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The Audiogram of the Goldfish Determined by a Heart Rate Conditioned Method

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Abstract

The audiogram of the goldfish was determined by using a change of the conditioned heart rate in a special test tank designed to minimize the near-field effect. A stable acoustic condition was obtained by suspending the fish in a small cage at a fixed position in the test tank. The sensitive frequencies ranged widely from 70 Hz to about 4,600 Hz, showing the best frequency around 600 Hz where the mean threshold value was -45.6 dB. A gradual rise below 600 Hz and a relatively sharp turn above 800 Hz were indicated in the audiogram. The method presented here seemed to be useful for a rapid determination of the audiogram of fishes.

Introduction

The audiogram furnishes fundamental data for understanding the hearing ability of an animal. The determination of the audiogram in the goldfish has been attempted by means of various conditioning methods by several investigators 1–6). They used different experimental procedures under different acoustic conditions of test tanks of their own devices. Avoidance behaviors1,3,6), the suppression of respiratory movements5), the feeding behavior2), and a change of the heart rate4) were adopted as conditioned responses.

The classical conditioning of the heart rate in combination with an electric shock as an unconditioned stimulus – the method described by Buerkle13) and Offutt4) – seems to be particularly useful in determining the audiogram because it is easy to condition experimental fish. This method also has the advantage of avoiding the effect of sound intensity variation due to a shift of the fish position in a test tank; the experiment can be conducted with a fish kept in a small cage fixed at a specific position. The sound field in the test tank used by Offutt4) was presumably influenced by the near-field effect, because the underwater projector was set 15 cm ahead of the fish where a considerable displacement of water particles must have been produced.

In the present study, an attempt was made to determine the audiogram of the goldfish by a heart rate conditioning method using a specially designed test tank in which the near-field effect was expected to be almost eliminated.

Material and Method

A total of 13 goldfish (*Carassius auratus*) of about 5 cm in body length were

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used as material. The experiment succeeded for 5 out of the total. Each fish was anesthetized with a 0.05% solution of MS 222 (Sandoz), and an enamel coated, copper wire electrode of 0.2 mm thick was implanted near the heart.

The test cistern was a hemisphere basket of iron mesh, 11 cm in diameter, covered with a thin rubber sheet (Fig. 1). The basket was hung by a string at the middle point of two loudspeakers facing each other at a distance of 55 cm in an experimental tank in which fine glass fibers were fixed. The experimental tank was lined inside with an iron mesh shield to intercept electrical noises. The whole tank was shaded to avoid light stimuli. An experimental fish was kept in a small cage of nylon mesh suspended at the center of the rubber coated basket and water was circulated through inlet and outlet tubes at the rate of 70 ml/min.

Sinusoidal waves at duration of 8–15 sec were given as sound stimuli. The rise time of the sound was 10 msec. No transient noise appeared at the start of the stimulus. The sound pressure (0 dB=1 μBar) was measured by a hydrophone set at the fish position. The sound pressure at lower intensities could not directly be measured because of a high level of electrical noise in the hydrophone. In that case it was estimated by using a linear relationship between the sound intensity and the out-put voltage of a power amplifier.

Cardiac potentials introduced by the implanted electrode were amplified by a pre-amplifier, filtered through a high pass filter, and displayed on an oscilloscope (VC–8, Nihon Kohden) (Fig. 2).

The training of the fish for conditioning with the pure tone of a selected frequency was initiated at the intensity of 0 dB or more. At the end of each tone, the fish was given an electric shock which brought about a body convulsion. The sound combined with the electric shock was repeated at 1–5 min intervals. When a delay or lack in one or more heart beats appeared while the sound was lasting (*i.e.* prior to the electric shock), the response was judged to be the positive conditioned response (Fig. 3). On decision of the alteration of the cardiac rhythm, the

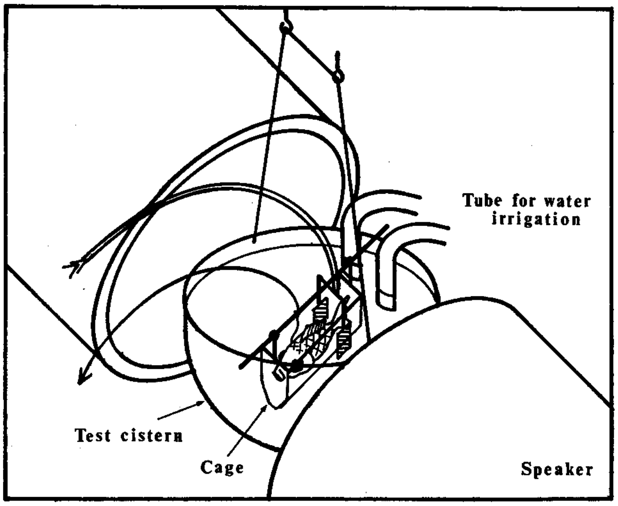

Fig. 1. A representation of the test tank.

