J Comp Physiol A (2002) 188: 577–587
DOI 10.1007/s00359-002-0333-7

ORIGINAL PAPER

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Temporal modulation transfer functions in auditory receptor fibres of the locust (*Locusta migratoria* L.)

Accepted: 2 July 2002 / Published online: 31 July 2002

Abstract The temporal resolution of auditory receptors of locusts was investigated by applying noise stimuli with sinusoidal amplitude modulations and by computing temporal modulation transfer functions. These transfer functions showed mostly bandpass characteristics, which are rarely found in other species at the level of receptors. From the upper cut-off frequencies of the modulation transfer functions the minimum integration times were calculated. Minimum integration times showed no significant correlation to the receptor spike rates but depended strongly on the body temperature. At 20°C the average minimum integration time was 1.7 ms, dropping to 0.95 ms at 30°C. The values found in this study correspond well to the range of minimum integration times found in birds and mammals. Gap detection is another standard paradigm to investigate temporal resolution. In locusts and other grasshoppers application of this paradigm yielded values of the minimum detectable gap widths that are approximately twice as large than the minimum integration times reported here.

Keywords Amplitude modulation · Auditory receptor · Gap detection · Temperature · Temporal resolution

Abbreviations *HFR* high-frequency receptor · *LFR* low-frequency receptor · *MIT* minimum integration time · *SPL* sound pressure level

Introduction

Acoustic signals are characterized by their carrier frequency content and by their pattern of amplitude modulations, i.e. their sound envelope. Rapid fluctuations in amplitudes often play a crucial role for signal recognition in various vertebrate and insect species (Michelsen 1985; Ewing 1989; Gerhardt 1994; Krodsma and Miller 1996; Bradbury and Vehrenkamp 1998). Hence, a prerequisite for signal recognition in these species is a sufficient temporal resolution of their auditory systems. Two standard paradigms to characterize temporal resolution are 'gap detection' and 'amplitude modulation transfer functions' (Green 1985; De Boer 1985). Both types of tests can be applied in psychophysical as well as in electrophysiological experiments. In addition, stimulus reconstruction, a procedure restricted to neurophysiological data, has recently been applied to the auditory channel (Rieke et al. 1995; Theunissen et al. 2000; Machens et al. 2001).

In this study we investigated the temporal resolution of a grasshopper at its most peripheral stage of auditory processing, the auditory receptor fibres. We employed an amplitude-modulation paradigm in order to complement earlier gap detection studies (von Helversen 1972, 1979; Ronacher and Römer 1985; Franz and Ronacher 2002), and a recent investigation using stimulus reconstruction methods (Machens et al. 2001).

Among the factors possibly influencing temporal resolution spike rates have to be considered. The question is whether higher spike rates will allow for smaller minimum integration times. In this context the observation may be important that high-frequency and low-frequency receptors differ in their maximum spike rates (Römer 1976). Another crucial factor influencing spike rates and temporal resolution is temperature. Grasshoppers are poikilotherms and their temperature can change by several degrees even within a few minutes. Among a neuron's response properties that may be relevant for temporal resolution, in particular spike rates and time constants of postsynaptic potentials are affected by temperature changes (Janssen 1992; Franz and Ronacher 2002). For this reason we investigated temporal resolution at two different temperatures. This study will be a further step in a comparative approach to

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temporal resolution across different taxa with different equipment of auditory receptors. Furthermore, it allows a comparison of different methods for measuring temporal resolution, both concerning the equivalence of values found with different procedures as well as the applicability of different paradigms.

Material and methods

Animals and electrophysiology

All experiments were performed on adult locusts (*Locusta migratoria* L) that were obtained from a commercial supplier. After removal of head, legs and wings the animals were fixed with wax with their dorsal side up onto a Peltier element (3 cm×1.5 cm) attached to a holder. The thorax was opened dorsally and the metathoracic ganglion was exposed and stabilized by a small Ni-Cr platform. The whole torso was filled with locust Ringer solution. The preparation was placed inside a Faraday cage (90 cm×74 cm×68 cm) lined with foam prisms to attenuate echoes. Recordings from single auditory receptor axons were performed (quasi-) intracellularly in the tympanal nerve with sharp glass microelectrodes (GC-100F, Clark Electromedical Instruments, Reading, UK; puller: Brown Flaming P 87) filled with 1 mol l^{-1} KCl (20–60 MΩ). For further details of the experimental procedures see Franz and Ronacher (2002). After successful penetration of a cell it was checked with sinus of different frequencies whether it was a high- or low-frequency receptor, whereafter the threshold for noise was determined using 500-ms pulses of broad-band noise at different intensities. The amplitude modulated stimuli were presented at 20 dB above threshold. Each 4.4-s stimulus was presented once. In a few cases, provided that the recording was stable enough, stimulus presentation was repeated at a lower intensity. The thresholds of the low frequency receptors ranged between 35 dB and 50 dB (median: 45 dB) for the low temperature (19–21°C) and between 20 dB and 50 dB (median: 44 dB) for the high temperature (30°C). For temperature influences on auditory receptor thresholds, see for example Abrams and Pearson (1982), Römer and Bailey (1986), and Oldfield (1988).

Since the responses of auditory neurons in the locust have been shown to be strongly temperature dependent (Wolf 1986; Franz and Ronacher 2002), experiments were performed at high (30°C) and low (19–22°C) temperatures to study the effect of temperature on temporal resolution for sinusoidally amplitude-modulated stimuli. The temperature of the preparation was adjusted by means of the Peltier element and monitored by a digital thermosensor that was inserted into the abdomen.

