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AUDITORY DISCRIMINATION BETWEEN COMPRESSIONS AND RAREFACTIONS BY GOLDFISH

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INTRODUCTION

The aim of this paper is to describe a special kind of auditory processing which is exhibited by teleost fish. Conditioned fish are shown to be capable of distinguishing between a given click and the same click inverted – that is with the compression and rarefaction phases reversed. Man cannot perform this discrimination.

In a previous paper it was suggested that phase analysis of the sounds made by other fish when swimming could be used by listening fish to discriminate between approach and recession (Piddington, 1971*b*). The present experiments indicate that, for a given tail flip, the first deflexion in the sound wave propagated backwards from a swimming fish is a compression, but is a rarefaction for the wave propagated forwards. Thus for a listening fish, an initial rarefaction would mean that the fish which flipped is approaching, whereas a compression would mean that it is receding.

Fish have been shown to be capable of real hearing (van Bergeijk, 1967) and to have a behavioural threshold comparable to that of man (0·001 Å water displacement (Enger, 1966); 10^{-16} W/cm^2 energy flux (Griffin, 1955)). However, relative to mammalian or amphibian auditory systems, that of the fish is not a good pitch-analysing system (Kleerekoper & Changnon, 1954; Enger, 1963).

The auditory and lateral-line systems in fish are excited by radically different types of water movement (e.g. see van Bergeijk, 1967; Piddington, 1971*a*). When an object moves through or vibrates in a fluid medium it creates both local hydrodynamic displacements and propagated pressure waves, designated by van Bergeijk (1967) as near-field and far-field sound respectively. The near-field falls off with distance more rapidly than the far-field. The lateral-line system is sensitive only to near-field displacements (Harris & van Bergeijk, 1962; van Bergeijk, 1964, 1967), but the ear (or sacculus) is sensitive to both fields (Enger, 1966, 1968). To stimulate only the ear but not the lateral-line requires, therefore, a far-field (with minimum displacement) and this can be achieved by using a loudspeaker in air rather than underwater (Enger, 1966; van Bergeijk, 1967; cf. Harris & van Bergeijk, 1962). This is the method adopted for the present study. Lateral-line units do not respond to clicks in air but auditory units do (Piddington, 1971*a*).

Recent reviews on the evolution of the acoustico-lateralis system have described the lateral line as a directional system for accurately locating nearby moving or vibrating

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objects, such as other organisms, and also for detecting water currents (e.g. Dijkgraaf, 1960; van Bergeijk, 1967). However, such reviews have not designated a specific function to the auditory system itself. The auditory system cannot locate sound sources in space (azimuth) (von Frisch & Dijkgraaf, 1935; van Bergeijk, 1964) and, except for species which have developed special sonic mechanisms for communication (e.g. Tavolga, 1964), the specific reason for its existence has appeared obscure.

Physiological evidence that fish have specific sensitivity to both compressions and rarefactions has existed for over 20 years (double-frequency microphonic, Zotterman, 1943) and the morphological basis is the organization of the auditory hair-cell receptors into two oppositely oriented populations (Hama, 1969; see also Lindeman, 1969, and review by Grinnell, 1969). Single nerve fibres from the auditory organ (sacculus) fire on the compression, rarefaction or both phases of a sound (Furukawa & Ishii, 1967*a*, *b*; P. S. Enger, personal communication), whereas in mammals, with only one orientation of hair cells, the auditory fibres fire only on the rarefaction (see review by Grinnell, 1969).

All of seven species of teleosts so far examined physiologically have exhibited separate coding for both sound phases, and these species include two freshwater specialized forms (with Weberian ossicles) (Furukawa & Ishii, 1967*a*, *b*; Groezinger, 1967; Hama, 1969), two freshwater unspecialized forms (Zotterman, 1943), two marine unspecialized forms (Enger & Andersen, 1967) and one marine form which produces special sounds for communication (Cohen & Winn, 1967). The separate coding of compressions and rarefactions thus appears to be a general physiological property of all teleost auditory systems.

This paper presents behavioural evidence of adaptive discrimination between compressions and rarefactions and attempts to link this receptive property to the production of compressions and rarefactions by flips of a fish's tail.

METHODS

Conditioning experiments

Nine goldfish (*Carassius auratus*, 10–15 g wt.) were maintained, conditioned and tested in a flat glass aquarium, 60 × 53 × 25 cm, which was shock-mounted on seven tennis balls and filled with water to a depth of 19 cm. The peak-to-peak noise level varied between 2 and 10 μbar.

High-frequency clicks (about 500 Hz, Fig. 1) were made by driving a 16 cm loudspeaker (Calrad S518) with 0·7 msec square pulses. The loudspeaker was held horizontally in the centre of a wooden baffle (66 × 53 × 1·5 cm), positioned 30 cm above the top of the aquarium, the bottom of which was covered with a 5 cm layer of acoustic-absorbing rubberized horse hair. The sound pressure was maximal in the top centre of the aquarium and fell off to a minimum (27 % max.) at the bottom corners. A hydrophone was routinely positioned at mid-water, 18 cm to the left of the centre, at the 75 % of maximum sound level. All values quoted are from this standard position.

