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Biophysics of Structure and Mechanism

V. Discussion* of Selected Topics about the Transduction Mechanism in Photoreceptors

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I. Intracellular Non-Localities (Cooperative Effects) in Photoreceptor Processes

A number of different observations suggest that the influence of the absorption of a photon in a single rhodopsin molecule has consequences which spread to at least neighboring areas of the photoreceptor. Although more than one mechanism is clearly involved, it was thought worth-while to summarize the various observations of "non-locality" in order to see what can be said about the mechanisms and their relationship to presently hypothesized receptor processes. Cone (Bochum Symposium, 1972) was the first to review *excitation* non-localities, in the context of an "internal transmitter" model.

A. Vertebrates (Fig. A)

1. Excitation: (a) In vertebrate rods, pigment molecules are located in discs isolated from the plasma membrane. (b) Far more than one channel is closed by single-photon absorption.

2. Adaptation: A definite sensitivity reduction outlives the conductance change following weak stimuli [Kleinschmidt and Dowling: J. Gen. Physiol. **66,** 617 (1975)]. It is not clear if it does so for very weak lights – if it does, an adaptation spread beyond a single disc is called for.

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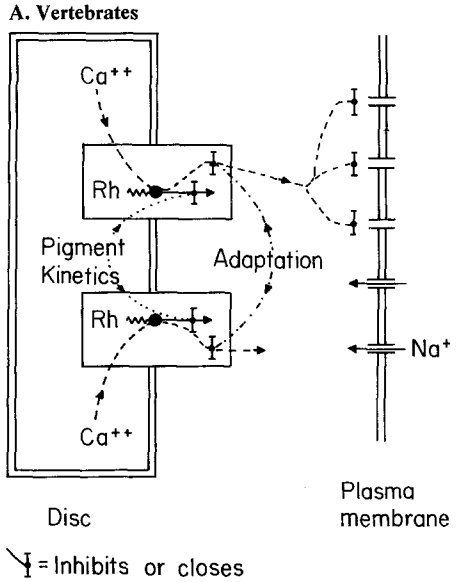
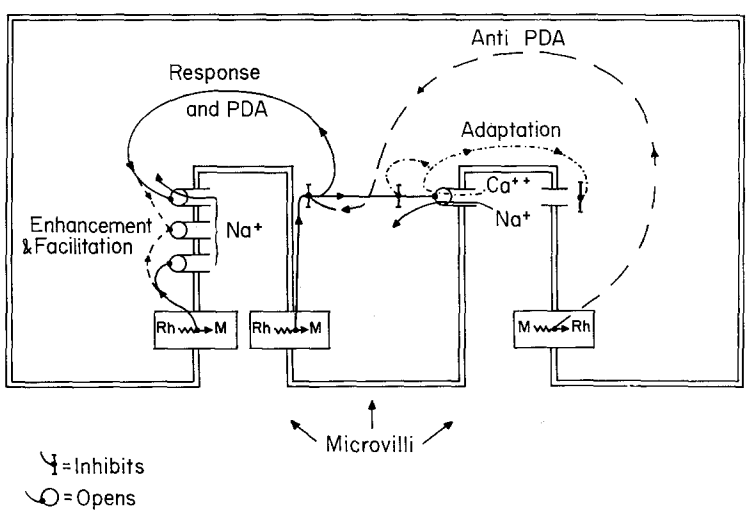


Fig. 1. A. The non-localities in the vertebrate photoreceptor (rod): Spread of excitation (blocking of plasma membrane channels); mutually inhibitory influence of pigment kinetics; and possibly spread of adaptation (perhaps to other discs). **B.** The non-localities in the invertebrate photoreceptor: Spread of excitation (opening of membrane channels); enhancement and facilitation of excitation; spread of adaptation; and spread of anti-PDA (or PDA)



3. Pigment Effects: Donner and Hemilä [Vision Res. **15**, 985 (1975)] showed that the pigment kinetics depend strongly on bleaching amount.

4. Mechanisms: All excitation-spread data appear consistent with the Ca^{++}-transmitter hypothesis. No adaptation-spread mechanism is known. Pigment-kinetics spread: Ca^{++} does affect pigment kinetics [Bäckstrom et al., Physica Norwegica **7**, 177 (1974)], but the effects appear to be in the wrong direction. Protons may be candidates.

