Modeling the One-Dimensional Propagation of the Caveolae-Inclusive Cardiac Action Potential

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Modeling the One-Dimensional Propagation of the Caveolae-Inclusive Cardiac Action Potential

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May 5, 2014

Abstract

Two models (based off of the Luo-Rudy 1 guinea-pig ventricular model) were produced to analyze the effects of caveolar sodium current on a single cardiac action potential. A separate model (based off of the Pandit et al. rat left ventricular model) was produced to observe the effect additional caveolar sodium current had on the one-dimensional propagation of a cardiac action potential in a line of cardiomyocytes. Evidence suggests that the opening of caveolae recruits additional sodium channels on the cardiomyocyte membrane that can affect both the peak voltage overshoot and the maximum upstroke velocity of the cardiac action potential—the change in maximum upstroke velocity in turn can alter the conduction velocity of an electrical signal. We examined two opening mechanisms of caveolae. The first opening mechanism simulated a 1-cm$^2$ patch of membrane perfused with a $\beta$-adrenergic agonist that opened a certain number of caveolae on the membrane. The second opening mechanism simulated a 1-cm$^2$ patch of membrane with stochastically opening caveolae that open according to a Poisson process. The effects of these two opening mechanisms of caveolae on a single cardiac action potential using the Luo-Rudy 1 model were compared to previous computational results using the Pandit et al. model. Our simulations (which incorporated varying membrane capacitance) revealed a 4.1% increase in peak voltage overshoot and a 19.1% increase in the maximum upstroke velocity for a 42% increase in sodium current due to $\beta$-adrenergic stimulation. Incorporating stochastically opening caveolae, we observed features such as delays in ventricular repolarization, early afterdepolarizations (characteristics of a serious heart condition called Long-QT Syndrome), and the absence of ventricular repolarization. Propagating single cardiac action potentials (modeled by the Pandit et al. model) revealed a nonlinear increase in conduction velocity as the total number of caveolae on each cell in a line of cardiomyocytes increased.
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References
1 Introduction and Background

1.1 An Overview of Cardiac Electrophysiology

1.1.1 Basics of the Cardiomyocyte

The human body is an amazing piece of biological and natural machinery composed of a multitude of components that work together to create a living, breathing organism. One of these important constituents, the heart, will be the focus of this research.

Our analysis begins at the smallest functional unit of the heart: an involuntary heart muscle cell called the cardiomyocyte. The cardiomyocyte membrane is a phospholipid bilayer that not only acts as a barrier to prevent the movement of certain compounds across the membrane but also possesses a special ability to generate electrical signals that causes the heart to contract (cells that possess this ability are known as excitable cells). These electrical signals arise due to action potentials—a rapid up-down change in electrical potential (or voltage) across the cell membrane. The membrane of a cardiomyocyte is naturally polarized because of a difference in ion concentrations between the intracellular and extracellular spaces. This natural potential difference is referred to as the resting membrane potential (ranging from about $-70 \text{ mV}$ to $-95 \text{ mV}$ in cardiac cells depending upon species) and can be thought of as a stable equilibrium of the system.

A stimulus voltage or current that is applied to the cardiomyocyte membrane can be thought of as a perturbation of the resting system. If the stimulus is strong enough, a cardiac action potential (CAP) will occur. During a CAP, movement of sodium ($\text{Na}^+$), potassium ($\text{K}^+$), and calcium ($\text{Ca}^{2+}$) ions across the membrane via voltage gated protein transporters—called ion channels—alters the membrane potential generating an electrical signal that proceeds from cell to cell through gap junctions which connect adjacent cells.

1.1.2 The Cardiac Action Potential

Let us take a moment to observe what happens during a CAP (see Figure 1.1). Our point of reference will be the resting membrane potential (Phase 4) of about $-85 \text{ mV}$ (as mentioned previously this value is different across different species). During Phase 4, the voltage gated sodium, potassium, and calcium channels assume their closed conformational states—meaning those ion species are prohibited from passing through their respective channels during this time.

As an electrical signal reaches and depolarizes the membrane to a certain threshold voltage, Phase 0 occurs. At this point, the sodium channels change to an active and open conformational state allowing a rapid influx of positively charge sodium ions. This flow of positive ions into the cardiomyocyte causes a
temporary shift in membrane polarity—the membrane voltage peaks at around +40 mV (this is the value for guinea pig ventricular myocytes and this value is different for different species).

By the time of the voltage peak, the sodium channels have assumed an inactive conformational state in which no amount of stimulus will open the channels for a given period of time—we call this the refractory period. We have now reached Phase 1. The sudden and short drop in membrane potential is due to the transient outward flow of positively charged potassium ions (as well as the outward flow of chloride ions) through open channel proteins.

We then observe a plateau in membrane voltage characteristic of Phase 2. The sustained voltage is the result of the balance between the efflux of potassium ions and the influx of calcium ions.

