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The Prolonged Depolarizing Afterpotential and its Contribution to the Understanding of Photoreceptor Function* **

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Abstract. Comparison of flies bred on vitamine A-poor and vitamine A-rich diets show the latter to exhibit, after blue illumination, 1) slight deviation from the linear relationship between stimulus intensity and receptor sensitivity and, 2) after intense blue illumination the phenomenon of the PDA. Both these effects could result from reduced pigment distances in such membranes. Maximum PDA was produced after about 20 s of illumination with blue light, and following this the resistance of the membrane was seen to stay low, returning to the resting value at the same rate as the PDA decline. The response to test flashes, repressed during illumination, gradually returned during the decline of the PDA, similar to the way the photoreceptor would respond to the sum of two stimuli: the test flash and a decreasing background illumination. Red light immediately following blue abolished the PDA and white light produced a small PDA. All these experiments corroborate a new model (without resorting to the concept of inhibitors) which links the photopigments with receptor excitation, the assumptions for which are the following: 1) PDA is produced after abnormally high primary quantum absorption by rhodopsin molecules, 2) PDA is a retarded membrane excitation by a substance in stored form, 3) the store is built up when production of this substance is larger than its consumption, and 4) time and energy are necessary for the regeneration of *excitatory* rhodopsin molecules.

Key words: *Calliphora* – Photoreceptors – Visual pigments – Prolonged depolarizing afterpotential – Light adaptation.

In invertebrate photoreceptors, in contrast to vertebrate eyes, the visual pigments are thermostable, i.e., the bleaching sequence ends with metarhodopsin, which can then be reconverted to rhodopsin by further quantum absorption. λ-specific equilibria between P and M can result from monochromatic illumination, and these equilibria depend only on the absorption probabilities of the two states of the pigments (α_P,

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α_M, at wavelenghts of illumination). Minimal rhodopsin concentrations are produced by wavelengths at which the ratio α_P/α_M is maximized. In the blowfly *P–M* system, red light photoreconverts *M* 580 into *P* 490, while 460 nm is the most effective wavelength for reducing the concentration of rhodopsin ($C_{Peq} \simeq 0.2\ C_{Po}$, Hamdorf et al., 1973; Hamdorf and Schwemer, 1975).

By systematically varying the adapting light and producing λ-specific *P* contents in the receptor, a linear proportionality was shown to exist between sensitivity and the rhodopsin content (within the range 0.2–1.0 C_{Po}), leading to the use of such responses in determining existing *P–M* concentrations in the blowfly (Hamdorf and Rosner, 1973; Rosner, 1975). However, a difference in larval diets from a vitamine A-poor (heart-fed) to a vitamine A-enriched one (liver-fed), results in a difference of from about 40 to about 2000 in the number of *P* molecules in the membrane of each microvillus (Schwemer, unpublished results; Boschek and Hamdorf, 1976). The liver-fed breed of blowflies (*L* flies) have exhibited the following two differences from the *H* flies:

1) while in the *H* flies a linear proportionality is seen to exist between sensitivity and C_{Peq} in all the possible *P–M* equilibrium states, in the *L* flies the sensitivity loss following blue adaptation is somewhat higher (Razmjoo and Hamdorf, 1976),

2) following intense blue illumination (a condition not normally provided in the natural habitat), the *L* flies exhibit a prolonged desensitized state, lasting many seconds, which has been termed the prolonged depolarizing afterpotential (PDA).

It could, therefore, be the greater packing density of the photopigments in the blowfly microvillus membrane which produces these anomalies. Having dealt with the above first effect elsewhere (Razmjoo and Hamdorf, 1976), it is the second of these anomalous symptoms which is the concern of this presentation.

In the species so far investigated which exhibit this phenomenon (e.g., Limulus, Balanus, Drosophila, Calliphora, etc.), there is common agreement that: 1) PDA is elicited by bright monochromatic stimuli which shift the photoequilibrium to maximum *M* content, and that 2) the PDA is abolished by coloured stimuli which shift the equilibrium in the opposite direction to maximum *P* content.

