A compartment model for vertebrate phototransduction predicts sensitivity and adaptation

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ABSTRACT

The different sensitivity and adaptation properties of vertebrate rods and cones are best explained by a compartment model where the number of compartments constitutes the essential difference between the two types of photoreceptors. For the cones, the natural compartment is a single fold of the plasma membrane; for the rods, the size of the compartment had to be defined empirically. The interesting aspect of this model is that the number of compartments controls the amplitude of the single photon response, the intensity response, and together with the decay time constant of the single photon response, the adaptation properties.

INTRODUCTION

Most vertebrate retinae are duplex in the sense that they contain both rods and cones. Cones have a high threshold, contain different visual pigments, support color vision, and can work under high ambient luminosities. On the other hand, rods have a low threshold, contain a single visual pigment, and operate only at low ambient luminosities. The biochemical machinery which supports visual phototransduction has been the focus of intensive studies over the past ten years and is still an active field of research [40, 54, 7, 59, 23, 30, 16, 28]. Although minor differences have been found between the proteins involved in the phototransduction cascade of rods and cones, none of these differences offers a satisfactory explanation of the above fundamental differential behavior. Furthermore, the rods are believed to have evolved from the cones [58], and possibly kept the same biochemistry. However, following the lead of Forti et al. [15] in simulation studies, Ichikawa [25, 26] has been able to mimic

important aspects of the differences in gain and kinetics between rods and cones by manipulating the rate constants of the reactions in the cascade.

This paper presents a compartment model of vertebrate phototransduction and proposes that the major difference between rods and cones is the number of compartments present in their respective outer segments and that this single difference not only explains the size of the single photon response and the shape of the intensity response curve, but also, together with the decay characteristic of the response, the adaptation behavior, that is the reduced sensitivity under non-bleaching background conditions. The only other requirements are that a single isomerization closes all the cationic channels associated with a compartment and that the current decay represents an underlying limiting process. It must, however, be understood that this is a first approximation model in the sense that a number of experimental results are ignored in order to make the model mathematically tractable. An initial account of this model has been presented elsewhere [46].

THE MODEL

The nature of the compartment

For the purpose of this model, a compartment does not necessarily need to be a physical compartment in the sense that physical barriers such as membranes would be required to define its boundaries, it can be an operational compartment. An example of such an operational compartment could be the length of tube where a bolus of radioactive nuclei, moving at a given speed, loose 90 % of its radioactivity. In this sense, any molecule or protein with a finite lifetime operates within a compartment. Thus excited rhodopsin, excited transducin, and excited phosphodiesterase all operate within compartments whose size depends on the diffusion properties of these molecules and on their respective lifetimes. It is therefore conceivable that the outer segment of both rods and cones is made up of compartments which are biochemically isolated from each other but electrically connected in a linear fashion such that current changes in different compartments can add algebraically.

The size of the response

The model makes the additional hypothesis that the action of a given photon which produces an isomerization is maximal. This means that two or more simultaneous isomerizations within the same compartment will not produce an effect greater than a single isomerization. The kinetics of the risetime of the

response may change with multiple isomerizations but the maximal amplitude will be the same as that of a single isomerization. The consequence of these two hypotheses is that, if an outer segment, made up of N compartments, carries a dark current of magnitude J, then the single photon response will be J/N. Furthermore, the shape of the intensity response will be of the exponential saturation type regardless of the shape of the single photon response.

This model was first proposed by Lamb et al. [35] and follows from the following argumentation. Suppose that a flash of intensity I photons/square microns affects n different compartments in an outer segment made of N compartments, the fractional response to this flash will be n/N. This number (n/N) also represents the probability that a compartment is the locus of one or more isomerizations, and $(1 - n/N)$ is the probability of no isomerization in a compartment or zero event. For a flash of light illuminating the whole outer segment, the absorption of photons can be considered a Poisson process because individual absorptions are independent of each other, and because the incremental probability of absorption is proportional to the incremental size of the photon flux density . This last requirement is met if the optical density is constant along the outer segment and the geometry of the outer segment is considered cylindrical. The probability of zero event in this Poisson process is $Exp[-kI]$. In this expression, k represents the cross-section in square microns for isomerization of the compartment and I the intensity of the flash expressed in photons per square micron. By simple substitution, one finds that the fractional response r/r_{max} is given by the following formula which is illustrated in Fig.1:

$$r/r_{max} = n/N = 1 - Exp[-kI]$$

The above results, that is the size of the single photon response and the shape of the intensity response curve are entirely independent of the shape of the single photon response. The presence of the exponential in this formula comes from the equation describing the Poisson statistics and not from the shape of the response as described by the kinetics of the biochemical cascade. This point is made because Pugh and Lamb [45] have shown that for $t \ll t_{peak}$, $r(t)/r_{max}(t)$ also shows exponential saturation under certain assumptions regarding the cascade kinetics. However this latest analysis does not yet give a solution when $t = t_{peak}$.

