THE MODE OF OPERATION OF THE ELECTRIC RECEPTORS IN *GYMNARCHUS NILOTICUS*

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The perception of objects by *Gymnarchus niloticus* and similar electric fish has been attributed to an electrical mechanism (Lissmann, 1951, 1958). In a previous paper (Lissmann & Machin, 1958, hereafter referred to as L & M) we confirmed this hypothesis experimentally, and postulated a possible mode of action of the electric receptors. In the present paper experimental results are given which appear to confirm this postulate.

TWO POSSIBLE MODES OF ACTION OF ELECTRIC RECEPTORS

The electric receptors which, following L & M, will be assumed to be the mormyromasts, must measure the amplitude of a signal consisting of 1 msec. pulses at a repetition frequency of about 300 cyc./sec.; these pulses are emitted by the electric organ. The information about the signal amplitude has to be coded and transmitted down a sensory nerve in the form of impulses with a maximum rate of the order of 500 cyc./sec. It was proposed in L & M that this could only be done by 'smoothing' the incoming pulses and transmitting a sensory nerve signal characteristic of their mean value. An integration time constant of about $\frac{1}{4}$ sec. was suggested for the smoothing mechanism.

One other possible mechanism had been overlooked; this has been pointed out in a private communication by T. H. Bullock. According to Bullock's hypothesis, each signal pulse initiates a nerve impulse after a delay; this delay is dependent on the amplitude of the signal pulse. Since the signal pulse derives from the fish's own electric organ, its time of occurrence is presumably available in the C.N.S.; the amplitude of signal at the receptor can be deduced by comparing the timing of the transmitted pulses from the electric organ with that of the receptor nerve impulses. The reduction of band-width, or temporal integration, necessary to achieve adequate signal-to-noise ratio would then be carried out in the C.N.S. after the comparison has been made. This mechanism also gives an effective improvement of $\sqrt{3}$ in signal-to-noise ratio over the alternative mechanism (L & M, p. 476).

The mechanism postulated in L & M will hereafter be called 'pulse-frequency-modulation', while Bullock's mechanism will be known as 'pulse-phase-modulation'; both are illustrated in Fig. 1. The experiments described in this paper were designed to distinguish between the two mechanisms.

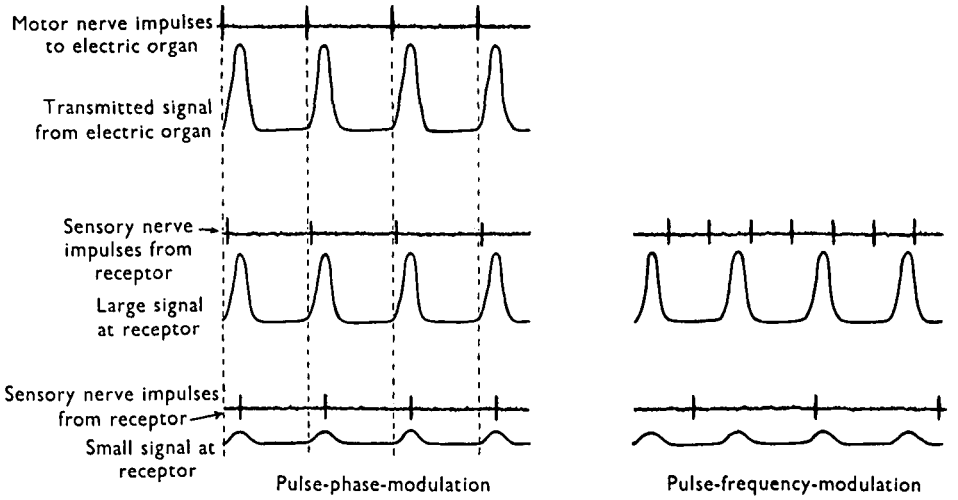


Fig. 1. Comparison of two possible modes of action of the electric receptors.

PLAN OF EXPERIMENTS: RESULTS TO BE EXPECTED ON THE TWO HYPOTHESES

It was estimated previously (L & M) that *Gymnarchus* was sensitive to d.c. potential gradients of about 0·03 μV./cm. It was concluded (L & M, p. 477) that the same receptors were probably responsible both for object location and for the sensitivity to small direct currents. The present experiments are founded on the assumption that the sensitivity of the fish to externally applied currents gives information about the electric receptors used for object location.

