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"Puff Dynamics in Oocytes: Buffers Regulation"

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Puff dynamics in oocytes: Buffers regulation

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Abstract

A particles system approach is presented to study the stochastic cluster model presented in (3). We focus in buffer regulation.

The spatio-temporal properties of signals arising through IP_3R 's have been extensively characterized by optical imaging in Xenopus laevis oocytes (1). These studies have revealed a hierarchical organization of release events, ranging from [Ca^{2+}] liberation from single IP3R's ("blips"), through the concerted opening of several IP3R's within a cluster ("puffs") to global waves involving cluster-cluster interactions via [Ca^{2+}]induced [Ca^{2+}] liberation (2). Puffs appear to represent ubiquitous "elementary events" of intracellular [Ca^{2+}] signaling, which can both have local signaling functions in their own right, and serve as building blocks from which global signals are constructed. It is, therefore, important to understand the mechanisms underlying the generation and modulation of puffs.

In our previous work (3), we extracted the main from experimental data, a sequence of amplitudes, A (maximum amplitude in the puff event), and inter-puff times, τ (time between events), $(A_1, \tau_1, A_2, \tau_2, , ,)$ and presented rigorous evidence that A_n and τ_n are not independent variables, neither τ_n and A_{n+1}. We have shown that for experiments the amplitude (A_n) modifies the next inter-puff time, moreover large A_n gives large τ_n in mean, while small A_n generate small τ_n, we will call this dependence "inhibitory". The same type of behavior is observed when conditioning the amplitudes (A_{n+1}) to the previous inter-puff times (τ_n). A_{n+1} increase as τ_n increases, this dependence will be denominated by "frustration".

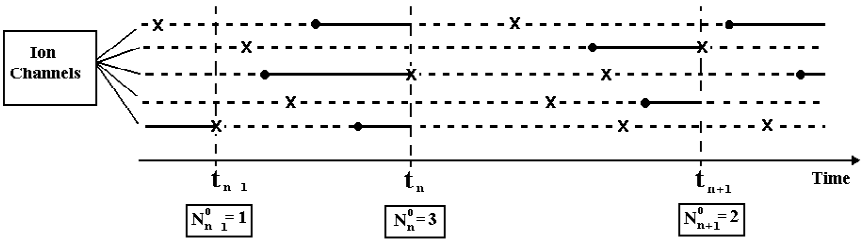

Fig. 1. Model of cluster dynamics in terms of individual channels presented in (3). Random binding events of Ca^{2+} ions to activating sites on IP3R are marked by crosses. If the channel is inhibited (depicted with dashed lines), nothing happens. If the channel is uninhibited (depicted with solid lines), it opens, resulting in opening of all other uninhibited channels in the cluster to generate a puff. During each puff, some of the uninhibited channels become inhibited, with a probability that is a (saturating) increasing function of the puff amplitude (characterized by the number of channels that opened during the puff, N^o). At any time, an inhibited channel may spontaneously become uninhibited (indicated with a solid black circle) with a probability per unit time, λ_2. The schematic illustrates a cluster of 5 channels, which generates 3 puffs involving varying numbers of open channels.

In this letter, we apply a particles system approach to study the properties of the stochastic cluster channels model presented in (3). In particular, we focus on the conditions at which the model capture the "inhibitory"and "frustration" dependencies, and study buffers regulation.

Based on fluorescent data, applying the algorithm in (13) is possible to convert amplitude in number of channels that participate in the event, N^o. In this way, is possible to construct a new sequence with $N^{o\prime}s,(N^o_1,\tau_1,N^o_2,\tau_2,,,)$, which is analogous to the amplitude one. In order to understand the conditions at which "inhibitory" dependence is expected in the cluster model of Fig. 1, we calculate the inter-puff time distribution, conditional to N^o_n channels opened previously, $F_{\tau_n/N^o_n}(t)=P(\tau_n<t/N^o_n)$. Eq. 1 shows this conditional distribution.

$$F_{\tau_n/N^o_n}(t)=1-P(Y>t)^N(p_i+(1-p_i)\frac{P(X>t)}{P(Y>t)})^{N^o_n} \tag{1}$$

