A stochastic model of the single photon response in *Drosophila* photoreceptors

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Abstract

We present a quantitative model for the phototransduction cascade in *Drosophila* photoreceptors. The process consists of four stages: (1) light absorption by Rhodopsin, (2) signal amplification phase mediated by a G-protein coupled cascade, (3) closed/open state kinetics of the transient receptor potential (TRP) ion channels which regulate the ionic current in/out of the cell and (4) Ca regulated positive and negative feedbacks. The model successfully reproduces the experimental results for: single photon absorption "quantum bump" (QB), statistical features for QB (average shape, peak current average value and variance, the latency distribution, etc), arrestin mutant behaviour, low extracellular Ca$^{2+}$ cases, etc. The TRP channel activity is modeled by a Monod-Wyman-Changeux (MWC) model for allosteric interaction, instead of using the usual ad hoc Hill equation. This approach allows for a plausible physical explanation of how Ca/calmodulin regulate the protein activity. The cooperative nature of the TRP channel activation leads to "dark current" suppression at the output allowing for reliable detection of a single photon. Stochastic simulations were produced by using the standard rate equations combined with the Poisson distribution for generating random events from the forward and reverse reaction rates. Noise is inherent to the system but appears to be crucial for producing such reliable responses in this complex, highly nonlinear system. The approach presented here may serve as a useful example how to treat complex cellular mechanisms underlying sensory processes.

Phototransduction, G-protein cascade, computational biology, stochastic simulations

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1 Introduction

Visual transduction in *Drosophila* represents a biological system of great interest. It has been studied for the last few decades, but all the complexities of the photo-cascade are still not yet fully understood. However, many interesting properties have been revealed as well as the underlying mechanisms by which the system generates high quantum efficiency, single photon response, huge signal amplification and fast recovery, as well as light adaptation to 11 orders of magnitude of light intensities, spanning the range of a day-night cycle. Our kinetic model of the *Drosophila*’s single photon transduction process is presented here. Although all the details of the phototransduction are not yet known, work on the modeling of this process has started and it produces some useful insights into the whole process. In addition, the understanding of this phototransduction process could serve as an inspiration for technological advancements in the area of light detection and noise reduction and could perhaps be used for designing bio-inspired imaging chips. Another motivation for modeling the cascade comes from the need to assess the effect of using melanopsin as a membrane protein for neural cells photosensitisation for applications in the rapidly expanding area of optogenetics in neuroscience. Melanopsin also utilises G-protein coupled cascade (GPCC) processes, presumably similar to *Drosophila*’s. In general, we have an engineering type of perspective to the problem, and analyse it mostly from the system point of view. Saying this, we emphasise that here we attempt to treat the problem in almost all its complexity, faithfully following the known biochemistry of the phototransduction process, and modeling it without using "engineering-type" approximations.

The details for the biological model of the phototransduction cascade come from the experimental work published in a number of papers over the last two decades, e.g. 2,10,11. In photoreceptor neurons, the elementary response to a single photon of light is known as a quantum bump. A quantum bump results from the regulation of the activation of some of the ion channels in response to one activated rhodopsin complex, and reflects the amplification of the entire visual cascade in *Drosophila* photoreceptors. The process of phototransduction (probably completely) occurs in the tightly packed cylindrically shaped microvilli, located on the side of the photoreceptor cell. In *Drosophila* photoreceptors, part of the phototransduction cascade is organised as a distinct signalling complex. The inactivation-no-afterpotential-D (INAD) protein, which consists of five PDZ domains, functions as a multivalent adaptor that brings together several components of the phototransduction cascade into a macromolecular complex, with a direct dynamic role in the cascade. The INAD scaffold protein assembles several components of this cascade, including TRP (and TRPL) channels, phospholipase C (PLC), eye protein kinase C (PKC), calmodulin, the myosin III NINAC, etc., into an organised protein complex. In summary, fly photoreceptors are considered to have the fastest known G protein-coupled signalling cascades: latency times of ~40 ms and bump halfwidths of ~20 ms. The kinetics is about a hundred times faster than in the amphibian rods (at similar temperatures) and about ten times faster than in the mammalian rods at 37°C.

The mathematical models that are generated invariably include a large number of variables with numerous parameters, many of which are unknown, or cannot be directly measured. With such highly complex systems there are often few direct measurements that can be made and limited access for inputs or perturbations. Some of the modeling paradigms used in our model were previously developed for modeling the phototransduction process in vertebrates. The vertebrate’s GPCC system was researched in details and extensive modeling can be found in the works of Lamb, Pough and others. Bisegna et al. expanded the vertebrate phototransduction modeling to use sophisticated mathematical approaches to integrate the known cytoarchitecture with the known biochemistry to account for the properties of the photoresponse. Regarding the invertebrate retina, very recently two very good stochastic modeling studies of the single photon response were published by Hardie and Postma and Pumir et al., which represent first attempts in modeling QB response in *Drosophila*. In the former model both temporal and spatial dependencies within a microvillus were incorporated. However, the approach is different from ours and based on the random walk encounter, with very few details about the simulation parameters and quantitative comparison with experimental QB statistics. There are a number of differences between our approach and the latter work. First, Pumir et al. describe a systems analysis, which means that hypothetical elements are introduced in the cascade, such as activator A or regulatory factor C. However, in our model we try to faithfully follow only the biochemical processes that were experimentally described in the literature, such as calcium-dependent activities of enzymes (e.g. DAG kinase (DGK), PKC, NINAC, etc.) or the calcium pump currents. Furthermore, we use MWC allosteric transition theory to describe the TRP channel activity and Goldman-Hodgkin-Katz (GHK) current equation to calculate currents, which should more closely and accurately follow the biophysics of the real processes. All our negative and positive feedbacks have individual physical explanations and models instead of one universal feedback factor with various coupling factors. In this way we are able to identify the effects of each of these processes on the overall...
kinetics.

The stochastic form of the model deals with actual numbers of molecules activated/deactivated in the system as well as the ion-channel noise. Our stochastic model, in contrast to the usual Master equation approach and Gillespie algorithm\textsuperscript{25}, offers easy switching between deterministic and stochastic versions of the rate equations. This approach allows for an independent analysis of the effects of noise for each component in a dynamical system. In this way we were able to identify a stochastic resonance process in the phototransduction which enhances the response.

2 Modeling of the G-protein phototransduction cascade

2.1 Biochemical Model of Phototransduction in \textit{Drosophila} photoreceptors

Phototransduction in \textit{Drosophila} is mediated by the ubiquitous phosphoinositide cascade, leading to the opening of the TRP and TRPL channels\textsuperscript{11,12,26}. It appears that in WT the current is dominated by the TRP channels\textsuperscript{11,12,26}. A summarised and simplified picture of the \textit{Drosophila} phototransduction process is presented in Fig. 1, where we point out the main variables of the system and their dependencies. We first divide the cascade into different modules (Fig. 1B). A brief description of each module is given below. Then we convert the biochemical model into our stochastic mathematical model and perform numerical simulations in order to determine optimal values for a number of parameters introduced in the model.

The first module (M1) corresponds to the rhodopsin cycle since the activation pathway in \textit{Drosophila} photoreceptors starts with the light activation of a rhodopsin. Initially rhodopsin is in its ground state \( R \). A photon absorption by rhodopsin complex generates a metarhodopsin (\( M^* \)), which activates the G-proteins very efficiently\textsuperscript{27}. \( M^* \) is rapidly modified into thermally stable metarhodopsin \( M^b \), by the process of arrestin binding\textsuperscript{28}. \( M^b \) is stable for several hours, but very inefficient in activating G-proteins, hence this process is neglected in the model. Here we do not need to consider the recycling from \( M^b \) back to the ground state \( R \), since we only simulate a single QB, i.e. response to a single photon absorption, but it is straightforward task to include this process in the case of modeling prolonged exposures to light. Thus for our purposes, module M1 represents the kinetics of \( M^* \) activity, which depends on the concentration of free \textit{Ar}r2. The amount of free \textit{Ar}r2 will depend on the NINAC buffer activity\textsuperscript{29}, which depends on the amount of Ca\(^{2+}\).

The second module (M2) is the cascade amplifier and corresponds to the kinetics of G proteins, PLC molecules and DAG generation. A G-protein (specifically \textit{G} in \textit{Drosophila}) binds to \( M^* \), Fig. 1A step (2), GDP exchanges for GTP bound on the \( \alpha \) subunit\textsuperscript{30} and the \( \alpha \) subunit with attached GTP dissociates from the rest of the protein (\( \beta \gamma \) subunits), Fig. 1A step (3). Theoretical considerations reveal that diffusion of G-proteins in the microvillar membrane is sufficient to account for rhodopsin-G-protein interaction in a microvillus in very dim light\textsuperscript{31}. The \( \text{G}_\alpha \) subunit works as a diffusible shuttle between activated rhodopsin and the PLC in the INAD complex\textsuperscript{2,3}. When \textit{G}_\alpha-GTP\( \equiv \text{G}^* \) binds to a PLC they create a complex which activates the effector protein PLC, Fig. 1A step (4). The active complex formed (GPLC*) then starts to hydrolyze phosphatidylinositol biphosphate (PIP\(_2\)) present in the microvillus, producing DAG (see Fig. 2). The GPLC* activity is rapidly quenched by the influx of Ca\(^{2+}\), which also accelerates the recycling of PIP\(_2\), preventing excessive depletion of substrate\textsuperscript{18}. The GPLC* complex decays due to the activity of a GTPase-activating protein (GAP), which is in this case PLC, the same protein\textsuperscript{32} which is the target of \( \text{G}^* \). GAP activity increases with Ca\(^{2+}\) (negative feedback)\textsuperscript{32}. DAG molecules are phosphorylated by DGK to produce phosphatidic acid (PA), and after several additional enzymatic steps, PIP\(_2\) is regenerated\textsuperscript{33}. The decay of DAG, induced by the activity of DGK, is also regulated by Ca\(^{2+}\), ref.\textsuperscript{34}.

