

THE CHANGE OF DISCHARGE FREQUENCY BY A.C. STIMULUS IN A WEAK ELECTRIC FISH

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INTRODUCTION

A South American gymnotid, *Eigenmannia*, is known to be a kind of weak electric fish, producing continuously the electric organ discharge with an intensity of about 1 V. and a constant frequency of about 300 cyc./sec. at 25° C. (Coates, Altamirano & Grundfest, 1954; Lissmann, 1958). The frequency is a function of temperature, with a Q_{10} of about 1.5. At a fixed temperature, however, the discharge frequency is extremely constant. Furthermore, the constancy is not affected by several kinds of artificial stimuli, including the ordinary electrical stimulus (cf. Grundfest, 1957). On the other hand the animal is apparently highly sensitive to the change of the electric field surrounding it; it escapes from a metallic conductor but is indifferent when the conductor is insulated (Grundfest, 1957). The exact measurement of the threshold has, however, not yet been made.

This paper is concerned with a peculiar response of the fish to an externally applied electrical current. The effective stimulus was an alternating current with a frequency very close to the discharge frequency of the fish, to which the latter changed slightly but significantly. It will be shown that the threshold of this response is very low (around 10 μ V./cm. in some fish), suggesting that some 'electric receptor' receiving the stimulus signal is at work.

METHODS

The experiments were performed on five fish supplied from a tropical fish dealer in Tokyo. The length of the fish ranged between 10 and 13 cm. They were kept in an aquarium at a regulated temperature of 24° C.

All experiments were done with the intact fish. It was kept in water in a trough of 1 cm. width, 14.5 cm. length and 2 cm. depth, made with two paraffin blocks in a Lucite box (Fig. 1). At both ends the trough was open to two pools. In each of them a silver plate stimulating electrode (2 \times 5 cm.) was immersed. At the opening of the trough some cotton was wadded to prevent the fish from escaping into the pool. The fish usually kept quiet in the trough during the experiment. The recording electrodes were two silver wires which were placed on the cotton waddings at both ends of the trough.

During the early experiments there was no water circulation. It was later found that the stability of the resting discharge frequency was greatly improved by water circulation. Aerated tap water, with a trace of sodium hyposulphate to remove

chlorine, was supplied to one of the pools at an end of the trough, and the excess was carried away by suction.

The discharge of the fish was picked up differentially by a cathode-follower input probe (Grass P6) with a grid current of less than 10^{-10} A. Under the present recording conditions the available potential was several hundred millivolts. The stimulating circuits were composed of an audio-frequency oscillator and an attenuator. The circuit was usually shunted by a key so as that the stimulating current could be applied at a definite time. To avoid possible stimulating effects due to shunting of the ends of

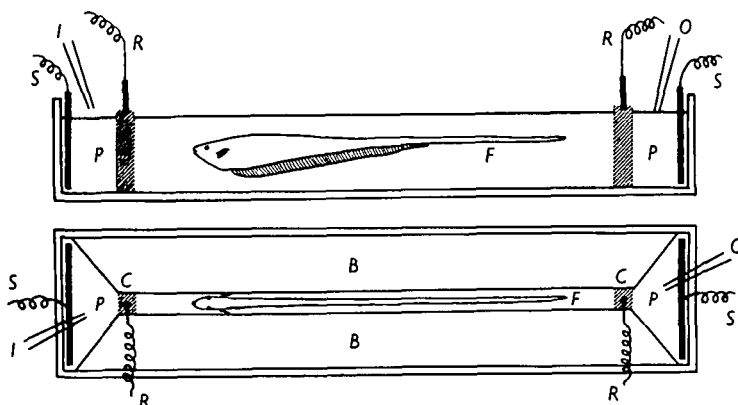


Fig. 1. General arrangement for the experiment. Upper: the side view. Lower: the top view. *B, B*: two paraffin blocks. *C, C*: cotton waddings. *F*: the electric fish. *I*: the inlet of circulation water. *O*: the outlet of circulation water. *P, P*: pools at both ends of the trough. *R, R*: two silver-wire recording electrodes. *S, S*: two silver-plate stimulating electrodes.

the trough, resistors of variable values ($25\text{ k}\Omega$ – $100\text{ M}\Omega$) were inserted in series with the stimulating electrodes. The applied current was monitored across a resistor of $12\text{ k}\Omega$. Usually the stimulus was applied for 30 sec., measured by a stop-watch.