Stimuli

The stimuli consisted of a broad-band noise carrier (3–30 kHz) that was sinusoidally amplitude modulated at different frequencies and modulation depths. Stimuli were generated in the Labview programming environment (National Instruments, Austin, Tex., USA). The modulation frequencies were 10 Hz, 20 Hz, 40 Hz, 83 Hz, 125 Hz, 167 Hz, 250 Hz, 333 Hz, and 500 Hz, and the modulation depths covered 0% (unmodulated noise), 12.5%, 25%, 50% and 100%, yielding a total of 45 different stimuli. Each stimulus consisted of a 200-ms segment of unmodulated noise, followed by a 4-s segment of constant modulation frequency and depth, and another segment of 200 ms of unmodulated noise. Details of different modulation depths of the 20-Hz stimuli are shown in Fig. 1. Stimuli were delivered via one of two speakers (D28–2, Dynaudio, Skanderborg, Denmark), which were placed at right angles to the longitudinal axis of the animal, and were oriented towards the animals' ears at a distance of 35 cm. Stimulus presentation was controlled with TurboLab (Stemmer, Puchheim, Germany). All stimuli were digitized with a sampling rate of 100 kHz (Data translation board DT 2821). From the computer, the stimuli were routed through a computer-controlled attenuator (Heinecke, Seewiesen, Germany) and an amplifier (Diora WS 502C, Conrad, Hirschau, Germany) to the speakers.

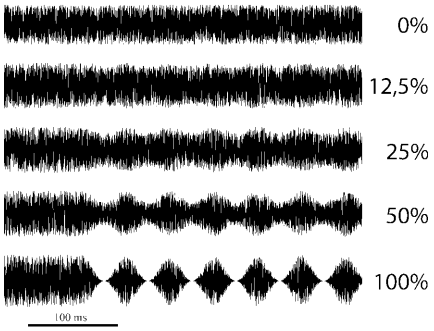

Fig. 1. Detail of the stimuli used: broad-band noise was sinusoidally amplitude modulated at 20 Hz. At the beginning of each stimulus there was a 200-ms section of unmodulated noise (100 ms of which are shown on the *left* of the signals), followed by 4 s of modulated noise. Five modulations depths are shown (*0%*, *12.5%*, *25%*, *50%* and *100%*). Scale bar 100 ms

Data evaluation and computation of amplitude modulations transfer functions

The recorded signal was amplified (intracellular amplifier, Heinecke) and stored on a four-channel DAT recorder (PC 204 A, Sony, Tokyo, Japan) at 20 kHz for off-line analysis with Neurolab (Hedwig and Knepper 1992). Lists of spike times were derived and period histograms (Fig. 2) were obtained starting at the onset of the amplitude modulated part of the stimulus (cf. Fig. 1). Each period histogram (360°) was divided into 32 bins, corresponding to a bin width of 11.25°. Note that this period corresponds to quite different absolute time scales, depending on the modulation frequency. The bin width was chosen so as to be larger than the minimum sampling interval. For constructing period histograms one can sample spikes across a fixed number of modulation periods, or over a fixed time period (in our case 4 s). However, when sampling across a constant number of modulation periods, the spike counts would differ substantially between a modulation frequency of 10 Hz and 500 Hz. We therefore decided to sample spikes over the whole 4-s range of amplitude modulation, which seems justified as the period histograms are now based on roughly equal spike numbers (Fig. 2), exceeding 200 spikes (cf. Zar 1984; Gleich and Klump 1995). From these period histograms the vector strength and Rayleigh's z were calculated (Batschelet 1965; Gleich and Klump 1995). Calculation of vector strength becomes problematic for multimodal distributions. However, this factor was not really relevant in our data sets, since multiple peaks in the period histograms occurred only rarely, and, if at all, at high modulation depths leading to high vector strengths (cf. Fig. 2, 40 Hz, 100% modulation depth). Hence, this possible artefact did not influence the determination of thresholds in our data. We tested thresholds of $z=3$, $z=5$, and $z=15$ ($z=3$ and $z=5$ correspond to a 5% and a 1% significance level for phase locking, respectively). The two criteria of $z=3$ and $z=5$ made little difference concerning the shape of the modulation transfer functions as well as the minimal integration times (see Results). Hence, a value of $z=3$ was selected as threshold criterion. However, since even for unmodulated noise stimuli (modulation depth 0%) z values up to 1 or 2 were found, we decided to increase our threshold criterion of $z=3$ by the highest z value observed with an unmodulated stimulus (see Fig. 3b).

For each stimulus frequency, Rayleigh's z was plotted against the modulation depth in percent (Fig. 3). From these plots, modulation thresholds were determined as that depth of modulation where z exceeded the threshold value (provided that all subsequent

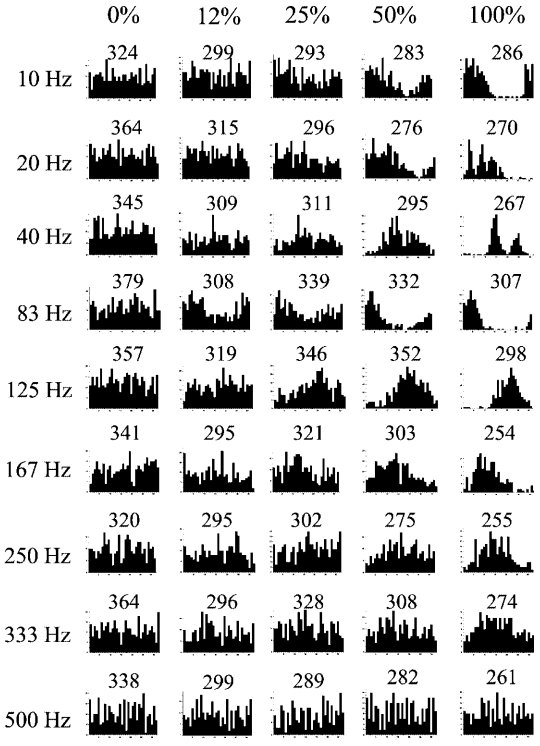

Fig. 2. Period histograms as derived from the spike trains of a low-frequency receptor tested at low temperature (20°C). Modulation frequencies are indicated on the *left*, modulation depths at the *top* of the Figure (0% = unmodulated noise; definition of modulation depth according to Gleich and Klump 1995). Smaller numbers indicate the total number of spikes contributing to each histogram

z values were also above threshold; if z crossed the threshold repeatedly, the largest modulation depth where the threshold was exceeded was taken as the threshold value). Temporal modulation transfer functions were obtained by plotting modulation thresholds in dB [20log(m/100), with m being the threshold modulation depth in percent] against the modulation frequency (Fig. 3c, d; cf. Gleich and Klump 1995). The cut-off frequency was then determined as the frequency where the curve reached the –3 dB point from the peak modulation threshold (Fig. 3c). The minimum integration time (MIT) was calculated according to Dooling and Searcy (1981) on the basis of the cut-off frequency f_c as: $\mathrm{MIT} = 1/(2\pi f_c)$.

