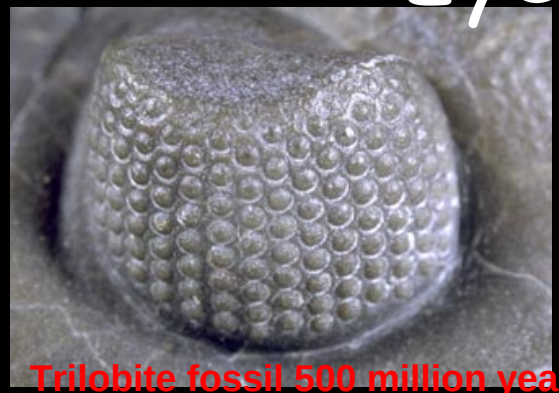
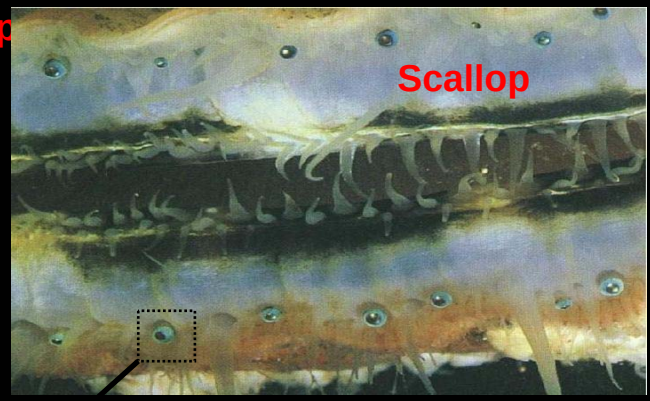
Eyes everywhere…



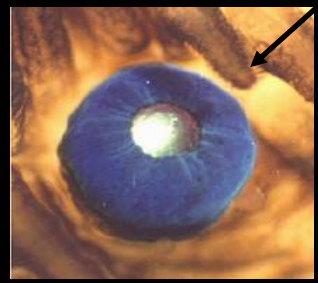
Trilobite fossil 500 million years

Copepod's 2 lens telescop



Scallop

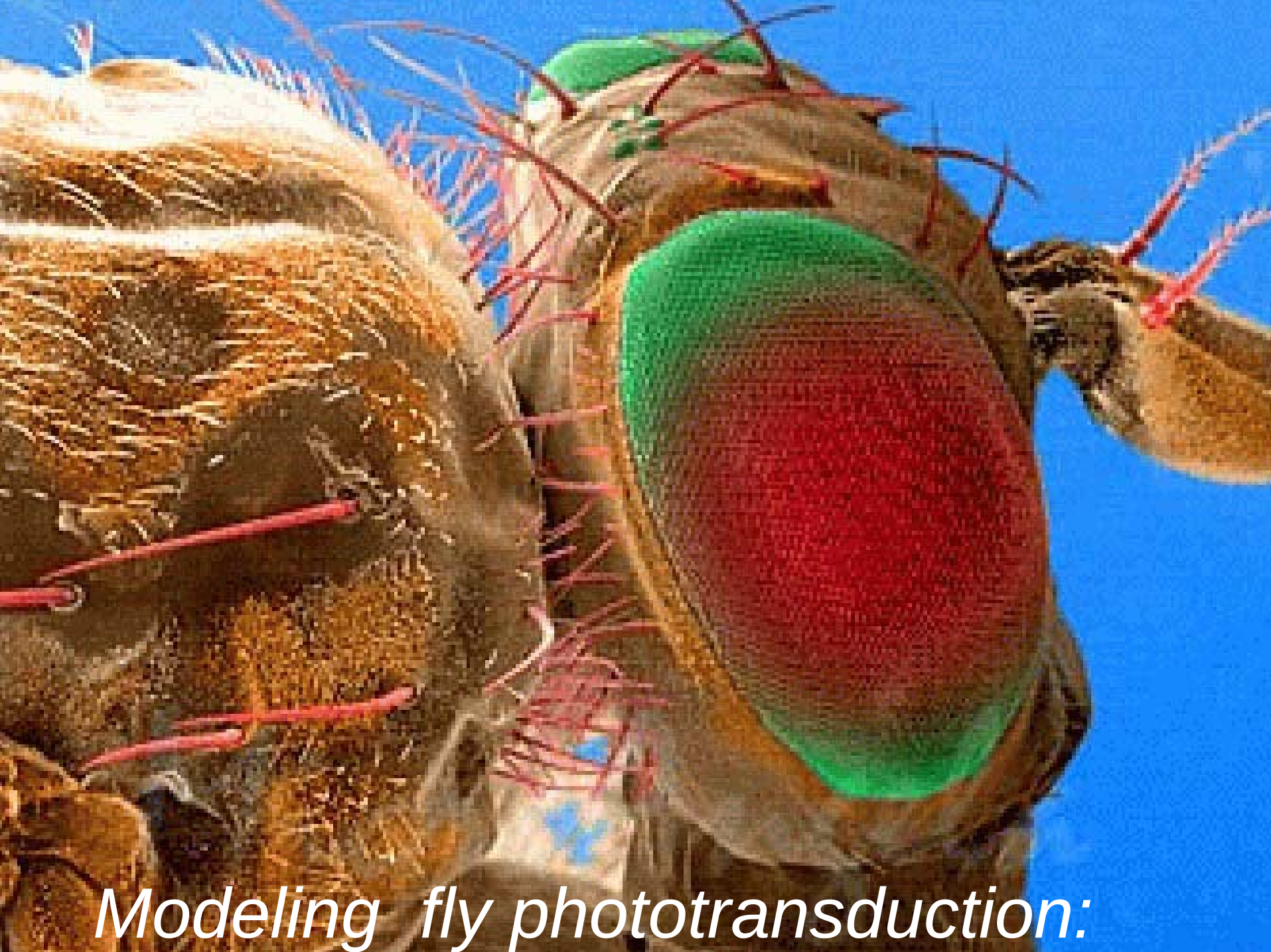
House Fly



Black ant

Octopus

Cuttlefish



Modeling fly phototransduction:

Limits of modeling?

Comparative systems biology?

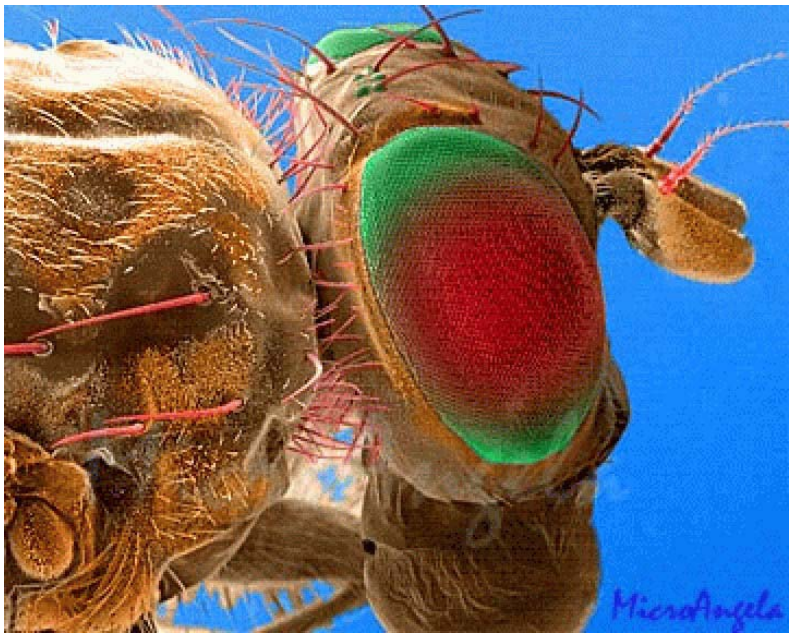
- vertebrate phototransduction (rods, cones)
- insect phototransduction
- olfaction, taste, etc…

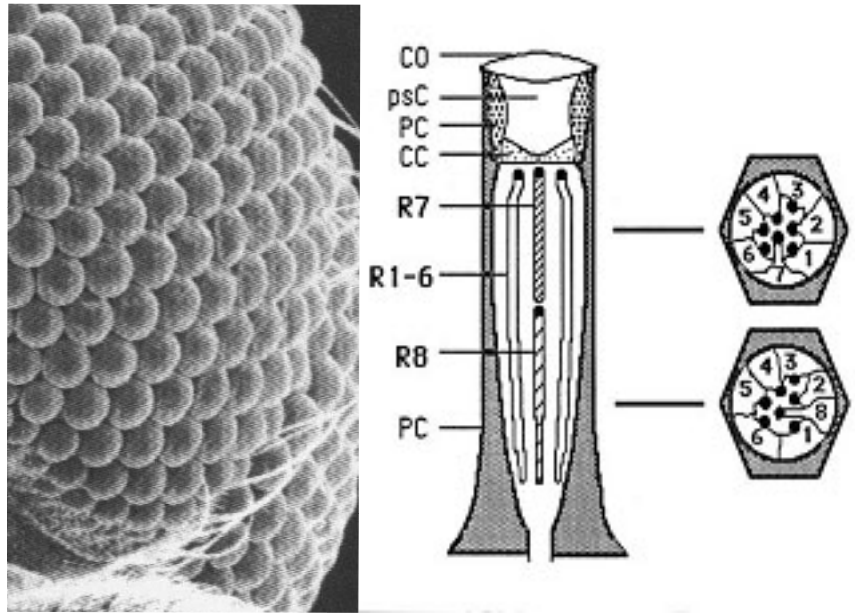
Fly photo-transduction

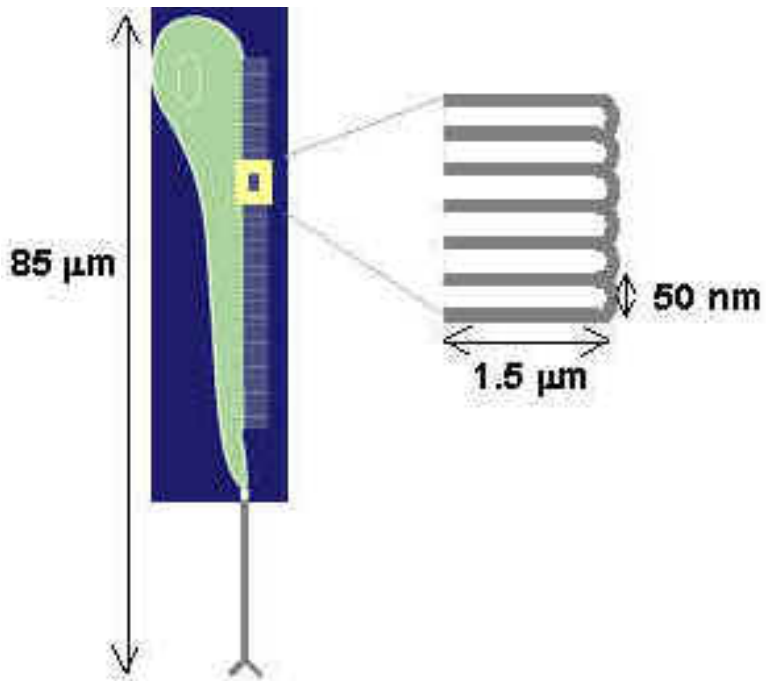
Outline:

- About the phenomenon
- Molecular mechanism
- Phenomenological Model
- Predictions and comparisons with experiment.

