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SIGNAL TRANSMISSION FROM RED CONES TO HORIZONTAL CELLS IN THE TURTLE RETINA

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SUMMARY

1. Intracellular recordings were made from L-type horizontal cells in the retina of the turtle *Pseudemys scripta elegans*. The responses were evoked by 500 msec pulses of 'white' light.

2. L-type horizontal cells were classified as either, 'small receptive field' s.r.f. or 'large receptive field' l.r.f. based upon (1) receptive field size and (2) kinetics of responses to test flashes covering small and big spots.

3. Constant illumination of the entire receptive field, with any intensity studied, evoked a response that reached a peak and then slowly sagged back to a steady-state level that was about half the peak response.

4. Termination of backgrounds resulted in a very fast recovery of the membrane potential that overshoot the dark-adapted potential. This 'off' response had faster kinetics in horizontal cells than in red cones.

5. The intensity–response curve measured around any background intensity was shifted along the log intensity axis toward higher test intensities. The curves obtained under light-adapted conditions were sharper than the curve measured in the dark-adapted state.

6. The photoresponses of red cones and s.r.f. horizontal cells were compared under similar states of adaptation. In the dark-adapted state the gain, expressed as the millivolt change in the horizontal cell per millivolt change in the cone, was not linearly related to cone potential, and was highest for dim stimuli. Light-adaptation modified the synaptic transmission to make the horizontal cells most sensitive to light modulation around the background illumination.

7. The mechanisms by which signal transmission can be modified by light-adaptation are discussed in terms of transmitter release by the presynaptic terminals and its binding to post-synaptic sites.

INTRODUCTION

From recent studies on the mechanisms of cone to horizontal cell synaptic transmission, a qualitative understanding of this process is emerging. It has been suggested that the transmitter in the cone to horizontal cell synapse is continuously released in the dark and that this release diminishes when the receptors are hyperpolarized by

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light (Trifonov, 1968; Toyoda, Nosaki & Tomita, 1969; Dowling & Ripps, 1973; Cervetto & Piccolino, 1974; Trifonov, Byzov & Chailahian, 1974). This transmitter appears to depolarize the horizontal cell by increasing the permeability of its membrane to sodium (Kaneko & Shimazaki, 1975; Waloga & Pak, 1976).

In this paper the mechanism of signal transmission from photoreceptors to horizontal cells in the turtle *Pseudemys scripta elegans* is examined by comparing the photoresponses of red cones and L-type horizontal cells under various states of adaptation. Such a comparison is justified because the L-type horizontal cells in the turtle retina are activated mainly by single red cones with some impingement from the red members of double cones (Fuortes & Simon, 1974). It will be shown that the signal transmission from red cones to L-type horizontal cells is non-linear, having the highest gain, expressed as the millivolt change in horizontal cell response per millivolt change in red cone response, for dim light stimuli. Background illumination modifies the characteristics of synaptic transmission to make the horizontal cells most sensitive to light modulation around the background intensity.

METHODS

The methods used for stimulating and intracellularly recording from retinal cells of the turtle, *Pseudemys scripta elegans*, are the same as described previously (Normann & Perlman, 1979). The photostimulator contained two beams of light originating from a single light source (General Electric Quartzline, 45W). The flux of the unattenuated light was 6.4×10^{15} effective quanta (640 nm) $\text{sec}^{-1} \text{cm}^{-2}$ for the test channel and 9.1×10^{15} effective quanta (640 nm) $\text{sec}^{-1} \text{cm}^{-2}$ for the background channel. Unless otherwise specified, all intensities described in the text refer to the density of the neutral density filters interposed in each beam. The test stimuli were 500 msec pulses delivered every 6 sec, which covered spots of diameters ranging from 0.3 to 3.2 mm. The background light covered the same retinal area as the test stimuli.

Cones and horizontal cells were identified and classified according to their receptive field and spectral properties following criteria described elsewhere (Baylor, Fuortes & O'Bryan, 1971; Simon, 1973). L-type horizontal cells were identified by the following criteria: (1) they have relatively large receptive fields (> 1 mm in diameter), (2) they respond with hyperpolarization to monochromatic stimuli of wave-lengths 400–700 nm, (3) they produce relatively large responses to saturating stimuli (approx. 50 mV).

Results were analysed only when the intensity–response series performed throughout a particular experiment demonstrated satisfactory stability of the cell in terms of both dark-adapted resting potential and the size of the responses to light stimuli of various intensities.

RESULTS

In order to characterize the cone to horizontal cell synapse by comparing cone and horizontal cell photoresponses, the dimensions of the light stimulus must be sufficiently large to insure that neither cone nor horizontal cell signals spread tangentially across the retina. Unless this criterion is met, these photoresponses cannot be compared in a meaningful manner since such a tangential spread of signals could alter the apparent sensitivity of these cell types as well as the temporal characteristics of their photoresponses (Fuortes, 1972; Simon, 1973). Thus, we have studied the spatial properties of red cones and L-type horizontal cells by increasing the diameter of the stimulating spot from 0.3 to 3.2 mm. The receptive field of the red cones was always much smaller than that of the horizontal cells. The latter were classified into two broad categories (small receptive field, s.r.f., and large receptive field, l.r.f.) based upon their spatial properties and the temporal characteristics of

their photoresponses. This classification supports previous findings (Simon, 1973) but, because of the large variation of the receptive field sizes in each category, it is also compatible with the idea of a continuous distribution of receptive field sizes (Lamb, 1976).

With the experimental apparatus in its present form, illumination of the entire receptive field could be achieved only for small receptive field horizontal cells. Thus, all the results described below were obtained from full field illumination (spot diameter of 3.2 mm) of s.r.f. horizontal cells unless otherwise stated.

