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Dynamics of mouse rod phototransduction and its sensitivity to variation of key parameters

L. Shen¹, G. Caruso², P. Bisegna³, D. Andreucci⁴, V.V. Gurevich¹, H.E. Hamm¹, and E. DiBenedetto⁵

H.E. Hamm: heidi.hamm@vanderbilt.edu

¹Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA

²Construction Technologies Institute, National Research Council, Rome, Italy

³Department of Civil Engineering, University of Rome Tor Vergata, Italy

⁴Department of Mathematical Methods and Models, University of Rome La Sapienza, Italy

⁵Department of Mathematics, Vanderbilt University, Nashville, TN, USA

Abstract

The deep understanding of the biochemical and biophysical basis of visual transduction, makes it ideal for systems-level analysis. A sensitivity analysis is presented for a self-consistent set of parameters involved in mouse phototransduction. The organising framework is a spatio-temporal mathematical model, which includes the geometry of the rod outer segment (ROS), the layered array of the discs, the incisures, the biochemistry of the activation/deactivation cascade and the biophysics of the diffusion of the second messengers in the cytoplasm and the closing of the cyclic guanosine monophosphate (cGMP) gated cationic channels. These modules include essentially all the relevant geometrical, biochemical and biophysical parameters. The parameters are selected from within experimental ranges, to obey basic first principles such as conservation of mass and energy fluxes. By means of the model they are compared to a large set of experimental data, providing a strikingly close match. Following isomerisation of a single rhodopsin R^* (single photon response), the sensitivity analysis was carried out on the photo-response, measured both in terms of number of effector molecules produced, and photocurrent suppression, at peak time and the activation and recovery phases of the cascade. The current suppression is found to be very sensitive to variations of the catalytic activities, Hill's coefficients and hydrolysis rates and the geometry of the ROS, including size and shape of the incisures. The activated effector phosphodiesterase (PDE^*) is very sensitive to variations of catalytic activity of G-protein activation and the average lifetimes of activated rhodopsin R^* and PDE^* ; however, they are insensitive to geometry and variations of the transduction parameters. Thus the system is separated into two functional modules, activation/deactivation and transduction, each confined in different geometrical domains, communicating through the hydrolysis of cGMP by PDE^* , and each sensitive to variations of parameters only in its own module.

1 Introduction

The control of signal transduction in cells is known to occur by precise, highly regulated localisation of key enzymes in subcellular-compartments [1–3]. Thus biochemistry, biophysics and the underlying geometry and the composite structure of the cytoplasm all contribute to a synergistic system behaviour of these processes. Conventional modelling is based on well-stirred Michaelis–Menten kinetics, thereby averaging concentrations within the volume of the cell, and losing any space-resolved information on the local dynamics of the process. Thus after many years of biochemical, molecular and electrophysiological

research on the mechanisms of signal transduction, there is an emerging need to expand the investigative tools into spatio-temporal mathematical modelling, which afford a more quantitative and global understanding of how cells respond to signals and their functioning as systems. This is increasingly recognised by cell biologists and biochemists, as evidenced by a number of recently proposed models describing certain aspects of cell signalling, such as cAMP generation [4] intracellular calcium [5] the dynamics of G protein activation [6, 7] and so on.

Mathematical models by their nature need a precise knowledge of the key parameters. These are biochemical (reaction rates), biophysical (diffusion coefficients, electrical constants) and geometrical (size and shape of cells). When these parameters are not measured precisely, but only known to be within a certain range, part of the task of mathematical modelling is also to assess the self-consistency of the parameters, and how sensitive the system is to variations of these parameters within their experimental ranges.

This issue is complex, as it involves, in addition to creation of a mathematical model, the compilation of parameter tables based on often very different published experimental estimates, which must be consistent with basic biophysical first principles (conservation of mass, fluxes, energy etc.).

In most cell types, available biochemical information, such as expression levels of signalling proteins, rates of their activation and deactivation, and their precise activities in active and inactive state, is insufficient for comprehensive modelling of complex interplay between different pathways involving numerous positive and negative feedback loops. Yet only models that describe multiple processes simultaneously occurring in the cell and take into account cellular geometry and compartmentalisation have a chance to capture the complex regulation of cell behaviour needed for a system-level understanding. Rod phototransduction is arguably the most studied signalling process, where the expression levels and biochemical and biophysical properties of the majority of players, their subcellular localisation, cross-talk mechanisms and cellular geometry are described more extensively than in any other cell. Therefore the rod provides a unique opportunity to develop a comprehensive quantitative spatio-temporal mathematical model of cell signalling, explore the capabilities of the model, and generate experimentally testable predictions.

The wealth of experimental data describing rod signalling also allows to generate a comprehensive set of parameters and test their consistency. The model allows to perform quantitative analysis of system sensitivity to parameter variation. This analysis is important for several reasons. It pinpoints the gaps in our knowledge base by identifying the most crucial parameters that must be measured with high precision. It determines the relative contributions of individual parameters to the consistent performance of the cell as a whole. It also reveals the biological significance of inter-species variations in size and shape of cells with the same function, activities of homologous enzymes, biophysical characteristics of homologous channels and so on. Finally, one would like to analyse how the resulting models identify the system synergies.

We are not aware of a general theory of parameter consistency and sensitivity analysis. The goal of this paper is to carry out this analysis for visual transduction in mouse rod photoreceptors. In addition to improving comprehensive quantitative description of mouse rod as a coherent functional system, this work paves the way to the development of tools necessary for the quantitative description of signalling in other cell types.

1.1 Modelling visual transduction

As shown in Fig. 1, the rod outer segment (ROS) of vertebrates can be approximated by a right-cylinder of height H and radius R , housing a parallel stack of equal, equispaced disc membranes, floating within the cytoplasm. The discs containing high concentrations of the light receptor rhodopsin (Rh), a prototypical G protein-coupled receptor, the rod G protein, Transducin or Gt, and the effector enzyme, cyclic guanosine monophosphate cGMP phosphodiesterase PDE ([8]). A photon of light captured by a molecule of rhodopsin induces its isomerisation into its activated state R^* . Activated rhodopsin, upon encounter with the transducer Gt, converts it into the activated state T^* . The latter, in its turn, converts the effector PDE into its activated state E^* . This cascade of events occurs on the disc where rhodopsin has been isomerised. Activated effector E^* hydrolyses the second messenger cGMP, freely diffusing in the cytoplasm. As a result, cGMP dissociates from the cGMP-gated channels on the plasma membrane, thereby causing their closure and a consequent hyperpolarisation of the cell. Channel closure prevents the entry of Na^+ and another second messenger, Ca^{2+} ions. The resulting drop in inward current and consequent hyperpolarisation reduces neurotransmitter release at the rod synapse. Thus, the signal is light, its receptor is rhodopsin, and the cellular response is a change in voltage at the photoreceptor's plasma membrane, which changes the cell's electrical output and leads to a signal being sent on to bipolar cells and then downstream retinal neurons and ultimately to the brain [9, 10]. Recovery from light excitation takes place through the decrease of Ca^{2+} , brought about by the closure of cGMP-gated channel combined with constant activity of $\text{Na}^+/\text{Ca}^{2+}, \text{K}^+$ exchanger, which results in the activation of guanylyl cyclase (GC) and resynthesis of cGMP [11, 12]. Rhodopsin turnoff proceeds through removal of recoverin (Rec) from rhodopsin kinase (RK), phosphorylation of rhodopsin and arrestin binding. G protein turnoff occurs via regulator of G-protein signalling 9 (RGS9) activation of intrinsic GTPase activity of Gt.

This signalling system has provided important insights into basic mechanisms of signal transduction by G protein-coupled receptors [9, 13]. Its input light can be presented in a precisely controlled way, the output, the current across the plasma membrane is easily measured, and the underlying protein components have been purified and recombined. The kinetics of underlying reactions was determined in vitro [14, 15]. Three-dimensional structures of many components have been solved [16–21]. The steps in this elegant cascade are well described, and estimates of enzyme and substrate concentrations and equilibrium constants can be found in the literature.

A glance at the cascade reveals that the control of the various steps occurs by precise, highly regulated localisation of key enzymes in sub-compartments of the ROS; for example, the activation part of the cascade occurs on a disc membrane, whereas the transduction occurs in the cytosol, and current drop occurs on the outer shell of the ROS. These are geometrically different domains, and the space-resolved information determined by their shape and dimensions plays a significant role in shaping the signal, along with the biochemistry and the biophysics of the process.

1.2 Mathematical model and sensitivity analysis of parameters

We have created a spatio-temporal mathematical model for rod phototransduction based on the mathematical theories of homogenisation and concentrated capacity, and a Matlab based, finite-element code has been developed for this system [22–27]. The model is capable of discriminating between membrane processes (two-dimensional), and cytoplasmic processes (three-dimensional), and links them through their flux exchange. This way, the biophysical properties of the system are captured by the flux-interaction between the membrane and the cytoplasm.

Populating the model with a consistent set of parameters is a major task. While a reasonably complete set of parameters is available for the salamander [28], no such set exists for mouse rods. Several biochemical and biophysical parameters are reported in the literature, however, the range of the values is large. Moreover, some parameters are experimentally derived for mouse, whereas others are determined for different species, such as bovine. Therefore the choice of parameters is not a trivial task. We have sought a selection criterion for these parameters, based on first principles (conservation of basic biophysical quantities), and functional affinities. The parameters are classified loosely in four basic but overlapping groups: (i) geometrical (size and shape of the ROS), (ii) electrophysiological (currents, electrical parameters, basal dark values), (iii) biochemical (rate constants of first order reactions and Hill coefficients, activity of enzymes and second messengers concentrations) and (iv) biophysical (diffusion coefficients). Here we investigate how the variation of these parameters affects the response, and compare the response predicted by the model with the available experimental data. Although we explicitly extract the system behaviour of the single photon response (SPR), the conclusions are qualitatively applicable to dim light responses of rods in general. For example, we test the effects of the change of some geometrical parameters (radius R of the ROS transversal cross section, height H of the ROS etc.), either alone or in combination with biochemical or biophysical parameters. We also test the effect on response of the change in Hill coefficients, and in the affinity of cGMP-gated channels for cGMP.

The role of the incisures, clefts that give the discs a scalloped or lobed appearance, has been unknown for a long time [29–32]. Their functional role was investigated in [25], and further insight is gained here, by investigating how the photoresponse is affected by changing the number of incisures and their shape, length, size and so on. It is worth pointing out that this capability of dissecting the contribution of the various components of the system, and exploring their concerted behaviour, is afforded only through a mathematical model. A final point is about the choice, made in Section 2.1, of the functionals against which one can measure the sensitivity of the system to the changes of various parameters. Some of these functionals are natural in that they are experimentally measurable. They include the total relative current suppression as a function of time $\tilde{I}^*(t)$ following an isomerisation, the time of peak response t_{peak} of such a current, and the photocurrent at peak time $I(t_{\text{peak}})$. Others are not, to date, directly accessible to experimental measurement, yet relevant to the process. They include the total number $E^*(t)$ of molecules of activated effector in the activated disc, as a function of time, the peak time t_{peak}^* and the value $E^*(t_{\text{peak}}^*)$ at peak time. As indicated earlier, the activation part of the cascade occurs on the disc, and the functional $E^*(t)$ reflects this module of the signalling cascade. The transduction part occurs in the cytoplasm and its output is the relative current drop $\tilde{I}^*(t)$. It is natural to ask, which part of these two modules is more sensitive to variations of parameters. Thus, our choice of functional is intended to separate the contribution of the activation module from that of the transduction module, in order to determine which one of these is more sensitive to parameter variations.

