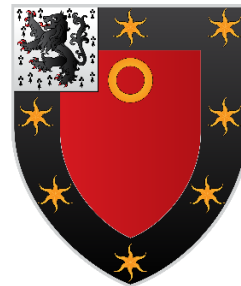


*MULTI-SCALE MODELLING AND
SIMULATIONS INTO THE MECHANISMS
LINKING NEURONAL NITRIC OXIDE
SYNTHASE AND ATRIAL FIBRILLATION*



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This thesis is submitted to the Department of Computer Science, University of Oxford, for the degree of Doctor of Philosophy. This thesis is entirely my own work, and, except where otherwise indicated, describes my own research.

*Moim rodzicom
oraz Jarkowi*

*To my parents
and Jarek*

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Doctor of Philosophy
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MECHANISMS LINKING NEURONAL NITRIC OXIDE
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ABSTRACT

Atrial fibrillation (AF) is the most common cardiac arrhythmia. Its incidence is projected to rise due to population ageing and increasing prevalence of associated risk factors. AF alters, or remodels, the affected atrial tissue, promoting future occurrences of itself and increasing resistance to treatment. Mechanisms underlying AF initiation and remodelling are not well understood. Recent experimental evidence indicates that decreased levels of the neuronal isoform of Nitric Oxide Synthase (nNOS) may be related to AF onset and precede remodelling. However, the potential mechanisms cannot be easily elucidated with experiments alone. Furthermore, experiments are complicated by inter-subject variability, which is particularly important in human studies due to the wide heterogeneity of the human population.

In this thesis, I use multi-scale modelling and simulations in synergy with experimental information to investigate mechanistic links between nNOS and AF at the level of ionic currents/cellular action potential/whole atria in human. First, I construct populations of models spanning experimentally-observed variability in human atrial myocytes under control conditions. Second, I use those populations of control models to identify key ionic mechanisms underpinning nNOS-mediated regulation of the cellular action potential in human atrial myocytes. I show that two of those currents – I_{Kur} and I_{K1} – play a key role in explaining the phenotypic shortening of the action potential observed under nNOS inhibition conditions and preceding AF-induced tissue remodelling. Finally, I build models of human whole atria and establish that this action potential shortening leads to the establishment of a vulnerable substrate, and hence is the main mechanism of pro-arrhythmia at this level. Overall, I provide a picture of nNOS-mediated mechanisms related to AF onset from ionic currents to the whole organ level.

PUBLICATIONS & PRIZES

Lists of publications, presentations, and prizes resulting from the work described in this thesis are presented below.

Publications

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X. Liu, S. Reilly, R. Carnicer, A. Recalde, **A. Muszkiewicz**, R. Jayaram, M. C. Carena, M. Stefanini, R. Wijesurendra, O. Lomas, R. Sayeed, G. Krasopoulos, A. Bueno-Orovio, B. Rodriguez, B. Casadei, Loss of Myocardial nNOS Mediated by Upregulation of miR-31 in Human Atria Contributes to Begetting of Atrial Fibrillation. *Biophysical Journal* 2016, 110 (3): 451a. DOI: 10.1016/j.bpj.2015.11.2421.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABC – Approximate Bayesian Computation

AF – Atrial Fibrillation

AFER – AF-induced electrical remodelling

AP – action potential

APA – action potential amplitude

APD_{x%} – action potential duration at x% repolarization

AVR – atrio-ventricular ring

BB – Bachmann bundle

CT – crista terminalis

CV – conduction velocity

ERP – effective refractory period

FO – fossa ovalis

IABs – inter-atrial bridges

LA – left atrium

LAPG – LA appendage

LHS – Latin Hypercube Sampling

NO – nitric oxide

nNOS – neuronal Nitric Oxide Synthase

PMs – pectinate muscles

PVs – pulmonary veins

RA – right atrium

RAI – right atrial isthmus

RAPG – RA appendage

RMP – resting membrane potential

SAN – sino-atrial node

SMC – Sequential Monte Carlo

SMTc – S-methylthiocitrulline (nNOS inhibitor)

SR – sinus rhythm

SVC – superior vena cava

1 INTRODUCTION

1.1 Introduction

The heart is a muscular organ whose function is to continually provide the body with an uninterrupted supply of vital nutrients and oxygen by pumping blood. The heart pumps blood by regular contractions, and this contractile motion requires an electrical signal to proceed. During one heartbeat, the upper chambers of the heart – the atria – receive an electrical signal to contract, and pass blood to the ventricles, the lower heart chambers. In turn, the ventricles also receive an electrical signal triggering contraction, and push the blood out into the body. This cycle is repeated uninterruptedly several billion times during our lifetimes, and its regularity is crucial for our survival. Cardiovascular diseases constitute the leading cause of mortality in the world (World Health Organization, 2004), with disturbances of the normal heart rhythm, or arrhythmias, underlying a large proportion of those.

Atrial fibrillation (AF) is the most common cardiac arrhythmia. It impacts 1% of the general population, and its incidence is rising due to population ageing and increasing prevalence of associated risk factors, such as obesity, diabetes and hypertension (Chugh et al., 2014; January et al., 2014). During an episode of AF, the atria are affected by a disorganized electrical activity and fail to contract; at the same time, ventricles contract

but do so irregularly. AF significantly raises the risk of morbidities such as stroke, and increases the risk of mortality (Alonso et al., 2009). The complicating factor is the ability of this arrhythmia to alter, or remodel, the properties of the affected atrial tissue, which promotes future occurrences of AF and increases resistance to treatment (Allessie et al., 2002). Understanding this remodelling process is a subject of intense investigation, as it could lead to improved therapies for the disease.

Nitric oxide (NO) is a signalling molecule known to regulate atrial electrical properties (Tamargo et al., 2010). One of the proteins that synthesises NO in atria is called the neuronal isoform of Nitric Oxide Synthase (nNOS). Recent experimental evidence suggests that nNOS depletion may be related to AF, and potentially to the AF-induced tissue remodelling process (Liu et al., 2013; Reilly et al., 2016). Human and murine atrial muscle cells (or ‘myocytes’) exposed to nNOS inhibitor adopt the electrical behaviour associated with AF. Furthermore, nNOS protein content is decreased in atrial tissue from humans and goats in AF. Finally, genetically-engineered mice lacking the nNOS gene are more likely to experience induced AF. However, the underlying mechanisms linking nNOS with AF are not yet well understood.

Experimental studies are the backbone of medical research, as they bring tremendous value to clinical investigations. There are, however, three significant limitations of experiments. First, it is hard to design and conduct experiments crossing multiple time- and length-scales, which is a necessary requirement for investigations into mechanisms underlying a complex disease such as AF. Secondly, restricted access to human tissue limits the number and types of experiments that are possible. The third complicating factor is the wide heterogeneity, or variability, observed in the human population. Variability manifests itself through differences in physiological function between individuals of the same species, and more often in drastic differences between individuals

when conditions change, e.g. in disease or under drug action (Britton et al., 2013). Even within a relatively-restricted patient cohort, variability can be significant. An example of this is depicted in Figure 1.1, which shows experimental recordings of cellular electrical signals, also known as action potentials (APs), from human atrial myocytes used as control in the study of nNOS-mediated cellular effects. Accounting for physiological variability is one of the highest priorities in arrhythmia research.

In addition to experiments, tools that can be used to investigate mechanisms of disease progression are computational modelling and simulation. The past fifty years have seen significant contributions to the field of cardiac modelling (Noble, 2002). Most recent biophysically-detailed models of isolated cardiomyocytes include descriptions of ten or more ionic processes underlying the generation of cellular action potential. Those single-cell models can be coupled to produce one-dimensional muscle fibres or two-dimensional tissue fragments, or even to populate three-dimensional patient-specific meshes of the human heart obtained in hospitals via medical imaging (Arevalo et al., 2016). Furthermore, within the last five to ten years, novel computational frameworks for studying physiological variability have been designed and applied in the field of arrhythmia research (Britton et al., 2013; Groenendaal et al., 2015; Sobie, 2009).

Computational modelling and simulation can overcome some of the limitations of experiments outlined previously. In simulations, crossing multiple spatio-temporal scales is not a restriction; indeed, one can study how changes at the level of one ionic current impact the cellular electrical signal, and how that in turn affects the behaviour of electrical wavefront at the level of whole atria. Models capturing experimentally-observed variability can be used to generate and probe clinically-relevant hypotheses, which can in turn be experimentally tested. As a result, by constructing computer models in synergy with experimental information, one can most effectively answer questions relevant to arrhythmia research.

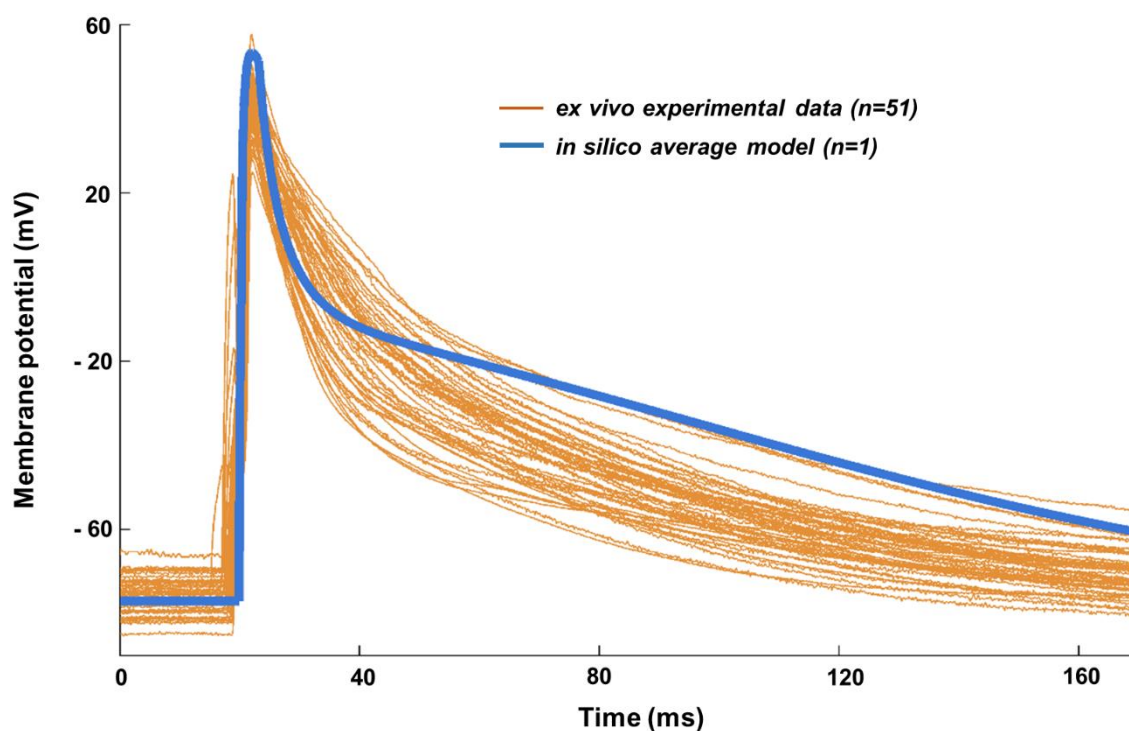


Figure 1.1 Wide variability in action potentials elicited from isolated human atrial myocytes becomes apparent when *ex vivo* results are compared with the output of a biophysically-detailed generic cell model (Maleckar et al., 2009). Experimental data were provided by Dr Xing Liu and are used in Chapters 4 and 5 of this thesis.

1.2 Thesis goals

In this thesis, I construct models of isolated human atrial myocytes as well as human whole atria, in synergy with experimental information, with the aim of shedding light onto some of the processes linking nNOS inhibition/ depletion and AF. As indicated previously, nNOS may play a significant role in AF, but the complete mechanisms are unclear. Simulations of atrial cardiomyocytes could help us understand the key ionic mechanisms underlying the effect of nNOS inhibition/depletion in isolated cells. Whole-atria simulations would allow us to assess the potential contribution of those single-cell effects to the generation and maintenance of AF. In this thesis, I contribute to answering the following research question: ‘*How does the loss of nNOS contribute to AF-related changes in human atria?*’ This central research question can be subdivided into three parts:

1. What ionic mechanisms underpin cell-to-cell variability observed in cellular and ionic processes in isolated human atrial myocytes under control conditions?
2. What are the key ionic currents underlying the nNOS-mediated effects in isolated human atrial myocytes?
3. What is the contribution of the single-cell effects induced by nNOS depletion/inhibition to the generation and maintenance of AF, and how pro-arrhythmic are electrophysiological alterations due to nNOS deficiency at the whole-atria level?

The first question involves building populations (Britton et al., 2013), or ensembles, of human atrial cell models under control conditions that will capture the variability seen in experiments (as shown in orange in Figure 1.1). Those populations of control models are then used to answer the second question. Experimentally-observed changes in ionic currents caused by nNOS inhibition are incorporated, either sequentially or concurrently, into the *in silico* control populations to understand which ionic currents are crucial for nNOS-mediated effects at the level of the cellular action potential. The third question is answered in whole-atria simulations that build on the knowledge of the cellular nNOS-inhibition phenotype obtained in the previous step.

1.3 Thesis outline

I begin in Chapter 2, where I introduce the basic concepts in cardiac electrophysiology. I explain the flow of ionic currents across the cellular membrane, the resultant cellular electrical activity, and the emergence of electrical wavefronts at the level of the whole organ. I then describe how the heart behaves in AF, including the progression of this disease. This is followed by a review of the known effects of nNOS in the heart. I conclude the chapter by describing existing evidence for physiological variability in cardiac electrophysiology and implications of this variability for arrhythmia research.