The action of background light on the sensitivity

In order to predict the sensitivity change in this system as a function of background light, it is important to known the time course which characterizes the recovery of the compartment after it has been hit by a photon; that is select a shape for the single photon response. I have chosen the simple exponential $Exp[-t/T]$, where T is the time constant of the exponential current decay. The implications of this choice is that the current response represents a limiting process. No other process having a slower time course should affect the recovery

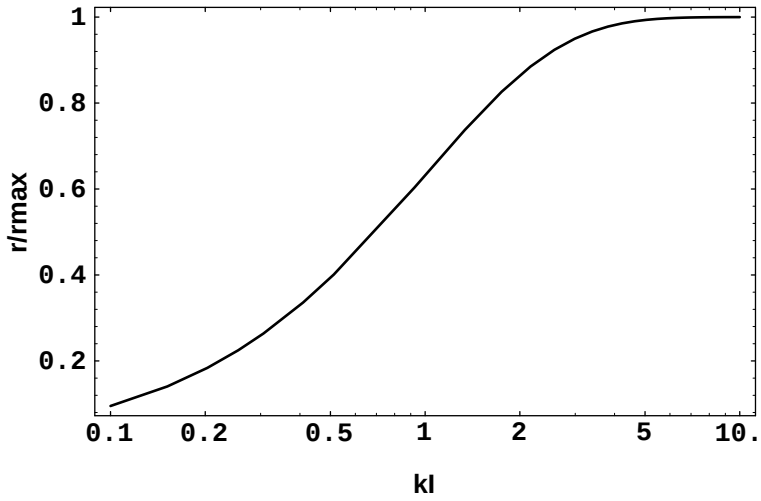

Figure 1: Normalized response as a function of stimulus intensity in units of kI

under this hypothesis. One such process is pigment regeneration which takes place with a time course of the order of minutes. Since I am considering a non-bleaching situation, it will not affect the model. I am also ignoring that the decay time changes to a certain extent as the background intensity increases [50].

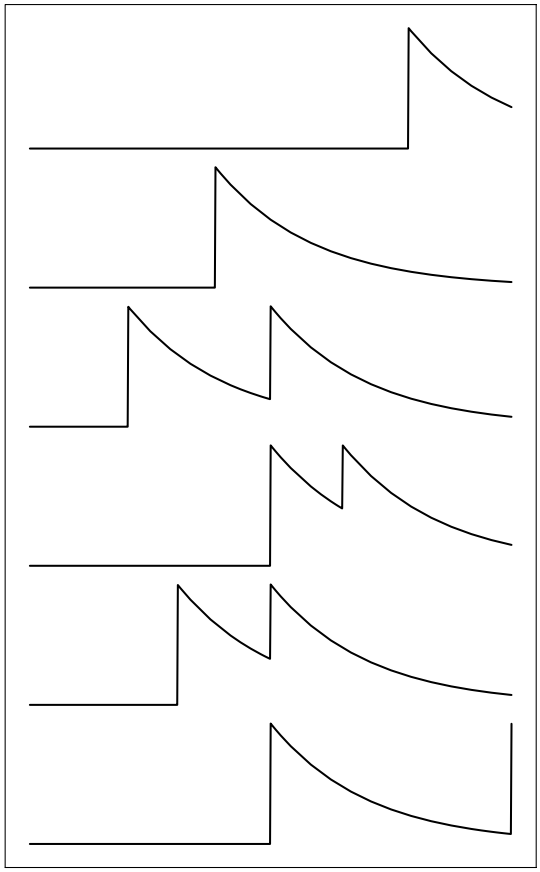

Figure 2: Background simulation

The approach used to analyse the effect of background light is novel and uses a theorem in statistical mechanics called the "*ergodic theorem or hypothesis*" (McQuarrie,[38]) which states that "*for a stationary random process, a large number of observations made on a single system at N arbitrary instants of time have the same statistical properties as observing N arbitrary chosen systems*

at the same time from an ensemble of similar systems" . If the action of a steady background light on an outer segment can be considered stochastic and stationary, I can apply the ergodic hypothesis by equating compartments to systems.

