

DIRECTIONAL HEARING OF A GRASSHOPPER IN THE FIELD

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Summary

An electrophysiological method for making long-term recordings from the tympanal nerve was developed in *Chorthippus biguttulus* (Gomphocerinae) to gain insight into the ecophysiological constraints of sound localization in acridid grasshoppers. Using this 'biological microphone', the directional dependence of auditory nerve activity was monitored both in the laboratory and in various natural habitats of this species. On gravel and in sparse vegetation, the overall patterns of directionality were found to be very similar to those in the free sound field in the laboratory, regardless of whether the animal was positioned horizontally or vertically. However, the differences between the ipsi- and contralateral sides were smaller in these habitats than in the laboratory. In dense vegetation, the directional patterns were greatly affected by the environment. Moreover, a minimum in nerve activity was not always reached on the contralateral side, as is

typical for the free sound field situation. On the basis of these data, predictions can be made about the ability of the animals to determine the correct side of a sound source. In the free sound field of the laboratory, correct lateralizations are expected at all angles of sound incidence between 20 and 160°, a prediction corresponding to the results of behavioural studies. In sparse vegetation, a similar accuracy can be anticipated, whereas on gravel and in dense vegetation directional hearing is expected to be severely degraded, especially if the animal is oriented horizontally. The predictions from our present electrophysiological investigations must now be confirmed by behavioural studies in the field.

Key words: directional hearing, biological microphone, sensory ecology, audition, grasshopper, *Chorthippus biguttulus*.

Introduction

During the course of evolution, the sense of hearing has developed several times independently in many orders and families. It serves to identify and to localize acoustically active predators, prey and conspecific mates (Busnel, 1963; Webster et al., 1992). The sensory and neural mechanisms underlying acoustic communication have been thoroughly investigated in several orthopteran insects (Elsner and Popov, 1978; Huber et al., 1989; Ewing, 1989; Bailey, 1991). For our present study on the directionality of hearing in natural biotopes, we performed experiments on the gomphocerine grasshopper *Chorthippus biguttulus*, a species in which both males and females produce species-specific songs during pair formation (von Helversen and von Helversen, 1997).

The ability of males of *Ch. biguttulus* to lateralize a singing female (i.e. to determine whether she is on his right or left side) can easily be tested in behavioural experiments because the males exhibit a precise turning reaction towards the side of the sound source: if stimuli are presented from both sides simultaneously, males are able to discriminate intensity differences of 1.5 dB (von Helversen and von Helversen, 1983; von Helversen, 1997). Such accuracy of lateralization is within the range of human capabilities, an astonishing fact because the ears of these grasshoppers are positioned only 2–3 mm apart, i.e. less than one-tenth of the wavelength of the signal

to be localized. Grasshoppers can achieve such accurate discrimination because they make use of a pressure gradient receiver mechanism (Michelsen and Rohrseitz, 1995). The sound pressure, which is non-directional, acts on the tympanal membrane, both on the outside of each tympanum and, with a phase delay and at decreased amplitude, also on the inside. How much the internal pressure is delayed and attenuated relative to the outside pressure is dependent on the frequency of the sound and its angle of incidence. The interaction between these two pressures and the tympanal membrane results in a driving force acting on the tympanal membrane which, in turn, stimulates the auditory receptors (Michelsen and Rohrseitz, 1995).

In contrast to field studies on bush-crickets (Rheinlaender and Römer, 1986, 1990), investigations on the directionality of the auditory system in grasshoppers and locusts have so far been performed exclusively under the ideal acoustic conditions of the laboratory (Römer, 1976; Wolf, 1986a,b; von Helversen and Rheinlaender, 1988). However, the natural habitat of acridid grasshoppers living close to the ground is acoustically very complex. The signals received by the animal arrive not only directly from the singing conspecific but also *via* multiple scattered sound paths (Michelsen, 1978; Michelsen and Larsen, 1983; Lang, 1999). Therefore, the acoustic cues

encoding the direction of a sound source, especially the amplitude of the signal, may differ substantially from the conditions in the free sound field of the laboratory (Michelsen and Rohrseitz, 1997). For these reasons, it is necessary to determine how far the directionality of the grasshopper ear is affected by the acoustic conditions in the natural environment.

On the basis of a first attempt made by Werner (1995), we developed an electrophysiological preparation for long-term recording of the tympanal nerve in the grasshopper *Ch. biguttulus*. Using this 'biological microphone', the directional dependence of tympanal nerve activity could be monitored in the laboratory and in various natural habitats to obtain data on the ecophysiological constraints of sound localization.

Materials and methods

Animals

All experiments employed adult male grasshoppers of the species *Chorthippus biguttulus* (L.) which had been caught in the field near Göttingen. Small groups of animals were kept in cages in the laboratory for up to 2 weeks.

Establishing the nerve recording

The grasshopper was mounted on an operation table, ventral side up. A window was cut into the cuticle of the metathoracic sternum, making the tympanal nerve accessible without destroying the main trachea and the air sacs between the ears. With the help of a micromanipulator, a hook electrode with an opening of approximately 50 µm made from steel wire (V2A, diameter 40 µm) was placed under the tympanal nerve close to the metathoracic ganglion. To prevent short-circuits, the electrical connection was isolated from the haemolymph of the animal using low-viscosity silicone (Baysilone, Bayer AG Leverkusen). The indifferent electrode, which made contact with the haemolymph, was placed close to the hook electrode.

The functioning of the nerve recording was tested by presenting acoustic stimuli and monitoring tympanal nerve activity on an oscilloscope. The wound was then closed using a mixture of beeswax and collophonium (2:1), which also fixed the electrodes. The wires could now be bent around the animal and waxed to the body surface in such a way that they were led away from the dorsal side. After removing the animal from the operation table, its legs and wings were fixed with wax/collophonium in a normal listening position. A holder was then waxed to the dorsal side of the animal at the level of its ears. The preparation was mechanically stable and could be transported to the field and back to the laboratory.

Recording arrangement

The holder with the animal was connected to a movable device (Fig. 1). It was possible to turn the animal on the spot without changing the sound path between the animal and the loudspeaker. A protractor was used to determine the angle of sound incidence. The whole apparatus could easily be moved to different experimental sites.

The chronically implanted electrodes were connected to a small difference preamplifier (Burr Brown INA116U). After further amplification and high-pass filtering (50 Hz), the recorded signals were stored on a DAT cassette recorder (Sony Instrumentation cassette recorder PC204 A). A trigger pulse, indicating the beginning of a signal, was also recorded.

Experimentation sites

In the laboratory, the experiments were performed (i) in a free sound field and (ii) 2 cm above the surface of a table on which a layer of foam simulated the situation in behavioural experiments (Gilbert, 1995). The distance between loudspeaker and animal was 30 cm.

