COMPUTER ASSISTED ANALYSIS
OF S-POTENTIALS

KEN-ICHI NAKA

From Willis H. Booth Computing Center, California Institute of Technology, Pasadena, California 91109

ABSTRACT   S-potentials from the channel catfish were analyzed using a high speed biological data processing system developed at this Institute. The analysis has shown that, when moderately dark adapted, the relation between the (maximal) amplitude of S-potential and log intensity of flash was the hyperbolic tangent throughout the whole visible spectrum. This is what one could expect if the S-potential in the fish was generated by signals from a single class of pigment and each signal had discrete action on the S-potential generating mechanism. The maximal absorption of the pigment involved was at around 625 nm.

INTRODUCTION

In a series of papers Naka and Rushton (1966 *a, b, c*, and 1967) have proposed that the relation between the intensity, I, of a light flash and the maximal amplitude, V, of the resulting S-potentials followed the relation

$$V/V\infty = I/(I + I_{1/2}) \tag{1}$$

where $V\infty$ is the value of V when I is very large and $I_{1/2}$ is the value of I required to make V half of its maximum value.

A simple formal model was proposed from the equation and this model fitted adequately the results obtained experimentally so long as only one pigment system was involved. If the light excited more than one class of cones, interactions of various kinds were observed to occur. The present paper describes a more exact numerical study of the relation between the amplitude of the S-potential and intensity of flash made in conditions improved in two respects. A. The former work (Naka and Rushton, 1966 *a, b, c*, and 1967) on the tench concerned a fish with three or four different kinds of cones which made isolation of the response of one class of cone rather difficult. The present study was on the channel catfish S-potential which (as will be described later) appeared to be generated by signals from one kind of cone. B. In the former work, records were photographed, measured up, plotted and compared with a fixed template curve that corresponded to equation 1. The correspondence

was obviously not bad but no attempt was made to enter into laborious questions as to the extent to which the divergences seen lay within experimental error, nor what other relation might fit the results better.

This type of analytical investigation can be quickly and precisely performed by the high speed Biological Data Processing System developed at this Institute (Lockemann and Knutsen, 1967). Applied to the present problem, it was found that experimental results deviate very slightly (but consistently) from equation 1. The results fit exactly a similar expression with an additional arbitrary parameter.

DATA ACQUISITION

The fish used were channel catfish (*Ictalurus punctatus*) obtained locally and kept in the laboratory. Preparation of the retina and recording techniques were practically the same as described in one of the papers previously published (Naka and Rushton, 1966 *a*). Briefly, the frontal half of the excised eyeball was removed and the vitreous humor was drained as thoroughly as possible. The eyecup preparation was placed in a chamber with a continuous, cool, moist oxygen supply. S-potentials were picked up by potassium citrate filled glass pipettes. Experiments were performed under moderately dark adapted conditions. The amplified potentials were recorded on magnetic tapes for later analysis and also by a pen writer for preliminary screening of results. The optical system was composed of two identical channels each composed of a Bausch & Lomb High Intensity Grating Monochrometer (with visible grating) (Bausch & Lomb, Inc., Rochester, N. Y.) coupled with a xenon arc light source, a Kodak (Kodak, New York City) four log annular neutral density wedge (M-type carbon), and a solenoid shutter. A potentiometer which was linked to the rotating axis of the annular wedge served to read off relative log intensity (J) of flash. Illumination was a diffused field over the retinal surface and a flash of 0.3 sec in duration was given once in every 3 sec. Relative energy of the spectral lights were calibrated by a Reeder Thermopile (Chas. Reeder & Co., Inc., Detroit, Mich.) and a Keithley 150B Microvoltmeter (Keithley Instruments, Cleveland, Ohio).

DATA ANALYSIS

Data analysis and subsequent curve fitting were done by use of the Biological Data Processing System of the California Institute of Technology. With use of the "Phase 2 System" (Lockemann and Knutsen, 1967) the entire data processing could be done without any manual interference. Three sets of data stored on the (analogue) magnetic tape, S-potential, position of the neutral density wedge and timing signal, were first transmitted to the discs of the IBM 360/44 computer through a multichannel AD converter (Lori I). Sampling rates of AD conversion were 50 points per second for S-potential and 10 points per second for the position of the neutral wedge. Timing signals were transmitted as TOE's (flash signal caused a digital clock ran at
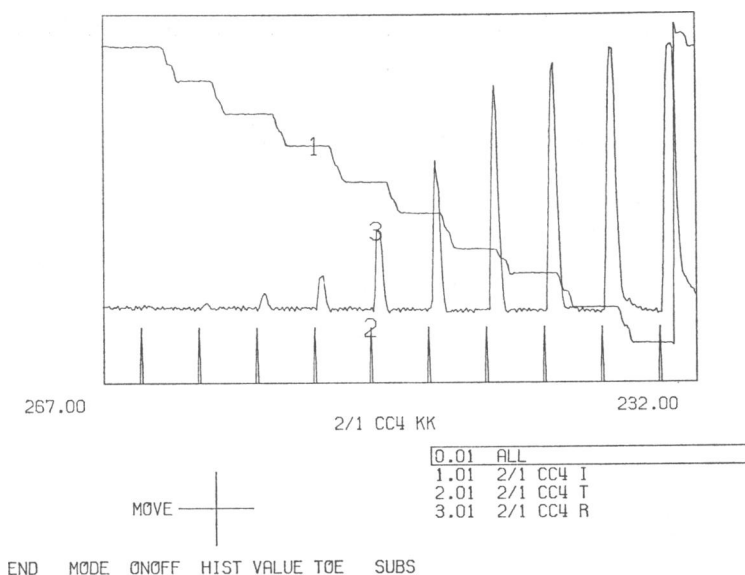
267.00 2/1 CC4 KK 232.00

