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AM representation in green treefrog auditory nerve fibers: neuroethological implications for pattern recognition and sound localization

Received: 8 January 2004 / Revised: 2 August 2004 / Accepted: 2 August 2004 / Published online: 5 October 2004
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Abstract In addition to spectral call components, temporal patterns in the advertisement-call envelope of green treefrog males (*Hyla cinerea*) provide important cues for female mate choice. Rapid amplitude modulation (AM) with rates of 250–300 Hz is typical for this species' advertisement calls. Here we report data on the encoding of these rapid call modulations by studying the responses of single auditory nerve fibers to two-tone stimuli with envelope periodicities close to those of the natural call. The free-field response properties of 86 nerve fibers were studied from 32 anesthetized males. The accuracy of stimulus envelope coding was quantified using both a Gaussian function fit to the interspike interval histograms derived from the first seven 20-ms stimulus segments, and the vector-strength metric applied to the phase-locked responses. Often, AM encoding in the initial stimulus segment was more faithful than that in its second half. This result may explain why conspecific females prefer calls in which the initial segment is unmasked rather than masked. Both the questions of pattern recognition and localization are discussed, and the data are related to behavioral obser-

uations of female choice and localization performance in this species.

Keywords Amphibian · Amplitude modulation · Anuran · Auditory nerve · Ear

Abbreviations AM: Amplitude modulation · CF: Characteristic frequency · FTC: Frequency-threshold curve · PSTH: Post-stimulus time histogram · SPL: Sound pressure level

Introduction

Many anuran (frog and toad) vocal signals exhibit amplitude modulation (AM) (for a review see Gerhardt 1988; Walkowiak 1988; Simmons and Buxbaum 1996). Moreover, the modulation rate of the signal envelope is often diagnostic of the species (e.g., in the grassfrog, *Rana temporaria*, see review in Walkowiak 1988; for the pair of sister species: barking treefrog, *Hyla gratiosa*, and green treefrog, *Hyla cinerea*, see Oldham and Gerhardt 1975), and may also distinguish different call types within the vocal repertoire of a single species. In the green treefrog, for example, advertisement calls and aggressive calls differ in their rate of modulation of the waveform envelope; the former signal is characterized by a 300-Hz AM, whereas the latter exhibits an additional 50-Hz AM component superimposed on the 300-Hz AM (Gerhardt 1978a, b). When 50-Hz AM was superimposed on the advertisement call, it was no longer attractive to female green treefrogs (Gerhardt 1978a). In addition, synthetic advertisement calls evoked more antiphonal calling by male green treefrogs than did synthetic aggressive calls, suggesting males can also discriminate between the two call types (Simmons et al. 1993a; Allan and Simmons 1994). These results clearly indicate that green treefrogs can detect the 50-Hz modulation component of the aggressive call. Even the

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300-Hz envelope modulation that is characteristic of the temporal fine structure of the advertisement calls seems to be salient to the green treefrogs. Gerhardt (1978b) showed that female green treefrogs prefer synthetic signals with 300-Hz beats in their envelope to signals with comparable spectral peaks and a 300-Hz periodicity, but lacking the 300-Hz beats. For example, a synthetic advertisement signal composed of tones of 900, 1,200, and 3,000 Hz is strongly preferred by female green treefrogs over a call composed of tones of 900 and 3,000 Hz alone. A similar preference for the three-component signal was observed when the beats were created by adding tones of 900, 2,700, and 3,000 Hz. These behavioral data suggest that the auditory system of green treefrogs should be able to accurately encode signal envelope information.

Besides serving to differentiate vocal signals, signal envelope modulation may also play an important role in the localization of conspecific callers. Female green treefrogs have been shown to accurately localize the source of advertisement calls in two and three dimensions (Rheinlaender et al. 1979; Gerhardt and Rheinlaender 1982). For example, the jump error (the average deviation from a straight line connecting the female with the sound source that it is approaching on the ground) can be as small as 11.8° when the frogs make head scanning movements prior to their jumps (Rheinlaender et al. 1979). Two acoustic cues are available to female frogs when choosing the jump angle: interaural intensity differences and interaural time differences. Physical measurements of the motion of the green treefrog's tympanum in relation to the angle of sound incidence using laser vibrometry (Michelsen et al. 1986) indicate that the available interaural intensity differences may be on the order of 2–3 dB for an incidence angle of 30° . Similar results have been obtained through physiological measurements of sensory thresholds in the green treefrog's torus semicircularis as a function of the angle of sound incidence (Rheinlaender et al. 1981). For the average error angles shown in phonotaxis experiments, this difference is likely to be even smaller, i.e., approximately 1–2 dB. Whereas this difference appears to be great enough to allow auditory lateralization (Rheinlaender et al. 1979), whether it can explain the observed localization accuracy has never been tested directly. The second acoustic cue available to female frogs is the interaural time difference. It is unlikely that green treefrogs can determine the interaural time differences solely from phase differences of the sine-wave components of their vocal signals. The dominant frequency peaks of the green treefrog's advertisement calls are found at about 900 and 3,000 Hz. Phase-locking in the frog auditory nerve is very weak at 900 Hz and does not occur at 3,000 Hz (e.g., see Narins and Hillery 1983; Rose and Capranica 1985; Hillery and Narins 1987; Narins and Wagner 1989; Ronken 1990; Feng et al. 1991). However, the periodicity of the call envelope may provide reliable interaural time cues. Phase-locking of frog auditory nerve fibers to periodic fluctuations in the signal envelope

has been observed in a variety of species up to modulation frequencies of 500 Hz (Rose and Capranica 1985; Dunia and Narins 1989; Narins and Wagner 1989; Feng et al. 1991, Simmons et al. 1992, 1993b; Simmons and Ferragamo 1993). Furthermore, in the bullfrog, auditory-nerve fibers tuned to low frequencies have been found that respond primarily to the quadratic difference tone $f_2 - f_1$ (Capranica and Moffat 1980). Since these fibers respond preferentially to low frequencies, they would be especially suited for encoding envelope periodicity. Finally, interaural intensity and time cues may converge onto the same code. In the leopard frog, *Rana pipiens*, Feng (1982) reported that a 1 dB intensity change may result in latency shifts of between 100 and 600 μ s. Latency shifts of this magnitude may allow frogs to analyze interaural cues in the time domain.

