Sobre la propagación de ondas intracelulares de Ca2+

Física Biológica/Biofísica.

Departamento de Física, FCEN-UBA

universidad de buenos aires - exactas
departamento de física
Juan José Giambiagi

FACULTAD DE CIENCIAS EXACTAS Y NATURALES
UNIVERSIDAD DE BUENOS AIRES

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This talk is about intracellular calcium signals.

Why calcium?

Calcium is a universal second messenger.

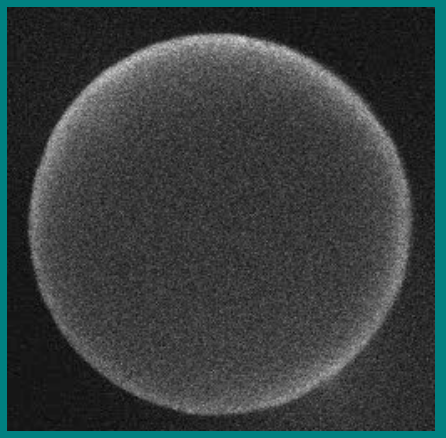
It is involved in processes as diverse as:

Fertilization

Cell death

Neuronal communication, excitability and memory

Muscle contraction and heart beat



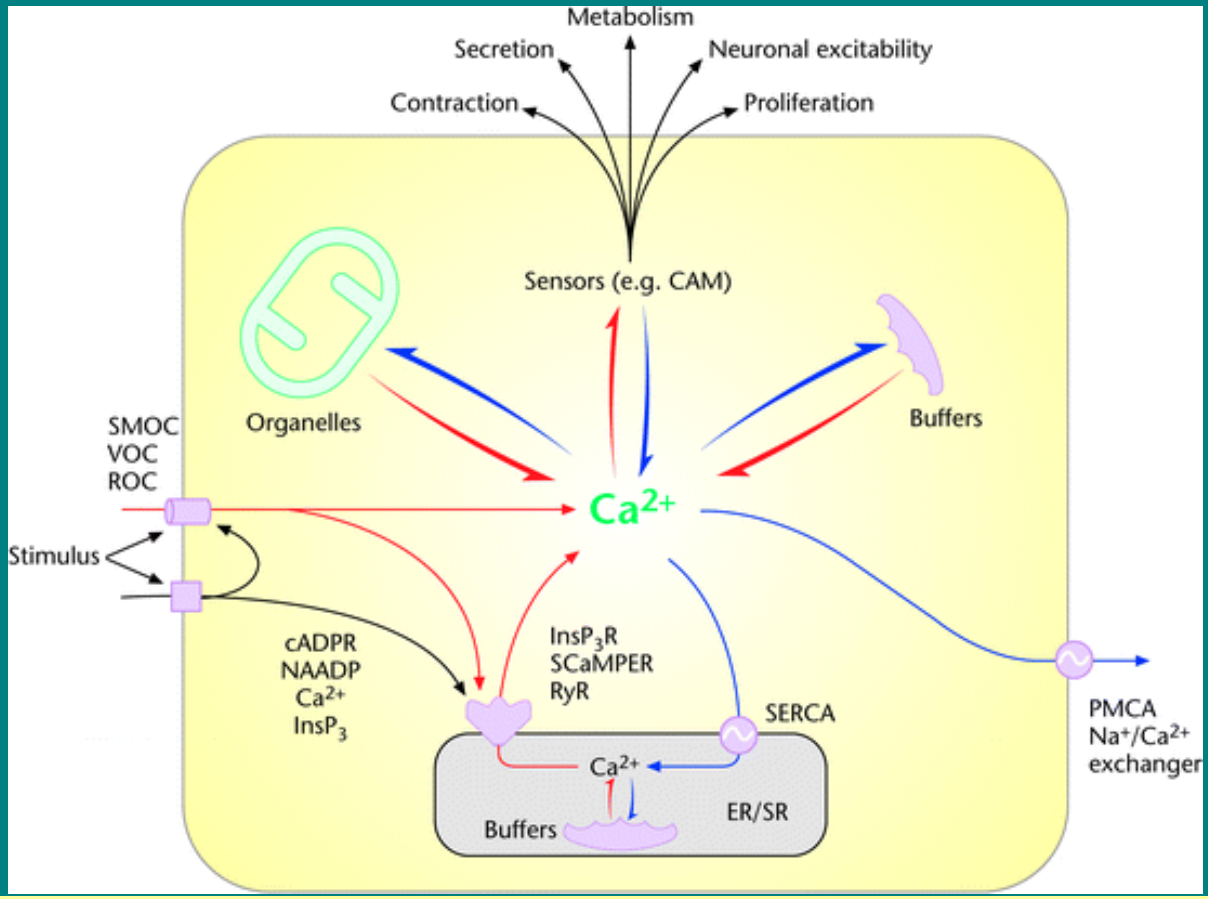
Intracellular calcium waves.
Fontanilla and Nuccitelli

How can it be universal and specific?

Different spatio-temporal patterns of the intracellular (cytosolic) Ca2+ distribution induce different end responses. The information they carry is encoded in space and time. This is key for the universality of the signals.

In particular, the signals can remain localized or propagate as waves. Example: the fertilization wave.

Calcium waves involve calcium release from internal stores such as the endoplasmic reticulum.



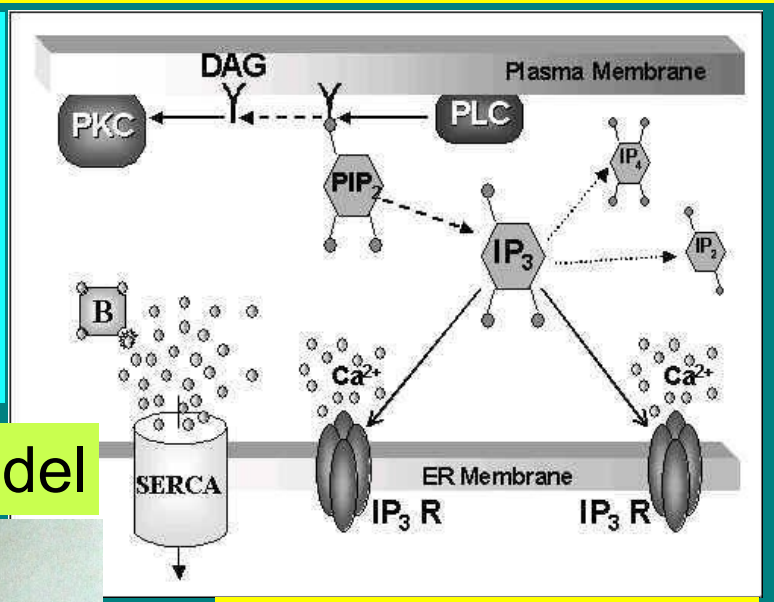
When calcium is released from the ER, it diffuses and interacts with other species (buffers, pumps, etc). Fig. from **Martin D Bootman** and **Peter Lipp**, **Calcium Signalling and Regulation of Cell Function**

Geometry, diffusion, channel kinetics and reactions are some of the factors that determine the resulting Ca2+ signal.

