

Age-related pacemaker deterioration is due to impaired intracellular and membrane mechanisms: Insights from numerical modeling

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Age-related deterioration of pacemaker function has been documented in mammals, including humans. In aged isolated sinoatrial node tissues and cells, reduction in the spontaneous action potential (AP) firing rate was associated with deterioration of intracellular and membrane mechanisms; however, their relative contribution to age-associated deficient pacemaker function is not known. Interestingly, pharmacological interventions that increase posttranslation modification signaling activities can restore the basal and maximal AP firing rate, but the identities of the protein targets responsible for AP firing rate restoration are not known. Here, we developed a numerical model that simulates the function of a single mouse pacemaker cell. In addition to describing membrane and intracellular mechanisms, the model includes descriptions of autonomic receptor activation pathways and posttranslation modification signaling cascades. The numerical model shows that age-related deterioration of pacemaker function is related to impaired intracellular and membrane mechanisms: HCN₄, T-type channels, and phospholamban functions, as well as the node connecting these mechanisms, i.e., intracellular Ca²⁺ and posttranslation modification signaling. To explain the restored maximal beating rate in response to maximal phosphodiesterase (PDE) inhibition, autonomic receptor stimulation, or infused cyclic adenosine monophosphate (cAMP), the model predicts that phospholamban phosphorylation by protein kinase A (PKA) and HCN₄ sensitivity to cAMP are altered in advanced age. Moreover, alteration in PKA and cAMP sensitivity can also explain age-reduced sensitivity to PDE inhibition and autonomic receptor stimulation. Finally, the numerical model suggests two pharmacological approaches and one gene manipulation method to restore the basal beating rate of aged pacemaker cells to that of normal adult cells. In conclusion, our numerical model shows that impaired membrane and intracellular mechanisms and the nodes that couple them can lead to deteriorated pacemaker function. By increasing posttranslation modification signaling, the deteriorated basal and maximal age-associated beating rate can be restored to adult levels.

INTRODUCTION

The U.S. Administration on Aging estimates that by the year 2050, 20% of the population will be 65 y and older. By the year 2025, the number of individuals age 85 and older is projected to double and reach 8 million in the United States alone. At the same time, chronic cardiovascular diseases, e.g., coronary artery atherosclerosis, hypertension, and chronic heart failure, have reached epidemic proportions, and their incidence is growing exponentially, along with risks of disability with increasing age (Ogawa et al., 1992; Ferrari, 2002). Importantly, sinoatrial node (SAN) dysfunction increases exponentially with age, reaching an incidence of 1 of every 600 people ages 65 and older (Kusumoto and Goldschlager, 1996).

The SAN rate is controlled by both the autonomic nervous system (ANS) and intrinsic pacemaker mechanisms forming the coupled-clock system (Yaniv et al., 2015b). The ANS controls the beating rate by balancing

the ratio of sympathetic to parasympathetic stimulation of G protein-coupled receptors: the sympathetic system stimulates β -adrenergic receptors (β -ARs), activating adenylyl cyclase (AC); the parasympathetic system stimulates cholinergic receptors, inactivating AC. In the cell, ATP is converted to cAMP by AC. The cAMP level controls PKA activity, and both cAMP and PKA determine the activation level of intrinsic pacemaker mechanisms: increased cAMP/PKA activity leads to an increase in the beating rate and vice versa (Yaniv et al., 2015a; Behar et al., 2016). In pacemaker cells, other types of AC exist (AC1 and AC8); these ACs are activated by intracellular Ca²⁺, which is balanced by the interaction between membrane channels, exchangers, and pumps (membrane clock or M clock) and by internal Ca²⁺ storage (Ca²⁺ clock; Yaniv et al., 2013b). Both cAMP/PKA and intracellular Ca²⁺ serve as the node connecting the two clocks. Thus, deterioration in either clock's molecules or in ANS receptors can affect pacemaker function.

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Abbreviations used: AC, adenylyl cyclase; ANS, autonomic nervous system; AP, action potential; β -AR, β -adrenergic receptor; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; DD, diastolic depolarization; HCN channel, hyperpolarization-activated cyclic nucleotide-gated channel; HR, heart rate; IBMX, 3-isobutyl-1-methylxanthine; ISO, isoproterenol; LCR, local Ca²⁺ release; NCX, Na⁺/Ca²⁺ exchanger; PDE, phosphodiesterase; PLB, phospholamban; SAN, sinoatrial node; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase.

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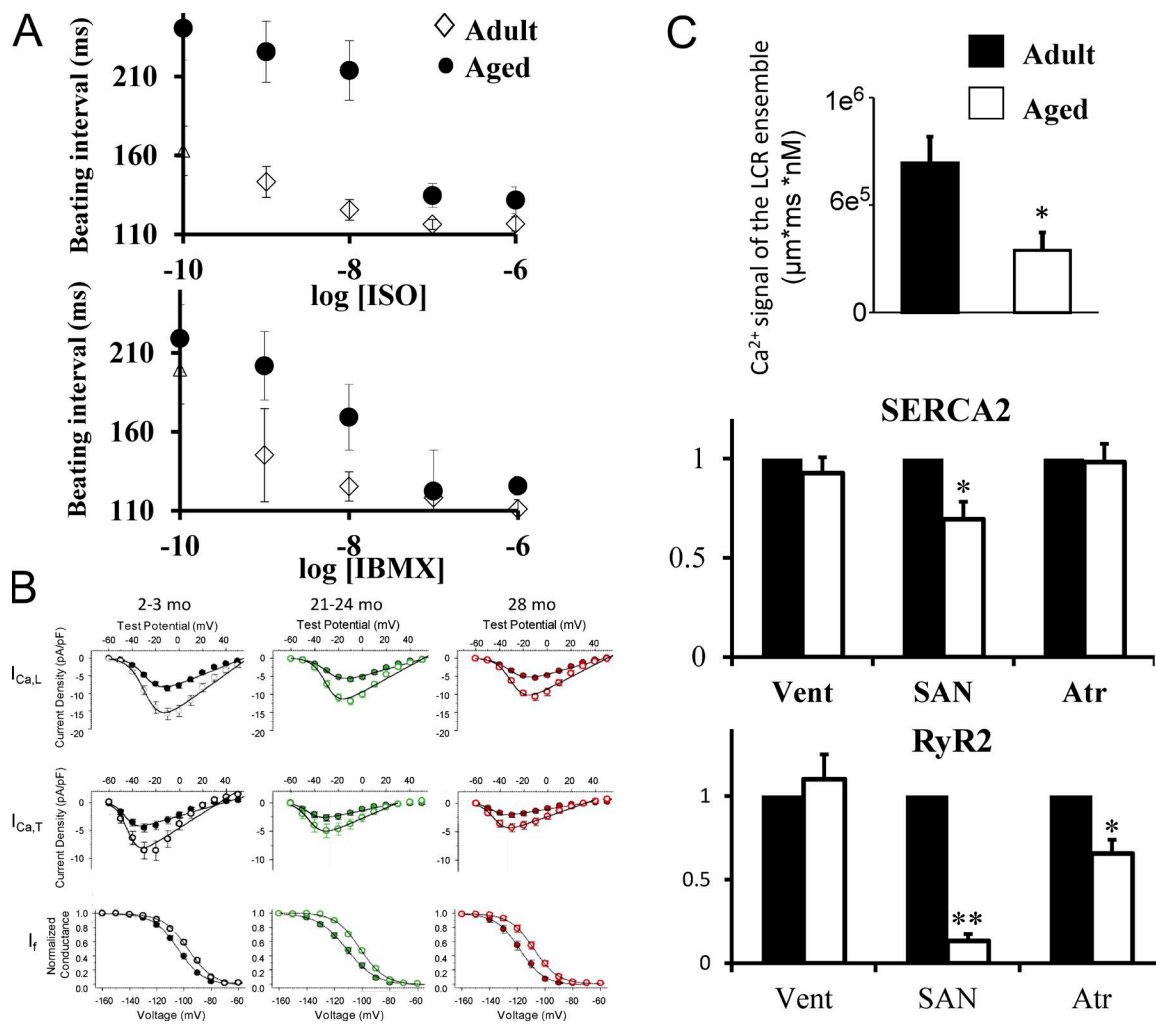


Figure 1. Age-associated deficient pacemaker function. (A) Age-associated reduction in basal beating interval and sensitivity to PDE inhibition by IBMX and β -AR stimulation by ISO. Note that the maximal beating rate is restored in response to maximal IBMX or ISO concentration (adapted from Yaniv et al. [2016]). Data are presented as mean \pm SD. (B) Age-associated reduction in L-type, T-type, and HCN current conductance (adapted from Larson et al. [2013]) shown for sinoatrial node myocytes in mice aged 2–3 mo (black), 21–24 mo (green), and 28 mo (red) in the absence (filled circles) and presence (open circles) of 1 μ M ISO. Data are presented as average \pm SEM. (C) Age-associated reduced LCR ensemble, SERCA, and RyR2 presence (adapted from Liu et al. [2014]). From top to bottom: mean amplitudes of Ca^{2+} signals of the LCR ensemble (integrated Ca^{2+} signal produced by each LCR); representative Western blots of SAN (protein expression), atrial and ventricular tissues from adult (2–4 mo) and aged (24–27 mo) mice normalized to sarcomeric actin levels; RyR protein levels. Data are presented as mean \pm SD. (top) *, $P < 0.05$ versus adult, mixed effects model for repeated measure ANOVA; (middle) *, $P < 0.05$ versus adult, two-tailed t test; (bottom) **, $P < 0.01$ versus adult; and *, $P < 0.05$, two-tailed t test.