For the production of low-frequency clicks (about 50 Hz, Fig. 1) a closed system was necessary. For this, the baffle was lowered to rest on the top of the aquarium and the horse hair was removed. The baffle had a 5 mm layer of silicone rubber around the edge which made a snug contact with the aquarium top. There was a 6·5 cm air space

between the baffle and water surface; small holes drilled in the baffle for the admittance of hydrophone and electrode wires and for the addition of food did not affect the click waveform (compare design by Harris in van Bergeijk, 1967). The speaker was driven by 13 msec square pulses, rounded off by an RC circuit of 100 msec time constant, and amplified ten times by a Krohn-Hite wide-band amplifier. It was necessary to put a 50 Ω resistor in series with the loudspeaker (8 Ω) to cut down on noise originating

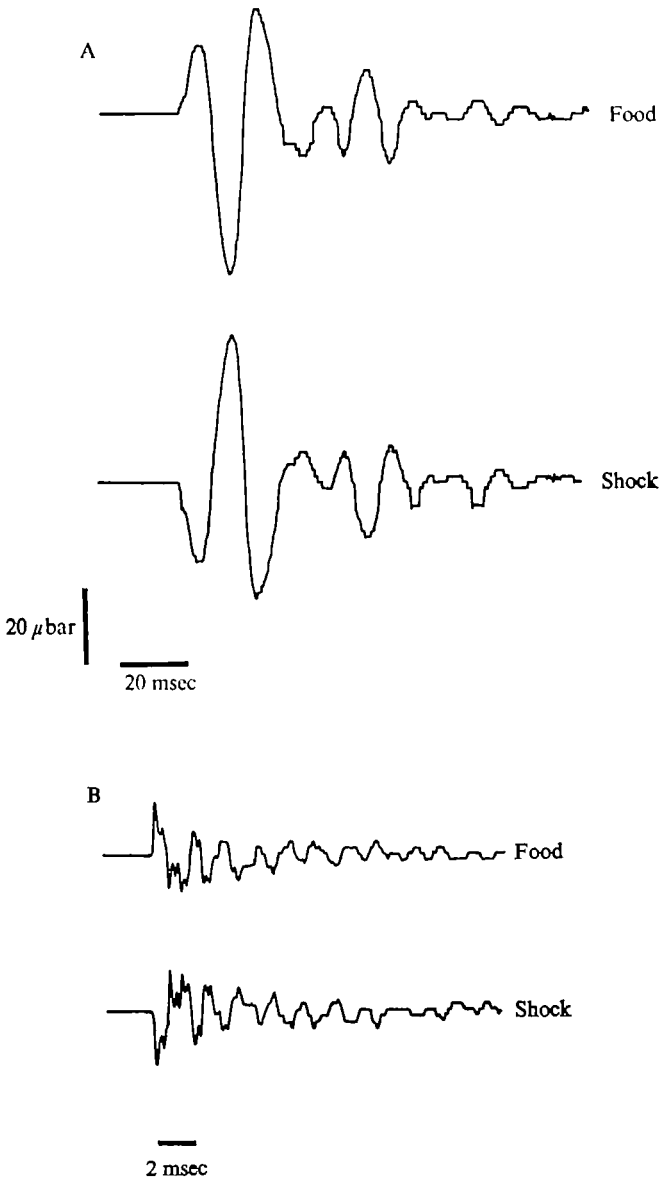

Fig. 1. Waveforms used to condition fish. The compression-first signal was reinforced with food and rarefaction-first with shocks. Note that the one signal is merely the inverse of the other. Small differences in waveform are due to noise, are not repeatable, and disappear with further averaging. A, low-frequency (*c.* 50 Hz) click; B, high-frequency (*c.* 500 Hz) click. Each trace is the average of 64 records: compression up, rarefaction down on this and following figures.

from the output of the amplifier. The sound pressure was maximal at the bottom centre of the aquarium and fell off to a minimum (47% of max.) at the top centre (cf. open system). The level was 66% of maximum at the standard hydrophone position.

The click polarity was inverted by means of a double-pole double-throw switch inserted between amplifier and loudspeaker. This method provided perfect inversion with no unwanted changes in waveform (Fig. 2).

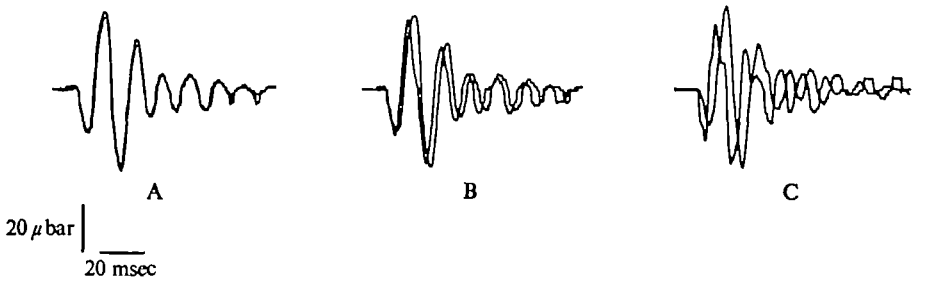

Fig. 2. Controls which show that phase is the important parameter. A, two superimposed traces, one rarefaction-first and the other compression-first but with oscilloscope input reversed as well (see text). Note reliability of click inversion; small differences not repeatable. B, C, also two traces, one trace same as in A (rarefaction-first), the other of altered rise-time and waveform (but still rarefaction-first). Fish responded correctly to both novel waveforms (B, C) when presented in either polarity; the only parameter consistent with behaviour was phase, i.e. the order of compressions and rarefactions (see text).

Recordings of click waveforms were made with a calibrated pressure-sensitive hydrophone (Clevite CH-17), which has a frequency range of from less than 1 Hz to 150 KHz. The hydrophone signal was directly amplified AC and displayed on a Tektronix 502 A oscilloscope (no pre-amplifying or filtering was used). The amplified signals from the oscilloscope were stored in a digital oscilloscope (Northern Scientific 544) and written out on an x-y plotter.

During conditioning sessions clicks of a given polarity were followed by positive reinforcement (food) and clicks of the inverse polarity by negative reinforcement (shocks). Ten clicks at 2/sec were given for each trial and reinforcement was given 1 sec after the last click. Food (in the form of live brine shrimp) was given at the right front corner of the tank (from the observer's point of view) and shocks were delivered via electrodes positioned in the same corner of the tank. The electrodes consisted of two tinned copper wires of 1·5 mm diameter dangling vertically to the bottom, 8 cm apart, each one equidistant from the corner of the tank and each 5 cm from the nearest wall.

