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CENTRAL CONTROL OF AUDITORY INPUT IN THE GOLDFISH

II. EVIDENCE OF ACTION IN THE FREE-SWIMMING ANIMAL

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INTRODUCTION

It was shown previously (Piddington, 1971) that shocks to the midbrain of anaesthetized goldfish reversibly reduced or abolished the neural input following a click without changing the receptor potential or microphonic. The control system so revealed must act by neuronal inhibition (sometimes facilitation), but not via muscle action which would have reduced the microphonic (Galambos & Rupert, 1958).

The aim of the experiments to be described here was to discover whether this control system actually operates in the awake, free-swimming goldfish.

In mammals the middle-ear muscles serve a protective function by contracting reflexly after loud sounds (Galambos & Rupert, 1958), and by controlled contraction during body movements and vocalizations serve to reduce stimulation during sound production (Grinnell, 1969).

The function of the mammalian cochlear efferents (olivocochlear bundle, OCB) is less well understood (Galambos, 1960; Fex, 1968). However, one major contrast to the middle-ear muscles is that the cochlear efferents can act discretely on sounds of different frequency (Klinke, Boerger & Gruber, 1969) and this should mean finer, more selective control over the input. One recent paper presents evidence that the cochlear efferents can operate in frequency discrimination (Capps & Ades, 1968), and others (Dewson, 1967, 1968; Trahiotis & Elliott, 1970; Nieder & Nieder, 1970) show evidence of action in the discrimination of signals in noise. Other independent studies have shown that the efferents may also mediate habituation; the click-evoked action potential, recorded from the auditory nerve at the round window of the cochlea, gets progressively smaller as a result of slow monotonous repetition of a click (Al'tman, 1960; Burgeat, Andrianjatovo & Burgeat-Menguy, 1963; Veselý, 1963; Buño *et al.* 1966). Habituation is an active process of suppression that is not equivalent to fatigue (Kandel & Spencer, 1968; Bruner & Kennedy, 1970) and its occurrence in primary neurones is at first startling and controversial (Wickelgren, 1968; cf. McKay, 1970). Buño *et al.* (1966) report habituation of the receptor potential itself (microphonic) and also show complex interference effects of other sense modalities and even conditioning. These results, though requiring replication, are important indications that the cochlear efferents are under direct cortical control and are not simply acting as a lower reflex pathway (see also Galambos, 1954, 1956; Fex, 1962, 1968; Veselý, 1963; Dewson, 1968).

In view of the controversy about whether or not habituation can occur at early input levels (see Wickelgren, 1968), part of the present study was designed to demonstrate neural habituation to clicks in the goldfish medulla whilst controlling for unwanted masking effects by simultaneously monitoring the input at the receptor or microphonic level. The use of slowly repeated 50/sec click trains (rather than single clicks) as an habituating stimulus revealed another kind of more-rapid feedback control that acts within the individual train rather than on the entire train (as does habituation). Other experiments, particularly those utilizing reward-conditioning, indicate that facilitatory control may also exist.

METHODS

A total of 20 chronically implanted goldfish, *Carassius auratus*, were used in this study, 13 yielding useful results.

The operation was performed with the animal under light anaesthesia (MS 222, 120 mg/l). The fish were held in a semi-stereotaxic holder (Piddington, 1971) that allowed continuous perfusion of the gills via a glass tube in the mouth. The skin was scraped from the skull and a small hole made in the midline, 0·5 mm wide, starting at the posterior border of the skull and extending 3 mm anteriorly. To help anchor the dental cement to the skull, three insect pins, 3 mm long, were inserted around the hole and a thin layer of Ethicon tissue adhesive was painted over the skull and allowed to dry.

Two bipolar pairs of electrodes were implanted, one for the microphonic and the other for the neural response. Each was constructed of two 25 μm insulated stainless-steel wires (California Fine Wire Co.) cut across at the tip, one wire ending 2 mm before the other. The bipolar pairs, 70 cm long, were coated with flexible Tygon paint, then glued together except for 5 cm at either end.

Each electrode was clipped to a micromanipulator 1 cm from its tip and inserted, while clicks were delivered from a 4 in. loudspeaker, 50 cm away in air. The positioning of the electrodes had been calculated geometrically from dissections and stereotaxic localizations made with the brain completely exposed (Piddington, 1971, fig. 1). The posterior electrode, for microphonics, was inserted first, fixed in with a little dental cement and unclipped from the holder. When the second was also in place, dental cement was added to fill in the hole and cover the pins. Finally, the two wires were joined by further coats of Tygon paint and a small polystyrene float was glued to the cement to provide neutral buoyancy.

The animals were revived with fresh water and, when not being tested, were kept in an aquarium, the wires supported by a long cotton thread which absorbed any twists.

The experimental aquarium, 60 × 53 × 25 cm, was rendered relatively anechoic by placing 3 in. of glass-fibre matting on the bottom plus further lining all around with 2 in. rubberized horsehair, which was dried out between experiments because water-logging abolished the acoustic absorption. The fish was restricted to the top centre portion of the tank by a small container, 20 × 20 × 8·5 cm, made by gluing thin plastic wrapping material over a flimsy Plexiglass frame – heavier materials gave unwanted sound reflexions.

Clicks were delivered from the 4 in. loudspeaker positioned 70 cm vertically above

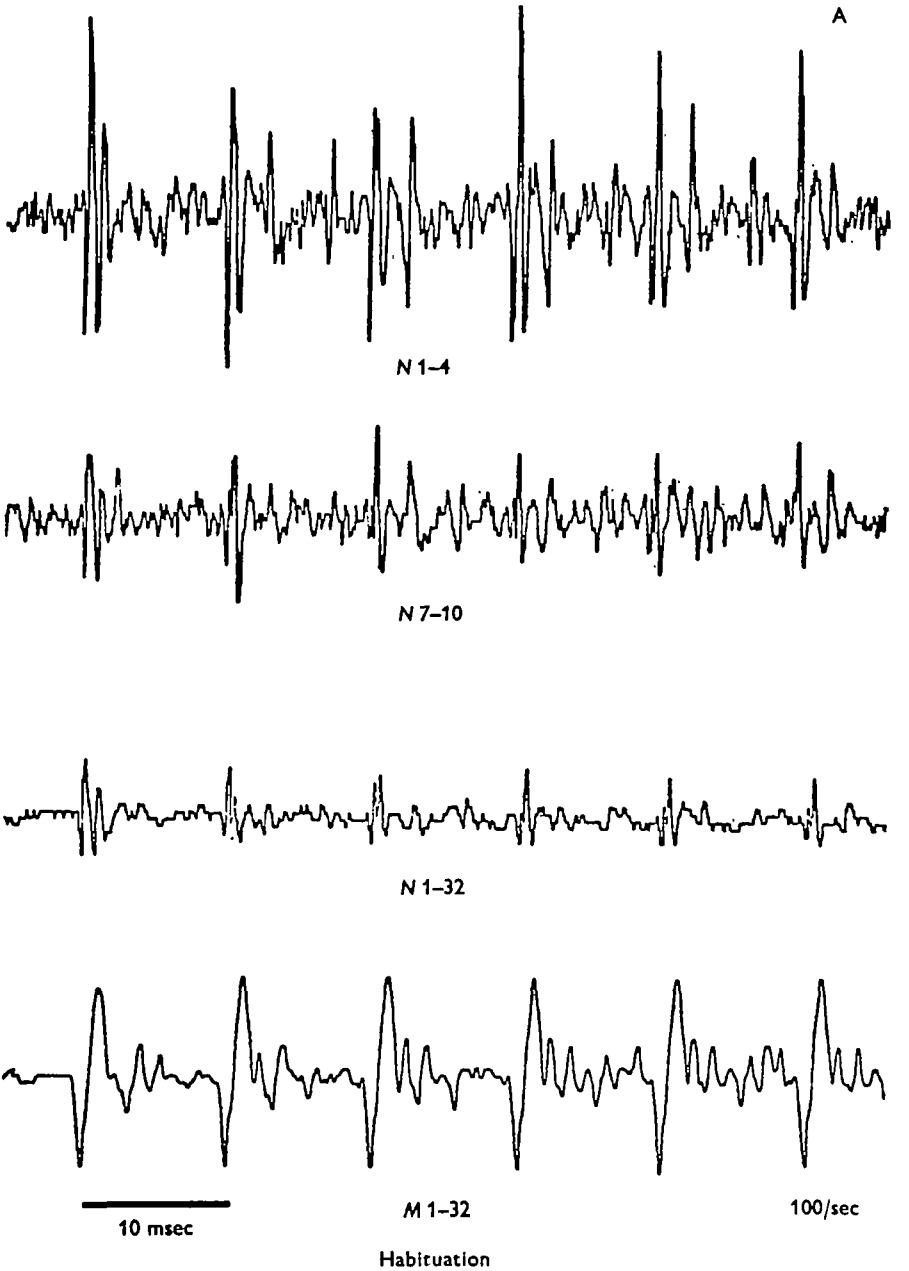


Fig. 1. Plasticity of neural responses in free-swimming fish.