Results

There are different ways of obtaining amplitude modulation transfer functions (see for example Krishna and Semple 2000). One type of modulation transfer function is based on spike rates as the criterion (rate modulation transfer functions), another on the phase locking of spikes to the period of the sinusoidally modulated stimulus (temporal modulation transfer functions). In general, rate-based transfer functions are applicable to the responses of neurons on higher levels of auditory processing. On the level of auditory receptors the frequencies of amplitude modulations usually have little effect on spike rates, while the timing of spikes is strongly affected (Joris and Yin 1992; Krishna and Semple 2000). This observation was confirmed by our data. Up to modulations depths of 50% the spike rates of the tympanal receptor cells were only marginally influenced by the sinusoidal amplitude modulations. In general, the spike rates differed by less than 15% (rarely up to 20%) from the mean spike rates of an unmodulated stimulus (see Fig. 2), and no consistent effect of the modulation frequency was found (cf. Joris and Yin 1992). For this reason we focus on temporal modulation transfer functions.

Choice of the $z = 3$ criterion

Period histograms (Fig. 2) were obtained from the spike trains as described in Materials and methods. The occurrence of a significant locking of spikes to the stimulus envelope was tested by applying circular statistics (Batschelet 1965; Gleich and Klump 1995). Plots of Rayleigh's z against modulation depth are shown in Fig. 3a, b for a low-frequency receptor recorded at 20°C (same cell as in Fig. 2). Fig. 3a gives an overview over the complete range of z values found for this cell, while Fig. 3b shows a magnification of the lower part (up to $z = 15$). The horizontal lines in Fig. 3b indicate the applied thresholds (note that our threshold criterion was added to the highest "base-line" z-value occurring for an unmodulated stimulus; in this case $z = 3 + 1.83$; cf. Materials and methods). As can be seen in this plot, the curves obtained at different modulation frequencies crossed the threshold at different modulation depths. The sole exception was the curve for the 500-Hz modulation (open circles), which did not come near the threshold even at 100% modulation depth; this was typical for all recordings obtained at low temperature. In Fig. 3c modulation transfer functions are depicted for three threshold values ($z = 3$, 5, and 15). The three curves exhibit a clear bandpass characteristic with a peak at 83 Hz. The roll-off is steeper at higher frequencies than at lower frequencies. In general, the shape of the modulation transfer curves was very similar for $z = 3$ and $z = 5$, except that the curves for $z = 5$ were shifted to lower decibel values. Note that a higher peak in this plot indicates more negative decibel values and thus the resolution of smaller modulation depths (see right ordinate in Fig. 3c, d), i.e. a lower modulation threshold. The cut-off frequencies and the corresponding minimal integration times hardly differed between the $z = 3$ and the $z = 5$ criteria, while for $z = 15$ larger deviations were observed (see insets in Fig. 3c). Since many cells, at several modulation frequencies, did not reach the threshold of $z = 15$, this criterion was not considered further. Subsequently, we applied a threshold criterion of $z = 3$ to all recordings, in order to facilitate the comparison with other investigations (e.g. Gleich and Klump 1995). From these curves the (upper) cut-off frequencies were determined as indicated in Fig. 3d and converted into minimum integration times as described at the end of Materials and methods.

