

HEARING THRESHOLD AND FREQUENCY DISCRIMINATION IN THE PURELY AQUATIC FROG *XENOPUS LAEVIS* (PIPIDAE): MEASUREMENT BY MEANS OF CONDITIONING

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Summary

Hearing threshold and frequency discrimination for underwater sound were measured in the clawed frog *Xenopus laevis* by means of conditioning. A go/no go discrimination procedure was used in which the test tone was presented concurrently with a wave on the surface of the water. The tone signalled whether or not the frog should respond to the wave. The hearing range of *X. laevis* was 200–4000 Hz. Similar thresholds of 92–96 dB re 1 μ Pa were found at 600 Hz, 1400–1800 Hz and 3200–3600 Hz. A high threshold at 1000–1300 Hz suggested that this was the frequency range between the sensitivities of the amphibian and basilar papillae. Relative frequency discrimination was approximately 5% at 400–800 Hz, 45% at 1000 Hz and

2.4–6% at 1600–2500 Hz. This last range encompasses the dominant frequencies of the advertisement call of this species. High discrimination acuity at these frequencies may be used in distinguishing between calling males. The threshold for a one-third-octave bandpass noise centred at 600 Hz was 27.6 dB lower than that for a pure tone of 600 Hz, suggesting that sound intensity was integrated within this bandwidth, possibly by a critical-band mechanism.

Key words: frog, hearing, audiogram, frequency discrimination, critical band, *Xenopus laevis*, conditioning.

Introduction

Frogs are favourite models for studying intraspecific acoustic communication and social behaviour (Wells, 1977; Arak, 1983; Rand, 1988; Gerhardt, 1988; Halliday and Tejero, 1995; Sullivan et al., 1995). A major issue in these studies is how females might use acoustic information to choose and find males that will give them higher reproductive success. In the field, Davis (1988) demonstrated acoustically mediated neighbour recognition in male bullfrogs (*Rana catesbeiana*), but its auditory basis remained unknown. A central shortcoming is the lack of information about the frogs' capabilities in acoustic discrimination, which is due to the general failure to condition amphibians and thus to test their sensory abilities directly (Macphail, 1982; Elepfandt, 1996a,b; Fay and Megela-Simmons, 1999). Only indirect methods that might not approach the limits of auditory abilities have been available, such as a spontaneous choice between acoustic modifications of mating calls (Gerhardt, 1988, 1994), a change in dermal conductivity (Brzoska et al., 1977; Brzoska, 1980), reflex modification (Megela-Simmons et al., 1985; Moss and Megela-Simmons, 1986; Megela-Simmons, 1988a,b) or electrophysiological recording (for reviews, see Zakon and Wilczynsky, 1988; Lewis and Narins, 1999).

Frogs have two separate auditory organs, the amphibian and basilar papillae (for a review, see Lewis and Lombard, 1988). The amphibian papilla responds to frequencies up to approximately 1000 Hz, with some interspecific variation. The basilar papilla responds to higher frequencies. Lewis and Lombard (1988) described a correlation between the form of an amphibian papilla and the range of its frequency response. According to this correlation, the amphibian papilla in *X. laevis* should respond up to approximately 800 Hz. The dominant frequency of the advertisement call in *X. laevis*, which is at 1600–2000 Hz, is thus within the sensitivity range of the basilar papilla. Electrophysiological recordings from basilar papilla afferents in several frog species have revealed that, in any given individual, these fibres have identical characteristic frequencies. Since phase-locking in auditory nerve fibres in amphibians is limited to frequencies below approximately 1000 Hz, Zakon and Wilczynsky (1988) concluded that frequency discrimination in frogs should be impossible in the frequency range of the basilar papilla. It might only be possible within the range of the amphibian papilla, because in afferents from this papilla different characteristic frequencies were found.

We succeeded in conditioning the South African clawed frog *Xenopus laevis* to respond to sounds to test its auditory abilities directly. *X. laevis* is completely aquatic and communicates acoustically while sitting on the bottom of bodies of water. The tests were therefore performed with underwater sound. For conditioning, we used the go/no go paradigm of our previous water wave discrimination tests (Elepfandt et al., 1985). However, in the present study, the stimuli were joint presentations of tones and waves on the surface of the water. The animals had to distinguish whether or not a test tone was presented together with the water wave. The form of the water wave itself was unimportant and was varied, so that only the tone would provide the reliable cue for learning.

We examined the thresholds of *X. laevis* for hearing pure tones and for discriminating between frequencies. In mammals and birds, an important feature of hearing is that neighbouring frequencies of a complex sound are processed through a common filter so that they can mask each other and their power is integrated to a joint loudness. The bandwidth of this filter is called critical band. Our results suggest the existence of critical-band processing in *X. laevis*. Finally, our data provide evidence that inputs from the two auditory papillae might be perceived differently.

Preliminary data have been published elsewhere (Elepfandt and Günther, 1986; Traub and Elepfandt, 1987; Günther et al., 1987; Elepfandt and Hainich, 1988a,b; Elepfandt et al., 1989a,b; Elepfandt, 1996b).

Materials and methods

Animals

Adult male and female *Xenopus laevis* Daudin (1–2 years old) were taken from our laboratory breeding colony, which originates from specimens imported from Cape Province, Republic of South Africa. [Systematists subdivide the species *Xenopus laevis* into five subspecies (Kobel et al., 1996). The animals generally named *X. laevis*, and used here, belong to the subspecies *X. laevis laevis*]. Their length was 5–7 cm from snout to vent. To exclude visual cues, animals were blinded by bulbus extirpation under anaesthesia (with MS222, ethyl-*m*-aminobenzoate, 0.3% w/v in water) at least 1 week before the start of testing. The animals were kept at room temperature (approximately 20°C) in aquaria (60 cm × 40 cm) filled with water 8 cm deep. In each aquarium, three animals of different sizes were kept so that they could be easily identified. During the training and testing periods, the animals were fed only in the test tank as the reward for a correct response to the positive test stimulus. Different animals were used for threshold determination, for frequency discrimination and for critical-band examination. Equal numbers of males and females were used in all tests. In total, 96 animals were used in the study over several years.