Measurements of intrinsic (measured during autonomic blockade, precluding ANS effects) and maximal beating/heart rate (HR) provide evidence for deterioration in pacemaker function associated with aging. Although the basal HR (i.e., at rest) does not change in advanced age, the intrinsic HR declines with age in humans (Jose and Collison, 1970) and other mammals (Yaniv et al., 2016; Fig. 1 A). Because the intrinsic heart rate precludes ANS effects, its reduction reflects impaired activity of intrinsic pacemaker mechanisms: cAMP-PKA/ Ca^{2+} signaling (Liu et al., 2014) and ionic channel currents (e.g., hyperpolarization-activated cyclic nucleotide-gated [HCN] channel and T-type and

L-type Ca^{2+} channels; Larson et al., 2013; Fig. 1, B and C). However, the relative contribution of each mechanism to age-associated deterioration of pacemaker function is debatable. In contrast to the in vivo basal HR, the maximal HR declines in advanced age (Heath et al., 1981; Hagberg et al., 1985). Indeed recent data on pacemaker tissue (Liu et al., 2014; Yaniv et al., 2016) and data at the heart level (Brodde and Leineweber, 2004) have shown that the sensitivity to β -AR stimulation or phosphodiesterase (PDE) inhibition (using 3-isobutyl-1-methylxanthine [IBMX]) was reduced in advanced age. However, data have also shown that, in response to maximal autonomic receptor stimulation

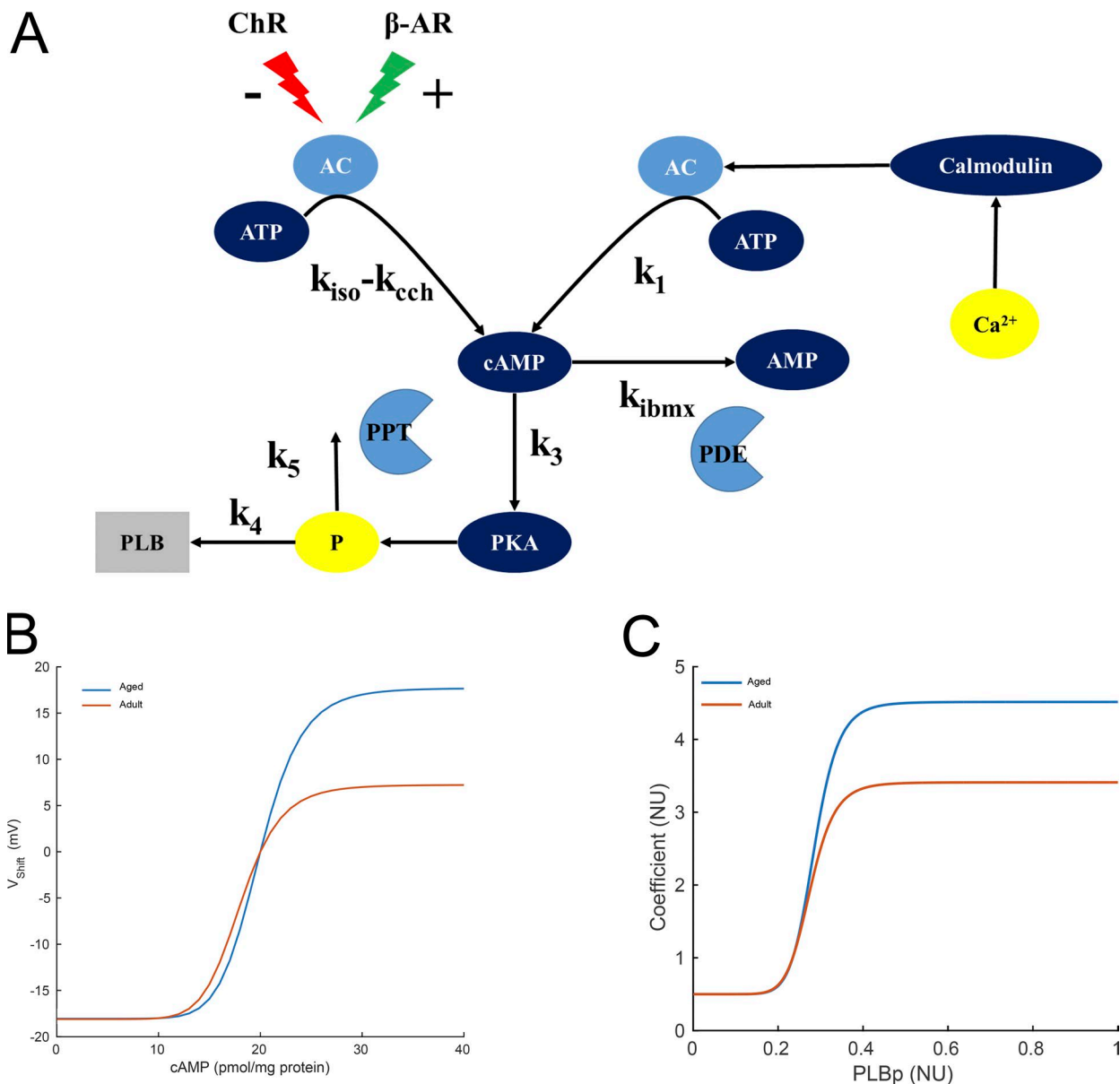


Figure 2. **Model parameters adjustment.** (A) Schematic illustration of the AC-cAMP-PKA signaling cascade. AC is activated by adrenergic receptors (β -AR) and calmodulin and deactivated by cholinergic receptor (ChR) stimulation. Activated AC converts ATP into cAMP, which itself is transformed into PKA. PKA phosphorylates several targets, including PLB proteins, whose phosphorylation level will regulate the activation of SERCA and thus the rate at which Ca^{2+} enters the SR. The model includes two restraining mechanisms that act like brakes: protein phosphatase (PPT), which removes phosphate groups from proteins, and PDE, which breaks the phosphodiester bond in cAMP and degrades its level. (B) I_f shift in the I–V curve as a function of cAMP in adult and aged mice. Basal state ($V_{shift} = 0$ and $[cAMP] = 20$ pmol/mg protein). The maximal shift for adult and aged mice was taken from the experimental results of Sharpe et al. (2017). (C) PLBp modulation of SERCA in adult and aged mice. The expressions for adult and aged mice are empirical.

or maximal increase in cAMP/PKA by PDE inhibition, the age-associated reduction in maximal beating rate can be compensated for. Nonetheless, the underlying biophysical mechanisms that allow this are not known. Numerical modeling is very useful, and sometimes it is the only feasible tool to explore such biophysical mechanisms. However, to date no aged pacemaker cell model exists.

Here, we used a numerical model of mouse pacemaker cells (Kharche et al., 2011) adapted to describe the effects of autonomic receptor stimulation. The model was used to answer three specific questions: (a) What is the relative contribution of each clock to the age-associated deterioration in pacemaker function? (b) What age-associated biophysical modulation makes it possible to compensate for

the reduction in maximal beating rate obtained in response to maximal PDE inhibition or β -AR stimulation? (c) Can the age-associated deterioration in pacemaker function be reversed by drug intervention or gene manipulation?

MATERIALS AND METHODS

Existing experimental results

Several groups have measured the reduction in the basal beating rate of either SAN tissue or single pacemaker cells from aged mice (Larson et al., 2013; Liu et al., 2014; Yaniv et al., 2016; Sharpe et al., 2017). Different mechanisms can cause the reduced pacemaker function: (a) reduced sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) activity (Liu et al., 2014); (b) reduced RyR function (Liu et al., 2014); (c) shift of the half-activation potential of the HCN current (Sharpe et al., 2017); or (d) reduced L-type and T-type currents (Larson et al., 2013). Although the action potential (AP) sensitivity to β -AR (studied using isoproterenol [ISO]) or PDE inhibition (studied using IBMX) was reduced in advanced age (Yaniv et al., 2016), the maximal AP firing rate in response to these drugs (Yaniv et al., 2016) or to cAMP (Sharpe et al., 2017) was not significantly different between aged and young mice.

