

Data Supplement

Synergism of coupled subsarcolemmal Ca^{2+} clocks and sarcolemmal voltage clocks confers robust and flexible pacemaker function in a novel pacemaker cell model

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Parameters and formulations of Basal state action potential firing model

PARAMETERS

Fixed ion concentrations, mM

$\text{Ca}_o = 2$: Extracellular Ca^{2+} concentration.

$\text{K}_o = 5.4$: Extracellular K^+ concentration.

$\text{K}_i = 140$: Intracellular K^+ concentration.

$\text{Na}_o = 140$: Extracellular Na^+ concentration.

$\text{Na}_i = 10$: Intracellular Na^+ concentration.

$\text{Mg}_i = 2.5$: Intracellular Mg^{2+} concentration.

Cell compartments

$C_m = 32$ pF: Cell electric capacitance.

$L_{\text{cell}} = 70$ μm : Cell length.

$R_{\text{cell}} = 4$ μm : Cell radius.

$L_{\text{sub}} = 0.02$ μm : Distance between jSR and surface membrane (submembrane space).

$V_{\text{cell}} = \pi \cdot R_{\text{cell}}^2 \cdot L_{\text{cell}} = 3.5185838$ pL: Cell volume.

$V_{\text{sub}} = 2\pi \cdot L_{\text{sub}} \cdot (R_{\text{cell}} - L_{\text{sub}}/2) \cdot L_{\text{cell}} = 0.035097874$ pL: Submembrane space volume.

$V_{\text{jSR_part}} = 0.0012$: Part of cell volume occupied by junctional SR.

$V_{\text{jSR}} = V_{\text{jSR_part}} \cdot V_{\text{cell}}$: Volume of junctional SR (Ca^{2+} release store).

$V_{\text{i_part}} = 0.46$: Part of cell volume occupied with myoplasm.

$V_i = V_{\text{i_part}} \cdot V_{\text{cell}} - V_{\text{sub}}$: Myoplasmic volume.

$V_{\text{nSR_part}} = 0.0116$: Part of cell volume occupied by network SR.

$V_{\text{nSR}} = V_{\text{nSR_part}} \cdot V_{\text{cell}}$: Volume of network SR (Ca^{2+} uptake store).

The Nernst equation

$E_X = (RT/F) \cdot \ln([X]_o/[X]_i) = E_T \cdot \ln([X]_o/[X]_i)$, where

$F = 96485$ C/M is Faraday constant,

$T = 310.15$ K° is absolute temperature for 37°C,

$R = 8.3144$ J/(M·K°) is the universal gas constant,

E_T is “RT/F” factor = 26.72655 mV,

and $[X]_o$ and $[X]_i$ are concentrations of an ion “X” out and inside cell, respectively.

Electric potentials, mV

$E_{\text{Na}} = E_T \cdot \ln(\text{Na}_o/\text{Na}_i)$: Equilibrium potential for Na^+ .

$E_{\text{K}} = E_T \cdot \ln(\text{K}_o/\text{K}_i)$: Equilibrium potential for K^+ .

$E_{\text{Ks}} = E_T \cdot \ln\{(\text{K}_o + 0.12 \cdot \text{Na}_o)/(\text{K}_i + 0.12 \cdot \text{Na}_i)\}$: Reversal potential of I_{Ks} .

$E_{\text{CaL}} = 45$: Apparent reversal potential of I_{CaL} .

$E_{\text{CaT}} = 45$: Apparent reversal potential of I_{CaT} .

$E_{st} = 37.4$: Apparent reversal potential of I_{st} .

Sarcolemmal Ion currents and their normalized conductances (g_x)