A. Habituation of the neural response by repeated sounds. Responses to the entire train reduced in amplitude. Averaged microphonic (*M*) and neural responses (*N*) to trains of clicks at 100/sec repeated at 1 train/sec. Numbers at right represent the trains averaged to give the respective traces: trials 1–4 are from the same habituating sequence as trials 7–10, but trials 1–32 are from a separate sequence. Recovery occurred between sequences. Each click response in trace 1–4 is larger than the responses in 7–10, which in turn are larger than in 1–32. Note short latency of neural response (1 msec).

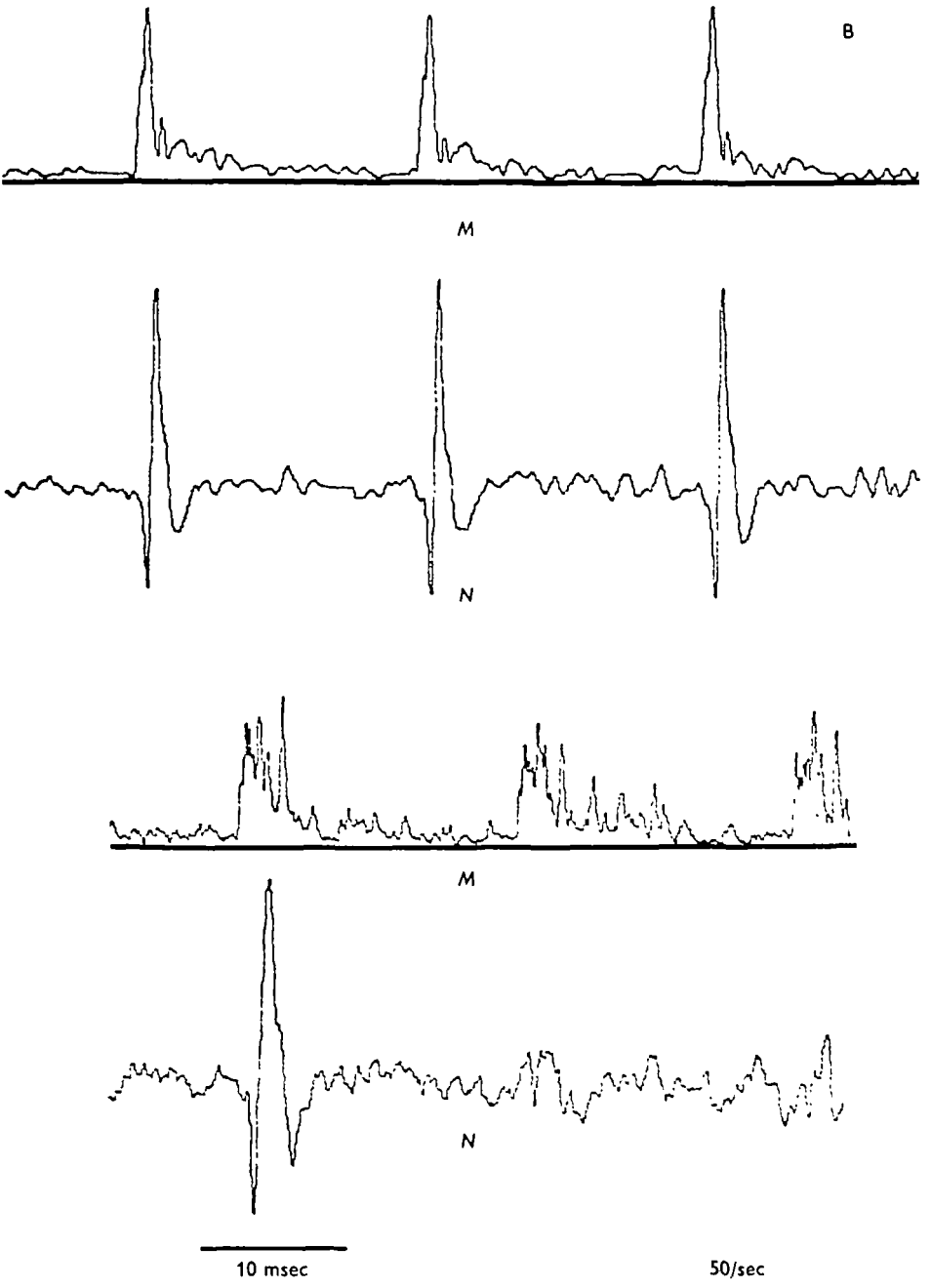


Fig. 1B

B. Rapid feedback during a 50/sec click train. Note almost complete obliteration of the second and third neural responses in the free-swimming animal. First click response (N_1) not reduced (cf. Fig. 1*a*). Top two traces, an anaesthetized animal; bottom two traces, a free-swimming animal. Top, average of eight sweeps at 1/sec; bottom, single sweep. *M*, rectified microphonic; *N*, neural.

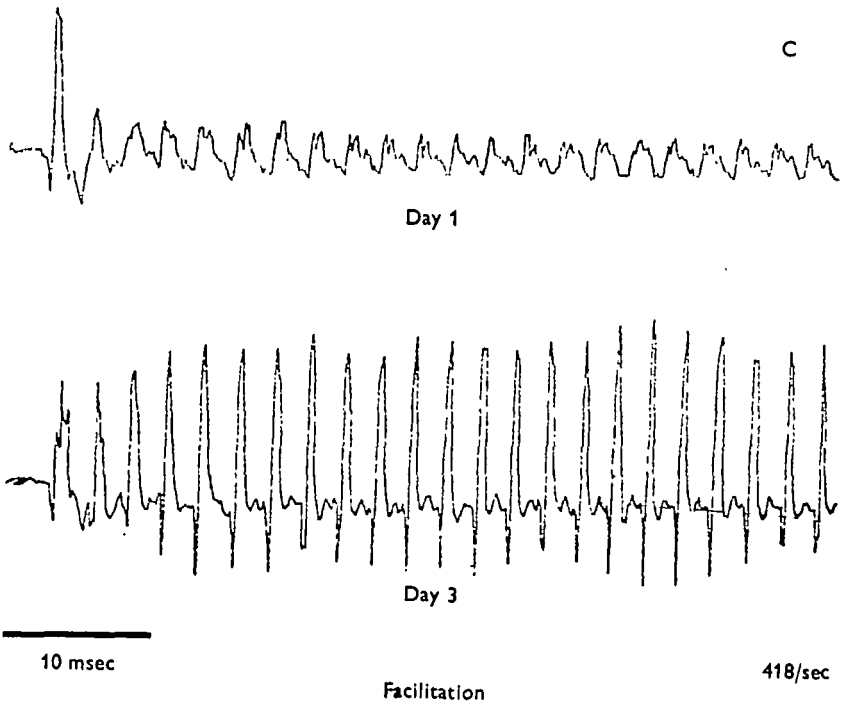


Fig. 1C

C. Facilitation, or enhancement in neural following ability with high-frequency click trains. Each trace, 32 trials averaged at 1/sec. Stimulus 1 train/ sec, each train 40 clicks at 418/sec. From days 1 to 3, the animal was rewarded with food after each block of 32 trials. Note the improvement in following on day 3.

the surface. For a constant click in air the intensity within the small container, measured with a hydrophone, varied from a maximum at the centre on the surface to 75% of this at the bottom corners. This variation was not serious because the neural amplitudes were referred to the amplitudes of the microphonic, and because the fish spent most of the time on the bottom anyway.

The tank was also checked by using an anaesthetized implanted fish as a hydrophone. The microphonic amplitude varied with lateral or vertical position in the tank in the same way as did the hydrophone output, but the amplitude did not depend on the orientation of the fish.

Records were taken with conventional oscillographic equipment together with averaging by a small digital computer with $x-y$ plotter.

RESULTS

The basic finding in this study is that, in the free-swimming goldfish, the afferent neural response to a constant auditory stimulus may undergo considerable changes which are never revealed in the same animal under anaesthesia (Fig. 1). Three kinds of response modifications were seen.

(1) Habituation: with slow (1/sec) repetition of a single click or brief click train

the evoked potentials progressively declined in amplitude and usually remained depressed for long periods (Fig. 1 *a*).

(2) Rapid negative feedback: the neural responses to the second and later clicks of a 50/sec click train were depressed relative to the first (Fig. 1 *b*).

(3) Facilitation: with certain rapid click trains the neural responses, instead of being depressed, were enhanced (Fig. 1 *c*, lower trace).

The results are consistent with the hypothesis that the afferent neural response is modified by the action of descending control fibres, the existence of which has been demonstrated physiologically (Piddington, 1971). However, there are numerous alternate explanations, and the rest of this section is devoted to their exclusion and to a detailed description of each particular phenomenon.

