Simulation of Cardiac Pacemaker Dysfunction Arising from Genetic Mutations

A thesis submitted to the University of Manchester for the degree of Doctor in Philosophy in the Faculty of Engineering and Physical Sciences

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Abstract

University of Manchester
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Degree Program: Doctor of Philosophy
Thesis Title: Simulation of Cardiac Pacemaker Dysfunction Arising from Genetic Mutations

The sinoatrial node (SAN) is the primary pacemaker in mammalian hearts and is vital to cardiac function. Genetic mutations in SAN can result in lose-of-function of ion channels, consequently arouse sinus node dysfunction (SND), Brugada syndrome (BrS) and progressive cardiac conduction disease (PCCD). The mechanisms underlying the he pathogenesis for cardiac pacemaker dysfunctions associated with genetic mutations has not been defined. In this project, by using computer modeling, mechanisms by which the HCN4 mutations impair cardiac pacemaking and possible pro-arrhythmic effects of ivabradine were investigated. Action potential (AP) models for rabbit sinoatrial node cells were modified to incorporate experimentally reported $I_f$ changes induced by HCN4 gene mutations. At both the cellular and intact SAN-atrium tissue level, $I_f$ reduction due to the HCN4 mutations slowed down pacemaking. At the tissue level, these mutations compromised the AP conduction across the SAN-atrium, leading to a possible sinus arrest or SAN exit block. Moreover, vagal nerve activity could amplify the bradycardiac effects of the HCN4 gene mutations, leading to sinus arrest and SAN exit block that was not observed with the mutations or ACh alone. Similarly, SND associated with SCN5A mutations and acquired cardiac conditions were studied. 1) Mathematical models of rabbit SAN cells and 2D tissue models were modified to investigate SAN function and intracardiac conduction in a murine model of long QT syndrome type 3. A prolonged tail current $I_{Na,l}$ was introduced and incorporated with a normal $I_{Na,T}$ to test the SAN pacemaker function and AP conduction from the SAN to atrial septum. Simulation results showed that a combined reduction in $I_{Na,T}$ and introduction of $I_{Na,l}$ achieved alterations in both pacemaking rate and conduction 2) Mathematical models of mouse SAN cells were modified to investigate the mechanisms underlies the SAN associated with SCN5A deficiency and aging. A coupled SAN-atrium cell model was developed to replicate the experimentally observed slowing of SAN conduction with aging and SCN5A-disruption The modelling studies reconstructed the physiological mechanisms by which both aging and SCN5A-disruption lead to SND, thereby drawing parallels between these and similar conduction changes in the ventricle that occur in the possibly related condition of PCCD. At last, a 2D anatomically based model of the SAN-atrium was constructed. This model successfully reproduced the effects of vagal nerve stimulation and SCN5A-E161K gene mutation on spontaneous activity of the SAN and AP conduction across the SAN-atrium.
The Author

Xinzhao Zhang completed a Bachelor of Physics (Bsc) degree in Astrophysics at the University of Manchester, School of Physics and Astronomy in 2007. In that year, he enrolled to do a Master of Physics (MPhys) leading to Doctor of Philosophy degree with the Biological Physics Group at the same institution.
Declaration

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Chapter 1

Introduction

Heart disease has become the biggest killer in the UK, causing over one third of all deaths [1]. In addition to the deaths, many more people are suffering from different kinds of heart diseases which include cardiac arrhythmia, ischaemia, angina, heart attack, congenital heart disease, heart failure, cardiomyopathy and so on. The treatments of heart disease are mainly restricted to drugs, artificial pacemaker, heart transplantation, coronary angioplasty and coronary bypass surgery. To some extent, these treatments have reduced risk of deaths. However, heart diseases can’t be cured fundamentally by these treatments. In order to cure heart disease, mechanisms underlying the pathogenesis for the heart disease must be fully understood. Genes that hold the information of cells and pass genetic traits to offspring show the most fundamental differences between healthy and diseased heart. Therefore, study of heart disease associated with gene mutations will be extremely helpful for the heart disease research.

1.1 The heart

1.1.1 Function of the heart

The heart is responsible for pumping blood to provide the oxygen and nutrients for human’s activities and remove the waste products that we do not need. The human heart is located in the left centre of the chest and consists of four chambers, the left and right atria and ventricles (Figure 1.1) [2]. The right and left atria are smaller than their respective ventricles and have much thinner walls. This is due to less pressure the atria have to work against. The right atrium and ventricle are separated from the
left atrium and ventricle by a muscular wall called septum which can prevent the blood mixing together from two sides. The upper two atria are also separated from the lower two ventricles by a layer of dense connective tissue called the fibrous skeleton [3]. This fibrous tissue can structurally and electrically isolate the atria from the ventricles. Two atroventricular valves, tricuspid valve (lies between the right atrium and ventricle) and mitral valve (lies between the left atrium and ventricle) on the fibrous skeleton can control the blood to flow from atria into ventricles and prevent any backflow. Besides, two semilunar valves, the aortic valve which is located between the left ventricle and aorta and the pulmonary valve which is located between the right ventricle and pulmonary artery have the same function as atroventricular valves [3].

Figure 1.1: Structure diagram of the heart. Taken from [4].
The blood flows around our body which is called circulation. It can be divided into two parts: systemic circulation and pulmonary circulation. For the systemic circulation, oxygen-poor blood from the body fills the right atrium via the vena cavae. The right atrium contracts and blood enters into the right ventricle through the tricuspid valve. The right ventricle contracts and pumps the blood into lungs via the pulmonary artery for oxygenation. For the pulmonary circulation, oxygenated blood returns from the lungs to the left atrium via the pulmonary veins. The left atrium contracts and the mitral valve opens up to allow blood to enter into the left ventricle. The left ventricle contracts to pump oxygenated blood through the aorta to all regions of the body.

1.1.2 Electrical activity of the heart

Heart relaxation and contraction result from cooperation between the electrical activities of the conduction system, which is composed of nodal tissue and communication between cells via gap junctions.

The electrical activity initiates from the pacemaker, sinoatrial node (SAN) and then spreads over left and right atrium via junctional electrical coupling between cells. Once the electrical pulse passes down the atrial septum with a speed of 0.8 to 1.0 m/s (meter per second), it is conducted to the atrioventricular node (AVN) which is situated in the inferior portion of the interatrial septum. The AVN slows down the electrical signal before it continues through the bundle of His (0.03 to 0.05 m/s). The bundle of His begins at the top of the interventricular septum, goes through the fibrous skeleton of the heart and goes down along the interventricular septum. It is divided into right and left bundle branches and terminates in an extensive network which is known as Purkinje fibers. Purkinje fibers distribute all over the ventricular walls and have the highest conduction velocity (5.0 m/s) so that the electrical impulse can conduct through the whole ventricles rapidly [5].
The propagation of electrical activity consists of 3 phases: Firstly, the SAN depolarizes. Via the conduction pathway, depolarization propagates to the atria leading to contraction of the atria. And then, depolarization arrives at the AVN. Secondly, the SAN completes repolarization. When the depolarization arrives at the AVN, it will stop for a moment. Then, His bundle, the bundle branches and Purkinje fibers depolarize in sequence. In this phase, the atria relax and ventricles contract. Thirdly, ventricles relax because of repolarization of the AVN, His bundle, the bundle branch and the Purkinje fibers [5].

1.2 The sinoatrial node (SAN)

1.2.1 The anatomy of the SAN

The SAN was firstly discovered in a mole’s heart by Martin Flack in the late summer of 1906 during his stay in the vacation farmhouse of Professor Arthur Keith near Bredgar [6]. It is generally located in the right atrium at the junction of the crista terminalis (CT), near the opening of superior vena cave (SVC) [7, 8] (Figure 1.2 & Figure 1.3).

![Figure 1.2: Topographical organization of the rabbit SAN. Adapted from [195].](image-url)
Figure 1.3 Histology of the rabbit SAN. A: Endocardial view of a SAN-atrial muscle preparation. B: Schematic diagram of a cross section through the crista terminalis (CT) and intercaval region. Dashed yellow line is the extent of SAN tissue overlying the atrial muscle of the CT. Red area stands for atrial cells. Pink part stands for no myocardial cells, only connective tissue. Stippled area stands for interweaving cells. Gray area stands for block zone. SVC, superior vena cava. SEP, interatrial septum. IVC, inferior vena cava. CT, crista terminalis. RA, right atrial appendage. Endo, endocardium. Epi, epicardium. Figure from [196].
1.2.2 Function of SAN

Under normal circumstances, the SAN automaticity is responsible for initiating the heart rhythm, so called the primary pacemaker. If the SAN does not function, the AVN will take over and become the pacemaker of the heart. Also, the Purkinje fibres are capable of acting as a pacemaker in the case the AVN fails.

The SAN is functionally, anatomically and electrophysiologically inhomogeneous. Normally, the action potential (AP) is initiated in a small area of the SAN, the leading pacemaker site [9]. This region is known as the centre of SAN. The cells in this region are small (compared with the cells in the atrial muscle) and poorly organised. When the AP is first initiated in the centre of the SAN, it is conducted from the centre to the surrounding atrial muscle via the transitional and peripheral regions of the SAN [9-10]. The cells in the periphery of the SAN are larger and contain well-organised myofilaments [9, 11-12]. Although the principle function of the periphery of the SAN is to conduct the AP from the leading pacemaker site in the centre to the atrial muscle, it does show pacemaker activity. In many cases the leading pacemaker site can shift from the centre toward the periphery in response to a wide range of different interventions [13].

The cells from different regions of the SAN also differ in terms of electrophysiology. In the centre, the AP upstroke velocity is lower, the takeoff potential is more positive, the AP is longer, the maximum diastolic potential and the resting potential in quiescent tissue are more positive (Table 1.1) [14, 23]. From the centre to the periphery of the SAN, the shape and arrangement of the cells are changing gradually. Despite there is no distinct border between the centre and periphery cells, but the cells can be distinguished on the basis of cell capacitance (C_m), i.e. experimentally measured larger cells with higher C_m (presumably from the periphery) and smaller cells with lower C_m (presumably from the centre). Besides the cell capacitance, the conduction velocities are also quite different from the centre and
periphery cells. In the mouse, the conduction velocity in the centre is about 4 cm/s parallel to the CT and 3 cm/s perpendicular to it [15] and the conduction velocity in the periphery is about 49 cm/s parallel to the CT and 36 cm/s perpendicular to it [15].

Table 1.1 AP feathers of SAN cell models [14, 23].

<table>
<thead>
<tr>
<th>Model</th>
<th>MDP (ms)</th>
<th>APA (mV)</th>
<th>CL (ms)</th>
<th>APD_{50} (ms)</th>
<th>APD_{100} (ms)</th>
<th>dV/dtmax (V/s)</th>
<th>DDR (mV/s)</th>
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</table>

1.3 Expression and role of ion channels in SAN

As mentioned in section 1.2, APs recorded from the central and peripheral of the SAN show a clear electrical heterogeneity. In order to investigate the ionic mechanisms responsible for these differences, whole-cell patch-clamp method has been used to measure specific membrane currents at cellular level. A voltage clamp that recorded the membrane potential from its initiation to a new desired value by an electronic feedback system has been extensively studied [197]. Recently, quantification of proteins responsible for ion channels has been measured at the molecular level. This can also be measured at mRNA levels. A combination of these two methods has largely enhanced our understanding of the regional variations in cardiac electrophysiology [197].
1.3.1 Na\(_{1.5}\) and \(I_{\text{Na}}\)

In the heart, the cardiac Na\(^+\) channel is responsible for the upstroke of the AP in atrial and ventricular cells and primarily underlines AP initiation and propagation. However, in the SAN, previous studies suggested the role of the cardiac Na\(^+\) channel is less important than the calcium and potassium channels [16]. The resting potential of SAN cells is about -40 mV and the maximum diastolic potential is between -50 mV and -60 mV, which implies that most of the sodium channels remain inactivated. Therefore, the fast inward Na\(^+\) current was considered small or absent [16].

However, more and more studies have shown that the cardiac Na\(^+\) channel plays critical role in the modulating pacemaker AP in the SAN pacemaker cells and also contributes to pacemaker activity [17-19]. Expression of Na\(_v\) 1.5 mRNA was discovered in rabbit SAN, both in center and periphery, by Tellez et al. [17] although it is significantly lower than in atrial muscle. Similar results were reported from a mouse [18]. Functional analysis manifested that the application of tetrodoxin (TTX) slows down pacemaking activity in both mouse [18] and rabbit [14], and even decelerates SAN conduction with higher concentrations [18]. Studies on human SAN have revealed that Na\(_{1.5}\) mRNA is present in the periphery, but not the center [20-21]. Functional analysis of the sodium current was observed in human SAN cells [22]. Despite that most Na\(^+\) channels may reside in the inactivated state because of the moderately negative membrane diastolic potential (MDP) and long diastolic depolarization phase of single SAN cell, Verkert et al. [22] still argued that \(I_{\text{Na}}\) may contribute to human SAN pacemaker activity for three reasons. These are: (1) Na\(_v\) 1.5 proteins are present in the periphery of the human SAN, (2) a large inward current with \(I_{\text{Na}}\) characteristics is present in human SAN cells, and (3) under in vivo condition, the atrium exerts an effective hyperpolarizing load on the periphery of SAN, thereby bringing the peripheral cells to a more hyperpolarized potential where not all Na\(^+\) channels are inactivated [22].
The upstroke velocity of the AP from the centre to the periphery is gradually increasing and the slowing down of activation of \( I_{Na} \) by TTX has little or no effect on the upstroke in the centre of the SAN, whereas it slows the upstroke velocity in the periphery [14, 42]. Therefore, Kodama et al. [14] suggested that \( I_{Na} \) may be unimportant in the centre for one of three reasons: (1) \( I_{Na} \) may be present in the centre, but it is inactivated because of the low diastolic potential in the centre. (2) Expression of \( Na^+ \) channels in sinoatrial node cells are regionally varying. (3) There may be a regional variation in the mix of SAN cells and atrial cells with SAN instead of a regional variation in the properties of SAN cells. Similarly, the reasons above can explain why \( I_{Na} \) plays a key role in pacemaker activity in the periphery.

### 1.3.2 \( Ca_{1.2}, Ca_{1.3}, Ca_{3.1} \) and \( I_{Ca} \)

It was widely accepted that \( Ca^{2+} \) current (\( I_{Ca} \)) plays a significant role in the last third of the pacemaker potential. It is responsible for the upstroke of the AP and blocking of \( I_{Ca} \) abolishes pacemaker activity [23]. However, this idea was challenged because the diastolic depolarization occurs between -60 mV and -40 mV, whereas the “activation threshold” of \( I_{Ca} \) is around -40 mV [24-25]. It remained to be clarified until another calcium current with lower-threshold was detected in various excitable cells in 1985 [26-27]. This calcium current was suggested to contribute to the pacemaker current, because the activation of this current overlaps with the pacemaker potential. The two types of calcium current, the transient type (\( I_{Ca,T} \)) and long-lasting type (\( I_{Ca,L} \)) were identified by different activation and inactivation kinetics, conductance properties and sensitivities to blockers [28].

L-type \( Ca^{2+} \) channels are multi-subunit complexes [29]. In the SAN cells, two isoforms, \( Ca_{1.2} \) and \( Ca_{1.3} \), have been detected to contribute to \( I_{Ca,L} \) [19, 30-32]. However, the expression of mRNA level shows that \( Ca_{1.2} \) has lower density throughout the SAN and atrial muscle region and \( Ca_{1.3} \) has high density in the SAN region and has higher density compared to atrial muscle [33-34]. The contribution of \( I_{Ca,L} \) in impulse generation in SAN cells was generally considered to be minor. This is
because the threshold for activation of $I_{Ca,L}$ is around -40 mV, whereas the pacemaker depolarization occurs between -65 and -40 mV [13]. However, this is not in agreement with the marked bradycardia effect of $I_{Ca,L}$ blocker [35-38]. Bounman et al. [38] suggested that the activated threshold $I_{Ca,L}$ was around -60 mV instead of -40 mV and recovery from the inactivation of $I_{Ca,L}$ occurred during repolarization, which make $I_{Ca,L}$ available already early in diastole.

T-type calcium channels may contain one of three $\alpha_1$ subunits, $\alpha_{1G}$ (Ca$_v$3.1), $\alpha_{1H}$ (Ca$_v$3.2) or $\alpha_{1I}$ (Ca$_v$3.3) [39], which have been found in the SAN of most species [28, 34, 40-41]. The major isoform is Ca$_v$3.1 [41] in the primary pacemaker region. $I_{Ca,T}$ is distinct from $I_{Ca,L}$ by its lower activation threshold (about -65 mV) and faster inactivation kinetics. Therefore, the activation of T-type calcium channels is earlier than L-type calcium channels, approximately the first third of the pacemaker potential, indicating that $I_{Ca,T}$ contributes to the generation of the pacemaker potential of SAN cells. However, Kameyama et al. [28] suggested that the role of $I_{Ca,T}$ in the pacemaker mechanism was significant but rather like a stabilizer of the rate of depolarization.

Similar to Na$^+$ channel, the Ca$^{2+}$ channels are not uniformly expressed in the SAN. The study of regional differences of the Ca$^{2+}$ current in pacemaker activity of the SAN showed that $I_{Ca}$ played the obligatory role in the centre of SAN but less important in the periphery [10]. Application of Ca$^{2+}$ channels blocker (nifedipine) abolished the AP in the centre, but had little effect on periphery, and so $I_{Na}$ plays the key role in the periphery.

### 1.3.3 HCN1, HCN2, HCN4 and $I_f$

In 1979, Brown et al. [43] recorded a current from small voltage-clamped SAN preparations. This current was defined as the “funny” current ($I_f$) because of several unusual features: 1) it is activated on hyperpolarization with a threshold of approximately -50 mV to -40 mV; 2) it is fully activated between -10 mV and -20 mV
in physiological solutions as a result of the channel’s mixed permeability to Na⁺ and K⁺ [44]; 3) it can bind to cyclic adenosine monophosphate (cAMP). Comparing with \( I_{-k2} \), which was thought to be the pacemaker current [45], the properties of \( I_t \) were more like a pacemaker current: it was inward, activated hyperpolarization in the diastolic range of potential, and increased by adrenaline. In fact, more and more experimental evidence indicate that \( I_t \) is the cardiac pacemaker current [51, 57-58]. It drives slow diastolic depolarization and is responsible for generation of spontaneous activity.

Three decades later, since \( I_t \) was described, the cloning of \( I_t \) was firstly achieved. A new family of channels known as hyperpolarization-activated cyclic nucleotide-gated (HCN) channels was successfully cloned from mouse brain [46]. These channels are classified as four different isoforms (HCN1-4) [47-50]. Up to now, three HCN (1, 2 and 4) isoforms have been found in the mammalian heart. In the SAN, HCN4 is the predominant isoform whilst HCN1 and HCN2 are less expressed [19, 30-32].

The role of \( I_t \) in pacemaker activity in the SAN is still controversial. In one hand, \( I_t \) is proposed as the principle pacemaker current [51, 57-58], but in the other hand, \( I_t \) is considered to play a minor role in generation of SAN pacemaker depolarization [52-56]. Recent clinical data on \( I_t \) gene mutations [59-62] have provided evidences that \( I_t \) contributed to human pacemaker activity. Moreover, recent experimental data [57, 63] have showed the foot of the activation curve of native human SAN \( I_t \) overlapped with that of diastolic depolarization in human SAN cells which implied that \( I_t \) activation contributed a depolarizing current during diastolic depolarization in human SAN cells.

1.3.4 ERG, \( K_r \),LQT1 and delayed rectifier potassium current, \( I_K \)

The delayed rectifier potassium current (\( I_K \)) of cardiac cells consists of two components: \( I_{Kr} \) and \( I_{Ks} \) [64]. The rapid activated potassium current, \( I_{Kr} \), that has been reported to exist in SAN cells of species such as the rabbit [65], guinea-pig [66-67]
and mouse [68], is considered to play an important role in repolarization and the resulting slow diastolic depolarization. In contrast, slowly delayed potassium current $I_{Ks}$ was recorded in species, such as porcine [69], appearing to be the predominant rectifier current and making a large contribution to repolarization [70]. The characteristics of two currents are also quite different from each other: $I_{Kr}$ is activated at around -40 mV and shows strong inward-rectification [71] whereas the threshold of $I_{Ks}$ is around -20 mV. Experimental results showed that in spontaneous active cells, partial inhibition of $I_{Kr}$ could shift the maximum diastolic potential and decrease the AP amplitude repolarization phase; complete block of $I_{Kr}$ would terminate automaticity quickly with a resting potential between -30 and -40 mV. Inhibition of $I_{Ks}$ has a minor effect in most species except guinea pig [66] and rabbit [81].

The distribution of $I_{Kr}$ and $I_{Ks}$ are heterogeneous in the SAN region [73-74]. The mRNA of ERG, which is largely expressed in the SAN region of the rabbit heart [75] and in the CT of the ferret heart including the SAN region [76], is largely responsible for $I_{Kr}$. Along with mink, an auxiliary β subunit, K$_v$LQT1 is considered to form $I_{Ks}$ channels. Experimentally measured mRNA levels showed that it was largely expressed in the SAN of the guinea-pig [77], ferret [78] and mouse [79].

1.3.5 $K_v4.2$, $K_v4.3$, $K_v1.4$, KChIP2 and $I_{to}$

According to different characteristics, transient outward potassium current ($I_{to}$) can be divided into two components: fast recovering transient outward potassium current ($I_{to,f}$) and slow recovering transient outward potassium current ($I_{to,s}$). $I_{to}$ channels are coded by the $K_v1$ and $K_v4$ gene family [82]. $K_v4.2$ and $K_v4.3$ are believed to be responsible for $I_{to,f}$, while $K_v1.4$ and KChIP2 are responsible for $I_{to,s}$. Blocking of $I_{to}$ by 4-aminopyridine causes a larger effect on pacemaker activity of SAN periphery than centre [83]. Normally, $I_{to}$ is considered to contribute to the AP repolarization phase of SAN peripheral cells, but play a minor role in SAN central cells.
1.3.6 $K_{ir2.1}$, $K_{ir2.2}$ and $I_{K1}$

$I_{K1}$, named inward rectifier time independent potassium current, has been observed in mouse and rat SAN cells [40, 68] but not rabbit [84]. $I_{K1}$ channels are coded by $K_{ir2.1}$, $K_{ir2.2}$ [68] and expressed much lower in SAN than that in atrium and ventricle. $I_{K1}$ is largely responsible for stabilizing the resting membrane potential as well as APD of the ventricle cells, but less important for SAN cells.

1.3.7 The sustained inward current, $I_{st}$

$I_{st}$, a novel subtype of the L-type channel current carried by Na$^+$ under physiological conditions, has been reported in several species [67-68, 85-86]. Since the genetic basis of $I_{st}$ channels remains unknown, the role of $I_{st}$ in pacemaker activity has not been established. Mathematical modelling study showed the possibility that $I_{st}$ contributed to pacemaking by virtue of its slow inactivation rate and low threshold of activation [87]. Zhang et al. [88] suggested that $I_{st}$ might significantly contribute to the centre cell of rabbit SAN but was less important in periphery because of the small current density in comparison with $I_{Na}$.

1.3.8 $K_{ir3.1}$, $K_{ir3.4}$ and $I_{K,ACh}$

$I_{K,ACh}$ is an inward rectifier K$^+$ current which can be activated by muscarinic and adenosine receptors [89-90]. Under normal circumstances, the $I_{K,ACh}$ remains inactive unless a neurotransmitter, acetylcholine (ACh) is applied. $I_{K,ACh}$ is strongly expressed in the SAN [91] and responsible for negative chronotropic effect with an increase of maximum diastolic potential [91-94]. $K_{ir3.1}$ and $K_{ir3.4}$ are the major isoforms that contribute to $I_{K,ACh}$ in the heart. However, as a result of lacking $K_{ir3.4}$ channels, $I_{K,ACh}$ current has not been found in mice [95].

1.3.9 SR Ca$^{2+}$ release

SR Ca$^{2+}$ release has been reported to contribute to the generation of pacemaker
activity by Rubenstein and Lipsius [96] and Li et al. [97]. Rubenstein and Lipsius [96] found that in cat, application of ryanodine slowed automaticity in atrial subsidiary pacemaker cells by reducing the slope of the late diastolic depolarization, which implied that SR Ca\(^{2+}\) release was important in generation of the late phase of the diastolic depolarization. Li et al. [97] also reported similar result from cultured rabbit SAN cells.

1.3.10 Na\(^{+}\)-Ca\(^{2+}\) exchanger current, \(I_{NCX}\)

\(I_{NCX}\), Na\(^{+}\)-Ca\(^{2+}\) exchanger current, was firstly described by Horachova et al. in 1979 [98]. Five years later, Brown et al. [99] found the evidence of presence of \(I_{NCX}\) in pacemaker tissue by using multicellular SAN preparations. After 30 years of study, \(I_{NCX}\) has been identified to be a major factor in regulation the electrophysiological function of cardiac cells. It is responsible for intervening in cardiac cell Ca\(^{2+}\) homeostasis, relaxation of the cells and excitation-contraction coupling. Functional analysis indicated that \(I_{NCX}\) was responsible for maintaining the automaticity of the pacemaker cells in both amphibians [100] and mammals [101-102].

1.3.11 Na\(^{+}\)-K\(^{+}\) pump current, \(I_{P}\)

In SAN cells, Na\(^{+}\)-K\(^{+}\) pump current (\(I_{P}\)) is responsible for the setting of the maximum diastolic potential in the rage of \(-60\) mV [103]. Under normal circumstances, for each pump cycle, three Na\(^{+}\) will be extruded and two K\(^{+}\) will be transported in the intracellular milieu. Consequently, an outward current generated by Na\(^{+}\)-K\(^{+}\) pump will influence cellular pacemaking.

1.3.12 Background current, \(I_{b}\)

In the SAN, background current (\(I_{b}\)) is an inward current carried by Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) contributing to the generation of the pacemaker depolarization [104].
1.4 Heart disease

1.4.1 Sick sinus syndrome (SSS)

Sick sinus syndrome (SSS) or sinus nodes dysfunction (SND) was firstly described by Lown in 1967 in human [105]. It is defined as the intrinsic inadequacy of the SAN to perform its pacemaking function due to a disorder of automaticity and/or inability to excite to the rest of the atrium. It denotes a collection of cardiac arrhythmias that includes several electrocardiographic features, such as sinus bradycardia, sinus arrest, SAN exit block, slow SAN atrium conduction, or alternating periods of bradycardia and tachyarrhythmias.[20, 106-108]. Although sick sinus syndrome most commonly occurs in older people (especially the older than 65), it can also occur at any age, including in the foetus, infant and child without heart disease or other contributing factors [109]. From mild to severe, patients with SSS present symptoms with fatigue, palpitations, anxiety, dizziness, fainting, and syncope in some cases. In the United States [110], more than half of SSS patients require pacemaker implantations.

SSS and HCN4 genetic mutations

Recent studies have screened the ion channel genes associated with SAN function and determined the relationship of SAN function with mutations of HCN4 [59-62]. SSS associated with four different types of HCN4 gene mutations that codes for the hyperpolarisation-activated pacemaking channel (the $I_f$ channel) have been studied so far [59-62]. The first study was reported by Scholze-Bahr et al. [59], who identified the HCN4-573X mutation in congenital SSS patient with idiopathic sinus bradycardia and chronotropic incompetence. Later on, a miss-sense HCN4 mutation was identified by Ueda et al. [60]. This HCN4 mutation is named as D553N mutation with replacement of Asp (GAC) with Asn (AAC) at codon 553 in a patient with SND. Another HCN4 gene mutation, the S672R mutation (a replacement of serine 672 by arginine) was identified by Milanesi et al. [61] in members of a large family with sinus bradycardia. Most recently, the HCN4 G480R (substitution of glycine to arginine at position 480 of the protein) in familial SSS patients was identified by Nof
et al. [62]. Modelling of the causative link between these HCN4 gene mutations and SSS is presented in Chapter 3.

