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# COMMENTS ON THE TESTING OF TWO PROMINENT DARK-ADAPTATION HYPOTHESES

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**Abstract**—There appear to be some logical errors in recent attempts to test the hypotheses, (1) that the process of dark adaptation is controlled by the concentration of a substance in the receptors (the photochemical hypothesis) and (2) that the state of the visual system during dark adaptation is equivalent to that produced by some stabilized steady background (the equivalent-background hypothesis). The various factors that should be considered in testing these hypotheses are discussed.

## INTRODUCTION

For several decades two hypotheses have played a central role in research on the mechanisms of dark adaptation. The first, the photochemical hypothesis, states that dark adaptation (or some part of it) is controlled by a substance (e.g. free opsin) in the receptors. The second is the equivalent-background hypothesis. The two hypotheses are completely independent, although in theories of dark adaptation they often appear together (Barlow, 1964; Rushton, 1965a). Considerable care must be taken in order to provide adequate tests of these hypotheses. Unfortunately, discussions of the various factors that should be considered are scattered throughout the literature. The purpose of this note is to collect together the most important ones. One motive for doing this is that there appear to be some logical errors in several recent attempts to test these hypotheses. The two hypotheses will be considered successively.

### Hypothesis 1

*The photochemical hypothesis: The process of dark adaptation is controlled by the concentration of a substance in the receptors*

Usually this hypothesis is stated as a relationship between the relative concentration (proportion) of the substance and relative threshold:

$$\frac{\Delta I}{\Delta I_\infty} = f_c(q) \qquad (1)$$

where $\Delta I$ is threshold at time $t$, $q$ is the proportion of the substance in the receptors at time $t$, and $\Delta I_\infty$ is threshold when $q = 0.0$. The subscript $c$ indicates that the relationship may depend on stimulus configuration. The most well-known special case of equation (1) is the Dowling–Rushton relation (Dowling, 1960; Rushton, 1961) which relates relative threshold to the proportion of bleached photopigment:

$$\frac{\Delta I}{\Delta I_\infty} = 10^{\alpha q} \qquad (2)$$

where $\alpha$ is a constant. The Dowling–Rushton relation describes the relation observed for relatively brief (but large) test fields and large uniform bleaching fields. Not surprisingly, it fails under other stimulus conditions (e.g. with fast flickering or small test fields). Unfortunately, several authors have taken these failures of the Dowling–Rushton relation as evidence contrary to the more general photochemical hypothesis. This line of reasoning is flawed. If dark adaptation is controlled by bleached photopigment, one would still expect some changes in the relationship between threshold and the concentration of the substance, especially given the variety of summative, inhibitory and nonlinear mechanisms in the visual system. In order to disprove Hypothesis 1 it is not enough to disprove equation (2), one must reject the more general formulation, equation (1).

#### *A correct test*

How then does one test the photochemical hypothesis? It appears that the only convincing test is one like those carried out by Dowling and Wald (1960), Rushton and Powell (1972), Hollins and Alpern (1973) and Pugh (1975). Basically, the test takes the following form: *If Hypothesis 1 is correct and the temporal and spatial configuration of the test and any auxiliary fields are held constant, then any pair of bleaching procedures which both yield the the same proportion of the substance and the same distribution of the substance across the receptors must have identical effects on sensitivity.* More formally, let $q$ be the proportion of the substance produced by procedure $A$ and let $q^*$ be the proportion produced by procedure $B$. Hypothesis 1 implies that if $q = q^*$, and the distributions of the substance across the receptors are identical, then $f_c(q) = f_c(q^*)$. This is a very powerful test which says that sensitivity must be predictable from the relative concentration of the substance no matter how that concentration is obtained. In its logical structure and power, the above test is similar to the use of action spectra and the univariance principle to determine

whether a given visual function is being mediated by a particular receptor system. The only difference is that in testing Hypothesis 1 the kinetics of the substance take the place of the absorption spectrum of the receptor photopigment. The discussion below will focus on the hypothesis that the substance controlling dark adaptation is bleached photopigment, since that version of the hypothesis has received the most attention.