In Chapter 3, I describe the computational and mathematical tools used to model isolated cardiomyocytes and whole atria. At the cellular level, I begin with the Hodgkin-Huxley model, which was the first electrophysiology model to describe the electrical flow of currents across a cellular membrane. I then review a selection of cardiac cellular electrophysiology models, concluding with the most recent biophysically-detailed models of human atrial myocytes. At the whole-atria level, I start with the description of the electrical wavefront propagation and geometrical models of whole atria. Following that, I describe how changes due to AF have been modelled in the literature. Finally, I review the approaches that enable us to study inter-subject variability in cardiac electrophysiology. Here, the focus is on the experimentally-calibrated population-of-models methodology, since this method is then extended and investigated in depth in the following chapter.

Chapter 4 constitutes the first of three simulation studies in this thesis. Here, I explore methodological considerations on constructing populations of human electrophysiology models from a specific patient cohort dataset. I compare results obtained with three different models of human cellular electrophysiology (Courtemanche et al., 1998; Grandi et al., 2011; Maleckar et al., 2009) and two methods of generating populations of models (Drovandi et al., 2016; McKay et al., 1979). Further, I explore the sources and modulators of cell-to-cell variability beyond the traditionally-considered ones (Britton et al., 2013; Groenendaal et al., 2015; Sobie and Sarkar, 2011), and show that a relatively-narrow range of cellular behaviours seen in cells from the restricted patient cohort can be obtained with a wide range of possible ionic profiles. The experimentally-calibrated populations based on (Grandi et al., 2011; Maleckar et al., 2009) models are found to best capture the variability in cellular and ionic processes observed in human atrial myocytes under

control conditions, i.e. in the absence of nNOS inhibition. Those populations of models are then utilized in the following chapter.

In Chapter 5, I use the experimentally-calibrated populations of models developed in the previous chapter to investigate the role of nNOS-mediated changes in ionic currents on the human atrial action potential, both in normal sinus rhythm and following the onset of AF. To mimic nNOS inhibition, I incorporate the experimentally-measured changes in four ionic currents (I_{to} , I_{Kur} , I_{CaL} , I_{K1}) sequentially and concurrently into the control populations of models. I find that the experimentally-observed changes in I_{to} , I_{Kur} , I_{CaL} , and I_{K1} currents are sufficient to explain the shortening of the cellular action potential seen under nNOS block, and that I_{Kur} and I_{K1} currents play a key role in this process. I also show that, under the effect of AF-induced remodelling, the absence of cellular AP shortening can be explained by reduced nNOS levels. These findings are replicated with two populations of models, highlighting the robustness of nNOS-mediated regulation of human atrial action potential as well as model independence of the results.

Chapter 6 contains the final simulation study in this thesis. Here, I investigate the importance of nNOS inhibition/depletion on inducibility and persistence of atrial arrhythmias including AF in human with the aid of whole-atria simulations. I build phenotypic whole-atria models to study the relative importance of two potential mechanisms of pro-arrhythmia identified experimentally: the shortening of the AP and the loss of the gradient in AP duration (APD) across the left and right atrial chambers. I observe that (i) shortening of the AP in the absence of the inter-chamber APD gradient increases inducibility and alters the dynamics of re-entries that arise, and (ii) abolishment of the right-to-left gradient in APD does not change re-entry inducibility or dynamics. Thus, the simulations show that the dominant mechanism of pro-arrhythmia due to nNOS inhibition is AP shortening.

In Chapter 7, I summarize the main findings of the thesis, and discuss my work in relation to others in the field. Limitations of the methodology and results are discussed, and future lines of research, beyond the scope of this thesis but likely to lead to fruitful discoveries, are suggested.

2 CARDIAC ELECTROPHYSIOLOGY: FROM CELLS TO WHOLE ORGAN

2.1 Introduction

This chapter contains the biological background of the thesis. I start by describing the anatomy and function of a healthy heart and atria. I introduce the core principles of cardiac electrophysiology, from the level of individual ionic currents flowing across cellular membranes, through action potential generation, to propagation of the electrical signal in the whole heart. Following that, I describe the pathophysiology of AF, with a focus on electrophysiological changes in the atria. Thus, I review the determinants of pro-arrhythmia linked to AF, and describe AF-induced electrical and structural changes to atrial tissue properties. Then, I shift the focus to the role of nitric oxide in the heart, and review known electrophysiological effects due to increased nNOS levels and nNOS inhibition/ gene deletion (in the absence of atrial-specific studies, I focus on ventricular myocytes for this purpose). I finish the chapter by describing existing evidence for physiological variability at the single-cell and whole-atria levels. I discuss causes and modulators of this variability as well as their importance in biology and medicine, with a particular focus on arrhythmia research.

2.2 Healthy heart

2.2.1 Anatomy and function of the heart

The heart is a muscular organ functioning as a pump, the size of a clenched fist, whose role is to circulate blood around the body. Cardiac anatomy is depicted in Figure 2.1. The

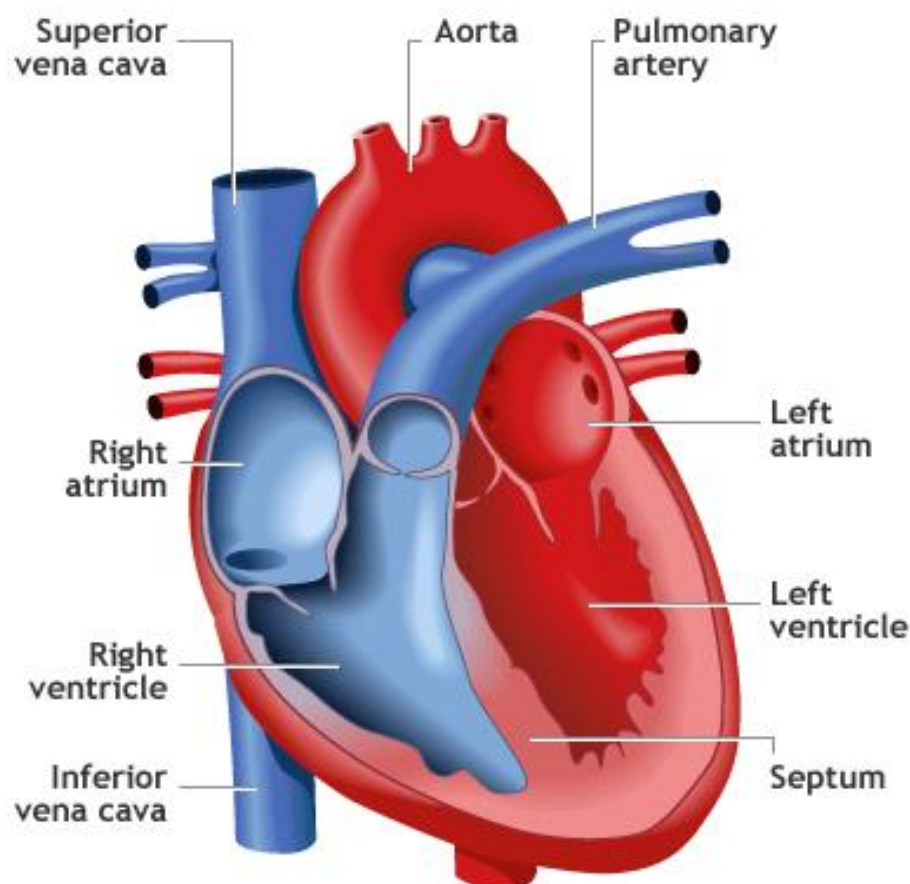


Figure 2.1 Cardiac anatomy. Blue and red colours indicate the right and the left sides of the heart, and correspond to the regions of the organ pumping the deoxygenated and oxygen-rich blood. Image was obtained from lucyz-human-wiki.wikispaces.com and is licenced under a Creative Commons Attribution Share-Alike 3.0 License.

heart consists of four chambers: the two smaller chambers are known as the atria, while the two larger ones are referred to as the ventricles. Deoxygenated blood flows into the right atrium via the superior and inferior venae cava, before being passed to the right ventricle. The contraction of the right ventricle sends the blood through the pulmonary artery and into the lungs, where it picks up oxygen before returning to the left atrium. In turn, the left atrium contracts, sending the oxygen-rich blood to the left ventricle. Following this, the left ventricle pumps the oxygenated blood into the body via the aorta.

2.2.2 Atrial anatomy

Atrial anatomy is illustrated in Figure 2.2. The right atrium (RA) passes blood to the right ventricle via the tricuspid valve (part of the atrio-ventricular ring; AVR). The left atrium (LA) collects blood from the pulmonary veins (PVs) before passing it to the right ventricle via the mitral valve (part of the AVR). Usually, four PVs empty into the LA. Figure 2.2 also highlights the location of the sino-atrial node (SAN), the primary pacemaker of the heart. Preferential conduction pathways, including crista terminalis (CT) and pectinate muscles (PMs) in RA, as well as the inter-atrial Bachmann bundle (BB), are also indicated. In addition to the BB, the left and right atrial chambers are connected electrically via inter-atrial bridges (IABs). Aside from those discrete regions of conducting tissue, the right and left atrial chambers are electrically separated by a layer of non-conducting tissue within the interatrial septum, i.e. the wall separating the atrial chambers (Anderson and Cook, 2007). Finally, Figure 2.2 also includes the right atrial isthmus (RAI), also known as cavo-tricuspid isthmus. The RAI is a region of fibrous tissue in RA, next to the tricuspid valve and inferior vena cava (the latter is not visible in Figure 2.2).

2.2.3 From cardiomyocytes to fibres, sheets, and whole organ

The basic building blocks of organs are cells. The three most common cell types in the heart are (i) cardiomyocytes comprising the muscular walls of the heart; (ii) fibroblasts (connective tissue cells) forming the extracellular matrix and abundantly present in scars; and (iii) endothelial cells covering the inner and outer surfaces of the organ. In this thesis, I focus on cardiomyocytes.

A cardiomyocyte is a rod-shaped cell, typically 50 – 100 μm in length and 10 – 20 μm in diameter. At the subcellular level, cardiomyocytes contain structures such as: the cell nucleus with the cellular DNA; mitochondria, which act as the powerhouses of the cell by producing the energy-storing molecule ATP; and sarcoplasmic reticulum, an organelle

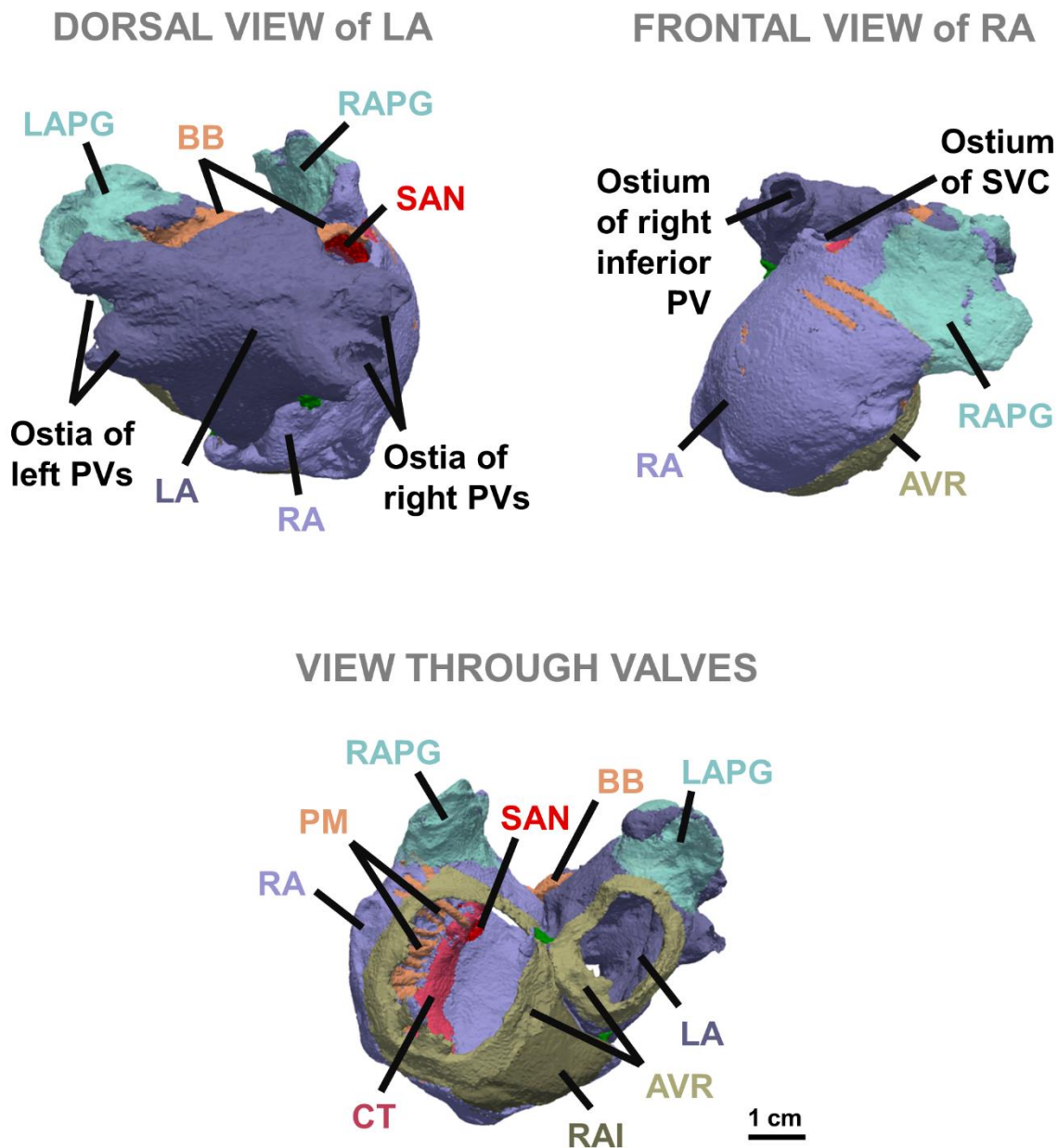


Figure 2.2 Anatomy of human atria shown in dorsal view of the left atrium (LA), frontal view of the right atrium (RA), and through valves separating atria from ventricles. Images generated with ParaView (Ayachit, 2015), using the whole-atria mesh of (Seemann et al., 2006), based on the Visible Female dataset (<http://www.ntis.gov/products/visible-human/>). Acronyms: LA – left atrium, RA – right atrium, LAPG/RAPG – LA/RA appendages, SAN – sino-atrial node, CT – crista terminalis, BB – Bachmann bundle, PMs – pectinate muscles, AVR – atrio-ventricular ring, RAI – right atrial isthmus, IABs – inter-atrial bridges (shown in green), PVs – pulmonary veins, SVC – superior vena cava.

responsible for the storage of calcium ions. The function of these sub-cellular compartments, as well as of the cell itself, is possible in a large part due to specialised proteins that reside in these structures' membranes. The proteins present in the membrane of the cardiomyocyte that are vital for the generation of the action potential and initiation of cellular contraction are known as ion channels, ion pumps and exchangers.