The end response depends on the type of the evoked signal.

A key component of intracellular Ca2+ waves is Ca2+ release from the ER through IP3 receptors

The IP3 receptor is a ligand gated channel located on the membrane of the endoplasmic reticulum. It is one of the main targets for the initiation of intracellular Ca2+ signals.



The IP3 receptor is "sensitive" to IP3

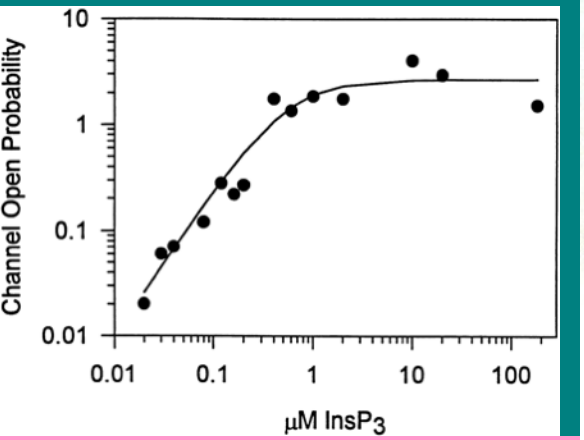
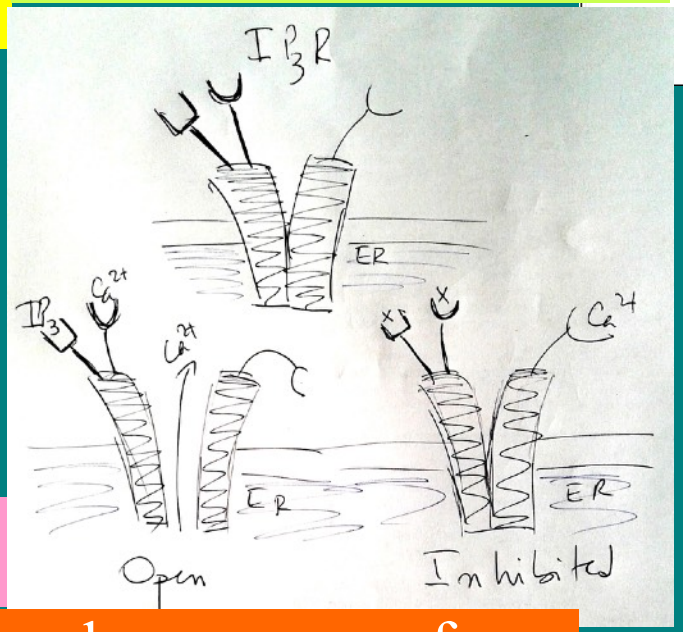
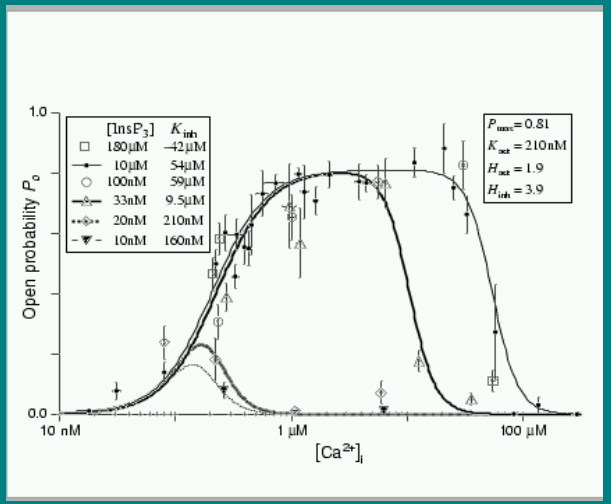


Fig. *Moraru et al, J Gen Physiol.* ***113****, 837 (1999)*

Simplest kinetic model



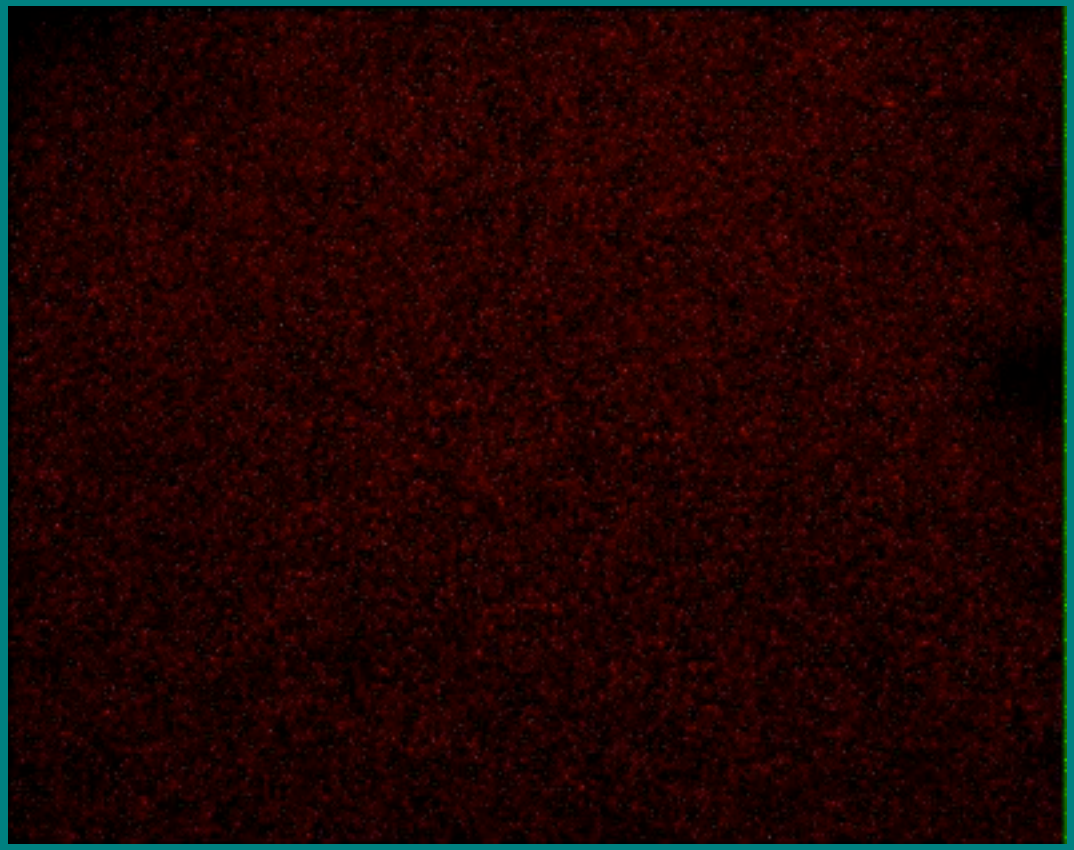
and to calcium



IP3Rs are subject to the phenomenon of Calcium Induced Calcium Release (CICR).

