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## Supplemental Information

# $Ca^{2+}$ Release via $IP_3$ Receptors Shapes the Cardiac $Ca^{2+}$ Transient for Hypertrophic Signaling

**Hilary Hunt, Agnė Tilūnaitė, Greg Bass, Christian Soeller, H. Llewelyn Roderick, Vijay Rajagopal, and Edmund J. Crampin**

# Supporting Information: $Ca^{2+}$ release via $IP_3$ receptors shapes the cytosolic $Ca^{2+}$ transient for hypertrophic signalling in ventricular cardiomyocytes

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## Model Equations

Model ODEs are described in the main text and as follows:

$$\frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{\mathrm{SR}}}{\mathrm{d}t} = \frac{V_{\mathrm{myo}}}{V_{\mathrm{SR}}} \cdot (-I_{\mathrm{RyR}} + I_{\mathrm{SERCA}} - I_{\mathrm{SRl}} - I_{\mathrm{IP_3R}}) \tag{1}$$

$$\frac{\mathrm{dTnC}}{\mathrm{d}t} = I_{\mathrm{TnC}} \tag{2}$$

### CaRU model

We use the reduced, Hinch et al. (2004), model of the CaRU as described in Yu et al. (2011). The CaRU is modelled as having four states, $z_1, z_2, z_3, z_4$, each describing a different combination of an LTCC and an RyR channel being either open or closed. $J_{Li}$ and $J_{Ri}$ are the total flux through the LTCCs and RyRs in state $i$ respectively. These equations are detailed below.

$$I_{\mathrm{CaL}} = \frac{N}{V_{\mathrm{myo}}} \cdot (z_1 \cdot J_{L1} + z_2 \cdot J_{L2}) \tag{3}$$

$$I_{\mathrm{RyR}} = \frac{N}{V_{\mathrm{myo}}} \cdot (z_1 \cdot J_{R1} + z_3 \cdot J_{R3}) \tag{4}$$

$$\frac{\mathrm{d}z_1}{\mathrm{d}t} = -(r_1 + r_5) \cdot z_1 + r_2 \cdot z_2 + r_6 \cdot z_3 \tag{5}$$

$$\frac{\mathrm{d}z_2}{\mathrm{d}t} = r_1 \cdot z_1 - (r_2 + r_7) \cdot z_2 + r_8 \cdot z_4 \tag{6}$$

$$\frac{\mathrm{d}z_3}{\mathrm{d}t} = r_5 \cdot z_1 - (r_6 + r_3) \cdot z_3 + r_4 \cdot z_4 \tag{7}$$

$$z_4 = 1 - z_1 - z_2 - z_3 \tag{8}$$

$$J_{L1} = J_{Loo} \cdot y_{oo} + J_{Loc} \cdot y_{oc} \tag{9}$$

$$J_{L2} = \frac{J_{Loc} \cdot \alpha_p}{\alpha_p + \alpha_m} \tag{10}$$

$$J_{R1} = y_{oo} \cdot J_{Roo} + J_{Rco} \cdot y_{co} \tag{11}$$

$$J_{R3} = \frac{J_{Rco} \cdot \beta_{pcc}}{\beta_m + \beta_{pcc}} \tag{12}$$

where the CaRU fluxes are described as

$$J_{Rco} = \frac{J_R \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_i\right)}{g_D + J_R} \tag{13}$$

$$J_{Roo} = \begin{cases} \dfrac{J_R \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_i + \frac{\frac{J_L}{g_D} \cdot \mathrm{FVRT_{Ca}}}{1 - e^{-\mathrm{FVRT_{Ca}}}} \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_o \cdot e^{-\mathrm{FVRT_{Ca}}}\right)\right)}{1 + \dfrac{J_R}{g_D} + \dfrac{\frac{J_L}{g_D} \cdot \mathrm{FVRT_{Ca}}}{1 - e^{-\mathrm{FVRT_{Ca}}}}} & |\mathrm{FVRT_{Ca}}| > 10^{-5} \\ \dfrac{J_R \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_i + \frac{\frac{J_L}{g_D} \cdot 10^{-5}}{1 - e^{-10^{-5}}} \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_o \cdot e^{-10^{-5}}\right)\right)}{1 + \dfrac{J_R}{g_D} + \dfrac{\frac{J_L}{g_D} \cdot 10^{-5}}{1 - e^{-10^{-5}}}} & \text{otherwise} \end{cases} \tag{14}$$

$$J_{Loc} = \begin{cases} \dfrac{\frac{J_L \cdot \mathrm{FVRT_{Ca}}}{1 - e^{-\mathrm{FVRT_{Ca}}}} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-\mathrm{FVRT_{Ca}}} - [\mathrm{Ca}^{2+}]_i\right)}{1 + \dfrac{J_L}{g_D} \cdot \dfrac{\mathrm{FVRT_{Ca}}}{1 - e^{-\mathrm{FVRT_{Ca}}}}} & |\mathrm{FVRT_{Ca}}| > 10^{-5} \\ \dfrac{\frac{J_L \cdot 10^{-5}}{1 - e^{-10^{-5}}} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-10^{-5}} - [\mathrm{Ca}^{2+}]_i\right)}{1 + \dfrac{J_L}{g_D} \cdot \dfrac{10^{-5}}{1 - e^{-10^{-5}}}} & \text{otherwise} \end{cases} \tag{15}$$

