THE FLICKER RESPONSE CURVE FOR FUNDULUS

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I

A number of vertebrates have been examined by us as to the form of the interdependence of flash frequency (F) and flash intensity (I) for reaction to visual flicker. The technic, apparatus, and procedure have been uniform in all these cases. There has been used, except in special experiments, a flash cycle in which light time and dark time are equal, and the temperature is the same (21.5°). We know, for several forms, that the *shape* of the F-log I curve (the flicker response contour) is not a function of temperature,[1] nor of the fraction of the flash cycle occupied by light.[2] Its position on the log I axis is governed by the temperature; its position and the magnitude of the F-scale units are dependent upon the light time fraction of the cycle time. Its shape is, however, a function of the nature of the animal tested.[3]

For typical vertebrates the flicker response contour comprises two parts or segments, so that the curve is a doubly inflected S.[4] The association of the smaller, low intensity part with the functional activity of retinal rods and of the larger, upper portion with that of retinal cones[5] is consistent with a large body of speculation and systematized information in visual physiology.[6]

In keeping with this conception, an animal which exhibits no histologically recognizable retinal rods, the turtle *Pseudemys*, and another with no cones, the gecko *Sphaerodactylus*, give flicker response curves which follow a

[1] Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*, *b*; 1937–38 *b*; 1938–39; Crozier and Wolf, 1940; 1939–40 *a*.

[2] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *c*, *e*; Crozier and Wolf, 1939–40 *b*.

[3] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *b*; 1937–38 *a*; 1937; 1938–39; Crozier, 1937; Crozier and Wolf, 1938–39 *a*; 1939–40 *a*.

[4] Wolf and Zerrahn-Wolf, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*; 1937–38 *a*, *b*, *e*.

[5] Hecht, 1937 (review); Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*; 1937–38 *b*, *e*; Crozier and Wolf, 1938–39 *a*, *b*, *c*.

[6] Hecht, 1937.

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simple, uncomplicated sigmoid course over its whole extent.[7]   To this
curve there can be fitted a probability integral

$$F = kF_m \int_{-\infty}^{\log I} e^{-(\log I/I_i)^2/2\sigma_{\log I}^2} \cdot d \log I_m$$

which describes the interdependence of F and I with excellent fidelity.[7]

This equation also serves to describe each segment of the compound
curve for other vertebrates (various teleosts,[8] and man[9]). The "rod"
and "cone" curves overlap in a particular way,[8, 9] which has been analyzed
by the assumption that after reaching a maximum value of F the rod curve
declines with increasing intensity. Various bits of evidence, in addition
to its descriptive utility, support this idea.[5] It is of some moment, how-
ever, that this general formulation be tested by applying it to a variety of
animals. We have pointed out[8] that different kinds of overlapping of the
rod and cone contributions to the F-log I contour are to be expected, and
some of these have since been found.[8, 9] The possibility cannot be lost
sight of that there might well be discovered in certain vertebrates evidence
of a third group of effects, in addition to those labelled as due to rods and
to cones respectively; such as might for example be traceable to a distinct
rôle of parafoveal cones, or of double or twin rods or cones in lower
forms. A distinct suggestion of this is given by our measurements with
the newt *Triturus*.[10]

Our immediate interest, however, is not with the bearing of such in-
formation upon the duplexity doctrine,[11] nor with ethological parallelisms
between habits and visual performance in response to flicker. The ele-
ments of excitation involved in threshold or marginal response to flicker
give evidence, in most vertebrates, that they form two groups or popula-
tions. This does not mean that the details of the form of the flicker con-
tour are due to or that they measure properties of the peripheral retina.
The response is a reaction of the whole organism, and there is every reason
to believe that the elements of excitation, whose effects are the basis of the
measurements, are central nervous elements. The initiation of their
activities is by way of their peripheral (retinal) representatives, and if these
are of two or more categories there could well be corresponding groups of

[7] Crozier, Wolf, and Zerrahn-Wolf, 1938–39; Crozier and Wolf, 1938–39 *b*.

[8] Crozier, 1937; Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *c*; Crozier and Wolf, 1938–39 *a*.

[9] Crozier, 1937; Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *b*.

[10] Crozier and Wolf, 1939–40 *b, c*.

[11] Crozier, Wolf, and Zerrahn-Wolf, 1938–39; Crozier and Wolf, 1938.

the central elements. Where rods and cones are distinguishable, the correlation with classes of elements concerned in the determination of the index-response to flicker is likely to be useful. But it is equally possible that instances should occur in which a composite flicker contour is obtained with an animal having no detected histological duplexity of retinal constitution.

The chief utility of further data upon the F-log I curves of diverse animals is to obtain a test of the generality of the method of analysis of the composite contours. Breeding experiments show pretty clearly[12] that the rod and cone populations of effects, as we may for convenience continue to label them, are independently determined. Since the shape of the F-log I curve is demonstrated to be a constitutional property of the organism[13] we can inquire as to the correlation of the properties of rod and cone effects in the different curves. This can be done quantitatively in terms of the three parameters of the probability integral describing each partial curve—the values of $F_{max.}$, log $I_{infl.}$, and $\sigma_{\log I}$—and of the degree of separation between the rod and cone curves.

Data on the teleost *Fundulus heteroclitus* were obtained with these purposes in mind, and also with the idea that it should be a form suitable for experiments seeking to modify the quantitative properties of the F-log I curve. While *Fundulus* has proved to be in some respects a difficult animal to work with, the measurements do give information upon some of the points in view.