Field experiments were performed close to the ground in three types of biotope. (i) Sparse vegetation, which is the natural habitat of *Ch. biguttulus*, but also of *Ch. albomarginatus* and *Omocestus viridulus*. This biotope is characterised by herbs and grasses with a height of up to 3–5 cm covering approximately 80% of the ground (for example *Thymian serpyllum*, *Poa trivialis*, *P. pratensis*, *Lolium perenne*). (ii) The margin of a gravel path where singing grasshoppers can also be found. Approximately 10–20% of the ground is covered by several species of *Poa*. (iii) Areas with dense grassy vegetation where normally mainly *Chorthippus parallelus*, but also few individuals of *Ch. biguttulus*, can be observed. The ground is totally covered by plants with a height of 40–80 cm (for example *Arrhenaterum elatius*, *Festuca rubra*, *Agropyron repens*, *Cirsium arvense*). Further information about the biotopes can be found in Gilbert (1995).

In the field, the preparation was placed 100 cm from the loudspeaker, 2–3 cm above the grass. In all experiments, the temperature close to the preparation was approximately 26–32 °C, but within any experiment the range was less than 2 °C.

Acoustic stimulation

Sound pulses of 10 ms duration were used, and these included a rising and falling ramp of 1 ms. The stimuli consisted of 7 kHz sine waves. They were generated by an external digital/analogue converter (E-DAC; Gilbert et al., 1997) connected to the parallel port of a notebook computer. The signals were amplified (Denon DCA 450) and transmitted via a loudspeaker (Dynaudio D21 AF). Calibration of the acoustic stimuli was performed in the laboratory using a Brüel & Kjær microphone (type 4133) with a measuring amplifier (type 2619). No corrections were made for the excess attenuation in the field (Lang, 1999). The absolute intensities of the signals presented to the animal therefore differed between laboratory and field conditions. The calculated driving forces of the plotted directional patterns are relative to the frontal direction (0°) of the animal.

For the measurement of nerve response curves, the amplitude of the signal was varied from 35 to 71 dB sound pressure level (SPL) with an increase of 2 dB per step. Below 35 dB SPL and above 71 dB SPL, the increase was 5 dB per step.

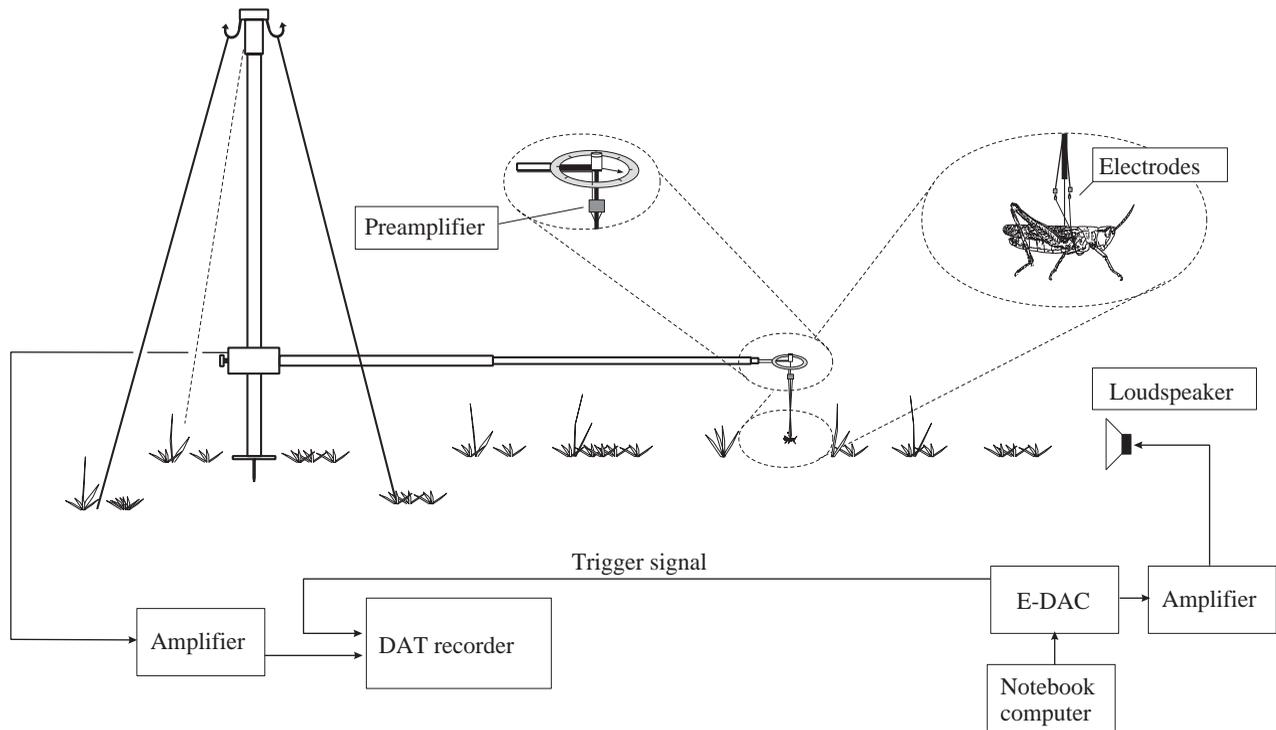


Fig. 1. Experimental arrangement for measuring the directional response pattern of the tympanal nerve of a grasshopper (*Chorthippus biguttulus*) in the field. The animal was fixed onto a holder and placed in the habitat without disturbing the acoustic properties. The response of the tympanal nerve was recorded extracellularly using electrodes connected to wires on the holder. The electrical responses were amplified and stored on a DAT recorder. Acoustic stimuli were generated by a notebook computer. After analogue conversion by an external device (E-DAC), the signals were amplified and played to the animal *via* a loudspeaker. At the same time, a trigger signal was recorded on the DAT recorder.

(see Fig. 2). For each signal amplitude, 100 stimuli were presented separated by pauses of 90 ms.

During the recording of the directional pattern of the tympanal nerve response, the position of loudspeaker remained constant. To change the angle of sound incidence, the animal was rotated in steps of 5 or 10°. For each angle of sound incidence, 100 stimuli of the same amplitude were presented separated by pauses of 90 ms. Because the dynamic range of the nerve response is limited, three different amplitudes were used for each angle.

In Figs 3, 4, 6 and 7, positive angles indicate that the loudspeaker was ipsilateral to the monitored tympanal nerve, whereas negative angles indicate that the sound source was on the contralateral side. For measurements in which the animals were in a horizontal position, an angle of sound incidence of 0° refers to the frontal direction, and an angle of sound incidence of 180° refers to the caudal direction of the animal. In the vertical position, 0° refers to the ventral side, and 180° to the dorsal side of the animal.

Digitization and analysis

For further analysis, the data were transferred to a computer using an analogue/digital translation board (Data Translation DT 2821) with corresponding software (Stemmer; Turbolab 4.2). Nerve activity was measured as the integral below the

summed action potential curve. Within an interval of 30 ms starting with the beginning of the presentation of the signal, the absolute values of the recorded voltages were integrated in a sliding window of 10 ms duration. The maximum of the values determined in this way was used as a measure of the nerve activity for the given stimulus. The activities evoked by 100 successive stimulations were in most cases normally distributed.

