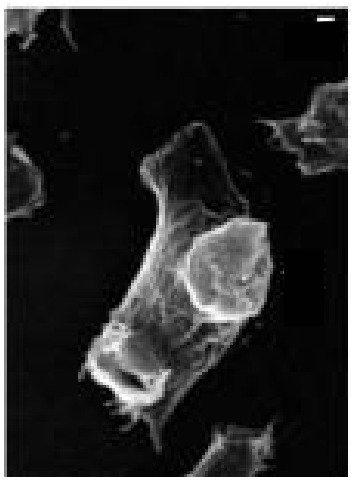
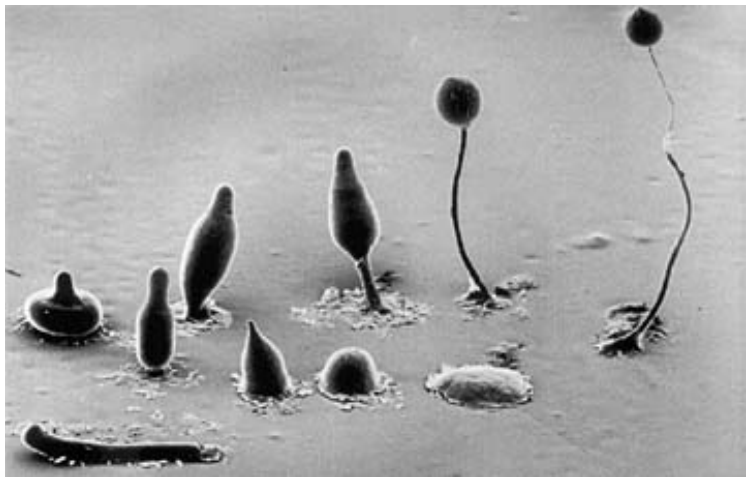
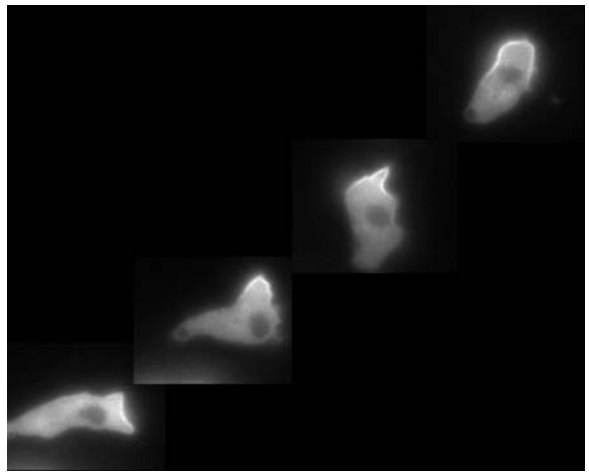
Cellular individuality in directional sensing

Azadeh Samadani (Brandeis University)
Jerome Mettetal (MIT)
Alexander van Oudenaarden (MIT)

How do cells make a decision?

A cell makes many decisions based on the cues from the external environment

presence of a gradient

absence of a gradient

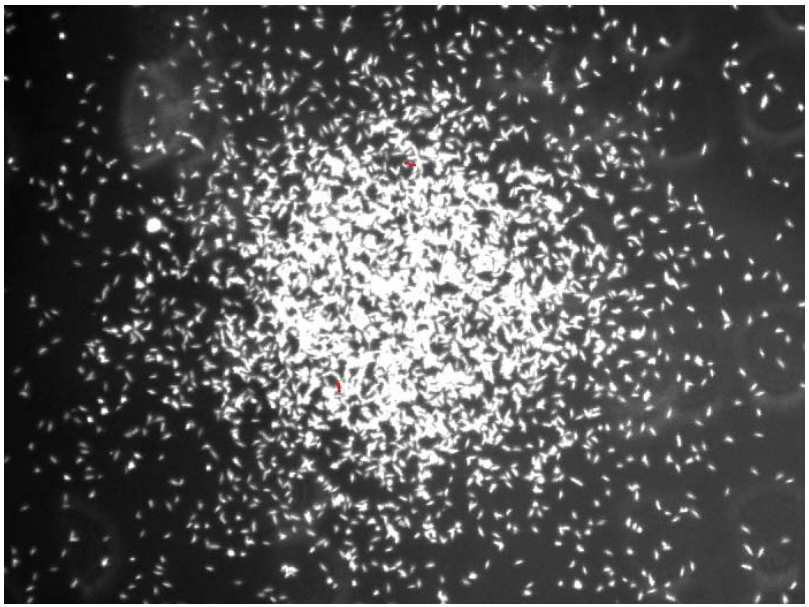
cue-dependent symmetry breaking

random symmetry breaking

How does the decision making vary from cell-to-cell?

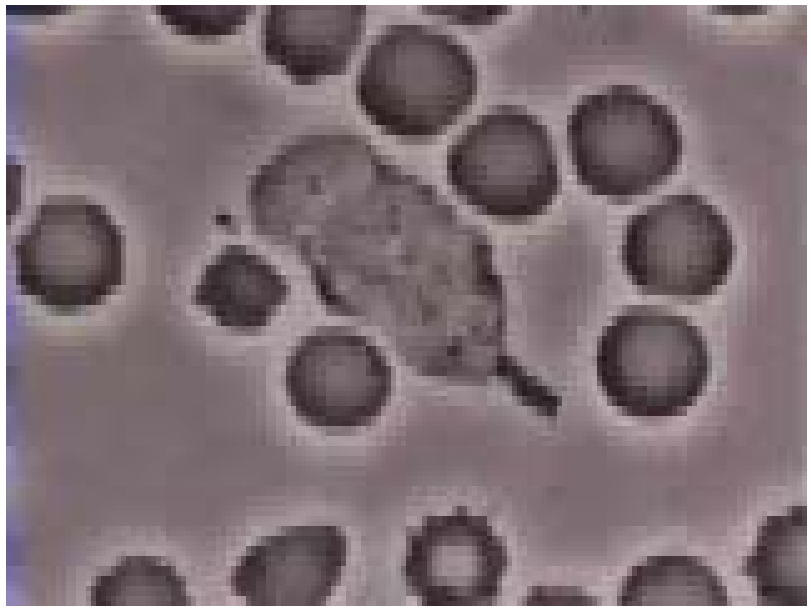
How do cells make a decision?

Bacteria (Prokaryote)



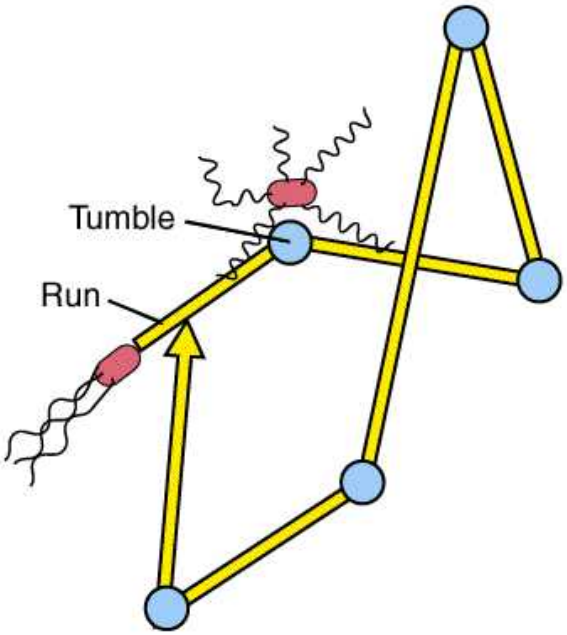
Movie by Nikhil Mittal & Elena Budrene

White blood cell (Eukaryote)

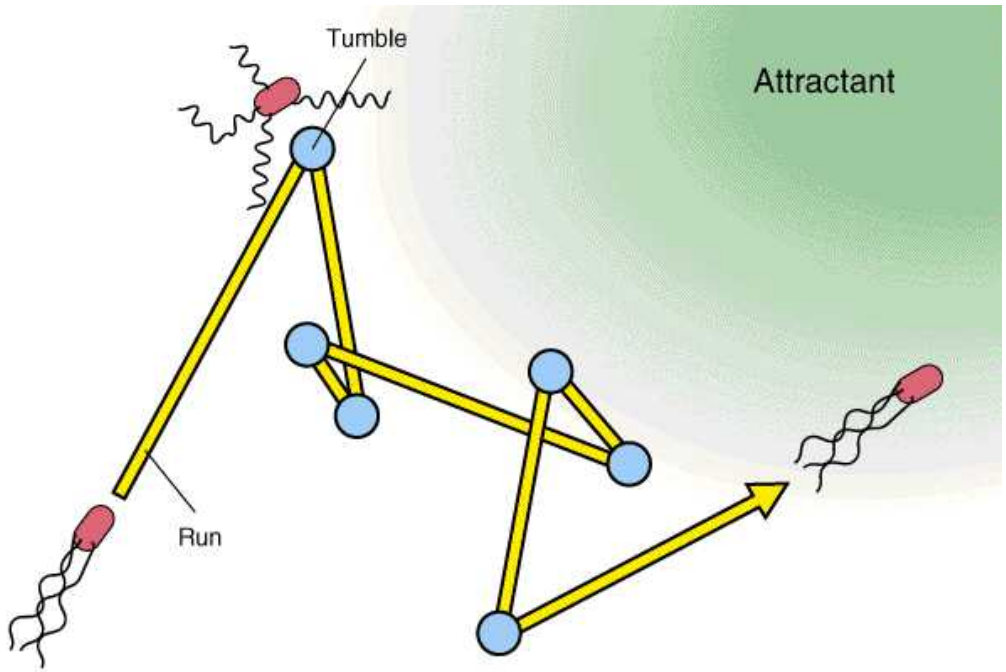


This movie is made by David Rogers.
Taken from website of Tom Stossel.

Absence of chemical attractant



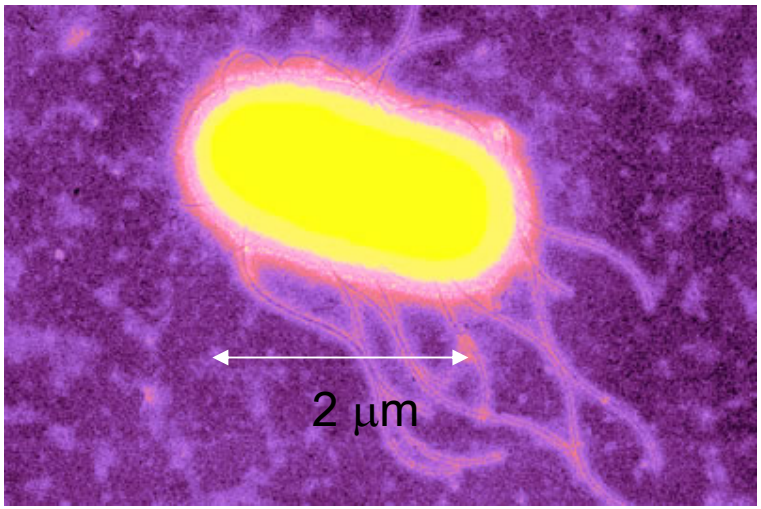
Presence of chemical attractant



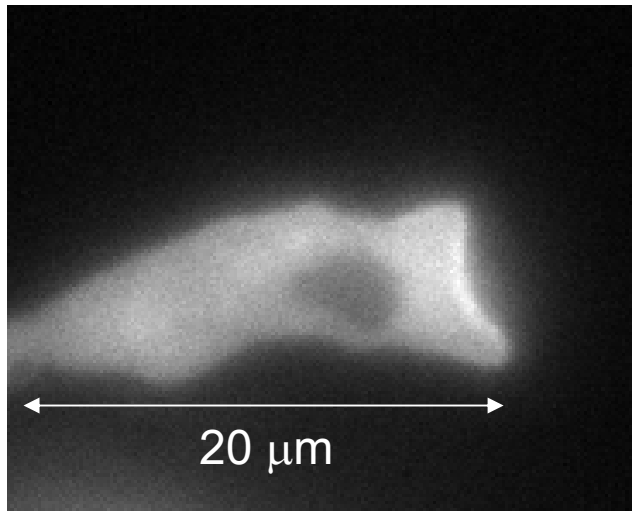
Temporal gradient sensing

Bacteria vs. Amoebae

E. coli



Slime mold amoeba



Bacteria (Prokaryote): Small

- Small compare to diffusion length
- Sample over time
- Biased random walk towards the food

Amoebae (Eukaryote): Large

- Larger cells
- Sample the periphery of the cell
- Directed motion towards the food

Objectives and long term goals:

1. By quantitatively exploring cue-dependent cell polarization, we will better understand the molecular mechanism of directed cell motility (chemotaxis)