II

The general procedure followed exactly the outlines of that which we have followed with other organisms we have tested. An important feature of this technic is the maintenance of the healthy condition of the individuals tested, and their continued activity after habituation to laboratory conditions and to the manipulations involved in the tests. From the standpoint of the observer *Fundulus* has been a rather more "difficult" animal to work with than certain other teleosts we have used.

About 50 *F. heteroclitus*, obtained from a dealer in Boston in September, 1937, were kept in three tanks with Southern exposure, for $3\frac{1}{2}$ months. They were maintained in fresh water, and tested in this medium. When bought they ranged in length from 1.75 to 3.0 inches. They grew considerably in the laboratory; about twelve died.

For observation, each *Fundulus* was first put into a separate jar. They were for some time hyperreactive to nearby movements. Each individual was tested in the apparatus, at a flash frequency of $F = 15$/sec. For the subsequent tests the ten most consistently reactive individuals were selected. At first the fishes were "restless," reacted to chance

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[12] Crozier, Wolf, and Zerrahn-Wolf, 1937; 1937–38 *a*; Crozier and Wolf, 1938–39 *a*.

[13] Wolf and Zerrahn-Wolf, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*; 1937–38 *a*; Crozier and Wolf, 1938–39 *a*.

vibrations, and scarcely came to rest after once being exposed to visual flicker. After continuance of the routine procedure for about a week—feeding at a fixed time, transferring to observation jars, placing in the thermostat for 2 hours' dark adaptation, and transference to the apparatus for exposure to flicker—the behavior of these individuals became more stabilized. The reactive state was found to be distinctly improved by keeping the individual aquaria in a room in which people were frequently moving about.

After being "broken in" in this way these fishes come to the surface of the water when the covers of their aquaria are lifted and jump at offered food. From this time onward they give, during tests of response to flicker, reactions which are as precise as those with any of the number of teleost species we have examined.

The test for critical flash illumination consists in exposing an individual in its aquarium, surrounded by a rotating striped cylinder, to a gradually increased illumination controlled by a manually operated diaphragm.[13] The speed of rotation of the striped cylinder determines the flash frequency of illumination of any fixed point. This speed is kept constant by observation of a sensitive voltmeter measuring the current produced by a suitable magneto geared to the shaft of the motor driving the cylinder. The motor speed can be controlled to a precision of 0.1 F/sec. under the most unfavorable conditions of stripe number on the cylinder.

From calibration curves giving the intensity of illumination at the position of the fish as a function of diaphragm opening, the illumination which is achieved at the moment of the fish's reaction to the movement of the encircling stripe system is read.

The response of the animal consists in a suddenly aroused swimming motion in the direction of the movement of the stripes. This response appears sharply as soon as the intensity of illumination (with F fixed) has been increased to the critical level. Backward swimming, observed more frequently with sunfishes, almost never occurs with *Fundulus*. Rarely, a *Fundulus* directly facing the wall of its container will "back off" a little and then follow the motion of the stripes. Should an individual already be in motion when the light diaphragm is opened, the direction of its movement is clearly altered when response to seen flashes occurs. In most such cases tests were repeated, and readings taken only when the fishes were quiet. The jerky response at threshold I is then unmistakable. Observations at low illuminations were preceded by about 1 hour of dark adaptation of the observer. In general, the ease of judgment of the occurrence of a threshold response to flicker was less above $F = 25$ than below. This condition is different from that obtaining with certain other forms. It does not affect the quantitative behavior of the scatter of the readings of mean critical intensities for the different individuals. This scatter, here measured by P.E.$_{r_1}$, obeys precisely the same law as that which we have found to hold for other organisms.

Three readings of critical illumination were obtained for each individual, in succession, at each fixed flash frequency. Ten individuals were used throughout. The mean of each set of three readings was taken as I_1, the mean of the ten averages being recorded as I_m. This is the procedure we have consistently employed in flicker response observations with lower organisms. The reasons for it are discussed in our earlier papers. The order of the levels of F selected was so arranged as to reveal any time-drift in sensitivity of the animals used, or in the observer's appreciation of the movements constituting the index response of the fish. The statistical examination of the records reveals no evidence that such effects are present in the data.

The measurements were made with *Fundulus* kept in fresh water. The temperature

was 21.5° during the tests. The flicker cycle used involved equality of light time and dark time (t_L/t_D = 1). Supplementary tests at several appropriate flash frequencies were made with *Fundulus* living and exposed in sea water. (These results are not entered in Table I or in Fig. 1.) No differences in the properties of I_m were detected for the two series.

TABLE I

Mean critical illuminations for reaction of *Fundulus heteroclitus* to flicker as a function of flash frequency, F/sec., computed as described in the text, with the measures of the dispersions of I_1. Temperature = 21.5° C. Equality of light time and dark time in the flash cycle (t_L/t_D = 1.0).

