Calcium puffs in *Xenopus* Oocytes: How many IP3 receptors and how are they distributed?

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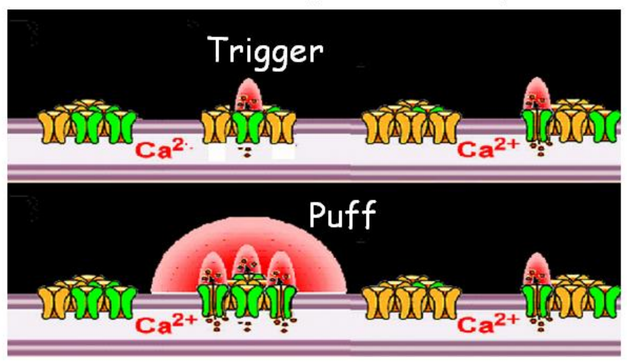
Introduction

Local calcium events, "puffs", are a fundamental building block of cell-wide calcium signals; additionally, puffs can function independently to generate local signals. Puffs are thought to arise through the concerted opening of several inositol 1,4,5- trisphosphate receptor-channels (IP3R) which are clustered together to operate as a functional unit. Activation of IP3Rs is dependent on the presence of both IP3 and calcium. Therefore calcium diffusing from an open channel will increase the opening probability of nearby channels within the cluster, thereby synchronizing their opening (Parker et al, 1996; Bootman et al, 1997). However, controversies remain regarding how puffs are initiated, how many IP3Rs contribute to a puff, and the spatial distribution of the IP3R/channels within a cluster (Swillens et al, 1999; Dupont et al, 2000; Niggli and Egger, 2002). Here we used a combination of high-resolution calcium imaging and computer modeling to address these issues. We have observed brief increases in local calcium that precede puff generation. We call these small calcium signals "trigger events" because it appears that these events may initiate puffs and, importantly, we can use these events to predict the number of IP3Rs involved in puff generation.

Working Hypothesis: Opening of one IP3R/channel triggers the opening of many more channels, resulting in a calcium puff.



Questions: How many channels open during a puff? What is the spatial distribution of these channels?

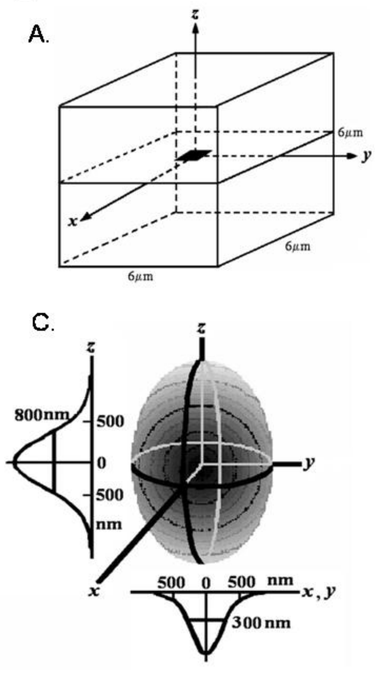
Methods (experimental)

Briefly, stage V-VI oocytes from *Xenopus laevis* were plucked and collagenase treated (0.5 mg/ml), stored in Barth's solution and used for up to 5 days. About 1 hour before confocal microscopy, oocytes were microinjected to a final concentration of 40 μM fluo-4-dextran, 8μM caged IP3, and 300μM of the slow calcium buffer EGTA to prevent puffs from initiating calcium waves. Flashes of ultraviolet light were used to photorelease IP3 and initiate calcium puffs which were monitored using a custom-built line-scan confocal scanner interfaced to an Olympus IX70 inverted microcscope (Parker et al, 1997). Recordings were from the granule layer of the animal hemisphere at room temperature in Ringer's solution. Linescan images were acquired at 2.2 ms per line, 0.06 μm/pixel, with a 25 μM scanline. Ca2+ images are displayed as ratios (ΔF/Fo), depicting the change in fluorescence at a pixel during the response (ΔF) relative to the resting fluorescence at the same pixel before stimulation (Fo). Data are corrected for autofluorescence of the oocyte.