Synaptic transmission in the dark-adapted retina

In Fig. 1A the mean of the peak responses of eighteen horizontal cells (circles) and ten red cones (squares) are plotted as a function of the log of the light intensity which evoked each response. The two curves saturate at about the same light intensity

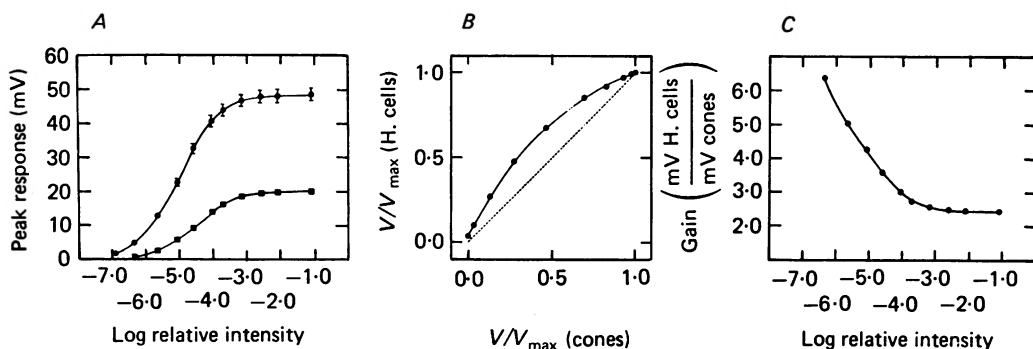


Fig. 1. Signal transmission from red cones to s.r.f. horizontal cells in the dark-adapted state. *A*, mean peak responses of eighteen s.r.f. horizontal cells (circles) and ten red cones (squares) evoked by light stimuli of intensities given in the abscissa. The mean maximum response of the horizontal cells is 44.7 mV (range 32–65 mV) and of the red cones is 20 mV (range 17.5–25 mV). The vertical bars show s.e. of mean. Error bars for cones were smaller than height of square data points. *B*, the transfer function between red cones and s.r.f. horizontal cells was calculated by normalizing the curves given in *A* and plotting the normalized horizontal cell response as a function of the normalized cone response evoked by a certain light stimulus. The dashed line has a slope of 1 and represents the expected transfer function if the cone–horizontal cell system was linear. *C*, the gain, defined as the millivolt change in horizontal cell per millivolt change in cone, was obtained from the data in *A*, by dividing the horizontal cell peak response by the cone peak response evoked by the same stimulus intensity. Stimulus given on the abscissa as log relative intensity.

indicating that all the cones within the receptive field of the horizontal cells were directly illuminated. However, the mean horizontal cell responses were more sensitive than the mean cone responses by at least 0.5 log unit; a finding similar to that reported in the skate retina (Green, Dowling, Siegel & Ripps, 1975). In a previous study on the turtle retina (Fuortes, Schwartz & Simon, 1973) it was reported that the L-type horizontal cells were less sensitive than either the red or the green cones. However, the light stimulus used in that study covered a spot of only 2.5 mm diameter and, because this is smaller than the receptive field of the l.r.f. cells (the most frequently impaled cells), it could have resulted in an apparent reduction in horizontal cell sensitivity.

The horizontal cells and red cones used to construct Fig. 1 were impaled in different retinæ. However, in some experiments a horizontal cell and a red cone were impaled in the same retina with the same electrode during a single penetration and their photoresponses were related in a way similar to the one illustrated in Fig. 1*A*. Moreover, in each eyecup similar retinal regions were always investigated (about midway between the optic nerve and the edge of the eyecup). Therefore, while using cells from different retinæ might cause subtle effects in the apparent red cone-horizontal cell interaction, the qualitative relationships described below should maintain.

The transfer function of the red cone - s.r.f. horizontal cell system was obtained by comparing the normalized peak horizontal cell response to the normalized peak cone response evoked by the same stimulus (Fig. 1*B*). The deviation of the data points from the identity line (interrupted line, Fig. 1*B*) suggests that signal transmission from the red cones to the horizontal cells is non-linear and that the system is most sensitive to dim stimuli. A similar non-linearity in the synaptic transmission between photoreceptors and post-synaptic cells (either horizontal cells or bipolar cells) was suggested by Falk & Fatt (1972) based on theoretical considerations and on the behaviour of known synapses. The mean maximal response of the s.r.f. horizontal cells was 48.7 mV (range 32–65 mV) while that of the cones was 20 mV (range 17.5–25 mV). Therefore, if the transfer function in Fig. 1*B* was linear, the gain in terms of the millivolt change in horizontal cell per millivolt change in cone should have been 2.44. The non-linearity in the transfer function is further illustrated in Fig. 1*C* where the gain of the system is plotted as a function of the log intensity of the light stimulus. The gain was highest for dim light and gradually decreased toward the asymptotic value (2.44) as the stimulus was made brighter.

It has been previously suggested that spatial summation can account for the increased sensitivity of horizontal cells compared to photoreceptors (Green *et al.* 1975). However, if synaptic transmission is linear and if the entire receptive field is illuminated, then the normalized intensity-response curves obtained from horizontal cells and photoreceptors should coincide except in the region of very dim stimuli (less than 1 photon absorbed per cone). Here the responses of the photoreceptor are attenuated by lateral interactions (Baylor, 1974; Baylor, Fuortes & O'Bryan, 1971) while the horizontal cell sums all the responses of the individual cones which are within its receptive field. In the experiments described in Fig. 1, the smallest cone response was of the order of 1–2 mV, which corresponded roughly to the absorption of 100 photons per cone per pulse (Baylor & Hodgkin, 1973). Therefore, spatial summation does not seem to be responsible for the non-linear transfer function described in Fig. 1*B*. Rod input to L-type horizontal cells could also account for the enhanced sensitivity to dim stimuli. However, the action spectrum of L-type horizontal cells measured at the low response region (less than 5 mV response) was found to have a peak around 630 nm which confirms previous reports that L-type horizontal cells in the turtle retina received input mainly from red cones (Fuortes *et al.* 1973; Fuortes & Simon, 1974). Considering the above arguments the non-linearity in the transfer function cannot be accounted for by such trivial reasons as spatial summation or rod input. It is therefore concluded that the synapse itself behaves in a non-linear way. A similar non-linearity in the relationship between the amplitude

of the presynaptic action potential and the amplitude of post-synaptic potential was found in the giant synapse in the stellate ganglion of the squid (Miledi & Slater, 1966).