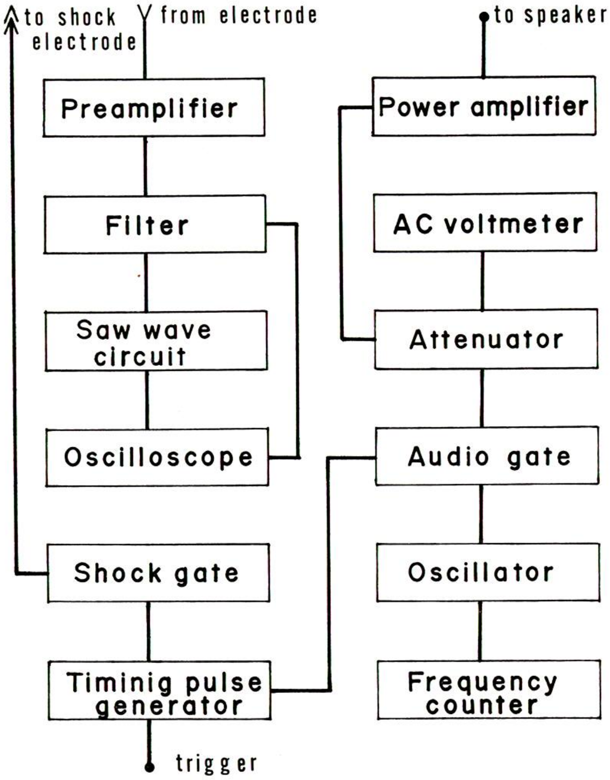

Fig. 2. Block diagram of the electric equipments.

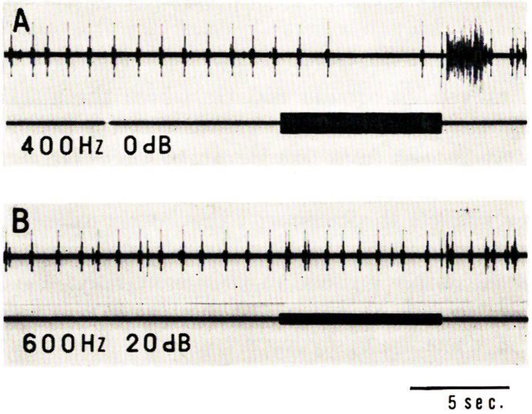

Fig. 3. Photographs of cardiac responses (upper trace) with sound stimulus (lower trace).
A: Positive responses showing a considerable delay of heart beats during the sound,
B: Negative response.

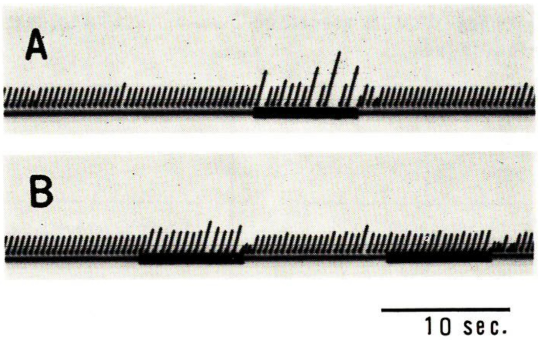

Fig. 4. A display of pulse intervals as a change in height of the spot on the oscilloscope, which is indicated by the length of an oblique line on the photograph. A: A display of the positive response. B: A display of the negative response.

intervals of the heart beats were transformed to linear voltage changes and displayed on the oscilloscope. When voltage raised above a standard level which represented the heart rate at 30–60 sec before the onset of the tone, the response was decided to be a positive response (Fig. 4). Once a distinct positive response was observed, the intensity of the sound was lowered by 5 dB. In each positive response attained this procedure was repeated until the negative response indicated by no change in the heart rate was observed. Then, the intensity was raised by 5 dB again. In this way, the up and down courses were carried out usually more than ten times. The threshold for a given frequency was determined as the average of sound pressures where the positive responses were obviously observed.

Results and Discussion

The individual and the mean thresholds for frequencies from 70 to 4,600 Hz with 5 goldfish are given in Table 1. The individual audiogram with each subject seems to represent a basically similar pattern and the audiogram obtained by plotting the average values is shown in Fig. 5. The best frequency, the most sensitive frequency range, lies around 600 Hz where the mean threshold is –45.6 dB. Below 600 Hz, the thresholds rise gradually. On the contrary, a relatively sharp rise of the curve is seen from 600 Hz up to 4,600 Hz.

