SUPPORTING MATERIAL

Parameter sensitivity analysis of stochastic models provides insights into cardiac calcium sparks

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Sticky Cluster Model equations

$$\frac{d[Ca^{2+}]_{ss}}{dt} = J_{release} + J_{efflux} + J_{buffer} \quad \text{(S1)}$$

$$J_{release} = \sum_{j=1}^{N_{RyR}} RyR_{open}^{j} J_{RyR} \quad \text{(S2)}$$

$$J_{RyR} = \frac{D_{RyR}}{V_{SS}}\left([Ca^{2+}]_{JSR} - [Ca^{2+}]_{SS}\right) \quad \text{(S3)}$$

$$J_{buffer} = \sum_{i=CaM,SR,SL} k_{on}^{i}[B_i][Ca^{2+}]_{ss} - k_{off}^{i}([B_i]_{Total} - [B_i]) \quad \text{(S4)}$$

$$J_{efflux} = \frac{\left([Ca^{2+}]_{myo} - [Ca^{2+}]_{SS}\right)}{\tau_{efflux}} \quad \text{(S5)}$$

$$\frac{d[Ca^{2+}]_{JSR}}{dt} = \beta_{JSR}\left[-J_{release}\frac{V_{SS}}{V_{JSR}} + J_{refill}\right] \quad \text{(S6)}$$

$$\beta_{JSR} = \left[1 + \frac{[CSQ]_{Total} K_{CSQ}}{(K_{CSQ} + [Ca^{2+}]_{JSR})^2}\right]^{-1} \quad \text{(S7)}$$

$$J_{refill} = \frac{\left([Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR}\right)}{\tau_{refill}} \quad \text{(S8)}$$

RyR gating equations

$$k_{close} = k_{close,\max} CF_{close} \quad \text{(S9)}$$

$$k_{open} = k_{open,\max} CF_{open} \frac{([Ca^{2+}]_{SS})^4}{([Ca^{2+}]_{SS})^4 + K_m^4} \quad \text{(S10)}$$

$$K_m = K_{\max} - \alpha[Ca^{2+}]_{JSR} \quad \text{(S11)}$$

$$K_{coup} = \exp\left(2E_J \big/ (N_{RyR} - 1)\right) \quad \text{(S12)}$$

$$CF_{open} = K_{coup}^{(2N_{open} + 1 - N_{RyR})} \quad \text{(S13)}$$

$$CF_{close} = K_{coup}^{(2N_{closed} + 1 - N_{RyR})} \quad \text{(S14)}$$

Table S1 – Geometry Parameters

Parameter	Definition	Value
V_{SS}	Sub-space volume	1.0×10^{-12} μL
V_{JSR}	Junctional SR volume	1.6×10^{-12} μL

Table S2 – Fixed Ionic Concentrations

Parameter	Definition	Value
$[Ca^{2+}_{Myo}]$	bulk myoplasmic Ca^{2+} concentration	0.1 μmol/L
$[Ca^{2+}_{NSR}]$	NSR Ca^{2+} concentration	1.0×10^{3} μmol/L

Table S3 - Buffering Parameters

Parameter	Definition	Value
$[B_{CaM}]_{Total}$	Total calmodulin concentration	24 μmol/L
k_{on}^{CaM}	Calmodulin Ca^{2+} on rate constant	100 μmol/L $^{-1}$ s^{-1}
k_{off}^{CaM}	Calmodulin Ca^{2+} on rate constant	38 s^{-1}
$[B_{SL}]_{Total}$	Total SL membrane buffer concentration	900 μmol/L
k_{on}^{SL}	SL membrane buffer Ca^{2+} on rate constant	115 μM^{-1} s^{-1}
k_{off}^{SL}	SL membrane buffer Ca^{2+} off rate constant	1000 s^{-1}
$[B_{SR}]_{Total}$	Total SR membrane buffer concentration	47 μM
k_{on}^{SR}	SR membrane buffer Ca^{2+} on rate constant	115 μM^{-1} s^{-1}
k_{off}^{SR}	SR membrane buffer Ca^{2+} off rate constant	100 s^{-1}
$[B_{Csq}]_{Total}$	Total calsequestrin concentration	30.0×10^{3} μmol/L
K_{Csq}	Calsequestrin Ca^{2+} dissociation constant	630 μmol/L

