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## Temperature effects on auditory nerve fiber response in the American bullfrog

P. van Dijk<sup>1</sup>, E.R. Lewis<sup>2</sup> and H.P. Wit<sup>1</sup>

<sup>1</sup> Institute of Audiology, University Hospital, Groningen, The Netherlands and <sup>2</sup> Electronics Research Laboratorium,  
University of California, Berkeley, California, U.S.A.

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Single fiber recordings were made from auditory nerve fibers of the American bullfrog (*Rana catesbeiana*). As temperature was raised: (1) Best frequencies of fibers from the amphibian papilla ( $N = 15$ ) increased. Below 600 Hz best frequency changes up to 0.06 oct/°C were found; above 600 Hz changes were less than 0.03 oct/°C. In the basilar papilla ( $N = 4$ ) no significant increase of best frequency was found. (2) Spike rates in response to fixed-RMS-amplitude stimuli increased considerably:  $Q_{10}$  of spike rate ranged from 5 to 10. (3) Spontaneous activity, found in basilar papilla fibers, increased with average  $Q_{10} = 1.6 (\pm 0.3)$ . (4) A conspicuous change of tuning quality factor  $Q_{10dB}$  was only observed in two fibers, that were taken to low temperatures ( $< 16^\circ\text{C}$ ). (5) the nearly linear frequency vs. phase relation in amphibian papilla shifts to higher frequency (along with shift of best frequency), while its average slope remains nearly unchanged.

Frog; Temperature; Auditory nerve fiber

### Introduction

The effect of temperature on neural response in auditory nerve fibers has been investigated in several species (American toad: Moffat and Capranica, 1976; Tokay gecko: Eatock and Manley, 1981; guinea pig: Gummer and Klinke, 1983; caiman: Smolders and Klinke, 1984; pigeon: Schermuly and Klinke, 1985). Investigation of temperature effects provides a reversible method to probe the physical mechanisms underlying signal processing in the inner ear. Comparisons of temperature effects offer a possibility to contrast tuning mechanisms in different species. Thresholds are found to be temperature dependent in all species so far investigated. Best frequency also changes in all species, except in the mammalian cochlea and the basilar papilla of the American toad. The other hearing organ of the American

toad, the amphibian papilla, was found to change its best frequency with temperature. Thus, the amphibian ear seems to use different tuning mechanisms within the same ear. This study was aimed at providing more data on the effect of temperature on anuran inner ear function. The American bullfrog (*Rana catesbeiana*) was used since a considerable amount of data already exists on both functional and morphological properties of the bullfrog's ear (for a review see Lewis et al., 1985).

Interest in temperature effects on neural response of auditory fibers in the frog was also prompted by temperature dependence of otoacoustic emission data in frogs (Wilson et al., 1986; van Dijk and Wit, 1987; Genossa, 1989; van Dijk et al., 1989). Frequency of an otoacoustic emission increases as temperature is increased and with reduced temperature a spontaneous otoacoustic emission disappears. Comparison of temperature effects on neural response and on otoacoustic emission, offers the possibility of investigating the relation between these two phenomena.

Correspondence to: P. van Dijk, PTT Telematics Laboratory, P.O. Box 15000, 9700 CD Groningen, The Netherlands.

## Material and Methods

Frogs were anesthetized with a combination of sodium pentobarbital (Nembutal® 50 mg/ml solution) and ketamine hydrochloride (Ketaset® 100 mg/ml). Each solution was injected intramuscularly in different hindleg of the frog, approximately 1 h prior to surgery (doses for both solutions: 66  $\mu$ l/100 g body weight). Surgery was done identical to Lewis et al. (1982b): after removal of a small patch of skin, a hole was drilled in the roof of the mouth in order to expose the VIIIth nerve. Surgery lasted at most 1 h and was done immediately prior to experiment. If during experiment the frog appeared to recover from anesthesia, an extra 10  $\mu$ l of the ketamine solution was injected. The condition of the animal could be checked during an experiment by observing blood circulation in small vessels on the VIIIth nerve. After surgery, the frog was placed in an acoustic chamber, on its back with mouth open.

An acoustic coupler was fitted around the frog's tympanic membrane and sealed with silicon grease. The coupler contained a Koss Pro4x driver and a Brüel and Kjaer 4166 condenser microphone to calibrate the sound pressure level of acoustic stimuli.

Single fiber recordings were made with glass micro electrodes filled with 2.0 M NaCl. After the nerve was penetrated, the frog ear was acoustically stimulated with noise bursts to identify acoustical fibers. As soon as a fiber was contacted, its best frequency was approximately determined using tone bursts. Then, the fiber's response was characterized at different ear temperatures. Temperature was measured by a small thermocouple carefully placed in the frogs middle ear, through the Eustachian tube. Temperature was lowered by putting iceblocs on the frog or raised by gently dripping warm water (approx. 35°C) on the frog's abdomen. Different protocols were used to characterize the fiber's response, depending on best frequency.