*Measuring the kinetics*

To test Hypothesis 1 by the above method, one must determine psychophysical sensitivity and measure (or know) the kinetics of photopigment bleaching and regeneration. The measurement of sensitivity during dark adaptation is in principle straightforward. Of course, care must be taken to ensure that complete recovery of sensitivity is reached, since the position of the lower asymptote greatly affects the estimated time constant of recovery (Pugh, 1975). Unfortunately, our knowledge of photopigment kinetics, at least in the living human retina, comes from the rather tricky technique of reflection densitometry. In densitometry it is difficult to obtain accurate estimates of the amount of reflected light *not* passing through the receptors (stray light) and, as a consequence, the optical density of the photopigment in the dark-adapted eye at the wavelength of the measuring beam. If the amount of stray light is sizable but unknown, the best one can do is measure the quantity $\gamma$ given by the equation:

$$\gamma = \frac{(1 - 10^{-2D_m p})}{(1 - 10^{-2D_m})} \tag{3}$$

where $D_m$ is the density of the photopigment at the wavelength of the measuring beam, and $p = 1 - q$, (e.g. see Alpern & Pugh, 1974). If $2D_m$ is less than about 0.2, the quantity on the right reduces approximately to $p$. But the wavelength of the measuring beam in most studies is near $\lambda_{max}$, thus it is likely that $2D_m$ is often considerably larger than 0.2. If the dark-adapted density of the photopigment at the wavelength of the measuring beam is underestimated then it is easy to show, using the above equation, that the half-bleaching constant will be overestimated and the time constant of regeneration will be underestimated (Geisler, 1978; Padmos, 1979).† Thus, for testing a proposed relationship between bleaching and sensitivity, like the Dowling–Rushton relation, it is important to consider the possible errors that would be introduced if the optical density at the wavelength of measuring beam were incorrectly estimated. Fortunately, for testing Hypothesis 1 by the method described above it is *not* necessary to know $D_m$, since for all values of $D_m$, $q = q^*$ if and only if $\gamma = \gamma^*$. In other words, one must know $D_m$ in order to test a particular proposed function relating bleaching to sensitivity, but not in order to test Hypothesis 1 by the above method.

*Invalid tests*

The discussion above has outlined what seems to be the only legitimate test of Hypothesis 1. In the literature there are numerous examples of incorrect reasoning concerning this matter, and it will prove useful to examine two instances. These studies are not unique in making this sort of error and were only singled out to aid the presentation. Baron *et al.* (1979) measured threshold late-receptor potential (LRP) during dark-adaptation, for different temporal modulation frequencies of the test field. They found faster recovery of sensitivity for the high frequencies than for low frequencies. They argued: "... prior electrophysiological and psychophysical studies have reported a linear relationship between log sensitivity and photopigment concentration; these results suggest a mechanism dependent upon photopigment concentration. If this were so, the process ought to be independent of the temporal characteristics of non-intrusive test stimuli." As mentioned above, this is not a reasonable analysis since there is good reason to expect that equation (1) will be different for different stimulus configurations. It is not possible to reject the hypothesis that the adaptation changes are dependent upon photopigment concentration simply by finding that the dark-adaptation curves for different test field parameters have a different shape.

Norren and Padmos (1974) measured psychophysical and ERG sensitivity during dark adaptation in the human and macaque cone systems. The primary purpose of their study was to compare the recovery rates of the short- and long-wavelength cone systems, but they also presented a brief analysis of the relationship of their data to densitometry measurements. This analysis and their experiments provide another good example of the problems that can arise if the effects of stimulus configuration are ignored. In one condition, they first bleached more than 90% of the red and green cone photopigments with a 5° yellow pre-adaptation field, and then measured threshold during dark adaptation with a 1.3° test field flickered at 1 Hz. They found that the dark-adaptation curve, plotted as log relative threshold as a function of time, could be fit with an exponential decay function with a time constant of about 90 sec. (Some other conditions showed even smaller time constants.) From this they concluded that, "The present results concerning R, G recovery in the fovea (time constant of about 90 sec) do not confirm the 'Rushtonian' literature in which