The third module (M3) corresponds to the ion channels. \textit{Drosophila}'s photoreceptor light activated conductance consists of two types of channels: TRP and TRPL\textsuperscript{11,35}. Their properties are described in the next paragraph. The channels were considered to be regulated by three components. First of all, their probability of opening depends on the amount of DAG in the microvillus. Second, the influx of Ca\(^{2+}\) at the very early stage of the phototransduction acts as a positive feedback and this process has been suggested\textsuperscript{2,26} to be calmodulin (CaM) mediated as TRP binds to CaM. So when Ca\(^{2+}\) first gets into the cell, it promotes the increase in activity of the channels via CaM. The third component regulating TRP is PKC, which phosphorylates the channel, inhibiting its activity. PKC is regulated by DAG and Ca\(^{2+}\). The combination of these three components acting on TRP allows an abrupt rise of the response as well as a fast recovery. Although the genetic evidence supports the role of DAG as an excitatory ligand for TRP channels, an explicit experimental proof of this assumption is still lacking\textsuperscript{11}. However, directly injected DAG metabolites (Polyunsaturated Fatty Acids, PUFA)s activate the channels. Regardless, the TRP channel opening probability depends on the DAG concentration and this factor
Figure 1: *Drosophila* phototransduction cascade model: A) biological model showing a microvillus membrane with the cascade participants (see text for details). B) A schematic representation of the cascade showing the positive (full, red lines) and negative (broken, blue lines) feedbacks. The cascade is separated into four modules, where each module represents a functional step in the phototransduction process: M1 - photon absorption, M2 - amplification cascade, M3 - ion channel opening/closing kinetics and M4 - intracellular calcium concentration.
is used in the model.

The last module (M4) follows the time evolution of the intracellular ion concentrations of Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\) and K\(^+\). Three mechanisms induce ion currents: the opening of the ion channels, the Ca\(^{2+}\) dependent pumps, which are Na\(^+\)/Ca\(^{2+}\) exchangers, and the passive diffusion from the microvillus to the cell body. TRP channels are highly selective for Ca\(^{2+}\) (the ratio of Ca\(^{2+}\) and Na\(^+\) permeabilities is \(\approx 1\), I\(_{\text{TRP}}\) \(~ 100 : 1\)) and have a small single-channel conductance in physiological solutions (mean open time of \(~ 1\) ms). The TRPL channels are relatively non-selective (P_{Ca}\(_{\text{TRPL}}\) : P_{Na}\(_{\text{TRPL}}\) \(~ 4 : 1\)) with a higher conductance (\(~ 35\) pS), and slower kinetics (mean open time of 1-2 ms). However, it is indeed the highly Ca\(^{2+}\) permeable TRP channels that carry more than 90% of the light-sensitive current, hence the TRPL-channel current is included as a correction to the TRP current.

### 2.2 Mathematical Model

#### 2.2.1 Stochastic model

The phototransduction process can be described by a set of chemical rate equations:

\[
\frac{dX}{dt} = g(X,t) - r(X,t)
\]

where \(X\) is the number of molecules and \(g\) and \(r\) are the rates of increasing (generation) and reducing (removal) the number of \(X\). Species \(X\) can also be taken to represent, for example, the activated form of an enzyme. In order to describe the time evolution of the variable \(X\) which takes only discrete set of values one possibility is to use the master equation for the probability of having exactly \(X\) molecules.

If the rate of generation of molecules of type \(X\) is \(g\), i.e. \(dX/dt = g\), the number of molecules produced during a discrete time step \(\Delta t\) is \(\Delta X = g\Delta t\). However, the processes of generation and removal are random processes. If the stochastic nature of the events is modeled as a Poisson process, the change in \(X\) at every time step will be Poisson distributed, such that on average it takes the time \(\tau = 1/g\) for generating a new molecule. Therefore we have, at each time step,

\[
\Delta X = \Pi(g\Delta t)
\]

where \(\Pi(\lambda)\) gives a Poisson distributed positive integer or zero with mean value \(\lambda\). The value of \(g\Delta t\) is usually very small, because the time step in the numerical calculations is small (\(\Delta t \leq 0.1\) ms), so eq. 2 gives zero almost all the time. Sometimes it gives one which represents the stochastic generation of a molecule \(X\) (values bigger than one have very small probability for small time steps). The assumption of the Poisson distribution is correct for rare events, i.e. for \(g\Delta t \ll 1\), what implies the time step must be \(\Delta t \ll 1/g\). Within each time-step, the probability of the event is very small, however the probability over a fixed period has a fixed value (the rate value). The results will not depend on the value of \(\Delta t\) (when \(\Delta t \ll 1/g\)) because the number of trials \((N)\) during a fixed period of time increases as \(1/\Delta t\). Hence the average time for the generation of a new molecule (which is proportional to \(N\Delta t\)) will be \(\tau = 1/g\). We can apply the same method to the removal process (with a rate \(r\)), and take the rates as time-dependent. Therefore, the full rate equation for the evolution (1), becomes:

\[
\Delta X(t) = \Pi(g(t)\Delta t) - \Pi(r(t)\Delta t)
\]

where the molecule generation and removal processes are independent, so the resulting distribution is a Skellam distribution. In this way randomness is introduced in the process of transduction without introducing any new parameters.

The specific algorithm for this stochastic scheme that we implemented is similar to the Monte-Carlo approach described by Koch for fast sodium ion channels (ref. 41, p.204) and it is essentially a version of the well-known “method of transformation” (ref. 42, chapter 2). In each time step, the values for \(\lambda\) and the probabilities for generation (removal) of 0, 1, 2, ... molecules are calculated. Then the segment \([0,1]\) is partitioned to represent each of these values and a random number \((\text{rand})\) is generated, drawn from a uniform probability distribution between 0 and 1, and the part of the \([0,1]\) segment on which \(\text{rand}\) falls gives the value for the generation (removal) process. Eventually the difference of these two numbers, gives the change of the number of molecules of the species \(X\) during the time step \(\Delta t\), eq. (3). In the code we ensure that the values of \(X\) never become less than zero. In practice the quantity \(\Delta X\) almost never comes out so large as to produce a negative number of molecules of \(X\) because the rate of removal is proportional to \(X\), (e.g. when \(X = 0\) the removal number \(\Pi(r(t)\Delta t)\) can only be zero, when \(X = 1\) the probability for \(\Pi(r(t)\Delta t) = 2\) is very small, etc.).
2.2.2 Metarhodopsin deactivation (module M1)

The process of rhodopsin activation is relatively fast on the time scale of other processes involved in the cascade. Typically we assume ~ 1 ms time delay between photon absorption by a rhodopsin complex and its activation i.e. conversion into metarhodopsin. Another important factor is the metarhodopsin deactivation. In both vertebrates and invertebrates, rhodopsin signaling is terminated by arrestin (Arr2) binding. In vertebrates, this binding requires prior rhodopsin phosphorylations by rhodopsin kinase (RK), but in invertebrates, phosphorylation does not play a direct role\(^{28,43}\). Hence the active metarhodopsin lifetime is regulated by the concentration of the surrounding arrestin. The single event of arrestin binding could be the sole determinant of activated rhodopsin lifetime in flies\(^{44}\). The degradation (i.e. deactivation) of metarhodopsin is described by a rate equation:

\[
\frac{dM^*}{dt} = -k_{MA}Arr_{\text{free}}(t)M^*(t)
\]  

(4)

where \(k_{MA}\) is the reaction rate for the binding of Arr2 to M*. \(Arr_{\text{free}}\) is the number of free Arr2 molecules, which depends on the activity of its buffer NINAC, regulated by Ca\(^{2+}\), as described in a recent paper by Liu et.al.\(^{29}\) The reaction corresponding to the sequestration/release of Arr2 by NINAC is:

\[
\text{NINAC} \cdot \text{Arr2} \leftrightarrow \text{NINAC} + \text{Arr2}.
\]

The equilibrium concentrations of NINAC ([N]), free Arr2 ([Arr\(_{\text{free}}\)]) and sequestered Arr2 ([N\(\text{A}\)]) are related by:

\[
\frac{[N\text{A}]_{\text{eq}}}{[N]_{\text{eq}}[Arr_{\text{free}}]_{\text{eq}}} = K_{\text{ninac}}(\text{Ca}^{2+}),
\]

(5)

where \(K_{\text{ninac}}\) is the equilibrium constant for this reaction. Note that \(K_{\text{ninac}}\) depends on Ca\(^{2+}\). When the intracellular Ca\(^{2+}\) concentration increases it triggers the CaM activity\(^{29}\) and the equilibrium will be shifted so that more arrestin is released from NINAC. \(K_{\text{ninac}}\) decreases with the Ca\(^{2+}\)-induced activity of CaM. This can be described by the following two equations:

\[
K_{\text{ninac}} = K_{\text{ninac,max}} e^{-\beta_1A_{\text{cam}}},
\]

(6)

\[
A_{\text{cam}} = \frac{[\text{Ca}^{2+}]_{\text{tot}}}{[\text{Ca}^{2+}]_{\text{tot}} + K_{\text{cam}}},
\]

(7)

where \([\text{Ca}^{2+}]_{\text{tot}}\) is the total intracellular calcium concentration (later on we will introduce the free calcium concentration as well). The rationale for using an exponential activation/inhibition function is explained bellow in the next subsection.

Therefore, if \([\text{Ca}^{2+}]_{\text{tot}}\) is known at time \(t\), it is possible to calculate (7) and (6), and then by using the conservation of total number of arrestin and NINAC molecules to solve eq. (5) and find \([Arr_{\text{free}}]\), in order to get the instantaneous decay rate of M* in eq. (4). Since the intracellular concentration of Ca\(^{2+}\) is roughly zero in physiological conditions, very few arrestin molecules are free at \(t = 0\) (\(Arr_{\text{free}}(t = 0) = Arr_{\text{min}}\)). Therefore M* will start decaying with a maximal time constant \(\tau_{M^*} = 1/(k_{MA}Arr_{\text{min}})\). Once intracellular Ca\(^{2+}\) increases, Arr2 is very rapidly released and \(\tau_{M^*}\) very quickly reduces to small values. The arrestin concentration is also affected by the prior light exposure through the process of protein translocation in photoreceptor light adaptation\(^{44}\). The regulation of arrestin concentration might be a dominant adaptation mechanism in *Drosophila*\(^{31}\).