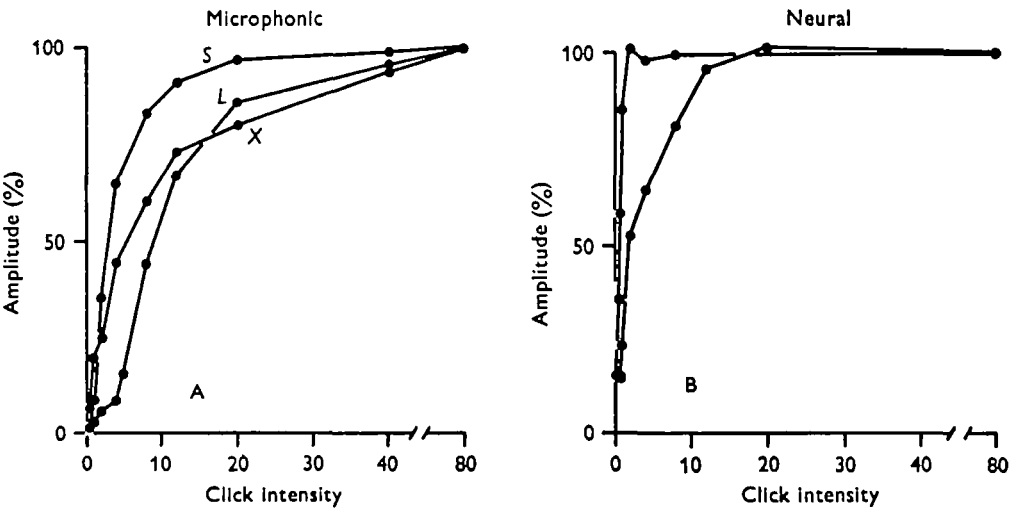


Fig. 2. A. Microphonic responses from an anaesthetized animal. Microphonic amplitude as a function of intensity for three regions of the inferior labyrinth. Recordings are from sagittal plane close to otoliths. *S*, anterior sacculus; *L*, posterior lagena; *X*, overlap region between *S* and *L*.

B. Neural responses in an anaesthetized animal from two points in the medullary auditory region, showing selectivity of electrode for populations that saturate at different intensities.

The microphonic response

In order to demonstrate modification of an early-order afferent response by a descending control system it is necessary to show that in each test instant the input remains constant and that any modifications of the evoked potential are reversible. The masking effect of noise must be eliminated along with other undesirable artifacts such as electrode drift and tissue damage. For these reasons the receptor response or microphonic was recorded simultaneously with neural response and MS 222 anaesthetic was used as a reversing agent.

Furukawa & Ishii (1967*a*) presented a detailed analysis of the saccular microphonic by direct recordings with microelectrodes. However, for this study it was impossible to observe the sacculus directly because the overlying brain structures had to be kept intact. The microphonic recorded was thus found by a vertical stereotaxic approach at the midline via the posterior cerebellum and medulla (Fig. 1 *a*).

There are four reasons for identifying these potentials as saccular microphonics:

(1) Mechanical artifacts were excluded because (*a*) the electrode did not touch the bone, (*b*) saturation occurred at a specific intensity (Fig. 2*a*), and (*c*) the potential exhibited compression predominance and double-frequency following of a sine wave (see Furukawa & Ishii, 1967).

(2) They were only found near the inferior labyrinth.

(3) The lagena is an unlikely source because (*a*) the microphonics responded to human speech and (*b*) they saturated at the same intensity as did the saccular nerve when recorded from directly. The lagena is activated only by the most intense sounds (Furukawa & Ishii, 1967*a*).

(4) They were unlikely to come from the saccular nerve because (*a*) the latency was zero, (*b*) there was no adaptation or latency shifts to continuous tones of 1000 Hz and (*c*) there was no spontaneous neural activity; when auditory feedback was set up with the audio monitor the sound was tonal with no crackle and no waxing and waning for a given position of the loudspeaker.

For the microphonic potential there were differences in the intensity function between animals but these were probably dependent on the exact longitudinal location of the electrode (Fig. 2*a*). In Fig. 2(*a*) the recording from the position between sacculus and lagena gives a curve that at first rises more steeply than the other two, flattens out and crosses them, and again rises more steeply at the end. Though requiring more direct confirmation, these records indicate that for both sacculus and lagena the posterior end may be more sensitive than the anterior and this may in part be because both otoliths are more massive anteriorly, a known fact (von Frisch, 1936) which I have confirmed (see also Furukawa & Ishii, 1967*a*).

For an anaesthetized fish held stationary at any point in the experimental tank, changes in the orientation of the fish had no effect on the amplitude of the microphonic, and this confirmed van Bergeijk's (1964) postulate that the fish auditory system is non-directional (see also von Frisch & Dijkgraaf, 1935; cf. lateral line, van Bergeijk, 1967; Piddington, 1971).

Controls

Because acoustic noise-masking has been considered the most dangerous artifact encountered in mammalian habituation experiments (Wickelgren, 1968), and because in chronic studies on the cochlear efferents the middle-ear muscles must always be eliminated (e.g. Galambos, 1960; Fex, 1968), special control studies directed at eliminating these possible explanations for the results were carried out.

The rectified records of Fig. 3 show that, in the anaesthetized animal, increasing noise raises the microphonic base-line, reduces the microphonic signal and reduces the neural amplitude. These results, when plotted, show a linear dependency of the neural amplitude on the microphonic amplitude (whole potential minus base-line noise). If the base-line level of the microphonic remains down, then the given experiment may be considered noise-free. If a full-sized microphonic gives a depressed neural response, then noise-masking is not the cause.

In the free animal it was found that swimming and breathing sounds made by the fish cruising about the experimental tank were insufficient to alter the amplitude of either the microphonic or the neural response, so long as the animal made no violent

escape-like movements. The crucial test was to add small doses of anaesthetic to the water so as to arrest selectively first the swimming and then the breathing and to measure the click response at each stage. No differences were seen.

When changes did occur in the neural response the rectified microphonic nevertheless maintained a constant base-line level and constant click-evoked amplitude (e.g.

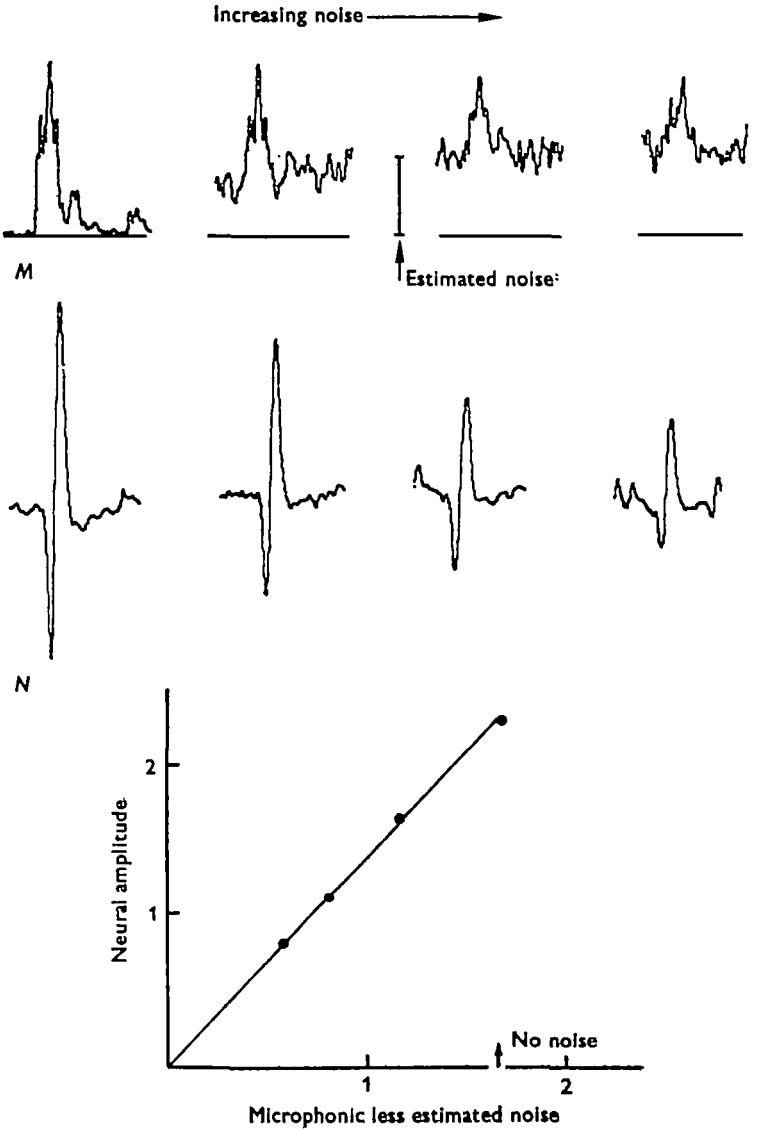


Fig. 3. Microphonic and neural responses from an anaesthetized animal; effects of acoustic noise-masking. Increasing noise raises the rectified microphonic baseline (*M*) and reduces the neural response (*N*). Intensity is at saturation of both *N* and *M*.

Fig. 4*a*), which is not to be expected for either noise-masking or muscular control (see Galambos & Rupert, 1958). Furthermore, when the neural amplitude was plotted as a function of the microphonic amplitude at different intensities after prolonged

habituation, the curve showed a reversible departure from linearity, saturating at a low intensity, and thereby showing that only the high-threshold fibres had been eliminated from the response (Fig. 4*b*). This is quite the opposite effect to that expected from noise-masking which, on increasing intensity, first eliminates the low-threshold fibres (Dewson, 1967; Nieder and Nieder, 1970).

The neural response, general

During both the anaesthetized and free-swimming phases, the response in the medulla to a single click was usually a triphasic action potential going positive–negative–positive (Fig. 1*b*). In the anaesthetized phase the latency from onset of the microphonic to the peak of the negative spike varied from 1 to 3 msec and the width of the negative spike from 0·5 to 2 msec. All quantitative features of the response, from exact waveform and amplitude to the dependence of these on click intensity and frequency, were critically dependent on the electrode placement during the implant (Fig. 2*b*) and all showed some kind of change during long-term chronic recording. The 25 μm electrode used in this study probably sampled from 1–10 % of the available fibres.