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0.01  ALL
1.01  2/1 CC4 I
2.01  2/1 CC4 T
3.01  2/1 CC4 R
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MOVE

END   MODE   ONOFF   HIST VALUE TOE   SUBS

FIGURE 1   Display of data on time domain. The trace marked "1" was the position of the neutral wedge (J) "2", timing signals (TOE's of 'ON' of flash), and "3", S-potentials (polarity was reversed).

500 KHz to be read and the resulting time, *Time of Event*, to be sent to the computer). Stored data was first inspected on the IBM 2250 display unit on a *"Scan"* mode (Lockemann and Knutsen, 1967) which is shown in Fig. 1. In this figure, the trace marked "1" was the position of the neutral density wedge, trace "2," timing signal sent as TOE's and trace "3," S-potential (negativity as upward swing). A set of runs (a run composed of 8–12 responses by a series of flashes covering three to four log units in intensity) was accepted for analysis if there was no appreciable change in the DC level during the set of runs and also if no changes in the maximal amplitude of the response were found.

DEFINITION OF TERMS

It is convenient to take V against "J" ($= \log I$) on the horizontal axis. Let $J = \log I$, then writing "b" for $I_{1/2}$ equation 1 becomes

$$V/V\infty = 1/(1 + b10^{-J}) \tag{2}$$

This is the tanh curve of Naka and Rushton (1966 *a*). A modification that fits the facts better is

$$V/V\infty = 1/(1 + b10^{-aJ}) \tag{3}$$

where "a" is slightly different from unity. This is a logistic function. From equation 3 the following results are easily obtained.

$$d/dJ(V/V\infty) = 2.3\, a(V/V\infty)(1 - V/V\infty) \qquad (4)$$

when

$$d^2/dJ^2(V/V\infty) = 0,\ (V/V\infty) = \tfrac{1}{2} \qquad (5)$$

The value of $d/dJ(V/V\infty)$ at this maximum slope when $V/V\infty = \tfrac{1}{2}$ is

$$d/dJ(V/V\infty)\mathrm{max} = 2.3a/4 \qquad (6)$$

When $(V/V\infty) = \tfrac{1}{2}$, the value of J is

$$J = \log b^{1/a} \qquad (7)$$

On actual plotting the horizontal axis was scaled as kJ and computations were done on this scaling. The value of "k" was different from experiment to experiment but was kept unchanged for a given series of experiments. From equation 6 it follows