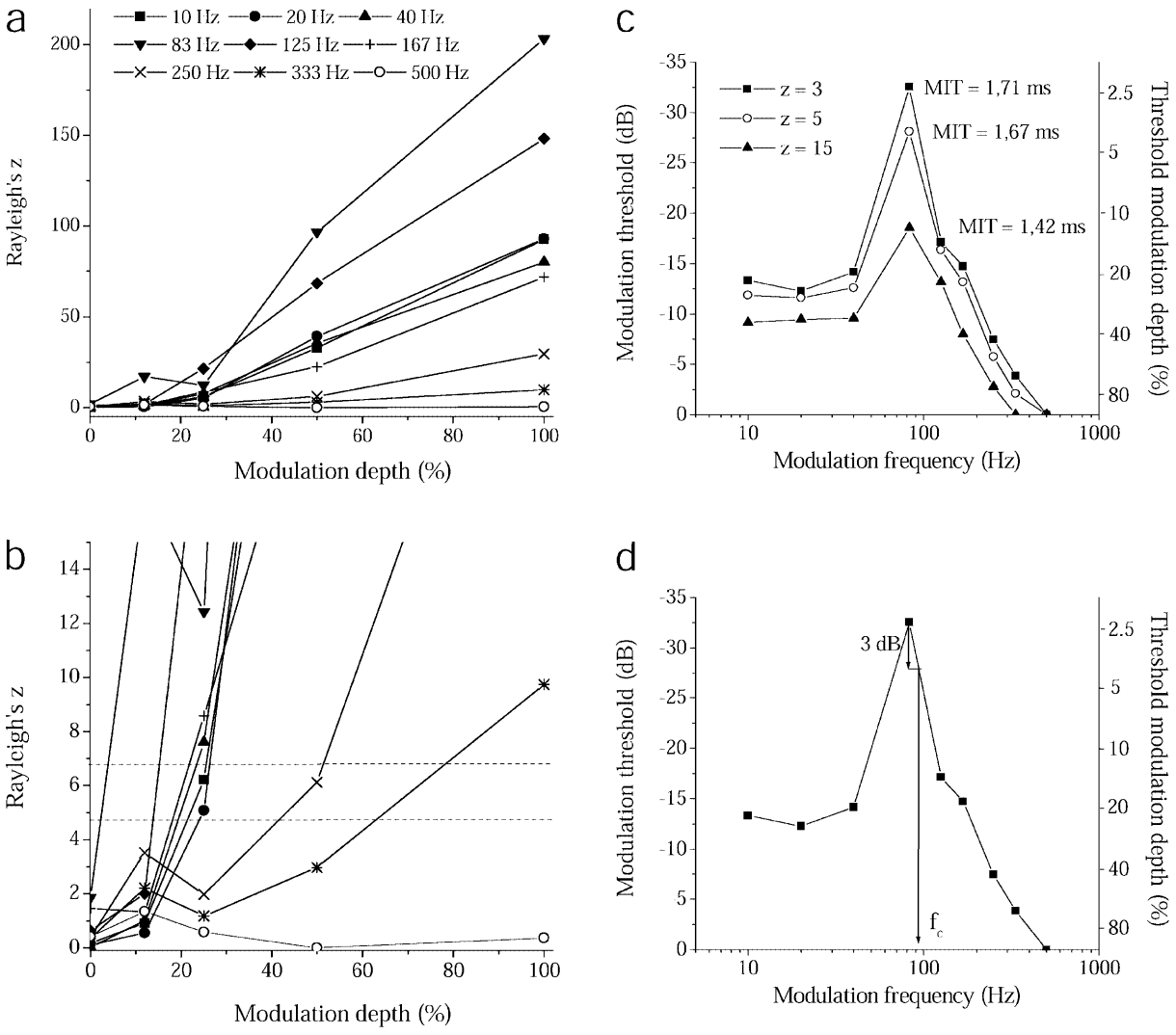

Fig. 3. **a** Dependence of Rayleigh's z on modulation frequencies and depths. **b** Detail of **a**; *horizontal lines* indicate the threshold values applied. Note that the highest "base-line" z value observed for an unmodulated stimulus, in this case 1.8, was added to the $z = 3$ or $z = 5$ criterion, cf. Materials and methods. The *intersection* of the individual curves with the horizontal lines yielded a modulation threshold, which then was transformed into a decibel value according to Dooling and Searcy (1981), and plotted against modulation frequency in Fig. 3c. **c** Influence of different threshold criteria on the shape of the modulation-transfer function. Also indicated are the "minimum integration times" (MIT) derived from the cut-off frequencies (cf. Fig. 3d and Materials and methods). **d** Ascertaining the cut-off frequency. The left and right ordinates in **c** and **d** give modulation thresholds in decibels and in percentage modulation depth

Influence of temperature on modulation transfer functions

An overview of the data obtained from low-frequency receptors is presented in Fig. 4. This figure illustrates the effects of temperature on the modulation transfer functions; Fig. 4a summarizing data obtained at 30°C, and Fig. 4b those obtained from a different sample of low-frequency receptors at 20°C (range 19–21°C). At 30°C the variation between cells is substantial, concerning both the range of peak values on the frequency scale (between 83 Hz and 250 Hz), as well as the lowest modulation thresholds attained (range –12 to –32 dB; see Fig. 4a). In spite of this variability most curves (eight out of nine) show a clear bandpass characteristic. In vertebrates there are indications that a bandpass characteristic is more likely to occur at higher intensities. We therefore tested three of these cells also at a lower intensity (10 dB or 15 dB instead of 20 dB above threshold). In none of these examples did the response change to a low pass (data not shown).

At 20°C the variability of peak frequencies appears to be smaller as most curves peaked at 83 Hz, and the widths of the individual curves tended to be smaller as well. Measured 5 dB below peak the average width of the curves was 55 ± 4 Hz for the low temperature and 136 ± 49 Hz for the high temperature (the two respective cells with lowpass characteristic, marked by arrows in Fig. 4a, b, were not included in this calculation of mean values). A consistent effect of the higher temperature is a peak shift towards higher modulation frequency values (Fig. 4c, d). The peak of the average curves shifted from

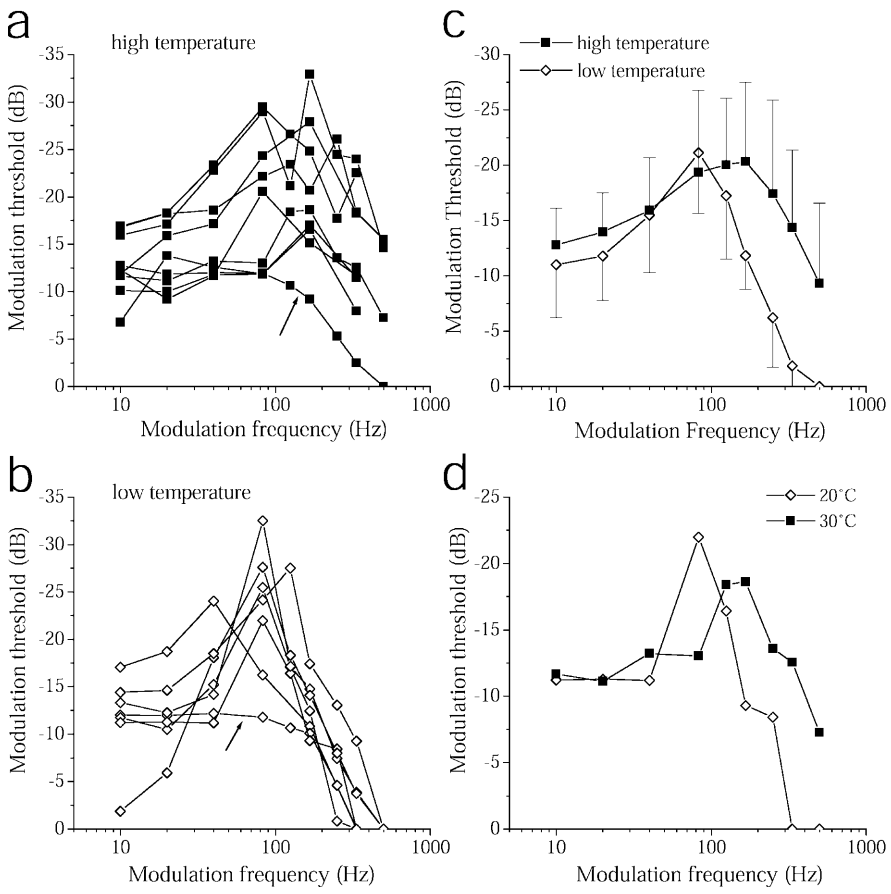

Fig. 4. **a** Modulation-transfer functions of nine low-frequency receptors tested at 30°C. **b** Modulation-transfer functions of seven different receptors obtained at low temperature (19–21°C). **c** Average modulation-transfer functions for both temperatures. **d** Modulation-transfer functions of a single cell tested at both temperatures (high temperature first)