Habituation of the neural response

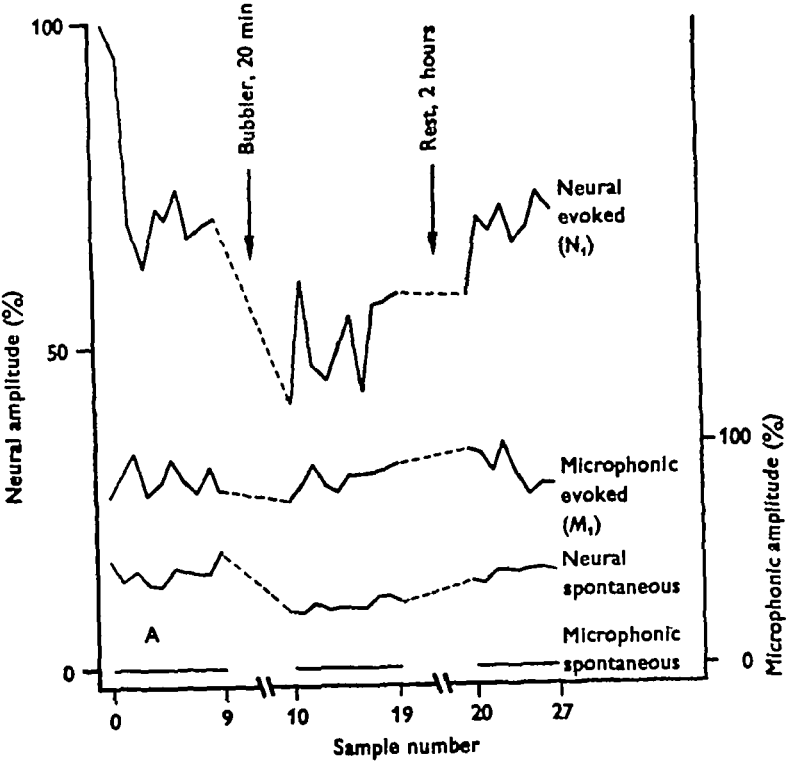
Slow repetition at 1/sec of a single click or brief click train was found to produce a cumulative suppression of the evoked potential with no effect on the microphonic (Figs. 1*a*, 4*a*). The response to each click in the train was diminished. Such habituation was found in 7 out of the 13 implanted animals; the fastest example required about ten presentations at 1/sec to produce a pronounced depression (Fig. 1*a*). Animals which became habituated did so on each day tested.

Fig. 4(*b*) shows that the neural amplitude recovers during anaesthesia, even when the stimulus is left on, and that only high-threshold fibres are habituated; there is actually an enhancement of the response at low intensity. Such lack of habituation of low-threshold fibres was detected in all three animals tested in this way, even if weak clicks were used as an habituating stimulus.

Long bursts of loud noise from a stone bubbler produced reversible suppression equivalent to habituation (Fig. 4*a*).

It was impossible to induce rapid dishabituation or interference with other modalities; changes in click amplitude or frequency, flashes, water disturbances either synchronized to the click or unsynchronized, addition of food or of a second fish to the tank – all failed to dishabituate. Single clicks injected at irregular times between regular habituating trains gave responses of identical amplitude (see also Rowell & McKay, 1969). These negative findings contrast with the rapid dishabituation, interference and attentional shifts described for the mammalian cochlea (Buño *et al.* 1966) for the cochlear nucleus (Hernandez-Peon *et al.* 1956) and for other habituating systems (Kandel & Spencer, 1968).

Fig. 4*b* indicates a dose-dependent action of MS 222 anaesthetic in preventing habituation (see also Webster, 1969). Though not repeated in other animals, it was found that slight but rapid habituation still occurred at 60 mg/l. Suppression was complete after only one brief click train (10 clicks at 50/sec); further click trains at 1/sec produced no further suppression, and recovery by rest was complete in less



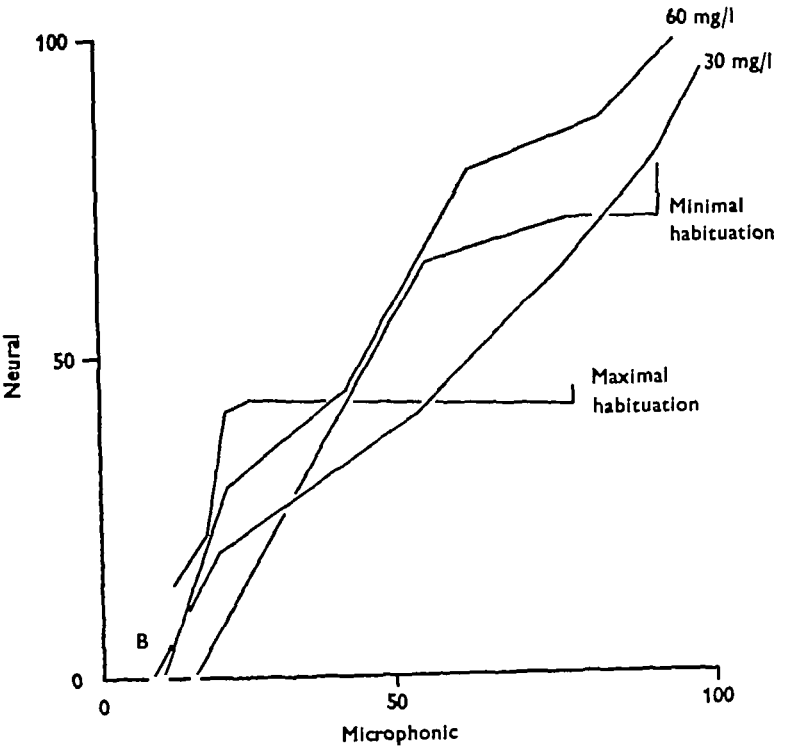


Fig. 4. For legend see facing page.

than 30 sec. This probably indicates that the early, more rapid phase of habituation (see Figs. 4(*a*), 7) is the last phase to be eliminated by the anaesthetic. Furthermore, habituation probably operates first on those fibres with highest threshold because in Fig. 4(*b*) the curve for minimal habituation saturates at a higher intensity (microphonic amplitude) than the curve for maximal habituation.

These results are consistent with the hypothesis that slow repetition of a constant brief auditory stimulus causes habituation, or the slow cumulative suppression of subsequent input (see Kandel & Spencer, 1968). Such suppression is not fatigue because anaesthetic restores the original amplitude even if the auditory stimulus is left on.

Noise-masking and muscular control have already been eliminated as alternative explanations for response decrement, and a close examination of the data will reveal that the following are also inconsistent explanations: inconstancy of the auditory stimulus, desynchronization between fibres, electrode drift or polarization, anoxia, hormones, changes in blood flow, phasic negative feedback giving an accumulation of inhibitory transmitter and phasic feedback that is anticipatory.

Rapid feedback action on the neural response

During habituation experiments in which trains of 10 clicks at 50/sec were presented regularly at 1 train/sec it was noted that some animals did not become habituated but rather showed a more rapid kind of suppression in which the responses to the second or later clicks of each train were depressed whilst the response to the first click (N_1) remained constant (Fig. 1*b*). (When habituation occurred N_1 was depressed; Fig. 1*a*.) These changes in the neural response were unaccompanied by changes in the microphonic (Fig. 1*b*) and were abolished by anaesthesia. Six of the 13 animals exhibited this kind of input suppression, and of the six, three showed habituation as well (N_1 depressed).

In the anaesthetized or curarized preparation the second click of a click pair gives a full-sized neural response if the interval between the clicks is 20 msec or greater (Fig. 1(*b*); Piddington, 1971, Fig. 4). Continuous repetition of clicks at the same interval still produces full-sized evoked responses, and so the grouping of only ten such clicks into a train presented at 1 train/sec constitutes an optimal stimulus. The finding that the second and later responses (or 'neural following responses') were obviously depressed in the awake animal (Fig. 1*b*) was thus surprising, and in order to lend credibility to the phenomenon a long series of samples was taken and plotted as percentages (N_2/N_1) in a histogram (Fig. 5). The values of N_2/N_1 pooled from the same animals under anaesthetic showed a tight distribution about 100% whereas

Fig. 4. A. Habituation of the N_1 neural response to repeated click trains. Long-term changes at constant sample size. Stimulus is a train of ten clicks at 50/sec, 1 train/sec. Each point is the average of the first click response (N_1) for each of eight such trains. There was a 2 min rest interval between each series of trains producing a given point. N_1, response to first click in each train. Note: relative constancy of microphonic (M_1), suppression of N_1 after bubbler, slow and partial recovery of N_1 after rest, reduction of spontaneous (rectified) neural activity after bubbler.

B. Selective habituation of high-threshold fibres, facilitation of low-threshold fibres, and reversal by anaesthetic. The neural amplitude is plotted against the microphonic for different click intensities. MS 222 anaesthetic was added after maximal habituation. Eight trials averaged per point. Same animal as Fig. 7. Variations in microphonic are due to tank acoustics.

the values taken during free-swimming showed lower means and broader distributions (Fig. 5).

