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Bilateral Coding of Sound Direction in the CNS of the Bushcricket *Tettigonia viridissima* L. (Orthoptera, Tettigoniidae)

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Summary. 1. By means of simultaneous recordings with hook-electrodes the response behavior of a pair of homologous auditory interneurons on either side of the CNS of the bushcricket *Tettigonia viridissima* L. was studied with particular emphasis on bilateral symmetry and directional coding.

2. In about 50% of the preparations the threshold curves of both interneurons were symmetrical, tested within a frequency range from 5 to 40 kHz (Fig. 4a). In the remaining cases significant bilateral asymmetries at frequencies above 20 kHz and/or below 12 kHz could be observed (Figs. 4 and 5). Therefore, in this bushcricket species symmetrical thresholds seem to be guaranteed only within a rather limited frequency band around 20 kHz. This frequency coincides with one (the higher) of the two spectral bands within the conspecific call (Fig. 2).

3. From recordings of unilaterally activated interneurons it can be inferred that the directional sensitivity of the single tympanic organ is as well tuned to this stimulus frequency of about 20 kHz. Maximal intensity differences of 15 to 20 dB between ipsi- versus contralateral stimulation could be found (Fig. 3). Above and below this frequency the directionality of the ear was much lower.

4. This frequency dependence of directional hearing could be confirmed on the level of the CNS. Only at a stimulus frequency of about 20 kHz the directional curves of both interneurons were very pronounced (Fig. 10) with precisely encoding the lateralization of the sound source starting at about 10 dB above neuronal thresholds (Figs. 7, 8 and 9).

5. Though in single preparations the intensity-response curves of the two interneurons were notably different (Fig. 6), with respect to right versus left discrimination no auditory 'handedness' could be observed.

6. As some insects already orient to a single or a small number of stimuli, individual neuronal responses of the right versus the left interneuron were compared at different sound directions (Figs. 11 and 12). Surprisingly, with frontal stimulation the probability of 'correct' (symmetrical) responses was only 17% which abruptly increased as soon as the sound source became lateral. These results are discussed in terms of the zig-zag walk of the localizing insect.

Introduction

As a rule, studies on sound localization are based on the assumption that both sides of the auditory pathway participate in the task of providing directional information. If one ear is blocked or destroyed, then sound localization is impaired or even lost. This could be demonstrated in many controlled behavioral experiments on different classes of animals and will here only be quoted for insects (Regen, 1913; Murphey and Zaretsky, 1972; Bailey and Thomsen, 1977; Wendler et al., 1980). Although there is no direct morphological or physiological evidence one can assume that the auditory information is mediated through two (more or less) identical, linked halves which have symmetrical transmission characteristics. The degree of symmetry finally determines how accurate an animal can face or – in the case of insects – lateralize the sound source.

In order to find out how symmetrically the two halves of the auditory pathway are activated, nervous activity from homologous pairs of cells or cell populations on both sides must be depicted. This bilateral recording situation can best be performed in insects where, beside units with small axonal diameters, giant fibres also conduct the sensory information within the CNS.

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For directional hearing Suga and Katsuki (1961) were the first to show inhibitory interaction between both sides of the CNS using this bilateral recording technique. Boyan (1979) studied two pairs of auditory interneurons in the ventral nerve cord of the cricket and demonstrated that these units were precisely tuned to each other in encoding sound direction, though most of the preparations exhibited a remarkable auditory 'handedness'. Unfortunately, Boyan did not control the degree of opening of the functional spiracles during his experiments, which – in the case of crickets – seems to influence auditory perception considerably (Larsen and Michelsen, 1978; Wendler et al., 1980).

In the present study we recorded simultaneously from a pair of homologous auditory interneurons in the auditory system of the European bushcricket *Tettigonia viridissima*. The bushcricket preparation is favoured since a highly symmetrical and 'well controlled' stimulus situation can be achieved simply by aligning body axis and forelegs into the sound field. Therefore in this species the question of auditory handedness in insects will be reviewed. In addition to conventionally presented averaged spike data we also compared simultaneous discharges of the right versus the left interneuron to individual auditory stimuli. As the localizing cricket already orients subsequent to a single or a small number of sound pulses (Murphey and Zaretsky, 1972), we expected this single event analysis to be more appropriate for correlation with the phonotactic behavior than statistically blended averaged responses.

Material and Methods

For the experiments 38 male and female bushcrickets of *Tettigonia viridissima* L. were used. They were captured in wild grassland and low shrubs near Bochum during the first half of the mating season in August and September 1979 and kept in an insect house for some days before the neurophysiological study. The experiments were performed only on those indiviuals which appeared healthy and undamaged especially with regard to the tympanic and tracheal system. But neither their age after the last moult nor the internal and functional state of the auditory system could be established precisely (see Results and Discussion).

Each animal under investigation was at first anaesthetized with CO_2 so that it remained immobilized for approximately 3 min. During this time the hind and middle legs and the wings were removed and the preparation was waxed upside down on a small, thin metal sheet (of the same surface size as the animal) mounted on a brass needle of 3 mm diameter. The tarsi of the forelegs were fixed with sticky wax in small forks (diameter 0.5 mm) in such a way that the legs were maintained in a natural posture and the openings of the tympanic slits were oriented perpendicular to the long axis of the body. This alignment of the legs could be simply done by optical control (previous neurophysiological test measurements had revealed that even angular deviations of at least up to 15° from the perpendicular leg position did not change the directional properties of the studied interneurons; see also Nocke, 1975). Thus we were confident that each preparation received symmetrical auditory stimuli relative to its geometrical configurations.