Figure from: *Mak et al, PNAS (1999)*

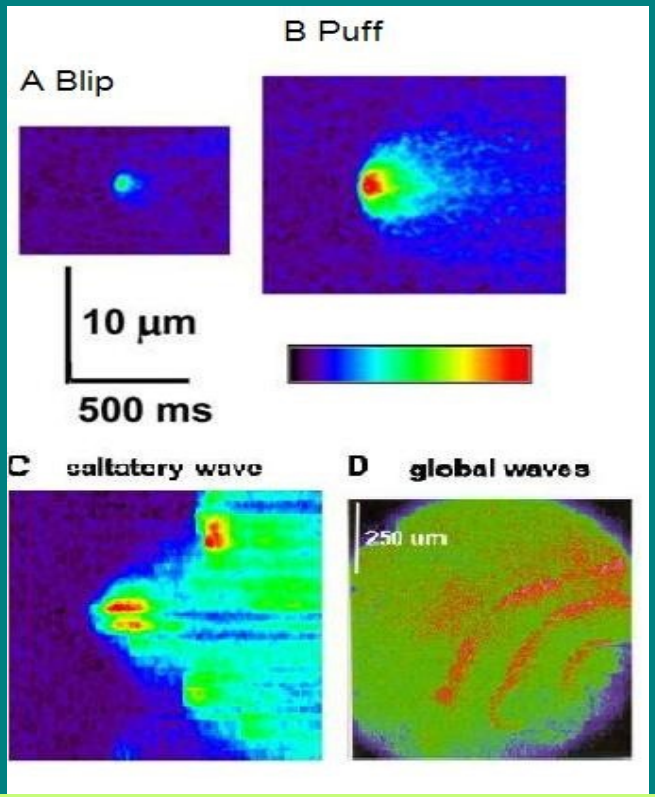
El fenómeno del CICR introduce un feedback positivo que hace que, dependiendo de la situación, la dinámica sea excitable. En particular, se observaron ondas espirales en señales de Ca2+.

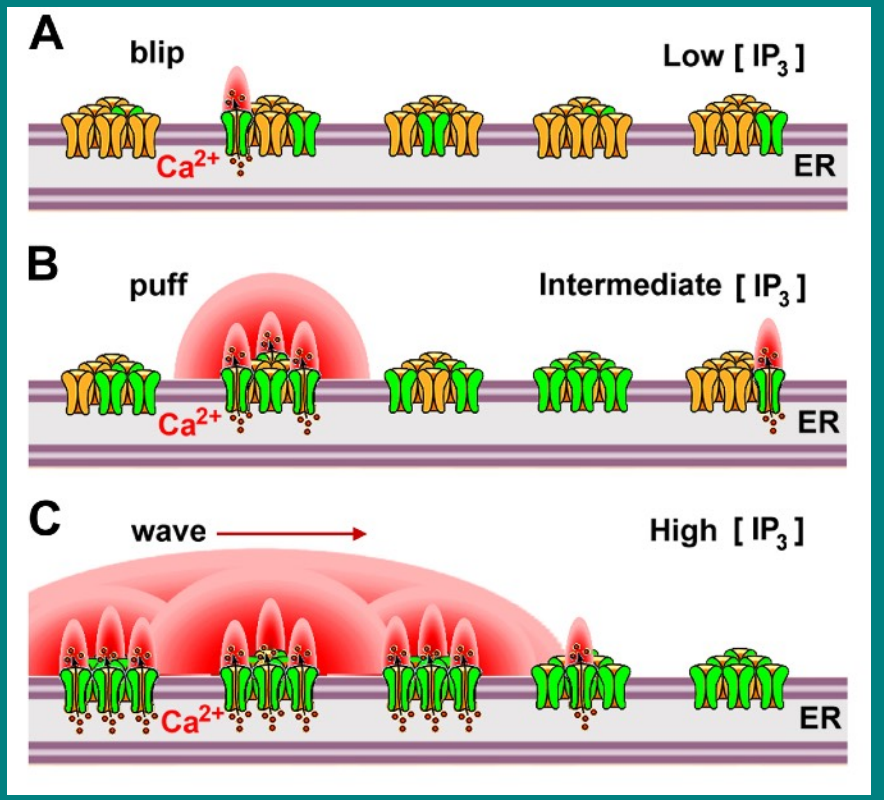


Experimento en el que se fotoliza IP3 enjaulado y así se induce la liberación de Ca2+ desde el ER. El Ca2+ citosólico se observa con un indicador que aumenta su fluorescencia al ligar Ca2+.

In most cell types, IP3 receptors are organized in clusters.

Different signals are evoked depending on the amount of IP3:





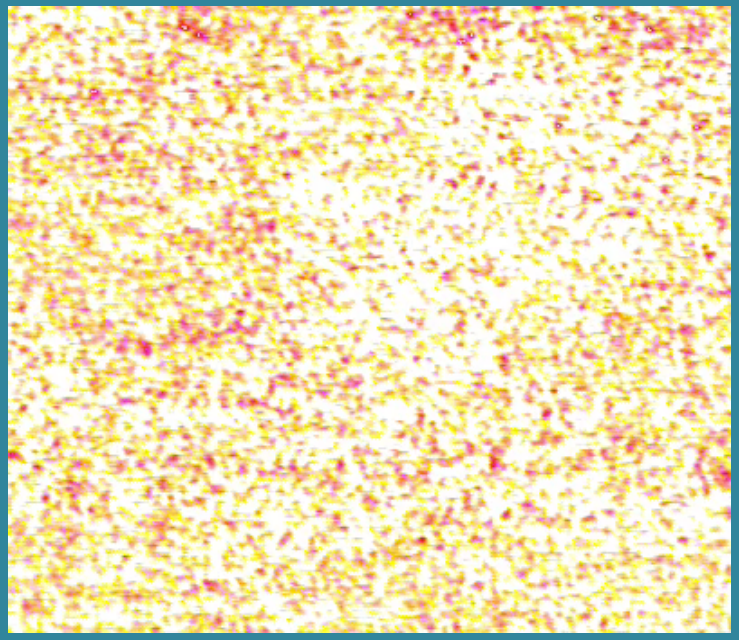
The signals are observed using Ca2+ dyes that increase their fluorescence when bound to Ca2+ and caged IP3 that is photolyzed with UV light.