2 Procedures and methods

2.1 Mathematical model and sensitivity functionals

We are using the spatio-temporal mathematical model previously created in [22–27]. The local currents generated across the ROS lateral surface, as function of space and time, are computed by the formula

$$J_{\text{ex}} = \frac{j_{\text{ex}}^{\text{sat}}}{\sum_{\text{rod}} K_{\text{ex}} + [\text{Ca}^{2+}]}, J_{\text{cG}} = \frac{j_{\text{cG}}^{\text{max}}}{\sum_{\text{rod}} K_{\text{cG}}^{\text{m}_{\text{cG}}} + [\text{cGMP}]^{\text{m}_{\text{cG}}}} \quad (1)$$

In the first of these, $j_{\text{ex}}^{\text{sat}}$ is the saturated exchange current, K_{ex} is the Ca^{2+} concentration at which the exchange rate is half maximal and Γ_{rod} is the surface area on the lateral boundary of the ROS. In the second, $j_{\text{cG}}^{\text{max}}$ is the maximal cGMP-current, m_{cG} is the Hill exponent, and K_{cG} is the cGMP concentration at which the exchange rate is half maximal. Notice that J_{ex} and J_{cG} are current densities (i.e. current per unit area, measured in $\text{pA}/\mu\text{m}^2$), and, in general, have different values at different points of the lateral boundary of the ROS, following the spatio-temporal distribution of $[\text{cGMP}]$ and $[\text{Ca}^{2+}]$. In the absence of light, J_{ex} and J_{cG} are constant and equal to their constant dark values $J_{\text{ex};\text{dark}}$ and $J_{\text{cG};\text{dark}}$ defined as in (1) with $[\text{Ca}^{2+}]$ and $[\text{cGMP}]$ replaced by $[\text{Ca}^{2+}]_{\text{dark}}$ and $[\text{cGMP}]_{\text{dark}}$, respectively.

Fig. 2 shows the cross-section of ROS with a stack of discs with one incisure. Introduce cylindrical coordinates (θ, z) on the lateral boundary of the ROS (with radius R and height H) where z ranges over $(0, H)$ and θ ranges over $[0, 2\pi)$. The local value of (total) current density J_{loc} and its dark value J_{dark} are defined as

$$J_{\text{loc}}(\theta, z, t) = J_{\text{ex}}(\theta, z, t) + J_{\text{cG}}(\theta, z, t), J_{\text{dark}} = J_{\text{loc}}|_{t=0} \quad (2)$$

The corresponding global quantities are defined as integrals over the lateral boundary of the ROS, that is

$$j_{\text{tot}}(t) = \int_{\Gamma_{\text{rod}}} J_{\text{loc}}(\theta, z, t) dS, j_{\text{dark}} = j_{\text{tot}}|_{t=0} \quad (3)$$

where dS is the surface measure of the lateral boundary of the ROS. While $J_{\text{loc}}(\theta, z, t)$ is point-wise current density defined on the lateral boundary of the ROS, experimentally what is measured is the global current $j_{\text{tot}}(t)$ as a function of time, and what is reported and graphed is the absolute and relative global current drop

$$I(t) = j_{\text{dark}} - j_{\text{tot}}(t) \text{ total current suppression at time } t$$

$$I^*(t) = 1 - \frac{j_{\text{tot}}(t)}{j_{\text{dark}}} \text{ total (relative) current suppression at time } t \quad (4)$$

The quantities $I(t)$ and $I^*(t)$ are the natural functionals of the photocurrent, whose sensitivity to variation of parameters that can be directly computed and compared with experimental data. Other experimentally measurable functionals are:

$$t_{\text{peak}}, \text{ time to peak of } I(t); I(t_{\text{peak}}), \text{ peak value of } I(t) \quad (5)$$

In our mathematical spatio-temporal model [27], the number of molecules of activated effector E^* is a function $(x, y, t) \rightarrow E^*(x, y, t)$ for (x, y) ranging over an activated disc. This quantity, which is the final output of the activation cascade, serves as an input to the transduction part of the cascade by hydrolysing cGMP. While not directly accessible to experimental measurements, it is then natural to ask how sensitive is the total number of activated effector molecules to variation of the parameters. The corresponding sensitivity functional is

$$E^*(t) = \int_{D_{\text{eff}}} E^*(x, y, t) dx dy \text{ total number of molecules of } E^* \text{ in } D_{\text{eff}} \text{ at time } t \quad (6)$$

Here D_{eff} is the effective domain of the activated disc, that is the transversal cross-section of the ROS, at the activated level, from which the incisures have been removed. Related, but experimentally inaccessible, functionals are

$$t_{\text{peak}}^*, \text{time to peak of } E^*(t); E^*(t_{\text{peak}}^*), \text{peak value of } E^*(t) \quad (7)$$

2.2 Methods of sensitivity analysis

The system parameters of a signalling pathway can be regarded as random variables since their values depend on unknown variations in the cell environment. Accordingly the dynamics of the system changes as the result of the variation of the parameter values. The sensitivity analysis is a general technique that analyses how sensitive a system is with respect to variation in parameter values. It depends on the existence of an underlying mathematical model of the process, whose behaviour is identified by n representative functionals y_i for $i = 1, \dots, n$ in terms of m parameters $x = (x_1, \dots, x_m)$. Suppose the parameters have been calculated at the set value $x^* = (x_1^*, \dots, x_m^*)$, say for example by some best fit process. The sensitivity of the i th functional y_i with respect to the j th parameter x_j at x^* is computed from

$$S_{ij} = \frac{x_j}{y_i} \frac{\partial y_i}{\partial x_j} \Big|_{x=x^*}, (y_i = y_i(x_1, \dots, x_m)) \quad (8)$$

Thus S_{ij} is the ratio of the relative change of y_i to the relative change of the parameter x_j about x_j^* . In our system, the functionals are those in (4)–(7) above, and the parameters are rate constants, Hill coefficients, Michaelis–Menten parameters, diffusion coefficients of the second messengers, electrical parameters, geometrical dimensions and shape of the ROS. These are listed and described in Table 1. The selection procedure is described in detail in the Appendix, which includes calibration against experimental data (Appendix E). Calibration exhibits an excellent agreement with the SPR data kindly provided by Dr. F. Rieke. The sensitivity analysis based on such an optimal set is carried by computing numerically the derivatives indicated in (8), for variations of the parameters not exceeding 5% from x^* .

3 Sensitivity analysis

The sensitivity analysis of the current response $I(t)$ and the total number of activated PDE molecules $E^*(t)$, defined in (4) and (6), with respect to the phototransduction parameters, is effected by introducing the quantities I_{act} , I_{peak} , I_{rec} referring to current, and E_{act}^* , E_{peak}^* , E_{rec}^* referring to the effector. Those denoted by the subscript $_{\text{act}}$ measure the rates of increase at early time points ($t \rightarrow 0$) during the activation phase. Those denoted by the subscript $_{\text{peak}}$, are peak values. Finally, those denoted by the subscript $_{\text{rec}}$ measure the rates of decay at later time points during the recovery phase.

Specifically, I_{act} is defined by first identifying $I(t)$, for times $t \rightarrow (0, 0.01 \text{ s})$, with the quadratic function $(1/2)A_I t^2$, and then by setting $I_{\text{act}} = A_I$. Thus I_{act} , in units of pA per s^2 , is not a current; it rather measures the rate of increase of the current at times $t \rightarrow 0$. Likewise, E_{act}^* is defined by identifying $E^*(t)$, for times $t \rightarrow (0, 0.01 \text{ s})$, with the quadratic function $(1/2)A_E t^2$, and then by setting $E_{\text{act}}^* = A_E$, with similar dimensional considerations. The quantities I_{rec} and E_{rec}^* are defined by identifying $I(t)$ and $E^*(t)$, for times $t > 0.8 \text{ s}$, with the exponential functions $C_I \exp(-t/\tau_I)$, and $C_E \exp(-t/\tau_E)$, respectively, and then by setting them equal to the respective exponential coefficients $1/\tau_I$ and $1/\tau_E$. As such, I_{rec} is not a current,

and it rather measures, in s^{-1} , the rate of decay of the current change. Similar considerations hold for E_{rec}^* . The quantities $I_{\text{peak}} = I(t_{\text{peak}})$ and $E_{\text{peak}}^* = E^*(t_{\text{peak}}^*)$ are the peak current and peak number of effectors.

The sensitivities of I_{act} , I_{peak} and I_{rec} and the analogous quantities E_{act}^* , E_{peak}^* , E_{rec}^* with respect to the phototransduction parameters, are computed by using the space-resolved model previously described [22, 26, 27], and are numerically evaluated by increasing each of the parameters by 5% of its nominal value, and are reported in Table 2.

3.1 Geometry

We commence by computing the sensitivity of the SPR with respect to the radius R and the length H of the mouse rod. The standard ranges for the experimental measurements of ROS geometry, as reported in Section 7.1, are 0.1–0.2 μm for diameter d and 0.4–1 μm for H , amounting to an uncertainty of about 15% for d and 5% for H . Moreover, a consistent variation in these parameters is introduced by transgenic techniques. For example, morphology of arrestin knockout mice in [74] showed that the length of ROS lacking arrestin was 25% shorter than that of wild type. In [65], it was also reported that the ROS lacking rhodopsin kinase were 50% shorter than in the wild type case. The radius of rod of transgenic mice may change accordingly, for example, Makino and coworkers obtained a transgenic mouse exhibiting a rod larger in radius with respect to normal mouse rods [84].

Any change of radius or height causes the change of the area of the outer shell where the cGMP-gated channels and the Ca^{2+} exchangers reside. Sensitivity to variations of R and H is performed at constant channel density. As a consequence, a percentage change in R or H results in the same change in j_{dark} , as can be seen from (1). Accordingly, S_{ij} for j_{dark} is 1.00 for both R and H , as reported in Table 2.

The variation of R has no effect on E^* , in fact a change in R does not change the total number E^* of activated PDE molecules at any time, even if it may slightly affect the local density of E^* on the activated disc.

A larger R implies a longer distance for transversal diffusion of cGMP and Ca^{2+} , and causes a response with smaller I_{act} (slower response at early time points), smaller I_{peak} , and bigger I_{rec} (faster recovery at late time points). A larger H does not affect either $E^*(t)$ (obvious), or the response $I(t)$, since the highly localised response spreads only within a narrow slice of the rod near the activated disc.