$$a = 4/2.3 \cdot k \cdot d/dJ(V/V\infty)\mathrm{max}. \qquad (8)$$

In the channel catfish, under ordinary conditions, lights of 400–700 nm, if they were strong enough, could bring the potential to a certain fixed ceiling, $V\infty$, the absolute amplitude of which was dependent on the particular recording conditions and was usually between 40 and 60 mv (negative going from the dark level). Consequently, in the channel catfish two parameters, "a" and "b" in equation 3 remain to be defined. Plotting of results and fitting of curve in equation 3 were done in the following sequence. A. To specify the portion of data to be plotted (*Begin, End of Cycle* = 267, 232 in Fig. 2). B. To plot the amplitude of S-potential against kJ as dots or as lines connecting dots on the IBM 2250 display unit. C. Fit equation 3 to the data points and to indicate on the screen three parameters; 1. $kJ_{1/2}$ which is the position of the curve on the log intensity axis, 2. slope of the curve at $kJ_{1/2}$, and 3. variance between the data points and fitted curve. Fig. 2 illustrates a typical display of data points by 600 nm flash (10 points connected by lines) and a curve fitted to the data points (smooth curve) with three parameters: *Intensity Displacement for* $kJ_{1/2}$, *Slope* for $d/dJ(V/V\infty)\mathrm{max}$ (in the subsequent description this will be referred to as a 'slope'), and *Variance* as usually defined. Figures other than 4, 5, and 8 were generated by copying the display picture to a Calcomp plotter. Fig. 2 shows an entire display, Fig. 1 a part of display, and other figures are composite pictures of several displays plotted by the plotter.
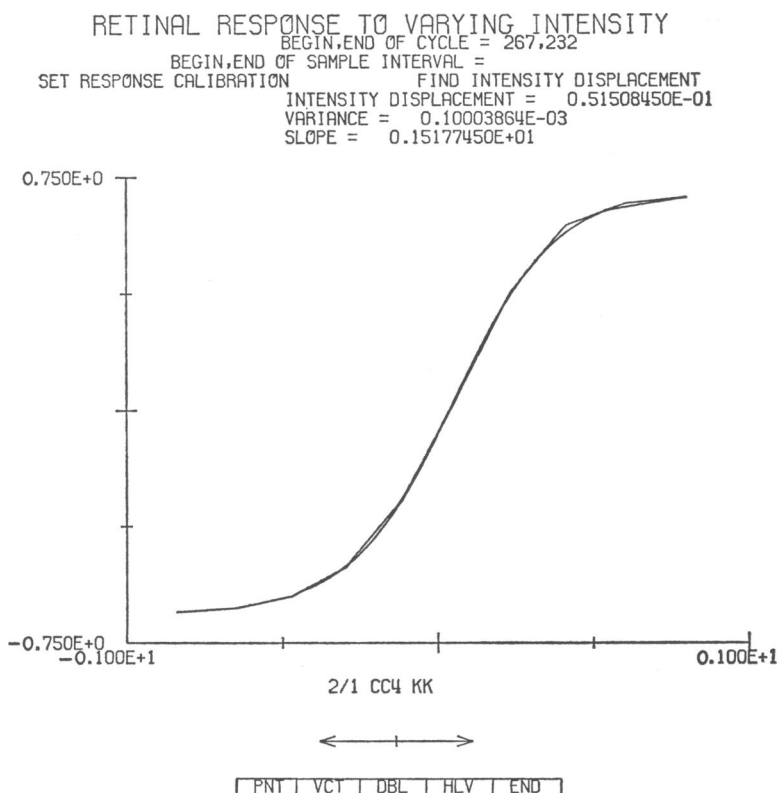
RETINAL RESPONSE TO VARYING INTENSITY
BEGIN,END OF CYCLE = 267,232
BEGIN,END OF SAMPLE INTERVAL =
SET RESPONSE CALIBRATION   FIND INTENSITY DISPLACEMENT
                          INTENSITY DISPLACEMENT =   0.51508450E-01
                          VARIANCE =   0.10003864E-03
                          SLOPE =   0.15177450E+01

0.750E+0

-0.750E+0
-0.100E+1   0.100E+1

2/1 CC4 KK

[PNT | VCT | DBL | HLV | END]

FIGURE 2   Data points (connected by lines) by flash of 625 nm and a logistic function fitted to the data points. The abscissa was in the kJ unit; i.e., the scaling was arbitrarily set as ±1, middle point taken as 0. "k" was the scaling factor to transform the arbitrary scale into $\log_{10}$ scale. The ordinate was the amplitude of S-potential (the maximal value for a given intensity). Variance of the order shown in this figure was a typical one. The numerical values were expressed by a fraction which had to be multiplied by the power of 10 which was indicated after "E".

RESULTS

Value of "a" at Different Wavelengths

S-potentials from the channel catfish were all negative going potentials which were of L-type of MacNichol and Svaetichin (1958). In the channel catfish no polarity reversal of the response due to change in the wavelength of flash took place. As already mentioned, under ordinary conditions, the maximal response amplitude by any spectral lights (including white light) was nearly identical throughout the whole spectrum. This maximum excursion or ceiling is $V\infty$ in equation 3 and was a fixed quantity for a given recording condition.

Records in Fig. 3 are V-J curves and logistic functions fitted to the data points. The four sets of data points which were obtained from the same unit were by flashes of

700NM   600   500   400

10/24 CC

VARIANCE = 0.10037528E-03   0.12527100E-03   0.16948900E-03   0.11217780E-03
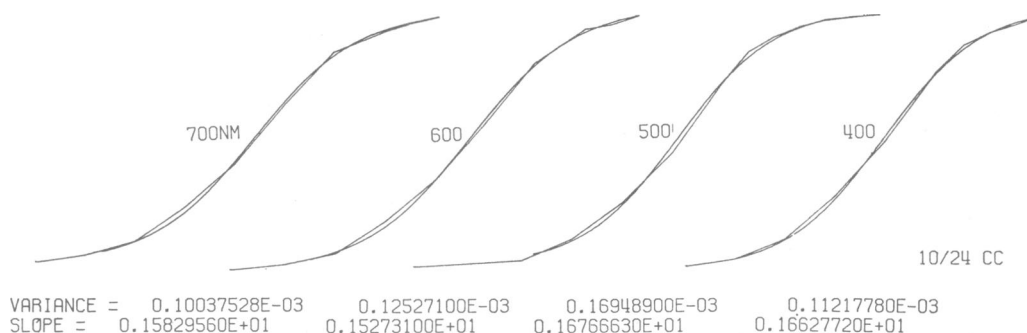SLOPE = 0.15829560E+01   0.15273100E+01   0.16766630E+01   0.16627720E+01

FIGURE 3   V-J curves by four different spectral lights. Records were from the same unit. Both data points and fitted curves were represented by lines. Extended curves were for data points and shorter curves were for fitted functions. Variance and 'slope' (as computed on the kJ scale) were also shown under corresponding records. In this experiment four log unit was 1.544 on the horizontal axis ($k = 1.544/4$). This value and equation 8 gave "a"s for four curves.