F/sec.	log I_m	log P.E.$_{I_1}$
1	$\bar{7}$.9777	$\bar{8}$.6371
2	$\bar{6}$.3808	$\bar{8}$.9267
3	$\bar{6}$.6833	$\bar{7}$.3516
4	$\bar{6}$.9353	$\bar{7}$.2418
5	$\bar{5}$.1623	$\bar{7}$.4788
6	$\bar{5}$.4012	$\bar{6}$.0792
7	$\bar{5}$.6688	$\bar{6}$.2250
8	$\bar{4}$.0913	$\bar{6}$.5943
9	$\begin{cases}\bar{4}.6310 \\ \bar{4}.5611\end{cases}$	$\begin{cases}\bar{5}.2732 \\ \bar{6}.9940\end{cases}$
10	$\bar{4}$.9268	$\bar{5}$.3890
11	$\bar{3}$.3477	$\bar{5}$.9267
12	$\bar{3}$.6807	$\bar{4}$.4501
15	$\bar{2}$.1755	$\bar{4}$.5001
17.5	$\bar{2}$.5161	$\bar{3}$.0390
20	$\bar{2}$.8214	$\bar{3}$.3073
25	$\bar{1}$.3664	$\bar{2}$.0881
30	$\bar{1}$.8543	$\bar{2}$.4997
35	0.2610	$\bar{1}$.0633
40	0.6719	$\bar{2}$.9972
45	$\begin{cases}1.1361 \\ 1.1281\end{cases}$	$\begin{cases}\bar{1}.3797 \\ \bar{1}.3747\end{cases}$
48	1.4746	$\bar{1}$.6910
50	1.8226	0.2151
52	2.2669	0.1230

III

The data secured by the procedure outlined in section II are summarized in Table I.

When log I_m is examined as a function of F it is seen to have the general form of the curve obtained with other fishes we have tested. The curve (Fig. 1) is a double S-shaped affair, but the slope of its greater part does not superficially have exactly the properties previously encountered. The analysis of this situation confirms in a rather striking way the validity of
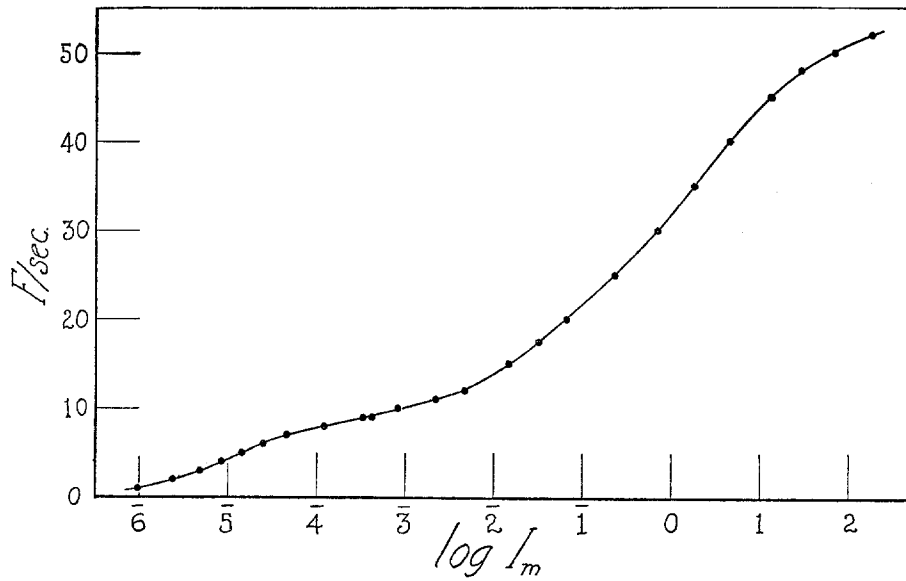
FIG. 1. The flicker response contour for *Fundulus heteroclitus*, log I_m vs. F: at 21.5°, in fresh water, with equality of light time and dark time in the flash cycle. Data in Table I.
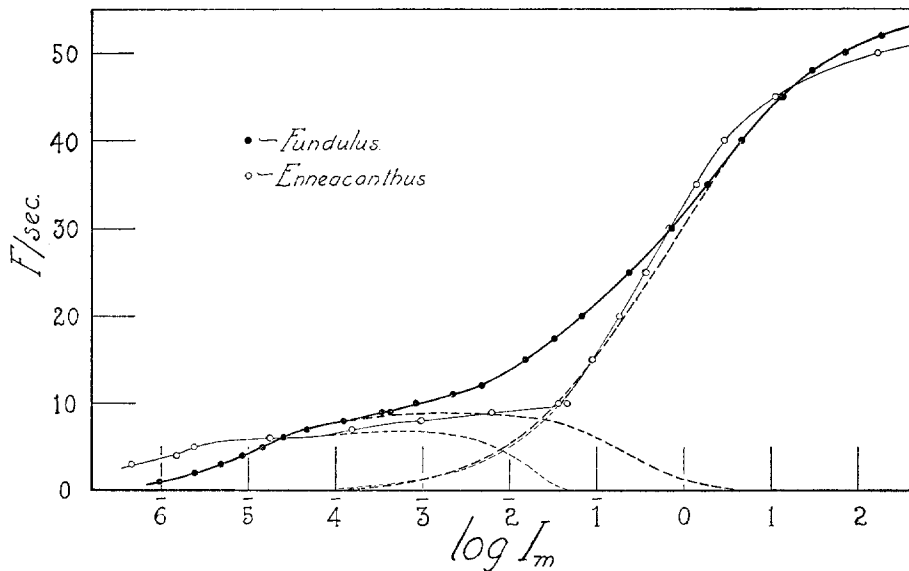

FIG. 2. Analysis of the flicker response curves for *Fundulus* and for the sunfish *Enneacanthus*,[3] under the same conditions. In each case the composite curve is resolvable into two parts: the rod portion at lower intensities, falling off as higher intensities are required, and the cone part. These parts separately adhere to probability integrals (Figs. 3 and 4). The rod part is large with *Fundulus*, and distorts the curve in a comparatively extreme way. The cone curve for *Fundulus* most nearly approaches that for *Enneacanthus* among the forms studied. The independence of rod and cone contributions is made evident by the fact that their properties vary quite independently in the different animals whose flicker response curves have been determined.

the analytical method we have used for the description of the various flicker response curves which have been measured. Among the known curves of this kind the graph for *Fundulus* provides a case which is important because of its rather extreme character.

