Computational Investigation of the Mechanisms Underlying the Cardiac Pacemaker and its Dysfunction

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Ruoxi Wang
School of Physics and Astronomy
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Abstract
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Ruoxi Wang
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Computational Investigation of the Mechanisms underlying the Cardiac Pacemaker and its Dysfunction
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The sinoatrial node is the primary cardiac pacemaker, which is responsible for generating spontaneous depolarisation of cellular membranes, leading to pacemaking action potentials that control the initiation and regulation of the rhythms of the heart. Previous studies in experimental electrophysiology have gathered a large amount of experimental data about the mechanisms of cardiac pacemaking activities at the molecular, ionic and cellular levels, however, the precise mechanisms underlying the genesis of spontaneous pacemaking action potentials still remain controversial. Mathematical models of the electrophysiology provide a unique alternative tool complimentary to experimental investigations, enabling us to analyse the fundamental physiological mechanisms of cardiac pacemaking activities in an efficient way that would be more difficult to conduct in experimental approaches. In this thesis, an integrated model, incorporating the detailed cellular ion channel kinetics, multi-compartment intracellular Ca\textsuperscript{2+} handling system and cell morphology, was developed for simulating the spontaneous pacemaking action potentials as well as the stochastic nature of local Ca\textsuperscript{2+} dynamics in the murine SA node cells. By using the model, the ionic mechanisms underlying the automaticity of primary cardiac pacemaking cells were investigated, the individual role of the ‘membrane clock’ (the cell membrane events) and ‘Ca\textsuperscript{2+} clock’ (intracellular Ca\textsuperscript{2+} activities) on generating the pacemaking action potentials were examined. In addition, the model also considered the regulation of the autonomic nervous systems on cardiac pacemaking action potentials. For the first time, competitive regulation of electrical action potentials of the murine SA node cells by the circadian sympathetic and parasympathetic systems during 24-hours were investigated. Furthermore, the individual role of the neurotransmitters, ACh- and ISO-induced actions on variant ion channel and Ca\textsuperscript{2+} handling in regulating cardiac pacemaking action potentials were also analysed. At the tissue level, an anatomically detailed 2D model of the intact SA node and atrium was developed to investigate the ionic mechanisms underlying sinus node dysfunctions in variant genetic defect conditions. Effects of these genetic defects in impairing cardiac pacemaker ability in pacing and driving the surrounding atrium as seen in the sinus node dysfunction were investigated.
Declaration

I declare that no portion of the work referred to in the thesis has been submitted in support of another degree or qualification of this or any other university or other institute of learning.

Ruoxi Wang
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Chapter 1
Introduction

1.1 The heart

The heart is the central part of the circulatory system in mammals. Through the rhythmical muscle mechanical contraction, it pumps the oxygenated, nutrient-rich blood throughout the whole body and the deoxygenated blood to the lungs via the pulmonary artery. In humans, the location of the heart is approximately in the centre of the chest between the two lungs. However, the accurate size and location of a human heart varies due to the age and gender (Oberman et al., 1967), for example, the weight of a man’s heart ranges from 300 to 350g, whereas a women’s heart weighs from 250 to 300g (Kumar et al., 2014).

1.1.1 Anatomy and physiology of the heart

Mammalian hearts are divided by septa into two different systems: the left heart and the right heart (Figure 1.1). The function of the right heart is to pump the deoxygenated blood to the lungs though the pulmonary artery and exchange carbon dioxide gas, dissolved in the blood from the body, with oxygen, and then return the newly oxygenated blood to the heart. Then the left heart moves the oxygenated blood around the body though the systemic circuit, delivering oxygen and nutrients. The left and right parts of heart are further divided into two chambers: the atria and ventricles. The blood enters the heart into the atria and is pumped out by the ventricles. The chamber walls of ventricles are thicker than that of the atria, which is mainly due to the greater blood pressure needed in ventricles to pump blood out (Bjork et al., 1954).
Figure 1.1 A schematic diagram of the structure of the human heart. Major structures and blood vessels are labelled, including the four active chambers: left/right atrium and left/right ventricle. Arrows represent the direction of blood flow. Adapted from Colman (2014).

The atria are the thin-walled chambers which receive the blood from the body and the lungs, and then send it down to the ventricle. They are located in the upper portion of the heart and occupy nearly one-third of the total volume. The right atrium receives deoxygenated blood from the body though the superior and inferior vena cava, while the left atrium receives the oxygenated blood from the lungs via the pulmonary vein. The right atrium and the right ventricle are separated by the tricuspid, which prevents
the backflow of blood into the right atrium. Similarly, the left atrium and left ventricle are separated by the mitral valve (Bjork et al., 1954).

The ventricles are the lower chambers of the heart, accounting for approximately two-thirds of the total volume. The deoxygenated blood flows into the right ventricle from the right atrium and is then pumped to the lungs via the pulmonary artery. The left ventricle receives the oxygenated blood from the left atrium and forces it around the whole body, under great pressure, through the aorta.

1.1.2 The sinoatrial (SA) node

Before the discovery of the SA node, the top right atrium had been considered to be the pacemaking region for centuries. It was also unclear if the heart’s beating was controlled by itself or controlled by the human brain. This controversy was not resolved for many years till the discovery of the SA node in a mole’s heart by Keith and Flack (1907). The relationship between function and anatomy structure of the SA node was reported by Trautwein and Uchizono (1963).

The location and size of the SA node was described as a relatively small region which is located at the top of the right atrium, at the junction between: the crista terminalis (CT), a thick band of atrial muscle at the border of the atrial appendage, composed of venous tissue, the superior and inferior venae cavae, and the intercaval region of two great veins, as shown in Figure 1.2. The published illustrations show that in humans the SA node is beneath the epicardial surface of the CT, and it may extend along the entire length, between the superior and inferior venae cavae (Anderson et al., 1978).
Figure 1.2 A schematic diagram of the location of the SA node in the heart. The centre and periphery of the SA node are illustrated in red and blue, respectively. Ao: aorta, CS: coronary sinus, IVC: inferior vena cava, PA: pulmonary artery, PV: pulmonary vein, RA: right atrium, RV: right ventricle, SVC: superior vena cava. Adapted from Dobrzynski et al. (2005).

In some mammals (humans, dogs, and pigs), there is a layer of atrial muscle between the SA node and the endocardium (Woods et al., 1976; Anderson et al., 1978; Opthof et al., 1987), while in the other animals (rabbits, guinea pigs, and monkeys) the SA node may account for the entire thickness between the endocardium and the epicardium (Bleeker et al., 1980; Opthof et al., 1985; Alings et al., 1990). It is clear that the function of the atrial muscle in the intercaval region and the extensive connective tissue found in large animals is to protect the region against the high wall pressure (Boyett et al., 2000). The connective tissue also has another function: to protect the SA node from being electrically suppressed by the large mass of atrial muscle surrounding it. Because of the SA node being connected to the atrial muscle
directly, the contact between the two tissues over such a large area will have a substantial suppressive effect on the SA node pacemaker activity, due to electronic interactions between the SA node and the surrounding atrial tissue (Boyett et al., 1995).

1.2 Cardiac electrophysiology

1.2.1 The cell membrane; ion channel; ion pumps and exchangers

The cardiac cell membrane (Figure 1.3) is a thin lipid bilayer, which separates the intracellular space from the extracellular environment. This lipid bilayer is formed by molecules consisting of a hydrophilic head and a hydrophobic tail (amphipathic molecules), which normally allows for the passive diffusion of hydrophobic molecules, but is able to prevent polar solutes, such as nucleic acids, proteins and ions, from diffusing across the cell membrane. The movement of these polar solutes is controlled by specialised transmembrane protein structures such as pores, channels and gates.

Figure 1.3 The physical structure of the cell membrane. Schematic diagram of the cell membrane or sarcolemma. The yellow polar head groups separate the brown hydrophobic tails from the aqueous cytosolic and the extracellular environment.
Ionic channels are macromolecular pores in the cell membrane, which enable the passive transport of ions because of an electrochemical gradient. Ion channels have selective permeability, which means that a specific channel can only transfer a specific type of ion. Therefore, channels are usually named by the ion which can flow through them, e.g. Na\(^+\) channel, K\(^+\) channel, and Ca\(^{2+}\) channel. An ion channel may have two different processes: activation and inactivation, referred to as gates (Grant, 2009). The event whether an ion can flow through the channel is determined by the state of these gates (Colman, 2014). In cardiac myocytes, most of the gates are voltage-dependent (Figure 1.4).

![Figure 1.4](image)

Figure 1.4 An example of an ion channel. An ion channel may be in three states: (i) closed, (ii) open, or (iii) inactivation. The direction of flow depends on the intracellular and extracellular ion concentrations.

Ionic pumps (including ion exchangers) require energy and can therefore transport an ion across the membrane, regardless of the intra- and extra-cellular concentrations. Ionic pumps use complex molecular structures to bind to the necessary ions and transfer them across the membrane before releasing them on the other side. Ion exchangers are a specific type of ionic pump, which can use the energy of one kind of ion moving across the membrane with its concentration gradient to transport another ion against its concentration gradient (Figure 1.5).
1.2.2 Cardiac action potential

In physiology, an action potential (AP) is a short-lasting event in which the electrical membrane potential of a cell membrane rapidly rises and falls, following a consistent trajectory. The morphology of the AP manifests itself in five phases (0-4):

**Phase 0**: Rapid upstroke of the AP. This is the depolarization of the cell, and activation of fast sodium channels, which lead to a rapid influx of sodium ions and the membrane potentially shifting into positive voltage range. This phase is central to the rapid propagation of the cardiac impulse.

**Phase 1**: Brief, early repolarization. The sodium channel is inactivated, and there is a brief repolarization of the membrane potential because of the activation of the transient outward potassium current. This phase sets the potential for the next phase of the AP.
**Phase 2**: Plateau. This is the longest phase. It is a plateau because of the balance between inward calcium current, through the L-type calcium channel, and outward potassium current, through the rectifier potassium channel. Both of the channels activate during the upstroke, but reach their peak during this phase.

**Phase 3**: Rapid repolarization. This is the repolarization of the cell. Due to the inactivation of the calcium channels and the activation of the delayed rectifier potassium at the end of the last phase, the outward potassium currents reduce the membrane potential.

**Phase 4**: Resting. This is the resting period of the AP. The membrane potential returns to its resting value, and the delayed rectifier potassium channels become inactive.

In order to quantify the action potential, some measurable AP characteristics are defined, which are summarized below:

- **Overshoot (OS)**: the maximum membrane potential measured during the time course of APs;
- **MDP**: the maximum diastolic membrane potential;
- **APA**: the amplitude of the membrane AP, measured as the difference between OS and MDP;
- **CL**: the cycle length of the action potential, measured as the time interval between two consecutive APs;
- **APD\(_{90}\)**: the action potential duration measured at 90 per cent depolarization;
- **Maximum upstroke velocity (dV/dt\(_{\text{max}}\)**): the maximal positive value of the differentiated membrane potential against time during phase 0.

The cardiac action potential differs significantly in different myocytes of the heart. For instance, the APD\(_{90}\) in the ventricle can be 3.5 times larger than that in atrial myocytes (Grant, 2009).
Figure 1.6 A schematic diagram of a typical cardiac action potential. Five main phases are labelled: resting (4), upstroke (0), early repolarization (1), plateau (2), and final repolarization (3). The inward currents (marked by ↑), $I_{Na}$, $I_{Ca}$ and $I_{f}$, are shown in the yellow boxes; the Na$^+$-Ca$^{2+}$ exchanger (NCX) is also shown in yellow. The outward currents (marked by ↓), $I_{Kach}$, $I_{K1}$, $I_{to}$, $I_{Kur}$, $I_{Kr}$ and $I_{Ks}$ are shown in grey boxes. Adapted from Grant (2009).

1.2.3 Intracellular Ca$^{2+}$ handling

The fluxes of ions through the channels on the cell membrane not only act to change the membrane potential, but also affect the variation of the intracellular ion concentrations. It is well-known that the Ca$^{2+}$ signalling in the cardiac and the skeletal muscle myocytes plays an important role in their actual contraction. This regulation of Ca$^{2+}$ ions inside the cell is governed by a series of sub-cellular structures (Li, 2012).
The sarcoplasmic reticulum (SR) is a kind of smooth endoplasmic reticulum found in myocytes, which stores a large quantity of Ca\(^{2+}\) ions. The membrane of the SR is structurally similar to that of a cell, consisting of many Ca\(^{2+}\) channels and pumps. The ryanodine receptors (RyR) form one of the Ca\(^{2+}\) channels located in the SR, which controls the release of Ca\(^{2+}\) ions from the SR. During cardiac action potential, the depolarization of the cell membrane activates the L-type Ca\(^{2+}\) channel, resulting in a Ca\(^{2+}\) influx into the cell to elevate the Ca\(^{2+}\) concentration around the cell membrane. This increase of Ca\(^{2+}\) concentration can trigger the release of Ca\(^{2+}\) ions from the SR through the RyRs, which are located close to the L-type Ca\(^{2+}\) channel (Bers, 2002). This is the Calcium-Induced-Calcium-Release (CICR) process (Bers, 2002). Then, the intracellular Ca\(^{2+}\) transients, produced by the CICR process, get involved in the...
contraction of the cardiac myocytes by binding with the contractile proteins. Finally, 
Ca\(^{2+}\) will be released from its binding sites on the contractile proteins, and pumped 
back to the SR through an ATP-dependent Ca\(^{2+}\) pump, SERCA. Ca\(^{2+}\) ions will also be 
extruded out of the cell via the sarcolemmal ATP-dependent Ca\(^{2+}\) pump and the 
Na\(^+\)-Ca\(^{2+}\) exchanger. The intracellular Ca\(^{2+}\) handling is illustrated in Figure 1.7.

1.2.4 The electrophysiological properties of pacemaker cells
The action potential of pacemaking cells in the SA node and atrioventricular node 
(AVN) shows different characteristics as compared to the APA in the ventricle 
myocardium. In SA node cells, the membrane potential in phase 4 is more depolarized 
(-50 to -65 mV), then it gradually enters into phase 0, after a slow diastolic 
depolarization (Figure 1.8). The rate of depolarization in phase 0 is much slower than 
that in the ventricle myocardial cells, which leads to slow propagation of the cardiac 
impulse in the nodal region.

Figure 1.8 An example of action potential for the SA node cell. The roles of 
pacemaking currents are labelled. The Phase 0 (rapid upstroke), Phase 3 (rapid 
repolarization) and Phase 4 (resting) of the action potential are marked as red. The 
membrane potential at the beginning of Phase 4 is around -60 mV. (Klabunde, 2005)
1.3 The conduction system of the heart

The electrical activity of the heart is a series of electrical excitation waves propagating in the cardiac myocardium (Zhang, 2012). A large amount of previous experimental data has shown that many components of the heart have the ability to generate spontaneous rhythmic action potentials (APs) and to act as a functional pacemaker (Fox, 2002). However, the heart will only follow the primary pacemaker, the sinoatrial node. The SA node is a small and special region of cardiac tissue, which is located in the right atrium, close to the opening of the superior vena cava.

The conduction system (Figure 1.9) of the action potential plays an important role in the proper function of the heart. The abnormal conduction of APs throughout the whole heart will impair the timing and strength of the contraction and affect the cardiac output. The propagation of the AP is not only controlled by the electrophysiology of the different cell types and is also affected by properties such as the size and orientation of cardiac myocytes. A description of the conduction system of APs in the heart is summarised below.

The SA node is capable of generating rhythmic pacemaker activity. Once generated, the pacemaker signal spreads from cell to cell via the gap junctions, through excitable tissue, such atrial muscle. The propagation of electrical impulse from the right atrium to the left atrium is primarily through Bachmann’s bundle, which is a fast conduction pathway leading from the crista terminalis. This pathway allows the right and left atrium to contract at the same time, although the cardiac electrical activity originates from the right atrium. However, the electrical impulse cannot be conducted directly from the atrium to the ventricle, since the two are separated from each other by the fibrous skeleton. Thus, a specialized conducting tissue is required. These tissues contain the atrioventricular node (AV node), bundle of His, and Purkinje fibres. The
atrium and ventricle are electrically coupled by the AV node, which is located in the inferior part of the inter-atrial septum. The AV node is continuous with the atrioventricular bundle, also known as the bundle of His, which allows the electrical impulse to quickly reach either side of the ventricular septum, as well as the Purkinje network. The Purkinje system is a vast, branching network of fibres, spreading to all parts of the ventricular myocardium. This conduction pathway delivers the APs throughout the ventricles almost simultaneously, which excites both ventricles, therefore causing them to contract and eject blood into pulmonary and systemic circulation (MR, 1990).

Figure 1.9 A schematic diagram of the cardiac conduction system. This pathway consists of five elements: the SA node, AV node, the bundle of His, the left and right bundle of branches and the Purkinje fibres. Adapted from Frazier et al. (2000).
1.4 Automatic regulation of the heart

Although the rhythmic contraction of the heart arises from the spontaneous activity of the SA node, the heart rate is also under the regulation of the automatic nervous system, which acts as a control system in an animal’s body by transmitting the signals to and from the body’s different organs (Kerin et al., 1983; Jä nig, 1989). These signals, normally known as hormones, can bind to a special receptor protein in the target cell and affect its physiological activities, thus controlling the behaviour of the target organ (Colman, 2014). The effects of hormones vary from one region to another. In the heart, the hormones related to cardiac activities are mainly released by the sympathetic and parasympathetic regulatory systems, both of which are branches of the automatic nervous system (Robinson et al., 1966). The autonomic regulation of the heart is illustrated in Figure 1.10.

The sympathetic nervous system refers to the release of adrenaline, which is known to increase the heart rate and the force of the contraction of cardiac myocytes (Jakob et al., 1988; Colman, 2014). In this study, we focus on the effect of isoprenaline (ISO), which is structurally and functionally similar to adrenaline. Experimentally, ISO is usually used as a sympathomimetic drug, mimicking the effects of adrenaline. It increases the spontaneous pacing rate and also affects many of the ion channels as well as intracellular Ca$^{2+}$ processes in the cardiac myocyte (Zhang et al., 2012). In this thesis, Chapter 4 provides a detailed description of the electrophysiological effect of ISO on the SA node. In contrast to ISO, the acetylcholine (ACh) released by the parasympathetic nervous system plays an opposite role in pacemaker activities, by reducing the pacing rate (Zhang et al., 2002). Furthermore, ACh also activates the acetylcholine-activated potassium channel, $I_{K,ACh}$, and influences the kinetics and the channel conductance of L-type Ca$^{2+}$ channels and the funny current. The exact effect of ACh on healthy and diseased hearts is discussed in Chapters 4 and 5.
Figure 1.10 A schematic diagram of the autonomic regulation on the heart. The red and blue lines represent its two branches: the sympathetic and parasympathetic regulatory pathways, respectively. CNS: Central nervous system; RA: Right atria; LA: Left atria; RV: Right ventricle; LV: Left ventricle; SA: Sino-atrial node; AV: Atrioventricular node; NE: Norepinephrine; ACh: Acetylcholine. Adapted from Gordan et al. (2015).

1.5 Aim of this study and thesis overview

The mechanisms underlying the initiation and regulation of the cardiac primary pacemaker remains controversial. Previous studies have shown that detailed computational models of cardiac action potentials can act as a fast and accurate tool in researching the underlying mechanisms of cardiac disease (Wilders, 2007; Fink et al., 2011). In addition, genetically modified mice are widely used to mimic and investigate human heart disease, and a large quantity of experimental data relating to the behaviour of murine pacemaker cells, under both healthy and diseased conditions, is available. Therefore, the biophysically detailed model for the murine SA node was chosen as the focus of this thesis to investigate the mechanisms underlying the cardiac pacemaker and its dysfunction. A detailed description of the individual chapters within this thesis is provided below.
Chapter 2: This chapter provides a discussion of how to use a set of ordinary and partial differential equations, to describe cell behaviour, on both single cells and tissue levels. The numerical methods used to solve these equations are discussed. A review of cardiac pacemaker modelling is also included.

Chapter 3: The development of a new computational model regarding the action potential of murine SA node is discussed. The multi-compartment intracellular calcium handling system and a morphologically reconstructed cell geometry were incorporated into an existing murine SA node model (Kharche et al., 2011). The stochastic nature of local Ca\(^{2+}\) dynamics in SA node cells was simulated by Markov chain models and solved by the Monte Carlo method. The model was validated by comparing the simulated results with the experimental observations on the behaviour of intracellular Ca\(^{2+}\) cycling. The mechanisms underlying the automaticity of the SA node were investigated by the model.

Chapter 4: The model developed in Chapter 3 was used as a basis for the simulation of the effects of the sympathetic and parasympathetic regulation on the murine SA node. The regional differences of the SA node were taken into account. The formulations describing the dose-dependency actions of ACh and ISO on various ionic channels and intracellular Ca2+ transients were implemented into the model based on experimental data from the murine SA node. The chronotropic effects of ACh and ISO on cardiac pacemaker cells were studied.

Chapter 5: The newly developed murine SA model was further updated with new isoform-specific formulations for the funny current, based on recent experimental data. Moreover, the single cell model was incorporated into an anatomically detailed 2D model for intact murine SA node-atrium tissue. The model successfully reproduced the initiation and propagation of rhythmic action potentials through the whole right
atrium of the murine model. By using the model, the consequences of the loss-of-function of $I_f$ caused by $HCNI$ deficiency, in generating sick sinus syndrome, were studied on both single cells and at the tissue level. The effect of automatic regulation of this cardiac disease was also taken into consideration.

Chapter 6: The novelty of the models and simulation results obtained in this thesis are summarised in this Chapter. A discussion of possible future work, such as directions for further model development and improvement, is also included.
Chapter 2
Mathematical and physical basis

Due to the limited ability of experimental approaches, a biophysically detailed mathematical model provides an alternative way to explain the relationship between the ionic channels of cardiac myocytes and the action potential, and how this relationship contributes to cardiovascular diseases. In this regard, knowledge of many physical laws and principles is required.

2.1 Mathematical modelling of cardiac action potential

2.1.1 Nernst equilibrium potential

The equilibrium potential is important in cardiac cell modelling, as it not only describes how a potential difference can arise across the cell membrane, even in a balanced state, but also determines the direction of the current flow. The equilibrium potential for an arbitrary ion can be calculated by the Nernst equation.

According to the Boltzmann equation, the relative probabilities \( p_2 \) and \( p_1 \) of state 1 and state 2 is thermal equilibrium:

\[
\frac{p_2}{p_1} = \exp \left( - \frac{u_2 - u_1}{kT} \right)
\]  

(2.1)

where \( T \) is the absolute temperature, \( k \) is the Boltzmann constant, and \( u_2-u_1 \) stands for the energy difference between the two states. The equation can be transferred to a chemical formulation with concentrations, \( c \), and molar energies, \( U \):

\[
\frac{c_2}{c_1} = \exp \left( - \frac{U_2 - U_1}{RT} \right)
\]  

(2.2)

where \( R \) is the molar gas constant.
Then, in the cardiac cell, for a mole of an arbitrary ion, \( X \), separated by the membrane potential difference, \( V_1-V_2 \), the energy difference, \( U_1-U_2 \), could be \( zF(V_1-V_2) \), where \( F \) is the Faraday’s constant and \( z \) stands for the charge of the ion, \( X \). Because the membrane potentials are measured inside and outside, the Nernst equation can be formulated by:

\[
V_X = V_1 - V_2 = \frac{RT}{zF} \ln \left( \frac{[X]_o}{[X]_i} \right)
\]

(2.3)

where \([X]_o\) and \([X]_i\) represent the extracellular and intracellular concentrations, respectively.

### 2.1.2 Hodgkin–Huxley equations

#### 2.1.2.1 Membrane potential

The concentrations of ions on either side of the cell membrane are different, and this disparity leads to an electrochemical gradient. The potential difference across the cell membrane is caused by this electrochemical gradient:

\[
V = \phi_i - \phi_o
\]

(2.4)

where \( \phi_i \) is the intracellular potential, while \( \phi_o \) is the extracellular potential.

The cell membrane is a lipid bilayer, whose width is approximately 5 nm (Lewis and Engelman, 1983). The separation of charge on either side of such a narrow insulating lipid bilayer can be treated as an electrical capacitor across the membrane (Figure 2.1):

\[
V = \frac{Q}{C_m}
\]

(2.5)

where \( C_m \) is the capacitance of the membrane, \( Q \) is the charge of the membrane and \( V \) is the potential difference across the cell membrane.
Figure 2.1 Schematic diagram of the structure of the electric circuit model of the membrane. $C_m$ represents the membrane capacitance. $I_{Na}$, $I_{Ca}$ and $I_K$ indicate the inward Na$^+$ current, Ca$^{2+}$ current and outward K$^+$ current, respectively. $I_b$ is a background current. Modified from Hodgkin and Huxley (1952).

**Resting potential**

At rest, an imbalance of intracellular and extracellular ion concentrations results in a negative potential difference across the membrane. The Nernst equation for a single ion type can be applied to several types of ions:

$$E = \frac{RT}{F} \ln \left( \frac{P_K [K^+]_o + P_{Na} [Na^+]_o + P_{Cl} [Cl^-]_o}{P_K [K^+]_i + P_{Na} [Na^+]_i + P_{Cl} [Cl^-]_i} \right)$$  \hspace{1cm} (2.6)

where $E$ is the equilibrium potential, $P_X$ is the permeability of the membrane for the particular ion, $X$. $[X]_o$ and $[X]_i$ represent the extracellular and intracellular concentrations, respectively.
When all of the ions are taken into consideration, the resting potential for a cardiac cell may vary from -40 mV to -90 mV (Hille, 1978) due to differences in the cell type.

**Action potential**

As the ions move across the cell membrane, through the ionic channel and ionic pump, the membrane potential rises and then rapidly returns to its resting state. That is the action potential. The rate of the change of the membrane potential, from equation (2.5), is given by:

\[
\frac{dV}{dt} = \frac{1}{C} \frac{dQ}{dt}
\]  
(2.7)

which can be rewritten as,

\[
C_m \frac{dV}{dt} = I
\]  
(2.8)

where, \( I = \frac{dQ}{dt}\), is the capacitive current across the membrane.

According to Figure 2.1, two types of current contribute to the total current: the capacitive current, \( I_{\text{cap}} \), and the ionic current, \( I_{\text{ion}} \), and the total current, over a period of time, must be zero,

\[
I_{\text{total}} = I_{\text{cap}} + I_{\text{ion}} = 0
\]  
(2.9)

Therefore, the ionic current should be:

\[
C_m \frac{dV}{dt} = -I_{\text{ion}}
\]  
(2.10)

**2.1.2.2 Membrane current**

Generally, ions move across the membrane either passively, through the ion channels, or actively, through the ion pump and exchangers. Therefore, each type of ion works with different kinetics and moves across the membrane at a different stage of the
action potential. In fact, the ionic current, $I_{\text{ion-total}}$, is the summation of all the ionic currents,

$$I_{\text{ionic-total}} = \sum_{\text{all-ionic}} I_{\text{ionic}}$$

(2.11)

An electrical membrane current is caused by the flow of the ions across the membrane. According to Ohm’s law, the current, $I$, between a potential difference, $V$, is:

$$I = gV$$

(2.12)

where $g$ is the reciprocal of the resistance, or the conductance of the conductor. However, for a special type of ion channel, because of the electrochemical gradient, the Nernst equilibrium potential must be taken into consideration when we calculate the current by Ohm’s law. In this condition, the Nernst equilibrium potential acts as a battery with an electromotive force. It was first introduced by Hodgkin and Huxley (1952), who established that for an ion, $X$, the current becomes:

$$I_X = g(V - E_X)$$

(2.13)

where $g$ is the conductance to a particular ion, $V$ is the membrane potential and $E_X$ is the equilibrium potential for the ion, $X$. Different from a simple Ohm conductor, in this equation, $g$ is nonlinear and the membrane conductance changes with the membrane potential.

Hodgkin and Huxley (1952) published their famous paper describing the ion channels of the squid giant axon, in which the currents from the various ion channels were described mathematically. They also made a sequence of important statements about ion channels which not only resulted in equations to describe action potentials, but also provided essential understanding of the behaviour of the channels themselves.

Hodgkin and Huxley decomposed the conductance, $g$, into the maximum conductance, $g_{\text{max}}$, and parameters representing the fraction, $\gamma$, of the ion channels that are “open”:

$$g = g_{\text{max}} \gamma$$

(2.14)
where the fraction, $\gamma$, may include the activation, deactivation and the inactivation ratios, depending on the gating mechanisms in the channel. The parameters all vary between 0 and 1. For any parameter, if the fraction of gates in a specific state (active or inactive), has a value, $\eta$, then the fraction of gates in the other state would be $(1-\eta)$:

$$
(1-\eta) \xrightarrow{\alpha} \eta \\
(1-\eta) \xleftarrow{\beta} \eta
$$

where $\alpha$ and $\beta$ are the transition rate constants (from one state to the other state).

Therefore, the time evolution of $\eta$ can be defined as:

$$
\frac{d\eta}{dt} = \alpha(1-\eta) - \beta\eta
$$

This equation forms the basis of single cell models used today, with the other ion channels being modelled by the same analogy and their equivalent parameter for $\eta$ being adjusted for the properties of each particular channel.

### 2.1.3 Markov chain models

In addition to the Hodgkin–Huxley models, the channel conductance, $g$, in equation (2.14), can also be expressed by a Markov chain model. It does this by multiplying the maximum channel conductance by a number in an interval [0, 1], representing the fraction of the maximum current, which is similar to the Hodgkin–Huxley model. However, instead of describing a channel varying with several different independent gates, which can change between the open and closed state, it is assumed that the ion channels behave like the Markov processes, which allow for more states and complex state transitions, thus the channel behaviour is more closely associated with the structure of ion channel protein (Wilders, 2007). With this advance, the channel is able to be described in terms of its chemical reaction by the Markov model. Figure 2.2 illustrates an example of a Markov model for L-type $\text{Ca}^{2+}$ channel developed by Jafri
et al. (1998). With this type of model, the channel conductance is calculated by using the sum of the gates in the open state (state O and state $O_{Ca}$ in the figure) instead of the gating parameters in equation (2.14). In this case, the Markov model successfully indicated the interaction between the voltage-dependent activation state and the $Ca^{2+}$-dependent inactivation state of the L-type $Ca^{2+}$ channel. However, these two states are introduced independently in traditional Hodgkin–Huxley equations (DiFrancesco and Noble, 1985). Although the Markov model is capable of describing the conformational state of the ion channel, it is not widely used in cardiac cell modelling as it usually consists of a large set of equations, thereby increasing the computational complexity. In general, the Markov model is only applied in some specific cases such as modelling an ion channel mutation. Clancy and Rudy (1999) developed a Markov model for the $Na^{+}$ channel mutations that are responsible for the long QT syndrome. In this thesis, the Markov chain model is employed to simulate the local $Ca^{2+}$ dynamics (solved by the Monte Carlo method) and the regulation of calsequestrin (CSQN) on RyR activities. A more detailed explanation of this will be provided in Chapter 3.

