

# Antagonistic Components of the Late Receptor Potential in the Barnacle Photoreceptor Arising from Different Stages of the Pigment Process

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**ABSTRACT** The late receptor potential (LRP) recorded in barnacle photoreceptor cells exhibits, at high light levels, a strong dependence on the color of the stimulus and of the preceding adaptation. Most strikingly, red illumination of a cell previously adapted to blue light results in a depolarization which may last for up to 30 min after the light goes off, while blue illumination of a cell previously adapted to red light cuts short this extended depolarization or prevents its induction by a closely following red light. Comparison of the action spectra for the stimulus-coincident LRP and for the extended depolarization and its curtailment with those previously measured for the early receptor potential (ERP) confirms that these phenomena derive from the same bi-stable pigment as the ERP. The stimulus-coincident response and the extended depolarization appear to arise from substantial activation of the stable 532 nm state of the pigment, while activation of the stable 495 nm state depresses or prevents the extended depolarization and probably also depresses the stimulus-coincident response. Since either process can precede the other, with mutually antagonistic effects, one is not simply the reversal of the other; they must be based on separate mechanisms. Furthermore, comparison with ERP kinetics shows that both processes involve mechanisms additional to the pigment changes, as seen in the ERP. A model is proposed and discussed for the LRP phenomena and their dependences on wavelength, intensity, and duration of illumination based on excitor-inhibitor interactions.

## INTRODUCTION

The central open question in photoreceptor transduction is: how does the pigment process ultimately induce the membrane conductance changes which underlie the (late) receptor potential response (LRP)? A major difficulty in the search for an answer has been the impossibility of isolating or even identi-

ifying the specific pigment stage(s) or transition(s) responsible for the membrane changes.

We have shown by studying the early receptor potential (ERP) (Minke et al., 1973) that the barnacle photoreceptor contains a visual pigment which has two stable (as well as several unstable) states called the 532 and 495 states for the positions of the peaks of their absorption spectra. We suggested in the preceding article that these may be the rhodopsin state and a metarhodopsin state of the pigment. We also showed that light interconverts the two states and that the ratio of the populations of the 495 and 532 states after saturating stimulation increases with increasing stimulus wavelength, most of this change taking place in the wavelength region 550–600 nm.

“Reddening” and “blueing” stimuli were defined in the preceding article as those which move pigment from the 532 to the 495 state and vice versa, respectively. The amount of pigment transferred is related to the extent that the adaptation-stimulus wavelength shift spans the 550–600 nm region, reddening and blueing stimuli being those for which the stimulus is of longer or shorter wavelength than the adaptation, respectively. Stimuli which result in little or no transfer of pigment between the two states, either because the stimulus and adaptation wavelengths are the same or because both are below 550 nm or both above 600 nm, are called “neutral.” We show in the present article that these manipulations lead to a dissection of the coupling mechanism of the conductance change, at least at high light levels, and that two antagonistic contributions can be related directly and quantitatively to the degree of reddening or blueing of the stimuli. The new LRP adaptation phenomenon presented in this article differs from the classical dark adaptation in depending essentially on *color* of adaptation and in being apparently completely stable in the dark.

The concept of antagonistic contributions to the LRP from different pigment states is not new. The history is summarized in Sillman et al. (1972); see also Mainster and White (1972). The present observations appear, however, to provide the most direct and specific evidence of such antagonistic contributions. Other studies of the pigment-LRP coupling are summarized in the preceding paper.

Previous studies of this preparation (Gwilliam, 1962, 1963, and 1965; Brown et al., 1970 and 1971; Stratten and Ogden, 1971; Shaw, 1972) did not deal with wavelength effects or did not see the present effects probably due to use of too low intensities.

Nolte, Brown, and Smith (1968), and Nolte and Brown (1972 *a* and *b*), however, have seen in some cells of the *median* eye of *Limulus*, wavelength-dependent phenomena somewhat resembling those we present here for the barnacle. They report that ultraviolet light induces in the *Limulus* cells an extended depolarization which is suppressed by visible light, the repolariza-

tion taking place shortly after the onset of the visible light. They speculate that the extended depolarization must be due to the UV-induced transfer of the visual pigment into a second "stable" state which decays slowly back to the original state, but whose return may be speeded by visible stimulation. We will show this model to be inapplicable to the barnacle pigment.

A preliminary description of the phenomena described in this article has been presented in Hillman, Hochstein, and Minke (1972).

#### METHODS

Intracellular recordings were made in excised photoreceptors of the barnacles *Balanus eburneus* and *amphitrite* as described in the preceding two articles (Hillman et al., 1973; Minke, Hochstein, and Hillman, 1973). As with the ERP, the observations for the two species were very similar. The wavelength and intensity characteristics of the illumination through the various filters are given in Table I of the preceding article. In all of the figures of the present article, the ERP is invisible on the scale displayed and with stimulus intensity reduced by color filters. All measurements were done at about 22°C. Many ERP measurements were made at low temperature in the preceding paper, but the phenomena were qualitatively the same, and the action spectra identical, at room temperature.

Cell membrane conductance was measured by use of a standard bridge. 1 nA square-wave depolarizing pulses were applied at the rate of two per second to the intracellular electrode through a 100 M  $\Omega$  resistor. The bridge was initially balanced in the dark and the degree and direction of imbalance were observed in various conditions.

#### RESULTS

##### 1. *Color-Dependent Phenomena*

The late receptor potential (LRP) in the barnacle photoreceptor, like the early receptor potential (preceding article), shows, at least at high light levels (approaching those normally reaching the receptors in vivo on a sunny day), a variety of phenomena dependent on wavelengths of adaptation and of stimulation. The most striking LRP effects occur in the period after stimulation. The terminology defined in the Introduction (neutral, reddening, and blueing stimuli) will be used. The different effects on the pigment as seen in the ERP will be shown to be reflected in different LRP phenomena. The quantitative agreement of the intensity, duration, and wavelength dependences of the ERP and the LRP effects will be shown in sections 3 and 4 below.

We find two phenomena: an extended post-stimulus depolarization ("tail") excited by reddening stimuli (trace *a* of Fig. 1) and a period during which no tail can be excited ("anti-tail") induced by blueing stimuli (trace *b*).