The flicker response contours of a variety of animals have been described with high fidelity by means of probability integrals

$$F = kF_{\max.} \int_{-\infty}^{\log I} e^{-(\log I/I_0)^2/2\sigma^2_{\log I}} \cdot d \log I,$$

where I_o is the intensity at the inflection point of the curve. For the turtle *Pseudemys*,[7] which has retinal elements of one type only (cones), such a curve adequately fits the data from end to end of the intensity scale. With arthropods such as *Apis*[14] and *Anax* (larva)[14] the gross morphology of the optic surface introduces complications which have been resolved by other experiments.[14, 15] The reasons for the applicability of a probability integral are connected with the interpretation of response to visual flicker as a limiting case of the discrimination of intensities.[16] The inclusive theory of intensive discrimination,[17] which embraces sensorial phenomena of tension,[18] superficial pressure,[19] and auditory[20] response, and presumably other kinds of sensory phenomena, appears to require that the discrimination of intensive effects involves essentially the statistical comparison of populations of elementary resultants for which the compared intensities are respectively responsible.

The flicker response curves for one organism, man, have been fitted by means of equations derived from the theory that the quantitative properties of such measurements are governed by the photochemistry of the retina.[6] The resulting equations do not describe the data as well as probability integrals do, and the properties of their parameters as revealed in appropriate additional experiments are inconsistent with the facts.[1, 2] The responses which are the basis of the measurements involved are reactions of the organism as a whole. The assumption that they are governed by the state of the peripheral retina is unnecessary, and in certain crucial cases[2] can be shown to be inadmissible. The equation derived on the basis of an immediate reflection of retinal events appears to give a reasonable description of the data for the human observer because it is formally identical with a logistic in log I;[21] the latter cannot usually be separated by

[14] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 c.

[15] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 e.

[16] Crozier, 1935; 1935–36.

[17] Crozier, 1936; Crozier and Holway, 1938.

[18] Crozier and Holway, 1937; Holway and Crozier, 1937 a.

[19] Holway and Crozier, 1937 a, b.

[20] Crozier and Holway, 1937; Upton and Crozier, 1936; Holway and Hurvich, 1937.

[21] Crozier, Wolf, and Zerrahn-Wolf, 1936–37 c.

curve fitting from a probability integral (in log I) except within 2 per cent or so distance of the asymptotes. For other organisms the assumptions underlying the derivation of the photostationary state equation, as applied to its adjustment to the observations, become unreasonable.[3] The retinal theory of the control of the properties of the flicker curve in addition fails to give a satisfactory account of the composition of the duplex curve for most vertebrates,[6, 7] whereas the analysis by probability integrals does.[7, 8]

Among lower forms thus far examined, the flicker response curve of *Fundulus* is comparatively extreme. At one end of the series we have the turtle *Pseudemys*,[7] for which the curve is a simple probability integral without complication. This is correlated with the absence of retinal rods. The presence of double or twin cones does not involve or lead to the activity of more than one detectable population of sensory effects. Several teleosts,[12] possessing retinal rods, exhibit increasing degrees of prominence of a subsidiary population of effects at lower intensities. The rising curve of these (rod) effects is also well fitted by a probability integral. By subtraction of the ordinates of the larger curve attributed to the exclusive rôle of retinal cones a curve of "decay of effect due to rods" is obtained; it is a reversed probability integral.[8] The contribution due to the participation of rod-connected elements concerned with response to flicker declines systematically, and in terms of a population of excitable elements, as the critical intensity for threshold total effect increases.

For the newt *Triturus*[10] and for *man*[9] the rod contribution curve (of different magnitude in the two cases) so to speak, sits upon the lower end of the cone curve.

In all of these cases the resolution of the duplex curve is obtained quantitatively on the basis that the fundamental function for each of the two groups or populations of effective elements is described as a probability summation.

The curve for *Fundulus* (Fig. 1) requires a comparatively considerable enlargement of the rod-controlled contribution to the total observable flicker sensitivity. The rod contribution also continues to be evident at higher intensities than with fishes earlier tested. This results in a very marked distortion of the curve in the region where presumptive cone effects are dominant. The uppermost part of the data is, however, very accurately fitted by a probability integral (Figs. 2 and 3). So also is the lowermost rising portion. For other fishes thus far tested the entrance of the effects due to cones produces a distinct "bump" on the composite graph.[8] With *Fundulus* the junction is on the flat region of maximum F for the rods, and is not perceptible as a distinct irregularity.

The difference between the curves for *Fundulus* and for other teleosts is strikingly shown in Fig. 2. Comparison is there made with the observa-