We have investigated the role of incisures in salamander rod phototransduction and found that the number, geometry and distribution of incisures significantly influence the response [26]. Since mouse rod has only one incisure, the SPR is not very sensitive to the geometry of that incisure (Table 2). However, the presence of the incisure favours longitudinal diffusion of second messengers by creating vertical cytoplasmic channels crossing the discs. An incisure with greater length l_a or width b_a promotes a larger spread of the response implying positive values for the sensitivity to I_{peak} in Table 2. However, a longer incisure yields a negative value for the sensitivity to I_{act} , as the presence of the incisure lets some of the initial drop of cGMP diffuse longitudinally lowering the magnitude of the initial drop of cGMP near the outer shell, thereby yielding a slower response at early time points. Variation of the geometry of the incisure has no effect on $E^*(t)$.

3.2 Catalytic activity and diffusion

We examine here the sensitivity of production of $E^*(t)$ (effector PDE *) and current response $I(t)$, as defined in Section 2.1, with respect to parameters related to the catalytic activity and diffusion.

3.2.1 Production of PDE—The total number of E^* is here computed by numerically integrating the nonlinear system of partial differential equations modelling the cascade [27]. This number can be shown to be accurately approximated by the formula

$$E^*(t) = \Phi\left(\frac{\nu_{RE}}{k_R - k_E}\right)(e^{-k_E t} - e^{-k_R t}), \text{ for } t > 0 \quad (9)$$

describing the number of activated PDE in terms of the number of isomerisations, the catalytic activity ν_{RE} of R^* , the deactivation rate k_R of R^* and the deactivation rate k_E of E^* . While this formula is mathematically well defined for all values of k_E and k_R , it has biochemical meaning only for $k_R > k_E$, since the lifetime of R^* is shorter than that of E^* [80]. The values of these parameters and their ranges (Table 1), are discussed in Sections 10.2.1 and 10.2.2 and reflect the indicated biochemical meaning.

Since, by this formula, $E^*(t)$ is linearly proportional to ν_{RE} , the sensitivity index of both E_{act}^* and E_{peak}^* with respect to ν_{RE} is 1.00, as reported in Table 2. However, a variation of ν_{RE} has no effect on the recovery rate E_{rec}^* of E^* . The sensitivity of E_{peak}^* to k_R and k_E are both negative, as expected, and the sensitivity with respect to k_R is larger, in absolute value, than the one to k_E , that is, the value of k_R is the most important in reducing the production of E^* .

Both k_R and k_E have little effect on the increase rate of E^* at early times (an increase of these quantities reduces only slightly E_{act}^*), whereas k_E plays an important role in the recovery phase of E^* ; as a matter of fact the sensitivity of E_{rec}^* to k_E is 1, showing that the exponential decay of E^* is exactly k_E , as can be inferred from (9).

3.2.2 Current response—Similarly to the production of E^* , a larger ν_{RE} causes a larger response, thus implying a positive value for the sensitivity to I_{act} and I_{peak} leaving almost unchanged the recovery rate of the current. Larger values of k_R or k_E decreases the peak value of current (implying negative sensitivities for I_{peak}) and reduces only slightly the increase rate of current at early times. It can be observed that the sensitivities of I_{act} and I_{peak} with respect to ν_{RE} , k_R and k_E have the same sign as the corresponding sensitivities for E_{act}^* and E_{peak}^* but they are lower in absolute value. More generally, a given percentage variation of E^* yields a smaller percentage variation of current I , because of diffusivity of the second messengers in the cytoplasm, which tends to dampen variations of the source E^* [27]. Such a dampening phenomenon does not seem to occur during the recovery phase. Indeed, while the recovery phase of E^* is governed by k_E , as experimentally demonstrated in Krispel *et al.* [89], I_{rec} exhibits a sensitivity greater than 1 with respect to k_E .

A larger Ca^{2+} buffer B_{Ca} leads to a smaller Ca^{2+} flux, which slows down the recovery phase and results in a larger peak response, that is, in a positive sensitivity for I_{peak} , whereas it does not affect the initial response and yields a small positive sensitivity for I_{rec} .

Diffusion coefficients D_E , and D_T , relevant to diffusion of E^* and T^* on the activated disc, have negligible effect both on E^* and I^* . An increase of the diffusion coefficient D_{cG} of cGMP in the cytosol results in a faster response, with higher peak value (i.e. positive

sensitivities for I_{act} and I_{peak}) whereas it affects the recovery phase less. Compared to D_{cG} , the SPR is less sensitive to variations in D_{Ca} .

3.3 cGMP synthesis and hydrolysis

Next, we discuss the sensitivity of the SPR and j_{dark} with respect to the parameters related to the cGMP synthesis and hydrolysis. Obviously, most of these parameters do not affect at all the production of E^* . Only the increase of the surface density of dark-activated PDE ($[\text{PDE}]$) results in a slight increase of initial response I_{act} .

An increase of the surface hydrolysis rate of cGMP by PDE in the dark (k_{hyd}) or of the surface density of dark-activated PDE ($[\text{PDE}]$) increases the depletion of cGMP in the dark state, closes more channels and, as a consequence, reduces dark current (j_{dark}), the initial response I_{act} , and the peak response I_{peak} as well. Moreover, this increase slightly increases the recovery rate I_{rec} .

A larger surface hydrolysis rate of cGMP by light-activated PDE ($k_{\sigma, \text{hyd}}^*$) increases the depletion of cGMP, and thus increases the current response. It is more effective at early times, yielding a consistent positive value for the sensitivity of I_{act} , and contributes also to a positive sensitivity of the peak response I_{peak} .

As shown in formula (11) in Appendix B, for a fixed level of $[\text{Ca}^{2+}]$, the increase of each of the parameters $\max$, $\min$ or K_{cyc} strengthens the activity of GC and increases the production of cGMP, thus increasing the dark current and the current response, thereby yielding a positive value for the sensitivities of I_{act} and I_{peak} .

In the process of the response, $[\text{Ca}^{2+}]$ drops from $[\text{Ca}^{2+}]_{\text{dark}}$ 462 nM to the lowest level 260 nM, and then recovers. For all the time, $[\text{Ca}^{2+}]$ is greater than K_{cyc} 129 nM, and thus, $[\text{Ca}^{2+}]/K_{\text{cyc}}$ is greater than 1. The increase of the Hill coefficient of GC (m_{cyc}) greatly decreases the activity of GC, and decreases dark current and current response, thereby yielding a negative value for the sensitivities of I_{act} and I_{peak} .

3.4 cGMP-gated channel and Ca^{2+} exchanger

Finally, we examine the sensitivity of the SPR and j_{dark} with respect to the parameters related to cGMP-gated channel and Ca^{2+} exchanger.

Increasing either the density of cGMP-gated channels ($j_{\text{cG}}^{\text{max}}/\sum_{\text{rod}}$) or Ca^{2+} exchangers ($j_{\text{ex}}^{\text{sat}}/\sum_{\text{rod}}$) leads to an increase of the current flux, and thus results in the increase of dark current and current response as well. The constant K_{cG} is much larger than $[\text{cGMP}]$, and thus $K_{\text{cG}}/[\text{cGMP}]$ is greater than 1. The increase of Hill coefficient of cGMP-gated channel m_{cG} greatly decreases the opening of cGMP-gated channels, thereby decreasing dark current and current response.

The increase of K_{ex} ($[\text{Ca}^{2+}]$ for half maximum exchanger channel opening) or K_{cG} ($[\text{cGMP}]$ for half maximum cGMP-gated channel opening) decreases the opening of cGMP-gated channel or Ca^{2+} exchanger, respectively, and leads to a decrease of dark current and current response.

Increasing f_{Ca} (the fraction of cGMP-activated current carried by Ca^{2+}) increases the Ca^{2+} flux, leading to a fast recovery, and as a consequence a decrease of dark current and current response.

4 Discussion

The spatio-temporal mathematical model for visual transduction [22, 25–27] systemically describes the relationship among various components in the signalling pathways. In this study, we collected and calibrated a set of parameters for modelling SPR in mouse ROS, and explored the sensitivity of the system response to the change of these parameters.

For a given mathematical model, sensitivity analysis in principle could be carried by relating any parameter to any other and at any given time. The complexity of the system presents so many intertwined connections that information might be lost without an organising principle. We have organised the parameters into broad, at times overlapping groups, including geometrical, biochemical and biophysical parameters. On the temporal side, the system might be more or less sensitive to a set of parameters at its inception, or near its peak response, or in its recovery phase. Accordingly, we have devised functionals capable of detecting the sensitivity of the system to each group of parameters in each of these time intervals (Section 3). Finally, each parameter is defined by its relevant biophysical, biochemical, or physiological concepts, so that any change in value for a parameter represents a physical disturbance in ROS.

The model is capable of separating the activation/deactivation module on the discs and the transduction module of the cascade. First the activation/deactivation components of the process are not affected by changes in the geometry of the ROS nor in the incisures (Table 2), nor by any biophysical process occurring in the transduction module. It is sensitive only to parameters involved in biochemical processes occurring on the activated disc. These include the catalytic rate r_{RG} of G protein activation by rhodopsin and the average lifetimes k_R^{-1} and k_E^{-1} of activated rhodopsin and activated effector PDE*. The transduction module, however, is sensitive to the activation/deactivation module, through the turnoff rates k_R and k_E of activated rhodopsin and effector.

There is a strong sensitivity of the photocurrent and effector to the parameter r_{RG} during the activation and peak time. However, neither recovery of the effector nor the current are sensitive to this parameter. All this is in agreement with the known biochemistry of visual transduction.

The rhodopsin turnoff rate k_R , has a surprisingly small effect on the activation rates and recovery of both E^* and I, but a large, negative effect on these quantities at peak time. The current recovery rate is highly sensitive to the turnoff rate k_E of PDE*.

The geometry of ROSs is striking, and its function depends on it. There has never been a systematic way of thinking about the role of different aspects of geometry of ROSs until this modelling approach [22, 25–27]. The sensitivity of visual parameters of the model to geometry allow us to understand what the system is sensitive to, and what it is not sensitive to. This is one of the most important outcomes of having a comprehensive 4D mathematical model that provides a systems view of phototransduction.

Table 2 shows that the effector activation parameters are independent of geometry. We believe this is so because the determinants of these parameters are two-dimensional encounters between the R^* on the activated disc and the disc-bound Gt and PDE.

It is only the transduction module, measured by the current suppression, that ‘feels’ the geometry of the ROS. The current output is highly sensitive to the geometry parameter of radius, but not height, and the geometry of the incisures. Here I_{act} and I_{peak} have a negative

sensitivity to the radius, that is, with a smaller radius these parameters increase, as discussed in Section 3.1.

