Supplemental Information

Mathematical Model

Computation

A modified version of the model of Sneyd et al. 2004 (1) was used to address the relationship between store depletion and SOCE. The model equations as well as the parameter values used for the simulations are given below. The differential equations and parameter values were integrated numerically with the CVODE solver in the software package XPPAUT (2). The ER compartment was separated into two parts - internal ER and subplasma membrane ER, which were connected by calcium diffusion. In our model equations, c denotes the free cytosolic calcium concentration, and c_{er} and c_{suber} represent the concentration of calcium in the internal and subplasma membrane ER respectively. Depletion of the subplasma membrane ER only was assumed to be directly responsible for activation of SOCE. The volume of subplasma membrane ER is assumed to be 2-5 % of the bulk ER volume. As shown in supplemental Figure 1, following agonist stimulation it empties much more rapidly compared to the internal ER. Further a slow inactivation (with time constant 5-6 min) of SOCE by cytosolic calcium is included in the model and represented by the variable h. We assumed a constant basal leak of calcium (J_{basal}) across the plasma membrane, which is necessary to balance the pump fluxes in the absence of agonist stimulation. The plasma membrane calcium pump and the SERCA pump are modeled by Hill equations. The rate of the SERCA pump depends on the amount of calcium in the store in such a way that the rate of SERCA pump increases as the concentration of calcium in the ER decreases. In addition, the maximum rate of SERCA pump is assumed to be an exponential function of time in order to simulate the diffusion of thapsigargin from the plasma membrane to the two store compartments; this is a discrete approximation to the continuous diffusion process. The different diffusion times for thapsigargin corresponding to the internal ER and subplasma membrane ER are thus approximated by using different time constants.

Model equations:

$$\frac{dc}{dt} = f(J_{in} - J_{pm} + J_{er} - J_{serca,er} + J_{suber} - J_{serca,suber})$$

$$\frac{dc_{er}}{dt} = \frac{f}{V_{er}}(V_{cyt}(J_{serca,er} - J_{er}) - V_{suber}J_D))$$

$$\frac{dc_{suber}}{dt} = \frac{f}{V_{suber}}(V_{cyt}(J_{serca,suber} - J_{suber}) + V_{suber}J_D)$$

$$\frac{dh}{dt} = \frac{(h_{inf} - h)}{\tau_h},$$

where $c = [Ca^{2+}]_{cyt}$, $c_{er} = [Ca^{2+}]_{er}$, $c_{suber} = [Ca^{2+}]_{suber}$, and h is the activation variable for calcium entry.

Calcium entry :

$$J_{in} = (J_{basal} + J_{in,suber})h,$$

where J_{basal} – constant and

$$J_{in,suber} = \frac{\nu_{suber}}{1+\exp\frac{c_{suber}-\kappa_{suber}}{\theta_{suber}}}\ ,\ h_{inf} = \frac{\nu_{h}}{1+\exp\frac{c-\kappa_{h}}{\theta_{h}}};$$

Calcium release :

$$J_{er} = p_{er}(c_{er} - c),\ J_{suber} = p_{er}(c_{suber} - c);$$

Calcium diffusion within ER :

$$J_{D} = p_{D}(c_{er} - c_{suber});$$

Calcium pumps :

SERCA pump - $J_{serca,er} = \frac{R_{er}(t)c}{K_{er}+c}\cdot\frac{1}{c_{er}},$

$J_{serca,suber} = \frac{R_{suber}(t)c}{K_{er}+c}\cdot\frac{1}{c_{suber}},$ where

$$R_{er}(t) = R_{er\,max}\exp\frac{-(t-T_{Tg})}{\tau_{er}},$$

$$R_{suber}(t) = R_{er\,max}\exp\frac{-(t-T_{Tg})}{\tau_{suber}},$$

Plasma membrane pump - $J_{pm} = \frac{V_{pm}c^2}{K_{pm}^2+c^2}.$

Table 1. Parameter values and initial conditions used in the model equations.

