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S-POTENTIAL AND DARK ADAPTATION IN FISH

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SUMMARY

1. The change in d.c. level of the S-space was investigated during dark adaptation to see if there is any correlation between the d.c. level and log sensitivity.

2. Except for a few minutes after strong adaptation the d.c. level of the S-space was found unchanged throughout dark adaptation.

3. The maximal excursion of the potential (the ceiling) also remained unchanged during the light and dark adaptation.

4. Changes in the sensitivity in dark adaptation were found formally equivalent simply to a change in the unit of light intensity.

5. Thus adaptations to bleaching or to the equivalent background are entirely different in the effect on S-potentials.

INTRODUCTION

Since Svaetichin's first description of S-potentials (1953) their function has been a puzzle. Their very large receptive fields suggest that they do not convey information perceived as fine detail. It seems more plausible to suppose that they are concerned rather with the regulation of adaptation. Thus Svaetichin, Laufer, Mitarai, Fatehchand, Vallecalle & Vellegas (1961) suggested that they might be responsible for the excitability change during dark adaptation, and Rushton (1962) that they might control the Weber-Fechner adaptation to background fields. In the vertebrate eye it is now established that after strong bleaching the log. threshold is raised in proportion to the fraction of visual pigment still unregenerated (Dowling (1960) in the rat, Rushton (1961) in human rods, (1963) in human cones). And Lipetz (1961) in the frog, Rushton & Westheimer (1962) in man have shown that when some rods are bleached and some spared in a receptive field, the threshold of the spared rods is raised more than can be accounted for by the scatter of the bleaching light. Consequently there

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must be some retinal organization that raises the visual threshold of rods that have been little bleached, when their neighbours have been bleached substantially.

In the study of dark adaptation, the concept of 'equivalent background' (Stiles & Crawford, 1932; Crawford, 1947) has been found very powerful. It looks as though the action of free opsin is to emit 'noise' or 'dark light' (Barlow, 1964) which generates a signal like that from stabilized real light and raises the threshold and looks bright (after image) in the same way that real light does (Barlow & Sparrock, 1964). Upon this view, light signals and after-image signals would travel along the same paths and be processed in the same way, and in consequence both would affect similarly the S-potential if that was an important part of the processing.

However, a somewhat different organization was suggested by A. L. Hodgkin (personal communication) and developed experimentally by Rushton (1965*a*, *b*, *c*) according to which the light signal and the after-image signal are processed in quite different ways. Experiments to decide between these two interpretations seem to favour Hodgkin's suggestion (Rushton, 1965*c*). One is reluctant, however, to postulate two different pathways from rods and cones, one for light signals and the other for after-image signals, even though the analysis seems to require it. It is at this point that S-potentials have something important to say. For if a real light background affects the S-potential, but the equivalent background of bleaching does not, then they cannot both be similar signals in the same pathways. This is precisely the difference that we now find to be the case.

METHODS

Two kinds of cyprinid fish, *Tinca tinca* and *Carassius carassius*, were used. Detailed description of experimental procedures were reported elsewhere (Naka & Rushton, 1966*a*). Some of the experiments were done in Cambridge, England, and some others in Osaka, Japan. In both cases the recording system was conventional. The light source used was either a 36 W tungsten lamp or a 150 W Xenon arc lamp. The test flash was monochromatic light of 540 or 680 nm (duration, 100 msec) and its intensity was controlled by a 6 or 4 log. unit neutral wedge; the position of which was electrically monitored. Adapting light was obtained from a tungsten lamp and it was always strong enough to clamp the voltage at the maximum of hyperpolarization or ceiling.

RESULTS

In this paper we try to answer two questions. (*a*) Does the S-space (cf. Naka & Rushton, 1967) change its resting level during dark adaptation? (*b*) If so, is the change correlated with change of threshold? The first question, though apparently simple, was experimentally hard to answer with confidence because there is often a drift in the recorded potential of impaled units not related to the dark adaptation studied. However, there were several cases of exceptional stability where the unit maintained a

very steady d.c. level for more than 20 min, and the results to be described are from these.