In both the *H* and *L* flies, after red adaptation which increases the *P* content, PDA is not observed, and superimposed responses to a constant test stimulus at the isobestic point (500 nm, 100 ms duration) which were repressed during the red illumination period, return rapidly to the previously high amplitude (Fig. 1). After bright blue adaptation, however, the *L* flies exhibit long-lasting PDAs, and during the decline of the PDA to the resting potential, the superimposed response amplitudes to the test flashes increase continuously to a certain maximum height, smaller than the original response, indicative of the reduced *P* concentration (Fig. 1, third row). These response amplitudes, when compared with the level of the resting potential, are seen not to be really increasing, but indicating a summation of these and the PDA level, and thus the declining PDA is comparable to the receptor response to a declining background illumination. Such a sustained illumination would be expected to keep the membrane depolarized, i.e., a sustained but slowly increasing membrane resistance. This is indeed found to be the case in the experimental results in Figure 2, where it is seen that the time course of the increase of membrane resistance (third trace) is identical with the time course of the PDA decline (second trace).

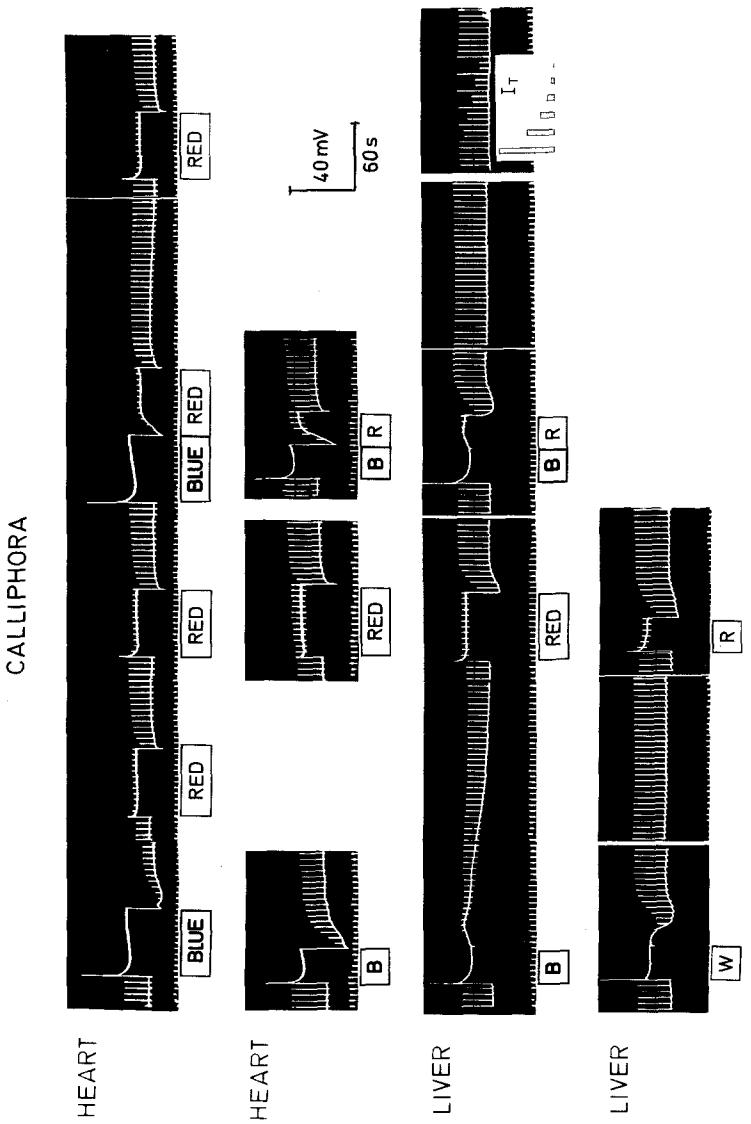

Fig. 1. The comparison of PDA induction and abolition in *H* and *L* flies. The adapting lights (white, blue = 473 nm and red = 615 nm) are indicated by the widths of the labels (W, B and R) below each trace. Throughout the experiments the receptor is subject to test flashes lasting 100 ms, every 5 s. At the end of the third row, the successive addition of 50% neutral density filters to the test light displays reduced response amplitudes. Also notice: 1) the absence of PDA in *H* flies and its presence in the *L* flies, 2) the identical effect of red adaptation in both flies, 3) that in *H* and *L* flies after blue adaptation, the reduction in response amplitude to test flashes is subsequently restored to the previous maximum value by red adaptation, 4) that in *L* flies red adaptation immediately following blue abolishes the PDA, 5) the small PDA response following white light and the rapid recovery of the receptor response to test flashes to the original value. This and the following figures are all intracellular recordings from the retinular cells R_{1-6}