The test tank

The test tank was carefully constructed to ensure reproduction of pure tones in the water (Fig. 1). It rested on a

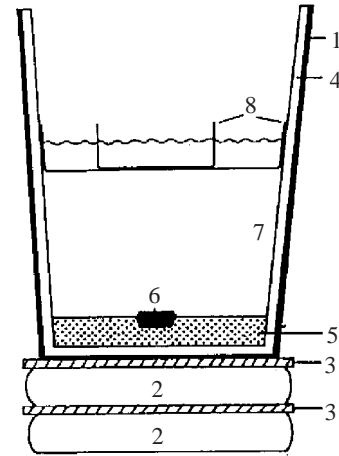


Fig. 1. Apparatus for the production of pure tones under water. The apparatus consisted of a cylindrical polyvinylchloride tank (1) (diameter 65–70 cm, height 90 cm) filled with water to a depth of 45 cm (7) and standing on tyre inner tubes (2) and plywood (3) to isolate it from ground vibrations. The inside of the tank was lined with plastic bubble wrap or styrofoam (4), and a further inner lining of thin plastic sheeting prevented contact between the water and the tank. A loudspeaker (6) was placed on a 5 cm thick layer of sand (5) at the bottom of the tank. A horizontal net (8) (mesh diameter 5 mm) was stretched 7 cm below the water surface to prevent deep diving by the frog. A circular net (8) (diameter 30 cm) placed centrally on the horizontal net contained the frog and prevented it from coming close to the edges of the tank. Within the central arena at depths of 5–7 cm, sound pressure varied by less than 2 dB for any frequency used.

double layer of plywood and tyre inner tubes to isolate it from ground vibrations. The inside of the tank was lined with plastic bubble wrap or with layers of styrofoam and an additional inner layer of thin plastic foil sheeting. Thus, the water in the tank had no contact with an external resonating substratum but was surrounded on all sides only by air (provided by the bubble wrap or the styrofoam). At the water-to-air interface, waterborne sound is completely reflected (but with a phase reversal, in contrast to airborne sound), so that the water in this tank was reverberant. During the test, the frog was restrained by netting within the top 7 cm of the water, where it could move around freely in a central circular test arena. The frog was stimulated by sound broadcast from below only when it was sitting quietly on the net, so that its head was located 5–6 cm below the water surface. Several tanks with minor modifications were used in different laboratories in Konstanz and Berlin. Although absolute sound intensity differed slightly between the tanks, the pure tone quality (as described below) was equal in all the tanks.

Tone production and measurement

Pure tones lasting 3 s and with rise and fall times of 100 ms were broadcast from the underwater loudspeaker (UW 30 Diatran, University Sound; Renkforce SA 9120 amplifier). Frequencies from 100 Hz to 4400 Hz were used. Previous experiments with standing waves have shown that the ear of

X. laevis is a pressure receiver (Hetherington and Lombard, 1982). Sound pressure was measured with a hydrophone (Brüel & Kjaer 8103 with Brüel & Kjaer 2635 amplifier) and is expressed in dB re 1 μ Pa, the standard reference for underwater sound. Measurements of sound pressure were taken all over the test arena (spacing was 5 cm). For all frequencies used in the experiments, oscillograms verified the temporal structure of the tone and Fast Fourier Transform (FFT) analysis verified that pure tones arrived throughout the test arena with signal-to-noise ratios of nearly 30 dB. At depths of 5–7 cm, the sound pressure at any frequency used in the tests differed maximally by 2 dB between the centre and the edge of the test arena. The maximum value was taken as the reference. These measurements were repeated every 2–3 weeks. In the tests for a critical band, a one-third-octave bandpass noise centred at 600 Hz (535–674 Hz) was presented (Wavetek 132, plus custom-made filters for the upper and lower frequency limits) instead of the pure tone; FFT analysis verified the flat spectrum of the noise. The width of the noise band was chosen on the basis of the widths of critical bands in mammals (Zwicker et al., 1957).

Conditioning

A discrete trial go/no go discrimination procedure was applied in which the frog had to learn to respond to the positive test stimulus (S+) but not to the other (S–). The principle was the same as in our previous conditioning of *X. laevis* for water wave discrimination (for details of testing and analysis, see Elepfandt et al., 1985); only the stimuli differed. S+ was the joint presentation of the test tone (or the noise) and a surface water wave produced by manually dipping a rod onto the water surface 6–8 cm away from the animal. In hearing threshold tests, S– was the presentation of a surface water wave alone. The presence or absence of the tone signalled whether or not the frog should respond to the wave. The tone started slightly earlier, so that it ended approximately when the wave arrived at the frog, to simulate an insect making sound and struggling on the water surface at some distance. A precise temporal relationship between the sound and water wave or a constant wave pattern was not intended. Rather, the slight irregularities of the wave pattern and timing made the tone the only reliable cue for discrimination between S+ and S–. The response criterion was the easily discernible orienting response of *X. laevis* towards the origin of the water wave. This response clearly differs from spontaneous initiations of swimming. In frequency discrimination tests, S– was a wave presented with a different tone frequency from that in S+. Thus, a complex stimulus was applied that affected the lateral line and the auditory systems more-or-less simultaneously. The lateral line system in *X. laevis* responds only up to 40–50 Hz (Kroese et al., 1978), whereas the lower limit of hearing was found to be 200 Hz. Thus, the frequency ranges of the two sensory modalities do not overlap. S+ and S– were presented in quasi-random alternation according to Fellows (1967). Interstimulus intervals were 1–3 min. Responses to S+ were rewarded with *Tubifex* worms or minced meat presented with forceps to the

frog's mouth. Responses to S– were followed by a sharp stroke with the rod on the frog's head (simulating an animal reacting in self-defence). When the animal did not respond, it was neither rewarded nor punished. When conditioning *X. laevis*, both reward and punishment must be applied to yield a discrimination (Elepfandt et al., 1985). The strength of the punishing stroke affects the general responsiveness of the frog. It was adjusted to make the animal's response rate approach approximately 50%, which gives best discrimination (Elepfandt et al., 1985).