The non-uniform distribution of IP3Rs can cause wave propagation failure

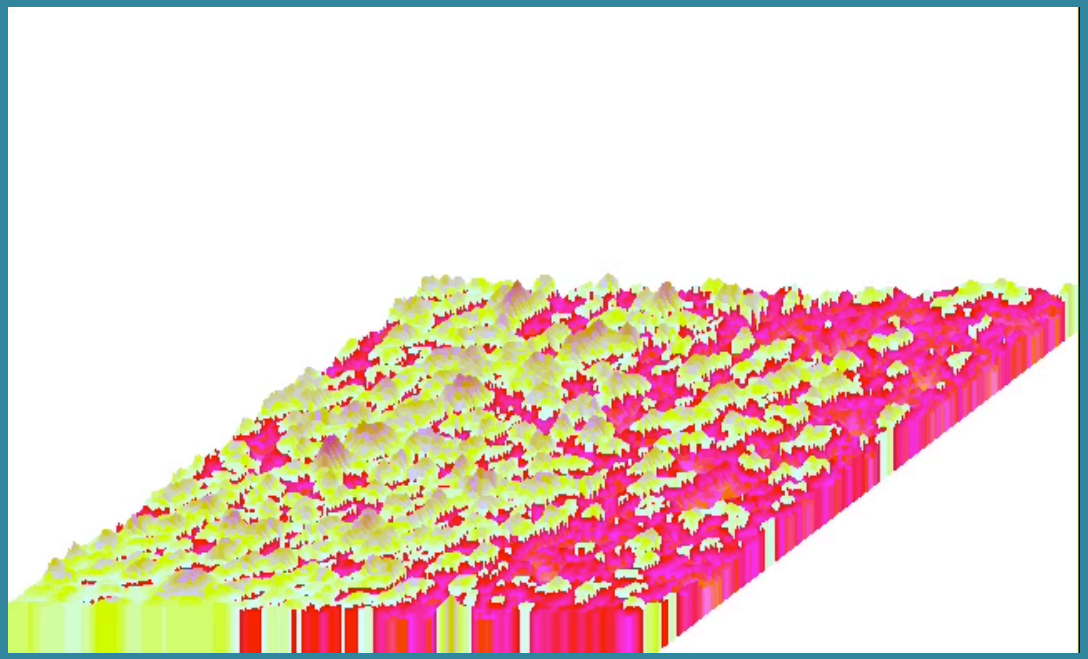
Figs. by I. Parker's group.

To propagate or not to propagate: different responses are evoked depending on the range of the signals

Calcium puffs



Calcium waves



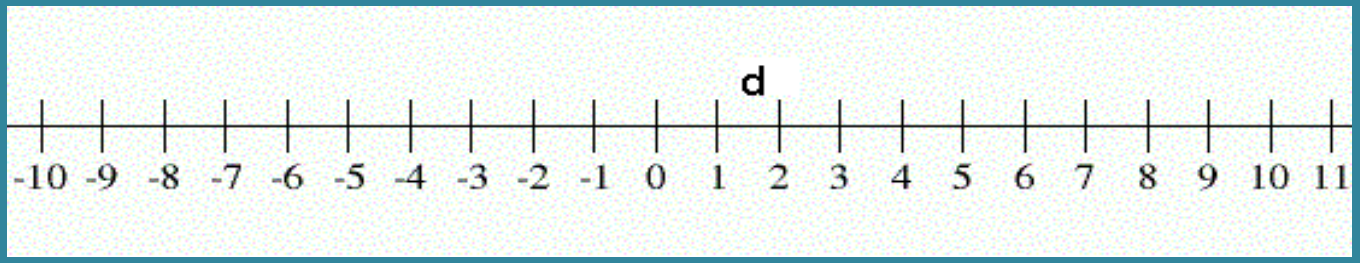
I. Parker’s group.

Understanding how the interplay of various processes affects the properties of Ca2+ waves with the

The fire-diffuse-fire model

(SPD, J Keizer & J Pearson, PNAS´99)

In this model, clusters of channels are represented by point sources separated by a distance **d**, which “turn on” or “fire” when the calcium concentration at the site is above a certain threshold. These sites keep on firing for a fixed amount of time, τ, during which they release a total of σ calcium ions. Once released, calcium diffuses with an effective diffusion coefficient, D , between clusters.

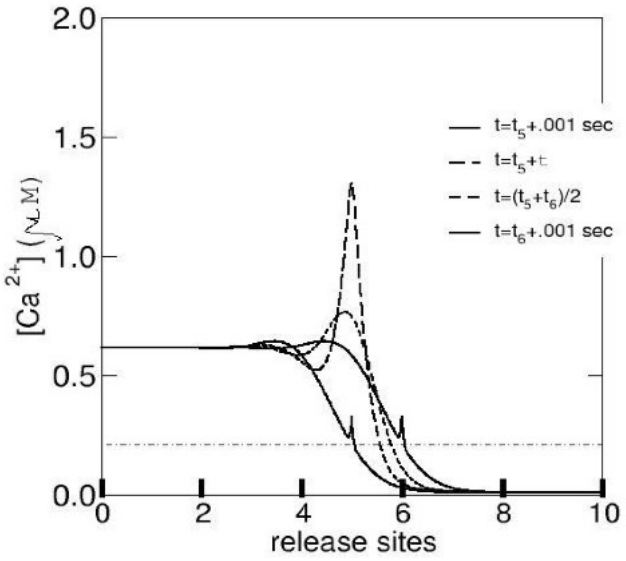


The model depends on two dimensionless parameters:

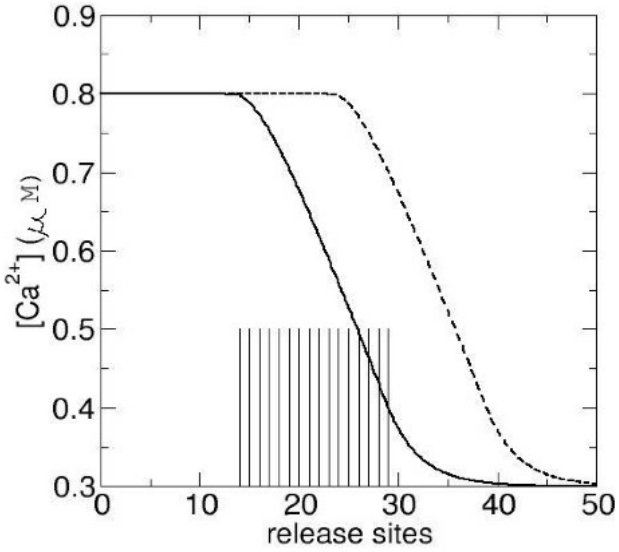
$\beta = D\,\tau / d^2$; $\Gamma = \sigma / d^3\,([Ca^{2+}]_{threshold} - [Ca^{2+}]_{basal})$

These parameters rule whether the wave is able to propagate (Γ) or whether it is saltatory or continuous (β).

The saltatory case.



The continuous case.



The saltatory limit holds when $\beta = D\,\tau / d^2 < 1$. In the saltatory example (which is very saltatory because $\beta \ll 1$) at most one site is "firing" at any given time. Individual release sites are clearly distinguishable.

