ROD-CONE INDEPENDENCE FOR SENSITIZING INTERACTION IN THE HUMAN RETINA

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SUMMARY

1. Illumination of a retinal area adjoining a tested area can cause either desensitization or sensitization through lateral interaction, depending on whether the distance separating the illuminated and tested areas is small or large. This interaction occurs both in the rod and the cone mechanisms of the retina when each is tested separately by selection of appropriate adapting and testing stimuli.

2. In the mixed rod/cone region of the near periphery of the human retina the spatial parameters of interaction within the rod and cone systems were different when mapped out by using only one kind of adapting stimulus and sampling the excitatory state of the rod and cone systems by different probing stimuli.

3. Sensitizing interaction signals generated by the rod system are incapable of causing sensitization of the cone system.

INTRODUCTION

The duplicity theory of vision, according to which there co-exist a rod and a cone system within the retina, is based on the fact that the separation of rod and cone responses is experimentally easy (Hecht, 1937). Yet there is abundant evidence for both rod and cone signals being funnelled through the same ganglion cell (Granit, 1944; Donner & Rushton, 1959; Gouras & Link, 1966; Barlow, Fitzhugh & Kuffler, 1957; Barlow & Levick, 1968).

Certain interaction phenomena between spatially adjoining retinal areas may reasonably be believed to be mediated by retinal structures proximal to the receptor cells and thus allow further insights into the nature of separateness of the rod and cone pathways.

Interaction between adjacent retinal regions is of two kinds. First there is a sharing of excitatory signals in what has been called excitation pools (Rushton & Westheimer, 1962; Rushton, 1963). There is, as it were, a spilling over of light-excited signals from adjoining areas. Since light

excitation in its simplest and most direct form causes a reduced sensitivity (desensitization) to further or incremental illumination, this kind of interaction may be called desensitizing interaction. There is, however, a further kind of interaction which is of the opposite nature. It manifests itself in an increased sensitivity of a retinal area when an adjoining region is being illuminated (Westheimer, 1965). It is best understood in terms of lateral inhibition by which in this context may be meant the spreading to adjoining areas of signals in the opposed sense from those engendered by light. As seen in human psychophysical experiments (Westheimer, 1965, 1967), desensitizing interaction exists predominantly between adjacent retinal areas and sensitizing interaction only at higher adaptation levels and over longer distances.

There is now satisfactory evidence that the desensitizing kind of interaction does not carry over from the rod to the cone system (Alpern, 1965). The experiments in this paper are directed to the question whether sensitizing signals produced by the rod system are capable of inducing sensitization in the cone system.

METHODS

The best way of sampling the state of sensitivity of a given retinal region in the intact human observer is by measuring the threshold for the detection of a small, brief probing flash. Increases in intensity of an adapting background light make themselves felt by increases in the threshold of the probing flash. In the experiments here reported, the illumination characteristics of the immediate background upon which the probing flash was superimposed remained constant. However, as the background illumination characteristics of the vicinity of the tested area were changed the threshold of the test flash changed and these changes may be interpreted as manifestations of interaction signals between adjoining retinal regions.

The adapting background consisted, in the first instance, of a uniform circular patch of light whose diameter could be varied. It was seen by the right eye in the near (8°) periphery of the temporal retina. Correct fixation was assured by a dim red fixation light. The test flash, 5 min of arc in diameter and 10 msec in duration, was presented every 1½ sec in the centre of the circular adaptation field which was exposed continuously.

The circular adapting or background field was placed on a large surround whose retinal illumination was about 1·5 log units less and which served to mask any stray light from the test flash when the background was small. All target configurations were seen in Maxwellian view in an apparatus that has been described previously (Westheimer, 1965). Figure 1*A* shows the subject's view of the target configuration in the initial set of experiments.