For training and testing, each frog was transferred individually, in a beaker filled with water from the aquarium, to the test arena in the tank. At the beginning of each session, the responsiveness of the frog was tested by presenting only S+ and offering the reward. If the frog reacted at least three times within 10 stimulus presentations, regular testing began. Otherwise, the frog was returned to its storage tank and the next frog was taken for testing. In the tests, the frogs were presented with up to eight 12-trial blocks in series. If the animal responded at least three times in any block, another 12-trial block was given; otherwise, testing of the animal was stopped. Usually only 1–3 training blocks were possible per session. Training and testing were repeated approximately every 2–3 days, but some animals required longer intersession intervals before they showed sufficient responsiveness.

In tests for hearing threshold, S+ was initially given with the tone at a high intensity, so that the sound audible outside the tank was louder than when a specimen of *X. laevis* was calling. Once the animal had learned the discrimination of S+ from S– to criterion (see *Statistical analyses*), tone intensity was attenuated (Hewlett Packard HP 350D) and discrimination tests were repeated at that new intensity to criterion, and so on, in a staircase method, until the threshold was reached. Attenuation steps were initially 6 dB and were reduced to 2 dB when discrimination was close to the threshold. Once the threshold to a particular frequency had been determined in this way, testing was started again with a new frequency starting at the high intensity.

In tests for frequency discrimination, tones were presented at approximately 20–30 dB above threshold, but tone intensities were modified by up to 10 dB during each session to exclude discrimination on the basis of loudness. Training started with widely separated frequencies, and the difference was reduced in a stepwise manner using a staircase method, as in the hearing threshold determination, until the discrimination threshold was attained. The final step size at the threshold was approximately 1% of the test frequency. Threshold was defined as the mean tone intensity between the lowest detected and the highest non-detected tone intensity, or between the smallest tone difference detected and the greatest difference not detected. The latter threshold is generally termed discrimination limen.

Statistical analyses

Discrimination of the stimuli was tested by sequential testing for differences between two binomial distributions

(Wald, 1945), represented here by the responses and non-responses to S+ and S-, respectively. In contrast to the usual tests, which use a null hypothesis H_0 and a fixed number of trials, this test makes use of two hypotheses, H_0 (no difference) and H_1 (difference), and the trials are continued until one of the two hypotheses can be rejected and, consequently, the other hypothesis accepted. The advantages of this procedure are (i) that the test always leads to a definite decision, (ii) that it can cope with changes in the animal's responsiveness that would make tests with fixed numbers of trials impossible, and (iii) that the results of successive test blocks can be concatenated irrespective of how much time has passed between the blocks. This last fact renders the number of blocks in any single session irrelevant, and tests may continue over days, until the statistical decision can be made. Our hypotheses were $H_0=50\%$ correct reactions for non-discrimination and $H_1=70\%$ correct reactions for discrimination. Acceptance of H_1 means that the frog has reached the discrimination criterion. The significance level was 5% for both hypotheses. 'Correct' reactions were 'orienting response to S+' and 'no orienting response to S-'.

Results

General activity and tone learning

Initially, each frog required 1–2 weeks of daily transfer to the tank for adaptation. Thereafter, training could be started within 5–10 min of the transfer. Later, occasional feeding movements of the frog with its front legs (a sort of flicking as if to bring food to its mouth) immediately after the transfer indicated that the animal had learned the relationship between transfer to the tank and food presentation. Learning the task took 10–20 sessions for approximately half the animals, and the rest were eliminated from further tests. The task of discriminating this combined wave/tone stimulus appeared to be more difficult for *X. laevis* than discriminating between frequencies of water waves, which was learned within 4–8 sessions by most animals (Elepfandt et al., 1985). Once a discrimination had been learned for this combined stimulus, however, the animals easily transferred the discrimination to the same task with modified tone intensity or frequency. No difference was seen in learning to distinguish between a wave presented with a tone and a wave without a tone or between waves presented with different tones.

To examine their memory for the complex stimulus, some animals that had performed a discrimination to criterion were not tested for 5 weeks but were maintained and fed in their aquarium. They were then retested in the tank. In this retesting, they were neither rewarded nor punished, to prevent new learning. Nevertheless, all 15 animals tested in this way reached the discrimination criterion on the first day of testing, and discrimination acuity was undiminished.

The animals showed considerable variation in responsiveness both among individuals and among sessions. Even after learning the task and regularly working with it for several weeks or months, an animal might suddenly completely cease to respond and had to be withdrawn from the procedure.

Because of this difficulty, a complete threshold curve could be obtained for only one animal. Some of the animals that had stopped responding were reintroduced to the procedure after a few months of rest, and they immediately responded correctly. This indicated that they had remembered the task and that relearning was not necessary.

After a few weeks of testing, some animals began to respond to the tone even before the wave arrived. Such responses were unoriented turns or feeding movements with their front legs. Such early responses could appear in up to 25% of all presentations of S+. In some of these early-responding animals, we tried to change the paradigm to responding to the tone alone by presenting the tone without the water wave and rewarding each tone response immediately. These attempts failed completely, and within 6–8 sessions no response to a tone occurred. This demonstrated that concurrent presentation of the tone and the water wave component was essential for maintaining the conditioned behaviour.

When the frequency difference approached the discrimination limen, many animals became restless and sometimes ceased to respond to the stimuli altogether. The frequency difference then had to be increased temporarily to regain correct responses. This was also observed occasionally in tests of hearing threshold.

