Color Processes in Single Retinal Elements

By

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In our preceding papers¹⁾²⁾³⁾⁴⁾ it was shown that the same mode of analysis as used on the human eye by Motokawa proved to be still useful on excised frog's eyes when action potentials of the optic nerve instead of electrical phosphenes were taken as the index. By this method three kinds of color processes were distinguished in the frog's retina. Each process has its own time constant and can be characterized by it. However, the question arises as to whether the color processes distinguished in this way may be considered as elementary retinal processes or represent merely some group averages. This question will be attacked most adequately by means of the micro-electrode technique recently developed by Granit⁵⁾⁶⁾ and others.

We went, therefore, to the single-spike technique not only to answer the question raised above, but also to be able to make the data obtained by Motokawa's method of analysis directly comparable with those obtained by Granit and his collaborators.

EXPERIMENTAL.

Method and Procedure.

As experimental materials excised eyes of Japanese toads were used. After removal of lens and cornea the eye was placed on a pad of Ringer-soaked cotton lying on a large plate of silver which served as a leading-off electrode (see Fig. 1). Micro-electrodes used in the present investigation consisted of a 40 micra silver wire, round which a glass capillary was fused in such a manner that the tip formed a well round point. The electrode was placed by means of a screw of a microscope-stage gently onto the retina. Discharges of retinal elements were detected by means of a four stage R-C coupled amplifier connected with a cathode ray oscillograph and a loudspeaker. The impulses were simultaneously listened to and observed on the screen of the oscillograph. When the tip of the micro-electrode came sufficiently close to a retinal elements, what was known by

Fig. 1. Experimental arrangements. Ag: silver plate. AMP: amplifier. C: Ringer-soaked cotton. ME: micro-electrode. R: retina. SE: stimulating electrodes of Zn-ZnSO₄-gelatine type. SV: stimulating voltage.

a sudden increase in the base-line noise of the loudspeaker, the whole retina was illuminated to see whether or not well-isolated spikes appeared in response to the illumination.

Stimulating electrodes used were non-polarizable electrodes of Zn-ZnSO₄-gelatine type. For stimulation a constant current was made to pass through the retina from outside to inside for two seconds, and impulses at the make of the current were chosen as the index. When the electrical sensitivity of the element was found too low, that element was abandoned to seek for another more excitable element.

The procedure of experimentation was as follows: The electrical threshold at the resting level was determined after a preliminary dark adaptation of about 15 minutes. The retina was thereafter exposed to white light for two seconds, and then stimulated electrically every 30 seconds till about 10 minutes after removal of the pre-illuminating light. In this series of experiments it was tried to adjust the intensity of each electrical stimulus to just the threshold value at each moment in order to obtain a just perceptible response. In determination of thresholds two persons were engaged, of whom one was watching the screen of the oscillograph and the other listening in the loudspeaker. They were ordered to inform the experimenter who was adjusting the intensity of stimulating currents, of each response with the following three kinds of expression: no response, just perceptible and above threshold. These were denoted by signs —, + and ++ respectively in the record. If the reports from the two persons did not agree with each other it was marked by two kinds of signs. When successful trials in which each applied voltage was found to be of just threshold strength at the moment, were numerous enough, we constructed an excitability curve from the data obtained in such a single series of measurements. If trials were not successful enough to allow the

construction of an excitability curve, the same procedure—pre-illumination and then electrical stimulation at intervals of 30 seconds—had to be repeated once more. Repetition of the same procedure proved useful in experiments on preparations with the whole optic nerves, for physiological conditions of most preparations remained fairly constant for a long time, but in experiments on single elements or a restricted number of elements conditions were not always favorable enough to allow repetition of the procedure. Therefore we endeavored to finish measurements in a single or at most two series of experiments. For a trained experimenter it was not so difficult, as it might appear, to adjust the intensity of each stimulus to the threshold strength.

RESULTS.

Fig. 2(a) shows well-isolated impulses from a single retinal element in response to illumination with white light, and three single spikes elicited by an electric stimulus are shown in Fig. 2(b). The noise-less part of this record is due to shock artefacts.

