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Effects of Background Illumination on the Horizontal Cell Responses in the Tiger Salamander Retina

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Synaptic transmission between photoreceptors and horizontal cells (HCs) was studied in the flat-mounted isolated retinas of the tiger salamander. Background illumination expedited the rise time of the HC light response, and the HC response rise time (HCRRT) reached steady state about 2 sec after the onset of the background illumination. The change in HCRRT is probably responsible for the background-induced enhancement of the HC responses to short light stimuli. The amplitude of the HC responses to 100 msec light steps in the presence of background illumination was 2–5 times larger than that measured under dark-adapted conditions. Background illumination exerted little effect on the response rise time in cones and bipolar cells, and thus it caused no significant response enhancement in those cells. The background-induced change in HCRRT correlated closely with the rod voltage but not with the HC voltage. These results suggest that the background-induced change in HCRRT is probably mediated by postsynaptic events in HCs because no significant time course change is observed in photoreceptors and bipolar cells (which share the same synapses with the HCs). A suppressive rod action on the cone inputs in HCs may be responsible for modulating the HCRRT. By shortening the HCRRT, background illumination regulates the frequency response of the photoreceptor–HC synapse and alters the capacity of spatial resolution of retinal bipolar cells.

In the vertebrate retina, photoreceptors continuously release neurotransmitters in darkness, which depolarizes the horizontal cells (HCs) (Byzov and Trifonov, 1968; Dowling and Ripps, 1973; Schacher et al., 1974). Light hyperpolarizes the photoreceptors and reduces neurotransmitter release, which results in a hyperpolarizing response in HCs (Dowling and Ripps, 1973; Dacheux and Miller, 1976). It is possible that the efficacy or gain of the photoreceptor–HC synapse is modulatable by light or darkness. In *Xenopus,* mudpuppy, and fish retinas, background illumination increased the amplitude of the HC responses to light steps and flickers (Hassin and Witkovsky, 1983; Frumkes and Eysteinsson, 1987; Yang et al., 1988a). It is not clear, however, whether these changes in HC responses are mediated by modification of the presynaptic light responses in the photoreceptors or by changes in efficacy of the photoreceptor–HC synapse. In addition to changing the response amplitude, background light also alters the time course and waveform of the HC responses: the rise of the HC responses under light-adapted condition is much faster than that in darkness, and an off-overshoot response can be observed in light-adapted HCs but not in dark-adapted HCs (Tauchi et al., 1984; Wu, 1987a, b). These light-dependent changes in response kinetics may affect the modulation of response amplitude in the following ways: (1) For short light stimuli, the amplitude of the HC response is larger if the rise time course is faster and (2) the amplitude of the HC responses to repetitive light flashes (flickers) is progressively larger when a depolarizing overshoot is present after each flash, i.e., the HC baseline is progressively depolarized by the repetitive overshoot responses (Wu, 1988).

The purpose of this study is to characterize the mechanisms underlying the light-dependent modulation of the rise time course of the HC response, and its relationship with the background-induced changes in the HC response amplitude. Since the time to peak of the HC response under dark-adapted condition is about 1–2 sec and the response kinetics during this period of light exposure may change significantly, it is therefore difficult to determine accurately the response kinetics by measuring the time to peak of the HC responses. In this study, the standard stimuli we used are short light steps (100 msec), during which the response kinetics do not change significantly. The interstimuli duration is set to be 5–10 sec so that HC off-overshoot responses under light-adapted conditions can have sufficient time to relax back to the baseline level before the next stimulus is turned on.

Materials and Methods

The preparations. Larval tiger salamander (*Ambystoma tigrinum*) purchased from the Lowrance Waterdog Farm (Tulsa, OK) were used in this study. Prior to an experiment, the animal was dark-adapted overnight and then decapitated under infrared illumination. The eyes were enucleated and hemisected. A piece of the posterior half of the eyecup was inverted over a piece of Millipore filter paper (HAO 0.45 μm) secured in the superfusion chamber. The sclera and the pigment epithelium were removed from the retina. Oxygenated Ringer's solution was added to the superfusion chamber so that the retina was immersed totally under solution. Flat-mounted isolated retinas and living retinal slices were used in this study, and the detailed description of these preparations were given in Werblin (1978) and Wu (1987c). The entire procedures were done under infrared illumination with a dual unit fine-R-scope (FJW Industry, Mount Prospect, IL). The retinal cells were viewed by a 40× Zeiss water immersion objective lens modified for the Hoffman modulation contrast optics (Hoffman Modulation Optics, Greenvale, NY). During the experiment, retinal cells as well as the 2 electrodes were clearly observed on the screen of a TV monitor con-

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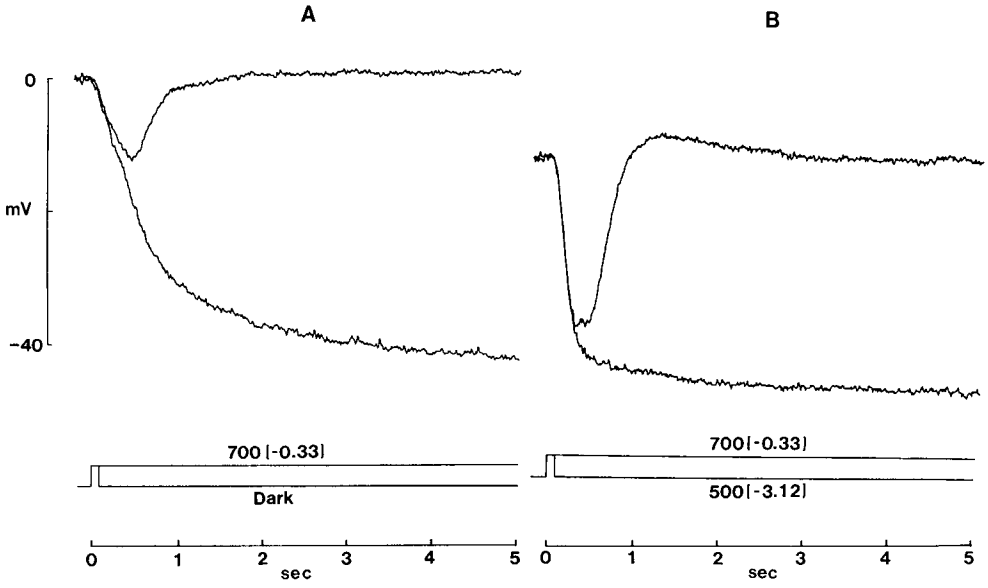

Figure 1. Voltage response of a HC to long (4 sec) and short (100 msec) light steps (*lower traces*) under dark-adapted condition (*A*) and in the presence of (500 nm/−3.12 background illumination (*B*). All test light steps used in this figure were of the same intensity and wavelength (700 nm/−0.33). The resting potential of the HC under dark-adapted condition (*A*) was about −20 mV.

nected to the infrared image converter (COHU, Palo Alto, CA, model 4415) attached to the microscope.

Recording and stimulation. Intracellular recording and current injection were made with micropipettes drawn out on a modified Livingston puller with omega dot tubing (1.0 mm OD and 0.5 mm ID). The micropipettes were filled with 2 M potassium acetate, and had tip resistances, measured in Ringer's solution, of 100–600 MΩ.

The membrane potential of photoreceptors was recorded as the potential difference between the intracellular recording electrode and the bath electrode. Two intracellular electrodes were sometimes inserted into 2 retinal cells to monitor the simultaneous light responses.

Retinal cells were impaled under visual control, in either the flat-mounted retinas or in the slices and the impalement was facilitated by adjusting the negative capacitance in the electrode headstage. Voltage and current traces were monitored with an oscilloscope (Tektronix 5500A) and stored in magnetic tapes (Racal 7DS).

Solutions. Preparations were maintained at room temperature (20–23°C) in an oxygenated Ringer's containing 108 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$ and 5 mM Hepes adjusted at pH 7.7.

Light source. The preparation was stimulated with a dual-beam photostimulator. Two independent light beams whose intensity and wavelength could be adjusted by neutral density filters and interference filters were provided by quartz halogen sources. The light was transmitted to the preparation via the epi-illuminator and the objective lens of the microscope, and the spot diameter on the retina could be adjusted by a diaphragm in the epi-illuminator. In most experiments described in the article, large field illumination (600–1200 μm in diameter) was used. The light sources were calibrated with a radiometric detector (United Detector Technology, Inc.). The intensity of unattenuated 600 nm light is 9.42×10^{17} photons sec^{-1} cm^{-2}. All light stimuli used in this article are described by pairs of numbers (e.g., 500 nm/−1.32), with the first number indicating the wavelength and the second number indicating the relative attention (in log units) with respect to the intensity of the 600 nm given above.