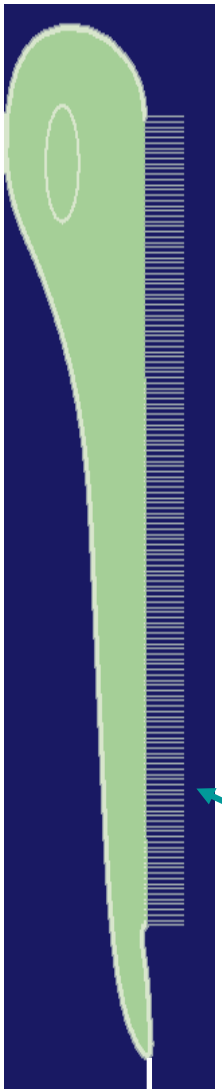
Compound eye of the fly

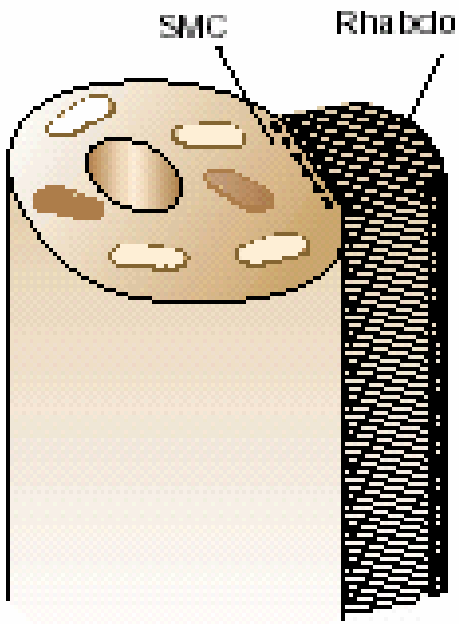


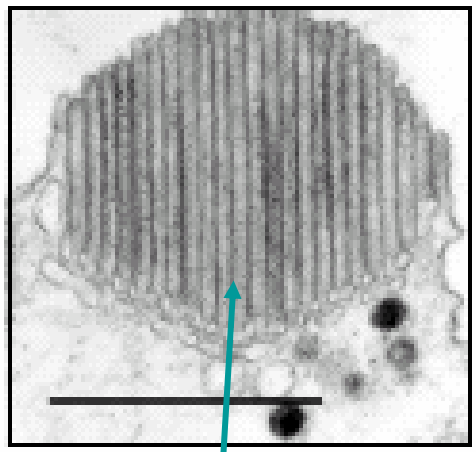




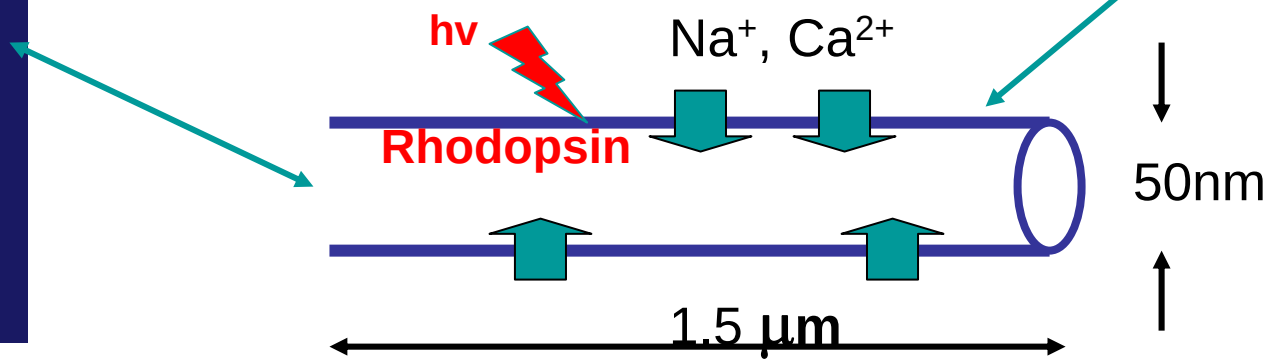
Fly photoreceptor cell



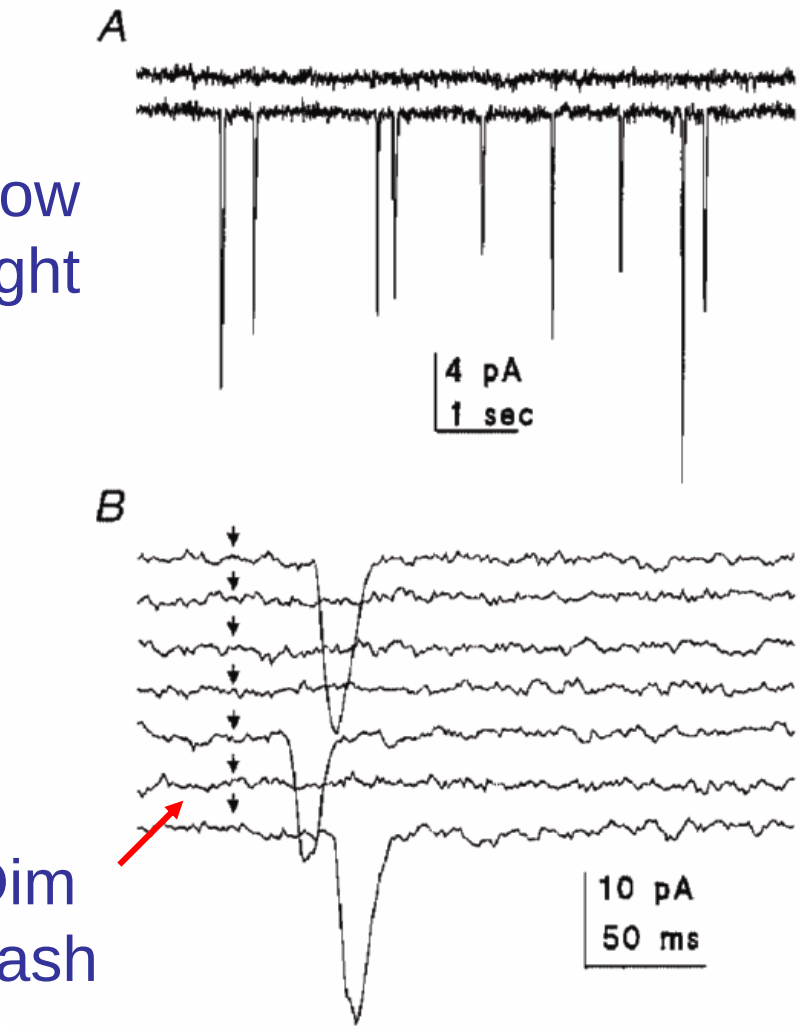




Microvillus



Single photon response in Drosophila: a Quantum Bump



Low light

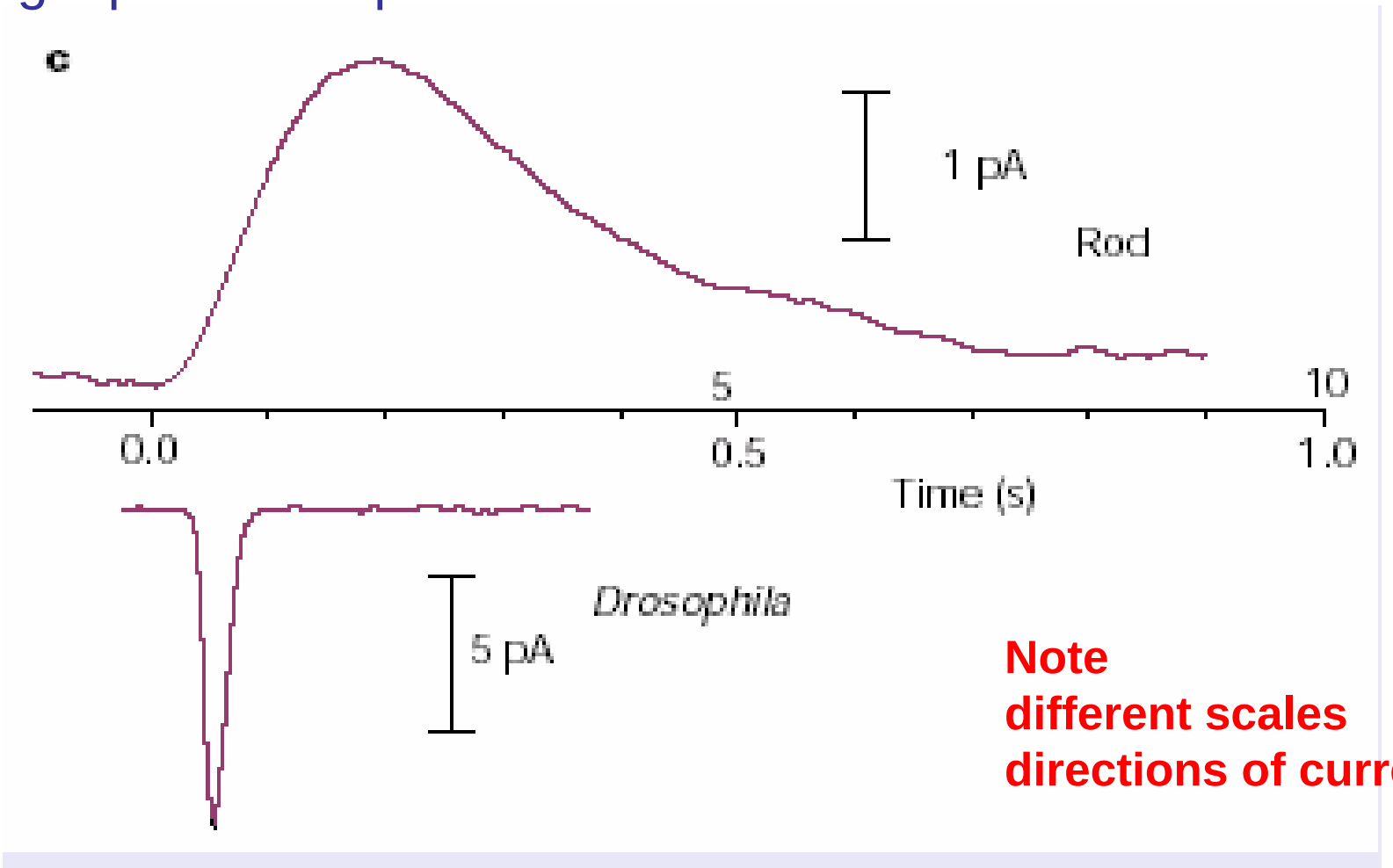
Dim flash

"All-or-none" response

Henderson and Hardie, J.Physiol. (2000) 524, 179

Comparison of a fly with a toad.

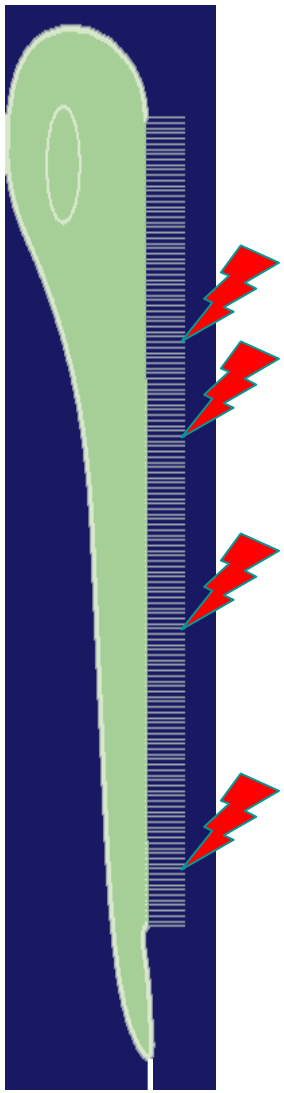
Single photon response:



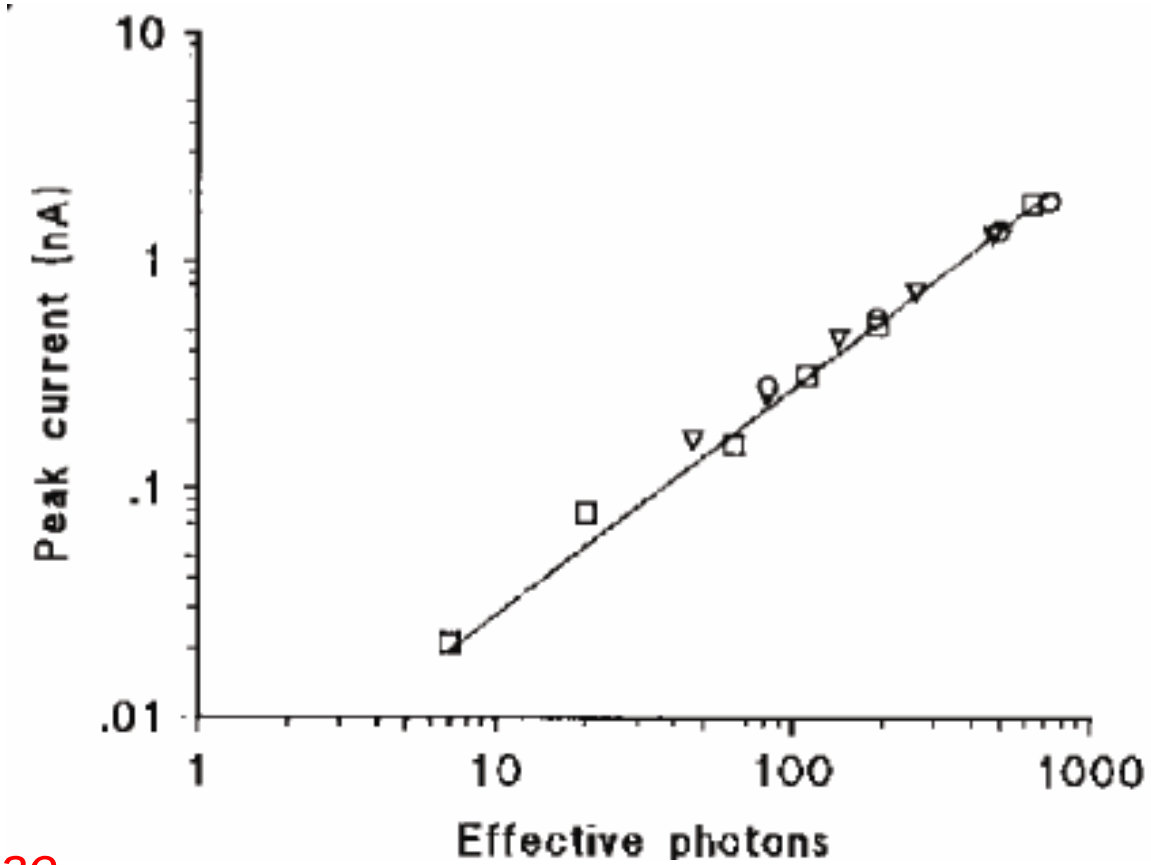
Note
different scales
directions of current!!

From Hardie and Raghu, Nature 413, (2001)

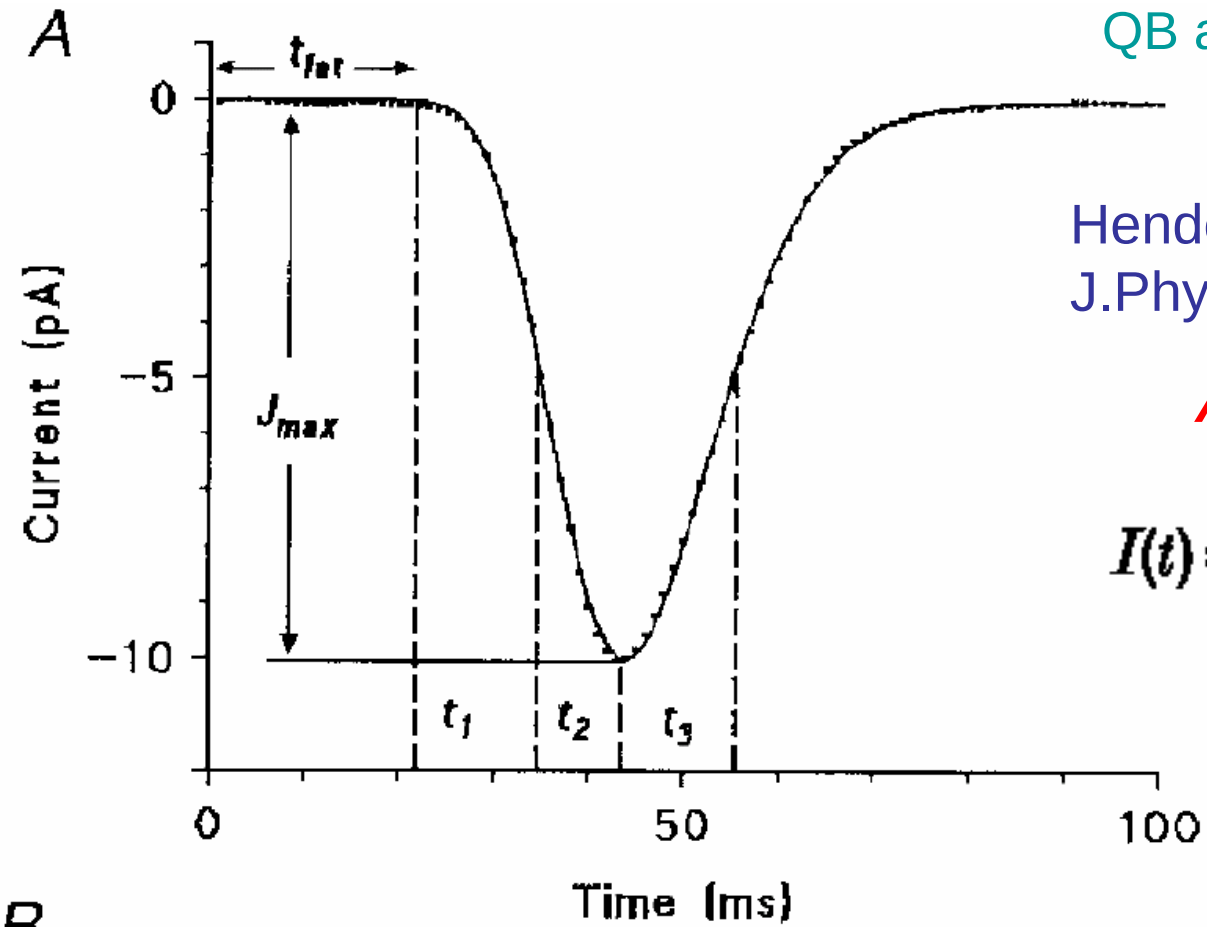
Linearity of macroscopic response



Linear summation over microvillae



Average QB wave-form



QB aligned at t_{max}

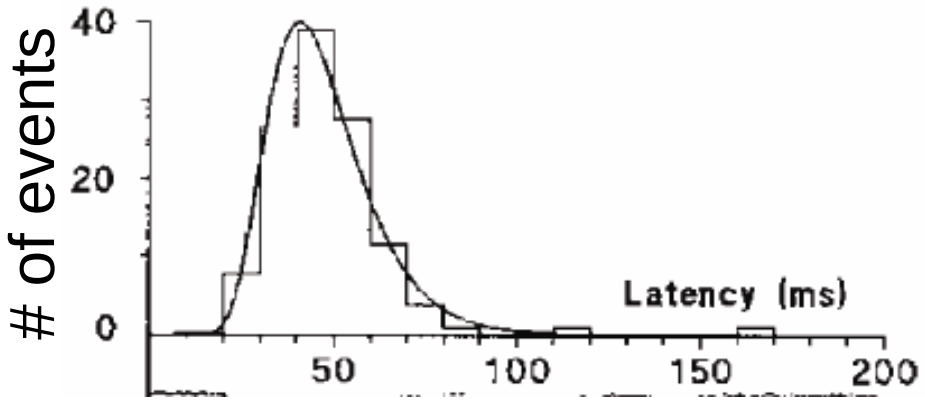
Henderson and Hardie,
J.Physiol. (2000) 524, 179

A miracle fit:

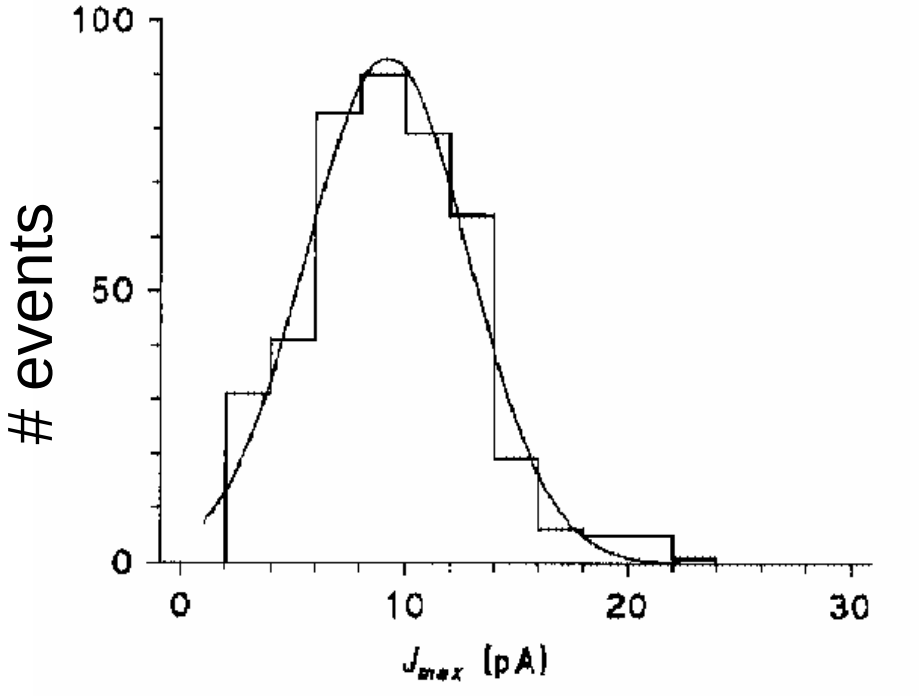
$$I(t) = \exp\left(\frac{-\ln(t/t_{pk})^2}{2\sigma^2}\right),$$

QB variability

Latency distribution



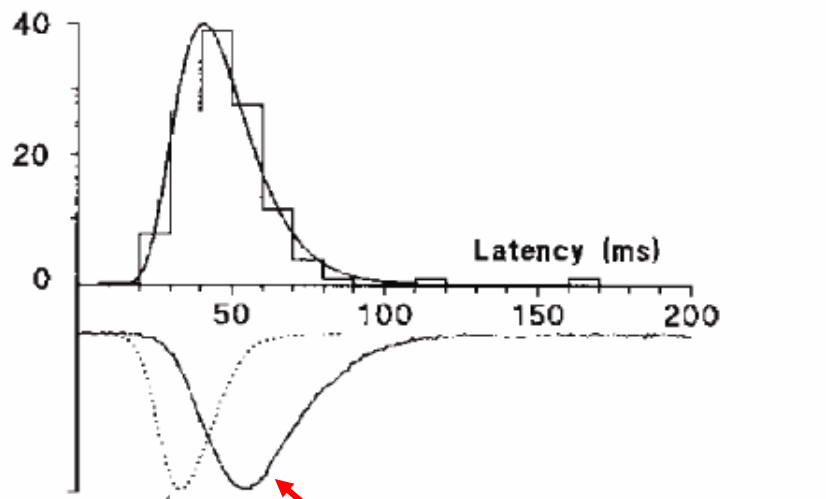
Latency (ms)



Peak current $J_{\max}$ (pA)

$$\frac{rms(J_{\max})}{mean(J_{\max})} \approx 0.4$$

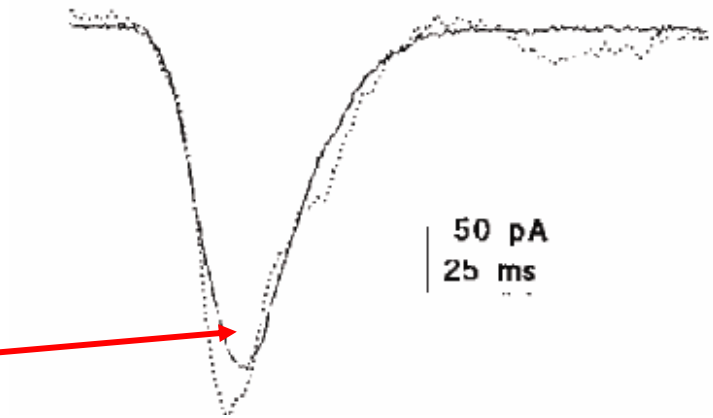
Multi-photon response



QB waveform

Convolution with latency distribution

Macroscopic response = average QB



Latency distribution determines the average macroscopic response

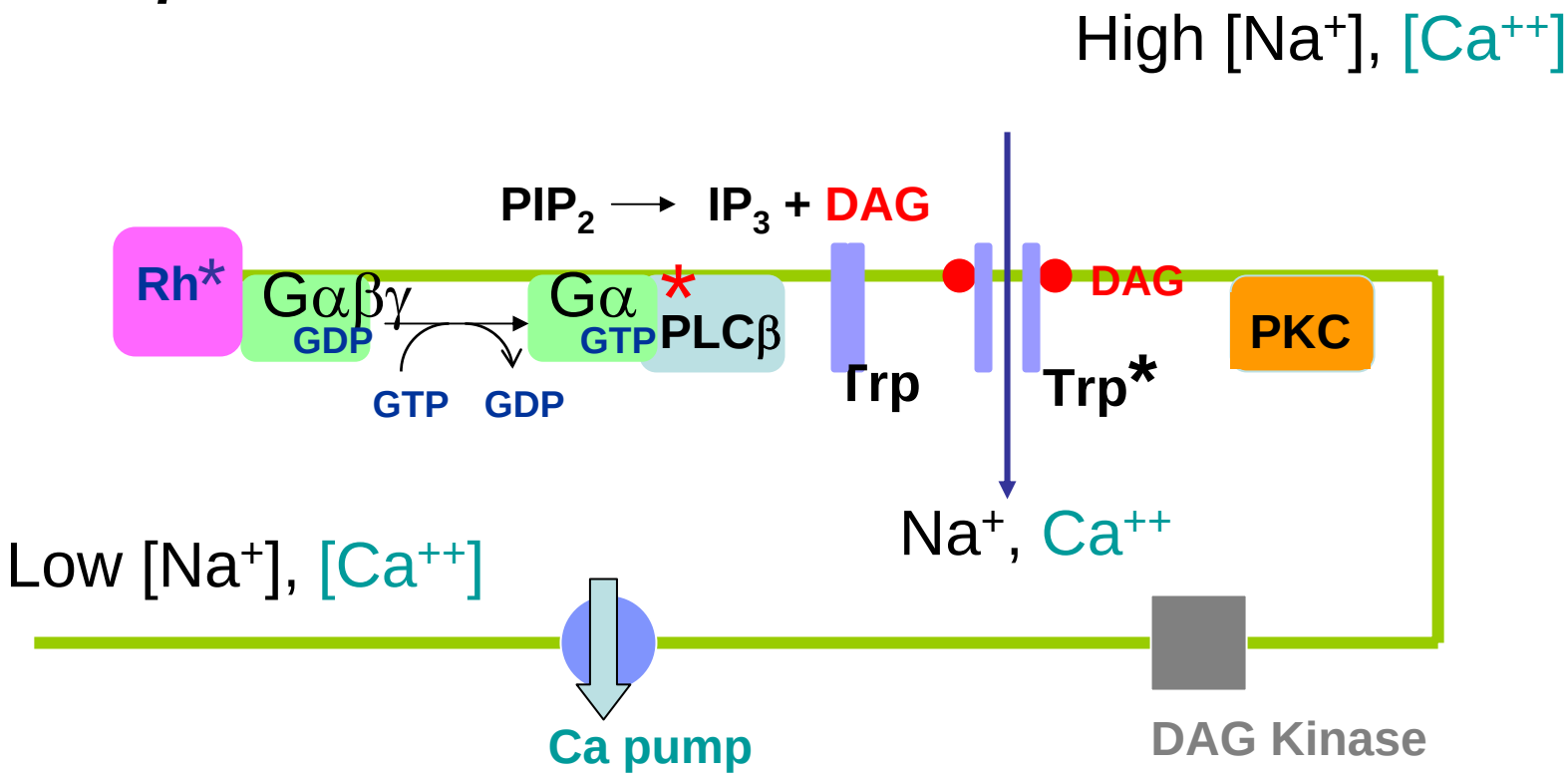
!!! **Fluctuations control the mean** !!!

Advantages of Drosophila photo-transduction as a model signaling system:

- Input: Photons
- Output: Changes in membrane potential
- Single receptor cell preps
- Drosophila genetics

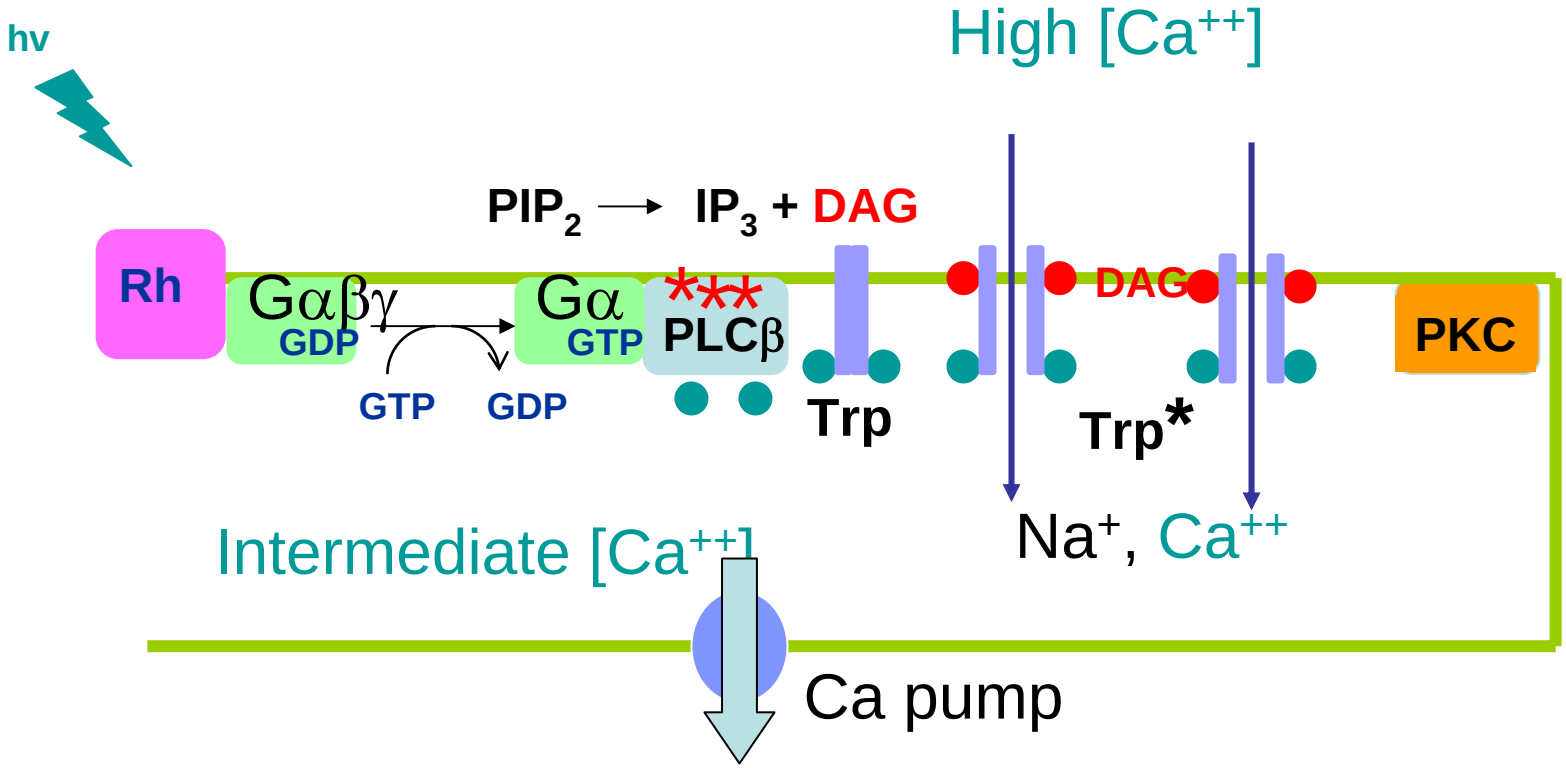
Molecular mechanism of fly phototransduction

Response initiation



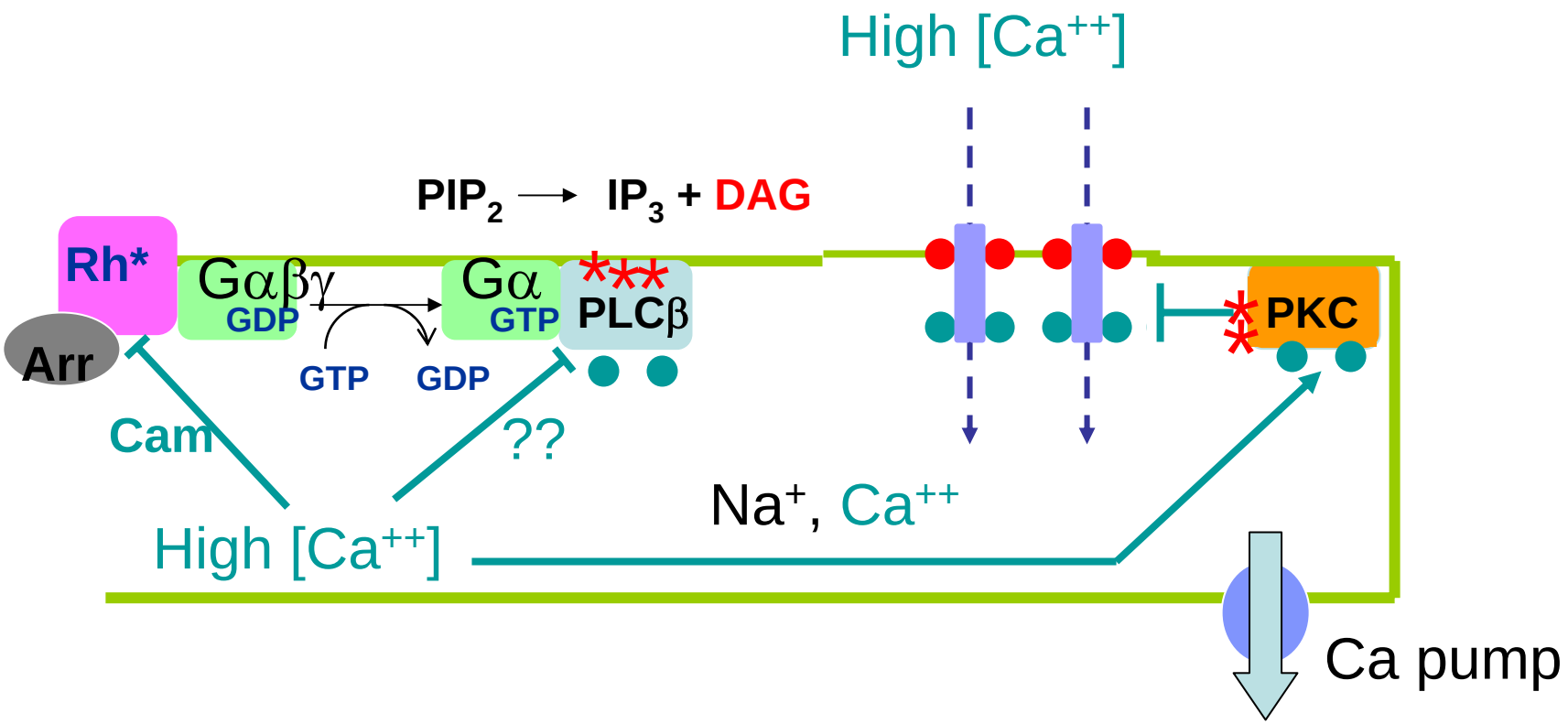
Cast: **Rh = Rhodopsin; $G_{\alpha\beta\gamma}$ = G-protein**
PIP_2 = phosphatidyl inositol-bi-phosphate
DAG = diacyl glycerol
PLCβ = Phospholipase C -beta ;
TRP = Transient Receptor Potential Channel

Positive Feedback



Intermediate [Ca] facilitates opening of Trp channels and accelerates Ca influx.