Here we comment further on the role of incisures and the connection between geometrical and biochemical parameters. In all cases, the photoresponse is not very sensitive to the width of the single incisure present in the mouse ROS. The photoresponse is mildly and negatively sensitive to the length of the incisure at early times only. A possible explanation is that molecules of cGMP in the vertical shaft created by the incisures are, on average, closer to the average location of the activation site. Thus, these are the first to migrate by diffusion to the activated PDE, while those bound to the channels are slowed, thereby lowering the response at early times. The response at peak time is relatively insensitive to variations of either width or length of the incisure. It should be pointed out that a similar analysis on salamander reveals a considerably larger response to the geometry of the incisures [25]. This is rather surprising since the total area exposed by the mouse incisure is $0.04 \mu\text{m}^2$, which amounts to 2.6% of the area of the disc it belongs to. For the salamander on the other hand, the total area of the incisures is about 0.84% of the area of its disc. Yet the salamander photoresponse is highly sensitive to the incisure geometry and the mouse photoresponse is not. On the other hand, the salamander ROS has up to 32 incisures and mouse has only one. This suggests that their distribution on the disc plays a functional role and underscores the role of the geometry in this signalling cascade.

The SPR is not sensitive to the length H of the ROS, since the response is localised in a small section of the ROS about the activation site [22, 26, 85]. The SPR is negatively sensitive to an increase of the radius R , since more distance has to be travelled (with the same diffusion coefficient) by the cGMP in the cytosol before the cGMP on the channels is removed. The dark current j_{dark} , however, is very sensitive to both H and R because it counts ions flowing into the ROS through its entire surface, which is linearly proportional to R and H as indicated in (3). Indeed the dark concentration of cGMP and Ca^{2+} are not affected by geometrical change, because there is no geometry related parameters in (13)–(15).

These results, however assume that the remaining biochemical quantities remain the same. Specifically, in Section 3.1, we have discussed the effect of geometrical change on SPR with an assumption by which channel density is kept constant. In fact, we also implicitly assumed that the concentrations of proteins (e.g. G, PDE, and GC) are kept constant. The model analyses changes of the photoresponse when R or H changes, by keeping the reaction rates constant, which amounts to keeping constant the concentrations of the underlying proteins modulating the cascade; for example, keeping constant the catalytic activity R_G , which is proportional to the concentration of the transducer G protein, means keeping the latter constant for changing R and H . In reality, a geometrical change results from concurrent biochemical configurations. For example, the transgenic Bouse of [84] is shorter than WT, it has larger radius and has no incisures. Its geometrical change is due to an overexpression of rhodopsin. Moreover, the number of molecules of GC and PDE per disc remains unchanged, and thus their concentration is smaller than in WT. It is not known, however, whether the channel density remains the same or decreases with increasing area of the outer shell. The combined changes of geometry and biochemistry lead to a series of consequent changes in ROS, which finally affect the SPR. It is of interest to look at the sensitivity under these conditions.

These remarks point to an essentially limitless number of ways of performing a sensitivity analysis of the SPR on the visual transduction parameters. While from a mathematical point of view, some of them can be changed by keeping the others fixed, the biological system, upon varying some parameters, will rearrange itself to a new biochemical state. The

advantage of the mathematical model we propose [22, 25–27] is in its capability of separating processes and analysing their individual impact on the system, analysing the behaviour of the whole system when an individual protein is knocked out in transgenic mice [33, 65, 69, 74, 86], and adapting itself to multiple combinations of synergistic variations of parameters, to reflect the underlying systems behaviour of the photoresponse.

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7 Appendixes A: geometrical parameters

7.1 Diameter (d) and length (H) of the ROS

The structure of mouse photoreceptors has been characterised by electron microscopy by several investigators [55–58]. It is reported a disc diameter of $1.4 \pm 0.1 \mu\text{m}$ [55], $1.32 \pm 0.12 \mu\text{m}$ [59], $1.22 \pm 0.12 \mu\text{m}$ [59], $0.9\text{--}1.5 \mu\text{m}$ [56, 58], and $1.4 \pm 0.2 \mu\text{m}$ [57]. The length of the ROS is reported to be $23.6 \pm 0.4 \mu\text{m}$ [55], $23.8 \pm 1 \mu\text{m}$ [59]. The work of [55] was cited in [60] where the diameter was reported as $1.4 \mu\text{m}$ and the length $24 \pm 0.4 \mu\text{m}$. In [40] the diameter is reported to be in the range $1\text{--}2 \mu\text{m}$ and the length $25 \mu\text{m}$. The same authors report a diameter of about $2 \mu\text{m}$ and length of about $20 \mu\text{m}$ in [28]. Thus, the range of the reported values for the diameter is $0.9\text{--}2 \mu\text{m}$ and the range of ROS length is $20\text{--}25 \mu\text{m}$. In our simulations, we used the value for the diameter $1.4 \mu\text{m}$ and for the length $23.6 \mu\text{m}$.

7.2 Disc thickness (ϵ), interdiscal space ($v\epsilon$), thickness of the outer shell ($\sigma\epsilon$)

The discs occupy about 50% of the envelop volume in rods, leaving about 50% of the volume as cytoplasmic space [28, 38, 40, 43]. Assuming that discs are equal, and equally spaced, and neglecting the volume of the outer shell, it follows that the disc thickness and

the width () of the interdiscal spaces are equal (i.e. 1). The packing density measured in [55] is 41.3 ± 0.9 discs/ μm , implying a disc-repeat distance + 24.2 nm. Direct measurements of disc thickness in [55] give 15.0 ± 0.6 nm and an interdiscal space 8.4 ± 0.9 nm, with a slightly different disc-repeat distance of 23.4 nm. This measurement of and differs slightly from our assumptions. However, the values reported in [55] ‘... should not be taken as absolute because fixation and orientation of the outer segment with regard to the plane of section can greatly alter the measurements’ [55]. An average number of 34 discs/ μm is reported in [59]), implying a disc-repeat distance of 29.4 nm. This measurement is close to 36 discs/ μm and a disc-repeat distance of 28 nm given in [40]). In [56], it is reported a thickness range of 7 nm for single-layered disc membrane and 16–17 nm for double-layered disc membrane. Thus we conclude that the reported range for disc-repeat distance is 23.4–29.4 nm. In our simulations, we have taken a disc-repeat distance of 29 nm. It follows from our previous assumption (1) that the disc thickness , and the interdiscal distance are both 14.5 nm. To further support these choices, we observe that the dimension of cGMP-PDE (bovine), is $150 \times 108 \times 60$ Å [87], and part of the molecule (about 7.5 nm) extrudes out of the disc membrane into the interdiscal space. When compared with an interdiscal space of 14 nm as reported in [40] and our previous simulations for salamander [25, 26], the interdiscal distance of 14.5 nm provides enough space for the diffusion of PDE. The thickness of the outer shell is reported to be 15 nm by [40, 61]. We used this value in our simulations.

7.3 Computed values for disc radius (R_{disc}), ratio between thickness of outer shell and disc thickness (σ), number of discs (n), envelop volume (v_{tot}), cytoplasmic volume (v_{cyt}), volume-to-surface ratio (η), lateral surface of the ROS (Σ_{rod})

$$\begin{aligned} R_{\text{disc}} &= \frac{1}{2}d - \sigma\varepsilon = 0.685\mu\text{m} & \sigma &= \frac{\sigma\varepsilon}{\varepsilon} = \frac{15}{14.5} \\ n &= \frac{H}{\varepsilon + \nu\varepsilon} = 814 & V_{\text{tot}} &= \frac{1}{4}\pi d^2 H = 36.33\mu\text{m}^3 \\ V_{\text{cyt}} &= \frac{1}{2}V_{\text{tot}} = 18.16\mu\text{m}^3 & \eta &= \frac{1}{2}\nu\varepsilon = 7.25\text{nm} \\ \Sigma_{\text{rod}} &= \pi d H = 103.8\mu\text{m}^2 \end{aligned}$$

7.4 Incisures

It is reported in [40] that mammalian discs have a single incisure. In [55] it is reported that discs in mouse ROS have a single deep cleft or incisure. We have been unable to find in the literature a direct measurement of the geometry of incisures for mouse. We have estimated the structure of incisures in mouse, by relying on a picture reported in [81], showing an intermediate level ROS (Fig. 3). First we measured the diameter by optical methods, obtaining a value of 54 μm . Given the indicated magnification this corresponds to a physical length of 1.5652 μm , which falls within the reported range 0.9–2 μm for diameter of ROS. An incisure is modelled as an isosceles triangle, of base ℓ_{base} and equal sides ℓ_{side} . We measured the base as 10 mm and the sides as 12 mm. Rescaling by the diameter of our choice ($d = 1.4$ μm), we obtained

$$\frac{54}{d} = \frac{10}{\ell_{\text{base}}} = \frac{10}{\ell_{\text{side}}} \Rightarrow \ell_{\text{base}} = 0.2593\mu\text{m}, \text{ and } \ell_{\text{side}} = 0.3111\mu\text{m}$$

We take the ℓ_{side} as the length of the incisure, denoted by ℓ_r , and we take the arc length of the incisure open as the width of the incisure, denoted by ℓ_b . One also computes the width ℓ_b of the incisure and its area A_{inc} from

$$\ell_b = 2\ell_r \arcsin\left(\frac{\ell_{\text{base}}}{2\ell_r}\right) = 0.2674\mu\text{m} \quad A_{\text{inc}} = \frac{1}{2}\ell_r \ell_b = 0.04\mu\text{m}^2$$

Remark 1

While there is only one incisure, the ratio of its area to the area of the geometrical disc it belongs to, is $4A_{\text{inc}}/\ell_d^2 = 2.6\%$. This is a much larger percentage than for the similar ratio for Salamander, which is 0.84% . The analysis in [26] shows that the incisures in mouse and salamander have different functional roles. This supports the idea that not only the relative area exposed by the incisure(s), but also their number, disposition and shape, play a significant role in phototransduction.

8 Appendix B: electro-physiological parameters

Let $F_j^\pm$ denote the upper and lower faces of the discs, and let F^* denote the lower face of the activated disc. It was shown in [22, 25] that the flux of cGMP across the faces $F_j^\pm$ and across F^* is given by

$$-D_{\text{cG}} \frac{\partial[\text{cGMP}]}{\partial z} \Big|_{F_j^\pm} = \frac{1}{2} \nu_{\text{RE}} \varepsilon (\pm \alpha \mp \beta_{\text{dark}}[\text{cGMP}]) - \delta_{z^*} k_{\sigma, \text{hyd}}^* [\text{PDE}^*]_{\sigma} [\text{cGMP}] \quad (10)$$

where z^* is the z -coordinate of the activated face and δ_{z^*} is zero for all z except for $z = z^*$, where it equals one. Moreover

$$\alpha \stackrel{\text{def}}{=} \alpha_{\min} + \frac{\alpha_{\max} - \alpha_{\min}}{1 + ([\text{Ca}^{2+}]/K_{\text{cyc}})^{m_{\text{cyc}}}} \quad (11)$$

This term expresses the synthesis of cGMP by GC, whereas the term $\beta_{\text{dark}}[\text{cGMP}]$ in (10) represents the reduction of cGMP because of hydrolysis by dark activated PDE. Both these terms will be discussed in detail in Section 9.

