LETTER TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in THE JOURNAL OF GENERAL PHYSIOLOGY are invited. Receipt of such letters will be acknowledged, and those containing pertinent scientific comments and scientific criticisms will be published.]

Antagonistic Process as Source of Visible-Light Suppression of Afterpotential in *Limulus* UV Photoreceptors

Dear Sir:

The relation between the visual pigment cascade and the membrane potential changes which result from the absorption of photons in photoreceptors is still unclear. Nolte and Brown (1), working on the UV cells of the median eye of *Limulus*, have recently established an interesting correlation between the induction and suppression by light of a prolonged depolarizing afterpotential (PDA) and the hypothetical photoconversion of the visual pigment from the primary state (VP360) to a metastable photoproduct (M480) and the reverse photoconversion, respectively. However, they offered a tentative model relating the depolarization directly to the presence of M480 or dark conversion of M480 into VP360. We present evidence suggesting that (*a*) no pigment change takes place during the dark decline of the PDA, and (*b*) the pigment does not return in the dark to its original state at least for hours at room temperature. (We assume, as do Nolte and Brown, that this cell contains only one visual pigment. No cells are known to contain more than one.)

The observations (1, 2) on which Nolte and Brown based their model relate to the very slow repolarization in the dark of a UV cell depolarized by strong UV stimulation (*see* trace A of Fig. 1). This PDA can be rapidly suppressed by visible light (as in trace D of Fig. 1). The model suggests that the dark decay and the suppression by light of the PDA are due to the return of the pigment to the VP360 state, by slow conversion in the dark, or by photoregeneration.

If the dark decay of the PDA were directly linked to the return of the pigment to its primary state, however, one would expect that further UV stimulation of a cell in which a maximal PDA has declined to base line would again induce a PDA. This turns out not to be the case.

Trace A of Fig. 1 (*see* reference 3 for Methods) shows the intracellular response to UV light (~360 nm) of a cell which had been exposed 5 min earlier to strong visible light (~550 nm), and resembles the third trace of Fig. 1 of reference (1). Trace B