B. Invertebrates (Fig. 1B)

1. Excitation: (a) Single-photon response in *Limulus* is so large that many channels, and in fact more than one microvillus, must be involved (Dodge, private communication). (b) The stimulus-response curve becomes sub-linear for flashes affecting only a very small fraction of the pigment molecules (this could be an adaptation spread). (c) Fein and Charlton (private communication) report a supra-linear portion of the response-stimulus (R-S)-curve in *Limulus* (Stieve: The supra-linear effect can be elicited by low external calcium). (d) Hanani and Hillman [J. Gen. Physiol. **67,** 235 (1976)] report a sensitivity enhancement, following relatively weak stimuli, which outlives the conductance change, in barnacle. Note that Hagins et al. [Nature (Lond.) **194,** 844 (1967)] and Fein and Charlton (private communication) report that the excitation does *not* spread throughout the cell.

2. Adaptation: A clear sensitivity reduction outlives the conductance change in many cells, even for stimuli affecting only a small fraction of the pigment molecules. This effect does *not* spread throughout the cell (Hagins et al., 1967).

3. PDA Affects: Hillman et al. [J. Gen. Physiol. **68,** 227 (1976)] have shown that the PDA and anti-PDA (see III.) interact mutually at a distance, that the PDA R-S-curve is supra-linear, and that even the spectator molecules may play a role. An enhancement of PDA sensitivity outlives the PDA conductance change. This facilitation may be related to the PDA supra-linearity and to the facilitation of the normal response.

4. Mechanisms: The adaptation spread may be due to Ca^{++} diffusion [Fein and Lisman, Science **187,** 1094 (1975)]. The excitation and PDA spread mechanisms are unknown and may be related.

A separate mini-symposium on bistable pigments and PDA phenomena was summarized to the meeting as follows:

II. Bistable Pigments

The following appear as acceptable generalizations *at the moment.*

1. No invertebrate "rhodopsins" go to dissociation after light absorption, and all are "bi-stable".

2. Rhodopsin: The chromophore is 11-cis (which is directly checked only for Ascalaphus – Hamdorf et al., Bochum Symposium, 1972); it is thermally stable; photon absorption gives a physiological response (conductance change) and a negative (intracellular) early receptor potential (ERP).

3. Metarhodopsin: Lifetime > 1 h; photon absorption gives no direct conductance change (possibly excepting scallop) but cancels or prevents PDA and gives a positive ERP.

4. The relative and absolute positions of the rhodopsin and metarhodopsin spectra vary all over the place. (Part of the metarhodopsin variation may be due to its possibly being in its acid form at physiological pH in some animals and in its alkaline form in others – Hamdorf).

5. There appears to be an incomplete correlation between spectrophotometric, ERP and LRP (late receptor potential) observations in some preparations (crayfish, barnacle, *Limulus*), which may suggest either that there is more than one pigment in these cells or that a single pigment sits in more than one type of membrane site.

6. Reduction of rhodopsin content by Vitamin A deprivation or by adaptation reduces cell sensitivity roughly proportionally, with possible exceptions (barnacle, crayfish, *Limulus*?).

III. The Basic PDA Phenomenology

1. The PDA (prolonged depolarizing afterpotential) appears in a wide variety of preparations, in fact in every invertebrate in which it has been sought (arthropods and crustacea) but in no vertebrate. In the single invertebrate preparation (scallop) in which the response is hyperpolarizing, a prolonged hyperpolarizing afterpotential (PHA) is seen (Gorman and Cornwall, private communication).

2. The PDA depends on the *net* transfer of pigment from the rhodopsin (Rh) to the metarhodopsin (M) state; pigment molecules which make "round trips" do not contribute (Fig. 2A). The PDA lasts seconds, minutes, or hours in different preparations. The decline of the PDA does *not* depend on the return of the pigment to the Rh state.

3. The dependence of the PDA amplitude on amount of pigment transferred is initially supra-linear, indicating a cooperative effect [Hillman et al., J. Gen. Physiol. **68,** 227 (1976)] (Fig. 2D, E). This (or a similar) cooperativity appears also as a "facilitation": A second PDA can be enhanced by a preceding PDA even when the first has declined to baseline (Hillman et al.) (Fig. 2F). An underlying, pre-PDA process is thus indicated (see Minke and Hamdorf, this symposium).

4. The PDA conductance appears to have the same ionic basis as that of the ordinary late receptor potential; both are dominantly Na^+-conductance increases [Brown and Cornwall, J. Physiol. **248,** 579 (1975)], except in the scallop, where both are K^+ increases (Gorman and Cornwall).

5. The PDA can be killed by *net* photo-return of the pigment to the Rh state (Fig. 2B). After such a killing, the PDA can immediately be re-instated by a net transfer to the M state, and the process can be repeated indefinitely. The killing process is however not just the reversal of the induction process because the same process is presumably responsible for the "anti-PDA":

6. If the pigment is transferred from the M to the Rh state in a *resting* cell (one in which the PDA has been allowed to die spontaneously), a PDA *cannot* now be immediately induced (Fig. 2C). This "anti-PDA" dies with a time course comparable to but different from the time course of the decline of the PDA. (Razmjoo and Hamdorf [private communication] have *failed* to find an anti-PDA in Calliphora, even for times as short as 1 s).