Let S denote the outer shell of the ROS and denote by $\mathbf{n}$ the unit normal on these surfaces, pointing outside the ROS. It was shown in [22, 25], that the outward flux of Ca^{2+} across S is

$$-D_{\text{Ca}} \nabla[\text{Ca}^{2+}] \cdot \mathbf{n} = \frac{1}{B_{\text{Ca}} \mathcal{F}} \left(J_{\text{ex}} - \frac{1}{2} f_{\text{Ca}} J_{\text{cG}} \right) \quad (12)$$

where the currents J_{ex} and J_{cG} are as in (1) and f_{Ca} is the fraction of cGMP-activated current carried by Ca^{2+} . These formulas, along with (1), involve several biochemical (ε , β_{dark} , $k_{\sigma, \text{hyd}}^*$, B_{Ca} , K_{ex} , K_{cG} , m_{cG}), electrophysiological ($\mathcal{F}$, f_{Ca} , $j_{\text{ex}}^{\text{sat}}$, $j_{\text{cG}}^{\text{max}}$) and biophysical (D_{cG} , D_{Ca}) parameters. Each of them will be discussed below and numerical ranges will be provided, along with a rationale for using these ranges. These equations involve also some geometrical parameters (ℓ_r , ℓ_d) that have already been discussed.

8.1 Dark concentrations and dark currents

Dark concentrations of calcium, $[Ca^{2+}]_{\text{dark}}$ and cGMP, $[cGMP]_{\text{dark}}$ and the dark current densities J_{dark} , $J_{cG;\text{dark}}$ and $J_{ex;\text{dark}}$ are computed from the first principle-based balance of fluxes as indicated below. In the dark, the last term on the right-hand side of (10) is zero, since no R^* is present. The system is in a uniform steady state and the synthesis of cGMP by GC is in equilibrium with the hydrolysis of cGMP by PDE activity in the dark. Thus also the first term in the right-hand side of (10) is zero, yielding

$$\alpha_{\text{dark}} = \beta_{\text{dark}} [cGMP]_{\text{dark}} \quad (13)$$

where α_{dark} is defined from (11) for $[Ca^{2+}]$ replaced by its dark value $[Ca^{2+}]_{\text{dark}}$. In the dark, the Ca^{2+} influx from the cGMP gated channels compensates the Ca^{2+} efflux via the exchanger. Thus the Ca^{2+} flux across S is zero, and $[Ca^{2+}]_{\text{dark}}$ and $[cGMP]_{\text{dark}}$ can be computed by setting to zero the right hand side of (12). Thus recalling the expressions (1) of J_{ex} and J_{cG}

$$j_{ex}^{\text{sat}} \frac{[Ca^{2+}]_{\text{dark}}}{K_{ex} + [Ca^{2+}]_{\text{dark}}} = \frac{1}{2} f_{Ca} j_{cG}^{\text{max}} \frac{[cGMP]_{\text{dark}}^{m_{cG}}}{K_{cG}^{m_{cG}} + [cGMP]_{\text{dark}}^{m_{cG}}} \quad (14)$$

Next, we compute $[cGMP]_{\text{dark}}$ from (13) and substitute it in (14). This gives an equation involving $[Ca^{2+}]_{\text{dark}}$ alone, of the form

$$\left(\frac{\beta_{\text{dark}} K_{cG}}{\alpha_{\text{dark}}} \right)^{m_{cG}} = \frac{1}{2} f_{Ca} \frac{j_{cG}^{\text{max}}}{j_{ex}^{\text{sat}}} \left(1 + \frac{K_{ex}}{[Ca^{2+}]_{\text{dark}}} \right) - 1 \quad (15)$$

A necessary condition for these relations, to admit a positive solution for $[cGMP]_{\text{dark}}$ and $[Ca^{2+}]_{\text{dark}}$, is

$$\left(\frac{\beta_{\text{dark}} K_{cG}}{\alpha_{\text{dark}}} \right)^{m_{cG}} > \frac{1}{2} f_{Ca} \frac{j_{cG}^{\text{max}}}{j_{ex}^{\text{sat}}} - 1 \quad (16)$$

We next begin discussing each of the parameters, by examining the available, published experimental data, and will identify a range of variability for them. For some, for example, K_{ex} , for which no published values and/or ranges are available, the determination is effected by fitting the mathematical model to the experimental data of Rieke (Appendix E), subject to the constraints (13)–(16).

8.1.1 $[Ca^{2+}]_{\text{dark}}$ and $[cGMP]_{\text{dark}}$

The reported experimental measurements of $[Ca^{2+}]_{\text{dark}}$ span a large range. In [28] a variability range of 200–670 nM is reported; in [51] such a range is given as 200–400 nM. Single values are also reported in the literature; for example, 220 nM [52], 273 nM [49], 400 nM [50], 554 nM [47, 48], 670 nM [53]. A value of 250 nM of $[Ca^{2+}]_{\text{dark}}$, specifically for mouse is reported in [54]. The value 400 nM is also used in numerical simulations of [37]. Thus the literature exhibits a range of 200–670 nM for $[Ca^{2+}]_{\text{dark}}$ and this is the range we use for our simulations. In the iterative process of fitting Rieke's experimental data (Appendix E), we start from a putative value of $[Ca^{2+}]_{\text{dark}} = 400$ nM in (13)–(15). Successive approximations to calibrate the parameters, yield a final computed value of $[Ca^{2+}]_{\text{dark}} = 462$ nM.

As reviewed in [40], the range of $[cGMP]_{\text{dark}}$ for mammals, is 0.5–4 μM . The most widely accepted range is 2–4 μM reported in [45]. The value 4 μM was indicated in [40] and the value 3 μM was reported in [35, 36, 39]. Thus, we take the range 2–4 μM and use $[cGMP]_{\text{dark}} = 3 \mu\text{M}$ as the starting value in (13)–(15), for the iterative process of calibrating the parameters. The final calibration yields the value $[cGMP]_{\text{dark}} = 2.93 \mu\text{M}$.

8.1.2 Total dark current j_{dark}

The literature contains a number of experimental measurements of this parameter. Reported values are 8.9 pA in [38], 9 pA in [73], 10.4 pA in [69], 11.3 pA in [68], 12.1 pA in [36], 12.3 pA in [36, 70, 71], 12.5 pA in [65], 14.7 pA in [37], 17 pA in [67], and 21 pA in [28]. In some contributions [66, 88], the circulating dark current was assumed to be equal to the value of the saturating light response, and estimated, by indirect simulations as 8.2 pA in [34], 8.8 pA in [72], 8.9 pA in [74], 14.1 pA in [66], 10.8 pA in [37] and 15 pA in [36]. Thus a range of 8.2–21 pA emerges, which is what we use in our simulations. The starting value in the calibration procedure is taken to be $j_{\text{dark}} = 12.3 \text{ pA}$, and the computed value after calibration is 13.8 pA.

8.1.3 Dark current $j_{\text{ex;dark}}$ carried by the exchanger, the dark current $j_{\text{cG;dark}}$ carried by the cGMP-gated channels, and the fraction f_{Ca} of cGMP-activated current carried by Ca^{2+}

In the dark state, the Ca^{2+} influx from the cGMP-gated channels compensates the Ca^{2+} flux extruded by the exchanger. Therefore the parameters $J_{\text{ex;dark}}$, $J_{\text{cG;dark}}$ and f_{Ca} are linked by

$$J_{\text{ex;dark}} = \frac{1}{2} f_{\text{Ca}} J_{\text{cG;dark}} \quad (17)$$

In analogy with the definition of $J_{\text{ex;dark}}$, $J_{\text{cG;dark}}$ we define

$$J_{\text{ex;dark}} = \int_{\text{rod}} J_{\text{ex;dark}} dS = J_{\text{ex;dark}} \sum_{\text{rod}}, J_{\text{cG;dark}} = \int_{\text{rod}} J_{\text{cG;dark}} dS = J_{\text{cG;dark}} \sum_{\text{rod}} \quad (18)$$

In [28] it is reported $j_{\text{cG;dark}} = 20 \text{ pA}$ and $j_{\text{ex;dark}} = 1 \text{ pA}$, from which it follows $f_{\text{Ca}} = 0.1$. In [38], a value of 0.53 pA is reported as exchanger ‘initial amplitude’ with a 8.9 pA dark current. Taking this ‘initial amplitude’ as $j_{\text{ex;dark}}$ one has

$$j_{\text{cG;dark}} = (8.9 - 0.53) \text{ pA} = 8.37 \text{ pA} \quad \text{and} \quad f_{\text{Ca}} = 2 \times 0.53 / 8.37 = 0.127$$

An investigation of the cation selectivity of cGMP-gated channels in bovine ROS is carried in [64]. The reported relative fluxes are

$$\text{Li:Na:K:Cs} = 0.83:0.88:1:0.59, \text{Mg:Mn:Ca:Sr:Ba} = 0.5:0.86:1:0.64:0.47$$

and $\text{Na} : \text{Ca} = 7 : 1$. Therefore

$$f_{\text{Ca}} = \frac{2}{7((0.83/0.88) + 1 + (1/0.88) + (0.59/0.88)) + 2(0.5 + 0.86 + 1 + 0.64 + 0.47)} = 0.06$$

In [34] it is reported that the exchange current is 7.7% of the suppressible current. From this

$$\frac{j_{\text{ex;dark}}}{j_{\text{dark}}} = \frac{(1/2)f_{\text{Ca}}j_{\text{cG;dark}}}{(1/2)f_{\text{Ca}}j_{\text{cG;dark}} + j_{\text{cG;dark}}} = \frac{f_{\text{Ca}}}{f_{\text{Ca}} + 2} = 0.077 \Rightarrow f_{\text{Ca}} = 0.17$$

It is also reported that the 10–15% of the cGMP-activated current is carried by Ca^{2+} [28, 63]. This implies a range of 0.06–0.17 for f_{Ca} and the value 0.06 is used in the simulation.

Finally, after calibration we compute $j_{\text{ex;dark}} = 0.4$ pA, and $j_{\text{cG;dark}} = 13.4$ pA.

8.2 Electrophysiological parameters of the exchanger and the cGMP-gated channels

8.2.1 $\text{Na}^+/\text{Ca}^{2+}$, K^+ exchanger – the saturated exchange current $j_{\text{ex}}^{\text{sat}}$ and the value of K_{ex} as the Ca^{2+} concentration for half maximum exchanger channel opening

The current due to Ca^{2+} exchange is given by the Michaelis–Menten relationship reported in (1). The value of K_{ex} is estimated from the dark state

$$j_{\text{ex;dark}} = j_{\text{ex}}^{\text{sat}} \frac{[\text{Ca}^{2+}]_{\text{dark}}}{K_{\text{ex}} + [\text{Ca}^{2+}]_{\text{dark}}}$$

For the values $j_{\text{dark}} = 12.3$ pA and $[\text{Ca}^{2+}]_{\text{dark}} = 400$ nM, compute

$$j_{\text{ex;dark}} = \frac{j_{\text{dark}}}{1 + 2/f_{\text{Ca}}} = \frac{12.3 \text{ pA}}{1 + 2/0.06} = 0.358 \text{ pA}$$

From this and the previous relation

$$K_{\text{ex}} = \frac{j_{\text{ex}}^{\text{sat}}}{j_{\text{ex;dark}}} [\text{Ca}^{2+}]_{\text{dark}} = [\text{Ca}^{2+}]_{\text{dark}} = 1.12 \frac{j_{\text{ex}}^{\text{sat}}}{\text{pA}} \mu\text{m} - 0.4 \mu\text{m}$$

The value of $j_{\text{ex}}^{\text{sat}}$ in bovine rods, is reported to be 1–2 pA [28, 76]. For the choice $j_{\text{ex}}^{\text{sat}} = 1.8$ pA we compute $K_{\text{ex}} = 1.6 \mu\text{M}$. This is the value used in our simulations.