Shocks were delivered in a 1·5 sec burst at 90/sec via the isolation transformer of a Grass S8 stimulator. The dial settings were 150 V and 1 msec; the actual voltage or current produced in the water was not measured. This shock strength, though not damaging, cleared all fish away from the corner whilst not affecting fish which were more than 15 cm from the wires.

During testing sessions click polarities were changed in accord with a predetermined pseudo-random order and trials were given in blocks of about 30 at four trials a minute. The blocks were separated by rest periods.

For testing single fish the right-front (or 'test') corner was defined as the cubic region bounded by two vertical lines, each drawn 15 cm from the corner on the front

(60 × 25 cm) and right (53 × 25 cm) plates of the aquarium. (The right plate was viewed with a mirror.) The fish scored a correct response to the food-reinforced signal if it was found in the corner during the second following the last click of the stimulus (that is in the second preceding reinforcement). The fish scored an incorrect response if it was out of the corner, and in this case no food was given. These designations were exactly the opposite for the shock-reinforced stimulus; the animal received a shock if it gave an incorrect response (remained in or swam to the test corner).

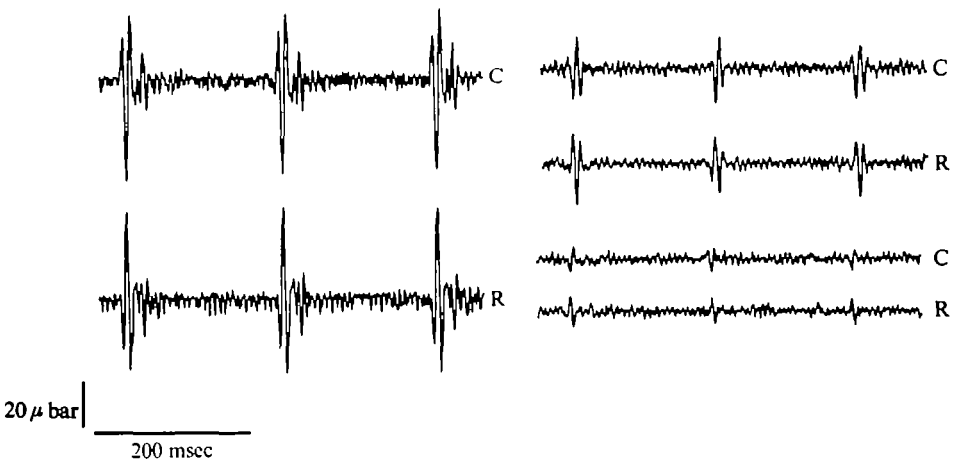

Fig. 3. Relationship of signals to noise level. Unaveraged single sweeps. Top trace in each pair compression-first click (C), bottom rarefaction-first (R). Fish originally trained at maximum amplitude (left, 60 μbar peak-to-peak), then at lower amplitudes (20 and 10 μbar respectively). Note at lower intensities smaller deflections in click waveform (e.g. the first) tend to disappear into the noise. Fully trained fish reacted correctly to C or R signals independent of intensity (see text).

In testing the nine fish together the vertical criterion lines were moved from 15 to 22 cm from the corner. For the food signal a correct response was three or more fish in the corner, a 'partial' response was one or two, and an incorrect response was zero. The designations were the opposite for the shock signal (zero in the corner correct, one or two partial, three or more incorrect).

The data were analysed by a standard χ^2 statistical test.

Tail-flip experiments

Controlled tail flips were produced by applying shocks to the (right) medullary motor area of a clamped anaesthetized fish. Hydrophone recordings were taken from various points around the fish to determine, for a given point, the order of compressions and rarefactions produced.

The fish was anaesthetized with tricane methane-sulphonate (MS 222, 120 mg/l) for electrode implantation (for details see Piddington, 1971*b*). The electrodes (60 μm stainless steel, insulated, tip separation 3 mm) were inserted into the brain whilst shocks (1 msec, 10 V, 0·5/sec) were delivered. When a locus giving a maximal twitch had been found, the electrodes were sealed in place.

The animal was revived, re-anaesthetized to a level at which swimming stopped but respiration persisted (60 mg/l), and placed in a thin-walled plastic chamber

(20 × 20 × 2·5 cm) in the centre of a shock-mounted anechoic aquarium (60 × 53 × 25 cm) (for details see Piddington, 1971*b*). The animal was then clamped by the dorsal fin with an 'alligator' clip attached to a light 10 cm stick on a pivot. When a shock was given, the resulting tail flip pushed the animal forward on the pivot which then allowed return to the original position by gravity.

The experiment was also performed by lightly anaesthetizing a fish with sodium pentothal (*c.* 30 mg/kg, i.p.) and applying a train of shocks directly across the head (near the gills) via a pair of 1 mm diameter external electrodes (stimulator dial settings: 10 msec train, 150 V, 0·1 msec, 500/sec). The results were essentially the same with either method.

The hydrophone (shielded from the shock artifact with copper mesh) was sequentially moved around the outside of the small plastic container in a circle of radius 15 cm and centre the base of the fish's tail. (For experiments with sodium pentothal the small chamber was not used.)

Hydrophone records taken at 30 cm distance showed that the recorded wave fell off in intensity as the square of the distance and this verified that the waveform recorded corresponded to sound pressure and not near-field displacement (which falls off as the cube, see Harris & van Bergeijk, 1962).

RESULTS

The basic finding in this study is that fish can learn to discriminate between two click signals which differ only in phase, that is in the order of compressions and rarefactions (Fig. 1). When clicks of the food-reinforced polarity were given the conditioned fish were at first aroused (if quiet) and they then clustered in the feeding corner of the tank. When the shock-reinforced polarity was given the fish vacated the corner or, if outside it already, remained so. Both arousal and correct orientation could be obtained with a single click and also with a variety of signals differing markedly in both amplitude and wave shape (frequency) but all having in common the same order of compressions and rarefactions (Figs. 2, 3).

