Cellular Mechanisms of Calcium-Mediated Triggered Activity

A dissertation presented

by

Zhen Song

to
The Department of Physics

In partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in the field of

Physics

Northeastern University
Boston, Massachusetts
April 22, 2013
CELLULAR MECHANISMS OF CALCIUM-MEDIATED TRIGGERED ACTIVITY

by

Zhen Song

ABSTRACT OF DISSERTATION

Submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Physics in the College of Science of Northeastern University April 22, 2013.
Abstract

Life-threatening cardiac arrhythmias continue to pose a major health problem. Ventricular fibrillation, which is a complex form of electrical wave turbulence in the lower chambers of the heart, stops the heart from pumping and is the largest cause of natural death in the United States. Atrial fibrillation, a related form of wave turbulence in the upper heart chambers, is in turn the most common arrhythmia diagnosed in clinical practice. Despite extensive research to date, mechanisms of cardiac arrhythmias remain poorly understood. It is well established that both spatial disorder of the refractory period of heart cells and triggered activity (TA) jointly contribute to the initiation and maintenance of arrhythmias. TA broadly refers to the abnormal generation of a single or a sequence of abnormal excitation waves from a small submillimeter region of the heart in the interval of time between two normal waves generated by the heart’s natural pacemaker (the sinoatrial node). TA has been widely investigated experimentally and occurs in several pathological conditions where the intracellular concentration of free Ca\(^{2+}\) ions in heart cells becomes elevated. Under such conditions, Ca\(^{2+}\) can be spontaneously released from intracellular stores, thereby driving an electrogenic current that exchanges 3Na\(^{+}\) ions for one Ca\(^{2+}\) ion across the cell membrane. This current in turn depolarizes the membrane of heart cells after a normal excitation. If this calcium-mediated “delayed after depolarization” (DAD) is sufficiently large, it can generate an action potential. While the arrhythmogenic importance of spontaneous Ca\(^{2+}\) release and DADs is well appreciated, the conditions under which they occur in heart pathologies remain poorly understood. Calcium overload is only one factor among several other factors that can promote DADs, including sympathetic nerve stimulation, different expression levels of membrane ion channels and calcium handling proteins, and different mutations of those proteins. How those various fac-
tors interact synergistically to promote DADs is not well understood. Furthermore, at an even more basic level, it remains unclear to what degree spontaneous Ca\(^{2+}\) release and the appearance of DADs are deterministic, meaning reproducible under identical conditions, or inherently stochastic like nucleation in the physical context of phase transitions.

In this thesis, we use and further develop a biologically detailed computational model to investigate basic aspects of TA in isolated heart cells (cardiac myocytes). Isolated cells can be obtained by enzymatic dissociation of heart tissue and studied experimentally using standard electrophysiological recording methods and confocal imaging of Ca\(^{2+}\) dynamics. Hence they provide a well controlled setting to investigate the generation of DADs under well controlled conditions. Our computational model captures essential aspects of the hierarchical architecture of ventricular myocytes, which consists of a large number of approximately 20,000 to 50,000 regularly spaced sub-micron regions containing clusters of 50-100 Ryanodine receptor (RyR) Ca\(^{2+}\) release channels. Each of those regions acts as a discrete “calcium release unit” (CRU). Therefore our model allows us to address for the first time quantitatively the fundamental question of whether Ca\(^{2+}\) release, which is highly stochastic at the level of a single calcium release unit, is stochastic or deterministic at the whole cell level where the Ca\(^{2+}\) signal is the summation of releases from a large number of units. Addressing this question is the focus of the first part of this thesis. Our results demonstrate that both the initiation and termination of TA are highly stochastic at the whole cell level due to the spatiotemporal organization of discrete release events into multiple Ca\(^{2+}\) waves. Our results allow us to characterize the probability distributions that govern the number of DADs preceding a triggered action potential and the number of triggered action potentials after termination of periodic stimulation. We show that a limit cycle underlies the bi-directionally coupled dynamics of membrane of voltage and Ca\(^{2+}\)
when TA is sustained for long intervals. Furthermore, we construct a simple theoretical model that allows us to relate the shape of those distributions to the statistics and properties of Ca$^{2+}$ waves. The second part of this thesis focuses on investigating TA in the context of a specific mutation of a calcium buffering protein calsequestrin (CSQN). This mutation underlies catecholaminergic polymorphic ventricular tachycardia (CPVT), which is a pathophysiological condition that affects a subset of the human population. Our results shed light on the mechanisms by which altered Ca$^{2+}$ buffering and altered kinetics of RyR Ca$^{2+}$ release channels as a direct and indirect effect of this mutation, respectively, promote TA.
ACKNOWLEDGEMENTS

I would like to thank Professor Alain Karma, for his guidance and continuous encouragement during my graduate career. He dedicates a tremendous amount of time to his students. There are countless times that we have been discussing science from the day to the midnight. It is an incredible honor to be one of his students. This dissertation would not have been possible without him. I would also like to thank the thesis committee members for their invaluable comments and discussions that resulted in the improvement of the dissertation.

I would like to thank Dr. Juan Restrepo, for his mentorship at the first beginning when I joined the group. He spent many hours teaching me the practical computational techniques and skills, and explaining detailed aspects of the computational models of our group.

I would also like to thank Dr. Robert Spatschek, Dr. Nan Wang, Dr. Ari Adland, Dr. Damien Tourret, Dr. Yechuan Xu, Colin Rees and Dr. Gabriel Facini for sharing knowledge of programming and computational plotting, and most importantly sharing their happiness with me. Especially, I want to thank Dr. Ari Adland and Dr. Damien Tourret for maintaining computer clusters of the lab.

I thank Helen Chu, Norbert Chu, Daniel Fu, Ari Adland and Damien Tourret for correcting grammatical issues and proofreading my thesis. It is difficult to describe how nice and kind they are. I am very lucky to have all these great friends.

I thank Selena Li and her family for giving me a great favor in my daily life. This dissertation would have been much harder to complete without them.

Northeastern University and American Heart Association are acknowledged for the financial support. I also thank Gordon Research Conferences for providing me with a good opportunity to interact with many people in this field.
Contents

Abstract 3
Acknowledgements 7
Table of contents 8
List of figures 11
List of tables 18

1 Introduction 19
  1.1 Cardiac arrhythmias ........................................ 19
  1.2 Cardiac excitation-contraction coupling .................... 21
  1.3 Underlying mechanisms of cardiac arrhythmias ............. 23
  1.4 Experimental observation of triggered activity ............ 24
  1.5 Open questions to address .................................. 28
  1.6 Computational modeling studies on triggered activity .... 34

2 Stochasticity in initiation and termination of Ca mediated triggered activity 39
  2.1 Ionic model .................................................. 39
  2.2 Incorporation of β-adrenergic stimulation and RyR hyperactivity into the physiologically detailed model .................. 46
  2.3 Combined effects of β-adrenergic stimulation and RyR hyperactivity on TA ........................................ 50
2.4 Stochastic initiation and termination of \( \text{Ca}^{2+} \) wave mediated triggered activity .................................................. 53

2.5 Bistable dynamics ................................................................. 57

2.6 Stochastic initiation and termination of TA are governed by geometric distributions ......................................................... 60

2.7 Dependence of the level of arrhythmogenicity on RyR leakiness and \( \text{Ca}^{2+} \) diffusivity ................................................................. 71

2.8 Simplified model of \( \text{Ca}^{2+} \) wave dynamics .................................................. 86

2.9 \( \text{Ca}^{2+} \) oscillation without coupling to action potential ......................... 96

2.10 Discussion ............................................................................ 101

3 CPVT linked to CSQN\(^{R33Q}\) mutation ........................................ 109

3.1 RyR Markov model ............................................................. 109

3.2 Alteration of \( \text{Ca}^{2+} \) cycling in R33Q mutant cells ......................... 114

3.3 RyR gating parameters .......................................................... 121

3.4 CSQN-independent luminal “brake” for SR \( \text{Ca}^{2+} \) release ................... 124

3.5 Spontaneous \( \text{Ca}^{2+} \) release at a low SR \( \text{Ca}^{2+} \) content in R33Q mutant cells 138

3.6 \( \text{Ca}^{2+} \)-mediated TA in heterozygous R33Q mutant cells .................. 147

3.7 \( \text{Ca}^{2+} \)-mediated TA at different R33Q expression level ..................... 155

3.8 Discussion ............................................................................ 158

4 Conclusion ............................................................................. 165

Appendices .................................................................................. 168

A Details of the ionic model .......................................................... 169

A.1 \( \text{Ca}^{2+} \) cycling and membrane voltage dynamics ......................... 169

A.2 Instantaneous cytosolic buffering ............................................ 171

A.3 Instantaneous luminal buffering ................................................. 171
A.4 Troponin C dynamic buffering ........................................ 172
A.5 Uptake current $I_{up}$ .................................................. 173
A.6 Leak current $I_{leak}$ .................................................... 174
A.7 Sodium-calcium exchange current $I_{NaCa}$ .................... 174
A.8 Calcium current, $I_{Ca}$ ............................................... 175
A.9 Release current, $I_r$ .................................................. 176
A.10 RyR gating Markov model ......................................... 177
A.11 Nearest-neighbor diffusive currents, $I_{ci}$, $I_{cs}$, and $I_{cNSR}$ .... 179

B Permissions ................................................................. 180
B.1 Permission for Fig. 1.2 ............................................... 180
B.2 Permission for Fig. 1.3 ............................................... 180
B.3 Permission for Fig. 1.4 ............................................... 181
B.4 Permission for Fig. 1.5 ............................................... 181
B.5 Permission for Fig. 1.7 ............................................... 182
B.6 Permission for Fig. 2.1 ............................................... 182
B.7 Permission for Fig. 2.2 ............................................... 183
B.8 Permission for Fig. 3.1 ............................................... 183

Bibliography ............................................................... 184
List of Figures

1.1 ECG recording of sinus rhythm, ventricular tachycardia (VT) and ventricular fibrillation (VF). ...................................................... 20
1.2 Illustration of excitation-contraction coupling [6] with permission. See text for details. ................................................................. 22
1.3 Large cell wide Ca$^{2+}$ waves can lead to sufficient membrane depolarization to elicit an action potential. ................................. 26
1.4 Typical example of spontaneous Ca$^{2+}$ waves after sudden cessation of pacing in a current clamped rabbit ventricular myocyte stimulated at a pacing cycle length of 400 ms. ...................................................... 27
1.5 Arrhythmogenic disturbances in Ca$^{2+}$ cycling in myocytes expressing CASQ$^{R33Q}$ ........................................................................ 29
1.6 Schematic diagram explaining how the combination of SR leakiness induced by caffeine (Caff) and SR load elevation induced by isoproterenol (Iso) promotes spontaneous SR Ca$^{2+}$ release and Ca$^{2+}$ waves from the experiments by [108]. ...................................................... 31
1.7 Simultaneous measurement of Ca$^{2+}$ changes during spontaneous Ca$^{2+}$ waves in the cytosolic and SR luminal compartments. .............. 38
2.1 Schematic representation of modeling Ca$^{2+}$ dynamics in the cell. . . . 41
2.2 Markov model of RyR gating kinetics. .................................................. 45
2.3 β-adrenergic stimulation effect on LCC and cytosolic Ca^{2+} with clamped 
AP. ........................................................................................................ 48
2.4 β-adrenergic stimulation effect on LCC and cytosolic Ca^{2+} under free 
running AP at a pacing cycle length of 1 sec. ...................................... 49
2.5 Ca^{2+}-mediated TA occur under combined effect of both isoproterenol 
and hyperactive RyRs. ........................................................................ 51
2.6 Initiation of Ca^{2+}-mediated TA under the administration of isoproterenol 
effect in a cell with hyperactive RyRs. .............................................. 55
2.7 Termination of Ca^{2+} wave mediated triggered activity. .............. 56
2.8 Termination trajectories of Ca^{2+} wave mediated TA in V_{m}-[Ca]_{i} phase-
plane. ...................................................................................................... 59
2.9 Diastolic intracellular Ca^{2+} evolution and distribution of the oscillation 
number during initiation and termination of Ca^{2+}-mediated TA. .... 61
2.10 Autocorrelation coefficient of DAD amplitudes. ......................... 63
2.11 Distribution of the time interval between the onset of AP and the peak 
of the following DAD. ...................................................................... 64
2.12 Time trace of [Ca]_{i} and V_{m} during a spontaneous Ca^{2+} release. .... 67
2.13 Distribution of DAD peaks in the simulation with Na^{+} current and  
LCCs blockade. ..................................................................................... 68
2.14 Distribution of [Ca]_{i} peaks during DADs in the simulation with Na^{+} 
channels and LCCs blockade method. ................................................ 69
2.15 Validation of Na^{+} channels and LCCs blockade method. ............ 70
2.16 α effect on the initiation of Ca^{2+}-mediated TA. ......................... 72
2.17 α effect on the termination of Ca^{2+}-mediated TA. ..................... 72
2.18 ξ effect on the initiation of Ca$^{2+}$-mediated TA. ................. 72
2.19 ξ effect on the termination of Ca$^{2+}$-mediated TA. ................. 73
2.20 Probability that a DAD induces a TAP as a function of α. .......... 76
2.21 Probability that a DAD induces a TAP as a function of $1/\xi$. ....... 77
2.22 Distribution of spontaneous [Ca]$_i$ peaks at different α values. ....... 78
2.23 Distribution of spontaneous [Ca]$_i$ peaks at different ξ values. ....... 79
2.24 Relationship of the average spontaneous [Ca]$_i$ peak and the standard
deviation of spontaneous [Ca]$_i$ peaks with α (A) and $1/\xi$ (B). .......... 80
2.25 Steep dependence of $p([\text{Ca}]_i)$ on $[\text{Ca}]_i$ at different σ values. ........ 81
2.26 Distribution of normal [Ca]$_i$ transients at a pacing cycling length of
0.3 sec and α=3. ................................................................. 82
2.27 Cytosolic Ca$^{2+}$ dynamics in the whole cell simulation for the detailed
model. ............................................................ 83
2.28 Transversal and longitudinal line scan images for the cell under a pac-
ing cycle length of 1 sec with Na$^+$ channels and LCCs blockade during
diastolic intervals between two normal paced beats for different RyR
hyperactivity. ........................................ 84
2.29 Transversal and longitudinal line scan images for the cell under a pac-
ing cycle length of 1 sec with Na$^+$ channels and LCCs blockade during
diastolic intervals between two normal paced beats for different Ca$^{2+}$
diffusivity. ........................................ 85
2.30 Time trace of a typical Ca$^{2+}$ spark during spontaneous Ca$^{2+}$ release
in the presence of isoproterenol effect. .......................... 88
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.31</td>
<td>Time traces of the whole cell average [Ca]$_i$ at different $k_f$ (A), and $L_p$ (B) for the simplified model.</td>
</tr>
<tr>
<td>2.32</td>
<td>Distribution of spontaneous [Ca]$_i$ peaks at different $k_f$ (A), and $L_p$ (B) obtained from the simplified model.</td>
</tr>
<tr>
<td>2.33</td>
<td>Coefficient of variation of the spontaneous [Ca]$_i$ peaks.</td>
</tr>
<tr>
<td>2.34</td>
<td>Relationship between $\log_{10}(N_f)$ and $\log_{10}(k_f)$ at different $L_p$ values in the simplified model.</td>
</tr>
<tr>
<td>2.35</td>
<td>Distribution of the number of Ca$^{2+}$ wave foci at different $k_f$ obtained from the simplified model.</td>
</tr>
<tr>
<td>2.36</td>
<td>Example of Ca$^{2+}$ oscillation in the cell with 20% of normal NCX.</td>
</tr>
<tr>
<td>2.37</td>
<td>Ca$^{2+}$ oscillation in the closed cells.</td>
</tr>
<tr>
<td>2.38</td>
<td>Characterization of Ca$^{2+}$ oscillation in the cell with normal NCX, attenuated NCX and 0 NCX (closed cell).</td>
</tr>
<tr>
<td>2.39</td>
<td>Same trajectories as in Fig. 2.38 shown in $T_{osc}$-[Ca]$<em>i$ (A) and $T</em>{osc}$-[Ca]$_{jsr}$ phase-planes, respectively.</td>
</tr>
<tr>
<td>2.40</td>
<td>Relationship of $N_{TAP}$ and $N_{DAD}$ to NCX level ($\beta$). The simulations were carried out at a pacing cycling length of 0.3 sec and in the presence of isoproterenol effect and hyperactive RyRs ($\alpha=3.3$). The cells were paced until the steady state and the electrical stimuli were ceased. A DAD was counted when the amplitude of the depolarization is above -85 mV.</td>
</tr>
<tr>
<td>3.1</td>
<td>Illustration of disruption of regulatory functions of CSQN by the CPVT CSQN$^{R33Q}$ [103].</td>
</tr>
<tr>
<td>3.2</td>
<td>Extended 6-state Markov model of RyR gating kinetics.</td>
</tr>
</tbody>
</table>
3.3 Example of experiment-like line scan images of cytosolic Ca\textsuperscript{2+} in the cell with expression of CSQN\textsuperscript{WT} .................................................. 115
3.4 Example of experiment-like line scan images of cytosolic Ca\textsuperscript{2+} in the cell with expression of CSQN\textsuperscript{R33Q} .................................................. 117
3.5 Diastolic cytosolic [Ca]_i in the simulation of permeabilized myocyte experiment. ................................................................. 118
3.6 Intracellular Ca\textsuperscript{2+} signals in the cell with expression of CSQN\textsuperscript{WT} (A), and CSQN\textsuperscript{R33Q} (B), respectively. ............................................. 119
3.7 Characteristics of intracellular Ca\textsuperscript{2+} signals and membrane voltage with the normal no flux boundary condition in the WT cell (A) and R33Q mutant cell (B), respectively. ............................................. 120
3.8 Characteristics of intracellular Ca\textsuperscript{2+} signals and membrane voltage with the normal no flux boundary condition in the WT cell (A) and R33Q mutant cell (B), respectively. ............................................. 122
3.9 Intracellular Ca\textsuperscript{2+} signals in the cell with the fixed boundary condition and in the WT cell (A) and R33Q mutant cell (B), respectively. .... 123
3.10 Extra luminal RyR regulation f([Ca]_{jsr}) as a function of [Ca]_{jsr} ............................................. 126
3.11 Characteristics of intracellular Ca\textsuperscript{2+} signals and membrane voltage with the normal no flux boundary condition and the extra luminal "brake" of SR release in a WT cell (A) and a R33Q mutant cell (B), respectively. ............................................. 127
3.12 Intracellular Ca\textsuperscript{2+} signals in the cell with the fixed boundary condition and the extra luminal "brake" of SR release in the WT cell (A) and R33Q mutant cell (B), respectively. ............................................. 128
3.13 Ca$^{2+}$ wave propagation length as a function of [Ca]$^B_i$ in the permeabilized cell simulation. .................................................. 130
3.14 Ca$^{2+}$ spark frequency vs. [Ca]$^B_i$ in the permeabilized myocyte simulation. 131
3.15 Experiment-like line scan images in the permeabilized myocyte simulation for the model with an extra luminal SR “brake”. .............. 132
3.16 Ca$^{2+}$ wave frequency as a function of [Ca]$^B_i$ in the permeabilized myocyte simulation. .................................................. 133
3.17 The amplitude of spontaneous Ca$^{2+}$ release vs. [Ca]$^B_i$ in the permeabilized myocyte simulation. ................................. 135
3.18 Termination of Ca$^{2+}$ wave mediated triggered activity with Sato et al. RyR gating parameters. ................................. 136
3.19 Distribution of the number of TAPs in the presence of isoproterenol effect and hyperactivity RyRs with large single RyR flux strength. ... 137
3.20 Ca$^{2+}$-mediated TA in WT (A) and R33Q mutant (B) cells in the presence of isoproterenol. .................................................. 139
3.21 Simulation of R33Q mutant cell by Model I (A) and Model II (B). ... 141
3.22 Example of Ca$^{2+}$ spark interval distribution in the R33Q mutant cell simulation. ................................................. 143
3.23 Change in diastolic [Ca]$_i$, SR Ca$^{2+}$ content, number of spontaneous Ca$^{2+}$ oscillations and Ca$^{2+}$ spark frequency at different $J_{\text{max}}$. .... 145
3.24 Comparison of Ca$^{2+}$ spark frequency in WT and R33Q mutant cells. ... 146
3.25 Whole cell Ca$^{2+}$ signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.2. ......................... 149
3.26 Whole cell Ca\textsuperscript{2+} signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.4. 150

3.27 Whole cell Ca\textsuperscript{2+} signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.6. 151

3.28 Whole cell Ca\textsuperscript{2+} signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.8. 152

3.29 Intracellular Ca\textsuperscript{2+} level under different ratios of R33Q to WT in the presence of isoproterenol effect after electrical stimuli are stopped. 153

3.30 Change in diastolic [Ca\textsubscript{i}], SR Ca\textsuperscript{2+} content, number of spontaneous Ca\textsuperscript{2+} oscillations and Ca\textsuperscript{2+} spark frequency with varying the ratio of R33Q to WT. 154

3.31 Number of TAPs as a function of the CSQN\textsuperscript{R33Q} expression level. 156

3.32 Intracellular Ca\textsuperscript{2+} level with different R33Q expression levels in the presence of isoproterenol effect after the electrical stimuli are stopped. 157
List of Tables

A.1 Buffering parameters [80] ................................. 172
A.2 Luminal buffering parameters [80] ................................. 173
A.3 Uptake and leak current parameters [80] ................................. 174
A.4 Sodium-calcium exchanger current parameters ................................. 175
A.5 Calcium current parameters ................................. 176
A.6 SR release current and RyR Markov model parameters ................................. 178
A.7 Diffusive timescales ................................. 179
Chapter 1

Introduction

1.1 Cardiac arrhythmias

Cardiovascular disease is the single most common cause of death. Over half of the mortality is attributed to sudden cardiac deaths (SCD). Approximately, there are 320,000 SCDs per year in the United States. According to Sudden Cardiac Arrest Foundation, this is approximately equivalent to the total number of people who die from Alzheimer’s disease, assault with firearms, breast cancer, cervical cancer, colorectal cancer, diabetes, HIV, house fires, motor vehicle accidents, prostate cancer and suicide combined [31] [51] [12]. In fact, cardiac arrhythmia, such as ventricular fibrillation, are one of the most common causes of SCD of hospital cases. Over 80% of SCDs are associated with the development of ventricular fibrillation (VF). VF is an immediately life-threatening arrhythmia in which the heart’s electrical activity and associated contraction become disordered and ineffective. It is characterized by rapid, irregular activation of the ventricles and therefore prevents an effective mechanical contraction. During a VT event, blood pressure instantaneously drops to zero. As a result, this may lead to death within minutes due to lack of cardiac output, unless successful electrical
Figure 1.1: ECG recording of sinus rhythm, ventricular tachycardia (VT) and ventricular fibrillation (VF). In sinus rhythm P wave, QRS complex and T wave represent for atrial contraction, ventricular contraction, and ventricular relaxation, respectively.

defibrillation is performed. At the normal heart rhythm, the heart beats regularly, producing a single coordinated electrical wave that can be seen as a normal electrocardiogram (ECG). During arrhythmias such as ventricular tachycardia (VT) and VF, this normal behavior is disrupted mainly because of high frequency spiral waves of electrical activity that repetitively stimulates the heart. As a result this produces contractions at a rapid rate [13] [74] [14] [23] [28] [34] [43] [118]. The ECG records rapid rates with increased complexity. Fig. 1.1 shows typical sinus rhythm, VT and VF seen in ECG recordings.
1.2 Cardiac excitation-contraction coupling

The normal mechanical response of the heart relies on well functioned excitation-contraction coupling of the cardiac muscle. At the cellular level, for instance, a ventricular myocyte is an excitable system, which contracts in response to electrical stimuli in a ventricle of the heart. Fig. 1.2 depicts how electrical stimuli result in cell contraction [1]. When the ventricular myocyte is stimulated from its resting potential, the depolarization is accumulated. An action potential is created when the membrane potential is up to threshold. The voltage then relaxes back to the resting potential after a short period, which is defined as the action potential period (APD). The slope of the action potential and APD are determined by ionic currents across the cell membrane. During the APD the ventricular myocyte is excited, which is also a refractory state because the myocyte can not be excited any further. When the electrical stimulus propagates to a ventricular myocyte, L-type Ca\(^{2+}\) channels (LCCs) open (I\(_{Ca}\) in the figure), resulting in calcium (Ca\(^{2+}\)) influx into the cytoplasm. The elevation of Ca\(^{2+}\) is sensed by ryanodine receptors (RyRs), which are located in the cell surface membrane and T-tubules [68] [113]. A large release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) occurs in response to the opening of RyRs. The Ca\(^{2+}\) release through this mechanism is called calcium-induced calcium release (CICR) [20], which involves positive feedback and is potentially unstable. The released Ca is then pumped back into the SR through a SERCA uptake pump current. Abnormal excitation-contraction coupling can then cause the heart behave irregularly, which in turn lead to cardiac arrhythmias such as VT and VF.
Figure 1.2: Illustration of excitation-contraction coupling [6] with permission. See text for details.
1.3 Underlying mechanisms of cardiac arrhythmias

Such tachyarrhythmias usually result from the addition of abnormal impulses to the normal cardiac rhythm. Abnormal impulses are reported to begin by one of three mechanisms: reentry, automaticity or triggered activity [84] [44].

Re-entry arrhythmias occur when an electrical stimulus recurrently travels in a circle within the heart, rather than moving from one end of the heart to the other and then stopping [2]. Every cardiac cell is able to transmit impulses once in every direction within a short time. In normal conditions, the action potential impulse spreads through the heart very quickly in order to make each cell only respond once. However, in an abnormal condition such as conduction block (conduction is abnormally slow in some areas, for example in heart damage) the myocardial cells are unable to activate the fast sodium channel, part of the impulse will arrive late and potentially be treated as a new impulse. Depending on the timing, this can produce a sustained abnormal circuit rhythm. During tachycardia, a single wave can rotate as a spiral wave producing fast rates and complexity. During fibrillation, a single spiral wave can degenerate into multiple waves.

Automaticity refers to a cardiac muscle cell firing off an impulse on its own. All cells in the heart have the ability to initiate an action potential. However, only some of these cells are designed to routinely trigger heart beats. These particular cells are found in the conduction system of the heart and include the sinoatrial node, atrioventricular node, bundle of His and Purkinje fibers. The sinoatrial node is a single specialized location in the atrium which has a higher automaticity than the rest of the heart. Therefore, it is usually responsible for setting the heart rate and initiating each heart beat. Any part of the heart that initiates an impulse without a signal from the sinoatrial node is considered as an ectopic focus. This may cause a single premature beat once in a
while. If the ectopic focus fires more frequently than the sinoatrial node it can produce a sustained abnormal rhythm [55] [72].

Research over the last several decades has identified other sources of ectopic foci that do not result from automaticity, but instead are due to a response to a preceding impulse. Such abnormal impulse was termed triggered activity (TA) because it required a preceding action potential as its initiator [16] [15]. TA can occur before or after full repolarization of the fiber, which are termed either early after depolarizations (EADs) or delayed after depolarizations (DADs). All after depolarizations may not reach threshold potential, but, if they do, they can trigger another after depolarization, and thus self-sustain.

1.4 Experimental observation of triggered activity

In the early 1970s, studies on isolated Purkinje fibers found that digitalis-like glycosides produced a DAD that could reach threshold and result in an action potential [22] [85]. A short while after, Rosen et al. [83] developed a cross-perfusion experiment where they infused a dog with ouabain and took its blood from this animal to superfuse an isolated Purkinje fiber. When the dog developed ventricular ectopic beats, DADs were seen in the isolated Purkinje fiber. This study demonstrate there is a link between DADs and ventricular arrhythmias. Subsequently, Lederer and Tsien [57] used the voltage clamp technique to investigate the membrane currents responsible for the DAD, their work demonstrated a transient inward current activated on repolarization in digitalis-intoxicated preparations. Studies by several groups then showed that the transient inward current occurred at about the same time as a after-contraction, thereby linking it to a rise of cytosolic Ca^{2+} [46] [45]. Later on, the transient inward current was shown to be the Na^{+}-Ca^{2+} exchanger current in response to spontaneous Ca^{2+} release [67] [21].
Of note, the diastolic elevation of Ca\textsuperscript{2+} propagates as a wave along the cell. Evidence for such Ca\textsuperscript{2+} waves was first inferred from tension changes \[45\] \[30\] \[56\] \[18\]. The direct cellular studies have measured the Ca\textsuperscript{2+} wave itself in single cells \[117\] \[115\] \[48\] \[119\]. Fig. 1.3 shows large cell-wide Ca\textsuperscript{2+} waves in an Purkinje cell that lead to sufficient membrane depolarization to trigger an action potential. Fig. 1.4 represents a confocal line scan images in a current clamped myocyte where spontaneous Ca\textsuperscript{2+} waves exhibited after sudden cessation of pacing. We see each Ca\textsuperscript{2+} wave causes a DAD. Specifically, an increase of Ca\textsuperscript{2+} influx and/or a decrease of Ca\textsuperscript{2+} efflux across the cell membrane leads to an increase of SR Ca\textsuperscript{2+} content which in turn produces Ca\textsuperscript{2+} waves. Some of the Ca\textsuperscript{2+} in the wave is extruded out of the cell through NCX causing a DAD. If the DAD is up to threshold, it can produce an action potential.