The purpose of this study is to explore the accuracy of envelope periodicity encoding in the green treefrog's auditory nerve. The results address questions about both pattern recognition and localization. In addition, the data will be related to behavioral observations of female choice and localization performance in the green treefrog.

Materials and methods

Animal preparation and recording procedures

Green treefrogs (*Hyla cinerea*, $n = 32$), were anesthetized with intra-muscular injections of pentobarbital sodium (Nembutal, Abbott Laboratories, 50–75 μ g per g body mass) approximately 1 hour prior to surgery. The experimental animal's right eighth cranial nerve was exposed following the surgical procedure outlined in detail in White et al. (1992). When the effect of the anesthetic weakened after completion of the surgery (in any case, Nembutal has little effect on auditory-nerve fiber responses—see Dodd and Capranica 1992) the frog was immobilized with an intramuscular injection of *d*-tubocurarine chloride (Squibb, 6–12 μ g per g body mass), oriented in a natural posture (upright, mouth closed, lungs deflated), and placed onto a vibration-isolated table (Backer-Loring) inside a sound-insulated room (IAC 1202-A). The upright posture coupled with free-field sound stimulation (see below) closely simulated the conditions in the behaving animal. During recordings, the temperature in the sound-insulated room ranged between 22 and 26°C. The frog's body was covered with moist cotton gauze to facilitate cutaneous respiration. Additional injections of partial doses of *d*-tubocurarine chloride were administered as needed to maintain immobility.

Prior to recording, a steel hook fashioned from an insect needle and attached to a micromanipulator (Narishige) was used to retract the vertebral artery and choroid plexus medially in order to expose the medial wall of the otic capsule and the eighth cranial nerve. Neural responses were recorded from individual axons within the eighth cranial nerve using glass micropipettes

filled with 3 mol^{-1} KCl having impedances of 25–80 M Ω (allowing both intracellular and extracellular recording). The electrode was advanced remotely using a hydraulic microdrive (Trent-Wells). Action potentials were fed to a unity-gain, high impedance ($10^{13} \Omega$) pre-amplifier (Winston 1090), a variable gain low-noise amplifier (Opamp Labs 423), and a window discriminator (Mentor N750). The high signal-to-noise ratio and the uniform height of action potential amplitudes observed in the recordings suggested that the recordings were from individual auditory-nerve fibers. The TTL-output of the window discriminator was connected to timing circuits of a minicomputer (Computer Automation LSI-2/20) or a PC-compatible notebook computer (Compaq LTE 25) that recorded the time of occurrence of the action potentials. Neuronal activity was monitored on an oscilloscope (Tektronix 5112) and a loudspeaker.

Sound presentation in the neurophysiological experiments

For acoustic stimulation the frog was positioned in the center of a circle described by a moveable arm that could be rotated 360° around the frog (Wang et al. 1996). A loudspeaker (Pyle Driver P499 W, 10.2 cm diameter) was attached to one end of the arm. The distance from the loudspeaker to the frog's tympanum was ca. 34 cm. At the start of each recording session, the sound field was initially calibrated with a 900-Hz tone using a random incidence 1/2-inch microphone (Brüel and Kjaer 4134) placed close to the right tympanum of the frog. The frequency response of the playback system (amplifier NAD 3220PE, equalizer Yamaha GQ1031B) was flat within ± 3 dB over the frequency range from 0.25–6.0 kHz as determined either with the 1/2-inch microphone or with a pair of miniature calibration microphones (Knowles EK 3033), one of which is placed at a distance of 3–5 mm from the center of each tympanum. The calibration microphone pair was also used to determine phase differences between the stimuli impinging on the left and right tympana. For the determination of frequency-threshold curves (FTCs), pure-tone stimuli (duration: 50 ms; linear rise-fall times: 5 ms; repetition period: 180 ms) were generated using a heterodyne analyzer (Brüel and Kjaer 2010) controlled by the minicomputer. A programmable attenuator adjusted the stimulus level in steps of 1 dB over the range from 0 to 128 dB SPL. Recordings of FTCs covering a 5-octave range followed the procedures described in White et al. (1992) and Benedix et al. (1994).

In all subsequent measurements, the stimuli were generated using the notebook computer and a custom-built D/A-converter board (8-Bit, 25-kHz sampling rate, total harmonic distortion less than 0.2%). The stimuli were attenuated (HP-350D) and low-pass filtered with a cutoff at 5 kHz (Krohn Hite 3550) for anti-aliasing. They were presented at a constant over-all level of 85 dB

SPL through the same playback system used for measuring the FTCs. To determine the periodicity encoding of the auditory-nerve fiber, the responses to 140 repetitions of a two-tone stimulus (repetition period 420 ms, equal sound-pressure level of the components, duration: 150 ms; rise/fall times: 20 ms, Fig. 1a) were recorded. This two-tone complex mimicked the envelope periodicity of the species' advertisement call (Fig. 1b) [Oldham and Gerhardt 1975, observed average envelope periodicities of 267 and 303 Hz (mean: 285.5 Hz) in green treefrog populations that were sympatric or allopatric to barking treefrog populations, respectively]. Depending on the fiber's characteristic frequency (CF), the stimulus consisted of two tones added with cosine phase that had frequencies of either (a) 858 and 1,144 Hz, (b) 1,716 and 2,002 Hz, or (c) 2,860 and 3,146 Hz, each pair resulting in a beat of 286 Hz. We chose the frequency pair that was the closest to the CF of each fiber. These beat stimuli were presented with the speaker centered in front of the animal (sound incidence angle: 0°). Following the recording of the nerve fiber's response to the two-tone stimulus, we determined the neuron's response pattern when stimulated with six different signals: (1) a digital representation of a natural call, (2) a 900-Hz tone, (3) a 3,000-Hz tone, (4) 900+1,200-Hz tones, (5) 1,800+2,100-Hz tones, and (6) 3,000+3,300-Hz tones. These were presented from up to 12 sound-incidence angles starting with 0° and continuing in the sequence of 15, 30, -15 , -30 , -45 , 45, 90, 135, -90 , -135 , 180° as long as the recording remained stable (positive and negative angles indicate speaker positions right and left of the frog's body axis, respectively). Since both digital stimulus generation and the individual spike timing in response to the stimuli were under the control of crystal-clock generators, temporal accuracy of $< 1 \mu\text{s}$ was achieved.