If pulses of voltage are applied across the water in a tank, on the pulse-frequency-modulation hypothesis the fish should be affected only by their mean level. Thus square pulses of voltage V, width W and frequency f will have an effect dependent only on the mean level, given by VWf. *All* pulse trains with the same value of VWf should give the same effect provided that W and $1/f$ are not comparable with the smoothing time constant; furthermore the threshold sensitivity V_t for pulses of constant frequency should be inversely proportional to their width, and for pulses of constant width should be inversely proportional to their frequency. For applied pulses at the fish's own transmitted frequency, there is no reason to expect an enhanced sensitivity.

In the case of the pulse-phase-modulation mechanism, there is at first sight no possibility of registering the amplitude of applied pulses, since the moment of occurrence of the pulse is not available in the C.N.S. However, we may postulate that there are receptors which give an immediate response (i.e. not delayed) to an incoming pulse; the output from these receptors may provide the time reference from which the amplitude of the pulses can be deduced in the C.N.S. If the delay in firing of the nerve impulses is merely determined by the amplitude of the incoming pulse, its width should have no effect on the response.

THE MEASUREMENT OF INTEGRATION TIME-CONSTANT

It will be shown later that the experimental results favour the pulse-frequency-modulation mechanism. Assuming that this is the operative one, an approximate experimental determination of the integration time-constant τ can be made. The response of the fish will be dependent approximately on the mean value of the stimulus over the previous τ seconds. With a *single* pulse of voltage V' and width W' used as stimulus, the response should depend on $V'W'/\tau$. If now the threshold for continuous stimulation is V_t, and that for single pulse stimulation is $(V'W')_t$, then the integration time-constant is given by $\tau = (V'W')_t \div V_t$. If the threshold is found to be dependent only on $V'W'$ and not on V' or W' separately, further support is given to the pulse-frequency-modulation hypothesis.

MATERIALS AND METHODS

The experiments were carried out with a 50 cm. specimen of *Gymnarchus niloticus*, under ordinary laboratory conditions, in water at a temperature between 25 and 28° C. The experimental tank measured $120 \times 75 \times 45$ cm. and contained two carbon plates 30×30 cm. mounted as shown in Fig. 2.

The electrical circuit providing pulses to the plates must have the following properties:

(i) It must be possible to switch the pulses on and off without changing the impedance of the circuit between the plates, so that any currents due to impurities in the plates (L & M, p. 453) are not altered at the instant of switching.

(ii) The frequency, amplitude and width of the pulses must be variable without changing the impedance in the plate circuit.

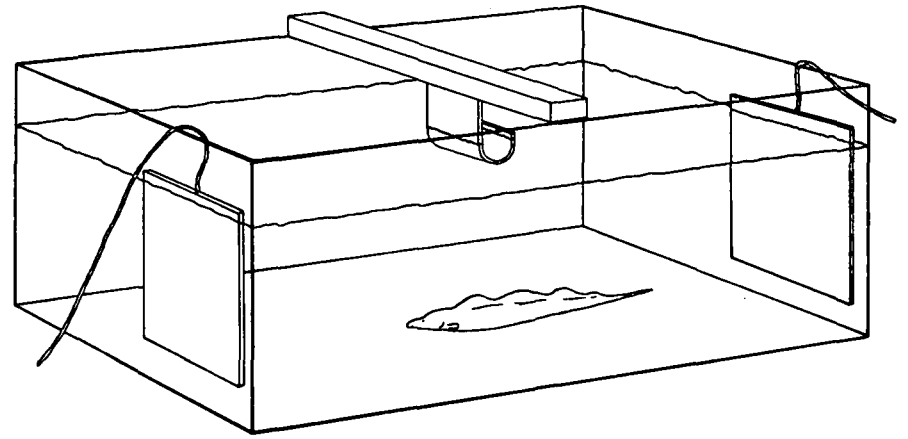
(iii) The plate circuit must be entirely insulated from earth, so that the effect of earth leakage from the water in the tank is minimized.

The circuit finally adopted is shown in Fig. 3. A standard electrophysiological stimulator provides pulses of variable frequency and width to modulate a radio-frequency signal generator. The signal generator has a piston attenuator, the output of which is fed to a transformer and rectifier circuit. Direct-current pulses isolated from earth and of widely variable amplitude are thereby generated. By switching the signal generator to the unmodulated condition, a d.c. voltage, variable by the attenuator, can be fed to the carbon plates.