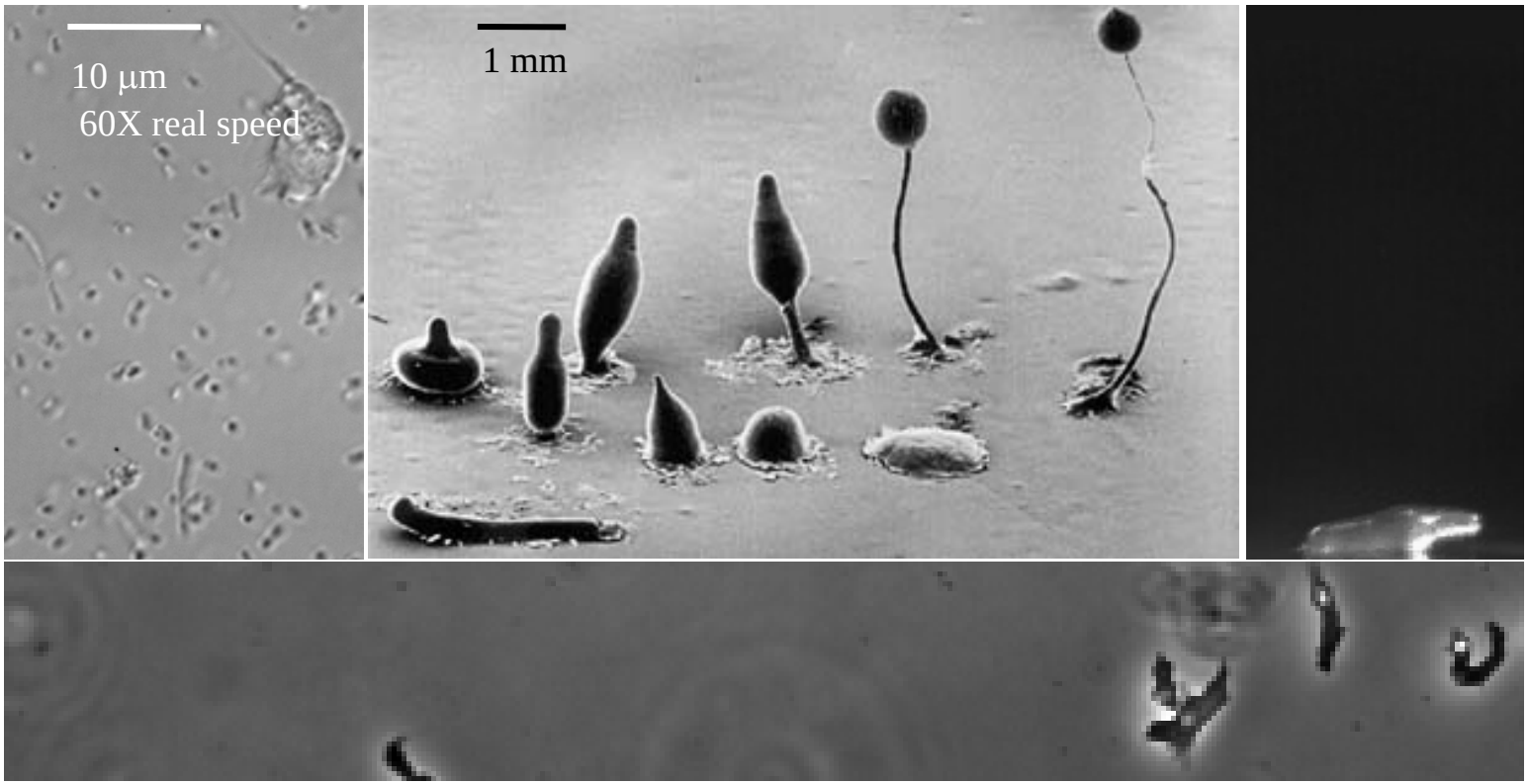
2. By understanding stochastic cellular behavior, we will improve our understanding of non-genetic individuality and its impact on the fitness of a population

Focus on ‘well characterized' biochemical networks in a ‘simple’ organism:

The model system: *Dictyostelium* (social amoeba)

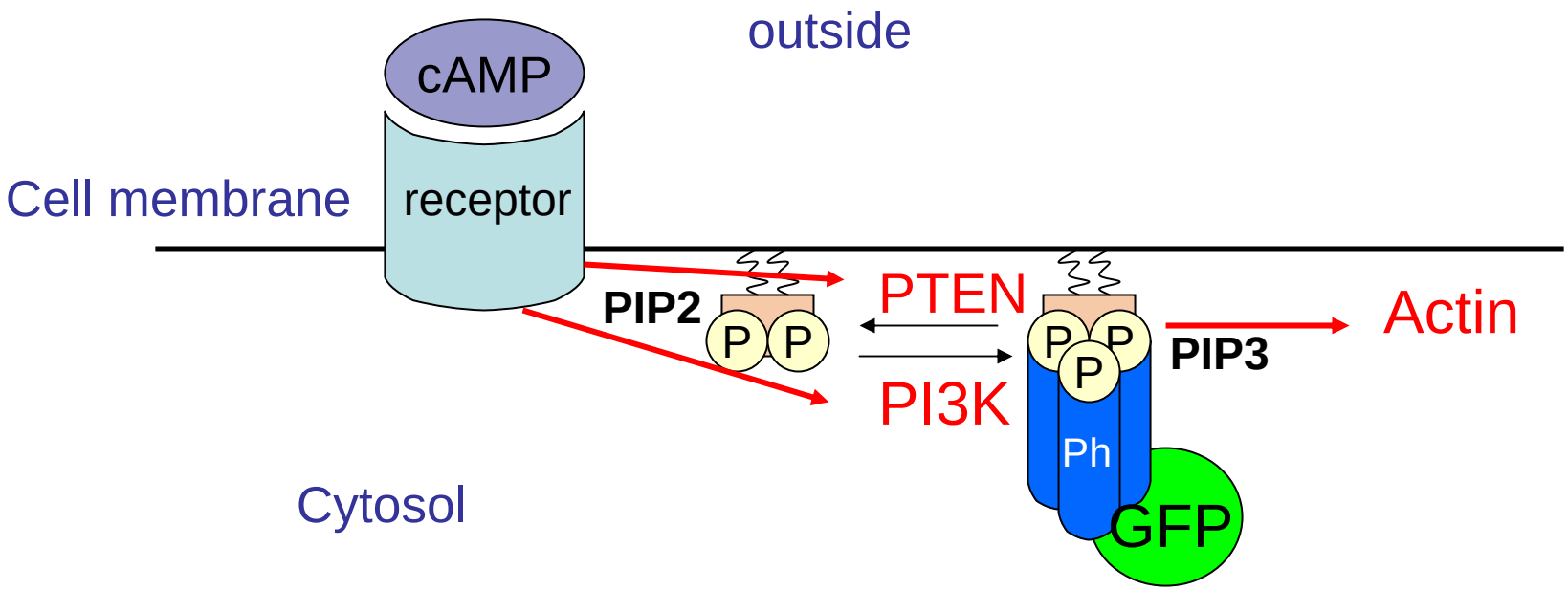
A model system: *Dictyostelium* (social amoeba)

An experimental model system for eukaryotic chemotaxis



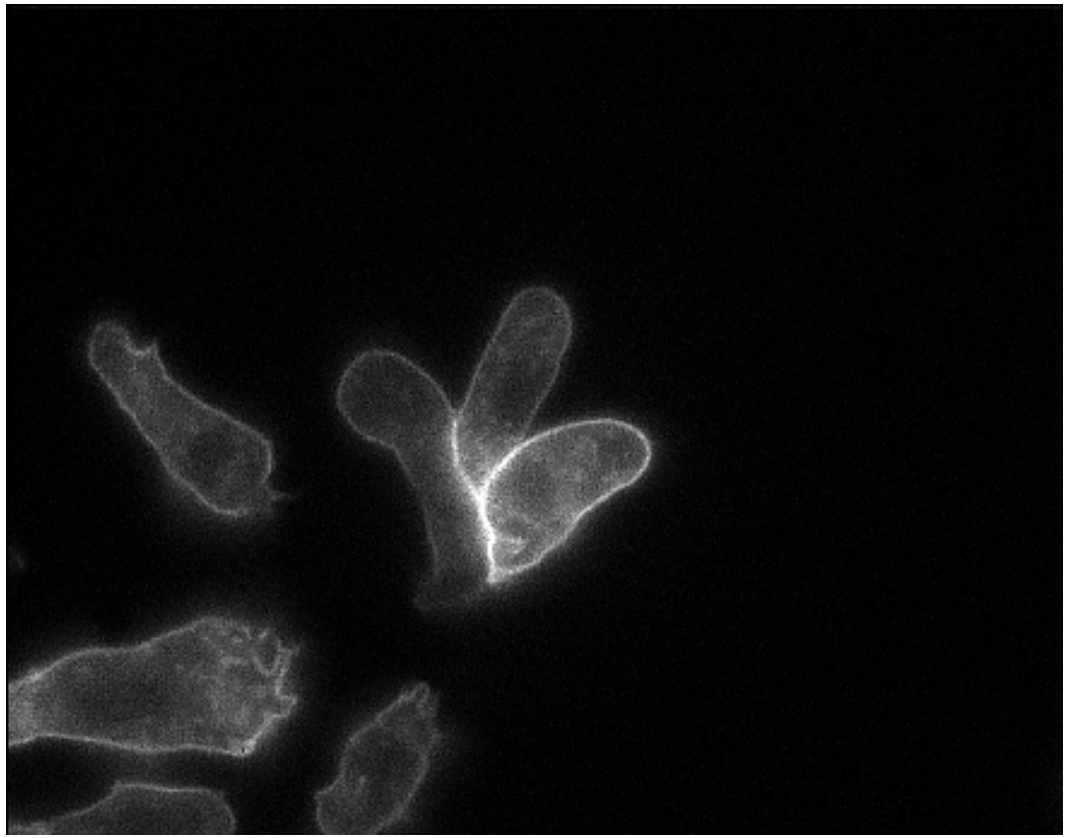
cAMP source

A well characterized biochemical networks



GFP indicates where the leading edge of a cell would be if the cell is able to move

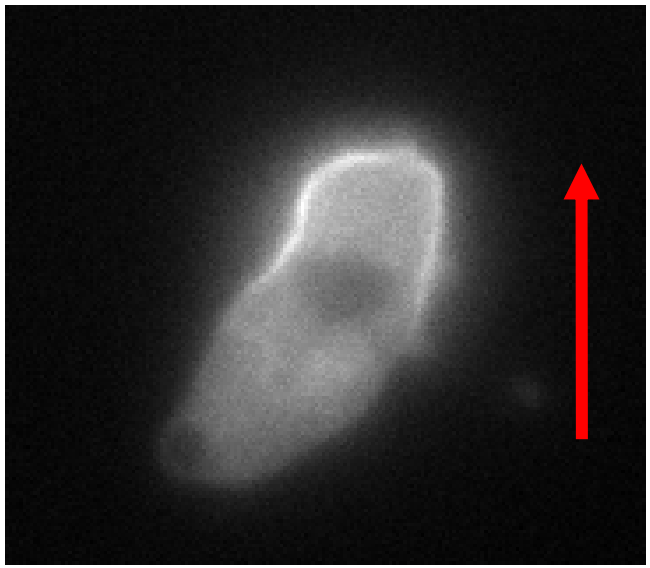
Receptor distribution is uniform around cell membrane



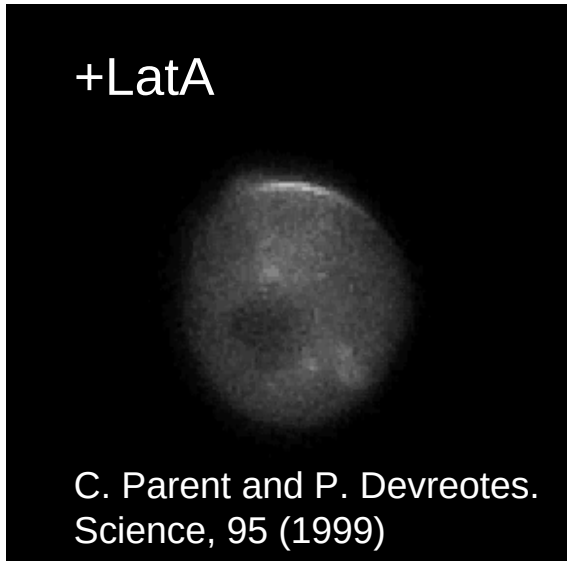
Movie taken from P. Devreotes website

Therefore asymmetric signaling must occur downstream of the receptors

PH-CRAC-GFP is a convenient reporter of the leading edge of a cell, even when cells are immobile



In a gradient, PH-CRAC-GFP accumulates to the leading edge of a cell

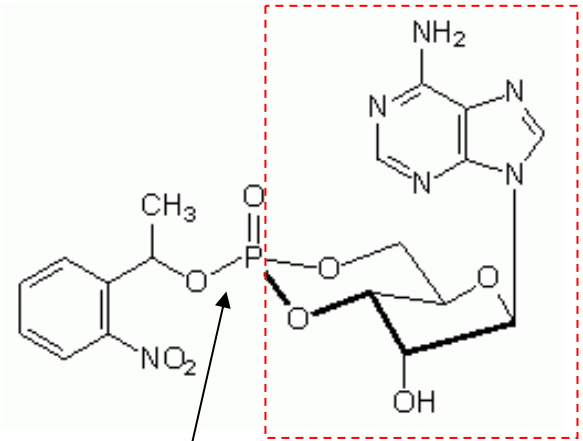


Gradient sensing can be separated from the movement

CRAC: Cytosolic Regulator of Adenylyl Cyclase

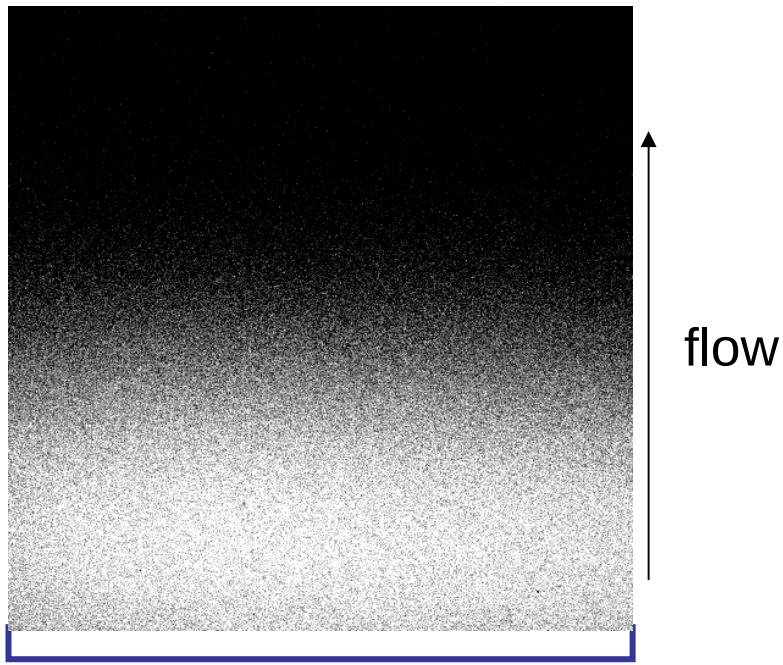
A different technology: UV induced uncaging of cAMP