Data analysis

For each fiber isolated, an FTC was generated using the automatic up-down tracking method described in Kiang et al. (1970) and Evans (1979), and adapted for the frog by Narins and Hillery (1983). Briefly, the number of spikes evoked by a 50-ms tone burst and captured within a 50-ms window was compared to the number of spikes occurring in a subsequent 50-ms tone-free window. The intensity of the tone burst was adjusted in 1-dB steps until the number of spikes in the first window exceeded the number of spikes in the second window by one (threshold criterion). In this manner, thresholds were estimated for frequencies in 0.05 octave steps over five octaves centered at the fiber's estimated CF. Subsequently, the FTCs were smoothed using a Hamming window of width 0.25 octave averaging two runs of the FTC-procedure.

Peristimulus-time histograms (PSTHs, bin width: $40 \mu\text{s}$, $n = 58$ units) and interval-histograms were constructed from the data collected for each fiber in the

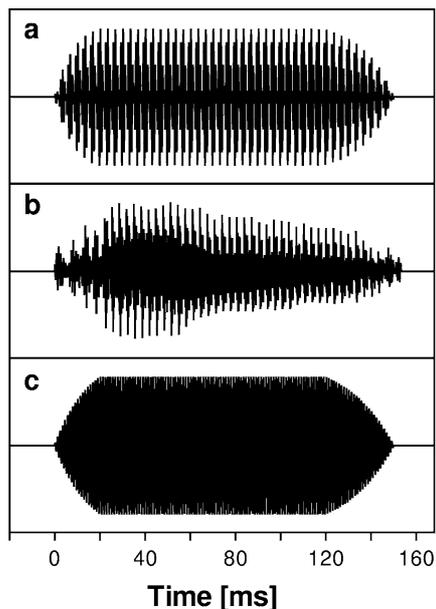


Fig. 1 Representative oscillograms of stimuli: **a** two-tone stimulus: 900 + 1,200-Hz pure tones that result in a beat frequency of 300 Hz, duration: 150 ms; **b** natural call, duration: 153 ms; **c** 900 Hz pure tone, duration: 150 ms

periodicity-encoding experiment. A normal distribution was fitted to the first peak, and its mean and standard deviation were calculated. A period histogram based on the stimulus period was constructed for the neural responses, and the vector strength of the response and the associated Rayleigh z statistics for significance were calculated (Zar 1999). All computations were carried out either for the ongoing response to the total stimulus or for a subset of 20-ms time intervals.

For the analysis of the neuron's directional response to each stimulus, PSTHs were constructed [bin width: 10 μ s ($n = 5$ units) or 50 μ s ($n = 2$ units)]. In a subsequent cross-correlation analysis, the PSTH recorded for the frontal position was used as a reference against which the spike timing observed in the PSTHs recorded at the other positions of the speaker were compared. To determine the relative time-shift between each pair of PSTHs, a raised-cosine function was fitted to the cross-correlation data and its phase shift was calculated. In this study, only data in which the cross-correlation function itself revealed a significant periodicity are reported.

Behavioral testing of sound-localization accuracy

Gravid female green treefrogs ($n = 32$) were collected near Savannah, Georgia, and brought into a dimly-lit semi-anechoic room (walls covered with sound absorbing wedges, cut-off frequency 300 Hz) for testing within 24 h. Single-tone and two-tone stimuli that had been pre-recorded onto tape (TEAC A2340SX) were amplified (Quad 303) and played back to the subjects via a loudspeaker (Analog-Digital Systems 200) placed on the floor of the

chamber at a distance of 1 m from the release point of the frog. The stimulus tape had been generated by recording the output of a custom-built 8-bit D/A-converter board (50-kHz sampling rate) in an AT&T 6300 personal computer that was low-pass filtered (Krohn Hite 3200) with a cut-off frequency of 12.5 kHz. The response to only one type of playback per individual treefrog was included in the statistical analysis. The playback signals were designed to mimic those used in the neurophysiological experiments (repetition period: 800 ms; duration: 150 ms; rise-fall times: 20 ms) and were composed of tones of 900, 3,000 Hz, and tone combinations of 900 + 1,200, 1,800 + 2,100, and 3,000 + 3,300 Hz. The playback levels were adjusted to 85 dB SPL at the release point of the frog. Levels were calibrated with a sound-level meter (General Radio 1900A) with a 1-inch condenser microphone, C-weighting and fast response.

A ceiling-mounted video camera (Panasonic WV-220P) recorded the sound localization behavior of the frogs (Panasonic model NV-3020 SD videotape recorder). An orthogonal 10-cm grid was drawn on a plastic cover on the floor of the chamber to facilitate subsequent analysis of orientation behavior from the video tape. Prior to the start of the observations, the frog was placed in a release cage in the middle of the arena and the sound playback was broadcast. Next, the videotape recorder was activated and the lid of the release cage was raised. The female frog usually climbed on the rim of the release cage and subsequently approached the speaker broadcasting the signal in a series of jumps, often punctuated by scanning movements and orienting turns of the body. Jump angles of the frog's trajectory relative to the direction of the sound source were determined by analyzing the position of the frog in a series of single video frames on a monitor (for the method used for calculating jump-errors see Rheinlander et al. 1979). Scanning and orienting movements during the approach to the speaker were counted. The quality of the video recording was not sufficient to measure the orientation of the frog's body axis in relation to the direction of the sound source.

Results

Frequency threshold curves (FTCs) were obtained from 86 eighth nerve fibers. Their characteristic frequencies (CFs) and best thresholds ranged from 210 to 3,330 Hz and from 40 to 89 dB SPL, respectively. The distribution of CFs generally conformed to published distributions for *H. cinerea* (Capranica and Moffat 1983).