The following detailed account will attempt to show that discrimination is based solely on the order of compressions and rarefactions in the auditory signal, that the lateral line is not stimulated, and that the sacculus is the organ responsible.

The tail-flip experiments show that a hydrophone sensor positioned directly behind a swimming fish always picks up a compression as the first deflexion in the pressure wave; from in front, the first deflexion is a rarefaction (Fig. 7). The angling of the tail fin during a flip appears crucial in determining the plane about which the recorded signal is inverted.

CONDITIONING EXPERIMENTS

The signal first and most often used to condition the fish is shown in Fig. 1*a*. This waveform, which was intended to approximate to that produced by a tail flip (e.g. Fig. 7), was composed of deflexions taking about 10 msec and thus corresponded to a frequency of 50 Hz. Most sounds made by swimming fish have dominant frequencies in this region, that is, below 100 Hz (Moulton, 1960). The intensity was calculated to

saturate the sacculus (see Enger, 1966; van Bergeijk, 1967; Furukawa & Ishii, 1967*a*; Piddington, 1971*b*).

Learning took place in stages. After preliminary familiarization with the tank and feeding technique the fish took 2 days to develop a conditioned arousal response to the food-reinforced signal (for which see Fig. 1).

The inverse polarity (shock-reinforced) was not used until after another 2 days training by which time a positive clustering response had been obtained. For the first few trials with the inverted signal the fish persisted in clustering and therefore received shocks, but by the end of 2 more days they had learned to avoid the shocks whilst still responding correctly to the positive signal.

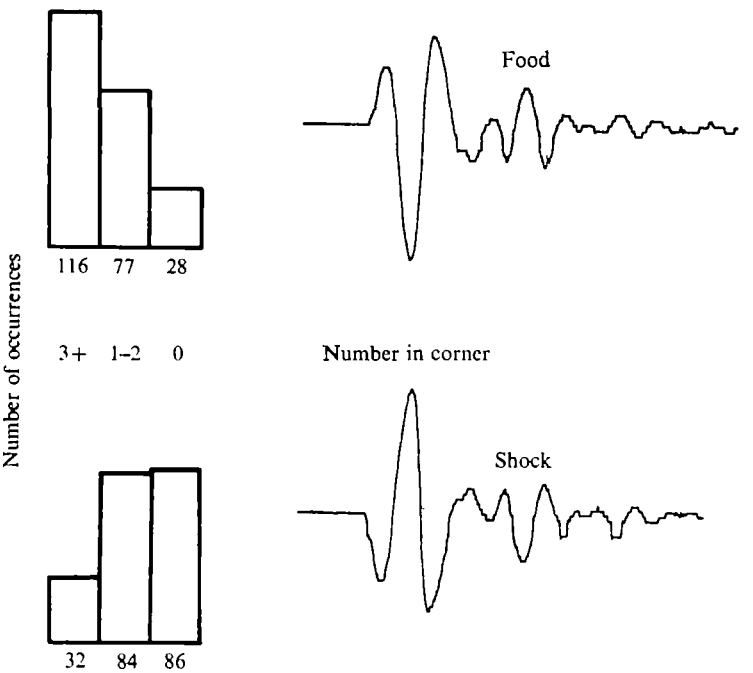

Fig. 4. Statistical comparison of group responses to signals of opposite polarity. The proportion of occurrences (trials) for which there were three or more fish (3 +) in the test corner is larger for the food-reinforced polarity (compression-first). Similarly, note that tendency to vacate corner (0 in corner) is greater following shock-reinforced polarity. Proportion of partial responses (1–2) about same. Result highly significant ($\chi^2 = 80$, d.f. = 2, $P < 0{\cdot}001$). Low-frequency click as in Fig. 1.

The response was statistical. There were individual differences in reaction to either stimulus, differences which seemed to depend on the amount of feeding prior to a given trial and other more subtle differences relating perhaps to mood or motivation. Similarly, the vigour with which the fish vacated the test corner on presentation of the shock-reinforced signal was dependent upon the shock voltage used during training. Even at the maximal voltage finally employed one individual received shocks quite frequently but also got more to eat by waiting persistently in the test corner. (When tested singly this animal avoided shocks much better, see Fig. 6.) By contrast, another individual did not feed at all but did show definite shock avoidance.

Large samples of randomised trials (see methods) were taken in order to show that the result was statistically significant. Fig. 4 shows that, for the group, both food-reinforced and shock-reinforced signals gave more correct than incorrect responses and that each yielded a similar proportion of partial responses. (This distribution is the pooled result of three separate testing sessions each of which yielded the same form of distribution. Distributions taken during the initial stages of learning were flatter.)

For statistical analysis the number of responses for which the same behaviour (e.g. clustering) occurred were compared for the two signals. (Thus correct responses to the positive signal were compared with incorrect responses to the negative signal, and so on.) The null hypothesis that the spatial distributions (Fig. 4) are the same for the two signals may be rejected at the 0·001 level of significance ($\chi^2 = 80$, d.f. $= 2$). We may conclude that the fish had indeed tended to react in the expected manner.

It was necessary to perform a number of control experiments which tested for (*a*) reliable phase inversion of the signal, (*b*) possible involvement of the lateral line, (*c*) individual reactivity to the signals (versus mere copying of a 'leader'), (*d*) reversal learning.

(*a*) *Phase analysis versus intensity or frequency*

Reliable phase inversion was confirmed by averaging 64 clicks of one polarity (e.g. rarefaction-first) and superimposing the resulting trace with one of the opposite polarity but with the input terminals to the oscilloscope also inverted (so as to produce a similar waveform on the screen). Fig. 2*a* shows that the two traces can be superimposed almost perfectly; this was true for any given hydrophone position in the tank. The small differences were not repeatable (and were smaller than the noise level and smaller than the differences in amplitude which occur within the tank – see methods).