The two are mutually antagonistic: a tail is not induced by a reddening

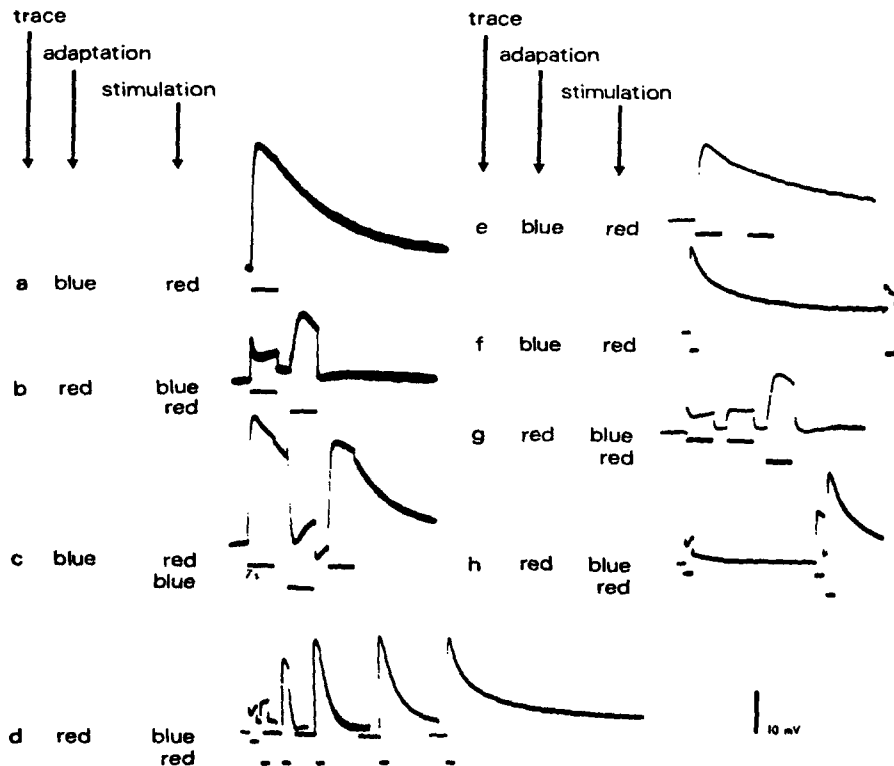


FIGURE 1. The late receptor potential tail and anti-tail phenomena. *B. amphitrite*. The cell was adapted to red or blue light as indicated. After 3 min in the dark, the cell was stimulated with red and/or blue lights as indicated and marked by the bars below each trace. Each bar corresponds to a 7 s stimulus pulse (traces *a*, *b*, *c*, *e*, and *g* are of 50 s duration, traces *f* and *h* are of 200 s duration, and trace *d* is at the same sweep speed as the latter). Trace *d* shows five superposed recordings, each of a blue stimulus (always giving the same response) and a red stimulus after various dark intervals. The adaptations and stimulations are to full intensity red (K6, 7 s) and blue (447 nm, 7 s) lights. (See Table I of preceding article for wavelength and intensity characteristics of these and all other filtered lights referred to in the figure legends. Where no intensity is given, or where  $\log I = 0$  is specified, the intensities are those given in Table I.) (*a*) A reddening stimulus induces a tail. (*b*) A blueing stimulus induces an anti-tail which prevents induction of a tail by a closely following red stimulus or (*c*) depresses a previously induced tail. These effects decay in the dark (*a*) and (*d*). Neutral stimuli do not affect these phenomena (*e*, *f*, *g*, and *h*). The smallness of the response to the second red stimulus in trace *f* compared with those of traces *b* and *g* is due to the fact that the tail had not yet declined to base line in this case. (Measured from base line, the amplitude is actually close to those of traces *b* and *g*.) See text for definitions of reddening, blueing, and neutral stimuli.

stimulation if that stimulus occurs during an anti-tail (trace *b* of Fig. 1); while an anti-tail is not induced by a blueing stimulation if that stimulus occurs during a tail (trace *c*). Trace *c* also shows that a blueing stimulation occurring during a tail is followed by suppression of the tail. (There is no simple way of

observing the parallel phenomenon, suppression of an anti-tail by a reddening stimulation.)

Both the tail and the anti-tail die away slowly in the dark. Trace *a* shows the decay of a tail, while trace *d* shows increasing tails induced by reddening stimulations at different times during an anti-tail. The durations of the tail and anti-tail varied from a few seconds to many minutes in different cells, but appeared to be stable in any given cell. The two durations were not directly related, although there was some suggestion of a negative correlation. In all the traces of Fig. 1, the cell was kept in the dark after the preceding adaptation for times long compared with the durations of the tail and the anti-tail.

Finally, neutral stimulations neither suppress tails (trace *e*) nor induce tails (trace *f*) nor suppress anti-tails (trace *g*) nor induce anti-tails (trace *h*).

The stimulus-coincident responses also show quite strong color dependences, but these will be referred to only briefly in the Discussion, as we have not yet clearly established the parameters of these variations. We note only that the blueing stimulus presented during the early part of a tail elicits a stimulus-coincident response which is sometimes considerably less depolarized than the preceding tail (as in trace *c* of Fig. 1), sometimes hardly affects the tail (Fig. 3), and sometimes even increases the depolarization (not illustrated).

Traces *b*, *h*, *c*, and *g* of Fig. 1 illustrate special cases of what turns out to be a general principle: the response after *any* stimulus series of duration short compared with those of the tail and anti-tail depends only on the initial and final states of adaptation. For instance, a final red saturating stimulus (adaptation) always induces a tail if the cell was initially blue-adapted, or never induces a tail in an initially red-adapted cell, no matter what other stimuli intervened during a time, preceding the red stimulus, short compared with the tail and anti-tail durations.

Bridge measurements show that all components of the LRP, including the tail and its modulations, are accompanied by membrane conductance increases which roughly follow the time-course of the depolarization. (The polarizing effect of the electrogenic pump demonstrated by Koike, Brown, and Hagiwara, 1971, undoubtedly distorts this correlation.) Neither the membrane potential nor the membrane conductance depart appreciably from the resting dark value during the anti-tail. It is interesting to note that in those cells in which blue light decreases the tail depolarization during the blue light, the conductance also decreases during the blue light—a characteristic reminiscent of the normal LRP response in vertebrates (Toyoda, Nosaki, and Tomita, 1969). Fig. 2 shows a sample of such an observation.

In the dark, the cell remains blue-adapted or red-adapted stably, and neither of these conditions may be exclusively termed the “normal” or the “dark-adapted” conditions of the cell. The same tail was elicited by red stimulation when presented 5 min or 60 min of darkness after blue adaptation.

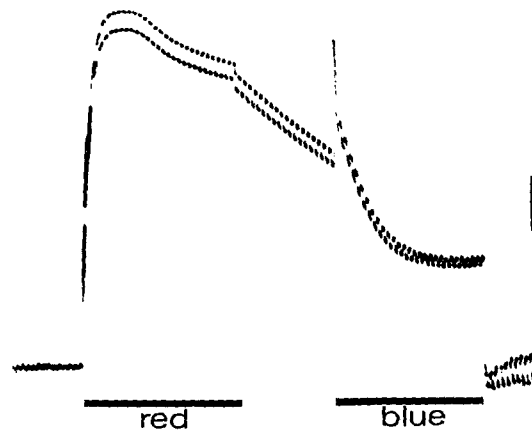


FIGURE 2. Bridge measurements of cell conductance variations. *B. amphitrite*, see Methods. The bridge was balanced in the dark. The underbalance seen during the stimuli corresponds to an increase in conductance. The cell had previously been adapted to blue light (447 nm, 15 s). After 5 min of darkness the cell was stimulated with red light (K6, 15 s, first bar under trace) and a tail was induced. After another 10 s in the dark, a blue stimulus was applied (447 nm, 15 s, second bar). This caused a reduction in depolarization and in conductance. After this the membrane potential returned to near (actually slightly beyond) its initial value, and the conductance became considerably *less* than its resting value (the trace broadening here arises from an *overbalance*). The vertical bar denotes 10 mV.

Similarly a tail was not produced by red stimulation when presented 5 min or 60 min after red adaptation.

As with the ERP, we have been unable to find any LRP phenomena in the ventral photoreceptors of *Limulus* which could not be reproduced at all other wavelengths of adaptation or stimulation by adjusting the intensity. The *Limulus* responses generally resemble those of the *Balanus* to neutral stimuli.

## 2. Psychophysical Tests

We have also investigated the tail phenomenology psychophysically in the intact animal. The observations are of the shadow reflex: the periodic opening of the opercula and extrusion of the cirri cease after dimming of the light. Gwilliam (1962, 1963, 1965) and Shaw (1972), have shown that this reflex arises from a rapid hyperpolarizing change in the photoreceptor potential. (H. M. Brown and W. Wilson, private communication, have noted that the shadow reflex sometimes failed to occur after red or simultaneous red and blue stimuli.)

We find that the shadow reflex fails to occur after red stimuli under the conditions of traces *a*, *c*, *e*, *h*, and last part of *d* of Fig. 1, but does occur after all blue stimuli and after red stimuli under the conditions of traces *b*, *g*, first part of *d*, and last part of *f*. The timings were, however, different from those of

Fig. 1 for *b*, *c*, *d*, *g*, and *h*, the red stimuli being switched off only after the periodic motion, stopped by cessation of the preceding stimulus, had re-started. The periods during which red stimuli failed to give the reflex after a fully reddening stimulus (as in trace *e*) or succeeded in giving the reflex after a fully blueing stimulus (as in trace *d*) corresponded to the durations of the tail and the anti-tail observed intracellularly in cells from other animals, namely seconds to minutes.