Because Ca\textsuperscript{2+} mediated arrhythmias begin with spontaneous release of Ca\textsuperscript{2+} from the SR, a considerable research effort has focused on the proteins that regulate CICR. For example, several studies have reported that in the context of heart failure (HF), the function of SR Ca\textsuperscript{2+} release channels RyRs, becomes abnormal due to different causes, such as improper phosphorylation \[65\] modifications in response to oxidative stress \[102\], and altered subcellular structure \[96\]. These changes are believed to be responsible for the increased risk of arrhythmia observed in HF. However, in the context of HF, additional changes are also present, including remodeling of other ionic channels and transporters \[42\], decreased responsiveness to \(\beta\)-adrenergic stimulation \[9\], and alterations in myocardial energetics \[69\]. Nowadays, substantial attention is focused on a monogenetic condition known as catecholaminergic polymorphic ventricular tachycardia (CPVT), which is a congenital arrhythmia disorder caused by mutations in either RyR or the calsequestrin-2 gene (CSQN). CPVT are often observed in young people. Patients with CPVT have structurally normal hearts but are at risk of developing arrhythmias during \(\beta\)-adrenergic stimulation, a state associated with rapid heart rates,
Figure 1.3: Fig. 7 in Boyden et al [8] with permission. Large cell wide Ca$^{2+}$ waves can lead to sufficient membrane depolarization to elicit an action potential. A, selected image frames of Ca$^{2+}$ from an IZPC (Purkinje cell aggregate from the infarcted heart) during the Ca$^{2+}$ induced electrical activity. B, transmembrane potential (black line) and Ca$^{2+}$ (multicolored lines) changes of this aggregate during the cell wide wave induced electrical activity.
In patients, either mutations in RYR or in CSQN can cause CPVT. Single channel studies characterizing mutant RyRs have consistently shown that they exhibit enhanced open probability compared with wild-type RyR [41] [40], especially after phosphorylation by protein kinase A [59] [116]. Therefore, CPVT can be studied in in vitro experiments by stimulating β-adrenergic receptors with isoproterenol, simulating the state of sympathetic activation under which arrhythmias occur, while simultaneously increasing RyR open probability with a drug such as caffeine. At the cellular level, Eisner’s group has extensively investigated how enhanced RyR open probability affects both normal CICR and the potentially arrhythmogenic CICR that occurs in the context of CPVT. More recently, this group has examined potentially arrhythmogenic spontaneous SR Ca$^{2+}$ release in ventricular myocytes [107] [108]. In their experiments, myocytes were exposed to caffeine and/or isoproterenol to study the cause of stronger contractions, and larger increases in intracellular Ca$^{2+}$. 

Figure 1.4: Fig. 3 in Xie et al [119]. Typical example of spontaneous Ca$^{2+}$ waves after sudden cessation of pacing in a current clamped rabbit ventricular myocyte stimulated at a pacing cycle length of 400 ms. Each Ca$^{2+}$ wave causes a delayed after depolarization (DAD), which become progressively smaller.
Ca\textsuperscript{2+}-mediated TA. They found that increasing RyR open probability by itself is not sufficient to induce spontaneous SR Ca\textsuperscript{2+} release. The increased SR Ca\textsuperscript{2+} load under the administration of isoproterenol is also required in order to obtain arrhythmogenic Ca\textsuperscript{2+} waves. These studies [107] [108] emphasize the importance of understanding alterations in RyR gating kinetics at the cellular level.

Meanwhile, there are also several studies investigating CPVT linked to CSQN mutations. CSQN is a major Ca binding and buffering protein within the SR. It plays two different roles in cardiac myocytes. It serves as a Ca storage reservoir in the SR and acts as an active modulator of the Ca release process. As Ca storage molecules, CSQNs can act as buffers by binding to Ca. CSQN modulates the RYR activity by interacting with the auxiliary proteins triadin-1 and junctin (T/J). The open probability of RYR steeply increases upon addition of T/J, and it decreases in the presence of CSQN [104] [33] [49]. Experiments have shown that mutations in CSQN are attributable to the disruption of the polymerization reaction required for high capacity Ca binding by CSQN\textsuperscript{2DEL} [103], reducing the CSQN expression while increasing RYR expression level as a compensatory effect by CSQN\textsuperscript{307} and CSQN\textsuperscript{ΔE9/ΔE9} [95] and compromising the ability of CSQN to control RYR channel activity by CSQN\textsuperscript{R33Q} [103]. Fig. 1.5 shows adenoviral-mediated expression of CSQN\textsuperscript{R33Q} in adult rat myocytes led to Ca\textsuperscript{2+} waves and TA as an example. These mutation-related effects contribute to the disruption of the SR release function through the CSQN-mediated RYR regulation and buffering ability within the SR.

1.5 Open questions to address

The experimental observations of TA in the cell with either mutant RyRs or CSQN support the hypothesized mechanism (Fig. 1.6) proposed by Eisner’s group for Ca\textsuperscript{2+} me-
Figure 1.5: Fig. 3 in Terentyev et al [101] with permission. Arrhythmogenic disturbances in Ca\(^{2+}\) cycling in myocytes expressing CASQ\(^{R33Q}\). Recordings of membrane potential (upper traces), along with line-scan images (middle traces) and averaged temporal profiles (lower traces) of fluo-3 fluorescence in myocytes infected with Ad-Control, Ad-CASQ\(^{WT}\), and Ad-CASQ\(^{R33Q}\) vectors. The myocytes were stimulated at 2 Hz in the presence of 1 \(\mu\)mol/L ISO.
diated arrhythmias, where both mutations in RyRs and CSQN result in the increased hyperactivity of RyRs and Ca$^{2+}$ overload in the presence of isoproterenol. However, it has also been suggested that the SR Ca$^{2+}$ content may not be the only major factor determining when arrhythmogenic Ca$^{2+}$ waves occur. Edgell et al [17] reported the observation of aftercontractions, when the stimulation frequency of guinea-pig ventricular myocytes was increased. However, the SR Ca$^{2+}$ content was not different when aftercontractions were observed compared to when the stimulation rate was just sub-threshold for generating aftercontractions. Similar results were obtained when aftercontractions were evoked in the presence of catecholamines [106]. It was found that when aftercontractions occur, the cytosolic Ca$^{2+}$ concentration is elevated. Thus these authors believed that it is the increase of cytosolic and SR Ca$^{2+}$ together that is important. Therefore, a consensus hypothesis could be that both SR and cytosolic Ca$^{2+}$ play important roles [109]. However, this hypothesis needs to be tested.

In experiment, TA can sometimes be isolated or occur in “bursts” of two, three, or an even larger number of subsequent triggered action potentials [88] [98] [123]. For instance, in the study of Sedej et al. [88], the number of triggered action potentials under the condition of SR Ca$^{2+}$ and intracellular Na$^{+}$ overload combined with hyperactive RyR was found to be surprisingly highly variable (191.4±58.1 min$^{-1}$). The question then arises as to what controls the number of triggered action potentials, i.e., the degree of TA? Different experimental conditions, such as temperature, drug dosage, heterogeneity in cell size and structure may be factors. However whether or not the intrinsic stochasticity of ion channel opening and closing by itself can result in the large variation of TA in identical cells is still an interesting open question. Furthermore, if that is the case, why do both Ca$^{2+}$ and voltage signals in the cell under normal conditions exhibit almost identical time traces from beat-to-beat as seen in both experiment and computer simulations, considering the intrinsic existence of ion channel stochasticity?
Figure 1.6: Schematic diagram explaining how the combination of SR leakiness induced by caffeine (Caff) and SR load elevation induced by isoproterenol (Iso) promotes spontaneous SR Ca\(^{2+}\) release and Ca\(^{2+}\) waves from the experiments by [108]. Addition of caffeine (Caff) only makes RyR calcium release channels more leaky thereby reducing the threshold SR load for spontaneous SR Ca release. However, enhanced leakiness also reduces SR load which results in falling below the release threshold with caffeine only. With addition of isoproterenol (Iso) only, enhanced SERCA2 increases the SR load, but this load still falls below the threshold for spontaneous release. Finally, with addition of both caffeine and isoproterenol, the threshold for spontaneous release is reduced at the same time that the SR load is increased, thereby creating the condition for spontaneous release and Ca\(^{2+}\) waves.
In the scenario of severe TA, it is usually observed sustained oscillations in both Ca\(^{2+}\) and voltage dynamics, which imply stable limit cycles. Thus, the initiation and termination of TA involve a dynamical transition between an excitable silent state and an oscillatory state. However, the underlying mechanism governing this state transition and the role of ion channel stochasticity in the state transition remain unknown. Moreover, in some experiments, it has also been reported that the Ca\(^{2+}\) dynamics can exhibit an oscillatory behavior by itself without representing V\(_m\) oscillations [92] [100] [75]. The question then rises as to what is the relative importance of Ca\(^{2+}\) and voltage oscillations in maintaining sustained TA in the context of CPVT? It also remains unknown whether Ca\(^{2+}\) signals can exhibit sustained oscillations without coupling to the membrane potential.

In addition, CSQN is believed to play a key role in regulating the RyR gating kinetics. However, a recent experiment [50] has reported that complete absence of CSQN in CSQN-null mice does not cause structural heart diseases. In fact, cardiac myocytes from CSQN-null mice still exhibit a steep and nonlinear relationship between the SR Ca\(^{2+}\) load and the SR Ca\(^{2+}\) release, suggesting that the RyR channels can sense luminal Ca\(^{2+}\) in the absence of CSQN. Furthermore, CSQN-null cardiac myocytes exhibit normal intracellular Ca\(^{2+}\) cycling at a low SR Ca\(^{2+}\) content, suggesting that the removal of CSQN does not result in a dramatic increase in RyR leakiness at a low SR Ca\(^{2+}\) load. Finally, CSQN-null cardiac myocytes show spontaneous Ca\(^{2+}\) waves in the presence of \(\beta\)-adrenergic stimulation, indicating that in the absence of CSQN, spontaneous SR Ca\(^{2+}\) releases can be still initiated and terminated. Taken together, these observations demonstrate that CSQN may modulate SR Ca\(^{2+}\) releases, but an extra RyR luminal regulation other than the CSQN-mediated luminal gating may exist as well.

Furthermore, mutations in CSQN in the context of CPVT either enhances the RyR
activity or reduces the buffering capacity of CSQN molecules. Specifically, both effects may be exhibited in a cell with certain CSQN mutations, such as CSQN$^{R33Q}$, which is one of a few well-characterized CSQN mutations at the cellular level. Thus, it is useful to study the relative importance of these alterations in inducing spontaneous Ca$^{2+}$ releases in the cell with the R33Q mutation. Although the characteristics of Ca$^{2+}$ handling in the R33Q mutant cell has been well-characterized, there are still a few key questions that need to be addressed. For instance, cells with expression of CSQN$^{R33Q}$ are seen to increase the rate of the SR release, which contributes to lower the SR Ca$^{2+}$ content. Thus, it remains unclear how spontaneous Ca$^{2+}$ releases occur at such a low SR Ca$^{2+}$ load.

In addition, humans contain two copies of each gene, one from the father and one from the mother. If a mutation occurs in just one copy of the gene then that individual is considered heterozygous. On the other hand if both copies of a gene are mutated then that individual is homozygous genotype. Clinically, only homozygous R33Q mutation carriers show TA, thus the effect of the ratio of R33Q to WT CSQN on spontaneous Ca$^{2+}$ releases becomes important to investigate.

All these open questions listed above remain challenging to address experimentally, because TA results from the complex interaction of a very large number of cardiac membrane ion channels and calcium cycling proteins. Thus it is generally extremely difficult, if not impossible, to predict the effect of one defective functional protein, taken in isolation, without considering its interaction with all the other normally functioning cardiac proteins. From this standpoint, insilico electrophysiological computer models of cardiac activity provide a powerful tool to study this complex interaction in order to gain basic insights into complex mechanisms of triggered activity and arrhythmias.
1.6 Computational modeling studies on triggered activity

Recently, a few modeling studies have examined how RyR or CSQN mutations may lead to arrhythmogenic spontaneous SR Ca$^{2+}$ release. Faber and Rudy [19] simulated the effects of the D307H point mutation in CASQ2 in the context of the guinea pig ventricular myocyte, and Iyer et al. [38] modeled generic CASQ2 and RyR2 mutations within a human cell. Both models predicted that the mutations resulted in spontaneous SR Ca$^{2+}$ release and DADs, which is consistent with experimental observation. These models computed the ionic currents that are responsible for the action potential. Thus such simulations provided quantitative benchmarks for understanding how inward ionic currents through NCX responded to the diastolic cytosolic Ca$^{2+}$ elevation, and in turn induced triggered action potential. However, the limitations of these studies reveals the challenges that must be overcome in the future for more mechanistic, quantitative predictions of Ca$^{2+}$-mediated arrhythmias.

Most important among the limitations, the two studies mentioned above [19] [38] are based on whole-cell common pool models of CICR in which a single model variable describes SR Ca$^{2+}$ release in the entire cell. It has been shown that such type of model can not give graded SR Ca$^{2+}$ release [99] (normal SR Ca$^{2+}$ release is smoothly graded as a function of the Ca$^{2+}$ influx provided by the inward current.). In addition, the characteristics of intracellular Ca$^{2+}$ dynamics under the government of common pool models are inconsistent with important, established experimental results. These results include that physiological CICR occurs as a spatial-temporal summation of sub-cellular elementary events known as Ca$^{2+}$ sparks, and spontaneous Ca$^{2+}$ release occurs in the form of Ca$^{2+}$ waves, in which sparks trigger neighboring sub-cellular units to fire sparks. Furthermore, the occurrence of Ca$^{2+}$ sparks is a probabilistic process, and this feature
were not considered in these models mentioned above. Because stochastic simulations of Ca\(^{2+}\) sparks [94] or Ca\(^{2+}\) waves [39] are computationally demanding, it is difficult to integrate detailed physiological intracellular Ca\(^{2+}\) cycling into formulations of ionic currents and membrane potentials.

In Chapter 2, in order to overcome these major limitations, a physiologically detailed model of membrane voltage developed by Mahajan et al. [63] is coupled to the intracellular Ca cycling model developed by Restrepo et al. [80]. It combines a physiologically detailed CRU structure with diffusive interaction of CRUs. This detailed model has been previously used to study bi-directionally coupled Ca\(^{2+}\) transient and repolarization alternans [79]. Here, it allows us to realistically investigate the effect of stochasticity and spatial structure on the genesis and termination of Ca\(^{2+}\) wave mediated TA. Furthermore, this model includes a new mathematical formulation of CSQN-mediated luminal gating of RyR activity that is critically important to study the effect of CSQN mutations linked to CPVT (see also [29]). It should be noted that Ca\(^{2+}\) waves and oscillations have also been studied with detailed models of spatially coupled CRUs in other systems such as Xenopus oocytes [112] [91] [93]. Synchronization of Ca\(^{2+}\) release from diffusively coupled CRUs has also been studied in cardiac pacemaker cells [64].

We study CPVT linked to RyR mutations by extending our model to mimic β-adrenergic stimulation effect on both Ca\(^{2+}\) and membrane voltage dynamics in the presence of hyperactive RyRs. We analyze the stochasticity in initiation and termination of triggered action potentials in order to determine the underlying mechanisms that controls the degree of Ca\(^{2+}\)-mediated TA. These results demonstrate a novel mechanism for the initiation and termination of TA. Furthermore, the role of diffusive coupling between CRUs in the genesis and termination of TA is studied. In addition, Ca\(^{2+}\) oscillation without coupling to membrane voltage is investigated in order to address the
role of Ca$^{2+}$ oscillation by itself in Ca$^{2+}$-mediated TA. These findings are interpreted in terms of dynamics of membrane voltage and intracellular Ca$^{2+}$ cycling.

Chapter 3 is devoted to investigate CSQN$^{R33Q}$ mutation related CPVT. Because within the mutations that were previously mentioned, the effects of the CSQN$^{R33Q}$ are somewhat better characterized in comparison to other mutations. The CSQN$^{R33Q}$ mutation is reported to impair the ability of CSQN to act as a luminal Ca sensor but has no effect on CSQN polymerization [101] [103]. In addition, other experimentalists claimed that reduction in the level of CSQN protein and Ca$^{2+}$ buffering capacity is a common defect of CPVT CSQNs mutations [78]. The question is if it is enough to induce TA without other compensatory effects, such as alterations in SR volume, RyR, calreticulin and SERCA expression levels. Also, clinically, only homozygous CSQN$^{R33Q}$ carriers show TA. At the cellular level, Terentyev et al. [101] reported no TA was observed when the ratio of CSQN$^{R33Q}$ to CSQN$^{WT}$ is 1:1, but if the ratio increased to 2:1, then TA exhibited.

These observation indicate that the mechanisms for DAD and TA is multifactorial, so that referring to a single cause is misleading. Therefore, the main focus in our CSQN$^{R33Q}$ mutation caused TA modeling study is to investigate the relative role of altered RyR gating regulated by CSQN$^{R33Q}$, the change of R33Q expression level in homozygous myocyte, the ratio of R33Q to WT expression in heterozygous myocyte, in inducing DADs and TA. The characteristics of spontaneous Ca$^{2+}$ waves in both CSQN$^{WT}$ and CSQN$^{R33Q}$ myocytes have been studied in simulating permeabilized cell experiments in order to compare with experimental findings, including Ca$^{2+}$ spark and/or wave frequency, Ca$^{2+}$ wave morphology, and cytosolic Ca$^{2+}$ and SR Ca$^{2+}$ concentration. Fig. 1.7 shows spontaneous Ca$^{2+}$ waves in permeabilized cell experiments for CSQN$^{WT}$ and CSQN$^{R33Q}$ myocytes. In addition, the strength of single RyR flux is linked to the occurrence of TA. We analyze its effect on the SR Ca$^{2+}$ load
and Ca^{2+} spark frequency. Furthermore, a simple Ca^{2+} spark model is developed to calculate spark frequency at different SR Ca^{2+} load and diastolic cytosolic Ca^{2+} level, such a simple model provides us a tool to investigate the relative importance of diastolic cytosolic Ca^{2+} level and SR Ca^{2+} load. These results are interpreted within the framework that increased spark frequency enhances Ca^{2+} diffusion to next CRUs, and therefore increases the probability for having Ca^{2+} waves [109].
Figure 1.7: Fig. 3 in Terentyev et al [103] with permission. Simultaneous measurement of Ca$^{2+}$ changes during spontaneous Ca$^{2+}$ waves in the cytosolic and SR luminal compartments, using the Ca indicators Rhod-2 and Fluo-5N. Representative images of Ca waves and intra-SR Ca$^{2+}$ recorded in permeabilized dog ventricular myocytes over-expressing WT or mutant forms of CSQNR33Q (48 h after adenoviral infection). Traces represent time-dependent profiles of cytosolic Rhod-2 and luminal Fluo-5N signals. Spontaneous Ca$^{2+}$ waves were induced by lowering [EGTA] from 200 mM to 50 mM (PCa7) in the internal solution.
Chapter 2

Stochasticity in initiation and termination of Ca mediated triggered activity

2.1 Ionic model

Generally, cells are categorized into two types, excitable cells and non-excitable cells. All cells (not just excitable cells) have a resting potential: an electrical charge across the plasma membrane, with the interior of the cell negative with respect to the exterior. The size of the resting potential varies depending on the cell’s type. In excitable cells the resting potential is in the range of -80∼-70 mV whereas in non-excitable cells, the membrane potential is held at a relatively stable value and will not respond to any current stimulus. However, if the cell is excitable, when the membrane potential reaches the threshold voltage (about -60 mV in mammalian ventricular myocytes), an action potential is generated. For instance, a ventricular myocyte is an excitable cell. Historically, the excitable cell was studied in a mathematical framework by A.
L. Hodgkin and A. F. Huxley in the squid giant axon in 1952 [37] [47] [73]. Their model consists of sodium, potassium and leakage currents. Extensions of this model to cardiac cells were developed by Noble in 1960 and 1962 [70] [71], and McAllister et al. in 1975 [66]. These early models are based on the Purkinje fiber conducting system. In 1977, Beeler and Reuter constructed a simple model of the ventricular myocyte action potential with only four currents, including Ca$^{2+}$ current, Na$^{+}$ current, time independent and both voltage and time dependent potassium currents [4]. Over the past two decades, the improvement of experimental techniques such as voltage clamp and patch clamp, makes accurate recordings of single cell or even single ion channel flux possible. In the early 1990s, Luo and Rudy established more accurate ventricular myocyte action potential models [60] [61] [62]. More recently, Shiferaw et al. [90] [89] proposed a new phenomenological model of excitation-contraction coupling in ventricular myocytes providing a theoretical framework to interpret various experimentally observed modes of instability ranging from electromechanically concordant and discordant alternans to quasi-periodic oscillations of voltage and calcium. Later on, Mahajan et al. [63] developed a detailed action potential model accurately reproduces the dynamics of the cardiac action potential and intracellular Ca$^{2+}$ cycling at rapid heart rates relevant to ventricular tachycardia and fibrillation.

However, as I discussed in Chapter 1, these type of models are so-called “whole-cell common pool” models. They cannot describe arrhythmogenic Ca$^{2+}$ waves that underlie spontaneous Ca$^{2+}$ release and play a key role in triggered activity. Also, most of these models did not include ion channel stochastic opening and closing (except for Mahajan et al. where those authors employed a Markov model to describe LCC stochasticity). However, ion channel flux are the build-blocks of membrane voltage dynamics and intracellular Ca$^{2+}$ cycling, and thereby play a role in Ca$^{2+}$-mediated arrhythmia. Several attempts have been made over the years. For instance, previous
Figure 2.1: Schematic representation of modeling Ca$^{2+}$ dynamics in the cell from [80] with permission. a). Schematic representation of the compartments that constitute a single CRU. b). Currents in a CRU. The arrows indicate the average direction of calcium flux during one beat. The three lower arrows indicate diffusive coupling between the compartments of adjacent CRUs. c). Schematic illustration of the heterogeneous orientation of the T-tubules inside the myocyte.
models by Winslow and co-workers [32] [36] were able to capture basic elementary Ca$^{2+}$ spark events, but did not include diffusive coupling between CRUs, while those models can reproduce the phenomenon of graded release (the gradedness of SR calcium release as a function of Ca$^{2+}$ entry via channels), they cannot describe arrhythmogenic Ca$^{2+}$ waves. At a different extreme, the model by Izu et al. [39] describes the diffusive coupling between CRUs, but does not model the underlying stochastic dynamics of RyR Ca$^{2+}$ release channels underlying Ca$^{2+}$ sparks and the bi-directional coupling between Ca$^{2+}$ and membrane voltage dynamics. To address these issues, a new physiologically detailed model of membrane voltage developed by Mahajan et al. [63] is coupled to the intracellular Ca$^{2+}$ cycling model developed by Restrepo et al. [80]. The Ca$^{2+}$ cycling model consists of a 3D grid of $\sim$20,000 CRUs, each one consisting of various compartments depicted in Fig. 2.1. The dynamics of the Ca$^{2+}$ concentration in each compartment is described by differential equations that include diffusive currents between the different compartments and Markov models for individual LCC and RyR channels. Neighboring CRUs are coupled by diffusive currents, and Ca$^{2+}$ released by a spark in one CRU may trigger sparks in neighboring CRUs. Here, we emphasize that this model is the first to combine a physiologically detailed CRU structure with diffusive interaction of CRUs. This model combines features that allow us to realistically investigate the effect of stochasticity and spatial structure on the genesis of Ca$^{2+}$-mediated TA: i) the release of Ca$^{2+}$ from the CRUs is stochastic, since individual RyR and LCCs are described with Markov models; ii) the model incorporates spatial structure and diffusive coupling between neighboring CRUs; and iii) the model includes physiologically detailed currents that allow us to explore the effect of pharmacological or genetic interventions on Ca$^{2+}$ wave mediated TA. This model has been used by Restrepo et al. to gain basic insights into cellular mechanisms of calcium transient alternans [80] [79]. Details of this ionic model are described in the Appendix. In this
chapter, I will give an overview of this model and explain how we extend this model to simulate the β-adrenergic stimulation effect and hyperactive RyRs in order to study Ca\textsuperscript{2+}-mediated TA in the context of CPVT linked to RyR mutations.

The transmembrane voltage is described by the standard equation for excitable cells:

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

(2.1)

where \(V_m\) is the transmembrane voltage, \(I_{\text{ion}}\) is the total membrane current, \(I_{\text{stim}}\) is the stimulus current, and \(C_m\) is the transmembrane capacitance. Following Mahajan et al. [63], all ionic currents are computed for 1\(\mu\)F of cell membrane capacitance and have units of \(\mu\)A/\(\mu\)F. The total membrane current is given by

\[
I_{\text{ion}} = I_{\text{Na}} + I_{\text{to, f}} + I_{\text{to, s}} + I_{\text{Kr}} + I_{\text{Ks}} + I_{\text{K1}} + I_{\text{NaK}} + I_{\text{Ca}} + I_{\text{NaCa}}.
\]  

(2.2)

The Ca\textsuperscript{2+} cycling of this model is described by a set of differential equations

\[
\dot{c}_i = \beta_i(c_i)(I_{\text{dsi}} \frac{V_s}{V_j} - I_{\text{up}} + I_{\text{leak}} - I_{\text{TCl}} + I_{ci}),
\]  

(2.3)

\[
\dot{c}_s = \beta_s(c_s)(I_{\text{dps}} \frac{V_p}{V_s} + I_{\text{NCX}} - I_{\text{dsi}} - I_{\text{TCS}} + I_{cs}),
\]  

(2.4)

\[
\dot{c}_p = \beta_p(c_p)(I_r + I_{\text{Ca}} - I_{\text{dps}}),
\]  

(2.5)

\[
\dot{c}_{NSR} = (I_{\text{up}} - I_{\text{leak}}) \frac{V_i}{V_{\text{NSR}}} - I_{\text{tr}} \frac{V_{\text{JSR}}}{V_{\text{NSR}}} + I_{c_{\text{NSR}}},
\]  

(2.6)

\[
\dot{c}_{\text{JSR}} = \beta_{\text{JSR}}(c_{\text{JSR}})(I_{\text{tr}} - I_r \frac{V_p}{V_{\text{JSR}}}),
\]  

(2.7)

where \(c_i\) is the free Ca\textsuperscript{2+} concentration in the bulk myoplasm, \(c_s\) is the free Ca\textsuperscript{2+} concentration in a thin layer just below the cell membrane, \(c_p\) is the free Ca\textsuperscript{2+} concentration in the proximal space, \(c_{\text{JSR}}\) is the free Ca\textsuperscript{2+} concentration in the junctional SR, \(c_{\text{NSR}}\)
is the free Ca\textsuperscript{2+} concentration in the network SR, $\beta$ terms account for instantaneous buffering in corresponding compartments using the rapid buffering approximation, $I_{\text{up}}$ is the SERCA uptake current representing total flux into the NSR, $I_{\text{leak}}$ is the leak current from NSR to cytosol, $I_{\text{NCX}}$ is Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange current, $I_{\text{Ca}}$ is the L-type Ca\textsuperscript{2+} influx, $I_{r}$ is the total Ca\textsuperscript{2+} efflux from the JSR, $I_{\text{dsi}}$, $I_{\text{dps}}$ and $I_{\text{tr}}$ are the diffusion currents from adjacent compartments, $I_{\text{TCi}}$ and $I_{\text{TCs}}$ are the troponin C dynamic buffering currents in cytosol and submembrane spaces, $I_{\text{ci}}$, $I_{\text{cs}}$ and $I_{\text{cNSR}}$ are the diffusion currents between neighboring CRUs in the corresponding compartments.

The CSQN-mediated luminal gating of the RyR channels which regulate Ca\textsuperscript{2+} release from the SR is overviewed as follows. There is experimental evidence showing that luminal Ca\textsuperscript{2+} controls SR Ca\textsuperscript{2+} release is regulated by CSQN through interactions with the RyR channels. CSQN is the main buffering protein within the SR [24]. In vitro experiment [77] shows that CSQN forms dimers when the SR Ca\textsuperscript{2+} load rises above $\sim$500$\mu$M, and forms higher order polymers at concentrations of a few millimolar. Lipid bilayer experiments [33] have shown that the activity of RyR channels is modulated by the interaction of CSQN with the auxiliary proteins triadin-1 and junctin (T/J). They observed that the open probability of purified RyR channels steeply increases with addition of T/J, and it decreases again in the presence of CSQN. Furthermore, when Ca\textsuperscript{2+} concentration on the luminal side is increased from 20 $\mu$M to 5 mM, the open probability of RyRs increases again. In addition, in vivo experiments [105] show that transgenic mice with over(under)-expressed CSQN result in Ca\textsuperscript{2+} sparks with longer (shorter) refractory periods. These experiments suggest that CSQN acts as a luminal Ca\textsuperscript{2+} sensor and regulates RyR channel activity by attaching to the T/J complex of the RyR channels. These observations indicate that polymerization of CSQN might modulate RyR sensitivity by inhibiting the interaction of CSQN with the T/J RyR complex. Therefore, Restrepo et al. [80] constructed a simple model of CSQN luminal
Figure 2.2: Fig. 2 of [80] with permission. (a) Schematic representation of the four-state Markov model representing the four possible states of a RyR channel: closed CSQN-unbound (1), open CSQN-unbound (2), open CSQN-bound (3), and closed CSQN-bound (4). (b) Fraction of monomers $\hat{M}$ (solid line), and equilibrium value of the CSQN-unbound fraction of RyR channels (dashed line), as a function of the JSR free $\text{Ca}^{2+}$ concentration $c_{\text{JSR}}$.

gating by assuming that only CSQN monomers can bind to and inhibit RyR channels. Although this might be an oversimplification, such a model captures the general features of CSQN modulation of RyR activity, providing a powerful tool study CSQN mutation related CPVT computationally.

Fig. 2.2 shows the schematic of the four-state Markov model of RyR channels, and the fraction of monomers $\hat{M}$ (solid line) and equilibrium value of the CSQN-unbound fraction of RyR channels (dashed line), as a function of the JSR free $\text{Ca}^{2+}$ concentration, $c_{\text{JSR}}$. RyR gating kinetics can be described as follows. When SR $\text{Ca}^{2+}$ content is high, CSQN molecules form dimers and therefore RyRs stay in the CSQN-unbound state (see Fig. 2.2 b), which has a higher open probability ($k_{12} \gg k_{43}$ in Fig. 2.2 a). During CICR, SR $\text{Ca}^{2+}$ content decreases and the fraction of CSQN monomers increases. CSQN monomers bind to T/J RyR complex, forcing RyRs to switch to the CSQN-bound state, which has a much lower open probability, and therefore terminates CICR process. Subsequently, part of cytosolic $\text{Ca}^{2+}$ is pumped back to SR by SERCA
uptake current, and CSQN monomers unbind from the T/J RyR complex after a period of time, which contributes to refractoriness of RyR channels and is believed to underly Ca\(^{2+}\) spark restitution.