Results

Effects of background illumination on the time course and amplitude of the HC responses

Figure 1 shows the voltage responses of a HC to long (4 sec) and short (100 msec) light steps under dark-adapted condition (*A*) and in the presence of background illumination (*B*). Under dark-adapted conditions (Fig. 1*A*), the amplitude of the HC response to the long light step (700 nm, −0.33) was about 40 mV and the time to peak was about 4 sec. Because of its slow rise, the HC response to the short light step of the same intensity (700 nm, −0.33) could only reach an amplitude of about 12 mV. In the presence of background illumination (Fig. 1*B*), the HC was steadily hyperpolarized for about 10 mV, and the response to the long light step reached the peak voltage of approximately the same level as Figure 1*A* but the time to peak was only about 1 sec. Because of this faster rise, the HC response to the short light step could reach an amplitude of about 26 mV (increment response measured from the ambient voltage). This result demonstrates that the rising time course of the HC light responses in dark-adapted retina can be shortened by background illumination, and the amplitude of the HC responses to short (100 msec) light steps is closely related with the response rise time. As it will be shown later in this paper, the background-induced change of the HC response rise time (HCRRT) occurs about 200 msec after the onset of background light. It is, therefore, inappropriate to use the time to peak of the HC responses to long light steps to characterize the rise time of the HC response at a given background level, because the long test light itself will induce changes of the response rise time. The amplitude of the HC responses to 100 msec light steps, on the other hand, provides a good index in characterizing the rise time of HC responses for the following reasons: (1) Within a certain range of background intensity and test light intensity, the amplitude of the HC response to 100 msec light steps is larger when the time to peak of the HC response is shorter; (2) the 100 msec light step is short enough so that it does not induce significant changes in response time course; and (3) it is convenient. For most of the rest of the experiments described in this article, we used 100 msec light step as the standard test flash and the amplitude of the response to this test flash as an indicator of the response rise time: a larger response amplitude represents a faster rise time.

Figure 2*A* shows the voltage responses of a dark-adapted HC to 100 msec light steps in the presence of background light of various intensities. In darkness, 100 msec flashes elicited small HC responses. In the presence of background illumination, the amplitude of the responses (increment responses measured from the ambient potential levels) to the same test flashes increased progressively with the background intensity. Figure 2*B* shows

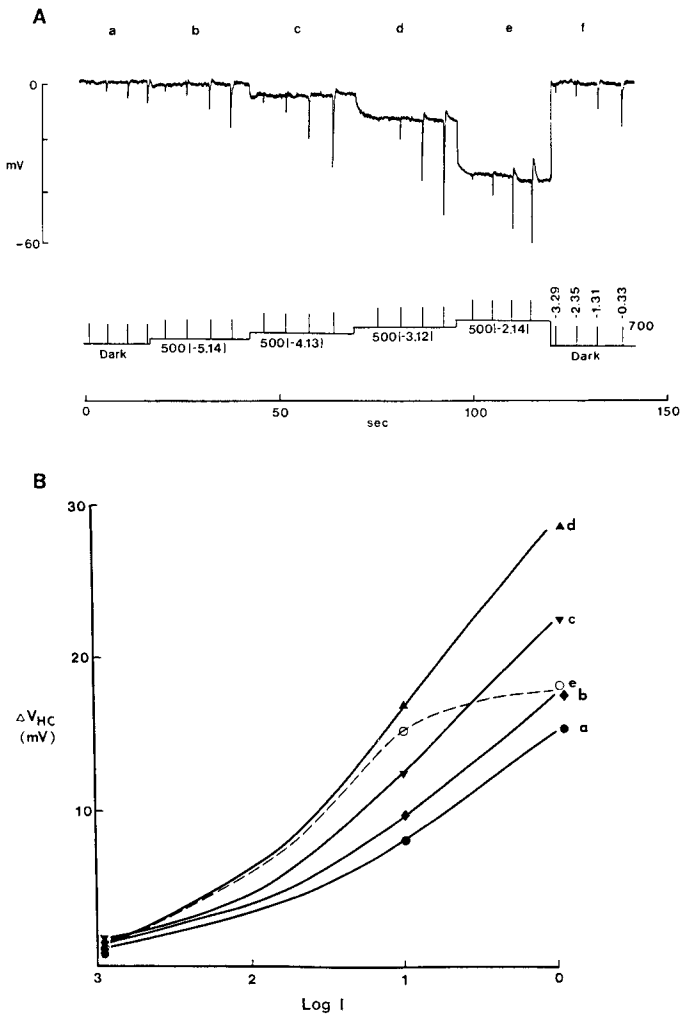

Figure 2. *A*, Voltage responses of a dark-adapted HC to 100 msec light steps (*upright bars* in *lower trace*) of various intensities (700 nm/−3.29, 700 nm/2.35, 700 nm/−1.31, 700 nm/−0.33) in the presence of background illumination of various intensities (*steps* in *lower trace*: *a*, dark; *b*, 500 nm/−5.14; *c*, 500 nm/−4.13; *d*, 500 nm/−3.12; *e*, 500 nm/−2.14; *f*, dark). The dark resting potential of the HC was about −19 mV. *B*, Voltage-intensity (*V*-log *I*) relations of the increment responses (measured as the response amplitude from the ambient levels) of the HC shown in *A* (*a–e* same as in *A*).

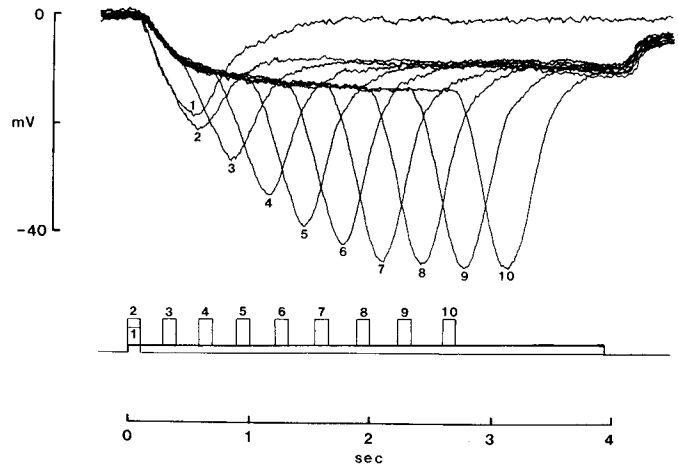

Figure 3. HC responses to 100 msec test light steps (700 nm, −1.31) at various instances after the onset of steady background illumination (500 nm, −4.13). Ten traces of the HC responses to 10 identical test light steps are superimposed. The HC was dark-adapted initially with a resting potential about −20 mV.

the voltage-intensity (*V*-log *I*) relations of the increment responses of the HC shown in Figure 2*A*. In the presence of dim and intermediate background illuminations (*a–d*), the response amplitude increased with the intensity of the background light, indicating that the rise time of the HC response became progressively faster as brighter background light was delivered to the retina. In the presence of bright background light (*e*), the increment responses to bright test flashes (−1.31 and −0.33) became smaller (dashed line in Fig. 2*B*), although the absolute levels of hyperpolarization of these responses (resultant responses = background response + increment response) were lower than those in *d*. This saturation of the HC increment responses is due to the fact that the sum of the background and test lights (−1.31 and −0.33) in *e* is bright enough to hyperpolarize the HC to the peak voltage (−80 mV). The results shown in Figure 2 suggest that the dynamic range of the background-induced changes of the HCRRT resides between 0 (darkness) and −3 log units (500 nm) of the background illumination.

Kinetics of the background-induced change of the HCRRT

The results described above have shown that steady background illumination expedites the rise time of the HC response. To study the mechanisms underlying this background-induced effect, it is important to determine the time course of the change of the HCRRT. Figure 3 shows the HC responses to 100 msec test light steps at various instances after the onset of a steady background light. Test step 1 was given without background illumination, the onset of step 2 was given at the same time as the onset of the background light, and the onset of step 3 to 10 were given 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4 sec after the onset of the background light, respectively. The response to step 2 was slightly larger than that to step 1 because the background light added more intensity to the test light step. As the background light preceded the test step for longer periods (steps 3–10), the response amplitude became progressively larger because the rise time became faster. The response amplitude (and thus the rise time) reached a steady state when the background preceded the test step for approximately 2 sec. Figure 4 shows the time course of the background-induced change of the HCRRT. The amplitude of the resultant response to the 100 msec test step was plotted as a function of the time delay between the onset of the background and the onset of the test light step (Fig. 4*A*). Background lights of 2 intensities were used in this experiment and the time courses were plotted in semilog scales in Figure 4*B*. The changes of responses amplitude (and thus the response rise time) induced by the 2 backgrounds followed approximately the same exponential time course. The time to reach steady state was about 2 seconds.