To find out the frequency change an audio-frequency oscillator was used as the standard. Two methods were adopted to compare the discharge frequency with that of the standard oscillator. The first was to utilize the Lissajous figure. The x -axis of a cathode-ray oscilloscope was driven by the oscillator, the fish discharge being fed to the y -axis. When the two frequencies were closely matched the Lissajous figure stood still. When the discharge frequency started changing the figure was disturbed at once. Although this method was preferable for detecting the occurrence of the response, it gave neither the amount nor the direction of the frequency change unless the frequency of the oscillator was moved to follow the discharge frequency. This inconvenience was overcome by using the second, 'beat' method. The fish discharge was fed into one of the two inputs of the differential y -axis amplifier of the oscilloscope, the output of the standard oscillator being fed into the other. The two signals were thus mixed, and when the two frequencies were comparable the difference of the frequencies could be obtained from the frequency of beating. This method allowed us to measure the time course, direction and magnitude of the change of discharge frequency.

All experiments were done at a room temperature of 22 – 26° C .

RESULTS

To make the description briefer, two simple notations will be introduced.

The difference between the stimulus frequency and the resting discharge frequency will be denoted as ΔS . If the stimulus frequency is higher than the resting discharge frequency, ΔS will take the positive sign. Thus $\Delta S = S - R_0$, where S is the stimulus frequency and R_0 the resting discharge frequency.

When an appropriate stimulus is applied to the fish, it changes its discharge frequency. The direction and magnitude of the change in discharge frequency will be represented by ΔR . If the discharge frequency increases, ΔR will take the positive sign. Thus $\Delta R = R - R_0$, where R is the discharge frequency during the response. The absolute value of ΔR will be called 'the response magnitude'.

Constancy of the discharge frequency in the resting condition

In Fig. 2A a record of the fish discharge is presented. The remarkable constancy of the resting discharge frequency has already been mentioned. At 26° C. the discharge frequency of a fish, swimming freely in the aquarium, was traced for 1 hr. by the Lissajous figure method and it was found to be between 320 and 321 cyc./sec. A variation of this order is well within the experimental error.

When the fish was put in the experimental trough the frequency change became more prominent. Often the frequency decreased gradually at a rate of 1 or 2 cyc./sec./min. It was difficult to explain this decrease by the temperature change only, because sometimes the discharge frequency continued falling even when the room temperature was gradually rising. It was probably due to the adverse experimental conditions, such as insufficient supply of oxygen. This frequency decline could almost be eliminated by circulating the water through the trough. When the resting discharge changed in a rate more than 0.5 cyc./sec./min., the response data was usually discarded.

Under these experimental conditions the constancy of the resting discharge frequency was not disturbed by several kinds of stimuli, such as tapping the experimental desk, turning on and off the light or moving a magnet outside the trough.

The response to alternating current with a frequency close to that of the fish discharge

When an alternating current with a frequency very close to that of the fish discharge was used as stimulus, the fish responded to it by changing its discharge frequency. In Fig. 2B one example is shown using the Lissajous method. At first the stimulating frequency was adjusted so that the Lissajous figure did not move very quickly (B_1). When the stimulus was applied to the fish, the Lissajous figure began to be disturbed, indicating that the fish discharge frequency had changed (B_2). After the termination of the stimulus the disturbance gradually disappeared, indicating that the frequency change was temporary and reversible (B_3). The fish did not manifest any violent movement during the response when the stimulating current did not exceed 10^{-7} A. In Fig. 2C the same effect is demonstrated by the 'beat method'. For determining the direction of the response, two audio-frequency oscillators were used. In Fig. 2C, the lower beam is the mixture of the fish discharge and the output of oscillator no. 1. The frequency of this oscillator was set about 3 cyc./sec. above the fish discharge

frequency, so that the beating was already taking place before stimulation. The upper beam is the mixture of the fish discharge and the output of oscillator no. 2, which was used as the stimulator. On application of the stimulating current to the animal the beat frequency on the lower beam started decreasing, whereas that on the upper beam started increasing. Since oscillator no. 1 was set above the discharge frequency, the decrease of the beat frequency on the lower beam indicated an increase of the discharge frequency.