The underwater sound has two kinds of physical characteristics, the pressure wave and the water particle displacement; the latter, called the near-field effect, grows up in a short distance from the sound source at lower frequencies[7,8)]. It has been assumed that the water particle displacement may efficiently stimulate the auditory receptors of the fish as well as do the pressure waves[2,9–11)]. The sound pressure can easily be measured by hydrophone, but the water particle displacement cannot be measured so easily. Therefore, it is desirable to conduct the study under an acoustic condition in which the near-field effect is eliminated as much as possible. One of the methods of eliminating the near-field effect in a small tank is to produce the sound in air[12)]. In the present study, an experimental fish kept in a small cistern was given sound stimuli projected from two loudspeakers placed in air at

Table 1. *Auditory thresholds in five goldfish.*

Frequency (Hz)	Threshold (dB/1 μbar)					Mean threshold (dB/1 μbar)	Standard deviation
	S–1	S–2	S–3	S–4	S–5		
70	−24.8	—	−29.4	−20.0	−24.0	−24.6	3.9
200	—	−23.9	−39.4	−38.6	−28.6	−32.6	7.6
400	—	−50.0	−40.5	−41.3	−33.1	−41.2	6.9
600	—	—	−47.3	−42.2	−47.0	−45.6	2.9
800	−33.5	−41.3	—	—	−39.4	−38.1	4.1
1000	−17.2	−6.7	−28.8	−16.4	−29.4	−19.7	9.5
2500	—	—	−7.5	+14.0	+7.5	+4.7	11.0
3000	−1.5	+6.2	—	—	—	+2.4	5.5
4600	+28.1	+28.3	+25.8	—	+30.0	+28.1	1.7

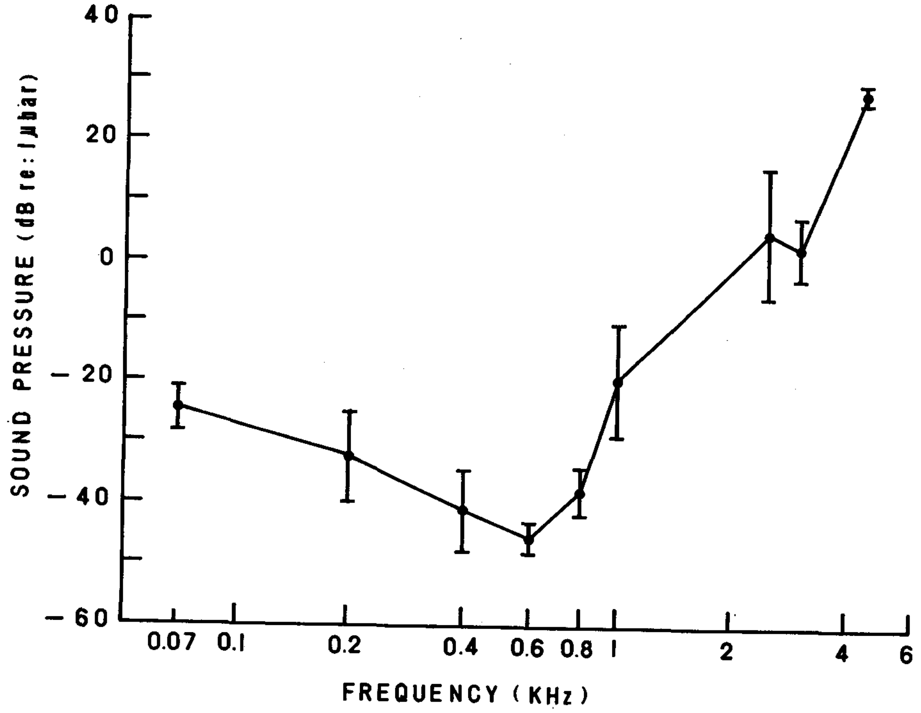

Fig. 5. Audiogram for the goldfish based on the mean values of the thresholds in Table 1. The bar length represents standard deviation about the mean.

both sides of the cistern. In this way, a room above the cistern could be utilized for neurophysiological procedures including the manipulation of the apparatuses and the handling and observation of the experimental fish. The acoustic impedance of a rubber sheet is close to that of water. Therefore, the water in the cistern enclosed by the thin rubber sheet is acoustically considered to be in direct contact with air. Even if a local displacement of water could occur at the surface of the rubber wall,