![Figure 2.2 Example of a Markov chain model for $I_{CaL}$. The channel is open when in state O or $O_{Ca}$. The upper rows represent the voltage dependent processes whereas the bottom states indicate the $Ca^{2+}$ dependent processes corresponding to the states in the upper row. $C_0$ - $C_4$ and $C_{Ca0}$ - $C_{Ca4}$ represent the close state. $\alpha$, $\beta$, $\alpha'$, $\beta'$, $f$, $g$, $f'$ and $g'$ are the transition rate. Adapted from Jafri et al. (1998). Detailed explanation of the symbols will be provided in section 3.2.2.3.](image-url)
2.2 Modelling of action potential propagation

At the tissue level, the electrophysiological interaction between adjacent myocytes relies on the gap junctional ion channels in the intercalated discs. Ions are able to pass into the neighbouring myocytes via these channels, which may cause a depolarization of neighbouring myocytes or may even lead to the initiation of an action potential (if the membrane potential of neighbouring myocytes reaches the threshold for the activation of \( I_{Na} \)). This process allows the propagation of cardiac electrical activity in myocardial tissue. The bidomain equations were developed to describe this process (Tung, 1978). However, the solution of these equations is exceptionally computationally intensive (Vigmond et al., 2008). For this reason the approximation of the bidomain model, the monodomain model, was chosen in this thesis.

2.2.1 Monodomain equation

In the bidomain model, both intracellular and extracellular potential fields are taken into consideration. However, in the monodomain model, the tissue is assumed to be isotropic, which means the anisotropy ratio between the intracellular and extracellular spaces is one (Colli Franzone et al., 2005). Therefore, the propagation of AP in myocardial tissue can be formulated by:

\[
\frac{\partial V}{\partial t} = \nabla \cdot (D \nabla V) - \frac{I_{\text{ion}}}{C_m}
\]  

(2.18)

where \( I_{\text{ion}} \) and \( C_m \) are the same as in equation (2.11), representing the total membrane current and membrane capacitance, respectively, \( \nabla \) is the spatial gradient operator, \( D \) is a tensor of diffusion coefficient, characterizing the spread of electrical potential. As the electrical propagation is related to the cell orientation within tissue fibres (Benson et al., 2008), the spread along the fibres is faster than in the case perpendicular to it,
and the local diffusion tensor, $\tilde{D}$, at a particular point in the local coordinate system, will be heterogeneous and indicated by:

$$\tilde{D} = \begin{pmatrix} D_\parallel & 0 \\ 0 & D_\perp \end{pmatrix}$$  \hspace{1cm} (2.19)$$

where $D_\parallel$ and $D_\perp$ represent the diffusion coefficients in directions along the fibres and perpendicular to the fibres, respectively, and can be obtained experimentally by measuring the conduction velocity in directions parallel to, and perpendicular to, a fibre. It is necessary to translate from the local diffusion tensor, $\tilde{D}$ to the global diffusion tensor, $D$, in equation (2.18), by the following relationship:

$$D = A \tilde{D} A^T$$  \hspace{1cm} (2.20)$$

where $A$ is a matrix made up of the eigenvectors representing the direction of the tissue fibres at each point, and can be given by:

$$A = \begin{pmatrix} f & 0 \\ 0 & s \end{pmatrix}$$  \hspace{1cm} (2.21)$$

where $f$ and $s$ are eigenvectors, corresponding to directions parallel to, and perpendicular to, the tissue fibres, respectively.

### 2.2.2 Boundary conductions

When solving differential equations, it is necessary to define the behaviour of the system at the boundary (Tinker and Lambourne, 2000). In cardiac modelling, the “no-flux” boundary conditions usually applied (Fenton et al., 2005):

$$n.D \nabla V = 0$$  \hspace{1cm} (2.22)$$

where $n$ is a vector normal to the tissue, $D$ and $V$ are the same as in equation (2.18). These conditions indicate that the change in membrane potential with the time is zero at the boundary ($dV/dt = 0$). It is easy to implement the boundary condition in an idealized square tissue, as the normal to the surface is in the coordinate direction.
However, for a realistic tissue, the direction of the normal vector is not unique. Therefore, it is necessary to approximate the normal vector firstly, and then set the change of membrane potential to zero in this direction.

### 2.3 A review of existing SA node cell models

In 1980, Yanagihara et al. (1980) firstly applied the Hodgkin and Huxley model to the cardiac primary pacemaker cells and developed the first cardiac model for the SA node. The Yanagihara et al. (1980) model was relatively simple, with only a few ion channels being considered, including $I_f$, $I_K$ and $I_{Na}$. Since then, more detailed models for the pacemaker cells have been developed, due to an increasing quantity of experimental data and the identification of new ion channels. In 1982, Irisawa and Noma (1982) modified the Yanagihara et al. (1980) model by incorporating the “slow inward current”, $I_{si}$. This current was further developed by being separated into two components, a $Ca^{2+}$-dependent inactivation $Ca^{2+}$ current ($I_{Ca}$) and the sodium-calcium exchanger ($I_{NaCa}$) in the Noble and Noble model (Noble and Noble, 1984). In addition, the sodium-potassium pump ($I_{NaK}$) and the variation of intracellular ion concentrations were also taken into account by Noble and Noble.

In the 1980s, most of the SA node models were multicellular, developed by adapting the Purkinje fibre model (Wilders, 2007). In 1989, the first cardiac model for a single isolated rabbit SA node cell was constructed by Noble et al. (1989). Later on, the pacemaking activity of the heart was described in a more quantitatively accurate way. The function of the $Ca^{2+}$ channel was separately modelled in the term of two components, $I_{CaL}$ and $I_{CaT}$, by Wilders et al. (1991). Demir et al. (1994) incorporated sarcolemmal $Ca^{2+}$ pump current and $Ca^{2+}$ buffering into the SA node model. Moreover, the effects of acetylcholine (ACh) were introduced by both Demir et al. (1994) and Dokos et al. (1996). At the same time, more ion channels were identified.
experimentally. The delayed rectifier potassium channel was also divided, based on different kinetics, into the rapidly activating $I_{Kr}$, and slowly activating $I_{Ks}$ (Lei and Brown, 1996).

In 2000, Zhang et al. (2000) developed the biophysical model for the centre and periphery of rabbit SA node, which successfully introduced regional differences, based on experimental observations of rabbit primary pacemaker myocytes with different cell capacitances, rather than cells isolated from the periphery and centre of the SA node. This is described as a ground-breaking model by Noble et al. (2012). Later on, the model was further developed by incorporating the chronotropic effect of acetylcholine (Zhang et al., 2002) and isoprenaline (Zhang et al., 2012).

The mathematical model for primary pacemaker cells was also improved by Kurata et al. (2002). In his model, several new formulations were implemented, including the incorporation of $I_g$, equations for the voltage- and Ca$^{2+}$-dependent inactivation of $I_{CaL}$, and formulations for the kinetics of $I_{Kr}$, $I_{to}$, $I_{sus}$ and $I_{NaK}$. The intracellular Ca$^{2+}$ dynamics was also included.

In addition, the cardiac primary pacemaker models for different species were also developed. The guinea pig and mouse SA nodes were introduced by Sarai et al. (2003) and Mangoni et al. (2006), respectively. Colman (2014) constructed the human SA node model, based on mRNA data. In 2011, Kharche et al. (2011) developed a model for the action potential of the mouse SA node, which, for the first time, included the molecular basis of the ion channel. In this model, the major ionic currents were expressed in an isoform-specific way, including the sodium channel (consisting of $I_{Na,1.1}$ and $I_{Na,1.5}$), the L-type Ca$^{2+}$ channel (consisting of $I_{CaL,1.2}$ and $I_{CaL,1.3}$) and the T-type Ca$^{2+}$ channel (consisting of $I_{CaT,3.1}$). The comparison of AP characteristics among previous SA node models is summarized in Table 2.1.
Table 2.1 Comparison of APs characteristics among previous SA node cell models

<table>
<thead>
<tr>
<th>Model</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>CL (ms)</th>
<th>APD_{50} (ms)</th>
<th>APD_{100} (ms)</th>
<th>dV/dt_{max} (V/s)</th>
<th>DDR (mV/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bristow and Clark (1982)</td>
<td>-61</td>
<td>73</td>
<td>361</td>
<td>93</td>
<td>181</td>
<td>2.2</td>
<td>69</td>
</tr>
<tr>
<td>Irisawa and Noma (1982)</td>
<td>-66</td>
<td>84</td>
<td>329</td>
<td>73</td>
<td>146</td>
<td>5.2</td>
<td>88</td>
</tr>
<tr>
<td>Noble and Noble (1984) central cell</td>
<td>-61</td>
<td>84</td>
<td>263</td>
<td>70</td>
<td>170</td>
<td>4.7</td>
<td>204</td>
</tr>
<tr>
<td>Noble and Noble (1984) peripheral cell</td>
<td>-73</td>
<td>102</td>
<td>254</td>
<td>55</td>
<td>122</td>
<td>8.1</td>
<td>247</td>
</tr>
<tr>
<td>Noble et al. (1989)</td>
<td>-74</td>
<td>106</td>
<td>169</td>
<td>45</td>
<td>80</td>
<td>13.8</td>
<td>439</td>
</tr>
<tr>
<td>Wilders et al. (1991)</td>
<td>-66</td>
<td>97</td>
<td>388</td>
<td>91</td>
<td>165</td>
<td>7.3</td>
<td>66</td>
</tr>
<tr>
<td>Demir et al. (1994)</td>
<td>-61</td>
<td>96</td>
<td>263</td>
<td>86</td>
<td>125</td>
<td>9.6</td>
<td>194</td>
</tr>
<tr>
<td>Dokos et al. (1996)</td>
<td>-65</td>
<td>81</td>
<td>385</td>
<td>78</td>
<td>135</td>
<td>8.8</td>
<td>53</td>
</tr>
<tr>
<td>Zhang et al. (2000) central cell</td>
<td>-58</td>
<td>79</td>
<td>327</td>
<td>139</td>
<td>214</td>
<td>2.7</td>
<td>183</td>
</tr>
<tr>
<td>Zhang et al. (2000) peripheral cell</td>
<td>-78</td>
<td>104</td>
<td>161</td>
<td>75</td>
<td>105</td>
<td>83.1</td>
<td>418</td>
</tr>
<tr>
<td>Kurata et al. (2002)</td>
<td>-59</td>
<td>75</td>
<td>307</td>
<td>107</td>
<td>186</td>
<td>6.4</td>
<td>170</td>
</tr>
<tr>
<td>Sarai et al. (2003)</td>
<td>-62</td>
<td>77</td>
<td>377</td>
<td>102</td>
<td>171</td>
<td>4.8</td>
<td>97</td>
</tr>
<tr>
<td>Mangoni et al. (2006)</td>
<td>-67</td>
<td>91</td>
<td>212</td>
<td>104</td>
<td>121</td>
<td>5.3</td>
<td>370</td>
</tr>
<tr>
<td>Kharche et al. (2011)</td>
<td>-64</td>
<td>87</td>
<td>212</td>
<td>35</td>
<td>62</td>
<td>9.2</td>
<td>179</td>
</tr>
</tbody>
</table>

For the definitions of the APs characteristics, see the Glossary.
2.4 Numerical methods

As described above, the membrane potential and ion channel currents in the single cell model are deterministic, represented by a series of ordinary differential equations (ODE). Moreover, the monodomain model, used to describe the tissue behaviour in higher dimensions, consists of a partial differential equation (PDE) coupled with a set of ODEs.

For the sake of solving both of these ODEs and PDEs from cardiac models, a number of numerical techniques have been developed, some of which are very simple while others are more complex. In general, a balance between accuracy and computational time is indispensable. In this section, numerical methods used in the model developed in this thesis are discussed.

2.4.1 The forward Euler method

The Euler method is a first-order numerical procedure for solving ordinary differential equations with a given initial value. It is the most basic common method for numerical integration of ordinary differential equations, for example the dynamics of the membrane potential in a cardiac cell. In an initial value ODE problem:

\[
\frac{dy}{dt} = f(t, y)
\]

\[
y(t_0) = y_0
\]

Choose a sufficiently small value, \( h \), for the size of the time step and set:

\[
t_n = t_0 + nh
\]

Now, we can find the value of \( y \) from \( t_n \) to \( t_{n+1} = t_n + h \) is:

\[
y_{n+1} = y_n + hf(t_n, y_n)
\]
Because of the simplicity of the method, solving the ODEs, given the previous state, is computationally light using equation (2.26) and with a sufficiently small \( h \). It is widely used in the solution of single cell level modelling.

### 2.4.2 The fourth order Runge–Kutta method

The fourth order Runge–Kutta method (RK4) is another important method for the approximation of solutions of ODEs. The RK4 method for the same initial problem, as defined for the forward Euler method, is given by the following equations:

\[
y_{n+1} = y_n + \frac{1}{6} (k_1 + 2k_2 + 2k_3 + k_4) \tag{2.27}
\]

\[
t_{n+1} = t_n + h \tag{2.28}
\]

where the variables are defined as:

\[
k_1 = hf(t_n, y_n) \tag{2.29}
\]

\[
k_2 = hf(t_n + \frac{h}{2}, y_n + \frac{h}{2}k_1) \tag{2.30}
\]

\[
k_3 = hf(t_n + \frac{h}{2}, y_n + \frac{h}{2}k_2) \tag{2.31}
\]

\[
k_4 = hf(t_n + h, y_n + hk_3) \tag{2.32}
\]

Thus, the next value \((y_{n+1})\) is determined by the present value \((y_n)\) plus the weighted average of 4 deltas, where:

- \( k_1 \) is the delta based on the slope at the beginning of the interval, using \( y_n \);
- \( k_2 \) is the delta based on the slope at the midpoint of the interval, using \( y_n + \frac{1}{2}k_1 \);
- \( k_3 \) is another delta based on the slope at the midpoint, but now using \( y_n + \frac{1}{2}k_2 \);
- \( k_4 \) is the delta based on the slope at the end of the interval, using \( y_n + k_4 \).
Although the fourth order Rung-Kutta model provides a more accurate solution, it is more computationally intensive than the Euler method. Because of that, it is widely used in single cell models, but is not common in models of high complexity.

### 2.4.3 Finite difference method

The finite difference method (FDM) is a relatively simple and common approach for approximating the solution to a partial differential equation over an N-dimensional space. In order to maintain the accuracy of the solution, the space is divided into a grid with sufficient small node spacings. For example, if a function \( f(x) \) varies with both time and 1D-cable space (with equal point spacing \( \Delta x \)), the approximation of \( f(x) \) around \( x = x_0 \) can be calculated based on Taylor’s theorem:

\[
 f(x_0 + \Delta x) = f(x_0) + \frac{f'(x_0)}{1!} \Delta x + \frac{f''(x_0)}{2!} \Delta x^2 + \ldots + \frac{f^{(n)}(x_0)}{n!} \Delta x^n + R(\Delta x^n)
\]

(2.33)

where \( R(\Delta x^n) \) represents the remain terms. The equation can be rewritten by taking the first two terms:

\[
 f(x_0 + \Delta x) = f(x_0) + f'(x_0) \Delta x + R(\Delta x^2)
\]

(2.34)

Rearranging this equation:

\[
 f'(x_0) = \frac{f(x_0 + \Delta x) - f(x_0)}{\Delta x} - \frac{R(\Delta x^2)}{\Delta x}
\]

(2.35)

If \( R(\Delta x) \) is sufficiently small, the approximation of the first derivative can be given by:

\[
 f'(x_0) \approx \frac{f(x_0 + \Delta x) - f(x_0)}{\Delta x}
\]

(2.36)

The second derivative can be calculated by using the central difference formula:

\[
 \delta[f](x_0) = f \left( x_0 + \frac{\Delta x}{2} \right) - f \left( x_0 - \frac{\Delta x}{2} \right)
\]

(2.37)
as
\[ f''(x_0) \approx \frac{\delta^2[f](x_0)}{\Delta x^2} \]  
(2.38)

Therefore:
\[ f''(x_0) = \frac{f(x_0 + \Delta x) + f(x_0 - \Delta x) - 2f(x_0)}{\Delta x^2} \]  
(2.39)

In this thesis, the FDM is used to update the membrane potential of a myocyte by discretizing the monodomain equation within a histologically reconstructed geometry. Applying equations (2.36) and (2.39) to monodomain formulation, the equation (2.18) can be given by:
\[ \Delta V + \Delta \frac{V_{x+1} + V_{x-1} - 2V_x}{\Delta^2} - \frac{I_{ion}}{C_m} \]  
(2.40)

where \( D, I_{ion}, \) and \( C_m \) are the same as in equation 2.18, \( \Delta x \) and \( \Delta t \) are space and time step, respectively. Therefore, the membrane potential of the \( x \)-th cell at time \( t+\Delta t \) can be calculated by:
\[ V_x^{t+\Delta t} = V_x^t + \Delta t \frac{D}{\Delta^2} (V_{x+\Delta x}^t + V_{x-\Delta x}^t - 2V_x^t) - \frac{\Delta t I_{ion}}{C_m} \]  
(2.41)

It is noteworthy that the choices of the time step (\( \Delta t \)), space step (\( \Delta x \)) and diffusion coefficient (\( D \)) are directly related to the stability of the solution (Iserles, 1996), thus the stability equation must be considered for the monodomain equation to be solved in a stable domain:
\[ \Delta t \leq \frac{\Delta x^2}{2^d D} \]  
(2.42)

where \( d \) represents the number of spatial dimensions.
Chapter 3
Dynamic interaction of membrane and Ca^{2+} clocks in cardiac pacemaking

3.1 Introduction

For the past 50 years, the mechanisms underlying the automaticity of the sinoatrial (SA) node have always been a major topic for cardiac researchers. The initiation of the heartbeat, whether due to intracellular Ca^{2+} activities, the ‘Ca^{2+} clock’ (C clock) or the cell membrane events, the ‘membrane clock’ (M clock), remains controversial.

The theory of the M clock suggests that a set of voltage- and time-dependent transmembrane ionic channels, such as the funny current ($I_f$), the L-type Ca^{2+} current ($I_{Ca,L}$) and the T-type Ca^{2+} current ($I_{Ca,T}$), not only generate the spontaneous action potential, but also determine when the next AP can occur (Lakatta and DiFrancesco, 2009). This hypothesis was validated by the discovery of $I_f$ in the late 1970s (Brown et al., 1979). Based on a wealth of experimental proofs, the $I_f$ is thought to be the most important ion channel responsible for heart rate regulation, even being specifically committed to this function (DiFrancesco and Noble, 2012). However, some recent studies have shown that the activity of the pacemaker originates from another physiological clock, the spontaneous Ca^{2+} release from the sarcolemma reticulum (SR) during the diastolic depolarisation phase that triggers the NCX current and ignites the action potential (Maltsev and Lakatta, 2007). This theory is advanced by some experimental observations of local Ca^{2+} sparks that occur before the initiation of the cell action potential in various mammals, including mice, rabbits and cats (Chen et al., 2009; Li, 2012). Schematic representations of these two theories are shown in Figure 3.1.
Figure 3.1 A schematic illustration of the mechanism underlying the spontaneous activity of the SA node. RyR: ryanodine receptor, SERCA: sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase, SR: sarcolemma reticulum. Adapted from Maltsev et al. (2011).

Based on the M and C clock theories, several mathematical models have been developed in order to identify the exact mechanisms underlying the automaticity of the SA node. Though these models have helped us better to understand the pacemaking mechanisms, they do have some limitations. For example, the C clock in the models does not consider the stochastic features of the intracellular Ca\textsuperscript{2+} handling. As the local Ca\textsuperscript{2+} release is a stochastic event, which occurs in a small reaction space (Cheng and Lederer, 2008), understanding the relationships between the local Ca\textsuperscript{2+} release events and whole-cell functioning in pacemaker cells requires a stochastic multiscale model of the intracellular Ca\textsuperscript{2+} handling dynamics. In addition, the Ca\textsuperscript{2+} release channel on the SR, the RyRs, plays a significant role in cardiac pacemaking. Previous studies have shown that the opening of RyRs is not only associated with cytoplasmic Ca\textsuperscript{2+} concentration, but is also controlled by the luminal Ca\textsuperscript{2+} sensor, such as calsequestrin (CSQN), a kind of SR luminal auxiliary protein (Györke and Györke, 1998). Among existing cardiac mathematical models, only a guinea-pig ventricle model by Gaur et al. (2011) has considered the regulation of CSQN in SR Ca\textsuperscript{2+} release; however, all of the SA node models only treat the CSQN as a Ca\textsuperscript{2+}
buffer in the SR. Therefore, a new generation of mathematical models of SA node cells are required in order to better simulate the pacemaking action potentials and underpin the mechanisms responsible for the automaticity of the SA node cells.

Motivated by this, in this chapter, I sought to: 1) update the existing murine SA model developed by Kharche et al. (2011) to take into account of the regulation of CSQN on RyRs activities; 2) develop a two-dimensional model for a SA node cell that incorporates the spatial distribution and stochastic nature of L-type calcium channels and RyRs channels, and local coupling between Ca\textsuperscript{2+} influx and SR release and cell morphology. To this end, by using this model, I investigated the mechanisms underlying the local Ca\textsuperscript{2+} release during the late phase of diastolic depolarisation and their effects on the spontaneous rhythmic action potential of SA node cells.
3.2 Methods and model development

In this chapter, a mathematical model for the murine primary SA node cell was developed, which considered the spatial geometry of a cardiac SA node myocyte, the spatial distribution of L-type calcium channels and RyRs channels within the cell and the local coupling between Ca\(^{2+}\) influx and SR Ca\(^{2+}\) release. In addition, the stochastic nature of local Ca\(^{2+}\) dynamics in the SA node cells was simulated by Markov chain models of the L-type calcium channels and RyRs channels, which were solved by the Monte Carlo method.

3.2.1 Stochastic simulation for a Markov process

It can be assumed that state transitions in ion channels behave like Markov processes. The probability of changing state does not depend on the amount of time spent in the current state (Li, 2012). Such a process can be illustrated by Figure 3.2 for a simple two-state Markov processes, in which O and C represent the open and closed states, respectively, and \(\alpha\) and \(\beta\) represent the transition rates between the two states. The probability of transition from the open state at time \(t\) to the closed state at time \(t+\Delta t\) is calculated by:

\[
P_{[O\rightarrow C]} = \beta \Delta t
\]  

(3.1)

In order to more accurately simulate the stochastic nature of the ion channel, the Monte Carlo algorithm can be employed to simulate the process of ion channel dynamics carried by Markov processes. In this chapter, the Monte Carlo algorithm was employed to simulate stochastic behaviours of L-type Ca\(^{2+}\) and RyRs channels.
Figure 3.2 Schematic diagram of a simple two-state Markov chain model. The model only consists of two states: open (O) and closed (C). $\alpha$ and $\beta$ represent the transition rates between these two states.

The Monte Carlo simulation for Markov processes operates as follows for one global time step:

**Initialisation:** it is necessary to set the total number of ion channels of each channel type and the initial state for each single channel. In this chapter, 2680 L-type Ca$^{2+}$ channels and 15,640 RyRs channels were employed to simulate the stochastic nature of Ca$^{2+}$ cycling, based on the stoichiometry of the coupling between them (Gaur et al., 2011; Li, 2012). A more detailed explanation will be provided in Section 3.2.2.

**Random number generation:** the essential aspect of the Monte Carlo algorithm is the generation of a random number, in the interval $[0, 1]$ with a uniform distribution, which decides the state of the channel in the next time step based on the probability calculated by Equation 3.1. For example, when a channel is in an open state, if the random number associated with the channel is in an interval $[0, \beta \Delta t]$, the channel will transit into a closed state in the next time step, whilst if the random number is in the interval between $\beta \Delta t$ and 1, the channel will stay in the open state.

**Update the state:** in this step, the time is advanced to $t+\Delta t$, and the state of the channel is updated based on the last step.

**Iterate:** unless the simulation is finished, the process returns to Step 2 (random number generation).

Therefore, the current density of the channel is calculated based on the fraction of open channels during the simulation time.
3.2.2 Model development

Based on the results of the previous chapter’s analysis of existing models of murine primary pacemakers, Kharche’s model (Kharche et al., 2011) was chosen as a base for the new murine SA node model developed in this part of the study. The detailed multi-compartment intracellular Ca\(^{2+}\) handling system and cell morphology were taken into account. The formulations of the L-type Ca\(^{2+}\) channel developed by Jafri et al. (1998) and RyRs channels developed by Gaur et al. (2011) were adapted in the model. The new model considered a two-dimensional Ca\(^{2+}\) handling lattice based on a real cell geometry.

3.2.2.1 Cell model

Figure 3.3 illustrates the schematic representation of the cell model developed in this chapter. The model considered 15 membrane ion channel currents, among which 13 were passive ionic channels, one was an ionic pump and the other was an ion exchanger. The membrane potential is computed by the following equation:

$$\frac{dV}{dt} = -\frac{1}{C_m} (I_{Na,1,1} + I_{Na,1,5} + I_{CaL,1,2} + I_{CaL,1,3} + I_{CaT} + I_{Kr} + I_{Ks} + I_{K1} + I_f + I_{sus} + I_{to} + I_{st} + I_{NaK} + I_{NaCa} + I_b)$$  \hspace{1cm} (3.2)

where \(C_m\) is the cell capacitance. Similar to other existing models, the membrane potential and most of the ion channel currents in this model are deterministic, represented by ordinary differential equations, except for the Ca\(_V\)1.3 L-type calcium current, which is processed separately and stochastically. Details of definitions of the variables, model equations and relevant parameters are provided in the Appendix.

In order to describe the detailed intracellular Ca\(^{2+}\) activity, the intracellular space of a murine SA node cell with a length of 70.2 \(\mu\)m and a width of 23.4 \(\mu\)m is discretised into a two-dimensional lattice by a spatial resolution of 1.3 \(\mu\)m. This formed a lattice.
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of 391 elements (Detailed explanation will be provided in section 3.2.2.5), each of which consisted of the following Ca\(^{2+}\) cycling components: myoplasmic space, submembrane space, junctional SR (JSR), and network SR (NSR). As the murine SA node cells do not have T-tubules, only the peripheral elements (orange part in Figure 3.3) that are adjacent to the cell membrane surface couple directly with the membrane ionic currents, pumps and exchangers, whereas the elements in the central region (blue part in Figure 3.3) do not. Each Ca\(^{2+}\) element communicates with its neighbouring elements via Ca\(^{2+}\) diffusion in the myoplasmic space and the NSR.

Figure 3.3 Schematic illustration of the murine SA node cell model with a detailed multi-compartment intracellular Ca\(^{2+}\) handling system. The model consisted of two types of Ca\(^{2+}\) release elements: peripheral couplings (blue part) and internal extended JSR (orange part). All of the Ca\(^{2+}\) release elements were made up of the following Ca\(^{2+}\) cycling components: myoplasmic space, submembrane space, JSR and NSR. Each Ca\(^{2+}\) element communicates with its neighbouring elements via Ca\(^{2+}\) diffusion in the myoplasmic space and the NSR. The ionic currents, pumps and exchangers on the cell membrane couple with the Ca\(^{2+}\) release elements (marked by the orange box) in peripheral regions of the cell.
3.2.2.2 Ca\textsuperscript{2+} release element

Previous studies have shown that ‘Ca\textsuperscript{2+} sparks’ in cardiac myocytes, generated by the opening of SR release channels (ryanodine receptors), are ‘elementary’ Ca\textsuperscript{2+} release events (Smith et al., 1998). It has also been reported that cardiac myocytes have three different Ca\textsuperscript{2+} release categories: 1) dyads, spaces between the JSR and T-tubules; 2) peripheral couplings, associations between the JSR and cell membrane; and 3) internal extended JSR, which are not linked with the cell membrane or T-tubules (Perni et al., 2012). In the first two situations, the Ca\textsuperscript{2+} fluxes through RyRs are mainly triggered by the activation of L-type calcium channels in response to the membrane potential. However, the Ca\textsuperscript{2+} release can also happen spontaneously without a trigger from the L-type calcium channel (Cheng and Lederer, 2008). In this chapter, our model considered two types of Ca\textsuperscript{2+} release elements – peripheral couplings (blue part in Figure 3.3) and internal extended JSR (orange part in Figure 3.3), because of the absence of T-tubules in murine SA node cells.

For L-type Ca\textsuperscript{2+} channels, Ca\textsubscript{V}1.2 and Ca\textsubscript{V}1.3 are expressed in murine SA nodes. A recent study showed that the subcellular distributions of these two isoforms in murine SA node cells are distinct: Ca\textsubscript{V}1.3 is closely co-localised with the RyRs in fluorescence microscopy, whereas Ca\textsubscript{V}1.2 is only limited on cell membranes, which may suggest a different contribution to the pacemaking function (Christel et al., 2012). In addition, the role of Ca\textsubscript{V}3.1-mediated T-type Ca\textsuperscript{2+} channels in SA node calcium handing also remains controversial. Mangoni et al. (2006) found a marked reduction of pacing rate in Ca\textsubscript{V}3.1-knockout mice, which was associated with a prolonged diastolic depolarisation phase as shown in some simulation results, which indicates that $I_{CaT}$ is associated with intracellular Ca\textsuperscript{2+} signalling and therefore contributes to cardiac pacemaking. Experiments in latent pacemaker cells by Hüser et al. (2000) support this view, as it was shown that diastolic Ca\textsuperscript{2+} sparks were observed by activation of T-type Ca\textsuperscript{2+} channels, and these disappeared when an $I_{CaT}$ blocker was applied. However,
similar experimental observations were not observed in rabbit SA node cells (Vinogradova et al., 2004).

To summarize, in consideration of the co-distribution between \( \text{Ca}_{V}1.3 \) channels and RyRs and the potential contribution of T-type \( \text{Ca}^{2+} \) channels, our model assumes, as illustrated in Figure 3.4, both \( I_{\text{CaL},1.3} \) and \( I_{\text{CaT}} \) act as triggers of RyRs that contribute to the \( \text{Ca}^{2+} \) concentration of the subspace in peripheral-coupling \( \text{Ca}^{2+} \) release elements, \( I_{\text{CaL},1.2} \) and \( I_{b\text{Ca}} \) (background \( \text{Ca}^{2+} \) current) contribute to the \( \text{Ca}^{2+} \) of the myoplasm in each peripheral \( \text{Ca}^{2+} \) element, which differs from the previous models (Kharche et al., 2011).

