

QUANTITATIVE ANALYSIS OF RESPONSES FROM LATERAL-LINE NERVES OF FISHES. II

BY HUDSON HOAGLAND

(From the Physiological Laboratory, Clark University, Worcester)

(Accepted for publication, December 23, 1932)

I

In a recent paper (Hoagland, 1932-33) it was pointed out that the lateral-line nerves of catfish *Ameiurus nebulosus* appear to be normally in a state of continuous activity, discharging impulses at a high frequency. These impulses were shown to be initiated by the sense organs (neuromasts) of the lateral-line system. The frequency of the discharge could be modified by various forms of mechanical stimulation and by altering the temperature of the receptors. The present paper is based upon a quantitative study of the effect of temperature on the impulse discharge from the lateral-line organs of the catfish, and also an analysis of the contribution which the individual receptors make to the total discharge in the nerve.

II

Tests of lateral-line nerves of a number of species of fresh water fish have recently been made, using the method previously described (Hoagland, 1932-33). In brief, this consists in baring the lateral-line nerve about a centimeter behind the head and dissecting it free for 1 or 2 cm. It is then tied, cut cephalad, and the freed length is drawn across silver, silver-chloride electrodes connected to the recording system. The action potentials of the nerve are amplified and recorded by an iron armature oscillograph (Matthews, 1928) used in conjunction with a camera and a standing wave screen. A loud speaker makes the amplified action potentials audible.

The lateral-line nerve of the brook trout, *Salvelinus fontinalis* (Mitchill), was also found to give a very vigorous spontaneous discharge of impulses in five out of seven nerves tested (*cf.* Fig. 6 *c* and *d*). Other fishes tested were: perch, four nerves; pickerel, eight nerves; blue-gilled and red-gilled sunfish, six nerves of each; *Fundulus*, five nerves; and goldfish, four nerves. No nerve impulses were obtained from the thirty-two lateral-line nerves of these other fishes. Not only were there no spontaneous impulses, but stroking the skin above the lateral-line canal and

using other forms of mechanical stimulation, effective on the catfish and trout, produced no responses.

There is an outstanding morphological difference between fishes giving responses and those which do not in the cases so far examined. Those of the former group either do not have scales (catfish) or have extremely small ones (trout), while those of the latter group are well protected by scales. The degree of myelination and mechanical strength of the very peripheral lateral-line nerves seems to be roughly an inverse function of the protection afforded by the integument. The scaly fishes examined, with the possible exception of the goldfish, have very much more delicate lateral-line nerves than have the catfish or trout, thus rendering surgical manipulation difficult. It seems probable that the more delicate nerves of the heavily scaled fishes may be damaged in the operation and thus silenced. It is also possible that shearing action of the scales while handling them may destroy the delicate connections of the nerve with the neuromasts.

III

If one cuts through a spontaneously discharging lateral-line nerve at a point several centimeters posterior to the original incision, an immediate decline in frequency of discharge results, owing to the severance of connections with neuromasts caudal to this incision. In this way, by successively cutting the lateral-line system along the flank at decreasing distances from the region where the nerve passes from the body to the recording electrodes, it is possible to study the contribution made to the total response by the individual receptor groups located between these cuts and the initial operation. This may be done by photographing the spontaneous discharge after each fresh cut, the distance between this cut and the original incision from which the nerve emerges being carefully measured. The number of end organs remaining after each cut may be counted as follows: After completion of the experiment the fish is turned over and the opposite nerve is exposed by an operation bilaterally symmetrical to the first one. This second nerve is then pulled very gently. In the case of the catfish, and quite frequently with trout, it is possible to draw the entire nerve out of the body without breaking it. As one pulls, a puckering is detected at each neuromast group as the fibers innervating it are broken. The breaking away from the neuromasts occurs in an anterior posterior direction along the flank, so that a count of the successive puckerings gives the number of groups of neuromasts caudal to the exit of the nerve to the electrodes. By such a count the distribution of

the neuromasts as a function of the distance from the incision may be obtained. Such a direct procedure is necessary since the neuromasts in *Ameiurus* are packed with increasing closeness as one approaches the tail and are not always marked externally by pores as are those more cephalad. Control tests showed a high degree of bilateral symmetry for distribution of sense organs in a given fish, indicating that the distri-