Where p_i is the probability of inhibition when N^o_n channels opened, $p_i=p_{inh}(N^o_n)$, $P(X>t)=e^{-\lambda_1 t}$, and $P(Y>t)$ given by Eq. 2

$$P(Y>t)=\begin{cases}\frac{\lambda_2 e^{-\lambda_1 t}-\lambda_1 e^{-\lambda_2 t}}{\lambda_2-\lambda_1}\ if\ \lambda_1\neq\lambda_2\\ (1+\lambda_1 t)e^{-\lambda_1 t}\ if\ \lambda_1=\lambda_2\end{cases} \tag{2}$$

The $F_{\tau_n/N^o_n}(t)$ calculus is based on three main points: 1) finding a minimum distribution of many random variables, 2) memoryless property for exponential distributions, and 3) Bayes total probability theorem. Fig. 2A) show an example of the conditional inter-puff time density, $f_{\tau_n/N^o_n}(t)=\frac{\partial F_{\tau_n/N^o_n}(t)}{\partial t}$, with

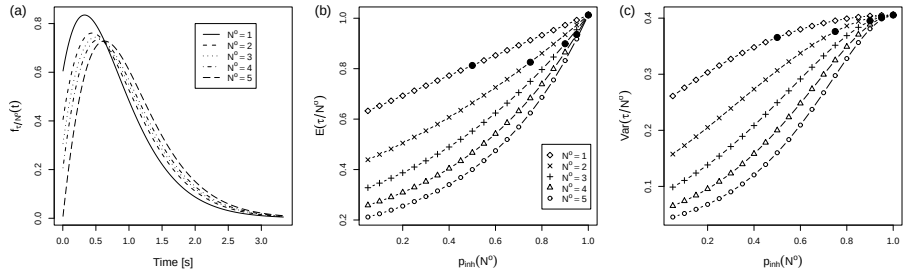

Fig. 2. Inter-puff time conditional: 1) density ($f_{\tau_n/N_n^o}(t)$) for a particular inhibitory probability mapping ($p_{inh}(1,2,3,4,5) = 0.4, 0.8, 0.9, 0.95, 1$), 2) expectation ($E(\tau_n/N_n^o)$), and 3) variance ($Var(\tau_n/N_n^o)$) respectively as a function of $p_{inh}(N^o)$. Dot points in B) and C) correspond to the A) case.All graphs with parameters N=5,$\lambda_1 = 1$ and $\lambda_2 = 0.5$.

$\lambda_1 = 1, \lambda_2 = 0.5, N = 5, p_{inh}(N_n^o) = 0.4, 0.8, 0.9, 0.95, 1$ for $N_n^o = 1, 2, 3, 4, 5$ respectively. We observe that by increasing N_n^o the density moves to the right, showing that a large amplitude puff, equivalent large N_n^o, gives a inter-puff time large in mean, as observed in experiments (3). Entering more in details, in the experiments not only $< \tau_n/N_n^o >$ increases with N_n^o, also the conditional standard deviation, σ_{τ_n/N_n^o}, increases. Fig. 2 B shows the τ_n/N_n^o expectation, $E(\tau_n/N_n^o)$, and in Fig. 2 C the variance, $Var(\tau_n/N_n^o)$, for various $p_{inh}(N^o)$. Choosing an appropriate $p_{inh}(N^o)$ mapping, as the one proposed for Fig. 2 A, both $E(\tau_n/N_n^o)$ and $Var(\tau_n/N_n^o)$ increases as N_n^o increases (black points in Fig. 2 B) and C), same as experimental data. As we will discuss later, this mapping is related to the buffers kinetic and concentrations in each experiment.

We first study the conditions for obtaining a maximum in the density, $f_{\tau_n/N_n^o}(t)$. It is not difficult to see that if:

$$\lambda_2 N - (N^o)^2 \lambda_1 (1-p_i)^2 - N^o(1-p_i)(\lambda_2 + \lambda_1 p_i) > 0 \tag{3}$$

the density has a maximum similar, and in the contrary case the $f_{\tau_n/N_n^o}(t)$ is monotonically decreasing, similar to a Gamma distribution. For $p_i = 1$ (for all N^o) the distribution have a maximum. This is expected because all channels will be inhibited immediately after a puff event in this case.