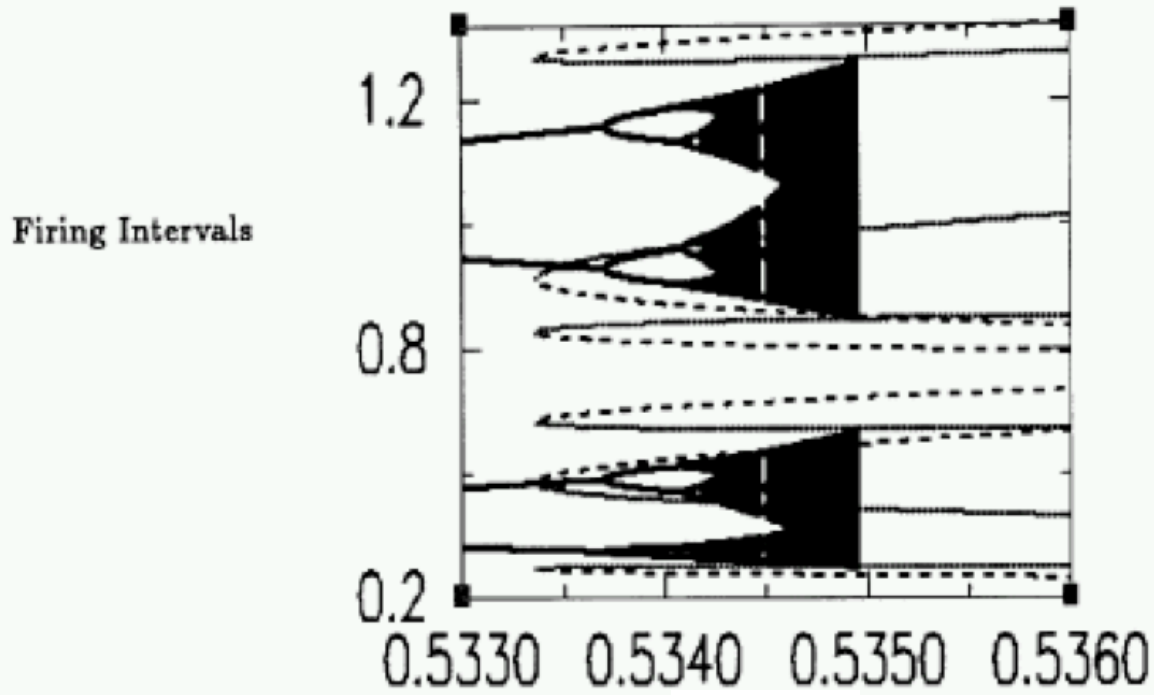
The continuous limit holds when $\beta = D\,\tau / d^2 \gg 1$ ($d \to 0$, σ/d^3=finite). The wave travels almost without deformation. Several sites are "firing" simultaneously and the individuality of the release sites goes unnoticed.

Changing β, the character of the propagation can be changed.

Propagation failure

In the very saltatory limit of the fdf model, we found a period doubling cascade to chaos in the map of time intervals and eventually a crisis that destroys the attractor (propagation failure).

J.E. Pearson, S. Ponce-Dawson / Physica A 257 (1998) 141–148



A “judicious” choice of β could prevent propagation failure from happening.

How to “switch” from propagating to non-propagating Ca2+ signals (or viceversa)?

Numerically it is pretty easy: changing parameters.

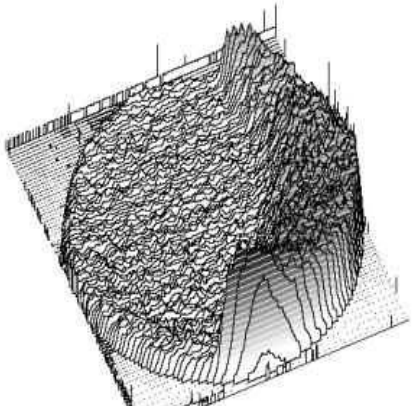
How do cells do it? (Do they do it? They do it and can do it over different timescales!)

In the case of intracellular Ca2+ signals we have found two strategies: software programming and hardware rewiring. Here I will discuss the latter.

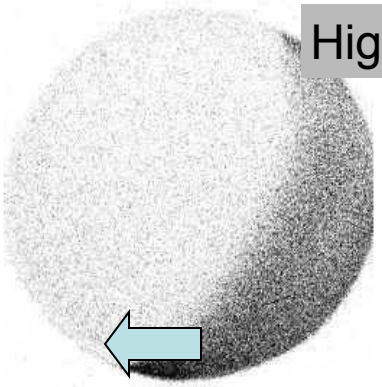
The Ca2+ signaling toolkit is flexible enough to allow the occurrence of these two types of strategies.