Cardiomyocytes are connected with one another via the so-called gap junctions. Gap junctions are protein complexes that allow ionic species to flow between individual cells, enabling inter-cellular electrical communication. A gap junction consists of two hemi-channel units called connexons, each of which is constructed of six proteins called connexins (Allessie et al., 2002). Two types of connexins have been located to the atrial tissue: connexin-40 and connexin-43 (Ausma et al., 2003; Ryu et al., 2007; van der Velden et al., 2000). While gap junctions can be found on the elongated sides of the rod-shaped cardiomyocyte, their density is highest at the ends of the cells. The implication is that the cells are effectively connected in an end-to-end fashion, yielding a fibre-like structure. The electrical conduction between cardiomyocytes happens preferentially along the direction of the fibre (Dössel et al., 2012). The individual muscle fibres are in turn arranged into bundles (such as the CT or BB) and sheets (as in the RA free wall). In human atria, multiple joining and separating muscle bundles and sheets comprise the cardiac muscle.

2.2.4 Generation of the cardiac electrical signal and contraction

A single cardiomyocyte is capable of generating a cellular electrical signal (also known as an action potential; AP) and of contraction, subject to a sufficiently strong external stimulus. The majority of myocytes are not capable of self-excitation, i.e. they do not, under normal conditions, produce APs in the absence of external triggers. The exception are cardiomyocytes of the SAN (the pace-making structure of the heart), as well as

Purkinje cells of the cardiac conduction system. Once the SAN myocytes produce an electrical signal, it spreads via gap junctions to the neighbouring cells, which respond by generating their own APs, in turn exciting myocytes in their vicinity. The electrical excitation acts as a trigger for the mechanical contraction of the individual cardiomyocytes. Efficient functioning of the heart is conditioned upon synchronous propagation of electrical excitation and subsequent contraction of 2-3 billions of cells.

Macroscopically, the first region of the heart to become excited is the SAN. From there, the excitation spreads into the RA, and – via BB – into the LA. Once both atrial chambers depolarize, the electrical excitation reaches the atrio-ventricular node (AVN), located in the tissue separating atria and ventricles. The AVN is the only structure capable of transmitting electrical signals from the upper to the lower heart chambers, which – under healthy conditions – are otherwise electrically isolated. From the AVN, the excitation is rapidly conducted to the ventricular chambers. There is a delay between the electrical excitation of the atria and ventricles due to the slower conduction at the AVN, and it is critical for cardiac function: it ensures that atria contract fully before the ventricles do so.

2.2.5 Electrophysiology of non-self-excitable atrial myocytes

In the absence of external stimuli, a cardiomyocyte maintains a negative resting membrane potential (RMP) with respect to its surroundings. This is a result of ionic concentration gradients across the cellular membrane. Upon sufficient stimulation, a cardiomyocyte responds by generating an action potential (AP), i.e. a voltage trace consisting of a rapid depolarization phase followed by a slower cellular repolarization back to the resting state. Multiple ionic currents underpin AP generation, with some currents involved in the depolarization of the cell, and others acting to repolarize the myocyte to make it ready for the next excitation. Ionic currents flow through ion channels, and whether an ion channel will open or close is determined by ion channel gating.

2.2.5.1 Ion channel gating

Ion channels can allow or block the flow of specific ion types across the cellular membrane. The behaviour of ion channels is controlled by gating mechanisms. Ion channels become activated/ deactivated when ion channel gates, controlling the behaviour of a particular current, open or close. Typically, an ion channel has at least two gates: an activation and an inactivation gate. The activation gate is closed until the conditions permitting it to open are met. By contrast, the inactivation gate is usually open, and closes only when appropriate criteria for channel opening are met. The conditions that determine whether a particular ion channel will open can involve a voltage-dependent gating mechanism (as is the case with the fast sodium current I_{Na} that initiates an AP) or a concentration-dependent mechanism (e.g. increase in intracellular Ca^{2+} concentration triggers inactivation of the I_{CaL} current). Non-linear interactions between different ionic currents can be seen, and are often mediated by membrane potential. For instance, one ionic current may act to alter the transmembrane potential of the cell, affecting the remaining transmembrane currents.

2.2.5.2 Action potential generation

A typical atrial action potential lasts c.a. 150 – 350 ms and is composed of four distinct stages (phases) shown schematically in Figure 2.3. In response to an increased membrane potential, sodium channels activate and the fast inward sodium current I_{Na} is injected into the cell, giving rise to the AP upstroke, or phase 0 of the AP. In this phase, the cardiomyocyte depolarizes rapidly to its fullest extent. Once the membrane is sufficiently depolarized, the transient outward potassium current I_{to} and the ultra-rapid potassium current I_{Kur} activate and partially repolarize the cell. This rapid early repolarization is labelled as phase 1, and it often manifests itself via the presence of a spike in the AP.

In phase 2, a slow repolarization of the cell takes place. Here, the outward potassium currents (typically I_{Kur} , as well as the fast and slow delayed rectifier potassium currents

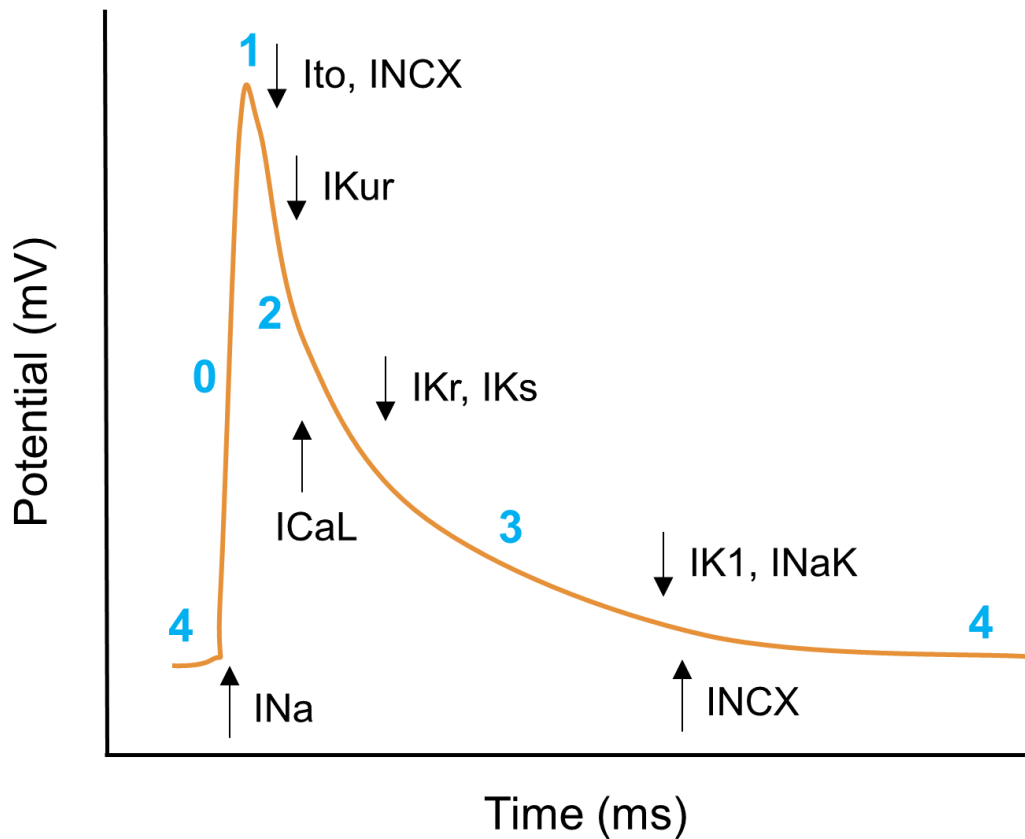


Figure 2.3 Effects of major ionic currents on the AP in atrial myocytes not capable of self-excitation. Arrows pointing upwards and downwards indicate inward and outward currents, respectively. Phases of the AP are indicated in light blue.

I_{Kr} and I_{Ks}) compete with the sarcolemmal L-type calcium channel (LTCC, generating the L-type calcium current I_{CaL}). I_{CaL} , activated as a result of the earlier membrane depolarization by the fast sodium current, allows the flow of calcium ions down the concentration gradient into the cytosol. Depending on the balance of these competing currents, the second phase of the AP may manifest via a slower repolarization, a plateau or even a dome in the voltage trace.

Following phase 2, a rapid late repolarization takes place in phase 3. Here, the repolarizing currents no longer compete with I_{CaL} . Additionally, the inward rectifier potassium current I_{K1} acts to lower the transmembrane potential further. Finally, the resting membrane potential is reached and maintained in phase 4, preparing the cell for the next excitation.

2.2.5.3 Calcium-induced calcium release

The calcium that enters the cell via I_{CaL} during phase 2 of the AP leads to the increase of the intracellular Ca^{2+} concentration. In turn, this induces the release of Ca^{2+} from the sarcoplasmic reticulum via ryanodine receptors (RyRs) in a process known as the calcium-induced calcium release, which triggers cellular contraction. This process is beyond the scope of this thesis, and the reader is referred to e.g. (Bers, 2008) for details. Following cellular contraction, the sarcoplasmic/ endoplasmic reticulum Ca^{2+} ATP-ase (SERCA), located in the membrane of the sarcoplasmic reticulum, re-fills the intracellular organelle with calcium ions. Separately, the sodium-calcium exchanger (NCX) and the plasma membrane Ca^{2+} ATP-ase (PMCA) extrude excess calcium out of the cell. The NCX expels one Ca^{2+} and introduces three Na^+ back into the cell. This influx of sodium is balanced by the Na^+/K^+ ATP-ase (NaK) pump, which expels three Na^+ out of and imports two K^+ into the cell. Aside from their role in ion homeostasis, I_{NCX} and I_{NaK} also influence the AP (Figure 2.3). The transient increase in $[Ca^{2+}]_i$ triggering cellular contraction is referred to as the intracellular calcium transient (CaiT).

2.2.6 Quantification of cardiac electrical properties

To quantify the cellular electrical properties, quantities known as the action potential biomarkers are used. These include the amplitude of the AP, abbreviated as APA, the resting membrane potential (RMP), and the AP duration at various degrees of repolarization. For example, APD_{90} refers to AP duration at 90% of repolarization, and is a measure of how quickly a cell will recover from previous excitation. Each AP biomarker is usually influenced by more than one ionic current. For example, the main determinants of APD_{90} are outward potassium repolarization currents and I_{CaL} (Sánchez et al., 2014).

To quantify electrical properties of the tissue, the effective refractory period (ERP) and conduction velocity (CV) are frequently used. The ERP determines how quickly the cell will be ready for next excitation. The ERP is governed by the recovery kinetics of I_{Na}

inactivation gates, which are in turn influenced by the membrane potential (Shaw and Rudy, 1997). Under normal conditions, the ERP is well approximated by the APD.

The CV is a measure of the speed of electrical wavefront propagation. Given two points, the CV is calculated by dividing the distance by the time it takes for the electrical excitation to travel between those points. There are two main determinants of the CV in cardiac tissue: cellular I_{Na} availability and gap junction density between cells (Dössel et al., 2012). Density of gap junctions is highest at the ends of cylindrically-shaped myocytes; as a result, conduction velocity is highest along the fibre direction than transverse to it (Section 2.2.3; (Dössel et al., 2012)).

2.2.7 Heterogeneities in atrial electrical properties

2.2.7.1 Action potential duration and effective refractory period

Electrical properties of atrial muscle tissue are heterogeneous. Several studies point towards a decrease in APD and ERP with increasing distance from the stimulation site at the SAN. In canine RA, (Feng et al., 1998) reported longest APD_{95} in CT (closest to the SAN) and shortest APD_{95} in AVR (furthest from the SAN). Separately, (Cha et al., 2005) described shorter APD_{90} in pulmonary veins than in LA in dogs. The ERP and APD are shorter in LA compared to RA, a phenomenon referred to as the RA/LA gradient in APD/ERP. In multi-cellular canine atrial preparations, (Li et al., 2001) reported a mean value of APD_{90} of 173 ms in RA and 140 ms in LA. In isolated mouse atrial myocytes, mean APD_{90} was 20 ms in RA and 17 ms in LA (Lomax et al., 2003a), a finding later reproduced in mouse atrial preparations (Nygren et al., 2004). In experiments with Langendorff-perfused rabbit hearts, mean ERP in RA and LA was 82 and 70 ms, respectively (Ravelli and Allessie, 1997). Recordings in open-chest dogs (Lee et al., 1999), pigs (Wirth et al., 2003), and conscious goats (M. C. Wijffels et al., 1995) also revealed a longer ERP in RA than in LA. A consequence of the RA/LA gradient in

APD/ERP is a small dispersion of repolarization, which in turn is thought to be cardio-protective against arrhythmias.