Due to the absorption of photons which have led to isomerization, a certain number of compartments are not in the dark-adapted state but rather in a process of exponential recovery during which their sensitivity is reduced. The contribution of these compartments to a response of a test flash will be different for each compartment and depend on how long ago each compartment has been the site of an isomerization due to background light. This is illustrated in Fig.2. This figure illustrates the effects of a test flash producing four isomerizations (identified by dots at the peak of the response) in four out of six different compartments. Background photons hit compartments #1 and #2, while the other compartments are hit by both background photons and test photons which occur in the middle of the trace. In compartments #4 and #6, which are completely dark-adapted, the response is maximal, in the other two (#3, #5), the response amplitude depends on how long ago each compartment has been hit by background light. The sum of these responses divided by four gives the mean response per isomerization for that very specific background situation. This represents "*observing N arbitrary chosen systems at the same time from an ensemble of similar systems"* in the ergodic hypothesis. If N is large, I would have a very good estimate of the single photon response of my system under a given background. The "*ensemble average*", as I have done above, can be substituted by a "*time average*" of the observations done on one compartment as described below.

In an outer segment, made of N identical compartments which are under a total background generating I_{bk} isomerizations per second, each compartment is subject to random isomerizations at a rate equal to I_{bk}/N. To measure the sensitivity of a given compartment, I only need to observe the responses to N isomerizations occurring randomly in time and to average the responses since all compartments are considered identical. Actually these isomerizations need not come from "test photons"; the photoreceptor cannot tell whether the photon comes from the background or the test flash. I only need to observe the response to N random izomerizations due to photons belonging to the background to test the sensitivity of the system. This part represents "*a large number of observations made on a single system at N arbitrary instants of time"* in the ergodic hypothesis. Because of the nature of light, the intervals between isomerizations in a given compartment are arbitrary and Poisson distributed, and the mean interval is N/I_{bk} second. After a given isomerization, a compartment will start its recovery process with a time constant T and will be the locus of another isomerization after, on the average, a time interval equal to N/I_{bk}. To find out the mean response to such a process, using Mathematica, I ran a series of computer simulations. Using the "ExponentialDistribution[ ]", I generated 5000 Poisson distributed random intervals having a mean rate of 5 per sec., each event gen-

erating a step of amplitude one decaying exponentially with a time constant of 200 msec. According to the model, the amplitude of the response to a given event is:

$$1 - Exp[-t_i/T].$$

where t_i represents the time since the previous event and T equals to 200 msec. The mean amplitude to the 5000 random events was then calculated, it was 0.4997. Keeping the same time constant of 200 msec., the simulation was repeated for mean frequencies of 0.5 to 500 isomerizations per sec. and the mean response to each frequency calculated. The points representing these mean responses were then plotted on a loglog graph as a function of frequency and are presented in Fig. 3, the continuous line is the Weber-Fechner $I_o/(I_o+I)$ function with I_o set to $1/T$. The superposition is perfect.

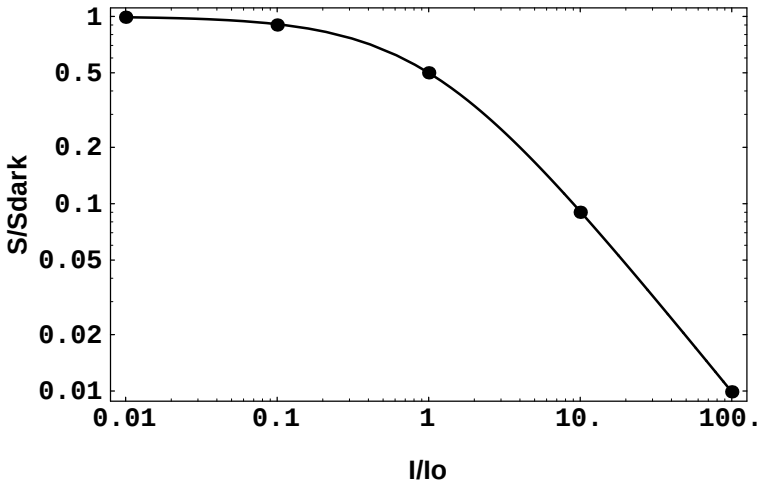

Figure 3: Sensitivity as a function of background light. The points are simulation results. The curve is the Weber-Fechner law.