The attenuator was calibrated in terms of voltage by amplifying the output of the rectifier and displaying it on a measuring oscillograph. Checks were carried out to ensure that the amplitude of the output pulses was independent of their frequency and width.

Before the experiments were started the fish normally either cruised about in the aquarium, or waited in one corner where it usually received its food. These activities were periodically interrupted by visits to the surface for a gulp of air.

When the carbon plates were first introduced into the aquarium the behaviour changed abruptly. The fish now rested most of the time on the bottom as far away



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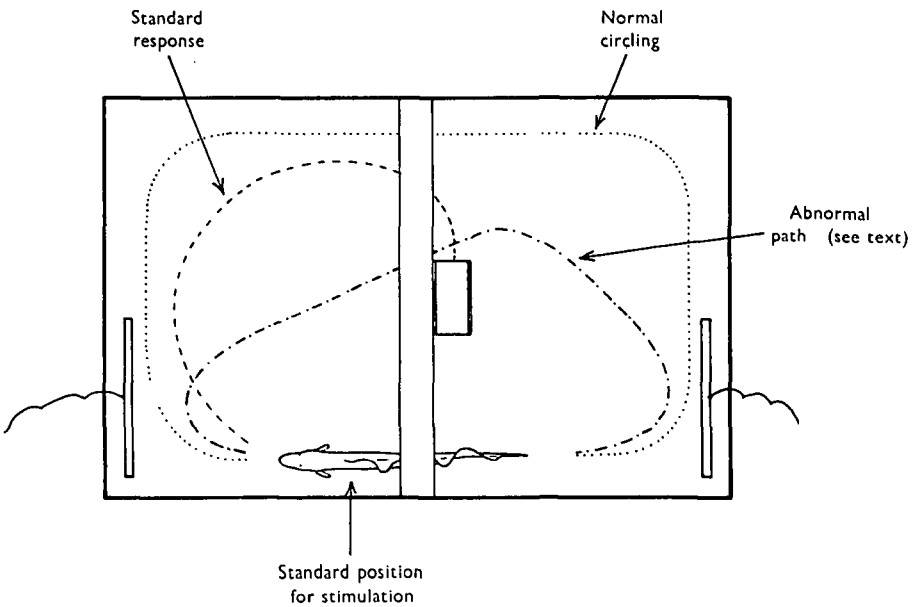

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Fig. 2. Arrangements of the experimental tank. (*a*) General view. (*b*) Plan view.

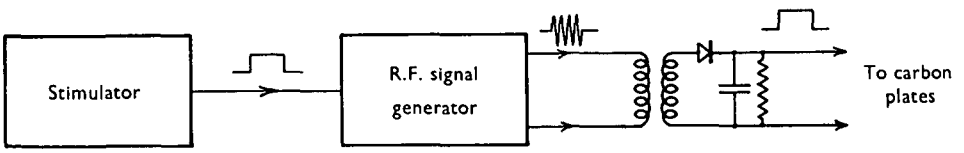

Fig. 3. Stimulating circuit.

from the carbon plates as possible. From time to time it undertook excursions towards the plates, swimming backwards and performing waving exploratory movements with the tail. After about 1 week it returned to its original pattern of behaviour and took no further notice of the plates.

When the fish was swimming between the carbon plates the application of an electric stimulus via these plates, if above a certain level, led to a sudden acceleration of the swimming movements. The stimulus consisted of either a single pulse or a pulse train about $\frac{1}{2}$ sec. long. In order to establish a more definite and standard response a series of training experiments was carried out. For this purpose a Perspex trough 10 cm. long and 5 cm. deep was placed close to the surface in the centre of the tank. After the application of the electrical stimulus, with the fish in a standard position between the plates, a piece of food suspended by a thread was presented. This reward was then used to lure the fish gradually nearer and nearer to the trough, and finally it was made to enter the trough before receiving the reward. From then on the fish was only fed when it had entered the feeding trough after the stimulus.

The normal course of an experiment was as follows: at first the fish was either stationary in one corner or cruising about. The introduction of the feeding trough led to steady circling along the walls of the tank. For reasons best known to itself the fish invariably circled clockwise on these occasions. Administration of an electrical stimulus above threshold brought about a marked increase in the swimming speed of the fish, a direct approach to the feeding trough and entry from the side furthest from the carbon plates. Thereupon the fish was fed. This is the 'standard response'. Application of a subthreshold stimulus caused no reaction and the fish continued to circle steadily.