It should be noted, however, that for $j_{\text{ex}}^{\text{sat}} = 1$ pA one has $K_{\text{ex}} = 0.72 \mu\text{M}$. Moreover, in [76] there is a reported that the value $K_{\text{ex}} = 0.9 \mu\text{M}$, corresponding to $j_{\text{ex}}^{\text{sat}} = 1.16$ pA.

8.2.2 cGMP-gated channel, – the maximum cGMP-gated channel current $j_{\text{cG}}^{\text{max}}$, the value of K_{cG} as the cGMP concentration for half maximum opening of the cGMP-gated channels, and the Hill exponents m_{cG} for cGMP-gated channels

The current carried by the cGMP-gated channels is given by the Hill-type relation reported in (1). Most contributions report $m_{\text{cG}} = 3$ [35–37, 40, 71], and this value is used as initial in our simulations. Also we take $K_{\text{cG}} = 20 \mu\text{M}$ as reported in [28]. The value of $j_{\text{cG}}^{\text{max}}$ is then computed from the dark state

$$j_{\text{cG;dark}} = j_{\text{cG}}^{\text{max}} \frac{[\text{cGMP}]_{\text{dark}}^{m_{\text{cG}}}}{K_{\text{cG}}^{m_{\text{cG}}} + [\text{cGMP}]_{\text{dark}}^{m_{\text{cG}}}}$$

With $j_{\text{dark}} = 12.3 \text{ pA}$, and $[\text{cGMP}]_{\text{dark}} = 3 \text{ }\mu\text{M}$ (Section 8.1.1), compute

$$j_{\text{cG;dark}} = \frac{j_{\text{dark}}}{1 + (1/2)f_{\text{Ca}}} = \frac{12.3 \text{ pA}}{1 + (1/2)0.06} = 11.94 \text{ pA}$$

From this and the preceding relation, we finally compute

$$j_{\text{cG}}^{\text{max}} = j_{\text{cG;dark}} \frac{K_{\text{cG}}^{\text{m}_{\text{cG}}} [\text{cGMP}]_{\text{dark}}^{\text{m}_{\text{cG}}}}{[\text{cGMP}]_{\text{dark}}^{\text{m}_{\text{cG}}}} = 11.94((20/3)^3 + 1) \text{ pA} = 3550 \text{ pA}$$

Finally, after calibration we use $m_{\text{cg}} = 2.9$.

9 Appendix C: biochemical parameters

9.1 cGMP synthesis

The rate of cGMP synthesis by GC is expressed as the change in concentration in the outer segment cytoplasmic volume over time (in μMs^{-1}) and is given by (11). In this formula, α_{min} is the minimum rate for high $[\text{Ca}^{2+}]$ (theoretically as $[\text{Ca}^{2+}] \rightarrow \infty$), and α_{max} is the maximum rate at low $[\text{Ca}^{2+}]$ (theoretically as $[\text{Ca}^{2+}] \rightarrow 0$). Denote also by α_{dark} the rate of cGMP synthesis in the dark, given by (11) with $[\text{Ca}^{2+}]$ replaced by its dark value $[\text{Ca}^{2+}]_{\text{dark}}$

$$\alpha_{\text{dark}} \stackrel{\text{def}}{=} \alpha_{\text{min}} + \frac{\alpha_{\text{max}} - \alpha_{\text{min}}}{1 + ([\text{Ca}^{2+}]_{\text{dark}}/K_{\text{cyc}})^{m_{\text{cyc}}}} \quad (19)$$

It follows from these definitions that the parameters α_{max} , α_{min} and α_{dark} must satisfy the compatibility conditions

$$\alpha_{\text{dark}} > \alpha_{\text{min}} \text{ and therefore } \frac{\alpha_{\text{max}}}{\alpha_{\text{min}}} > \frac{\alpha_{\text{max}}}{\alpha_{\text{dark}}} \quad (20)$$

The parameter K_{cyc} in (11) is the half-saturating $[\text{Ca}^{2+}]$, and m_{cyc} is the Hill coefficient. In [34] it is reported that

$$\begin{aligned} \alpha_{\text{max}} &= 55.9 \mu\text{Ms}^{-1} & \Delta\alpha &= \alpha_{\text{max}} - \alpha_{\text{min}} = 47.5 \mu\text{Ms}^{-1} \\ K_{\text{cyc}} &= 73.0 \text{ nM} & m_{\text{cyc}} &= 2.4 \end{aligned} \quad (21)$$

With these values, and the value $[\text{Ca}^{2+}]_{\text{dark}} = 462 \text{ nM}$ computed in Section 8.1.1, using formula (19) one computes $\alpha_{\text{dark}} = 8.96 \mu\text{Ms}^{-1}$. In [35] it is reported

$$\begin{aligned} \alpha_{\text{max}} &= 7.65 \mu\text{Ms}^{-1} & \alpha_{\text{min}} &= 5.5 \mu\text{Ms}^{-1} \\ K_{\text{cyc}} &= 129 \text{ nM} & m_{\text{cyc}} &= 2.45 \end{aligned} \quad (22)$$

Using these values, and again $[\text{Ca}^{2+}]_{\text{dark}} = 462 \text{ nM}$, one computes $\alpha_{\text{dark}} = 8.49 \mu\text{Ms}^{-1}$. Thus the value of α_{dark} computed from either set of parameters is roughly the same. Of the two sets of parameters we will take the latter for two reasons. First, the value of K_{cyc} is closer to that reported in most contributions ($\approx 200 \text{ nM}$, [36]). Second, it is linked to the compatibility

condition (20). The ratio $\alpha_{\max}/\alpha_{\text{dark}}$ has been reported in [36] to be in the range 11–12. Using the set of parameters in (22) we compute

$$\frac{\alpha_{\max}}{\alpha_{\min}} \simeq 13.9 > 11 - 12 (\text{as in [36]}) > 9.01 \simeq \frac{\alpha_{\max}}{\alpha_{\text{dark}}} ([35])$$

The same ratio for the values in (21) gives

$$11 - 12 (\text{as in [36]}) > 6.65 \simeq \alpha_{\max} / \alpha_{\min} > 6.24 \simeq \frac{\alpha_{\max}}{\alpha_{\text{dark}}} ([34])$$

Therefore while both data sets satisfy the compatibility condition (20), those of [34] are not compatible with those of [36]. Although our choice is based on a consistency analysis, it should be pointed out that wide ranges of these parameters are reported in the literature. For example, [37], reports $m_{\text{cyc}} = 2.3$ and $K_{\text{cyc}} = 400\text{nM}$. In [36], $m_{\text{cyc}} = 3.7$ –4, and in [38], $m_{\text{cyc}} = 3.7$ and $K_{\text{cyc}} = 250\text{nM}$.

9.2 cGMP hydrolysis by dark-activated PDE

9.2.1 Basal PDE activity with rate β_{dark}

The rate β_{dark} of basal PDE activity is the rate of cGMP hydrolysis by PDE activity in the dark. The simulations of [37, 38] use the values $\beta_{\text{dark}} = 9, 10$. From the *in vivo* investigations in [35], and Fig. 2 of that paper we estimate

$$\beta_{\text{dark}} \simeq 0.03 \frac{\text{mol cGMP}}{\text{mol Rhodopsin}} \text{s}^{-1}$$

The ratio of Rhodopsin per PDE is 50 for mammals [28] (64.5 for bovine [83]). Therefore we estimate the basal PDE activity β_{dark} to be either 1.5 s^{-1} , or 1.935 s^{-1} . A much higher value of 45 s^{-1} for bovine is reported in [89]. Discarding such an outlier, we find for β_{dark} a range 1.5 – 10 s^{-1} . The parameter β_{dark} is linked to $\alpha_{\min}, \alpha_{\max}, [\text{Ca}^{2+}]_{\text{dark}}$ and $[\text{cGMP}]_{\text{dark}}$ by the relationships (13) and (19), and it is subject to the compatibility condition (16).

Regarding β_{dark} as a function of the indicated parameters, from the estimated ranges of $[\text{Ca}^{2+}]_{\text{dark}}$ and $[\text{cGMP}]_{\text{dark}}$ reported in Section 8.1.1, and the values of $\alpha_{\max}, \alpha_{\min}$ and $K_{\text{cyc}}, m_{\text{cyc}}$ in (22), one computes from (13) and (15) a range $\beta_{\text{dark}} = [1.68$ – $11.79] \text{ s}^{-1}$. This is close and consistent with the experimental and computational sources discussed above.

Using the starting calibrating choices $[\text{Ca}^{2+}]_{\text{dark}} = 400 \text{ nM}$ and $[\text{cGMP}]_{\text{dark}} = 3 \text{ }\mu\text{M}$, we find that the compatibility condition (16) is satisfied for $\beta_{\text{dark}} > 1.72 \text{ s}^{-1}$. As a starting value for β_{dark} in the parameter calibration process, we take $\beta_{\text{dark}} = 3.2 \text{ s}^{-1}$. The computed value after calibration is $\beta_{\text{dark}} = 2.9 \text{ s}^{-1}$.

9.2.2 Surface hydrolysis rate $k_{\sigma;\text{hyd}}$ of cGMP by PDE in the dark; surface density $[\text{PDE}]_{\sigma}$ of dark-activated PDE

There exist two hydrolytic subunits (subunit) per PDE molecule. Transducin binds to the hydrolytic subunits and activate the PDE molecule, which then catalyses the hydrolysis of cGMP. We assume that, in the dark active PDE molecules have one transducin bound with partial activity ($k_{\sigma;\text{hyd}}$) and that in the light active PDE molecules have two transducin bounds with full activity ($k_{\sigma;\text{hyd}}^*$). The surface density $[\text{PDE}]_{\sigma}$, of dark-activated PDE (in number of molecules per μm^2), is 500 [28, 40]. The density of Rh in mouse ROS is 30 000–55 000 Rh monomers per μm^2 , with an average density of $48\,300 \pm 8300$ Rh monomers per

μm^2 [56, 59]. The PDE concentration in bovine rod disc membranes is $1/64.5$ of the Rh concentration, as reported in [83]. Thus $[\text{PDE}]$ is $48\,300/64.5 = 749$ molecules of PDE per μm^2 . The ratio of Rh monomer to PDE heterotetramer is 50, as reported in [28, 40]. In such a case $[\text{PDE}] = 48\,300/50 = 966$ PDE/ μm^2 . Thus we have a range of 500–1000 molecules of PDE per μm^2 . In our simulations we use the value 750 PDE/ μm^2 .