† For example, unpublished densitometry measurements obtained on the author's right fovea were analyzed assuming that $D_m$ is negligible and 0.5. For $D_m$ negligible the least-squares estimates were: $t_0 = 90$ sec, and $I_0 = 4.5$ log td. For $D_m = 0.5$ the estimates were: $t_0 = 169$ sec, and $I_0 = 4.0$ log td. It should also be added that small errors in measuring the final asymptote of the pigment regeneration curve can seriously affect the estimated time constant.

always a time constant of about 120 sec is mentioned. Nevertheless, our experimental set up closely resembled the one described by Rushton and Henry (1968)...." The reasoning behind this conclusion seems to be as follows. It is known that bleached photopigment regenerates exponentially. If one then assumes the Dowling–Rushton relation holds, then log threshold should recover exponentially with the same time constant as the photopigment:

$$\log \frac{\Delta I}{\Delta I_x} = \alpha q_0 e^{-t/t_0} \qquad (4)$$

where $q_0$ is the initial level of bleaching and $t_0$ is the time constant of regeneration. Since equation (4) fits the data with a time constant of 90 sec, there is a discrepancy with the "Rushtonian" literature. Norren and Padmos had to implicitly assume that the Dowling–Rushton relation holds in order to compare their results to the densitometry results of Rushton and Henry. Under this assumption there are only two possible interpretations of their findings; either the time constants of regeneration for their subjects are faster than those reported by Rushton and Henry, or else Hypothesis 1 is incorrect under their conditions. The difficulty with these interpretations is again the assumption that the relationship between sensitivity and photopigment concentration will not change when stimulus conditions are changed. As pointed out by Kooijman *et al.* (1978) the stimulus conditions used by Norren and Padmos differed from those under which the Dowling–Rushton relation has been found to hold. In particular, Norren and Padmos presented a steady 1.3 log *td* background following the bleaching field instead of having total darkness. Adding a dim auxiliary background changes the shape of the dark-adaptation curve (DuCroz and Rushton, 1966), thus one cannot employ the Dowling–Rushton relation in estimating the time constant of regeneration. Nor can one use the discrepant time constant obtained by this analysis to reject Hypothesis 1. To illustrate this argument an alternative interpretation of Norren and Padmos' data will be given. It appears to be more plausible and leads to exactly the opposite conclusion; namely, that their *R, G* data are perfectly consistent with the "Rushtonian" literature.

DuCroz and Rushton (1966) found that the equivalent-background hypothesis held under the conditions used by Norren and Padmos (i.e. for added backgrounds). Let us make the following plausible assumptions: (1) the Dowling–Rushton relation holds in the without-background condition, (2) the equivalent-background hypothesis holds, and (3) the generalized Weber's law would describe the increment-threshold function obtained with their 1-Hz test field. Under these assumptions it is easy to derive the version of equation (1) that would be expected if the dark adaptation process *is* controlled by the proportion of bleached pigment. This derived relationship is,

$$\log \frac{\Delta I}{\Delta I_x} = \log\left(\frac{I}{I_x} + 10^{\alpha q}\right) \qquad (5)$$

where $q = q_0 e^{-t/t_0}$, $I$ is the intensity of the fixed background, and $I_x$ is the dark–light constant in the generalized Weber's law. Note that in the present application $I$ is a constant, so that equation (5) is an equation describing threshold as a function only of the proportion of bleached pigment. Equation (5) fits the dark-adaptation curve obtained by Norren and Padmos just as well as equation (4) (which they used) except that the time constant needed with equation (5) is 120 sec, not 90 sec.

The similarity of the predictions of equations (4) and (5) is illustrated in Fig. 1. (Note that equation (5) reduces to equation (4) when $I = 0$.) The solid circles are given by equation (4) for $t_0 = 120$ sec, $q_0 = 1.0$, and $\alpha = 3.5$. The open circles are given by equation (5) for $t_0 = 120$ sec, $q_0 = 1.0$, $\alpha = 3.5$, $I = 20$ (1.3 log *td*) and $I_x = 10$. The value of 10 for $I_x$ is a typical one, derived from increment-threshold curves that were obtained under conditions similar to those of Norren and Padmos (Geisler, 1979). The ×'s show the predictions of equation (4) for $t_0 = 90$ sec, $q_0 = 1.0$ and $\alpha = 3.0$. The close correspondence of the ×'s and open circles shows that being able to fit a dark-adaptation curve with an exponential function provides very little, if any, support for the Dowling–Rushton relation, since a very different relation [equation (5)] is also consistent with the approximately exponential decay.