The width of these distributions appeared to depend on the waxing and waning in strength of whatever mechanism was causing response decrement. As already explained, when habituation occurred, restoration to full amplitude could only be obtained if the sound was turned off or if anaesthetic was given. However, for the

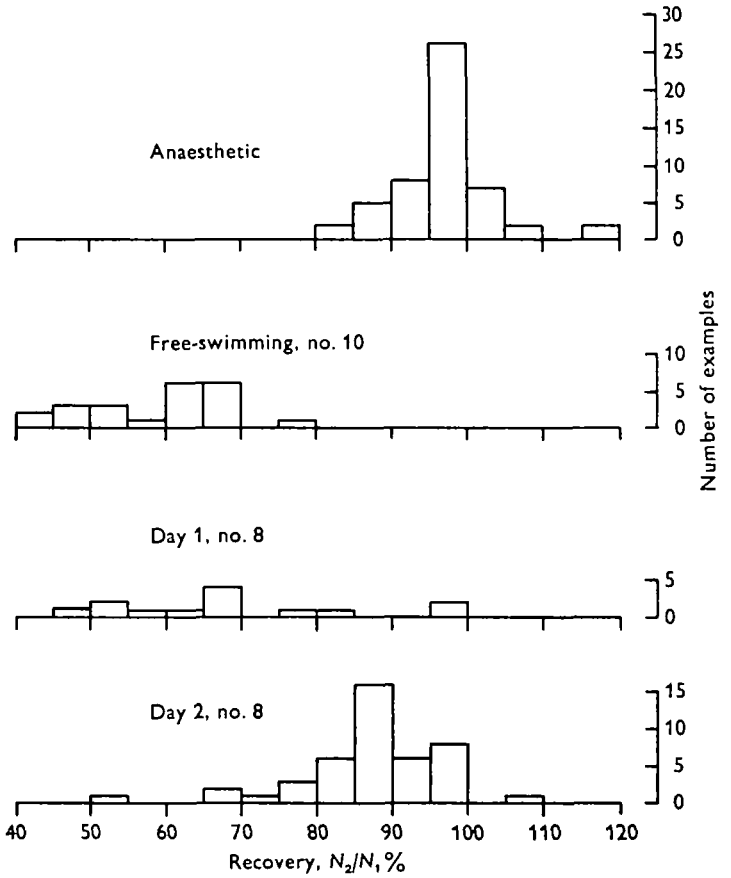


Fig. 5. Feedback. Recovery after a previous click at 20 msec click-separation for two free-swimming animals (8 and 10) is depressed compared to recovery in same animals under anaesthetic. The ordinate is the neural response to the second click (N_2) expressed as a percentage of the first (N_1) and the abscissa is the number of occurrences of a given value. Each value was calculated from the computer average of eight sweeps at 1/sec repetition.

present more rapid form of suppression, full-sized responses were often encountered several times during regularly maintained stimulation. The amplitudes were not uniformly depressed but exhibited wide fluctuations.

In one animal observed continuously for over 1 h the fluctuations occurred with a period of about 2 min, and the example in Fig. 1(*b*) was taken during one of the periods of maximal depression.

Fluctuations in neural following may possibly occur on a longer time base as well. Fig. 6(*b*) shows a clear depression at 100–200/sec (relative to the anaesthetized condition) for the first day after revival but not for the tenth. More remarkable is the

finding that the following at 400/sec on day 10 is actually enhanced or facilitated relative to the value for the initial anaesthetized condition (see Results, facilitation).

The waxing and waning in response amplitude made it rather difficult to measure the temporal properties of the mechanism, and for this reason only one extensive experiment was performed (Fig. 6). By taking large samples (64 trials averaged), and by changing the click frequency between each reading, it was possible to average out the fluctuations and to reveal a definite notch in the N_2 recovery curve at 10 msec after N_1 (Fig. 6*a*). The steady-state curve for the same data showed its maximum deviation from the corresponding curve taken during anaesthesia to be at 100–200/sec. Thus 10 msec appears to be the optimum interval for N_2 suppression and 5–10 msec for steady-state depression.

These results are consistent with the hypothesis that brief auditory stimuli activate a feedback loop which inhibits subsequent input. The loop probably goes through the midbrain, is subject to more central control, and is broken at some point by MS 222 or curare. Central control of this loop is inferred from waxing and waning in the strength of feedback action. Under anaesthetic no feedback occurs and the loop may be considered broken. The breakage point is probably at the torus semicircularis or higher; afferent evoked activity can still be recorded at the torus, and shocks there and higher cause inhibition of the evoked response at the medulla. The 'more peripheral' ascending and descending paths are thus working but the loop as a whole is broken (see Piddington, 1971, fig. 1). The latency from click to response at the torus semicircularis is about 4 msec (see also Groezinger, 1967) and the latency of shock-induced inhibition is about 5 msec, and so a latency of 10 msec is a reasonable figure for the timing of a complete feedback loop passing through the midbrain.

The following are alternate but unlikely explanations for the data: antifacilitation, fatigue or adaptation, electrode drift, noise-masking, reflex muscular control, inconstancy of the auditory stimulus, and desynchronization between fibres. The controls are similar to the controls for habituation. (1) The constancy of the rectified microphonic, with constant base-line level, excludes inconstancy of the auditory stimulus, noise-masking and reflex muscular control (Fig. 1*b*). (2) The constancy of the onset or first click response, which is maintained during fluctuations in the later responses, excludes electrode drift. (3) The demonstration of a finite latency of 10 msec excludes antifacilitation and also fatigue or adaptation. (4) Reversible waxing and waning, going from zero to full following within minutes, further excludes fatigue and adaptation, as does the reviving effect of anaesthetic. (5) The relative constancy in width and waveform of the depressed responses excludes desynchronization. Even the smallest potentials are still sharp in outline.

Interaction of feedback with habituation

Three animals exhibited both habituation and feedback action to sound stimuli. In such animals feedback action became less powerful during habituation, especially during the first trials where habituation was most rapid (trials 1–3, Fig. 7). The simplest explanation is that both habituation and feedback act predominantly on a common population of afferent fibres which at first tend to respond only to the first click (feedback) and then not at all (habituation). The common population consists of high-threshold fibres.

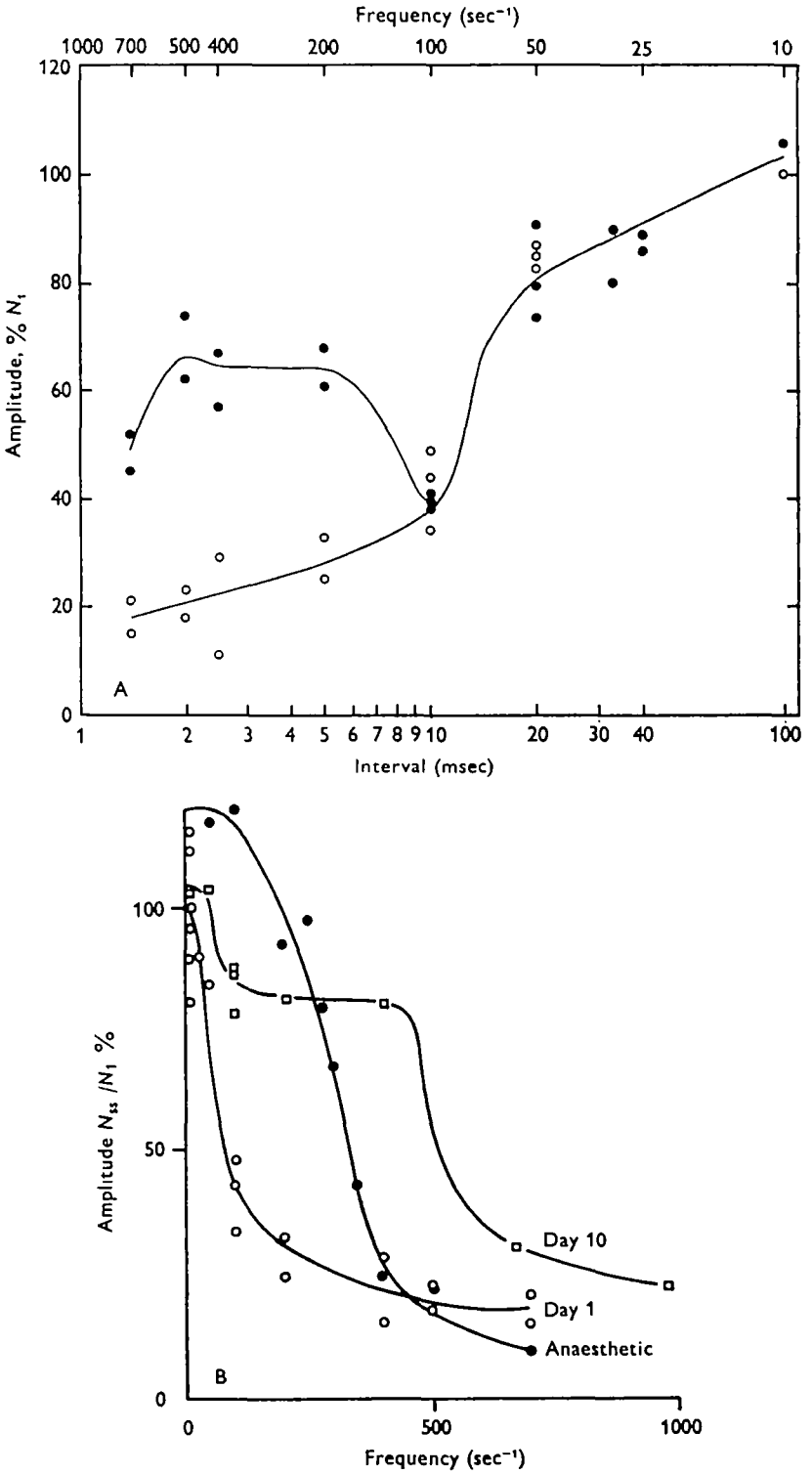


Fig. 6. For legend see facing page.