Caged cAMP-inactive



Active cAMP

UV (360 nm) cleaves this bond

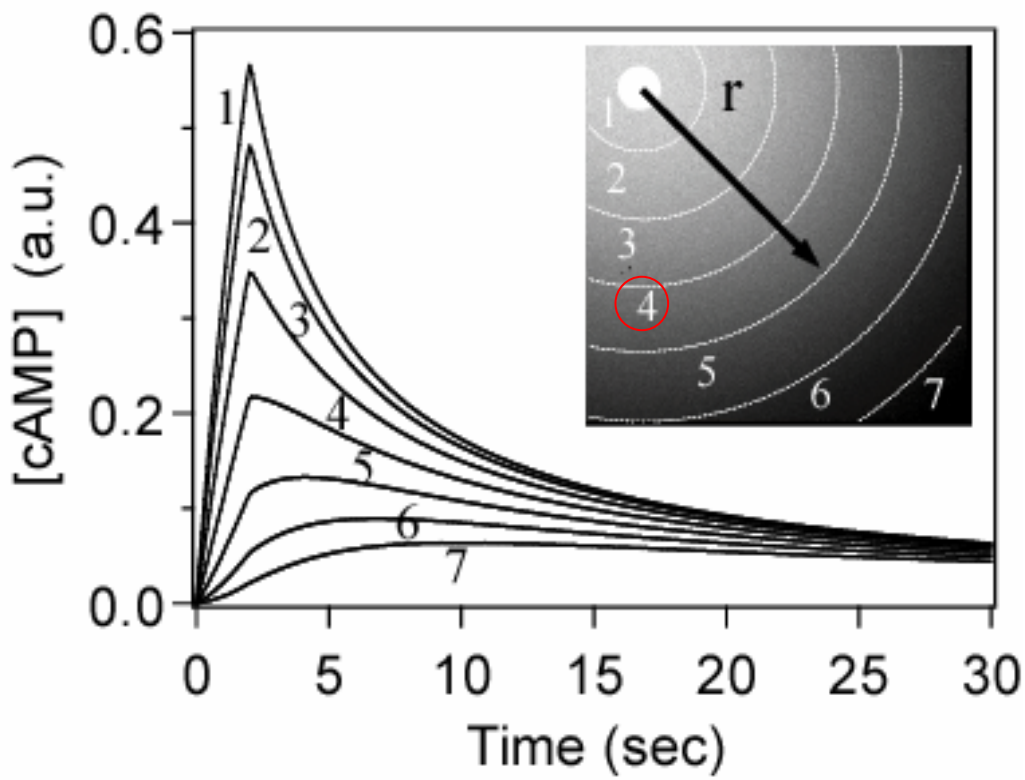


Main advantages:

-allows well defined cAMP pulses

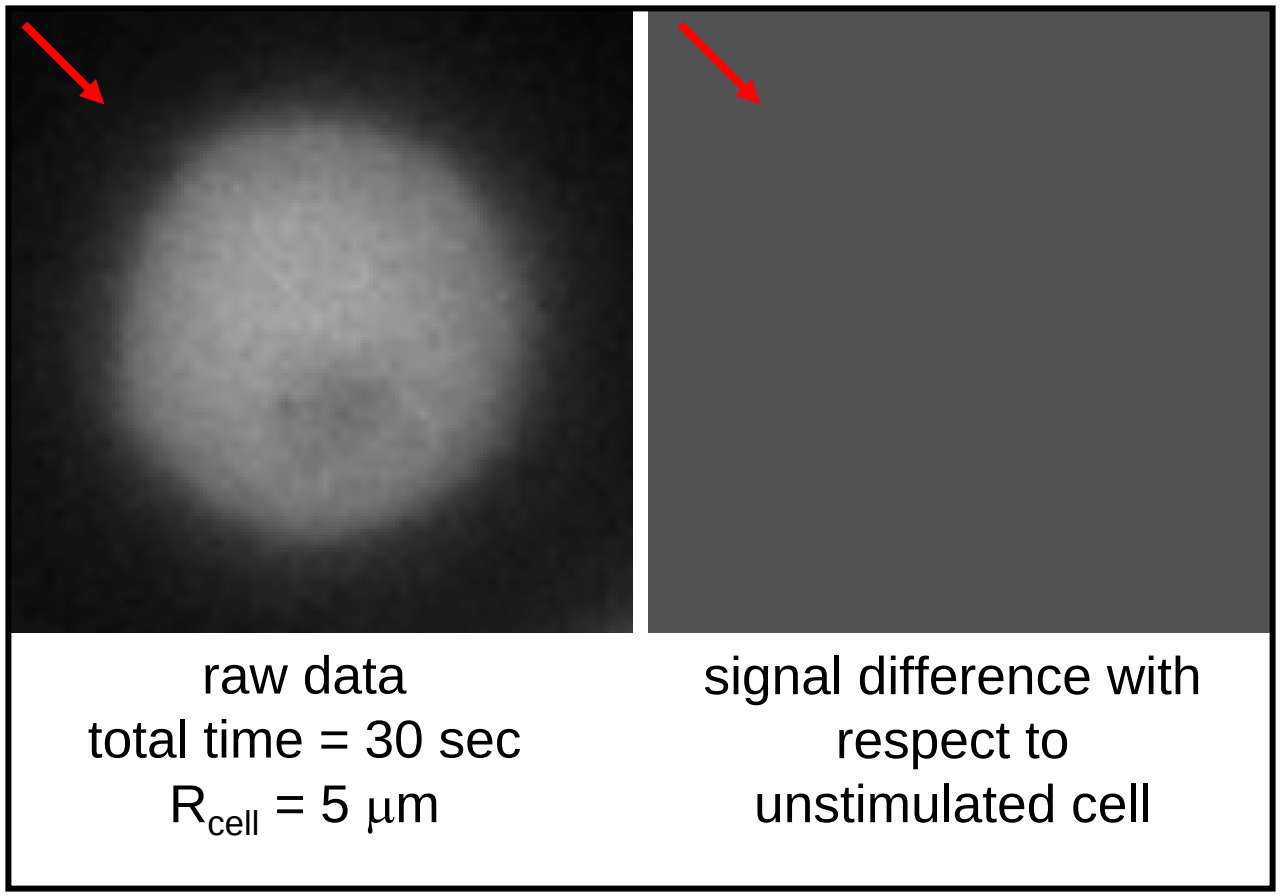
-pulses are reproducable

spatio-temporal cAMP concentration



$D_{cAMP} \sim D_{fluorescein} = 3.0 \times 10^{-6}\ cm^2/s$

Response of a single cell to a pulse

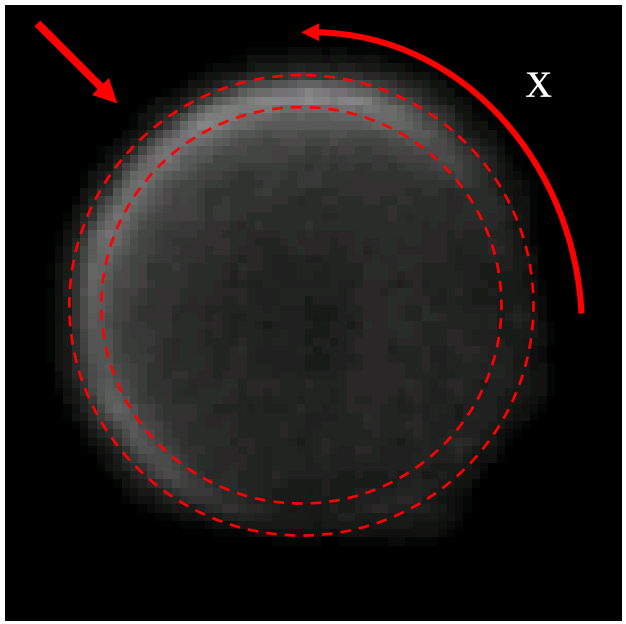


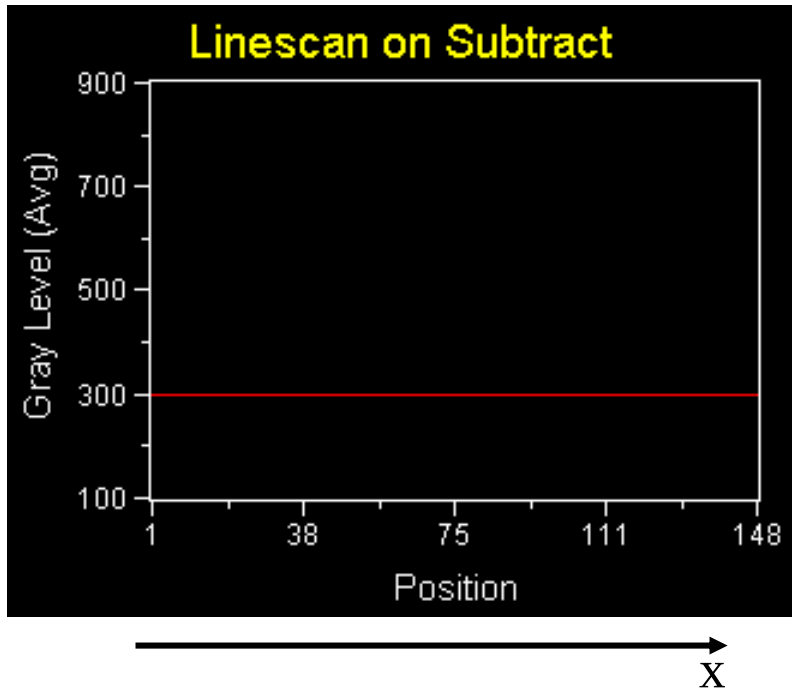
raw data
total time = 30 sec
R_{cell} = 5 μm