Now by converting the rate equation (4) into our stochastic model equation (3), the probability of switching off M* between time \(t\) and \(t + \Delta t\) is: \(p(t) = 1 - \exp[-k_{MA}Arr_{\text{free}}(t) \Delta t]\), i.e. the Poisson probability for a non-zero value. For small time steps: \(p(t) \approx k_{MA}Arr_{\text{free}}(t) \Delta t\), which gives the average lifetime of M* (\(\tau_{M^*}\)) independent of the time step since the number of calculations of \(p\), i.e. the attempts \(n\) to switch off M* during the time \(\tau_{M^*}\) is \(n = \tau_{M^*}/\Delta t\) (so shorter \(\Delta t\) gives smaller \(p\) but \(n\) is proportionally bigger). Hence the activation state of the rhodopsin is modeled as a two-state Markov chain, where M* switches abruptly from 1 to 0. This is in agreement with our understanding of the biochemistry of the process, explained above, which describes the process of metarhodopsin deactivation as a single event process entirely described by arrestin binding.

The activation function

The Ca\(^{2+}\) dependence of the reaction rates used in eq. (6) and the other similar equations here was modeled by using the form:

\[
1/\tau = k(\text{Ca}^{2+}) = k_{\text{dark}} \exp(-\beta A(\text{Ca}^{2+}))
\]

(8)
where \( k_{\text{dark}} \) is the rate constant in "dark", i.e. for \( Ca^{2+} \)-free solution, \( \beta \) is a constant and \( A(Ca^{2+}) \) represents the saturation function of the enzyme with calcium (hence we use a Hill equation form given by eq. (7)). The exponential type of dependency on calcium in eq. (8) we obtain by starting from the Ahrrenius law for chemical reaction rates:

\[
k = k_o \exp(-E_a/k_BT),
\]

where \( E_a \) is the activation energy for the reaction and \( k_B \) is the Boltzmann constant and \( T \) temperature. Given constant \( T \), let \( E_a/k_BT = B \). The activation barrier can be modulated by an enzyme or by binding calcium. Hence \( B \) will depend on \( A \), so we can write \( B(A) = B(0) + (dB/\partial A) \cdot A + O(A^2) \), and approximate it with linear term: \( B(A) = B(0) + \beta A \), where \( \beta = dB/\partial A = \text{const} \). Now we get from eq. (9):

\[
k = k_o \exp(-B(0) - \beta A) = k_o' \exp(-\beta A),
\]

where \( k_o' = k_o \exp(-B(0)) \).

We can compare eq. (8) with more conventional algebraic forms, for example Nikonov et.al\(^{20} \) use a power function for the calcium dependency of the reaction rates:

\[
k = k_{\text{max}} \left( 1 - \frac{k_{\text{max}} - k_{\text{min}}}{1 + ([Ca^{2+}] / K)^n} \right),
\]

where \( K \) is the \( Ca^{2+} \) concentration for half-maximal effect and \( n \) is the Hill coefficient for the activation by \( Ca^{2+} \). The two forms are similar in the sense that both represent the rate constant changing between its maximal and minimal values and these values are the same for both models. It is only that our form may allow for faster transitions between these extremes, which we needed in the case of very confined space which a microvillus is and where all processes develop faster. For example, in the case of metarhodopsin deactivation, experiments show shorter average lifetimes for \( M^* \) (\( \sim 25 \) ms) than the average latency times for the quantum bump (\( \sim 40 \text{–} 45 \) ms). In other words, the metarhodopsin was deactivated almost before any ion channel has been open to let calcium into the microvillus and hasten the \( M^* \) deactivation. This indicates that even very small amounts of calcium entering the microvillus will very rapidly deactivate \( M^* \) and hence we need exponential dependencies for the activation function for the NINAC*Arr2 complex. Generally speaking the exponential form of eq. (8) reduces to something like eq. (11) in the case when the values of the exponent \( \beta A < 1 \), when the effect of interactions takes more conventional algebraic form: \( k \approx k_o'(1 - \beta A) \).

### 2.2.3 The Cascade Amplification (module M2)

Initially, all the G proteins are in the inactive form \( G_{\alpha \beta' \gamma} \cdot GDP \). They randomly move on the two-dimensional membranes of microvilli. The rate at which G proteins get activated depends on the kinetics of a three-step process:\(^{19} \) (i) collision of a G-protein with \( M^* \) and binding, (ii) exchange of GDP to GTP and unbinding of the complex, and (iii) release of the \( \alpha \) subunit, \( G_{\alpha} \cdot GTP \) (or \( G^* \)). During the process (ii), no other G-protein can bind to \( M^* \). If an active G-protein has just been released from \( M^* \), the time for the next G-protein to be released is the sum of the times for processes (i) \( \tau_{\text{coll}} \), i.e. the average time for a G-protein to collide with \( M^* \), and the average time for process (ii), \( \tau_{\text{GDP}} \). The microscopic diffusion theory\(^{45} \) gives that the time for a molecule to travel the average squared distance \( \langle \delta^2 \rangle \) in a two-dimensional space is \( t = \langle \delta^2 \rangle / (4D) \), where \( D \) is the diffusion constant of the molecule. Lamb and Pugh in\(^{19} \) gave a detailed theory of enzymatic activation with two-dimensional diffusion. According to eq. (A1) in\(^{19} \) the collision rate between an active metarhodopsin and G-proteins is:

\[
v_{\text{coll}} \approx \alpha_1 (D_{M^*} + D_G) C_G ,
\]

where \( C_G \) is the area density of \( G \), i.e. \( C_G = N_G / S_{mv} \) (\( N_G \) is the number of G-molecules in a microvillus and \( S_{mv} \) is the area of a microvillus membrane). The factor \( \alpha_1 \) is defined in Appendix 1. Since the number of available G-proteins reduces over the time, we slightly alter relation (12) to:

\[
v_{\text{coll}} \approx \alpha_1 (D_{M^*} + D_G) \frac{G_{\text{tot}} - G^* \cdot GPLC^*}{S_{mv}} ,
\]

where \( G_{\text{tot}} \) is the total number of G proteins and \( GPLC^* \) is the number of \( G^*-\text{PLC} \) active complexes in the microvillus. The average time between two collisions is: \( \tau_{\text{coll}} = 1 / v_{\text{coll}} \). The rate of generating \( G^* \) is then

\[
v_1(t) = \frac{1}{\tau_{\text{coll}}(t) + \tau_{\text{GDP}}}.
\]
where \( \tau_{\text{GDP}} \) is the average time for the GDP/GTP exchange. If every generated \( G_{\alpha \beta \gamma} \) GTP molecule would produce an active \( G_\alpha \) GTP \( \equiv G^* \) instantaneously, then the rate of activation of \( G \) proteins would be

\[
\frac{dG^*}{dt} = v_1(t)M^*(t) .
\] (15)

where the collision efficiency of 100% is assumed (it is possible to introduce this new parameter, but in order to reduce the parameter space the collision efficiency was effectively absorbed in the value for the diffusion constant which is anyhow only an estimate at the moment – similar reasoning will apply to other reactions).

However, the release of the \( \alpha \) subunit happens after some random delay time, which is on average \( \tau_1 \). This delay is introduced into the model by the so-called delay stage function \( f_{\text{act}}(t, \tau) \). This model can be used in any process where a molecule takes some time \( \tau \) to get activated from the moment it received the stimulus. This function is defined as

\[
f_{\text{act}}(t, \tau) = 1 - e^{-t/\tau}
\] (16)

and gives the probability for a molecule to be active after time \( t \) if the stimulus was at \( t = 0 \). The way to use it is to convolve its derivative with the rate of activation in order to obtain the generation term in eq. (15) (see Appendix 2 for details and examples):

\[
\frac{dG^*}{dt} = v_1(t)[M^*(t) \otimes \frac{\partial}{\partial \tau} f_{\text{act}}(t, \tau_1)] - v_2(t)G^*(t)
\] (17)

where the second term describes the decrease of free active \( G \) proteins due to \( G^* \) binding to PLC (and forming GPLC* complex) – the rate of this process is \( v_2(t) \). Eq. (12) can be used again to estimate the collision rate of a diffusing \( G^* \) with PLC molecules:

\[
v_2(t) = \alpha_2D_{G^*}\frac{PLC_{\text{tot}}/S_{\text{tot}}}{(1 + \sqrt{GPLC^*(t)/\pi})^2}
\] (18)

Here we assumed that PLC molecules are practically stationary in comparison to \( G^* \), since they are anchored to the large INAD complexes. The expression in the denominator is a geometrical factor due to the fact that active \( G \) proteins have to explore increasingly longer distances as the number of GPLC* increases, since they all start diffusing from the same point (where the \( M^* \) is). Generally speaking, reaction and diffusion on spaces with complex geometries can give rise to kinetics that depart radically from mass-action form.

The kinetics of GPLC* is given by:

\[
\frac{dGPLC^*}{dt} = v_2(t)G^*(t)\frac{GPLC^*(t)}{\tau_P(Ca^{2+})}
\] (19)

The decay of an active complex GPLC* is described by the time constant \( \tau_P \), which represents the action of a GTPase-activating protein (GAP). Cook et al.\(^{32}\) showed that the PLC functions as a GAP as well, and its activity is also regulated by \( Ca^{2+} \). Qualitatively speaking, \( \tau_P \) decreases with the \( Ca^{2+} \) induced activity of GAP. The \( Ca^{2+} \) dependence of \( \tau_P \) is modeled as (see eq. (A6), Appendix 2):

\[
\tau_P(Ca^{2+}) = \tau_{P,\text{dark}}e^{-\beta_2A_{\text{gap}}(Ca^{2+})}
\] (20)

\[
A_{\text{gap}}(t) = \frac{\partial}{\partial t} \left( \frac{[Ca^{2+}]_{\text{tot}}}{[Ca^{2+}]_{\text{tot}} + K_{\text{gap}}} \right) \otimes f_{\text{act}}(t, \tau_2)
\] (21)

where \( \tau_{P,\text{dark}} \) is the GAP activity for low calcium concentrations and \( \tau_2 \) is the activation delay parameter.