2.2.7.2 Heterogeneities in ionic currents

Within and between atrial chambers, heterogeneities in ionic currents underlie the gradients in APD and ERP. However, unlike the intra-atrial APD and ERP differences, the heterogeneities in ionic currents appear to be species-dependent. Table 9.1 (Appendix 1) provides detailed information on intra-atrial heterogeneities in ionic currents measured to date (Caballero et al., 2010; Cha et al., 2005; Ehrlich et al., 2003; Feng et al., 1998; Gong et al., 2008; González de la Fuente et al., 2013; Knobloch et al., 2002; Li et al., 2001; Lomax et al., 2003a, 2003b; Melnyk et al., 2005; Nakamura et al., 2010; Nygren et al., 2004; Sarmast et al., 2003; Swartz et al., 2009; Van Wagoner et al., 1997; Voigt et al., 2010; Yamashita et al., 1995). In dogs, the main ionic currents thought to underpin the APD/ERP gradient are I_{to} , I_{CaL} and I_{Kr} , with no regional differences seen in I_{Kur} and I_{K1} (Feng et al., 1998; Li et al., 2001). In mouse, I_{K1} and I_{CaL} appear to play a key role, with no differences in I_{to} and I_{Kr} (Lomax et al., 2003a; Nakamura et al., 2010). Some studies implicate I_{Kur} in intra-atrial APD gradients in mouse (Lomax et al., 2003a; Nakamura et al., 2010); however, those are usually concluded on the basis of low cell number ($n=5$), or through the use of non-specific ion channel blockers. In pigs, evidence exists for differential levels of I_{Kur} and I_{Kr} in atrial chambers (Knobloch et al., 2002); however, other currents have not yet been measured. Intra-atrial gradients in ionic currents in human tissue (particularly in control conditions) remain largely unknown.

2.2.7.3 Conduction velocity

Conduction velocity within the atria is also heterogeneous. As discussed previously, the individual cardiomyocytes are preferentially linked in an end-to-end fashion, giving rise to muscle fibres. Atrial muscle bundles (such as CT or BB) can be highly anisotropic. For instance, the ratio of longitudinal to transverse CV can be as high as 8:1 in CT (Saffitz et

al., 1994). Experimental measurements of conduction velocity outside of the highly anisotropic bundle regions are scarce. (Spach et al., 1988) reported a ratio of longitudinal to transverse CV as 4.76:1 in the human atrium; however, this measurement included conduction pathways. Therefore, it is likely that the CV anisotropy in atrial myocardium is not as large (in fact, modellers tend to assume a ratio of 2:1 (Sánchez et al., 2013; Seemann et al., 2006)). In absolute terms, the longitudinal CV measured in human atrium ranges between 51 and 120 cm/s (Dimitri et al., 2012; Feld et al., 1997; Hansson et al., 1998; Lin and Wikswo, 1999; Spach et al., 1988). Regions of slow conduction, such as in RAI or around PVs, correlate with complex fibre architectures or abrupt changes in fibre orientation (Betts et al., 2002; Feld et al., 1997; Markides et al., 2003). Non-conducting tissue (characterised by absence of myocytes) is present in atrial septal wall and electrically separates the LA and RA.

2.3 Atrial fibrillation

Figure 2.4 compares the electrical activity in the heart in normal sinus rhythm (SR) and in AF. In SR, the electrical activity of the heart is controlled by the SAN. The SAN cardiomyocytes trigger electrical excitation in the heart at a frequency between 1 Hz (at rest) up to 3 Hz (during exercise); the exact value is controlled by metabolic processes (Nattel, 2002). In SR, atrial contraction is followed by ventricular contraction at the same rate. In AF, the electrical excitation and contractile activity of the heart are no longer controlled by the pace-making function of the SAN. Instead, dyssynchronous activation of atrial myocytes at frequencies between 5.8-10 Hz overrides the SAN signals (Goodacre and Irons, 2002; Nattel, 2002). The atria do not contract regularly but quiver chaotically, failing to effectively pass blood to the ventricles. In contrast to atria, the ventricular chambers continue to contract in AF; however, they do so irregularly and at a faster-than-normal rate of c.a. 2.5 Hz (Nattel, 2002). Not all electrical activity originating in the atria

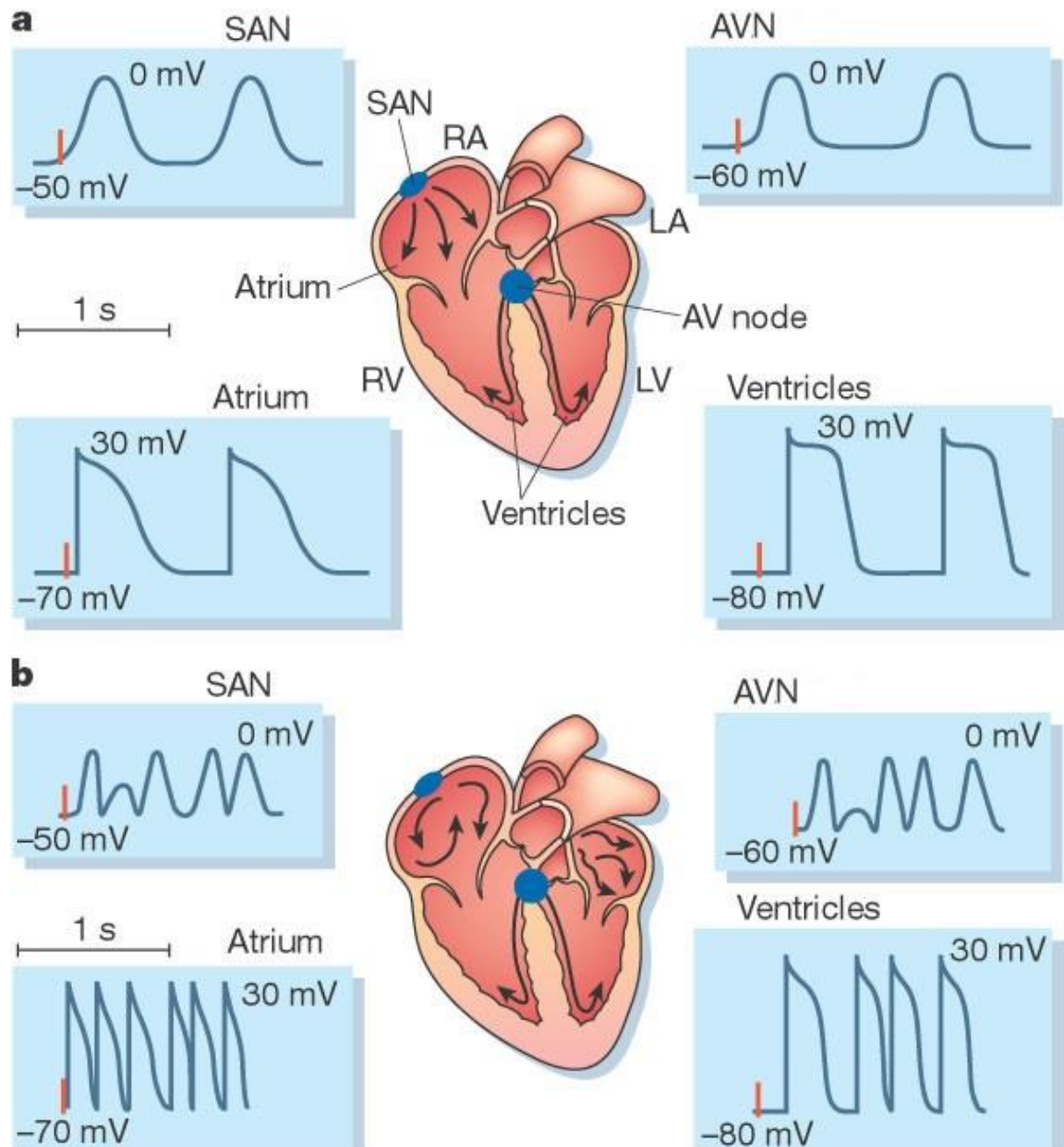


Figure 2.4 Comparison of the electrical signals in the heart in normal sinus rhythm (A) and in AF (B). Representative cellular APs from the SAN, AVN, atrium and ventricles are shown. The red vertical line on each graph indicates a common time reference. Acronyms: left ventricle – LV, right ventricle – RV. Image reproduced with permission from (Stanley Nattel, ‘New ideas about atrial fibrillation: 50 years on’, *Nature*, 415(6868), 219-226, 2002. Website URL: <https://www.nature.com/nature/journal/v415/n6868/full/415219a.html>).

propagates into the ventricles; instead, the AVN partly filters the electrical signals. Nonetheless, the electrical impulses that do propagate via AVN into the ventricles are irregular and inappropriately timed.

2.3.1 Risk factors, incidence, and morbidities

AF is the most common cardiac arrhythmia (January et al., 2014) and its incidence increases with age. It affects c.a. 1% of the general population and c.a. 10% of people above 80 years of age (Benjamin et al., 1998; Ho et al., 1993). Symptoms of AF vary between individuals and range from none to severe (January et al., 2014). Most common symptoms include fatigue, palpitations and heart failure (Hsu et al., 2004). AF is associated with a five-fold increase in risk of stroke (Alonso et al., 2009) and a three-fold increase in risk of heart failure (Krahn et al., 1995; Miller et al., 2005; Wang et al., 2003). AF also doubles the risk of mortality (Alonso et al., 2009).

A number of risk factors predisposing to AF development have been identified. The most prominent one is associated heart disease, present in over 70% of AF patients (Kozlowski et al., 2010; Wakili et al., 2011). Obesity, diabetes, hyperthyroidism as well as genetic mutations have been linked to AF (Brugada et al., 1997; Chugh et al., 2014). Additionally, patients undergoing surgery (including, but not limited to, cardiac operations such as coronary artery bypass graft or valve replacement) are likely to develop AF, with the incidence rate between 10% and 60% depending on surgery type (Maesen et al., 2012). Interestingly, a number of AF antecedents (including ageing, obesity, diabetes, inflammation following surgery) are associated with oxidative stress (Keaney et al., 2003; Maesen et al., 2012; Watanabe et al., 2008), which is the phenomenon whereby the body does not manage to neutralize excess oxidants, leading to cell damage. The mechanisms linking risk factors with AF development are a focus of intense investigation.

2.3.2 Classification of atrial fibrillation

According to AF episode duration, frequency of occurrence, and treatment success, an internationally-recognized classification of AF into four types has been devised (Fuster et al., 2011; Lévy, 2000; Levy et al., 2003):

1. Recently diagnosed (acute-onset): this type of AF occurs when the arrhythmia is diagnosed in a patient for the first time. Once detected, AF is then classified into one of the three categories below;
2. Paroxysmal AF: this type of AF is self-terminating, with the typical episode lasting less than 24 hours, although a single episode can last for up to 7 days. If the patient experiences more than one paroxysm of AF, they may be diagnosed as having persistent AF;
3. Persistent AF: episodes of AF lasting for more than a week are classified as persistent AF. Persistent AF can self-terminate or it can be stopped with cardioversion (a process of terminating an abnormally fast heart rate with drugs or by delivering an electric shock). Persistent AF is more likely to be recurrent than paroxysmal AF;
4. Chronic AF (Permanent AF): in this type of AF, the normal sinus rhythm either cannot be restored with treatment, or the AF episodes recur within 48 hours of cardioversion. AF is also classified as chronic if the long-lasting episode has never been treated.

While the above classification is internationally recognized (Levy et al., 2003), some authors modify these criteria (Allessie et al., 2002; Markides, 2003) or introduce new classification methods based on patient characteristics. For instance, some studies further subdivide the paroxysmal AF category into ‘sustained AF’ (episode lasting more than 24 hours but less than 7 days) and ‘paroxysmal AF’ (episode lasting less than 24 hours) (Wijffels et al., 1995). Other frequently used terms in the literature include lone AF (i.e. AF episodes occurring in the absence of any other cardiovascular diseases (Brand, 1985)); secondary AF (i.e. AF resulting from primary conditions such as myocardial infarction (Lévy, 2000)); post-operative AF (occurring as an aftermath of cardiac and non-cardiac surgery (Maesen et al., 2012)); and ‘non-valvular AF’ (i.e. AF occurring in the absence

of valvular diseases (Lévy, 2000)). Given this complexity, it may be the case that ‘atrial fibrillation’ is an umbrella term encompassing different pathological processes but manifesting via similar symptoms (Bonilla et al., 2012).

2.3.3 Initiation and maintenance of atrial fibrillation

Arrhythmias are generated in the presence of a vulnerable substrate (i.e. a fragment of the tissue which, upon initial excitation, depolarizes and repolarizes of its own accord, i.e. sustains a re-entrant wave or re-entry) and/or a trigger (i.e. an electrical discharge whose origin may be external to the SAN) (Shimizu and Centurion, 2002).

