Theoretical Modeling of Triggered Activity in Cardiac Arrythmias

by Mingwang Zhong

B.S. in Physics, Jilin University

A dissertation submitted to

The Faculty of
the College of Science of
Northeastern University
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

December 6, 2019

Dissertation directed by

Alain Karma
Arts and Sciences Distinguished Professor
I would like to express my sincere gratitude to my advisor, Professor Alain Karma, for his continuous support along this journey of the doctoral program. He spent much time working with me through the details when I ran into difficulties and guiding me to break the problems into small parts that can be solved more easily. I am also inspired by his passion for scientific research. Although he is busy, he still spared a lot of time discussing with other graduate students and me about the research, which he enjoyed a lot. In addition, I would like to thank him for his persistence, without which my thesis and papers cannot be improved significantly. Besides my advisor, I also would like to thank the committee members: Dr. Bum-Rak Choi, Dr. Mark Williams, and Dr. Dapeng Bi for spending time on reviewing my thesis.

I also would like to thank the National Institute of Health and Northeastern University for providing funding over these years. The past years to pursue the doctoral degree would be a memorable time in my life, and it would not be possible without their funding.

My sincere thanks also go to my collaborators, Dr. Bum-Rak Choi, Dr. Anatoli Kabakov, Dr. Dmitry Terentyev, Dr. Tae Yun Kim, Dr. Peter Bronk, and Dr. Gideon Koren at Brown University. Their experimental work has profoundly improved the thesis. The numerous discussions with them were helpful, which deepened my understanding of cardiology. In particular, I thank Dr. Bum-Rak Choi for providing useful suggestions to perform research and to revise my thesis.

It was a pleasant time working with the members of Professor Karma’s group. I thank Dr. Colin M. Rees and Dr. Yechuan Xu for teaching me the basic knowledge of cardiac electrophysiology and computational techniques. Dr. Colin M. Rees spent plenty of time talking with me about my research and giving helpful suggestions. I also would like to thank Longhai Lai, who shared invaluable knowledge of computational skills and other research areas. Finally, I thank Saroj Dhakal for beneficial talks with him about ion channel modeling and for proofreading my thesis.
Abstract of Dissertation

Cardiovascular disease is a leading cause of death in the world and accounts for annual deaths over 800,000 in the United States. Triggered activity, describing abnormal depolarizations interrupting normal action potentials or diastole, is a mechanism underlying cardiac arrhythmias. Underlying triggered activity is the early afterdepolarization (EAD) during an action potential and the delayed afterdepolarization (DAD) during diastole. This thesis aims to investigate: 1) how Ca\(^{2+}\) waves present in many heart diseases, such as heart failure, emerge from Ca\(^{2+}\) sparks, 2) why EADs occur in the long QT syndrome types 1 and 2 (LQT1 and LQT2), 3) how antiarrhythmic targets, including the small-conductance Ca\(^{2+}\)-activated K\(^{+}\) (SK) current and the late sodium current, prevent EADs, and 4) how cellular triggered activity evolves into cardiac arrhythmias at the tissue level. I primarily perform the studies by using an improved physiologically multi-scale model, which incorporates spatially distributed Ca\(^{2+}\) release units and the bi-directional interplay between Ca\(^{2+}\) and membrane voltage. In addition, the studies of LQT syndromes are carried out using experimentally measured ion currents as input into the model, which turns out to be crucial to uncover the mechanisms underlying arrhythmias. Investigation of DADs reveals that strong coupling between ryanodine receptors (RyR) is critical to induce Ca\(^{2+}\) waves, and the low incidence of DADs in the healthy heart with strong RyR coupling is because of the low Ca\(^{2+}\) sensitivity of RyR. Moreover, EADs in the transgenic LQT2 rabbit model are caused by enhanced Ca\(^{2+}\) sensitivity of RyR, reduced Ca\(^{2+}\) spark refractoriness, and nonlinearity of Na\(^{+}\)/Ca\(^{2+}\) exchanger as a function of Ca\(^{2+}\) concentration. Blocking of late sodium current in LQT2 has been shown to prevent EADs by removing a depolarizing current and reducing intracellular Na\(^{+}\) load. Moreover, we propose a four-state model of the SK channel, which efficiently eliminates EADs. For the transgenic LQT1 rabbits, we use an improved whole-cell model to study EAD formation. Heterogeneity of transient outward K\(^{+}\) current is found to be essential to explain EAD initiation in right ventricles while no EADs but prolonged action potential in left ventricles. This heterogeneity is also critical to initiating premature ventricular contractions in a one-dimensional cable consisting of LQT1 myocytes. These studies advance the current understanding of triggered activity initiation and suggest that the SK current and the late sodium current could be therapeutic targets to prevent cardiac arrhythmias.
Key words: Modeling • Cardiac arrhythmia • Triggered activity • Early afterdepolarization • Delayed afterdepolarization • Long QT syndrome • Ca$^{2+}$ wave • Ryanodine receptor • Antiarrhythmic targets • SK channel
Table of Contents

Acknowledgements 2
Abstract of Dissertation 3
Table of Contents 5
List of Abbreviations 10
List of Figures 12
List of Tables 15
Chapter 1: Introduction 16
  1.1 Cardiac electrophysiology 17
    1.1.1 Cardiac excitation-contraction coupling 17
    1.1.2 Cardiac arrhythmias and long QT syndromes 21
    1.1.3 Rabbit model of heart disease 23
  1.2 Triggered activity as a mechanism of cardiac arrhythmias 24
    1.2.1 Early afterdepolarizations 26
    1.2.2 Delayed afterdepolarizations 27
  1.3 Mathematical modeling of cardiac myocytes 28
  1.4 Open questions related to triggered activity 32
  1.5 Thesis outline 36
Chapter 2: Ca$^{2+}$ waves and delayed afterdepolarizations 40
  2.1 Mathematical modeling 42
  2.2 Ca$^{2+}$ waves in the permeabilized cells 44
    2.2.1 Ca$^{2+}$ sparks 44
    2.2.2 Ca$^{2+}$ wave formation 47
    2.2.3 The role of RyR cooperativity 50
    2.2.4 The effect of molecular crowding 52
  2.3 Delayed afterdepolarizations in the intact cells 55
    2.3.1 Addition of caffeine and ISO induce delayed afterdepolarizations 55
2.3.2 Delayed afterdepolarizations occur at an intermediate strength of RyR cooperativity

2.4 Discussion

2.5 Supporting Information

Chapter 3: Early afterdepolarizations in long QT syndromes

3.1 Na\(^+\)/Ca\(^{2+}\) exchanger mediated EADs in long QT syndrome type 2

3.1.1 Methods

3.1.2 Role of RyR refractoriness and open probability on AP phenotype

3.1.3 Mechanism of increased whole-cell forward mode NCX current without increasing whole-cell cytosolic calcium concentration

3.1.4 Suppression of EAD formation via reduction of NCX current

3.1.5 EAD mechanism in LMC myocytes

3.1.6 The effect of pacing frequency

3.1.7 Discussion

3.2 Transient outward K\(^+\) current underlies EAD formation in long QT syndrome type 1

3.2.1 \(I_{to}\) is larger in RV cardiomyocytes

3.2.2 Fitting of \(I_{Kr}\) using experimental data

3.2.3 \(I_{to}\) facilitates EAD Formation in RV cardiomyocytes

3.2.4 Role of \(I_{Kr}\) in EAD formation

3.2.5 Contribution of \(I_{to}\) inactivation to EAD onset

3.2.4 Ultra-long APDs in LV myocytes

3.2.5 Discussion

Chapter 4: Antiarrhythmic targets in long QT syndromes

4.1 Small-conductance Ca\(^{2+}\)-activated K\(^+\) channel

4.1.1 Methods

4.1.2 Steady-state SK currents
4.1.3 Regulation of extracellular $K^+$ on the binding affinity of $Mg^{2+}$

4.1.4 Time-dependent SK currents in voltage-clamp mode

4.1.5 SK channel as an antiarrhythmic target

4.1.6 Discussion

4.2 Late sodium channel blocker GS967 suppresses PVT in a transgenic rabbit model of long QT syndrome type 2

4.2.1 Larger $I_{NaL}$ in long QT syndrome type 2 modulates APDs via altering $[Na]_i$

4.2.2 $I_{NaL}$ blockade prevents EADs through modulating $[Na]_i$ and $I_{NCX}$

4.2.3 Discussion

Chapter 5: Bridging the gap between the cell and the tissue: how do EADs propagate?

5.1 Initiation of premature ventricular complexes in a cable of LQT1 myocytes

5.2 Electronic current flow through cell-cell coupling regulates the APD prolongation of PVCs during propagation

5.3 PVCs have longer APDs than normal action potentials because of reduced $I_{to,s}$

5.4 Discussion

Chapter 6: Conclusion

Appendix A: Multi-scale model for rabbit ventricular myocytes

A.1 Cell architecture

A.2 $Ca^{2+}$ buffering

A.3 RyR model

A.4 Modification of luminal gating

A.5 $Na^+$ dynamics

A.6 Ionic currents

A.7 SERCA uptake current

A.8 SR leak current

A.9 The $Na^+ / Ca^{2+}$ exchanger current ($I_{NCX}$)
A.10 L-type Ca\(^{2+}\) channel (\(I_{\text{Ca,L}}\)) 160
A.11 RyR-mediated SR Ca\(^{2+}\) release 162
A.12 The fast sodium current (\(I_{\text{Na}}\)) 163
A.13 Inward rectifier K\(^{+}\) current (\(I_{\text{K1}}\)) 164
A.14 The rapidly activating delayed rectifier K\(^{+}\) current (\(I_{\text{Kr}}\)) 164
A.15 The slowly activating delayed rectifier K\(^{+}\) current (\(I_{\text{Ks}}\)) 164
A.16 The fast component of the transient outward K\(^{+}\) current (\(I_{\text{to,f}}\)) 166
A.17 The slow component of the transient outward K\(^{+}\) current (\(I_{\text{to,s}}\)) 167
A.18 The Na\(^{+}\)-K\(^{+}\) pump current (\(I_{\text{NaK}}\)) 167

Appendix B: Multi-scale model for Ca\(^{2+}\) waves 168
B.1 RyR model 168
B.2 Mobile buffers (flu-3, EGTA, ATP) 170
B.3 Diffusion time of Ca\(^{2+}\) and mobile buffers 171
B.4 Uncoupled sub-membrane spaces in the longitudinal direction 171
B.5 Optical blurring of sparks by the confocal microscope 171
B.6 Ca\(^{2+}\) spark detection 172
B.7 SR Ca\(^{2+}\) pump (uptake) 172
B.8 High-resolution Ca\(^{2+}\) spark model 173

Appendix C: Multi-scale model and experimental conditions for LQT2 myocytes 176
C.1 Experiments 176
   C.1.1 Myocyte isolation 176
   C.1.2 Cell electrophysiology and Ca\(^{2+}\) imaging 177
C.2 Mathematical modeling 177

Appendix D: Multi-scale model to study the late sodium currents in LQT2 myocytes 178
D.1 Cytosolic volume 178
D.2 RyR gating 179
D.3 Allosteric Ca\(^{2+}\) activation of NCX 179
D.4 Voltage-dependent inactivation time scale $\tau_f$ of $I_{Ca,L}$ 179

Appendix E: Whole-cell model for LQT1 myocytes 181

E.1 Model of $I_o$ 182
E.2 Model of $I_{Kr}$ 185
E.3 Model of $I_{Ca,L}$ 187
E.4 Calcium cycling 189
E.5 Modification of $I_{Ks}$ 190
E.6 Modification of $I_{Na}$ 191

References 192
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC</td>
<td>Littermate control</td>
</tr>
<tr>
<td>LQT1</td>
<td>Long-QT type 1</td>
</tr>
<tr>
<td>LQT2</td>
<td>Long-QT type 2</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>ISO</td>
<td>Isoproterenol</td>
</tr>
<tr>
<td>LCC</td>
<td>Long-type Ca$^{2+}$ channel</td>
</tr>
<tr>
<td>RyR</td>
<td>Ryanodine receptor</td>
</tr>
<tr>
<td>EAD</td>
<td>Early afterdepolarization</td>
</tr>
<tr>
<td>DAD</td>
<td>Delayed afterdepolarization</td>
</tr>
<tr>
<td>CSQN</td>
<td>Calsequestrin</td>
</tr>
<tr>
<td>JSR</td>
<td>Junctional sarcoplasmic reticulum</td>
</tr>
<tr>
<td>NSR</td>
<td>Network sarcoplasmic reticulum</td>
</tr>
<tr>
<td>CRU</td>
<td>Ca$^{2+}$ release unit</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>FDHM</td>
<td>Full duration at half maximum</td>
</tr>
<tr>
<td>$F$</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>$N_{CU}$</td>
<td>Number of RyRs in the CSQN unbound state</td>
</tr>
<tr>
<td>$N_{CB}$</td>
<td>Number of RyRs in the CSQN bound state</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

$I_{rel}$ Releasing current via CRU
$I_{Ca,L}$ Current of LCC
$I_{Kr}$ Current of the rapid component of the delayed rectifier $K^+$ channel
$I_{Ks}$ Current of the slow component of the delayed rectifier $K^+$ channel
$I_{NCX}$ Current of $Na^+/Ca^{2+}$ exchanger
$I_{Na}$ Current of the fast sodium channel
$I_{NaL}$ Current of the late sodium channel
$I_{SK}$ Current of the small-conductance $Ca^{2+}$-activated $K^+$ channel
NCX $Na^+/Ca^{2+}$ exchanger
AP Action potential
APD Action potential duration
PVT Polymorphic ventricular tachycardia
VF Ventricular fibrillation
PVC Premature ventricular complex
SCD Sudden cardiac death
SK channel Small conductance $Ca^{2+}$-activated $K^+$ channel
NaL channel Late sodium channel
K$_{ir}$ channel Inward rectifier $K^+$ channel
CaM Calmodulin
CaMBD Calmodulin binding domain
$[Ca^{2+}]_i$ (or $c_i$) $Ca^{2+}$ concentration in the cytosolic space
$[Ca^{2+}]_{JSR}$ (or $c_j$) $Ca^{2+}$ concentration in the JSR space
$[Ca^{2+}]_{NSR}$ (or $c_n$) $Ca^{2+}$ concentration in the NSR space
$[Ca^{2+}]_s$ (or $c_s$) $Ca^{2+}$ concentration in the sub-membrane space
$[Ca^{2+}]_p$ (or $c_p$) $Ca^{2+}$ concentration in the dyadic space
$V_m$ (or $V$) Membrane voltage
# List of Figures

1.1 Cardiac conduction system and action potentials ........................................ 17
1.2 Subcellular structure and ion transportations in the cardiac myocytes ........... 19
1.3 Long QT syndromes and cardiac arrhythmias ............................................. 21
1.4 Ca\(^{2+}\) fluxes and action potentials in rabbit and rat ventricular myocytes .......... 24
1.5 Afterdepolarizations and reentry ................................................................. 25
1.6 DADs generated by spontaneous diastolic Ca\(^{2+}\) waves, which results from the regenerative initiation of Ca\(^{2+}\) sparks ................................................................. 28
1.7 The Hodgkin-Huxley model ........................................................................ 29
1.8 Simplified diagram of subcellular spaces ..................................................... 31
1.9 Proposed mechanisms of PVT initiation in long QT syndromes ....................... 36

2.1 RyR gating model and transition rates .......................................................... 43
2.2 Ca\(^{2+}\) sparks in the permeabilized cell ......................................................... 45
2.3 Effect of ATP on Ca\(^{2+}\) sparks in the permeabilized cells at 0.1 \(\mu M\) (top) and 0.2 \(\mu M\) (bottom) .............................................................. 46
2.4 Transition from Ca\(^{2+}\) sparks to Ca\(^{2+}\) waves in the absence of ATP ............. 47
2.5 Ca\(^{2+}\) wave formation in the permeabilized cell .......................................... 48
2.6 Transition from spontaneous Ca\(^{2+}\) sparks to propagating waves ................... 49
2.7 Effect of RyR cooperativity \((H)\) on Ca\(^{2+}\) wave formation .............................. 51
2.8 Statistics of \(N_{CU}\) and \(N_{CB}\) for different Hill coefficients and Ca\(^{2+}\) loads ....... 53
2.9 Effect of molecular crowding ..................................................................... 54
2.10 Longitudinal line-scan images of Ca\(^{2+}\) concentration in SR \(([Ca^{2+}]_{JSR})\) ....... 55
2.11 Effect of buffers’ binding rate .................................................................... 56
LIST OF FIGURES

2.12 Combination of caffeine and ISO initiates DADs in LMC myocytes ............ 57
2.13 Effect of SR load and Hill coefficient on DAD formation ....................... 58
2.14 Effect of transition time $\tau_u$ ................................................. 59
2.15 Dependence of afterdepolarization initiation on RyR activity ................. 60

3.1 Schematic illustration of the 4-state model of RyR gating ......................... 73
3.2 Effect of altering RyR gating parameters ........................................ 74
3.3 Effect of RyR parameters on EAD initiation ..................................... 77
3.4 Effect of the sudden increase of RyR Ca$^{2+}$ sensitivity .......................... 80
3.5 Effect of RyR gating parameters on the number of EADs, $c_j$, and $c_i$ .......... 82
3.6 Role of nonlinear $I_{NCX}$ on EAD formation .................................... 83
3.7 Effect of reducing NCX conductance .............................................. 84
3.8 Contribution of $V_m$ and $Ca^{2+}$ to $I_{NCX}$ that initiates EADs ............... 85
3.9 Effect of LCC and NCX conductance on the number of EADs .................. 86
3.10 Elimination of EADs by NCX blocker SEA0400 in experiments ............... 87
3.11 Experimental recordings of $V_m$ showing the effect of SEA0400 ............ 88
3.12 Effect of RyR activity for LMC myocytes ..................................... 89
3.13 Effect of stimulation frequencies on EAD initiation ............................... 90
3.14 Comparison of amplitude and inactivation of $I_{to}$ between RV and LV ........ 95
3.15 State diagram of MGWMN model for $I_{Kr}$ .................................... 96
3.16 Comparison of different models of $I_{Kr}$ ...................................... 97
3.17 EADs regulated by $I_{to}$ in LQT1 myocytes under ISO stimulation ........... 98
3.18 LQT1 myocytes in RV and LV using an alternative HH model of $I_{Kr}$ ....... 100
3.19 The contribution of $g_{to,si}$ and $\tau_{si}$ to EAD onset ........................... 101
3.20 Variability of APD in LV obtained by changing $g_{Kr}$, $g_{Ca,L}$, and $g_{to,si}$ .... 102

4.1 Schematic diagram for the model of the SK channel ............................. 107
4.2 Fits of the SK channel currents ................................................... 111
4.3 Analysis of the four-state model .................................................. 113
4.4 A five-state model to include regulation of extracellular K$^+$ ................. 116
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>Ca$^{2+}$-dependent activation of SK channels.</td>
<td>117</td>
</tr>
<tr>
<td>4.6</td>
<td>Blockade only by Mg$^{2+}$ under the voltage-clamp mode</td>
<td>118</td>
</tr>
<tr>
<td>4.7</td>
<td>Blockade only by Ca$^{2+}$ under the voltage-clamp mode</td>
<td>119</td>
</tr>
<tr>
<td>4.8</td>
<td>Effects of various drugs of the SK channel on voltage dynamics under application of ISO</td>
<td>120</td>
</tr>
<tr>
<td>4.9</td>
<td>Reducing the blocking effect enhances $I_{SK}$ peak during the depolarization phase</td>
<td>122</td>
</tr>
<tr>
<td>4.10</td>
<td>Effect of $I_{NaL}$ on APDs</td>
<td>130</td>
</tr>
<tr>
<td>4.11</td>
<td>$I_{NaL}$ effect on APDs in LMC myocytes</td>
<td>131</td>
</tr>
<tr>
<td>4.12</td>
<td>$I_{NaL}$-dependent EADs in LQT2 myocytes under 50 nM ISO</td>
<td>132</td>
</tr>
<tr>
<td>5.1</td>
<td>PVC initiation in a cable of LQT1 myocytes by increasing $I_{Ca,L}$</td>
<td>137</td>
</tr>
<tr>
<td>5.2</td>
<td>Effect of enhanced $g_{Ca,L}$ in PVC initiation</td>
<td>139</td>
</tr>
<tr>
<td>5.3</td>
<td>Normal $g_{Ca,L}$ with $I_{Na}$ activation shifted by -10 mV results in PVCs</td>
<td>140</td>
</tr>
<tr>
<td>5.4</td>
<td>Voltage diffusion is critical to prolonging APD of PVC</td>
<td>141</td>
</tr>
<tr>
<td>5.5</td>
<td>Unrecovered $I_{\alpha,i}$ is responsible for APD prolongation in PVCs</td>
<td>143</td>
</tr>
<tr>
<td>A.1</td>
<td>Multi-scale model of ventricular myocytes</td>
<td>149</td>
</tr>
<tr>
<td>A.2</td>
<td>Effect of altering the interaction between CSQN and Ca$^{2+}$</td>
<td>156</td>
</tr>
<tr>
<td>A.3</td>
<td>Schematic diagram of the 16-state LTCC model</td>
<td>161</td>
</tr>
<tr>
<td>D.1</td>
<td>Effect of GS967 on steady-state Na$_i$</td>
<td>180</td>
</tr>
<tr>
<td>E.1</td>
<td>HH model of $I_{Ca,L}$ fits experimental recordings under 50 nM ISO</td>
<td>187</td>
</tr>
</tbody>
</table>
List of Tables

3.1 Instantaneous values at the whole-cell level. .............................. 78

4.1 Parameters of the SK channel models ......................................... 112

A.1 General parameters of the cell .................................................. 153
A.2 Parameters of buffers ............................................................... 154
A.3 Parameters for $I_{up}, I_{leak}, I_{NCX}$, RyR, and $I_{Ca.L}$ ..................... 159

B.1 Parameter changes under the effect of ISO ................................. 169
B.2 Parameters of RyR gating under different conditions ....................... 170
B.3 Parameters of the SR Ca$^{2+}$ pump in the thermodynamical model .......... 174
B.4 Modified parameters for high-resolution Ca$^{2+}$ spark model ............... 174
B.5 Modified parameters from Zhong et al. [1] .................................. 175

D.1 Modified parameters from Zhong et al. [1] .................................. 180

E.1 Modified parameters from Mahajan et al. [2] ................................. 182
E.2 Parameters of $I_{lo}$ from experiments ......................................... 183
In humans and other mammals, the heart has four chambers: lower left and right ventricles and upper left and right atria (Fig. 1.1 A). From the systemic circulation, the right atrium receives blood low in oxygen and high in carbon dioxide, and uni-directly pumps the blood to the right ventricle. Then blood is pumped through the pulmonary artery to the lung where it receives oxygen and releases carbon dioxide. The oxygenated blood in the pulmonary vein then returns to the left atrium, flows to the left ventricle, and is pumped via the aorta to the systemic circulation where oxygen is used. The beating frequency of the heart is close to 72 beats per minute, meaning that the human heart beats 2-3 million times in a normal life span.

Cardiovascular disease, the leading cause of deaths, is responsible for 840,768 deaths in 2016, which accounts for \( \sim 27\% \) of overall deaths in the United States [3, 4]. Such a high death rate leads to the cost in the United States at $351.2 billion in 2014 - 2015. Of the deaths due to cardiovascular disease, about 50% are attributable to sudden cardiac deaths. While much has been done to understand, prevent, and treat SCD, there is still much unknown, because it is associated with not only biology and medicine but also mathematics and physics. Unlike many other organs in humans, the heart is an excitable medium. Under some conditions, the transition from sinus rhythm to arrhythmias does not require the change in the biological properties of the heart. Therefore, it is challenging not only for mathematicians and physicists to understand the
physiological nature of the heart but also for biologists and cardiologists to grasp the nonlinear dynamics.

**Figure 1.1: Cardiac conduction system and action potentials.** A The structure and conduction system of the heart (modified from Karma [5]). SA node: sinoatrial node, AV node: atrioventricular node. B Action potential of the cells at different locations of the heart. 0: Depolarization (upstroke) phase, 1: early-fast repolarization phase, 2: plateau phase, 3: repolarization phase, 4: diastole phase. C Schematic diagram of normal electrocardiogram (ECG) showing the P-wave, the QRS complex, and the T-wave. Different colors correspond to the action potentials in B. Panel B and C are modified from textbookofcardiology.org.

## 1.1 Cardiac electrophysiology

### 1.1.1 Cardiac excitation-contraction coupling

The contraction of the heart is initiated by the excitation of the electrical conduction system (Fig. 1.1). The conduction system is a set of specialized heart cells that can transmit signals throughout the heart at high speed (2 - 3 m/s) [6], such that the chambers contract in a coordinated way and the contractions of the cells in a chamber are synchronous. The excitation of the
conduction system starts from the sinoatrial node (SA node) in the wall of the right atrium. The SA node can spontaneously produce an action potential. This signal instantly travels throughout the atria and stimulate atria to contract. Then the signal travels to the atrioventricular node (AV node). After a delay, allowing the blood to fill the ventricles, the signal travels through the His bundle, diverges at the bundle branches, and travels to the Purkinje fibers in the inner walls of the ventricles, leading to the ventricular contractions.

The membrane potential behaviors differ in different types of heart cells. In the cells from the SA node and AV node, the membrane potentials are oscillatory (Fig. 1.1 B), spontaneously varying between -60 mV and 40 mV. The AV node has a slower oscillatory frequency, such that under the normal condition, the AV node is forced to oscillate at the same frequency as the SA node. The atrial and ventricular myocytes are excitable. As a short impulse above a threshold transmits to these cells, they instantly depolarize from the resting potential \(-80 \text{ mV}\) to the positive voltage range (phase 0 in Fig. 1.1 B) and then repolarize to the resting potential after several hundred milliseconds (phase 3 in Fig. 1.1 B). Such a depolarization and repolarization event is called an action potential. During an action potential, intracellular Ca\(^{2+}\) concentration also rises and decays, triggering the myocytes to contract and relax. The process of cardiac contraction by the electrical stimulus is called excitation-contraction coupling.

Clinically, action potentials can not be directly measured. Instead, the electrocardiogram (ECG) detected by the body surface electrodes is introduced to represent the electrical activity of the heart. The ECG is a weighted measurement of the electrical activity of different heart cells. The normal ECG (Fig. 1.1 C) has a P-wave, a QRS complex, and a T-wave. The P-wave is a small peak that reflects the electrical activity of atria. The QRS complex is a large sharp spike occurring during phase 0 of the ventricular action potential. The T-wave is also a small peak occurring during phase 3 of the ventricular action potential. The electrical activity of ventricles causes the QRS complex and the T-wave because ventricles have the largest volume compared to other parts of the heart. The time interval between the start of the QRS complex and the end of the T-wave is called QT interval, which is a rough measurement of action potential
duration (APD) of the ventricular myocytes. The QT interval is widely used clinically because the prolonged QT interval (long QT syndrome) frequently results in cardiac arrhythmias.

![Subcellular structure and ion transportations in the cardiac myocytes.](image)

**Figure 1.2:** Subcellular structure and ion transportations in the cardiac myocytes. 
A Schematic diagram of SR, T-tubules, mitochondria, and myofilaments in ventricular myocytes (modified from Fawcett et al. [7]). Note that T-tubules are co-located with JSR (or subsarcolemmal cisternae), distributed in the discrete planes called Z-lines. B Ion transportations in the myocytes (modified from Bers [8]). Red arrows show the Ca\(^{2+}\) influx to bind myofilaments and green arrows show the Ca\(^{2+}\) efflux to remove Ca\(^{2+}\) from myofilaments. Inset shows time traces of membrane voltage, Ca\(^{2+}\) transient, and contraction force in the rabbit ventricular myocyte.

The contraction of the heart muscle is achieved by cytosolic Ca\(^{2+}\) binding myofilaments, which is related to the subcellular Ca\(^{2+}\) dynamics. Fig. 1.2 shows the subcellular structure and Ca\(^{2+}\) transportation in the cardiac myocytes. A single myocyte is a rod-like cell, and so the myofilaments which pack together to form myofibrils. The cell membrane, called sarcolemma, penetrates the cell to form a T-tubule system, which typically forms a set of discrete planes (called Z-lines) perpendicular to the longitudinal direction, with the average distance between Z-lines about ~ 1.8 µm [9, 10]. Between the Z-lines there are sarcoplasmic reticulums (SR). The ends of the SR form saclike cisternae, called junctional SR (JSR), which serve as the Ca\(^{2+}\) store and are located close to the T-tubules.
Chapter 1 Introduction

During the depolarizing phase of the AP, \( \text{Ca}^{2+} \) enters the cell through the long-type calcium channels (LCCs) localized in the sarcolemma and in close proximity to JSR. \( \text{Ca}^{2+} \) entry significantly increases the local space beneath the sarcolemma, called the dyadic cleft (proximal space), which on the other side against the SR membrane with a RyR cluster embedded in it. RyRs sense \( \text{Ca}^{2+} \) in the dyadic cleft and release \( \text{Ca}^{2+} \) from SR, further elevating dyadic calcium concentration. High \( \text{Ca}^{2+} \) concentration in the dyadic cleft results in \( \text{Ca}^{2+} \) diffusion to the vicinity called submembrane space, which is also beneath the T-tubule membrane. \( \text{Ca}^{2+} \) then diffuses to the cytosolic space, and binds myofilament proteins, leading to the contraction of the cell. During the plateau phase and thereafter phases of the AP, \( \text{Ca}^{2+} \) is removed from the cytosolic space by four routes. In the human ventricle, which is similar to rabbit, dog, and cat, the SR ATPase pumps 70\% \( \text{Ca}^{2+} \) back to the NSR, and \( \text{Na}^+/	ext{Ca}^{2+} \) exchanger extrudes 28\% \( \text{Ca}^{2+} \) outside of the cell, leaving 1\% each to be pumped to mitochondria by \( \text{Ca}^{2+} \) uniporter and extruded outside of the cell by sarcolemma \( \text{Ca}^{2+}\text{-ATPase} \) [8] (Fig. 1.4 B).

The ventricular action potential is generated by the ions passing through the channels in the cell membrane. As neighboring cells are excited, the voltage of the ventricular myocyte is elevated through gap junctions above a threshold that activates the sodium channels. The large sodium current (\( I_{\text{Na}} \)) rapidly depolarizes the cell and bring the membrane voltage to a high level. The elevation of membrane voltage activates the long-type \( \text{Ca}^{2+} \) current (\( I_{\text{Ca},L} \)), which allows extracellular \( \text{Ca}^{2+} \) to enter the cell. At the end of this depolarization phase, the transient outward \( \text{K}^+ \) current (\( I_{\text{to}} \)) is activated, which brings down the voltage and forms a notch (phase 1). Shortly after this phase, the slow component of the delayed rectifier \( \text{K}^+ \) current (\( I_{\text{Ks}} \)) is activated, counterbalancing the depolarizing current \( I_{\text{Ca},L} \), to maintain the plateau (phase 2). During this phase, the fast component of the delayed rectifier \( \text{K}^+ \) current (\( I_{\text{Kr}} \)) is inactivated. At the end of phase 2, \( I_{\text{Ks}} \) reaches to a higher value and \( I_{\text{Kr}} \) recovers from the inactivation. These two repolarizing currents work together to bring down the membrane voltage (phase 3) significantly. As voltage decreases below -30 mV, the inward rectifier \( \text{K}^+ \) current (\( I_{\text{K1}} \)) starts to be activated, further lower the membrane voltage. Finally, the ventricular myocytes stay at the resting potential \( \sim -86 \text{ mV} \).
Chapter 1 Introduction

1.1.2 Cardiac arrhythmias and long QT syndromes

Cardiac arrhythmias occur when the electrical activity that coordinates the heartbeat does not work properly, causing the heart to beat too slow, too fast, or irregularly. When the heart rate is slower than 60 beats per minute, it is called bradycardia, and when the heart rate is faster than 100 beats per minute, it is called tachycardia. Arrhythmias may not have any signs or symptoms. When arrhythmias are noticeable, the signs and symptoms include a fluttering in the chest, sweating, or even passing out and shortness of breath. The tachycardia in the ventricles is shown in Fig. 1.3 A. As the heartbeat becomes more irregular, ventricular tachycardia (VT) evolves into ventricular fibrillation (VF), which breaks the synchronization of contraction in the ventricle and results in loss of ability to pump blood throughout the body.

![Image of sinus rhythm, VT, and VF](image)

**Figure 1.3:** Long QT syndromes and cardiac arrhythmias. A Initiation of polymorphic ventricular tachycardia (VT) and ventricular fibrillation (VF) by the R-on-T phenomenon (modified from Oksuz et al. [11] and Chen et al. [12]). The arrows denote R-on-T events. B Differences of T-wave in genotyped patients with LQT1, LQT2, and LQT3 (modified from Morita et al. [13]).

Another important form of heart rhythm disorder is long QT syndrome (LQTS), which may predispose an individual to tachyarrhythmias. LQTS manifests a prolonged QT interval in the surface ECG and is associated with the prolongation between ventricular depolarization and repolarization (Fig. 1.1). LQTS may be congenital or acquired later in life. The inherited LQTS
are due to genetic mutations in proteins forming Na\(^+\) and K\(^+\) channels that delay repolarization. The onset of LQTS later in life results from reduced repolarization reserve in situations of low blood K\(^+\), heart failure, or certain medications [13]. Classified by the genes encoding cardiac ion channels, the congenital LQTS can be grouped in 16 subtypes [14]. Of all patients with LQTS, over 90% are identified as LQT1, LQT2, and LQT3 [15, 16].

The most common form is the LQT1, which accounts for 42% to 54% of all genotyped LQTs [15, 16]. LQT1 results from the loss-of-function mutations in the KCNQ1 gene, encoding the \(\alpha\) subunit of the KvLQT1 potassium channel that carries the slowly activating delayed rectifier K\(^+\) current (\(I_{Ks}\)). \(I_{Ks}\) reduction increases transmural dispersion of repolarization between the epicardium and midmyocardium, thus resulting in a broad-based T-wave in the surface ECG (Fig. 1.3) and acting as a proarrhythmic substrate. Patients with LQT1 suffering sudden cardiac death can be triggered by physical or emotional stress. In particular, swimming and diving are LQT1-specific triggers [17].

The second-most common form is the LQT2 and is responsible for 35% to 45% of all genotyped LQTs [15, 16]. Clinically, LQT2 manifests a low amplitude, frequently bifid (with two peaks), T-wave in the surface ECG (Fig. 1.3). LQT2 arises from the loss-of-function of KCNH2 that encodes the \(\alpha\) subunit (HERG) of the \(I_{Kr}\) channel [18, 19]. A large proportion of death in LQT2 patients occurs as a result of triggered activity and polymorphic ventricular tachycardia evoked by emotional stress or exercise [18–20]. Features of the ECG, such as triggered activity and torsade de pointes, are thought to be caused by early afterdepolarizations (EADs) [20, 21].

LQT3 accounts for 1.7% to 8% of all genotyped LQTs [15, 16]. In the surface ECG, LQT3 has a flat ST segment and a narrow-based tall T-wave (Fig. 1.3). This LQTS is caused by SCN5A mutations that result in the gain-of-function of Na\(^+\) channels, including a persistent late current (\(I_{NaL}\)), slowed inactivation, and faster recovery from inactivation [22]. These cardiac events usually occur at rest without arousal and occasionally under sympathetic stimulation (emotion 19% and exercise 13%) [17].
1.1.3 Rabbit model of heart disease

A variety of animal models of heart disease have been employed to understand the mechanism of cardiac arrhythmias and to develop novel antiarrhythmic therapies. Small animal models, such as mouse and rat, are beneficial because of low cost and short gestation time. Over the decades, a tremendous amount of data has been collected using rat models to study cardiovascular disease. On the other hand, large animal models, such as pig and dog, provide distinct advantages. For example, hemodynamic assessment and imaging are easier in larger hearts, and large size allows human-scale interventions to be practicable [23]. More importantly, cellular electrophysiology and Ca\textsuperscript{2+} transportation in large animals are more like humans than in small animals. In particular, sustained cardiac arrhythmias in small animals are less likely to occur due to the small heart size. If ventricular tachycardia or ventricular fibrillation occur in the small animals, the usual consequence is a spontaneous transition to sinus rhythm. However, the purchase cost and daily allowance of large animals can be inhibitory for studies of cardiac disease.

The practical alternative to large animals is the rabbit, which is a medium-sized animal. Rabbit heart is large enough to perform surgical interventions at a moderate cost, and it has cellular and molecular characteristics still very like humans [24]. In particular, rabbit ventricles have action potential phenotype the same as that in humans, while action potential duration in rat and mouse ventricles is considerably shorter (Fig. 1.4 A). This difference is because that rat and mouse almost entirely depend on transient outward current \( I_{\text{to}} \) to repolarize, and lack the delayed rectifier currents \( I_{Ks} \) and \( I_{Kr} \). Moreover, in the cytosolic space of rabbit ventricles, 70% Ca\textsuperscript{2+} is removed by SR ATPase and 28% by NCX, while SR almost removes all cytosolic Ca\textsuperscript{2+} in rat ventricles ([8], Fig. 1.4 B & C).

In the past decades, transgenic rabbit models have been developed to over-express and knock-out genes of interest [26]. Similar to the regular rabbit model, the transgenic rabbit models offer many advantages because the results from transgenic and knock-out mice models for some cardiac diseases cannot be extrapolated to humans. To date, the transgenic rabbit models are
Chapter 1 Introduction

**Figure 1.4:** Ca$^{2+}$ fluxes and action potentials in rabbit and rat ventricular myocytes. 
**A** Action potentials of normal rabbit and rat ventricular myocytes (modified from Bassani *et al.* [25]). **B** and **C** show the percentage of cytosolic Ca$^{2+}$ in rabbit and rat ventricular myocytes removed by SR, NCX, and slow component, including sarcolemma Ca$^{2+}$-ATPase (SL Ca-ATPase) and mitochondria uniporter (Mito; modified from Bers [8]). Action potential and Ca$^{2+}$ flux of human ventricles are more like a rabbit.

used to study LQTS [27–29], hypertrophic cardiomyopathy [30, 31], phospholamban overexpression [32], and $G_{\text{s}\alpha}$ overexpression [33].

### 1.2 Triggered activity as a mechanism of cardiac arrhythmias

The mechanisms underlying cardiac arrhythmias could be divided into two groups: (1) abnormal impulse formation, (2) impulse conduction disturbance, or the combination of them.

There are two forms of abnormal impulse formation. The first one is automaticity, the property of a cell to spontaneously generate action potentials. All of the heart cells in the conduction system can fire off on their own. However, the normal heart rhythm is controlled by the SA node because it has the fastest intrinsic heart rate than others. Abnormal automaticity includes increased and reduced automaticity, leading to tachycardia and bradycardia, respectively. Tachycardia and bradycardia due to abnormal automaticity can occur in a variety of conditions, including damage in the pacemakers and the impulse conduction, and mutations of the ion channels in the structurally healthy heart [34, 35].

The second form is triggered activity. Under certain abnormal conditions, unexpected depolarizations occur during an action potential or after it, and the manifestation of these unexpected depolarizations
depends on the preceding action potentials. Such events are referred to as afterdepolarizations. Based on their temporal relationship, afterdepolarizations are traditionally reorganized into two classes: early afterdepolarizations (EADs), which take place during phase 2 or phase 3 of an AP (Fig. 1.5 A), and delayed afterdepolarizations, which take place after an AP but before the next normal one (Fig. 1.5 A). Extra action potentials, called triggered activity (TA), can occur when the amplitudes of EADs or DADs are sufficient to bring the membrane potential above a potential threshold.

The impulse conduction disturbance is associated with reentry, which describes the phenomenon that electrical activity persistently travels within a circle in the heart, rather than spreading out the heart and then stopping (Fig. 1.5 B). Reentry can occur when there is an anatomic or functional obstacle, and the electrical waves manifest as circus movement [38, 39]. In this case, the cells around the obstacle take turns to recover from refractoriness and are ready to excite before the next wave arrives. Reentry can also occur without circus movements, such as reflection or phase 2 reentry. The reflection reentry takes place in a linear segment of the
tissue (e.g., a Purkinje fiber), where waves travel in both directions over an area of impaired conduction [40]. The phase 2 reentry occurs when voltage waves propagate from the site with a high dome of action potential during phase 2 (commonly in epicardium) to the site without a dome [41, 42].

In the following paragraphs, we will specifically talk about afterdepolarizations that can lead to triggered activity.

1.2.1 Early afterdepolarizations

A critical condition underlying EADs formation is AP prolongation, which accompanies most EADs, but not all, regardless of the specific mechanisms. The repolarization is prolonged because the balance of currents during phase 2 or phase 3 of an action potential deviates to the inward direction. If the change of current balance continues to result in the net inward current, the action potential stops to repolarize and starts with a second depolarization; that is, an EAD. From the perspective of the ECG, the AP prolongation of ventricles manifests as the long QT syndrome. Some antiarrhythmic drugs, primarily class IA and III, predispose to the development of EADs, because they inhibit potassium channels or augment inward currents [43, 44].

Most EADs associated with pharmacologic treatments or pathophysiological conditions could be categorized into two types. The first one is the voltage-driven EADs, which directly results from the reduction of repolarization currents or the increase in depolarization currents. The repolarization currents involved in EAD development are mainly potassium currents ($I_{Kr}$, $I_{Ks}$, and $I_{K1}$). The depolarization currents include calcium current ($I_{Ca,L}$), Na$^+$/Ca$^{2+}$ exchanger current ($I_{NCX}$), and late sodium current ($I_{NaL}$). The second category is Ca$^{2+}$-driven EADs [28, 45]. Under the conditions of sarcoplasmic reticulum (SR) Ca$^{2+}$ overload and/or enhanced RyR activity, a large amount of spontaneous Ca$^{2+}$ releases from SR during phase 2 or phase 3, activating $I_{NCX}$ and thereby initiating EADs.
Chapter 1 Introduction

EAD-induced triggered activity is dependent on heart rate. Some antiarrhythmic drugs, such as β-adrenergic agonists, generally induce EADs at a fast heart rate [46]. In contrast, other drugs, such as class III, typically induce EADs at a slow heart rate [47]. This is because class III drugs rely on blocking the potassium channels, which prolongs the action potential duration and refractoriness, to prevent re-entrant arrhythmias. Under the conditions of LQT1 or LQT2, in which the repolarizing current $I_{K_s}$ or $I_{Kr}$ is absent, β-adrenergic agonist (such as isoproterenol) facilitates to elicit EADs at a slow heart rate [28, 29].

1.2.2 Delayed afterdepolarizations

DADs are observed under conditions that build up the intracellular Ca$^{2+}$ load, such as heart failure [48], hypertrophy [49], and myocardial infarction [50]. They are also observed in the hearts exposed to the toxic levels of cardiac glycosides (digitalis) [51] and catecholamines [52]. The toxic level of digitalis was the first identified cause of DADs [51] and was found to be associated with the inhibition of Na$^+$/K$^+$ pump and Ca$^{2+}$ overload. Catecholaminergic polymorphic ventricular tachycardia (CPVT) was later found to stem from the mutations of the ryanodine receptor gene ($hRyR2$) [53] and the cardiac calsequestrin gene ($CSQ2$) [54]. In contrast to EADs, DADs generally occur at a relatively fast heart rate.

Previous experiments showed that increased external Ca$^{2+}$ concentration [55, 56], intracellular sodium concentrations [57] and the addition of isoproterenol (ISO) [58] facilitate the generation of Ca$^{2+}$ waves and DADs. Under these conditions, the elevated sarcoplasmic reticulum (SR) Ca$^{2+}$ concentrations ($[Ca^{2+}]_{JSR}$) were measured to reach a certain threshold. There are also studies demonstrating that modulations of RyRs have an impact on the generation Ca$^{2+}$ waves and its frequency, including the acquired modulations, such as the addition of caffeine [58] or tetracaine [59], and the pathological modulations, such as mutation of RyR [60] and phosphorylation [61] in heart failure. Moreover, there is evidence showing that inhibition of the SR Ca$^{2+}$ pump reduces Ca$^{2+}$ waves frequency and propagating speed [62, 63]. However, the roles of SR Ca$^{2+}$ content and RyR activity have not been tested in mathematical modeling.
Ca\textsuperscript{2+} waves during the diastolic phase have been recognized to initiate DADs and triggered activity [64, 65] (Fig. 1.6). Ca\textsuperscript{2+} waves are the consequence of sequential activation of Ca\textsuperscript{2+} sparks, which are local Ca\textsuperscript{2+} releases from individual Ca\textsuperscript{2+} release units (CRU). As a Ca\textsuperscript{2+} spark occurs, Ca\textsuperscript{2+} concentration at the neighboring CRU site can be elevated. If such a fire-diffuse-fire pattern sustains, a Ca\textsuperscript{2+} wave occurs.

**Figure 1.6:** DADs generated by spontaneous diastolic Ca\textsuperscript{2+} waves, which results from the regenerative initiation of Ca\textsuperscript{2+} sparks. A Representative recording of membrane action potential (top) with the corresponding confocal line-scan image (bottom) in ventricular fibrillation myocytes in the presence of 100 nM isoproterenol. Asterisks denote the subthreshold DADs, and arrows denote the super-threshold DADs. B Discrete Ca\textsuperscript{2+} sparks in patch-clamped cardiac myocytes at 10 s after application of 0.5 mM caffeine. Panel A is modified from Belevych et al. [66], and panel B is modified from Lukyanenko et al. [67].

### 1.3 Mathematical modeling of cardiac myocytes

A quantitative description of the action potential is not available until Hodgkin and Huxley (HH) in 1952 developed the first mathematical models of membrane currents of a squid giant axon and put them in a unified framework [68]. The basic principle of the HH model is to consider the excitable cell as an electrical circuit, in which the lipid bilayer is considered as a capacitor (\(C_m\)), ion channels across the cell membrane are considered as electrical conductors (\(G_i\)), and the electrochemical gradients driving the flow of ions are considered as batteries (\(E_i\)).
Therefore, Kirchoff’s law gives

\[ C_m \frac{dV_m}{dt} = -(I_{\text{ion}} + I_{\text{ext}}) \]

(1.1)

\[ I_{\text{ion}} = G_{\text{Na}}(V_m - E_{\text{Na}}) + G_{\text{K}}(V_m - E_{\text{K}}) + G_{\text{L}}(V_m - E_{\text{L}}) \]

where \( I_{\text{ext}} \) is the externally applied current, \( G_{\text{Na}} \) and \( G_{\text{K}} \) depend on \( V_m \) and time, and \( G_{\text{L}} \) is the conductance of the small leak current. \( E_i \) is called the Nernst potential, which is determined by the ion concentrations across the cell membrane

\[ E_i = -\frac{RT}{zF} \ln \frac{c_i}{c_o} \]

(1.2)

where \( c_i \) is the intracellular ion concentration, \( c_o \) is the extracellular ion concentration, \( T \) is the temperature, \( R \) is the universal gas constant, \( z \) is the valence of the ion, and \( F \) is the Faraday’s constant.