This small set up (consisting of preparation, holder and brass needle) could be turned to a rock-wool clad metal block in the centre of a home-made anechoic chamber (1.5 m in diameter) so that the preparation was elevated 5 cm above the rock-wool floor at the same height as the acoustical axis of the speaker (Audax TW8 spz.). The angular accuracy of the animal's alignment in the sound field was $\pm 2°$. From the centre of the ceiling a steel cylinder (10 mm in diameter) was suspended, in the inner tube of which the two cathode followers and the pin connectors for the two different microelectrodes were inserted. As the hook-electrodes used were about 5 cm long, the steel cylinder ended about 5 cm above the preparation. Thus a 'free' sound field situation for the animal was achieved (it was homogeneous to within 3 dB in the frequency range from 5 to 40 kHz). The speaker could be rotated at a distance of 50 cm around the animal in the horizontal plane so that far field conditions predominated. Its angular position was varied in 15° or 30° steps by remote control from outside the chamber.

Previous control measurements with hook-electrodes from the ascending and descending connectives relative to the prothoracic ganglion had revealed that on each side of the CNS of *Tettigonia viridissima* there exists one large acoustic fibre mediating auditory information to the brain and to the abdomen (compare Suga and Katsuki, 1961).

The yielded recording amplitudes of 2–3 mV furthermore indicated that this pair of interneurons could be assigned to the giant fibre system (similar to findings of Kalmring et al., 1979, in the bushcricket *Decticus verrucivorus*). For further functional proof that the two elements recorded in each preparation comprised a pair of homologous interneurons in the auditory pathway of *Tettigonia viridissima* see Results.

In the usual experiments a small flap of cuticle covering the cervical connectives was removed to allow access of the two different electrodes to the recording sites. The cervical connectives which were severed just posterior to the suboesophageal ganglion were hooked by electrolytically sharpened steel electrodes providing good electrical contact to the dorsomedial part of the connectives. By this approach spike amplitudes around 2 mV could be recorded for one hour and longer with a signal-to-noise ratio of at least a factor of 10 (see e.g. spike display in Fig. 1a). After placing the two electrodes the operating hole was coated with petroleum jelly to prevent desiccation of the nervous tissue.

In order to study the directional characteristic of the tympanic organ in 8 experiments the opposite tympanic nerve was dissected. By means of this operation a monaural pathway respectively to one of the two auditory interneurons was achieved and thus – by recording the threshold sensitivity of a single unit – the directional characteristics of the tympanic organ could be simply inferred (without for example recording summated potentials by hooking the tympanic nerve). Care was taken not to interfere with the prothoracic tracheal system whose integrity should be of elementary importance for the directional hearing in bushcrickets (Lewis, 1974; Nocke, 1975; Michelsen and Larsen, 1978).

Sound stimuli were produced by an amplitude modulated generator (Akustischer Stimulator II, Burchard) and thus pure sound pulses of 5 ms flat-top duration with a rise and fall time of 5 ms could be produced. The envelope of this signal approximating an individual sound pulse of the conspecific mating call (see Fig. 2) was used in all experiments. Stimulus frequency was varied from 5 up to 40 kHz, sound intensity was attenuated in 10 or 1 dB steps. Sound pressure levels at the position of the preparation were calibrated by a Brüel and Kjaer $^1/_2$ in. condenser microphone to an accuracy of ± 1 dB. They are expressed in dB re 2×10^{-5} N/m^2. The ambient temperature inside the chamber was 22 ± 2 °C.

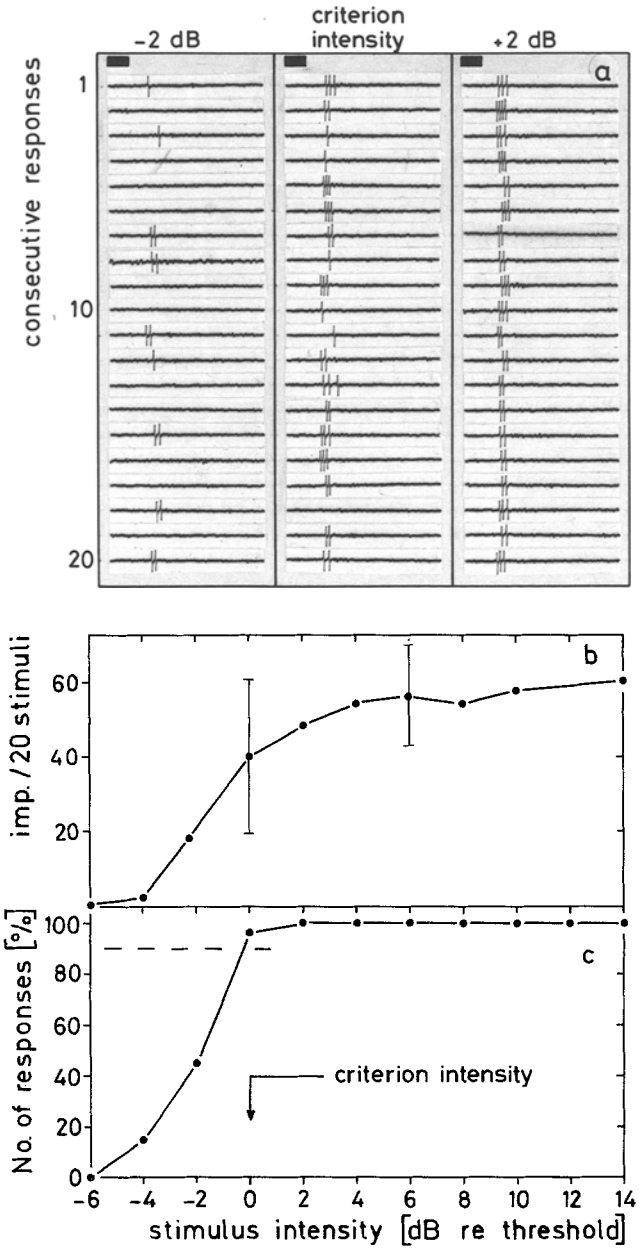

Fig. 1 a–c. Response behavior of an auditory interneuron near the threshold value. **a** 20 consecutive responses at criterion intensity and 2 dB above and below, respectively; filled marks on top represent sound stimulus (20 kHz, flat-top duration 5 ms, rise and fall time 5 ms). **b** Intensity response curve of the same neuron at 20 kHz (in this and subsequent figures standard deviations are indicated by vertical bars). **c** Probability of firing for 20 consecutive responses to define the neuronal threshold. Criterion intensity is achieved starting at the 90% level (dashed line)

Action potentials were amplified in the usual way (cathode follower and AC-coupled amplifier, Tektronix, 122) displayed on an oscilloscope and stored on magnetic tape (Racal Store 4 D). For statistical analysis each stimulus configuration was repeated at least 20 times. The spike data were later analyzed by a minicomputer (Intertechnique, Didac 800) in terms of number of spikes per stimulus and latency of the first discharge relative to the arrival of the sound signal at the insect's ears.