In a single \( \text{Ca}^{2+} \) release element, \( \text{Ca}^{2+} \) concentrations in myoplasm and NSR are supposed to be spatially uniform. \( \text{Ca}^{2+} \) fluxes within the element are shown in Figure 3.3. \( \text{Ca}^{2+} \) diffusion from subspace to myoplasm is modelled by:

\[
J_{\text{Cadiff}_\text{sub}} = \frac{[\text{Ca}^{2+}]_{\text{sub}} - [\text{Ca}^{2+}]_{\text{i}}}{\tau_{\text{Cadiff}_\text{sub}}} \quad (3.3)
\]

Similarly, the \( \text{Ca}^{2+} \) transition between the JSR and the NSR is modelled by:

\[
J_{\text{Cadiff}_\text{jsr}} = \frac{[\text{Ca}^{2+}]_{\text{jsr}} - [\text{Ca}^{2+}]_{\text{jsri}}}{\tau_{\text{Cadiff}_\text{jsr}}} \quad (3.4)
\]

where \( \tau_{\text{Cadiff}_\text{sub}} \) and \( \tau_{\text{Cadiff}_\text{jsr}} \) are the time constants for \( \text{Ca}^{2+} \) diffusion.

Inter-element coupling occurs via \( \text{Ca}^{2+} \) diffusion in both myoplasm and NSR, as shown in Figure 3.3. The inter-element \( \text{Ca}^{2+} \) diffusion is modelled by:

\[
J_{\text{Cadiff}_\text{i}} = D_{i} \nabla^{2}[\text{Ca}^{2+}]_{i} \quad (3.5)
\]

\[
J_{\text{Cadiff}_\text{nsr}} = D_{\text{nsr}} \nabla^{2}[\text{Ca}^{2+}]_{\text{nsr}} \quad (3.6)
\]
where $D_i$ and $D_{nsr}$ represent the diffusion coefficient for free $\text{Ca}^{2+}$ in myoplasm and NSR, respectively. Because of the rapid diffusion of $\text{Ca}^{2+}$ in SR, $D_i$ is assumed to be much smaller than $D_{nsr}$ in the model.

Figure 3.4 Schematic diagram of a single $\text{Ca}^{2+}$ release element in the peripheral region of the SA node cell. As the peripheral elements are adjacent to the cell membrane surface, these elements couple directly with the membrane ionic currents, pumps and exchangers. The CSQN is assumed to act not only as a SR $\text{Ca}^{2+}$ buffer, but also as a SR $\text{Ca}^{2+}$ release regulator in the model.

### 3.2.2.3 L-type $\text{Ca}^{2+}$ channel, $I_{\text{CaL}}$

The L-type calcium channel in murine SA nodes consists of two different isoforms, which are expressed in low quantities by $\text{CaV1.2}$ and highly expressed by $\text{CaV1.3}$ (Mangoni et al., 2003, 2006). The electrophysiological properties of these two isoforms and their contributions to the whole-cell action potentials have been investigated by using the Hodgkin–Huxley model in previous studies (Kharche et al., 2011). In this chapter, the L-type calcium channel was simulated by a 12-state Markov chain model, which is adapted from Jafri et al. (1998) for $\text{CaV1.2}$- and $\text{CaV1.3}$-mediated $I_{\text{CaL}}$, respectively. Specifically, as described above, $I_{\text{CaL,1.2}}$ was represented by a set of ODEs, whereas the whole-cell $I_{\text{CaL,1.3}}$ was computed by the summation of individual unitary $\text{CaV1.3}$ L-type calcium currents.
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Figure 3.5 Schematic diagram of the 12-state Markov chain mode of an L-type Ca^{2+} channel. \( I_{CaL} \) is activated via a series of voltage- and Ca^{2+}-induced processes from closed states to open states. The upper row indicates the six state transitions under the normal mode, representing the voltage-dependent activation processes, whereas the bottom row of states represent the Ca^{2+}-dependent inactivation processes corresponding to the normal mode in the upper row. With the increase of [Ca^{2+}]_{i} or [Ca^{2+}]_{sub}, the state of the L-type Ca^{2+} channel will switch from the upper row to the bottom row.

The developed model of the L-type Ca^{2+} channel current consisted of three components: the voltage-dependent activation process; the voltage-dependent inactivation process; and the Ca^{2+}-dependent inactivation process (Rice et al., 1999). Specifically, in the model, the voltage-dependent activation and Ca^{2+}-dependent inactivation processes were described by a 12-state Markov chain model, which is illustrated in Figure 3.5. The Model Normal (the upper rows of the states) represents the voltage-dependent activation of the L-type Ca^{2+} channels, consisting of five closed states (C_{0} to C_{4}) and one open state (O). The five closed states (C_{0} to C_{4}) on the top and a subset of closed states (C_{Ca0} to C_{Ca4}) on the bottom correspond to four independent subunits of L-type Ca^{2+} channels (Jafri et al., 1998), which can open and close the channel. The transition rate of the closed states, \( \alpha \) and \( \beta \), are formulated by:

\[
\alpha_{CaL,1,2} = 1.0085e^{(-0.022(V-0.0504))} \quad (3.7)
\]
\[
\beta_{CaL,1,2} = 0.224e^{(-0.076(V-9.68))} \quad (3.8)
\]
\[ \alpha_{Ca_{1.3}} = 4.732e^{(0.011(V+98.65))} \quad (3.9) \]
\[ \beta_{Ca_{1.3}} = 0.193e^{(-0.0616(V-25.828))} \quad (3.10) \]

where \( V \) is the membrane potential. In addition, \( f \) and \( g \) represent the rate constants for transitions between the closed state and open state.

Similarly, the \( Ca^{2+} \)-dependent inactivation process is illustrated by the bottom row of the states, Model Ca. The transition rate of the closed states, \( \alpha' \) and \( \beta' \), are based on the rate in Model Normal:

\[ \alpha' = a\alpha \quad (3.11) \]
\[ \beta' = \frac{\beta}{b} \quad (3.12) \]

where \( a \) and \( b \) are model transition constants. \( f' \) and \( g' \) represent the rate constants between the closed state and open state for \( Ca^{2+} \)-dependent inactivation. Significantly, \( f' \) is much smaller than \( f \), which means Model Ca opens rarely, greatly increasing the \( Ca^{2+} \)-dependent inactivation of the channel (Rice et al., 1999).

**Table 3.1 Parameters of L-type Ca\(^{2+}\) channels.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>( \text{Ca}_1.2 )</th>
<th>( \text{Ca}_1.3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a )</td>
<td>Mode transition parameter</td>
<td>2.027</td>
<td>1.994</td>
</tr>
<tr>
<td>( b )</td>
<td>Mode transition parameter</td>
<td>1.936</td>
<td>1.936</td>
</tr>
<tr>
<td>( P_{Ca} )</td>
<td>The permeability of the unitary L-type ( Ca^{2+} ) channel</td>
<td>0.098</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Modified from Jafri et al. (1998)
The transition between Model Normal and Model Ca is controlled by rate $\gamma$ and $\omega$, which were formulated by:

$$\gamma_{CaL,1.2} = 0.0234 [Ca^{2+}]_i$$  \hspace{1cm} (3.13)  

$$\gamma_{CaL,1.3} = 0.0533 [Ca^{2+}]_{sub}$$  \hspace{1cm} (3.14)  

$$\omega_{CaL,1.2} = \omega_{CaL,1.3} = 0.02158$$  \hspace{1cm} (3.15)  

where $\gamma_{CaL,1.2}$ and $\omega_{CaL,1.2}$ represent transition rate for $I_{CaL,1.2}$; $\gamma_{CaL,1.3}$ and $\omega_{CaL,1.3}$ are transition rate for $I_{CaL,1.3}$; the $[Ca^{2+}]_i$ and $[Ca^{2+}]_{sub}$ are the Ca$^{2+}$ concentrations in the myoplasm and local subsarcolemmal space, respectively. With the increase of $[Ca^{2+}]_i$ or $[Ca^{2+}]_{sub}$, the state of the L-type Ca$^{2+}$ channel will switch from Model Normal to Model Ca.

In addition, the voltage-dependent inactivation ($y_{CaL,1.2}$ and $y_{CaL,1.3}$) is simulated by a Hodgkin and Huxley-type gate model:

$$\frac{dy}{dt} = \frac{y_{\infty} - y}{\tau_y}$$  \hspace{1cm} (3.16)  

$$y_{CaL,1.2}^{\infty} = \frac{1.0}{1.0 + e^{\frac{[V+36.0/4.6]}{\sqrt{2}}} \frac{0.6}{0.00512e^{-V/39.8} + 0.00653}}$$  \hspace{1cm} (3.17)  

$$\tau_{CaL,1.2} = 7.4 + 45.77e^{-0.5(V+28.4/11.0)}$$  \hspace{1cm} (3.18)  

$$y_{CaL,1.3}^{\infty} = \frac{1.0}{1.0 + e^{\frac{[V+35.0/7.3]}{\sqrt{2}}}}$$  \hspace{1cm} (3.19)  

$$\tau_{CaL,1.3} = 7.4 + 45.77e^{-0.5(V+28.4/11.0)}$$  \hspace{1cm} (3.20)  

where $\tau_{CaL,1.2}$ and $\tau_{CaL,1.3}$ are the time constant for $y_{CaL,1.2}$ and $y_{CaL,1.3}$, respectively. The steady state of Cav1.2 is based on experimental data (Mangoni et al., 2003). The steady state of Cav1.3 and the time kinetics are adopted from a study by Kharche et al. (2011). Values for other parameters are listed in Table 3.1.
Therefore, the whole-cell Ca\textsubscript{V.1.2} L-type current is formulated by:

\[ I_{\text{Cal.1.2}} = P_{\text{Cal.1.2}} O_{\text{Cal.1.2}} (O_{\text{Cal.1.2}} + O_{\text{Cal.1.2}}) \frac{4VF^2}{RT} [\text{Ca}^{2+}] e^{\frac{3V}{RT}} - 0.34 [\text{Ca}^{2+}]. \]  

(3.21)

and each unitary Ca\textsubscript{V.1.3} L-type Ca\textsuperscript{2+} current can be computed by:

\[ i_{\text{Cal.1.3}} = P_{\text{Cal.1.3}} O_{\text{Cal.1.3}} (O_{\text{Cal.1.3}} + O_{\text{Cal.1.3}}) \frac{4VF^2}{RT} [\text{Ca}^{2+}]_{\text{sub}} e^{\frac{3V}{RT}} - 0.34 [\text{Ca}^{2+}]. \]  

(3.22)

where \( P_{\text{cal}} \) is the permeability of the unitary L-type Ca\textsuperscript{2+} channel. \( O_y, O \) and \( O_{\text{Ca}} \) represent the open state of the voltage-dependent inactivation, voltage-dependent activation and Ca\textsuperscript{2+}-dependent inactivation, respectively.

The whole-cell Ca\textsubscript{V.1.3} L-type Ca\textsuperscript{2+} current is the summation of all unitary Ca\textsubscript{V.1.3} L-type Ca\textsuperscript{2+} currents, which can be computed by:

\[ I_{\text{Cal.1.3}} = \sum_{i=1}^{N_{\text{tot}}} \frac{N_{\text{tot}}}{N_{\text{channel}}} \sum_{i=1}^{N_{\text{channel}}} I_{\text{Cal.1.3}} \]  

(3.23)

where \( N_{\text{tot}} \) is the total number of the L-type Ca\textsuperscript{2+} channels and \( N_{\text{channels}} \) represents the number of L-type Ca\textsuperscript{2+} channels employed in the simulation.

**Simulation results**

The unitary Ca\textsubscript{V.1.3} L-type Ca\textsuperscript{2+} current model was solved by the Monte Carlo method (described in the previous section), which determines the state of the current in the next step based on the transition rates shown above. Figure 3.6 shows the simulation results for the current traces of five unitary L-type Ca\textsuperscript{2+} channels (I to V), recorded under two different voltages. The downward trace represents the channel that activates from a closed state to an open state. It also shows that the activation of the unitary channel is discontinuous and stochastic. At -10 mV, the channel activates frequently but with a small amplitude (Figure 3.6B). By contrast, at more negative potential (-30 mV), the amplitude of the current is higher and the re-opening of the
Chapter 3 Dynamic interation of membrane and Ca\(^{2+}\) clocks in cardiac pacemaking

channel is infrequent (Figure 3.6A). Similar experimental observations were found in previous studies (Josephson et al., 2010; Rose et al., 1992).

![Figure 3.6 Simulated unitary Ca\(_V\)1.3 L-type Ca\(^{2+}\) currents corresponding to varying testing potentials from a holding potential of -60 mV. (A & B) Time traces of unitary Ca\(^{2+}\) currents in response to 300-ms test pulses of -30 mV and -10 mV, respectively.](image)

The whole-cell current trace and I–V relationships for both Ca\(_V\)1.2 and Ca\(_V\)1.3 are illustrated in Figure 3.7. In the simulations, the L-type Ca\(^{2+}\) current was recorded from a holding potential of -60 mV, by applying depolarising voltage steps lasting 300 ms and varying from -50 mV to 50 mV with a 10-mV increment. Similar to the experimental results of Christel et al. (2012), both of the two isoforms demonstrated rapid activation and relatively slow inactivation (Figure 3.7A(ii) and Figure 3.7B(ii)). Meanwhile, the Ca\(_V\)1.3 currents inactivated more slowly than the Ca\(_V\)1.2 currents. The I–V relationships for the whole-cell L-type current is shown in Figure 3.7A(i) and Figure 3.7B(i). The maximal value (negative) of Ca\(_V\)1.2 is at approximately 10 mV,
whilst Cav1.3 reaches the peak at a testing potential of -10 mV, both of which are also consistent with the experimental observations by Mangoni et al. (2003).

Figure 3.7 Simulated I–V (i) relationship of whole-cell L-type Ca$^{2+}$ currents under voltage clamp protocols (ii). In this study, the L-type Ca$^{2+}$ current was recorded from a holding potential of -60 mV by applying a series of testing potentials lasting 300 ms, varying from -50 mV to 50 mV with a 10-mV increment. The time traces and I–V relationships of $I_{CaL,1.2}$ and $I_{CaL,1.3}$ are shown in (A) and (B), respectively. Simulation data were validated against experimental data by Mangoni et al. (2003).

3.2.2.4 SR Ca$^{2+}$ release channel, RyRs

The RyRs is a cluster of Ca$^{2+}$ release channels located in the JSR. The molecular basis of RyRs regulation is complicated. Previous studies have demonstrated that the activity of the RyRs can be controlled not only by Ca$^{2+}$, but also by a series of cytosol ligands, including CSQN and Mg$^{2+}$ (Győrke and Győrke, 1998).
As a Ca\(^{2+}\)-binding protein, the CSQN has two different forms in the SR, which are free and Ca-bound CSQN. At a low \([\text{Ca}\(^{2+}\)]_{\text{SR}}\), the Ca\(^{2+}\)-free CSQN inhibits the RyRs channel, which is mediated by another two SR luminal auxiliary proteins, triadin 1 and junctin (Györke et al., 2004). During the \([\text{Ca}\(^{2+}\)]_{\text{SR}}\) release, a large quantity of RyRs channels may switch from a CSQN-uninhibited state to a CSQN-inhibited state, as the Ca\(^{2+}\)-free CSQN level increases. At the same time, the CSQN can also act as a buffer in the SR (Gaur and Rudy, 2011; MacLennan and Wong, 1971). Apart from the CSQN, the magnitude of intracellular \([\text{Mg}\(^{2+}\)]\) also affects the activities of the RyRs channel. Research shows that the adaptation of RyRs could be very slow in the absence of Mg\(^{2+}\) (Valdivia et al., 1995).

Figure 3.8 Schematic diagram of a four-state Markov chain mode of a RyRs channel. The upper and lower tiers indicate the activation and refractory processes. The transition between the upper tier and the lower tier relies on the concentration of CSQN. O1 and O2 are the open states, C1 and C2 are the closed states. Image from Gaur and Rudy (2011).

In this chapter, the functional mechanisms of a single RyR schannel are simulated by a four-state Markov chain model, which is based on a formulation by Gaur et al. (2011). The dynamics of a single RyRs, illustrated in Figure 3.8, include four states and two tiers of model gating. The upper and lower rows of the states represent the activation tier and refractory tier, respectively. The transition rates between the open state (O1 and O2) and closed state (C1 and C2) were adapted from Sobie et al. (2002).
The activation rates \( k_{C1O1} \) and \( k_{C2O2} \) are a function of the local subsarcolemmal Ca\(^{2+}\) concentrations, which are formulated by:

\[
k_{C1O1} = (1 + \frac{[Mg]^{1.1}}{(Km_{Mg})^{1.1}})([Ca^{2+}]_{sub})^{3.87} + (Km_{upper})^{3.87}
\] (3.24)

\[
k_{C2O2} = (1 + \frac{[Mg]^{1.1}}{(Km_{Mg})^{1.1}})([Ca^{2+}]_{sub})^{1.87} + (Km_{lower})^{1.87}
\] (3.25)

where \( Km_{upper} \) and \( Km_{lower} \) are the constants for Ca\(^{2+}\)-dependent activation for SR Ca\(^{2+}\) release. \( Km_{Mg} \) represents the constant for Mg\(^{2+}\)-dependent activation for SR Ca\(^{2+}\) release.

The transition between the activation tier and refractory tier is mainly controlled by CSQN. The transition rate from the activation to the refractory tier corresponds to the magnitude of Ca\(^{2+}\)-free CSQN, whereas the rate from the refractory to the activation tier is related to the proportion of Ca\(^{2+}\)-bound CSQN:

\[
k_{C1C2} = k_{O1O2} = 0.1 \frac{csqn_{free}}{csqn_{total}}
\] (3.26)

\[
k_{C2C1} = k_{O2O1} = 0.1 \frac{csqn_{Ca}}{csqn_{total}}
\] (3.27)

The dynamics of CSQN, with a total capacity of absorbing 10 mM Ca\(^{2+}\) ions, is adapted from Kharche et al. (2011):

\[
\frac{df_{csqn,Ca}}{dt} = k_{f_{csqn,Ca}} [Ca^{2+}]_{JSR} \left(1.0 - f_{csqn-Ca}\right) - k_{b_{csqn,Ca}} f_{csqn-Ca}
\] (3.28)

The state of individual RyRs channels is determine by the Monte Carlo method based on the transition rate describe above. The Ca\(^{2+}\) fluxes via RyRs channels are
computed based on the number of open RyRs. Therefore, the Ca\textsuperscript{2+} fluxes of individual RyRs channels can be calculated by:

\[ J_{\text{rr}} = k s(O_1 + O_2) ([Ca^{2+}]_{\text{JSR}} - [Ca^{2+}]_{\text{sub}}) \] (3.29)

where \( k s \) is the conductance of the RyRs channel and \([Ca^{2+}]_{\text{sub}}\) and \([Ca^{2+}]_{\text{JSR}}\) represent the subspace Ca\textsuperscript{2+} concentration and JSR Ca\textsuperscript{2+} concentration, respectively.

**Simulation result of RyRs responses to cytosolic and SR Ca\textsuperscript{2+}**

The activation of a single RyRs channel is tested at different SR Ca\textsuperscript{2+} concentrations. Figure 3.9A shows simulated single RyRs channel records for 20 μM and 1.0 mM \([Ca^{2+}]_{\text{SR}}\). The upwards trace in the figure represents the transition of a single RyRs channel from a closed state to an open state. At a higher \([Ca^{2+}]_{\text{SR}}\) (1.0 mM), the RyRs channel is activated rapidly and can remain in the open state for a while. However, the open probability (\( P_o \)) of a single RyRs is lower at 20 μM \([Ca^{2+}]_{\text{SR}}\). Figure 3.9B shows the relationship between the open probability of a single RyRs and \([Ca^{2+}]_{\text{SR}}\) under a fixed Ca\textsuperscript{2+} concentration in the cytosol. In a certain range of \([Ca^{2+}]_{\text{SR}}\), the open probability increases with the growth of \([Ca^{2+}]_{\text{SR}}\). The simulation results are consistent with previous experimental observations (Györke and Györke, 1998).

---

**Figure 3.9** Properties of a single RyRs channel. (A) Simulated single-channel current records of RyRs in response to 20 μM and 1.0 mM of \([Ca^{2+}]_{\text{SR}}\). The \([Ca^{2+}]_{\text{i}}\) or \([Ca^{2+}]_{\text{sub}}\) was held at 0.1 μM. (B) Open probability of a single RyRs channel in response to \([Ca^{2+}]_{\text{SR}}\) varying from 0.001 to 10 mM.
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The activity of a cluster of RyRs channels is assessed by varying the cytosolic Ca\(^{2+}\) concentration (0.1, 0.3, 0.5, 1.0 and 2.0 μM) and the simulation results are shown in Figure 3.10. The model behaviour is similar to that observed in a previous experiment (Györke and Fill, 1993), as the RyRs channels activate rapidly to the peak, followed by a slow inactivation (Figure 3.10A). Figure 3.10B shows the simulation and experimental results (Györke and Fill, 1993) for the open probabilities of the RyRs channels at peak and steady state with different [Ca\(^{2+}\)]\(_{cyto}\). The channel reaches the maximum peak open probability (approximately 0.95) at 1.0 μM [Ca\(^{2+}\)]\(_{cyto}\) or above, while the number for the steady state is close to 10.0 μM.

![Figure 3.10 Properties of macroscopic Ca\(^{2+}\) release from the SR. (A) Simulated timetraces of macroscopic Ca\(^{2+}\) release in response to different concentrations of intracellular Ca\(^{2+}\) (0.1, 0.3, 0.5, 1.0 and 2.0 μM). The time traces were recorded and shown as the open probability for 10,000 RyRs channels. (B) The open probabilities of the RyRs channels at peak and steady state with the different [Ca\(^{2+}\)], in (A). The black solid and red dashed lines represent the peak and steady state generated by the model, respectively. Experimental data by Györke and Fill (1993) are represented by black filled circles and red open circles.](image)

### 3.2.2.5 Cell architecture and morphology

The shape of an isolated murine SA node cell can vary from one to another, which has also been observed in the rabbit and the guinea-pig heart (Mangoni and Nargeot, 2001). Figure 3.11 illustrates four different morphological types of murine SA node
cell: ‘spindle’, ‘elongated spindle’, ‘spider’ and atrial-like cells. The range of the cell volume is from 0.9 pL to 5.0 pL (Kharche et al., 2011).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length (μm)</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho et al. (2003)</td>
<td>87.4 ± 1.0</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>Mangoni et al. (2003)</td>
<td>51.9 ± 1.6</td>
<td>3.0 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>107.6 ± 1.6</td>
<td>2.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>88.5 ± 1.6</td>
<td>3.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>62.7 ± 1.6</td>
<td>14.9 ± 1.6</td>
</tr>
<tr>
<td>Lei et al. (2004)</td>
<td>65.0 ± 1.8</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>200.0 ± 1.8</td>
<td>20.1 ± 1.8</td>
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<tr>
<td></td>
<td>54.6 ± 1.8</td>
<td>7.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>84.1 ± 1.8</td>
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<td></td>
<td>87.2 ± 1.8</td>
<td>8.7 ± 1.8</td>
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<tr>
<td></td>
<td>104.5 ± 1.8</td>
<td>5.6 ± 1.8</td>
</tr>
<tr>
<td>Lei et al. (2005)</td>
<td>61.8 ± 1.0</td>
<td>3.1 ± 1.0</td>
</tr>
</tbody>
</table>

In this chapter, the two-dimensional model of Ca$^{2+}$ cycling in the murine SA node cell is based on a morphological geometry reconstructed from a confocal microscopy image of a ‘spindle’-like SA node cell, as shown in Figure 3.12. The image was discretised by a spatial resolution based on the distance between two neighbouring Ca$^{2+}$ release sites. Previous studies have shown that the average distance between two Ca$^{2+}$ release units in the sarcolemma of atrial and ventricular myocytes is 1.69 ± 0.41 μm and 1.87 ± 0.18 μm, respectively (Chen-Izu et al., 2006). The immuno fluorescence staining images by Maltsev et al. (2011) showed that the value in rabbit SA node cells is approximately 1.3 μm. Therefore, the geometry was discretised by a spatial
resolution of 1.3 μm, forming a regular Cartesian grid of 54 × 18 nodes, each of which representing a Ca^{2+} release element in the SA node cell.

Figure 3.11 Different cell morphologies of murine SA nodes. (a) Spindle-shaped cell; (b) elongated spindle-shaped cell; (c) spider cell; and (d) atrial-like-shaped cell. Reproduced from Mangoni and Nargeot (2001).

Figure 3.12 Cell geometry. Upper panel: confocal whole-cell image (Maltsev and Lakatta, 2007). Lower panel: morphologically reconstructed cell geometry.
3.3 Results

The newly developed model of murine SA node cells was used to investigate the ionic mechanisms underlying the initiation of cardiac pacemaking action potentials and the effects of the stochastic nature of local Ca\(^{2+}\) dynamics on the whole-cell action potential. In this section, simulation results will be presented for: 1) the normal electrophysiological properties of Ca\(^{2+}\) releases at unitary element and whole-cell levels; 2) the study of the mechanisms responsible for spontaneous rhythmic activities in the SA node cells.

3.3.1 Whole-cell Ca\(^{2+}\) currents and Ca\(^{2+}\) concentrations during AP

Figure 3.13 shows the simulated results of the whole-cell Ca\(^{2+}\) currents and Ca\(^{2+}\) concentration during an AP. The model can generate spontaneous rhythmic APs and Ca\(^{2+}\) transients. The primary characteristic parameters of an AP are: 166.98 ms of CL, 60.70 ms of APD\(_{90}\), -64.21 mV of MDP, 21.21 mV of OS potential and 14.23 of dV/dt\(_{\text{max}}\), which are all consistent with experimental data for murine SA node cells (Kharche et al., 2011; Mangoni et al., 2003, 2006). As shown in Figure 3.13B, the whole-cell L-type Ca\(^{2+}\) presents a noise-like fluctuation during the AP, because of the stochastic openings of each unitary L-type Ca\(^{2+}\) channel. Similarly, the profiles of [Ca\(^{2+}\)]\(_{\text{sub}}\) and [Ca\(^{2+}\)]\(_{i}\) also demonstrate noise-like fluctuations, which is due to the combined effects of stochastic activation of L-type Ca\(^{2+}\) and RyRs channels. The peak amplitude of simulated [Ca\(^{2+}\)]\(_{i}\) oscillation is 1.53 times higher than the basal level, which is consistent with experimental findings (as shown in Table 3.3). Though direct experimental data in mouse SA node cells are limited, our simulation results are comparable with previous observations from rabbit, guinea-pig and frog SA node cells (Chen et al., 2009; Li, 2012).
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Figure 3.13 Simulated results of action potentials and global intracellular Ca$^{2+}$ transients. (A) Model-generated APs. (B): Whole-cell $I_{Ca,L,1.3}$. (C) Global intracellular Ca$^{2+}$ concentration. (D) Global Ca$^{2+}$ concentration in subspace. (E) Global Ca$^{2+}$ concentration in JSR. (F) Global Ca$^{2+}$ concentration in NSR.

Table 3.3 Characteristics of simulated whole cell Ca$^{2+}$ transient.

<table>
<thead>
<tr>
<th>[Ca$^{2+}$]i Amplitude (folds)</th>
<th>Species</th>
<th>Region</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.53</td>
<td>Mouse</td>
<td>SA node</td>
<td>Model</td>
</tr>
<tr>
<td>1.50 ± 0.5</td>
<td>Rabbit</td>
<td>SA node</td>
<td>Vinogradova et al. (2002)</td>
</tr>
<tr>
<td>1.30</td>
<td>Rabbit</td>
<td>SA node</td>
<td>Vinogradova et al. (2006)</td>
</tr>
<tr>
<td>1.48 ± 0.5</td>
<td>Mouse</td>
<td>SA node</td>
<td>Wu et al. (2009)</td>
</tr>
<tr>
<td>1.75 ± 0.5</td>
<td>Mouse</td>
<td>SA node</td>
<td>Liu et al. (2011)</td>
</tr>
<tr>
<td>2.30</td>
<td>Mouse</td>
<td>Atrium</td>
<td>Li et al., (2005)</td>
</tr>
<tr>
<td>2.36</td>
<td>Mouse</td>
<td>Atrium</td>
<td>Xie et al., (2012)</td>
</tr>
</tbody>
</table>
Figure 3.14A illustrates the two-dimensional results for the details of the Ca$^{2+}$ dynamics during the APs. The model demonstrates its ability to reproduce spatial Ca$^{2+}$ wave propagation in murine SA node cells. Because of the absence of the T-tubules in SA node cells, Ca$^{2+}$ diffusion plays an important role in the generation and sustainment of the Ca$^{2+}$ waves. As shown in Figure 3.14A, along with the upstroke of spontaneous APs, the CICR was initiated in the peripheral region of the cell due to the Ca$^{2+}$ influx in response to the activation of Ca$^{2+}$ channels on the cell membrane, resulting in a marked increase of $[\text{Ca}^{2+}]_i$ in this region. Then, the peripheral $[\text{Ca}^{2+}]_i$ diffused into the central region owing to the spatial gradient of Ca$^{2+}$ concentration, which triggered the Ca$^{2+}$ release inside the cell, generating intracellular Ca$^{2+}$ wave propagation. However, the inward-propagating Ca$^{2+}$ wave did not completely spread inside the whole cell, as shown in Figure 3.14A(v) (i.e., the central region of the SA node cell remained blue while the peripheral part was red). A possible explanation for this was that, during the Ca$^{2+}$ diffusion, $[\text{Ca}^{2+}]_i$ showed a gradual decrease in its amplitude. When its amplitude fell below the concentration required for the activation of non-junctional RyRs, the Ca$^{2+}$ release in the adjacent RyRs stopped, leading to wave termination. The simulated Ca$^{2+}$ dynamics in SA node cells showed a spatially heterogeneous $[\text{Ca}^{2+}]_i$ wave propagation, which was similar to experimental observations in atrial myocytes (Bootman et al., 2006).

The model also successfully reproduced the local Ca$^{2+}$ release events (Ca$^{2+}$ sparks) in murine pacemaker cells during the action potential, which are attributable to the stochastic features of the L-type Ca$^{2+}$ and RyRs channels. The local Ca$^{2+}$ sparks occurred across the whole cell prior to the beginning of the action potential (Figure 3.14A), especially during the diastolic depolarisation phases. This is consistent with experimental observations in mouse and other animal SA node cells (Chen et al., 2009). The frequency of Ca$^{2+}$ sparks in the peripheral region of the cell was higher than that in the central region. In simulations, spontaneous Ca$^{2+}$ sparks occurred
almost exclusively near the cell membrane of the SA node (Figure 3.14B), which is consistent with experimental Ca\(^{2+}\) images from a quiescent SA node cell loaded with the Ca\(^{2+}\) indicator, Fluo-4 AM (Chen et al., 2009).