If best frequency was below 1 kHz, we characterized the fiber's response by determining a reverse correlation function (de Boer, 1967, 1968; de Boer and de Jongh, 1978; Møller, 1977; Evans, 1977; for a review see Eggermont et al., 1983). Gaussian noise was chosen as acoustic stimulus.

The noise was low pass filtered (RC filter, cutoff frequency 2 kHz, 6 dB/oct), before supplying it to the acoustical driver. The power spectral density of the noise, as recorded by the B and K microphone inside the coupler was constant within  $\pm 5$  dB between 50 and 800 Hz. Stimulus level was set at 10 to 20 dB above the fiber's threshold for the noise. The stimulus levels used (for the entire noise band from 50 Hz to 2 kHz) ranged from 60 to 100 dB SPL. With two exceptions (see Results), stimulus level was left unchanged while the electrode contacted the fiber. A homemade data acquisition board was used to compute a reverse correlation function: triggered by spikes, the board averages the noise in a fixed time window (length between 120 and 30 ms) immediately before each spike. The reverse correlation function is obtained by reversing the time axis of the resulting function. Averaging proceeded until a reverse correlation function was clearly visible on the oscilloscope screen. A fast Fourier transform was applied to the reverse correlation function, yielding magnitude and phase as a function of frequency. From the magnitude function (tuning curve) best frequency and  $Q_{10dB}$  were determined. Best frequency was defined as the frequency for which the magnitude function has its maximum. In addition to the reverse correlation function, driven and spontaneous spike rates were determined.

Two units also responded to vibratory stimuli. Because these units had high thresholds for airborne sound, they were stimulated with 110 dB SPL noise.

If best frequency was above 1 kHz, logarithmic frequency sweeps were used to determine the fiber response. Typically, frequency was swept from 1000 to 1800 Hz with a rate of 4 octave per minute; one or two times up and down. Stimulus levels ranged from 60 to 80 dB SPL. The sound pressure level of the sweep tone, as measured with the B and K microphone, was constant within  $\pm 5$  dB over the entire frequency range. For each fiber, the same stimulus level was used at different body temperatures. At fixed body temperatures, a histogram was accumulated of sweep tone frequency versus number of spikes. The frequency axis was usually divided in 50 bins. The histogram was normalized to 1 s measuring time per bin. Since histograms were rather noisy, a Gaussian curve

$n(A, B, C, f_{BF} | f) = A \exp[-B(\log f - \log f_{BF})^2] + C$  was fitted to them. This curve yielded best frequency ( $= f_{BF}$ ),  $Q_{10dB} (= 10^{+\sqrt{(\ln 10)/B}} - 10^{-\sqrt{(\ln 10)/B}})$  stimulus driven spike rate at best frequency ( $= A$ ) and spontaneous spike rate ( $= C$ ). The choice of a Gaussian curve was motivated solely by the fact that it usually fitted well to the histogram.

## Results

Fig. 1a shows three reverse correlation functions from a fiber, for the same stimulus level, at different temperatures. With one exception, the trend illustrated in the figure was present in all fibers with best frequency below 1 kHz: the frequency of the transient oscillation decreased as temperature decreased, and the onset delay of the oscillation increased as temperature decreased; opposite effects were observed as temperature increased. Onset delay typically was about 3 ms. The decrease of frequency with decreasing temperature is clearly illustrated by Fig. 1b. This figure shows the magnitude of the fast Fourier transform of the reverse correlation functions in Fig. 1a. Fig. 1c shows the phase of the fast Fourier transform of the reverse correlation functions in Fig. 1a.

Along with the magnitude plot, the phase plot shifted to lower frequencies. In most fibers, phase displayed a linear relation with frequency. Slopes of phase plots were determined by linear least-squares fit. Slopes ranged from  $-0.5 \times 10^{-2}$  to  $-1.3 \times 10^{-2}$  full cycles per Hz in the acoustical fibers. In the low frequency fibers that also responded to seismic stimuli, phase slopes were about  $-1.2 \times 10^{-2}$  cycles per Hz. Standard deviation of slope, obtained from difference between phase plot and fit, was typically  $5 \times 10^{-4}$  cycles per Hz. For individual fibers, phase plots showed a tendency to become slightly less steep as temperature was increased. The difference between slopes, obtained at the highest and the lowest temperature, was significant in 12 out of 17 fibers tested. Averaged across all fibers, the magnitude of the slope decreased by  $0.01 \times 10^{-2}$  cycles per Hz per °C. In a few fibers, the phase plot curved to a less steep slope for higher frequencies.

