Seeing single photons

What makes rod cells so reliable?

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Physics/Neuro 141

Week 4

Multiple Phosphorylation Sites Confer Reproducibility of the Rod's Single-Photon Responses

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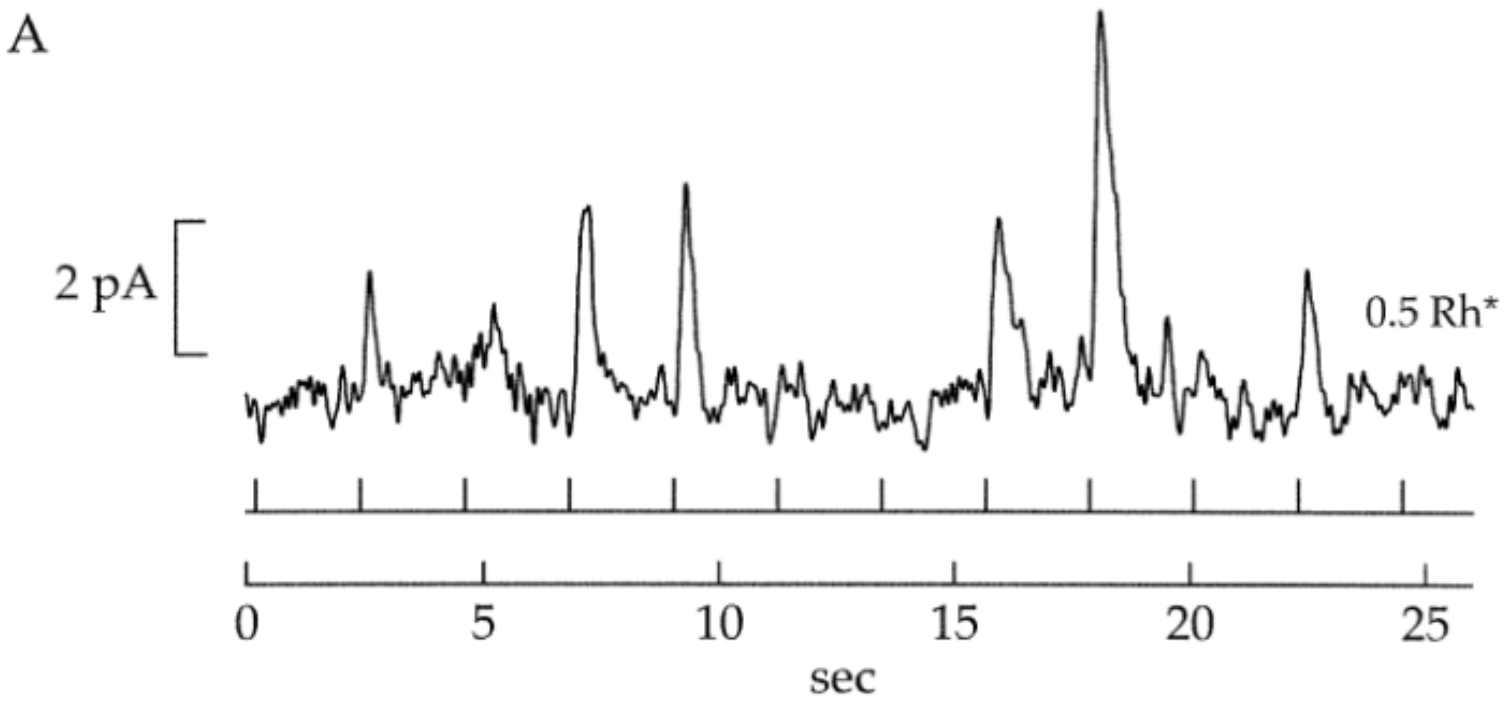
Although signals controlled by single molecules are expected to be inherently variable, rod photoreceptors generate reproducible responses to single absorbed photons. We show that this unexpected reproducibility—the consistency of amplitude and duration of rhodopsin activity—varies in a graded and systematic manner with the number but not the identity of phosphorylation sites on rhodopsin's C terminus. These results indicate that each phosphorylation site provides an independent step in rhodopsin deactivation and that collectively these steps tightly control rhodopsin's active lifetime. Other G protein cascades may exploit a similar mechanism to encode accurately the timing and number of receptor activation.