$$J_{Loo} = \begin{cases} \dfrac{\frac{J_L \cdot \mathrm{FVRT_{Ca}}}{1 - e^{-\mathrm{FVRT_{Ca}}}} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-\mathrm{FVRT_{Ca}}} - [\mathrm{Ca}^{2+}]_i + \frac{J_R}{g_D} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-\mathrm{FVRT_{Ca}}} - [\mathrm{Ca}^{2+}]_{\mathrm{SR}}\right)\right)}{1 + \dfrac{J_R}{g_D} + \dfrac{J_L}{g_D} \cdot \dfrac{\mathrm{FVRT_{Ca}}}{1 - e^{\mathrm{FVRT_{Ca}}}}} & |\mathrm{FVRT_{Ca}}| > 10^{-5} \\ \dfrac{\frac{J_L \cdot 10^{-5}}{1 - e^{-10^{-5}}} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-10^{-5}} - [\mathrm{Ca}^{2+}]_i + \frac{J_R}{g_D} \cdot \left([\mathrm{Ca}^{2+}]_o \cdot e^{-10^{-5}} - [\mathrm{Ca}^{2+}]_{\mathrm{SR}}\right)\right)}{1 + \dfrac{J_R}{g_D} + \dfrac{J_L}{g_D} \cdot \dfrac{10^{-5}}{1 - e^{-10^{-5}}}} & \text{otherwise} \end{cases} \tag{16}$$

where

$$\mathrm{FVRT} = \frac{FV}{RT} \tag{17}$$

$$\mathrm{FVRT_{Ca}} = 2\mathrm{FVRT} \tag{18}$$

$F$ being the Faraday constant, $V$ the voltage across the cell membrane, described later, $R$ the gas constant and T the temperature.

CaRU reduced states:

$$r_1 = y_{oc} \cdot \mu_{poc} + y_{cc} \cdot \mu_{pcc} \tag{19}$$
$$r_2 = \frac{\alpha_p \cdot \mu_{moc} + \alpha_m \cdot \mu_{mcc}}{\alpha_p + \alpha_m} \tag{20}$$
$$r_3 = \frac{\beta_m \cdot \mu_{pcc}}{\beta_m + \beta_{pcc}} \tag{21}$$
$$r_4 = \mu_{mcc} \tag{22}$$
$$r_5 = y_{co} \cdot \epsilon_{pco} + y_{cc} \cdot \epsilon_{pcc} \tag{23}$$
$$r_6 = \epsilon_m \tag{24}$$
$$r_7 = \frac{\alpha_m \cdot \epsilon_{pcc}}{\alpha_p + \alpha_m} \tag{25}$$
$$r_8 = \epsilon_m \tag{26}$$

and

$$\mathrm{expVL} = e^{\frac{V - V_L}{del_{VL}}} \tag{27}$$
$$t_R = 1.17 \cdot t_L \tag{28}$$
$$\alpha_p = \frac{\mathrm{expVL}}{t_L \cdot (\mathrm{expVL} + 1)} \tag{29}$$
$$\alpha_m = \phi_L / t_L \tag{30}$$
$$\beta_{poc} = \frac{C_{oc}^2}{t_R \cdot (C_{oc}^2 + K_{\mathrm{RyR}}^2)} \tag{31}$$
$$\beta_{pcc} = \frac{[\mathrm{Ca}^{2+}]_i}{t_R \cdot ([\mathrm{Ca}^{2+}]_i^2 + K_{\mathrm{RyR}}^2)} \tag{32}$$
$$\beta_m = \frac{\phi_R}{t_R} \tag{33}$$

$$\epsilon_{pco} = \frac{C_{co} \cdot (\mathrm{expVL} + a)}{\tau_L \cdot K_L \cdot (\mathrm{expVL} + 1)} \tag{34}$$
$$\epsilon_{pcc} = \frac{[\mathrm{Ca}^{2+}]_i \cdot (\mathrm{expVL} + a)}{\tau_L \cdot K_L \cdot (\mathrm{expVL} + 1)} \tag{35}$$
$$\epsilon_m = \frac{b \cdot (\mathrm{expVL} + a)}{\tau_L \cdot (b \cdot \mathrm{expVL} + a)} \tag{36}$$

$$\mu_{poc} = \frac{(C_{oc}^2 + c \cdot K_{\mathrm{RyR}}^2)}{\tau_R \cdot (C_{oc}^2 + K_{\mathrm{RyR}^2)})} \tag{37}$$
$$\mu_{pcc} = \frac{[\mathrm{Ca}^{2+}]_i^2 + c \cdot K_{\mathrm{RyR}}^2}{\tau_R \cdot ([\mathrm{Ca}^{2+}]_i^2 + K_{\mathrm{RyR}}^2)} \tag{38}$$
$$\mu_{moc} = \frac{\theta_R \cdot d \cdot (C_{oc}^2 + c \cdot K_{\mathrm{RyR}}^2)}{\tau_R \cdot (d \cdot C_{oc}^2 + c \cdot K_{\mathrm{RyR}}^2)} \tag{39}$$
$$\mu_{mcc} = \frac{\theta_R \cdot d \cdot ([\mathrm{Ca}^{2+}]_i^2 + c \cdot K_{\mathrm{RyR}}^2)}{\tau_R \cdot (d \cdot [\mathrm{Ca}^{2+}]_i^2 + c \cdot K_{\mathrm{RyR}}^2)} \tag{40}$$