Table S4 – RyR Parameters

Parameter	Definition	Value
D_{RyR}	RyR Permeability constant	2.2×10^{-9} μL s^{-1}
α	RyR luminal dependence factor	1.0×10^{-3} (unitless)
N_{RyR}	Number of RyR channels in a cluster	28
$k_{open,max}$	maximum RyR opening rate	3 x 10^{4} s^{-1}
$k_{close,max}$	maximum RyR closing rate	480 s^{-1}
K_{max}	sensitivity of opening to subspace [Ca]	19.87 μmol/L

Table S5 – Initial Conditions

Parameter	Definition	Value
$[Ca^{2+}_{ss}]$	subspace Ca^{2+} concentration	0.1 μmol/L
$[Ca^{2+}_{JSR}]$	JSR Ca^{2+} concentration	1.0×10^{3} μmol/L

Multivariable regression analysis

The parameter sensitivities calculated from multivariable regression analysis (Fig. 3B) indicate how much each model parameter influences Ca^{2+} spark amplitude and Ca^{2+} spark duration. Here we illustrate through an example how these parameter sensitivities can be understood in quantitative terms.

The multivariable regression procedure generates a linear model:

$$\hat{Y} = XB \qquad \text{(S17)}$$

where **X** is a matrix of parameters, **Ŷ** the matrix of predicted model outputs, and **B** the regression matrix. For simplicity, here we consider the predicted change in a single model output ($\Delta\hat{y}$) produced by a change in a single model parameter (Δx):

$$\Delta\hat{y} = \Delta x b \qquad \text{(S18)}$$

where b is the relevant parameter sensitivity.

Before performing the regression, parameters in **X** and outputs in **Y** are log-transformed. Then the regression procedure converts the log-transformed values into z-scores by subtracting the mean and dividing by the standard deviation on a column-by-column basis. Thus, to predict the value ŷ resulting from an arbitrary value of a parameter x, we compute:

$$\frac{\log(\hat{y}) - \log(\mu_y)}{\sigma_y} = b\frac{\log(x) - \log(\mu_x)}{\sigma_x} \qquad \text{(S19)}$$

or:

$$\log(\frac{\hat{y}}{\mu_y}) = \frac{b\sigma_y}{\sigma_x}\log(\frac{x}{\mu_x}) \qquad \text{(S20)}$$

where μ_y and μ_x are the mean values of the output and the parameter, respectively and σ_y and σ_x are the respective standard deviations. Each parameter sensitivity shown in Fig. 3B is a scaled value $b' = b\cdot\sigma_y/\sigma_x$.

These scaled parameter sensitivities allow us to compute the percentage change in an output through the formula:

$$\frac{\hat{y}}{\mu_y} = e^{\left(b'\log(x/\mu_x)\right)} \qquad \text{(S21)}$$

or, equivalently:

$$\frac{\hat{y}}{\mu_y} = \left(\frac{x}{\mu_x}\right)^{b'} \qquad \text{(S22)}$$

For example, the sensitivity coefficient b' describing how **CSQ** affects Ca^{2+} spark amplitude is equal to 0.45. This implies that a 50% increase in **CSQ** leads to a 20% increase in spark amplitude because $(1.5)^{0.45} = 1.20$.

Logistic regression analysis

The parameter sensitivities calculated from logistic regression analysis (Fig. 4A) indicate how much each model parameter influences Ca^{2+} spark probability. Here we illustrate through an example how these parameter sensitivities can be understood in quantitative terms.

As described in the main text, the logistic regression model describes spark probability as a function of changes in the 18 model parameters:

$$\log\left(\frac{P}{1-P}\right) = b_0 + b_1 x_1 + \ldots + b_{18} x_{18} \qquad \text{(S23)}$$

where each variable x_i represents a deviation from the baseline value. Thus, when all parameters are equal to the baseline values, we have:

$$\log\left(\frac{P_0}{1-P_0}\right) = b_0 \qquad \text{(S24)}$$

where we define P_0 as the probability that a single RyR opening will trigger a Ca^{2+} spark with the baseline set of parameters. P_0 is equal to 976/2000 = 0.48 in this model.