2.3.3.1 Trigger

An ectopic focus gives rise to a series (a ‘burst’) of rapid electrical discharges that are capable of triggering an arrhythmia episode. In paroxysmal AF, over 90% of ectopic foci have been reported in and around pulmonary veins in LA (Chen et al., 1999a, 2001; Haïssaguerre et al., 1998; Jaïs et al., 1997; Lu et al., 2001); by contrast, ectopic activity originating in the RA, usually around superior vena cava, has been reported in 4.5 – 6.2% of patients (Chen et al., 1999b; Haïssaguerre et al., 1998; Tsai et al., 2000). The intervals between consecutive ectopics in both atrial chambers were irregular and ranged from 110 to 270 ms, with a mean $\pm$ standard deviation of 175 ± 30 ms (Haïssaguerre et al., 1998). However, abnormally fast ectopic firing originating outside of the SAN can trigger fibrillation even if the ectopic impulses are regular (Berenfeld et al., 2011), for instance when the excitation wave breaks up in different regions of the atria (Nattel et al., 2008). In chronic AF, multiple ectopic foci distributed throughout the atrial tissue have been reported (Lin et al., 2001).

At the cellular level, potential mechanisms underlying spontaneous ectopic activity are (i) enhanced automaticity, (ii) early after-depolarizations (EADs), and (iii) delayed after-depolarizations (DADs), depicted in Figure 2.5. **Enhanced automaticity** can rely on

atrial myocytes external to the SAN but capable of self-excitation to fire at a faster rate than that generated by the SAN. The ionic mechanism that would cause such behaviour is hypothesised to be due to the so-called funny current, I_f . This current is normally present in atrial cardiomyocytes within the SAN, where it is responsible for the spontaneous depolarization in phase 4 of the AP (Figure 2.5A). Aside from the SAN, I_f is also present in non-self-excitable myocytes (Hoppe and Beuckelmann, 1998; Thuringer et al., 1992; Yu et al., 1993), and increased levels of HCN mRNA have been reported in right and left atrial appendages and free wall tissue from patients in AF (Lai et al., 1999b). In principle, this could lead to enhanced automaticity (as shown in Figure 2.5A). (Chen et al., 2000) reported APs with spontaneous phase 4 depolarization in canine pulmonary vein sleeves (the ‘sleeves’ are formed of the myocardial tissue extending from the LA into the PVs over distances of 10–40 mm; (Chen et al., 1999a; Hassink et al., 2003; Saito et al., 2000)). This suggests a link between AF and enhanced automaticity due to mechanisms active in phase 4 of the AP.

A mechanism behind enhanced automaticity unrelated to I_f , but linked to the difference in membrane potentials of neighbouring cells, has also been proposed. Pulmonary vein myocytes have been reported to have a resting membrane potential of c.a. -65 mV, compared to -88 mV in LA myocytes. Such a potential difference could lead to a current injection into the LA myocytes, triggering ectopic electrical activity (Cherry et al., 2007).

DADs are depolarizations of the membrane occurring after the full repolarization of the cell has taken place (Figure 2.5B; (Nattel et al., 2008)). They usually appear as small ‘bumps’ in the voltage trace; however, if the DAD exceeds the threshold potential, it will trigger a premature AP (Figure 2.5B). DADs are linked to abnormalities in intracellular calcium handling, with the main mechanism being a spontaneous calcium release from the sarcoplasmic reticulum and the resultant increased I_{NCX} activity. I_{NCX} expels one Ca^{2+} ion out of the cell while importing three Na^+ ions into the cytosol, thereby generating a

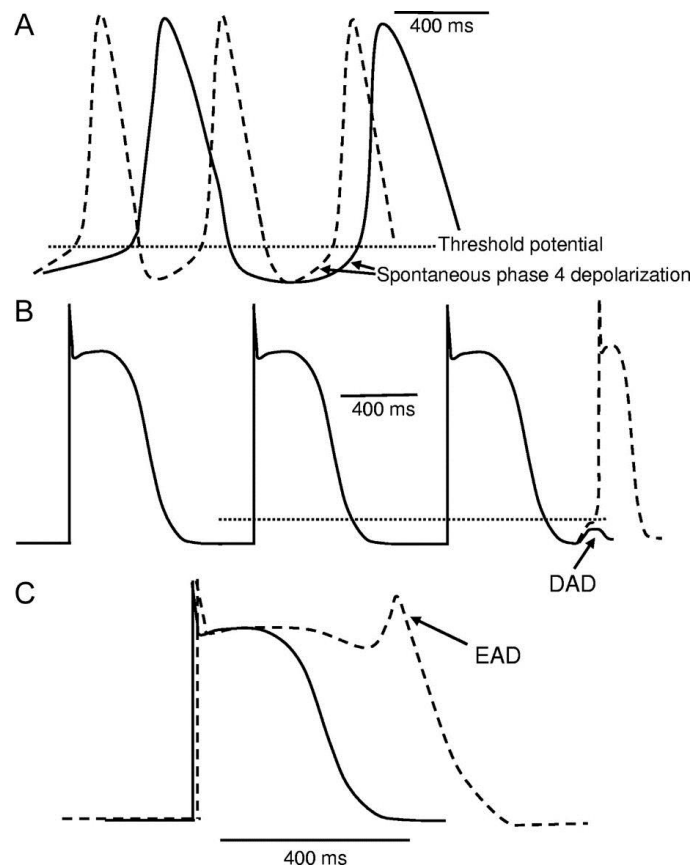


Figure 2.5 Principal cellular mechanisms that could give rise to triggered electrical activity. **A** Enhanced automaticity, **B** delayed after-depolarizations (DADs), **C** early after-depolarizations (EADs). Reproduced with permission from (Stanley Nattel, Brett Burstein, Dobromir Dobrev, ‘Atrial Remodeling and Atrial Fibrillation: Mechanisms and Implications’, *Circulation: Arrhythmia and Electrophysiology*, 1(1), 62-73, 2008. Website URL: <http://circep.ahajournals.org/content/1/1/62.long>).

net inward current capable of depolarizing the cellular membrane. Abnormal calcium handling linked to DADs can be a result of rapid cell pacing. Ca^{2+} enters the cell on each action potential; therefore, rapid pacing increases Ca^{2+} entry and may trigger spontaneous release of calcium from the sarcoplasmic reticulum, leading to DADs. Abnormal calcium handling leading to DADs and spontaneous triggered activity has been documented in canine models of AF and atrial tachycardia (Stambler et al., 2003; Yeh et al., 2008). Within LA, DADs have been reported in pulmonary vein sleeves (Chen et al., 2001).

EADs are abnormal depolarizations of the membrane potential and can be triggered in phase 2 or phase 3 of the cellular AP (Figure 2.5C). They have traditionally been linked

to I_{CaL} reactivation (Nattel et al., 2008; James N Weiss et al., 2010). Under normal conditions, the I_{CaL} current becomes activated by depolarized membrane potential quickly after the initiation of the AP, and injects calcium into the cell, often leading to the AP plateau (Figure 2.5C). Increased intracellular Ca^{2+} concentration then causes channel closing. However, depolarized membrane voltage for prolonged periods of time can again trigger I_{CaL} activation, leading to EADs. The mechanisms underpinning I_{CaL} reactivation can range from enhanced inward currents or suppressed outward currents (Passini et al., 2016; Weiss et al., 2010). More recently, a mechanism of EAD generation separate from I_{CaL} reactivation, and instead linked to non-equilibrium reactivation of I_{Na} has been proposed by (Edwards et al., 2014; Morotti et al., 2016). EADs are capable of triggering arrhythmias in atria, including AF (Burashnikov and Antzelevitch, 2003) and atrial tachycardia (Satoh and Zipes, 1998), and have been reported in pulmonary vein sleeves (Chen et al., 2001, 2000).

2.3.3.2 Vulnerable substrate

A trigger is necessary to initiate an arrhythmia, while a vulnerable substrate contributes to maintaining it. The substrate is a fragment of the atrial tissue which, upon initial excitation, is capable of sustaining a single or multiple re-entrant waves. A common mechanism behind re-entry establishment is a uni-directional block, pictured schematically in Figure 2.6. The uni-directional block relies on tissue fragments with variable ERPs being electrically connected. Then, an excitation wavefront is able to propagate in the part of the tissue that has had enough time to recover from previous excitation, but will encounter a block in the tissue that is still refractory (i.e. that has a longer ERP). The excitation wavefront travels along the path connecting the two regions and gives rise to a re-entry, provided that the time it takes for the excitation wave to travel along the path is greater than the refractory period of the tissue. This mechanism is favoured by short refractory periods, slow conduction, and long pathways linking tissue

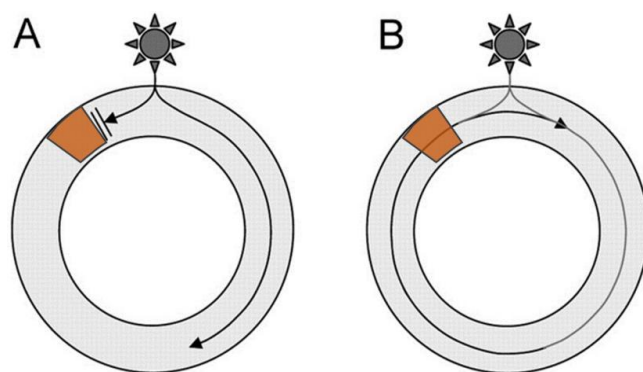


Figure 2.6 Schematic diagram illustrating the establishment and maintenance of re-entry through a uni-directional conduction block. **A** Trigger initiates an excitation wavefront which sees a uni-directional block in the part of the tissue that is refractory (orange). **B** The excitation travels along a path connecting the region of long and short ERP and forms a re-entrant pattern, provided that the time taken to travel the path is less than the refractory period of the tissue. Reproduced with permission from (Stanley Nattel, Brett Burstein, Dobromir Dobrev, ‘Atrial Remodeling and Atrial Fibrillation: Mechanisms and Implications’, *Circulation: Arrhythmia and Electrophysiology*, 1(1), 62-73, 2008. Website URL: <http://circep.ahajournals.org/content/1/1/62.long>).

regions with variable ERPs (Nattel et al., 2008). Anatomical boundaries within healthy atria (due to abrupt changes in fibre orientation, such as around PVs (Betts et al., 2002; Markides et al., 2003)) as well as changes to the atria due to past diseases (such as scars) can contribute to pro-arrhythmia by providing anchors for re-entry. Additionally, AF causes tissue remodelling which impacts the vulnerable substrate. This is discussed in the following section.

2.3.4 Atrial-fibrillation-induced remodelling of the atrial tissue

AF is a progressive disease whose recurrent episodes alter, or remodel, the affected atrial tissue, promoting future occurrences of itself. The process of AF-induced remodelling spans multiple time- and length-scales and impacts the generation of triggers and vulnerable substrate. Changes to atrial electrical properties are referred to as AF-induced electrical remodelling (AFER), and take place on the time scale of minutes to days after AF onset (Allessie et al., 2002). AFER is accompanied by changes to metabolic (Opacic et al., 2016) and contractile tissue properties (Allessie et al., 2002). Termination of AF

within the first few days results in reversal of electrical (Wijffels et al., 1995; Yue et al., 1997) and contractile tissue properties. An episode of AF lasting more than a week impacts on structural properties of the tissue (Allessie et al., 2002), which are not as readily reversible as electrical changes. Prolonged episodes of AF result in irreversible changes to the affected atria (such as atrial dilation and fibrosis (Verheule et al., 2014)). Description of metabolic and contractile remodelling is beyond the scope of this thesis; instead, I focus on electrical changes due to AF-induced electrical and structural remodelling processes.

2.3.4.1 Electrical remodelling

At the single-cell level, electrical remodelling manifests itself via the decrease in the action potential duration (APD) and in the loss of the APD rate adaptation, i.e. the remodelled cells cannot adjust the generated APD to the stimulation rate (Allessie et al., 2002). Table 2.1 summarizes the changes to ionic currents at the cell level due to AFER in human.

Within the first minutes of AF onset, a partial inhibition of the L-type calcium current occurs. It results from a drastic increase in the intracellular calcium concentration due to an increase in pacing rate (Allessie et al., 2002; Nattel, 2002). AF episodes lasting for several hours to days cause a decrease in translation of the I_{CaL} channel, attenuating the current by anywhere between 40 and 70% (Bosch et al., 1999; Christ et al., 2004; Skasa et al., 2001; Van Wagoner et al., 1999; Voigt et al., 2009; Workman et al., 2001). One study also reports a +4 mV shift in the I-V curve of I_{CaL} (Christ et al., 2004), while another notes a change in the kinetics of the current (Bosch et al., 1999). The mechanisms responsible for the decrease of I_{CaL} also cause an upregulation of the I_{K1} current by anywhere between 47% and 137% (Bosch et al., 1999; Dobrev et al., 2005, 2002, 2001; Gaborit et al., 2005; Van Wagoner et al., 1997; Workman et al., 2001), which results in hyperpolarization of the myocyte membrane and in ERP shortening.

In chronic AF, 44% to 80% reduction in the transient outward potassium current I_{to} has been observed (Bosch et al., 1999; Brandt et al., 2000; Caballero et al., 2010; Grammer et al., 2000; Van Wagoner et al., 1997; Workman et al., 2001), with one study also reporting a +16 mV shift in the I-V curve of I_{to} (Bosch et al., 1999). Sustained outward potassium current I_{sus} (which includes I_{Kur}) is unchanged in some studies (Bosch et al., 1999; Grammer et al., 2000; Workman et al., 2001), and decreased in others (Caballero et al., 2010; Van Wagoner et al., 1997). Decrease in I_{Kur} has been reported by (Brandt et al., 2000; Christ et al., 2008). Intra-atrial heterogeneities in ionic currents are affected by AFER. (Caballero et al., 2010) reports that I_{to} and I_{Kur} currents are decreased differentially in each atrium: I_{to} decrease is smaller in RA than in LA, while the opposite is observed for I_{Kur} .