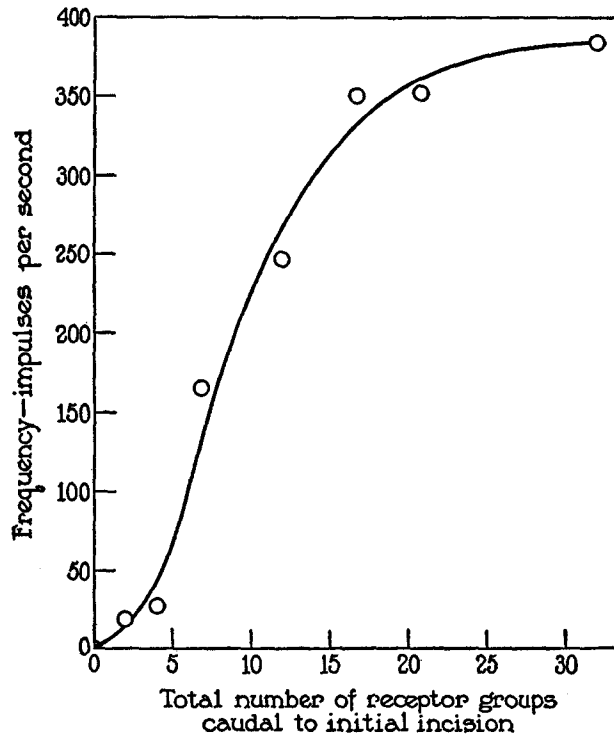


FIG. 1. Relation between frequency of nerve impulse discharge and number of contributory lateral-line receptor groups.

bution on one side could serve as a measure for that on the opposite, tested side.

Fig. 1 shows a typical curve indicating the relation between frequency of spontaneous discharge of nerve impulses from the lateral-line nerve of a catfish and the number of neuromasts caudal to the point of emergence of the nerve from the body. The curve is sigmoid—the

flattened upper part indicating that the more densely packed organs towards the tail contribute a smaller quota of impulses to the total discharge. As will be seen more clearly presently, the curvature at

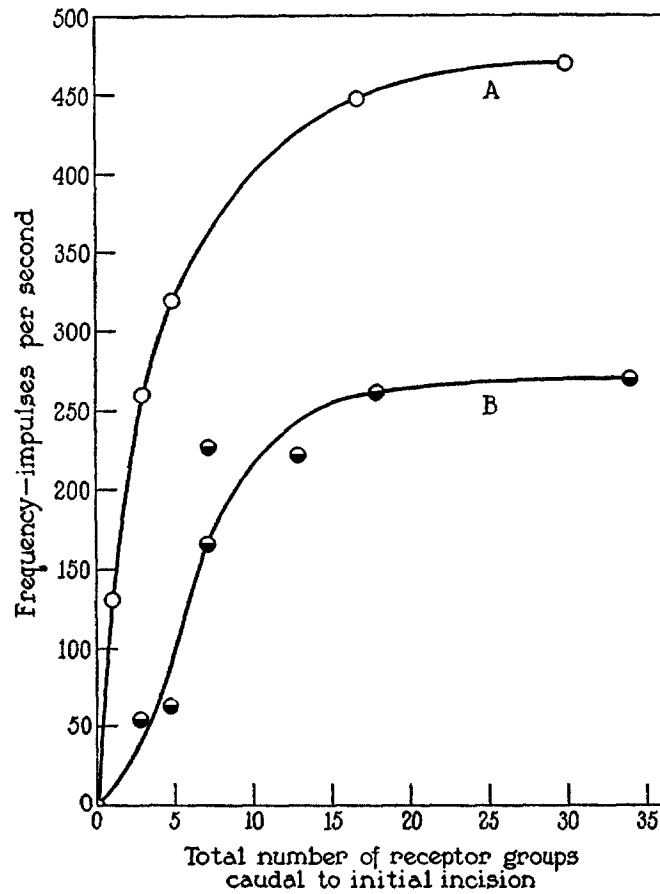


FIG. 2. Properties of the curves are discussed in the text.

the bottom of the figure may be accounted for by the fact that the first few receptor groups caudal to the initial incision are damaged by the operation, so that a full discharge from their neuromasts is not obtained.¹

¹ The initial operation exposing the nerve involves not only cutting away a patch of skin, but also the freeing of from 1 to 2 cm. of nerve. A certain amount of

In some experiments one gets results shown by Curve A of Fig. 2, where there is an almost linear relation between frequency of impulse and the first five neuromasts posterior to the incision. This indicates, in such cases, the probable absence of damage to sense organs near the initial incision. Six out of eight experiments plotted gave sigmoid curves like those of Fig. 1. Only two curves were obtained of the type of Curve A, Fig. 2. Curve B of Fig. 2 is an example from another experiment yielding a typical sigmoid curve similar to that of Fig. 1. Unless otherwise designated the figures in this paper refer to experiments with the lateral-line nerves of catfish. The trout lateral-line nerve has not as yet been studied systematically.

Fig. 3 is the differential curve of Fig. 1, made by plotting the slope of that curve against the number of sense organ groups. It is essentially a skewed distribution curve, showing the relative frequency contributed by each of the sense organ groups caudal from the region of the initial operation.

Each point in Figs. 1 and 2 was obtained by counting the number of impulses in each of fifteen tenth-of-a-second intervals on the photographs in which 0.1 second = approximately 15 mm. The frequencies for the fifteen intervals were averaged and their probable errors determined.