**SSS and SCN5A genetic mutations**
The SCN5A gene was found to localize on the short arm of chromosome 3 [111], encoding on the α subunit of cardiac voltage-gated sodium channel. The first SCN5A mutation related to SSS was reported by Benson et al.[107], who identified a “loss-of-function” SCN5A mutation in patients with rare familial sinus bradycardic syndrome. Later on, familial SSS associated with such loss of function mutations was extensively studied. Up to now, fourteen loss-of-function, SSS-associated, SCN5A mutations have been identified [135]. Loss of function has been characterized as a major biophysical phenotype of SSS-related SCN5A mutation, but the detailed mechanisms of the phenotype have not been defined. However, the causative link between SCN5A gene mutations and SSS was reported by my colleagues Butters et al. [142].

### 1.4.2 Long-QT syndrome subtype 3
Long-QT syndrome subtype 3 (LQT3) is one type of LQT syndrome usually arising from “gain-of-function” SCN5A gene mutations. It is characterized by delayed cardiac depolarization, a prolonged QT interval on ECG and lethal ventricular arrhythmias and presents clinically as ventricular tachycardia, cardiac arrest, and sudden death [157]. To date, more than 80 SCN5A mutations have been identified to link with LQT3. Studying of SAN function and intracardiac conduction in a murine model of LQT3 is presented in Chapter 4.

### 1.4.3 Brugada syndrome
Brugada syndrome (BrS), firstly described by Brugada and Brugada [184], is a genetic disease characterized by ST-segment elevation in the right precordial leads, with incomplete or complete bundle branch block, and an episode of ventricular
fibrillation. It is also known as “Sudden Unexpected Death Syndrome” (SUDS) which commonly appears amongst young men without known underlying cardiac disease. Approximately 20% of clinical affected individuals carry the SCN5A mutation leading to a loss-of-function of sodium channel.

1.4.4 Isolated cardiac conduction disease

Isolated cardiac conduction disease (CCD) is a life threatening disorder of the heart, which is characterized by the progressive alteration of cardiac conduction through the His-Purkinje system with right or left bundle block and a wide QRS complex. SCN5A had been the only gene found to be involved in the pathogenesis of CCD until Watanabe et al. [185] reported a defect of SCN1B linking it with CCD. Another important factor in CCD, fibrosis, have been recently observed extensively in old Scn5a +/- mice, but not young Scn5a c/c. Therefore, combination of loss-of-function of sodium channel and age-related increase in fibrosis is believed to be the underlying cause of SCN5A-relation CCD.

1.5 Thesis overview

Chapter 1: An introduction to the physiological background refers to this thesis. A description of the heart, with emphasis on the SAN, is given, from ion channel expressions to role of each current in cardiac pacemaking. Moreover, a brief introduction of heart disease associated with genetic mutations is presented.

Chapter 2: A description of how to develop a mathematical model based on experimental results, from single cell level to the whole heart. The numerical methods applied to compute the mathematical formulae of cardiac cells are also discussed.

Chapter 3: Investigation of SSS associated with HCN4 gene mutations. AP models of rabbit SAN cells (central and peripheral) were modified to incorporate experimentally reported I_f changes induced by HCN4 gene mutations. The cell models were also incorporated into an anatomically detailed two-dimensional (2D) tissue model of the
intact SAN-atrium. Effects of the mutations and vagal nerve activity on cardiac pacemaking at the single cell and tissue levels were studied. Experimental validation was performed to verify simulation results by using multielectrode extracellular recordings of AP initiation and conduction pattern in isolated rabbit SAN-atrium tissue.

**Chapter 4:** In section 4.1, mathematical models of rabbit SAN cells (central and peripheral) and 2D tissue models were modified to investigate SAN function and intracardiac conduction in a murine model of long QT syndrome type 3. A prolonged tail current $I_{\text{Na,L}}$ was introduced and incorporated with a normal $I_{\text{Na,T}}$ to test the SAN pacemaker function and AP conduction from the SAN to atrial septum. In section 4.2, mathematical models of mouse SAN cells (central and peripheral) were modified to investigate the mechanisms underlies the SAN associated with SCN5A deficiency and aging. A coupled SAN-atrium cell model was developed to replicate the experimentally observed slowing of SAN conduction with aging and SCN5A-disruption. In section 4.3, the effects of pulmonary hypertension (PHT) on SAN were investigated. Mathematical models of mouse SAN cells (central and peripheral) were modified to evaluate the possible consequences of changes in ion channels and intracellular Ca$^{2+}$ handling at the mRNA level induced by PHT.

**Chapter 5:** The description of the development of biophysically detailed 2D SAN-atrium model of rabbit is presented. The response of the model to ACh and gene mutations is analyzed. Mechanisms of pacemaker shift are discussed.

**Chapter 6:** The novelty of the models and results presented in this thesis are outlined and future plans for further model development and improvement are discussed.
Chapter 2

From physiology to computational modeling

Assembled from equations, computational models have been introduced to cardiac simulation for about fifty years [112]. An integrative model of cardiac myocytes and their coupling to each other would ultimately allow us to construct a virtual heart which can be used to investigate various aspects of heart function and dysfunction [112]. For example, drug actions can be modeled, resulting in a quantitative model of drug safety and risk, accelerating novel drug development and reducing the frequency of potentially dangerous drug trials. As a research tool, computational models can also help us understand cardiac mechanics, mechanism of arrhythmias, mechanisms of defibrillation and genetic mutations etc [122]. Because computational modelling is easy to handle, low cost, safe and efficient, it has become more and more popular in cardiac research. Furthermore, based on increasing availability of both detailed experimental data and high-performance computers, computational models have begun to be much more valuable and accurate in reproducing electrophysiology of the heart [25, 122]. From sub-cellular and cellular to the whole organ level, to date, hundreds of computational models have been adopted to do cardiac research.

In this chapter, a description of developing a computational model that can simulate the electrophysiology of a cardiac cell and using mathematical equations to describe the propagation of the AP through cardiac tissue is presented. Moreover, numerical methods that are used for solving the respective equations are discussed.
2.1 Cell-level model

2.1.1 Cardiac myocytes

Cardiac myocytes are muscle cells that comprise the vast bulk of cardiac tissue. They are responsible for conducting electrical signals and contracting to provide the force needed to pump the blood around the body. Cardiac myocytes are bound by a membrane which separates the intra- and extra-cellular environments [113]. The membrane contains a large number of specialized structures, such as passive channels, active pumps and gated channels, which are responsible for allowing a regulated flow of ions in and out of cells, thus to modulate the ionic composition of the intercellular space and electrical activity of the cell [114].

2.1.2 Myocyte electrical action potential

The electrical activity of a cardiac myocyte is caused by current flow through ion channels and transporters in the cell membrane. The cardiac AP has various configurations in different cell types (Figure 2.1). This is due to the spatial heterogeneity of channel expression which allows the different electrical characteristics of the different portions of the heart [115].

Fig. 2.1: AP configuration in different parts of the heart.

Taken from [115].
Once myocytes are excited, ions such as Ca\(^{2+}\), Na\(^+\), K\(^+\), Cl\(^-\) will begin to move in or out of the cell. These effects work together to form the AP. The typical AP of ventricular myocyte is usually divided into 5 phases (Figure 2.2).

![Figure 2.2 A: The five phases of AP [116]. B: AP from the rabbit SAN cell [135].](image)

**Phase 0:**

In fast response cells (atria, His bundle, Purkinje fibers and ventricular cells), the Na\(^+\) current leads to this rapid upstroke which means rapid depolarization. While the membrane potentials are stimulated over the threshold of Na\(^+\) channels, Na\(^+\) channels are open leading to net influx of cations, which inclines the membrane to depolarize. As activation of Na\(^+\) channels are fast, the depolarization activates a large amount of Na\(^+\) channels arousing a large transient Na\(^+\) current. With the AP approaching reversal potential of Na\(^+\), Na\(^+\) current starts to decline after reaching a peak and finally becomes inactivation. At the same time, some outward currents are activated such as \(I_{to}\). Both of these two effects slow down the depolarization.

**Phase 1:**

The repolarization of cardiac cell appears following the peak of depolarization. A secondary depolarization could occur in several types of cardiac cells leading to spike-dome shaped AP. In phase 1, the Na\(^+\) current is inactive and the outward currents are activated. Both factors determine a slight repolarization of cardiac AP.
Normally, $I_{to}$ ($I_{to1}, K^+$) takes up a majority of transient outward currents, whereas, a Ca$^{2+}$-activated Cl$^-$ current ($I_{to2}$) is also involved. It is proposed that the different shapes of the ventricular AP result from different expression of $I_{to}$ in different sort of cardiac cells [117].

Phase 2:

This phase is called “plateau” phase. During this phase, the inward currents and outward currents balance with each other to maintain the “plateau”. In ventricular myocytes, the major inward current is L-type calcium current, $I_{Ca,L}$. $I_{Ca,L}$ does not play a key role in phase 1 due to its slower activation and smaller amplitude in comparison with the Na$^+$ current. The delayed rectifier potassium currents $I_{Kt}$ and $I_{Ks}$ predominate in outward currents. The details of these currents have been introduced in Chapter 1. The L-type calcium current gradually declines during the plateau and, conversely, the delayed rectifier potassium currents increase in this process, finally result in an end of the plateau.

Phase 3:

During this phase, the L-type calcium current becomes inactive. Meanwhile, K$^+$ efflux via the delayed rectifier potassium currents drives the AP to a more negative voltage. In ventricular myocytes, the inward rectifier potassium current $I_{K1}$ is activated during this phase. It contributes to a relatively fast repolarization [117].

Phase 4:

During this period, heart is diastolic. The K$^+$ flux governs the resting membrane potential. $I_{K1}$ brings the membrane potential back to resting state [118]. Moreover, several types of background currents are involved in this stage. The inward background current is mainly carried by Na$^+$. While, the Na$^+$.K$^+$ pump currents are also contributing to a small outward background current.

The AP upstroke (phase 0) will not be initiated until the potential difference across
the cellular membrane is raised above a particular level. This is known as the threshold of the excitation. Following the AP initiation, there is a time interval called the absolute refractory period. During this period, a second AP cannot be triggered by a stimulus of any strength. The absolute refractory phase lasts from phase 0 to phase 2, then the AP is in a period known as relative refractory. During this period, a second AP can be triggered but only by a stimulus with higher amplitude than the threshold. After the relative refractory, the AP remains at the resting potential, waiting for the next stimulus [119].

2.1.3 Cell Model development

The Nernst Equilibrium

The Nernst equilibrium describes how a difference in ionic concentrations between two phases can result in a potential difference between the phases [120]. Suppose there are two reservoirs, separated by a semi-permeable membrane, containing different types of ions at each side at different concentrations. The solutions on each side of the membrane are assumed to be electrically neutral. The concentration difference across the membrane results in a flow of ions from one side to another. Therefore, this charge imbalance will set up an electric field that opposes the further diffusion of ions through the membrane. When equilibrium is reached the potential difference, $V_s$, across the membrane is given by

$$V_s = \frac{RT}{zF} \log \left( \frac{[S]_e}{[S]_i} \right),$$  

(2.1)

here $S$ is the common ion symbol, where subscripts $i$ and $e$ denote internal and external concentrations respectively, $R$ is the universal gas constant, $T$ is the absolute temperature, $F$ is Faraday’s constant, and $z$ is the charge of the ion $S$.

The Electrical Circuit Model of the Cell Membrane
Since the cell membrane separates charge, it can be viewed as a capacitor (Figure 2.3). The capacitance of any insulator is defined as the ratio of the charge across the capacitor to voltage potential necessary to hold that charge, and is denoted by

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

(2.2)

Since there can be no net buildup of charge on either side of the membrane, the sum of the ionic and capacitive currents must be zero, and so

\[ C_m \frac{dV}{dt} + I_{ion} = 0, \]  

(2.3)

here \( C_m \) is capacitance, \( V \) is the membrane potential difference between the internal potential, \( V_i \) and the external potential, \( V_e \), (so that \( V = V_i - V_e \)).

![Figure 2.3 Electrical circuit model of the cell membrane. Taken from [121].](image)

**The Hodgkin-Huxley Model**

In 1950’s, Hodgkin and Huxley [121] described quantitatively three different types of ion current (sodium, potassium, and “leak current” consists mainly of chlorine) in the squid giant axon and observed a linear instantaneous current-voltage relation:
\[
C_m \frac{dV}{dt} = -g_{Na}(V - V_{Na}) - g_k(V - V_k) - g_L(V - V_L) + I_{app},
\]

where \(I_{app}\) is the applied current, \(g\) is the channel conductance; \(V\) is membrane potential and \(V_{Na}, V_k,\) and \(V_L\) are the Nernst potentials for the corresponding ions.

The sodium current is transient when the membrane potential is held constant under voltage clamp. The sodium channels consist of two processes at work, one that turns on the sodium current, say, activation and one that turns it off, say, inactivation. Hodgkin and Huxley realized that it would be easier to write channel conductance as a function of two different variables. One is responsible for turning on and the other for turning off the channel. Therefore, Hodgkin and Huxley proposed that the sodium conductance is of the form:

\[
g_{Na} = g_{Na}m^3h,
\]

where \(m\) is the activation variable, and \(h\) is the inactivation variable. The reason of raising \(m\) to the third power is based on time course of \(I_{Na}\) during voltage clamp observation. Interestingly, when the structure of \(I_{Na}\) is examined in detail, it shows that the sodium channel has three structures which open to allow current to flow.

Hodgkin and Huxley also fitted the time-dependent behavior of \(m\) and \(h\) to a first order process with dynamics:

\[
\frac{dw}{dt} = \alpha_w(1-w) - \beta_w w,
\]

where \(w\) equals to \(m\) or \(h\). Because \(m\) is small at rest and first increase, it is called the sodium activation, and because \(h\) shuts down, or inactivates the sodium current, it is called the sodium inactivation. When \(h\) equals to 0, the sodium current is completely inactivated. The overall procedure is similar to that used in the specification of \(g_{Na}\).
Different channels have different activation and inactivation variables. These variables are modulated by different $\alpha_w$ and $\beta_w$. The unknown functions of $\alpha_w$ and $\beta_w$ are determined by fitting to the experimental curves [121].

**First-generation cardiac cell model**

The first generation cell model was firstly developed by Noble [122], who based it on the Hodgkin-Huxley [121] model of the squid giant axon and adapted it for Purkinje fiber cell. Later on, Noble’s model was used as a basis for various cardiac cell models to reproduce the AP based on available experimental information about the voltage and time dependence of ion channel conductance data. The general form of these models is shown as follow:

\[
C_m \frac{dV_m}{dt} = -\sum I,
\]  \hspace{1cm} (2.7)

\[
I = g y_1 y_2 \cdots y_n (V_m - V_E),
\]  \hspace{1cm} (2.8)

\[
\frac{dy}{dt} = \frac{y_\infty - y}{\tau},
\]  \hspace{1cm} (2.9)

where $I$ is the current, $g$ is the maximal conductance of the ion channel, $y$ are the gating variables varying between 0 and 1, describing the activation, inactivation and recovery of the ion channel, $V_E$ is the Nernst potential, $y_\infty$ is the steady-state value of a single gating variable $y$ (i.e. its value for $dv/dt = 0$), and $\tau$ is a time constant.

**Second-generation cardiac cell models**

The second-generation models not only include a biophysical description of ion channel, pump and exchange currents, but also include a detailed description of intracellular Na$^+$, Ca$^+$ and K$^+$ concentrations [112]. In addition to the general form of these models described above, the second generation models also include an equation to balance the intracellular and extracellular ion concentrations:
where $S$ describes different types of ions, $[N]$ is the concentration of ions which is affected by $S$ different types of ion channels, pumps and exchanger currents.

It is important to understand the advantages and limitations of each model so that we can make an appropriate choice. First-generation models perform a good balance between numerical efficiency and biophysically important detail and have been widely used for studies of ventricular re-entry and fibrillation in simplified geometries as well as the whole ventricle simulation. Second-generation models include more details of ion channels and exchanger currents. Therefore, they are suitable for a detailed mechanistic study of phenomena, such as action of drugs on specific ion channels and consequences of channelopathies, the mechanisms of after-depolarizations and the development of regional ischaemia.

2.2 Tissue-level model development

At the tissue level, the electrical activity of a cardiac myocyte is a sequence of electrical excitation waves propagating in cardiac myocardium. Cardiac cells communicate with neighbouring cells electrically via gap junction coupling. The junctions connect the cytoplasm of each cell to its neighbour’s and allow the efficient transmission of electrical signals along the fiber orientation, thus activate the whole tissue. The tissue model can be constructed by coupling various single cell models to take into account gap junction coupling and anatomical heterogeneity. A continuum, monodomain approximation to the propagation of AP in cardiac tissue can be described as:
\[
\frac{\partial V}{\partial t} = \nabla \cdot (D \nabla V) - \frac{I_{\text{tot}}}{C_m},
\] (2.11)

where \( V \) is the membrane potential, \( t \) time, \( \nabla \) the spatial gradient operator, \( D \) the diffusion coefficient that characterises electrotonic spread of voltage via gap junctions, \( C_m \) the cell membrane capacitance and \( I_{\text{tot}} \) the total ionic current.

### 2.2.1 The anisotropic model

As described above, the membrane potential \( V \) is the membrane potential difference between the intracellular potential, \( V_i \) and the extracellular potential, \( V_e \). For the anisotropic tissue, in Cartesian coordinate system, the diffusion coefficient \( D \) can be described as:

\[
D = \frac{\partial}{\partial x} + \frac{\partial}{\partial y},
\] (2.12)

\( D \) is a symmetrical matrix given by:

\[
D = \begin{pmatrix}
D_{xx} & D_{xy} \\
D_{yx} & D_{yy}
\end{pmatrix}
\] (2.13)

In anisotropic tissue, the diffusion of voltage can be divided into two directions—along the fiber axis and perpendicular to the fiber axis. The diffusion tensor at the particular point in a local coordinate system is given by [123]:

\[
\bar{D} = \begin{pmatrix}
D_{\parallel} & 0 \\
0 & D_{\perp}
\end{pmatrix}
\] (2.14)

where \( D_{\parallel} \) and \( D_{\perp} \) are diffusion coefficients along the fiber axis and perpendicular to the fiber axis respectively. Assuming the eigenvectors along the fiber axis and perpendicular to the fiber axis are given as \( f \) and \( s \). In the global Cartesian coordinate
system, we can translate the diffusion tensor $D$ from local tensor $\mathbf{D}$ by the following relationship:

$$D = A\mathbf{D}A^T,$$  \hspace{1cm} (2.15)

where matrix $A$ is given by:

$$A = \begin{pmatrix} f & 0 \\ 0 & s \end{pmatrix},$$  \hspace{1cm} (2.16)

where $A^T$ is the transpose of $A$. $A$ and $A^T$ are the transformation matrices from the local to global coordinate systems and vice versa [123].

Substituting equation 2.16 into equation 2.15 gives

$$D = D_\parallel ff^T + D_\perp ss^T,$$  \hspace{1cm} (2.17)

where $A$ is an orthogonal matrix, (i.e. $AA^T = I$), so we get:

$$ff^T + ss^T = I,$$  \hspace{1cm} (2.18)

where $I$ is the identity matrix. The substitution of equation 2.18 into equation 2.17, allows the diffusion coefficient $D$ to be written as:

$$D = D_\perp I + (D_\parallel - D_\perp)AA^T.$$  \hspace{1cm} (2.19)

The anisotropic model consists of a coupled set of parabolic and elliptic differential equations. Boundary conditions for the equations may vary. The most appropriate
boundary conditions can be described as: the body is assumed to be a passive conductor that is isolated at the other surface and no intracellular current leaves the heart [124].

2.2.2 The monodomain model

The bidomain model [123] considers both intracellular and extracellular potential fields, representing a good tool for modelling complex cardiac electrophysiological waves. However, it is difficult to find the solutions of a system of two nonlinear partial differential equations coupled to a system of ordinary differential equations. Besides, the bidomain equations are computationally expensive as they have to solve a large linear system at each computational time-step. The monodomain model [112] is an approximation of the bidomain model. For the monodomain model, we assume the tissue is isotropic so the diffusion coefficient $D$ becomes a scalar. The propagation of AP in cardiac tissue can then be simplified as:

$$\frac{\partial V}{\partial t} = D V^{2V} - \frac{I_{\text{tot}}}{C_m}. \quad (2.20)$$

2.3 Whole-heart characteristics

ECG is a standardized noninvasive method of recording and presenting the electrical activity of the whole heart. The resulting waveform exhibits certain characteristic features which in comparison to normal ECG waveforms can aid in the diagnosis of cardiac abnormalities [125].

The schematic ECG shown in Figure 2.4 depicts the characteristic features generally preset in a typical ECG waveform recorded from a healthy heart. There are five standard waves in the ECG. Each wave is related to a specific electrical event.
The P-wave is caused by the depolarization of the atria, representing time interval from initial SAN impulse to pass through AVN. Normally, a P-wave tends to last 0.1 to 0.12 seconds.

The PR interval is measured from the beginning of P wave to beginning of the QRS complex, representing the time from initial SAN impulse to pass through AVN. In a healthy heart, it tends to last 0.12 to 0.2 seconds. A PR interval longer than 0.2 seconds suggests a delay at AVN, while shorter than 0.12 seconds is associated with arrhythmia syndromes [126].

The QRS complex is associated with the ventricular depolarization, representing the time taken for activation of the left and right ventricles via the specialized His-Purkinje system and bundle branches. The QRS complex has the largest
magnitude in the ECG and tends to last less than 0.12 seconds. If the duration of QRS is too small, it reflects ventricular hypertrophy. If the QRS complex is longer than 0.12 second, it suggests a problem with conduction through specialized conduction tissue below the AVN, such as bundle branch block (BBB) [126].

The ST segment connects the QRS complex and the T wave. The depression of the ST segment suggests ischaemia and the elevation of ST segment suggests a myocardial infarction [126].

The T wave is associated with the ventricular repolarization. Approximately, the peak of T wave represents the end of absolute refractory of the ventricles and the end of T-wave corresponds to the final stages of ventricular repolarization.

The QT interval reflects the time taken for the ventricular depolarization and repolarization. Both LQT and SQT predispose to dangerous arrhythmias, including ventricular tachyarrhythmias and ventricular fibrillation [126].

2.4 Numerical Methods

For the mathematical models, we usually explore the function of the heart by solving ordinary differential equations (ODEs) and partial differential equations (PDEs) [128]. Several numerical techniques including explicit or forwards Euler’s method [129], fourth-order Runge Kutta method [130-131] and finite difference method (FDM) [132] are mostly used to estimate the real solutions of ODEs and PDEs. These techniques vary in complexity and accuracy. In this section, the advantages and disadvantages of these methods will be discussed.

2.4.1 Explicit Euler’s method

The explicit Euler’s method [128] is perhaps the simplest and the most commonly used numerical integration technique in cardiac cell modelling. It is a first order
numerical procedure for solving ODEs with a given initial value. It is best presented by the following relationship:

\[ t_{n+1} = t_n + h , \]  
\[ y_{n+1} = y_n + hf(t_n, y_n) + O(h^2) , \]  
\( (2.21) \)  
\( (2.22) \)

here \( t \) is time, \( y \) is position, \( h \) is the step size in the same unites as \( t \), \( n \) is the step number, \( O(h^2) \) is the truncation (local) error. In going from \( y_0 \) to \( y_n \) the global truncation error is \( O(h) \). As long as the interval \( h \) is small enough (in cardiac system, \( h \) is typically 0.02 ms or smaller which is close to the length of individual myocytes), the sequence \( y_1, y_2, \cdots, y_n \) should be a good approximation of the time evolution of \( y \).

### 2.4.2 Runge-Kutta method

The Runge-Kutta method [130] is the use of a “trial” step at the midpoint of the interval to compute the “real” step across the whole interval. For example, use the value of both \( t \) and \( y \) at the midpoint; we can rewrite equation 2.22 as:

\[ k_1 = hf(y_n, t_n) , \]  
\[ k_2 = hf(y_n + \frac{1}{2}k_1, t_n + \frac{1}{2}h) , \]  
\[ y_{n+1} = y_n + k_2 + O(h^3) , \]  
\( (2.23) \)  
\( (2.24) \)  
\( (2.25) \)

here \( k_1 \) is the slope at the beginning of the interval and \( k_2 \) is the slope at the midpoint of the interval. The value of \( y \) can be determined by the slope \( k_1 \). This is called the second-order Runge-Kutta method. This method has a local error of \( O(h^3) \) and a global error of \( O(h^2) \) which is one order more accurate than Euler’s method.

In the same way, the Runge-Kutta method can be increased to higher orders. The
fourth-order Runge-Kutta method, which is the most commonly used by far, can be described as:

\[
k_1 = hf(y_n, t_n),
\]

\[
k_2 = hf(y_n + \frac{1}{2}k_1, t_n + \frac{1}{2}h),
\]

\[
k_3 = hf(y_n + \frac{1}{2}k_2, t_n + \frac{1}{2}h),
\]

\[
k_4 = hf(y_n + k_3, t_n + h),
\]

\[
y_{n+1} = y_n + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4) + O(h^5),
\]

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

For the fourth-order Runge-Kutta method, the derivative is evaluated four times in each step: once at the initial point \((k_1)\), twice at the trial midpoints \((k_2, k_3)\) and once at a trial endpoint \((k_4)\).

Both Euler’s method and fourth-order Runge-Kutta method have advantages and disadvantages. The comparisons of two methods are shown as below:

1. Euler’s method is much simpler and more straightforward in solving ODEs.
2. Running at the equivalent step size, the fourth-order Runge-Kutta method is more accurate than Euler’s method, but requires more computational time.
3. The fourth-order Runge-Kutta method is more stable than Euler’s method, especially with a relative large time step.

Despite the fourth-order Runge-Kutta method offering a more accurate and stable approach for exploring the solution of ODEs, most of cardiac models are using explicit Euler’s method. This is because the explicit Euler’s method is more suitable for calculating APs based on Hodgkin-Huxley type equations; easy to implement, computational load is low for cell models and PCs are fast. Moreover, the
reaction-diffusion equation 2.11 is demanded in step size for solving AP propagation in excitable media.

2.4.3 Finite difference method

The finite difference method is a way of calculating the solution to PDEs, such as the bidomain and monodomain equations described above. For anisotropic tissue $D$ is a diffusion tensor. Therefore, the diffusion term of the equation 2.11 can be written for a Cartesian coordinate system as:

$$
\nabla \cdot (D \nabla V_m) = \sum_{i=1}^{2} \sum_{j=1}^{2} \frac{\partial}{\partial x_i} \left( D_{ij} \frac{\partial V_m}{\partial x_j} \right) = \sum_{i=1}^{2} \sum_{j=1}^{2} \left( \frac{\partial D_{ij}}{\partial x_j} \frac{\partial V_m}{\partial x_i} + D_{ij} \frac{\partial^2 V_m}{\partial x_i \partial x_j} \right). \tag{2.32}
$$

We assume the cardiac tissue is cut into a number of nodes. The spacing between each node is $\Delta x$ in the $x$ direction, $\Delta y$ in the $y$ direction. Using the centered difference, the first order derivative in the $x$ direction can be approximated as:

$$
\frac{\partial V_m}{\partial x} \approx \frac{V(x + \Delta x, y) - V(x - \Delta x, y)}{2\Delta x}. \tag{2.33}
$$

The second order derivative in $x$ and $y$ directions can be approximated as:

$$
\frac{\partial^2 V_m}{\partial x \partial y} \approx \frac{[V(x + \Delta x, y + \Delta y) + V(x + \Delta x, y - \Delta y)] - [V(x - \Delta x, y + \Delta y) + V(x - \Delta x, y - \Delta y)]}{2\Delta x 2\Delta y}. \tag{2.34}
$$

In other directions, the expression for the derivatives is similar.

