Description and Use of *Fhf2*^{WT} and *Fhf2*^{KO} Cardiomyocyte Strand Models

DESCRIPTION: The *Fhf2*^{WT} and *Fhf2*^{KO} ventricular cardiomyocyte models described previously (Park et al, *Nature Comm.* 7:12966, 2016) were scripted onto the NEURON software platform (Hines and Carnevale, *Neuroscientist* 7:123, 2001) with several modifications and then linked into strands with gap junctional conductances.

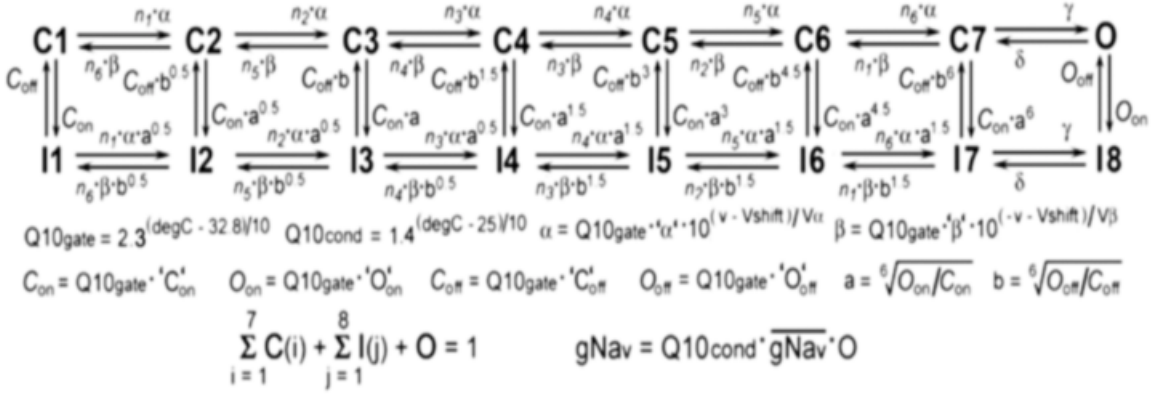
Cardiomyocyte Dimensions and Gap Junction Connectivity into Strands: The cardiomyocyte model cells (myocyte.hoc) are cylinders with length (cell.L) of 100 microns and diameter (cell.diam) of 22.34 microns. With standard membrane unit capacitance (cell.cm) of 1 μF/cm^2, each cell's membrane capacitance is 70 pF. The number of cells in the strand can be selected in the Graphical User Interface (GUI); all published data analysis was on strands comprising 111 cells (1.11 cm length).

Gap junctions are modeled as reciprocal and equivalently weighted conductances between adjacent cells n and n+1. The conductance in "source" cell n is gap_sources.o[n].g, while the matching conductance in "sink" cell n+1 is gap_dests.o[n+1].g, with the currents driven by the voltage differential between source cell n and sink cell n+1. These conductances are absolute set values (in pS) multiplied by Q10 = $1.43^{\{(^{o}C - 37)/10\}}$, unlike transmembrane ion conductances (below), which are expressed as conductance densities (S/cm^2). The normal physiological setting for junctional conductance is 772.8 nS at 37ºC. The junctional conductances can be manipulated equally between all cell pairs in the GUI, or specific cell pair conductances can be varied by text commands.

Ion Conductances in Cardiomyocytes: All ion conductance densities are set equivalently in all cells of the strand. Each ion conductance density can be manipulated equally throughout the strand in the GUI, or specific cell ion conductance densities can be varied by text commands.

Voltage-gated sodium conductances: The myocytes include two 16-state Markov model voltage-dependent sodium conductances termed NAV_withF and NAV_noF (Scheme 1).

Scheme 1



Fhf2^{KO} cardiomyocytes only have a functional NAV_noF conductance (i.e. $\overline{gNav}$ for NAV_withF = 0), while *Fhf2*^{WT} cardiomyocytes contain a mixture of NAV_withF and NAV_noF. Employing this mixture does not imply knowledge that wild-type ventricular cardiomyocytes necessarily bear a mixture of sodium channels with and without associated FHF2, but rather the mixture of models was employed to achieve a closer modeling of voltage dependent inactivation to recorded values, as presented in Online Table VII in Park et al., Circ Res 127, in press, 2020. It is also important to emphasize that for each sodium channel model, the maximum available sodium conductance upon simulated step depolarization from -135 mV to -30 mV is not equal to $\overline{gNav}$, but is equal to $\overline{gNav}$ * (C_{off}/[C_{on}+ C_{off}]). For the NAV_noF