Encoding of the temporal structure of the signals

We observed a wide range of temporal response patterns across fibers, both in adaptation time course and in representation of the envelope periodicity of the two-tone

stimuli. Adaptation ratios, defined here as the ratio of the average response rate during the first 40 ms of stimulus presentation to that in the subsequent 40-ms time period, ranged from 0.5 to 28.7 (mean \pm SD = 2.1 ± 3.0). Examples of adaptation patterns from representative units are presented in Fig. 2. The tonic response of the unit shown in Fig. 2a contrasts with the responses of the other units in Fig. 2, which exhibit weak (Fig. 2b) or strong (Fig. 2c) adaptation. On average, the spike rate during the first 20–40 ms was about twice that of the sustained response (see Fig. 3 for boxplots summarizing responses from 58 units). Some tonically responding units faithfully represented the periodicity of the stimulus envelope over the total duration of the stimulus (Fig. 4a), whereas other units followed the periodicity of the stimulus best during the first 40 ms of the response (Fig. 4b) or not at all (Fig. 4c).

Inter-spike interval histograms often exhibited multiple peaks corresponding to multiples of the per-

iod of the stimulus envelope. On average, fibers encode the period of the stimulus envelope (3.49 ms) precisely by the mean inter-spike interval of the first peak in the histogram (Fig. 5a). In contrast, some units did not show an accurately time-locked response to the stimulus envelope (Fig. 5c); units with such a response characteristic were found similarly in auditory-nerve fibers with low or high characteristic frequency. Occasionally, units produce more than one spike per cycle of the envelope period in response to the stimulus onset (Fig. 5a, b); in these cases, the mean inter-spike interval appears to be slightly shorter during the first 20 ms of the stimulus presentation (Fig. 6a). The standard deviation of the first peak in the interval histogram changes relatively little over time indicating that the accuracy of encoding of the envelope period by individual spikes does not decline throughout the duration of the stimulus (Fig. 6b). The vector strength of the response also remains unchanged throughout the stimulus (Fig. 7a). Constant vector strength coupled with a decreasing spike rate, however, will force a reduction in the Rayleigh z statistic (z depends on n , the number of spikes), thus decreasing the accuracy of periodicity encoding throughout the duration of the stimulus (Fig. 7b).

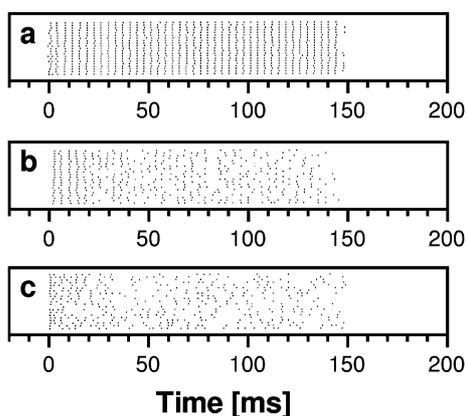


Fig. 2a–c Rasterplots showing responses of three auditory nerve fibers to 21 repetitions of a two-tone (860 + 1,146 Hz) stimulus with a beat frequency of 286.5 Hz. **a** CF: 500 Hz, best threshold: 50 dB SPL. **b** CF: 1,111 Hz, best threshold: 64 dB SPL. **c** CF: 2,898 Hz, best threshold: 50 dB SPL

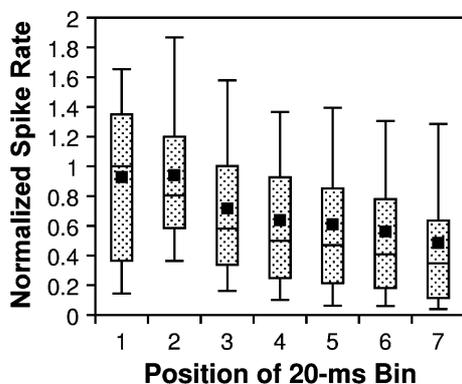


Fig. 3 Spike rates normalized to the median value of the rate during the first 20-ms period following stimulus onset (*bin No. 1*). The *boxes* represent the inter-quartile range, the *horizontal bars* in the boxes represent the median values, and the *squares* indicate the mean normalized firing rates. The *vertical lines* show the extent of 10–90% of the normalized values

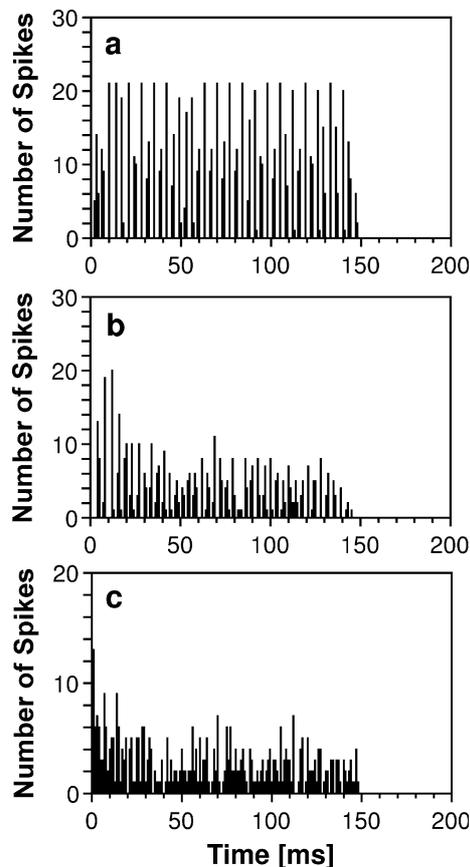


Fig. 4 PST histograms showing the responses of the same neurons (**a–c**) to the same stimuli as in Fig. 2. A maximum response of 21 spikes is obtained if the neuron fires at each cycle of the beat frequency. Bin width: 1 ms

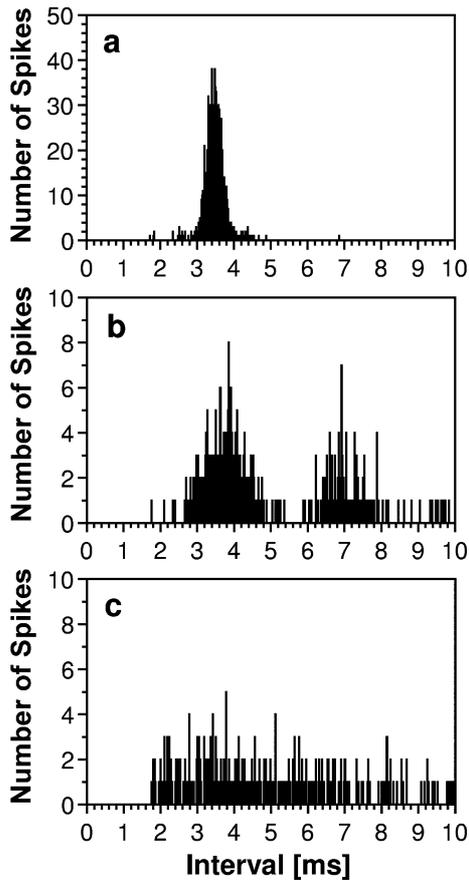


Fig. 5 Inter-spike interval histograms of the same neurons (a–c) in response to the same stimuli as in Fig. 2. **a** Low-frequency neuron fired once per beat cycle throughout the stimulus, with a mean inter-spike interval of 3.49 ms representing the beat period exactly. **b** Mid-frequency neuron fires at a lower rate, often skipping one beat cycle, resulting in a second peak at two times the beat period. **c** High-frequency neuron firing exhibits only weak association with the beat cycle. Bin width: 20 μ s