The element $D_{ij}$ that has been described above is the diffusion coefficient for longitudinal and transverse propagation $D_{\parallel}$ and $D_{\perp}$. Assuming the local fiber
orientation are cosines \( n_1 \) and \( n_2 \). The derivatives of these elements are given by [134-135]:

\[
\frac{\partial D_{ij}}{\partial x_i} = (D_{ii} - D_{ij}) \left( n_i \frac{\partial n_j}{\partial x_i} + n_j \frac{\partial n_i}{\partial x_j} \right). \tag{2.35}
\]

In a numerical scheme, these derivatives can be precomputed and stored at the beginning of simulation.

The two terms of the right hand side of equation 2.32 can be treated individually and expanded as:

\[
\sum_{i=1}^{2} \sum_{j=1}^{2} \left( \frac{\partial D_{ij}}{\partial x_i} \frac{\partial V_m}{\partial x_j} \right) = \frac{\partial D_{xx}}{\partial x} \frac{\partial V_m}{\partial x} + \frac{\partial D_{xy}}{\partial x} \frac{\partial V_m}{\partial y} + \frac{\partial D_{yx}}{\partial y} \frac{\partial V_m}{\partial x} + \frac{\partial D_{yy}}{\partial y} \frac{\partial V_m}{\partial y} \tag{2.36}
\]

\[
\sum_{i=1}^{2} \sum_{j=1}^{2} \left( D_{ij} \frac{\partial^2 V_m}{\partial x_i \partial x_j} \right) = D_{xx} \frac{\partial^2 V_m}{\partial x^2} + D_{yy} \frac{\partial^2 V_m}{\partial y^2} + 2D_{xy} \frac{\partial^2 V_m}{\partial x \partial y}. \tag{2.37}
\]

In an isotropic monodomain model, the diffusion term of the equation 2.11 can be written for a Cartesian coordinate system as:

\[
\nabla \cdot D \nabla V_m = \nabla \cdot \nabla^2 V_m = D \left[ \frac{\partial^2 V_m}{\partial x^2} + \frac{\partial^2 V_m}{\partial y^2} \right]. \tag{2.39}
\]

Using the centered difference, the first and second order derivative in the \( x \) direction can be written as:

\[
\frac{\partial V_m}{\partial x} \approx \frac{V(x + \Delta x, y) - V(x - \Delta x, y)}{2\Delta x}, \tag{2.40}
\]

\[
\frac{\partial^2 V_m}{\partial x^2} \approx \frac{V(x + \Delta x, y) - 2V(x, y) + V(x - \Delta x, y)}{\Delta x^2}. \tag{2.41}
\]
The choice of time step ($\Delta t$), space step ($\Delta x$) and diffusion coefficient ($D$) will directly affect not only the stability of a particular scheme but also the computational time it will take to solve. For an explicit finite difference solution to the monodomain model with isotropic diffusion, a standard linear stability analysis leads to the criterion that

$$\Delta t < \frac{1}{D} \frac{\Delta x^2}{2d}, \quad (2.42)$$

here $d$ is the dimension of the simulation. If the parameter choices do not satisfy this relationship, inaccuracies may arise.

### 2.5 Limitations of modelling

Despite computational modelling acts as a powerful tool for cardiac research, it does have disadvantages that we must realize. In fact, some of the disadvantages are intrinsic to models, and others emerge from choices made.

One of the most important limitations of modelling is how much physiological information the model can show by comparison with the real physiology. The mechanical activity of the heart is not included in purely computational models. Therefore, model validation based on the experimental measurements becomes extremely important. Secondly, a choice must be made. Choosing phenomenological models offer very fast solution times, but do so by sacrificing physiological details. Inversely, biophysically detailed models can offer more insight, but can be very slow to solve, especially for tissue and whole heart simulations. Moreover, extrapolation made by modelling might be wrong. Models based on incomplete data or instabilities assumptions are unlikely to produce acceptable results.
Chapter 3

Sinus node dysfunction due to reduced $I_f$

3.1. Introduction

Sick sinus syndrome (SSS) is defined as the intrinsic incompetence of the cardiac pacemaker, SAN to perform its pacemaking functions [105]. It denotes a collection of cardiac arrhythmias which includes disordered SAN automaticity, inability of SAN to drive the rest of the atrium (i.e., SAN exit block), sinus bradycardia, sinus arrest, slow SAN-atrium conduction, or alternating periods of bradycardia and tachycardia arrhythmias [20, 106, 108, 135]. From mild to severe, patients with SSS present symptoms with syncope, pre-syncope, palpitations, dizziness or sudden death [106, 136]. Approximately half of SSS patients have to be fitted with an electronic pacemaker [110].

Mechanisms underlying the pathogenesis for sinus node dysfunction (SND) in SSS have not been defined. SSS most commonly occur in elderly people [137]. It can also occur at any age of healthy people without any evident structural heart disease, but with genetic defects [109]. SSS associated with four different types of HCN4 gene mutations that codes for the hyperpolarisation-activated pacemaking channel (the $I_f$ channel) have been identified so far [59-62]. The first study was reported by Scholze-Bahr et al. [59], who identified the HCN4-573X mutation in congenital SSS patient with idiopathic sinus bradycardia and chronotropic incompetence. This mutation causes a heterozygous 1-bp deletion (1631delC) in exon 5 of the human HCN4. Functional analysis (electrophysiological patch studies on expressed channels) manifested that the mutation shifted the activation curve of $I_f$ channel to more
hyperpolarization potentials with a steeper slope in comparison with that of WT channels. Later on, a missense HCN4 mutation, the HCN4 D553N mutation with replacement of Asp (GAC) with Asn (AAC) at codon 553 in a patient with SND was identified by Ueda et al. [60]. Functional analysis revealed that this mutation reduced membrane channel expression resulting in a decreased $I_f$ current, together with a faster activation and slower deactivation kinetics, but without change to the voltage-dependence of the activation curve. Milanesi et al. [61] identified another HCN4 gene mutation, the S672R mutation (a replacement of serine 672 by arginine) in members of a large family with sinus bradycardia. Functional analysis showed that the mutation shifted the activation curve of $I_f$ channels to more hyperpolarisation potentials, together with a remarkable faster deactivation process. Most recently, Nof et al. [62] identified the HCN4 G480R (substitution of glycine to arginine at position 480 of the protein) in familial SSS patients. This mutation also shifts the activation curve of $I_f$ channel to more repolarization potentials with a slower activation kinetics.

As described above, the four HCN4 mutations (-573X, D553N, S672R, G480R) share in common a reduction in $I_f$. However, they are intrinsically different in their functional impacts on $I_f$ channels. Some of them reducing $I_f$ by altering the $I_f$ voltage-dependence of activation (i.e., HCN4-573X, S672R and G480R) and the other (D553N) reducing $I_f$ by reducing channel membrane expression. Therefore, these mutations may have different impacts on modulating the cardiac pacemaking activity, and thus, differentially affect the ability of the SAN to pace and drive the surrounding atrial muscle.

The expression of $I_f$ channels is not just constrained in the SAN region of the heart, therefore, reduction in $I_f$ may also be associated with other cardiac diseases such as progressive cardiac conduction system disease [60] in a similar way as a defective sodium channel current ($I_{Na}$). However, on the other hand, blocking $I_f$ by ivabradine has been suggested to slow heart rates for patients with heart failure or coronary
vascular diseases. Though preliminary clinical trials [138-141] have shown its promising safety, it is still unclear whether or not blocking $I_f$ by ivabradine may produce SND that is analogous to the functional impacts of HCN4 gene mutations in SSS as both share in common a reduced $I_f$.

It is well known that $I_f$ channels are modulated by both sympathetic and para-sympathetic nerve systems by interactions of cAMP with the cyclic nucleotide-binding domain of $I_f$ channels [43]. Activity of para-sympathetic systems slows the pacemaking ability of the SAN by acetylcholine (ACh), the neurotransmitter released by the vagal nerves upon stimulation. This is primarily via the actions of the ACh-activated potassium channel current ($I_{K,ACh}$) and inhibition of $I_f$ by ACh, and the later has been shown [88] to play an important facilitative role by reducing the ability of $I_f$ to curtail the chronotropic effect caused by activation of $I_{K,ACh}$, such that a depolarizing current reservoir provided by $I_f$ is reduced. Therefore, it is likely that such a negative chronotropic effect of vagal tone activity on SAN pacemaking activity will be amplified when the depolarizing $I_f$ channels are defected (shift the activation curve to more negative potential) by the HCN4 mutations or ivabradine, in a similar way to defected $I_{Na}$ due to SCN5A gene mutations [142].

Due to a lack of phenotypically accurate experimental models, the mechanisms by which the loss-of-function of $I_f$ arising from HCN4 mutations slows down the heart rate and impairs AP conduction across the SAN-atrium (i.e., the ability of the SAN to drive the surrounding atrium) have not been fully elucidated, neither possible pro-arrhythmic effects of ivabradine given that its action is to block $I_f$ channel. The aim of this study was to investigate by computer modelling of the functional effects of this mutation and consequences of the loss-of-function of HCN4 channel in generating SAN dysfunctions, and possible pro-arrhythmic effects of $I_f$ channel blocking mimicking the actions of ivabradine. Simulation predictions of the effect of ivabradine were then tested by in vitro experimentations on intact tissue of rabbit
sinoatrial node and atrium [80, 84].

3.2 Methods
The consequences of the impaired $I_f$ channel function due to several HCN4 mutations (HCN4-573X, D533N, S672R and G480R) in generating SND were investigated by using (i) previously developed electrophysiologically detailed mathematical models of the central and peripheral SAN cells [84], (ii) two-dimensional (2D) anatomical models of the intact SAN-atrium tissue, incorporating accurate single cell models of the SAN [84] and the right atrium (RA) [143], and histologically reconstructed tissue geometry [80], and (iii) multi-electrode extracellular potential recordings of the activation pattern from isolated intact rabbit SAN-atrium tissue to verify model predictions.

A full list of the model equations and parameters used for the central and peripheral SAN cells and RA cells under control conditions, HCN4 mutation conditions and ACh conditions, as well as 2D tissue model of the intact SAN-atrium, is provided in Appendix 2. The computational model was written in C program shown in Appendix 6.

3.2.1 HCN4 mutation model
In order to model HCN4 mutations (HCN4-573X, D533N, S672R and G480R), parameters of the $I_f$ current, were modified based on experimental data (Figure 3.1). Note that prior experimental data were recorded from cultured cells transfected with HCN4 genes, whereas our models are for the native current in rabbit SAN cells. Thus, in simulations we relatively shifted the steady-state activation curve by the same amount (-5.4, 0, -8.4 and -30mV for HCN4-573X, D533N, S672R and G480R respectively) as observed experimentally (Figure 3.1A) and implemented the same percentage changes to the activation time constants as observed experimentally (Figure 3.1B and C). We scaled the maximal $I_f$ channel conductance for variant HCN4
mutant channels to reproduce the normalised I-V relationship and the reduction in $I_f$ as observed experimentally (Figure 3.1D). The heterozygous HCN4 mutation was modeled by dividing $I_f$ into two components – one for the WT $I_f$ and another for mutant $I_f$. The WT component had a maximum conductance of 50% of control, whereas the mutant component had a maximum conductance of varied percentage of control. Parameters used for WT and mutant $I_f$ equations were listed in Table 3.1 and full equations are presented below.

The activation curves and the shift of the half activation voltages (Figure 3.1A) for HCN4 currents recorded under whole-cell conditions were obtained by standard activation and deactivation protocols and analyzed by the Boltzmann equation:

$$y = \frac{1}{1 + e^{\left(\frac{V - V_{0.5}}{s}\right)}},$$

where $y$ is the fractional activation, $V$ is the voltage in millivolts, $V_{0.5}$ is the half activation voltage in millivolts and $s$ is the inverse slope factor in millivolts. The shift of the half activation voltage and inverse slope factor were measured from the experiments.

The traces of average activation time shown in Figure 3.1B were fitted from experimental data. The formulae of activation time for the WT are given by:

$$\alpha_{WT} = e^{-\left(\frac{V+118.9}{26.63}\right)}$$

$$\beta_{WT} = e^{\left(\frac{V+45.13}{21.25}\right)}$$

$$\tau_{WT} = \frac{1000.0}{2.3\times(\alpha_{WT} + \beta_{WT})}.$$
The formulae of activation time for the S672R mutation are given by:

\[
\alpha_{S672R} = e^{-\left(V+12191\right)/30} \tag{3.5}
\]

\[
\beta_{S672R} = e^{\left(V+5813\right)/1625} \tag{3.6}
\]

\[
\tau_{S672R} = \frac{1000.0}{2.3 \times (\alpha_{S672R} + \beta_{S672R})}, \tag{3.7}
\]

where \( \tau_{WT} \) and \( \tau_{S672R} \) represent the time constant of WT and S672R mutation respectively.

**Table 3.1 Values for mutation parameters**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Shift in ( V_{0.5} ) (mV)</th>
<th>Slope factor</th>
<th>Normalized current density</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCN4-573X</td>
<td>-5.8</td>
<td>7.98</td>
<td>1</td>
</tr>
<tr>
<td>D533N</td>
<td>0</td>
<td>10.2</td>
<td>0.125</td>
</tr>
<tr>
<td>S672R</td>
<td>-8.4</td>
<td>11.2</td>
<td>0.9</td>
</tr>
<tr>
<td>G480R</td>
<td>-30</td>
<td>10.2</td>
<td>0.111</td>
</tr>
</tbody>
</table>
Figure 3.1 Simulated effect of the HCN4 mutations on \( I_f \) channel current. A: Steady-stated activation curves for WT channels (black line), HCN4-573X (blue line), D553N (black line), S672R (red line) and G480R (green line) mutation channels. B: Time constant curves of activation and deactivation for WT channels (Black line) and the S672R (red line) mutant channels. C: Slope factors of steady-state activation curves – simulation and experimental data. D: Normalized (to WT \( I_f \) channels) I-V relationship. E: Time traces of simulated \( I_f \) channel currents during voltage clamps for WT and mutant channels.
3.2.2 Two-dimension slice model (This section refers to my colleague Butters et al. ’s paper [142])

Using the standard nonlinear cable, the dynamics of initiation and conduction of electrical APs in the heart can be modeled as in Eqn. 2.20. Here, $V$ is the membrane potential, $t$ is the time in second, $\nabla$ is the spatial gradient operator, $D$ is the diffusion coefficient that characterises electrotonic spread of voltage via gap junctions, $C_m$ is the cell membrane capacitance and $I_{tot}$ is the total ionic current. Electrophysiologically-detailed models for $I_{tot}$ – and hence, single cell APs – have been developed for rabbit SAN [84] and RA [143] cells.

The intact SAN-atrium tissue model was based on the histologically reconstructed [80] geometry of a single slice of the rabbit SAN and RA that cut from the atrial muscle of the crista terminalis and the intercaval region with central and peripheral SAN areas (Figure 3.2). The geometry was presented with a high spatial resolution (40 μm, which corresponds to 2 to 4 diameters of a cardiac myocyte). i.e. regular Cartesian grid of 210 × 45 nodes. For each node, using immunohistochemistry mapping data [80] to identify whether it belongs to the SAN or RA cell type based on. We used Zhang et al. equations [84] to model the central and peripheral SAN cells, and used the Aslanidi et al. equations [143] to model the atrial cells, with each model producing different AP morphology (Figure 3.2A).

The experimentally observed non-conductive region (“block zone”) which is next to the SAN towards the atrial septum was also introduced in this 2D model (Figure 3.2A). The cells in this region have low excitability and $I_{tot}$ in Equation 3.8 was described as:

$$I_{tot} = I_B = G_m (V - V_R),$$

(3.8)

here $I_B$ is the passive membrane current in block zone, $G_m$ is the membrane
conductance, and \( V_R \) is the resting membrane potential. \( V_R \) and \( G_m \) were the value which could be considered to correspond to the resting potential and membrane conductance of the surrounding atrial tissue.

In the 2D tissue model, the AP conduction was caused by intercellular electrical coupling via gap junctions. It was modeled through the diffusion coefficient, \( D \). The regional differences were also considered in the model as well as the gap junctional coupling between the SAN centre, periphery and atrial tissue as observed experimentally [80] and the electrophysiological properties. \( D \) is the spatial gradient (Figure 3.2F) which has been introduced as in the previous study [144]:

\[
D(x) = D_c + D_p \left( \frac{1.0}{1.0 + e^{-0.5(x-x_1)}} + \frac{1.0}{1.0 + e^{0.5(x-x_2)}} \right),
\]

(3.9)

where \( x \) is the horizontal coordinate across the 2D slice, \( x_1 \) and \( x_2 \) are assumed to correspond to the positions of the SAN boundaries within the tissue, and \( D_c \) and \( D_p \) are the diffusion coefficients of central and peripheral SAN cells, respectively.

The regional differences in the cellular electrical properties of SAN cells were also considered in the 2D model. We modeled the gradient distributions of current densities from the central to the peripheral SAN by correlating them to cell membrane capacitance (\( C_m \)), i.e small in the centre and large in the periphery [84, 144]. The spatial gradient distribution of \( C_m \) (Figure 3.2E) was described as:

\[
C_m(x) = C_m^c + C_m^p \left( \frac{1.0}{1.0 + e^{-0.1(x-x_1)}} + \frac{1.0}{1.0 + e^{0.1(x-x_2)}} \right),
\]

(3.10)

here \( C_m^c \) is the capacitances of central SAN cells and \( C_m^p \) is the capacitances of peripheral cell.
As found experimentally, spatial variations in all ion channel conductances were described as a function of $C_m$ [84]:

$$g_Z(x) = \frac{C_m(x) - C_m^C}{C_m^P - C_m^C} g_Z^C + \frac{C_m^P - C_m(x)}{C_m^P - C_m^C} g_Z^P,$$  \hspace{1cm} (3.11)

where $g_Z(x)$ is the conductance of one of the currents $Z \in \{\text{Na, Ca, K, ...}\}$, and $g_Z^C$ and $g_Z^P$ are the conductances for central and peripheral SAN cells, respectively.
Figure 3.2 Model of the rabbit SAN and surrounding atrial tissue [142]. A: distribution of cell types throughout the 2D tissue slice (SAN centre-red, SAN periphery-yellow, RA-blue, Block Zone-green), insets show respective single cell APs. B: spatial distribution of the activation time during normal AP conduction through the 2D slice (rainbow palette), lines and number show isochrones of the activation. C: activation tie profile through the middle of the 2D slice, an open circle shows respective experimental data. D: AP profile during conduction through the slice. E: gradient in cell capacitance along the slice. F: gradient in the diffusion coefficient along the slice.
3.2.3 ACh effects (This section refers to my colleague Butters et al. ’s paper [142])

With the effect of ACh, The heart rate will decrease by slowing down the spontaneous diastolic depolarization rate of the SAN. ACh-activated K$^+$ current, $I_{K,ACh}$, which has been introduced in Chapter 1 was introduced as part of $I_{tot}$ [145]:

$$I_{K,ACh} = g_{K,ACh} \left( \frac{[K^+]_o}{10 + [K^+]_o} \right) \left( \frac{V - E_K}{1 + e^{(V - E_K - 140)F/2.5RT}} \right), \quad (3.12)$$

where $[K^+]_o$ is the extracellular K$^+$ concentration, $g_{K,ACh}$ is the maximum channel conductance, $E_K$ is the equilibrium potential for K$^+$ and $F$, $R$ and $T$ have their usual meanings. $g_{K,ACh}$ which is ACh dose-dependent is the conductance as below:

$$g_{K,ACh} = g_{K,ACh,max} \frac{[ACh]^n_{K,ACh}}{K_{0.5,K,ACh} + [ACh]^n_{K,ACh}}, \quad (3.13)$$

where $j$ and $k$ are inactivation variables, $g_{K,ACh,max}$ is the maximum value of $g_{K,ACh}$, $n_{K,ACh}$ is the Hill coefficient, $[ACh]$ is the ACh concentration, and $K_{0.5,K,ACh}$ is the ACh concentration that produces half-maximal activation of $g_{K,ACh}$. In both the SAN and atrial cell models, $I_{K,ACh}$ is included with $g_{K,ACh,max}$ described by the same spatial gradient function as the other ion channel conductances, as described in Equation 3.12.

ACh also effects two other currents, i.e. the L-type Ca$^{2+}$ current, $I_{Ca,L}$ and the pacemaker current, $I_f$ by shifting the activation curve of the hyperpolarization-activated current to more negative potentials.

For $I_{Ca,L}$, the fractional block, $b$, is given by [145]:
\[ b = b_{\text{max}} \frac{[ACh]}{K_{0.5,\text{Ca}} + [ACh]}, \]  
\[ (3.14) \]

where \( K_{0.5,\text{Ca}} \) is the ACh concentration that produces a half maximal block of \( I_{\text{Ca,L}} \) and \( b_{\text{max}} \) is the maximum fraction of \( I_{\text{Ca,L}} \) block. The shift in \( I_f \) activation curve is given by [145]:

\[ s = s_{\text{max}} \frac{[ACh]^{n_f}}{K_{0.5,f}^{n_f} + [ACh]^{n_f}}, \]
\[ (3.15) \]

where \( n_f \) is the Hill coefficient, \( s_{\text{max}} \) is the maximum shift of the \( I_f \) activation curve and \( K_{0.5,f} \) is the ACh concentration that produces a half maximal shift of the activation curve of \( I_f \).

### 3.2.4 Isolated tissue experiments

Six male adult rabbits (2 to 3 months old) were used for experimental study. The sinoatrial preparations were set up as described previously from animals killed by anesthetic overdose with venous injection of sodium phenobarbital (in accord with UK Home Office Legislation) [18]. After excision of the SAN and surrounding atrial muscle, the preparation was placed endocardial surface upwards in a tissue bath and superfused with modified Tyrode’s solution (in mmol/L: NaCl 120, NaHCO₃ 25.2, NaH₂PO₄ 1.2, MgCl₂ 1.3, glucose 5, KCl 4.0, CaCl₂ 1.8, gassed with 95% O₂/5% CO₂) at 37°C and at a flow rate of ≈5 mL/min. Electric signals were obtained from the surface of this preparation by apposition of a custom-made extracellular multielectrode array that allowed electrograms to be monitored at multiple sites in the tissue as excitation passed under the array. The electrode array held 30 separate silver electrodes in a 5×6 configuration. The inter-electrode distance was 0.55 mm; thus the total array dimensions were approximately 3 mm length and 4 mm width. The electrodes were Teflon coated silver wires with a coated outer diameter of 0.2 mm and an inner diameter of 0.125 mm (Science Products, Millville, NJ, USA). The spacing
of the electrodes was obtained with 0.33 Teflon coated silver wires. The silver tips were chlorided. The 30 recording electrodes were connected through shielded wires to a 32-channel amplifier (SCXI-1102C, National Instruments Corporation UK Ltd, Newbury, UK). The sampling frequency for each channel was set at 1 kHz. The signals were continuously sampled and stored on disk and displayed on screen using a custom-developed program, written in Labview 7.0 (National Instruments Corporation UK Ltd). Experiments were performed under the conditions of control, application of ivabradine (IVB) (0.2 μmol/L, 1.5 μmol/L, 10 μmol/L and 20 μmol/L; IC$_{50}$ ≈0.1μmol/L [146]) or a nondegrading ACh equivalent, carbachol (CCh) (200 nmol/L; IC$_{50}$≈100 nmol/L [147]), and a combination of IVB (0.2 μmol/L, 1.5 μmol/L, 10 μmol/L and 20 μmol/L) and CCh (200 nmol/L). Propagation maps were then derived during off-line analysis. The signals were displayed on screen in sets of 8 to 16 electrograms. The activation time was denoted as the point of maximal negative slope and marked with a cursor. After marking all significant waveforms in all leads, the activation times were then displayed in a grid representing the layout of the original recording array. All activation times, in milliseconds, were related to the timing of the first detected waveform.

3.2.5 Statistical analysis

All data are reported as means ± S.E.M. Comparisons were performed by a one-way analysis of variance (ANOVA). A value of p<0.05 was considered significant difference.

3.3 Results

3.3.1 Mutation effects on single cells

Firstly, the functional effects of the HCN4 mutations (HCN4-573X, D533N, S672R and G480R) on pacemaking APs at the single cell level under control and ACh conditions was investigated. Figure 2A-C show simulated APs with the WT and
S672R mutant channels, along with underlying $I_f$ and $I_{CaL}$ channel currents in the central (left panels) and peripheral (right panels) cells. These simulations illustrate that the S672R mutation slowed down pacemaking APs in both of the central (Figure 3.3Ai) and the peripheral cell (Figure 3.3Aii) models. The measured pacemaking cycle length (PCL) increased from 338 ms to 357 ms (by 5.6%) for the central cell, and from 170 ms to 190 ms (by 11.8%) for the peripheral cell models. The slowing down in SAN pacemaking APs as manifested by an increase in PCL in both of the central and peripheral cell models supports the hypothesis of Milanesi et al. [61] on that the S672R mutation impairs $I_f$ channels, resulting in less inward current during the diastolic depolarization phase that slows down the heart rate. In simulation, the S672R mutation showed a greater effect on increasing PCL in the peripheral cell model than in the central cell model. Such a regional different response to reduced $I_f$ arising from the S672R mutation is similar to experimental observations of Nickmaram et al. [148], who showed that blocking $I_f$ by $\text{Cs}^{2+}$ caused an increased PCL by 7% in central and 24% in peripheral tissue of rabbit SAN. In the model, the resultant slowing down of the pacemaking was primarily due to the mutation-induced decrease in $I_f$ (Figure 3.3B), as other major underlying currents (such as $I_{CaL}$, $I_{Na}$ (not shown) and $I_{Kf}$ (not shown)) were not greatly affected (Figure 3.3B and C). Functional effects of other considered HCN4 mutations pacemaking APs of central and peripheral cells were similar to those of the S672R mutation, and were summarised quantitatively in Figure 3.3D. All of these HCN4 mutations caused a slowing down in pacemaking APs, in both central and peripheral cells and with a more dramatic effect in the peripheral cell. Among the four HCN4 variants, the D553 (reducing membranous channel expression) and the G480 (the one shifting the most of the activation curve leftward) have relatively greater impacts on slowing pacemaking APs as both have greater effects on reducing $I_f$ current density.
Figure 3.3 Effect of HCN4 mutations on pacemaking APs (from t=0). Simulated APs and underlying currents for the central (left panels) and the peripheral (right panels) SAN cells. (A) Effects of the HCN4 S672R mutation on APs. (B) Time traces of $I_f$. (C) Time traces of $I_{Ca,L}$. (D) Effects of the HCN4-573X, D553N, S672R and the G480R mutations on the cycle lengths (PCLs) of APs.
3.3.2 ACh effects on single cells under WT and HCN4 mutations

Figure 3.4 shows the simulated effects of $5 \times 10^{-8}$ mol/L ACh on pacemaking APs under WT and the HCN4 S672R mutation condition. Under the WT condition, ACh slowed down pacemaking by increasing the PCL in both central and peripheral SAN cells, with a greater effect on the central cell model as seen previously [145]. At a concentration of $5 \times 10^{-8}$ mol/L, ACh resulted in the PCL increasing from 338 ms to 445 ms (Figure 3.4A), an increase of 31.7% for the central SAN cell, and from 170 ms to 191 ms (Figure 3.4B), an increase of 12.3% for the peripheral SAN cell. In simulations, the central model was more sensitive to ACh than the peripheral model, which is consistent with experimental observations [148].