- I_{CaL} : L-type Ca^{2+} current ($g_{CaL} = 0.464$ nS/pF).
 I_{CaT} : T-type Ca^{2+} current ($g_{CaT} = 0.1832$ nS/pF).
 I_f : Hyperpolarization-activated current ($g_{if} = 0.15$ nS/pF).
 $V_{If,1/2} = -64$ mV: Half activation voltage for I_f current.
 I_{st} : Sustained non-selective current ($g_{st} = 0.003$ nS/pF).
 I_{Kr} : Delayed rectifier K^+ current rapid component ($g_{Kr} = 0.08113973$ nS/pF).
 I_{Ks} : Delayed rectifier K^+ current slow component ($g_{Ks} = 0.0259$ nS/pF).
 I_{to} : 4-aminopyridine sensitive transient K^+ current ($g_{to} = 0.252$ nS/pF).
 I_{sus} : 4-aminopyridine sensitive sustained K^+ current ($g_{sus} = 0.02$ nS/pF).
 I_{NaK} : Na^+/K^+ pump current ($I_{NaKmax} = 2.88$ pA/pF).
 I_{NCX} : Na^+/Ca^{2+} exchanger (NCX) current ($k_{NCX} = 187.5$ pA/pF).
 I_{bCa} : Background Ca^{2+} current ($g_{bCa} = 0.0006$ nS/pF).
 I_{bNa} : Background Na^+ current ($g_{bNa} = 0.00486$ nS/pF).

Modulation of sarcolemmal ion currents by ions

- $K_{mfCa} = 0.00035$ mM: Dissociation constant of Ca^{2+} -dependent I_{CaL} inactivation.
 $K_{mKp} = 1.4$ mM: Half-maximal K_o for I_{NaK} .
 $K_{mNap} = 14$ mM: Half-maximal Na_i for I_{NaK} .
 $\beta_{fCa} = 60$ mM $^{-1} \cdot$ ms $^{-1}$: Ca^{2+} association rate constant for I_{CaL} .
 $\alpha_{fCa} = 0.021$ ms $^{-1}$: Ca^{2+} dissociation rate constant for I_{CaL} , ms $^{-1}$.

NCX function, mM

- $K_{1ni} = 395.3$: intracellular Na^+ binding to first site on NCX.
 $K_{2ni} = 2.289$: intracellular Na^+ binding to second site on NCX.
 $K_{3ni} = 26.44$: intracellular Na^+ binding to third site on NCX.
 $K_{1no} = 1628$: extracellular Na^+ binding to first site on NCX.
 $K_{2no} = 561.4$: extracellular Na^+ binding to second site on NCX.
 $K_{3no} = 4.663$: extracellular Na^+ binding to third site on NCX.
 $K_{ci} = 0.0207$: intracellular Ca^{2+} binding to NCX transporter.
 $K_{co} = 3.663$: extracellular Ca^{2+} binding to NCX transporter.
 $K_{cni} = 26.44$: intracellular Na^+ and Ca^{2+} simultaneous binding to NCX.
 $Q_{ci} = 0.1369$: intracellular Ca^{2+} occlusion reaction of NCX.
 $Q_{co} = 0$: extracellular Ca^{2+} occlusion reaction of NCX.
 $Q_n = 0.4315$: Na^+ occlusion reactions of NCX.

Ca^{2+} diffusion

- $\tau_{difCa} = 0.04$ ms: Time constant of Ca^{2+} diffusion from the submembrane to myoplasm.
 $\tau_r = 40$ ms: Time constant for Ca^{2+} transfer from the network to junctional SR.

SR Ca^{2+} ATPase function

- $K_{up} = 0.6 \cdot 10^{-3}$ mM: Half-maximal Ca_i for Ca^{2+} uptake in the network SR.
 $P_{up} = 0.012$ mM/ms: Rate constant for Ca^{2+} uptake by the Ca^{2+} pump in the network SR.

RyR function

$k_{oCa} = 10 \text{ mM}^{-2} \cdot \text{ms}^{-1}$; $k_{om} = 0.06 \text{ ms}^{-1}$; $k_{iCa} = 0.5 \text{ mM}^{-1} \cdot \text{ms}^{-1}$; $k_{im} = 0.005 \text{ ms}^{-1}$; $EC_{50_SR} = 0.45 \text{ mM}$; $k_s = 250 \cdot 10^3 \text{ ms}^{-1}$; $MaxSR = 15$; $MinSR = 1$; $HSR = 2.5$;