The positive reactions were usually quite definite and left little doubt about the position of the threshold.

However, in the course of training several new patterns of behaviour emerged and some of these caused difficulties.

(1) Particularly during experimental sessions, the path of the fish along the wall of the aquarium furthest from the carbon plates was shortened by cutting off the corners, so that the fish finally swam on a more triangular course with the apex just beyond the trough (Fig. 2*b*).

(2) Between the carbon plates the fish often slowed down—as if waiting for the stimulus. Along this stretch of the path the tail was often waved markedly from side to side.

(3) Even in the absence of a preceding stimulus the fish at times hesitated or stopped near the entrance to the feeding trough.

(4) A disturbing feature appeared in the course of prolonged series of trials near the threshold level. The fish sometimes showed the very characteristic standard response (acceleration and entering the feeding trough) without any previous application of an electrical stimulus. Attempts to punish the fish on these occasions seemed to produce a neurotic state which took the form of aggressive behaviour, with the fish careering on the surface, its head out of water and snapping in all

directions. However, such apparently spurious positive responses appeared quite often to coincide with loud noises and vibrations in the building, for example, banging of distant doors or passing heavy traffic. Critical experiments were therefore undertaken at night, and generally the determination of the threshold was not only unambiguous but remained constant over a period of months.

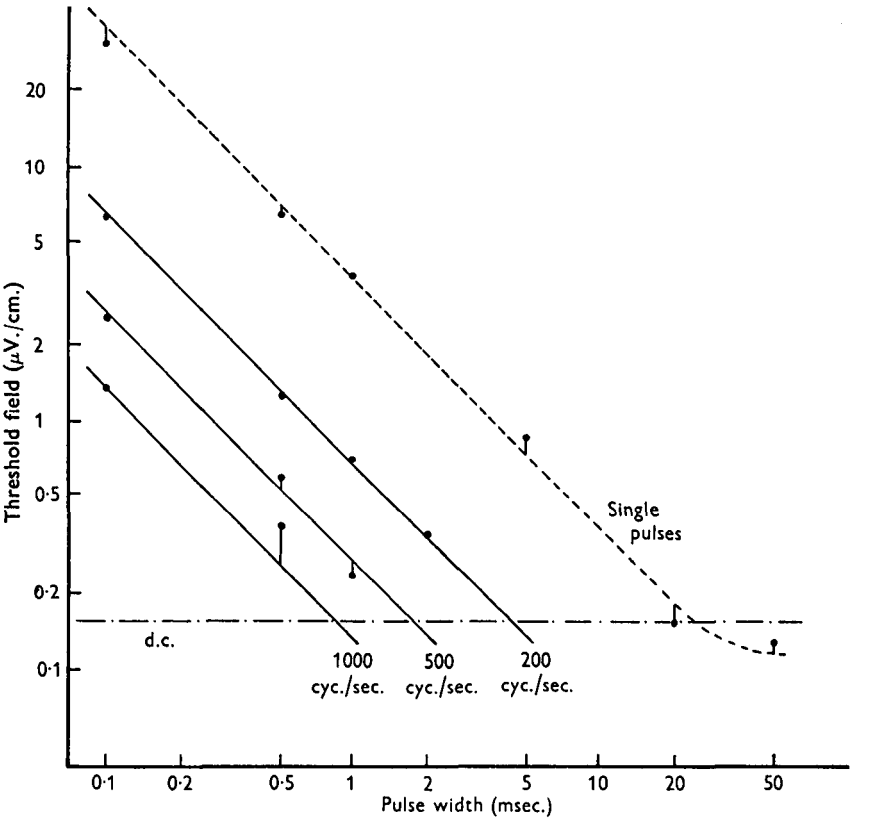

Fig. 4. Threshold field as a function of pulse width and frequency.

RESULTS

The threshold field for the standard response is plotted in Fig. 4 as a function of pulse width for three frequencies. The straight lines of slope -1 indicate the relation to be expected on the pulse-frequency-modulation hypothesis; their absolute position is chosen to give the best fit to all the results displayed. The horizontal line representing the threshold to d.c. agrees well with the pulse results, each sloping line cutting it at the appropriate point. Thus d.c. can be regarded as 1 msec. pulses at 1000 cyc./sec., or 5 msec. pulses at 200 cyc./sec., etc. It is clear that the results are consistent with the pulse-frequency-modulation mechanism.