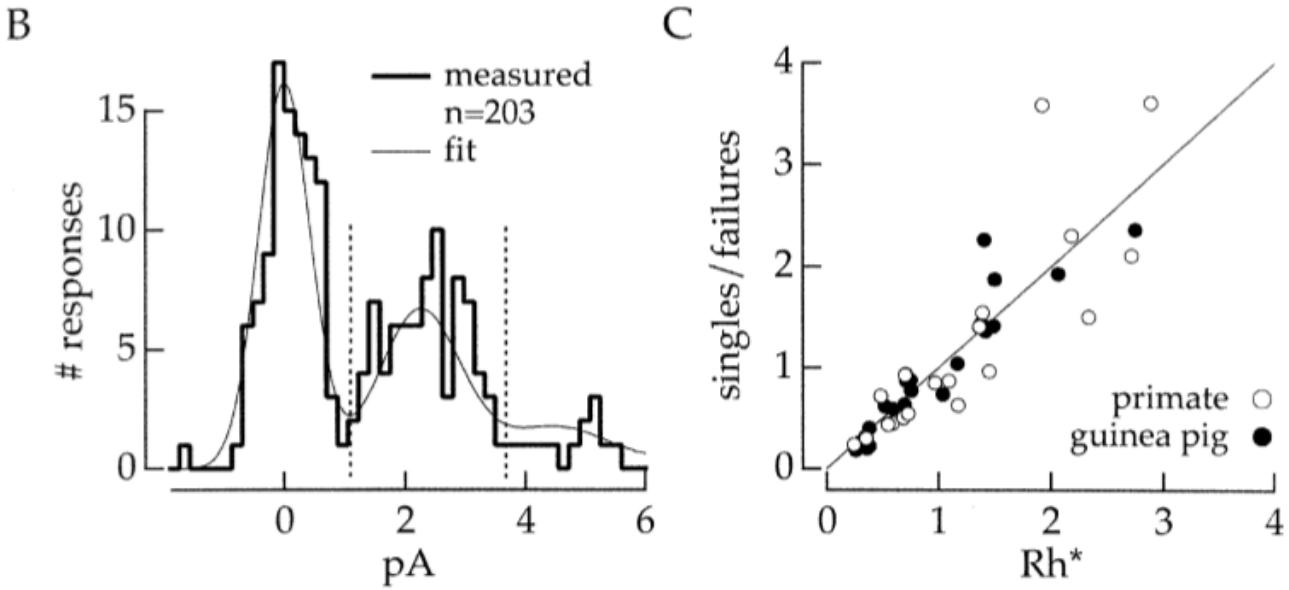
Isolation of single photon responses



Responses of a primate rod to a series of fixed-strength flashes are shown. Dark current was 25 pA