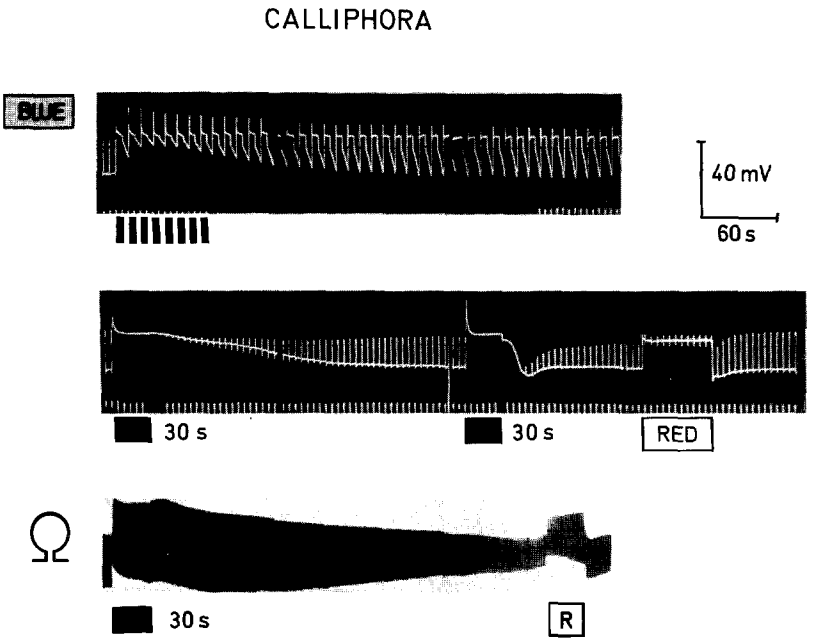

Fig. 2. The PDA in an *L* fly receptor. 1st trace: PDA induction by 5 s blue stimuli interrupted by 5 s dark periods. 2nd trace: After the decline of a large PDA, the same stimulus produces a much smaller PDA. 3rd trace: Wheatstone bridge resistance measurement during maximum PDA production. Stimuli: blue = 473 nm, red = 615 nm, test flash = 498 nm, 100 ms

In the experiments shown in Figure 3, it is observed that the maximum PDA production occurs with about 20 s of illumination (in this particular case), and that further illumination does not greatly alter this. This could mean that PDA is induced by a substance which becomes accumulated to a maximum value during the initial 20 s, which is then subsequently incapacitated *in the dark as well as during further illumination,* within about 3 min, and after the decline of the PDA to the resting potential, a further exposure to blue light induces only a small PDA (Fig. 2, second trace). Further, the time course of the accumulation and decline of this "PDA-inducing substance" is shown by an experiment where the blue adaptation was interrupted by alternating 5 s dark periods (Fig. 2, upper trace). The level of the PDA increases during the first 4–5 blue exposures and then declines during the following stimuli. It is also observed that red illumination following blue adaptation, which returns all *M* back into *P,* abolishes the PDA response (Fig. 1, third row), or "turns off" the hypothetical background light.

With the removal of the blue filter, the very bright white light produced (about 300 times the intensity of the blue light), caused only a small PDA (Fig. 1, bottom trace). This is because the red component of the white light absorbed by metarhodopsin causes the back reaction $M \rightarrow P$. The number of transitions during white light, being a function of intensity, are much greater than with blue light, and yet the PDA is comparatively small. This means that the PDA response is strongly correlated with the resulting high *M* concentration, *and not simply the result of the number of forward transitions!*