The threshold for 1 msec. 200 cyc./sec. pulses was checked with the direction of field reversed. No significant change of threshold occurred.

The dotted line on Fig. 4 gives the threshold for single pulses; again the pulse-frequency-modulation hypothesis is confirmed. From the position of this line relative to those for repetitive stimulation, the integration time constant of the receptors is found to be 25 msec.

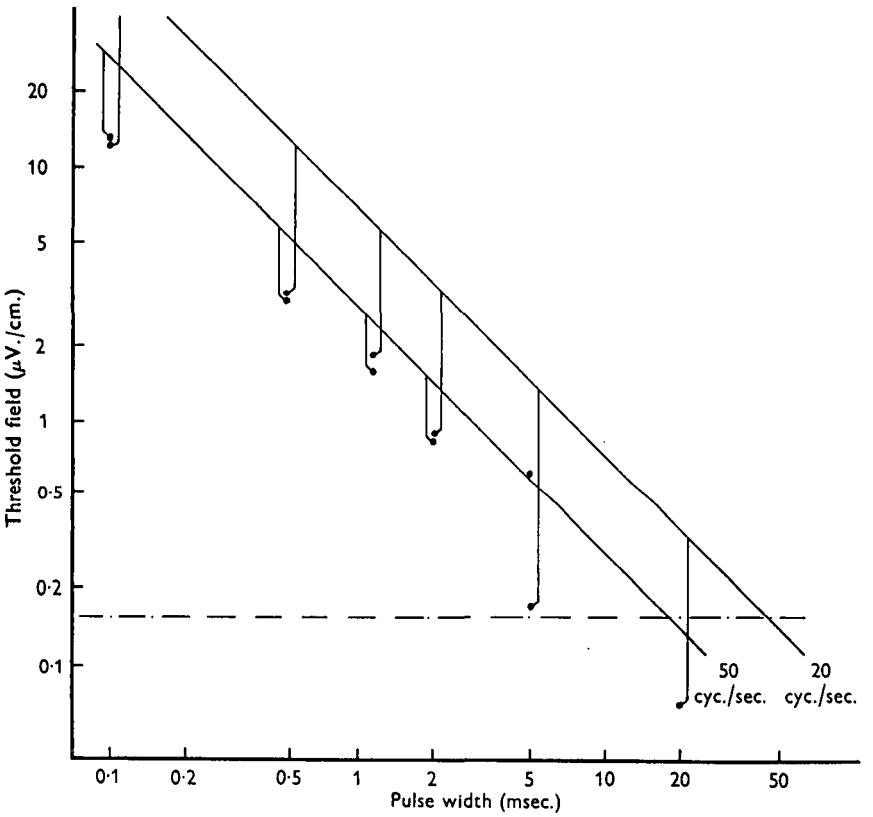

Fig. 5. Threshold field for 20 and 50 cyc./sec. pulses.

The results for a pulse repetition frequency of 50 and 20 cyc./sec. are shown separately in Fig. 5, together with the lines on which they should lie. When the pulse width or interval becomes comparable with the integration time constant it is apparent that the threshold is no longer inversely proportional to pulse frequency. Indeed the fish is more sensitive to 20 msec. pulses at 20 cyc./sec. than to d.c. Possible reasons for this will be discussed in the next section.

DISCUSSION OF RESULTS

Although the experimental results clearly favour our pulse-frequency-modulation hypothesis, it may be interesting to see whether the pulse-phase-modulation mechanism could give the same results. Each nerve impulse must now give information about the charge (current × time) passed during the pulse; therefore the impulse cannot be released until the whole of the stimulating pulse has occurred. The

receptor must then comprise an integrating mechanism to measure current × time, an 'off' indicator to give the moment of termination of the pulse, a delay mechanism initiated by the 'off' indicator and of duration determined by the stored value of current × time, and finally a trigger for the nerve impulse. The time reference in the C.N.S. must be based on the 'off' indication. In this way a pulse-phase-modulation mechanism could be designed to give the observed experimental results, but only at the expense of extreme complexity of the receptors. No compensating advantages are obtained for the process of obstacle location, except possibly an improvement of $\sqrt{3}$ in the signal-to-noise ratio. It is still, however, impossible to explain the good fit of the d.c. stimulation results to those with pulse stimulation.