Field & Rieke, 2002

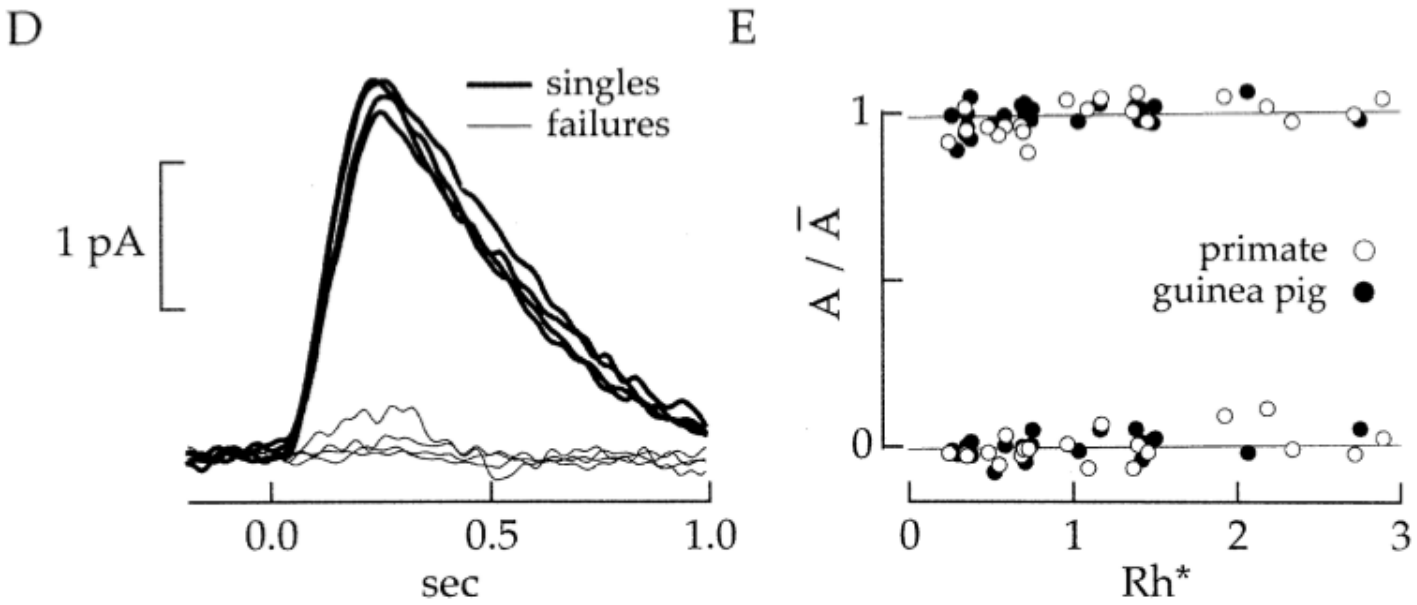
Is a single photon event a single photon event?



- Does the number of identified singles and failures agreed with expectations?
- In Poisson statistics, the number of singles divided by the number of failures estimates the mean number of successes.
- In Poisson statistics, if the mean number of successes is μ, then the probability of k successes is $P(k;\mu) = \frac{\mu^k e^{-\mu}}{k!}$ so:

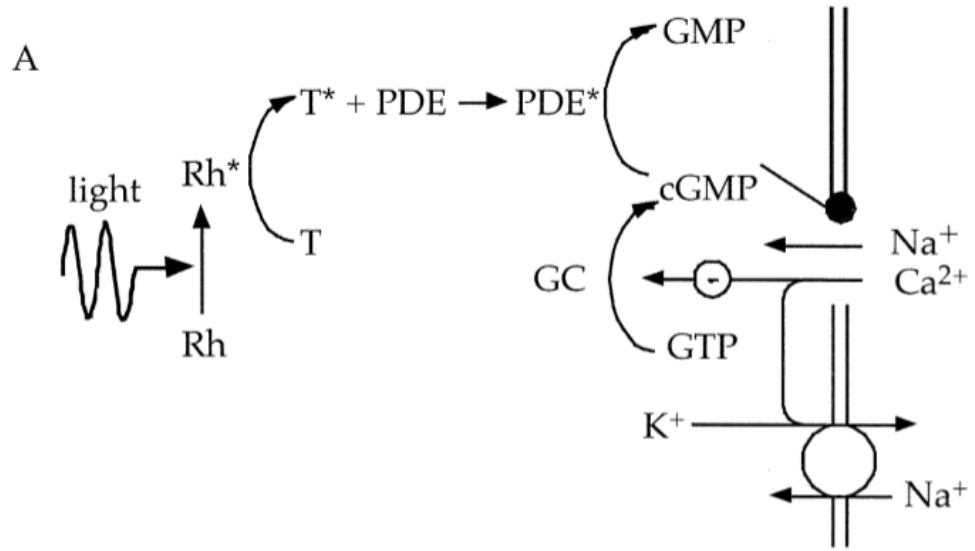
$$\frac{P(1)}{P(0)} = \mu$$

Is a single photon event a single photon event?