Determination of Neuronal Threshold

Some of the essential findings in this study are based on the determination of neuronal thresholds. For this purpose a statistical method was chosen which permitted a highly accurate and reliable definition of the neuronal threshold value without using an on-line computer. In Fig. 1 b a typical example of the conventional intensity response curve is shown for the interneuron under investigation. With increasing sound intensity the spike number per stimulus increased but started to saturate within 5–10 dB above the foot of the curve. This rather steep slope of the curve near "threshold" and the comparatively high variability between responses of repetitive stimuli (see standard deviations) are common characteristics of this type of interneuron. The variability of the responses can be seen in greater detail in Fig. 1 a where 20 individual discharge patterns of a repeated stimulus are shown together. From right to left in each column the sound intensity was increased in steps of 2 dB. At a sound intensity of 2 dB below criterion intensity the neuron responded only in 9 cases with one or sometimes even with two impulses whereas it was 'silent' in the remaining 11 cases. Increasing sound intensity by only 2 dB changed the response activity significantly. At this stimulus level the neuron remained 'silent' only once whereas it responded in the remaining 19 cases with one up to three impulses. Finally, increasing the stimulus intensity by another 2 dB led to absolutely regular response behavior in as much as each given stimulus elicited a nervous reaction.

Based on this response characteristic which was found to be persistent for one or two hours, we defined the neuronal threshold as shown in Fig. 1 c. In a series of 20 repeated stimuli we counted those which caused the neuron to discharge. We defined the criterion (threshold) intensity as that at which the 90% level (in our cases 18 responses) was achieved. This point coincided with the steepest slope of the 'conventional' intensity characteristic (compare Fig. 1 c and b). In each preparation this threshold determination was applied to the right and left interneuron at various frequencies and the corresponding starting values were rechecked at the end of the experiment. In only one out of 38 cases did the experimental results have to be rejected as the rechecked threshold value differed from the starting measurement by more than 2 dB.

Controls of Directional Symmetry Within the Chamber

As shown later in several preparations the auditory thresholds of the right and left interneuron were remarkably different at certain frequencies. To indicate that these asymmetries were attributable to the respective preparation but not a peculiarity of the chamber's acoustics we rechecked in 4 preparations the threshold differences after changing the lateralization of the animal and recoupling the electrodes to the previously opposite connectives. By this procedure the threshold differences in the 'usual' recording situation could be compared to those after horizontally turning the animal by 180°. In all control measurements the sign of the threshold differences changed with changing the lateralization of the preparation (e.g. neuron at the right recording channel more sensitive than that one at the left side or vice versa), but the size of the differences did not change by more than 1 dB. This proves the symmetry of the sound field inside the recording chamber with regard to the impingement of the sound from right or left.

Further control of the sound field was performed by measuring the sound pressure level at different speaker positions with a $^1/_2$ in. condenser microphone which in each case pointed to the sound source. At no frequency of interest did the sound intensity differ by more than 1 dB at different speaker locations. Thus we are confident that the directional characteristics found in the preparations are due to the physical or neuronal processes and not to any acoustical deficiency of the recording chamber.

Analysis of the Stridulatory Sounds

In order to correlate some neuronal findings with the spectrum of the conspecific stridulatory sounds we recorded the calling song of 5 individual males of *Tettigonia viridissima* in a sound proof chamber ($3 \times 4 \times 3$ m) at ambient temperature of 22 °C and at a distance of 1 m from the insect ($^1/_2$ in. condenser microphone, 2606 Brüel and Kjaer measuring amplifier, IV L Nagra tape). By means of computer analysis on a PDP 12 mini computer (Digital Equipment; 32 k core memory, 256 k disk) we determined the power spectrum of the stridulatory sounds within a frequency range from 2 to 40 kHz.

Results

To correlate our neurophysiological results with the temporal and spectral features of the conspecific call we first recorded and analyzed the stridulatory song of 5 males at 22 °C ambient temperature. A typical example is given in Fig. 2. In contrast to many bushcricket species which produce songs with broad spectra, the call of *Tettigonia viridissima* exhibits two distinct energy bands, one around 10 kHz and the second one around 20 kHz, which is slightly broader and lower in amplitude. As proved by sectionwise analysis (within time windows of 5.2 ms) the spectral composition seems to be persistent throughout the whole song which consists of double pulsed elements (inset of Fig. 2). Studying the properties of the auditory system of this species we therefore have to focus our attention on these two frequency bands.