Fig. 2 shows two histograms of frequency vs. spikes rate for a fiber with best frequency above 1 kHz. Histograms were determined with the same stimulus level, but at different temperatures. No conspicuous increase of best frequency with temperature was observed in this fiber, but spike rate at best frequency shows a considerable increase with temperature.

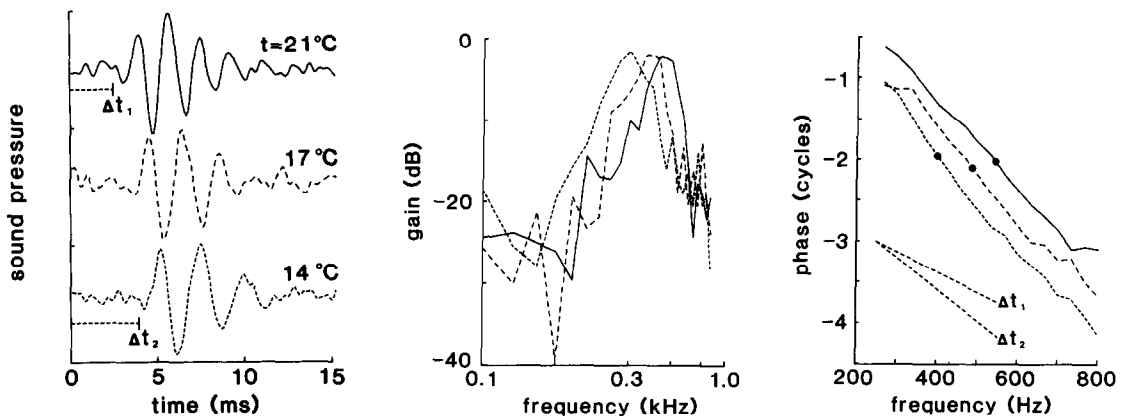


Fig. 1. (a) Reverse correlation functions of a nerve fiber from the amphibian papilla, at three different temperatures. Stimulus was a low pass noise,  $f_{cutoff} = 2$  kHz. RMS stimulus level was 80 dB SPL. Dashed bars indicate time delays:  $\Delta t_1 = 2.5$  ms and  $\Delta t_2 = 3.9$  ms. Line types (solid, dashed) indicate corresponding data in (a), (b) and (c). (b) Tuning curves, i.e. magnitude of fast Fourier transform of reverse correlation functions in (a). Best frequencies determined from these tuning curves were 400 Hz at  $t = 14^\circ\text{C}$ , 490 Hz at  $t = 17^\circ\text{C}$  and 540 Hz at  $t = 21^\circ\text{C}$ . (c) Phase response, i.e. argument of the fast Fourier transform of the reverse correlation functions in (a). Filled dots (●) indicate phase at best frequency. Slopes of phase responses that correspond to the time delays indicated in (a) are displayed by straight dashed lines (see Discussion).

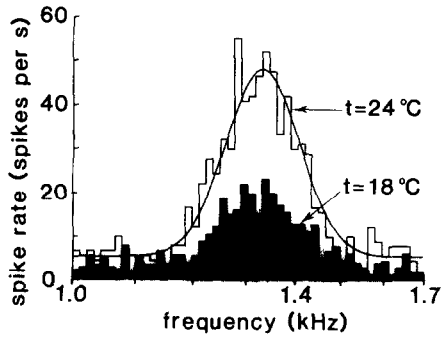


Fig. 2. Frequency vs. spike rate for a basilar papilla fiber at two different temperatures. Stimulus was 80 dB SPL sinusoid, swept up and down from 1.0 to 1.7 kHz with 4 oct/min. Histograms were normalized to 1 s measuring time per frequency bin. The smooth solid curve is a Gaussian fit to the 'open' histogram. Fits to both histograms revealed that best frequency was 1320 Hz for both histograms. At  $t=18^{\circ}\text{C}$  and  $t=24^{\circ}\text{C}$ , stimulus driven spike rate at best frequency was respectively  $17\text{ s}^{-1}$  and  $42\text{ s}^{-1}$ , and spontaneous activity was respectively  $3\text{ s}^{-1}$  and  $5\text{ s}^{-1}$ .