The negative chronotropic effect of ACh was due to an integral action of reduced $I_f$ (Figure 3.4B), $I_{CaL}$ (Figure 3.4C) and ACh-activated $I_{K,ACH}$ (Figure 3.4D). With the S672R mutation, the negative chronotropic effect of ACh was amplified (Figure 3.4A). The augmented negative chronotropic effects of ACh with the mutant $I_f$ channel can be attributed to the reduced inward depolarizing $I_f$ during the diastolic depolarization period, which counter-balances the ACh-activated outward repolarizing current, $I_{K,ACH}$, leading to a greater slowing of the diastolic depolarization phase. Simulations with other HCN4 gene mutations showed similar results. In two cases (D553N and G480R), simulated addition of ACh even ceased the spontaneous pacemaking APs in the peripheral cell model (not shown) due to $I_f$ in the two cases was almost completely varnished. Figure 3.4E summarized the measured PCLs of central (Figure 3.4Ei) and peripheral (Figure 3.4Eii) cell models for WT and all considered HCN4 mutation conditions with and without ACh. In the central cell model, with a combined action of ACh and HCN4 mutations, the measured PCL was increased by 47.1%, 66.3%, 38.2% and 125.8% respectively (Figure 3.4Ei) for the HCN-573X, D553N, S672R and G480R mutation as compared to the WT condition. In the peripheral cell model, the measured PCL was increased by 24.6% and 22.1% for the -573X and S672R mutations respectively. In other two cases (D553N and G480R), the measured PCL
was infinitely long as the pacemaking APs in the peripheral models were abolished.

Figure 3.4 Effect of ACh on the SAN pacemaking APs with HCN4 gene mutations. Simulated APs and underlying ionic channel currents for the central (i) and the peripheral (ii) SAN cells. (A) Effect of the S672R mutations and ACh ([ACh]=5×10^{-8} mol/L) on APs. (B) Time traces of $I_{f}$. (C) Time traces of $I_{Ca,L}$. (D) Time traces of $I_{K,ACh}$. (E) Effects of HCN4 gene mutations and ACh on PCLs.
The dose-dependent effects of ACh on pacemaking APs with the WT and mutant \( I_f \) channels were also simulated. Figure 3.5 presents results obtained from the central (Figure 3.5Ai-Aiii) and the peripheral (Figure 3.5Bi-Biii) cell models for different ACh concentrations with the WT and the S672R mutant \( I_f \) channels. It shows that increase in ACh concentration led to a greater increase in PCL, and the negative chronotropic effect of ACh was amplified by the S672R mutation at all ACh concentrations. Such an augmented negative chronotropic effect of ACh is also indicated by the leftward shift of the dose-dependent effect of ACh on pacemaking APs by the mutation for the central (Figure 3.5D) and the peripheral (Figure 3.5E) cells. Similar results were obtained for all other HCN4 mutations, indicating a more suppressive effect of ACh on SAN cells incorporating ‘mutant’ \( I_f \) (Figure 3.5D and E). It is of note that among the four considered HCN4 mutation, ACh has greatest suppressive effects in the G480R and the D553N mutation conditions, with greater effects on the peripheral than on the central cell models.

Figure 3.6 shows the effects of various ACh concentrations on the characteristics of pacemaking APs for WT cell (open symbols) and the S672R mutation cell models (filled symbols). Figure 3.6A-D show the changes of AP amplitude (Figure 3.6A), AP duration (Figure 3.6B; measured at -30mV), maximal diastolic potential (Figure 3.6C), cycle length (Figure 3.6D) and the maximal upstroke velocity (Figure 3.6E and F). All of these changes in AP characteristics except for the maximal upstroke velocity illustrated that the central cell shows more sensitive to ACh than the peripheral cell, and such sensitivity is augmented by the S672R mutation. However, for the maximal upstroke velocity, the peripheral cell (Figure 3.6F) shows more sensitive to ACh than the central cell (Figure 3.6E), and such sensitivity is also augmented by the S672R mutation. Note that the maximal upstroke velocity of peripheral SAN cells plays an important role for assuring successful AP conduction from the SAN to the surrounding atrial muscle. Such a dramatic reduction of the maximal upstroke velocity of peripheral APs by the combined action of the mutant \( I_f \) channel and ACh may lead to a conduction failure at the border between the SAN and atrium, leading to conduction
exit block – a common feature of SSS. Other HCN4 gene mutations show similar
effects on modulating the effects of ACh on altering AP characteristics (data not
shown).
Figure 3.5 Dose-dependent effect of ACh on SAN pacemaking with HCN4 mutations. 
Ai-Aiii: Effects of varying ACh concentrations on central SAN APs with WT (black line) and mutant (red line) \( I_f \) channels. Bi-Biii: Effects of varying ACh concentrations on peripheral SAN APs with WT (black line) and mutant (red line) \( I_f \) channels. D: Dose-dependent effects of ACh on PCLs of central SAN cells. E: Dose-dependent effects of ACh on PCLs of peripheral SAN cells.
Figure 3.6 Dose-dependent effects of ACh on the characteristics of pacemaking APs for central and peripheral SAN cells. (A): AP amplitude. (B) AP duration. (C) Maximal diastolic potential. (D) PCL. (E) Maximal upstroke velocity of APs for central SAN cells. (F) Maximal upstroke velocity of APs for peripheral SAN cells.
3.3.3 Effects in 2D tissue

Figure 3.7 shows the functional consequences of the mutations on AP conduction across the intact SAN-atrium under control and ACh conditions by using 2D intact model of intact SAN and atrial tissue. In the figure, profiles of APs recorded from representative cells across the SAN-atrium model were presented in spatial (running horizontally) and temporal (running vertically) scales as in our previous study. Figure 3.7A presents the normal conduction in the normal tissue (with the WT channel and no ACh). Simulation of ACh addition (1.5 × 10⁻⁸ mol/L) slowed down the pacemaking rate (i.e., led to an increase in the PCL) (Figure 3.7B). Figure 3.7C shows the AP profile with the HCN4-573X mutation. Without ACh, the mutation slowed down the pacemaking rate, but the SAN was still able to drive the atrium. Addition of ACh further slowed down SAN pacemaking AP, and caused a conduction block in the direction towards the septum (Figure 3.7D) where cells have less excitability [146]; however, the AP conduction in the direction towards the CT was sustained. Figure 3.7E shows the effects of the D553N mutation. This mutation caused a reduction of SAN pacemaking rate by 33.3%. With addition of ACh (1.5x10⁻⁸ mol/L), the SAN pacemaking activity was further suppressed with a much slower pacemaking rate. The SAN was able to drive the atrium through the CT, but AP conduction towards the septum was blocked (Figure 3.7F). This may explain why the patients with the D553N mutation showed severe bradycardia and recurrence of syncope. Figure 3.7G shows the AP profile with the S672R mutation. This mutation also slowed down the SAN pacemaking rate, which was further slowed down by ACh though the SAN was able to drive the atrial muscle (Figure 3.7H) in both of the CT and the septum directions. Figure 3.7I shows the effects of the G480R mutation. This mutation caused a reduction of SAN pacemaking rate by 66.7%. With addition of ACh (1.5x10⁻⁸ mol/L), the SAN pacemaking activity was suppressed leading to SAN arrest [Figure 3.7J]. This suggests that the G480R mutation might be asymptomatic in absence of ACh (severe sinus bradycardia without sinus rest or block as observed in patients), but with ACh the G480R mutation could potentially impair the ability of the SAN,
leading to SAN arrest.

Detailed simulations were also performed to investigate the effects of the HCN4 mutations and ACh on the characteristics of AP initiation and conduction in the intact SAN-atrium tissue model. Figure 3.8A presents the measured activation timing sequence across the tissue with the WT and S672R mutant channels, with and without ACh. In the model, ACh or the S672R mutation alone increased the time required for the AP to propagate from the centre of the SAN to the atrium in both direction of the CT and the septum. With a combined action of the mutation and ACh, the time taken for the AP to reach the atrial septum was increased by 41.2%. Results with the G480R mutation are presented in Figure 3.8B for comparison purposes as it caused SAN arrest. The G480R mutation alone or ACh alone also resulted in a dramatic increase in the AP conduction time. But with a combined action of the mutation and ACh, AP conduction failed in both of the directions. Furthermore, a combined action of the G480R mutation and ACh caused a dramatic delay in SAN AP initiation. After a short period of transition, the AP initiation was abolished, leading to SAN arrest.

The computed AP conduction velocity in SAN-atrium tissue simulations also reflected the above observations. In the normal tissue with the WT channel, addition of ACh reduced the conduction velocity by ~12% from SAN to atrium (on average) (Figure 3.8C). In tissue with the HCN4 mutant $I_f$ channel, the conduction velocity was decreased by ~14.4% in the SAN (on average) for the S672R mutation (Figure 3.8C). Subsequent addition of ACh to the tissue with the S672R mutant channel further reduced the AP conduction velocity in the SAN, and resulted in a decrease in the conduction velocity by ~20.3% in the SAN (on average). In tissue with the G480R mutant channel, the AP conduction velocity was decreased by ~28.6% in the SAN (on average) (Figure 3.8D); subsequent addition of ACh caused a conduction block in both of the CT and the septum direction, before the SAN pacemaking was abolished (Figure 3.8D).
Changes in the measured conduction velocity across the SAN were attributed to the changed maximum upstroke velocity (dV/dt\text{max}), as shown in Figure 3.8E and F. In the normal tissue with the WT channel, simulated addition of ACh reduced dV/dt\text{max} in both the RA and the SAN. In tissue with the S672R and G480R mutant channels, there was a decrease in dV/dt\text{max} throughout the tissue, not only in the periphery of SAN, but also in the centre of the SAN. Subsequent addition of ACh further reduced dV/dt\text{max} in the SAN. With the G480R mutant channels, a more dramatic effect on decreasing dV/dt\text{max} in the SAN periphery, primarily at the border between the SAN and the atrium was observed as compared to the S672R mutation condition. This resulted in the SAN failing to drive the atrium, leading to a SAN exit block. Combined effects of the G480R mutation and ACh also reduced dV/dt\text{max} markedly in the SAN region that eventually abolished SAN pacemaking (Figure 3.8F) and dV/dt\text{max} was zero throughout the SAN (data not shown).
Figure 3.7 Effects of ACh and HCN4 mutations on AP conduction. AP profiles in the 2D tissue with [ACh]=0 (left) and [ACh]=1.5×10⁻⁸ mol/L (right) are shown. A and B: tissue with the WT channel. C and D: tissue with the HCN4-573X channel. E and F: tissue with the D553N channel. G and H: tissue with the S672R channel. I and J: tissue with the G480R channel.
Figure 3.8 Effects of ACh and the HCN4 mutations on AP conduction parameters. Activation times with the S672R mutation and ACh (A) and the G480R mutation (B) as compared to the WT channel; conduction velocities with the S672R mutation and ACh (C) and the G480R mutation (D) as compared to the WT channel; dV/dt max with the S672R mutation and ACh (E) and the G480R mutation (F) as compared to the WT channel. [ACh]=1.5×10⁻⁸ mol/L.
3.3.4 Effects of reduced $I_f$ by altering channel properties and by ivabradine

Experimental data have shown a wide range of the mutation-induced shift in the steady state of the $I_f$ channel activation curve among the -573X, D535N, S672R and S480R mutations. Besides, these HCN4 mutations in patients are heterozygous. In order to investigate the general effect of a negative shift of the $I_f$ channel activation curve, further studies were performed with a linear shift of the activation curve from 0 to -30 mV. Results are shown in Figure 3.9. Figure 3.9A and B present the measured PCL for the central and peripheral cell models, respectively. For example, in the central cell model, shift by -10 mV can induce a prolongation of PCL by 5.3% in comparison with WT. In both models, the measured PCL increases monotonically with the increased extent of the negative shift of the channel activation curve and such a negative effect was augmented by ACh.

Effects of dose-dependent blocking of $I_f$ by ivabradine (from 0.1 to 100 μmol/L) were also studied by using single cell and 2D tissue models. Figure 3.9C presents the dose-dependent fractional blocking of $I_f$ implemented in the models and its comparison to experimental data [149]. In both central (Figure 3.9D) and peripheral (Figure 3.9E) cell models, the measured PCL increased with increase of ivabradine concentration. Addition of ACh enhanced the negative effects of ivabradine, with greater effects at high doses. Similar results were also observed from the 2D tissue model (Figure 3.9F). Moreover, at high doses (i.e., over 100 mol/L) of ivabradine that blocks $I_f$ over 90%, without ACh, the SAN was pacing and driving the surrounding atrium. However, with ACh (1.5 x 10^{-8} mol/L), the pacemaking APs of the SAN was abolished, leading to AP conduction failure and SAN arrest.
Figure 3.9 Effects of a linear shift of the steady-state activation curve and dose-dependent block of $I_f$ by ivabradine on PCLs with ([ACh] = $1.5 \times 10^{-8}$ mol/L; closed circles) and without ACh ([ACh] = 0; open circles). A and B: Effects of a linear-shift of the steady-state activation curve of $I_f$ channels on PCLs for central (A) and peripheral (B) cell models. C: Dose dependent block of $I_f$ by ivabradine in simulation (solid line) in comparison with experimental data [149]. D and E: Simulated dose-dependent effect of blocking $I_f$ by ivabradine in the central (D) and the peripheral (E) cell models. F: Simulated dose-dependent effect of blocking $I_f$ by ivabradine in the 2D model.
3.3.5 Experimental validation

Multi-electrode extracellular recordings of AP initiation and conduction pattern in isolated rabbit SAN-atrium tissue were performed experimentally to verify simulation results with the 2D tissue slice model (Figure 3.10). Due to the lack of phenotypically appropriate rabbit models, defective \( I_f \) by the HCN4 variants was mimicked by reducing \( I_f \) through the application of ivabradine. Figure 3.10 shows the electrical activation maps of the intact SAN-atrium tissue reconstructed from extracellular potentials recorded from the endocardial surface of the SAN-atrium preparation (see Methods). In control, ivabradine produced an increase in pacemaking cycle length (PCL), which was dose-dependent - higher concentration of IVB resulted in a greater PCL increase. This is consistent with simulation results (Figure 3.9). However, combined application of IVB and 200 nmol/L CCh resulted in a much more substantial increase of PCLs than IVB did alone. In 2 out of 6 tissue preparations, sinus arrest was observed with combined application of ivabradine ([IVB]= 20 \( \mu \)mol/L) and CCh (200 nmol/L). This is also in good agreement with the simulation results (Figure 3.9), i.e., application of ACh enhanced the effect of block of \( I_f \) on pacemaking and had risk of stopping pacemaking. Correspondingly, AP conduction and activation patterns were also altered by IVB and ACh. In control, the impulse was generated in the SAN centre and propagated toward the RA with an average AP conduction velocity in the direction transverse to the CT of 0.23±0.02 m/s (n=6) (Figure 3.10Ci). Application of 1.5\( \mu \)mol/L and 10\( \mu \)mol/L IVB slowed the AP conduction velocity to 0.168±0.003m/s (n=6) and 0.145±0.007m/s (n=6) respectively (Figure 3.10Cii and Ciii). Application of CCh (200 nmol/L) also slowed pacemaking and AP conduction. In addition, it caused a shift in the leading pacemaking site. Compared with application of IVB alone, combined actions of IVB and CCh not only resulted in an even larger increase in pacemaking cycle length (Figure 3.10B), but also a much more substantial slowing of the AP conduction velocity (Figure 3.10Dii and Diii). Figure 3.11 and Table 3.2 summarized quantitatively the effects of IVB and CCh on PCL and averaged SAN conduction velocity on intact rabbit SAN-atrium.
It is also of note that applications of IVB and CCh also produced pacemaking alternans, during which the measured PCL alternant between large and small values (Figure 3.12 and 3.13). In some of species, multiple leading pacemaking sites were also observed (Figure 3.14). Irregular heart beats either arising from pacemaking alterans or multiple leading pacemaking sites is also a feature of SND.

Fig.3.10 Effect of IVB, CCh and their combination on PCLs and AP initiation and conduction in rabbit SAN-atrium tissue. A and B: representatives of surface ECG under various conditions. C and D: isolated SAN-atrium tissue with superimposed activation maps. IVC indicated inferior vena cava; SVC, superior vena cava. The SAN-leading pacemaking site in control is shown with an asterisk.
Figure 3.11 Effect of IVB, CCh and their combination on PCLs and AP conduction velocity if in rabbit SAN. (A) PCL measured under control and ivabradine. (B) Dose-dependent effect of ivabradine on PCLs. (C) PCL measured under control and CCh. (D) PCL measured with CCh together with various concentrations of ivabradine. (E) Comparison of dose-dependent effects of ivabradine on PCL with and without CCh. The linear CCh+ IVB donates the summation of PCLs measured with CCh and IVB condition alone. (F) Effects of CCh and ivabradine of AP conduction velocity across the SAN.
### Table 3.2 Summary of statistically significant changes (P<0.05) in isolated tissue experiments

<table>
<thead>
<tr>
<th></th>
<th>Cycle Length(ms) (n=5-6)</th>
<th>Increase in CL vs control (%)</th>
<th>Conduction velocity (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>428.7±50.6</td>
<td>0</td>
<td>0.230±0.028</td>
</tr>
<tr>
<td>1.5μM IVB</td>
<td>514.8±35.3</td>
<td>20.0</td>
<td>0.168±0.004</td>
</tr>
<tr>
<td>10μM IVB</td>
<td>564.1±45.6</td>
<td>31.8</td>
<td>0.145±0.007</td>
</tr>
<tr>
<td>20μM IVB</td>
<td>645.5±71.8</td>
<td>50.7</td>
<td>0.113±0.011</td>
</tr>
<tr>
<td>200 nM CCh</td>
<td>498.4±48.8</td>
<td>16.3</td>
<td>0.135±0.020</td>
</tr>
<tr>
<td>200nM CCh+1.5μM IVB</td>
<td>641.5±65.4</td>
<td>49.6</td>
<td>0.110±0.014</td>
</tr>
<tr>
<td>200nM CCh+10μM IVB</td>
<td>738.8±71.2</td>
<td>72.3</td>
<td>0.080±0.014</td>
</tr>
<tr>
<td>200nM CCh+20μM IVB</td>
<td>Abolished</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 3.12 Representative recordings of surface ECG obtained by oesophageal pacing in anaesthetized rabbit. Effects of various concentrations of IVB on PCLs were showed to compare with control condition. Effects of combining IVB and 200 nmol/L CCh on PCLs were also illustrated to compare with application of IVB alone. PCL alternans showed up after application of 20 μmol/L IVB or 200 nmol/L CCh.
Figure 3.13 A: The distribution of irregular PCLs with application of 20 μmol/L IVB. B: Recording of ECG corresponding to A.

Figure 3.14 Multiple leading pacemakers with application of combining 10 μmol/L IVB and 200 nmol/L CCh.
3.4 Discussion

In this study, we have investigated the functional impacts of HCN4 mutations in impairing the ability of the SAN to pace and drive the surrounding atrial muscle. Our major findings are as follows: (1) At the cellular level, $I_f$ reduction due to the HCN4 mutations slowed down pacemaking in both of the central and peripheral cell models, with a greater effect on the latter. At the intact SAN-atrium tissue level, these mutations also slowed down the pacemaking of the SAN. In addition, they compromised the AP conduction across the SAN-atrium, leading to a possible sinus arrest or SAN exit block. As slowing down in SAN pacemaking and impaired AP conduction are major features of SSS, our simulation results provide evidence substantiating the causative link between familial SSS and the identified HCN4 gene mutations. (2) The bradycardiac effects of the HCN4 gene mutations are likely to be amplified by vagal nerve activity: simulated addition of ACh to the tissue with a mutant channel not only slowed down pacemaking and AP conduction, but also compromised the ability of the SAN to pace and drive the atrium, leading to sinus arrest and SAN exit block that was not observed with the mutations or ACh alone. (3) The considered four HCN4 mutations show common features in impairing the pacemaking ability of the SAN and the AP conduction across the SAN-atrium, but have differences in affecting the ability of the SAN to drive the surrounding atrium, especially when ACh is considered. This is due to the different impacts of each of the individual HCN4 gene mutations on altering $I_f$ channel properties. (4) The functional impacts of ivabradine, the $I_f$ channel blocker, are analogous to those of the considered HCN4 gene mutations at both of the cellular and the multicellular tissue levels on impairing the SAN pacemaking ability and the AP conduction across the SAN-atrium. As such the bradycardiac effects of ivabradine are also amplified by ACh, leading to possible sinus arrest and SAN exit block that is pro-arrhythmic. (5) Our simulation results are supported by experimental data, collectively indicating an important role of defective $I_f$ channels on impairing cardiac pacemaking, which is accentuated by the cholinergic activity. Taken together, these findings enhance the current understanding
of the mechanistic links between the studied HCN4 gene mutations and the resultant anomalies of cardiac pacemaker. They also provide novel insights towards possible pro-arrhythmic effects of ivabradine that is used for controlling the heart rates of patients with heart failure [150-152] and coronary circulation diseases [153-154].

**HCN4 mutations and pacemaker abnormalities**

At the isolated single cell level, our study has shown that all the considered HCN4 mutations slowed down pacemaking APs in both central and peripheral SAN cells. Their effect was greater on the peripheral cells than the central SAN cells that normally initiate and control the heart rhythm. At the intact SAN-atrium tissue level, these mutations slowed down the intact SAN pacemaking rate, with effect much greater than in the isolated central and peripheral cells. For example, the S672R mutation increased the PCL by 5.6% and 11.8% for the central and the peripheral cell models respectively, but increased the PCL of the intact SAN by ~23%. The greater functional impacts of the HCN4 gene mutations on slowing pacemaking AP rate can be attributed to the electrotonic interaction between the SAN and the atrium. Due to a more hyperpolarized resting potential (more negative than the maximal diastolic potential of the SAN) and lack of pacemaking activity, the atrium acts as an electrical load suppressing the pacemaker activity of the SAN [155]. Such a suppressive action is not just located in peripheral SAN cells at the border between the SAN and the atrium, but can also be mapped onto the central region of the SAN through cell-to-cell electrical coupling, although its effects here are weaker than in the periphery. With such a suppressive action from the atrium, the effect of the HCN4 gene mutations on impairing SAN becomes greater as less $I_f$ is available to counterbalance the suppressive effect of adjacent atrial tissue [155].

It is of interest that both simulation and experimental data have shown that the reduction in $I_f$ arising from either the HCN4 mutations or ivabradine slowed down AP conduction across the SAN. As shown by the activation time profiles (Figure 3.8 for
Chapter 3 Sinus node dysfunction due to reduced $I_f$

simulations and Fig.8 for experimentations), the AP conduction through the SAN is slower with all mutations or with ivabradine as compared to the WT or control condition respectively. Slowing down in the AP conduction across the SAN is one of major features of SSS patients [105]. The slowed AP conduction can be attributed to the combined action of reduced $I_f$ and the electrotonic interaction between the SAN and the atrium. In a normal setting, the electrotonic interaction between the SAN and the atrium suppresses the pacemaking of SAN cells, resulting in a reduced maximal upstroke velocity ($dV/dt_{\text{max}}$) of APs of SAN cells. With a reduced $I_f$, such a suppressive effect from the atrium to the SAN become enhanced as manifested by the decreased $dV/dt_{\text{max}}$ as shown in Figure 3.8F that contributes to the slowing down of AP conduction across the SAN.

**Acetylcholine effects**

In both simulations and experiments, the bradycardiac effect of reduced $I_f$ arising from either the HCN4 gene mutations or ivabradine was accentuated by ACh. Single cell and 2D tissue simulation results were supported by applications of ivabradine together with CCh in experiments. The augmented bradycardiac effect of HCN4 gene mutations by ACh can be attributed to the associated reduction of $I_f$, which is affected by both of the mutations and the ACh. Without ACh, reduced $I_f$ arising from the HCN4 gene mutation leads to a decreased inward depolarising current during the diastolic phase, resulting in a slowing down in cardiac pacemaking rate. With ACh, in the control case, the activation curve of $I_f$ is shifted leftward, resulting in a decreased $I_f$ that contributes to the negative chronotropic effects of ACh. However, on the other hand, the ACh-activated $I_{K,ACh}$ causes a more hyperpolarized membrane potential in SAN cells, which activates more $I_f$ to counter-balances the ACh-activated $I_{K,ACh}$ (an outward repolarising current), as well as the leftward shift of the $I_f$ activation curve. Such a dedicated dynamics interactions between the increased repolarising current ($I_{K,ACh}$) and the reduced depolarising current ($I_f$) produces the negative chronotropic effects of ACh. Meanwhile, these interactions also protect the SAN from arrest by
ACh. However, as $I_f$ is defected by the HCN4 gene mutations, though the ACh-activated $I_{K,ACH}$ causes a more negative maximal diastolic potential of SAN cells, but there is less $I_f$ to counter-balance the effect of $I_{K,ACH}$, which results in the observed augmented negative chronotropic effect.

Our simulations demonstrate that ACh not only augments the functional impacts of HCN4 gene mutations on slowing down SAN pacemaking as observed in single cell studies [145], but also impairs the ability of the SAN to drive the surrounding atrial muscle – as revealed by reduced AP conduction velocity in our 2D simulations (Figure 3.8D), and verified in isolated SAN-atrium tissue experiments (Figure 3.10B). The augmented effects of the HCN4 gene mutations on SAN AP conduction by ACh (CCh) can be primarily attributed to the activation of $I_{K,ACH}$, which suppresses the pacemaking APs by reducing their $dV/dt_{max}$, with a greater effect on peripheral SAN cells than on central cells. It can also be partially attributable to the reduction of $I_{CaL}$ by ACh that is associated with a PKA-dependent reduction in L-type Ca$^{2+}$ channel phosphorylation, which can occur either during β-adrenoreceptor stimulation [156], or in its absence. In particular, the great reduction in the $dV/dt_{max}$ of peripheral cells by ACh impairs the ability for SAN to drive the surrounding atrial muscle, leading to a sinus exit block as seen in both simulations (Figure 3.8F) and in experimentations.

Thus, from both simulation and experimental results, increased vagal nerve activity (leading to greater ACh release onto the SAN) can be anticipated to augment the bradycardiac effects of the HCN4 mutations. This result is consistent with clinical observations that significantly lower heart rates occur at night, when vagal tone is high.

**Limitations**

Limitations of cardiac cell and tissue models are well documented [84, 143]. In the present study, we modified the equations for $I_f$ based on available experimental data.
from cultured cells [61]. As our models are for rabbit cells and tissue, we implemented a relative shift of the steady-state activation curves and changes of the activation and deactivation time constants based on experimental data. Here we assumed that rabbit $I_f$ channel characteristics would show changes in response to HCN4 mutations similar to those of human $I_f$ channels. In addition, in simulations heterozygous HCN4 mutant $I_f$ channels were modelled by dividing $I_f$ into two equivalent components of WT and mutant $I_f$. All of these limitations may affect quantitatively the effects of the HCN4 mutations on impairing the SAN pacemaking and driving ability. For example, with the condition of G480R, the mutation itself slows remarkably the pacemaking. This is consistent with clinical data as patient of the mutation carrier shows a prominent slow heart rate (with a minimal heart rate $< 36$ bpm). However, with a combined action of the ACh, the mutation abolished the pacemaking activity of the SAN leading to SAN arrest. This is different to clinical data as no cases of sudden cardiac deaths, no complaint of syncope during rest or physical exercises were reported in the affected members of the family, from which the mutation was identified.

Another notable limitation of the present study is that, due to the lack of phenotypically accurate rabbit models, in our in vitro experiments defective $I_f$ channel function with HCN4 variants was mimicked pharmacologically – by reducing $I_f$ through the application of ivabradine. In addition, the 2D anatomical tissue model is only a portion of the whole atria of the rabbit, which lacks consideration of the 3D geometrical structure of the SAN and the atrium, the electrical heterogeneity and anisotropy of the tissue. Whilst it is worthwhile to make explicit the limitations of the study, however, these limitations do not alter our conclusions on the mechanisms by which the considered HCN4 gene mutations impair the cardiac pacemaker. Besides, there was good concordance between experimental and simulation findings (see Figure 3.9).
Mechanisms of SSS associated with HCN4 gene mutation

Our simulations show that the considered HCN4 gene mutations can lead to different modes of SAN dysfunction: abnormally slow pacemaking (with all mutations; enhanced by ACh), conduction exit block (HCN4-573X with ACh), failure of the SAN to pacemake and conduction (G480R with ACh. All these behaviours are typical of SSS [106]. Although an exaggeration of the normal ageing process through factors such as degenerative fibrosis could also be an important cause, in the present study it is clear that $I_f$ channel mutations underlie the familial disease as only defected $I_f$ channels were considered.

