

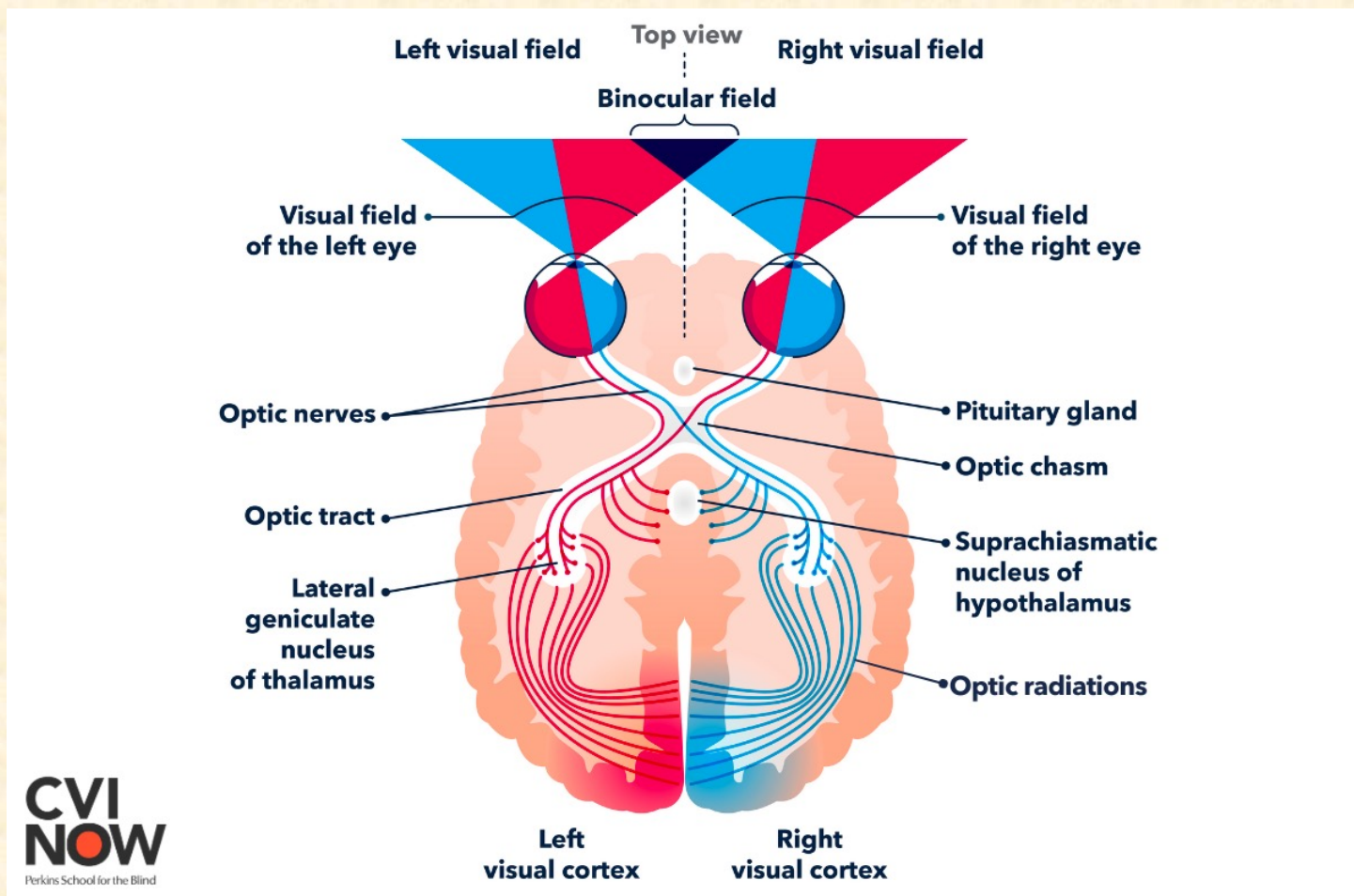
Modelling the transduction of light into an electrical signal by rod and cone photoreceptors in the retina

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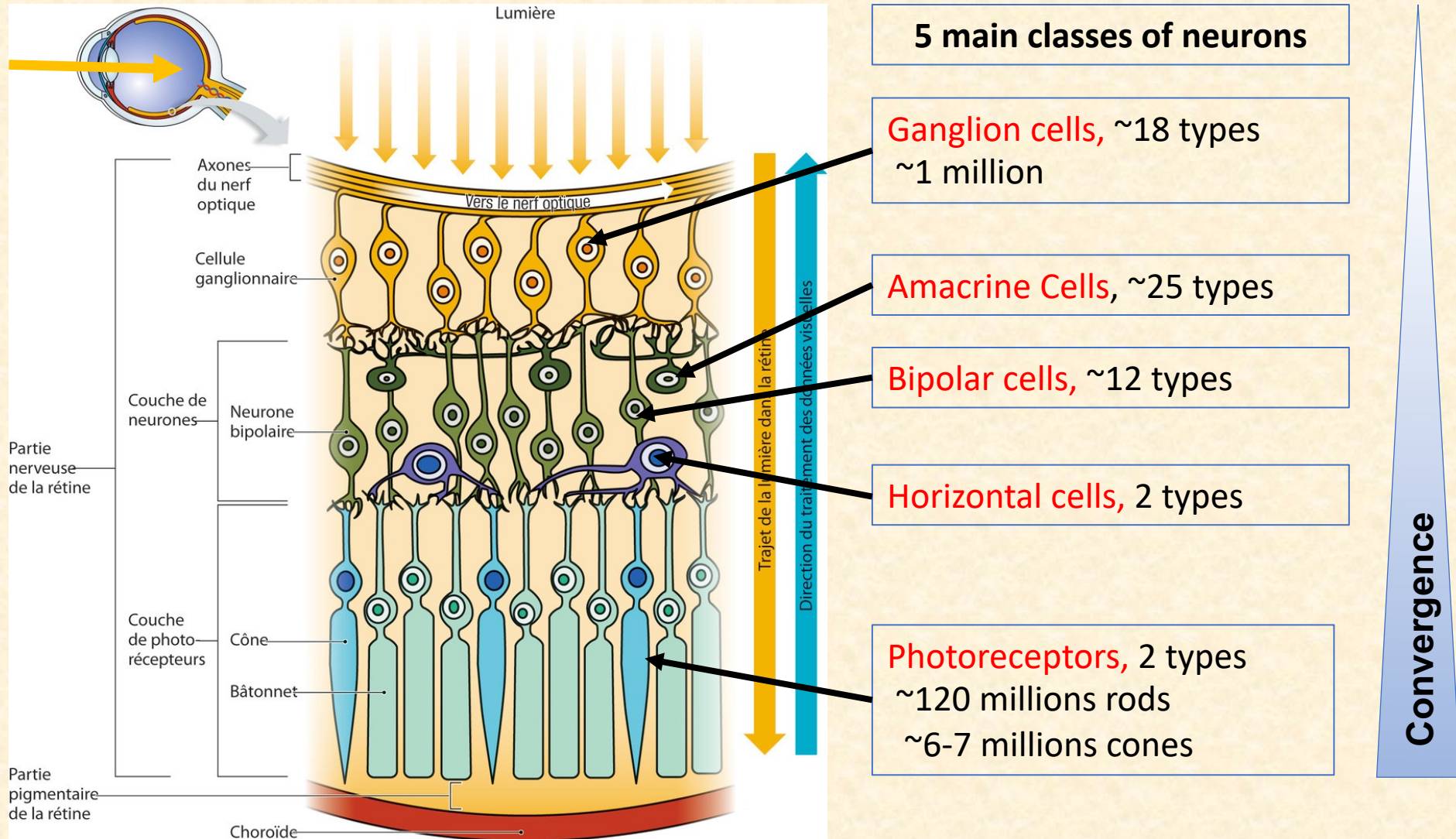
Outline

1. Biological introduction
 - Duplex vision in vertebrates
 - Photoreceptor properties
2. How to use modeling to understand photoreceptor functioning
 - Single Photon Response in Rods
 - Identification of main differences between rods and cones
 - Derivation and application of analytic results
3. Modelling challenges
 - How do cones adapt to bright light ?
 - What is the physiological role of the variety of ion channels in the inner segment ?
 - What is the voltage dependent dynamics of glutamate release at the ribbon synapse ?

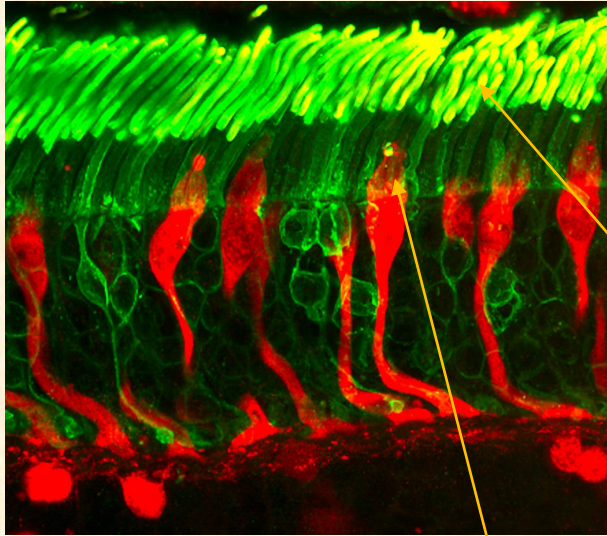
The signaling pathway for binocular vision



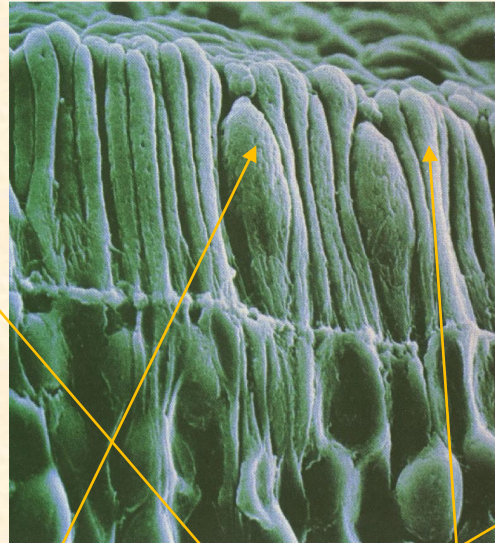
The Retina is a Complex Neuronal Network



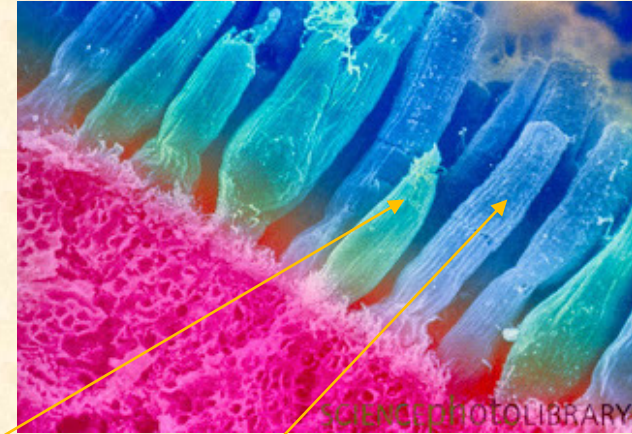
Duplex vision: Rods for night vision (scotopic vision) and cones for daylight vision (photopic vision)



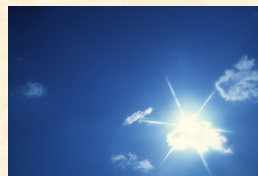
Confocal microscopy image with fluorescent probes.
(Robert Fariss, NIH)



EM of Retinal Rod and Cone



Colored EM of Retinal Rod and Cone photoreceptors (Science photo library)

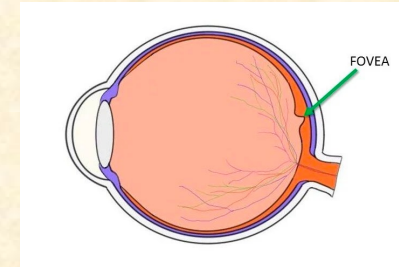
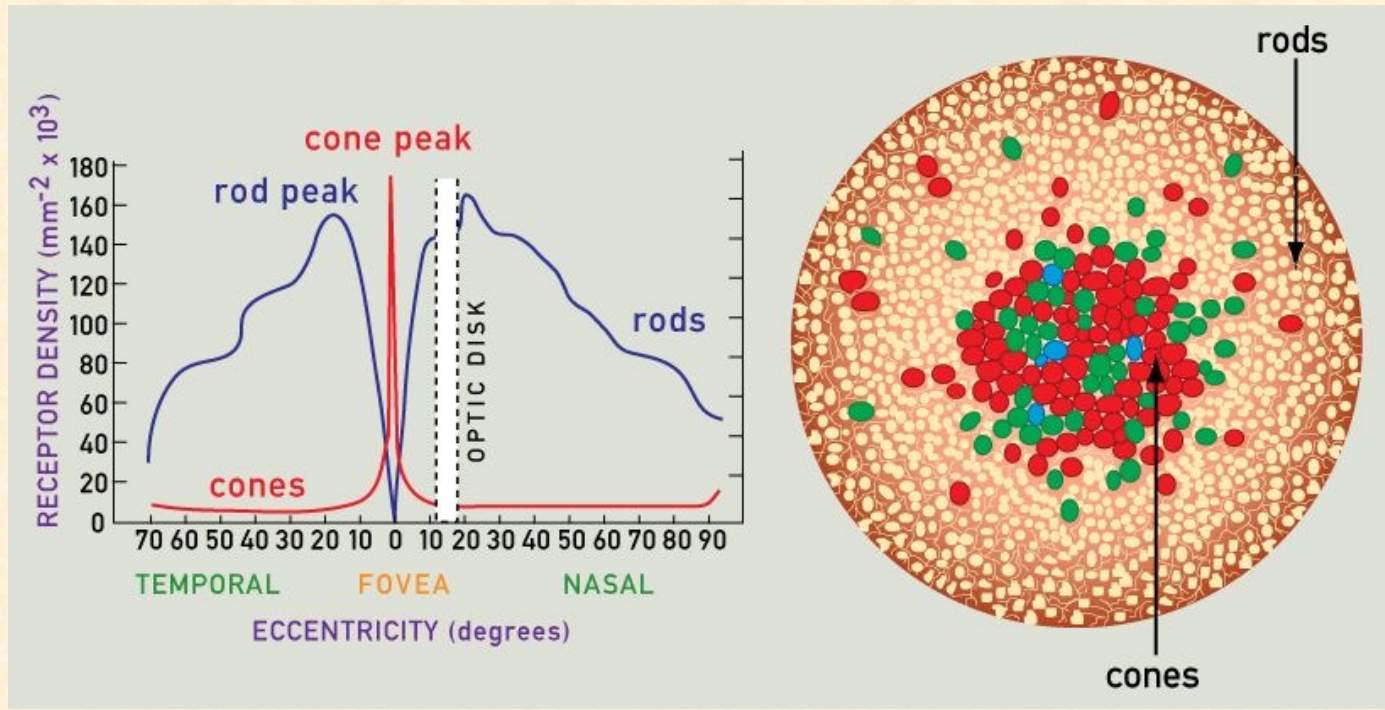


Cone



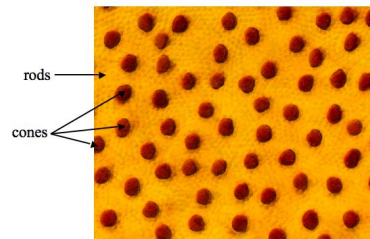
Rod

Distribution of rods and cones in the retina

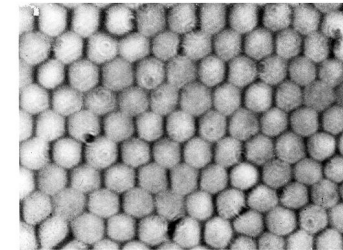


~120 millions rods
~6-7 millions cones
(red 64%, green 34%, blue 2%)

Near periphery photoreceptor mosaic

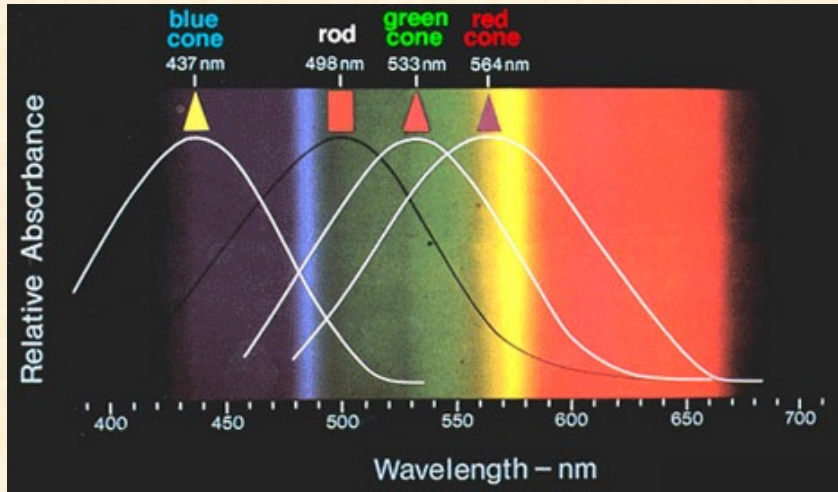


Foveal cone mosaic

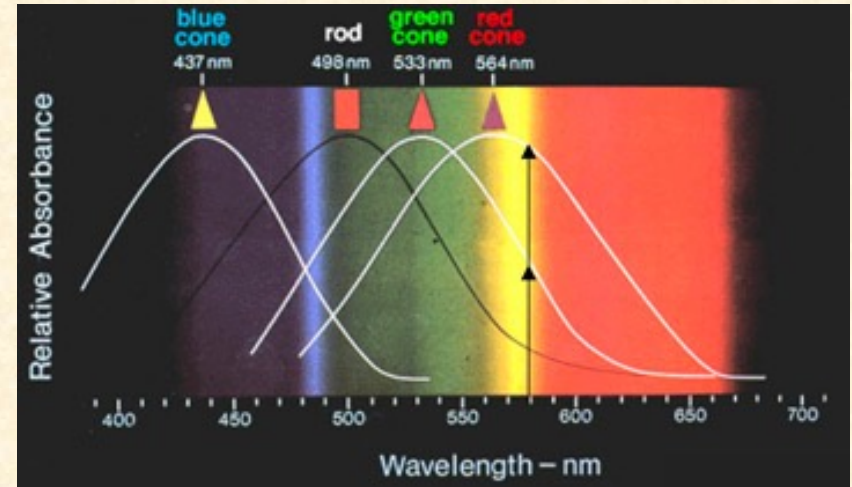


Human color vision is based on the spectral sensitivity and activation patterns of 3 types of cones