The next objective is to respond: How many channels opens in a puff event? For this, we define a stochastic process $N^{act}(t)$ as the number of activable channels at time t from the last event. Eq. 4 (see Appendix for details) describes the probability of having k ($k = \{0, 1, 2, , , N\}$) activables channels at time t, conditional that at t=0 there was a event in where N^o channels participate.

$$P(N^{act}(t) = k/N^o) = (e^{\lambda_2 t})^{N-k}(1-e^{\lambda_2 t})^{k-N^o}(1-p_i)^j \sum_{j=max\{0,j-k\}}^{N^o} \binom{N^o}{j}\binom{N-N^o+j}{N-k}\left(\frac{p_i(1-e^{-\lambda_2 t})}{1-p_i}\right)^j \tag{4}$$

From Eq. 4, we obtain the conditional variance and expectation of $N^{act}(t)$: .

The following equation shows these expressions:

$$\begin{aligned} E(N^{act}(t)/N^o) &= N - (N - N^o(1-p_i))e^{-\lambda_2 t} \\ Var(N^{act}(t)/N^o) &= N - E(N^{act}(t)/N^o) - h(t, N^o) \end{aligned} \tag{5}$$

with $h(t, N^o) = (N - N^o(1-p_i^2))e^{-2\lambda_2 t}$. The $N^{act}(t)$ process is independent of λ_1, this is expected, because the number of activable channels, or the opposite, the number of inhibited channels, $N^{inh}(t)$ ($N^{inh}(t) = N - N^{act}(t)$), can only depend on the number of channels that opened previously (N^o), the probability of inhibition and the time to get out from inhibition.

$N^{act}(t)$ process is the foundation process for responding how many channels opened in a puff event. In the same way as $N^{act}(t)$, we define the $N^o(t)$ process, like the number of channels that opens in a puff event at time t from the last event. $N^o(t)$ take values between 1 and N, while $N^{act}(t)$ between 0 and N. However, $N^{act}(t)$ and $N^o(t)$ will be very similar, if there exist a puff event at time t, in this one all the activable channel will participate. Eq. 6 describes the probability of having a event of j ($j = \{1, 2, , , N\}$)channels, knowing that the previous event of N_n^o channels occur at an arbitrary time, and the inter-puff time is τ_n.

$$P(N_{n+1}^o(\tau_n) = j/N_n^o) = \frac{P(N^{act}(\tau_n) = j/N_n^o)}{1 - P(N^{act}(\tau_n) = 0/N^o n)} \tag{6}$$

The $N_{n+1}^o(\tau_n)$ conditional variance and expectation are presented in Eq. 7.

$$\begin{aligned} E(N_{n+1}^o(\tau_n)/N_n^o) &= \frac{E(N^{act}(\tau_n)/N_n^o)}{1 - g(\tau_n, N_n^o)} \\ Var(N_{n+1}^o(\tau_n)/N^o) &= \frac{Var(N^{act}(\tau_n)/N_n^o)}{1 - g(\tau_n, N_n^o)} - g(\tau_n, N_n^o)(\frac{E(N^{act}(\tau_n)/N_n^o)}{1 - g(\tau_n, N_n^o)})^2 \end{aligned} \tag{7}$$

Where $g(\tau_n, N^o) \equiv P(N^{act}(\tau_n) = 0/N_n^o) = p_i^{N^o} e^{-N\lambda_2 \tau_n}$. Fig. 3 shows $P(N_{n+1}^o(\tau_n) = j/N_n^o = 2)$ as a function of the inter-puff time, for the same parameters used in Fig. 2. For small times, lesser that 0.5s, the most probable is that only one channel contribute to the event, while for longer times (greater than 8s) the most probable is that all N channels that build the cluster participate. In times of the order of 8s all inhibited channels become activable, that is way the N_{n+1}^o conditional expectation is a monotonically increasing function of τ_n (from 1 to N), and the conditional variance (eq. 7) is a convex function of τ_n (with $\lim_{t \to 0} Var(N_{n+1}^o(t)/N^o) = Var(N_{n+1}^o(1/t)/N^o) = 0$)

The next is to try to advance in the direction of the stationary or marginal distributions. With all the information presented above, we construct a Markov chain in discrete time for the number of channels that open in the events. The state space is $N^o = \{1, 2, , , N\}$, and the transition probabilities are given by