Negative feedback and inactivation

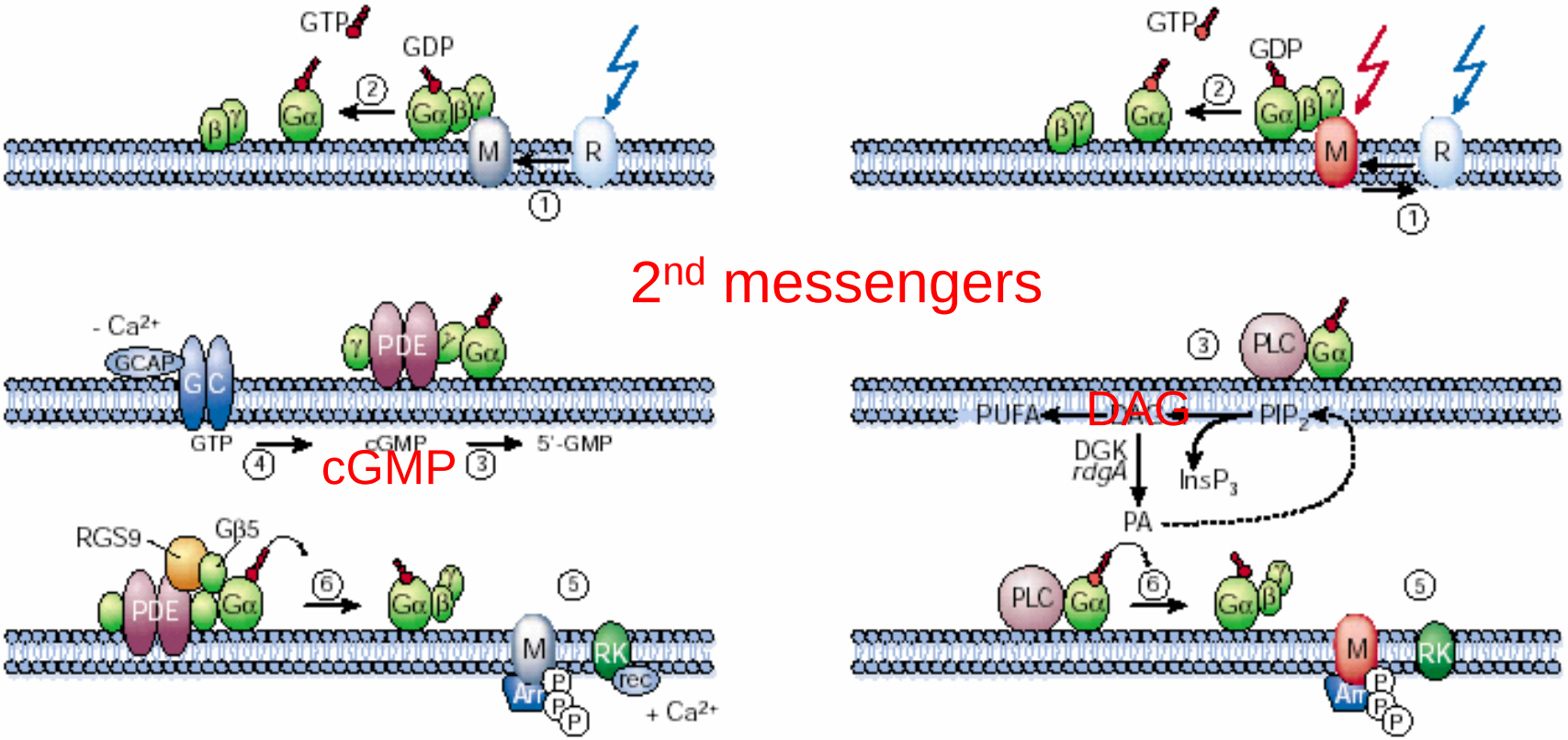


Cast: Ca^{++} acting directly and indirectly
e.g. via PKC = Protein Kinase C
and Cam = Calmodulin
Arr = Arrestin (inactivates Rh*)

Comparison of early steps

Vertebrate

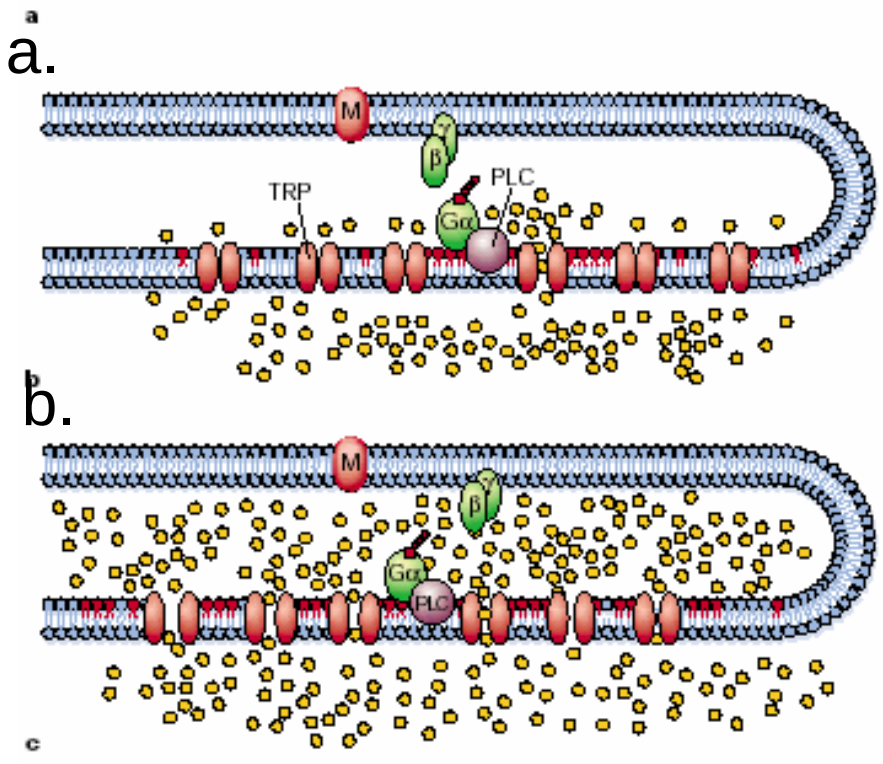
Drosophila

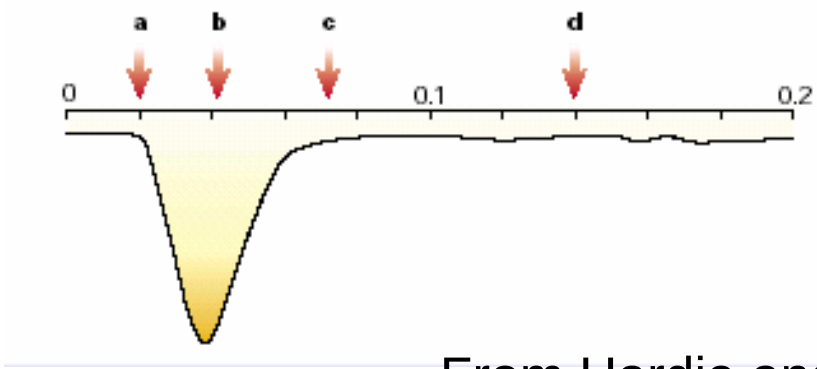


2nd messengers

From Hardie and Raghu, Nature 413, (2001)

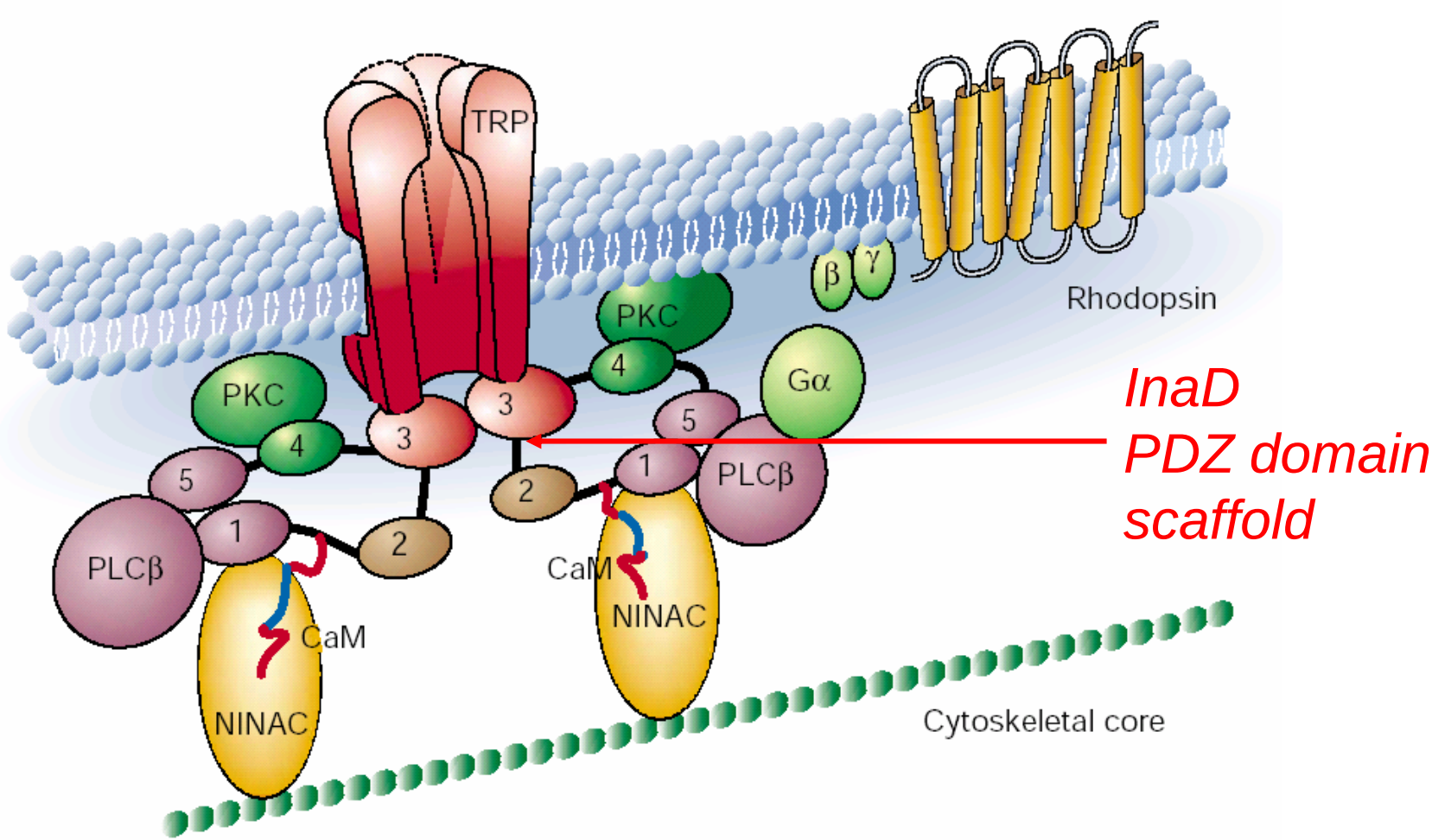
…and another cartoon





From Hardie and Raghu, Nature 413, (2001)

InaD signaling complex



From Hardie and Raghu, Nature 413, (2001)

Speed and space: the issue of localization and confinement.

Order of magnitude estimate of activation rates:

$\dot{G}^* \sim \quad \dot{PLC}^* \sim k\,[G] \sim 10\mu m^2/s \quad 100 / .3\ \mu m^2 > 1\ ms^{-1}$

Diffusion limit on reaction rate

Protein (areal) density

! Fast Enough !

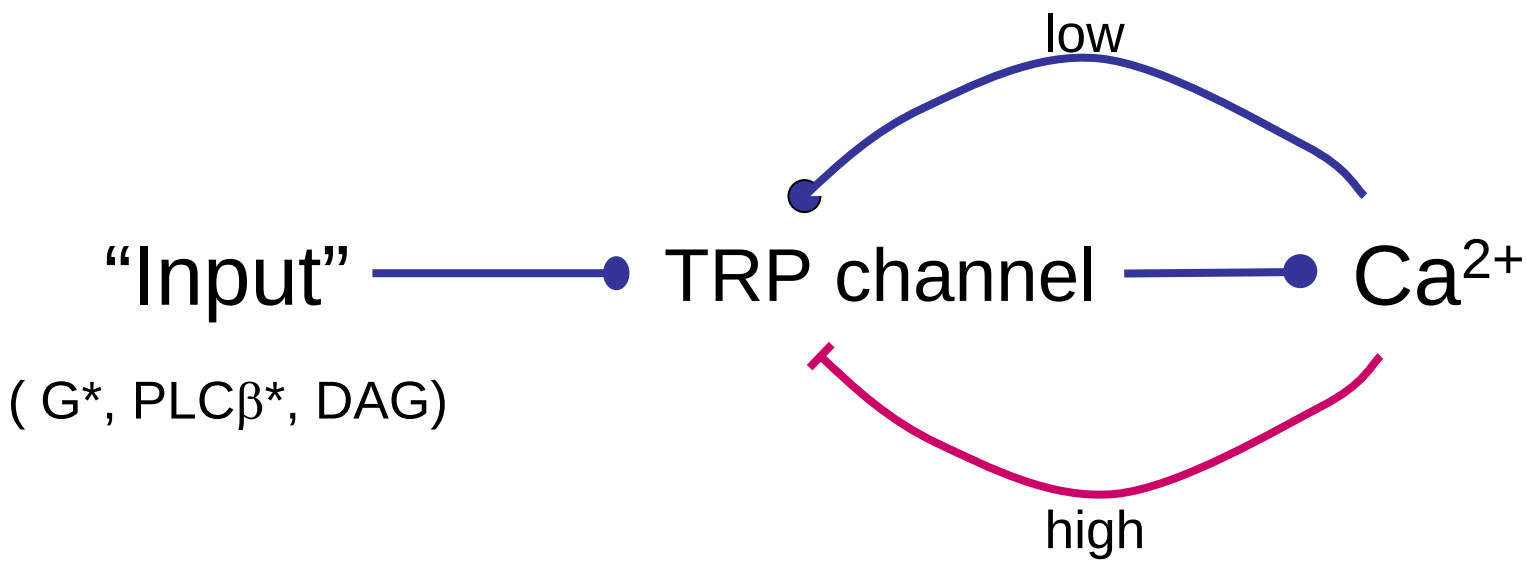
However if: $\sim 1\mu m^2/s \quad 10 / .3\ \mu m^2 = .03 ms^{-1} << 1\ ms^{-1}$

! Too Slow !

Possible role for InaD scaffold !

How "complex" should the model of a complex network be?