2.2 Incorporation of \(\beta\)-adrenergic stimulation and RyR hyperactivity into the physiologically detailed model

In the context of CPVT, TA is often observed in the presence of \(\beta\)-adrenergic stimulation. The proposed hypothesis by Venetucci et al [109] is that the increased SR Ca load by \(\beta\)-adrenergic stimulation goes above the lowered SR release threshold by hyperactive RyRs (mutation effect) causing spontaneous Ca\(^{2+}\) waves, which in turn induce DAD and TA. \(\beta\)-adrenergic stimulation primarily has effects on L-type Ca channels, SR Ca pumps, \(I_{\text{ks}}\), sarcolemmal Na/K pumps, and sodium current. However, experimental observations on certain effects are somewhat controversial. Some results indicate that \(\beta\)-adrenergic agonists could simulate Na/K-ATPase and reduce intracellular Na concentration [52] [58] [114], while Gao et al [27] [26] provided compelling evidence for Na/K-ATPase inhibition by \(\beta\)-adrenergic agonists. Gadsby et al [25] and Walsh et al [110] [111] reported that \(\beta\)-adrenergic agonists increased cardiac K conductance, while Bennett et al [5], showed accelerated decline in K conductance. Experiments also showed that AP duration can be increased, decreased, or unchanged depending on the species and experimental conditions. In this dissertation, \(\beta\)-adrenergic stimulation will be incorporated into our model to study the mechanism of DADs in the setting of CPVT. In view of the controversial observation of the effect of \(\beta\)-adrenergic stimulation on \(I_{\text{ks}}\), Na/K pump and sodium current, we will only incorporate the effect on L-type
calcium channels and SR Ca pump into the insilico model.

In the presence of the β-adrenergic agonist, both the Ca transient and LCC increase in amplitude. In particular, previous studies [54] [76] reported a 2~3-fold increase of peak calcium transient and a 30%~40% increase in the rate of decay of the calcium transient, as well as a 1.5~8-fold increase in basal LCC [86] [122] in ventricular myocyte under β-adrenergic stimulation. It has been shown that a shift toward mode 2 gating of LCC is involved in enhancing LCCs by β-adrenergic stimulation [121] [11] [10]. Mode 2 is the LCC state in which long stable openings occur. Mode 1 refers to the normal state where the openings are shorter. Mode 0 is characterized by the channel being unavailable to open.

To quantitatively model these effect, we increase the maximal uptake rate of SERCA to twice the original value, recruit 4 more LCC channels, decrease both Ca-induced and Vm-induced inactivation rates by 50%, and increase the C1 state to O state transition rate to 3 fold for the LCC 7-state Markov model taken from Mahajan et al. [63] to simulate a shift toward greater mode 1 and mode 2 gating (vs. mode 0, and mode 1). After these modifications, the simulation results (Fig. 2.3 and Fig. 2.4) of Ca\(^{2+}\) transient and LCC currents are comparable to experimental observations. In Fig. 2.3, cells are stimulated from a holding potential of -80 mV to indicated test potentials in absence (black) and presence (red) of β-adrenergic stimulation. Peak I\(_{Ca}\) as a function of clamped AP is plotted in the top of panel B, and the time required for the decay of [Ca\(_i\)] from peak to 50% is shown in the bottom of panel B. These results demonstrate that under AP clamped condition, this computer model produced 2-5 times increase in peak I\(_{Ca}\) and a 40% increase in [Ca\(_i\)] decay rate. Furthermore, the simulation results under free running AP condition are shown in Fig. 2.4, where panel A shows time traces of AP, I\(_{Ca}\) and [Ca\(_i\)] in absence (black) and presence (red) of β-adrenergic stimulation, and panel B shows peak I\(_{Ca}\), the integral of I\(_{Ca}\) within one
Figure 2.3: β-adrenergic stimulation effect on LCC and cytosolic Ca$^{2+}$ with clamped AP. (A) Traces show $I_{Ca}$ (left) and [Ca]$^{i}$ recorded from a holding potential of -80 mV to indicated test potentials in absence (black) and presence (red) of β-adrenergic stimulation. (B) Peak $I_{Ca}$ as a function of clamped AP is plotted on top, and the time required for the decay of [Ca]$^{i}$ from peak to 50% is shown at the bottom.
Figure 2.4: β-adrenergic stimulation effect on LCC and cytosolic $Ca^{2+}$ under free running AP at a pacing cycle length of 1 sec. (A) Traces show AP, $I_{Ca}$ and $[Ca]_i$ in absence (black) and presence (red) of β-adrenergic stimulation at a pacing cycle length of 1 s. (B) peak $I_{Ca}$ (a), the integral of $I_{Ca}$ within one beat (b), peak $[Ca]_i$ (c), and the $[Ca]_i$ decay time from peak to 50% (d) in absence (Ctrl) and presence (Iso) of β-adrenergic stimulation.
beat, peak [Ca]_i, and the [Ca]_i decay time from peak to 50%. These results show that under the current clamp condition, peak I_{Ca} is increased to 1.27 times in the presence of β-adrenergic stimulation as much as in control, total Ca^{2+} influx by I_{Ca} is increased to 1.62 times, peak [Ca]_i is increased to 2.85 times, and [Ca]_i decay rate is increased by 31%. Simulation results shown in Fig. 2.3 and Fig. 2.4 validate the ability of our computer model to mimic the effect of β-adrenergic stimulation.

In the context of CPVT, RyR channels are found to be hyperactive in almost every type of RyR mutations as we discussed in Chapter 1. To mimic such hyperactive effect that most RyR mutations represent, we introduce a dimensionless parameter into RyR gating kinetics such that the transition rate of RyRs from closed state to open state takes the following form:

\[ k_{12} = \alpha K_u c_p^2, \]

\[ k_{43} = \alpha K_b c_p^2. \]

Increasing α promotes the transition rate of RyRs from the closed state to the open state. Thus varying α allows us to change the hyperactivity of RyR channels to different levels as needed.

### 2.3 Combined effects of β-adrenergic stimulation and RyR hyperactivity on TA

To examine the hypothesis proposed by Venetucci et al [108] [109] that the increased SR Ca^{2+} load by β-adrenergic stimulation goes above the lowered SR release threshold by hyperactive RyRs (mutation effect) causing spontaneous Ca2+ waves, which in turn induce DAD and TA, we stimulated the cell under four different conditions, which
Figure 2.5: Ca$^{2+}$-mediated TA occur under combined effect of both isoproterenol and hyperactive RyRs. From top to bottom, experiment-like line scan of $[Ca]_i$, time courses of the whole cell averaged $[Ca]_i$ and $[Ca]_{jsr}$, as well as AP at pacing cycling length of 1sec are shown for control (a), hyperactive RyR only (b), isoproterenol only (c), and hyperactive RyR plus isoproterenol (d). The experiment-like line scan was recorded through the center axis in transversal direction (same axis for the rest line scan plot in this letter). The marked numbers on the top of black arrows in the time course of $[Ca]_i$ indicate the diastolic $[Ca]_i$ values for the corresponding case. The marked numbers in the time course of $[Ca]_{jsr}$ represent the diastolic SR Ca$^{2+}$ load (upper) and the nadir (lower) for each case. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of $\mu$M), and black color indicates lowest Ca$^{2+}$ concentration.
are control, presence of leaky RyRs, presence of isoproterenol effect, and presence of both leaky RyRs and isoproterenol effect together. According to the experimental observation in Ref [108], spontaneous Ca\(^{2+}\) waves should only occur in the cell with both \(\beta\)-adrenergic stimulation and hyperactive RyRs. Our computational simulation results are shown in Fig. 2.5. In the control case, RyR channels have the normal leakiness, i.e. \(\alpha=1\), and the effect of isoproterenol is absent. Therefore, no spontaneous Ca\(^{2+}\) waves occur. In the cell with only leaky RyRs (\(\alpha=4\) here), SR Ca\(^{2+}\) content is decreased by \(\sim 25\%\) in the steady state and we observe a few Ca\(^{2+}\) sparks in the experimental-like line scan image. However, there are still no Ca\(^{2+}\) waves exhibited. In the cell with normal RyRs and isoproterenol effect, the SR Ca\(^{2+}\) content is increased to \(\sim 135\%\) of the normal load, but spontaneous Ca\(^{2+}\) waves are still not exhibited. In the cell with both leaky RyRs and isoproterenol effect, spontaneous Ca\(^{2+}\) waves mediated TA finally occurs in between every two consecutive paced beats in the steady state. These results appear to support the hypothesis proposed by Venetucci et al. that either increasing RyR leakiness or promoting Ca\(^{2+}\) cycling by \(\beta\)-adrenergic stimulation alone is not sufficient to induce spontaneous Ca\(^{2+}\) waves. Ca\(^{2+}\)-mediated TA is obtained under the combined effects of RyR hyperactivity and \(\beta\)-adrenergic stimulation. However, we found that the SR Ca\(^{2+}\) content was only \(\sim 10\%\) higher than in the cell with leaky RyRs, and lower than in the control cell. This slight alteration in SR Ca\(^{2+}\) load does not seem to account for the severe TA condition as we see in the simulation results. In fact, we notice that the diastolic [Ca\(_i\)] in the presence of both leaky RyRs and isoproterenol effect is about 2-fold of all the other three cases. These results suggest that the effect of isoproterenol is more complex than simply increasing SR Ca\(^{2+}\) content above the Ca\(^{2+}\) wave threshold, since Ca\(^{2+}\) wave activity increases despite almost constant SR load. It seems that the diastolic [Ca\(_i\)] plays a more important role in inducing Ca\(^{2+}\)-mediated TA. In the next section, we will further explore statistical properties of the
initiation and termination of Ca\textsuperscript{2+} mediated TA, and the mechanisms underlying the degree of arrhythmogenicity.

### 2.4 Stochastic initiation and termination of Ca\textsuperscript{2+} wave mediated triggered activity

Experiments have shown that DADs do not always trigger APs, and the timing for DADs and/or triggered AP to occur is not deterministic under periodic AP pacing in the context of CPVT (see Fig. 1.5). Similar stochasticity is observed in another pacing protocol, where the cell was paced to the steady state in the presence of \(\beta\)-adrenergic stimulation and then the electrical stimulus was suddenly ceased. Under this pacing protocol, TA can be isolated, in a burst of several beats or even sustained. The number of triggered APs before the final termination has been reported highly variable [88]. The origin of the stochasticity is certainly multi-factors. The fluctuation of experimental environment, such as temperature and the heterogeneity of cell properties, such as cell size, geometrical structure, protein level, and drug dosage may all contribute to the high level of stochasticity. However, the ionic channels involved in membrane voltage dynamics and the intracellular Ca\textsuperscript{2+} cycling inherently open and close in a probabilistic manner. Therefore, it is important to investigate how the stochastic ion channel opening and closing links to stochastic initiation and termination of Ca\textsuperscript{2+}-mediated TA.

To address this question, we simulate the cell with hyperactive RyRs (controlled by \(\alpha\)) in the presence of isoproterenol effect under the two pacing protocols used in above experiments. To study the stochasticity in the initiation of Ca\textsuperscript{2+}-mediated TA, cell were stimulated at a pacing cycle length of 1 s for 40 beats to reach the steady state, then the isoproterenol effect was turned on in the model for another 60 beats. The reason
to pace cells at a pacing cycle length of 1 s is to have a long enough diastolic interval for TA to occur during pacing. In Fig. 2.6, examples of the simulation results are illustrated. After the addition of isoproterenol effect, spontaneous Ca\(^{2+}\) release appears following each paced beat. As shown in the enlarged line scan images (top panel in Fig. 2.6), these spontaneous Ca\(^{2+}\) release events are manifested as wave generation and propagation. In addition, the morphology of Ca\(^{2+}\) signal reflected by the line scan images reveals that Ca\(^{2+}\) waves precede the more synchronous \([\text{Ca}^2_]_i\) change, which supports the view that slow action potentials are initiated by Ca\(^{2+}\) waves, leading to subsequent activation of LCCs to trigger synchronized SR Ca\(^{2+}\) releases. Moreover, the number of DADs before the initiation of triggered AP varies in independent identical trials (6 DADs in trial 1, 3 DADs in trial 2, and 8 DADs in trial 3, see Fig. 2.6).

As the next step, we investigate how Ca\(^{2+}\)-mediated TA terminates. Cells were paced under a cycle length of 300 ms for 40 beats in the presence of isoproterenol effect followed by a sudden cessation of electrical stimulus. Under this pacing protocol, the pacing frequency is higher than that of triggered APs, therefore triggered APs only exhibit after the paced stimulus is stopped. Thus, the interaction between triggered APs and paced APs is avoided, providing the same onset of triggered APs in multiple trials. This makes the statistical analysis of triggered APs convenient. Similar to the previous results in the study of the initiation of Ca\(^{2+}\)-mediated TA, the terminations are found to take place randomly (11 triggered APs in trial 1, 8 triggered APs in trial 2, and 6 triggered APs, see Fig. 2.7) with the same parameters. In addition, the time traces of both Ca\(^{2+}\) and voltage signals show nearly periodic dynamics during TA in the absence of paced impulses. In physics, such oscillatory behavior is often interpreted as a limit cycle in a two-dimensional phase space. In the next section, we will analyze this limit cycle and propose a novel mechanism governing the initiation and termination of Ca\(^{2+}\)-mediated TA.
Figure 2.6: Initiation of Ca\(^{2+}\)-mediated TA under the administration of isoproterenol effect in a cell with hyperactive RyRs (α=3.5). For trial 1, from top to bottom, the experiment-like line scan recorded through the center axis in transversal direction, time courses of the whole cell averaged [Ca]\(_i\) and [Ca]\(_{jsr}\), and time trace of membrane voltage. Only the time trace of membrane voltage is shown for trial 2 and 3. The pacing cycle length is 1 sec. The red arrow indicates when the isoproterenol effect is turned on in the simulation. The green arrows mark DADs, blues star symbols label TAPs, and black filled circles tag periodic stimuli. In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of µM), and black color indicates lowest Ca\(^{2+}\) concentration.
Figure 2.7: Termination of Ca\(^{2+}\) wave mediated triggered activity. For trial 1, from top to bottom, the experiment-like line scan recorded through the center axis in transversal direction, time courses of the whole cell averaged \([\text{Ca}]_i\) and \([\text{Ca}]_{\text{jsr}}\), and time trace of membrane voltage. Only the time trace of membrane voltage is shown for trial 2. The pacing cycle length is 300 ms and \(\alpha=3.3\). The green arrows mark DADs, blues star symbols label TAPs, and black filled circles tag periodic stimuli. In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of \(\mu\text{M}\)), and black color indicates lowest Ca\(^{2+}\) concentration.
2.5 Bistable dynamics

In mathematics, in the area of dynamical systems, a limit cycle on a plane or a two-dimensional manifold is a closed trajectory in phase space. In the case where all the neighboring trajectories approach the limit cycle as time approaches infinity, it is called a stable limit cycle. Stable limit cycles imply self-sustained oscillations. Here, the sustained TA forms stable limit cycles in the cytosolic Ca\(^{2+}\)-membrane potential phase-plane diagram (Fig. 2.8). The limit cycles plotted in this figure correspond to the last two oscillations for the two independent identical trials shown in Fig. 2.7. It shows in Fig. 2.8 that they superimpose on top of each other, which indicates that they reach the same steady state. In addition to the triggered APs, the final termination of TA is also plotted in this phase-plane. As expected, the termination occurs at the critical point marked by the horizontal arrow where \(V_m\) is \(\sim 60\) mV, i.e. the activation threshold for Na\(^+\) channels. However, after the cell system falls off the critical point, it does not go back to the diastolic state of the limit cycle. Instead, it reaches and stays at an even lower level of diastolic state, which is far away from the stable limit cycle. In summary, the Ca\(^{2+}\) cycling dynamics stays in the stable oscillatory state during sustained TA and approaches a stable silent state when TA terminates. Although, this limit cycle diagram is drawn for the termination of Ca\(^{2+}\)-mediated TA, we believe it is the same mechanism controlling the initiation of Ca\(^{2+}\)-mediated TA, where the periodic paced electrical impulses help shorten the gap between the bistable states by elevating the intracellular Ca\(^{2+}\) level, and the initiation of TA occur when the cell system overcomes the critical point to activate Na\(^+\) channels. Of note, once a DAD succeeds in inducing a triggered AP, it is possible that the Ca\(^{2+}\) cycling dynamics readjusts the Ca\(^{2+}\) level due to LCC reopening, and gradually evolves into the steady state limit cycle. The evolution of diastolic Ca\(^{2+}\) levels during the initiation
and termination will be further investigated later in this section. In summary, these results suggest that Ca\(^{2+}\)-mediated TA is governed by bistable dynamics between an oscillatory state and an excitable silent state. In addition, this underlying mechanism of Ca\(^{2+}\)-mediated TA explains the reason why the cell normally needs a preceding stimulus to induce TA. Namely, without a stimulus, resting cells stay in the silent state, which is a dynamically stable state. The initiation and termination of Ca\(^{2+}\)-mediated TA are closely linked to the intracellular Ca\(^{2+}\) dynamics. Thus, we measured the diastolic [Ca\(_i\)] and the SR Ca\(^{2+}\) load during TA. These results explain the mechanism of escape from the limit cycle to the silent state.

For the initiation (Fig. 2.6), one DAD follows each paced AP before the genesis of TAPs. The diastolic [Ca\(_i\)] and SR Ca\(^{2+}\) load within the diastolic interval between the paced AP and the following DAD were measured. When TAPs were initiated, both the diastolic [Ca\(_i\)] and SR Ca\(^{2+}\) load that precede a TAP were recorded as well. These diastolic [Ca\(_i\)] and [Ca\(_{jsr}\)] values are plotted vs. the corresponding oscillation event, denoted as \(n\), in panel (A. b) and (A. c) of Fig. 2.9, respectively. The oscillation events include both DADs and TAPs. The data show that the intracellular Ca\(^{2+}\) level during DADs exhibits transient relaxation (\(~2\)-3 oscillations) to the steady state. TAPs can randomly occur at a different time with nearly the same intracellular Ca\(^{2+}\) levels in identical independent trials. The distribution of the number of DADs, denoted as \(N_{DAD}\), preceding a TAP is plotted in panel (A. a) of Fig. 2.9, which appears broad. It is noticed that the peak of the distribution is at \(N_{DAD}=2\), which is the same number of DADs that the intracellular Ca\(^{2+}\) requires to reach steady state.

Following the same manner, during the termination of TA, the diastolic [Ca\(_i\)] and [Ca\(_{jsr}\)] right before TAPs and DADs are plotted vs. the oscillation number, \(n\), in panel (B. b) and (B. c) of Fig. 2.9. Likewise, the Ca\(^{2+}\) intracellular level during sustained TAPs shows transient relaxation to the steady state. TAPs can terminate
Figure 2.8: Termination trajectories of $\text{Ca}^{2+}$ wave mediated TA in $V_m$-$[\text{Ca}]_i$ phase-plane. Note that these trajectories correspond to the trial 1 and trial 2 as shown in Fig. 2.7. Only the part of each time trace from the last two TAPs to the end are plotted, respectively. The escape from the limit cycles occurs at the critical point indicated by the black arrow representing the point for $\text{Na}^+$ current activation. Once the cell system terminates from the sustained TAPs, it reaches the silent state marked by the black arrow on the left of the plot.
at a different time with the similar intracellular Ca\(^{2+}\) level in identical independent trials. The distribution of the number of TAPs, denoted as \(N_{\text{TAP}}\), preceding a DAD is shown in panel (B. a) of Fig. 2.9, which is broad. Similarly as in the initiation, the distribution frequency of \(N_{\text{TAP}}\) reaches the peak value after the same number of TAPs as for the intracellular Ca\(^{2+}\) level to approach the steady state. In the next section, we study what kind of distributions that \(N_{\text{DAD}}\) and \(N_{\text{TAP}}\) follow and how these distributions are affected by key parameters of Ca\(^{2+}\) cycling dynamics.

### 2.6 Stochastic initiation and termination of TA are governed by geometric distributions

For the initiation of Ca\(^{2+}\)-mediated TA, we want to know whether the DADs preceding a TAP are independent events. To study that, a large enough data set for statistical analysis is needed. However, in the parameter regime of our simulations (\(\alpha=3.5\)), the number of DADs preceding a TAP is typically less than 20. To obtain an enough number of DADs for statistical analysis, we simulated the cells in the presence of isoproterenol effect with hyperactive RyRs at a pacing cycle length of 1 sec, but Na\(^+\) currents and LCCs were blocked during the diastolic interval between two consecutive paced beats, so that the DAD between the two consecutive paced APs can not induce triggered APs no matter what amplitude the DAD can reach. The LCCs were blocked because when the depolarization driven by NCX during spontaneous Ca\(^{2+}\) releases is up to threshold for LCC activation, LCCs can open without the activation of Na\(^+\) currents. The cell was paced for 5000 beats at given parameters, which provides enough data for the purpose of computing statistical averages.

The correlation between these DADs is studied by calculating the autocorrelation
Figure 2.9: Diastolic intracellular Ca\textsuperscript{2+} evolution and distribution of the oscillation number during initiation and termination of Ca\textsuperscript{2+}-mediated TA. For the initiation, we show in panel A: a) Distribution of the number of DADs before initiating a TAP; b) Diastolic cytosolic Ca\textsuperscript{2+} concentration, denoted as [Ca\textsubscript{i} \textsuperscript{D}], vs. the number of oscillations (DAD/TAP); c) Diastolic JSR Ca\textsuperscript{2+} concentration, denoted as [Ca\textsubscript{jsr} \textsuperscript{D}], vs. the number of oscillations (DAD/TAP). Note that the cell exhibited a sequence of DADs first, then stochastically initiate TAPs. The blue arrows indicate the first TAP in each trial. It shows that the first TAP has the same diastolic Ca\textsuperscript{2+} level as preceding DADs. For the termination, we shown in panel B: a) Distribution of the number of TAPs before termination; b) Relation between [Ca\textsubscript{i} \textsuperscript{D}] and the number of oscillations; c) Relation between [Ca\textsubscript{jsr} \textsuperscript{D}] and the number of oscillations. The blue arrows indicate the last oscillation event in each trial is a DAD. After that the cell reached the silent state (the last point of each trajectory).
coefficient of the amplitude of DADs. The equation is

\[ c(j) = \frac{1}{N-j} \sum_{j=1}^{N-j} A_k A_{k+j} - \left( \frac{1}{N} \sum_{k=1}^{N} A_k \right)^2, \]

where \( A_k \) is the amplitude of the \( k \)th DAD, \( j \) is the interval between two DADs and \( N \) is the total number of DADs. The autocorrelation coefficient is plotted as a function of \( j \). We also calculate the autocorrelation coefficient of a set of uniform distributed random numbers in the same graph (see Fig. 2.10). The results show that these DADs almost behave as independent random variables, despite a slight dependence on the previous events (\( c(1) \sim 0.15 \)). Of note, TAPs occur when the depolarization of membrane potential is up to the threshold for the activation of \( \text{Na}^+ \) currents, denoted as \( V_{th} \). After each AP, \( \text{Na}^+ \) channels require repolarization to recover from inactivation before another AP can occur. The time required depends on the diastolic level. For instance, the recovery period, \( \tau \sim 10 \text{ ms at } -100 \text{ mV, } 30 \text{ ms at } -80 \text{ mV or } 100 \text{ ms at } -72 \text{ mV before the cell can fire another AP} \) [1]. In our simulation, the diastolic level is \( \sim -85 \text{ mV} \), and the time interval between the complete repolarization of a paced AP and the occurrence of the following DAD is about \( \sim 250 \text{ ms} \) (Fig. 2.6), which is even much longer than the recovery time required at -80 mV. Thus, we can assume that the \( \text{Na}^+ \) channel is fully recovered before the occurrence of each DAD. In addition, we measured the time interval between the peak of a DAD and the onset of the previous paced AP, denoted as \( T_{DAD} \) (Fig. 2.11). The width of \( T_{DAD} \) distribution is about \( \sim 20 \text{ ms} \), which is a narrow range, indicating that each DAD occurs after nearly the same time interval following a paced AP. Taken together, the threshold for activating the \( \text{Na}^+ \) current for each DAD during the initiation process of \( \text{Ca}^{2+} \)-mediated TA can be assumed constant, and each DAD is independent on other DADs. Therefore, the number of DADs preceding a TAP should follow a geometric distribution. Let \( p \) be
the probability that a DAD induces a TAP, then in the steady state, the probability of having a TAP after $k$ DADs can be written as

$$f(k) = p(1 - p)^k,$$  
(2.11)

which is the probability mass function of a geometric distribution. Here, this equation is valid because all the $k$ DADs are independent and the transition rate $p$ is a constant, i.e. the threshold for activating the Na$^+$ current remains constant for all the $k$ DADs.

To obtain the value of $p$, the threshold value ($V_{th}$) of the membrane voltage for activating the Na$^+$ channel is required. The Na$^+$ current activates rapidly. Thus, $V_{th}$ is the value where the slope of a TAP dramatically increases as shown in Fig. 2.12.
Figure 2.11: Distribution of the time interval between the onset of AP and the peak of the following DAD. The cell was paced at a pacing cycle length of 1 sec in the presence of isoproterenol effect and leaky RyRs (α=3.5) for 5000 beats. Na⁺ channels and LCCs were blocked when spontaneous Ca²⁺ release occurs.
where $V_{th} = -61.5 \text{ mV}$, and the corresponding $[\text{Ca}]_i$ threshold for activating the $\text{Na}^+$ current, denoted as $[\text{Ca}]_i^{th}$ is $0.7 \mu\text{M}$.

Thus, in the simulation where $\text{Na}^+$ currents and LCCs were blocked during the diastolic interval between two consecutive paced APs, $p$ can be determined by the ratio of the number of DADs with a peak value of $V_m$ that exceeds $V_{th}$ to the total number of DADs (see Fig. 2.13). In fact, $p$ can also be determined by the ratio of the number of DADs with the corresponding $[\text{Ca}]_i$ peak value exceeding $[\text{Ca}]_i^{th}$ to the total number of DADs (see Fig. 2.14). Once the $p$ value is known, the distribution of $N_{\text{DAD}}$ as shown in Fig. 2.9 A. a) can be analytically predicted. Of note, the natural logarithm of a geometric probability mass function takes the following form

$$\ln f(k) = k \cdot \ln (1 - p) + \ln p,$$

(2.12)

where $\ln (1 - p)$ is the slope of $\ln f(k)$ vs. $k$. By substituting the $p$ value obtained above, $\ln (1 - p)$ is determined. To prove the distribution of $N_{\text{DAD}}$ follows a geometric distribution, the logarithm of the frequency of $N_{\text{DAD}}$ is plotted vs. the DAD number in the same graph. As we discussed above, the $\text{Ca}^{2+}$ cycling dynamics takes a few oscillations to reach the steady state after the addition of isoproterenol effect, which gives rise to the initial drop-off phase of the distribution of $N_{\text{DAD}}$. To avoid the effect of the transient relaxation of the $\text{Ca}^{2+}$ cycling dynamics on the distribution of $N_{\text{DAD}}$, we only fit the natural logarithm of the distribution of $N_{\text{DAD}}$ from the peak to the tail, since the position of the peak frequency of the distribution indicates the time when the intracellular $\text{Ca}^{2+}$ cycling dynamics reaches the steady state (see Fig. 2.9). The slope of the linear fitting curve shows good agreement with the theoretical slope value, $\ln (1 - p)$.

In addition, we measured the number of DADs between two subsequent DADs that
have peak values larger than $V_{th}$ in the simulation where both Na$^+$ currents and LCCs are blocked during DADs. We define this number as $N'_{DAD}$, which should exhibit the same distribution as $N_{DAD}$ with the same given parameters. To avoid the transient effect of Ca$^{2+}$ cycling dynamics on the distribution of $N'_{DAD}$, we begin to record $N'_{DAD}$ after the first 20 DADs. In Fig. 2.15, the natural logarithm of the frequency of $N'_{DAD}$ is plotted as well. The slope of the corresponding linear fitting curve also perfectly matches the theoretical slope value, $\ln(1 - p)$.

These results validate that, after reaching the steady state, the number of DADs preceding a TAP follows a geometric distribution, because DADs exhibited in the simulation are independent and the probability that a DAD induces a TAP is a constant. This constant probability that a DAD induces a TAP is determined by the threshold for the Na$^+$ current activation, which is a constant value (-61.5 mV). In a cardiac cell, each paced AP resets the phase of Ca$^{2+}$ cycling dynamics of the cell by synchronizing local Ca$^{2+}$ sparks in CRUs through CICR. After firing Ca$^{2+}$ sparks, these CRUs require a refractory period before being able to excite again, which sets up the time for a DAD to occur. Thus, each DAD after a paced AP occurs at nearly the same condition where most of CRUs just come out of the refractory state. This explains why the time interval between the complete repolarization of the paced AP and the occurrence of DAD is about $\sim250$ ms, which is the typical refractory period. Consequently, these DADs behave independently in terms of the nearly uncorrelated amplitude of DADs. However, the CRUs fired during a DAD also need a similar refractory period to recover. If these CRUs are not fully recovered by the time the next paced AP comes, they may not be triggered, which would have slight effect on the amplitude of the DAD following the upcoming paced AP. That explains why the autocorrelation coefficient $c(1)$ is $\sim0.15$.

Likewise, during sustained TAPs, each TAP plays the similar role as the paced AP
in synchronizing \( \mathrm{Ca}^{2+} \) sparks in CRUs. Thus, each TAP occurs at nearly the same condition where most of CRUs just come out of the refractory state. That explains why the TAPs oscillate almost periodically in the steady state, and the shape of TAPs is nearly identical (Fig. 2.8). In summary, these TAPs behave independently, and the threshold for the activation of \( \mathrm{Na}^+ \) currents is constant during each TAP. Therefore, the number of TAPs before the final termination follows a geometric distribution as well. In the next section, the dependence of distributions of \( N_{\text{DAD}} \) and \( N_{\text{TAP}} \) on key parameters and how such dependence is linked to the degree of arrhythmogenicity in the cell would be discussed.
Figure 2.13: Distribution of DAD peaks in the simulation with Na\(^+\) current and LCCs blockade. The cell was paced at a pacing cycle length of 1 sec in the presence of isoproterenol effect and leaky RyRs (\(\alpha=3.5\)) for 5000 beats. Na\(^+\) channels and LCCs were blocked within the diastolic interval between two consecutive APs. \(V_{th}\) is the threshold voltage for Na\(^+\) channels to activate. The probability \(p\) that a DAD induces a TAP is calculated by the ratio of the number of DADs with a peak value of \(V_m\) that exceeds \(V_{th}\) to the total number of DADs. Here, \(p=0.2306\).
Figure 2.14: Distribution of $[Ca]_i$ peaks during DADs in the simulation where Na$^+$ channels and LCCs are blocked within the diastolic interval between two consecutive APs. $\xi=0.7$ and $\alpha=3.5$. The Gaussian fitting parameters are: mean $\lambda=0.6577$; standard deviation $\sigma=0.0569$. The probability $p$ that a DAD induces a TAP is calculated by the ratio of the number of DADs with the corresponding $[Ca]_i$ peak value exceeding $[Ca]_{i}^{th}$ to the total number of DADs. Here, $p=0.2339$. 
Figure 2.15: Logarithm of the distribution frequency, denoted as $\ln(F)$ vs. the number of DADs before the occurrence of TAPs in the simulation without Na$^+$ channels and LCCs blockade (black square); vs. the number of DADs between two up-threshold DADs, i.e. DADs with the peak values larger than $V_{th}$ (red triangle). The corresponding linear fit is plotted with black and red solid lines, respectively. The analytical prediction fit from geometric distribution is plotted with blue solid lines as well.
2.7 Dependence of the level of arrhythmogenicity on RyR leakiness and Ca\(^{2+}\) diffusivity

The major effect of RyR mutation in the context of CPVT is increased leakiness of RyR channels. The cell with hyperactive RyRs under the administration of \(\beta\)-adrenergic stimulation causes Ca\(^{2+}\) waves, which in turn drives NCX to bring voltage up to the threshold for Na\(^{+}\) channels activation resulting in an AP. Therefore, in the following study, we look into how the level of RyR hyperactivity affects the degree of Ca\(^{2+}\) mediated TA. In addition, the role of diffusive coupling strength between CRUs is also investigated, since it directly affects Ca\(^{2+}\) wave properties. These properties include the probability that Ca\(^{2+}\) sparks generate Ca\(^{2+}\) waves, Ca\(^{2+}\) wave amplitude, and wave propagation length, which are essential for understanding Ca\(^{2+}\)-mediated TA.

