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# Transduction in Photoreceptors: Determination of the Pigment Transition or State Coupled to Excitation*

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**Abstract.** Several recent reports have shown that light absorption by metarhodopsin does not contribute to the excitation of invertebrate photoreceptors at low intensities. Where the pigment transition scheme is known, this result may be used to exclude some or most of the transitions and states of the pigment as sources of the coupling to excitation. The methodology of this approach is described and illustrated.

**Key words:** Photoreceptor – Visual pigment – Transduction.

## Introduction and Methodology

The search for an understanding of the transduction process in photoreceptors goes on. A vital component is clearly the knowledge of the exact characteristics of the state(s) or transition(s) of the pigment molecule serving as the source of the coupling of the pigment process to the ionic conductance change underlying the late receptor potential (LRP). The purpose of this article is to present a general methodology for assigning the coupling to a specific state or transition (or limited group of states or transitions) in a known pigment transition scheme.

The identification of the coupling source is facilitated in invertebrates by there being in most (or all) invertebrate pigment systems a long-lived photoproduct called metarhodopsin. Normally, both the rhodopsin (R) and the metarhodopsin (M) states of the pigment are substantially excited by light, leading to two different but interlocked and (possibly) overlapping series of thermal transformations. This means that some transitions are activated only by light absorption by R, others by M, and still others by both R and M to varying relative degrees, depending on the scheme and parameters of the pigment system and on the relative populations of the two states, $c_R$ and $c_M$, which may be manipulated by adaptation with light of appropriate wavelength.

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The approach is based on a measurement of the action spectrum of the LRP for two widely differing $c_R/c_M$. If a dependence of the shape and position of the spectrum on $c_R/c_M$ is found, the direction and degree of that dependence should pinpoint the source of the coupling, as in general only one transition will be excited from R and M to just that relative degree. (Combinations of couplings from other transitions can of course in general not be excluded. Furthermore, if a dependence on $c_R/c_M$ is found, one must consider the possibility that *different* processes are activated by different transitions – as is the case for the PDA phenomenology at stimulus intensities higher than those considered here; see Hochstein et al., 1973.) If no dependence on $c_R/c_M$ is found, the coupling must be from those transitions which come from R or M alone. Whether it is R or M can be resolved either by determining if LRP sensitivity rises or falls as, for instance, $c_R$ rises and $c_M$ falls (and assuming that sensitivity increases with population); or more directly by comparing the LRP action spectrum with the photosensitivity spectra of the R and M states found spectrophotometrically or electrophysiologically through the early receptor potential (ERP).

In the next part of this article we discuss briefly how $c_R/c_M$ may be varied and its value determined. We then illustrate the calculation of the expected dependence of the LRP action spectrum on $c_R/c_M$ for each transition putatively coupled to the LRP. Finally we review briefly our published result (Atzmon et al., 1978) in the context of this methodological exposition.

Throughout this article we shall refer mainly to "transitions" for brevity, since we need remember only that any conclusions about a thermal transition also apply to the state from which it originates and to whose population its rate is always proportional.

## *The Manipulation and Determination of the Relative Pigment State Populations*

Since both the R and M states are photosensitive, constant light of a particular wavelength will set up a photoequilibrium between the two states which will persist when the light is turned off (Hamdorf, 1970). The equilibrium populations will de-

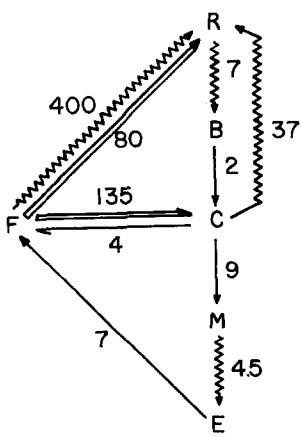


**Fig. 1.** The transition scheme of the visual pigment of the barnacle photoreceptor, taken from Minke et al. (1974) with minor modifications. The numbers are rate constants: For the thermal transitions (straight arrows), at 20° C; for the phototransitions (zig-zag arrows), at full intensity white light. Atzmon et al. (1978) have used the approach described in the text to show that only the transitions R⇝ B and B → C and the state B can serve as the primary source of coupling to the late receptor potential

pend on the relative photosensitivities and also on the weighting factors which express the probability that photoisomerization of a molecule in one state will convert it to the other state (that is, it will not return to the same state) (Hochstein et al., 1978). The dependence of the population of either state on wavelength of equilibrating (saturating) light is known as the photo-equilibrium spectrum (Hillman, 1979). The populations are determined by spectrophotometry (Hamdorf, 1970), by early receptor potential measurements (Hillman et al., 1976) or, if the LRP sensitivity is known to be proportional to R population (as it is in many but not all invertebrates), by LRP sensitivity variations (Hamdorf and Razmjoo, 1979). The populations may also be calculated from the photosensitivity spectra of R and M (if the relative amplitudes of these spectra are known) by calculating the weighting factors from the known pigment scheme and parameters (Hochstein et al., 1978).