Ca²⁺ and Mg²⁺ buffering

$k_{bCM} = 0.542 \text{ ms}^{-1}$: Ca²⁺ dissociation constant for calmodulin.
 $k_{bCQ} = 0.445 \text{ ms}^{-1}$: Ca²⁺ dissociation constant for calsequestrin.
 $k_{bTC} = 0.446 \text{ ms}^{-1}$: Ca²⁺ dissociation constant for the troponin-Ca²⁺ site.
 $k_{bTMC} = 0.00751 \text{ ms}^{-1}$: Ca²⁺ dissociation constant for the troponin-Mg²⁺ site.
 $k_{bTMM} = 0.751 \text{ ms}^{-1}$: Mg²⁺ dissociation constant for the troponin-Mg²⁺ site.
 $k_{fCM} = 227.7 \text{ mM}^{-1} \cdot \text{ms}^{-1}$: Ca²⁺ association constant for calmodulin.
 $k_{fCQ} = 0.534 \text{ mM}^{-1} \cdot \text{ms}^{-1}$: Ca²⁺ association constant for calsequestrin.
 $k_{fTC} = 88.8 \text{ mM/ms}$: Ca²⁺ association constant for troponin.
 $k_{fTMC} = 227.7 \text{ mM/ms}$: Ca²⁺ association constant for the troponin-Mg²⁺ site.
 $k_{fTMM} = 2.277 \text{ mM/ms}$: Mg²⁺ association constant for the troponin-Mg²⁺ site.
 $TC_{tot} = 0.031 \text{ mM}$: Total concentration of the troponin-Ca²⁺ site.
 $TMC_{tot} = 0.062 \text{ mM}$: Total concentration of the troponin-Mg²⁺ site.
 $CQ_{tot} = 10 \text{ mM}$: Total calsequestrin concentration.
 $CM_{tot} = 0.045 \text{ mM}$: Total calmodulin concentration.

FORMULATIONS FOR MEMBRANE CLOCK**Membrane potential**

$$dV_m/dt = - (I_{CaL} + I_{CaT} + I_f + I_{st} + I_{Kr} + I_{Ks} + I_{to} + I_{sus} + I_{NaK} + I_{NCX} + I_{bCa} + I_{bNa}) / C_m$$

Gating variables and their 14 differential equations

All 14 membrane ion current variables (y_{16} - y_{29}) are listed in main text Table 1.

$$dy_i/dt = (y_{i,\infty} - y) / \tau_{yi}$$

$$(y_i = d_L, f_L, f_{Ca}, d_T, f_T, p_{aF}, p_{aS}, p_i, n, q, r, y, q_a, q_i)$$

τ_{yi} : Time constant for a gating variable y_i .

α_{yi} and β_{yi} : Opening and closing rates for channel gating.

$y_{i,\infty}$: Steady-state curve for a gating variable y_i .

Ion currents**L-type Ca²⁺ current (I_{CaL})**

$$I_{CaL} = C_m \cdot g_{CaL} \cdot (V_m - E_{CaL}) \cdot d_L \cdot f_L \cdot f_{Ca}$$

$$d_{L,\infty} = 1 / \{1 + \exp[-(V+13.5)/6]\}$$

$$f_{L,\infty} = 1 / \{1 + \exp[(V+35)/7.3]\}$$

$$\alpha_{dL} = -0.02839 \cdot (V_m + 35) / \{ \exp[-(V_m+35)/2.5] - 1 \} - 0.0849 \cdot V_m / [\exp(-V/4.8) - 1]$$

$$\beta_{dL} = 0.01143 \cdot (V - 5) / \{ \exp[(V_m - 5)/2.5] - 1 \}$$

$$\tau_{dL} = 1 / (\alpha_{dL} + \beta_{dL})$$

$$\tau_{fL} = 257.1 \cdot \exp\{ -[(V_m + 32.5)/13.9]^2 \} + 44.3$$

$$f_{Ca,\infty} = K_{mfCa} / (K_{mfCa} + Ca_{sub})$$

$$\tau_{fCa} = f_{Ca,\infty} / \alpha_{fCa}$$

T-type Ca²⁺ current (I_{CaT})

$$I_{CaT} = C_m \cdot g_{CaT} \cdot (V_m - E_{CaT}) \cdot d_T \cdot f_T$$

$$d_{T,\infty} = 1 / \{1 + \exp[-(V_m + 26.3)/6.0]\}$$

$$f_{T,\infty} = 1 / \{1 + \exp[(V_m + 61.7)/5.6]\}$$

$$\tau_{dT} = 1 / \{1.068 \cdot \exp[(V_m + 26.3)/30] + 1.068 \cdot \exp[-(V_m + 26.3)/30]\}$$

$$\tau_{fT} = 1 / \{0.0153 \cdot \exp[-(V_m + 61.7)/83.3] + 0.015 \cdot \exp[(V_m + 61.7)/15.38]\}$$