Cellular sites where background-induced changes of HCRRT may occur

We have shown in the previous sections that the rise time of the HC responses, represented by the amplitude of the responses to 100 msec light steps, becomes faster in the presence of background illumination. This background-induced change in HC

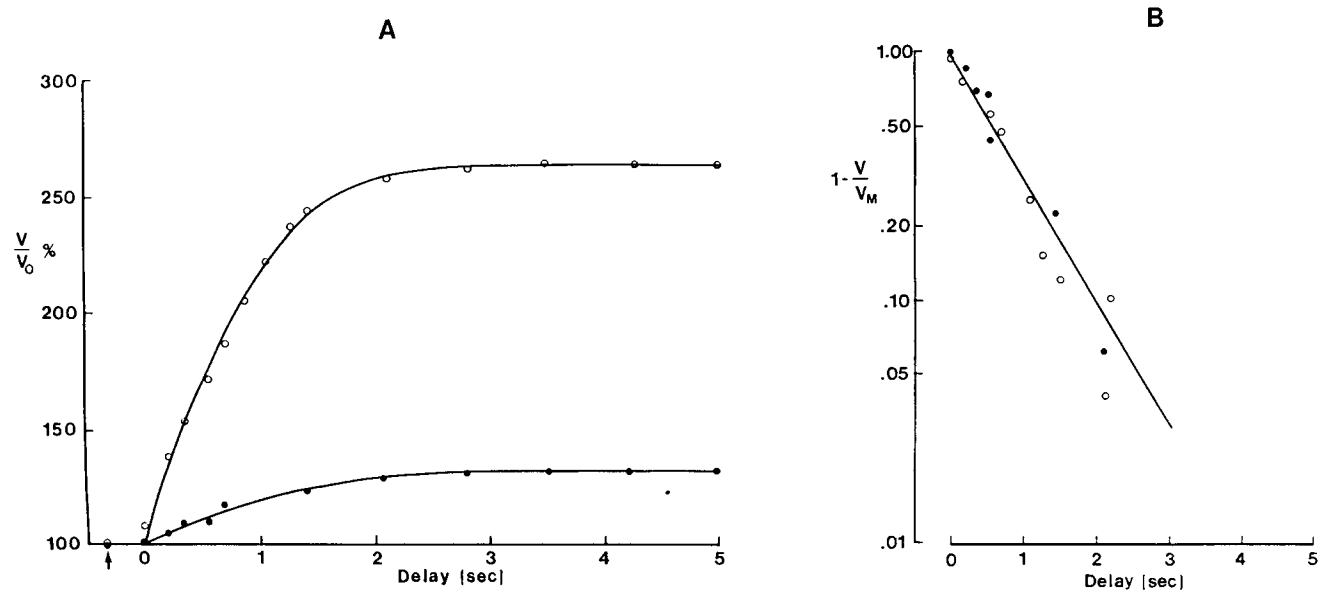

Figure 4. Time course of the background-induced change of the HCRRT. V is the amplitude of the HC responses to 100 msec test lights (700 nm, −1.31) at various times after the onset of background illumination (500 nm, −4.13: *filled circles*; 500 nm, −3.12: *open circles*). V_0 is the amplitude of the HC response to the same test light without background illumination. V/V_0 is plotted against the delay (time between the onsets of the background light and the test light) in *A*. $1\text{-}V/V_M$ is plotted in a semilog scale against the delay in *B*, where V_M is the maximum amplitude of the HC responses in the presence of the background illumination. This plot shows that the changes of HCRRT induced by the 2 backgrounds follow the same time course with a time-to-peak of about 2 sec.

responses may originate from several cellular sites in the retina.

1. *Photoreceptors*: Because HC light responses are mediated by the light responses of rods and cones in this retina (Hanani and Vallerga, 1980), changes in photoreceptor responses may alter the HC responses.

2. *Photoreceptor terminals*: Background illumination may alter the properties of the photoreceptor terminals which may be electronically insulated from the cell bodies where recordings are made.

3. *Synaptic cleft*: Background illumination may affect events occurring in the synaptic cleft such as the removal and uptake of the photoreceptor transmitters.

4. *Postsynaptic membrane in HCs*: Background illumination may alter the properties of the postsynaptic receptors and/or other proteins in the HC membrane.

To determine at which of the above sites background-induced changes of HCRRT occur, the effects of the background illumination on the photoreceptors and bipolar cell responses were examined. Figure 5 shows voltage responses of a rod, a cone, a HC, and a hyperpolarizing bipolar cell (HBC) to 100 msec light steps in the absence and presence of background illumination. The HC increment responses, similar to those shown in Figure 2, was enhanced by background light. The amplitude of the increment responses of the rod, the cone, and the HBC, on the other hand, was reduced by the background light. This result indicates that the background-induced change in rise time, represented by the amplitude of the responses to short test flashes seen in HC, is not present in photoreceptors or bipolar cells. This is not a surprising conclusion when we examine the rise time of the photoreceptor, HC, and bipolar cell responses to longer test light steps. Figure 6 shows the voltage responses of a cone, a rod, a HC, and a HBC under dark-adapted condition to a 1 sec light step. The time to peak of the rod, the cone, and the HBC was 50–150 msec, whereas that of the HC was about 1.5 sec. The time to peak of rod_c—a small population of rods strongly coupled with cones (Wu and Yang, 1988)—and that of the depolarizing bipolar cells (DBC; Fig. 15) was also about 100–150 msec under dark-adapted condition, a 100 msec light step could evoke maximum responses in photoreceptors and bipolar cells but not in HCs. Background illumination shortened the time to peak of HC and thus enhanced the HC responses to 100 msec flashes. The rise time of the photoreceptors and bipolar cells was not significantly expedited by background illumination [10–25 msec changes, compared with the 1–3 sec changes in HCs (see Fig. 15, and also Baylor and Hodgkin (1974) and Wu (1987b)] and thus the resultant responses to 100 msec flashes reached the same level as the responses recorded in darkness (Fig. 5).

Results described above suggest that the background-induced changes in HCRRT is unlikely to be the result of changes of the presynaptic light responses in photoreceptors because the amplitude of the rod and cones increment responses to 100 msec flashes decrease in response to background illumination. Moreover, because the background-induced changes of responses rise time is not observed in either the HBCs or DBCs (Fig. 5, 15), it is unlikely that the changes in HCs are mediated by events occurring in the photoreceptor terminals or in the synaptic cleft. This is because bipolar cells and HCs in the tiger salamander retina share the same synapses in the rod and cone terminals and their dendrites are adjacent to each other in the diad or triad regions (Lasansky, 1973, 1978). Changes in photoreceptor terminals or in the synaptic cleft should have been recorded in both the bipolar cells and the HCs.

Background-induced modulation of HC responses depends on the rod, but not the HC, voltage

Our results in the previous section have suggested, by eliminating other cellular sites, that the background-induced changes of the HC response time course probably occur in the HC mem-

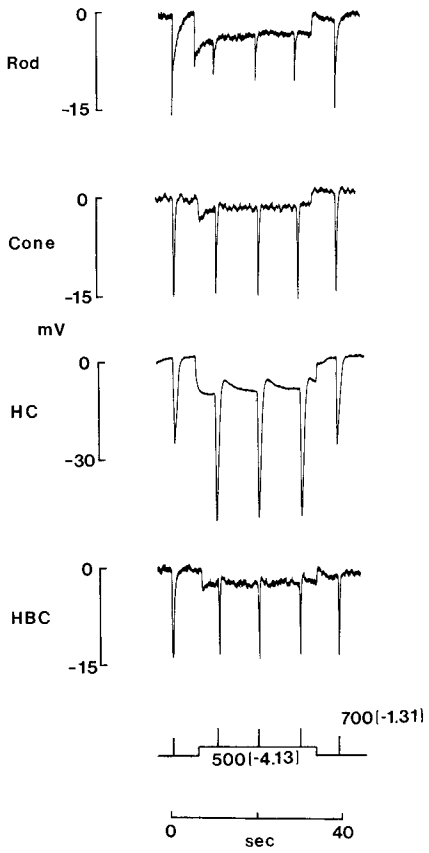

Figure 5. Voltage responses of a rod, a cone, a HC, and a HBC to 100 msec light steps (*upright bars* in *lower trace*: 700 nm, −1.31) in the absence and presence of background illumination (500 nm, −4.13). The resting potentials of the rod, the cone, the HC, and the HBC were −40, −41, −20, and −40 mV, respectively.