The conductance represents the production of the permeability of a single channel and the number of open channels. To determine the conductance, experiments are performed to measure membrane currents (e.g., sodium current) under the voltage-clamp mode, where voltage is clamped at a resting value and then suddenly clamped at a different value. By fitting the current to a hypothesized function, the gating of the channel could be determined. The gating of the channel can be generally separated into activation, which causes the channel to open, and
inactivation, which causes the channel to close. For the sodium channel, the conductance can be written as

\[
G_{Na} = g_{Na} m^3 h \\
\frac{dm}{dt} = m_\infty - m \frac{1}{\tau_m} \\
\frac{dh}{dt} = h_\infty - h \frac{1}{\tau_h}
\]  

(1.3)

where \(g_{Na}\) is the maximum conductance, \(m\) is the activating variable, and \(h\) is the inactivating variable. The above equations show that \(m\) and \(h\) dynamically approach to \(m_\infty\) and \(h_\infty\) in the time scales of \(\tau_m\) and \(\tau_h\), respectively. \(m_\infty\) and \(h_\infty\) represent the activation and inactivation in the steady states and can be measured in the experiments. Typically the activation \(m_\infty\) is an ascending sigmoidal function of \(V_m\), meaning that the channel tends to open at the higher voltage, and the inactivation \(h_\infty\) is a descending sigmoidal function of \(V_m\), meaning that the channel tends to close at the lower voltage. For the sodium channel, it has a similar form of activation, but it does not have the inactivation in the original HH model.

\[
G_K = g_K n^4 \\
\frac{dn}{dt} = n_\infty - n \frac{1}{\tau_n}
\]  

(1.4)

The original HH model for the squid giant axon plays a pivotal role in developing mathematical models for other excitable media such as cardiac myocytes. Since Denis Noble developed the first cardiac action potential model in 1962 [70], there have been over 100 models present for a variety of cell types from a variety of species (summarized in Noble et al. [71]). An important model is the whole-cell model developed by Mahajan et al. [2] for rabbit ventricular myocytes. In addition to nine types of sarcolemmal ion channels, this whole-cell model incorporates intracellular Ca\(^{2+}\) dynamics that regulate the gating of the long-type Ca\(^{2+}\) channel (described by a seven-state Markovian model), Na\(^+\)/Ca\(^{2+}\) exchanger, and the slow component of the delayed rectifier K\(^+\) channel. Adapted from Shiferaw et al. [72], this model phenomenologically incorporates the spatially localized nature of Ca\(^{2+}\) releases from SR triggered by LCCs (Fig. 1.8).
Figure 1.8: **Simplified diagram of subcellular spaces.** The diagram shows three Ca\(^{2+}\) release units (CRUs). LCCs, JSR, and RyRs are co-located, with a small space, dyadic cleft, confined by T-tubule and SR membrane. NCX is located in the submembrane space, and NSR is distributed between two neighboring Z-lines. Embedded in the NSR membrane is the SERCA2a that pumps Ca\(^{2+}\) from the cytosol to NSR.

The above models can study electrophysiological features at the whole-cell level, but some features arise from local events, such as Ca\(^{2+}\) sparks, Ca\(^{2+}\) waves, DADs, and spark induced Ca\(^{2+}\) alternans. Recently, multiscale models have been developed to explicitly include Ca\(^{2+}\) diffusion and the spatial distribution of Ca\(^{2+}\) dynamics inside the cell [56, 73–78]. As shown in Fig. 1.8, L-type Ca\(^{2+}\) channels are colocalized with the ryanodine receptors. Both of them sense dyadic Ca\(^{2+}\) concentration and release Ca\(^{2+}\) to the dyadic junction. Hence, the local dyadic Ca\(^{2+}\) concentration can be elevated from 0.1 \(\mu\)M to > 100 \(\mu\)M, which dramatically influences the activity of LCC, RyR, and NCX (driven by Ca\(^{2+}\) in the neighboring submembrane space).

The multiscale models aim to deal with the local Ca\(^{2+}\) dynamics and ion channel activity. However, current multiscale models longitudinally couple submembrane compartments, and use Ca\(^{2+}\) diffusion coefficients much larger than experimental measurements. To understand the mechanism of Ca\(^{2+}\) wave formation and thus DAD formation, a physiologically realistic model is required.

In the following chapters, all the multiscale models are improved from the Restrepo’s model [73], and we adopt a 16-state model for 4 LCCs introduced by Terentyev et al. [28], and the original 4-state model for 100 RyRs in each CRU. The 16-state LCC model is the natural
extension of the HH model that includes flickering ($\sim 0.1$), voltage-dependent activation, voltage-dependent inactivation, and Ca\textsuperscript{2+}-dependent inactivation. In addition to the regulation by dyadic Ca\textsuperscript{2+}, the 4-state RyR model also includes the regulation of luminal Ca\textsuperscript{2+} (Ca\textsuperscript{2+} in JSR) that regulates both the open rates and the refractoriness of RyR. Most importantly, the improved detailed model only couples submembrane compartments transversely. To study the formation of Ca\textsuperscript{2+} waves and DADs, we also use the Ca\textsuperscript{2+} diffusion coefficients consistent with the experimental observation [79]. To study the formation of EADs, the settings of RyRs and other sarcolemma currents are modified.

Details of the multiscale models and the simple whole-cell model are given in the following chapters and appendices.

1.4 Open questions related to triggered activity

Phosphorylation of RyRs – for example, in heart failure – has been suggested to result in Ca\textsuperscript{2+} waves and DADs. However, many aspects of it are still controversial. Planar lipid bilayer experiments of RyRs show that RyRs are clustered in near-crystalline arrays, and these channels tend to open and close together with the coupling mediated by a regulatory protein known as FKBP 12.6 [80]. Under the condition of phosphorylation, experiments exhibit increased RyR open probability and dissociation of FKBP 12.6 from the RyR macromolecular complex [81, 82]. The natural expectation based on these results would be an increased Ca\textsuperscript{2+} leak from SR and an increased Ca\textsuperscript{2+} spark rate in quiescent ventricles from failing hearts. However, experiments of Ca\textsuperscript{2+} sparks cannot detect an increased Ca\textsuperscript{2+} spark rate both in heart failure [83] and in the setting of fixing the SR load upon protein kinase A phosphorylation [84]. Moreover, other experiments demonstrate that over-expression of FKBP12.6 in cultured adult myocytes decreases SR Ca\textsuperscript{2+} leak [85], and mice robustly survive the absence of FKBP 12.6 [86].

In an attempt to address this problem, Sobie et al. [87] proposed a hypothesis that the coupling between RyRs, called RyR cooperativity, increases the Hill coefficient in the steady state open probability of the RyR cluster. This hypothesis can explain that phosphorylation of RyRs cannot
increase the Ca\textsuperscript{2+} spark rate in Li et al. [84], which is under the condition of low [Ca\textsuperscript{2+}] (10 and 50 nM), while phosphorylation of isolated RyRs increases the open probability of a single RyR at an increased [Ca\textsuperscript{2+}] in Valdivia et al. [88].

From the perspective of modeling, the understanding of Ca\textsuperscript{2+} wave formation is also fundamentally incomplete. Two types of models are involved in producing Ca\textsuperscript{2+} waves. The first is the simple models that only consider cytosolic space and the Ca\textsuperscript{2+} buffers in it. However, it either depends on the huge release current (over 10 pA) to produce Ca\textsuperscript{2+} waves or cannot produce normal Ca\textsuperscript{2+} spark size [89, 90]. The huge release current was introduced to produce normal Ca\textsuperscript{2+} spark width by Izu et al. [91]. In contrast, the release current is 1 ~ 3 pA in the experiments [9, 92–94]. The consequence is that the local Ca\textsuperscript{2+} transient during waves is over 300 \(\mu\)M, which is over 100 times larger than the experimental measurements [8, 95, 96]. The other type is the multi-scale detailed models that consider the submembrane space and the dyadic cleft between RyRs and LCCs [56, 75, 77, 78, 89, 90]. However, these models unrealistically couple compartments between neighboring CRUs. The coupling facilitates to produce Ca\textsuperscript{2+} waves because it enables more Ca\textsuperscript{2+} to travel to the neighboring CRUs, initiating and sustaining the fire-diffuse-fire behavior.

EADs, another type of afterdepolarizations that result in triggered activity, are widely observed in long QT syndromes. By expressing pore mutants of gene KCNQ1 (KvLQT1-Y315S) to eliminate \(I_{Ks}\), previous studies produced transgenic rabbits with LQT1 syndrome [27, 97, 98]. These studies show that EADs and PVTs occur in transgenic LQT1 rabbits under sympathetic stimulation [97, 98]. Optical mapping shows that most of EADs originate from RV myocytes, and triggered activity propagation frequently encounters the long APD region in LV myocytes, which leads to conduction blocks and reentry formation [97]. However, it is still unknown why triggered activity preferentially originate from RV myocytes, although APD in RV is shorter than that in LV [97].

By overexpressing pore mutants of the human gene KCNH2 (HERG-G628S) to eliminate \(I_{Kr}\), Brunner et al. [27] created transgenic rabbits with LQT2 syndrome. As a result, these
rabbits exhibit prolonged QT interval and high incidence of SCD (>50% at 1 year of age) due to PVT. The optical mapping used to investigate the substrate of arrhythmia revealed a prominent spatial dispersion of APD and discordant APD alternans \cite{27, 99}. The observation of EADs in isolated myocytes under β-adrenergic stimulation has supported the hypothesis that arrhythmia originates at the single-cell level \cite{98, 100}. Another study shows that Ca\textsuperscript{2+}-mediated communication between RyRs and other Ca\textsuperscript{2+} transport complexes play a critical role in EAD formation \cite{28}. The RyR activity in ventricular myocytes of the LQT2 rabbit is enhanced, resulting in a large number of late aberrant SR Ca\textsuperscript{2+} release events. This study proposes that these protracted Ca\textsuperscript{2+} releases from SR drive the depolarizing NCX current, which suffices to maintain the membrane potential (\(V_m\)) for a prolonged time in a window permissive for LTCCs reactivation, thereby causing EADs. However, the mechanism is still poorly understood why forward mode NCX current can be increased without significantly elevating the whole-cell cytosolic calcium concentration during the vulnerable period of repolarization. Furthermore, the role of NCX in EAD formation in LQT2 has not been investigated experimentally.

Moreover, the late sodium current \(I_{NaL}\) in transgenic LQT2 rabbits facilitates to initiate EADs and thus PVTs \cite{101, 102}. Because of this role, \(I_{NaL}\) can be a potential therapeutic target to improve contractility \cite{103}. \(I_{NaL}\) is present when a small number of Na\textsuperscript{+} channels persistently open during the plateau phase of the AP. Although \(I_{NaL}\) is small relative to the peak \(I_{Na}\) (less than 0.1%), its magnitude is nonnegligible compared to other depolarizing currents \cite{104}. Because the presence of \(I_{NaL}\) prolongs APD and therefore is arrhythmogenic, studies have been carried out to identify drugs to suppress this current. So far, many drugs have been tested to suppress \(I_{NaL}\). The drugs include tetrodotoxin \cite{105}, ranolazine \cite{106, 107}, and GS-458967 (GS967) \cite{102}, and each one has a different selectivity to \(I_{NaL}\) compared to \(I_{Na}\). However, the quantitative understanding of how blocking \(I_{NaL}\) eliminates EADs in the LQT2 setting is still lacking. The proposed hypothesis is that \(I_{NaL}\) can act as a depolarizing current and can alter intracellular Na\textsuperscript{+} level, which modulates NCX activity.

Another potential antiarrhythmic target of interest is the small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (SK) channel, which has a single channel conductance from 10 to 20 pS \cite{108}. As an outward
Chapter 1 Introduction

current, the SK current mitigates the loss of repolarization reserve and cardiac arrhythmias. The SK channel is known to be activated by intracellular Ca\textsuperscript{2+} [109], which means that the steady-state SK current is a linear function of voltage. However, there is evidence showing the steady-state SK current is inwardly rectified [109–111], which is regulated by intracellular divalent cations, such as Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. The inward rectification indicates that the SK channel current, which is a repolarization current, is suppressed at high Ca\textsuperscript{2+} concentration during an action potential, facilitating the occurrence of cardiac arrhythmias. This behavior means that releasing the inward rectification is expected to prevent cardiac arrhythmias. Other studies performed in the voltage-clamp mode show that the SK current peaks earlier than the submembrane Ca\textsuperscript{2+} concentration, indicating the significant role of the blocking effect [112]. However, current models of the SK channel only consider Ca\textsuperscript{2+}-dependent activation [113, 114] and do not incorporate the blocking effect by intracellular divalent cations. In addition, the behavior of the SK current during an action potential is unknown, and the relative contributions of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} are still under debate [111, 112].

As mentioned above, EADs at the cellular scale are critical to inducing cardiac arrhythmias at the tissue scale. A classical mechanism to bridge the gap between EADs and arrhythmias is associated with the R-on-T phenomenon, introduced to describe "R-waves interrupting T-waves". The R-on-T phenomenon occurs because of external stimuli, such as sports accidents, leading to EADs or triggered activity at the end of a T-wave. [115, 116]. In this view, the trigger and the substrate are independent. However, this explanation of the mechanisms underlying PVTs is incomplete. Regarding the transition from EADs at the cellular scale to the PVTs at the tissue scale, Weiss et al. [21] raised five critical questions to answer. (1) What is the crucial mechanism underlying EADs when the repolarization reserve decreases? (2) How do cellular EADs overcome the source-sink mismatch between coupled cells to form premature ventricular complex? The source-sink mismatch exists because the repolarizing current of the neighboring cells has a significant influence on the cells with EADs, such that EADs tend to vanish. (3) If EADs are focal, why the QRS electrical axis shifts during torsades des pointes (TdP), which suggests that the foci of EADs change the positions? (4) Why is TdP triggered
by both focal and reentrant mechanisms suggested by the mapping experiments? (5) How do triggered activity occurs at fast heart rates, given that EADs favor the slow heart rates?

**Figure 1.9:** Proposed mechanisms of PVT initiation in long QT syndromes. Credit to Bum-Rak Choi.

Recently, Huang *et al.* [117] demonstrated that both the trigger and the vulnerable substrate could coincide to spontaneously trigger PVC by using a one-dimensional cable of LQT2 myocytes. The occurrence of the trigger and substrate is because of the steep spatial repolarization gradient and increased $I_{Ca,L}$. This mechanism of arrhythmia is distinct from the classical "R-on-T" mechanism because the trigger and the substrate are not independent. Based on this result, Choi *et al.* (unpublished work) propose a hypothesis that the dispersion of repolarization, acting both as a trigger and an arrhythmogenic substrate, is the universal mechanism underlying arrhythmias in LQTS (see Fig: 1.9 for LQT1 and LQT2). To confirm this hypothesis, it is necessary to study the source-sink effect in the LQT1 setting at the tissue level. In particular, it is unknown how PVTs and PVCs originate from regional EADs due to $I_{Kr}$ heterogeneity in the LQT1 setting. In addition, it is also necessary to overcome the limitation of the significantly enhanced $I_{Ca,L}$ in Huang *et al.* [117].

### 1.5 Thesis outline

This thesis is to investigate triggered activity underlying cardiac arrhythmias. The following chapters will begin with $Ca^{2+}$ wave formation by introducing RyR cooperativity into a physiologically
Chapter 1 Introduction

The results in Chapter 2 demonstrate that strong RyR cooperativity is critical to producing propagating Ca^{2+} waves at high cytosolic Ca^{2+} load in the permeabilized cell. To generate normal Ca^{2+} spark size with a reasonably small release current, we consider the fact that the myofilaments and mitochondria occupy a large fraction of the cytosol, which is called molecular crowding. With the addition of caffeine (enhances RyR activity) and isoproterenol (enhances the long type Ca^{2+} channel current, sarcoplasmic reticulum (SR) Ca^{2+} pump, and the slow component of the delayed rectifier K\(^+\) current \(I_{Ks}\), and phosphorylates RyR ) to the intact cells, this model can generate delayed afterdepolarizations (DADs) during the diastolic phase. Overall, this study highlights the role of RyR cooperativity in forming Ca^{2+} waves and, thereby, cardiac arrhythmias.

Chapter 3 focuses on another type of afterdepolarizations leading to triggered activity; that is, EADs. This chapter describes the mechanisms of EAD formation in LQT2 and LQT1 myocytes. We use the modified physiologically multiscale model to study EAD formation in LQT2 myocytes. The results show that because the local NCX current is a saturating nonlinear function of the local submembrane calcium concentration, a larger number of smaller-amplitude discrete Ca^{2+} release events can produce a large increase in whole-cell forward-mode NCX current without increasing the whole-cell cytosolic calcium concentration significantly. Furthermore, this study brings insights into how alterations of stochastic RyR activity at the single-channel level cause late aberrant Ca^{2+} release events. Experimental measurements in transgenic LTQ2 rabbits confirm the critical arrhythmogenic role of NCX and identify this current as a potential target for antiarrhythmic therapies in LQT2.

In addition, we use a whole-cell model to study EAD formation in LQT1 myocytes. The results demonstrate that the larger conductance of the slow component of \(I_{to}\) (\(I_{to,s}\)) in RV brings down the membrane voltage to the vulnerable window, allowing LCCs to reactivate and thus promote EADs. In the LV, however, the smaller conductance of \(I_{to,s}\) allows the membrane voltage to stay at a high plateau, which leads to the sufficient activation of the rapidly activating delayed rectifier K\(^+\) current \(I_{Kr}\) during the repolarization phase and thus suppresses EADs.
Chapter 1 Introduction

It is of practical importance to suppress EADs. Therefore, Chapter 4 includes the studies of two antiarrhythmic targets: the SK current and the late sodium current. In this chapter, we present a biophysically based kinetic model of the SK channel to incorporate the blocking effect. The binding and unbinding of divalent cations to amino acid residue within the channel are considered as ions jumping over a free-energy barrier, which leads to the transition rates being exponential functions of membrane voltage. Optimization of the model parameters using experimental data favors comparable contributions of Ca\(^{2+}\) and Mg\(^{2+}\) to the blocking effect when Ca\(^{2+}\) concentration is at the high end of the physiological range. We subsequently explore two extremes that blocking is achieved only by Mg\(^{2+}\) or Ca\(^{2+}\). Both models with constant Mg\(^{2+}\) or dynamical changing Ca\(^{2+}\) capture the feature in experiments that SK current peaks earlier than Ca\(^{2+}\) concentration in sub-membrane space where SK channels are located. Integration of the SK channel model to a physiological multiscale model for long QT syndrome type 2 (LQT2) myocytes shows that both SK current peak during the depolarization phase and the repolarization phase contribute to eliminating EADs. The ability to capture critical features from different data sets makes this model useful to investigate the antiarrhythmic role of SK current under various pathological conditions.

In addition, in this chapter, the late sodium current is modeled by altering the inactivation gates \(h\) and \(j\) of the standard sodium channel. The integration of the late sodium channel to the physiologically multiscale model shows that the late sodium current promotes EADs in LQT2 myocytes in two pathways. One is that \(I_{NaL}\) provides an additional depolarization current during the plateau phase. The other is that \(I_{NaL}\) elevates intracellular Na\(^{+}\) concentration, which reduces the repolarizing \(I_{NCX}\), brings down the action potential plateau, and suppresses the slowly activating delayed rectifier K\(^{+}\) current (\(I_{Ks}\)).

Finally, Chapter 5 studies how cellular EADs in the LQT1 setting evolve into premature ventricular complexes and ventricular tachycardia at the tissue scale. Specifically, we study PVC initiation and propagation in a one-dimensional cable consisting of LQT1 myocytes, given that \(I_{to}\) heterogeneity is present between RV and LV myocytes. The results show that PVCs originate under the condition of \(I_{to,s}\) heterogeneity and enhanced \(g_{Ca,L}\) for RV myocytes. In this case, the trigger,
Chapter 1 Introduction

EADs, and the tissue substrate for arrhythmogenesis originate from the same source. In addition, the prolongation of the APD during PVC propagation is observed, and is associated with the electronic current flow between cells. Although this study is still preliminary, it provides insights in the following investigation.
So far, two types of mathematical models have been developed to generate Ca$^{2+}$ waves. The first type is the simple models [89–91, 118, 119], which do not consider the sub-cellular architecture – including SR and dyadic space, and only consider the cytosolic space and Ca$^{2+}$ buffers in it. Izu et al. [89] developed the first stochastic model of Ca$^{2+}$ sparks and waves in which the activation probability of a Ca$^{2+}$ spark is dependent on local $[Ca^{2+}]_i$. CRUs are asymmetrically distributed, which is 2 $\mu$m in the longitudinal direction and 0.4 $\mu$m or 0.8 $\mu$m in the transverse direction, to match the sub-cellular structure of the real heart cell. By introducing a large release current (> 10 $pA$), this model is able to produce a reasonable Ca$^{2+}$ spark with width at 2 $\mu$m. Soeller et al. [90] later adopted the real distribution, instead of regularly arrangement, of RyR clusters on Z-lines and found that this feature doubled the propagating speed of Ca$^{2+}$ waves in the longitudinal direction.

Another type is the multi-scale detailed models developed to investigate diastolic Ca$^{2+}$ waves which initiate DADs and triggered action potentials. Based on the detailed model developed by Restrepo et al. [73], Wasserstrom et al. [56] studied the cell as a linear array of 200 diffusively coupled CRUs. This model demonstrated that increased external Ca$^{2+}$ concentration decreases the variability of latency distribution of the first spontaneous Ca$^{2+}$ spark after the action potential stops. Gaur et al. [75] developed a multi-scale model to study the whole-cell level consequences of the microscopic dyadic modulation, especially the dyadic volume and
Chapter 2 Ca$^{2+}$ waves and delayed afterdepolarizations

impaired calsequestrin (CSQN) buffering and regulation. This model contains 10,000 CRUs, and couples dyadic spaces and network SR spaces (NSR) between adjacent CRUs, whereas the cytosolic spaces remain uncoupled. Song et al. [76, 77] improved Restrepo’s model to investigate DADs and EADs under the modulation of RyR activity, pacing cycle length, intracellular sodium concentration, etc. The initiation and termination of DADs triggered by Ca$^{2+}$ waves turn out to be stochastic. Recently Xie et al. [78] produced Ca$^{2+}$ waves by introducing the spatially heterogeneous distribution of RyR cluster size into the Restrepo’s model. This model shows that Ca$^{2+}$ waves initiate at the large RyR cluster site because the SR load is elevated due to the small Ca$^{2+}$ releases by small RyR clusters.

Although the models listed above are able to produce propagating Ca$^{2+}$ waves, the underlying mechanisms are not realistic, i.e., either the release current is too large or the coupling between neighboring CRUs cannot be supported by experiments. Izu’s [89] model requires huge release current to produce underlying Ca$^{2+}$ sparks with reasonable width; otherwise, the spark width is only half of the experimental observations. In addition, the spark amplitude (peak fluorescence ratio, $F/F_0$) and local $[Ca^{2+}]_i$ during waves are much larger than the experimental measurements. Soller’s model [90] adopted a small release current, but the spark profile is not in agreement with the experiment. For the multi-scale detailed models, a group of them [56, 76–78] are based on Restrepo et al. [73], in which the diffusion constant of Ca$^{2+}$ in the cytosolic space is largely increased (5 fold in the longitudinal direction and 2 fold in the transverse direction). More importantly, in these models, the sub-membrane compartments are diffusively coupled longitudinally, which facilitates the propagation of Ca$^{2+}$ waves by a standard fire-diffuse-fire mechanism. While the introduction of such a coupling was motivated by the observation that deep T-tubule invaginations of the sarcolemma may not always be perpendicular to the long axis of the cell [73], and thus not fully contained within Z-planes, this observation alone does not seem to justify diffusively coupling all submembrane compartments longitudinally, and this class of models does not produce Ca$^{2+}$ waves when this longitudinal coupling is removed. For other multi-scale models, such as Gaur et al. [75], only the dyadic spaces between neighboring couplons are coupled, whereas the cytosolic spaces remain uncoupled. If the spaces are coupled
realistically, Ca\(^{2+}\) waves should be suppressed because Ca\(^{2+}\) concentration in the cytosol is much smaller than in dyadic spaces ([Ca\(^{2+}\)]\(p\)), leading to a much smaller spark frequency at the wavefront, which cannot sustain the waves. As to Walker \textit{et al.} [120], this model used a single cytosolic space for the whole-cell, and the Ca\(^{2+}\) diffusion in the longitudinal direction is achieved by coupling submembrane spaces. In summary, all of the existing spatially distributed Ca\(^{2+}\) dynamics models that have so far reproduce Ca\(^{2+}\) waves appear to rely on simplifications of the cell architecture that are not fully justified. A major challenge taken up in the present thesis is the development of a model that reproduces quantitatively the salient features of both individual Ca\(^{2+}\) spark profiles and Ca\(^{2+}\) waves with realistic descriptions of the Ca\(^{2+}\) release current and the subcellular architecture with, in particular, longitudinal Ca\(^{2+}\) transport only mediated by diffusion inside the cytosol.

\section{Mathematical modeling}

To produce propagating Ca\(^{2+}\) waves with the above challenges addressed, in this study, we presented a physiologically multi-scale detailed model improved from Restrepo \textit{et al.} [73]. The schematic diagram of the model is shown in Fig. A.1. Since the T-tubules are located in Z-lines, we only coupled the submembrane spaces in Z-lines. In the longitudinal direction, the coupling is achieved by Ca\(^{2+}\) diffusion in the cytosol and NSR. We also adopted the realistic diffusion time constants in the cytosol for both Ca\(^{2+}\) and mobile buffers. Most importantly, we introduced the coupling between RyRs, called RyR cooperativity, into the model to suppress Ca\(^{2+}\) spark frequency at low [Ca\(^{2+}\)]\(_i\) and enhance it at high [Ca\(^{2+}\)]\(_i\), ensuring Ca\(^{2+}\) sparks to stay quiescent, and to dominantly occur at the wavefront. RyR cooperativity arises from the fact that RyRs are organized as a crystal array in the SR membrane, each touching neighbors with a protein, FKBP 12.6 [121–123]. This arrangement enables coupled gating between RyRs, meaning that RyRs tend to open and close collectively [80, 124]. From the perspective of modeling, stronger collective behavior implies a larger Hill coefficient in the gating function [87].
Chapter 2 Ca\(^{2+}\) waves and delayed afterdepolarizations

Besides RyR cooperativity, we considered the fact that mitochondria and myofilaments occupy a large fraction of the cell volume \([24]\), called molecular crowding. With the effective cytosolic space smaller than assumed in Izu et al. \([89]\), small release current is sufficient for Ca\(^{2+}\) to explore a large space, which produces normal Ca\(^{2+}\) spark profile and facilitates to initiate Ca\(^{2+}\) waves. Moreover, as discussed in the Introduction section, SR load and RyR activity are critical to producing Ca\(^{2+}\) waves and thereby DADs in the intact cells. Therefore, we followed Venetucci et al. \([65]\) to add caffeine and ISO to the virtual myocyte. Caffeine enhances RyR activity, which results in increased SR Ca\(^{2+}\) leak \([24, 28, 125]\). ISO enhances \(I_{Ca,L}\), SR pump, and \(I_{Ks}\) \([98]\), which increases Ca\(^{2+}\) influx via LTCCs and elevates SR load via SR pump.

**Figure 2.1: RyR gating model.** A. Schematic diagram of the four-state RyR model. RyR gating is regulated by CSQN in JSR binding to the RyR/T/J complex. When bound with CSQN, a RyR has a lower open probability. B Transition time scales between CSQN-unbound states and CSQN-bound states (\(\tau_u = 1/r_u\) and \(\tau_b = 1/r_b\)). C opening rates of RyR in the CSQN-unbound state (\(k_u\)) and CSQN-bound state (\(k_b\)). D Open probability of a single RyR (\(P_0\)) at various SR loads and Hill coefficients. The channel is assumed to be in the steady-state. The open probability is given by \(P_0 = 1/(1 + k^-/k_{CRU})\), where \(k_{CRU} = N_{CU}k_u + N_{CB}k_b\), and \(N_{CU}\) represents the number of RyRs in the CSQN-unbound closed state and \(N_{CB}\) represents the number in the CSQN-bound closed state. Here, \(\alpha = 0.12 ms^{-1}\)
Chapter 2 Ca\textsuperscript{2+} waves and delayed afterdepolarizations

The key element of this multiscale detailed model is 100 RyRs collocated with LCCs in each CRU [73]. Each RyR is described by a four-state model (Fig. 2.1 A). Luminal Ca\textsuperscript{2+} regulates the RyR sensitivity via auxiliary proteins triadin/junctin (T/J) interacting with the luminal Ca\textsuperscript{2+} buffer calsequestrin (CSQN) [126]. This mechanism is incorporated into this model by allowing CSQN to bind the RyR/T/J complex when SR Ca\textsuperscript{2+} is depleted and by choosing the RyR closed-to-open transition rate in the CSQN bound state (\(k_b\)) to be much smaller than the one (\(k_u\)) in the CSQN unbound state (Eq. 2.1, Fig. 2.1 C, and Table B.2). As a result, the RyR cluster has a larger firing probability at a higher SR Ca\textsuperscript{2+} load (Fig. 2.1 D). The reduced sensitivity in the CSQN bound state both contributes to Ca\textsuperscript{2+} spark termination and induces a refractory state whereby CSQN must unbind from RyRs before future sparks are possible [127, 128].

\[
k_u = \frac{\alpha}{1 + (c^*/c_p)^H} + \gamma_u
\]
\[
k_b = \frac{\beta}{1 + (c^*/c_p)^H} + \gamma_b
\]

Based on Zhong et al. [1], changes of the RyR model and other changes are detailed in Appendix B. Other changes include: 1) the addition of mobile buffers including ATP, dye, and EGTA, 2) realistic diffusion time constants for Ca\textsuperscript{2+} and mobile buffers, 3) uncoupled submembrane spaces in the longitudinal direction.

2.2 Ca\textsuperscript{2+} waves in the permeabilized cells

2.2.1 Ca\textsuperscript{2+} sparks

Following Bovo et al. [125], we investigated the Ca\textsuperscript{2+} spark profile in the permeabilized cells which are bathed at different \([Ca^{2+}]_i\). In these cells, Ca\textsuperscript{2+} ion is free to diffuse across the cell boundary so that \([Ca^{2+}]_i\) remains at a constant. Fig. 2.2 shows the statistics of the spark characteristics. With Ca\textsuperscript{2+} load increased from 0.1 \(\mu M\) to 0.2 \(\mu M\), the spark frequency increases by 3-fold, and the spark amplitude \(F/F_0\), spark width (full width at half maximum, FWHM), and spark duration (full duration at half maximum, FDHM) stays the same (Fig. 2.2 B-D).
Chapter 2 Ca\textsuperscript{2+} waves and delayed afterdepolarizations

Ca\textsuperscript{2+} spark profile is determined by measuring the concentration of Ca\textsuperscript{2+} bound fluorescence indicator dye after convolving with a point spread function (see Appendix B). The analysis of the underlying Ca\textsuperscript{2+} release current (Fig. 2.2 E) demonstrates that the integrated current at 0.2 \(\mu\text{M}\) is \(\sim 70\%\) larger than at 0.1 \(\mu\text{M}\), and both of them are reasonably small compared to the experiments and other models [129, 130].

**Figure 2.2:** Ca\textsuperscript{2+} sparks in the permeabilized cell. Summarized data of Ca\textsuperscript{2+} spark frequency (A), amplitude (\(F/F_0\), B), width (FWHM, C), and duration (FDHM, D). Spark number: \(n = 110\) (0.1 \(\mu\text{M}\) with ATP), \(n = 412\) (0.2 \(\mu\text{M}\) with ATP), \(n = 123\) (0.1 \(\mu\text{M}\) without ATP), and \(n = 384\) (0.2 \(\mu\text{M}\) without ATP). Mean ± standard error. The following panels are without ATP. E. Average (lines) and standard deviation (shaded colors) of Ca\textsuperscript{2+} currents underlying sparks. The fitting results are \(I = 10e^{-t/4.14}(1 - e^{-t/9})\) for 0.1 \(\mu\text{M}\) and \(I = 20e^{-t/3.244}(1 - e^{-t/6.333})\) for 0.2 \(\mu\text{M}\). F. Longitudinal line-scan image of Ca\textsuperscript{2+} spark at 0.1 \(\mu\text{M}\). The image was obtained by smoothing each spark data and then averaging all sparks. The spark data is the signal of fluorescence indicator dye after convolving with a point spread function (PSF), which is to simulate the optical blurring effect in the experiment. G. Longitudinal line-scan image of Ca\textsuperscript{2+} spark at 0.1 \(\mu\text{M}\) obtained from a high-resolution model. There are 10 × 5 × 5 lattices in the longitudinal and transverse directions, respectively. This image is for one spark, with release current from the red line in panel E.

Since the model only contains 2 × 2 × 2 lattices in each CRU space, it is coarse in terms of the Ca\textsuperscript{2+} spark imaging. To improve the resolution, we interpolated each Ca\textsuperscript{2+} spark data using...
Figure 2.3: Effect of ATP on Ca\(^{2+}\) sparks in the permeabilized cells at 0.1 µM (top) and 0.2 µM (bottom). Aa. Average release current over all Ca\(^{2+}\) sparks. Ab. Average Ca\(^{2+}\) concentration in the dyadic space. Ac. Average SR load. Ba - Bc Same as Aa-Ac but is for 0.2 µM.

The cubic method and then averaged all sparks. Fig. 2.2 F shows the result. The size is \(F/F_0 = 1.73\), FWHM = 1.96 µm, and FDHM = 14.1 ms. To validate this coarse resolution model, we constructed a high-resolution model to generate a single Ca\(^{2+}\) spark at 0.1 µM using the release current obtained from the red line in Fig. 2.2 E. This model contains 10 lattices in the longitudinal directions and 5 \(\times\) 5 lattices in the transverse directions. The spark size generated by this model (Fig. 2.2 G) is \(F/F_0 = 1.71\), FWHM = 2.22 µm and FDHM = 14.4 ms. This spark size is in agreement with that in Fig. 2.2 F, validating the detailed model which has a coarse resolution.

We also studied the effect of ATP on the Ca\(^{2+}\) spark profile. Here, ATP only acts as a mobile buffer in the dyadic cleft, submembrane space, and cytosolic space. The diffusion constant of ATP is around half of that of Ca\(^{2+}\) [131, 132]. Fig. 2.2 A-D shows that removing ATP does not affect Ca\(^{2+}\) spark frequency, increases spark amplitude, decreases spark width, and significantly increases spark duration at 0.1 µM while slightly at 0.2 µM. To explain these behaviors, we investigated the release current and Ca\(^{2+}\) concentrations underlying the sparks. The results (Fig. 2.3) show that, in contrast to reducing the release current amplitude, removing
ATP substantially increases \([Ca^{2+}]_p\) amplitude. In addition, ATP increases the SR depletion time, release current duration, and \([Ca^{2+}]_p\) decay time. The effect of ATP on the spark duration is achieved by prolonging the tail current, which is significant at 0.1 \(\mu M\). However, at 0.2 \(\mu M\) in which the release current increases by \(\sim 100\%\), the prolonged tail current is smaller compared to the peak current, which explains why removing ATP at 0.2 \(\mu M\) only slightly increases the spark duration. Although ATP significantly affects \(Ca^{2+}\) spark characteristics, Fig. 2.4 shows that the effect of ATP on \(Ca^{2+}\) wave formation is small.

![Figure 2.4](image-url)  

**Figure 2.4:** Transition from \(Ca^{2+}\) sparks to \(Ca^{2+}\) waves in the absence of ATP. The mobile buffer ATP has no significant effect on \(Ca^{2+}\) wave formation.

### 2.2.2 \(Ca^{2+}\) wave formation

Similar to \(Ca^{2+}\) sparks, we studied \(Ca^{2+}\) wave formation in the permeabilized cells. Following the experiments [125] showing that \(Ca^{2+}\) waves occur at a high \(Ca^{2+}\) load, we varied \(Ca^{2+}\) load to study the transition from \(Ca^{2+}\) sparks to \(Ca^{2+}\) waves. In addition, we varied the Hill coefficient in RyR opening rates (Eq. 2.1) to modify the strength of RyR cooperativity. Fig. 2.5 A summarizes the results at different \(Ca^{2+}\) loads and Hill coefficients. At low \(Ca^{2+}\) load or small Hill coefficient, only \(Ca^{2+}\) sparks occur (blue colors). At large \(Ca^{2+}\) load and large Hill coefficient, \(Ca^{2+}\) waves occur (pink colors). In the transition region from sparks to waves are mini-waves (green color and open circle) and broken waves (orange colors and open square).
Chapter 2 \( \text{Ca}^{2+} \) waves and delayed afterdepolarizations

**Figure 2.5:** \( \text{Ca}^{2+} \) wave formation in the permeabilized cell. A. Parameter space diagram of \( \text{Ca}^{2+} \) waves versus cytosolic \( \text{Ca}^{2+} \) load (\( \left\lbrack \text{Ca}^{2+}\right\rbrack_i \)) versus RyR cooperativity (\( H \)). Blue: isolated \( \text{Ca}^{2+} \) sparks, Green: sequential \( \text{Ca}^{2+} \) sparks in neighboring regions, Yellow: Broken waves, Pink: propagating waves. B. Longitudinal line-scan images of sparks in neighboring regions (open circle) and broken waves (open square). C. Longitudinal line-scan images at \( H = 8 \) for different loads. At the positions marked by the black bars, the temporal profiles of fluorescence of \( \text{Ca}^{2+} \) sparks and waves are shown in D.

The examples of longitudinal line-scan images of mini-waves and broken waves are presented in Fig. 2.5 B, and the examples of \( \text{Ca}^{2+} \) sparks and waves at \( H = 8 \) are presented in Fig. 2.5 C. In some cases, \( \text{Ca}^{2+} \) waves initiate at the cell boundary (broken waves in Fig. 2.5 B and 0.4 \( \mu M \) in Fig. 2.5 C), whereas in other cases \( \text{Ca}^{2+} \) waves initiate within the cell (0.3 \( \mu M \) in Fig. 2.5 C). The local peak fluorescence ratio is \( \sim 2 \) for \( \text{Ca}^{2+} \) sparks, and \( 4 \sim 5 \) for \( \text{Ca}^{2+} \) waves (Fig. 2.5 D). At 0.4 \( \mu M \), the propagating speed of \( \text{Ca}^{2+} \) waves is \( \sim 102 \mu m/s \), and the wave frequency is \( \sim 1.1 \text{ Hz} \). Both the fluorescence profiles and wave speed are consistent with experimental observations [125].

More information on Fig. 2.5 C at 0.4 \( \mu M \) is shown in Fig. 2.6 A&B, where Fig. 2.6 A is the longitudinal line-scan image of \( \left\lbrack \text{Ca}^{2+}\right\rbrack_i \) from the beginning and Fig. 2.6 B is the cross-section.
Chapter 2 $\text{Ca}^{2+}$ waves and delayed afterdepolarizations

**Figure 2.6:** Transition from spontaneous $\text{Ca}^{2+}$ sparks to propagating waves. A. Spatial-temporal line-scan image of $[\text{Ca}^{2+}]_i$ corresponding to Fig. 2.5 C. B. Cross-section images (longitude versus transverse) of $[\text{Ca}^{2+}]_i$ showing the transition from random opening to propagating waves. C. Same as panel A, except for increasing RyR activity of $5 \times 3 \times 3$ CRUs at the center of the cell. The increase of RyR activity is obtained by increasing $\alpha$ to 0.6 (Eq. 2.1).

Images of $[\text{Ca}^{2+}]_i$ at different times. At the beginning where all CRUs are at the same state, $\text{Ca}^{2+}$ waves randomly initiate at different sites and formed multiple mini-waves (at 0.64 s in Fig. 2.6 B). Then the CRU firings become increasingly controlled by waves initiated from the cell boundaries (middle two columns in Fig. 2.6 B). Finally, waves only initiate at the boundary and propagate throughout the cell (Fig. 2.6 B). Although it can be clearly seen that $\text{Ca}^{2+}$ waves propagate in the longitudinal direction, it is notable that $\text{Ca}^{2+}$ waves also propagate in the transverse direction. By counting the number of CRUs the waves cross in 10 ms (11.4 s and 11.41 s in Fig. 2.6 B), the wave speed in the transverse direction could be estimated, which is $\sim 270 \ \mu m/s$ and much faster than that in the longitudinal direction.

In the most right panel of Fig. 2.5 C, $\text{Ca}^{2+}$ waves originate from one side of the cell boundary.
Chapter 2 \( \text{Ca}^{2+} \) waves and delayed afterdepolarizations

and propagate to another. This is because SR uptake lowers \( \text{Ca}^{2+} \) concentration in the middle of the cell. In many experiments, \( \text{Ca}^{2+} \) waves initiate within the cell instead of at the boundary [125, 133, 134]. In this case, several \( \text{Ca}^{2+} \) sparks occasionally occur in small spaces, serve as the trigger to generate propagating \( \text{Ca}^{2+} \) waves. To roughly capture this feature, we increased RyR activity by increasing \( \alpha \) from 0.12 to 0.6 (Eq. 2.1) for a block of CRUs at the center of the cell. Such block contains \( 5 \times 3 \times 3 \) CRUs in the longitudinal and transverse directions, respectively. The result in Fig. 2.6 C demonstrates that \( \text{Ca}^{2+} \) waves initiate at the position with enhanced RyR activity. The \( \text{Ca}^{2+} \) wave speed is \( \sim 94 \mu m/s \), and the wave frequency is 1.05 Hz, which is similar to Fig. 2.6 A.

2.2.3 Role of RyR cooperativity

To quantitatively understand why RyR cooperativity (Hill coefficient) is critical to producing \( \text{Ca}^{2+} \) waves in the permeabilized cells, in this section we analyzed the CRU opening rate \( k_{\text{CRU}} \) defined below by Eq. 2.2 for large and small Hill coefficients. The CRU opening rate is controlled by the gating of 100 RyRs, which is regulated by the Hill coefficient, \( [\text{Ca}^{2+}]_p \), and \( [\text{Ca}^{2+}]_{\text{JSR}} \) (Fig. 2.1 and Eq. 2.1). Fig. 2.7 A shows the histogram of \( [\text{Ca}^{2+}]_p \) just before a spark firing for small and large \( H \) at cytosolic \( \text{Ca}^{2+} \) load 0.3 \( \mu M \) and 0.4 \( \mu M \). At \( H = 2 \) where no \( \text{Ca}^{2+} \) waves occur, \( [\text{Ca}^{2+}]_p \) just before the spark is around the basal value (0.3 \( \sim \) 0.5 \( \mu M \)). At \( H = 8 \), however, the wavefronts were significantly elevated \( [\text{Ca}^{2+}]_p \) at the neighboring CRU sites to as high as 0.6 \( \sim \) 1.2 \( \mu M \), which is sufficient to trigger \( \text{Ca}^{2+} \) sparks in a short time and sustains the propagation of the waves. Notice that not all the sparks are triggered by the wavefront, and CRUs can occasionally fire spontaneously (the red bars at \( \sim 0.3 \mu M \) in Fig. 2.7 A). In this case, \( [\text{Ca}^{2+}]_p \) just before these sparks is close to the basal values. Similarly, we also measured \( [\text{Ca}^{2+}]_{\text{JSR}} \) just before \( \text{Ca}^{2+} \) spark firing, which is shown in Fig. 2.7 B. The result demonstrates that local \( [\text{Ca}^{2+}]_{\text{JSR}} \) at \( H = 2 \) is much lower than that at \( H = 8 \) (550 \( \mu M \) for \( H=2 \) versus 800 \( \mu M \) for \( H=8 \)).

\[
k_{\text{CRU}} = \frac{N_{\text{spark}}}{N_{\text{spark}} + N_{\text{non-spark}}} \left( N_{\text{CU}}k_u + N_{\text{CB}}k_b \right) \tag{2.2}
\]


**Figure 2.7:** Effect of RyR cooperativity \( (H) \) on \( \text{Ca}^{2+} \) wave formation. A. Histogram of \( [\text{Ca}^{2+}]_p \) just before the \( \text{Ca}^{2+} \) sparks initiate. Number of events: 7359 (\( H = 2, 0.3 \text{ µM} \)), 8502 (\( H = 2, 0.4 \text{ µM} \)), 418 (\( H = 8, 0.3 \text{ µM} \)), and 760 (\( H = 8, 0.4 \text{ µM} \)). B. Similar to A, the histogram of \( [\text{Ca}^{2+}]_{\text{JSR}} \). C. Summary of data for \( \text{Ca}^{2+} \) sparks and non-spark release events. D. CRU opening rate \( (k_{\text{CRU}}) \) as a function of \( [\text{Ca}^{2+}]_p \). The red lines are for \( H = 2 \), and the blue lines are for \( H = 8 \), obtained by using Eq. 2.1 and Eq. 2.2. The dot-dashed red line is obtained by assuming that the SR load is \( 800 \text{ µM} \) which is sufficient to initiate a spark when a single RyR open, and the transition between CSQN-bound and CSQN-unbound states is in equilibrium.