400, 500, 600, and 700 nm. In the figure "slope" and variance for each fitted curve are shown under corresponding record as they appeared on the display unit. The $V\infty$ and "a" (obtained from equation 8) were 1012 and 1.08 for 400 nm data points, 1069 and 1.11 for 500 nm data points, 1072 and 0.99 for 600 nm data points and 1008 and 1.02 for 700 nm data points (the maximal amplitudes were in relative units). As seen from both the fitted curves and also from variance for each record, the fit of theoretical curve to data points was nearly perfect at four wavelengths chosen in this figure. There seems to be no reason to make any further elaboration or to try to fit another function until there is any further evidence to do so. The maximal amplitudes of the response ($V\infty$) were practically identical and "a"s were nearly 1 for all curves fitted. Similar data so far obtained in the fish all agreed well with the results shown in Fig. 3. Thus, V-J curves from the channel catfish S-potential elicited by any spectral flash could be fitted to a logistic function of equation 3 if proper "a" was chosen. In the original formula (equation 2) developed in the tench experiment, "a" was chosen as 1 and this was assumed as the V-J curve generated by signals from a single class of receptor (Naka and Rushton 1966 a, b, c, and 1967). Therefore, "a" in equation 3 bears considerable importance on the description of V-J curve and also on the validity of the formal models presented in the tench papers. If it is unity for all wavelengths as presumed in the tench, equation 3 has only one variable which determines the position of V-J curve on the J axis. If "a" is not unity, V-J is no longer a tanh curve and a simple formal model has to be abandoned. If "a" is wavelength dependent, the Principle of Univariance (Naka and Rushton, 1966) leads to a conclusion that the S-potential must be generated by signals from more than one class of receptors. In the present curve fitting, "a" was not arbitrarily taken as 1 as in the tench experiment; "a" for the best fitting logistic function could be precisely defined by the data points. Therefore, "a"s at 7 wave

lengths from 400 to 700 nm were carefully computed and the results are tabulated in Table I. As seen from Table I "a"s for the best fitting logistic function differed slightly from 1 but they were reasonably close to 1 for all spectral lights. Consequently, V-J curves of S-potentials from the channel catfish had, at least as the first approximation, only one degree of (formal) freedom which was the lateral shift of the curve along the intensity axis (J). It should be mentioned here that "a" tends to be slightly greater for lights of shorter wavelength. This has been consistently found but, as of now, we have no explanation for this small but consistent deviation.

Position of V-J curves on the J axis is a dual function of wavelength of flash and also of the state of adaptation (Naka and Rushton, 1968). If the retina becomes more sensitive to a flash of light either due to change in the wavelength or by dark adaptation, the curve shifts towards smaller J. In the present experiment it was confirmed that in dark adaptation the V-J curve was shifted along the J axis, and the only change involved was $J_{1/2}$. Other parameters remained unchanged. This agrees well with the results obtained in the tench and carp by Naka and Rushton (1968), namely that the increase in the sensitivity of S-space (Naka and Rushton, 1967) was a shift of whole V-J curve along the log intensity axis without involving any change in the DC level of the space. If the state of adaptation was held reasonably constant, $J_{1/2}$ at each wavelength plotted against wavelength gives the log spectral sensitivity of pigment involved in generation of S-potential. The log spectral sensitivity curves thus derived are shown in Fig. 4 in which $J_{1/2}$'s from five runs were plotted (dots). Though points from individual runs were not exactly identical, the spectral sensitivity curve had a well defined contour with a peak at around 625 nm. The shape of the curve in the blue region, however, was not well established due to the small number of data points available.

In the frog retina, Donner and Rushton (1959) have shown that under a condition in which only a single class of receptors was involved a flash of one wavelength could be replaced by a flash of another wavelength without producing any response if the intensities of two spectral lights were properly balanced. Similarily, if