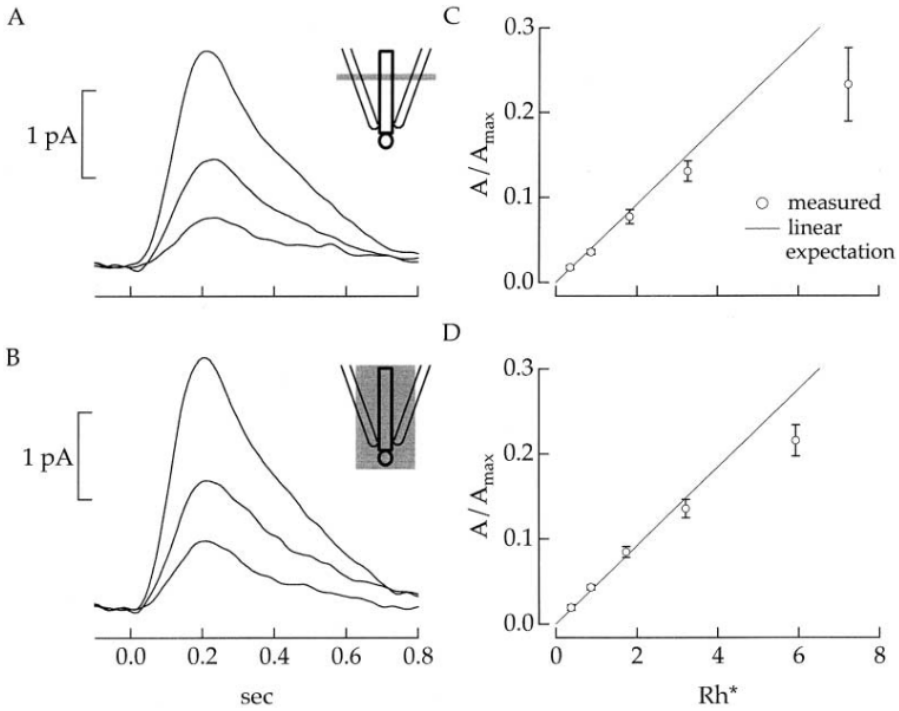
- Isolating singles and failures across flash strengths provides a test for contamination.
- Contamination would cause the average single or failure isolated from flashes of one strength to differ from the averages for another flash strength.
- If the identified singles contained many multiphoton responses, the number of these contaminating responses would increase at higher flash strengths.
- The average failures show little structure at all flash strengths, indicating that they are not substantially contaminated with single photon responses.

The phototransduction cascade



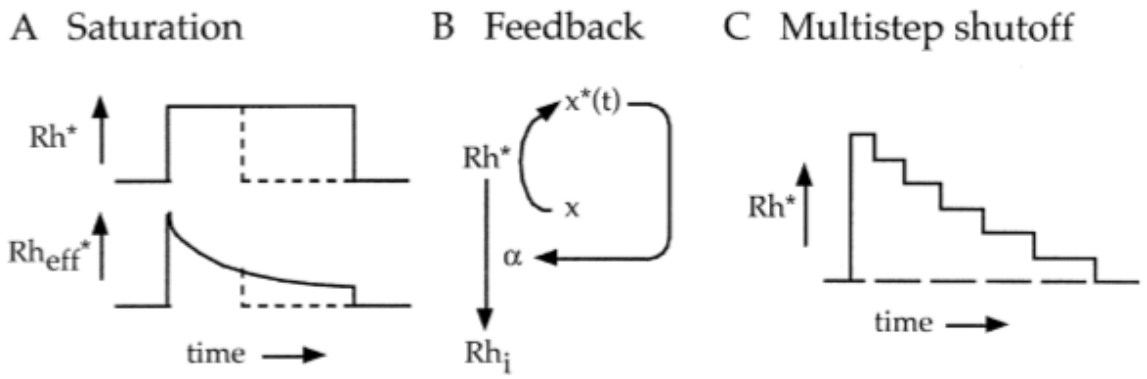
The first step in amplification is the number of activated transducin per Rh*, which increases with Rhodopsin lifetime

Does Rh* saturate the local biochemistry?



- If the single photon response significantly depleted cGMP or open cGMP-gated channels near the site of photon absorption, the response to two absorbed photons falling in the same region of the outer segment should be less than twice the single photon response.
- To test for such a saturation, we delivered dim flashes that either illuminated the entire outer segment or were restricted to a narrow transverse strip.