Aside from the differences of \( [\text{Ca}^{2+}]_p \) and \( [\text{Ca}^{2+}]_{\text{JSR}} \) between \( H = 2 \) and \( H = 8 \), we still need to link the single RyR opening rate (Eq. 2.1) to the CRU opening rate. Consider that at the beginning all RyRs are in the closed states (CSQN bound or CSQN unbound in Fig. 2.1), then the opening rate of the first RyR in the cluster should be \( N_{\text{CU}}k_u + N_{\text{CB}}k_b \), where \( N_{\text{CU}} \) and \( N_{\text{CB}} \) are the number of RyRs in the CSQN-unbound closed state and CSQN-bound closed state, respectively. Once a RyR in the cluster fires, either it triggers a \( \text{Ca}^{2+} \) spark, which requires > 2 RyRs to open, or it cannot trigger a \( \text{Ca}^{2+} \) spark but becomes a non-spark release event. Therefore, the CRU opening rate \( k_{\text{CRU}} \) can be expressed as Eq. 2.2. To obtain \( k_{\text{CRU}} \) for different Hill coefficients, we first measured the number of sparks and the number of non-spark events.
Fig. 2.7 C shows that, at \( H = 8 \) where \([Ca^{2+}]_{JSR}\) before depletion is \( \sim 800 \mu M\), over 99% of the release events are \( Ca^{2+} \) sparks, whereas at \( H = 2 \) where \([Ca^{2+}]_{JSR}\) before depletion is \( \sim 550 \mu M\), only 50% \( \sim 60\% \) of the events are \( Ca^{2+} \) sparks. Such a result is in agreement with Sato et al. [135], which revealed that a single RyR opening at lower SR load had a smaller possibility to initiate a spark. Similar to \([Ca^{2+}]_p\) and \([Ca^{2+}]_{JSR}\) distribution before a spark, we then measured \( N_{CU} \) and \( N_{CB} \) distribution (Fig. 2.8). Using the typical values of \([Ca^{2+}]_{JSR}, N_{CU}, \) and \( N_{CB} \) (Fig. 2.8), we obtained the curves of \( k_{CRU} \) as a function of \([Ca^{2+}]_p\) (Fig. 2.7 D). The result illustrates that at large Hill coefficient (\( H = 8 \)), the CRU opening rate is \( \sim 0.0002 \text{ms}^{-1} \) for the basal \([Ca^{2+}]_j \) (0.3 \( \sim 0.4 \mu M\)) and it steeply increases to 0.6 \text{ms}^{-1} at 1 \( \mu M\) which can be elevated to by the wavefront at the neighboring CRU sites (red and green bars in Fig. 2.7 A).

At \( H = 2 \), the CRU opening rate is 0.01 \text{ms}^{-1} to 0.02 \text{ms}^{-1} for the basal \([Ca^{2+}]_j \), meaning that the average latency is 50 ms \( \sim 100 \text{ms} \) for all available CRUs in the whole cell. To have a better comparison, we also showed the curve for \( H = 2 \) at \( c_j = 800 \mu M \) (the dot-dashed red curve in Fig. 2.7 D). This curve shows that the CRU opening rate at basal \( Ca^{2+} \) load is comparable to that at 1 \( \mu M \) (\( \sim 0.5 \text{ms}^{-1} \) at 0.4 \( \mu M \) versus \( \sim 2 \text{ms}^{-1} \) at 1 \( \mu M \)).

### 2.2.4 Role of molecular crowding

In the cardiac myocytes of a diversity of animals, the cell volume occupied by myofilaments and mitochondria is 34% \( \sim 91\% \) [24]. Previous models also used 0.34 \( \sim 0.68 \) as the ratio of the cytosolic volume to the cell volume [73, 75]. Under the constraint of reasonable \( Ca^{2+} \) spark profile, in this study, we mainly use 0.34 as the ratio of the cytosolic volume \( (V_i) \) to the whole-cell volume excluding SR, T-tubules, nucleus, and other small organelles \( (V_{CRU}) \). The proportion of nucleus and other organelles in the ventricles is typically less than \( \sim 15\% \) [24], meaning that 0.34 is a reasonable value for \( V_i/V_{CRU} \). In this section, we assessed the effect of molecular crowding on both \( Ca^{2+} \) spark profile and \( Ca^{2+} \) wave formation. As \( V_i \) varies, \([Ca^{2+}]_{JSR}\) could also change, which may affect the \( Ca^{2+} \) release current underlying \( Ca^{2+} \) sparks. To eliminate this factor, we applied the same release current from the red curve in Fig. 2.2 E to the high-resolution model that included only one CRU. Fig. 2.9 A-C demonstrate that \( V_i \) only
**Figure 2.8:** Statistics of $N_{CU}$ and $N_{CB}$ for different Hill coefficients and Ca$^{2+}$ loads. $N_{CU}$ for $H = 8$ is much larger than that for $H = 2$, whereas $N_{CB}$ for $H = 8$ is much smaller than that for $H = 2$.

Influences the spark amplitude, and has no significant effect on spark width and spark duration. To explain this behavior, we also present the longitudinal line-scan images of spark for different $V_i/V_{CRU}$ values, shown in Fig. 2.9 D. We then examined the effect of $V_i$ on Ca$^{2+}$ waves using the detailed model which has 2 compartments in each direction of the cytosol and NSR. The simulations are carried out at cytosolic Ca$^{2+}$ load 0.4 μM in the permeabilized cells. The results (Fig. 2.9 E) show that the time interval between consecutive waves is minimal at $V_i/V_{CRU} \sim 0.4$. When $V_i/V_{CRU}$ increases above 0.6, more spontaneous sparks occur, Ca$^{2+}$ waves become less organized, and the time interval between waves is longer. On the other hand, when $V_i/V_{CRU}$ decreases below 0.4, the time interval between waves also increase. Moreover, the local $[Ca^{2+}]_i$ peak decreases from 10 μM to 3 μM as $V_i/V_{CRU}$ increases from 0.1 to 1. Notably, at $V_i/V_{CRU} = 0.4$, which is close to the value 0.34 used in most of the simulations in this paper, the local $[Ca^{2+}]_i$ peak is $5 \sim 7$ μM. Such a result is larger than the experiment measurements ($1 \sim 3$ μM,
Chapter 2 \( \text{Ca}^{2+} \) waves and delayed afterdepolarizations

\[ \text{Figure 2.9: Effect of molecular crowding.} \] Summary data of \( \text{Ca}^{2+} \) spark amplitude (A), width (FWHM, B), and duration (FDHM, C) for different values of \( V_i/V_{CRU} \), where \( V_i \) is the cytosolic volume, and \( V_{CRU} \) is the volume of space including cytosol, myofilaments, and mitochondria. The simulations are carried out using the high-resolution model, which contains only one CRU. The release current is from the red line in Fig. 2.2 E. D. Longitudinal line-scan images of sparks for different values of \( V_i/V_{CRU} \). E. Longitudinal line-scan images of \([\text{Ca}^{2+}]_p\) in the permeabilized cell with \( \text{Ca}^{2+} \) load at 0.4 \( \mu M \).

For the case without molecular crowding effect \( (V_i/V_{CRU} = 1) \), however, the local \([\text{Ca}^{2+}]_i\) peak is in agreement with some experiments. To explain the change of local \([\text{Ca}^{2+}]_i\) peak as \( V_i \) changes, we also present the luminal \( \text{Ca}^{2+} \) content \([\text{Ca}^{2+}]_{JSR}\) for different \( V_i/V_{CRU} \) (Fig. 2.10). In contrast to local \([\text{Ca}^{2+}]_i\), local \([\text{Ca}^{2+}]_{JSR}\) increases with the increase of \( V_i \).

An alternative way to interpret molecular crowding is to note that the average binding rates of buffers are affected. This is because myofilaments are compacted in the form of myofibers, and not all of them are accessible by \( \text{Ca}^{2+} \) on the time scale of diffusion from one CRU to the neighbors. Notice that the buffer of the SR \( \text{Ca}^{2+} \) pump is only located in the space [8, 95, 96].
Chapter 2 $\text{Ca}^{2+}$ waves and delayed afterdepolarizations

**Figure 2.10:** Longitudinal line-scan images of $\text{Ca}^{2+}$ concentration in SR ($[\text{Ca}^{2+}]_{JSR}$). The numbers on top of the images are $V_i/V_{CRU}$. The images show that increasing cytosolic volume results in an SR load increase.

around myofibers, and the buffers in the submembrane space are also not inside of myofibers. Therefore, the binding rates of these buffers remain the same. Fig. 2.11 shows that changes in buffers’ binding rates have a similar effect as the changes of cytosolic volume $V_i$.

2.3 Delayed afterdepolarizations in the intact cells

2.3.1 Addition of caffeine and ISO induce delayed afterdepolarizations

So far, we have focused on the $\text{Ca}^{2+}$ waves in the permeabilized cells. $\text{Ca}^{2+}$ waves could also occur in the intact cells during diastole to activate DAD as well as cardiac arrhythmias. Venetucci et al. [58] revealed that in addition to the enhanced RyR activity (by caffeine), SR $\text{Ca}^{2+}$ overload (by ISO to enhance SR $\text{Ca}^{2+}$ pump) was also required to produce $\text{Ca}^{2+}$ waves in the intact cells. To produce the DADs in the present study, we mimicked the effect of caffeine by decreasing $c^*$ from 3 $\mu M$ to 1.4 $\mu M$, and mimicked the effect of ISO by increasing SR $\text{Ca}^{2+}$ pump strength by 100%, enhancing $I_{Ks}$ conductance by 33% and modifying $I_{Ca,L}$ parameters as in Zhong et al. [1]. Moreover, we mimicked the phosphorylation of ISO on RyRs by decreasing the Hill coefficient from 12 to 8. The effects of caffeine and caffeine with ISO on CRU open probability are shown in Fig. 2.12 D. The virtual cells are paced at 0.5 Hz, and after they reach the steady-states, the pacing stopped. The results are shown in Fig. 2.12.
Chapter 2 Ca$^{2+}$ waves and delayed afterdepolarizations

**Figure 2.11: Effect of buffers’ binding rate.** Summary data of Ca$^{2+}$ spark amplitude (A), width (FWHM, B), and duration (FDHM, C) under different values of $f$, where $f$ is the multiplier of buffers’ binding rate. D. Longitudinal line-scan images of the sparks in A-C. E. Line-scan images of $[\text{Ca}^{2+}]_i$ in the permeabilized cell with Ca load at 0.4 $\mu M$. For all cases, $V_i/V_{CRU} = 0.68$.

Compared to the permeabilized cells, here RyR has a larger RyR opening rate ($\alpha = 0.3 \text{ ms}^{-1}$ vs $\alpha = 0.12 \text{ ms}^{-1}$ in the permeabilized cell). Under the condition of control (Fig. 2.12 A), which has a strong RyR cooperativity (H = 2) and low Ca$^{2+}$ sensitivity ($c^* = 3 \mu M$), only spontaneous Ca$^{2+}$ sparks occur during diastole. The release current and spark profile are consistent with those in the permeabilized cells at 0.1 $\mu M$ (Fig. 2.12 E). The addition of caffeine decreases Ca$^{2+}$ transient (2.1 $\mu M$ for control vs. 1.5 $\mu M$ for caffeine) and whole-cell averaged $F/F_0$ (12 for control versus 10 for caffeine; Fig. 2.12 B). Because RyR is leakier, the SR load decreases from 740 $\mu M$ (control) to 690 $\mu M$ (Fig. 2.12 B). With the additional application of ISO, SR load is restored and elevated to 780 $\mu M$. In this case, propagating Ca$^{2+}$ waves occur (line-scan...
Chapter 2 \( \text{Ca}^{2+} \) waves and delayed afterdepolarizations

**Figure 2.12:** Combination of caffeine (enhance \( \text{Ca}^{2+} \) sensitivity of RyR) and ISO (phosphorylates RyR, enhances \( I_{c,L} \), SR pump, and \( I_{Ks} \)) initiates DADs in littermate control myocytes pacing at 0.5 \( Hz \). Here, \( \alpha \) is increased to 0.3 \( ms^{-1} \). Before the simulations terminate, the virtual cells stop pacing for 6 s. **A.** Top to bottom: Longitudinal line-scan image of fluorescence \( (F/F_0) \), whole-cell averaged fluorescence profile \( (F/F_0) \), membrane voltage \( (V_m) \), whole-cell averaged \( [\text{Ca}^{2+}]_i \) (or \( c_i \)), and the whole-cell averaged \( [\text{Ca}^{2+}]_{JSR} \) (or \( c_j \)). The RyR parameters of interest are \( H = 12 \) and \( c_\text{V} = 3 \) \( \mu M \). **B.** Similar to A, with RyR phosphorylated by caffeine. \( H = 12 \) and \( c_\text{V} = 1.4 \) \( \mu M \). **C.** Similar to B, with additional application of ISO. \( H = 8 \) and \( c_\text{V} = 1.4 \) \( \mu M \). Asterisks denote DADs. The propagating speed is 87 \( \mu m/s \) for the wave denoted by the white arrow. **D.** CRU open probabilities for control, caffeine, and caffeine+ISO. **E.** \( \text{Ca}^{2+} \) release current underlying a spark in **A** and in the permeabilized cell at 0.1 \( \mu M \). **F.** Statistics of spark profile corresponding to **A**. \( n = 71 \). Mean ± standard error.

image in Fig.2.12 C, and 2.5) with propagating speed 87 \( \mu m/s \) (white arrow). As a result of \( \text{Ca}^{2+} \) waves in the diastolic phase, DADs occur (asterisks in Fig.2.12 C). The line-scan image shows that \( \text{Ca}^{2+} \) waves initiate at multiple sites, and some of them collide with each other.

To understand the mechanism of DAD initiation under ISO, we dissected the roles of SR load
Chapter 2 $Ca^{2+}$ waves and delayed afterdepolarizations

**Figure 2.13: Effect of SR load and Hill coefficient on DAD formation.** Higher $[Ca^{2+}]_{SR}$ and $[Ca^{2+}]_i$ facilitate to produce DADs (blue lines vs. red lines), whereas stronger Hill coefficient suppresses DADs (green lines vs. blue lines). For all lines, $c^* = 1.4 \mu M$.

and RyR cooperativity. Fig. 2.13 shows that ISO elevates $Ca^{2+}$ both in SR and in the cytosol (blue lines vs. red lines), which work together to initiate $Ca^{2+}$ waves. In addition, reducing the Hill coefficient promotes $Ca^{2+}$ wave formation, although diastolic SR load is reduced (blue line vs. the green line). This result indicates that there is an optimal range of Hill coefficient to initiate $Ca^{2+}$ waves in the intact cells, and this behavior is in agreement with the result in the permeabilized cells, where $Ca^{2+}$ waves occur for Hill coefficient in the range of 5 to 7 at 0.2 $\mu M$ (Fig. 2.5).

In addition, the transition time $\tau_u$, which characterizes the time scale from the CSQN-bound closed state to CSQN-unbound closed state, plays a role in DAD formation. The results in Fig. 2.14 show that a small constant of $\tau_u$ (150 ms) does not trigger $Ca^{2+}$ waves, while a function of $c_j$ does when $\tau_u$ has a large value at low SR load (Fig. 2.1 B). This is because when $\tau_u$ is 150 ms, the spark refractoriness is small, and RyR is so leaky that SR load stays at a low level during the plateau phase of the action potential. As a result, $Na^+/Ca^{2+}$ exchanger removes much more $Ca^{2+}$ from the cell, while $Ca^{2+}$ influx through $I_{Ca,L}$ remains the same, leading to a lower $Ca^{2+}$ content in the SR during diastole.
2.3.2 Delayed afterdepolarizations occur at an intermediate strength of RyR cooperativity

In Fig. 2.12, several factors other than RyR gating are involved in the presence of ISO to induce DADs: $I_{Ca,L}$, SR pump, and $I_{Ks}$. To have a clearer understanding of the role of RyR gating, we investigated the effects of $c^*$ and Hill coefficient in this section. Fig. 2.15 A shows the behaviors of the action potential in the parameter space $c^*$ versus H in the presence of ISO.
Chapter 2 \( \text{Ca}^{2+} \) waves and delayed afterdepolarizations

**Figure 2.15:** Dependence of afterdepolarization initiation on RyR gating with the application of ISO. A Initiation of afterdepolarizations in the parameter space \( c^* \) vs. H. EAD\(_2\): phase 2 EADs; EAD\(_3\): phase 3 EADs; RF: repolarization failure. The open black circle at H = 8 and \( c^* = 1.4 \, \mu M \) corresponds to Fig. 2.12 C. B - F Representative time traces of action potentials and \( \text{Ca}^{2+} \) transients from panel A: H = 12 and \( c^* = 3 \, \mu M \) (B), H = 6 and \( c^* = 1.4 \, \mu M \) (C), H = 4 and \( c^* = 2.6 \, \mu M \) (D), H = 6 and \( c^* = 1 \, \mu M \) (E), H = 6 and \( c^* = 1.8 \, \mu M \) (F). The filled arrows denote external stimulus, the open arrows denote EADs, and the asterisks denote DADs. At the bottom of D, E, and F show the corresponding longitudinal line-scan images.

Here, the addition of ISO is assumed to have no effect on RyR gating. Fig. 2.5 previously showed that decreasing H below 8 at a \( \text{Ca}^{2+} \) concentration of 0.2 \( \mu M \) produced \( \text{Ca}^{2+} \) waves in permeabilized myocytes, consistent with the results of Fig. 2.15 A showing that decreasing H promotes DADs. This figure clearly shows that at a very large Hill coefficient, only normal action potentials occur (Fig. 2.15 B). On the other hand, if the Hill coefficient is small, action potentials oscillate and not fully repolarize (Fig. 2.15 D). Underlying the voltage oscillations are repeatedly whole-cell \( \text{Ca}^{2+} \) releases. In the transition region are the complex behaviors of the action potential. In this region, \( \text{Ca}^{2+} \) waves during the diastolic phase result in DADs (Fig.
Chapter 2 $Ca^{2+}$ waves and delayed afterdepolarizations

2.15. If $Ca^{2+}$ waves occur earlier, for instance, during phase 2 or phase 3 of an action potential, EADs are generated. In addition, enhanced SR leak by increasing $Ca^{2+}$ sensitivity of RyR prolongs the plateau phase, leading to the voltage-dependent EAD (left open arrow in Fig. 2.15 E), and the prolonged action potential duration by EAD, in turn, sustains $Ca^{2+}$ leak. We also observed that in the region of DADs, as Song et al. [77] shows, sub-threshold and super-threshold DADs randomly occur. For example, at $H = 8$ and $c^* = 1.4 \mu M$, both sub-threshold and super-threshold DADs can occur if the initial conditions are altered.

2.4 Discussion

In the present study, we considered RyR cooperativity to produce $Ca^{2+}$ waves using the physiologically detailed model that contains $\sim 16,000$ spatially distributed CRUs. We showed that strong RyR cooperativity is critical to initiating propagating $Ca^{2+}$ waves in the permeabilized cells (Fig. 2.5 A). Strong RyR cooperativity means that RyRs behave collectively; that is to say, when most of the neighbors are closed, the RyR tends to close, and when most of the neighbors are open, the RyR tends to open. From the perspective of modeling, RyR cooperativity increases the Hill coefficient in the gating functions Eq. 2.1. With a larger Hill coefficient, RyR has a much smaller open probability at small $Ca^{2+}$ concentration, indicating that the spark rate at the basal load ($0.3 \mu M$ and $0.4 \mu M$) is sufficiently low to elevate $[Ca^{2+}]_{JSR}$ (Fig. 2.7 D). However, the spark rate is not too small so that several CRUs in a confined space can fire simultaneously to generate mini-waves, which brings the $Ca^{2+}$ concentration at the neighboring CRUs to be around $1 \mu M$ (Fig. 2.7 D). At this concentration, together with the elevated SR load, the large Hill coefficient results in a large RyR opening rate of $\sim 0.1 ms^{-1}$ at the adjacent CRUs. Thus, the CRUs stay quiescent until the mini-wavefront arrives, sustaining the waves to propagate (Fig. 2.5 and 2.6) as in the classical fire-diffuse-fire mechanism [90, 119].

On the other hand, if the Hill coefficient is very small (2, for example), RyR opening rate is so high at the basal $Ca^{2+}$ concentration that most CRUs randomly open in $50 ms \sim 100 ms$. Even though some mini-waves may occur, most of the quiescent CRUs throughout the cell will open before the wavefront arrives. In this case, no well-defined $Ca^{2+}$ waves occur. In the
intact cells, the increased Ca\(^{2+}\) spark rate at relatively low Ca\(^{2+}\) concentration due to decreased Hill coefficient results in sustaining Ca\(^{2+}\) leak from SR (Fig. 2.15 A&D). The large Ca\(^{2+}\) leak persistently drives Na\(^+\)/Ca\(^{2+}\) exchanger to extrude Ca\(^{2+}\), which depolarizes the membrane voltage and forms the EAD-like oscillations.

The significance of this study is that it reveals the critical role of RyR cooperativity in Ca\(^{2+}\) wave formation. It has been reported that in heart failure, RyR becomes hyperphosphorylated [80, 81], which is intimately related to the dissociation of regulatory protein FKBP 12.6 by protein kinase (PKA) and thus increase of SR Ca\(^{2+}\) leak. Two interpretations are present to explain the role of hyperphosphorylation in RyR gating. One is that RyR hyperphosphorylation increases steady-state open probability \(P_o\) and thus increases SR Ca\(^{2+}\) leak, supported by both experiments [80, 81] and simulation [136]. The other is that the RyR gating function becomes less steep by hyperphosphorylation [87]. Both of them indicate that strong RyR cooperativity in healthy cells stabilizes RyR and thus prevents arrhythmogenic Ca\(^{2+}\) waves, which is in agreement with the finding in the present study. Simulations of both the permeabilized cells and the intact cells at low cytosolic Ca\(^{2+}\) load show that Ca\(^{2+}\) waves occur when RyR cooperativity is reduced to an intermediate large value (from 12 to \(\sim 8\); Fig. 2.5 A, Fig. 2.13, and Fig. 2.15).

The reason is that, when RyR cooperativity is too strong, CRU firing probability is too small at low cytosolic Ca\(^{2+}\) load (Fig. 2.12 D), and not enough spontaneous sparks occur simultaneously in a confined space to serve as a Ca\(^{2+}\) wave trigger. If the mini-waves occur, which is rare, the strong RyR cooperativity also causes CRU firing probability to be too small at the wavefront, where cytosolic Ca\(^{2+}\) concentration is 0.6 \(\sim 1.2 \mu M\) (Fig. 2.12 D and Fig. 2.7 A). Previous theoretical studies indicate that the Hill coefficient can be as large as 10 [87, 137], supporting the present study.

Similar to the RyR cooperativity (Hill coefficient), Ca\(^{2+}\) sensitivity of RyR also regulates membrane voltage. When Ca\(^{2+}\) sensitivity is high, CRU firing probability is also high during diastolic Ca\(^{2+}\) concentration (Fig. 2.12 D), allowing more Ca\(^{2+}\) sparks to spontaneously fire in a confined space, which serve as the Ca\(^{2+}\) wave triggers (Fig. 2.15 A). In addition, high Ca\(^{2+}\) sensitivity significantly alters Ca\(^{2+}\) releases during the plateau phase of the action potential.
Chapter 2 Ca$^{2+}$ waves and delayed afterdepolarizations

This aberrant Ca$^{2+}$ release drives NCX to the forward mode and thereby prolongs action potential (Fig. 2.15 E). When Ca$^{2+}$ sensitivity is too low, however, CRU firing probability is small in the range of cytosolic Ca$^{2+}$ concentration at the wavefront. As a result, Ca$^{2+}$ waves rarely initiate and cannot propagate.

Besides RyR gating, Ca$^{2+}$ overload also facilitates to produce Ca$^{2+}$ waves. In the permeabilized cells, Bovo et al. [125] showed that spontaneous Ca$^{2+}$ sparks evolve to Ca$^{2+}$ waves as the cytosolic Ca$^{2+}$ load increases. Here, we observed the same phenomenon in the simulations (Fig. 2.5). Several factors synergize to produce this behavior. First, higher cytosolic Ca$^{2+}$ load results in higher spark frequency, and a thus higher probability that a small number of CRUs open simultaneously, which can largely elevate [Ca$^{2+}$]$_i$ at the neighboring CRUs and form propagating waves. Second, at high Ca$^{2+}$ load, the SR Ca$^{2+}$ pump activity is enhanced, which elevates [Ca$^{2+}$]$_{JSR}$ to a higher level, additionally promoting the spark frequency. Third, because the basal [Ca$^{2+}$]$_i$ is higher, [Ca$^{2+}$]$_i$ at the neighboring CRUs could be elevated to an even higher value, facilitating the waves to propagate. On the other hand, in the intact cell, SR Ca$^{2+}$ overload is required to trigger Ca$^{2+}$ waves and thus DADs. Fig. 2.13 shows that with the same RyR activity, an increment of [Ca$^{2+}$]$_{JSR}$ triggers Ca$^{2+}$ waves. This result suggests that there is a threshold of SR load, which confirms the experimental findings [55, 58, 138–140]. The increment of SR load is achieved by adding ISO, which promotes Ca$^{2+}$ influx through long-type Ca$^{2+}$ channels so that more Ca$^{2+}$ is present in the cell and simultaneously enhances SR Ca$^{2+}$ pump so that more Ca$^{2+}$ is stored in SR. In this way, more RyRs in the quiescent CRUs move from the CSQN-bound state to the CSQN-unbound state that has a larger opening rate (Eq. 2.1), which increases the CRU open probability at the wavefront and thus sustains the Ca$^{2+}$ waves and DADs.

Another feature of this model is that it produces a realistic Ca$^{2+}$ spark profile with reasonably small Ca$^{2+}$ release current consistent with experimental measurements ($F/F_0 = 1.75$, FWHM = 2.2 µm, FDHM $\sim$ 13 ms, $I_{rel}$ of 1 $\sim$ 3 pA, Fig. 2.2) [9, 92–94]. This is achieved by incorporating molecular crowding. To implement molecular crowding, we assumed that myofilaments and mitochondria are uniformly distributed throughout the cytosolic space, and reduced the
Chapter 2 Ca$^{2+}$ waves and delayed afterdepolarizations

effective cytosolic volume to be 0.5 µm$^3$ while not affecting the diffusion time constants of Ca$^{2+}$ and mobile buffers. This effective cytosolic volume is within the experimental estimation which reveals that myofilaments and mitochondria occupy 31% ~ 91% of the cell volume, considering that the nucleus and other organelles occupy a small fraction of cell volume [24]. The investigation of the molecular crowding effect shows that spark amplitude decreases with the increase of the effective cytosolic volume, yet the spark width and duration do not change significantly (Fig. 2.9 A-C). Reducing cytosolic volume proportionally reduces the amount of buffers in the cytosol, leading to reduced spark amplitude. As the spark amplitude decreases, the half maximum at which the spark width and duration are measured, proportionally decreases as well, giving rise to unchanged spark width and duration.

As to Ca$^{2+}$ waves, molecular crowding modulates the time interval between consecutive waves by the competition of two effects. Increased cytosolic volume enhances buffers’ ability to trap Ca$^{2+}$ spark, which hinders Ca$^{2+}$ From diffusing to the neighboring CRUs and prevents Ca$^{2+}$ waves from occurring. On the other hand, more Ca$^{2+}$ trapped by the buffers in the cytosol reduces the decrease of local $[Ca^{2+}]_i$ by SR Ca$^{2+}$ pump, which elevates $[Ca^{2+}]_{JSR}$ to a higher level (Fig. 2.10), elevates local $[Ca^{2+}]_i$ peak, and facilitates Ca$^{2+}$ waves to propagate. As a result of the competition, there is a minimal time interval between waves at $V_i/V_{CRU} \approx 0.4$.

Another interpretation of molecular crowding is the altered binding rates of buffers in the cytosol. Because myofilaments are compacted in the form of myofibers, which occupy most space of the cell, not all buffers in the cytosol are accessible to Ca$^{2+}$ in the time scale for Ca$^{2+}$ to spread to the neighboring Z-lines. Therefore, the overall binding rate is effectively reduced. It should be noted that the SR Ca$^{2+}$ pump buffer is not affected, because this buffer is located at the network SR surrounding myofibers, meaning that all the SR Ca$^{2+}$ pump buffer is accessible to Ca$^{2+}$. The same reason applies to the buffers in the submembrane space. Fig. 2.11 shows that the decrease in binding rates increases spark amplitude, while the spark width and duration stay unchanged. In addition, a minimal time interval between Ca$^{2+}$ waves exists at $f \sim 0.5$. These results suggest that the proposed two interpretations of molecular crowding are essentially the same.
Chapter 2 Ca\textsuperscript{2+} waves and delayed afterdepolarizations

In addition to molecular crowding, one mobile buffer, ATP, also modulates Ca\textsuperscript{2+} spark profile in the permeabilized cell. Fig. 2.2 B-D shows that ATP significantly increases spark amplitude and duration while decreases spark width. Subsequent investigation (Fig. 2.3) and analysis reveal that ATP influences Ca\textsuperscript{2+} spark in two ways. One way is to carry Ca\textsuperscript{2+} out of the dyadic space, which reduces [Ca\textsuperscript{2+}]\textsubscript{p} and thus reduces spark duration. With spark duration reduced, the SR load is elevated, such that the release current peak reaches to a higher value. The other way is that ATP serves as a buffer, further reducing [Ca\textsuperscript{2+}]\textsubscript{p}. Even though ATP has a large dissociation constant (\(K_d = 200 \mu M\) [131, 141]), the total concentration of ATP is also large (4 mM), meaning that the amount of Ca\textsuperscript{2+}-bound ATP is comparable to other buffers.

Limitations

Although this model demonstrates its ability to produce Ca\textsuperscript{2+} waves with reasonable propagating speed, Ca\textsuperscript{2+} sparks with normal size and underlying release current, and complex DAD behaviors in the intact cells, it is important to acknowledge that the local [Ca\textsuperscript{2+}]\textsubscript{i} during waves are still too high. Experiments estimate that local [Ca\textsuperscript{2+}]\textsubscript{i} during waves are in the range of 1 \~ 3 \mu M [8, 95, 96]. In this model, however, the local [Ca\textsuperscript{2+}]\textsubscript{i} during waves are in the range of 5 \~ 7 \mu M (Fig. 2.9 E and 2.14 A). The large [Ca\textsuperscript{2+}]\textsubscript{i} during waves is a long-standing problem. Here, we proposed two possible explanations. First, some pathways are connecting neighboring Z-lines. The schematic diagram of the sub-cellular structure of cardiac myocytes (Fig. 1.2) indicates that myofilaments are packed in the form of myofibers, between which there is a long and narrow space in the longitudinal direction [24]. Ca\textsuperscript{2+} may diffuse much faster in this long and narrow space than in myofibers where myofilaments block the way. If it is true, the molecular crowding effect should be modeled more realistically, rather than varying the cytosolic volume (Fig. 2.9) or associate constant of buffers in the cytosolic space (2.11). Second, the irregular structure of Z-lines and the existence of corbular SRs between Z-lines may be of great importance. Experiments [10, 90, 142] show that Z-lines are not evenly spaced in the longitudinal direction. At some points, neighboring Z-lines merge together and form a spiral ladder structure. Since the distance between RyR clusters in Z-lines is about half of those in neighboring Z-lines, this irregular structure facilitates Ca\textsuperscript{2+} wave propagation. Similarly, the existence of corbular SRs
Chapter 2 Ca\(^{2+}\) waves and delayed afterdepolarizations

[143, 144] effectively shortens the distance between neighboring Z-lines and facilitates Ca\(^{2+}\) wave propagation. However, my attempts show that incorporation of the corbular SR and the complex structure of Z-lines do not facilitate Ca\(^{2+}\) wave formation.

2.5 Supporting Information

The following videos are available at the public repository:

https://github.com/mingwang-zhong/Calcium-Wave

**Movie S1** Transition from spontaneous Ca\(^{2+}\) releases to propagating Ca\(^{2+}\) waves. The movie shows the cytosolic Ca\(^{2+}\) concentration in a 3-dimensional virtual myocyte, which is permeabilized and bathed at 0.4 \(\mu M\) (Fig. 2.6 A).

**Movie S2** Ca\(^{2+}\) wave initiation during the diastolic phase in the intact cell. The movie shows the cytosolic Ca\(^{2+}\) concentration in a 3-dimensional virtual myocyte, which is pacing at 0.5 \(Hz\) (Fig. 2.12 C). During the action potential, Ca\(^{2+}\) releases are synchronized throughout the cell. During the diastolic phase, Ca\(^{2+}\) waves initiate from local sparks and propagate around.
 Early afterdepolarizations in long QT syndromes

3.1 Na+/Ca\(^{2+}\) exchanger mediated EADs in long QT syndrome type 2

(This section is adapted from the published work: Zhong et al. [1].)

To study the phenotype of human arrhythmia, Brunner et al. [27] created a transgenic rabbit model of long QT syndrome type 2 that overexpresses a pore mutant of the human gene KCNH2 (HERG-G628S) in cardiomyocytes to eliminate \(I_{Kr}\) currents. As a result, these rabbits exhibited a prolonged QT interval and a high incidence of SCD (>50% at 1 year of age) due to PVT [27]. Ex vivo optical mapping used to investigate the underlying substrate of arrhythmia revealed a prominent spatial dispersion of AP duration (APD) and discordant APD alternans [27, 99]. The observation of triggered activity in the form of EADs in isolated myocytes under \(\beta\)-adrenergic stimulation has supported the hypothesis that arrhythmia originates at the single-cell level [98, 100].

Normal Ca\(^{2+}\) cycling in cardiomyocytes is maintained by the interplay between the AP and Ca\(^{2+}\) release from the sarcoplasmic reticulum mediated by the ryanodine receptors. Upon depolarization, a small amount of Ca\(^{2+}\) enters the cell through sarcolemmal L-type calcium channels (LCCs) to activate RyR clusters that release a much larger amount of Ca\(^{2+}\) to the
cytosolic compartment. The sum of those localized Ca\(^{2+}\) release events (Ca\(^{2+}\) sparks) contributes to the transient rise of the whole-cell cytosolic calcium concentration \([Ca^{2+}]_i\) (Ca\(^{2+}\) transient). Subsequent removal of intracellular Ca\(^{2+}\) by the electrogenic sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchanger current (NCX) provides additional depolarizing current and can prolong the AP duration (APD), especially when RyR-mediated SR Ca\(^{2+}\) release termination is not robust.

Previous studies of LQT2 rabbit models showed that Ca\(^{2+}\)-mediated communication between RyRs and other Ca\(^{2+}\) transport complexes play a critical role in EAD formation \([28, 145]\). In rabbit LQT2 ventricular myocytes, the RyR activity is enhanced, resulting in a large number of late aberrant SR Ca\(^{2+}\) release events. These studies suggest that the protracted Ca\(^{2+}\) releases from individual RyR clusters provide a basis for an increased depolarizing NCX current, which suffices to maintain the membrane potential \((V_m)\) for a prolonged time in a window permissive for reactivation of LCCs, thereby causing EADs. The computational modeling \([28]\) validated this hypothesis by demonstrating that late aberrant Ca\(^{2+}\) releases result in a larger magnitude of NCX current during late phase 2 and early phase 3 of the AP, thereby slowing the repolarization and enabling L-type Ca\(^{2+}\) current reactivation during this vulnerable time window, which initiates EADs.

Most computer modeling studies to date have related EADs to the instability of \(V_m\) dynamics driven by reactivation of the L-type Ca\(^{2+}\) current \(I_{Ca,L}\) during the plateau phase of the AP \([146–151]\). The role of RyR-mediated Ca\(^{2+}\) release in EAD formation was also modeled in the setting of pharmacologically-induced LQT2 with a generic block of \(I_{Kr}\) \([152]\). This study found that reduced SR Ca\(^{2+}\) release can protect against EADs by reducing the Ca\(^{2+}\) transient amplitude and NCX current. In contrast, another study of transgenic LQT2 rabbits showed that stabilization of RyR activity by a CAM kinase inhibitor (KN93) increased Ca\(^{2+}\) transient amplitude while at the same time eliminating EADs \([28]\). The computer modeling study of LQT2 ventricular myocytes under \(\beta\)-adrenergic stimulation reproduced experimental observations \([28]\) and further showed that, in the presence of RyR hyperactivity, the depolarizing NCX current could be significantly larger during late phase 2 and early phase 3 of the AP, thereby promoting reopening of LCCs and EADs. Importantly, modeling revealed that NCX
current is enhanced despite SR depletion and reduced $\text{Ca}^{2+}$ transient amplitude (i.e. reduced $[\text{Ca}^{2+}]_i$ peak during early phase 2 of the AP).

However, the mechanism why forward mode NCX current can be enhanced without significantly increasing the whole-cell cytosolic calcium concentration during the vulnerable period of repolarization is still not fully understood. Furthermore, the role of NCX in triggered activity in the setting of LQT2 has not been investigated experimentally. Here we use a physiologically detailed ventricular myocyte model with spatially distributed $\text{Ca}^{2+}$ release to further elucidate the mechanism of NCX-mediated triggered activity in LQT2 ventricular myocytes, and our collaborators validate experimentally the computer modeling predictions by pharmacological partial block of NCX.

The modeling results show that the local NCX current is a saturating nonlinear function of the local submembrane calcium concentration. Therefore, a larger number of smaller amplitude $\text{Ca}^{2+}$ release events generated by hyperactive RyRs and reduced SR load, can increase whole-cell forward mode NCX about two-fold as a smaller number of larger amplitude $\text{Ca}^{2+}$ release events generated by stable RyRs and normal SR load, without significantly increasing calcium transient. The modeling study further dissects the arrhythmogenic role of two different RyR channel gating mechanisms that may underlie the increased spark rate observed in permeabilized myocyte experiments: increased sensitivity to cleft calcium concentration leading to increased RyR open probability ($P_o$), and shortened refractoriness leading to faster recovery of local RyR clusters. This study reveals that the combination of both effects is most effective at potentiating NCX and EADs. Finally, this study highlights the role of NCX in triggered activity by demonstrating computationally that partial NCX blockade eliminates EADs. To validate this prediction, our collaborators use cellular electrophysiology and confocal $\text{Ca}^{2+}$ imaging study of ventricular myocytes derived from LQT2 hearts with pharmacologically suppressed NCX. The observations confirm the critical role of NCX in EAD initiation.
3.1.1 Methods

3.1.1.1 Experiments

Myocyte Isolation

All procedures were approved by the Rhode Island Hospital Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Ventricular myocytes were isolated from the hearts of transgenic LQT2 (n=3) female NZW rabbits (3.5 kg) using standard enzymatic digestion procedures. In brief, the heart was excised from euthanized rabbits and perfused for 5 to 7 minutes with a nominally Ca\textsuperscript{2+}-free solution containing (in mM): 140 NaCl, 4.4 KCl, 1.5 MgCl\textsubscript{2}, 0.33 NaH\textsubscript{2}PO\textsubscript{4}, 16 taurine, 5 HEPES, 5 pyruvic acid, and 7.5 glucose. Next, the heart was perfused for 10 to 15 minutes with the same solution to which 0.65% collagenase type II (Worthington Biochemical, L), and 0.1% BSA were added. The LV was minced, and the cells were dispersed with a glass pipette for 3-5 minutes in a solution containing (in mM): 45 KCl, 65 K-glutamate, 3 MgSO\textsubscript{4}, 15 KH\textsubscript{2}PO\textsubscript{4}, 16 taurine, 10 HEPES, 0.5 EGTA, and 10 glucose and 1% BSA (pH 7.3). The cell suspension was filtered through a 100-\textmu m nylon mesh, and plated on laminin coated coverslips in medium M199, and used within 6 to 8 hours.

Cell Electrophysiology and Ca\textsuperscript{2+} Imaging

Action Potentials (APs) were recorded using the whole-cell patch-clamp technique at 35±2°C using Axopatch 200B amplifier and DIGIDATA 1322A interface (Axon Instruments, CA) [28]. The external solution contained (in mM): 140 NaCl, 5.4 KCl, 1.85 CaCl\textsubscript{2}, 0.5 MgCl\textsubscript{2}, 10 HEPES, and 5.6 glucose (pH 7.4). Patch pipettes were filled with the following solution (in mM): 90 K-aspartate, 50 KCl, 5 MgATP, 5 NaCl, 1 MgCl\textsubscript{2}, 0.1 Tris GTP, 10 HEPES, and 0.1 Rhod-2 (Molecular Probes, OR (pH 7.2). APs were evoked by the application of 3-ms-long voltage pulses with amplitude 20% above the threshold level. Intracellular Ca\textsuperscript{2+} imaging was performed using the Leica SP5 confocal system in a line scan mode. Rhod-2 was excited by
543 nm laser and the fluorescence was acquired at 560-660 nm wavelengths. Calcium transients were analyzed using Leica Software, Origin 8.2 (OriginLab, Northampton, MA) and Image J (NIH, Bethesda, MA).

3.1.1.2 Mathematical modeling

**Rabbit ventricular myocyte model**

We use a physiologically detailed ventricular myocyte model with Ca\(^{2+}\) cycling coupled to membrane voltage (\(V_m\)) dynamics \cite{28} to investigate the ionic mechanisms of EAD formation and elimination in LQT2 myocytes under different conditions. This multi-scale model, originally developed by Restrepo et al \cite{73,154}, bridges the submicron scale of individual Ca\(^{2+}\) release units (CRUs) and the whole-cell by simulating a realistically large number of 20,000 diffusively-coupled CRUs spatially distributed throughout the cell, with 4 LCCs colocated with 100 RyRs in each CRU (Fig. A.1 in the Supporting Material). It accounts for the stochastic nature of the trigger and releases Ca\(^{2+}\) fluxes by using a Markov description of channel kinetics for both LCCs and RyRs. In addition, it describes the bidirectional coupling of Ca\(^{2+}\) and \(V_m\) dynamics by incorporation of a full set of sarcolemmal currents including \(I_{Ca,L}\), \(I_{NCX}\), \(I_{Ks}\), \(I_{to,f}\), \(I_{K1}\), and \(I_{NaK}\). We exclude \(I_{Kr}\) in order to model LQT2 myocytes. This model, therefore, has the unique capability to explore the effects of altering RyR activity at the single-channel level on EAD formation at the whole-cell level.

The model we use is an extension of the model of Restrepo et al. \cite{73,154} that includes a higher spatial resolution of the diffusive coupling between CRUs, a more quantitative description of Ca\(^{2+}\) buffers, and a 16-state Markov model of LCCs. The \(I_{Ca,L}\) model reduces at the whole-cell level to a Hodgkin-Huxley formulation with voltage- and Ca\(^{2+}\)-dependent inactivation. This Markov model of LCCs provides more flexibility for fitting whole-cell experimental measurements of \(I_{Ca,L}\) including a larger window \(V_m\) range of this current. We have also modified the Ca\(^{2+}\)-dependent activation of the NCX current to be time-dependent to account for more recent experiments showing a slower time scale of allosteric Ca\(^{2+}\) activation \cite{155}. These changes are detailed in the online supplement of Zhong et al. \cite{1}.
Chapter 3 Early afterdepolarizations in long QT syndromes

To mimic the effect of β-adrenergic stimulation with isoproterenol (ISO), the model was used to fit the experimental data that results in an increase in the SERCA uptake rate as well as increases in $I_{Ca,L}$ and $I_{Ks}$ currents [28]. The effect of β-adrenergic stimulation under steady-state conditions was modeled to produce non-transient EADs that persist on an experimental time scale longer than the time scales of ISO-induced $I_{Ca,L}$ and $I_{Ks}$ phosphorylation [98].

Computer simulations were carried out by pacing myocytes at 0.25 Hz in accordance with experiments [28] until a steady state was reached. During pacing, whole-cell cytosolic and SR calcium concentrations were recorded, denoted by $[Ca^{2+}]_i$ and $[Ca^{2+}]_{JSR}$, respectively, along with $V_m$ and individual sarcolemmal currents. In addition, experimental confocal line scans were emulated by recording the local $[Ca^{2+}]_i$ along a longitudinal row of CRUs passing through the center of the myocyte and parallel to its long axis.

Model of hyperactive RyRs

SR release is mediated by 100 RyRs collocated with LCCs in each CRU. Each RyR is described by a four-state model [73] (also shown in Fig. 3.1), which assumes that luminal Ca$^{2+}$ regulates the sensitivity of the RyR channels via auxiliary proteins triadin/junctin (T/J) interacting with the luminal Ca$^{2+}$ buffer calsequestrin (CSQN) [126]. This mechanism is incorporated by allowing CSQN to bind to the RyR/T/J complex when Ca$^{2+}$ is depleted in the JSR and by choosing the RyR closed to open transition rate in the CSQN bound state ($k_b$) to be much smaller than the one ($k_u$) in the CSQN unbound state. The reduced sensitivity in the CSQN bound state both contributes to Ca$^{2+}$ spark termination and induces a refractory [87, 127] state whereby CSQN must unbind from RyRs before future sparks are possible. Experiments reported a large RyR unitary calcium current under near-physiological ionic conditions (0.35–0.6 pA at 1 mM $[Ca^{2+}]_{JSR}$) [156, 157], which is ~10-fold larger than the value assumed in Restrepo et al. [73]. Therefore, we follow Sato et al. [135] by using RyR closed-to-open transition rates that produce maximum open probability around 0.1 (Figure 2C). We also introduce an SR load dependence of the closed-to-open rates ($k_u$ and $k_b$) to model the increase of RyR open probability at high $[Ca^{2+}]_{JSR}$, which is not captured by the original Restrepo et al. model.
In that model, luminal gating of RyR is solely mediated by CSQN binding to the RyR/T/J complex and the RyR open probability becomes independent of SR load when all RyRs occupy the CSQN unbound state, which occurs for $[Ca^{2+}]_{JSR}$ larger than $\approx 600 \mu M$ (Fig. A.2). In the present model, RyRs are sensitive to luminal Ca$^{2+}$ (in addition to being regulated by CSQN binding) as supported by a wide range of experiments (see [158] and references therein).

![Figure 3.1: Schematic illustration of the 4-state model of RyR gating.](image)

The rates $k_u$ and $k_b$ (corresponding to $k_{12}$ and $k_{43}$, respectively, Restrepo et al. [73]) are given by

$$k_u = \frac{\alpha}{\left[1 + (c^*/c_p)^2\right] \left[1 + \left(c^*_{jsr}/c_{jsr}\right)^2\right]},$$

$$k_b = \frac{\beta}{\left[1 + (c^*/c_p)^2\right] \left[1 + \left(c^*_{jsr}/c_{jsr}\right)^2\right]},$$

respectively, where $c_p = [Ca^{2+}]_{cleft}$ and $c_{jsr} = [Ca^{2+}]_{JSR}$ are the calcium concentrations in the proximal space (dyadic cleft) and junctional SR (JSR) compartment, respectively. We model RyR hyperactivity both by shifting the open probability of RyRs towards lower cleft calcium concentration by reducing $c^*$ as further explained below and by shortening RyR refractoriness. The increase in open probability is consistent with lipid bilayer studies of hyperactive RyRs.