An explanation of the anomalous results at 50 and 20 cyc./sec. (Fig. 5) can be given on the pulse-frequency-modulation hypothesis by assuming that adaptation takes place before the transmission of information up the nerve to the C.N.S. When the stimulus is switched on the receptors integrate with a time constant of about 25 msec., then send a signal to the C.N.S. characteristic of the charge passed during that time. If the stimulus is continued the signal gradually ceases. Thus the sensitivity of the fish to single 20 and 50 msec. pulses is about the same as to d.c. (Fig. 4).

If, however, the stimulus is 20 msec. pulses at 20 cyc./sec., the receptors will send out a signal on the first pulse, 're-set' during the inter-pulse interval and send out a further signal for each subsequent pulse. Thus the effect at the C.N.S. of pulses spaced sufficiently far apart in time will be greater than that for a d.c. stimulus of the same voltage.

The adaptation and re-setting time is not likely to be less than the integration time-constant, and the results of Fig. 5 indicate that it is probably of the same order. Further experiments on this effect are being undertaken, and will be reported in a subsequent paper.

Adaptation at the receptors is clearly of advantage to the fish, as the process of obstacle location entails the detection of small changes in a standing current flowing in the receptors. The experiment described earlier, whereby the direction of the field was changed without changing the threshold, implies either that the receptors are sensitive to current of either polarity, or that two sets of receptors are provided, each sensitive to current of one polarity. When considered with the effect of adaptation, this suggests that changes in either direction of the standing current in the receptors could probably be detected.

It is possible that additional, less sensitive, receptors operating on the pulse-phase-modulation system are provided; the present experiments merely indicate that when the fish is operating near the threshold, this mechanism is not the operative one. It might, in fact, be advantageous to have some receptors which give information about the frequency of incoming pulses. 'Interference' with the locating mechanism due to stray currents in the water, or to the emissions of other nearby electric fish could then be identified. The problem of mutual interaction of electric fish in shoals, which could have been explained on a pulse-phase-modulation system, remains inexplicable on the present results. However, few field

observations on the behaviour of social electric fish are available, and it is not clear whether or not mutual interaction confuses their locating mechanism.

The mechanism of temporal integration in the receptors

The smoothing of electrical stimuli by the receptors might be achieved by a simple resistance-capacity filter. The resistance could be identified with the jelly-filled canal leading to the mormyromasts, while one could postulate a relatively insulating membrane surrounding the mormyromast itself, giving it a capacity to the surrounding tissue. In L & M (p. 484) the resistance of a jelly-filled canal was estimated at 300 kΩ; assuming the mormyromast to be a sphere of 0·1 mm. diameter, and the membrane capacity to be 3 μF. cm.$^{-2}$ (Katz, 1952), the capacity would be 0·001 μF. The time-constant of such an integrating circuit would be about 0·3 msec.

It is conceivable that errors of a factor of 100 could have been made in the estimates of resistance and capacity, and that the smoothing time-constant is of structural origin, but this must remain speculative until accurate measurements on the jelly-filled canals and mormyromasts are possible.

The sensitivity of an electric receptor

Previous crude experiments (L & M, p. 451) suggested that *Gymnarchus niloticus* would respond to d.c. fields of about 0·03 μV./cm. The present results give a value of about 0·15 μV./cm., but are much more reliable. Previously there was a discrepancy of a factor of ten between the sensitivity derived from d.c. experiments and that inferred from object-location thresholds (L & M, p. 477). This discrepancy is reduced to a factor of two if the figure of 0·15 μV./cm. is used.

In L & M it was shown that the threshold sensitivity of *Gymnarchus* corresponds to a change of current in a receptor of 0·003 $\mu\mu$A. (This conclusion was based on the results of object-location experiments, and therefore corresponds to within a factor of two with the present results.) For a receptor integration time assumed there to be $\frac{1}{4}$ sec., this current will be only about 1/60 of the random noise current flowing in the receptor circuit. To explain this discrepancy it was assumed that spatial integration took place in the C.N.S. over about 5000 receptors, whereupon the signal-to-noise ratio would exceed unity. Averaging of the output of 5000 receptors would produce negligible spatial 'blurring' of the information.