CaRU states

$$C_{cc} = [\mathrm{Ca}^{2+}]_i \tag{41}$$

$$C_{co} = \frac{[\mathrm{Ca}^{2+}]_i \cdot g_D + J_R \cdot [\mathrm{Ca}^{2+}]_{\mathrm{SR}}}{g_D + J_R} \tag{42}$$

$$C_{oc} = \begin{cases} \dfrac{g_D \cdot [\mathrm{Ca}^{2+}]_i + \dfrac{J_L \cdot [\mathrm{Ca}^{2+}]_o \cdot \mathrm{FVRT}_{\mathrm{Ca}} \cdot e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}{1 - e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}}{g_D + \dfrac{J_L \cdot \mathrm{FVRT}_{\mathrm{Ca}}}{1 - e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}} & |\mathrm{FVRT}_{\mathrm{Ca}}| > 10^{-9} \\ \dfrac{g_D \cdot [\mathrm{Ca}^{2+}]_i + J_L \cdot [\mathrm{Ca}^{2+}]_o}{g_D + J_L} & \text{otherwise} \end{cases} \tag{43}$$

$$C_{oo} = \begin{cases} \dfrac{g_D \cdot [\mathrm{Ca}^{2+}]_i + J_R \cdot [\mathrm{Ca}^{2+}]_{\mathrm{SR}} + \dfrac{J_L \cdot [\mathrm{Ca}^{2+}]_o \cdot \mathrm{FVRT}_{\mathrm{Ca}} \cdot e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}{1 - e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}}{g_D + J_R + \dfrac{J_L \cdot \mathrm{FVRT}_{\mathrm{Ca}}}{1 - e^{-\mathrm{FVRT}_{\mathrm{Ca}}}}} & |\mathrm{FVRT}_{\mathrm{Ca}}| > 10^{-9} \\ \dfrac{g_D \cdot [\mathrm{Ca}^{2+}]_i + J_R \cdot [\mathrm{Ca}^{2+}]_{\mathrm{SR}} + J_L \cdot [\mathrm{Ca}^{2+}]_o}{g_D + J_R + J_L} & \text{otherwise} \end{cases} \tag{44}$$

$$denom = (\alpha_p + \alpha_m) \cdot ((\alpha_m + \beta_m + \beta_{poc}) \cdot (\beta_m + \beta_{pcc}) + \alpha_p \cdot (\beta_m + \beta_{poc})) \tag{45}$$

$$y_{oc} = \frac{\alpha_p \cdot \beta_m \cdot (\alpha_p + \alpha_m + \beta_m + \beta_{pcc}}{denom} \tag{46}$$

$$y_{co} = \frac{\alpha_m \cdot (\beta_{pcc} \cdot (\alpha_m + \beta_m + \beta_{poc}) + \beta_{poc} \cdot \alpha_p)}{denom} \tag{47}$$

$$y_{oo} = \frac{\alpha_p \cdot (\beta_{poc} \cdot (\alpha_p + \beta_m + \beta_{pcc}) + \beta_{pcc} \cdot \alpha_m)}{denom} \tag{48}$$

$$y_{cc} = \frac{\alpha_m \cdot \beta_m \cdot (\alpha_m + \alpha_p + \beta_m + \beta_{poc})}{denom} \tag{49}$$

## Extracellular exchange and the cell membrane

$$I_{\mathrm{NCX}} = \frac{g_{\mathrm{NCX}} \cdot \left(e^{\eta \cdot \mathrm{FVRT}} \cdot [\mathrm{Na}^+]_{\mathrm{i}}^3 \cdot [\mathrm{Ca}^{2+}]_{\mathrm{e}} - \mathrm{e}^{(\eta-1) \cdot \mathrm{FVRT}} \cdot [\mathrm{Na}^+]_{\mathrm{e}}^3 \cdot [\mathrm{Ca}^{2+}]_{\mathrm{i}}\right)}{([\mathrm{Na}^+]_{\mathrm{e}}^3 + \mathrm{K}_{\mathrm{mNa}}^3) \cdot \left([\mathrm{Ca}^{2+}]_e + K_{\mathrm{mCa}}\right) \cdot \left(1 + k_{sat} \cdot e^{(\eta-1) \cdot \mathrm{FVRT}}\right)} \tag{50}$$

$$I_{\mathrm{PMCA}} = \frac{g_{\mathrm{PMCA}} \cdot [\mathrm{Ca}^{2+}]_i}{K_{\mathrm{PMCA}} + [\mathrm{Ca}^{2+}]_i} \tag{51}$$

$$I_{\mathrm{CaB}} = g_{\mathrm{CaB}} \cdot (E_{\mathrm{Ca}} - V) \tag{52}$$

where

$$E_{\mathrm{Ca}} = \frac{RT}{2F} \cdot \ln\left(\frac{[\mathrm{Ca}^{2+}]_o}{[\mathrm{Ca}^{2+}]_i}\right) \tag{53}$$

$$V = \begin{cases} -0.4 \bmod(t, T_{osc}) & \text{if } \bmod(t, T_{osc}) \leq 200 \text{ ms} \\ V_0 & \text{otherwise} \end{cases} \tag{54}$$

where t is the time since the start of the simulation and $T_{\mathrm{osc}}$ is the period of the driving voltage. The shape of the driving voltage was altered from that described in the original Hinch et al model. Examination of the individual

fluxes in simulations with reduced SERCA revealed that the original step function caused LTCCs to play a larger role than expected. As SERCA function was restricted, flux through LTCCs increased on a scale that resulted in peak amplitude increasing with reduced SERCA solely because of the influx of $Ca^{2+}$ through LTCCs. This was deemed unlikely to be physiologically plausible and the voltage function reduced to its current form. The effects of this change $V$ on the base model are negligible.