Spectral sensitivity

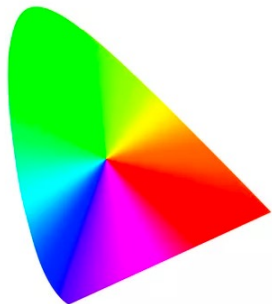


Activation pattern for light of 580 nm

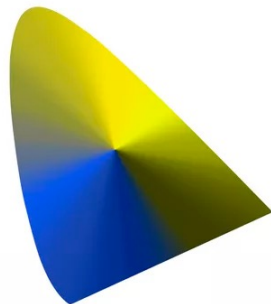


Colorblindness

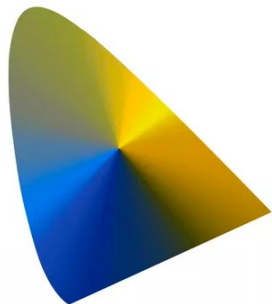
Normal vision



Protanopia ✖



Deuteranopia ✖



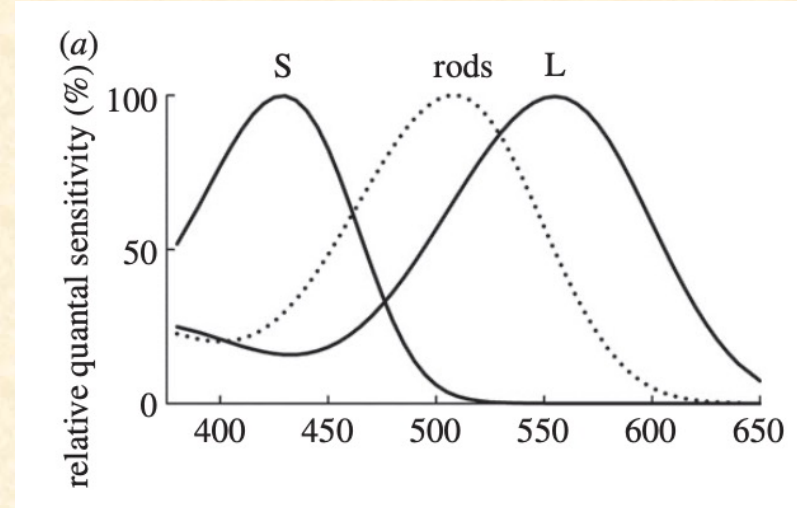
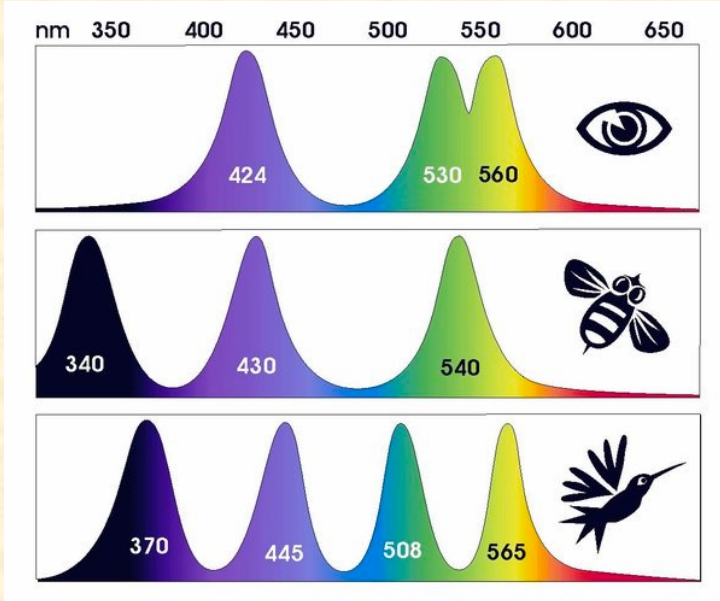
Tritanopia ✖



Color is a perception but not a physical property of light !

- <https://www.sas.upenn.edu/~scottds/vision/colorvis.html>
- <https://www.verywellhealth.com/what-do-color-blind-people-see-5092522>

How do animals see colors ?



Spectral sensitivity of dog



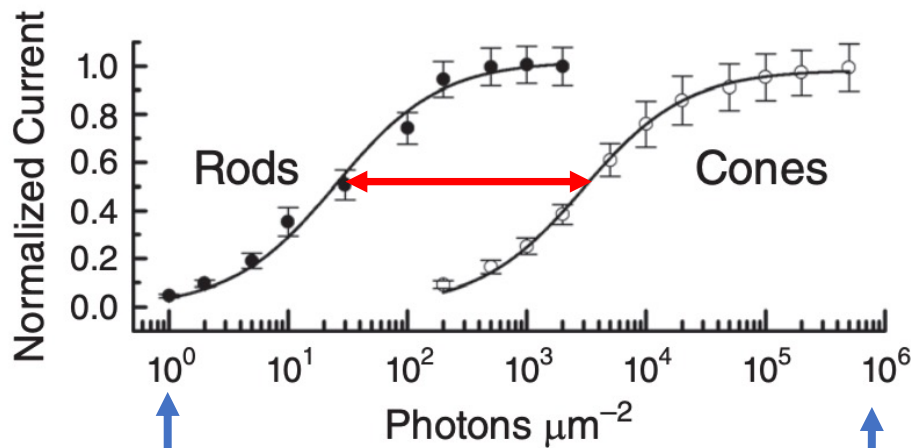
Mantis shrimp: 12 photoreceptors !



- <https://www.quora.com/What-color-can-most-animals-not-see>
- Kasparson et al (2013)

Rods are much more sensitive to light than cones

Responses of dark adapted mouse rod and cones to flashes of light



Moonlight

$\sim 10^{-1}$ Lux $\sim 10^2$ photons/ $\mu\text{m}^2/\text{s}$

Bright sunlight

$\sim 10^5$ Lux $\sim 10^8$ photons/ $\mu\text{m}^2/\text{s}$

Sunset/sunrise

Rods and cones cover a
Luminance Range $\sim 10^9$ lux !

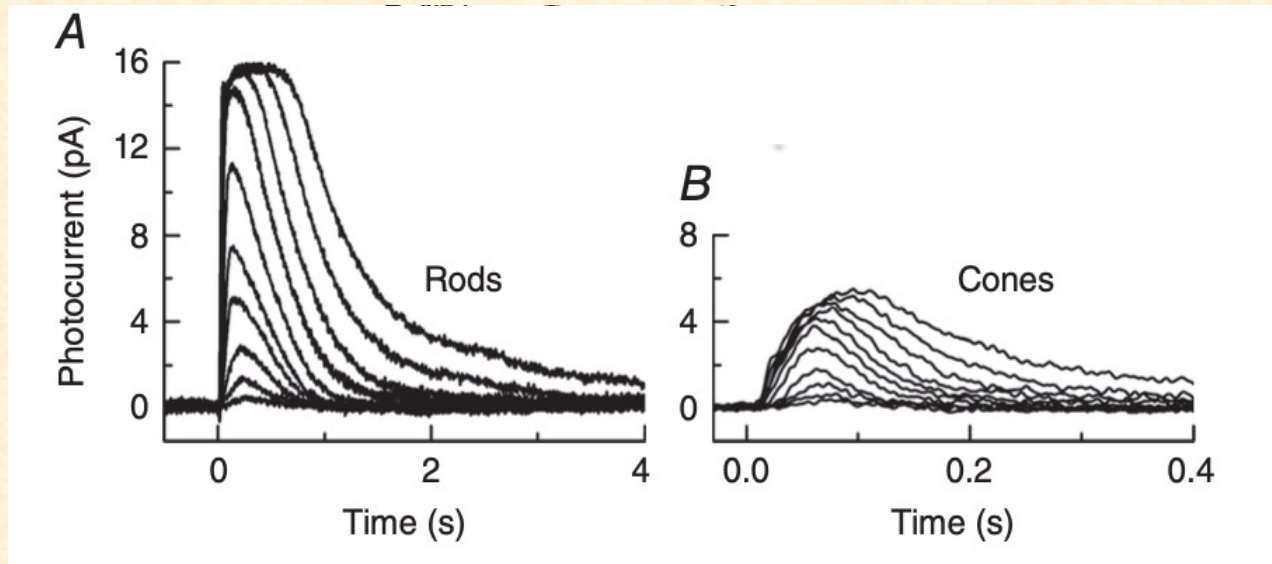
Illuminance	Example
120,000 lux	Brightest sunlight
111,000 lux	Bright sunlight
20,000 lux	Shade illuminated by entire clear blue sky, midday
1,000 - 2,000 lux	Typical overcast day, midday
<200 lux	Extreme of darkest storm clouds, midday
400 lux	Sunrise or sunset on a clear day (ambient illumination).
40 lux	Fully overcast, sunset/sunrise
<1 lux	Extreme of darkest storm clouds, sunset/rise

For comparison, nighttime illuminance levels are:

Illuminance	Example
<1 lux	Moonlight ^[3]
0.25 lux	Full Moon on a clear night ^{[4][5]}
0.01 lux	Quarter Moon
0.002 lux	Starlight clear moonless night sky including airglow ^[4]
0.0002 lux	Starlight clear moonless night sky excluding airglow ^[4]
0.00014 lux	Venus at brightest ^[4]
0.0001 lux	Starlight overcast moonless night sky ^[4]

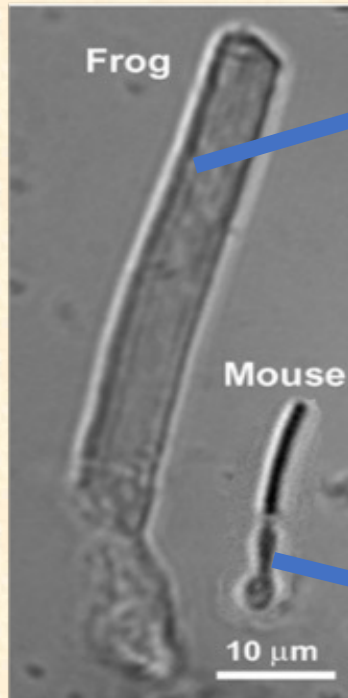
1 Lux $\sim 10^3$ photons/ $\mu\text{m}^2/\text{s}$

Cones are less sensitive to light but have a higher temporal resolution compared rods

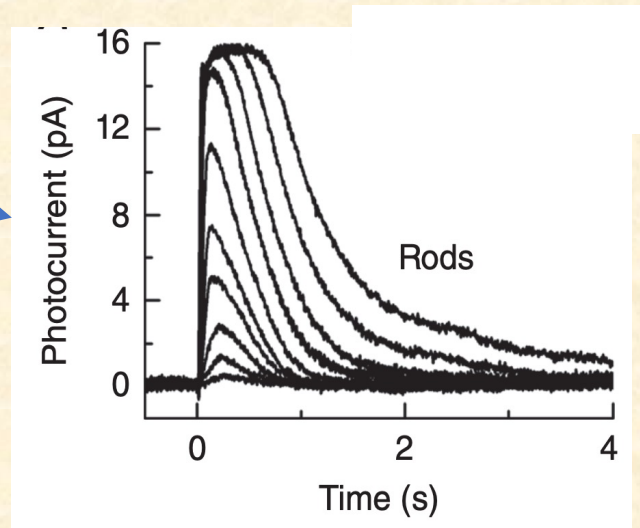
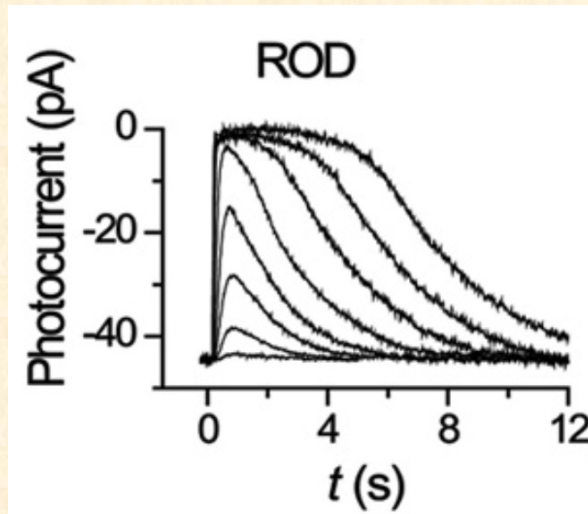


Responses of mouse rods and cones to flashes of light

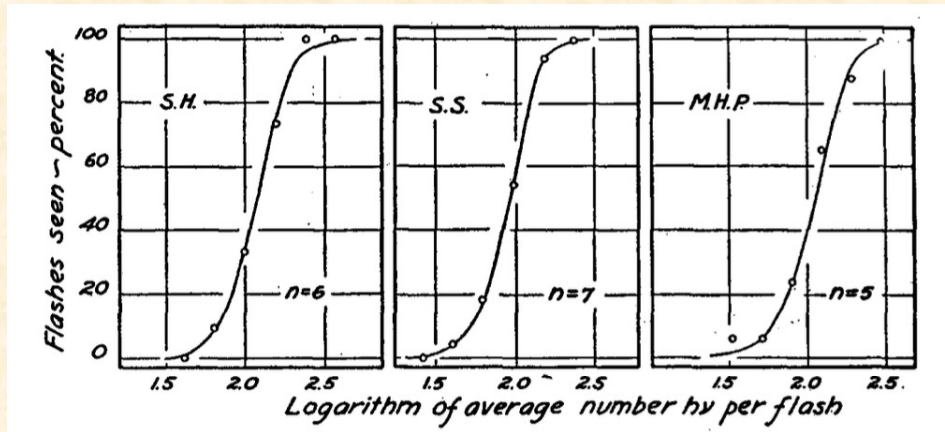
Temporal resolution depends on the photoreceptor size



Frog and mouse rod



First evidence that rods can detect the absorption of single photons from behavioral experiments in humans (1942)



Percentage of flashes that have evoked a visual response as a function of the log of the flash intensity

Flashes illuminated an area of the retina containing ~500 rods

Assumptions:

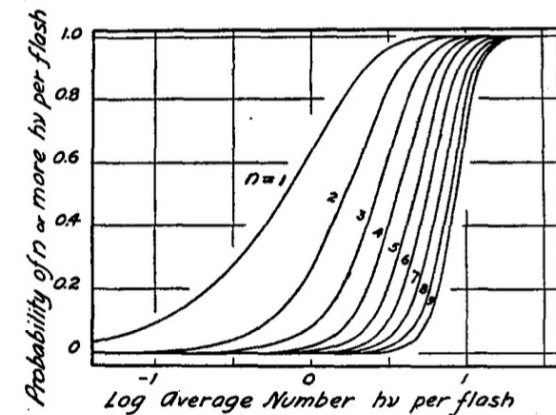
1. The stochastic number of absorbed photons per flash is Poisson distributed
2. Minimum of n photon absorptions are necessary to see a flash

I : flash intensity qI : average number of photon absorptions

$$p_{see}(n, qI) = \sum_{k=n}^{\infty} \frac{e^{-qI} (qI)^k}{k!} = 1 - \sum_{k=0}^{n-1} \frac{e^{-qI} (qI)^k}{k!}$$

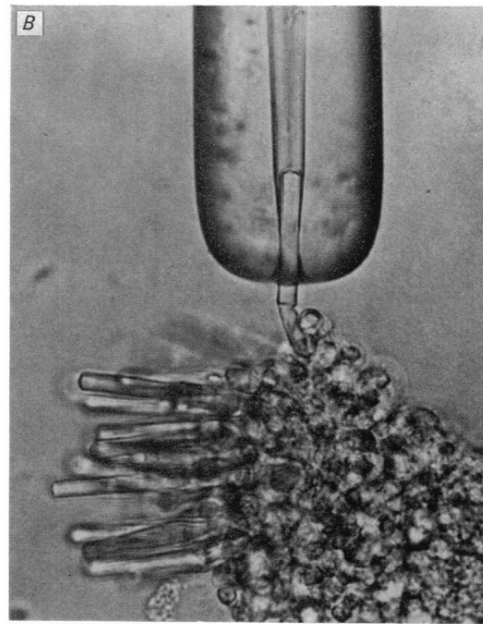
$$\ln qI = \ln q + \ln I$$

$p_{see}(n, qI)$

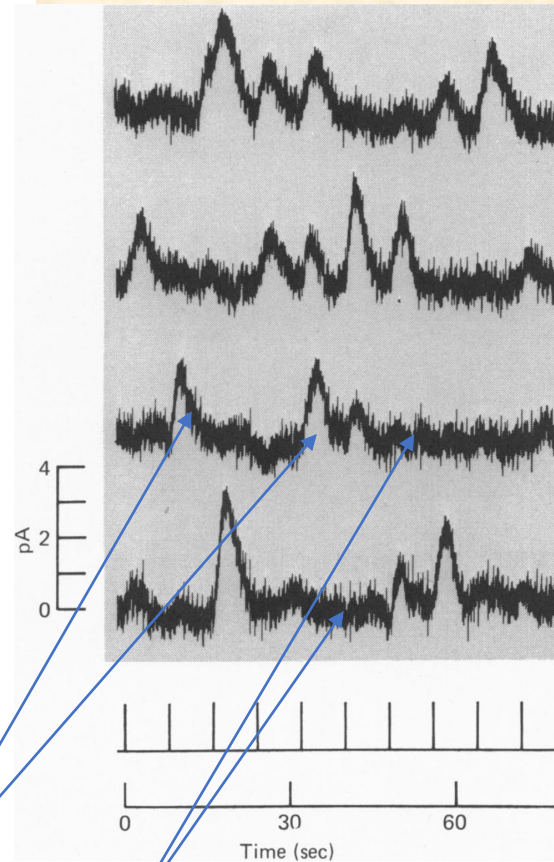


$\ln I$

Direct experimental evidence that rods detect the absorption of a single photon (1979)



Suction electrode recordings in toad rods



Photon Absorptions

No absorption, only background noise

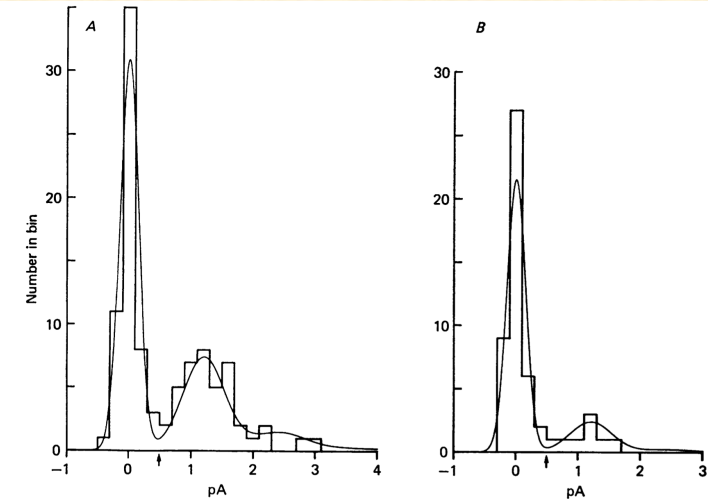


Fig. 3. Amplitude histograms for flash responses. Amplitudes were measured by the least squares method described on p. 614. Ordinate is number of occurrences of amplitude shown on abscissa, bin width 0.2 pA. A, from same cell and same flash intensity (0.029 photons μm^{-2}) as in Fig. 2; ninety-nine trials. Response amplitude had mean $\mu = 0.56$ pA and variance $\sigma^2 = 0.58$ pA². The curve is eqn. (10) with $m = 0.53$, $a = 1.2$ pA, $\sigma_0 = 0.15$ pA and $\sigma_1 = 0.3$ pA. B, same cell, lower intensity (0.014 photons μm^{-2}); fifty-two trials. Mean response $\mu = 0.17$ pA, variance $\sigma^2 = 0.21$ pA². Curve is for same parameters, except $m = 0.25$. Arrows mark the criterion level of 0.5 pA used to separate responses from failures.