One can therefore conclude that the model adapts according to the Weber-Fechner law. The obvious advantage of this model is that from the knowledge of

the number of compartments and of the decay time constant of the photocurrent, one can localize the adaptation curve on the background intensity axis; that is determine I_o as being equal to N/T in a system made of N compartments. Up to now, the Weber-Fechner equation was simply a mathematical fit to a series of experimental observations. In vertebrate phototransduction, if the model is true, it is now linked intimately to the structure of the outer segment and the kinetics of the biochemistry responsible for the current recovery.

Does this model fit reality?

Up to now I have tried to establish that a system constructed of identical compartments, which are chemically isolated but electrically connected, carrying the same fraction of the total current and responding maximally to a single event, will saturate exponentially regardless of the shape of the single event in time. Furthermore, if the shape of the single event can be approximated to a sharp rise followed by an exponential decay, then the sensitivity of that system as a function of background will follow the Weber-Fechner function. However, one can legitimately ask to which extent does this apply to vertebrate phototransduction. In other words, are the vertebrate rod and cone outer segments constructed of biochemically isolated compartments which are maximally excited by a single isomerization?

The case of the vertebrate cone

As it is well known the major anatomical difference between rods and cones is that, in the cones, the outer segment is made of infoldings of the plasma membrane while in the rods this is true only for the first micron or so. Can I define a credible compartment in the cone outer segment? One of the most recent anatomical studies of the cone outer segment is that of Eckmiller [10]. It clearly shows that each fold hangs out in extracellular space and its interior is in communication with the rest of the outer segment through only a small part of the circumference adjacent to the ciliary structure. From the above article [10], I estimate this arc to be of the order of 20 degrees. This small part is called here a "neck". Can the cone fold therefore constitute an isolated biochemical compartment bound by the membranes which define its geometry? Electrically, this small neck has a small electrical resistance because its length is minute. The cone fold is thus connected to the ciliary structure of the outer segment through a low resistance path.

The biochemical isolation of the cone fold becomes more evident when one considers that any molecule in order to exit or enter the fold has to pass through this neck opening. For example, an activated opsin molecule in a given fold would first have to find the exit, move up or down in the ciliary section, and

enter into an adjacent fold in order to activate a transducin molecule located there. These motions would have to take place before the opsin became inactive.

Random walk in two dimensions is described by the following formula:

$$< r^2 >= 4Dt$$

where $< r^2 >$ is the mean square distance traveled, D is the diffusion coefficient taken here to be similar to that of rhodopsin,circa 0.5 $\mu\mathrm{m}^2 - \mathrm{sec}^{-1}$ [44, 37], and t the time in second. Given that the risetime of the small flash cone response is in the order of 35 msec. in mammals [52] and assuming that the opsin is inactivated at the time of the peak, one obtains a value of r equal to 0.26 micron. Opsin molecules, which are further than this distance away from the opening, have little chance of leaving the fold during the risetime of the cone response.

Clearly, these numbers indicate that the excited visual pigment molecule is restricted in its action to the fold it belongs to. Similar calculations can be done for transducins and phosphodiesterases attached to the membrane. The diffusion coefficients of these proteins are not believed to be more than twice as large as that of the visual pigment [36]. Their action is also estimated to be restricted to the fold that they were belonging to when activation took place.

Molecules, which are diffusing in solution such as cGMP or Ca^{++}, have diffusion coefficients which are much greater than membrane bound proteins. One can ask to which extent cGMP molecules, present in neighbouring folds, diffuse into the fold which is the site of photoactivation. It is true that reduction of cGMP concentration in a given fold will generate a concentration gradient and a number of cGMP molecules present in adjacent folds will diffuse out of theses folds and enter the active fold to be hydrolysed there. However, the rod disk arrangement constitutes a system of baffles which reduces the effective diffusion coefficient of cGMP in the range of 1.4- 5.5 $\mu\mathrm{m}^2 - \mathrm{sec}^{-1}$ [42]. More recent results give higher values in the range of 60 to 70 $\mu\mathrm{m}^2 - \mathrm{sec}^{-1}$, but these values are still six to seven times lower than in aqueous solutions [33]. After 35 msec., the mean distance travelled is only 3 μm for a molecule diffusing at 70 $\mu\mathrm{m}^2 - \mathrm{sec}^{-1}$. Although a similar analysis has not been done for the cone outer segment, the effect should be even stronger. The conclusion is thus that, over the time course of the single photon event, considering the folds of the outer segment of cones as chemically isolated compartments is probably a good first approximation.