The above analysis illustrates the rather surprising fact that one can be seriously misled by simply comparing exponential time constants for different conditions even when exponential-decay functions fit reasonably well in all cases. Again, the heart of the

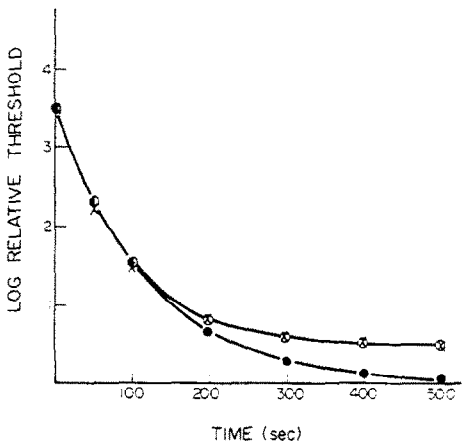


Fig. 1. Theoretical dark-adaptation curves. ●—● equation (4) $t_0 = 120$ sec. O—O equation (5) $t_0 = 120$ sec. × — × equation (4) $t_0 = 90$ sec. What is plotted is the log of threshold relative to threshold in the dark-adapted eye when there is no steady background.

present argument is that differences in the rate of recovery of sensitivity with different test and/or auxiliary field parameters are perfectly consistent with the hypothesis that the recovery of sensitivity is controlled by a single substance whose decay rate is unaffected by the changes in the parameters. Changes in the test and/or auxiliary field parameters may change the rate of recovery of sensitivity simply by changing the relationship between threshold and the concentration of the substance [equation (1)]. Similar arguments can be generated to produce alternative accounts of the "fast" decay rates observed under other conditions, such as with fast flickering test fields (Norren and Padmos, 1974; Baron *et al.*, 1979). In conclusion, the only valid test of Hypothesis 1 seems to be the one described earlier.

It is important to note that the above criticism is not unique to the problem of testing the photochemical hypothesis. In general, it is incorrect to reject the hypothesis that a given mechanism is driving the temporal change in some aspect of perception simply because it is found that the rate of change depends on stimulus configuration. For example, the difference in visual persistence for low and high spatial-frequency gratings (Meyer and Maguire, 1977) does not contradict the hypothesis that the persisting signal originates from the photoreceptors.

### Hypothesis 2

*The equivalent-background hypothesis: The effect of a given state of adaptation following bleaching is identical to that produced by some stabilized background of light of the same spatial configuration as the bleaching field.*

The traditional method of testing Hypothesis 2 is to obtain dark-adaptation curves and increment-threshold curves (with continuous background) under the same set of stimulus conditions. Then equivalent-background functions (background intensity as a function of time) are calculated from the data for each stimulus condition by reading off the continuous background intensity that produced the same threshold as that observed at each point in time after bleaching. If the equivalent-background functions obtained under the different conditions are identical it is interpreted as confirmation of the hypothesis. Below are listed what seem to be the major factors that should be considered before attempting the analysis.

#### *Alignment of functions*

The data must satisfy certain common sense properties. For example, the lower asymptotes of the dark-adaptation and increment-threshold curves obtained with the same stimulus configurations must agree. This seems to be an obvious point, but it is seriously violated in a recent study reported by Kooijman *et al.* (1978). For example, in one of their main conditions the asymptote of the dark-adaptation and increment-threshold curves differ by at least 0.6 log units and in another by more than 0.3 log units. Clearly, with such large discrepancies one can place little faith in their equivalent-background analysis.