tions on the sunfish *Enneacanthus*,[22] at the same temperature. The cone curve for *Enneacanthus* most closely approaches that for *Fundulus*, among the others available. The contrast in the character of the rod contributions
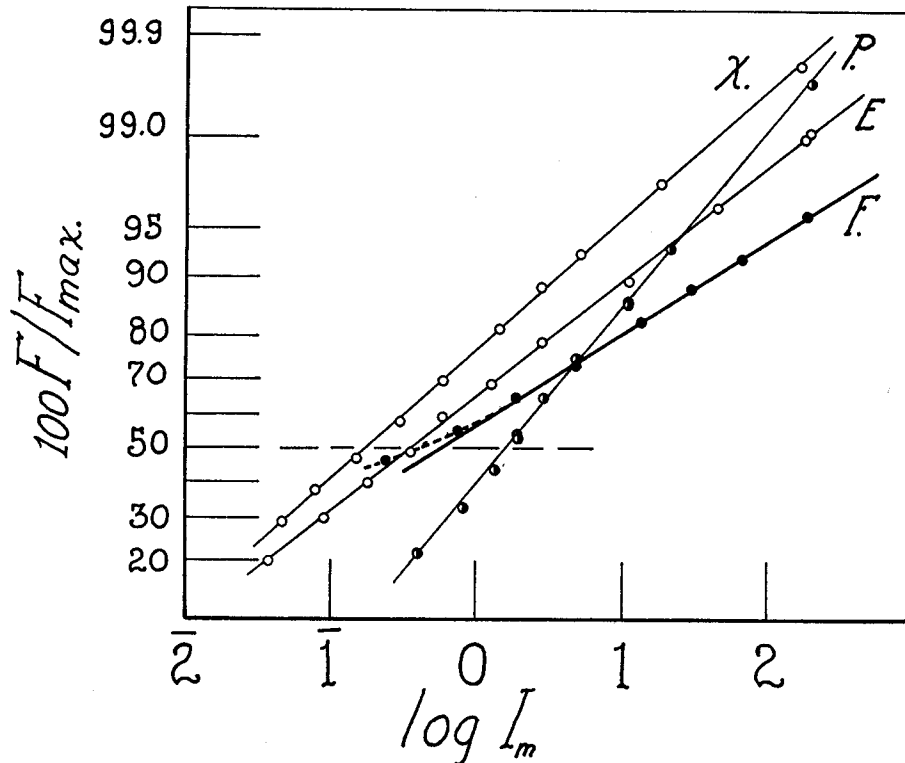

FIG. 3. The cone portions of the flicker response curves for several teleosts[3] are compared upon a probability grid. For *Fundulus* (*F.*) only the uppermost 40 per cent of the *F.* range is described by a probability integral; for the others the degree of overlapping with the rod contribution is less extensive, so that with *Enneacanthus* (*E.*) some 80 per cent of the *F.* range is uncomplicated. The lines drawn have been extrapolated to give (Fig. 2) a means of dissecting the composite graph into rod and cone contributions. (In all cases, temperature $= 21.5°$ and $t_L/t_D = 1$.) *F.*, *Fundulus*; *E.*, *Enneacanthus*; *X.*, *Xiphophorus*; *P.*, *Platypoecilius*. The values of F_{max} are: *F.*, 54.2; *E.*, 52.1; *X.*, 43.1; *P.*, 46.1.

is pronounced. Each of the duplex curves has been analyzed (Fig. 2) by the procedure used for other forms. The result is given in Fig. 2. The ascending and descending rod curves and the cone curve are calculated probability integrals. The degree of adherence of the data to the corresponding equations is adequately shown by the graphs in Figs. 3 and 4.

[22] Wolf and Zerrahn-Wolf, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *a*, *c*, *d*; 1937–38 *d*.

The parameters of the probability integral are rationally related to (1) the genetic composition of the animal,[8] (2) the temperature,[1] and (3) the proportion of light time in the flash cycle[23] $(t_L/(t_L + t_D))$. They are therefore proper indices of the properties of the flicker contour. For log I_m vs. F these parameters differ in a characteristic way from one animal to another (Figs. 3 and 4). It is natural to inquire if correlations can be established between parameters of the rod and cone functions. This might well be a
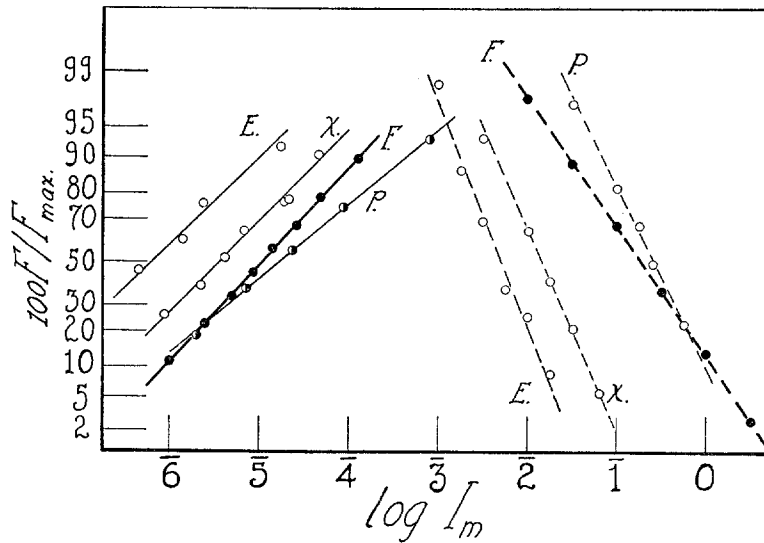

FIG. 4. The rising rod curves, F vs. log I_m, for the teleosts considered in Fig. 3 are shown to be well described by probability integrals. The comparatively large size of the *Fundulus* curve ($F.$) permits a more precise test than with the curves of other fishes. The declining curves, at the right, are the difference curves as in Fig. 2; the plotted points were read from these curves.

matter of some interest for interpretive ethology. Apparently, however, no such correlations obtain. This is consistent with the independent behavior of the two in a genetic experiment.[8]

IV

The variation of critical intensity has a number of important aspects.[24] As shown in Fig. 5, the scatter of I_1, measured by the dispersion of the ten mean values of I_e at each F and computed as $P.E._{1_{I_1}}$, obeys precisely the

[23] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 d, e.