Hearing threshold

The hearing range of *X. laevis* was 200–4000 Hz (Fig. 2). Frequencies outside this range yielded no response even at the maximal sound intensities possible with the equipment. Three dominant hearing optima were observed at 600 Hz, 1400–1800 Hz and 3200–3600 Hz. Their thresholds were 92–96 dB re 1 μ Pa. There was a high threshold at 1000–1300 Hz and a

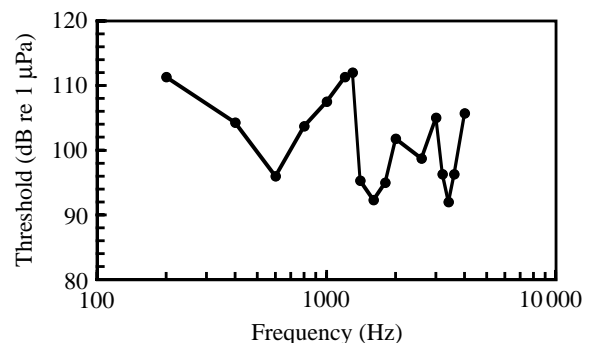


Fig. 2. Hearing threshold of *Xenopus laevis* for pure underwater tones. The hearing range was 200–4000 Hz. There was a sensitivity optimum at 600 Hz (within the range of sensitivity of the amphibian papilla), poor hearing at 1000–1300 Hz (probably delineating the range between the sensitivities of the two types of auditory papilla) and two further optima at 1400–1800 Hz and 3200–3600 Hz. The optimum at 1400–1800 Hz overlaps partially with the main frequency domain of the advertisement call of the species. Underwater sound at 92–96 dB re 1 μ Pa corresponds in power to airborne sound at 30–34 dB SPL. Each point is a mean value for 3–6 individuals (see Table 1). Standard deviations of inter-individual differences were generally less than 3 dB and are therefore not shown.

second, minor threshold elevation at 2600 Hz. The frequency range of the middle optimum corresponds roughly to the lower range of the dominant frequency in the advertisement call of the species, whereas the upper optimum extends to approximately double that frequency. Inter-individual threshold differences were small, and standard deviations at given frequencies were generally less than 3 dB (Table 1). Larger differences were found only at 1400 Hz and 1800 Hz, two frequencies close to a steep change in threshold. No threshold difference was found between sexes.

Frequency discrimination

Conditioning for frequency discrimination demonstrated that *X. laevis* is capable of distinguishing between tones on the basis of frequency. At 1000–1300 Hz, discrimination was poor (Fig. 3). At lower frequencies, the relative discrimination limen was 4.5–6%. Also, between 1600 and 2500 Hz, discrimination was better than 6%, having an optimum of 2.4% at 2000 Hz. That is, 1950 Hz could be distinguished from 2000 Hz. At 3000 Hz, discrimination acuity decreased to 20%. Inter-individual differences were remarkably small, with rare exceptions (Table 2). Often, the limen was quite similar for most animals, except for one specimen with poorer discrimination. No difference was found between sexes.

In three specimens, an additional experiment was performed to determine whether the high sensitivity at 3400 Hz might be due to an artefactual subharmonic at 1700 Hz, so that the animals heard 1700 Hz instead of the 3400 Hz that was presented. The tone presented did not itself contain the subharmonic, so that if the subharmonic was heard it would be an effect of the animal. In the control experiments, the animals were tested for discrimination between 1700 Hz and 3400 Hz. This discrimination was easily made by the frogs, indicating that the two frequencies are heard differently. If the frogs heard

Table 1. Hearing thresholds in *Xenopus laevis* for pure underwater tones

Frequency (Hz)	<i>N</i>	Individual threshold (dB re 1 μ Pa)	Mean threshold (dB re 1 μ Pa)
200	3	110, 110, 114	111.3 \pm 2.3
400	3	104, 104, 105	104.3 \pm 0.6
600	6	93, 93, 93, 96, 100, 101	96.0 \pm 3.7
800	3	103, 104, 104	103.7 \pm 0.6
1000	4	106, 107, 108, 109	107.5 \pm 1.3
1200	4	109, 109, 113, 114	111.3 \pm 2.6
1300	3	111, 112, 113	112.0 \pm 1.0
1400	6	89, 91, 91, 97, 99, 105	95.3 \pm 6.1
1600	3	90, 92, 95	92.3 \pm 2.5
1800	6	88, 91, 91, 96, 101, 103	95.0 \pm 6.0
2000	4	99, 102, 103, 103	101.8 \pm 1.9
2600	3	97, 99, 100	98.7 \pm 1.5
3000	3	103, 104, 108	105.0 \pm 2.6
3200	3	94, 96, 99	96.3 \pm 2.5
3400	3	89, 92, 95	92.0 \pm 3.0
3600	3	96, 96, 97	96.3 \pm 0.6
4000	3	105, 105, 107	105.7 \pm 1.2

Values are means \pm S.D.

N indicates the number of individuals examined.

Inter-individual differences were small; standard deviations greater than 4 dB occurred only at two frequencies close to steep changes in hearing threshold (1400 Hz and 1800 Hz).

a 3400 Hz tone as being 1700 Hz, a discrimination from a tone presented at 1700 Hz should not have occurred.

Discrimination between distant frequencies such as 400 Hz and 2000 Hz involves a discrimination between tones that probably stimulate different sensory epithelia, i.e. the amphibian and the basilar papillae, respectively, whereas

Table 2. Frequency discrimination limens in *Xenopus laevis* for pure underwater tones

Frequency (Hz)	<i>N</i>	Individual threshold (Hz)	Mean threshold (Hz)	Discrimination limen	
				Absolute (Hz)	Relative (%)
400	8	420, 421 (2), 422 (3), 423, 441	424.0 \pm 6.9	24	6.0 \pm 1.7
600	6	575 (3), 572.5 (2), 565	572.5 \pm 3.9	27.5	4.6 \pm 0.6
800	7	760 (3), 755 (3), 745	755.7 \pm 5.3	44.3	5.5 \pm 0.7
1000	4	1250, 1450, 1550 (2)	1450.0 \pm 141.4	450	45.0 \pm 14.1
1300	3	1550, 1560, 1560	1556.7 \pm 5.8	256.7	19.7 \pm 0.4
1400	3	1600, 1612.5, 1625	1612.5 \pm 12.5	212.5	15.2 \pm 0.8
1600	3	1520, 1510, 1510	1513.3 \pm 5.8	86.7	5.5 \pm 0.4
1800	3	1730 (3)	1730.0	70	3.9
2000	4	1960, 1955 (2), 1940	1952.5 \pm 8.7	47.5	2.4 \pm 0.4
2500	4	2360 (4)	2360.0	140	5.6
3000	4	2440, 2425, 2415, 2410	2422.5 \pm 13.2	577.5	19.3 \pm 0.4

Values are means \pm S.D.