Synaptic transmission in the light-adapted retina

Horizontal cell hyperpolarization produced by background illumination

In response to a long step of light, the horizontal cells initially hyperpolarized to a peak value and then gradually depolarized until a steady-state level was achieved as illustrated in Fig. 2*A*. The number to the left of each record indicates the log relative intensity of the background illumination. The cell shown in Fig. 2*A* was particularly stable but typical of all horizontal cells studied. The steady-state level of membrane potential under constant illumination was usually about half way between the dark-adapted level and the peak of the response to the background light and was relatively independent of the intensity of the background light. In Fig. 2*B* data from forty-six backgrounds studied in fourteen horizontal cells are presented. The ratio between the steady-state hyperpolarization and the peak response is plotted against the log of the effective background (log background-log threshold, where threshold was defined as the light stimulus evoking a peak response which was 10% of the saturating response) for l.r.f. horizontal cells (squares) and s.r.f. cells (triangles). Most of the data points fall around the value of 0.5 for the steady-state to peak ratio, irrespective of the background intensity. The mean value is 0.45 ± 0.09 (s.d.).

This observed fall in horizontal cell hyperpolarization during steady illumination may be a consequence of similar behaviour observed in the red cones (Normann & Perlman, 1978). They showed that all backgrounds studied caused the cones to reach a steady-state hyperpolarization of about $0.44 V_{cp}$ where V_{cp} was the cone peak response to the background light. If the horizontal cells were simply following the cones, then from the dark-adapted transfer function (Fig. 1*B*) the steady-state hyperpolarization of these cells produced by bright backgrounds should have been $0.66 V_{hp}$, where V_{hp} was the horizontal cell peak response to the background light. As this was not the case (Fig. 2*B*), a continuous exposure to light, therefore, either modifies the mechanism of synaptic transmission from cones to horizontal cells or induces some changes in the horizontal cell membrane in addition to the direct effects of the synaptic transmitter.

The effect of background illumination on horizontal cell photoresponses

The background illumination was maintained until the retina had reached approximately a steady-state of light-adaptation as judged by the stability of the horizontal cell potential and its responses to test pulses (at least 2 min). The background light was then substituted with 500 msec pulses of different intensities ranging from full darkness to bright unattenuated light. The responses of a horizontal cell (the same one used in Fig. 2*A*) to light stimuli delivered while light-adapted to backgrounds of different intensities are shown in Fig. 3. Throughout the experiment shown in this figure, the membrane potential was very stable so that between background applications the resting potential did not change. The responses obtained at each level of light-adaptation (Fig. 3*B, C, E, F*) are drawn relative to the dark-

adapted resting potential (dotted line). The steady-state potential due to the background can be measured as the difference between the dotted line and the potential before the response. Between each period of light-adaptation the cell was allowed to dark-adapt, and the photoresponses to a series of light stimuli were recorded as a control (Fig. 3D) for comparison with the dark-adapted photoresponses obtained just after impaling the cell (Fig. 3A).

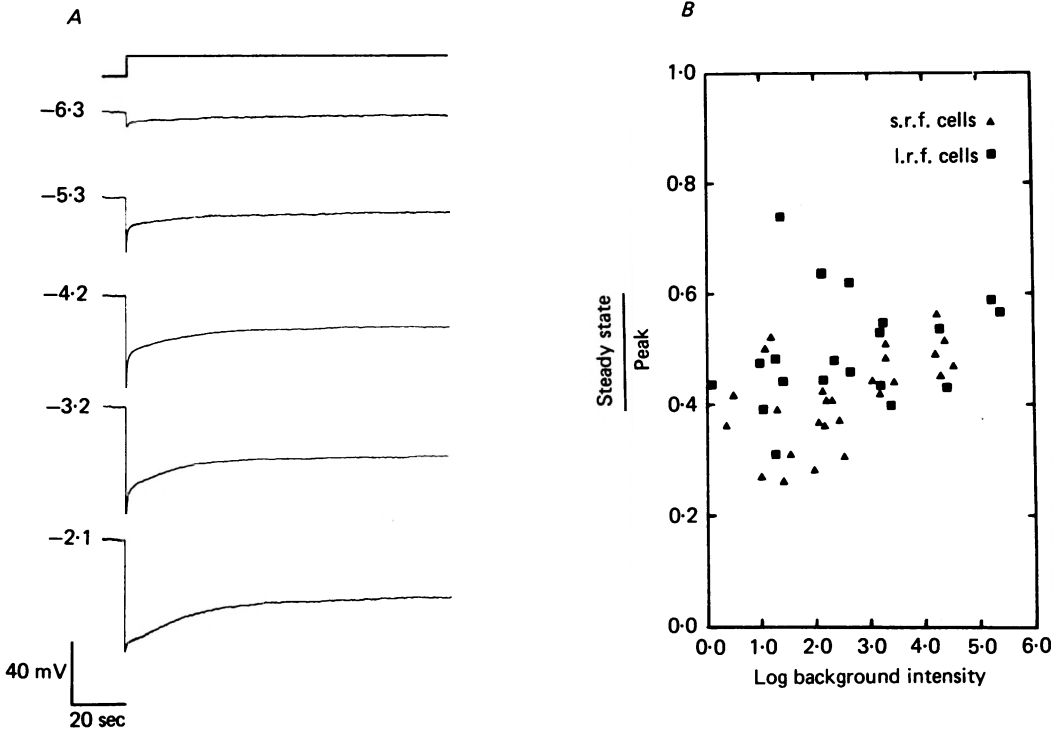


Fig. 2. The effect of background illumination on the membrane potential of horizontal cells. *A*, responses of a s.r.f. horizontal cell to 2 min illumination of the entire receptive field. At the left of each trace, the log relative intensity of the background is given. *B*, the ratio between steady-state membrane polarization, measured 2 min after background onset, and the peak response to the background light as a function of the background intensity. The effective background was calculated relative to threshold intensity, defined as the stimulus intensity evoking a peak response which was 10% of the saturating response. The data were obtained from eight s.r.f. cells (triangles) and six l.r.f. cells (squares).