Example of hardware rewiring: oocyte to egg transition

In eggs Ca2+ waves are involved in fertilization



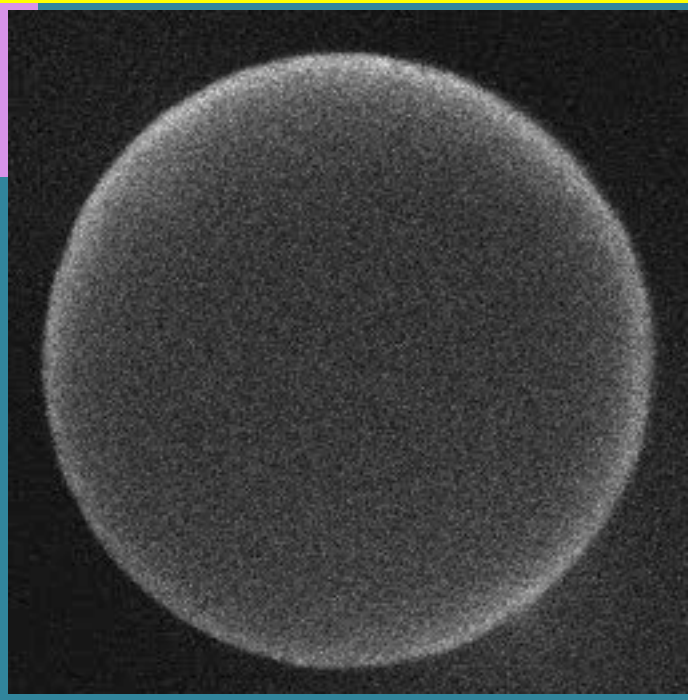
Low calcium



High calcium

Wave propagation

Diameter ~ 2.2 mm
Wave speed ~ 5 um/sec



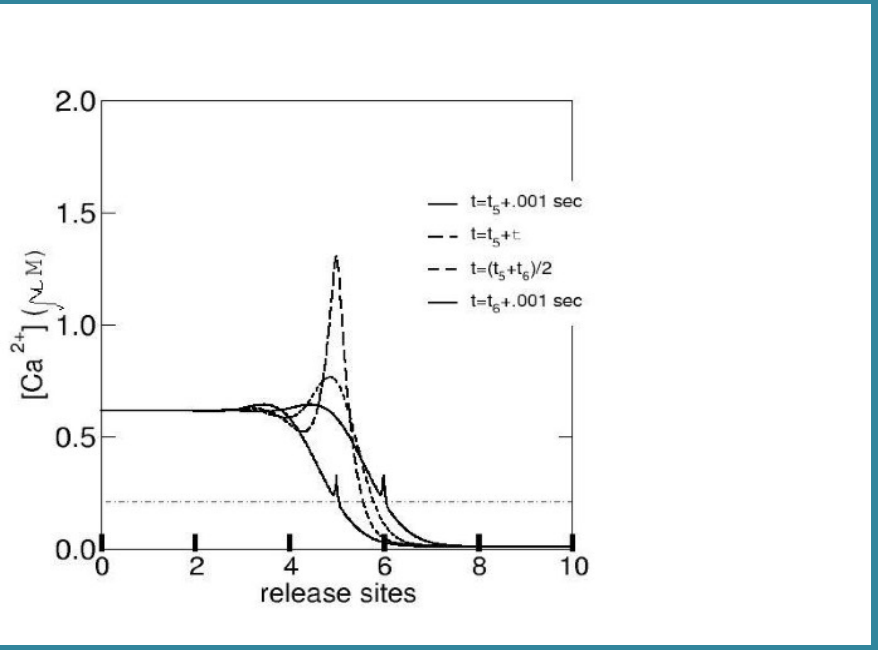
Fontanilla and Nuccitelli

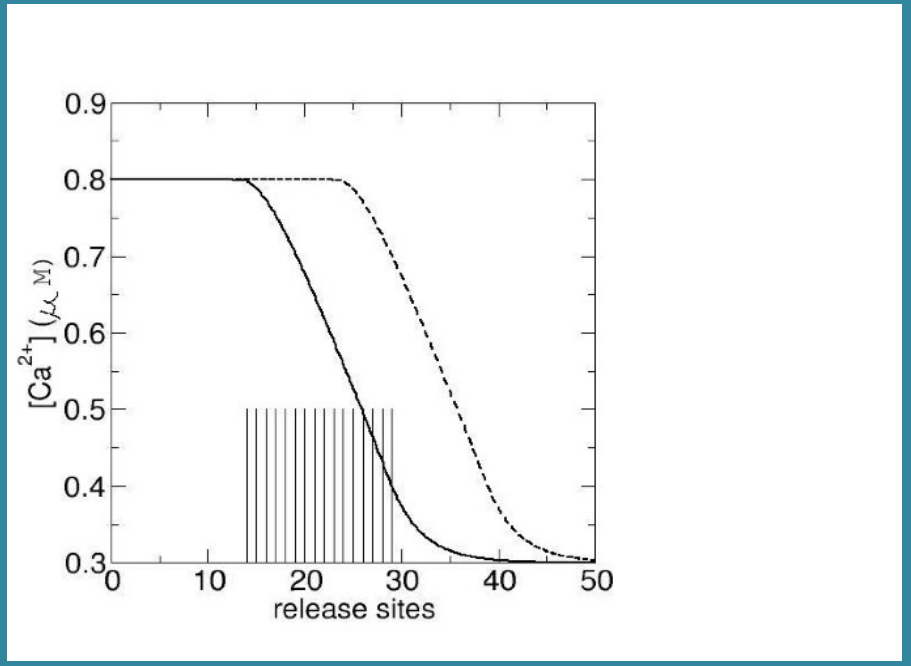
The fertilization wave in (mature) Xenopus eggs is not saltatory.

Left figure from John Wagner's homepage
Experiment by R. Nuccitelli (UCDavis)

In *Xenopus* eggs, the calcium concentration remains elevated over several minutes. This starts the enzymatic machinery involved in cell division.

In fact, the parameters we used for the saltatory and continuous waves in the fdf model corresponded to immature oocytes and eggs, respectively.



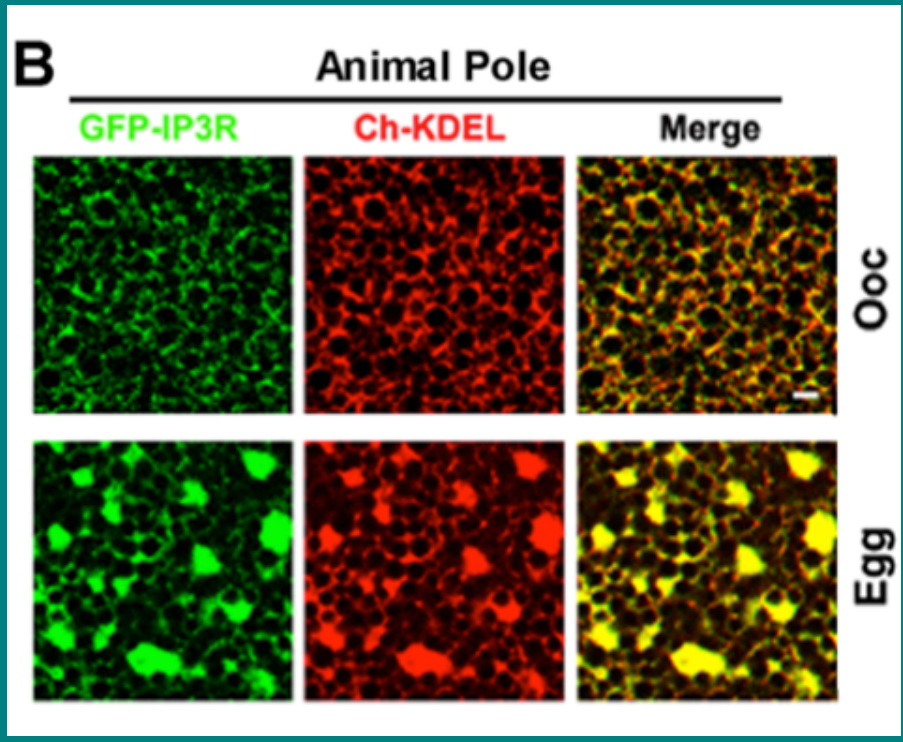


From oocytes to eggs

ER and IP3Rs are spatially redistributed with maturation (from oocyte to egg). Ca2+ pumps are also ”inactivated”.

This restructuring underlies the differences observed in the Ca2+ signals elicited in oocytes and eggs (e.g., saltatory vs continuous waves).

Maturation can be induced incubating oocytes for 12-16hs with progesterone



ER (red) and IP3Rs (green)
Sun et al, PLoS ONE, 2011

The comparison of signals in oocytes and eggs can shed light on how the interaction between geometry and dynamics modulates the resulting Ca2+ signal. It can also show how cells can “reduce” signaling “randomness”.