Envelope cues provide for large interaural time differences

The measurements of the variation of the phase of tone stimuli at the right and left tympana relative to the angle of sound incidence in general showed a maximum interaural time difference of 77 μ s when the sound was presented from +90° (median value for the frequencies of 900, 1,200, 1,800, 2,100, 3,000, and 3,300 Hz). The directional pattern followed a sine function (e.g., see Woodworth 1962) as would be expected if no standing waves affect the sound field. The maximum interaural time difference measured with the pair of probe microphones varied slightly with the frequency of the signal, ranging from 65 to 94 μ s for frequencies in the range of 900–3,300 Hz. In all cases except one the maximum time difference was observed at +90°, with the exception being at +135°. The variation in time differences was sufficiently small to be unimportant for the interpretation of the data on neural interaural differences.

The response of auditory-nerve fibers that encoded the stimulus periodicity varied with the direction of

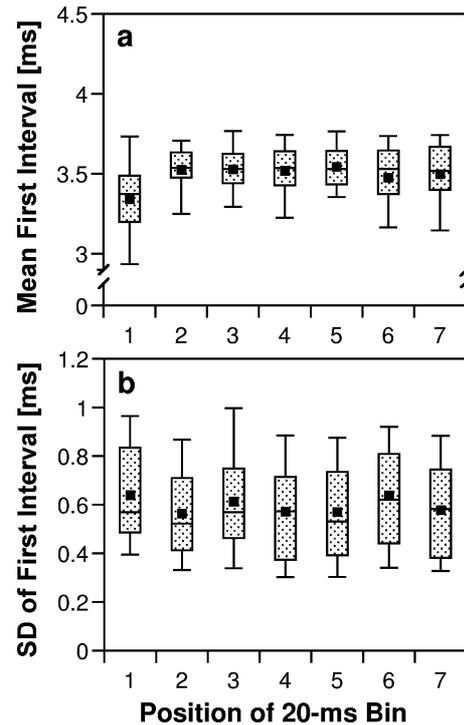


Fig. 6 Box plots showing **a** mean values of the Gaussian function fits to the first peak in the interval histogram calculated separately for each 20-ms bin for the ongoing response to the stimulus and **b** their standard deviations

sound incidence. Figure 8 shows cross-correlograms of the PSTH of a tonically-responding auditory-nerve fiber obtained when stimulating from a specific angle with the PSTH obtained with stimulation from the front (0°). The peaks in the correlogram are very sharp and allow an accurate estimate of the relative delay in the neural response. The delays of the response were asymmetrical with respect to the body axis of the frog resulting in a relatively large change when moving the speaker ipsilaterally (Fig. 9a). This is also evident in the average response of seven auditory nerve fibers in which the delays in the cross-correlograms were determined (Fig. 10). Assuming that the two ears exhibit delay patterns of the response that are mirror images of each other, one can compute the resulting interaural-time differences in the response and compare them to those of the physical acoustic signal (Fig. 9b). The neural interaural time differences are more than an order of magnitude larger than the acoustic interaural time differences.

Behavioral measurement of the accuracy of sound localization

We selected those individuals in behavioral tests that indicated (by at least three head-scanning movements) that they were actively localizing the pure-tone and 300-Hz-beat stimuli that were presented to them. The best

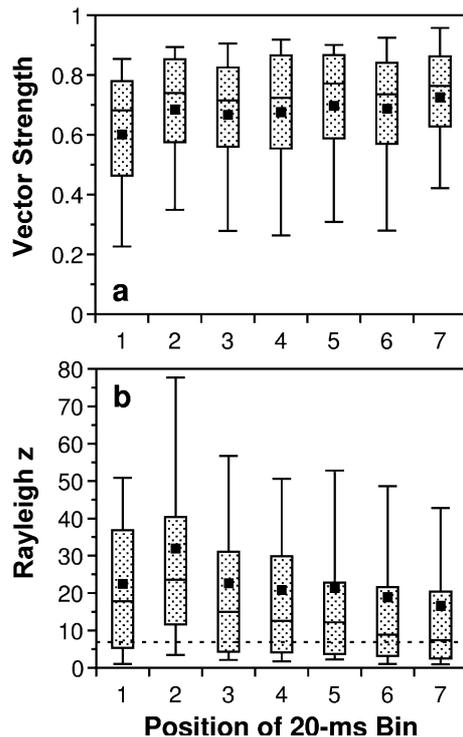


Fig. 7 Box plots showing **a** vector strength of the phase-locking of the response to the 3.49-ms beat period calculated separately for each 20-ms bin during the ongoing response to the stimulus, and **b** the Rayleigh z statistic as a function of the bin position. Dashed line indicates the z value for statistically-significant phase-locking ($z = 6.89$, $P < 0.001$, assuming a mean number of spikes per interval of ca. 300)

localization performance in the horizontal plane was observed for the stimuli with 900 Hz components. The mean jump error angles were 19.3 and 18.9° for the 900 Hz pure tone and 900 + 1,200 Hz (i.e., the 300-Hz-beat) stimulus, respectively. For the 3,000-Hz pure tone and the 3,000 + 3,300-Hz beat stimuli, the mean jump error angles were 31 and 28°, respectively. The performance for the beating stimulus created by adding tones of 1,800 and 2,100 Hz was intermediate (mean jump error angle 25.6°). Due to the large variation in the jump error angles, however, none of the observed differences was statistically significant (Table 1).