### *Calculation of LRP Action Spectrum Dependence on Putative Coupling Source*

If the coupling source is unique and if there are no loops in the transition scheme – that is, if each transition can be reached only from R or M – no dependence of the relative LRP action spectrum on $c_R/c_M$ is expected. (This is only true to the extent that self-screening is negligible – as it is in the barnacle.) If there are such loops, a dependence will appear if the coupling source is any transition accessible from both M and R. Figure 1 illustrates such a pigment scheme. This example is derived from ERP data on the barnacle photoreceptor by Minke et al. (1974). The ten transitions and four unstable states of the scheme may be assigned as shown in Table 1. If the coupling source is any of the transitions or states in the first two rows, no dependence of the LRP action spectrum on $c_R/c_M$ is expected, and the action spectrum should agree with the photosensitivity spectrum of either R or M. If the source is any of the states or transitions in the third row, the LRP action spectrum is expected to be a weighted sum of the R and M photosensitivity spectra, and to depend on the initial $c_R/c_M$. The relative weightings of the R and M spectra will be the respective products of the relative R and M populations $c_R$ and $c_M$ and the fractions of those molecules leaving R or M which pass through the transition under consideration.

Take, for instance, the transition C → M of the scheme of Figure 1. Of the molecules leaving R a fraction $f_1$ will pass through C → M, the remainder returning to R via F. Similarly, of the molecules leaving M, a fraction $f_2$ will return to M via

**Table 1**

| Accessible from | States | Transition |
|---|---|---|
| R | B | R⇝ B, B → C |
| M | E | M⇝ E, E → F |
| R and M | C, F | C⇝ R, F⇝ R, C → M, C → F, F → C, F → R |

States and transitions of the barnacle photoreceptor visual pigment (transition scheme shown in Figure 1) accessible to absorption of light by *R* or *M* or both

$C \rightarrow M$, the remainder proceeding to R. The LRP action spectrum will then be the sum of the R and M photosensitivity spectra with the relative weightings $c_R f_1 : c_M f_2$.

We now proceed to illustrate the calculation of $f_1$ for $C \rightarrow M$. For algebraic simplicity we shall exclude from consideration the transitions $F \rightsquigarrow R$ and $C \rightsquigarrow R$. Since for the weak test stimuli used in this experiment $c_F$ and $c_C$ will always be small, the effect of this exclusion is totally insignificant (calculated to be less than a thousandth of one percent).

We define $k_{xy}$ as the rate constant of the transition $x \rightarrow y$. Then the fraction of molecules leaving R which arrive at M is the total number of molecules arriving at M divided by the total leaving R;

$$f_1 \equiv \int c_C(t) k_{CM} dt / \int c_B(t) k_{BC} dt = k_{CM} \int c_C(t) dt / k_{BC} \int c_B(t) dt.$$

Now the population of C is zero before and after the illuminations, so the total input to C must be equal to the total output:

$$\int [c_B(t) k_{BC} + c_F(t) k_{FC}] dt = \int c_C(t)(k_{CF} + k_{CM}) dt$$

or

$$k_{BC} \int c_B(t) dt + k_{FC} \int c_F(t) dt = (k_{CF} + k_{CM}) \int c_C(t) dt.$$

Finally, the total output of R must be equal to sum of the total input to M and the total return to R:

$$\int c_B(t) k_{BC} dt = \int c_C(t) k_{CM} dt + \int c_F(t) k_{FR} dt$$

or

$$k_{BC} \int c_B(t) dt = k_{CM} \int c_C(t) dt + k_{FR} \int c_F(t) dt.$$

By straightforward algebraic manipulation of these three equations we obtain

$$f_1 = \frac{k_{CM}(f_{FR} + k_{FC})}{k_{CM}(k_{FR} + k_{FC}) + k_{CF} k_{FR}}.$$

We may similarly obtain

$$f_2 = \frac{k_{CM} k_{FC}}{k_{CM}(k_{FR} + k_{FC}) + k_{CF} k_{FR}}.$$

*Application to the Barnacle*

In the barnacle, the photosensitivity spectra are known from ERP measurements, the state populations are determined as described in an earlier section, and the ERP-derived rate constants needed for calculating the fractions $f$ are given in Figure 1. Calculation of the predicted LRP action spectrum for each putative transition is then straightforward. The results of these calculations have been presented in our earlier publication (Atzmon et al., 1978) and compared there with the experimentally determined relative LRP action spectra after red and blue adaptations which give the maximum difference in $c_R/c_M$. No dependence of the relative action spectrum on the

adaptation was found, and the upper limit set on an undetected dependence was found to be well below the effect calculated for all of the thermal transitions of the third row of Table 1. Since (as noted in the Introduction) any conclusions about thermal transitions also apply to their states of origin, the states of this row are also excluded.

The phototransitions C $\rightsquigarrow$ R and F $\rightsquigarrow$ R can also easily be excluded as primary coupling sources: For weak stimuli, $c_C$ and $c_F$ are both very small (as noted above) and therefore very weakly photo-excited. Furthermore, both the populations and the phototransition rate constants depend linearly on light intensity, so the actual rates are proportional to the *squares* of the light intensity. In contrast, the LRP amplitude depends initially linearly, and at higher intensities sub-linearly, on intensity. The LRP is therefore unlikely to arise from these transitions.

The choice between the first and second rows of Table 1 is also easy: The LRP action spectrum is found to match the ERP-determined photosensitivity spectrum of R adequately and to be far from that of M.

Accordingly, the only *transitions* remaining as candidates for the primary coupling are R $\rightsquigarrow$ B and B $\rightsquigarrow$ C; and so the *state B* is also a candidate (R and M are of course not candidates as they are permanently present).

## Conclusion

We have described a general method by which, if the transition scheme of a visual pigment is known, it is possible to identify the transitions(s) or state(s) responsible for the pigment-conductance coupling, or at least to limit the possible candidates. It is therefore now important to identify the pigment states biochemically in the barnacle and in other candidate preparations.

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