The experimental procedure was usually first to find a suitable L-unit using the light flash of moderate intensity given once in every 2 or 3 sec. When a stable unit had been found, a V–log I curve was obtained for the somewhat light-adapted state existing. The preparation was then more strongly light adapted by an exposure of up to 30 sec to white light from a 36 W tungsten lamp. The adapting light clamped the potential to a fixed level of hyperpolarization that was usually between 30 and 60 mV below the resting value, depending upon the unit. During the subsequent dark adaptation the sensitivity was monitored by recording the responses either to flashes of fixed intensity or the whole V–log I relation was obtained. The fixed flash was given every few seconds at the beginning, but later only once in each 10 sec so as not to disturb much the course of dark adaptation. Care was always taken to exclude the effects due to extracellular potential (probably P III of Granit's component) resulting from the adapting light. Though this potential was in some cases as large as 10 mV it could easily be distinguished from the S-potential by its slower rising phase.

Figure 1 shows four portions of a pen-writing record taken from an experiment in which the potential was stable for more than 20 min. This tracing shows the general course of the experiment; photographs of oscilloscope records taken simultaneously were used for accurate measurement. In Fig. 1 the tracings in each set from above downwards are as follows: camera signals, time dots at 10 sec intervals, a horizontal reference line drawn in afterwards (= dark potential level), S-potentials, position of photometric wedge (= log intensity when light was exposed), reference line drawn afterwards. In the first set (0–3 min 40 sec) the potential tracing shows the change in d.c. level during light adaptation and the return to the initial level during the next 3 min. The remaining sets show that this initial level is held nearly constant for the rest of the experiment. Slight fluctuations are indicated by thickening of the horizontal reference line. They were less than 3 mV and showed no fixed pattern in relation to dark adaptation.

The procedure in Fig. 1 was as follows. First the V–log I relation was obtained before the record of Fig. 1 begins. This relation appears in Fig. 2 (triangles). Then the eye was exposed to the adapting light for 20 sec, during which time the potential is seen to be depressed to a fixed extreme level of hyperpolarization nearly 40 mV below the resting value. When the bleaching light was suddenly turned off the S-potential did not suddenly return to the resting value (as happens when the same intensity of light is exposed for short duration or when the intensity of the light is

low); the return was somewhat exponential and nearly reached the resting value in 3 min. In similar experiments it was found that the time for return depended upon both the intensity and the duration of the bleaching light.