Fig. 4 is a plot of the variability (*cf.* Crozier, 1929), expressed as per cent probable error of the mean frequency for fifteen tenth-of-a-second intervals, against the number of active sensory groups. The variability is seen to be constant from approximately the seventh sense organ group caudal to the initial incision, while it increases rapidly as one approaches the region of exit of the nerve. The constancy of variability beyond the seventh receptor group indicates that the flattened upper parts of the curve of Fig. 1 and Curve B of Fig. 2 are not due to inability to distinguish and count the higher frequencies.

pulling on the nerve is inevitable. The subsequent incisions severing the lateral-line canal and nerve at intervals are made with a very thin sharp knife thrust but once vertically into the side from above the lateral-line canal. Spread of damage from these knife thrusts would be slight compared to that from the initial operation where, aside from the effects of mechanical manipulation, diffusion of tissue extracts might produce inhibition of the neuromasts in an amount proportional to their concentrations.

Were this the case one would expect the relative variability to increase with increasing numbers of sense organs owing to the coincidence of impulses on the records which might escape counting.

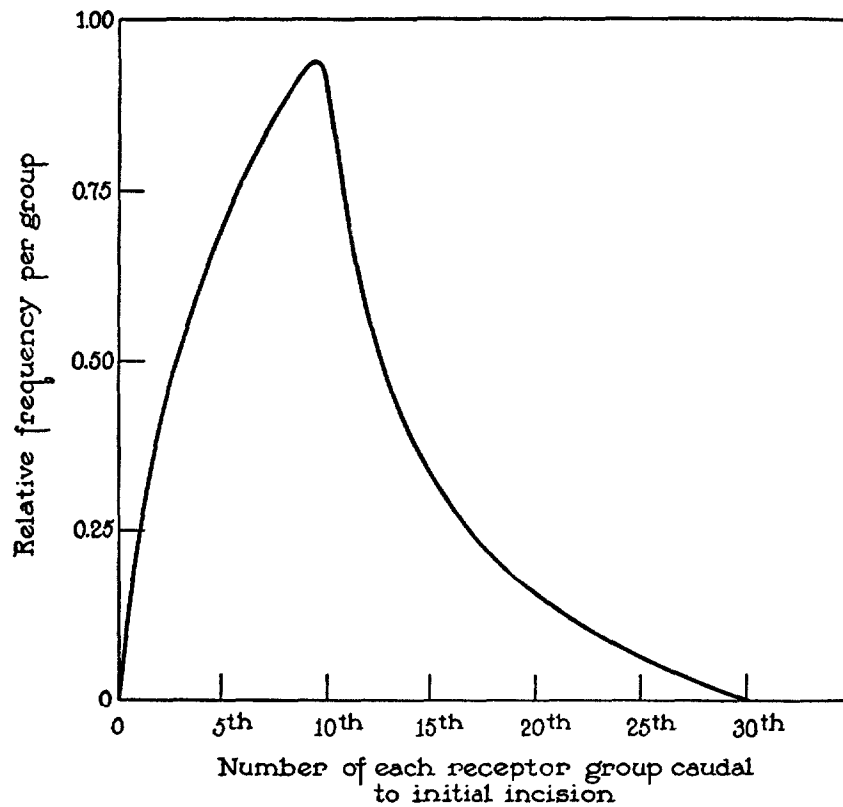


FIG. 3. The differential curve derived from Fig. 1, made by plotting the slope of that curve against the number of receptor groups. The curve shows the relative contribution made by each receptor group to the total response.

Fig. 5 is an example of the data from which the curves have been made. It shows segments of typical records of impulses corresponding to thirty-two, seventeen, seven, four, and two receptor groups.

The frequency and uniformity of the potential magnitudes of the discharge from one or two receptor groups was often so low (cf. Fig. 1,

Curve B of Fig. 2, and Fig. 3) as to indicate that only a few fibers were functioning. It is possible to remove the entire lateral-line nerve and tease apart the branches going to the neuromasts. When this is done one can count under the microscope ten to fifteen fibers going to each group of sense organs, excepting in the closely packed groups near the tail where the number of fibers declines to about half of this number. This paucity of fibers supplying the caudal groups gives a basis for the