## Other fluxes across the SR membrane

$$I_{\mathrm{SERCA}} = \frac{g_{\mathrm{SERCA}} \cdot [\mathrm{Ca}^{2+}]_i^2}{K_{\mathrm{SERCA}}^2 + [\mathrm{Ca}^{2+}]_i^2} \tag{55}$$

$$I_{\mathrm{Sl}} = g_{\mathrm{Sl}} \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_i\right) \tag{56}$$

$$I_{\mathrm{IP_3R}} = \frac{k_f \cdot N_{\mathrm{IP_3R}}}{V_{\mathrm{myo}}} \cdot P_{\mathrm{IP_3R}} \cdot \left([\mathrm{Ca}^{2+}]_{\mathrm{SR}} - [\mathrm{Ca}^{2+}]_i\right) \tag{57}$$

$$\tag{58}$$

where, as described in Sneyd et al. (2017)

$$P_{\mathrm{IP_3R}} = \frac{\beta}{\beta + k_\beta \cdot (\beta + \alpha)} \tag{59}$$

Where $\alpha$ describes the rate of inactivation, $\beta$ the rate of activation. The parameter $k_\beta$ is used to fit to data, as in Sneyd et al. (2017)

$$\alpha = (1 - B) \cdot (1 - m \cdot h_\alpha) \tag{60}$$

$$\beta = B \cdot m \cdot h \tag{61}$$

Here $B$ describes the dependence on $\mathrm{IP_3}$, $m$ the dependence on $Ca^{2+}$, and $h$ and its limit $h_\alpha$ is a delay factor which also has a dependence on $Ca^{2+}$. In this study, we are primarily interested in the dependence of $\mathrm{IP_3R}$ channels on $Ca^{2+}$ so we fix $B$ and focus on the other parameters.

$$m = \frac{[\mathrm{Ca}^{2+}]_i^4}{K_c^4 + [\mathrm{Ca}^{2+}]_i^4} \tag{62}$$

$$\frac{dh}{dt} = \frac{(h_\alpha - h) \cdot \left(K_t^4 + [\mathrm{Ca}^{2+}]_i^4\right)}{t_{max} \cdot K_t^4} \tag{63}$$

$$h_\alpha = \frac{K_h^4}{K_h^4 + [\mathrm{Ca}^{2+}]_i^4} \tag{64}$$

The values $m$ and $h_\alpha$ are Hill functions. Together they are controlled by two parameters which determine the $Ca^{2+}$ dependence of $\mathrm{IP_3R}$ channels: $K_c$ and $K_h$. $\mathrm{IP_3R}$ channels are active at the intersection of these two functions.

## Cytosolic buffers

$$I_{\mathrm{TnC}} = k_{m_{\mathrm{TnC}}} \cdot (B_{\mathrm{TnC}} - \mathrm{TnC}) - k_{p_{\mathrm{TnC}}} \cdot \mathrm{TnC} \cdot [\mathrm{Ca}^{2+}]_i \tag{65}$$

$$\beta_{\mathrm{fluo}} = \left(1 + \frac{K_{\mathrm{fluo}} \cdot B_{\mathrm{fluo}}}{\left(K_{\mathrm{fluo}} + [\mathrm{Ca}^{2+}]_i\right)^2}\right)^{-1} \tag{66}$$

$$\beta_{\mathrm{CaM}} = \left(1 + \frac{K_{\mathrm{CaM}} \cdot B_{\mathrm{CaM}}}{\left(K_{\mathrm{CaM}} + [\mathrm{Ca}^{2+}]_i\right)^2}\right)^{-1} \tag{67}$$

### NFAT Coupling

Total cellular NFAT is split between four states: nuclear phosphorylated, $A_{pn}$, and dephosphorylated, $A_n$, and cytosolic phosphorylated, $A_{pc}$, and dephosphorylated, $A_c$ and cycles between them.

$$\frac{dA_n}{dt} = J_2 C_{cn} - J_3 \tag{68}$$
$$\frac{dA_{pn}}{dt} = J_3 - J_4 \tag{69}$$
$$\frac{dA_c}{dt} = J_1 - J_2 \tag{70}$$
$$\frac{dA_{pc}}{dt} = \frac{J_4}{C_{cn}} - J_1 \tag{71}$$

$C_{cn}$ is a scaling factor that accounts for the difference in volume between the cytosol and the nucleus while $J_1$, $J_2$, $J_3$, and $J_4$ are flux terms that describe the movement of NFAT between these four states.

$$J_1 = k_{f,1} A_{pc} N_{\mathrm{NFAT}} f_{\mathrm{CnA}} - k_{r,1} A_c (1 - f_{\mathrm{CnA}}) \tag{72}$$
$$J_2 = k_{f,2} A_c \tag{73}$$
$$J_3 = k_{f,3} A_n (1 - f_{\mathrm{CnA}}) - k_{r,3} A_n N_{\mathrm{NFAT}} f_{\mathrm{CnA}} \tag{74}$$
$$J_4 = k_{f,4} A_{pn} \tag{75}$$