Rapidly activating delayed rectifier K⁺ current (I_{Kr})

$$I_{Kr} = C_m \cdot g_{Kr} \cdot (V_m - E_K) \cdot (0.6 \cdot p_{aF} + 0.4 \cdot p_{aS}) \cdot p_i$$

$$p_{a,\infty} = 1 / \{1 + \exp[-(V_m + 23.2)/10.6]\}$$

$$p_{i,\infty} = 1 / \{1 + \exp[(V_m + 28.6)/17.1]\}$$

$$\tau_{paF} = 0.84655354 / [0.0372 \cdot \exp(V_m/15.9) + 0.00096 \cdot \exp(-V_m/22.5)]$$

$$\tau_{paS} = 0.84655354 / [0.0042 \cdot \exp(V_m/17.0) + 0.00015 \cdot \exp(-V_m/21.6)]$$

$$\tau_{pi} = 1 / [0.1 \cdot \exp(-V_m/54.645) + 0.656 \cdot \exp(V_m/106.157)]$$

Slowly activating delayed rectifier K⁺ current (I_{Ks})

$$I_{Ks} = C_m \cdot g_{Ks} \cdot (V_m - E_{Ks}) \cdot n^2$$

$$\alpha_n = 0.014 / \{1 + \exp[-(V_m - 40)/9]\}$$

$$\beta_n = 0.001 \cdot \exp(-V_m/45)$$

$$n_\infty = \alpha_n / (\alpha_n + \beta_n)$$

$$\tau_n = 1 / (\alpha_n + \beta_n)$$

4-aminopyridine-sensitive currents (I_{4AP} = I_{to} + I_{sus})

$$I_{to} = C_m \cdot g_{to} \cdot (V_m - E_K) \cdot q \cdot r$$

$$I_{sus} = C_m \cdot g_{sus} \cdot (V_m - E_K) \cdot r$$

$$q_\infty = 1 / \{1 + \exp[(V_m + 49)/13]\}$$

$$r_\infty = 1 / \{1 + \exp[-(V_m - 19.3)/15]\}$$

$$\tau_q = 39.102 / \{0.57 \cdot \exp[-0.08 \cdot (V_m + 44)] + 0.065 \cdot \exp[0.1 \cdot (V_m + 45.93)]\} + 6.06$$

$$\tau_r = 14.40516 / \{1.037 \cdot \exp[0.09 \cdot (V_m + 30.61)] + 0.369 \cdot \exp[-0.12 \cdot (V_m + 23.84)]\} + 2.75352$$

Hyperpolarization-activated, “funny” current (I_f)

$$I_f = I_{fNa} + I_{fK}$$

$$y_\infty = 1 / \{1 + \exp[(V_m - V_{f,1/2})/13.5]\}$$

$$\tau_y = 0.7166529 / \{\exp[-(V_m + 386.9)/45.302] + \exp[(V_m - 73.08)/19.231]\}$$

$$I_{fNa} = C_m \cdot 0.3833 \cdot g_{f} \cdot (V_m - E_{Na}) \cdot y^2$$

$$I_{fK} = C_m \cdot 0.6167 \cdot g_{f} \cdot (V_m - E_K) \cdot y^2$$

Sustained inward current (I_{st})