Figure 3.14 Intracellular Ca\(^{2+}\) dynamics  (A) Simulated intracellular Ca\(^{2+}\) transients together with corresponding APs. (B) Intracellular Ca\(^{2+}\) images (loaded with Fluo-4 AM) from a quiescent SA node cell (Chen et al., 2009). The nucleus of this SA node cell is shown by the red dashed lines. Both simulated and experimental observations indicated that the Ca\(^{2+}\) sparks occurred primarily near the periphery of the SA node cell.
3.3.2 Ca\textsuperscript{2+} current and Ca\textsuperscript{2+} concentration in a single Ca\textsuperscript{2+} element during AP

As described above, \([\text{Ca}^{2+}]_i\) wave propagation in a murine SA node cell shows spatially heterogeneous features. In order to better understand the behaviours of such a heterogeneous intracellular Ca\textsuperscript{2+} wave in the murine SA node cell, a line scan analysis was implemented so as to compare the differences between the Ca\textsuperscript{2+} transients in the central and the peripheral regions. Figure 3.15 shows a simulated line scan image of \([\text{Ca}^{2+}]_i\) in the vertical direction (red line in the insert figure) and the corresponding time traces of Ca\textsuperscript{2+} transients in both the central and the peripheral single Ca\textsuperscript{2+} elements, marked by the dots in the figure. It was shown that during Ca\textsuperscript{2+} propagation from the peripheral to the central region, there was a gradual decrease in the \([\text{Ca}^{2+}]_i\) transient amplitude. The Ca\textsuperscript{2+} transients in the peripheral region showed a higher systolic concentration and decayed rapidly, whereas the Ca\textsuperscript{2+} transients in the central region showed a lower amplitude and decayed slowly. However, spontaneous Ca\textsuperscript{2+} sparks were observed in both the central and the peripheral regions of the cell.

Lateral line scan (along the cell) images and the spatial average of \([\text{Ca}^{2+}]_i\), for the central and peripheral regions are shown in Figure 3.16. Similarly to the results described above, the averaged Ca\textsuperscript{2+} transients in the central region remained stable, although they demonstrated higher diastolic, but lower systolic concentrations as compared with those from the peripheral region. Similarly, less frequent Ca\textsuperscript{2+} sparks were observed in the central region of the SA node cell as compared with the peripheral region. Frequent Ca\textsuperscript{2+} sparks occurred near the cell membrane during the diastolic depolarisation phase, which preceded the initiation of the pacemaking action potentials. Furthermore, the Ca\textsuperscript{2+} transients in the peripheral region of the cell were highly synchronised with spontaneous APs from beat to beat. These simulation results are consistent with experimental findings in mouse SA node cells by Chen et al.
(2009), which suggested a close relationship between the membrane potential and \([\text{Ca}^{2+}]_i\) oscillations.

Figure 3.15 Simulated line scan image in the vertical direction. The red line in the insert indicates that the scan line runs vertically across the cell. The \(\text{Ca}^{2+}\) transient in the central and peripheral regions of the cell are shown in (B) and (C), respectively. \(\text{Ca}^{2+}\) sparks can be observed in the peripheral region.
Figure 3.16 Simulated line scan image in the lateral direction. The red lines in the inserts indicate that the scan lines run laterally along the cell. (B) Spatial average Ca$^{2+}$ concentration in (A). Black and green lines represent the results of the peripheral and central regions, respectively. (C) Corresponding membrane potentials as a function of time.
3.3.3 Dynamic interaction of the membrane and Ca\(^{2+}\) clocks

The interactions of the membrane and Ca\(^{2+}\) clocks are sophisticated and remain controversial. In this section, the roles of the membrane and Ca\(^{2+}\) clocks in generating cardiac pacemaking APs are analysed by using the murine SA node cell model developed above.

**Role of the funny current (If)**

The role of $I_f$ on the pacemaking potentials of SA node cells has been investigated in previous experimental and simulation studies in various species, and all of them have shown that reducing the $I_f$ can lead a slowing down of spontaneous pacemaking activities.

![Figure 3.17 Effect of blocking $I_f$ on spontaneous APs and intracellular Ca\(^{2+}\) transients.](image)

(A) Model-generated APs, (B) global $[\text{Ca}^{2+}]_i$, (C)$I_{\text{NCX}}$ and (D) SR Ca\(^{2+}\) uptake. $J_{\text{SERCA}}$ under control conditions (black traces) and with $I_f$ blocked (red traces) are shown.
Simulation results of blocking $I_f$ in this model are presented in Figure 3.17. It was shown that blocking $I_f$ increased the cycle length of the SA node cell by 26.9%, from 167 ms to 212 ms. In simulations, blocking $I_f$ also produced a reduction of the MDP (from -64.21 mV to -67.5 mV). It can also be found that the cycle length of intracellular Ca$^{2+}$ oscillations rise due to blocking $I_f$. Meanwhile, the diastolic [Ca$^{2+}$]$_i$ decreased, but the averaged amplitude of [Ca$^{2+}$] remained the same (nearly 0.08 μM) when $I_f$ was blocked. A decreased diastolic [Ca$^{2+}$]$_i$ level may be attributable to the contribution of the Na$^+/Ca^{2+}$ exchanger current, which extruded more intracellular Ca$^{2+}$ ions during the prolonged diastolic depolarisation phase, as shown in Figure 3.17C. In addition, the frequency of local Ca$^{2+}$ releases was slightly increased (control: 24 sparks per AP vs $I_f$ blocking: 26 sparks per AP), which might be due to the prolongation of the diastolic depolarisation phase when $I_f$ was blocked.

**Role of T-type Ca$^{2+}$ current ($I_{CaT}$)**

Previous experimental studies suggested a critical role of $I_{CaT}$ in SA node pacemaking. In their study, Mangoni et al. (2006) found that in Ca$V_{3.1}$-knockout mice, the cellular beating rate of the heart was dramatically slower as compared with the wild-type mice. In this section, the newly developed model was implemented in order to analyse the functional impacts of blocking $I_{CaT}$ on the pacemaking action potentials. Figure 3.18 illustrates the effect of blocking $I_{CaT}$ on the action potential and the intracellular Ca$^{2+}$ dynamics. By blocking $I_{CaT}$ completely, the pacemaking activity slowed down, with the cycle length increasing from 167 ms to 198 ms (by 18.6%). Similarly to the action of blocking $I_f$, blocking $I_{CaT}$ also prolonged the diastolic depolarisation phase, which was associated with a reduced diastolic [Ca$^{2+}$]$_i$, due to more Ca$^{2+}$ ions being extruded out via the Na$^+/Ca^{2+}$ exchanger channel during the prolonged diastolic phase. Meanwhile, the amplitude of intracellular Ca$^{2+}$ transients was also seen to decrease, possibly due to the inhibition of Ca$^{2+}$ influx through the T-type Ca$^{2+}$ channels, which decreased the trigger for the SR Ca$^{2+}$ release.
Figure 3.18 Effect of blocking $I_{CaT}$ on spontaneous APs and intracellular Ca$^{2+}$ transients. (A&B) Model-generated APs and global [Ca$^{2+}$]. (C&D) Lateral line scan images of intracellular Ca$^{2+}$ transients as well as the average Ca$^{2+}$ concentrations (iii) for the central (C) and peripheral (D) regions under control (i) and $I_{CaT}$-blocked (ii) conditions.

At the subcellular level, blocking $I_{CaT}$ also reduced the Ca$^{2+}$ transients in the central and the peripheral regions, as shown in Figure 3.18C and Figure 3.18D. Though some local Ca$^{2+}$ releases were still present near the cell membrane during the diastolic depolarisation phase, they seemed to be less frequent as compared with the control condition. The measured frequency of Ca$^{2+}$ sparks in the peripheral region decreased from 24 (sparks per AP) under control conditions to 14 (sparks per AP) when $I_{CaT}$ was blocked. In the central region, local Ca$^{2+}$ release was almost absent during the diastolic depolarisation phase with $I_{CaT}$ being blocked, which was mainly due to the fact that [Ca$^{2+}$], in this region fell below the threshold concentration required to activate non-junctional RyRs.
Role of Cav1.2 L-type Ca\(^{2+}\) current (\(I_{\text{Cal,1.2}}\))

Figure 3.19 shows the effect of blocking \(I_{\text{Cal,1.2}}\) on the action potential and the Ca\(^{2+}\) transients, which marginally increased the cycle length by 0.5%. Experimental data on the effects of blocking \(I_{\text{Cal,1.2}}\) on cardiac pacemaking APs are limited, but our simulation results are similar to those in the study of Kharche et al. (2011).

![Figure 3.19](image)

Figure 3.19 Effect of blocking \(I_{\text{Cal,1.2}}\) on spontaneous APs and intracellular Ca\(^{2+}\) transients as a function of time.

Role of Cav1.3 L-type Ca\(^{2+}\) current (\(I_{\text{Cal,1.3}}\))

The study by Mangoni et al. (2003) has shown that Cav1.3-knockout mice present with significantly slower pacemaking activity with prolonged cycle lengths or even abolished spontaneous activity as compared with wild-type mice. In this study, the functional role of \(I_{\text{Cal,1.3}}\) in cardiac pacemaking APs was also investigated. The results are shown in Figure 3.20.

Complete block of \(I_{\text{Cal,1.3}}\) caused an arrest in the pacemaking action potential, leading to a stable resting potential at -39.76 mV (blue dashed line in Figure 3.20A). Blocking \(I_{\text{Cal,1.3}}\) by 45% produced a 9.5% increase in the cycle length, which changed from 167 ms to 183 ms. Blocking \(I_{\text{Cal,1.3}}\) by 45% also caused a reduction in the amplitude of the action potential, a more depolarised MDP and a lower OS potential. Meanwhile, the
systolic $[\text{Ca}^{2+}]_i$ was seen to decrease dramatically from 0.23 μM to 0.17 μM when $I_{\text{CaL,1.3}}$ was blocked by 40%, whereas the diastolic $[\text{Ca}^{2+}]_i$ remained the same.

Figure 3.20 Effect of blocking $I_{\text{CaL,1.3}}$ on spontaneous APs and intracellular Ca$^{2+}$ transients. (A&B) Model-generated APs and global $[\text{Ca}^{2+}]_i$. (C&D) Lateral line scan images of intracellular Ca$^{2+}$ transients as well as the average Ca$^{2+}$ concentrations (iii) for the central (C) and peripheral (D) regions under control (i) and part-blocking $I_{\text{CaL,1.3}}$ (ii) conditions.

At the subcellular level, partial block of $I_{\text{CaL,1.3}}$ dramatically decreased the systolic Ca$^{2+}$ concentration in both of the central and the peripheral regions (Figure 3.20C & Figure 3.20D), which is mainly due to the reduction of Ca$^{2+}$ influxes via Ca$^{2+}$ channels on the cell membrane inhibiting the Ca$^{2+}$ release from the SR. Specifically, the local Ca$^{2+}$ release during the diastolic depolarisation phase almost disappeared in
the peripheral region when $I_{CaL,1.3}$ was blocked. The measured frequency of Ca$^{2+}$ sparks was only 8 (sparks per action potential). This suggested that the occurrences of local Ca$^{2+}$ releases near the cell membrane are primarily triggered by Ca$V_{1.3}$ L-type Ca$^{2+}$ channels in the mouse SA node cell.

**Role of RyRs**

Experimentally, the effect of SR Ca$^{2+}$ release was investigated by blocking the RyRs with 30 μM ryanodine, which has extremely high affinity for the open-form RyRs. However, an important issue related to the ryanodine effect is that ryanodine may also reduce the activities of some other sarcolemmal ionic channels under some experimental conditions (Maltsev et al., 2004). Moreover, the available experimental data of ryanodine effects are limited and some of them are even contradictory to each other. Research by Satoh (1997) found that 10 μM ryanodine decreased the $I_f$ by 45\% at -90 mV, whereas another study by Li et al. (1997) showed no significant change of $I_f$ under a relatively high concentration of ryanodine. Satoh (1997) also indicated in the same paper that the $I_{CaL}$ was decreased by 10\% and 8\% by 1 μM and 10 μM ryanodine, respectively, whilst others observed that $I_{CaL}$ remained the same when ryanodine was applied (Bogdanov et al., 2001; Li et al., 1997). In addition, it has also been reported that 1 μM and 10 μM of ryanodine reduce the delayed rectifier K$^+$ current ($I_{Kr}$ and $I_{Ks}$) by 6\% and 26\%, respectively (Satoh, 1997). Li et al. (1997) also observed that $I_{CaT}$ was decreased by approximately 17\% by 10 μM of ryanodine; however, the authors did not clearly indicate whether the reduction was the result of the change of [Ca$^{2+}$], or a direct effect of ryanodine on T-type Ca$^{2+}$ channels. The effects of ryanodine on the ionic channels are summarised in Table 3.4. In this part of the study, the effects of 30 μM ryanodine are simulated based on experimental data via the following: 1) setting the Ca$^{2+}$ release constant $K_s$ to 0; 2) decreasing the current density of $I_{CaT}$ by 20\%; and 3) reducing the conductance of $I_{Kr}$ and $I_{Ks}$ by 25\%.
Chapter 3 Dynamic interaction of membrane and Ca\(^{2+}\) clocks in cardiac pacemaking

Figure 3.21 Effect of 30 μM ryanodine on spontaneous APs and intracellular Ca\(^{2+}\) transients. (A&B) Model-generated APs and global [Ca\(^{2+}\)], (C&D) Lateral line scan images of intracellular Ca\(^{2+}\) transients as well as the average Ca\(^{2+}\) concentrations (iii) for the central (C) and peripheral (D) regions under control conditions (i) and with the presence of 30 μM RyRs (ii).

Figure 3.21 illustrates the effect of 30 μM ryanodine on action potentials and intracellular Ca\(^{2+}\) dynamics. On blocking RyRs, the pacemaking cycle length was slightly increased by 7.8%, from 167 ms to 180 ms, which is comparable to the experimental data of rabbit SA node cells by Lancaster et al. (2004). Research by Lancaster et al. (2004) has shown that 30 μM ryanodine caused a percentage change of cycle length of -4.7±11% for small cells (projected cell area <400 μm\(^2\)) and 12.4±10% for intermediate cells (projected cell area <700 μm\(^2\)). The OS potential was slightly decreased from 21.56 mV to 19.4 mV when 30 μM ryanodine was applied, together with a more depolarised MDP, from -65.21 mV to -58.56 mV. In addition,
under 30 μM ryanodine, the diastolic [Ca^{2+}]_{i} slightly increased as compared with the control condition; however, the amplitude of intercellular Ca^{2+} transients was dramatically decreased by 58.5% (Figure 3.21B), which is consistent with previous experimental observations (Lancaster et al., 2004). At a subcellular level, as shown in Figure 3.21C & Figure 3.21D, both the amplitudes of the Ca^{2+} transients in the central and the peripheral regions were reduced when 30 μM ryanodine was applied. Significantly, from the line scan image of [Ca^{2+}]_{i} in the peripheral region of the cell, as shown in Figure 3.21C(ii), the local Ca^{2+} release during the depolarisation phase completely disappeared, which was due to the block of SR Ca^{2+} release when applying 30 μM ryanodine.

Table 3.4 Effects of ryanodine on the ionic channels

<table>
<thead>
<tr>
<th>Channel type</th>
<th>Experimental observation</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_f)</td>
<td>45% ↓ by 10 μM ryanodine at -90 mV*</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td>No changes#</td>
<td></td>
</tr>
<tr>
<td>(I_{Ca,L})</td>
<td>10% ↓ by 1 μM ryanodine*</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td>8% ↓ by 10 μM ryanodine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No changes#</td>
<td></td>
</tr>
<tr>
<td>(I_{Ca,T})</td>
<td>17% ↓ by 10 μM ryanodine#</td>
<td>20% ↓</td>
</tr>
<tr>
<td>(I_K)</td>
<td>6% ↓ by 1 μM ryanodine*</td>
<td>(I_{Kr}) and (I_{Ks}): 25% ↓</td>
</tr>
<tr>
<td></td>
<td>26% ↓ by 10 μM ryanodine*</td>
<td></td>
</tr>
</tbody>
</table>

* Satoh (1997); # Li et al. (1997). ↓ means decrease.

Although both the experimental and simulation results suggest that 30 μM ryanodine prolongs the cycle length of SA node cell pacemaking APs and significantly reduces the Ca^{2+} sparks during the diastolic depolarisation phase, it is still unclear whether the increase in cycle length is due to the decrease of the local Ca^{2+} release or the
inhibition of some ionic channels when ryanodine is applied. The computational model allows us to distinguish between these two causes.

Figure 3.22 Effect of blocking RyRs on spontaneous APs and intracellular Ca\(^{2+}\) transients. (A&B) Model-generated APs and global [Ca\(^{2+}\)]\(_i\). (C&D) Lateral line scan images of intracellular Ca\(^{2+}\) transients as well as the average Ca\(^{2+}\) concentrations (iii) for the central (C) and peripheral (D) regions under control (i) and RyRs-blocked (ii) conditions.

The role of the inhibition of Ca\(^{2+}\) SR release by ryanodine was investigated by studying the effect of blocking RyRs alone on the spontaneous activity of the models. The simulated effects of the inhibition of RyRs alone caused by ryanodine on the rate of spontaneous action potential are shown in Figure 3.22. The rhythmic cycle length was not significantly changed, as it only increased by 2.9%, from 167 ms to 172 ms. The MDP remained the same when only RyRs was blocked, whereas the OS potential
was reduced slightly. Similarly to the effect of 30 μM ryanodine, blocking RyRs dramatically decreased the amplitude of intracellular Ca$^{2+}$ transients by 62.5%. At a subcellular level, both the amplitudes of Ca$^{2+}$ transients in the central and peripheral regions were reduced as a result of blocking RyRs (Figure 3.22C & Figure 3.22D). Undoubtedly, the local Ca$^{2+}$ release could not been seen when the RyRs was totally blocked.

The contribution of the inhibition of ionic channels by ryanodine was also investigated by blocking each channel separately. Partial blocking of $I_{CaT}$ by 20% slightly increased the cycle length by 2.69%, while partial blocking of $I_K$ ($I_{Kr}$ and $I_{Ks}$) by 25% resulted in minor cycle length increases of 2.1%. However, the intracellular Ca$^{2+}$ dynamics were almost unchanged.

Thus, the experimental observations and simulation results above suggest that SR Ca$^{2+}$ releases have a significant effect on intracellular Ca$^{2+}$ cycling, but play a limited role in generating spontaneous rhythmic pacemaking activity.
3.4 Discussion and conclusion

In SA node cells, the intracellular Ca\textsuperscript{2+} dynamics and local Ca\textsuperscript{2+} release, occurring mainly near the cell membrane during the diastolic depolarisation phase, are believed to play a significant role in generating spontaneous pacemaking activity in the heart. As an experimental approach has only limited ability to elucidate the relative role of Ca\textsuperscript{2+} clocks in the rhythmic action potentials of SA node cells, in this study we took a computational approach by developing a biophysically detailed mathematical model for the action potentials of murine SA node cells, which incorporated detailed intracellular Ca\textsuperscript{2+} cycling dynamics with stochastic openings of unitary L-type Ca\textsuperscript{2+} channels and single RyRs channels.

Comparison with previous models for SA nodes

In the last decade, several numerical models of SA node cells have been constructed for various species. Most of them are deterministic, represented by a set of ordinary differential equations and not formulated at the microscopic level of Ca\textsuperscript{2+} release elements. In parallel, several models have been developed in order to analyse single Ca\textsuperscript{2+} sparks at the scale of Ca\textsuperscript{2+} release elements (Smith et al., 1998; Sobie et al., 2002). In previous studies, though some attempts have been made to couple the two-level model behaviours together, they have some limitations. These included a common pool model developed by Maltsev et al. (2011), in which it was shown that the local Ca\textsuperscript{2+} release events were associated with an ensemble-average open probability of the RyRs. Another study was the three-dimensional cylinder model of Ca\textsuperscript{2+} dynamics developed by Stern et al. (2014), who showed that the local Ca\textsuperscript{2+} release only occurred near the cell membrane as there were no non-junctional RyRs assumed in the cell. The present model has made some progress as compared with the previous models.
The major differences between the present model and those of previous studies are: 1) the regulation of CSQN on the SR Ca\(^{2+}\) release was taken into consideration; 2) the stochastic nature of local Ca\(^{2+}\) dynamics was simulated; and 3) a morphologically reconstructed geometry of a SA node cell was incorporated. A key achievement of the present model is that the simulated APs are consistent with experimental recordings from mouse SA node cells, in terms of all of the primary characteristic parameters of the AP, including CL, APD\(_{90}\), MDP, OS potential and dV/dt\(_{\text{max}}\), all of which match with experimental data. In addition, the whole-cell Ca\(^{2+}\) transients and intracellular Ca\(^{2+}\) cycling are also comparable with the experimental observations in mouse SA node cells.

**Different dynamics between unitary and whole-cell L-type Ca\(^{2+}\) and RyRs channels**

The opening of unitary L-type Ca\(^{2+}\) channels is discontinuous in time and stochastic, which is reproduced by the model, as shown in Figure 3.6, while the whole-cell current indicates a bell-shaped I–V relationship (Figure 3.7). The simulation results suggest that the current density of macroscopic L-type Ca\(^{2+}\) channels is linked with the open probability and amplitude of unitary channels.

The open probability of unitary and whole-cell RyRs channels is associated with the Ca\(^{2+}\) concentration in the JSR and subspace, as shown in Figure 3.9 and Figure 3.10. The model behaviour is similar to that observed in previous experiments (Györke and Fill, 1993; Györke and Györke, 1998).

**Ca\(^{2+}\) waves in SA nodes**

The Ca\(^{2+}\) wave, initiated from the peripheral region near the cell membrane and terminated at the central region of the cell, has been observed in experimental studies. This phenomenon has been reproduced by the model (Figure 3.14). The mechanisms
responsible for Ca\(^{2+}\) wave termination at the central region remain controversial. From our simulation data, as described above, it seems that one of the possible reasons is that during the Ca\(^{2+}\) diffusion inwards to the central region of the cell, \([\text{Ca}^{2+}]_i\) falls below the concentration that is required for the activation of non-junctional RyRs. Research by Bootman et al. (2006) suggested that a functional difference between the junctional and non-junctional RyRs might restrict the Ca\(^{2+}\) wave in the peripheral region of the cell, and the non-junctional RyRs might have a relatively low sensitivity and require a high \([\text{Ca}^{2+}]_i\) before the SR Ca\(^{2+}\) release. Stern et al. (2014) reported that some of the SA node cells even have no non-junctional RyRs in the interior of the cell. Another reason is that the Ca\(^{2+}\) wave may be interfered with by some cellular organelles, such as the nucleus. However, in our model, the junctional and non-junctional RyRs are supposed to have the same activation kinetics and are formulated by the same equations, which suggests that the phenomenon that central Ca\(^{2+}\) waves do not always occur is mainly due to the Ca\(^{2+}\) diffusion process, resulting in a gradual decrease in \([\text{Ca}^{2+}]_i\).

**Effects of M or C clocks on the spontaneous pacemaking activity of SA node cells**

*Role of the M clock*

The contribution of the M clock to the spontaneous pacemaking activity of SA node cells was investigated by blocking related transmembrane ionic channels, including the \(I_f\), the T-type Ca\(^{2+}\) current and the L-type Ca\(^{2+}\) current. Simulation results suggest that blocking \(I_f\), \(I_{CaT}\) and \(I_{CaL}\) significantly slows down the spontaneous activity of the SA node cell model, or even causes an arrest in auto-rhythmic action potentials. In simulations of blocking \(I_f\), the cycle length of APs is increased by 26.9%, which is in agreement with previous experimental observations (Fenske et al., 2013). Though such a slowing down in pacemaking is accompanied by a decrease of diastolic intracellular Ca\(^{2+}\) concentration, this is due to the fact that during the prolonged diastolic depolarisation phase, more intracellular Ca\(^{2+}\) ions are moved out through the
NCX channel (Figure 3.17). It has also been shown that blocking $I_{CaT}$ increases the cycle length by 18.6% (Figure 3.18), which is also accompanied by a decrease in the amplitude of the intracellular Ca$^{2+}$ transients. However, the decreased $[\text{Ca}^{2+}]_i$ amplitude is due to the inhibition of Ca$^{2+}$ influx through the T-type Ca$^{2+}$ channels, which reduces the SR Ca$^{2+}$ release. In simulations, though $I_{CaL,1.2}$ has no obvious effect on the rhythmic action potentials (Figure 3.19), the Cav1.3 L-type channel plays a crucial role in them. The spontaneous activity of the pacemaker is directly abolished when $I_{CaL,1.3}$ is completely blocked. Furthermore, partially blocking $I_{CaL,1.3}$ by 45% produces a 9.5% increase in the pacemaking cycle length (Figure 3.20).

However, some of the ionic channels, such as $I_{CaT}$ and $I_{CaL}$, not only play important roles in rhythmic action potentials, but are also able to mediate the intracellular Ca$^{2+}$ dynamics. Collectively, our simulation data suggest that the effect of the C clock on cardiac pacemaking is important.

**Role of the C clock**

The contribution of the C clock to the spontaneous pacemaking activity of SA node cells is analysed in two sections regarding the role of SR Ca$^{2+}$ releases and the effect of Ca$^{2+}$ sparks during the diastolic depolarisation phase. Simulated line scan images show that the SR Ca$^{2+}$ releases are highly synchronised with the spontaneous action potential (Figure 3.16), suggesting a high correlation between the two (i.e., the SR Ca$^{2+}$ releases are mainly triggered by action potentials). Experimentally, the role of SR Ca$^{2+}$ releases has been investigated by blocking the RyRs with 30 μM ryanodine. In the model, based on previous experimental data (Li et al., 1997; Satoh, 1997), the effect of 30 μM ryanodine is simulated by the combined contribution of completely blocking RyRs and partly reducing $I_{CaT}$, $I_{Kr}$ and $I_{Ks}$. Simulation results show that the cycle length is slightly increased by 7.8%, while the intracellular Ca$^{2+}$ concentration is dramatically decreased (Figure 3.21), which is comparable with previous experimental observations of rabbit SA node cells (Lancaster et al., 2004). Moreover,
simulations have been performed in order to determine the relative roles of RyRs blocking and inhibition of some ionic channels in generating the observed bradycardia via 30 μM ryanodine. The results indicate that the ryanodine-induced reduction of $I_{CaT}$ and $I_K$ ($I_{Kv}$ and $I_{Ks}$) increases the cycle length by 2.69% and 2.1%, respectively. When only RyRs is blocked, the cycle length is increased by 2.9%, suggesting a limited role of SR Ca$^{2+}$ releases in the regulation of rhythmic pacemaker activity.

Table 3.5 Simulated results for APs and local Ca$^{2+}$ release.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cycle length (ms)</th>
<th>Frequency of Ca$^{2+}$ sparks (per AP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>167</td>
<td>24</td>
</tr>
<tr>
<td>$I_f$ blocked</td>
<td>212</td>
<td>26</td>
</tr>
<tr>
<td>$I_{CaT}$ blocked</td>
<td>198</td>
<td>14</td>
</tr>
<tr>
<td>Part of $I_{CaL,1.3}$ blocked</td>
<td>183</td>
<td>8</td>
</tr>
<tr>
<td>30 μM ryanodine</td>
<td>180</td>
<td>0</td>
</tr>
<tr>
<td>RyRs blocked</td>
<td>170</td>
<td>0</td>
</tr>
</tbody>
</table>

As a consequence of the lack of T-tubules, it is observed in both experiments and simulations that the local Ca$^{2+}$ release (Ca$^{2+}$ sparks) primarily occurred close to the cell membrane (Figure 3.14). These spontaneous and stochastic events are supposed to be the core of the C clock, which can trigger the NCX current and maintain the action potential (Maltsev and Lakatta, 2007). The frequency of Ca$^{2+}$ sparks in the peripheral region of the cell during the diastolic depolarisation phase and the cycle length of APs under different conditions are summarised in Table 3.5. The Ca$^{2+}$ sparks occur infrequently when Ca$^{2+}$ influx via transmembrane Ca$^{2+}$ channels (T- or L-type Ca$^{2+}$ channels) is inhibited, especially when $I_{CaL,1.3}$ is blocked. This result suggests that the local Ca$^{2+}$ release during the diastolic depolarisation phase is primarily triggered by Ca$^{2+}$ influx originating from T- and L-type Ca$^{2+}$ channels, especially the stochastic behaviour of the L-type Ca$^{2+}$ channel. In addition, there is no obvious linear relationship between the frequency of local Ca$^{2+}$ release events and the pacing rate of
rhythmic action potentials. All of these results suggest there being a limited effect of local Ca\textsuperscript{2+} release events and the C clock on the spontaneous pacemaking activity of SA node cells.

**Limitations of the Model**

The effect of phosphorylation of Ca\textsuperscript{2+} cycling proteins and β-adrenergic regulation on the Ca\textsuperscript{2+} clock and therefore on spontaneous pacemaking activity were not taken into consideration due to the limited experimental data on murine SA node. A more detailed model, incorporating the intracellular signalling pathways, is needed for further study. Another limitation is the lack of detailed consideration of subcellular structures. The Ca\textsuperscript{2+} wave may be interfered with by some cellular organelles, such as the nucleus.

**Conclusion**

In this study, we have developed a biophysically detailed mathematical model of murine SA node cells, which incorporates a multi-compartment intracellular calcium handling system and a morphologically reconstructed cell geometry. The stochastic nature of local Ca\textsuperscript{2+} dynamics in SA node cells is simulated by Markov chain models and solved by the Monte Carlo method. The model successfully reproduces the rhythmic action potentials and the behaviour of intracellular Ca\textsuperscript{2+} cycling that are consistent with previous experimental observations (Chen et al., 2009; Kharche et al., 2011; Mangoni et al., 2003, 2006). The mechanisms underlying the automaticity of the SA node are investigated by the model. Simulation suggests that intracellular Ca\textsuperscript{2+} dynamics (the C clock) play a minor role in the spontaneous activity of murine SA node cells.
Chapter 4
Modelling of the chronotropic effect of autonomic regulation on the murine sinoatrial node

4.1 Introduction

Although the spontaneous activity that causes the heart’s rhythmic contractions originates within the cardiac muscle itself, the rate of this contraction can also be influenced by the autonomic nervous system. As a branch of the nervous system, the autonomic nervous system (ANS) acts as a control system in the body, responsible for the regulation of bodily involuntary and unconscious activities, such as the heart rate (Jänig, 1989). It has two divisions, the sympathetic nervous system and the parasympathetic nervous system, which serve respectively to accelerate and decelerate the heart rate (Robinson et al., 1966). The fibres from these two divisions terminate in the sinoatrial (SA) node and can release some endogenous chemicals, known as neurotransmitters, which can vary the impulse traffic of the SA node, thus mediating the cardiac pacemaker activities (Borell et al., 2007). It has been reported that some neurotransmitters, such as acetylcholine and adrenaline, have a strong influence on action potential (AP) morphology and the pacing rate of SA node cells (Colman, 2014).