The underlying mechanisms for SSS associated with the considered HCN4 gene mutations share some commons with those of SCN5A mutations as revealed in our previous study [142]. With the SCN5A mutations considered in our previous study, the pacemaking ability of peripheral SAN cells is impaired, rather than central SAN cells that initiate and control the heart rate. With SCN5A mutations, a defective $I_{Na}$ channel currents accentuates the electrotonic interaction between the SAN and the atrium as the excitability of the atrium is reduced such that the electrical load from the atrium is increased, which contributes to impaired AP conduction through the SAN and atrium. With the considered HCN4 gene mutations, both of the central and peripheral SAN cells are impaired, resulting in slow pacemaking rates. The depressive effect of the electrotonic interaction between the SAN and the atrium become more prominent when $I_f$ is defective, such that both of the SAN pacemaking ability and AP conduction are impaired. However, with the HCN4 gene mutations, the excitability of the atrium is not affected as atrial cells do not have $I_f$ channels, the more depressive effect of the atrium is due to a weaker SAN with a reduced $I_f$. This is different to the SCN5A gene mutations, in which the SAN becomes weaker and the electrical load from the atrium to the SAN becomes greater.
Chapter 4

Simulation of sinoatrial node dysfunction associated with genetic mutations

Addendum: The work presented in this chapter has been submitted or published in journals [217-219]. I was involved in to do the simulation study. The text presented here has been reworded where possible.

4.1 Sino-atrial node function and intracardiac conduction in a murine model of long QT syndrome type 3 (Simulation part)

4.1.1 Background

Long QT syndrome type 3 (LQT3) is characterized by delayed cardiac depolarization, prolonged QT interval on ECG and lethal ventricular arrhythmias [157]. Functional analysis reveals that the common mechanism in LQT3 is the impairment of the fast inactivation that leads to increased persistent or late sodium current, $I_{Na,L}$. This current has been shown to prolong the AP plateau in animal models [158-161]. In heterologous expression systems, elevation of late sodium current in human LQT3 mutation sodium channel [162-164] may be directly responsible for the disease. Thus far, more than 80 SCN5A mutations have been identified to link with LQT3. Of these
mutations that associated with the nine base pair deletion, ΔKPQ is the most severe [157, 163-166].

“Loss-of-function” has been characterized as a major biophysical phenotype of sinus node dysfunction (SND)-related SCN5A mutants [107], however, SND has also been reported in patients with “gain-of-function” mutations of SCN5A associated with the LQT3 [167]. This syndrome presents clinically as a significant decrease in mean heart rates [168] and episodes of sinus pause and arrest [169]. Decrease in mean heart rate (i.e. sinus bradycardic arrhythmias) could contribute to the risk factors predisposing to lethal arrhythmias in LQT3 patients. Episodes of sinus pause and arrest could predispose to the ventricular arrhythmias that potentially lead to sudden cardiac death. Moreover, SND often occurs with other heart conditions such as heart failure and cardiac ischaemia that often accompany QT prolongation [170].

Similar clinical outcomes caused by different SCN5A mutations are consistent with reports of overlap syndrome in patients with LQT3 and BrS. Conversely, some SCN5A mutations have been implicated in more than one phenotype in particular patients [171]. For example, a single Na$^+$ channel mutation involving deletion of lysine 1500, ΔK1500, is simultaneously associated with LQT3, Brugada syndrome (BrS) and conduction system disease [169]. In addition, the 1795insD mutation, leading to generation of a persistent component of Na$^+$ current during maintained depolarization, has been linked to both LQT3 and BrS and results in a 62% reduction of channel expression [172]. Finally, clinical phenotypes that overlap with those observed in BrS have also been identified in patients carrying the SCN5A+/ΔKPQ mutation [173].

In this study, an established mouse model that carried the mouse equivalent of the human SCN5A-ΔKPQ mutation [174-176] was used to examine SAN function and
intracardiac conduction. Mathematical modelling was used to explore the consequences of LQT3-associated $\text{Scn}5\alpha+/\Delta$ mutations.

### 4.1.2 Model development

The electrophysiological behavior of the SAN cell can be described by differential equation as described in Eqn. 2.7. Here $V$ (in mV) is the membrane potential, $t$ is the time (in s), $I_{\text{ion}}$ (in pA) is the sum of all transmembrane ionic currents and $C_m$ (in pF) is the cell capacitance per unit surface area. $I_{\text{ion}}$ is given by the following equation:

$$I_{\text{tot}} = I_{\text{NaT}} + I_{\text{Ca,T}} + I_{\text{Ca,L}} + I_{\text{to}} + I_{\text{sus}} + I_{K,s} + I_{K,r} + I_{b} + I_{\text{NaCa}} + I_{p} + I_{\text{NaL}}, \quad (4.1)$$

here $I_{\text{NaT}}$ is the TTX-sensitive Na$^+$ current. This single cell model is based on Zhang et al. [84] (both centre and periphery) but additionally includes the late Na$^+$ current ($I_{\text{NaL}}$), a small component of Na$^+$ current that persisted after termination of the initial transient. The steady-state activation and availability curves for $I_{\text{NaL}}$ (in terms of $V_{1/2}$ and slope) are similar to those of $I_{\text{NaT}}$ in the human ventricle. The voltage-dependent kinetics of $I_{\text{NaL}}$ inactivation was employed from the experimental data of Maltsev et al. [177] whilst the kinetics of the voltage-dependent steady-state activation of $I_{\text{NaL}}$ was the same as that of $I_{\text{NaT}}$ following the study of Hund and Ruby [178]. A full list of equations and parameters used for late sodium current is shown in Appendix 4. The voltage clamps of $I_{\text{NaL}}$, 6% $I_{\text{NaL}}$ and their combination are shown in Figure 4.1A, B and C. Figure 4.2D shows the AP with applying 6% $I_{\text{NaL}}$. 

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Figure 4.1 Simulated TTX-sensitive current ($I_{\text{Na,T}}$) (A), Late Sodium current ($I_{\text{Na,L}}$) (B) and combination of these two currents (C) during 20-ms voltage clamp pulses from -140 mV to -40 mV. (D) the AP with applying 6% $I_{\text{Na,L}}$.

**Two-dimension tissue model**

At tissue level, the previous developed two-dimensional (2D) tissue model [143] of the intact SAN-atrium was modified to explore the AP initiation and conduction under control and SCN5A-ΔKPQ mutation condition, respectively. Details of this model has been described in Chapter 3, a full list of model equations and parameters is shown in Appendix 2. In order to simulate the AP initiation and conduction under SCN5A-ΔKPQ mutation condition, $I_{\text{Na,L}}$ was introduced into the 2D model.
4.1.3 Results

Effects of Scn5a+/Δ on SAN pacemaker function

The consequences of Scn5a+/Δ mutation observed in the experiment can be summarized as [218]:

1) Electrocardiographic QTc prolongation.
2) Bradycardia and increases in sinus node recovery times (SNRTs).
3) Impaired conduction in the SAN and atrial tissue.

The following mathematical studies demonstrated a possible physiological basis for these findings arising from altered Na⁺ function.

Figure 4.2 shows effects of various $I_{Na,L}$ on the SAN AP characteristics at the single cell level. Both central (Figure 4.2i) and peripheral (Figure 4.2ii) cells are presented. The simulations initially tested the consequences of adding a prolonged tail current $I_{Na,L}$ as previously known to occur in Scn5a+/Δ [175], with its magnitude varying between 0, 3, 6 and 18% of the normal (100%) total WT Na⁺ current, $I_{Na,T}$ (Figure 4.2). They were then also performed for the peripheral SAN cell (Figure 4.4) with a reduction of $I_{Na_T}$ to 70% of its WT level, in combination with an absence (0%) (Figure 4.3 (i)) and presence (6%) (Figure 4.3 (ii)) of the prolonged tail current $I_{Na,L}$. For both Figure 4.2 and 4.3, the simulations quantified these effects through computations of the resulting AP waveforms (A), cycle length (CL) (B) AP duration (APD) (C), peak AP amplitude (D), maximum rate of voltage change during the AP, $(dV/dt)_{max}$ (E) and minimum diastolic potential (MDP) (F).

Figure 4.2 demonstrates that progressive increases in $I_{Na,L}$ in the presence of a normal $I_{Na,T}$ correspondingly increased CL, APD, AP peak and $(dV/dt)_{max}$, but not MDP in central SAN cells (Figure 4.2(i)). They similarly increased CL and APD but not MDP in peripheral SAN cells. They exerted less marked effects on AP peaks and
(dV/dt)\text{max} in the SAN periphery (Figure 4.2(ii)). Nevertheless, such a manoeuvre could replicate the slowed HR and prolonged APDs observed experimentally. In contrast, use of a peripheral SAN model, demonstrated that reducing $I_{Na,T}$ in an absence of $I_{Na,L}$ did not produce such prolongation in CL, APD and reduction of AP peak and MDP, although it did reduce $(dV/dt)\text{max}$ (Figure 4.3(i)). When combined with the presence of $I_{Na,L}(6\%)$, there was an increase in CL. APD and AP peak were not affected, but $(dV/dt)\text{max}$ was reduced (Figure 4.3(ii)).

The results in Figure 4.2 and Figure 4.3 collectively suggest that simulations achieving both increased CL and reduced $(dV/dt)\text{max}$ require both a reduction in $I_{Na,T}$ and an introduction of $I_{Na,L}$. These predictions are explored further in the 2D simulations.
Figure 4.2 Effects of various $I_{\text{Na,L}}$ on the SAN pacemaking rate. Simulated results recorded from central (i) and peripheral (ii) SAN cells. Effects of various $I_{\text{Na,L}}$ on APs (A), CL (B), APD (C), Peak Amplitude (D), Maximal Diastolic Potential (E), $dv/dt_{\text{max}}$ (F).
Figure 4.3 Effects of 70% $I_{\text{Na,T}}$ without (i) or with (ii) $I_{\text{Na,L}}$ on the SAN pacemaking rate. Simulated results recorded from peripheral SAN cell. Effects of the $I_{\text{Na,L}}$ and reduced $I_{\text{Na,T}}$ on APs (A), CL (B), APD (C), Peak Amplitude (D), Maximal Diastolic Potential (E), $dV/dt_{\text{max}}$ (F).
Simulations of the effects of Scn5a+/Δ on 2D tissue model

In simulations, the AP was first initiated in the centre of the SAN and then propagated in both directions towards the atrial septum and the right atrium and crista terminalis. These simulations explored the effect on conduction of the following different situations are illustrated from Figure 4.4 to Figure 4.7.

In Figure 4.4 panels A-D, $I_{Na,T}$ was maintained at normal (100%) values. However, the magnitude of $I_{Na,L}$ was progressively increased from 0% (A) to 3% (B), 6% (C) and 18% (D) respectively. This demonstrated that such alterations in $I_{Na,L}$ alone did not affect conduction time. Nor did it produce large changes in CL (Figure 4.4 panels E and F). In Figure 4.5 panels A-D, $I_{Na,T}$ was progressively reduced from 100% (A) to 80% (B), 70% (C) and 35% (D), but without $I_{Na,L}$ (0%). This demonstrated prolongation in both conduction times and CL. (Figure 4.5 E and F). Figure 4.6 panel A-D showed that $I_{Na,T}$ was progressively reduced from 100% (A) to 80% (B), 70% (C) and 35% (D), but $I_{Na,L}$ was maintained constant at 6% of the control $I_{Na,T}$. This demonstrated a more significant prolongation in both conduction times and CL in comparison with 0% $I_{Na,L}$ (Figure 4.6 E and F). Finally in Figure 4.7 panel A-D showed that $I_{Na,T}$ was reduced to 35% of its normal value, together with $I_{Na,L}$ being increased from 0% (A), to 3% (B), 6% (C) and 18% (D) of control $I_{Na,T}$. This showed that combined reduction in $I_{Na,T}$ with small increases in $I_{Na,L}$ would result in a SA exit block leading to large increases in conduction time from SAN to atrial septum, though less from the SAN to the right atrium. It also produced marked changes in CL. Together these results thus demonstrated that both marked reductions in $I_{Na,T}$ and a presence of low (~3-6%) levels of $I_{Na,L}$ were required to simulate the changes in both conduction times and CL reported by the present experiments.
Figure 4.4 Effects of various $I_{Na,L}$ on the AP conduction. AP profiles are shown in the 2D tissue without $I_{Na,L}$ (A), 3% $I_{Na,L}$ (B), 6% $I_{Na,L}$ (C) and 18% $I_{Na,L}$ (D). Figure (E) shows the conduction time from SAN centre to atrial septum (white bars) or atrial muscle (black bars); Figure (F) illustrates the CLs with different $I_{Na,L}$. 
Figure 4.5 Effects of reducing $I_{\text{Na,T}}$ on the AP conduction. AP profiles are shown in the 2D tissue with control (A), with 80% $I_{\text{Na,T}}$ (B), 70% $I_{\text{Na,T}}$ (C) and 35% $I_{\text{Na,T}}$ (D). Figure (E) shows the conduction time from SAN centre to atrial septum (white bars) or atrial muscle (black bars); Figure (F) illustrates the CLs with different $I_{\text{Na,T}}$. 
Figure 4.6 Effects of 6% $I_{Na,L}$ and various $I_{Na,T}$ on the AP conduction. AP profiles are shown in the 2D tissue with 100% $I_{Na,T}$ (A), with 80% $I_{Na,T}$ (B), 70% $I_{Na,T}$ (C) and 35% $I_{Na,T}$ (D). Figure (E) shows the conduction time from SAN centre to atrial septum (white bars) or atrial muscle (black bars); Figure (F) illustrates the CLs with different $I_{Na,T}$. 
Figure 4.7 Effects of 35% \( I_{Na,T} \) and various \( I_{Na,L} \) on the AP conduction. AP profiles are shown in the 2D tissue with control (A), 3% \( I_{Na,L} \) (B), 6% \( I_{Na,L} \) (C), 18% \( I_{Na,L} \) (D). Figure (E) shows the conduction time from SAN centre to atrial septum (white bars) or atrial muscle (black bars); Figure (F) illustrates the CLs with different \( I_{Na,L} \).
4.1.4 Discussion

The experimental findings showed that: 1) Scn5a+/∆ mice showed significant changes in SAN pacemaker activity. 2) Scn5a+/Δ mice showed a significant longer SNRT following burst pacing than WT, reflecting a relative suppression of SAN pacemaker function following overdriven conditions. 3) Both intact animals and isolated Scn5a+/Δ SA preparations showed a depression of impulse conduction suggested by comparisons with WT.

Our mathematical simulation performed the possible functional effects of augmenting prolonged $I_{Na,L}$ or reducing total, $I_{Na,T}$ whether alone or in combination on both SAN pacemaker function and AP conduction from the SAN to atrial septum. This involved modelling at both the single cell and two-dimensional SA node-atrial tissue model. This approach followed from previous experimental studies reporting that variants in Scn5a associated with LQT3 resulted in (a) a loss-of-function in the Na$^+$ channel produced by negative shifts in the steady-state inactivation curve, and/or positive shifts in the steady-state activation curve, of $I_{Na,T}$. Either change would reduce the $I_{Na,T}$ window current. (b) an augmented late persistent $I_{Na,L}$ [162, 169, 175, 179-182]. The latter was computationally shown to increase pacemaker cycle length for the 1995insD mutation [179] in a single cell SAN model though this did not investigate the ability of the SAN to pace surrounding atrial muscle. We also complemented previous simulations of the effects of reducing $I_{Na,T}$ on SAN function [144] that had not clarified either the mechanistic links between the gain-of-function of $I_{Na,L}$ or the combined effects of reduced $I_{Na,T}$ and augmented $I_{Na,L}$.

These simulations then demonstrated that alterations in $I_{Na,L}$ failed to affect $(dV/dt)_{max}$, a major determinant of conduction, in the SAN periphery. Conversely, reducing $I_{Na,T}$ alone did reduce $(dV/dt)_{max}$, but did not produce such increases in CL. However, a combined reduction in $I_{Na,T}$ and introduction of $I_{Na,L}$ achieved alterations
in both pacemaking rate and reduced \((dV/dt)_{\text{max}}\). These findings were then extended in a 2D model of intact SAN and atrial tissue, which provided detailed predictions concerning conduction characteristics to both the atrial septum and the atrial tissue. These similarly demonstrated that both marked reductions in \(I_{\text{Na,T}}\) and a presence of low (~3-6%) levels of \(I_{\text{Na,L}}\) could simulate the changes in both conduction times and CL reported by the present experiments.

Systematic simulation studies developed a simple explanation for overlapping phenotypic characteristics shown by human LQT3 and SND in terms of a combination of reduced total, \(I_{\text{Na,T}}\) that might reflect reduced Na\(^+\) channel expression in combination with the late persistent \(I_{\text{Na,L}}\) associated with \(Scn5a^{+/-}\Delta\).

### 4.2 TGF-β1 Mediated Fibrosis and Ion Channel Remodelling Are Key Mechanisms Producing the Sinus Node Dysfunction Associated with SCN5A Deficiency and Aging (Simulation part)

#### 4.2.1 Background

Mutations in the cardiac Na\(^+\) channel gene (SCN5A) can result in lose-of-function of sodium channels, consequently arouse sinus node dysfunction (SND) [107-108, 186-187], Brugada syndrome (BrS) [184] and progressive cardiac conduction disease (PCCD) [169]. Since SND, BrS and PCCD are all associated with loss-of-function SCN5A mutation, phenotypic overlapping is expected as they are sharing a common pathogenesis. For example, genetic defect in SCN5A that associated with PCCD is often accompanied by SND [188-189]. Furthermore, it also has been reported that the effects of SCN5A mutations on electrical function in the heart are age-dependent [190-193].
Despite the clinical severity of such arrhythmic syndromes among SCN5A mutation carriers varies with genetic background, the presence or absence of molecular modifiers and particular physiological conditions, but of these factors, age appears to be the most important influence. The slowed conduction in PCCD progressively worsens with age ultimately leading to an atrioventricular block [189]. Moreover, degenerative fibrotic cardiac abnormalities to extents varying with age have also been observed in several familial arrhythmic syndromes associated with SCN5A. However, whether or not this acting through the resulting phenotype or directly participates in the pathogenic process itself remains unclear.

In this study, a heterozygous Scn5a knockout \((Scn5a^{+/−})\) mouse model with a 50% background haploinsufficiency in the wild type (WT) Na,1.5 protein was developed. The characteristics of the model are in common with human PCCD, presenting cardiac conduction slowing with a pattern consistent with age-dependent degenerative cardiac abnormalities. Sinus bradycardia, slowed SAN conduction and SAN exit block that replicating major feathers of clinically observed SND in patients are also presented in this \(Scn5a^{+/−}\) mice model. Moreover, according to the level of myocardial remodelling, aging, and functional Na,1.5 protein, two distinct phenotypes are classified- a milder and a more severe one. Use this model in an investigation for interactions between aging and \(Scn5a\)–disruption in producing SND and the possible cellular and molecular mechanism producing this condition is presented. Mathematical models are used to investigate the SAN cell function and sinoatrial conduction based on the experiment measured gene expression level in young WT, young \(Scn5a^{+/−}\), old WT, and old \(Scn5a^{+/−}\) mice.

4.2.2 Model development

Single cell model

A mouse sinus node myocyte model from Kharche et al. [194] was used to simulate the SAN AP of the young WT mouse. To simulate the SAN AP of the old WT mouse
and young and old Scn5a+/- mice, the ionic conductance for each of the major ionic currents in the Kharche et al. [194] model were modified based on measured in ion channel expression at the mRNA level (Appendix 5). In the old WT mouse and young and old Scn5a+/- mice, the percentage change in the level of mRNA (with respect to that in the young WT mouse) for the ion channels responsible for a particular ionic current was used to adjust the ionic conductance for the ionic current in the Kharche et al. model [194]. Note that in the Kharche et al. model [194], both Ca\(_v\)1.2 and Ca\(_v\)1.3 were considered, but in experiment, only Ca\(_v\)1.2 was measured. Therefore, we adjusted the \(I_{\text{Ca,L}}\) current by modifying the Ca\(^{2+}\) conductance of Ca\(_v\)1.2 but keeping conductance of Ca\(_v\)1.3 unchange. Currents \(I_{\text{Ca,T}}, I_{\text{to}}\) and \(I_{\text{k1}}\) were modified in the same way.

**Coupled nodal and atrial cells model**

Coupling conductance between nodal and atrial cells was calculated in an analogous way – in this case, the expression levels of mRNAs for four connexin isoforms (Cx30.2, Cx40, Cx43 and Cx45) were summed after correction for (multiplication by) the single channel conductance (Appendix 5). The single channel conductances of the connexins are taken from Boyett et al. [13]. The coupling conductance between the nodal and atrial cells of the young WT mouse was set to obtain a reasonable conduction time and this was then scaled (depending on the connexin mRNA expression) for the other mice. The conduction time between the nodal and atrial cells (measured as the time delay (\(\Delta t\)) between the excitation of the SAN and atrial cell) were computed. As there is no murine atrial cell model available, the Kharche et al. model for mouse SAN was modified by removing the pacemaking currents (\(I_f\) and \(I_{\text{CaT}}\)) to reproduce atrial AP. The coupled sinus node and atrium system was as:

\[
C_s \frac{dV_s}{dt} = -I_{\text{tot}_s} + I_j
\]  \hspace{1cm} (4.2)

\[
C_a \frac{dV_a}{dt} = -I_{\text{tot}_a} - I_j
\]  \hspace{1cm} (4.3)
\[ I_J = g(V_a - V_s) \]  \hspace{1cm} (4.4)

where subscripts \( s \) and \( a \) denote SAN and atrial cell respectively. \( C \) is the membrane capacitance (\( \mu F/cm^2 \)), \( V \) the membrane potential (in \( mV \)), \( t \) the time (in \( s \)) and \( I_{tot} \) the total current (in \( nA \)). The coupling between the SAN and the atrial cell is through the junction current \( I_J \); and \( g \) is the junction (or coupling) conductance (in \( nS \)) specifying the coupling strength between these two cells.

The modelling above assumes that there is a linear relationship between mRNA and function and it is known that this is not necessarily the case. However, the aim of the modelling is not to produce a definitive biophysically-detailed AP model - instead it is to consider the possible consequences for the AP of the changes in expression levels of the ion channels etc. It is, therefore, a form of bioinformatics. We have previously used an analogous approach to compute the human SAN AP.

4.2.3 Results
The experimental findings showed that both aging and \( Scn5a \)-disruption significantly prolonged both intrinsic CL and sinoatrial conduction time (SACT). Interaction of the two effects in old \( Scn5a^{+/} \) hearts produced the greatest functional changes, i.e. slowest automaticity and slowest conduction. Simulated results are shown as below.

**Effects of ion channel remodelling and aging on intrinsic CL**
SAN APs in young WT mice were simulated using a previously developed AP model of a mouse SAN myocyte from Kharche *et al.* [194] (Figure 4.8A). This model then led to similar simulations for the remaining groups of old WT and young and old \( Scn5a^{+/} \) mice (Figure 4.8 Panel B to Panel D). The SAN AP of the old WT mice and young and old \( Scn5a^{+/-} \) mice were simulated based on the experimental measurements (Appendix 5). The ionic conductance for each major ionic current in the Kharche *et al.*
model were modified on the basis of changes in ion channel expression at the mRNA level. Figure 4.8 illustrated the effects of ion channel remodelling and aging on AP of the SAN cell. Either the ion channel remodelling or aging resulted in a prolongation of CL. Interaction of the two effects produced the slowest pacemaker activity. The experimental results also demonstrated that Na,1.5 was down-regulated with both the Scn5a+/- and aging conditions. In order to investigate the general effects of Scn5a+/- and aging on intrinsic CL, further studies were performed with modelling of 10% increments block of Na,1.5 channel conductance against CL. Results are shown in Figure 4.9. The computed CL increased monotonically with the increments block of conductance.

![Figure 4.8](image_url)

**Figure 4.8** Effects of ion channel remodelling and aging on intrinsic CL. A: AP of young WT. B: AP of young Scn5a+/- in comparison with young WT. C: AP of old WT. D: AP of old Scn5a+/- in comparison with old WT.
Chapter 4 Simulation of sinoatrial node dysfunction associated with genetic mutations

Figure 4.9 Effects of a gradual blocking of Na_\text{v}1.5 channel conductance on pacemaking CL.

**Effects of aging and Scn5a disruption on SAN conduction**

Coupled atrial SAN cells model was used for replicating the observed slowing of SAN conduction with aging and Scn5a disruption. Figure 4.10 showed that increasing in junctional conductances g_{ij} resulted in shorter conduction times. Under control condition (g_{ij}=0), the SAN cell was active but the atrial cell was resting (Figure 4.10A). When junctional conductance g_{ij} was raised to 0.5 nS, the atrial cell was activated (Figure 4.10B). The conduction time was measured as the time interval between two successive APs. As a result of the small junctional conductance (Figure 4.10B), the conduction time was relatively long. With the coupling strength (junctional conductance g_{ij}) was increased, the conduction time decreased. (Figure 4.10 Panel B to Panel F).

The experiment results showed that there was a slower conduction (within SAN and from SAN to surrounding atrium) in Scn5a^{+/−} compared to WT mice and in old compared to young mice, with the old Scn5a^{+/−} mice showing the slowest conduction. In order to investigate the general effects of Scn5a^{+/−} and aging on SAN conduction, further studies were performed with modelling of conduction time against junctional...
conductance $g_j$ (Figure 4.11A). The results showed that the conduction was the lowest in old $Scn5a^{+/−}$ mice, followed by old WT, young $Scn5a^{+/−}$ and young WT. Figure 4.11B illustrated the conduction times in young WT, young $Scn5a^{+/−}$, old WT, and old $Scn5a^{+/−}$ mice, respectively, with a junctional conductance $g_j = 1.5 \, nS$.

![Atrial SAN cell coupling model with increasing junctional conductance](image)

Figure 4.10 Atrial SAN cell coupling model with increasing junctional conductance.
Comparisons with experimental results

The models that incorporated the alterations in ion channel expression replicated the experimentally observed slowed pacemaker activity, as evidenced in their predicting increased CLs in separating successive pacemaking APs in the young Scn5a+/−, old WT, and old Scn5a+/− mice (Figure 4.12). The old Scn5a+/− mice that showed the slowest pacemaker activity was also in agreement with experimental findings (Figure 4.12B). The effect of reducing the junctional conductance gj between cells (Figure 4.12C) further demonstrated prolongations of conduction times most obvious in the old Scn5a+/− mice. When gj = 1.2, the simulation results successfully replicated the experimental conduction pattern in which SAN-atrium conduction was slowed in the old WT and young Scn5a+/− conditions compared to young WT mice, with the greatest slowing in old Scn5a+/− mice (Figure 4.12D).
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4.2.4 Summary

The experimental results suggest that both electric remodelling and tissue degeneration, detected as TGF-β1-mediated fibrosis, affect pacemaker and conduction function in SND associated with Scn5a disruption or aging, with a combination of both aging and Scn5a disruption producing the most severe phenotype. In implicating Na\(_{\text{v}}\)1.5 deficiency in such changes, we also suggest a novel regulatory role for Na\(_{\text{v}}\)1.5 in cellular biological processes extending beyond its electrophysiologic function. The modelling studies at least partially reconstructed the physiological mechanisms by which both aging and Scn5a disruption lead to SND, thereby drawing parallels between these and similar conduction changes in the ventricle that occur in the possibly related condition of PCCD.