### 3. Gradation of the Phenomena with Wavelength, Intensity, and Duration

Various combinations of wavelength and intensity of adapting and stimulating lights can produce various amounts of reddening and blueing effects as illustrated in Figs. 3–5. In each case, for stimuli short compared with the durations of the tail and the anti-tail, the response was found to be determined by the *product* of intensity and duration of stimulus or adaptation (see below). We call this product the “amount” of light. The tail is generally measured by the height, above base line, of the depolarization shortly (0.5 s) after the relevant (tail-inducing or -depressing) stimulus.

Fig. 3 (upper part) shows the responses of a fully blue-adapted cell to saturating stimuli of various wavelengths (top row of figure), and to various amounts of red light (right column). A saturating stimulus is one to which the response is the same as for stimuli of greater amounts. The height of the tail induced by a stimulus of saturating strength is wavelength dependent, growing from zero for any stimulation below 550 nm, to a maximum height for saturating stimuli above 600 nm. The tail height is also amount-dependent, growing from zero for weak stimulation to maximum for that wavelength for saturating stimulation (right column shows example at 620 nm).

The degree to which a decaying tail is depressed after cessation of a blueing light (Fig. 1, *c*) also depends on the wavelength and amount of this light. Fig. 3 (lower part) shows the effects of saturating lights of various wavelengths (bottom row) and of various amounts of blue light (left column). Any sufficiently strong light of wavelength below 550 nm suppresses the tail completely. For wavelengths above 550 nm, even saturating lights can only shorten the tail, and above 600 nm, illumination has little effect on the tail. The tail depression is also amount-dependent, growing from zero for weak stimulation, to maximum for that wavelength for saturating stimulation (left column shows example for 543 nm).

The tail and the anti-tail also depend on the wavelength and intensity history of the adaptation *preceding* the red or blue stimulus (Figs. 4 and 5, respectively). Fig. 4 shows the tail height induced by fixed red stimuli after various adaptations, and Fig. 5 shows the degree of anti-tail induced by fixed blue stimuli after various adaptations—as tested by the tail height induced by a fixed reddening test flash immediately after the blue stimulus.

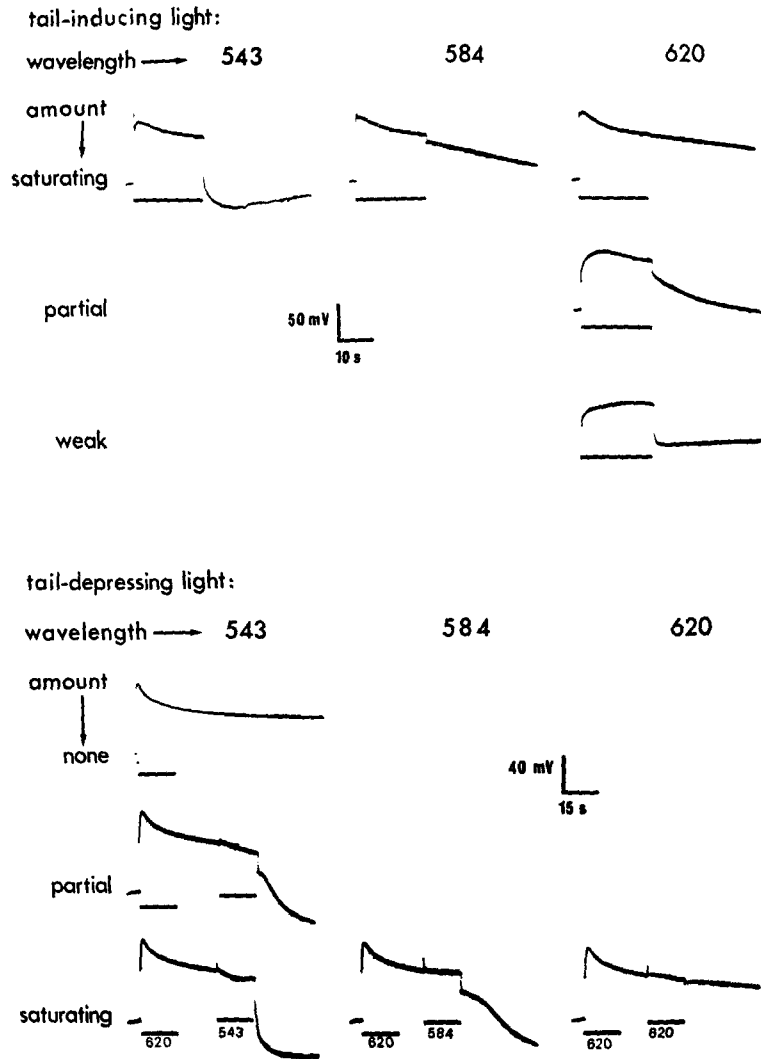


FIGURE 3. *Upper part*, The induction of a tail. *B. eburneus*. The responses to 20-s stimuli, of intensities and wavelengths indicated, of a single cell adapted to full intensity blue light (447 nm, 15 s) 3 min before each stimulus. The length of a tail induced by a saturating stimulus (top row) grows from zero for stimuli below 550 nm to a maximum length for stimuli above 600 nm. The saturating stimuli were  $\log I = 0$ ,  $-0.5$ , and  $0$  for the 543, 584, and 620 nm responses, respectively. The tail length increases with stimulus intensity up to this saturation value (shown for 620 nm stimuli, right column, with  $\log I = -1.3$  ["partial"] and  $-2.0$  ["weak"]). Calibration bars apply to all traces in the figure. *Lower part*, The depression of the tail. *B. eburneus*. The effects on the tail of 15-s stimuli of various wavelengths and intensities. A tail is induced by strong red stimulation (620 nm, 15 s; the first black bar under each column, marked "620," shows stimulus duration) in a cell adapted 3 min earlier to strong blue light (447 nm, 15 s). Roughly 15 s after cessation of the tail-inducing stimulus the trial stimulus is switched on as shown by second black bar under each trace (except the first trace). The degree of depression of the tail after a saturating stimulus (bottom row) grows from near zero for stimuli above 600 nm to a complete suppression for stimuli below 550 nm. The saturating stimuli were  $\log I = -1.3$ ,  $-0.5$ , and  $0$  for the 543, 584, and 620 nm stimuli, respectively. The depression increases with stimulus intensity up to this saturation value, as shown for 543 nm stimuli, left column ("partial" is  $\log I = -1.8$ ).



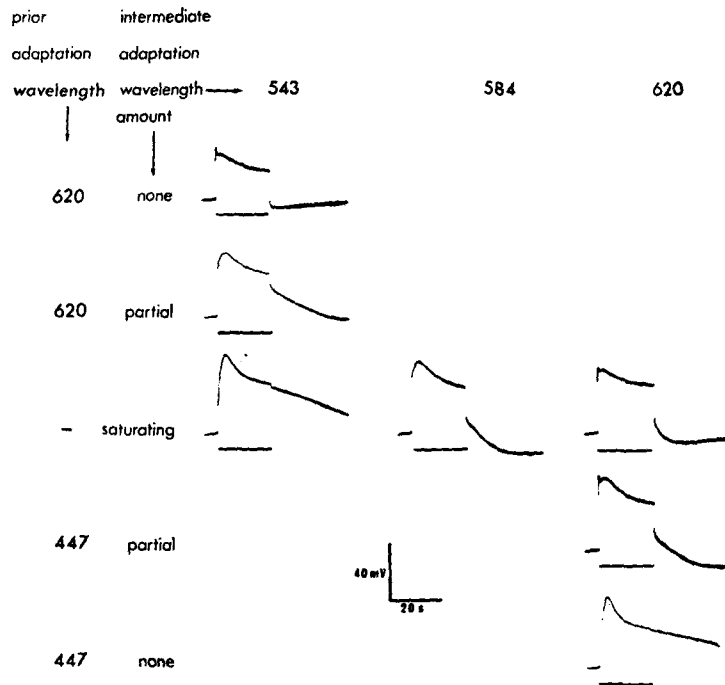


FIGURE 4. The dependence of the tail on adaptation. *B. eburneus*. The responses of a single cell prior-adapted to full red (620 nm, 20 s) or blue (447 nm, 15 s) light, and re-adapted 3 min later to 30 s of "intermediate adaptation" at 543 nm ("partial" and "saturating" are  $\log I = -1.8$  and 0, respectively), 584 nm ( $\log I = 0$ ), and 620 nm ("saturating" and "partial" are  $\log I = 0$  and  $-0.5$ , respectively), as indicated. All stimuli were of strong red light (620 nm, 20 s; duration shown by black bar under each response) presented an additional 3 min in the dark after the intermediate adapting light. The responses in the center row, for saturating intermediate adaptation, were independent of color of prior adaptation. The tail is maximum for any saturating intermediate adaptation of wavelength below 550 nm and zero for wavelengths above 600 nm. For prior red adaptation (left column) the tail increases with amount of intermediate blue (543 nm) adaptation, and for prior blue adaptation (right column) the tail decreases with amount of intermediate red (620 nm) adaptation.