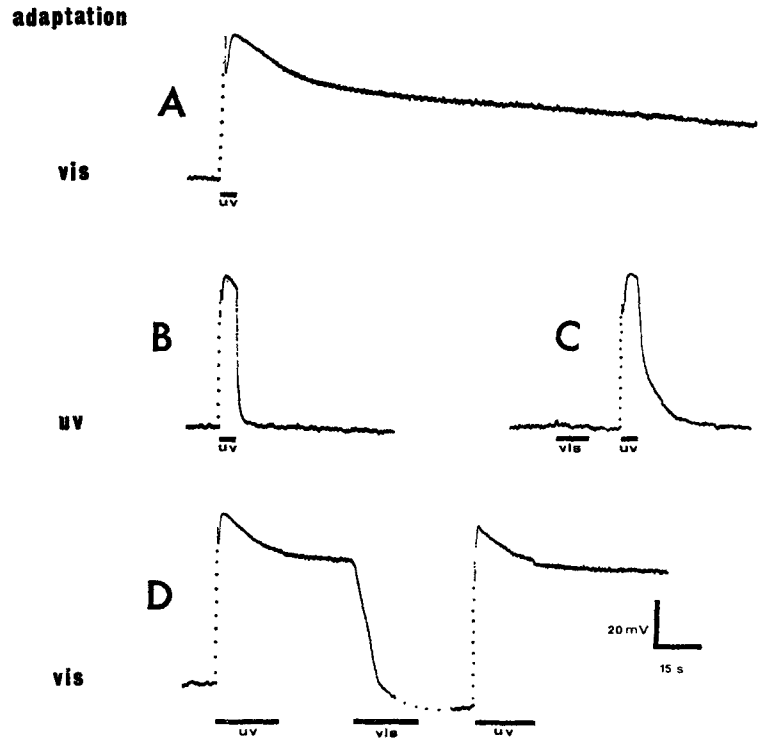

FIGURE 1 Dependence of PDA on state of adaptation. (A) The response to strong UV light of a cell adapted five min earlier to strong visible light (~550 nm). A nearly saturated PDA is induced. (B) The response of the cell to the same stimulus after a full PDA had declined to base line. No PDA is induced. (C) The responses of the cell, after a PDA had declined, to a strong visible light and a closely following UV stimulus. Little, PDA is induced. Compare the response to the same UV stimulus in trace A which differs only in the dark interval between the visible light (response not shown in trace A) and the UV light. (D) The response to a succession of UV and visible lights, as indicated, of a cell adapted 3 min earlier to a visible light. Compare the response to a similar succession of stimuli in trace C: the stimuli are of longer duration here, but the significant difference is in the dark interval between the first UV light (response not shown in trace C) and the visible light. In trace C this interval was long enough for the PDA to decline. The bars give the time and potential calibrations for all traces. All stimuli were saturating, or nearly so, both in terms of the responses they induced, and in terms of their adapting effects on the cell, that is, increasing the stimuli further had little effect on the responses either to these stimuli or to the following stimuli. Portions of the traces missing either because of rapid potential changes or because of film frame switching have been filled in with dots to guide the eye.

shows the response to the same UV stimulus 20 min later, after the PDA seen in trace A had declined to base line. The response is *not* followed by any PDA. Further waiting in the dark, for up to several hours, does not change the response in any way. Thus a UV stimulus induces a PDA only in a cell adapted to visible light.

This directly indicates that the cell does *not* return to its initial state during the

decline of the PDA, so that there are at least two cell states thermally stable for times long compared with those of the measurements, and the PDA in particular. The cell can be returned to its initial state only by visible light (trace D).

Since a PDA can immediately be reinduced (trace D), with the same spectral and intensity requirements as originally, this "disinhibition" presumably involves return of the *pigment* to its original state. However, Fig. 2 shows that stimuli at two different wavelengths which depress (partially suppress) the PDA to the same extent (trace pair A) also disinhibit the cell to the same extent (trace pair B), that is, equal PDAs result from equal following UV stimuli. This strongly suggests that the disinhibition arises from the same pigment transition as the depression, that is, the M480 → VP360 transition, as shown by Nolte and Brown. Furthermore, Fig. 2 shows that the equality of disinhibition is preserved if the stimuli are presented after rather than during dark decay of the PDA (trace pair C). Thus the pigment apparently does not change its state during the dark decay of the PDA and remains in the M480 state until photo-returned to the VP360 state. Unless there is a state change which does not manifest itself in a change either of the absorption spectrum or of the inhibitory contribution of the M480 → VP360 transition, the PDA must thus be an extrapigmental process,

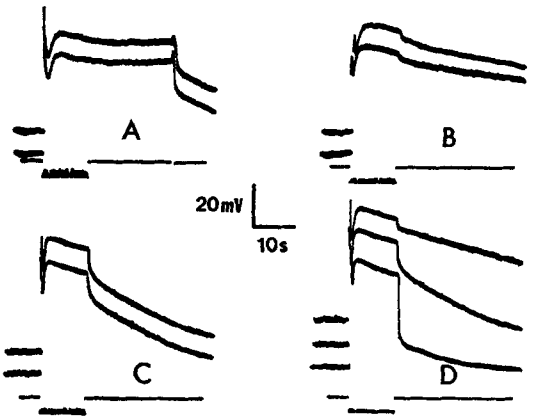

FIGURE 2 Dependence of PDA depression and cell "disinhibition" on wavelength. Color filters used were Balzers K2 and K4 broad-band interference filters (Balzers Aktiengesellschaft, Fürstentum, Liechtenstein) with transmission peaks at 455 nm and 555 nm, respectively. In each trace set, traces are displaced vertically to avoid overlap. Calibration bars apply to all traces. Trace pair A: identical PDAs depressed by 1 s of K4 light (upper trace) and 333 ms of K2 light (lower trace). Trace pair B: the responses of the cell to identical (relatively weak) UV stimuli, after decline of the residual PDAs of the two traces of trace pair A, respectively. Trace pair C: preceding the traces shown, the cell had been exposed, after decay of a PDA, to 150 ms of K4 light (upper trace) and 50 ms of K2 light (lower trace). 5 min later, UV stimuli were presented, giving the responses shown. Trace set D: as in trace pair C, but showing the effects of changing the duration of the K2 light from 0 (bottom trace) to 50 ms (middle trace) to 80 ms (top trace). The equality of the pairs of PDAs in trace pairs A, B, and C shows that (with an accuracy of better than ±10%, as judged by trace set D) the ratios of sensitivities at the chosen wavelengths for PDA depression, and for cell "disinhibition" during and after the PDA, are all the same.

and the suppression of the PDA must involve another, independent and antagonistic, extrapigmental process. The exciting process is activated by the VP360 → M480 transition and the suppression process by the reverse transition; both transitions can be induced only by light.