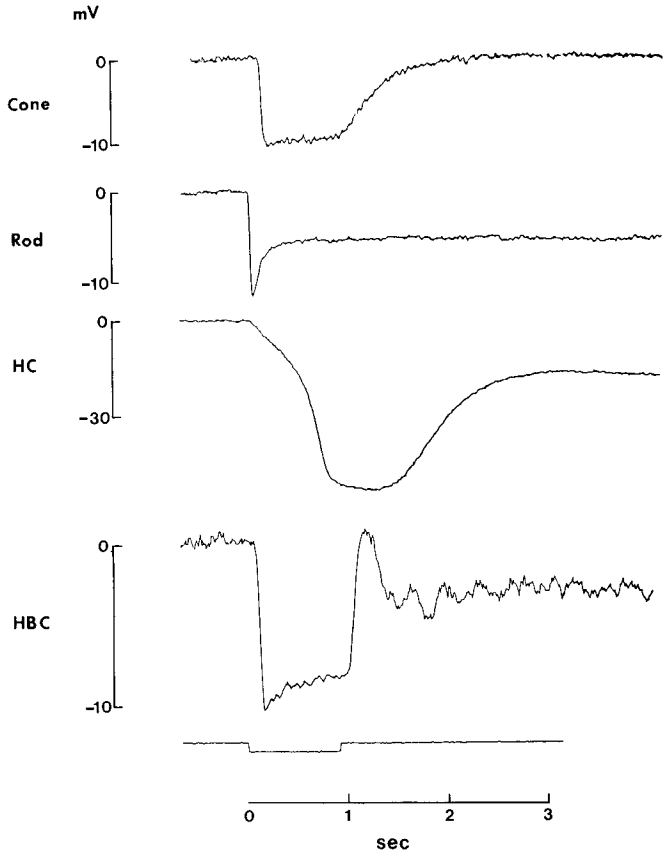

Figure 6. Voltage responses of a cone, a rod, a HC, and a HBC under dark-adapted condition to a 1 sec light step (520 nm, −3.35). The times-to-peak of the 4 cells were about 100 msec, 60 msec, 1.5 sec, and 150 msec, respectively, and the resting potentials were −38, −37, −17, and −40 mV, respectively.

brane. Although HC light responses are mediated by the reduction of transmitter flow from photoreceptors, some voltage-dependent currents in HC (e.g., Tachibana, 1983) may limit the rising rate of the HC light responses. Figure 7 shows the effect of depolarizing currents on the light responses of a HC in the presence of background illumination. The lowest trace ($I = 0.0$ nA) is the voltage response of a HC recorded in the presence of background illumination and the time to peak of this response is about 0.3 sec. Depolarizing current steps of various intensities (indicated by the numbers in nA on the left of each trace) was injected into the HC and the HC responses to the same test light in the presence of the same background light are shown in the upper traces. The amplitude of the responses became progressively larger for larger currents because the HC was depolarized further away from the peak light voltage. The slope of the rise of these responses, however, was approximately constant. This suggests that the HC response rise is probably independent of the HC voltage, and the background-induced modulation is probably not mediated by voltage-dependent currents in the HCs.

Figure 8 shows the effects of 500 and 700 nm background light on the HC responses to 100 msec test flashes. Background light of 700 nm/−4.89, 700 nm/−3.95, 500 nm/−8.14, and 500 nm/−7.14 resulted in a steady hyperpolarization in the HC for 3.5, 7, 1, and 3 mV, respectively (Fig. 8, *A–D*). The degree of change of response rise time, as indicated by the ratio of the resultant response (background response + increment response) to the dark response, was 1.27, 1.48, 1.30, and 1.45 for Figure 8, *A, B, C,* and *D,* respectively. It is clear from these data that the degree of change of HC response rise time does not correlate with the HC membrane potential, which is consistent with the conclusion drawn from the results shown in Figure 5; however, it correlates very well with the potential changes in rods. The 700/−4.89 and 500/−8.14 background lights (Fig. 8, *A, C*) hyperpolarize the rods to approximately the same voltage, and so do the 700/−3.95 and 500/−7.14 background lights (Fig. 8, *B, D*) (Wu and Yang, 1988). The degree of rise time changes in Figure 8*A* (1.27) is very close to that in *C* (1.30), and that in *B* (1.48) is very close to that in *D* (1.45). This correlation is further demonstrated in Figure 9, in which the sensitivity of the change of HCRRT is plotted against the wavelength of the background light. Data points (solid circles) were derived from the intensities of the background light required to elicit HC response of a given degree of change of HCRRT (represented by the ratio of the resultant response to dark response to 100 msec flashes, see Fig. 8). The solid curves are the spectral sensitivities of the rod and the cone. The data points agree very well with the rod spectral sensitivity, suggesting that the background-induced modulation of the HCRRT is probably mediated by changes of the rod voltage.

To examine further the role of rods in modulating the HC response, we studied the effects of bleaching light on the back-

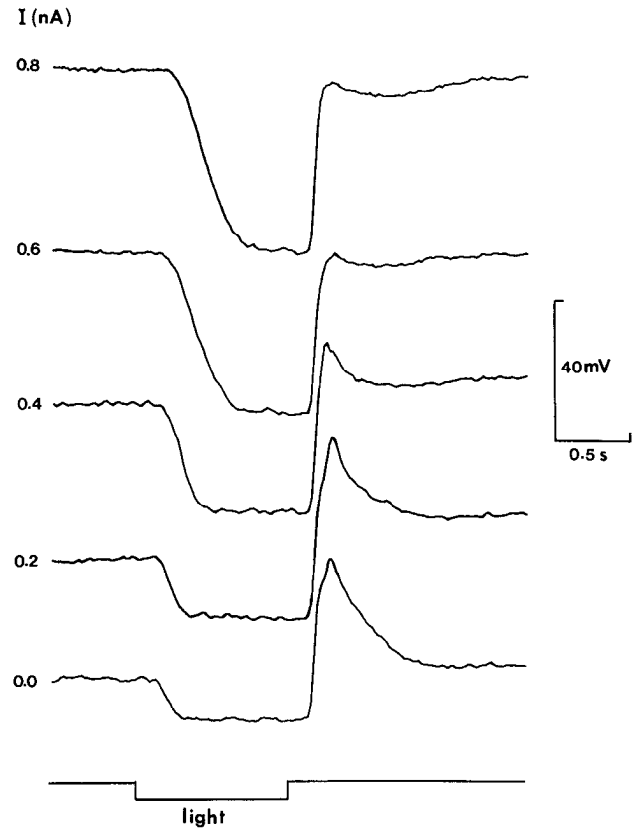

Figure 7. Effect of current injection on the HC responses to a 1 sec light step (520 nm, −1.3) in the presence of background illumination (500 nm, −2.3). Current steps (values given at the *left* of each trace) were passed into the HC through the recording electrode and a bridge circuit. The same light step was delivered to the cell when it was depolarized by the current at various levels. The voltage scale indicates the voltage of each trace but not the intertrace spaces. The ambient potential of the HC without current (0.0 nA) was about −72 mV.

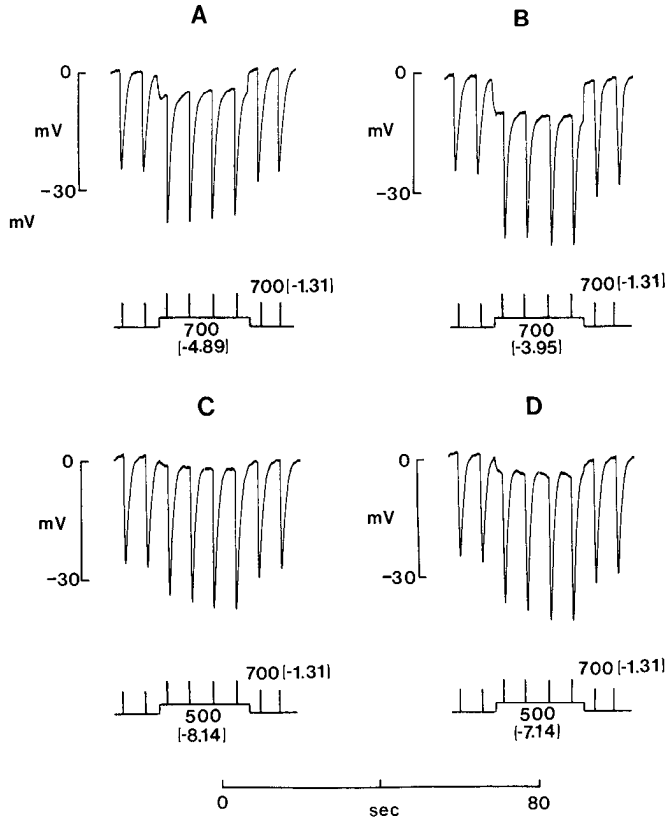

Figure 8. Effects of 500 and 700 nm background illumination on the HC responses to 100 msec test light steps (700 nm, −1.31). Backgrounds of 700 nm, −4.89; 700 nm, −3.95; 500 nm, −8.14; and 500 nm, −7.14 were used in *A, B, C,* and *D,* respectively. The experiments were performed under dark-adapted condition, and the HC resting potential was −20 mV.