Basal numerical model

We adapted the Kharche et al. (2011) model for mouse pacemaker cells. This model was primarily based on experimental data from isolated mouse SAN cells. We updated the model based on Ca^{2+} measurements from Liu et al. (2014). For some ionic current parameters and biochemical signaling for which no mouse experimental data were available, we used the formulation from rabbit SAN cell models. The Kharche et al. (2011) model was updated to include the equations of cAMP-PKA signaling cascades and autonomic receptor stimulation (Yaniv et al., 2015a; Behar and Yaniv, 2016; Fig. 2 A). In addition, the following changes were made to the original model: the extracellular concentration of Ca^{2+} was changed to 2 mM according to Maltsev and Lakatta (2009); the intracellular concentration of Na^+ was changed from 8.1 to 10 mM as in the Maltsev–Lakatta model; the release rate parameter of the Ca^{2+} from the SR, k_s , was updated to $250,000 \text{ ms}^{-1}$, similar to the Maltsev–Lakatta model; the formulation of j_{up} to describe the Ca^{2+} pump rate was changed according to Kurata et al. (2002); and the conductance of I_{CaL} was increased by 18% and 38% for $g_{\text{CaL1.2}}$ and for $g_{\text{CaL1.3}}$, respectively. Finally, K^+ and Na^+ intracellular concentrations were kept constant, and I_{st} was removed from the model because it has no molecular basis (Maltsev et al., 2014). All model equations can be found in the supplement.

Model strategy

The effects of aging were simulated by varying the following model parameters: SERCA activity was decreased by 30%, as observed experimentally in Liu et al. (2014) (experimental data are presented in Fig. 1 C and are taken into account in Eqs. 20 and 22), and the conductance of I_{CaT} was decreased by 20% to simulate current density reduction during diastolic depolarization (DD), as found in Larson et al. (2013) (experimental data are presented in Fig. 1 B and are taken into account in Eqs. 23 and 24). In addition, Liu et al. (2014) showed changes in RyR function with a decrease in RyR activity in aged cells and a decrease in Ca^{2+} release of the local Ca^{2+} release (LCR) ensemble. To simulate these results, the release rate parameter of the Ca^{2+} from the SR, $k_s = 250,000 \text{ ms}^{-1}$, was decreased to $k_s = 87,500 \text{ ms}^{-1}$, i.e., decreased by a factor of 65%, corresponding to the experimental decrease in Ca^{2+} release of the LCR ensemble found in Liu et al. (2014) (Eqs. 29 and 30 and Fig. 1 C). The half-activation potential $V_{0.5}$ was changed in the HCN current equations from 104.2 mV in adult cells to 110 mV in aged cells (Sharpe et al., 2017; taken into account in Eqs. 14 and 17). The resulting decrease in the AP firing rate caused by aging was compared with published experimental data under basal conditions. In addition, the expression for the I–V curve of the HCN current was modified in the aged model (Fig. 2 B) to match the maximal shift found experimentally in the I–V curve under cAMP saturation (Sharpe et al., 2017), which was shown to be different from that of adults; $\sim 17 \text{ mV}$ in aged cells versus $\sim 7 \text{ mV}$ in adult cells (Sharpe et al., 2017). These values defined the maximal activation of $V_{0.5}$ by cAMP in adult and aged cells in our model (Fig. 2 B).

Ca₂ release of cAMP was modeled as in our previous work (Behar and Yaniv, 2016). The effect of ISO was simulated using the equations developed in Yaniv et al. (2015a) and updated in Behar and Yaniv (2016) (Fig. 2 A). The effect of IBMX was simulated by decreasing the basal degradation rate of cAMP by PDE.

Model equations

cAMP activity

$$\frac{d[\text{cAMP}]}{dt} = \left(\frac{k_{\text{iso}} \cdot [\text{ATP}] + k_1 \cdot [\text{ATP}] - k_{\text{ibmx}} \cdot [\text{cAMP}] - k_{\text{ech}} \cdot [\text{ATP}]}{[\text{cAMP}] - k_3 \cdot [\text{cAMP}] - k_{\text{ech}} \cdot [\text{ATP}]} \right) / 60,000. \quad (1)$$

Our numerical model predicts that k_{iso} and k_{ibmx} are different for the adult and aged cases (see Results for further details).

Adult

$$k_{\text{iso,adult}} = 0.1599 \cdot \frac{[\text{ISO}]^{0.6238}}{76.5441^{0.6238} + [\text{ISO}]^{0.6238}}, \quad (2)$$

$$k_{\text{ibmx,adult}} = -0.8730 \cdot \frac{[\text{IBMX}]^{0.8395}}{4.0550^{0.8781} + [\text{IBMX}]^{0.8395}} + 1. \quad (3)$$

Aged

$$k_{iso,aged} = 0.1434 \cdot \frac{[ISO]^{2.0704}}{334.2799^{2.0704} + [ISO]^{2.0704}}, \quad (4)$$

$$k_{ibmx,aged} = -0.9118 \cdot \frac{[IBMX]^{0.8781}}{47.6705^{0.8781} + [IBMX]^{0.8781}} + 1, \quad (5)$$

$$k_{cch} = 0.0146 \cdot \frac{[CCh]^{1.4402}}{51.7331^{1.4402} + [CCh]^{1.4402}}, \quad (6)$$

$$k_1 = K_{AC,T^+} \cdot \frac{K_{AC}}{1 + \exp\left(\left(K_{Ca} - k_{bCM} \cdot \frac{f_{CM}}{k_{jCM} \cdot (1 - f_{CM})}\right) / K_{AC,Ca}\right)}, \quad (7)$$

$$k_2 = k_{ibmx} \cdot 265.3512 \cdot \frac{[cAMP]^{5.7343}}{24.7290^{6.7343} + [cAMP]^{6.7343}}, \quad (8)$$

$$k_3 = k_{PKA} \cdot \frac{[cAMP]^{(n_{PKA}-1)}}{k_{PKA,cAMP}^{n_{PKA}} + [cAMP]^{n_{PKA}}}. \quad (9)$$

In addition, in the aged-model equations that follow, two additional changes were made to the cAMP activation curve for I_f and the phospholamban (PLB) sensitivity to PKA phosphorylation (see Results and Fig. 2 C for further details).

Hyperpolarization activated "funny" current, I_f

$$I_{fNa} = 0.3833 \cdot g_{fj} \cdot (V_m - E_{Na}) \cdot y, \quad (10)$$

$$I_{fK} = 0.6167 \cdot g_{fj} \cdot (V_m - E_K) \cdot y. \quad (11)$$

Adult

$$K_{if} = 25.3403, K_{0.5if} = 18.1115, n_{if} = 9.6383, \quad (12)$$

$$V_{shift} = K_{if} \cdot \frac{[cAMP]^{n_{if}}}{K_{0.5if}^{n_{if}} + [cAMP]^{n_{if}}} - 18.1040, \quad (13)$$

$$y_{\infty} = \frac{1}{1 + \exp((V_m + 104.2 - V_{shift}) / 16.3)}. \quad (14)$$

Aged

$$K_{if} = 35.7355, K_{0.5if} = 19.9587, n_{if} = 9.6383, \quad (15)$$

$$V_{shift} = K_{if} \cdot \frac{[cAMP]^{n_{if}}}{K_{0.5if}^{n_{if}} + [cAMP]^{n_{if}}} - 18.0457, \quad (16)$$

$$y_{\infty} = \frac{1}{1 + \exp((V_m + 110 - V_{shift}) / 16.3)}, \quad (17)$$

$$\tau_y = 1.5049 / \left(\frac{\exp(-(V_m + 590.3) \cdot 0.01094) +}{\exp((V_m - 85.1) / 17.2)} \right). \quad (18)$$

The rate of Ca^{2+} uptake (pumping) by the SR, j_{up}
Adult

$$f([PLB_p]) = 2.9102 \cdot \frac{[PLB_p]^{9.5517}}{0.2763^{9.5517} + [PLB_p]^{9.5517}} + 0.4998, \quad (19)$$

$$j_{up} = P_{up,basal} \cdot \frac{f([PLB_p])}{1 + K_{up} / [Ca^{2+}]_i}. \quad (20)$$

Aged

$$f([PLB_p]) = 4.0152 \cdot \frac{[PLB_p]^{9.9463}}{0.2853^{9.9463} + [PLB_p]^{9.9463}} + 0.4999, \quad (21)$$

$$j_{up} = 0.7 * P_{up,basal} \cdot \frac{f([PLB_p])}{1 + K_{up} / [Ca^{2+}]_i}. \quad (22)$$

T-type Ca^{2+} current, I_{CaT}

Adult

$$I_{CaT} = g_{CaT} \cdot (V_m - E_{CaT}) \cdot d_T \cdot f_T. \quad (23)$$

Aged

$$I_{CaT} = 0.8 \cdot g_{CaT} \cdot (V_m - E_{CaT}) \cdot d_T \cdot f_T. \quad (24)$$

$$d_{T\infty} = \frac{1}{1 + \exp(-(V_m + 26) / 6)}, \quad (25)$$

$$f_{T\infty} = \frac{1}{1 + \exp((V_m + 61.7) / 5.6)}, \quad (26)$$

$$\tau_{dT} = 1 / \left(\frac{1.068 \cdot \exp((V_m + 26.3) / 30) +}{1.068 \cdot \exp(-(V_m + 26.3) / 30)} \right), \quad (27)$$

$$\tau_{fT} = 1 / \left(\frac{0.0153 \cdot \exp(-(V_m + 61.7) / 83.3) +}{0.015 \cdot \exp((V_m + 61.7) / 15.38)} \right). \quad (28)$$

RyR function

Adult

$$j_{SRCa} = k_s \cdot OO \cdot ([Ca^{2+}]_{jSR} - [Ca^{2+}]_{sub}). \quad (29)$$