Facilitation

Facilitation as well as inhibition was detected in several animals and was seen with high-frequency trains (Figs. 1(*c*), 6(*b*)) or as a result of habituation (Fig. 4*b*).

All of five animals tested with click trains at 400/sec showed changes in neural following ability from depressed to enhanced relative to the initial control values obtained during anaesthesia (e.g. Figs. 1(*c*), 6(*b*)). These results were somewhat

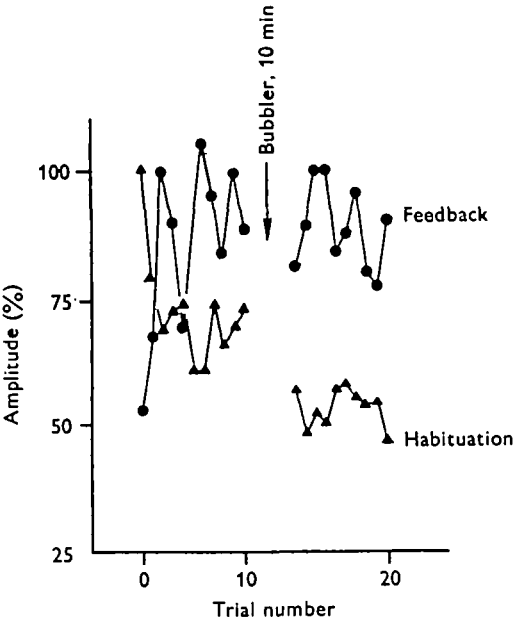


Fig. 7. Effect of habituation to repeated click trains on rapid feedback acting within the train. Note inverse relationship. Stimulus 1/sec trains of 5 clicks at 50/sec. Each point is the average of eight trials at 1/sec separated by rest periods of 30 sec. Feedback is $N_2(x)/N_1$, or amplitude of the response to the second click of train as a percentage of the response to the first click (20 msec earlier) at the given trial (x). Habituation is $N_1(x)/N_1(0)$, or the amplitude of the response to first click of the xth train as percentage of the response to the first click of the first train (zeroth). Same animal as Fig. 4(*b*), same data as Fig. 5 (no. 8, day 2) now plotted in sequence.

difficult to interpret because of synchronization changes between afferent fibres, but the example in Fig. 1(*c*) is relatively free of such changes. (The small difference in N_1 between days 1 and 3 is an example of a synchrony effect; Fig. 1(*c*).)

When facilitation had occurred over long periods, as in Fig. 1(*c*), subsequent anaesthesia caused slow or even incomplete reversal to the value obtained during the implant. Anaesthesia was complex; after only 10 min at 120 mg/l of MS 222 the

Fig. 6. Feedback. A. Latency of action. Amplitude of second click response (N_2, filled circles) and of 'steady-state' level (40 msec after N_1, open circles) as a function of click separation or frequency. Each point, average of 64 trials. Note notch in second-click recovery curve at 10 msec.

B. 'Steady-state' neural following on different days, same animal. Note on day 1: greatest depression relative to anaesthetic is at 100–200/sec. Compare enhanced following at 400/sec on day 10. Day 1 curve is same data as A (steady-state); anaesthetic equals during operation, day 0.

first click response had returned to 'normal' (reversal of habituation) yet both the amplitude and synchronization of the following responses took 90 min to reach their new steady level. (Each of two animals tested with anaesthetic gave this result.)

In an habituation experiment, habituation with high-intensity clicks produced an enhancement in the response to low-intensity clicks, and this enhancement was removed by light anaesthesia (Fig. 4*b*).

There are several possible explanations for these results: (1) changes between facilitation and antifacilitation in the afferent synapses, (2) electrode drift, (3) tonic inhibitory selection of different afferent populations which have different properties, (4) changes in synchrony, (5) control of, or drift in, the strength of a local negative feedback circuit.

Of these explanations only the first (facilitation versus antifacilitation) is consistent with all the data including also the facilitatory effects reported by Piddington (1971). However, for the experiment in Fig. 1(*c*) electrode drift is more likely to have occurred than in the other experiments because the recordings were made over a period of days rather than hours. Neverthess, the kind of rapid neural following exemplified in Fig. 1(*c*) (day 3) was never detected in anaesthetized animals and so is more likely to be caused by physiological conditions in the awake animal rather than by changes in the electrode *per se*. Furthermore, electrode drift cannot be responsible for the slow changes in neural following induced by anaesthesia; for the 80 min period following the first 10 min of anaesthesia (120 mg/l, MS 222) N_1 remains constant while the following responses adjust to a new level. (N_1 is critically dependent on electrode position.)

The specific evidence which collectively indicates that descending control fibres mediate facilitatory effects may be summarized as follows. (1) Fast-acting reversible facilitation by shocks to the midbrain of acute preparations has been demonstrated (Piddington, 1971). (2) The highest neural following ability was found in free-swimming animals; all animals under anaesthesia showed less than 100% following at 400/sec. (3) The greatest enhancements were found in animals rewarded with food (Fig. 1(*c*); see next section). (4) The enhancements were abolished or altered by anaesthesia (Fig. 4*b*) which appears to break the feedback loop in the inhibitory system (see Feedback section).

Conditioning experiments

Large fluctuations in the amplitude of the evoked response were a conspicuous feature of this study, and it was desirable to show that these fluctuations had a meaningful rather than an artifactual basis. Positive reinforcement with food after auditory stimulation was devised as a technique to bias the control system experimentally, and training employing both positive and negative reinforcement of stimuli at different click frequencies was used to find out whether the animal can manipulate the control system in an adaptive manner.

In the first experiments the animals were implanted, revived, and then trained separately. Live brine shrimp were given after 32 repetitions at 1/sec of a 100 msec click train consisting of 40 clicks at 418/sec; samples of the evoked neural activity were taken at intervals. All three animals tested this way showed improvement in neural following, and Fig. 1(*c*) shows an example which was most nearly free of

unwanted synchronization changes. In each animal the level of facilitation reached was higher than in a naive animal (for which see Fig. 6*b*).

The enhancement in Fig. 1 (*c*) correlates with the training but it cannot be concluded that learning had taken place; it was difficult to see a clear-cut behavioural response to the sound, and extinction was not achieved. The application of the stimulus without reinforcement for a period of $\frac{1}{2}$ h on each of 3 days (during which over 6000 click trains were delivered) was insufficient to extinguish the response, which was also unaffected after 1 h of anaesthesia and after 3 days in a sound-proof room. Pseudo-conditioning or mere sensitization could have been responsible, and so the second experiment was designed to control for this by the use of both positive and negative reinforcement of stimuli at different click frequencies. In this experiment positive reinforcement still followed the 418/sec stimulus, but in addition there was negative reinforcement at 100/sec (10 clicks per 100 msec train). The 100/sec stimulus was left on for long periods and no food was given.

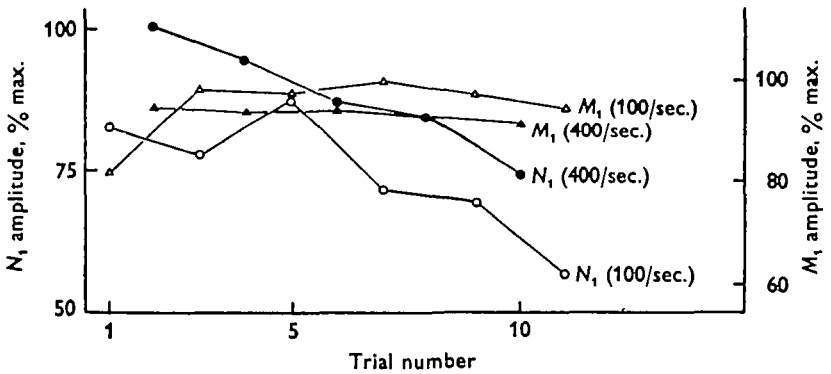


Fig. 8. Effect of prior conditioning on the rate of habituation of N_1; 'boredom' versus food. Responses to a sound associated with 'boredom' (100 clicks/sec) become habituated faster than responses to a sound associated with food (400 clicks/sec). (See text.) The effect is opposite to that of fatigue because the stimulus at 400/sec contains four times the energy of that at 100/sec. Each point or trial is the average of 32 stimuli at 1/sec, with no reinforcement and with 2 min rest periods between trials. The click frequency (100 or 400/sec) is switched between trials. M_1, microphonic response to first click; N_1, neural response to first click. Same animal as in Fig. 1 (*a*).

Training this time was carried out on a group of 10 fish living together, and one fish which most obviously developed the desired behaviour was selected for study.