model, $C_{off}/[C_{on}+ C_{off}] = 0.1667$, while for the NAV_withF model, $C_{off}/[C_{on}+ C_{off}] = 0.9259$. Since *Fhf2*^{WT} and *Fhf2*^{KO} model cardiomyocytes were tuned to generate the same peak sodium current upon step depolarization from -135 mV, consistent with our recorded cardiomyocyte data, $\overline{gNav}$ is greater in the *Fhf2*^{KO} model cardiomyocyte. As stipulated above, that the maximum available conductance in NAV_noF model is far less than its $\overline{gNav}$ is not meant to necessarily imply that most sodium channels in real *Fhf2*^{KO} cardiomyocytes are inactivated under all conditions.

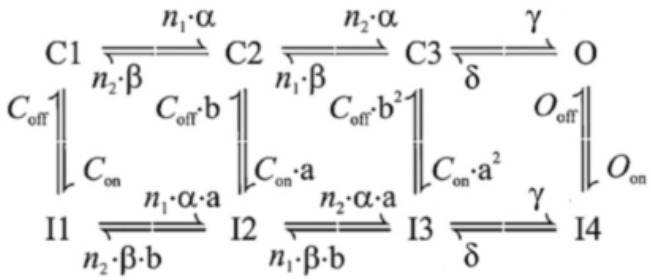
In the Markov models, α and β are voltage(v)-dependent rate constants, Q10gate is the thermodyamic scaling factor for rate constants, and Q10cond is the thermodynamic scaling factor for conductance. Parameters with equivalent values for NAV_withF and NAV_noF models are: n1 = 100, n2 = n3 = 20, n4 = 3, n5 = 1.5, n6 = 0.75, 'α' = 2.44375 (*ms*$^{-1}$), 'β' = 0.01325 (*ms*$^{-1}$), Vα = Vβ = 9 (*mV*), γ = 150 (*ms*$^{-1}$), δ = 40 (*ms*$^{-1}$), 'O'$_{off}$ = 0.0005 (*ms*$^{-1}$). Parameters with different values for NAV_withF vs NAV_noF models are: 'C'$_{on}$ = 0.004 *vs* 0.025 (*ms*$^{-1}$), 'C'$_{off}$ = 0.05 *vs* 0.005 (*ms*$^{-1}$), 'O'$_{on}$ = 0.85 *vs* 1.3 (*ms*$^{-1}$), V_{shift} = -54 *vs* -57.5 (*mV*). In the *Fhf2*^{KO} cardiomyocyte, $\overline{gNav}$_noF = 25 nS/pF, while in the *Fhf2*^{WT} cardiomyocyte, $\overline{gNav}$_noF = 8.83 nS/pF and $\overline{gNav}$_withF = 2.94 nS/pF.

Online Table VII in Park et al., *Circ. Res.* 127, in press, 2020 presents the Na_V inactivation and activation characteristics and generated currents of the *Fhf2*^{WT} and *Fhf2*^{KO} cardiomyocyte models, which are in close agreement with sodium current recordings from *Fhf2*^{WT} and *Fhf2*^{KO} ventricular cardiomyocytes (Park et al, *Nature Comm.* 7:12966, 2016; Wang et al, *J. Mol. Cell. Cardiol.* 104:63, 2017; Park et al, Circ. Res. *Circ. Res.* 127, in press, 2020). The $\overline{gNav}$ densities for the *Fhf2*^{WT} cardiomyocyte were estimated to generate action potential amplitude in isolated cardiomyocyte model with amplitude similar to prior recordings and conduction velocity in model strand comparable to velocity reported by optical mapping, while $\overline{gNav}$ for NAV_noF in *Fhf2*^{KO} cells allowed *Fhf2*^{WT} and *Fhf2*^{KO} model cardiomyocytes to generate same peak sodium current upon depolarization from -135 mV holding potential, as previously demonstrated empirically[1]. A third cardiomyocyte model termed *Fhf2*^{WT}*Na*$_V^{HYPO}$ has the same Na_V gating parameters as the *Fhf2*^{WT} model, but the Nav densities are reduced by a factor of 0.49 so that the *Fhf2*^{WT}*Na*$_V^{HYPO}$ and *Fhf2*^{KO} models generate the same I-Na_{peak} when depolarized from a -87mV resting potential (Online Table VII in Park et al., *Circ. Res.* 127, in press, 2020).