Methods (computer model)

The model consists of two main parts, the calcium release source through IP3R channels from the endoplasmic reticulum, and the subsequent calcium propagation through cytosolic space. We simulate the propagation of calcium through a 6x6x6μm cytosolic space divided into cubic (40 nm side) grid elements. IP3 channels are distributed on a square plane (B) located at the center of the cube in A (below). The IP3 channels are evenly distributed across the plane; "L" represents the total distance across the cluster of channels. The center channel is assumed to generate the trigger event. The simulation of the point spread function (p.s.f) of the confocal microscope as Gaussian functions is shown in C. Other model parameters are shown in D.

Fig.1



D.

Free Calcium	
Diffusion coefficient	200 μm^2 sec^{-1}
Resting concentration	0.05 μM
Calcium Indicator (Fluo-4 dextran)	
Diffusion coefficient	15 μm^2 sec^{-1}
Total Concentration	40 μM
On-rate	150 μM^{-1} sec^{-1}
Off-rate	300 sec^{-1}
Stationary Buffer	
Total concentration	300 μM
On-rate	400 μM sec^{-1}
Off-rate	800 sec^{-1}

Results (experimental)

"Trigger Events" Precede a Subset of Calcium Puffs

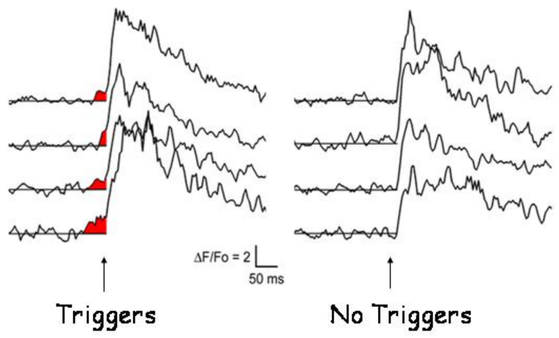


Fig. 2. Each trace represents the temporal profile through the peak of a puff. Trigger events (low amplitude, brief increases in fluorescence) preceded puffs about 40% of the time (n = 96 puffs).

Confocal Image of Puff With Trigger Event

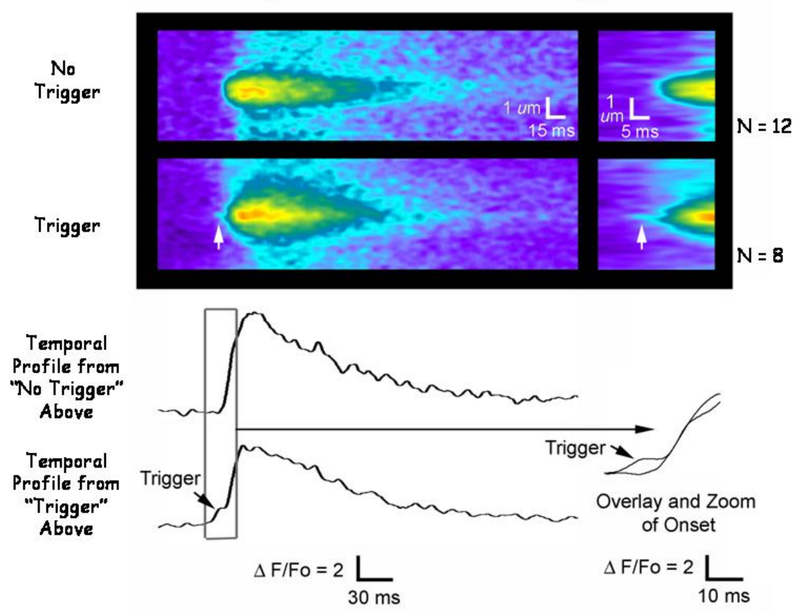


Fig. 3. While a trigger event is generally not discernable in an individual confocal image of a puff, averaging puffs with triggers (as determined by their temporal profile, Fig. 2) revealed a small but clear increase in calcium before the fast onset of the puff. This trigger event is visible in both the confocal image (labeled "trigger") and a temporal profile taken through the center of the puff (bottom panel). Arrows indicate triggers.