N indicates the number of individuals tested, and the numbers in parentheses indicate the number of animals yielding that particular threshold.

Inter-individual differences were remarkably small at most frequencies, often only one animal with poorer discrimination deviating from the general discrimination limen.

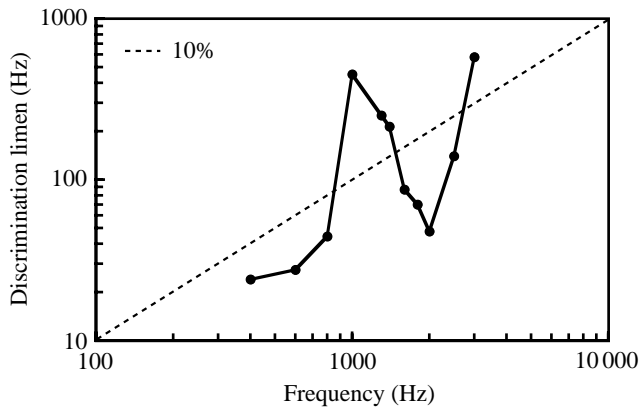


Fig. 3. Frequency discrimination limens of *Xenopus laevis* for underwater sound. Frequency discrimination was found in the range of both the amphibian (range 200–800 Hz) and the basilar (range 1300–4000 Hz) papillae. In the range of the amphibian papilla, the relative discrimination limen was approximately 5%. In the range of the basilar papilla, the relative discrimination limen reached an optimum of 2.4% within the range of the dominant frequency of the advertisement call of the species. For reference, the dashed line indicates a relative discrimination limen of 10%. Each point is a mean for 3–8 individuals (see Table 2). Standard deviations are not shown because they were generally less than 1% of the test frequency.

discrimination between 400 Hz and 600 Hz is a discrimination between tones presumably stimulating the same papilla. Therefore, we examined whether the transition from discrimination of widely separated tones to discrimination of tones that stimulate the same papilla would make relearning difficult. For testing, inexperienced animals that initially learned to discriminate 400 Hz (S+) from 2000 Hz were then each successively trained to discriminate 400 Hz (S+) from 1700 Hz, 1300 Hz, 1100 Hz, 900 Hz, 700 Hz, 600 Hz, 500 Hz and 450 Hz. Tests for each frequency were performed to criterion before the next frequency was used. Each animal learned the discriminations of 400 Hz from 1700 Hz and 1300 Hz within one session, then required two or three sessions for the discrimination of 400 Hz from 1100 Hz and/or 900 Hz, and finally again required only one session for discrimination of 400 Hz from each of the other lower frequencies (Fig. 4). This relearning pattern was found in all five animals examined. Clearly, the transition from discrimination between inputs to the two papillae to discrimination between inputs to the same papilla involved more than just learning a new frequency.

Critical-band testing

The usual method of testing critical bands involves analysing the masking of a pure tone by bandpass noise centred on the tone frequency. Alternatively, one can compare the hearing threshold for bandpass noise with the hearing threshold for a tone at its centre frequency (Gässler, 1954). In our tests, we compared the hearing threshold for a 600 Hz tone with that of a one-third-octave bandpass noise centred on that frequency. Our data on hearing threshold and frequency discrimination

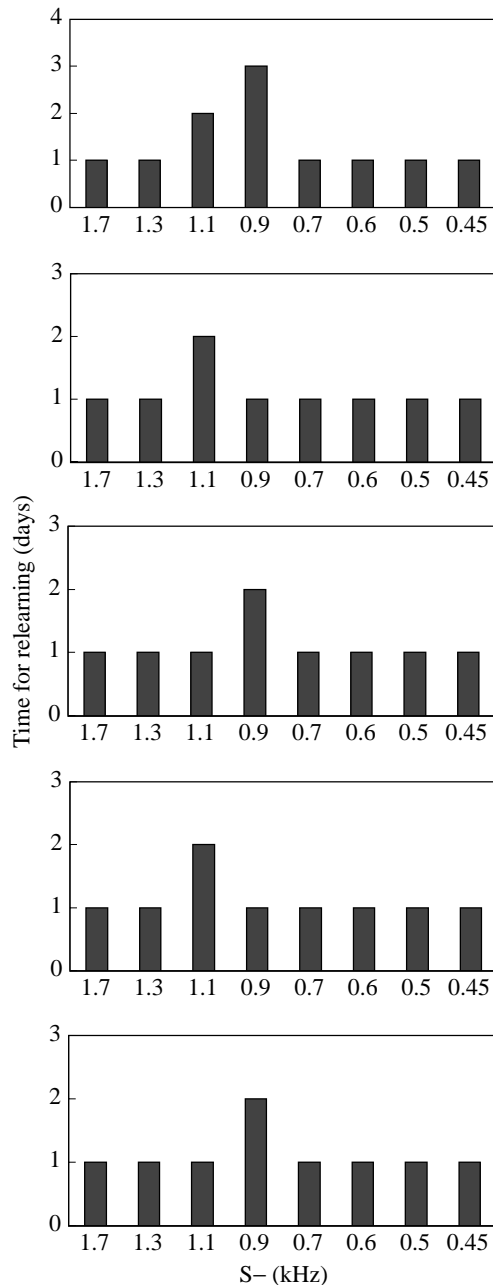


Fig. 4. The duration of discrimination learning in *Xenopus laevis* at several frequencies when the task was shifted from discriminating tones designed to stimulate different auditory papillae to discriminating tones that should both stimulate the same papilla. Data are presented individually for the five animals tested. The animals were initially trained to discriminate 400 Hz from the alternative frequency 2000 Hz. After this discrimination had been learned, the frequency of S- was reduced in a stepwise manner, and discrimination for each frequency was tested to criterion. The sequence of alternative frequencies was 1700 Hz, 1300 Hz, 1100 Hz, 900 Hz, 700 Hz, 600 Hz, 500 Hz and 450 Hz, and the columns show, from left to right, the number of days required to learn each of the new discriminations. In each animal, learning the discrimination of 400 Hz from 1100 Hz and/or 900 Hz took more time than learning the discrimination of 400 Hz from the other frequencies.

suggest that the frequency range of the noise (535–674 Hz) is completely within the frequency range of the amphibian papilla. In four specimens, hearing thresholds were determined for both the pure tone and the bandpass noise. The thresholds were 100.0 ± 6.0 dB re $1 \mu\text{Pa}$ for the pure tone and 72.4 ± 3.8 dB re $1 \mu\text{Pa Hz}^{-1}$ for the bandpass noise (means \pm S.D.). These thresholds indicate a total power difference of only 6.2 dB, which suggests that the effective intensity of the noise was integrated within this band, as would be expected for an organism possessing a critical band.