$$I_{st} = C_m \cdot g_{st} \cdot (V_m - E_{st}) \cdot q_a \cdot q_i$$

$$q_{a,\infty} = 1 / \{1 + \exp[-(V_m + 57)/5]\}$$

$$\alpha_{qa} = 1 / \{0.15 \cdot \exp(-V_m/11) + 0.2 \cdot \exp(-V_m/700)\}$$

$$\beta_{qa} = 1 / \{16 \cdot \exp(V_m/8) + 15 \cdot \exp(V_m/50)\}$$

$$\tau_{qa} = 1 / (\alpha_{qa} + \beta_{qa})$$

$$\begin{aligned}\alpha_{qi} &= 1 / \{3100 \cdot \exp(V_m/13) + 700 \cdot \exp(V_m/70)\} \\ \beta_{qi} &= 1 / \{95 \cdot \exp(-V_m/10) + 50 \cdot \exp(-V_m/700)\} + 0.000229 / [1 + \exp(-V_m/5)] \\ \tau_{qi} &= 6.65 / (\alpha_{qi} + \beta_{qi}) \\ q_{i,\infty} &= \alpha_{qi} / (\alpha_{qi} + \beta_{qi})\end{aligned}$$

Na⁺-dependent background current (I_{bNa})

$$I_{b,Na} = C_m \cdot g_{bNa} \cdot (V_m - E_{Na})$$

Na⁺-K⁺ pump current (I_{NaK})

$$I_{NaK} = C_m \cdot I_{NaKmax} \cdot \{1 + (K_{mKp}/K_o)^{1.2}\}^{-1} \cdot \{1 + (K_{mNap}/Na_i)^{1.3}\}^{-1} \cdot \{1 + \exp[-(V_m - E_{Na} + 120)/30]\}^{-1}$$

Ca²⁺- background current (I_{bCa})

$$I_{bCa} = C_m \cdot g_{bCa} \cdot (V_m - E_{CaL})$$

Na⁺-Ca²⁺ exchanger current (I_{NCX})

$$\begin{aligned}I_{NCX} &= C_m \cdot k_{NCX} \cdot (k_{21} \cdot x_2 - k_{12} \cdot x_1) / (x_1 + x_2 + x_3 + x_4) \\ d_o &= 1 + (Ca_o/K_{co}) \cdot \{1 + \exp(Q_{co} \cdot V_m/E_T)\} + (Na_o/K_{1no}) \cdot \{1 + (Na_o/K_{2no}) \cdot (1 + Na_o/K_{3no})\} \\ k_{43} &= Na_i / (K_{3ni} + Na_i) \\ k_{41} &= \exp[-Q_n \cdot V_m / (2E_T)] \\ k_{34} &= Na_o / (K_{3no} + Na_o) \\ k_{21} &= (Ca_o/K_{co}) \cdot \exp(Q_{co} \cdot V_m/E_T) / d_o \\ k_{23} &= (Na_o/K_{1no}) \cdot (Na_o/K_{2no}) \cdot (1 + Na_o/K_{3no}) \cdot \exp[-Q_n \cdot V_m / (2E_T)] / d_o \\ k_{32} &= \exp[Q_n \cdot V_m / (2E_T)] \\ x_1 &= k_{34} \cdot k_{41} \cdot (k_{23} + k_{21}) + k_{21} \cdot k_{32} \cdot (k_{43} + k_{41}) \\ d_i &= 1 + (Ca_{sub}/K_{ci}) \cdot \{1 + \exp(-Q_{ci} \cdot V_m/E_T) + Na_i/K_{cni}\} + (Na_i/K_{1ni}) \cdot \{1 + (Na_i/K_{2ni}) \cdot (1 + Na_i/K_{3ni})\} \\ k_{12} &= (Ca_{sub}/K_{ci}) \cdot \exp(-Q_{ci} \cdot V_m/E_T) / d_i \\ k_{14} &= (Na_i/K_{1ni}) \cdot (Na_i/K_{2ni}) \cdot (1 + Na_i/K_{3ni}) \cdot \exp[Q_n \cdot V_m / (2E_T)] / d_i \\ x_2 &= k_{43} \cdot k_{32} \cdot (k_{14} + k_{12}) + k_{41} \cdot k_{12} \cdot k_{34} + k_{32} \\ x_3 &= k_{43} \cdot k_{14} \cdot (k_{23} + k_{21}) + k_{12} \cdot k_{23} \cdot (k_{43} + k_{41}) \\ x_4 &= k_{34} \cdot k_{23} \cdot (k_{14} + k_{12}) + k_{21} \cdot k_{14} \cdot (k_{34} + k_{32})\end{aligned}$$