The peak responses to both increment and decrement stimuli were measured from the dark-adapted membrane potential and these values were used to construct the intensity-response curves for each state of light-adaptation shown in Fig. 4. The 0 potential refers to the dark-adapted resting potential. The horizontal bar in each curve represents the steady potential level caused by the background. Increasing the background illumination caused the intensity-response curve to shift to the right along the log intensity axis similar to the effect of light-adaptation on the S-potential in the retina of the skate (Dowling & Ripps, 1971). However, unlike the skate, the curves illustrated in Fig. 4 did not fit a single template. The dark-adapted curve is broad, covering about 3.8 log units while the intensity-response curves measured in

the light-adapted states are sharper, covering about 2.5 log units. A possible explanation for the differences between the S-potential in the skate and the horizontal cell responses in the turtle, besides species differences, is that in this study the entire receptive field of the horizontal cells was illuminated and the whole dynamic range of the horizontal cells response was studied by recording responses to both increments and decrements of light.

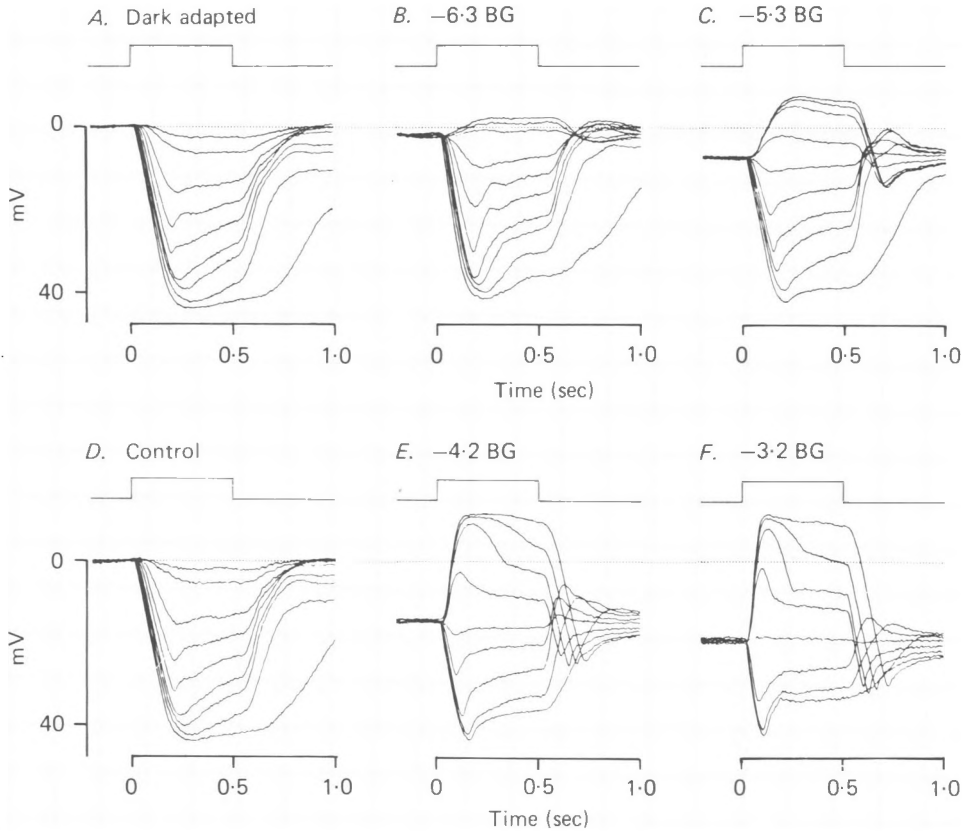


Fig. 3. Photoreponses of a s.r.f. horizontal cell (the same one used in Fig. 5A) to 500 msec pulses of light during various levels of adaptation. During background (BG) illumination, the test pulse was substituted for the background for 500 msec. Test pulses were delivered 6 or 12 sec apart. The dotted line represents the dark-adapted resting potential which was stable throughout the experiment. All the light-adapted responses were plotted with the actual d.c. shift compared to the dark-adapted resting potential. *A*, dark-adapted responses to light stimuli ranging in intensity from -6.9 to -2.1 log units separated by about 0.5 log units. *B*, *C*, *E*, *F*, responses of the horizontal cell when light-adapted to background intensities of -6.3 , -5.3 , -4.2 , -3.2 log units respectively. The range of intensities given in log relative units used to evoke graded responses are as follows: *B*: -6.9 to -2.1 ; *C*: -6.9 to -2.1 ; *E*: -5.6 to -1.0 ; *F*: -4.6 to -0.5 . In each set of responses a response to a dark pulse is also shown. *D*, photoreponses obtained by 500 msec test flashes of increasing intensity (from -6.9 to -2.1 log units in about 0.5 log unit steps), recorded between periods of light-adaptation. In general, following each period of background illumination, a series of responses similar to the one shown in *D* were obtained to ensure complete recovery of the cell and stability of the recordings, before the next background was applied.

Light-adaptation had different effects on the intensity-response curves of red cones and horizontal cells. In the cones, the curve shifted along the intensity axis and slightly downward as background illumination was increased, but all the curves fitted a single template (Normann & Perlman, 1979). Thus, at each state of adaptation the intensity-response curve of the cones spanned the same (but shifted) dynamic range of intensities (3–3.5 log units) and had the same total excursion of potential ($V_{\max} - V_{\min}$). In the horizontal cells, on the other hand, light-adaptation shifted the intensity-response curve to the right along the log intensity axis but also caused the curve to become steeper as the background illumination was increased (Fig. 4).