The next question is whether or not a single isomerization closes all the open cationic channels associated with a given fold? The inside volume of a mammalian cone fold is estimated to be $1.6 * 10^{-2}$ femtoliter (assuming an inside height of 5 nm and a radius of 1 μm), if the resting concentration of free cGMP is approximatively 5 μM, then the number of cGMP molecules in the fold is circa 50. Knowing that the PDE surface density is approximatively $1000/\mu\mathrm{m}^2$ [9], the number of PDE molecules in the fold is circa 6000. This means that, if fully activated, there are 120 molecules of PDE for each molecule

of free cGMP to hydrolyse. Actually it is interesting to note that there are two bound molecules of cGMP to each PDE [17] for a total of 12, 000 cGMP bound to PDE non-catalytic sites. If the total dark current of 40 picoamp. is equally divided among the 800 folds of a typical cone outer segment and the channel conductance is 0.1 ps, at the resting potential of 40 mv., the number of open channels is 13. Since 3 cGMP molecules are required to keep a channel open [14, 19] the total number of cGMP molecules bound to the channel proteins is 39, neglecting the channels to which only one or two cGMP are bound. At 50, the molecules of free cGMP are nearly equal in number to the molecules bound to the channel proteins. Given that PDE has an effective K_m of $600\mu M$ [9], a concentration of $22\mu M$ [9], and a turnover number of 1000 per sec.[16], the initial number of 50 cGMP would be reduced to $1/e^2$ of its value or to 7 in less than 54 msec. This leaves little time for cGMP from neighboring folds to come in, or for the cGMP cyclase to react in order to prevent the closing of all the open channels.Therefore, I am confident that the hypothesis of total closure of all cationic channels associated with a given fold is a possible one.

The consequences of accepting the above hypotheses are as follows:

1- the single photon fractional current response of cones should be $1/N$, where N is the number of folds or disks in the outer segment. It also indicates that, although increasing the length of the outer segment (that is N) increases the probability of photon capture by increasing the overall optical density, it decreases the size of the single photon response. Actual length of cone outer segments therefore represents an engineering compromise between the size of the single photon response and the probability of photon capture.

2- from the knowledge of the thickness of the fold ($1/33$ μm), the optical density per unit thickness (0.016 OD/μm, [20]), and the quantum efficiency for isomerization of the visual pigment (0.67, [8]), and using Equation 3 of Baylor et al. [4], k for cones in the total occlusion exponential equation becomes $0.00059d^2$, where d is the cone diameter in microns.

3- I_o, in the Weber-Fechner law, is given by N/T, where N is the number of folds in the outer segment and T the time constant of the exponential which can be fitted to the decay of the small flash response.

The case of the vertebrate rod

Considering the geometry of the rod outer segment, it is not evident that we are facing a system made up of compartments. Although it is true that a given rhodopsin molecule, being a transmembrane protein, cannot leave the disk to which it belongs, and that PDE is in the same situation being a permanently membrane attached protein, the case of transducin is more ambiguous since there have been reports of two types of transducin, membrane attached and soluble [7]. The soluble kind could leave its native disk and possibly excite a PDE molecule located on an adjacent disks or on the cylindrical plasma membrane.

However , to a modeler, the most perplexing fact in rod phototransduction is that a single isomerization produces the same 3- 5 % reduction in dark current in the Bufo rod [3] which is one of the largest rods (6μm X 50 μm) and in the smallest rod (2μm X 25 μm) in monkey [4]. This small size rod is also found in rat, bovine, and human. The ratio of the volumes of these two rods is 27. Following an isomerization in Bufo rod, the biochemistry activated has therefore to hydrolyse 27 times more cGMP than in the monkey rod in order to reduce the cGMP concentration to the same level in the two rods. Since the proteins involved in the cascades are all initially membrane bound, their number only increases as the square of the dimensions. Thus there is, per disk, only 9 times more rhodopsin, transducin , and phosphodiestrerase in a bufo rod as compared to a monkey rod, assuming a constant surface density across species for these molecules. Soluble molecules such as Ca^{++} and cGMP increase in number as the cube of the dimensions. Larger rods would be expected to be less sensitive than smaller ones, but this is not the case as found experimentally.

The approach taken had therefore to be empirical. Analyzing in many rods the amount of plasma membrane involved in the total occlusion model [35], it was found that this area was equal to the area of membrane present in one disk. That is, the occlusion length l could be predicted by equating the cylindrical area of plasma membrane ($\pi * d * l$) to the amount of membrane present in one disk ($2 * \pi * d^2/4$) where d is the diameter of the rod. The solution being $l = d/2$. It was as if in terms of membrane area affected by an isomerization, the effects were the same in rods and in cones of the same diameter, only the site of action was different; in the cones, it is the membrane of the disk shaped fold, while in the rods, it is the plasma membrane whose area is equal to that of the disk.