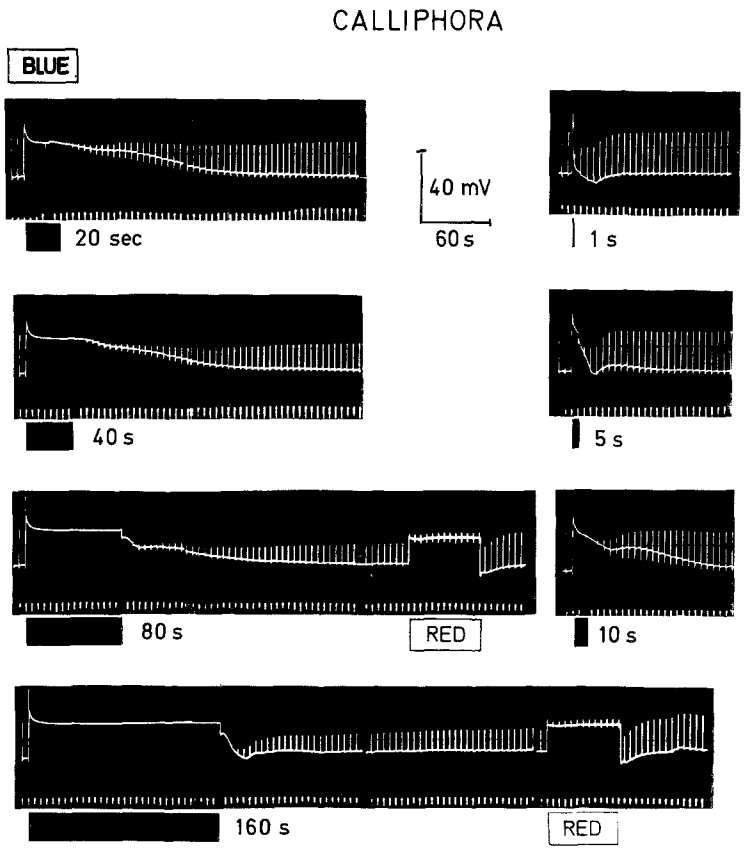

Fig. 3. PDA response as a function of the duration of blue adaptation. Notice that: 1) the response amplitude to test flashes after the decline of PDA is reduced, depending on the length of the adapting period, 2) maximum PDA is produced at about 20 s of blue adaptation, and seemingly reduced with further adaptation times, 3) during the 80 s and 160 s blue adaptations, the PDA effect is masked by the sustained membrane depolarization to the stimulus. Stimuli: blue = 473 nm, red = 615 nm, test flash = 498 nm, 100 ms

The characteristics of the PDA-inducing substance "X" may then be summarized as follows:

1) X is maximally produced and accumulated when blue stimulus is incident on a P-loaded and dark-adapted membrane,

2) X production depends on the number (and possibly on the molecular distances) of pigment molecules in the microvillus membrane,

3) X is inactivated by photoreconversion ($M \rightarrow P$ transitions),

4) X has a different decay time than that of the substance which triggers the normal receptor response.

Further supporting evidence for points 1 and 2 are the following. From Figure 3 it can be seen that with increasing $I_a \times t$, response amplitudes to constant test flashes measured $\simeq 3$ min after the cessation of blue adaptations become increasingly depressed, reaching an end value after about 80 s of blue adaptation, indicating the almost end point of the M-side-shifted equilibrium. A depression to half of this final