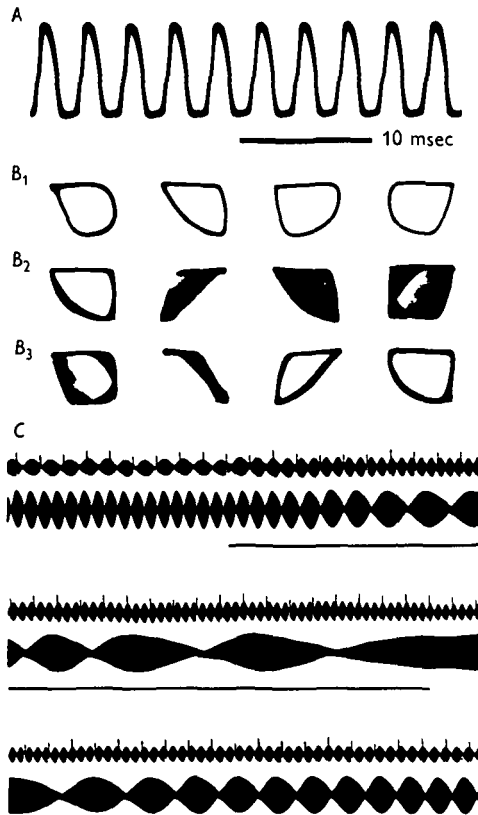


Fig. 2. *A*: the spontaneous discharge of the electric organ. *B*: Lissajous's figures obtained by combining the fish discharge (*Y*-axis) and the stimulating sinusoidal signal (*X*-axis). Records are taken at about 8 sec. intervals. *B*₁: before stimulus application. *B*₂: during stimulus application. *B*₃: after stimulus application. *C*: demonstration of the frequency change of fish discharge by the 'beat method' (see text). The spikes superimposed on the upper beam are the time signals at 1 sec. intervals. The period of stimulus application is shown by a bar under the two beams.

In typical cases the frequency change started within 1 sec. after the beginning of the stimulus, increased gradually and attained to a steady level in about 10 sec. (see Fig. 6*B, E*). After the cessation of the stimulus it slowly subsided, and in about 1 min. the frequency recovered almost completely to its original value.

Although this response was elicited with unexpected regularity, it was found that sometimes it failed to occur. Furthermore, even when it was elicited, the directions and magnitudes of the response were not constant. The rates of rise were also variable. These diverse effects were mainly due to the fact that ΔS was by no means constant

in each case of stimulation. It was found that the direction and magnitude of the response was extremely critically influenced by the sign and magnitude of ΔS , as will be described below.

The relation between the direction and magnitude of the response and the stimulus frequency

When ΔS was more than ± 20 cyc./sec., ΔR was, if present, very small. Weak responses could sometimes be recognized when ΔS was around ± 10 cyc./sec. The smaller ΔS the larger was the response magnitude, except when ΔS was very close to zero, where the response magnitude again became very small. These features will be seen in Fig. 3 in which the relation between ΔS and ΔR is plotted.

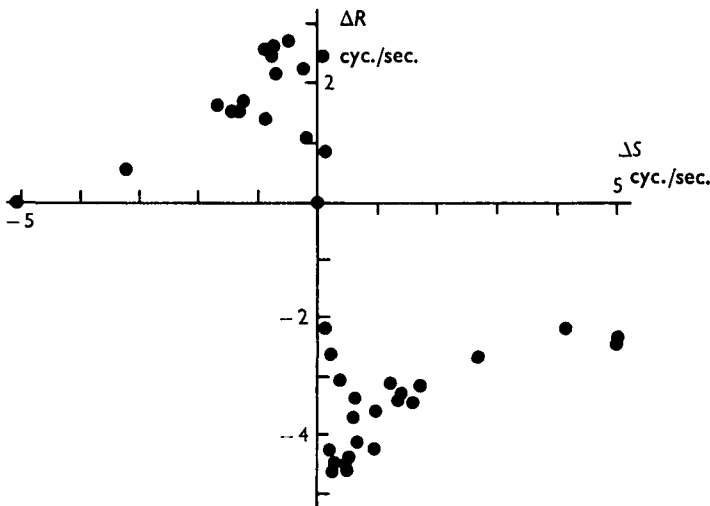


Fig. 3. The relation between ΔS and ΔR of one fish.