Eventually the calcium channels close and inactivate initiating Phase 3. The potassium channels remain open and allow the continued efflux of potassium ions resulting in a quick repolarization. It is important to note that the depolarization step (Phase 0) happens most rapidly, followed by the repolarization step (Phase 3), and the plateau (Phase 2). Repolarization occurs until the membrane voltage returns to its resting potential. By this point, we have cycled through one complete CAP. As the electrical signal travels across the membranes of various cardiomyocytes, the muscle cells coordinate to produce an overall contraction of the heart.
1.2 Mathematical Modeling of Cardiac Electrophysiology

1.2.1 The Nernst Potential

Before delving more deeply into the modeling aspects of cardiac electrophysiology, we must understand how and why ions tend to move across the membrane via voltage gated ion channels. For an ion species $S$, there are two main mechanisms that drive the movement of this particle. First, differences in concentration of $S$ create a chemical gradient that applies a force on $S$ causing it to diffuse from an area of high concentration to an area of low concentration. Second, an electrical gradient is established that drives the movement of charged particles by placing them in an electric field (known as electrophoresis). Because we are concerned with charged particles, concentration differences create a potential difference between two points—in our case between the intracellular and extracellular environments. When the influx and efflux of $S$ across the membrane are in equilibrium, the potential difference is given by the Nernst equation

$$E_S = \frac{RT}{qF} \ln \left( \frac{[S]_e}{[S]_i} \right)$$  \hspace{1cm} (1.1)

where $E_S$ is the Nernst potential of ion species $S$ in mV, $R$ is the universal gas constant in J/(mol·K), $T$ is the absolute temperature in K, $q$ is the charge on ion species $S$ in Coulombs, $F$ is Faraday’s constant in Coulombs/mmol, and $[S]_e$ and $[S]_i$ are external and internal concentrations of $S$ respectively in mM. The difference between the membrane potential and the Nernst potential at any time (i.e. $(V_m - E_S)$) is the strength of the electrochemical gradient acting on that ion and is called the driving force associated with ion species $S$.

1.2.2 Representing the Cardiomyocyte Membrane as an RC Circuit

Representing the CAP as a mathematical model can augment and support the process of physical experimentation by generating several simulations in a short amount of time thus creating new hypotheses for researchers to explore on live subjects. Note the similarities between the cardiomyocyte membrane and a simple electrical RC (resistor-capacitor) circuit. The lipid bilayer acts as a capacitor by separating charged particles in the intracellular and extracellular space while the separate ion channels act as variable resistors (see Figure 1.2).

First, recall the classical relationship

$$CV = Q$$  \hspace{1cm} (1.2)

where $C$ is the capacitance, $V$ is the voltage, and $Q$ is the total charge accumulated on the capacitor. Because
Figure 1.2: The cardiomyocyte membrane and an equivalent RC circuit[1].
the cardiomyocyte membrane acts as a capacitor, we note that \( C_m V_m = Q_m \) where \( C_m, V_m, \) and \( Q_m \) are the membrane’s capacitance, voltage, and total charge accumulated respectively. Applying Kirchoff’s current law to the RC membrane circuit, we observe that the sum of the individual currents equals zero.

\[
I_{Na} + I_{Ca} + I_{K} + I_{C_m} = 0
\]  

Or more generally,

\[
I_{C_m} = - \sum_{S \in \Omega} I_S
\]  

where the sum is taken over the set, \( \Omega \), of all ion species involved in the transmembrane currents. Differentiating (1.2) with respect to time and noting that \( I_{C_m} = \frac{dQ_m}{dt} \), we can derive the following relationship.

\[
\frac{dV_m}{dt} = \left( \frac{1}{C_m} \right) \left( \frac{dQ_m}{dt} \right) = - \left( \frac{1}{C_m} \right) \left( \sum_{S \in \Omega} I_S \right)
\]  

Equation (1.5) is the basis of the Hodgkin-Huxley model that has paved the way for modeling cardiac electrophysiology.

### 1.2.3 The Hodgkin-Huxley Model

Nearly all CAP models have a common foundation in the Hodgkin-Huxley model. Alan Hodgkin and Andrew Huxley developed the first quantitative model of the propagation of an electrical signal along a squid giant axon[2]. In the squid giant axon (as with many neuronal cells) the principal ionic currents are the sodium and potassium currents. Although there exist other minor currents, the Hodgkin-Huxley theory combines these minor currents into one so-called leakage current.

As discussed previously, the movement of ions across the membrane is analogous to current flowing through a variable resistor. The voltage drop across these variable resistors, or voltage gated ion channels, has two components. First, the voltage drop due to concentration differences is given by the Nernst potential in Equation (1.1). Second, the voltage drop due to an electrical current flowing through a resistor is given by Ohm’s law \( R_S I_S \) where \( R_S \) is the resistance of the ion channel and \( I_S \) is the current due to the movement of ion species \( S \). Thus, the total voltage drop is given by

\[
V_m = R_S I_S + E_S
\]  

and solving for \( I_S \) we see
\[ I_S = g_S (V_m - E_S) \] (1.7)

where \( g_S = 1/R_S \) is the bulk membrane conductance of the ion channels for species \( S \). The current \( I_S \) and conductance \( g_S \) are generally specified per unit area of the membrane, being the product of the single channel conductance times the number of channels per unit area of the membrane.

In terms of the Hodgkin-Huxley model, we see that Equation (1.5) becomes

\[
\frac{dV_m}{dt} = \left( \frac{1}{C_m} \right) \left[ -g_{Na} (V_m - E_{Na}) - g_K (V_m - E_K) - g_L (V_m - E_L) + I_{app} \right] \quad (1.8)
\]

where \( I_{app} \) is an applied current. The work of Hodgkin and Huxley aimed to determine how the conductances of each ion channel depended on voltage.