Peripheral Directional Sensitivity

To get an idea of the peripheral directional coding in *Tettigonia viridissima* we measured the threshold sensitivity of one of the two paired interneurons after dissection of the contralateral auditory input at various angles of incidence and stimulus frequencies (see Material and Methods). In Fig. 3 the shifts of the neuronal thresholds are shown when the sound source was moved in distinct intervals from the ipsilateral to the contralateral side in front of the preparations. Whereas at a frequency of 5 and 10 kHz the total change in threshold was in the order of about 5 dB and thus very small, the directional sensitivity increased at around 15 kHz and became very pronounced at a frequency of 20 kHz. At this frequency maximal differences of 15–20 dB between ipsi-versus contralateral stimulation were found. A further increase in stimulus frequency again reduced the directional sensitivity of the tympanic organ which is shown here only for 30 kHz (these findings are corroborated by our later bilateral and suprathreshold measurements on the intact auditory system, compare e.g. Fig. 3 with Fig. 10). Thus it becomes evident that only one (the higher) of the two frequency bands

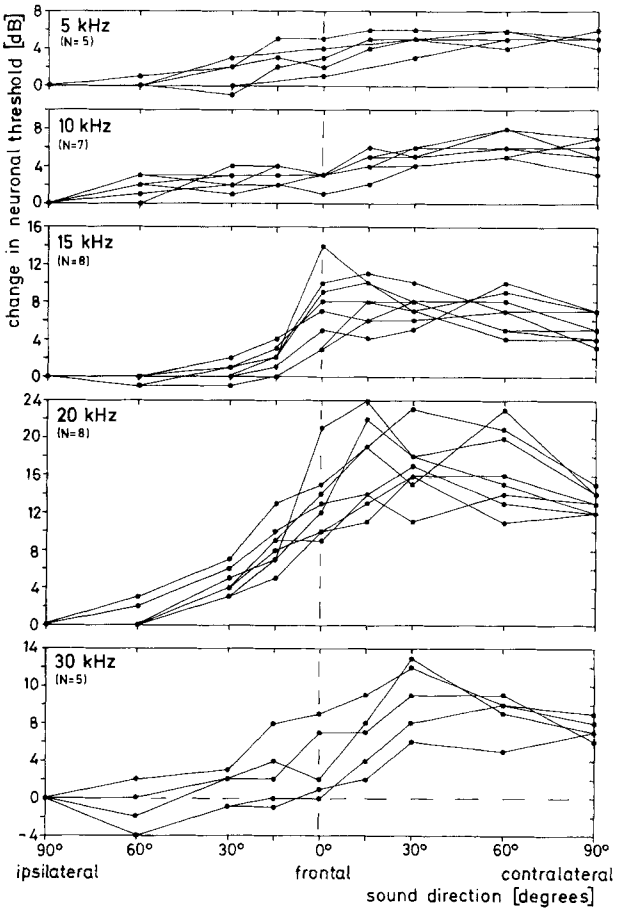

Fig. 3. Directional sensitivity of the tympanic organ of *T. viridissima* at different stimulus frequencies when moving the sound source from ipsi- to contralateral (inferred from changes in threshold of one of the two interneurons after dissection of the contralateral tympanic nerve; for further explanations see text). N: number of preparations at each given stimulus frequency. Note the pronounced directional sensitivity at 20 kHz

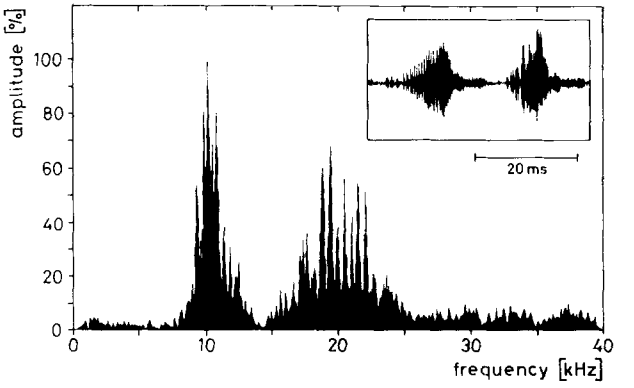

Fig. 2. Power spectrum of the male's conspecific calling song of *Tettigonia viridissima* recorded at 22 °C ambient temperature and evaluated by digital Fourier analysis. Inset: gross temporal structure of one chirp consisting of two pulses

of the conspecific call provides pronounced directional hearing in *Tettigonia viridissima* (see Discussion for the consequences of this phenomenon under natural conditions).

The plots at 20 kHz furthermore reveal that the steepest slopes of the directional curves appeared when the sound source simply switched the longitudinal axis of the animal. Between, for example, the two sound source positions 15° 'right' and 15° 'left' the tympanic thresholds changed by about 10 dB and thus an extremely high lateral sensitivity is provided. This is a prominent feature of pressure gradient receivers (Olson, 1943; Fletcher and Thwaites, 1979) and its meaning for the animals' phonotactic behavior will be discussed below.

Bilateral Symmetry Within the CNS

As a first step in the study of bilateral symmetry in the auditory system of *Tettigonia viridissima* we compared the two threshold curves of the right and left interneuron (at ipsilateral sound stimulation), respectively. As at threshold values the contralateral ear cannot be excited (see Fig. 3) we were confident of measuring the compound frequency sensitivity of the ipsilateral receptor fibres driving each interneuron.

A common characteristic of all our 15 preparations was the comparatively low threshold values within the frequency range from 10 to 20 kHz (see e.g. Fig. 4a) which corresponds to the spectral characteristics of the conspecific call. In this frequency range the absolute sensitivity was of the order of 30 dB SPL which means that in all preparations this pair of auditory interneurons was fully capable of managing long distance communication. Surprisingly in only about 50% of the preparations did the two interneurons exhibit more or less identical threshold curves over the whole frequency range tested. One example for this configuration is given in Fig. 4a, where neither threshold curves deviated by more than 3 dB from the other. However, in the remaining 8 (out of 15) cases this 'total' congruence of both threshold curves could not be found. In these preparations the threshold values of both interneurons overlapped only within a rather limited 'midfrequency' band. Figure 4b shows an example where both curves diverge by more than 10 dB above 25 kHz and below 10 kHz. Similar to this result was the finding in Fig. 4c, but with the exception that in the high frequency part both curves again coincided at 35 kHz to diverge again above 35 kHz. Consequently there seems to be no systematic deficit of the right or the left tympanic organ as a whole (or the correlated synaptic processes) but it is more likely that single receptor cells or neighbouring groups fail to function (see Discussion).