Characteristics of Puffs and Trigger Events that are Relevant to the Model

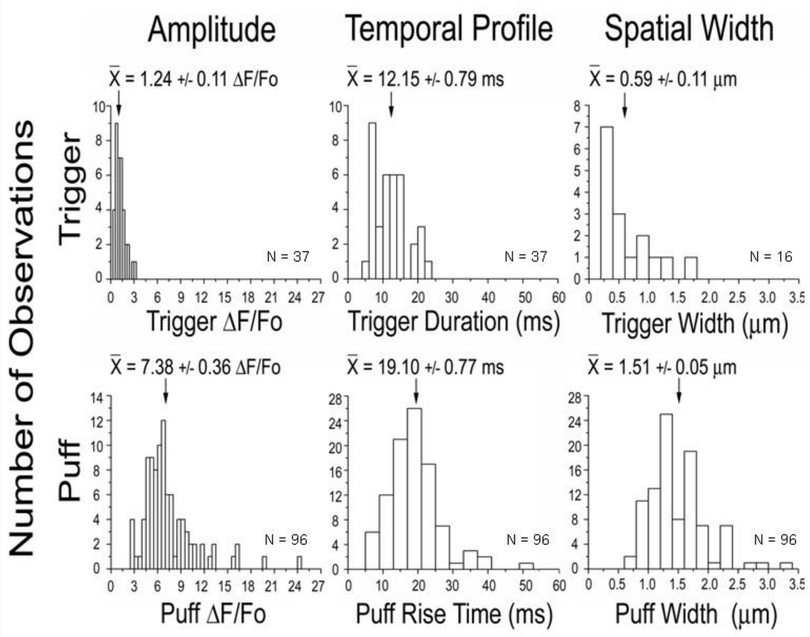


Fig. 4. Amplitude, temporal characteristics, and spatial characteristics of puffs and triggers. Mean +/- SEM are noted above each panel. These values are used in the following computer simulation to predict the number and distribution of IP3Rs that contribute to a puff. An important parameter for the model is the ratio between puff ΔF/Fo and trigger ΔF/Fo, this ratio is 6.

Results (model)

Simulated Linescan Image

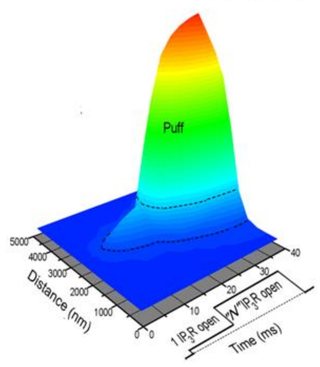


Fig. 5. A single IP3R with a Ca^{2+} current of 0.4 pA opens for 12 ms, followed by the opening of some greater number of channels for 19 ms. By varying the channel number and width of the channel cluster ("L") we can determine which parameters result in a trigger/puff simulation that most closely matches the experimental data. (In this example N= 25 and L = 520 nm.)

Results (model, continued)

Simulation of Event Amplitudes

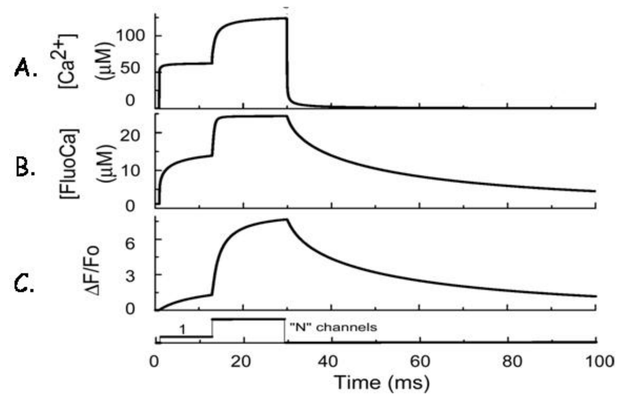


Fig. 6. Graphs show trajectories of A. local free cytosolic calcium at the center grid element (located over the trigger channel), B. fluo-bound calcium at the center grid element, and C. fluorescence ratio change averaged over a confocal spot centered on the "trigger" IP3R during the opening of a single IP3R channel followed by the opening of some greater number of channels. (N = 25 channels in this example).