---

73
Figure 3.2: Effect of altering RyR gating parameters. (A) Spark restitution curves obtained by measuring the magnitude of SR Ca blinks in steady-state with a holding membrane potential $V_m = 0 \text{ mV}$. Blink magnitude is determined by taking the difference of the minimum $[\text{Ca}^{2+}]_{\text{JSR}}$ and $[\text{Ca}^{2+}]_{\text{JSR}}$ just before the spark begins. Blink magnitudes are binned into 20 ms intervals. The average values and standard errors are plotted as a function of time since the last spark. The blue and red lines are corresponding to $\beta/\alpha = 1$ and $\beta/\alpha = 0.01$, respectively, with $\alpha = 4 \text{ ms}^{-1}$ and $c^* = 7 \mu \text{M}$. (B) Spark recovery time as a function of $\beta/\alpha$. The data points are extracted from a single exponential fit of spark restitution curves as in (A). (C) Steady-state open probability of RyRs for $\beta/\alpha = 1$ and $c^* = 20 \mu \text{M}$ (red), $c^* = 3 \mu \text{M}$ (blue), and for high (solid lines) and low (dashed lines) $[\text{Ca}^{2+}]_{\text{JSR}}$. (D) Peak magnitude of $I_{\text{Ca,L}}$ current (red squares) and SR Ca$^{2+}$ release transients (blue circles) under voltage-clamp steps from holding potential -80 mV to step voltages between -40 and 40 mV for $c^* = 20 \mu \text{M}$ (red) and $c^* = 3 \mu \text{M}$ (blue). $I_{\text{Ca,L}}$ current peak did not differ between $c^* = 20 \mu \text{M}$ (solid red line) and $c^* = 3 \mu \text{M}$ (dashed red line). CICR response has a wider $V_m$ range for $c^* = 3 \mu \text{M}$ compared to $c^* = 20 \mu \text{M}$. From Zhong et al. [1].

[153] and the observed increased frequency of Ca$^{2+}$ sparks in permeabilized LQT2 myocytes where RyRs are hyperphosphorylated [28].

We can reduce refractoriness by increasing $k_b$ relative to $k_u$. The ratio $\beta/\alpha$ controls the relative reduction of the RyR opening rate in the CSQN bound state to the unbound state. By increasing this ratio, the channels that have transitioned to the bound state fail to keep the small opening rate, thus fail to suppress the continued sparking of the same CRU.
Chapter 3 Early afterdepolarizations in long QT syndromes

Refractoriness has previously been characterized by taking repeated nanoscopic measurements of SR Ca\(^{2+}\) blinks (local JSR depletions) \([159]\). This study found that the characteristic timescale for the magnitude of subsequent SR Ca\(^{2+}\) blinks to recover to the original magnitude was much longer than the timescale of the Ca\(^{2+}\) refilling. By replicating these experiments using our model, we produce a quantitative measurement of spark restitution. Fig. 3.2A shows that simulations with a larger value of \(\beta/\alpha\) have reduced refractoriness. When \(\beta/\alpha\) is smaller than 0.01 (0.01 corresponding to normal RyR activity), the magnitude of SR Ca\(^{2+}\) blinks recovers on a time scale determined by the rate of CSQN unbinding, whereas when \(\beta/\alpha\) is larger than 0.5, the refractory state does not inhibit RyR opening and so the magnitude of SR Ca\(^{2+}\) blinks recovers on a time scale determined by the SR refilling time. Fig. 3.2B illustrates this trend for values of \(\beta/\alpha\) ranging between 0.001 and 1.

In order to model increased Ca\(^{2+}\) sensitivity, we reduce \(c^*\) in Eqs. 3.1 and 3.2, which reduces the EC\(_{50}\) of RyR open probability, thereby increasing the transition rate to the open state at low cleft calcium concentration \(c_p\). This effect is illustrated in Fig. 3.2C where we plot the RyR open probability as a function of cleft calcium concentration \(c_p\) for \(c^* = 3 \, \mu M\) and \(c^* = 20 \, \mu M\). For the purpose of illustration in Fig. 3.2C, we restrict our attention to the limiting case \(\beta/\alpha = 1\) (corresponding to \(\alpha = \beta = k_p\)), where the refractory state does not inhibit RyR openings and the EC\(_{50}\) is given by

\[
EC_{50} = \frac{c^*}{\sqrt{1 + k_p \left[ 1 + \left( \frac{c^*_{jsr}}{c_{jsr}} \right)^2 \right]^{-1}}}. \tag{3.3}
\]

By varying the EC\(_{50}\) of RyR opening in this way, there is a measurable effect on the trend of Ca\(^{2+}\)-induced-Ca\(^{2+}\)-release (CICR) gain. When \(c^*\) is lower, the rate of spark recruitment due to \(I_{Ca,L}\) is increased above and below 5 mV (the peak voltage for RyR recruitment). The increase of RyR activity when \(c^*\) is reduced is much more potent in the voltage range of EAD onset than for the majority of the AP plateau (Fig. 3.2D).

We have previously shown \([28]\) that the mechanism for EAD initiation in this model is largely caused by downstream remodeling of cytosolic Ca\(^{2+}\) cycling, manifested by increased spark
frequency in permeabilized myocytes, and has less to do with the direct effect of reduced repolarization current due to the absence of $I_{Kr}$. To investigate the effect of altered Ca$^{2+}$ cycling, it is useful to distinguish between two extreme cases. The first corresponds to a choice of model parameters with enhanced RyR-mediated SR Ca$^{2+}$ leak due to both increased sensitivity of RyRs to cytosolic Ca$^{2+}$ and shortened refractoriness, referred to as the “hyperactive” RyR model. This model best reproduces the experimentally observed electrophysiological phenotype of LQT2 under β-adrenergic stimulation with hyperphosphorylated RyRs. The second corresponds to a choice of parameters where RyR activity is stabilized via Ca$^{2+}$-calmodulin dependent protein kinase II (CaMKII) inhibitor KN93 [28], referred to as the “stabilized” RyR model. We have deliberately chosen to only include the stabilizing effect of CaMKII inhibition on RyR activity. While KN93 may potentially influence other sarcolemmal currents, we are primarily interested here in exploring the effect of abnormal Ca$^{2+}$ handling on NCX current and EAD formation. In addition to the two above cases of stabilized and hyperactive RyRs, we investigate intermediate cases in which RyR activity is enhanced by increasing channel sensitivity to cytosolic Ca$^{2+}$ (decreasing $c^*$) or shortening the refractory period (increasing $\beta/\alpha$). While there is no known simple way to vary those two parameters individually in the experiment, we exploit the ability to vary them independently in the computational model to gain basic insights into the individual arrhythmogenic roles of increased RyR open probability and shortened refractoriness, which have not been clearly dissected to date.

### 3.1.2 Role of RyR refractoriness and open probability on AP phenotype

Fig. 3.3 compares results of simulations of LQT2 myocytes under β-adrenergic stimulation for model parameters corresponding to stabilized RyRs (A), and increased RyR sensitivity (B), reduced RyR refractoriness (C), and both increased sensitivity and reduced refractoriness denoted as hyperactive RyRs (D). For all the simulations, the intracellular sodium concentration reaches a steady-state value that varies by less than 3%.

Fig. 3.3B shows the effect of increased Ca$^{2+}$ sensitivity by reducing $c^*$ from 7 $\mu M$ to 3 $\mu M$. AP traces in the top panel show that the increased sensitivity causes only a minor deflection
in the AP waveform and is insufficient to evoke EADs. The increased RyR open probability results in a reduction in the diastolic SR Ca\(^{2+}\) load (middle panel). Since there is no difference in the luminal dependence of RyR gating, CSQN will bind to the channel and prevent SR Ca\(^{2+}\) release below 550 µM. As a result, the nadir of the SR depletion during systole remains
unchanged. This results in a reduced $\text{Ca}^{2+}$ transient amplitude (2nd panel). Confocal line scan equivalents show that the number of late $\text{Ca}^{2+}$ sparks during repolarization does not significantly increase when $\text{Ca}^{2+}$ sensitivity is increased in this way (Fig. 3.3A vs Fig. 3.3B bottom panels). Consequently, the NCX current is largely unchanged during the repolarization phase (Fig. 3.3B 4th panel).

We then compare the LQT2 model with stabilized RyRs to a model in which RyR refractoriness is hindered by increasing the RyR open rate in the bound state from $\beta/\alpha = 0.01$ to $\beta/\alpha = 0.6$. The top panel of Fig. 3.3C shows that without RyR refractoriness, AP repolarization is interrupted with a large EAD, consistent with the results of Terentyev et al. [28]. Without CSQN-mediated inactivation of RyRs at low SR load, both the diastolic and systolic SR loads are reduced dramatically (middle panel). $\text{Ca}^{2+}$ transient amplitudes are slightly smaller when refractoriness is reduced, while the rate of decay of the $\text{Ca}^{2+}$ transient is noticeably slower (second panel). This is due to the late aberrant $\text{Ca}^{2+}$ sparks that occur during repolarization when refractoriness is reduced which can be seen in the confocal line scan (bottom panel). When this late $\text{Ca}^{2+}$ release activity occurs, the NCX current shifts towards forward-mode (4th panel), which slows repolarization and allows $I_{\text{Ca,L}}$ to recover from inactivation.

When we model both increased $\text{Ca}^{2+}$ sensitivity and reduced RyR refractoriness, the $V_m$ traces show a significantly elongated AP with many EADs (top panel in Fig. 3.3D). As before, this model has a reduced SR $\text{Ca}^{2+}$ load (middle panel) but with a much greater reduction in $\text{Ca}^{2+}$ transient amplitude (2nd panel) and a slower rate of $\text{Ca}^{2+}$ transient decay. Due to the increased $\text{Ca}^{2+}$ sensitivity (Fig. 3.2D), CICR during the repolarization phase (-20 mV to 0 mV) is much

---

**Table 3.1:** Instantaneous values at the whole-cell level.

<table>
<thead>
<tr>
<th>RyR gating model</th>
<th>$[\text{Ca}^{2+}]_i$ ($\mu$M)</th>
<th>$[\text{Ca}^{2+}]_s$ ($\mu$M)</th>
<th>$J_{\text{rel}}$ ($\mu$M$_{\text{cyt}}$/ms)</th>
<th>Spark Rate (sparks/ms)</th>
<th>$I_{\text{NCX}}$ (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 20 ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stabilized</td>
<td>0.69</td>
<td>2.41</td>
<td>2.31</td>
<td>8951</td>
<td>-0.44</td>
</tr>
<tr>
<td>Hyperactive</td>
<td>0.25</td>
<td>0.98</td>
<td>1.29</td>
<td>7671</td>
<td>-0.13</td>
</tr>
<tr>
<td>t = 350 ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stabilized</td>
<td>0.09</td>
<td>0.15</td>
<td>0.04</td>
<td>107</td>
<td>-0.26</td>
</tr>
<tr>
<td>Hyperactive</td>
<td>0.1</td>
<td>0.2</td>
<td>0.11</td>
<td>609</td>
<td>-0.55</td>
</tr>
</tbody>
</table>
more sensitive to $I_{Ca,L}$ current, increasing the rate of late aberrant $Ca^{2+}$ releases during this period (bottom panel of Fig. 3.3D). There is, therefore, an even greater increase in the forward mode NCX current that slows repolarization and allows reactivation of $I_{Ca,L}$ (4th panel). This results in the repolarization being interrupted multiple times, leading to multiple EADs.

The interruption to repolarization occurs in a vulnerable $V_m$ window where LCCs begin to recover from inactivation but have not yet fully deactivated [149, 160]. For time $t > 300 ms$ after initial depolarization, corresponding to late phase 2 of the AP, $V_m$ enters the critical window where $I_{Ca,L}$ begins to recover from inactivation but is still sufficiently activated to allow the current to increase. It is inside this window that $V_m$ in the hyperactive RyR model begins to diverge from the stabilized model towards the first EAD (top panel of Fig. 3.3D). The whole-cell NCX current during this time window is larger in the hyperactive RyR model by 110\% (-0.55 pA/pF vs -0.26 pA/pF at $t \sim 350$ ms, Table 3.1). While the forward mode NCX current increase clearly follows the increase in SR $Ca^{2+}$ release, it is not accompanied by an increase in whole-cell $[Ca^{2+}]_i$, which is traditionally assumed to determine NCX magnitude. Table 1 shows that $[Ca^{2+}]_i$ have comparable magnitudes in the hyperactive and stabilized models at $t \sim 350 ms$ (Table 1) and that the average submembrane calcium concentration $[Ca^{2+}]_s$ is only 30\% larger in the hyperactive model compared to the stabilized one.

Fig. 3.5A maps the effects of varying independently and jointly the two RyR gating parameters $1/c^*$ and $\beta/\alpha$ ($\alpha$ fixed) that control the sensitivity of RyRs to cleft $Ca^{2+}$ and refractoriness, respectively. The number of EADs increases when both $\beta/\alpha$ and $1/c^*$ are increased. When we examine the diastolic SR $Ca^{2+}$ load over the same range of $\beta/\alpha$ and $1/c^*$ (Fig. 3.5B), the load tends to be depleted when these parameters are increased. Fig. 3.5C shows that reducing $c^*$ reduces the $Ca^{2+}$ transient magnitude, which is consistent with experimental observations [28]. This is because the increased RyR open rate will deplete the diastolic load without allowing the SR nadir during systole to deplete. When $\beta/\alpha$ is increased, the effect on $Ca^{2+}$ transient amplitude is similar to pronounced because it also increases RyR open probability in the bound state.
3.1.3 Mechanism of increased whole-cell forward mode NCX current without increasing whole-cell cytosolic calcium concentration

As noted earlier, the forward mode NCX current in the hyperactive RyR model has an approximately 110% larger magnitude than in the stabilized RyR model at the time of EAD onset. This increase in NCX current is present even though $[Ca^{2+}]_i$ is only slightly increased in the hyperactive model (2nd panel of Fig. 3.3D at $t \sim 350 \text{ ms}$ and Table 1). Because RyRs are located in dyads along the t-tubules in the submembrane space, the Ca$^{2+}$ flux generated by late aberrant releases will preferentially increase $[Ca^{2+}]_s$ compared to $[Ca^{2+}]_i$ (Fig. 3.6A). The inset of Fig. 3.6A shows that at $t \sim 350$ ms, the hyperactive RyR model has a larger total SR Ca$^{2+}$ release current than the stabilized RyR model by a factor of 3 ($0.11 \mu M_{cyt}/ms$ vs $0.04 \mu M_{cyt}/ms$). This larger release current causes the whole-cell averaged $[Ca^{2+}]_s$ to be $\sim 33\%$ larger in the hyperactive RyR model compared to the stabilized RyR model at $t \sim 350 \text{ ms}$ shown in Table 1 (the increase in $[Ca^{2+}]_s$ is smaller than the increase in SR release current because $[Ca^{2+}]_s$
has a finite background value). Importantly, this \( \sim 33\% \) increase in \([Ca^{2+}]_s\) cannot account for \( \sim 110\% \) increase in forward mode NCX current in a simplistic common pool model where whole-cell \([Ca^{2+}]_s\) is assumed to drive the whole-cell NCX current. In the more realistic spatially distributed model of Ca\(^{2+}\) release used in this study, the local \([Ca^{2+}]_s\) that determines the local NCX current is different in each compartment, varying spatially based on stochastic LCC openings and SR Ca\(^{2+}\) sparks.

To understand the relationship between the increased SR Ca\(^{2+}\) release and the increased NCX current, we examine the spatially distributed nature of Ca\(^{2+}\) release underlying NCX-mediated Ca\(^{2+}\) to \(V_m\) voltage signal transduction. For this purpose, we first compare the total spark firing rates in the hyperactive and stabilized RyR models (Fig. 3.6B), and also give their values at the times \( t = 20\ ms \) and 350 ms in Table 1, corresponding to the peak of calcium transient and EAD onset, respectively. The whole-cell spark rate at \( t \sim 350\ ms \) is around 6 fold larger in the hyperactive RyR model (609 sparks/ms) than the stabilized RyR model (107 sparks/ms), even though the whole-cell SR Ca\(^{2+}\) release current is only about 3 fold larger. This implies that there are a smaller number of large sparks in the stabilized RyR model compared to a greater number of small sparks in the hyperactive RyR model. A smaller number of larger sparks occur in the stabilized model because the larger SR Ca\(^{2+}\) load causes a larger Ca\(^{2+}\) flux during each spark and sparks are less abundant because RyR channels are less active.

In order to understand why a larger number of smaller sparks contribute to a larger NCX current in the hyperactive model, we note that the local NCX current is a nonlinear function of the local \([Ca^{2+}]_s\). This nonlinearity stems from the ubiquitous physical limitation on ion transport rate through ion channels, which causes here the NCX current to saturate above \([Ca^{2+}]_s \sim 4\ \mu M\) to a current of \(-11\ \mu M/ms\) at \(V_m = -20\ mV\) and \(A_{NCX} = 0.5\) (Fig. 3.6C). As a direct result of this nonlinearity, the whole-cell NCX current cannot be determined by the spatial average of \([Ca^{2+}]_s\) compartments as it would be in a "common pool" model. Furthermore, because NCX saturates at larger \([Ca^{2+}]_s\), a smaller number of large sparks could potentially contribute less to the total NCX current than a greater number of small sparks, even though both sets of sparks
Figure 3.5: Effect of RyR gating parameters on the number of EADs, $c_j$, and $c_i$. Effect of altering RyR gating on EAD formation and SR release characteristics. A: number of EADs as a function of RyR Ca$^{2+}$ sensitivity, which is increased by decreasing $c^*$ (increasing $1/c^*$), and refractoriness, which is reduced by increasing the ratio $\beta/\alpha$ of RyR transition rates from closed to open states with CSQN bound ($k_b$) and unbound ($k_u$) to T/J following the 4-state model of Restrepo et al. [73] shown in Fig. 3.1. Both reducing refractoriness and increasing Ca$^{2+}$ sensitivity produce unstable phenotypes with many EADs. Red diamond, blue triangle, blue circle, and blue diamond denote parameters used in Figs. 3.3A-D, respectively. $\alpha$ is fixed at 4 ms$^{-1}$. (B) SR Load as a function of $1/c^*$ and $\beta/\alpha$. (C) Ca$^{2+}$ transient amplitude as a function of $1/c^*$ and $\beta/\alpha$. From Zhong et al. [1].

Contribute to the same whole-cell [Ca$^{2+}$]$_s$. For this to be true, at least a subset of the larger sparks needs to fall in the range of [Ca$^{2+}$]$_s$, where NCX saturates (Fig. 3.6C).

Therefore, we constructed histograms of local [Ca$^{2+}$]$_s$ and $I_{NCX}$ current at $t = 20$ ms corresponding to the peak of the calcium transient (Figs. 3.6D and 4E, respectively) and $t \sim 350$ ms corresponding to the critical time of EAD onset (Figs. 3.6G and 4H, respectively). At $t = 20$ ms, SR Ca$^{2+}$ releases are highly synchronized, and a large fraction of the CRUs have elevated [Ca$^{2+}$]$_s$. In the stabilized RyR model, the CRUs have [Ca$^{2+}$]$_s$ as high as 7 $\mu$M (the tail of the red distribution in Fig. 3.6D), whereas in the hyperactive RyR model the peak [Ca$^{2+}$]$_s$ is 3 $\mu$M (the tail of the blue distribution in Fig. 3.6D). At $t = 20$ ms, the stabilized RyR model has more CRUs with larger peak local NCX current than the hyperactive RyR model (Fig. 3.6E), resulting in a larger whole-cell NCX current. The direct relation of the local NCX current to the local [Ca$^{2+}$]$_s$ is shown in Fig. 3.6F, which shows the saturation effect for [Ca$^{2+}$]$_s$ larger than 4 $\mu$M.

At $t \sim 350$ ms, during the critical period where $V_m$ enters the $I_{Ca,L}$ window, the heightened [Ca$^{2+}$]$_s$ in the hyperactive RyR model is being driven by 3 fold larger release current from
Figure 3.6: Role of nonlinear $I_{NCX}$ on EAD formation. A Calcium concentration in the submembrane space ($[Ca^{2+}]_s$) as a function of time for the stabilized RyR model (red) and the hyperactive RyR model (blue). Arrows indicate the peak calcium transient at $t = 20$ ms and the EAD onset point at $t \sim 350$ ms. Inset is the calcium release current as a function of time. B The Ca$^{2+}$ spark frequency as a function of time, computed by summing all the events satisfying $N_o \geq 3$, where $N_o$ is the number of open RyRs in a CRU. C The local NCX current as a function of local Ca$^{2+}$ for $V_m = -20 \text{ mV}$, 0 mV and 20 mV, with $[Na^+]_i = 6 \text{ mM}$ and allosteric Ca$^{2+}$ activation $A_{NCX} = 0.5$. The current saturates at $[Ca^{2+}]_s \sim 4 \text{ µM}$. D Distribution of local submembrane calcium concentration in each CRU at $t = 20$ ms. E Distribution of local NCX currents in each CRU at $t = 20$ ms. F The relation of the local NCX current to local $[Ca^{2+}]_s$ for the stabilized RyR model (red squares) and the hyperactive RyR model (blue circles) at 20 ms, with bin width 0.1 µM. G-I The similar results at $t \sim 350$ ms, with membrane voltage at -15.5 mV. The vertical arrow in (G) indicates the Ca$^{2+}$ threshold of allosteric activation (0.3 µM), and the vertical arrow in (H) indicates the corresponding NCX current (1.2 µM/ms). From Zhong et al. [1].

junctional SR, which results from 6 fold larger spark rate (Table 1). This implies that in the stabilized RyR model the SR Ca$^{2+}$ flux elevating $[Ca^{2+}]_s$ is highly localized to the CRUs undergoing large sparks, whereas in the hyperactive RyR model there are more sparks with smaller SR Ca$^{2+}$ flux. With a greater number of CRUs with $[Ca^{2+}]_s$ in the range 0.3 µM to 1.5
Chapter 3 Early afterdepolarizations in long QT syndromes

Figure 3.7: Effect of reducing NCX conductance to 20% of its normal value in the computational model of LQT2 under β-adrenergic stimulation. NCX reduction suppresses EADs despite hyperactive RyRs causing late aberrant Ca\(^{2+}\) releases. A. \(V_m\) traces for 4 consecutive beats in steady-state with reduced (red) and normal (blue) NCX conductance. B to I detailed comparison for the first beat in A of \(V_m\) traces (B), the sarcoplasmic reticulum calcium concentration (C), confocal line scans for reduced NCX (D) and normal NCX (E), the cytosolic calcium concentration (F), and 3 key sarcolemmal currents including \(I_{Ca,L}\) (G), \(I_{NCX}\) (H), and \(I_{Ks}\) (I). The origin of time in B to I corresponds to the start of the first beat in A. The scale bar is 30 µM. From Zhong et al. [1].

µM (Fig. 3.6G), more CRUs have NCX current in the range from -1.2 µM/ms to -6 µM/ms (blue bars in Fig. 3.6H), which results in a larger whole-cell average NCX current. In contrast, the stabilized RyR model has a greater number of CRUs with high values of \([Ca^{2+}]_s\) that extend into the range where the nonlinearity of the NCX versus \([Ca^{2+}]_s\) relation becomes significant (red squares vs. blue squares in Fig. 3.6I). We note that the small difference between the red and blue squares in Fig. 3.6I, which is due to different amounts of time-dependent allosteric Ca\(^{2+}\) activation of NCX (\(A_{NCX}\)), implies that this nonlinearity is predominantly responsible for the 110% larger whole-cell average NCX current for hyperactive compared to stabilized RyRs.
3.1.4 Suppression of EAD formation via reduction of NCX current

To investigate the effect of reducing NCX conductance on EAD formation, we modify the hyperactive RyR model such that the strength of the NCX current ($g_{NCX}$) is 20% of the normal value (10.5 $\mu$M/ ms vs 52.5 $\mu$M/ms). Fig. 3.7A shows that, in this reduced NCX model, EADs are consistently suppressed. Suppression occurs despite the fact that late aberrant Ca$^{2+}$ release events during repolarization are still present when NCX conductance is reduced (Fig. 3.7D).

Fig. 3.7B shows that cytosolic Ca$^{2+}$ content in the reduced NCX model is elevated through all stages of the AP until it repolarizes. This is to be expected as reduced NCX conductance will slow the rate of Ca$^{2+}$ extrusion from the myocyte and increase the SR Ca$^{2+}$ load (Fig. 3.7 C).

While we find no evidence to suggest that the suppression of EAD onset is due to differences in other Ca$^{2+}$-dependent sarcolemmal currents including $I_{Ca,L}$ (Fig. 3.7 G) and $I_{Ks}$ (Fig. 3.7 I), we see only a modest decrease in forward mode depolarizing NCX current in the reduced NCX conductance model during the critical time window of EAD onset (Fig. 3.7 H).

Figure 3.8: Contribution of $V_m$ and Ca$^{2+}$ to $I_{NCX}$ that initiates EADs. The results correspond to Fig. 3.7 B-I. Those plots are intended to show that partial blockade of NCX leads to smaller $I_{NCX}$ magnitude together with higher [Ca$^{2+}]_s$ and higher allosteric activation of $I_{NCX}$ for $V_m$ in the $I_{Ca,L}$ window range ($V_m \sim -20 mV$). Regions of the plots outside this $V_m$ range are included to represent a complete cycle length but are not directly relevant to the mechanism of EAD suppression by NCX blockade. A The dynamic $I-V$ plot of NCX. The hyperactive model with reduced NCX conductance (red) simply repolarizes, whereas the one without reduced NCX conductance (blue) loops back on itself once it reaches -30 mV. The NCX current at the point of EAD onset is larger in the model with normal NCX conductance. B The whole-cell average submembrane calcium concentration as a function of $V_m$. The hyperactive model with reduced NCX conductance has a steep peak because of impaired Ca$^{2+}$ extrusion leading to increased SR Ca$^{2+}$ load. C Allosteric activation of NCX channels as a function of $V_m$. Because of the Ca$^{2+}$ buildup in the reduced conductance NCX model, Ca$^{2+}$ will activate the channel more rapidly and compensate for the reduced conductance. From Zhong et al. [1].
When EADs are suppressed by stabilizing RyR activity, $V_m$ follows a very similar time course to the hyperactive RyR model, slowly deviating only during the critical window after $t > \sim 300$ ms (Fig. 3.3D). In contrast, when EADs are suppressed via reducing NCX conductance, the time course of $V_m$ begins to deviate from the full NCX model very early in the AP. As a result, it is misleading to compare the magnitude of the NCX current at the same time. When comparing NCX currents by comparing the magnitude of the current at a given time, it is difficult to quantitatively distinguish the effects of $V_m$ and submembrane Ca$^{2+}$ on the NCX current. Additionally, as the time course of AP differs, the time differs at which the $V_m$ is within the critical window for $I_{Ca,L}$ reactivation. I, therefore, compare the NCX currents as a function of $V_m$ during pacing (Fig. 3.8A). Using this dynamic $I-V$ plot to represent the NCX current, we find that within the critical window of $V_m$ (around -20 mV), the reduced conductance NCX model has $\sim 70\%$ of the magnitude of the normal conductance NCX model. The curves at high voltage represent the fast change of voltage and NCX current during phase 1, which is beyond the scope of our interest. When the NCX current is reduced the forward mode of this current responsible for Ca$^{2+}$ extrusion is unable to keep up with Ca$^{2+}$ influx through $I_{Ca,L}$. As a result, the SR load increases (Fig. 3.7C). When NCX conductance is reduced, the higher
SR load increases \([Ca^{2+}]_s\) (Fig. 3.8B) during systole and hence both NCX driving force and \(Ca^{2+}\)-dependent allosteric activation of NCX (Fig. 3.8C). This model predicts that reduction in NCX conductance is an effective approach to suppress EAD onset for a wide range of \(I_{Ca,L}\) conductances (Fig. 3.9). When NCX conductance is reduced in the model, \(I_{Ca,L}\) conductance must be increased further (from 182 mmol/cm/C to 240 mmol/cm/C) to reach the threshold for EAD onset.

**Figure 3.10:** Experimental validation that NCX suppression using NCX blocker SEA0400 eliminates EADs in LQT2 myocytes exposed to 50 nM isoproterenol. Membrane potential traces (black) and corresponding averaged time-dependent profiles (red) and confocal line-scan images of \(Ca^{2+}\) transients recorded in current clamped LQT2 myocytes with (A) and without (B) SEA0400. Despite shortened AP and absence of EADs in myocytes with SEA0400, abnormal \(Ca^{2+}\) activity is evident during repolarization and diastole. From Zhong et al. [1].

To validate the predictions of computer modeling, we recorded \(Ca^{2+}\) transients in LQT2 myocytes exposed to 50 nM of isoproterenol, a \(\beta\) -adrenergic agonist, for 10-15 mins before and after application of 350 nM SEA0400 to partially block NCX exchanger (Fig. 3.10). Without the blocker, myocytes paced at 0.25 Hz experienced significant prolongation of AP and EADs, and a protracted tail component of \(Ca^{2+}\) transients [28]. Under SEA0400, the peak \(Ca^{2+}\) transient amplitude tends to increase (\(F/F_0 = 4.04\pm0.44\) after vs \(3.37\pm0.69\) before SEA0400, \(n=3\)), with confocal line scans continuing to show late intracellular \(Ca^{2+}\) cycling activity.
However, AP prolongation was suppressed (from 1404±335 ms to 158±43 ms) and EADs were fully abolished, in agreement with our computational modeling results with reduced NCX conductance (Fig. 3.7). AP traces of all LQT2 myocytes treated with SEA0400 are shown in Fig. 3.11.

### 3.1.5 EAD mechanism in LMC myocytes

The important role of RyR activity in EAD initiation can also be shown in LMC myocytes. Similar to Fig. 3.3, Fig. 3.12 in the Supporting Material shows the simulation results under β-adrenergic stimulation for stabilized RyRs, increased Ca\(^{2+}\) sensitivity, reduced RyR refractoriness, and hyperactive RyRs. Increased RyR activity resulting from the addition of caffeine induces aberrant late Ca\(^{2+}\) releases, which drives the depolarizing forward mode NCX current, prolongs the action potential duration and reactivates the \(I_{Ca,L}\) channels. Moreover, caffeine reduced RyR refractoriness, which allows RyR to reopen after the \(I_{Ca,L}\) channels are reactivated, generating an EAD.
Figure 3.12: Effect of RyR activity for LMC myocytes. The simulation results are undergoing repetitive stimulation at 0.25 Hz in the presence of 50 nM ISO. The four cases from left to right are for the stabilized RyR, increased Ca$^{2+}$ sensitivity, reduced refractoriness, and the hyperactive RyR. The hyperactive RyR corresponds to the addition of 250 µM caffeine. The results show the same EAD generation mechanism as in LQT2 myocytes (Fig. 3.3). From Zhong et al. [1].

3.1.6 The effect of pacing frequency

Even though $I_{Ks}$ in the hyperactive RyR model tends to eliminate EADs instead of initiating them (Fig. 3.13 C), it plays an important role in rate-dependent EAD elimination (Fig. 3.13 A & B). The activation timescale $I_{Ks}$ is 0.5 s. Therefore, this current starts to accumulate for stimulation frequency faster than 0.5 Hz. At 1 Hz, EADs are only intermittent. At 2 Hz, the diastolic interval is about 0.1 s and the large $I_{Ks}$ accumulation suffices to eliminate all EADs.

3.1.7 Discussion

Previous computational modeling studies have revealed many important ionic mechanisms of EAD formation. However, those studies have focused primarily on alterations of sarcolemmal
membrane currents. Furthermore, they used common pool models [2, 161], where both the Ca\(^{2+}\) flux through LCCs and the release flux through RyRs feed into the same (whole-cell) compartment, and Ca\(^{2+}\) cycling is described by a deterministic set of equations. Our work uses a multiscale ventricular myocyte model to directly probe how alterations of stochastic RyR activity at the single-channel level affect the Ca\(^{2+}\) and voltage dynamics at the whole-cell level. Smaller Ca\(^{2+}\) transient amplitudes have traditionally been associated with a smaller forward mode NCX current, which would shorten the AP and would contribute to suppressing EADs as predicted in a computer modeling study of EAD formation in pharmacological LQT2 [152]. In contrast, our detailed computational model was able to reproduce the experimental observation that RyR hyperactivity promotes EAD formation while reducing the Ca\(^{2+}\) transient amplitude, but promoting late aberrant Ca\(^{2+}\) releases during repolarization [28]. Here we show that those late sparks are a product of modified RyR gating at the single-channel level, which is currently

---

**Figure 3.13:** Effect of stimulation frequencies on EAD initiation. A The membrane voltage traces at different stimulation frequencies for hyperactive RyR. Top to bottom: 0.25 Hz, 0.5 Hz, 1 Hz, and 2 Hz. The RyR parameters: 1/c* = 0.26 \(\mu\)M\(^{-1}\), \(\beta = 1.6\) ms\(^{-1}\). B \(I_{Ks}\) for the four different stimulation frequencies in A. C \(I_{Ks}\) for the stabilized RyR model (red) and hyperactive RyR model (blue) in Fig. 3.3 D. From Zhong et al. [1].
not reproducible in any single pool model. This modified gating requires both increased RyR sensitivity to cleft Ca\(^{2+}\) and reduced RyR refractoriness.

Currently, the mechanisms underlying Ca\(^{2+}\) spark refractoriness are still a matter of debate. Some experiments claim that Ca\(^{2+}\) spark refractoriness depends primarily on local SR refilling\([66, 162]\), whereas others support that some time-dependent inhibitory mechanisms, such as RyR, also contribute to this behavior\([159]\). Based on the agreement between experimental observations of hyperactive RyR behavior and our computational model, which relies on reduced refractoriness to produce RyR hyperactivity, we believe that the RyR inhibitory mechanism is required to produce spark refractoriness.

Our modeling results show that NCX current is less depolarizing with hyperactive than with stable RyRs in early phase 2 when \([Ca^{2+}]_i\) peaks (Fig. 3.3D). However, the NCX current becomes more depolarizing in the hyperactive case within a critical interval of time during late phase 2 to early phase 3, during which the voltage traverses the window for voltage-dependent reactivation of LCCs. We have previously demonstrated that the higher rate of SR Ca\(^{2+}\) release throughout the vulnerable time window increases Ca\(^{2+}\) flux through the submembrane region, which drives forward-mode NCX current \([28]\). Here we have shown that increased NCX current may not be predictable using only whole-cell measurements of cytosolic Ca\(^{2+}\) content, as the whole-cell average \([Ca^{2+}]_i\) in Fig. 3.3 D insufficiently differs between the hyperactive and stabilized models.

Even in a case of equal total SR Ca\(^{2+}\) release flux, we expect NCX current to be driven more in a hyperactive RyR model with increased RyR activity and depleted SR load than in the stabilized RyR model with higher SR load. In a hyperactive model where the SR Ca\(^{2+}\) content is depleted, a greater number of smaller individual Ca\(^{2+}\) release events drive a greater NCX current compared to a case with fewer, larger individual sparks, in which NCX current is limited by local current saturation due to limited ion transport rates \([163]\). This NCX-mediated Ca\(^{2+}\) to voltage signal transduction mechanism explains how altered Ca\(^{2+}\) handling can promote EAD formation in the setting of reduced SR load with NCX driven primarily by late aberrant
Ca\(^{2+}\) releases induced by LCCs, as opposed to spontaneous Ca\(^{2+}\) waves in the setting of Ca\(^{2+}\) overload where both early and delayed afterdepolarizations can coexist [164–166].

The LQT2 model predicts that reducing NCX conductance will result in complete suppression of EADs despite late aberrant Ca\(^{2+}\) releases occurring throughout the repolarization phase. We found that a factor of five reductions in conductance causes only 20% decrease in the resulting current (Fig. 3.7). This self-tuning compensatory effect is due to the build-up of cytosolic Ca\(^{2+}\) content, which both increases the driving force of forward mode NCX and also increases the Ca\(^{2+}\)-dependent allosteric activation of the channel.

Identifying changes in currents that are both voltage- and time-dependent is difficult when investigating the root cause of altered electrophysiological behavior in the setting of a ventricular myocyte with nonlinearly coupled voltage and Ca\(^{2+}\) dynamics. Comparing NCX current at any given instant during repolarization in Fig. 3.7H appears to suggest no significant difference between NCX currents in the reduced NCX and full NCX models. By examining NCX behavior as a dynamic I – V plot as in Fig. 3.8, we exploited the ability to compare the NCX current in each model at equal \(V_m\), revealing a difference in the degree of Ca\(^{2+}\)-dependent potentiation of the current. This is critical as the vulnerable window for EAD formation is the voltage range in which \(I_{Ca,L}\) under \(\beta\) -adrenergic stimulation has the propensity to spontaneously recover from inactivation and reanimate.

Experiments using SEA0400 to reduce NCX current in LQT2 rabbit myocytes are consistent with computer modeling and validate the role of NCX in EAD onset. This provides a potential pharmacological intervention to treat arrhythmia caused by reduced repolarization reserve exacerbated by irregular SR Ca\(^{2+}\) release. While we have shown that this arrhythmia may be common in hereditary LQT2, they may also play a role in other human cardiac diseases exhibiting RyR hyperphosphorylation such as heart failure [81]. NCX antagonists may, therefore, be viable as a general intervention for suppressing premature ventricular depolarizations in a variety of human cardiac pathologies.
Targeting NCX current is a novel approach and has distinct advantages. Since hearts are contracting muscles, blocking LCCs or RyRs have severe side effects because they induce depressed contractility and bradyarrhythmias. Targeting other ion channels on cell membrane, such as \( I_{KS} \) by class III drugs, increases tissue heterogeneity and thus results in proarrhythmias. In this study, we present that partial block of NCX current in LQT2 rabbits eliminates EADs without altering Ca\(^{2+} \) transients significantly (Fig. 3.7 and 3.10).

**Limitations**

We used a model of CSQN-dependent RyR refractoriness [73, 154], which is supported by experiments showing reduced RyR refractoriness in transgenic mice under-expressing CSQN [167]. However, the biological origin of RyR refractoriness is still not fully understood[66, 162]. Additionally, we assumed that each CRU in the network has the same number of LCC and RyRs, thereby neglecting heterogeneities in CRU properties that may be present [136, 142] and could potentially affect the distributions of local Ca\(^{2+} \) spark amplitudes and local NCX currents. Moreover, we have only considered one specific NCX model and whether similar effects occur with other available NCX models remain to be investigated [168]. Finally, we have neglected the spatial variation of Ca\(^{2+} \) within the submicron dyadic space. While the impact of this variation can in principle be investigated using higher-resolution models [169–171], we do not expect that its incorporation will fundamentally change the basic mechanism of NCX-mediated subcellular Ca\(^{2+} \) to voltage signal transduction elucidated in the present study.

### 3.2 Transient outward K\(^+ \) current underlies EAD formation in long QT syndrome type 1

(This section is adapted from the published work: Choi et al. [29].)

Long QT syndrome type 1 (LQT1) is hereditary heart disease and has an increased risk of giving rise to ventricular fibrillation (VF) and sudden cardiac death [172]. LQT1 is the most common form of long QT syndromes (LQTS) and is responsible for 42% to 54% of all cases [15, 16].
LQT1 results from the loss-of-function mutations in the KCNQ1 gene, encoding the α subunit of the KvLQT1 potassium channel that carries the slowly activating delayed rectifier K⁺ current (\(I_{Ks}\)). \(I_{Ks}\) blockers, such as chromanol HMR 1556 and 293B, used in experimental models have been proven helpful to elucidate the role of \(I_{Ks}\) in regulating action potential duration \[173, 174\]. Despite chromanol perfusion did not significantly prolong APD due to limited activation of \(I_{Ks}\) in control, \(I_{Ks}\) becomes prominent during β-adrenergic receptor stimulation and \(I_{Kr}\) block. In another investigation, this combination of chromanol 293B and β-adrenergic receptor stimulation increases transmural repolarization dispersion and can cause polymorphic ventricular tachycardia \[175\]. Clinical data have also shown that the interval between the peak and the end of the T-wave significantly increases during exercise in LQT1, but not in LQT2 \[176\], supporting the role of APD dispersion in PVT initiation. Although chromanol enhances the dispersion of repolarization in experimental models, it often fails to induce EADs \[177\]. In addition, a high concentration of chromanol 293B effectively suppresses transient outward K⁺ current (\(I_{to}\)) \[178\], limiting the connection between APD prolongation by blocking \(I_{Ks}\) and LQT1-related arrhythmias.

The present study is to elucidate the mechanisms underlying EAD formation from the RV despite shorter APD than in the LV in LQT1 rabbits. Analysis of the experimental data \[29\] reveals that loss of \(I_{Ks}\) unmasks the central role of \(I_{to,s}\) heterogeneity, with the collaboration of \(I_{Ca,L}\) and \(I_{Kr}\), in developing EADs preferentially in the RV.

### 3.2.1 \(I_{to}\) is larger in RV cardiomyocytes

Given the action potential phenotype and the rapid repolarization during the notch, we hypothesize that \(I_{to}\) is responsible for the difference between RV and LV of LQT1 myocytes. \(I_{to}\) was measured in the voltage-clamp mode under isoproterenol (50 nM). The results show that the peak \(I_{to}\) amplitude in RV myocytes is significantly larger than LV myocytes (Fig. 3.14 A - C).

\[
I_{to,\text{total}} = I_{to,fi}e^{-t/\tau_{fi}} + I_{to,si}e^{-t/\tau_{si}}
\]  

(3.4)
Figure 3.14: Comparison of amplitude and inactivation of $I_{to}$ between RV and LV cells. Representative traces of $I_{to}$ are shown in A for RV and B for LV myocytes. The traces can be fitted with two exponential functions: the fast component ($f_i$) and the slow component ($s_i$). C, The total amplitude of $I_{to}$ ($I_{to,fi} + I_{to,si}$) in RV is 28\% larger than LV myocytes at 50 mV (17.1 ± 2.1 pA/pF for RV versus 12.4 ± 1.4 pA/pF for LV; ANOVA $P<0.05$; RV (n=16) and LV (n=15) are from 5 hearts). D, $I_{to,fi}$ and $I_{to,si}$ for RV and LV myocytes at various voltage. Both fast and slow components are larger in RV. E, The time scale of fast components ($t_{fi}$) are the same in RV and LV myocytes, whereas the time scale of slow component ($t_{si}$) in RV is longer (Table E.2). F, Activation and inactivation of $I_{to}$ for LQT1 (solid lines) and LMC (dotted lines) myocytes. The normalized data were fitted with the Boltzmann functions. From Choi et al. [29].

The $I_{to}$ inactivation kinetics could be best described with two exponential functions (see Eq. 3.4 and Fig. 3.14 A & B): a fast component denoted as $I_{to,fi}$ with a time scale of $\tau_{fi}$, and a slow component denoted as $I_{to,si}$ with a time scale of $\tau_{si}$. Further analysis of these two components reveals that $I_{to,fi}$, and $I_{to,si}$ are 30\% and 83\% greater in RV ($g_{to,fi} = 0.068 \pm 0.005 mS/\mu F$ and $g_{to,si} = 0.033 \pm 0.005 mS/\mu F$) than LV myocytes ($g_{to,fi} = 0.052 \pm 0.005 mS/\mu F$ and $g_{to,si} = 0.018 \pm 0.002 mS/\mu F$, Fig. 3.14 D), respectively. $\tau_{fi}$ are slightly different between RV and LV, but $\tau_{si}$ is 25\% smaller in RV myocytes ($\tau_{si} = 73 \pm 6.7 ms$ in RV versus 59 ±6.9 ms in LV at high voltage, Fig. 3.14 E).
3.2.2 Fitting of $I_{Kr}$ using experimental data

Initial modeling was unsuccessful in producing $I_o$ dependent EAD onset in LQT1 myocytes using the standard formulation of $I_{Kr}$ by Zeng et. al.[179]. Voltage clamp studies of the $I_{Kr}$ current have suggested that Hodgkin-Huxley (HH) type models do not sufficiently reproduce observed the behavior in $I_{Kr}$ experiments [180–182]. A meta-analysis of multiple $I_{Kr}$ models [183] shows that models that accurately portray the behavior of $I_{Kr}$ have both deeper closed states of inactivation, and inactivation pathways which are not independent of the activation gate (a channel will typically need to activate before it can transition to the inactivated conformation).

In accordance with these findings we chose to model $I_{Kr}$ with 5 states based on the formulation of Mazhari et al. [184], depicted in Fig. 3.15.

![State diagram of MGWMN model for $I_{Kr}$](image)

**Figure 3.15**: State diagram of MGWMN model for $I_{Kr}$. $C_1$, $C_2$, and $C_3$ are the closed states, $I$ is the inactivated state, and $O$ is the open state. From Choi et al. [29].

Comparing to the standard formulation of $I_{Kr}$ by Zeng et al. [179], the 5-state model can accurately fit the experimental measurements (Fig. 3.16 D, see Appendix E for the details to fitting the data), due to the existence of deep closed states that produce double exponential $I_{Kr}$ traces. With mono-exponential traces generated by HH type model, only the slow activation feature can be captured, shown in Fig. 3.16 A. Importantly, as discussed below, this feature suffices to produce EADs (Fig. 3.18 A) even though it does not reproduce the two exponential time scales $I_{Kr}$ activation kinetics (Fig. 3.16 A).
Figure 3.16: Comparison of different models of $I_{Kr}$.  
A) A representative trace of $I_{Kr}$ activation at 30 mV. Double exponential fit to time course of $I_{Kr}$ activation suggesting a single independent activation gate cannot reproduce the time-dependent gating of $I_{Kr}$.  
B) Model originally formulated by Luo and Rudy16 using independent activation and inactivation gates.  
C) Similar Hodgkin-Huxley type 2-state model with parameters tuned to best fit experimental recordings.  
D) $I_{Kr}$ modeled using the 5-state model described here. The error bars are standard deviation to show the scatter of data. From Choi et al. [29].

3.2.3 $I_{to}$ facilitates EAD Formation in RV cardiomyocytes

Then we validate the hypothesis by reproducing the AP phenotypes using computational modeling, in which we only modify $I_{to}$. Fig. 3.17 A shows that the $I_{to}$ conductances and time scales close to those in LV myocytes elevate voltage to a high value and prolong APD to ~700 ms (solid blue curve; $g_{to,si} = 0.025 \, mS/\mu F$, $g_{to,fi} = 0.08 \, mS/\mu F$, $\tau_{si} = 50 \, ms$, and $\tau_{fi} = 8.4 \, ms$).

In contrast, the $I_{to}$ conductances and time scales close to those in RV myocytes bring down membrane voltage to a low level, generate EADs, and prolong APD (solid red curve; $g_{to,si} = 0.050 mS/\mu F$, $g_{to,fi} = 0.08 \, mS/\mu F$, $\tau_{si} = 80 \, ms$, $\tau_{si} = 8.4 \, ms$). When the model includes the $I_{ks}$ to model RV and LV myocytes in LMC, the APDs are significantly reduced, and no EAD occurs (blue and red dashed lines for LV and RV, respectively). To further clarify the role of $I_{to}$ in EAD formation, we present the time traces of the fast and slow components of $I_{to}$ in Fig.
Chapter 3 Early afterdepolarizations in long QT syndromes

Figure 3.17: EADs regulated by $I_{to}$ in LQT1 myocytes under ISO stimulation. A. Voltage traces for the right ventricular (RV) and the left ventricular (LV) in the setting of LQT1 and LMC. The parameters in $I_{to}$ model are from the results of fitting the voltage-clamp measurements. Only RV from LQT1 (solid red line) exhibited EADs. B, $I_{to}$ traces corresponding to A are broken into the fast component (thick red in RV, thick blue in LV) and the slow component (thin red in RV, thin blue in LV). C, $I_{Ca,L}$ traces during action potentials. D, $I_{Kr}$ traces during action potentials. E, $I_{Kr}$ as a function of $V_m$ during action potentials. Note that in the range of $V_m$ during EADs, $I_{Kr}$ from RV (red) is around half of that from LV (blue). F, EAD formation in the parameter space of $g_{to,sl}$ (y-axis) vs. $g_{Ks}$ (x-axis). Other parameters: $g_{to,fi} = 0.074 \text{ mS/µF}$, $\tau_{si} = 69 \text{ ms}$, and $\tau_{fi} = 8.4 \text{ ms}$. The green curves show the representative voltage traces for each region, with parameters denoted by the green squares. The right-most green square corresponds to LMC cells. From Choi et al. [29].