TABLE I
VALUE OF a AT SEVEN WAVELENGTHS

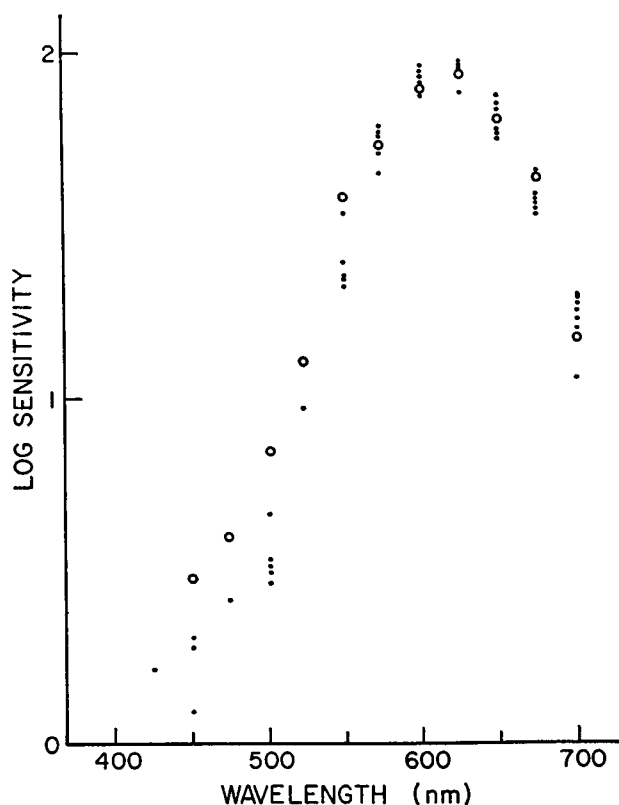| Wavelength	Number of Measurements	Mean of "a"	Variance
nm			
700	11	0.999	0.003
650	9	0.975	0.0004
600	13	0.999	0.002
550	10	1.039	0.003
500	11	1.039	0.004
450	10	1.041	0.002
400	9	1.015	0.007

FIGURE 4   Log spectral sensitivity curves by $J_{1/2}$ (dots) and by the color matching by silent substitution (open circles).

only one pigment was involved in the generation of the catfish S-potential a flash of one wavelength followed by a second flash of another wavelength (at a particular intensity) should not evoke any transient response. Records in Fig. 5 show results of experiments in which a fixed intensity flash of 625 nm was followed immediately (substituted) by a second flash of either 625 nm (A) or 475 nm (B). The intensity of the second flash was varied to show, (a) that if it was not properly balanced in intensity a transient could be seen; (b) that it was possible by a fine adjustment of the intensity of the second flash to obtain a silent substitution (cf. Donner and Rushton, 1959). As seen from the records a silent substitution can be made and this held true for flash of other wavelengths. Knowing the log intensity of the second flash which could be matched to a fixed intensity flash of a given wavelength (a flash of 625 nm was chosen as the reference) it was possible to obtain a log spectral sensitivity of the pigment involved. A typical spectral sensitivity curve obtained by this method is shown in Fig. 4 by open circles. Two sets of curves obtained by two different methods, one by $J_{1/2}$ and the other by color matching, agreed well substantiating the assump-
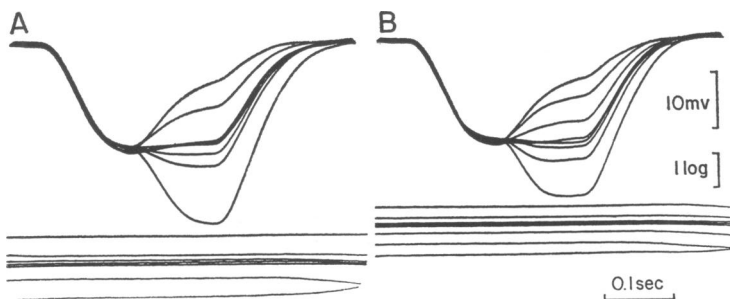
FIGURE 5 Matching of responses by two successive flashes. A flash of 625 nm (0.3 sec in duration) was followed by another flash of the same duration. The second flash was 625 nm in "*A*" and 475 nm in "*B*". Intensity of the second flash was varied to obtain a silent substitution.

tion that the channel catfish S-potential was generated by signals from a single class of pigment.

INTERACTION OF TWO SPECTRAL LIGHTS

The results described strongly suggest, (*a*) that the channel catfish S-potentials were generated by signals from a single class of pigment with the absorption maximum at 625 nm; (*b*) that the *V-J* curve follows very closely the tanh relation of equation 2. If this is true it is possible to predict all the results of the following type of experiment. Suppose the flash is not a single monochromatic light of variable log intensity *J*, but a mixture of two monochromatic lights, say 625 nm and 475 nm both presented in the same single flash. One component is kept at fixed intensity I_0 and the other I_1 varied over a wide intensity range. The S-potential, *V*, is measured in response to this flash ($I_0 + I_1$) and plotted against $J_1(\log I_1)$.

The expectations were predicted by Naka and Rushton (1966 *c*, equation 2). The curve should still be tanh curves with the following properties which define them.

(*a*) The maximum of all curves is the same and is simply the S-potential ceiling, $V\infty$.

(*b*) The minimum is naturally the value of *V* for I_0 alone.

(*c*) The midpoint of the curve (*I'* of Table II) is given by

$$I' = I_0 + I_{1/2}$$

where I_0 is the intensity of the fixed component in the flash and $I_{1/2}$ corresponds to the midpoint of the curve when only one component is in the flash (i.e. when $I_0 = 0$).