ground-induced change of the HC responses. Figure 10 shows the effects of background illumination on the HC responses to 100 msec test flashes before and after bleaching. Under dark-adapted condition (Fig. 10*A*), a 500 nm/−4.13 background light caused a change in response rise time (represented by the ratio of resultant response to dark response) of 2.2. About 3 min after bleaching (Fig. 10*C*), when rods were still irresponsive to light (Wu, 1987b), the same background light caused no change in the response rise time (or enhancement of the HC response to 100 msec flashes). This result is again consistent with the notion that the background-induced modulation of the HC response rise time is mediated by changes of the rod voltage.

Background illumination primarily modulates postsynaptic receptors of cone transmitters in the HC membrane

Results described so far have suggested that the background-induced modulation of HCRRT is probably mediated by voltage-independent mechanisms in the HC membrane, and the modulation depends closely on the rod voltage. Since the HC light response results from the reduction of photoreceptor transmitters, it is reasonable to postulate that the rise of the HC light response is mediated by the dissociation of the photoreceptor transmitters and the postsynaptic receptors. In the tiger salamander retina, HCs receive inputs from both rods and cones (Hanani and Vallerga, 1980; Yang and Wu, 1989a). It is important to determine which population of the postsynaptic receptors in the HCs is modulatable by background illumination. Figure 11 shows the effect of background illumination on the spectral sensitivity of a dark-adapted HC. In darkness, the HC showed a spectral sensitivity which represented a mixture of rod and cone inputs (Wu and Yang, 1988; Yang and Wu, 1989a). In the presence of background illumination (500 nm/−3.12) that induced large changes in HCRRT (represented by the large enhancement of the HC response to 100 msec flashes; Fig. 2), the HC exhibited a spectral sensitivity that closely resembled that of the cones. This result suggests that the HC responses in the presence of background light is primarily mediated by cone inputs and the background-induced modulation of the HCRRT is probably acted mainly on the postsynaptic receptors to the cone transmitters.

Other evidence supporting this notion is given in Figure 12, which shows the effects of 700 nm background light on the HC responses to 500 nm test flashes. Similar to the results shown in Figures 2 and 3, where the wavelengths of the background and test lights were reversed, the HC responses to 100 msec test flashes were enhanced by background illumination. However, the HC responses during the first 50 sec after the termination of background light in Figure 12 were smaller than the responses before background was turned on. The HC responses after the termination of background light in Figures 2 and 3 were similar

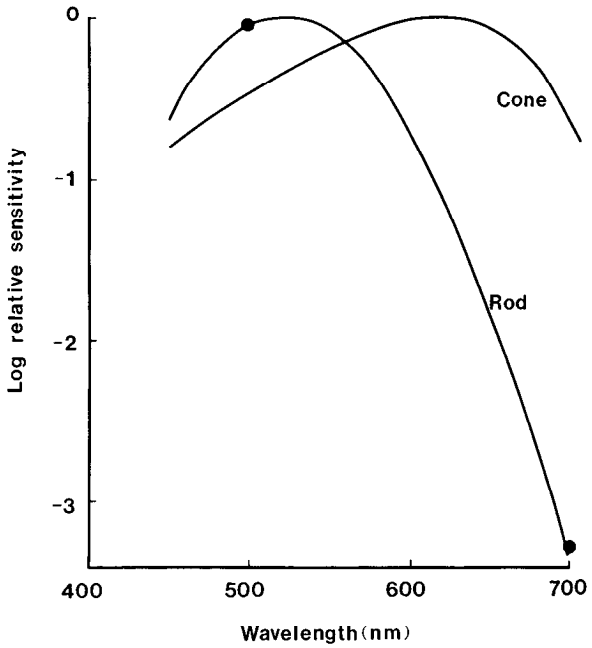

Figure 9. Spectral sensitivity of the change of HCRRT (*solid circles*) and the spectral sensitivity of the rod and the cone (*solid lines*). The *solid circles* were obtained by plotting the relative intensity of the *background light* of 2 wavelengths needed to produce a criterion degree of change in HC response amplitude to 100 msec light steps.

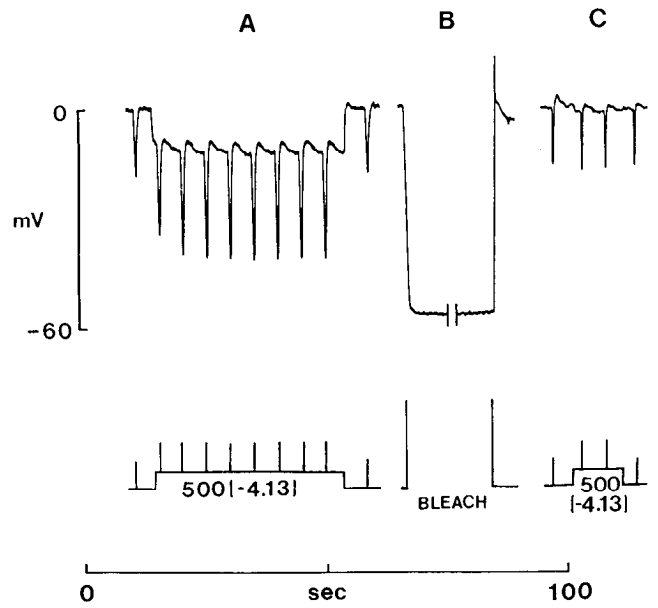

Figure 10. Effects of background illumination (500 nm/−4.13) on the HC responses to 100 msec light steps (700 mn/−1.31) before (*A*) and after (*C*) bleaching. The bleaching light (white light, about 43 $\mu W/\mu m^2$) was applied to the retina for about 1 min (*B*). The HC membrane potential returned to its dark-adapted level about 3 min after bleaching light was turned off (*C*).

to or larger than the responses before background light. Since the HC responses to 700 nm flashes consists of more cone input than those to 500 nm flashes, the greater reduction of response amplitude after background light, as shown in Figure 12, suggests that the background-induced changes in HCRRT (represented by the enhancement of responses to 100 msec flashes) is probably mediated by events mainly associated with the cone inputs.

Effects of pharmacological agents on the background-induced changes of the HCRRT

Results described in previous sections have suggested that background probably modulates the cone inputs in dark-adapted HCs and the modulation depends on the rod voltage. It is of great interest to examine what pharmacological agents, especially the putative transmitters and compounds released from the photoreceptors and HCs, affect this modulation. Therefore, we studied the effects of L-glutamate, L-aspartate, GABA, bicuculline, glycine, and strychnine on the HC responses. Moreover, various studies have demonstrated that cadaverine, putrescine, histidine, and melatonin are released from photoreceptors (Miller and Schwartz, 1983; Wiechmann, 1986) and that dopamine exerts modulatory action on HCs in the fish and turtle (Witkovsky and Stone, 1987; Yang et al., 1988b). We thus examined the effects of these compounds on the HC response time courses as well.