83 Hz at 20°C to 167 Hz at 30°C (Fig. 4c), and a similar picture was found in a cell for which complete modulation transfer functions could be obtained at both temperatures (Fig. 4d). In the range of low modulation frequencies the two curves attain similar values. The roll-off in the high-frequency range was somewhat flatter at 30°C (Fig. 4c), but this is mainly due to the averaging of curves peaking at different frequencies (cf. Fig. 4a). The cut-off frequencies of the individual curves, measured 3 dB below the peak (cf. Fig. 3d) ranged from 55 Hz to 180 Hz for the low temperature (Fig. 4b), and from 100 Hz to 275 Hz for the high temperature (Fig. 4a). The two distributions are significantly different ($P < 0.002$, U-test).

Is spike rate a limiting factor?

In order to explore the influence of total spike count on the shape and reliability of these modulation transfer functions we divided the 4-s stimulus traces into two segments of 2 s, or into four segments of 1 s, and performed the same calculations as shown in Figs. 2 and 3 on these smaller data sets. The transfer functions obtained from the 1-s and 2-s segments are, of course, based on a smaller total number of spikes, compared to those of the 4-s segments. We compared modulation transfer functions of the first and the second 2-s segments with respect to the peak frequencies and the peak threshold values with those of the 4-s segments. In 32 out of 46 cases (70%) the peak frequencies did not shift between the 2-s and the 4-s segments, in 11% the peak shifted towards lower frequencies, in 19% towards higher frequencies (Fig. 5a). Comparing the results of the 1-s segments, 55% of the curves peaked at the same frequency as found for the 4-s segment (24% and 21% showed shifts to lower and higher frequencies, respectively; data not shown). These results demonstrate that the peak (and cut-off) frequencies are a robust indicator of the cell's temporal resolution capacity. In Fig. 5b the minimum modulation thresholds measured for the first and the second 2-s segment at the peaks of the respective curves are plotted against each other. Although indicating a certain variability in the height of the peaks, these data are clustered around the 45°line. Hence, also in this respect the data evaluation was stable across different spike train segments. While the peak frequencies did not shift by much in most cases (Fig. 5a), the minimal modulation thresholds measured at the peaks of the respective curves differed on average by 3.6 dB and 4.2 dB for spike train segments of 4 s, 2 s and 1 s duration (Fig. 5c). Not unexpectedly, on the basis of fewer spikes modulation detection threshold was shifted towards somewhat larger modulation depths, although the individual variability is quite large (the respective mean modulations thresholds were 7.5%, 11% and

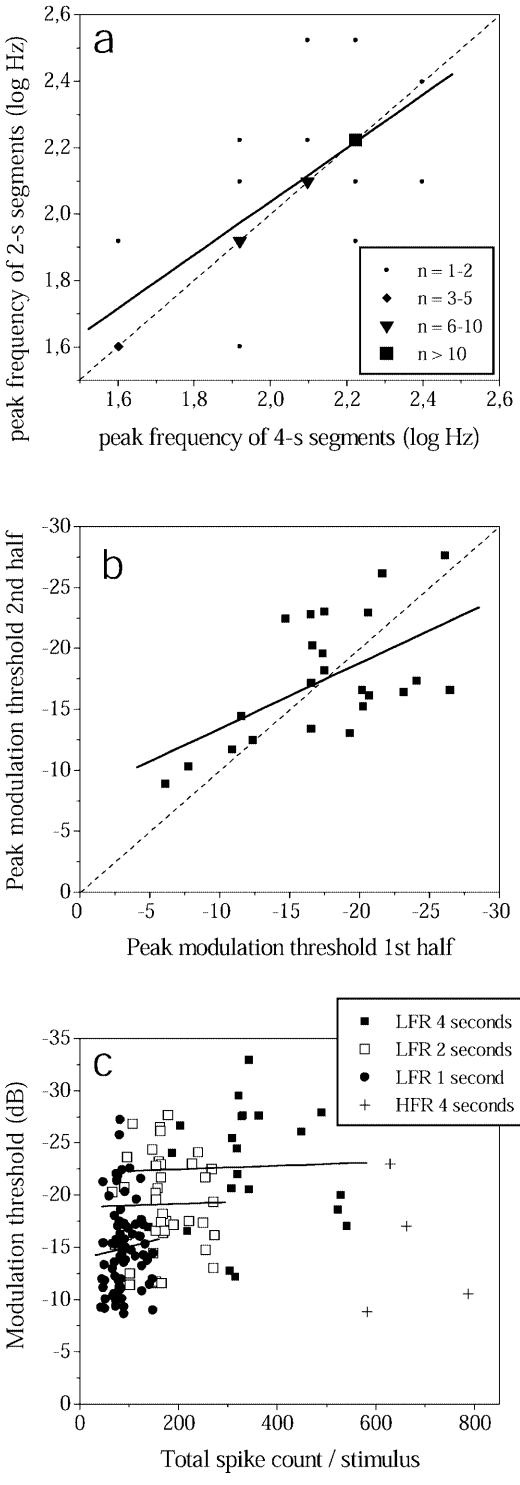

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Fig. 5. a Comparison of peak frequencies of the modulation transfer functions obtained for 4-s segments (*abscissa*) and 2-s segments (*ordinate*), frequencies on logarithmic scales. *Broken line*: 45° line. Regression line $y = 0.803(\pm 0.116)x + 0.431(\pm 0.243)$; $r = 0.72 \pm 0.154$, $n = 46$; slope different from slope 1 at $P < 0.05$). **b** Comparison of peak modulation depths of the first (*abscissa*) and the second 2-s segment (*ordinate*); $r = 0.59$. **c** Plot of the peak modulation depths against the total number of spikes present in the respective 4-s, 2-s and 1-s segments (*filled squares*, *open squares*, *filled circles*, respectively). For each condition, the regression lines are not significantly different from zero ($r = -0.03$, -0.02, and -0.08, respectively). The respective mean values differ by 3.6 dB and 4.2 dB. Included are the data of low-frequency receptors at both temperatures (*squares* and *circles*). Also shown are data for the 4-s segments of the four high-frequency receptors (*crosses*). *LFR* low-frequency receptor, *HFR* high-frequency receptor

18%, corresponding to -22.7 ± 5.6 dB, -19.1 ± 4.6 dB and -14.9 ± 4.2 dB; cf. Materials and methods).