Records in Fig. 6 show data points obtained by two flashes given simultaneously and logistic curves fitted to the data points (data points were connected by straight lines). Curves shown in this figure were recorded in sequence from "*A*" to "*H*" from
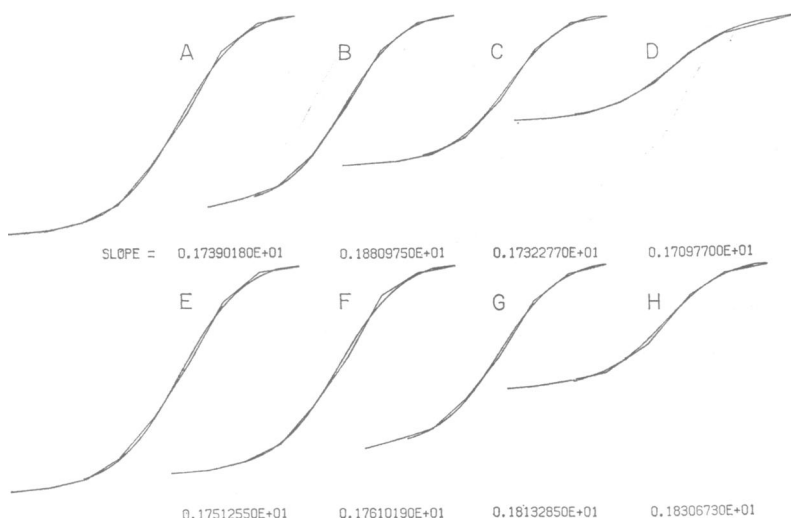
FIGURE 6   *V-J* curves by two spectral flashes given simultaneously. One component, 625 nm, was varied and its intensity was plotted as kJ_1. "*A*" and "*E*" were control obtained by flashes of 625 nm alone. The fixed intensity components (I_0) were 625 nm in "*B*", "*C*", and "*D*" and 475 nm in "*F*", "*G*", and "*H*". The first data point (the beginning of the curve) was the dark level in "*A*" and "*E*" and was the amplitude, V_o, of the response by the fixed intensity flash alone. In the curve fitting the first data point was taken as *0* and *V* was measured from this level. The "slope" for each curve is shown. In this experiment four log unit was 1.262 on the abscissa ($k = 1.262/4$). Parameters of curves in this figure are tabulated in Table II.

the same unit. "*A*" and "*E*" were controls obtained by flash of 625 nm (I_1) alone and a fixed intensity flash (I_0) of three different intensity levels as added in "*B*", "*C*" and "*D*" (I_0 was 625 nm) and in "*F*", "*G*" and "*H*" (I_0 was 475 nm). Values of "slope" as computed (on kJ scale) are also shown with each curve; in Table II they were converted into "*a*" from equation 8. As seen from this figure, regardless of the wavelength and intensity of the added flash the ceiling of the response, $V\infty$, was the same (see also Table II) and the data points fitted very closely to the logistic function (variance was of the order of 10^{-4} or less). The "slopes" of the fitted curves were very comparable. Parameters of the data points and fitted curves are tabulated in Table II. The ceilings, $V\infty$, were almost identical and the computed "*a*" s were nearly unity for all curves. Similar results have been obtained in other experiments in which the variable flash (I_1) was of 475 nm instead of 625 nm as shown in this figure. These results clearly demonstrate that *V-J* curves by flash composed of two components, whether one component was of the same wavelength or not, still fitted a logistic function with comparable slopes as those obtained by single flashes.

To test the validity of prediction (*c*), the intensity of the added flash, I_0, had to be known. It was convenient to calculate, not to measure experimentally, the intensity of added flash from *V-J* curve. If the recording condition was stable and the state

TABLE II*

Corresponds to "A" to "H" in Fig. 6	$V\infty$	V_o	$V_0/V\infty$	a	I_o	I'	I''
A	0.886			1.03			
B	0.864	0.154	0.178	1.04	0.230	1.241	1.214
C	0.864	0.292	0.338	1.05	0.528	1.534	1.770
D	0.879	0.490	0.557	0.94	1.285	2.300	2.480
E	0.840			0.94			
F	0.884	0.080	0.095	0.94	0.095	1.093	1.070
G	0.872	0.188	0.216	0.98	0.260	1.258	1.313
H	0.868	0.412	0.475	1.01	0.898	1.897	1.763

* Column A to H corresponds to records "A" to "H" in Fig. 6. V's were in arbitrary unit. $V\infty$, the amplitude of the response by the maximal intensity flash (either one or two components). V_o, the amplitude of the response by the fixed intensity flash. "a", computed from equation 8 using value of "slope" in Fig. 6. I_o, intensity of fixed component computed from equation 9 using value of $V_0/V\infty$. $I' = I_{1/2} + I_0$, $I_{1/2}$ being the midpoint intensity of V-J curve by single flash. $I_{1/2}$ was 10.11 (computed from record "A") for B, C, and D and was 0.998 (computed from record "E") for F, G, and H. I'', the midpoint intensity of V-J curve by two component flash (experimental).

of adaptation was held reasonably constant, the intensity of added flash can be obtained either from equation 2 or 3. Let V_0 be the amplitude of the response by the added flash, $V\infty$, the ceiling of the response (which was a fixed value for a given recording condition) and J_0 (log I_0) the intensity of flash to give V_0, equation 3 becomes

$$V_0/V\infty = 1/(1 + b10^{-aJ_0})\tag{9}$$

From equation (9), I_0 could be calculated and the control run by single flash ("A" or "E" in Fig. 6) gives $I_{1/2}$. Table II lists the actual and computed results. Agreement between I' (expected) and I'' (experimental) is reasonably good and prediction (c) seems to be held.