When a single parameter changes, the magnitude of the change and the corresponding parameter sensitivity b determine the new spark probability, as follows. Remember that parameters are log-transformed and converted into z-scores before performing the logistic regression. Thus, for a new value x of a particular parameter:

$$\log\left(\frac{P}{1-P}\right) = b_0 + b\frac{\log(x) - \log(\mu_x)}{\sigma_x} \qquad \text{(S25)}$$

Subtracting Eq. S24 from Eq. S25, we obtain:

$$\log\left(\frac{P}{1-P}\right) - \log\left(\frac{P_0}{1-P_0}\right) = b\frac{\log(x) - \log(\mu_x)}{\sigma_x} \qquad \text{(S25)}$$

or:

$$\log\left(\frac{\frac{P}{1-P}}{\frac{P_0}{1-P_0}}\right) = \frac{b}{\sigma_x}\log\left(\frac{x}{\mu_x}\right) \qquad \text{(S26)}$$

As with the multivariable linear regression, we rescale parameter sensitivities, i.e. $b' = b/\sigma_x$. Thus:

$$\frac{\frac{P}{1-P}}{\frac{P_0}{1-P_0}} = \left(\frac{x}{\mu_x}\right)^{b'} \qquad \text{(S27)}$$

and:

$$\frac{P}{1-P} = \frac{P_0}{1-P_0}\left(\frac{x}{\mu_x}\right)^{b'} \qquad \text{(S27)}$$

This shows how we can calculate a new probability from the baseline probability and the percentage change in a parameter. For instance, the unscaled parameter sensitivity corresponding to parameter Ca_{NSR} (Fig. 4B) is equal to 1.26, and the standard deviation σ_x is equal to 0.29, so the scaled sensitivity b' is equal to 4.34. At baseline, $P_0/(1-P_0)$ = 0.48/0.52 = 0.92. Thus a 20% increase in Ca_{NSR} results in $P/(1-P)$ = 2.03, or P = 0.67. This is consistent with experimental results indicating that changes in SR $[Ca^{2+}]$ have a large effect on the rate of spontaneous Ca^{2+} sparks.

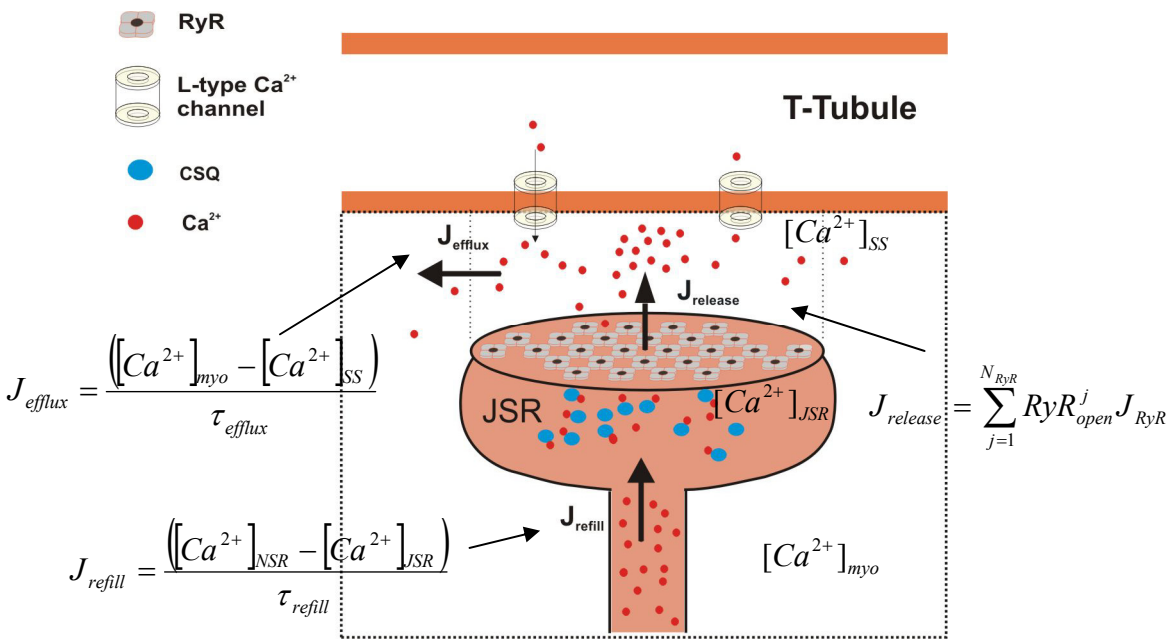
Generation of Receiver Operator Characteristic (ROC) curves

From an initial set of 800 trials, 7 unique sets of 700 trials each were generated. A logistic regression model built from each set of 700 trials was used to predict the probability that Ca^{2+} sparks would be produced in the remaining 100 trials. In this way, the model was built and predictions were performed on different sets of simulation results. The threshold for whether not a particular trial generated a spark was systematically varied from 0 to 1. At each threshold we compared the logistic regression predictions with the simulation results by computing the true positive rate and the false positive rate as follows. Each trial can be categorized as true positive (TP: both simulation and regression indicate that a spark was triggered), a true negative (TN: both simulation and regression indicate that a spark was not triggered), a false positive (FP: regression predicts a spark whereas simulation indicates no spark), or a false negative (FN: regression predicts no spark whereas simulation produces a spark). True positive rate (TPR) and false positive rate (FPR) were then calculated as:

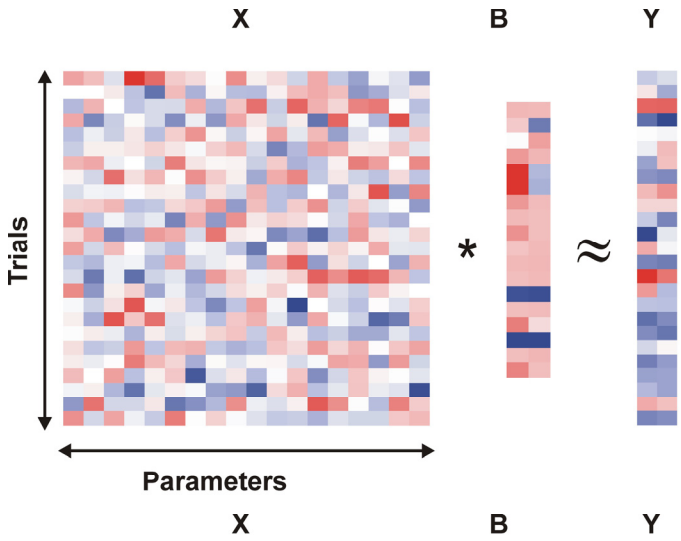
$$TPR = \frac{TP}{TP + FN} \tag{3}$$

$$FPR = \frac{FP}{FP + TN} \tag{4}$$

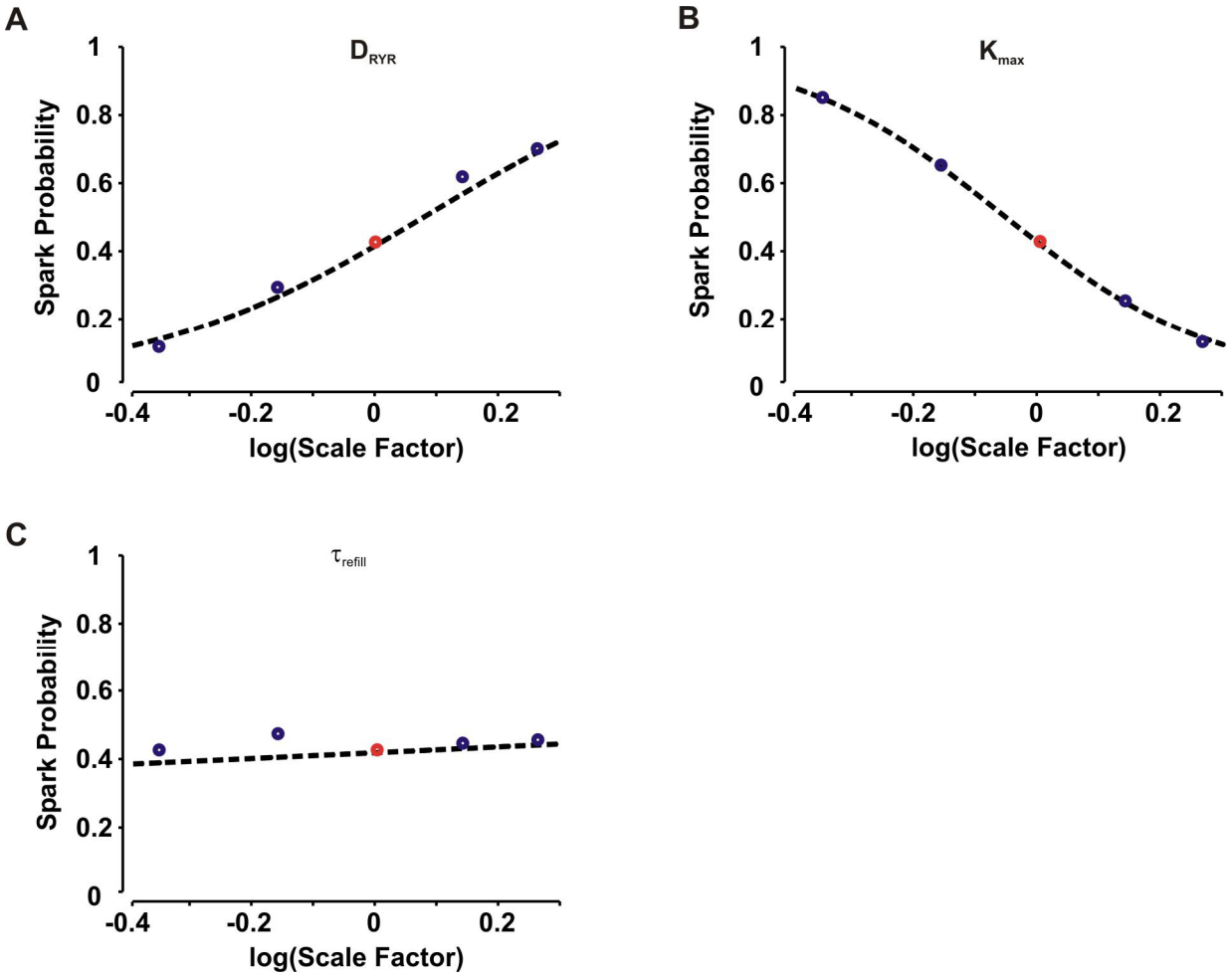
These two quantities were plotted against one another to produce the ROC curves shown in Fig. 4A. Prediction strength was assessed by computing the area under the curve (AUC). a random predictor has AUC=0.5 whereas a perfect predictor has AUC = 1.0.