Parameter values			
V_{cyt}	0.54	V_{er}	0.0856
V_{suber}	0.004275	f	0.01
ν_{suber}	$20\mu M.s^{-1}$	$R_{er\ max}$	$1800\mu M.s^{-1}$
κ_{suber}	$250\mu M$	K_{er}	$0.001\mu M$
θ_{suber}	$9\mu M$	V_{pm}	$26\mu M.s^{-1}$
ν_h	1	K_{pm}	$0.18\mu M$
κ_h	$0.05\mu M$	p_{er}	$0.02s^{-1}$
θ_h	$0.6\mu M$	p_D	$0.012s^{-1}$
τ_h	300s	τ_{suber}	100s
J_{basal}	$1.8s^{-1}$	τ_{er}	500s

Initial conditions	
c	$0.05\mu M$
c_{er}, c_{suber}	$297.01\mu M$
h	0.99

References:

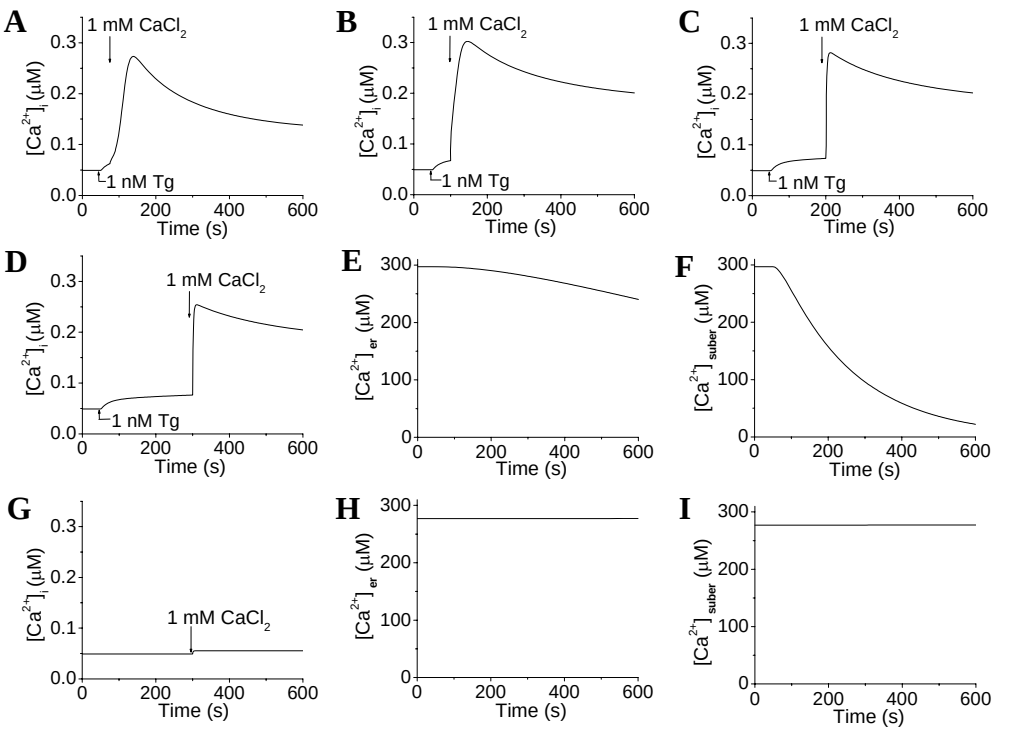
1. Sneyd, J., Tsaneva-Atanasova, K., Yule, D. I., Thompson, J. L., and Shuttleworth, T. J. (2004) *P Natl Acad Sci USA* **101**(5), 1392-1396
2. Ermentrout, B. (2002) *Simulating, Analyzing, and Animating Dynamical Systems: a guide to XPPAUT for researchers and students*, Society for Industrial and Applied Mathematics, Philadelphia

Supplemental Figure Legends

Supplemental Figure 1. Modeling of Tg-stimulated changes of Ca^{2+} in subplasma membrane and internal ER. (A – C) Computer simulations show that model accounts for calcium measurements (Fig 3 A – C) in 1 nM Tg-stimulated HSG cells. Simulated response of cytosolic Ca^{2+} to Tg and $CaCl_2$ additions (D) and model prediction that decrease in [Ca^{2+}] in internal (er) (E) is slower than in subplasma membrane ER (suber) stores (F). Compare with measurements in Fig 6 A. In control simulation without Tg (G - I), both ER stores remain stable. Compare Fig 6C.

Supplemental Figure 2. Redistribution of YFP-STIM 1 to the subplasma membrane region following carbachol stimulation. **(A)** Redistribution of YFP-STIM1 into punctae was observed at epifluorescence following stimulation of cells with 100 μM CCh (bottom panel) but not with 1 μM (top panel). Images were taken at 0 and 6 min after CCh addition. **(B)** Redistribution of YFP-STIM1 into punctae following stimulation with 1 (top panel) and 100 μM (bottom panel) CCh in Ca^{2+}-free media.

SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2