Calibration of the nerve activity

A nerve response curve recorded immediately before measuring the directional pattern was used to calibrate tympanal nerve activity. The response was recorded with the sound source at 90° (30–85 dB) ipsilateral to the monitored tympanal nerve. In the range of stimulus intensities between 47 and 72 dB, the activity of the tympanal nerve increases linearly with the logarithm of the amplitude of the acoustic signal. The regression line through this linear region of the response curve was used to determine the force driving the tympanal membrane (Fig. 2).

As mentioned above, the sound level of the stimulus was kept constant during the recording of the directional pattern. Depending on the angle of sound incidence, the tympanal nerve activity varied systematically. Using the linear regression of the nerve response curve, each value of nerve

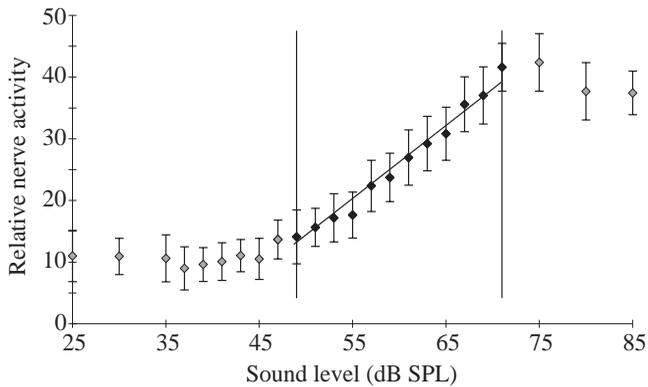


Fig. 2. Nerve response curve of tympanal nerve activity over a range of sound pressure levels. The regression line through the region of linearly increasing nerve activity is used for calibration (see Materials and methods). The driving forces of the directional pattern curves are calculated from the nerve activity. Each point represents the mean \pm S.D. of 100 stimuli.

activity calculated for the stimulation during the directional pattern could be transformed into a driving force (in dB). The frontal direction (0°) was taken as reference: the value of 0 dB results from the regression between -30° and 30° in the directional pattern. The resulting driving forces were then plotted *versus* the respective angle of sound incidence (see Figs 3, 4, 6, 7). By doing this, it is possible not only to compare the directional patterns of the same individual measured in different environments but also to compare directional patterns between different individuals. In addition, the data can be compared with behavioural experiments performed in the laboratory, as will be discussed below.

For a valid calibration, it was necessary that the nerve activity did not depart from the linear range of the response curve. To ensure that the variation did not deviate from the linear region, more than one signal amplitude had to be used

to record the directional pattern. This was taken into consideration in the final evaluation.

To illustrate the presentation of data, the directional pattern of one individual placed in the free sound field is shown in Fig. 3. The pattern is not far from a sinusoidally shaped curve: the tympanal nerve activity and, thus, the calculated driving force increased when the animal was turned from facing the sound source (0°) to a position 90° ipsilateral to the loudspeaker. In contrast, the driving force decreased when the preparation was turned the other way, exposing the contralateral side to the loudspeaker. A minimum activity was reached at an angle of sound incidence between -80° and -90° .

To characterize and compare different directional patterns, the following two variables were defined. (i) The 'maximal range of sensitivity', which is defined as the difference between the average of the three maximal and the three minimal driving forces. In most cases, this value indicates the interaural difference at these angles of sound incidence. For the individual shown in Fig. 3, the range is approximately 22 dB. (ii) The 'angle of 1.5 dB difference', which is a measure of the ability of the animal to lateralize sound when the intensity difference between the two sides is at least 1.5 dB (see von Helversen and von Helversen, 1983; von Helversen, 1997). To determine this angle, the differences in driving force between ipsi- and contralateral sound were calculated from 0° to 30° . A linear regression through these data was used to calculate the angle of 1.5 dB difference (Fig. 3). For the animal shown in Fig. 3, this angle is 5° .

Results

General aspects

Free sound field

The directional patterns of 14 individuals placed in the free sound field and rotated in the horizontal plane are shown in

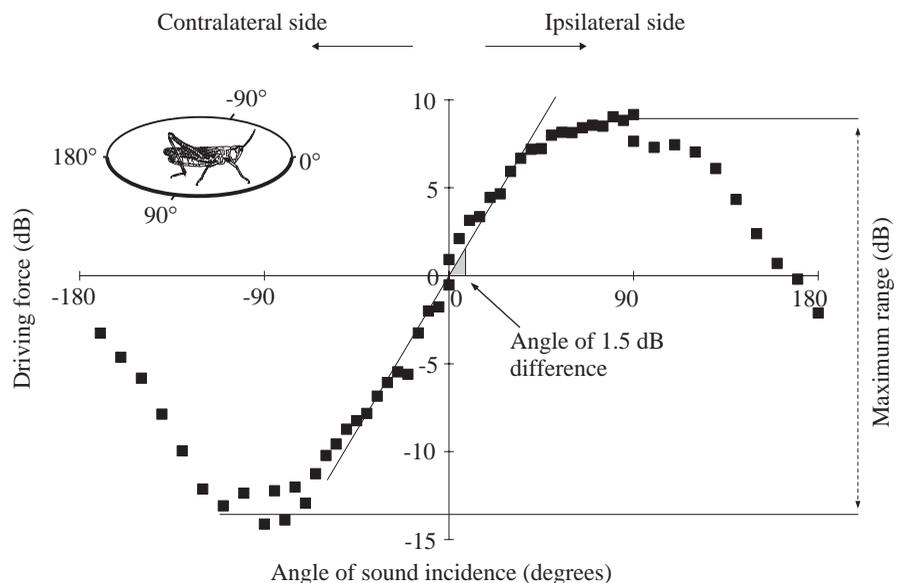


Fig. 3. The directional response pattern of the tympanal nerve of the grasshopper *Chorthippus biguttulus*. Driving forces (dB) are given relative to that at an angle of 0° , at which the animal is facing the sound source. Moving the sound source to the ipsilateral side results in an increase in the driving force corresponding to increased nerve activity; moving it to the contralateral side results in a decrease in the driving force. Two variables are indicated: the maximal range and the angle of 1.5 dB difference (see text for more details).

Fig. 4Ai. In most animals, these patterns have an almost sinusoidal shape. The driving force is maximal at angles of sound incidence of approximately 90° , and a minimum is reached at angles of sound incidence of approximately -90° on the contralateral side. Very similar directional patterns were obtained when the animals were placed vertically (Fig. 4Aii).

Two of the 14 animals had deviant directional patterns (see inset in Fig. 4A): although there was no major difference from the pattern for the other animals on the ipsilateral side, a local maximum in the driving force was present on the contralateral side at angles at which the driving force was minimal in the other animals. The driving force at -90° was equal to or higher than that in the frontal direction. Unfortunately, these animals could not be tested in the vertical plane.

Natural habitats

In sparse vegetation (Fig. 4B) and on gravel (Fig. 4C), the overall appearance of most directional patterns recorded was identical to those in the free sound field situation, except for some individuals showing drastic deviations for unknown reasons (e.g. the pattern marked by 'x' in Fig. 4C). The driving force was highest with the sound source on the ipsilateral side and lowest on the contralateral side. The differences in driving force between the ipsi- and contralateral sides were, however, smaller in both these habitats than in the free sound field.