Changes in I_{Ks} , I_{Kr} and I_{NCX} due to AFER have also been observed. I_{Ks} was upregulated by 100% in LA and by more than 100% in RA (Caballero et al., 2010). When it comes to data on I_{Kr} changes in persistent and/ or chronic AF, levels of hERG mRNA were decreased in three studies by values ranging between 27% and 38% (Brundel et al., 2001b; Lai et al., 1999a; Oh et al., 2010). It is important to note that the information in mRNA levels is only an indirect measure of the number of ion channels and the density of the current. Lastly, an increase in I_{NCX} levels has been observed in a number of studies, although the magnitude of the increase ranges between a 43% increase to 116% (El-Armouche et al., 2006; Neef et al., 2010; Schotten et al., 2002; Voigt et al., 2012). The intracellular calcium handling is also altered in persistent/ chronic AF. An increase in Ca^{2+} uptake by the sarcoplasmic reticulum (via the SERCA pump) has been reported by (Shanmugam et al., 2011), while (Hove-Madsen et al., 2004; Voigt et al., 2012) observed an increase in the open probability and Ca^{2+} leak, as well as an increase in Ca^{2+} release from the sarcoplasmic reticulum.

Table 2.1 Changes in ionic currents due to AF-induced electrical remodelling in human. Unless indicated otherwise, ionic currents were elicited in isolated myocytes. Updated from (Colman et al., 2013; Koivumäki et al., 2014).

Current	Experimental observation
I_{CaL}	-73% & change in kinetics (Bosch et al., 1999)
	-63% (Van Wagoner et al., 1999)
	-72% (Skasa et al., 2001)
	-63% (Workman et al., 2001)
	-50% & +4 mV shift (Christ et al., 2004)
	-64% (Gaborit et al., 2005)
	-51% (Greiser et al., 2007)
	-43% (Voigt et al., 2009)
	-27% mRNA, -55% protein (Brundel et al., 2001a)
	-57% mRNA, -43% protein (Brundel et al., 1999)
	-33% in mRNA expression (Oh et al., 2010)
I_{Kur}	-55% at 50 mV (Brandt et al., 2000)
	-22% (Christ et al., 2008)
	-47% protein (Brundel et al., 2001a)
	Decrease mRNA (Lai et al., 1999a)
	No change mRNA (Brundel et al., 2001a, 2001b)
I_{to}	-66% RA, -61% LA (Van Wagoner et al., 1997)
	-84% at +50 mV and +16 mV shift (Bosch et al., 1999)
	-44% (Brandt et al., 2000)
	-65% (Workman et al., 2001)
	-50% RA, -75% LA (Caballero et al., 2010)
	-49% (Christ et al., 2008)

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	-80% (Grammer et al., 2000)
	Decreased mRNA expression and protein level (Brundel et al., 2001a, 2001b)
I _{K1}	+106% LA, no change RA (Van Wagoner et al., 1997)
	+102% at -90 mV (Bosch et al., 1999)
	+137% at -100 mV (Dobrev et al., 2001)
	+73% at -100 mV (Dobrev et al., 2002)
	+47% at -100 mV (Dobrev et al., 2005)
	+96% at -100 mV (Gaborit et al., 2005)
	+75% at -115 mV (Workman et al., 2001)
I _{Ks}	+100% LA (Caballero et al., 2010)
	> 100% RA (Caballero et al., 2010)
	-30% mRNA (Lai et al., 1999a)
	-56% protein (Brundel et al., 2001a)
I _{NCX}	+43% ± 14% (Neef et al., 2010)
	+67% (Schotten et al., 2002)
	+116% protein level (El-Armouche et al., 2006)
	+49% (Voigt et al., 2009)
	Increase protein (Gaborit et al., 2005)
	No change mRNA (Brundel et al., 1999)
	Current, protein, mRNA increase (Voigt et al., 2012)
I _{Kr}	-27% mRNA (Lai et al., 1999a)
	-38% in mRNA expression (Brundel et al., 2001b)
	-30% in mRNA expression (Oh et al., 2010)
	No change in mRNA (Brundel et al., 2001a, 2001b)
SERCA	Increased uptake (Shanmugam et al., 2011)

	-28% mRNA, -28% protein (Brundel et al., 1999)
	No change protein (Neef et al., 2010; Schotten et al., 2002; Shanmugam et al., 2011)
	-25% protein (El-Armouche et al., 2006)
RyR	Open probability increase 4x (Voigt et al., 2012)
	Increased release (Hove-Madsen et al., 2004)
	No change mRNA (Brundel et al., 1999)
SR Ca ²⁺ leak	Increased (Neef et al., 2010; Voigt et al., 2012)

2.3.5 Structural remodelling

AFER is closely followed by the structural remodelling of the individual myocytes and the atrial tissue (Allessie et al., 2002). In the first week of AF, myocytes start losing sarcomeres, i.e. the structural elements responsible for cellular contraction (Allessie et al., 2002; Nattel and Dobrev, 2012). Dedifferentiation (i.e. reversal to a more foetal phenotype) of some myocytes is also seen, leading to increased tissue heterogeneity (Allessie et al., 2002). At the tissue level, changes in levels of the gap junction proteins have been observed. In goats, four months of AF resulted in a heterogeneous decrease in the levels of connexin 40 (Ausma et al., 2003; van der Velden et al., 2000). In a dog model of sterile pericarditis (a condition predisposing to AF), levels of connexin 40 and connexin 43 were strongly reduced in the epicardium (Ryu et al., 2007). Heterogeneous conduction in fibrillating atrium is also promoted by an increasing degree of fibrosis (Wakili et al., 2011). At the whole-atria level, enlargement of the left atrial chamber has been reported.

2.3.6 AF-induced remodelling promotes re-entry inducibility and maintenance

Figure 2.7 summarizes the principal mechanisms by which AF-induced atrial remodelling promotes re-entry inducibility and maintenance. AFER shortens APD in individual cells

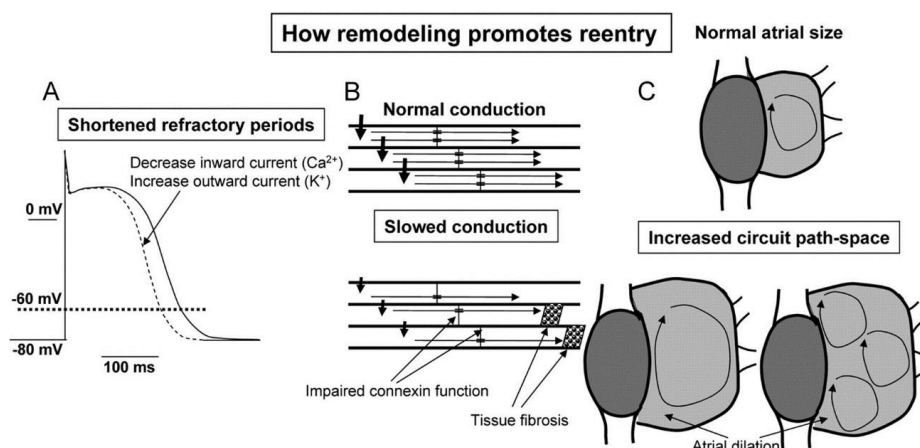


Figure 2.7 Mechanisms by which AF-induced remodelling promotes re-entry. **A** shortened refractory periods, **B** slowed conduction, **C** increased circuit path-space due to atrial dilatation. Reproduced with permission from (Stanley Nattel, Brett Burstein, Dobromir Dobrev, 'Atrial Remodeling and Atrial Fibrillation: Mechanisms and Implications', *Circulation: Arrhythmia and Electrophysiology*, 1(1), 62-73, 2008).

and the ERP at the tissue level. Structural remodelling impairs electrical communication between the cells due to reduced levels of connexin proteins. Together with the increasing degree of fibrosis, these changes promote tissue heterogeneity and slow CV. In turn, this increases the likelihood of the excitation wavefront breaking up into multiple small wavelets (Gharaviri et al., 2012; Verheule et al., 2014) and minimises the chances of AF self-termination. Permanent AF is associated with a dilation of LA, which is in turn able to support a greater number of re-entrant circuits. Additionally, AFER promotes DADs by increased I_{NCX} and increased open probability of RyR (Voigt et al., 2012). In a canine model of rapid atrial pacing, an increased incidence of EADs and DADs around pulmonary veins has been reported (Chen et al., 2001, 2000). This demonstrates the impact of AF on triggered activity.

2.4 Nitric oxide and neuronal Nitric Oxide Synthases in the heart

Nitric oxide (NO) is a signalling molecule regulating atrial electrical properties (Tamargo et al., 2010). The NO molecule is a reducing agent, and is produced from L-arginine in a biochemical reaction catalysed by a family of enzymes known as Nitric Oxide Synthases

(NOSs). The NOS family includes the ‘endothelial’, ‘neuronal’, and ‘inducible’ isoforms, also referred to as eNOS, nNOS and iNOS. Under normal conditions, eNOS and nNOS are expressed in cardiac tissue; iNOS is only expressed during inflammatory and pathological states (such as heart failure; (Haywood et al., 1996; Vejlstrup et al., 1998)). Short-term AF has been linked with a profound decrease in levels of atrial NO and with inconsistent changes of eNOS content. (Cai et al., 2002) demonstrated a decrease, and (Shiroshita-Takeshita et al., 2007) reported an increase in eNOS atrial content in animal models, respectively. By contrast, decreased nNOS levels have been linked to AF (Liu et al., 2013; Reilly et al., 2016). As a result, this review focuses on the nNOS protein.

Much of what is known about nNOS in the heart comes from studies focusing on ventricles (as in the case of myocardial infarction and ventricular arrhythmias) or ventricular myocytes, typically isolated from various animal models (such as mouse, rat, and dog). This section focuses on reviewing the current knowledge on nNOS in the context of such studies.

2.4.1 Neuronal isoform of Nitric Oxide Synthase

Compartmentalized to both the sarcoplasmic reticulum (Xu et al., 1999) and the plasma membrane (Ueda et al., 2008) of ventricular myocytes, nNOS is located in close proximity to calcium-handling proteins, including LTCC (Sears et al., 2003), SERCA (Sears et al., 2003; Xu et al., 1999; Zhang et al., 2008), RyR (Lim et al., 2008; Xu et al., 1999), and PMCA (Ueda et al., 2008), as well as the sodium channel (Ueda et al., 2008). nNOS regulates the intracellular calcium fluxes as well as Ca^{2+} and Na^{+} homeostasis in the cell, and is involved in the excitation-contraction coupling in cardiomyocytes. In fact, nNOS levels and activity are elevated in early stages of heart disease, where NO plays a cardioprotective role (Burger et al., 2009; Jin et al., 2013; Loyer et al., 2008). This is due to its regulation of the calcium-handling proteins (LTCC, SERCA, RyR). For instance, a

study by (Burger et al., 2009) reveals that in mouse hearts suffering from myocardial infarction, nNOS (and hence NO) levels are elevated, and nNOS-generated NO S-nitrosylates LTCC, SERCA, and RyR, and reduces both the influx of Ca^{2+} into the cell and the intracellular calcium transient amplitude (S-nitrosylation is a post-translational modification of a protein whereby NO is covalently attached to a thiol moiety of a cysteine amino acid). This prevents calcium accumulation in ventricular myocytes, and lowers the risk of ventricular arrhythmias. In contrast, reduced levels of nNOS in myocardial infarction hearts promote arrhythmogenesis (Burger et al., 2009).

However, nNOS action is not limited to the calcium-handling subsystem: amongst others, nNOS-derived NO was found to inhibit one of the most crucial cellular processes in cardiac cells, namely mitochondrial respiration (Chouchani et al., 2013; Torres et al., 1995; Welter et al., 1996). nNOS also regulates cardiac oxidases expressed in the heart (including NADPH oxidase and xantine oxidase) (Zhang et al., 2014, 2009), impacting the levels of reactive oxygen species. Additionally, nNOS regulates kinase and phosphatase-dependent phosphorylation (Zhang et al., 2014, 2009). The aspects of nNOS functionality relevant to cardiac electrophysiology are summarized in Table 2.2.

SERCA is inhibited by the protein phospholamban (PLM) when the latter is not phosphorylated. nNOS controls PLM phosphorylation, influencing the activity of SERCA: decreased levels of nNOS cause a drop in the numbers of phosphorylated PLM (Roof et al., 2012; Wang et al., 2008), thus increasing the amount of PLM bound to SERCA (Zhang et al., 2008). Inhibition of SERCA results in a prolongation of the intracellular calcium transient. On the other hand, elevated levels of nNOS localized to the sarcoplasmic reticulum result in an increased phosphorylation of PLM and enhanced SERCA activity (Loyer et al., 2008). Additionally, there is evidence for S-nitrosylation of SERCA (Burger et al., 2009), but the functional significance of this is unclear.

Table 2.2 Impact of increased nNOS levels (nNOS↑) and nNOS inhibition/gene deletion (nNOS ↓) on the ionic currents in mouse ventricular myocytes.