Probability distribution for the peak amplitude:

$$p(r) = \sum_{k=0}^{\infty} \frac{e^{-m} m^k}{k!} \underbrace{\frac{1}{(2\pi(\sigma_0^2 + k\sigma_1^2))^{1/2}}}_{\text{Gaussian distribution for the amplitude}} \exp\left(-\frac{(r - ka)^2}{2(\sigma_0^2 + k\sigma_1^2)}\right)$$

Poisson distribution for the number of absorbed photons

Gaussian distribution for the amplitude

Ability to detect single photon absorptions is crucial for the visual sensitivity under very dim light conditions

Consider a very dim flash with mean photon absorptions $qI \ll 1$:

$$p(n = 0) = e^{-qI} \approx 1$$

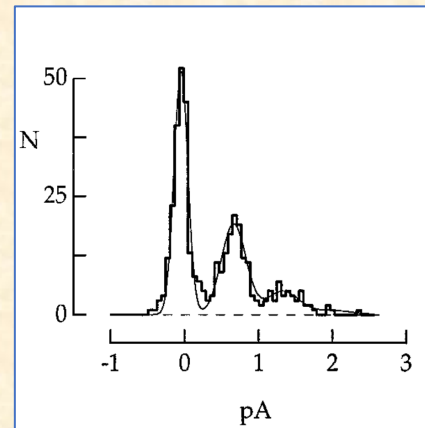
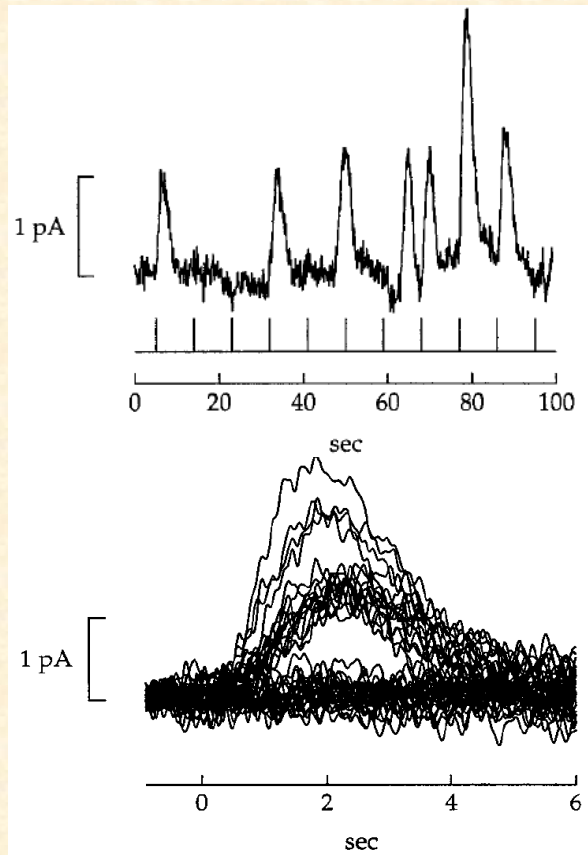
$$p(n = 1) = e^{-qI} qI \sim I$$

$$p(n = 2) = e^{-qI} \frac{(qI)^2}{2} \sim I^2$$



- On average I^{-1} rods are necessary to see the flash if rods can signal single photon absorption
- On average $I^{-2} \gg I^{-1}$ rods are necessary if rods would need 2 photon absorptions to generate a signal

Reproducibility of the single photon response (SPR)



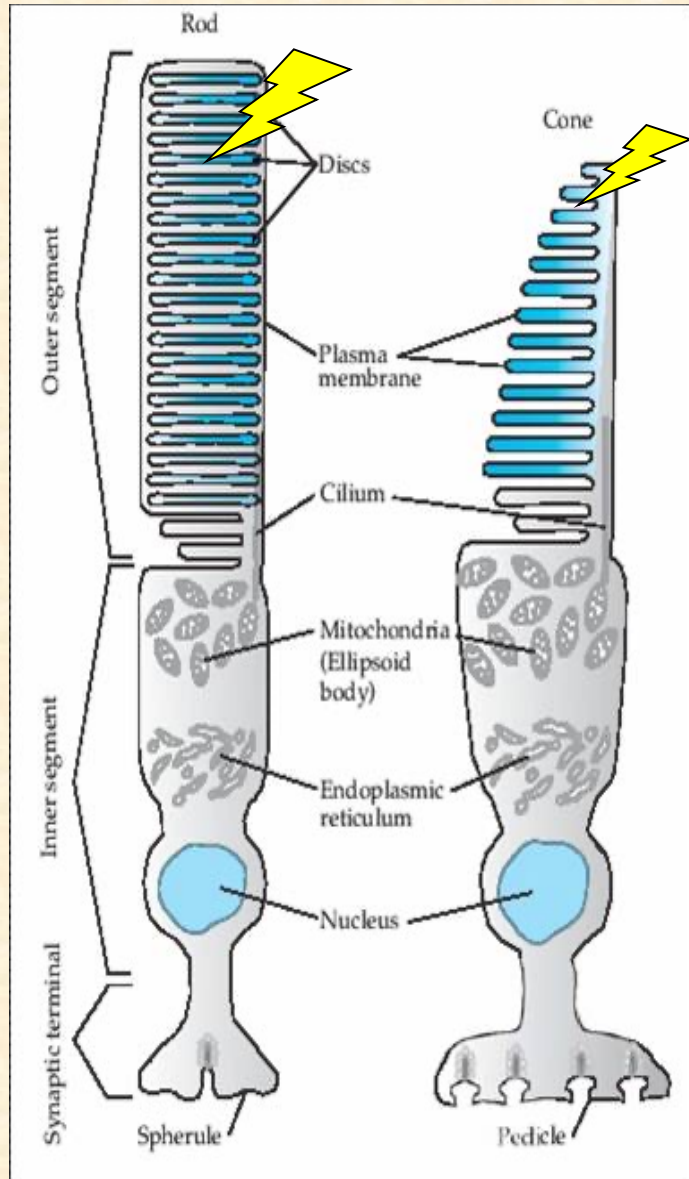
$$CV_{area} \sim 0.4 \ll 1$$

CV = coefficient of variation = mean/SD

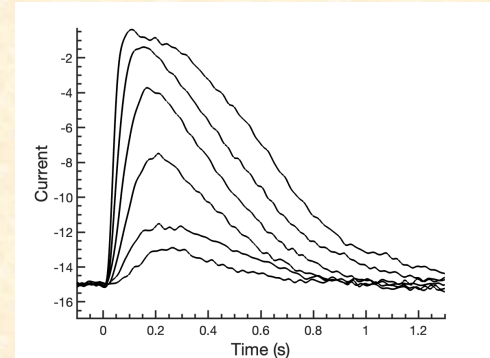
Why is the cellular response generated from a single molecular event so reproducible ?

How can we use modelling to understand how the geometry and the biochemistry of the signaling pathway determine the photoreceptor response ?

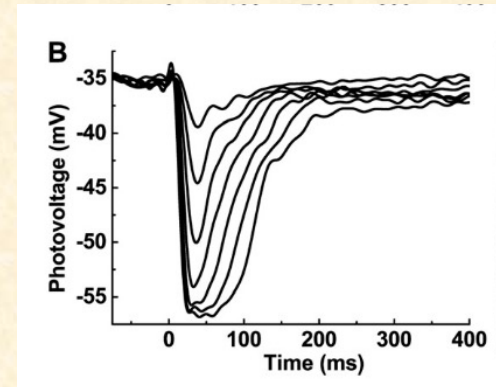
Photoreceptor physiology



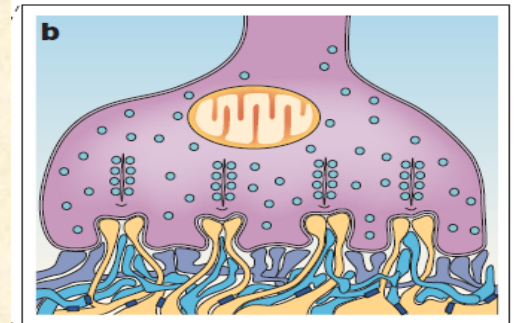
Outer Segment
Light Sensory Unit
Current Response



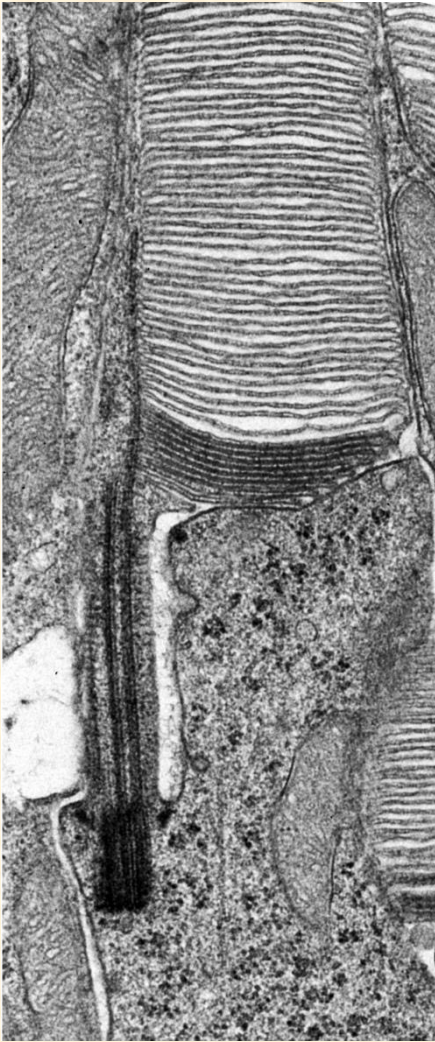
Inner Segment
Voltage response



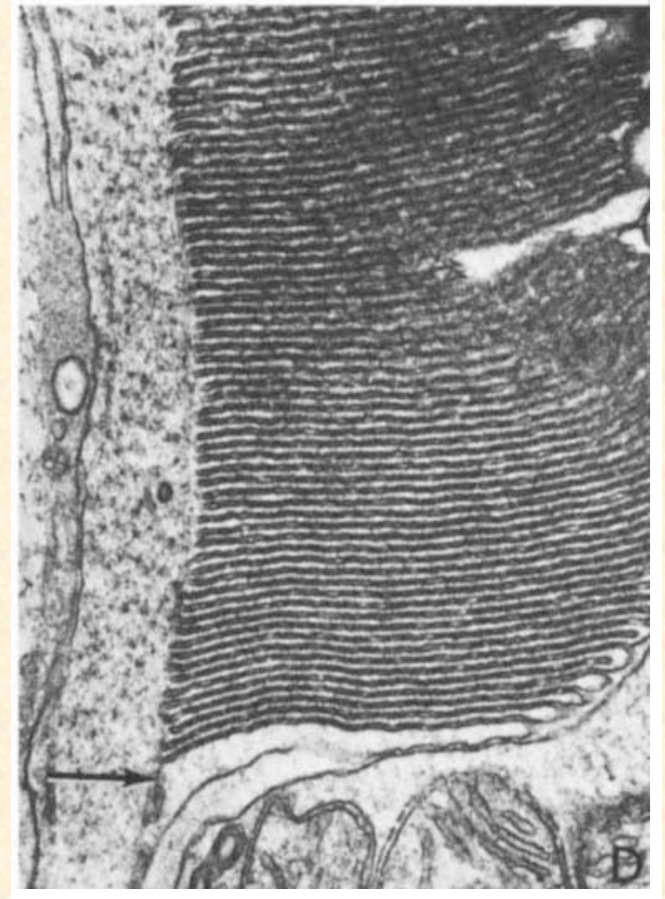
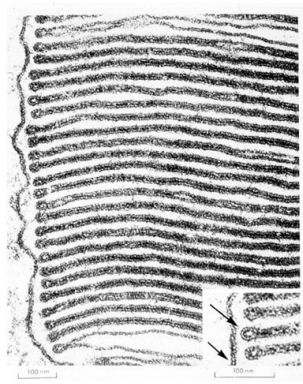
Ribbon Synapse
Glutamate release is maximal in darkness!



Structure of rod and cone outer segments



Rod outer segment with
internal discs



Cone outer segment with
invaginations

The diagram illustrates the molecular mechanisms of PDE activation and inactivation in a rod cell. On the left, a schematic of a rod cell shows the outer segment with photoreceptor discs and the inner segment with the nucleus and mitochondria. Two lines from the outer segment point to the detailed molecular diagrams on the right.

PDE Activation: This diagram shows the sequence of events starting from light absorption. Light ($h\nu$) hits the rhodopsin (R), causing it to activate (R*). Activated rhodopsin then activates the G-protein (G), which in turn activates the PDE (G* α -E*). The activated PDE then hydrolyzes cGMP (cG) into GMP, leading to the closure of the cation channel (cG-gated channel). This process is coupled with the exchange of $4Na^+$ for Ca^{2+} and K^+ via an exchanger. The closure of the channel leads to a decrease in Ca^{2+} levels, which is sensed by GCAP (guanylate cyclase activating protein) to activate guanylate cyclase (GC), which then synthesizes cGMP to reopen the channel.

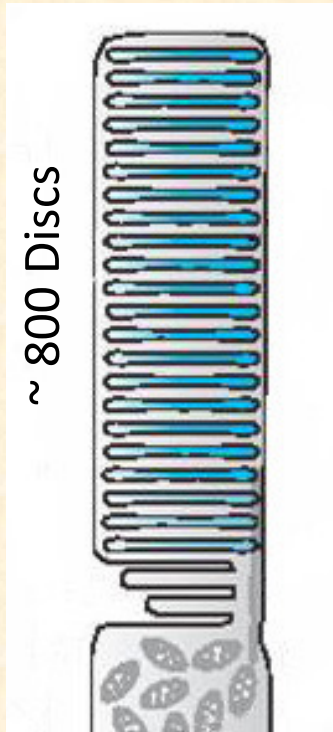
Rhodopsin Inactivation: This diagram shows the process of rhodopsin being deactivated. Activated rhodopsin (R*) is converted back to the inactive state (R) by the action of the arrestin (Arr) and the recovery kinase (RK). The recovery kinase (RK) is activated by the G-protein (G* α -E*) and the arrestin (Arr). The recovery kinase (RK) then phosphorylates the arrestin (Arr), leading to its release from the rhodopsin (R) and the restoration of the inactive rhodopsin (R).

PDE Inactivation: This diagram shows the process of PDE being inactivated. The activated PDE (G* α -E*) is inactivated by the action of the RGS-G β 5 complex. The RGS-G β 5 complex is activated by the G-protein (G* α -E*) and the RGS protein. The RGS-G β 5 complex then binds to the G-protein (G* α -E*), leading to its inactivation (G α -E*) and the restoration of the inactive PDE (G α -E*).

Amplification: The diagram highlights the amplification of the signal. A single photon of light ($h\nu$) can activate a single rhodopsin (R), which can then activate multiple G-proteins (G), which in turn can activate multiple PDEs (G* α -E*). This leads to a large number of cGMP molecules being hydrolyzed, resulting in a significant change in the cGMP concentration and the closure of the cation channel.

Amplification

Important parameters for a mouse rod model



Geometry:

Radius $\sim 0.7 \mu\text{m}$

Length $\sim 24 \mu\text{m}$

Cytoplasmic Volume: $\sim 18 \mu\text{m}^3$

Protein abundancies:

Rhodopsin. : $\sim 25000/\mu\text{m}^2$, $\sim 6 \times 10^7$

G-protein : $\sim 3000/\mu\text{m}^2$, $\sim 7 \times 10^6$

PDE : $\sim 500/\mu\text{m}^2$, $\sim 1 \times 10^6$

Spontaneous pigment activations
have to occur extremely seldom
in order to allow for reliable
single photon detection !