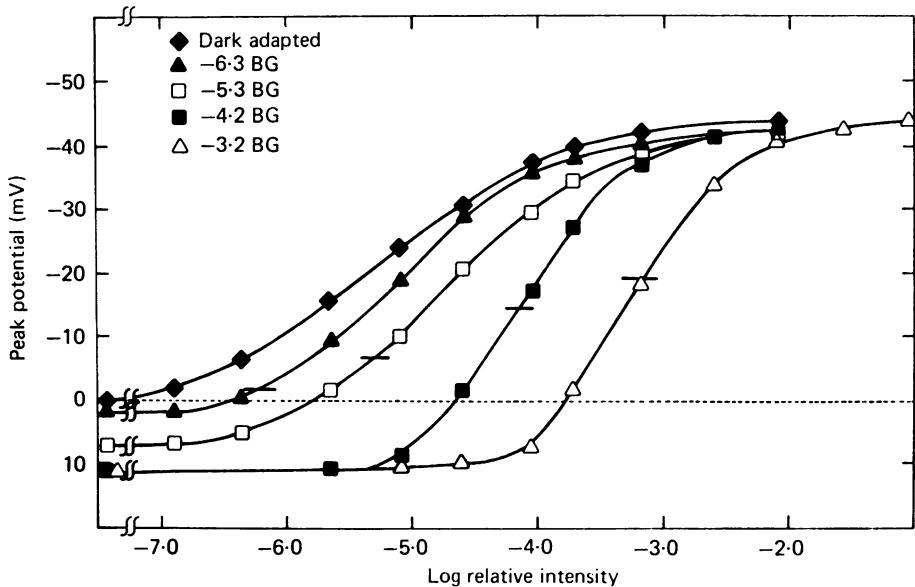


Fig. 4. The peak responses shown in Fig. 3 were measured from the dark-adapted resting potential and plotted as a function of the test intensity. The dashed line represents the dark-adapted resting potential. The curves through the data points were drawn by eye. The horizontal bar in each curve indicates the steady-state membrane potential measured at least 2 min after the background onset. Light-adaptation caused the curves to shift along the intensity axis and to become steeper. Note that during light-adaptation the peak potential evoked by a saturating stimulus is unchanged while the response to dark pulses overshoots the dark-adapted resting potential.

Moreover, the total excursion in potential grew as the background was made brighter (Fig. 4). Similar sharpening of the intensity-response curve by background illumination was observed in horizontal cells in the *Necturus* retina (Werblin, 1974). However, Werblin (1974) attributed this sharpening to the transition from rod dominance to cone dominance while in the turtle the L-type horizontal cells receive input mainly from red cones, even at low light levels (Fuortes & Simon, 1974; and unpublished observations). These differences between red cones and horizontal cells further demonstrate that although the horizontal cells' behaviour is a direct consequence of the red cones' behaviour, the relationship between these cells can be modified by changes in the background intensity.

In response to 500 msec pulses of darkness substituted for the background, the

membrane potential of the horizontal cells reached a peak which was more depolarized than the dark-adapted level. This could be accounted for by a relatively slow voltage dependent conductance mechanism similar to that described by Werblin (1975). However, as the absolute potential of the peak response to a supersaturating flash was independent of the background intensity, such a mechanism seems unlikely. Moreover, during the dimmest background studied, the steady-state membrane potential was only about 2 mV more hyperpolarized than the dark-adapted potential and thus should activate a negligible portion of the voltage dependent conductance. Yet the response to a 500 msec pulse of darkness still overshoot the dark-adapted potential. An alternative explanation of the overshoot to decrements is as follows. In the dark-adapted state only a fraction of the transmitter modulated channels are open. A background light modifies synaptic transmission in such a way that when the background is turned off all the available post-synaptic sites bind to transmitter. This results in a greater sodium conductance than in the dark-adapted state, and causes the membrane potential to become more positive.

Termination of the background elicited a recovery of the horizontal cell membrane potential. After an initial delay of from 20 to 50 msec, this recovery could be adequately described by a single exponential time course. The time constant of the recovery phase became shorter as the background light was made brighter as illustrated in Fig. 5A for both l.r.f. cells (squares) and s.r.f. cells (triangles). The time constants measured ranged from 100 msec for the dimmest background to 15 msec for the brightest one. A somewhat similar behaviour was observed in the red cones of the turtle but their response to the termination of the dimmest background had a time constant of about 140 msec while termination of the brightest background evoked a depolarization with a time constant of about 40 msec.

This paradoxically faster 'off' response of the horizontal cells than the red cones to the substitution of the background illumination with 500 msec pulses of darkness is further illustrated in Fig. 5B. In this Figure the responses of a s.r.f. horizontal cell (continuous) and a red cone (dotted) were normalized to have the same amplitude 500 msec after the termination of the background. For every background studied, the horizontal cell potential recovered faster than the cone potential when the background was turned off even though the cones started to respond slightly before the horizontal cells. Partial illumination of the receptive field (which explains the faster 'off' response of horizontal cells than of cones to saturating stimuli delivered to the dark-adapted retina) cannot account for the behaviour described in Fig. 5B. Incomplete background illumination of the receptive field will cause peripheral cones within the receptive field to be illuminated by dim scattered light. These peripheral cones will be light-adapted to dimmer light than the cones directly illuminated and, therefore, their response to the termination of the background will be slower than that of the central cones. Thus, the effect of the peripheral cones should be to slow down the horizontal cell response compared to the central cones. Fig. 5B shows that this was not the case. Moreover, the faster recovery of membrane potential of the horizontal cell when the background was terminated (Fig. 5B) was observed in s.r.f. cells where the entire receptive field was illuminated by the light stimuli.