signal difference with
respect to
unstimulated cell

Response of the cell is polarized towards the direction of the pulse

Response of a single cell to a pulse



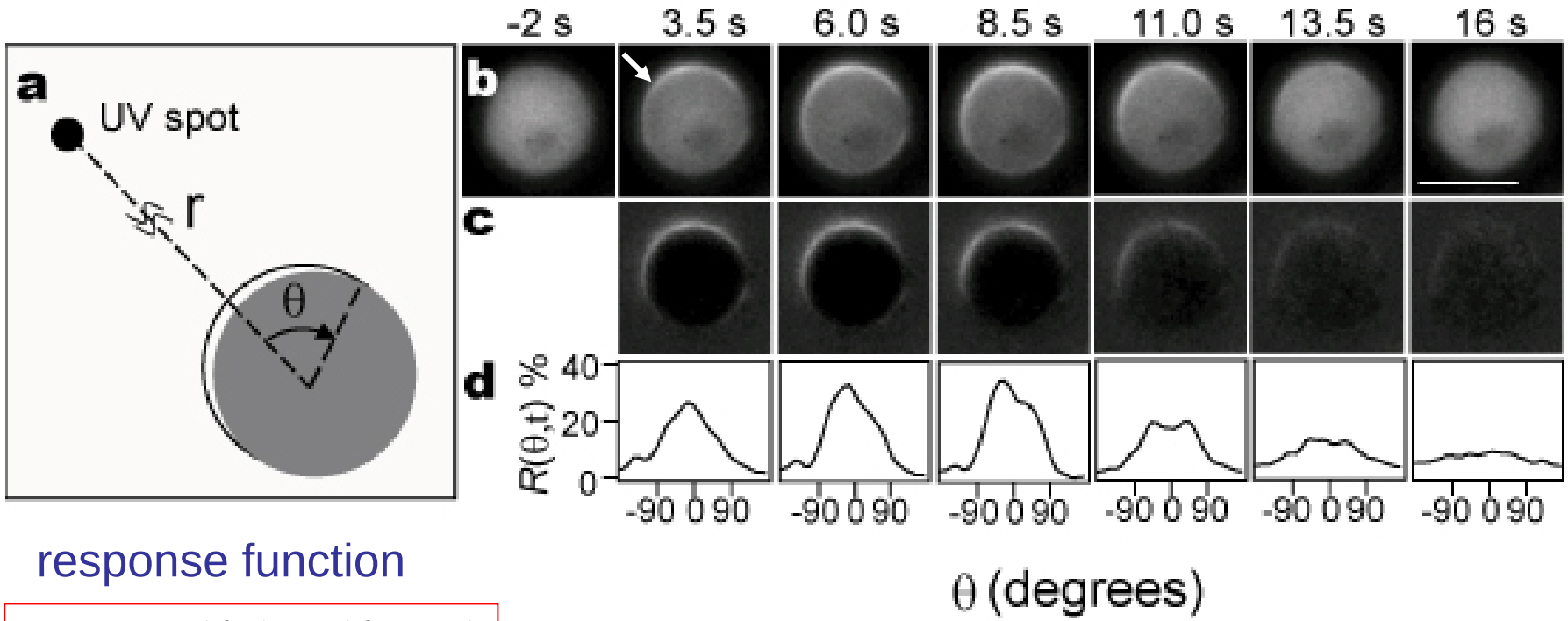


signal difference with respect to unstimulated cell

quantifying GFP concentration Along cell membrane

Maximum of the response ~ 8 seconds

Response of a single cell to a pulse

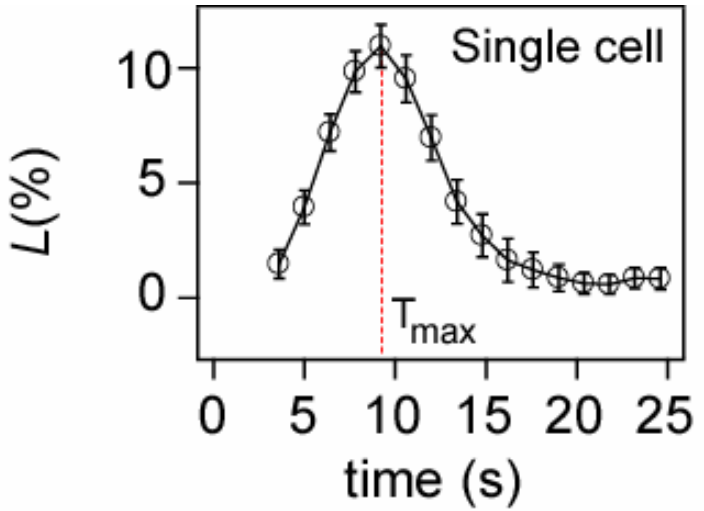


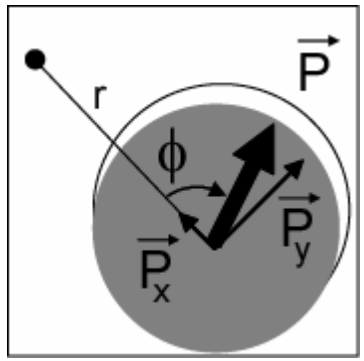
response function

$$R(\theta,t) = \frac{I(\theta,t) - I(\theta,t=0)}{I_{total}}$$

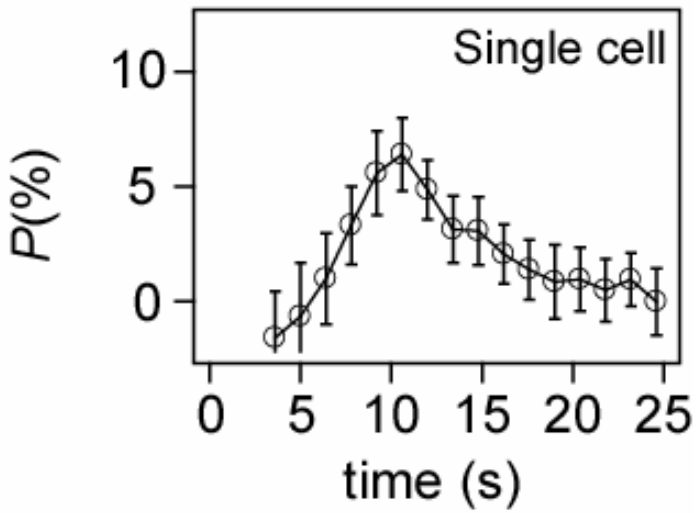
maximum of the response ~ 8 seconds

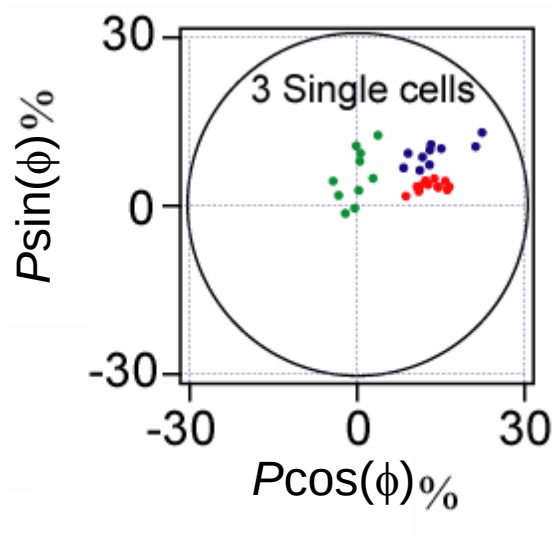
A single cell responds reproducibly to multiple pulses



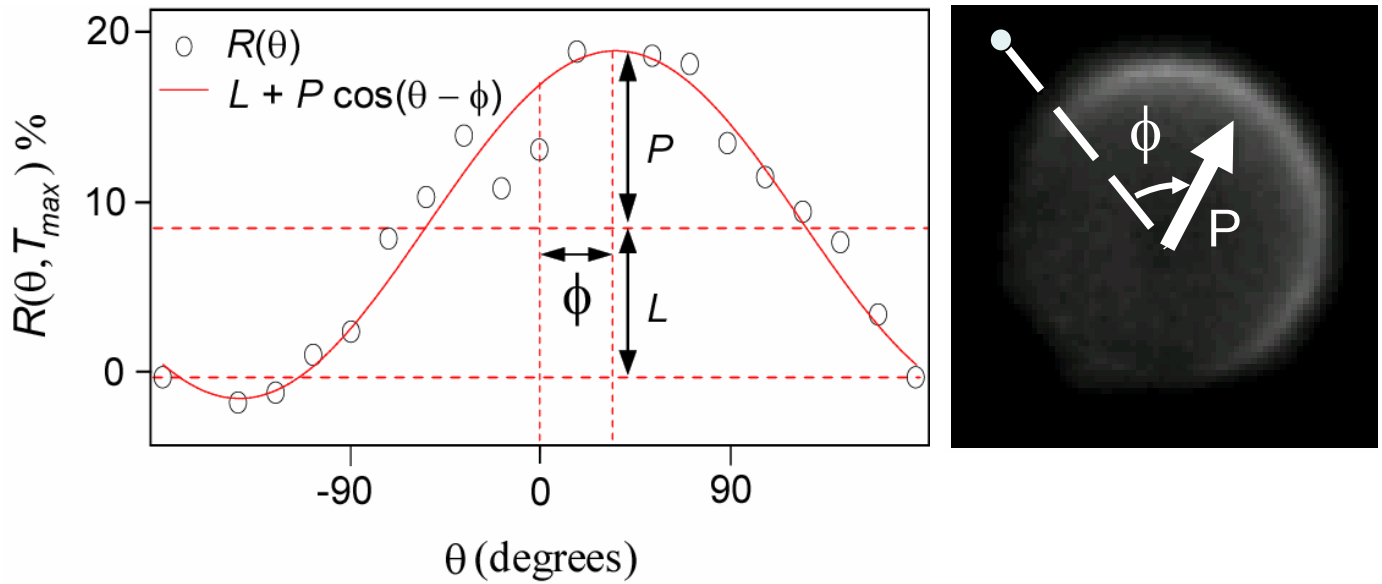


10 repeated stimulation for three single cells





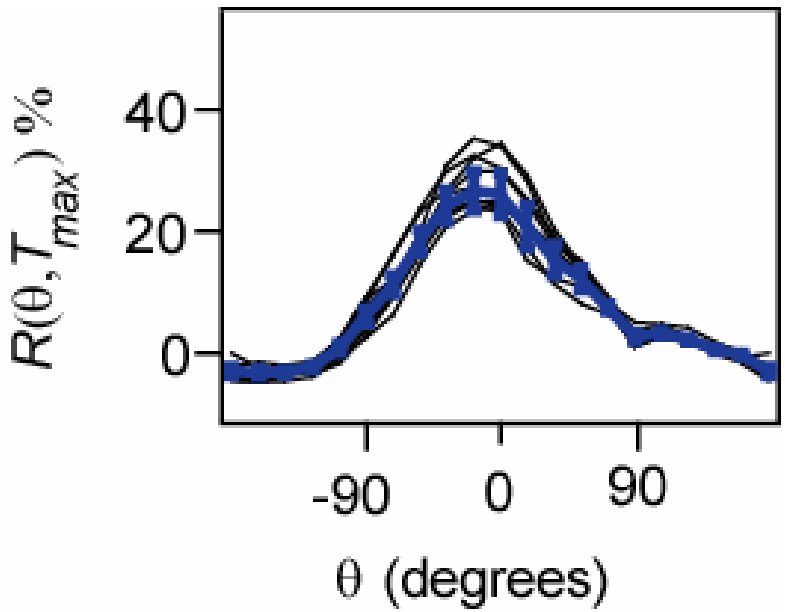
The response function can be characterized with 3 parameters



1) Localization mean of the response function
2) Polarization amplitude of the response function
3) Polarization angle direction of the maximum response

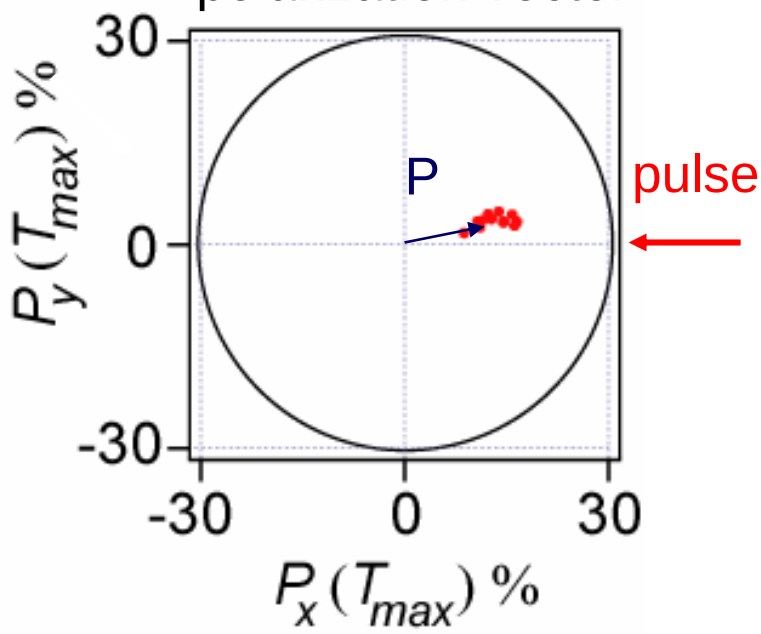
A single cell responds reproducibly to multiple pulses

10 repeated stimulation of the same cell



the error bars denote standard deviations

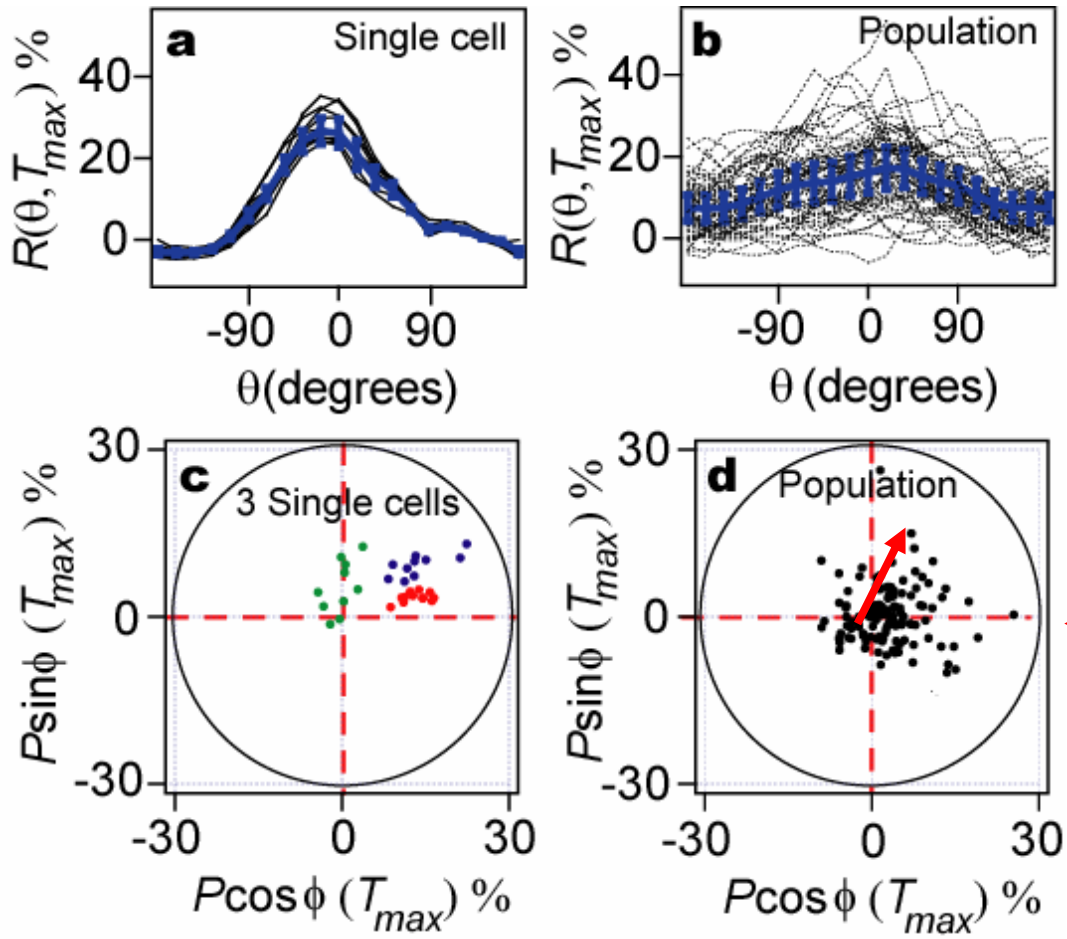
polar plot of the polarization vector



the pulses are separated by 2 minutes

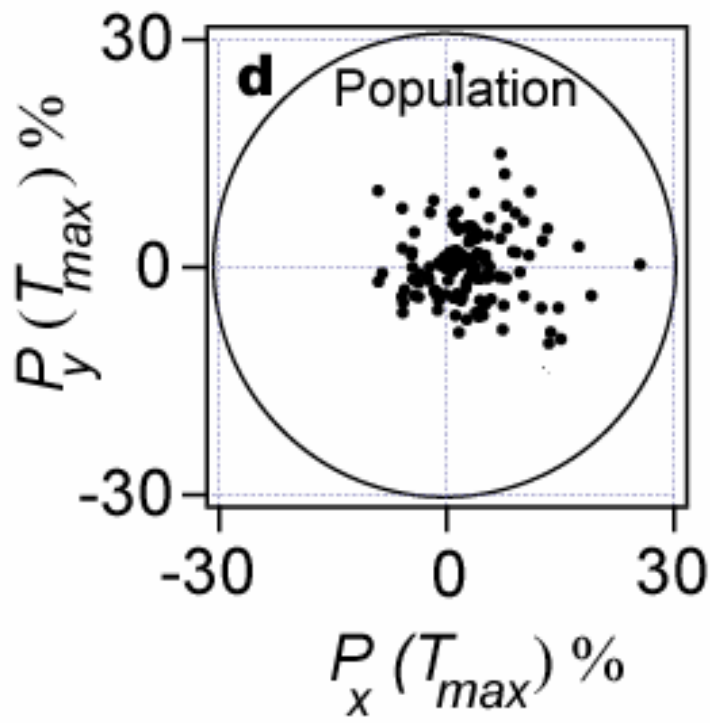
Response to the same pulse vary significantly from cell-to-cell