Acetylcholine

Acetylcholine (ACh) is one of the neurotransmitters released by the parasympathetic nervous system. It is well known that ACh has a negative chronotropic effect on the heart rate, slowing down the spontaneous activity of the SA node, the pacemaker of
the heart (Zhang et al., 2002). Previous studies have shown that the effects of ACh on cardiac APs are complex: 1) activating the acetylcholine-activated potassium channel, \( I_{KAC} \); 2) affecting the kinetics of \( I_f \) (by shifting its activation curve toward more hyperpolarised potential); and 3) inhibiting the magnitude of \( I_{CaL} \) (DiFrancesco et al., 1989; Honjo et al., 1992; Zaza et al., 1996).

**Isoprenaline**

Isoprenaline (ISO) is one of the sympathomimetic drugs, used to mimic the effects of neurotransmitters released by the sympathetic nervous system (Verloop et al., 2015). It is structurally similar to adrenaline, which is a neurotransmitter and also known as a medication for the treatment of cardiac arrest. In the SA node, ISO can significantly accelerate the electrical activity (Fedorov et al., 2010; Zhang et al., 2012).

Comparing with ACh, ISO has more complex effects on SA node APs, including affecting the channel conductance and kinetics of many ion channels and intracellular \( Ca^{2+} \) handling. Previous experiments have shown that ISO augments the funny current (\( I_f \)) by shifting its activation curve to positive potentials, which has been considered to be the primary mechanism underlying the effect of ISO on cardiac pacemaker activity (Alig et al., 2009; Liao et al., 2010; Baruscotti et al., 2011; Zhang et al., 2012; Larson et al., 2013). Moreover, ISO also increases the current density of the L-type \( Ca^{2+} \) channel (\( I_{CaL} \)) (Zaza et al., 1996; Alig et al., 2009; Wu et al., 2009; Neco et al., 2012, Gao et al., 2013; Larson et al., 2013), and shifts the I-V curve to more hyperpolarisation potential (Kharche et al., 2011; Li et al., 2012; Colman, 2014). Similarly, it has been observed that ISO largely raises the magnitude of the T-type \( Ca^{2+} \) current (\( I_{CaT} \)) (Li et al., 2012; Larson et al., 2013); however, the activation kinetics of \( I_{CaT} \) remains the same after the application of ISO (Li et al., 2012). Furthermore, it is reported that both of the delayed rectifier potassium currents (\( I_{Kr} \) and \( I_{Ks} \)) are affected by ISO. To be more specific, ISO increases \( I_{Kr} \) density (Walsh et
al., 1988; Freeman and Kass, 1993; Vinogradova et al., 2008) and causes a shift of its activation curve toward more negative potentials (Lei et al., 2000, Ke et al., 2007), whereas the density of $I_{Ks}$ is also increased after the addition of ISO (Walsh et al., 1988; Freeman and Kass, 1993; Lei et al., 2002; Vinogradova et al., 2008). In addition, ISO has been found to affect sarcoplasmic reticulum (SR) uptake, resulting in the enhancement of the amplitude and minimal diastolic level of intracellular Ca$^{2+}$ concentration (Kharche et al., 2011; Zhang et al., 2012).

The chronotropic effects of ACh and ISO have been confirmed to affect the rhythmic spontaneous activity of the SA node (Zhang et al., 2002; Zhang et al., 2012). Sympathetic and parasympathetic regulation have also been linked to the cardiac diseases, such as sinus node dysfunction (Butters et al., 2010). Therefore, the aim of this study is to construct a biophysically detailed model to simulate the functional effect of autonomic regulation on murine pacemaker activity. The model will be used for the investigation of sick sinus syndrome (Chapter 5).
4.2 Methods

The effects of ACh and ISO were incorporated into the newly developed model of action potentials in the centre and periphery of a murine SA node. The experimentally based equations developed by Zhang et al. (2002) were implemented to simulate the effect of ACh, with some parameters being updated based on the experimental data of Mesirca et al. (2013). Formulations for the effects of ISO were adapted from the previous modelling studies on rabbit SA node cells by Zhang et al. (2012) and human atrial cells by Colman et al. (2014). The combined effects of ACh and ISO were used to mimic the regulation of the autonomic nervous systems on cardiac pacemaking action potentials. Details of definitions of variables, model equations and relevant parameters are provided in the Appendix.

4.2.1 Development of the central and peripheral murine SA node model

The computational model of APs for the primary murine SA node (developed as shown in Chapter 3) was further modified to take into account the regional differences of the cardiac pacemaker. For the sake of computing efficiency, the model was simplified with all of the ionic currents represented by deterministic ordinary differential equations. The central cell model was directly adapted from the simplified model presented in Chapter 3, with the absence of $I_{Na,1.5}$, as the Na$_{v}1.5$ is not expressed in the centre of the murine SA node (Lei et al., 2005). The peripheral model was constructed based on the central cell model, with similar gating properties of ionic channels and intracellular $[Ca^{2+}]_i$ handling mechanisms. Fifteen membrane ion channel currents were considered in the model: 13 are passive ionic channels, one is an ionic pump and one is an ion exchanger. The membrane potential is represented by the following equation:
\[
\frac{dV}{dt} = -\frac{1}{C_m} (I_{Na,1,1} + I_{CaL,1,2} + I_{CaL,1,3} + I_{CaT} + I_{Kr} + I_{ks} + I_{K1} + I_j + I_{sus} + I_{to} + I_{NaK} + I_{NaCa} + I_b)
\]

where \(C_m\) is the cell capacitance. The values of 25 and 50 pF were chosen for central and peripheral \(C_m\) respectively, based on previous experimental observation (Lei et al., 2005).

Moreover, the dynamic variations of intracellular ionic concentrations of \(\text{Ca}^{2+}\), \(\text{Na}^+\) and \(\text{K}^+\) were also inherited from the model developed as shown in Chapter 3. Modifications of the central cell model, primarily in the current densities via changes in ionic current conductance, were implemented to reproduce the faster pacemaking activities of peripheral SA node cells as recorded by lei et al. (2005). Because of the limited availability of experimental data from murine SA node cells, the kinetics of ionic channels were minimally modified based on previous SA node modelling studies (Zhang et al., 2000; Yu, 2009). Table 4.1 summarises all ionic current conductance and the scaling ratio used to produce the central and peripheral SA node cell model.
Table 4.1 The modifications in ionic current conductance from central to peripheral models. Previous studies by Zhang et al. (2000) and Yu (2009) are shown for comparison.

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>Central model</th>
<th>Peripheral model</th>
<th>Ratio*</th>
<th>Zhang et al. Ratio*</th>
<th>Yu et al. Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$, pF</td>
<td>25.0</td>
<td>50.0</td>
<td>2.0</td>
<td>3.25</td>
<td>2.0</td>
</tr>
<tr>
<td>$g_{b,Ca}$, μS</td>
<td>$1.3 \times 10^{-5}$</td>
<td>$2.6 \times 10^{-5}$</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{b,Na}$, μS</td>
<td>$7.4 \times 10^{-5}$</td>
<td>$1.48 \times 10^{-4}$</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{b,K}$, μS</td>
<td>$2.2 \times 10^{-5}$</td>
<td>$4.4 \times 10^{-5}$</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{CaL,1.2}$, μS</td>
<td>$8.23 \times 10^{-3}$</td>
<td>$2.8 \times 10^{-2}$</td>
<td>3.4</td>
<td>$3.45$</td>
<td>$2.50$</td>
</tr>
<tr>
<td>$g_{CaL,1.3}$, μS</td>
<td>$2.37 \times 10^{-2}$</td>
<td>0.128</td>
<td>5.4</td>
<td>3.44</td>
<td></td>
</tr>
<tr>
<td>$g_{CaT}$, μS</td>
<td>$1.06 \times 10^{-2}$</td>
<td>$1.06 \times 10^{-2}$</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{Kl}$, μS</td>
<td>$9.34 \times 10^{-4}$</td>
<td>$5.19 \times 10^{-3}$</td>
<td>5.56</td>
<td>-</td>
<td>$2.78$</td>
</tr>
<tr>
<td>$g_{Kr}$, μS</td>
<td>$2.96 \times 10^{-3}$</td>
<td>$5.92 \times 10^{-2}$</td>
<td>2.0</td>
<td>6.17</td>
<td>$2.24$</td>
</tr>
<tr>
<td>$g_{Ks}$, μS</td>
<td>$2.99 \times 10^{-4}$</td>
<td>$1.61 \times 10^{-3}$</td>
<td>5.4</td>
<td>6.17</td>
<td>$5.63$</td>
</tr>
<tr>
<td>$g_{Na,1.1}$, μS</td>
<td>$7.99 \times 10^{-3}$</td>
<td>$7.99 \times 10^{-3}$</td>
<td>1.0</td>
<td>$\infty$</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{Na,1.5}$, μS</td>
<td>0.0</td>
<td>$2.23 \times 10^{-2}$</td>
<td>$\infty$</td>
<td>$\infty$</td>
<td></td>
</tr>
<tr>
<td>$g_{st}$, μS</td>
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<td>$3.36 \times 10^{-3}$</td>
<td>2.0</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{sus}$, μS</td>
<td>$7.0 \times 10^{-4}$</td>
<td>$2.03 \times 10^{-3}$</td>
<td>2.9</td>
<td>$54.55$</td>
<td>$14.47$</td>
</tr>
<tr>
<td>$g_{to}$, μS</td>
<td>$4.97 \times 10^{-4}$</td>
<td>$1.15 \times 10^{-3}$</td>
<td>2.30</td>
<td>2.33</td>
<td>2.30</td>
</tr>
<tr>
<td>$g_{fK}$, μS</td>
<td>$1.5 \times 10^{-3}$</td>
<td>$2.55 \times 10^{-4}$</td>
<td>1.50</td>
<td>3.93</td>
<td>0.73</td>
</tr>
<tr>
<td>$g_{fNa}$, μS</td>
<td>$1.0 \times 10^{-3}$</td>
<td>$1.41 \times 10^{-4}$</td>
<td>1.50</td>
<td>3.93</td>
<td>1.03</td>
</tr>
<tr>
<td>$I_{NaK_{max}}$, nA</td>
<td>$6.04 \times 10^{-2}$</td>
<td>0.19</td>
<td>3.06</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_{NaCa}$, nA</td>
<td>$3.3 \times 10^{-5}$</td>
<td>$7.2 \times 10^{-5}$</td>
<td>2.18</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Ratio of normalised conductance or current in peripheral to central SA node cell model.
4.2.2. Modelling of acetylcholine

The effects of ACh have not been considered by previous modelling studies in murine pacemaker cells (Mangoni et al., 2006; Kharche et al., 2011). This Chapter presents the modification of the model to include: 1) the formulation of \( I_{K,Ac} \); 2) the fractional block of \( I_{CaL} \); and 3) the shift of activation curve of \( I_f \).

ACh-activated potassium channel, \( I_{K,Ac} \)

The time-dependent formulation of \( I_{K,Ac} \), based on equations given by Zhang et al. (2002), was modelled as an additional part of \( I_{tot} \) in the model:

\[
I_{K,Ac} = g_{K,Ac} \left( \frac{K^+}{10.0 + [K^+]_o} \right) \left( \frac{V - E_K}{1 + e^{(V-E_K-140)RT/2.5RT}} \right)
\]  

(4.2)

where \( g_{K,Ac} \) is the maximum channel conductance, \([K^+]_o\) is the extracellular \( K^+ \) concentration and \( E_K \) is the equilibrium potential for \( K^+ \). The expression for \( g_{K,Ac} \) is given by

\[
g_{K,Ac} = g_{K,Ac,max} j_k \frac{[ACh]^{n_{K,Ac}}}{K_{0.5,K,Ac}[ACh]^{n_{K,Ac}}} \]  

(4.3)

where \( g_{K,Ac,max} \) is the maximum value of \( g_{K,Ac} \), \( j \) and \( k \) are inactivation variables, \([ACh]\) is the concentration of ACh, \( n_{K,Ac} \) is the Hill coefficient, and \( K_{0.5,K,Ac} \) is the concentration of ACh producing half-maximal activation of \( g_{K,Ac} \).

It is known that the ACh binds to the M-2 muscarinic receptor on the cell membrane, resulting in the activation of \( I_{K,Ac} \) (Grant, 2009); thus the Hill equation was employed to quantify the dependency of \( g_{K,Ac} \) on ACh concentration. Values of \( n_{K,Ac} \) and \( K_{0.5,K,Ac} \) were achieved by fitting this equation to experimental data of Mesirca et al. (2013) from murine SA node cells. Figure 4.1A illustrates the relationship between fractional activation of \( I_{K,Ac} \) and ACh concentration.
Figure 4.1 Simulation of $I_{K,ACh}$. (A) Dose dependency of activation of $I_{K,ACh}$, filled circles indicate experimental data from the murine SA node by Mesirca et al. (2013). (B) Time trace of simulated $I_{K,ACh}$ corresponding to a series of testing potentials from -115 mV to +45 mV with 10 mV increments from a holding potential of -75 mV, $I_{K,ACh}$ was activated by 10 μM acetylcholine. (C) I-V relationship curve for $I_{K,ACh}$. Black line represents the simulated result; solid circles indicate experimental data from the murine SA node by Lomax et al. (2003).
The voltage dependent inactivation values $j$ and $k$ were computed by equations:

$$
\frac{dj}{dt} = \alpha_j (1 - j) - \beta_j j \tag{4.4}
$$

and

$$
\frac{dk}{dt} = \alpha_k (1 - k) - \beta_k k \tag{4.5}
$$

where $\alpha_j$, $\beta_j$, $\alpha_k$, and $\beta_k$ are rate constants ($s^{-1}$). In Zhang et al.’s (2002) model, $\alpha_j$ and $\alpha_k$ were assumed to be constants, whereas $\beta_j$ and $\beta_k$ were voltage dependent. In this study, values were modified to fit the murine data provided by Lomax et al. (2003). Simulation results for voltage clamp and I-V relationships are shown in Figure 4.1B and Figure 4.1C.

$$
\alpha_j = 111.65 \tag{4.7}
$$

$$
\alpha_k = 27.91 \tag{4.6}
$$

$$
\beta_j = \frac{248.55}{1.0 + e^{-(V + 64.78)/13.01}} \tag{4.8}
$$

$$
\beta_k = \frac{62.14}{1.0 + e^{-(V + 64.78)/15.01}} \tag{4.9}
$$

**Depression of $I_{CaL}$**

The partial depression of $I_{CaL}$ due to the addition of ACh is given by a dimensionless quantity,

$$
b = b_{max} \frac{[ACh]}{K_{0.5,Ca} + [ACh]} \tag{4.10}
$$

where $b_{max}$ represents the maximum fractional block of $I_{CaL}$, and $K_{0.5,Ca}$ is the concentration of ACh producing half-maximal depression of $I_{CaL}$.

**Shift of $I_f$ activation curve**

The shift factor of $I_f$ activation curve due to ACh is calculated by
\[ s = s_{\text{max}} \frac{[ACh]^{n_f}}{K_{0.5,f} + [ACh]^{n_f}} \]  

(4.11)

where \( s_{\text{max}} \) is the maximal shift of the \( I_f \) activation curve, \( n_f \) is the Hill coefficient and \( K_{0.5,f} \) is the ACh concentration at which the \( I_f \) activation curve is shifted to half of its maximal value. Thus, the shift factor was implemented to shift the \( I_f \) activation curve to more negative potentials, reducing the density of \( I_f \). Due to the lack of experimental observation for murine SA node, it is assumed that the block of \( I_{CaL} \) and the shift of the activation curve of \( I_f \) due to ACh are the same as in the rabbit.

### 4.2.3 Modelling of isoprenaline

In order to model the effect of ISO, the dose dependency of each ISO-affected ion channel, including \( I_{CaL} \), \( I_{CaT} \), \( I_f \), \( I_{Kr} \), \( I_{Ks} \), and \( I_{st} \), were considered independently and incorporated into our newly developed murine SA node model. Figure 4.2 demonstrates the concentration-dependent effects of ISO.

**Increase of \( I_{CaL} \)**

Experimentally, ISO has been found to have complex effects on the L-type Ca\(^{2+}\) channel: it not only increases the density of \( I_{CaL} \) (Alig et al., 2009; Larson et al., 2013), but also changes its kinetics (Li et al., 2012). In this Chapter, this dose-dependent increase of \( I_{CaL} \) is described by an equation developed by Zhang et al. (2012) for rabbit SA node cells, and modified to fit murine data (Alig et al., 2009; Wu et al., 2009; Neco et al., 2012; Gao et al., 2013; Larson et al., 2013), which is formulated by:

\[ f_{\text{CaL}} = f_{\text{CaL, max}} \frac{[ISO]}{K_{0.5,\text{CaL}} + [ISO]} \]  

(4.12)

where \( f_{\text{CaL}} \) represents the percentage of increase of \( I_{CaL} \) density, \( f_{\text{CaL, max}} \) is the maximum value of \( f_{\text{CaL}} \) (usually obtained when ISO concentration reaches saturation), and \( K_{0.5,\text{CaL}} \) represents the ISO concentration producing a half-maximal increase of
$I_{CaL}$. This is incorporated into the model by changing the channel conductance, thus augmenting the current density. Figure 4.2A illustrates the relationship between the increase of $I_{CaL}$ and the concentration of ISO.

### Shift of activation and inactivation of $I_{CaL}$

The formulation for the shift of activation and inactivation of $I_{CaL}$ was based on the experimental data by Li et al. (2012) from mouse ventricular myocytes. Due to the lack of available data for the concentration dependence of this effect, it was assumed that the dose dependency of the shift in activation and inactivation of $I_{CaL}$ was the same as that of the increase in $I_{CaL}$ density, which is described by:

$$S_{CaL} = S_{CaL,\text{max}} \frac{[ISO]}{K_{0.5,SCaL} + [ISO]}$$

where $S_{CaL}$ represents the shift of activation and inactivation of $I_{CaL}$, $S_{CaL,\text{max}}$ is the maximum of $S_{CaL}$, obtained at the saturating concentration of ISO from Li et al. (2012), and $K_{0.5,SCaL}$ indicates the ISO concentration producing the half-maximal shift of the curve.

### Increase of $I_{CaT}$

Two recent studies have shown that ISO dramatically augmented the density of $I_{CaT}$ in murine SA node, without changing its kinetics (Li et al., 2012; Larson et al., 2013). However, both studies indicate only the increase of the $I_{CaT}$ at 1 μM of ISO, which is not enough for the concentration dependency of this effect. Therefore, the equation describing the increase of $I_{CaT}$ was also assumed to be the same as the dose-dependent formulation for the increase of $I_{CaL}$:

$$f_{CaT} = f_{CaT,\text{max}} \frac{[ISO]}{K_{0.5,fCaT} + [ISO]}$$
where \( f_{CaT} \) represents the percentage of increase of \( I_{CaT} \) density, the value of 0.96 was chosen as \( f_{CaT,\text{max}} \), the maximum percentage of increase of \( I_{CaT} \), based on experimental data from murine SA node cells (Li et al., 2012; Larson et al., 2013).

**Shift of activation of \( I_f \)**

In contrast to the effect of ACh, ISO can cause a shift in the voltage-dependent activation curve of \( I_f \) toward more positive potentials, resulting in an increase of \( I_f \) (Alig et al., 2009; Liao et al., 2010). In this Chapter, the shift of \( I_f \) activation curve has been described by the equation as shown below:

\[
S_f = S_{f,\text{max}} \left( \frac{[ISO]^n_f}{K^{n_f}_{0.5,f} + [ISO]^n_f} \right)
\]

where \( S_{f,\text{max}} \) indicates the maximum shift of \( I_f \) activation curve, \( K^{n_f}_{0.5,f} \) is the ISO concentration for the half-maximal shift, and \( n_f \) represents the Hill coefficient. In the model, values of 13.6 mV, 13.5 nM and 0.392 were chosen as \( S_{f,\text{max}} \), \( K^{n_f}_{0.5,f} \), and \( n_f \), respectively, obtained by fitting the equation to the experimental data from murine SA node cells (Alig et al., 2009; Liao et al., 2010; Baruscotti et al., 2011; Larson et al., 2013). The relationship between the shift of \( I_f \) activation curve and ISO concentration is illustrated in Figure 4.2C.

**Effects on \( I_{Kr} \), \( I_{Ks} \) and \( I_{st} \)**

Previous studies have shown that ISO had complex effects on \( I_K \) in pacemaker cells. The current densities of \( I_{Kr} \) and \( I_{Ks} \) were increased with the addition of ISO (Walsh et al., 1988; Freeman and Kass, 1993; Lei et al., 2002; Vinogradova et al., 2008). Moreover, ISO has also been found to shift the activation curve of \( I_{Kr} \) toward more negative potentials (Lei et al., 2000, Ke et al., 2007) and increase the rate of \( I_{Kr} \) deactivation (Lei et al., 2000). Research by Guo et al. (1995) indicated that ISO also increased \( I_{st} \). In this model, because of the limited availability of experimental data from murine SA node cells, the effects of ISO on \( I_{Kr}, I_{Ks} \) and \( I_{st} \) were assumed to be
the same as in the rabbit. The formulations were inherited from Zhang’s (2012) model as shown below:

\[
f_K = f_{K, \text{max}} \frac{[ISO]}{K_{0.5,k} + [ISO]} \quad (4.16)\]

\[
S_K = S_{K, \text{max}} \frac{[ISO]}{K_{0.5,K_{\text{act}}} + [ISO]} \quad (4.17)\]

\[
f_{st} = f_{st, \text{max}} \frac{[ISO]}{K_{0.5, \text{st}} + [ISO]} \quad (4.18)\]

where \( f_K, S_K, \) and \( f_{st} \) represent the percentage of the increase of \( I_K \) (\( I_{Kr} \) and \( I_{Ks} \)), shift of \( I_{Kr} \) activation curve and the percentage of the increase of \( I_{st} \), respectively. The relationship between the changes of these three channels and the ISO concentration is illustrated in Figure 4.2D-F.

**Actions of ISO on Ca\(^{2+}\) handling**

It has been reported that the addition of ISO in SA node cells can cause an elevation of the amplitude and diastolic level of \([\text{Ca}^{2+}]_i\) (Wu et al., 2009). Previous studies have also shown that ISO increased SR uptake and release by stimulation of calmodulin kinase II (Maltsev and Lakatta, 2009). Due to the lack of electrophysiological data, Colman (2014) provided an alternative way to simulate the actions of ISO on Ca\(^{2+}\) handling: that is, by using the dose-dependency of phosphorylation level (a number between 0 and 1) of the RyR and phospholamban (PLB, an inhibitor of SR Ca\(^{2+}\) pump). Thus, Colman’s (2014) approach was implemented in this project,

\[
f_{\text{RyR}} = \left(1.0 + e^{-2.55(\log[\text{ISO}]+1.21)}\right)^{-1} \quad (4.19)\]

\[
f_{\text{PLB}} = \left(1.0 + e^{-3.0(\log[\text{ISO}]+2.2)}\right)^{-1} \quad (4.20)\]

where \( f_{\text{RyR}} \) and \( f_{\text{PLB}} \) represent phosphorylation level of the RyR and PLB, respectively. Then, \( f_{\text{RyR}} \) and \( f_{\text{PLB}} \) were applied to describe the percentage of the maximum effect of ISO on SR Ca\(^{2+}\) release and uptake. Figure 4.2G&H illustrates the relationship between the ISO concentrations and phosphorylation level of the RyR and PLB.
Figure 4.2 Dose-dependent effects of ISO on ionic channels. (A) Fractional increase of $g_{Ca,L}$. (B) Shift of $I_{Ca,L}$-V curves. (C) Fractional shift of $I_f$. (D) Fractional increase of $g_{Kr}$ and $g_{Ks}$. (E) Shift of $I_Kr$ activation curve. (F) Fractional increase of $g_{st}$. (G) Phosphorylation level of RyR. (H) Phosphorylation level of PLB.
4.2.4 Modelling of the circadian rhythm of the autonomic regulation

Previous studies have shown that the behaviour of the autonomic nervous system in the heart demonstrates a circadian pattern (Burgess et al., 1997; Kontopoulos et al., 1999). To be more specific, the sympathetic nervous system indicated a high activity level in the morning and late evening, while parasympathetic nervous system activity was high during sleeping time (Somers et al., 1993; Calandra-Buonaura et al., 2015). Figure 4.3 indicates the experimental observation of the circadian rhythm of the autonomic activities by Kontopoulos et al. (1999). High frequency power (HFP) represents the activity of the parasympathetic nervous system (Figure 4.3A), whereas low frequency power (LFP) reflects the activity of the sympathetic nervous system (Figure 4.3B). As ACh plays a primary role in the parasympathetic nervous system’s regulation of cardiac activity (DiFrancesco et al., 1989), the activity of the parasympathetic nervous system in the heart is assumed to positively relate to the concentration of released ACh. Similarly, the concentration of adrenaline can be used to evaluate the activity of the sympathetic nervous system (Verloop et al., 2015).

In order to simulate the time-of-day variation of heart rate under autonomic regulation, the combined effects of ACh and adrenaline were incorporated into the model in this study. The effect of ISO was applied in the model to mimic the action of adrenaline, as ISO is functionally and structurally similar. The circadian rhythms of ACh and ISO concentrations were based on the circadian activities of parasympathetic and sympathetic nervous systems observed by Kontopoulos et al. (1999) (Figure 4.3). The peak and minimum points in Figure 4.3A were assumed to be the highest and lowest concentrations of ACh in the model over 24 hours. The value of 0 (nM) was set as the lowest concentration, while the highest concentration was chosen to produce the minimum heart rate over 24 hours. A similar approach was applied to ISO. The highest concentration of ISO was assumed to generate the maximum heart rate over 24 hours.
Figure 4.3 The circadian variation of the autonomic nervous system. Adapted from Kontopoulous et al. (1999).
4.3 Results

The model developed above was used to investigate the chronotropic effect of autonomic regulation on the murine sinoatrial node. In this section, simulated results will be presented for: 1) individual effects of ACh and ISO on spontaneous pacemaking activity; 2) functional roles of each action caused by ACh and ISO; and 3) the combined effect of ACh and ISO.

4.3.1 Simulated results for murine SA node cells

The simulated time course of the APs and the dynamic variation of the major ionic currents are illustrated in Figure 4.4. Both the central and peripheral models of the murine SA node indicated a similar AP morphology, with a rapid depolarising upstroke, short APD and fast pacemaking rate, which are consistent with the unique features of isolated murine SA node cell APs (Kharche et al., 2011). However, compared with the central version, the AP generated by the peripheral model demonstrated a more negative take-off point, a more positive overshoot, a more negative maximum diastolic potential, a greater amplitude, and a shorter duration. Moreover, the peripheral model also indicated faster spontaneous activity: the cycle length of the peripheral model was 164 ms, whereas that of the central model was 194.4 ms. The difference between central and peripheral SA node cells is primarily based on their size and capacitance (Yu, 2009). In this study, the cell capacitances of the central and peripheral models are 25 and 50 pF, respectively.

4.3.2 Effect of ACh

Figure 4.5A&B illustrates the simulated action potentials of both central and peripheral murine SA node under control conditions and with the addition of varying concentrations of ACh. It can be seen that ACh slowed down the spontaneous pacemaking activity of both central and peripheral cell models. The simulated results
Figure 4.4 Simulated APs and ionic currents for central and peripheral murine SA node cells. (A) Model-generated APs. (B)-(L): Ionic currents during APs – $I_{Na,1.1}$, $I_{Na,1.5}$, $I_{CaL,1.2}$, $I_{CaL,1.3}$, $I_{CaT}$, $I_f$, $I_{to}$, $I_{Kr}$, $I_{NaK}$, and $I_{NaCa}$. Black solid line represents the central model; red dashed line indicates the peripheral model.
also suggest that the chronotropic effect of ACh depended on its concentrations, and this dose dependency differed for central and peripheral models. The cycle length of APs was prolonged to 0.8 seconds with the presence of 0.04 μM ACh in the central model, which is approximately four times that under control conditions (194.5 ms). However, the effect of this concentration on the peripheral model was relatively small: it increased the cycle length of APs by only 42%, from 127.6 ms (control condition) to 180.8 ms. The increase in cycle length has also been observed in an experimental study of the mouse SA node (Mesirca et al., 2013), as well as in a previous modelling study (Zhang et al., 2002).

In addition, the model in the presence of ACh demonstrated a small overshoot potential and shortened action potential duration. The changes in primary AP characteristics, including the overshoot (OS), the action potential duration, maximal diastolic potential (MDP) and cycle length, in both central (filled circles) and peripheral (open triangles) models are shown in Figure 4.5C-F. The regional difference of the effect of ACh can be observed: the central model had a higher sensitivity to ACh than the peripheral model.