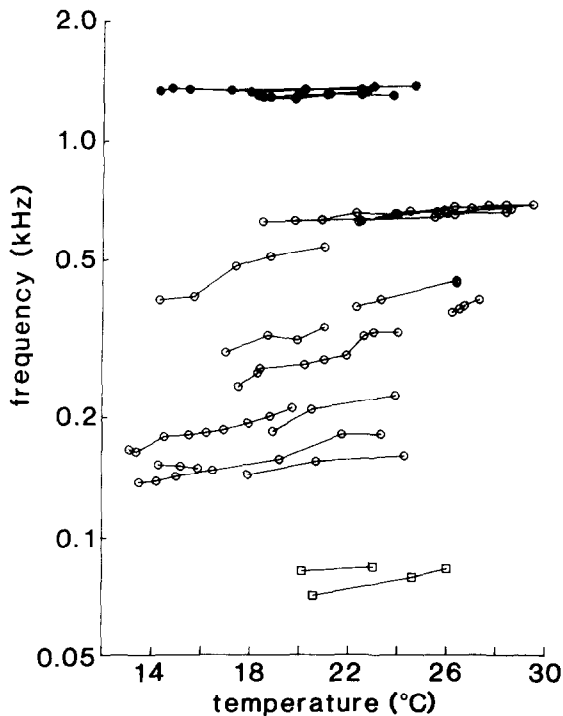


Fig. 3. Temperature vs. best frequency for fibers from the amphibian papilla ( $\circ$ ), basilar papilla ( $\bullet$ ) and sacculus ( $\square$ ). Data points from the same fiber are connected by solid lines.

Fig. 3 shows temperature vs. best frequency for all 21 fibers. This figure combines results from experiments where temperature was increased and with those where it was decreased. Increase of best frequency of a fiber with temperature was determined with a linear least-squares fit to the data. Below 1 kHz results were obtained from 17 fibres. For 16 of these fibers, best frequency increased with temperature with 0.006 to 0.065 oct/ $^{\circ}\text{C}$ . In one case, frequency decreased with temperature ( $-0.005$  octave/ $^{\circ}\text{C}$ ). Above 1 kHz results were obtained in 4 fibers. For these fibers, increase of best frequency ranged from 0.0007 to 0.01 oct/ $^{\circ}\text{C}$ . Accuracy of frequency slopes were estimated from accuracy in best frequency and temperature. Typically, this accuracy was 0.01 oct/ $^{\circ}\text{C}$ . The increase of best frequency with temperature was found to be significant in 15 of the 17 fibers with best frequency below 1 kHz. For none of the high frequency fibers ( $>1$  kHz) increase was found to be significant.

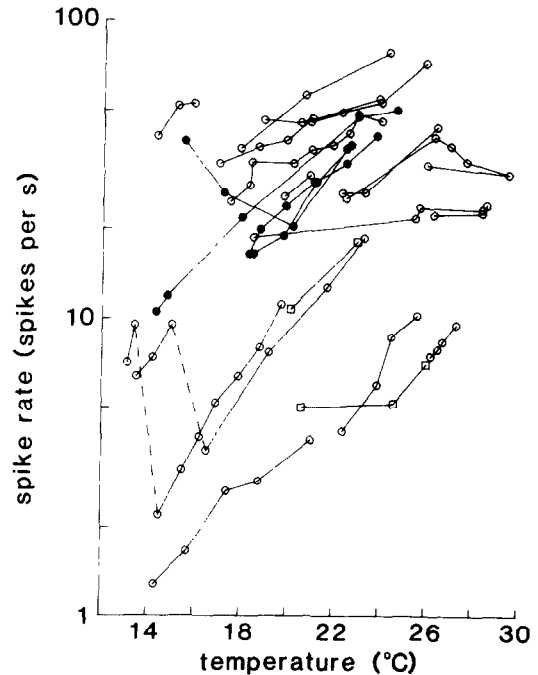


Fig. 4. Temperature vs. stimulus driven spike rate. Data points from the same fiber, with the same stimulus level, are connected by solid lines. Dashed lines also connect data points from the same fiber, but stimulus level differed 10 dB. Data symbols are the same as in Fig. 3.

Fig. 4 shows temperature vs. stimulus driven spike rate. Generally, spike rate increased with temperature. The increases of spike rate with temperature was higher for lower spike rates. At  $t = 21^\circ\text{C}$ , for spike rates below  $20\text{ s}^{-1}$ , (thermal)  $Q_{10}$  was 10; above  $20\text{ s}^{-1}$ ,  $Q_{10}$  was 5.

With one exception, we did not find spontaneous activity in fibers with best frequency below 1 kHz. In the exceptional fiber we found a low rate (approx. 1 spike/s) at high temperature ( $t = 26^\circ\text{C}$ ). Fibers with best frequency above 1 kHz typically had a spontaneous activity of 10 spikes/s. In these fibers, spontaneous activity tended to increase slightly with temperature ( $Q_{10} = 1.6 (\pm 0.3)$ ).