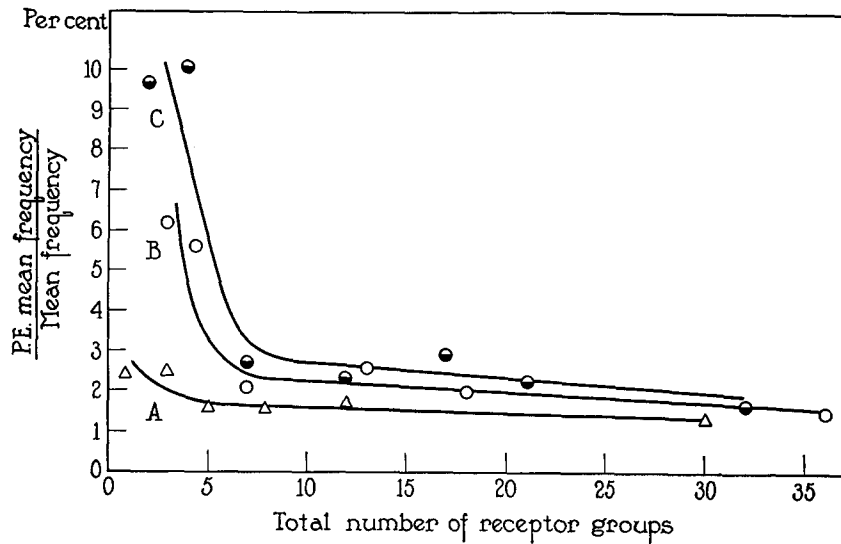
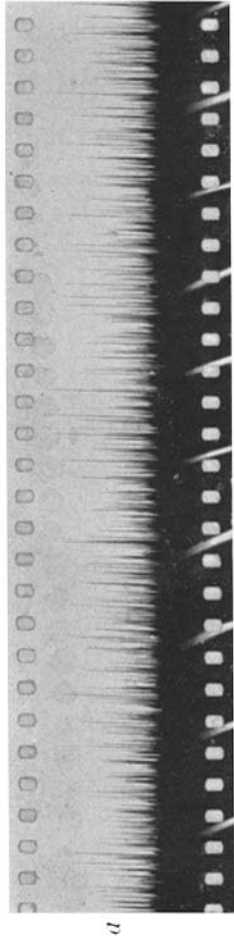


FIG. 4. Plot of relative variation against the total number of receptor groups. The significance of the curves is discussed in the text. Curves A, B, and C correspond respectively to the experiments recorded in Curves A and B of Fig. 2 and Fig. 1.

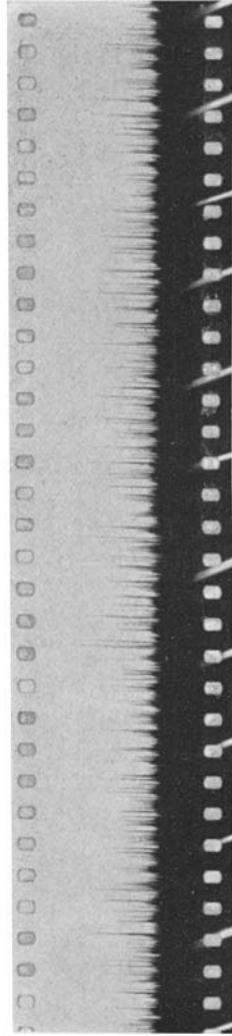
flattened upper parts of the curves of Figs. 1 and 2 and for the declining right half of the curve of Fig. 3. The frequencies are evidently less for the caudal groups because there are fewer fibers supplying them. The fact that the relative number of *active* fibers is few for receptors near the operation compared to those in the middle region of the trunk, while the number of fibers innervating the groups is as large or larger than for those more caudal groups, indicates that some of the fiber connections to the neuromasts near the point of incision are inactive