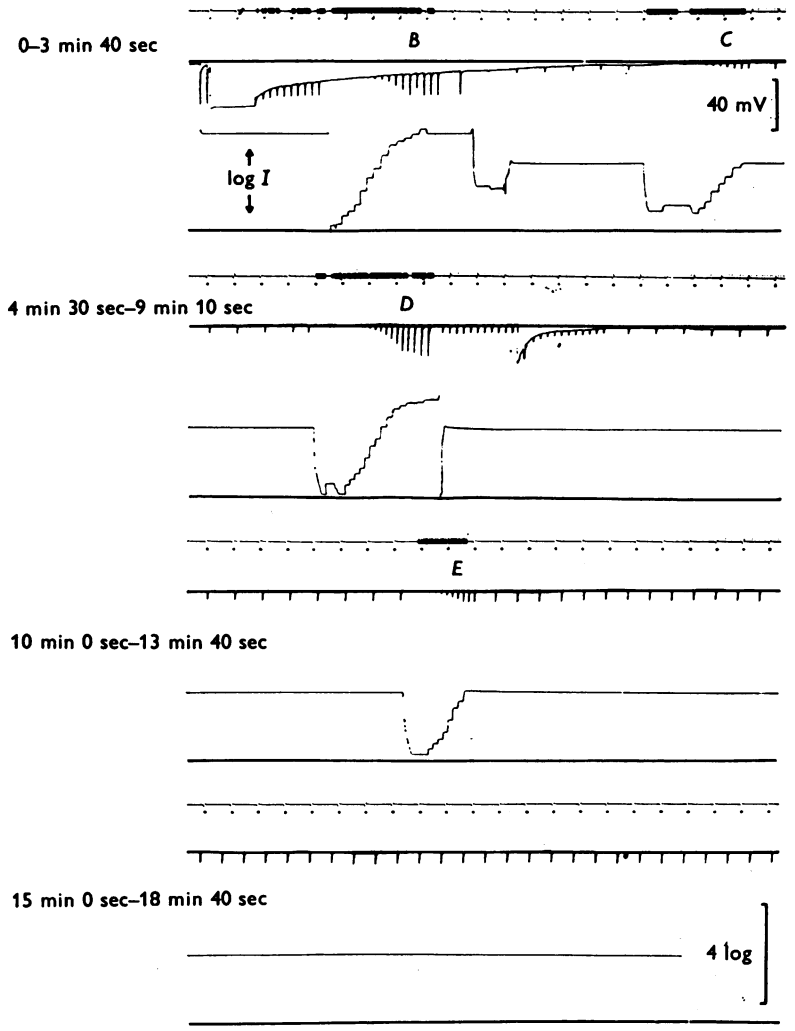

Fig. 1. Pen-writer records of S-potentials during light adaptation and subsequent change in d.c. level during dark adaptation. Six traces in each record are: camera signals, time (10 sec), reference line for the d.c. level (or dark level), S-potential, the position of the neutral-density filter and the reference line for the position of the filter. The two reference lines were drawn on the record later. *B*, *C*, *D* and *E* indicate the time when *V*–log *I* curves in Fig. 2 were obtained. After the middle of uppermost record, test flashes of a fixed intensity were given in every 10 sec.

During the bleaching exposure and for 20 sec subsequently the eye was stimulated by flashes of full energy available. During the exposure when hyperpolarization was maximal the flashes were without effect; during the first 20 sec of dark adaptation the fixed intensity flash produced a hyperpolarization which was increasing if measured from the d.c. level obtaining at that moment (4·1–11·5 mV), but decreasing if measured absolutely from the horizontal base line (32·8–28·7 mV). At times indicated by letters *B*, *C*, *D* and *E* the *V*–log *I* relation was redetermined. These results are

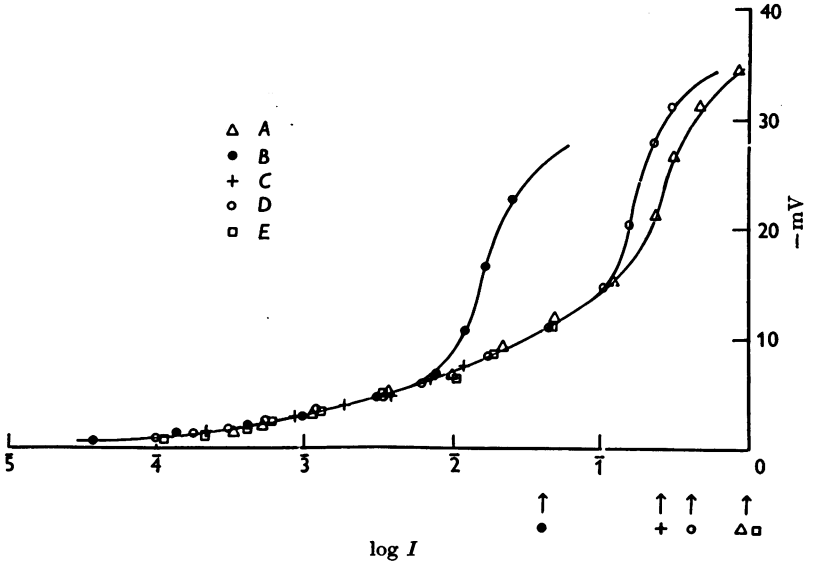

Fig. 2. *V*–log *I* curves from the unit shown in Fig. 1. *A* was obtained before the start of the pen-writer record of Fig. 1 and other letters correspond to those shown in Fig. 1. All curves were laterally displaced on the log *I* axis to superpose the lower branches. The amounts of lateral shifts of each curve are shown by arrows with corresponding signs. Each upper curve is the same curve displaced along the lower curve. All measurements were made from oscilloscope records.

plotted in Fig. 2 as curves *B*, *C*, *D* and *E*. During the rest of the time, flashes 1·2 log. units weaker than the maximum flash were presented once every 10 sec. The fact that the response to light of this fixed intensity increases steadily long after the point where the S-potential had returned to the base line means that there is some component of adaptation that changes independently. The nature of this change is displayed in the *V*–log *I* curves of Fig. 2.