In dense vegetation (Fig. 4D), the directional patterns were greatly affected by the environment. Moreover, in this biotope, the driving force did not always reach a minimum on the contralateral side, and a large scatter was observed in the data, especially in the vertical plane.

To quantify the general effects of the habitat on the directional patterns, two variables were analyzed (Fig. 5): (i) the maximal range, defined as the difference between the average maximal and the average minimal driving force; and (ii) the angle of 1.5 dB difference, which is an indicator of the ability of the grasshopper to make correct left/right decisions when the intensity difference is at least 1.5 dB.

In the horizontal plane, directional patterns recorded in the free sound field of the laboratory showed the greatest maximal range and the smallest angles of 1.5 dB difference (Fig. 5A,B). The maximal range differed significantly ($P < 0.001$, Student's *t*-test) from those determined in all three natural habitats (Fig. 5A), where it was only two-thirds of the value in the free sound field. There were no significant differences between the three natural habitats. The angles of 1.5 dB difference (Fig. 5B) were only slightly larger ($P < 0.05$) in sparse vegetation than in the free sound field. However, in dense vegetation, the 1.5 dB angle doubled. In the vertical plane, the differences between measurements in the free sound field and in dense vegetation were highly significantly smaller than in the horizontal plane, both for the maximal range ($P < 0.001$) and for angle of 1.5 dB difference ($P < 0.001$) (Fig. 5A,B).

Influence of the habitat on the directional sensitivity of a single individual

The directional patterns shown in Fig. 4 illustrate the general

influence of the habitat on the hearing of *Ch. biguttulus* males. To investigate directional hearing in a single individual, directional patterns were recorded from one animal in different types of surroundings. The example shown in Fig. 6 clearly demonstrates that the directional pattern was greatly influenced by the vegetation: the maximal range clearly decreased in the natural habitat compared with the free sound field.

In the free sound field, the directional pattern had a prominent maximum at angles of sound incidence of $80\text{--}100^\circ$ and a minimum with the sound source on the contralateral side (-80° to -100°). In sparse vegetation, where only the frontal part of the directional pattern was recorded, the driving forces on the ipsilateral side were smaller than those in the free sound field. In contrast, the directional pattern measured in dense vegetation was very different: the driving force varied greatly with the angle of sound incidence, and the curve was not sinusoidal.

Directional patterns in the laboratory

For behavioural tests of lateralization performance in the laboratory, the grasshoppers are usually placed on a table (von Helversen, 1972; von Helversen and von Helversen, 1983; von Helversen and Rheinlaender, 1988). Therefore, directional patterns were recorded not only in the free sound field but also from an animal placed close to the surface of a table. The directional patterns of three animals recorded both in the free sound field and above a table in the laboratory were averaged (Fig. 7A). The slope of the curves at an angle of 0° was less steep when the animals were positioned above the table compared with being placed in a free sound field. This is also expressed in the lower maximal range (10 ± 1.6 dB, Fig. 7B) and the wider angle of 1.5 dB difference ($7.8 \pm 1.2^\circ$, Fig. 7B) (means \pm S.D.), but it should be noted that only three animals were tested.

Discussion

General remarks

The directional hearing of *Ch. biguttulus* has been studied thoroughly using both electrophysiological and behavioural approaches in the laboratory (e.g. von Helversen, 1997; Wolf, 1985; von Helversen and Rheinlaender, 1988; von Helversen and von Helversen, 1995). However, neither in this species nor in any other acridid grasshopper has any attempt been made so far to investigate the patterns of directional hearing in the field. Therefore, the present study was performed to investigate the influence of the habitat on the directional hearing of *Ch. biguttulus*. For this purpose, a movable and mechanically stable 'biological microphone' was developed, i.e. an electrophysiological preparation enabling long-term recordings of tympanal nerve activity both in the laboratory and in the natural habitat.

Pure tones of 7 kHz were used for stimulation, a frequency that is within the range of the maximum of the song spectrum of *Ch. biguttulus* females. Although a pure tone does not resemble the natural non-resonant song, it has the advantage