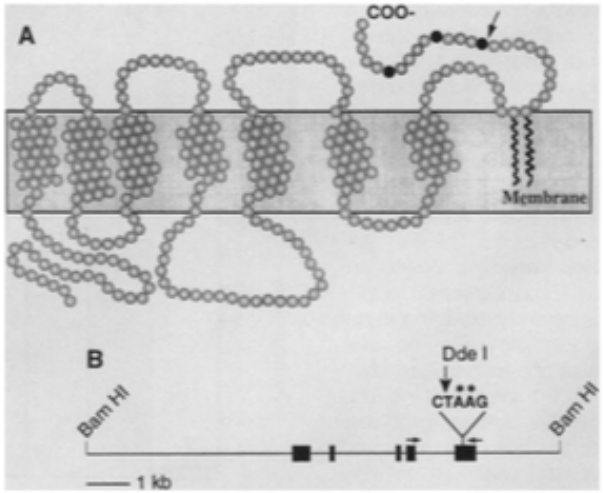
Different models for rod reliability



- Insensitivity of the activity on the disc to variations in rhodopsin's activity could occur through depletion of transducin or PDE
- Variability in rhodopsin's activity could be small due to feedback
- Variability in rhodopsin's activity could be small if shutoff occurs through a series of steps

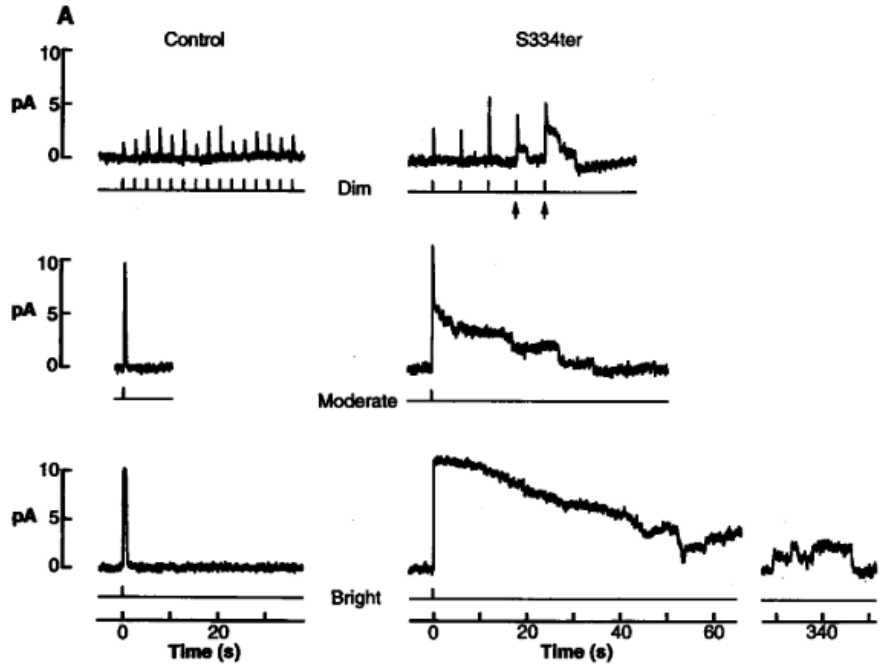
Chen et al., 1995
Doan et al., 2006

Mechanisms of Rhodopsin Inactivation Require the COOH terminus



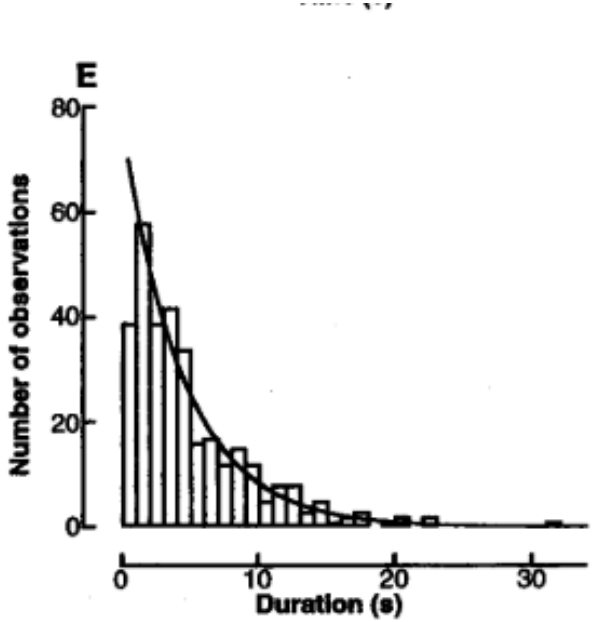
- In rod photoreceptors, photoexcitation of rhodopsin triggers activation of a G protein that leads to a decrease in the intracellular concentration of cGMP