7. The PDA exists *in vivo*, that is, manifests itself behaviorally for an appropriate stimulus regime [Hochstein et al., J. Gen. Physiol. **62,** 105 (1973)], but it is unlikely that such a regime ever occurs naturally.

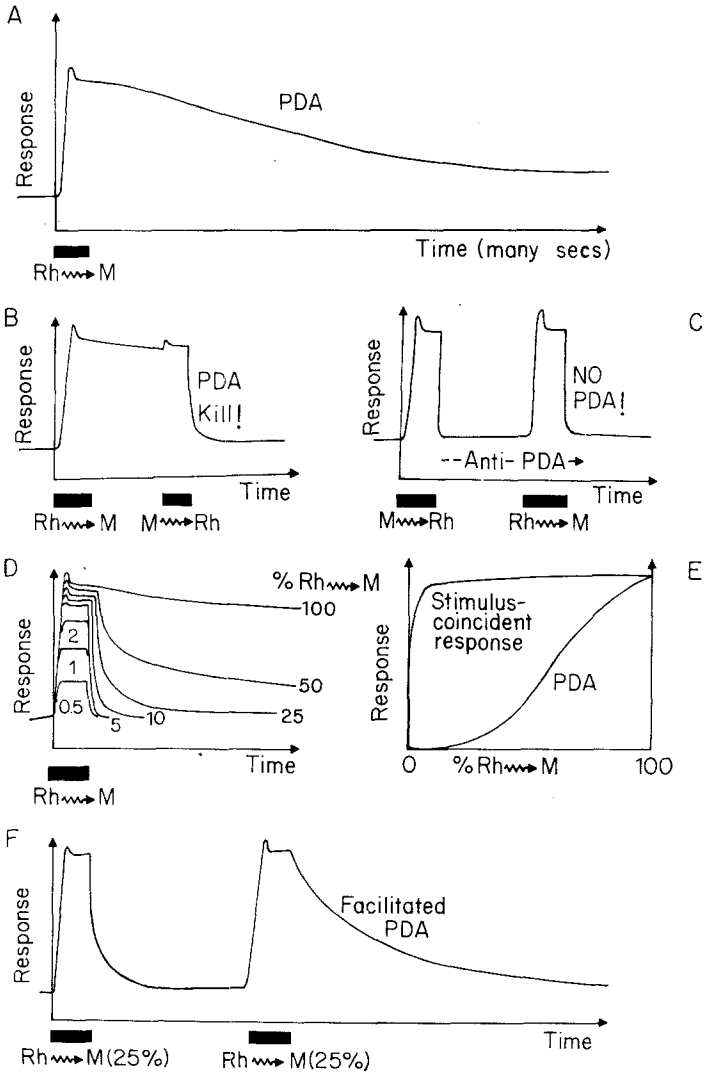
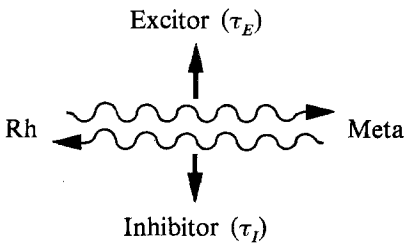


Fig. 2. The basic PDA phenomena: **A.** The full PDA. **B.** Killing the PDA. **C.** The anti-PDA. **D.** Stimulus-coincident and PDA responses for various intensities. **E.** Dependence of the amplitudes of the stimulus-coincident and PDA responses on amount of pigment transferred from the Rhodopsin to the Metarhodopsin state, taken from D. **F.** Facilitation of a PDA by a preceding stimulus

8. As a mnemonic for this complex phenomenology, an "excitor/inhibitor" model is suggested (Hochstein et al., 1973). There is no external evidence for the excitor and inhibitor process and it is likely that different alternative models (such as that of Hamdorf, this symposium, where, however, the "anti-PDA" is not cited and so no inhibitor is needed) can be reconciled with the data. The hypotheses of the model are: (a) Each Rh → M transition produces one unit of excitor, and each M → Rh transition one unit of inhibitor. (b) Excitor opens membrane ionic channels,

inhibitor does nothing to membrane but excitor and inhibitor mutually annihilate 1 : 1. (c) There is a positive cooperative effect among excitors. (d) If not annihilated, excitor and inhibitor die slow natural deaths. All the phenomena described above would arise from these properties. In addition, if one assumed that the excitor-inhibitor annihilation takes a finite time, the normal response to neutral light (one which results in no net pigment transfer) would be predicted, but other properties of this response do not fit the model. The sketch shows the basic excitor-inhibitor model (τ_E and τ_I indicate the finite lifetimes of the excitor and inhibitor).