For fibers with best frequency below 1 kHz, the quality factor ( $Q_{10\text{dB}}$ ) for tuning ranged from 0.6 to 2.2. Low and high frequency slope of magnitude plots of reverse correlation functions were typically  $+20\text{ dB/octave}$  and  $-30\text{ dB/octave}$ . Among these fibers we found conspicuous temperature dependence of  $Q_{10\text{dB}}$  in only two. In both cases the variation in  $Q_{10\text{dB}}$  occurred at temperatures below  $16^\circ\text{C}$ . Cooling the frog in each case resulted in a drop of  $Q_{10\text{dB}}$  from about 1.7 to 1.0 between  $16^\circ\text{C}$  and  $13^\circ\text{C}$ . This increase of  $Q_{10\text{dB}}$  was mainly due to flattening of the high frequency slope of about  $10\text{ dB/oct}$ . Unfortunately, we could not hold these fibers long enough to determine whether this effect could be reversed by raising the temperature again. Fibers with best frequency above 1 kHz had  $Q_{10\text{dB}}$ 's ranging from 0.2 to 0.5. In these fibers  $Q_{10\text{dB}}$  did not change with temperature.

## Discussion

In this study, the response of auditory fibers in the VIIIth nerve was characterized using the reverse correlation technique (de Boer, 1967, 1968; de Boer and de Jongh, 1978; Møller, 1977; Evans, 1977; Eggermont et al., 1983) with white noise stimuli and using frequency sweeps. Only a single stimulus level was used for each fiber (at different temperatures). This limited stimulus ensemble was used because fibers could be held for a limited time. During experiments most time was spent by changing the animals body temperature. Thus, at each temperature the fiber response had to be

characterized quickly. The reverse correlation technique is very suitable for this purpose. The reverse correlation function equals the input-output correlation function of the peripheral auditory system. Input is sound pressure at the tympanic membrane and output is obtained by characterizing the spike train in an auditory nerve fiber as a train of delta-function pulses. If we model the system between tympanic membrane and auditory fiber as linear system followed by a nonlinear stochastic spike generator, and use white Gaussian noise as stimulus, then the reverse correlation function is proportional to the impulse response of the linear system. Obviously, in the ear the hypothetical linear system is a bandpass filter (de Boer, 1967, 1968; de Boer and de Jongh, 1978; Møller, 1977; Evans, 1977; Eggermont et al., 1983). By examining changes of the reverse correlation function with temperature, we focus our attention only on this linear filter. By fast Fourier transforming the reverse correlation function we can determine the amplitude and phase responses of the filter. A reverse correlation function can only be obtained for fibers that phase-lock to the stimulus (Eggermont et al., 1983). Since fibers in the frog ear do not phase-lock to stimulus frequencies above 1 kHz (Shofner and Feng, 1981; Hillery and Narins, 1983), we used frequency sweeps to characterize the response of fibers most sensitive to those frequencies.

### Best frequency

In the bullfrog inner ear, auditory fibers with best frequency above 1 kHz innervate the basilar papilla. Fibers with best frequencies below 1 kHz innervate the amphibian papilla. The sacculus is also sensitive to low frequency ( $< 300\text{ Hz}$ ) airborne sound with high intensity and to seismic stimuli (Lewis et al., 1982b). For fibers from the amphibian papilla and the sacculus we found that best frequency increases with temperature. Fibers from the basilar papilla ( $> 1\text{ kHz}$ ) showed no significant increase. We do not know to what extent the different temperature effects found in fibers with best frequency above and below 1 kHz can be attributed to the different stimuli used. However, our results are similar to those of Mof-fat and Capranica (1976) in the American toad and of Stiebler and Narins (1988) in *H. regilla*

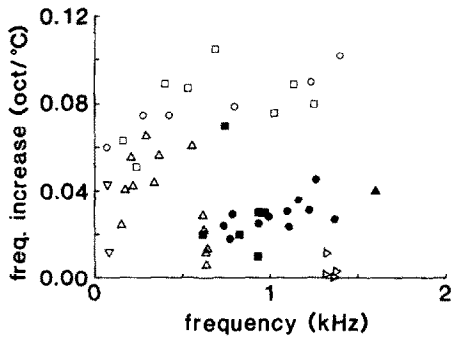


Fig. 5. Frequency vs. increase of frequency by change of body temperature. Open symbols refer to best frequency in auditory nerve fibers: seismic ( $\nabla$ ), amphibian papilla ( $\Delta$ ) and basilar papilla ( $\triangleright$ ) in the bullfrog ( $t = 21^\circ\text{C}$ , this work); pigeon ( $\square$   $t = 33^\circ\text{C}$ , Schermuly and Klinke, 1985); caiman ( $\circ$   $t = 21^\circ\text{C}$ , Smolders and Klinke, 1984). Closed symbols refer to frequency of otoacoustic emissions in frogs: *R. temporaria* ( $\blacksquare$   $t = 20^\circ\text{C}$ , Wilson et al., 1986), bullfrog ( $\blacktriangle$  Genossa, 1989) and *R. esculenta* ( $\bullet$   $t = 21^\circ\text{C}$ , van Dijk et al., 1989). Temperatures at which the frequency increase was determined are indicated.