Discussion

Our data demonstrate the first successful auditory conditioning of a frog and the first direct examination of the limits of hearing sensitivity and frequency discrimination in an amphibian. Our success was presumably at least partly due to the choice of a new, specially adapted, training and testing paradigm. This paradigm made use of a response that can easily be elicited: the orienting response to a water wave, which is made during feeding. The response could be conditioned using a combination of reward and moderate punishment. Auditory conditioning of *X. laevis* turned out to be more difficult than the water wave conditioning that we carried out previously (Elepfandt, 1985). Apparently, associating the combined lateral line and auditory input was more difficult than learning a response to a water wave stimulus alone. Once learned, however, the task was easily generalised by the frog to other frequencies and remembered for many weeks.

In our tests, the water wave stimuli were produced by hand. Therefore, the experimenter might have inadvertently produced different waves when S+ or S- was given, and the animal could possibly have used these cues for discrimination. However, the nearly identical thresholds in several animals, even when trained by different experimenters who could not know the thresholds for the frog, clearly indicate that the animals used the tone as the cue for discrimination.

The range of hearing in *X. laevis*, being 200–4000 Hz, is within the range of that of other frogs (Fay, 1988; Fay and Megela-Simmons, 1999). The threshold curve of *X. laevis* differed markedly from those of non-amphibian vertebrates in that it contained three hearing optima of nearly equal sensitivity. The optimum at 400–800 Hz probably corresponds to the range of the amphibian papilla. This would corroborate the suggestion of Lewis and Lombard (1988) that this papilla should only have fibres with best frequencies up to 800 Hz. The high threshold and poor frequency discrimination at 1000–1300 Hz suggest that this frequency range lies between the optima of the tuning curves of amphibian and basilar papillae. Accordingly, the optima at 1400–1800 Hz and 3200–3600 Hz should represent the sensitivity of fibres from the basilar papilla. To our knowledge, the occurrence of two optima at such distant frequencies has not been reported for the basilar papilla of any other frog species. These two optima were also found, however, in our studies of tympanic motion

(Christensen-Dalsgaard et al., 1990; Christensen-Dalsgaard and Elepfandt, 1995) and of evoked potentials (N. G. Bibikov and A. Elepfandt, unpublished observations) and have therefore now been confirmed in *X. laevis* using three independent methods. The frequencies of best hearing sensitivity agreed with those of tympanic vibrations (Christensen-Dalsgaard et al., 1990; Christensen-Dalsgaard and Elepfandt, 1995), indicating that the spectral positions of the optima are largely determined by the mechanics of tympanic vibration. The central optimum at 1400–1800 Hz overlaps partially with the dominant frequency, 1600–2000 Hz, of the advertisement call.

The pressure threshold at the three optima was 92–96 dB re $1 \mu\text{Pa}$. Underwater sound with this pressure is equal in power to an airborne sound of 30–34 dB SPL (Elepfandt, 1996b). This is within the range of hearing thresholds in terrestrial frogs (Fay, 1988).

Determination of hearing thresholds in *Rana catesbeiana* and *Hyla cinerea* by the reflex modification technique (Megela-Simmons et al., 1985) yielded essentially U-shaped threshold curves with, in *H. cinerea*, a minor second minimum at 3000 Hz. A gap between the ranges of the two auditory papillae was not found. These results, however, showed considerable variability, so it is difficult to decide to what degree the difference from our findings in *X. laevis* reflects an interspecies difference or whether it might be due to limitations of the reflex modification method. In our study, despite the use of several experimental tanks in different laboratories, inter-individual differences were found to be remarkably small, which provides strong support to the form of the hearing threshold in *X. laevis*.

Frequency discrimination was detected within the ranges of both auditory papillae. This is in contrast to the expectations based on electrophysiological recordings (Zakon and Wilczynski, 1988). Those recordings, however, were all made on neobatrachians, so our results may indicate a difference between *X. laevis* and this group of frogs. Shallow water with depths below 1 m acts as an acoustic high-pass filter (Rogers and Cox, 1988). This makes it advantageous for species such as *X. laevis*, which call at the bottom of shallow bodies of water, to have their main power of sound production and sound analysis at higher frequencies. The frequency discrimination in the range of the basilar papilla is in good agreement with neuroanatomical data that indicate an enlarged basilar papilla in *X. laevis*. In *X. laevis*, the number of afferent fibres from the basilar papilla is 65% of the number of fibres from the amphibian papilla; in frogs communicating using sound in air, this percentage is less than 30% (Will and Fritzsche, 1988). Also, the number of afferent fibres per sensory hair cell in *X. laevis* is approximately 5–6 in the basilar papilla compared with only 0.7 in its amphibian papilla (Elepfandt, 1996b; López-Anaya et al., 1997). Thus, anatomy suggests the possibility of a more refined sound analysis in *X. laevis* in the range of the basilar papilla, as was found in our tests.

Frequency discrimination acuity at 400–800 Hz was approximately 5%, which is within the range of frequency

discrimination in otophysan fish (Fay, 1988). Discrimination acuity is considerably higher between 1400 Hz and 2500 Hz. The relative frequency discrimination of 2.4% at 2000 Hz is superior to that in ectothermic vertebrates examined so far (see Fay, 1988). This high acuity may enable discrimination of individual calling males. Between males, the dominant frequency may vary from 1600 to 2200 Hz. In any given male, however, the dominant frequency is very constant: it is not affected by variation in sexual hormone levels and changes by less than $5 \text{ Hz } ^\circ\text{C}^{-1}$ with water temperature (Jansen and Elepfandt, 1992). On the bottom of water bodies, where *X. laevis* calls during the night, the temperature changes little and very slowly (A. Elepfandt, unpublished observations), so that under natural conditions the dominant frequency of the call of a given male is constant relative to the auditory frequency resolution of 50 Hz.