Here $k_{f,i}$ are forward rate constants in the NFAT phosphorylation reaction, $k_{r,i}$ are reverse rate constants, and $f_{\mathrm{CnA}}$ is the fraction of activated calcineurin, given by

$$f_{\mathrm{CnA}} = \frac{[\mathrm{Ca}^{2+}]_i^n}{[\mathrm{Ca}^{2+}]_i^n + K_{m,N}^n \left(1 + \frac{K_{d,1}}{M}\right)} \tag{76}$$

where $n$ is the Hill coefficient for calcineurin activation, $K_{m,N}$ is the half-maximal activation conentration of cytosolic $Ca^{2+}$ for calmodulin activation for the expected cellular concentration of calmodulin, and $K_{d,1}$ is the calcineurin-calmodulin dissociation constant. Parameter values for these constants can be found in Table S3.

## Code Availability

CellML code is available on the Physiome Repository at:
https://models.physiomeproject.org/workspace/5ee

Matlab code is available on github at:
https://github.com/CellSMB/compartmental_ECC_ETC

## Parameter sensitivity analysis: Equations for main and total effects

As described in Saltelli et al. (2010), the main and total effects were calculated by generating two sampling matrices $A$ and $B$ of model parameter values and then, for each parameter, a matrix $A_B^{(i)}$ for which all but the $i$th column match those of $A$ and the $i$th column is the $i$th column of $B$.

The main effect of parameter $i$ is then:

$$S_i = V_{X_i}(E_{X_{\sim i}}(Y|X_i)) \Big/ V(Y) \tag{77}$$
$$= V(Y) - \sum_{j=1}^{N} \left(f(B)_j - f(A_B^{(i)})_j\right)^2 \Big/ 2V(Y)N \tag{78}$$

**Parameter values used in modelled $Ca^{2+}$ currents**

| Parameter | Description | Value |
|---|---|---|
| $\mathbf{N}$ | **Number of CaRUs in the cell** | **50 000** |
| $\mathbf{V_{myo}}$ | **Myocyte volume** | **25.84×10$^3$ $\mu$m$^3$** |
| $\mathbf{N_{IP_3R}}$ | **Number of IP$_3$R channels in the cell** | **20 000** |
| $\mathbf{g_{SERCA}}$ | **Maximum pump rate of SERCA** | **0.45 $\mu$M ms$^{-1}$** |
| $\mathbf{K_{SERCA}}$ | **Half saturation constant of SERCA** | **0.5 $\mu$M** |
| $\mathbf{g_{NCX}}$ | **Maximum pump rate of NCX** | **38.5 $\mu$M ms$^{-1}$** |
| $\eta$ | **Voltage dependence of NCX** | **0.35** |
| $\mathbf{K_{mNa}}$ | **Na$^+$ half saturation of NCX** | **87.5 mM** |
| $\mathbf{K_{mCa}}$ | **Ca$^{2+}$ half saturation of NCX** | **1.380 mM** |
| $\mathbf{k_{sat}}$ | **Low potential saturation factor of NCX** | **0.1** |
| $\mathbf{g_{PMCA}}$ | **Maximum pump rate of Ca$^{2+}$-ATPase** | **3.5 nM ms$^{-1}$** |
| $\mathbf{K_{PMCA}}$ | **Half saturation constant of Ca$^{2+}$-ATPase** | **38.5 $\mu$M ms$^{-1}$** |
| $\mathbf{g_{CaB}}$ | **Conductance of background Ca$^{2+}$ current** | **2.32 ×10$^{-5}$ $\mu$M ms$^{-1}$ mV$^{-1}$** |
| $\mathbf{g_{SRl}}$ | **Pump rate of NCX** | **1.8951 ×10$^{-5}$ ms$^{-1}$** |
| $\mathbf{K_t}$ | **IP$_3$R delayed response parameter** | **0.1 $\mu$M** |
| $\mathbf{t_{max}}$ | **IP$_3$R recovery time parameter** | **1000 s$^{-1}$** |

Table S1: $N_{IP_3R}$ from Harzheim et al. (2009). All other values from Hinch et al. (2004)

The total effect of parameter $i$ is then:

$$S_{T_i} = E_{X_{\sim i}}(V_{X_i}(Y|X_{\sim i}))\Big/V(Y) \tag{79}$$

$$= \sum_{j=1}^{N}\left(f(A)_j - f(A_B^{(i)})_j\right)^2\Big/2V(Y)N \tag{80}$$

Here we denote $f(A)_j$ the results of the simulation with parameter in row $j$ of the sampling matrix $A$; $V(Y)$ the variance in simulation results across all rows of $A$ and $B$; and $N$ the number of rows in $A$ and $B$.

In our parameter analysis, we generated parameter values within the range [0, 100] using the MATLAB sobolset function with Skip $1 \times 10^3$ and Leap $1 \times 10^2$, scrambled with the Mattousek-Affine-Owen algorithm. $N$ was set to $1 \times 10^6$.