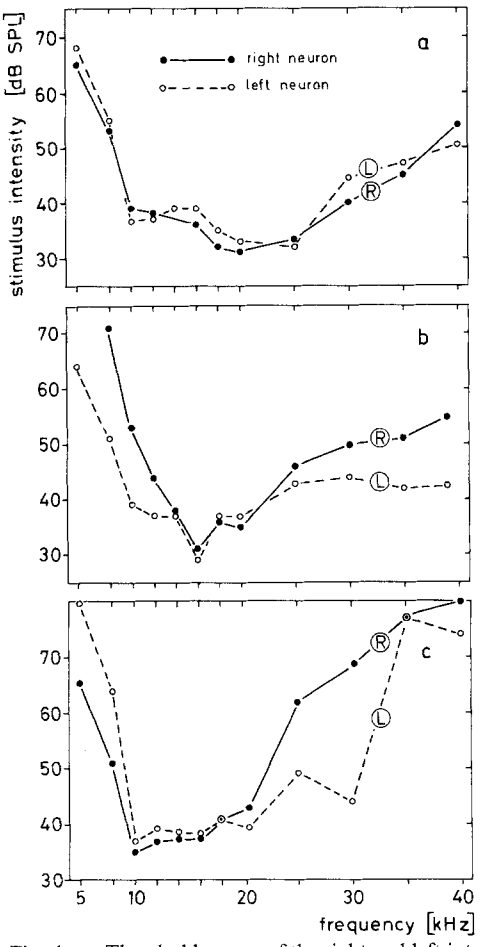

Fig. 4a–c. Threshold curves of the right and left interneuron respectively at ipsilateral sound stimulation (**a, b, c** three different preparations). For further explanations see text

The most important results concerning the asymmetries in thresholds between the 15 pairs of auditory interneurons are summarized in Fig. 5 where the maximal threshold differences measured at each stimulus frequency are plotted. The data emphasize once again that in the low (5 up to 10 kHz) and high (25 up to 40 kHz) frequency range the bilateral threshold curves could differ by as much as 20 dB (or even more) whereas in the midfrequency range from 12 up to 20 kHz the differences were maximally 5 dB, normally less. This means that the auditory pathway of *Tettigonia viridissima* certainly provides symmetrical thresholds only in a very limited frequency range whereas above and below significant asymmetries may occur. It is noteworthy that this frequency band coincides with the frequency of optimal directional sensitivity (see e.g. Figs. 3 and 10) which matches only the 20 kHz component of the conspecific call.

Therefore in 9 preparations the corresponding intensity-response characteristics were determined at this prominent frequency of 20 kHz. The example

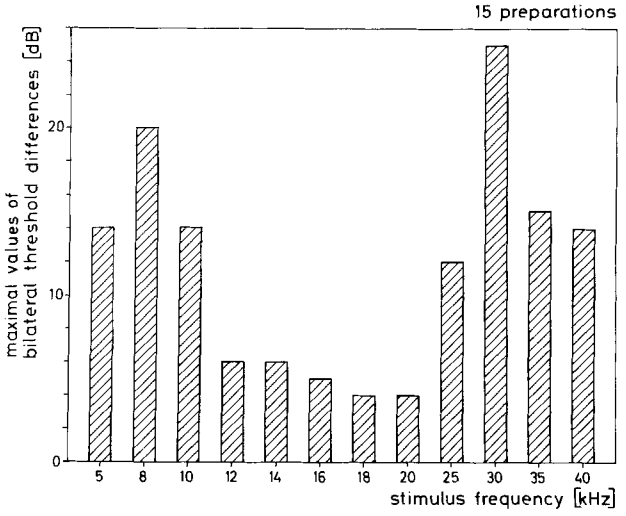

Fig. 5. Maximal values of bilateral threshold differences between the pairs of interneurons at different stimulus frequencies (collected from 15 preparations)

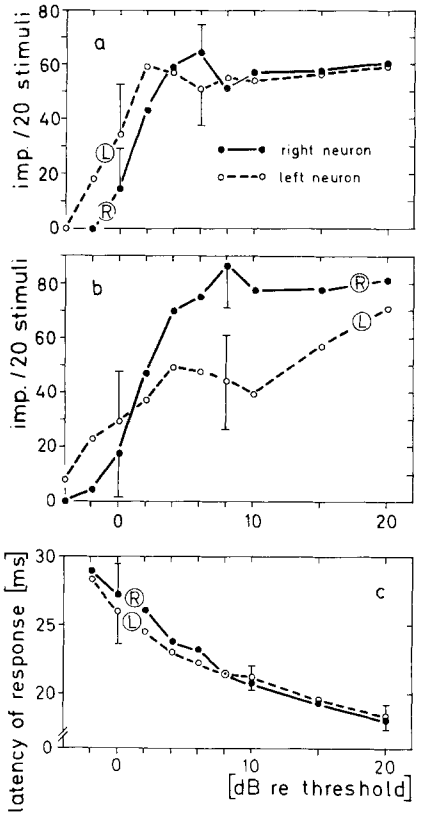

Fig. 6a–c. Intensity-response curves of a pair of interneurons from two preparations (**a**, **b**) at a stimulus frequency of 20 kHz. **c** Latency characteristics of the same pair of interneurons as shown in **b**

in Fig. 6a shows fairly symmetrical response characteristics of both interneurons for the whole intensity range tested. Similar relationships were found in three more preparations. In the remaining 5 (out of 9) cases, however, remarkably asymmetric intensity-response characteristics between the right and left interneuron were discovered. Figure 6b shows one example of this, and a glance would suggest that the plots come from two unpaired neurons. These differences did not so much concern threshold values but, more interestingly, slopes. At a sound intensity of 10 dB above threshold, for example, the right interneuron fired twice as often as the left one (Fig. 6b).

This impression of an unpaired recording situation disappeared however in all 5 cases as soon as the latency characteristics and, as shown later, the directional sensitivities of both interneurons were studied. As a typical example, from the same pair of interneurons in Fig. 6b the corresponding latencies of responses are shown in Fig. 6c. Surprisingly, the two latency functions are found to be highly symmetrical over the whole intensity range tested which contrasts with the two intensity functions. Thus, intensity-response curve and latency characteristic need not to be correlated with each other. Comparing the corresponding latency functions in all 9 preparations, very symmetrical curves similar to the findings in Fig. 6c were observed.