The surface hydrolysis rate $k_{\sigma;\text{hyd}}$ of cGMP by dark-activated PDE in [$\mu\text{m}^3 \text{s}^{-1}$ per PDE molecule] can be calculated from [[25], formula (16)]

$$k_{\sigma;\text{hyd}} = \frac{\eta\beta_{\text{dark}}}{[\text{PDE}]_{\sigma}} = \frac{0.00725\mu\text{m} \times 2.9\text{s}^{-1}}{750\# \text{ of PDE molecules per } \mu\text{m}^2} = 2.8 \times 10^{-5} \mu\text{m}^3 \text{s}^{-1} \quad (23)$$

where the parameter η is defined and reported in Section 8.1. In the simulations we use

$$k_{\sigma;\text{hyd}}[\text{PDE}]_{\sigma} = \eta\beta_{\text{dark}} = 0.021\mu\text{m} \cdot \text{s}^{-1}$$

9.3 cGMP hydrolysis by light-activated PDE

9.3.1 Amplification constant A ; the rate ν_{RE} of PDE formation by fully activated Rh; the surface hydrolysis rate $k_{\sigma;\text{hyd}}^*$ of cGMP by light-activated PDE

The amplification constant A is defined as in [28], by

$$A = \nu_{\text{RE}} \left(\frac{(1/2)k_{\text{cat}}/K_{\text{m}}}{N_{\text{Av}} V_{\text{cyt}} B_{\text{cG}}} \right) m_{\text{cG}} \quad (24)$$

where ν_{RE} is the rate of PDE formation per fully activated Rh, $k_{\text{cat}}/K_{\text{m}}$ is the hydrolytic efficacy of activated PDE dimer, N_{Av} is Avogadro number and B_{cG} is the buffering power of the cytoplasm for cGMP. As in [25] set

$$k_{\sigma;\text{hyd}}^* = \frac{k_{\text{cat}}/K_{\text{m}}}{N_{\text{Av}} B_{\text{cG}}} \text{ so that } A = \nu_{\text{RE}} \left(\frac{(1/2)k_{\sigma;\text{hyd}}^*}{V_{\text{cyt}}} \right) m_{\text{cG}}$$

The value of the parameter A for mammals is reported to be 12 s^{-2} in [40], 5.6 s^{-2} in [28], 6.2 s^{-2} in [60], and 13.6 s^{-2} in [34]. This gives a range of 5.6 – 13.6 s^{-2} . We take $A = 13.6 \text{ s}^{-2}$ as reference value in our simulations. For such a choice

$$\nu_{\text{RE}} k_{\sigma;\text{hyd}}^* = 2AV_{\text{cyt}}/m_{\text{cG}} = 2 \times 13.6\text{s}^{-2} \times 18.16\mu\text{m}^3/3 = 164.65\mu\text{m}^3 \text{s}^{-2}$$

As discussed in [25], the range of ν_{RE} can be taken as 120 – 220 s^{-1} ([28, 39, 43, 82], see also Section 10.1). Therefore the range of $k_{\sigma;\text{hyd}}^*$ is 0.75 – $1.37 \mu\text{m}^3 \text{s}^{-1}$. These two parameters are used to adjust the simulations to fit the experimental data. After calibration we compute $\nu_{\text{RE}} = 190\text{s}^{-1}$ and $k_{\sigma;\text{hyd}}^* = 0.9\mu\text{m}^3 \text{s}^{-1}$.

9.3.2 Buffering power B_{cG} of cytoplasm for cGMP; hydrolytic efficacy $k_{\text{cat}}/K_{\text{m}}$ of activated PDE dimer

The buffering power of cGMP describes the relationship between the total and the free concentration of cGMP, which is defined as $B_{\text{cG}} = d[\text{cG}]_{\text{tot}}/d[\text{cG}]$. More than 90% of the total cGMP in the ROS tightly bind to the two non-catalytic sites of PDE [28]. In contrast to

the sites on the channels, these binding sites equilibrate exceedingly slowly, over a time course of many minutes [28], so that we consider them as permanent stores of cGMP. Thus, within a range of 1–2 in [28, 39, 40], we take $B_{cG} = 1$ in our simulation as in [43].

The parameter k_{cat} is the turnover rate of doubly-activated PDE holomer with two transducin bound, and K_m is the Michaelis constant. In [40], the value of k_{cat} for bovine ROS is reported in the range 600–7400 s^{-1} . The range of K_m for PDE is reported as 6–80 $M s^{-1}$ in [78]. Thus, the range of k_{cat}/K_m is estimated as 7.5–1200 $\mu M s^{-1}$. Since this is quite large, we compute it instead from the previous formula

$$k_{cat}/K_m = k_{\sigma,hyd}^* N_{Av} B_{cG}$$

Using the value $N_{Av} = 6.02 \times 10^{23}$, we deduce a range of 450–820 $\mu M s^{-1}$ for k_{cat}/K_m . When compared with the range 340–600 $\mu M s^{-1}$ in amphibians [43], the higher range we have estimated is reasonable in view of the higher value of k_{cat} for bovine, as observed in [40]. Thus for k_{cat}/K_m we take the range 450–820 $\mu M s^{-1}$. Within it, we select the value 540 $\mu M s^{-1}$ and then select the values $k_{cat} = 5400 s^{-1}$ and $K_m = 10 \mu M$.

9.4 Diffusion coefficients D_{cG} of cGMP and D_{Ca} of Ca^{2+} ; buffering power B_{ca} for Ca^{2+}

A detailed discussion on how to interpret the diffusion coefficients is in [22, 25, 62]. As we could not find an experimental measurement of D_{cG} for mouse rod, we take the range of D_{cG} , $50 \times 196 \mu m^2 s^{-1}$, for salamander in [25] as our reference. Taking into account the fact that mouse has a higher body temperature than that of salamander, we take a value of 150 $\mu m^2 s^{-1}$ in our simulation.

The parameter $D_{ca} = 15 \mu m^2 s^{-1}$, is taken from [41]. The buffering power $B_{ca} = 20$ for Ca^{2+} is taken from [41, 42]. This is consistent with the aqueous free diffusion of Ca^{2+} with the range of 140–300 $\mu m^2 s^{-1}$ reported in [41]. A range of 17.5–44 is also reported in [39, 42].

10 Appendix D: activation and deactivation parameters

10.1 Activation parameters

The value of the rate R_G and R_E of T^* and E^* formation per fully activated Rh have been reviewed by several authors [11, 90]. These two parameters are linked by the coefficient of coupling efficiency c_{TE} through the formula

$$\nu_{RE} = c_{TE} \nu_{RG}$$

Such a coefficient is estimated to be 0.7 in [91], in the range 0.8–0.9 in [40] and 1 in [43]. It is remarked in [28] that it increases to unity at lower rates. In our simulations we take $c_{TE} = 1$. For this choice, the discussion below about R_G , applies equally well to R_E .

Both [11, 90] accept the range 120–150 s^{-1} reported in [43]. The value $R_G = 1000 s^{-1}$ estimated from the AT signal, and reported in [92], are in conflict with the value $R_G = 150 s^{-1}$, measured from GTP S binding assay, and reported in [92]. An analysis is produced in [90] (pages 165–7), by which the two measurements can, at least in part, be reconciled by dividing AT-based measurements by a factor of 10. This brings the two measurements into reasonable agreement. An estimate of R_G , measured from the dissociation of AT signal is in [93]. From this R_G , for bovine rod at 22°C is calculated in [90] to yield a value of 220

s^{-1} . To our knowledge there are no published values of ν_{RG} , for mouse. We will estimate it in three ways. First, by an analysis similar to that in [90, 93], the rate ν_{RG} , for bovine at 34°C is predicted to be

$$\nu_{RG} = \nu_{RG, \max} \frac{[T]}{[T] + K_T}$$

, where the maximum rate $\nu_{RG, \max} = 1300 s^{-1}$, and $[T]$ is the surface concentration (in # of molecules per μm^2) of the membrane-bound transducer, and K_T , is the concentration of T that would yield half maximal rate. Therefore for bovine rod at 37°C, $\nu_{RG} = 650 s^{-1}$.

Second, an estimate based on AT signal as in [94] yields $\nu_{RG} = 4000 s^{-1}$, for bovine rod at 37°C. By the rescaling suggested in [90], we estimate $\nu_{RG} = 400 s^{-1}$.

Third, we seek to determine ν_{RE} , from the formula for amplification constant A introduced in (24). As discussed in Section 9.3 with $N_{Av} = 6.022 \times 10^{23}$, we use $V_{cyt} = 18.16 \mu m^3$, $B_{cG} = 1$, $m_{cG} = 3$, and A in the range 5.6–13.6 s^{-2} .

The temperature of the mouse (37°C), higher than the temperature of amphibians (22°C), is expected to increase the values of the parameters k_{cat} and ν_{RE} [90]. The value of k_{cat} for amphibian, reported in [95] is 4400 s^{-1} , and it seems to be widely accepted [43]. The value of k_{cat} for bovine reported in [40] is as high as 7400 s^{-1} . Thus, we take 4400–7400 s^{-1} as a reference range of k_{cat} for mouse rod. As discussed in [43], the value of K_m is 10 μM . From these ranges we compute from (24), a range for ν_{RE} , of 55–225 s^{-1} . For the choice $m_{cG} = 2$, the other parameters and ranges remaining fixed, we compute a range of 85–340 s^{-1} for ν_{RE} . In calibrating the parameters, we select a range of 120–220 s^{-1} for ν_{RE} . The selected value after calibration is $\nu_{RE} = 190 s^{-1}$.

10.2 Decay parameters

10.2.1 Decay rate k_R and average lifetime τ_R^* of R^*

Two critical events, phosphorylation and arrestin binding, participate in the deactivation of R^* . The parameters k_R and τ_R^* , are mutually reciprocal ($k_R = \tau_R^{*-1}$). In the simulations of [37] the value $k_R = 11 s^{-1}$ was used. On the other hand, the average lifetime τ_R^* cannot exceed 200 ms. Indeed a value of 150 ms is estimated in [91], implying $k_R = 6.7 s^{-1}$. In recent studies, Krispel *et al.* [80] reported that the average lifetime for R^* is 80 ms, which means that the deactivation rate of R^* is $k_R = 12.5 s^{-1}$ [80]. Such, a range of 6.7–12.5 s^{-1} is set as a reference for k_R , and $k_R = 12.5 s^{-1}$ is taken in our simulation.

10.2.2 Decay rate k_E and average lifetime τ_E^* of E^*

These parameters are linked by $k_E = \tau_E^{*-1}$. Formouse rod, in vivo measurements of [37] report $k_E = 5 s^{-1}$. The simulations of [38] use $k_E = 7 s^{-1}$. In [28], for mammalian rods, a value $\tau_E^* = 0.2 s$ is reported, implying $k_E = 5 s^{-1}$. In recent studies, Krispel *et al.* [80] reported that the G protein deactivation is normally at least 2.5 times slower than R^* deactivation, which means $\tau_E^* = 2.5 \tau_R^* = 2.5 \times 80 ms = 0.2 s$, implying that the deactivation rate of E^* is $k_E < 5 s^{-1}$ [80]. A range of 5–7 s^{-1} is set as a reference for k_E , and $k_E = 5 s^{-1}$ is taken in our simulation.