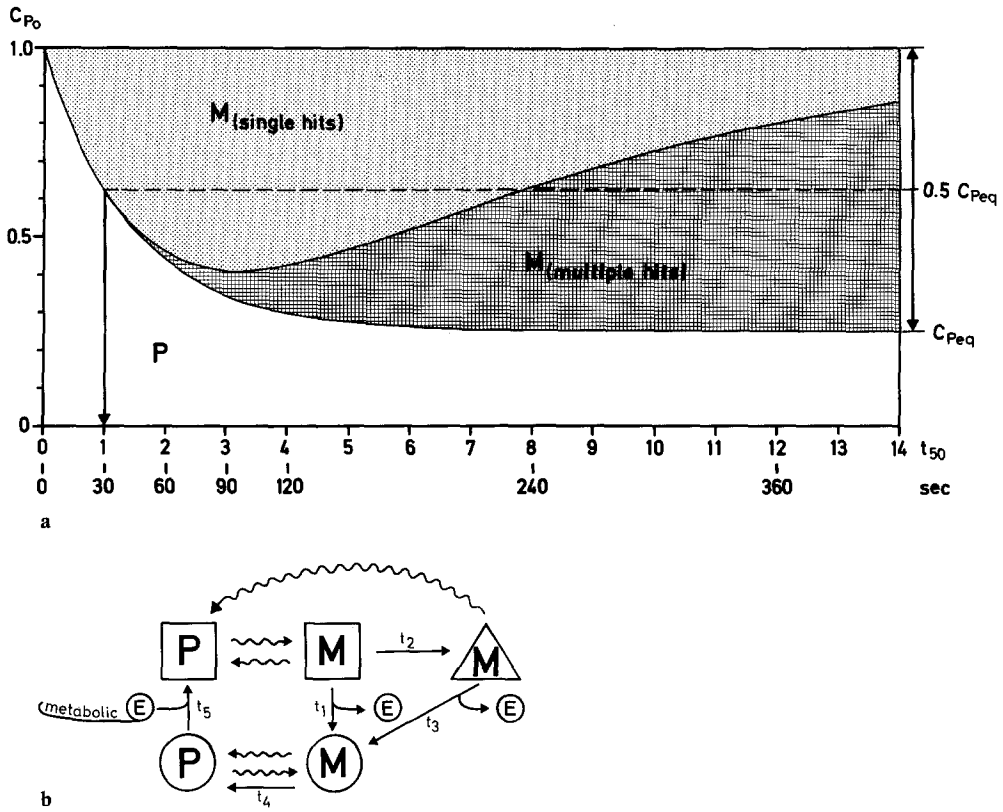

Fig. 4. a Time course of photoequilibration during blue adaptation. The kinetics have been drawn to scale by the amplitudes of test flash responses from Figure 3. Notice the coincidence of the decline of *P* concentration with the production of *M* by single quantum absorption in the initial 30 s phase, and thus the absence of back reactions during this period, and their contribution (due to multiple hits) thereafter. **b** The schematic model of the coupling of photopigment states and receptor excitation in an insect photoreceptor. For explanation see text

value is seen to occur after $\simeq$ 20–40 s of blue adaptation, giving an estimate of the speed of the photoequilibration (Fig. 4a), and the number of *P* molecules which will absorb a single quantum for the first time during the adaptation process. Since the response amplitudes reveal that about half of the final *P* concentration is reached after $\simeq$ 30 s exposure to blue, the time scale of the kinetics can be determined. Calculations show that the steep decline of the *P* concentration is caused by *primary* quantum hits to dark-adapted *P* molecules, and that secondary hits to *M* molecules are negligibly small (shown in the diagram by the area allotted to the concentration of *M* produced by multiple hits). Since PDA induction reaches its maximum at about 20–40 s, we may conclude that PDA originates from primary quantum absorption by dark-adapted *P* molecules.

The characteristics of the photoreceptor response can be explained by various kinetic models, based on various assumptions, e.g., by complex enzymatic systems triggered by quantum absorption, controlling the activation and inhibition of an intracellular transmitter, and/or by the competition of light-activated transmitters for

a limited number of membrane sites. However, in all these models, additional assumptions become necessary to explain the new experimental results, namely that during PDA the responses to test flashes are superimposed on what appears to be mimicing a declining background illumination. Furthermore, models based on the assumption that quantum absorption by M molecules compensate the excitatory output of absorbing P molecules by the release of an inhibitory substance or by triggering other inhibitory mechanisms seem improbable, at least in the blowfly, as in H flies a *linear*, and in L flies an *almost linear* proportionality between P content and sensitivity has been found, even after blue adaptation, when quanta absorption by M molecules is 4—5 times greater than by P molecules.

Thus, while the validity of all models offered so far appear to be highly doubtful, the PDA and other related phenomena *can* be adequately explained by a new model, based on completely different assumptions, namely that:

1) PDA production is a retarded membrane excitation by a substance X in a stored form,

2) this store is built up when the production of X is larger than its consumption rate,

3) the photoregenerated P molecules (or adjacent structures) need longer times — possibly for energy uptake — to become membrane excitatory again.