The key step to understanding the dynamics of the conductances came from the development of the voltage clamp[2]. The use of the voltage clamp allowed Hodgkin and Huxley to fix the membrane potential while observing the amount of transmembrane current passing through specific ion channels as a function of time. By selectively blocking certain types of ion channels, Hodgkin and Huxley were able to analyze individual ionic currents for various fixed or “clamped” voltages.

The voltage clamp experiments performed by Hodgkin and Huxley suggested that \( g_{Na} \) and \( g_K \) were functions of time and membrane potential while the leakage conductance was taken as constant[3]. In fact, they were able to characterize the time and voltage dependencies of \( g_{Na} \) and \( g_K \)

\[
g_K = \overline{g}_K n^4 \quad (1.9)
\]

\[
g_{Na} = \overline{g}_{Na} m^3 h \quad (1.10)
\]

where \( \overline{g}_K \) and \( \overline{g}_{Na} \) are constants known as the maximum potassium and sodium conductances and \( n, m, \) and \( h \) are what have become known as gating variables. Each gating variable \( z \) obeys a differential equation of the form

\[
\frac{dz}{dt} = \frac{z_\infty (V_m) - z}{\tau_z (V_m)} \quad (1.11)
\]

where \( z_\infty (V_m) \in [0,1] \) (the steady-state value of \( z \)) and the time constant \( \tau_z (V_m) \) are non-linear functions of membrane voltage and are determined experimentally[1].

What is remarkable about the work of Hodgkin and Huxley[3] is that while they only sought to fit experimental data, they also stumbled upon a feature of ion channels that would later be verified with the
development of sophisticated channel protein imaging. Recent imaging has shown that sodium and potassium channels each have four substructures which act as physical gates regulating the flow of ions through the channel pore[1]. This is the same number of gating variables that show up in each conductance of potassium and sodium in the Hodgkin-Huxley model. In general, the Hodgkin-Huxley model can be summarized as follows

\[
\frac{dV_m}{dt} = - \left( \frac{1}{C_m} \right) \left( \sum_{S \in \Omega} I_S \right) \tag{1.12}
\]

where the sum is taken over the set, \( \Omega \), of all ion species involved in the transmembrane currents given by

\[
I_S = g_S z_1^{k_1} z_2^{k_2} \ldots z_p^{k_p} (V_m - E_S) \tag{1.13}
\]

and where each gating variable \( z_i \) obeys the following differential equation.

\[
\frac{dz_i}{dt} = \frac{z_{i\infty}(V_m) - z_i}{\tau_{z_i}(V_m)} \tag{1.14}
\]

1.2.4 A Summary of Cardiac Action Potential Models

Since the formulation of the Hodgkin-Huxley model, several CAP models have adapted the Hodgkin-Huxley model to include other currents such as the calcium current. The first successful ventricular model was published by Beeler and Reuter in 1977. The Beeler-Reuter model has a total of eight variables and included four of the eight ionic currents known at the time to be present in cardiac muscle. These ionic currents were formulated mathematically in terms of Hodgkin-Huxley type equations. This model incorporated a fast inward sodium current, a slow inward current due mainly to calcium ions, a time-independent outward potassium current, and a voltage- and time-dependent outward current. The Beeler-Reuter model simulates the prolongation, or plateau, of the action potential rather successfully. However, the Beeler-Reuter model does not predict action potential behavior during conduction disturbances or arrhythmias[5]. More recent work has been done to improve this model.

One model that followed the development of the Beeler-Reuter model was the model developed by Pandit et al. and includes a system of 26 coupled ordinary differential equations that was based off experimental data from rat left ventricular myocytes[4]. This model incorporates a total of twelve different ionic currents for sodium, calcium, potassium combined. The Pandit-Clark-Giles-Demir model was modified by Besse to incorporate additional sodium current attributed to open caveolae on the cardiomyocyte membrane and thus seemed a reasonable model on which to base this research. However, despite accurately modeling a rat CAP,
the amount of unknown variables in this model prevent a reasonable analysis of the system.

In 1991, Ching-hsing Luo and Yoram Rudy published an article that discussed their improvements and alterations to the Beeler-Reuter model. The Luo-Rudy 1 model (based off of data from guinea pig ventricular cells) added three new currents: the plateau potassium current, a background current with constant conductance, and an additional potassium current\cite{6}. This model also has a total of eight variables and can model certain influences on propagation and arrhythmogenesis. The Luo-Rudy 1 model will form the basis of the research discussed in this paper due to the relatively small number of variables and the ability of this model to incorporate pathophysiological conditions.

1.2.5 A Summary of Propagation Models

Up until this point, we have discussed models of a single CAP. Our next order of business is to summarize the similarities in CAP one-dimensional propagation models. There are two main ways to analyze one-dimensional propagation. The first (and more realistic) approach is to assume that propagation of a CAP is discontinuous or discrete. At the cellular level and macroscopic level, there are boundaries that separate each individual cardiomyocyte. On the cellular level, we see that the cardiomyocyte membranes form physical barriers between adjacent cells. One example of such a barrier are gap junctions. A gap junction is a specialized intercellular connection that directly connects the cytoplasm of two adjacent cells through channel proteins. The specialized gap junction channel proteins allow various molecules and ions to pass freely between the cells. On the macroscopic level, connective tissue forms a barrier between groupings of cardiomyocytes. In each case, we would expect these barriers to affect the passage of a signal across separate cells—and in fact these barriers do. But, adding realistic qualities to a model also introduces model complexity and more difficult mathematical analysis.