Tests of spontaneous preferences for advertisement calls in terrestrial frogs required frequency differences of 20% or more to elicit a preference (Gerhardt, 1988). Our tests with pure tones demonstrate considerably better frequency discrimination ability. This difference may indicate an interspecies difference or that the frequency criterion for preferences between advertisement calls may be less precise than auditory discrimination of pure tones.

For *X. laevis*, transfer from one frequency discrimination task to the discrimination of new frequencies typically required only one session. However, changes from discrimination of tones that stimulate different auditory papillae to discrimination of tones that stimulate the same papilla always required more time for relearning. This suggests that the inputs from the two types of auditory papilla may be perceived differently. The delay in learning was not long, but previous tests in water wave pattern discrimination have shown that *X. laevis*, once it has learned a task and its generalisation, can learn a new related task within 2–3 days (Elepfandt, 1985). The possibility that stimulus intensities at 900 Hz and 1100 Hz were significantly closer to the hearing threshold and, therefore, that the discrimination of these frequencies from 400 Hz was made on the basis of intensity cannot be excluded. However, the animals had been trained to distinguish between tone frequencies irrespective of their intensity. If the inputs from the basilar and amphibian papillae formed a perceptual auditory continuum, there would be no reason why the animals should give up the familiar frequency discrimination and resort, for just two frequencies, to a new cue for discrimination. The more plausible explanation for the delay in relearning is that discrimination between stimuli that affect different auditory papillae and discrimination between stimuli that affect the same auditory papilla are perceptually different tasks.

We have demonstrated the possible existence of a critical band in *X. laevis* within the sensitivity range of the amphibian papilla. At present, it is unclear whether critical-band processing also exists within the range of the basilar papilla and whether the bandwidth is narrower in the range of best frequency discrimination. Tests using the reflex modification technique in *Rana catesbeiana* and *Hyla cinerea*, two

neobatrachians, have shown a complex relationship between frequency and the width of critical bands (Moss and Megela-Simmons, 1986; Megela-Simmons, 1988a). Our tests suggest that critical-band processing may not be limited to neobatrachians, but might be common among amphibians.

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References

- Arak, A.** (1983). Male–male competition and mate choice in anuran amphibians. In *Mate Choice* (ed. P. Bateson), pp. 181–210. Cambridge: Cambridge University Press.
- Brzoska, J.** (1980). Quantitative studies on the elicitation of the electrodermal response by calls and synthetic acoustical stimuli in *Rana lessonae* Camerano, *Rana r. ridibunda* Pallas and the hybrid *Rana 'esculenta'* L. (Anura, Amphibia). *Behav. Process* **5**, 113–141.
- Brzoska, J., Walkowiak, W. and Schneider, H.** (1977). Acoustic communication in the grass frog (*Rana temporaria temporaria*): calls, auditory thresholds and behavioral responses. *J. Comp. Physiol. A* **118**, 173–186.
- Christensen-Dalsgaard, J., Breithaupt, T. and Elepfandt, A.** (1990). Underwater hearing in the clawed frog, *Xenopus laevis*: Tympanic motion studied with laser vibrometry. *Naturwissenschaften* **77**, 135–137.
- Christensen-Dalsgaard, J. and Elepfandt, A.** (1995). Biophysics of underwater hearing in the clawed frog, *Xenopus laevis*. *J. Comp. Physiol. A* **176**, 317–324.
- Davis, M. E.** (1988). Acoustically mediated neighbor recognition in the North American bullfrog, *Rana catesbeiana*. *Behav. Ecol. Sociobiol.* **21**, 185–190.
- Elepfandt, A.** (1985). Naturalistic conditioning reveals good learning in a frog (*Xenopus laevis*). *Naturwissenschaften* **72**, 492–493.
- Elepfandt, A.** (1996a). Sensory perception and the lateral line system in the clawed frog, *Xenopus*. In *The Biology of Xenopus* (ed. R. C. Tinsley and H. R. Kobel), pp. 97–120. Oxford: Clarendon Press.
- Elepfandt, A.** (1996b). Underwater acoustics and hearing in the clawed frog, *Xenopus*. In *The Biology of Xenopus* (ed. R. C. Tinsley and H. R. Kobel), pp. 177–193. Oxford: Clarendon Press.
- Elepfandt, A., Fleig, A., Hainich, M. and Traub, B.** (1989a). Good tone–frequency discrimination in a frog (*Xenopus laevis*, Pipidae). In *Dynamics and Plasticity in Neuronal Systems* (ed. N. Elsner and W. Singer), p. 275. Stuttgart: Thieme Verlag.
- Elepfandt, A., Fleig, A., Hainich, M. and Traub, B.** (1989b). Frequency discrimination in frog auditory system. *Eur. J. Neurosci. (Suppl.)* **2**, 259.
- Elepfandt, A. and Günther, E.** (1986). Bestimmung der Hörschwelle des Krallenfrosches (*Xenopus laevis* Daudin) durch Verhaltenstests. In *Sensomotorik identifizierte Neurone* (ed. N. Elsner and W. Rathmayer), p. 216. Stuttgart: Thieme Verlag.
- Elepfandt, A. and Hainich, M.** (1988a). Determination of low-frequency tone discrimination in the clawed frog, *Xenopus laevis*, by conditioning. *Eur. J. Neurosci. (Suppl.)* **1**, 163.