A naïve model



Kinetic equations:

Activation stage (G-protein; PLCβ; DAG):

$$\frac{d}{dt}A = -\tau_A^{-1}A + I$$

Input (Rh activity)

QB "generator" stage (Trp, Ca^{++}):

open channels

Positive and negative feedback

$$\frac{d}{dt}Trp^* = A^n F_+(Ca)Trp - \tau_{Trp^*}^{-1} F_-(Ca)Trp^*$$

$$\frac{d}{dt}[Ca] = \sigma Trp^*([Ca_{ex}] - [Ca]) - \tau_{Ca}^{-1}([Ca] - [Ca_0])$$

Ca^{++} influx via Trp*

Ca^{++} outflow/pump

Feedback Parameterization

$$F_\alpha(Ca) = 1 + g_\alpha \frac{([Ca]/K_{D\alpha})^{m_\alpha}}{1+([Ca]/K_{D\alpha})^{m_\alpha}}$$

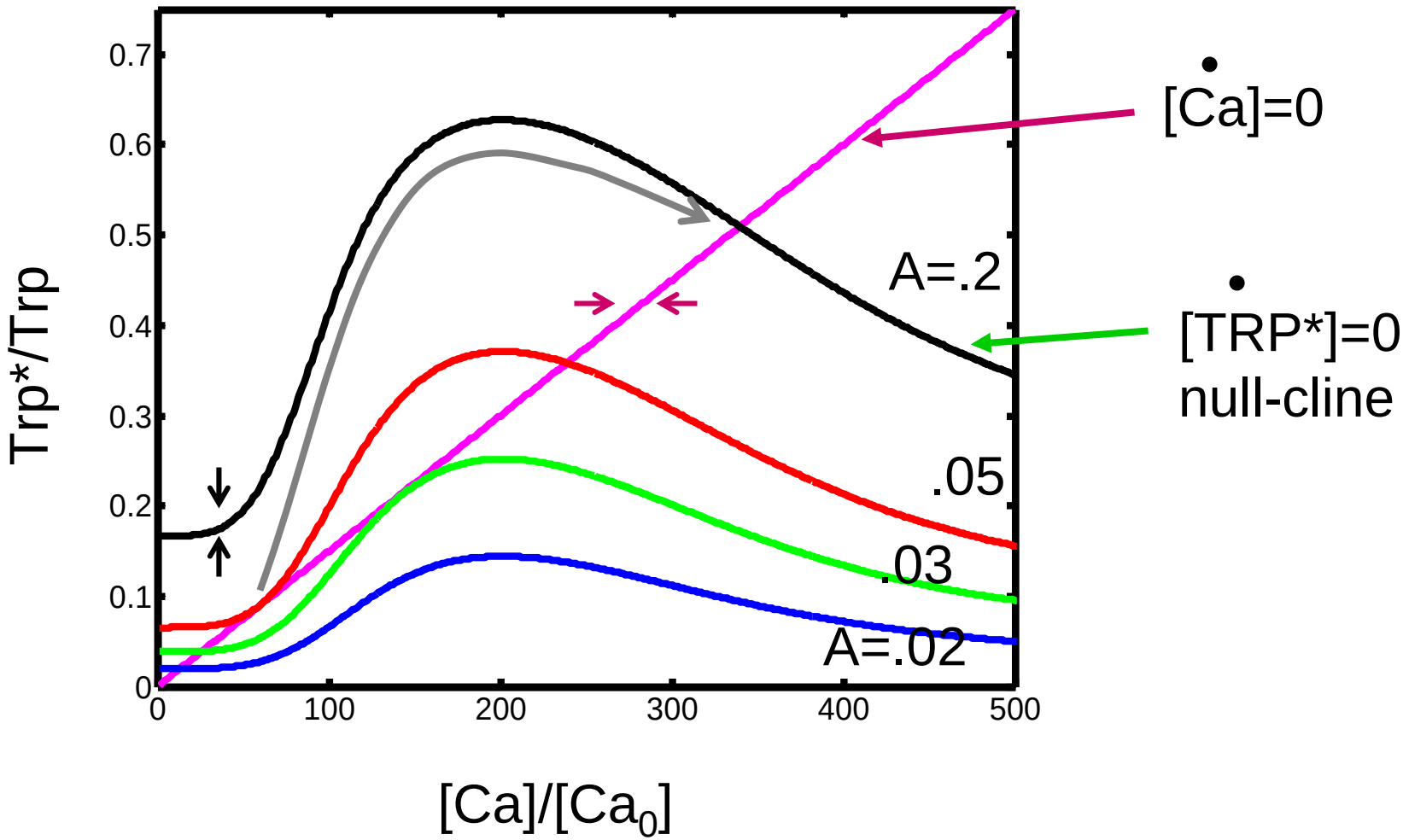
Parameterized by the "strength" g_α (~ ratio at high/low [Ca])

Characteristic concentration $K_{D\alpha}$

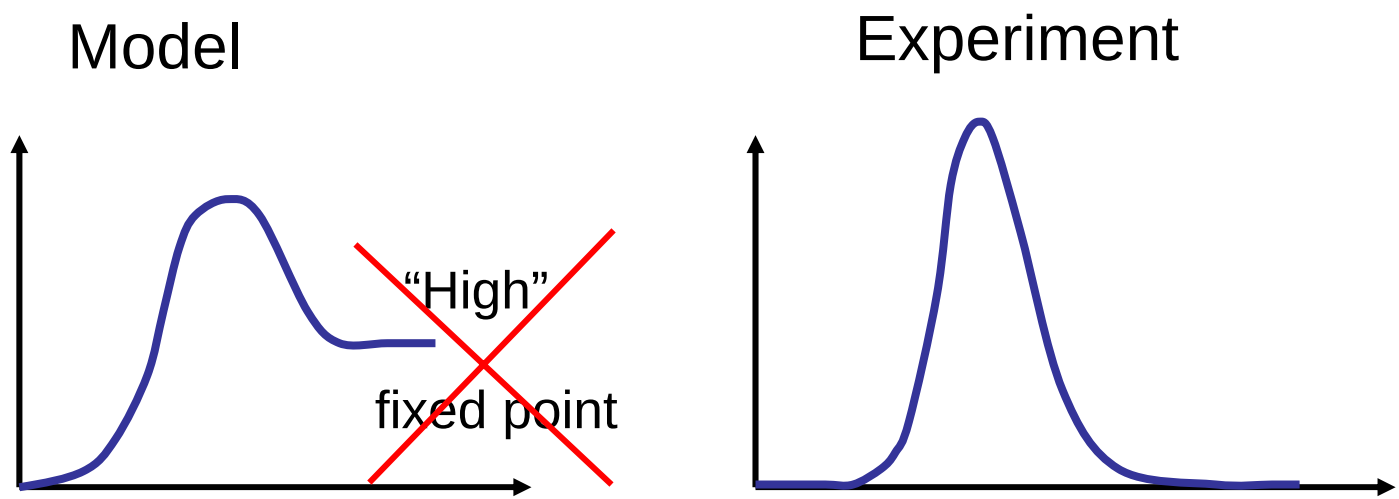
and Hill constant m_α

Note: this has assumed that feedback in instantaneous…

Null-clines and fixed points



Problems with the simple model



- In response to a *step* of Rh* activity (e.g. in Arr mutant) QB current relaxes to zero
- Ca dynamics is fast rather than slow ⇨ no “overshoot”
- Long latency is observed

Order of magnitude estimate of Ca fluxes

$[Ca]_{dark} \sim .2\mu M$ ⟺ 1 Ca ion / microvillus

$[Ca]_{peak} \sim 200\mu M$ ⟺ 1000 Ca ion / microvillus

Note: μ–villus volume ~ $5*10^{-12}$ μl

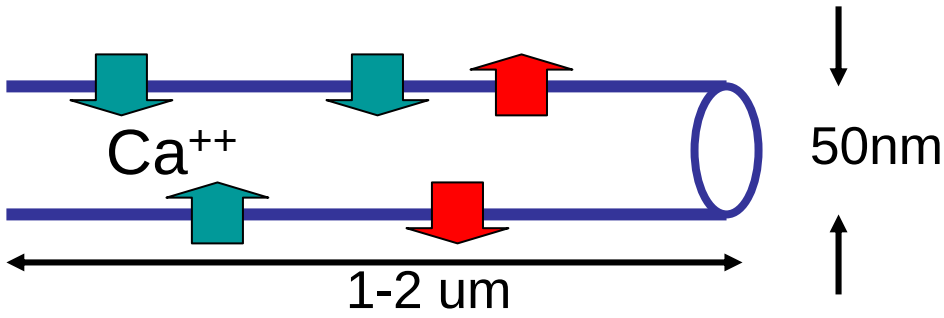
30% of 10pA ⟺ Influx 10^4 Ca^{2+} / ms

Hence, Ca is being pumped out very fast ~ 10 ms^{-1}

↓

[Ca] is in a quasi-equilibrium

Microvillus as a Ca compartment



Compare 10 ms^{-1} with diffusion rate <u>across</u> the microvillus:

$$\tau^{-1} \sim D_{ca} / d^2 \sim 1\ \mu m^2/ms \ / .0025\ \mu m^2 = 400\ ms^{-1}$$

But diffusion <u>along</u> the microvillus:

$$\tau^{-1} \sim 1\ \mu m^2/ms \ / 1\ \mu m^2 = 1\ ms^{-1}$$

is too slow
compared to $10ms^{-1}$

Hence it is decoupled from the cell.

Note: microvillae could not be > .3μm in diameter,
i.e it is possible the diameter is set by diffusion limit

Slow negative feedback

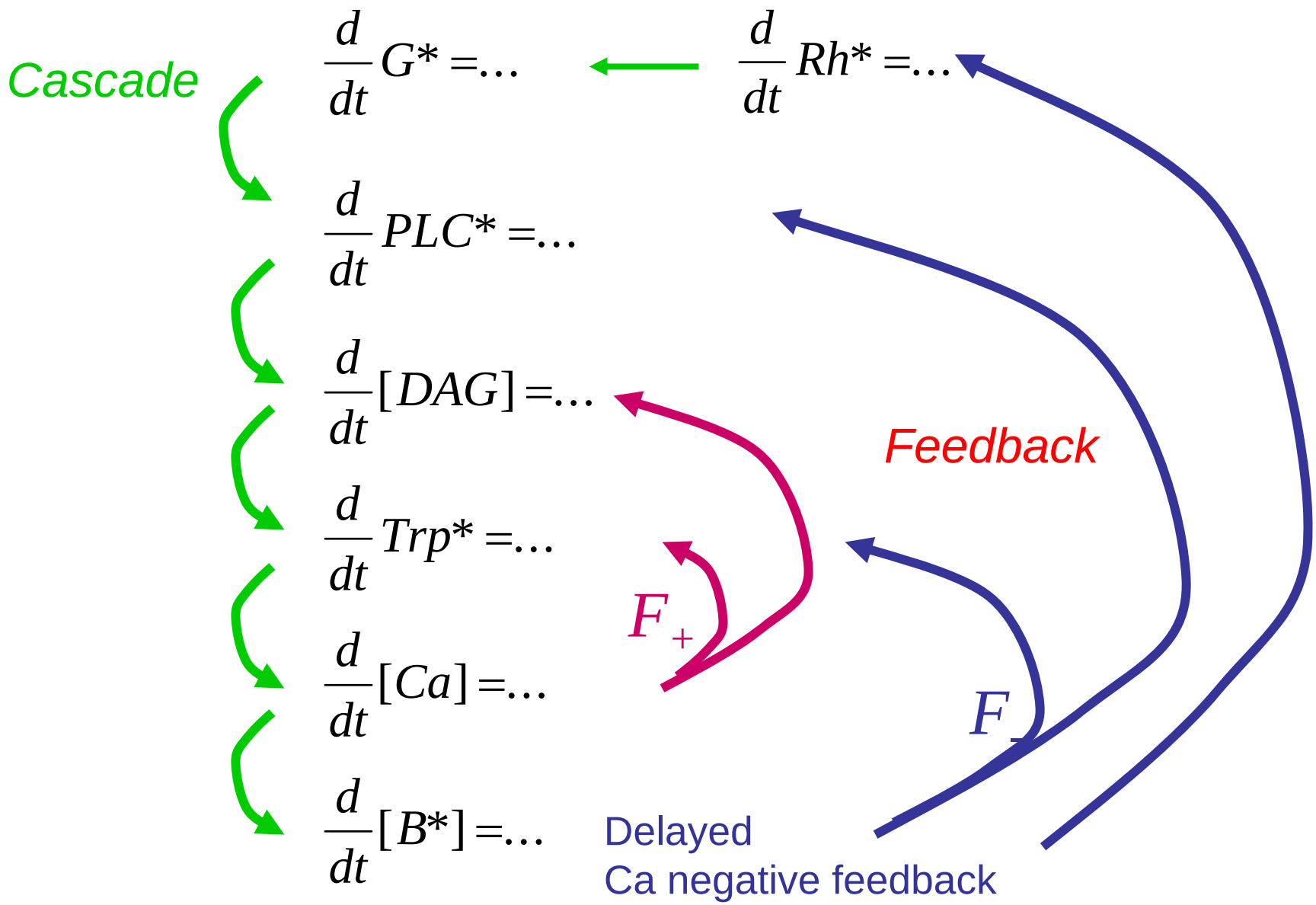
Assume negative feedback is mediated by a Ca-binding protein (e.g. Calmodulin??)

$$\frac{d}{dt}B^* = k_x[Ca]B - \tau_x^{-1}B^*$$

Slow relaxation

$$F_-(B^*) = 1 + g_- \frac{([B^*]/K_{D-})^{m_-}}{1 + ([B^*]/K_{D-})^{m_-}}$$

A more 'biochemically correct' model:



Stochastic effects

Numbers of active molecules are small !

e.g. 1 Rh*, 1-10 G* & PLC*, 10-20 Trp*

Chemical kinetics Reaction “shot” noise.

Master equation

Numerical simulation

Gillespie, 1976, J. Comp. Phys. 22, 403-434
see also
Bort,Kalos and Lebowitz, 1975, J. Comp. Phys. 17, 10-18

Stochastic simulation

Event driven Monte-Carlo simulation
a.k.a. Gillespie algorithm

Gillespie, 1976, J. Comp. Phys. 22, 403-434
see also
Bort,Kalos and Lebowitz, 1975, J. Comp. Phys. 17, 10-18

Numbers of molecules (of each flavor) $\#X_a(t)$ are updated

$$\#X_a(t) \rightarrow \#X_a(t) \text{ +/- } 1 \quad \text{at times} \quad t_{a,i}$$

distributed according to independent Poisson processes with transition rates $\Gamma_{a,+/-}$. Simulation picks the next "event" among all possible reactions.

Note: simulation becomes very slow if some of the Reactions are much faster then others. Use a "hybrid" method.