Novel waveforms (of either polarity) which were either slightly different or markedly different from the standard waveform (Fig. 2*b*, *c*) elicited correct responses from the fish provided that the order of compressions and rarefactions was used as the criterion. The response was thus independent of frequency (waveform) over the range indicated in Fig. 2.

Further evidence that the fish were discriminating phase rather than amplitude or frequency was obtained by testing at lower click intensities and also at a frequency 10 times higher (Figs. 3, 5).

When the click intensity was lowered in stages it was observed that the correct responses still persisted but were graded to a less distinct level. Further retraining strengthened this response (Fig. 5*a*) which was still present at 10 μbar peak-to-peak sound pressure. The threshold for discrimination was not determined. However, 10 μbar was close to the noise level (Fig. 3) and this value is in the order of 20 db above the behavioural threshold for goldfish at 50 Hz (-5 db RMS re 1 μbar; Enger, 1966).

When clicks of higher intensity were re-presented it was found that the response had become generalized throughout and above the intensity range employed during training (10–60 μbar), that is, it was intensity-independent. These figures suggest that the sacculus was the organ involved (cf. Enger, 1966; van Bergeiik, 1967; Furukawa & Ishii, 1967*a*).

To obtain the 500 Hz click shown in Fig. 1*b*, the open system was required. (Because of resonance in the closed system it was not possible to grade the signal in frequency

(waveform) beyond the limits indicated in Fig. 2*a–c*.) With this faster waveform the fish had to be completely retrained before a positive discrimination could be demonstrated (Fig. 5*b*). The upper frequency limit for discrimination was not determined.

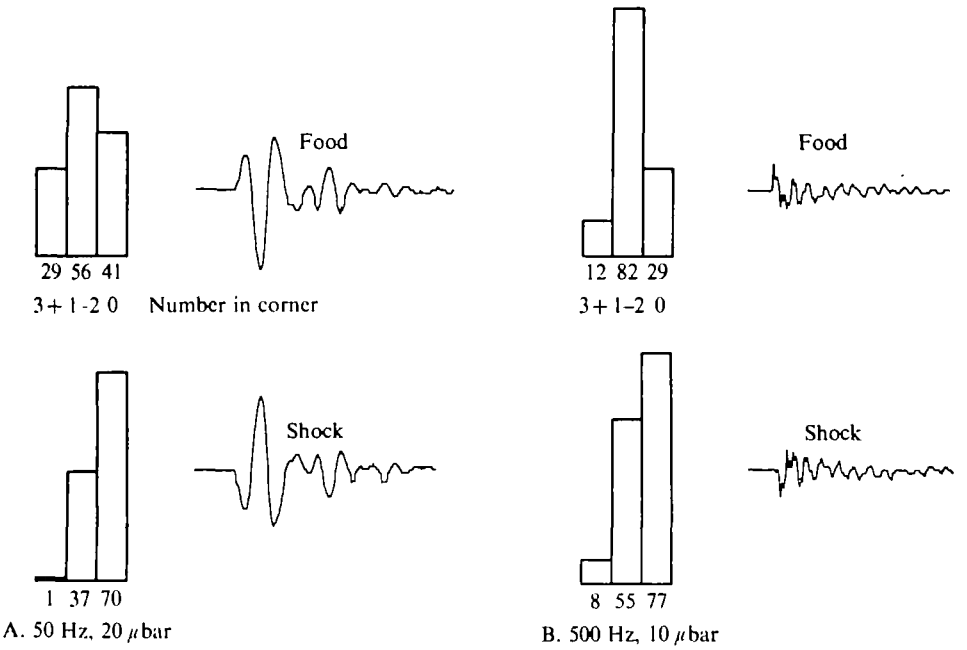

Fig. 5. Group responses at low intensity and high frequency. A, low intensity. Tendency for animals to be in corner is significantly greater for food-reinforced signal ($\chi^2 = 34$, d.f. $= 2$, $P < 0{\cdot}001$). Compared to Fig. 4, animals show less tendency to feed but a better tendency to avoid shock. B, high frequency. Distributions are significantly different ($\chi^2 = 28$, d.f. $= 2$, $P < 0{\cdot}001$). Note for A and B similarities in shape of distribution for the given treatment (food or shock).

It may be concluded from this section that phase, or the order of compressions and rarefactions, was the relevant parameter used in discrimination. Intensity or frequency cues were not used, and in any case were not available parameters because the waveform was reliably inverted at all points within the tank.

(*b*) *Lateral line not stimulated*

The lateral line is known to be directionally sensitive to water displacements (e.g. van Bergeijk, 1964; Cahn, 1967) and for these experiments it must be shown that the lateral line was not being used.

The experimental system was designed so as to stimulate only the auditory receptors, or sacculus. For the open system, the 500 Hz click was at too high a frequency to stimulate the lateral line (e.g. van Bergeijk, 1967; Cahn, 1967); Piddington (1971*a*) showed that clicks in air do not activate lateral-line nerve fibres. However, for the closed system, the loudspeaker was closer to the water surface and operating at a frequency (50 Hz) to which the lateral line could potentially respond. Lacking a displacement-sensitive hydrophone (see Banner in Cahn, 1967), it was essential to provide special controls to show that the lateral line was not excited by the click.

At the lowest intensity used (10 μbar) the motion of the speaker cone could not be seen, nor felt with the finger tips. At the highest intensity (60 μbar) no vibration of the baffle could be felt and, with oblique illumination, no ripples could be seen on the water surface.