From the cut-off frequencies (see Fig. 3d) of the individual modulation-transfer curves the minimum integration times were calculated and plotted against the mean spike rate of the receptor neurons (Fig. 6). Data for low-frequency receptors at low and high temperature are shown separately (open and filled symbols, respectively). There was a weak tendency towards minimum integration times being smaller at higher spike rates, but the correlation was not significant for the high-temperature data ($P > 0.2$). For the low temperature the significance of the regression line ($P = 0.03$) depended on a single point (arrow), and disappeared if this data was excluded from the analysis. Thus, contrary to the naïve expectation no strong relation between higher spiking rates and higher temporal resolution seems to exist. This is corroborated by the minimum integration times fond in four high-frequency receptors which are also included (Fig. 6). The minimum integration times of high-frequency receptors were not substantially smaller than those obtained from low-frequency receptors at the same temperature, in spite of the lower spike rates of the latter. The corresponding modulation transfer functions of high-frequency receptors – measured at high temperature – were similar to those shown in Fig. 4, with a tendency

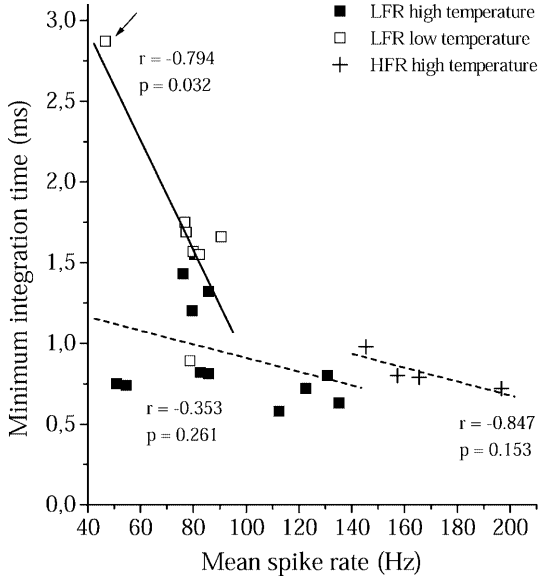

Fig. 6. Minimum integration times in dependence of the cells' spike rate (*abscissa*). Data obtained on LFRs at low (*open squares*) and high temperature (*filled squares*) are plotted separately. In this figure the data of three cells are included that were tested at a second intensity. Also shown are the minimum integration times of four HFRs

to lower peak modulation values (data not shown). The respective average minimum integration times were 1.7 ± 0.6 (mean $\pm$ SD, $n = 7$) for low-frequency receptors at low temperature, 0.95 ± 0.33 ($n = 12$) for this receptor type at high temperature, and 0.82 ± 0.11 ($n = 4$) for high-frequency receptors at high temperature (the corresponding medians were 1.66, 0.8, and 0.8).

Discussion

This study had two main goals: (1) to determine the temporal resolution of auditory receptors of a grasshopper in an experimental paradigm frequently used in investigations of vertebrate (but not insect) hearing in order to allow for a direct comparison of these differently organized hearing systems (see Introduction), and (2) to further explore the influence of temperature on temporal resolution, since various neuronal functions of these poikilothermic animals are strongly affected by temperature changes (Janssen 1992; Franz and Ronacher 2002).

Comparison with modulation transfer functions of vertebrates

From the modulation transfer functions a cut-off frequency can be estimated (conventionally measured 3 dB below peak – see Fig. 3d), which then can be transformed into an indicator of temporal resolution, the "minimum integration time", MIT (cf. Materials and methods). At 20°C and 30°C locust receptors yielded minimum integration times of 1.7 ± 0.6 ms and 0.95 ± 0.33 ms, respectively. These values fall in the lower range of minimum integration times found in psychophysical experiments on vertebrates (see Table 1). In a comparison with electrophysiological results our high temperature data are similar as those obtained on starling auditory nerve fibres (Gleich and Klump 1995, mean MIT: 1.14 ± 0.86, median: 0.97), and approximately twice as high as those found in auditory nerve fibres of the coqui frog (median: 0.42 ms). Surlykke et al. (1988) tested tympanal receptors of a moth with amplitude-modulated noise stimuli; however, these authors do not give cut-off frequencies and values for minimum integration time. They report that significant spike locking was found up to a maximal modulation frequency of 200 Hz (at 20°C). However, as can be extrapolated from their Fig. 4, the transition to non-significance occurs at about 270 Hz, and thus the temporal resolution of moth receptors appears to be similar to that of the locust (cf. Fig. 4b).