Darkness:

cGMP $\sim 4 \mu\text{M}$, $\text{Ca}^{2+} \sim 0.3 \mu\text{M}$, Current $\sim -15 \text{ pA}$, Voltage $\sim -40 \text{ mV}$

Physical Units: mV (voltage), pA (current), μm (length), μM (concentrations)

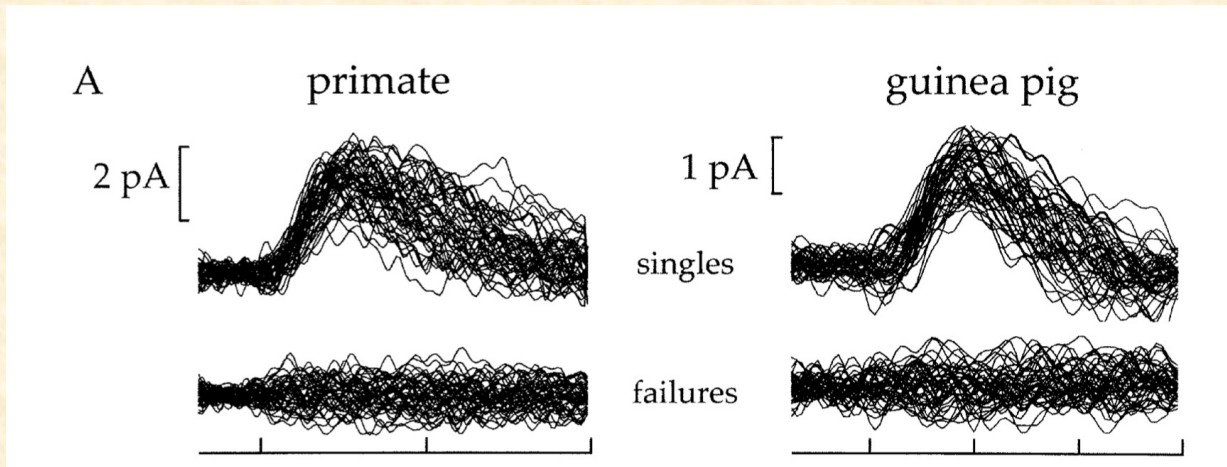
$$\mathcal{N} = 6.02 \times 10^{23} \frac{1}{\text{mol}} = 6.02 \times 10^{23} \frac{1}{\text{M l}} = 602 \frac{1}{\mu\text{M} \mu\text{m}^3}$$

Avogadro constant

$$\mathcal{F} = e^+ \mathcal{N} = 9.6 \times 10^4 \frac{\text{C}}{\text{mol}} = 9.6 \times 10^{-5} \frac{\text{spA}}{\mu\text{M} \mu\text{m}^3}$$

Faraday constant

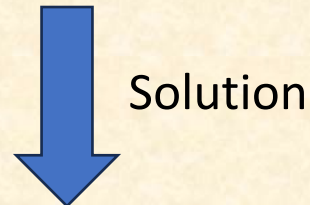
Why is the SPR so reproducible ?



$$CV_{area} \sim 0.4 \ll 1$$

One activated Rhodopsin $\xrightarrow{\text{Amplification}}$ Many activated PDE

If Rhodopsin is deactivated in a single stochastic event, its lifetime is exponentially distributed and one expects a CV of the order 1.



Sequential deactivation via by multiple phosphorylation steps

Effect of sequential deactivation via multiple stochastic steps

1) Deactivation occurs through a single stochastic event with rate μ . The lifetime of activated rhodopsin τ has an exponential Probability Density Function (PDF):

$$p(\tau) = \mu e^{-\mu\tau}, \quad \bar{\tau} = \frac{1}{\mu}, \quad \sigma^2 = \frac{1}{\mu^2}, \quad CV = \frac{\sigma}{\bar{\tau}} = 1$$

2) Deactivation occurs through 2 sequential stochastic events with rates $\nu=2\mu$. The PDF of the deactivation time $\tau = \tau_1 + \tau_2$ is

$$p(\tau) = \int_0^\tau \underbrace{\nu e^{-\nu s}}_{\substack{\text{1. event} \\ \text{after time } s}} \underbrace{\nu e^{-\nu(\tau-s)}}_{\substack{\text{2. event after} \\ \text{time } \tau-s}} ds = \nu^2 \tau e^{-\nu\tau}, \quad \bar{\tau} = \frac{2}{\nu} = \frac{1}{\mu}, \quad \sigma^2 = \frac{2}{\nu^2} = \frac{1}{2} \frac{1}{\mu^2}, \quad CV = \frac{1}{\sqrt{2}}$$

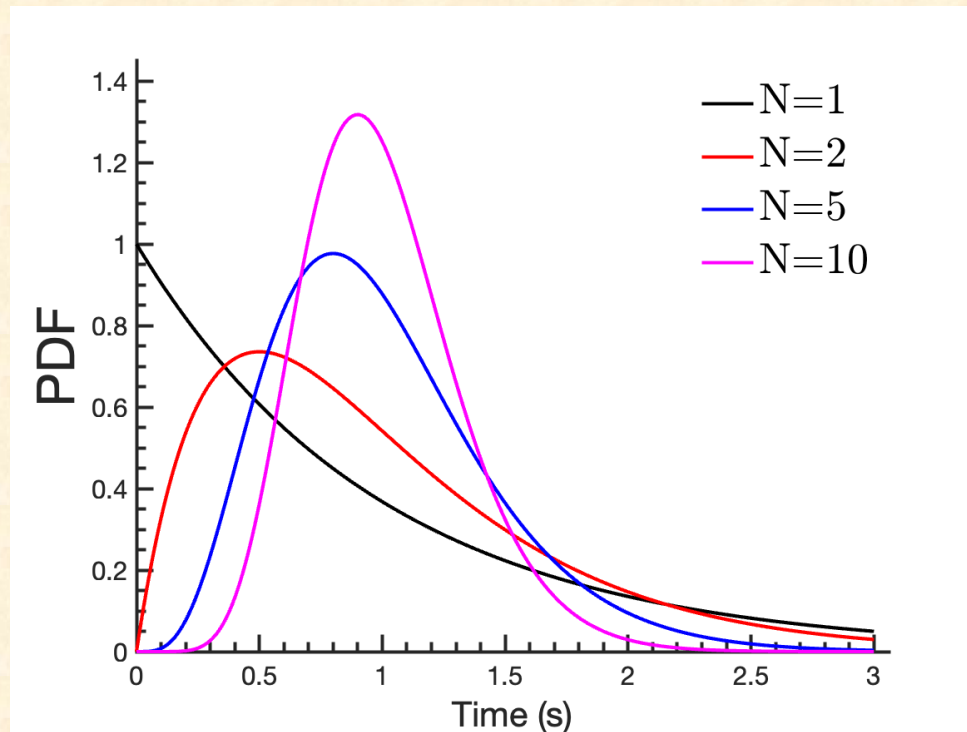
3) Deactivation occurs through N sequential stochastic events with rates $\nu=N\mu$. The PDF for $\tau = \tau_1 + \tau_2 + \dots \tau_N$ is

$$p(\tau) = \frac{\nu^N \tau^{N-1} e^{-\nu\tau}}{(N-1)!}, \quad \bar{\tau} = \frac{N}{\nu} = \frac{1}{\mu}, \quad \sigma^2 = \frac{N}{\nu^2} = \frac{1}{N} \frac{1}{\mu^2}, \quad CV = \frac{1}{\sqrt{N}}$$

$$\Gamma(\tau; N, \nu) = \frac{\nu^N \tau^{N-1} e^{-\nu\tau}}{(N-1)!}$$

Erlang distribution

Examples of Erlang distributions $\Gamma(N, N\mu)$ with $\mu = 1s^{-1}$



Sequential deactivation coupled to stochastic PDE activation

Assume that rhodopsin activates PDE with rate λ (we neglect the intermediate step of transducin activation)

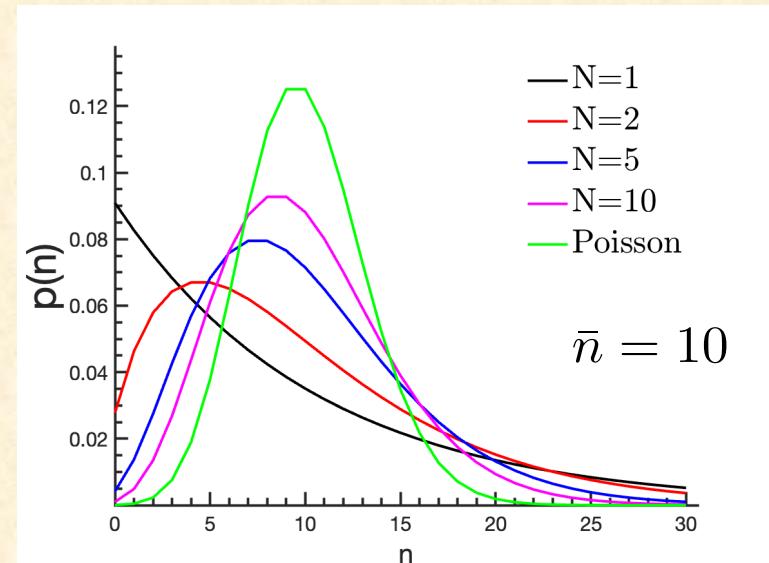
What is probability distribution for the number of activated PDEs ?

$$p(n) = \int_0^\infty p(n, \tau) d\tau = \int_0^\infty p(n|\tau) \Gamma(\tau; N, N\mu) d\tau = \int_0^\infty \frac{e^{-\lambda\tau} (\lambda\tau)^n}{n!} \frac{(N\mu)^N \tau^{N-1} e^{-N\mu\tau}}{(N-1)!} d\tau$$

$$\bar{n} = \frac{\lambda}{\mu} = \frac{N^N \bar{n}^n}{n!(N-1)!(N+\bar{n})^{N+n}} \int_0^\infty x^{N+n-1} e^{-x} dx = \frac{N^N \bar{n}^n (N+n-1)!}{n!(N-1)!(N+\bar{n})^{N+n}}$$

$$N=1: \quad p(n) = \frac{1}{1+\bar{n}} \left(\frac{\bar{n}}{1+\bar{n}} \right)^n$$

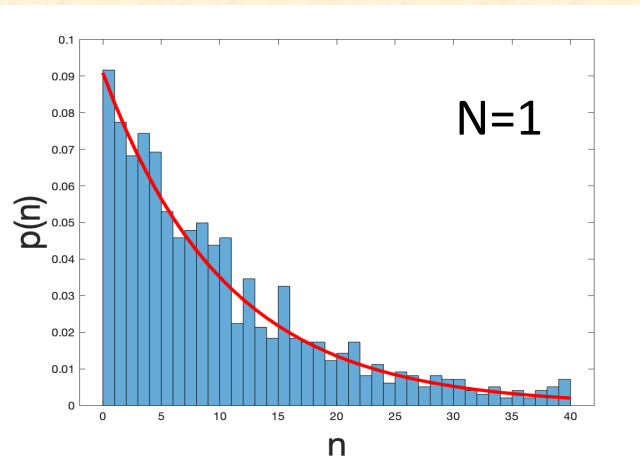
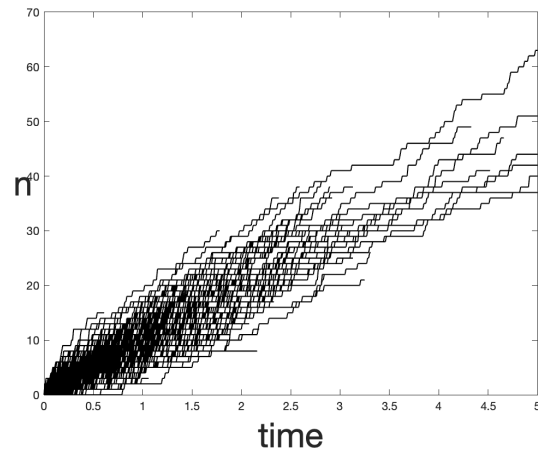
$$N \rightarrow \infty: \quad p(n) = \frac{e^{-\bar{n}} \bar{n}^n}{n!}$$



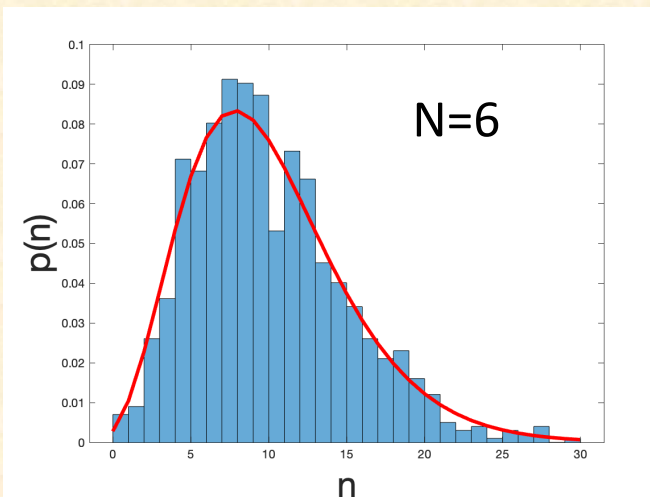
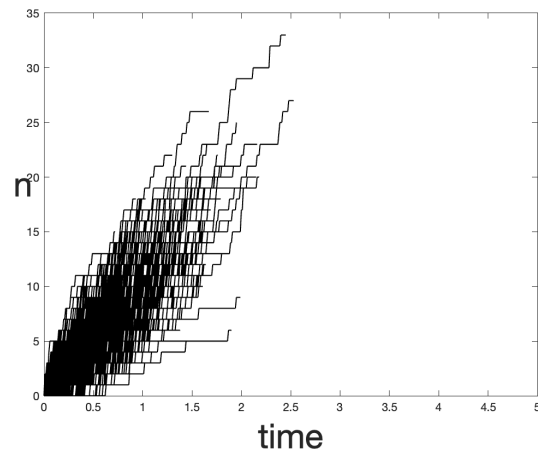
Sequential rhodopsin deactivation coupled to stochastic PDE activation

$$p(n) = \frac{N^N \bar{n}^n (N + n - 1)!}{n! (N - 1)! (N + \bar{n})^{N+n}} \quad \bar{n} = \frac{\lambda}{\mu}, \quad \sigma^2 = \bar{n} \left(1 + \frac{\bar{n}}{N}\right), \quad CV = \sqrt{\frac{1}{\bar{n}} + \frac{1}{N}}$$

Stochastic simulations (Gillespie algorithm) with $\lambda=10^{-1}$, $\mu=1s^{-1}$ ($\bar{n} = 10$)

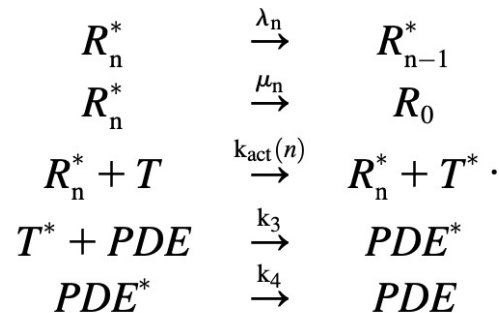


$$CV \approx 1$$



$$CV \approx \frac{1}{\sqrt{6}} \approx 0.4$$

Stochastic model of time dependent PDE activation



Set of reactions modelled by Continuous time Markov chain

$P(n, l, k, t)$: Probability to find at time t the system in configuration (n, l, k)

l : number of activated transducin
 k : number of activated PDE

Master Equation:

$$\begin{aligned}
 \partial_t P(n, l, k, t | n_0, l_0, k_0, t_0) &= \sum_{n', l', k'} W(n, l, k | n', l', k', t) P(n', l', k', t | n_0, l_0, k_0, t_0) \\
 &\quad - P(n, l, k, t | n_0, l_0, k_0, t_0) \sum_{n', l', k'} W(n', l', k' | n, l, k, t),
 \end{aligned}$$

Transition probabilities

$$\begin{aligned}
 W(n, l, k | n', l', k', t) &= \lambda_{n'} \delta_{n, n'+1} \delta_{l, l'} \delta_{k, k'} + \mu_{n'} \delta_{n, n'} \delta_{l, l'+1} \delta_{k, k'} \\
 &\quad + k_{act}(n) \delta_{n, n'} \delta_{l, l'+1} \delta_{k, k'} + \delta_{n, n'} \delta_{l, l'-1} \delta_{k, k'} \\
 &\quad + k_3 l' \delta_{n, n'} \delta_{l, l'-1} \delta_{k, k'+1} + k_4 k' \delta_{n, n'} \delta_{l, l'} \delta_{k, k'-1},
 \end{aligned}$$

$$\begin{aligned}
 \frac{\partial}{\partial t} P(n, l, k, t) &= \lambda_{n+1} P(n+1, l, k, t) + \delta_{n,0} \sum_{n'=1}^N \mu_{n'} P(n', l, k, t) \\
 &\quad + k_{act}(n) P(n, l-1, k, t) + \\
 &\quad + k_3(l+1) P(n, l+1, k-1, t) + k_4(k+1) P(n, l, k+1, t) \\
 &\quad - (\lambda_n + \mu_n + k_{act}(n) + k_3 l + k_4 k) P(n, l, k, t).
 \end{aligned}$$

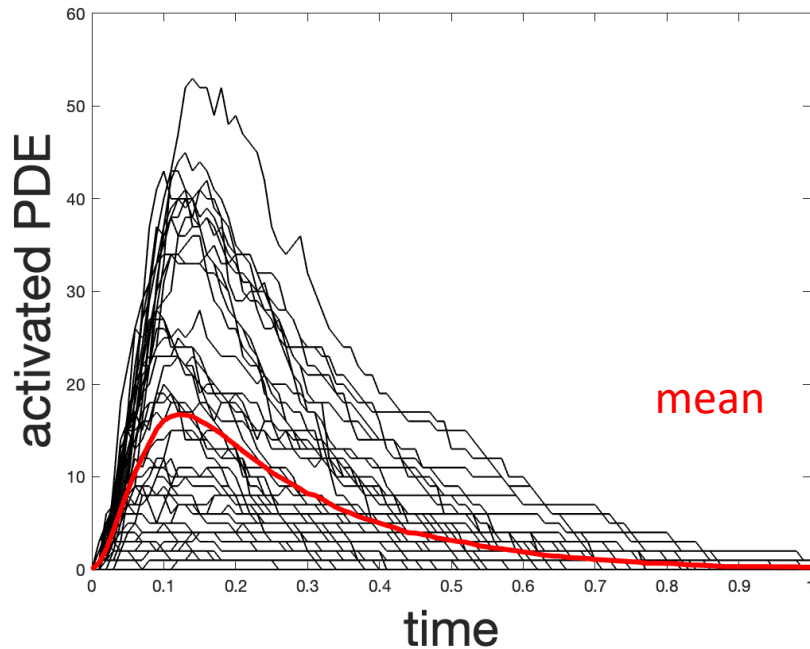
Time dependent mean and variance of activated PDE:

$$\bar{P}(t) = \sum_{n, l, k} k P(n, l, k, t).$$

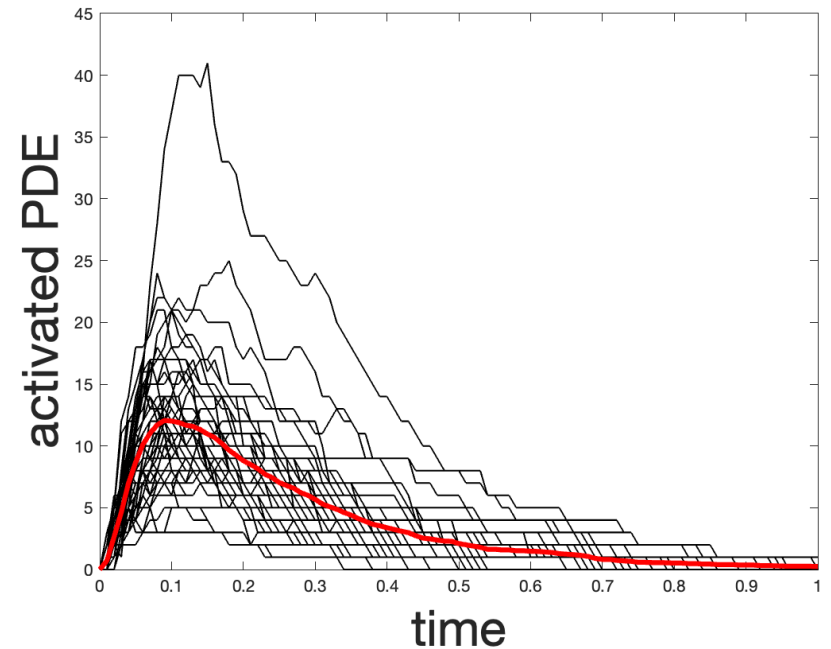
$$\Sigma_P^2(t) = \sum_{n, l, k} k^2 P(n, l, k, t) - \bar{P}(t)^2.$$

Stochastic simulation of PDE activation

Rhodopsin deactivation in a single step

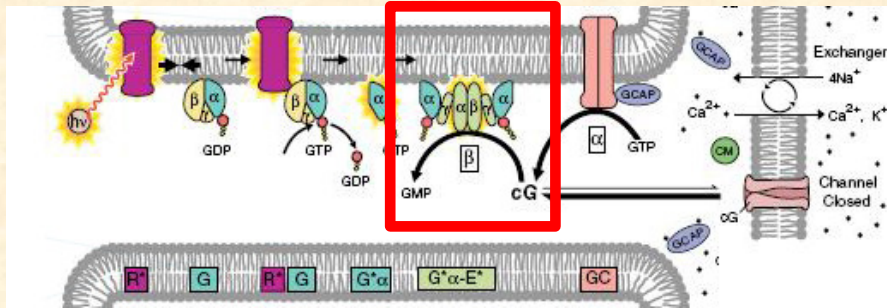


Rhodopsin deactivation after 6 phosphorylation steps



But how do we know that around 10-15 PDEs become activated during a SPR ?