Table 1 summarizes the effects of the various pharmacological agents on the HCs in the dark-adapted tiger salamander retina. Some of these agents caused depolarization, some caused hyperpolarization, and some caused no change in the HC potential. None of these agents significantly affected the background-induced changes of the HC response rise time. The effect of GABA on the HC responses is given as an example in Figure 13. In normal Ringer's solution (Fig. 13*A*), background light expedited the HC rise time and the degree of this change, as represented by the ratio of the resultant response to the dark response, was 2.2. In the presence of 100 μM GABA (Fig. 13*B*), the membrane potential of the HC did not change, but the response became slower and thus the response to 100 msec flashes became smaller. Nevertheless, background light under this condition still ex-

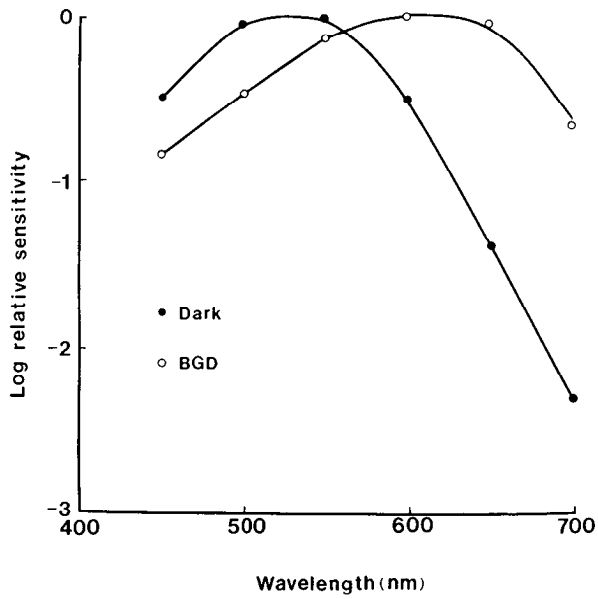

Figure 11. Effects of background illumination (500 nm, −3.12) on the spectral sensitivity of the dark-adapted HC. *Filled circles* represent the spectral sensitivity of the HC under dark-adapted condition and *open circles* represent the spectral sensitivity of the same cell in the presence of background illumination. The criterion voltage used to determine these spectral sensitivity curves was 2.5 mV.

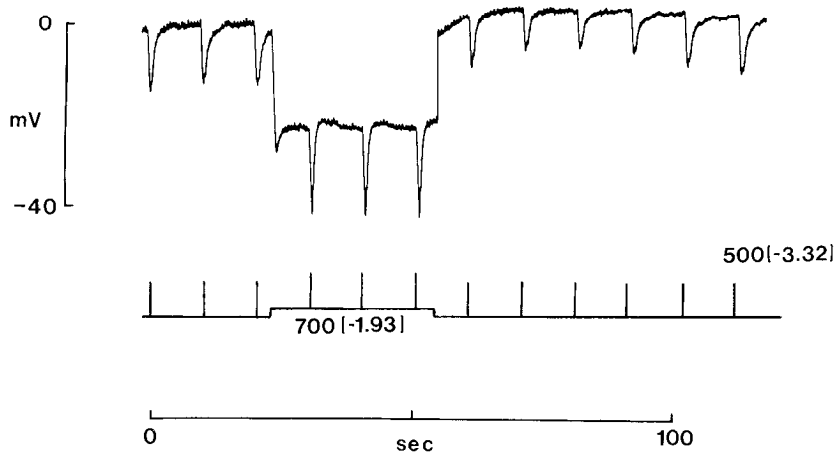

Figure 12. Effects of 700 nm background light (−1.93) on the HC responses to 500 nm short (100 msec) light steps (−3.32). The HC was initially dark-adapted, and the resting potential was about −21 mV.

pedited the HCRRT and the degree of this change is even larger (3.0). This result demonstrates that although GABA can affect the time course of HC response (this will be discussed in a later paper, and also see Witkovsky and Stone, 1987), it does not significantly affect the background-induced changes of HCRRT in the tiger salamander retina.

Effects of background illumination on the gain of the photoreceptor–HC synapses

We have shown in the previous sections that background illumination expedites the HCRRT and enhances the responses to short test flashes. Figure 14*A* shows the effect of background light on the HC responses to light steps of various durations. The ratio of the response amplitude in the presence of background to that in darkness (the relative gain) is plotted against the light duration in Figure 14*B*. Both the increment and the resultant responses to short light steps were greatly enhanced by background illumination, but those to long light steps were not much affected. The cutoff duration (T_c, defined as the longest duration of light step to which HC responses exhibit background-induced enhancement) for the HC, shown in Figure 14, was about 600 msec. T_c varied from HC to HC depending on the state of adaptation. The most dark-adapted HC exhibited a T_c around 800 msec and the partially light-adapted HC had T_c around 200 msec. This result indicates that the background-induced change in HCRRT can modify the relative gain of the photoreceptor–HC synapse, and the modification depends on the duration (or frequency) of the light stimuli.

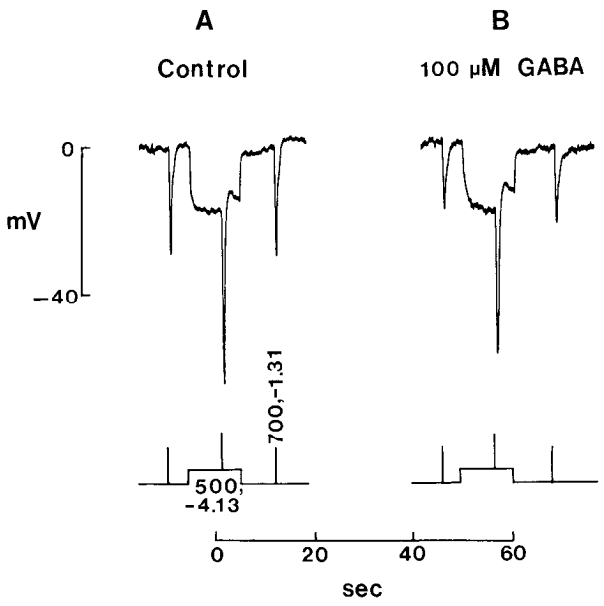

Figure 13. Effects of GABA on the background-induced changes of HCRRT. The same test light steps (700 nm, −1.31, 100 msec) and background light (500 nm, −4.13, 10 sec) were used to stimulate the HC in normal Ringer's solution (*A*) and in the presence of 100 μM GABA (*B*). GABA did not cause significant changes in the HC membrane potential, but it did slow the response rise time. GABA did not significantly affect the background-induced change in response time and amplitude.

Effects of background illumination on the response waveforms of the photoreceptors and second-order neurons

In the vertebrate retina, HCs exert inhibitory actions on cones and bipolar cells (Baylor et al., 1971; Naka, 1972; Toyoda and Tonosaki, 1978). Changes in HC responses lead to changes in responses of the cones and bipolar cells. Figure 15 shows the effects of background light on the response waveforms of the dark-adapted photoreceptors and second-order neurons in the tiger salamander retina. Figure 15*A* shows the voltage responses of a rod, a cone, a HC, a DBC, and a HBC to a 1 sec light step under dark-adapted condition; Figure 15*B* shows the voltage

Table 1. Effects of pharmacological agents on HCs

Test substance	HC membrane potential	Background-induced change in HC response
L-Glutamate (0.1–2 mM)	dep.	None
L-Aspartate (0.1–2 mM)	dep.	None
GABA (0.1–1 mM)	No change	None
Bicuculline (0.05–1 mM)	No change	None
Glycine (0.01–10 mM)	No change	None
Strychnine (0.05–1 mM)	No change	None
Cadaverine (0.1–1 mM)	dep.	None
Putresine (0.1–1 mM)	No change	None
N-Acetylhistidine (0.1–1 mM)	hyp.	None
Melatonin (0.1–1 mM)	hyp.	None
Dopamine (0.1–1 mM)	hyp.	None