32 Receptor Groups



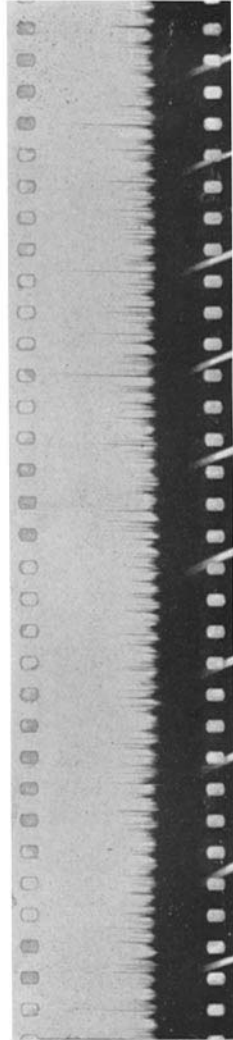
a

17 Receptor Groups



b

7 Receptor Groups



c

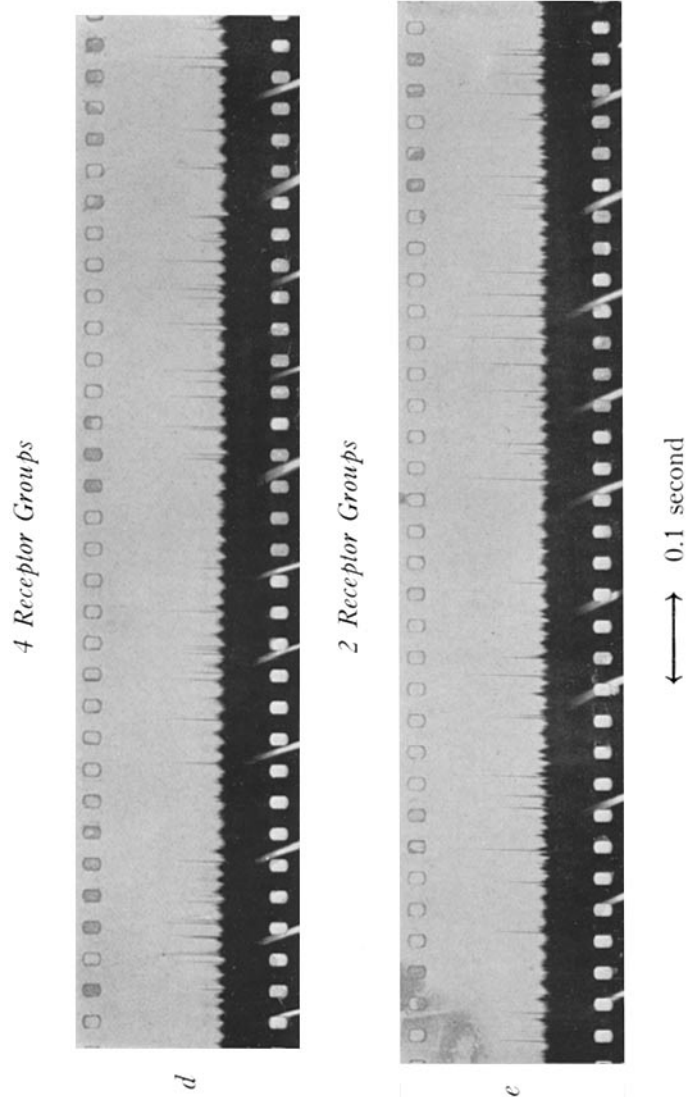
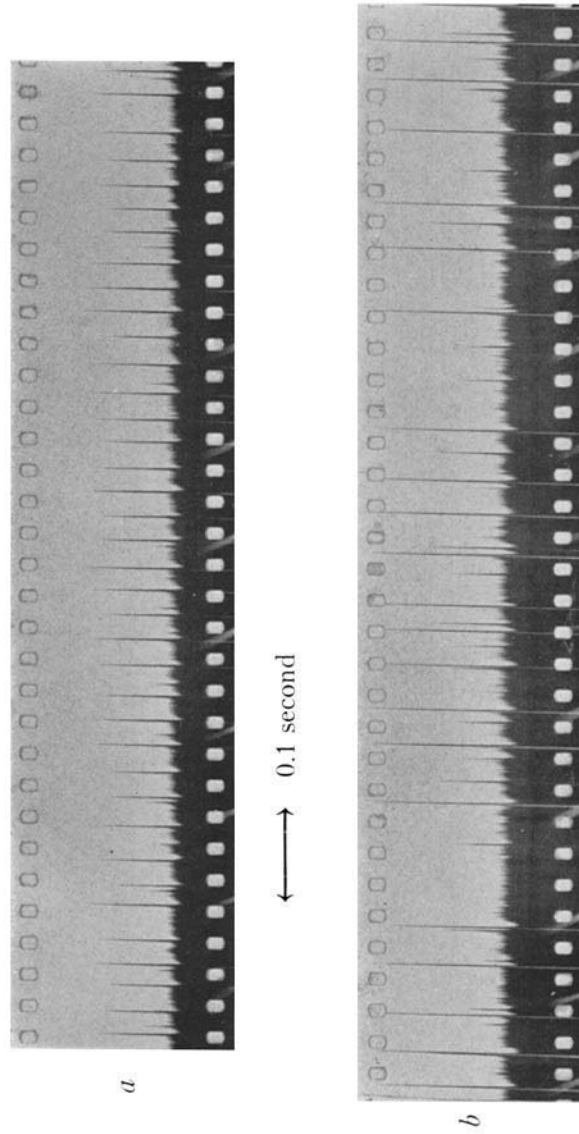


FIG. 5. Sample photographic records of the response for varying numbers of receptor groups. These are samples of the data actually used in determining the curve of Fig. 1. *d* and *e* show responses from only two or three active fibers. Since there are ten to twenty fibers supplying each of the more cephalad receptor groups it is clear that most of these fibers are inactive. Reasons for this inactivity are discussed.



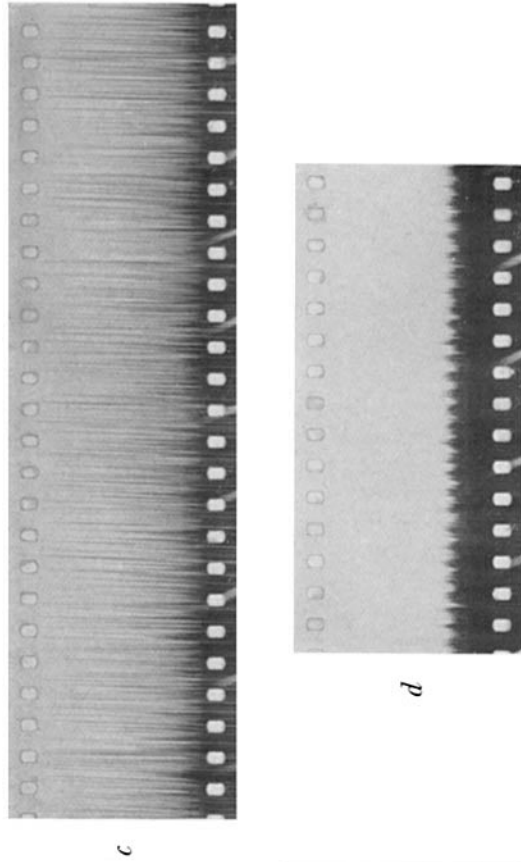


FIG. 6. (a) Rhythmic response of a single fiber obtained by cutting so as to leave only one receptor group and then chilling the skin to 8°C. The photograph was made after 10 minutes at 8°C.
 (b) Preparation made as in a. Temperature 5°C. Two fibers are seen to be active. The waxing and waning of frequency is very characteristic. Cf. also Fig. 5, d and e.
 (c) Full spontaneous discharge at room temperature of the lateral-line nerve of a brook trout.
 (d) Base line immediately after crushing nerve between the electrodes and the body of the fish. Same nerve as in c.