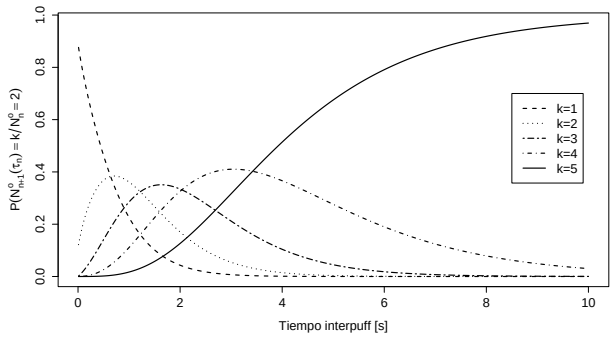

Fig. 3. Number of channels that participate in a event: $P(N^o_{n+1}(\tau_n) = k/N^o_n = 2)$ as a function of τ_n For $N = 5$, $\lambda_1 = 1$, $\lambda_2 = 0.5$, and $p_{inh}(1, 2, 3, 4, 5) = 0.4, 0.8, 0.9, 0.95, 1$

Eq. 8.

$$P(N^o_{n+1} = k/N^o_n = j) = \int_o^\infty P(N^o_{n+1}(t) = k/N^o_n = j) f_{\tau/N^o=j}(t)dt \qquad (8)$$

Once we have the transition probabilities, solving (finding a eigenvector of eigenvalue 1) Eq. 9, we obtain the stationary measure, $P(N^o = k)$.

$$P(N^o = k) = \sum_{j=1}^{N} P(N^o_n = k/N^o_{n-1} = j) P(N^o = j) \qquad (9)$$

From the N^o stationary probability given by Eq. 10 we calculate the inter-puff time marginal distribution.

$$f_\tau(t) = \sum_{j=1}^{N} f_{\tau/N^o=j}(t) P(N^o = j) \qquad (10)$$

Now with the stationary probabilities we explore some "buffers scenarios". In all puff experiments reported, experimentalists use some exogenous buffers to impede wave propagation. As a buffer, basically is a loosing term in the $[Ca^{2+}]$ reaction diffusion equation, it is expected that as the concentration is increase the fluorescence amplitude decreases, that is way the result presented in (12) was surprising. Basically, they observed that if they increase a little the buffer concentration the amplitude increases. In the theoretical argument, exposed before, in favor of the contrary effect, no channels dynamics was taken into account. In order to try to reveal this "paradox" we explore the particle model for different buffers scenarios. In particular we propose various scenarios, or equivalent various $p_{inh}(N^o)$ mappings (see Fig 4 A)) and calculate the expectation of N^o, τ and their respectively variance. As the [Buffer] increases the Ca^{2+} free microdomain ($[Ca^{2+}]$ in the mouth of the channel) decreases, and as the IP_3R inhibits at high $[Ca^{2+}]$ (4; 7) the probability of inhibition will be smaller. In this way a $p_{inh}(N^o)$ mapping as the one depicted with circles in Fig. 4 A) will be a scenario with a cytosol with less [Buffer] than the one with

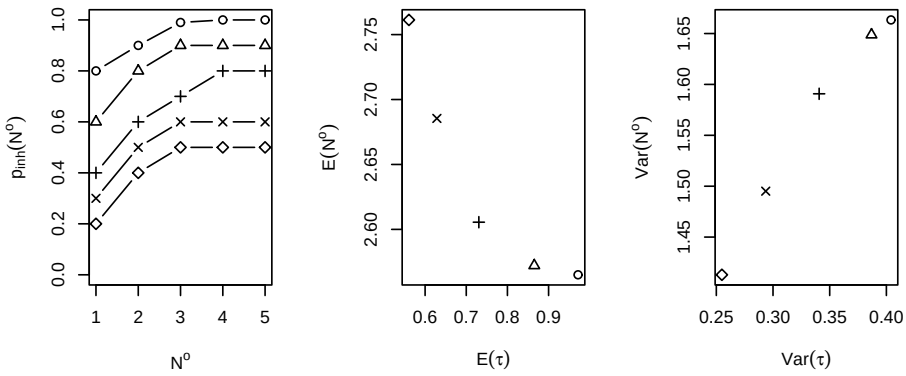

Fig. 4. Buffers scenarios: A) Probability of inhibition ($p_{inh}(N^o)$) as a function of N^o , B) N^o Expectation (E(N^o)), and C) N^o Variance ($Var(N^o)$) for the different $p_{inh}(N^o)$ scenarios of A). With $\lambda_1 = 1$, $\lambda_2 = 0.5$ and N=5