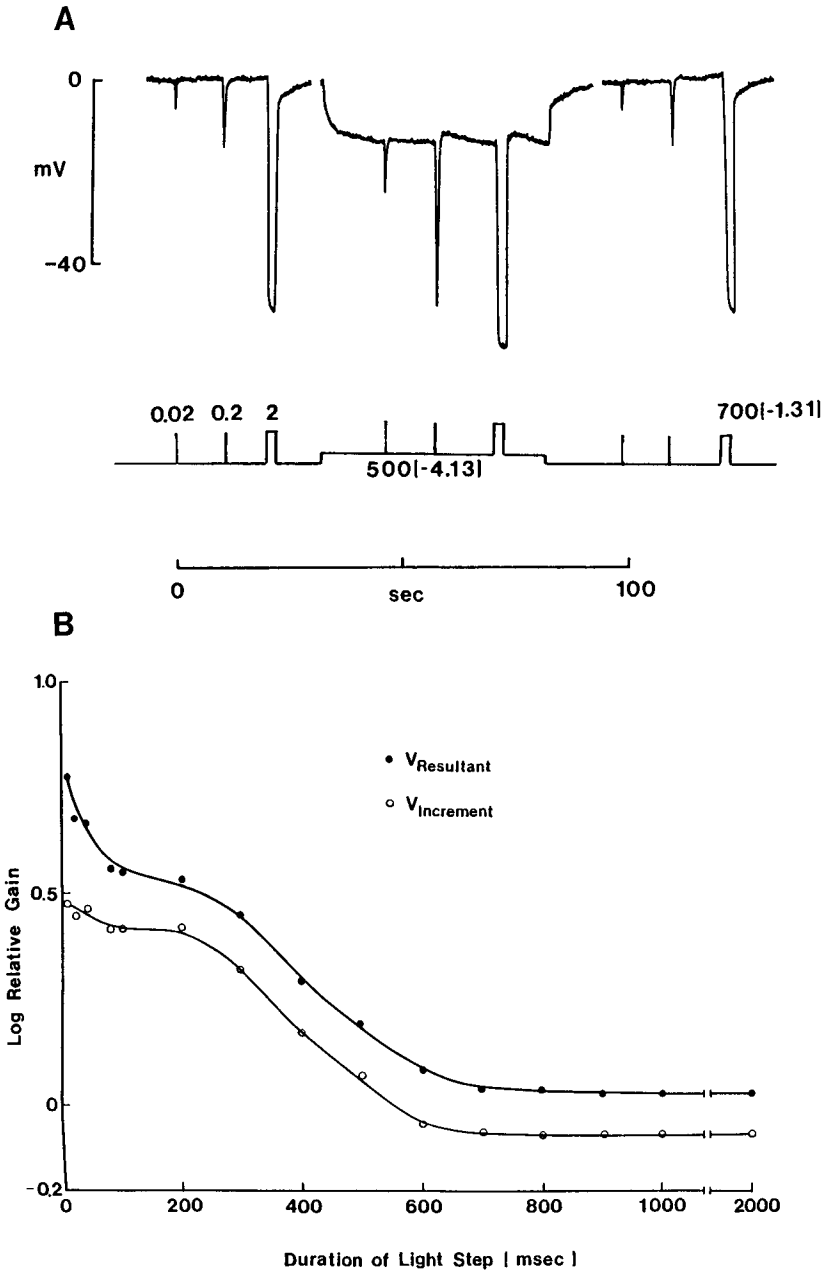

Figure 14. Effects of background illumination on the HC responses to light steps of various durations. *A*, HC responses to light steps 0.02, 0.2, and 2 sec (700 nm, 1.31) in duration in the absence and presence of background illumination (500 nm, −4.13). *B*, Relations of the relative gain (HC responses with background/HC responses without background, in log scale) and the duration of the light steps. *Open circles* give the relative gain of the increment responses, and filled circles give that of the resultant responses (increment responses + background responses).

responses of these cells to the same light steps in the presence of background illumination. Note that the HC response in Figure 15*B* was much larger than that in *A* because its rising phase was faster. The responses of the cone and bipolar cells in the 2 panels exhibited approximately the same amplitude but different waveforms. The responses are more transient (arrows) in Figure 15*B* than in *A*. It is possible that part of these transient responses in *B* is attributable to the faster and larger HC response which sags the cone and bipolar cell responses at a faster rate through inhibitory synapses (Baylor et al., 1971; Toyoda and Tonosaki, 1978; Attwell et al., 1983).

Discussion

Background-induced change of the HCRRT

In this paper, we have demonstrated that background illumination expedites the rise time course of the HC light response, and this change in response time course is more then 10 times the change in rise time occurring in cones and bipolar cells. The change correlates very well with the changes in rod voltage but not with changes in HC voltage. We postulate that the postsynaptic receptors to cone transmitters in the HC membrane are the modulation sites. This is reasonable because our experiments demonstrated that the background-induced modulation does not occur in photoreceptors or bipolar cells. The response rise times of these cells are much faster than that of the horizontal cells under dark-adapted condition, and they exhibit little change in response to background illumination (Fig. 15). Bipolar cells share the same photoreceptor synapses with the HC in this retina (Lasansky, 1973, 1978), and hence the absence of modulation in bipolar cells eliminates the presynaptic terminals and synaptic clefts as the modulation sites. Our experiments show that the HCRRT is independent of the HC voltage, and thus voltage-dependent ionic currents in the HC membrane are unlikely to limit the HC response rise time. This conclusion is

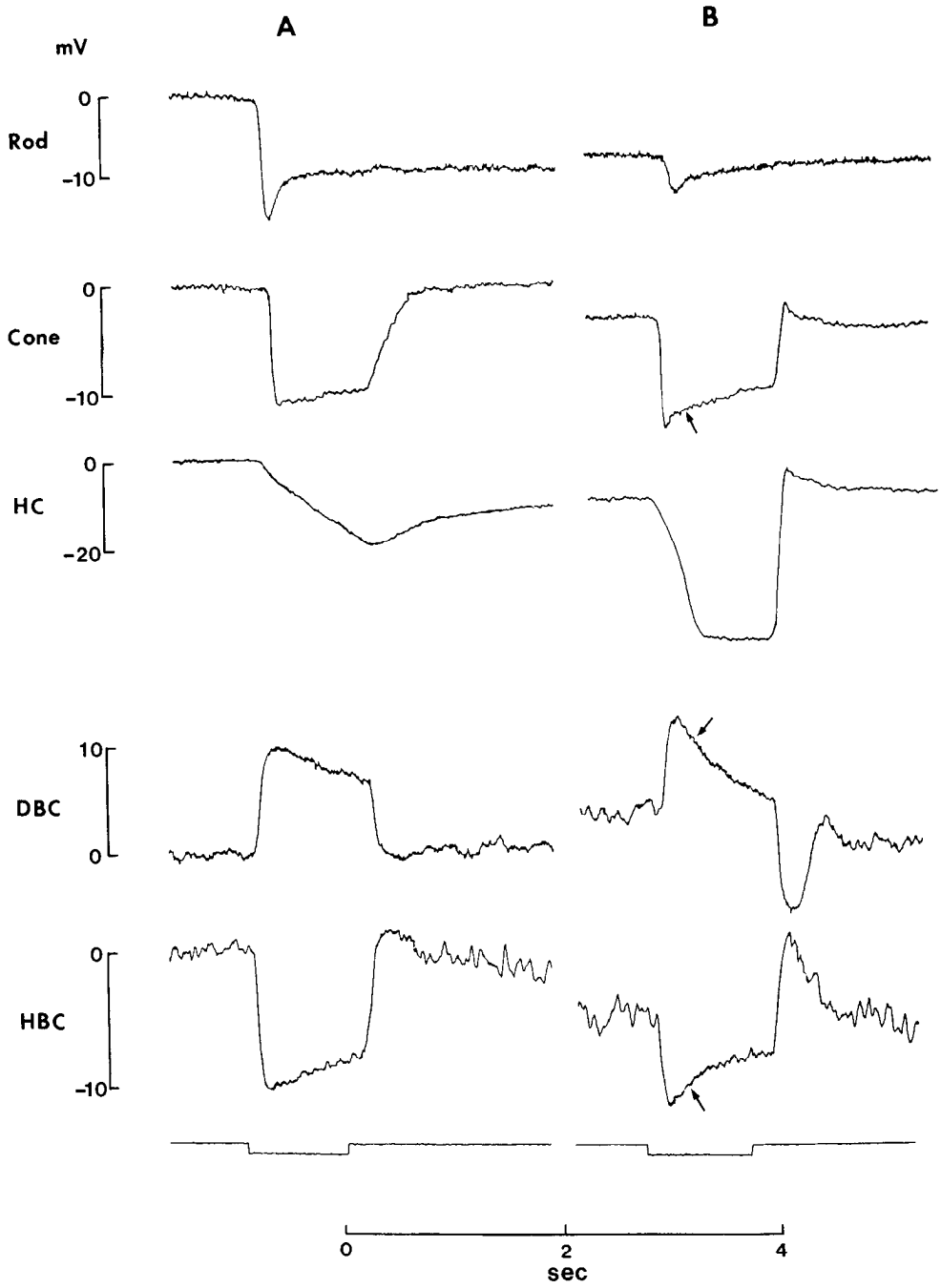

Figure 15. Voltage responses of a rod, a cone, a HC, a DBC, and a HBC under dark-adapted condition (*A*) and in the presence of background illumination (*B*). The test step used in both *A* and *B* was 700 nm, −1.35 and the background in *B* was 520 nm, −4.06. Note that the HC response to the 1 sec light step is larger in *B*, and the cone and bipolar cell responses are more transient (*arrows*) in *B*.

consistent with our previous study showing that the HCRRT under dark-adapted condition is much slower than that under light-adapted condition at the *same* resting potential (Wu, 1987b, figure 2). We have shown in this article that background light modulates the efficacy and kinetics of the transmitter-receptor complexes in the HCs. In the fish retina, it has been shown that dopamine, whose concentration is controlled by light, regulates the transmitter-gated conductances in the HC membrane (Knapp and Dowling, 1987). Similar mechanisms may be responsible for the background-induced change in the HC response time in the tiger salamander retina.