Post-Script

K. Hamdorf and S. Razmjoo, Bochum, Fed. Rep. Germany

The existence of an anti-PDA substance has never been the subject of our investigations. After the decline of a maximally produced PDA, the duration and intensity of a P-regenerating illumination are important and critical [Rosner, J. Comp. Physiol. **102,** 269 (1975)], and there are indications in the cases of manduca, blowfly, honey bee, and Ascalaphus [Hamdorf and Kaschef, J. Vergl. Physiol. **48,** 251 (1964); Baumann and Mauro, Nature New Biol. **244,** 146 (1973); Hamdorf and Schwemer, in Photoreceptor Optics, eds. Snyder and Menzel, p. 263, Springer Verlag 1975] that *even after* adequate regeneration, possibly an electrical adaptation mechanism may account for a further increase in sensitivity in the dark, the time course of which has been shown to depend on metabolic parameters such as energy supply by glucose, oxygen and temperature (see also Hamdorf and Razmjoo, this symposium).

In our experiments, following the decline of a maximal PDA and the return of the potential to the resting value, a regenerating stimulus is followed almost immediately ($\simeq$ 250 ms later) by the same maximal PDA-producing stimulus, with the production, once again, of the same maximal PDA. We would like to further point out that when our regenerating stimuli were not adequate, then the same PDA-producing stimulus evoked correspondingly much smaller or even no PDAs! (See Fig. 3)

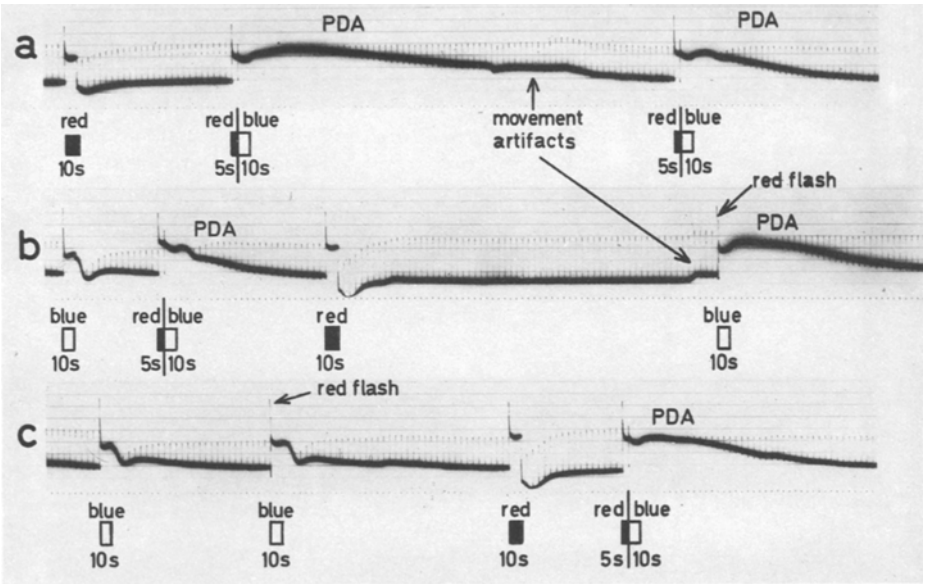


Fig. 3. Intracellularly recorded PDA as a function of immediate adaptation in Calliphora photoreceptors.

Trace a: after an initial 10s of rhodopsin-regenerating red adaptation, a further 5s of red is immediately followed by 10s of blue, eliciting a large PDA, after the decline of which the sequence is again repeated, eliciting a PDA not quite as large as the first, due to the incompleteness of regeneration by only 5s of red adaptation. At the point of filter exchange, note that the downward spike is the off response after red, while the upward spike is the transient response to blue.

Trace b (cont. of **a**): due to the exhaustion of rhodopsin, a further 10s of blue elicits a very small PDA, but a redblue sequence elicits a larger PDA. A further 10s of red adaptation restores the receptor response to the test flashes to maximum, after which a red flash is followed by 10s of blue, eliciting a larger PDA.

Trace c (cont. of **b**): after PDA decline, again a further blue light elicits a very small PDA, and after a red flash followed by 10s of blue, the PDA is only very slightly larger, indicating the inadequacy of rhodopsin regeneration by a short-duration flash, but after 10s of red followed by the sequence a larger PDA is again obtained.

Conditions: test pulses, 498 nm, 30 ms every 5s
red adaptation: edge filter admitting 580 nm to greater wavelength
blue adaptation: bandpass filter admitting 396–467 nm
ordinate: 10 mv per division