Discussion

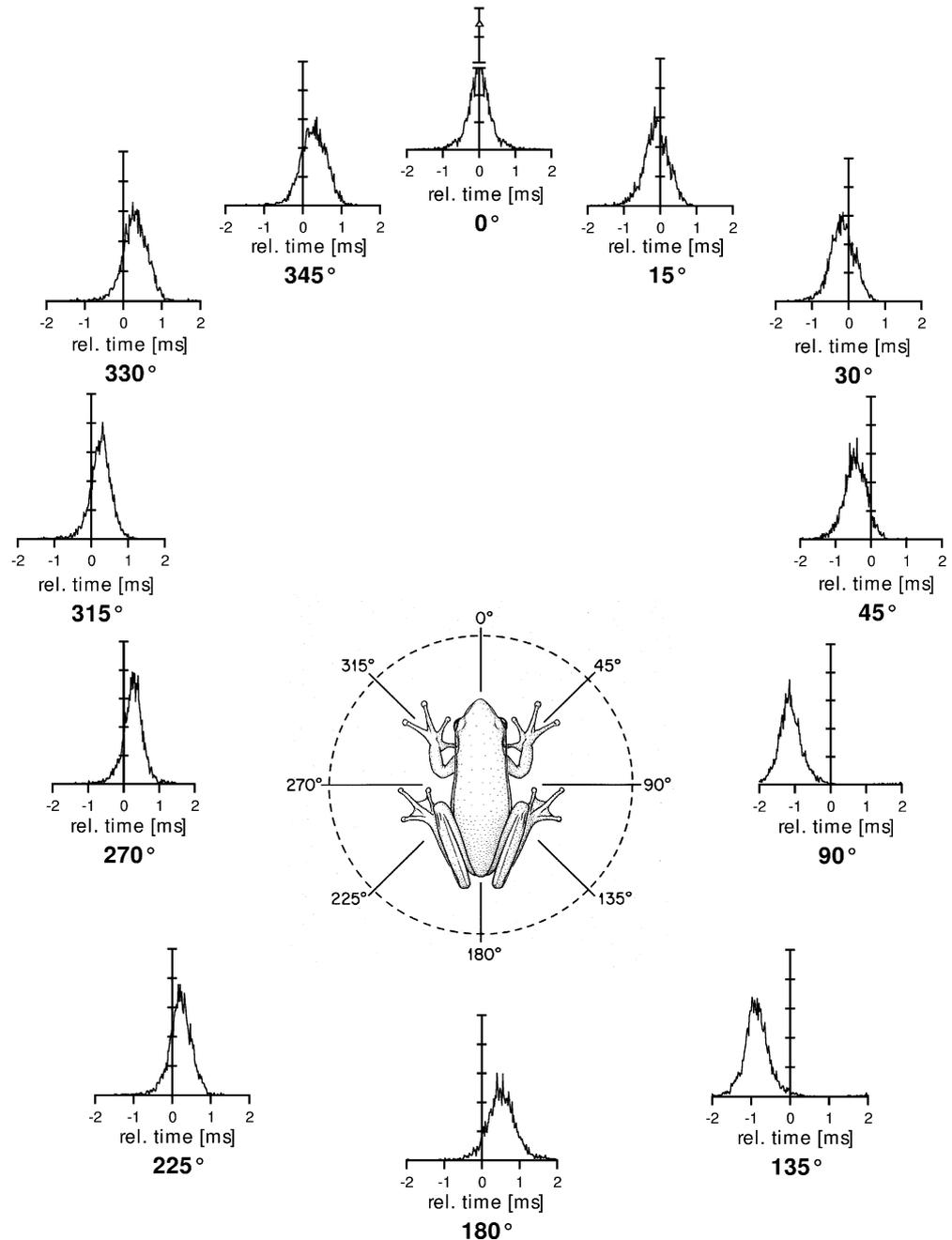
Encoding of the temporal fine structure of the call

The accurate response synchronization of green treefrog auditory-nerve fibers to the envelope of two-tone stimuli suggests that the auditory periphery is able to reflect the species-specific rate of amplitude modulation of between 260 and 300 Hz typical for the advertisement calls in its temporal response pattern. This parallels results from studies of auditory nerve responses to calls of the bullfrog, *Rana catesbeiana* (e.g. Schwartz and Simmons 1990; Simmons and Ferragamo 1993;

Simmons et al. 1992) and the leopard frog, *Rana pipiens* (Feng et al. 1991) that have lower envelope-modulation frequencies. Similar to the finding of Simmons et al. (1993b) in the bullfrog, the temporal envelope of the fibers is encoded despite the fact that there is little entrainment of the fibers' responses to the phase of the component frequencies. This ability could be explained by the response to a difference tone by an auditory-nerve fiber that was tuned to frequencies well below the component frequencies (e.g., Capranica and Moffat 1980). However, in general such envelope-locked responses were found in fibers tuned to the frequency of components making up the signal rather than to the frequency of the difference tone. Thus, it appears that the fibers respond to the repetitive transients in the amplitude-modulated signal presented within the limits of the auditory-nerve fiber's excitatory FTC. This is in agreement with the results from studies by Simmons et al. (1993b) and Bodnar and Capranica (1994) in the bullfrog showing that the phase relationship between components of a multi-frequency signal and the resulting "peakedness" will affect the occurrence of time-locked responses.