Estimation of the cGMP hydrolysis rate of an activated PDE

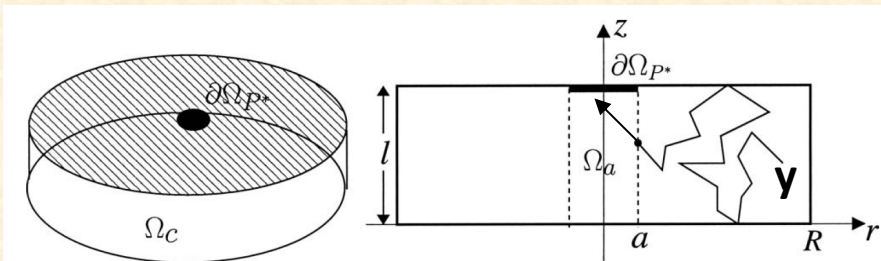


Activated PDE is highly efficient enzyme



cGMP hydrolysis is determined by the diffusion limited encounter rate between activated PDE and cGMP

Mean first passage time (MFPT) analysis to compute the encounter rate:



$\tau(\mathbf{y})$ MFPT for a diffusing cGMP to encounter activated PDE on the surface when starting at position $\mathbf{y}$

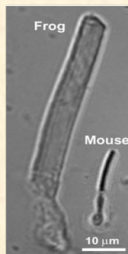
$\partial_t p(\mathbf{x}, t | \mathbf{y}) = D \Delta_y p(\mathbf{x}, t | \mathbf{y})$ Backward Fokker-Planck Equation



$D \Delta \tau(\mathbf{y}) = -1$ MFPT equation

$$\frac{1}{\tau} = k_{enc} = \frac{D}{R^2} \left(\pi \frac{l}{a} \frac{a_0(l/a)}{\sqrt{2}} + \frac{4 \ln(R/a) - 3}{8} \right)^{-1}$$

Spatially averaged encounter rate



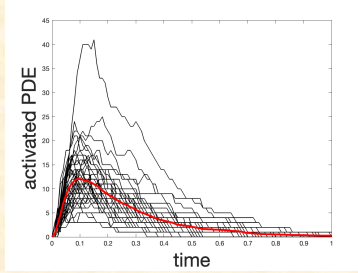
Mouse: $\sim 60 \text{ s}^{-1}$

Toad: $\sim 3 \text{ s}^{-1}$

Impact of the geometry !

Computational framework for the current response

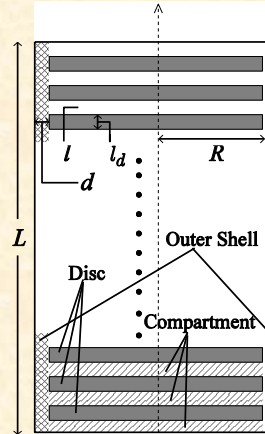
Simulations of PDE activation in the compartment where the photon is absorbed



Adjust PDE activation to obtain correct current amplitude
(Exp confirmation in 2019 by group of KW Yau at JH)

+

Model for the OS with 800 comp



+

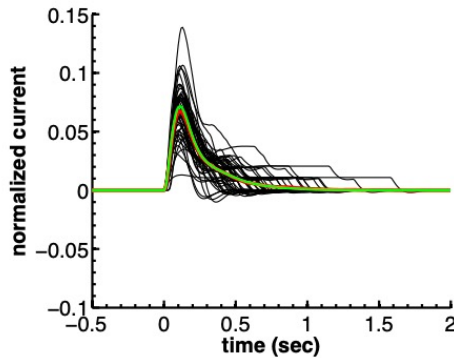
Coarse grained effective 1D model with longitudinal diffusion of cGMP and calcium

+

Dynamics of cGMP synthesis and hydrolysis

+

Current dynamics based on CNG Channels and Ca-Exchangers

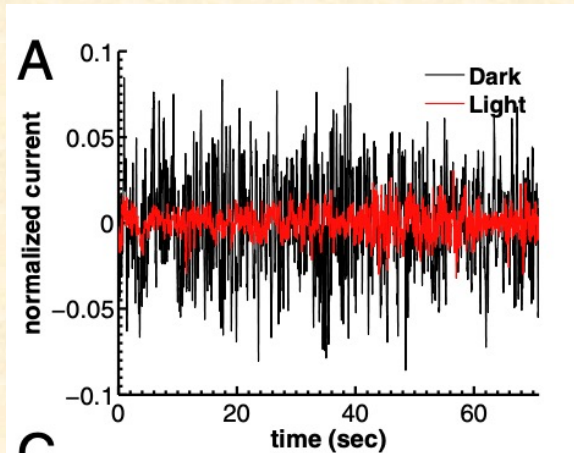


$$\begin{aligned} \frac{d\hat{g}(n,t)}{dt} &= d_g(\hat{g}(n+1,t) + \hat{g}(n-1,t) - 2\hat{g}(n,t)) \\ &\quad + \beta_d \frac{r_\alpha + (1-r_\alpha) \frac{k_\alpha^{n_\alpha}}{k_\alpha^{n_\alpha} + \hat{c}a(n,t)^{n_\alpha}}}{r_\alpha + (1-r_\alpha) \frac{k_\alpha^{n_\alpha}}{k_\alpha^{n_\alpha} + 1}} - (k_{sp}P_{sp}^*(n,t) + k_{li}P_{li}^*(n,t)) \hat{g}(n,t) \\ \frac{d\hat{c}a(n,t)}{dt} &= d_{ca}(\hat{c}a(n+1,t) + \hat{c}a(n-1,t) - 2\hat{c}a(n,t)) + \gamma_d \left(\frac{(1+k_{ch}^{n_{ch}})\hat{g}(n,t)^{n_{ch}}}{\hat{g}(n,t)^{n_{ch}} + k_{ch}^{n_{ch}}} - \frac{(1+k_{ex})\hat{c}a(n,t)}{\hat{c}a(n,t) + k_{ex}} \right) \\ \hat{I}(n,t) &= \frac{2}{f_{ca} + 2} \frac{(1+k_{ch}^{n_{ch}})\hat{g}(n,t)^{n_{ch}}}{\hat{g}(n,t)^{n_{ch}} + k_{ch}^{n_{ch}}} + \frac{f_{ca}}{f_{ca} + 2} \frac{(1+k_{ex})\hat{c}a(n,t)}{\hat{c}a(n,t) + k_{ex}} \\ \hat{I}_{os}(t) &= \frac{I_{os}(t)}{I_{os,d}} = \frac{1}{N_{comp}} \sum_{n=1}^{N_{comp}} \hat{I}(n,t). \end{aligned}$$

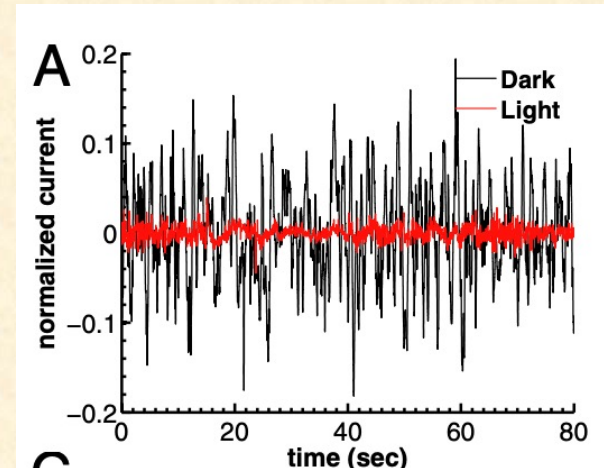
But what about the background noise due to spontaneous PDE activations and deactivations ?

Dark noise analysis to estimate the biophysical properties of spontaneous PDE activation

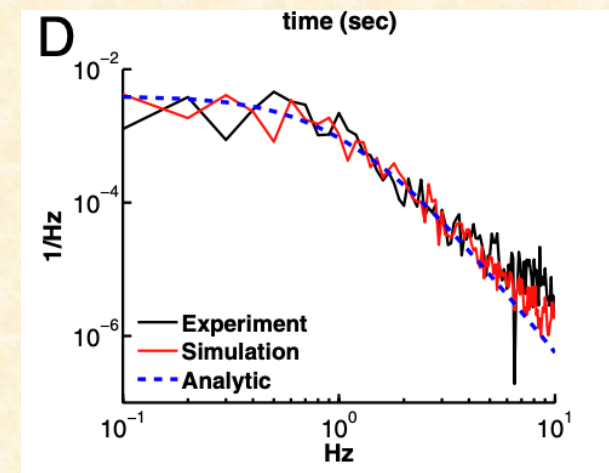
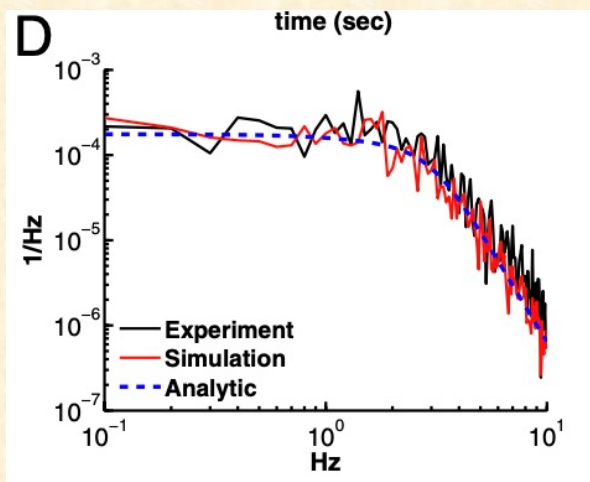
Recordings of dark noise from a WT mouse rod



Recordings of dark noise from a GCAPs KO mouse rod

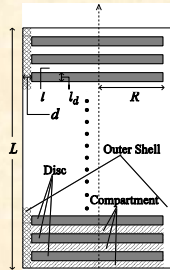


Power spectrum



Dark noise analysis to estimate the biophysical properties of spontaneous PDE activation

Analytic result for the GCAPs Ko PS:



$$S_{\hat{I}_{os}}(\omega) = \frac{4\xi_{ch}^2}{N_{comp}\bar{P}_{sp,comp}^*} \frac{\beta_d^2\mu_{sp}}{(\beta_d^2 + \omega^2)(\mu_{sp}^2 + \omega^2)}$$

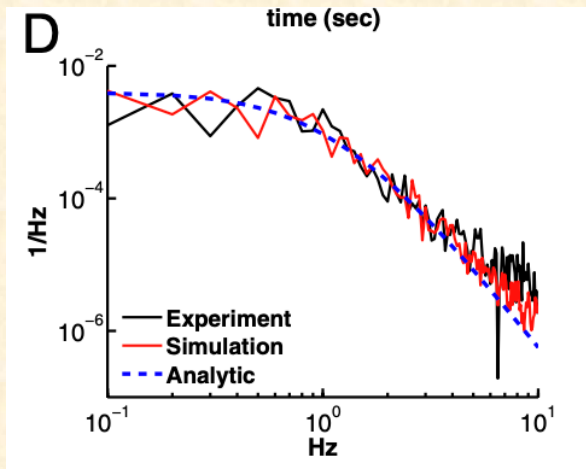
$$\bar{P}_{sp,comp}^* = \frac{P_{tot,comp}\lambda_{sp}}{\mu_{sp}}$$

$$\lambda_{sp}, \mu_{sp}$$

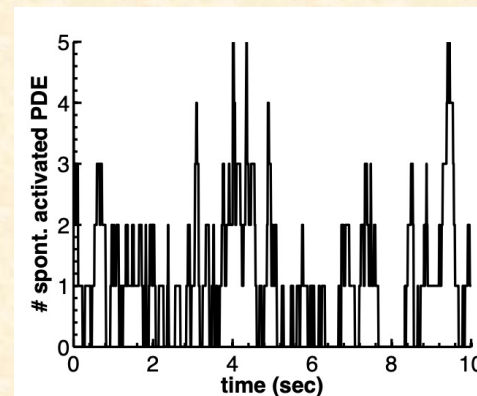
Mean number of spont.
activated PDEs per
compartment

Spont. activation and
deactivation rates of a
single PDE

Extract parameters from fitting:

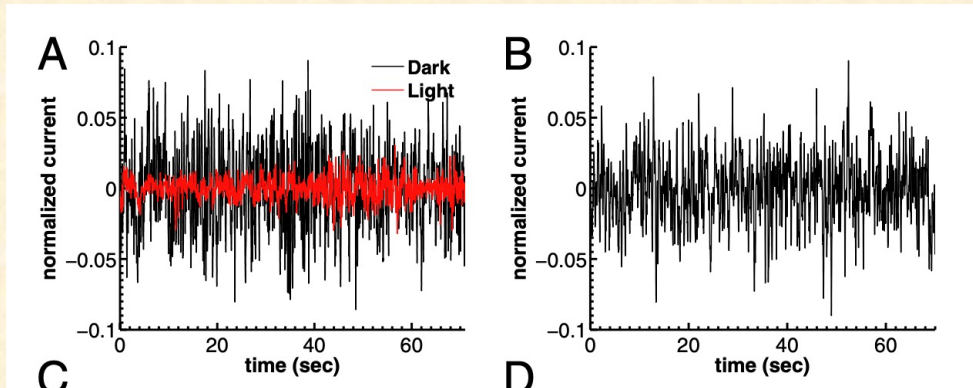


$$\bar{P}_{sp,comp}^* \approx 0.9 \quad \mu_{sp} \approx 12s^{-1}$$

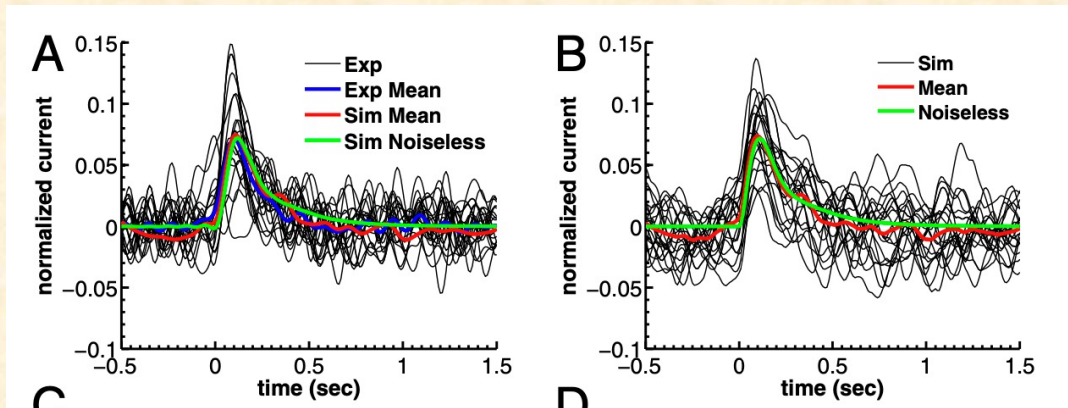


Stochastic simulations
of spont PDE
activations in a
compartment

Simulations of single photon responses with dark noise



Recordings and simulations of dark noise in a Wt mouse rod



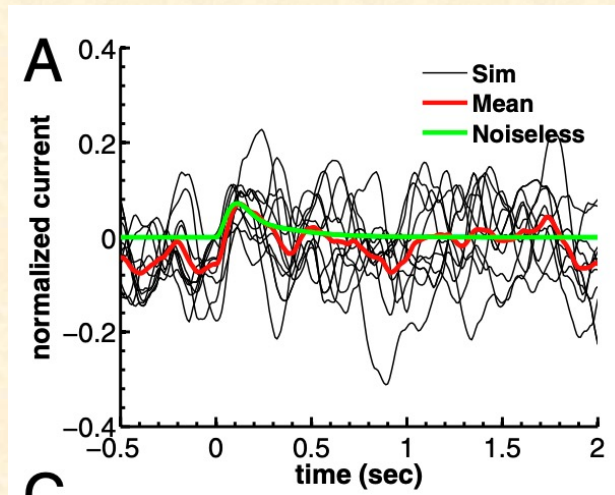
Recordings and simulations of SPR in a Wt mouse rod

Prediction: PDE density has to adapt to rod geometry

Why is the PDE density in mouse rod around 5-fold higher than in a toad rod ?