As mentioned in Chapter 1, the hyperactivity of RyR is controlled by the parameter \(\alpha\), the pre-factor of the close-to-open rate of RyRs. To change the strength of diffusive coupling between CRUs, the diffusion time constants in cytosol, submembrane and network SR are multiplied by a parameter \(\xi\). Increasing \(\xi\) indicates slow diffusion, i.e. weak coupling strength. To investigate the role of RyR leakiness in Ca\(^{2+}\)-mediated TA, we simulate the cell with different \(\alpha\) in the presence of isoproterenol. At each given \(\alpha\), we carried out 500 independent simulations under the same pacing protocol as in Section 2.3 to study both the initiation and termination process of Ca\(^{2+}\)-mediated TA, respectively.

For the initiation, in Fig. 2.16, we plot \(N_{\text{DAD}}\) as a function of \(\alpha\), and the logarithm of the distribution of \(N_{\text{DAD}}\) for different \(\alpha\) values is shown as well. We observe that the average value of \(N_{\text{DAD}}\) is decreased remarkably with increasing \(\alpha\) in panel A. More visually, the slope of ln F vs. \(N_{\text{DAD}}\) is dramatically changed with small shift of \(\alpha\) (0.1). Similarly, for the termination of Ca\(^{2+}\)-mediated TA, the change of \(N_{\text{TAP}}\) with \(\alpha\) is also
Figure 2.16: $\alpha$ effect on the initiation of Ca$^{2+}$-mediated TA. A) $N_{\text{DAD}}$ as a function of $\alpha$. B) Logarithm of normalized distribution of $N_{\text{DAD}}$ for different $\alpha$. Cells were paced at a pacing cycle length of 1 sec in the presence of isoproterenol effect with different $\alpha$.

Figure 2.17: $\alpha$ effect on the termination of Ca$^{2+}$-mediated TA. A) $N_{\text{TAP}}$ as a function of $\alpha$. B) Logarithm of normalized distribution of $N_{\text{TAP}}$ for different $\alpha$. Cells were paced at a pacing cycle length of 300 ms in the presence of isoproterenol effect with different $\alpha$. $N_{\text{TAP}}$ was counted after stopping the electrical stimulus.

Figure 2.18: $\xi$ effect on the initiation of Ca$^{2+}$-mediated TA. A) $N_{\text{DAD}}$ as a function of $\xi$. B) Logarithm of normalized distribution of $N_{\text{DAD}}$ for different $\xi$. 
very steep as seen in Fig. 2.17.

Similarly, \(N_{\text{DAD}}\) and \(N_{\text{TAP}}\) show a strong dependence on the diffusive coupling coefficient \(\xi\) in the initiation and termination of Ca\(^{2+}\)-mediated TA as seen in Fig. 2.18 and 2.19. Qualitatively, we understand that either increasing RyR hyperactivity or the strength of diffusive coupling promotes the probability of spontaneous Ca\(^{2+}\) releases, which in turn increase the degree of arrhythmogenicity in the cell. However, quantitatively why the degree of Ca\(^{2+}\)-mediated TA is remarkably sensitive to RyR hyperactivity and the diffusivity of Ca\(^{2+}\) between CRUs in a nonlinear manner remains unclear.

To address these issues, we start with investigating the initiation process of Ca\(^{2+}\)-mediated TA. The effect of RyR hyperactivity and the Ca\(^{2+}\) diffusivity on the degree of Ca\(^{2+}\)-mediated TA can be quantified by the calculating the probability \(p\) that a DAD induces a TAP with different \(\alpha\) and \(\xi\) values, respectively. In Fig. 2.15, we have shown that when the Ca\(^{2+}\) cycling dynamics reaches the steady state, the number of DADs preceding a TAP follows a geometric distribution, where the probability \(p\) that a DAD induces a TAP can be calculated by the ratio of the number of DADs with a peak value of \(V_m\) that exceeds \(V_{th}\) to the total number of DADs (see Fig. 2.13), or by the ratio of the number of DADs with the corresponding \([\text{Ca}]_i\) peak value exceeding \([\text{Ca}]_i^{\text{th}}\) to the
total number of DADs (see Fig. 2.14). In addition, $p$ can also be obtained from the slope of a linear fitting curve as shown in Fig. 2.15 by

$$p = 1 - e^s,$$  \hspace{1cm} (2.13)

where $s$ is the slope of the linear fitting curve. As we have shown in Fig. 2.15, all these methods give rise to nearly the same $p$ value at fixed parameters. Here, $p$ is determined by calculating the ratio of the number of DADs with the corresponding $[\text{Ca}]_i$ peak value exceeding $[\text{Ca}]_i^{\text{th}}$ to the total number of DADs for different $\alpha$ values (Fig. 2.20) and $\xi$ values (Fig. 2.21), respectively. These results show that the probability $p$ that a DAD induces a TAP is quite sensitive to the change of $\alpha$ and $\xi$ values. It should be noted that in Fig. 2.14, the distribution of the $[\text{Ca}]_i$ peak value corresponding to each DAD is well fitted by a Gaussian distribution. To further understand the origin of this sigmoid-like steep dependence of $p$ on the parameters $\alpha$ and $\xi$, we plot the spontaneous $[\text{Ca}]_i$ peak distribution for different $\alpha$ values in Fig. 2.22, and different $\xi$ values in Fig. 2.23. These distributions can all be well fitted by a Gaussian distribution. It appears that the average spontaneous $[\text{Ca}]_i$ peak increases with increasing $\alpha$ or decreasing $\xi$, but the widths of these distributions do not alter much with changing $\alpha$ and $\xi$ values (Fig. 2.24). Therefore, we assume that these Gaussian distributions have different average values but the same standard deviation values within our parameter regime. Therefore, $p$ is only a function of the average spontaneous $[\text{Ca}]_i$ peak, which takes the
following form

\[ p([Ca]_i) = \int_{[Ca]_i^{th}}^{\infty} \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x-[Ca]_i)^2}{2\sigma^2}} \, dx \]

\[ = \frac{1}{\sqrt{\pi}} \int_{\frac{[Ca]_i^{th} - [Ca]_i}{\sqrt{2\sigma}}}^{\infty} e^{-x^2} \, dx \]

\[ = \frac{1}{2} \text{erfc} \left( \frac{[Ca]_i^{th} - [Ca]_i}{\sqrt{2\sigma}} \right), \quad (2.14) \]

where \([Ca]_i^{th}\) is the threshold \([Ca]_i\) peak for triggering an AP, \(\sigma\) is the standard deviation of the spontaneous \([Ca]_i\) peak values, \([Ca]_i\) is the average value of spontaneous \([Ca]_i\) peaks, and \text{erfc} is the complementary Gauss error function, which is a special function (non-elementary, sigmoid shape). Numerically, we can calculate \(p([Ca]_i)\) at a given \([Ca]_i\) value. In Fig. 2.20 and Fig. 2.21, \(p([Ca]_i)\) as a function of \(\alpha\) and \(1/\xi\) are plotted, respectively. These curves perfectly match the ones obtained from calculating the ratio of the number of DADs with the corresponding \([Ca]_i\) peak value exceeding \([Ca]_i^{th}\) to the total number of DADs. Note that the steepness of a complementary Gauss error function \text{erfc}(x)\) on the variable \(x\) is controlled by the width of a Gaussian distribution. When the width of a Gaussian distribution is small, the dependence of \text{erfc}(x)\) on the variable \(x\) becomes steep as shown in Fig. 2.25.

The data demonstrate that the sigmoid shape relationship of the probability that a DAD induces a TAP with \(\alpha\) and \(\xi\) is underlain by the Gaussian distributed spontaneous \([Ca]_i\) peaks, and the width of the Gaussian distribution changes the steepness of the dependence of \(\text{Ca}^{2+}\)-mediated TA on \(\alpha\) and \(\xi\). However, these distributions of spontaneous \([Ca]_i\) peaks during TA have much broader widths (\(\sim 0.15 \mu M\)) than that of the distribution of normal \([Ca]_i\) transient as shown in Fig. 2.26, where the width of the distribution is about \(\sim 0.007 \mu M\). Therefore, the fundamental behavior of \(\text{Ca}^{2+}\) cycling dynamics in the cell under the arrhythmogenic condition is very different from
Figure 2.20: Probability that a DAD induces a TAP as a function of $\alpha$. Each $p$ value is determined by the ratio of spontaneous $[Ca]_i$ peaks that are larger than $[Ca]_i^{th}$. $\xi=0.7$. The cell was paced at a pacing cycle length of 1 sec in the presence of isoproterenol effect and leaky RyRs with Na$^+$ channels and LCCs being blocked during spontaneous Ca$^{2+}$ release.
Figure 2.21: Probability that a DAD induces a TAP as a function of $1/\xi$. Each p value is determined by the ratio of spontaneous $[Ca]^i$ peaks that are larger than $[Ca]^{th}_i$, $\alpha=3.5$. The cell was paced at a pacing cycle length of 1 sec in the presence of isoproterenol effect and leaky RyRs with Na$^+$ channels and LCCs being blocked during spontaneous Ca$^{2+}$ release.
Figure 2.22: Distribution of spontaneous \([\text{Ca}]_i\) peaks at different \(\alpha\) values. Pacing protocol is the same as described in Fig. 2.20.

that in the cell under the normal condition.

Generally, the spontaneous \([\text{Ca}]_i\) is the summation of multiple \(\text{Ca}^{2+}\) waves in our simulations Fig. 2.27. Thus, the number of \(\text{Ca}^{2+}\) wave foci, how fast the foci propagate to neighboring CRUs, and how far the foci propagate before they terminate all affect the morphology of the \(\text{Ca}^{2+}\) waves, which is attributable to the amplitude of spontaneous \([\text{Ca}]_i\). Therefore, the broad width of the distribution of the spontaneous \([\text{Ca}]_i\) peaks is directly linked to the number of \(\text{Ca}^{2+}\) wave foci, denoted as \(N_f\), the wave propagation speed, denoted as \(v_f\), and the critical propagation length before a \(\text{Ca}^{2+}\) wave diminishes, denoted as \(L_p\).

When the leakiness of RyRs, i.e. \(\alpha\), increases, the probability of firing \(\text{Ca}^{2+}\) sparks is increased, which in turn promotes the probability of generating \(\text{Ca}^{2+}\) wave foci. The wave speed \(v_f\) and the critical wave propagation length \(L_p\) may also be affected by \(\alpha\),
Figure 2.23: Distribution of spontaneous $[Ca]_i$ peaks at different $\xi$ values. Pacing protocol is the same as described in Fig. 2.21

but these effects do not seem to be as strong as in the change of $N_f$. Both the transversal and longitudinal line scan images (Fig. 2.28) show that the $v_f$ maintains as a relative constant (roughly estimated as $\sim 200$ $\mu$m/s) at different $\alpha$ values. In addition, the longitudinal line scan images (Fig. 2.28) reveal that $N_f$ increases when $\alpha$ is increased. It is noted that although the wave propagation length $L_p$ is almost the same ($\sim$5-10 CRUs) at different $\alpha$ values, the Ca$^{2+}$ waves become more connected because of the increased $N_f$. According to these observations, we assume that the elevation in the amplitude of spontaneous $[Ca]_i$ with increasing $\alpha$ is primarily caused by the increased number of Ca$^{2+}$ wave foci.

Furthermore, when Ca$^{2+}$ diffusivity between CRUs is enhanced, $L_p$ seems to be increased, but $N_f$ remains relatively the same (Fig. 2.29). The Ca$^{2+}$ wave propagation speed $v_f$ appears relatively the same (roughly estimated as $\sim 200$ $\mu$m/s) under different
Figure 2.24: Relationship of the average spontaneous $[Ca]_i$ peak and the standard deviation of spontaneous $[Ca]_i$ peaks with $\alpha$ (A) and $1/\xi$ (B). Pacing protocol is the same as described in Fig. 2.20.
diffusive coupling strength, which may be the compensatory result of the lowered SR Ca\textsuperscript{2+} load at strong diffusive coupling strength. Based on these observations, the strong diffusive coupling strength is assumed to elevate the spontaneous [Ca\textsubscript{i}] amplitude primarily by increasing the Ca\textsuperscript{2+} wave propagation length.

To further investigate how changing $\alpha$ or $\xi$ affects Ca\textsuperscript{2+} cycling dynamics, which in turn causes broad distributed spontaneous [Ca\textsubscript{i}] peaks, we constructed a simplified model that links the basic features of spontaneous Ca\textsuperscript{2+} waves to the number of Ca\textsuperscript{2+} wave foci, the wave propagation speed and the size of Ca\textsuperscript{2+} waves, which are the fundamental properties that govern Ca\textsuperscript{2+} cycling dynamics in the cell.
Figure 2.26: Distribution of normal [Ca]$_i$ transients at a pacing cycling length of 0.3 sec and $\alpha$=3. The red curve is fitted by a Gaussian distribution with the mean, denoted as $\mu$, is equal to 1.6105 and the standard deviation, denoted as $\sigma$, is equal to 0.0037.
Figure 2.27: Cytosolic Ca\(^{2+}\) dynamics in the whole cell simulation for the detailed model. From panel a) to f), we show a series of snapshots during spontaneous Ca\(^{2+}\) waves in the whole cell. Time indicated in each figure is in the unit of millisecond. Here, \(\alpha=3.5\), \(\xi=0.7\). Na\(^{+}\) currents and LCCs are blocked during the diastolic interval between two consecutive APs.
Figure 2.28: Transversal (a) and longitudinal (b) line scan images for the cell under a pacing cycle length of 1 sec with Na$^+$ channels and LCCs blockade during diastolic intervals between two normal paced beats for different RyR hyperactivity. The time traces of the fraction of CSQN-unbound RyRs ($f_{ub}$) are also plotted in each panel, respectively. Panel A represents the simulation results carried out at $\alpha=3.5$, while panel B is for $\alpha=4.0$. Here, $\xi=0.7$. The color bar of line scan images has the unit of $\mu$M. Note that spontaneous Ca$^{2+}$ release occurs when most of RyRs are recovered from the CSQN-bound state, i.e. $f_{ub} \sim 1$. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of $\mu$M), and black color indicates lowest Ca$^{2+}$ concentration.
Figure 2.29: Transversal (a) and longitudinal (b) line scan images for the cell under a pacing cycle length of 1 sec with Na$^+$ channels and LCCs blockade during diastolic intervals between two normal paced beats for different Ca$^{2+}$ diffusivity. The time traces of the fraction of CSQN-unbound RyRs ($f_{ub}$) are also plotted in each panel, respectively. Panel A represents the simulation results carried out at $\xi=0.7$, while panel B is for $\alpha=0.85$. Here, $\alpha=3.5$. The color bar of line scan images has the unit of $\mu$M. Note that spontaneous Ca$^{2+}$ release occurs when most of RyRs are recovered from the CSQN-bound state, i.e. $f_{ub} \sim 1$. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of $\mu$M), and black color indicates lowest Ca$^{2+}$ concentration.
2.8 Simplified model of Ca\(^{2+}\) wave dynamics

The cell geometry maintains the same as in the original detailed Restrepo et al. model, i.e. we kept the same number of CRUs in both longitudinal and transversal directions as in the detailed model. Although, it is believed that several CRUs firing at the same time causes a Ca\(^{2+}\) wave foci, it is assumed that the size of Ca\(^{2+}\) wave foci is not a dominant factor in determining the amplitude of the spontaneous \([Ca]_i\). Therefore a spontaneous Ca\(^{2+}\) spark is equivalent to a Ca\(^{2+}\) wave focus in this study. In addition, we ignored the anisotropy of the geometric shape of CRUs, and assumed the length of CRU to be 1 µm in both longitudinal and transversal directions.

As discussed in the previous section, three parameters control the Ca\(^{2+}\) wave morphology in the cell, including \(N_f\), \(v_f\) and \(L_p\). Thus, in the simplified model, we introduced a parameter \(k_f\), which represents the probability of having a Ca\(^{2+}\) wave focus per unit volume per unit time. At each time step \(\Delta t\), the probability that a CRU fires a Ca\(^{2+}\) spark (focus) is \(k_f \cdot \Delta t \cdot \Delta V\), where \(\Delta V=1 \ \mu m^3\), which is the volume of one CRU. The number of Ca\(^{2+}\) foci generated in the cell within each time step is then determined by Monte Carlo methods. When a Ca\(^{2+}\) wave focus is generated, it propagates to neighboring CRUs at a velocity of \(v_f\). The furthest distance that a Ca\(^{2+}\) wave focus can propagate is determined by \(L_p\). Once a CRU fires, it can not fire again in the rest of the simulation time. For simplicity, the time course of a Ca\(^{2+}\) spark in each CRU is given by

\[
Ca_i^{sp} = A_1 \cdot (e^{-(t-t_0)/\tau_1} - e^{-(t-t_0)/\tau_2}) + A_0,
\]

where \(A_1=1.7 \ \mu M\), \(A_0=0.15 \ \mu M\), \(\tau_1=38 \ \text{ms}\) and \(\tau_2=4 \ \text{ms}\). These parameters are chosen to fit a typical Ca\(^{2+}\) spark of the detailed model at \(\alpha=3.5\) and \(\xi=0.7\) in the presence of isoproterenol effect (Fig. 2.30). The whole cell \([Ca]_i\) is then the summation of all
these local Ca$^{2+}$ sparks. Of note, the time step $\Delta t$ is chosen such that a Ca$^{2+}$ wave focus needs $10\Delta t$ to propagate to the nearest neighboring CRUs.

To study the effect of $\alpha$ and $\xi$ on the degree of Ca$^{2+}$-mediated TA, and more fundamentally how they cause broad distributed spontaneous $[Ca]_i$ peaks, we need to first find a set of parameter values of $k_f$, $v_f$ and $L_p$ that corresponds to a given $\alpha$ or $\xi$ values. The Ca$^{2+}$ wave propagation speed $v_f$ is set to be the same in both longitudinal and transversal directions, and the value is 200 $\mu$m/s, which is the estimation value of the Ca$^{2+}$ wave propagation speed in the detailed model and is assumed to be constant when varying $\alpha$ and $\xi$. As shown in Fig. 2.27, 2.28, and 2.28, when $\alpha=3.5$ and $\xi=0.7$, the number of Ca$^{2+}$ wave foci is roughly about $\sim$50 and each focus typically propagates $\sim$5 CRUs away from itself in the detailed model. Therefore, we chose $L_p=5$ $\mu$m and $k_f=0.000015$ $\mu$m$^{-3}$ms$^{-1}$, such that the simplified model reproduces the similar number of Ca$^{2+}$ wave foci and spontaneous $[Ca]_i$ amplitude as seen in the detailed model. Of note, the total volume of a Ca$^{2+}$ wave propagated across before it diminishes is $\sim (2L_p)^3$. In summary, in this simplified model, $v_f$ is assumed to be constant and each CRU fires identical Ca$^{2+}$ sparks, then the Ca$^{2+}$ dynamics in the simplified model is only controlled by $k_f$ and $L_p$.

As the next step, we examine how these two parameters individually affect the spontaneous $[Ca]_i$ amplitude in the simplified model. When $k_f$ is increased, the number of Ca$^{2+}$ wave foci increases, thus the the amplitude of $[Ca]_i$ increases as $k_f$ is increased (Fig. 2.31, panel A). When $L_p$ is increased, Ca$^{2+}$ waves propagate further, involving more CRUs to fire, which in turn increases the amplitude of $[Ca]_i$ as well (Fig. 2.31, panel B). Of note, once the $[Ca]_i$ peak is up to threshold where NCX drives enough depolarization on the membrane voltage to activate Na$^+$ channels, TAPs can be induced if a set of ionic currents is incorporated into this simplified model. To fundamentally understand the reason of having broad widths in distributions of spontaneous $[Ca]_i$
Figure 2.30: Time trace of a typical Ca\textsuperscript{2+} spark during spontaneous Ca\textsuperscript{2+} release in the presence of isoproterenol effect with \(\alpha=3.5\) and \(\xi=0.7\) (black curve), which is fitted by Eq. 2.15 with \(A_1=1.7\ \mu\text{M}, A_0=0.15\ \mu\text{M}, \tau_1=38\ \text{ms}\) and \(\tau_2=4\ \text{ms}\).
Figure 2.31: Time traces of the whole cell average $[\text{Ca}]_i$ at different $k_f$ (A), and $L_p$ (B) for the simplified model. $k_f$ has the unit of $\mu m^{-3} \cdot ms^{-1}$, and $L_p$ has the unit of $\mu m$. When $k_f$ is varied, $L_p$ is equal to 3 $\mu m$. When $L_p$ is varied, $k_f=0.00005 \mu m^{-3} \cdot ms^{-1}$. 
peaks during TA, it is useful to study the spontaneous $[\text{Ca}]_i$ distribution in this simplified model with different $k_f$ and $L_p$. For a given $k_f$ or $L_p$ value, 1000 independent trials are performed in the simplified model. In Fig. 2.32, the spontaneous $[\text{Ca}]_i$ peak values appear to follow a Gaussian distribution for given $k_f$ and $L_p$ values. Increasing either $k_f$ or $L_p$ increases the average value of $[\text{Ca}]_i$ peak. Similar to what is seen in the detailed model, the standard deviations of these Gaussian distributions vary slightly when increasing $k_f$ or $L_p$. Of note, the widths of the distribution of spontaneous $[\text{Ca}]_i$ peaks are $\sim 0.1 \mu\text{M}$, which is similar as in the detailed model ($\sim 0.15 \mu\text{M}$). In addition, the coefficient of variation ($\sigma/\mu$) is found to decrease when increasing $k_f$ or $L_p$. Of note, increasing $k_f$ or $L_p$ increases the average value of the spontaneous $[\text{Ca}]_i$ amplitude in the detailed model (Fig. 2.31). Therefore, $\sigma/\mu$ decreases as $\mu$ is increased. This result is similar as seen in the detailed model, where the ratio decreases with increasing $\alpha$ and $1/\xi$ (Fig. 2.33), which are shown to increase the average spontaneous $[\text{Ca}]_i$ amplitude monotonically (Fig. 2.24). The data demonstrate that the simplified model can quantitatively reproduce the results obtained from the detailed Restrepo et al. model. More importantly, the simplified model reveals that the broad distributed spontaneous $[\text{Ca}]_i$ peaks are governed by the number of $\text{Ca}^{2+}$ wave foci and the $\text{Ca}^{2+}$ wave size. In fact, the amplitude of the spontaneous $[\text{Ca}]_i$ can be estimated as

$$[\text{Ca}]_i \sim N_f \cdot \Delta V \cdot \text{Ca}^*_i, \quad (2.16)$$

where $\text{Ca}^*_i$ is the average amplitude of a $\text{Ca}^{2+}$ spark in a CRU, and $\Delta V$ is the size of a $\text{Ca}^{2+}$ wave. In the simplified model, $N_f$ and $\Delta V$ are controlled by the probability of generating a $\text{Ca}^{2+}$ wave focus ($k_f$) and the critical wave propagation length ($L_p$). The $\text{Ca}^{2+}$ wave size is simply governed by $L_p$. When $L_p$ is small, spontaneous $\text{Ca}^{2+}$ releases are manifested as isolated wave-lets, whereas in the cell with large $L_p$, well propagated
Figure 2.32: Distribution of spontaneous \([\text{Ca}_i]\) peaks at different \(k_f\) (A), and \(L_p\) (B) obtained from the simplified model. \(k_f\) has the unit of \(\mu\text{m}^{-3} \cdot \text{ms}^{-1}\), and \(L_p\) has the unit of \(\mu\text{m}\). When \(k_f\) is varied, \(L_p\) is equal to 5 \(\mu\text{m}\). When \(L_p\) is varied, \(k_f=0.000015 \mu\text{m}^{-3} \cdot \text{ms}^{-1}\).
continuous Ca$^{2+}$ waves are exhibited. The number of Ca$^{2+}$ wave foci is determined by $k_f$. When $k_f$ increases, the number of Ca$^{2+}$ wave foci is increased. In fact, at the small $L_p$ limit, the number of Ca$^{2+}$ wave foci can be estimated as

\[ N_f \sim k_f \cdot \tau_p \cdot V, \quad (2.17) \]

where $V$ is the volume of the cell and $\tau_p$ is the Ca$^{2+}$ spark life time, which quantifies the time interval within which the multi-foci waves contribute to the amplitude of [Ca]$$_i$.

At the large $L_p$ limit, the number of Ca$^{2+}$ wave foci is self-limited by $L_p$, because Ca$^{2+}$ wave tips are in close contact with each other. Thus, the average size of a Ca$^{2+}$ wave at such case is

\[ \Delta V \sim \frac{1}{k_f \cdot \tau}, \quad (2.18) \]

and

\[ \tau \sim \frac{\Delta V^{\frac{1}{2}}}{v_f}. \quad (2.19) \]

By substituting Eq. 2.19 into Eq. 2.18, we obtain

\[ \Delta V \sim \frac{1}{k_f} \cdot \frac{v_f}{\Delta V^{\frac{1}{2}}} \quad (2.20) \]

\[ \Delta V \sim \left( \frac{v_f}{k_f} \right)^{\frac{3}{4}}. \quad (2.21) \]

Consequently, the number of Ca$^{2+}$ wave foci can be estimated as

\[ N_f \sim \frac{V}{\Delta V} \sim V \cdot \left( \frac{k_f}{v_f} \right)^{\frac{3}{4}}. \quad (2.22) \]
In order to check the validity of this analytical prediction of the number of Ca^{2+} wave foci at the two extreme limit of \( L_p \), the relationship between \( \log_{10}(N_f) \) and \( \log_{10} k_f \) is shown in Fig. 2.34 at different \( L_p \) values. Of note, the total number of CRU is a fixed number. Thus, when \( k_f \) increases, \( N_f \) eventually would saturate, causing the slope of \( \log_{10}(N_f) \) vs. \( \log_{10} k_f \) to decrease with increasing \( k_f \). However, when \( k_f \) is not too large (\(< \sim 0.00005 \, \mu m^{-3}ms^{-1}\)), \( N_f \) is relatively small. The linear fitting curve of the data points that have \( k_f \) values less than 0.00005 \( \mu m^{-3}ms^{-1} \) demonstrates that the above analytical prediction is valid. At \( L_p=1 \, \mu m \), the slope of such a linear fitting curve of \( \log_{10}(N_f) \) vs. \( \log_{10} k_f \) is 0.99, and is 0.74 at \( L_p=70 \, \mu m \) (large \( L_p \) limit). As seen in Eq. 2.17 and Eq. 2.22, the analytical predicted slope for the small \( L_p \) limit is equal to 1, and 0.75 for the large \( L_p \) limit. In addition, when \( L_p=3 \, \mu m \), it is noted that the slope of \( \log_{10}(N_f) \) vs. \( \log_{10} k_f \) for the data points that have \( k_f \) values less than 0.00005 \( \mu m^{-3}ms^{-1} \) is larger than 0.75 and less than 1, because this propagation length is in between the two extreme limits of the analytical prediction. However, when \( k_f \) increases, the data points for all three \( L_p \) values in Fig. 2.34 are superimposed on top of each other, which indicates that at this \( k_f \) regime, Ca^{2+} waves start to overlap each other.