The center rows of these two figures are for saturating adaptation to the wavelengths indicated. The tail is approximately maximal and the anti-tail zero (nearly maximal tail on the response to the following reddening test stimulus) for any saturating adaptation up to about 550 nm. Above 600 nm, the tail becomes zero and the anti-tail maximal (that is, allowing no tail on the response to the reddening test stimulation).

For weaker, nonsaturating adaptations, the tail and the anti-tail also depend on the preceding state of adaptation ("prior adaptation"). For a given state of prior adaptation, the dependence of the two effects on amount of "intermediate adaptation" is illustrated in the left and right columns of Figs. 4 and 5.

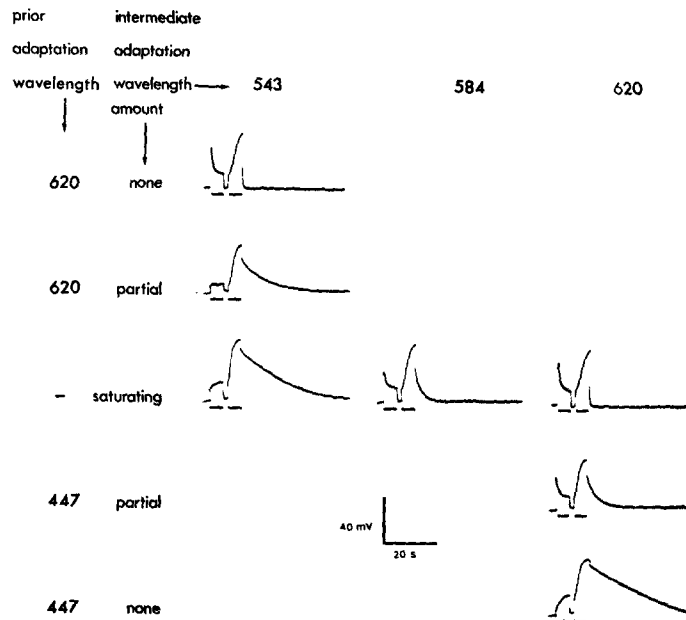


FIGURE 5. The dependence of the anti-tail on adaptation. *B. amphitrite*. The responses of a single cell prior-adapted to saturating red (620 nm, 20 s) or blue (447 nm, 15 s) light and readapted 3 min later to 15 s of "intermediate adaptation" at 543 nm ("partial" and "saturating" are  $\log I = -1.5$  and 0, respectively), 584 nm ( $\log = 0$ ), and 620 nm ("saturating" and "partial" are  $\log I = 0$  and  $-0.3$ , respectively), as indicated. An additional 3 min in the dark later, a pair of stimuli was presented consisting of a strong blue light (447 nm, 5 s) followed 1.3 s later by strong red light (620 nm, 5 sec, duration as shown by bars under each response). The blue lights induce anti-tails which are measured by their effects on the tails of the responses to the red lights. The center row is for saturating intermediate adaptation and the responses were independent of color of prior adaptation. The anti-tail is maximum (that is, no tail is induced by following red light) for any saturating intermediate adaptation of wavelength above 600 nm, and the anti-tail is approximately zero (full tail on following red light) for wavelengths below 550 nm. For prior blue adaptation (right column) the anti-tail increases with amount of intermediate red (620 nm) adaptation, and for prior red adaptation (left column) the anti-tail decreases with amount of intermediate blue (543 nm) adaptation. Note that the cell was declining during the experiment, and at least part of the nonsystematic variation of the stimulus-coincident response amplitude (the measurements were not made in the order shown) is due to this.

The top left trace of each figure shows the effect of a saturating red prior adaptation with no intermediate adaptation. The trace below it is for partial blueing intermediate adaptation, and the third trace in the column is for saturating blueing intermediate adaptation. Similarly, the bottom trace in the right column is for saturating blue prior adaptation, the next trace above it is for partial reddening intermediate adaptation and the top trace in the column is for saturating reddening intermediate adaptation.

In summary of this rather complex set of phenomena one can say that: (a) A maximum tail is the result of saturating red stimulation after saturating blue adaptation. A maximum anti-tail is the result of saturating blue stimulation after saturating red adaptation. (b) If the red stimulation occurs during an anti-tail, a tail is not induced. If the blue stimulation occurs during a tail, an anti-tail is not induced, but the tail is cut short. (c) The transition from "red" to "blue" occurs mainly in the range 550–600 nm. All saturating stimuli above 600 nm are approximately equally "red." All saturating stimuli below 550 nm are approximately equally "blue." Intermediate wavelengths or nonsaturating amounts produce intermediate effects.

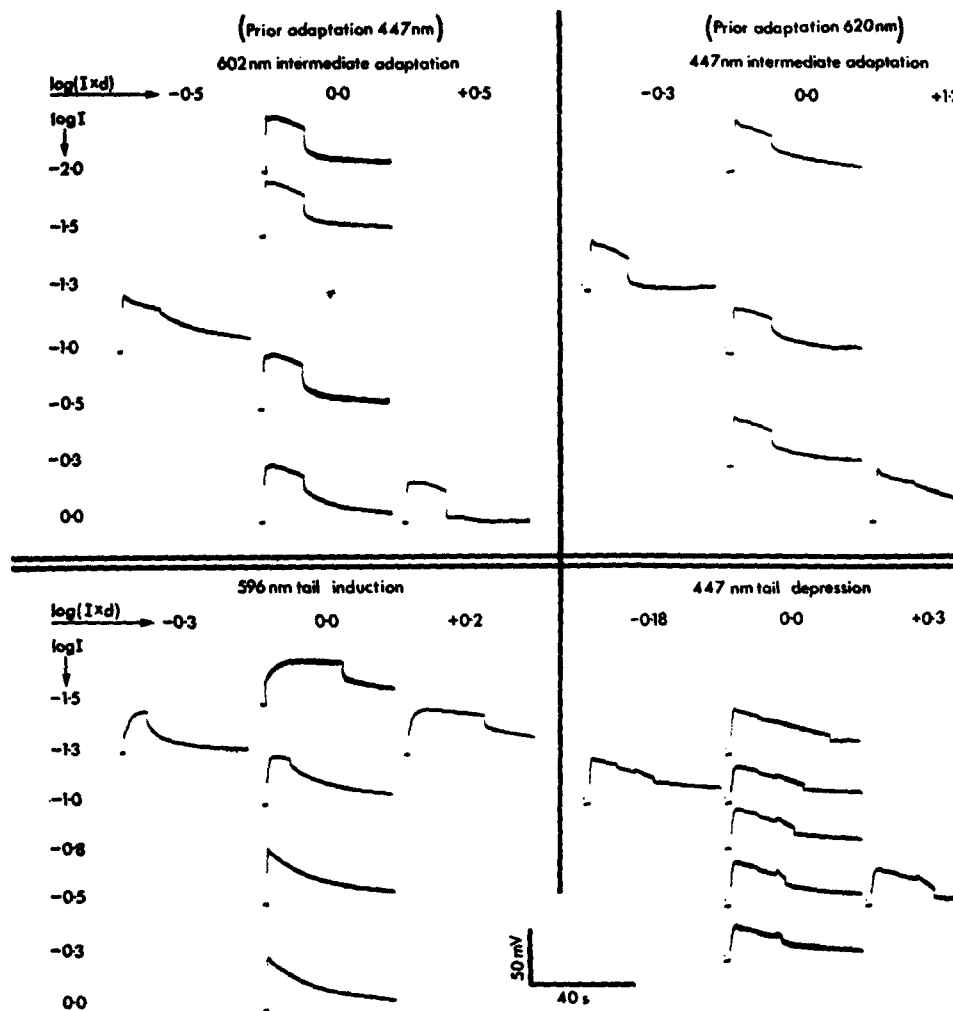
In examining the quantitative dependences of the tail height on adaptation and stimulation intensity and duration, we found a kind of "Bloch law" applicable—that is, that the response depends only on the product of intensity and duration as noted above. Fig. 6 illustrates some of the observations. The bottom two quadrants are designed to show that the tail height depends, to a good approximation, only on the product of intensity and duration of the tail-inducing reddening stimulus (bottom left quadrant, compare top of Fig. 3) or of the tail-depressing blueing stimulus (bottom right quadrant, compare bottom of Fig. 3) for fixed stimulus wavelength and fixed state of adaptation. This law was found to hold at least for stimuli of duration reasonably short compared with the tail length. The central column in each quadrant shows the responses to stimuli of various intensities  $I$  as indicated on the left, but with durations  $d$  chosen to make amount  $I \times d$  constant (defined as  $\log [I \times d] \equiv 0$ ) for this column. The traces to the left and right of the center columns, for lesser and greater amounts, demonstrate the sensitivity to differing amounts, as against the insensitivity to differing intensities and durations for fixed amounts. (Because most of the responses illustrated were for stimuli not *very* short compared with the tail length, the tail height should *not* be measured a fixed time after the stimulus, but after some weighted mean stimulus time—but this uncertainty does not appreciably affect the conclusion.)