FORMULATIONS FOR SR Ca²⁺ CLOCK**Ca²⁺ release flux ($j_{SRCarel}$) from SR via RyRs**

$$\begin{aligned}j_{SRCarel} &= k_s \cdot O \cdot (Ca_{jSR} - Ca_{sub}) \\ k_{CaSR} &= MaxSR - (MaxSR - MinSR) / (1 + (EC_{50_SR}/Ca_{jSR})^{HSR}) \\ k_{oSRCa} &= k_{oCa} / k_{CaSR} \\ k_{iSRCa} &= k_{iCa} \cdot k_{CaSR} \\ dR/dt &= (k_{im} \cdot RI - k_{iSRCa} \cdot Ca_{sub} \cdot R) - (k_{oSRCa} \cdot Ca_{sub}^2 \cdot R - k_{om} \cdot O) \\ dO/dt &= (k_{oSRCa} \cdot Ca_{sub}^2 \cdot R - k_{om} \cdot O) - (k_{iSRCa} \cdot Ca_{sub} \cdot O - k_{im} \cdot I) \\ dI/dt &= (k_{iSRCa} \cdot Ca_{sub} \cdot O - k_{im} \cdot I) - (k_{om} \cdot I - k_{oSRCa} \cdot Ca_{sub}^2 \cdot RI) \\ dRI/dt &= (k_{om} \cdot I - k_{oSRCa} \cdot Ca_{sub}^2 \cdot RI) - (k_{im} \cdot RI - k_{iSRCa} \cdot Ca_{sub} \cdot R)\end{aligned}$$

Intracellular Ca²⁺ fluxes

Ca²⁺ diffusion flux from submembrane space to myoplasm: $j_{Ca_dif} = (Ca_{sub} - Ca_i)/\tau_{difCa}$

Ca²⁺ uptake (pumping) by the SR: $j_{up} = P_{up}/(1 + K_{up}/Ca_i)$

Ca²⁺ flux between (network and junctional) SR compartments: $j_{tr} = (Ca_{nSR} - Ca_{jSR})/\tau_{tr}$

Ca²⁺ buffering

$$\begin{aligned} df_{TC}/dt &= k_{fTC} \cdot Ca_i \cdot (1 - f_{TC}) - k_{bTC} \cdot f_{TC} \\ df_{TMC}/dt &= k_{fTMC} \cdot Ca_i \cdot (1 - f_{TMC} - f_{TMM}) - k_{bTMC} \cdot f_{TMC} \\ df_{TMM}/dt &= k_{fTMM} \cdot Mg_i \cdot (1 - f_{TMC} - f_{TMM}) - k_{bTMM} \cdot f_{TMM} \\ df_{CMi}/dt &= k_{fCM} \cdot Ca_i \cdot (1 - f_{CMi}) - k_{bCM} \cdot f_{CMi} \\ df_{CMs}/dt &= k_{fCM} \cdot Ca_{sub} \cdot (1 - f_{CMs}) - k_{bCM} \cdot f_{CMs} \\ df_{CQ}/dt &= k_{fCQ} \cdot Ca_{jSR} \cdot (1 - f_{CQ}) - k_{bCQ} \cdot f_{CQ} \end{aligned}$$

Dynamics of Ca²⁺ concentrations in cell compartments

$$\begin{aligned} dCa_i/dt &= (j_{Ca_dif} \cdot V_{sub} - j_{up} \cdot V_{up}) / V_i - (CM_{tot} \cdot df_{CMi}/dt + TC_{tot} \cdot df_{TC}/dt + TMC_{tot} \cdot df_{TMC}/dt) \\ dCa_{sub}/dt &= j_{SRCarel} \cdot V_{jSR} / V_{sub} - (I_{CaL} + I_{CaT} + I_{bCa} - 2 \cdot I_{NCX}) / (2 \cdot F \cdot V_{sub}) - (j_{Ca_dif} + CM_{tot} \cdot df_{CMs}/dt) \\ dCa_{jSR}/dt &= j_{tr} - j_{SRCarel} - CQ_{tot} \cdot df_{CQ}/dt \\ dCa_{nSR}/dt &= j_{up} - j_{tr} \cdot V_{jSR} / V_{nSR} \end{aligned}$$