The illumination level of the adapting background was 200 photopic td of red light, i.e. light from a 2750° K tungsten source filtered by a glass passing radiation only beyond 620 nm. The same physical radiation flux was simultaneously stimulating the rod system to the extent that about 5 quanta were being absorbed in each rod per second. When the excitation level of the cone system was being assayed, the test flash contained a red filter; for testing the rod system, a blue-green filter (Ilford 623). This is patterned on the method of separating rod and cone function used by Aguilar & Stiles (1954).

After preliminary adaptation, the subject viewed the steady background and adjusted the setting of a neutral wedge to place the rhythmically flashing test stimulus at threshold. The experiments reported here were carried out with the author as subject; the salient features were repeated on two other subjects with similar results.

The important feature of the experiments is that the increment thresholds of the rod and cone systems were measured independently but under stimulus conditions that desensitized them both by light adaptation. How was it assured that the two increment test flashes, the red and blue-green, did in fact test only the cone and rod systems, respectively?

As can be seen in Fig. 2, for large background conditions, the difference between the threshold neutral wedge settings for the red and the blue-green test flashes was about 1·2 density units. This means that in the mixed rod-cone retina of the near periphery 1·2 log units more tungsten light was required to place the test flash at
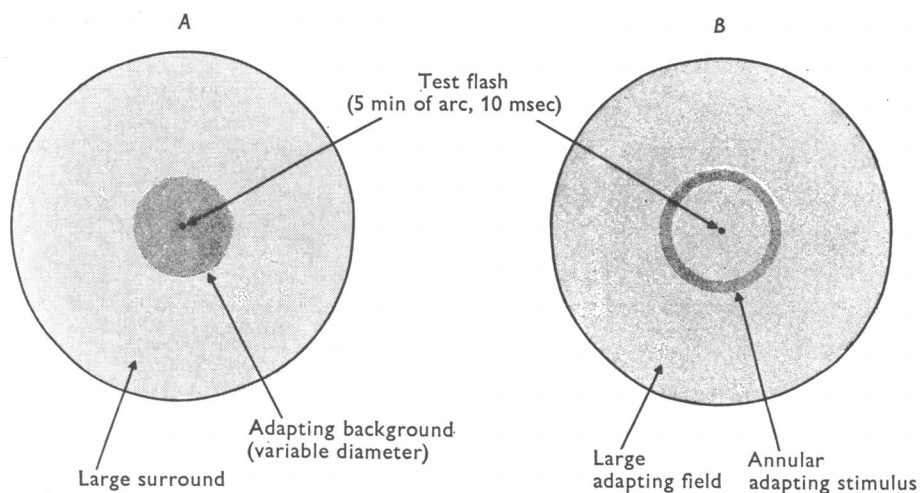

Fig. 1. *A*. Subject's view of the stimulus in the main experiment. Variable size circular adapting field, 200 photopic td of red light, seen steadily on temporal retina of right eye 8° from fovea. In its centre, a 5 min of arc diameter, 10 msec probing test flash is presented every 1½ sec. A large dim surround serves to mask stray light from test flash.

B. Target configuration for confirming experiment. The steady adapting background, 200 photopic td of red light, has been enlarged to fill available field (8°). An annulus, 64 min of arc inner diameter, 86 min of arc outer diameter, 0·7 log units brighter than background, is also seen steadily. Retinal location and test flash as before.

threshold when seen through the red filter than when seen through the blue-green filter. When the two filters were used for foveal presentation of the test flash, where only cones are present, the neutral wedge settings were about equal. This means that under identical background conditions, the mixed rod-cone retina is 1·2 log units more sensitive to blue-green test flashes than to red flashes, whereas the cone retina is about equally sensitive. Since this shift is appropriate, it is concluded that the

particular selection of filters in the ΔI flash has achieved the purpose of individually displaying the adaptation levels of the rod and the cone systems.