The top two quadrants illustrate that a similar law is applicable to the (intermediate) adaptation for tail-induction. The top left quadrant is for intermediate red adaptation after prior blue adaptation (compare right column of Fig. 4) and the top right quadrant is for intermediate blue adaptation after prior red adaptation (compare left column of Fig. 4). In both quadrants, the response shown was elicited by a fixed red saturating stimulus. Here there appeared to be no upper limit (at least in the range of many minutes) to the adaptation duration for which the law remained valid.

Similar but less detailed experiments indicate that the same law applies also to anti-tail induction stimuli (of duration short compared with the anti-tail) and intermediate adaptation for anti-tail induction.

The quantitative dependence of the tail and anti-tail effects on stimulus or adaptation amount is displayed in Fig. 7. In each graph, the abscissa is linear absolute light amount and the ordinate is the logarithm of  $[R(A) - R(\infty)] / [R(0) - R(\infty)]$ , where  $R(A)$  is the tail height for the given (stimulus or adaptation) amount, and  $R(0)$  and  $R(\infty)$  are the heights for zero and saturating amounts. Note that  $R(0)$  is zero for tail induction and near zero for blue intermediate adaptation for tail induction and anti-tail induction, while  $R(\infty)$  is near zero for the remaining cases.

The circles in Fig. 7a show the dependence of tail height on amount of tail-inducing red (620 nm) stimuli (as in upper part of Fig. 3), and the squares and diamonds the tail-height dependence on amount of red intermediate adaptation for tail induction (as in Fig. 4) and anti-tail induction (as in Fig.



5), respectively, all starting with saturating blue (prior) adaptation. Similarly, Figs. 7 *b* and 7 *c* (for 543 and 447 nm, respectively) show the dependence of tail height on amount of tail-depressing blue stimuli (triangles) (as in lower part of Fig. 3) and the tail-height dependence on amount of blue intermediate adaptation for tail-induction (squares) (as in Fig. 4) and anti-tail induction (diamonds) (as in Fig. 5), all starting with saturating *red* (prior) adaptation. Data from experiments in four separate cells are presented.

It would probably be more significant to plot conductance rather than potential, but we have not attempted the correction. Further uncertainties arise from the effects of the electrogenic pump (Koike et al., 1971) and from the uncertainty of a factor of 2 in the absolute light intensities.

In each graph a straight line is introduced which represents the dependence of the early receptor potential (ERP) amplitude on amount of intermediate adaptation of the same wavelength, and with the same prior adaptation, as for the LRP. The ordinate is the same function of change of ERP amplitude as given above for the LRP tail amplitude and the lines are taken from graphs like those of Fig. 3 of the preceding article.

FIGURE 6. Demonstration that the tail height depends only on the product of intensity and duration of tail-inducing stimulus, of tail-depressing stimulus, or of intermediate adaptation. *B. amphitrite*. All responses in this figure are from the same cell. In each quadrant, all responses in the central column are for the same amounts (products of intensity  $I$  and duration  $d$ ) of intermediate adaptation (upper quadrants) of tail-inducing stimulation (lower left quadrant), or of tail-depressing stimulation (lower right quadrant). The side traces in each quadrant are for light amounts  $I \times d$  smaller and larger than those of the central column by the ratios whose logarithms are given above those traces. The logarithms of the corresponding intensities ( $\log I$ ) are shown on the left.  $\log I = 0$  is the intensity given in Table I of the preceding article for the 596 nm filter (bottom left quadrant), 447 nm filter (right quadrants), and 602 nm filter (top left quadrant).  $\log (I \times d) = 0$  is defined as 1 s of  $\log I = 0$  light except for the tail depression quadrant, where  $\log (I \times d) = 0$  is defined as 1.5 s of  $\log I = 0$  light. The light amounts used for the central columns were chosen to make the test most sensitive, that is, the dependence of tail height on  $I \times d$  is steepest around these values. In the top left (right) quadrants the cell was first fully prior-adapted to 447 nm (620 nm) illumination. The cell was then exposed to various combinations of intensities and durations of intermediate-adapting 602 nm (447 nm) light as indicated. A fixed strong red stimulus (620 nm) then induced the responses shown. In the bottom left quadrant ("tail induction") the responses are of the cell, when fully blue-adapted, to strong red stimuli (596 nm) of various intensity-duration combinations as indicated. In the bottom right quadrant ("tail depression") the responses are to two successive stimulations of a previously fully blue-adapted cell. A fixed strong red tail-inducing stimulus (620 nm) is followed by 447 nm stimuli of various intensity-duration combinations as indicated. The response had begun to decline during this final set of runs on this cell (this quadrant). The calibration axes apply to all responses. The initial base line has been strengthened for clarity in some traces.