The model is phenomenological…

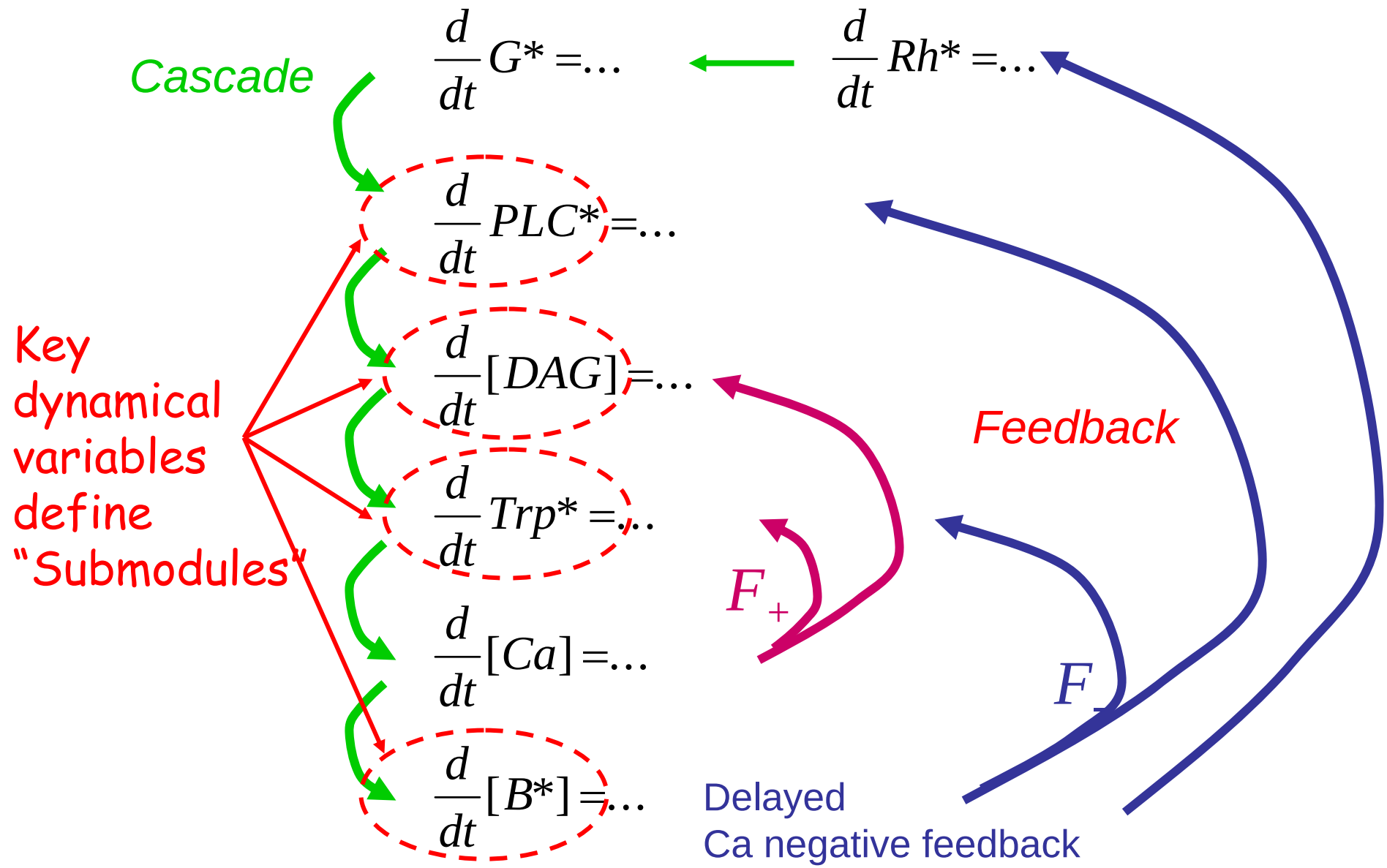
Many (most?) details are unknown:

e.g. Trp activation may not be directly by DAG,
but via its breakdown products;
Molecular details of Ca-dependent feedback(s)
are not known;
etc, etc

BUT

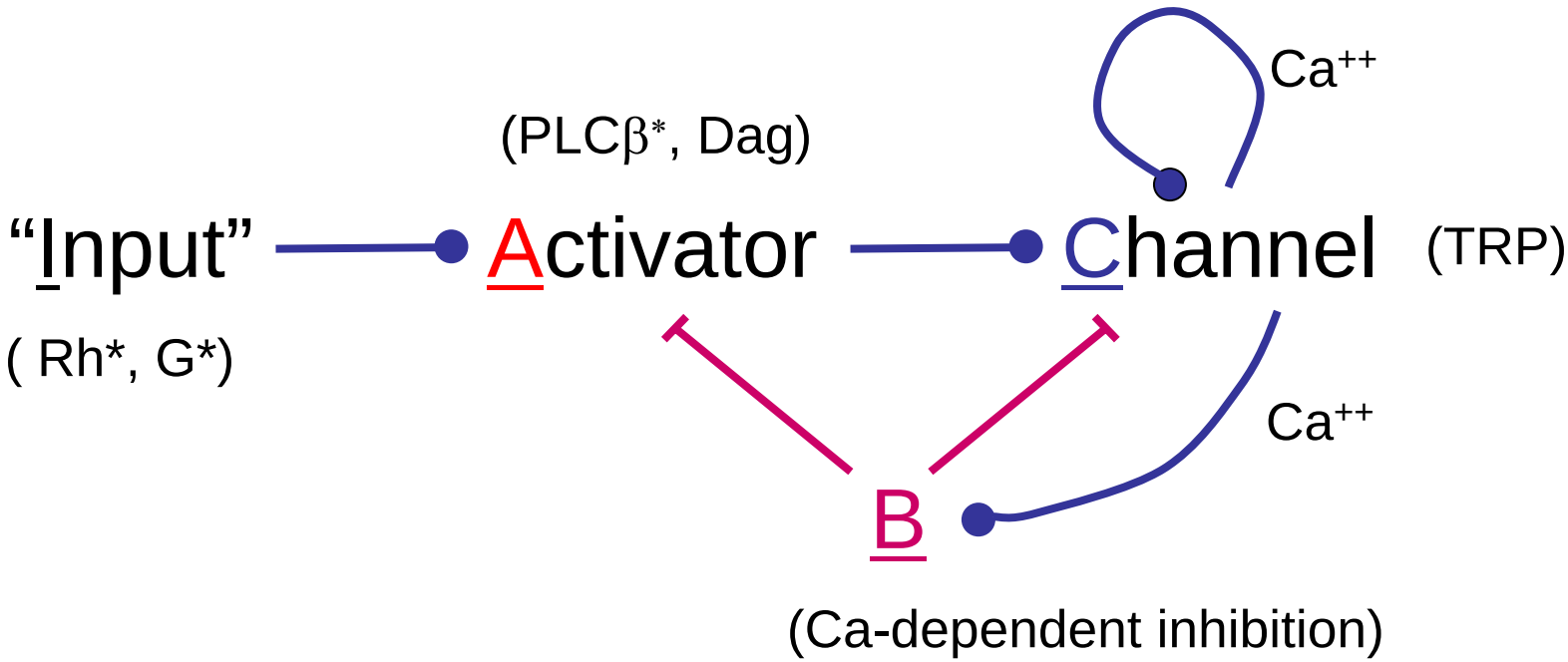
there's much to be explained on a qualitative and quantitative level…

Identifying "submodules"

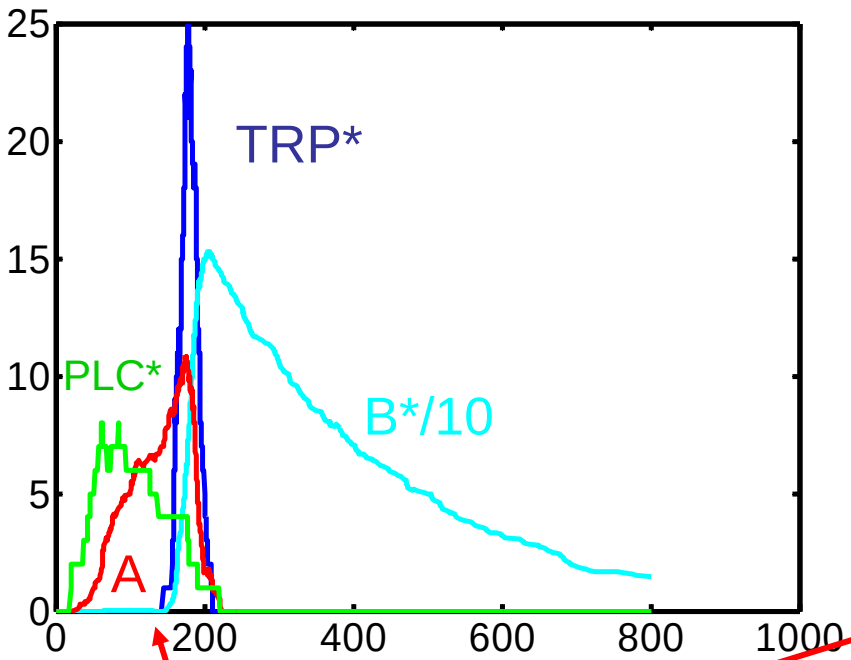


Rephrased in a "Modular" form:
the "ABC model"

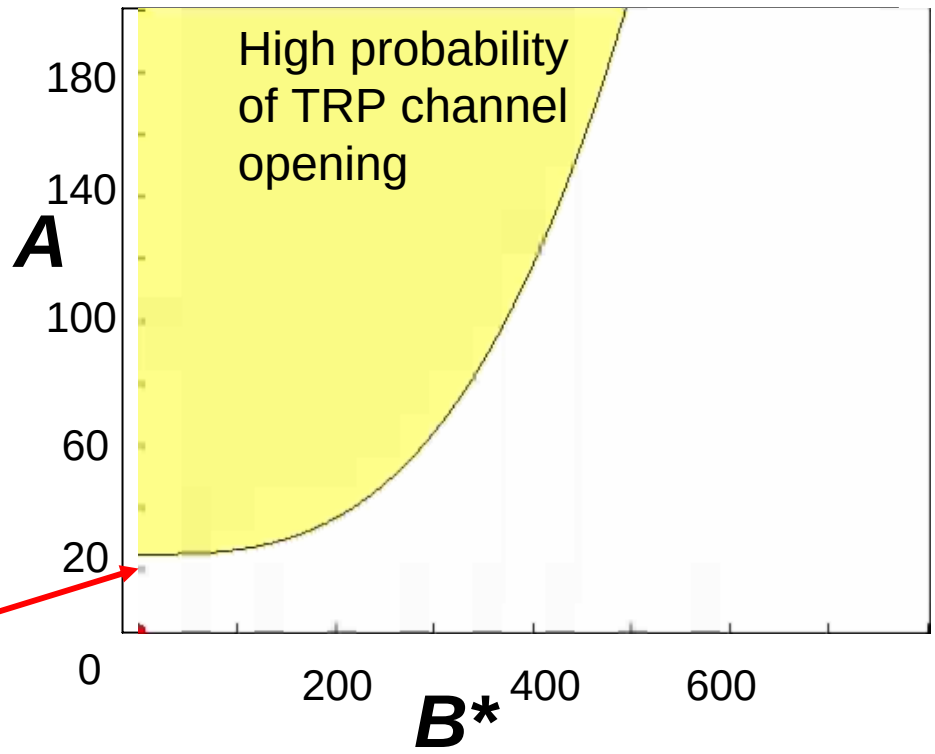
Activator – Buffer – Ca-channel



Quantum Bump generation



(A,B) - "phase" plane



Threshold for QB generation

"INTEGRATE & FIRE" process

What about null-cline analysis?

Problems:

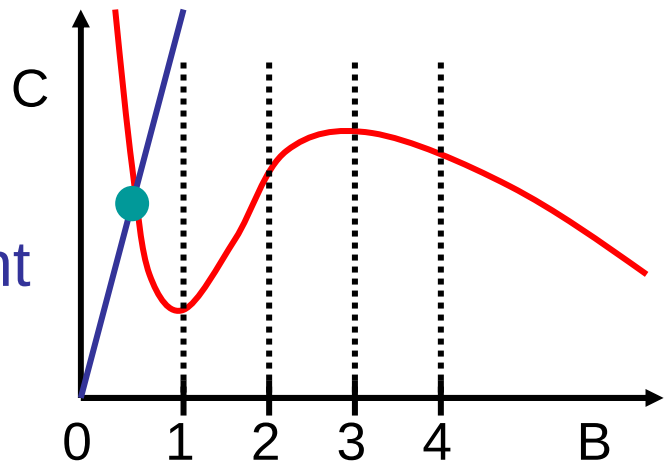
- 3 variables A,B,C
- Stochasticity

Generalized "Stochastic Null-cline"

$$\frac{dX}{dt} = 0 \quad \rightarrow \quad \text{Prob}\,(x \rightarrow x+1) = \text{Prob}\,(x \rightarrow x-1)$$

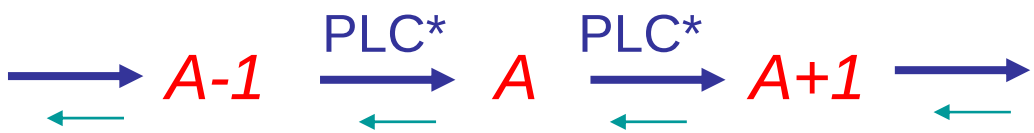
- Discreteness

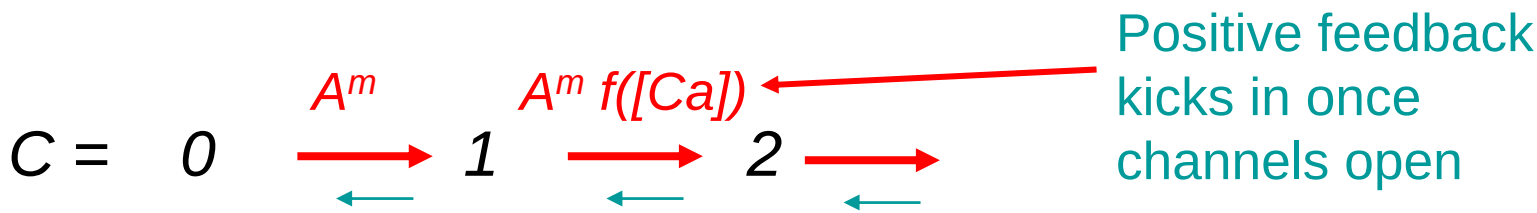
"Ghost" fixed point



Can one calculate anything?

E.g. estimate the threshold for QB generation:





Threshold A = A_T such that

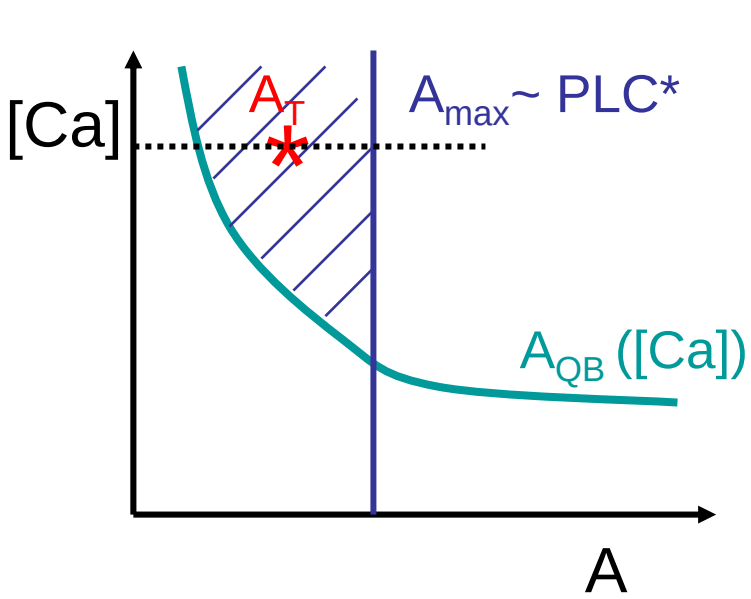
Prob (A_T -> A_T +1) = Prob (C=0 -> C=1)

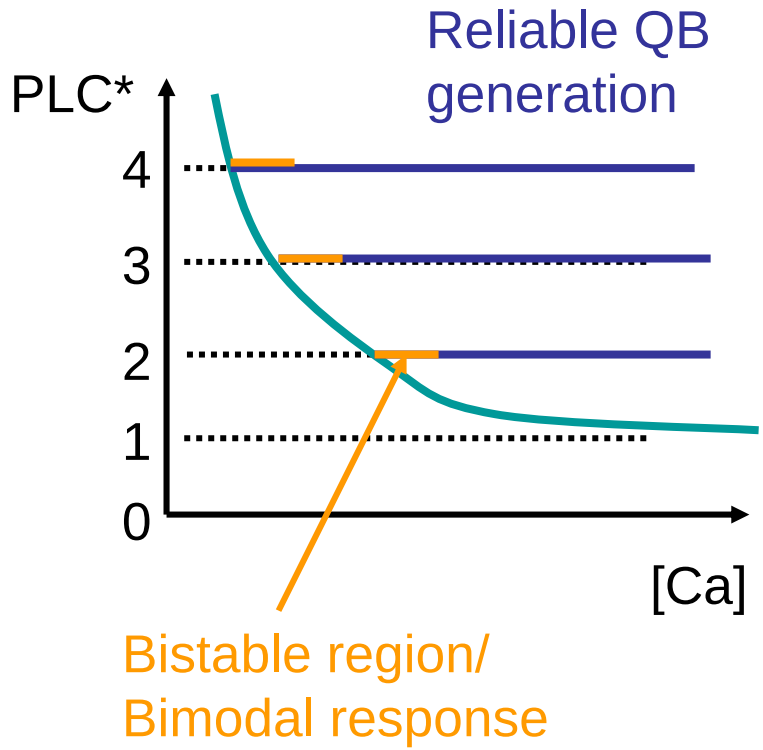
NOTE: Better still to formulate as a "first passage" problem