The second approach to analyzing propagation is to treat the passage of a signal as continuous. And in most cases, we see that this is a valid assumption. The analysis of a continuous system is also much simpler than a discrete system so in this paper we will focus on continuous one-dimensional propagation in a line of cardiomyocytes.

We first note the conservation law regarding diffusive systems

$$\frac{\partial \phi}{\partial t} = f - \nabla \cdot J$$

(1.15)

where $\phi$ is the amount of some chemical species, $f$ represents the local production of $\phi$ per unit volume, and $J$ is the diffusion flux of $\phi$\cite{2}. According to Fick’s second law of diffusion, $J$ (dimensions of amount of substance per unit area per unit time) is given by
\[ J = -D \nabla \phi \] (1.16)

where \( D \) is the diffusion coefficient (dimensions of length squared per time) and \( \phi \) has dimensions of amount of substance per unit volume. The gradient operator \( \nabla \) is interpreted as a vector of partial derivative operators of the chemical species \( \phi \) with respect to the location of the chemical species at some point in time. Combining Equations (1.15) and (1.16) we see that

\[ \frac{\partial \phi}{\partial t} = \nabla \cdot (D \nabla \phi) + f \] (1.17)

and if \( D \) is constant

\[ \frac{\partial \phi}{\partial t} = D \nabla^2 \phi + f \] (1.18)

we have what is known as a reaction-diffusion system. Generally, \( D \nabla^2 \phi \) is known as the diffusion term and \( f \) is known as the reaction term. Also, we are concerned with the movement of particles in one-dimension so Equation (1.18) reduces to

\[ \frac{\partial \phi}{\partial t} = D \frac{\partial^2 \phi}{\partial x^2} + f \] (1.19)

where \( x \) is the location of the diffusive particle(s) at a specific time.

An analysis of two different CAP propagation models—Li-Alexiades-Buchanan[7] and Kléber-Rudy[8]—revealed a common foundation derived from Equation (1.19). For this research, we will make use of the continuous one-dimensional propagation model given by

\[ C_m \left( \frac{\partial V_m}{\partial t} \right) + \sum I_S = \left( \frac{a}{2\rho_i} \right) \left( \frac{\partial^2 V_m}{\partial x^2} \right) \] (1.20)

where \( a \) is the fiber radius (cm) and \( \rho_i \) is its axial resistivity (kΩcm). Note that \( V_m \) is now a function of both time \( t \) and space \( x \) and thus the currents \( I_S \) will also be functions of time and space because they are functions of \( V_m \). Observe that the right hand side of Equation (1.20) is the diffusion term and we can think of the sum of the currents as the reaction term.
1.3 Significance of Caveolae to the Cardiac Action Potential

1.3.1 Caveolae Structure and Function

Caveolae are small invaginations of the plasma membrane that have a nearly spherical shape with a diameter of 50-100 nm and can occur in clusters called rosettes (see Figure 1.3)[9]. These pits in the cell membrane are generally closed off from the extracellular environment with their caveolar necks pinched together tightly. As a result, the intracaveolar environment may be drastically different from the intracellular and extracellular environment.

![Figure 1.3: The structure of a caveola][9]

Caveolae also contain several different membrane proteins. The signature protein, caveolin, has been found to play an important role in maintaining the pit-like structure of the caveola. Also, recent investigations of caveolar structure have shown that caveolae contain Na\(_{v}\)1.5 channels—the sodium channels responsible for the current driving the action potential upstroke[10]. In fact, stimulation of rat cardiomyocytes perfused with isoproterenol (a β-adrenergic agonist) causes the caveolar necks to open thus significantly increasing the sodium current[11][12]. Considering the large caveolar density—about 25000 total per cell or 5 caveolae/µm\(^2\) of membrane[1]—one would expect that caveolae play a crucial role in the dynamics of the CAP.

1.3.2 Significance of Caveolae to the Cardiac Action Potential

Clearly caveolae have the potential to significantly alter the CAP. Simulations of action potentials with a caveolae-inclusive model have shown that the additional sodium current observed when caveolae open increases the peak voltage overshoot and maximum upstroke velocity[1]. These simulations, along with findings in other literature, suggest a linear relationship between sodium current (open caveolae) and maximum upstroke velocity. Another important result of these simulations is that the overall CAP morphology changed only slightly with an increase in sodium current. But, changes in maximum upstroke velocity are known to
have significant effects on conduction velocity of the excitatory wave in cardiac tissue[1]. Furthermore, introducing stochastic caveolae (caveolae that open in a flickering manner) into the same simulations produces action potentials with delays in repolarization and early afterdepolarizations[1].

1.4 Specific Objective

1.4.1 Development of a Caveolae-Inclusive Cardiac Action Potential Model

The first goal of this research is produce similar results to those observed by the simulations performed by Besse[1]. We will use a different CAP model—the Luo-Rudy 1 model of guinea pig ventricular myocytes[6]—and incorporate the additional sodium current due to open caveolae. That is, we aim to accurately model the situation in which the caveolae necks are completely open with no stochasticity. We expect to see increases in both the peak voltage overshoot and maximum upstroke velocity of a CAP. We would also like to incorporate stochastic caveolae into our model and observe its effects on the CAP.