A possible explanation of the paradoxical response of horizontal cells and red cones to the termination of background illumination is that a slight recovery of the cone

potential is sufficient to cause release of large amounts of transmitter which in turn can cause a complete return of the horizontal cell potential to its dark-adapted level. The burst of transmitter release does not appear to be due to an accumulation of transmitter in the presynaptic terminal during the period of light-adaptation, for it has been shown in the frog retina that during light-adaptation there is no accumulation of synaptic vesicles in the cone terminals (Schacher, Holtzman & Hood, 1976). Therefore, it is postulated that light-adaptation modifies synaptic transmission between cones and horizontal cells. This modification can either sensitize the transmitter release mechanism so that a larger amount of transmitter is released by small

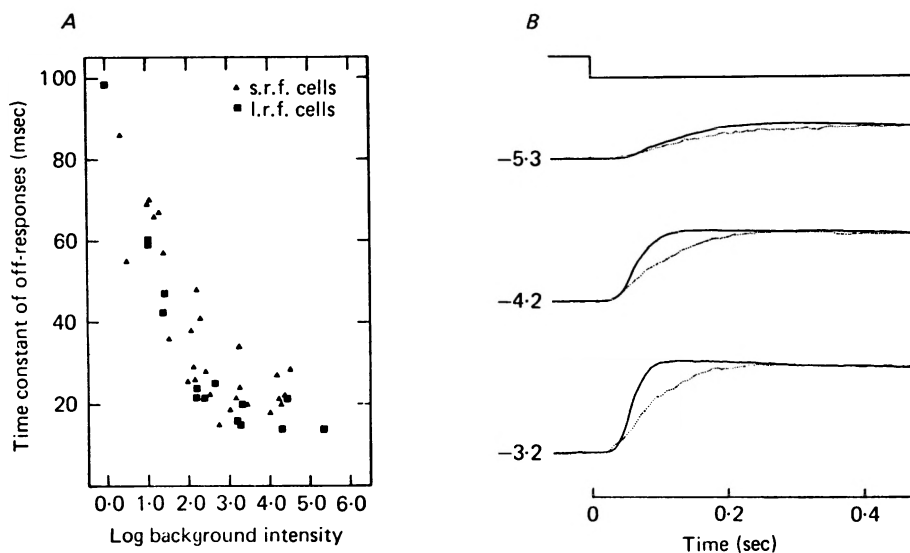


Fig. 5. The kinetics of the response to a 500 msec pulse of darkness delivered at different background intensities. *A*, the responses of horizontal cells to dark pulses fitted reasonably an exponential time course. The ordinate gives the time constant of the response to dark pulses, obtained from eight s.r.f. cells (triangles) and six l.r.f. cells (squares), while light-adapted to the intensities given in the abscissa. Log effective background was calculated relative to the threshold intensity as in Fig. 2*B*. *B*, the time course of the responses to 500 msec pulses of darkness substituted for background illumination of intensities given in log units at the left of each pair of traces. The responses of a s.r.f. horizontal cell (continuous traces) and those of a red cone (dotted traces) were compared by normalizing them to have the same amplitude 500 msec after the onset of the dark pulse.

recovery of the cone potential, or increase the affinity of the post-synaptic binding sites to the transmitter so that more binding will occur for a fixed amount of transmitter present.

The light-adapted cone-horizontal cell transfer function

In order to further investigate the effect of light-adaptation on synaptic transmission between cones and horizontal cells, the over-all transfer function was constructed by comparing peak responses of a horizontal cell and a cone, during different levels of background illumination. Fig. 6 shows the gain of the cone-horizontal cell system, defined as mV change in horizontal cell per millivolt change in red cone,

plotted as a function of the log light intensity for three different states of adaptation. In the dark-adapted state (filled squares) the highest gain is for dim stimuli. During continuous illumination the highest gain was calculated for light modulations around the background intensity (vertical arrows). Thus, the turtle retina adjusts to steady illumination in at least two ways: (1) light-adapted cones can respond in a graded manner to light intensities which saturate the dark-adapted cones (Normann & Perlmann, 1979), and (2) the cone to horizontal cell synapse is modified to make it most sensitive to light modulations around the background intensity.

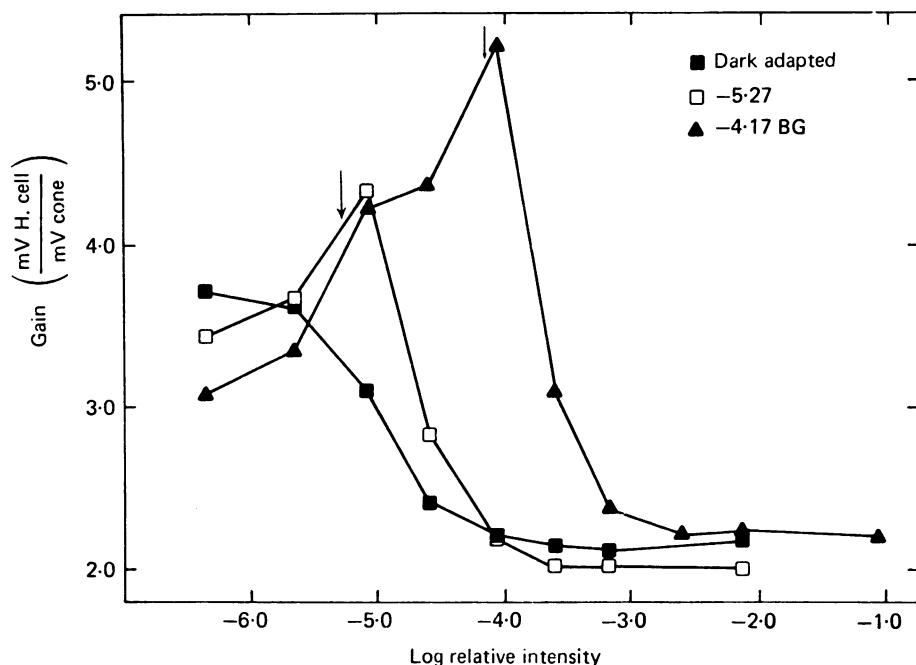


Fig. 6. The gain of the red cone-horizontal cell system was calculated by dividing the peak response amplitude of a s.r.f. horizontal cell by the peak response amplitude of a red cone evoked by the stimulus intensity given on the abscissa. The gain of the system in three different states of adaptation is shown. In the dark-adapted state (filled squares) the highest gain is for dim light stimuli. In states light-adapted to background intensities of -5.27 (open squares) and -4.17 (triangles) log units, the highest gain is calculated for light modulations around the background intensities (vertical arrows).