$$S_{\hat{I}_{os}}(\omega) = \frac{4\xi_{ch}^2}{N_{comp} \bar{P}_{sp,comp}^* (\beta_d^2 + \omega^2) (\mu_{sp}^2 + \omega^2)} \beta_d^2 \mu_{sp}$$

$$\bar{P}_{sp,comp}^* = \frac{P_{tot,comp} \lambda_{sp}}{\mu_{sp}} \sim \frac{R^2 \rho_{pde} \lambda_{sp}}{\mu_{sp}}$$



SPR for a mouse rod
with reduced PDE
density as in a toad
rod

2180 • The Journal of Neuroscience, March 16, 2022 • 42(11):2180–2189

Cellular/Molecular

Reproducibility of the Rod Photoreceptor Response Depends Critically on the Concentration of the Phosphodiesterase Effector Enzyme

Ala Morshedian,¹ Gabriela Sendek,¹ Sze Yin Ng,¹ Kimberly Boyd,² Roxana A. Radu,¹ Mingyao Liu,³ Nikolai O. Artemyev,² Alapakkam P. Sampath,¹ and Gordon L. Fain^{1,4}

¹Stein Eye Institute, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, 90095-7000, ²Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, Iowa, 52242, ³Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, China, 200062, and ⁴Department of Integrative Biology and Physiology, University of California Los Angeles, Los Angeles, California 90095-7239

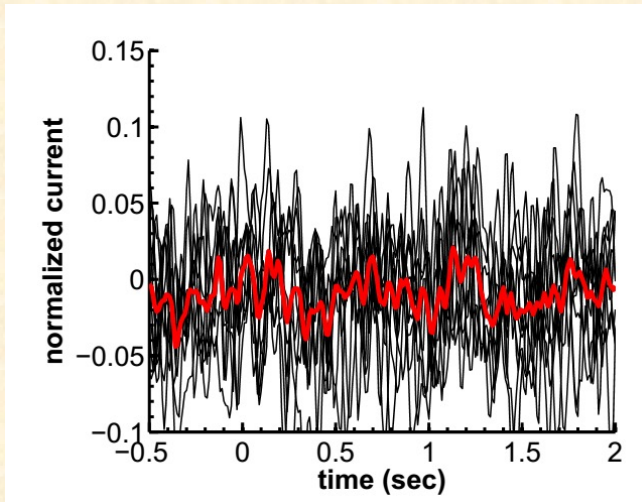
Prediction: Catalytic activity of spontaneous and G-protein activated PDE are different

Diffusion limited hydrolysis rate of
PDE activated by transducin:

$$\sim 60 \text{ s}^{-1}$$

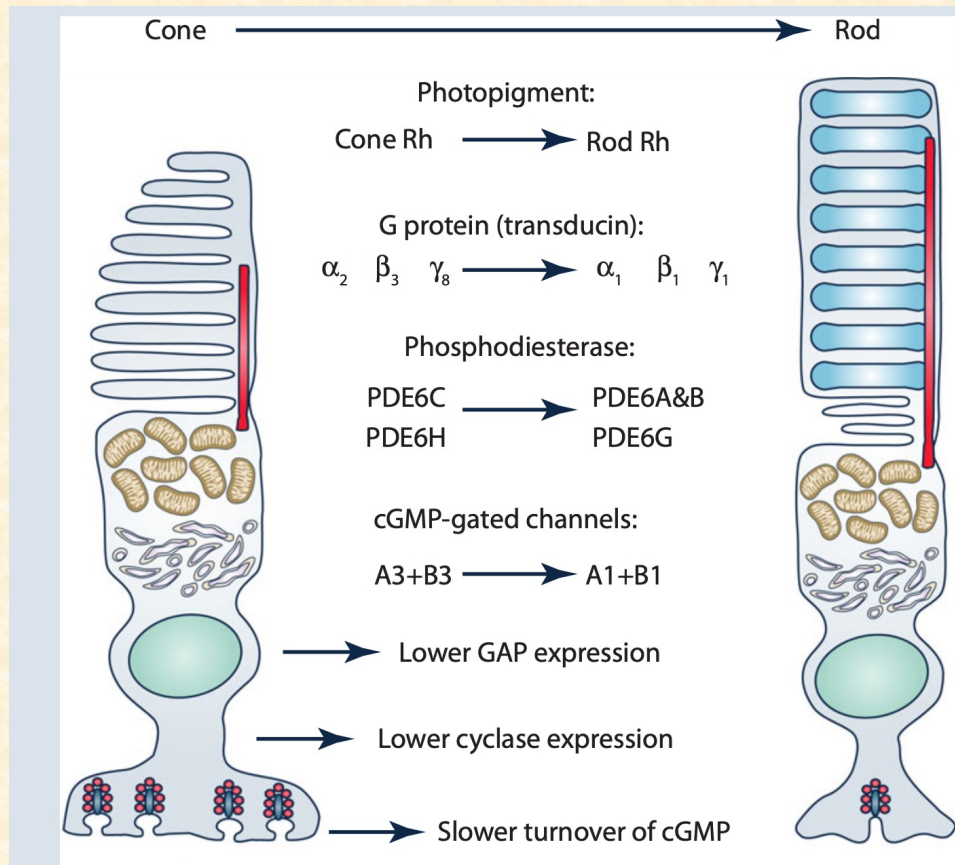
Hydrolysis rate of spont. activated PDE:

$$\frac{\beta_d}{\bar{P}_{sp,comp}^*} = \frac{4.1 \text{ s}^{-1}}{0.9} \sim 4.5 \text{ s}^{-1}$$



SPR for an mouse rod where also the
activity of spontaneously activated
PDE would be high and diffusion
limited

What distinguishes rods from cones (except photopigments) ?



Similar pathways but
many adaptations

Ingram, Fain, Sampath (2016)

- Which differences are most relevant ?
- How to modify parameters to transform a rod into a cone ?

One possible approach: Detailed models based on a large system of equations + sensitivity analysis

Mathematical analysis of phototransduction reaction parameters in rods and cones

Yukari Takeda^{1,5}, Kazuma Sato^{2,5}, Yukari Hosoki², Shuji Tachibanaki³, Chieko Koike⁴ & Akira Amano^{2✉}

Retinal photoreceptor cells, rods and cones, convert photons of light into chemical and electrical signals as the first step of the visual transduction cascade. Although the chemical processes in the phototransduction system are very similar to each other in these photoreceptors, the light sensitivity and time resolution of the photoresponse in rods are functionally different than those in the photoresponses of cones. To systematically investigate how photoresponses are divergently regulated in rods and cones, we have developed a detailed mathematical model on the basis of the Hamer model. The current model successfully reconstructed light intensity-, ATP- and GTP-dependent changes in concentrations of phosphorylated visual pigments (VPs), activated transducins (Tr*s) and phosphodiesterases (PDEs) in rods and cones. In comparison to rods, the lower light sensitivity of cones was attributed not only to the lower affinity of activated VPs for Trs but also to the faster desensitization of the VPs. The assumption of an intermediate inactive state, MII^i , in the thermal decay of activated VPs was essential for inducing faster inactivation of VPs in rods, and possibly also in cones.

Takeda Et al, Scientific Rep (2022)

$$[RK_{free}] = [RK_{total}] \quad (\text{Eq. S73})$$

$$- ([MII_1^i \cdot RK_{post}] + [MII_1^i \cdot RK_{post}] + [MII_0^i \cdot RK_{pre}] + [MII_0^i \cdot RK_{pre}] + [MII_0^i \cdot RK_{pre} \cdot ATP] + [MII_0^i \cdot RK_{pre} \cdot ATP])$$

$$[PDE_{free}] = [PDE_{total}] \quad (\text{Eq. S74})$$

$$- ([PDE \cdot G_{\alpha} \cdot GTP] + [PDE^* \cdot G_{\alpha} \cdot GTP] + [cG \cdot PDE^* \cdot G_{\alpha} \cdot GTP] + [RG9 \cdot PDE^* \cdot G_{\alpha} \cdot GTP])$$

$$[T_{free}] = [T_{total}] \quad (\text{Eq. S75})$$

$$- ([MII_0^i \cdot G \cdot GTP] + [MII_0^i \cdot G] + [MII_0^i \cdot G \cdot GTP] + [G \cdot GTP] + [G_{\alpha} \cdot GTP] + [PDE \cdot G_{\alpha} \cdot GTP] + [PDE^* \cdot G_{\alpha} \cdot GTP] + [cG \cdot PDE^* \cdot G_{\alpha} \cdot GTP] + [RG9 \cdot PDE^* \cdot G_{\alpha} \cdot GTP])$$

$$[RG9_{free}] = [RG9_{total}] - [RG9 \cdot PDE^* \cdot G_{\alpha} \cdot GTP] \quad (\text{Eq. S76})$$

$$[GC_{free}] = [GC_{total}] - [GC \cdot GTP] \quad (\text{Eq. S77})$$

$$[GTP_{free}] = [GTP_{total}] \quad (\text{Eq. S78})$$

$$- ([MII_0^i \cdot G \cdot GTP] + [G \cdot GTP] + [G_{\alpha} \cdot GTP] + [PDE \cdot G_{\alpha} \cdot GTP] + [PDE^* \cdot G_{\alpha} \cdot GTP] + [cG \cdot PDE^* \cdot G_{\alpha} \cdot GTP] + [RG9 \cdot PDE^* \cdot G_{\alpha} \cdot GTP] + [GC \cdot GTP])$$

$$\frac{d}{dt}[ATP] = k_{RK4}[MII_0^i \cdot RK_{pre} \cdot ATP] + k_{RK4}^i[MII_0^i \cdot RK_{pre} \cdot ATP] - k_{RK3}[ATP][MII_0^i \cdot RK_{pre}] - k_{RK3}^i[ATP][MII_0^i \cdot RK_{pre}] \quad (\text{Eq. S79})$$

$$\frac{d}{dt}[cGMP] = \frac{k_{GC3}}{1 + \left(\frac{[Ca^{2+}]}{K_c}\right)^m}[GC \cdot GTP] - (\beta_{dark}[cGMP] + \beta_{sub}[cG \cdot PDE^* \cdot G_{\alpha} \cdot GTP]) \quad (\text{Eq. S80})$$

$$\frac{d}{dt}[Ca^{2+}] = -b \frac{I_{photo}}{2F \cdot v_{ol}} - \gamma_{Ca}([Ca^{2+}] - c_0) - k_{b1}[Ca^{2+}](e_T - [Cab]) + k_{b2}[Cab] \quad (\text{Eq. S81})$$

$$\frac{d}{dt}[Cab] = k_{b1}[Ca^{2+}](e_T - [Cab]) - k_{b2}[Cab] \quad (\text{Eq. S82})$$

$$I_{photo} = G_{max} \frac{[cGMP]^{n_h}}{[cGMP]^{n_h} + K_m^{n_h}} (V_m - E_p) \quad (\text{Eq. S83})$$

Problems: Most parameters are unknown, only simulations, derivation of conceptual insight from a sensitivity analysis of such complex models is difficult !

Minimal model for rod and cone with only the most relevant features: Less is more

Phototransduction equations assuming a spatially uniform outer segment :

$$\frac{d}{dt}R^* = \phi(t)\kappa - \mu_{rh}R^* \quad \text{Photopigment activation and deactivation}$$

$$\frac{d}{dt}T^* = k_{act}R^* - \mu_{tr}T^* \quad \text{Transducin activation and deactivation}$$

$$\frac{d}{dt}P^* = k_{tr}T^* - \mu_{pde}P^* \quad \text{PDE activation and deactivation}$$

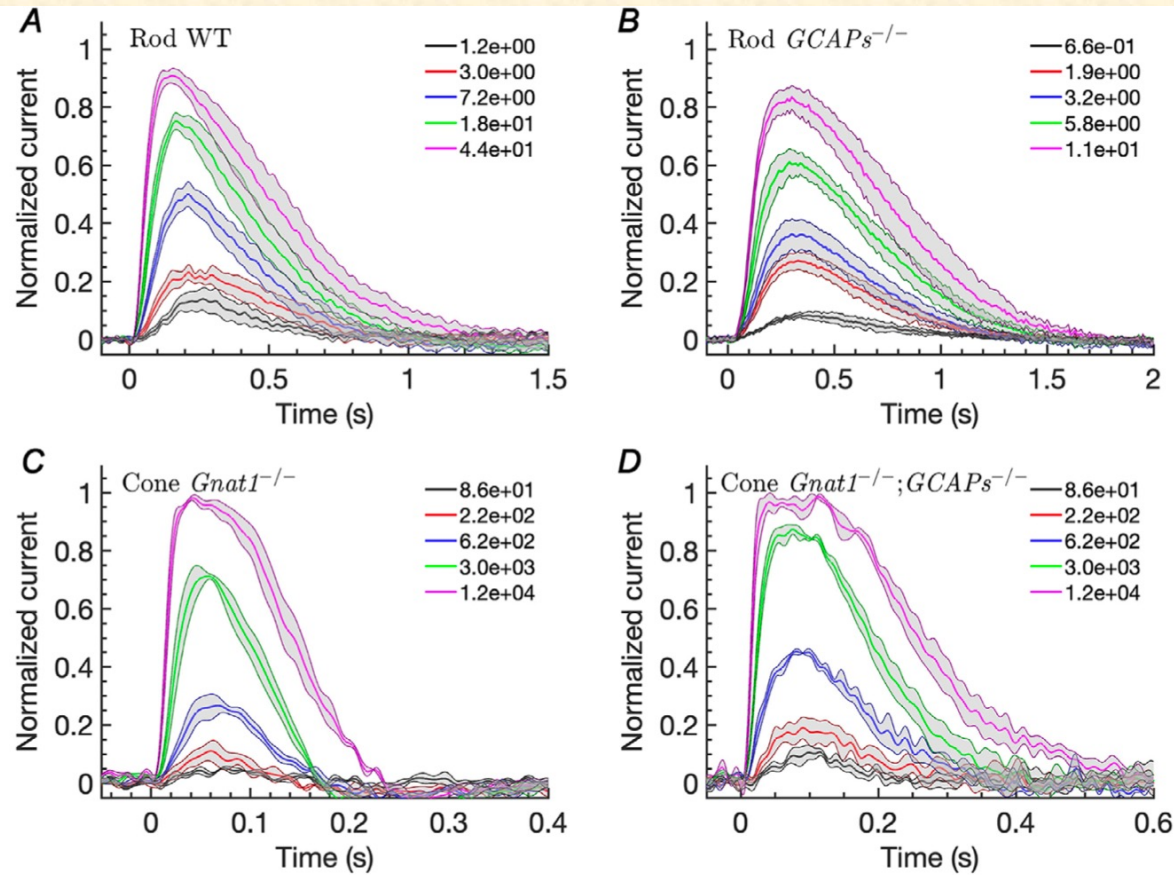
$$\frac{d}{dt}c_{cg} = \alpha_{max} \frac{r_{\alpha}c_{ca}^{n_{\alpha}} + K_{\alpha}^{n_{\alpha}}}{c_{ca}^{n_{\alpha}} + K_{\alpha}^{n_{\alpha}}} - (\beta_d + \beta_{sub}P^*)c_{cg} \quad \text{cGMP concentration with synthesis and hydrolysis}$$

$$\text{Current: } I = I_d \left(\frac{c_{cg}}{c_{cg,d}} \right)^{n_{ch}} \quad \text{Calcium dynamics is proportional to the current: } \frac{c_{ca}}{c_{ca,d}} = \frac{I}{I_d}$$

$$\text{Normalized quantities: } \hat{c}_{cg} = \frac{c_{cg}}{c_{cg,d}} \quad \hat{c}_{ca} = \frac{c_{ca}}{c_{ca,d}}, \quad \hat{I} = \frac{I}{I_d}$$

$$\text{Current Change (zero in darkness)} \quad \hat{i} = \frac{I_d - I}{I_d} = 1 - \hat{I}$$

Experimental data for mouse rods and cones to calibrate the model



Elimination of non-identifiable parameters

$$\begin{aligned}
 \frac{d}{dt}R^* &= \phi(t)\kappa - \mu_{rh}R^* \\
 \frac{d}{dt}T^* &= k_{act}R^* - \mu_{tr}T^* \\
 \frac{d}{dt}P^* &= k_{tr}T^* - \mu_{pde}P^* \\
 \frac{d}{dt}\hat{c}_{cg} &= \beta_d \frac{1 + \hat{K}_\alpha^{n_\alpha}}{r_\alpha + \hat{K}_\alpha^{n_\alpha}} \frac{r_\alpha \hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}}{\hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}} - (\beta_d + \beta_{sub}P^*)\hat{c}_{cg}
 \end{aligned}$$

Initial conditions in darkness:

$$R^* = T^* = P^* = 0$$

$$\hat{c}_{cg} = \hat{I} = 1$$

Current:

$$\hat{i} = 1 - \hat{I} = 1 - \hat{c}_{cg}^{n_{ch}}$$

Variable
Transformation



$$\tilde{P}^* = \beta_{sub}P^* \quad \tilde{T}^* = \frac{\beta_{sub}k_{tr}}{\mu_{pde}}T^* \quad \tilde{R}^* = \frac{\beta_{sub}k_{act}k_{tr}}{\mu_{pde}\mu_{tr}}R^*$$

$$\begin{aligned}
 \frac{d}{dt}\tilde{R}^* &= \mu_{rh}(\phi(t)\kappa\xi - \tilde{R}^*) \\
 \frac{d}{dt}\tilde{T}^* &= \mu_{tr}(\tilde{R}^* - \tilde{T}^*) \\
 \frac{d}{dt}\tilde{P}^* &= \mu_{pde}(\tilde{T}^* - \tilde{P}^*) \\
 \frac{d}{dt}\hat{c}_{cg} &= \beta_d \frac{1 + \hat{K}_\alpha^{n_\alpha}}{r_\alpha + \hat{K}_\alpha^{n_\alpha}} \frac{r_\alpha \hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}}{\hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}} - (\beta_d + \tilde{P}^*)\hat{c}_{cg}
 \end{aligned}$$