Aged

$$j_{SRCa} = 0.35 \cdot k_s \cdot OO \cdot ([Ca^{2+}]_{jSR} - [Ca^{2+}]_{sub}), \quad (30)$$

$$k_{CaSR} = \frac{MaxSR - MinSR}{1 + (EC_{50SR} / [Ca^{2+}]_{jSR})^{H_{SR}}}, \quad (31)$$

$$k_{oSRCa} = k_{oCa} / k_{CaSR} \quad (32)$$

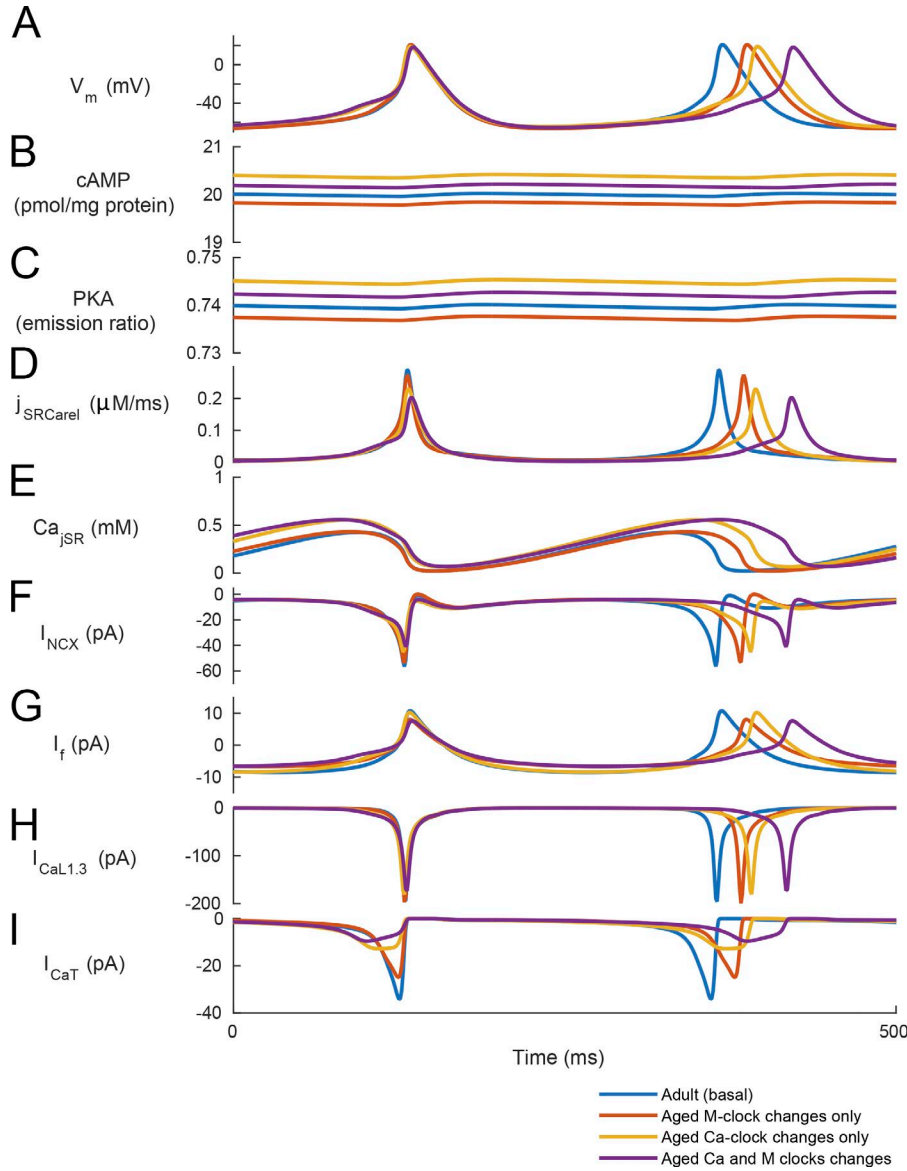


Figure 3. Model simulation in response to changes occurring with aging. Coupled-clock function of adult mice (blue), aged mice when only changes in the M clock mechanisms were taken into account (red), aged mice when only changes in the Ca²⁺ clock mechanisms were taken into account (yellow), and aged mice when changes in both M and Ca²⁺ clock mechanisms were included (purple). Membrane voltage, main membrane currents, AC-cAMP-PKA signaling, and Ca²⁺ cycling (flux and concentration) in the SR in adult and aged mice: membrane voltage (V_m ; A); cAMP level (B); PKA activity level (C); flux of Ca²⁺ existing in the SR ($j_{SRCaRel}$; D); Ca²⁺ concentration in the junctional SR compartment (Ca_{jSR} ; E); the Na⁺-Ca²⁺ exchanger current (I_{NCX} ; F); HCN current (I_f ; G); L-type current (I_{CaL} ; H); and T-type Ca²⁺ current (I_{CaT} ; I).

$$k_{iSRCa} = k_{iCa} \cdot k_{CaSR}, \quad (33)$$

$$\frac{dR}{dt} = (k_{im} \cdot RI - k_{iSRCa} \cdot [Ca^{2+}]_{sub} \cdot R) - (k_{oSRCa} \cdot [Ca^{2+}]_{sub}^2 \cdot R - k_{om} \cdot OO), \quad (34)$$

$$\frac{dOO}{dt} = (k_{oSRCa} \cdot [Ca^{2+}]_{sub}^2 \cdot R - k_{om} \cdot OO) - (k_{iSRCa} \cdot [Ca^{2+}]_{sub} \cdot OO - k_{im} \cdot S), \quad (35)$$

$$\frac{dS}{dt} = (k_{iSRCa} \cdot [Ca^{2+}]_{sub} \cdot OO - k_{im} \cdot S) - (k_{om} \cdot S - k_{oSRCa} \cdot [Ca^{2+}]_{sub}^2 \cdot RI), \quad (36)$$

$$\frac{dRI}{dt} = (k_{om} \cdot S - k_{oSRCa} \cdot [Ca^{2+}]_{sub}^2 \cdot RI) - (k_{im} \cdot RI - k_{iSRCa} \cdot [Ca^{2+}]_{sub} \cdot R). \quad (37)$$

The software was developed in Matlab (MathWorks). Numerical integration was performed using the Matlab ode15s stiff solver, and the model simulations were run for 2,000 s to ensure that steady state was reached. Computation was performed on an Intel Core i7-4790 CPU at 3.60 GHz with 8 GB of RAM.

All model equations and parameters (Tables S1 and S2) appear in the supplement. The source code of the numerical model is available at <http://bioelectric-bioenergetic-lab.net.technion.ac.il/programs>.

Online supplemental material

Table S1 shows coupled differential equation variables in the initial conditions. Table S2 shows model constants. The supplemental text shows all model equations.

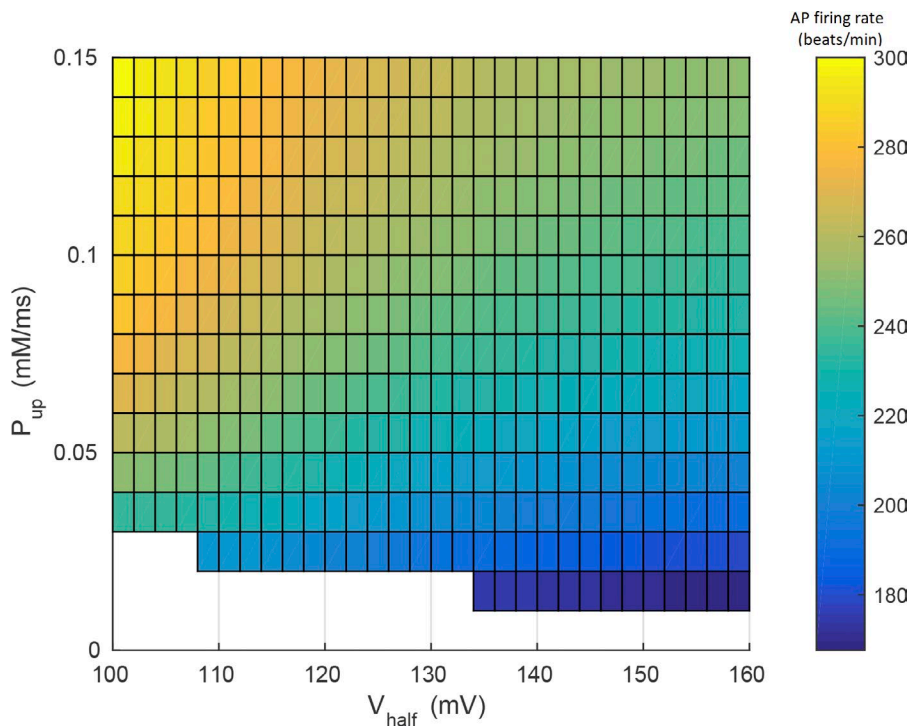


Figure 4. **Parameter sensitivity to P_{up} and $V_{0.5}$.** The effect of changes in P_{up} and $V_{0.5}$ on AP firing rate. $V_{0.5}$ refers to the half activation voltage of the HCN current, I_f .