In this study linear responses at each state of adaptation were not recorded and, therefore, the values of the gain obtained around each background might not reflect true gain. However, the data in Fig. 6 suggest that the gain for small light modulation is increasing when the retina is progressively adapted to background intensities of -5.27 and then to -4.17 log units. Based on theoretical consideration and on the behaviour of neuromuscular junctions, Falk & Fatt (1972) concluded that the maximum sensitivity would be attained when the steady membrane potential of the post-synaptic cell was depolarized to a level one-half its maximum. It is feasible that in the dark-adapted turtle retina, the horizontal cells are even more depolarized than one-half maximum and thus exhibit less than maximum gain. When background

light is applied, the membrane potential hyperpolarizes toward a level which is associated with maximum gain. The maximum sensitivity calculated for the photo-receptor-post-synaptic cell system was 2.7 (Falk & Fatt, 1972), based on assumed values for synapse parameters. In Fig. 6, a gain of at least 5 was determined for the red cone-horizontal cell system in the turtle retina.

DISCUSSION

Synaptic transmission in the dark-adapted retina

The relationship between horizontal cell peak response and cone peak response is determined by the mechanisms of synaptic transmission from cones to horizontal cells. Under dark-adapted conditions, the cone-horizontal cell synapse is non-linear; its gain is higher for dim stimuli than for bright stimuli (Fig. 1*B, C*). In order to localize the non-linearity in the cone-horizontal cell synapse to pre- or post-synaptic elements, it was assumed that the over-all synaptic transfer function is a product of the transmitter release function and the binding between transmitter and post-synaptic sites (binding function). The following assumptions (similar to those of Falk & Fatt (1972)) were used to derive a qualitative description of the binding function. (1) The binding between transmitter and post-synaptic channels exhibits saturation kinetics analogous to the equilibrium kinetics assumed for the interaction between acetylcholine molecules and receptor sites located on the end-plate membrane of the sartorius muscle (Kordas, 1972). (2) The sodium conductance of the post-synaptic membrane is linearly related to the fraction of total channels bound to transmitter. (3) When the horizontal cell membrane hyperpolarizes by light to the saturating level, the amount of transmitter present in the synapse is negligible compared to the dark-adapted level. (4) In the dark-adapted state the concentration of transmitter in the synapse is finite and the fraction of post-synaptic channels bound to transmitter is smaller than 1. The last assumption is based on the overshoot above dark-adapted potential seen when the backgrounds were terminated.

Fig. 7 shows hypothetical transmitter release (7*A*) and binding (7*B*) functions. The assumed relationship between horizontal cell potential and transmitter (Fig. 7*B*, upper curve), together with the measured transfer function between cones and horizontal cells (Fig. 1*B*), leads to the non-linear relationship between transmitter release by the presynaptic terminal and the cone membrane potential (Fig. 7*A*, lower curve). Similar non-linearity between transmitter release and membrane potential of the presynaptic terminal was observed in the rat diaphragm preparation (Liley, 1956) and in the frog sartorius (Katz & Miledi, 1967).

Synaptic transmission in the light-adapted retina

It was shown in the previous section that the behaviour of the horizontal cells during exposure to background illumination could not be explained solely by the behaviour of the red cones under the same conditions (Figs. 2-6). Thus, light-adaptation apparently modifies either the transmitter release by the presynaptic terminals or the binding characteristics between transmitter and post-synaptic sites or both. Both speculative mechanisms are based on transitions from one operating curve to another when the state of adaptation is changed.

A possible presynaptic mechanism is described in Fig. 7 *A*. In the dark, the amount of transmitter released is described by point (1). When a saturating background is turned on, the cone hyperpolarizes to the saturating level, V_L , where transmitter release is minimal. As light adaptation progresses, the cone membrane potential gradually falls toward $V_{1/2}$. Simultaneously, the release function changes to that described by the upper curve. At the steady-state, the transmitter release mechanism is operating at point (4) rather than at point (3) releasing more transmitter than expected from the dark-adapted curve (lower curve). This causes the horizontal cell to depolarize more toward the dark-adapted level. The higher slope of the release