(only two or three fibers are active in *d* and *e* of Fig. 5).² This susceptibility to damage further strengthens the notion that the inactivity of the lateral-line organs of scaly fishes may be due to destruction of the very delicate nerve fibers characteristic of these animals.

The photographic records at room temperatures of responses from only one or two receptor groups, while indicating a marked decline in frequency as compared to the response from many groups, usually gives a discharge at a frequency too high to enable one to tell the number of active fibers by noting the characteristic ("all or nothing") height of the potential spikes (*d* and *e* of Fig. 5 are exceptions to this). If one chills the skin (*cf.* Hoagland, 1932-33) the frequency declines considerably, due in part to the elimination of certain neuromasts at characteristic temperature thresholds, and it is often possible in this way to get responses below 10°C. which are clearly indicative of the activity of only one or two nerve fibers. The discharge of such single fibers is not perfectly rhythmical but usually waxes and wanes in frequency. A quantitative study of the single fiber response is now in progress. In general, the frequency varies roughly between 40 to 10 per second, the periodicity of the fluctuation from maximum to maximum ranging from 0.5 to 2 seconds. Very rarely a fiber is found which beats quite rhythmically for several minutes without changing its frequency. Fig. 6*a* is an example of this latter response while Fig. 6*b* along with Figs. 5*d* and 5*e* show the waxing and waning type of response found in nine out of eleven cases in which single action potentials were individually distinguishable. Two fibers are shown active in Fig. 6*b*. Fig. 6*c* shows the spontaneous discharge from the lateral-line nerve of a brook trout while 6*d* shows the base line for this same response immediately after crushing the nerve between the electrodes and its exit from the body. The response from the trout is generally more vigorous than that from the catfish.

The falling part of the variability curve shown in Fig. 4 may now be understood, since the increase in the variability evidently depends

² The reduced contribution to the total frequency, as indicated by the lower part of the curve of Fig. 1, and Curve B of Fig. 2, and by the differential curve of Fig. 3, is not a characteristic of any particular anatomically fixed group of receptors, but depends only on the proximity of the receptors to the region of the original operation.

upon the fact that the discharge is irregular and fluctuating when few sensory groups are involved at room temperature. This interpretation is borne out by Curve A of Fig. 4 which rises very little when few receptor groups are involved. This curve corresponds to the data of Curve A of Fig. 2, which gives an almost linear rise in frequency for the first five receptor groups. In this experiment enough fibers were active, even from the group nearest the initial incision, to obliterate the uneven discharge due to the individual fibers. Curves B and C of Fig. 4 correspond to the data of Curve B of Fig. 2 and Fig. 1 respectively. The amount of curvature corresponding to the first five receptor groups in Curve B of Fig. 2 and Fig. 1 parallels the corresponding amount of curvature of the variability curves in Fig. 4.

The correspondence of the three variability curves with the three frequency curves serves as an internal check on the analysis. The constancy of variability after the first five or six receptor groups beyond the operation indicates that the method of counting impulses at high frequencies does not introduce an error. The relatively large decline in frequency and the high variability shown in two typical cases, representative of six out of eight experiments, indicates that destructive effects of the operation may extend to the first five or six receptor groups—a distance of roughly $2\frac{1}{2}$ cm. from the actual operation.

IV

If one examines the response of two or three receptor groups as a function of the temperature of the skin, by the method previously described (Hoagland, 1932-33), one gets curves as shown in Fig. 7. The data of two typical experiments out of eleven are plotted. The points are numbered in the order of their determination. It is clear that the decline at low temperatures is not produced by irreversible changes in the tissue, since full recovery occurs on raising the temperature—as is indicated by the points numbered 4 and 5.

Fig. 8 shows data of four experiments plotted according to the Arrhenius equation, $\frac{K_1}{K_2} = e^{\mu/R (\frac{1}{T_2} - \frac{1}{T_1})}$, where K_1 and K_2 are frequencies at the absolute temperatures, T_1 and T_2 , R is the gas constant, and μ is the temperature characteristic. A mean temperature characteristic of 5050 calories is obtained.