RESULTS

Mechanisms associated with both the M and Ca^{2+} clock contribute to the age-deteriorated pacemaker function. To explore the relative role of M and Ca^{2+} clock components in age-associated deterioration of pacemaker function, we used our numerical model. Fig. 3 shows the coupled-clock function of adult mice, aged mice when only changes in the M clock mechanisms were taken into account (Fig. 1 B), aged mice when only changes in the Ca^{2+} clock mechanisms were taken into account (Fig. 1 C), and aged mice when changes in both M and Ca^{2+} clock mechanisms were included. The numerical model simulations show that only when mechanisms associated with both the M and Ca^{2+} clocks are taken into account does the reduction in spontaneous AP firing rate match the experimental results (Larson et al., 2013). Fig. 3 shows that age-reduced pacemaker function was associated with reduced release of Ca^{2+} from the RyR (reduced $j_{SR_{Ca^{2+}}}$), small elevation in SR load (less release compared with sequestration of Ca^{2+}), and reduction in I_f , I_{NCX} , $I_{Ca,L}$, and $I_{Ca,T}$. Quantitatively, the peak amplitude of $j_{SR_{Ca^{2+}}}$ changed from 0.291 $\mu\text{M}/\text{ms}$ in adult cells to 0.20 $\mu\text{M}/\text{ms}$ in aged cells, and the amount of Ca^{2+} ejected over one cycle was 8.14 μM in adult cells and 7.69 μM in aged cells.

Although we changed SERCA activity, HCN current conductivity, and RyR kinetics in accordance with experimental data (Fig. 1), we also performed parameter sensitivity analysis to prove that the model predictions are not dependent on only one set of parameters. Fig. 4 shows the changes in AP firing rate in the aged model

in response to changes in $V_{1/2}$ of the HCN channel or in response to changes in P_{up} of the SERCA. Moreover, the range we chose for P_{up} allowed us not only to reproduce the experimental results, but also to provide a stable beating rate (Fig. 4).

Reduction in I_{NCX} , I_{Na} , I_K , or I_{NaK} can deteriorate age-dependent pacemaker function

Although age-dependent changes of RyR, SERCA, PLB, $I_{Ca,L}$, $I_{Ca,T}$, and I_f have been reported, no data exist on changes of I_{NCX} , I_{Na} , I_K , or I_{NaK} . However, the numerical model can illustrate the indirect result of other pacemaker functions caused by the coupled-clock effect. Fig. 5 illustrates all currents for the basal adult and aged cases. I_{NCX} amplitude decreases significantly with aging (Fig. 5 J), I_{K1} and I_{NaK} density (Fig. 5, D and K) are higher during early DD in aged cells, and the density of I_{Na} (Fig. 5, G and H) is lower during DD, but with a peak amplitude more shifted to the early DD. The net effect of all coupled-clock changes is a slower AP upstroke during DD in aged cells than in adult cells, thus leading to a lower AP firing rate.

Age-associated modification in I_f sensitivity to cAMP and PLB sensitivity to PKA can explain the restoration of age-deteriorated pacemaker function

We next simulated the changes in spontaneous AP firing rate in response to maximal ISO, IBMX, or cAMP concentration. Fig. 6 shows that in response to any of these drugs (which bring the beating rate to its maximal value), the spontaneous AP firing rate in aged pacemaker cells is lower than in adult cells. However, these results contra-

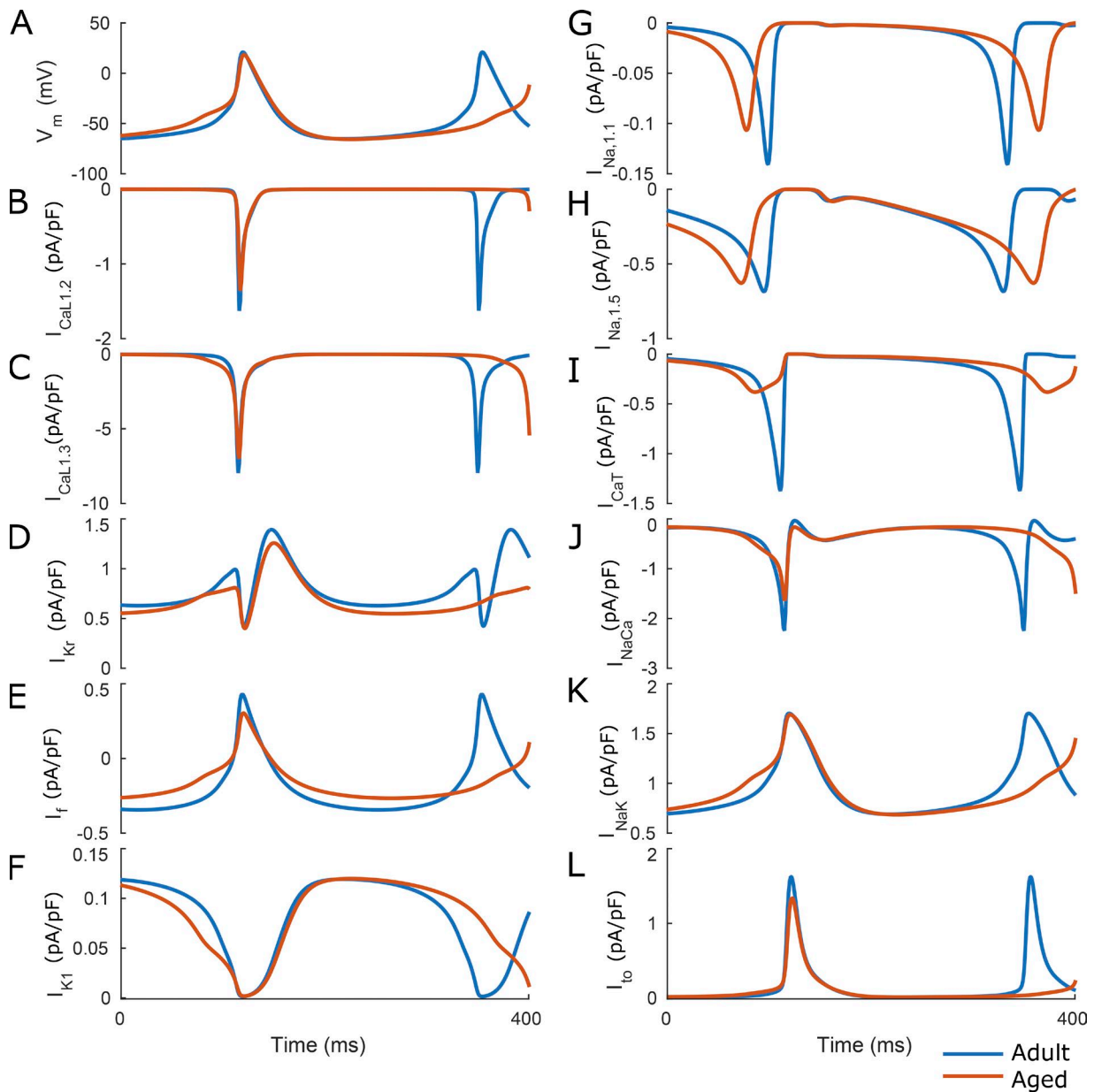


Figure 5. **Major currents in adult and aged mice.** Modification in specific membrane and sarcoplasmic proteins (I_{CaT} , RyR, I_f) modified the other proteins. (A) Membrane voltage (V_m). (B) 1.2 L-type current ($I_{CaL1.2}$). (C) 1.3 L-type current ($I_{CaL1.3}$). (D) Rapidly activating delayed rectifier K^+ current (I_{Kr}). (E) HCN current. (F) Inward rectifying K^+ current (I_{K1}). (G) Sodium current ($I_{Na1.1}$). (H) Sodium current ($I_{Na1.5}$). (I) T-type Ca^{2+} current (I_{CaT}). (J) Na^+ - Ca^{2+} exchanger current (I_{NaCa}). (K) Na^+ - K^+ pump current (I_{NaK}). (L) 4-aminopyridine-sensitive currents (I_{to}).

dict recently published data (Liu et al., 2014; Yaniv et al., 2016). The model was modified to fit the experimental data: we searched for modifications in clock mechanisms that could bring the maximal spontaneous AP firing rate of aged cells to the one simulated for adults. We focused on two targets, age-dependent modification in HCN4 sensitivity to cAMP and PLB sensitivity to PKA. Fig. 6 shows that modification of HCN4 sensitivity to cAMP in aged cells can partially restore the maximal age-deteriorated pacemaker AP firing rate to the adult level, but not to the level documented experimentally. We modified

PLB sensitivity to PKA so that the maximal heart rate in response to ISO or IBMX stimulation will fit the experimentally published levels (Liu et al., 2014; Yaniv et al., 2016). Only when the modification in PLB sensitivity to PKA (see Materials and methods, Eqs. 19, 20, 21, and 22) was also taken into account could the maximal AP firing rate of aged pacemaker cells be restored to the adult level. Note that although there is a slight difference between the final predicted maximal heart rate of adult and aged pacemaker cells, this difference is not significant and cannot not be measured experimentally.