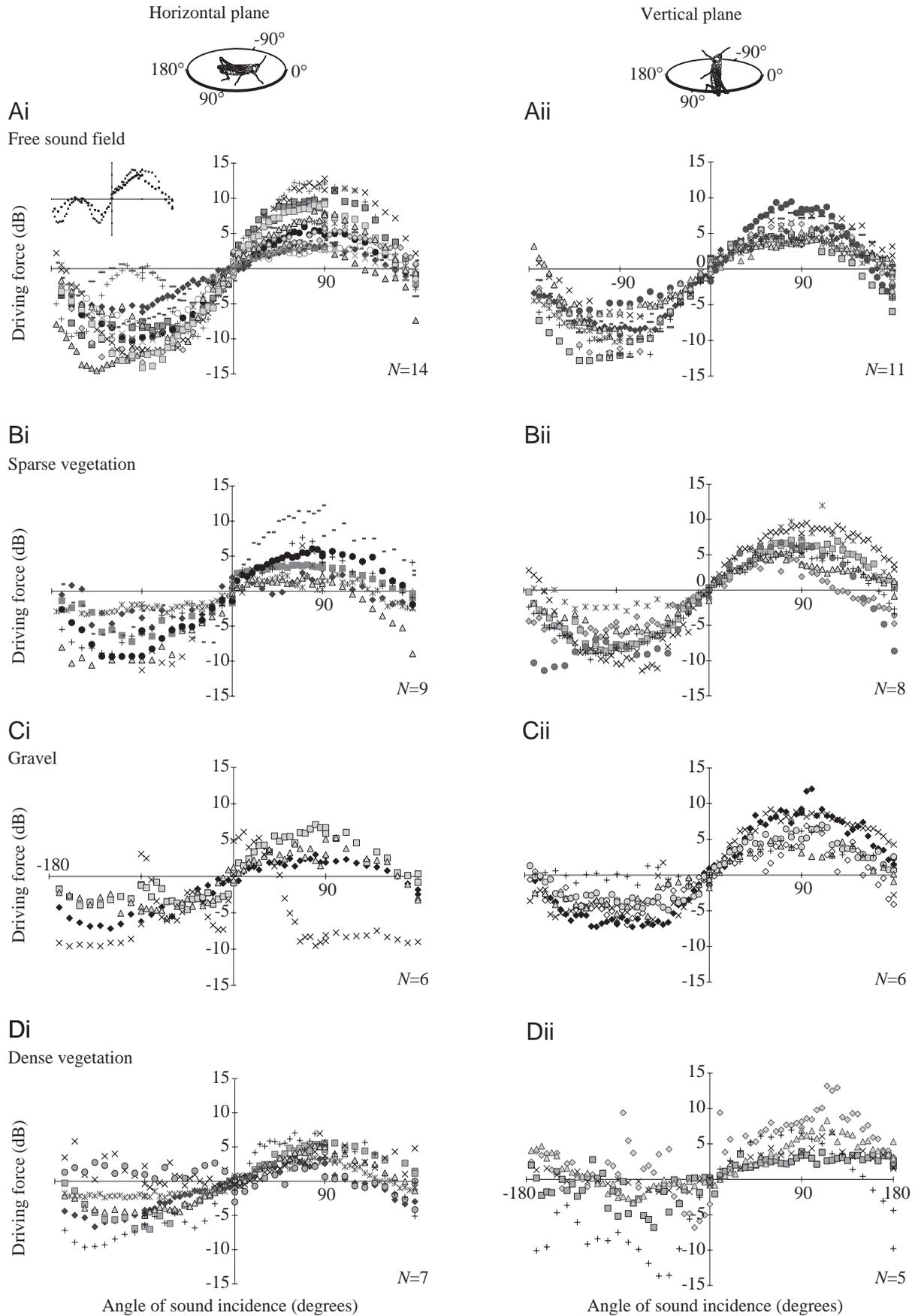


Fig. 4

Fig. 4. Directional patterns of the tympanal nerve response in *Chorthippus biguttulus* recorded from individual grasshoppers placed horizontally (Ai–Di) or vertically (Aii–Dii) in the free sound field (A), in sparse vegetation (B), on gravel (C) or in dense vegetation (D). Each type of symbol represents a single animal. The inset in A shows the directional patterns of two animals with a local maximum on the contralateral side. It can be seen that the directional patterns are more scattered when animals are positioned above gravel or in dense vegetation than in the free sound field. The effect is more pronounced in the horizontal than in the vertical plane (N is the number of animals). It was not possible to study the same individuals under all the different conditions, so particular symbols do not refer to particular animals.

that, in the natural habitat, no frequency-dependent changes in the spectrum interfere with the directionality of the driving force.

Directional hearing in the laboratory

The directional patterns measured in the free sound field (Fig. 4A) show some interindividual variability. The maximal range is 15–16 dB on average. Generally, there is very little difference between the directional patterns measured in the horizontal plane and in the vertical plane. This is to be expected because, at a frequency of 7 kHz, the directional pattern is determined mainly by the pressure difference mechanism in the ear and only to a much smaller extent by the diffraction of sound by the animal (A. Michelsen, personal communication).

In the desert locust *Locusta migratoria*, directional patterns of the tympanal organ have been measured in the laboratory by several authors (Autrum et al., 1961; Adam, 1977a,b; Miller, 1977; Wolf, 1986a,b; von Helversen and Rheinlaender, 1988). The left/right differences found by Autrum et al. (1961) at 7 kHz of 15–22 dB, and the differences of 8–24 dB measured for frequencies between 4 and 25 kHz by Römer (1976), fit well with the present data for *Ch. biguttulus* in the free sound field.

In two out of 14 recordings, directional patterns with a local maximum for sounds played from the contralateral side were observed. This is consistent with the directional patterns recorded by Autrum et al. (1961) in older individuals of *Locusta migratoria*. A similar type of variation in directional pattern has also been observed at 8 kHz at the tympanal membrane in *Ch. biguttulus* using laser vibrometry (A. Michelsen, personal communication). These findings are not surprising because, depending on the sound transmission parameters (attenuation and delay), a directional pattern with a second maximum can be calculated (Michelsen and Rohrseitz, 1995).

Directional patterns measured over a table (Fig. 7) are analogous to the situation during behavioural tests of animals in the laboratory. The maximal range is reduced to approximately 10 dB (compared with 15–16 dB in the free sound field), which is close to the left/right difference of 9–10 dB found in behavioural tests with both male and female *Ch. biguttulus* (von Helversen, 1984; Lakes-Harlan and Pfahler, 1995). Using electrophysiological techniques, Wolf

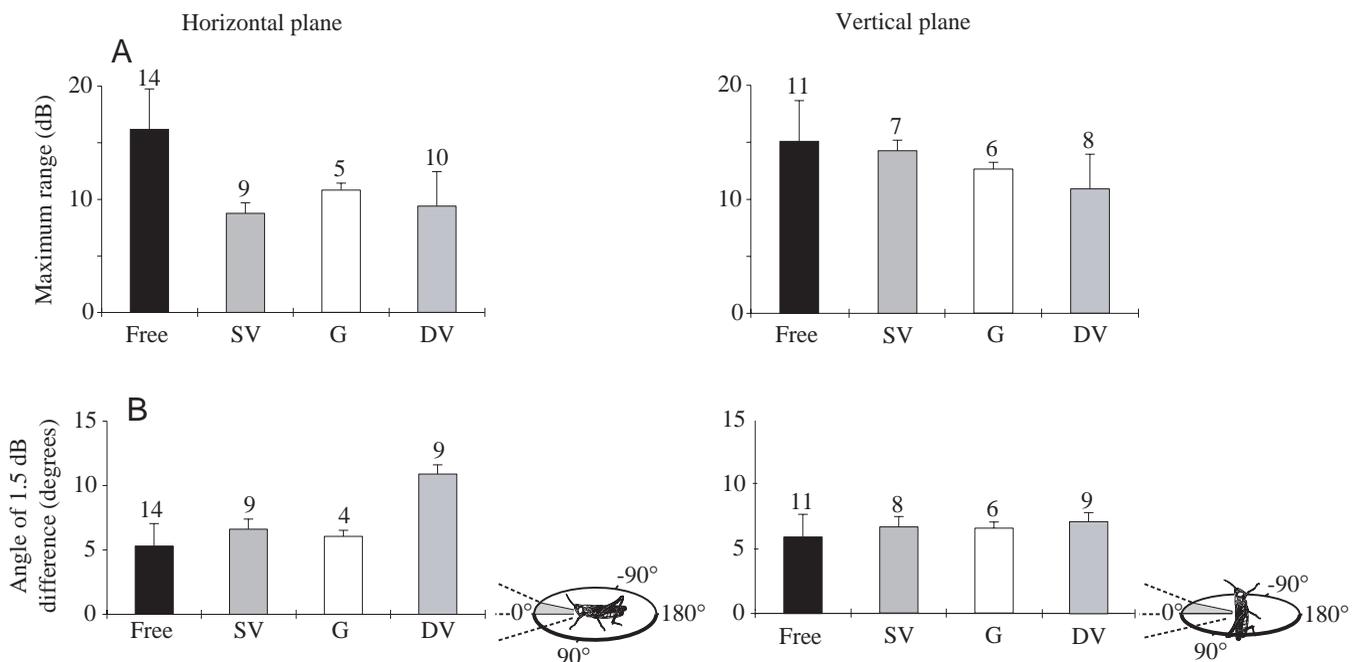


Fig. 5. Effects of habitat on the maximal range (A) and the angle of 1.5 dB difference (B) (see Fig. 3). The maximal range in the free sound field (Free) is significantly greater ($P < 0.001$) than in all the natural habitats when the grasshopper is placed horizontally. In vertically positioned animals, the maximal range differs significantly ($P < 0.001$) from that in the free sound field only in dense vegetation (DV). The angle of 1.5 dB difference is significantly larger ($P < 0.001$) in dense vegetation than in the other two natural habitats and in the free sound field for grasshoppers positioned both horizontally and vertically. Values are means + S.D.; values of N are given above the columns. G, gravel; SV, sparse vegetation.