From our detailed study of directional characteristics two distinct examples are given below. In Fig. 7 the directional sensitivity of a pair of interneurons is shown, the intensity characteristics of which are symmetrical but insignificant different at threshold values (see Fig. 6a). Thus, at low sound intensities

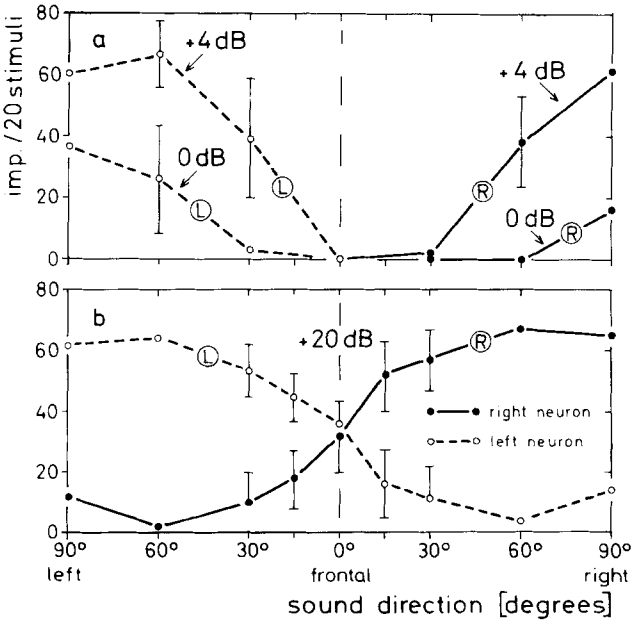

Fig. 7. Directional characteristics of the same pair of interneurons as shown in Fig. 6a at different sound intensities above threshold as indicated (stimulus frequency 20 kHz)

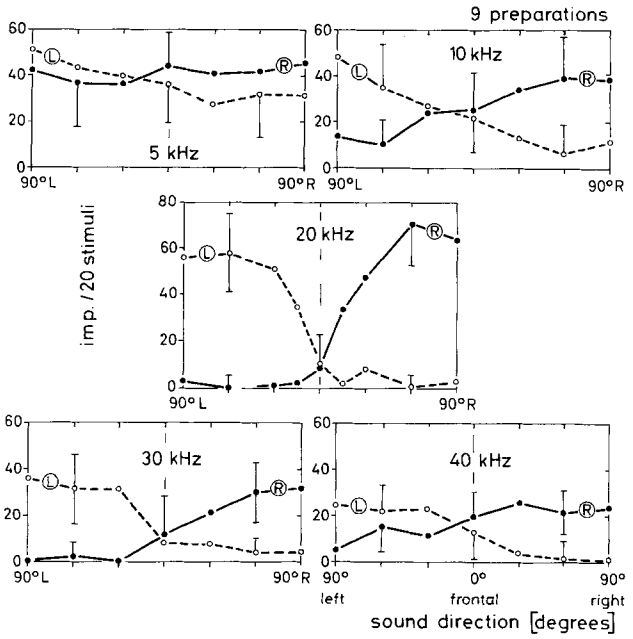

Fig. 10. Averaged directional characteristics of 9 pairs of interneurons at different stimulus frequencies as indicated (sound intensity 10 dB above threshold)

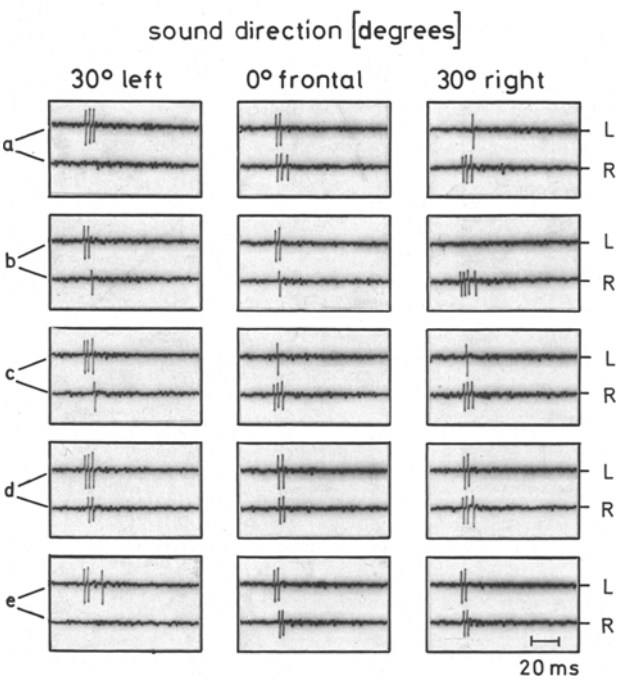

Fig. 11. 5 consecutively recorded examples (line *a–e*) of coincidental responses of the left (*L*) and right (*R*) interneuron at different sound directions (stimulus frequency 20 kHz, sound intensity 10 dB above threshold). Note the asymmetry in spike count and/or latency response with frontal (symmetrical) stimulation

up by the dashed line. By this procedure an artificial 'handedness' was induced in our analysis to avoid the equalizing effect of averaging. But surprisingly, only near threshold (Fig. 9, 0 and 4 dB re threshold) did this 'handedness' reappear in the averaged directional plots. Starting at 8 dB above threshold the 'identification' of accurate lateralization was again performed as it was previously observed with individual preparations.

Until now the directional coding by this pair of auditory interneurons has been studied solely at a stimulus frequency of 20 kHz which coincides with the higher of the two spectral bands within the conspecific call. Figure 10 documents the directional sensitivity of both interneurons at different stimulus frequencies (averaged data from 9 preparations, here representatively shown for a sound intensity of 10 dB above threshold). Clearly at 20 kHz the directional sensitivity was more pronounced than at lower and higher stimulus frequencies where as well the accuracy of lateral coding as the amount of bilateral spike differences were reduced. Thus by this pair of auditory interneurons optimal directional information is provided only within a rather limited frequency range.