diamonds. Fig. 4 B) shows the $E(N^o)$ versus $E(\tau)$ for the different buffers scenarios, the expected N^o ("amplitude") increases as the buffer concentration is increased, while the expected inter-puff time decreases. Although we are not talking about fluorescence amplitude, only number of channels that participate of a puff event, it seems reasonable that for some regime (no se como ponerlo pero es muy importante) the effect of the increment in the N^o (calcium flux) will be more important than the buffering effect (less Ca^{2+} free and less Ca^{2+} bound to the fluorescent indicator) and so the amplitude will increase. Besides the buffer effect over p_{inh}, in the case where [Buffer] is increase with no calcium free added, the change in the basal $[Ca^{2+}]$ contribute to have greater $E(N^o)$ and $E(\tau)$. Es decir, a decrease only in the basal concentration (3) result to be similar to increase only the [Buffer]. Quizas decir algo de que asi no fueron hechos los experimentos, pero es la mejor manera de amplificar el efecto. Fig. 4 C) we show the respectively variances for each buffer scenario, interestingly both variance decreases when the [buffer] is increased.

We believe that the process studied here can have many other applications, such as infection processes. From a modeling point of view, we see our model as a necessary step from which more sophisticated models may be built for understanding buffers regulation in puff events.

References

[1] Y. Yao, J. Choi, I. Parker (1995). **..**, ...
[2] X. Sun, N. Callamaras, I. Parker (1998). **..**, ...
[3] D. Fraiman, B. Pando, S. Dargan, I. Parker, S. Ponce Dawson (2005). *Puff dynamics in oocytes: interdependence*
[4] D.O. Mak, S. McBride, J.K. Foskett (1998) *P.N.A.S. USA* **269**,7238-7242

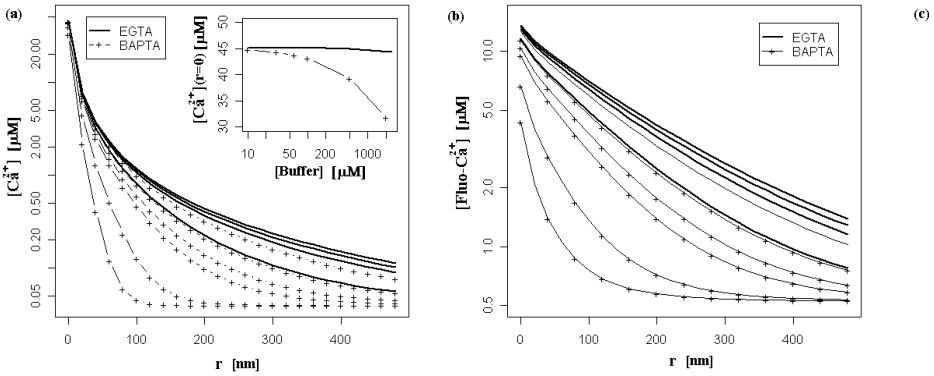

Fig. 5. A) Ca^{2+} free, B) Ca^{2+} bound. [EGTA]=10,200,500,2500, [BAPTA]=10,30,60,100,500,2000

[5] T. Harris (). **23**, 607-615
[6] D.O. Mak,
[7] I. Bezprozvanny, J. Watras, B.E. Ehrlich, (1991) *Nature* **351**,751-754
[8] J. Ramos-Franco, M. Fill, G.A. Mignery (1998) *Biophysical J.* **75**,834-839
[9] M. Iino (1990) *J. Gen. Physiol* **267**, 1103-1122
[10] E. A. Finch, T.J. Turner,et al., (1991) *Science* **252**, 443-446
[11] D. Fraiman, S. Ponce Dawson (2005). *Statistical model*
[12] S. Dargan, I. Parker ().
[13] A.C. Ventura, L. Bruno, A. Demuro, I. Parker, S. Ponce Dawson (). *Biophysical J.* **88**, 2403-2421