The consequences of accepting the above are as follows:

1- The fractional current response of a rod to a single isomerization is equal to $d/(2 * l)$, where d and l are respectively the diameter and the length of the outer segment.

2- Since $d/2$ defines the occlusion length, again using Equation 3 of Baylor et al. [4] the value of k in the intensity-response relation becomes: $0.01d^3$, where d is the rod diameter in μm. The dimensions of the constant 0.01 are μm^{-1}.

3- I_o, in the Weber-Fechner law, is N/T where N is the number compartments the outer segment and T the time constant of the exponential which can be fitted to the decay part of the small flash response. N is calculated by dividing "l" by "$d/2$" which should be the same as dividing the total dark current by the amplitude of the rod response to a single isomerization.

Comparison with experimental results

Predictions obtained from the model can be tested against values obtained from experiments for the following three parameters: the single photon response,

the value of "k" in the total occlusion formula, and the value of I_0 in the Weber-Fechner formula. Although the single photon response and the value of "k" are not independent of each other, these two parameters will be used in the comparison tables since experimental results have been reported both ways. Data relating to both rods and cones will be combined.

Table I summerizes the data concerning the single photon response of various rods and cones in a number of species. The theoretical response is "$1/N$" for the cones where N is the number of folds in the outer segment; for the rods, the theoretical response is "$d/(2 * l)$" where d is the diameter and l the length of the outer segment.

Table 1 Single Photon Response: Model vs Experiments

Species	Photoreceptor type	Observed %	Predicted %	Ref.
Turtle	Cone	0.16	0.125	[1]
idem	Cone	0.05	0.125	[51]
Walleye	Cone	0.14	0.2	[6]
idem	Twin cone	0.14	0.2	[6]
Salamander	Red cone	0.76	0.33	[43]
idem	Blue cone	18	0.39	[43]
idem	Rod	1	23	[24]
Squirrel	Cone	0.02	0.5	[31]
Toad	Rod	5	6	[3]
Monkey	Rod	3-5	4	[4]
Rabbit	Rod	5	5	[41]

Table 2 compares the observed "k" in the intensity-response plot obtained by various experimenters to the "k" calculated using the theoretical "occlusion length", the quantum efficiency, the optical density of the visual pigment, and the dimensions of the outer segment.

Table 2 Intensity Response: Model vs Experiments

Species	Photoreceptor type	"k" observed x 10^3	"k" predicted x 10^3	Ref.
Human	Cone	1	1	[49]
Macaque	Red Cone	0.38	1	[50]
idem	Green Cone	0.42	1	[50]
idem	Blue cone	0.40	1	[50]
idem	Rod	$6-20$	80	[32]
Human	Rod	18	80	[32]
Monkey	Rod	68	80	[4]
Bass	Rod	24	40	[39]
idem	Single Cone	3	9.4	[39]
idem	Twin Cone	0.5	15	[39]
idem	Fast Twin	0.07	15	[39]

It can be observed that the predicted sensitivity, expressed either as percent of total current or voltage reduction due to a single isomerization (Table 1) or as the value of "k" in the total occlusion formula (Table 2), is in general agreement with the experimentally obtained values. There are exceptions, notably in the sensitivity of the Salamander rod and blue cone, and that of the Bass twin and fast twin cones where the model does poorly. For the Salamander rod, the presence of deep incisures (see Fig. 5 in Olson and Pugh [42]) in the rod disk represents diffusion barriers to the cascade proteins moving in or on the membrane and this could be an effective mean of reducing sensitivity. For the Salamander blue cone and the Bass twin and fast twin cones, I have no explanation. However, in the Salamander, I have difficulty in seeing how a blue cone, which has a sensitivity 24X greater than that of a red cone, can operate in a color opponent system to generate color vision. The same argument can be used in the case of the Bass cones where the ratio of the sensitivities of the single and fast twin cones is 43. From an engineering point of view, the best color system would be one in which all cones would have the same dark-adapted sensitivity and the same behavior under light adapting conditions. These criteria seem to have been achieved in the Macaque retina [50]. The sensitivity of the cones in the ground squirrel retina is also much lower than predicted. Using the value of 50 pa for the dark current, a channel conductance of 0.1 ps, a resting potential of -40 mv, and 200 folds in the outer segment, a single photon response of 0.02 % change in dark current [31] corresponds to the closing of only 2.5 channels in a fold. However, in a psychological study, Jacobs and Yolton [27] have found that ground squirrel dichromatic color vision has the same threshold as trichomatic human vision. I take this to indicate a possible problem with the electrophysiological data reporting the low sensitivity of ground squirrel cones.