**Contribution of each Ach-affected process to the chronotropic effect**

The actions of ACh on \( I_{K,ACh} \), \( I_{CaL} \), and \( I_f \) were analysed separately by simulating the activation of \( I_{K,ACh} \), the inhibition of \( I_{CaL} \), and the shift of activation curve of \( I_f \) alone in the model. The simulated results are shown in Figure 4.6. It can be seen that the chronotropic effect of ACh on cardiac pacemaking activity was primarily due to the activation of \( I_{K,ACh} \). In the central model, 0.05 μM ACh increased the cycle length by 43.8% from 194.5 ms to 279.6 ms when the ACh activated only the \( I_{K,ACh} \), whereas the increase for the other two effects was 5.3% (\( I_{CaL} \) affected only) and 12.9% (\( I_f \) affected only), respectively. The dose dependency of the percentage increase of cycle length by ACh in the central and peripheral models (illustrated in Figure 4.7) also
Figure 4.5 Simulated effects of ACh on murine SA node. (A-B): The effect of different concentrations of ACh on the APs of the central (A) and peripheral (B) models. (C-F): Concentration-dependent changes of APs characteristics in central model (filled circles) and peripheral model (open triangles). (C) overshoot potential, (D) action potential duration, (E) maximal diastolic potential, and (F) cycle length.
Figure 4.6 Analysis of each ACh-affected action on APs of central (i) and peripheral (ii) murine SA node models as a function of time. (A) Effect of activation of $I_{K,ACH}$. (B) Effect of ACh-induced depression of $I_{CaL}$. (C) Effect of ACh-induced inhibition of $I_f$. Control conditions (with the absence of ACh, black solid line) are shown for reference.
confirmed the major contribution of the activation of $I_{K,ACh}$ to the effect of ACh (open square). With ACh activating $I_{K,ACh}$ only, the cycle length could be significantly increased by 100% (at the concentration of 0.055 μM), while the actions of ACh on $I_f$ or $I_{CaL}$ were unable to produce similar effects. However, the effect of ACh on $I_f$ was also important. As shown in Figure 4.6A(ii) and Figure 4.6C(ii), the increase of cycle length caused by ACh activating $I_{K,ACh}$ and affecting $I_f$ were approximately the same (15.9% vs 16.0%) in peripheral model. The simulated results also suggested a limited role for the action of ACh on $I_{CaL}$. As shown in Figure 4.7B, the inhibition of $I_{CaL}$ due to the ACh caused only a negligible change in cycle length in the central model. With the peripheral model, the inhibition of $I_{CaL}$ even accelerated the spontaneous pacemaking activity. The experimental observation on a peripheral rabbit SA node cell indicated similar results. Complete block of $I_{CaL}$ by nifedipine also accelerated the pacing rate (Kodama et al., 1997).

![Figure 4.7](image)

Figure 4.7 Concentration dependency of percentage increase of the cycle length by ACh in the central model (A) and the peripheral model (B) under different conditions. Filled circles: normal condition (ACh-affected $I_{K,ACh}$, $I_{CaL}$ and $I_f$). Open squares: ACh-activated $I_{K,ACh}$ only. Open triangles: ACh-affected $I_f$ only. Open diamonds: ACh-affected $I_{CaL}$ only.
4.3.3 Effect of ISO

Figure 4.8A&B illustrates the simulated APs and the intracellular Ca$^{2+}$ concentration for both the centre and the periphery of the murine SA node cell under control conditions and in the presence of 0.1 μM ISO. It can be seen that ISO had an effect on the morphology of APs in both cell models. It produced a shortened cycle length and action potential duration, and a large amplitude of action potentials, which are comparable with the experimental data on murine SA node cells (Alig et al., 2009; Baruscotti et al., 2011). ISO also raised the diastolic depolarisation rate (DDR) by 21.7%, from 23 to 27 V/s in the central model, and by 17% from 36 to 42 V/s in the peripheral model. This increment of DDR is also observed by Wu et al. (2009) on a murine SA node (Wu et al., 2009). In addition, the amplitude of intracellular Ca$^{2+}$ concentration was increased by 12% and 55% in the central and peripheral models, respectively. The dynamic variation of ionic currents affected by ISO is shown in Figure 4.8C-F. It can be seen that, in both cell models, $I_{CaL}$, $I_{CaT}$, and $I_f$ were increased during the diastolic depolarisation phase, which is the main reason for the increment of DDR; this therefore accelerated the rate of rhythmic activity. The reduction in APD can be attributed to the increase in the outward current, $I_K$, (Figure 4.8E).

The simulated APs with the presence of varying concentrations of ISO are shown in Figure 4.9. Similar to the effect of ACh on pacemaking activity, the chronotropic effect of ISO depended on its concentrations. In the central model, the pacing rate of the cell was increased by 16% with 0.001 μM ISO, whereas the increase of pacing rate by 0.1 μM ISO was 27%. In the peripheral model, 0.001 μM and 0.1 μM of ISO accelerated the pacing rate by 7% and 9%, respectively. The simulated effect of ISO on pacing rate is compared with experimental data from murine SA node cells (Wu et al., 2009) in Figure 4.9C. Both central and peripheral models indicated the dose dependency, but the regional difference can be observed; the increase in pacing rate in the central model is larger than that in the peripheral model.
Figure 4.8 Simulated effect of ISO on murine SA node. Data of central (i) and peripheral (ii) models in control (black solid line) and with 0.1 μM ISO (red dashed line) are shown: (A) APs; (B) intracellular \([\text{Ca}^{2+}]\); (C) \(I_{\text{CaL}}\); (D) \(I_{\text{CaT}}\); (E) \(I_f\); and (F) \(I_{Kr}\).
Chapter 4 Modelling of the chronotropic effect of autonomic regulation on murine SA node

Figure 4.9 Dose-dependent effect of ISO on APs and pacing rate of murine SA node model. Simulated APs produced by murine SA node central (A) and peripheral (B) models in control and different concentrations of ISO. (C): Concentration dependence of ISO effect on pacing rate for central (black) and peripheral (red) models. Experimental data from murine SA node by Wu et al. (2009) are shown for reference. Pacing rates are normalised to the value under control condition (no ISO) for each model.

**Contribution of each ISO-affected process to the chronotropic effect**

The relative roles of each action caused by ISO were analysed separately by simulating this action only. The resultant change in pacing rate is illustrated in Figure 4.10. It can be seen that the chronotropic effects of ISO were primarily contributed by the actions on $I_{CaL}$, $I_{CaT}$ and $I_f$. In the central model, the dose dependencies of the ISO
Figure 4.10 Dose dependence of ISO effect on pacing rate in central (A) and peripheral (B) models under different simulated conditions. Open circles: experimental data from mouse SA node cells by Wu et al. (2009). Black solid line: normal condition (ISO affected $I_{CaL}$, $I_{CaT}$, $I_f$, $I_K$, $I_{st}$, RyR and SERCA). Red solid line: $I_{CaL}$ affected only. Blue dashed line: $I_{CaT}$ affected only. Green solid line: $I_f$ affected only. Red dashed line: $I_K$ affected only. Blue solid line: $I_{st}$ affected only. Grey dashed line: RyR affected only. Green dashed line: SERCA affected only.
effect on $I_{Ca,L}$ (solid red line) and $I_{Ca,T}$ (dashed blue line) were similar: both reached the maximal effect at approximately 0.1 μM of ISO. However, in the peripheral model, the contribution of the action on $I_{Ca,T}$ was relatively larger than that of the ISO effect on $I_{Ca,L}$. The contribution of ISO-affected $I_f$ was also important. The maximal increase of pacing rate caused by ISO action on $I_f$ was almost the same in both central and peripheral models (increasing the pacing rate by around 8%). In the central cell, the chronotropic effect of ISO at low concentrations of $< 10^{-3}$ μM is primarily the result of action on $I_f$. In the peripheral cell, the action on $I_f$ provided the major contribution to the effect of ISO on pacing rate. In contrast to the role of the ISO-affected process on $I_{Ca,L}$, $I_{Ca,T}$ and $I_f$, the action on $I_{Kr}$ reduced the pacing rate of the murine SA node (dashed red line). Furthermore, the simulated results also suggested a limited contribution of ISO effect on $I_{st}$ (solid blue line), RyR (dashed grey line) and SECRA (dashed green line) to the chronotropic effect on the pacing rate, as shown in Figure 4.10.

4.3.4 Combined effect of ACh and ISO on murine SA node

As models for the effects of both ACh and ISO have been developed, the investigation for the combined effects of ACh and ISO on pacemaker activity can be performed by the model. Figure 4.11 illustrates the influence of 0.02 μM of both ACh and ISO on the AP of the centre and the periphery of the murine SA node model. The control conditions, the effects of 0.02 μM of ACh and 0.02 μM of ISO, are shown in Figure 4.11A-C for reference. It can be seen that the shape of the action potential was changed with the presence of ACh and ISO; a shortened action potential duration and more depolarised MDP were observed in both cell models. The cycle length was also prolonged by 6.5% and 17.8% in the central and peripheral cells respectively. As ACh and ISO have an ‘opposite’ influence on the heart rate, it is important to determine the relative roles of ISO and ACh on cardiac pacemaker activity.
Figure 4.11 Effect of ACh and ISO on APs of central (left) and peripheral (right) models. Simulated APs under control (A), ACh induced (B), and ISO induced (C) conditions are shown for reference.

Figure 4.12 illustrates the change of pacing rate of the central cell model with the addition of varying concentrations of ACh and ISO. The black line in the figure represents that the pacing rate is equal to that under control conditions (308 bpm); thus, the effect of ACh was compensated for by ISO. It can be seen that the effects on pacing rate were dominated by ACh, at a relatively higher concentration of ACh (>0.02 μM).
4.3.5 Circadian rhythm of the heart rate under autonomic regulation

The combined effects of ACh and ISO were incorporated into the model to simulate the time-of-day variation of heart rate under autonomic regulation. As discussed in section 4.2.3, the minimum and maximum heart rates were assumed to be produced by the highest concentrations of ACh and ISO respectively over 24 hours. Ho et al.’s (2011) experimental data from mice indicated that the minimum heart rate is approximately 82% of the mean heart rate over a day, whereas the maximum heart rate is 114% of the mean. Based on the concentration dependency of the change in heart rate by ACh and ISO (as shown in Figure 4.7 and Figure 4.9), the values of 12
(nM) and 10 (nM) were chosen as the highest concentration of ACh and ISO, respectively. The circadian variations of concentrations of ACh and ISO are illustrated in Figure 4.13A and Figure 4.13B. It can be seen that ACh concentration was higher between midnight and 7am whereas ISO concentration was higher in the morning (8am to 2pm) and late evening (7pm to 9pm).

Figure 4.13C demonstrates the time-of-day variation of simulated pacing rate in murine SA node models. The simulated results for models individually affected by ACh (filled triangles) and ISO (filled squares) are shown for reference. It can be seen that the pacing rate was relatively low when only ACh affected the model, whereas the model with the addition of ISO demonstrated a high pacing rate. The pacing rate was relatively low (approximately 260 bpm) for the model dually influenced by ACh and ISO during the early morning (1am to 6am), which is close to the value of the model affected by ACh only. Then it increased between 7am and 11am. After peaking at 11am, the pacing rate reduced to 310 bpm at 5pm, which was followed by another increase to 355 bpm during late evening (7pm to 9pm). Then it reduced to 275 bpm at midnight. In addition, the pacing rate in the late morning (7am to 11am) and late evening (7pm to 9pm) was similar to the value of the model affected by ISO only.
Figure 4.13 The time-of-day variation of ACh concentration (A), ISO concentration (B), and heart rate under different conditions (C&D). Open circles: the model dually influenced by ACh and ISO. Filled triangles: ACh affected only. Filled squares: ISO affected only. Filled dimonds: Huikuri et al. (1990). Filled stars: Krauchi et al. (2010).
4.4 Summary

In this chapter, the murine SA node model developed in Chapter 3 was further modified to take into consideration the regional differences of the SA node. The peripheral model demonstrated faster pacemaking activity than the central model, which is consistent with previous experimental and modelling studies (Zhang et al., 2000; Lei et al., 2005). Then, detailed electrophysiological formulations for sympathetic and parasympathetic regulation were constructed, based on the experimental data from murine SA node cells, and implemented in the newly developed murine model. By using the model, the chronotropic effects of ACh and ISO, together with each affected action for spontaneous pacemaking activity, were investigated. Simulated results suggested: 1) the activation of $I_{K,ACh}$ and the shift of $I_f$ activation curve caused by ACh were the major contributions to the effect of ACh in slowing down the pacing rate; 2) the increase of ionic current $I_{CaL}$, $I_{CaT}$ and $I_f$ associated with ISO were primarily responsible for ISO accelerating the pacing rate; 3) the combined effects of ISO and ACh on pacing rate were dominated by ACh when ACh was at a higher concentration; and 4) both effects of ACh and ISO indicated regional differences, the central model having a higher sensitivity to both ACh and ISO than the peripheral model. In addition, the circadian rhythm of the autonomic regulation was also investigated by the model. The simulated results suggest that the autonomic nervous system’s regulation of cardiac pacemaking activity results from a balance between the sympathetic and parasympathetic regulatory pathways. The heart rate was high in the late morning and late evening, suggesting the sympathetic nervous system remained dominant, while the value was relatively low during sleeping time, meaning the parasympathetic nervous system plays an important role. This is consistent with clinical observations that significantly lower heart rates occur between 1am and 7am (Martínez-Lavín et al., 1998; Carandente et al., 2006). The model provided a fast and accurate way to calculate the circadian rhythm of heart rate and to further understand the underlying mechanisms governing the heart.
Comparing with previous modelling studies on the murine SA node (Mangoni et al., 2006; Kharche et al., 2011), the key achievements of this model include: 1) incorporating the regional differences of the cardiac pacemaker cell; 2) considering the independent dose dependency of all affected actions caused by ISO and ACh; 3) analysing the contribution of each ACh/ISO-affected process to the chronotropic effect, and 4) investigating the circadian rhythm of the autonomic regulation. The model will be used for the investigation of sick sinus syndrome due to the deficiency of \textit{HCN1} (Chapter 5).
Chapter 5
Biophysical modelling of sinus node dysfunction due to *HCN1* deficiency

5.1 Introduction

Sick sinus syndrome (SSS), also known as sinus node dysfunction (SND), refers to a collection of abnormal heart rhythms, presumably due to the dysfunction of the heart’s primary pacemaker, the sinoatrial (SA) node. It is always, pathologically speaking, “the parent in crime” (Monfredi and Boyett, 2015), which usually results in many cardiac arrhythmias, such as intermittent sinus bradycardia, sinus pause, sinus arrest, slow SA node-atrium conduction, sinus exit block, alternating bradycardia and chronic atrial tachycardia (Dobrzynski et al., 2007). Although the syndrome has a relatively higher incidence in the elderly, it can also occur in other age groups, and even in new-borns (Dobrzynski et al., 2007). In addition, both genders are affected equally. Patients with SND may present with a wide range of symptoms, including syncope, chest pain, shortness of breath, dizziness or light-headedness (Butters et al., 2010). At present, the common treatment of SSS is the implantation of an artificial cardiac pacemaker.

However, the mechanisms underlying the pathogenesis of SND are unclear. A previous study has shown that healthy people without any evident structural heart disease can also be affected by SND because of particular genetic defects (Benson et al., 2003). Over the last decade, the molecular basis of the ionic channels related to SND has been investigated in various mammals. Familial SND is associated with several gene mutations, including the fast sodium current gene *SCN5A*, the funny current gene *HCN*, the T-type Ca$^{2+}$ current gene *CACNA1G* and the L-type Ca$^{2+}$
current gene \textit{CACNA1C} (Monfredi and Boyett, 2015). Among them, \textit{HCN} is considered to be of particular importance.

The hyperpolarization-activated cyclic nucleotide-gated (\textit{HCN}) channel family is widely expressed throughout the heart, and previous studies have shown that three of its four members (\textit{HCN1}, \textit{HCN2} and \textit{HCN4}) exit in SA node cells (Fenske et al., 2013). Quantitatively, \textit{HCN4} is identified as the major isoform in pacemaker cells, counting approximately 70-80\% of the total \textit{I_f}, whereas \textit{HCN1} and \textit{HCN2} are expressed at relative low levels (Hoesl et al., 2008). Research by Stieber et al. (2003) indicated that \textit{HCN4} plays an important role in the formation of mature SA node cells during embryogenesis. Furthermore, a number of studies on human, rabbit and genetic mouse models suggested that this channel is also essential for the automaticity of the heart rate, as mice lacking \textit{HCN4} display sinus bradycardia (Baruscotti et al., 2011; Zhang, 2012). The role of \textit{HCN2} in spontaneous pacemaker activity is relatively obscure. However, Ludwig et al. (2003) observed that, compared with control conditions, \textit{HCN2}-deficient mice do not show significantly different heart rates, but these mice exhibit cardiac dysrhythmia.

In contrast to \textit{HCN2} and \textit{HCN4}, previous studies regarding the contribution of \textit{HCN1} to the SA node are limited. \textit{HCN1} was originally considered to exist mainly in the brain; however, increasing evidence has shown that \textit{HCN1} is not only expressed in the heart, but also involved in the control of rhythmic cardiac activity. Several studies have indicated a considerable level of \textit{HCN1} transcription in the SA node, counting approximately 20–35\% of the total \textit{HCN} (Herrmann et al., 2011; Marionneau et al., 2005). However, corresponding results were not observed in some other studies (Baruscitti et al., 2011; Liu et al., 2007). Recently, an expression study in mouse hearts confirmed the specific \textit{HCN1} expression at the protein level and suggested an essential role of \textit{HCN1} in the native \textit{I_f} and spontaneous pacemaker activity (Herrmann...
et al., 2012). In addition, another recent study by Fenske et al. (2013) observed sinus bradycardia in HCN1-knockout mice.

Although previous experimental studies have shown that SND is closely associated with the HCN1 mutations (Fenske et al. 2013), the mechanisms underlying the relationship between the loss-of-function of $I_f$ caused by HCN1 mutations and the abnormality of cardiac pacemaking have not been fully clarified, especially from the perspective of cell electrophysiology and intercellular electrical couplings at tissue levels. Biophysically detailed computational models can provide a platform for the analysis of the initiation and conduction of cardiac electrical activity. Butters et al. (2010) investigated the causative relationship between SCN5A gene mutations and SND, and successfully demonstrated the mechanisms by which the mutations impair the driving ability of the SA node by using an anatomically detailed 2D model of intact rabbit SA nodes-atria.

Based on this, in this Chapter, the aim was to: 1) update the newly developed murine SA model to consider the molecular basis of the funny current; and 2) develop a 2D tissue model of the murine SA node and the surrounding atrial muscle. To these end, by using the model, I investigated the mechanisms underlying the loss-of-function of the $I_f$ due to HCN1 deficiency and how this impairs the spontaneous activity of the heart and weakens cardiac impulse propagation from the SA node to the atrial tissue.
Chapter 5 Biophysical modelling of sinus node dysfunction due to *HCNI* deficiency

5.2 Methods

In this chapter, the consequences of the loss-of-function of *I_f* caused by *HCNI* deficiency on generating SND were investigated by using: 1) electrophysiologically detailed computational models of the central and peripheral murine SA node cells; and 2) 2D models of the intact SA node-atrium tissue, incorporating accurate single-cell models of the SA node and right atrium (RA) and histologically reconstructed tissue geometry.

5.2.1 New formulations for *I_f*

The murine SA node model developed in Chapter 4, based on the Kharche et al. (2011) model, was further modified for studying the effects of *HCN* mutations. *I_f* is a mixed current, which is permeable to Na\(^+\) and K\(^+\) ions (Zhang et al., 2000); in the Kharche et al. (2011) model, the *I_f* has two components: *I_{f,Na}* and *I_{f,K}*. However, Kharche et al. (2011) only presented a single native *I_f* in the murine SA node cell model due to the limited experimental data. In this chapter, an isoform-specific *I_f* was introduced, consisting of *I_{f,HCNI}*, *I_{f,HCN2}* and *I_{f,HCN4}*, each of which considered the channel permeability to Na\(^+\) and K\(^+\) ions. Thus, the newly developed *I_f* is given by:

\[
I_f = I_{f,HCN1} + I_{f,HCN2} + I_{f,HCN4} \tag{5.1}
\]

\[
I_{f,HCN1} = I_{f,Na,HCN1} + I_{f,K,HCN1} \tag{5.2}
\]

\[
I_{f,HCN2} = I_{f,Na,HCN2} + I_{f,K,HCN2} \tag{5.3}
\]

\[
I_{f,HCN4} = I_{f,Na,HCN4} + I_{f,K,HCN4} \tag{5.4}
\]

Experimentally, the basic properties of *I_f* are investigated by the whole-cell patch-clamp technique in isolated SA node cells and described by the equations developed by Noble and DiFrancesco (1989). For example, formulations for *I_{f,HCNI}* are:

1. **I_{f,HCNI}**
2. **I_{f,HCN2}**
3. **I_{f,HCN4}**
Chapter 5 Biophysical modelling of sinus node dysfunction due to HCN1 deficiency

\[ I_{fNa,HCN1} = g_{fNa,HCN1} y_{HCN1} (V - E_{Na}) \]  \hspace{1cm} (5.5)

\[ I_{fK,HCN1} = g_{fK,HCN1} y_{HCN1} (V - E_{K}) \]  \hspace{1cm} (5.6)

where \( g_{fNa,HCN1} \) and \( g_{fK,HCN1} \) are channel conductance, and \( E_{Na} \) and \( E_{K} \) are the channel reversal potentials arising from Na\(^+\) and K\(^+\) gradients. The activation curves are fitted by the Boltzmann function:

\[ y = \frac{1}{1 + e^{(V - V_{0.5})/k}} \]  \hspace{1cm} (5.7)

where \( V_{0.5} \) is the voltage for current half activation and \( k \) is the slope factor.

<table>
<thead>
<tr>
<th>Reference</th>
<th>HCN-knockout ( I_f )</th>
<th>Control ( I_f )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_{0.5} ) (mV)</td>
<td>( k )</td>
</tr>
<tr>
<td>Baruscotti et al. (2011)*</td>
<td>-76.0</td>
<td>11.7</td>
</tr>
<tr>
<td>Herrmann et al. (2007)*</td>
<td>-90.4±3.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Ludwing et al. (2003)#</td>
<td>-92.2±1.4</td>
<td>14±1.4</td>
</tr>
<tr>
<td>Alig et al. (2009)</td>
<td>-101±3</td>
<td>15.8±1.7</td>
</tr>
<tr>
<td>Cho et al. (2003)</td>
<td>-107±5.6</td>
<td>15.4±3.6</td>
</tr>
<tr>
<td>Mangoni et al. (2001)</td>
<td>-87±1</td>
<td>12±1</td>
</tr>
</tbody>
</table>

*Represents HCN4-knockout; #Represents HCN2-knockout.

The electrophysiological properties of individual HCN channel isoforms are experimentally studied by using genetically modified mice. As shown in Table 5.1, previous experimental studies demonstrated a wide range in measured \( V_{0.5} \) of \( I_f \) in murine SA node cells. Meanwhile, mutants and control mice exhibit very similar \( V_{0.5} \) of \( I_f \). However, a recent research by Zong et al. (2012) showed that the activation kinetics of individual isoforms are different to each other; HCN1 has a more positive \( V_{0.5} \) compared with HCN2 and HCN4. Another study by Fenske et al. (2013) indicated a similar observation. Based on Zong et al.’s (2012) results, in this study, we chose...
the value of $V_{0.5} = -75.2$ mV for $I_{f,HCN1}$, -92.0 mV for $I_{f,HCN2}$ and -91.2 mV for $I_{f,HCN4}$. The experimental observation and model for $I_f$ steady-state activation are shown in Figure 5.1A and Figure 5.1C.

![Figure 5.1](image)

Figure 5.1 Activation kinetics for different $HCN$ isoforms. (A) Steady-state activation curve as a function of membrane voltage; open circles, open triangles and filled squares represent experimental data for $I_{f,HCN1}$, $I_{f,HCN2}$ and $I_{f,HCN4}$, respectively, by Zong et al. (2012). (B) Time kinetics for $I_{f,HCN1}$, $I_{f,HCN2}$ and $I_{f,HCN4}$; open circles: experimental data by Herrmann et al. (2007). (C) Summarised $V_{0.5}$ data of three $HCN$ channels; filled circles: experimental data by Zong et al. (2012). (D) Time constants ($\tau_{act}$) at -100 mV; filled circles: experimental data by Herrmann et al. (2007).

Individual $HCN$ channel isoforms also illustrate different voltage-dependent time kinetics: $HCN1$ and $HCN2$ activate more rapidly than $HCN4$ channels in murine SA node cells. Moosmang et al. (2001) found that the $HCN1$ channel activates 16-times faster than the $HCN2$ channel and 25-times faster than the $HCN4$ channel. Similar results have also been observed in rabbit SA nodes (Altomare et al., 2003). In this
Chapter 5 Biophysical modelling of sinus node dysfunction due to HCN1 deficiency

Chapter, the activation time constants of the $I_f$ were fitted according to the experimental data measured from cultured mammalian cell lines (HEK293), which stably expressed murine HCN1, HCN2 and HCN4 (Herrmann et al., 2007). The experimental data and the model for $I_f$ time kinetics are shown in Figure 5.1B and Figure 5.1D.

Table 5.2 Current density percentages among $I_{f,HCN1}$, $I_{f,HCN2}$ and $I_{f,HCN4}$.

<table>
<thead>
<tr>
<th>Reference</th>
<th>HCN1</th>
<th>HCN2</th>
<th>HCN4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ludwing et al. (2003)</td>
<td>30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herrmann et al. (2007)</td>
<td>25%</td>
<td></td>
<td>75%</td>
</tr>
<tr>
<td>Hoesl et al. (2008)</td>
<td></td>
<td></td>
<td>75-80%</td>
</tr>
<tr>
<td>Ludwing et al. (2008)</td>
<td></td>
<td></td>
<td>75%</td>
</tr>
<tr>
<td>Baruscoetti et al. (2011)</td>
<td></td>
<td></td>
<td>~80%</td>
</tr>
<tr>
<td>Herrmann et al. (2011a)</td>
<td></td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td>Fenske et al. (2013)</td>
<td>30%</td>
<td>10%</td>
<td>60%</td>
</tr>
<tr>
<td>Gaborit et al. (2007)*</td>
<td>20.5%</td>
<td>16%</td>
<td>63.5%</td>
</tr>
<tr>
<td>Chandler et al. (2009)*</td>
<td>40%</td>
<td>10%</td>
<td>50%</td>
</tr>
<tr>
<td>Herrmann et al. (2011b)*</td>
<td>34%</td>
<td>7%</td>
<td>59%</td>
</tr>
<tr>
<td>Model</td>
<td>28.4%</td>
<td>10.7%</td>
<td>60.9%</td>
</tr>
</tbody>
</table>

* Represents mRNA data.

The relative contributions of HCN1, HCN2 and HCN4 to the whole-cell $I_f$ were also considered in the model. Previous experimental observations for current density ratios among $I_{f,HCN1}$, $I_{f,HCN2}$ and $I_{f,HCN4}$ are summarised in Table 5.2. Based on these, the maximum ionic current conductance were assigned proportionally to the various isoforms: HCN4 was considered to be the largest component of the native $I_f$ and the ratio among these three isoforms ($g_{f,HCN1}$:$g_{f,HCN2}$:$g_{f,HCN4}$) is approximately 3:1:6. Model equations and parameters for $I_f$ were validated by quantitatively comparing the simulated I-V relationship with experimental data (Baruscoetti et al., 2011; Herrmann
et al., 2007; Khoury et al., 2013). Current traces of three different isoforms during voltage clamp simulations are shown in Figure 5.2.

Figure 5.2 Simulated patch clamp and I–V relationships. $I_f$ was activated by depolarising voltage steps (varying from -130 to -50 mV) from a holding potential of -40 mV. (A) Current trace of voltage clamp recordings of $I_f$. (B) I–V relationships for $I_f$; solid line: model-generated I–V curves, open circles: Baruscotti et al. (2011), open squares: Herrmann et al. (2008), open triangles: Khoury et al. (2013). (C) Model-generated I–V curves for each $HCN$ channel. (D) Current densities at -130 mV for different $HCN$ channels.

### 5.2.2 Modelling of heart rate variability

In this Chapter, the effects of $HCNI$ mutations on heart rate (cycle length) and heart rate variability (HRV) were also studied by incorporating the stochastic fluctuations in autonomic nerve activity in the control and the mutant murine SA node models.
As discussed in Chapter 4, the spontaneous pacemaking activity of the heart is also influenced by the autonomic nervous system. Previous studies have shown that HRV is usually attributed to cardiac autonomic nerve fluctuations; autonomic blockade abolished HRV in the conscious human (Craft and Schwartz, 1995; Pomeranz et al., 1985). Therefore, the pulse-like random fluctuation was intended to simulate the stochastic behaviour of the autonomic modulation of cardiac pacemaking by the release of ACh and ISO. In the model, the concentration of ACh and ISO was calculated by:

\[
[ACh] = [ACh]_0 + [ACh]\xi(t)
\]  

and

\[
[ISO] = [ISO]_0 + [ISO]\xi(t)
\]

where \([ACh]_0\) and \([ISO]_0\) were assumed to have constant values of \(9 \times 10^{-3} \text{M}\), which reproduced the average chronotropic effect of parasympathetic and sympathetic nervous regulation, respectively. \([ACh]\xi(t)\) and \([ISO]\xi(t)\) indicate the random fluctuation term, which is given by:

\[
[ACh]_{\xi(t)} = \begin{cases} 
C_1(p_1 - 0.5) & \text{if } (p_1 \geq 0.5); \\
C_1(-p_1) & \text{otherwise};
\end{cases}
\]  

and

\[
[ISO]_{\xi(t)} = \begin{cases} 
C_2(p_2 - 0.5) & \text{if } (p_2 \geq 0.5); \\
C_2(-p_2) & \text{otherwise};
\end{cases}
\]

where \(C_1\) and \(C_2\) represent the constant concentrations of ACh and ISO, respectively. \(p_1\) and \(p_2\) are pseudo-random numbers between 0 to 1, generated using uniform distribution (Zhang et al., 2009).

### 5.2.3 Development of a 2D SA node-atrium tissue model

For the purposes of investigating the effects of the HCN1 mutation on the behaviours of cardiac tissue, a 2D anatomical model of the intact SA node-atrium tissue was
developed, considering the heterogeneity of action potential characteristics, the anisotropy of tissue conducting properties and the complex geometry of the RA.

5.2.3.1 Anatomical SA node-atrium geometry
Due to the limited availability of experimental observations on murine SA node cells, the tissue slice used in this Chapter was adapted from Zhang’s (2012) results, an immunohistologically reconstructed geometry of the rabbit RA tissue meshed from the endocardiac surface (Dobrzynski et al., 2005). Zhang’s geometry not only indicates a high resolution (40 μm × 40 μm) and a regular Cartesian grid of 385 × 250 nodes, but also identifies the distribution of different cell types throughout the RA; each node is considered as either a SA node or RA cells. Because of the differences between the species, Zhang’s geometry was rescaled by reducing nodes and adjusting the resolution to match the size of the murine RA. In addition, the geometry was further modified to match the distribution of SA node in murine RA based on experimental observation by Liu et al. (2007). Therefore, the geometry used in the model was discretised by a spatial resolution of 15 μm, forming a regular Cartesian grid of 192 × 125 nodes, each of which represents the murine SA node or RA.