Condition for QB generation

Prob (C=1 → C=2) > Prob (C=1 → C=0)

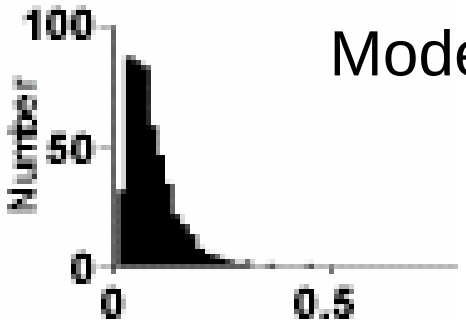
→ $A_T > A_{QB}([Ca])$





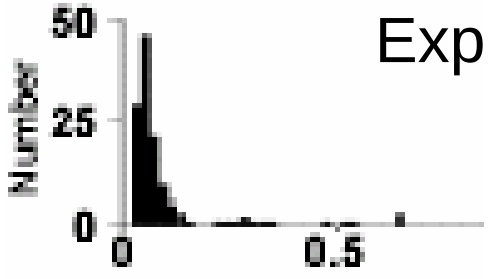
Quantum Bump theory versus reality

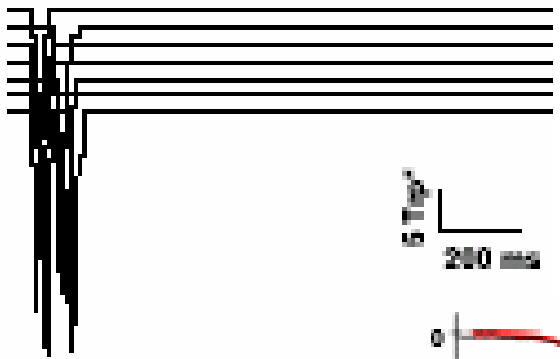
Model

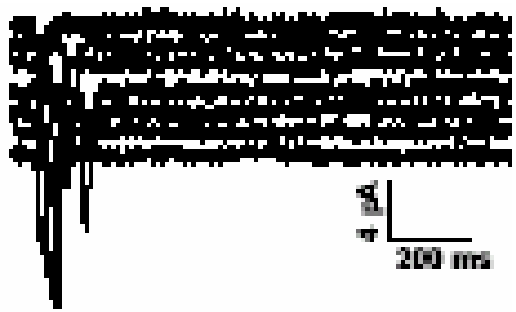


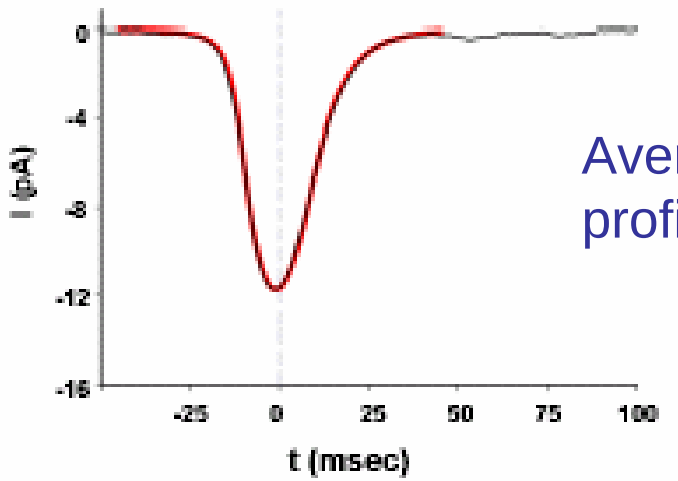
Latency histogram

Experiment



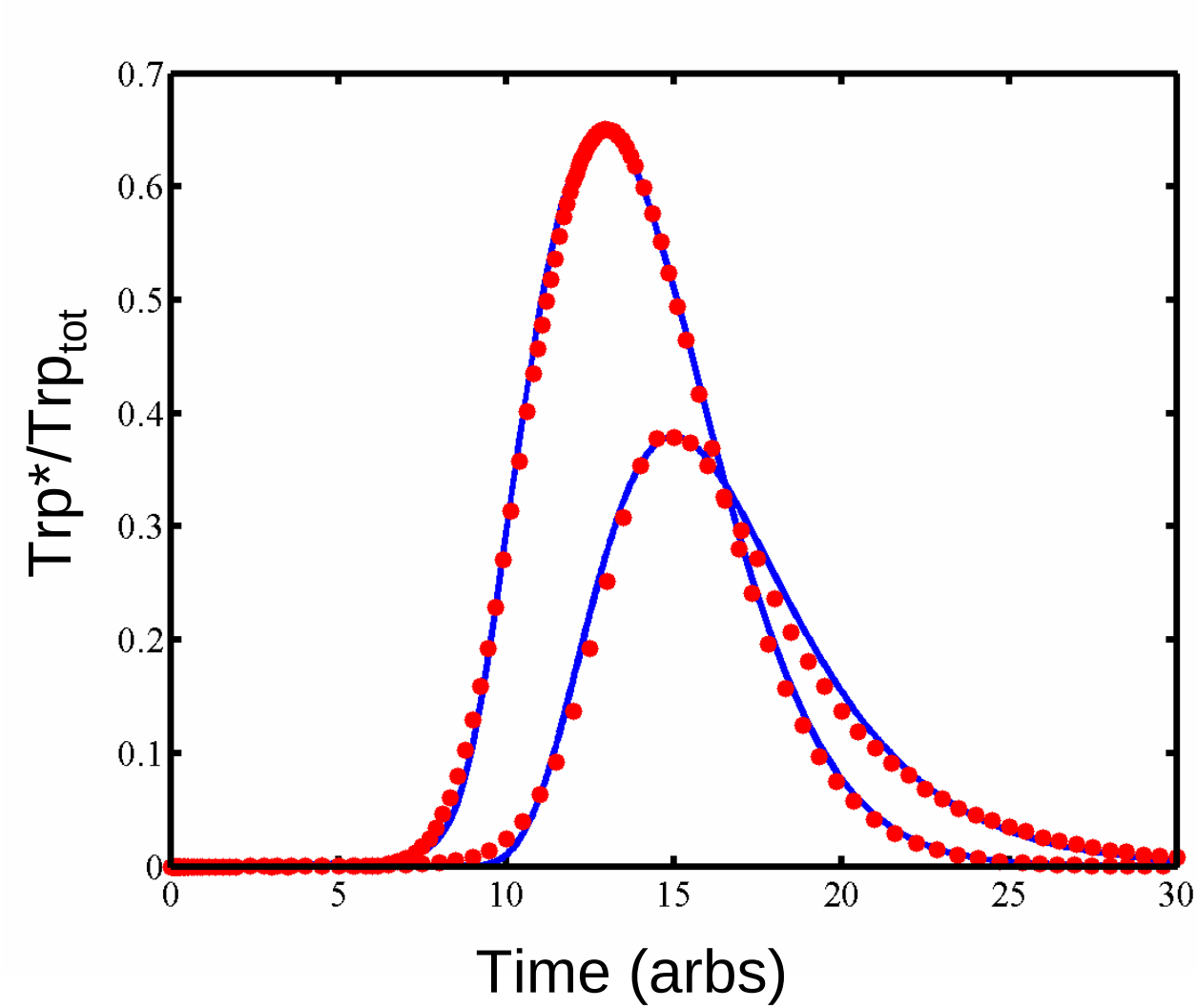






Average QB profile

Fitting the data: QB wave-form

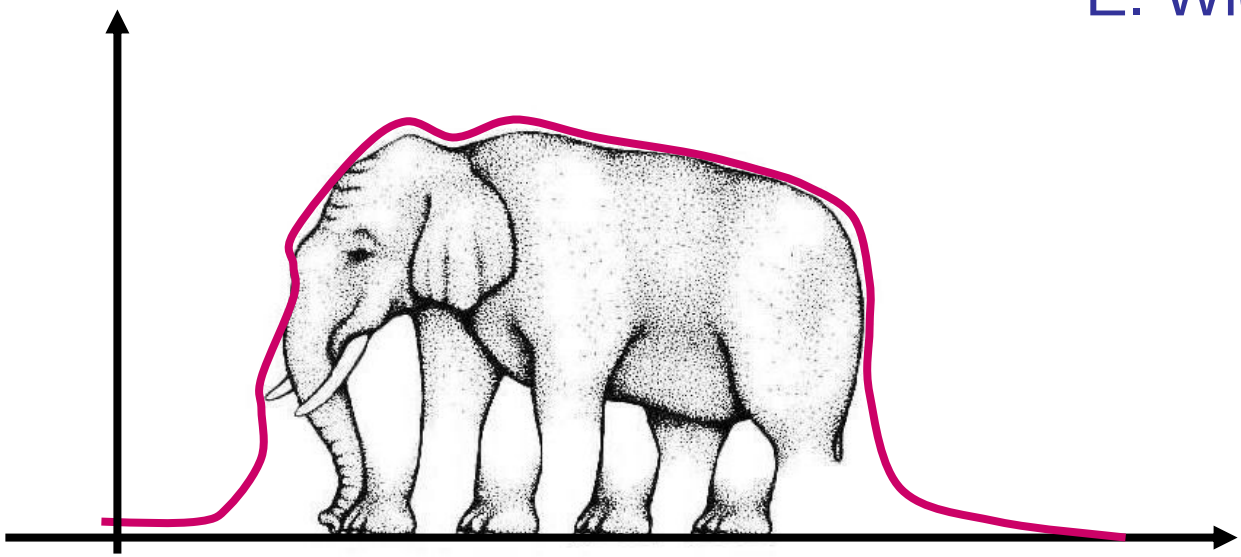


There is a manifold of parameter values providing good fit for < QB > shape !!

So what ???

"With 4 parameters I can fit an elephant and with 5 it will wiggle its trunk."

E. Wigner



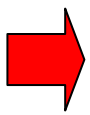
Non-trivial “architectural” constraints

Despite multiplicity of fits, certain constraints emerge:

- Trp activation must be cooperative
- Activator intermediate must be relatively stable:
  “integrate and fire” regime.
- Negative feedback must be delayed
- Multiple feedback loops are needed

Etc, …

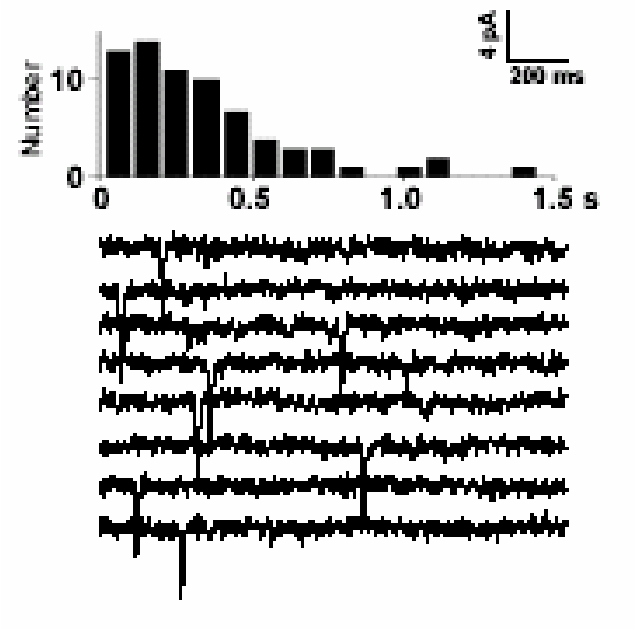
Furthermore:

Fitting  certain relation between parameters:
“phenotypic manifold”
- the manifold in parameter space corresponding to the same quantitative phenotype.

Many more features to explain quantitatively!

Constraining the parameter regime…

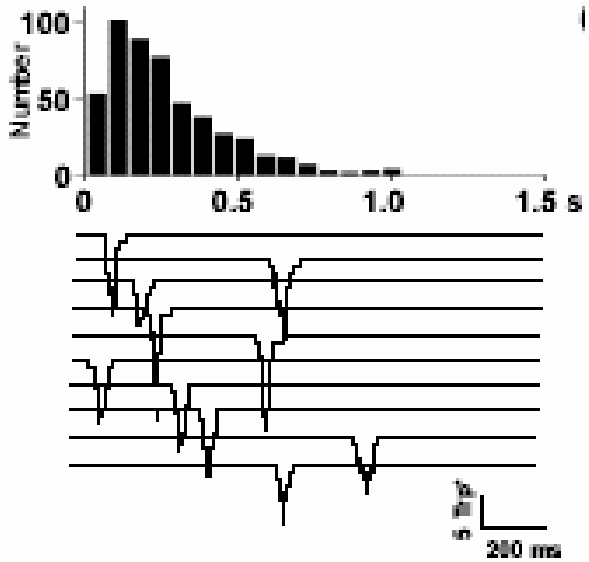
Help from the data on *G*-protein hypomorph flies:



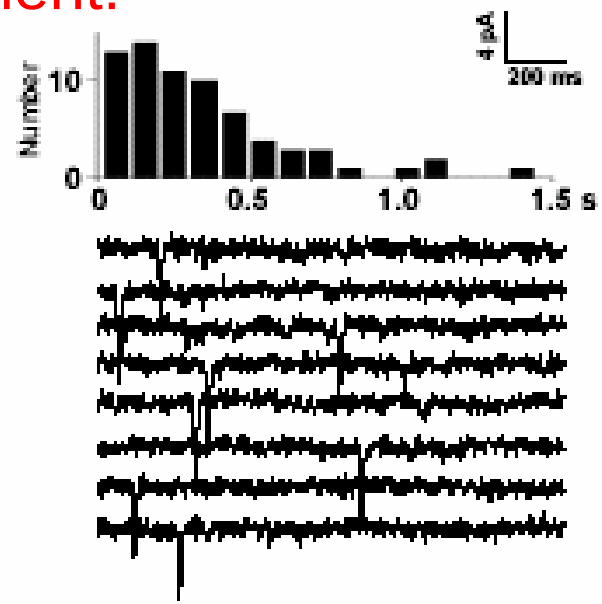
- # of G-proteins reduced by ~100
- QB “yield” down by factor of 10^3
- Increased latency (5-fold)
- Fully non-linear QB with amplitude reduced about two-fold

G-protein hypomorph

Model:



Experiment:



- Single G* and PLC* can evoke a QB !!
- Reduced yield explained by PLC* deactivating before *A* reaches the QB threshold
- Relation between yield reduction and increased latency. # PLC* ~ 5 for WT

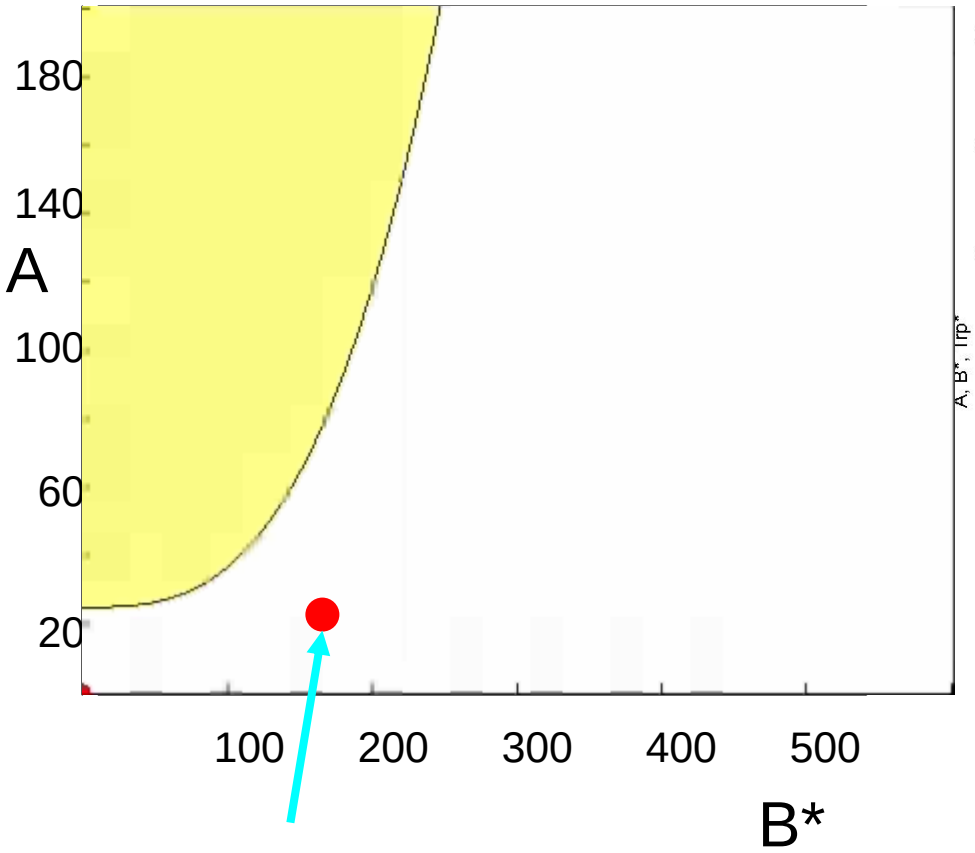
What happens in response to continuous activation ?