and *E. coqui*. These authors used the same stimuli for all fibers investigated. Fig. 5 makes a comparison between species. It is a scatter diagram, displaying best frequency vs. increase of best frequency by increase of body temperature (open symbols). (Closed symbols refer to otoacoustic emission data; see below). In auditory fibers of caiman (Smolders and Klinke, 1984), Tokay gecko (Eatock and Manley, 1981) and pigeon (Schermuly and Klinke, 1985) the temperature dependence of best frequency is similar to that in frog seismic and auditory fibers with best frequencies below 500 Hz. The slope of best frequency vs. temperature for those fibers is larger for higher best frequencies. At best frequencies in the neighborhood of 100 Hz the average slope was  $0.04 \text{ oct}/^\circ\text{C}$ , while at 1500 Hz it was  $0.10 \text{ oct}/^\circ\text{C}$ . Amphibian papillar fibers with best frequencies above 600 Hz increased on average with  $0.02 \text{ oct}/^\circ\text{C}$  and basilar papilla fibers increased only  $0.004 \text{ oct}/^\circ\text{C}$  on average. In the guinea pig, Gummer and Klinke (1983) found no dependence of best frequency on temperature. Several authors (Eatock and Manley, 1981; Gummer and Klinke, 1983; Smolders and Klinke, 1984; Schermuly and Klinke, 1985; Oldfield, 1988) have pointed out that differences in temperature dependence of best frequency could indicate the presence of different

tuning mechanisms in different hearing organs. Thus, the mammalian cochlea and the anuran basilar papilla may use a tuning mechanisms that is different from that used by the reptilian ear, the bird ear and amphibian papilla and (evidently) the sacculus in the anuran ear.

Within the amphibian papilla the temperature experiments indicate two populations of fibers, possibly using different tuning mechanisms. For fibers with best frequencies below 600 Hz (at  $t = 21^\circ\text{C}$ ) the slopes of best frequency vs. temperature ranged up to  $0.06 \text{ oct}/^\circ\text{C}$ ; above 600 Hz the slope was less than  $0.03 \text{ oct}/^\circ\text{C}$ . Our data are insufficient to decide whether the change at 600 Hz is really a discontinuity.

Additional physiological evidence for two populations of fibers in the amphibian papilla exists. Fibers with best frequency below 550 Hz exhibit two-tone suppression and response to the quadratic distortion tone  $f_1 - f_2$  (Feng et al., 1975; Capranica and Moffat, 1980). Above 550 Hz they do not. Thus, there is a correlation between the nonlinear response properties and temperature dependence of best frequency, indicating that frequency tuning and nonlinear response may be closely linked. This correlation extends towards the basilar papilla, which shows no two-tone suppression (Feng et al., 1975) and small (if any) temperature dependence of best frequency. Capranica and Moffat suggested that the lack of two-tone suppression above 550 Hz in the amphibian papilla is merely a consequence of the fact that the best suppressing tone would have been outside the sensitivity region of the papilla. A suppressing tone would thus apparently not be able to reach the sensory epithelium. Our data suggest a more profound difference between the operation associated with fibers with best frequencies above and below 550 Hz.

There is also morphological evidence for two populations in the amphibian papilla. Since the papilla is tonotopically organized (Lewis et al., 1982b), fibers with best frequency below and above 550 Hz innervate different parts of the papilla. These two regions lie approximately on different sides of the tectorial curtain spanning the chamber in which the papilla is located (the curtain is part of the tectorium covering all hair cells in the papilla, Lewis et al., 1982b). Hair cell orientation

differs on the two sides of the curtain. Rostral to the curtain hair cells (sensitive to frequencies below 500 Hz) are orientated parallel to the length of the papilla. On the other side of the curtain, hair cell orientation is perpendicular to the long axis of the papilla. Thus, the amphibian papilla is divisible into two regions on bases of hair cell orientation, temperature dependence of best frequency, and nonlinear response properties. In each case, the dividing line occurs where best frequencies fall in the 500 to 600 Hz range.