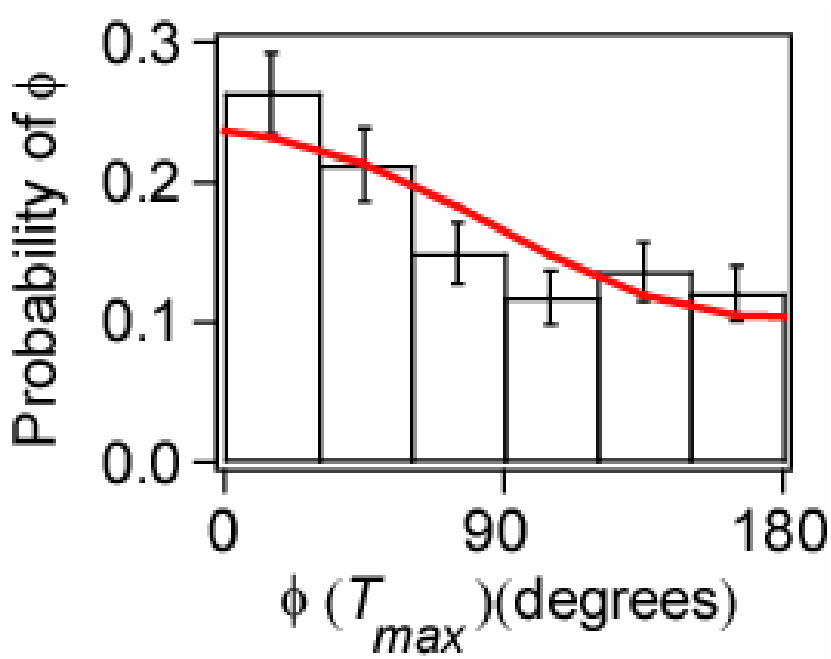
Single cell vs. Population



Single cell - 10 pulses 100 cells - 1 pulse

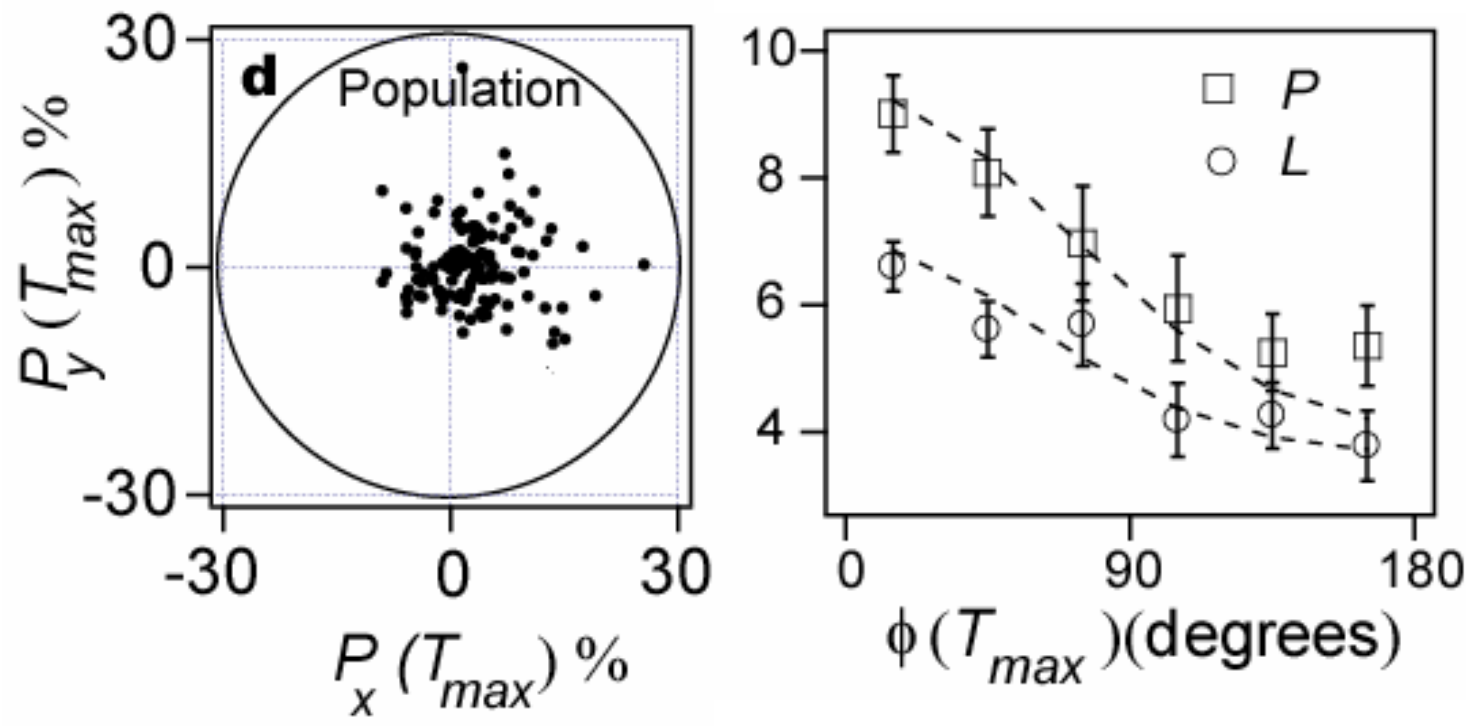
The population correctly detects the pulse direction





more cells polarize in the direction of the pulse $\phi = 0$

The magnitudes of *L* and *P* correlate with ϕ



"Right cells" (ϕ = 0) larger localization; stronger polarization

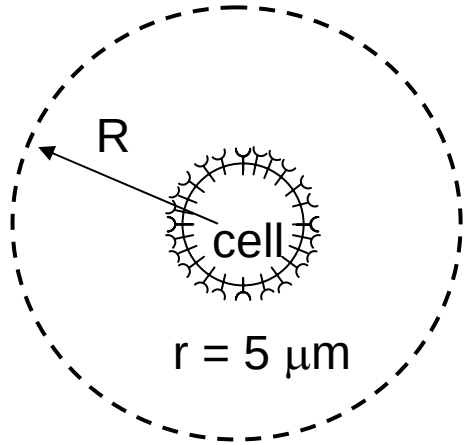
"wrong" cells (ϕ = 180) smaller localization; weaker polarization

Can we reduce the noise by increasing the signal?

If the bound state of the receptor t ~ (1- 2 sec)

D (of cAMP) = 10^{-6} cm^2/s
R ~ $(D\ t)^{1/2}$ = 10^{-3} cm ~ 10 μm

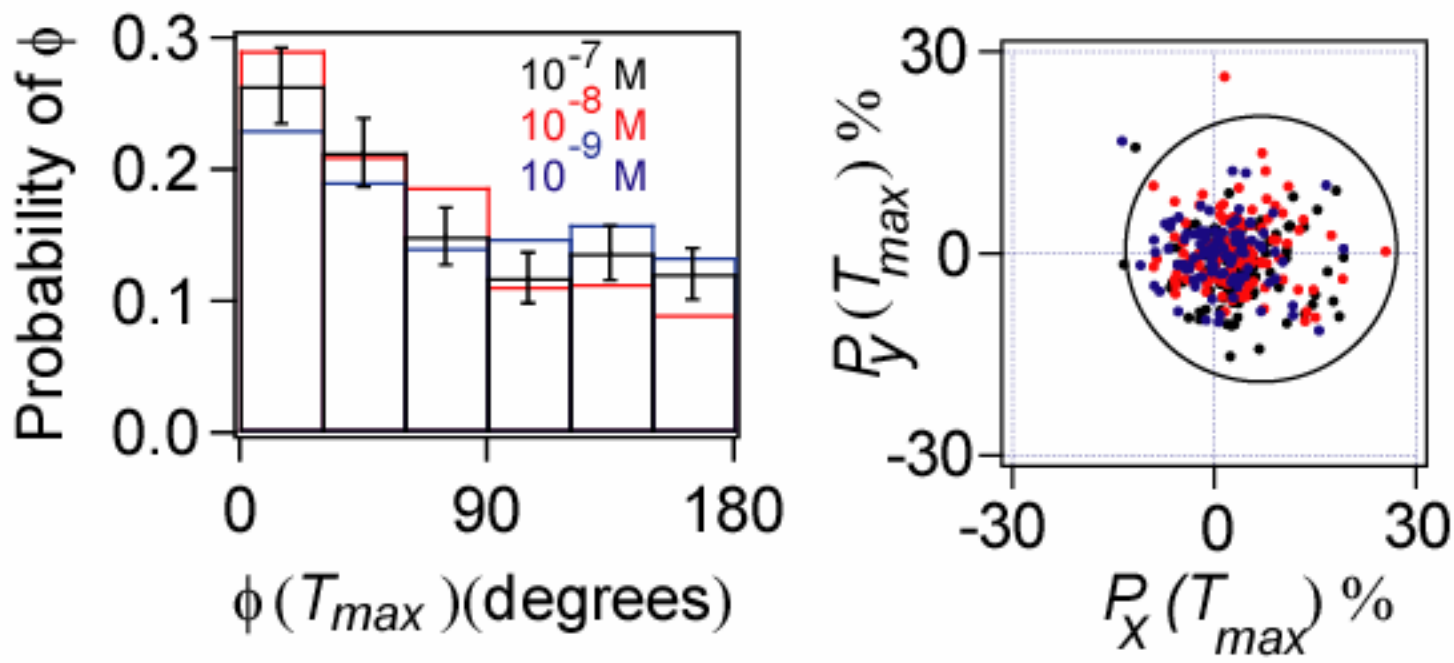
There are between $5x10^4$ to 10^5 receptors/cell



In the sampling volume there are:

C	molecules	molecule/receptor	Noise/signal $\frac{1}{\sqrt{N}}$
10^{-10} M	6 x 10^2	0.01	cells do not respond
10^{-9} M	6 x 10^3	0.1	1%
10^{-8} M	6 x 10^4	1	0.5%
10^{-7} M	6 X 10^5	10	0.1%
10^{-6} M	6 X 10^6	100	0.01%

The noise in directional sensing does not decrease by increasing the external concentration



The origin of symmetry breaking must be interacellular

Summary of the main experimental observations

- The response of a single cell is reproducible from pulse-to-pulse
- The response of cells within population vary greatly from cell -to-cell
- On average the population finds the correct direction of the pulse
- Individual cells polarizing in the right direction have about two-folds larger localization and polarization than cells that polarization in the wrong direction
- The origin of the noise must be intaracellular

How can we explain the variability?

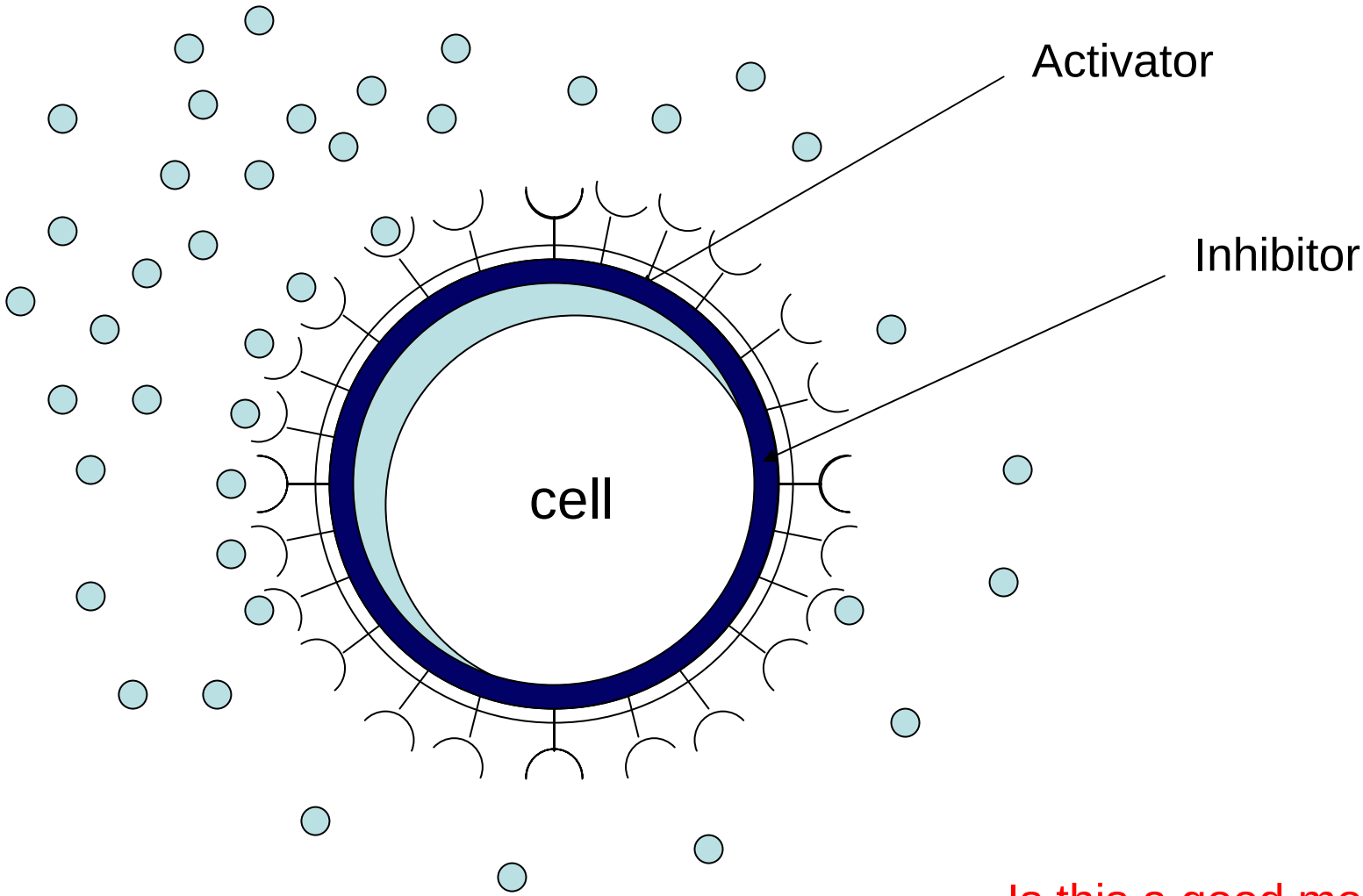
Models

Local excitation and global inhibition of the signal

Activator	Inhibitor
Diffuses slowly	Diffuses rapidly
Local (leading edge)	Global (front, back and sides)

- Diffusion-Translocation, Postma, van Haastert, Biophys. J. (2001)
- Receptor-Regulated phospholipid dynamics, Narang, Subramanian and Laufenberger, Annals of Biomed. Eng. (2001)
- Inhibitor-Diffusion, Rappel, Thomas, Levine and Loomis, Biophys. J. (2002)
- Local excitation- Global Inhibition, Iglesias and Levchenco, Biophys. J. (2002)

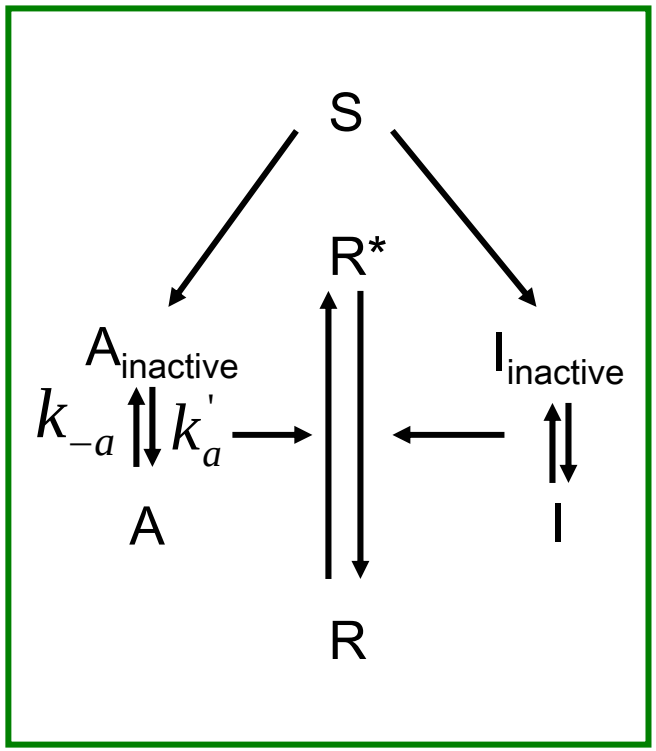
Mechanism: Local Excitation-Global Inhibition



Is this a good model?