By the end of 1 week of training (at 5–10 sessions per day) the fish responded positively to both 100/sec and 400/sec stimuli (generalization). The conditioned response was a general increase in swimming rate plus congregation at the particular corner of the tank where the food was routinely added. By the end of a second week, however, the fish had learned to ignore the 100/sec stimulus whilst still responding vigorously to 400/sec.

The fish which was selected gave no behavioural responses on the first day after implantation, but did so after 2 days of re-training in the presence of two other experienced fish from the original 10.

In this animal the neural following at 400/sec was better than at 100/sec, and this

relation was reversed by anaesthesia to the 'normal' condition (for which see Fig. 6*b*). Unfortunately the records were greatly complicated by changes in synchrony of the neural following potentials and also by habituation of the whole train.

Fig. 8 compares the habituation rate of N_1 at the two different frequencies when tested alternately. In Fig. 8 each point is the average amplitude of 32 responses to the first click in the given train (N_1). The microphonic, M_1 (which varies a little as the animal moves to different points in the tank), stays relatively constant while successive averages of the neural response show a slow decline which is habituation. The line linking the N_1 values for 400/sec is above the line for 100/sec throughout its length, and this result is statistically significant. We can reject the null hypothesis that the paired differences between the two lines is zero at the 0·01 level of significance (two-tailed Student's t test). It is therefore concluded that the 400/sec line lies above the 100/sec line, and this indicates that during each habituating series of 32 trains the stimulus associated with food caused slower habituation of the input.

Two further points need to be made. At 400/sec the click train contains 40 clicks; at 100/sec, 10 clicks. If fatigue occurred, the neural responses would be expected to decline faster at 400/sec, yet they do not (Fig. 8). Furthermore, the microphonic (M_1) at 100/sec tends to be slightly bigger than M_1 at 400/sec, and this difference is opposite in sign to the neural difference (N_1). Thus the smaller microphonic gives a larger neural response; the stimulus containing less clicks causes faster decrement. This paradoxical result can be explained by assuming that the animal's habituation rate had become significantly modified by the training procedure, the animal having 'learned' to habituate faster to the negatively reinforced stimulus.

DISCUSSION

This paper presents the first recordings of auditory evoked activity from free-swimming fish subjected to far-field sound (see Piddington (1971) for description of near-field and far-field).

The results show that the neural evoked response can exhibit considerable modification in the absence of changes in the microphonic or receptor potential, and that such modifications are removed by anaesthesia. This is evidence that the descending control system described by Piddington (1971) indeed operates in the awake animal, that masking by noise is not an important factor, and that muscular control of the input does not occur. Anaesthetic appears to break the feedback loops responsible for control action.

Auditory control operates in three distinct modes: (1) rapid inhibitory feedback, (2) slow inhibitory feedback (habituation) and (3) facilitation (Fig. 1). Combinations of these may occur in a given situation (Figs. 4(*b*), 6(*b*), 7), and pilot conditioning experiments indicate that the animal may be able to use these mechanisms adaptively (Figs. 1(*c*), 8).

On the basis of conspicuous summation effects of shocks in the acute preparation (Piddington, 1971), and by comparison with recent results on the mammalian cochlear efferents (Klinke & Gruber, 1969), I postulated that the control system in the goldfish could act selectively on the input; that is, it could inhibit different fibre populations at different times (Piddington, 1971). Is there any evidence that the system operates

thus in the free animal? is the system really useful and, if so, is it an automatic regulating system or can it also be commanded?

Habituation

With slowly repeated intense stimulation, only high-threshold fibres became habituated; the sensitive fibres were even enhanced (Fig. 4*b*). Reversible depression equivalent to habituation was obtained by creating noise with a bubbler (Figs. 4(*a*), 7), but dishabituation was never obtained. Rowell & McKay (1969) reported lack of dishabituation in an insect auditory interneurone.

Some workers report no habituation at lower neural levels in mammals (Wickelgren, 1968), but by mixed results show that chance selection of animals and recording sites can be crucial.

Underwater sounds with minimal near-field (as in this study) are probably heard as distant sounds (the lateral line is not stimulated; see Piddington, 1971), and lack of habituation in sensitive fibres (Fig. 4*b*) could mean that these fibres are for alerting the fish to weak sounds (see also Rowell & McKay, 1969). Conversely, the high-threshold alarm fibres (S_1 of Furukawa, 1966) might well be expected to become habituated because the present click stimulus (an intense, repeated far-field sound that completely saturates the sacculus) could certainly be called useless and also monotonous (avoidance of 'false alarm'). Furthermore, in these experiments a stone bubbler produced habituation, and this could mean that fish living in surf zones or in areas near river rapids keep their high-threshold fibres habituated for long periods.

As evidence of a non-automatic or commanded component of habituation a pilot conditioning experiment suggested that responses to a sound associated with food became habituated slightly slower than responses to a sound associated with boredom (Fig. 8), although the number of experiments is not sufficient to establish this. In mammals centrally commanded action of the cochlear efferents would account for conditioning at the cochlea, complex interference by vision (Buño *et al.* 1966), habituation (Veselý, 1963) and for their recently postulated role in frequency sharpening (Capps & Ades, 1968).

Negative feedback action

Prominent negative feedback or depression of subsequent input after a click (latency 10 msec, decay-time less than 100 msec) was found to exhibit pronounced waxing and waning in strength, acting most strongly on high-threshold fibres (Results, interaction with habituation). Feedback is itself an automatic regulatory function but control of its strength is more likely to be commanded centrally.

The latency of 10 msec was shown to be consistent with the action of a feedback loop involving the midbrain (see Fig. 1, Piddington, 1971), and reasons were given in support of the idea that anaesthetic breaks the loop at the midbrain level. However, anaesthetic could alternatively be removing central permissive influences which normally control the strength of the loop.

The fish has no muscular control over the input, but in mammals special middle-ear muscles act 10–15 msec after a sound to suppress subsequent input (Galambos & Rupert, 1958). The middle-ear reflex also appears to be under more central control (see Grinnell, 1969).

Facilitation

The evidence for descending facilitatory control is less consistent than for inhibition but if verified would supplement recent work on descending facilitation in the mammalian medulla (Whitfield, 1968).

Pre-synaptic control of high-frequency potentiation (a type of facilitation) has never been postulated (e.g. Eccles, 1964, 1969) but could explain the data on high-frequency following (Figs. 1(*c*), 6(*b*)). Both frequency potentiation and habituation can occur in a single motor synapse (Bruner & Kennedy, 1970), and in the cochlea habituation is controlled by the crossed efferents which act pre-synaptically on the outer hair cells (Fex, 1968; see also Hama, 1969). Thus if both frequency potentiation and habituation occur in single afferent synapses, then in principle central control could act on both functions.

In summary, there are indications from several different kinds of experiments that the descending control system in fish can act selectively on the input and that it may do so either automatically after certain sounds (feedback, habituation) or as a result of central commands (waxing and waning, conditioning).

Conditioning experiments

The results indicated that conditioning procedures may operate in biasing the animal's control system. Positive reinforcement after 400/sec click trains appeared to give an enhancement in neural following (Fig. 1*c*). An experiment in which two different frequencies were reinforced differently (400/sec positively and 100/sec negatively) indicated that the habituation rate to the positive frequency had become significantly slower relative to the habituation rate to the negative frequency (Fig. 8).

These experiments were designed to show that the wide variability in afferent responses encountered in the free animal occurred as a result of changes in the influences exerted by the control system. The experiments bear this out but need to be repeated. The system as a whole appears to be complex rather than unreliable (see review by Bullock, 1970) and the conditioning results confirm the thesis of Buño *et al.* (1966) that auditory efferents can mediate conditioning effects at the periphery.

Functions of the descending control system

(*a*) *Prevention of self-excitation during sound production.* Mammals can blank out self-made sounds by contracting the middle-ear muscles (Grinnell, 1969) but fish have no such mechanism and would have to rely on neuronal inhibition to achieve the same result. Inhibitory feedback in the fish is equal in latency to the mammalian middle-ear reflex (10–15 msec, Galambos & Rupert, 1958) and so appears temporally suitable for rapid blanking.

Circumstantial evidence for blanking does exist: Hama (1969) has proposed that saccular efferents mediate the inhibition of primary auditory fibres which occurs as a consequence of discharges in the giant Mauthner cell (Furukawa, 1966). The M-cell tail flip probably produces considerable sound energy both directly by the rapid distortion of the musculature and indirectly by the tail pressing on the water (see Tavolga, 1964). If this is so, then the simultaneous inhibition of auditory fibres would function as a neural blanking process diminishing self-stimulation during sound pro-

duction and especially preventing re-excitation of the M-cell. Neural blanking seems essential in species that produce sounds for communication (see Tavolga, 1964, 1967; Cohen & Winn, 1967; Salmon, 1967).

(*b*) *Attentional control.* Habituation, which appears to act selectively on high-threshold fibres (Fig. 4*b*), could be a mechanism for shifting the animal's attention between loud (alarm) and soft (alerting) sounds. Similarly, the feedback mechanism, which appears to be under more central control, would appear to be shifting the coding emphasis from transmission of only the onset of a given complex sound (maximum feedback action) to transmission of the fine structure of the sound (minimal feedback action plus frequency following).