What is the advantage that accurate time-coding of the signal envelope in the auditory nerve confers on an individual animal? At the high sound-pressure levels that are typical for the background noise created by a frog chorus (Narins 1982; Gerhardt and Klump 1988) thresholds that are based on a discharge rate increase may be higher than thresholds based on the synchronization to a temporal feature of the stimulus (e.g., Rose et al. 1967; Fay 1978; Sachs et al. 1980; Narins and Hillery 1983; Hillery and Narins 1987). Furthermore, temporal coincidence detection mechanisms that have been invoked to explain the temporal tuning properties of neurons in the brainstem and midbrain of the grass frog could be used to extract the periodicity of the species-specific call from a noisy background (van Stokkum and Gielen 1989; van Stokkum 1990). Filters in the amphibian midbrain that are tuned to a species-specific rate of modulation can provide the basis for conspecific call recognition (e.g., Rose and Capranica 1985). The envelope periodicity of the call is an important character that is evaluated by the male green treefrogs in call interactions (Simmons et al. 1993a). The observation by Gerhardt (1978b) that green treefrog females prefer calls exhibiting a 300-Hz beat over calls with components in similar frequency ranges that lack the 300-Hz beat confirms the importance of the envelope periodicity for mate choice. We show that although the accuracy of synchronization is similar throughout the duration of the signal, the first 40 ms of the signal provides the most reliable cues for the amplitude modulation. Due to the higher spike rate at the beginning of the stimulus, auditory nerve fibers can more reliably encode the stimulus period as reflected by the Rayleigh- z statistic. Behavioral observations by Klump and Gerhardt (1992) support the notion that

Fig. 8 Example of the neural directionality pattern for a two-tone stimulus (900 + 1,200 Hz). PSTHs were generated from the response of the auditory nerve to the stimulus presented from the angles shown; each PSTH was cross-correlated with the PSTH derived from the response to the stimulus presented from the reference angle (0°). Cross-correlation functions are shown (bin width: 22 μ s) for each angle of sound incidence. The *curve* at 0° is an autocorrelation function. The *vertical scales* of the crosscorrelation and autocorrelation functions span the range from 0 to 0.25 and 0 to 0.7, respectively, with tic intervals of 0.05. The *triangle* on the *vertical axis* of the autocorrelation function indicates its maximum value of 0.67



the onset of the mating call is potentially more important for recognition and localization. In a two-speaker choice experiment, green treefrogs clearly preferred the leading call over the lagging call when the lead was only 40 ms and the two calls overlapped (Klump and Gerhardt 1992).

Sound localization cues and localization performance

Both interaural time and intensity cues are available to the green treefrogs for azimuth sound localization. Using binaural click stimuli, Feng (1975) found neurons in the green treefrog's torus semicircularis that were

sharply tuned to interaural time differences (the neuron's response probability could be halved by changing the interaural time difference in the click presentation by 200–300 μ s, see Fig. 62 in Feng 1975). In the superior olivary nucleus of the green treefrog, Feng and Capranica (1978) reported that the binaural responsiveness of cells was graded over a range of interaural time differences of $\pm 500 \mu$ s. Our observation of a high accuracy of spike timing in the response of auditory-nerve fibers with respect to the periodic variation of the envelope of a two-tone stimulus suggests that time-locked responses of auditory-nerve fibers to the signal envelope may provide a basis for the representation of the azimuth angle of sound incidence by interaural spike time differences.

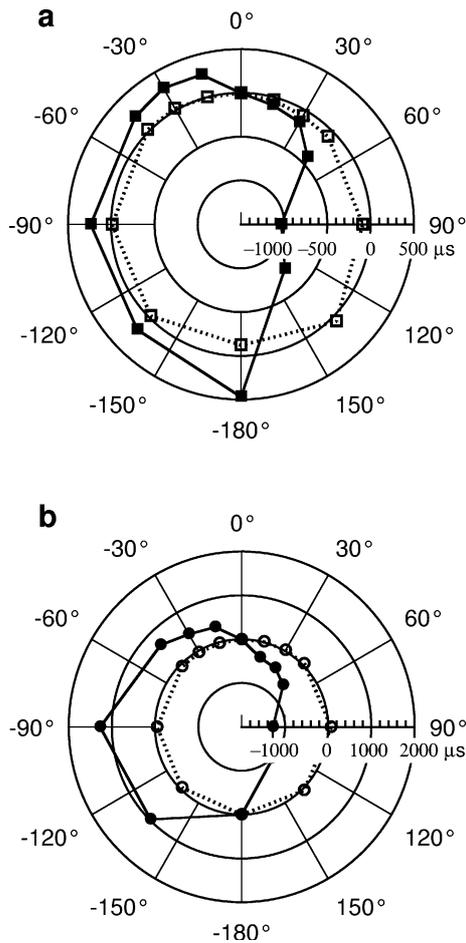


Fig. 9 **a** *Open square* shows delay of the acoustic waveform relative to the time of sound incidence from the front (0°) and *filled square* shows delay of the neural response pattern relative to the pattern observed when the sound source is located at 0° in front of the frog as inferred from the position of the peak in the cross-correlogram. **b** Interaural time differences of the acoustic signal (*open circle*) and the cross-correlation of the neural response (*filled circle*) as shown in **a**. Assuming similar patterns in the left and right ear, interaural differences were calculated by subtracting the time delays of the sound or the neural responses of the right and left ears, respectively

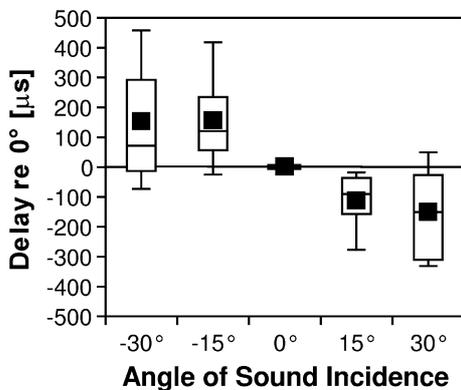


Fig. 10 Box plots of delays of the neural response patterns ($n=7$) observed at various sound incidence angles relative to the delay of the pattern observed when the sound source is located at 0° in front of the frog as inferred from the position of the peaks in the cross-correlogram

Table 1 Jump error (i.e., the difference between the angle of sound incidence and the direction of the jump of the frog from the starting point to the endpoint) in response to different tones and tone combinations