The measured time-constant is one-tenth of the postulated value; it is therefore necessary to assume spatial integration over about 50,000 receptors. Mere averaging over such a large number would destroy some of the detail in the pattern of information from the receptor field. Accordingly, it must be assumed that the integration must take the form of a 'pattern recognition' process, whereby the pattern of information from the receptors is identified with one of a series of stored patterns, either innate or previously learned. Such a process is formally identical with spatial integration over a large number of receptors.

Even with spatial integration in the C.N.S., an individual receptor must transmit

information about a current change of 0·003 $\mu\mu$A. This signal will be very 'noisy'; in terms of impulse transmission in the sensory nerve, it may represent an indiscernibly small change in the firing due to standing current and to noise. It is only when the outputs of 50,000 such information channels are correlated that a clear indication, free from noise, can be obtained.

The minimum detectable current is at first sight improbably small; it is of interest therefore to compare the sensitivity of electric receptors with that of other sense organs which respond to electrical stimuli. Such a comparison must inevitably be somewhat speculative. The present view about mechano-receptors involves the causal sequence stimulus → receptor potential → nerve impulses. It is tempting to identify the electric stimulus to an electric sense organ as the 'receptor potential', which initiates nerve impulses by a similar process. However, the values of receptor potential measured for mechano-receptors (Gray & Sato, 1953; Eyzaguirre & Kuffler, 1955) are of the order of millivolts at threshold; the corresponding currents are probably of the order of microamperes. It is, perhaps, not surprising that the currents involved in the operation of a mechano-receptor, which measures relatively gross mechanical changes, are much larger than those of electric receptors, which occur in very large numbers to provide essential orientation information for the fish. It would be more appropriate to compare electric receptors with the receptors of the ear or eye, where again essential orientation information is derived from the output of a very large number of receptors.

Tasaki, Davis & Legouix (1952) find in the ear of a guinea-pig cochlear microphonics which are linearly related to sound pressure. Extrapolating their results, the level of cochlear microphonic potential at the threshold of hearing should be about 0·01 μV. If the cochlear microphonics are the receptor potentials for the sense organs of the ear (although this is not universally accepted) and assuming that the resistance in the circuit to a sense organ is of the order of 1000 Ω, a current of about 10 $\mu\mu$A. should produce an output from the sense organ.

Direct evidence about the threshold of the eye to electric current is provided by Brindley (1955) who finds that a 50 cyc./sec. current of 20 μA. causes the sensation of light ('phosphene') of about 15° diameter. This corresponds to the excitation of about 3×10^6 rods and cones; the current per receptor is thus about 6 $\mu\mu$A.

The sensitivity attributed to the electric receptors of *Gymnarchus* is thus about a factor of 1000 better than the sense organs of the ear and eye. Since the electric receptors are presumably specialized for detecting electric currents while those of the ear and eye are not, such a factor is not unreasonable.

A current of 0·003 $\mu\mu$A. flowing for 25 msec. (the integration time of the electric receptor) corresponds to a movement of 1000 univalent ions. This figure is about a factor of ten lower than Brindley's (1955) value per rod and cone cell for the threshold for phosphenes produced by short electrical pulses. Such a threshold appears more than adequate when compared with a visual receptor, where the bleaching of one molecule of rhodopsin by one quantum can give rise to a signal (Brindley, 1960), or with an olfactory receptor, which can be stimulated by a single molecule (Neuhaus, 1953).

The mechanism by which such minute current changes can control the frequency of nerve impulses remains totally unexplained. However, even for the ear and eye, which have been studied for centuries, the situation is similar.

SUMMARY

1. Two possible modes of action of the electric receptors of *Gymnarchus niloticus* are described. In one, received pulses are 'smoothed' and a sensory discharge occurs dependent only on the smoothed level ('pulse-frequency-modulation'). According to the other mechanism, sensory nerve impulses are produced at the same frequency as the received pulses, but after a delay dependent on their amplitude ('pulse-phase-modulation').

2. An experiment has been devised to distinguish between the two mechanisms, and, if the pulse-frequency-modulation mechanism is the operative one, to measure the smoothing time-constant.

3. The results favour the pulse-frequency-modulation mechanism, and indicate a smoothing time-constant of 25 msec. It appears also that adaptation takes place in the electric receptors.

4. The origin of the smoothing action is discussed.

5. The sensitivity of electric receptors is compared with that of other sense organs.

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