So far, we have studied the bi-directional oscillation under the coupling between \( V_m \) and Ca^{2+} signals. However, in some experiments, it has been reported that Ca^{2+} signal can exhibit oscillatory behavior by itself without representing \( V_m \) oscillation [92] [100] [75]. In the next section, we will explore the underlying mechanism of Ca^{2+} oscillation without coupling to membrane voltage.
Figure 2.33: Coefficient of variation of the spontaneous [Ca]i peaks with changing $\alpha$ (A), $1/\xi$ (B) for the detailed model, and with changing $k_f$ (C) $L_p$ (D) for the simplified model. For the detailed model, the cell was stimulated at a pacing cycle length of 1 sec in the presence of isoproterenol effect, and the Na$^+$ channels and LCCs were completely blocked during spontaneous Ca$^{2+}$ release. When changing $\alpha$, $\xi$ is fixed at 0.7. When changing $\xi$, $\alpha$ is fixed at 3.5. For the simplified model, when $k_f$ is changed, $L_p=5$ µm, and when $L_p$ is varied, $k_f$ is held at 0.000015 µm$^{-3} \cdot$ ms$^{-1}$.)
Figure 2.34: Relationship between $\log_{10}(N_f)$ and $\log_{10}(k_f)$ at different $L_p$ values in the simplified model. $y_1$ is the curve fitting for the data points that have $k_f$ values less than $0.00005 \mu m^{-3} \cdot ms^{-1}$ at $L_p=1 \mu m$. $y_2$ is the curve fitting for all the data points at $L_p=1 \mu m$. $y_3$ is the curve fitting for the data points that have $k_f$ values less than $0.00005 \mu m^{-3} \cdot ms^{-1}$ at $L_p=3 \mu m$. $y_4$ is the curve fitting for all the data points at $L_p=3 \mu m$. $y_5$ is the curve fitting for the data points that have $k_f$ values less than $0.00005 \mu m^{-3} \cdot ms^{-1}$ at $L_p=70 \mu m$. $y_6$ is the curve fitting for all the data points at $L_p=70 \mu m$. 
2.9 \textbf{Ca}^{2+} \text{ oscillation without coupling to action potential}

During spontaneous \text{Ca}^{2+} \text{ release, NCX extrudes intracellular \text{Ca}^{2+} \text{ out of the cell, which tends to suppress TA, because the \text{Ca}^{2+} \text{ extrusion tends to lower intracellular \text{Ca}^{2+} \text{ level. However, during the process of \text{Ca}^{2+} \text{ extrusion, the membrane voltage is elevated as a result of NCX's stoichiometry (3 Na}^{+} \text{ entering the cell in exchange for 1 \text{Ca}^{2+} \text{ leaving). At the point where } V_m \text{ is up to the threshold for \text{Na}^{+} \text{ current to activate, the cell can exhibit a triggered AP. During the triggered AP, the intracellular \text{Ca}^{2+} \text{ is refilled by LCC opening, which maintains the condition of intracellular \text{Ca}^{2+} \text{ overload to induce TA. For instance, as seen in Fig. 2.7, intracellular \text{Ca}^{2+} \text{ level is maintained during sustained TA. Therefore, in \text{Ca}^{2+} \text{ wave mediated TA, \text{Na}^{+}-\text{Ca}^{2+} \text{ exchanger

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Distribution of the number of \text{Ca}^{2+} \text{ wave foci at different } k_f \text{ obtained from the simplified model. The unit of } k_f \text{ is } \mu \text{m}^{-3} \cdot \text{ms}^{-1}. \text{Here, } L_p=5 \mu \text{m.}}
\end{figure}
current certainly plays a key role, because it bridges spontaneous Ca\textsuperscript{2+} release to the transmembrane voltage. Continuously decreasing the strength of NCX should eliminate the electrical oscillation ultimately, but the function of extruding Ca\textsuperscript{2+} is also decreased, which may cause increased Ca\textsuperscript{2+} oscillatory activity because of the higher intracellular Ca\textsuperscript{2+} level. These Ca\textsuperscript{2+} oscillations by themselves can cause irregular cell contractions in the absence of oscillations on the membrane voltage dynamics.

In Fig. 2.36, we show an example of simulation results with 20% of normal NCX strength. The pacing protocol is the same as we used to study the termination of Ca\textsuperscript{2+}-mediated TA in Section 2.3. It shows that after the electrical stimulus stops, the intracellular Ca\textsuperscript{2+} exhibits oscillatory behavior, which is accompanied by almost undetectable electrical reflection. In addition, we see the oscillatory period increases with time until the oscillation vanishes (Fig. 2.38 A). The corresponding line scan images show that the Ca\textsuperscript{2+} oscillation becomes less synchronized with time, while the oscillation period is increasing. Of note, the Ca\textsuperscript{2+} oscillatory activity is accompanied by the net loss of intracellular Ca\textsuperscript{2+} through NCX extrusion (although slower than in control cell) during the whole oscillation.

Note that in the cell with the normal NCX, Ca\textsuperscript{2+}-mediated TA can exhibit sustained TAPs, and these Ca\textsuperscript{2+}-V\textsubscript{m} coupled oscillations show a fairly stable oscillation period, denoted as T\textsubscript{osc}, in the steady state (Fig. 2.7 and Fig. 2.38). In addition, in the cell with normal NCX, intracellular Ca\textsuperscript{2+} concentration maintains the same value from beat-to-beat (Fig. 2.38 B and C). Therefore, both the inherent desynchronization among Ca\textsuperscript{2+} oscillators and the net loss of intracellular Ca\textsuperscript{2+} can potentially contribute to the increased oscillation period in the cell with attenuated NCX.

To separate the role of these two factors in increasing Ca\textsuperscript{2+} oscillation period, we performed the following simulations. The cell was simulated under the pacing protocol we used to study the termination of Ca\textsuperscript{2+}-mediated TA in Section 2.3, except that
Figure 2.36: Example of Ca$^{2+}$ oscillation in the cell with 20% of normal NCX. From top to bottom, the experiment-like line scan recorded through the center axis in transversal (A) and longitudinal (B) direction, time courses of the whole cell averaged $[\text{Ca}_i]$ (C) and $[\text{Ca}_{\text{jsr}}]$ (D), and time trace of membrane voltage (E), $\alpha=3.3$. In the line scan images, the color represents the level of cytosolic Ca$^{2+}$ concentration. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of µM), and black color indicates lowest Ca$^{2+}$ concentration.
Figure 2.37: Ca$^{2+}$ oscillation in the closed cells. The cells were paced at a pacing cycle length of 300 ms in the presence of isoproterenol and leaky RyRs ($\alpha=3.3$). After 40 beats, the electrical stimulus was ceased, and NCX was also removed from the model simultaneously. From top to bottom, we show the Ca$^{2+}$ oscillation in 4 independent identical simulations.

NCX is blocked for all subsequent times. Under this condition, the intracellular Ca$^{2+}$ exhibits sustained oscillatory activity in a closed cell, i.e. the total intracellular Ca$^{2+}$ is conserved in such cell. In Fig. 2.37, we show examples of [Ca]$_i$ oscillation in 4 independent trials. It is noticed that the Ca$^{2+}$ oscillations sustain much longer than in the cell with 20% of normal NCX. In addition, the Ca$^{2+}$ oscillation period is observed to increase more slowly in the closed cell than in the cell with attenuated NCX (Fig. 2.38 A), which indicates that without coupling to membrane voltage, the termination of Ca$^{2+}$ oscillations does not necessarily require the net loss of intracellular Ca$^{2+}$. But the net loss of intracellular Ca$^{2+}$ can accelerate the termination of Ca$^{2+}$ oscillations. In fact, when the Ca$^{2+}$ oscillation period changes with time, both corresponding [Ca]$_i$ and [Ca]$_{jsr}$ alter with time as well (Fig. 2.38).
To better understand this behavior, the relations between the oscillation period and diastolic \([\text{Ca}_i]\) as well as diastolic \([\text{Ca}_{\text{jsr}}]\) are plotted in Fig. 2.39, respectively. In the \(T_{\text{osc}}-\text{[Ca}_i\) phase-plane, we see the trajectories corresponding to the cell with the attenuated NCX are superimposed on the one related to the closed cell. \(T_{\text{osc}}\) monotonically decreases as the diastolic \([\text{Ca}_i]\) is increased. However, in the \(T_{\text{osc}}-\text{[Ca}_{\text{jsr}}\) phase-plane, the trajectories corresponding to the cell with the attenuated NCX and the closed cell do not well superimposed as in the \(T_{\text{osc}}-\text{[Ca}_i\) phase-plane. In addition, \(T_{\text{osc}}\) does not change monotonically with \([\text{Ca}_{\text{jsr}}]\). In fact, the relation between \(T_{\text{osc}}\) and \([\text{Ca}_{\text{jsr}}]\) is not one-to-one mapping. Therefore, it appears that it is the diastolic \([\text{Ca}_i]\), rather than the diastolic \([\text{Ca}_{\text{jsr}}]\) that strongly affects the period of \(\text{Ca}^{2+}\) oscillation in the cell where \(\text{Ca}^{2+}\) cycling dynamics is not coupled to the membrane voltage.

In addition, the \(\text{Ca}^{2+}\) oscillation trajectories in the cell with the normal NCX also form a dense thin cluster in the \(T_{\text{osc}}-\text{[Ca}_i\) phase-plane, and a loose expanded cluster in the \(T_{\text{osc}}-\text{[Ca}_{\text{jsr}}\) phase-plane. These results suggest that the period of the \(\text{Ca}^{2+}-V_m\) coupled oscillation is controlled by diastolic \([\text{Ca}_i]\) more than the diastolic \([\text{Ca}_{\text{jsr}}]\). The relative importance of cytosolic and luminal \(\text{Ca}^{2+}\) is extensively studied in the next chapter in the context of CPVT linked to CSQN mutations.

Moreover, we measured the number of \(N_{\text{TAP}}\) and \(N_{\text{DAD}}\) at different NCX levels in the simulation where we first pace the cell to the steady state and stop pacing in the presence of isoproterenol effect and hyperactive RyRs \((\alpha=3.3)\) at a pacing cycling length of 0.3 sec. The level of NCX is controlled by multiplying NCX current by a dimensionless parameter \(\beta\). Increasing \(\beta\) increases NCX level. As seen in Fig. 2.40, as NCX is decreased from the control level \((\beta=1)\), \(N_{\text{TAP}}\) first increases and then decreases, but \(N_{\text{DAD}}\) monotonically increases. The reason that \(N_{\text{TAP}}\) exhibits a maximum value when varying \(\beta\) is probably the following. When NCX strength decreases, the cytosolic \(\text{Ca}^{2+}\) level is elevated. The elevation of cytosolic \([\text{Ca}_i]\) may drive more depolarization.
current via NCX despite the decreased NCX strength (small $\beta$).

2.10 Discussion

The study in this chapter 2 focused on understanding the underlying mechanisms of $\text{Ca}^{2+}$-mediated TA in the context of CPVT linked to RyR mutations. We have found $\text{Ca}^{2+}$-mediated TA is obtained under the combined effects of RyR hyperactivity and $\beta$-adrenergic stimulation. These results support the hypothesis proposed by Venetucci et al. [109] that either increasing RyR hyperactivity or promoting $\text{Ca}^{2+}$ cycling by $\beta$-adrenergic stimulation alone is not sufficient to induce spontaneous $\text{Ca}^{2+}$ waves. It appears that the diastolic $[\text{Ca}^2+]_i$ is elevated under the combined effect of leaky RyRs and isoproterenol (panel d of Fig. 2.5). The elevated diastolic $[\text{Ca}^2+]_i$ therefore promotes the $\text{Ca}^{2+}$ spark probability when the SR $\text{Ca}^{2+}$ load remains almost the same as in the cell with the same RyR hyperactivity. Our results clearly demonstrated that the elevated diastolic $[\text{Ca}^2+]_i$ played a key role in inducing $\text{Ca}^{2+}$-mediated TA. Therefore the SR $\text{Ca}^{2+}$ load exceeding a threshold can not be the sole explanation of TA.

TA occurs spontaneously in the presence of both hyperactive RyRs and isoproterenol effect, but the number of TAPs does not necessarily need to be different in the identical cells. Our simulation results demonstrated that during the initiation of $\text{Ca}^{2+}$-mediated TA, the numbers of DADs preceding a TAP in identical cells could be dramatically different. Likewise, the numbers of TAPs before the termination in identical cells also showed high variability. The data demonstrated that both the initiation and termination of $\text{Ca}^{2+}$-mediated TA were stochastic processes.

In fact, spontaneous $\text{Ca}^{2+}$ waves are caused in the condition that isoproterenol increases the intracellular $\text{Ca}^{2+}$ level up to the lowered threshold for spontaneous $\text{Ca}^{2+}$ waves by hyperactive RyRs. When the amplitude of membrane voltage driven by
Figure 2.38: Characterization of Ca\textsuperscript{2+} oscillation in the cell with normal NCX, attenuated NCX and 0 NCX (closed cell). A. Ca\textsuperscript{2+} oscillation period as a function of oscillation number. B. Diastolic [Ca\textsubscript{i}] of each oscillation vs. oscillation number. C. Relation between the diastolic [Ca\textsubscript{jsr}] of each oscillation and the oscillation number. For the cell with normal NCX, two independent trajectories are shown for $\alpha=3.0$, 3.2, 3.3 and 3.4, respectively. At $\alpha=3.3$, we show two independent oscillation trials with 0, 10%, and 20% of normal NCX.
Figure 2.39: Same trajectories as in Fig. 2.38 shown in $T_{osc}$-$[Ca]_i$ (A) and $T_{osc}$-$[Ca]_{jsr}$ phase-planes, respectively.
Figure 2.40: Relationship of $N_{\text{TAP}}$ and $N_{\text{DAD}}$ to NCX level ($\beta$). The simulations were carried out at a pacing cycling length of 0.3 sec and in the presence of isoproterenol effect and hyperactive RyRs ($\alpha=3.3$). The cells were paced until the steady state and the electrical stimuli were ceased. A DAD was counted when the amplitude of the depolarization is above -85 mV.
spontaneous Ca\textsuperscript{2+} releases through NCX is up to the threshold for Na\textsuperscript{+} currents to activate, a triggered AP is induced. However, the timing for a triggered AP to initiate appears stochastic in our results. In these simulations, cells have the same geometric structure and simulations were carried out with the same parameters. It is noticed that the stochastic property of this model is incorporated through Markov model of both RyR and LCC gating kinetics. Therefore, they are the two candidates that cause the stochastic initiation of Ca\textsuperscript{2+}-mediated TA. However, the threshold of LCC activation is $\sim$40 mV, which is higher than the threshold of Na\textsuperscript{+} channel activation ($\sim$60 mV). Thus, LCC can not be activated during DADs. Consequently, the stochastic initiation of triggered APs is only caused by the stochasticity from the Markov model of RyR gating kinetics. Depending on the number of open RyRs, the amplitude of spontaneous Ca\textsuperscript{2+} release varies. Another interesting finding is that triggered APs can exhibit a higher rhythm than the paced APs for the parameters used here (see Fig. 2.6). Thus, the triggered APs are able to continuously inhibit the paced APs, which can underly tachyarrhythmias.

The sustained oscillations represented in both Ca\textsuperscript{2+} and the membrane potential dynamics during TA imply stable limit cycles. Therefore, TA is governed by a bistable dynamics between an oscillatory state and an excitable silent state. The superimposed limit cycles in the [Ca]\textsubscript{i}-V\textsubscript{m} phase-plane suggest that sustained TA reaches the steady state after the first several TAPs. The relationship between the initiation and termination of TA and the corresponding intracellular Ca\textsuperscript{2+} level was studied. The results suggest that the TAPs in identical cells can be stochastically initiated at the same intracellular Ca\textsuperscript{2+} level, and terminated in the same manner. Therefore, the initiation and termination of Ca\textsuperscript{2+}-mediated TA can be probabilistic processes at the exactly same conditions.

The number of TAPs during TA is highly variable in identical cells. Specifically
in the termination, the diastolic intracellular Ca\(^{2+}\) level for a DAD is nearly the same as for the previous TAP (the last TAP right before the termination), indicating the escape out of the limit cycle to the silent state can be independent on the diastolic intracellular Ca\(^{2+}\) level. As shown in Fig. 2.8, the termination occurs when spontaneous Ca\(^{2+}\) releases fail to produce sufficient cytosolic Ca\(^{2+}\) that drives NCX up to the threshold for activating Na\(^{+}\) currents. Likewise, the initiation of a TAP occurs when the amplitude of spontaneous Ca\(^{2+}\) releases exceeds the threshold of Na\(^{+}\) current activation. Therefore, both initiation and termination of Ca\(^{2+}\)-mediated TA are governed by the stochastic transition between an oscillatory state and an excitable silent state. It appears that increasing the diastolic Ca\(^{2+}\) level promotes the probability of generating a TAP, whereas decreasing the diastolic Ca\(^{2+}\) level helps terminate TAPs (panel (B. b) and (B. c) of Fig. 2.9). However, when to induce or terminate the Ca\(^{2+}\)-mediated TA is independent on the diastolic Ca\(^{2+}\) level, and it occurs in a probabilistic manner, which gives rise to broad distributions of both \(N_{\text{DAD}}\) and \(N_{\text{TAP}}\).

The degree of arrhythmogenicity in the cell is steeply dependent on the RyR hyperactivity and Ca\(^{2+}\) diffusivity. This steep relationship is underlain by Gaussian distributed spontaneous [Ca]\(_i\) peaks, and the width of the Gaussian distribution changes the steepness of the dependence of Ca\(^{2+}\)-mediated TA on the RyR hyperactivity and Ca\(^{2+}\) diffusivity. The distribution of spontaneous [Ca]\(_i\) peaks during TA has much broader width (~0.15 \(\mu\)M) than that of the distribution of normal [Ca]\(_i\) transient (~0.007 \(\mu\)M). In the cell under the normal condition, during CICR, CRUs nearly simultaneously fire by the activation of LCCs. The “fire-diffuse-fire” (one CRU firing triggers neighboring CRUs to fire) process in the cell is reasonable rare during the opening of LCCs. Thus, the probability \(f(m)\) that \(m\) CRUs fire Ca\(^{2+}\) sparks in the cell
can be calculated by

\[ f(m) = \binom{M}{m} \cdot p^m f \cdot (1 - p f)^{M-m}, \]

(2.23)

where \( M \) is the total number of CRUs in the cell, and \( p_f \) is the probability that one CRU fires a \( Ca^{2+} \) spark. This is simply a binomial distribution, of which the mean number of \( Ca^{2+} \) sparks \( \mu_{sp} \) is \( M \cdot p_f \), the standard deviation \( \sigma_{sp} \) is \( \sqrt{M \cdot p_f \cdot (1 - p_f)} \), and the ratio of the standard deviation to the mean value is \( \sqrt{(1 - p_f) / (M \cdot p_f)} \). During the normal CICR, \( p_f \) is nearly equal to 1 due to LCC openings, thus \( \mu_{sp} \sim M \), and \( \sigma_{sp} \sim 0 \), which explains the observation that the distribution of the normal \([Ca]_i\) transient amplitude has a very narrow width. In the parameter regime of TA, \( Ca^{2+} \) waves are not caused by LCC openings, and \( Ca^{2+} \) wave foci are generated randomly after CRUs recovery from the refractoriness. These waves propagate to neighboring CRUs through the “fire-diffuse-fire” process. After firing, these neighboring CRUs can not fire again to generate \( Ca^{2+} \) wave foci due to the refractoriness of the SR release. Thus, the region in the cell where \( Ca^{2+} \) wave foci can be potentially generated decreases as the number of \( Ca^{2+} \) wave foci is increased, i.e. the number of \( Ca^{2+} \) wave foci is self-limited. In fact, the results from the simplified model demonstrate that the number of \( Ca^{2+} \) wave foci within our parameter regime is around \( \sim 100 \) (Fig. 2.35), which is about 2 orders of magnitude smaller than the number of CRUs that fire during the normal CICR. Ion channel stochasticity exists in both the normal and arrhythmogenic conditions, but the large number of CRUs in cells under the normal condition appears to average out the fluctuation in the amplitude of \([Ca]_i\) transient. That explains why the fluctuation in the spontaneous \([Ca]_i\) amplitude is much larger than that of the normal \([Ca]_i\) transient.

We demonstrated that \( Ca^{2+} \) cycling dynamics could oscillate without coupling to membrane voltage dynamics, but the amplitude of these oscillations decreased with
time. This is mainly caused by the desynchronization of local Ca\textsuperscript{2+} cycling dynamics. Moreover, we found that all the Ca\textsuperscript{2+} oscillations in closed cells terminated at almost the same time. This is different from what is seen in the cell with the normal NCX, where the termination of oscillations occurred randomly. In the cell with the normal NCX, Ca\textsuperscript{2+} oscillations drive sufficient membrane voltage depolarization to activate LCCs, which synchronize the Ca\textsuperscript{2+} release in each CRU through CICR. In the steady state, the synchronization by LCCs resets the phase of local Ca\textsuperscript{2+} sparks in each triggered beat, such that the next TAP is induced with almost the same phase configuration of the local Ca\textsuperscript{2+} cycling dynamics as in inducing the preceding TAP. Therefore, each Ca\textsuperscript{2+}-\textit{V}_m coupled oscillation in the steady state in these cells can be considered as independent identical events (superimposed limit cycles in the steady state). Therefore the termination of these oscillations is a stochastic process. However, in the closed cell, LCCs can not be activated during Ca\textsuperscript{2+} oscillations. Therefore, the local Ca\textsuperscript{2+} releases in CRUs lack the external force to periodically reset the phase of individual Ca\textsuperscript{2+} cycling dynamics in CRUs. Consequently, Ca\textsuperscript{2+} cycling dynamics in the CRUs gradually becomes out of phase. The cytosolic and luminal Ca\textsuperscript{2+} adjusts their concentrations and eventually the Ca\textsuperscript{2+} oscillations terminate. The time constant of the termination is determined by the inherent properties of Ca\textsuperscript{2+} cycling dynamics such as Ca\textsuperscript{2+} diffusivity, SERCA pump strength and SR Ca\textsuperscript{2+} release flux, which are the same in identical cells. Therefore, the Ca\textsuperscript{2+} oscillations in independent identical cells nearly terminate at the same time.
Chapter 3

CPVT linked to CSQN$^{R33Q}$ mutation

In the previous chapter, we investigated the underlying mechanisms of Ca$^{2+}$-mediated TA in the context of CPVT linked to RyR mutations, where the study was carried out under the general effect of RyR mutations, which is increased hyperactivity of RyR channels. In this chapter, we extend the investigation to a well-characterized pathology condition of CPVT linked to the CSQN$^{R33Q}$ mutation. Our main goal is to explore how the CSQN$^{R33Q}$ mutation affects RyR gating kinetics, causing Ca$^{2+}$-mediated TA in the presence of isoproterenol effect.

3.1 RyR Markov model

As discussed in Chapter 1, CSQN has been shown to modulate the functional activity of the RyR channel complex. At a high SR Ca$^{2+}$ concentration, CSQN molecules form dimers or even a higher degree of polymers, and RyR channels show a faster transition rate from the closed state to the open state. During a SR Ca$^{2+}$ release
event, the SR Ca\(^{2+}\) content decreases, and CSQN molecules form monomers through Ca\(^{2+}\)-dependent depolymerization. CSQN monomers binding to RyR/T/J complex causes a much slower closed-to-open transition rate of RyRs. It takes a certain time period for CSQN monomers to unbind from the RyR/T/J complex, which contributes to the refractoriness of RyR channels, preventing the cell from spontaneous SR Ca\(^{2+}\) releases.

Experiments done by Terentyev et al. [101] [103] reported that CSQN\(^{R33Q}\) altered cytosolic and intra-SR Ca\(^{2+}\) activity by mainly affecting the modulation of RyR activity. The disruption of regulatory functions of CSQN by CPVT CSQN mutation R33Q is illustrated in Fig. 3.1. The single RyR channel bilayer experiment in [101] shows that the R33Q mutation impairs the ability of CSQN to modulate the RYR activity. Therefore, these authors proposed the following hypothesis for Ca\(^{2+}\)-mediated TA linked to the CSQN\(^{R33Q}\) mutation in the context of CPVT. It is the lowered threshold for spontaneous Ca\(^{2+}\) waves caused by the impaired ability of CSQN\(^{R33Q}\) to inhibit RyR activities together with the increased SR Ca\(^{2+}\) load in the presence of isoproterenol that induce spontaneous Ca\(^{2+}\) waves, which in turn give rise to DADs and/or TAPs. In addition, experiments have also reported that CSQN mutations are usually accompanied by a reduced protein level and consequently less buffering capacity [78]. This effect is believed to elevate free SR Ca\(^{2+}\) that in turn contributes to Ca\(^{2+}\)-mediated TA. Moreover, CPVT does not develop in the heterozygous carriers of the R33Q mutation [101]. This lack of a clinical phenotype in the heterozygous carriers could be attributable to the less-profound changes in Ca\(^{2+}\) handling than in the homozygous carriers. Therefore, how the CSQN\(^{R33Q}\) ratio affects Ca\(^{2+}\) mediated TA becomes important to investigate as well.

To address these issues, we extended the original 4-state RyR Markov model of Restrepo et al. [80] to a 6-state model as shown in Fig. 3.2. In the modified RyR
Figure 3.1: Illustration of disruption of regulatory functions of CSQN by the CPVT CSQN\textsuperscript{R33Q} [103] with permission. CSQN is the primary Ca\textsuperscript{2+} buffering protein in the junctional SR (left-hand scheme). When the SR Ca\textsuperscript{2+} load is high, CSQN molecules form polymers (dimers in our simulation), which have high Ca\textsuperscript{2+} binding capacity. During the process of CICR, SR Ca\textsuperscript{2+} load decreases. CSQN molecules in turn form monomers through the Ca\textsuperscript{2+} dependent depolymerization mechanism. CSQN monomers bind to the RyR/T/J complex, regulating RyR activity in a way that RyR channels are shut off when the SR Ca\textsuperscript{2+} load reaches a certain threshold. After the deactivation, RyRs stay refractory until the SR Ca\textsuperscript{2+} load is refilled. Expression of CSQN\textsuperscript{R33Q} displaces WT CSQN from the RyR/T/J complex without interfering with polymerization and Ca\textsuperscript{2+} buffering (right). This impairs the ability of CSQN to act as a luminal Ca\textsuperscript{2+} sensor, i.e., inhibit RyR activity at low luminal Ca\textsuperscript{2+}, resulting in a lowered SR Ca\textsuperscript{2+} load and nadir, as well as shortened Ca\textsuperscript{2+} signaling refractoriness.
Figure 3.2: Extended 6-state Markov model of RyR gating kinetics. The left panel shows the original RyR kinetics in our model, and the right panel shows the altered RyR gating dynamics due to the R33Q mutation effect on RyR activity. CU and OU represent for the closed-unbound and open-unbound states. CB\textsuperscript{1} and OB\textsuperscript{1} represent for the WT closed-bound and open-bound states. CB\textsuperscript{2} and OB\textsuperscript{2} represent for the mutant type (R33Q) close-bound and open-bound states. The determination of transition rates between states is discussed in detail in the main text.

model, the state 1, 2, 3, and 4 are kept the same as in the original model, therefore the state transition rates in the loop from state 1 to 4 maintains the same as in the original RyR kinetics as well. The remaining state transition rates in the modified RyR model are determined as follows. According to the single channel bilayer experiment carried out in [103], a single RyR channel in the presence of CSQN\textsuperscript{R33Q} does not alter its activity in a way that is experimentally detectable. Thus, we assume that CSQN\textsuperscript{R33Q} completely loses the function of compromising RyR activities, i.e., the transition rate from the closed CSQN\textsuperscript{R33Q}-bound state to the open CSQN\textsuperscript{R33Q}-bound state, $k_{56}$, is equal to the transition rate from the closed CSQN-unbound state to the open CSQN-unbound state, $k_{12}$. As in the original 4-state model, the average opening time is assumed to be independent on whether RyRs are CSQN bound or unbound. Thus, the transition rate from the open CSQN\textsuperscript{R33Q}-bound state to the closed CSQN\textsuperscript{R33Q}-unbound state, $k_{65}$, is assumed to be equal to $k_{21}$ and $k_{34}$. 

112
The transition rates from CSQN-unbound states to CSQN$^{R33Q}$-bound state are determined by the CSQN$^{R33Q}$ polymerization, which is SR Ca$^{2+}$ dependent. Terentyev et al. [103] showed that CSQN$^{R33Q}$ polymerized at a relatively low Ca$^{2+}$ concentration compared to CSQN$^{WT}$ in Fig. 5C of [103]. However, Bal et al. [3] showed the opposite effect in Fig. 3 of their paper, where CSQN$^{R33Q}$ polymerized at a relatively high Ca$^{2+}$ concentration compared to CSQN$^{WT}$. The experiments in [103] were conducted in vivo, with CSQN expressed in the endoplasmic reticulum (ER) of cultured cells, while Bal et al. [3] conducted their experiment in solution. Therefore the different results of the two sets of experiments can be attributed to the different conditions of those experiments (in vivo versus in vitro) as well as to the possible alteration of polymerization kinetics by the large acceptor and donor molecules in the FRET experiments.

It should be noted that the dominant effect of the R33Q mutation is to disrupt the binding of WT CSQN to RyR/T/J thereby making RyR channels more hyperactive. Therefore, in view of this dominant effect and of the contradictory results of the above experiments regarding Ca$^{2+}$-dependent polymerization properties, we assume that in our model that CSQN$^{R33Q}$ and CSQN$^{WT}$ have identical Ca$^{2+}$-dependent polymerization kinetics. Thus, the transition from the CSQN-unbound state to the CSQN$^{R33Q}$-bound state is modeled with the rate constants

$$k_{15} = k_{26} = \tau_b^{-1}[M^{R33Q}]/B_{CSQN}^0,$$  \hspace{1cm} (3.1)

where $[M^{R33Q}]$ is the CSQN$^{R33Q}$ monomer concentration, and $B_{CSQN}^0$ is the total CSQN molecule concentration. Note that the transition rate from the CSQN-unbound state to the CSQN$^{WT}$-bound state is given by

$$k_{14} = k_{23} = \tau_b^{-1}[M^{WT}]/B_{CSQN}^0,$$  \hspace{1cm} (3.2)
where \([M^{WT}]\) is the CSQN\(^{WT}\) monomer concentration. Therefore, this model has the ability to study how the ratio of CSQN\(^{R33Q}\) to CSQN\(^{WT}\) affects the RyR gating kinetics. The CSQN\(^{R33Q}\)-unbinding rate \(k_{51}\) is assumed to be equal to the CSQN\(^{WT}\)-unbinding rate \(k_{41}\). Finally, to satisfy the detailed balance, we set the transition rate from the open CSQN\(^{R33Q}\)-bound to the open CSQN\(^{R33Q}\)-unbound to be \(k_{62} = k_{32}\). Of note, when the cell only expresses CSQN\(^{WT}\), \([M^{R33Q}]\) is equal to 0 and the 6-state Markov model reverts to the 4-state original Markov model, whereas in the cell with CSQN\(^{R33Q}\) alone, \([M^{WT}]\) is equal to 0 and the 6-state Markov model is reduced to a 2-state model with only the closed state and the open state, because CSQN\(^{R33Q}\)-bound RyRs have the same close-to-open transition rate as that of CSQN-unbound RyRs.