**Fixed ionic concentrations and buffer parameters**

| Ion | Description | Value |
|---|---|---|
| $[Na^+]_i$ | Intracellular sodium | 10 mM |
| $[Na^+]_e$ | Extracellular sodium | 140 mM |
| $[Ca^{2+}]_e$ | Extracellular calcium | 1 mM |
| $B_{CaM}$ | Total cytosolic concentration of calmodulin | 50 x $10^{-3}$ mM |
| $K_{CaM}$ | Half saturation constant of calmodulin | 2.38 x $10^{-3}$ mM |
| $B_{TnC}$ | Total cytosolic concentration of troponin | 70 x $10^{-3}$ mM |
| $k_{m_{TnC}}$ | Dissociation rate of $Ca^{2+}$ to troponin | 0.04[3] $mM^{-1}$ $ms^{-1}$ |
| $k_{p_{TnC}}$ | Binding rate of $Ca^{2+}$ to troponin | 0.04 $\mu M^{-1}$ $ms^{-1}$ |
| $B_{fluo}$ | Concentration of Fluo-4AM dye | 1 x $10^{-3}$ mM |
| $K_{fluo}$ | Dissociation constant of Fluo-4AM dye | 1 mM |
| $V_0$ | Resting membrane potential | −80 mV |

Table S2: $K_{fluo}$ and $B_{fluo}$ from Thomas et al. (2000). All other values from Hinch et al. (2004)

**Parameter values used to model NFAT4**

| Parameter | Description | Value |
|---|---|---|
| $C_{cn}$ | Volume difference between nucleus and cytosol | 50 |
| $k_{f,1}$ | Rate constant | 7.69 × $10^{-6}$ $nM^{-1}s^{-1}$ |
| $k_{f,2}$ | Rate constant | 1.44 × $10^{-3}$ $s^{-1}$ |
| $k_{f,3}$ | Rate constant | 3.62 × $10^{-4}$ $s^{-1}$ |
| $k_{f,4}$ | Rate constant | 4.45 × $10^{-4}$ $s^{-1}$ |
| $k_{r,1}$ | Rate constant | 1.93 × $10^{-2}$ $s^{-1}$ |
| $k_{r,3}$ | Rate constant | 4.71 × $10^{-5}$ $nM^{-1}s^{-1}$ |
| $n$ | Hill coefficient of calcineurin | 2.92 |
| $K_{m,N}$ | Calcium-calcineurin constant | 535 nM |
| $K_{d,1}$ | calcineurin-calmodulin dissociation constant | 1760 nM |
| $M$ | Expected cellular calmodulin concentration | 6000 nM |

Table S3: Parameters for the parsimonious NFAT model by Cooling et al. (2009)

## Supplementary Figures

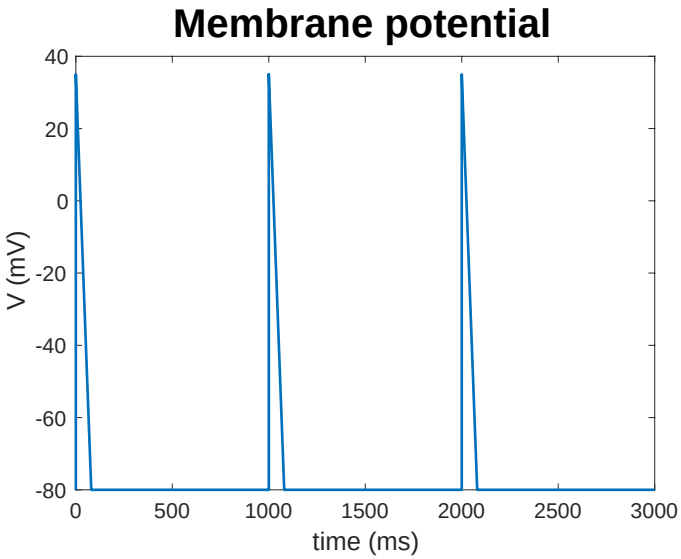


Figure S1: Membrane depolarisation initiating each calcium transient.