Fig. 9 is a plot of the per cent variability against the temperature in degrees Centigrade, for four experiments. Two of the four curves show no change in variability of response with temperature, while the variability is seen to increase at low temperatures in two cases. Since

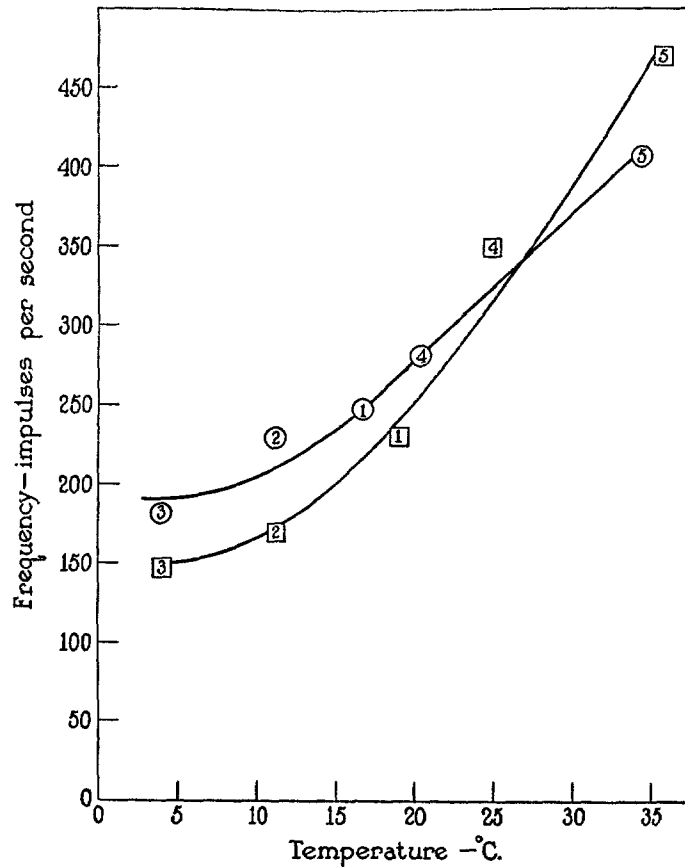


FIG. 7. The numbers of the experimental points indicate the order in which they were determined.

temperature causes a decline in frequency, one would expect the variability to increase at low temperatures if the lowered frequency depended, in part, on the cessation of activity of increasing numbers of receptors with declining temperature. The situation would be similar

to that discussed in connection with the rise in variability of response with the operative removal of functional units illustrated in Figs. 4

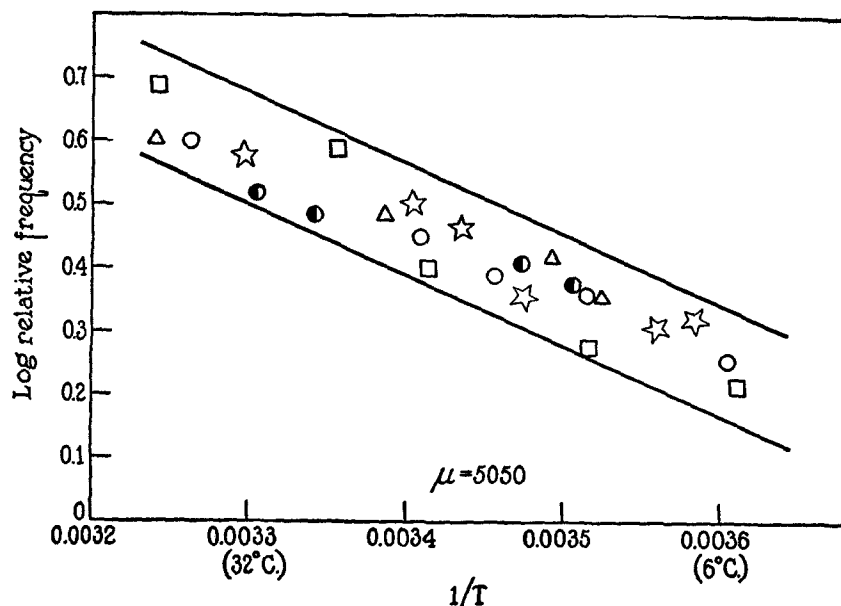


FIG. 8. Plot of temperature vs. frequency according to the Arrhenius equation.

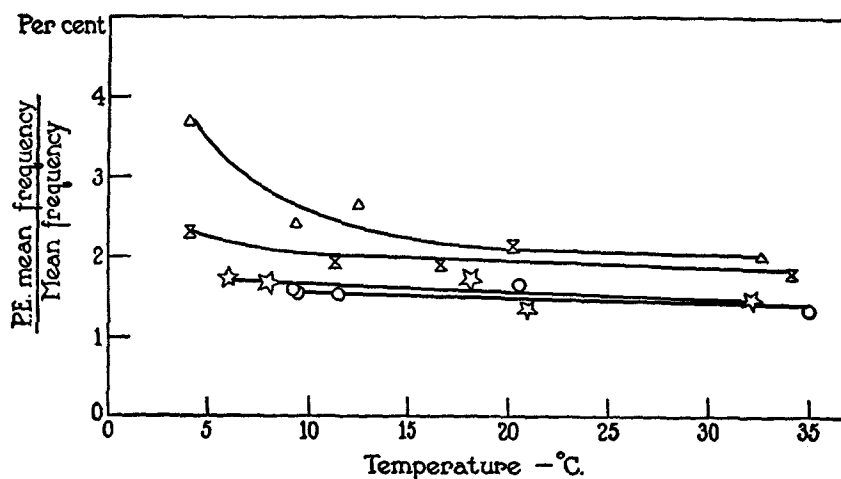


FIG. 9. Relative variability as a function of temperature.