The suppressing process is also effective when induced after decay of a PDA. When the visible stimulus is presented to the cell after decay of a PDA, it is followed by a period during which little or no PDA can be induced (trace C of Fig. 1). This inhibition decays slowly in the dark, that is, larger intervals between the visible stimulation and the UV stimulation result in longer PDAs; sufficiently long intervals (greater than 5 min in this cell) result in full PDAs. The interval required varies from cell to cell and is generally shorter than the PDA. If the visible light is presented during a PDA (trace D of Fig. 1) or to a visible-adapted cell (not illustrated) no inhibition period results.

The fact that visible light does not induce a response even in a UV-adapted cell suggests that excitation of the UV-adapted state of the pigment (M480) does not induce a substantial voltage response. The fact that a UV light does induce a response even in a UV-adapted cell suggests therefore that UV adaptation leaves an appreciable fraction of the pigment in the second, or response-inducing, state (VP360). The PDA does not appear in UV stimulation of a UV-adapted cell because equal amounts of pigment are transferred in both directions in this case (as in any case of stimulation by the same wavelength as the adaptation) and the PDA induction and suppression cancel; the existence of a response implies that the two antagonistic processes take time to cancel each other, so that an induction effect is present during, but not after, the stimulus.

We note that the phenomena described above are closely parallel to those observed by us in the barnacle (*Balanus*) (3). However, the PDA in the barnacle is induced by *red* light; the bulk of the suppression by blue light follows *cessation* of the light; and blue light alone gives an appreciable depolarizing response. In the barnacle, we have also examined the early receptor potential (4) as a direct manifestation of pigment transitions (5). We found that the pigment has two thermally stable states in addition to a number of unstable states, but we observed *no* transitions with time constants in the range of the decay times of the PDA and of its inhibition. We concluded that the processes responsible for the PDA and for its suppression or inhibition are in some sense separated from the pigment. Accordingly, we developed in (3) a detailed model based on antagonistic "excitor" and "inhibitor" processes activated in proportion to pigment transitions. These processes have long but finite lifetimes in isolation and mutually neutralize rapidly but not instantaneously. This model accounts for all the various phenomena described above for *Limulus* as well as for *Balanus*.

The fact that these phenomena appear in such unrelated animals as *Limulus* (Arachnida) and *Balanus* (Crustacea) suggests that there may be antagonistic components of the coupling mechanisms in all photoreceptors, at least of invertebrates. There are other species (vertebrate as well as invertebrate) in which a second visual pigment state is known to exist with a lifetime sufficiently long and an absorption spectrum sufficiently different from that of the primary state (6) as to make it possible to check the presence of the PDA-related phenomena in these species.

We are very grateful to Prof. R. Werman for a critical reading of the manuscript.
The work was supported in part by grants from the Central Research Fund of the Hebrew University and from the Israel Academy of Science and the Humanities.

Received for publication 10 April 1973.

B. MINKE
S. HOCHSTEIN
P. HILLMAN
The Institute of Life Sciences
The Hebrew University of Jerusalem
Jerusalem, Israel

REFERENCES

1. NOLTE, J., and J. E. BROWN. 1972. Ultraviolet-induced sensitivity to visible light in ultraviolet receptors of *Limulus*. *J. Gen. Physiol.* **59**:186.
2. NOLTE, J., and J. E. BROWN. 1972. Electrophysiological properties of cells in the median ocellus of *Limulus*. *J. Gen. Physiol.* **59**:167.
3. HOCHSTEIN, S., B. MINKE, and P. HILLMAN. 1973. Antagonistic components of the late receptor potential arising from different stages of the pigment process. *J. Gen. Physiol.* **62**:105.
4. HILLMAN, P., F. A. DODGE, S. HOCHSTEIN, B. W. KNIGHT, and B. MINKE. 1973. Rapid dark recovery of the invertebrate early receptor potential. *J. Gen. Physiol.* **62**:77.
5. CONE, R. A. 1967. Early receptor potential: photoreversible charge displacement in rhodopsin. *Science* (*Wash. D.C.*). **155**:1128.
6. Handbook of Sensory Physiology. Vol. VII/1, Photochemistry of Vision, H. J. A. Dartnall, editor. Springer-Verlag Gmbtt., Berlin, 1972.