3.17 B. The results show two main differences of $I_{to}$ between LV and RV. First, the larger $I_{to}$ peak amplitude in RV gives rise to faster initial repolarization during phase 1 of the AP (i.e., a larger notch). Second, the larger conductance of the slow component of $I_{to}$ ($I_{to,sl}$) in RV gives
rise to the larger $I_{to,si}$ value in the subsequent phase 2 of the AP ($t < 200 \text{ ms}$).

Fig. 3.17 C shows that $I_{Ca,L}$ reactivates at the end of phase 2 and coincidently EADs occur in RV, whereas in LV, the repolarization is so fast at the end of phase 2 that $I_{Ca,L}$ fails to recover from inactivation, and EADs fail to occur. The faster rate of repolarization during the end of phase 2 and all range of phase 3 in LV is caused by $I_{Kr}$ (Fig. 3.17 D). Because of the long AP plateau at high $V_m$ in LV, $I_{Kr}$ activates to a significantly larger level than RV. Fig. 3.17 E explicitly compares $I_{Kr}$ at the same voltage. This result shows that $I_{Kr}$ is doubled in LV than RV during the range of $-20$ to $10 \text{ mV}$ that is relevant to EAD onset.

Fig. 3.17 F shows that a smaller number of EADs are present with increasing $I_{Ks}$ conductance ($g_{Ks}$). EADs vanish at a normal value of $g_{Ks}$ corresponding to LMC myocytes (green square at the white region). Fig. 3.17 F also shows the importance of varying $I_{to,si}$ conductance. In absence of $I_{Ks}$, when $g_{to,si}$ is small than $0.04 \text{ ms}/\mu \text{F}$, as in LV myocytes, the high $V_m$ during phase 2 prevents EAD formation despite the prolonged APD. For large $g_{to,si}$, as in RV myocytes, multiple EADs occur (green square on the y-axis). In this case, $g_{to,si}$ is large enough to bring down $V_m$, but also small enough to avoid rapid repolarization by $I_{Kr}$ activation, allowing $V_m$ to traverse the critical window to reactivate $I_{Ca,L}$ and initiate EADs.

### 3.2.4 Role of $I_{Kr}$ in EAD formation

To further elucidate the critical role of $I_{Kr}$ in EAD formation, we also use a Hodgkin-Huxley model of $I_{Kr}$ with parameters fitted to the voltage-clamp data (see Appendix E). The simulation results (Fig. 3.18) show that the Hodgkin-Huxley model of $I_{Kr}$ reproduces the AP phenotypes in the RV and LV (compare Fig. 3.17 A & D with Fig. 3.18 A & D). Furthermore, in Fig. 3.18 E and F, we compared the $I_{Kr}$ model in the present study with the commonly used Hodgkin-Huxley model from Zeng et al. [179]. The results show that the slow time scale of activation from $-30 \text{ mV}$ to $0 \text{ mV}$ is critical to promote EAD in RV. Without the slow activation timescale, as $V_m$ traverses the EAD window, $I_{Kr}$ current becomes so large that $V_m$ spends a very short time in the window, allowing no sufficient time for $I_{Ca,L}$ to recover. In the present study, all parameters in the Hodgkin-Huxley model of $I_{Kr}$ were fitted to the voltage-clamp...
Figure 3.18: LQT1 myocytes in RV and LV using an alternative HH model of $I_{Kr}$. A Sample $V_m$ traces for different $I_{to}$ conductances including experimentally measured values from RV and LV cells. B $I_{to}$ traces broken into fast inactivating component (red thick in RV, blue thick in LV) and slowly inactivating component (red thin in RV, blue thin in LV) for action potentials in panel A. C $I_{Ca,L}$ from RV (red) and LV (blue) during action potentials in panel A. D $I_{Kr}$ during action potentials in panel A. E Activation and inactivation curves with Boltzmann fits used in HH model. Dashed lines denote the equivalent curves used in Zeng et al. [179]. Experimental data is processed using procedures described in E. F $I_{Kr}$ activation time constant function fitting to activation data (black squares) and deactivation data (green circle). The dashed line denotes activation time used in Zeng et al. [179] Data in E and F are from recordings in both RV and LV ($n=7$). From Choi et al. [29].

data. This comparison demonstrates that the accurate modeling of $I_{Kr}$ is critical to elucidate arrhythmogenic mechanisms in LQT1 myocytes.
3.2.5 Contribution of $I_{to}$ inactivation to EAD onset

The experiment measurements (Fig. 3.14) and simulation data (Fig. 3.17) indicate that $I_{to}$ is a major contributor to RV vs. LV AP characteristics and EAD genesis. Both $I_{to}$ peak amplitude and its inactivation kinetics can contribute to phase 1 repolarization and determine EAD formation. Fig. 3.19 shows EAD dynamics in the parameter space of inactivation time constant ($\tau_{si}$) and conductance ($g_{to,si}$) of the slowly inactivating component of $I_{to}$. Panel A shows a heat map of EAD numbers and the parameter sets used for the representative computer simulations for RV and LV are marked with red and blue squares and experimental measurements are shown by red (RV) and blue (LV) filled circles together with standard errors. As shown in panels A, if $I_{to}$ inactivation is slow (larger $\tau_{si}$), less $I_{to}$ conductance (smaller $g_{to,si}$) is needed to generate EADs. The RV and LV ranges of parameters fall well inside and outside the EAD window, respectively, consistent with observed AP phenotypes.

3.2.6 Ultra-long APDs in LV myocytes

The experimental recordings from LV myocytes show a large variability of APD in LV with several seconds of long APD [29]. Since there are cell-to-cell variations in ion channel expressions,
we investigated whether variations in $I_{Kr}$ and $I_{Ca,L}$ can reproduce large variations in APD in LV cells. Fig. 3.20 shows that APD in LQT1 setting lacking $I_{Ks}$ are highly sensitive to $I_{Kr}$ and $I_{Ca,L}$ conductances. Only 15% reduction in $g_{Kr}$ or increase in $g_{Ca,L}$ produces ultra-long APDs in LV setting (red and green lines in Fig. 3.20). When $I_{to}$ alone is decreased by 15%, its effect on APD prolongation was moderate, suggesting that the balance between $I_{Kr}$ and $I_{Ca,L}$ is a major determinant of ultra-long APDs. It is important to note that ultra-long APDs still could not initiate EADs in all cases with LV setting, supporting that $I_{to}$ is a dominant current to modulate EAD dynamics.

### 3.2.7 Discussion

The present modeling study demonstrates that $I_{to,si}$ plays a pivotal role in EADs formation and the AP phenotype (Fig. 3.17 & 3.18). Experiments in Choi et al. [29] revealed that at moderately slow heart rate $> 600 ms$, $I_{to,si}$ in RV of rabbits recovers to a larger amplitude than LV. This result may explain why LQT1 rabbits exhibit dramatically more sudden cardiac deaths after atrioventricular node ablation that causes a slow heart rate[185].

Choi et al. [29] also show the degree of $I_{to}$ heterogeneity in LMC myocytes is similar to LQT1 myocytes. However, this heterogeneity in LQT1 has a greater impact on PVT initiation than

Figure 3.20: Variability of APD in LV obtained by changing $g_{Kr}$, $g_{Ca,L}$ and $g_{to,si}$. The blue line corresponds to the LV trace shown in Fig. 3.8, using the 5-state $I_{Kr}$ model. The parameters were varied within 15% of the mean values based on the standard error of voltage-clamp data. From Choi et al. [29].
in LMC, where the presence of $I_{Ks}$ abolishes the arrhythmogenic effect of $I_{to}$ at the cellular level (Fig. 3.17 F). Previous computer modeling studies of LQT1 uncover the critical role of $I_{Ks}$ in APD prolongation [186] and EAD formation under the condition of reduced $I_{Kr}$ [187]. In particular, the study by O’Hara and Rudy [187] showed that a partial block of $I_{Kr}$ in addition to isoproterenol was needed to provoke EADs when $I_{Ks}$ kinetics is altered by the mutation of Q357R KCNQ1. In the present study, however, isoproterenol suffices to provoke EADs without blocking $I_{Kr}$ in the absence of $I_{Ks}$. Because $I_{to}$ heterogeneity is present even in normal myocardium between RV and LV as well as epi- and endocardium [188–190], this study suggests that $I_{Ks}$ has an important and protective role beyond repolarization reserve by serving as a safety mechanism that counterbalances the arrhythmogenic effect of $I_{to}$ heterogeneity. Hence, the main consequence of the $I_{Ks}$ malfunction in LQT1 hearts is to expose the arrhythmogenic role of $I_{to}$ heterogeneity.
4.1 Small-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel

SK channels are not activated by membrane voltage because they do not have voltage sensors. Compared to the family of voltage-gated K\(^{+}\) channels that share similar topology, SK channels retain two out of seven positively charged amino acids in the S4 segment [191]. Instead, the activation of SK channels is achieved by intracellular Ca\(^{2+}\) [109]. In the absence of Ca\(^{2+}\), four subunits of S6 with each of them linking to calmodulin (CaM) binding domain (CaMBD) are monomeric, and the channel is closed. In the presence of Ca\(^{2+}\), however, Ca\(^{2+}\) binds EF-hands in the N-terminal lobe of CaM, and Ca\(^{2+}\) bound CaM then binds CaMBD of the channel subunit, forming the dimerization between two Ca\(^{2+}\)-CaM/CaMBD complexes. The dimeric complexes result in the rotary movement of the S6 helices and thus drive the gate to open [192]. Ca\(^{2+}\)-response curves show that SK channels have a high sensitivity to Ca\(^{2+}\) (0.3-1 \(\mu\)M) with an activation time scale in the range of 5 -15 ms and Hill coefficient in the range of 2.5 - 5 [109, 112].

Although the activation of SK channels is voltage-independent, the steady-state current is not a linear function of voltage but is inwardly rectified [109–111]. With divalent cations present, such as Ca\(^{2+}\), Mg\(^{2+}\), Sr\(^{2+}\), and Ba\(^{2+}\), SK currents are suppressed in a concentration-dependent manner. Since the SK current is a repolarization current, the inward rectification indicates that
this current, thus the repolarization reserve, is reduced at the high Ca\(^{2+}\) concentration during an action potential, which facilitates the occurrence of cardiac arrhythmias. Conversely, the alleviation of the inward rectification is expected to prevent cardiac arrhythmias.

The inward rectification results from the binding of intracellular divalent cations to an amino acid residue, Ser-359, in the pore-forming region of rSK2. Comparison with a KcsA K\(^+\) channel in bacteria \[193\] revealed that the binding site is likely to be near the K\(^+\)-selectivity filter, which is different from the Mg\(^{2+}\) binding site in the central cavity of Kir channels \[194\]. The binding affinity of Mg\(^{2+}\) and Ca\(^{2+}\) in rSK2 are \(~0.18\) \(mM\) and \(~19.3\) \(\mu M\) \[111\], respectively, which are in the physiological range (0.14 \(mM\) - 2 \(mM\) for Mg\(^{2+}\) \[24\] and 0.1 \(\mu M\) - 30 \(\mu M\) for Ca\(^{2+}\) \[112\] in the sub-membrane space where SK channels are located). This observation indicates that the blocking effects by Ca\(^{2+}\) and Mg\(^{2+}\) are significant under physiological conditions, and the influence of Ca\(^{2+}\) on SK current is complex in the intact cells. Furthermore, similar to the Kir channel \[195\], extracellular K\(^+\) also modulates SK channel gating. Increased extracellular K\(^+\) concentration reduces the apparent binding affinity of Mg\(^{2+}\) while increasing its voltage dependence.

The first mathematical model of the SK channel was based on the detailed kinetic analysis, which demonstrated that Ca\(^{2+}\)-dependent gating could be best described by a model with two open and four closed states, in which the transition rates from deeper closed states to closed states are Ca\(^{2+}\) dependent, and other rates are constants \[113\]. This model was later reduced to the Hodgkin-Huxley form and introduced to the action potential model, as described by Kennedy et al. \[114\]. However, these models fail to incorporate the voltage-dependent inward rectification by intracellular divalent cations, which has been found to play a critical role in regulating \(I_{SK}\) and thus in preventing cardiac arrhythmias \[112\].

In this study, we proposed a four-state Markov model for the SK channel to include the blocking effects by Ca\(^{2+}\) and Mg\(^{2+}\). This model is a simplified model in that Mg\(^{2+}\) has been suggested to bind both the open and the closed states \[110\] and that the SK channel has a intrinsic inward rectification which is independent of blocking by Ca\(^{2+}\) and Mg\(^{2+}\) \[196\]. Based on
the barrier model [197], we treated the binding and unbinding of divalent cations as jumping over a free-energy barrier, and the local minima correspond to intracellular space and the binding site within the channel. Fitting data from Soh et al. [110, 111] shows that this model reproduces inward rectification of SK currents and the voltage-dependent binding affinity of \( \text{Mg}^{2+} \). We also simplified the model to three-state models to explore two extremes where the SK channel is blocked only by \( \text{Mg}^{2+} \) or \( \text{Ca}^{2+} \). We found that both models with constant \( \text{Mg}^{2+} \) and dynamically changing \( \text{Ca}^{2+} \) reproduces the key experimentally observed behavior that SK current peaks earlier than the local \( \text{Ca}^{2+} \) concentration. Finally, we incorporated the \( \text{Mg}^{2+} \) dependent blocking model to the LQT2 myocyte model and find that the pharmacological enhancement of SK current by NS309, increased channel expression level, and protein kinase A phosphorylation of SK channels lead to the suppression of EADs.

4.1.1 Methods

4.1.1.1 Steady-state SK currents

Soh et al. [111] found that an amino acid residue, Ser-359, is present in the pore-forming region of rSK2, and such a residue is responsible for binding intracellular divalent cations including \( \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Sr}^{2+}, \text{and Ba}^{2+} \). Therefore, the binding process can be depicted as the process that the cations jump from intracellular space to the binding site by overcoming a potential energy barrier [197]. Fig. 4.1 A shows a schematic diagram of the potential energy profile, where the local minimum is the binding site Ser-359 and \( \Delta G \) is the barrier height. Because the SK channel is not permeable to divalent cations, there are only transitions between intracellular space and the binding site. Since the binding site is inside the membrane lipid bilayer, positive membrane voltage facilitates the binding. According to the chemical reaction rates, the jumping rate from the intracellular space to the binding site is governed by an exponential function of the height of the barrier \( \Delta G_1 \), and the electrical potential change \( \xi zFV \), where \( \xi zV \) represents the voltage difference. Therefore, the binding rate can be written as \( k \sim \kappa e^{(-\Delta G_1 + \xi zFV)/(RT)} \), and the unbinding rate can be written as \( r \sim \kappa e^{(-\Delta G_2 - \eta zFV)/(RT)} \), which can be simplified to
be the forms in Eq. 4.1. For the Ca$^{2+}$-dependent activation rate, we adopted a simple function, 
\[ \alpha_0 [Ca]^{b_0}. \]

**Figure 4.1:** Schematic diagram for the model of the SK channel. (A) Schematic diagram of the barrier model with only one binding site to describe the blocking effect by divalent cations. The rate at which the ion jumps from cytoplasm to the binding site is \( k \), which is governed by the energy barrier \( \Delta G_1 \) and the potential change \( \xi zFV \). Similarly, the transition rate of the ion to leave the binding site \( r \) is governed by the energy barrier \( \Delta G_2 \) and the potential change \( \eta zFV \). Since the divalent cations cannot traverse the SK channel, the energy barrier between the binding site and the outside of the cell is infinitely high. (B) State diagram of the four-state model for the SK channel, where \( C \) is closed state, \( O \) is open state, and \( B_{Mg} \) and \( B_{Ca} \) are blocking states. Except for \( k_0 \) and \( r_0 \), which are a function of [Ca] or a constant, all other transition rates are exponential functions of membrane voltage (Eq. 4.1).

\[
\begin{align*}
  k_0 &= \alpha_0 [Ca]^{b_0} \\
  k_1 &= \alpha_1 e^{v_m/b_1} \\
  k_2 &= \alpha_2 e^{v_m/b_2} \\
  r_0 &= \text{Constant} \\
  r_1 &= \gamma_1 e^{-v_m/\delta_1} \\
  r_2 &= \gamma_2 e^{-v_m/\delta_2}
\end{align*}
\]

(4.1)

Therefore, the whole-cell SK current in the steady-state is given by

\[
\begin{align*}
  I_{SK,ss} &= \frac{N_iSK}{1 + \frac{k_0}{\alpha_0} [Ca]^{-b_0} + \frac{k_1}{r_1} [Ca]^{b_1} + \frac{k_2}{r_2} [Mg]^{b_2}} \\
  i_{SK} &= P_{SK} zF \left[ K \right]_i - \left[ K \right] e^{-z} \frac{1 - e^{-z}}{1 - e^{-z}}, z = FV/(RT)
\end{align*}
\]

(4.2)
where $N$ is the number of channels in a cell, and $i_{SK}$ is the single-channel current. We adopted the electrodiffusion model for single-channel current because outwardly rectification occurs in the range from -80 mV to 20 mV as extracellular K$^+$ concentration reduces from 120 mM to 4 mM [110]. Instead of increasing proportionally to voltage, the single-channel current in this model deflects to a higher value at moderately high voltage.

To quantitatively characterize parameters in the four-state model, and to assess the capability of the model to reproduce the influence of the Ca$^{2+}$ and Mg$^{2+}$ concentrations on the inward rectification of the SK current, we used a set of data from Soh et al. [110, 111] where cloned SK channels, rSK2, are expressed in Xenopus oocytes. We obtained data by using the online tool https://apps.automeris.io/wpd/ to digitize the figures. Eq. 4.2 suggests that not all the parameters can be uniquely determined. Instead, the data can only impose several constraints, which are listed in Table 4.1. The values of $h_0$, $h_1$, and $h_2$ are obtained by fitting the normalized SK currents in Soh et al. [111] using Eq. 4.5. The K$^+$ permeability $P_{SK}$ is based on the assumption that the single-channel conductance is 14 pS [113] with symmetrical K$^+$ at 150 mM. Other constraints in Table 4.1 are estimated by using a genetic algorithm to minimize $\sum w(I_{SK, data} - I_{SK, model})^2$ ($w$ is the weighting factor) that quantifies the difference between model results and experimental measurements. Experimental data (Fig. 4.2, [110]) show that SK currents at $[Ca] = 20 \mu M$ are larger than those at $[Ca] = 2 \mu M$, which is out of our expectation. To reduce the impact from this data set, we chose $w = 1$ for $[Ca]$ at 0.6 and 2 $\mu M$, and $w = 0.1$ for $[Ca]$ at 20 $\mu M$. The fitting process was written and carried out with Matlab.

4.1.1.2 Time-dependent SK currents

To calibrate the parameters in the voltage-clamp mode with the Ca$^{2+}$ concentration unclamped, we introduced the SK channel model to a physiologically multi-scale model for ventricular myocytes developed by Restrepo et al. [73] and improved by Zhong et al. [1]. This multi-scale model includes a realistic number of 16,120 spatially distributed Ca$^{2+}$ release units (CRU) and links the whole-cell level membrane voltage dynamics to the local sub-cellular Ca$^{2+}$ dynamics.
Experiments [112, 198] show that SK channels in mouse myocytes are colocalized with LCCs, and both of them are located outside the dyadic space. Without loss of generality, the Ca$^{2+}$ concentration sensed by SK channels can be expressed as $[Ca]_{ps} = 0.9[Ca]_p + 0.1[Ca]_s$, where $[Ca]_p$ is the Ca$^{2+}$ concentration in the dyadic space, and $[Ca]_s$ is the Ca$^{2+}$ concentration in the sub-membrane space. The different weights chosen for the proximal and submembrane compartments is motivated by the observation that SK channels are primarily co-localized with LCC channels [112] and hence sense the proximal Ca$^{2+}$ concentration predominantly. Previous studies [1, 112] showed that local $[Ca]_p$ could reach a high level, which requires a very small time step if we used $k_0$ in Eq. 4.1. Therefore, we adopted Eq. 4.3 as the Ca$^{2+}$-dependent activation rate.

$$k_0 = \frac{\alpha_0}{1 + (e^*/[Ca])^{h_0}} \quad (4.3)$$

The parameters are constrained by a set of data from Hamilton et al. [112]. To reproduce $I_{Ca,L}$ versus voltage curve for sham cell in the presence of isoproterenol (ISO) consistent with experimental measurements [112], we modified the 16-state $I_{Ca,L}$ model in Zhong et al. [1]:

$$d_{\infty} = \frac{1}{(1 + e^{-(V_m+20)/5.5})^2}$$
$$\tau_d = \frac{2}{1 - e^{-(V_m+20)/10} + 0.6(V_m+21)/e^{(V_m+21)/10}}$$
$$f_{\infty} = 0.01 + \frac{0.99}{1 + e^{(V_m+30)/8.5}} + \frac{1}{1 + e^{-(V_m-60)/11}}$$
$$\tau_f = 0.02 - 0.007e^{0.001136(V_m+10.5)^2}$$
$$r_1 = 0.15 \text{ ms}^{-1} \quad (4.4)$$

Under two extremes where Ca$^{2+}$ or Mg$^{2+}$ could be solely responsible for the blocking effect, parameters are estimated to be the values listed in Table 4.1, where $h_0$ and $e^*$ are chosen to produce an activation curve in agreement with that in Hamilton et al. [112]. Similar to the
previous subsection, here we also assumed the single SK channel conductance to be $14\, pS$ \cite{113, 199}. In terms of the voltage dynamics, what matters is the total number of SK channels in a cell, instead of the number of channels in each couplon. Analysis shows that the required number of SK channels is much smaller than the number of couplons in the cell. Therefore, it is safe to assume that the maximum number of SK channels in each couplon is 1.

4.1.1.3 Models of LQT2 and LMC myocytes

Our previous model for LQT2 myocytes produces early afterdepolarizations (EADs) in the absence of $I_{SK}$ \cite{1}. To integrate $I_{SK}$ into this model without eliminating EADs, we made the following modifications to decrease the repolarization currents and increase the depolarization currents: $g_{KS} = 0.1386\, mS/\mu F$ (conductance of the slowly activating delayed rectifier K$^+$ channel), $P_{Ca} = 12.5\, \mu mol/C/ms$ (L-type Ca$^{2+}$ channel permeability), and $c^* = 2.94\, \mu M$ (constant for the Ca$^{2+}$ sensitivity of the ryanodine receptor). For the model for LMC myocytes, we only modified $g_{KS}$ and $P_{Ca}$ to be $0.1386\, mS/\mu F$ and $12.5\, \mu mol/C/ms$, respectively.

4.1.2 Steady-state SK currents

Soh \textit{et al.} \cite{110, 111} measured steady-state SK currents as a function of membrane voltage at various Ca$^{2+}$ and Mg$^{2+}$ concentrations under symmetrical $120\, mM$ K$^+$. Fitting this set of data using the four-state model gives optimized parameters listed in Table 4.1 and steady-state SK currents shown in Fig. 4.2. Comparison between Fig. 4.2 A and B shows that the four-state model captures the main feature, which is that the SK current is inwardly rectified in the full range of voltage. The fitting results are quantitatively in agreement with the experimental data for $[Ca]$ at both 0.6 and 2 $\mu M$, but not at $[Ca] = 20\, \mu M$. The experimental data show that SK current at $[Ca] = 20\, \mu M$ is larger than that at 2 $\mu M$, whereas the fitting results show that SK current is strongly suppressed at $[Ca] = 20\, \mu M$.

With the extracellular K$^+$ concentration reduced to 4 $mM$, however, SK currents in the experiment are apparently outwardly rectified in the moderate range of voltage above the Nernst potential, and slightly inwardly rectified at the high voltage (open circles in Fig. 4.2 C). With the single
SK channel current described by the electrodiffusion model (Eq. 4.2), the four-state SK channel model produces currents consistent with the experimental observations at various [Mg] (solid lines in Fig. 4.2 C).

**Figure 4.2:** Fitting of the SK channel currents using the four-state model described by Fig. 4.1. (A) Experimental measurements at various Ca\(^2+\) and Mg\(^2+\) concentrations under symmetrical 120 mM K\(^+\) [110]. (B) Corresponding fitting results. (C) Experimental measurements with superimposed fitting results under the condition that the extracellular K\(^+\) is reduced to 4 mM. All data are fitted with the steady-state SK current described by Eq. 4.2 using the genetic algorithm. The parameters of the model are listed in Table 4.1.

Based on the analytical steady-state SK current, we derived the normalized current \(I/I_{\text{max}}\) as a function of Mg\(^2+\) concentration (Eq. 4.5). With \(h_2 = 1\), Eq. 4.5 fits well the experimental measurements at small Ca\(^2+\) concentrations, shown in Fig. 4.3 A. The analytical result also reproduces the feature that the binding affinity of Mg\(^2+\) decreases with the increase in Ca\(^2+\) concentration. Based on the normalized current \(I/I_{\text{max}}\), we also derived \(K_{d,Mg}\), which is defined as the value of [Mg] at half maximum of \(I/I_{\text{max}}\) (Eq. 4.6). The result in Fig. 4.3 B shows that at a specific Ca\(^2+\) concentration, \(K_{d,Mg}\) is approximately an exponential function of membrane voltage, which is the same as the experimental result. However, the four-state model produces a slope in Fig. 4.3 B much smaller than in the experiment. This is because, in the negative range of voltage, the steady-state current at [Ca] = 2 \(\mu\)M and [Mg] = 1 mM from the model is smaller than that in the experiment (Fig. 4.2 A and B), which results in an underestimated \(K_{d,Mg}\) for the negative voltage.

\[
I/I_{\text{max}} = \frac{1}{1 + [Mg]^{h_2}/K_{d,Mg}} \tag{4.5}
\]
### Table 4.1: Parameters of the SK channel models

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4-State model</th>
<th>Extended 5-state model</th>
<th>Parameter</th>
<th>Simplified 3-state model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ca(^{2+})</td>
<td>Mg(^{2+})</td>
</tr>
<tr>
<td>(N)</td>
<td>567,500</td>
<td>4,054</td>
<td>Expression(^{\ddagger})</td>
<td>7%</td>
</tr>
<tr>
<td>(P_{SK}) ((\mu m^3/\text{ms}))</td>
<td>0.00002467</td>
<td>0.00002467</td>
<td>(P_{SK}^{\ddagger}) ((\mu \text{mol}/(\text{Cms})))</td>
<td>0.2</td>
</tr>
<tr>
<td>(h_0)</td>
<td>3.88</td>
<td>3.88</td>
<td>(\alpha_0) ((\text{ms}^{-1}))</td>
<td>1.2</td>
</tr>
<tr>
<td>(r_0/\alpha_0)</td>
<td>0.9077</td>
<td>9.6211</td>
<td>(c^+) ((\mu \text{M}))</td>
<td>2.6</td>
</tr>
<tr>
<td>(h_1)</td>
<td>0.65</td>
<td>0.65</td>
<td>(r_0) ((\text{ms}^{-1}))</td>
<td>0.08</td>
</tr>
<tr>
<td>(\alpha_1/\gamma_1)</td>
<td>249.88</td>
<td>20.2565</td>
<td>(h_0)</td>
<td>2.5</td>
</tr>
<tr>
<td>(1/\beta_1 + 1/\gamma_1)</td>
<td>0.00476</td>
<td>0.01837</td>
<td>(\alpha_1) ((\text{ms}^{-1}))</td>
<td>0.14</td>
</tr>
<tr>
<td>(h_2)</td>
<td>1</td>
<td>1</td>
<td>(\beta_1) ((\text{mV}))</td>
<td>7</td>
</tr>
<tr>
<td>(\alpha_2/\gamma_2)</td>
<td>0.5924</td>
<td>0.1017</td>
<td>(\gamma_1) ((\text{ms}^{-1}))</td>
<td>0.004</td>
</tr>
<tr>
<td>(1/\beta_2 + 1/\delta_1)</td>
<td>0.0145</td>
<td>0.031</td>
<td>(\delta_1) ((\text{mV}))</td>
<td>30</td>
</tr>
<tr>
<td>(h_3)</td>
<td>-</td>
<td>1</td>
<td>(h_1)</td>
<td>0.65</td>
</tr>
<tr>
<td>(\alpha_3/\gamma_3)</td>
<td>-</td>
<td>0.001769</td>
<td>(\alpha_2) ((\text{ms}^{-1}))</td>
<td>-</td>
</tr>
<tr>
<td>(1/\beta_3 + 1/\delta_1)</td>
<td>-</td>
<td>0.0102</td>
<td>(\beta_2) ((\text{mV}))</td>
<td>-</td>
</tr>
<tr>
<td>(\gamma_2) ((\text{ms}^{-1}))</td>
<td>-</td>
<td>-</td>
<td>(\delta_2) ((\text{mV}))</td>
<td>-</td>
</tr>
<tr>
<td>(h_2)</td>
<td>-</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Units of [Ca], [Mg], and [K]\(_d\): \(\mu \text{M}\).

\(^{\ddagger}\)The total number of couplons is 16,120, and each couplon contains 0 or 1 SK channel.

\(^{\ddagger\ddagger}\)\(P_{SK}\) has a different unit because \(i_{SK}\) in this model is in the unit of flux: \(\mu \text{M}/\text{ms}\).

\[ K_{d,Mg} = \frac{r_2}{k_2} \left( 1 + \frac{r_0}{\alpha_0} [Ca]^{-h_0} + \frac{k_1}{r_1} [Ca]^{h_1} \right) \]  

\[ \frac{B_{Ca}}{B_{Mg}} = \frac{k_1 r_2 [Ca]^{h_1}}{k_2 r_1 [Mg]^{h_2}} \]  

Based on the fitting results, a natural question arises, that is, which is the dominant divalent cation between Ca\(^{2+}\) and Mg\(^{2+}\) in the blocking effect? To address this question, we investigated the fraction of channels in Ca\(^{2+}\) binding state \(B_{Ca}\) and in Mg\(^{2+}\) binding state \(B_{Mg}\). Eq. 4.7 shows
Chapter 4 Antiarrhythmic targets in long QT syndromes

Figure 4.3: Analysis of the four-state model. (A) Normalized current ($I/I_{\text{max}}$) as a function of Mg$^{2+}$ concentrations at 90 mV. The squares, circles, and triangles are from Soh et al. [110] and correspond to 0.6, 2, and 20 µM, respectively. The solid lines are plotted using Eq. 4.5. (B) $K_{d,Mg}$ as a function of voltage at 2 µM Ca$^{2+}$. The squares are from Soh et al. [110], and the solid line is plotted using Eq. 4.6. (C) compared with Mg$^{2+}$ at 1 mM, the relative contribution of Ca$^{2+}$ to the blockade at various Ca$^{2+}$ concentrations. The results are plotted using Eq. 4.7.

The analytical result of the ratio of $B_{Ca}$ to $B_{Mg}$. With transition rates described by Eq. 4.1, Eq. 4.7 can be expressed as a function of membrane voltage at specific concentrations of Ca$^{2+}$ and Mg$^{2+}$. The results are plotted in Fig. 4.3 C, which shows that the relative contribution of Ca$^{2+}$ to the blocking effect exponentially decreases as membrane voltage increases. In addition, the relative contribution of Ca$^{2+}$ increases with the increase in Ca$^{2+}$ concentration. At high Ca$^{2+}$ concentration (20 µM) and low membrane voltage (< 0 mV), Ca$^{2+}$ is dominant in blocking. However, at low Ca$^{2+}$ concentration (0.1 ~ 1 µM) in the full range of voltage, Mg$^{2+}$ is dominant.

4.1.3 Regulation of extracellular K$^+$ on the binding affinity of Mg$^{2+}$

Using the single-vacancy two sites model, Soh et al. [110] analytically reproduced the linear dependence of $K_{d,Mg}$ on extracellular K$^+$ concentration, as observed in experiments. Therefore, a natural way to include the regulation of extracellular K$^+$ concentration on $K_{d,Mg}$ would be integrating the single-vacancy two-site model [195, 200] to the present four-state model. Fig. 4.4 A shows the state diagram assuming that the internal K$^+$ binding site is always saturated (refers to Soh et al. [110] and Spassova et al. [195]). In the original two-site single-vacancy model, the transportation of an intracellular K$^+$ ion to the extracellular side is achieved by
circulation between states \(O_K, O_{KK},\) and \(B_{Mg},\) where \(O_K\) denotes the state that \(K^+\) only occupies the intracellular binding site, \(O_{KK}\) denotes the state that \(K^+\) occupies both intracellular and extracellular binding sites, and \(B_{Mg}\) denotes the state that \(Mg^{2+}\) occupies the intracellular binding site and \(K^+\) occupies the extracellular binding site. Here, we considered both \(O_K\) and \(O_{KK}\) as the open states. Opposite to the binding rate of \(Ca^{2+}\) or \(Mg^{2+},\) the binding rate of \(K^+\) is in the form of \(\alpha_3 e^{-V_m/\beta_1},\) which increases with the decrease of voltage. Similarly, the unbinding rate \(r_3\) is \(\gamma_3 e^{V_m/\delta_3}.\) Under the condition of detailed balance, this model gives

\[
K_{d,Mg} = \frac{r_2}{k_2} (1 + \frac{r_0}{k_0} + \frac{k_1}{r_1}[Ca]^{h_1} + \frac{k_3}{r_3}[K]_e)
\]

(4.8)

which is a linear function as \([K]_e.\) In the experiment, the results of the above equation are fitted with the Woodhull equation [201]

\[
ln(K_{d,Mg}) = ln(K_{d,Mg}(0mV)) - M_g(z\delta)FV_m/(RT)
\]

(4.9)

Therefore,

\[
M_g(z\delta) = \frac{RT}{100F} \ln\left( \frac{K_{d,Mg}(V_m = 0)}{K_{d,Mg}(V_m = 100mV)} \right)
\]

(4.10)

\[
= \frac{RT}{100F} \ln\left( \frac{1 + \lambda [K]_e}{1 + \rho [K]_e} \right) + \sigma
\]

The above equation can fit well the experimental data in Soh et al. [110] with \(\lambda = 0.05564,\) \(\rho = 0.003175,\) and \(\sigma = 0.3252.\) Note that the above equation is different from that in Soh et al. [110]. When intracellular \(Ca^{2+}\) concentration is at 2 \(\mu M,\) most of the SK channels are activated, and most of them are in the blocking states, meaning that \(1 + r_0/k_0\) is negligible compared to other terms. With this approximation, the fitting of Eq. 4.10 using the experimental data gives
three following constraints to the parameters.

\[
\frac{\alpha_1}{\gamma_1} = 11.4536, \frac{\alpha_3}{\gamma_3} = 17.4\frac{\alpha_2}{\gamma_2}
\]

Moreover, fitting experimental data using Eq. 4.8 gives one more constraint to the slope,

\[
\frac{1}{\beta_2} + \frac{1}{\delta_2} - \left( \frac{1}{\beta_1} + \frac{1}{\delta_1} \right) = 0.0126
\]

\[
\frac{1}{\beta_1} + \frac{1}{\beta_3} + \frac{1}{\delta_3} = 0.0286
\]

The analytical results of Eq. 4.8 and 4.10 under constraints of Eq. 4.11 and 4.12 are shown as the solid lines in Fig. 4.4 B and C, which verifies the approximation that \(1 + r_0/k_0\) is negligible. However, under these constraints, the five-state model cannot produce steady-state current as good as the four-state model (Fig. 4.4 D versus Fig. 4.2 A and B). The five-state model still captures the feature that SK currents are inwardly rectified at positive voltages, but at negative voltages, this model only produces a linear relationship between current and voltage.

### 4.1.4 Time-dependent SK currents in voltage-clamp mode

The data of SK currents in Soh et al. [110] are in steady-state, which means that not all parameters can be uniquely determined. To give optimized values for all parameters, time-dependent SK currents are required. To achieve this goal, and given that the relative contributions of Mg\(^{2+}\) and Ca\(^{2+}\) to the blocking effect are comparable (Fig. 4.3 C), in this section we explored two extremes corresponding to cases where the SK current is blocked either by Mg\(^{2+}\) or by Ca\(^{2+}\). We investigated SK currents in the voltage-clamp mode with LCCs present and Ca\(^{2+}\) concentrations unclamped. Because the SK channels are located at couplons, we integrated the simplified three-state models to the physiologically multi-scale model that incorporates sarcolemma ion channels and 16,120 spatially distributed CRUs [1].
We first studied the Ca$^{2+}$-dependent activation. Previous works showed that the Hill coefficient has a wide range from 2.5 to 5 [109, 112]. Here we adopted 2.5 as measured in Hamilton et al. [112]. To measure EC$_{50}$, activation, and deactivation time scales, we clamped Ca$^{2+}$ concentration at various values with the voltage clamped at -90 mV to entirely suppress the blocking effect. We then fitted the corresponding SK current time traces (Fig. 4.5 A) with exponential functions. With values of $\alpha_0$ and $c^*$ in Eq. 4.3 chosen from Table 4.1, this model produces EC$_{50} = 0.86 \mu M$, which is consistent with the result (EC$_{50} \sim 1 \mu M$) of the wild type SK channel in Hamilton et al. [112]. The activation time is 1 - 10 ms and decreases with the increase of Ca$^{2+}$ concentration. The deactivation time is a constant at 12 ms.
Chapter 4 Antiarrhythmic targets in long QT syndromes

**Figure 4.5**: Ca$^{2+}$-dependent activation of SK channels. (A) Time traces of SK channel current at various Ca$^{2+}$ concentrations. On top is the protocol. During the time range from 0 to 100 ms, Ca$^{2+}$ concentration is clamped to the target values. In the other time range, Ca$^{2+}$ concentration is clamped at 0.1 µM. $[Ca]_{ps}$ is the Ca$^{2+}$ concentration at the position between the dyadic space and the submembrane space. (B) Activation of the SK channel versus Ca$^{2+}$ concentration. The simulated results are fitted using the Hill equation with EC$_{50}$ and Hill coefficient at 0.86 µM and 2.5, respectively. (C) Time scales of activation and deactivation versus Ca$^{2+}$ concentration.

We then calibrated parameters in the Mg$^{2+}$-dependent blocking model, which only contains states of $C$, $O$, and $B_M$. Fig. 4.6 shows the results of the model together with the experimental data from Hamilton et al. [112], which demonstrates that LCC current ($I_{Ca,L}$), the SR Ca$^{2+}$ release trigger, peaks at $\sim -5$ mV, while the Ca$^{2+}$-activated SK current peaks at $\sim -15$ mV. Applying the changes to $I_{Ca,L}$ as described in Eq. 4.4, the physiologically multi-scale model produces $I_{Ca,L}$ in agreement with the experimental measurements (Fig. 4.6 A). Based on this result, incorporation of the three-state SK channel model to the multi-scale model with parameters listed in Table 4.1 yields SK currents as a function of voltage quantitatively in agreement with the experimental observations (Fig. 4.6 B). The time traces of SK channel currents (Fig. 4.6 C) show that SK currents decay to the steady-state current in 70 ms, except for -30 mV in which case the decay time is about 200 ms. At -10 mV, the time trace of the SK current produced by the model is quantitatively in agreement with the experimental measurement (Fig. 4.6 D), which shows that the time to peak is 5 ms and the time to decay is 20 - 30 ms. Another essential feature of this model is that it captures the behavior from the experiment that at -10 mV, the SK current peaks earlier than Ca$^{2+}$ concentration in the sub-membrane space where SK channels are located (Fig. 4.6 E). This feature sustains in a
Figure 4.6: Blockade only by Mg$^{2+}$ under the voltage-clamp mode. The model only contains three states: C, O, and B$_{Mg}$. (A) $I_{Ca,L}$ versus voltage with holding potential at -40 mV. The data of sham cell treated with ISO is from Hamilton et al. [112]. (B) Peak value of the SK current versus voltage. Also included is the result with the SK model from Kennedy et al. [114], which does not consider blockade by divalent cations. For Kennedy’s model, the Ca$^{2+}$ sensitivity is 1 µM, and the conductance is 0.027 µS/µF. (C) Time traces of the SK current at various voltages. (D) Comparison of normalized SK current traces between the experimental measurement and the model result at -10 mV. (E) SK current peaks earlier than the Ca$^{2+}$ concentration sensed by SK channels ([Ca]$_{ps}$) at -10 mV. As a comparison, the Kennedy et al. model only yields the SK current that peaks during the decay phase of [Ca]$_{ps}$. (F) Pooled data of time to peak for [Ca]$_{ps}$ and SK currents at various voltages.

A wide range of voltage from -20 mV to 20 mV (Fig. 4.6 F).

As a comparison, we employed the SK channel model from Kennedy et al. [114], which only contains Ca$^{2+}$-dependent activation. The integration of this model to the physiologically multi-scale model shows that the SK current peaks at a much higher voltage compared to Hamilton et al. [112] (blue circles versus red squares in Fig. 4.6 B). In addition, the time trace of the SK current has a much longer decay time than Ca$^{2+}$ concentration in the sub-membrane space (dashed blue line versus solid red line in Fig. 4.6 E), and most importantly, the SK current peaks at the time when the sub-membrane Ca$^{2+}$ concentration is in the decay phase.
(dashed blue line versus solid red line in Fig. 4.6 E), which is opposite to the results produced by the Mg\(^{2+}\)-dependent blocking model and to the experimental measurements. The pooled data demonstrate that such a feature exists for all voltages (green bars vs. red bars in Fig. 4.6 F).

On the other hand, Ca\(^{2+}\) can also be solely responsible for the blocking effect, in which case the model only contains states of C, O, and B\(_{Ca}\). The parameters are listed in Table 4.1, where the Ca\(^{2+}\) dependent activation is the same as the Mg\(^{2+}\)-dependent blocking model, and the Hill coefficient of blocking is constrained by Soh et al. [111]. The results of SK currents are shown in Fig. 4.7, which demonstrates that the Ca\(^{2+}\)-dependent blocking model yields similar results to the Mg\(^{2+}\)-dependent blocking model, although Ca\(^{2+}\) concentration changes
dynamically while Mg\(^{2+}\) concentration stays at a constant. The main differences are decay times and steady-state currents. With the SK channel blocked only by Ca\(^{2+}\), the decay time at -30 mV is \(\sim 400\) ms, and the largest steady-state SK current is 0.7 pA/pF at -20 mV (Fig. 4.7 B). Both of them are larger than the values of the Mg\(^{2+}\)-dependent blocking model (Fig. 4.7 C).

**Figure 4.8:** Effects of various drugs of the SK channel on voltage dynamics under application of ISO. A Perfusion of apamin provokes more EADs in LQT2 myocytes while it has no effect on the APD in LMC myocytes. The addition of apamin is mimicked by reducing the single channel current by 15%. B Perfusion of NS309 suppresses EADs in LQT2 myocytes while it has no effect on the APD in LMC myocytes. NS309 is mimicked by reducing EC\(_{50}\) of Ca\(^{2+}\)-dependent activation from 0.86 \(\mu M\) to 0.05 \(\mu M\). C Enhanced expression level by 100% eliminates EADs in LQT2 myocytes and slightly shortens the APD in LMC myocytes. For all these cases, drugs have small effect on Ca\(^{2+}\) transient.
4.1.5 SK channel as an antiarrhythmic target

The SK current is an outward current that repolarizes the action potential and tends to eliminate EADs, suggesting that the SK current can be a target to treat cardiac arrhythmias. Apamin, a bee venom toxin, is a specific blocker of the SK channel and has been shown to prolong action potentials at the cellular level and to increase the dispersion of repolarization at the tissue level [108, 112, 202]. Conversely, there is a family of drugs that enhance the SK current, including 1-EBIO [203], NS309 [204], benzimidazolinone [205], and ISO (by protein kinase A phosphorylation)[112]. In addition, the SK channel has been found to be dormant in a healthy heart and become active in cardiac disease [206, 207]. These studies found that active SK channels in cardiac disease are because of increased expression levels and increased Ca$^{2+}$ sensitivity. In this section, we studied the effect of the apamin, NS309, enhanced expression level, and protein kinase A (PKA) phosphorylation on SK channel gating by integrating the simplified Mg$^{2+}$-dependent blocking model to the physiologically multi-scale model in the presence of isoproterenol.

We modeled the effect of apamin by reducing the single channel conductance. Fig. 4.8 Aa shows that via reducing the single channel current by 15%, perfusion of apamin increases the number of EADs significantly in LQT2 myocytes. The time traces of $I_{SK}$ show that the blocking effect by Mg$^{2+}$ is so strong that $I_{SK}$ only has a small peak during the depolarization phase, and it is highly suppressed during the plateau phase. However, during the repolarization phase, $I_{SK}$ recovers to a higher level. Apamin affects the voltage dynamics via reducing $I_{SK}$ during the repolarization phase, which results in a larger number of EADs. In LMC myocytes, apamin also reduces $I_{SK}$ during the repolarization phase, but the APD is not altered (Fig. 4.8 Ab). Time traces of Ca$^{2+}$ transient (blue lines in Fig. 4.8) show that $I_{SK}$ only affects membrane voltage and has no effect on Ca$^{2+}$ dynamics. This behavior applies not only to apamin, but also to other drugs shown in Fig. 4.8 and 4.9.

NS309 has been found to increase Ca$^{2+}$ sensitivity of the SK channel activation and has no effect on voltage-dependent behavior [208]. Therefore, we modeled the effect of a particular
concentration of NS309 by reducing EC$_{50}$ of the SK channel activation from 0.86 $\mu$M to 0.05 $\mu$M. Fig. 4.8 Ba shows that increased Ca$^{2+}$ sensitivity enhances $I_{SK}$ during the repolarization phase, which eliminates EADs. NS309 also enhances $I_{SK}$ in LMC myocytes, but it has no effect on the APD (Fig. 4.8 Bb).