1.4.2 Development of a One-Dimensional Propagation Model

The second and primary goal of this research will be to develop a one-dimensional caveolae-inclusive propagation model. We will assume a continuous propagation for a more thorough mathematical analysis. The continuous propagation model that we will modify will be the one given by Equation (1.20). The caveolae-inclusive one-dimensional propagation model will allow us to observe the effects caveolar sodium current will have on the passage of an electrical signal along the membranes of separate cardiomyocytes. If time permits, we will attempt to incorporate and observe the effects of stochastic caveolae on CAP morphology and signal propagation.
2 The Caveolae-Inclusive Models

2.1 The Caveolae-Inclusive Model

2.1.1 Introduction

As mentioned previously, Besse developed a model that accounts for the additional currents resulting from the opening of caveolae. Besse also quantified the density of caveolae on the typical ventricular myocyte membrane because not much was known about caveolae at the time. We will utilize the results of Besse’s work to formulate the caveolae-inclusive model based off of the Luo-Rudy 1 model. However, one major difference exists between Besse’s model and the one presented in this paper. The model in this paper will build its foundations on a model based off of experimental data from guinea pig ventricular myocytes—not rat ventricular myocytes.

2.1.2 Assumptions of the Model

We will build off of Besse’s work and assume that caveolae open instantaneously and exist in one of two states—open or closed. The caveolar density is estimated to be about 5 caveolae/µm² or equivalently 500 caveolae/pF. This yields an approximation of 25000 caveolae/cell. Single-caveolae patch clamp experiments suggest that most caveolae contain only a single sodium channel. Therefore, it will also be assumed that each closed caveolae sequesters exactly one sodium channel, and no other ion channels, pumps, or exchangers (although there is literature which suggests there are other channels embedded in caveolae).

We will also follow the quasi-three compartment framework suggested by Besse in which there are two possible orientations (or modes) of the different compartments depending on whether a β-agonist is present or not. When no β-agonist is present, the caveolae are closed and are separated and isolated from the extracellular environment and this situation is modeled with a normal Luo-Rudy 1 CAP model. When a β-agonist is present, the caveolae are open and the caveolar membrane may interact with the extracellular space. Extra sodium channels are recruited that may contribute to the sodium current important for the initial upstroke of the CAP.

One final modification we will make will be to account for changes in membrane capacitance due to the increase in surface area attributed to open caveolae. We will assume each caveola is a is a sphere with a diameter of 75 nm and that the capacitance is proportional to surface area.
2.1.3 Formulation of the Caveolae-Inclusive Model

Relevant to the development of the caveolae-inclusive model is the sodium current from the Luo-Rudy 1 model.

\[ I_{Na} = g_{Na} m^3 h j (V_m - E_{Na}) \]  \hspace{1cm} (2.1)

Recall from the Hodgkin-Huxley formalism that only the \( m \) (activation variable) and \( h \) (inactivation variable) gating variables were present in the sodium current. The Luo-Rudy 1 model includes a slow inactivation gate \( j \) in order to model the property of slow recovery of sodium channels. Under the assumptions discussed above, incorporating the additional caveolar current involves modifying the sodium current in Equation (2.1). Besse asserts that

\[ I_{cav} = n \gamma_{Na} m^3 h j (V_m - E_{Na}) \] \hspace{1cm} (2.2)

where \( I_{cav} \) is the current due to sodium channels in the open caveolae, \( n \) is the number of open caveolae, and \( \gamma_{Na} = 18 \) pS is the single sodium channel conductance.

Noting that the caveolar current will increase the sodium current by a certain percentage, in this research we incorporate the caveolar current into our model by simply multiplying the sodium current by \( C_{cav} \geq 0 \). \( C_{cav} \) represents the percent increase in sodium current attributed to open caveolae. A value of \( C_{cav} = 0 \) represents a membrane patch with no open caveolae and will be the baseline for our simulations. For example, \( C_{cav} = 0.3 \) represents a membrane patch with the appropriate number of open caveolae to cause a 30% increase in sodium current. We simply add \( I_{cav} = C_{cav} I_{Na} \) to the transmembrane currents.

The simulations will be ran under voltage clamp conditions with a stimulus current applied at \( t = 25 \) ms for a duration of 0.5 ms. All currents are calculated for a 1-cm\(^2\) patch of membrane with an initial capacitance of 1 \( \mu \)F/cm\(^2\). We solve our system of differential equations using a Fourth Order Runge-Kutta numerical method. We will then observe the action potential morphologies for several different values of \( C_{cav} \) and compare our results to those found by Besse.

2.1.4 Results

Simulations of guinea pig action potentials with the caveolae-inclusive model show that additional caveolar sodium current leads to increases in peak voltage overshoot and maximum upstroke velocity. This is consistent with the results Besse observed. However, the maximum upstroke velocity and peak voltage overshoot appear to increase in a nonlinear fashion with respect to the percent increase in sodium current.
Figure 2.1: Action potential morphology using the Luo-Rudy 1 model with several different values for $C_{cav}$. Capacitance was allowed to vary.

Figure 2.2: The blue plot shows the relationship between the percent increase in peak voltage overshoot versus percent increase in sodium current due to open caveolae. The red plot shows the relationship between the percent increase in maximum upstroke velocity versus the percent increase in sodium current due to open caveolae. Both were fit with a polynomial function.
due to open caveolae (see Figure 2.2).