5.2.3.2 Mathematical 2D slice model
To simulate the dynamics of the initiation and propagation of action potentials, the monodomain equation was employed in this study (Zhang, 2012; Zhang et al., 2000):

\[
\frac{\partial V}{\partial t} = \nabla \cdot (D \nabla V) - \frac{I_{tot}}{C_m}
\]  

(5.12)

where \(D\) is the tensor of diffusion coefficients characterising the spread of electrical activity, \(\nabla\) is the spatial gradient operator, \(I_{tot}\) represents the total ionic membrane current calculated by different cell models and \(C_m\) is the cell capacitance. In the tissue model, \(I_{tot}\) for SA node cells was computed using murine central and peripheral SA
node cell models developed in this study, whereas for atrial cells the biophysically
detailed mouse atrium model by Shen et al. (unpublished) was incorporated.

As described above, there are regional differences in cell electrophysiology and
electrical couplings between the SA node centre, periphery and the surrounding
atrium (Butters et al., 2010; Zhang, 2012). Therefore, to simulate the propagation of
the action potential throughout the RA, the spatial gradient in cellular
electrophysiology and intercellular electrical coupling was introduced. In the model,
the differences in electrical couplings were modelled by a gradient of diffusion
coefficient (Butters et al., 2010), which is formulated by:

\[
D_{(x)} = D_c + D_p \left[ \left( \frac{1}{1 + e^{-0.3(x-x_1)}} + \frac{1}{1 + e^{-0.3(x-x_2)}} \right)^2 \\
+ \left( \frac{1}{1 + e^{-0.3(y-y_1)}} + \frac{1}{1 + e^{-0.3(y-y_2)}} \right)^2 \right]^{0.5} \quad (5.13)
\]

where \(x\) and \(y\) are the transverse and longitudinal coordinates of the 2D slice,
respectively, \(x_1, x_2, y_1\) and \(y_2\) represent the approximate boundaries of the SA node and
\(D_c\) and \(D_p\) are the diffusion coefficients of central and peripheral SA node cells,
respectively. The gradient in diffusion coefficients are illustrated in Figure 5.3.

Meanwhile, in order to simulate the regional differences in electrophysiological
properties, the gradient in current densities from the centre to the periphery of the SA
node was incorporated by correlating the cells with the cell capacitance (Butters et al.,
2010). Similarly to the diffusion coefficient, the gradient of the cell capacitance was
formulated by:

\[
C_m(x) = C_m^c + C_m^p \left[ \left( \frac{1}{1 + e^{-0.1(x-x_1)}} + \frac{1}{1 + e^{-0.1(x-x_2)}} \right)^2 \\
+ \left( \frac{1}{1 + e^{-0.1(y-y_1)}} + \frac{1}{1 + e^{-0.1(y-y_2)}} \right)^2 \right]^{0.5} \quad (5.14)
\]
where $C_m^c$ and $C_m^p$ represent the cell capacitances of central and peripheral SA node cells, respectively. The gradient in membrane capacitance is demonstrated in Figure 5.3. In addition, according to the experimental observations, the spatial variations of ionic current conductances were controlled by $C_m$ (Butters et al., 2010):

$$g_z(x, y) = g^c_z + C_m^p - C_m^c \frac{C_m^p - C_m^c}{C_m^p - C_m^c} g^p_z$$  \hspace{1cm} (5.15)$$

where $g_z(x, y)$ represents the conductance for one of the ionic currents $z \in \{\text{Na, Ca, K...}\}$ and $g^c_z$ and $g^p_z$ are the conductances for central and peripheral SA nodes, respectively.

Figure 5.3 Gradients in diffusion coefficients and cell capacitances along the transverse and longitudinal directions of the SA node–atrium. (A & B) Gradients in diffusion coefficients along the transverse and longitudinal directions. (C & D) Gradients in cell capacitances along the transverse and longitudinal directions.
5.3 Results

In this Chapter, the newly developed model of murine SA nodes and the surrounding atrial cells were used to investigate SND generated by the loss-of-function of $I_f$ due to $HCNI$ deficiency. In this section, simulation results will be presented for: 1) normal electrophysiological properties at the single-cell and tissue level; and 2) the effect of $HCNI$ mutation on the spontaneous rhythmic pacemaking activity of the heart.

5.3.1 Comparison with the experimental data

Due to the different experiment environments, the experimentally observed APs of murine SA nodes are variable. For example, the cycle length is given by Verheijck et al. (2001) to be 144 ms; by Cho et al. (2003) to be 204 ms; by Mangoni et al. (2003) to be 190±22 ms; by Lei et al. (2004) to be 157±2 ms; by Lei et al. (2005) to be 170±7 ms; and by Mangoni et al. (2006) to be 256 ms. Obviously, the experimental data have a large range, with the longest being more than double that of the shortest. However, the model’s results are 194.5 ms for the centre and 127.5 ms for the periphery of the SA node, lying within this range. The other main characteristics of simulated APs, such as $APD_{90}$, maximal diastolic potential (MDP) and $dV/dt_{\text{max}}$, are also comparable with the experimental observations. The quantitative results are summarised in Table 5.3.

<table>
<thead>
<tr>
<th></th>
<th>CL* (ms)</th>
<th>APD$_{50}$* (ms)</th>
<th>APD$_{90}$* (ms)</th>
<th>OS* (mV)</th>
<th>MDP* (mV)</th>
<th>$dV/dt_{\text{max}}$* (V/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>106 to 256</td>
<td>26 to 57</td>
<td>46 to 107</td>
<td>23 to 42</td>
<td>-70 to -52</td>
<td>8 to 32</td>
</tr>
<tr>
<td>Centre</td>
<td>194.50</td>
<td>29.5</td>
<td>56.4</td>
<td>23.9</td>
<td>-66.37</td>
<td>8.97</td>
</tr>
<tr>
<td>Periphery</td>
<td>127.50</td>
<td>27.70</td>
<td>51.90</td>
<td>35.35</td>
<td>-71.32</td>
<td>23.98</td>
</tr>
</tbody>
</table>

Modified from Kharche et al. (2011). * please see the Glossary.
5.3.2 Effects of HCN1 deficiency on the single-cell model

The functional effects of HCN1 mutations on pacemaking APs at a single-cell level were investigated by isoform-specific ionic current blocking simulations. Figure 5.4 illustrates the simulated APs, together with the main AP characteristics, under control and mutation conditions in the central (left panels) and peripheral (right panels) cells. The simulation results have shown that all of the HCN isoform deficiencies slowed down the pacemaker activity in both the central and peripheral cell models. To be more specific, the measured cycle length increased from the control value of 194.5 ms to 221.2 ms (13.7%) for the central model, and from 127.5 ms to 143.5 ms (12.5%) for the peripheral model when \(I_{f,HCN1}\) was blocked. Lacking \(I_{f,HCN2}\) caused a slight increase of the cycle length by 1.1% (from 194.5 ms to 196.6 ms) for the central cell, and 1.0% (from 127.5 ms to 128.8 ms) for the peripheral cell. Blocking \(I_{f,HCN4}\) increased the cycle length by 2.6% (from 194.5 ms to 199.6 ms) for the central cell, and 4.2% (from 127.5 ms to 132.8 ms) for the peripheral cell. As shown in Figure 5.4B, the simulation results for the change of the cycle length are comparable with experimental observations of HCN-knockout mice. Research by Fenske et al. (2013) has shown that the HCN1 deficiency prolonged the cycle length of isolated murine SA node cell by 13 ± 4% and Ludwig et al. (2003) indicated that the cycle length of HCN2-konclout mice was increased by 2%.

Apart from slowing the pacemaker activity, the HCN mutations also caused changes in other characteristics of AP morphology. As shown in Figure 5.4C, in both central and peripheral models, lacking \(I_f\) produced a more hyperpolarised MDP, with a greater effect being observed in the peripheral cells. In particular, compared with the control condition (-71.32 mV in peripheral cells), blocking \(I_f\) (\(I_{f,HCN1}, I_{f,HCN2}\) and \(I_{f,HCN4}\)) generated a MDP of -74.5 mV. As the \(I_f\) mainly activates during the diastolic depolarisation phase of the AP, lacking \(I_f\) decreased the diastolic depolarisation rate (DDR) (Figure 5.4D). This reduction was observed specifically when \(I_{f,HCN1}\) was
Figure 5.4 Functional roles of different HCN channels on central (left) and peripheral (right) murine SA node models. AP profiles (A) and main AP characteristics: CL (B), MDP (C), DDR (D) and dV/dt_{max} (E) under control (black), I_{f,HCN1} (red), I_{f,HCN2} (green), I_{f,HCN4} (blue) and native I_{f} (dark red) blocked conditions. Open circles: experimental data by Fenske et al. (2013), Ludwig et al. (2003) and Herrmann et al. (2007).
In addition, the HCN deficiency also affected the upstroke of the AP, and it was observed that the dV/dt\textsubscript{max} was increased when I_f was blocked (Figure 5.4E). This is because the more negative MDP, caused by HCN deficiency, increased the availability of activation of voltage-gated Na\textsuperscript{+} channels, thus increasing the upstroke velocity. A more detailed explanation is illustrated in Figure 5.5. It can be seen that compared with the control condition, I_{Na} increased when I_{f,HCN1} was blocked (the inactivation gating variable increased due to the more negative MDP), whereas another major underlying current, I_{CaL}, was not greatly affected.

Figure 5.5 Analysis of increased dV/dt\textsubscript{max} in a HCN1-deficiency model. (A) AP profiles. (B–D) Current density, activation and inactivation of I_{Na} (i) and I_{CaL} (ii) during AP. Black: control conditions; red: HCN1 deficiency (I_{f,HCN1} blocked).
The addition of ACh and ISO

In this chapter, the consequences of HCN1 deficiency on cardiac pacemaker activity were also investigated by incorporating the effects of ACh and ISO. Figure 5.6A and Figure 5.6B illustrate the simulated APs with different concentrations of ACh under both control and HCN1-deficiency condition in the murine central SA node cell model. The results suggested that the addition of ACh slowed the spontaneous pacemaker activity under both control and mutation conditions. Moreover, it can be seen that the dose dependency of the chronotropic effect of ACh differed for control and HCN1-deficiency mice.

Figure 5.6 Effect of ACh on control and HCN1-deficiency models. AP profiles under control (A) and $I_{f,HCN1}$ (B) blocked conditions with different concentrations of ACh. (C & D) Cycle length and change in pacing rate under control and $I_{f,HCN1}$ blocked conditions.
Under control conditions, the cycle length in the absence of ACh was approximately 194 ms, and ACh concentrations of 0.01 μM and 0.03 μM increased the cycle length to 235 ms and 389 ms, respectively. The latter corresponds to a decrease in heart rate of 50%, which is considered to be sinus bradycardia (Zhang et al., 2002). With the deficiency of \( HCN1 \), the cycle length in the absence of ACh was 218 ms. An ACh concentration of 0.01 μM prolonged the cycle length to 297 ms, whilst a concentration of 0.03 μM protracted the cycle length to 1.7 s, a decrease that most likely represents sinus pauses.

The simulated dose-dependent effects of ACh on central SA node cells under both control and \( HCN1 \)-deficiency conditions, together with the percentage slowing of the heart rate, were illustrated in Figure 5.6C and Figure 5.6D. Under control conditions, ACh concentrations greater than 0.03 μM cause a sharp increase in the cycle length. For \( HCN1 \) deficiency, the curve is not as sharp, although it can be seen that the steep part of the curve starts at a lower ACh concentration, which is approximately half that of control conditions. The dose-dependency graph displayed in Figure 5.6C shows that ACh has a more pronounced chronotropic effect when lacking \( I_{HCN1} \), since for a given concentration, the percentage slowing of the rate is higher. This lower intrinsic heart rate is indicative of sinus bradycardia (Fenske et al., 2013), suggesting a link between the \( HCN1 \) channel and SND.

Figure 5.7A and Figure 5.7B illustrate the simulated effects of varying concentrations of ISO on APs under both control and \( HCN1 \)-deficiency conditions in the murine central SA node cell model. It can be seen that ISO accelerated the pacemaking activity of both control and \( HCN1 \)-deficiency models. In control conditions, 0.001 μM and 1.0 μM of ISO increased the pacing rate of the model by 16% (from 308 to 357 bpm) and 27% (from 308 to 391 bpm), respectively. With the deficiency of \( HCN1 \), the pacing rate was accelerated by 8% (from 271 to 292 bpm) and 19% by 0.001 μM
and 1.0 μM of ISO, respectively. Figure 5.7C illustrates the effects of ISO on changes in pacing rates for both control and HCN1-deficiency conditions. The experimental observations from murine SA nodes are shown in Figure 5.7D (Fenske et al., 2013). The simulated results suggest that the effects of ISO were reduced in the HCN1-deficiency model.

Figure 5.7 Effects of ISO on control and HCN1-deficiency models. AP profiles under control (A) and \( I_{\text{f,HCN1}} \) (B) blocked conditions with 0.001 μM (upper panel) and 1.0 μM (lower panel) of ISO. (C) Simulated pacing rate with 0.001 and 1.0 M of ISO. (D) Experimental data from murine SA nodes by Fenske et al. (2013).
Figure 5.8 The time-of-day variation of heart rate under different conditions. (A & B) The circadian variation of ACh and ISO concentration. (C) Open circles: control condition, Filled circles: $I_{f,HCNI}$ blocked.

**Effects of HCNI deficiency on circadian rhythm of the heart rate**

The effects of HCNI deficiency on the circadian rhythm of the heart rate were also simulated by the model. The results of the time-of-day variation of the heart rate under control and HCNI-deficiency conditions are shown in Figure 5.8. It can be seen that both of models demonstrated a low pacing rate during the period from 1am to 6am and a relatively high pacing rate in the late morning (7am to 11am) and late evening (7pm to 9pm), which resulted from the balance between the sympathetic and
parasympathetic nervous systems. The low heart rate in the early morning (1am to 6am) was primarily attributed by the increase in the activity of parasympathetic nervous systems. Comparing with the control condition, the HCN1-deficiency model indicated a lower heart rate, especially during the early morning time. The lowest heart rate during that period was 175 bmp, which is only 0.6 of the average heart rate under control condition. Therefore, the circadian rhythm of autonomic nerves activity can be anticipated to be a good predictor of the bradycardic effects of the HCN1 deficiency.

**Effects of HCN1 deficiency on HRV**

In order to investigate the effects of HCN1 deficiency on HRV, a stochastically varying ACh and ISO concentration was implemented. The simulated results of HRV under control and HCN1-deficiency conditions are illustrated in Figure 5.9. It can be seen that the model generated a concentration of ACh and ISO that fluctuates about a central value and within a given range (Figure 5.9A). Figure 5.9B and Figure 5.9C show that the model generated APs corresponding to these fluctuating ACh and ISO concentrations over the time course of 10,000 ms. The amplitude and cycle length of these APs were no longer regular, unlike when the ACh and ISO concentrations were constant, and fewer APs were generated by the SA node cells lacking $I_{f,HCN1}$, which is consistent with the longer cycle lengths expected from Figure 5.9B.

The measured cycle lengths of the APs displayed in Figure 5.9B and Figure 5.9C were summarised in Figure 5.9D. It can clearly be seen that there is a much larger variation in the cycle lengths for APs generated under HCN1-deficiency conditions than under control conditions. This can be quantified by calculating the cycle length standard deviation. For the 10,000-ms period, the average cycle length of the APs under control conditions (Figure 5.9B) was 197.5 ms, whereas that of HCN1-deficiency conditions (Figure 5.9C) was 225.7 ms. Furthermore, the cycle
Figure 5.9 Simulated HRV for control and \textit{HCNI}-deficiency models. (A) The fluctuations of [ACh] (upper panel) and [ISO] (lower panel). (B & C) Simulated APs under control (B) and $I_{f,HCNI}$ blocked (C) conditions with stochastically varying concentrations of ACh and ISO. (D & E) The cycle length variability and SDNN under both control (black) and $I_{f,HCNI}$ blocked (red) conditions.
length standard deviations were computed for control (6.30 ms) and $HCNI$-deficiency conditions (16.37 ms), respectively, which are shown in Figure 5.9D. The dramatic fluctuations in the cycle length when $I_{f,HCNI}$ was blocked, as shown by the larger cycle length standard deviation value, are characteristic of sinus dysrhythmia. These simulated results are comparable with the experimental observations by Fenske et al. (2013).

5.3.3 The contribution of individual $HCN$ isoforms to cardiac pacemaker activity

As described above, blocking $I_{f,HCNI}$ caused a significant enhancement of the cycle length, even greater than the increase under the complete block of $I_{f,HCN4}$, which is considered the major isoform of the $I_f$. However, this result is not expected by the relative current densities. The simulated voltage clamp results for the central model, illustrated in Figure 5.10, have shown that $I_{f,HCNI}$ made up only 28% of the native $I_f$, whereas the value for $I_{f,HCN4}$ was close to 61%.

The mechanism contributing to this complex phenomenon can be explained by carrying out the simulated voltage clamp experiments at two different time intervals. Figure 5.10 illustrates the simulated current trace and I-V relationships of $HCN$ currents for a long period of 5 s (upper panel) and a short period of 300 ms (lower panel). In both conditions, the voltage was clamped from a holding potential of -40 mV to a testing potential varying from -130 to -50 mV with 10-mV increments. It can be seen that the maximum $I_f$ current density under the short time interval is smaller than that of the long time interval, which is mainly because the $I_f$ cannot fully activate during a short period and fails to reach the maximum level. As the $I_f$ regulates the pacemaker activity by primarily acting during the DDR, the detailed I-V relationship around the MDP are shown in the insets of Figure 5.10B(i) and Figure 5.10B(ii). It can be seen that $HCN4$ made the largest contribution to the whole $I_f$, whereas the
Chapter 5 Biophysical modelling of sinus node dysfunction due to \textit{HCN1} deficiency

Figure 5.10 Simulated patch clamp and I–V relationships under different conditions. Simulated current trace (A) and corresponding I–V curves for 5 s (i) and 300 ms (ii) of patch clamp time intervals. Insets in B(i) and B(ii) indicate the current densities of each \textit{HCN} channel around the potential of MDP.

Contributions of \textit{HCN1} and \textit{HCN2} were relatively small when the voltage clamp time was long. However, for a short clamping time, the current density of \textit{HCN1} is larger than that of the other two isoforms. This is due to the time kinetics of \textit{HCN1} being faster than other \textit{HCN} channels; for a short clamping time, \(I_{f,HCN1}\) can fully activate, whereas the others are unable to reach the maximum value. Therefore, during a relatively short period of spontaneous AP of murine SA node cells, \(I_{f,HCN1}\), with its fast time kinetics, is capable of fully activating and making a substantial contribution to rhythmic pacemaking. The dynamic variations of the three different isoforms of \(I_f\) during the spontaneous AP are shown in Figure 5.11. It can be seen that the current density of \(I_{f,HCN1}\) was larger than for the other two \textit{HCN} isoforms.
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Figure 5.11 Dynamic variations of different \textit{HCN} channels during APs. (A) Simulated APs. (B) Dynamic variation of $I_{\text{f,HCN1}}$ (red), $I_{\text{f,HCN2}}$ (green) and $I_{\text{f,HCN4}}$ (blue). Enlarged area marked by red box in (i) is shown in (ii).

5.3.4 Action potential propagation on the 2D SA node-atrium tissue

Action potential conduction through the reconstructed SA node-atrium tissue slice was simulated by using the finite difference method (discussed in Section 2.4.3) with a space step of 0.015 mm and a time step of 0.0025 ms. Figure 5.12 illustrates the simulated AP propagations under control conditions. It can be seen that the electrical signal was initiated from the centre of the SA node, then propagated via peripheral regions towards the atrium. The RA was activated in approximately 30 ms, with conduction velocities of 40 cm/s. This is comparable with experimental measurements from murine atria (Lei et al. 2005). Figure 5.13A shows the activation sequence of the tissue, which demonstrates the time at which each cell reached -20 mV. The activation sequence of the murine RA obtained experimentally by Lei et al. (2005) is shown in Figure 5.13B for reference.
Figure 5.12 AP propagation in 2D models of murine SA nodes and surrounding atrial tissue. (A) Distribution of cell types through the tissue. (SA node centre: blue; SA node periphery: red), Modified from Zhang (2012). (B–I) Simulated AP propagation through the SA node–atrium tissue. (CT: crista terminalis, IVC: inferior vena cava, RA: right atrium, SEP: interatrial septum, SVC: superior vena cava).

5.3.5 Effects of HCN1 deficiency on the tissue model

The 2D model was used for studying how HCN1 deficiency affects the action potential propagation through the RA. Figure 5.14 illustrates the action potential activation times for the propagation across the tissue (the red dashed line in Figure 5.14A) under control and HCN1-deficiency conditions. In control conditions, APs
originated from the central region of the SA node with a cycle length of 206 ms, whereas the cycle length in the \textit{HCNI}-deficiency model was 238.9 ms. It can be seen that the negative effects of \textit{HCNI} deficiency on the spontaneous pacemaking activity of the heart was enhanced in the tissue model; this is due to the electrotonic interactions between the SA node and its surrounding atrium. In addition, the 2D model also indicated a reduced conduction velocity in the SA node with \textit{HCNI} deficiency, as shown in Figure 5.14B; the conduction time in the \textit{HCNI}-deficiency model was slightly longer than that of the control model. This is consistent with experimental observations from \textit{HCNI}-knockout mice (Fenske et al., 2013). The reduction in conduction velocity was attributed to the more negative MDP and the reduction in DDR, as shown in Figure 5.14C and Figure 5.14D. With these two effects, a SA node cell required more time and current to charge the cell membrane of the neighbouring cell, therefore prolonging the time required in order to reach the threshold potential for the initiation of an AP, thereby slowing the AP propagation.

The effects of sympathetic and parasympathetic regulation on SND due to \textit{HCNI} deficiency were also studied using the 2D model. The simulated cycle lengths under different conditions are shown in Table 5.4. In normal tissue (without \textit{HCNI}
deficiency), the addition of 0.01 μM ACh increased the cycle length by 18.9%, whereas 0.01 μM ISO reduced the cycle length by 17.1%. With *HCNI* deficiency, the cycle length was increased to 310 ms by 0.01 μM ACh, while it was 212 ms with the presence of 0.01 μM ISO. The effects of ACh and ISO on AP propagation were also assessed. It can be seen in Figure 5.14C and Figure 5.14D that ACh slowed AP conduction, whereas ISO increased it.

<table>
<thead>
<tr>
<th>Table 5.4 Simulated cycle lengths under different conditions (ms).</th>
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<tr>
<td></td>
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<tr>
<td>Control</td>
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<tr>
<td><em>HCNI</em> deficiency</td>
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</table>
Figure 5.14 Effects of HCN1 deficiency, ACh and ISO on AP conduction. (A) SA node–atrium tissue. Simulated recordings were measured from the cell along the red dashed line. (B) Activation times of control and HCN1-deficiency models. (C) Activation times of the control model with ACh and ISO. (D) Activation times of the HCN1-deficiency model with ACh and ISO. (CT: crista terminalis, IVC: inferior vena cava, RA: right atrium, SAN: SA node, SEP: interatrial septum, SVC: superior vena cava.)
5.4 Discussion and conclusion

The $HCN$ gene, encoding for the funny current, has been confirmed as a major contributor to the spontaneous pacemaker activity and is associated with SND, which is usually described as “the parent in crime” (Monfredi and Boyett, 2015). A recent study by Fenske et al. (2013) has shown that mice lacking $HCN1$ displayed many abnormal cardiac activities characterised by the impaired initiation and conduction of cardiac electrical activity, resulting in bradycardia, sinus arrhythmia and recurrent sinus pause. However, due to the limited utility of the experimental approach, the mechanisms contributing to this complex phenomenon have not been fully clarified. In this Chapter, in order to address this issue, we take inspiration from a computational approach by developing a biophysically detailed mathematical model of action potentials for both central and peripheral murine SA node cells with isoform-specific formulations for the funny current, incorporating it into a 2D model of the murine SA node and its surrounding atrium.

Comparison with previous models

In the last decade, several numerical models of SA node cells have been generated for various species. However, the functional roles of isoform-specific ionic channels were not taken into account in most of these studies, as each channel was only represented by single-channel voltage clamp experimental data. Only Kharche et al.’s (2011) model considered two different isoforms for sodium channels and L-type calcium channels. At the tissue level, several studies (Butters et al., 2010; Unudurthi et al., 2014) have successfully simulated the cardiac electrical propagation within the SA node and surrounding atrium. However, these studies only focused on the behaviours of the cardiac electrical activity in a restricted portion of the whole atrium; the geometrical structure of the SA node and the atrium, the electrical heterogeneity and the anisotropy of the tissue were not considered.
Compared with the models discussed above, the major advantages of this model are: 1) the introduction of the isoform-specific \( HCN \) channels; and 2) the incorporation of an immunohistologically reconstructed geometry of the intact SA node-atrium tissue.

Another primary achievement of this model is that the morphology and conduction of the simulated APs are consistent with experimental observations on murine SA nodes and atria, with all of the main characteristic parameters, including CL, APD\(_{90}\), MDP, OS potential, dV/dt\(_{\text{max}}\) and the activation sequence, matching the experimental data.

**\( HCN1 \) contribution to the native \( I_f \) and murine pacemaker activity**

In this chapter, our simulation results suggest that \( HCN1 \) makes a substantial contribution to the native \( I_f \) in the murine SA node. The simulated voltage clamp results, shown in Figure 5.2, indicate that \( I_{f,HCN1} \) made up approximately 28% of the native \( I_f \), whereas \( I_{f,HCN2} \) and \( I_{f,HCN4} \) made up close to 11% and 61%, respectively, which are also consistent with the experimental results of Fenske et al. (2013). An expression study by Herrmann et al. (2012) supports these voltage clamp results as well, with similar expressions of \( HCN1 \) (34%) and \( HCN4 \) (59%) on an mRNA as well as a protein level in mouse SA nodes being reported.

In addition, current blocking simulation indicates that the functional role of \( HCN1 \) in the murine pacemaker activity is significant, being even greater than that of \( HCN4 \). As shown in Figure 5.4, complete blocking of \( I_{f,HCN1} \) increased the cycle length by 13.6% (from 194.5 ms to 221.2 ms) and 12.5% (from 127.5 ms to 143.5 ms) in central and peripheral murine SA node cells, respectively, while the increases in the cycle lengths under \( I_{f,HCN4} \) block conditions were only 2.6% and 4.2% in central and peripheral cells, respectively. This is mainly due to the fast time kinetics of \( HCN1 \). As shown in Figure 5.10, the simulated voltage clamp results illustrate that the distribution of \( HCN \) current densities was not the same under the different clamping periods. For a long clamping period, the current density of \( I_{f,HCN4} \) was largest, compared with the other
HCN isoforms, whereas $I_{f,HCN1}$ contributed most to the native $I_f$ when the clamping time interval was short. Therefore, during a short period of time, such as the cycle length of murine SA node APs, $I_{f,HCN4}$ does not have sufficient time to fully activate and modulate pacemaking, whereas $I_{f,HCN1}$, with its fast time kinetics, plays an important role in murine pacemaker activity. This mechanism was originally described by Fenske et al. (2013), but they did not provide a detailed explanation of it. This study provides mechanistic insights for understanding the time kinetic basis underlying the $HCN1$ contribution to the pacemaking action potentials of murine SA nodes.

**HCN1 deficiency and SND**

At a single-cell level, as described above, our study has indicated that $HCN1$ deficiency slowed down pacemaker activity in both central and peripheral SA node cells. The increase of cycle length on the peripheral cells was greater than that on the central SAN cells, which usually initiate and control the heart rate. In addition to the slowing APs, blocking $I_{f,HCN1}$ also produced a more hyperpolarised MDP and an increased $dV/dt_{\text{max}}$, which are comparable with experimental recordings from murine SA nodes by Fenske et al. (2013). Furthermore, the simulated results suggest that the $HCN1$-deficiency model demonstrated a larger cycle length standard deviation value, which is a characteristic of sinus dysrhythmia. This is consistent with experimental observations (Fenske et al., 2013) that $HCN1$-knockout mice had increased HRV.

At the intact SA node–atrium tissue level, the $HCN1$ deficiency slowed down the pacemaking rate, with the effect being much greater than in the isolated central and peripheral cells. Blocking $I_{f,HCN1}$ produced an increase in the cycle length of 12.5% and 13.6% for the central and the peripheral models, respectively, but increased the cycle length of the intact SA node–atrium tissue by approximately 16.1%. These great impacts of $HCN1$ deficiency can be attributed to the electrotonic interactions between
the SA node and the atrium. As the deficiency produced a more hyperpolarised MDP and a weaker pacemaking activity (low DDR; see Figure 5.4), the atrium acted as an electrical load (Boyett et al., 1995), which inhibited the pacemaking activity of the SA node. This inhibition not only affected the peripheral region of the SA node at the boundary between the SA node and the atrium, but also was able to be applied to the centre of the SA node via cell-to-cell electrical coupling. The inhibition in the central SA node cells was weaker than that in the periphery. Therefore, with the inhibition from the atrium, the effect of HCN1 deficiency on impairing pacemaking activity becomes greater as less $I_f$ is available to counterbalance the suppressive effect of adjacent atrial tissue (Boyett et al., 1995).

The effects of HCN1 deficiency were also studied by incorporating ACh and ISO in both single-cell and 2D tissue models. Simulated results suggested that ACh enhanced the effects of HCN1 deficiency on impairing pacemaking activity and AP conductions.

**Limitations of the Model**

In this study, the functional effect of $I_{f,HCN4}$ was relatively limited, compared to some experimental findings (Stieber et al., 2003; Baruscotti et al., 2011). This is mainly due to the equations for $I_f$ being based on available experimental data from different groups (Herrmann et al., 2007; Zong et al., 2012; Fenske et al., 2013; Khoury et al., 2013); some of the data were obtained from cultured cells (Herrmann et al., 2007; Zong et al., 2012). Another possible reason could be that the window current of $I_{f,HCN4}$ was small compared with other dominant isoforms. The electrophysiological properties of this current will be improved as experimental data become available. Another limitation of this study is that the histologically reconstructed geometry of intact SA node-atrium tissue used in the model was adapted and rescaled from experimental observation on rabbit hearts. In addition, a recent research by Bueno-Orovio et al. (2014) has shown
that structural heterogeneity in cardiac muscle may pose possible limitations on monodomain models as conclusive representations of cardiac tissue. Although it is worthwhile to elucidate the limitations of the study, these limitations do not revise our conclusions on the mechanisms underlying the sick sinus syndrome, caused by $HCNI$ deficiency.