Additional evidence for this conclusion is contained in the following test carried out in the peripheral retina. It utilizes the fact that the cone retina has a Stiles–Crawford effect while the rod retina for all practical purposes does not (Crawford, 1937; Flamant & Stiles, 1948). The beam containing the increment-threshold stimulus was brought into the eye in two different positions within the pupil, one near the centre and the other near the edge. In shifting from one of these entry points to the other, the blue-green increment stimulus needed little change in neutral wedge setting for threshold visibility, but the red stimulus had to be attenuated less when it entered the eye near the periphery of the pupil where a beam is less effective in stimulating cones. This is further proof that seen at threshold against the red background, the particular blue-green increment stimulus was detected by the rod system, while the red incremental stimulus was detected by the cone system.
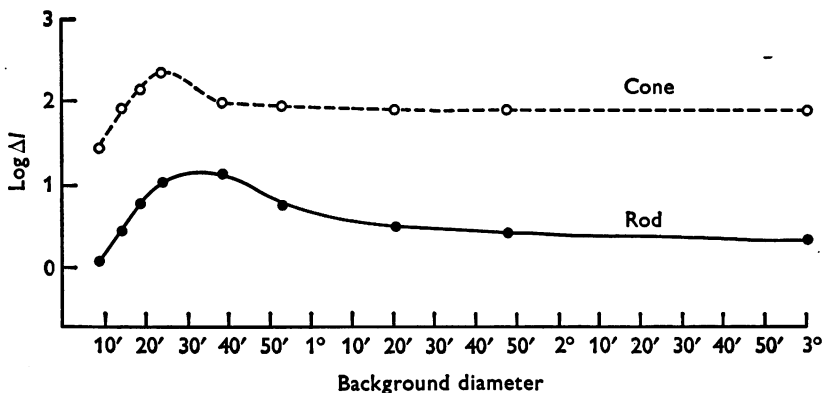

Fig. 2. Incremental threshold for small, brief test flash seen in peripheral vision against a constant, circular, red adapting background as a function of background diameter. Upper curve: test flash red, verified to give cone response. Lower curve: test flash blue-green (rod vision).

RESULTS

The influence of increasing the area of the background on the threshold of a probing flash is shown in Fig. 2. Up to a certain critical area, an increase in diameter of the circular background increases the light quantity required for the threshold of a brief, small probing flash in its centre, i.e. desensitizes the retina. Enlarging the diameter of the background area further produces a fall in threshold, i.e. a sensitization of the retina. Both curves in Fig. 2 were obtained in the mixed rod/cone periphery about 8° from the fovea under identical conditions except that for the upper curve there was a red filter in the ΔI stimulus beam and for the lower curve, a blue-green filter. The evidence for the conclusion that the red ΔI stimulus measures cone function, and the blue-green ΔI stimulus measures rod function, was presented in the Methods section.

The two curves in Fig. 2 are in excellent agreement with the separate experiments on rod interaction (Westheimer, 1965) and cone interaction (Westheimer, 1967) reported earlier. Here, however, we have displayed the spatial parameters of rod and cone interaction in a single retinal region under identical background conditions. The critical diameter, i.e. the diameter of the adapting background beyond which lateral interaction signals becomes sensitizing rather than desensitizing, is smaller for the cone retina than for the rod retina. This, in itself, is compatible with the view that separate interacting pathways exist for the rod and cone retinas, but it has yet to be clarified whether laterally interacting signals originating in one system do not in fact produce sensitization of the other.

The confirming experiments consisted in observing the effects of super-imposing an annulus on a large uniform adapting field. The annulus outer and inner diameters were chosen to cover that retinal region which is suspected, from inspection of Fig. 2, to send sensitizing signals to the threshold-detecting mechanism of the rod system only and not the cone system. The relevant questions are: (1) do the sensitizing signals originating in this annulus affect the rod threshold only and not the cone threshold? and if so, (2) do sensitizing signals from the annulus which affect only rods originate from excitation of rods? Positive answers to both these questions would prove that rod sensitization originates and is mediated entirely within the rod system.