Figure 4.12 A: Simulated APs in the 4 groups of mice. B: Simulated CL by comparison with experimental data. C: Simulation of sinoatrial conduction by SAN cell and atrial coupling. D: Simulated sinoatrial conduction time when \(g_j\) was 1.2 nS and its comparison to experimental data.
Chapter 5

Constructing a SAN-atrium tissue model

5.1 Background
In Chapter 3, a previous developed 2D anatomical model of the intact SAN-atrium tissue [143] has been used to explore the consequences of the impaired \( I_f \) channel function due to several HCN4 mutations in generating SND. The model provided valuable insight into the behaviors of cardiac tissue in health, disease and drug actions, but as discussed in Chapter 3, this model had limitations. It is only a portion of the whole atria of the rabbit, which is lacking consideration of the geometrical structure of the SAN and the atrium, the electrical heterogeneity and anisotropy of the tissue. As a result of these limitations, the previous developed 2D model is unable to simulate some experimental observations, such as pacemaking alternans (Figure 3.12 and 3.13) and multiple leading pacemaking sites (Figure 3.14) in response to vagal nerve stimulation or application of ivabradine. In this Chapter, we addressed these limitations and developed an electrophysiologically detailed two dimension model of the rabbit SAN and intact atria tissue. The new model incorporated the heterogeneity of action potential characteristics, anisotropy of tissue conducting properties and complex geometry of the SAN, therefore it provided a powerful computational tool for simulating vagal nerve stimulation, gene mutations or drug actions.

5.2 Model development
5.2.1 Anatomical SAN-atrium geometry
The SAN-atrium geometry used for the model development was extracted from the anatomical model of the rabbit SAN [80]. The extracted anatomical geometry is
segmented into different tissue types, with distinct classifications for central and peripheral SAN, the pectinate muscles, the crista terminalis, the intercaval region, right atrium and atrial septum, as shown in figure 5.1.

Figure 5.1 Views of 3D model of SAN adapted from [80]. A: Outer surfaces of model. B: View of myocytes after removing connective tissue. C: SAN centre. D Model section (from line a-a in B).
The 2D SAN-atrium model used in Chapter 3 was based on the geometry of figure 5.1D, a slice of whole atrium, whereas in this Chapter, the 2D model was based on the whole SAN-atria tissue meshing from endocardiac surface (Figure 5.1B). Considering the cell spatial distribution, the geometry of the tissue was discretised via a finite differences approach, which allowed the SAN-atrium tissue to be divided into 385×250 nodes in a Cartesian grid at a high spatial resolution of 40 μm.

### 5.2.2 Simulation methods

Single cell models, including models of SAN (centre and periphery) [84] and right atrium [143], are used for constructing the 2D SAN-atrium model. To simulate the propagation of AP, the monodomain equation is used to describe the changes in the transmembrane potential with time.

\[
\frac{\partial V}{\partial t} = D \nabla^2 V - \frac{I_{\text{tot}}}{C_m}
\]  

(5.1)

where \( D \) is a tensor representing the diffusivity of the electrical potential, \( C_m \) is the membrane capacitance. All other symbols have their usual meanings.

As the SAN-atrium tissue is anisotropic, the diffusion coefficient and membrane capacitance are considered. The diffusion coefficient in transverse and longitudinal directions are shown in figure 5.2A and B, respectively. Figure 5.2C and D show the gradients in membrane capacitance in transverse and longitudinal directions. Equation 5.1 was solved numerically using a finite difference PDE solver based on the explicit Euler method (section 2.4.4) with time and space steps \( \Delta t = 0.01 \) ms, \( \Delta x = 0.04 \) mm, \( \Delta y = 0.04 \) mm, respectively. The boundary conditions used in the simulation were the no-flux conditions, i.e. nodes on the boundary had the differential of the voltage set to zero in the direction of nodes outside the tissue [13].
Figure 5.2 Diffusion coefficient and membrane capacitance along the transverse and longitudinal directions of the SAN-atrium. A: gradient in cell capacitance along the transverse direction. B: gradient in cell capacitance along the longitudinal direction. C: gradient in the diffusion coefficient along the transverse direction. D: gradient in the diffusion coefficient along the longitudinal direction. The distances are same as Figure 5.1.
5.2.3 Model validation

Figure 5.3 shows the simulation of propagating electrical activity through the SAN-atrium model. Figure 5.3A is the anatomical model of the SAN-atrium with myocyte classification. Pink zone indicates atrial muscle; blue zone is classified as peripheral SAN and red zone is central SAN. Figure 5.3B shows endocardial view of the model surface, where the pectinate muscles, the CT and the interval region are clearly marked. The computed activation sequence under control condition is shown in Figure 5.3B to H. The activation time through the SAN-atrium is shown in Figure 5.3I (lines and number show isochrones of the activation) and the shade area is the conduction block zone.

The 2D rabbit SAN-atrial model is based on electrophysiologically accurate reconstruction of the ionic, cellular and tissue properties of the rabbit SAN and atrium and the simulation results are qualitatively in agreement with a variety of experimental observations [198-200]: 1) in the SAN, the conduction is slow, whereas in the surrounding atrial muscle, the conduction becomes much faster; 2) in the SAN, conduction occurs preferentially parallel to the CT; 3) conduction goes around the top and bottom of the block zone to reach the atrial septum.

The computed pacemaking CL of the model is ~346 ms, corresponding to an endogenous rate of ~180 beats/min. Complete activation of the whole atrium takes approximately 70 ms (Figure 5.3). After the activation there is an extended plateau phase, which lasts for about 100 ms. During this plateau phase, the whole SAN-atrium has a very uniform potential (Figure 5.3). Repolarization begins at SAN, following a very similar pattern to the spread of excitation. Average conduction velocities were calculated from surface time information. Under control condition, the conduction velocities along the CT is approximately 0.20 ms⁻¹ and in the atrium is approximately 0.10 ms⁻¹. This is in agreement with the experimental measurements [198-200].
Figure 5.3 Model of the rabbit SAN and surrounding atrial tissue. A: distribution of cell types throughout the 2D tissue. (SAN centre-red, SAN periphery-blue, atrial muscle-pink) B-H: Simulated AP propagation through the SAN-atrium tissue. (CT-crista terminalis, IVC-inferior vena cava, SVC-superior vena cave, RA-right atrium; SEP-interatrial septum) I: Simulated activation sequence of the SAN-atrium tissue.

5.2.4 Comparisons with existing models

Several multi-cellular SAN-atrium models have been developed [201-204]. The Michaels et al. [201] model is based on a resistively coupled cellular network, and is constrained to a highly idealized 2D rectangular Cartesian geometry. The Michaels et al. [201] model is the first multi-cellular model coupled both SAN and atrial cells, which is useful for understanding of SAN form and function. However, this model lacks heterogeneity in action potential characteristics, anisotropy of tissue conductance properties or a complex geometry. Consequently it has limitations for simulation studies. Cloherty et al. [204] developed a monodomain model of rabbit SAN. Despite the heterogeneity of electrical activity, anisotropic of tissue properties and geometry of the SAN were considered in this model, but there was only one SAN central cell acting as the pacemaker which means all spontaneous activities would...
initiate from this point. Moreover, Cloherty \textit{et al.} [204] model considered the SAN as a highly idealized geometry lacking in anatomical model from experiment. Although this model can produce a number of characteristics observed experimentally, it is restricted for case studies, such as for simulation of vagal never stimulation, mutations and drug actions.

The 2D model developed in this Chapter addresses the limitations above, each of which can significantly impact on pacemaking activity, AP propagation and spatial migration of the primary pacemaker site in response to various external influences.

5.3 Results

5.3.1 Simulation of ACh effects on cardiac pacemaking

It is well known that ACh, a neurotransmitter released from parasympathetic vagal nerves terminals, can slow down the spontaneous activity of the SAN and AP conduction across the SAN-atrium [13, 205-206]. Such effects of ACh is mediated primarily via the ACh-activated K$^+$ current, $I_{K,ACh}$ [13, 211]. The pacemaker shift within the SAN in response to vagal nerve stimulation has been studied [208-212]. These studies demonstrated that application of ACh could result in the leading pacemaker site shifted alongside the CT, usually towards the superior vena cava but occasionally towards the interior vena cave. Moreover, multiple leading pacemaker sites triggered by high concentration of ACh have also been reported [210, 213-214]. In this section, various concentrations of ACh are used to investigate the possible effects of ACh on SAN-atrium tissue.

Figure 5.4 shows the AP propagation under the influence of $8\times10^{-8}$ mol/L ACh. Compare with control condition in Figure 5.3, it is obvious that the wave propagation within the SAN and atrium is much slower when ACh is applied. Moreover, the computed pacemaker CL (the time interval between successive spontaneous action
potential) is prolonged by 17.3%. Such decrease in conduction velocity and increase in CL have been observed experimentally in the rabbit SAN [215].

Figure 5.4 Simulated AP propagation under the influence of $8 \times 10^{-8}$ mol/L ACh. Snapshots of activation pattern at various timings were shown.
Figure 5.5 shows the AP propagation under the influence of $15 \times 10^{-8}$ mol/L ACh. Interestingly, two leading pacemakers show up. One is the normal leading pacemaker locating in the centre of SAN and the other is in the SAN periphery region. Figure 5.5 shows that the AP initiation and propagation from the central leading pacemaker is faster than the peripheral one. At 80 ms, two waves collide, leading to a merged wave propagation.

Figure 5.5 Simulated AP propagation under the influence of $15 \times 10^{-8}$ mol/L ACh. Snapshots of activation patten at various timings were shown.
Figure 5.6 shows the AP propagation under the influence of $21 \times 10^{-8}$ mol/L ACh. Instead of two leading pacemakers under the influence of $15 \times 10^{-8}$ mol/L ACh, the pacemaker in peripheral region dominates the pacemaking, although the pacemaker in the SAN centre is still active. As the new leading pacemaker is far from the original region, the AP conduction pathway is extremely different from the control condition. Moreover, the new leading pacemaker situates at the edge of SAN periphery, resulting in the unstable pacemaking. From the third cycles of the pacemaking at 560 ms, a counterclockwise spiral wave shows up. The wave rotates round the SAN with a period of 160 ms and lasts forever (Figure 5.6).

With a higher concentration of ACh ([ACh] > $21 \times 10^{-8}$ mol/L), pacemaking activity is abolished (not shown). This might be because [ACh] > $21 \times 10^{-8}$ mol/L causes the spontaneous of the peripheral cell model to cease.
Figure 5.6 Simulated AP propagation under the influence of $23 \times 10^{-8}$ mol/L ACh. Snapshots of activation pattern at various timings were shown.
5.3.2 Simulation of SCN5A (E161K) mutation

Familial sick sinus syndrome (SSS) has been linked to loss-of-function mutations of the SCN5A gene, which result in decreased inward Na\(^+\) current, \(I_{Na}\). In previous study [142], it has been reported that the SCN5A mutations not only slowed down pacemaking, but also compromised AP conduction across the SAN-atrium, leading to a possible SAN exit block or sinus arrest, the major features of SSS. However, this study used the same model as in Chapter 3 and the limitations of this model have been described above. In this section, the effects of SCN5A mutation on SAN-atrium was investigated by using the model developed in this Chapter.

Figure 5.7 shows the effects of SCN5A-E161K mutation on AP propagation. Due to the impaired \(I_{Na}\), the initiation of pacemaking is slower in comparison with control and AP fails to conduct across the atrium, leading to a SAN exit block. The simulation result is consistent with the previous report but shows more details in AP conduction.
Figure 5.7 Effects of SCN5A-E161K mutation on AP propagation. Snapshots of activation pattern at various timings were shown.
5.4 Summary

A 2D model of the rabbit SAN-atrium was constructed. The model includes biophysically detailed SAN (centre and periphery) and atrial cell models as well as the geometric representation of the anatomy of the rabbit SAN from Dobrzynski et al. [80]. Heterogeneity of action potential characteristics and anisotropy of tissue conductance properties are also considered in this model.

The model successfully reproduces the activation sequence of SAN-atrium under control condition. The computed cardiac pacemaking CL and individual tissue conduction velocities are also consistent with experimental observations.

The 2D model has also been used to investigate the effects of vagal nerve stimulation on spontaneous activity of the SAN and AP conduction across the SAN-atrium. Simulation results show that different concentrations of ACh might cause several possible consequences: 1) when the concentration of ACh is low (< 13×10^{-8} mol/L), it slows down pacemaking and reduces AP conduction velocity; 2) when the concentration of ACh is raised to 15×10^{8} mol/L, two leading pacemakers show up. One is the normal leading pacemaker locating in the centre of SAN and the other is in the SAN periphery region; 3) when the concentration of ACh reaches 21×10^{8} mol/L, the leading pacemaker shifts from the centre of the SAN to the periphery, but with unstable pacemaking activities; 4) when the concentration of ACh is higher than 21×10^{-8} mol/L, the spontaneous activity is abolished. All phenomena above have been observed in experiments [210, 213-214]. Various consequences caused by different concentrations of ACh indicate that the mechanism underlying the pacemaker shift within the SAN in response to vagal nerve stimulation are complex. It has been reported that the regional difference in sensitivity to ACh is the key role result in pacemaker shift [145]. It also has been reported that the distance of the pacemaker shift in response to ACh is dose-dependent [212] (higher concentration of ACh leads to a larger distance of the pacemaker shift). In this Chapter, the possible
The 2D model has also been used to investigate the effects of SCN5A-E161K gene mutation on spontaneous activity of the SAN and AP conduction across the SAN-atrium. The simulation results show that SCN5A-E161K gene mutation not only slowed down pacemaking, but also compromised AP conduction across the SAN-atrium, leading to a possible SAN exit block, which is consistent with previous study [142].
Chapter 6

Discussion and Conclusions

Presented in this thesis includes an investigation of cardiac pacemaker dysfunction arising from genetic mutations. Firstly, mechanisms by which HCN4 mutations associated with sick sinus syndrome impairs cardiac pacemaker and pro-arrhythmia of ivabradine were investigated. Then, case studies on cardiac pacemaker dysfunction associated with SCN5A mutations, aging and ion channels remodelling in various heart diseases were presented. At last, a 2D model of the SAN-atrium based on biophysically detailed myocyte models, including tissue heterogeneity, the anisotropy of tissue conductance properties and the complex geometry of the SAN, was constructed. Using this model to investigate effects of vagal tone stimulation and gene mutations on SAN-atrium tissue were also presented.

6.1 Mechanisms of SSS associated with HCN4 gene mutation

In Chapter 3, simulation results showed that the considered HCN4 gene mutations could lead to different modes of SAN dysfunction: abnormally slow pacemaking (with all mutations; enhanced by ACh), conduction exit block (HCN4-573X with ACh), failure of the SAN to pacemake and conduction (G480R with ACh). All these behaviours are typical of SSS [106]. Although an exaggeration of the normal ageing process through factors such as degenerative fibrosis could also be an important cause, in the present study it is clear that $I_f$ channel mutations underlie the familial disease as only defected $I_f$ channels were considered.

The underlying mechanisms for SSS associated with the considered HCN4 gene mutations share some commons with those of SCN5A mutations as revealed in our
previous study [143]. With the SCN5A mutations considered in our previous study, the pacemaking ability of peripheral SAN cells is impaired, rather than central SAN cells that initiate and control the heart rate. With SCN5A mutations, a defective $I_{Na}$ channel currents accentuates the electrotonic interaction between the SAN and the atrium as the excitability of the atrium is reduced such that the electrical load from the atrium is increased, which contributes to impaired AP conduction through the SAN and atrium. With the considered HCN4 gene mutations, both of the central and peripheral SAN cells are impaired, resulting in slow pacemaking rates. The depressive effect of the electrotonic interaction between the SAN and the atrium become more prominent when $I_f$ is defective, such that both of the SAN pacemaking ability and AP conduction are impaired. However, with the HCN4 gene mutations, the excitability of the atrium is not affected as atrial cells do not have $I_f$ channels, the more depressive effect of the atrium is due to a weaker SAN with a reduced $I_f$. This is different to the SCN5A gene mutations, in which the SAN becomes weaker and the electrical load from the atrium to the SAN becomes greater.

6.2 Mechanisms of pacemaker shift

Mechanisms underlying the pacemaker shift within the SAN in response to vagal nerve stimulation are complex. In the cellular level, the concentration of ACh ([ACh]) $> 13 \times 10^{-8}$ mol/L can cause the spontaneous activity of the central model to cease, whereas [ACh] $> 21 \times 10^{-8}$ mol/L can cause the spontaneous of the peripheral model to cease [80]. This regional difference in sensitivity to ACh has been considered as a key role in pacemaker shift [212]. If the concentration of ACh is low (<$13 \times 10^{-8}$), the spontaneous activity of the SAN and AP conduction across the SAN-atrium will be slowed down, but there will not be a pacemaker shift because the central cells are capable of pacing and still acting as the leading pacemaker. However, if the concentration of ACh is sufficient high, the spontaneous activity of the central model will be terminated, but the peripheral cells are capable of pacing, which will induce the leading pacemaker shift from the SAN centre to periphery. In some cases, two
leading pacemakers appear, the causes of this phenomenon might be that the SAN contains one “ACh site” and one “control site”. Without application of ACh, the “control site” acts as the leading pacemaker and with high concentration of ACh, “ACh site” dominates the pacing. However, with certain concentration of ACh, both sites have similar automaticity, result in two leading pacemakers appear. The distance of the pacemaker shift in response to ACh has also been reported as dose-dependent (higher concentration of ACh leads to a larger distance of the pacemaker shift) [212]. Our model also showed that with sufficient high concentration of ACh, the leading pacemaker might shift to the edge of the SAN periphery, leading to an unstable pacemaking.

6.3 The 2D SAN-atrium model

The 2D rabbit SAN-atrium model which was developed is capable of modelling the electrophysiological behaviours of the SAN at tissue level. It includes both tissue heterogeneity and conduction anisotropy. The use of biophysically detailed myocyte model as the basis makes the model flexible. Diffusion of voltage was altered to produce conduction velocities in the SAN-atrium tissue comparable with those measured experimentally and to reproduce clinical measurement of total activation time under control condition. The model is based on an anatomical geometry from Dobrzynski et al.[80], which includes central and peripheral SAN, the pectinate muscles, the crista terminalis, the intercaval region, right atrium and atrial septum. All these features make the model suitable for modelling a variety of conditions, such as vagal nerve stimulation, genetic defects or drug actions.

6.4 Limitations of the 2D model

Whilst the presented 2D model of the rabbit SAN-atrium provides a good reproduction of the experienmental observations and is suitable for modelling a variety of conditions, it does omit several factors that could be considered. First of all, this model is lacking in 3D anatomical details. Anatomical model of SAN-atrium tissue is a
complex 3D structure, but the 2D model developed in Chapter 5 is a sheet model extracted from the 3D structure. On one hand, this approximation is reasonable because of two reasons. 1) In the rabbit, the SAN is known to occupy the entire thickness from the endocardium to epicardium. 2) The atrial wall in the region of the SAN is relatively thin [216]. On the other hand, the heterogeneity across the transmural wall is sacrificed. This is a limitation of using a 2D model. Using 3D models may provide a deeper insight into the SAN-atrial function on the whole organ level. In addition, the anatomical geometry is based on one heart only; therefore, no account is taken of interindividual variation.

6.5 Future work

In this thesis, mechanisms underlying sinus node dysfunction arising from genetic mutations have been investigated at both of the cellular and the multicellular tissue levels. In order to explore the effects of gene mutations on the whole organ level, a 3D model of the SAN-atrium is required. The work in future is to base on the 3D anatomically detailed model and extend the 2D model to three spatial dimensions.
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References


[56] Harzheim D, Pfeiffer KH & Fabritz L. Cardiac pacemaker function of HCN4 channels in mice is confined to embryonic development and requires cyclic AMP. EMBO J. 27:692–703, 2008.


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[126] (www.ecglibrary.com/ecghome.html).


Appendix 1

Glossary

AF          Atrial fibrillation
AM          Atrial muscle
AP          Action potential
APA         AP amplitude
APD         AP duration
APD50       AP duration at 50% repolarization
APD90       AP duration at 90% repolarization
BBB         Boundle brance block
BrS         Brugada syndrome
Ca\textsubscript{v}1.2  Voltage-gated L-Type Ca\textsuperscript{+} channel alpha\textsubscript{1} subunit
Ca\textsubscript{v}1.3  Voltage-gated L-Type Ca\textsuperscript{+} channel alpha\textsubscript{1} subunit
Ca\textsubscript{v}3.1  Voltage-gated T-Type Ca\textsuperscript{+} channel alpha\textsubscript{1} subunit
Ca\textsubscript{v}3.2  Voltage-gated T-Type Ca\textsuperscript{+} channel alpha\textsubscript{1} subunit
CCD         Isolated cardiac conduction disease
CL          Cycle length
C\textsubscript{m}    Cell membrane capacitance
CT          Crista terminalis
C\textsubscript{X40}  Connexin-40
C\textsubscript{X43}  Connexin-43
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX45</td>
<td>Connexin-45</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>DDR</td>
<td>Diastolic depolarization rate</td>
</tr>
<tr>
<td>dV/dt_{max}</td>
<td>Maximum upstroke velocity of AP</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>E_{Ca}</td>
<td>Reversal potential of Ca^{2+} ion</td>
</tr>
<tr>
<td>E_{CaL}</td>
<td>Reversal potential of $I_{CaL, 1.2}$ and $I_{CaL, 1.3}$</td>
</tr>
<tr>
<td>E_{CaT}</td>
<td>Reversal potential of $I_{CaT}$</td>
</tr>
<tr>
<td>E_{Ks}</td>
<td>Reversal potential of $I_{Ks}$</td>
</tr>
<tr>
<td>E_{Na}</td>
<td>Reversal potential of Na^{+} ion</td>
</tr>
<tr>
<td>E_{Na, 1.5}</td>
<td>Reversal potential of sodium $I_{Na, 1.5}$</td>
</tr>
<tr>
<td>E_{st}</td>
<td>Reversal potential of $I_{st}$</td>
</tr>
<tr>
<td>ERG</td>
<td>Voltage-gated K^{+} channel alpha subunit, rapid delayed rectifier</td>
</tr>
<tr>
<td>F</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>FDM</td>
<td>Finite difference method</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite element method</td>
</tr>
<tr>
<td>HCN</td>
<td>Hyperpolarization-activated cyclic nucleotide gated</td>
</tr>
<tr>
<td>HCN4</td>
<td>Hyperpolarization-activated channel type 4</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human embryonic kidney 293</td>
</tr>
<tr>
<td>HF</td>
<td>Heart failure</td>
</tr>
<tr>
<td>g_{Na,1.5}</td>
<td>Conductance of late sodium current</td>
</tr>
<tr>
<td>hNa,1.5</td>
<td>human voltage-gated sodium channel protein type 5 subunit alpha</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$I_b, I_b,Na, I_{b,K}, I_{b,Ca}$</td>
<td>Background Na$^+$, K$^+$, and Ca$^{2+}$ currents respectively</td>
</tr>
<tr>
<td>$I_{CaL,1.2}, I_{CaL,1.3}$</td>
<td>L-type Ca$^{2+}$ current isoforms Ca$\alpha_{1.2}$ and Ca$\alpha_{1.3}$ respectively</td>
</tr>
<tr>
<td>$I_{CaT}$</td>
<td>T-type Ca$^{2+}$ current</td>
</tr>
<tr>
<td>$I_f, I_{fNa}, I_{fK}$</td>
<td>Hyperpolarization-activated current, with Na$^+$ and K$^+$ components respectively</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>Time-independent K$^+$ current</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>Rapid delayed rectifying K$^+$ current</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>Slow delayed rectifying K$^+$ current</td>
</tr>
<tr>
<td>$I_{Na,1.1}, I_{Na,1.5}$</td>
<td>Isoforms Na$\alpha_{1.1}$ and Na$\alpha_{1.5}$ of Na$^+$ currents respectively</td>
</tr>
<tr>
<td>$I_{NaCa}$</td>
<td>Na$^+$-Ca$^{2+}$ exchanger current</td>
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<tr>
<td>$I_{NaK}$</td>
<td>Na$^+$-K$^+$ pump current</td>
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<td>$I_{Na,L}$</td>
<td>Late sodium current</td>
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<tr>
<td>$I_{st}$</td>
<td>Sustained inward Na$^+$ current</td>
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<td>$I_{to}, I_{sus}$</td>
<td>Transient and sustained components of 4-AP-sensitive currents respectively</td>
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<tr>
<td>LQT3</td>
<td>Long-QT syndrome type 3</td>
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<tr>
<td>LQT</td>
<td>Long-QT syndrome type</td>
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<tr>
<td>K$^+$</td>
<td>Potassium ions</td>
</tr>
<tr>
<td>K$_{ir}2.1$</td>
<td>inwardly rectifying potassium channel subunit</td>
</tr>
<tr>
<td>K$_{v}1.4$</td>
<td>Voltage-gated K$^+$ channel alpha subunit, fast transient outward</td>
</tr>
<tr>
<td>K$<em>{v}4.2, K</em>{v}4.3$</td>
<td>Voltage-gated K$^+$ channel alpha subunit, transient outward</td>
</tr>
<tr>
<td>K$_{v}LQT1$</td>
<td>Voltage-gated K$^+$ channel alpha subunit, delayed rectifier</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>$[K^+]_i$, $[Na^+]_i$, $[Ca^{2+}]_i$</td>
<td>Intracellular $Na^+$, $K^+$ and $Ca^{2+}$ concentrations respectively</td>
</tr>
<tr>
<td>$[K^+]_o$, $[Na^+]_o$, $[Ca^{2+}]_o$</td>
<td>Extracellular $Na^+$, $K^+$ and $Ca^{2+}$ concentrations respectively</td>
</tr>
<tr>
<td>MDP</td>
<td>Maximum diastolic potential</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<td>$Na_v1.1$</td>
<td>Voltage-gated $Na^+$ channel protein type 1 alpha subunit</td>
</tr>
<tr>
<td>$Na_v1.5$</td>
<td>Voltage-gated $Na^+$ channel protein type 5 alpha subunit</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary differential equation</td>
</tr>
<tr>
<td>OS</td>
<td>Overshoot of AP</td>
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<td>PDE</td>
<td>Partial differential equation</td>
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<td>PF</td>
<td>Purkinje fibre</td>
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<td>PM</td>
<td>Pectinate muscle</td>
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<td>$Q_{10}$</td>
<td>Functional change in a variable with a 10 K increase in temperature</td>
</tr>
<tr>
<td>R</td>
<td>Universal gas constant</td>
</tr>
<tr>
<td>SAN</td>
<td>Sinoatrial node</td>
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<tr>
<td>SND</td>
<td>Sinoatrial node dysfunction</td>
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<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
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<tr>
<td>SSS</td>
<td>Sick sinus syndrome</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
</tr>
<tr>
<td>$T$</td>
<td>Absolute temperature in Kelvins (K)</td>
</tr>
<tr>
<td>TOP</td>
<td>Take off potential</td>
</tr>
<tr>
<td>TTX</td>
<td>Tetrodotoxin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TTX-R</td>
<td>TTX resistant</td>
</tr>
<tr>
<td>TTX-S</td>
<td>TTX sensitive</td>
</tr>
<tr>
<td>V</td>
<td>Membrane potential</td>
</tr>
<tr>
<td>$V_{1/2}$</td>
<td>Voltage of half-activation or half-inactivation ion channel gate</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
</tr>
</tbody>
</table>
Appendix 2

General equations

\[
\frac{dV}{dt} = -\frac{I_{\text{tot}}}{C_m}
\]

Equilibrium potentials

\[
E_{Na} = \frac{RT}{F} \ln \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i},
\]

\[
E_K = \frac{RT}{F} \ln \frac{[\text{K}^+]_o}{[\text{K}^+]_i},
\]

\[
E_{Ca} = \frac{RT}{2F} \ln \frac{[\text{Ca}^{2+}]_o}{[\text{Ca}^{2+}]_i},
\]

\[
E_{Cl} = \frac{RT}{F} \ln \frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i},
\]

SAN cell model

\[
I_{\text{tot}} = I_{Na} + I_{Ca,L} + I_{Ca,T} + I_{t_o} + I_{t_s} + I_{K,r} + I_{K,s} + I_f + I_{K,b} + I_{Na,b} + I_{Ca,b} + I_{NaCa} + I_{NaK} + I_{ACh}
\]