**Conclusion**

In this study, we have developed mathematical models of the action potentials in the centre and periphery of murine SA node cells, with isoform-specific formulations for the funny current, and then incorporated them into an anatomically detailed 2D model of intact murine SA node–atrium tissue. The model successfully reproduced the initiation and propagation of rhythmic action potentials through the murine whole RA, consistent with experimental observation (Cho et al., 2003; Kharche et al., 2011; Lei et al., 2005; Mangoni et al., 2006). The consequences of the loss of function of $I_f$ caused by $HCNI$ deficiency are investigated by the model. Our simulation suggests that selective block of $HCNI$ could be a causative factor of SND, and demonstrates the mechanisms by which this deficiency weakens the pacemaking activity of the SA node.
The cardiac pacemaker, the sinoatrial node, plays an important role in controlling the heart rhythm. The unique feature of the SAN cells is their ability to produce spontaneous depolarisation leading to pacemaking action potentials. Though advances in experimental electrophysiology have gathered a large amount of experimental data in relation to the molecular, ionic and cellular bases of cardiac pacemaking activities, the exact ionic mechanisms underlying the genesis of spontaneous pacemaking action potentials at normal and abnormal physiological/pathological conditions remain incompletely understood. In this study, a family of biophysically detailed mathematical models of murine SA node at cellular and tissue levels have been developed based on extant experimental data. The developed models have been used to investigate the mechanisms underlying the cardiac pacemaking activities at variant normal and abnormal physiological/pathological conditions.

The first part of this thesis is focused on the investigation of ionic mechanisms underlying the automaticity of primary cardiac pacemaking cells in the murine heart. In this part of the study, an integrated model, incorporating the detailed cellular ion channel kinetics, multi-compartment intracellular Ca$^{2+}$ handling system and cell morphology, was developed for simulating the electrical action potentials of murine SA node cells. The model was able to reproduce the spontaneous pacemaking action potentials as well as the stochastic nature of local Ca$^{2+}$ dynamics in the SA node cells. The characteristics of the simulated action potentials, such as the pacemaking CL, maximal diastolic potential, upstroke velocity, action potential duration and amplitude of the action potentials all matched to experimental data, validating the model development. Using the computational model, the individual role of the ‘membrane
Chapter 6 Discussion and conclusions

clock’ (the cell membrane events) and ‘Ca\(^{2+}\) clock’ (intracellular Ca\(^{2+}\) activities) on generating the pacemaking action potentials were examined in a detailed way that would not be possible by using traditional experimental approaches.

Further, the model also considered the regulation of the autonomic nervous systems on cardiac pacemaking action potentials, which forms the second part of this thesis. In this part of the thesis, for the first time, detailed models of the sympathetic and parasympathetic regulation on the murine SA node cells were developed. Using the model, individual role of the neurotransmitters, ACh- and ISO-induced actions on variant ion channel and Ca\(^{2+}\) handling in regulating cardiac pacemaking action potentials were analysed.

In the last part of this thesis, an electrophysiologically and anatomically detailed mathematical model of the intact SA node and atrium was developed to investigate the ionic mechanisms underlying sinus node dysfunctions in variant genetic defect conditions. Effects of these genetic defects in impairing cardiac pacemaker ability in pacing and driving the sounding atrium as seen in the Sick Sinus Syndrome were investigated.

In the following sections, the main findings obtained in this thesis are summarised.

6.1 Dynamic interaction of membrane and Ca\(^{2+}\) clocks in cardiac pacemaking

In this study, the biophysically detailed model of murine SA node provides a powerful platform for studying the mechanisms underlying the automaticity of the sinoatrial node cells. The role of the M clock in the spontaneous pacemaking activity of SA node cells is studied by blocking relevant transmembrane ionic channels, including...
the $I_f$, $I_{CaT}$ and $I_{Cal}$. Simulation results suggest that blocking $I_f$, $I_{CaT}$ and $I_{Cal}$ significantly slows down the spontaneous activity of the SA node cell model, or even causes an arrest in auto-rhythmic action potentials. However, some of the ionic channels, such as $I_{CaT}$ and $I_{Cal}$, not only play important roles in rhythmic action potentials, but are also able to mediate the intracellular Ca$^{2+}$ dynamics. Collectively, our simulation data suggest that the effect of the C clock on cardiac pacemaking is important.

The role of the C clock in the automaticity of SA node cells is analysed in two sections that examine the role of SR Ca$^{2+}$ releases and the effect of Ca$^{2+}$ sparks during the diastolic depolarisation phase. The simulated results indicated that blocking RyR dramatically reduced intracellular Ca$^{2+}$ transient, but failed to largely increase the cycle length. In addition, the model suggests that the local Ca$^{2+}$ release, during the diastolic depolarisation phase, is primarily triggered by Ca$^{2+}$ influx, originating from T- and L-type Ca$^{2+}$ channels, especially from the stochastic behaviour of the L-type Ca$^{2+}$ channel. There is no obvious linear relationship between the frequency of local Ca$^{2+}$ release events and the pacing rate of rhythmic action potentials. All of these results suggest that there is a limited effect of local Ca$^{2+}$ release events and the C clock on the spontaneous pacemaking activity of SA node cells.

### 6.2 Analysis of the chronotropic effect of autonomic regulation

In this study, detailed electrophysiologically formulations for the sympathetic and parasympathetic regulation were constructed based on the experimental data from murine SA node cells and were implemented in the newly developed murine model. By using the model, the chronotropic effect of ACh and ISO, together with each of the affected ion channels to spontaneous pacemaking activity, were investigated.
Simulated results suggested 1) the activation of $I_{K, ACh}$ and the shift of $I_f$ activation curve, caused by ACh, were the major contributions to the effect of ACh in slowing down the pacing rate; 2) the increase of ionic current $I_{CaL}$, $I_{CaT}$ and $I_f$ associated with ISO was primarily responsible for ISO accelerating the pacing rate; 3) the combined effect of ISO and ACh on the pacing rates was dominated by ACh, when ACh was at a higher concentration; 4) both the effects of ACh and ISO showed regional differences, the central model having a higher sensitivity to both ACh and ISO than the peripheral model; 5) the autonomic nervous system’s regulation of cardiac pacemaking activity results from a balance between the sympathetic and parasympathetic regulatory pathways. The heart rate was high in the late morning and late evening, suggesting the sympathetic nervous system remained dominant, while the value was relatively low during sleeping time, meaning the parasympathetic nervous system played an important role.

### 6.3 Investigating the sinus node dysfunction

Our simulations suggest the functional importance of $HCN1$ for spontaneous cardiac pacemaking, as $HCN1$ deficiency can result in abnormalities in cardiac pacemaking and electrical conduction, including sinus bradycardia, sinus dysrhythmia (with the addition of ACh) or even sinus pause (at a high concentration of ACh). All of these behaviours are typical of SND (Butters et al., 2010; Monfredi and Boyett, 2015). In addition, mutations in other $HCN$ isoforms also indicate a causative link with SND; previous studies have shown that mice lacking $HCN4$ display sinus bradycardia (Baruscotti et al., 2011; Zhang, 2012), whereas $HCN2$-deficient mice exhibit cardiac dysrhythmia (Ludwig et al., 2003). Apart from $HCN$ mutations, other ionic channel defects, such as mutations in the $SCN5A$ gene encoding the pore-forming $\alpha$-subunit of the fast Na$^+$ channel $I_{Na}$, can lead to impairments in heart rate and action potential conduction as well (Butters et al., 2010). In this case, the mutation mainly weakens
the pacemaking activity of the peripheral SA node, while the defective $I_{Na}$ reduces the excitability of the atrial cell, which increases the electrical load from the atrium to the SA node, thus impairing action potential propagation (Butters et al., 2010). This is different to $HCN1$ deficiency, in which both the centre and periphery of the SA node were impaired, and the prolonged conduction is because of the weaker SA node with a reduced $I_f$. Although some studies have reported the importance of the normal ageing process (such as degenerative fibrosis) for SND, it is clear that genetic defects in ion channels underlie this familial disease.

### 6.4 Future work

Although the presented platform provides a useful tool for the study of cardiac primary pacemaker and its dysfunction, it could be improved by addressing some of the limitations described above. The primary focus of this thesis is in modelling the electrophysiological properties of the SA node cell. Previous studies have shown that several signalling pathways play an important role in intracellular Ca$^{2+}$ handling, by affecting the phosphorylation of some key messenger proteins in Ca$^{2+}$ cycling in SA node cell, such as CaM and CaMKII (Bers, 2002; 2008). For the sake of studying the complete patterns of the Ca$^{2+}$ clock, an extension to the present model would involve incorporating the details of the signalling pathways. In addition, the 2D anatomical tissue model used in this study only represents a portion of the murine atria. In order to explore the effect of sinus node dysfunction on the whole organ level, a 3D model of the SAN-atrium is required. This work in future is to be based on the 3D anatomically detailed model and to extend the 2D model to three spatial dimensions.
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a mouse model of catecholaminergic polymorphic ventricular tachycardia. Circulation 126, 392–401. doi:10.1161/CIRCULATIONAHA.111.075382


Appendix

Glossary

4-AP 4-Aminopyridine
APD Action potential duration
APD50 APD at 50% repolarization
APD90 APD at 90% repolarization
[Ca$^{2+}$]$_i$ Intracellular Ca$^{2+}$ concentration
[Ca$^{2+}$]$_{sub}$ Ca$^{2+}$ concentration in subspace compartments
[Ca$^{2+}$]$_{jsr}$ Ca$^{2+}$ concentration in junctional SR
[Ca$^{2+}$]$_{nsr}$ Ca$^{2+}$ concentration in network SR
[Ca$^{2+}$]$_o$ Extracellular Ca$^{2+}$ concentrations
CL Cycle length
Cm Cell capacitance
[CM]$_{tot}$ Total calmodulin concentration
[CQ]$_{tot}$ Total calsequestrin concentration
$d_T$ Activation variable for $I_{CaT}$
$d_{NaCa}$ Denominator constant for $I_{NaCa}$
$E_{Ks}$ Reversal potential for $I_{Ks}$
$E_{Ca}$ Equilibrium potential for Ca$^{2+}$
$E_K$ Equilibrium potential for K$^+$
$E_{Na}$ Equilibrium potential for Na$^+$
$E_{CaL}$ Reversal potential for $I_{CaL}$
$E_{CaT}$ Reversal potential for $I_{CaT}$
$F$ Faraday’s constant
$F_{Na}$ Fraction of inactivation of $I_{Na}$ that occurs slowly
$f_{CMI}$ Fractional occupancy of calmodulin by [Ca$^{2+}$]$_i$
$f_{CMS}$ Fractional occupancy of calmodulin by [Ca$^{2+}$]$_{sub}$
Appendix

\( f_{CQ} \) Fractional occupancy of calsequestrin by \([Ca^{2+}]_{sr}\)

\( f_T \) Inactivation variable for \(I_{CAT}\)

\( f_{TC} \) Fractional occupancy of the troponin \(Ca^{2+}\) site by \([Ca^{2+}]_i\)

\( f_{TMC} \) Fractional occupancy of the troponin \(Mg^{2+}\) site by \([Ca^{2+}]_i\)

\( f_{TMM} \) Fractional occupancy of the troponin \(Mg^{2+}\) site by \(Mg^{2+}\)

\( g_{b,Ca} \) Conductance of \(I_{b,Ca}\)

\( g_{b,K} \) Conductance of \(I_{b,K}\)

\( g_{b,Na} \) Conductance of \(I_{b,Na}\)

\( g_{Ca,T} \) Conductance of \(I_{Ca,T}\)

\( g_{Kr} \) Conductance of \(I_{Kr}\)

\( g_{Ks} \) Conductance of \(I_{Ks}\)

\( g_{KI} \) Conductance of \(I_{KI}\)

\( g_{Na1.1} \) Conductance of \(I_{Na1.1}\)

\( g_{Na1.5} \) Conductance of \(I_{Na1.5}\)

\( g_{st} \) Conductance of \(I_{st}\)

\( g_{sus} \) Conductance of \(I_{sus}\)

\( g_{to} \) Conductance of \(I_{to}\)

\( h_{1.1} \) Fast inactivation variable for \(I_{Na1.1}\)

\( h_{1.5} \) Fast inactivation variable for \(I_{Na1.5}\)

\( h_{s1.1} \) Slow inactivation variable for \(I_{Na1.1}\)

\( h_{s1.5} \) Slow inactivation variable for \(I_{Na1.5}\)

\( I_{b,Ca} \) Background \(Ca^{2+}\) current

\( I_{b,K} \) Background \(K^+\) current

\( I_{b,Na} \) Background \(Na^+\) current

\( I_{Ca,T} \) \(T\)-type \(Ca^{2+}\) current

\( I_{Kr} \) Rapid delayed rectifying \(K^+\) current

\( I_{Ks} \) Slow delayed rectifying \(K^+\) current

\( I_{KI} \) Inward rectifier \(K^+\) current
Appendix

\( I_{Na1.1} \) Na⁺ channel isoform \( Na_v1.1 \) current
\( I_{Na1.5} \) Na⁺ channel isoform \( Na_v1.5 \) current
\( I_{NaCa} \) Na⁺-Ca\(^{2+}\) exchanger current
\( I_{NaK} \) Na⁺-K⁺ pump current
\( I_s \) Sustained inward Na⁺ current
\( I_{sus} \) Sustained component of 4-AP-sensitive current
\( I_{to} \) Transient component of 4-AP-sensitive current
\( j_{1.1} \) Slow inactivation gating variable of \( I_{Na1.1} \)
\( j_{1.5} \) Slow inactivation gating variable of \( I_{Na1.5} \)
\( k_bCM \) Ca\(^{2+}\) dissociation constant for calmodulin
\( k_bCQ \) Ca\(^{2+}\) dissociation constant for calsequestrin
\( k_bTC \) Ca\(^{2+}\) dissociation constant for troponin-Ca complex
\( k_bTMC \) Ca\(^{2+}\) dissociation constant for troponin-Mg complex
\( k_bTMM \) Mg\(^{2+}\) dissociation constant for troponin-Mg complex
\( k_fCM \) Ca\(^{2+}\) association constant for calmodulin
\( k_fCQ \) Ca\(^{2+}\) association constant for calsequestrin
\( k_fTC \) Ca\(^{2+}\) association constant for troponin
\( k_fTMC \) Ca\(^{2+}\) association constant for troponin-Mg complex
\( k_fTMM \) Mg\(^{2+}\) association constant for troponin-Mg complex
\( k_{NaCa} \) Scaling factor for \( I_{NaCa} \)
\( K_{mf} \) Forward-mode Ca\(^{2+}\) affinity of the SERCA pump
\( K_{m,K} \) Dissociation constant of K⁺ activation of \( I_{NaK} \)
\( K_{m,Kp} \) Half-maximal [K⁺]\(_i\) for \( I_{NaK} \)
\( K_{m,Na} \) Dissociation constant of Na⁺ activation of \( I_{NaK} \)
\( K_{mNaP} \) Half-maximal [Na⁺]\(_i\) for \( I_{NaK} \)
\( K_{mr} \) Reverse-mode Ca\(^{2+}\) affinity of the SERCA pump
\( K_s \) Ca\(^{2+}\) release constant
\( K_{up} \) Half-maximal [Ca\(^{2+}\)]\(_i\) of Ca\(^{2+}\) uptake by \( j_{up} \) in the NSR
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[K^+]_i$</td>
<td>Intracellular $K^+$ concentration</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>Extracellular $K^+$ concentration</td>
</tr>
<tr>
<td>$m_{1.1}$</td>
<td>Activation gating variable for $I_{Na1.1}$</td>
</tr>
<tr>
<td>$m_{1.5}$</td>
<td>Activation gating variable for $I_{Na1.5}$</td>
</tr>
<tr>
<td>$MDP$</td>
<td>Maximum diastolic potential</td>
</tr>
<tr>
<td>$[Mg^{2+}]_i$</td>
<td>Intracellular $Mg^{2+}$ concentration</td>
</tr>
<tr>
<td>$n_{up}$</td>
<td>SR uptake and hill coefficient</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>Intracellular $Na^+$ concentrations</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>Extracellular $Na^+$ concentrations</td>
</tr>
<tr>
<td>$NSR$</td>
<td>Network SR</td>
</tr>
<tr>
<td>$OS$</td>
<td>Overshoot of the AP</td>
</tr>
<tr>
<td>$p_a$</td>
<td>General activation variable for $I_{Kr}$</td>
</tr>
<tr>
<td>$p_i$</td>
<td>Inactivation variable for $I_{Kr}$</td>
</tr>
<tr>
<td>$P_{up}$</td>
<td>Rate constant for $Ca^{2+}$ uptake by $j_{up}$ of the NSR</td>
</tr>
<tr>
<td>$q$</td>
<td>Inactivation variable for $I_{to}$</td>
</tr>
<tr>
<td>$q_a$</td>
<td>Activation gating variable for $I_{st}$</td>
</tr>
<tr>
<td>$q_i$</td>
<td>Inactivation gating variable for $I_{st}$</td>
</tr>
<tr>
<td>$r$</td>
<td>Activation gating variable for $I_{to}$ and $I_{sus}$</td>
</tr>
<tr>
<td>$R$</td>
<td>Universal gas constant</td>
</tr>
<tr>
<td>$RyR$</td>
<td>Ryanodine receptor</td>
</tr>
<tr>
<td>$SERCA$</td>
<td>Sarcoplasmic reticulum $Ca^{2+}$-ATPase</td>
</tr>
<tr>
<td>$SR$</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>$T$</td>
<td>Absolute temperature (in K)</td>
</tr>
<tr>
<td>$[TC]_{tot}$</td>
<td>Total concentration of the troponin $Ca^{2+}$ site</td>
</tr>
<tr>
<td>$[TMC]_{tot}$</td>
<td>Total concentration of the troponin $Mg^{2+}$ site</td>
</tr>
<tr>
<td>$TOP$</td>
<td>Take off point</td>
</tr>
<tr>
<td>$V$</td>
<td>Voltage</td>
</tr>
<tr>
<td>$V_{cell}$</td>
<td>Cell volume</td>
</tr>
</tbody>
</table>
### Appendix

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_l$</td>
<td>Myoplasmic volume available for Ca$^{2+}$ diffusion</td>
</tr>
<tr>
<td>$V_{JSR}$</td>
<td>Volume of the JSR</td>
</tr>
<tr>
<td>$V_{sub}$</td>
<td>Subspace volume</td>
</tr>
<tr>
<td>$V_{NSR}$</td>
<td>Volume of NSR</td>
</tr>
<tr>
<td>$x_s$</td>
<td>Activation gating variable of $I_{Ks}$</td>
</tr>
</tbody>
</table>
Appendix

Cell Model Equations

Ion Concentrations

\[
\frac{d_{\text{Na}^+}}{dt} = - \frac{I_{\text{b,Na}} + I_{\text{Na1.1}} + I_{\text{Na1.5}} + 3I_{\text{NaK}} + 3I_{\text{NaCa}} + I_{\text{st}}}{FV_i}
\]

\[
\frac{d_{K^+}}{dt} = - \frac{I_{b,K} + I_{K_1} + I_{K_2} + I_{K_3} - 2I_{\text{NaK}} + I_{\text{nsr}} + I_{\text{st}}}{FV_i}
\]

\[
\frac{d_{\text{Ca}^{2+}}}{dt} = \frac{j_{\text{Ca,df}} V_{\text{sub}} - j_{\text{up}} V_{\text{nsr}} - (I_{\text{Ca12}} + I_{b,\text{Ca}} - 2I_{\text{NaCa}}) / (2F)}{V_i}
\]

\[
-((C)M\text{ot})_{\text{tot}} \frac{d_{\text{f,cma}}}{d_i} + (T)C\text{tot} \frac{d_{\text{f,mc}}}{d_i} + (T)M\text{Ctot} \frac{d_{\text{f,mac}}}{d_i}
\]

\[
\frac{d_{\text{Ca}^{2+}}}{dt}_{\text{sub}} = -\frac{(I_{\text{Ca1r}} + I_{\text{Ca13}} - 2I_{\text{NaCa}}) / (2F) + j_{\text{rel}} V_{\text{jsr}} - j_{\text{Ca,df}} - (C)M\text{ot}}{V_{\text{sub}}}_{\text{tot}} \frac{d_{\text{f,ca}}}{d_i}
\]

\[
\frac{d_{\text{Ca}^{2+}}}{dt}_{\text{jsr}} = -j_{\text{r}} - j_{\text{rel}} (C)Q \frac{d_{\text{f,ca}}}{d_i}
\]

\[
\frac{d_{\text{Ca}^{2+}}}{dt}_{\text{nsr}} = j_{\text{up}} - j_{\text{rel}} \frac{V_{\text{jsr}}}{V_{\text{nsr}}}
\]

Equilibrium Potentials

\[
E_K = \frac{RT}{F} \ln\left(\frac{[K^+]_{\text{o}}}{[K^+]_i}\right)
\]

\[
E_{Na} = \frac{RT}{F} \ln\left(\frac{[Na^+]_{\text{o}}}{[Na^+]_i}\right)
\]

\[
E_{Ca} = \frac{RT}{2F} \ln\left(\frac{[Ca^{2+}]_{\text{o}}}{[Ca^{2+}]_i}\right)
\]

\[
E_{Ks} = \frac{RT}{F} \ln\left(\frac{[K^+]_{\text{o}} + 0.12[Na^+]_{\text{o}}}{[K^+]_i + 0.12[Na^+]_i}\right)
\]
Appendix

Total Ionic Membrane Current

\[
\frac{dV}{dt} = -\frac{1}{C_m}(I_{Na,1.1} + I_{Na,1.5} + I_{Ca,L,1.2} + I_{Ca,L,1.3} + I_{Ca,T} + I_{Kr}) + I_{Ks} + I_{K1} + I_{f} + I_{surr} + I_{c} + I_{NaK} + I_{Na,Ca} + I_{p})
\]

Na\(^+\) current (\(I_{Na,1.1}\) and \(I_{Na,1.5}\))

\[
I_{Na,1.1} = g_{Na,1.1} m_{1.1}^3 h_{1.1} [Na^+] V e^{(V-E_{Na})F/RT} - 1 \frac{F^2}{e^{F/RT} - 1} \frac{RT}{F^2}
\]

\[
I_{Na,1.5} = g_{Na,1.5} m_{1.5}^3 h_{1.5} [Na^+] V e^{(V-E_{Na})F/RT} - 1 \frac{F^2}{e^{F/RT} - 1} \frac{RT}{F^2}
\]

\[
m_{1.1c} = \frac{1.0}{[1.0 + e^{-(V+31.09)/5.0}]^{1/3}}
\]

\[
h_{1.1c} = \frac{1.0}{1.0 + e^{-(V+56.0)/3.0}}
\]

\[
j_{1.1c} = h_{1.1c}
\]

\[
m_{1.5c} = \frac{1.0}{[1.0 + e^{-(V+45.21)/7.22}]^{1/3}}
\]

\[
h_{1.5c} = \frac{1.0}{1.0 + e^{-(V+62.58)/6.01}}
\]

\[
j_{1.5c} = h_{1.5c}
\]

\[
F_{Na} = 0.0952 e^{0.003(V+34.4)} [1.0+1.66 e^{-0.225(V+63.7)}]^{-1} + 0.0869
\]

\[
h_{s1.1} = (1 - F_{Na}) h_{1.1} + F_{Na} j_{1.1}
\]

\[
h_{s1.5} = (1 - F_{Na}) h_{1.5} + F_{Na} j_{1.5}
\]

\[
\tau_m = \frac{0.63 e^{-0.34(V+56.7)}}{0.83 e^{0.34(V+56.7)} + 0.63 e^{0.08(V+65.03)}} + 0.0492
\]

\[
\tau_h = \frac{0.113}{[13475.07 e^{(V-59.40)/15.65}]^{-1} + [1.11 + 0.044 e^{-(V-68.93)/18.24}]^{-1}}
\]

\[
\tau_j = \frac{0.125}{[140557.23 e^{(V-59.46)/17.38}]^{-1} + [2.47 + 0.77 e^{-(V-68.93)/18.24}]^{-1}}
\]
T-type Ca$^{2+}$ current ($I_{CaT}$)

\[ I_{CaT} = g_{CaT} d_{T} f_{T} (V - E_{CaT}) \]

\[ d_{T} = \frac{1.0}{1.0 + e^{-(V + 26.0)/6.0}} \]

\[ \tau_{dT} = \frac{1.0}{1.068 e^{(V + 26.3)/30.0} + 1.068 e^{-(V + 26.3)/30.0}} \]

\[ f_{T} = \frac{1.0}{1.0 + e^{(V + 61.7)/5.6}} \]

\[ \tau_{fT} = \frac{1.0}{0.0153 e^{(V + 61.7)/83.3} + 0.015 e^{(V + 61.7)/15.38}} \]

Rapid delayed rectifying K$^{+}$ current ($I_{Kr}$)

\[ I_{Kr} = g_{Kr} P_{a} P_{i} (V - E_{K}) \]

\[ P_{a} = \frac{1.0}{1.0 + e^{-(V + 21.17)/9.76}} \]

\[ \tau_{Pa} = \frac{0.7}{0.0036 e^{V/15.34} + 0.0018 e^{-V/25.87}} \]

\[ P_{i} = \frac{1.0}{1.0 + e^{(V + 16.76)/19.0}} \]

\[ \tau_{Pi} = 0.2 + \frac{0.9}{0.1 e^{V/54.65} + 0.67 e^{V/106.16}} \]

Slow delayed rectifying K$^{+}$ current ($I_{Ks}$)

\[ I_{Ks} = g_{Ks} x_{s}^{2} (V - E_{Ks}) \]

\[ x_{s} = \frac{1.0}{1.0 + e^{-(V - 20.88)/11.85}} \]

\[ \tau_{xs} = 1000.0 \frac{1.0 + e^{-(V - 48.91)/10.63}}{13.1} + e^{-V/35.32} \]
Inward rectifier $K^{+}$ current ($I_{Kt}$)

$$I_{Kt} = g_{Kt}[K^{+}]rac{(V - E_K)}{[1.0 + e^{0.071(V - E_K)}](K^{+})_{o} + 0.229]}$$

Sustained inward $Na^{+}$ current ($I_{st}$)

$$I_{st} = g_{st}q_{st}(V - E_{st})$$

$$q_{st} = \frac{1.0}{1.0 + e^{-(V+67.0)/5.0}}$$

$$\alpha_{qs} = \frac{1.0}{0.15e^{-V/11.0} + 0.2e^{-V/700.0}}$$

$$\beta_{qs} = \frac{1.0}{16.0e^{V/8.0} + 15.0e^{V/50.0}}$$

$$\tau_{qs} = \frac{1.0}{\alpha_{qs} + \beta_{qs}}$$

$$\alpha_{qs} = \frac{0.15}{3100.0e^{(V+10.0)/13.0} + 700.3e^{(V+10.0)/70.0}}$$

$$q_{qs} = \frac{\alpha_{qs}}{\alpha_{qs} + \beta_{qs}}$$

$$\tau_{qs} = \frac{1.0}{\alpha_{qs} + \beta_{qs}}$$

4-AP-sensitive currents ($I_{to}$ and $I_{sus}$)

$$I_{to} = g_{to}r_{to}(V - E_{K})$$

$$I_{sus} = g_{sus}r_{sus}(V - E_{K})$$

$$q_{ss} = \frac{1.0}{1.0 + e^{(V+49.0)/13.0}}$$

$$\tau_{q} = 1.5\times[6.06 + \frac{39.1}{0.57\times e^{-0.08(V+44.0)} + 0.07\times e^{0.1(V+45.95)}]}$$

$$r_{ss} = \frac{1.0}{1.0 + e^{-(V-19.3)/15.0}}$$

$$\tau_{r} = \{2.75 + 14.41/[1.04e^{0.09(V+30.61)} + 0.37e^{-0.12(V+23.84)}]\}/0.303$$
**Na⁺-K⁺ pump current (I\textsubscript{NaK})**

\[ I_{NaK} = I_{NaK} \left( 1.0 + \left( \frac{K_{mKp}}{[K^+]_o} \right)^{1.2} \right)^{-1} \left( 1.0 + \left( \frac{K_{mNap}}{[Na^+]_o} \right)^{1.3} \right)^{-1} \left( 1.0 + e^{-(V-E_{Na}+120)/30} \right)^{-1} \]

**Na⁺-Ca²⁺ exchanger current (I\textsubscript{NaCa})**

\[ I_{NaCa} = k_{NaCa} \frac{[Na^+]_o^3 [Ca^{2+}]_o e^{0.0374(V-E_{Na})} - [Na^+]_o^3 [Ca^{2+}]_o e^{0.0374(V-E_{NaCa})-1}}{1 + d_{NaCa} ([Ca^{2+}]_o [Na^+]_o^3 + [Ca^{2+}]_o [Na^+]_o^3)} \]

**Background currents (I\textsubscript{b})**

\[ I_{bNa} = g_{bNa} (V - E_{Na}) \]

\[ I_{bK} = g_{bK} (V - E_{K}) \]

\[ I_{bCa} = g_{bCa} (V - E_{Ca}) \]

**Intracellular Ca²⁺ dynamics**

\[ j_{up} = P_{sp} \frac{[Ca^{2+}]_o^{n_p} - [Ca^{2+}]_{arr}^{n_p}}{1 + \frac{[Ca^{2+}]_o^{n_p} - [Ca^{2+}]_{arr}^{n_p}}{K_{mf}}} \]

\[ j_{ir} = \frac{[Ca^{2+}]_{arr} - [Ca^{2+}]_{jsr}}{\tau_{ir}} \]
Ca\textsuperscript{2+} buffering

\[
\frac{d_{j_{TC}}}{d_t} = k_{j_{TC}} [Ca^{2+}](1.0 - f_{TC}) - k_{b_{TC}} f_{TC}
\]

\[
\frac{d_{j_{TMC}}}{d_t} = k_{j_{TMC}} [Ca^{2+}](1.0 - f_{TMC} - f_{TMM}) - k_{b_{TMC}} f_{TMC}
\]

\[
\frac{d_{j_{TMM}}}{d_t} = k_{j_{TMM}} [Mg^{2+}](1.0 - f_{TMC} - f_{TMM}) - k_{b_{TMM}} f_{TMM}
\]

\[
\frac{d_{j_{CMi}}}{d_t} = k_{j_{CMi}} [Ca^{2+}](1.0 - f_{CMi}) - k_{b_{CMi}} f_{CMi}
\]

\[
\frac{d_{j_{CMs}}}{d_t} = k_{j_{CMs}} [Ca^{2+}]_{sub} (1.0 - f_{CMs}) - k_{b_{CMs}} f_{CMs}
\]

\[
\frac{d_{j_{CQ}}}{d_t} = k_{j_{CQ}} [Ca^{2+}]_{j_{CQ}} (1.0 - f_{CQ}) - k_{b_{CQ}} f_{CQ}
\]