The most notable character of this relation is the fact that the direction of the response is determined by the sign of ΔS . When the stimulus frequency is higher or lower than the discharge frequency, the response is a decrease or an increase of the discharge frequency, respectively. In other words, when ΔS is positive, ΔR is negative, and vice versa. The discharge frequency changes in such a direction that the difference becomes bigger.

When ΔS is very close to zero, either the direction of the response does not follow to the above simple law, or the response does not take place at all. It can be said that a finite value of ΔS is necessary for the determination of the direction, and when ΔS is zero, the state may be regarded as being at an unsteady equilibrium. Probably a spontaneous fluctuation of the discharge frequency can trigger the response, because even if the fluctuation is very small, the frequency difference will be increased once the response is elicited. Otherwise, however, the response cannot occur.

Examination of the possible roll of the phase relation between two signals

For explaining the ineffectiveness of stimulation when $\Delta S = 0$, it may be proposed that a certain phase relation is necessary between the two signals to elicit the response. This proposed explanation is, however, not likely to be true as is shown by the following experiments.

A square-pulse wave was also found effective in eliciting the response when the frequency was close to that of the fish discharge (Fig. 4*A*). When, however, the square pulse was triggered by the amplified fish discharge signal, the response did not take place at all (Fig. 4*B*). This is reasonable, because in this case ΔS is strictly zero. It was further possible to change the phase relation between the two signals by changing the delay time of the square-pulse generator or by reversing the sign of the pulse. Regardless of these procedures, the stimulus was found to be completely ineffective.

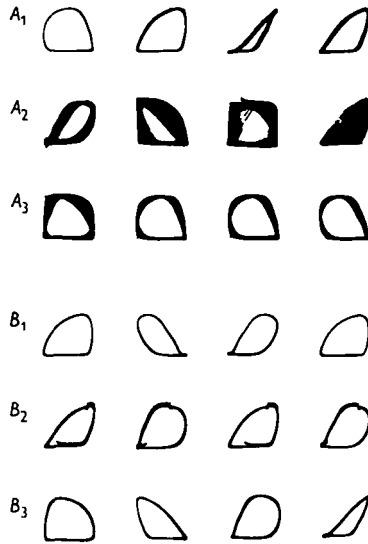


Fig. 4. The ineffectiveness of the stimulus when the frequency difference between two signals is strictly zero. Lissajous's method. Records are taken at 8 sec. intervals. *A*, the stimulus frequency is very close to the fish discharge frequency. *B*, the stimulus is synchronized with the fish discharge frequency by the electronic triggering device. *A*₁, *B*₁: before stimulation. *A*₂, *B*₂: during stimulation. *A*₃, *B*₃: after stimulation.

Effect of 'chasing' the discharge frequency by changing the stimulus frequency

Since the response seems to be an 'escape' from the applied stimulus frequency, attempts were made to 'chase' the discharge frequency by changing the stimulus frequency slowly in the direction of the response. In Fig. 5 one example is presented. At first ΔS was $+0.8$ cyc./sec. so that the response was a decrease of the discharge frequency. After 30 sec. the response gradually attained a steady level, but a decrease of the stimulus frequency caused a further decrease of the discharge frequency. The same kind of response occurred several times, but the frequency change gradually attained a limiting value of about 6.5 cyc./sec. which made further decrease of the stimulus frequency ineffective. Eventually a further decrease of the stimulus frequency caused an increase of the discharge frequency, which thus moved towards the

stimulus frequency, crossed it, and returned to the original level quickly. It may be concluded from this type of experiment that there is a definite range of frequency over which the response can occur.