Protein/Channel	Current	Effect of nNOS ↓	Effect of nNOS ↑
SERCA	J_{up}	↓ (Zhang et al., 2008)	↑ (Loyer et al., 2008)
LTCC	I_{CaL}	↑ (Sears et al., 2003)	↓ (Burkard et al., 2007)
RyR	J_{rel}	↑ (Gonzalez et al., 2007), ↓ (Wang et al., 2008)	–
NaK pump	I_{NaK}	↓ (Zhou et al., 2002)	–
NCX	I_{NCX}	–	–
SCN5A	I_{Na}	–	↑ (Ueda et al., 2008) (SCN5A mutation)

RyR response to variations in nNOS levels is a contentious issue in the literature. Gonzalez *et al.* used transgenic mice with nNOS gene deletion to show that reduced levels of nNOS result in a decrease in S-nitrosylation of RyR and to an increase in RyR oxidation, thus resulting in an *increased* diastolic calcium leak (Gonzalez et al., 2007). Contrastingly, Wang *et al.* used the same mouse model to demonstrate that while RyR S-nitrosylation decreases upon both nNOS inhibition and gene deletion, the probability of RyR being open *decreases* (Wang et al., 2008). Studies also report conflicting evidence on nNOS association with RyR (Barouch et al., 2002; Burkard et al., 2007; Loyer et al., 2008). Interestingly, Loyer *et al.* note the co-immunoprecipitation of nNOS and RyR (but not SERCA and LTCC) (Loyer et al., 2008), while Burkard *et al.* contradict these findings reporting nNOS co-immunoprecipitation with SERCA and LTCC but not RyR (Burkard et al., 2007). At present, the reason for such discrepancies is unclear. However, RyR activity depends on multiple factors which include its phosphorylation, oxidation (especially by xanthine oxidase), the level of S-nitrosylation, as well as the concentrations

of Ca^{2+} and NO (Lim et al., 2008; Zhang et al., 2014). Those factors could be behind the experimental differences described above.

NCX activity is left unaffected by a reduction in nNOS levels (Gonzalez et al., 2007; Sears et al., 2003), although Gonzalez *et al.* report an increase in NCX levels in their mouse model of nNOS gene deletion (Gonzalez et al., 2007). It is worth noting that Zhou *et al.* demonstrate that nNOS knockout decreases the activity of the mouse **NaK pump** (Zhou et al., 2002), suggesting that nNOS could influence NCX indirectly through its influence on Na^+ homeostasis. However, the experiments were performed on cells with low levels of both neuronal and endothelial NOS isoforms, so it is not clear to what extend nNOS impacts I_{NaK} or I_{NCX} .

PMCA inhibits nNOS (Ueda et al., 2008). This is a result of alpha-syntrophin docking nNOS in sarcolemma and subsequently forming a complex with PMCA and the sodium channel SCN5A (Ueda et al., 2008). **Sodium channel SCN5A** is stimulated by nNOS-derived NO. Absence of alpha-syntrophin results in dissociation of the complex formed by nNOS, PMCA and SCN5A (Ueda et al., 2008). Uninhibited nNOS S-nitrosylates the SCN5A channel, causing an increase in the two components of the sodium current: the peak sodium current I_{Na} and the late sodium current I_{NaL} (Ueda et al., 2008). This has been observed in the long QT syndrome with a mutation in the SCN5A gene (Ueda et al., 2008; Zhang et al., 2014).

nNOS function in atria is less well understood than in ventricles. This is chiefly because studies to date focus on the effects of two NOS isoforms distinct from nNOS, i.e. eNOS and iNOS (Bonilla et al., 2012). As already mentioned, elevated levels of nNOS are cardioprotective in ventricles in early stages of heart disease (Burger et al., 2009; Jin et al., 2013; Loyer et al., 2008), thus decreasing nNOS protein content (and activity) can be expected to be pro-arrhythmic. (Liu et al., 2013; Reilly et al., 2016) confirm this by noting

that nNOS depletion in atrial myocytes correlates with increased AF inducibility and sustainability. However, the question remains about the specific mechanisms by which nNOS exerts its action in atria. In ventricular myocytes, increased nNOS levels are anti-arrhythmic in a large part due to the impact of nNOS on the calcium subsystem. Unlike ventricular myocytes, atrial myocytes lack a prominent t-tubular network (Brette and Orchard, 2003; Smyrniak et al., 2010). Therefore, the cardio-protective effects of nNOS in the upper and lower chambers of the heart likely operate via different mechanisms. Electrophysiological effects of nNOS in relation to AF in atria are explored in (Liu et al., 2013; Reilly et al., 2016) as well as in Chapter 5 of this thesis.

2.5 Biological variability

Biological variability, also referred to as physiological or inter-subject variability, is seen at all scales in biological systems. It manifests via differences in physiological function between individuals of the same species, and more often in drastic differences between individuals when conditions change, e.g. in disease or under drug action (Britton et al., 2013; Marder and Taylor, 2011; Sarkar et al., 2012). Physiological variability has significant implications for treating and managing heart diseases. For instance, drugs that are designed to have anti-arrhythmic properties in diseased tissue, at certain heart rates, and with a particular acid-base balance, can become pro-arrhythmic at different heart rates or in less diseased tissue (Savelieva and Camm, 2008). Likewise, susceptibility to pathological conditions such as arrhythmias can also differ from individual to individual or depending on the condition of the patient (Severi et al., 2009; Vincenti et al., 2014). By studying variability, one can explore and improve the current understanding of the mechanisms that lead to differences in outcomes when different individuals have the same condition or are given the same treatment. Physiological variability is difficult to investigate using experimental methods alone (Carusi et al., 2012; Sarkar et al., 2012) due

to the need to average data to control experimental error. As a result, variability has often been ignored in studies to date. However, recent developments in computer modelling used in synergy with experimental data enable investigations into causes and implications of physiological variability (Britton et al., 2013; Groenendaal et al., 2015; Sarkar et al., 2012). In what follows, I review experimental evidence for the importance of biological variability at different spatio-temporal scales in the heart, and discuss implications of variability in medicine and arrhythmia research.

2.5.1 Variability in cardiac electrophysiology is observed at different scales

In cardiac electrophysiology, there are significant inter-subject and intra-subject differences in the electrical activity of cardiac tissue from the same region of the heart (Feng et al., 1998; Walmsley et al., 2015). Figure 2.8A-B illustrates experimentally-observed variability in cardiac electrophysiology at the level of ionic current density and action potentials elicited from different isolated myocytes, while Figure 2.8C shows body surface electrical activity recorded in the ECG lead V5 in two patients. In addition to inter-patient variability, Figure 2.8C also highlights temporal variability within each patient (shown as overlapping curves following the same morphology). Table 2.3 provides a summary of the AP properties and ranges of values reported in recent studies, emphasising consistent variability in the AP across multiple species and sample/tissue types.

2.5.2 Causes and modulators of variability

At the level of an isolated cell, one cause of variability is the biophysical processes responsible for the flow of ionic currents across the cellular membrane. Multiple proteins regulate the sarcolemmal flow of ionic species vital for electrophysiological function, including sodium, calcium, and potassium ions, and an alteration in the balance of these ionic currents would give rise to differences in the AP. Crucially, these currents are affect-

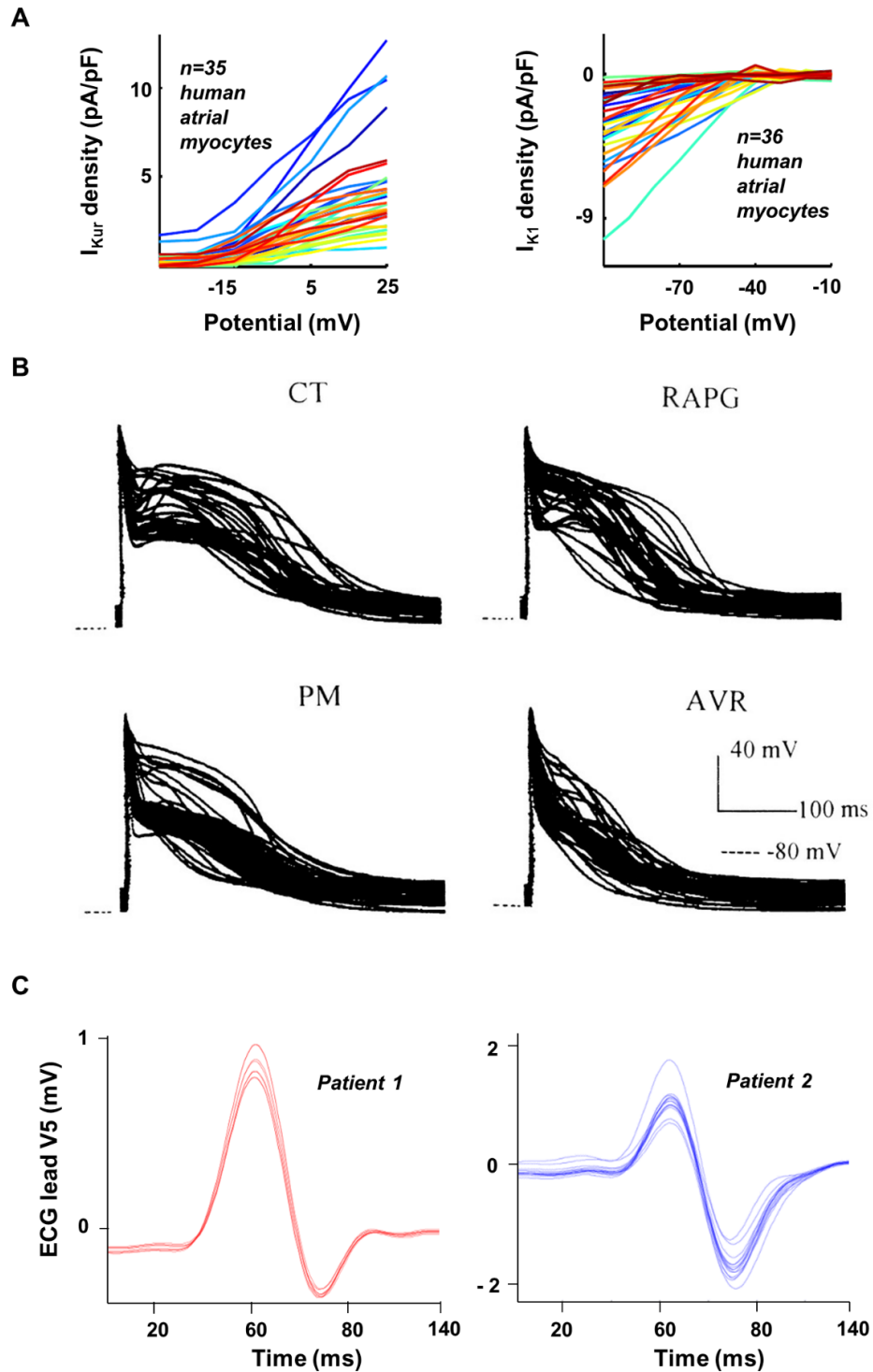


Figure 2.8 Variability in cardiac electrophysiological properties at the level of **A** ionic currents, **B** action potentials, and **C** body surface activity measured in the ECG lead V5. Data in **A** were obtained from human atrial myocytes and are used in Chapter 4 in this thesis. Plots in **B** were reproduced with permission from (Jianlin Feng, Lixia Yue, Zhiguo Wang, Stanley Nattel, 'Ionic Mechanisms of Regional Action Potential Heterogeneity in the Canine Right Atrium', *Circulation Research*, 83(5), 541-551, 1998) and show individual AP traces in CT, RAPG, PM and AVR regions of canine RA. Data in **C** illustrate inter-subject and temporal differences (the latter shown as overlapping curves following the same morphology) and were provided by Aurore Lyon.

Table 2.3 Variability in physiological properties across species, experiment type, tissue/cell type, investigated electrophysiological properties, measurement frequencies (for AP measurement only). Minimum and maximum values of APD₉₀ at 1 Hz are used as illustration of variability (with the exception of (Zhou et al., 2015, 2013), where data at 1.67 Hz are reported). Acronyms: APD_{xx} – action potential duration at xx% repolarization, RMP – resting membrane potential, APA – action potential amplitude, dV/dt_{MEAN} and dV/dt_{MAX} – mean and maximum slope of the AP upstroke, V₂₀ – voltage measured at 20% of APD₉₀ time, ARI – activation-recovery interval. n/N refers to the number of samples/patients. Reproduced from (Muszkiewicz et al., 2016).

Species	Experiment type; Tissue/ cell type and cell number	Properties investigated	Frequencies at which APs were elicited	APD ₉₀		Reference
				Min (ms)	Max (ms)	
Human	Whole-cell patch-clamp on isolated atrial cardiomyocytes (n=29 cells)	APD ₂₀ , APD ₅₀ , APD ₉₀ , RMP, APA	0.25, 0.5, 1, 2, 3 Hz	63.4	131.6	(Liu et al., 2013; Muszkiewicz et al., 2014)
Human	Micro-electrode recordings on atrial trabeculae from patients in SR (n=254; N=214)	APD ₂₀ , APD ₅₀ , APD ₉₀ , RMP, APA, V ₂₀ , dV/dt _{MAX}	1 Hz	193	467	(Sánchez et al., 2014)
Human	Micro-electrode recordings on atrial trabeculae from patients with chronic AF (n=215; N=149 patients)	APD ₂₀ , APD ₅₀ , APD ₉₀ , RMP, APA, V ₂₀ , dV/dt _{MAX}	1 Hz	141	349	(Sánchez et al., 2014)
Human	Whole-cell patch-clamp on isolated ventricular non- diseased cardiomyocytes (n=25)	APD ₂₀ , APD ₅₀ , APD ₉₀ , RMP, APA, dV/dt _{MEAN}	0.2, 0.5, 1 Hz	105	687	(Coppini et al., 2013; Passini et al., 2016)

Human	Whole-cell patch-clamp on isolated ventricular hypertrophic cardiomyopathy cardiomyocytes (n=80)	APD ₂₀ , APD ₅₀ , APD ₉₀ , RMP, APA, dV/dt _{MEAN}	0.2, 0.5, 1 Hz	238	997	(Coppini et al., 2013; Passini et al., 2016)
Human	Micro-electrode recordings on human right ventricular non-diseased papillary and trabeculae samples (n=62; N=38)	Peak voltage, Time of peak voltage, APD ₄₀ , APD ₅₀ , APD ₉₀ , Triangulation 90-40, RMP	1 Hz	178	442	(Britton et al., 2014)
Human	In vivo epicardial sock yielding in vivo electrograms (240 sites in n=41 patients)	ARI (as surrogate of APD ₉₀)	1.67, 1.82, 2, 2.22, 2.5, 2.86 Hz	149*	391*	(Zhou et al., 2015, 2013)
Rabbit	Micro-electrode recordings on isolated rabbit Purkinje fibres (n = 12)	APD ₉₀ , RMP, Peak voltage, dV/dt _{MAX} , Plateau Duration, Peak Dome	0.2, 1, 2 Hz	188	342	(Britton et al., 2013)
Rabbit	Data from isolated ventricular myocytes, LV tissue, Langendorff-perfused hearts from 13 sources, as identified in systematic literature review	APD ₉₀ , APD ₅₀	1, 1.667, 2.5 Hz	167	230	(Gemmell et al., 2014)

ted by varying protein levels (Schulz et al., 2006), cell environment (Severi et al., 2009; Vincenti et al., 2014), and circadian rhythms (Jeyaraj et al., 2012; Ko et al., 2009). Therefore, even for a specific cell, the balance of ionic currents will change in time or under drug action and following the onset of disease, altering the AP. Differences in cellular APs combine with structural differences between hearts (such as in scar location), resulting in differing functional behaviour between organs. These can manifest as different re-entry patterns, and may require personalized interventions (Arevalo et al., 2016).