Transduction gain:

$$\xi = \frac{k_{act}\beta_{sub}k_{tr}}{\mu_{rh}\mu_{pde}\mu_{tr}}$$

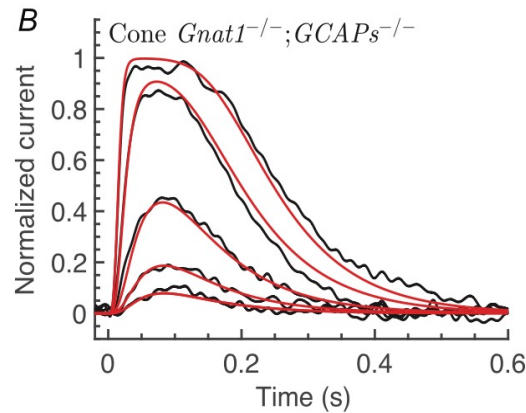
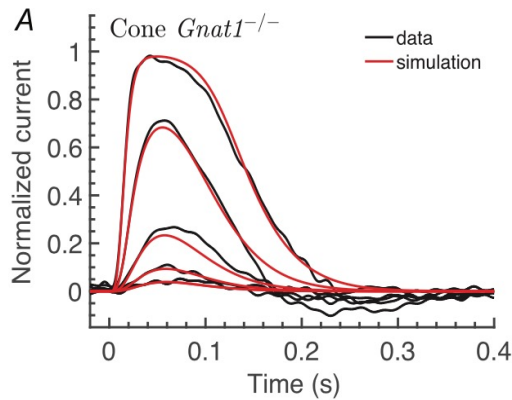
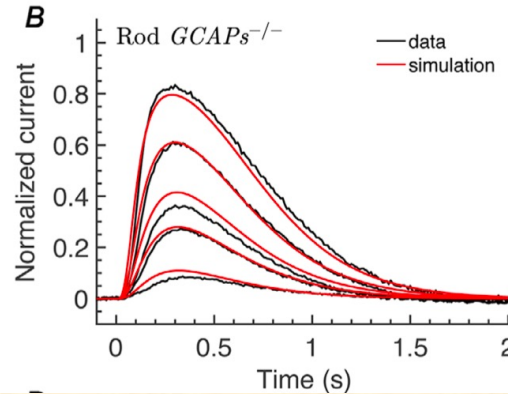
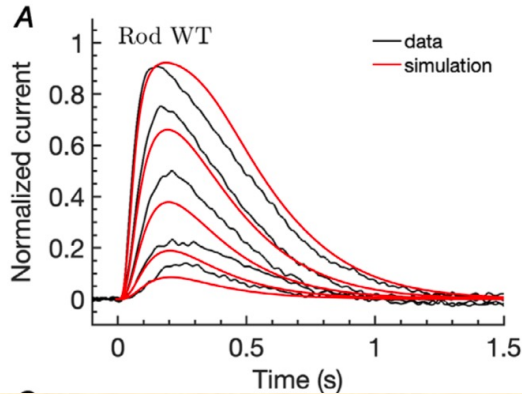


Analysis for the difference between rods and cones is reduced to 5 parameters:

$$\xi, \mu_{rh}, \mu_{tr}, \mu_{pde}, \beta_d$$

Most significant differences between rods and cone

Extracting parameters from data

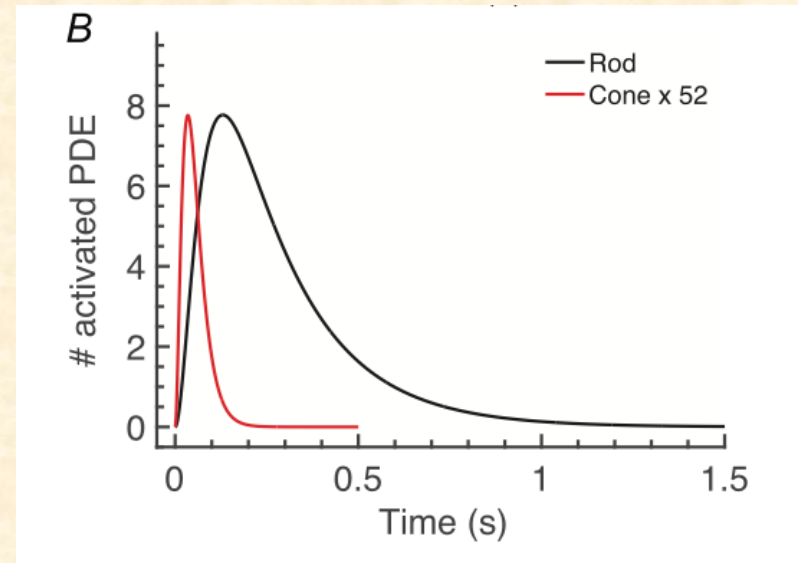
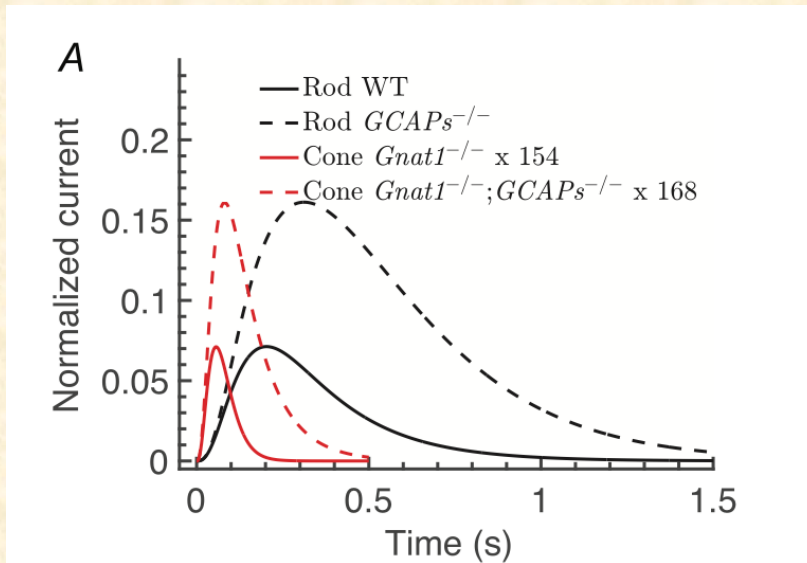


	Rod	Cone
$\mu_{pde} (s^{-1})$	5	37.8
$\beta_d (s^{-1})$	4.1	12
ξ	0.18	0.0007
$\kappa (\mu m^2)$	0.28	0.013

Dynamics

Sensitivity

Single photon response for rod and cone



No amplification in cones !

Experimental evidences for the differences between rods and cones

- PDE deactivation rate ~ 7 fold increased in cones
~10x higher GAP concentration in cones (Cowan *et al.* 1998; Zhang *et al.* 2003)
- Dark cGMP turnover rate ~ 3 fold faster in cones
Factor of 3 in salamander (Cornwall & Fain 1994, Cornwall *et al.* 1995)
increased spontaneous activity of cone PDE (Majumder *et al.* 2015)
- Rate of transducin activation ~ 3 fold reduced in cones
 - Amplification constant is smaller in cones (Nikonov *et al.*, 2006; Cao *et al.*, 2014, Ingram *et al.* 2016)
 - 3 fold reduced sensitivity for rods with cone transducin (Chen *et al.*, 2010)
- PDE activity ~ 2 fold reduced in cones
Decreased sensitivity in mouse rods with exchange of cone for rod PDE6 (Majumder *et al.* 2015)

Analytic solutions for SPR and dim light responses

$$\begin{aligned}
 \frac{d}{dt} \tilde{R}^* &= \mu_{rh}(\phi(t)\kappa\xi - \tilde{R}^*) \\
 \frac{d}{dt} \tilde{T}^* &= \mu_{tr}(\tilde{R}^* - \tilde{T}^*) \\
 \frac{d}{dt} \tilde{P}^* &= \mu_{pde}(\tilde{T}^* - \tilde{P}^*) \\
 \frac{d}{dt} \hat{c}_{cg} &= \beta_d \frac{1 + \hat{K}_\alpha^{n_\alpha}}{r_\alpha + \hat{K}_\alpha^{n_\alpha}} \frac{r_\alpha \hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}}{\hat{c}_{cg}^{n_{ch}} + \hat{K}_\alpha^{n_\alpha}} - (\beta_d + \tilde{P}^*) \hat{c}_{cg}
 \end{aligned}$$

Linear response
analysis



$$\hat{c}_{cg} = e^{-y} \quad y \ll 1$$

$$\begin{aligned}
 \frac{d}{dt} \tilde{R}^* &= \mu_{rh} \left(\phi(t)\kappa\xi - \tilde{R}^* \right) \\
 \frac{d}{dt} \tilde{T}^* &= \mu_{tr} \left(\tilde{R}^* - \tilde{T}^* \right) \\
 \frac{d}{dt} \tilde{P}^* &= \mu_{pde} \left(\tilde{T}^* - \tilde{P}^* \right) \\
 \frac{d}{dt} y &= \tilde{P}^* - \beta_d (1 + n_{ch} \alpha') y
 \end{aligned}$$



Strength of calcium feedback to cGMP synthesis

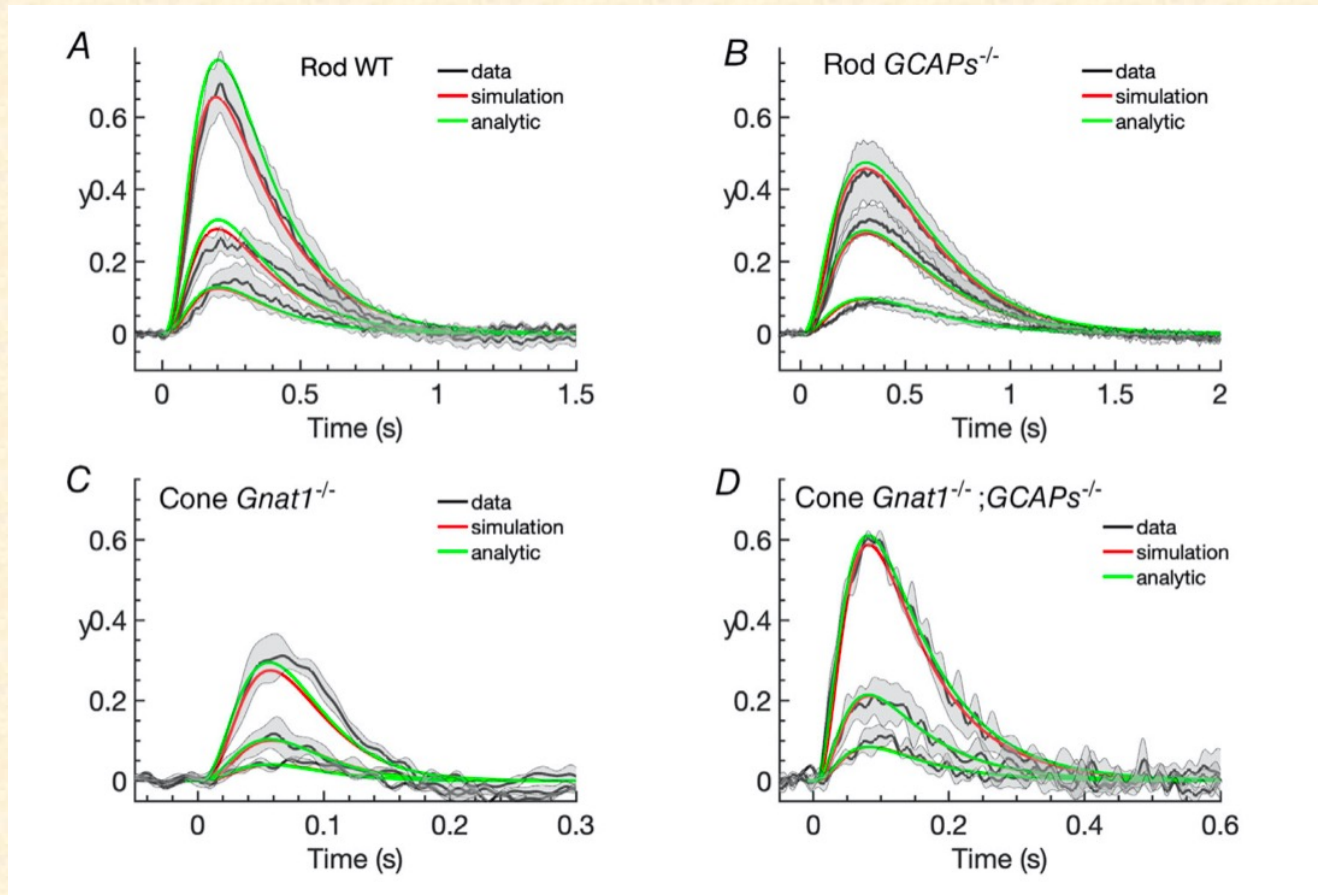
Analytic solution:

$$y(t) = \kappa\xi \int_0^t \phi(s) g_y(t-s) ds$$

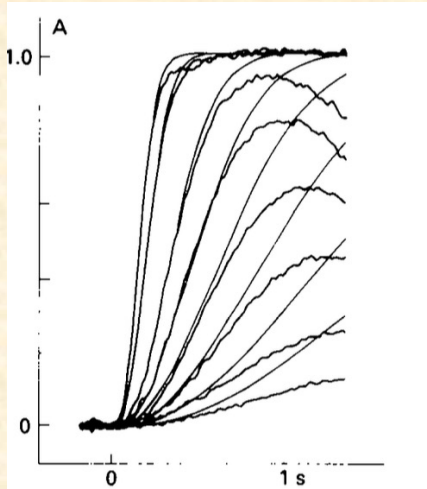
$$\hat{i}(t) = 1 - \hat{c}_{cg}^{n_{ch}} \approx n_{ch} y(t)$$

$$g_y(t) = \frac{\mu_{rh}\mu_{pde}\mu_{tr} \left(-\frac{e^{-\mu_{rh}t}}{(\mu_{rh}-\mu_{tr})(\mu_{rh}-\mu_{pde})(\mu_{rh}-\tilde{\beta}_d)} - \frac{e^{-\mu_{tr}t}}{(\mu_{tr}-\mu_{rh})(\mu_{tr}-\mu_{pde})(\mu_{tr}-\tilde{\beta}_d)} \right)}{-\frac{e^{-\mu_{pde}t}}{(\mu_{pde}-\mu_{rh})(\mu_{pde}-\mu_{tr})(\mu_{pde}-\tilde{\beta}_d)} - \frac{e^{-\tilde{\beta}_d t}}{(\tilde{\beta}_d-\mu_{rh})(\tilde{\beta}_d-\mu_{tr})(\tilde{\beta}_d-\mu_{pde})}}.$$

Analytic solution vs simulation



Application: Revisiting formula for the amplification constant characterizing the rising phase of flash response

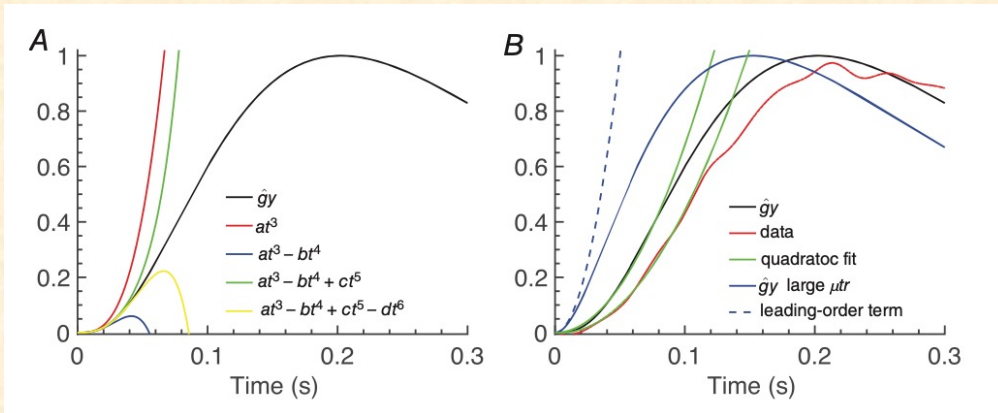


$$\hat{i}(t) \approx \frac{1}{2} R_0^* n_{ch} k_{act} \beta_{sub} t^2$$

$$A = n_{ch} k_{act} \beta_{sub}$$

Claim: Initial rising phase is quadratic and is determined by activation rates (Pugh & Lamb (1992,1993)):

Use analytic result to analyze the rising phase:



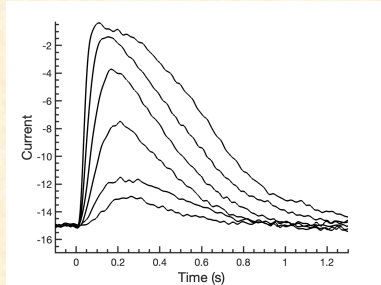
$$A = n_{ch} k_{act} \beta_{sub} \delta$$

Depends only on
deactivation rates

Mouse rod: $\delta \sim 0.11$

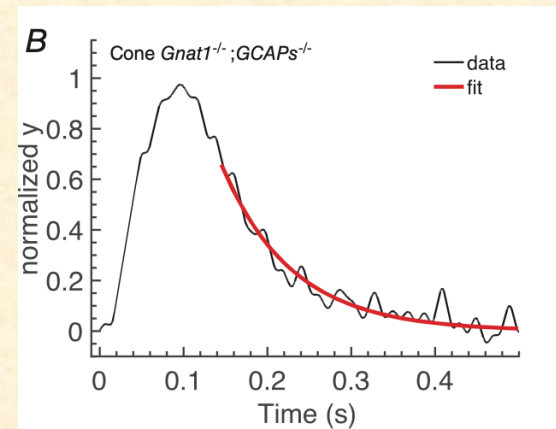
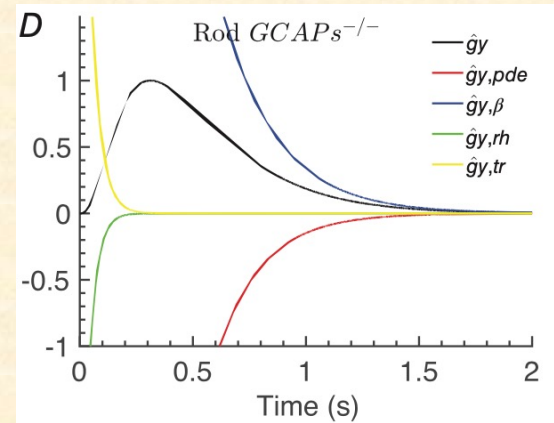
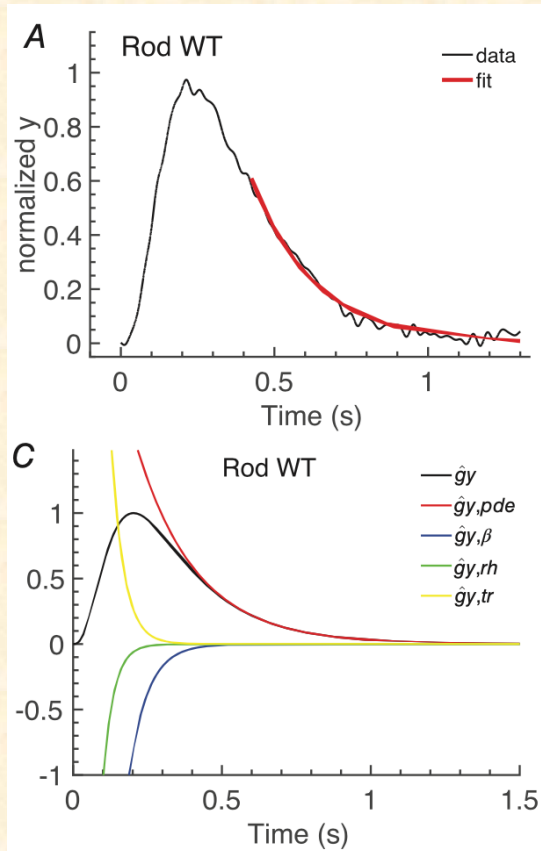
$$\hat{i}(t) = R_0^* \xi n_{ch} g_y(t)$$