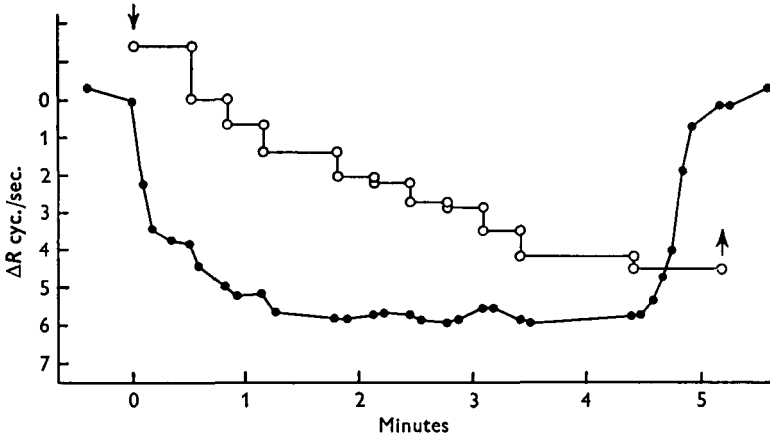


Fig. 5. Effect of 'chasing' the discharge frequency by changing the stimulus frequency towards the discharge frequency. ○, stimulus frequency; ●, discharge frequency. Arrows mark the beginning and end of the period of stimulation.

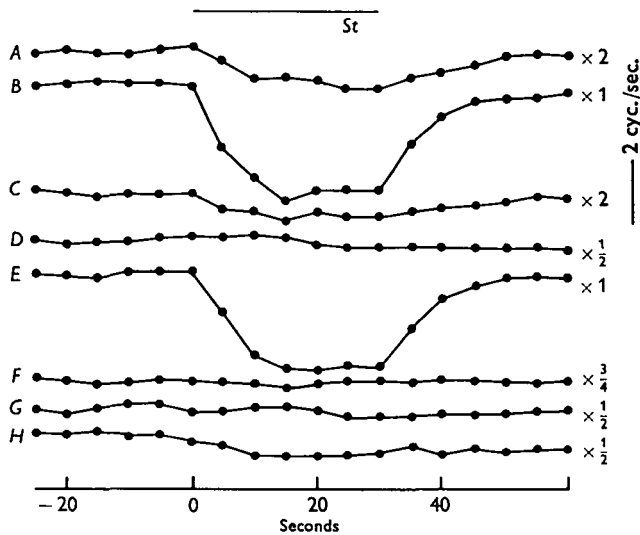


Fig. 6. Responses on stimulation with frequencies of various multiples of the discharge frequency. Successive records from one animal. The multiples are indicated at right-hand ends of the curves. The bar *St* indicates the period of stimulation. The stimulus frequencies are always set slightly higher than the exact multiples of the discharge frequency to avoid ineffectiveness due to too-close matching.

Stimulation with double-frequency signals

When the stimulus frequency was twice the discharge frequency, a weaker effect could be observed (Fig. 6). The amount of the response can roughly be taken as one-third of that in which the stimulus and discharge frequencies are roughly the same. Other multiple frequencies were also tried, but the effects were far weaker. The effect of half-frequency stimulation was also very weak, if present. It is improbable

that the harmonics in the stimulating oscillator are responsible for eliciting the response. The oscillator (Yokokawa Co. OV-CR-201) is supposed to have a content of higher harmonics of 1% and the content of lower harmonics is far less. If the above response had been elicited by harmonics, half-frequency stimulation should have caused a stronger effect than double-frequency stimulation. The actual results were, however, to the contrary.