Analysis of Single Neuronal Events

In this study the data analysis has so far been based upon the statistical comparison of averaged discharges. But as mentioned before the phonotactic response of some crickets can occur as a result of a single sound stimulus. Therefore it is also extremely important simply to compare coinciding individual responses on the right and left side of the auditory pathway (without averaging successive responses).

In Fig. 11 5 typical pairs of coinciding responses are given for 3 different sound directions. The middle row represents the symmetrical stimulus situation as the sound source was precisely in front of the animal on its longitudinal axis. Looking at the spike count, in only two cases (line d, e) did both interneurons discharge symmetrically with two spikes each (as it is known from our preceding statistical analysis). In the remaining 3 cases either the left or the right neuron discharged more strongly than the opposite one (in line c the spike counts are even as different as 3 to 1). This high probability of unequal and thus 'wrong' spike counts with frontal stimulation shows that on the basis of these individual neuronal events a 'correct' prediction of frontal sound stimulation can hardly be performed. However, shifting the sound source to a lateral position (in Fig. 11 shown for 30° right and left) significantly increased the probability of 'correct' discharges as now the respective ipsilateral neuron reliably responded more strongly than the contralateral one (for one exceptional case of 'wrong' discharges see line e, 30° right).

In Fig. 12 these findings concerning the analysis

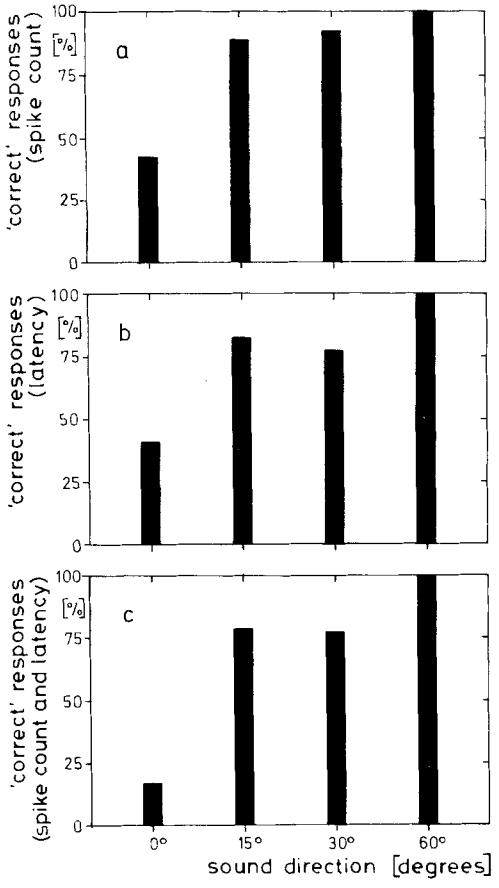

Fig. 12. Probability of 'correct' responses between the right and left interneuron at different sound directions (6 preparations, stimulus frequency 20 kHz, sound intensity 20 dB above threshold). Each column of the diagram is based on the analyis of about 270 paired neuronal events. For further explanations see text

of single coinciding pairs of neuronal responses are summarized for various angles of incidence and from different points of view. In Fig. 12a 'correct' bilateral response for frontal sound stimulation (0°) is defined by equal spike counts in both interneurons; for a lateral stimulus angle (15°, 30°, 60°) a 'correct' response is defined by unpaired discharges, the respective ipsilateral neuron leading. Surprisingly, with the symmetrical (frontal) stimulus configuration both neurons were excited symmetrically only in 43% of the cases. But as soon as the animal's body axis deviated from the target axis, this probability for 'correct' paired discharges increased for 15° to about 89% and for 30° to about 92%. When the sound source was 60° to the right or left side (or more lateral which is not shown in Fig. 12) then at all given stimuli 'correct' discharge patterns were observed.

In Fig. 12b this kind of analysis is repeated but on the basis of comparing the latencies of the two responses. On the assumption that time differences less than 1 ms are not distinguished in the insects' auditory pathway (see Rheinlaender and Mörchen, 1979), with frontal sound stimulation (0°) in only 41% of the cases did symmetrical latency responses occur (Fig. 12b), which is similar to our analysis based on spike counts. Again with lateral stimulus angles the probability of correct temporal relationships improved with the respective ipsilateral neuron leading by shorter latency responses (these latency differences observed were in the order of 3 to 5 ms). It may certainly be assumed that both bilateral spike counts *and* latency differences influence successive neurons in the bushcricket's CNS. Therefore in Fig. 12c both probabilities of Fig. 12a and b are combined. But as already known from Fig. 11 (see frontal stimulation, lines d, e) symmetrical spike counts need not to be correlated with identical latency responses and therefore this kind of analysis must lead to a different conclusion. Consequently in Fig. 12c with frontal sound stimulation the probability of 'correct' discharge patterns was much lower than the analysis of spike count *or* latency differences (compare 17% with 41% and 43%). Again the sound source had to be as lateral as 60° to be correctly encoded by both neuronal parameters. With regard to directional coding these data suggest that both interneurons operate as more 'reliably' the more the sound direction diverges from the animal's longitudinal axis (for the consequences of these findings at phonotactic approach see Discussion).

Discussion

Autrum (1940) indicated that insect ears may act as pressure gradient receivers. This is especially true for the ensiferan ear (gryllids and bushcrickets) where both the inside and outside of the auditory organ are exposed to the sound. Depending on the degree of interaction between the two sound waves such a sound receiver can be directionally sensitive (Michelsen and Nocke, 1974). As the sound reaches the inside of the tympanic organ by a rather complex system of tracheal tubes (Lewis, 1974; Nocke, 1975) having frequency dependent transmission characteristics (Michelsen and Larsen, 1978; Seymour et al., 1978), this is one of the reasons that the bushcricket ear provides optimal directional sensitivity only within a limited frequency band as shown first by Zhantiev and Dubrovin (1973). For the cricket, Hill and Boyan (1977) demonstrated that this band coincides with the species' song frequency.