Three methods were used to deliberately increase the near-field displacement whilst decreasing the pressure field. (i) When the baffle edges were propped up by 2 cm no pressure pulse was recorded and the fish did not become alerted or oriented even when the voltage to the speaker was doubled. No ripples could be seen, but the near-field

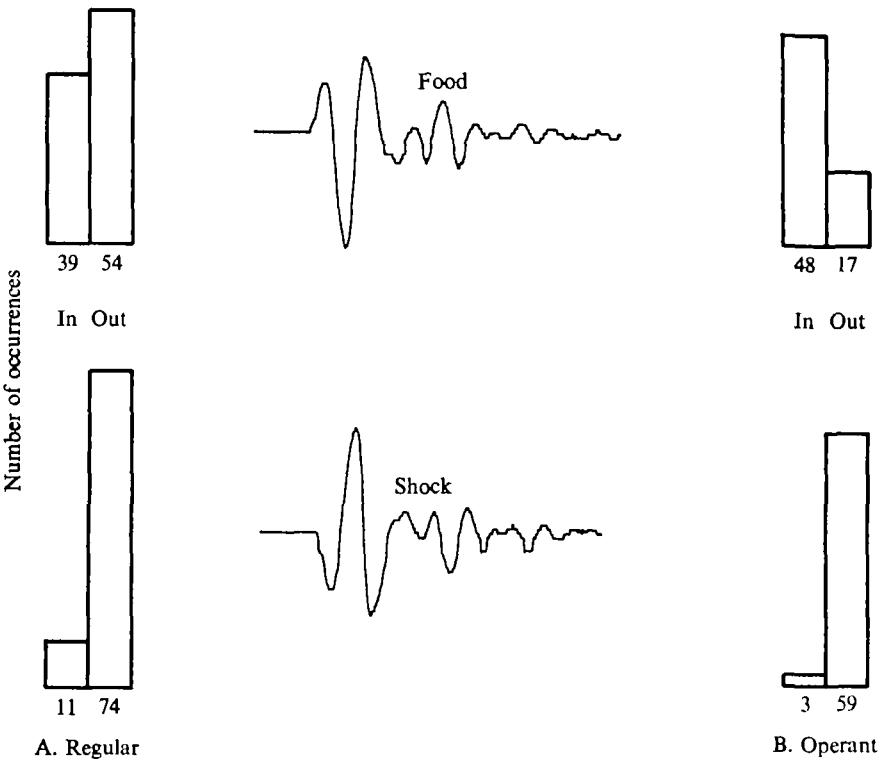

Fig. 6. Responses of an individual fish to low-frequency clicks (60 μbar, as in Fig. 1). Tendency to be in corner is greater for the food-reinforced polarity. A, Trials presented regularly at 4/min ($\chi^2 = 17$, d.f. = 1, $P < 0{\cdot}001$). B, trials presented only when fish in corner ($\chi^2 = 60$, d.f. = 1, $P < 0{\cdot}001$). Operant technique enhances correct responses to both polarities.

in the air near the surface must have been larger (see von Bekesy in Cahn, 1967). (ii) A thin stick was glued to the speaker cone, the other end dipping 5 cm into the water and causing ripples on the surface when the loudspeaker was activated. No response occurred at the human visual threshold for surface ripples. When the amplitude was raised to four times this value the fish still failed to become alerted or oriented to the stimulus except if they happened to be within about 10 cm of the tip of the rod, in which case they turned sharply and attacked the rod. The same behaviour occurred for either polarity of the loudspeaker signal. This was a typical lateral-line response (see Dijkgraaf in Cahn, 1967), but was entirely different from the response under consideration here. (iii) Puffs of air at 2/sec directed at the water surface created ripples but did not influence the fish.

These controls verify that the response in question was a farfield auditory response which involved the sacculus and not the lateral-line.

(*c*) *Responses by individuals*

There was no obvious leader of the group, but four fish appeared to be consistently more responsive than the rest. These four fish were tested singly; all four had to be retrained before giving positive responses. Each fish appeared to be 'lost' on its own and at first 'tried to escape' from the aquarium. A statistical test was only employed for one fish because (*a*) the method was laborious, and (*b*) once the response had been established it appeared in a more clear-cut and obvious form than with the group observed together.

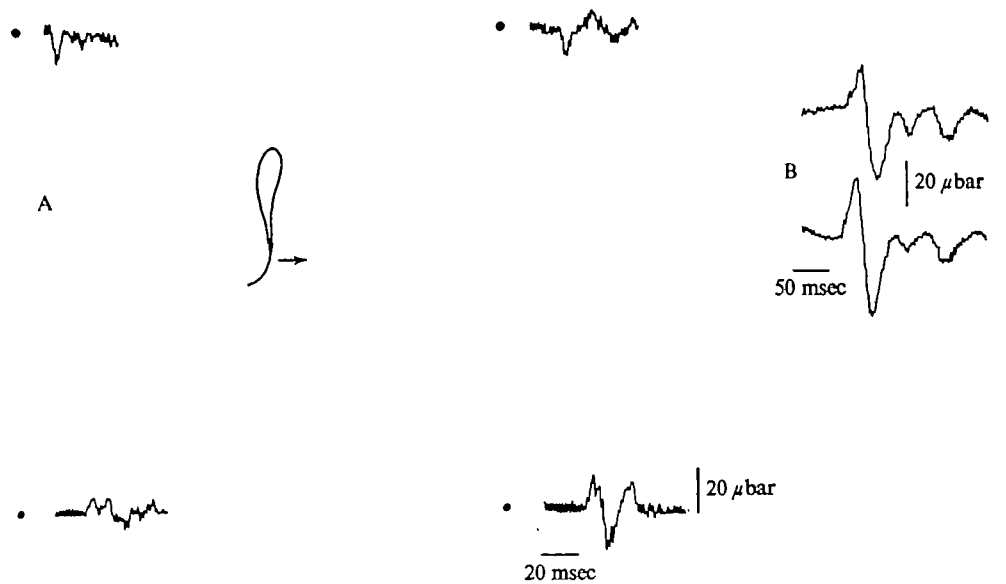

Fig. 7. Tail-flip recordings. External shock electrodes, single sweeps, compression up, rarefaction down. A, sample records from four positions around animal. Note that waveforms are variable but that from in front the first deflection is a rarefaction; and from behind, a compression. Tail motion indicated by arrow. B, two traces taken from directly behind a (different) fish. Both waveforms are compression-first.