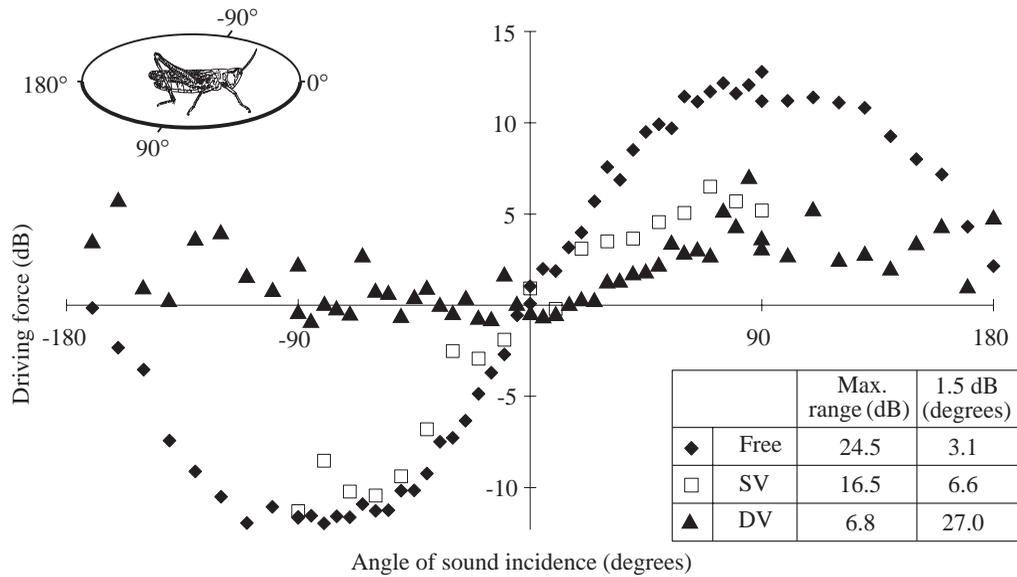


Fig. 6. Comparison of the directional patterns of the tympanal nerve response of an individual grasshopper in different habitats. Dense vegetation (DV; filled triangles), in particular, influences the directional pattern. The sinusoidally shaped curve in the free sound field (Free; filled squares) was recorded after those in sparse (SV; open squares) and dense vegetation.

1.5 dB refers to the angle of 1.5 dB difference (see Fig. 3). (1986a,b) measured a left/right difference of 8 dB in *Ch. biguttulus*. Taking into consideration that these experiments took place 3 cm above a rock wool surface, the result is consistent with the present experiments.

Directional hearing in the habitat

In the natural habitat, the sound field is considerably more complex than in the laboratory: grass and herbs usually grow between the singing animal and its listening mate, the surface is uneven and gravel stones may be scattered around. For good reasons, most studies dealing with sound propagation outdoors are performed at least 1 m above the surface and over larger distances (Cosens and Falls, 1984; Forrest, 1994; Larom et al., 1997). During sound propagation, the signal is changed not only as a result of geometric spreading, but also because there is frequency-dependent excess attenuation (Michelsen, 1978; Lang, 1999). The tendency for high frequencies to be affected more than low frequencies gets stronger the closer the sender and receiver are to the ground (Embleton, 1996).

In their natural environment, acridid grasshoppers such as *Ch. biguttulus* sit on the ground or close to it on vegetation. The directional patterns of the tympanal nerve in the field were measured 2–3 cm above the ground at a distance of 100 cm from the loudspeaker. Hence, the recorded directional pattern is dependent not only on the internal properties of the animal but also on the distortion of the sound field created by direct and indirect sound paths. The general influence of the environment on different individuals can be seen in Fig. 4B–D. In sparse vegetation, the directional pattern is in most cases similar to that in the free sound field. The maximal range is reduced in all types of habitat compared with that in the optimal conditions in the free sound field (see Fig. 5). The surroundings may have a large influence on the directional pattern of a single animal,

as can be seen in Fig. 6. The almost sinusoidally shaped directional curve recorded in the free sound field is only slightly degraded in sparse vegetation, whereas in dense vegetation there is very little similarity to the directional pattern under free

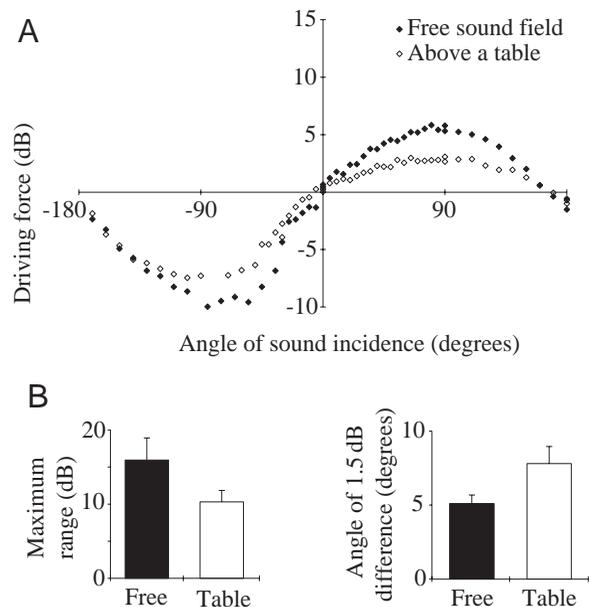


Fig. 7. (A) Averaged directional patterns of the tympanal nerve response of three individual grasshoppers in the laboratory. The same animals were positioned in the free sound field (Free; filled symbols) and above a table (Table; open symbols). (B) The maximal range is reduced above a table ($P < 0.01$) and the angle of 1.5 dB difference (see Fig. 3) is slightly larger than in the free sound field ($P < 0.05$). Values are means + s.d., $N = 3$.