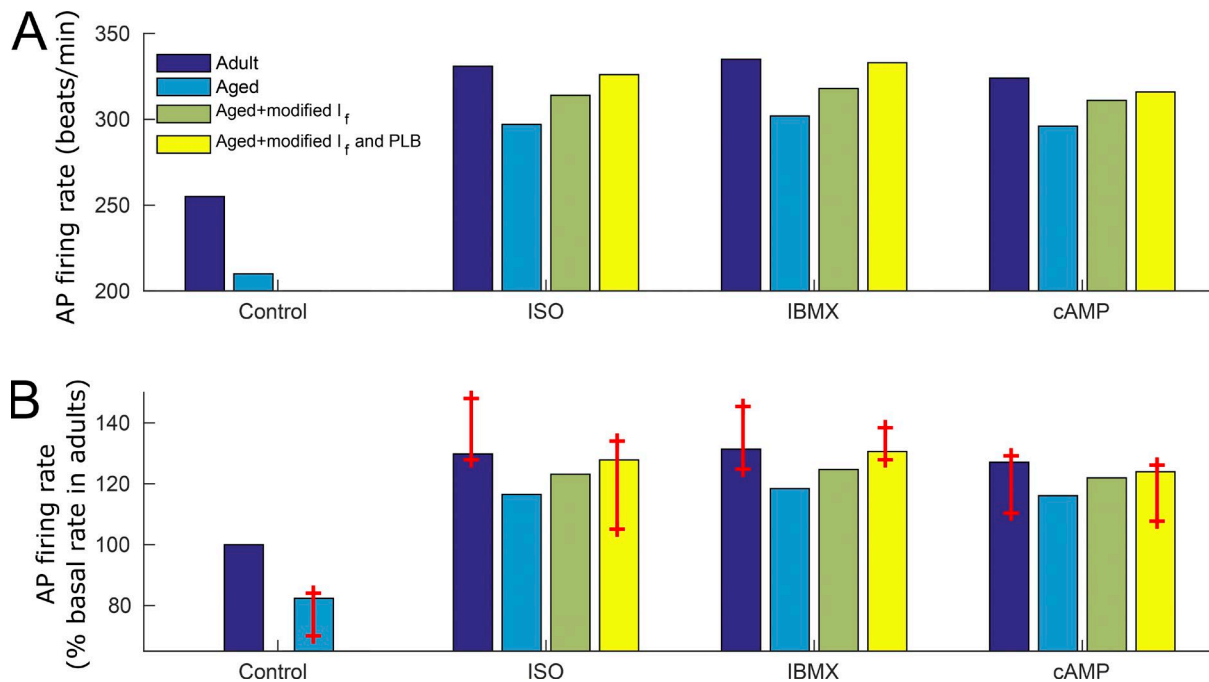


Figure 6. **Mean AP firing rate changes in response to drug perturbations.** Simulated AP firing rate in absolute numbers (A) and percentage change from the adult basal firing rate (B) of young and adult mouse SAN cells under ISO (1,000 μM), caged cAMP release, or IBMX treatment (3,000 μM). In B, the red bars show the experimental range (defined as mean \pm twice the SEM) of the decrease in AP firing rate in aged cells with respect to the adult basal rate, as reported in Larson et al. (2013), Liu et al. (2014), and Sharpe et al. (2017).

Figs. 7 and 8 show how the coupled-clock mechanisms lead to restored maximal pacemaker function. In response to maximal concentration of ISO (Fig. 7), cAMP and PKA are restored, the release of Ca^{2+} is compensated for, more Ca^{2+} is accumulated in the SR because the rate of Ca^{2+} sequestration is higher than the rate of release, and I_f , I_{NCX} , $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$ are all completely or partly restored. In response to maximal concentration of IBMX (Fig. 8), similar changes in coupled-clock mechanisms occur.

Age-associated modification in I_f sensitivity to cAMP and PLB sensitivity to PKA can explain the reduced sensitivity of pacemaker function to external and internal stimuli

Experimental data show that the sensitivity to either PDE inhibition (by IBMX) or β -AR receptor stimulation (by ISO) is reduced in advanced age (Yaniv et al., 2016). Our numerical model could reproduce these results only when $k_{\text{iso,aged}}$ and $k_{\text{ibmx,aged}}$ (Eqs. 4 and 5) were modified with respect to their adult expression. Thus, the model predicts that in aged cells, the response to ISO or IBMX stimulation is different than in adult pacemaker cells. This age-related modification was generated to match the drug dose-dependent results that were reported experimentally (Yaniv et al., 2016). Fig. 9 shows that higher concentrations of ISO or IBMX are needed to achieve the same percentage of increase in spontaneous AP firing rate. Based on the numerical model re-

sults, the age-associated EC_{50} was ~ 203 nM for ISO and 26 μM for IBMX compared with ~ 11 nM and ~ 6.3 μM in adults in response to ISO and IBMX, respectively. Note that these results can be obtained only by age-associated modification in HCN_4 sensitivity to cAMP and PLB sensitivity to PKA.

The age-associated basal AP firing rate can be reversed
The numerical model was used to predict whether the age-associated decrease in basal beating rate can be reversed using drug perturbations or gene manipulation. Fig. 10 A shows that 167 nM ISO can reverse the aged basal AP firing rate to that of adult mouse pacemaker cells. Fig. 10 B shows that 50 μM IBMX can also reverse the aged basal AP firing rate to that of adult mouse pacemaker cells. Thus, using pharmacological interventions, the old heart can be made adult again. A recent patent (Maltsev et al., 2016) suggests that overexpression of AC1 or AC8 can lead to restoration of normal beating rate. Fig. 11 shows that increasing the presence of AC1 and AC8 (by increasing K_{AC}) reverses the age-associated decrease in beating rate and restores it to the adult level.

DISCUSSION

Our first major conclusion is that both the M and Ca^{2+} clocks contribute to the deterioration of basal pacemaker function in advanced age (Fig. 3). This con-

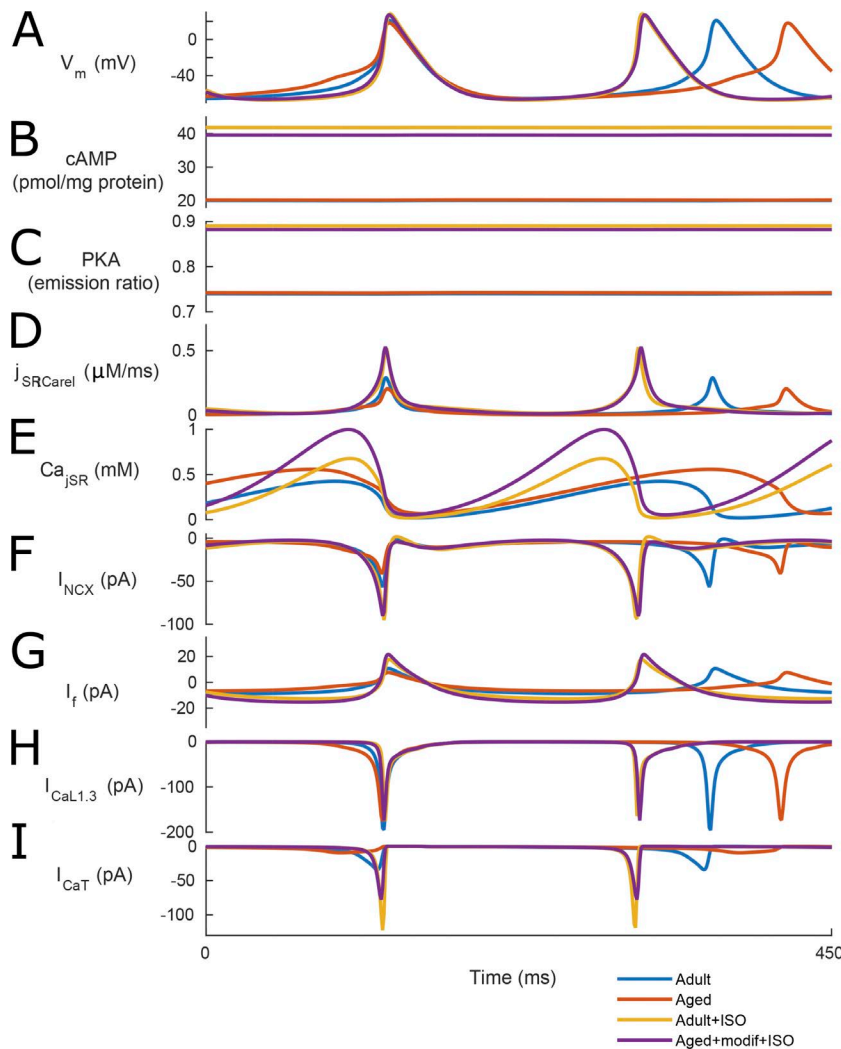


Figure 7. Model simulation in response to pacemaker function changes occurring in response to ISO in adult and aged mice. In response to maximal concentration of ISO, AP firing rate (A), cAMP (B), and PKA activities (C) are restored; the flux of Ca^{2+} in the SR ($j_{\text{SR}^{\text{Careli}}}$; D) and Ca^{2+} concentration in the junctional sarcoplasmic reticulum compartment (Ca_{jSR} ; E) are compensated for; and the Na^+ - Ca^{2+} exchanger current (I_{NCX} ; F), HCN current (I_f ; G), L-type current (I_{CaL} ; H), and T-type Ca^{2+} current (I_{CaT} ; I) are completely or partly restored.