- *In vitro* rhodopsin activity can be quenched by the phosphorylation of the COOH-terminus and the subsequent binding of arrestin
- We generated transgenic mice that produced a form of rhodopsin in which the COOH-terminal sites that are phosphorylated were deleted

Electrical recordings from single rods with normal and truncated rhodopsin



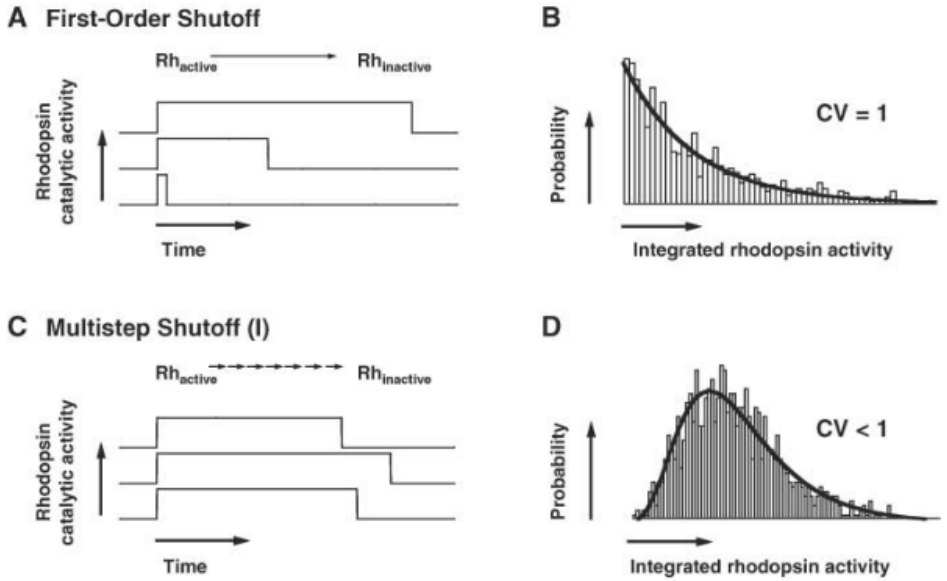
- Electrical recordings to a series of dim flashes gave some normal responses
- Stronger flashes always gave prolonged responses

Single-step inactivation



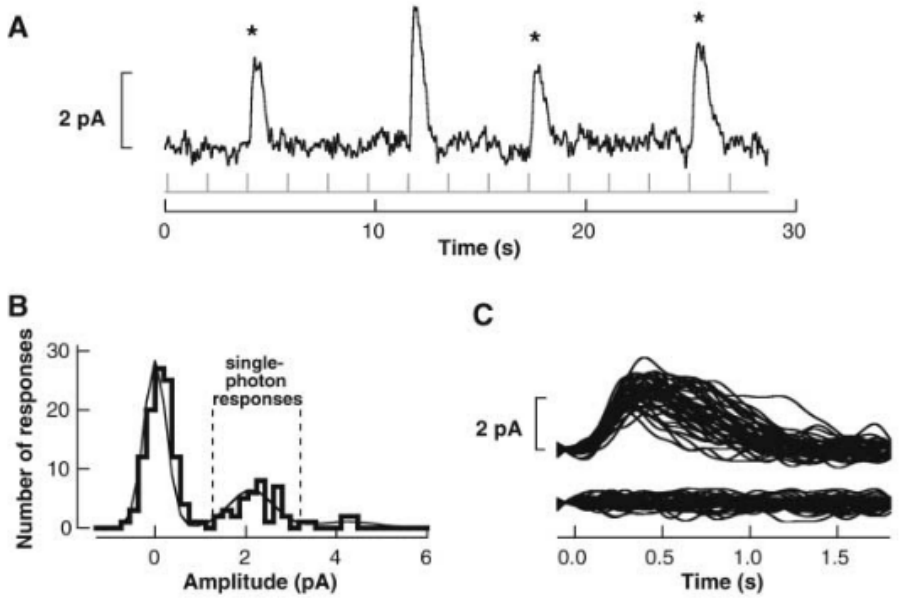
- Without C-terminus, inactivation still occurs, but with a single-exponential time course of the prolonged responses
- Poisson interval distribution: $P(t) = \lambda e^{-\lambda t}$