Curves *A*, *B*, and *D* in Fig. 2 consist of two parts, one rising gradually, the other rather steeply. It is likely that the gradual rise is due to the scotopic mechanism and the steep rise to the photopic, for the former

alone is present at lowest light levels, it is not seen when a test flash of 680 nm is used (Fig. 5) instead of the 540 nm of Fig. 2, and it recovers relatively slowly. Moreover the V–log I curves of the colour mechanisms (Naka & Rushton, 1966*b*) have the shape of the quickly rising branch but not of the other.

There is some doubt as to whether *rods* can contribute to the S-potential. Orlov & Maksimova (1965) claim that they do, but Witkovsky (1967) finds the contrary and draws support from the histological studies of Stell (1965) in the goldfish, and Yamada & Ishikawa (1965) in the carp, where no horizontal cell makes contact both with cones and rods. The only way to settle which *receptor* is involved is to investigate the Stiles–Crawford effect as Donner & Rushton (1959) did with the green rods in the retina of the frog. But this experiment has not yet been performed with S-potentials. Whatever be the scotopic receptors, the lower branch of the curves of Fig. 2 is clearly an entity, distinct from the upper branches.

In all the curves of Fig. 2 the lower branch is of the same shape but laterally displaced to various extents along the log I axis. Thus it is possible by suitable lateral shift of each curve to bring the lower parts into coincidence. In Fig. 2 the curves are plotted after shifting in this way by amounts indicated by the arrows with corresponding marks. It is possible that with a different shift (along the lower curve, not along the horizontal) the upper branches may also be brought into coincidence.

If the effect of adaptation upon the V–log I curve is simply to cause a lateral shift of components of the curve, we should expect that the ceiling of hyperpolarization would remain unchanged in value. This is confirmed in the earliest part of Fig. 1 where the ceiling is seen to be maintained throughout the adapting exposure (though in some units hyperpolarization was found to decline with further adaptation). In Fig. 3 the ceiling level is examined throughout some cycles of bleaching and dark adaptation, and found to remain almost exactly at -28 mV throughout. All the measurements in Fig. 3 were made when the d.c. potential had returned to within 4 mV of the initial resting value. The horizontal arrows show the ceiling of hyperpolarization using a short exposure of the white adapting light. The circles show the S-potential resulting from a weaker flash of fixed intensity, namely the maximum brightness of the 540 nm stimulus. In the initial (dark-adapted) state this gave an S-potential of about half ceiling height. A lateral shift of the V–log I curve would cause change in circles but no change in arrows, and this is what is shown in Fig. 3. The actual procedure was as follows.

First (time A, Fig. 3); a V–log I relation was determined using the 540 nm flashes up to the full strength available which only reached halfway to full hyperpolarization; 40 sec later this was repeated (B, Fig. 3). The two sets of results agree and are plotted together in Fig. 4 (filled circles and triangles). The eye was then exposed to the adapting light for

30 sec as shown by the black rectangle (Fig. 3), and later further adapting exposures are given, either very briefly simply to check the ceiling voltage (horizontal arrows) or more extended as shown by the thickness of the