Simulation of Spatial Distributions

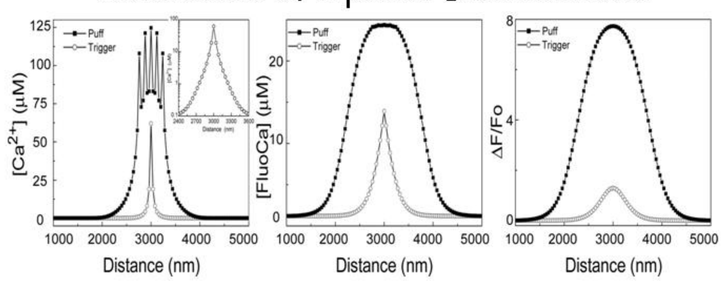


Fig. 7 shows the same parameters as above, but plotting the spatial width of the trigger event and puff at times immediately before the initiation and termination of the puff. In this manner we can determine the number of channels that must open to result in a simulated puff and trigger with spatial widths and amplitudes (above) that correspond to experimental values.

20 - 30 IP3R Channels Distributed as a Cluster ~600 nm Across Offer the Best Fit to the Data

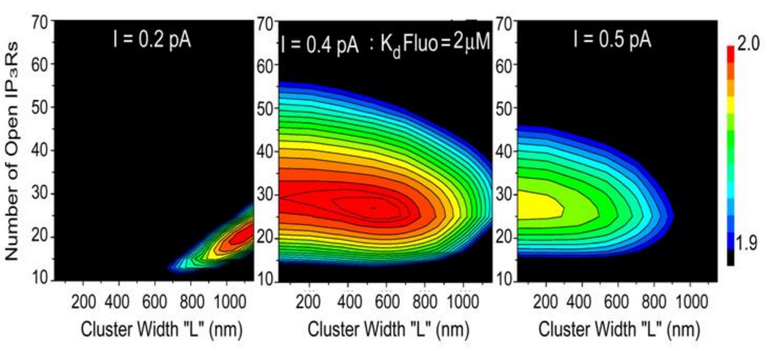
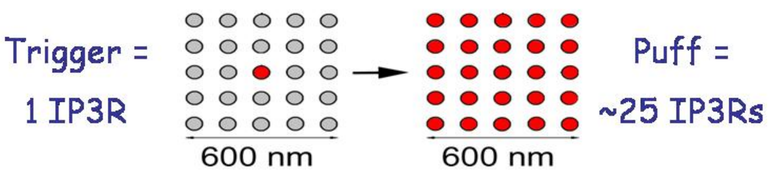


Fig. 8. The channel cluster width (x-axis) and the number of open IP3Rs (y-axis) was varied and the model generated a puff with trigger for each combination of these two parameters (for example, Fig 5). A matching degree function then determined the fit of each simulated puff to the experimental values. A best fit is indicated by red, a single channel current of 0.4pA, a channel cluster width of ~600 nm and a channel number of ~ 25 IP3Rs resulted in a simulated puff that best fit the experimental data (center panel). The channel current value of 0.4pA is based on experimental data (Bezprozvanny and Ehrlich, 1994), using values of 0.2pA and 0.5pA generated simulated puffs that do not match well with experimental values (left and right panels).

Conclusions

Using experimentally measured kinetics (12 ms trigger and a 19 ms risetime of the puff) we were able to build a model that closely approximates the spatial profile and amplitude of experimentally acquired trigger events and puffs.

Open Channel Number During a Puff = 20 – 35 IP_3Rs
Cluster Width = 400 – 650 nm
Single Channel Current = 0.4 pA



Citation and Support:
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