and 5. The rising part of the curves of both Figs. 4 and 9 are evidently the result, therefore, of the elimination of functional units leaving only a few fibers active which, as we have seen, normally beat irregularly. The variation in frequency is, therefore, relatively great. In the experiments illustrated in Fig. 4 the elimination is the result of the effect of the operation on adjacent neuromasts, while in the case of Fig. 9 the elimination is evidently due to the inactivation of neuromasts at low temperature (*cf.* also Hoagland, 1932-33, Fig. 4).

The photographic records of the response also bear this out. For experiments in which the variability is highest one finds that the records indicate that only a few nerve fibers are active at the lowest temperature—the action potential magnitudes can be classified into two or three distinct groups. The variability analysis, therefore, appears to offer an effective approach to the measurement of the number of units which are contributing to the summed response of the nerve trunk. It may also serve as a delicate quantitative indicator of the spread of the effect of surgical manipulation through nervous tissue.

DISCUSSION

In attempting to account for the spontaneous discharge from the lateral-line nerve, I suggested (Hoagland, 1932-33) that the hair cells of the neuromasts projecting into the lumen of the lateral-line canal might actually be cilia, which by beating excite the nerve fibers of the neuromasts. As yet it has not been possible to examine the living neuromasts optically. Bowen (1932) has, however, recently reported that the hair cells of the crista of the ear of catfish may be seen to beat as cilia. The sensory region of the crista is made up of sensory cells and supporting cells, the hair cells projecting into the lymph of the ampulla. There is a striking correspondence between the anatomy of the lateral-line receptors and that of the receptors of the inner ear. This close relationship is extended by considerations of the neuroanatomy of the two systems and also by their ontogeny (Herrick, 1901; 1903). According to Bowen the amount and frequency of movement of the hair cells of the crista vary considerably in different preparations. The activity was found to be greatly affected by a variety of reagents, small amounts of which were observed to produce a complete

cessation of movement and a retraction of the hairs. The application of distilled water, for example, caused the complete disappearance of the hairs in from 2 to 3 seconds. In some preparations only one or two hairs were active while in others many were seen to be beating vigorously. Individual hairs were found to beat with waxing and waning frequencies. The average frequency for beating, of four hairs, over a period of some minutes, was 5 beats per second—a frequency somewhat less than that usually found for the spontaneous discharge of fibers of the lateral-line nerve (*cf.* Figs. 5 and 6).

All of the foregoing facts are suggestive if the hair cells of the neuromasts may be considered as cilia. The sensitivity of the hair cells of the crista to reagents suggests a possible parallel with the susceptibility of the neuromasts to operative shock. The character of the beating of individual cilia is to a large degree consistent with what one might expect if similar activity were determining the emission of impulses from the lateral-line nerve.

There is a possible resemblance between the anatomical arrangement of the Purkinje cells of the mammalian cerebellum and the organs of the lateral-line system. In a sense, the latter system may be regarded as a model of a battery of Purkinje cells. In both cases the individual units contribute fibers to a common path. The Purkinje cells are thought to be internuncial neurones which continually receive and transmit impulses serving to regulate posture and coordination by way of tonic excitatory and inhibitory effects on a variety of centers. It has been suggested that they may act tonically by discharging spontaneously. The lateral-line system, in addition to its receptor function, is also known, by its continuous action, to exert a central inhibitory influence (Parker and Van Heusen, 1917, and Hoagland, 1932–33). The quantitative analysis of impulses from the lateral-line system may possibly shed some light on the operation of internuncial neurones.

SUMMARY

1. The lateral-line nerves of trout as well as those of catfish are found to discharge impulses spontaneously at a high frequency.
2. The frequency of nerve impulse discharge is measured as a function of the number of participating receptor groups (lateral-line sense

organs). A quantitative analysis is made of the contribution to the total response made by each group of sense organs.

3. An analysis of the variability of the response is presented which makes it possible to estimate quantitatively the longitudinal extent of damage to the neuromasts due to surgical manipulation.

4. A method is described for recording the response of a single nerve fiber in the lateral-line trunk.

5. The frequency of the spontaneous discharge from the lateral-line nerve trunk when plotted as a function of temperature according to the Arrhenius equation yields a temperature characteristic of approximately 5000 calories.

6. The variability of the frequency of response as a function of temperature indicates the existence of temperature thresholds for the spontaneous activity of the neuromasts.

7. A possible basis for the spontaneous activity is considered.

It is pointed out that the lateral-line system may serve as a model of the Purkinje cells of the cerebellum.

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