Fast Na\(^+\) current

\[
I_{Na} = g_{Na} m^3 h [\text{Na}^+]_o \frac{VF^2}{RT} \left(\frac{e^{(V-E_{Na})}F/RT}{e^{VF/RT}} - 1\right)
\]

\[
\frac{dm}{dt} = \frac{m_m - m}{\tau_m}
\]

\[
m_m = \left(\frac{1}{1 + e^{-V/15.46}}\right)^{1/3}
\]

\[
\tau_m = 1000 \left(\frac{0.6247 \times 10^{-2}}{0.832e^{-0.33(V+56.7)}} + 0.627e^{0.082(V+65.01)} + 4 \times 10^{-5}\right)
\]

\[
h = (1 - F_{Na}) h_1 + F_{Na} h_2, \quad F_{Na} = \frac{9.52 \times 10^{-2} e^{-6.3 \times 10^{-2}(V+34.4)}}{1 + 1.66e^{-0.22(V+63.7)}} + 8.69 \times 10^{-2}
\]
\[
\frac{dh_1}{dt} = h_{1\infty} - h_1, \quad \frac{dh_2}{dt} = h_{2\infty} - h_2
\]
\[
h_{1\infty} = h_{2\infty} = \frac{1}{1 + e^{(V+6.6)/6.4}}
\]
\[
\tau_{h_1} = 1000 \left( \frac{3.717 \times 10^{-6} e^{-0.281(V+17.1)}}{1 + 3.732 \times 10^{-3} e^{-0.342(V+37.76)}} + 5.977 \times 10^{-4} \right)
\]
\[
\tau_{h_2} = 1000 \left( \frac{3.186 \times 10^{-8} e^{-0.621V+18.8}}{1 + 7.189 \times 10^{-8} e^{-0.668V+34.07}} + 3.556 \times 10 \right)
\]

**L-type Ca\(^{2+}\) current**
\[
I_{\text{Ca},L} = g_{\text{Ca},L} \left[ d_L f_L + \frac{0.006}{1 + e^{(V+14.1)/6.0}} \right] V - E_{\text{Ca},L} (1-b)
\]
\[
\frac{dd_L}{dt} = \frac{d_{L\infty} - d_L}{\tau_{d_L}}
\]
\[
d_{L\infty} = \frac{1.0}{1 + e^{(V+23.1)/6.0}}, \quad \tau_{d_L} = \frac{1000.0}{\alpha_{d_L} + \beta_{d_L}}
\]
\[
\alpha_{d_L} = \frac{3.12(V+28.0)}{e^{(V+28.0)/4.0} - 1}, \quad \beta_{d_L} = \frac{25.0}{1 + e^{-4(V+28.0)/4.0}}
\]

\[
\frac{df_L}{dt} = \frac{f_{L\infty} - f_L}{\tau_{f_L}}
\]
\[
f_{L\infty} = \frac{\alpha_{f_L}}{\alpha_{f_L} + \beta_{f_L}}, \quad \tau_{f_L} = \frac{1000.0}{\alpha_{f_L} + \beta_{f_L}}
\]
\[
\alpha_{f_L} = \frac{3.12(V+28.0)}{e^{(V+28.0)/4.0} - 1}, \quad \beta_{f_L} = \frac{25.0}{1 + e^{-4(V+28.0)/4.0}}
\]
\[
b = b_{\text{max}} \frac{[\text{ACh}]}{K_{0.5,\text{Ca}} + [\text{ACh}]}
\]

**T-type Ca\(^{2+}\) current**
\[
I_{\text{Ca},T} = g_{\text{Ca},T} d_T f_T (V - E_{\text{Ca},T})
\]
\[
\frac{\text{d}d_T}{\text{d}t} = \frac{d_{Te} - d_T}{\tau_{d_T}},
\]
\[
d_{Tc} = \frac{1.0}{1 + e^{-(V+37.0)/6.8}}, \quad \tau_{d_T} = \frac{1000.0}{\alpha_{d_T} + \beta_{d_T}},
\]
\[
\alpha_{d_T} = 1068e^{(V+26.3)/30.0}, \quad \beta_{d_T} = 1068e^{-(V+26.3)/30.0}
\]
\[
\frac{\text{d}f_T}{\text{d}t} = \frac{f_{Te} - f_T}{\tau_{f_T}},
\]
\[
f_{Te} = \frac{1.0}{1 + e^{(V+71.7)/83.33}}, \quad \tau_{f_T} = \frac{1000.0}{\alpha_{f_T} + \beta_{f_T}},
\]
\[
\alpha_{f_T} = 15.3e^{-(V+71.7)/83.33}, \quad \beta_{f_T} = 15^{(V+71.7)/15.3}
\]

**Transient outward K\(^+\) current**

\[
I_{\text{to}} = g_{\text{to}}qr(V - E_K)
\]
\[
\frac{\text{d}r}{\text{d}t} = \frac{r_\infty - r}{\tau_r},
\]
\[
r_\infty = \frac{1.0}{1 + e^{-(V-10.93)/19.7}}, \quad \tau_r = 1000\left(2.98 \times 10^{-3}\right) + \frac{15.59 \times 10^{-3}}{1.037e^{0.09(V+30.68)} + 0.369e^{-0.12(V+23.84)}}
\]
\[
\frac{\text{d}q}{\text{d}t} = \frac{q_\infty - q}{\tau_{q_1}},
\]
\[
q_\infty = \frac{1.0}{1 + e^{(V+59.37)/13.1}}, \quad \tau_q = 1000\left(10.1 \times 10^{-3}\right) + \frac{65.17 \times 10^{-3}}{0.57e^{-0.08(V+498)} + 0.24 \times 10^{-4} e^{0.9(V+50.93)}}
\]

**Sustained outward current**

\[
I_{\text{sus}} = g_{\text{sus}}(V - E_{\text{sus}})
\]
Fast delayed rectifier $K^+$ current

\[ I_{K,r} = g_{K,r} P_a P_i (V - E_K) \]

\[ P_a = (1 - F_{K,r}) P_{a,f} + F_{K,r} P_{a,s} \]

\[ \frac{dp_{a,f}}{dt} = \frac{p_{we} - p_{a,f}}{\tau_{p,a,f}}, \quad \frac{dp_{a,s}}{dt} = \frac{p_{we} - p_{a,s}}{\tau_{p,a,s}} \]

\[ P_{we} = \frac{1.0}{1 + e^{-(V+14.2)/10.6}} \]

\[ \tau_{p,a,f} = 1000 \left( \frac{1.0}{37.2e^{(V-9)/15.9} + 0.96e^{-(V-9)/22.5}} \right) \]

\[ \tau_{p,a,s} = 1000 \left( \frac{1.0}{4.2e^{(V-9)/15.9} + 0.15e^{-(V-9)/21.6}} \right) \]

\[ \frac{dP_{we}}{dt} = \frac{P_{we} - P_{ic}}{\tau_{p_i}} \]

\[ P_{ic} = \frac{1.0}{1 + e^{(V+18.6)/10.1}}, \quad \tau_{p_i} = 2.0 \]

Slow delayed rectifier $K^+$ current

\[ I_{K,s} = g_{K,s} x_s^2 (V - E_K) \]

\[ \frac{dx_s}{dt} = \frac{x_{we} - x_s}{\tau_{x_s}} \]

\[ x_{we} = \frac{\alpha_{x_s}}{\alpha_{x_s} + \beta_{x_s}}, \quad \tau_{x_s} = \frac{1000.0}{\alpha_{x_s} + \beta_{x_s}} \]

\[ \alpha_{x_s} = \frac{14.0}{1 + e^{-(V-40)/9}}, \quad \beta_{x_s} = e^{-V/45} \]

“Funny” current

\[ I_f = I_{f,Na} + I_{f,K} \]

\[ I_{f,Na} = g_{f,Na} y (V - E_{Na}) \]

\[ I_{f,K} = g_{f,K} y (V - E_K) \]
\[
\frac{dy}{dt} = \frac{y_x - y}{\tau_y}
\]

\[
y_x = \frac{\alpha_y}{\alpha_y + \beta_y}, \quad \tau_y = \frac{1000.0}{\alpha_y + \beta_y}
\]

\[
\alpha_y = e^{-(V + 78.9 + s)/26.2}, \quad \beta_y = e^{(V + 75.131 + s)/21.25}
\]

\[
s = s_{\text{max}} \frac{[\text{ACh}]}{K_{0.5, f} + [\text{ACh}]}^p
\]

**Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger current**

\[
I_{\text{NaCa}} = k_{\text{NaCa}} \left( \frac{[\text{Na}^+]_h / [\text{Ca}^{2+}]_e e^{\gamma_{\text{NaCa}}VF / RT} - [\text{Na}^+]_e / [\text{Ca}^{2+}]_i e^{\gamma_{\text{NaCa}}-1)VF / RT}}{1 + d_{\text{NaCa}}([\text{Na}^+]_h / [\text{Ca}^{2+}]_e + [\text{Na}^+]_e / [\text{Ca}^{2+}]_i)} \right)
\]

**Na\textsuperscript{+}-K\textsuperscript{+} pump current**

\[
I_{\text{NaK}} = I_{\text{Na}} \left( \frac{[\text{Na}^+]_i}{K_{m, \text{Na}} + [\text{Na}^+]_i} \right)^3 \left( \frac{[\text{K}^+]_o}{K_{m, \text{K}} + [\text{K}^+]_o} \right)^2 1.6 \frac{1.5 + e^{-(V + 60) / 40}}{1.6}
\]

**Background currents**

\[
I_{\text{Na,b}} = g_{\text{Na,b}}(V - E_{\text{Na}}), \quad I_{\text{Ca,b}} = g_{\text{Ca,b}}(V - E_{\text{Ca}}), \quad I_{\text{K,b}} = g_{\text{K,b}}(V - E_{\text{K}})
\]

**Acetylcholine activated K\textsuperscript{+} current**

\[
I_{\text{K,ACH}} = g_{\text{K,ACH}} \left( \frac{[\text{K}^+]_o}{10 + [\text{K}^+]_o} \right) \frac{(V - E_{\text{K}})}{1 + e^{(V - E_{\text{K}} - 140)F / 2.5RT}}
\]

\[
g_{\text{K,ACH}} = g_{\text{K,ACH}} j_{kK, ACH} \frac{[\text{ACh}]}{K_{0.5, K, ACH} + [\text{ACh}]}^{j_{kK, ACH}}
\]

\[
\frac{dj}{dt} = \frac{j_x - j}{\tau_j}
\]

\[
\frac{j_x}{\alpha_j + \beta_j}, \quad \tau_j = \frac{1000.0}{\alpha_j + \beta_j}
\]

\[
\alpha_j = 73.1, \quad \beta_j = \frac{120}{1 + e^{-(V + 50) / 15}}
\]
\[
\frac{dk}{dt} = \frac{k_x - k}{\tau_k}
\]

\[
k_x = \frac{\alpha_k}{\alpha_k + \beta_k}, \quad \tau_k = \frac{1000.0}{\alpha_k + \beta_k}
\]

\[
\alpha_k = 3.7, \quad \beta_k = \frac{5.82}{1 + e^{-(V_{150})/15}}
\]

**Table A1. SAN Model parameter values**

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<thead>
<tr>
<th>Parameter</th>
<th>Center</th>
<th>Periphery</th>
</tr>
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<tbody>
<tr>
<td>(b_{\text{max}})</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>(C_m)</td>
<td>20 pF</td>
<td>65 pF</td>
</tr>
<tr>
<td>(d_{\text{NaCa}})</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>(E_{\text{Ca,L}})</td>
<td>46.4 mV</td>
<td>46.4 mV</td>
</tr>
<tr>
<td>(E_{\text{Ca,T}})</td>
<td>45 mV</td>
<td>45 mV</td>
</tr>
<tr>
<td>(E_{\text{sus}})</td>
<td>-</td>
<td>-</td>
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<tr>
<td>(g_{\text{Na}})</td>
<td>0 (\mu S/pF)</td>
<td>1.85 (\times 10^{-8}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{Ca,L}})</td>
<td>2.9 (\times 10^{-4}) (\mu S/pF)</td>
<td>1.0 (\times 10^{-3}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{Ca,T}})</td>
<td>2.14 (\times 10^{-4}) (\mu S/pF)</td>
<td>2.14 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{to}})</td>
<td>2.5 (\times 10^{-4}) (\mu S/pF)</td>
<td>5.6 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{sus}})</td>
<td>3.3 (\times 10^{-6}) (\mu S/pF)</td>
<td>1.8 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{K,ACH}})</td>
<td>3.53 (\times 10^{-10}) (\mu S/pF)</td>
<td>1.218 (\times 10^{-9}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{Kf}})</td>
<td>3.99 (\times 10^{-3}) (\mu S/pF)</td>
<td>2.46 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{Ks}})</td>
<td>2.59 (\times 10^{-3}) (\mu S/pF)</td>
<td>1.6 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{Kl}})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(g_{\text{f,Na}})</td>
<td>0.27 (\times 10^{-4}) (\mu S/pF)</td>
<td>1.05 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{f,K}})</td>
<td>0.27 (\times 10^{-4}) (\mu S/pF)</td>
<td>1.05 (\times 10^{-4}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{b,Na}})</td>
<td>2.91 (\times 10^{-6}) (\mu S/pF)</td>
<td>2.9 (\times 10^{-6}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{b,Ca}})</td>
<td>6.62 (\times 10^{-7}) (\mu S/pF)</td>
<td>6.61 (\times 10^{-7}) (\mu S/pF)</td>
</tr>
<tr>
<td>(g_{\text{b,K}})</td>
<td>1.3 (\times 10^{-6}) (\mu S/pF)</td>
<td>1.3 (\times 10^{-6}) (\mu S/pF)</td>
</tr>
<tr>
<td>(I_{\text{Na,K}})</td>
<td>2.46 (\times 10^{-3}) nA/pF</td>
<td>2.46 (\times 10^{-3}) nA/pF</td>
</tr>
<tr>
<td>(k_{\text{NaCa}})</td>
<td>1.36 (\times 10^{-7}) (\mu S/pF)</td>
<td>1.36 (\times 10^{-7}) (\mu S/pF)</td>
</tr>
<tr>
<td>([\text{Na}^+]_o)</td>
<td>140 mM</td>
<td>140 mM</td>
</tr>
<tr>
<td>Symbol</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>8 mM</td>
<td>8 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>2 mM</td>
<td>2 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_i$</td>
<td>0.0001 mM</td>
<td>0.0001 mM</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>5.4 mM</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>140 mM</td>
<td>140 mM</td>
</tr>
<tr>
<td>$K_{m,K}$</td>
<td>0.621</td>
<td>0.621</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
<td>5.64</td>
<td>5.64</td>
</tr>
<tr>
<td>$K_{0.5,Ca}$</td>
<td>0.09 µM</td>
<td>0.09 µM</td>
</tr>
<tr>
<td>$K_{0.5,f}$</td>
<td>$1.26 \times 10^{-2}$ µM</td>
<td>$1.26 \times 10^{-2}$ µM</td>
</tr>
<tr>
<td>$K_{0.5,K,ACh}$</td>
<td>0.28 µM</td>
<td>0.28 µM</td>
</tr>
<tr>
<td>$n_{K,ACh}$</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>$n_f$</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>$s_{max}$</td>
<td>-7.2 mV</td>
<td>-7.2 mV</td>
</tr>
<tr>
<td>$\gamma_{NaCa}$</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Appendix 3

Atrial cell model

\[ I_{\text{tot}} = I_{Na} + I_{Ca,L} + I_{Ca,T} + I_{t_o} + I_{K_s} + I_{K_t} + I_{NaCa} + I_{NaK} + I_{Na,b} + I_{Ca,b} + I_{Ca,p} \]

Fast Na\(^+\) current

\[ I_{Na} = g_{Na} m^3 h \left[ \text{Na}^+ \right] \frac{VF^2 e^{(V-E_{Na})} F / RT}{e^{VF / RT} - 1} \]

\[ \frac{dm}{dt} = \frac{m_v - m}{\tau_m} \]

\[ m_v = \frac{\alpha_m}{\alpha_m + \beta_m}, \quad \tau_m = \frac{1000.0}{\alpha_m + \beta_m} \]

\[ \alpha_m = \frac{460.0(V + 44.4)}{1 - e^{-(V+44.4)/12.673}}, \quad \beta_m = 18400.0e^{-(V+44.4)/12.673} \]

\[ h = 0.635h_1 + 0.365h_2 \]

\[ \frac{dh_1}{dt} = \frac{h_{1v} - h_1}{\tau_{h_1}}, \quad \frac{dh_2}{dt} = \frac{h_{2v} - h_2}{\tau_{h_2}} \]

\[ h_{1v} = h_{2v} = \frac{\alpha_h}{\alpha_h + \beta_h} \]

\[ \alpha_h = 44.9e^{-(V+66.9)/5.57}, \quad \beta_h = \frac{1491.0}{1 + 323.3e^{-(V+94.0)/12.9}} \]

\[ \tau_{h_1} = 1000 \left( \frac{0.03}{1 + e^{(V+40.0)/6.0}} + 0.00015 \right) \]

\[ \tau_{h_2} = 1000 \left( \frac{0.12}{1 + e^{(V+55.0)/2.0}} + 0.00045 \right) \]

L-type Ca\(^{2+}\) current

\[ I_{Ca,L} = g_{Ca,L} \left( d_L f_L + \frac{1.0}{1 + e^{-(V-E_{Ca,L})/12.0}} \right) (V - E_{Ca,L}) \]

\[ \frac{dd_L}{dt} = \frac{d_{Lv} - d_L}{\tau_{d_L}} \]
\[
d_{L\infty} = \frac{1.0}{1 + e^{-(V+10.95)/6.6}}, \quad \tau_{d_L} = \frac{1000.0}{\alpha_{d_L} + \beta_{d_L}}
\]
\[
\alpha_{d_L} = 16.72\frac{V + 45.0}{1 - e^{-\frac{(V + 45.0)}{2.5}}} + \frac{50.0(V + 10)}{1 - e^{-\frac{(V + 10)}{4.808}}} \quad \beta_{d_L} = \frac{4.48(V + 5.0)}{e^{\frac{(V + 5.0)}{2.5}} - 1}
\]
\[
d_{f_L} = \frac{f_{L\infty} - f_L}{\tau_{f_L}}
\]
\[
f_{L\infty} = \frac{1.0}{1 + e^{-(V+10.95)/6.6}}, \quad \tau_{f_L} = \frac{1000.0}{\alpha_{f_L} + \beta_{f_L}}
\]
\[
\alpha_{f_L} = \frac{8.49(V + 18.0)}{e^{\frac{(V + 18.0)}{4.0}} - 1}, \quad \beta_{f_L} = \frac{67.922}{1 + e^{-(V+18.0)/4.0}}
\]

**T-type Ca^{2+} current**

\[
I_{\text{CaT}} = g_{\text{CaT}} d_T f_T (V - E_{\text{CaT}})
\]
\[
d_{T\infty} = \frac{1.0}{1 + e^{-(V+23.0)/6.1}}, \quad \tau_{d_T} = \frac{1000.0}{\alpha_{d_T} + \beta_{d_T}}
\]
\[
\alpha_{d_T} = 674.173 e^{-(V+23.0)/30.0}, \quad \beta_{d_T} = 674.173 e^{(V+23.0)/30.0}
\]
\[
d_{f_T} = \frac{f_{T\infty} - f_T}{\tau_{f_T}}
\]
\[
f_{T\infty} = \frac{1.0}{1 + e^{-(V+23.0)/6.1}}, \quad \tau_{f_T} = \frac{1000.0}{\alpha_{f_T} + \beta_{f_T}}
\]
\[
\alpha_{f_T} = 9.637 e^{-(V+75.0)/83.33}, \quad \beta_{f_T} = 9.637 e^{(V+75.0)/15.38}
\]

**Transient outward K⁺ current**

\[
I_{\text{Ko}} = g_{\text{Ko}} r_{\infty} \left(0.59 s_1^3 + 0.41 s_2^3 \right) \left[0.6 s_3^6 + 0.4 \right] (V - E_{\text{K}})
\]
\[
dr = r_{\infty} - r
\]
\[
dt = \frac{1.0}{1 + e^{-(V+15.0)/5.63}}, \quad \tau_r = 1000 \left(\frac{1.0}{\alpha_r + \beta_r} + 0.0004\right)
\]
\[ \alpha_r = 386.6e^{V/12.0}, \quad \beta_r = 8.011e^{-V/7.2} \]

\[
\frac{ds_1}{dt} = \frac{s_{10} - s_1}{\tau_{r_1}}, \quad \frac{ds_2}{dt} = \frac{s_{20} - s_2}{\tau_{r_2}}
\]

\[
s_{10} = s_{20} = \frac{1.0}{1 + e^{(V+28.29)/7.06}} = 1000 \left( \frac{0.189}{1 + e^{(V+32.8)/0.1}} + 0.0204 \right)
\]

\[
\tau_{r_1} = 1000 \left( \frac{0.189}{1 + e^{(V+32.8)/0.1}} + 0.45e^{-V-13.54/13.97} \right)
\]

\[
\frac{ds_3}{dt} = \frac{s_{30} - s_3}{\tau_{r_3}}
\]

\[
s_{30} = \left[ \frac{1.0}{1 + e^{(V+50.67)/27.38}} + 0.666 \right] / 1.666
\]

\[
\tau_{r_3} = 1000 \left( \frac{7.5}{1 + e^{(V+23.0)/0.5}} + 0.5 \right)
\]

**Sustained outward current**

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

**Fast delayed rectifier K⁺ current**

\[ I_{K_r} = g_{K_r}p_a p_{i}(V - E_K) \]

\[
\frac{dp_a}{dt} = \frac{p_{a0} - p_a}{\tau_{p_a}}
\]

\[
p_{a0} = \frac{1.0}{1 + e^{-(V+5.1)/7.4}}, \quad \tau_{p_a} = 1000.0 \frac{1000.0}{\alpha_{p_a} + \beta_{p_a}} \]

\[
\alpha_{p_a} = 9.0e^{-V/25.371}, \quad \beta_{p_a} = 1.3e^{-V/13.026}
\]

\[
\frac{dp_i}{dt} = \frac{p_{i0} - p_i}{\tau_{p_i}}
\]

\[
p_{i0} = \frac{\alpha_{p_i}}{\alpha_{p_i} + \beta_{p_i}}, \quad \tau_{p_i} = 1000.0 \frac{1000.0}{\alpha_{p_i} + \beta_{p_i}}
\]
\[ \alpha_{p_i} = 100.0 e^{-V/54.645}, \quad \beta_{p_i} = 656.0 e^{V/106.157} \]

**Slow delayed rectifier K\(^+\) current**

\[ I_{K_s} = g_{K_s} n(V - E_K) \]

\[ \frac{dn}{dt} = \frac{n_\infty - n}{\tau_n} \]

\[ n_\infty = \frac{1.0}{1 + e^{-(V + 0.9)/13.8}}, \quad \tau_n = 1000 \left( \frac{1.0}{\alpha_n + \beta_n} + 0.060 \right) \]

\[ \alpha_n = 1.66 e^{V/69.452}, \quad \beta_n = 0.3 e^{-V/21.826} \]

**Inward rectifier K\(^+\) current**

\[ I_{K_I} = g_{K_I} \left( \frac{[K^+]_o}{K_{m,K} + [K^+]_o} \right)^3 \frac{V - E_K}{1 + e^{1.39(V - E_K + 3.6)/F/RT}} \]

**Na\(^+\)-Ca\(^2+\) exchanger current**

\[ I_{NaCa} = k_{NaCa} \frac{[Na^+]_o^3 [Ca^{2+}]_o e^{3NaCaVF/RT} - [Na^+]_o^3 [Ca^{2+}]_o e^{3NaCa-1}VF/RT}{1 + d_{NaCa}([Na^+]_o^3 [Ca^{2+}]_o + [Na^+]_o^3 [Ca^{2+}]_o)} \]

**Na\(^+\)-K\(^+\) pump current**

\[ I_{NaK} = I_{NaK} \left( \frac{[Na^+]_o^{1.5}}{K_{m,Na}^{1.5} + [Na^+]_o^{1.5}} \right) \left( \frac{[K^+]_o}{K_{m,K} + [K^+]_o} \right) \frac{1.6}{1.5 + e^{-V - 60}/40} \]

**Ca\(^2+\) pump current**

\[ I_{Ca,p} = I_{Ca,p} \frac{[Ca^{2+}]_o}{[Ca^{2+}]_o + 0.0002} \]

**Background currents**

\[ I_{Na,b} = g_{Na,b} (V - E_{Na}), \quad I_{Ca,b} = g_{Ca,b} (V - E_{Ca}) \]
Table A2. Atrial Model parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Na}$</td>
<td>0.028 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Ca,L}$</td>
<td>144.0 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Ca,T}$</td>
<td>120.0 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{to}$</td>
<td>200.0 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{sus}$</td>
<td>26.00 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Kr}$</td>
<td>70.00 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Ks}$</td>
<td>50.00 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{K1}$</td>
<td>203.2 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Na,b}$</td>
<td>0.400 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$g_{Ca,b}$</td>
<td>0.400 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$k_{NaCa}$</td>
<td>0.400 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$I_{NaK}$</td>
<td>1.288 x 10^{-3} nA/pF</td>
</tr>
<tr>
<td>$I_{Ca,p}$</td>
<td>190.0 x 10^{-6} μS/pF</td>
</tr>
<tr>
<td>$d_{NaCa}$</td>
<td>0.0003</td>
</tr>
<tr>
<td>$\gamma_{NaCa}$</td>
<td>0.4500</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
<td>11.00 mM</td>
</tr>
<tr>
<td>$K_{m,K}$</td>
<td>1.000 mM</td>
</tr>
<tr>
<td>$K_{m,K1}$</td>
<td>0.590 mM</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>140.0 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>2.500 mM</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>5.000 mM</td>
</tr>
<tr>
<td>$E_{sus}$</td>
<td>-70 mV</td>
</tr>
<tr>
<td>$E_{Ca,L}$</td>
<td>50 mV</td>
</tr>
<tr>
<td>$E_{Ca,T}$</td>
<td>38 mV</td>
</tr>
<tr>
<td>$R$</td>
<td>8314 mJ/mol °C</td>
</tr>
<tr>
<td>$F$</td>
<td>96487 C/mol</td>
</tr>
<tr>
<td>$T$</td>
<td>35°C</td>
</tr>
</tbody>
</table>
Late Na$^+$ current