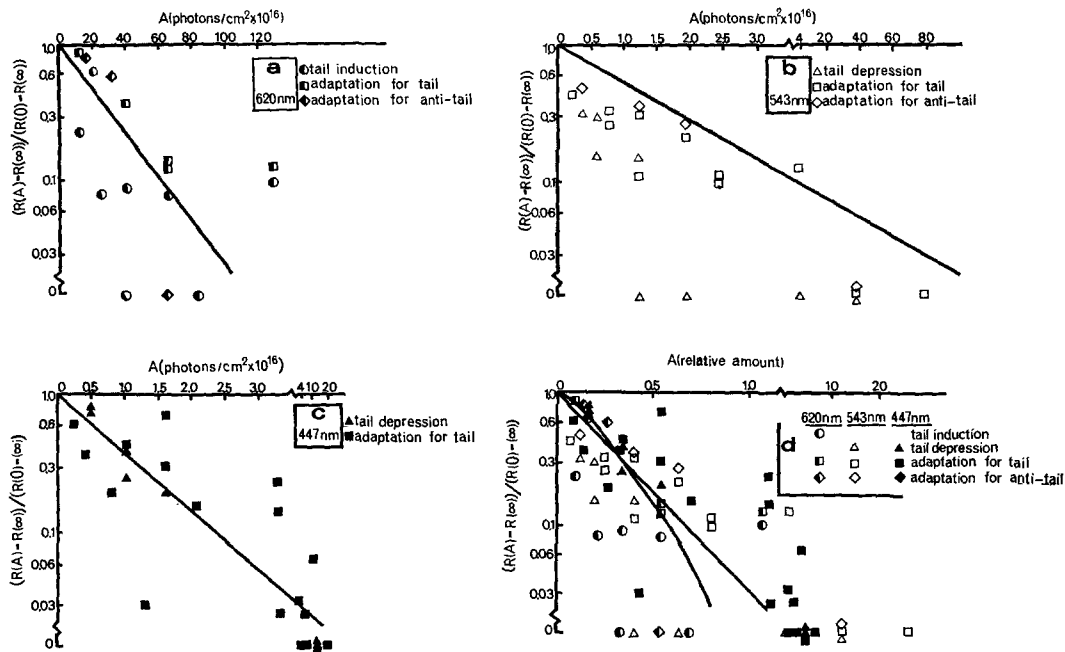


FIGURE 7. The dependence of tail amplitude on stimulus or adaptation amount. *Abscissa*, linear light intensity (absolute in *a*, *b*, and *c*, relative in *d*). Ordinate:  $[R(A) - R(\infty)]/[R(0) - R(\infty)]$ , logarithmic scale, where  $R(A)$  is the tail height as a function of amount  $A$  of (1) tail-inducing red (620 nm) light, procedure exactly as in upper part of Fig. 3: Circles, graph *a*. (2) intermediate-adapting red (620 nm) light for tail induction, blue prior-adapted cell, procedure as in right column of Fig. 4: Squares, graph *a*. (3) intermediate-adapting red (620 nm) light for anti-tail induction, blue prior-adapted cell, procedure as in right column of Fig. 5: Diamonds, graph *a*. (4) tail-depressing blue light, procedure as in lower part of Fig. 3: Triangles, graphs *b* and *c* (543 and 447 nm tail-depressing light, respectively). (5) intermediate-adapting blue light for tail induction, red prior-adapted cell, procedure as in left column of Fig. 4: Squares, graphs *b* and *c* (543 and 447 nm intermediate-adapting light, respectively). (6) intermediate-adapting blue (543 nm) light for anti-tail induction, red prior-adapted cell, procedure as in left column of Fig. 5: Diamonds, graph *b*. Thus, all the points in graph *a* are for 620 nm stimulation or adaptation, after saturating 447 nm light, and in graphs *b* and *c* for 543 and 447 nm, respectively, after saturating 620 nm light. The dependence of the same function of ERP amplitude on intermediate adaptation amount (Fig. 3 of preceding article) for the same combination of wavelengths of prior and intermediate adaptation is shown as a straight line in each graph. Graph *d* reproduces all the points from graphs *a-c*, with amount scales adjusted for greatest overlap of the sets of points. An approximately best-fit straight line is drawn corresponding to a linear dependence of response height on pigment change, and also a best-fit square dependence of response on pigment. See text. Note the breaks in the abscissae, from where the scales are multiplied by a factor 40, and the break to 0 at the bottom of the ordinate scale.

In order to display the shape of the amount dependence as clearly as possible, the points from graphs *a*, *b*, and *c* are replotted together in Fig. 7 *d* after adjusting their relative amount scales to bring the sets of points most closely into coincidence (see Discussion).

The amplitude of the steady state of the stimulus-coincident LRP for neutral stimuli depends, of course, on intensity and not on amount. On a graph like those of Fig. 7, but with intensity replacing amount, this amplitude would plot as an L-shaped curve, downwards near the left axis and then across near the bottom of the graph.

#### 4. Criterion Action Spectra

We have measured the criterion action spectra for the stimulus-coincident LRP, for the tail induction, and for the tail depression.

The stimulus-coincident response criterion action spectrum is measured by finding, for a cell in a given state of adaptation, the *intensity* of stimulation at each wavelength needed to induce a response with the same criterion steady-state amplitude. This action spectrum in our preparation has previously been carefully measured, in cells adapted to white light, by Stratten and Ogden (1971) and by Shaw (1972), using techniques slightly different from ours. They found spectra approximating Dartnall nomograms (Dartnall, 1953) peaked, respectively, at 535 and 530 nm. Our measurements for red- and blue-adapted cells are in substantial agreement with each other and with their results.

The tail-induction criterion action spectrum is the wavelength-dependence of the reciprocal of the *amount* of light needed at each wavelength to induce a small criterion height and duration of tail in a fully blue-adapted cell. The cell was always left in the dark, after the adaptation, for a time long compared with the duration of the anti-tail. The results of three experiments on different cells were averaged and then normalized for best fit to the ERP 532 state action spectrum taken from Fig. 5 of the preceding article. The open squares in Fig. 8 are the tail-induction criterion action spectrum points, while the triangles are the ERP 532 state action spectrum and the curve is a 532 nm Dartnall nomogram, both of the latter normalized as in Fig. 5 of preceding article. The inset shows superposed responses illustrating the criterion used in one of the experiments and the responses to stimuli 0.2 log stronger and 0.3 log weaker than that eliciting the criterion tail.

The tail-depression criterion action spectrum is determined by measuring the amount of light needed at each wavelength to produce a small criterion depression of an always identically prepared tail. The procedure consisted of inducing a maximal tail by strong reddening light. 10 s later, a brief test flash was given at the wavelength under examination, and the degree to which the tail was depressed after the flash was measured. The reciprocals of

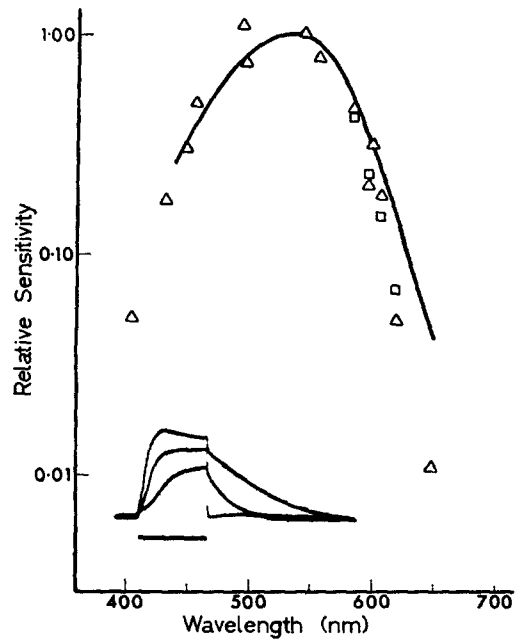


FIGURE 8. Tail induction criterion action spectrum. *B. eburneus*. The relative sensitivity, the reciprocal of the relative number of photons required to elicit a criterion tail, is plotted on a logarithmic scale against stimulus wavelength (open squares). Only red and orange stimulation could elicit any tail. The cell was first blue-adapted (447 nm, 15 s). After 3 min of darkness, the cell was stimulated at each wavelength with a particular intensity and the procedure repeated with different intensities until a criterion tail height was obtained. All points are averages of two independent measurements on different cells. The insert shows the criterion response chosen for one of the sets of measurements, and responses to stimuli 0.2 log stronger and 0.3 log weaker. (Note that the upper trace after the stimulus is a continuation of the upper trace during the stimulus, etc.) For comparison, the action spectrum for the ERP of the 532 state is shown (triangles, taken from Fig. 5 of the preceding article) as well as a Dartnall 532 nm nomogram, both normalized as in preceding article. The tail induction points are normalized for best fit to the ERP points.

the light amounts giving a criterion depression at each wavelength form the action spectrum. The results of two different experiments normalized to 1 at their peaks were averaged and the averages are shown as filled squares in Fig. 9. Also shown are the ERP 495 state criterion action spectrum (open circles, taken from Fig. 5 of the preceding article) and a 495 nm Dartnall nomogram, both also normalized to 1 at their peaks. The inset shows superposed responses illustrating the criterion used in one of the experiments and the responses to stimuli 0.3 log stronger and 0.2 log weaker than that eliciting the criterion tail depression. In the measurements of both the tail-induction and the tail-depression spectra, the stimulus duration was necessarily short compared with the tail decay time.