Application: What limits the recovery of flash responses ?



$$\hat{i} = R_0^* \xi n_{ch} g_y = \hat{i}_{rh} + \hat{i}_{tr} + \hat{i}_{pde} + \hat{i}_{\beta}$$

Decomposition
of the response

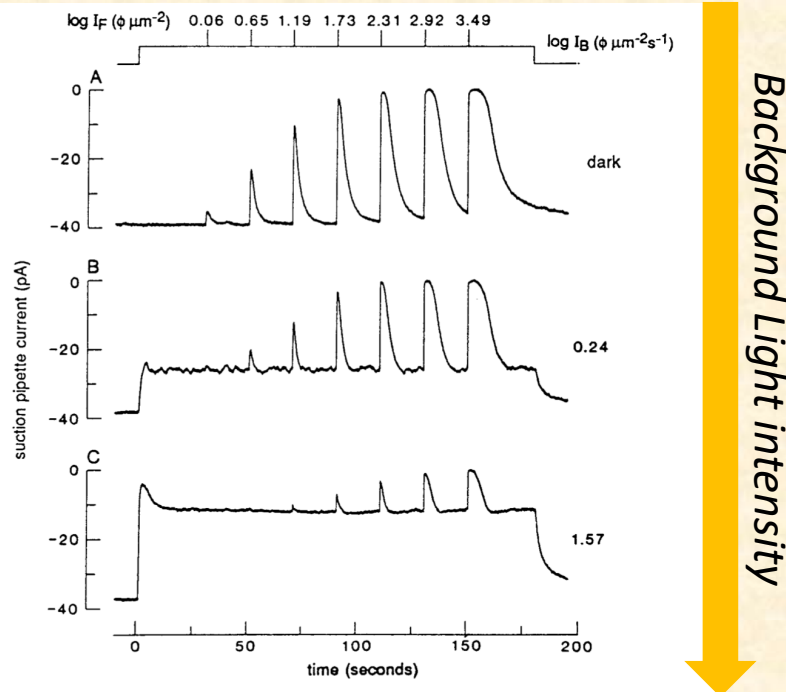


Challenges for future work

1. Adaptation to an increasing background light
2. Voltage response in the inner segment
3. Dynamics of glutamate release at the synaptic terminal

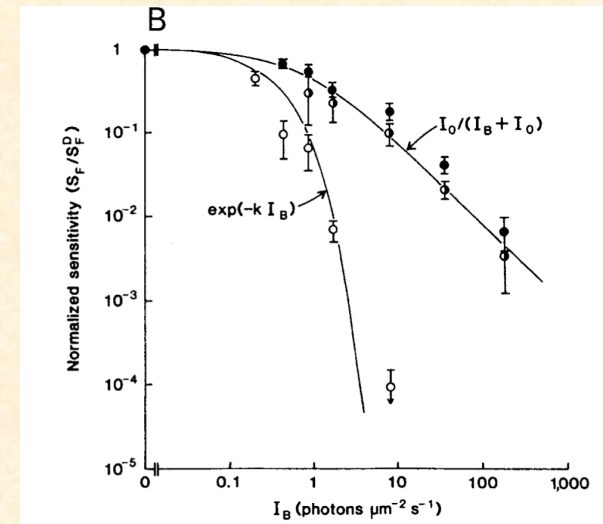
Photoreceptor functioning in background light and adaptation

Data from salamander rod



Matthews (1990)

Sensitivity decreases with background intensity (Adaptation)



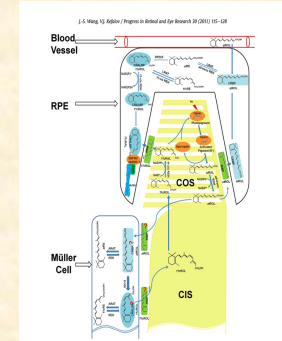
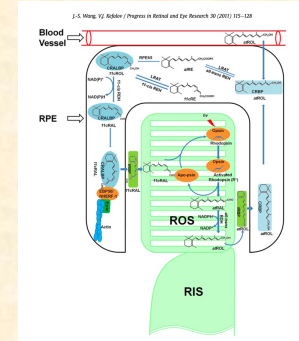
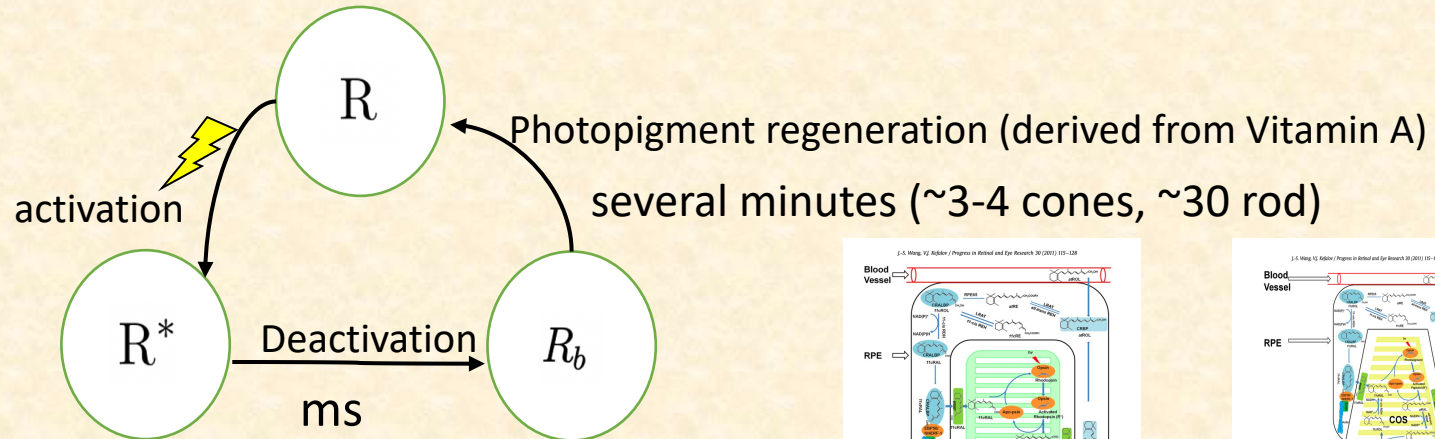
Weber-Fechner relation

$$\frac{S}{S_d} = \frac{I_0}{I_0 + I_B} \sim \frac{I_0}{I_B}$$

Most important features for adaptation:

1. Ca^{2+} dynamics and feedback processes that occur on long time scale
2. Visual pigment regeneration and bleaching

Implications of slow visual pigment regeneration

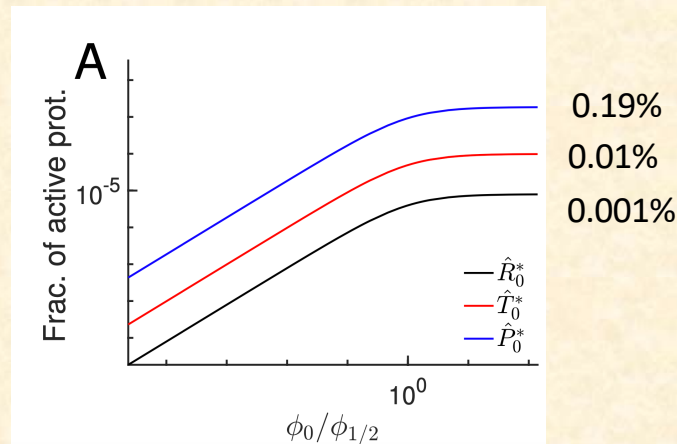


Fractions of activated proteins at steady state

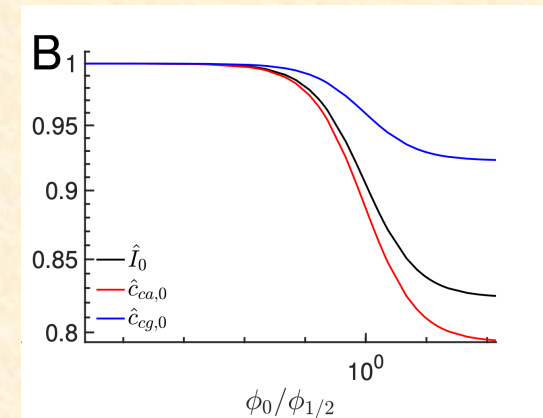
$$\hat{R}_0 = \frac{\phi_{1/2}}{\phi_{1/2} + \phi_0}$$

$$\hat{R}_0^* = \frac{\tau_{rh}}{\tau_{cyc}} \frac{\phi_0}{\phi_0 + \phi_{1/2}}$$

$$\hat{P}_0^* = \hat{R}_0^* \frac{R_{tot} k_{act}}{P_{tot} \mu_{pde}}$$



Current and steady state concentrations



Extended model with protein conservation

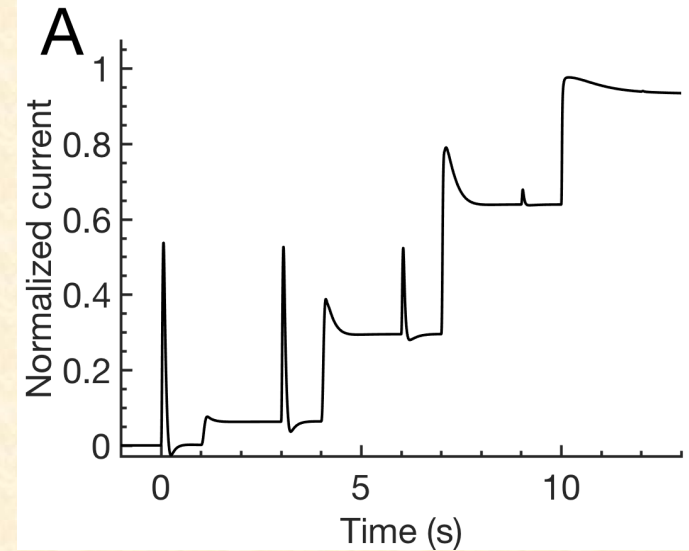
Conservation equations

$$\begin{aligned} R_{tot} &= R + R^* + R_d = R_{tot}(\hat{R} + \hat{R}^* + \hat{R}_b) \\ T_{tot} &= T + T^* + P^* = T_{tot}(\hat{T} + \hat{T}^* + r_{p,t}\hat{P}^*) \\ P_{tot} &= P + P^* = P_{tot}(\hat{P} + \hat{P}^*) . \end{aligned}$$

Transduction equations

$$\begin{aligned} \frac{d}{dt}R^* &= \phi(t)\kappa\hat{R} - \mu_{rh}R^* \\ \frac{d}{dt}R_b &= \mu_{rh}R^* - \mu_{rh,b}R_b \\ \frac{d}{dt}T^* &= k_{act}R^*\hat{T} - \mu_{tr}T^*\hat{P} \\ \frac{d}{dt}P^* &= \mu_{tr}T^*\hat{P} - \mu_{pde}P^* \\ \frac{d}{dt}c_{cg} &= \alpha - (\beta_d + \beta_{sub}P^*)c_{cg} \\ \frac{d}{dt}c_{ca} &= \frac{1}{\mathcal{F}V_{os}(1 + B_{ca})} \left(\frac{f_{ch,ca}}{2} I_{ch,max}p_{ch} - I_{ex,max}p_{ex} \right) \end{aligned}$$

Simulations



Development of a molecular model for the inner segment

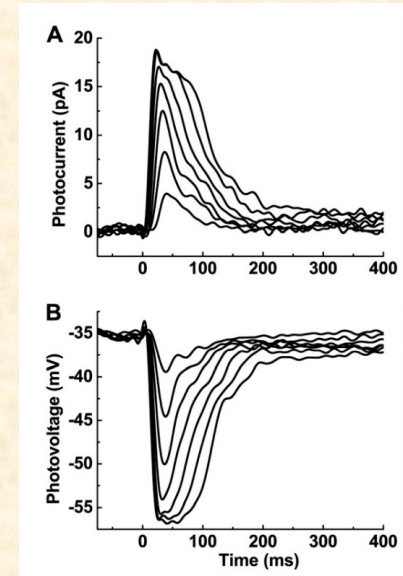
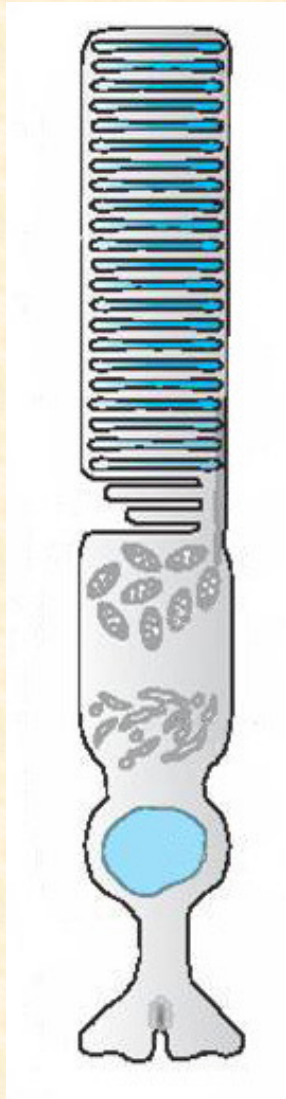
OS

- CNG channels
- NCKX Exchanger

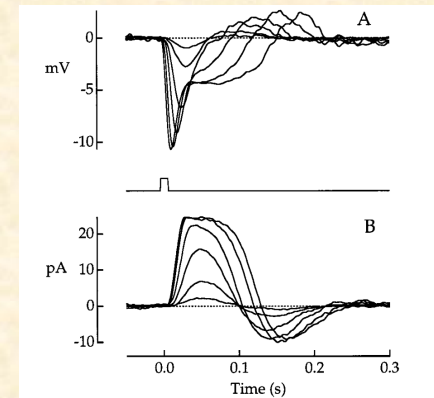
IS

- Voltage and Ca^{2+} gated K^+ channels
- Hyperpolarization-activated HCN channels
- Ca^{2+} activated Cl^- channels
- Voltage gated Ca^{2+} channels
- Ca^{2+} pump (PMCA)
- $\text{Na}^+ \text{K}^+ \text{Cl}^-$ pump

ST



Mouse cone

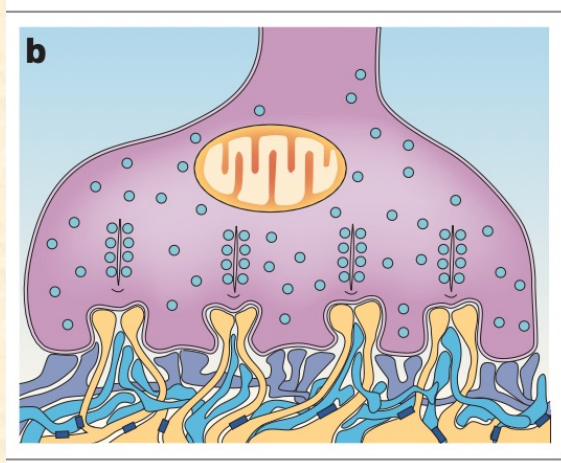


Macaque cone

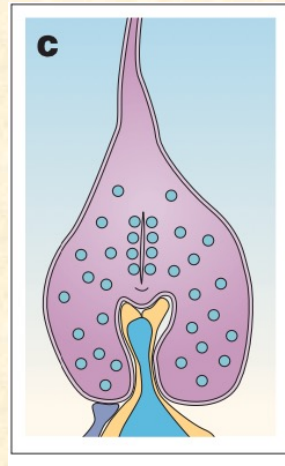
What are the contributions and physiological roles of the different channels in the inner segment ?

- Ingram et al (2019)
- Schneeweis&Schnapf (2000)

Development of a molecular model for vesicular release at rod and cone ribbon synapses



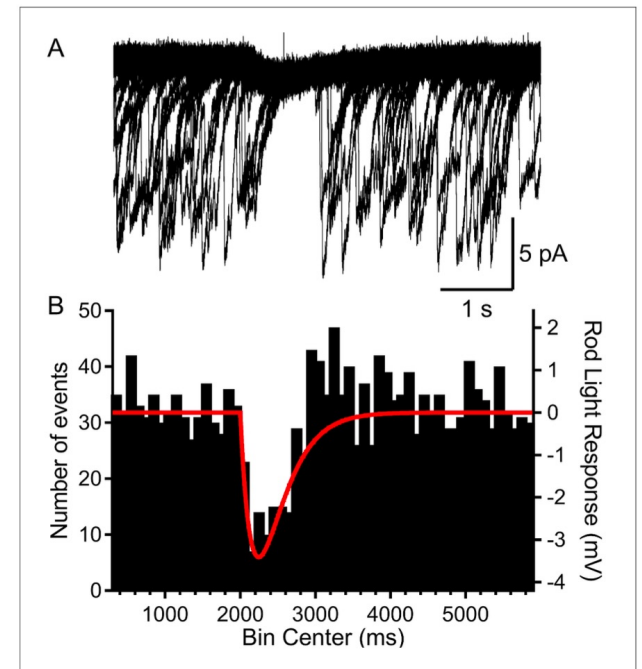
Cone Synapse



Rod Synapse

What is the voltage dependent dynamics of Ca^{2+} and vesicular release ?
How does the synapse signal a SPR with 1mV voltage change ?

Multivesicular release with Erlang statistics at -40 mV



- Hays et al (2021)
- Waessle (2004)