With regular trial presentation at four per minute (see Methods) a statistically significant result was obtained ($P < 0{\cdot}001$; Fig. 6*a*). However, in Fig. 6(*a*) shock avoidance appeared to be more pronounced than approach for food (which nevertheless occurred more often than chance since the test or corner area was 7 % of the total area of the tank). All four fish were less motivated to feed on their own than in the group.

An operant test was devised whereby the stimulus was only delivered when the fish appeared in the test corner (as if wanting food). Fig. 6*b* shows that this technique led to an enhanced proportion of correct responses to the food-reinforced stimulus and also, surprisingly, to the shock-reinforced stimulus.

The ability to discriminate phase was thus present in at least four individuals of the group; copying was not essential in explaining the group results but seemed to be responsible for elevating the motivation to feed.

(*d*) *Reversal training*

It was possible to reverse-train the fish so that the rarefaction click (the first deflexion was a rarefaction) became associated with food and the compression click with the shock (cf. Fig. 1).

Reversal learning was accomplished in stages. At first, rarefaction clicks were delivered during normal feeding so as to teach the fish that this signal was no longer harmful. The clicks were 60 μbar peak-to-peak (as in Fig. 1) and were given singly or in a group of 2–3. Gradually, trial by trial, the number of clicks was increased and moved forward in time so as finally to precede feeding by the standard amount. Once this feeding response had been obtained it was relatively easy to extinguish the feeding response to the compression click by the use of shocks. Further training resulted in the development of shock-avoidance to the compression clicks.

A statistical sample was not taken; reversal training was performed merely to show that true learning had occurred. Pseudo conditioning or activation of an innate behaviour had not taken place.

TAIL-FLIP EXPERIMENTS

Fig. 7 shows that sample records taken from behind the fish have a compression as the first deflexion, whereas records from in front have a rarefaction first. These records are single traces and they exhibit variability in waveform. This was mainly caused by variation in the strength of the tail flip which did not, however, affect the order of compressions and rarefactions found for a given hydrophone position.

For animals implanted with electrodes the results with respect to the sound phases were comparable to those expressed in Fig. 7, but the tail flips were weaker and recordings required considerable averaging.

It can be seen that the waveforms produced in Fig. 7 are comparable in duration to the stimulus used in the conditioning experiments (Fig. 1). However, it is obvious that the sounds produced were quite weak. Nevertheless, it was shown that the recorded wave corresponded to acoustic pressure rather than near-field displacement (see Methods).

DISCUSSION

Phase discrimination

The results showed that conditioned fish are capable of discriminating between a given click and the same click inverted. It was shown that the discrimination is based solely on phase information, or the order of compressions and rarefactions in the auditory stimulus. The experimental responses which developed were learned rather than innate, were independent of amplitude or frequency over a specified range, and were shown to be far-field auditory responses involving the sacculus. The lateral line was not involved. Though trained with a burst of 10 clicks at 2/sec, the fish nevertheless exhibited correctly oriented responses to isolated single clicks.

As outlined in the introduction, and in Piddington (1971*b*), there is clear anatomical and physiological evidence for separate coding of compressions and rarefactions in

seven species of teleosts. The sensory hair cells in the sacculus are arranged in two oppositely oriented populations; one set depolarizes on the compression phase and the other on the rarefaction (Furukawa & Ishii, 1967*a*; Hama, 1969). Two opposing orientations of the hair cells are found also in the lateral line and vestibular-otolith systems (Lindeman, 1969; see review by Grinnell, 1969).

It is clear that the present experiments need to be repeated (especially with unspecialized fishes). The range of waveforms tested was restricted, and the limits to which the phase discrimination can be taken were not determined. Nevertheless, a few preliminary comments are in order.

The results of these experiments appear to have a sound physiological basis (see Piddington, 1971*b*) yet they are somewhat remarkable because man cannot perform the same phase discrimination. Other persons besides myself listened intently to the clicks being presented and nobody could tell whether the click was 'normal' or inverted (see waveforms in Figs. 1, 2), even after 'training sessions' in which we had prior knowledge of the polarity.

It was similarly impossible for us to discriminate the click sounds produced by an audio monitor connected to the amplified output of the hydrophone.

This is extra evidence that the fish were discriminating phase and not frequency or intensity. Man is about ten times as good as goldfish at frequency discrimination (see review by Grinnell, 1969) and so if man could not detect any frequency differences then it is most improbable that the fish could have.

Mammals have only one orientation of the cochlear hair cells and the nerve fibres fire only on the rarefaction (see review by Grinnell, 1969). However, this does not explain the lack of phase discrimination in man because discrimination could theoretically be achieved by analysing increases (rarefaction) as opposed to decreases (compression) in the spontaneous firings of the auditory nerve fibres. Evidently, this kind of analysis does not take place. It could occur in fish, but a more likely mechanism is the comparison of 'times of arrival' in the two channels, compression and rarefaction respectively (see Perkel & Bullock, 1968). However, regardless of the mechanism, it would appear that the onset or first few deflexions of a given signal are the most important (in the next section I report that for a model tail flip only the sign of the first deflexion can be reliably predicted; other features of the wave are highly variable). The rapid-feedback system (10 msec latency) described by Piddington (1971*b*) would appear to facilitate onset analysis by obliterating the rest of the given signal.

In most mammals time differences between the two ears are used in locating sound sources (e.g. Galambos, Schwartzkopff & Rupert, 1959; Grinnell, 1969); the firing of binaural units is influenced markedly by time differences down to less than 0·5 msec (Galambos, *et al.* 1969). Fish cannot locate far-field sound sources (van Bergeijk, 1964) but if comparison of the order of compressions and rarefactions is the analogous neural mechanism then one would predict that phase discrimination might extend to above 1 KHz. This would probably be suitable for the analysis of most sounds made by swimming fish since these are mainly below 100 Hz, though sometimes extending to 2 KHz (Moulton, 1960).