clusion is in agreement with experimental data from mouse SAN tissue and pacemaker cells, which have shown deterioration in ionic current (decrease in HCN, L-type, and T-type currents; Larson et al., 2013), Ca^{2+} -related mechanisms (decrease in the amount of RyR and SERCA; Liu et al., 2014), and the node signaling that coupled the clocks (Ca^{2+} global and local release parameters; Liu et al., 2014). Note that only when changes in SERCA, I_f , and RyR (i.e., both M-related and Ca^{2+} -related clock mechanisms) are taken into account can we simulate both the age-associated decrease in basal mean AP firing rate and the changes in other experimentally measured coupled-clock mechanisms (Fig. 1). Because the two clocks are coupled (Yaniv et al., 2013b), changes in one mechanism related to one clock can indirectly affect mechanisms related to the other clock (Behar et al., 2016). Thus, even when changes in only one clock mechanism are taken into account (either M or Ca^{2+} in Fig. 3), the clock entrainment leads to indirect changes in the other. Consequently, an explicit change in the numerical model equations for a specific mechanism is not always necessary to describe known

experimental modifications observed in that mechanism's function. For example, the Na^+ / Ca^{2+} exchanger (NCX) current decreases in aged simulations as a consequence of changes in other mechanisms related to Ca^{2+} signaling (Fig. 3). Importantly, entrainment between the clocks means that restoring one mechanism may partially restore the others; thus, even if a particular drug is not known to restore a specific mechanism, it can restore it indirectly by acting on other mechanisms.

Our second major conclusion is that the sensitivity to β -AR stimulation or PDE inhibition is reduced in advanced age (Fig. 9). These results are in accordance with recently published data (Liu et al., 2014; Yaniv et al., 2016). This reduction in sensitivity can be the result of a reduction in the number of receptors or in the sensitivity of each receptor. Thus, a higher dose of drugs is needed to induce the desired response.

Our third major conclusion is that the reduced maximal beating rate can be reversed using maximal ANS stimulation, cAMP application, or PDE inhibition (Figs. 6, 7, and 8). These results are in accordance with recent publications in which the reduced maxi-

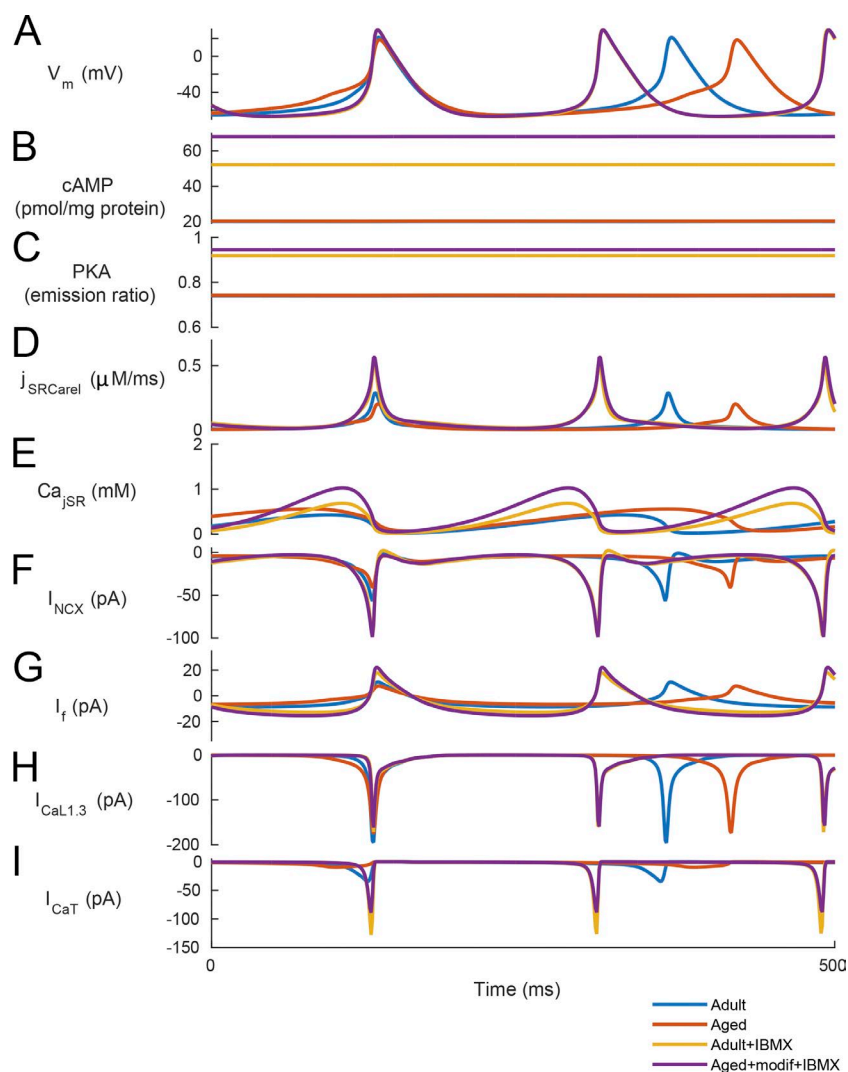


Figure 8. Model simulation in response to pacemaker function changes occurring in response to IBMX in adult and aged mice. In response to maximal concentration of IBMX, AP firing rate (A), cAMP (B), and PKA (C) activities are restored; the flux of Ca^{2+} exiting the SR (j_{SRCarel} ; D) and Ca^{2+} concentration in the junctional sarcoplasmic reticulum compartment (Ca_{jSR} ; E) are compensated for; and the Na^{+} - Ca^{2+} exchanger current (I_{NCX} ; F), HCN current (I_f ; G), L-type current (I_{CaL} ; H), and T-type Ca^{2+} current (I_{CaT} ; I) are completely or partly restored.

mal HR of aged pacemaker tissue can be reversed by maximal β -AR stimulation by using ISO or a high level of PDE inhibition using IBMX (Liu et al., 2014; Yaniv et al., 2016). In addition, the maximal AP firing rate could be restored in single isolated pacemaker cells by cAMP (Sharpe et al., 2017). Our model predicts that the age-associated reduction in maximal beating rate can be reversed only if both the relationship between cAMP and HCN_4 and the relationship between PKA and PLB phosphorylation are different in aged mice than in adult mice (Figs. 6, 7, and 8). Our model also predicts that, at maximal β -AR stimulation or PDE inhibition, the cAMP/PKA activity of aged pacemaker tissue is similar to that of adults. It is important to note that restoring the maximal beating rate is not equivalent to reversing the aged cell back to adult cell function because, although the AP firing rate was restored (i.e., the end effect “maximal beating rate”), the intrinsic currents and ion channel conductances are not all the same between adult cells and aged cells that were treated with one of these drugs (Figs. 7 and

8). In other words, a restored maximal beating rate is not equivalent to a restoration of the coupled-clock mechanisms to the adult beating rate but only to its end effect.

Our numerical model equations of the membrane and Ca^{2+} components are in general based on the model of Kharche et al. (2011) (see details in Materials and methods under Basal numerical model). However, to study whether age-deteriorated AP firing is caused by membrane or Ca^{2+} molecules, a description of the node signaling that couples them is critical. Our numerical model is the first to include a description of AC-cAMP-PKA signaling and updated Ca^{2+} dynamics based on recent data (Liu et al., 2014). Thus, only our adaptation to the Kharche et al. (2011) model can be used to answer this study’s major question: “What is the relative contribution of each clock to the age-associated deterioration in pacemaker function?” To further elaborate on this question and prove that our conclusions are robust against the choice of the model parameters, we per-

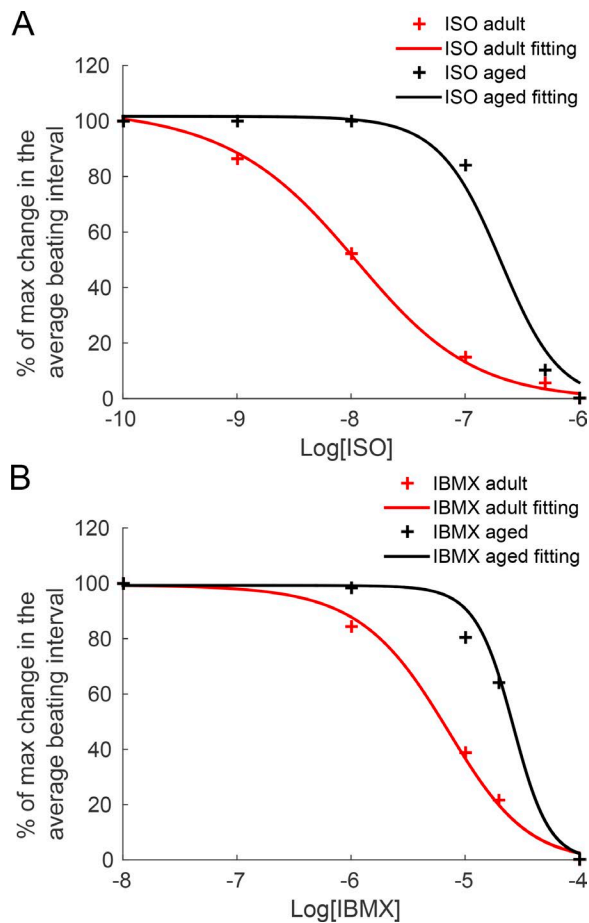


Figure 9. **Age-associated basal AP firing rate can be reversed.** (A) 167 nM ISO can reverse the aged basal AP firing rate to that of adult mouse pacemaker cells. (B) 50 μM IBMX can also reverse the aged basal AP firing rate to that of adult mouse pacemaker cells.

formed parameter sensitivity analysis (Fig. 4). The AP firing rate in the aged model depends on both mem-

brane and Ca^{2+} clock components when changes in $V_{1/2}$ of the HCN channel or in response to changes in P_{up} of the SERCA are taken into account.

