. That time delays in the order of a ms are indeed relevant in the lateralization process could be demonstrated in behavioural experiments on the acuity of lateralization in *Ch. biguttulus;* lateralization of a sound source was possible entirely on the basis of temporal differences in the tympanal nerves (v. Helversen and Rheinlaender 1988).

The neuronal mechanism of lateralization based on time dependent inhibition and excitation may be very similar to the one discussed for steepness detection. In both mechanisms a time delay is evaluated; for lateralization, between receptor elements of the same threshold but on different body sides, and for steepness detection, between receptor elements of different thresholds on the same body side.

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References

Adam LJ (1977) The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae) I. Its original form and time constancy. Biol Cybern 26:241–247

Busnel RG (1963) On certain aspects of animal acoustic signals. In: Busnel RG (ed) Acoustic behaviour of animals. Elsevier, Amsterdam London New York, pp 69–133

Busnel RG, Loher W (1961) Declenchement de phonoréponses chez *Chorthippus brunneus* (Acrididae). Acustica 11:65–70

Dumortier B (1963) Ethological and physiological study of sound emissions in Arthropoda. In: Busnel RG (ed) Acoustic behaviour of animals. Elsevier, Amsterdam London New York, pp 583–654

Elsner N (1974) Neuroethology of sound production in gomphocerine grasshoppers. I. Song patterns and stridulatory movements. J Comp Physiol 88:72–102

Elsner N, Popov AJ (1978) Neuroethology of acoustic communication. Adv Insect Physiol 13:229–355

Ewing AW (1989) Arthropod bioacoustics. Neurobiology and behaviour. Edinburgh Univ Press, Edinburgh, pp 167–174

Hall JC, Feng AS (1988) Influence of envelope rise time on neural responses in the auditory system of anurans. Hearing Res 36:261–276

Helversen D v (1972) Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* (Orthoptera, Acrididae). J Comp Physiol 81:381–422

Helversen D v, Helversen O v (1983) Species recognition and acoustic localization in acridid grasshoppers: a behavioural approach. In: Huber F, Markl H (eds) Neuroethology and behavioural physiology. Springer, Berlin Heidelberg New York Tokyo, pp 95–107

Helversen D v, Rheinlaender J (1988) Interaural intensity and time discrimination in an unrestraint grasshopper: a tentative approach. J Comp Physiol A 162:333–340

Helversen O v, Helversen D v (1987) Innate receiver mechanisms in the acoustic communication of orthopteran insects. In: Guthrie V (ed) Aims and methods in neuroethology. Manchester Univ Press, Manchester, pp 104–150

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

Mörchen A, Rheinlaender J, Schwartzkopff J (1978) Latency shift in insect auditory nerve fibers. A neuronal time cue of sound direction. Naturwissenschaften 65:656–657

Rheinlaender J, Mörchen A (1979) "Time-intensity-trading" in locust auditory interneurones. Nature 281:672–674

Römer H (1976) Die Informationsverarbeitung tympanaler Rezeptorelemente von *Locusta migratoria*. J Comp Physiol 109:101–122

Römer H, Marquart V (1984) Morphology and physiology of auditory interneurons in the metathoracic ganglion of the locust. J Comp Physiol A 155:249–262

Römer H, Rheinlaender J, Dronse R (1981) Intracellular studies on auditory processing in the metathoracic ganglion of the locust. J Comp Physiol 144:305–312

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

Stumpner A, Ronacher B (1991) Auditory interneurones in the metathoracic ganglion of the grasshopper *Chorthippus biguttulus*. I. Morphological and physiological characterization. J Exp Biol 158:391–410

Stumpner A, Ronacher B, Helversen O v (1991) Auditory interneurones in the metathoracic ganglion of the grasshopper *Chorthippus biguttulus*. II. Processing of temporal patterns of the song of the male. J Exp Biol 158:411–430