Modulation-transfer functions obtained in behavioural or psychophysical tests as well as in electrophysiological investigations on vertebrate auditory nerve fibres usually exhibit a lowpass characteristic (humans:

Table 1. Comparison of minimum integration times (MIT) and minimum detectable gap widths obtained in different species. *E*, *P* denote electrophysiological or psychophysical experiments, respectively. Values in parentheses are medians; all values in ms

Species	MIT (ms) Mean (median) or range	Gap width (ms) Mean (median) or range	Authors
Starling	1.14 (0.97) E		Gleich and Klump (1995)
	1.3 P		Klump and Okanoya (1991)
		(12.8) E	Klump and Maier (1989)
		(1.8) P	Klump and Gleich (1991)
Barn owl	1.7 P	1.5 P	Dent et al. (2002)
Budgerigar	1.2 P		Dooling and Searcy (1981)
		~2.5	Okanoya and Dooling (1990)
Chinchilla	1–1.4 P		Salvi et al. (1982)
		~3 P	Giraudi et al. (1980)
		2–3 P	Salvi and Arehole (1985)
		~1.5 E	Zhang et al. (1990)
Monkey	0.7–1.0 P		Moody (1994)
Humans	2.5–2.8 P		Viemeister (1979)
	2.1 P	3.1 P	Formby and Muir (1988)
	3.8 P		Salvi et al. (1982)
	5.8 P		Dooling and Searcy (1981)
		2.4 P	Shailer and Moore (1983)
		2.8–3 P	Buus and Florentine (1985)
		2.2 P	Snell et al. (1994)
Coqui frog	(0.42) E		Dunia and Narins (1989)
Locust (20°C)	1.7 (1.66) E		This study
(30°C)	0.95 (0.8) E		This study
(30°C)		1.7 E	Franz and Ronacher (2002)
C. biguttulus (20°C)		3.0 E	Ronacher and Römer (1985)
(30°C)		1.5 E	Ronacher and Römer (1985)
(35°C)		1.2–1.7 P	von Helversen (1972)
(30°C)		2 P	von Helversen and von Helversen (1997)
(23–25°C)		2.2–3.3 P	Ronacher and Stumpner (1988)
Noctuid moth		~2 E	Surlykke et al. (1988)

Viemeister 1979; Dooling and Searcy 1981; Formby and Muir 1988; cat: Rhode and Greenberg 1994; chinchillas: Salvi et al. 1982; budgerigars: Dooling and Searcy 1981; starling: Klump and Okanoya 1991). In contrast, many neurons of the cochlear nucleus and on higher levels of the vertebrate auditory pathway show bandpass characteristics (Rhode and Greenberg 1994; Kuwada and Batra 1999; Krishna and Semple 2000). In our sample only 3 out of 20 receptors exhibited a lowpass modulation transfer function, while all others had clear bandpass characteristics (Fig. 4). Interestingly, several authors also reported a bandpass characteristic – in psychophysical experiments – if the signal was gated instead of being continuous sinusoidally modulated noise (Viemeister 1979; Klump and Okanoya 1991; Gleich and Klump 1995; Dent et al. 2002). However, as our stimuli had a duration of 4 s, which is longer than the "continuous" stimulus used by Gleich and Klump (1995), this factor is unlikely to have caused the bandpass characteristics depicted in Fig. 4. Also there was no consistent change of the shape of the modulation transfer functions in the second half of the 4-s stimulus. There are indications that higher intensities incur a transition from lowpass to bandpass (Frisina et al. 1990; Kuwada and Batra 1999), but our data gave no hint that our choice of intensities (20 dB above a cell's threshold) could be responsible (see Results). Note that the intensities used caused firing rates between 50 Hz and 140 Hz (Fig. 6) at which the low-frequency receptors were clearly not at their upper limits of response. One possible explanation for the bandpass characteristic could lie in the rather slow amplitude rise at low modulation frequencies. For example, at 20 Hz the rise-time of a sine half-wave is 12.5 ms, and earlier investigations have shown that long rise-times lead to a less accurate triggering of spikes in locust auditory receptors (see Fig. 2 in Krahe and Ronacher 1993). This in turn must deteriorate the locking of spikes to the stimulus envelope and thus the z values. An interesting parallel to the bandpass characteristic reported here was found in a stimulus reconstruction study on the responses of auditory receptors of locusts (recorded at 30°C). These experiments yielded lower coding efficiencies and information rates for stimuli with cut-off frequencies of the amplitude modulation between 25 Hz and 50 Hz, and maxima for cut-off frequencies around 200 Hz (Machens et al. 2001). This optimum curve has been taken as an indication that locust auditory receptors are optimized for stimuli with corresponding statistics of amplitude modulations.

Comparison with results obtained in a gap-detection paradigm

Another frequently used paradigm to investigate temporal resolution is gap detection. The values for the minimum detectable gap widths (in broad-band noise carriers) are rather consistent across species, with one exception: for auditory nerve fibres and neurones of the nucleus cochlearis of the starling Klump and Gleich (1991) found a rather high median value for the detectable gap width of 12.8 (Table 1). However, since a few cells in their sample exhibited minimum detectable gap widths of 3.2 ms and 1.6 ms, the discrepancy from the 1.8-ms value found in psychophysical tests probably can be resolved. If both methods, gaps and modulation-transfer functions, were applied to the same species (or subjects), the minimum integration times generally tended to be smaller than the minimum detectable gap widths (Table 1). Gleich and Klump (1995) concluded that the durations of the detectable gap widths would be around twice as large as the minimum integration times determined from modulation transfer functions. This relation appears to hold for budgerigars and chinchillas (see Table 1), but for the starling only if the high median for electrophysiology is ignored. Humans and barn owls may be exceptions to this "rule", as their minimum integration times are in the same range or even larger than the minimum detectable gap widths (Table 1).

Applying a gap-detection paradigm to tympanal receptors of locusts yielded a mean detectable gap width of 1.7 ± 0.35 ms at 30°C (Franz and Ronacher 2002), i.e. around twice the value of the minimum integration time at that temperature (mean 0.95; median 0.8). In another grasshopper species (*Chorthippus biguttulus*) tympanal receptor cells were also investigated in a gap-detection paradigm (Ronacher and Römer 1985), at similar temperatures as tested here. The minimum detectable gap width in this study was 1.5 ± 0.6 ms (for temperatures between 29.5°C and 31.5°C). Thus, for the tympanal receptors of these insects roughly a 1:2 relation between minimum integration times and detectable gap widths seems to hold as well, although the scolopidial structure of their sensory cells differs fundamentally from that of the inner ear hair cells of vertebrates; whether there are similarities between insect and vertebrate transduction mechanisms is a matter of debate (Eberl 1999).