### 3.2 Alteration of Ca\(^{2+}\) cycling in R33Q mutant cells

In the experiment by Terentyev et al. \([101]\) \([103]\), the intracellular Ca\(^{2+}\) cycling with expression of CSQN\(^{R33Q}\) was studied in a permeabilized myocyte. Permeabilization involves treatment of cells with a mild surfactant. This treatment dissolves cell membranes, and allows larger dye molecules into the cell’s interior. High capacity Ca\(^{2+}\) buffers (EGTA) were added in the experimental solution to adjust the intracellular Ca\(^{2+}\) concentration. By decreasing the concentration of EGTA in the internal solution of the permeabilized myocyte, the Ca\(^{2+}\) cycling in the myocyte exhibited a transition from Ca\(^{2+}\) sparks to waves due to the elevation of the free Ca\(^{2+}\) level in cytosol. In some other experiments \([7]\), Ca\(^{2+}\) buffers (EGTA) are kept the same, but the free Ca\(^{2+}\) in the experimental solution is adjusted by adding a certain amount of CaCl\(_2\). Both methods induce Ca\(^{2+}\) waves by increasing the cytosolic free Ca\(^{2+}\) concentration. To study Ca\(^{2+}\)-mediated TA in R33Q mutant cells, the validity of our model to reproduce basic features of Ca\(^{2+}\) handling as seen in permeabilized myocyte experiment needs to
Figure 3.3: Example of experiment-like line scan images of cytosolic $\text{Ca}^{2+}$ in the cell with expression of CSQN$^{\text{WT}}$. These images were recorded through the center axis of the cell in the transversal direction. From top to bottom, $[\text{Ca}]_i = 0.08$, 0.1 and 0.2 µM, respectively. The key RyR gating parameters are $J_{\text{max}}=1$, $\tau_u=165$ ms and $\tau_b=5$ ms. In the line scan images, yellow color corresponds to highest $\text{Ca}^{2+}$ concentration (in unit of µM), and black color indicates lowest $\text{Ca}^{2+}$ concentration.
be verified.

As the first step, we simulated the cells under the conditions similar to the permeabilized myocyte experiment. To adjust \([Ca]_i\) to different levels, we simply fixed the cytosolic \(Ca^{2+}\) concentration in the CRUs located on the 6 boundary surfaces of the cell to be the desired value, denoted as \([Ca]_i^B\). Of note, the steady state luminal \(Ca^{2+}\) concentration is governed by the balance between the SERCA pump and the SR leak current. Thus, the luminal SR \(Ca^{2+}\) self-adjusts to the steady state level according to the given value of \([Ca]_i^B\). By increasing \([Ca]_i^B\), we observed a transition from \(Ca^{2+}\) sparks to waves in simulations of both wild type (WT) and R33Q mutant cells as shown in Fig. 3.3 and Fig. 3.4. These results are consistent with experimental observations. It is useful to mention that the average diastolic \([Ca]_i\) in the simulation of a permeabilized myocyte is lower than the fixed boundary cytosolic \(Ca^{2+}\) concentration \([Ca]_i^B\) (Fig. 3.5), because the SERCA pump in the CRUs inside the cell uptakes cytosolic \([Ca]_i\) into the SR. This behavior is observed in experiments (Fig. 6 of [100]), where the experimental solution \(Ca^{2+}\) concentration is higher than the diastolic \([Ca]_i\).

However, it is noticed that the SR \(Ca^{2+}\) content in the R33Q mutant cell maintains at almost the same level as in the WT cell at the same given \([Ca]_i^B\) for both types of cells (Fig. 3.6), which contradicts the experimental observations [103] where the SR \(Ca^{2+}\) content of the R33Q mutant cell is remarkably less than that of the WT cell. The SR \(Ca^{2+}\) content in the cell with expression of CSQN\textsuperscript{R33Q} is almost at the level of the SR \(Ca^{2+}\) nadir of the cell with expression of CSQN\textsuperscript{WT} [101] [103]. In fact, in the simulation where the cells were periodically paced with electrical stimuli, both types of cells (WT vs. R33Q) still showed almost the same SR \(Ca^{2+}\) concentrations (Fig. 3.7).

These results suggest that in this model, RyR leakiness is not altered dramatically. In a WT cell, when the pacing cycle length is long (> 300 ms), most RyRs are recovered from the CSQN-bound state to the CSQN-unbound state before the next stimulus
Figure 3.4: Example of experiment-like line scan images of cytosolic Ca\textsuperscript{2+} in the cell with expression of CSQN\textsuperscript{R33Q}. These images were recorded through the center axis of the cell in the transversal direction. From top to bottom, [Ca\textsubscript{i}]\textsuperscript{B}=0.08, 0.1 and 0.2 µM, respectively. The key RyR gating parameters are J\textsubscript{max}=1, \tau_\text{u}=165 ms and \tau_\text{b}=5 ms. In the line scan images, yellow color corresponds to highest Ca\textsuperscript{2+} concentration (in units of µM), and black color indicates lowest Ca\textsuperscript{2+} concentration.
Figure 3.5: Diastolic cytosolic $[\text{Ca}]_i$ in the simulation of permeabilized myocyte experiment. The red bar represents the fixed cytosolic $\text{Ca}^{2+}$ concentration value in the CRUs of the 6 boundary surfaces of the cell. The green bar represents the corresponding average value of the diastolic $[\text{Ca}]_i$ of the whole cell at a given boundary $[\text{Ca}]_i$ value. These simulations are carried out with the original RyR gating parameters from Restrepo et al. [80] for the WT cell.
Figure 3.6: Intracellular Ca$^{2+}$ signals in the cell with expression of CSQN$^{\text{WT}}$ (A), and CSQN$^{R33Q}$ (B), respectively. From top to bottom of each panel, it shows transversal line scan, time traces of the whole cell average $[\text{Ca}]_i$ and $[\text{Ca}]_{\text{JSR}}$. To simulate permeabilized myocyte experiment, the cell was simulated with fixed boundary condition. Here, $[\text{Ca}]_i^B$ is 0.2 µM. The cell exhibits periodic Ca$^{2+}$ waves. The key RyR gating parameters are $J_{\text{max}}=1$, $\tau_u=165$ ms and $\tau_b=5$ ms. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of µM), and black color indicates lowest Ca$^{2+}$ concentration.
Figure 3.7: Characteristics of intracellular Ca$^{2+}$ signals and membrane voltage with the normal no flux boundary condition in the WT cell (A) and R33Q mutant cell (B), respectively. The cell was paced at a pacing cycle length of 1 sec. The RyR gating parameters are from Restrepo et al. [80]. From top to bottom of each panel, it shows transversal line scan of the ratio of open RyRs to total RyRs, time traces of the whole cell average ratio of open RyR, denoted as $P_o$, $[\text{Ca}]_i$, $[\text{Ca}]_{jsr}$ and $V_m$.

because of the relatively short unbinding time constant ($\sim 125$ ms in the model). As we see in Fig. 3.7, the ratio of open RyRs to the total RyRs in CRUs is $\sim 90\%$ during CICR in the WT cell, thus the room to increase RyR hyperactivity is limited to $\sim 10\%$. Therefore, the model with the original parameters of RyR gating kinetics cannot reproduce the experimental observation that SR Ca$^{2+}$ content is dramatically decreased in the cell with the expression of CSQN$^{R33Q}$. 

120
3.3 RyR gating parameters

Recently, Sato et al. [87] explored another set of RyR gating parameters of Restrepo et al. [80] model based on new experimental data. The major modifications consist of i) an increased RyR flux strength, which is $\sim 18$ times as large as in the original model; ii) a larger CSQN unbinding time constant, which is $\sim 7$ times longer than in the original model; and iii) a $\sim 10$ times faster CSQN binding rate. The details of parameter modifications are listed in Appendix. These changes limit the number of RyR openings during a Ca$^{2+}$ spark to $\sim 15$ (vs. $\sim 80$-90 in the original Restrepo et al. model) in control cells, but the whole cell [Ca]i transient is kept almost the same as in the original model due to the larger single RyR flux. In fact, the feature that only a few RyRs can open during CICR in the WT cell model provides us with a much greater range to increase RyR hyperactivity in R33Q mutant cells. Of note, CSQN$^{R33Q}$-bound RyRs are assumed to have the same leakiness as those CSQN-unbound RyRs. Thus, RyRs in R33Q mutant cells can effectively all open during CICR, causing a dramatic decrease in the SR Ca$^{2+}$ content. In Fig. 3.8, it is observed that when the R33Q mutant cell is stimulated with periodic electrical stimuli, both SR Ca$^{2+}$ content and [Ca]i transient are dramatically decreased, compared to the WT cell.

In the simulation of a permeabilized myocyte, in addition to the observation of the dramatically lowered SR Ca$^{2+}$ content ($\sim 180$ µM), the cytosolic Ca$^{2+}$ line scan images for R33Q mutant cells show clustered randomly occurring broken wave-lets (Fig. 3.9), whereas the experimental line scan images for a R33Q mutant cell show well propagated periodic Ca$^{2+}$ waves. These results demonstrate that the simulated R33Q mutant cell cannot terminate SR Ca$^{2+}$ release normally, while in a real R33Q mutant cell SR Ca$^{2+}$ release can still be terminated regularly, although the wave frequency is higher than in the WT cell. These different wave morphologies exhibited in the line
Figure 3.8: Characteristics of intracellular Ca\(^{2+}\) signals and membrane voltage with the normal no flux boundary condition in the WT cell (A) and R33Q mutant cell (B), respectively. The cell was paced at a pacing cycle length of 1 sec. The key RyR gating parameters are \(J_{\text{max}}=18\), \(\tau_u=1250\) ms and \(\tau_b=0.5\) ms. From top to bottom of each panel, we show transversal line scan of the ratio of open RyRs to total RyRs, time traces of the whole cell average ratio of open RyR, denoted as \(P_o\), \([Ca_i]\), \([Ca_{jsr}]\) and \(V_m\).
Figure 3.9: Intracellular Ca\(^{2+}\) signals in the cell with the fixed boundary condition and in the WT cell (A) and R33Q mutant cell (B), respectively. The key RyR gating parameters are \(J_{\text{max}}=18\), \(\tau_u=1250\) ms and \(\tau_b=0.5\) ms. From top to bottom of each panel, we show transversal line scan, time traces of the whole cell average \([\text{Ca}]_i\) and \([\text{Ca}]_{\text{jsr}}\). Here, \([\text{Ca}]_i^B\) is 0.2 \(\mu\text{M}\). In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of \(\mu\text{M}\)), and black color indicates lowest Ca\(^{2+}\) concentration.
scan images between simulation and experiment suggest that in real myocytes, CSQN binding to the RyR/T/J complex may not be the only mechanism to terminate SR Ca\(^{2+}\) release. Physiologically, cells may have extra mechanisms to regulate the termination of SR Ca\(^{2+}\) release, which are covered by the CSQN-mediated mechanism in normal conditions. For instance, in the experiment by [49], CSQN knock-out cells showed normal SR Ca\(^{2+}\) release at a low SR Ca\(^{2+}\) load. In addition, these cells still showed a steep nonlinear SR load-SR release relationship. Moreover, purified RyRs are reported to be still sensitive to the luminal Ca\(^{2+}\) concentration [120] [53]. These experimental observations indicate the existence of an extra SR release “brake” other than the CSQN-mediated RyR regulation. In the next section, we discuss how we incorporate this extra luminal RyR regulation into our model and its effect on intracellular Ca\(^{2+}\) cycling.

### 3.4 CSQN-independent luminal “brake” for SR Ca\(^{2+}\) release

To incorporate an extra luminal RyR regulation, we assume that the RyR channels have luminal Ca\(^{2+}\) binding sites and that binding and unbinding to and from those sites occurs sufficiently rapidly for binding and unbinding to be in steady-state equilibrium. We further assume that Ca\(^{2+}\) binding is cooperative and that RyR channels closed to open transition rates are increased when Ca\(^{2+}\) is bound to the luminal sites. Thus, the modified transition rates take the following forms

\[
k'_{12} = k_{12} \cdot f ([Ca]_{jsr}) = K_u \cdot ([Ca]_p)^2 \cdot f([Ca]_{jsr}) \quad (3.3)
\]
\[
k'_{43} = k_{43} \cdot f ([Ca]_{jsr}) = K_b \cdot ([Ca]_p)^2 \cdot f([Ca]_{jsr}) \quad (3.4)
\]
\[
k'_{56} = k_{56} \cdot f ([Ca]_{jsr}) = K_u \cdot ([Ca]_p)^2 \cdot f([Ca]_{jsr}), \quad (3.5)
\]
where $K_u=3.8\times10^{-4}\mu M^{-2}ms^{-1}$, $K_b=10^{-6}\mu M^{-2}ms^{-1}$, and $[\text{Ca}]_p$ is the Ca$^{2+}$ concentration in the proximal space. The formula of $f([\text{Ca}]_{jsr})$ is

$$f([\text{Ca}]_{jsr}) = \frac{1}{1 + ([\text{Ca}]^*_{jsr}/[\text{Ca}]_{jsr})^H},$$

(3.6)

where $[\text{Ca}]^*_{jsr}=0.5$ mM, and the Hill coefficient $H=4$. These parameters are chosen to make $f([\text{Ca}]_{jsr})$ steeply dependent on $[\text{Ca}]_{jsr}$ (Fig. 3.10), such that the incorporation of $f([\text{Ca}]_{jsr})$ provides SR Ca$^{2+}$ release in R33Q mutant cells with an extra “brake”, but the RyR regulation in WT cells is almost unaffected. Because $[\text{Ca}]^*_{jsr}$ is chosen comparable to the concentration threshold for CSQN dimer formation ($\sim$400-500 $\mu$M) in the Restrepo et al. model.

In WT cells, when $[\text{Ca}]_{jsr}$ is high, $f([\text{Ca}]_{jsr})$ is close to 1, thus $k'_{12}$ has a value close to $k_{12}$. During CICR, $[\text{Ca}]_{jsr}$ decreases and CSQN molecules form monomers, which bind to the RyR/T/J complex, inhibiting RyRs from opening. Because of the steep relationship between $f([\text{Ca}]_{jsr})$ and $[\text{Ca}]_{jsr}$, $f([\text{Ca}]_{jsr})$ sharply decreases at a low SR Ca$^{2+}$ concentration, which makes the inhibition of the RyR activity even more effective. Therefore, this extra luminal RyR regulation does not make much change on the Ca$^{2+}$ cycling in WT cells as shown in panel A of Fig. 3.11. Namely, the time traces of $[\text{Ca}]_i$ and $[\text{Ca}]_{jsr}$ as well as $P_o$ in the WT cell maintain similar as in the WT cell without the extra “brake” for SR Ca$^{2+}$ release (Fig. 3.8).
Figure 3.10: Extra luminal RyR regulation $f([\text{Ca}]_{\text{jsr}})$ as a function of $[\text{Ca}]_{\text{jsr}}$. $f([\text{Ca}]_{\text{jsr}}) = \frac{1}{1 + ([\text{Ca}]_{\text{jsr}}^*/[\text{Ca}]_{\text{jsr}})^H}$, where $[\text{Ca}]_{\text{jsr}}^* = 0.5$ mM and $H = 4$. 

126
Figure 3.11: Characteristics of intracellular Ca\(^{2+}\) signals and membrane voltage with the normal no flux boundary condition and the extra luminal “brake” of SR release in a WT cell (A) and a R33Q mutant cell (B), respectively. The cell was paced at a pacing cycle length of 1 sec. The key RyR gating parameters are \(J_{\text{max}}=18\), \(\tau_u=1250\) ms and \(\tau_b=0.5\) ms. From top to bottom of each panel, we show transversal line scan of the ratio of open RyRs to total RyRs, time traces of the whole cell average ratio of open RyR, denoted as \(P_o\) \([\text{Ca}]_i\), \([\text{Ca}]_{\text{jsr}}\) and \(V_m\).
Figure 3.12: Intracellular Ca\(^{2+}\) signals in the cell with the fixed boundary condition and the extra luminal “brake” of SR release in the WT cell (A) and R33Q mutant cell (B), respectively. The RyR gating parameters are taken from Sato et al. [87]. The key RyR gating parameters are \(J_{\text{max}}=18\), \(\tau_u=1250\) ms and \(\tau_b=0.5\) ms. From top to bottom of each panel, we show transversal line scan, time traces of the whole cell average \([\text{Ca}]_i\) and \([\text{Ca}]_{\text{jsr}}\). Here, \([\text{Ca}]_B\) is 0.2 \(\mu\)M. In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of \(\mu\)M), and black color indicates lowest Ca\(^{2+}\) concentration.
In R33Q mutant cells, the RyR leakiness increases in the model by making $k_{12} = k_{34}$, but the extra luminal RyR regulation provides RyR channels with the ability to sense the SR $\text{Ca}^{2+}$ concentration, such that the SR $\text{Ca}^{2+}$ load still decreases but not as much as in the model without this extra luminal RyR regulation. In fact, the SR $\text{Ca}^{2+}$ load of the R33Q mutant cell is almost at the level of the SR nadir of the WT cell in the simulation (Panel B of Fig. 3.11), which is consistent with experimental observations. More importantly, the extra RyR regulation prevents spontaneous $\text{Ca}^{2+}$ releases when the SR $\text{Ca}^{2+}$ load is too low in the R33Q mutant cell. The incorporation of the extra luminal RyR regulation gives rise to well propagated periodic $\text{Ca}^{2+}$ waves in the simulation of permeabilized myocytes (Fig. 3.12), which is consistent with experimental observation as well.

In summary, the $\text{Ca}^{2+}$ cycling in R33Q mutant cells was investigated by three different models, consisting of:

I . The 6-state Markov model extended from the original Restrepo et al. [80] model as discussed in Section 3.1.

II . The same 6-state Markov model in I with a 18 times larger single RyR flux strength, a $\sim$7 times longer RyR unbinding timescale, and a 10 times shorter RyR binding timescale. These modifications of RyR gating parameters are referred from Sato et al. [87].

III . The model in II with an extra luminal RyR regulation described in Section 3.4. Here, for simplicity, we denote them as Model I, Model II and Model III in the following part of this chapter.

It is demonstrated that both Model I and Model III are able to reproduce the similar $\text{Ca}^{2+}$ wave morphology as seen in experiments that $\text{Ca}^{2+}$ waves in both WT and R33Q mutant cells propagate continuously (Fig. 3.13). However, Model I fails to show a
dramatically decreased SR Ca\textsuperscript{2+} load in R33Q mutant cells. Model III has shown the ability to reproduce important, established experimental observations for R33Q mutant cells, but the validity of this model to simulate Ca\textsuperscript{2+} cycling in WT cells still needs to be tested. As the next step, the characteristics of Ca\textsuperscript{2+} cycling in both WT and R33Q mutant cells are further studied in the simulation of permeabilized myocytes with all three models. These results are compared with experimental observations to validate the ability of Model III in capturing the basic features of Ca\textsuperscript{2+} handling in both types of cells.

![Figure 3.13: Ca\textsuperscript{2+} wave propagation length as a function of [Ca\textsubscript{i}]\textsuperscript{B} in the permeabilized cell simulation. Here, Ca\textsuperscript{2+} wave size is obtained by averaging the propagation length of the continuous Ca\textsuperscript{2+} waves in the unit of number of CRUs. The simulations are carried out with all three models in both types of cells: Model I for WT cell (red), Model I for R33Q mutant cell (green), Model II for WT cell (blue), Model II for R33Q mutant cell (purple), Model III for WT cell (cyan), Model III for R33Q mutant cell (yellow).]
Figure 3.14: $\text{Ca}^{2+}$ spark frequency vs. $[\text{Ca}]_i^B$ in the permeabilized myocyte simulation. The simulations were done in the cell with fixed boundary $\text{Ca}^{2+}$ to mimic permeabilized myocyte experiments. Both WT and R33Q mutant cells were simulated by three different models (color bar is in the same order as in Fig. 3.13) for 1000 sec. The simulations are carried out with all three models in both types of cells: Model I for WT cell (red), Model I for R33Q mutant cell (green), Model II for WT cell (blue), Model II for R33Q mutant cell (purple), Model III for WT cell (cyan), Model III for R33Q mutant cell (yellow).
Figure 3.15: Experiment-like line scan images in the permeabilized cell simulation for the model with an extra luminal SR “brake”. The key parameters are \( J_{\text{max}} = 18 \), \( \tau_u = 1250 \text{ ms} \) and \( \tau_b = 0.5 \text{ ms} \). From the top to bottom, \( [\text{Ca}]_i = 0.06 \), 0.1, and 0.3 \( \mu \text{M} \), respectively. In the line scan images, yellow color corresponds to highest \( \text{Ca}^{2+} \) concentration (in unit of \( \mu \text{M} \)), and black color indicates lowest \( \text{Ca}^{2+} \) concentration.
Figure 3.16: Ca$^{2+}$ wave frequency as a function of $[\text{Ca}]_i^B$ in the permeabilized myocyte simulation. The simulations are carried out with all three models in both types of cells (color bar is in the same order as in Fig. 3.13). The simulations are carried out with all three models in both types of cells, except for R33Q cells with Model II. Because, in that case, spontaneous Ca$^{2+}$ releases are manifested as clustered broken wave-lets, and it is difficult to define the wave frequency. For all the other cases, Model I for WT cell (red), Model I for R33Q mutant cell (green), Model II for WT cell (blue), Model II for R33Q mutant cell (purple), Model III for WT cell (cyan), Model III for R33Q mutant cell (yellow).
The Ca\textsuperscript{2+} spark frequencies obtained from all three models in the simulation of permeabilized myocytes are shown in Fig. 3.14. It appears that the Ca\textsuperscript{2+} spark frequency increases when [Ca\textsuperscript{B}] is increased in any of the three models. In addition, the simulation results by Model III for the WT cell show great agreement with the experimental data. In fact, our results (Fig. 3.15) show a transition of intracellular Ca\textsuperscript{2+} dynamics from Ca\textsuperscript{2+} sparks to waves when the cytosolic Ca\textsuperscript{2+} level is increased within the similar range as in the experiment [7]. In Fig. 3.16, the Ca\textsuperscript{2+} wave frequencies are summarized from the simulations of both WT and R33Q mutant cells. Mode III shows \(~2\sim3\) times larger wave frequency in the R33Q mutant cell than in the WT cell, which is consistent with experimental results (Fig. 2 in [103]). In addition, the Ca\textsuperscript{2+} wave amplitude (Fig. 3.17) in the R33Q mutant cell is found to be \(~50\%\) of that in the WT cell, which is similar to the experimental observation (Fig. 2 in [103]). These results suggest that Model III is able to reproduce experimentally well characterized properties of Ca\textsuperscript{2+} cycling in both WT and R33Q mutant cells. Thus, this model can be further used to investigate the underlying mechanisms of Ca\textsuperscript{2+}-mediated TA in the context of CPVT linked to the R33Q mutation. It is also important to emphasize that simulations of the WT cell with Sato et al. RyR gating parameters show stochasticity in the initiation and termination of Ca\textsuperscript{2+}-mediated TA in the presence of hyperactive RyRs and the isoproterenol effect, which is consistent with what we have observed in Chapter 2. In Fig. 3.18. We show examples of Ca\textsuperscript{2+}-mediated TA terminating at different time in two independent identical trials. The number of TAPs follows a geometric distribution (Fig. 3.19). These results indicate that stochasticity in initiation and termination of Ca\textsuperscript{2+}-mediated TA is an inherent property of ventricular myocytes. These results also provide us with another evidence for the model validation.
Figure 3.17: The amplitude of spontaneous $\text{Ca}^{2+}$ release vs. $[\text{Ca}]_i$ in the permeabilized myocyte simulation. Both WT and R33Q mutant cells were simulated by three different models (see text) under experiment-like permeabilized myocyte conditions. The simulations are carried out with all three models in both types of cells, except for R33Q cells with Model II. Because, in that case, spontaneous $\text{Ca}^{2+}$ releases are manifested as clustered broken wave-lets, and it shows no obvious spontaneous $[\text{Ca}]_i$. For all the other cases, Model I for WT cell (red), Model I for R33Q mutant cell (green), Model II for WT cell (blue), Model II for R33Q mutant cell (purple), Model III for WT cell (cyan), Model III for R33Q mutant cell (yellow).
Figure 3.18: Termination of Ca\textsuperscript{2+} wave mediated triggered activity with Sato et al. RyR gating parameters. For trial 1, from top to bottom, the experiment-like line scan recorded through the center axis in transversal direction, time courses of the whole cell averaged [Ca]\textsubscript{i} and [Ca]\textsubscript{jsr}, and time trace of membrane voltage. Only the time trace of membrane voltage is shown for trial 2. The pacing cycle length is 500 ms, and α=3.5. The solid black circles mark the electrical stimuli. In the line scan images, yellow color corresponds to highest Ca\textsuperscript{2+} concentration (in unit of µM), and black color indicates lowest Ca\textsuperscript{2+} concentration.
Figure 3.19: Distribution of the number of TAPs in the presence of isoproterenol effect and hyperactivity RyRs (A) for 500 independent trials conducted by the same simulation as in Fig. 3.18. Linear fitting of the natural logarithm of the distribution frequency (B) demonstrate a geometric distribution for the number of TAP in the steady state. Here, $\alpha=3.5$. 
3.5 Spontaneous Ca\(^{2+}\) release at a low SR Ca\(^{2+}\) content in R33Q mutant cells

Experiments have reported that under the administration of \(\beta\)-adrenergic stimulation, WT cells show no TA [101], or an isolated DAD [82] or TAP [97], whereas R33Q mutant cells exhibit severe Ca\(^{2+}\)-mediated TA [81] [101], which is presumably attributable to the compromised ability of CSQN\(^{R33Q}\) in inhibiting RyR openings. However, the increased RyR leakiness also decreases the SR Ca\(^{2+}\) load as seen in experiments [101] [103], and in our simulations (Fig. 3.12 3.15). Intuitively, lowering SR load should decrease the probability of generating Ca\(^{2+}\) waves. The question then arises as to how does spontaneous Ca\(^{2+}\) waves occur at such a low SR load?

As the first step to answer this question, both WT and R33Q mutant cells are simulated by Model III. The cells were paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect for 60 beats, followed by a sudden cessation of electrical stimuli. Consistent with the experiment, the WT cell exhibits one TAP, but the R33Q mutant cell shows sustained TAPs (Fig. 3.20). In addition, the spontaneous Ca\(^{2+}\) release in the R33Q mutant cell occurs at a very low SR Ca\(^{2+}\) content (at the level of a normal SR nadir for a WT cell).

However, when the R33Q mutant cell was simulated by Model I in the presence of isoproterenol effect, the spontaneous Ca\(^{2+}\) release was not exhibited even at a much higher SR Ca\(^{2+}\) load (Panel A of Fig. 3.21). At another extreme, the R33Q mutant cell simulated by Model II exhibits a sequence of TAPs at the SR Ca\(^{2+}\) load of \(\sim 180\) \(\mu\)M (Panel B of Fig. 3.21). Although, Model I and II have their limits in reproducing physiologically reasonable Ca\(^{2+}\) cycling in R33Q mutant cells, these results still suggest that the high SR Ca\(^{2+}\) load is not necessary for Ca\(^{2+}\)-mediated TA. In fact, the difference between the two models in the simulation of the R33Q mutant cell is only
Figure 3.20: Ca$^{2+}$-mediated TA in WT (A) and R33Q mutant (B) cells in the presence of isoproterenol. In each panel, from top to bottom, we show experiment-like line scan images of cytosolic Ca$^{2+}$ in the transversal direction, time traces of [Ca]$_i$, [Ca]$_{jsr}$ and $V_m$. Black solid circles indicate paced electrical stimuli. Cells were paced at a pacing cycle length of 0.5 s in the presence of isoproterenol effect. A sudden cessation was followed after the 60th beat. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of $\mu$M), and black color indicates lowest Ca$^{2+}$ concentration.
the RyR flux strength $J_{\text{max}}$. Because in the R33Q mutant cell, the transition rate from the closed CSQN$^{R33Q}$-bound state to the open CSQN$^{R33Q}$-bound state is the same as the rate from the closed CSQN-unbound state to the open CSQN-unbound state, the RyR Markov model reverts to a 2-state model in the simulation of R33Q mutant cells. Therefore, the CSQN binding and unbinding time constants do not affect the RyR activity.

The SR Ca$^{2+}$ release current used in the model is the following

$$I_{\text{re}} = J_{\text{max}} \cdot \frac{n_{\text{ryr}}}{N_{\text{ryr}}}(\left[\text{Ca}\right]_{\text{jsr}} - \left[\text{Ca}\right]_{\text{p}})/v_{p}, \quad (3.7)$$

where $J_{\text{max}}$ is the RyR flux strength, $n_{\text{ryr}}$ is the number of open RyR channels, $N_{\text{ryr}}$ is the total number of RyRs (100) in each CRU, and $v_{p}$ is the volume of the proximal space. When $J_{\text{max}}$ is large, one RyR channel opening may release more Ca$^{2+}$ ions from the SR despite the expected lowered SR load. Note that in the R33Q mutant cell, all the RyRs have a fast closed-to-open rate. Thus, the R33Q cell with large $J_{\text{max}}$ may have a high probability of firing Ca$^{2+}$ sparks, which in turn increases the probability of inducing spontaneous Ca$^{2+}$ waves. However, in the WT cell, the recruited number of RyRs during CICR is remarkably limited. In fact, most RyRs are in the closed CSQN-bound state. Thus, without the Ca$^{2+}$ influx from LCCs, it is difficult for CRUs to fire Ca$^{2+}$ sparks, even with a large $J_{\text{max}}$.

To verify this hypothesis, we investigate Ca$^{2+}$ spark frequency in CRUs with different $J_{\text{max}}$ values, and link the Ca$^{2+}$ spark frequency to the number of TAPs exhibited in the whole cell simulation at the same $J_{\text{max}}$. The simulations of the R33Q mutant cell are performed at different $J_{\text{max}}$ in the presence of isoproterenol effect. The pacing protocol maintains the same as in the simulation shown in Fig. 3.21. The average diastolic $[\text{Ca}]_{i}$ and SR Ca$^{2+}$ content are measured when the electrical stimuli are stopped. To calcu-
Figure 3.21: Simulation of R33Q mutant cell by Model I (A) and Model II (B). From top to bottom of each panel, we show experiment-like line scan images of cytosolic Ca\(^{2+}\) in the transversal direction, time traces of \([\text{Ca}]_i\), \([\text{Ca}]_{\text{jsr}}\) and \(V_m\). Black solid circles indicate paced electrical stimuli. Cells were paced at a pacing cycle length of 0.5 s in the presence of isoproterenol effect. A sudden cessation was followed after the 60th beat. In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of µM), and black color indicates lowest Ca\(^{2+}\) concentration.
late the Ca\textsuperscript{2+} spark frequency in a CRU at a given J\textsubscript{max} value with the corresponding diastolic [Ca]\textsubscript{i} and SR Ca\textsuperscript{2+} content, we constructed a Ca\textsuperscript{2+} spark model adapted from the whole cell model. The Ca\textsuperscript{2+} diffusion between CRUs is removed from the whole cell model, thus each CRU behaves independently. Within each CRU, at a given J\textsubscript{max}, [Ca]\textsubscript{nsr} and [Ca]\textsubscript{i} are held to the SR Ca\textsuperscript{2+} content and diastolic [Ca]\textsubscript{i} measured from the whole cell model, respectively. This adapted model can then simultaneously simulate Ca\textsuperscript{2+} sparks at different J\textsubscript{max} values in a large number of independent CRUs.