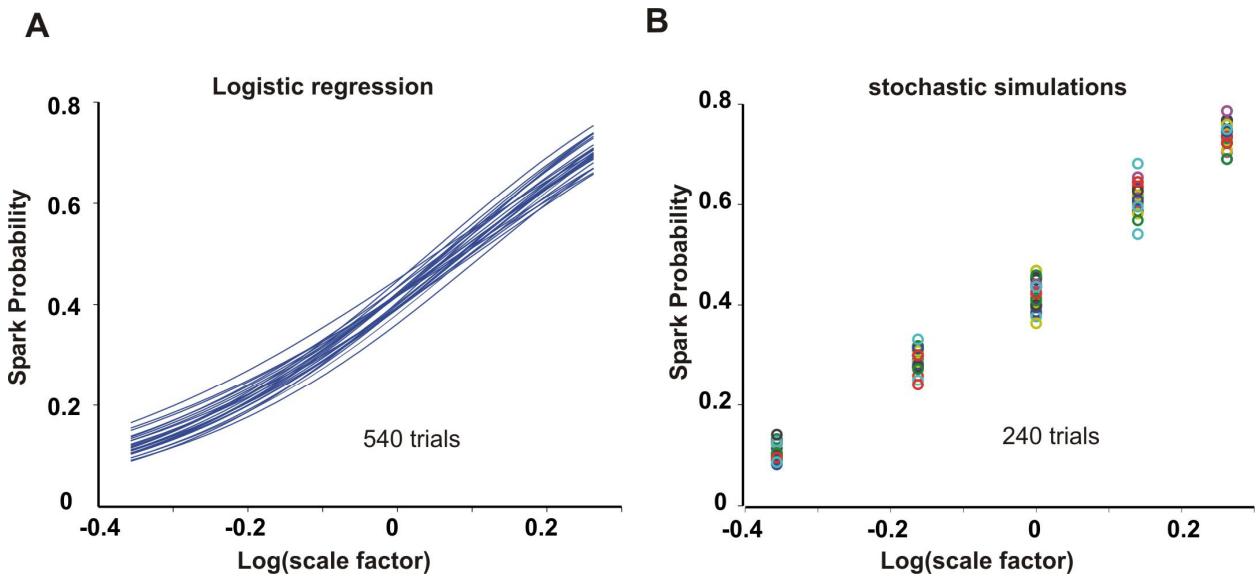
Supplementary Figure S1. Schematic diagram of the sticky cluster model. RyR channels are present in the JSR apposed to the T-tubule, and opening of one RyR triggers Ca^{2+} release ($J_{release}$) from the RyR cluster. This released Ca^{2+} binds to buffers in the subspace and diffuses into the myoplasm (J_{efflux}). Release leads to local depletion of JSR $[Ca^{2+}]$, and this concentration returns to its original level by Ca^{2+} transfer from the NSR (J_{refill}).