Both Ca$^{2+}$ sensitivity and expression level of the SK channel are found to be upregulated in heart failure (HF) [206], and other studies reveal that the SK current becomes larger 10 min after acute myocardial infarction or 30 min after ischemia [209, 210]. These results suggest that the transcription or translation processes related to the SK channel could be interfered in HF. Those results suggest that over-expression of SK channels by gene therapy could offer a possible antiarrhythmic strategy. Fig. 4.8 Ca shows that increasing the expression level by 100% significantly increases $I_{SK}$ during the repolarization phase, which eliminates EADs. In the LMC myocytes, increasing the expression level slightly shortens the APD. This is different from Fig. 4.8 Ab and Bb because in this case, $I_{SK}$ is enhanced during rising, instead of during the peak (Fig. 4.8 C vs. A and B).

In addition, experiments show that protein kinase A (PKA) can phosphorylate the SK channels at the N-terminal serine-136, which attenuates the rectification without changing Ca$^{2+}$ sensitivity.
of activation [112, 211]. Therefore, a method to incorporate this effect is to reduce $\alpha_2$ in the Mg$^{2+}$-dependent blocking model. Fig. 4.9 A shows that reducing $\alpha_2$ enhances $I_{SK}$ peak and shifts the maximum value to a higher voltage, which means that reducing $\alpha_2$ attenuates the rectification. In the LQT2 myocytes model, reducing $\alpha_2$ eliminates EADs and shortens the APD by enhancing the underlying $I_{SK}$ during the depolarization phase (Fig. 4.9 B and C). When $\alpha_2$ is small enough such that the blocking effect by Mg$^{2+}$ is weak, the large $I_{SK}$ during the plateau phase brings down the dome significantly (green lines in Fig. 4.9 B and C).

### 4.1.6 Discussion

#### 4.1.6.1 Steady-state SK currents

In this study, we proposed a four-state model of the SK channel that produces currents quantitatively in agreement with experimental measurements [110]. The critical component of this model is the states standing for blocking by Ca$^{2+}$ and Mg$^{2+}$. By considering the binding and unbinding of the divalent cations as a process of jumping over energy barriers, the transition rates between the open state and binding states can be described as an exponential function of membrane voltage. Given that positive voltage facilitates intracellular cations to overcome the energy barrier, to bind the amino acid residue, and hence to block the channel, the outward current at the positive voltage is reduced. Conversely, at the negative voltage, more cations leave the binding site such that more channels are unblocked, leading to increased inward current. Incorporation of the electrodiffusion model of the single-channel current is also important because this model enables the four-state model to produce the rectified currents in the voltage range from $-85 \text{ mV}$ to $40 \text{ mV}$ under the condition that intracellular K$^+$ concentration is 120 $\text{ mM}$ while extracellular K$^+$ concentration is 4 $\text{ mM}$ (Fig. 4.2 C, [110]). Conversely, if the linear model of the single channel current is used, the steady-state current in Fig. 4.2 C would resemble Fig. 4.2 A and B, which have a larger degree of inward rectification at a higher voltage.

The four-state model also captures the behavior that increased intracellular Ca$^{2+}$ concentration reduces the binding affinity of Mg$^{2+}$ (Fig. 4.3 A&B). This is because Ca$^{2+}$ and Mg$^{2+}$ are competitive to bind the amino acid residue in the pore-forming region of the SK channel.
As Ca$^{2+}$ concentration increases, the number of channels available for blocking by Mg$^{2+}$ decreases while the total number of channels stay the same, leading to the reduced binding affinity of Mg$^{2+}$ (Eq. 4.6). Moreover, both experimental measurements and model results demonstrate that $K_{d,Mg}$, characterizing the binding affinity of Mg$^{2+}$, exponentially decreases with the increase of membrane voltage (Fig. 4.3 B). This is because the binding rate of Mg$^{2+}$ exponentially decreases with the decrease of voltage, and the decreasing rate of Mg$^{2+}$ is larger than that of Ca$^{2+}$, which means that Ca$^{2+}$ becomes more dominant in the blocking effect as membrane voltage decreases. From the perspective of mathematical analysis, with [Ca] at 2 $\mu$M, most of the channels are in the blocking states of $B_{Ca}$ and $B_{Mg}$. Therefore, $1 + r_0/\alpha_0[Ca]^{-h_0}$ in Eq. 4.6 is negligible. Moreover, the fitted value of $1/\beta_2 + 1/\gamma_2$ is larger than $1/\beta_1 + 1/\gamma_1$, resulting in a minus sign of the exponent in Eq. 4.6.

Since the amino acid residue, Ser-359, is accessible to both Mg$^{2+}$ and Ca$^{2+}$, it is natural to compare the relative contribution of Mg$^{2+}$ and Ca$^{2+}$ to the blocking effect. Soh et al. [111] revealed that Mg$^{2+}$ has a much smaller binding affinity than Ca$^{2+}$ for the wild type SK channels. However, intracellular Mg$^{2+}$ concentration is much larger than Ca$^{2+}$ concentration (1 mM for Mg$^{2+}$ versus 0.1 $\sim$ 100 $\mu$M for Ca$^{2+}$ [1, 112]) during the action potential, indicating that the role of Mg$^{2+}$ could be comparable to Ca$^{2+}$. This is confirmed in the present study. Fig. 4.3 C shows that as Ca$^{2+}$ concentration increases above 20 $\mu$M during the depolarization phase of the action potential that reaches 40 $\sim$ 60 mV, the fraction of channels in $B_{Ca}$ is over twice of that in $B_{Mg}$. However, during the late phase of the action potential where voltage is in the range of 0 mV $\sim$ -60 mV and Ca$^{2+}$ concentration is below 1 $\mu$M, the fraction of channels in $B_{Ca}$ is less than that in $B_{Mg}$. Therefore, there is no dominant divalent cation contributing to blocking throughout the action potential.

The framework of the proposed SK channel model also provides an insight to explain behaviors in other studies under the condition that the extracellular K$^+$ is lower than intracellular K$^+$ [212–214]. In these studies, when the voltage is below the Nernst potential, the SK current is a linear function of voltage, and when the voltage is moderately larger than the Nernst potential, the SK current is highly suppressed. Interestingly, when the voltage is far above the Nernst
potential, SK current increases exponentially with voltage. These behaviors can be explained by the present four-state model, which suggests that the underlying mechanism of these behaviors may rely on the single-channel current modeling, rather than channel gating.

4.1.6.2 Regulation of extracellular K\(^+\) on the binding affinity of Mg\(^{2+}\)

The four-state model (Fig. 4.1 B) has no feature of modulation by extracellular K\(^+\). To capture this feature, we used an extended five-state model (Fig. 4.4 A) to include the two-site single-vacancy model. The two-site single-vacancy model has one intracellular binding site accessible to intracellular K\(^+\) and divalent cations, and one extracellular binding site accessible to extracellular K\(^+\). When the blocking ion binds to the binding site, it electrostatically knocks off the intracellular K\(^+\) ion and forces it to move outward \[195\]. In the present model, however, the outward movement is described by electrodiffusion, which is necessary because it is critical to producing the rectification with extracellular K\(^+\) reduced to 4 mM (Fig. 4.2 C). Moreover, both the single and double K\(^+\) bound states (\(O_K\) and \(O_{KK}\)) should be considered as the open states. If \(O_{KK}\) were not an open state, the fraction of \(O_K\) would decrease while \(O_{KK}\) would increase as voltage reduces to the negative range, because \(k_3\) has a form of \(\alpha_3 e^{-V_m/\beta_3}\) and \(r_3\) has a form of \(\gamma_3 e^{V_m/\delta_3}\). The consequence is that the SK current would be outwardly rectified at negative voltage under the condition of symmetrical K\(^+\), inconsistent with experimental measurements. With both \(O_K\) and \(O_{KK}\) are considered as the open states, the SK current at negative voltage under symmetrical K\(^+\) is not inwardly rectified but is a linear function of voltage (Fig. 4.4 D). The reason is that a large fraction of channels is in the state of \(O_{KK}\) in the negative range. As a result, the relationship between the SK current and the voltage is dominated by the single-channel current, which is a linear function when K\(^+\) is at symmetrical concentrations. Therefore, the two-site single-vacancy model, which is useful to describe K\(_{ir}\) channel currents showing a linear relationship at the negative voltage \[195\], may not be the candidate to describe the regulation of extracellular K\(^+\) on SK channel gating.
4.1.6.3 Time-dependent SK currents in voltage-clamp mode

By integrating the simplified three-state models to the physiologically multi-scale model, we found that blockade solely by Mg\(^{2+}\) or Ca\(^{2+}\) is sufficient to fit the experimental data from Hamilton et al. [112]. Both models capture the feature that the SK current peaks earlier than Ca\(^{2+}\) concentration sensed by SK channels. In contrast, the model only with Ca\(^{2+}\)-dependent activation produces time to peak of the SK current much larger than sub-membrane Ca\(^{2+}\) concentration, strengthening the necessity of introducing the blocking effect. The difference between the results of the Ca\(^{2+}\)-dependent blockade model (Fig. 4.7) and the Mg\(^{2+}\)-dependent blockade model (Fig. 4.6) stems from the dynamical change of Ca\(^{2+}\) concentration. We calibrated the parameters by the data of current versus voltage and time traces in the decay phase of \(I_{SK}\) at -10 mV where Ca\(^{2+}\) concentration is above 5 µM (Fig. 4.6 D). However, Ca\(^{2+}\) concentration is much lower in the steady-state, leading to weaker blocking by Ca\(^{2+}\) and thus higher steady-state currents than in the Mg\(^{2+}\)-dependent blockade model. The same reason applies to the longer decay time at -20 mV for the Ca\(^{2+}\)-dependent blockade model, where Ca\(^{2+}\) concentration is smaller than that at -10 mV.

4.1.6.4 Antiarrhythmic role of the SK channel

Since the SK current is a repolarization current, it can serve as the repolarization reserve to suppress abnormal behaviors of the action potential and prevent cardiac arrhythmias. Integration of the Mg\(^{2+}\)-dependent blockade model to the LQT2 myocyte model reveals that enhancing SK current by applying NS309, increasing expression level, and applying ISO eliminates EADs (Fig. 4.8 and 4.9), whereas inhibiting SK current by applying apamin increases the number of EADs (Fig. 4.8). These simulation results suggest that the present SK channel model is useful to study the mechanism of drugs acting on the SK channel under various pathological conditions.

It is of great importance to point out that the electrophysiological mechanisms of the drugs are not well understood. The previous study found that apamin inhibits the SK channel by acting on an amino acid between transmembrane segments S3 and S4, which is outside the pore region.
Chapter 4 Antiarrhythmic targets in long QT syndromes

[215]. In addition, the steady-state SK current is a Hill function of the apamin concentration, with Hill coefficient ranging from 0.7 to 0.9. However, the experimental data are lacking to uncover the detailed mechanism related to the present model. The reduced single-channel current in the present study is still a simplifying assumption. For the effect of NS309, reducing EC$_{50}$ to 0.05 $\mu$M of the Ca$^{2+}$-dependent activation is required to eliminate EADs (Fig. 4.8 Ba) because the channels are almost fully activated under the large local Ca$^{2+}$ concentration during the depolarization phase. In addition, the blocking effect by Mg$^{2+}$ is so strong that the $I_{SK}$ peak during the depolarization phase is small and narrow, leading to an even smaller effect of EC$_{50}$ on voltage dynamics. For the expression level enhanced by 100% (Fig. 4.8 Ca), there is a study showing that this degree of enhancement is present in HF [206], which means that 100% enhancement of the expression level is insufficient to eliminate EADs and thereby cardiac arrhythmias. However, this does not per say contradicts the results of the present study showing elimination of EADs by over-expression of ISK since we do not model other HF induced remodeling effects. The present study still provides insight into the mechanism underlying EAD elimination by increasing the expression level of the SK channel. The result shows that larger $I_{SK}$ during the repolarization phase is responsible for suppressing EADs.

In the above situations, $I_{SK}$ modulates voltage dynamics by the peak during the repolarization phase, which results from the strong blocking effect. However, this blocking effect can be altered by ISO, protein kinase inhibition, or other drugs related to PKA phosphorylation [112, 209, 211, 212, 216]. In this case, $I_{SK}$ affects the membrane voltage via the peak during the depolarization phase (Fig. 4.9 B). Hamilton et al. [112] suggest that PKA phosphorylation attenuates $I_{SK}$ rectification in a Ca$^{2+}$/voltage-dependent manner because the phosphorylation is at N-terminal serine-136, which is within the CaMBD at serine-465, and at C-terminal serine-568 to serine-570 (rat SK2) [211]. This analysis implies that $\alpha_1$ should be responsible for this mechanism. However, the possibility of regulation in the Mg$^{2+}$/voltage-dependent manner cannot be ruled out because the phosphorylation may have an impact on the channel conformation and thereby affect the voltage-dependent blocking, regardless of the specific divalent cations. In addition, the blocking effect by Mg$^{2+}$ is comparable to that by Ca$^{2+}$ during
the Ca\textsuperscript{2+} transient (Fig. 4.3), which means that the relief of Ca\textsuperscript{2+}-dependent blocking by ISO is insufficient to straighten the \( I - V \) curve in Hamilton et al. [112], supporting that PKA phosphorylation reduces both \( \alpha_1 \) and \( \alpha_2 \).

### 4.1.6.5 Limitation

Although block by intracellular divalent cations contributes to the inward rectification, Li et al. [196] show that the inward rectification can also be intrinsic, independent of intracellular divalent cations. The underlying mechanism is the voltage-dependent reduction of the single channel conductance. The present model does not incorporate this feature. However, compared to block by Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, this intrinsic property is a secondary effect, which will not have a great impact on our conclusion.

Another limitation is that the extended five-state model cannot capture the behaviors of SK currents regulated by external K\textsuperscript{+} concentrations. However, this study suggests that the extended five-state model can be used to describe the K\textsubscript{ir} channel. Therefore, a novel electrophysiological investigation of the K\textsuperscript{+}-dependent regulation is required to shed light on how Ca\textsuperscript{2+} and K\textsuperscript{+} movements are different from the K\textsubscript{ir} channel.

### 4.2 Late sodium channel GS967 suppresses PVT in a transgenic rabbit model of long QT syndrome type 2

The purpose of this study is to model the effect of \( I_{\text{NaL}} \) blocker GS967 in suppressing EADs in transgenic LQT2 rabbits, which have been shown previously to suffer SCD induced by frequent EADs and PVTs [27, 98, 145]. The reason that EADs occur in LQT2 rabbits is the enhanced activity of RyRs, the SR Ca\textsuperscript{2+} release channel, which causes elevation of intracellular Ca\textsuperscript{2+} ([Ca\textsubscript{i}]) and \( I_{\text{NCX}} \) during the plateau phase of APs [28]. Previous studies indicated that inhibiting \( I_{\text{NaL}} \) can reduce Ca\textsuperscript{2+} overload and suppress EADs by reducing Na\textsuperscript{+} overload, which enhances Ca\textsuperscript{2+} extrusion via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX1) [102, 217]. Here, using a multi-scale computational model, in this study we found that blocking \( I_{\text{NaL}} \) eliminates EADs through
modulating NCX1 as well as \( I_{Ks} \). This study suggests that the selective \( I_{NaL} \) blocker GS967 could be a candidate as a novel effective therapy for treating arrhythmias in LQT2 syndrome.

The computational model is based on the rabbit ventricular myocyte detailed model in Zhong et al. [1] with dynamically changed [Na]_i. \( I_{NaL} \) is modeled based on the standard sodium channel model, with modification of inactivation gates \( j \) and \( h \) (Eq. 4.13) to quantitatively fit the patch-clamp measurements in the present study for LMC and LQT2 myocytes. Other model parameters were also modified to reproduce the experimental results. Details of the model are provided in Appendix D.

\[
I_{Na} = g_{Na} m^3 h' j' (V_m - E_{Na})
\]

\[
h' = \alpha_{NaL} + (1 - \alpha_{NaL}) h
\]

\[
j' = \alpha_{NaL} + (1 - \alpha_{NaL}) j
\]

(4.13)

### 4.2.1 Larger \( I_{NaL} \) in long QT syndrome type 2 modulates APDs via altering [Na]_i in addition to directly depolarizing \( V_m \)

Experimental data (not published) demonstrated that, while \( I_{NaL} \) blockade slightly shortens APDs at physiological heart rate, it significantly shortens APDs at the slow heart rate in which EADs occur. The data also showed that \( I_{NaL} \) blockade suppresses PVTs robustly through the remodeling of Ca\(^{2+}\) handling in LQT2 rabbits. To understand the mechanism, experiments have been carried out to measure the \( I - V \) curve of \( I_{NaL} \) using the patch-clamp technique. Fig. 4.10 A showed \( I_{NaL} \) is 29% greater in LQT2 than in LMC. We then used the computer model to investigate the effect of \( I_{NaL} \) on action potential dynamics. We developed \( I_{NaL} \) equations based on the standard Na channel model (Eq. 4.13), with parameters chosen to fit the patch-clamp data in Fig. 4.10 A. The simulation results show that the effect of \( I_{NaL} \) on APDs is greatly dependent on [Na]_i. When the pacing cycle length is at 350 ms, the steady-state [Na]_i is around 10.5 mM, and APDs are similar for \( \alpha_{NaL} = 0.025 \) vs. 0.022, masking the effect of additional depolarizing currents by \( I_{NaL} \) (Fig. 4.10 C). When the pacing cycle length is set to 2 s, blockade of \( I_{NaL} \)
by reducing $\alpha_{Na,L}$ to 0.022 shortens APDs significantly (Fig. 4.10 D). These computational modeling results demonstrate the critical roles of $I_{Na,L}$ in regulating APD through modulating $[Na]_i$. Similar behaviors were also observed in LMC myocytes as shown in Fig. 4.11.

### 4.2.2 $I_{Na,L}$ blockade prevents EADs through modulating $[Na]_i$ and $I_{NCX}$

To dissect the two contributions of $I_{Na,L}$ to EAD formation through $[Na]_i$ modulation and additional depolarizing current, we first obtained $[Na]_i$ values from simulations with $[Na]_i$ unclamped for different $\alpha_{Na,L}$. We then carried out computational simulations with those fixed $[Na]_i$ values. Fig. 4.12 shows EAD formation in the parameter space of $\alpha_{Na,L}$ vs. $[Na]_i$ under 50 nM ISO. Multiple EADs were observed when $\alpha_{Na,L}$ is greater than 0.022 in the range of 8
Figure 4.11: $I_{NaL}$ effect on APDs in LMC myocytes. A AP traces and map of APD without application of ISO at PCL = 350 ms. Top panel: AP traces under the condition of normal level $I_{NaL}$ ($\alpha_{NaL} = 0.22$), partially blocked $I_{NaL}$ and fully blocked $I_{NaL}$. Bottom panel: APDs in the parameter space vs with results of the top panel shown as the red square, black circle, and green diamond. B AP traces and map of APD without the application of ISO at PCL = 2000 ms. C AP traces and map of APD with application of ISO at PCL = 2000 ms.

$\sim 13 \text{ mM}$ of $[\text{Na}]_i$. The voltage traces corresponding to the squares and circles in Fig. 4.12 A are shown in Fig. 4.12 B, where the squares and thick lines represent normal $I_{NaL}$ in LQT2 at different $[\text{Na}]_i$ levels, and the circles and thin lines represent partially blocked $I_{NaL}$ by 100 nM GS967. When $[\text{Na}]_i$ was fixed at 10.5 mM, blockade of $I_{NaL}$ alone reduces EADs (thin red line). The complete abolishment of EADs was obtained when $[\text{Na}]_i$ was also reduced to 5.2 mM (thin blue line). Note that in this case, the $V_m$ during plateau the phase is much higher (blue vs. red lines in panel B), suggesting the critical role of $I_{NCX}$ behind the EAD formation. Panel D shows the outward NCX current at 10.5 mM $[\text{Na}]_i$ (red lines). $I_{NCX}$ becomes an inward current (blue lines) throughout plateau when $[\text{Na}]_i$ was reduced to 5.2 mM, which elevates $V_m$ during the initial plateau phase, causing rapid activation of $I_{Ks}$ (panel E, blue lines) to repolarize APs quickly and prevent EADs. This modeling study emphasizes the critical roles of $I_{NaL}$ in $[\text{Na}]_i$ homeostasis: the blockade of $I_{NaL}$ alleviates $[\text{Na}]_i$ overload in addition to depolarizing $V_m$. 

131
Chapter 4 Antiarrhythmic targets in long QT syndromes

during the plateau phase.

**Figure 4.12:** $I_{NaL}$-dependent EADs in LQT2 myocytes under 50 nM ISO. A EAD formation in the parameter space of $\alpha_{NaL}$ vs. $[Na]_i$. Representative traces corresponding to the squares and circles are shown in panels B-F. The $[Na]_i$ values are chosen in the way described in the text. B $V_m$ traces for different $\alpha_{NaL}$ and $[Na]_i$. Lowering $\alpha_{NaL}$ from 0.025 (thick red line) to 0.022 reduced EADs (thin red line). Complete suppression of EADs was achieved by lowering $[Na]_i$ to 5.2 mM (blue lines). C-F Similar to panel B, $I_{Na}$, $I_{NCX}$, $I_{Ks}$, and $I_{Ca,L}$ during pacing. Lowering $[Na]_i$ to 5.2 mM increases forward mode $I_{NCX}$ (blue lines), which elevates $V_m$ during plateau and largely activates $I_{Ks}$, leading to rapid repolarization without EADs.

### 4.2.3 Discussion

Despite its small amplitude, $I_{NaL}$ can contribute to the slowing of repolarization during the plateau phase of action potentials. In addition, Na$^+$ entry through $I_{NaL}$ leads to Na$^+$ overload, which in turn causes Ca$^{2+}$ overload and promotes triggered responses such as EADs and DADs [104, 218]. Indeed, the arrhythmogenic role of $I_{NaL}$ has been implicated in many pathological conditions in which $I_{NaL}$ is enhanced such as in the ischemic and/or failing heart and LQT3 [103, 106, 218, 219]. $I_{NaL}$ is also thought to influence $V_m$ when repolarization reserve such as $I_{Kr}$ is reduced [220, 221]. Ranolazine, an antianginal drug, is known to block $I_{NaL}$ preferentially relative to the peak of $I_{Na}$ and effectively alleviate arrhythmias [104, 221]. However, the efficacy of ranolazine has been interpreted with caution due to a) non-specific blockade such as
Chapter 4 Antiarrhythmic targets in long QT syndromes

$I_Kr$ [220, 222], b) use-dependent blockade of peak $I_{Na}$ [222], and c) anti-adrenergic effect [223].

Studies with the more-selective $I_{NaL}$ blocker GS967 showed promising results in preventing arrhythmia with negligible effects on peak $I_{Na}$ and $I_Kr$ [102, 224]. In the present study testing GS967 on a transgenic rabbit model of LQT2, the results clearly demonstrated that $I_{NaL}$ is an essential contributor to triggering EAD and PVT that can be effectively abolished by selective inhibition of $I_{NaL}$ with GS967.

Despite effective suppression of EADs and PVTs by GS967, it is worth noting that in a few cases, the PVTs morphed into a transient monomorphic VTs caused by transient single rotors in the field of view. This phenomenon is most likely caused by GS967 suppression of EADs (and thereby multi-focal activity) allowing single reentry formation. In the structurally normal heart such as in LQT2 rabbits, this single rotor moved out of boundary to terminate the monomorphic VTs. Further studies are required to evaluate the advantages and risks of inhibiting $I_{NaL}$ in other cardiac diseases.

The minimal effect on APD under basal conditions and conduction velocity restitution is a major advantage of GS967. Earlier studies in rabbits reported that GS967 did not alter QRS duration or activation time in the wide range of physiological heart rates [224, 225]. Further examinations using S1S2 and the ramp pacing protocol revealed that APD and APD dispersion were not affected by GS967. Previous experiments in drug-induced LQT2 models using E4031 demonstrated that inhibition of $I_{NaL}$ by ranolazine or GS967 shortens APDs, and this phenomenon was associated with suppression of EADs [221]. GS967 also shortened APDs in transgenic LQT2 rabbit hearts but only during slow heart rate and ISO perfusion. This computational study demonstrates that inhibiting $I_{NaL}$ have two competing roles in modulating APDs, prolongation of APDs via reducing $[Na]_i$ and shortening of APDs via reducing $I_{NaL}$ during AP plateau. As a result, when the heart rate increases, the overall effect of $I_{NaL}$ blockade on APDs is negligible. The discrepancy between drug-induced vs. transgenic LQT2 rabbit model is likely due to the remodeling of cardiac excitation in transgenic LQT2 rabbits: 1) $I_{NaL}$ is 29% greater in LQT2 than LMC, which allows more significant roles in modulating $[Na]_i$, 2) prolongation of APD in LQT2 rabbits is much smaller compared to drug-induced LQT2 model (18% vs. 40%),
which is associated with down-regulation of $I_{Ca,L}$ and $I_{Ks}$ [27]. 3) E4031 perfusion in isolated rabbit hearts triggers EADs and PVTs without sympathetic stimulation while transgenic LQT2 rabbits (similar to humans) did not show EADs and PVT at baseline and required ISO or low K$^+$ to trigger EADs or PVTs [98, 145, 226], 4) LQT2 rabbits have remodeling of Ca$^{2+}$ handling proteins [28]. These unique features of transgenic LQT2 rabbits may have caused APDs and EAD formation more sensitive to [Na]$^+$ and Ca$^{2+}$ homeostasis shown in the present computational modeling.
Bridging the gap between the cell and the tissue: how do EADs propagate?

EADs and increased dispersion of repolarization are two key factors associated with arrhythmogenesis in LQT syndrome [20, 27, 227]. However, it is also well known that increased dispersion of repolarization tends to suppress EADs through the source-sink effect, which prevents the formation of premature ventricular complexes (PVC) and polymorphic ventricular tachycardia (PVT) [228, 229]. Therefore, how EADs at the cellular level evolve into PVCs and PVTs at the tissue level is not fully understood. In the LQT2 setting, Huang et al. [117] demonstrate that EADs evolve into PVCs because of enhanced $I_{Ca,L}$ and steep repolarization gradient due to $I_{Ks}$ heterogeneity. This study reveals that in tissue, reentry could occur at the same position where PVCs were initiated; that is to say, the trigger and the substrate for arrhythmogenesis could originate from the same source.

Recently, Choi et al. [29] demonstrate that in the LQT1 setting, the key factor of EAD formation in RV of the transgenic rabbits is the large conductance of the slow component of $I_{to}$, $g_{to,s}$, which brings down membrane voltage to the critical window of $I_{Ca,L}$ reactivation. On the other hand, the smaller $g_{to,s}$ in LV, together with a smaller activation time of $I_{Kr}$ at high membrane voltage, is the underlying mechanism of long APDs but no EADs. Further investigation (unpublished work by Choi et al.) shows that EADs in RV of the transgenic LQT1 rabbits elevate membrane voltage in neighboring cells, which form PVCs propagating towards
LV. These behaviors suggest that the $I_{to,s}$ heterogeneity between RV and LV is responsible for PVC and PVT formation in the LQT1 setting.

To understand PVC initiation in the LQT1 setting, we develop a one-dimensional tissue consisting of coupled 450 RV and 50 LV myocytes. The myocyte model is based on the whole-cell model that we have published to study EAD formation in transgenic LQT1 rabbits [29] (also described in Appendix E). 5 myocytes at the end are subject to regular stimulation at 0.25 Hz. We assume that the cell length is 100 $\mu$m. The voltage diffusion is introduced to mimic the electronic current flow through cell-cell coupling with diffusion constant being 1 $\mu$m$^2$/ms. The results show that the premature ventricular complex (PVC) occurs when $g_{Ca,L}$ for the RV myocytes is increased by 57%. In addition, the results demonstrate that the APD of PVC is longer than the preceding action potential and the APD of PVC persistently increases as the PVC propagates. Further analysis revealed that significantly reduced $I_{to,s}$ in PVCs due to long refractoriness accounts for prolonged APDs compared to the preceding action potentials, and the voltage diffusion between cells is responsible for prolonging the APD of the PVC during propagation.

### 5.1 Initiation of premature ventricular complexes in a cable of LQT1 myocytes

Since $g_{to,s}$ is critical to initiating EADs in RV of the transgenic LQT1 rabbits [29] and enhanced $I_{Ca,L}$ facilitates PVC initiation in the LQT2 tissue [117], we first investigate the behavior of action potentials of the single cell in the parameter space of $g_{to,s}$ vs. $g_{Ca,L}$. Fig. 5.1 A and B show that as $g_{to,s}$ increases, EADs occur, which is the same as the behavior in Chapter 3. These results also show that as $g_{Ca,L}$ increases, infinite plateau occurs. These behaviors apply to $\tau_{si}$ at both 80 ms (Fig. 5.1 A) and 50 ms (Fig. 5.1 B). The voltage traces corresponding to the symbols on the heat maps are shown in the top panels of Fig. 5.1 C and D.

If 500 RV myocytes (red squares) are coupled to form a cable (middle top in Fig. 5.1 C), the number of EADs significantly increases (from $\sim$ 3 to $\sim$ 13) because neighboring cells bring down the voltage during the critical window of EAD initiation. This effect is similar to
increasing the number of EADs in RV myocytes by increasing \( g_{\text{to},s} \) (Fig. 5.1 A). In addition, alternans of EAD number occur. The reason is that the diastolic interval is significantly reduced if a large number of EADs occur, leading to partial recovery of \( I_{\text{to},s} \) from inactivation. On the
other hand, if 500 LV myocytes are coupled to form a cable, only normal APs are present (the panel across Fig. 5.1 C and D). However, if the cable consists of 450 RV and 50 LV myocytes, the number of EADs is smaller than in a cable consisting of only RV myocytes (the bottom in Fig. 5.1 C). More importantly, the repolarization phase propagates along the cable in the form of a wave at 0.85 cm/s, termed "repolarization wave". This is because there is voltage diffusion between neighboring cells and the APDs of LV myocytes are short due to no EADs, which results in the full repolarization of RV myocytes close to the LV-RV boundary shortly after phase 2, and the fully repolarized RV myocytes repolarize more adjacent RV myocytes.

With $g_{Ca,L}$ of the individual RV myocyte is increased by 57% (from 70 to 110 mmol/cm/C), voltages cannot repolarize once it is elevated during the depolarization phase (top in Fig. 5.1 D). When 500 RV myocytes with increased $g_{Ca,L}$ are coupled to form a cable, the voltages also cannot repolarize once it is elevated at the first beat (middle top in Fig. 5.1 D). While these APDs are infinitely long, they are significantly reduced when they are coupled with 50 LV myocytes with normal $g_{Ca,L}$. Besides, EADs in the RV region initiates a propagating PVC at a speed of 1.85 cm/s (asterisk in the bottom of Fig. 5.1 D). As the PVC propagates, the membrane voltages stay at a high level, and their APDs persistently increase.

The membrane voltages over a long time reveal that PVCs occur in every 5 beats (Fig. 5.2 B). If $g_{Ca,L}$ of the RV myocytes is normal (70 mmol/cm/C), only EADs occur with propagating repolarization (Fig. 5.2 A). On the other hand, if $g_{Ca,L}$ is increased to 120 mmol/cm/C, complex behaviors take place. For some beats, PVCs originate from the RV side and propagate to the LV side while the APD is still longer at the RV side. The enhancement of $g_{Ca,L}$ leads to the APD of the PVC so long that it suppresses the next external stimulus. In addition, the beat after the next beat cannot produce EADs at the RV end. Instead, these cells have a high voltage plateau for $\sim 0.4$ s.

In addition to enhanced $g_{Ca,L}$ for RV myocytes, PVCs can also originate in a cable of 450 RV + 50 LV myocytes with the $I_{Na}$ activation shifted by -10 mV (Fig. 5.3 A). In this case, the period of the PVC is 3 beats, and the propagating speed is 3.4 cm/s. Although we have not
found experimental support for this shifting of activation, it provides a clue to understanding the mechanism of PVC initiation; that is, PVCs are initiated by sodium channel activation.
Chapter 5 Bridging the gap between the cell and the tissue: how do EADs propagate?

**Figure 5.3:** Normal $g_{Ca,L}$ with $I_{Na}$ activation shifted by -10 mV gives rise to PVCs. A Activation and inactivation curves of the sodium channel. B PVCs occur in every 3 beats on a cable with 50 LV and 450 RV myocytes. Triangles denote the boundary between LV and RV myocytes.

### 5.2 Electronic current flow through cell-cell coupling regulates the APD prolongation of PVCs during propagation

Fig. 5.2 shows that the long APD of PVCs affect the behavior of the following action potentials. More importantly, when $g_{Ca,L}$ is above a threshold, the APD of the PVC is longer than the pacing cycle length, which fully blocks the normal conduction of the next beat and thus cause arrhythmias. To understand the mechanism of APD prolongation when the PVC propagates, we extract the data of two cells showing significantly different APDs. Fig. 5.4 shows the analysis of the results, in which the cell number counts from the RV end to the LV end. Because the time interval between the AP and the PVC is longer at the RV side (#20), both the SR load ($c_j$) and $I_{lo}$ recover to a higher level. Higher $c_j$ results in more Ca$^{2+}$ release and higher Ca$^{2+}$ concentration, which leads to the stronger forward mode $I_{NCX}$ shortly after the depolarization. However, the larger $I_{lo}$ over wins the larger depolarization current. As a result, cells at the RV end have a smaller total depolarization current (left panel of Fig. 5.4 C) during the plateau.
Chapter 5 Bridging the gap between the cell and the tissue: how do EADs propagate?

phase of action potential ($V_m$ from 40 mV to 60 mV, Fig. 5.4 A). Such effect is overbalanced by
the diffusion current during the critical voltage range (middle panel of Fig. 5.4 C), resulting in
the overall larger depolarization current at the RV end (right panel of Fig. 5.4 C) and thus APD
prolongation.

The critical role of voltage diffusion between neighboring cells in lengthening APD indicates
that the voltage diffusion may also regulate PVC repolarization. If it is true, and because
the depolarization wave speed is controlled by coupling between cells, the following equation
should be satisfied

$$v \sim \sqrt{\frac{D}{\tau}} \Rightarrow \frac{v_1}{v_2} = \sqrt{\frac{\tau_2}{\tau_1}}$$

(5.1)
where the subscripts 1 and 2 denote the depolarization and repolarization waves, respectively, \( v \) is the wave speed, \( D \) is the diffusion constant of voltage, and \( \tau \) is the diffusion time between adjacent cells.

For the depolarization wave, the time from -30 mV to -80 mV is 0.6 ms, and the propagating speed is 59.3 cm/s. For the repolarization wave, the decay time is \( \sim 116.5 \) ms, and the wave speed is 1.85 cm/s. Therefore,

\[
32.1 = \frac{v_1}{v_2} \neq \sqrt{\frac{\tau_2}{\tau_1}} = 13.9
\]  

(5.2)

This equation indicates that the time to decay for the repolarization wave is not as long as predicted by Eq. 5.1. However, the qualitative consistency of Eq. 5.2 shows that Eq. 5.1 still sheds insights on quantitative understanding of APD prolongation during PVC propagation. This is important in our present theoretical modeling study because ultra-long APDs of PVCs will interrupt the following normal beats to potentially cause steeper repolarization gradient and thereby leading to cardiac arrhythmias. To fully understand the mechanism, further investigation is required.

### 5.3 PVCs have longer APDs than normal action potentials because of reduced \( I_{to,s} \)

Fig. 5.2 shows that APDs of PVCs are longer than the preceding action potentials, and the voltage during the plateau phase of PVCs is around 60 mV. To explain this behavior, we investigate the underlying currents involved in repolarization and depolarization. Fig. 5.5 shows that the repolarization currents \( I_{to,s} \) and \( I_{to,f} \) are smaller during the PVC, which prolongs the APD of the PVC. For the depolarization currents, \( I_{Ca,L} \) and \( I_{NCX} \) are also smaller during the PVC, which means that these two current do not contribute to APD prolongation during the PVC. For \( I_{Kr} \), the high peak of the red curve in Fig. 5.5 E is mainly because of the lower membrane voltage during the repolarization phase. Since \( I_{to,f} \) does not affect APDs significantly, the mechanism underlying the prolonged APD during the PVC is the reduced
Chapter 5 Bridging the gap between the cell and the tissue: how do EADs propagate?

\( I_{to,s} \). The reason that \( I_{to,s} \) is reduced during the PVC is that \( I_{to,s} \) has a recovery time scale of 3 s, much longer than the time interval between the normal action potential and the PVC, which means that \( I_{to,s} \) is barely recovered during the PVC.

Given that the electronic current flow between cells prolongs the APD during PVC propagation (Fig. 5.4), the APD of the PVC is regulated both by the reduced \( I_{to,s} \) and the electronic current flow between cells.

\[ \begin{align*}
V_m &= -60, 0, 60 \\
I_{to,s} &= 0, 1, 2, 3 \\
I_{to,f} &= 0, 2, 4, 6 \\
I_{Kr} &= 0, 10, 0 \\
I_{Ca,L} &= 1, 0, -1 \\
I_{NCX} &= 0, 0.3, 0.6, 0.9 \\
c_i &= 60, 90, 120 \\
c_j &= 120, 60, 0
\end{align*} \]

**Figure 5.5**: Unrecovered \( I_{to,s} \) is responsible for APD prolongation in PVCs. A Voltage traces of the cell #190 where the PVC originates. B Superimposed voltages for the normal action potential and the PVC in A. Also shown are \( I_{to,s} \) (C), \( I_{to,f} \) (D), \( I_{Kr} \) (E), \( I_{Ca,L} \) (F), \( I_{NCX} \) (G), \( c_i \) (H), and \( c_j \) (I).

### 5.4 Discussion

Although this study is still preliminary, it still gives insights into the mechanism of PVC initiation in a one-dimensional cable consisting of LQT1 myocytes. The results show that \( I_{to} \) heterogeneity and enhanced \( g_{Ca,L} \) for RV myocytes are critical to triggering PVCs and thereby give rise to both the trigger and the substrate for spontaneous initiation of cardiac rhythms.
arrhythmias (Fig. 5.1). The enhanced \(g_{Ca,L}\) is in agreement with the study that PVCs originate in a cable of LQT2 myocytes [117]. However, the present study requires a lower degree of \(g_{Ca,L}\) enhancement than in Huang et al. [117] (57% vs. over 100% in Huang et al.), which is physiologically more realistic. To understand the mechanism of PVC initiation, we have an attempt to shift the \(I_{Na}\) activation by \(-10\) \(mV\), which also gives rise to PVCs every 3 beats. The current study indicates that the key to understanding the mechanism underlying PVC initiation is the interplay between \(I_{Ca,L}\) and \(I_{Na}\). We find that in the bottom panel of Fig. 5.1 D, if PVCs cannot occur, only a region of cells have EADs and the others fully repolarize, which is similar to the rightmost beat. Therefore, we hypothesize that with an increased \(g_{Ca,L}\), cells during the repolarization phase are able to initiate a relatively high amplitude of EAD, whereas one side of the close neighbors cannot initiate EADs. This difference between cells leads to an abrupt change of voltage along the cable, allowing the activation of sodium channels that launches a PVC.

Although reduced \(I_{Na,s}\) prolongs APDs of PVCs compared to the preceding action potentials (Fig. 5.5), the large difference of APDs during PVCs between cells #190 and #20 demonstrates that the prolongation of APDs is predominantly determined by the electronic current flow between cells (Fig. 5.4). The following attempt of analysis shows that the PVC propagating speed does not quantitatively support the dominant role of voltage diffusion, but the values on both sides of Eq. 5.2 are qualitatively consistent, indicating that both sarcolemmal ionic currents and voltage diffusion between neighboring cells play roles in prolonging APDs of the PVCs. It is important to note that the persistently prolonged APDs during PVC propagation are not observed in the experiment (unpublished work). The possible reason for the ultra-long APDs in the modeling is that the inactivation curve of \(I_{Ca,L}\) is too large in the range of high voltage.
Conclusion

This thesis presents studies on the underlying mechanisms of triggered activity, which leads to cardiac arrhythmias and, thus, sudden cardiac death. Chapter 2 shows that the formation of Ca$^{2+}$ waves and DADs at low cytosolic Ca$^{2+}$ load is associated with a strong, but not too strong, RyR cooperativity. These results create a causal link between two phenomena observed in heart failure: higher probability of Ca$^{2+}$ wave genesis, and RyR phosphorylation that dissociates FKBP 12.6 from RyRs. This study reveals that RyR phosphorylation reduces cooperativity and thus initiates Ca$^{2+}$ waves and DADs. This study also confirms the essential role of increased SR Ca$^{2+}$ content and RyR activity in DAD formation, as suggested by experiments. In addition, the fact that myofilaments and mitochondria occupy a large fraction of cytosolic space may be the solution to the long-standing problem that the small Ca$^{2+}$ release current from SR cannot produce the normal Ca$^{2+}$ spark size in the modeling study. However, despite this improvement, the local Ca$^{2+}$ concentration during Ca$^{2+}$ waves may still be potentially higher in the model than in the experimental observations.

Chapter 3 includes studies of EADs, which is another mechanism of triggered activity. In LQT2 transgenic rabbits, the study shows that the Ca$^{2+}$-driven EADs originate when Ca$^{2+}$ sensitivity of RyR is increased, and spark refractoriness is reduced. Subsequent investigation of NCX reveals that the nonlinear function of NCX as a function of local Ca$^{2+}$ concentration is critical to enhancing NCX during the vulnerable voltage window of $I_{Ca,L}$ reactivation. Moreover,
Chapter 6 Conclusion experimental measurements in transgenic LTQ2 rabbits confirm the critical arrhythmogenic role of NCX and identify this current as a potential target for antiarrhythmic therapies in LQT2.

In LQT1 transgenic rabbits, however, larger $I_{to,s}$ in RV myocytes plays a pivotal role in initiating EADs by bringing down the voltage to the vulnerable window of $I_{Ca,L}$ reactivation. In contrast, smaller $I_{to,s}$ in LV myocytes is responsible for prolonging APD by elevating membrane voltage, which allows $I_K$ activation before EAD initiates. This study suggests that $I_{to}$ heterogeneity may account for regional EAD formation and the initiation of PVTs.

Chapter 4 focuses on the antiarrhythmic targets: the SK current and the late sodium current. This chapter presents a four-state Markovian model of SK channel to include the activation by intracellular Ca$^{2+}$ and blockade by intracellular Ca$^{2+}$ and Mg$^{2+}$. The integration of the simplified three-state model to the physiologically detailed model captures the feature observed in experiments that the SK current peaks earlier than submembrane Ca$^{2+}$ concentration in the voltage-clamp mode. Moreover, this model is able to model the effect of apamin, ISO, and 1-EBIO on the SK channel. Based on the investigation of SK channel in heart failure, the present study proposes two potential electrophysiological mechanisms of drugs that increase Ca$^{2+}$ sensitivity and expression level. The abilities to capture features from different data sets and to suppress EADs make this model useful to investigate the antiarrhythmic role of SK current under various pathological conditions.

The late sodium current is modeled by introducing a pedestal value to the inactivation gates $h$ and $j$ of the standard sodium channel. The study reveals that the late sodium current blocker GS967 eliminates EADs and PVTs by reducing both the depolarization current and the intracellular sodium concentration that regulates $I_{NCX}$. This study demonstrates that $I_{NaL}$ is a potential therapeutic target to prevent triggered activity and PVTs in LQTS. This study also demonstrates that $I_{NaL}$ blockade yields only a modest reduction of APD in control myocytes due to the knock-on effect of the change of intracellular sodium concentration on $I_{NCX}$.

Finally, Chapter 5 presents an attempt to understand the hypothesized universal mechanism for PVC initiation in tissue. To carry out simulations, we use the myocyte model from our
published work for EAD formation in transgenic LQT1 rabbit and then couple RV and LV myocytes in a one-dimensional cable. The results show that $I_{ho}$ heterogeneity and enhanced $g_{Ca,L}$ are two key factors to initiate PVCs, in which case both the trigger and the substrate for arrhythmogenesis originate from the same source. Moreover, persistent APD prolongation during PVC propagation is observed and is the consequence of the electronic current flow between cells. Although the results are still preliminary, they bring up insights to elucidate the mechanism of PVT initiation in the LQT1 setting.
I use the rabbit ventricular myocyte model originally developed by Restrepo et al. [73] and further improved in Terentyev et al. [28]. The schematic of the model is shown in Fig. A.1. Combining together a realistic number of 16,120 Ca$^{2+}$ release units (CRU), this multi-scale model links the whole cell level Ca$^{2+}$ dynamics to the local sub-cellular Ca$^{2+}$ dynamics in each CRU. The CRUs are coupled by calcium diffusion in the cytosolic space and network sarcoplasmic reticulum (NSR). The distance between CRUs are 1.8 $\mu$M and 0.9 $\mu$M in the longitudinal and transverse directions, respectively. Each CRU contains five sub-spaces: cytosolic space, sub-membrane space, dyadic space, junctional SR (JSR) space, and NSR space. The cytosol and NSR are divided into 8 compartments. The interaction between SR network and cytosolic space is implemented by Ca$^{2+}$ release from JSR to the dyadic space via a cluster of 100 ryanodine receptors (RyRs), and Ca$^{2+}$ uptake from the cytosol to NSR via Ca-ATPase. Each RyR is described by a 4-state random Markov model.

In addition, this model incorporates the bi-directional coupling of Ca$^{2+}$ with membrane voltage. I considered a full set of sarcolemmal ion channel currents, including calcium current ($I_{Ca,L}$), sodium current ($I_{Na}$), Na$^+$/Ca$^{2+}$ exchanger current ($I_{NCX}$), rapid component of the delayed rectifier K$^+$ current ($I_{Kr}$), slow component of the delayed rectifier K$^+$ current ($I_{Ks}$), fast component of the rapid inward K$^+$ current ($I_{to,f}$), slow component of the rapid outward K$^+$ current ($I_{to,s}$), Na-K pump current($I_{NaK}$), and inward rectifier K$^+$ current ($I_{K1}$). I described all currents at the
whole cell level except for $I_{Ca,L}$, which is localized at the couplon of each CRU via 4 long-type calcium channels (LTCC), and $I_{NCX}$ which is localized at the submembrane space of each CRU. Similar to RyRs, LTCCs are also described by a Markov model with 16 states, which could be reduced to the standard Hodgkin-Huxley model.

To model the effect of $\beta$-adrenergic stimulation with ISO, I fit the experimental data that results in an increase in the SERCA uptake rate as well as increases in $I_{Ca,L}$ and $I_{Ks}$ currents. I incorporate the effect of $\beta$-adrenergic stimulation under steady-state conditions in order to model non-transient EADs that persist on an experimental time scale longer than the time scales of ISO-induced $I_{Ca,L}$ and $I_{Ks}$ phosphorylation [98].