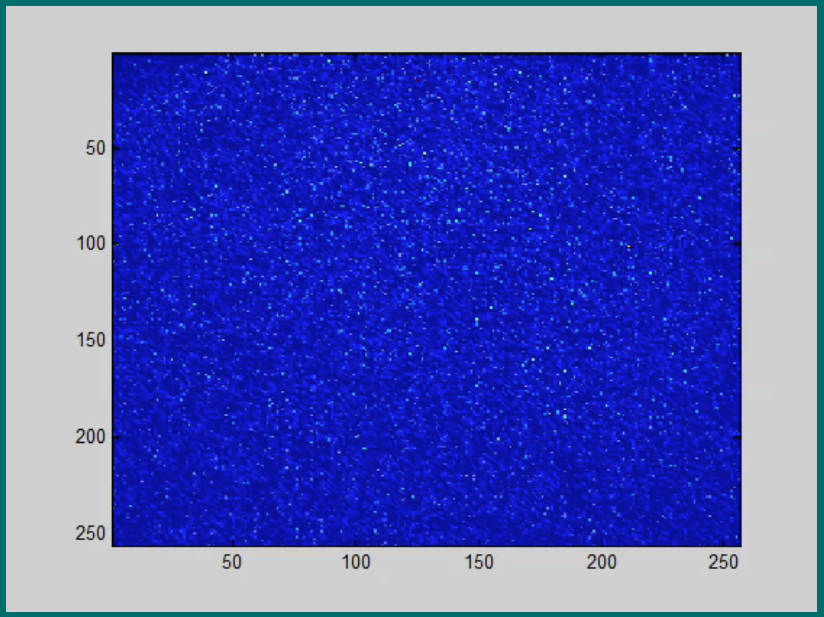
From oocytes to eggs

Signal Propagation

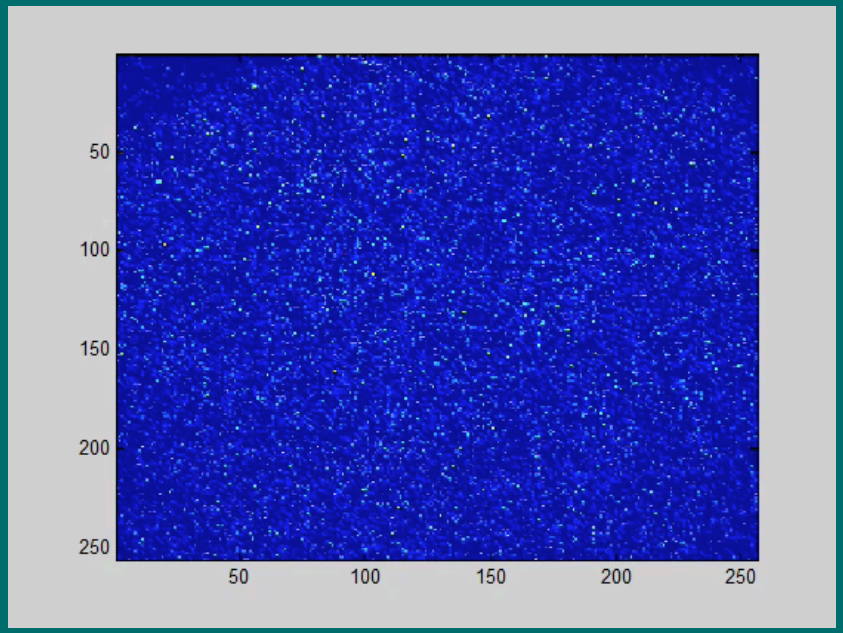
[Fluo-4]=36μM in immature oocytes and in eggs incubated in progesterone

207μm x 207μm frames, acquisition time: 0.56s. (Estefania Piegari, PhD thesis)

Oocyte



Egg



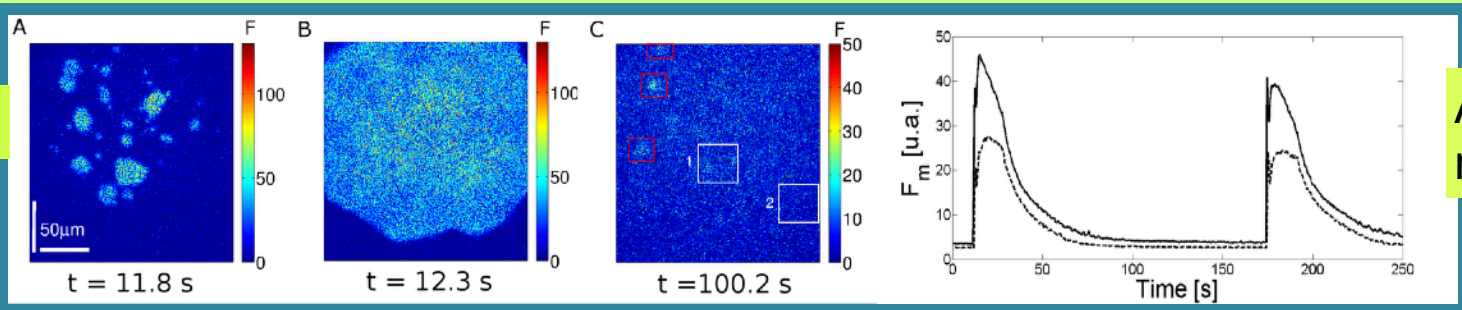
Two UV pulses

t_1 = 11.2 s
t_2 = 173.6 s

t_1 = 11.2 s
t_2 = 112 s

Observation of signals in oocytes and in eggs (maturated with progesterone)

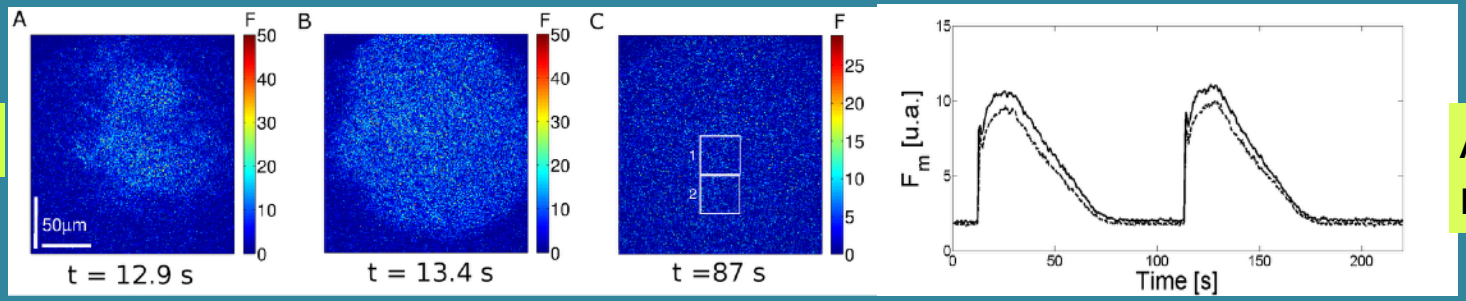
Ooc



Averaged over regions 1 and 2

- Second pulse response of smaller amplitude and slower decay than 1st
- Differences between regions
- Spatially localized events

Egg



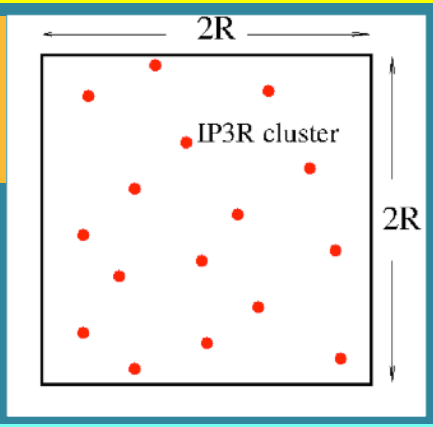
Averaged over regions 1 and 2

- No differences between pulses and regions.
- Smaller amplitude and slower decay than in oocyte.
- Decay: almost linear with time

Experiments: Signals in eggs are more uniform (consistent with a more uniform IP3R distribution) than in oocytes.