Effect of mixing two signals with different frequencies

The fish seems to detect a 'significant' signal from a mixed signal. In an experiment the stimulating signals were composed of two a.c.'s with different frequencies and intensities. No. 1 had a frequency close to the fish discharge ($\Delta S \approx +1$), but the

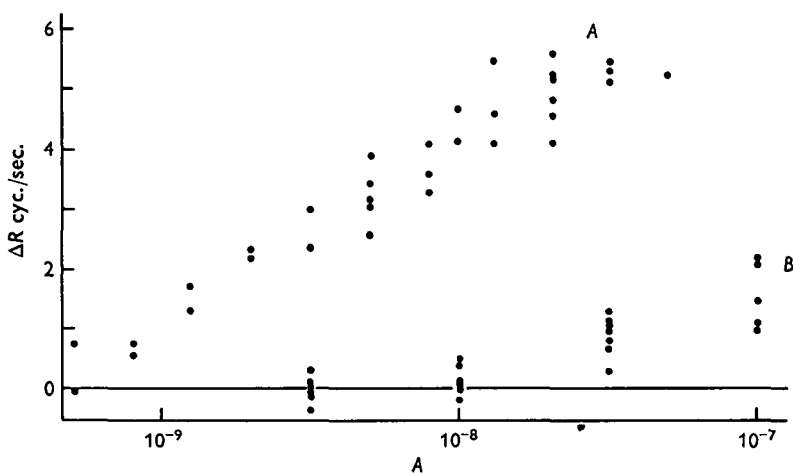


Fig. 7. The relation between the stimulus intensity and the magnitude of response. ΔS is set about +1 throughout. Data from two different animals (A and B).

intensity was weaker. No. 2 had a frequency far from the fish discharge ($\Delta S \approx +35$), but the intensity was about six times stronger. As usual, no. 1 alone was effective, whereas no. 2 alone was not. When the two signals were mixed and applied to the fish, the latter responded as if no. 1 was applied alone, even though there was a very strong 'insignificant' signal in the background.

The relation between the stimulus intensity and the response

When the intensity of the applied current is increased, the magnitude of the response increases. In Fig. 7, an intensity/response diagram is presented. The values obtained at a fixed intensity suffered rather large variations. One obvious reason for this is the difficulty of adjusting the stimulus frequency to maintain a constant ΔS . It can, however, be concluded that the magnitude of the response is roughly linearly related to the logarithm of the stimulus intensity.

From Fig. 7 a rough estimate of the thresholds for this response may be made. They are 10^{-8} A. and 3×10^{-10} A. To obtain the potential gradient around the fish it was necessary to measure the resistance of the water in the trough. When the experimental trough was filled with aquarium water, the resistance along its length was about 3 k Ω /cm. When the fish was put in the trough, this value increased. With one fish, it was about 10 k Ω /cm., which was adopted as the basis for the calculation.

Multiplying this value by the threshold current intensity, one obtains 3–100 $\mu\text{V./cm.}$ as the threshold potential gradient for this response.

When the stimulating current was strong, a strong decrease of the discharge frequency could be elicited, regardless of the sign of ΔS . The recovery from this type of response was slow and irregular; it took more than 2 min. and thereafter the resting discharge was different from that before stimulation. This type of response seems to belong to a different type. In one animal, stimulation with 10^{-6} A. caused this type of response. Detailed observations, however, were not made.

DISCUSSION

It was found that a peculiar response exists in this electric fish. When a sinusoidal (or a square pulse) electric signal with a frequency similar to that of the fish's own discharge is applied to the fish the latter's discharge frequency changes as if to escape from the applied signal frequency. The effectiveness of the stimulus depends on the difference between the two frequencies (ΔS); when ΔS is more than 10 cyc./sec. the response is barely recognizable. The smaller ΔS , the more effective the stimulus, except when ΔS is very small, where the response again fails to occur.

One of the remarkable characters of this response is its regularity. Its constant appearance, and its obedience to simple laws, seem to suggest that the centre of this response is a part of the C.N.S. with relatively little plasticity.

The threshold for this response is very low. Obviously the fish has to receive the applied signal by some means, and the threshold for perception can still be lower than the threshold for response. Perception cannot, however, depend upon direct stimulation of nerve fibres in the fish. The potential gradient which is necessary to elicit a clear response can be less than 10 $\mu\text{V./cm.}$ in some fish. Even if this value of the potential were directly imposed upon the membrane of some neuron, it is very unlikely to cause any excitatory effect, because the threshold depolarization of the known excitable membranes is of the order of 10^{-2} V. Admittedly, there are nerve fibres which, under some conditions, can detect smaller voltage gradients around them (see Terzuolo & Bullock, 1956). The sensitivity which the present experiments indicate is, however, still far too high to be explained on such a basis. It seems that an electrical receptor of some kind must be postulated. Lissmann (1958), Lissmann & Machin (1958) and Machin & Lissmann (1960) made the same supposition to explain the behaviour of another electric fish, *Gymnarchus niloticus*. It was supposed that the receptor is innervated by the lateral line nerve, and in fact Hagiwara, Kusano & Negishi (1962) and Hagiwara & Morita (1962) picked up the afferent impulses from that nerve in several kinds of electric fish. The impulse frequency changed on placing a piece of metal near the body. The estimated 'discrimination threshold' was about 50 $\mu\text{V./cm.}$, which agrees well with the threshold determined in the present work.