In the vertebrate retina, the electrotonic coupling between HCs is modulatable by dopamine and possibly other agents (Lasater and Dowling, 1985; Witkovsky et al., 1987; Tornqvist et al., 1988). However, HC coupling is unlikely to play a significant role in mediating the background-induced change in the HC responses. In our experiments, we used whole-field illumination, which results in no net lateral current flowing across electrical synapses between HCs. Based on the dimensions of the HC dendrites and somas in the tiger salamander retina (Lasansky, 1973), the cable characteristics of HCs are unlikely to be responsible for a time course difference on the order of seconds as we described in this article (Jack et al., 1975). Moreover, dopamine and related agents caused no significant change in the HC response time course.

Another factor that may affect the HCRRT is the light-dependent change of potassium concentration in the extracellular space [K_o] (Oakley et al., 1979). Instead of the dissociation of

the transmitter-receptors complexes in the HC membrane, the rate-limiting factor of the rise of the HC light responses may be limited by the downward drift of the potassium equilibrium potential (E_K) in response to lowering [K_o]. However, assuming all neurons in the outer retina have similar permeability to K^+, this factor is unlikely to be significant in mediating the HCRRT. This is because bipolar cells, whose dendrites and somas are adjacent to the HCs, do not exhibit either the slow response rise time in darkness or the background-induced change in their responses.

In the retina, horizontal cells make inhibitory feedback synapses onto cones (Baylor et al., 1971), and modification of this negative-feedback synapse may result in changes in the kinetics and gain of the forward synapses from photoreceptors to second-order cells (Marmarelis and Naka, 1973). In the fish and tiger salamander retinas, GABA is believed to be the primary neurotransmitter used by the HCs in the feedback synapse (Lam and Steinman, 1971; Tachibana and Kaneko, 1984; Wu, 1986). Our experiments show that neither GABA nor bicuculline blocks the background-induced changes in HCRRT (Fig. 11, Table 1). These results suggest that *if* GABA is the feedback transmitter used by the HCs in the tiger salamander retina, then the feedback synapses probably play little role in mediating the background-induced changes of the HCRRT.

Our failure in identifying the blocking agent for the background-induced change of the HCRRT prevents us from suggesting what modulator(s) is (are) involved in modulating the inactivation of the transmitter–receptor complexes in the cone–HC synapse. None of the substances listed in Table 1 significantly affected the background-induced *changes* of the HCRRT, although some of these substances altered the membrane potential and/or the response amplitude of the HCs. Although the identity of the modulator involved is unknown, our experiments provide important clues on the mechanisms of the background-induced modulation. Figure 3 and 4 show that the time course of inactivation of the modulators induced by background illumination is only about 2 sec. This short time course implies that complex biochemical processes may not be involved in the background-induced modulation of the cone–HC synapse. In a later article, we will present data showing that a long-term (time course, 5–10 min) modulation of the photoreceptor–HC synapse also exists in the tiger salamander retina (Yang and Wu, 1989b).

Rod–cone interaction in the tiger salamander retina

Recent psychophysical studies have described a suppressive rod–cone interaction, and it is attributed to a tonic, inhibitory influence of dark-adapted rods on cone-mediated pathways (Alexander and Fishman, 1984; Arden and Frumkes, 1986). Physiologically, Hassin and Witkovsky (1983) have demonstrated that rod-stimulating lights enhance the cone-mediated HC responses in the *Xenopus* retina, and Frumkes and Eysteinsson (1987) have shown that backgrounds enhance the peak-to-peak amplitude of the responses to sinusoidal flickers in cones, HCs, and bipolar cells of the mudpuppy and *Xenopus*. Our results in the tiger salamander retina support the notion that backgrounds modulate the cone-mediated HC responses through modulators associated with the rods. However, we have shown that the mechanisms of the suppressive rod–cone interaction in the tiger salamander retina differ from those in the *Xenopus* and mudpuppy in at least 2 respects: (1) We have shown that the background-induced modulation of the cone-mediated response is primarily observed in HCs but not in cones or bipolar cells, and (2) the background-induced modulation of the cone–HC synapse is primarily in the time domain. Background lights expedite the HCRRT for about 0.5–1 sec but do not affect the peak amplitude of the HC response significantly. These differences between our results and those described by Hassin and Witkovsky, and Frumkes and Eysteinsson, may be due to species differences. It is worth noticing that background illumination also expedites the response rise time in cones and bipolar cells in the tiger salamander retina (Fig. 15), but this change is no more than 50 msec, and thus the amplitudes of the cone and bipolar cell responses to 100 msec test flashes are affected little.

Functional implications of the response rise time in neurons in the outer retina

In the tiger salamander retina, we have shown that the response rise time of the HCs is much slower than that of the photoreceptors or bipolar cells and that background illumination expedites the HC response much more than expediting the photoreceptors or bipolar cell responses. These results suggest that the photoreceptor–bipolar cell (R-BC) synapse and the photoreceptor–horizontal cell (R-HC) synapse have different frequency responses, and the R-HC synapse is more plastic, with a frequency response modulatable by light (Fig. 14). Bipolar cells are the output neurons of the outer retina, and they receive central input from the photoreceptors and antagonistic lateral input from HCs whose responses are mediated by the R-HC synapse. The difference in frequency response and plasticity between the R-BC and R-HC synapses have several consequences in bipolar cell signaling.

In the time domain, the HC responses mediate a delayed sign-inventing signal in bipolar cells and make the bipolar cell response more transient (Werblin, 1977). The delay between the HC signal and the photoreceptor signal in bipolar cells is partially mediated by the delay of signal passing through the HC-BC synapse and partially by the difference in response rise time between the HCs and bipolar cells. Our results in this article show that background illumination expedites the HCRRT and thus it shortens the delay between the HC input and the photoreceptor input in bipolar cells. This makes the bipolar cell responses more transient in the presence of background illumination (Fig. 15). Even in the presence of background, the time-to-peak of the HC responses is still about 200 msec longer than that of the bipolar cell responses. Consequently, the amplitude of bipolar cell peak responses (or responses to short light steps) is not significantly affected by background illumination (Fig. 15). The amplitude of the bipolar cell plateau responses, on the other hand, is reduced in a faster rate with background light than in darkness, because the HC responses reach their peaks at a faster rate. In other words, the bipolar cell responses to short (or high-frequency) light stimuli can be transmitted to the inner retina with little inhibition from the HCs. The amplitudes of bipolar cell responses to long (or low-frequency) light stimuli are reduced by the HC inhibitory signal whose time course is adjustable by background illumination: background speeds up the HC signal and makes the inhibition stronger for shorter (or higher-frequency) light stimuli. Based on this view, the HC responses are probably involved in reducing the sustained (or low-frequency) signals in bipolar cells. It is probably economical, if not advantageous, for the retina to transmit only information on the changes of images, than to constantly transmit the same information on the unchanged image (Srinivasan et al., 1982; Attwell, 1986). In the presence of background light,

the need for reducing sustained information is probably even more critical than under dark-adapted condition because background light sets up a sustained signal level itself and *changes* of signals about this sustained level become more important (Srinivasan et al., 1982).

In the spatial domain, HC responses give rise to the antagonistic surround responses in cones and bipolar cells (Werblin and Dowling, 1969; Baylor et al., 1971; Naka, 1972). The spatial frequency sensitivity of bipolar cells to visual images can be obtained as the Fourier transform of the center–surround receptive field of the cells (Campbell and Robson, 1968). Horizontal cells average the light intensity over a much larger area than do the bipolar cell dendrites and this results in a fall of the bipolar cell spatial frequency sensitivity curve at low frequencies (Attwell and Wilson, 1983). Because of the slow rise time and the modulability of the HC responses, the antagonistic surround responses and the spatial frequency sensitivity of the bipolar cells becomes duration (or temporal frequency) dependent. For short (or high temporal frequency) light stimuli, the surround responses in bipolar cells are small, which may be partially responsible for the disappearance of the surround responses of retinal ganglion cells following prolonged dark adaptation (Barlow et al., 1957). The spatial frequency sensitivity of bipolar cells does not fall off significantly at low frequencies, resulting in a lower sensitivity to edges in the visual image (Attwell and Wilson, 1983). For long (or low temporal frequency) light stimuli, the bipolar cell surround responses are larger and the spatial frequency sensitivity of bipolar cells falls off sharply at low frequencies, resulting in a higher sensitivity to edges in the visual image. Background illumination expedites the HC rise time and therefore pushes the capability for higher spatial resolution to a lower temporal level (shorter stimulus duration, higher stimulus frequency) in bipolar cells.

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