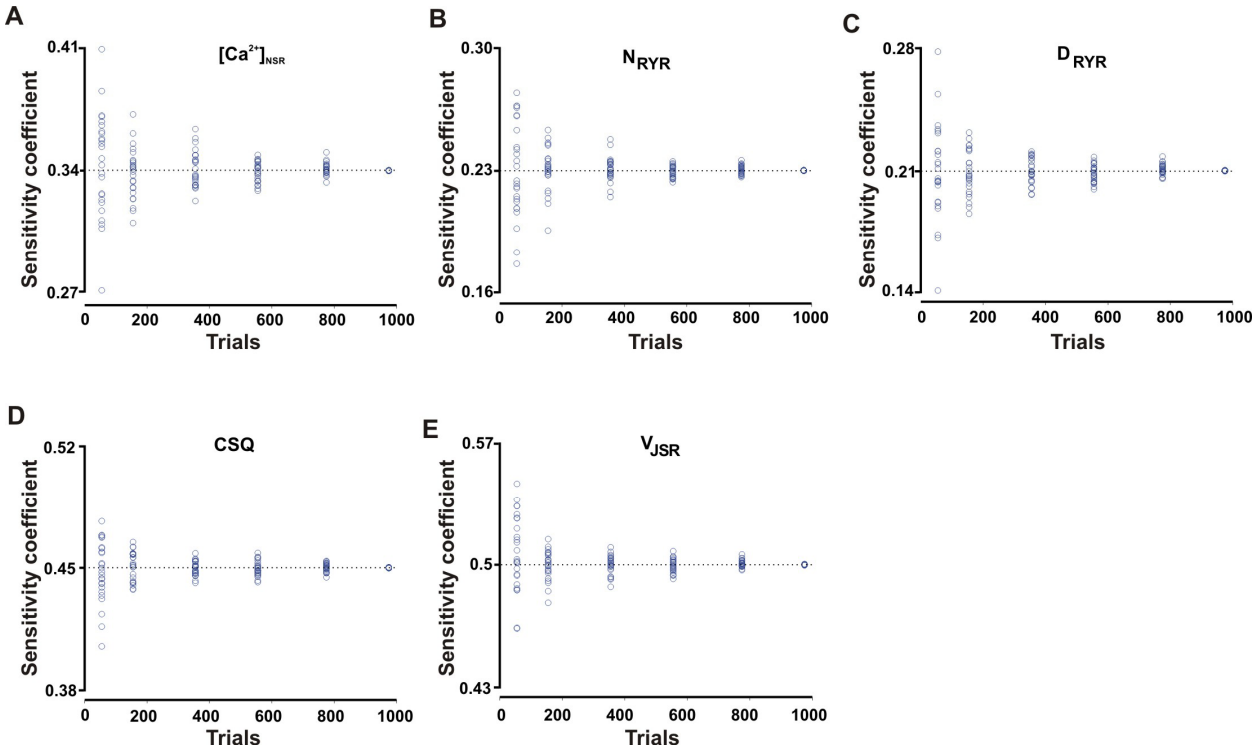
Supplementary Figure S2. Schematic representation of input, sensitivity coefficient, and output matrices in the multivariable regression analysis. Input matrix (**X**) consists of the number of trials (row) and the number of z-scored and log-transformed parameters (column), and z-scored and log-transformed output matrix (**Y**). Sensitivity coefficient matrix (**B**) makes the product **X*B** a close approximation to **Y.**



Supplementary Figure S3. Prediction by logistic regression at different parameters were compared with stochastic simulation with (A) D_{RYR}, (B) Kmax, (C) τ_{refill}. Each parameter is scaled by -30%, -15%, 0%, 15% and 30% for calculation and displayed as logarithm of scale factor for plotting.



Supplementary Figure S4. Computational performance with Ca_{NSR}. (A) Prediction curves (540 trials) of logistic regression with a standard deviation of 0.03. (B) Stochastic simulation data (240 trials: circles) with a standard deviation of 0.03.



Supplementary Figure S5. We estimated how sensitivity coefficients depend on the number of simulation (trials). We ran 2000 trials of stochastic simulations with randomly perturbed parameters. 976 subsets out of 2000 trials showed Ca^{2+} sparks. Parameter sensitivity coefficients on the spark amplitude were calculated at subsets of 976 trials. Each subset was randomly selected with trial size 55, 155, 355, 555, 775, and 975. This process was repeated for 25 times to display at each trial. We chose five most important parameters for spark amplitude (A) $[Ca^{2+}]_{NSR}$, (B) N_{RYR}, (C) D_{RYR}, (D) CSQ, and (E) V_{JSR}. This result shows that sensitivity coefficient converges as the trial size increases.