#### *Effects of stabilization*

Since the afterimage due to bleaching is stabilized on the retina, the most appropriate test of Hypothesis 2 requires that the continuous background also be stabilized. Nonetheless, in many tests of the hypothesis this has not been done. Fortunately, stabilization does not always seem to be necessary. Burkhardt (1966) and Sparrock (1969) have shown that the increment thresholds obtained on stabilized and unstabilized backgrounds are almost identical for large uniform backgrounds and relatively smaller flashed increment fields. This seems reasonable since the unstabilized background is effectively stabilized in the neighborhood of the test flash. For conditions where small eye movements can cause the illumination to fluctuate (e.g. small backgrounds) the stabilization problem becomes much more serious (Barlow and Andrews, 1973; Barlow and Sakitt, 1973). An example of this problem is the study of Rushton (1965b), in which he found that the equivalent-background functions for uniform and spotted fields were far from being identical. However, the potential problem in the study was that the steady backgrounds were presented under normal viewing conditions, whereas the bleaches were produced by an electronic flash. Because of small unavoidable eye movements, it is very reasonable to suppose that when the spotted backgrounds are presented continuously their adaptation effects are not localized (as in the flash bleaching conditions) but are distributed, perhaps uniformly. When testing Hypothesis 2 under conditions like those used by Rushton, it is probably necessary to stabilize the steady background on the retina.

#### *Effects of photopigment depletion*

In the cone system, unlike the rod system, photopigment depletion accounts for a sizable component of the dark-adaptation curves and only a much smaller component of the increment-threshold curves (Rodieck, 1974; Geisler, 1978, 1979). Thus, before testing Hypothesis 2 for cones, all the data should be corrected for the effects of photopigment depletion. This is not necessary if the increment-threshold functions obtained under the different conditions are superimposable by vertical translation (Geisler, 1979). The appropriate correction for depletion is given by,

$$I_c = \frac{I(1 - 10^{-D \cdot p})}{(1 - 10^{-D})} \tag{6}$$

where $I_c$ is the corrected intensity, $I$ the uncorrected intensity, $p$ is the proportion of unbleached pigment,

and $D$ is the effective optical density for the stimulus under consideration in the dark-adapted eye.*

*Short- and long-term mechanisms*

Dowling (1960), Hollins and Alpern (1973), and Rushton and Powell (1972), among others, have shown that it is probably useful to distinguish between the initial phase of dark adaptation which is controlled by some relatively quickly-recovering mechanism and the later phase of dark adaptation apparently controlled by the bleached photopigment (or a closely related substance in the receptors). Since the equivalent-background hypothesis may hold for either or both phases, one should attempt to determine which of the two mechanisms is being tested. For example, the results of Rinalducci *et al.* (1970) indicate that Hypothesis 2 fails with respect to spatial summation in the human cone system for the short-term mechanism, while the results of Geisler (1979) indicate that it may hold for the long-term mechanism.

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* If $D$ is small equation (6) reduces to,

$$I_c = I \cdot p \qquad (7)$$

Pugh and Mollon (1979) have suggested that since the kinetic descriptions of human (cone) photopigments have all assumed low optical densities then the error introduced by assuming that $D$ is small will be cancelled out. However, this is not entirely correct, since assuming the optical density is negligible leads to larger errors in deriving the kinetic description from densitometry than it does in applying the correction for depletion. This is illustrated below by a numerical example. Suppose for simplicity that the stimulus to be corrected for depletion and the measuring light used in the reflection densitometry are of the same wavelength, and that the optical density of the photopigment in the dark-adapted eye at this wavelength is 0.4. Equation (3) with $D_m = 0.4$ gives the quantity ($\gamma$) measured with the densitometer. Suppose we want to correct the stimulus for depletion after a bleach that resulted in $\gamma = 0.5$. The correct procedure would be as follows. First, we solve equation (3) and find that $p = 0.3$. Then we substitute $p$ into equation (6) and find that $I_c = 0.4 \cdot I$. The usual (incorrect) procedure is to assume that the density is negligible throughout. Thus, we find that $p = \gamma = 0.5$, and by equation (7), $I_c = 0.5 \cdot I$. Therefore, in this case the net effect of assuming the density is low is to overestimate $I_c$. The degree of overestimation is even larger if $D$ is less than $D_m$. Usually, $D$ is less than or equal to $D_m$ since the measuring beam in densitometry is almost always near $\lambda_{max}$. Thus, underestimating the density throughout generally leads to underestimating the effects of photopigment depletion.

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