[24] Crozier, 1935; 1935–36; 1936; Crozier, Wolf, and Zerrahn-Wolf, 1936–37 a, b; 1937–38 a.

rules found with other vertebrates.[1, 2, 3, 24] P.E.$_{I_1}$ is directly proportional to I_m, up to a rather high intensity at which the graph assumes a lower slope. The portion of slope < 1 has been interpreted as one of direct pro-
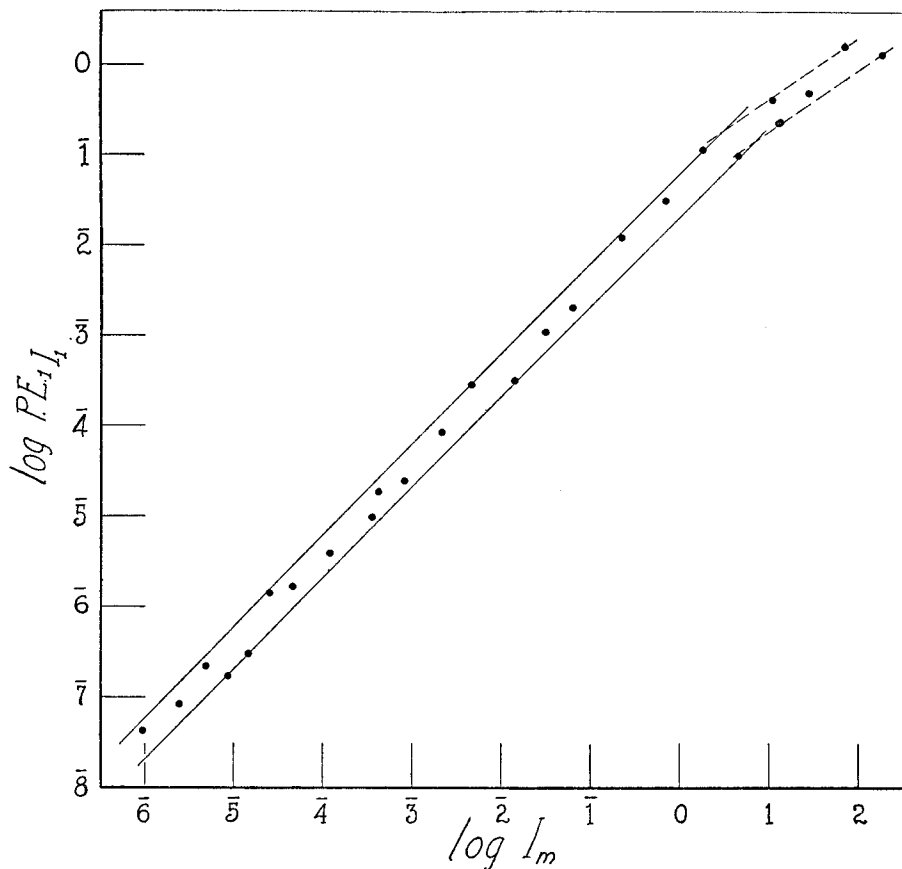
FIG. 5. The variation (σ_{I_1}) of critical intensity (I_1) for response to flicker (Table I) is a rectilinear function of I_m, with a slope of 1. Beyond a certain intensity (log I_m = 0.5) a new origin must be taken, so that the plot "breaks" at this point. This is the intensity at which the declining rod curve approaches the log I axis (Fig. 2). Above this intensity only cone effects are concerned.

portion to I_m but with a new origin.[24] The break has been correlated with the disappearance of rod contributions to the determination of the response.[24] It will be observed that the break occurs with *Fundulus* at log $I_m = 0.5$. This is precisely the level of intensity at which (Fig. 3) the declining rod contribution curve is required to fuse asymptotically with the abscissa axis. This constitutes an independent test of the analysis.

Table II contains a summary of the corresponding facts for other animals. The correlation is precise, as in each instance, the break in the plot of log $P.E._{I_1}$ comes exactly a little above the disappearance of imputed rod effect on the composite F-log I curve. This simply cannot be a matter of error due to the use of the instrument producing flicker, which was the same in all cases. Three animals which do not show retinal duplexity,

TABLE II

Values of log I_m at which a break occurs in the graph of σ_{I_1} vs. log I_m in the flicker response data of various animals.[3] In each case there is correspondence with the intensity beyond which cone-originating effects are exclusively involved in the determination of the critical illumination for response, as shown by the suppression of the contribution from rods required to give a quantitative dissection of the composite graph. With three forms (bee; $Anax$ larva; the turtle $Pseudemys$) there is involved only one class of retinal elements, and no break in the σ_{I_1} vs. log I_m curve is detectable although the range of intensities covered is the same for all forms. Thus the break cannot be attributed to "experimental errors" and their rôle in contributing to σ_I, but is a specific resultant of the fact that the measured variation of critical intensity is primarily an index of the variability of the reacting organism.

Animal	log I at the break
Fundulus	0.5
Platypoecilius	0.0
Xiphophorus	$\bar{1}.0$
Hybrid of *X.* and *P.*	0.0−
Enneacanthus	$\bar{1}.0$
Rana	$\bar{3}.5$
Triturus	0.3
Man	$\bar{2}.7; \bar{2}.8$
Apis	
Anax larva	None
Pseudemys	
Gecko	

$Anax$, $Pseudemys$, and $Sphaerodactylus$, show no break in the graphs of log $P.E._{I_1}$ vs. log I_m, although covering the whole range of measured intensities. These facts are obviously consistent with the view that the variation measured is a property of the reacting organisms,[24, 25] and is not an expression of manipulative "error."