Figure 2.1 shows the CAP time course of simulated cardiomyocytes with enough open caveolae to cause a 20, 40, 60, 80, and 100 percent increase in sodium current (or equivalently $C_{\text{cav}} = 0.2, 0.4, 0.6, 0.8,$ and 1.0). One obvious difference between the simulated guinea-pig action potential and the simulated rat action potential observed by Besse is the action potential duration (APD). The rat APD is approximately 125 ms compared to the guinea pig APD of approximately 425 ms. But, the action potentials shown in Figure 2.1 appear to have nearly identical action potential durations. However, we are concerned with other features of the CAP such as peak voltage overshoot and maximum upstroke velocity.

As seen in Figure 2.1, the number of open caveolae clearly have an effect on the peak voltage overshoot of the CAP. Besse observed that a 42% increase in sodium current (25000 open caveolae/cell) results in a 5.2% increase in peak voltage overshoot. Our simulations reveal a 5.1% increase in peak voltage overshoot for a sodium current increase of 42% and a constant capacitance—in strong agreement with Besse’s results (see Figure 2.2). With varying capacitance and a 42% increase in sodium current the peak voltage overshoot increased 4.1%.

Less evident from the graph in Figure 2.1 is a 26% increase in the maximum upstroke velocity for a 42% increase in sodium current and constant capacitance (see Figure 2.2). Comparing these values to those obtained by Besse—42% increase in sodium current resulting in a 29% increase in maximum upstroke velocity—we see that these results are also in good agreement. With varying capacitance and a 42% increase in sodium current the maximum upstroke velocity increased 19.1%.

2.2 The Stochastic Caveolae-Inclusive Model

2.2.1 Introduction

With the aid of the caveolae-inclusive model, we have seen that caveolae play crucial roles in cardiac electrodynamics. Strong evidence suggests that caveolin-associated pathologies may contribute to cardiac arrhythmogenesis and other cardiac pathological conditions such as Long-QT Syndrome (LQTS)[13]. One possible explanation for these caveolin-linked cardiac pathologies was explored by Besse and related to the opening dynamics of caveolae. More specifically, Besse looked at stochastic opening of caveolae and how this affects CAP morphology.

Consider a single stochastically opening caveola and the sodium channel it sequesters. If the caveola is open when a depolarizing stimulus arrives, the sodium channel acts as a normal channel on the sarcolemma. If the caveola is closed when a stimulus arrives, the sequestered sodium channel is still isolated from the extracellular environment and thus is in its closed state. However, if this same caveola opens and the
cardiomyocyte membrane is still sufficiently depolarized, the sodium channel within the caveola may open, conduct, and inactivate in the middle of the CAP. In each case described above, if the caveolar membrane is still depolarized and the caveola closes, the sodium channel is still inactive. Thus any subsequent openings of this particular caveola will not effect the CAP and we must only consider the first post-stimulus opening of the stochastic caveolae.

We seek a model that keeps track of each separate grouping of caveolae that open at a specific time during the CAP and their associated sodium currents. Thus, a more complex model than the caveolae-inclusive model that represents the gating variables as functions of both time since stimulus and time since caveolar opening is required for stochastic caveolae.

### 2.2.2 Assumptions of the Model

We will assume that caveolar opening occurs as a Poisson process having rate $\lambda$. In the context of our model, a large $\lambda$ means most caveolae open for the first time within a short time of the stimulus current and a small $\lambda$ means most caveolae have not opened late in the CAP. Let $N(t)$ represent the number of times a caveola experiences an opening event in a time interval of length $t$. The probability that the event happens $k$ times ($N(t) = k$) is given by the following well known formula.

$$P(N(t) = k) = e^{-\lambda t} \frac{(\lambda t)^k}{k!}$$  \hspace{1cm} (2.3)

Let $X$ denote the time since the stimulus current was applied until the caveola first opens. Notice that

$$P(X > t) = P(N(t) = 0) = e^{-\lambda t}$$  \hspace{1cm} (2.4)

and thus the cumulative distribution function for $X$ is given by the following formula.

$$P(X \leq t) = P(N(t) \geq 0) = 1 - e^{-\lambda t}$$  \hspace{1cm} (2.5)

Differentiating Equation (2.5) yields the probability density function of caveolar first opening.

$$\rho(t) = \lambda e^{-\lambda t}$$  \hspace{1cm} (2.6)

If the number of caveolae $n$ is large, then

$$n \rho(t) \Delta t$$  \hspace{1cm} (2.7)
provides a good approximation of caveolae that first open in a small time interval $\Delta t$.

Because $\lambda$ gives no information about how fast caveolae close, we must also assume that caveolae close slower than the time it takes for a sodium channel to open, conduct, and inactivate. This means that the opening behavior of caveolae after the first post-stimulus opening is of no consequence to a single CAP.