Recent experimental data demonstrate the inability to restore the maximal beating rate of isolated mouse pacemaker cells by means of PDE inhibition (Sharpe et al., 2017). However, these data contradict experimental results at the tissue level (Liu et al., 2014; Yaniv et al., 2016) and our numerical model predictions. Moreover, it is well known that heterogeneity among single SAN cells exists (Boyett et al., 2000) and, importantly, that the heterogeneity of the beating rate increases in the aged model (Lowsky et al., 2014), specifically at the single-cell level (Glauche et al., 2011). Furthermore, during isolation, some receptors and molecules can be impaired, especially in the aged model. Thus, the validity of experiments performed on single cells isolated from a small sample of aged mammals is debatable. To strengthen the findings from single cells, data must also be collected at the SAN tissue level. Note, however, that although pacemaker cell heterogeneity exists in the SAN tissue, the neighborhood effect (Michaels et al., 1986) synchronizes all the cells to follow the strongest one (i.e., the cell that beats the fastest).

Limitations

Our model does not incorporate the previously documented age-associated reduction in the number of NCXs (Liu et al., 2014). Although a decrease in the number of NCXs should result in a lower entrainment level in the coupled-clock model and lead to a decrease in AP firing rate, the numerical model that we used here causes the AP firing rate to increase: reducing the number of NCXs allows more Ca^{2+} to accumulate in the cytosol and thus to increase the AP firing rate. Thus, we did not decrease the conductance of NCX in the

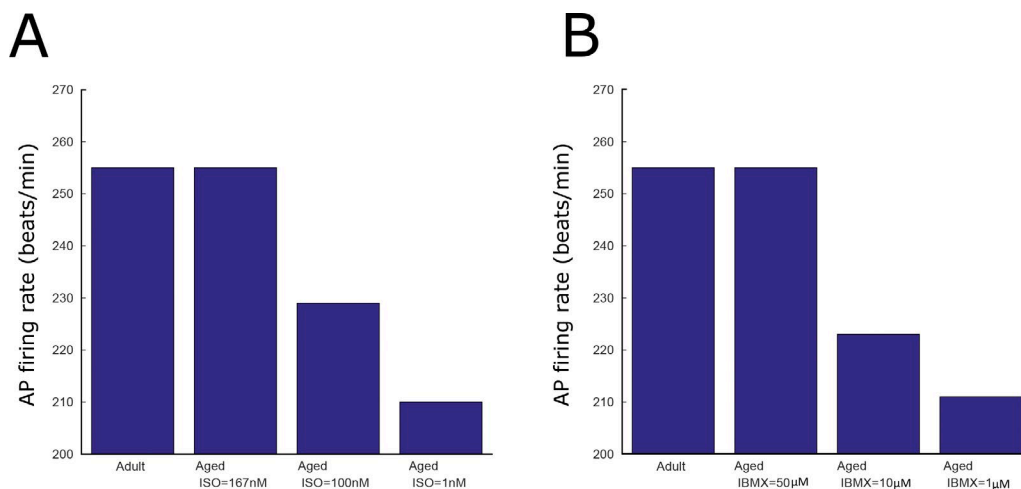


Figure 10. **Sensitivity of AP firing rate to drug perturbations.** (A and B) Simulation of beating interval dynamics in response to PDE inhibition by ISO (A) or IBMX (B).

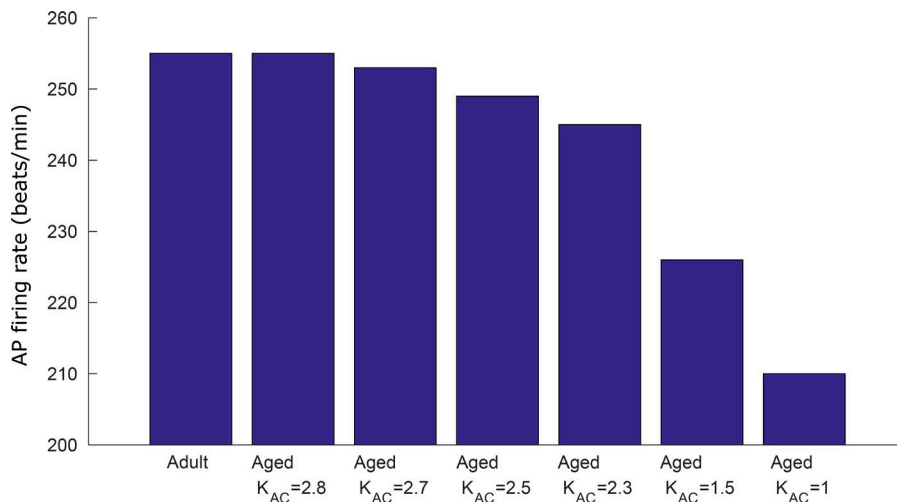


Figure 11. Overexpression of AC1 or AC8 restores the age-associated reduction in AP firing rate. Increase in K_{AC} can restore the age-associated reduction in AP firing rate.

numerical model. However, because of the reduction in SERCA, RyR, and HCN channel function, there is a significant decrease in NCX current, which reduces pacemaker function. This is caused by the coupling between the clocks (see above).

Because of the lack of experimental data on Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) activity in both adult and aged mice (Maltsev et al., 2014; Yaniv and Maltsev, 2014), we did not take into account age-related changes in CaMKII activity. Future experimental data on CaMKII activity in adult and aged pacemaker cells will allow us to explore its function. Moreover, no data exist for both adult and aged mice on local Ca^{2+} concentration and cAMP concentrations. Future experimental data will allow us to develop a local numerical model instead of our common pool model.

We showed in Fig. 6 B that the model predicted an 18% decrease in AP firing rate in aged pacemaker cells. Thus, the model prediction is on the lower bound of the experimental data. This suggests that other mechanisms, although playing a more minor role than the ones modeled, may be involved in further reducing the AP firing rate in aged cells.

The model was designed to describe mouse pacemaker function. Because of its short life span, the mouse is the ideal animal model to explore aging. Thus, the large majority of data on age-deteriorated pacemaker function were reported for mice. However, the end effect of aging in humans and mice is the same: in both models, there is no change in the basal beating rate, but only in the intrinsic beating rate (Jose and Collison, 1970). Future data from SAN tissue and signal pacemaker cells from larger aged mammals, including humans, will make it possible to develop computational models for mammals larger than mice.

The effect of PKA on I_{Ks} was documented in ventricular and atrial myocytes (Sampson et al., 2008). How-

ever, because no experimental data exist for sinoatrial cells, it is ambiguous to model PKA effect on I_{Ks} . Therefore, the current numerical model does not include the PKA effect on I_{Ks} .

Clinical aspects

Maintaining the basal heart rate when there is a decrease in the intrinsic beating rate of SAN cells implies that the ANS system must overcompensate for the deteriorated pacemaker function. Chronic stimulation of the β -AR can lead to cardiac hypertrophy and other damage to the heart (Kudej et al., 1997). We showed here that the PDE inhibitor can reverse the age-associated intrinsic beating rate, and thus may be a therapeutic tool if applied specifically only to the SAN area to prevent chronic stimulation of β -AR.

Overexpression of AC1 and/or AC8 has been suggested as a method to restore normal heart rate in the case of bradycardia (Maltsev et al., 2016). We showed here (Fig. 11) that overexpression of Ca^{2+} -activated AC can reverse the age-associated intrinsic beating rate. Thus, our numerical model shows for the first time the feasibility of such an approach for age-associated deficient pacemaker function.

Although we showed here that the maximal intrinsic beating rate can be restored under in vivo conditions, additional mechanisms, such as the preload and afterload, also affect the heart rate. Because the skeletal muscles contribute to the afterload and preload, they, too, affect the heart rate changes. Thus, even if the maximal action potential firing rate can be restored, aging-associated changes in skeletal muscle may prevent the restoration of maximal HR. Moreover, the ability of the heart to reach its maximal rate in response to pharmacological interventions implies that these drugs also restore the energetic balance in the heart (Yaniv et al., 2013a).

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Author contributions: Y. Yaniv and J. Behar conceived and designed the research; J. Behar performed simulations and analyzed the simulated data; J. Behar and Y. Yaniv drafted the manuscript; J. Behar prepared the figures; and J. Behar and Y. Yaniv edited and revised the manuscript and approved the final version.

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