In contrast to gryllids and many bushcrickets the calling song of *Tettigonia viridissima* exhibits two distinct frequency bands differing by approximately one octave (10 kHz and 20 kHz) but which are similar

in amplitude (see Fig. 2). The pair of auditory interneurons investigated is well adapted to these two frequencies in terms of absolute thresholds which are in the order of about 30 dB SPL (compare Fig. 4). However, with regard to directional cues both frequency bands have very different effects upon the directional hearing in this species. Our neurophysiological measurements show that a sound at 20 kHz provides best directional information whereas a 10 kHz signal is much less directionally effective (Figs. 3 and 10). Thus, the two spectral components within the calling song – probably important for species specific recognition and motivation – do not participate in directional hearing to the same degree. This must complicate conspecific phonotaxis in the natural environment as sound propagation in the field is extremely frequency dependent (for review see Michelsen and Nocke, 1974; Michelsen, 1978) and thus the 20 kHz component will be much more attenuated than the 10 kHz one. Consequently, at long interindividual distances the localizing *Tettigonia viridissima* has to make do with poor directional cues (see Fig. 10) which will only improve when the 20 kHz signal can be received.

Comparing the auditory thresholds of the right versus the left interneuron it is remarkable that in only 50% of our sample were the thresholds more or less the same in the whole frequency range (tested from 5 to 40 kHz, see Fig. 4a). In the remaining 50% this symmetry was restricted to frequencies from 12 to 20 kHz, whereas above and below this "midfrequency" region threshold asymmetries of more than 20 dB could occasionally be observed (see Fig. 4b, c and 5). We ascribe these threshold differences to the loss of function in single receptor cells (rather than to unbalanced synaptic processes). Considering that the receptor fibres (about 37 in each tympanic organ; Schumacher, 1973) exhibit V-shaped tuning curves with different best frequencies (Rheinlaender, 1975), this suggestion is supported by our findings in Fig. 4c where the threshold of the right interneuron reflects a missing sensory input at a specific frequency (for a first behavioral hint that in the late mating season bushcrickets can even be unilaterally deafened; see Baier, 1930). As the asymmetries revealed never occurred in the midfrequency band around 20 kHz, a concentration of receptor cells in this range can be assumed.

From our statistical analysis on the suprathreshold behavior of both interneurons we can conclude that at a frequency of 20 kHz a very precise right – left discrimination is provided (Figs. 7, 8 and 9) starting with sound intensities at about 10 dB above threshold. The directional curves are steepest near frontal sound stimulation (up to about 30° off the midline) and more or less angular independent when the sound source is lateral. These neuronal characteristics should be found again in the phonotactic orientation behavior, which is unknown for the bushcricket. It is, however, well established in crickets which are apparently specialized in recognizing on which side of their body axis the sound source is located (Murphey and Zaretsky, 1972), rather than in distinguishing between different angles on one side. With precise frontal sound stimulation both interneurons discharged symmetrically (at least from a numerical point of view) and in no preparation could an 'auditory handedness' be observed (as reported for crickets, Boyan, 1979). As the state of the spiracular openings in the cricket experiments were uncontrolled (Boyan, pers. communication) – and thus probably from preparation to preparation bilaterally uneven – the corresponding measurements have to be revised.

In contrast to this accurate midline 'identification' the two intensity curves of both interneurons were found to be different in about 50% of our preparations. In one example the right interneuron discharged even twice as much as the left one at a given sound intensity (Fig. 6b). This is a remarkable fact and means that coding of sound direction and coding of sound intensity need not be correlated (as found on the level of the receptor fibres; Mörchen et al., 1978). These results reveal that bilateral symmetry does not have to be a general feature of a pair of sensory neurons (see Chapple, 1977) but, more specifically, can be evolved for single, behaviorally relevant signal parameters.

To compare neuronal data with the behavior we should not only study averaged nervous responses, which are based on longer sequences of constant stimulus configurations. On the one hand we don't know the averaging capacities of the auditory centre in an insect; on the other hand we *do* know that some species of crickets – such as *Scapsipedus marginatus* – even respond phonotactically to single sound pulses (which does not seem to impair the accuracy of their acoustic orientation, compare Murphey and Zaretsky, 1972 with Bailey and Thomson, 1977). In this context a comparison of individual responses of the right and left interneuron to a given sound stimulus reveal some interesting facts. First of all, with frontal and thus symmetrical sound stimulation both interneurons rarely discharged symmetrically (the responses could be as different as if the animal were stimulated at an angle of 30°; see Fig. 11). Comparing more than about 250 paired neuronal events we found the probability of symmetrical discharges to be as low as about 17% (Fig. 12). This means that only at a very low rate the frontal position of the sound source is transmitted correctly by this pair of auditory interneurons.

But as soon as the sound source deviated from the animal's longitudinal axis by about 15°, the probability of 'correct' (now unpaired) discharge patterns increases significantly and at an angle of 60°, for example, no 'wrong' information was encoded.

Assuming that this kind of instability at frontal stimulus configuration is a common feature of auditory interneurons in insects, these neurophysiological results can be correlated well to the phonotactic behavior of the cricket. With frontal sound stimulation the crickets rarely walk straight ahead (Murphey and Zaretsky, 1972; Bailey and Thomson, 1977; Wendler et al., 1980) which would be the only correct behavioral response. Our neuronal data suggest that the cricket has to avoid aligning its body axis to the sound source in order to operate outside this angular (frontal) window of uncertainty. Consequently, deviating by small angular rates from the 'correct' course and thus avoiding frontal stimulation seems to be a useful strategy within the phonotactic walk which leads to the well known zig-zag approach (for details see Huber, 1977).

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