### 2.2.3 Formulation of the Stochastic Caveolae-Inclusive Model

In order to keep track of the time courses of separate caveolae, the gating variables of sequestered sodium channels will be dependent on the time since a stimulus current was applied, $t$, and the time of the caveolar first opening, $\tau$. Thus, the sodium current due to caveolae that have opened at time $\tau$ is given by

$$n\rho(\tau) \Delta \tau \gamma_{Na} m^3(t, \tau) h(t, \tau) j(t, \tau) (V_m(t) - E_{Na})$$

where the gating variables $z$ satisfy the following boundary conditions.

$$\begin{cases}
\frac{\partial z}{\partial t} = \frac{z_\infty (V_m(t)) - z}{\tau_z (V_m(t))} : 0 \leq \tau < t \\
z = z_\infty (V_m(0)) : t = \tau
\end{cases}$$

The total caveolar sodium current is the sum of the sodium currents due to caveolae that have opened since the time of the stimulus. Because our probability density function is continuous, the total caveolar current can be represented by the following integral.

$$I_{cav}(t) = \left(\int_0^t n\gamma_{Na} \lambda e^{-\lambda \tau} m^3(t, \tau) h(t, \tau) j(t, \tau) \, d\tau\right) (V_m(t) - E_{Na})$$

The simulations will be ran under voltage clamp conditions with a stimulus current applied at $t = 25$ ms for a duration of 0.5 ms. All currents are calculated for a 1-cm$^2$ patch of membrane with a capacitance of 1 $\mu$F/cm$^2$. We solve our system of differential equations using a Fourth Order Runga-Kutta numerical method. We will then observe the action potential morphologies for several different values of $\lambda$ and $n$ and compare our results to those found by Besse.

### 2.2.4 Results

The effect of stochastically opening caveolae on CAP morphology is highly dependent on our choice of $\lambda$ and $n$ values. For representative time courses see Figure 2.3. Simulations of guinea pig action potentials with the stochastic caveolae-inclusive model show that additional caveolar sodium current can lead to an increase
in action potential duration (APD), which is equivalent to a delay in ventricular repolarization. For some combinations of $\lambda$ and $n$, the inward calcium current may be reactivated leading to early afterdepolarizations. Both of these features are associated with Long-QT Syndrome.

Interestingly, some choices for $\lambda$ and $n$ cause a complete lack of ventricular repolarization. Instead, the cardiomyocyte membrane seems to settle into a new equilibrium potential different from the normal resting membrane potential. The new equilibrium value appears to be near the plateau potential.

In general, for each $n$, the increase in APD is relatively small for large $\lambda$ and very small $\lambda$ values whereas for intermediate $\lambda$ values we observe a substantial increase in APD. Recall that $\lambda$ represents the rate at which the stochastic caveolae open. A small $\lambda$ indicates that few caveolae open during the CAP. As a result, there is very little additional sodium current. Conversely, a large $\lambda$ value indicates that most caveolae open for the first time within a short time of the stimulus current. As a result, nearly all the caveolae open early in the CAP leaving very few caveolae to open for the first time late in the CAP. Both of these cases result in a relatively small increase in APD—these observations are consistent with the results of our simulations.

We have chosen to display representative time courses and APD dependence on $\lambda$ for three values of $n$ in Figure 2.3. Notice that for each $n$, significant increases in APD occur at intermediate $\lambda$ values. Part A of Figure 2.3 represents a patch of membrane with $1.0 \times 10^9$ stochastic caveolae. Notice that for each representative CAP, the membrane potential monotonically decreases after the peak voltage overshoot regardless of our choice for $\lambda$. Part B of Figure 2.3 represents a patch of membrane with $2.0 \times 10^9$ stochastic caveolae. The choice of $\lambda = 0.1$ results in a secondary spike in membrane potential known as an early afterdepolarization (EAD)—this feature elongates the CAP resulting in a delay in ventricular repolarization. Part C of Figure 2.3 represents a patch of membrane with $3.0 \times 10^9$ stochastic caveolae. We not only observe elongation of the CAP and a single EAD for $\lambda = 0.5$, but we also stumbled upon an additional feature. For $\lambda = 0.6$, we observe a complete disappearance of ventricular repolarization due to what appears to be several EADs occurring right after one another. As a result, the system appears to tend toward a new equilibrium value at about the plateau potential. Whether or not this is a true physiological feature of cardiomyocytes still remains a topic for future investigation[1]. These results and observations are similar to those observed by Besse using the Pandit et al. model.
Figure 2.3: Representative CAP time courses displaying the effects of stochastically opening caveolae on CAP morphology and early afterdepolarization formation. The break in the APD dependence on $\lambda$ graph for $3.0 \times 10^9$ stochastic caveolae is due to an absence of ventricular repolarization before 1200 msec.
2.3 The Caveolae-Inclusive Propagation Model

2.3.1 Introduction

The caveolae-inclusive model for a single cardiac action potential demonstrated that increasing the total number of open caveolae on the membrane lead to an increase in maximum upstroke velocity. Recall that changes in maximum upstroke velocity are known to cause significant changes in the conduction velocity of a propagating signal in cardiac tissue[1]. We now propagate a single CAP (modeled by the Pandit et al. rat left ventricular model) and observe the effects that caveolar sodium current have on the conduction velocity of the propagating signal.

2.3.2 Assumptions of the Model

For this model, we will again assume that there are two possible orientations of caveolae. When no β-agonist is present, the caveolae are closed and are separated and isolated from the extracellular environment and this situation is modeled with a normal Pandit et al. CAP model. When a β-agonist is present, the caveolae are open and the caveolar membrane may interact with the extracellular space. Extra sodium channels are recruited that may contribute to the sodium current important for the initial upstroke of the CAP.

Additionally, we assume that the extracellular space is extensive meaning that the resistance per unit length of the external medium for our line of cardiomyocytes is negligible and may be considered zero.

2.3.3 Formulation of the Caveolae-Inclusive Propagation Model

In order to model a propagating CAP, we must consider how both the transmembrane current and the axial current in our line of cardiomyocytes affects the membrane potential (see Figure 2.4).