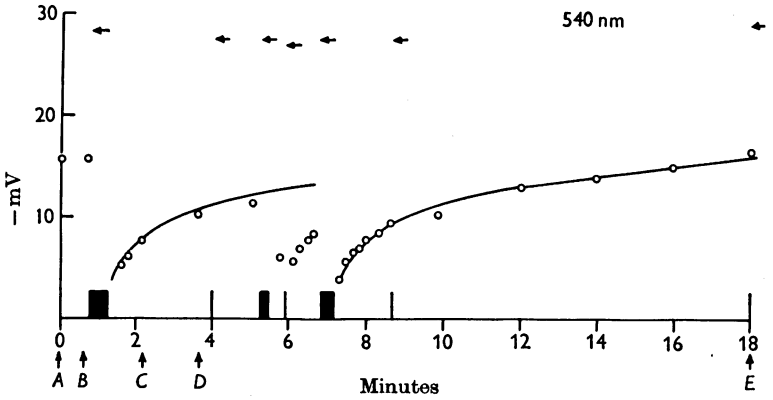

Fig. 3. Plotting of ceiling of potentials produced by strong lights during dark adaptation. The ceilings are shown by horizontal arrows and duration of the lights by black rectangles. The amplitudes of responses to fixed intensity test flash of 540 nm are shown by circles. Records were from a luminosity unit.

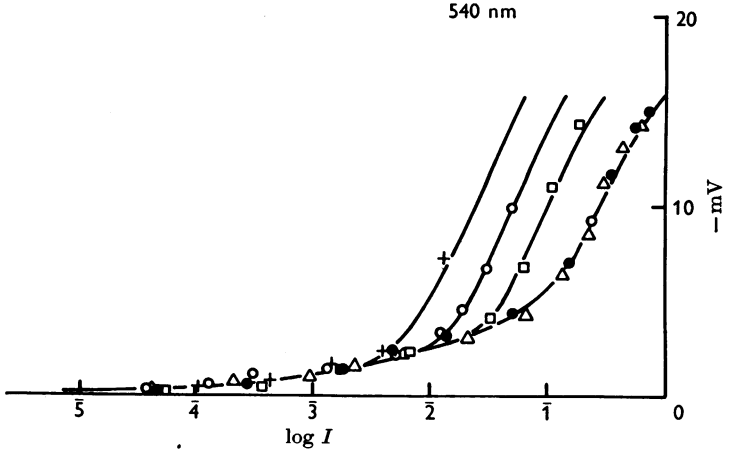

Fig. 4. V–log I curves from the record shown in Fig. 3. Plotted as in Fig. 2. Filled circles and triangles were obtained at A and B, crosses at C, open circles at D and squares at E as indicated in Fig. 3.

rectangles to change again the level of adaptation. At C, D and E further V–log I relations were measured, as plotted in Fig. 4. In between these measurements the fixed test flash elicited S-potentials plotted as circles in Fig. 3 which give information about the course of recovery. Dark adaptation is clearly far from complete and is steadily improving though the

S-potential has long returned to its dark-adapted d.c. level and though its ceiling is undergoing no change. The change associated with this recovery appears to be simply described by the lateral shift of V–log I curves. In Fig. 4 are plotted the V–log I relations at the times marked A, B, C, D and E in Fig. 3. As in Fig. 2, each curve has been displaced horizontally by amounts required to bring the lower branches into coincidence. It is clear that a further independent sliding of the upper branches upon lower branch will make these also nearly coincide.