One product of the PLC* action on a PIP2 is DAG, Fig. 2. DAG is a simple lipid consisting of a glycerol molecule linked through ester bonds to two fatty acids and has numerous roles in the cell apart from being a second messenger.\(^{34,46}\) Its small size and simple composition give exceptional properties to DAG.\(^{34}\) The time evolution of DAG is given by:

\[
\frac{dDAG}{dt} = v_3(t,Ca^{2+})GPLC^*(t) - \frac{DAG(t)}{\tau_{\text{diss}}(Ca^{2+})}
\] (22)

This equation contains a negative feedback factor in the rate function \( v_3 \) which describes the rate of conversion of PIP2 into DAG and InsP3. This rate includes the average time for PIP2 collision with an active GPLC*
Figure 2: Active Phospholipase C (the G*-PLC complex) hydrolyzes the phosphodiester link in the membrane phospholipid PIP$_2$, forming InsP$_3$ and DAG.

($\tau_{\text{coll,PIP}_2}$) and the time for the reaction PIP$_2$ → DAG + InsP$_3$, $\tau_{\text{reac,plc}}(\text{Ca}^{2+})$, i.e. the PLC activity, which is inhibited by calcium:\(^{18}\)

$$\nu_3(t, \text{Ca}^{2+}) = \frac{1}{\tau_{\text{coll,PIP}_2}(t) + \tau_{\text{reac,plc}}(\text{Ca}^{2+})}$$  \(23\)

From eq. (12):

$$\frac{1}{\tau_{\text{coll,PIP}_2}(t)} = \alpha_3 D_{\text{PIP}_2} \frac{\text{PIP}_2_{\text{tot}} - \text{PIP}_2_{\text{used}}(t)}{S_{\text{env}}}$$  \(24\)

where $\text{PIP}_2_{\text{used}}(t)$ is equal to the cumulative number of generated DAG molecules until the moment $t$. $\tau_{\text{reac,plc}}$ is a function of the PLC enzymatic activity, regulated by calcium (the activity decreases as the Ca$^{2+}$ concentration increases:\(^{2,18}\)):

$$\tau_{\text{reac,plc}}(\text{Ca}^{2+}) = \tau_{\text{pi}} e^{\beta_4 A_{\text{plc}}(\text{Ca}^{2+})}$$  \(25\)

$$A_{\text{plc}}(\text{Ca}^{2+}) = \frac{[\text{Ca}^{2+}]_{\text{free}}}{[\text{Ca}^{2+}]_{\text{free}} + K_{\text{pi}}}$$  \(26\)

where $\tau_{\text{pi}}$ represents PLC activity for low calcium concentrations. The decay of DAG molecules is induced by DAG kinase (DGK), which converts DAG into phosphatidic acid,\(^{34}\) which is then in turn used to resynthesise PIP$_2$. DGK is an ATP-dependent kinase - removing ATP the bump amplitude can increase\(^{47}\). The activity of DGK is regulated by calcium\(^{34}\) and we modeled the DAG lifetime as:

$$\tau_{\text{DGK}}(\text{Ca}^{2+}) = \tau_{\text{Ddark}} e^{-\beta_4 A_{\text{dgk}}(\text{Ca}^{2+})}$$  \(27\)

$$A_{\text{dgk}}(t) = \frac{\partial}{\partial t} \left( \frac{[\text{Ca}^{2+}]_{\text{free}}}{[\text{Ca}^{2+}]_{\text{free}} + K_{\text{dgk}}} \right) \otimes f_{\text{act}}(t, \tau_3) .$$  \(28\)

The rate equations for the number of active free G-proteins (17), the number of active G-PLC complexes (19) and the number of DAG molecules (22), were converted into their stochastic forms as described in section 2.2.1 and explained in detail on the example of the metarhodopsin deactivation.

2.2.4 TRP channel model (module M3)

The kinetics of TRP channels is controlled by three components:\(^{26}\) DAG, Ca$^{2+}$ and PKC. The model assumes that a TRP channel can be in the active state (the number of channels in this state will be $N_{\text{act}}$), which can be either open or closed, or in the inactive state, which is closed and unresponsive to any stimulus. DAG binding to an active channel will increase its probability of being open. Ca$^{2+}$ acts in a bimodal way (positive and negative feedback). First it increases the basal activity of channels, then it activates PKC, inducing the phosphorylation and closing of the channel. In addition, after the influx of calcium, the DAG concentration falls as well. In order for a channel to activate again, it needs to wait for the action of phosphatases which dephosphorylate the channel.\(^{17,48}\) On average, the number of open channels will be the product between $N_{\text{act}}$ and the probability of a channel being in the open state $A_{\text{trp}}$:

$$N_{\text{open}}(t) = N_{\text{act}}(t) A_{\text{trp}}(t)$$  \(29\)

We first calculate $A_{\text{trp}}(t)$ as a function of DAG.
TRP channel close/open state - the MWC model

We assume that an active TRP channel can be in two functional global states: open (O) and closed (C). The molecular transitions between the two states of a TRP molecule are induced or stabilised when it binds a ligand. We can assume that the ligand molecules in this case are DAG molecules, but this is still not confirmed and some alternative hypotheses have been suggested (e.g. ref. 49). However, this assumption is not crucial for the model, since the TRP activity is known to be regulated by DAG concentration. The allosteric transitions can be described by the MWC model.50,51 This model considers a protein with n identical subunits. The two states have different affinities for the ligand, hence different binding equilibria. The cooperativity is no longer an assumption (as in the Hill equation) but arises from the concerted (global) allosteric transition: all the subunits of the protein make the transition together. So if the protein is in the C state and some of the binding sites are occupied, the protein will switch more often to the O state, for which the empty binding sites will then have a higher affinity.

Let C and O represent protein states C and O with k occupied binding sites and K_C and K_O describe the binding to individual sites, so that the equilibrium condition for each multiple binding step of the ligand L is given by50

\[ \frac{[C_{i+1}]}{[C_i][L]} = \frac{(n-i)}{(i+1)}K_C \] and \[ \frac{[O_{i+1}]}{[O_i][L]} = \frac{(n-i)}{(i+1)}K_O \] (30)

where n is the total number of available sites. The equilibrium constant for each allosteric transition is given by

\[ Y_i = \frac{[C_i]}{[O_i]} \] (31)

and Y_0 is the basal activity, which reflects the probability of channel opening in the 'dark' (when no ligand is bound). Now if we measure the biological activity of the protein as the fraction of proteins in the O state, then the dose-response curve will have a sigmoidal shape similar to Hill equation. By using equations (30) and (31) we derive:

\[ A_{up}(t) = \frac{(1 + K_O[DAG(t - \tau_{DAGdelay})])^n}{(1 + K_O[DAG(t - \tau_{DAGdelay})])^n + (1 + K_C[DAG(t - \tau_{DAGdelay})])^n}/Y_0(Ca^{2+}) \] (32)

Our numerical simulations have shown that the [DAG] value that goes in eq. (32) is the value at the moment t - \tau_{DAGdelay} (where \tau_{DAGdelay} is an empirical delay constant for TRP channel activation). Only when \tau_{DAGdelay} \sim 10 ms was introduced, good agreement was achieved with experimental results regarding latency times and some other parameters. Apart from the activation time of the TRP channel one of the reasons for this delay might be, as is now thought to be the case, DAG rapidly flips from inner side of the bilayer to the oily or hydrophobic lipid rafts areas on the outside, where it temporarily resides. The outer lipid rafts now act as a subtle moderator on the rate of the translocation back to the inner membrane where it can reactivate its signalling pathway.

The TRP channels have recently been shown to be tetramers of four TRP proteins forming a selective pore in the centre.52 Therefore we can assume n = 4 and take K_C, K_O and Y_0 as parameters. In order to implement the Ca^{2+} induced positive feedback on the activity of the channels, we can imagine that the basal activity is Ca^{2+} dependent. The Ca^{2+} dependence of Y_0 is modeled as:

\[ Y_0(t) = Y_{0, dark} + (Y_{0, max} - Y_{0, dark})A_{cam}(Ca^{2+}) \] (33)

\[ A_{cam}(Ca^{2+}) = \frac{[Ca^{2+}]_{tot}}{[Ca^{2+}]_{tot} + K_{camtrp}} \] (34)

Qualitatively, the basal activity is minimal in the dark and increases when Ca^{2+} gets into the cell. Fig. 8 shows a typical dose-response curve for the activity of TRP channels.

Channel inhibition

The activity of TRP channels will decrease due to the degradation of DAG molecules by DAG kinase (DGK). However, PKC is critical for termination of the channel activity and correct recovery of the response.17 The rate at which PKC will inactivate the channels depends on the amount of DAG and Ca^{2+}. Hence, the process of TRP deactivation can be modeled as

\[ V_{pkc}(t) = V_{pkc,max} \left( \frac{[DAG]}{[DAG] + K_{pkc1}} \right) \left( \frac{[Ca^{2+}]_{free}}{[Ca^{2+}]_{free} + K_{pkc2}} \right) \] (35)

10
but the activation of PKC can introduce a delay time which is implemented by again using a delay stage function $f_{act}(t, \tau_4)$.