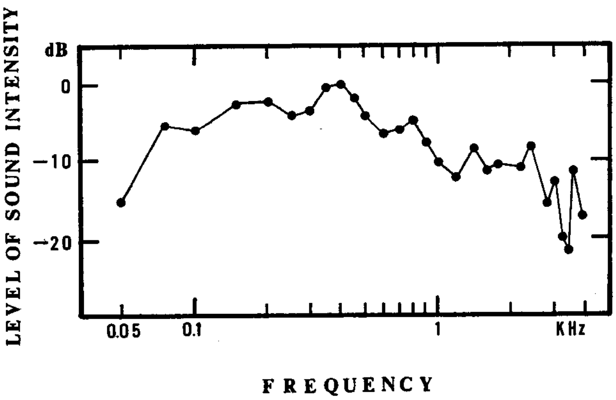

Fig. 6. Sound intensity-frequency relationship for the test tank under a constant input voltage at the power amplifier.

it must be conceivably cancelled at the center of the cistern because the two loudspeakers are driven at the identical phase. The frequency characteristic of the test tank is shown in Fig. 6. The sound pressure variation from 70 Hz to 800 Hz ranged as less than 10 dB. Above 800 Hz, however, a whole decline of the intensity was found showing that considerable peaks and dips may have been caused by a phase difference of waves at such higher frequencies. Below 70 Hz, the intensity fell down by the performance of the loudspeakers.

Figure 7 shows audiograms determined by the behavioral conditioning methods by previous investigators in comparison with the present study. Electric shocks were used as unconditioned stimuli by those workers except Enger[2)] who adopted the feeding. As conditioned responses, avoidance behaviors (curves A[1)], D[6)], and G[3)]), the suppression of respiration (curve E[5)]), and the delay of heart rate (curve C[4)] and the present study) were respectively observed. As to the acoustic conditions, the test tanks used by Weiss[1)] and Offutt[4)] are supposed to have emitted a considerable displacement of water particles, while the other workers essencially avoided the near-field effects by producing the sound from loudspeakers it air. Some differences in sensitivity or in pattern may be found among the audiograms. But a tendency showing a gradual rise of thresholds in the lower frequency range is noticed in most curves except that of Weiss[1)] which shows a steep slope below 200 Hz. However, the degree and the turning point are different. The gradual rise of the threshold in the curve C by Offutt[4)] is smilar in pattern to the present curve particularly below 200 Hz, although the sound field in his test tank at lower frequencies must have been different from that in mine. The sharp turn below 200 Hz in the Weiss' curve is contrary to that expected under the near-field acoustic condition. It is puzzling to see that the audiogram could not be more influenced by the near-field effect.

The ambient noise has an important effect on the determination of threshold values depending on the total and spectrum levels of the noise[3,13-15)]. In other words, the audiogram may be partly determined by the total level and frequency components of the ambient noise. Although the measurement of the level and spectrum of the ambient noise in the water was not possible in this study, it may

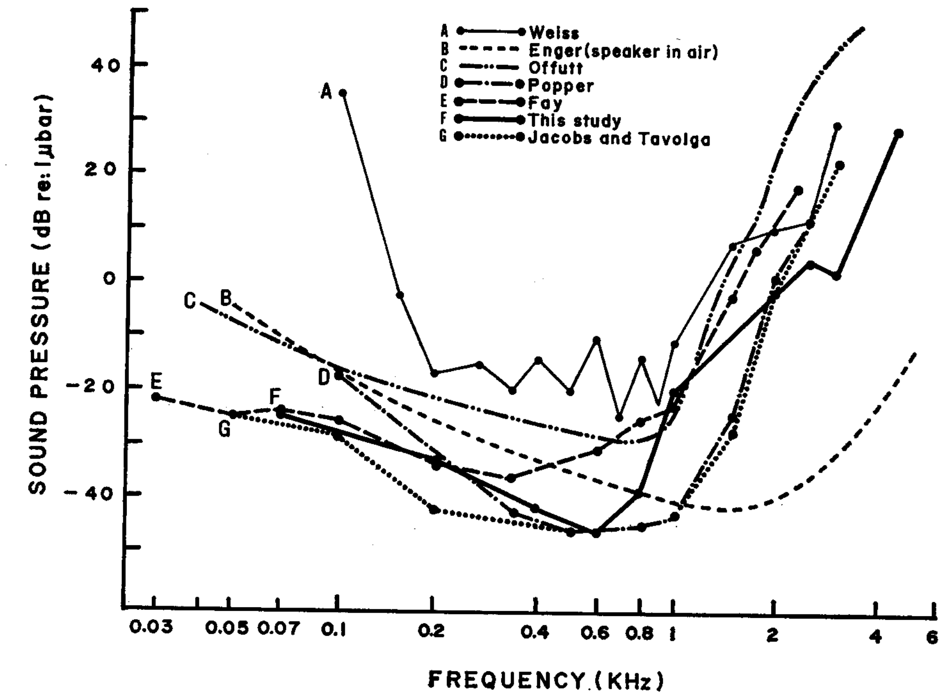

Fig. 7. Comparison of audiograms for the goldfish determined by the present study and previous investigations.