Table 3 compares the experimentally obtained value for I_o in various Weber-

Fechner plots to our predictions based on the number of compartments and the decay time constant of the small flash response. Predicted I_o is N/T, for the cones, the number of compartments is equal to the length of the outer segment in μm multiplied by 33 which represents the number of folds per μm. For the rods, the number of compartments is equal to the length of the outer segment divided by the occlusion length $d/2$ except for the Salamander rod as described below. The decay time of the single photon response or small flash response was measured from the figures in the referred publications with the help of a micrometer and represents the time from the peak to an amplitude equal to 37% of the peak.

Table 3 Background adaptation: Model vs Experiments

Species	Photoreceptor type	Io Observed Rh*/sec	Io Predicted Rh*/sec	Ref.
Turtle	Cone	2000	7273	[2]
Toad	Rod	4	11	[13]
Cat	Rod	35	83	[55]
Bass	Single Cone	8,500	3,800	[39]
Bass	Twin Cone	33,700	13,200	[39]
Bass	Rod	3	75	[39]
Human	Rod	120	110	[32]
Monkey	Rod	100	133	[4]
Rabbit	Rod	42	83	[41]
Newt	Rod	80	65	[57]

The formulation of the adaptation behavior where I_o is related to the time constant T offers an explanation of a phenomena which was considered bizarre. J.L. Schnapf [48] studying the sensitivity, kinetics and adaptation along the length of toad rods found that rods have a lower sensitivity and slower kinetics at the tip than at the base, but were bizarrely adapting at lower background intensities at the tip than at the base,seemingly at odds with the lower sensitivity of that region. Similar findings had been described [21] from electroretinogram recordings. However, from the above formula, slower kinetics means an increased value of T and therefore a reduced value for N/T which represents the background isomerization rate at which the sensitivity is reduced by 50%. Using the ratio of the time constants at the tip and at the base to predict the ratio of I_o's one obtains 1.35. The actual value, calculated from the data [48], is 1.66 in good agreement with the prediction. This approach offers a way of testing

the model. Kinetics of the photoresponse are known to vary with temperature [34], adaptation properties can be tested as a function of temperature to see if the variation of the decay time has the effect on the adaptation parameter I_o as predicted by the N/T formula.

DISCUSSION

In this paper, I have presented a model which addresses the differential behavior of vertebrate rods and cones with respect to sensitivity and adaptation. This differential behavior has been the focus of many investigations both experimental and theoretical. In 1981, Lamb et al. [35] proposed the total occlusion model in order to explain the shape of the intensity-response curve of photoreceptors; if the light intensity was expressed in photons per square microns, then the single parameter k was representing the isomerization cross-section in microns square sustained by the compartment where the total occlusion was taking place. This expression was providing an adequate fit to experimental data and could be used in place of the Michaelis-Menten type of formula. Here I have extended this approach in identifying the compartments by an analysis of the confinement of the biochemistry by the ultrastructure of the outer segment of cones and rods and I have obtained a value for the size of these compartments. This analysis led to the a numerical value for the parameter k in the total occlusion model [35] for both types of receptors,thereby localizing, in an absolute fashion, the intensity response curve on the intensity axis.

The hypothesis that a single isomerization closes all the ionic channels associated with a single cone fold receives support from human cone threshold experiments. Psychophysical experiments indicate that a test spot of 1 min of arc must deliver 600 photons (550 nm) at the cornea in order to be perceived reliably [22]. According to Schnapf et al. [50], this corresponds to 32 isomerizations per cone. At this intensity, the probability of two or more isomerizations in the same fold is negligible, this means that 32 folds have been the site of an isomerization. Since there are approximately 825 folds in a 25 μm cone, and if each fold carries the same fraction of dark current, the total occlusion of 32 folds represents a 4% (32/ 825) change in the dark current. This would mean that, at perception threshold, both rods and cones must see their dark current reduced by the same fraction (circa 4%). In a sense, this is not surprising since the anatomy at the synaptic junction located at the cone pedicule or at the rod spherule is very similar as seen in electron microscopy. In order to carry the message across the synaptic junction reliably, the same current change at the level of the outer segment would be required in the two types of receptors.