Consider the following (Fig. 4b). After red and subsequent dark adaptation ($\simeq$ 3 min), all the pigment molecules are in the [P] state. Quantum absorption (e.g., blue light) will convert [P] to [M], and the latter by the release of energy for membrane excitation will be converted to an ineffective form (M). The absorption of quanta by this (M) will not excite the membrane, but will yield (P) which will need metabolic energy (the equivalent of which was lost for excitation) to achieve the membrane-excitable form [P] (it is proposed — Stavenga, 1974 — that parallel to the photo back-reaction (M)→(P), there is also a very slow thermal reaction: $t_4 \ll t_1$, t_2, t_3, t_5, Fig. 4b).

Under extreme conditions of illumination (e.g., the intense blue light which produces the PDA), the number of [P]→[M] transitions exceed a certain extent, and some [M] will enter a stored form $\triangle_M$, in greater amounts than may normally occur. This $\triangle_M$ releases its membrane-excitatory energy much more slowly ($t_3 < t_2$), thus producing the PDA, the sustained "background illumination" effect. In experiments where the PDA in mimiced by a declining background light, the integration of a declining curve gives an estimate of the extent of the PDA-producing $\triangle_M$, which in these cases seem to be in the order of about 20%.

Red adaptation, when quickly following blue, will photoreconvert both the excitatory forms [M] and $\triangle_M$ back into [P], whereupon a further blue illumination can lead to a PDA. However, after the exhaustion of the PDA, when (M) is the main existing specie, further blue illumination will elicit a very small PDA, out of comparison with the previous one. Under such conditions the release of energy by [M] occurs simultaneously with the uptake of energy by (P), and thus a transfer between neighbouring M and P could possibly explain the unavailability of energy for excitation. Such an effect would be expected to be greater with decreasing distance between P and M molecules, that is, in more densely-packed membranes, but it should also be born in mind that the probability of two neighbouring [P] and (M) molecules capturing quanta almost simultaneously must be *extremely low!*

Finally, it should be made clear that the various forms of the pigment presented in the model are not necessarily new identities with different absorption spectra, but symbols for the function of the pigment states possibly in association with their neighbouring structures.

References

Boschek, C. B., Hamdorf, K.: Rhodopsin particles in the photoreceptor membranes of an insect. Z. Naturforsch. **31 c,** 763 (1976)

Hamdorf, K., Rosner, G.: Adaptation und Photoregeneration im Fliegenauge. J. comp. Physiol. **86,** 281–292 (1973)

Hamdorf, K., Schwemer, J.: Photoregeneration and the adaptation process in insect photoreceptors. In: Photoreceptor optics (eds. A. W. Snyder, R. Menzel), pp. 263–289. Berlin-Heidelberg-New York: Springer 1975

Hamdorf, K., Paulsen, R., Schwemer, J.: Photoregeneration and sensitivity control of photoreceptors of invertebrates. In: Biochemistry and physiology of visual pigments (ed. H. Langer), pp. 155–166. Berlin-Heidelberg-New York: Springer 1973

Razmjoo, S., Hamdorf, K.: Visual sensitivity and the variation of total photopigment content in the blowfly photoreceptor membrane. J. comp. Physiol. **105,** 279–286 (1976)

Rosner, G.: Adaptation und Photoregeneration im Fliegenauge. J. comp. Physiol. **102,** 269–295 (1975)

Stavenga, D. G.: Dark regeneration of invertebrate visual pigments. In: Photoreceptor optics (eds. A. W. Snyder, R. Menzel), pp. 290–295. Berlin-Heidelberg-New York: Springer 1975

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Discussion

Peter Hillman, Jerusalem, Israel

I point out that your observation of proportionality between receptor sensitivity and rhodopsin content does not appear to be universal.

Minke et al. [Biol. Bull. **147,** 491 (1974)] showed that in the barnacle, ERP observations show a large change in rhodopsin for red/blue adaptation which results in little or no change in LRP sensitivity.

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On the contrary. For although the existence of such a proportionality has been clearly demonstrated by parallel spectrophotometric measurements for a number of species, the ERP amplitude as an index of the P or M states has never been proven by parallel spectrophotometry, not even for the single case of the barnacle which you suggest as an exception.