It is very difficult to conceive the central mechanism of this reflex. The problem which should come first is how the constancy of the resting discharge frequency can be maintained at such a high level. It is tempting to assume that some controlling mechanism is at work, such as negative feedback devices. For example, the electric organ discharge is received by the fish itself, and if the frequency of the discharge

happens to be too high, the frequency of impulses in the afferent nerve fibres increases and in turn produces some inhibitory effect upon the pace-maker neurons.

If a mechanism like this did exist in the nervous system of this fish, then the effect of a.c. stimulation could be partially explained. It is possible that the externally applied signal 'simulates' the fish's own organ discharge, causing the number of afferent impulses to increase when, for example, ΔS is positive. The pace-maker is accordingly inhibited, and the discharge frequency becomes lower. Although such an idea is purely speculative, it has one merit that the constancy of the resting discharge frequency and the direction of the response can be explained on the same basis.

The utility of this reflex can easily be surmised. It is reasonable to assume that, like *Gymnarchus*, this fish is using its discharge to detect objects surrounding it. When many fish live together in a limited space, it would be very important for each of them to distinguish between its own signal and those of its neighbours. The frequency range allowed to this kind of fish is relatively restricted, so it may frequently happen that two fish close together discharge with similar frequencies. In such circumstances one of the fish can change its discharge frequency, to avoid a mutual confusion of the signals, in such a direction that the frequency difference is increased.

SUMMARY

1. A South American gymnotid *Eigenmannia*, changes the discharge frequency of its electric organ when a weak electric signal is applied to it with a frequency very close to that of its own discharge. Otherwise, the discharge frequency is extremely constant at a fixed temperature.
2. When the frequency of the applied signal is higher or lower than that of the fish discharge, the response is a decrease or increase of the discharge frequency, respectively. When the two frequencies are exactly the same, the response fails to occur.
3. The threshold of this response is very low. In one fish it was about $3 \mu\text{V./cm}$.

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REFERENCES

- COATES, C. W., ALTAMIRANO, M. & GRUNDFEST, H. (1954). Activity in electrogenic organs of knife-fishes. *Science*, **120**, 845-6.
- GRUNDFEST, H. (1957). The mechanisms of discharge of the electric organs in relation to general and comparative electrophysiology. *Prog. Biophys.* **7**, 3-85.
- HAGIWARA, S., KUSANO, K. & NEGISHI, K. (1962). Physiological properties of electroreceptors of some gymnotids. *J. Neurophysiol.* **25**, 430-49.
- HAGIWARA, S. & MORITA, H. (1962). Coding mechanism of electroreceptor impulses in *Gymnotus*, *Steatogenes* and *Eigenmannia*. *J. Neurophysiol.* (in the Press).
- LISSMANN, H. W. (1958). On the function and evolution of electric organs in fish. *J. Exp. Biol.* **35**, 156-91.
- LISSMANN, H. W. & MACHIN, K. E. (1958). The mechanism of object location in *Gymnarchus niloticus* and similar fish. *J. Exp. Biol.* **35**, 451-86.
- MACHIN, K. E. & LISSMANN, H. W. (1960). The mode of operation of the electric receptors in *Gymnarchus niloticus*. *J. Exp. Biol.* **37**, 801-11.
- TERZUOLO, C. A. & BULLOCK, T. H. (1956). Measurement of imposed voltage gradient adequate to modulate neuronal firing. *Proc. Nat. Acad. Sci., Wash.*, **42**, 687-94.