In Fig. 5 the increase of frog emission frequency with temperature is also indicated (closed symbols). Emission data were taken from Wilson et al. (1986), Genossa (1989) and van Dijk et al. (1989). The emission results range from 600 Hz to 1600 Hz. Generation of otoacoustic emissions is assumed to be related to frequency tuning mechanisms in the ear. In humans a close relation between minima in pure tone threshold audiogram and emission frequencies supports this view (Schloth, 1983, Long and Tubis, 1988, Horst et al., 1983). More evidence is supplied by the similar shape of neural tuning curves and iso-suppression tuning curves resulting from suppression of an emission by a pure-tone stimulus (Kemp, 1979, Wilson, 1980, Wit et al., 1981, Schloth and Zwicker 1983, Ziss and Glatke, 1988). Thus, since frequency tuning and otoacoustic emissions seem to be related, one would expect best frequency of nerve fibers and emission frequency to change with temperature in a similar way (i.e. with equal slopes). Below 600 Hz no emission data are available. The fact that only few emissions are found below 600 Hz could be due to reduced sensitivity of the microphone used in emission experiments (van Dijk et al., 1989). However, it could also be related to the duality of function and morphology of the amphibian papilla discussed above. For frequencies in the sensitivity range of the amphibian papilla above 600 Hz (see Fig. 5) slopes of best frequency vs. temperature and those of emission frequency vs. temperature are comparable. However, in the frequency range of the basilar papilla (1 to 2 kHz), the slopes of emission frequencies are significantly greater than those of best frequencies. This is also true within the same frog species (open and closed triangles are bullfrog results) and can thus not be attributed to

species differences of the sensitivity ranges of both papillas. Therefore, the hypothesis that the mechanisms that determine fiber frequency tuning and emission frequency are closely related, is not supported by the temperature experiments.

### $Q_{10dB}$

We did not observe a conspicuous dependence of  $Q_{10dB}$  on temperature, except in two fibers at low temperature. Also in reptilians, birds and mammals  $Q_{10dB}$  did not change with temperature (Smolders and Klinke, 1984; Schermuly and Klinke, 1985; Gummer and Klinke, 1983). Thus, this seems to be a property present in all species. However, some caution should be taken in comparing our  $Q_{10dB}$  to those from other reports. Usually,  $Q_{10dB}$  is defined as  $f_{BF}/\Delta f_{10dB}$  where  $\Delta f_{10dB}$  is the width of a threshold tuning curve. We defined  $\Delta f_{10dB}$  from an iso-intensity response histogram ( $> 1$  kHz) or a reverse correlation function ( $< 1$  kHz). The fast Fourier transform of a reverse correlation is also best interpreted as an iso-intensity response curve (Eggermont et al., 1983). From macro electrode recordings from the torus semicircularis in frogs, it is known that auditory threshold increases as temperature decreases (Hubl et al., 1977; Hubl and Schneider, 1979; Mohnke and Schneider, 1979; Walkowiak, 1980). Thus, when the frog is cooled while the stimulus level is kept constant, the stimulus approaches threshold. Using the reverse correlation technique, Møller (1977) found that in the rat ear tuning sharpened as the noise stimulus was lowered. This effect occurred also at levels only just above threshold. In cat (de Boer and de Jongh, 1978; Evans, 1977), however, the reverse correlation function did not change much until the stimulus was taken up to 45 dB above threshold, although there is prominent nonlinear response to tonal stimuli (Sachs and Kiang, 1968). Thus, although the auditory system is known to respond highly nonlinearly to tonal stimuli, its response to noise can be fairly linear. In the frog no data are available on linearity of neural response to noise stimuli. If, for example, in the frog lowering of the noise stimulus down to threshold turns out to narrow the tuning, then our results could mean that a  $Q_{10dB}$  defined from a threshold tuning curve will increase as temperature increases. This would

then be different from result in reptiles, birds and mammals.

### *Spike rates*

Although the tuning characteristics of seismic (presumably saccular) fibers, amphibian papilla fibers and basilar papilla fibers show a certain variety in their dependence on temperature, the stimulus driven spike rate behaves in the same way in all three organs as temperature is changed: iso-intensity spike rate increases with temperature. Macro electrode recordings from the torus semicircularis in several frog species (Hubl et al., 1977; Hubl and Schneider, 1979; Mohnke and Schneider, 1979; Walkowiak, 1980), have shown that threshold decreases as temperature increases. A threshold shift was also found by Moffat and Capranica (1976) in single fiber recordings in the American toad, although threshold in basilar papilla fibers changed only a little. However, the macro electrode torus recordings show that temperature change of threshold in the frequency range of the basilar papilla varies considerably among species. We did not determine threshold, but determined spike rate in response to a fixed-RMS-amplitude stimulus. Increase of spike rate with constant stimulus level could be due to (1) decrease of threshold, (2) reduction of refractory period (as reflected in an increase of maximum firing rate), or (3) a combination of (1) and (2). Our observation that spike rates in amphibian and basilar papilla show the same temperature dependence thus is consistent with American toad results: with increasing temperature Moffat and Capranica report (1) decrease of threshold and approximately constant dynamic range for amphibian papilla fibers, and (2) little change of threshold but increase of maximum firing rate for basilar papilla fibers.