In effect, we are introducing here a third allosteric state, which accounts for the channel desensitisation, an intrinsic braking mechanism which prevents channel from being open for an excessive period of time. A channel that has been phosphorylated will recover to the active state by the action of phosphatases that dephosphorylate the channel. This "relaxation" can be described with a time constant representing the action of phosphatases, which is modeled to be CaM/Ca$^{2+}$ dependent. The idea is that the action of phosphatases can be blocked, until the system stops being in a refractory period and can recover safely. The time evolution of the number of active channels $N_{act}(t)$ is given by:

$$\frac{dN_{act}}{dt} = -\nu_{pke}(t)PKC_{tot}N_{act}(t) + \nu_{dph}(Ca^{2+})(N_{tot} - N_{act}(t)),$$

where $PKC_{tot}$ and $N_{tot}$ are the total numbers of PKC proteins and TRP channels in the microvillus respectively, and $\nu_{dph}$ is the rate of dephosphorylation of TRP channels. The $Ca^{2+}$ dependence is:

$$\nu_{dph}(t) = \nu_{ph}e^{-\beta A_{cam}(Ca^{2+})}.$$

\[ (37) \]

### Channel fluctuations

The patch-clamp experiments on single ion channels show that they randomly fluctuate between the closed (C) and open (O) states. If $\alpha$ is the probability per unit time of $C \to O$ transition and $\beta$ is the probability for reverse transition, and assuming that the single-channel kinetics represents a Markov process (i.e. it does not matter how long a channel has been open) the probability that a closing transition occurs depends only on the intrinsic rate, $\beta$, and the duration of the time interval, $dt$. Now, if we call $P_o(t)$ the opening time distribution, namely the probability that the opening will have a duration longer than $t$, it is easy to show that

$$P_o(t) = e^{-\beta t}$$

and similarly for the closing time distribution,

$$P_c(t) = e^{-\alpha t}.$$

Therefore by knowing $\alpha$ and $\beta$, we can consider the channels individually and compute their stochastic behavior. A closed channel will be given a value 0, and at each time step $\Delta t$, a Poisson distributed number will be generated, $\Pi(\alpha\Delta t)$. When this number is non zero, the channel will open and set to a value of 1. Proof for using Poisson distribution for eqs. (38) and (39) is given in Appendix 3. Equation (32) gives the activity of TRP channels, $A_{trp}(t)$, which can be interpreted as the equilibrium probability of a channel to be open. Thus

$$A_{trp}(t) = \frac{\alpha}{\alpha + \beta}.$$

\[ (40) \]

Experiments were done on TRP channels from which the mean opening time $\tau$ was estimated by using channel noise analysis. The mean opening time gives an estimate of $\beta$, as $\beta \sim 1/\tau$. Now knowing the value for $\beta$ and calculating $A_{trp}$ using eq. (32) we get the expression for $\alpha$ in terms of the number of delayed DAG molecules $DAG_d = DAG(t - \tau_{DAG_{delay}})$ and calcium concentration:

$$\alpha(DAG_d) = \frac{\beta A_{trp}(DAG_d, Ca^{2+})}{1 - A_{trp}(DAG_d, Ca^{2+})}.$$

\[ (41) \]

### 2.2.5 Modeling Currents – module M4

Here are described equations used to calculate the currents through TRP channels ($I_{TRP}$), the current leaking out of a microvillus into the cell body ($I_{leak}$) and the current due to the calcium pumps ($I_{CaX}$). The time evolution of these variables is given by a set of equations, which were not modeled as explicitly stochastic, but they can contain variables which are stochastic such as the number of the open TRP ion channels.
Ionic currents through TRP channels

The TRP channel current \( I_{\text{TRP}} \) consists of ionic currents of \( \text{Ca}^{2+}, \text{Na}^+, \text{Mg}^{2+} \) and \( \text{K}^+ \). The "intracellular concentration" in this case is the ionic concentration inside the microvillus and may change significantly since the microvillus volume is very small \( (\approx 4 \times 10^{-18} \text{m}^3) \). Since the concentration gradients may change, the Goldman-Hodgkin-Katz current equation was used:

\[
I_{\text{TRP},q}(t) = N_{\text{open}}(t) w_q z_q F \beta_q P_m \frac{V - C_{q,\text{in}}(t) e^{-\beta q V_{m}}}{1 - e^{-\beta q V_{m}}},
\]

where \( P_m \) is the permeability of an open TRP channel to ion \( q \) (which is either \( \text{Ca}^{2+}, \text{Na}^+, \text{Mg}^{2+}, \) or \( \text{K}^+ \)), \( z_q \) its valence and \( C_q \) its concentration, and \( \beta_q = z_q F/RT \). The permeability \( P_m(t) \) can be obtained from the total permeability of the microvillus \( P(t) \) if we know the permeability ratios for the different ions, \( w_q = P_q(t)/P(t) \). These ratios have been measured\(^3\) and are given in Table 1. A small correction due to TRPL channels will be included in these ratios, using the values from\(^3\). If \( P_1 \) is the permeability of a single open TRP channel, then the total permeability of the microvillus is given by \( P(t) = N_{\text{open}}(t) P_1 \). The membrane voltage \( V_{m} \) is held constant as in the voltage-clamp experiments. Finally the total current through TRP channels is:

\[
I_{\text{TRP}}(t) = \sum_q I_{\text{TRP},q}(t).
\]

The intracellular calcium concentration is

\[
\frac{d[\text{Ca}^{2+}]_{\text{tot}}}{dt} = \frac{-I_{\text{Ca}}(t)/2 + I_{\text{CaX}}(t) - I_{\text{Ca, leak}}(t)/2}{F V_{mv}}
\]

For other ionic species we have equivalent equations. \( I_{\text{CalX}} \) and \( I_{\text{Ca, leak}} \) are defined below.

Current through the calcium pumps

The \( \text{Ca}^{2+} \) extrusion pumps were shown to be localized in the microvilli.\(^{33}\) Recently \( \text{CalX} \) exchanger has been identified as a dominant \( \text{Ca}^{2+} \) pump,\(^{37}\) which is a kind of \( \text{Na}^+/\text{Ca}^{2+} \) pump for which the ATP-driven activity depends on the intracellular concentration of \( \text{Ca}^{2+} \). This pump extrudes one \( \text{Ca}^{2+} \) ion for the entry of three \( \text{Na}^+ \) ions.\(^{36}\) The \( \text{Ca}^{2+} \) binding to the transport site of the exchanger is usually modeled\(^{53}\) with a Hill function with \( n = 1 \) (Michaelis-Menten kinetics). Therefore the current through the pumps will be

\[
I_{\text{CalX}}(t) = I_{\text{CalX, sat}} \frac{[\text{Ca}^{2+}]_{\text{free}}}{[\text{Ca}^{2+}]_{\text{free}} + K_{\text{calx}}}
\]

where \( I_{\text{CalX, sat}} \) is the saturation current of the pumps in a microvillus. Rapid, but well timed extrusion of calcium is crucial for a proper function of the cell and creation of a QB. Calcium regulates both positive and negative feedbacks in the transduction mechanism and it is necessary both for signal amplification and for rapid response termination.\(^2\) Hence the concentration of \( \text{Ca}^{2+} \) has to change in a such manner so that raise of \( \text{Ca}^{2+} \) during the initial stage of the QB allows amplification to develop further, before the onset of, again calcium activated, inactivation. In the model, we try to achieve this through a careful choice of the half-activity constants \( K \), which represent calcium effect on the various components, such as eq. (33) and (34) for the TRP channel activity and eq. (37) for the channel deactivation. The values of the exchange current are according to experiments in\(^{37}\) of the same order as the QB peak currents, therefore we choose \( I_{\text{CalX, sat}} \sim 12 \text{pA} \), which corresponds to the calcium extrusion currents observed in the experiments.\(^{37}\)

Diffusion from a microvillus into the cell body

All ions will tend to diffuse from the microvillus to the soma, due to the concentration gradients through a narrow connecting ‘neck’. We will simplify the problem by assuming homogenous distribution of ions in the microvillus, since the \( \text{Ca}^{2+} \) diffusion length over the period of \( t = 1 \text{ms} \) is \( \sim \sqrt{D_{\text{Ca}^{2+}} t} \approx 1 \mu\text{m} \) and we only consider the concentration gradient between a microvillus and the cell body. The two are connected through a narrow neck\(^{53}\) of the length \( L_{\text{nk}} = 0.06 \mu\text{m} \) and diameter \( d_{\text{nk}} = 0.035 \mu\text{m} \). The diffusion particle current density in one dimension is:

\[
J(x,t) = -D \frac{\partial C(x,t)}{\partial x}
\]
where \(C(x,t)\) is the concentration in mol/\(\mu\)m\(^3\) and \(J(x,t)\) the ion-particles current in mol/(\(\mu\)m\(^2\) s). If we assume a constant gradient for the diffusion along the length of the neck, we get

\[
I_{q,\text{leak}}(t) = z_q D_q \frac{C_{q,\text{mv}}(t) - C_{q,\text{soma}}}{L_{nk}} (\pi d_{nk}^2/4),
\]

(45)

with the initial condition \(C_{q,\text{mv}}(0) = C_{q,\text{soma}}\).

**Calcium buffering**

In the case of Ca\(^{2+}\), we need to compute the fraction of intracellular concentration that is buffered by CaM (Calmodulin). Calmodulin in general is believed to participate in a variety of intracellular transduction processes by modulating signaling molecules in response to calcium changes\(^{54}\). CaM is highly concentrated in fly microvilli\(^{53}\) and has four bindingsites with different affinities\(^{3,55}\). The steady-state binding can be described by an Aldai-Klotz equation.\(^{53}\) If we define \(x\) as the free Ca\(^{2+}\) concentration, we have

\[
[Ca^{2+}]_{\text{free}} \equiv x = [Ca^{2+}]_{\text{tot}} - [CaM] \frac{K_1 x + 2K_1 K_2 x^2 + 3K_1 K_2 K_3 x^3 + 4K_1 K_2 K_3 K_4 x^4}{1 + K_1 x + K_1 K_2 x^2 + K_1 K_2 K_3 x^3 + K_1 K_2 K_3 K_4 x^4}
\]

(46)

where \([CaM]\) denotes the total concentration of CaM in the microvillus, and \(K_i\) (\(i = 1, 2, 3, 4\)) are the macroscopic binding constants in mM\(^{-1}\) (given in Table 1). We assume fast equilibration of all \(Ca^{2+}\) association and dissociation processes, since these processes are of the order of few milliseconds or less\(^{55}\). The graph of the corresponding buffering power values \((B = [Ca^{2+}]_{\text{tot}}/[Ca^{2+}]_{\text{free}})\) as a function of total calcium concentration, calculated using eq. (46), is given in Fig. 3.