Nomenclature clarification: The names of rate parameters with units *ms*$^{-1}$ above that are flanked by apostrophes in Park et al., *Circ. Res.* 127, in press, 20 are named differently in the uploaded Nav models, where the rate parameters are instead preceded by the prefix A. As examples, 'α' in the publication is equivalent to Aα in the uploaded model, 'C'$_{off}$ is equivalent to AC_{off}, etc. Additionally, $\overline{gNav}$ in the publication is equivalent to gnabar in the uploaded model.

Voltage-gated calcium conductance: *Fhf2*^{WT} and *Fhf2*^{KO} cardiomyocyte models now have an equivalent L-type voltage-gated calcium conductance expressed through an 8-state Markov model (Scheme 2) based upon the equivalent calcium current density, voltage dependence of activation and steady-state inactivation, and voltage-dependent rate of inactivation measured empirically in *Fhf2*^{WT} and *Fhf2*^{KO} cardiomyocytes (Figure 2 and Table 1 in Park et al., *Circ. Res.* 127, in press, 20).

Scheme 2



The kinetic parameters are: $Q10 = 3^{\{(^{O}C - 32.76)/10\}}$, α = Q10 * 11.74 * $10^{\{(V_m + 17)/50\}}$ (*ms*$^{-1}$), β = Q10 * 0.0324 * $10^{\{(-V_m - 17)/5.5\}}$ (*ms*$^{-1}$), n_1 = 32.532, n_2 = 0.123, γ = Q10 * 150 (*ms*$^{-1}$), δ = Q10 * 40 (*ms*$^{-1}$), C_{on} = Q10 *

0.001 *(ms^{-1})*, C_{off} = Q10 * 10 *(ms^{-1})*, O_{on} = Q10 * 0.2 *(ms^{-1})*, O_{off} = Q10 * 0.001 *(ms^{-1})*, a = $(O_{on}/C_{on})^{0.5}$, b = $(O_{off}/C_{off})^{0.5}$, where V_m is membrane voltage.

Potassium conductances: The potassium conductances are taken from Bondarenko et al., *Am. J. Physiol. Heart. Circ. Physiol*. 287:H1378, 2004, and include the time-dependent conductances fast transient outward conductance (g_Kto_f), noninactivating ultrarapid delayed rectifer (g_Kurdr), noninactivating rapid delayed rectifier (g_Krdr), noninactivating slow delayed rectifier (g_Ksdr), and steady-state conductance (g_Kss), along with time-independent conductance (g_Kti) that has both leak and inward rectifier components. The weights of these conductances were adjusted 1) to give passive property ΔV as function of injected current similar to empirically recorded dissociated ventricular cardiomyocytes (Park et al, *Nature Comm.* 7:12966, 2016), and 2) to give a decay in the action potential in cardiomyocyte strand models similar to measured action potential decay optically recorded in paced ventricular myocardium (Online Fig II in Park et al., *Circ. Res.* 127, in press, 2020). These conductance values (S/μF) are g_Kti = 0.00021, g_Kss = 0.00007, g_Kto_f = 0.0000235, g_Kurdr = 0.000025, g_Krdr = 0.000468, g_Ksdr = 0.00000575.

Other time-independent currents: Two other small currents were incorporated to maintain cardiomyocytes at -87 mV resting potential at all temperatures. Background sodium conductance (g_Nabg = 0.0000018 S/μF) is taken from Bondarenko et al., *Am. J. Physiol. Heart. Circ. Physiol*. 287:H1378, 2004, while a temperature-dependent nonspecific current (i_ITEMP) was incorporated to offset small temperature variations in conductances near the resting potential, set at i_ITEMP = 0.0322 * {43 – (°C)} (pA/pF).

Cardiomyocyte strand simulations. The cardiomyocyte strands have a resting membrane potential of -87 mV. In all simulations, a 100 msec delay was employed to allow the Na_V Markov models to reach steady state prior to injecting the first cell with two 0.5 msec current stimuli at 10 Hz. Injected current amplitude was adjusted to achieve maximal induced sodium current in the first cell. The generated currents and voltages throughout the strand were analyzed only following the second stimulus, which takes into account the states of dynamic conductances at sinus rhythm. All simulations were conducted using Cvode multi-order variable time step integration method. For each model, simulations were run after either elevating temperature in 1°C increments or reducing junctional, sodium, or calcium conductances in 1% increments. Conduction safety was defined as propagation of action potentials through the entire strand, with regenerative sodium current reaching steady cell-to-cell amplitude. Conduction failure was defined as a failure to generate sodium current in all cells throughout the strand with accompanying fall-off in depolarization amplitudes. In most simulations, conductance parameters were altered equivalently in all cells within the strand. However, to investigate how calcium conductance contributes to conduction safety in the *Fhf2^{KO}* strand, we conducted simulations where calcium conductance was deleted from cells 51-111 only.