Local Excitation-Global Inhibition Model (LEGI)
Activator Equations

Iglesias and Levchenco (2002)



Local Activator

$$\frac{dA}{dt} = k_a' S(A_{tot} - A) - k_{-a} A$$

$$A_{inactive} + A = A_{tot}$$

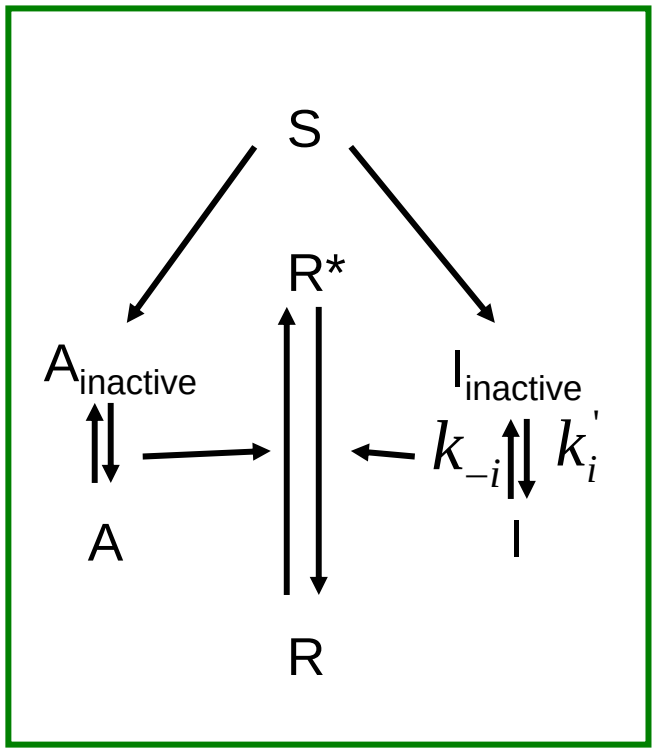
$$A << A_{tot}$$

$$k_a = k_a' A_{tot}$$

$$\frac{dA}{dt} = -k_{-a} A + k_a S$$

Local Excitation-Global Inhibition Model (LEGI): Inhibitor Equations

Iglesias and Levchenco (2002)



Global Inhibitor

$$\frac{dI}{dt} = -k_{-i} I + k_i' S (I_{tot} - I)$$

$$I_{inactive} + I = I_{tot}$$

$$I << I_{tot}$$

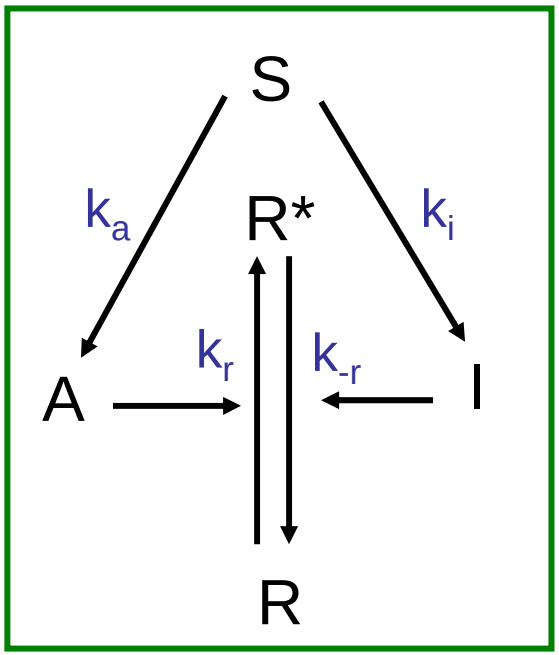
$$k_a = k_a' I_{tot}$$

diffusion

$$\frac{dI}{dt} = -k_{-i} I + k_i S + k_d \frac{d^2 I}{dx^2}$$

Local Excitation-Global Inhibition Model

Iglesias and Levchenco (2002)



$$\frac{dA}{dt} = -k_{-a}A + k_a S$$

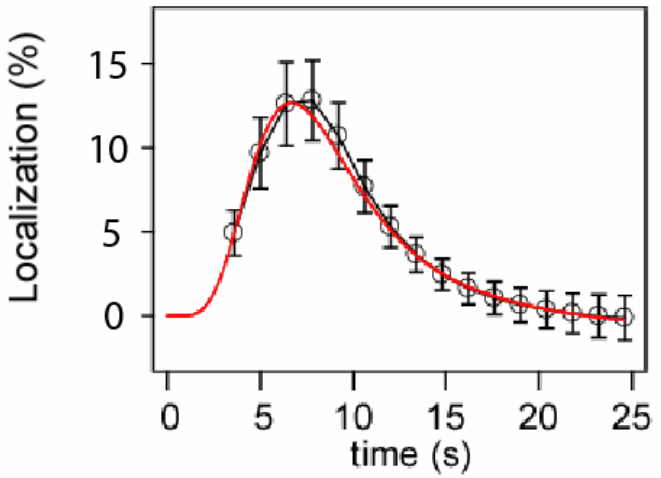
$$\frac{dI}{dt} = -k_{-i}I + k_i S + k_d \frac{d^2 I}{dx^2}$$

$$\frac{dR^*}{dt} = -k_{-r}IR^* + k_r RA$$

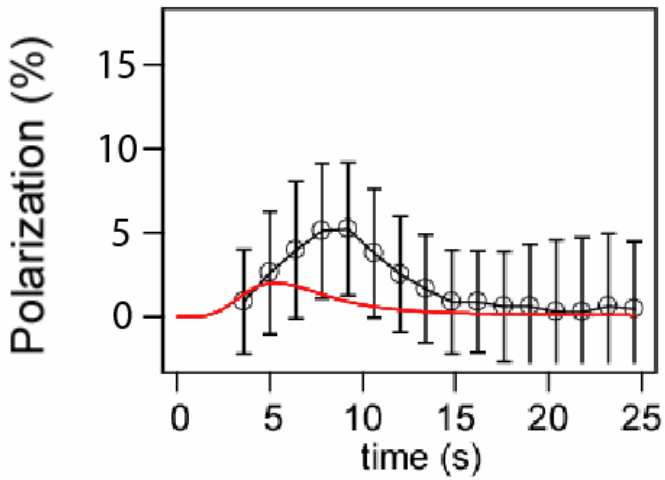
Activator
Inhibitor

Slow diffusion
Fast diffusion

Localization dynamics can be reproduced by the LEGI model



LEGI model fits the average and the dynamics of the localization fairly well



LEGI predicts a smaller polarization than observed experimentally

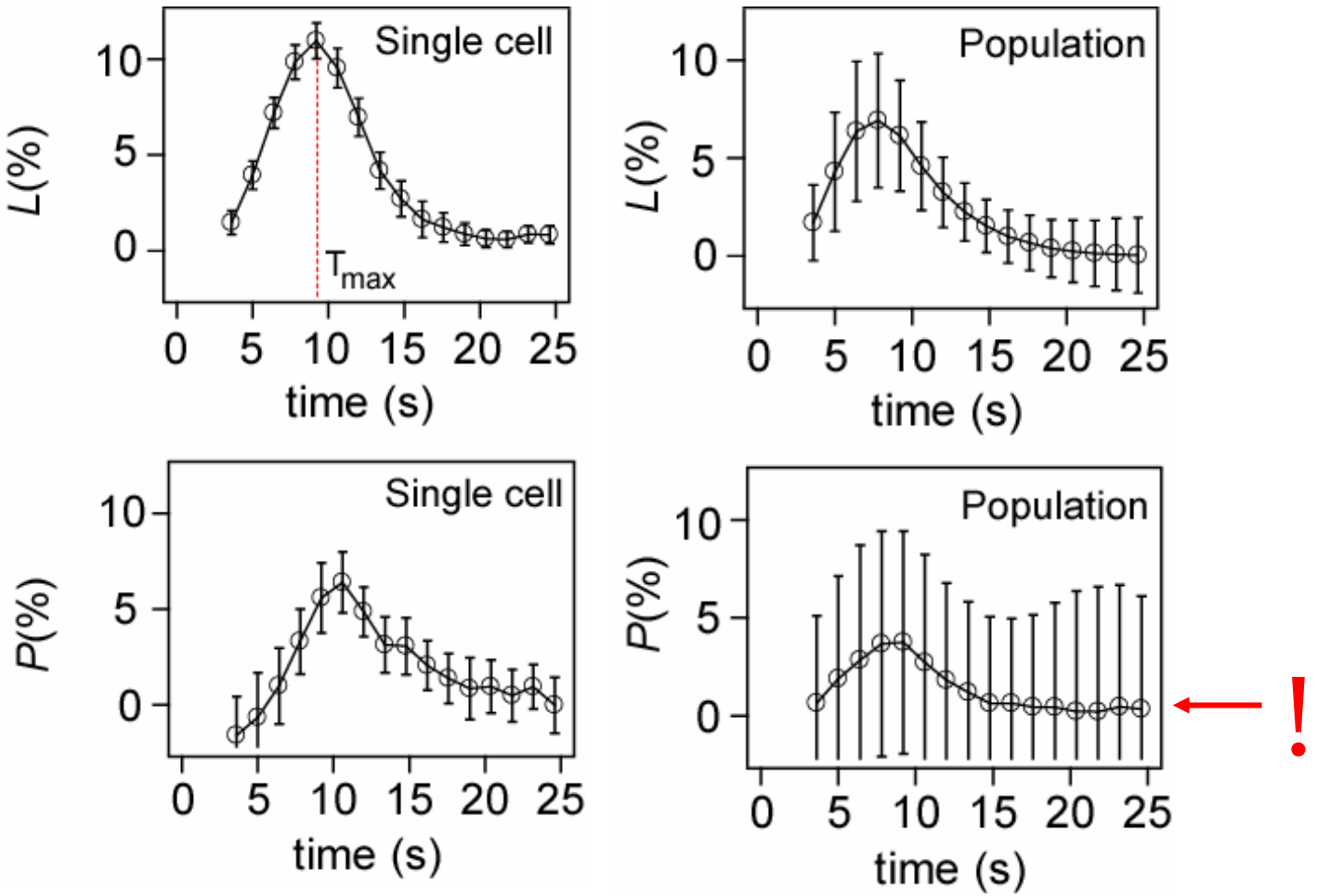
Problems with the LEGI models

- The model reproduce the average and dynamics of localization (not polarization) fairly well.
- Every single cell (according to the model) will polarize in the direction of the external gradient
- There is no allowance for stocasticity in the LEGI model

What can we do to improve on LEGI models?

pulse-to-pulse variability of a single cell

cell-to-cell variability of a population

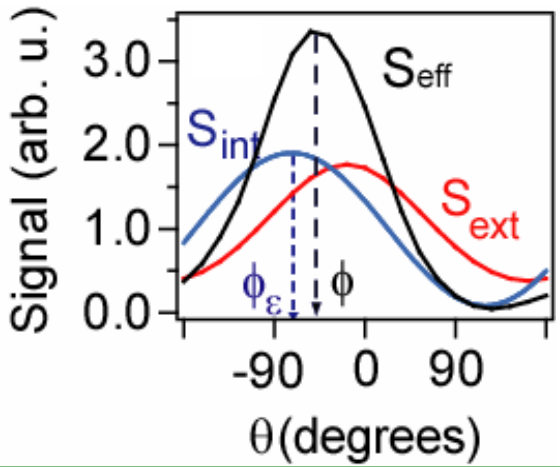


The error bars denote standard deviations, which increase 5 fold from single cell to population

The geometric Model

Proposal: $S = S_{ext} \times S_{\text{int}}$

$$S_{ext} = S_0 + S_1 \cos(\theta)$$
$$S_{\text{int}} = 1 + \varepsilon \cos(\theta + \phi_\varepsilon)$$

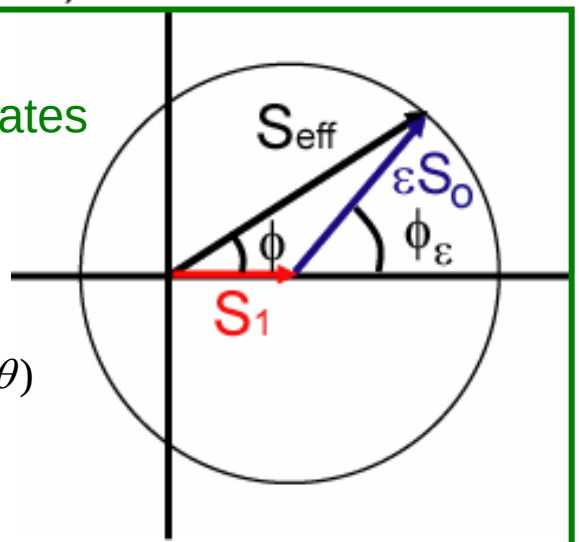


external (dynamic) **internal (static)**

$$S = \underbrace{\left(S_0 + S_1 \cos(\theta)\right)}_{\text{external (dynamic)}} \times \underbrace{\left(1 + \varepsilon \cos(\theta + \phi_\varepsilon)\right)}_{\text{internal (static)}}$$