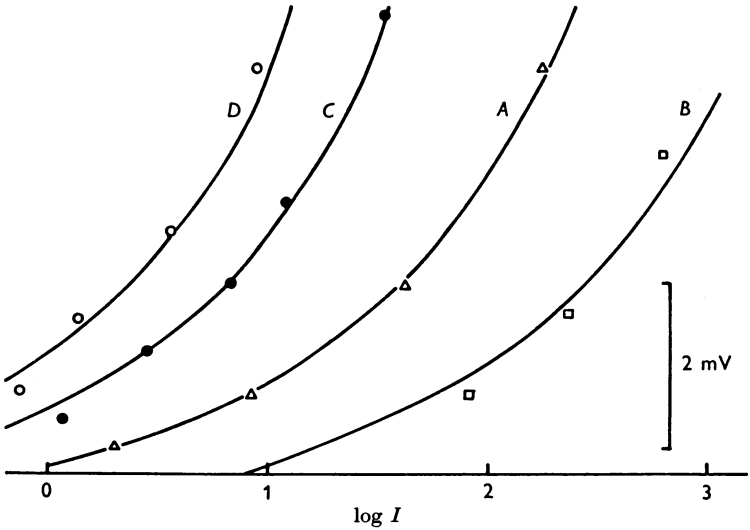

Fig. 5. V–log I curves produced by deep red flashes of 680 nm. A was before, B, 30 sec, C, 13 min and D, 30 min after strong adaptation by white light. Curves are the same one displaced laterally.

Figure 5 shows V–log I curves at various stages of adaptation, when instead of a test flash of wave-length 540 nm, one of 680 nm was used. In a recent paper (Naka & Rushton, 1966*c*) it was found that flashes of this wave-length excited only the deep red pigments (maximum at 680 nm), at any rate when only small S-potentials were investigated. Thus in Fig. 5 where V does not exceed 5 mV, we should expect a single V–log I curve of fixed shape laterally displaced to various extents during adaptation. This is seen to be the case, for the same curve has been drawn through each set of points, B 30 sec after adapting, C 13 min and D 30 min after. A was before adapting, when the retina was obviously rather light adapted.

As seen in Fig. 1, the adapting light hyperpolarized the unit to a fixed maximal extent from which it took a few minutes to return to the resting d.c. level again. All the V–log I measurements of this paper were made

when the d.c. level had fully returned (or nearly so), for in the very early stages, when both the potential level and the excitability to light were changing at once and rather fast, it was hard to interpret the records satisfactorily.

DISCUSSION

Though far from satisfactory, the experiments of this paper seem clear enough and consistent enough to point to a rather surprising conclusion. It is that the great change in sensitivity that accompanies dark adaptation is not caused by activity of S-units but appears to have already occurred before the S-units are reached. It is not caused by their activity, since throughout most of the course of dark adaptation the changes in S-potential are very small and irregular, whereas the changes in excitability are large and progressive. It occurs before the S-units are reached because their changes in excitability may be represented by a horizontal sliding of the V–log I curve; this means that the effect of adaptation is to attenuate the receptor signals in some fixed manner before they ever reach the S-units. It is as though some filter had been interposed in the light beam and was being gradually removed. Can this be what is actually happening?

It is well known that prolonged strong light adaptation of fish eyes causes the black pigment granules to move from behind so as to invade the receptor layer. Is it possible that the change, 'as though some neutral density had been interposed', is simply the interposition of these granules? Though we have not excluded pigment movement from being one contributory cause, it cannot have been the main cause. For, observing in histological preparations the amount of light adaptation required to produce pigment migration, it was found that the 30 sec of light adaptation we used was quite insufficient. Moreover, when pigment migration does occur the movement is slow, taking minutes to reach the new position; but the light adaptation we studied was instantaneous.

We have seen that the S-potential is affected very differently by a background of real light and by the after effects of strong light adaptation that leaves the threshold raised to some extent, for the real background always and essentially produces a maintained change of S-potential whereas the after-effect of bleaching essentially produces no change. Thus whatever be the physiological nature of the equivalent background of bleaching it cannot be a signal identical in quality and conduction path with that generated by the real background. If the receptor 'noise' or after-image signal was a message physiologically indistinguishable from that generated by the real background which raised the threshold equally, it would be distributed everywhere in the same way and act similarly

wherever investigated. But it does not act similarly upon S-units and therefore the two kinds of message must be different. This supports the alternative view suggested by Hodgkin and developed by Rushton (1965*a*, *b*, *c*), where the signals generated by real light and by free opsin though both acting upon the 'gain box' and 'turning the single knob there' that controls adaptation, differ essentially in their mode of action, distribution and turning power. They may, however, both bring the 'knob' into the same position, and then adaptation will be the same in the two cases—the equivalence of backgrounds and bleachings. But as to the physical nature of what is propagated, little is known of the background signal, and nothing of the free-opsin signal.

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