We could in this experiment confirm the fact found by Hartline⁷⁾ and Granit⁸⁾ that retinal elements fall into the three main types, i.e. on-element, off-element and on-off-element. Since the number of our observations on

Fig. 2. Spikes from single retinal elements in response to an optic (a) and to an electric stimulus (b). Uppermost and lowermost records controls showing noise-level.

pure on- and off-elements is not sufficient, only the data obtained from on-off-elements will be reported in the following. In Fig. 3 some typical excitability curves are illustrated. As can be seen in the figure, the curves obtained from single elements or a restricted number of elements show much simpler time courses than those obtained from preparations with the whole optic nerves. The curves for white light obtained from such preparations with the whole optic nerves show usually three humps R, G and B at about 1.5, 3 and 5 minutes at room temperature. And we see that the curves at single elements have crest times just corresponding to any one of the crest times of the three elevations R, G and B. As illustrated in the lowermost curve of Fig. 3, some elements, however, showed distinct two elevations, although their responses to stimuli consisted of at least apparently well-isolated spikes. In such cases, the crest time of one elevation was always found about the same as that of the crest time of the B elevation, while the crest time of the other elevation

Fig. 3. Excitability curves for white light of some retinal elements. Ordinate: percentage increases in electrical excitability above resting level. Abscissa: time in minutes after cessation of pre-illumination.

corresponded to that of the R or G, or took a value intermediate between R and G. We have not yet encountered such elements as showed separate two elevations corresponding to R and G. This may partly be due to the circumstances that the error of measurements is too great to distinguish the two elevations from each other which are located temporally so little apart, but there is some evidence to show a strong fusion tendency of the two components R and G; for example, the two components frequently appear fused together in the excitability curve for white light obtained from a preparation with the whole optic nerve, where accuracy

Fig. 4. Histograms concerning crest times of excitability curves of single retinal elements. Rectangles drawn in broken lines were constructed by crest times of elevations of compound excitability curves with two distinct maxima. (A): white pre-illuminating light. (B): red pre-illuminating light.

of measurement is much higher than in experiments on single elements.

The compound nature of the curve manifested itself in some elements also in such a fashion that the curve took a long lasting time course, though it had a single maximum. For example, a curve showing a distinct maximum at 3 minutes took a considerably protracted course in its descending branch, as if a weak B component were participating in that part of the curve. Such an element probably consisted of two components G and B of which the G component was apparently predominant. In the following statistical treatment, however, we treated such an element as belonging to the G group without taking the accompanying B component into account, because the criterion of the treatment was the crest time.

The statistical data are shown graphically in Fig. 4(A). The histo-

gram in full line was constructed by crest times of the excitability curves obtained from 51 retinal elements which showed single distinct maxima, and the rectangles drawn in broken line were constructed by the data obtained from 7 elements yielding compound curves with clearly separate two maxima. In the histogram there can be seen three maxima at 1.5, 3 and 5 minutes which may be denoted by R, G and B. Thus retinal elements obviously fall into the three main groups, the time constant of individual elements varying around the three fixed values. In this diagram it is also noteworthy that the two groups G and B are separated by a gap, while between R and G there is no such distinct separation.

In this point too, we see incomplete differentiation or a strong fusion tendency of the two components R and G, as pointed out above, and these findings seem to conform to the scheme of the Ladd-Franklin's theory advocating that the color substances R and G derive from their precursor Y which is assumed to be produced, together with the substance B, by decomposition of the white substance.

In order to see the distribution of retinal elements among the three groups, elements with crest times within the limits from 1.25 to 2.25 min. were defined as R elements, those from 2.25 to 3.75 min. as G elements and those from 4.25 to 5.75 min. as B elements. We have then 23 R elements, 21 G elements and 21 B elements; the three kinds of elements appeared thus in about the same proportion in our experiments. Since the probability of any kind of elements to be picked up by the micro-electrode inserted at random should be proportional to the number of elements of this kind existing in the retina, the above finding suggests that in the retina there exist the three kinds of elements in about equal number. We used white light as pre-illuminating light in the experiment stated above. If any colored light is used for pre-illumination the three kinds of elements will never appear in the same proportion. This is shown in the following experiment, in which we illuminated the whole retina with red light from a color filter transmitting a range of wave-lengths from 620 to 740 m μ , and determined excitability curves as before on 19 isolated single elements. The data are shown as a histogram in Fig. 4(B). As can be seen in this figure, 16 elements out of 19 have crest times ranging from 1.25 to 1.75 minutes, and the crest times of the other three elements fall into the range between 1.75 and 2.25 min., thus all the elements isolated under this condition belong to the R group.