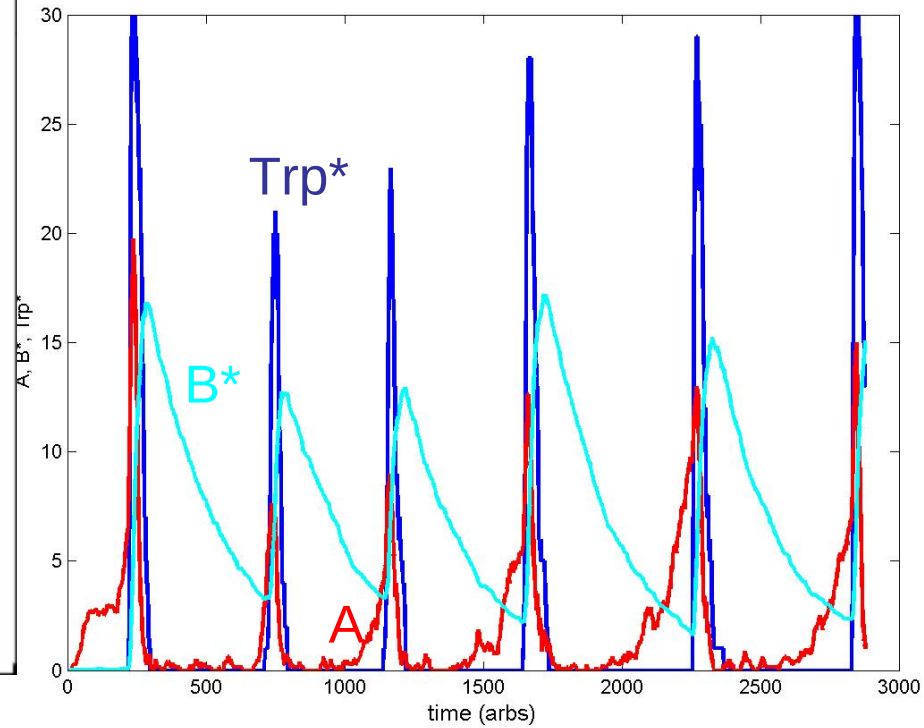
e.g. if Rh* fails to deactivate

Persistent Rh activity*

⇨ *Relaxation Oscillator*

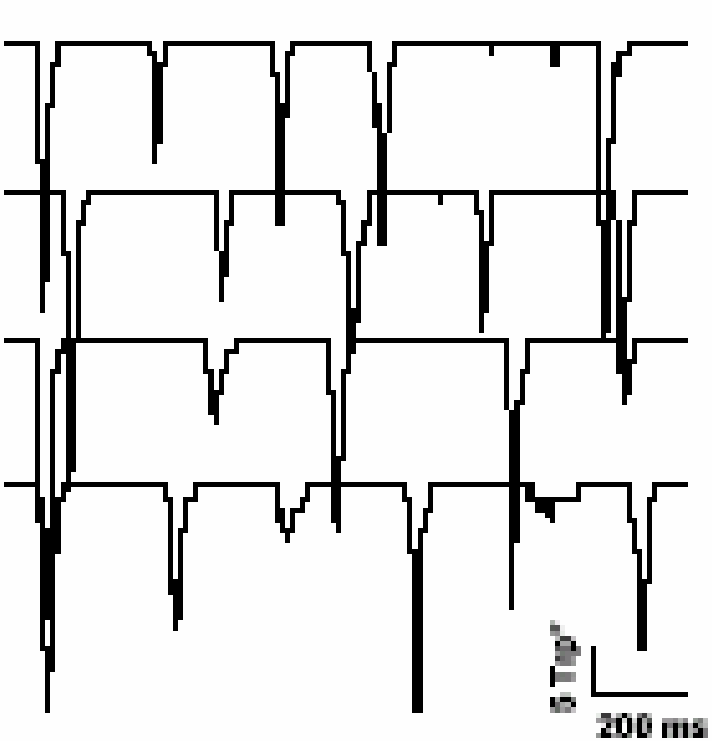
(A,B*) phase plane



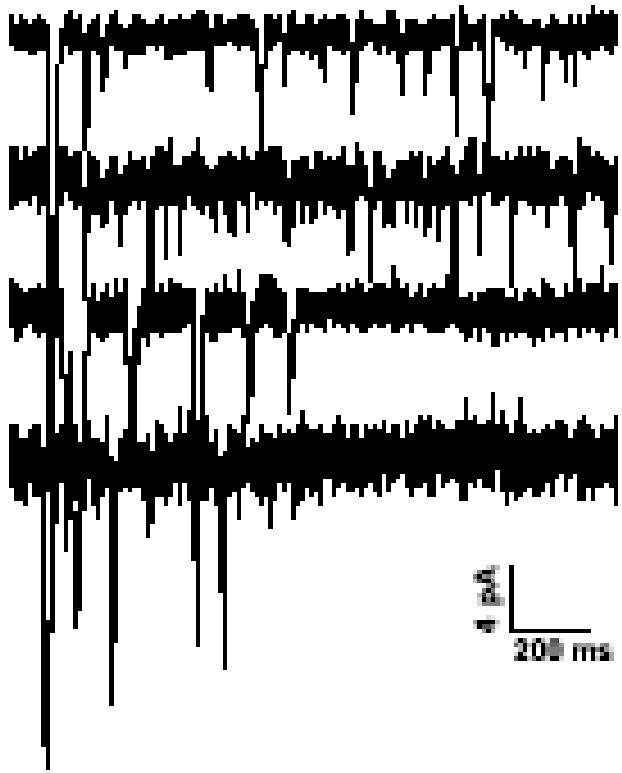


QB trains: theory versus experiment

Model:

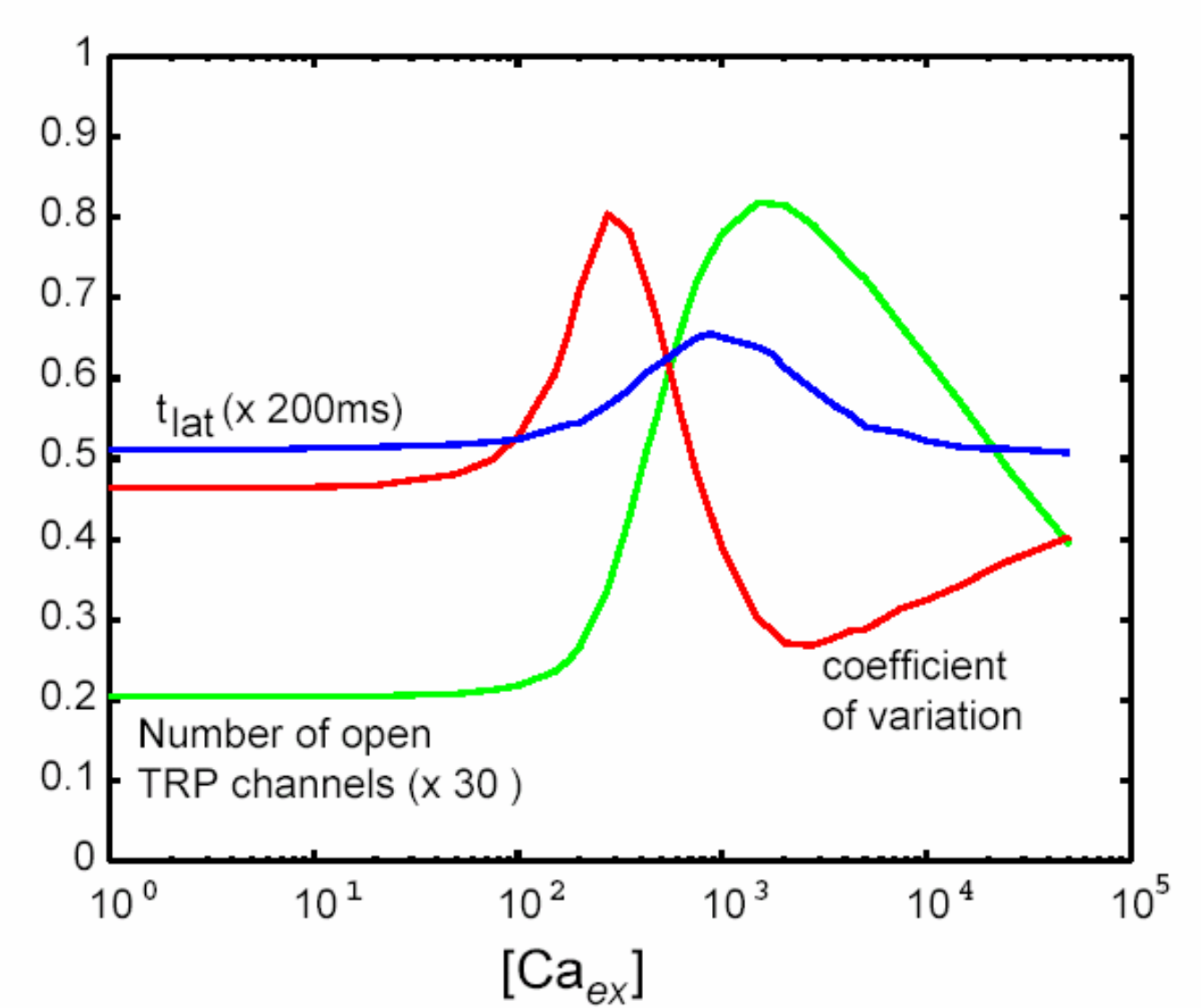


Arrestin mutant
(deficient in Rh* inactivation):

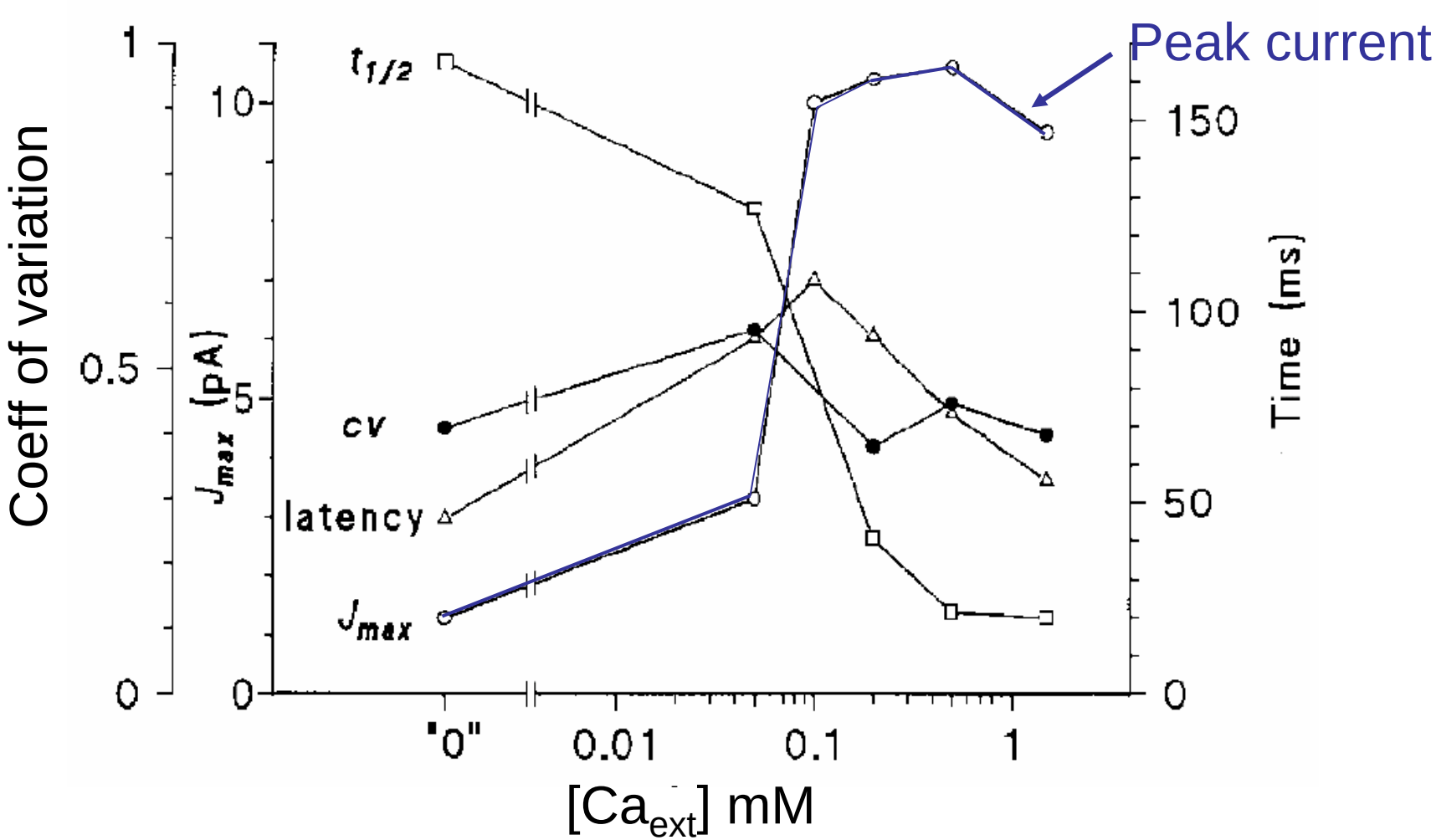


Qualitative but not quantitative agreement so far…

Predicted [Ca_{ex}] *dependence*



Observed external [Ca^{2+}] *dependence*



Henderson and Hardie, J.Physiol. (2000) 524.1, 179

What does one learn from the model?

e.g. Mechanisms/parameters controlling:

Threshold for QB generation.

QB amplitude fluctuations.

Latency.

Yield (or response failure rate)

Latency distribution.

Functional dependences:

e.g. dependence of everything on $[Ca^{++}]_{ext}$

Modeling methodology questions

- Need an intelligent method of searching the parameter space and of characterizing the parameter manifold ??? How does Evolution search the parameter space?

- Characterizing the “space of models”??

- “Convergence proof”??
  Given a model that fits N measurements can we expect that it will fit N+1 (even with additional parameters)?

- How accurate should a prediction be for us to believe that the model is correct ?? Unique??

Summary and Conclusion

A phenomenological model can explain observations and make numerous falsifiable predictions (especially for the functional dependence on parameters).

Insight into HOW the system works from understanding the most relevant parameters and processes.

?????

Can one get any insight into WHY the system is constructed the way it is (e.g. vertebrate versus insect)

?????

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