The membrane potential is now not only a function of time but also of position on the muscle fiber. Assuming $R_e$ is negligible, our membrane potential satisfies the partial differential equation given in Equation (1.20). We let $k = \frac{\mu}{2\pi}$ and thus our membrane potential satisfies the following partial differential equation.

$$C_m \left( \frac{\partial V_m}{\partial t} \right) + \sum I_S = k \left( \frac{\partial^2 V_m}{\partial x^2} \right) \tag{2.11}$$

The caveolar current for each cell in the muscle fiber is similar to what we have seen previously

$$I_{cav} = n\gamma_{Na} m^{3} h_j (V_m - E_{Na}) \tag{2.12}$$

where $n$ is the total number of open caveolae on each cell and $\gamma_{Na}$ is the single sodium channel conductance.
Our simulations will be ran with a stimulus current being applied at $t = 0$ ms from $x = 0$ cm to $x = 0.1$ cm. We use $k = 10^{-4}$ because the concern of this research is not to collect physiological accurate quantities but is to demonstrate visually how caveolae affect the conduction velocity in cardiac tissue. We solve our system of differential equations using the built in MATLAB partial differential equation solver pdepe. We now observe the propagated CAP morphologies and conduction velocities for several different values of $n$.

### 2.3.4 Results

Simulations of a propagated rat CAP in a one-dimensional muscle fiber show that additional caveolar sodium current leads to an increase in conduction velocity of the propagating signal. The conduction velocity appears to increase in a slightly nonlinear fashion with respect to the total number of open caveolae (see Figure 2.6).

Figure 2.5 shows the wavefront profiles (best fit lines of the peak voltages of each propagated CAP) of a propagated CAP in six different muscle fibers—each individual cell in each fiber contains a specific number of open caveolae. Using $n = 0$ as our baseline, the percent increases in conduction velocity are shown in the table below.

<table>
<thead>
<tr>
<th>$n$</th>
<th>Percent Increase in Conduction Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10000</td>
<td>5.18</td>
</tr>
<tr>
<td>20000</td>
<td>9.83</td>
</tr>
<tr>
<td>30000</td>
<td>14.15</td>
</tr>
<tr>
<td>40000</td>
<td>17.88</td>
</tr>
<tr>
<td>50000</td>
<td>21.44</td>
</tr>
</tbody>
</table>
Figure 2.5: Wavefront profiles of propagated cardiac action potentials. The peak voltage overshoot values for each position on the muscle fiber were fit with a best fit regression line. The slope of each line is the conduction velocity for that particular muscle fiber.

Figure 2.6: The conduction velocity for a propagating CAP has a slightly nonlinear dependence on the number of open caveolae on each cell. Clearly increasing the number of open caveolae increases the conduction velocity of the excitatory wave in a given muscle fiber.
Again, we note that these may not be physiologically accurate values. The purpose of this work is to demonstrate that additional caveolar sodium current leads to an increase in conduction velocity. We observed similar results using a more physiological accurate value for our diffusion coefficient $k$. However, using a larger $k$ allowed us to better visually represent our observations.
3 Conclusions and Future Considerations

3.1 Summary of Results

3.1.1 Single Cardiac Action Potential Models

Perfusing a cardiomyocyte with a $\beta$-adrenergic agonist opens caveolae recruiting additional sodium channels whose additional current can increase MUV and PV in a nonlinear fashion. Effects of stochastic caveolae on CAP morphology are highly dependent on the rate at which the caveolae open and the number of stochastic caveolae present on the cardiomyocyte membrane. We observed features such as increases in APD (delay in ventricular repolarization), early afterdepolarizations, and a complete disappearance of ventricular repolarization. These features are consistent with a serious heart condition called Long-QT Syndrome.

3.1.2 Propagating Cardiac Action Potential Model

Perfusing a muscle fiber with a $\beta$-adrenergic agonist opens a certain number of caveolae on each individual cell. The recruitment of the additional caveolar sodium channels increase the total transmembrane sodium current and thus the maximum upstroke velocity of a single CAP (as demonstrated in the caveolae-inclusive model). The increase in maximum upstroke velocity appears to affect the conduction velocity of a propagating CAP in a muscle fiber. Using the caveolae-inclusive propagation model, we observed an overall increase in conduction velocity when caveolae open.

3.2 Future Considerations

3.2.1 My Future Work

The caveolae-inclusive propagation model only models one specific opening mechanism of caveolae. My advisor and I are in the process of developing a propagation model that incorporates stochastically opening of caveolae. We expect that the features observed in the stochastic caveolae-inclusive model would be propagated to neighboring cells in a single muscle fiber.

3.2.2 Extension of the Models

There are few ideas to consider that are beyond the scope of this research. The first future consideration would be to incorporate other known channels present in caveolae (such as calcium channels) that could effect a CAP[14]. A second consideration would be to extend the caveolar propagation models into two- and three-dimensions. This would model the propagation of a CAP in a sheet of cells and a thick sheet of cells—three-dimensional propagation is important physiologically because the heart is a thick muscular
organ. Lastly, an interesting topic for further research would be to explore the anomaly we observed with the stochastic caveolae-inclusive model in which the membrane potential equilibrates at a value different than the resting membrane potential. We would suspect that this is strictly a feature of our system of differential equations and something that is not seen biologically. This would suggest that an individual could survive without ventricular repolarization.
References