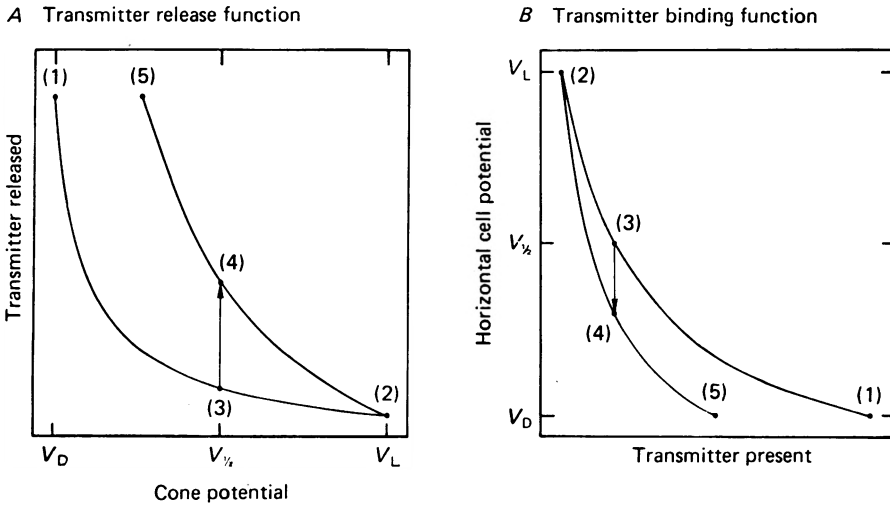


Fig. 7. Speculative mechanisms which could account for the effects of light-adaptation on the synaptic transmission between red cones and L-type horizontal cells. *A*, pre-synaptic mechanism. Transmitter release by the presynaptic terminal as a function of the cone membrane potential in the dark-adapted (lower curve) and light-adapted (upper curve) states. In response to the onset of a saturating background, the cone membrane hyperpolarizes from the dark-adapted level (point (1)) to point (2), then slowly sags back to reach a steady-state level, $V_{1/2}$ (point (3)). However, the operating curve of the release mechanism moves to the light-adapted one (upper curve) so that the presynaptic terminal operates at point (4). Point (5) represents the level of cone potential needed to release sufficient transmitter to cause the horizontal cell membrane to reach its dark-adapted resting potential. *B*, post-synaptic mechanism. The relationship between transmitter present and the membrane potential of the horizontal cell in the dark-adapted state (upper curve) and in the light-adapted state (lower curve). When a saturating background is applied, the horizontal cell hyperpolarizes from its dark-adapted potential (point (1)) to reach a peak (point (2)) and then sags to a steady-state level, $V_{1/2}$ (point (3)) due to the sag of the red cones. Simultaneously the curve is shifted down so that the horizontal cell operates at point (4) while light-adapted. Point (5) represents the amount of transmitter needed to cause complete recovery of the horizontal cell potential back to its dark-adapted level.

curve at point (4) compared to point (3) explains the sharpening of the intensity-response curve measured in horizontal cells during light-adaptation. Moreover, the cone membrane potential has to recover only partially when the background is turned off in order to release enough transmitter (represented by point (5)) to cause

complete recovery of the horizontal cell potential. A similar presynaptic mechanism was reported to exist in the rat diaphragm preparation (Hubbard & Willis, 1962*a, b*).

The 'desensitization' produced by acetylcholine at the motor end-plate of the frog sartorius (Katz & Thesleff, 1957) suggests a possible post-synaptic mechanism which could explain the effects of light-adaptation on the cone-horizontal cell synapse. In the dark-adapted state there is a large concentration of transmitter and the post-synaptic receptors are desensitized. In this state the relationship between the membrane potential of the horizontal cells and the levels of transmitter is described by the upper curve in Fig. 7*B*. When a bright background is applied the amount of transmitter present in the synaptic cleft is reduced resulting in post-synaptic resensitization and transition to a new operating curve (lower curve). In the steady-state, the horizontal cell membrane will operate at point (4). When operating at point (4) the change in horizontal cell potential induced by a constant change in transmitter is bigger than in point (3) giving rise to a sharper intensity-response curve. Moreover, in order to reach the dark-adapted potential, a smaller amount of transmitter has to be released (represented by point (5)) which can be induced by only partial recovery of the cone membrane potential.

Both the pre- or post-synaptic mechanisms can account for (1) the faster recovery of horizontal cell potential than cone potential in response to the termination of background, (2) the overshoot of the horizontal cell potential above the dark-adapted membrane potential in response to the termination of the background and, (3) the steeper intensity-response curve measured in light-adapted horizontal cells compared to that measured in the dark-adapted cells. Such a system could efficiently make horizontal cells most responsive to light modulation around the background level (Fig. 6) and create fast 'off' responses when receptors are sluggish (Fig. 5*B*).

Further support for the hypothesis that light-adaptation modifies the synaptic transmission between the red cones and the L-type horizontal cells is obtained from careful examination of the responses of these cells to the background illumination. The red cones hyperpolarize to a peak and then sag quite fast to reach the steady-state. The horizontal cells, on the other hand, have two components to the sag toward the steady-state. The sag is initially similar to the one observed in cones but then continues at a much slower pace. This second slow phase of change in the horizontal cell potential, which is most evident at bright background levels, might reflect a slow modification of the synaptic transmission mechanism. The recovery of the synapse from its light-adapted properties to the dark-adapted ones occurs quite fast. As an example, when a background of -4.0 log units is turned on, steady-state is reached after 2 min of continuous exposure. Following termination of the background, the horizontal cell regains its dark-adapted characteristics within 30 sec. Similar differential time courses between the desensitization and resensitization processes were also found in the end-plate of the frog sartorius muscle (Scubon-Mulieri & Parsons, 1977).

The above analysis of synaptic transmission deals only with peak responses and not with the kinetics of the responses. Saturating stimuli delivered at any state of adaptation evoked 'on' responses with similar kinetics suggesting that the horizontal cell response is determined by a limiting step which involves the removal of transmitter or unbinding of channels. The horizontal cell response to the termination of

the background became faster when the intensity of the background was increased (Fig. 5A). If the cone terminal responds to the termination of the background with a burst of transmitter release then the horizontal cell response reflects the rate at which the transmitter binds to the post-synaptic channels. Therefore, the horizontal cell response to pulses of darkness substituted for background illumination indicates an increase in the affinity of post-synaptic sites to transmitter induced by the light-adaptation. This supports the suggestion that light-adaptation changes the binding characteristics (Fig. 7B). Unfortunately, the cone–horizontal cell system lacks the advantages of the neuromuscular junction, namely size and knowledge of the identity of the transmitter. Therefore, the possible effects of light-adaptation discussed above are indistinguishable and might occur simultaneously.

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Signal transmission from red cones to horizontal cells in the turtle retina.

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