conceivably have consisted of relatively low frequency components since the main sources were a pump for the aeration and a motor for the water circulation. At least, a part of the gradual rise in thresholds below 600 Hz may have been affected by the ambient noise. The present audiogram does not show such a sharp rise at more than 1,000 Hz as compared with those of Offutt[4], Popper[6], Fay[5], and Jacobs and Tavolga[3]. All of the previous curves, except Enger's, seem to show the upper limit of hearing to be approximately 3,000 Hz. The sensitivity limit in the present curve, however, extends even over 4,600 Hz. It is not possible to specify a factor or any factors which caused this difference in the high frequency range where the ambient noise must have exerted little influence on the curve.

The threshold value at the best frequency determined by the heart rate conditioning by Offutt[4] reads about -28 dB on the graph. This is nearly 18 dB higher than those obtained by Jacobs and Tavolga[3] and Popper[6] who adopted the avoidance conditioning method. In the present study, the value was -45.6 dB that is close to those of the latter investigators. Therefore, the high threshold value by Offutt should not be attributed to the experimental method but to some other factors. The method of heart rate conditioning has been used by Chapman[15], and Chapman and Sand[16] in field studies of some marine fishes. This method seems to be available to observe responses to sound when the observer is far away from the fish. Avoidance behaviors have been generally used in laboratory studies of hearing in fishes, but the heart rate seems to be a more useful parameter as conditioned response.

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References

1) Weiss, B.A. (1966). Auditory sensitivity in the goldfish. *J. Aud. Res.* **6**, 321–335.
2) Enger, P.S. (1966). Acoustic threshold in goldfish and its relation to the sound source distance. *Comp. Biochem. Physiol.* **18**, 859–868.
3) Jacobs, D.W. and Tavolga, W.N. (1967). Acoustic intensity limens in the goldfish. *Animal Behavior* **15**, 324–335.
4) Offutt, G.C. (1968). Auditory response in the goldfish. *J. Aud. Res.* **8**, 391–400.
5) Fay, R.R. (1969). Behavioral audiogram for the goldfish. *J. Aud. Res.* **9**, 112–121.
6) Popper, A.N. (1971). The effects of size on auditory capacities of the goldfish. *J. Aud. Res.* **11**, 239–247.
7) Harris, G.G. and Bergeijk, W.A. van (1962). Evidence that the lateral-line organ responds to near-field displacements of sound sources in water. *J. Acoust. Soc. Am.* **34**, 1831–1841.
8) Harris, G.G. (1964). Considerations on the physics of sound production by fishes. p. 233–247. In Tavolga, W.N. (ed.), *Marine Bio-Acoustics*, Vol. 1. 413p. Pergamon Press, Oxford.
9) Enger, P.S. (1967). Effect of the acoustic near-filed on the sound threshold in fishes. p. 239–247. In Chan, P.H. (ed.). *Lateral Line Detectors.* 496 p. Indiana University Press, Blomington Indiana.
10) Chan, P.H., Siler, W. and Wodinsky, J. (1969). Acoustico-lateralis system of fishes: test of pressure and particle velocity in grunts, *Haemulon sciurus* and *Haemulon parrai*. *J. Acoust. Soc. Am.* **46**, 1572–1578.
11) Chapman, C.J. and Sand, O. (1974). Field studies of hearing in two species of flatfish. *Comp. Biochem. Physiol.* **47**, 371–385.
12) Parvulescu, A. (1967). Acoustics of small tanks. p. 7–13. In Tavolga, W.N. (ed.), *Marine Bio-Acoustics*, Vol. 2. 353 p. Pergamon Press, Oxford.
13) Buerkle, U. (1967). An audiogram of the Atlantic cod, *Gadus morhua* L. *J. Fish Res. Bd. Canada* **24**, 2309–2319.
14) Sand, O. (1971). An electrophysiological study of auditory masking of clicks in goldfish. *Comp. Biochem. Physiol.* **40**, 1043–1053.
15) Chapman, C.J. (1973). Field studies of hearing in teleost fish. *Helgoländer Meeresunters* **24**, 371–390.