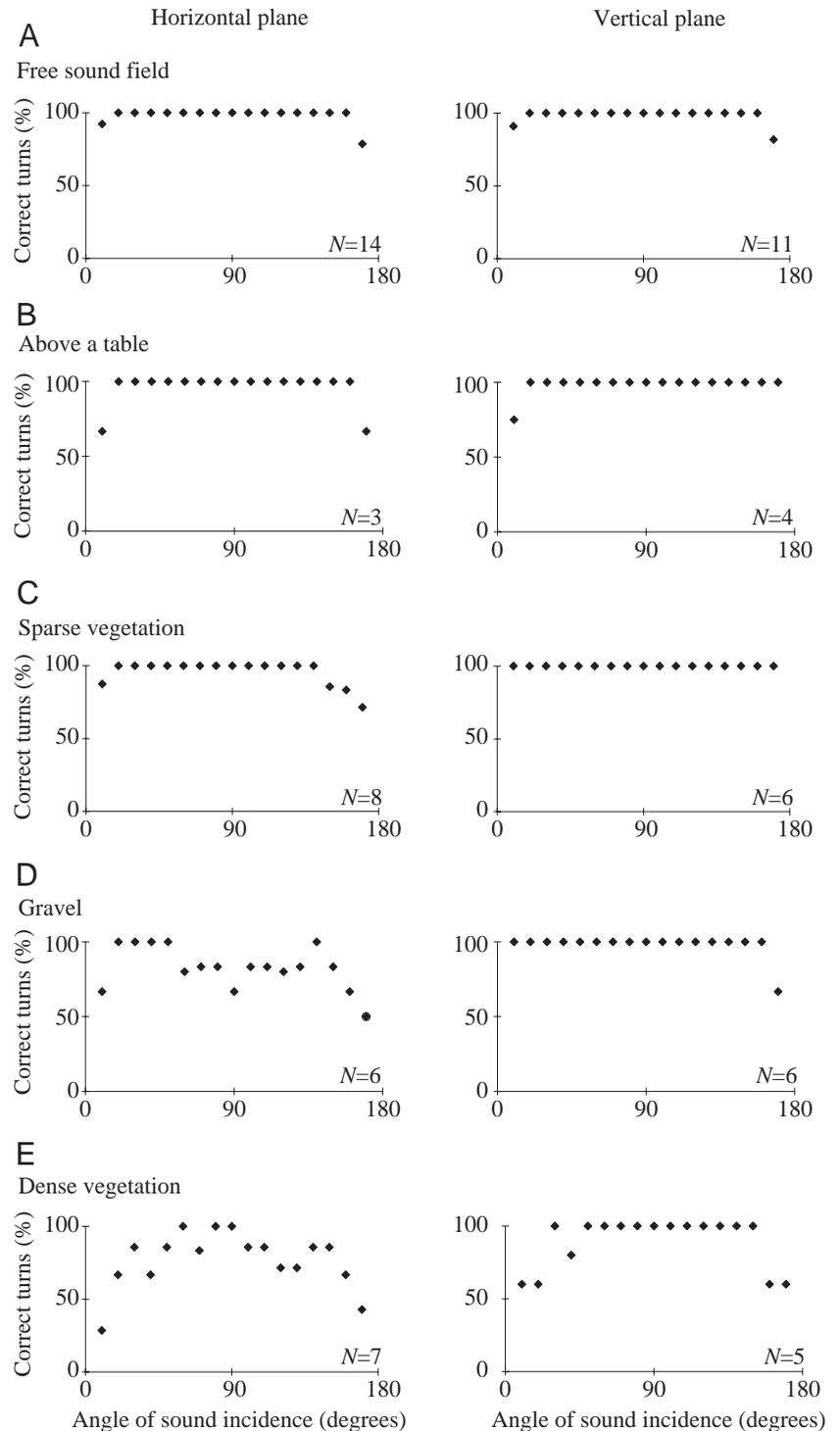


Fig. 8. Calculated phonotactic turning behaviour of the grasshopper *Chorthippus biguttulus* based on the directional patterns recorded electrophysiologically. The percentage of correct turns was calculated for different angles of sound incidence. In behavioural experiments, *Ch. biguttulus* males turn to the louder side if the driving force difference at the ears is at least 1.5 dB. The effect of the habitat is greater in horizontally positioned animals than in those placed vertically. *N* is the number of animals.

field conditions. The question remains, however, as to how much the ability of the animals to lateralize a sound source is reduced by the influence of the surroundings.

Predictions concerning behavioural performance

Using our method, it was possible to monitor the activity of one tympanal organ only, but the animal uses both ears to lateralize a sound source. If we assume that the auditory system is symmetrical, then the interaural difference between the driving

forces at a particular angle of sound incidence can be calculated: it is the difference between the ipsi- and contralateral responses for the angle in question. For this calculation, the averaged nerve responses were used, although it is doubtful whether, in the natural situation, averaging is used by the animals to increase the signal-to-noise ratio for the lateralization of a natural song. Ronacher and Krahe (1997) have shown that male *Ch. biguttulus* are able to lateralize sound even if extremely short female songs consisting of merely 18 pulses are used as stimuli.

Male *Ch. biguttulus* turn to the louder side of a sound source correctly if the interaural difference between the driving forces is at least 1.5 dB (100% correct decisions; 75% correct decisions with only 0.6 dB difference; von Helversen und Rheinlaender, 1988; von Helversen, 1998). Therefore, interaural differences larger than 1.5 dB calculated from the directional patterns presented in our study would represent a correct lateralization of a sound source (Fig. 8).

In the free sound field and above a table in the laboratory, 100% correct lateralizations are predicted for angles of sound incidence of 20–160° since the difference between the ipsi- and contralateral sides was greater than 1.5 dB in all recordings. Only with angles of sound incidence 10° away from the midline of the animal are there a few cases for which the interaural driving force difference is less than 1.5 dB. These cause a slightly reduced rate of correct turns (Fig. 8A,B). The angle of 1.5 dB difference (see Fig. 7) is slightly larger above the table (8±1°) than in the free sound field (5±0.6°) (means ± s.d., N=3). Although we applied 7 kHz stimuli, these findings fit well with the angle of 8–10° determined behaviourally, at which males correctly lateralize a female's song with an accuracy of at least 75% when stimulated by bandpass-filtered noise (von Helversen, 1997).

In sparse vegetation, the situation is only slightly changed compared with that in the laboratory (Fig. 8C), whereas above gravel and in dense vegetation directional hearing is much more degraded, at least in the horizontally placed animals (Fig. 8D,E). In dense vegetation, differences of less than 1.5 dB occur at several angles of sound incidence, and tympanal nerve activity is greater when the sound arrives from the contralateral side (not shown). The vertically positioned animals show a higher percentage of differences above +1.5 dB for all angles of sound incidence.

The directional patterns recorded in the natural habitat suggest that lateralization ability is affected most in dense vegetation: differences of less than 1.5 dB between ipsi- and contralateral sound occur at all angles of sound incidence, and the calculated angle of 1.5 dB difference is increased compared with that in the free sound field (see Fig. 5). In sparse vegetation, the results are similar to those recorded in the laboratory above a table, whereas the situation over gravel is intermediate. These findings nicely match the observation that *Ch. biguttulus* can often be found sitting on gravel and in sparse vegetation, but only rarely in dense vegetation. However, our knowledge of the behavioural ability of *Ch. biguttulus* to localize a sound source in the natural habitat is still rather limited. Therefore, the predictions of our present electrophysiological investigations must now be confirmed by behavioural studies in the field.