One of the beams of the Maxwellian view apparatus provided a large (8°) homogeneous adapting field of red light at retinal illuminance of 200 photopic troland, and for the rods, about 5 quanta (507 nm) absorbed per rod per second. On this another beam superimposed an annulus, inner diameter 67 min of arc, outer diameter 84 min of arc (Fig. 1*B*). Both the uniform field and the annulus, which was 0·7 log units brighter, remained on constantly. The test flash appeared in the centre of the annulus for 10 msec every 1½ sec. Ilford filters no. 608 and no. 623 together with neutral filters could be interposed in both the annulus and the test flash beams.

The addition of a red annulus to the large adapting field reduced the increment threshold about ¼ log unit for a blue-green test flash (rod vision) and left it unaffected for red test flash (cone vision). The signals origi-nating in the annular zone produce sensitization only of rod thresholds and not of cone thresholds.

To investigate whether these sensitizing signals originated from rod excitation or cone excitation, the following observation was made. In the immediately previous experiment, both the large adapting field and the annulus were red. Now the annulus was made interchangeably red (608 filter) or bluish-green (632 filter), and in each case its influence on the rod

threshold (blue-green filter in ΔI beam) was ascertained. The question was whether, in order to produce an equal sensitizing effect on the rod threshold, the relative luminances of the red and blue-green annuli had to conform to the rod luminosity curve or the cone luminosity curve. For rod vision tungsten light filtered through the 623 (blue-green) Ilford glass is about 1·2 log units more effective than through the 608 (red filter) whereas these two stimuli are about equally effective for cone vision. It turned out that, compared to the red annulus, equal threshold-reducing effects are observed when a 1·1 neutral filter is added to the blue-green annulus. This proves that the sensitizing signals sent to the rod-threshold determining structures by an annular zone are induced by rod stimulation.

<div align="center">DISCUSSION</div>

It has been demonstrated (Westheimer, 1967) that the sensitizing interaction that is the subject of this study does not occur when the central adapting field is presented to one eye and the inhibiting annulus to the other. The capacity of primate retinal ganglion cells to respond separately and individually to rod and to cone stimuli has been emphasized by the experiments of Gouras & Link (1966). It may, therefore, be safely assumed that the structures responsible for sensitizing interaction are retinal and situated distal to the ganglion cells. Since lateral interaction involves distances of the order of a degree (300 μ on the retina) it is most appropriate to think of amacrine or horizontal cells in this connexion. Now, recent experiments in *Necturus*, the only vertebrate in which systematic electrophysiological exploration of all types of retinal cells has been achieved, point to the horizontal cells as the structures mediating sensitizing lateral interaction (Werblin & Dowling, 1969). These cells seem to act to reduce the excitation transmitted by receptors to bipolar cells when an annular surround is present. This is lateral inhibition manifesting itself in a lessening of the light-engendered response from a retinal area when the surrounding area is also illuminated.

In the intact human observer, this lessening of effectiveness of adapting light is demonstrated and measured by the change in threshold of a probing light flash. To the extent that the above quoted electrophysiological results carry over to man, the experiments reported in this study suggest that in man there are horizontal cells that clearly connect within the rod system: a certain surrounding annulus produces sensitization only of rod threshold and the receptors that engender such rod-inhibitory signals are themselves rods. Unfortunately it has so far proved impossible to drive the cone threshold below its level for uniform illumination. Thus, whereas a surrounding annulus, superimposed on a uniform field, sensitizes the

rod threshold (Westheimer, 1965, Fig. 7; and also this paper), the equivalent cone experiment (Westheimer, 1967, Fig. 8) fails to show a reduction of the cone threshold below its uniform-field value. This fact does not permit the distinction to be drawn between a possible incapacity of the cone system to yield to sensitizing influences from rods, and the absence of such an influence. At any rate, there is a functional separation which confines the sensitizing, or laterally inhibiting, signals to the rod system and prevents the manifestation of cone sensitization by rod-generated lateral influences.

In this study, spatial and spectral stimulus parameters were designed to separate rod from cone signals. The enquiry into the possibility that in man laterally inhibitory pathways within the cone system are also separated into colour subsystems, something that is strongly suggested by the experimental results of Daw (1968) in the goldfish, forms the subject of another communication (McKee & Westheimer, 1969).

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