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

\[ \alpha_{m,L} = \frac{0.32(V_m + 47.13)}{1 - \exp(-0.1(V_m + 47.13))} \]

\[ \beta_{m,L} = 0.08 \exp \left( \frac{-V_m}{11.0} \right) \]

\[ \tau_{m,L} = \frac{1}{1000(\alpha_{m,L} + \beta_{m,L})} \]

\[ mL_x = \frac{\alpha_{m,L}}{\alpha_{m,L} + \beta_{m,L}} \]

\[ \frac{dm_x}{dt} = \frac{mL_x - m_L}{\tau_{m,L}} \]

\[ hL_x = \frac{1}{1 + \exp((V_m + 91)/6.1)} \]

\[ \tau_{h,L} = 0.6s \]

\[ \frac{dh_L}{dt} = \frac{hL_x - h_L}{\tau_{h,L}} \]

\[ g_{Na,L} = 1.6 \times 10^{-6} \mu S \]

\[ I_{Na,L} = g_{Na,L} m_L^3 h_L (V_m - E_{Na}) \]
## Appendix 5

The mRNAs expressed as a percentage of that in the SAN of the young WT mice.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Young WT (%)</th>
<th>Young HZ (%)</th>
<th>Old WT (%)</th>
<th>Old HZ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{Na}}$ (TTX-sensitive)</td>
<td>Nav1.1</td>
<td>100</td>
<td>2258</td>
<td>190</td>
</tr>
<tr>
<td>$I_{\text{Na}}$ (TTX-resistant)</td>
<td>Nav1.5</td>
<td>100</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>$I_{\text{Ca,L}}$</td>
<td>Cav1.2</td>
<td>100</td>
<td>94</td>
<td>69</td>
</tr>
<tr>
<td>$I_{\text{Ca,T}}$</td>
<td>Cav3.1</td>
<td>100</td>
<td>109</td>
<td>61</td>
</tr>
<tr>
<td>$I_{\text{Ca,L}}$</td>
<td>Cav3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{\text{Ca,T}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{\text{Na}}$ (fast recovering)</td>
<td>Kv4.2</td>
<td>100</td>
<td>70</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Kv4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{\text{K,ur}}$</td>
<td>Kv1.5</td>
<td>100</td>
<td>74</td>
<td>40</td>
</tr>
<tr>
<td>$I_{\text{K,r}}$</td>
<td>ERG (Kcnh2)</td>
<td>100</td>
<td>65</td>
<td>31</td>
</tr>
<tr>
<td>$I_{\text{K,s}}$</td>
<td>KvLQT1</td>
<td>100</td>
<td>75</td>
<td>39</td>
</tr>
<tr>
<td>$I_{\text{f}}$ (HCN1)</td>
<td>Hcn1</td>
<td>100</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Hcn2</td>
<td>100</td>
<td>113</td>
<td>59</td>
</tr>
<tr>
<td>$I_{\text{f}}$ (HCN4)</td>
<td>Hcn4</td>
<td>100</td>
<td>261</td>
<td>175</td>
</tr>
<tr>
<td>$I_{\text{K,1}}$</td>
<td>Kir2.1</td>
<td>100</td>
<td>119</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Kir2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{\text{NaCa}}$</td>
<td>NCX1</td>
<td>100</td>
<td>90</td>
<td>51</td>
</tr>
<tr>
<td>SRCa release</td>
<td>Ryr2</td>
<td>100</td>
<td>120</td>
<td>84</td>
</tr>
<tr>
<td>SRCa uptake</td>
<td>SERCA2A</td>
<td>100</td>
<td>64</td>
<td>55</td>
</tr>
</tbody>
</table>
Appendix 6

C++ Code for HCN4 mutation model

```cpp
#include <iostream>
#include <cstdlib>
#include <cmath>
#include <fstream>

double F[45], Y[40];

void f(double[int] [40], double[int] [40], double);

int main(int argc, char* argv)
{
    std::ofstream Data1("Data1.dat", std::ios::out);
    if(!Data1.good())
        std::cerr<<"File error in Data1.dat"<<std::endl;
    std::ofstream Data2("Data2.dat", std::ios::out);
    if(!Data2.good())
        std::cerr<<"File error in Data2.dat"<<std::endl;
    Data2.width(21);
    Data2<<"Cycle Length (ms)";
    Data2.width(21);
    Data2<<"APD at -30mV (ms)";
    Data2.width(21);
    Data2<<"Peak Amplitude (mV)";
    Data2.width(21);
    Data2<<"MDP (mV)"<<std::endl;
    Data2<<"=========================================================================
    =============================================================================="<<std::endl;

    double simduration=5.0;
    double t=0.0;
    double dt=0.00001;
    int counter=100;
    double Y2[40], Y3[40], Y4[40], K1[40], K2[40], K3[40], K4[40];
    double Y10=0., Y20=0., Y30=0., Y40=0., time1=0, APD, MDP, PA;

    Y[0] = 24.647264112113;
    Y[1] = 0.99996467893831;
```
Y[2] = 2.2468936714682e-09;
Y[3] = 3.2345730327880e-09;
Y[4] = 0.99959008461102;
Y[5] = 0.18674768111085;
Y[6] = 0.99988473099370;
Y[7] = 2.4205561901364e-05;
Y[8] = 0.69634991108725;
Y[9] = 7.1310805232061e-02;
Y[10] = 2.5987640388606e-03;
Y[12] = 141.00787373512;
Y[14] = 4.0325101594249e-02;
Y[15] = 1.9645983271737e-02;
Y[16] = 0.26273286617687;
Y[17] = 0.65070785620679;
Y[18] = 37.850083294438;
Y[19] = 37.631959763367;
Y[20] = 0.70949691548594;
Y[21] = 6.7039463006894e-03;
Y[22] = 0.28379895961342;
Y[23] = 0.97835751992561;
Y[24] = 142.06624412656;
Y[25] = 4.4287264001756;
Y[26] = 1.4498274724985;
Y[27] = 0.66877478466198;
Y[28] = 8.9460617212214e-03;
Y[29] = 0.0;
Y[30] = 0.81763459992821;
Y[31] = 0.86761738587680;
Y[32] = 0.14489681306392;
Y[33] = 8.6479803790433e-04;
Y[34] = 1.5267710087365e-03;
Y[35] = 0.88936441955979;
Y[36] = 0.54416675702551;
Y[37] = 1.7584796306003e-02;
Y[38] = 0.38030237413008;
Y[39] = 0.48075923797918;

for(t=0; t<simduration; t+=dt)
{
    Y40=Y30;
    Y30=Y20;
    Y20=Y10;
Y10 = Y[0];

for (int i = 0; i < 40; ++i)
{
    Y2[i] = Y[i];
    Y3[i] = Y[i];
    Y4[i] = Y[i];
}
f(Y, F, t);

for (int i1 = 0; i1 < 40; ++i1)
    K1[i1] = dt * F[i1];

for (int i2 = 0; i2 < 40; ++i2)
    Y2[i2] += 1/2 * K1[i2];
f(Y2, F, t);

for (int i3 = 0; i3 < 40; ++i3)
    K2[i3] = dt * F[i3];

for (int i4 = 0; i4 < 40; ++i4)
    Y3[i4] += 1/2 * K2[i4];
f(Y3, F, t);

for (int i5 = 0; i5 < 40; ++i5)
    K3[i5] = dt * F[i5];

for (int i6 = 0; i6 < 40; ++i6)
    Y4[i6] += K3[i6];
f(Y4, F, t);

for (int i7 = 0; i7 < 40; ++i7)
    K4[i7] = dt * F[i7];

for (int i8 = 0; i8 < 40; ++i8)
    Y[i8] = (Y[i8] + (K1[i8] + 2*K2[i8] + 2*K3[i8] + K4[i8])/6);

    counter--;

    if (!counter)
    {

Data1<<"t"<<Y[0]<<"t"<<F[40]<<"t"<<F[41]<<"t"<<F[42]<<std::endl;
    counter=100;
}

if(Y10<-30. && Y[0]>=-30.)
{
    std::cout<<"Cycle Length "<<(t-time1)*1000<<" ms"<<std::endl;
    Data2.width(21);
    Data2<<(t-time1)*1000;
    time1=t;
    Data2.width(21);
    Data2<<APD;
    Data2.width(21);
    Data2<<PA;
    Data2.width(21);
    Data2<<MDP<<std::endl;
}

if(Y10>=-30 && Y[0]<-30)
{
    std::cout<<"APD measured at -30mV "<<(t-time1)*1000<<" ms"<<std::endl;
    APD=(t-time1)*1000;
}

if(Y20<Y10 && Y[0]<Y10)
std::cout<<"Peak amplitude is "<<Y10<<" mV"<<std::endl;
PA=Y10;
}
if(Y20>Y10 && Y[0]>Y10)
{
    std::cout<<"MDP is "<<Y10<<" mV"<<std::endl;
    MDP=Y10;
}
} //End of for loop
Data1.close();
Data2.close();
return 0;
} //End of main

void f(double Y[40], double F[41], double t)
{
    double Temp = 310.0;
    double Cm = 20e-06;
    double Faraday = 96487.0;
    double R = 8314.0;
    double Vcell = 3.497e-06;
    double Vi = 0.465*Vcell;
    double VCa = Vi;
    double Vc = 0.136*Vcell;
double Vup = 0.01166*VCa;

double Vrel = 0.001296*VCa;

double gCaL = 2.115e-02;

double gCaT = 2.521e-02;

double gbNa = 1.60e-04;

double gbCa = 3.64e-05;

double gbK = 6.94e-05;

double gf = 1.963e-02;

double gk = 0.0187;

double gama = 0.5;

double kNaCa = 0.00001248;

double dNaCa = 0.0001;

double kmNa = 5.46;

double kmk = 0.621;

double INakmax = 0.2192;

double ICapmax = 0.02869;

double Nab = 140.0;

double kb = 5.4;

double Cab = 2.0;

double tp = 10e-03;

double alpha_up = 0.08;

double beta_up = 0.9*alpha_up;
double kcyca = 5e-05;

double kSRCa = 22.0;

double kxcs = 0.9;

double alpha_rel = 0.5;

double krel = 0.004;

double Mgi = 2.5;

double ACh = 0;

/*
//double V_half=-76.1;   //wild type   //S672R
//double V_half=-84.5;   //mutant      //S672R
//double S=10.2;         //wild type   //S672R
//double S=11.2;         //mutant      //S672R

//double V_half= -77.98;  //wild type   //D553N
//double V_half= -78.33;  //mutant
//double S=9.11;         //wild
//double S=8.29;         //mutant
//double Ach =2.4e-7;

//double V_half=-70.2; //wild //573X
double V_half=-76.0; //mutant
//double S=9.1; //wild
double S=7.2; //mutant

//double V_half=-60.0; //G480
//double V_half=-100.0; //mutant
//double S=9.1; //wild
//double S=7.2; //mutant
*/

/*Normalized*/

double V_half=-76.1;   //wild type
double S=10.2;         //wild type

//double V_half=-81.9;  //573X
//double S=7.98;
//double V_half=-84.5;  //S672R
//double S=11.2;          //S672R

//double V_half=-76.1;  //D553N
//double S=10.2;

//double V_half=-106.1;  //G480
//double S=10.2;

double RTONF = Temp*0.08554;

double INa, ICa, ICaL, ICaT, IK, Ib, IbNa, IbCa, IbK, IKp, If, IfNa, IfK, INaK,
ICap, INaCa, Iup, Itr, IRel, IAct, Ito, IKr, IKs;

double pNak, Eks, ENa, ECa, PNak, sh_new, d_1, Gks, V, bar_m, b1m, c1m, d1m,
adl,b2m, c2m, d2m, em, tm, ah, bh, bar_h, th1, th2, bar_h1, bar_h2, bar_r, bar_q, tr,
tq,bdl, afl, tdl, bar_dl, bfl, tfl, bar_fl, adt, bdt, tdt, bar_dt, bar_ft, aft, bft, tft,bar_pa, tpa,
api, bpi, bar_y, by, ay, ty, fab, ract, rinact, ar1, ar2, br1, br2, as, bs,tyr, tys, dkr_shift,
bar_Paf, bar_Pas, tPaf, tPas, bar_Pii, tPii, b, shift_y, Gkr1, Gkr2;

//dshift, a1, b1, a2, b2, d1, d2, e, rat,


Y[12]=140.0;

Y[13]=1.0e-04;

Y[24]=140.0;

Y[25]=5.4;

Y[26]=2.0;

if(Y[11]<1.0e-10)
     Y[11]=1.0e-10;

if(Y[12]<1.0e-10)
     Y[12]=1.0e-10;
if(Y[13]<1.0e-10)
    Y[13]=1.0e-10;
if(Y[24]<1.0e-10)
    Y[24]=1.0e-10;
if(Y[25]<1.0e-10)
    Y[25]=1.0e-10;
if(Y[26]<1.0e-10)
    Y[26]=1.0e-10;
if(Y[18]<1.0e-10)
    Y[18]=1.0e-10;
if(Y[19]<1.0e-10)
    Y[19]=1.0e-10;

double SE = Y[0];
double SM = Y[1];
double SH1 = Y[2];
double SH2 = Y[3];
double SdL = Y[4];
double SfL = Y[5];
double SdT = Y[6];
double SfT = Y[7];
double Spa = Y[8];
double Spi = Y[9];
double Sy = Y[10];

double SNai = Y[11];

double Ski = Y[12];

double SCai = Y[13];

double Sfac = Y[14];

double SfaTc = Y[15];

double SfaTmgc = Y[16];

double SfaTMgm = Y[17];

double SCaup = Y[18];

double SCarel = Y[19];

double Sf1 = Y[20];

double Sf2 = Y[21];

double Sf3 = Y[22];

double SfaCalse = Y[23];

double SNac = Y[24];

double Skc = Y[25];

double SCac = Y[26];

double SR = Y[27];

double SQ = Y[28];

// New for Ikr, Iks

double SXr1 = Y[30];

double SXr2 = Y[31];
double SXs = Y[32];

// New for Ifr and Ifs

double SYr = Y[33];

double SYs = Y[34];

// New for IKr, data from Ito & Ono

double SPaf = Y[35];

double SPas = Y[36];

double SPii = Y[37];

// New for IAch

double Sj = Y[38];

double Sk = Y[39];

double Ek = 26.71*log(Skc/Ski);

RTONF=Temp*0.08554;
Ek=26.71*log(Skc/Ski);

pNak= 0.03;

Eks=RTONF*log((Skc+pNak*SNac)/(Ski+pNak*SNai));

ENa=26.71*log(SNac/SNai);

ECa=13.35*log(SCac/SCai);

PNa=1.237e-07;

sh_new=0.635*SH1+0.365*SH2;

// New version of INa

//PNa=1.2e-6;
//dshift=15.0;
//a1=9.518e-2;
//b1=6.306e-2;
//d1=49.40-dshift;
//a2=1.662;
//b2=0.2251;
//d2=78.70-dshift;
//e=0.08693;
//rat=a1*exp(-b1*(SE+d1))/(1+a2*exp(-b2*(SE+d2)))+e;
//sh_new=(1-rat)*SH1+rat*SH2;
if(fabs(SE)>1.0e-4)
INa=PNa*pow(SM,3)*sh_new*SNac*Faraday/RTONF*(exp((SE-ENa)/RTONF)-1)/(exp(SE/RTONF)-1)*SE;
else
INa=PNa*pow(SM,3)*sh_new*SNac*Faraday/RTONF*(exp((SE-ENa)/RTONF)-1)*RTONF;
INa=0;

//ICaL, ICaT

  d_1=1/(1+exp(-(SE+14.1)/6.));

  ICaL=0.7604e-2*(SdL*SfL+0.006*d_1)*(SE-46.4);

  //blocking ICa by ACh

  b=0.14*(ACh/(1.2e-7+ACh));
ICaL=(1-b)*ICaL;

ICaT=0.214E-2*SdT*SfT*(SE-45.);

ICa=ICaL+ICaT;

// IKr,IKs
Gkr1=0.0020;
Gkr2=0.0012;
Gks=0.0003445;
IKr=7.382e-4*(0.6*SPaf+0.4*SPas)*SPii*(SE-Ek);
IKs=Gks*pow(SXs,2)*(SE-Eks);
IK=IKr+IKs;

// IKp and Ito (IKp=Isus)
IKp=26.5820e-5*SR*(SE-Ek);
Ito =4.905e-3*SQ*SR*(SE-Ek);

// Ib
IbNa=0.5818e-4*(SE-ENa);
IbCa=1.3236e-5*(SE-ECa);
IbK=2.5236E-5*(SE-Ek);
Ib=IbNa+IbCa+IbK;

// formulation of If
IfNa=6.25e-2*Sy*(0.8744e-2*(SE-77.6));
IfK=6.25e-2*Sy*(0.8744e-2*(SE+102));

//If=IfNa+IfK;       //WT 573X S672R
If=(IfNa+IfK);

//If=(1/9)*IfNa+IfK; //533N
//If=(1/8)*IfNa+IfK; //480R

//Another new formula for If
//If=16.2*0.001*(SE+27.7)*(0.15*SYr+0.55*SYs)/6.

//Formulation of IK,Ach
IAch=0.00705*Sj*Sk*(ACh/(2.0e-7+ACh))*(Skc/(10+Skc))*(SE-Ek)/(1+exp(1.*(SE-Ek-140)/(2.5*RTONF)));

// INaK (ip),ICap,INaCa
INaK=4.782E-2*pow((SNai/(SNai+5.64)),3)*pow((Skc/(Skc+0.621)),2)*1.6/(1.5+exp(-(SE+60)/40));
ICap=4.17E-3*SCai/(SCai+0.0004);
INaCa=(0.272e-5)*(pow(SNai,3)*SCac*exp(0.03743*SE*0.5)-pow(SNac,3)*SCai*exp(0.03743*(SE*(0.5-1))))/(1+0.0001*(SCai*pow(SNac,3)+SCac*pow(SNai,3)));

F[0]=-(INa+ICaT+ICaL+IK+Ito+IKp+If+Ib+INaK+INaCa+ICap+IAch)/Cm;
F[40]=If;
F[41]=ICaL;
F[42]=IAch;

// INa
V=SE+25.32;
bar_m=pow((1.0/(1+exp(-V/5.46))),(1./3.));
b1m=0.8322166;
c1m=0.33566;
d1m=56.7062;

b2m=0.6274;
c2m=0.0823;
d2m=65.0131;
em=0.04569e-3;
tm=0.6247E-3/(b1m*exp(-c1m*(SE+d1m))+b2m*exp(c2m*(SE+d2m)))+em;
F[1]=(bar_m-SM)/tm;

ah=44.9*exp(-(SE+66.9)/5.570);
bh=1491./(323.3*exp(-(SE+94.6)/12.9)+1);
bar_h=ah/(ah+bh);

th1=0.03/(1+exp((SE+40.0)/6))+0.00035;

th2=0.12/(1+exp((SE+60.0)/2))+0.00295;

bar_h1=bar_h;
F[2]=(bar_h1-SH1)/th1;

bar_h2=bar_h;
F[3]=(bar_h2-SH2)/th2;

//New formulation for I_to

bar_r=1./(1+exp(-(SE-10.93)/19.70));

tr=2.9775+19.595/(1.037*exp(0.09012*(SE+30.61))+0.369*exp(-0.1190*(SE+23.84)));

tr=tr*1.0e-3;
\[ F[27] = (\bar{r} - SR)/tr; \]

//\[ \bar{r}_1 = 1/((1+\exp(-(SE-10.93)/19.70))); \]

//\[ tr_1 = 10.4808 + 17.2436/(1.037\exp(0.09012*(SE+30.61-00)) + 0.369\exp(-0.1190 *(SE+23.84-00))); \]

//\[ tr_1 = tr_1 * 1.0e-3; \]

//\[ F[38] = (\bar{r}_1 - SR)/tr_1; \]

\[ \bar{q} = 1/((1+\exp((SE+59.37)/13.1)); \]

\[ t_q = 10.103 + 65.167/(0.5686\exp(-0.08161*(SE+19.0+20)) + 0.7174\exp(0.2719*(SE+20.93+20))) \]

\[ t_q = t_q * 1.0E-3; \]

\[ F[28] = (\bar{q} - SQ)/t_q; \]

//Formulation for ICaL, ICaT

\[ \text{if}\ (\text{fabs}(SE+35) <= 1.0e-04) \]

\{ 
\[ \text{adl} = 14.20*2.5-42.45*SE/(\exp(-0.208*SE)-1.); \]

\}

\[ \text{else if}\ (\text{fabs}(SE) <= 1.0e-4) \]

\{ 
\[ \text{adl} = -14.20*(SE+35)/((exp((-SE+35)/2.5)-1)+42.45*0.208; \]

\}

\[ \text{else} \]

\{ 
\[ \text{adl} = -14.20*(SE+35)/((exp((-SE+35)/2.5)-1)-42.45*SE/(exp(-0.208*SE)-1.); \]
if(fabs(SE-5.)<=1.0e-4)
    bdl=5.715/0.4;
else
    bdl=5.715*(SE-5)/(exp(0.4*(SE-5))-1.);

tdl=1/(adl+bdl);
bar_dl=1./(1+exp(-(SE+22.1)/6.));
F[4]=(bar_dl-SdL)/tdl;
if(fabs(SE+28)<=1.0e-4)
    afl=3.125*4;
else
    afl=3.125*(SE+28)/(exp((SE+28)/4)-1.);

bfl=25/(1+exp(-(SE+28)/4));
tfl=1/(afl+bfl);
bar_fl=1./(1+exp((SE+43)/5.));
F[5]=(bar_fl-SfL)/tfl;

adt=1068*exp((SE+26.3)/30.);
bdt=1068*exp(-(SE+26.3)/30.);
tdt=1/(adt+bdt);

bar_dt=1./(1+exp(-(SE+37.0-00)/6.8));
F[6]=(bar_dt-SdT)/tdt;
aft=15.3*exp(-(SE+71.0)/83.3);
bft = 15. * exp((SE + 71.0) / 15.38);

tft = 1. / (aft + bft);

bar_ft = 1. / (1 + exp((SE + 71.0) / 9.0));

F[7] = (bar_ft - SfT) / tft;

// Formulation for IK

bar_pa = 1. / (1 + exp(SE + 5.1) / 7.4);

tpa = 17. * exp(0.0398 * SE) + 2.11 * exp(-0.0510 * SE);

tpa = 1. / tpa;

F[8] = (bar_pa - Spa) / tpa;

api = 100 * exp(-0.0183 * SE);

bpi = 656 * exp(0.00942 * SE);

F[9] = api * (1 - Spi) - bpi * Spi;

// Formulation for If

shift_y = -7.5 * (pow(ACh, 0.69) / (pow(1.26e-8, 0.69) + pow(ACh, 0.69)));

// ay = exp(-(SE + 78.91 - shift_y) / 26.63);

// ay = exp(-(SE + 78.91) / 26.63);

// by = exp((SE + 75.13 - shift_y) / 21.25);

// by = exp((SE + 75.13) / 21.25);

// bar_y = ay / (ay + by);

// ay = exp(-(SE + 121.91 - shift_y) / 30); // S672R mutant

// by = exp((SE + 58.13 - shift_y) / 16.25);

ay = exp(-(SE + 118.91 - shift_y) / 26.63); // wild 573X 480R D533N
by = \exp((SE+45.13-shift_y)/21.25);

//bar_y = ay/(ay+by);

\[ ty = (1.0/(ay+by))/2.3; \]

bar_y = 1/(1+\exp((SE-V_{half})/S));

//\[ ty = (2.11*\exp(-0.5*\text{pow}(((SE+80.90-shift_y)/27.02), 2.0))/2.3; \]  //wild type

//\[ ty = (1.878*\exp(-0.5*\text{pow}(((SE+86.38-shift_y)/25.32), 2.0))/2.3; //S672R \] mutant

F[10] = (bar_y - Sy)/ty;

F[11] = 0.;

F[12] = 0.;

F[14] = (1.29e5 \times SCai \times (1-Sfac) - 307 \times Sfac)/1.0;

F[15] = (5.05e4 \times SCai \times (1-SfaTc) - 252 \times SfaTc)/1.0;

F[16] = (1.29e5 \times SCai \times (1-SfaTmgc-SfaTMgm) - 4.25 \times SfaTmgc)/1.0;

F[17] = (1.290 \times 2.5 \times (1-SfaTmgc-SfaTMgm) - 429 \times SfaTMgm)/1.0;

I_{up} = 1./1.00*(0.08 \times SCai-0.9*0.08*SCaup*5.e-5*0.9/22./(SCai+SCaup*5.e-5*0.9/22 +5.e-5*0.9+5.e-5);

I_{rel} = 0.5/1.00*\text{pow}((Sf2/(Sf2+0.25)),2)*SCarel;

I_{tr} = 1./1.00*(SCaup-SCarel)*2.*Faraday*V_{up}/0.06418;

ract = 240.*\exp(\text{SE}-40.)/12.5 + 240*\text{pow}((SCai/(SCai+0.0004)),4);

rinact = 40+400*\text{pow}((SCai/(SCai+0.0004)),4);

fab = (0.09*F[14]+0.031*F[15]+0.062*F[16])/6.00;

F[13] = 1.00*(2.*INaCa-ICaL-ICaT-ICap-IbCa-Iup+Irel)/(2.*V_{Ca} \times \text{Faraday})-fab;

F[13] = 0;
\[ F[18] = 1.00 \times \frac{(I_{up} - I_{tr})}{(2. \times \text{Faraday} \times V_{up})}; \]

\[ F[19] = 1.00 \times \frac{(I_{tr} - I_{rel})}{(2. \times \text{Faraday} \times V_{rel}) - 11.48 \times (770 \times S_{\text{Carel}} \times (1 - S_{\text{faCalse}}) - 641 \times S_{\text{faCalse}})}; \]

\[ F[20] = 0.96 \times S_{3\text{ract}} - S_{1\text{f1}}; \]

\[ F[21] = r\text{act} \times S_{1\text{f1}} - r\text{inact} \times S_{2\text{f1}}; \]

\[ F[22] = r\text{inact} \times S_{2\text{f1}} - 0.96 \times S_{3\text{f1}}; \]

\[ F[23] = (770 \times S_{\text{Carel}} \times (1 - S_{\text{faCalse}}) - 641 \times S_{\text{faCalse}})/2.75; \]

\[ // F[24] = (140. - S_{\text{Na}})/10. + 1.00 \times (I_{\text{Na}} + 3. \times I_{\text{NaCa}} + 3. \times I_{\text{NaK}} + I_{\text{bNa}} + I_{\text{fNa}})/(\text{Faraday} \times V_{\text{c}}); \]

\[ // F[25] = (5.4 - S_{\text{Kc}})/10. + 1.00 \times (-2. \times I_{\text{NaK}} + I_{\text{K}} + I_{\text{to}} + I_{\text{Kp}} + I_{\text{fK}} + I_{\text{bK}})/(\text{Faraday} \times V_{\text{c}}); \]

\[ // F[26] = (2. \times S_{\text{Ca}})/10. + 1.00 \times (I_{\text{CaL}} + I_{\text{CaT}} - 2. \times I_{\text{NaCa}} + I_{\text{cap}} + I_{\text{bCa}})/(2 \times \text{Faraday} \times V_{\text{c}}); \]

\[ F[24] = 0.; \]

\[ F[25] = 0.; \]

\[ F[26] = 0.; \]

\[ // \text{Formulation of } I_{K,S} \]

\[ a_{1r} = 50./((1+\exp(-(S_{E} - 5)/9.))); \]

\[ a_{2r} = a_{1r}; \]

\[ b_{1r} = 0.05 \times \exp(-(S_{E} - 20)/15.); \]

\[ b_{2r} = 0.4 \times \exp(\text{pow}((-S_{E} + 30)/30),3)); \]

\[ a_{s} = 14. / ((1+\exp(-(S_{E} - 40)/9.))); \]

\[ b_{s} = \exp(-(S_{E} - 00.)/45.); \]

\[ F[30] = a_{1r} \times (1 - S_{Xr1}) - b_{1r} \times S_{Xr1}; \]

\[ F[31] = a_{2r} \times (1 - S_{Xr2}) - b_{2r} \times S_{Xr2}; \]
F[32]=as*(1-SXs)-bs*SXs;

//new for Ifr and Ifs
bar_y=1.0/(1+exp((SE+77.2)/4.2));
ty_r=1./((0.32*exp(-0.03*SE)+100.*exp(0.07*SE))+0.07);
ty_s=1./((0.00001*exp(-0.12*SE)+100.*exp(0.07*SE))+1.0);
F[33]=(bar_y-SYr)/ty_r;
F[34]=(bar_y-SYs)/ty_s;

//Formulation of IKr, data from Ito & Ono
dkr_shift=10.;
bar_Paf=1.0/(1+exp(-(SE+23.2-dkr_shift)/10.6));
bar_Pas=bar_Paf;
t_Paf=1./(37.2*exp((SE-dkr_shift)/15.9)+0.96*exp(-(SE-dkr_shift)/22.5));
t_Pas=1./(4.2*exp((SE-dkr_shift)/17.0)+0.15*exp(-(SE-dkr_shift)/21.6));
F[35]=(bar_Paf-SPaf)/t_Paf;
F[36]=(bar_Pas-SPas)/t_Pas;
bar_Pii=1./(1+exp((SE+28.6-dkr_shift)/10.1));
t_Pii=0.006;
F[37]=(bar_Pii-SPii)/t_Pii;

//new for IAch
double aj=73.1;
double ak=3.7;
double bj=120./(1+exp(-(SE+50)/15.));
double bk=5.82/(1+exp(-(SE+50)/15.));

F[38]=aj*(1-Sj)-bj*Sj;

F[39]=ak*(1-Sk)-bk*Sk;

} // End of f()