Under any simulation condition, the *Fhf2^{KO}* cardiomyocytes generate substantially less sodium current than *Fhf2^{WT}* cardiomyocytes for two reasons: 1) at resting potential, approximately 74% of the sodium conductance is inactivated in *Fhf2^{KO}* cells, while there is only ~50% inactivation of the sodium conductance in *Fhf2^{WT}* cells (Figure 4E,F, Online Table VII in Park et al., *Circ. Res.* 127, in press, 2020), and 2) the Na_V model in *Fhf2^{KO}* cardiomyocytes has faster rates of closed-state and open-state inactivation than does the Na_v model in *Fhf2^{WT}* cells (Online Table VII in Park et al., *Circ. Res.* 127, in press, 2020). Action potential amplitudes, conduction velocity, $[dV/dt]_{max}$, safety factor (SF), and conduction safety or failure thresholds in the *Fhf2^{WT}*and *Fhf2^{KO}* strands in response to variations of temperature, Gj, gNa_V, or gCa_V are summarized in Online Table VI in Park et al., *Circ. Res.* 127, in press, 2020. SF values were calculated based upon its originally described formulation (Shaw and Rudy, *Circ. Res.* 81:727, 1997),

except that all membrane currents (sodium, calcium, potassium, capacitive) were incorporated into the calculation for determining when a cell transitioned from being predominantly a sink to a source.

USE OF THE MODELS

1) Download the Ventricular_GUI.zip file of the model. Extract the embedded files.
2) Open NEURON. Run the mknrn program, and use it to select the Ventricular_GUI folder and convert the .mod files into compiled .o files.
3) Launch the model by double-clicking Start_GUI_3. This will open the neuron.exe terminal and several GUI windows: "Main Menu", "Set model paramters", and "Set Sim Structure".

The "Set Sim Structure" window allows for the selection of the $Fhf2^{WT}$ or $Fhf2^{KO}$ cardiomyocyte models, the temperature (37°C default), the number of myocytes in the strand (default 111), and the gap junctional conductance between cells along the strand (default 772,800 pS). Toggling between the WT and KO models using the "Activate KO Mutation" button alters the conductance densities for the Nav_withF and Nav_noF sodium channel models, and these densities are seen in the "Set Model Parameters" window. The selected strand model can be launched from the button "Linear Propagation Along Strand", which generates additional windows, including stimulus electrodes positioned within the first cell myocyte.o[0] preset to generate 0.5 millisecond pulses of current at 100 msec and 200 msec after simulation initiation. The "Propagation Protocol" window allows selection of voltage vs. time, sodium current vs. time, and sodium channel states vs. time, and the simulation is initiated from the Run button.

The "Set Sim Structure" window has other buttons as well. For user convenience, other buttons in the "Set Sim Structure" window launch action potential propagation simulations present in Figures 4B, 4C, 5A, 5B, 5F, 5G, 6A, 6B, 6D, or 6E from Park et al., *Circ. Res.* 127, in press, 2020. These Figure Panel buttons each open a figure panel window and a voltage vs. time graph, and the simulation can be initiated from the Run button within the figure panel window. *It is highly recommended that when wishing to open different configuration buttons from the "Set Sim Structure" window, the model should be fully closed by closing the NEURON terminal window, and then relaunching the model from Start_GUI_3.*

In order to facilitate sodium current voltage clamp protocols, the "Set Sim Structure" window also has buttons to select for protocols to assay Na_v voltage dependence of activation, voltage dependence of steady-state inactivation, and sodium currents in response to variable-rate voltage ramps. The launch of any of these protocols creates a single cardiomyocyte with the selected genotype and temperature parameters, along with a protocol control window, from which the simulation is initiated with the Run button.

The densities of all ionic conductances can be changed equivalently in all cells of the strand using the "Set Model Parameters" window. This window can also be used to modify kinetic parameters for the sodium channel models. Changes to parameters of ionic and junctional conductances in a subset of cells within the strand can be made through hoc code commands in the terminal window. As examples,
1) The command:

```
for i=50,110 {prop_myo.myocytes.o[i].cell.gcabar_Ca_L = 0}
```

sets the calcium conductance to zero in cells 51-111 of the strand (note that first cell in model is myocytes.o[0])
2) The pair of commands:

```
prop_myo.gap_sources.o[10].g = 10000
prop_myo.gap_dests.o[10].g = 10000
```

resets the gap junctional conductance between cells 11 and 12 in the strand to 10000 pS.