The torus semicircularis recordings indicate that hearing threshold in a given frog species is optimal at temperatures at which the species usually mates. Thus, the ear is designed in a way which optimizes the chance for a frog to get in contact with a mating partner. For the bullfrog this preferred mating temperature apparently is in the 20°C's (Duellman and Trueb, 1986). We found higher spike rate in the 20°C's as compared to the 10°C's. This could be due to decrease of threshold. Al-

though we did not take temperature high enough to reveal an optimum, our result would then be in agreement with the finding that the sensitivity of the hearing organ of a frog species is optimal at temperatures at which the species usually searches for a mating partner.

Strong dependence on temperature of spike rate in response to fixed-amplitude stimuli is present in both warm and cold blooded species. In the pigeon (Schermyly and Klinke, 1985) and guinea pig (Gummer and Klinke, 1983) spike rates increased with increasing temperature. Smolders and Klinke (1984) found a maximal response around 30°C in the caiman. In these species, increase of spike rate coincides with decrease of threshold.

Compared to the stimulus driven spike rate, spontaneous activity shows only a small increase with increasing temperature. This is similar to results in the pigeon (Schermyly and Klinke, 1985) and guinea pig (Gummer and Klinke, 1983). Also in the American toad (Moffat and Capranica, 1976) and caiman (Smolders and Klinke, 1984) spontaneous activity increased with temperature. The increase found in the caiman was however consistently larger than in our experiments.

### *Phase response*

For fibers with best frequency below 1 kHz, the reverse correlation technique reveals the phase response of the auditory filter. Only the phase corresponding to the upper 20 dB of the frequency response curve (Fig. 1b) can be determined accurately. In most fibers, phase displayed a linear dependence on frequency. The frequency vs. phase slopes were similar to those found from cycle-histograms using sinusoidal stimuli (Lewis, 1984, 1988; Hillery and Narins, 1984). Linear phase is a characteristic of a linear time delay (an infinite-order dynamic system). It also can be approximated by a system with dynamics whose order is high but finite, such as a high-order linear filter. The impulse response of a high-order filter yielding truly linear phase would be symmetrical about its peak. This is not possible in causal systems, but it can be approximated. It is approximated quite well in the reverse correlation functions from the frog (e.g., top waveform in Fig. 1a). The impulse response of a second-order system, such as an



electrical or mechanical resonance, is maximally asymmetrical (with the peak amplitude occurring at the first half cycle of oscillation).

The reverse correlation functions (Fig. 1a) show that the impulse response of the auditory filter seems to include a time delay of the order of 3 ms. Such a time delay could effectively be in cascade with the high-order filter that gives rise to the oscillatory portion of the impulse response. In Fig. 1c we plotted slopes of phase plots that correspond to the time delays of 2.5 and 3.9 ms in Fig. 1a. These slopes differ considerably from the slopes found by the fast Fourier transform of the reverse correlation function. Thus, the linear phase plots can not be fully explained by a time delay, external to the high-order filter (such as signal travel time in inner ear fluids or axons).

The temperature experiments show even more clearly that the measured phase response is a property inherent to the high-order auditory filter. Since, in Fig. 1a the apparent time delay changes from 2.5 ms to 3.9 ms, the slope of the phase plot would have to change from  $-0.25 \times 10^{-2}$  to  $-0.39 \times 10^{-2}$  cycles per Hz (see Fig. 1c), if the delay was caused by a linear time delay in cascade with the filter. However, the measured phase slope changes only slightly with temperature. Therefore, the apparent time delays visible in the reverse correlation functions evidently is not the result of a separate linear time delay, but a property of the high-order filter itself. This conclusion is supported by the fact that phase at best frequency remains relatively unchanged; the phase plots shifts along with the frequency response (Fig. 1b) as temperature is changed, indicating that both are tightly linked. Lewis (1984, 1988) showed that the linear phase could extend over several full cycles. By estimating time delays external to the auditory filter, such as travel time in ear fluids and axonal travel time, he concluded that this linear phase must be a property of the filter. He concluded that the bullfrog ear makes use of high order filtering. The present study shows that linear phase is indeed a property inherent to the filtering process. Thus Lewis' conclusion is reconfirmed by our temperature experiments.

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