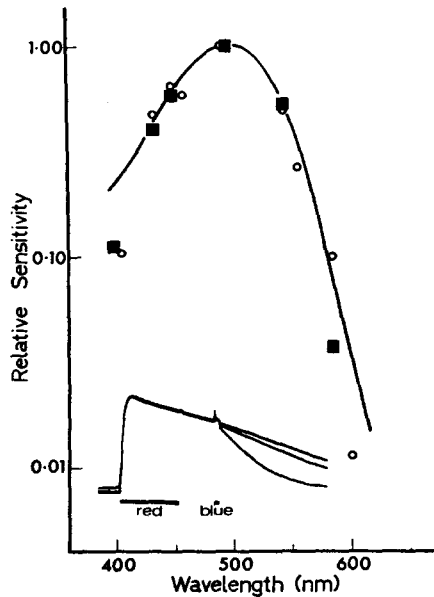


FIGURE 9. Tail depression criterion action spectrum. *B. amphitrite*. The relative sensitivity, the reciprocal of the relative number of photons required to elicit a criterion depression of a tail, is plotted on a logarithmic scale against stimulus wavelength (filled squares). The cell was initially adapted to blue light (447 nm, 15 s). After 3 min in the dark, a tail was elicited by reddening stimulation (620 nm, 15 s). 7 s after cessation of this red stimulus, the cell was illuminated at a particular wavelength and intensity, and the degree of depression of the tail after this was observed. The procedure was repeated at each wavelength until a criterion depression was obtained. All points are averages of two independent measurements on different cells. The insert shows the criterion response chosen for one of the sets of measurements, and the responses to stimuli 0.3 log stronger and 0.2 log weaker. For comparison, the action spectrum for the ERP of the 495 state is shown (circles, taken from Fig. 5 of the preceding article) as well as a Dartnall 495 nm nomogram. The two sets of points and the curve are all normalized to 1 at 495 nm.

In all criterion action spectra, selected measurements showed the spectrum shape to be independent of the criterion chosen as long as the stimulus was weak. Stimulus weakness insures that the state of the cell not change appreciably during the stimulus. This is necessary since stimuli of different wavelengths will in general affect the cell in different ways, and a criterion action spectrum requires that the cell be in the same state for all wavelengths of test stimulus, throughout the stimulation. We showed that this requirement was fulfilled by checking that, if the same stimulus was applied twice in succession, the second response (tail or tail-depression) was nearly the same as the first.

All measurements were reproducible within  $\pm 0.1$  log unit in any one experiment. The scatter among different experiments, after normalization at their peaks, was up to  $\pm 0.2$  log unit.

## DISCUSSION

1. *Antagonistic Pigment-LRP Coupling Processes*

The observations described in the results show that, with respect to the LRP, the barnacle photoreceptor can exist in more than one dark-stable state, depending on the wavelength of the adapting light. Since the same was shown to be true for the ERP in this preparation (preceding article) we wish to determine whether the same pigment is responsible for both sets of phenomena and to establish as far as possible the character of the contribution to the LRP of each part of the pigment process as seen in the ERP.

We first note that the stimulus and adaptation wavelength region over which most of the LRP changes occur is the same as that for the ERP, namely 550–600 nm (ERP, preceding article, Fig. 2; LRP tail-induction and depression, Fig. 3, this article; intermediate adaptation for tail and for anti-tail, Figs. 4 and 5, respectively). Below 550 nm and above 600 nm, both the ERP and the LRP effects appear to be nearly independent of wavelength.

Further proof for the identity of the pigment responsible for the ERP and the LRP phenomena comes from a comparison of the action spectra. The ERP shape dichotomy depends on the pigment molecules having two stable states, called the 532 and 495 states after the wavelengths (in nanometers) of the peaks of their Dartnall nomogram-shaped absorption spectra. The weak-stimulus criterion action spectrum for the tail depression by blueing light agrees well with the 495 state spectrum (Fig. 9). Since the stimulus must be blueing, points are only measurable below 600 nm, but this is enough to establish well the identity of the spectra. The action spectrum of the stimulus-coincident LRP of weak neutral light (Stratten and Ogden, 1971; Shaw, 1972), agrees well with the 532 state spectrum. Because of the limited range in which the tail induction action spectrum can be measured (the requirement for reddening stimuli limits it to points above 550 nm), its agreement with the ERP action spectrum of the 532 state (Fig. 8) is not too significant. (Note that it is not possible to measure the criterion action spectra of the induction and depression of an anti-tail, since the response to the test red stimulus would depend not only on the strength of the anti-tail, but also on the state of the pigment. These both vary with degree of blueing and one cannot separate their effects.) Nevertheless, the good agreement between the stimulus-coincident and the tail-depression action spectra with the 532 and the 495 ERP spectra, respectively, together with the correspondence of the critical 550–600 nm wavelength range noted above, may be taken as adequate proof that the same pigment is responsible for the ERP and for all the LRP phenomena.

The action spectra then clearly assign the stimulus-coincident response to activation of the 532 state, and the tail depression to activation of the 495

state of the pigment. The tail induction cannot arise from activation of the same state as is responsible for its depression, since one requires reddening and the other blueing, so tail induction must be assigned to activation of the 532 state. Similarly the anti-tail must arise from activation of the 495 state.

Thus the tail and stimulus-coincident LRP must arise from the same process, modified by the continued presence of stimulation in the latter case; and the tail-depression and anti-tail must also arise from the same process, differing only in the presence or absence of a tail as the stimulus is applied.

Now we have noted that neutral stimuli of any intensity do not modify either tails or anti-tails. Such stimuli of course do activate both stable pigment states, but in such a way as not to change the final population distribution. A tail is thus induced only when a *net* change occurs in pigment state populations in the direction of increased 495 population. An anti-tail or a tail depression is the result of a reverse change in pigment population.

The existence of two antagonistic effects (the tail on the one hand and the anti-tail and tail-depression on the other) as well as the dependence on *net* change imply a balance between two antagonistic processes. That is, at some stage between the pigment activation and the appearance of a response, the processes responsible for the tail and the anti-tail (and tail-depression) must be capable of mutual neutralization (see "Model" below).

The net change in the populations of the stable states in general depends on the degree of activation of both states. The reason the action spectra for the tail induction and depression agree with those for the two stable states, respectively, is that in each case only one of the two states is activated (see the Discussion of the preceding article). The output of whichever state is activated is then proportional to the net change of the populations of both states and this change thus has the action spectrum of the activated state.

Note that the stimulus-coincident LRP cannot depend on net pigment change, since it is also excited by neutral stimuli, and since its steady-state amplitude depends on intensity and not on amount. The latter statement is equivalent to saying that the response integration time for the stimulus-coincident LRP is very short (cf. Bloch's law); the integration time for the tail phenomena, in contrast, are at least of the order of many seconds, as shown in Fig. 6. The integration time for the *adaptation* for the tail appears to be very long (Fig. 6) linking the state of adaptation to the pigment, which also has a very long adaptation integration time (Fig. 3 of preceding article); that is, the pigment states are very stable.

We have thus demonstrated two new phenomena in the barnacle photo-receptor which we have called the tail and the anti-tail. We have shown that (a) these have different action spectra; (b) either can precede the other with reciprocal antagonistic effects; (c) each has a separate duration; and (d) neither duration is related to the pigment (ERP) kinetics. From this it is clear

that the two processes are separate, that one is not simply the reversal of the other, and that neither is purely a pigment process.

## 2. *The Functional Dependences of the Tail Phenomena*

We divide the analysis of the functional dependence of response (tail-height or anti-tail strength) on stimulus or adaptation amount into stages: (a) the dependence of the pigment change on stimulus (adaptation) amount; (b) the dependence of the antagonistic processes on pigment change; and (c) the dependence of the response amplitude on process strength.

(a) We demonstrated in the preceding article (Fig. 3) that the amplitude  $R(A)$  of the ERP at any specific time after onset of a fixed stimulus approaches its saturated value  $R(\infty)$  exponentially as a function of amount  $A$  of adapting light. That is,  $R(\infty) - R(A) = [R(\infty) - R(0)] e^{-KA}$ , where  $K$  is a constant. Since the ERP is a direct manifestation of the pigment state populations, the same dependence must apply to the populations of each of the two stable pigment states and hence also to the integrated difference between the input and output (the net change) of each. (It can be shown theoretically that this should apply to most bistable systems).