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References

- Adam, L.-J.** (1977a). 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.
- Adam, L.-J.** (1977b). The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae). II. Underlying spike pattern and causes of spike synchronization. *Biol. Cybern.* **28**, 109–119.
- Autrum, H., Schwartzkopff, J. and Swoboda, H.** (1961). Der Einfluß der Schallrichtung auf die Tympanal-Potentiale von *Locusta migratoria* L. *Biol. Zentralbl.* **80**, 385–402.
- Bailey, W. J.** (1991). *Acoustic Behaviour of Insects: An Evolutionary Perspective*. London: Chapman & Hall.
- Busnel, R.-G.** (1963). *Acoustic Behaviour of Animals*. Amsterdam, London: Elsevier.
- Cosens, S. E. and Falls, J. B.** (1984). A comparison of sound propagation and song frequency in temperate marsh and grassland habitats. *Behav. Ecol. Sociobiol.* **15**, 161–170.
- Elsner, N. and Popov, A.** (1978). Neuroethology of acoustic communication. *Adv. Insect Physiol.* **13**, 229–355.
- Embleton, T. F. W.** (1996). Tutorial on sound propagation outdoors. *J. Acoust. Soc. Am.* **100**, 31–48.
- Ewing, A.** (1989). *Arthropod Bioacoustics: Neurobiology and Behaviour*. Edinburgh: Edinburgh University Press.
- Forrest, T. G.** (1994). From sender to receiver: Propagation and environmental effects on acoustic signals. *Amer. Zool.* **34**, 644–654.
- Gilbert, F.** (1995). Partnerfindung und akustische Orientierung bei *Chorthippus biguttulus* im natürlichen Lebensraum. Diplom Thesis, University of Göttingen.
- Gilbert, F., Schütze, H., Glahe, M. and Conrad, U.** (1997). A simple hard- and software solution for generation and emission of high frequency signals. *XVI IBAC Conference*. Texas A&M University College Station, Texas, USA.
- Huber, F., Moore, T. E. and Loher, W.** (1989). *Crickets Behavior and Neurobiology*. Ithaca, London: Cornell University Press.
- Lakes-Harlan, R. and Pfahler, C.** (1995). Regeneration of axotomized tympanal nerve fibres in the adult grasshopper *Chorthippus biguttulus* (L.) (Orthoptera: Acrididae) correlates with regaining the localization ability. *J. Comp. Physiol. A* **176**, 797–807.
- Lang, F.** (1999). Acoustic communication distances of a gomphocerine grasshopper. *Bioacoustics* (in press).
- Larom, D., Garstang, M., Payne, K., Raspet, R. and Lindeque, M.** (1997). The influence of surface atmospheric conditions on the range and area reached by animal vocalizations. *J. Exp. Biol.* **200**, 421–431.
- Michelsen, A.** (1978). Sound reception in different environments. In *Sensory Ecology* (ed. M. A. Ali), pp. 345–373. New York, London: Plenum Press.
- Michelsen, A. and Larsen, O. N.** (1983). Strategies for acoustic communication in complex environments. In *Neuroethology and Behavioural Physiology* (ed. F. Huber and H. Markl), pp. 321–331. Berlin, Heidelberg: Springer.
- Michelsen, A. and Rohrseitz, K.** (1995). Directional sound processing and interaural sound transmission in a small and a large grasshopper. *J. Exp. Biol.* **198**, 1817–1827.
- Michelsen, A. and Rohrseitz, K.** (1997). Sound localisation in a

- habitat: an analytical approach to quantifying the degradation of directional cues. *Bioacoustics* **8**, 1–23.
- Miller, L. A.** (1977). Directional hearing in the locust *Schistocerca gregaria* Forskål (Acrididae, Orthoptera). *J. Comp. Physiol.* **119**, 85–98.
- Rheinlaender, J. and Römer, H.** (1986). Insect hearing in the field. I. The use of identified nerve cells as 'biological microphones'. *J. Comp. Physiol. A* **158**, 647–651.
- Rheinlaender, J. and Römer, H.** (1990). Acoustic cues for sound localization and spacing in orthopteran insects. In *The Tettigoniidae: Biology, Systematic and Evolution* (ed. W. J. Bailey and D. C. Rentz), pp. 248–264. Bathurst: Crawford House Press.
- Römer, H.** (1976). Die Informationsverarbeitung tympanaler Rezeptorelemente von *Locusta migratoria* (Acrididae, Orthoptera). *J. Comp. Physiol.* **109**, 101–122.
- Ronacher, B. and Krahe, R.** (1997). Long songs of a short-horned grasshopper: How important is song duration for pattern recognition and localization? In *Göttingen Neurobiology Report 1997* (ed. N. Elsner and H. Wässle), p. 317. Stuttgart, New York: Thieme.
- von Helversen, D.** (1972). Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* (Orthoptera: Acrididae). *J. Comp. Physiol.* **81**, 381–422.
- von Helversen, D.** (1984). Parallel processing in auditory pattern recognition and directional analysis by the grasshopper *Chorthippus biguttulus* L. (Acrididae). *J. Comp. Physiol. A* **154**, 837–846.
- von Helversen, D.** (1997). Comparison of male innate releasing mechanism for female song and female innate releasing mechanism for male song in *Chorthippus biguttulus*. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 301–341. Basel: Birkhäuser.
- von Helversen, D.** (1998). Is the ramped shape of pulses in the song of grasshoppers adaptive for directional hearing? *Naturwissenschaften* **85**, 186–188.
- von Helversen, D. and Rheinlaender, J.** (1988). Interaural intensity and time discrimination in an unrestrained grasshopper: a tentative behavioural approach. *J. Comp. Physiol. A* **162**, 333–340.
- von Helversen, D. and von Helversen, O.** (1983). Species recognition and acoustic localization in acridid grasshoppers: a behavioural approach. In *Neuroethology and Behavioural Physiology* (ed. F. Huber and H. Markl), pp. 95–107. Berlin, Heidelberg, New York: Springer.
- von Helversen, D. and von Helversen, O.** (1995). Acoustic pattern recognition and orientation in orthopteran insects: parallel or serial processing? *J. Comp. Physiol. A* **177**, 767–774.
- von Helversen, D. and von Helversen, O.** (1997). Recognition of sex in the acoustic communication of the grasshopper *Chorthippus biguttulus* (Orthoptera, Acrididae). *J. Comp. Physiol. A* **180**, 373–386.
- Webster, D. B., Fay, R. R. and Popper, A. N.** (1992). *The Evolutionary Biology of Hearing*. Heidelberg, New York: Springer.
- Werner, A.** (1995). Entwicklung einer Methode zur Tympanalnervableitung im Freiland bei der Feldheuschrecke *Chorthippus biguttulus* (L.). Diplom thesis, University of Göttingen.
- Wolf, H.** (1985). Monitoring the activity of an auditory interneuron in a free-moving grasshopper. In *Acoustic and Vibrational Communication in Insects* (ed. K. Kalmring and N. Elsner), pp. 51–60. Hamburg: Paul Parey.
- Wolf, H.** (1986a). Response patterns of two auditory interneurons in a freely moving grasshopper (*Chorthippus biguttulus* L.). I. Response properties in the intact animal. *J. Comp. Physiol. A* **158**, 689–696.
- Wolf, H.** (1986b). Response patterns of two auditory interneurons in a freely moving grasshopper (*Chorthippus biguttulus* L.). II. Representation of stimulus parameters relevant in behavior. *J. Comp. Physiol. A* **158**, 697–703.