This simple addition, however, began to break down when the intensity of the added flash was very high (if $V_0/V\infty$ was more than 0.6). Under these extreme conditions I' was always less than I''. This is what one would expect if equation 9 could only be applied to those responses obtained under comparable adaptational states. If repeated high intensity flash made "b" (or $I_{1/2}$) larger (which is equivalent to sliding the whole curve along J axis) the equation can no longer be applied to estimate I_0.

DISCUSSION

In the tench retina, Naka and Rushton (1966 a, b, c, and 1967) have proposed that the relation between the height of S-potential and intensity of light was simple hyper-

bolic tangent if the S-potential was generated by signals from a single class of cones. If the (maximal) height of the potential was plotted against log $I(J)$ it was a form of logistic function shown in equation 2. Though their conclusions were well supported by experimental evidences available at that time, the situation in the tench was complicated by the fact that the tench S-potential (L-type) was apparently receiving signals from more than one class of cones. Under normal conditions it was not possible to isolate S-potentials from a single class of cones over a wide range of experimental conditions. The curve fitting was also done by sliding a template which was of the form of equation 2. Though the fit was as good as one could expect from the accuracy of measurements in the series of experiments the evidence was not compelling nor could accuracy of fit be assessed.

In view of the facts that the proposed relation is very attractive and bears important implication on the mechanism of S-potential generation, we decided to reexamine the case under a much simpler situation and also with the use of the high speed digital computing system developed at this Institute. These two improvements over the tench experiment were expected to give more conclusive evidences on the validity of the proposed relation.

As described in the results we have obtained substantial evidences to prove that the logistic function of the form of equation 3 could fit the results with great accuracy and no further elaboration of the equation seemed necessary unless other strong evidence against the conclusion be brought forth. The most crucial question was, then, whether "a" in equation 3 is unity or not. The results were not satisfactory in that "a" in equation 3 had to be slightly different from unity to get the best fit. However, the deviation was very small and, as the first approximation, "a" could be taken as unity. The equation 2 seems adequate to describe V-J curves of S-potential from the channel catfish. In the crayfish photoreceptor (retinula cell), Glantz (1968) could describe the relation between the response voltage and the log intensity of flash by a (formally) identical function (equation 1 of this paper and equation 7 of Glantz's paper).

The Principle of Univariance (Naka and Rushton, 1965) requires that if the potential is generated by signals from a single class of pigment "a" should remain unchanged throughout the visible spectrum. Again, the results were not completely satisfactory as "a" was slightly larger for flashes of shorter wavelength. The log spectral sensitivity of the pigment involved had its maximum at around 625 nm and the results of color matching by the silent substitution (cf. Donner and Rushton, 1959) substantiated the conclusion. The experiments with two flashes also proved that predictions from equation 2 could be fulfilled with a small deviation.

This simple relation began, however, to break down when V-J curves were obtained in the presence of a strong steady background light. This is equivalent to making I_0 constant in the two flash experiment. One such example is shown in Fig. 7 in which three sets of V-J curves were recorded in the presence of a (also in absence of) strong steady illumination and the logistic functions were fitted to the
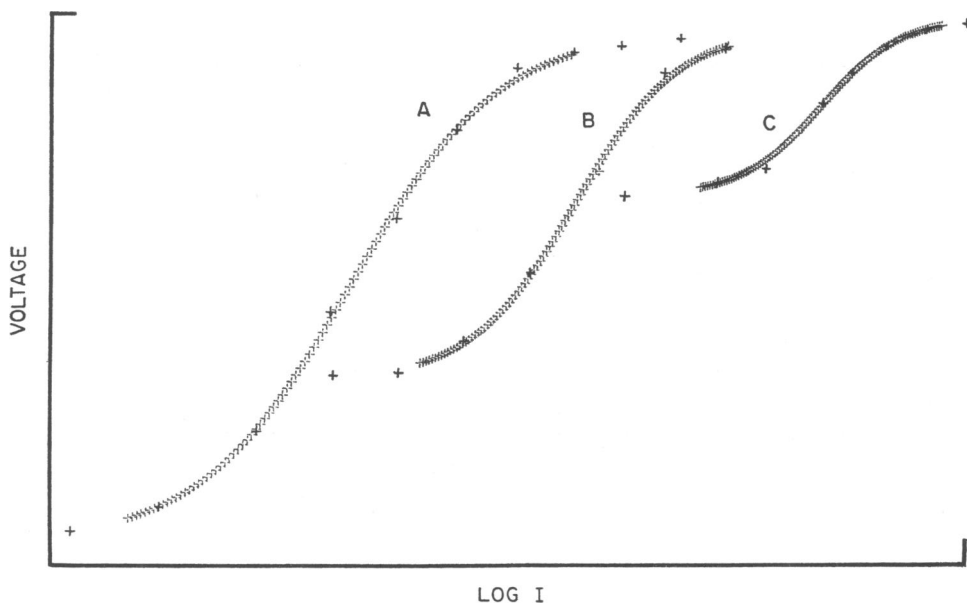
FIGURE 7 Logistic functions fitted to the data points obtained in the presence of steady illuminations (white light) of two different intensity levels. "*A*" was without (control) and "*B*" and "*C*" were in the presence of steady illumination. Three curves were displaced laterally by arbitrary amounts. Number of data points were 10 in "*A*", 7 in "*B*", and 8 in "*C*." The first data point (the far left cross for each curve) was the dark level in "*A*" and the raised level of potential by steady background illumination in "*B*" and "*C*." "*a*" was 0.99 for "*A*," 1.40 for "*B*," and 1.80 for "*C*." Midpoint in log unit ("*A*" taken as the reference) was 0.45 for "*B*" and 0.98 for "*C*." Variance was 1.05×10^{-4} for "*A*," 8.41×10^{-5} for "*B*," and 4.51×10^{-5} for "*C*."