The interval between two consecutive Ca\textsuperscript{2+} sparks in the same CRU is usually named as an inter-spark interval, denoted as T\textsubscript{sk}. Theoretically, the distribution of inter-spark intervals in the CRU model with fixed [Ca]\textsubscript{i} and [Ca]\textsubscript{nsr} is proved to follow an exponential distribution \cite{35}. In Fig. 3.22, we show the distribution of inter-spark intervals in the R33Q mutant cell simulation with J\textsubscript{max}=1 as an example, which follows an exponential distribution. The slope of ln F vs. ln T\textsubscript{sk} represents the average Ca\textsuperscript{2+} spark frequency at a given J\textsubscript{max} value.

In Fig. 3.23, the SR Ca\textsuperscript{2+} content, the diastolic [Ca]\textsubscript{i}, and the number of Ca\textsuperscript{2+} oscillations, denoted as N\textsubscript{wave}, obtained from the whole cell simulation, as well as the Ca\textsuperscript{2+} spark frequency obtained from the adapted Ca\textsuperscript{2+} spark model are plotted together against the change of J\textsubscript{max}. It appears that N\textsubscript{wave}, the SR Ca\textsuperscript{2+} load and the Ca\textsuperscript{2+} spark frequency all increase when J\textsubscript{max} is increased. The relationship between J\textsubscript{max} and the diastolic [Ca]\textsubscript{i} is not monotonic. The diastolic [Ca]\textsubscript{i} first slightly decreases (J\textsubscript{max}<3) and then increases (J\textsubscript{max}>3) as J\textsubscript{max} is increased. It is because at J\textsubscript{max}=3, the cell is still in the Ca\textsuperscript{2+} spark regime, which is verified by the fact that N\textsubscript{wave}=0, and with the Ca\textsuperscript{2+} spark frequency being higher than in the cell with J\textsubscript{max}=1, the intracellular Ca\textsuperscript{2+} level is further decreased via Ca\textsuperscript{2+} extrusion by NCX. However, when J\textsubscript{max} is further increased, the cell approaches the Ca\textsuperscript{2+} wave regime (N\textsubscript{wave}>0), and the reopening of LCCs during TAPs brings in more Ca\textsuperscript{2+} ions, which contribute to the elevation of
Figure 3.22: Example of Ca$^{2+}$ spark interval distribution in the R33Q mutant cell simulation. Panel A. Ca$^{2+}$ spark interval distribution with J$_{\text{max}}=1$. Panel B. The relationship between the natural logarithm of the distribution frequency, denoted as ln F, and the natural logarithm of the inter-spark interval, denoted as ln T$_{sk}$. The slope of the linear fitting curve is equal to -0.000025 ms$^{-1}$. The inverse of the slope gives rise to the averaged inter-spark interval, which is 40 s. The red curve represents the simulation results, and the black curve is the linear fitting curve of the red one. Note the fitting is processed by omitting the last 4 points. Because these points represent long inter-spark intervals, which are rare events in finite-time simulations. Here, [Ca]$_{\text{nsr}}=1.0$ mM, and [Ca]$_{i}=0.15$ µM.
diastolic \([\text{Ca}]_i\). In fact, the elevation of diastolic \([\text{Ca}]_i\) also increases the probability of spontaneous \(\text{Ca}^{2+}\) waves through CICR. These results suggest that increasing \(J_{\text{max}}\) increases the \(\text{Ca}^{2+}\) spark frequency in the simulation of R33Q mutant cell, and it is the increase in the \(\text{Ca}^{2+}\) spark frequency at large \(J_{\text{max}}\) that underlies the enhanced arrhythmogenicity in the cell. In addition, these results suggest that cytosolic \(\text{Ca}^{2+}\) plays a more important role than the SR \(\text{Ca}^{2+}\) load in increasing the degree of \(\text{Ca}^{2+}\)-mediated TA in R33Q mutant cells, where the SR \(\text{Ca}^{2+}\) content is much lower than in WT cells.

The comparison of the \(\text{Ca}^{2+}\) spark frequencies between WT and R33Q mutant cells is shown in Fig. 3.24. It is noticed that at a small \(J_{\text{max}}\) value, both WT and R33Q mutant cells exhibit similar low \(\text{Ca}^{2+}\) spark frequencies (high spark separation time as seen in the plot), whereas at a large \(J_{\text{max}}\) value, both types of cells show increased spark frequencies, but the spark frequency in the R33Q mutant cell is much higher than in the WT cell. These results explain that it is difficult to induce \(\text{Ca}^{2+}\) waves in the simulation of R33Q mutant cells with the original Restrepo et al. RyR gating parameters. Because the small \(J_{\text{max}}\) gives rise to the low \(\text{Ca}^{2+}\) spark frequency. In addition, in the simulation of WT cells with a large \(J_{\text{max}}\) value and a long RyR refractory period, an isolated TAP can be exhibited (Fig. 3.20). However, no TA is observed in the simulation of WT cells with a small \(J_{\text{max}}\) value and a short RyR refractory period. This is because the large \(J_{\text{max}}\) is required to increase the \(\text{Ca}^{2+}\) spark frequency up to the threshold for inducing \(\text{Ca}^{2+}\) waves. It is useful to emphasize that in some experiments, the WT myocyte under the administration of \(\beta\)-adrenergic stimulation does not show TA [101], but in other experiment, WT cells can exhibit an isolated DAD or TAP [82] [97]. Our simulation results suggest that the variation of the RyR flux strength may contribute to the different experimental observations.
Figure 3.23: Change in diastolic [Ca]$_i$, SR Ca$^{2+}$ content, number of spontaneous Ca$^{2+}$ oscillations and Ca$^{2+}$ spark frequency at different $J_{\text{max}}$. The average number of spontaneous Ca$^{2+}$ oscillations is obtained from 50 independent trials (right Y-axis). The average diastolic [Ca]$_i$ during Ca$^{2+}$ waves is plotted along the bottom X-axis. The corresponding [Ca]$_{\text{nfr}}$ is plotted along the left Y-axis. At each $J_{\text{max}}$, diastolic [Ca]$_{\text{nfr}}$ and [Ca]$_i$ are used to calculate Ca$^{2+}$ spark frequency (right Y-axis) in the single CRU model. The number labelled on the top X-axis is the $J_{\text{max}}$ values varied in the simulation. Note that $N_{\text{wave}}$ values indicate whether the cell is in the spark regime or in the wave regime. When $N_{\text{wave}}$ is equal to 0, it clearly shows that the cell is in the spark regime, whereas when $N_{\text{wave}}$ is larger than 0, the cell reaches the spontaneous Ca$^{2+}$ regime.
Figure 3.24: Comparison of Ca$^{2+}$ spark frequency in WT and R33Q mutant cells. These results are obtained from the adapted CRU model for both types of cell, with $J_{\text{max}}=1$ and 18, respectively.
3.6 \( \text{Ca}^{2+} \)-mediated TA in heterozygous R33Q mutant cells

Experiments have reported that heterozygous carriers of the R33Q mutation do not exhibit CPVT [101]. These authors have proposed that the lack of clinical phenotype in the heterozygous carriers could be attributed to the lower ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) in these human subjects (~1:1). Here, we test this hypothesis by stimulating cells with different CSQN\(^{R33Q} \) to CSQN\(^{WT} \) ratios in the presence of isoproterenol effect. Of note, the total CSQN concentration remains a constant when the ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) is varied. Our simulation does not take into account the compensatory effect that the CSQN protein level may be reduced with expression of the R33Q mutation. Therefore, we examine whether the ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) alone is attributable to \( \text{Ca}^{2+} \)-mediated TA in the heterozygous R33Q mutation carriers. We find that \( \text{Ca}^{2+} \)-mediated TA is more severe, in terms of more \( \text{Ca}^{2+} \) oscillations, when the ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) is increased (Fig. 3.25-3.28) in the presence of isoproterenol effect.

In addition, it is noticed that when the ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) increases, the SR \( \text{Ca}^{2+} \) content during \( \text{Ca}^{2+} \) oscillations is decreased but the corresponding diastolic [Ca]\(_i \) is increased (Fig. 3.29), which shows the similar behavior as we have seen in the above study of varying \( J_{max} \). Following the same method used there, the \( \text{Ca}^{2+} \) spark frequency is calculated at the corresponding [Ca]\(_i \) and [Ca]\(_{nsr} \) measured from the whole cell model simulation for a given CSQN\(^{R33Q} \) to CSQN\(^{WT} \) ratio. It is noticed that when the ratio of CSQN\(^{R33Q} \) to CSQN\(^{WT} \) is increased from 0 to 0.2, the \( \text{Ca}^{2+} \) spark frequency decreases, which is accompanied by a decrease in both the diastolic [Ca]\(_i \) and the SR \( \text{Ca}^{2+} \) content (Fig. 3.30). These results suggest that a threshold increase in RyR leakiness by increasing the CSQN\(^{R33Q} \) to CSQN\(^{WT} \) ratio is required to increase the
Ca\textsuperscript{2+} spark frequency and induce Ca\textsuperscript{2+} waves. If the increase in RyR leakiness is not up to the threshold, the intracellular Ca\textsuperscript{2+} level decreases (via NCX extrusion) with the enhanced SR leak through a few RyR openings, which may cause a lowered Ca\textsuperscript{2+} spark frequency. Once the RyR leakiness further increases (here by increasing the ratio of CSQN\textsuperscript{R33Q} to CSQN\textsuperscript{WT}) above the threshold, the Ca\textsuperscript{2+} spark frequency is increased, which is presumably because the SR leak by a few RyR opening is enough to trigger Ca\textsuperscript{2+} sparks. The increase in Ca\textsuperscript{2+} spark frequency in turn contributes to increasing the probability of inducing spontaneous Ca\textsuperscript{2+} waves. These results demonstrate that a critical ratio of CSQN\textsuperscript{R33Q} to CSQN\textsuperscript{WT} is required to induce Ca\textsuperscript{2+}-mediated TA in heterozygous carriers. In addition, this study shows that the Ca\textsuperscript{2+} spark frequency is not necessarily increased when the RyR activity is enhanced.
Figure 3.25: Whole cell Ca\textsuperscript{2+} signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.2. From top to bottom, we show transversal line scan image of cytosolic Ca\textsuperscript{2+}, time traces of the whole cell average [Ca]\textsubscript{i}, [Ca]\textsubscript{jsr} and the recording of membrane voltage V\textsubscript{m}. The cell was paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect. The electrical stimulus were stopped after the cell reaches the steady state. In the line scan images, yellow color corresponds to highest Ca\textsuperscript{2+} concentration (in unit of \(\mu\)M), and black color indicates lowest Ca\textsuperscript{2+} concentration. In the line scan images, yellow color corresponds to highest Ca\textsuperscript{2+} concentration (in unit of \(\mu\)M), and black color indicates lowest Ca\textsuperscript{2+} concentration.
Figure 3.26: Whole cell Ca$^{2+}$ signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.4. From top to bottom, we show transversal line scan image of cytosolic Ca$^{2+}$, time traces of the whole cell average [Ca]$^i$, [Ca]$^j_{sr}$ and the recording of membrane voltage $V_m$. The cell was paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect. The electrical stimulus were stopped after the cell reaches the steady state. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of $\mu$M), and black color indicates lowest Ca$^{2+}$ concentration.
Figure 3.27: Whole cell Ca\(^{2+}\) signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.6. From top to bottom, we show transversal line scan image of cytosolic Ca\(^{2+}\), time traces of the whole cell average [Ca]\(_i\), [Ca]\(_{jsr}\) and the recording of membrane voltage V\(_m\). The cell was paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect. The electrical stimulus were stopped after the cell reaches the steady state. In the line scan images, yellow color corresponds to highest Ca\(^{2+}\) concentration (in unit of \(\mu\)M), and black color indicates lowest Ca\(^{2+}\) concentration.
Figure 3.28: Whole cell Ca$^{2+}$ signal and membrane voltage recording of the cell with the ratio of R33Q to WT equal to 0.8. From top to bottom, we show transversal line scan image of cytosolic Ca$^{2+}$, time traces of the whole cell average [Ca]$_i$, [Ca]$_{jsr}$ and the recording of membrane voltage $V_m$. The cell was paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect. The electrical stimulus were stopped after the cell reaches the steady state. In the line scan images, yellow color corresponds to highest Ca$^{2+}$ concentration (in unit of µM), and black color indicates lowest Ca$^{2+}$ concentration.
Figure 3.29: Intracellular Ca\(^{2+}\) level under different ratios of R33Q to WT in the presence of isoproterenol effect after electrical stimuli are stopped. The top panel represents the SR Ca\(^{2+}\) content, and the bottom one shows the diastolic cytosolic Ca\(^{2+}\) level. The cell was paced at a pacing cycle length of 0.5 sec in the presence of isoproterenol effect. The electrical stimulus were stopped after the cell reaches the steady state.
Figure 3.30: Change in diastolic \([Ca]_i\), SR Ca\(^{2+}\) content, number of spontaneous Ca\(^{2+}\) oscillations and Ca\(^{2+}\) spark frequency with varying the ratio of R33Q to WT. The average number of spontaneous Ca\(^{2+}\) oscillations is obtained from 50 independent trials (right Y-axis). The average diastolic \([Ca]_i\) during Ca\(^{2+}\) waves is plotted along the bottom X-axis. The corresponding \([Ca]_{nsr}\) is plotted along the left Y-axis. At a given CSQNR\(^{R33Q}\) ratio, diastolic \([Ca]_{nsr}\) and \([Ca]_i\) are used to calculate Ca\(^{2+}\) spark frequency (right Y-axis) in the adapted CRU model. The number labelled on the top X-axis is the CSQNR\(^{R33Q}\) ratio values varied in the simulation. Note that \(N_{wave}\) values indicate whether the cell is in the spark regime or in the wave regime. When \(N_{wave}\) is equal to 0, it clearly shows that the cell is in the spark regime, whereas when \(N_{wave}\) is larger than 0, the cell reaches the spontaneous Ca\(^{2+}\) regime.
3.7 Ca$^{2+}$-mediated TA at different R33Q expression level

Experiments have reported that most CSQN mutations are accompanied by reduced CSQN protein levels and consequently reduced SR Ca$^{2+}$ buffering capacity [78], which are believed to increase the free SR Ca$^{2+}$ content and contribute to the occurrence of Ca$^{2+}$-mediated TA. This hypothesis is tested by stimulating R33Q mutant cells with different expression levels of CSQN$^{R33Q}$. In the parameter regime of our simulation, the R33Q mutant cell already exhibits sustained TAPs at the control expression level of CSQN$^{R33Q}$. Thus, further decreasing the expression level of CSQN$^{R33Q}$ does not have obvious effect on the degree of arrhythmogenicity. However, by increasing the expression level of CSQN$^{R33Q}$, the degree of Ca$^{2+}$-mediated TA is dramatically reduced (Fig. 3.31). When the CSQN$^{R33Q}$ expression level increases, the SR free Ca$^{2+}$ concentration is decreased, and with the same RyR leakiness, the diastolic [Ca]$\text{i}$ level in turn decreases as well (Fig. 3.32). Therefore, the lowered intracellular Ca$^{2+}$ concentration in the presence of the large CSQN$^{R33Q}$ expression level is not able to induce Ca$^{2+}$-mediated TA. These results suggest that the buffering capacity of CSQN indeed plays a key role in the genesis of Ca$^{2+}$-mediated TA. Note that, increasing the CSQN$^{R33Q}$ expression level does not affect the leakiness of RyR channels, thus the reduced Ca$^{2+}$-mediated TA is purely due to the lowered intracellular Ca$^{2+}$ level.
Figure 3.31: Number of TAPs as a function of the CSQN$^{R33Q}$ expression level. Here, when $N_{TAP}$ is larger than 20, it is denoted as sustained TA. The red bars represent $N_{TAP}$ with the diffusive coupling coefficient $\xi=0.4$, whereas the green bars are for $\xi=0.6$. The strong diffusive coupling strength enhances Ca$^{2+}$-mediated TA.
Figure 3.32: Intracellular Ca\(^{2+}\) level with different R33Q expression levels in the presence of isoproterenol effect after the electrical stimuli are stopped. The top panel represents for SR Ca\(^{2+}\) content, and the bottom one shows the diastolic cytosolic Ca\(^{2+}\) level. The simulations were carried out at \(\xi=0.4\) (red) and 0.6 (green).
3.8 Discussion

In chapter 2 we have demonstrated that the RyR channel stochastic closing or opening affects the amplitude of spontaneous Ca\(^{2+}\) waves, which plays a key role in the initiation and termination of Ca\(^{2+}\)-mediated TA. In chapter 3 we have studied the effect of RyR channel kinetics and the flux strength on Ca\(^{2+}\)-mediated TA in the context of CPVT linked to the CSQN\(^{R33Q}\) mutation. Experiments [101] [103] reported that CSQN\(^{R33Q}\) alters the cytosolic and SR Ca\(^{2+}\) dynamics by mainly affecting the modulation of RyR activity. More precisely, the R33Q mutation impairs the ability of CSQN to act as a luminal Ca\(^{2+}\) sensor, i.e., inhibits RyR activity at low luminal Ca\(^{2+}\), resulting in a lowered SR Ca\(^{2+}\) load and nadir, as well as shortened Ca\(^{2+}\) signaling refractoriness. The mutated effect of R33Q increases the hyperactivity of RyRs, which is incorporated into the Restrepo et al. [80] model as described in Section 3.1.

With a small value of the single RyR flux strength (\(J_{\text{max}}=1\)), the SR load is not decreased and no TA is exhibited in the simulation of the R33Q mutant cell in the presence of isoproterenol effect (Fig. 3.6, 3.7, and 3.21). In experiments, spontaneous Ca\(^{2+}\) releases in the cell are manifested as wave generation and propagation, which is governed by the “fire-diffuse-fire” process, i.e., one CRU firing triggers neighboring CRUs to fire. Since no Ca\(^{2+}\)-mediated TA at \(J_{\text{max}}=1\) was observed, it suggests that there needs to be an increase in the single RyR flux strength in the original Restrepo et al. model. This increase will help facilitate the “fire-diffuse-fire” process in causing Ca\(^{2+}\) waves. In fact, when the single RyR flux strength is large (\(J_{\text{max}}=18\)), Ca\(^{2+}\)-mediated TA is exhibited in the paced R33Q cell under the administration of isoproterenol (Fig. 3.21). However, the spontaneous Ca\(^{2+}\) releases are manifested as clusters that randomly occur as broken wave-lets (Fig. 3.9) in the simulation of permeabilized R33Q mutant cells. The morphology of Ca\(^{2+}\) waves is different from what is
seen in experiments, where Ca\(^{2+}\) waves are generated nearly periodically and propagate across the cell continuously. The different Ca\(^{2+}\) wave morphologies in simulation and experiment suggest that in the real R33Q mutant cell, SR Ca\(^{2+}\) releases can still be terminated regularly. In fact, cells with CSQN knock-out can still terminate SR Ca\(^{2+}\) releases and have a nearly normal [Ca]\(_{i}\) transient [50]. In addition, CSQN knock-out mice still show a nonlinear SR load-SR release relationship. Moreover, purified RyRs are still sensitive to the luminal Ca\(^{2+}\) load. These experimental observations indicate that an extra SR Ca\(^{2+}\)-dependent mechanism that regulates the RyR activity may exist in R33Q mutant cells. Thus, we incorporated an extra luminal RyR regulation into the original RyR gating kinetics. Our results demonstrated that with the extra luminal RyR regulation, the R33Q mutant cell exhibits the similar Ca\(^{2+}\) wave morphology as seen in permeabilized myocyte experiments (Fig. 3.15), and Ca\(^{2+}\)-mediated TA in the paced cell simulations (Panel B of Fig. 3.20).

With the extra luminal RyR regulation, the SR Ca\(^{2+}\) load is higher in comparison to without the extra regulation. Intuitively, the high SR Ca\(^{2+}\) load should facilitate the “fire-diffuse-fire” process, causing an increased Ca\(^{2+}\) spark frequency. However, in Fig. 3.14, the spark frequency in the R33Q mutant cell without the extra luminal RyR regulation is twice as higher compared to that in the R33Q mutant cell with the extra regulation at the same single RyR flux strength (\(J_{\text{max}}=18\)). This is because the extra luminal RyR regulation provides CRUs with the extra refractoriness, such that the previously fired CRUs cannot be fired again until the SR load is refilled up to threshold [Ca]\(_{jsr}^*\) for activating RyRs (Eq. 3.6).

This effect is essential for forming periodic Ca\(^{2+}\) waves in CSQN\(^{R33Q}\) mutant cells as seen in permeabilized myocyte experiments. In the R33Q mutation cell without the extra luminal RyR regulation, the refractoriness of RyR is removed because the CSQN\(^{R33Q}\)-bound RyR has the same opening rate as the CSQN-unbound RyR. There-
Therefore, the next spontaneous Ca$^{2+}$ spark in the same CRU can occur briefly after the previous firing, which causes disordered clustered broken waves-lets in the cell. These results suggested the refractoriness of RyR channels played a key role in determining the occurring time of spontaneous Ca$^{2+}$ waves after a complete repolarization of the membrane voltage.

In the WT CSQN cell, the refractoriness is mainly dominated by the RyR unbinding timescale ($\tau_u$) and the SR load refilling timescale. When the RyR unbinding timescale, $\tau_u$, is equal to 165 ms in the original Restrepo et al. model, the time interval between the occurrence of spontaneous Ca$^{2+}$ releases and the complete membrane potential repolarization is about 200 ms (Fig. 2.7). When $\tau_u$ is increased to 1250 ms, the time interval between the occurrence of spontaneous Ca$^{2+}$ releases and the complete membrane potential repolarization is about 1 s (Fig. 3.20 panel A). In both cases the SR Ca$^{2+}$ already reaches the diastolic level before the occurrence of spontaneous Ca$^{2+}$ releases. This suggests that the RyR unbinding timescale is the dominant factor of refractoriness of the SR Ca$^{2+}$ release.

In the R33Q mutant cell with the extra luminal RyR regulation, we notice that the time interval between the occurrence of spontaneous Ca$^{2+}$ releases and the complete membrane potential repolarization is about $\sim$50 ms (Fig. 3.20), which is the time needed for refilling SR Ca$^{2+}$ to the corresponding diastolic level as seen in the time trace of [Ca]$_{jsr}$ in Fig. 3.20 panel B. These results indicate that the timing of generating spontaneous Ca$^{2+}$ releases and the morphology of spontaneous Ca$^{2+}$ waves are governed by the refractoriness of the SR Ca$^{2+}$ releases. It is necessary to have both the large single RyR release flux strength and an extra luminal RyR regulation in the R33Q mutant cell for generating continuous spontaneous Ca$^{2+}$ waves in the permeabilized myocyte experiment and exhibiting TA in the paced cell experiment.

In addition, in the R33Q mutant cell, APD is longer than that in the WT cell
During the APD, we see many Ca\(^{2+}\) sparks exhibited in the line scan image of the R33Q mutant cell (Panel B of Fig. 3.20), whereas no detectable Ca\(^{2+}\) sparks are observed in the line scan image of the WT cell during the APD (Panel A of Fig. 3.20). This is because the refractoriness of SR Ca\(^{2+}\) releases in R33Q mutant cells is dramatically decreased as we discussed above. Therefore, in the R33Q mutant cell, LCCs can constantly trigger Ca\(^{2+}\) sparks in CRUs during the APD (see line scan image in Panel B of Fig. 3.20). The occurrence of these Ca\(^{2+}\) sparks elevates the cytosolic [Ca]\(_i\) concentration driving more NCX. As a result, APD is increased. In the real R33Q mutant cell, the prolonged APD may also contribute to induce EADs.

The relative importance of [Ca]\(_i\) and [Ca]\(_{jsr}\) in inducing Ca\(^{2+}\)-mediated TA is investigated in this study. In the presence of isoproterenol effect, the R33Q mutant cell with a small J\(_{\text{max}}\) value can not exhibit Ca\(^{2+}\)-mediated TA despite a very high SR Ca\(^{2+}\) load (∼0.8 mM) (Fig. 3.21), whereas with a large J\(_{\text{max}}\) value, Ca\(^{2+}\)-mediated TA is exhibited in the R33Q mutant cell at a much lower SR Ca\(^{2+}\) load (∼0.4 mM for the cell with the extra luminal RyR regulation, and ∼0.2 mM for the cell without the extra luminal RyR regulation). These results clearly demonstrate that SR Ca\(^{2+}\) load is not the cause of spontaneous Ca\(^{2+}\) releases in the context of CPVT linked to the CSQN\(_{\text{R33Q}}\) mutation.

In fact, we observe that the diastolic [Ca]\(_i\) is increased when J\(_{\text{max}}\) increases (Fig. 3.23). It is presumably because the large J\(_{\text{max}}\) facilitates the “fire-diffusive-fire” process causing spontaneous Ca\(^{2+}\) waves. It is noted that when J\(_{\text{max}}\) is increased from 1 to 3, the Ca\(^{2+}\) spark frequency is increased, but both the diastolic [Ca]\(_i\) and the SR Ca\(^{2+}\) load decrease. This is because the cell is still in the spark regime (N\(_{\text{wave}}\)=0). The increased spark frequency is not enough to generate Ca\(^{2+}\) waves, but the enhanced RyR activity contributes to lower the SR Ca\(^{2+}\) content via the Ca\(^{2+}\) extrusion by NCX. Therefore, the intracellular Ca\(^{2+}\) level is decreased despite the increased spark frequency. Further
increasing $J_{\text{max}}$ brings the cell into the Ca$^{2+}$ wave regime ($N_{\text{wave}} > 0$). The occurrence of spontaneous Ca$^{2+}$ waves induces TA, which contributes to bring extracellular Ca$^{2+}$ into the cell through LCC influx. Although, the Ca$^{2+}$ extrusion through NCX may be enhanced as well, the overall effect is to increase the diastolic [Ca]$\text{[i]}$ level. As seen in Fig. 3.23, the elevated diastolic [Ca]$\text{[i]}$ level is accompanied by an increased degree of Ca$^{2+}$-mediated TA. This result suggests that the level of Ca$^{2+}$-mediated arrhythmogenicity is strongly affected by the single RyR flux strength.

The effect of the ratio of R33Q to WT CSQN on inducing Ca$^{2+}$-mediated TA in the heterozygous carriers is investigated. Our results show that a threshold ratio (between 0.2 and 0.4) is required for the heterozygous myocyte to exhibit Ca$^{2+}$-mediated TA. If the ratio of R33Q to WT CSQN is below the threshold, the cell is still in the spark regime ($N_{\text{wave}} = 0$). Only when the overall RyR hyperactivity is increased up to the threshold, the cell approaches the spontaneous Ca$^{2+}$ wave regime ($N_{\text{wave}} > 0$) as seen in Fig. 3.30. This explains the experimental observation that Ca$^{2+}$-mediated TA only occurs in homozygous R33Q mutant carriers and not in heterozygous R33Q mutant carriers. The ratio of R33Q to WT CSQN in heterozygous R33Q mutant carriers is $\sim 0.5$, which may not be up to the threshold for bringing the real cell into the wave regime. Although, the threshold ratio value in our simulation does not exactly match that in the real cell, these results still bring insights of the underlying mechanism of Ca$^{2+}$-mediated TA in heterozygous R33Q carriers. In fact, with the leakiness of RyR being increased by increasing the ratio of R33Q to WT CSQN, the cell eventually exhibits sustained TA (Fig. 3.30) in the presence of isoproterenol effect. This result is consistent with what is seen in Fig. 2.17, where increasing the closed-to-open rate of RYRs leads to sustained TA in the presence of isoproterenol effect. This demonstrates that both RyR mutations and CSQN mutations share a common mechanism in inducing Ca$^{2+}$-mediated TA in the presence of isoproterenol effect.
Note that the initiation of Ca\textsuperscript{2+}-mediated TA in the study of varying the ratio of R33Q to WT CSQN is still governed by the diastolic [Ca\textsubscript{i}] level. This is similar to what is seen in the study of \(J_{\text{max}}\) in the homozygous R33Q mutant cell. It is important to emphasize that when varying the ratio of R33Q to WT CSQN, the hyperactivity of RyRs is only enhanced by effectively changing the closed-to-open rate of RyRs at a fixed large single RyR flux strength (\(J_{\text{max}} = 18\)). In fact, if \(J_{\text{max}}\) is small, for instance, if \(J_{\text{max}}\) is equal to 1, even the homozygous R33Q mutant cell cannot exhibit Ca\textsuperscript{2+}-mediated TA (Panel A of Fig. 3.21). This result indicates that the threshold ratio of R33Q to WT CSQN depends on the single RyR flux strength, \(J_{\text{max}}\). When \(J_{\text{max}}\) increases, the threshold ratio value of R33Q to WT CSQN is decreased.

Another interesting finding of this study is that when the ratio of R33Q to WT CSQN increases from 0 to 0.2, the cell is actually moving out of the Ca\textsuperscript{2+} wave regime (Fig. 3.30). Namely, \(N_{\text{wave}} = 1\) when the ratio is equal to 0, and \(N_{\text{wave}} = 0\) when the ratio is increased to 0.2. This is probably caused by increased Ca\textsuperscript{2+} spike events. Ca\textsuperscript{2+} spikes are nearly undetectable Ca\textsuperscript{2+} releases through the opening of a few RyR channels. This result suggests that increasing the R33Q to WT CSQN ratio does not always increase the probability of inducing Ca\textsuperscript{2+}-mediated TA.

In addition, we find that the interval between the occurrence of spontaneous Ca\textsuperscript{2+} releases and the complete membrane voltage repolarization decreases as the ratio of R33Q to WT is increased (Fig. 3.25 3.26 3.27 3.28). This is because the increased ratio of R33Q to WT CSQN decreases the effective RyR unbinding timescale \(\tau_u\) in the heterozygous R33Q mutant cell. Therefore, these results also support the view that it is the refractoriness of the SR Ca\textsuperscript{2+} release that dominates the timing of the occurrence of spontaneous Ca\textsuperscript{2+} releases.