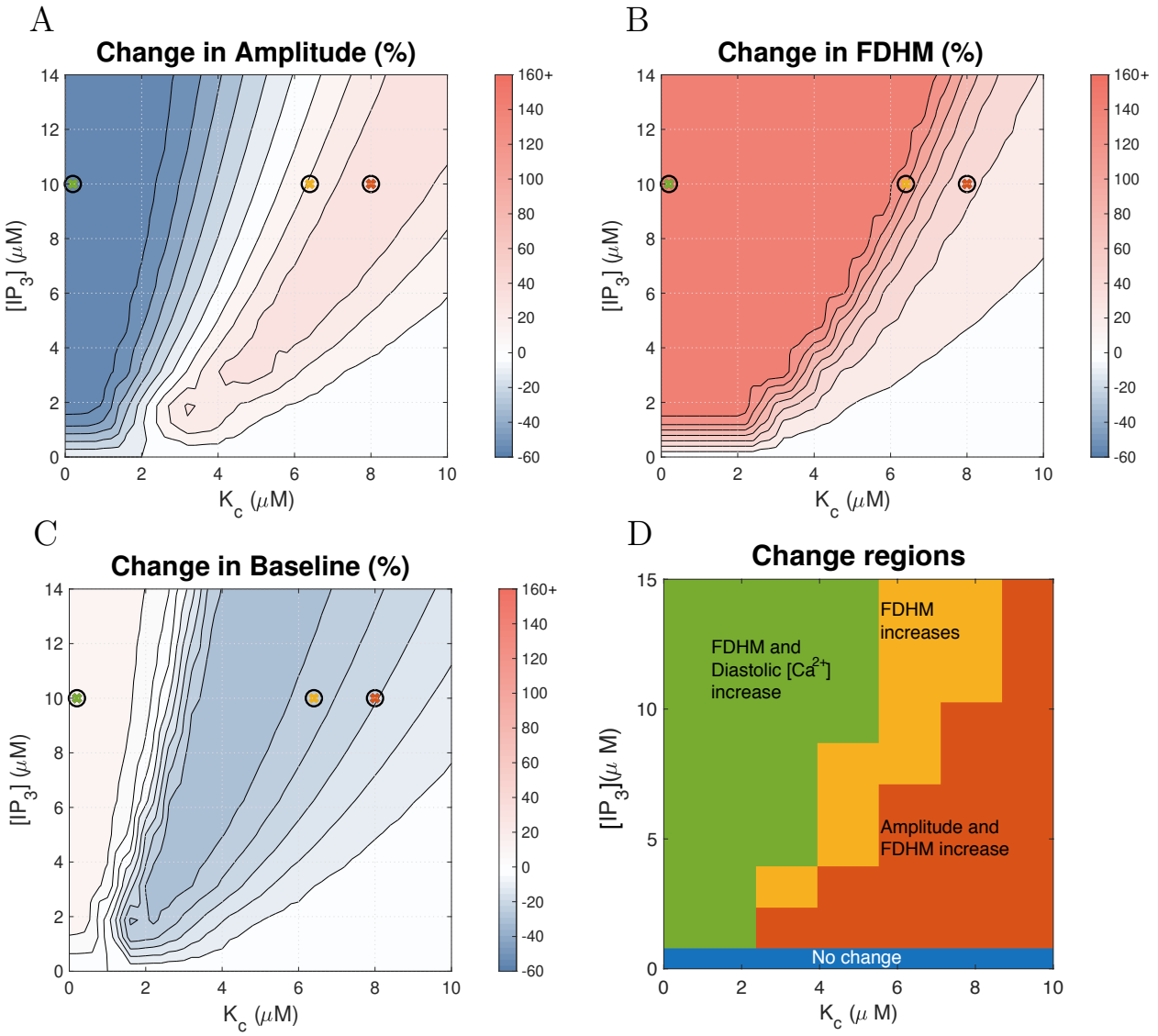


Figure S2: Effect of $IP_3$ concentration and the parameter $K_c$ on the calcium transient with large delay and pacing frequency of 0.3 Hz. These two parameters, along with maximum $IP_3R$ flux, have the greatest impact when considering the effect of $IP_3R$ activation on the calcium transient.