(b) We noted in the Results that the response after a stimulus series of duration short compared with the durations of the tail and anti-tail depends only on the initial and final states of adaptation. It is difficult to establish this completely quantitatively, since some tail and anti-tail decay always occurs during the course of the series, but the approximate validity is seen in Fig. 1 and also in a comparison of corresponding traces of Figs. 4 and 5. Although the adaptation conditions are only approximately the same in the latter two figures, the tail may be seen to be at least qualitatively unaffected by interjection of blue stimuli (Fig. 5). In any case, no cumulative effect on the final response was seen no matter how many, and what kind of, changes of adaptation were interjected.

This apparently exact cancellation of the effects of all intermediate pigment changes strongly suggests that both antagonistic LRP processes are linear with pigment change and also that their mutual neutralization is in the same ratio as their activation per unit pigment population shift.

(c) By plotting the effect change on a logarithmic scale against linear light amount in Fig. 7, we unfold the exponential dependence of the pigment change on light amount from the overall result, leaving the dependence of the response amplitudes on the processes. The result is fully consistent with linearity, the scatter of the points allowing adequate fits to power dependences with any power  $n$  between about 0.3 and 2.0. There appear to be no systematic differences in either the scale or the shape of the dependences of the various types of experiments.

A linear dependence is physiologically reasonable. It is not clear what

physiological mechanism could account for a power dependence with non-integral  $n$ . An integral  $n > 1$  could presumably arise from a cooperative mechanism. A representative curve for  $n = 2$  is displayed in Fig. 7. The fit of the points to this curve appears less good than to a linear dependence ( $n = 1$ ). Curves for  $n > 2$  give much worse fits.

An alternative source of nonlinearity would be a *saturation* of the process-response coupling (for instance, a saturation of the conductance channels). If we assume this saturation to manifest itself in an exponential dependence of response on process, its effect would be to shift the points in Figs. 7 *a*, *b*, and *c* towards the bottom left with respect to the ERP line. We have calculated the quantitative effects of such saturations. Taking into account the uncertainty of a factor 2 in the absolute scale, and the fact that the points in one of the graphs do fall below the ERP line (though not by significantly more than a factor 2), we conclude from the degree of absolute agreement between the points and the lines that the process-response coupling does not saturate at least up to light levels half those needed to saturate the pigment. Of course, a saturation occurring just in this range would be coincidental (although teleologically appealing). We therefore conclude that the dependence of tail height (actually, probably membrane conductance) on the strength of the exciting process, and hence on pigment change, is probably linear and non-saturating in the range up to pigment saturation.

### 3. A Model

The conclusions of section 1 of the Discussion make Nolte and Brown's model (see Introduction) inapplicable to the barnacle. The two critical observations on which these conclusions are based and which are not reported by Nolte and Brown are: (*a*) the requirement that the tail-inducing stimulus be non-neutral, and (*b*) the existence of the anti-tail phenomenon. In the case of the *Limulus* median ocellus, the experiments would be: (*a*) does a further UV stimulus after the decay of a saturated UV-induced extended depolarization induce a further extended depolarization? and (*b*) if not, does an extended depolarization result from a UV stimulation closely following a visible adaptation which in turn follows the decline of an extended depolarization? Negative answers to the first or to both these questions would invalidate their model for their own preparation as well.

Based on the conclusions of sections 1 and 2, we are able to construct the following model for the barnacle eye:

The simplest basis for a linear dependence of the processes responsible for the tail and the anti-tail on net pigment *change* would be that these processes are, respectively, proportional to the input and output of the 495 state of the pigment (or the output and input of the 532 state) and that they neutralize each other in equal quantities. (The outputs of both states would be less suit-

able since neutral stimuli do not in general induce equal outputs.) We therefore propose that flipping the pigment molecules *into* the 495 state releases an "excitor," while flipping molecules *out* of that state releases an "inhibitor." The excitor opens a membrane conductance channel, while the inhibitor has no direct effect on the conductance, but only neutralizes, and is neutralized by, the excitor in equal quantities. (The inhibitor should therefore perhaps more properly be called an "excitorase.") This neutralization is assumed to take place rapidly but not instantaneously. In the absence of neutralization, each material has a (different) long but finite lifetime.

The tail then arises when a reddening stimulus produces an excess of excitor. The anti-tail is induced by a blueing stimulus producing an excess of inhibitor. If the reddening stimulus occurs during an anti-tail, that is in the presence of a large quantity of inhibitor, the excitor produced will be neutralized by (and will neutralize) the inhibitor, and no tail will result. If the blueing stimulus occurs during a tail, that is in the presence of a large quantity of excitor, the inhibitor produced will neutralize (and be neutralized by) the excitor, depressing the tail but producing no anti-tail. The tail and the anti-tail decay in the dark with the lifetimes of the excitor and the inhibitor, respectively.

Neutral stimuli produce equal quantities of excitor and inhibitor. These rapidly neutralize each other, so that no tail or anti-tail is produced or depressed. During and shortly after the stimulus, however, because of the finite neutralization time, a certain quantity of excitor (and of inhibitor) is always present. Thus all stimuli will induce stimulus-coincident LRP's. Blueing stimuli produce little excitor but much inhibitor and should give smaller stimulus-coincident LRP's than if the same stimulus were neutral (that is, after blue adaptation) (compare first and second responses of trace *h* of Fig. 1). Similarly, any stimulus occurring during an anti-tail should produce depressed stimulus-coincident LRP's (compare second with later responses in trace *d* of Fig. 1, and also second responses of traces *g* and *h*).

Finally, the stimulus-coincident LRP due to a reddening stimulus (producing mainly excitor) should be larger than for a neutral blue stimulus (third and second responses of trace *h* of Fig. 1) even though the amount of 532 state activation by the latter is similar to that by the former as calculated from the action spectrum of that state.

If the membrane conductance is now assumed to be proportional to excitor concentration, the model predicts a linear dependence of the conductance during the tail on net pigment change, which is a linear dependence on light amount for not too large amounts. *During* a neutral stimulus, however, the steady-state response to lights not running through an appreciable fraction of the pigment will clearly *not* be linear with light intensity: the production rates of both excitor and inhibitor will be proportional to light intensity, but the

average excitor lifetime will decrease with increasing inhibitor concentration, so the excitor concentration will increase less than linearly with light intensity.

This model therefore explains the presence of the LRP with and without a tail according to different states of color adaptation and stimulation, and predicts a linear dependence of the tail amplitude on light amount (for not too large amounts) and a nonlinear dependence on intensity of the amplitude of the steady-state stimulus-coincident LRP, in agreement with experiment.

#### CONCLUSION

While we cannot firmly identify the 532 and 495 states of the barnacle visual pigment with conventional names, we suggested in the preceding article that they may be rhodopsin and a metarhodopsin, respectively. If so, our model suggests that the input to the metarhodopsin is responsible for the excitation, while the output from the metarhodopsin, which is proportional to the amount of the metarhodopsin present, is responsible for the inhibition. If the metarhodopsin involved is Meta II, this is in good agreement with previous proposals that the Meta I  $\rightarrow$  II transition is the source of visual excitation (Abrahamson and Ostroy, 1967; Falk and Fatt, 1968; Bonting and Daemen, 1969; Poincelot et al., 1969), and that the inhibitory contribution to the LRP arises from the presence of metarhodopsin II (see Introduction).

We note that a barnacle probably never sees a tail or an anti-tail in nature. The necessary intensities are present, but it is difficult to find possible sources of the necessary successions of colors.

We point out finally that this preparation offers a possible model of memory processes, as it appears to be the first time a fast, reversible, permanent, multi-stable single cell "memory" unit activated under physiological conditions has been seen in an animal, and its basis as a sum of molecular flip-flops demonstrated. (The bi-stable phytochrome system in plants [Borthwick et al., 1952; summary in Gardner et al., 1971] is the closest previous parallel, but that system has not yet been extensively investigated at the cellular level.) Clarification of the structural basis of this molecular bi-stability by biochemical, photometric, and other methods seems highly desirable.

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