Furthermore, we studied the effect of the expression level of CSQN\textsuperscript{R33Q} on inducing Ca\textsuperscript{2+}-mediated TA in homozygous CSQN\textsuperscript{R33Q} mutant carriers. Experiments report
that most CSQN mutations are accompanied by a reduced CSQN expression level and consequently reduced Ca$^{2+}$ buffering capacity. Our simulation results show that without the reduction on the CSQN$^{R33Q}$ expression level, the homozygous CSQN$^{R33Q}$ mutant cell can exhibit severe Ca$^{2+}$-mediated TA. Thus, further decreasing the expression level of CSQN$^{R33Q}$ does not increase the degree of Ca$^{2+}$-mediated TA dramatically. However, when the expression level of CSQN$^{R33Q}$ is increased, the degree of Ca$^{2+}$-mediated TA is decreased remarkably. With the Ca$^{2+}$ buffering capacity being enhanced with more CSNQ molecules present in the SR, the free Ca$^{2+}$ concentration decreases in the SR. The leakiness of RyRs remains the same when varying the CSQN$^{R33Q}$ expression level. As a result, the diastolic [Ca]$\text{_{i}}$ decreases with decreasing the SR Ca$^{2+}$ load. Therefore, the lowered intracellular Ca$^{2+}$ level at the high expression level of CSQN$^{R33Q}$ contributes to diminish Ca$^{2+}$-mediated TA. It should be noted that these studies are only conducted at a high $J_{\text{max}}$ value ($J_{\text{max}}=18$). The effect of reducing the expression level of CSQN$^{R33Q}$ on inducing Ca$^{2+}$-mediated TA may become dramatic when the single RyR flux strength is decreased. There may be an important region in the $J_{\text{max}}$ and CSQN concentration phase-plane where the expression level of CSQN becomes critical for inducing Ca$^{2+}$-mediated TA. Future work of extensive computational simulations is needed to address this issue.
Chapter 4

Conclusion

TA can be the cause of life-threatening reentrant cardiac arrhythmias and sudden cardiac arrest. TA has been linked with mutations of one or several cardiac membrane ion channel, as in the setting of CPVT. Even though TA has been extensively studied both experimentally and theoretically, its basic mechanisms remain poorly understood. TA results from the complex interaction of a very large number of cardiac membrane ion channels and calcium cycling proteins. Thus it is generally extremely difficult, if not impossible, to predict the effect of one defective functional protein, taken in isolation, without considering its interaction with all the other normally functioning cardiac proteins.

In the first part of this thesis, we used and further developed a physiologically detailed model to investigate basic aspects of TA in ventricular myocytes. We addressed several key questions on characterizing triggered action potentials. These questions include i) under what conditions triggered action potentials occur and terminate; ii) whether the appearance of spontaneous Ca\(^{2+}\) waves and TA is deterministic or stochastic; iii) what governs the number of delayed after depolarizations before a triggered action potential is induced, and the number of triggered action potentials before the
final termination. The main finding is that Ca$^{2+}$-mediated TA is strongly influenced by ion channel stochasticity in terms of a broad distribution for the number of triggered beats. In addition, triggered activity is governed by bistable dynamics between a metastable oscillatory state and a stable excitable silent state. The transition between the bistable dynamics occurs stochastically at the same intracellular Ca$^{2+}$ level. During the initiation process, when Ca$^{2+}$ cycling dynamics reaches the steady state in the presence of isoproterenol, the distribution of the number of DADs preceding a TAP follows a geometric distribution. Likewise, in the termination process, once Ca$^{2+}$ cycling dynamics reaches the steady state after the electrical stimuli are stopped, the distribution of the number of TAP before the final termination follows a geometric distribution as well. We found that the distribution of spontaneous [Ca]$^{2+}$ peaks followed a Gaussian distribution. We demonstrated that the number of Ca$^{2+}$ wave foci is self-limited to a relatively small value (∼100) compared to the number of CRUs involved in normal CICR (∼10,000). The small number of Ca$^{2+}$ wave foci explains that the width of the distribution of the spontaneous [Ca]$^{2+}$ peaks is much broader than the width of the distribution of the normal [Ca]$^{2+}$ transient peaks. This result explains that the appearance of Ca$^{2+}$ waves and TA is stochastic at a more basic level. These results provide a striking example of bistable dynamics in a biological context, as well as a potential link between ion channel stochasticity on sub-cellular scales and life-threatening cardiac disorders.

In the second part of this thesis, I have investigated the underlying mechanism of Ca$^{2+}$-mediated TA in the context of CPVT linked to the CSQNR33Q mutation. I tested the proposed hypothesis that CSQNR33Q alters the cytosolic and SR Ca$^{2+}$ dynamics by mainly affecting the modulation of RyR activity. In addition, I studied the effect of RyR channel kinetics and the flux strength on Ca$^{2+}$-mediated TA. The results show that it is necessary to have both a large strength of the single RyR flux
and an “extra” luminal RyR regulation in order for RyR hyperactivity to induce TA in paced cells and for Ca\(^{2+}\) waves to propagate continuously in permeabilized cells at low SR Ca\(^{2+}\) load. The occurrence of spontaneous Ca\(^{2+}\) waves are strongly dependent on the refractoriness of the SR Ca\(^{2+}\) release. When the refractory period is long, the time interval between the occurrence of spontaneous Ca\(^{2+}\) waves and the complete repolarization of the previous AP is prolonged. In addition, we demonstrated that the strength of the single RyR flux plays a key role in inducing spontaneous Ca\(^{2+}\) waves. When the strength is large, the “fire-diffuse-fire” process is facilitated, which in turn increases the probability of generating Ca\(^{2+}\) waves. In fact, we found that when the strength of the single RyR flux increased, the SR Ca\(^{2+}\) load was decreased and the diastolic [Ca]_i was increased. These results suggest that SR Ca\(^{2+}\) load may not be the dominant factor in inducing Ca\(^{2+}\)-mediated TA. In fact, the elevation in the diastolic cytosolic Ca\(^{2+}\) concentration plays a key role in generating Ca\(^{2+}\) waves, because the closed-to-open rate of the RyR is more critical than the SR Ca\(^{2+}\) load for firing Ca\(^{2+}\) sparks when the strength of the single RyR flux is large. We demonstrated that in the R33Q heterozygous cells Ca\(^{2+}\)-mediated TA is induced when the ratio of R33Q to WT CSQN exceeds a threshold value, which is consistent with experimental observations [101]. This threshold ratio value appears to decrease as the strength of the single RyR flux is increased. The effect of CSQN buffering capacity on Ca\(^{2+}\)-mediated TA has been investigated. We demonstrated that when the Ca\(^{2+}\) buffering capacity is enhanced with more CSNQ molecules present in the SR, both the SR Ca\(^{2+}\) load and the diastolic [Ca]_i level decrease, which tends to diminish Ca\(^{2+}\)-mediated TA. The investigation sheds light on the genesis of TA and arrhythmias in the setting of a genetic disorder such as CPVT. While this investigation is limited to a cellular level, we expect the insights to provide a basis to understand mechanisms of triggered activity at the organ level, in particular the synchronization of TA across many cells to generate a
propagated AP. Ventricular fibrillation remains a major cause of sudden death in the
US and worldwide. The novel insights into mechanisms of triggered activity from this
study provides an improved basis for risk stratification in a broad population and the
development of reliable antifibrillatory drug therapies.
Appendix A

Details of the ionic model

A.1 Ca\(^{2+}\) cycling and membrane voltage dynamics

Computer simulations were performed using a rabbit ventricular myocyte model that was constructed by combining mathematical formulations of sarcolemmal currents from Mahajan et al. [63] together with a mathematical model of Ca handling from Restrepo et al. [80]. The dimension of this model is 121 \(\mu\text{m} \times 25 \mu\text{m} \times 11 \mu\text{m}\) and there are 19,305 (65×27×11) CRUs. The separations of CRU are 1.84 \(\mu\text{m}\) and 0.9 \(\mu\text{m}\) in the longitudinal direction and transverse direction, respectively. These CRUs are coupled by cytosolic and NSR Ca\(^{2+}\) diffusion.

This combined model reproduces the characteristic rabbit action potential shape and captures essential aspects of the bi-directionally coupled dynamics of \(V_m\) and \([\text{Ca}]_i\) in the presence of both hyperactive RyRs and isoproterenol effect underlying Ca\(^{2+}\)-mediated TA. The dynamics of membrane voltage is described by the equation

\[
\frac{dV_m}{dt} = -\frac{1}{C_m}(I_{\text{ion}} + I_{\text{stim}}),
\]

where \(I_{\text{ion}}\) is the total membrane current density, \(I_{\text{stim}}\) is the stimulus current, and
where $C_m$ is the cell membrane capacitance. Following Mahajan et al., all ion currents are computed for $1 \mu F$ of cell membrane capacitance and have units of $\mu A/\mu F$.

The total membrane current is given by

$$I_{\text{ion}} = I_{\text{Na}} + I_{\text{K1}} + I_{\text{Kt}} + I_{\text{Ks}} + I_{\text{to}} + I_{\text{NaK}} + I_{\text{Ca}} + I_{\text{NaCa}}. \quad (A.2)$$

$\text{Ca}^{2+}$ cycling was simulated using a model developed by Restrepo et al. [80]. The equations for $\text{Ca}^{2+}$ cycling are:

$$\dot{c}_i = \beta_i (c_i) (I_{\text{dsi}} \frac{V_s}{V_i} - I_{\text{up}} + I_{\text{leak}} - I_{\text{TCi}} + I_{\text{ci}}), \quad (A.3)$$

$$\dot{c}_s = \beta_s (c_s) (I_{\text{dps}} \frac{V_p}{V_s} + I_{\text{NCX}} - I_{\text{dsi}} - I_{\text{TCs}} + I_{\text{cs}}), \quad (A.4)$$

$$\dot{c}_p = \beta_p (c_p) (I_t + I_{\text{Ca}} - I_{\text{dps}}), \quad (A.5)$$

$$\dot{c}_{\text{NSR}} = (I_{\text{up}} - I_{\text{leak}}) \frac{V_i}{V_{\text{NSR}}} - I_{\text{tr}} \frac{V_{\text{JSR}}}{V_{\text{NSR}}} + I_{c\text{NSR}}, \quad (A.6)$$

$$\dot{c}_{\text{JSR}} = \beta_{\text{JSR}} (c_{\text{JSR}}) (I_{\text{tr}} - I_{\text{r}} \frac{V_p}{V_{\text{JSR}}}), \quad (A.7)$$

where $c_i$ is the free $\text{Ca}^{2+}$ concentration in the bulk myoplasm, $c_s$ is the free $\text{Ca}^{2+}$ concentration in a thin layer just below the cell membrane, $c_p$ is the free $\text{Ca}^{2+}$ concentration in the proximal space, $c_{\text{JSR}}$ is the free $\text{Ca}^{2+}$ concentration in the junctional SR, $c_{\text{NSR}}$ is the free $\text{Ca}^{2+}$ concentration in the network SR, $\beta$ terms account for instantaneous buffering in corresponding compartments using the rapid buffering approximation, $I_{\text{up}}$ is the SERCA uptake current representing total flux into the NSR, $I_{\text{leak}}$ is the leak current from NSR to cytosol, $I_{\text{NCX}}$ is $\text{Na}^+-\text{Ca}^{2+}$ exchange current, $I_{\text{Ca}}$ is the L-type $\text{Ca}^{2+}$ influx, $I_{\text{r}}$ is the total $\text{Ca}^{2+}$ efflux from the JSR, $I_{\text{dsi}}$, $I_{\text{dps}}$ and $I_{\text{tr}}$ are the diffusion currents from adjacent compartments, $I_{\text{TCi}}$ and $I_{\text{TCs}}$ are the troponin C dynamic buffering currents in cytosol and submembrane spaces, $I_{c_i}$, $I_{c_s}$ and $I_{c\text{NSR}}$ are the diffusion currents between neighboring CRUs in the corresponding compartments.
A.2 Instantaneous cytosolic buffering

The factors $\beta_i(c_i)$ and $\beta_s(c_s)$ describe instantaneous buffering to Calmodulin, SR sites, Myosin (ca), and Myosin (Mg). Note that the concentration of the proximal space rapidly equilibrates, so we do not require knowledge of the instantaneous buffers in the proximal space. The equation of $\beta_i(c_i)$ is

$$\beta_i(c_i) = \left[ 1 + \sum \frac{B_bK_b}{(c_i + K_b)^2} \right]^{-1},$$

(A.8)

where the sum is over the instantaneous cytosolic buffers Calmodulin, SR sites, Myosin (ca$^{2+}$), and Myosin (Mg$^{2+}$), with buffer dissociation constants $K_{\text{CAM}}$, $K_{\text{SR}}$, $K_{\text{MCA}}$, and $K_{\text{MMg}}$ and total concentration of buffering sites $B_{\text{CAM}}$, $B_{\text{SR}}$, $B_{\text{MCA}}$, and $B_{\text{MMg}}$, respectively. In addition to Calmodulin, instantaneous buffering in the submembrane space includes the subsarcolemmal sites of high affinity with total concentration of sites and dissociation constant $B_{\text{SLH}}$ and $K_{\text{SLH}}$, respectively. The parameters for instantaneous cytosolic buffering are in Table. A.1

A.3 Instantaneous luminal buffering

$\beta_{\text{JSR}}(c_{\text{JSR}})$ describes instantaneous luminal Ca$^{2+}$ buffering to CSQN. The expression of $\beta_{\text{JSR}}(c_{\text{JSR}})$ is

$$\beta(c) = \left( 1 + \frac{K_CB_{\text{CSQN}}n(c) + \partial_n(c)(cK_C + c^2)}{(K_C + c)^2} \right)^{-1},$$

(A.9)
Table A.1: Buffering parameters [80].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{CAM}$</td>
<td>7.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$B_{CAM}$</td>
<td>24.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$K_{SR}$</td>
<td>0.6</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$B_{SR}$</td>
<td>47.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$K_{MCa}$</td>
<td>0.033</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$B_{MCa}$</td>
<td>140.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$K_{SLH}$</td>
<td>0.3</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$B_{SLH}$</td>
<td>13.4</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$B_T$</td>
<td>70.0</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$k_{on}^T$</td>
<td>0.0327</td>
<td>$(\mu$M ms)$^{-1}$</td>
</tr>
<tr>
<td>$k_{off}^T$</td>
<td>0.0196</td>
<td>$(\text{ms})^{-1}$</td>
</tr>
</tbody>
</table>

where

$$n(c_{JSR}) = \hat{M}n_M + (1 - \hat{M})n_D, \quad (A.10)$$

$$\hat{M} = \frac{(1 + 8\rho B_{CSQN})^{1/2} - 1}{4\rho B_{CSQN}}, \quad (A.11)$$

and

$$\rho(c_{JSR}) = \frac{\rho\infty c_{JSR}^h}{K^h + c_{JSR}^h} \quad (A.12)$$

The parameters for Luminal buffering are in Table. A.2.

### A.4 Troponin C dynamic buffering

These currents describe the rate of change in the concentration of $\text{Ca}^{2+}$ bound to Troponin C in the cytosolic and submembrane compartments, $[\text{CaT}]_i$ and $[\text{CaT}]_s$. These
Table A.2: Luminal buffering parameters [80].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;CSQN&lt;/sub&gt;</td>
<td>Concentration of CSQN molecules</td>
<td>400 µM</td>
</tr>
<tr>
<td>K&lt;sub&gt;C&lt;/sub&gt;</td>
<td>Dissociation constant of CSQN</td>
<td>600 µM</td>
</tr>
<tr>
<td>n&lt;sub&gt;M&lt;/sub&gt;</td>
<td>Buffering capacity of CSQN monomers</td>
<td>15</td>
</tr>
<tr>
<td>n&lt;sub&gt;D&lt;/sub&gt;</td>
<td>Buffering capacity of CSQN dimers</td>
<td>35</td>
</tr>
<tr>
<td>ρ&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>Asymptotic ratio of dimers to monomers</td>
<td>5000</td>
</tr>
<tr>
<td>K</td>
<td>Dimerization constant</td>
<td>850 µM</td>
</tr>
<tr>
<td>h</td>
<td>Dimerization exponent (steep)</td>
<td>23</td>
</tr>
</tbody>
</table>

quantities satisfy

\[
\frac{d[CaT]_i}{dt} = I_{TCi},
\]

(A.13)

with

\[
I_{TCi} = k_{on}^{T}c_i(B_T - [CaT]_i) - k_{off}^{T}[CaT]_i,
\]

(A.14)

and analogous expressions apply for the submembrane compartments, replacing the subscript i by s. Here, \(k_{on}^{T}\) and \(k_{off}^{T}\) are the on- and off-rate constants for Ca\(^{2+}\)-Troponin C binding, and \(B_T\) is the total concentration of Troponin C buffering sites. The values of these parameters are list in Table. A.1.

**A.5 Uptake current \(I_{up}\)**

\[
I_{up} = \nu_{up} \frac{(c_i/K_i)^H - (c_{NSR}/K_{NSR})^H}{1 + (c_i/K_i)^H + (c_{NSR}/K_{NSR})^H}.
\]

(A.15)

See Table. A.3 for the parameters.
Table A.3: Uptake and leak current parameters [80].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_{up}$</td>
<td>0.3</td>
<td>$\mu$M ms$^{-1}$</td>
</tr>
<tr>
<td>$K_i$</td>
<td>0.123</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$K_{NSR}$</td>
<td>1700</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$H$</td>
<td>1.787</td>
<td></td>
</tr>
<tr>
<td>$g_{\text{leak}}$</td>
<td>$1.035 \times 10^{-5}$</td>
<td>ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{\text{JSR}}$</td>
<td>500</td>
<td>$\mu$M</td>
</tr>
</tbody>
</table>

A.6 Leak current $I_{\text{leak}}$

\[
I_{\text{leak}} = g_{\text{leak}} \frac{c_{\text{JSR}}^2}{c_{\text{JSR}}^2 + K_{\text{JSR}}^2} (c_{\text{NSR}} - c_i). \tag{A.16}
\]

See Table A.3 for the parameters.

A.7 Sodium-calcium exchange current $I_{\text{NaCa}}$

\[
I_{\text{NaCa}} = \frac{K_a \nu_{\text{NaCa}}(e^{\eta z}[\text{Na}]_i^3[\text{Ca}]_o - e^{(\eta - 1)z}[\text{Na}]_o^3 c_s)}{(t_1 + t_2 + t_3)(1 + k_{\text{sat}} e^{(\eta - 1)z})}, \tag{A.17}
\]

where

\[
t_1 = K_{\text{mCa}}[\text{Na}]_o^2 \left[ 1 + \left( \frac{[\text{Na}]_i}{K_{\text{mNa}}} \right)^3 \right], \tag{A.18}
\]

\[
t_2 = K_{\text{mCa}}^3[\text{Na}]_i^3 + [\text{Na}]_i^3[\text{Ca}]_o + [\text{Na}]_o^3 c_s, \tag{A.19}
\]

\[
K_a = \left[ 1 + \left( \frac{K_{\text{da}}}{c_s} \right)^3 \right]^{-1}, \tag{A.20}
\]

\[
z = \frac{VF}{RT}, \tag{A.21}
\]
Table A.4: Sodium-calcium exchanger current parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_{NaCa}$</td>
<td>21</td>
<td>$\mu$M ms$^{-1}$</td>
</tr>
<tr>
<td>$K_{mCai}$</td>
<td>3.59</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$K_{mCao}$</td>
<td>1.3</td>
<td>mM</td>
</tr>
<tr>
<td>$K_{mNaI}$</td>
<td>12.3</td>
<td>mM</td>
</tr>
<tr>
<td>$K_{mNaO}$</td>
<td>87.5</td>
<td>mM</td>
</tr>
<tr>
<td>$k_d$</td>
<td>0.11</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>$k_{sat}$</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>$\eta$</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>$[Ca]_o$</td>
<td>1.8</td>
<td>mM</td>
</tr>
<tr>
<td>$[Na]_o$</td>
<td>136</td>
<td>mM</td>
</tr>
</tbody>
</table>

and the values of parameters are listed in Table A.4. The internal sodium concentration $[Na]_i$ adjusts slowly upon a change in pacing rate, reflecting the new equilibrium between sodium influx due to the sodium current, and sodium extrusion by the sodium-calcium exchanger and the sodium-potassium pump. This process occurs over many beats. Therefore, we fix $[Na]_i$ to the initial value of 12 mM in the study of Ca$^{2+}$-mediated TA. This $[Na]_i$ concentration is within the physiological range.

### A.8 Calcium current, $I_{Ca}$

Each CRU is assumed to have $N_L$ L-type channels under control condition. The Ca$^{2+}$ flux into the proximal space of a CRU is given by

$$I_{Ca} = i_{Ca} L_k,$$  \hspace{1cm} (A.22)

where the single channel current is

$$i_{Ca} = 4P_{Ca} zF \frac{\gamma_0 c_{Ca} e^{2z} - \gamma_0 [Ca]_o}{e^{2z} - 1},$$ \hspace{1cm} (A.23)
z = VT/(RT). The gating of each L-type channel is simulated by stochastically evolving the 7-state Markov model developed in Mahajan et al. [63]. The value $L_k$ is the number of channels in the open state, $1 \leq L_k \geq N_L$. The parameter values are in Table A.5.

### A.9 Release current, $I_r$

$$I_r = J_{\text{max}} \sigma_r P_o (c_{\text{JSR}} - c_p)/v_p, \quad (A.24)$$

where $P_o$ is the fraction of RyR channels that are in the open states, i.e. states OU and OB\(^1\) of RyRs in wild type cells (plus OB\(^2\) if we model R33Q mutant cells), $\sigma_r$ is the maximum RyR flux strength, and $J_{\text{max}}$ is the pre-factor we used to vary the RyR flux strength in Chapter 3. The parameters are in Table A.6. $P_o$ is determined by the RyR gating Markov model.
A.10 RyR gating Markov model

As described in the main text, for the wild type CSQN cell, the RyR gating Markov model includes the following 4 states: closed CSQN-unbound, open CSQN-unbound, open CSQN-bound, and closed CSQN-bound. For the heterozygous CSQN$^{R33Q}$ mutant cell, the RyR gating Markov model consists of 6 states: closed CSQN-unbound, open CSQN-unbound, open CSQN-bound, closed CSQN-bound, open CSQN$^{R33Q}$-bound, and closed CSQN$^{R33Q}$-bound. The rates of transition from state i to state j, $k_{ij}$, are

\[ k_{12} = K_uc_p^2, \]
\[ k_{14} = \hat{M}(c_p)\tau_b^{-1}B_{CSQN}/B_{CSQN}^0, \]
\[ k_{21} = \tau_c^{-1}, \]
\[ k_{23} = \hat{M}(c_p)\tau_b^{-1}B_{CSQN}/B_{CSQN}^0, \]
\[ k_{43} = K_GC_p^2, \]
\[ k_{41} = \tau_u^{-1}, \]
\[ k_{34} = \tau_c^{-1}, \]
\[ k_{32} = k_{41}k_{12}/k_{43}, \]
\[ k_{15} = \hat{M}^{R33Q}(c_p)\tau_b^{-1}B_{CSQN}/B_{CSQN}^0, \]
\[ k_{51} = \tau_u^{-1}, \]
\[ k_{26} = \hat{M}^{R33Q}(c_p)\tau_b^{-1}B_{CSQN}/B_{CSQN}^0, \]
\[ k_{62} = k_{41}k_{12}/k_{43}, \]
\[ k_{65} = \tau_c^{-1}, \]
\[ k_{56} = K_uc_p^2. \]
Table A.6: SR release current and RyR Markov model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{\text{max}}$</td>
<td>Pre-factor of RyR release strength</td>
<td>1 in Restrepo et al. [80] 18 in Sato et al. [87]</td>
</tr>
<tr>
<td>$\sigma_r$</td>
<td>Maximum RyR flux strength</td>
<td>$1.47 \times 10^{-2} , \mu m^3 ms^{-1}$</td>
</tr>
<tr>
<td>$K_u$</td>
<td>CSQN-unbound opening rate</td>
<td>$3.8 \times 10^{-4} , \mu M^{-2} , ms^{-1}$</td>
</tr>
<tr>
<td>$K_b$</td>
<td>CSQN-bound opening rate</td>
<td>$5 \times 10^{-5} , \mu M^{-2} , ms^{-1}$</td>
</tr>
<tr>
<td>$\tau_u$</td>
<td>CSQN-unbinding timescale</td>
<td>165.0 ms in Restrepo et al. [80] 1250.0 ms in Sato et al. [87]</td>
</tr>
<tr>
<td>$\tau_b$</td>
<td>CSQN-binding timescale</td>
<td>5.0 ms in Restrepo et al. [80] 0.5 ms in Sato et al. [87]</td>
</tr>
<tr>
<td>$\tau_c$</td>
<td>RyR closing timescale</td>
<td>1.0 ms</td>
</tr>
<tr>
<td>$B_{\text{CSQN}}^0$</td>
<td>Normal CSQN concentration</td>
<td>400 $\mu$M</td>
</tr>
</tbody>
</table>

The parameters are in Table. A.6. Note that $B_{\text{CSQN}}/B_{\text{CSQN}}^0$ is only different from 1 when the CSQN concentration is modified.

In Chapter 3, we introduced an extra SR Ca$^{2+}$ release regulation. We assume that the RyR channels have luminal Ca2+ binding sites and that binding and unbinding to and from those sites occurs sufficiently rapidly for binding and unbinding to be in steady-state equilibrium. We further assume that Ca2+ binding is cooperative and that RyR channels closed to open transition rates are increased when Ca2+ is bound to the luminal sites. Thus, the modified transition rates take the following forms

\[
\begin{align*}
    k'_{12} &= k_{12} \cdot f([Ca]_{\text{jsr}}) = K_u \cdot ([Ca]_{p})^2 \cdot f([Ca]_{\text{jsr}}) \quad (A.39) \\
    k'_{43} &= k_{43} \cdot f([Ca]_{\text{jsr}}) = K_b \cdot ([Ca]_{p})^2 \cdot f([Ca]_{\text{jsr}}) \quad (A.40) \\
    k'_{56} &= k_{56} \cdot f([Ca]_{\text{jsr}}) = K_u \cdot ([Ca]_{p})^2 \cdot f([Ca]_{\text{jsr}}) , \quad (A.41)
\end{align*}
\]

where,

\[
    f([Ca]_{\text{jsr}}) = \frac{1}{1 + ([Ca]_{\text{jsr}}/([Ca]_{\text{jsr}})^{\text{II}})} , \quad (A.42)
\]
and $[\text{Ca}]^*_{\text{jsr}} = 0.5 \text{ mM}$ and the Hill coefficient $H=4$.

### A.11 Nearest-neighbor diffusive currents, $I_{ci}, I_{cs}$, and $I_{c\text{NSR}}$

The diffusive currents in cytosol, submembrane and NSR are given by

$$I_{ci}^{(n)} = \sum_m \left( \frac{c_{i}^{(m)} - c_{i}^{(n)}}{\tau_{mn}} \right),$$

(A.43)

where the sum is over the six nearest neighbors. The values of the timescales ($\tau_{mn}$) are in Table. A.7. The Ca\(^{2+}\) diffusion timescales are multiplied by a pre-factor $\xi$ to study the effect of Ca\(^{2+}\) diffusivity on Ca\(^{2+}\)-mediated TA.
Appendix B

Permissions

B.1 Permission for Fig. 1.2

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135020419578
Publication: Nature
Title: Cardiac excitation-contraction coupling
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD

B.2 Permission for Fig. 1.3

Order Details
Licensee: Zhen Song
B.3 Permission for Fig. 1.4

Permission for using figures of [119] in thesis or dissertation is not required according to Copyright Clearance Center’s RightsLink service.

B.4 Permission for Fig. 1.5

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135030712738
Publication: Circulation Research
Title: Abnormal Interactions of Calsequestrin With the Ryanodine Receptor Calcium Release Channel Complex Linked to Exercise-Induced Sudden Cardiac Death
Type Of Use: Dissertation/Thesis
Total: 0.00 USD
B.5  Permission for Fig. 1.7

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135030845627
Publication: Biophysical Journal
Title: Modulation of SR Ca Release by Luminal Ca and Calsequestrin in Cardiac Myocytes: Effects of CASQ2 Mutations Linked to Sudden Cardiac Death
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD

B.6  Permission for Fig. 2.1

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135031083827
Publication: Biophysical Journal
Title: Calsequestrin-Mediated Mechanism for Cellular Calcium Transient Alternans
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD
B.7 Permission for Fig. 2.2

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135031083827
Publication: Biophysical Journal
Title: Calsequestrin-Mediated Mechanism for Cellular Calcium Transient Alternans
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD

B.8 Permission for Fig. 3.1

Order Details
Licensee: Zhen Song
License Date: Apr 23, 2013
License Number: 3135030845627
Publication: Biophysical Journal
Title: Modulation of SR Ca Release by Luminal Ca and Calsequestrin in Cardiac Myocytes: Effects of CASQ2 Mutations Linked to Sudden Cardiac Death
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD
Bibliography


[55] E. G. Lakatta, V. A. Maltsev, and T. M. Vinogradova. A coupled system of intracellular ca2+ clocks and surface membrane voltage clocks controls the time-


[81] N. Rizzi. Unexpected structural and functional consequences of the r33q homozygous mutation in cardiac calsequestrin: a complex arrhythmogenic cascade in a knock in ....


[94] E. Sobie, K. Dilly, J. dos Santos Cruz, and W. Lederer. Termination of cardiac 
ca2+ sparks: An investigative mathematical model of calcium-induced calcium 

[95] L. Song, R. Alcalai, M. Arad, C. M. Wolf, O. Toka, D. A. Conner, C. I. Berul, 
M. Eldar, C. E. Seidman, and J. Seidman. Calsequestrin 2 (casq2) mutations 
increase expression of calreticulin and ryanodine receptors, causing catecholamin-
egergic polymorphic ventricular tachycardia. JOURNAL OF CLINICAL INVESTI-

Orphaned ryanodine receptors in the failing heart. Proc. Natl. Acad. Sci. USA, 

afterdepolarizations and triggered activity in guinea pig ventricular myocytes. 

ca2+ release on action potentials and afterdepolarizations in guinea pig ventric-

[99] M. D. Stern. Theory of excitation-contraction coupling in cardiac muscle. Bio-

[100] S. C. W. Stevens, D. Terentyev, A. Kalyanasundaram, M. Periasamy, and S. Gy-
orke. Intra-sarcoplasmic reticulum ca2+ oscillations are driven by dynamic reg-
ulation of ryanodine receptor function by luminal ca2+ in cardiomyocytes. The 