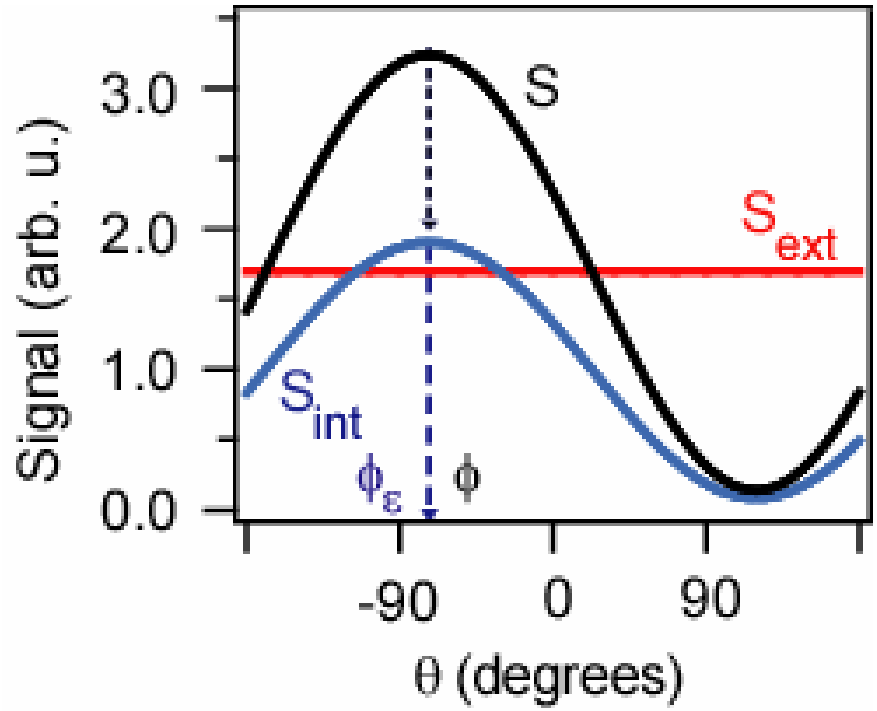
$$S = \left(S_0 + \frac{\varepsilon}{2} S_1 \cos(\phi_\varepsilon)\right) + \underbrace{\left(S_1 + \varepsilon S_0 \cos(\phi_\varepsilon)\right)}_{\sim P_x} \cos(\theta) - \underbrace{\left(\varepsilon S_0 \sin(\phi_\varepsilon)\right)}_{\sim P_y} \sin(\theta)$$

polar coordinates



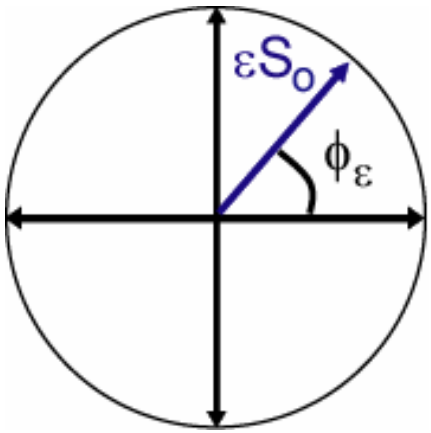
What happens in the case of a uniform external stimulation? $S_1 = 0$

First order prediction of the geometric model

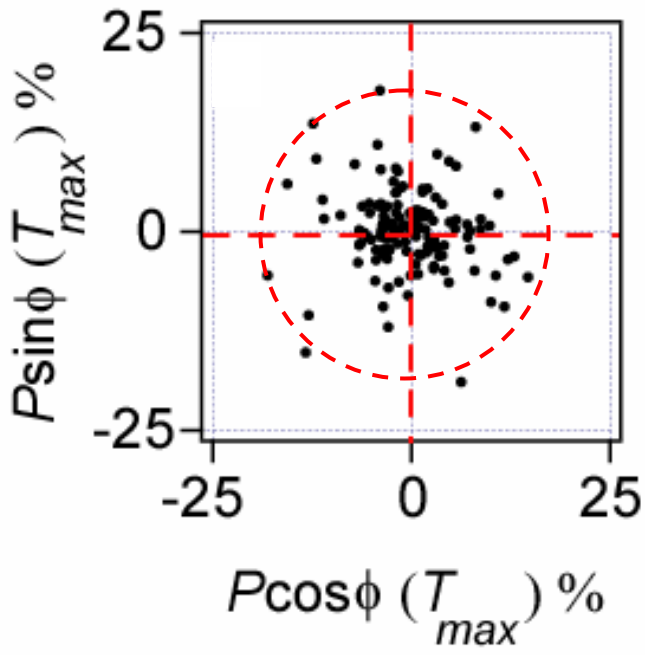


Geometric model allows for symmetry breaking even in the case of uniform stimulation

A uniform external stimulation

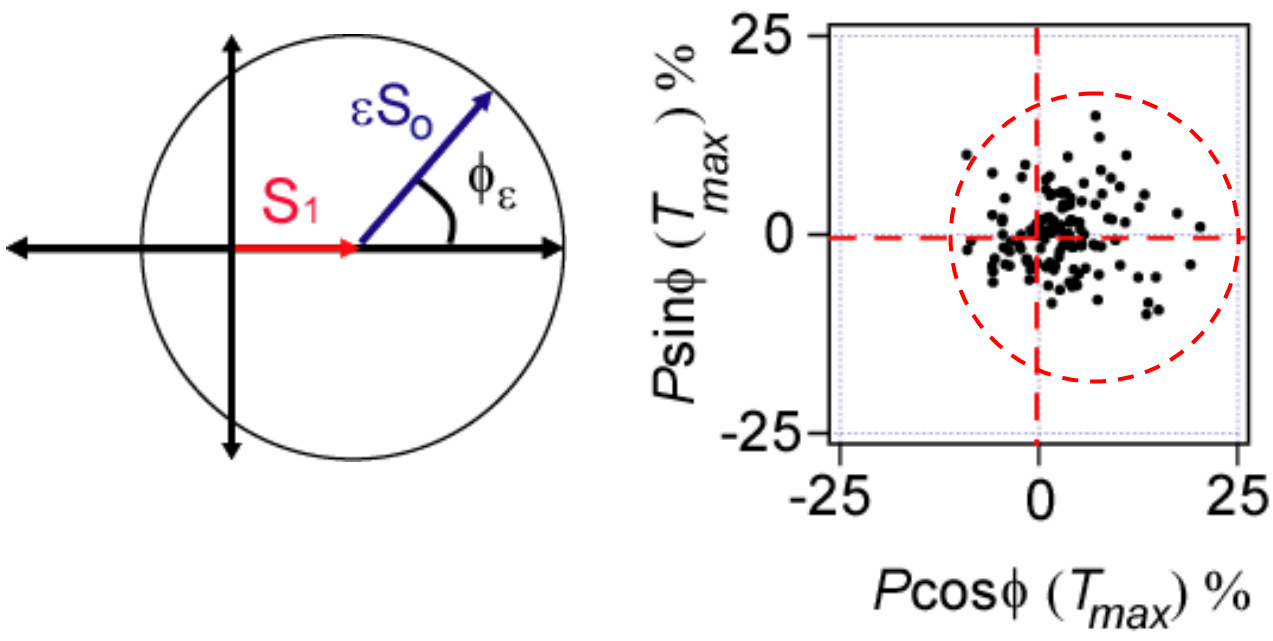


$S_1 = 0$



The distribution of polarizations are uniform as predicted by the geometric model

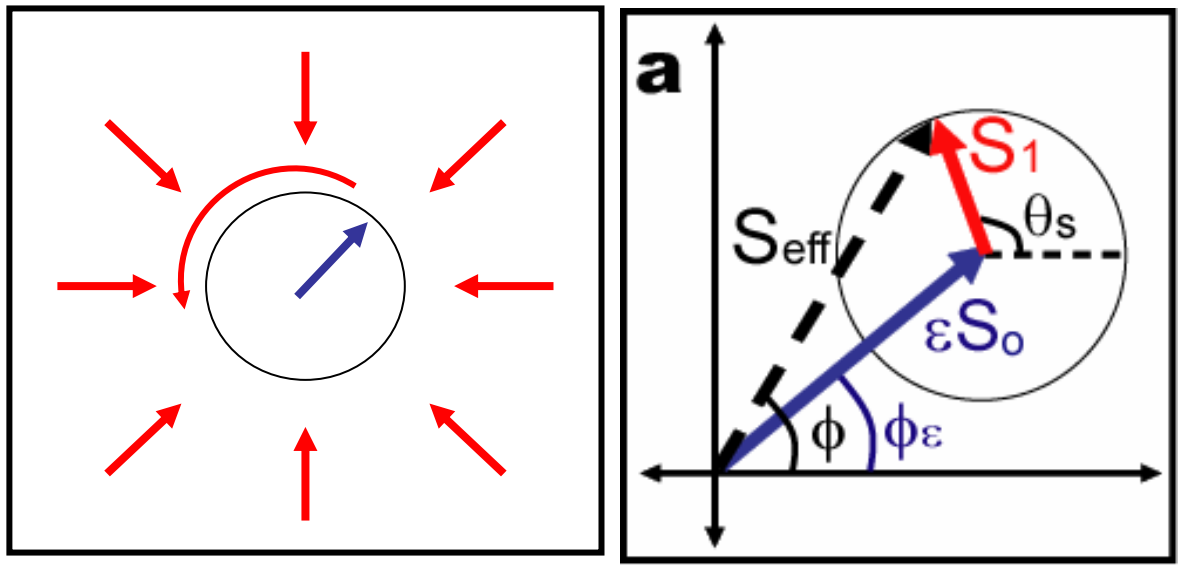
A directed pulse



The distribution of polarizations are shifted toward the direction of the external pulse

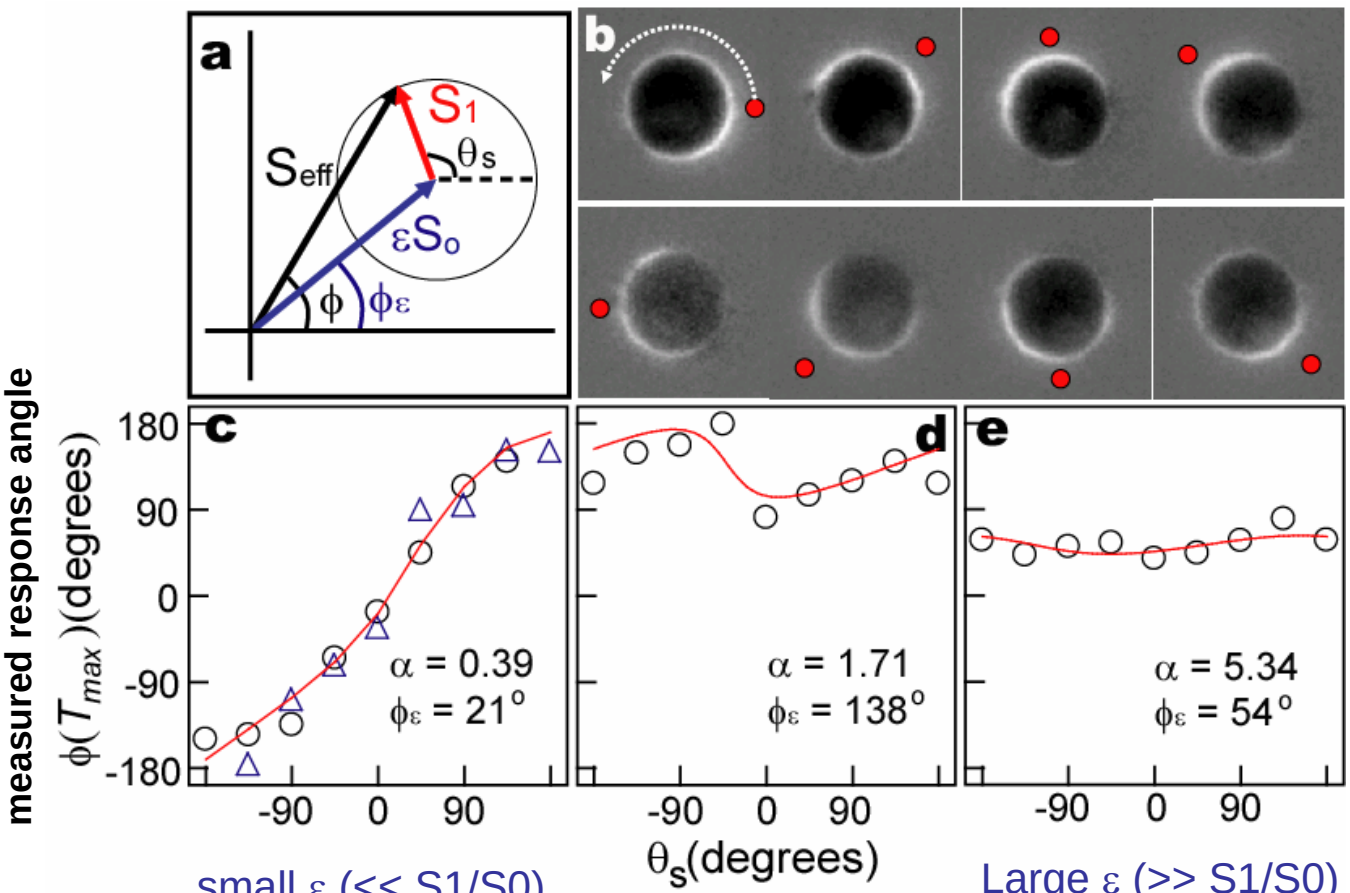
Proposed Experiments:

Moving the external source around the cell



internal signal (static)
external signal (dynamic)

Geometric model fits the data with only two fitting parameters $\alpha = \varepsilon S0/S1$ and ϕ_ε