**Figure A.1:** Multi-scale model of ventricular myocytes. A. Geometry of a myocyte. The myocyte consists of $62 \times 26 \times 10$ CRUs. B. Diffusive coupling of Ca$^{2+}$ between compartments of CRUs. CYT: Cytosolic space. SUB: Submembrane space. DYAD: Dyadic space. JSR: Junctional SR. NSR: Network SR. C. Geometry of a CRU. CYT and NSR are divided into 8 compartments. T-tubule (light blue), SUB (pink) and DYAD (yellow) which attach to the T-tubule, pass through two compartments $n = 0, 4$. 
Appendix A Multi-scale model for rabbit ventricular myocytes

A.1 Cell architecture

The myocyte structure is comprised of a lattice structure of 16120 diffusively coupled elementary calcium release units (CRUs). This structure is depicted in Fig. A.1. The model has a finer grid spacing in order to increase the spatial resolution of the diffusive coupling between CRUs. Specifically, the cytosol is divided into two compartments along \( x \), \( y \) and \( z \). As a result, each CRU with position denoted by the superscript indices \( i, j, k \) contains 8 cytosolic and NSR compartments denoted by subscript indices \( n = 0, 1, \ldots, 7 \) (Fig A.1). The time constants of diffusion between adjacent volumes are given in Table A.1.

The calcium cycling dynamics is governed by

\[
\frac{d}{dt}c_{i,j,k}^{i,j,k} = J_{\text{leak},n}^{i,j,k} - J_{\text{up},n}^{i,j,k} - J_{\text{buffer},n}^{i,j,k} + J_{d,ni,n}^{i,j,k} \frac{V_s}{v_i} + J_{d,pi,n}^{i,j,k} \frac{V_p}{v_i} + J_{ci,n}^{i,j,k}
\]

\[
\frac{d}{dt}c_{s,j,k}^{i,j,k} = J_{\text{up},s}^{i,j,k} V_p + J_{\text{buffer},s}^{i,j,k} + J_{\text{NCX},s}^{i,j,k} - J_{d,si,0}^{i,j,k} - J_{d,si,4}^{i,j,k}
\]

\[
\frac{dc_{p}^{i,j,k}}{dt} = \beta_p(c_{p}^{i,j,k}) \left( J_{\text{rel},s}^{i,j,k} + J_{\text{Ca},s}^{i,j,k} - J_{d,ps}^{i,j,k} - J_{d,pi,0}^{i,j,k} - J_{d,pi,4}^{i,j,k} \right)
\]

(A.1)

The Restrepo model considered the diffusion in all directions for the submembrane compartments. Here I only consider the diffusion in one of the traverse directions, which is given by

\[
J_{\text{cs}}^{i,j,k} = \frac{c_{i,j,k+1}^{i,j,k+1} + c_{i,j,k-1}^{i,j,k-1} - 2c_{i,j,k}^{i,j,k}}{\tau_s^{i,j,k}}
\]

(A.2)
Appendix A Multi-scale model for rabbit ventricular myocytes

Diffusion between the cytosolic, submembrane, and proximal compartments is given by

\[
\begin{align*}
J_{d_{pi,n}}^{i,j,k} &= \frac{\epsilon_p n - \epsilon_{i,j,k}}{2\tau_{ps}}, \quad n = 0, 4 \\
&= 0, \quad n \neq 0, 4 \\
J_{d_{st,n}}^{i,j,k} &= \frac{\epsilon_{i,j,k} - \epsilon_{i,j,k}}{2\tau_{si}}, \quad n = 0, 4 \\
&= 0, \quad n \neq 0, 4 \\
J_{d_{ps}}^{i,j,k} &= \frac{\epsilon_p - \epsilon_{i,j,k}}{\tau_{ps}}
\end{align*}
\]

(A.3)

and between nearest neighbor cytosolic compartments

\[
\begin{align*}
J_{ci,0}^{i,j,k} &= \frac{c_{i,1}^{i,j,k} + c_{i,1}^{i,j,k-1} - 2c_{i,0}^{i,j,k}}{\tau_i^1} + \frac{c_{i,2}^{i,j,k} + c_{i,2}^{i,j,k-1} - 2c_{i,0}^{i,j,k}}{\tau_i^1} + \frac{c_{i,4}^{i,j,k} + c_{i,4}^{i,j,k-1} - 2c_{i,0}^{i,j,k}}{\tau_i^1} \\
J_{ci,1}^{i,j,k} &= \frac{c_{i,0}^{i,j,k} + c_{i,0}^{i,j,k-1} - 2c_{i,1}^{i,j,k}}{\tau_i^1} + \frac{c_{i,3}^{i,j,k} + c_{i,3}^{i,j,k-1} - 2c_{i,1}^{i,j,k}}{\tau_i^1} + \frac{c_{i,5}^{i,j,k} + c_{i,5}^{i,j,k-1} - 2c_{i,1}^{i,j,k}}{\tau_i^1} \\
J_{ci,2}^{i,j,k} &= \frac{c_{i,1}^{i,j,k} + c_{i,1}^{i,j,k-1} - 2c_{i,2}^{i,j,k}}{\tau_i^1} + \frac{c_{i,2}^{i,j,k} + c_{i,2}^{i,j,k-1} - 2c_{i,2}^{i,j,k}}{\tau_i^1} + \frac{c_{i,6}^{i,j,k} + c_{i,6}^{i,j,k-1} - 2c_{i,2}^{i,j,k}}{\tau_i^1} \\
J_{ci,3}^{i,j,k} &= \frac{c_{i,2}^{i,j,k} + c_{i,2}^{i,j,k-1} - 2c_{i,3}^{i,j,k}}{\tau_i^1} + \frac{c_{i,3}^{i,j,k} + c_{i,3}^{i,j,k-1} - 2c_{i,3}^{i,j,k}}{\tau_i^1} + \frac{c_{i,7}^{i,j,k} + c_{i,7}^{i,j,k-1} - 2c_{i,3}^{i,j,k}}{\tau_i^1} \\
J_{ci,4}^{i,j,k} &= \frac{c_{i,3}^{i,j,k} + c_{i,3}^{i,j,k-1} - 2c_{i,4}^{i,j,k}}{\tau_i^1} + \frac{c_{i,4}^{i,j,k} + c_{i,4}^{i,j,k-1} - 2c_{i,4}^{i,j,k}}{\tau_i^1} + \frac{c_{i,0}^{i,j,k} + c_{i,0}^{i,j,k-1} - 2c_{i,4}^{i,j,k}}{\tau_i^1} \\
J_{ci,5}^{i,j,k} &= \frac{c_{i,4}^{i,j,k} + c_{i,4}^{i,j,k-1} - 2c_{i,5}^{i,j,k}}{\tau_i^1} + \frac{c_{i,5}^{i,j,k} + c_{i,5}^{i,j,k-1} - 2c_{i,5}^{i,j,k}}{\tau_i^1} + \frac{c_{i,1}^{i,j,k} + c_{i,1}^{i,j,k-1} - 2c_{i,5}^{i,j,k}}{\tau_i^1} \\
J_{ci,6}^{i,j,k} &= \frac{c_{i,5}^{i,j,k} + c_{i,5}^{i,j,k-1} - 2c_{i,6}^{i,j,k}}{\tau_i^1} + \frac{c_{i,6}^{i,j,k} + c_{i,6}^{i,j,k-1} - 2c_{i,6}^{i,j,k}}{\tau_i^1} + \frac{c_{i,2}^{i,j,k} + c_{i,2}^{i,j,k-1} - 2c_{i,6}^{i,j,k}}{\tau_i^1} \\
J_{ci,7}^{i,j,k} &= \frac{c_{i,6}^{i,j,k} + c_{i,6}^{i,j,k-1} - 2c_{i,7}^{i,j,k}}{\tau_i^1} + \frac{c_{i,7}^{i,j,k} + c_{i,7}^{i,j,k-1} - 2c_{i,7}^{i,j,k}}{\tau_i^1} + \frac{c_{i,3}^{i,j,k} + c_{i,3}^{i,j,k-1} - 2c_{i,7}^{i,j,k}}{\tau_i^1}
\end{align*}
\]

(A.4)
The equations for SR calcium cycling are given similarly as

\[
\frac{d}{dt} c_{i,j,k} = \beta(c_{i,j,k}) \left( J_{tr,b}^{i,j,k} + J_{tr,4}^{i,j,k} - J_{rel}^{i,j,k} \frac{V_p}{v_{JSR}} \right)
\]

\[
\frac{d}{dt} c_{n}^{i,j,k} = (J_{up,n}^{i,j,k} - J_{leak,n}^{i,j,k}) \frac{v_i}{v_{NSR}} - J_{tr,n}^{i,j,k} \frac{v_{JSR}}{v_{NSR}} + J_{cNSR,n}^{i,j,k}
\]

(A.5)

and the nearest neighbor diffusive currents between NSR compartments, \(J_{cNSR,n}^{i,j,k}\), have the same form as the diffusive currents between cytosolic compartments given by Eq. (A.4).

### A.2 Ca\textsuperscript{2+} buffering

All buffers are modeled in a time-dependent manner, which is described by Eq. A.6. Because of fast calcium changing in the submembrane space and proximal space, I introduce 10 fine-steps. Buffering parameters for the cytosolic, submembrane and proximal compartments are given in Table A.2.

\[
\frac{d}{dt} [Ca \cdot B_i] = k_{on}^{i} [Ca] [B_i] - k_{off}^{i} [Ca \cdot B_i]
\]

(A.6)

### A.3 RyR model

SR release is mediated by 100 RyRs collocated with LCCs in each CRU. Each RyR is described by a four-state model [73] (also shown in Fig. 2.1 A), which assumes that luminal Ca\textsuperscript{2+} regulates the sensitivity of the RyR channels via auxiliary proteins triadin/junctin (T/J) interacting with the luminal Ca\textsuperscript{2+} buffer calsequestrin (CSQN) [126]. This mechanism is incorporated by allowing CSQN to bind to the RyR/T/J complex when Ca\textsuperscript{2+} is depleted in the JSR and by choosing the RyR closed to open transition rate in the CSQN bound state (\(k_b\)) to be much smaller than the one (\(k_u\)) in the CSQN unbound state. The reduced sensitivity in the CSQN
### Table A.1: General parameters of the cell

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical constants and ionic concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_m )</td>
<td>Cell capacitance</td>
<td>45 pF</td>
</tr>
<tr>
<td>( v_i )</td>
<td>Local cytosolic volume*</td>
<td>0.0625 ( \mu m^3 )</td>
</tr>
<tr>
<td>( v_s )</td>
<td>Local submembrane volume</td>
<td>0.025 ( \mu m^3 )</td>
</tr>
<tr>
<td>( v_p )</td>
<td>Local proximal volume</td>
<td>0.00126 ( \mu m^3 )</td>
</tr>
<tr>
<td>( v_{JSR} )</td>
<td>Local junctional SR volume</td>
<td>0.02 ( \mu m^3 )</td>
</tr>
<tr>
<td>( v_{NSR} )</td>
<td>Local network SR volume*</td>
<td>0.003125 ( \mu m^3 )</td>
</tr>
<tr>
<td>( F )</td>
<td>Faraday Constant</td>
<td>96.5 C/mmol</td>
</tr>
<tr>
<td>( R )</td>
<td>Universal gas constant</td>
<td>8.315 J/mol/K</td>
</tr>
<tr>
<td>( T )</td>
<td>Temperature</td>
<td>308 K</td>
</tr>
<tr>
<td>([Na]_o)</td>
<td>External Na(^+) concentration</td>
<td>140 mM</td>
</tr>
<tr>
<td>([K]_i)</td>
<td>Internal K(^+) concentration</td>
<td>140 mM</td>
</tr>
<tr>
<td>([K]_o)</td>
<td>External K(^+) concentration</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>([Ca]_o)</td>
<td>External Ca(^{2+}) concentration</td>
<td>1.8 mM</td>
</tr>
<tr>
<td><strong>Diffusive time scales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \tau_i )</td>
<td>Transverse cytosolic</td>
<td>0.462 ms</td>
</tr>
<tr>
<td>( \tau_l )</td>
<td>Longitudinal cytosolic</td>
<td>0.98 ms</td>
</tr>
<tr>
<td>( \tau_n )</td>
<td>Transverse NSR</td>
<td>1.26 ms</td>
</tr>
<tr>
<td>( \tau_r )</td>
<td>Longitudinal NSR</td>
<td>4.2 ms</td>
</tr>
<tr>
<td>( \tau_s )</td>
<td>Transverse submembrane</td>
<td>1.42 ms</td>
</tr>
<tr>
<td>( \tau_{tr} )</td>
<td>JSR refilling time</td>
<td>6.25 ms</td>
</tr>
<tr>
<td>( \tau_{ps} )</td>
<td>Proximal to submembrane</td>
<td>0.0283 ms</td>
</tr>
<tr>
<td>( \tau_{si} )</td>
<td>Submembrane to cytosol</td>
<td>0.04 ms</td>
</tr>
<tr>
<td>( \tau_{pi} )</td>
<td>Proximal to cytosol</td>
<td>0.1 ms</td>
</tr>
<tr>
<td><strong>Ionic current conductances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( g_{Na} )</td>
<td>Na(^+) current conductance</td>
<td>12 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{K1} )</td>
<td>( I_{K1} ) conductance</td>
<td>0.6 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{NaK} )</td>
<td>( I_{NaK} ) conductance</td>
<td>1.5 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{Kr} )</td>
<td>( I_{Kr} ) conductance</td>
<td>0.0125 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{Kr} )</td>
<td>( I_{Kr} ) conductance</td>
<td>0 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{Ks} )</td>
<td>( I_{Ks} ) conductance</td>
<td>0.2 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{Io,f} )</td>
<td>( I_{Io,f} ) conductance</td>
<td>0.1 mS/( \mu F )</td>
</tr>
<tr>
<td>( g_{Io,s} )</td>
<td>( I_{Io,s} ) conductance</td>
<td>0.04 mS/( \mu F )</td>
</tr>
</tbody>
</table>

Each CRU contains a 2×2×2 lattice of cytosolic and NSR compartments.
TABLE A.2: Parameters of buffers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytosolic buffers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B_{Myo}$</td>
<td>Myosin concentration</td>
<td>140 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+\text{Myo}}$</td>
<td>On rate for Myosin Ca$^{2+}$ binding</td>
<td>0.0138 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-\text{Myo}}$</td>
<td>Off rate for Myosin Ca$^{2+}$ binding</td>
<td>0.00046 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{+\text{Myo}}$</td>
<td>On rate for Myosin Mg$^{2+}$ binding</td>
<td>0.0000157 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-\text{Myo}}$</td>
<td>Off rate for Myosin Mg$^{2+}$ binding</td>
<td>0.000057 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$B_{Ts}$</td>
<td>Slow Troponin C concentration</td>
<td>134 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+Ts}$</td>
<td>On rate for Slow Troponin C binding</td>
<td>0.00254 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-Ts}$</td>
<td>Off rate for Slow Troponin C binding</td>
<td>0.000033 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$B_{Tf}$</td>
<td>Fast Troponin C concentration</td>
<td>70 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+Tf}$</td>
<td>On rate for Fast Troponin C binding</td>
<td>0.0327 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-Tf}$</td>
<td>Off rate for Fast Troponin C binding</td>
<td>0.0196 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$B_{SR}$</td>
<td>SR binding site concentration</td>
<td>19 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+SR}$</td>
<td>On rate for SR binding</td>
<td>0.1 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-SR}$</td>
<td>Off rate for SR binding</td>
<td>0.06 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$B_{Cd}$</td>
<td>Calmodulin binding site concentration</td>
<td>24 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+Cd}$</td>
<td>On rate for Calmodulin binding</td>
<td>0.0543 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-Cd}$</td>
<td>Off rate for Calmodulin binding</td>
<td>0.238 (ms)$^{-1}$</td>
</tr>
<tr>
<td><strong>Submembrane space and proximal space buffers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B_{SLH}$</td>
<td>Membrane high binding site concentration</td>
<td>18 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+SLH}$</td>
<td>Membrane high binding site on rate</td>
<td>0.1 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-SLH}$</td>
<td>Membrane high binding site off rate</td>
<td>0.03 (ms)$^{-1}$</td>
</tr>
<tr>
<td>$B_{SL}$</td>
<td>Sarcolemma binding site concentration</td>
<td>50 $\mu$mol/l cyt</td>
</tr>
<tr>
<td>$k_{+SL}$</td>
<td>Sarcolemmal binding site on rate</td>
<td>0.1 ($\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{-SL}$</td>
<td>Sarcolemmal binding site off rate</td>
<td>1.3 (ms)$^{-1}$</td>
</tr>
</tbody>
</table>

Bound state both contributes to Ca$^{2+}$ spark termination and induces a refractory [87, 127] state whereby CSQN must unbind from RyRs before future sparks are possible. Experiments reported a large RyR unitary calcium current under near-physiological ionic conditions (0.35 - 0.6 $pA$ at 1 mM $[Ca^{2+}]_{JSR}$) [156, 157], which is $\sim$ 10-fold larger than the value assumed in Restrepo et al. [73]. Therefore, I follow Sato et al. [135] by using RyR closed-to-open
transition rates that produce maximum open probability around 0.1 (Fig. 3.2 C). I also introduce a SR load dependence of the closed-to-open rates \( k_u \) and \( k_b \) to model the increase of RyR open probability at high \([Ca^{2+}]_{JSR} [230]\), which is not captured by the original Restrepo model [73]. In that model, luminal gating of RyR is solely mediated by CSQN binding to the RyR/T/J complex and the RyR open probability becomes independent of SR load when all RyRs occupy the CSQN unbound state, which occurs for \([Ca^{2+}]_{JSR}\) larger than \(\approx 600 \mu M\) (Fig. A.2). In the present model, RyRs are sensitive to luminal \(Ca^{2+}\) (in addition to being regulated by CSQN binding) as supported by a wide range of experiments (see [158] and references therein).

I model RyR hyperactivity both by shifting the open probability of RyRs towards lower cleft calcium concentration by reducing \(\rho^+\) as further explained below and by shortening RyR refractoriness. The increase in open probability is consistent with lipid bilayer studies of hyperactive RyRs [153] and the observed increased frequency of \(Ca^{2+}\) sparks in permeabilized LQT2 myocytes where RyRs are hyperphosphorylated [28].

The transition rates in the RyR model are given by

\[
\begin{align*}
  k_u &= \frac{\alpha}{1 + \left(\frac{c^+}{c_p}\right)^2} \frac{1}{1 + \left(\frac{c_j}{c_j}\right)^2} \\
  k_b &= \frac{\beta}{1 + \left(\frac{c^+}{c_p}\right)^2} \frac{1}{1 + \left(\frac{c_j}{c_j}\right)^2} \\
  k^- &= 1/\tau_-
\end{align*}
\]

\[
\begin{align*}
  r_u &= 1/\tau_u \\
  r'_u &= r_u k_u/k_b \\
  r_b &= \left(1 + \left(\frac{c_B}{K_B}\right)^{24}\right)^{-1} \tau_b^{-1} \\
  c_B &= \frac{nc_jB_{CSQN}}{K_C + c_j}
\end{align*}
\]
Appendix A Multi-scale model for rabbit ventricular myocytes

All the parameters used are given in Table A.3.

A.4 Modification of luminal gating

\[ \beta(c) = \left( 1 + \frac{nB_{CSQN}K_C + \partial_c nB_{CSQN}(cK_c + c^2)}{(K_C + c)^2} \right)^{-1}. \]  

(A.8)

Due to a missing quantity \( B_{CSQN} \) in the original formulation Eq. 18 in Restrepo et al. [73], which is also highlighted in the equation above, \( \text{Ca}^{2+} \) is not conserved in the cell. With the error corrected and other parameters slightly changed, the Restrepo et al. model retains its ability to model calcium transient alternans [231]. However, a subsequent experimental study using FRET[232] showed that CSQN dimerization occurs on a time scale of minutes, thereby invalidating the assumption that the transition between monomers and dimers is instantaneous. Consequently, based on those experimental findings, I model here CSQN as a mixture of monomers and dimers with a fixed ratio of numbers of monomers and dimers. Furthermore, I assume that the average number of binding sites per CSQN molecule in this mixture \( n = 22 \).
Then the instantaneous luminal Ca\(^2+\) buffering reads

\[
\beta(c) = \left(1 + \frac{nB_{CSQN}K_C}{(K_C + c)^2}\right)^{-1}.
\] (A.9)

The original fractional occupancy \(c_B/B_{CSQN}\) and buffering factor \(\beta\) are shown as the red lines in Fig. A.2 B and C, and the modified functions are shown as the blue lines.

In addition, I assume that the rate of CSQN binding to the RyR/T/J complex \((r_b, \text{Fig. A.3})\) is dependent on the amount of Ca\(^2+\) bound to CQSN, instead of on the number of CQSN monomers present that is now fixed in the model. I model this dependence as shown in Eq. A.3 with parameter values are listed in Table A.3. The high Hill coefficient is chosen so that the experimentally known nonlinear relationship between fractional SR release and SR load is quantitatively reproduced by the model [233]. A comparison of the SR load dependence of \(k_b\) given by Eq. A.3 and the original Restrepo et al. model is given Fig. A.2 A.

### A.5 Na\(^+\) dynamics

Whole-cell intracellular Na\(^+\) dynamics is governed by

\[
\frac{d[Na^+]_i}{dt} = \frac{C_m}{F_{V_i}}(I_{Na} + 3I_{NCX} + 3I_{NaK})
\] (A.10)

### A.6 Ionic currents

The rate of change of the membrane voltage \(V\) is described by the equation

\[
\frac{dV}{dt} = -(I_{stim} + I_{Ca,L} + I_{NCX} + I_{Na} + I_{to,f} + I_{to,s} + I_{K1} + I_{NaK} + I_{Kr} + I_{Ks})
\]

\[
I_{Ca,L} = \frac{-2F_{Vp}}{C_m}J_{Ca}
\]

\[
I_{NCX} = \frac{F_{V_j}}{C_m}J_{NCX}
\] (A.11)

where \(I_{stim}\) is the stimulus current driving the cell.
A.7 SERCA uptake current

The SERCA uptake rate in each compartment is given by

\[ J_{up} = v_{up} \frac{(c_i/K_i)^H - (c_n/K_{NSR})^H}{1 + (c_i/K_i)^H + (c_n/K_{NSR})^H} \]  \hspace{1cm} (A.12)

The maximum SERCA uptake current \( (v_{up}) \) is increased both due to homeostatic upregulation in LQT2 myocytes, and pharmacological phosphorylation of PLB under ISO. The parameter under each condition is given in Table A.3.

A.8 SR leak current

The SR leak current in each compartment is given by

\[ J_{leak} = g_{leak} \frac{c_n^2}{c_n^2 + K_{NSR}} (c_n - c_i). \]  \hspace{1cm} (A.13)

A.9 The Na\(^+\)/Ca\(^{2+}\) exchanger flux (NCX)

To have a more realistic NCX current model, I consider the allosteric Ca\(^{2+}\) activation to be time-dependent, which was reported by Ginsburg et al. [155], with the activation timescale \( \tau_{NCX} \sim 150 \text{ ms} \). The exchange current flux in each CRU is given by,

\[ J_{NCX} = g_{NCX} A_{NCX} e^{\frac{\xi VF/RT}{H}} \left[ \frac{[Na]_i^3}{K_m,Na_o c_s} + e^{\left(\frac{\xi - 1}{\xi VF/RT}\right)H} \right] \]  \hspace{1cm} (A.14)

where

\[ H = K_{m,Ca_o} [Na]_i^3 + K_{m,Na_o} c_s \left( 1 + \frac{c_s}{K_{m,Ca_i}} \right) \]

\[ + K_{m,Ca_i} [Na]_o^3 \left( 1 + \frac{[Na^{+}]_i^3}{K_{m,Na_i}} \right) + [Na]_i^3 [Ca]_o + [Na]_o^3 c_s, \]
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uptake and SR leak parameters</strong></td>
<td></td>
<td><strong>L-type Ca(^{2+}) current parameters</strong></td>
<td></td>
</tr>
<tr>
<td>(v_{up}) (LMC)</td>
<td>0.3 ((\mu M) ms(^{-1}))</td>
<td>(P_{Ca})</td>
<td>11.4 (\mu)mol/C/ms</td>
</tr>
<tr>
<td>(v_{up}) (LMC with ISO)</td>
<td>0.525 ((\mu M) ms(^{-1}))</td>
<td>(\gamma_l, \gamma_o)</td>
<td>0.341</td>
</tr>
<tr>
<td>(v_{up}) (LQT2)</td>
<td>0.375 ((\mu M) ms(^{-1}))</td>
<td>(r_1)</td>
<td>0.22 ms(^{-1})</td>
</tr>
<tr>
<td>(v_{up}) (LQT2 with ISO)</td>
<td>0.525 ((\mu M) ms(^{-1}))</td>
<td>(\alpha_{fc})</td>
<td>0.006 ms(^{-1})</td>
</tr>
<tr>
<td>(K_i)</td>
<td>0.123 (\mu M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K_{NSR})</td>
<td>1700 (\mu M)</td>
<td>(r_2)</td>
<td>4 ms(^{-1})</td>
</tr>
<tr>
<td>(H)</td>
<td>1.787</td>
<td>(d_{s0})</td>
<td>5 mV</td>
</tr>
<tr>
<td>(g_{leak})</td>
<td>1.035 (\times 10^{-5}) ms(^{-1})</td>
<td>(f_{s0})</td>
<td>-22.8 mV</td>
</tr>
<tr>
<td>(K_{JSR})</td>
<td>500 (\mu M)</td>
<td>(f_{vk})</td>
<td>9.1 mV</td>
</tr>
<tr>
<td><strong>Na(^+)/Ca(^{2+}) exchanger parameters</strong></td>
<td></td>
<td><strong>With ISO</strong></td>
<td></td>
</tr>
<tr>
<td>(g_{NCX})</td>
<td>52.5 (\mu M) ms(^{-1})</td>
<td>(r_2)</td>
<td>2 ms(^{-1})</td>
</tr>
<tr>
<td>(K_{mCai})</td>
<td>0.00359 mM</td>
<td>(d_{s0})</td>
<td>0 mV</td>
</tr>
<tr>
<td>(K_{mCaO})</td>
<td>1.3 mM</td>
<td>(f_{s0})</td>
<td>-28 mV</td>
</tr>
<tr>
<td>(K_{mNaO})</td>
<td>12.3 mM</td>
<td>(f_{vk})</td>
<td>8.5 mV</td>
</tr>
<tr>
<td>(K_{mNaO})</td>
<td>87.5 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_{sat})</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\eta)</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\tau_{NCX})</td>
<td>150 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Luminal gating parameters</strong></td>
<td></td>
<td><strong>Parameters for RyR opening rates</strong></td>
<td></td>
</tr>
<tr>
<td>(J_{max})</td>
<td>0.169 (\mu m^3) ms(^{-1})</td>
<td>(\alpha)</td>
<td>4 ms(^{-1})</td>
</tr>
<tr>
<td>(\tau_u)</td>
<td>1100 ms</td>
<td>Stable</td>
<td></td>
</tr>
<tr>
<td>(\tau_b)</td>
<td>1 ms</td>
<td>(\beta)</td>
<td>0.04 ms(^{-1})</td>
</tr>
<tr>
<td>(\tau_c)</td>
<td>1 ms</td>
<td>(c^*)</td>
<td>3.1 (\mu M)</td>
</tr>
<tr>
<td>(n)</td>
<td>22</td>
<td>Increased Ca(^{2+}) sensitivity</td>
<td></td>
</tr>
<tr>
<td>(K_C)</td>
<td>600 (\mu M)</td>
<td>(\beta)</td>
<td>0.04 ms(^{-1})</td>
</tr>
<tr>
<td>(BCSQN)</td>
<td>460 (\mu M)</td>
<td>(c^*)</td>
<td>3.1 (\mu M)</td>
</tr>
</tbody>
</table>

\textbf{Table A.3: Parameters for} \(I_{up}, I_{leak}, I_{NCX}, RyR,\) and \(I_{Ca,L}\)
Appendix A Multi-scale model for rabbit ventricular myocytes

and where

\[
\frac{dA_{NCX}}{dt} = \frac{A_{NCX,\infty} - A_{NCX}}{\tau_{NCX}}
\]

\[
A_{NCX,\infty} = \frac{1}{1 + (0.3/c_s)^3}.
\]

(A.15)

A.10 L-type Ca\(^{2+}\) channel (\(I_{Ca,L}\))

Following Terentyev et al. [28], I adopt the 16-state Markov model for the L-type Ca\(^{2+}\) channel (LCC) (Fig. A.3), with 4 channels in the dyadic space of each CRU. The Ca\(^{2+}\) flux through LCCs into the dyadic space is determined by the number of open channels (\(N_O\)) in the CRU.

\[
J_{Ca} = i_{Ca} N_O
\]

(A.16)

where the single channel current flux is given by

\[
i_{Ca} = 4P_{Ca}zF \frac{\gamma_0[Ca]_o e^{2z} - \gamma_0[Ca]_o}{e^{2z} - 1},
\]

\[
z = VF/(RT).
\]

(A.17)

The 16 states, regulating the open probability of each channel, represent combinations of 4 independent binary gating mechanisms: 2 transitions modeling activation, one modeling voltage-dependent inactivation, and one modeling calcium-dependent inactivation. There are 4 states of activation \(O, C, C',\) and \(CC'\). The voltage-dependent component of activation represents transitions between \(O\) and \(C\) (or \(C'\) and \(CC'\)) with rates given by \(\alpha_d\) and \(\beta_d\). The voltage-independent component of activation represents transitions between \(O\) and \(C'\) (or \(C\) and \(CC'\)) with rates given by \(r_1\) and \(r_2\). For each state of activation, an LTCC can additionally be in a voltage-inactivated state \((I_V)\), a calcium-inactivated state \((I_C)\) or both \((I_VI_C)\). The rates of transitions to and from voltage inactivated states are given by \(\alpha_{I_v}\) and \(\beta_{I_v}\) respectively, and the rates of transitions to and from calcium inactivated states are given by \(\alpha_{I_c}\) and \(\beta_{I_c}\) respectively.

This model can be reduced to a simple 3-gate Hodgkin-Huxley form
Appendix A Multi-scale model for rabbit ventricular myocytes

Figure A.3: Schematic diagram of the 16-state LTCC model. The model includes one open state $O$, combinations of closed states ($C$ or $C'$), voltage- and calcium-dependent inactivated states ($I_V$ and $I_C$, respectively). The 4 states in each square may each transition to the corresponding states in the adjacent squares via the voltage-dependent ($\alpha_{fV}$, $\beta_{fV}$) and Ca$^{2+}$-dependent ($\alpha_{fC}$, $\beta_{fC}$) transition rates. From Zhong et al. [1].

\[ P_O = C d f_V f_C \]  \hspace{1cm} (A.18)

with a constant prefactor $C = r_1/(r_1 + r_2)$. $d$ and $f$ are standard gating parameters for voltage-dependent activation and inactivation respectively. The Ca$^{2+}$-dependent inactivation $f_C$, however, cannot be expressed with a simple mean-field ODE because $c_p$ is generally different in each CRU.

The rates of transitions between states (in ms$^{-1}$) are given by:

\[
\begin{align*}
\alpha_d &= \frac{d_{\infty}}{\tau_d} \\
\beta_d &= \frac{1 - d_{\infty}}{\tau_d} \\
\alpha_f &= \frac{f_{\infty}}{\tau_f}
\end{align*}
\]
The parameters for LTCC gating are given in Table A.3. The parameters are altered under the effect of ISO to match experimental measurements of increased peak magnitude, longer open duration and larger window current (Terentyev et al. [28]).

## A.11 RyR-mediated SR Ca\(^{2+}\) release

The SR release is mediated by the 100 RyR channels in each CRU. The flux is determined by the fraction of open RyRs, \( P_o \), and given by

\[
J_{rel} = J_{max} P_o (c_j - c_p) / v_p
\]  

(A.20)
A.12 The fast sodium current ($I_{Na}$)

As in the [161] model,

$$I_{Na} = g_{Na}m^3hj(V - E_{Na})$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h$$

$$\frac{dj}{dt} = \alpha_j(1 - j) - \beta_j j$$

$$\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m$$

$$\alpha_m = 0.32 \frac{V + 47.13}{1 - e^{-0.1(V + 47.13)}}$$

$$\beta_m = 0.08e^{-V/11}$$

(A.21)

For $V \leq -40$ mV,

$$\alpha_h = 0$$

$$\alpha_j = 0$$

$$\beta_h = \frac{1}{0.13(1 + e^{(V+10.66)/-11.1})}$$

(A.22)

$$\beta_j = 0.3 \frac{e^{-2.535 \times 10^{-7}V}}{1 + e^{-0.1(V+32)}}$$

For $V \geq -40$ mV,

$$\alpha_h = 0.135e^{(V+80)/-6.8}$$

$$\beta_h = 3.56e^{0.079V} + 3.1 \times 10^5 e^{-0.04391V}$$

$$\alpha_j = \frac{(-1.2714 \times 10^5 e^{0.2444V} - 3.474 \times 10^{-5} e^{-0.04391V})(V + 37.78)}{1 + e^{0.311(V+79.23)}}$$

(A.23)

$$\beta_j = \frac{0.1212e^{-0.01052V}}{1 + e^{-0.1378(V+40.14)}}$$


A.13 Inward rectifier $K^+$ current ($I_{K1}$)

As in the [161] model,

$$I_{K1} = g_{K1} \sqrt{\frac{[K^+]_o}{5.4}} \frac{A_{K1}}{A_{K1} + B_{K1}} (V - E_K)$$

$$A_{K1} = \frac{1.02}{1 + e^{0.2385(V - E_K - 59.215)}}$$

$$B_{K1} = \frac{0.49124 e^{0.08032(V - E_K + 5.476)} + e^{0.061750(V - E_K - 59.41)}}{1 + e^{-0.5143(V - E_K + 4.753)}}$$

$$E_K = \frac{RT}{F} \ln \left( \frac{[K^+]_o}{[K^+]_i} \right).$$

(A.24)

A.14 The rapidly activating delayed rectifier $K^+$ current ($I_{Kr}$)

While this current is excluded for all simulations in this study, I include it here for completeness.

$$I_{Kr} = g_{Kr} R(V) x_{Kr} \left[ V - \frac{RT}{F} \ln \left( \frac{0.98[K^+]_o + 0.02[Na^+]_o}{0.98[K^+]_i + 0.02[Na^+]_i} \right) \right]$$

$$R(V) = \frac{1}{1 + e^{(V+52)/17.8}}$$

$$\frac{dx_{Kr}}{dt} = \frac{x_{Kr}^\infty - x_{Kr}}{\tau_{Kr}}$$

$$x_{Kr}^\infty = \frac{1}{1 + e^{-(x+35)/5.2}}$$

$$\tau_{Kr} = \left( \frac{11.262(V - 159)}{1 - \exp(-(V - 159)/11.46)} + \frac{0.0002(V + 32)}{\exp((V + 32)/2.75) - 1} \right)^{-1}.$$

(A.25)

A.15 The slowly activating delayed rectifier $K^+$ current ($I_{Ks}$)

I use a formulation of $I_{Ks}$ based on Mahajan et al. [2], with slow kinetics for $Ca^{2+}$-dependent activation, which is given by,
\[ I_{K_s} = g_{K_s} x_{s1} x_{s2} Q_{Ks} (V - E_{Ks}) \]  \hspace{1cm} (A.26)

\[
\frac{dx_{s1}}{dt} = \frac{x_s^{\infty} - x_{s1}}{\tau_{xs1}}
\]

\[
\frac{dx_{s2}}{dt} = \frac{x_s^{\infty} - x_{s2}}{\tau_{xs2}}
\]

\[
x_s^{\infty} = \frac{1}{1 + e^{-(V-1.5)/16.7}}
\]

\[
\tau_{xs1} = \left( \frac{0.0000719(V + 30)}{1 - e^{-0.148(V+30)}} + \frac{0.00031(V + 30)}{-1 + e^{0.0687(V+30)}} \right)^{-1}
\]

\[
\tau_{xs2} = \tau_{xs1}
\]

\[
\frac{dQ_{Ks}}{dt} = \frac{Q_{Ks,\infty} - Q_{Ks}}{1000}
\]

\[
Q_{Ks,\infty} = 0.2 + \frac{0.16}{1 + \left( \frac{0.28}{c_r} \right)^3}
\]

\[
E_{Ks} = \frac{RT}{F} \ln \left( \frac{[K^+]_o + 0.01833[Na^+]_o}{[K^+]_i + 0.01833[Na^+]_i} \right).
\]
A.16  The fast component of the transient outward K⁺ current

\((I_{to,f})\)

As in Shannon et al. \[161\],

\[
I_{to,f} = g_{to,f}X_{to,f}Y_{to,f}(V - E_K)
\]

\[
\frac{dX_{to,f}}{dt} = \frac{X_{to,f}^\infty - X_{to,f}}{\tau_{X_{to,f}}}
\]

\[
\frac{dY_{to,f}}{dt} = \frac{Y_{to,f}^\infty - Y_{to,f}}{\tau_{Y_{to,f}}}
\]

\[
X_{to,f}^\infty = \frac{1}{1 + e^{-(V+3)/15}}
\]

\[
Y_{to,f}^\infty = \frac{1}{1 + e^{(V+33.5)/10}}
\]

\[
\tau_{X_{to,f}} = 3.5e^{-(V/30)^2} + 1.5
\]

\[
\tau_{Y_{to,f}} = \frac{20}{1 + e^{(V+33.5)/10}} + 20
\]
A.17 The slow component of the transient outward K$^+$ current ($I_{to,s}$)

As in Shannon et al. [161],

$$I_{to,s} = g_{to,s}X_{to,s}(Y_{to,s} + 0.5R_S^\infty)(V - E_K)$$

$$\frac{dX_{to,s}}{dt} = \frac{X_{to,s}^\infty - X_{to,s}}{\tau_{X_{to,s}}}$$

$$\frac{dY_{to,s}}{dt} = \frac{Y_{to,s}^\infty - Y_{to,s}}{\tau_{Y_{to,s}}}$$

$$X_{to,s}^\infty = \frac{1}{1 + e^{-(V+3)/15}}$$

$$Y_{to,s}^\infty = \frac{1}{1 + e(V+33.5)/10}$$

$$R_S^\infty = \frac{1}{1 + e(V+33.5)/10}$$

$$\tau_{X_{to,s}} = \frac{9}{1 + e(V+3)/15} + 0.5$$

$$\tau_{Y_{to,s}} = \frac{3000}{1 + e(V+60)/10} + 30$$

(A.28)

A.18 The Na$^+$-K$^+$ pump current ($I_{NaK}$)

As in Shannon et al. [161],

$$I_{NaK} = g_{NaK}f_{NaK}\frac{1}{1 + (K_{m,Nai}/[Na^+])} \frac{[K^+]_o}{[K^+]_o + K_{m,Ko}}$$

$$f_{NaK} = \frac{1}{1 + 0.1245e^{-0.1VF/(RT)} + 0.0365\sigma e^{-VF/(RT)}}$$

$$\sigma = \frac{1}{7}(e^{[Na^+]_o/67.3} - 1)$$

(A.29)
The model described here is based on the model in Appendix A, which is the same as that in Zhong et al. [1]. To mimic the β-adrenergic stimulation of isopretorenel (ISO), we modified Ca\(^{2+}\) uptake and \(I_{Ca,L}\) parameters as described in the Zhong et al [1]. Besides, following Liu et al [98], we increased the \(I_{Ks}\) conductance by 33% (Table B.1). To mimic the effect of caffeine, we modified the parameters in the RyR gating functions \(k_u\) and \(k_b\) (Table B.2).

Computer simulations were carried out using Graphic Processor Unit with CUDA and C language. We collected data after the cells reach steady states, which are after 10 s for the permeabilized cell, and after 8 s the intact cells. For the high-resolution model of the Ca\(^{2+}\) spark, we collected data from the beginning. The source codes of the models are available at https://github.com/mingwang-zhong/Calcium-Wave.

The main changes we have made, based on Zhong et al [1], are described in the following sections. Modifications of parameters and other minor changes are listed in Table B.1 - B.5.

### B.1 RyR model

The first modification is the Hill coefficient in RyR gating functions (Eq. 1 in the main text). Considering that there is a regulatory protein FKBP 12.6 connecting neighboring RyRs, the coupling between RyRs should be considered [81]. From the perspective of modeling, the RyR cooperativity leads to a larger Hill coefficient (>10) [87, 137]. Without loss of generality,
Appendix B Multi-scale model for Ca$^{2+}$ waves

**Table B.1: Parameter changes under the effect of ISO**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>No ISO</th>
<th>ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{uptake}$ (mM)</td>
<td>Strength of SR Ca$^{2+}$ pump</td>
<td>0.376</td>
<td>0.752</td>
</tr>
<tr>
<td>$g_{Ks}$ (mS/µF)</td>
<td>Conductance of $I_{Ks}$ channel</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>$r_2$ (ms$^{-1}$)</td>
<td>Ca$^{2+}$-dependent inactivation rate of $I_{Ca,L}$</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$d_{s0}$ (mV)</td>
<td>Constant in activation of $I_{Ca,L}$</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>$f_{s0}$ (mV)</td>
<td>Constant in $V_m$-dependent inactivation of $I_{Ca,L}$</td>
<td>-22.8</td>
<td>-28</td>
</tr>
<tr>
<td>$f_{vK}$ (mV)</td>
<td>Constant in $V_m$-dependent inactivation of $I_{Ca,L}$</td>
<td>9.1</td>
<td>8.5</td>
</tr>
</tbody>
</table>

The formula of $I_{Ks}$ is the same as that in Mahajan et al [2]. The parameters for $I_{Ca,L}$ are the same as that in Zhong et al [1].

we adopted $H = 8$ in the present study. The exposure of the cell to ISO may lead to RyR phosphorylation by protein kinase. However, if the phosphorylation is not strong enough to fully break RyR cooperativity, the change of $H$ might be small such that RyR cooperativity still facilitate Ca$^{2+}$ wave initiation. This can be seen from Fig. 2 in the main text which shows that Ca$^{2+}$ waves still occur for $H$ as low as 5.

Under the exposure to caffeine, the RyR activity is altered. We simulated this effect by reducing $c^*_u$ and $c^*_b$ in Eq. 1 in the main text. The changes of values are listed in Table B.2.

$$\tau_u (ms) = \frac{2000}{1 + ([Ca^{2+}]_{JSR}/670\mu M)^{24}} + 150$$

(B.1)

For the intact cells exposed to caffeine, $c^*_u$ is so small that RyRs could be activated by long-type Ca$^{2+}$ currents during the plateau phase of the action potential. RyR leakiness leads to a longer time for SR to refill, meaning that the diastolic SR load could not be high enough to activate spontaneous Ca$^{2+}$ waves. Therefore, we suppressed RyR leakiness during the plateau phase by increasing the transition time ($\tau_u$) at low SR load from calsequestrin(CSQN)-bound closed state to CSQN-unbound state. At high SR load, to ensure a large open probability for the RyR cluster, we set $\tau_u$ to be small. The expression of $\tau_u$ as a function of Ca$^{2+}$ concentration in the
Appendix B: Multi-scale model for Ca\textsuperscript{2+} waves

junctions SR ([Ca\textsuperscript{2+}]\textsubscript{JSR}) is shown in Eq. B.1. The Hill coefficient is chosen to be the same as that in the transition time $\tau_b$.

**Table B.2: Parameters of RyR gating under different conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Permeabilized cell</th>
<th>LMC</th>
<th>LMC + caffeine</th>
<th>LMC + caffeine + ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H$</td>
<td>2~8</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>$\alpha$ ($ms^{-1}$)</td>
<td>0.12</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>$\beta$ ($ms^{-1}$)</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>$c^+$ ($\mu M$)</td>
<td>1.4</td>
<td>3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>$\gamma_a$ ($ms^{-1}$)</td>
<td>$2 \times 10^{-6}$</td>
<td>$2 \times 10^{-6}$</td>
<td>$2 \times 10^{-6}$</td>
<td>$2 \times 10^{-6}$</td>
</tr>
<tr>
<td>$\gamma_b$ ($ms^{-1}$)</td>
<td>$2 \times 10^{-9}$</td>
<td>$2 \times 10^{-9}$</td>
<td>$2 \times 10^{-9}$</td>
<td>$2 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

B.2 Mobile buffers (fluo-3, EGTA, ATP)

To simulate the Ca\textsuperscript{2+} spark profile as in the experiments, we added the fluorescent indicator dye, fluo-3, to the cytosolic space [91]. A large amount of dye can bind to the proteins that reduce the mobility. Moreover, in many experimental studies of Ca\textsuperscript{2+} sparks and waves [125, 133], a large amount of slow mobile buffer, EGTA, was added to the cell to inhibit Ca\textsuperscript{2+} waves at low cytosolic Ca\textsuperscript{2+} load, such that Ca\textsuperscript{2+} sparks occur in a larger cytosolic Ca\textsuperscript{2+} load range. Following Bovo et al [125], we added the same amount of EGTA, 350 $mM$, to the cytosol. For the association and dissociation rate constant, we used the values from Smith et al [234]. In addition, we took into account the fast endogenous mobile buffer ATP. The dissociation rate constant seems to be much larger than association constant (30 $ms^{-1}$ and 0.15 $\mu M^{-1}ms^{-1}$, respectively) [131, 141], indicating that the amount of Ca\textsuperscript{2+} bound ATP might be small. However, there is a huge amount ATP in the cytosol (5$mM$ [24]), which results in the amount of Ca\textsuperscript{2+} bound ATP comparable to other buffers. Beside Ca\textsuperscript{2+}, ATP can also bind to Mg\textsuperscript{2+}, but we found that because Mg\textsuperscript{2+} binding rate is very small (0.00195 $\mu M^{-1}ms^{-1}$) [132], the change of Mg\textsuperscript{2+} bound ATP concentration is less than 0.1% as Ca\textsuperscript{2+} concentration changes from 0.1 $\mu M$ to 5 $\mu M$. Therefore, we ignore Mg\textsuperscript{2+} in the buffering dynamics, and reduce the effective ATP concentration to be 4 $mM$ (the other 1 $mM$ ATP only binds to Mg\textsuperscript{2+}).
Appendix B Multi-scale model for Ca$^{2+}$ waves

B.3 Diffusion time of Ca$^{2+}$ and mobile buffers

Following Izu et al [91], we adopted the realistic Ca$^{2+}$ diffusion time in the cytosolic space. In the longitudinal direction, the distance between two neighboring compartments is 0.9 µm, so the Ca$^{2+}$ diffusion time is $l^2/D = 2.7$ ms, corresponding to the diffusion coefficient 0.3 µm$^2$/ms. Similarly, in the transverse direction the distance is 0.45 µm, so the Ca$^{2+}$ diffusion time is 1.35 ms, corresponding to the diffusion coefficient 0.15 µm$^2$/ms. In addition, we adopted the diffusion time of ATP and dye about twice of free calcium [132]. Comparing to ATP and dye, the diffusion time of the mobile buffer EGTA is much larger (~ 2.6-fold) [234].