A simple model to describe the observations (G.S, LL, EP, SPD)

Geometry & parameters



-N IP3R-clusters uniformly distributed over the 2Rx2R square with mean separation **dm**

-N: Poisson distributed with mean $\lambda_N = R^2/dm^2$

-# of (IP_3-bound) IP_3Rs in each cluster: Poisson distributed with mean N_{RIP3}.

-All IP_3Rs are IP_3-bound; can be active or not.

Dynamics

Event: a cascade of IP_3R openings. It occurs instantaneously.

-One active IP_3R becomes open and all the active IP_3Rs of the cluster become open.

-These n_o open IP_3Rs induce the opening of all active IP_3Rs in clusters within a distance of the first one that depends on the level of Ca^{2+} in the medium and on n_o.

-This process is repeated until no more IP_3Rs fulfill the conditions.

-All IP_3Rs that participate of the event become inactive immediately

Total number of open IP3Rs during the event: $\mathbf{N_o}$.

Between events: $[Ca^{2+}]$ starts from high (homogeneous) level that depends on N_o and decreases exponentially with timescale $\mathbf{1/\delta_{Ca}}$. Inactive IP_3Rs become active after an exponentially distributed time of mean $\mathbf{t_{inh}}$=2.5s. Time step: dt=0.05s.

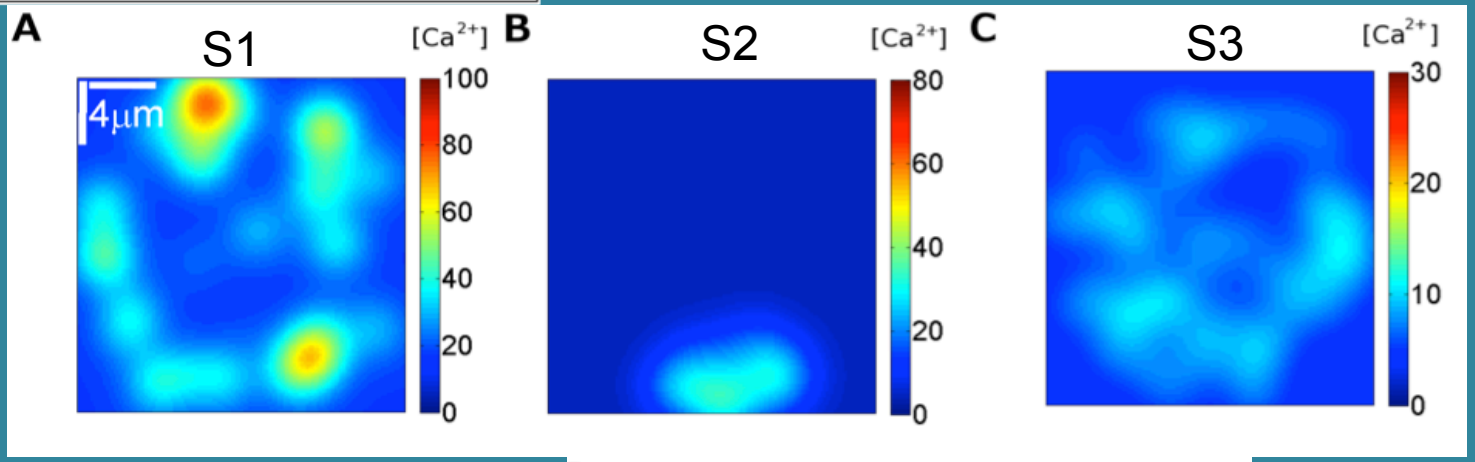
Event trigger: New event starts at a cluster with probability per unit time that depends on the number of active IP_3Rs at the cluster and on $[Ca^{2+}]$

Simulations

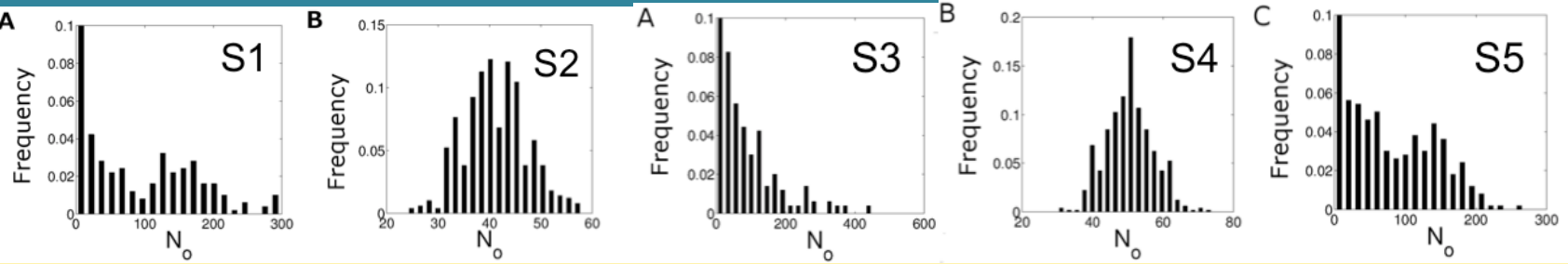
Parameters	S1	S2	S3	S4	S5	Units
dm	4	4	0.4	0.4	0.2	μm
λ_N	25	25	2500	2500	2500	$a.u.$
N_{RIP3}	75	75	1	1	1	$a.u.$
$N_{total-RIP3}$	2095	2972	2383	2567	2524	$a.u.$
δ_{Ca}	200	20	200	20	200	s^{-1}

Effect of varying mean cluster separation, dm, or rate of Ca2+ removal, δ_{Ca}, keeping mean total number of IP3Rs fixed

Examples of events



Histograms of event sizes



Changes in the behavior of the event size distributions are dominated by the rate of Ca^{2+} removal, δ_{Ca}. The distributions are exponential at small events for high δ_{Ca}. They are symmetric around $N_o \sim 0.02 N_{total-RIP3}$ for small δ_{Ca}.

Summary

Intracellular calcium (Ca2+) signals are ubiquitous

In most instances they involve Ca2+ release from the ER through IP_3 receptors (IP_3Rs)

IP_3Rs need to bind Ca2+ on the cytosolic side to become open. IP_3Rs are then subject to Calcium Induced Calcium Release (CICR)

In most cell types IP_3Rs are organized in clusters separated by ~2um.

Ca2+ signals can propagate throughout the cell or not depending on whether they can induce successive IP_3R openings with CICR.

The geometry of the IP_3R distribution and the processes that modulate the IP_3Rs activation and inhibition determine the type of signal that is eventually evoked.

Over their life time, cells can change the geometry of the IP_3R distribution and the efficacy or timescales of operation of the various processes involved in the modulation of Ca2+ signals.

The fact that Ca2+ is involved in both the opening and the inhibition of IP_3Rs allows for a fast “reprogramming” of the channels (not today!).