(*c*) *Frequency analysis and discrimination.* Pitch discrimination in fish can be as good as 3 % (Dijkgraaf & Verkeijen, 1950; Enger, 1963) but the analysing mechanisms are poorly understood. Basically two theories have been considered – the volley theory (phase locking) and the place theory (different tuning curves) – and evidence can be found favouring either (e.g. Enger, 1963; Groezinger, 1967; Furukawa & Ishii, 1967*a*; van Bergeijk, 1967; Grinnell, 1969; Page, 1970; Andersen & Enger, 1970). However, Tavolga's (1966) demonstration of critical masking bands excludes the volley theory, according to van Bergeijk in the discussion of that paper. The complex structure of the goldfish sacculus may allow for as yet unmeasured resonance effects. (See Furukawa & Ishii, 1967*a*; van Bergeijk in Tavolga, 1966; Andersen & Enger, 1970; and note the effect of intensity in Fig. 2*a*.)

Recent behavioural experiments on the mammalian cochlear efferents reveal that these fibres may sharpen frequency differences (Capps & Ades, 1968). The present experiments show that neural following, or the probability of firing during a click train, tends to decrease as frequency is increased (Fig. 6*b*). In an untrained animal the steep sections of the frequency curve may cover different ranges on different occasions. On day 1 (Fig. 6*b*) frequency analysis by neural following differences could theoretically be best between 50 and 100/sec, but on day 10, best at around 500/sec (Fig. 6*b*).

The shape of the frequency curve could be controlled by the descending fibres; the striking low-frequency cut-off at 100/sec in the day 1 curve (Fig. 6*b*) is probably caused by negative feedback, and the enhanced following at 400/sec on day 10 is probably caused by facilitation. The fish may be able to bias the afferent system so as to code preferentially for particular frequency differences. In a conditioned fish the biasing may be so strong as to cause a reversal in slope of the frequency curve (note Fig. 1*c*) and such reversal is not seen in anaesthetized or untrained animals. The physiological mechanism is obscure but similar frequency reversals can be demonstrated in single synapses (Bruner & Kennedy, 1970).

Biological significance of hearing in fish

It should be emphasized that we cannot determine the natural use of the control system, or of the fish auditory system as a whole, by using pure tones, clicks, and conditioning techniques in the laboratory (see Loewenstein, 1957; van Bergeijk, 1967). What is an interesting sound for a fish? Ethologists have not yet discovered a biologically relevant sound (e.g. Loewenstein, 1957; Dijkgraaf, 1960; Nicol, 1960; Tavolga, 1964, 1967; van Bergeijk, 1967) except for those species which have developed special

sonic mechanisms for communication (e.g. Moulton, 1963; Tavolga, 1964; Cohen & Winn, 1967; Salmon, 1967; Salmon, Winn & Sorgente, 1968).

In this vacuum of suggestions for uses of hearing in fish it may be worth while to propose one. This is that fish can tell whether other swimming fish are approaching or receding or moving tangentially by analysing the proportions of time in compression and rarefaction phases of the 'infrasonic' sound waves made by the swimming motions. Evidence that fish have specific sensitivity for both compressions and rarefactions has existed for over 20 years (double-frequency microphonic, Zotterman, 1943), yet the biological significance has never been explained nor even speculated upon. Single nerve fibres from the sacculus fire on the compression, rarefaction, or both phases of a sound (Furukawa & Ishii, 1967*a*, *b*; P. S. Enger, personal communication), whereas auditory fibres in the mammal fire only on the rarefaction (Grinnell, 1969). I think, therefore, that an unexplored function of hearing in fish is to be found in the fundamental domain of compressions and rarefactions.

A full explanation of this hypothesis is out of place in this paper but five main points will be considered. (1) The sound field of a swimming fish is probably complex (van Bergeijk, 1964) and probably asymmetrical with respect to compressions and rarefactions. (2) Extensive evidence exists for separate neural coding of compressions and rarefactions. (3) Although the swimbladder resonates between about 100 and 2000/sec, it nevertheless responds to lower frequencies, including static pressure (Furukawa & Ishii, 1967*b*; compare low-frequency hearing in man, von Bekesy, 1967). (4) For low frequencies, the underwater near-field extends to large distances (van Bergeijk, 1967), but the swimbladder auditory system responds to the near-field as well as the far-field (Enger, 1966, 1967). (5) The lateral line can distinguish between right and left symmetrical sources but the auditory system cannot (von Frisch & Dijkgraaf, 1935; van Bergeijk, 1964, 1967).

Swimming organisms are probably the most prevalent, biologically significant sources of sound underwater (Cousteau, 1953; Haas, 1958; Nicol, 1960; van Bergeijk, 1964, 1967); and the sounds of fish swimming have indeed been recorded by hydrophones (Moulton, 1960; Tavolga, 1964). Most sounds are of low frequency, but some are directly audible to a human diver (Moulton, 1960; Cousteau, 1963; original), ranging in sound quality from soft fluttering to thumps and whip cracks (cavitation).

Van Bergeijk (1967) has related the near-field of a swimming fish to the lateral line, but no one has described the pressure field at a distance. Swimming fish and whales slice through the water, undulating to and fro. Like skating or skulling, the main feature of this kind of motion is that the fin or blade applies pressure at right angles to the surface while the water flows along parallel (Parry, 1949). The undulations of the body and tail probably produce a spatially complex pressure field.

In fish and in whales the tail fin rapidly reverses its angle of attack at the end of each half cycle of the tail beat (Breder, 1926; Parry, 1949) and thus should produce a compression to the rear during both phases of the movement. The pressure waveform from behind should be asymmetrical, with more time spent in the compression phase, and should be at double the tail-beat frequency. From in front of the animal the waveform may be less asymmetrical due to the smoothed motions of the head, but should still be of double frequency and probably with rarefaction predominant. By contrast, the waveform to either side of the fish should be symmetrical, and at the

same frequency as the tail beat (though probably not a pure sinusoid). (Compare the asymmetric waveforms of a flying fruit fly Williams & Galambos, 1950.)

With appropriate analysis of the asymmetry, or ratio of compression time to rarefaction time, the listening fish should be able to determine if the particular swimming organism is coming, going, or passing, and, having done so, it could suitably alert the visual and lateral-line systems to be on the lookout (cf. Pumphrey, 1950).

Evidence of differential sensitivity to compressions and rarefactions exists for seven species of teleosts, and the basis is in the organization of the hair cells into two oppositely orientated populations (Hama, 1969; see also Grinnell, 1969; Lindeman, 1969). The physiological consequences are: (1) a double-frequency microphonic (Zotterman, 1943; Enger & Andersen, 1967; Cohen & Winn, 1967; Furukawa & Ishii, 1967*a*, *b*), (2) discrete coding by units in the auditory nerve (Enger, 1963, and personal communication; Furukawa & Ishii, 1967*a*, *b*), and (3) discrete double-frequency evoked potentials up to the midbrain (Groezinger, 1967). There is no behavioural evidence that fish can discriminate compressions from rarefactions.

The tail beat of fish and of whales can be at 1 or 2/sec (Parry, 1949; Gero, 1952) and at such low frequency the near-field extends to large distances in the order of 1 km (see van Bergeijk, 1967). Enger (1966) has shown that the swimbladder-auditory system responds to both near-field and far-field and that the threshold in terms of particle displacement is relatively constant at widely different distances from the underwater loudspeaker. (This is a relatively new discovery in fish hearing.) For goldfish the threshold is 1 Å at 50/sec (Enger, 1966), and this is equal to the lateral-line threshold (1–20 Å, van Bergeijk, 1967). However, the lateral line does not respond to the pressure component in a sound field (van Bergeijk, 1967), and so at a sufficiently large distance from the source only the swimbladder system will respond.

A full mathematical treatment is outside the scope of the present, and the reader is referred to Parry (1949), Gero (1952), Harris & van Bergeijk (1962), Enger (1966), van Bergeijk (1967) and U.S. Navy (1969). Further assessment of this hypothesis requires recordings of the exact waveforms made by swimming fish (pressure and displacement) and also evidence that fish can read the phase information.

SUMMARY

1. In the free-swimming electrode-implanted goldfish, the neural response in the medulla to a constant auditory stimulus may exhibit reversible fluctuations in amplitude which are abolished by anaesthesia.

2. The results are consistent with the action of an auditory control system which can reduce or enhance the input following a click.

3. Noise-masking effects and reflex muscular control were excluded by demonstrating the relative constancy of the rectified microphonic during simultaneous changes in the click-evoked action potential at the medulla.

4. There are three kinds of response modification: habituation, rapid inhibitory feedback, and facilitation.

5. Both feedback and habituation act predominantly on high-threshold auditory fibres. Low-threshold fibres do not become habituated, and dishabituation does not occur.

6. As in the mammal, anaesthetic reduces the tendency of the system to become habituated by an amount which depends on the dosage. Auditory fibres with highest threshold have the greatest tendency to become habituated and are the least affected in this respect by anaesthetic.

7. Simple conditioning experiments indicate that control influences exerted over the input can be biased by positive or negative reinforcement which follows the auditory stimulus.

8. The control system may work in attention, in frequency analysis, or in suppressing input to self-made sounds.

9. A new hypothesis is made on the biological significance of hearing in fish. A fish may be able to tell if other swimming fish are approaching, receding, or moving tangentially by analysing the proportions in time of the compressions and rarefactions present in the swimming sounds, which are proposed to be asymmetrical.

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