This paper, and the previous paper (Piddington, 1971*b*), propose that phase analysis in fish is used to detect the orientation of swimming fish as distinct from their location. Reptiles and amphibians may also discriminate phase since both appear to have the two

sets of hair cells (see review by Grinnell, 1969). If so, the mechanism would appear to be limited to animals which cannot locate far-field sound sources because of the high velocity of sound in the medium (amphibians underwater, reptiles underwater, or contacting the ground) (see van Bergeijk, 1964; Hartline, 1971).

Earthquake studies have shown that when the ground slips towards the observer the first deflexion measured is always a compression, and so phase information could also be present in the earth following disturbances caused by moving animals.

The phase mechanism appears somewhat superfluous for animals which can locate sound sources in air and there may be a distinct reason (such as better signal-to-noise detection) for the mammals to have lost one set of hair cells. Whether the remaining hair-cell orientation (rarefaction) is critical or arbitrary remains to be seen.

Sound production by fish tails

The results showed that a tail flip created sound waves which registered a rarefaction for the first deflexion if the hydrophone were in front of the fish but a compression for positions behind the fish. This was to be expected because the fish must push back on the water in order to move forward. The exact plane about which phase inversion occurred was not determined, and in fact the tail flips were too variable to make this possible.

In a previous paper (Piddington, 1971*b*) I suggested that phase analysis of the sounds made by a swimming fish could be used to discriminate between the approach or recession of that fish. The same basic arguments about the angling of the tail fin apply, but the implication that all swimming motions radiate enough sound is certainly wrong.

Objects small relative to a wavelength are inefficient radiators of acoustic energy (see Morse, 1948). Tail flips, or other swimming motions fast enough to radiate sound, are probably rare (see Tavolga, 1964) but when they do occur they can sound like loud thumps or even whip cracks (see Moulton, 1960; Cousteau, 1953, 1963; Tavolga, 1964; Piddington, 1971*b*). Tail-flip sounds though rare are probably biologically important (Piddington, 1971*b*), much as the rare snap of a twig by a stalking jaguar would be vitally important to its prey. The analysis of tail flips probably involves the order of compressions and rarefactions (as explained) rather than 'the proportions in time' of low-frequency swimming sounds proposed by Piddington (1971*b*).

The mechanism by which tail flips radiate high frequencies (up to 2 KHz, Moulton, 1960) is not known, but rapid motion of the tail tip is probably required (cf. Bainbridge, 1963). Force alone is certainly not sufficient. From Gero's (1952) figures it can be estimated that a large fast-swimming fish can push with 0·1 atmosphere (bar) pressure on the water (see also Parry, 1949). I carried out an experiment with a model underwater in which 20 kg force was imparted abruptly by a spear gun to a board, 10 cm square which was fixed to the spearhead. This created a pressure of 0·2 bar yet I could hear no sound and small schooling fish beyond a range of 3 m were not startled whilst those within 3 m were. Direction of motion of the board relative to the fish was without noticeable effect, and the 3 m fall-off is indicative of a purely near-field effect. The board had not moved fast enough to radiate acoustic energy (see again Morse, 1948).

The central proposition to the tail-flip theory is that the angling of the tail sends a

compression wave (the first deflexion is compression) backward from the animal and a rarefaction wave forward. The angling of tail fins has been described by Breder (1926), Parry (1949) and Bainbridge (1963). No such measurements were taken here but an experiment with a model fish-tail was performed in the labroatory. A stick ('fish body') with a flat board ('fish tail') attached at 45 degrees to the end was 'flipped' abruptly by hand under the water and hydrophone records were taken. The waveforms (shown to be sound pressure) were varied but the order of compressions and rarefactions could always be predicted. On the side of the board which was pushing on the water the hydrophone registered a compression first and on the pulling side it registered a rarefaction first. As expected, the plane dividing the phases corresponded to the plane of the board ('tail') and not to the stick ('body'). (Dipole objects small relative to a wavelength make hemispheres of compression and rarefaction on either side; see Morse, 1948; van Bergeijk, 1964.) For a real fish the plane about which phase inversion occurs would at any instant depend on the angling of the tail relative to the body.

A little reflexion will show that if a fish picks up a rarefaction there is only a certain probability that the fish producing the sound is oriented towards it. The probability depends on the angle of the tail relative to the body. It is simple to show that if a rarefaction occurs the probability of approach is greater than if a compression occurs (and vice-versa for the probability of recession).

For a tail angle of 45° the ratio of probabilities is 3:1, and this would indicate that the mechanism has survival value.

These experiments need to be repeated with larger fish reacting in a larger body of water (cf. field study by Enger & Anderson, 1967).

Biological significance

The sensitivity to propagated pressure waves gives the auditory system a longer range than either the visual, lateral line, or electro-receptor systems and so its use could be as a distant warning or alerting system (see Pumphrey, 1950). The present theory proposes that the swimming motions of a fish produce an appropriately propagated signal, and that to a listening fish the signal changes in a specific way which depends on the angle at which the swimming fish is oriented toward or away from the listening fish. The signal codes orientational information in the order of compressions and rarefactions and not as changes in intensity or frequency. Rapid changes in intensity or frequency would be interpreted independently as speed changes.

The advantage to the fish of rapid discrimination between approach and recession of a distant swimming fish would to an extent offset the disadvantage of being incapable of azimuthal or directional discrimination.

SUMMARY

1. Goldfish were taught to discriminate between a given click and the same click inverted, that is, with the compression and rarefaction phases reversed.

2. The responses were true auditory responses involving the sacculus but not the lateral line.

3. The responses were independent of both waveform (frequency) and intensity and could be elicited with single clicks. Phase was the relevant parameter.

4. Tail flips were found to send a rarefaction wave (rarefaction is the first deflexion) forward from the fish and a compression wave backward.

5. It is proposed that phase analysis of tail-flip sounds is used to tell whether a swimming fish is approaching or receding.

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