data points (both data points and fitted curves were represented by crosses). These three data sets were obtained from the same unit, "*A*" as a control without any background light, "*B*", and "*C*" in the presence of steady illuminations. All data sets could be fitted by logistic functions with variance of less than 10^{-4}. However, there were two points where the results contradicted our simple assumption: 1. The maximal amplitude of the response in the presence of a very strong steady illumination ("*C*") exceeded the maximal response obtained either by flash alone ("*A*") or by flash in the presence of a medium intensity steady illumination ("*B*"). Though the deviation was less than 10% this has been consistently observed under similar experimental conditions. 2. The logistic functions fitted to "*B*" or "*C*" had "*a*" of 1.48 and 1.96 respectively. These values are too large to be accounted for by any technical error or to be attributed to changes in the state of adaptation. This phenomenon deserves further investigation.

It is worth mentioning here that, as shown in this paper, the *V-J* curves of the catfish S-potential can be characterized by a few parameters. Therefore a well
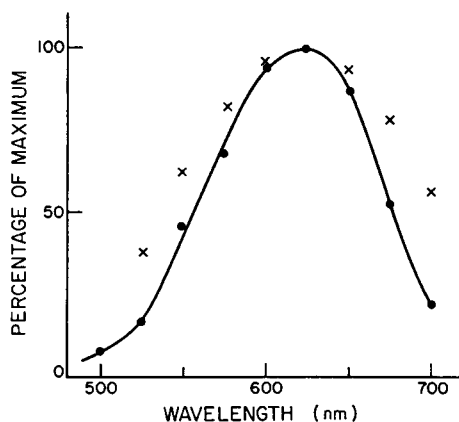
FIGURE 8 Comparison of the spectral sensitivity curve of the catfish 625 nm cone (derived from S-potentials) with the absorption of retinene₂-based nomogram pigment (Munz and Schwanzara, 1967). The smooth curve is for the catfish 625 nm cone plotted on linear scale and crosses are absorption of the retinene₂-based visual pigment calculated from Munz-Schwanzara nomogram.

designed data processing system can be a very useful tool in the further study of the functional significance of S-potentials.

The presence of cones in the fish retina with an absorption maximum at or around 625 nm was observed by Marks (1964) using microspectrometry, and by Tomita et al. (1967) by recording the electrical response from single cones. Naka and Rushton (1966 c) have also obtained evidence that some of the tench S-potentials are generated by signals from 625 nm cones. In Fig. 8 the absorption of a retinene₂-based nomogram pigment with a maximum absorption at 625 nm (Munz and Schwanzara, 1967) is compared with the spectral sensitivity curve of the catfish 625 nm pigment which was derived from S-potentials. In this figure, the sensitivity curve plotted on a linear scale was calculated from the log sensitivity curve in Fig. 4. The nomogram for the retinene₂-based visual pigments (Munz and Schwanzara, 1967) was used instead of Dartnall's nomogram since it is most likely that the channel catfish has retinene₂-based pigments. As seen from Fig. 8, the sensitivity curve derived from the S-potential is sharper than that of the nomogram pigment. The spectral sensitivity curve of the tench cones by Naka and Rushton (1966 c) was also narrower than the nomogram pigment. However, it is worth mentioning that the spectral sensitivity curve of single cones by Tomita et al. (1967) showed a discrepancy of similar magnitude from that of Dartnall's nomogram pigment.

CONCLUSION

When moderately dark adapted the relation between the amplitude of S-potential from the channel catfish and log intensity of flash is a hyperbolic tangent. This leads to two conclusions: 1. S-potential is generated by discrete signals which equally

contribute to changes responsible for S-potential. 2. The channel catfish S-potentials are, under present experimental conditions, generated by signals from a single class of pigment with the absorption maximum at 625 nm. However, this simple relation began to break down in the presence of strong steady background light. Under such experimental conditions, though the relation was still a logistic function, a power function had to be introduced to produce the best fit.

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