Stimulus frequency (Hz)	Mean error ($^\circ$)	SD	n
900	19.3	8.6	5
3,000	31.0	13.4	9
900 + 1,200	18.9	7.8	11
1,800 + 2,100	25.6	18.71	9
3,000 + 3,300	28.0	15.4	11

n number of individual frogs tested

Spike timing in the green treefrog's auditory-nerve fibers changed on average by $269 \mu\text{s}$ with a change in the angle of sound incidence from 15° left to 15° right of the body axis (Fig. 10) providing sufficient interaural time differences to allow an accurate sound localization. To achieve the mean accuracy observed in the behavioral tests of between 19° (at 900 Hz) and 31° (at 3,000 Hz), the frogs must be able to discriminate frontal angles resulting in interaural time differences of about 170 and $280 \mu\text{s}$, respectively. This estimate (based on a linear interpolation of the interaural time differences of fibers in the left and right auditory nerve) assumes that the accuracy observed in the behavioral response is limited by the perceptual accuracy. If the accuracy of the behavioral response is influenced significantly by motor accuracies in jumping, the perceptual accuracy could even be better. Interaural time differences of between 170 and $280 \mu\text{s}$ in the auditory-nerve fibers would be sufficient to considerably modify the response of more central auditory neurons in the green treefrog (Feng 1975; Feng and Capranica 1978). The maximum interaural time difference reflected in the timing of the action potentials was in fact much larger than 0.2–0.3 ms and could reach 1.3 ms when the sound was presented from $\pm 90^\circ$ (Fig. 9). This is far more than the variation of the interaural time difference in the acoustic signal impinging on the treefrog's left and right tympana, which was below 0.1 ms. Thus, the peripheral auditory system provides interaural time differences that are sufficient as a cue for accurate sound localization. The spike time differences are generated in response to the envelope of the signal and thus do not depend on phase locking to the individual tones making up the carrier. Similarly, it has been suggested for the mammalian auditory system that interaural time differences derived from the time shift in the envelope of the sound reaching the two ears may be used as a cue by the auditory system to evaluate the azimuth angle of sound incidence (see Yin 2002 for a review). The change in the Rayleigh z statistics during the ongoing stimulus suggests that interaural time differences could be represented more accurately in the first 40 ms than in the remainder of the stimulus. Thus, the preference for a leading over a lagging call observed in female green treefrogs could in part be due to a better ability to locate the position of the caller in addition to recognizing the species-specific envelope pattern.

A large variation in the interaural phase and time difference with azimuth angle of sound incidence has also been reported in studies of the peripheral auditory system of the grass frog (Jørgensen and Christensen-Dalsgaard 1997) and the leopard frog (Schmitz et al. 1992). In both species, large interaural phase differences were evident in the phase-locked firing of auditory-nerve fibers stimulated with low-frequency tones. A change of the angle of sound incidence away from the ear from which auditory-nerve fibers were recorded resulted in a phase advance. The maximum shift in the interaural time differences (derived from the phase shift) that occurred with a change in the azimuth angle of sound incidence in the grass frog was between 0.4 and about 2 ms (Jørgensen and Christensen-Dalsgaard 1997). This was in the same range as that observed in the green treefrog in the present study. However, the time shift was reduced with increasing frequency (frequencies up to 700 Hz were tested) in the grass frog and depended on the phase locking of the fibers to the period corresponding to the tone frequency. In contrast, in the green treefrog an interaural time difference of up to 1.3 ms was observed for signals containing higher frequency tonal components (900 and 1,200 Hz) and in an auditory nerve fiber that did not show substantial phase locking at the two frequencies of the component tones but only to the envelope of the waveform. The time-locking of spikes to the signal envelope of the two-tone stimulus was observed up to the highest frequencies tested (3,000 and 3,300 Hz) suggesting that interaural time-difference cues could be used also at such high frequencies. In contrast to the results in the grass frog and leopard frog auditory-nerve fibers that in general exhibited a phase advance when the angle of sound incidence was shifted to the contralateral side (only in two exceptional cases a phase delay was observed, see Jørgensen and Christensen-Dalsgaard 1997), in the green treefrog tested with tone combinations of 900 and 1,200 Hz a shift to the contralateral side in general resulted in a time delay in the firing of action potentials.

Interaural intensity difference is the second cue that has been proposed to be used in sound localization. Physiological and physical investigations using laser vibrometry suggest that in the green treefrog interaural intensity-difference cues in the range of between 4 and 9 dB are provided by the pressure-gradient mechanisms of the acoustically coupled ears (Rheinlaender et al. 1981; Michelsen et al. 1986). Rheinlaender et al. (1981) have suggested that the size of the intensity difference cues could be affected by the frog varying the configuration of the interaural air space. This could provide the frogs with a sufficient change in the interaural intensity difference with a shift in the angle of sound incidence to achieve localization that is as accurate as that allowed by temporal cues (see Feng and Capranica 1978). Similar to the temporal cues based on the encoding of the signal envelope, the interaural intensity difference cues that are derived from the change of tympanum vibration relative to the angle of sound incidence are rather similar at the low and high

frequencies of the tonal components comprising the green treefrog's calls (Michelsen et al. 1986).

Since physiological and physical studies provide evidence suggesting that the green treefrog's sound-localization ability should not be limited to low frequencies, it appeared worthwhile to compare the accuracy of sound localization for narrow-band stimuli to those that provide cues from the total frequency range of the species-specific calls. Similar to results of a previous study by Rheinlaender et al. (1979), the green treefrogs in this study were able to accurately localize a sound source in the horizontal plane when only low frequencies (900 Hz tones or a two-tone stimulus composed of 900- and 1,200-Hz tones) were presented. The localization accuracy was somewhat (although not significantly) deteriorated if the localization stimuli only contained frequencies of 1,800 and 2,100 Hz or of 3,000 and 3,300 Hz. The increased localization error evident in the frogs' jump angle when tested with 3-kHz pure tones was only about 50% larger than the localization error determined with 900-Hz pure tones in the present study (Table 1). Thus, green treefrogs may obtain sufficient interaural cues for accurate sound localization throughout the frequency range that is characteristic for the spectrum of their calls and the previous failure to elicit effective phonotaxis in green treefrog females by sounds containing only high frequencies (3 kHz) cannot be due to a lack of localization performance (e.g., Gerhardt 1981). It may well be that by selecting those frogs that performed head-scanning movements, only animals motivated to approach the source were included in our sample. Thus, we provide an unbiased estimate of their localization performance across frequencies from 900 to 3,300 Hz for which good localization cues appear to be available either in the form of interaural time differences of spikes time-locked to the signal envelope maxima or in the form of interaural intensity differences.

Acknowledgements We thank Kit Murphy who supplied frogs for this project and Knowles Electronics, Itasca, Illinois for kindly providing the microphones used for determining the binaural cues. M. Kowalczyk and S. Groß aided with the production of Fig. 8. This study was funded by grants from the Deutsche Forschungsgemeinschaft to GMK and by NIDCD grant No. DC-00222 to P.M.N.

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