Multi-step and single-step models of rod inactivation



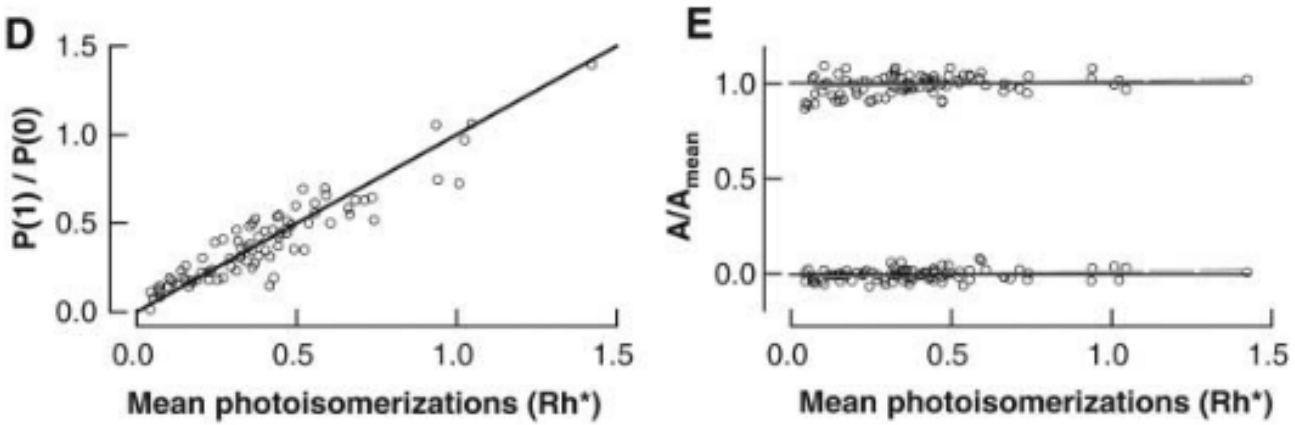
- The essence of the multi-step shutoff model is averaging
- The integrated rhodopsin activity averaged over multiple stochastic steps varies less than the activity controlled by a single step
- The coefficient of variation ($CV = \sigma/\mu$) diminishes with the number of independent steps (N): $CV = 1/\sqrt{N}$