It seems to be almost unnecessary to carry out similar troublesome experiments with other colored lights, because the relation between crest times and wave-lengths of lights used for pre-illumination has already been well-established in the preceding experiment on excised frog's eyes as well as in the human experiments. These experiments are sufficient to answer

the question raised above whether excitability curves obtained in human experiments represent an average of elemental processes. Yes, they are averages and that averages obtained in the most natural way without resort to any arithmetics.

DISCUSSION.

The fact that three groups of retinal elements appear in the histogram of crest times, seems to correspond to the important experimental fact provided by Granit⁽⁹⁾⁽¹⁰⁾ that there are three groups of modulators in the mammalian retina. In Granit's experiment on frog's retina⁽⁶⁾ the "blue" elements were found exceedingly difficult to isolate, whereas in our experiment the isolation of the B elements was found much easier than that of the other kinds of elements. In contrast with the incompletely differentiated "blue" maximum in the spectral distribution curve reported by Granit, the B group in our histogram stands clearly isolated from other groups. This difference in the behavior of the B modulators is presumably due to the difference in the mode of analysis. By the Granit's method of measuring photosensitivity of retinal elements it must be difficult to isolate the "blue" modulators from the most photosensitive scotopic dominators. Our method of analysis, however, does not suffer from such difficulties, because the modulators show in general such a high degree of electrical excitability against the scotopic dominators that the modulators always predominate over the scotopic dominators, unless the intensity of illumination is below the cone-threshold.

There is another minute but none the less important discrepancy between the results by Motokawa and by Granit: The R spectral locus of the human eye determined by Motokawa⁽¹⁾ shows a second hump in the violet end of the spectrum, while there is no such second rise in the R locus of the cat's eye determined by Granit.⁽⁹⁾⁽¹⁰⁾ The question now arises as to whether this discrepancy is due to the difference of species or to the difference of the methods. In this respect, Donner's work⁽¹¹⁾⁽¹²⁾ on the cat's retina is much worthy of attention. This author has revealed that the discharge frequency of retinal elements as a function of time is different according to the wave-lengths, but depends little on intensities of light. His three curves obtained by plotting the frequency maxima for red, green and blue agree with the spectral loci obtained by Motokawa from the human retina even to the extent of reproducing the hump in the violet part of the spectrum. Donner and we are, so to speak, looking on both sides of one and the same shield, measuring spike-frequencies on one hand and electrical excitability on the other hand. In view of the situation that the second rise of our R locus in the violet end well conforms to the results of color mixing experiments performed by a number of investigators, there

is no doubt that our methods utilizing time constants of retinal elements for analysis provide the most reliable results.

SUMMARY.

Color processes at single retinal elements or at a restricted number of elements of Japanese toads were investigated with the aid of micro-electrodes, taking well-isolated spikes as the index. The electrical excitability of a single retinal element as a function of time after cessation of pre-illuminating white light shows a maximum at 1.5, 3 or 5 minutes. These values of crest time correspond respectively to those of the R, G and B elevations which are found on the excitability curve for white light determined with action potentials of the whole optic nerve as the index. From retinal elements responding with isolated spikes to optic and electric stimuli sometimes an excitability curve having two distinct humps was obtained.

A histogram concerning crest times of excitability curves of these isolated elements shows that there are three groups of retinal elements. The number of elements belonging to each group was 23 for the R group, 21 for the G group and 21 for the B group. When red light was used instead of white light for pre-illumination all the 19 elements tested in this series of experiments showed excitability curves of the R type. The retinal elements so far studied are to be identified with Granit's modulators.

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