V

The retina of $Fundulus$ has been described as exhibiting a regional differentiation into a dorsal part (70 per cent of the total) containing rods and

[25] Crozier and Holway, 1938.

two types of cones (one type with an oil globule), and a ventral portion with rods and one type of cone (small, and lacking oil globule).[26] The rods are about twice as numerous as the cones. This might correspond to the large share taken by the rod function in the composite flicker contour (Fig. 2), but for the fact that data on *Rana* and other forms show that no quantitative ratio of this kind can be relied upon. So far as the data on reaction to flashes go, the several kinds of cones described by Butcher[26] form parts of a unified population. If, as Butcher's observations suggest, the dorsal cones with oil globule are concerned with chromatophore response to backgrounds, they, of course, might not be chiefly implicated in flicker effects at all. The horizontal direction of the light responsible for flashes in our experiments should result in equivalent illumination of dorsal and ventral halves of the retina.

The temptation is always great to stress one-to-one correlations between rods and cones and duplexity of visual function. The correlation has pitfalls. The effects measured are not immediately retinal. The presence of two chief kinds of retinal sensory cells is suggestive in connection with a double F-log I curve, particularly when animals with no rods or no cones have a simple curve. But it cannot be assumed that the presence of two histological kinds of cones will necessarily signify a further subdivision of the classes of central nervous elements responsible for a given particular type of response.

VI

When the F-log I_m curve for a given animal is determined at various temperatures of the organism it is found that the maximum to which the curve rises is not changed. For each change of temperature the curve is simply moved to a different position on the log I axis, without change of shape.[27] The shift is such that for a vertebrate whose curve exhibits two distinct sections the temperature characteristics for change in position are the same for the two parts. For fixed F we find that $1/I_m$ is related to temperature, within the range where free reversibility of effect is obtainable, by the Arrhenius equation;[28] hence (with the support of other relevant considerations) we may assume that $1/I_m$ behaves as if controlled by the velocity of an underlying system of chemical changes in which one or another catalytic link may be the pace-making step.

The question thus arises as to whether it is possible to decide if the

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[26] Butcher, 1938.

[27] Crozier, Wolf, and Zerrahn-Wolf, 1936–37 *c*, *d*; 1938–39; Crozier and Wolf, 1938–39 *c*.

chemical change appealed to is directly responsible for the obedience of $1/I_m$ to the Arrhenius equation, or whether it does so by secondary control of the number of effective available units or neural elements. Since F_{max}. is not affected,[27, 28] it must be decided that the total number of available neural elements is not the factor influenced by temperature.

It is well known that by exposing certain organisms to sufficiently high temperatures, above a "critical temperature" (or above the mislabelled "optimum" in ordinary experiments) for a sufficient time, one may produce changes which are only slowly reversible ("injury" and the like). If such changes are produced in the flicker response contour of a vertebrate, we may obtain information as to whether (1) the total number of neural elements available for discrimination of flicker has then been affected, or (2) whether a chemical system common to all these elements has been altered in a persistent way; and thus (3) whether this system is different in the rod and cone populations of elements. We already know[28] that the differing shapes of the rod and cone segments of the F-log I contour cannot be regarded as signifying the operation of chemically different systems in the respective elements. The effect of a durable modification of the F-log I curve by heat treatment shows that the "rod" and "cone" elements in *Fundulus* cannot be differentiated by this means.

For such an experiment *Fundulus* has peculiar advantages. Its F-log I curve is exceptional in the large contribution made by presumptive rod-excited elements. It is known that with *Fundulus* by careful adaptation to supranormal temperatures persisting physiological alterations may be induced.[29] We find that by exposure of winter-caught *F. heteroclitus* to 25–27° for 4 days the F-log I curve of individuals then kept for a week or longer at 21.5° has been "permanently" shifted in the direction of lower intensities, so that, although measured at 21.5°, its position relative to that for the unheated individuals is still the one expected for normal animals at 25–27°.

As Fig. 6 demonstrates, the induced shift in the curve shows no change in F_{max}., and no change in shape. The rod part is displaced to the same extent as the cone, and its maximum is likewise unaffected. Therefore it cannot be maintained that the number of neural elements available for recognition of flicker has been affected. Since this number is clearly a function of peripheral sensory area involved,[30] and is also appropriately

[28] Crozier, 1939; Crozier and Wolf, 1939 *a*, *b*; 1939–40 *a*.

[29] Loeb, 1913; Loeb and Wasteneys, 1912; Crozier, 1919.

[30] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *c*.

related to the proportion of light time in a flash cycle,[31] it must be admitted to be a sensitive index. The persisting change resulting from exposure to supranormal temperature must be conceived as governed by an enduring change in the chemical system regulating the excitability of all these
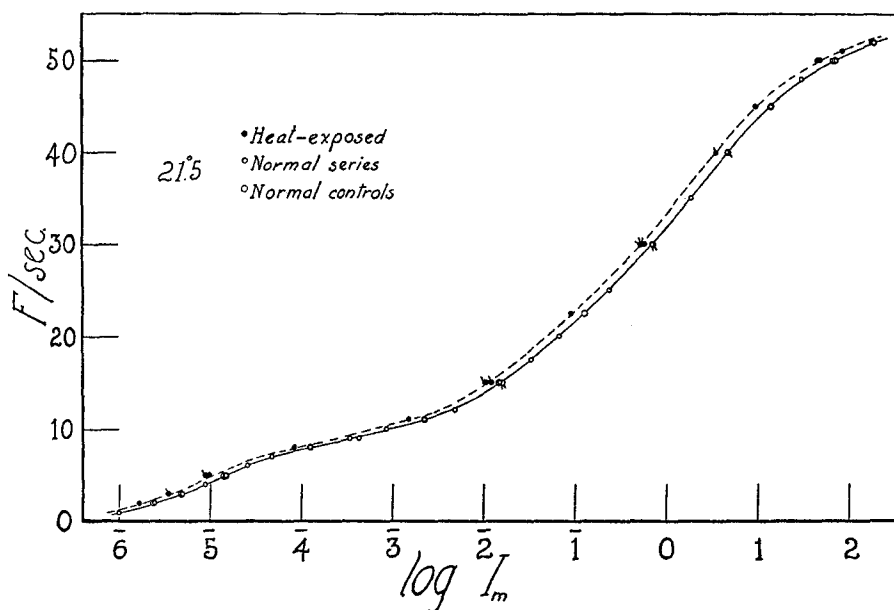