B.4 Uncoupled sub-membrane spaces in the longitudinal direction

The original Restrepo model [73] includes the coupling between sub-membrane spaces in the longitudinal direction. However, the sub-cellular structure of the cardiac myocytes show that the T-tubule and triad system are located in the z-lines [24], which are typically discretely distributed in the longitudinal direction. Therefore, we removed the longitudinal coupling of sub-membrane spaces.

B.5 Optical blurring of sparks by the confocal microscope

In the experiments, the recording of Ca$^{2+}$ spark is not Ca$^{2+}$ concentration itself. Instead, what is recorded is the brightness signal produced by Ca$^{2+}$ bound fluorescence indicator dye. During the measurement, the confocal microscope has an optical blurring effect. Following Smith et al [130], we introduced a point spread function (PSF) to simulate the optical blurring. This is done by convolving the three dimensional PSF $G(x,y,z)$ with the three dimensional Ca$^{2+}$ bound dye concentration $[CaF](x,y,z)$, as described by Eq. B.2. Typically, PSF is a three dimensional Gaussian function with reported FWHM of 0.7 - 1.4 µm in the longitudinal direction ($\lambda_x$), and ~ 0.4 µm in the transverse direction ($\lambda_y, \lambda_z$) [93, 132, 235]. Here, we adopted $\lambda_x = 1.3$ µm and
\( \lambda_y = \lambda_z = 0.48 \mu m \). To implement the convolution, we used the FFTW package for CUDA.

\[
[CaF]_{out pu}(x, y, z) = \iiint [CaF](x', y', z')G(x - x', y - y', z - z')dx'dy'dz'
\]  

(B.2)

### B.6 Ca\(^{2+}\) spark detection

We used two compatible ways to define a spark. For most of the cases (Fig. 1A&E and Fig. 4 in the main text), if the Ca\(^{2+}\) release current via a single RyR cluster is larger than 0.5 \( pA \), such an event is defined as a Ca\(^{2+}\) spark. For the spark statistics in Fig. 1B-D in the main text, if the local blurred fluorescence signal peak \( F \) is above a threshold, such an event is defined as a Ca\(^{2+}\) spark. The threshold is determined by comparing the results of these two methods. If these methods give the same spark rate, we defined the detected fluorescence signals as sparks, otherwise we changed the threshold until these two methods give the same spark frequency.

To determine the spark frequency, we followed the same procedure as in Izu et al \[89\]. A confocal sample of area 0.5 \( \mu m \times 1 \mu m \) contains 2 CRUs at the z-line. Therefore, in a confocal line-scan of length 100 \( \mu m \), there are 2 CRUs/\( z \)-line \( \times 100/1.8 \) \( z \)-lines = 111.1 CRUs. Therefore, the spark frequency is

\[
f_{\text{spark}} = f_{\text{CRU}}(#/\text{CRU/s}) \times 111.1(\text{CRU}/100\mu m) = 111.1 f_{\text{CRU}}(#/100\mu m/s)
\]

(B.3)

### B.7 SR Ca\(^{2+}\) pump (uptake)

We used a thermodynamical model of SR Ca\(^{2+}\) pump developed by Tran et al. \[236\]. Since the three-state model and simplified two-state model give essentially the same result when [MgATP] is in the physiological range, we adopted the two-state model. The formula for uptake...
Appendix B Multi-scale model for Ca\(^{2+}\) waves

is given by Eq. B.4. The parameters are listed in Table B.3. \(J_{\text{up}}\) is in unit of \(\mu M/\text{ms}\).

\[
T_{\text{MgATP}} = \frac{k_1^+ [\text{MgATP}]}{k_1^-}
\]

\[
T_{c_i} = \frac{c_i}{K_{d,c_i}} \quad T_{c_j} = \frac{c_j}{K_{d,c_j}}
\]

\[
T_{H_i} = \frac{[H^+]^2}{K_{d,H_i}} \quad T_{H_j} = \frac{[H^+]^2}{K_{d,H_j}}
\]

\[
T_H = \frac{[H^+]}{K_{d,H}} \quad T_H = \frac{[H^+]}{K_{d,H}}
\]

\[
\alpha_1^+ = \frac{k_2^+ T_{\text{MgATP}} T_{c_i}^2}{T_{\text{MgATP}} T_{c_i}^2 + T_{H_i} (1 + T_{\text{MgATP}} (1 + T_{H_1} + T_{c_i}^2))}
\]

\[
\alpha_2^+ = \frac{k_2^- T_{H_j}}{T_{H_j} (1 + T_{H_j}) + T_{H_j} (1 + T_{c_j})}
\]

\[
\alpha_1^- = \frac{k_2^- [\text{MgADP}] T_{H} T_{c_j}^2}{T_{H_j} (1 + T_{H_j}) + T_{H_j} (1 + T_{c_j}^2)}
\]

\[
\alpha_2^- = \frac{k_3^- [\text{Pi}] T_{H_i}}{T_{\text{MgATP}} T_{c_i}^2 + T_{H_i} (1 + T_{\text{MgATP}} (1 + T_{H_1} + T_{c_i}^2))}
\]

\[
J_{\text{up}} = v_{\text{up}} \frac{\alpha_1^+ \alpha_2^+ - \alpha_1^- \alpha_2^-}{\alpha_1^+ + \alpha_2^+ + \alpha_1^- + \alpha_2^-}
\]

B.8 High-resolution Ca\(^{2+}\) spark model

In the high-resolution model, there are 10 \(\times\) 5 \(\times\) 5 lattices for the cytosol (longitude \(\times\) transverse \(\times\) transverse). The diffusion time constants of Ca\(^{2+}\) and mobile buffers in the cytosol are modified following the law: \(\tau = l^2 / D\). All the interplays between cytosol and SR are blocked, and the Ca\(^{2+}\) release current is forced to be the red line in Fig. 1E in the main text. Ca\(^{2+}\) release still feeds the dyadic space, and the diffusion time constant between dyad and submembrane space remains unchanged. The dyadic space and submembrane space are still coupled to two neighboring compartments of the cytosol, with the diffusion time constants modified. All the modified parameters are listed in Table B.4.
**Appendix B Multi-scale model for Ca\textsuperscript{2+} waves**

**Table B.3: Parameters of SR Ca\textsuperscript{2+} pump**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1^+$</td>
<td>25900 mM\textsuperscript{-1}s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$k_2^+$</td>
<td>2540 s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$k_3^+$</td>
<td>20.5 s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$k_1^-$</td>
<td>2 mM\textsuperscript{-1}s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$k_2^-$</td>
<td>67200 mM\textsuperscript{-1}s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$k_3^-$</td>
<td>149 mM\textsuperscript{-1}s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$[\text{MgATP}]$</td>
<td>5 mM</td>
</tr>
<tr>
<td>$[\text{MgADP}]$</td>
<td>0.0363 mM</td>
</tr>
<tr>
<td>$[H^+]$</td>
<td>10\textsuperscript{-4} mM</td>
</tr>
<tr>
<td>$[P_i]$</td>
<td>1 mM</td>
</tr>
<tr>
<td>$K_{d,ci}$</td>
<td>0.91 mM</td>
</tr>
<tr>
<td>$K_{d,cj}$</td>
<td>2.24 mM</td>
</tr>
<tr>
<td>$K_{d,Hi}$</td>
<td>1.09×10\textsuperscript{-5} mM</td>
</tr>
<tr>
<td>$K_{d,Hj}$</td>
<td>3.54×10\textsuperscript{-3} mM\textsuperscript{2}</td>
</tr>
<tr>
<td>$K_{d,H}$</td>
<td>1.05×10\textsuperscript{-8} mM\textsuperscript{2}</td>
</tr>
<tr>
<td>$K_{d,H}$</td>
<td>7.24×10\textsuperscript{5} mM</td>
</tr>
</tbody>
</table>

**Table B.4: Modified parameters for high-resolution Ca\textsuperscript{2+} spark model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{ix}$</td>
<td>Number of cytosolic lattices in the longitudinal direction</td>
<td>10</td>
</tr>
<tr>
<td>$N_{iy}$</td>
<td>Number of cytosolic lattices in the transverse direction</td>
<td>5</td>
</tr>
<tr>
<td>$N_{iz}$</td>
<td>Number of cytosolic lattices in the transverse direction</td>
<td>5</td>
</tr>
<tr>
<td>$\tau_{nl}$</td>
<td>Diffusion time between longitudinal NSR</td>
<td>0.168 ms</td>
</tr>
<tr>
<td>$\tau_{nt}$</td>
<td>Diffusion time between transverse NSR</td>
<td>0.208 ms</td>
</tr>
<tr>
<td>$\tau_{il}$</td>
<td>Diffusion time between longitudinal cytosol</td>
<td>0.108 ms</td>
</tr>
<tr>
<td>$\tau_{it}$</td>
<td>Diffusion time between transverse cytosol</td>
<td>0.216 ms</td>
</tr>
<tr>
<td>$\tau_{pi}$</td>
<td>Diffusion time between dyad and submembrane space</td>
<td>0.025 ms</td>
</tr>
<tr>
<td>$\tau_{si}$</td>
<td>Diffusion time between cytosol and submembrane space</td>
<td>0.01 ms</td>
</tr>
</tbody>
</table>
### Table B.5: Modified parameters from Zhong et al. [1]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{JSR}$</td>
<td>Junctional SR volume</td>
<td>0.03 $\mu m^3$</td>
</tr>
<tr>
<td>$l_x$</td>
<td>Distance between z-lines</td>
<td>1.8 $\mu m$</td>
</tr>
<tr>
<td>$\tau_d$</td>
<td>Diffusion time between sub-membrane spaces</td>
<td>1.5 ms</td>
</tr>
<tr>
<td>$\tau_{nl}$</td>
<td>Diffusion time between longitudinal NSR</td>
<td>4.2 ms</td>
</tr>
<tr>
<td>$\tau_{nt}$</td>
<td>Diffusion time between transverse NSR</td>
<td>1.3 ms</td>
</tr>
<tr>
<td>$\tau_l$</td>
<td>Diffusion time between longitudinal cytosol</td>
<td>2.7 ms</td>
</tr>
<tr>
<td>$\tau_t$</td>
<td>Diffusion time between transverse cytosol</td>
<td>1.35 ms</td>
</tr>
<tr>
<td>$f_{ATP}$</td>
<td>Diffusion time factor of ATP compared to $Ca^{2+}$</td>
<td>2.148</td>
</tr>
<tr>
<td>$f_{dye}$</td>
<td>Diffusion time factor of dye compared to $Ca^{2+}$</td>
<td>2</td>
</tr>
<tr>
<td>$f_{EGTA}$</td>
<td>Diffusion time factor of EGTA compared to $Ca^{2+}$</td>
<td>2.59</td>
</tr>
<tr>
<td>$J_{max}$</td>
<td>RyR releasing constant</td>
<td>0.27 $\mu m^2$/ms</td>
</tr>
<tr>
<td>$\lambda_x$</td>
<td>FWHM of PSF in longitudinal direction</td>
<td>1.3 $\mu m$</td>
</tr>
<tr>
<td>$\lambda_y, \lambda_z$</td>
<td>FWHM of PSF in transverse direction</td>
<td>0.48 $\mu m$</td>
</tr>
<tr>
<td>$B_{dye}$</td>
<td>Total concentration of dye</td>
<td>50 $\mu mol/l$ cyt</td>
</tr>
<tr>
<td>$k_{dye}^+$</td>
<td>Associate constant of dye</td>
<td>0.08 ($\mu M$ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{dye}^-$</td>
<td>Dissociate constant of dye</td>
<td>0.09 ms$^{-1}$</td>
</tr>
<tr>
<td>$B_{ATP}$</td>
<td>Total concentration of ATP</td>
<td>4 mmol/l cyt</td>
</tr>
<tr>
<td>$k_{ATP}^+$</td>
<td>Associate constant of ATP</td>
<td>0.15 ($\mu M$ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{ATP}^-$</td>
<td>Dissociate constant of ATP</td>
<td>30 ms$^{-1}$</td>
</tr>
<tr>
<td>$B_{EGTA}$</td>
<td>Total concentration of EGTA</td>
<td>350 $\mu mol/l$ cyt (Permeabilized cell)</td>
</tr>
<tr>
<td>$B_{EGTA}^+$</td>
<td>Total concentration of EGTA</td>
<td>0 (Intact cell)</td>
</tr>
<tr>
<td>$k_{EGTA}^+$</td>
<td>Associate constant of EGTA</td>
<td>0.0015 ($\mu M$ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{EGTA}^-$</td>
<td>Dissociate constant of EGTA</td>
<td>0.003 ms$^{-1}$</td>
</tr>
<tr>
<td>$B_{SLH}$</td>
<td>Total concentration of membrane/high binding site</td>
<td>15 $\mu mol/l$ cyt</td>
</tr>
<tr>
<td>$B_{SL}$</td>
<td>Total concentration of sarcolemma binding site</td>
<td>42 $\mu mol/l$ cyt</td>
</tr>
<tr>
<td>$[Mg]_i$</td>
<td>Intracellular magnesium concentration</td>
<td>1 mM</td>
</tr>
<tr>
<td>$g_{Kr}$</td>
<td>Conductance of $I_{Kr}$</td>
<td>0.0078 $mS/\mu F$</td>
</tr>
<tr>
<td>$g_{K1}$</td>
<td>Conductance of $I_{K1}$</td>
<td>0.3 $mS/\mu F$ [2]</td>
</tr>
<tr>
<td>$V_{leak}$</td>
<td>Strength of SR leak current</td>
<td>0.00212 ms$^{-1}$</td>
</tr>
<tr>
<td>$V_{NCX}$</td>
<td>Strength of $I_{NCX}$</td>
<td>20 $\mu M$ms</td>
</tr>
<tr>
<td>$\tau_{NCX}$</td>
<td>Allosteric $Ca^{2+}$ activation time</td>
<td>0</td>
</tr>
<tr>
<td>$n_{Ca}$</td>
<td>Number of $Ca^{2+}$ binding site on CSQN molecule</td>
<td>31</td>
</tr>
<tr>
<td>$n_{LTCC}$</td>
<td>Number of $Ca^{2+}$ channels per couplon</td>
<td>6</td>
</tr>
<tr>
<td>$P_{Ca}$</td>
<td>LTCC permeability</td>
<td>11.9 $\mu mol/(msC)$</td>
</tr>
<tr>
<td>$f_{pace}$</td>
<td>Pacing frequency</td>
<td>0.5 Hz</td>
</tr>
<tr>
<td>$[Na]_i$</td>
<td>Intracellular sodium concentration</td>
<td>10 mM</td>
</tr>
</tbody>
</table>

The $Ca^{2+}$ leak current is only located in the junctional cleft [161], instead of cytosol.
C.1 Experiments

C.1.1 Myocyte isolation

All procedures were approved by The Rhode Island Hospital Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Ventricular myocytes were isolated from the hearts of transgenic LQT2 ($n=3$) female NZW rabbits (3.5 kg) using standard enzymatic digestion procedures. In brief, the heart was excised from euthanized rabbits and perfused for 5 to 7 minutes with a nominally Ca$^{2+}$-free solution containing (in mM): 140 NaCl, 4.4 KCl, 1.5 MgCl$_2$, 0.33 NaH$_2$PO$_4$, 16 taurine, 5 HEPES, 5 pyruvic acid, and 7.5 glucose. Next, the heart was perfused for 10 to 15 minutes with the same solution to which 0.65% collagenase type II (Worthington Biochemical), and 0.1% BSA were added. The LV was minced, and the cells were dispersed with a glass pipette for 3-5 minutes in a solution containing (in mM): 45 KCl, 65 K-glutamate, 3 MgSO$_4$, 15 KH$_2$PO$_4$, 16 taurine, 10 HEPES, 0.5 EGTA, and 10 glucose and 1% BSA (pH 7.3). The cell suspension was filtered through
Appendix C Multi-scale model and experimental conditions for LQT2 myocytes

a 100-µm nylon mesh, and plated on laminin coated coverslips in medium M199, and used within 6 to 8 hours.

C.1.2 Cell electrophysiology and Ca\textsuperscript{2+} imaging

Action Potentials (APs) were recorded using the whole-cell patch clamp technique at 35±2°C using Axopatch 200B amplifier and DIGIDATA 1322A interface (Axon Instruments, CA) \cite{28}. The external solution contained (in mM): 140 NaCl, 5.4 KCl, 1.85 CaCl\textsubscript{2}, 0.5 MgCl\textsubscript{2}, 10 HEPES, and 5.6 glucose (pH 7.4). Patch pipettes were filled with the following solution (in mM): 90 K-aspartate, 50 KCl, 5 MgATP, 5 NaCl, 1 MgCl\textsubscript{2}, 0.1 Tris GTP, 10 HEPES, and 0.1 Rhod-2 (Molecular Probes, OR (pH 7.2). APs were evoked by application of 3-ms-long voltage pulses with amplitude 20% above the threshold level. Intracellular Ca\textsuperscript{2+} imaging was performed using Leica SP5 confocal system in a line scan mode. Rhod-2 was excited by 543 nm laser and the fluorescence was acquired at 560-660 nm wavelengths. Calcium transients were analyzed using Leica Software, Origin 8.2 (OriginLab, Northampton, MA) and Image J (NIH, Bethesda, MA).

C.2 Mathematical modeling

The model described here is the same as the model in Appendix A, which is the same as that in Zhong \textit{et al.} \cite{1}. Computer simulations were carried out by pacing myocytes at 0.25 Hz in accordance with experiments \cite{28} until a steady state was reached. During pacing, whole-cell cytosolic and SR calcium concentrations were recorded, denoted by [Ca\textsuperscript{2+}]\textsubscript{i} and [Ca\textsuperscript{2+}]\textsubscript{SR}, respectively, along with \textit{V}_m and individual sarcolemmal currents. In addition, experimental confocal line scans were emulated by recording the local [Ca\textsuperscript{2+}]\textsubscript{i} along a longitudinal row of CRUs passing through the center of the myocyte and parallel to its long axis.
The model described here is based on the model in Appendix A, which is the same as that in Zhong et al. [1]. The computer simulations were carried out by pacing the myocytes at 0.5 Hz or 2.8 Hz (350 ms), where the myocytes reached the steady state before the data were collected. By varying the sodium potassium pump ($I_{NaK}$) conductance, $[Na]_i$ dynamically changes in the model to reach reasonable levels during the steady-states for different strength of $I_{NaL}$. The change of $I_{NaK}$ conductance is listed in Table D.1.

All the parameter changes from the model of Zhong et al. [1] are described in the following paragraphs and Table D.1. The formulation of $I_{NaL}$ that was added here is given in the main text. In addition, the code to produce the simulation results is available at https://github.com/MingwangZhong/Late-Sodium.

### D.1 Cytosolic volume

The data in D.M. Bers [8] and Sato et al. [237] show that 30% of cytosolic space is occupied by Mitochondrial, which means that the effective cytosolic space for Ca$^{2+}$ diffusion is 1 $\mu m^3$ considering that the average distance between z-lines is 1.8 $\mu m^3$ and the average distance between dyadic spaces in the transverse direction is 0.9 $\mu m^3$. Therefore, I increased the effective cytosolic space volume per CRU from 0.5 to 1 $\mu m^3$ to make the model more realistic.
D.2 RyR gating

With increase of cytosolic space volume, more amount of buffer is present in the cell, which results in smaller amplitude of Ca\(^{2+}\) transient during pacing and less Ca\(^{2+}\) dependent inactivation of LTCC. In order to counterbalance this effect, I modified the RyR open probability and Ca\(^{2+}\) sensitivity so that Ca\(^{2+}\) transient is reasonable and the late aberrant Ca\(^{2+}\) release driven EADs mechanism still holds in LQT2 myocytes. The modified parameters are listed in Table D.1.

D.3 Allosteric Ca\(^{2+}\) activation of NCX

I use the simpler formulation of NCX current given in Mahajan et al. [2] with time-independent allosteric Ca\(^{2+}\) activation since the more complex formulation with time-dependent allosteric Ca\(^{2+}\) activation of NCX used in Zhong et al. [1] is not required for EAD formation. Both formulations of NCX yield similar results in the context of the present study.

D.4 Voltage-dependent inactivation time scale \(\tau_f\) of \(I_{Ca,L}\)

The equation of \(\tau_f\) is as follows, with \(\tau_f\) increased from 1 to 1.2.

\[
\tau_f = \frac{f_{\tau_f}}{0.02 - 0.007e^{-0.001136(V_m+10.5)^2} \quad (D.1)}
\]

179
**Appendix D Multi-scale model to study the late sodium currents in LQT2 myocytes**

**Table D.1:** Modified parameters from Zhong et al. [1]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{K_s}$</td>
<td>$I_{K_s}$ conductance</td>
<td>0.4 mS/µF (No ISO)</td>
</tr>
<tr>
<td>$g_{K_s}$</td>
<td>$I_{K_s}$ conductance</td>
<td>0.5 mS/µF (ISO)</td>
</tr>
<tr>
<td>$g_{NaK}$</td>
<td>$I_{NaK}$ conductance</td>
<td>1.35 mS/µF</td>
</tr>
<tr>
<td>$g_{NCX}$</td>
<td>Strength of $I_{NCX}$</td>
<td>21 µM/ms</td>
</tr>
<tr>
<td>$g_{leak}$</td>
<td>Strength of SR leak</td>
<td>0.0000207 ms⁻¹</td>
</tr>
<tr>
<td>$P_{Ca}$</td>
<td>L-type Ca²⁺ channel permeability</td>
<td>9.8 µmol/(cmC)</td>
</tr>
<tr>
<td>$f_{t_f}$</td>
<td>Constant of $I_{Ca,L}$ inactivation time scale</td>
<td>1.2</td>
</tr>
<tr>
<td>$\tau_{u}$</td>
<td>Time scale of CSQN unbinding</td>
<td>1100 ms</td>
</tr>
<tr>
<td>$\tau_{b}$</td>
<td>Time scale of CSQN binding</td>
<td>1 ms</td>
</tr>
<tr>
<td>$\tau_{il}$</td>
<td>Diffusion time of $c_i$ in longitudinal direction</td>
<td>0.7 ms</td>
</tr>
<tr>
<td>$\tau_{it}$</td>
<td>Diffusion time of $c_i$ in transverse direction</td>
<td>0.33 ms</td>
</tr>
<tr>
<td>$B_{SAR}$</td>
<td>Concentration of sarcolemma Ca²⁺ binding site</td>
<td>42 µmol/l cyt</td>
</tr>
<tr>
<td>$B_{MH}$</td>
<td>Concentration of membrane/high Ca²⁺ binding site</td>
<td>15 µmol/l cyt</td>
</tr>
<tr>
<td>$V_i$</td>
<td>Cytosolic space volume</td>
<td>1 µm³</td>
</tr>
<tr>
<td>$k_{p,U}$</td>
<td>Maximum open rate of CSQN-unbound RyR</td>
<td>80 ms⁻¹</td>
</tr>
<tr>
<td>$k_{p,B}$</td>
<td>Maximum open rate of CSQN-bound RyR</td>
<td>35.5 ms⁻¹</td>
</tr>
<tr>
<td>$c^*$</td>
<td>Ca²⁺ sensitivity parameter of RyR</td>
<td>19</td>
</tr>
</tbody>
</table>

**Figure D.1:** Effect of 100 nM GS967 on steady-state [Na]ᵢ. Simulations are carried out under ISO at PCL = 2000 ms with (left panel) and without (right panel) 100 nM GS967. The [Na]ᵢ values of the blue and red markers in Fig. 4.12 are chosen from the steady states of the blue and red lines in this figure, respectively.
We use a ventricular myocyte model modified from Mahajan et al. [2]. In this model, Ca\(^{2+}\) cycling couples the membrane voltage \(V_m\) dynamics [2] to investigate the ionic mechanisms of EAD formation in LQT1 myocytes under conditions corresponding to the experiments. The model describes the bi-directional coupling of Ca\(^{2+}\) and \(V_m\) dynamics by incorporation of a full set of sarcolemmal currents including \(I_{Ca,L}\), \(I_{NCX}\), \(I_{Kr}\), \(I_{to}\), \(I_{K1}\), and \(I_{NaK}\), and also including (excluding) \(I_{Ks}\) for LMC (LQT1) myocytes.

To model the effect of β-adrenergic stimulation with isoproterenol (ISO), we increased the SERCA uptake rate by 50% and decreased the threshold by 40% (Table E.1). We used the modified Hodgkin-Huxley model of LTCC, with releasing currents under voltage clamp fitted to experimental measurements under 50 nM ISO.

Computer simulations were carried out by pacing myocytes at 0.25 Hz until a steady state was reached. This protocol, and the external electrolyte concentrations (Table E.1) were chosen to reproduce the conditions of isolated myocytes under patch clamp. This pacing protocol was applied to LMC and LQT1 myocytes from both LV and RV.

The gating kinetics of most of the ionic currents were largely unchanged, however, their conductances were adjusted to better reproduce experimental measurements. Additionally, extracellular ionic concentrations were changed to match experimental conditions. A list of these parameters is given in Table E.1.
Appendix E Whole-cell model for LQT1 myocytes

Table E.1: Modified parameters from Mahajan et al. [2]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Kr}$</td>
<td>$I_{Kr}$ conductance</td>
<td>$0.096 \text{ mS/µF (MGWMN)}$</td>
</tr>
<tr>
<td>$g_{Kr}$</td>
<td>$I_{Kr}$ conductance</td>
<td>$0.16 \text{ mS/µF (HH)}$</td>
</tr>
<tr>
<td>$g_{Ks}$</td>
<td>$I_{Ks}$ conductance</td>
<td>$0.17 \text{ mS/µF (RV)}$</td>
</tr>
<tr>
<td>$g_{Ks}$</td>
<td>$I_{Ks}$ conductance</td>
<td>$0.14 \text{ mS/µF (LV)}$</td>
</tr>
<tr>
<td>$g_{Ca,L}$</td>
<td>$I_{Ca,L}$ conductance</td>
<td>$70 \text{ mmol/(cmC)}$</td>
</tr>
<tr>
<td>$g_{NCX}$</td>
<td>Strength of $I_{NCX}$</td>
<td>$0.756 \mu M/s$</td>
</tr>
<tr>
<td>$\nu_{up}$</td>
<td>Strength of SERCA</td>
<td>$0.6 \mu M/s$</td>
</tr>
<tr>
<td>$c_o$</td>
<td>Extracellular Ca$^{2+}$ concentration</td>
<td>$1 \text{ mM}$</td>
</tr>
<tr>
<td>$[Na]_i$</td>
<td>Intracellular Na$^+$ concentration</td>
<td>$5.1 \text{ mM}$</td>
</tr>
<tr>
<td>$\tau_s$</td>
<td>Submembrane-cytosol diffusion time constant</td>
<td>$1.5 \text{ ms}$</td>
</tr>
<tr>
<td>$\tau_p$</td>
<td>RyR refractory period</td>
<td>$500 \text{ ms}$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Constant</td>
<td>$80$</td>
</tr>
<tr>
<td>$g_{RyR}$</td>
<td>Release current strength</td>
<td>$1.29 \text{ sparks cm}^2/\text{mA}$</td>
</tr>
</tbody>
</table>

E.1 Model of $I_{to}$

Fig. 3.17 A & B demonstrate examples of measured $I_{to}$ current and double exponential fit to these curves, characterized by the equation

$$I_{to} = I_{to,fi}e^{-t/\tau_{fi}} + I_{to,si}e^{-t/\tau_{si}} + I_{SS} \quad (E.1)$$

We model this current using a Hodgkin-Huxley type model of the form:

$$I_{to} = g_{fi}X_{to,fi}(V - E_K) + g_{si}X_{to,si}(V_m - E_K)$$

$$\frac{dX_{to}}{dt} = \frac{X_{to}^\infty - X_{to}}{\tau_{to,X}}$$

$$\frac{dY_{to,fi}}{dt} = \frac{Y_{to,fi}^\infty - Y_{to,fi}}{\tau_{fi}}$$

$$\frac{dY_{to,si}}{dt} = \frac{Y_{to,si}^\infty - Y_{to,si}}{\tau_{si}}$$

$$\tau_{to,X} = \frac{9}{1 + e^{(V_m+3)/15}} + 5 \quad (E.2)$$
### Table E.2: Parameters of $I_{to}$ from experiments [29]

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>LV</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVT origin</td>
<td>8/9 hearts</td>
<td>1/9 hearts</td>
<td>Fisher exact test, p&lt;0.05</td>
</tr>
<tr>
<td>PVT origin after 4-AP</td>
<td>1/5 hearts</td>
<td>4/5 hearts</td>
<td>Fisher exact test, p&lt;0.05</td>
</tr>
<tr>
<td>Cell capacitance</td>
<td>119 ± 25 (n=43)</td>
<td>168 ± 52 (n=38)</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>EAD incidences</td>
<td>8/12 myocytes</td>
<td>2/11 myocytes</td>
<td>Fisher exact test, p&lt;0.05</td>
</tr>
<tr>
<td>$I_{to}$ amplitude</td>
<td>17.1 ± 2.1 pA/pF (n=13)</td>
<td>12.4 ± 1.4 pA/pF (n=12)</td>
<td>ANOVA, p&lt;0.05</td>
</tr>
<tr>
<td>(at 50 mV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g_{to,fi}$</td>
<td>0.068 ± 0.0049 mS/µF</td>
<td>0.052 ± 0.0045 mS/µF</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>$\tau_{fi}$</td>
<td>8.8 ± 0.4 ms</td>
<td>7.8 ± 0.5 ms</td>
<td>Not significant</td>
</tr>
<tr>
<td>$g_{to,si}$</td>
<td>0.033 ± 0.0052 mS/µF</td>
<td>0.018 ± 0.0021 mS/µF</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>$\tau_{si}$</td>
<td>73 ± 6.7 ms</td>
<td>59 ± 6.9 ms</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>$I_{to,f}$ amplitude</td>
<td>8.9 ± 1.7 pA/pF</td>
<td>5.3 ± 1.6 pA/pF</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>$\tau_{fast}$</td>
<td>19.5 ± 1.1 ms</td>
<td>19.5 ± 1.9 ms</td>
<td>Not significant</td>
</tr>
<tr>
<td>$I_{to,s}$ amplitude</td>
<td>22.4 ± 1.8 pA/pF</td>
<td>14.8 ± 0.44 pA/pF</td>
<td>t-test, p&lt;0.05</td>
</tr>
<tr>
<td>$\tau_{slow}$</td>
<td>3.41 ± 0.33 ms</td>
<td>3.13 ± 0.12 ms</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

where $V_m$ is membrane voltage.

Since activation is much faster than inactivation, we calculate the steady state activation curve $X_{to}^\infty$ by dividing the total peak $I_{to}$ current at each voltage by the $K^+$ driving force $F(V_m) = V_m - RT/F \ln(K_o/K_i)$, where $K_o = 5.4 \text{ mM}$ under patch clamp conditions used to record this current. The conductance $g_{fi}$ is determined using the relation $[I_{to,fi}/F(V_m)]_{50\text{mV}} = g_{fi}$ where $I_{to,fi}$ is determined from the double exponential fit given by Eq. E.1, and similarly for $g_{si}$. The inactivation is calculated by dividing the steady state current $I_{SS}$ by the total peak $I_{to}$ current. These curves are shown in Fig. 3.14 F. Fitting to these curves yields the following

$$X_{to}^\infty = \frac{1}{1 + e^{-(V_m+10.06)/13.75}}$$

$$Y_{to}^\infty = \frac{1}{1 + e^{-(V_m+41.97)/5.14}}$$

(E.3)

In RV:
Appendix E Whole-cell model for LQT1 myocytes

\[ g_{fi} = 0.08 \text{ mS/\mu F} \]
\[ g_{si} = 0.05 \text{ mS/\mu F} \]  
\[ \tau_{fi} = 8.4 + \frac{100}{1 + e^{(V_m+60)/10}} \]
\[ \tau_{si} = 80 + \frac{3000}{1 + e^{(V_m+60)/10}} \]  

(E.4)

In LV:

\[ g_{fi} = 0.08 \text{ mS/\mu F} \]
\[ g_{si} = 0.025 \text{ mS/\mu F} \]  
\[ \tau_{fi} = 8.4 + \frac{100}{1 + e^{(V_m+60)/10}} \]
\[ \tau_{si} = 50 + \frac{3000}{1 + e^{(V_m+60)/10}} \]  

(E.5)

In both LV and RV, all time scales are in ms. \( g_{fi} \) and \( g_{si} \) are chosen to be different from experimental fitting shown in Fig. 3.14, because the later measurements showed that \( I_{to} \) in Fig. 3.14 was not fully recovered. Instead, the conductances are from the measurement of \( I_{to} \) recovery [29]. The measurement of \( I_{to} \) recovery also shows that the fast components of \( I_{to} \) in RV and LV are the same, and the slow component in LV is half of that in RV. The forms of \( \tau_{fi} \) and \( \tau_{si} \) are taken from Mahajan et al. [2]. The values of \( \tau_{fi} \) and \( \tau_{si} \) at high voltage are chosen to match the experimental measurements within standard error shown in Fig. 3.14 E. The values at the resting voltage are consistent with the measurements of recovery kinetics using a double-pulse protocol. The recovery kinetics was found to be well fitted by a double exponential with time constants approximately equal to 20 ms and 3 s. Examination of the inactivation time course of following the double pulse reveals that most of the fast recovering component inactivate quickly. However, a non-negligible fraction of the slowly recovering component inactivates rapidly (80% in LV, 40% in RV, [29]). Consequently, the current mathematical formulation of \( I_{to} \), decomposed as the sum of \( I_{to,fi} \) and \( I_{to,si} \), provides a reasonably good approximation to both the inactivation and recovery kinetics of measured \( I_{to} \), even though neither \( I_{to,fi} \) nor \( I_{to,si} \)
individually models $I_{t_0,f}$ or $I_{t_0,s}$ (traditionally associated with the fast and slowly recovering components of $I_{t_0}$).

**E.2 Model of $I_{Kr}$**

Based on the multi-state model given by Fig. 3.15, the rapidly activating delayed rectifier K$^+$ current is given by

$$I_{Kr} = g_{Kr} \sqrt{\frac{K_o}{5.4}} O(V_m - E_K)$$  \hspace{1cm} (E.6)

with the rates of reactions satisfy

$$\frac{dC_1}{dt} = \beta_1 C_2 - \alpha_1 C_1$$
$$\frac{dC_2}{dt} = k_b C_3 + \alpha_1 C_1 - (k_f + \beta_1) C_2$$
$$\frac{dC_3}{dt} = \beta_2 O + \psi I + k_f C_2 - (\alpha_2 + \alpha_{i2} + k_b) C_3$$  \hspace{1cm} (E.7)
$$\frac{dI}{dt} = \beta_i O + \alpha_{i2} C_3 - (\alpha_i + \psi) I$$
$$\frac{dO}{dt} = \alpha_i I + \alpha_{i2} C_3 - (\beta_i + \beta_2) O$$
where the transition rates are given by:

\[
\begin{align*}
\alpha_1 &= 0.007953e^{V_m/30.6312} \\
\beta_1 &= 0.083063e^{V_m/69.176} \\
\alpha_2 &= 0.008511e^{V_m/21.167} \\
\beta_2 &= 0.003242e^{V_m/95.0202} \\
\alpha_t &= 0.044965e^{V_m/25.1972} \\
\beta_t &= 0.187367e^{V_m/1467.5695} \\
\alpha_{t2} &= 0.000035e^{V_m/496052.4399} \\
\psi &= \frac{\alpha_2 \beta_2 \alpha_t}{\alpha_2 \beta_t} \\
k_f &= 0.300789 \\
k_b &= 0.135702
\end{align*}
\] (E.8)

A total of 17 parameters including \( g_{Kr} \) were fitted to \( I_{Kr} \) voltage clamp data averaged over 7 cells. E4031-sensitive current (\( I_{Kr} \)) was recorded with voltage steps between -30 and 30 mV from a holding potential of -40 mV. The initial parameters were chosen based on experimental data and previously published parameter sets in Mazhari et al. \([184]\) and calculated \( \tau \) values. The initial \( g_{Kr} \) was chosen based on the inactivation curve estimated from the tail current (see Fig. 3.18 E). The optimization of 17 parameters were done using a simultaneous perturbation stochastic optimization algorithm \([238]\) to accelerate initial fitting. Further optimization was performed using the genetic algorithm built in Matlab R2015b to search in parameter space for the best global minimum of the cost function defined as \( \Sigma(I_{Kr, data} - I_{Kr, fitting})^2 \). We obtained an accurate fit of experimental measurements with this formulation, depicted in Fig. 3.16.

To demonstrate that the significant aspect of this new model to the time course of repolarization and the onset of EADs is the slow time scale of activation, we reproduced the modeling results using an alternative HH-type formulation of \( I_{Kr} \). This model approximately fits the experimental recordings of \( I_{Kr} \) under voltage clamp (Fig. 3.16 C). Equations of this model are
Appendix E Whole-cell model for LQT1 myocytes

as follows.

\[ I_{Kr} = g_{Kr} \sqrt{\frac{K_0}{5.4}} X_{Kr} R(V_m)(V_m - E_K) \]

\[ R(V_m) = \frac{1}{1 + e^{(V_m + 53.5)/24.06}} \]

\[ \frac{dX_{Kr}}{dt} = \frac{X_{Kr}^{\infty} - X_{Kr}}{\tau_{Kr}} \]

\[ X_{Kr}^{\infty} = \frac{1}{1 + e^{-(V_m + 7.78)/7.14}} \]

\[ \tau_{Kr} = \left( \frac{1.466(V_m - 214.2)}{1 - e^{-(V_m - 214.2)/17.18}} + \frac{0.0175(V_m + 58.93)}{e^{(V_m + 58.93)/3.78}} \right)^{-1} \]

(E.9)

E.3 Model of \( I_{Ca,L} \)

The original Mahajan et al. [2] model of \( I_{Ca,L} \) has a very small window, and the parameters of this model are difficult to adjust to fit the voltage clamp data obtained under isoproterenol (ISO) stimulation. Therefore, we adopted the Hodgkin-Huxley model of \( I_{Ca,L} \). Even though this model has a recovery kinetics from inactivation that is predominantly voltage-dependent, it can be used to incorporate the contribution of \( Ca^{2+} \) to rapid inactivation as well as to reproduce both the steady-state activation and inactivation curves, and therefore the size of the \( I_{Ca,L} \) window.

**Figure E.1:** HH model of \( I_{Ca,L} \) fits to experimental recordings under 50 nM ISO. A) \( I_{Ca,L} \) current recording for \( V_m \) step to 0 mV with 50 nM ISO. The error bars are standard deviation to show the scatter of data. B) Activation and inactivation curves. The blue curve is only for voltage-dependent inactivation. \( Ca^{2+} \)-dependent inactivation is shown in Eq. (10). C) Simulation generated steady-state \( I_{Ca,L} \) window current in HH model. From Choi et al. [29].
Appendix E Whole-cell model for LQT1 myocytes

The HH model of $I_{Ca,L}$ was fit to experimental recordings utilizing holding potential of -50 mV, followed by a P1 pulse from -40 to +40 mV in 5 mV steps lasting 400 ms, followed by a P2 pulse to 0 mV. This procedure was performed with ISO. The peak of the P1 pulse provided a measurement of steady-state activation (black squares in Fig. E.1 B) and the peak of the P2 pulse provided a measure of steady-state inactivation (black circles in Fig. E.1 B). The time course of the P1 pulse constrained the kinetics of inactivation (Fig. E.1 A). The window current of LTCC occurs during repolarization of the action potential, typically peaking between 0 and -30 mV. A critical feature of this model is that the window current remains high at $V_m$ above 30 mV, which is the key in LV to maintain $V_m$ at a high level, allowing $I_{Kr}$ to be fully activated.

To improve the HH model of $I_{Ca,L}$, we used the same voltage clamp protocol as in the experiment to produce activation and inactivation simulation data, which were used to fit the experiment results shown in Fig. E.1 B. The steady-state activation and inactivation curves were mainly changed during this procedure. Then Ca$^{2+}$-dependent inactivation was carefully chosen so that the simulation-generated traces fitted the experimental traces within standard deviation shown in Fig. E.1 A. The equations we found from fitting are as follows
Appendix E Whole-cell model for LQT1 myocytes

\[ I_{Ca,L} = g_{Ca,L} \cdot f \cdot C \cdot i_{Ca} \]
\[ \frac{dd}{dt} = \frac{d^\infty - d}{\tau_d} \]
\[ \frac{df}{dt} = \frac{f^\infty - f}{\tau_f} \]
\[ \frac{df}{dt} = \frac{f^\infty_{Ca} - f_{Ca}}{\tau_{f,Ca}} \]

\[ i_{Ca} = \frac{4P_{Ca}\cdot V_m^2 \cdot c_s \cdot e^{2V_mF/(RT)} - 0.341c_o}{RT} \]
\[ d^\infty = \frac{1}{(1 + e^{-(V_m+17.6)/8.1})^2} \]
\[ f^\infty = \frac{0.98}{1 + e^{(V_m+27.66)/4.0432}} + \frac{0.5}{1 + e^{-(V_m-41)/8.93}} + 0.02 \]
\[ f_{Ca}^\infty = 0.7e^{-c_s/0.42} + 0.3e^{-c_s/9} \]
\[ \tau_f = \frac{75}{1 + e^{-(V_m-30)/4}} + 25 \]
\[ \tau_{f,Ca} = 3 \text{ ms} \]
\[ \tau_d = 0.8 \text{ ms} \]

(E.10)

For the simulations that HH model of \( I_{Kr} \) were used, the modified expressions are

\[ f^\infty = \frac{0.98}{1 + e^{(V_m+27.66)/4.0432}} + \frac{0.5}{1 + e^{-(V_m-46)/8.93}} + 0.02 \]
\[ \tau_f = \frac{43}{1 + e^{-(V_m-30)/6}} + 25 \]

(E.11)

This is because HH model of \( I_{Kr} \) has a longer activation time scale at high voltage (Fig. 3.16 & 3.18), therefore less depolarizing current is needed to reproduce the same behavior as MGWMN model of \( I_{Kr} \).

E.4 Calcium cycling

Calcium cycling in the rabbit myocyte is based on the model by Shannon et al. [161], and improved by Mahajan et al. [2]. In addition, the present model has two additional modifications.
First, the function \( g_{RyR}(V_m) \) was modified to obtain an experimentally realistic bell shape relationship between SR Ca\(^{2+}\) release amplitude and peak \( I_{Ca,L} \) as a function of \( V_m \) (graded release). Second, we introduced a dynamical variable \( f_s \) that keeps track at the whole cell level of the fraction of Ca\(^{2+}\) release units (with colocation of LCCs and RyRs in each unit) where a discrete release event (calcium spark) has occurred. This latter addition enables us to model phenomenologically RyR refractoriness. Together, those two modifications allow us to reproduce calcium transients that are similar to those observed in the RV and LV [29]. The modified Ca\(^{2+}\) cycling equations are:

\[
\frac{dJ_{rel}}{dt} = \frac{N'_s(t)Q(c'_j)c_j}{c^*} - \frac{J_{rel}}{T} \\
N'_s(t) = -g_{RyR}(V)P_0i_{Ca}f_s \\
\frac{df_s}{dt} = -\alpha N'_s(t) + \frac{1 - f_s}{\tau_s} \\
g_{RyR}(V_m) = \frac{g_{RyR}}{(1 + e^{(V_m+25)/25})(1 + e^{-(V_m+37)/5})}
\]  

(E.12)

where \( c_j \) and \( c'_j \) are free Ca\(^{2+}\) concentrations in the junctional SR and the network SR. In this phenomenological model, \( N'_s(t) \) is the spark rate in response to L-type Ca current, \( Q(c'_j) \) describes the relationship of calcium release to SR calcium load, \( T \) is the relaxation time of junctional SR (JSR) release current to its steady-state value, and denotes the refractory period of RyRs after a spark. The units of \( c_j \) and \( c'_j \) are \( v_{SR}/v_i \text{ mol}/l \text{ cyt} \), where \( v_{SR}/v_i \) is considered to be around 50, following Weber et al. [239]. The parameter values are summarized in Table E.1.

### E.5 Modification of \( I_{Ks} \)

We used the formulation based on Mahajan et al. [2], with \( \tau_{xs2} \) equal to \( \tau_{xs1} \), and calcium dependent activation threshold shifted to 0.3 \( \mu M \).
E.6 Modification of $I_{Na}$

The formulation for the fast sodium current is based on Mahajan et al. [2], with the off rate of activation gate shifted by -16 mV. This shifting is introduced accidentally, but it has no impact on the results.

$$\beta_m = 0.08e^{-(Vm+16/11)}$$  \hspace{1cm} (E.13)


Bibliography


[29] Bum-Rak Choi, Weiyan Li, Dmitry Terentyev, Anatoli Y Kabakov, Mingwang Zhong, Colin M Rees, Radmila Terentyeva, Tae Yun Kim, Zhilin Qu, Xuwen Peng, et al. Transient outward k+ current (ito) underlies the right ventricular initiation of


[36] Rajesh B Sekar, Eddy Kizana, Hee C Cho, Jared M Molitoris, Geoffrey G Hesketh, Brett P Eaton, Eduardo Marbán, and Leslie Tung. $i_{K_1}$ heterogeneity affects genesis and


[53] Silvia G Priori, Carlo Napolitano, Natascia Tiso, Mirella Memmi, Gabriele Vignati, Raffaella Bloise, Vincenzo Sorrentino, and Gian Antonio Danieli. Mutations in


[60] Silvia G Priori, Carlo Napolitano, Mirella Memmi, Barbara Colombi, Fabrizio Drago, Maurizio Gasparini, Luciano DeSimone, Fernando Coltorti, Raffaella Bloise, Roberto.


Bibliography


Bibliography


Bibliography


[185] L Gravelin, O Ziv, G Liu, K Hartmann, D Patel, L Schofield, L Chaves, M Shearer, G Koren, and BR Choi. Transgenic lqt1 animal model reveals shifting focus of eads


Bibliography


218


2+
-activated k+


[214] Angelo G Torrente, Rui Zhang, Heidi Wang, Audrey Zaini, Brian Kim, Xin Yue, Kenneth D Philipson, and Joshua I Goldhaber. Contribution of small conductance k+ channels to sinoatrial node pacemaker activity: insights from atrial-specific na+/ca2+

219


220


222