- Elepfandt, A. and Hainich, M.** (1988b). Low-frequency tone discrimination in the clawed frog, *Xenopus laevis*. *Soc. Neurosci. Abstr.* **14**, 1098.
- Elepfandt, A., Seiler, B. and Aicher, B.** (1985). Water wave frequency discrimination in the clawed frog, *Xenopus laevis*. *J. Comp. Physiol. A* **157**, 255–261.
- Fay, R. R.** (1988). *Hearing in Vertebrates: A Psychophysics Databook*. Winnetka, IL: Hill-Fay Associates.
- Fay, R. R. and Megela-Simmons, A. M.** (1999). The sense of hearing in fishes and amphibians. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 269–318. New York: Springer.
- Fellows, B. J.** (1967). Chance stimulus sequences for discrimination tasks. *Psychol. Bull.* **67**, 87–92.
- Gässler, G.** (1954). Über die Hörschwelle für Schallereignisse mit verschieden breitem Frequenzspektrum. *Acustica* **4**, 408–414.
- Gerhardt, H. C.** (1988). Acoustic properties used in call recognition by frogs and toads. In *The Evolution of the Amphibian Auditory System* (ed. B. Fritzsche, M. J. Ryan, W. Wilczynski and W. Walkowiak), pp. 455–483. New York: John Wiley & Sons.
- Gerhardt, H. C.** (1994). The evolution of vocalization in frogs and toads. *Annu. Rev. Ecol. Syst.* **25**, 293–324.
- Günther, E., Traub, B. and Elepfandt, A.** (1987). Determination of auditory and frequency discrimination thresholds in a frog, *Xenopus laevis*, by conditioning. *Soc. Neurosci. Abstr.* **13**, 321.
- Halliday, T. R. and Tejedo, M.** (1995). Intrasexual selection and alternative mating behaviour. In *Amphibian Biology*, vol. 2 (ed. H. Heatwole and B. K. Sullivan), pp. 419–468. Chipping Norton: Surrey Beatty & Sons.
- Hetherington, T. E. and Lombard, R. E.** (1982). Biophysics of underwater hearing in anuran amphibians. *J. Exp. Biol.* **98**, 49–66.
- Jansen, S. and Elepfandt, A.** (1992). Dominant frequency of mating call: A possible cue for interindividual discrimination in the clawed frog, *Xenopus laevis*. In *Rhythmogenesis in Neurons and Networks* (ed. N. Elsner and D. Richter), p. 200. Stuttgart: Thieme Verlag.
- Kobel, H. R., Loumont, C. and Tinsley, R. C.** (1996). The extant species. In *The Biology of Xenopus* (ed. R. C. Tinsley and H. R. Kobel), pp. 9–33. Oxford: Clarendon Press.
- Kroese, A. B. A., van der Zalm, J. M. and van den Bercken, J.** (1978). Frequency response of the lateral line organ of *Xenopus laevis*. *Pflügers Arch.* **375**, 167–175.
- Lewis, E. R. and Lombard, R. E.** (1988). The amphibian inner ear. In *The Evolution of the Amphibian Auditory System* (ed. B. Fritzsche, M. J. Ryan, W. Wilczynski and W. Walkowiak), pp. 93–123. New York: John Wiley & Sons.
- Lewis, E. R. and Narins, P. M.** (1998). The acoustic periphery of amphibians; anatomy and physiology. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 101–154. New York: Springer.
- López-Anaya, V. L., López-Maldonado, D. and Serrano, E. E.** (1997). Development of the *Xenopus laevis* cranial nerve: increase in number and area of axons of the saccular and papillar branches. *J. Morph.* **234**, 263–276.
- Macphail, E. M.** (1982). *Brain and Intelligence in Vertebrates*. Oxford: Clarendon.
- Megela-Simmons, A.** (1988a). Masking patterns in the bullfrog (*Rana catesbeiana*). I. Behavioral effects. *J. Acoust. Soc. Am.* **83**, 1087–1093.
- Megela-Simmons, A.** (1988b). Selectivity for harmonic structure for complex sounds by the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.* **162**, 397–403.
- Megela-Simmons, A., Moss, C. F. and Daniel, K. M.** (1985). Behavioral audiograms of the bullfrog (*Rana catesbeiana*) and the green treefrog (*Hyla cinerea*). *J. Acoust. Soc. Am.* **78**, 1236–1244.
- Moss, C. F. and Megela-Simmons, A.** (1986). Frequency selectivity of hearing in the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.* **159**, 257–266.
- Rand, A. S.** (1988). An overview of anuran acoustic communication. In *The Evolution of the Amphibian Auditory System* (ed. B. Fritzsche, M. J. Ryan, W. Wilczynski and W. Walkowiak), pp. 415–431. New York: John Wiley & Sons.
- Rogers, P. H. and Cox, M.** (1988). Underwater sound as a biological stimulus. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 131–149. New York: Springer-Verlag.
- Sullivan, B. K., Ryan, M. J. and Verrel, P. A.** (1995). Female choice and mating system structure. In *Amphibian Biology*, vol. 2 (ed. H. Heatwole and B. K. Sullivan), pp. 469–517. Chipping Norton: Surrey Beatty & Sons.
- Traub, B. and Elepfandt, A.** (1987). Konditionierung auf Tondiskrimination beim Krallenfrosch (*Xenopus laevis* Daudin). In *New Frontiers in Brain Research* (ed. N. Elsner and O. D. Creutzfeldt), p. 93. Stuttgart: Thieme Verlag.
- Wald, A.** (1945). Sequential tests of statistical hypotheses. *Ann. Math. Statist.* **16**, 117–186.
- Wells, K. D.** (1977). The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666–693.
- Will, U. and Fritzsche, B.** (1988). The eighth nerve of amphibians: peripheral and central distributions. In *The Evolution of the Amphibian Auditory System* (ed. B. Fritzsche, M. J. Ryan, W. Wilczynski and W. Walkowiak), pp. 159–183. New York: John Wiley & Sons.
- Zakon, H. and Wilczynski, W.** (1988). The physiology of the anuran eighth nerve. In *The Evolution of the Amphibian Auditory System* (ed. B. Fritzsche, M. J. Ryan, W. Wilczynski and W. Walkowiak), pp. 125–155. New York: John Wiley & Sons.
- Zwicker, E., Flottorp, G. and Stevens, S. S.** (1957). Critical bandwidth in loudness summation. *J. Acoust. Soc. Am.* **29**, 548–557.