The buffering is very strong \((B \sim 100)\) for smaller calcium concentrations and reaches maximum around 0.5 mM (which is the calmodulin concentration), then sharply drops around 2 mM. Since the normal intracellular calcium concentration is very low, the rush of calcium ions upon the opening of TRP ion channels is first met with high buffering, effectively keeping the free calcium concentration very low and thus delaying a fast triggering of the PKC shut down mechanism for ion channels.

**Figure 3:** The free calcium and calcium buffering power for 0.5 mM of CaM vs. total Ca\(^{2+}\) concentration.
In our model, some of the Ca\textsuperscript{2+}-mediated processes will take $[Ca^{2+}]_{\text{free}}$ as an argument, others $[Ca^{2+}]_{\text{tot}}$, depending on whether they are directly mediated by CaM or not.

2.3 Numerical Method and Parameters

The model described in the previous section gives the time evolution of the main phototransduction components, starting from the moment a photon has been absorbed by the retinal of a rhodopsin complex. The initial condition of the system corresponds to one activated rhodopsin, so $M^*(t = 0) = 1$. All numerical calculations were performed in MATLAB 7.8.0 (The Mathworks, R2009a).

The calculated average quantum bumps were compared to the experimental average, obtained from Roger C. Hardie, Cambridge University, in order to estimate parameters. In order to make a consistent comparison, the same steps were followed: produce a set of QBs with membrane voltage $V_m = -70$ mV, filter them with a 100-200 Hz low pass filter, align them and average (as described in ref. 12). For the filtering, a low pass filter was coded in MATLAB by using Fourier transforms.

The standard parameters, Table 1, were either directly taken from the literature or estimated on the basis of the published literature and our simulation results. The rest of the parameters, Table 2, were manually optimized using the following cost functions or constraints extracted from experiments: (i) a good fit to the experimental average QB, as well as to the peak current and latency time distribution histograms, (ii) an average lifetime of M* $\tau_M \sim 25 - 30$ ms, ref. 29,56 in normal physiological conditions and $\tau_M \sim 200$ ms in Ca\textsuperscript{2+}-free solutions, (iii) an average total number of GPLC* active complexes $\geq 5$, ref. 4, (iv) an average number of $\sim 15$ open TRP channels at the peak of the bump, and (v) a peak concentration for the total intracellular Ca\textsuperscript{2+} of the order of a few mM. It is probably possible to have more than one set of parameters fitting the experimental average Qb, but we have chosen the one with realistic values. A dedicated GUI was designed, see Fig. 9, which allows for much better overview of all parameters and simplifies the conductance of simulations.

3 Results

3.1 Quantum Bump generation

An example of temporal evolution of the key dynamical variables during a QB is shown in Fig. 4. In this example, M* decays after ca. 25 ms, producing 6 GPLC* complexes, at peak, which in turn generate DAG molecules, with peak value of around 200 and the total number of approximately 250. The total number of molecules of type X created during a QB, can be calculated as: $X_{\text{tot}} = \int_0^\infty \frac{1}{\tau_X} X(t) \, dt$, where $\tau_X$ is the average lifetime of X.

TRP channels start to open after DAG has reached a threshold value $DAG_T \approx 100$. The opening of a channel induces the Ca\textsuperscript{2+} influx and calcium increases the basal activity ($Y_0$ in eq. (33)) of the TRP ion channels. Now other TRP channels become more sensitive to DAG and easier to open, which could be the positive feedback at the initial stage of the phototransduction process noticed in the experiments. At the peak of the response, typically about 15 channels are open (on average). At this point the recovery mechanisms, driven by the high concentration of Ca\textsuperscript{2+} in the cell, start inhibiting the stimulus and inactivating the channels. The overall time of the response is $\sim 40$ ms. The photocurrent response (filtered with a 100 Hz low pass filter) representing the corresponding QB is shown in Fig. 4 panel five. The QB is produced after a latency time of about 40 ms. The peak amplitudes of the model was $I_{\text{max}} \sim 13$ pA.

The time evolution of Ca\textsuperscript{2+} is shown in Fig. 4 (bottom panel). Ca\textsuperscript{2+} first gets slowly into the cell (and deactivates the metarhodopsin first), then jumps abruptly to high concentration values. At the peak of the response, the total calcium concentration reaches values of about 2.5 mM and the free calcium peak is about 0.5 mM. Once the response is terminated, Ca\textsuperscript{2+} takes some time to be extruded. During this period, the system is recovering, and it is insensitive to any stimulus. The refractory period $T_{\text{ref}}$ is determined by the times of reactivation of channels and extrusion of Ca\textsuperscript{2+}. $T_{\text{ref}}$ can be estimated by simulating a second photon absorption at certain time $\tau$ after the recovery of the response. When $\tau$ is large enough so that a second QB is generated, then $T_{\text{ref}} = \tau$. This estimation was performed (data not shown) and gave $T_{\text{ref}} \lesssim 200$ ms. Another estimation of $T_{\text{ref}}$ was performed when QB trains were generated, e.g. see Fig. 6B. $T_{\text{ref}}$ was experimentally measured to be $\sim 100 - 200$ ms.
Figure 4: An example of the time evolution of the main cascade components for a random single quantum bump: a metarhodopsin in active ($M^* = 1$) and inactive (0) state, the number of active G-proteins ($G^*$) and active G-PLC complexes ($G_{PLC}^*$), the number of DAG molecules and the cumulative number of created DAG molecules which is equal to the number of decomposed PIP$_2$ molecules, the number of open TRP ion channels (which can take discrete values between 0 and 25), filtered photocurrent, and the last panel shows total Ca$^{2+}$ (red) and free Ca$^{2+}$ (black). For the parameters see Tables 1 and 2 and Fig. 8.
3.2 Quantum bump statistics

In order to test if the model produces an average QB behavior comparable to the real bumps, experiments of Henderson et al.\textsuperscript{12}, a statistical analysis was performed. Series of QBs were simulated, e.g. Fig. 5A, and the data was processed in such a way to mimic the experimental conditions: physiological extracellular ionic concentrations, constant membrane voltage, filtering of bumps, and QBs were aligned and averaged. A photoresponse was considered to be a QB when the peak amplitude was $\geq 3$ pA, in order to combine all the criteria for a QB as described in\textsuperscript{12}. Otherwise a response was considered as "failed" and not counted in the average. Some parameters of the model were manually optimized such that the average QB fitted reasonably well with the experimental average, while keeping some constraints on the dynamics of the cascade components, as described before. Fig. 5B shows the results for the average QB.

From these simulations, we can also extract the QB latency time ($t_L$) distribution. The time $t_L$ is defined as the time between the light pulse and the start of the QB defined as the first point rising two standard deviations above the baseline noise (taken to be 0.6 pA as in\textsuperscript{12} where experimental baseline noise is estimated to be 0.3 pA). Henderson et al. deduced the latency distribution by deconvolving the macroscopic impulse response to flashes with the average QB\textsuperscript{12}. Fig. 5C compares the experimental\textsuperscript{12} and theoretical latency distributions. The results match quite well, and this is one of the tests of the model. The average latency is $< t_L > \approx 45$ ms.

3.3 Model tests - Special cases: non-physiological Ca$^{2+}$ concentrations and various mutants

There are two additional independent ways to test the model. The first one concerns the response of the system for different extracellular Ca$^{2+}$ concentrations. Since calcium is regulating all the positive and negative feedbacks we expect the response will rise and fall more slowly if [Ca$^{2+}$]$_{out}$ decreases. The simulation results of the average QB for different values of extracellular Ca$^{2+}$ concentrations are shown in Fig. 6 and they approximately follow the experimental results from ref.\textsuperscript{12}. As expected, the quantum bump increases in duration as the [Ca$^{2+}$]$_{out}$ decreases. The rising phase becomes slower since it takes longer to build sufficient calcium concentration to trigger positive feedback.

The model allows virtual mutagenesis, namely simulations of the cases when a certain gene is knocked out. For instance, in arr2 mutants, there is only a reduced amount of arrestin in the microvillus, which is crucial for the decay of metarhodopsin. Fig. 7A shows a simulated event of single photon response in the case when there is no arrestin available. A series of QBs is generated as a response to a single rhodopsin activation. The first QB is usually higher in amplitude than a typical QB. The time between the QBs fluctuates. These results are in qualitative agreement with the experimental results of Pumir et al.\textsuperscript{4} shown in Fig. 7B. Experiments of Liu et al.\textsuperscript{29} also showed that, in arr2 mutants, a single photon produces a train of QBs, which lasts for about 1-2 s. The decay and eventual disappearance of QBs reflects the depletion of PIP$_2$. PIP$_2$ is recycled from DAG, however the recovery time constant for PIP$_2$ (about 1 s) is relatively long in comparison to the timescale that we use to observe quantum bumps. Hence the PIP$_2$ recovery is not included in the code, in order to speed it up, although that would be straightforward. From these trains of QBs it is also possible to estimate the refractory period. From our simulations, we can predict a value of $T_{ref} \sim 100$ ms, in agreement with the experimental estimates in ref.\textsuperscript{18}.