A temperature rise improves temporal resolution

A shift in temperature of 10°C had a pronounced influence on the values of the best modulation frequencies of the transfer functions, and consequently on the cut-off frequencies and the minimum integration time (Figs. 4, 6). The mean minimum integration times were 1.7 ms and 0.95 ms at 20°C and 30°C, respectively ($P < 0.002$; the corresponding medians were 1.66 and 0.8). At low temperature, for a modulation frequency of 500 Hz no cell showed a significant spike coupling to any modulation depth, while at high temperature the responses of three out of the four cells tested with 500 Hz were still significantly locked to the modulation period (responses between –10 dB and –15 dB). The results presented here fit nicely to the data obtained with a gap-detection paradigm from receptors and auditory interneurons of locusts (Franz and Ronacher 2002), as well as to receptor data and behavioural results obtained

in another grasshopper species, *Chorthippus biguttulus*. In the gap-detection paradigm, the low temperature led to a much inferior temporal precision of spike responses (Franz and Ronacher 2002). As with the minimum integration times, a temperature shift from 30°C to 20°C led to roughly a doubling of the minimum detectable gap width (1.5 ± 0.6 ms and 3.0 ± 0.8 ms, respectively; Ronacher and Römer 1985).

As the spike rates generally increase with higher temperature (for the cell shown in Fig. 4d, e.g. from 80 Hz to 130 Hz), the temperature-induced shift in minimum integration times could have been due to the higher spike rates (and hence decreased inter-spike-intervals). Contrasting to this expectation, there seems to be no strong correlation between minimum integration time and spike rate (Fig. 6). Note that several of the low-frequency receptors tested at high temperature covered the same range of spike rates as those tested at low temperature, nonetheless exhibiting clearly lower minimum integration times (Fig. 6). High-frequency receptors generally exhibit somewhat higher firing rates than low-frequency receptors (Fig. 6, cf. also Table 1 in Römer 1976), but their minimum integration times covered the same range as those of the low-frequency receptors (at the same temperature), confirming a small effect of spike rates upon temporal resolution. These results are corroborated by a recent investigation, which indicated that the crucial effect upon temporal resolution of a temperature rise seems to consist of a more precise locking of spikes to the stimulus envelope, rather than in an increase of firing rate (Franz and Ronacher 2002).

A similar shift of the best modulation frequency was also observed in midbrain neurons of frogs that exhibited bandpass characteristics: with a temperature rise from 14°C to 22°C the peak of the modulation transfer function shifted from 15 Hz to 25 Hz (Brenowitz et al. 1985; cf. also Rose et al. 1985; their data evaluation, however, focused on spike rates). In the visual system of insects rising temperatures also lead to an improved temporal resolution (Warzecha et al. 1999; Tatler et al. 2000), hence this influence of temperature on temporal resolution seems to be common.

No correlation between minimum detectable amplitude modulations and firing rate

The average modulation transfer functions (Fig. 4d) attained peak values of around –20 dB, corresponding to a detectable modulation depth of 10%. This value is comparable to those of other studies (e.g. Viemeister 1979: 5–6%; Klump and Okanoya 1991: 9%). A few receptors reached very high peak values of – 27 dB to – 32 dB (corresponding to detection of extremely small modulation depths of 4% and 2.5%). Such high values were only rarely reported, e.g. by Salvi et al. (1982) for humans in psychophysical tests, and recently for barn owls (Dent et al. 2002). Our choice of stimuli may have contributed to such extreme peak values: our smallest modulation depth (apart from 0%) was 12.5%, and the curves were linearly interpolated between the unmodulated and the 12% modulation depth stimulus. Perhaps inclusion of a modulation depth between 12.5% and 0% would have reduced the very steep slopes in some cells (cf. Fig. 3b).

The minimum modulation thresholds of the 4-s stimuli showed virtually no dependence on the total number of spikes available, and hence on the firing rate (see Fig. 5c, black squares; regression $r = -0.03$, $P = 0.89$). When the same data evaluation was performed on smaller (2-s or 1-s) segments of the stimulus, the peak values of the curves were shifted by 3.5–4 dB to lower (absolute) values when the stimulus duration was halved. Not unexpectedly, on the basis of fewer spikes modulation detection threshold was shifted towards somewhat larger modulation depths (the respective mean modulations thresholds were 7.5%, 11% and 18%; see Results). However, with the 2-s and the 1-s segments as well there was no significant correlation between modulation threshold and firing rates (Fig. 5c). With 1-s segments in the majority of cases only between 40 and 100 spikes were available for deriving the transfer functions. Nevertheless, a modulation depth (mean) of 18% could be detected (range 40–7%). Similar numbers of spikes could be available for more central elements of the auditory pathway from the set of (around 50–60) low-frequency receptors even for short time segments. Hence, a "counterpart" to the period histograms could possibly be found in the synchronisation of spikes across different receptor fibres.

Conclusions

This study aimed at providing data on temporal resolution of an insect's auditory system that can be directly compared with studies on temporal resolution of vertebrates. Earlier studies found that the gap-detection capacities of grasshoppers appear to be similar to those of vertebrates (von Helversen 1979; Ronacher and Römer 1985; Stumpner and Ronacher 1994; Franz and Ronacher 2002). However, since the grasshopper behavioural (and electrophysiological) tests always used stimuli that contained several gaps, the comparison with vertebrate data, in which commonly a single gap had to be detected, might be biased. The data obtained here in a modulation-transfer function paradigm are better comparable across species since the procedures are more similar (cf. Gleich and Klump 1995). The locust data show a good congruence with electrophysiological data obtained from the starling, and with psychophysical data obtained from mammals and birds. For humans, several reports assert rather high minimum integration times (see Table 1); however, these are often accompanied by a resolution of extremely small modulation depths (thresholds for amplitude modulations of 5% and less). In summary, this study confirm that the tem-

poral resolution of insects is comparable to that of vertebrates – although the sensory cells are quite differently organized in these taxa (see also Michelsen et al. 1985; Surlykke et al. 1988; Eberl 1999).

Acknowledgements We thank Astrid Franz and Matthias Hennig for many stimulating discussions and for valuable comments on the manuscript. This research was supported by a grant from the Deutsche Forschungsgemeinschaft to B.R. All experiments complied with the current German legislation concerning animal care.

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