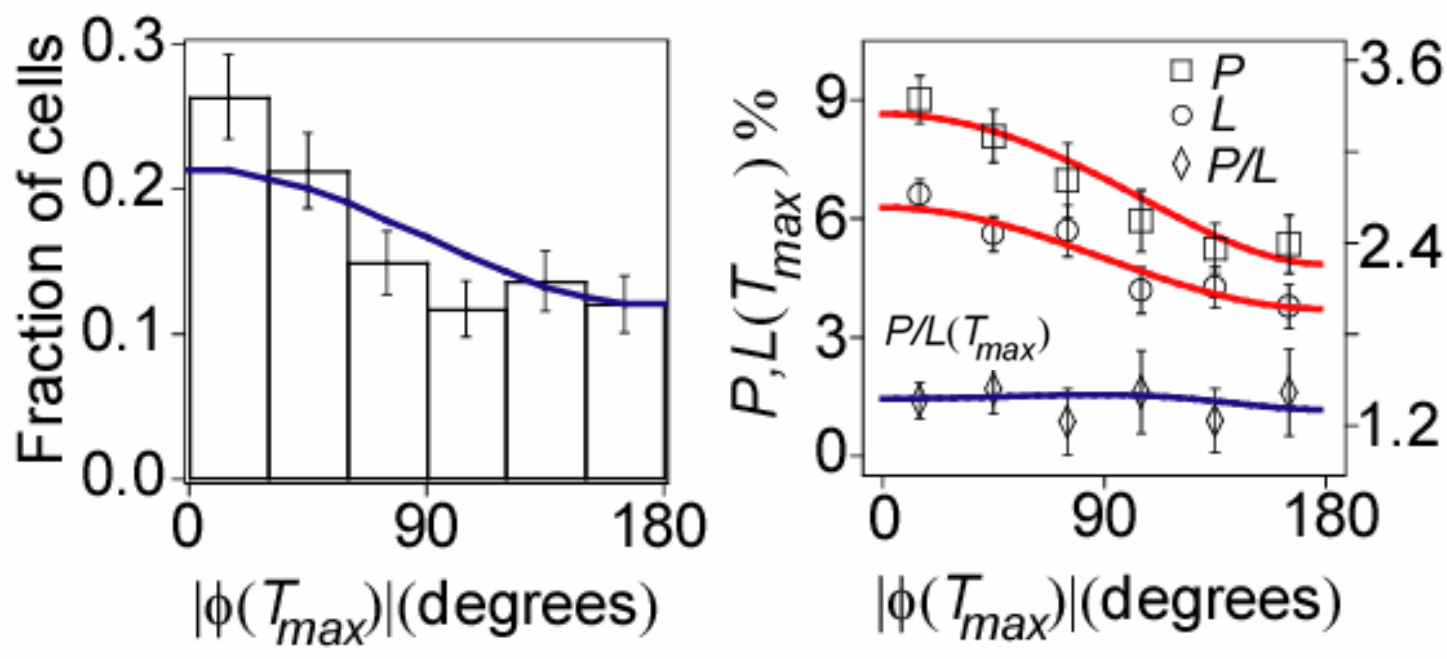


small ε (<< S1/S0) S_{eff} will follow the extracellular signal exactly ($\phi \approx \theta s$)

Intermediate ε (~ S1/S0)

Large ε (>> S1/S0) the S_{eff} stays in the direction of the internal signal ignoring the extracellular signal ($\phi \approx \phi\varepsilon$)

Geometric model can quantitatively predicts the fraction of cells that polarize in a specific direction



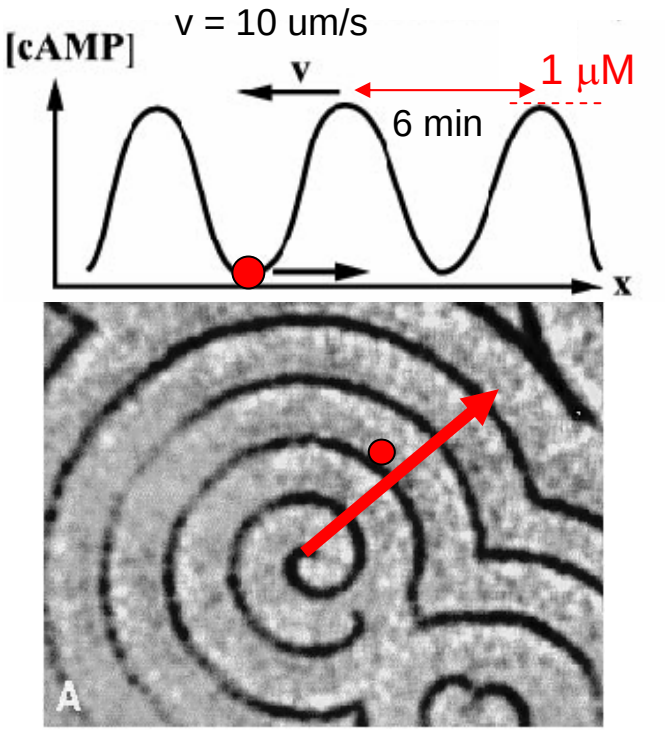
Using measured average value of α from our population measurements geometric model quantitatively predicts the relation between mean localization and polarization, with the polarization angle

Summary

- The response of a single cell is highly reproducible from pulse-to-pulse
- In contrast, a large variability is observed from cell-to-cell
- Geometric model successfully predicts the observed variability
- This observed variability is the results of variation in the spatial localizations of the proteins inside a cell and cannot be explain only by the fluctuations in the number of signaling molecules from cell-to-cell

Other interesting questions:

- Single *dictyostelium* cells communicate with each other through pulses of cAMP
- Cells demonstrate rectified motion in response to traveling pulses of cAMP



Dark field waves of Dictyostelium cells (Lee, Goldstein and Cox)

1- Why do cells show rectified motion?

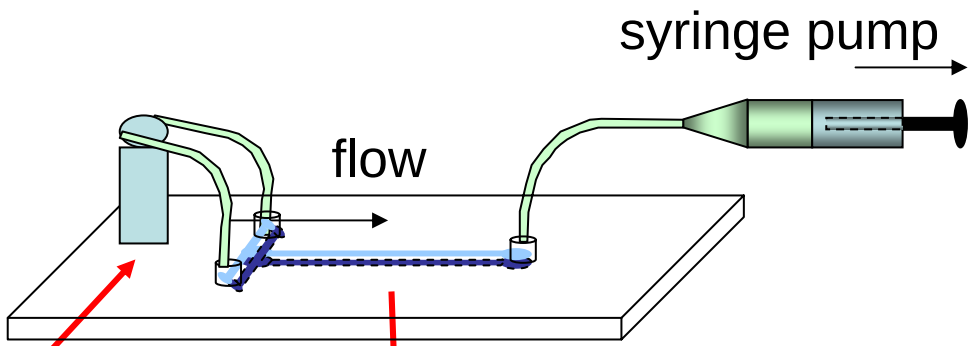
2- How does the response of cells vary as a function of pulse frequency?

3- how do cells respond to periodic vs. chaotic or aperiodic stimuli?

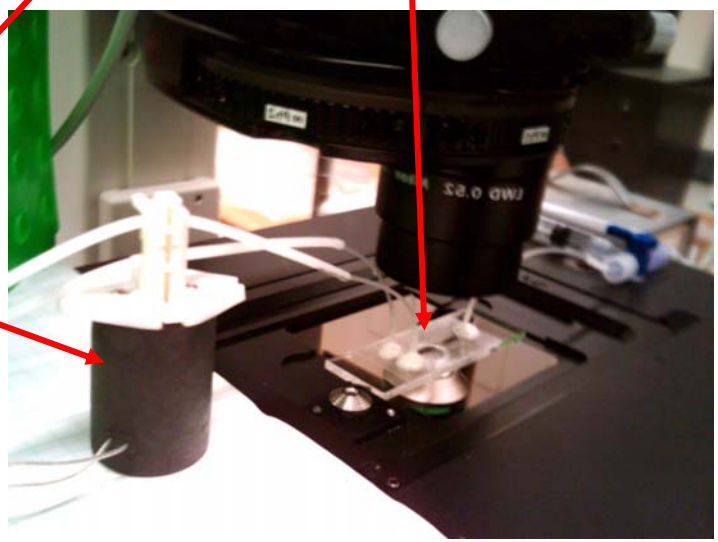
4- How does the chemotactic response vary by changing the adaptation time?

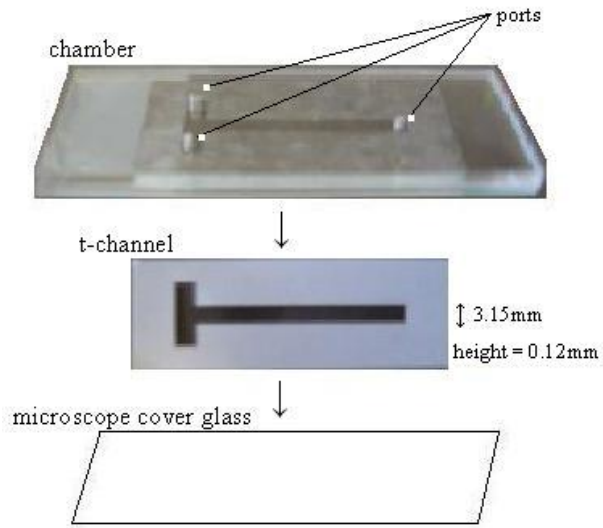
Sam Rauhala
Mike Desantis
Department of Physics Brandeis University

Experimental set up:
Making cAMP waves with different frequencies



computer controlled actuator

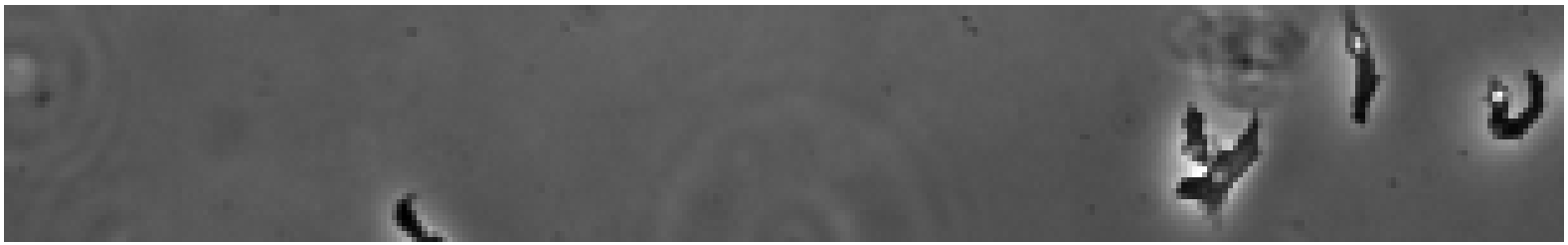




Jay Mettetal (MIT), Mike DeSantis and Samuel Rauhal (senior thesis at Brandeis)

Chemotaxis toward pulses of cAMP

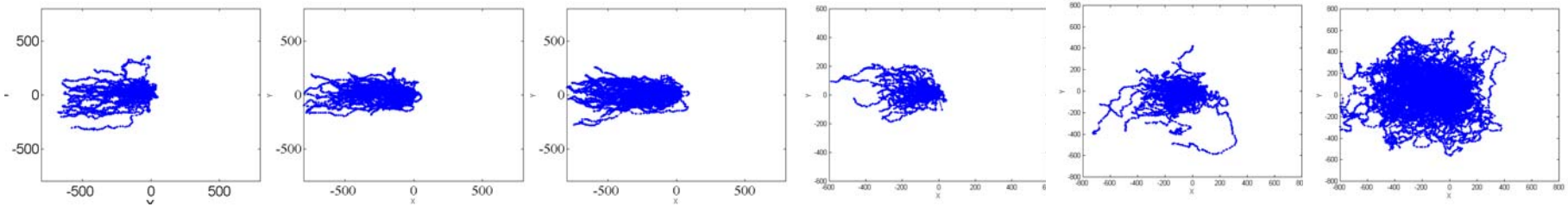
1sec pulse every min. Flow



~ 20 pulses 350 mm

Cell tracks as a function of wave frequency

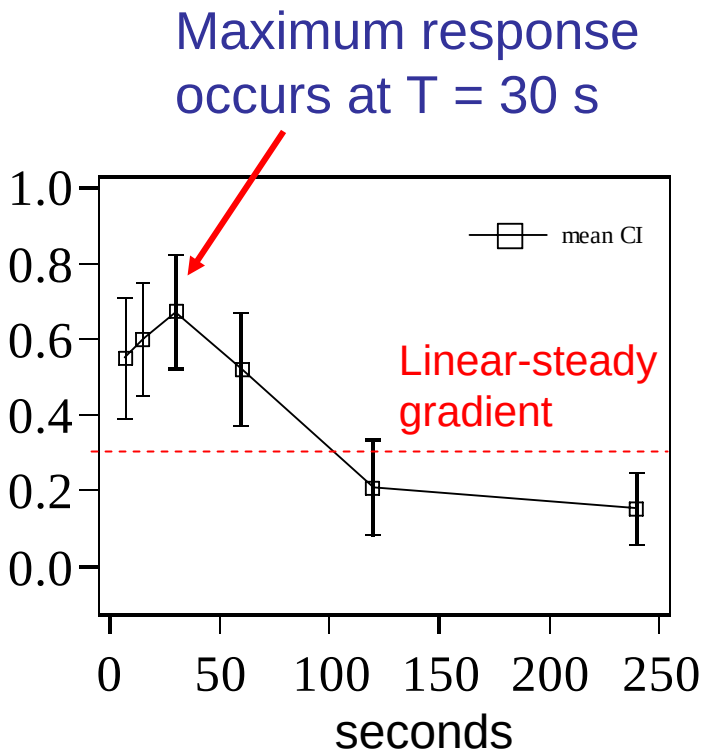
Δt = 7 sec 15 sec 30 sec 60 sec 120 sec 240 sec



Preliminary results with tracking motile cells shows that:

1 – At least within a certain range of frequencies, time varying stimuli are more efficient than continuous stimuli

2- Maximum response occurs for T = 30 sec



Wild type dictyostelium cells produces pulses with period of 6 min