Other mutations can be simulated, for example norpA hypomorphs, which display a huge reduction in the number of PLC molecules in the cell. The experimental consequence is a significant reduction in the amplitude of the response, and this result can be reproduced by the model (data not shown). Hypomorphs showing a reduction in the number of G proteins display an increase in the latency of the responses, a consequence that can be understood by looking at the dynamics described by the model. Another simulated mutation is in the exchanger calx which is responsible for the calcium pump CalX. The reduction of CalX activity causes a strong decrease of the QB amplitude and duration, as found in the experiments of Wang et al.\textsuperscript{37}. Simulation results faithfully reproduce this behaviour as well.

4 Discussion

Although the biochemistry of the phototransduction process is quite complex, seemingly it is still possible to create a model which follows most of the experimental details of the enzymatic amplification cascade, ion-channel kinetics and ubiquitous calcium regulation. The size of the parameter space of our model is relatively large, however, a number of parameters were measured or estimated and reported in the open literature (e.g.
Figure 5: Simulated individual QBs with 100 Hz filtering. Arrows indicate moments of single M* activation, approximately corresponding to brief light flashes in the experiments. Some M* activations elicited no response (failures). B) The average quantum bump for aligned QBs for: experimental 79 QBs from Henderson et al.\textsuperscript{12} (data obtained from R.C. Hardie, University of Cambridge, shown by red circles, standard deviation shown for the maximum current) and the model fit for a set of 1000 QBs (line). QB events were filtered (100 Hz), aligned and averaged. QBs are aligned as in Henderson et al.\textsuperscript{12} at the time \((t_2 − t_1)/2\) where \(t_1\) is the 50% rise time and \(t_2\) is the 50% decay time. C) Left panel: Latency distribution from experimental measurements in\textsuperscript{12} (solid black curve) compared to the latency distribution obtained by simulating 1200 QB events (bars). Right panel: the peak current distribution. Other results: the average lifetime of active metarhodopsin \(τ_{M^*} = 29\) ms, the average peak values for the number of active PLC molecules = 4.5, average number of activated PLC molecules during one QB = 5.4, average peak \(N_{trp} = 15.1\), average latency time =43 ms.
diffusion constants, physiological concentrations of ions, geometry of the microvillus, permeability ratios, stoichiometry of the cascade components, etc.). These are shown in Table 1. We are then left with $\sim 30$ parameters that can be adjusted. But all these "free" parameters have a physical meaning, and so cannot take any arbitrary values. Most of them can be given a lower/upper bound. For example, the predicted permeability of an open TRP channel is $P_1 \approx 1 \mu m \ s^{-1}$, and the experimentally measured permeabilities of TRP channels are often of the order of $1 \mu m \ s^{-1}$. Another estimate concerns the calcium pumps: the saturation current in a wild type *Drosophila* microvillus is suggested to be $I_{\text{sat, sat}} \sim 10 \text{pA}$, and we have used approximately the same value. Hence the available size of the parameter space is reduced and the number of restrictions on the results are such that it is unlikely that two very distinct sets of parameters give the same results. A set of optimized parameters is shown in Table 2.

The parameters concerning the TRP channels can give some insights into the nature and energetics of these proteins. In fact, the MWC model provides a thermodynamical interpretation which allows measuring the free energy change for the allosteric transition (opening and closing). The basal activity $Y_0$ represents the equilibrium constant for the allosteric transition $C \rightarrow O$ when no ligands are bound to the protein. So the free energy change for the opening of the unliganded channel is

$$\Delta G^0 = -RT \ln Y_0$$

which gives $\Delta G^0 \approx 37 \text{kJmol}^{-1}$ at room temperature (or 0.38 eV per molecule) in dark ($Y_{\text{min}} = 3 \times 10^{-7}$), and $\Delta G^0 \approx 29 \text{kJmol}^{-1}$ (0.30 eV) with the Ca$^{2+}$ induced positive feedback ($Y_{\text{max}} = 7 \times 10^{-6}$). These values are positive and slightly above typical single hydrogen bond energies, which are in the region of 0.2-0.3 eV, therefore there is a small probability for the transition to happen. However, if a ligand is bound to the channel, an allosteric transition will change the binding strength by $-RT \ln (K_O/K_C)$, which reflects the fact that the affinity is higher in the open state than in the close state. So for $j$ occupied binding sites, the free energy change for the allosteric transition is

$$\Delta G^0 = -RT \ln Y_0 - jRT \ln (K_O/K_C)$$

which becomes negative above $j = 3$. When $j = n_{up} = 4$, the change in free energy is $\Delta G^0 = -18 \text{kJmol}^{-1}$ for $Y_{\text{max}}$ and $\Delta G^0 = -9 \text{kJmol}^{-1}$ in the dark adapted case. Now the allosteric transition corresponds to a spontaneous favorable reaction. This explains why the binding of the ligand stabilises the open state and increases the probability of the channel opening. These considerations were taken into account in the fitting procedure for the QB.

The Ca$^{2+}$ dependence of $Y_0$ was given by eq. (33). Qualitatively, the basal activity is minimal in the dark and increases when Ca$^{2+}$ ions enter the microvillus. Fig. 8 shows the typical dose-response curve for the activity of TRP channels. The system will first follow the curve with minimum basal activity, then jumps to the curve with maximal basal activity, which is much steeper (here we neglect the intermediate values for
Figure 7: Quantum bumps in arrestin mutants - A) examples of the simulated single photon response in an 
arr2 mutant and B) the experimental results recorded using whole-cell voltage clamp - from Pumir et.al.4, 
reproduced with permission.

$\gamma_0$($Ca^{2+}$)). The recovery will be symmetric. The use of MWC theory in our model has allowed us to introduce 
a positive feedback mechanism into the TRP channel activity in a natural way, through the $\gamma_0$ dependence on 
calcium concentration. Furthermore, the sigmoidal shape of the cooperative binding defined by MWC model, 
provides a noise suppression mechanism for the phototransduction. The sigmoidal shape is effectively working 
as a threshold gate, preventing a random opening of TRP channels in dark due to some residual DAG which is 
spontaneously created in the microvillus by thermal excitations. Equally the effect of a spontaneously activated 
G-protein will be also dampened. Fig. 4 shows how up to $\sim$100 DAG molecules can be tolerated in a dark 
adapted microvillus before a TRP channel was opened. If PIP$_2$ concentration is low and the PIP$_2$ $\rightarrow$ DAG 
activation energy is high there will be very few thermally activated DAGs, but the response will be very slow 
due to slow rate of DAG creation. On the other side, if the PIP$_2$ concentration is high and the activation barrier 
low, the thermal creation of DAG might trigger false QBs. Hence it is crucial for a fast and reliable single-
photon response to have an optimum stoichiometry of the molecular species involved in the cascade and also 
some way of blocking the effect of the DAG created thermally. These mechanisms give the opportunity for the 
insect’s eye to detect a single photon. Note that the ability to create a perception of a single photon event in 
living organisms requires, apart from an exceptionally sensitive biomolecular amplification cascade, that the 
activated neuron is able to make a mark in the noisy neural system at the higher levels. The phototransduction 
process is not able to suppress every form of molecular noise, however sometimes it is possible to utilise noise 
for the sake of enhanced performance through the process of stochastic resonance.

**Conclusion**

Here we have presented our biochemistry based model and simulations of the single photon response in 
*Drosophila* photoreceptor cells. This amplification process allows a transduction from single photons to milli-
volts of electrical potential change across the cell membrane, with a gain of about 10$^6$, at relatively low power 
consumption and very good suppression of "dark current" noise. In addition, the continuous integrating ef-
flect of the membrane current eliminates any high frequency (>$1-10$kHz) noise. The model developed here 
gives not only a good simulation for the experimental results but also a number of new insights about the sys-
tem. They include dark current suppression, noise reduction, TRP allostery, TRP energetics, identification of 
channels as the most noise-sensitive component, parameters such as channel permeability $P_1$, etc.
Figure 8: Activity of TRP channels. Here $n = 4$, $K_O = 0.34$, $K_C = 0.0025$, $Y_{0,\min} = 3 \times 10^{-7}$ and $Y_{0,\max} = 7 \times 10^{-6}$. The arrows indicate a flow in the DAG-TRP activity space from the initial, dark-adapted relationship, to the maximum sensitivity for high intracellular calcium.

Acknowledgments

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Appendix

Appendix 1: Estimations for the diffusion constants

The collision rate between an active metarhodopsin molecule and G-proteins is given by eq. (12): $\nu_{\text{coll}} \approx \alpha_1 (D_{M^*} + D_G) C_G$. The factor $\alpha_1$ as defined in eq. (A1) is:

$$\alpha_1 = \frac{4 \pi}{\ln[4(D_{M^*} + D_G)t/\rho_1^2] - 1.15},$$

where $t$ is the time after metarhodopsin becomes active and $\rho$ is the encounter distance for $M^*$ and $G$, equal to the sum of the radiiuses of these two molecules. In our case we assume a typical time of $t \sim 10$ ms and $\rho \approx 5$ nm. Rhodopsin in microvillar photoreceptors is essentially non-diffusible, hence $D_{M^*} \approx 0$. The lateral diffusion coefficient for G-protein movement in the tissue culture cells was found to be $D_{G\text{uffy}} \equiv D_G \sim 0.1 \mu m^2/s$, whereas an estimate for the amphibian rods was $D_G \sim 1.2 \mu m^2/s$. The value of $D_G \approx 1.2 \mu m^2/s$ was chosen so that the average number of the activated G-proteins is about 5. Eventually from eq. (A1): $\alpha_1 \approx 2$.

The collision between an active G-protein ($G^*$) and a PLC molecule was given by eq. (18) and contains the constant $\alpha_2$ which is again given by an equation similar to eq. (A1):

$$\alpha_2 = \frac{4 \pi}{\ln[4(D_{G^*} + D_{PLC})t/\rho_2^2] - 1.15},$$

where $\rho_2$ is the encounter distance for $G^*$ and PLC. Since PLCs are anchored at the large INAD complexes they are virtually fixed and a reasonable estimation of $D$ for a subunit of the G-protein would be a slightly higher value than $D_G$, i.e. $D_{G^*} \equiv D_G \approx 1.5 \mu m^2/s$, hence $\alpha_2 \approx 2$.