Fig. 6. Mean critical intensity (I_m) as a function of flash frequency for *Fundulus heteroclitus;* means of three observations on each of eight individuals at all points, at 21.5° C., with a flash cycle having equal durations of light and darkness. The "heat-exposed" series, measured after exposure to 25–27° for 4 days; the "normal series," and the "normal controls," were not exposed to temperatures above that of the room. Duplications of points (shown by tags when overlapping) are based on repetitions of the experiment with another lot of ten individuals. The same curve is drawn for both sets of measurements, with displacement by 0.16 log units to the left for the heat-exposed series.

neural elements. The analysis of duplex flicker contours has shown that in the region of overlapping of rod and cone effects there is involved a declining rod contribution; the *Fundulus* curve is important because of the prominence of this feature, and the absence of any change in the form of the curve for the heat-exposed individuals shows that the "decay curve" for the presumptive rod contribution is likewise unaffected as to its shape.

[31] Crozier, Wolf, and Zerrahn-Wolf, 1937–38 *c, d.*

The *variability*[32] of critical intensity is precisely the same function of the intensity as with normal *Fundulus* (Fig. 7).
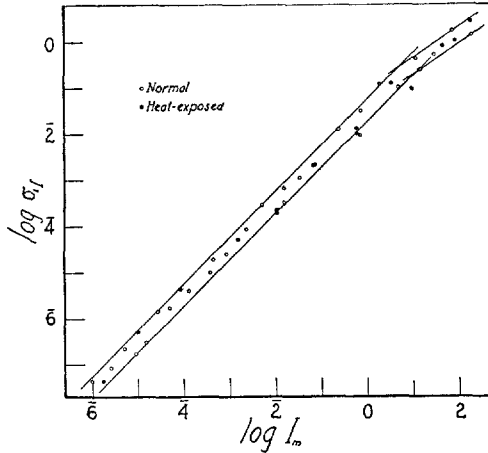

FIG. 7. The dispersion of the individual determinations of I_1, as a function of I_m, is identical for the normal and the heat-exposed series of *Fundulus*. Up to I_m = antilog 0.5, the variation and the mean are directly proportional.

The shift in the curve cannot be explained by enlargement of the iris aperture, as result of the exposure, since direct observation of these animals in a thermostat shows that no measurable change is produced. In the intact fish the iris diameter is independent of temperature (12–28°) and of illumination. Moreover, enlargement of the iris might not raise the curve vertically, nor shift it to the left (Fig. 6). It is not reasonable to suppose that the ocular media become persistently more transparent with heat treatment; any effect is more likely to be in the direction of increased opacity, if anything. Most of our heat-treated individuals died in about 2 weeks after return to room temperature; one was alive after 4 weeks. After a week at 21°, critical intensity determinations with previously heated *Fundulus* slowly drifted after 10 to 14 days up to the levels obtained before exposure to 27°, showing that the effect is reversible.

VII

SUMMARY

After *Fundulus heteroclitus* have been for some time in the laboratory, under conditions favorable for growth, and after habituation of the fishes to the simple routine manipulations of the observational procedure required, they are found to give reproducible values of the mean critical flash illumination (I_m) resulting in response to visual flicker. The measurements were made with equality of light time and dark time in the flash cycle, at 21.5°C.

Log I_m as a function of flash frequency F has the same general form as

[32] Crozier, 1936; 1935–36; Upton and Crozier, 1936; Crozier and Holway, 1937; 1938–39.

that obtained with other fishes tested, and for vertebrates typically: the curve is a drawn-out S, with a second inflection at the low I end.

In details, however, the curve is somewhat extreme. Its composite form is readily resolved into the two usual parts. Each of these expresses a contribution in which log I, as a function of F, is accurately expressed by taking F as the summation (integral) of a probability distribution of d log I, as for the flicker response contour of other animals.

As critical intensity I increases, the contribution of rod elements gradually fades out; this decay also adheres to a probability integral.

The rod contribution seen in the curve for *Fundulus* is larger, absolutely and relatively to that from the cones, than that found with a number of other vertebrates. The additive overlapping of the rod and cone effects therefore produces a comparatively extreme distortion of the resulting F-log I curve.

The F-log I_m curve is shifted to lower intensities as result of previous exposure to supranormal temperatures. This effect is only very slowly reversible. The value of F_{max} for each of the components of the duplex curve remains unaffected. The rod and cone segments are shifted to the same extent. The persisting increase of excitability thus fails to reveal any chemical or other differentiation of the excitability mechanism in the two groups of elements.

Certain bearings of the data upon the theory of the flicker response contour are discussed, with reference to the measurements of variation of critical intensity and to the form of the F-log I curve. The quantitative properties of the data accord with the theory derived from earlier observations on other forms.

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