The finding that, in rods, the area of plasma membrane occluded is related to the disk area, may be more than fortuitous. It has been proposed a few years ago [37], that the disk size is a major factor controlling rod sensitivity. Furthermore, it is surprising that the surface density of PDE is smaller than that

of transducin by a factor of 9 in mammalian rods [36]. Since transducin remains bound to PDE when this one is activated, there would be an overproduction of transducin assuming that all the disk transducins are activated. One could argue that we are in the presence of a gain loss in the sense that more PDE's could be activated if they were present. Another problem with the current rod model, in which the PDE is located on rod disk membrane, is that the minimum cGMP concentration is located at the center of the disk [9]. The optimum location for this minimum would be at the plasma membrane where the cGMP activated channels are located. One solution to that problem would be to locate rod PDE on the plasma membrane close to the channels. This new location would explain why Roof et al. [47] failed to find PDE on rod disk membranes with the electron microscope when, according to surface density numbers and to its size larger than transducin, it should have been clearly visible. This was not due to elution since PDE was present in SDS PAGE columns of the same preparation. That the full biochemistry responsible for phototransduction is present on the rod plasma membrane is evident from the work of Ertel [11, 12] where plasma membrane patches attain a light sensitivity and kinetics close to the intact outer segment (Fig. 3B in [12]). This new location would also equate the surface density of PDE on the plasma membrane in amphibian and mammals to 16,500 PDE per μm^2, a value calculated with the data given below and based on 33 disks per μm of outer segment) taking into consideration that the diameter of toad rod is about three times that of bovine or human rod. Current values based on disk location give a surface density of PDE three times lower in amphibian (167 per μm^2) [18] when compared to mammals (500 per μm^2) [53]. A lower surface density of PDE in toad rod should normally results in a lower transduction gain but it does not since a single isomerization produces the same percent current reduction in these two rods.

An hypothesis which would accommodate the model would be that phototransduction is a surface phenomena. A single isomerization would results in the closure of channels located over an area determined by the area of the disk. For the cone, the area would the cone disk itself. For the rod, activated transducin, in number proportional to the size of the disk, would migrate from the disk to plasma membrane where PDE would be located. Activated PDE molecules would then sweep the plasma membrane hydrolyzing cGMP close to membrane or as it becomes unbound from the channel. Because of the finite lifetime of activated PDE, the area affected would be limited and would define the rod compartment.

The most striking feature of the model resides in its ability to predict the isomerization rate required to reduce the sensitivity by 50% of both rods and cones in warm as well as in cold blooded species as shown in Table 3. It does that by making use of the "ergodicity theorem". The result of this analysis is that the single parameter I_o in the Weber-Fechner adaptation formula can now be calculated. It is simply N/T where N is the number of compartments in the outer segment used to predict the intensity-response curve and T is the time

constant of the decaying exponential which can be fitted over the decay part of the small flash response. When comparing rods and cones, the model offers an explanation for the different sensitivity and adaptation by proposing that the number of compartments supporting the dark current is much greater in cones than in rods. The effect of this difference is much stronger when comparing the adaptation properties because of the way the number of compartments appears in the formulae.

When comparing rods of warm blooded species to cold blooded ones, the difference is not in the sensitivity since a toad rod and a monkey rod show the same 3-5% change in dark current following an isomerization, but in the adaptation properties. These are shown here to be related to the time constant T of the small flash response which is much longer in cold blooded vertebrates, thus offering an explanation for the much lower background intensities required to reduce the sensitivity by 50% in these species.

Regarding the intensity-response curve, the data for the Salamander indicates that the size of the compartment is much smaller than predicted. This could be due to the presence of lobules in the outer segment disks which restrict the diffusion of membrane bound proteins leading to a smaller number of excited transducins and/or phosphodiesterases molecules in a given amount of time. This is equivalent to raising the number of compartments. A 1% current reduction [24] corresponds in this model to the presence of 100 compartments. When this number is used in the N/T formula, using 1.54 sec. as the decay time constant of the Salamander rod small flash response, one predicts a value for I_o close to that found experimentally [57], validating in a sense the approach.

The model does not preclude a role for calcium or any other molecules in adaptation [56, 29, 28] though it suggests that their action must result in changing the value of the decay time constant T and possibly changing the value of N for the rods where the size of the compartment could be affected by factors such as diffusion coefficients, or lifetimes of various excited proteins. Perturbations of the biochemical cascade, which render the decay of the photocurrent no longer the rate limiting process, kill this model since this hypothesis is, with "total occlusion" [35] , one of the two pillars on which rests the model.

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