Identifying single-photon responses



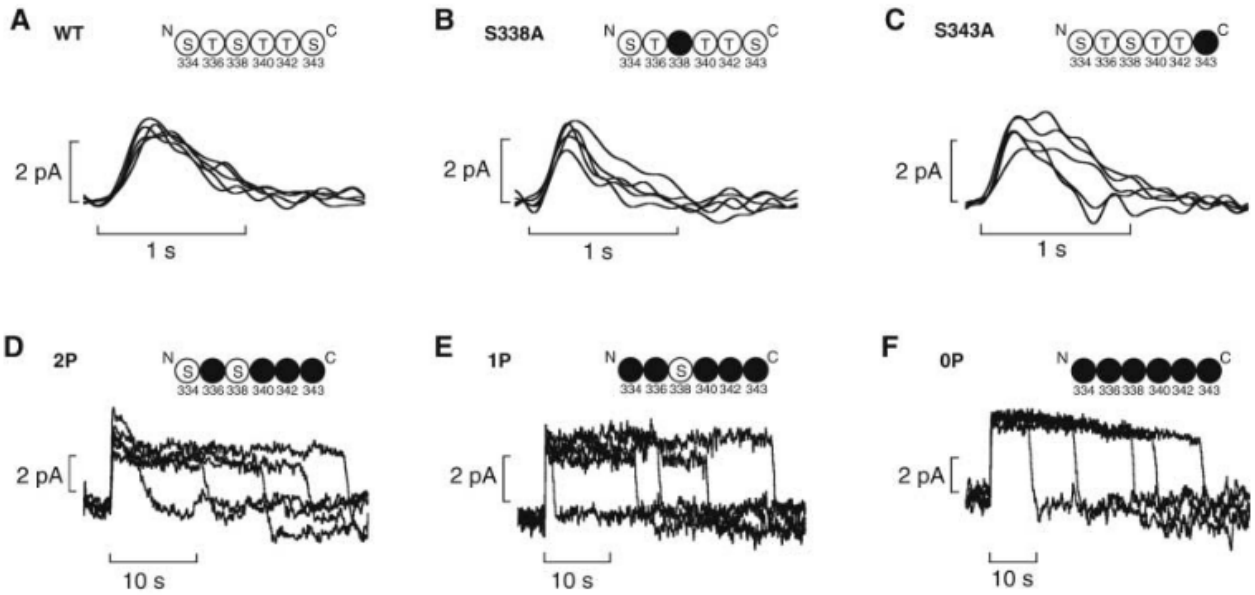
- Current responses to fixed strength flashes (vertical bars) that produced on average 0.4 isomerizations. Asterisks indicate identified single-photon responses.
- Histogram of response amplitudes from the same rod and flash strength. The fit is based on Poisson statistics. Vertical dashed lines represent thresholds used to identify single-photon responses
- Graph of 50 consecutive single-photon responses and responses to zero absorbed photons isolated from the same rod.

Are singles singles? Are zeros zeros?



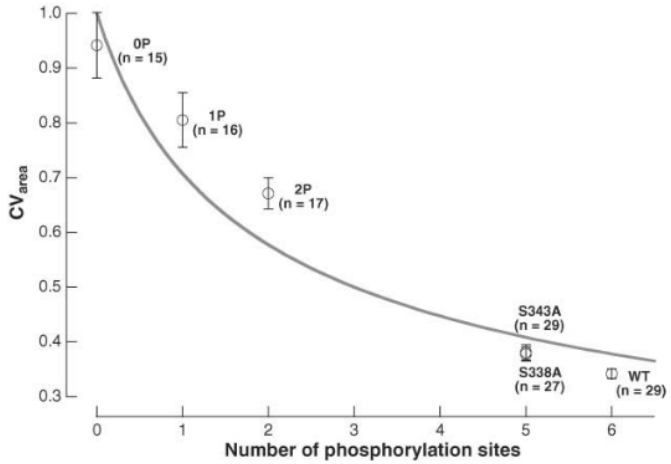
- Average Rh* estimated from the ratio of the number of identified single-photon responses *P*(1) and responses to zero absorbed photons *P*(0) plotted against the average Rh* estimated from the collecting area and flash strength. T
- The points fall near the line of unity slope, indicating that the number of identified single-photon responses and responses to zero photons were consistent with expectations from Poisson statistics.
- Amplitudes of single-photon responses and responses to zero absorbed photons plotted against the strength of the flash (in Rh*) from which responses were identified.
- Error-free identification predicts no dependence of the amplitude of the mean single-photon responses and responses to zero absorbed photons on flash strength

The effect of removing phosphorylation sites on the unitary response



- Examples of single-photon responses produced by wild-type and mutated rhodopsin with five, two, one, or zero phosphorylation sites.

The effect of removing phosphorylation sites on the unitary response



- Correlation of single-photon response variability with number of rhodopsin phosphorylation sites.
- Circles and vertical bars plot the mean *CV*. The smooth curve is the *CV* predicted by $1/\sqrt{N_p + 1}$ where N_p is the number of phosphorylation sites and 1 represents arrestin binding.