An estimation for $D_{PIP_2}$ and $\alpha_3$ for $\tau_{coll,PIP_2}$ in eq. (24) comes from the experimental values for the PLC enzymatic activity $v_3$, which is known to be high. For example, flashes containing one photon per microvillus create PLC activity which is sufficient to hydrolyze all the PIP$_2$ in the microvillus within $t_p = 1$ second. Hence our estimation for $v_3$, for zero calcium case, is $v_3 = (N_{PIP_2}/t_p)/N_{\text{tot,PLC}} \approx 3000/5 = 750$ s$^{-1}$, since the estimated number of PIP$_2$ molecules in a microvillus is about several thousand and the total average number of activated PLC molecules per one metarhodopsin ($N_{\text{tot,PLC}}$) is five. The minimum value for $D_{PIP_2}$ has to be in the region set out by the diffusion limit for the PIP$_2$ molecules for $v_3$. The average number of
### Table 1: Parameters of the QB model - literature based.

<table>
<thead>
<tr>
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<th>Value</th>
<th>Units</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_T$</td>
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</tr>
<tr>
<td>$PLC_T$</td>
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<td>Number of PLC molec. in microv.$^2$</td>
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<tr>
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<td>Frac. of channel permeab. due to Na$^{+}$</td>
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# Table 2: Parameters of the QB model - from the fitting procedures.

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<td>$k_{MA}$</td>
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<td>30</td>
<td>ms</td>
<td>Delay time for the action of PKC on TRP</td>
</tr>
<tr>
<td>$v_{\text{ph}}$</td>
<td>0.4</td>
<td>ms$^{-1}$</td>
<td>Rate of TRP dephosphorylation in dark</td>
</tr>
<tr>
<td>$\beta_5$</td>
<td>4</td>
<td></td>
<td>Deactivation constant for the relaxation of TRP</td>
</tr>
<tr>
<td>$I_{\text{calx, sat}}$</td>
<td>12</td>
<td>pA</td>
<td>Saturation current for the CalX pumps</td>
</tr>
<tr>
<td>$K_{\text{calx}}$</td>
<td>0.20</td>
<td>mM</td>
<td>Concentration of Ca$^{2+}$ for 50% activity of the pumps</td>
</tr>
</tbody>
</table>
Figure 9: The Graphical User Interface designed for running computer simulations of the Drosophila phototransduction events.
PIP2 molecules colliding with an active G-PLC complex depends on the product of diffusion constant of PIP2 ($D_{\text{pip2}}$) and the concentration (i.e., surface density) $\rho_{\text{pip2}}$ of PIP2 molecules, eq. (12). The diffusion coefficient of a typical membrane lipid is of the order of 1 $\mu$m$^2$/s, however in the case of PIP2 in the plasma membrane of atrial myocytes extremely low values were measured by ref. 58: $D_{\text{pip2}} = 0.00039$ $\mu$m$^2$/s. Therefore the range of possible values for $D_{\text{pip2}}$ could be very wide, spanning 3-4 orders of magnitude. The experimental and theoretical results in ref. 58 clearly show that if the mobility of PIP2 is very low, the PIP2 depletion is very localised around an active G-PLC, whereas the PIP2 elsewhere remains almost unaffected. This can be important in cells which utilise PIP2 for several different signalling paths, allowing for (de)activation of only the targeted ion channels. However in the invertebrate’s photoreceptor cells PIP2 might have much less versatile functions, therefore they could allow greater mobility. In any case the PIP2 concentration has to be high enough to provide enough molecules in the close proximity of an G-PLC. Therefore, our estimation is $D_{\text{pip2}} \sim 5$ $\mu$m$^2$/s, assuming nearly a 100% efficiency of hydrolysis of every PIP2 molecule per 140 nm$^2$, which implies average distance of $\sim$ 12 nm between molecules. The PIP2 molecules are long but narrow, see Fig. 2, with diameter of the InsP3 part of less than 1.6 nm, so they can fit comfortably. Finally, an equation similar to eq. (A1), gives $\alpha_5 = 1.5$.

**Appendix 2: The delay stage function**

Consider a certain molecule A. When this molecule receives a stimulus S, it can get activated,

$$A \rightarrow A^*$$

If the molecule becomes active instantaneously after the stimulus, then the rate at which $A^*$ are generated will be proportional to the stimulus $S(t)$,

$$\frac{dA^*}{dt} = \nu S(t),$$

where $\nu$ can depend on the amount of A, on geometry, etc. In this case, the amount of $A^*$ as a function of time will be given by:

$$A^*(t) = \int_0^t \nu S(t') \, dt'$$

(A3)

In reality, some molecules take a certain average time $\tau$ from the time of stimulus in order to get activated. In some cases, this extra time $\tau$ can be included in the rate as $1/\nu' = \tau + 1/\nu$, but not always. For example, in the case of G protein, the stimulus is the binding to $M^*$, and $\nu$ corresponds to the rate at which G proteins collide, bind and exchange (GDP/GTP). But once they unbind, it takes some time $\tau$ before they release the $\alpha$ subunit and create an active $G^*$. We can not include $\tau$ in the rate $\nu$ because during this time other G proteins can bind to $M^*$. It is easy to see that the result is not the same if we take $\tau$ separately.

The way to implement this delay time $\tau$ is to use a delay stage function $f_{\text{act}}(t, \tau)$, defined as

$$f_{\text{act}}(t, \tau) = 1 - e^{-t/\tau}$$

(A4)

which gives the probability of a molecule being active after the time $t$ from the stimulus. So a stimulus at time $t'$, $S(t')$, will contribute to the amount of $A^*$ at time $t$ with a factor $S(t') f_{\text{act}}(t - t', \tau)$, which tends to $S(t')$ when $t \rightarrow \infty$. Therefore equation (A3) will be corrected to

$$A^*(t) = \int_0^t \nu S(t') f_{\text{act}}(t - t', \tau) \, dt'$$

(A5)

Equation (A5) is the convolution between the stimulus $S(t)$ and the delay stage function $f_{\text{act}}(t, \tau)$. So we can write it as

$$A^*(t) = \nu S(t) \otimes f_{\text{act}}(t, \tau).$$

If we want the rate equation again, we have to find the first derivative of equation (A5) with respect to $t$, which gives:

$$\frac{dA^*}{dt} = \int_0^t \nu S(t') \frac{2}{\tau} f_{\text{act}}(t - t', \tau) \, dt'.$$
Figure 10: Example of a signal function $S(t)$ (top box), the rate of generation (non-delayed and delayed, middle box) and the output function $A^*(t)$ (non-delayed and delayed, bottom box. Parameters: $T = 60$ ms, $\tau = 5$ ms, $S_0 = 1$, $\nu = 1$ ms$^{-1}$.

which again can be written as

$$\frac{dA^*}{dt} = \nu S(t) \otimes \frac{\partial}{\partial t} f_{\text{act}}(t, \tau) = \nu \frac{\partial}{\partial t} S(t) \otimes f_{\text{act}}(t, \tau),$$

where the last relation comes from the well known property of convolution integral.

We present a demonstration for the delayed activation using the exponential kernel eq. (A4) on the example of a square stimulus: $S(t) = S_0$ for $t$ between $t = 0$ and $t = T$, otherwise $S(t) = 0$ (for example $S(t)$ could be the number of active metarhodopsins when $S_0$ is large or $S(t)$ could be the ensemble average). The rate of generating $A^*$ (this could be the active G proteins for the same example) will be according to eq. (A6):

$$g(t) = \int_0^T \nu S_0 e^{-\frac{(t-t')}{\tau}} dt' = \nu S_0 \left(1 - e^{-\frac{t}{\tau}}\right), \quad 0 \leq t \leq T$$

and

$$g(t) = \int_T^T \nu S_0 e^{-\frac{(t-t')}{\tau}} dt' = \nu S_0 \left(1 - e^{-\frac{T}{\tau}}\right) e^{-\frac{t-T}{\tau}}, \quad t \geq T$$

The corresponding time evolution of $A^*(t)$ is given by: $A^*(t) = \int_0^T g(t') dt'$. Hence:

$$A^*(t) = \nu S_0 \left[t - \tau(1 - e^{-\frac{t}{\tau}})\right], \quad 0 \leq t \leq T$$

$$A^*(t) = \nu S_0 \left[T - \tau(1 - e^{-\frac{T}{\tau}}) e^{-\frac{(t-T)}{\tau}}\right], \quad t \geq T$$

Fig. 10 shows these functions for some specific parameters illustrating the delay effect.
Appendix 3: Channel noise and Poisson distribution

Use of the Poisson distribution leads to eqs. (38) and (39), used for the probability that the channel is not open/closed up to time $t = T$, e.g.:

$$P_c(T) = \exp(-\alpha T),$$

where $\alpha$ is the rate of the channel opening. We can show this in the following way. Let us divide this time interval $T$ into $N$ very small intervals of the length $\tau = T/N$. The probability that the channel opening will happen during one particular $\tau$ is very small, but constant: $\lambda = \alpha \cdot \tau$ and hence Poisson distribution can be used. The probability that $k = 0$ channels are open during a time interval $\tau$ is according to the Poisson distribution:

$$P(k = 0) = \frac{\lambda^k \exp(-\lambda)}{k!} = \exp(-\lambda) = \exp(-\alpha \tau). \quad (A7)$$

Now if we start at $t = 0$, for every time interval $\tau$ there is the same probability that the channel is not open which is $P_i = \exp(-\alpha \tau)$. The total probability until time $t = T = N \tau$ will be product of all these probabilities:

$$P(\text{ch. closed until } t = T) = P_1 \cdot P_2 \cdot \ldots \cdot P_N = \exp(-\alpha T) \cdot \exp(-\alpha T) \cdot \ldots \cdot \exp(-\alpha T) = \exp(-\alpha N \tau) = \exp(-\alpha T).$$

References