11 Single photon response

We report in Table 3 the values of the SPR from several investigators. We will use as a benchmark the experimental data kindly provided by *F. Rieke* and reported in Fig. 4. The curves are each an average of about 100 SPRs on a single ROS, normalised by its own J_{dark} . Their average (i.e. the average of the averages) is reported in Fig. 5. Such a cumulative average exhibits a relative response of 5.42% and a time to peak of 184 ms. It is observed that the first non-negative value in the average of the experimental data occurs at time 37 ms. This is used as this ‘initial time’ of the simulated phenomenon. In other words, the average of the experimental data is moved to the left along the time axis of a time 37 ms, as shown in Fig. 5.

The calibration process is done by fitting such an average, assuming that a single photon, falls, at time $t = 0$, on the middle disc of the ROS. The simulation results are shown in Fig. 5.

Figure 1.
(Left) diagram depicting ROS structure, reproduced from [31]
(Right) geometrical description of the ROS

Figure 2.
Cross-section of ROS with a stack of discs with one incisure

Figure 3.
Intermediate level ROS cross-section $\times 34\,500$, reproduced from [81]

Figure 4.
Experimental data from 21 sets of SPRs kindly provided by Dr. F. Rieke

Figure 5.

Mouse SPRs by simulation (black) and experiment (grey)

The simulation is conducted by the parameters shown in Table 1

The wild type mouse shows a current response with maximum of 5.33% at 188 ms after the activation

Experimental data is an average of 21 sets of SPRs kindly provided by Dr. F. Rieke

Table 1

Parameters of the model for mouse ROS

Symbol	Units	Definition	Range	Value	References
$\mu_{\max}$	μMs^{-1}	maximum rate of cGMP synthesis at low Ca^{2+} concentration	76.5; 55.9	76.5	[34, 35]
$\mu_{\max}/\min$	–	suppression ratio of a from high to low Ca^{2+} concentration	6.7–13.9	13.9	[34–36]
A_{inc}	μm^2	area of the incisure		0.04	
dark	s^{-1}	rate of cGMP hydrolysis by dark activated PDE	1.5–10	2.9	[35, 37, 38]
B_{cG}	–	buffering power of cytoplasm for cGMP	1–2	1	[28, 39, 40]
B_{Ca}	–	buffering power of cytoplasm for Ca^{2+}	17.5–44	20	[39, 41, 42]
σ_{TE}	–	coupling coefficient from G^* to E^*	<1	1	[28, 43]
$[\text{cGMP}]_{\text{dark}}$	μM	concentration of cGMP in the dark	2–4	2.93	[28, 35, 36, 39, 40, 44–46]
$[\text{Ca}^{2+}]_{\text{dark}}$	mM	concentration of Ca^{2+} in the dark	200–670	462	[28, 37, 47–54]
d	μm	diameter of ROS	0.9–2	1.4	[28, 40, 55–60]
D_{cG}	$\mu\text{m}^2 \text{ s}^{-1}$	diffusion coefficient for cGMP	50–196	150	[25, 61, 62]
D_{Ca}	$\mu\text{m}^2 \text{ s}^{-1}$	diffusion coefficient for Ca^{2+}	15	15	[41]
D_{E}	$\mu\text{m}^2 \text{ s}^{-1}$	diffusion coefficient for activated PDE	1.2	1.2	[40]
D_{T}	$\mu\text{m}^2 \text{ s}^{-1}$	diffusion coefficient for activated G protein	2.2	2.2	[40]
D_{R}	$\mu\text{m}^2 \text{ s}^{-1}$	diffusion coefficient for activated Rh	1.5	1.5	[40]
	nm	disc thickness	14–17	14.5	[40, 55, 56]
	nm	volume-to-surface ratio		7.25	
$\mathcal{F}$	$\mathcal{C} \text{ mol}^{-1}$	Faraday's constant	96 500	96 500	
f_{Ca}	–	fraction of cGMP-activated current carried by Ca^{2+}	0.06–0.17	0.06	[28, 34, 38, 63, 64]
H	μm	length of ROS	20–25	23.6	[28, 55, 59, 60]
j_{dark}	pA	dark current	8.2–21	13.8	[28, 34, 36, 37, 65–74]
$j_{\text{cG}}^{\max}$	pA	maximum cGMP-gated channel current		3550	
$j_{\text{ex}}^{\text{sat}}$	pA	saturated exchanger current	1–2	1.8	[75–77]
$k_{\text{cat}}/K_{\text{m}}$	$\mu\text{M}^{-1} \text{ s}^{-1}$	hydrolytic efficiency of activated PDE dimer	450–820	540	[40, 43, 78]

Symbol	Units	Definition	Range	Value	References
k_{hyd}	$\mu\text{m}^3 \text{ s}^{-1}$	surface hydrolysis rate of cGMP by dark-activated PDE		2.8×10^{-5}	
$K_{\sigma, \text{hyd}}^*$	$\mu\text{m}^3 \text{ s}^{-1}$	surface hydrolysis rate of cGMP by light-activated PDE	0.75–1.37	0.9	
k_E	s^{-1}	rate constant for inactivation of PDE	5–7	5	[37, 38, 79, 80]
k_R	s^{-1}	rate constant for inactivation of Rh	6.7–12.5	12.5	[37, 42, 80]
K_{cyc}	nM	half-saturating $[\text{Ca}^{2+}]$ for GC activity	73–400	129	[34, 35, 37, 38]
K_{cG}	μM	$[\text{cGMP}]$ for half maximum cGMP-gated channel opening	20	20	[28]
K_{ex}	μM	$[\text{Ca}^{2+}]$ for half maximum exchanger channel opening	0.9–1.6	1.6	[28, 76]
b	μm	width of the incisure		0.2674	[81]
r	μm	length of the incisure		0.3111	[81]
	–	ratio between interdiscal space and disk thickness	0.56–1	1	[28, 40, 43, 55]
	nm	interdiscal space		14.5	[40, 55, 59, 60]
RE	s^{-1}	rate of PDE formation per fully activated Rh	120–220	190	[28, 39, 43, 82]
RG	s^{-1}	rate of transducin formation per fully activated Rh	120–220	190	[28, 39, 43, 42]
n	#	number of discs		814	
n_{inc}	#	number of incisures	1	1	[40, 55, 81]
N_{Av}	# mol ⁻¹	avogadro number	6.02×10^{-3}	6.02×10^{-3}	
m_{cyc}	–	Hill coefficient for GC effect	2.3–4	2.45	[34–38]
m_{cG}	–	Hill coefficient for cGMP-gated channel	~3	2.9	[35–37, 40, 71]
[PDE]	# μm^{-2}	surface density of dark activated PDE	500–1000	750	[28, 40, 56, 59, 83]
R_{disk}	μm	radius of disc		0.685	
	–	ratio between outer shell thickness and disk thickness		15/14.5	
	nm	distance between the disk rim and the plasma membrane (outer shell thickness)	15	15	[61, 40]
rod	μm^2	lateral surface area of ROS		103.8	
V_{cyt}	μm^3	cytoplasmic volume		18.16	

Table 2

Sensitivity index

S_{ij}	I_{act}	I_{peak}	I_{rec}	E_{act}^*	E_{peak}^*	E_{rec}^*	j_{dark}
Geometry							
R	-0.31	-0.30	0.13	0.00	0.00	-0.00	1.00
H	0.00	-0.00	-0.00	0.00	0.00	-0.00	1.00
b	-0.03	0.06	0.01	0.00	0.00	-0.00	0.00
r	-0.32	0.06	0.01	-0.00	0.00	-0.00	0.00
Catalytic activity and diffusion							
R_G	0.93	0.52	-0.04	1.00	1.00	-0.00	0.00
k_R	-0.03	-0.37	0.07	-0.05	-0.63	0.01	0.00
k_E	-0.01	-0.22	1.17	-0.02	-0.34	0.99	0.00
B_{Ca}	-0.00	0.22	0.08	0.00	0.00	-0.00	0.00
D_{cG}	0.08	0.27	0.02	0.00	0.00	-0.00	0.00
D_{Ca}	-0.00	-0.01	-0.00	0.00	0.00	-0.00	0.00
D_E	0.02	0.03	0.00	0.00	-0.00	-0.00	0.00
D_I	0.01	0.00	0.00	0.00	-0.00	-0.00	0.00
cGMP synthesis and hydrolysis							
[PDE]	-0.56	-1.57	0.09	0.07	0.00	0.00	-0.67
k_{-hyd}	-0.67	-1.57	0.09	0.00	0.00	-0.00	-0.67
$k_{\sigma_i hyd}^*$	0.93	0.52	-0.04	0.00	0.00	-0.00	0.00
$_{max}$	0.26	0.16	0.03	0.00	0.00	-0.00	0.26
$_{min}$	0.45	1.04	-0.07	0.00	0.00	-0.00	0.46
m_{eye}	-0.70	-1.04	-0.04	0.00	0.00	-0.00	-0.71
k_{cyc}	0.58	0.44	0.06	0.00	0.00	-0.00	0.58
cGMP-gated channel and Ca^{2+} exchanger							

S_{ij}	I_{act}	I_{peak}	I_{rec}	E_{act}^*	E_{peak}^*	E_{rec}^*	J_{dark}
J_{cG}^{max}	0.25	0.40	-0.01	0.00	0.00	-0.00	0.24
m_{cG}	-0.30	-1.64	0.07	0.00	0.00	-0.00	-1.23
K_{cG}	-0.66	-1.07	0.03	0.00	0.00	-0.00	-0.67
J_{ex}^{sat}	0.75	0.37	-0.06	0.00	0.00	-0.00	0.75
K_{ex}	-0.56	-0.21	0.02	0.00	0.00	-0.00	-0.56
I_{c_a}	-0.72	-0.55	-0.01	0.00	0.00	-0.00	-0.69

S_{ij} : sensitivity index; I_{act} : growth rate of current response at early time ($t = 0.01$ s); I_{peak} : peak value of current response at its time to peak t_{peak} ; I_{rec} : recovery rate of current response at late times ($t > 0.8$ s); E_{act}^* : growth rate of the production of effectors at early time ($t = 0.01$ s); E_{peak}^* : peak value of the number of effectors at its time to peak t_{peak}^* ; E_{rec}^* : decay rate of effectors at late times ($t > 0.8$ s); J_{dark} : dark current

Table 3

Single photon response

Dark current, pA	Response, pA	Relative response %	Time to peak, ms	Reference
8.2	0.66	8.0	140	[13]
11.3	0.34	3.0	89	[43]
10.4	0.56	5.4	108	[56]
12.5	0.527	4.2	109	[19]
8.9	0.39	4.4	138	[54]
12.3	0.46	3.7	104	[18]
8.9	0.39	4.4	137	[95]
8.8	0.53	6.0	128	[90]
12.3	0.50	4.1	114	[57, 58]
		5.42	184	Rieke's