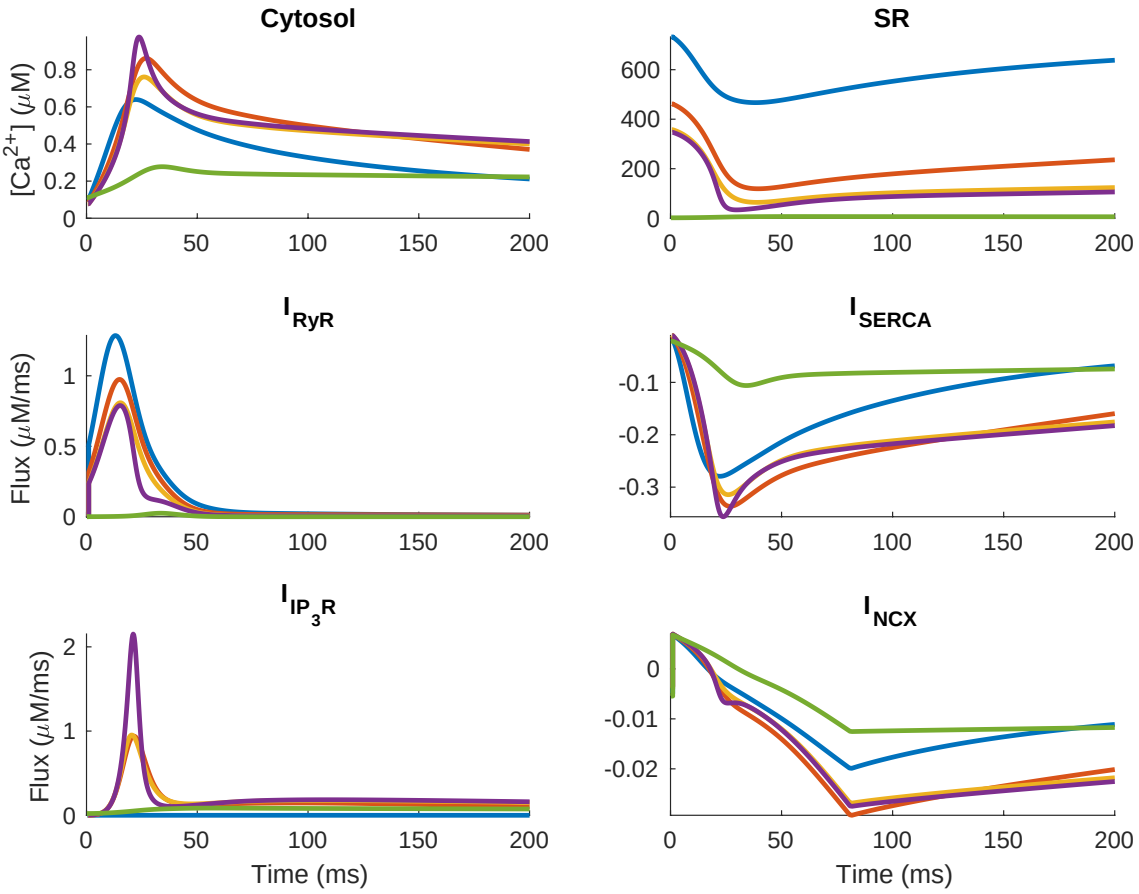


Figure S3: Simulated hypertrophic ECC transient and fluxes with varying $k_f$, $K_c$. The sign of $I_{NCX}$ indicates whether calcium is moving into (positive) or out of (negative) the cell. Parameters here are chosen to show the system behaviour at each region illustrated in Figure 7. The crosses in each of Figures S2 and 7 match the colours of the corresponding transients in this figure. $IP_3$ concentration is 10 $\mu$M in all simulations. The model is paced at 0.3 Hz.

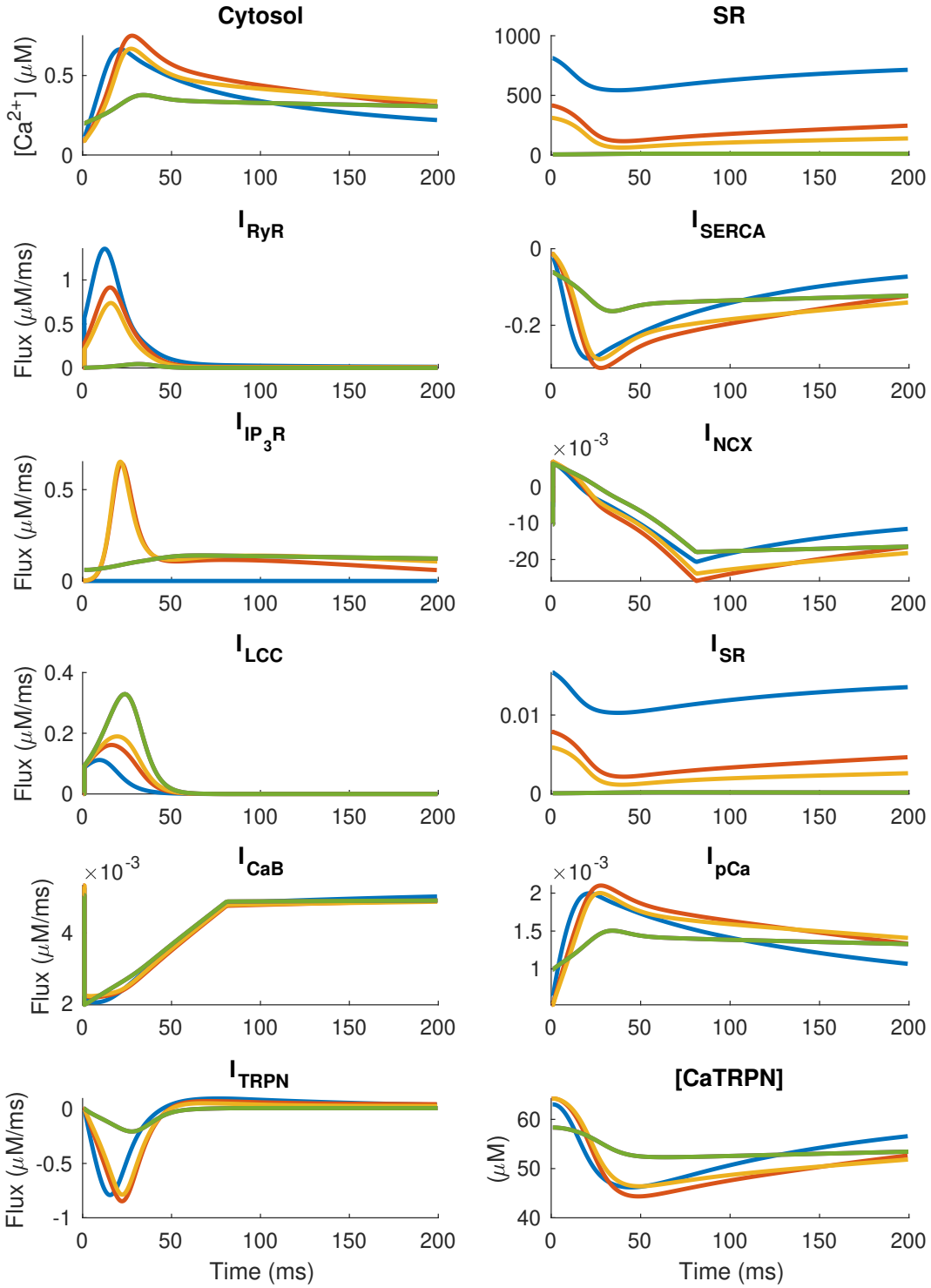


Figure S4: Simulated hypertrophic ECC transient and fluxes with varying [IP$_3$], $K_c$. The sign of $I_{NCX}$ indicates whether calcium is moving into (positive) or out of (negative) the cell. Parameters here are chosen to show the system behaviour at each region illustrated in Figure 4. The crosses in each of Figures 4 and 6 match the colours of the corresponding transients in this figure. IP$_3$ concentration is 10 $\mu$M in all simulations. The model is paced at 1 Hz.

## Supporting References


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