The biological basis for variability in ionic currents and the cellular AP stems in part from the multitude of regulatory processes internal to the cell. Specifically:

- **DNA mutations** can result in changes to ion channel structure and function. For instance, (Chen et al., 2003) described a gain-of-function mutation in KCNQ1 gene encoding the pore-forming subunit of the I_{Ks} channel, relating it to hereditary persistent AF.
- **Epigenetic changes** such as DNA methylation or histone deacetylation are also important determinants of variability. (Liu et al., 2008) showed that inhibition of an enzyme catalysing histone deacetylation decreases AF inducibility in an animal model of cardiac hypertrophy.
- In the process of **DNA transcription into RNA**, variability manifests itself in e.g. the overall number of RNA copies produced as well as in temporal variations of gene transcription (Jeyaraj et al., 2012; Ko et al., 2009). For instance, (Jeyaraj et al., 2012) linked circadian-rhythmic variation in messenger RNA that codes for an important I_{to} -forming subunit with the variability in the levels of the corresponding protein as well as variation in cardiac repolarization.

- **Translation of RNA gene messages into proteins** is impacted by e.g. the accessibility of messenger RNA molecules for translation by the ribosome as well as messenger RNA lifetime (stability). Processes such as RNA silencing by small non-coding RNAs repress translation and thus decrease protein levels; an example of this is described in Chapter 5 of this thesis.
- **Post-translational modification** of already synthesised proteins, leading to a change in protein function, is another manifestation of variability at the cellular level. Post-translational modification reactions include phosphorylation and S-nitrosylation, and examples of their impact on cellular function via the LTCC, SERCA and RyR proteins have been described in the previous section of this chapter.
- **Protein stability and degradation** also constitute causes and modulators of cellular variability.

In addition to impacting selected ionic currents, the processes outlined above could also modify the cellular surface-to-volume ratio and membrane capacitance. Variability in membrane capacitance in control human ventricular myocytes ranges between 96 ± 1 pF to 227 ± 15 pF, and increases with age (Polak and Fijorek, 2012). Certain diseases are also known to alter both cellular volume and membrane capacitance. For instance, increased protein content in cardiac hypertrophy leads to an increase in cell volume (Pagan et al., 2013). In heart failure, (Crossman et al., 2015) reported loss of t-tubules ('detubulation') in ventricular cardiomyocytes, which could impact the cellular surface-to-volume ratio.

Aside from the intra-cellular regulatory processes discussed above, factors external to the cell can act as sources and determinants of cell-to-cell variability. Examples include signalling molecules produced elsewhere in the body, such as hormones or neurotransmitters (e.g. acetylcholine secretion leads to the activation of the acetylcholine-

sensitive potassium repolarization current and shortening of the atrial AP; (Kovacs and Bailey, 1985)). Variability in ionic concentrations can also influence cellular electrical properties. (Severi et al., 2009) reported an inverse relationship between APD and extracellular calcium concentration *in vivo* and *in vitro*, while (Vincenti et al., 2014) related a drop in K^+ plasma concentration during a haemodialysis session to AF onset, partly due to slowed cellular depolarization and a decreased refractory period.

Each of the processes described above can impact the flow of ionic currents across the cellular membrane and for the generation of the AP, yielding variability at the ionic and cellular levels. From the standpoint of molecular cellular biology, one may expect some degree of covariation in the cellular regulating networks. For instance, in the case of the post-translational modification of proteins by phosphorylation, different proteins can become phosphorylated at once, altering each protein's function. Biological variability in cardiac electrical activity has so far been studied at the level of ionic currents and cellular APs rather than in molecular regulating networks (Britton et al., 2013; Groenendaal et al., 2015; Sobie, 2009). With the exception of the study by (Milstein et al., 2012), who demonstrated a reciprocal modulation of I_{K1} and I_{Na} within a macromolecular complex, covariation in e.g. ionic current densities in cardiac myocytes has not been studied or measured. For that reason, it is acceptable to assume a lack of covariation between ionic currents in studies of variability in cardiac myocytes, as in (Britton et al., 2013; Groenendaal et al., 2015; Sobie, 2009).

Inter-subject variability in controlled animal breeds is likely to be smaller than in the heterogeneous human population. Typically, the genetic background of the animals used for laboratory experiments is known; furthermore, the animals are kept under controlled conditions (not only in terms of diet and exercise, but in some cases also e.g. hormonal factors). By contrast, the human population – with varying genomes and exposed to a vast

range of environmental stimuli – will exhibit a greater degree of variability. This is expected to hold even in specific patients' cohorts, and is due to the difficulties in controlling for the extremely large number of potential causes and modulators of inter-subject variability. Additionally, experimental measurements of variability in humans come from patients who have different and often disease-related backgrounds, and thus represent only a subset of the 'true' variability in the human population.

An adequate control of measurement error is an important aspect of investigations into biological variability as well as its sources and determinants. The contribution of measurement error to experimentally-measured variability depends on the type of experiment used to elicit the data as well as on the number of data points available. For instance, a train of regular APs elicited from a specific cell is typically averaged over several beats to yield a mean AP trace, from which the associated biomarkers are computed (as in (Reilly et al., 2016)). The impact of measurement error on the AP trace is diminished due to this averaging, in turn made possible by applying the stimulation protocol repeatedly to the same cell. By contrast, the voltage-clamp protocol used to elicit a specific ionic current is applied to the cell only once, potentially leaving a wider window of opportunity for measurement error to affect experimental results. For studies of variability involving small datasets, measurement error can be controlled using intuition and experience (i.e. by manually excluding a (subset of) data point(s) judged as outliers). Larger datasets may allow for an application of rigorous statistical tools to this purpose. This is discussed in more detail in Chapter 3 of this thesis.

2.5.3 Importance of variability in biology and medicine

Understanding inter-subject and intra-subject variability in physiological and pathological function is one of the biggest challenges in biology and medicine. Sex and age differences have been identified as important determinants of inter-subject variability

(Abi-Gerges et al., 2006, 2004; Gaborit et al., 2010; Ganjehei et al., 2011; Herraiz-Martínez et al., 2015; Sims et al., 2008; Villareal et al., 2001) For instance, (Abi-Gerges et al., 2006, 2004) discussed differences in cardiac electrophysiology and inducibility of ventricular arrhythmias between females and males, while (Rosano et al., 1996) showed that susceptibility to paroxysmal supraventricular tachycardia in women varies with changes in hormonal levels during menstrual cycle. Additionally, (Sims et al., 2008) demonstrated that sex, age, and regional differences in levels of I_{CaL} ionic current are determinants of arrhythmia phenotype in rabbit with drug-induced long QT syndrome, while (Herraiz-Martínez et al., 2015) demonstrated that the amplitude of intracellular calcium transient in atrial cardiomyocytes decreased with age.

Intra-subject variability in electrophysiology manifests itself as spatial heterogeneities (including differences in repolarization from different regions of the heart, as in section 2.2.7). This variability within the same individual can also be modulated temporally by, for example, heterogeneous nervous innervation or circadian rhythms. In mice, circadian rhythms were found to alter protein levels of an important I_{to} subunit by approximately $\pm 40\%$ from the midpoint over a 24 hr cycle (Jeyaraj et al., 2012). Beat-to-beat variability in the AP is another manifestation of temporal variability, and processes such as alternans and the stochastic nature of ion channel dynamics have been implicated (Heijman et al., 2013; Pueyo et al., 2011; Walmsley et al., 2015). mRNA expression of genes that code for ion channel proteins is influenced by post-transcriptional regulation and buffering through processes that translate mRNA to functional ion channels (Nattel et al., 2010). There is evidence that mRNA expression can vary by large amounts, from almost complete downregulation up to increases of at least four-fold compared to baseline (Borlak and Thum, 2003) in the most extreme cases of remodelling such as heart failure.

At the level of single cells, physiological variability may manifest itself not only via differences in ionic current densities, but through differences in other variables impacting ionic current balance. For instance, inter-cellular variability in cardiomyocyte volume and membrane capacitance has been linked to patients' age (Polak and Fijorek, 2012). Variability in extracellular and intracellular ionic concentrations has been shown to have an effect on the AP-level properties such as the resting membrane potential and APD_{90} (Muszkiewicz et al., 2014, Passini et al., 2014, Vincenti et al., 2014).

3 MULTI-SCALE MODELLING AND SIMULATION IN CELLULAR AND WHOLE-ATRIA CARDIAC ELECTROPHYSIOLOGY

3.1 Introduction

In this chapter, I describe computational methods used for modelling and simulations in cellular and whole-atria cardiac electrophysiology. I start at the single-cell level, where I present the Hodgkin-Huxley model – the first action potential model to be published – and describe the mathematical equations controlling ionic currents and the cellular AP. I then provide a brief history of single-cell cardiac models, finishing with a comparison of the most recent models of human atrial electrophysiology. Following this, I move on to describe whole-atria modelling and simulation. Equations governing electrical excitation propagation in tissue are provided, and an overview of the geometrical models of whole atrial anatomy is described. I also describe how changes due to AF have been modelled in the literature. Finally, I shift gears to discuss computational approaches used to study variability, focussing on the experimentally-calibrated population-of-models methodology used in later chapters of this thesis.

3.2 Models of single-cell electrophysiology

3.2.1 The Hodgkin-Huxley model

The model of squid giant axon electrophysiology by (Hodgkin and Huxley, 1952) was the first action potential model to be published. While it describes the electrophysiological processes giving rise to an action potential in a neuronal cell, the concepts established by Hodgkin and Huxley are readily applicable to other excitable cells, including cardiac myocytes. Under the Hodgkin and Huxley formulation, the elements of the cell are treated as components of an electrical circuit. The membrane of the cell acts as a capacitor in parallel with resistors representing individual ionic currents. The interplay between the major currents involved in the AP generation is described with a system of non-linearly coupled ordinary differential equations. The total current I_{tot} through the membrane is:

$$I_{tot} = C_m \frac{dV_m}{dt} + I_{ion} \quad (3.1)$$

where C_m is the membrane capacitance, V_m is the transmembrane potential, and I_{ion} represents the sum of all transmembrane currents that arise as a result of ionic species flowing through the membrane (with inward currents, where positively charged ions flow into the cell, having a negative sign). In the original Hodgkin and Huxley model, I_{ion} consists of three terms: the sodium current I_{Na} , the potassium current I_K , and a leakage current I_l to account for other ionic species (e.g. chloride). For a particular ion type x , the ionic current I_x has the formulation:

$$I_x = G_x \prod_i \gamma_i (V_m - E_x)$$

with G_x , γ_i and E_x being the maximal conductance of the current, the gating variables describing the channel open probability, and the Nernst (reversal) potential for ion x . In what follows, I describe the roles of those three terms.

The maximal conductance of a particular current is a constant value that scales that current's magnitude. It can be thought of as the maximal current that could flow through one ion channel, multiplied by the number of channels of that particular type in the cellular membrane.

The gating variables are the most complicated terms in the equation for I_x . The behaviour of one current can be expressed as a product of different gating variables, describing activation, deactivation ('recovery from activation'), inactivation, and recovery from inactivation. For instance, three independent activation gates m and one inactivation gate h are present in the Hodgkin-Huxley formulation of the I_{Na} current, meaning that the product of gating variables becomes $\prod_i \gamma_i = m^3 h$. Similarly, the I_K current is controlled by four independent activation gates n (the gating variables in the case of the leakage current I_l are set to 1). Thus, in the Hodgkin-Huxley model of squid giant axon, the formulae for the three ionic currents are:

$$I_{Na} = G_{Na} m^3 h (V_m - E_{Na})$$

$$I_K = G_K n^4 (V_m - E_K)$$

$$I_l = G_l (V_m - E_l)$$

Gating variables are typically time- and voltage-dependent (although concentration-dependent processes can also be described, e.g. (C. Luo and Rudy, 1994; C. H. Luo and Rudy, 1994)). The general formula for the time dependence of the gating variable γ is:

$$\frac{d\gamma_i}{dt} = \alpha_{\gamma_i}(1 - \gamma_i) - \beta_{\gamma_i}\gamma_i$$

where α_{γ_i} and β_{γ_i} are rate constants describing the transition from open to closed state of the channel and vice versa. For the m , h , and n gates of the Hodgkin and Huxley model, the rate constants are given by the formulae obtained by fitting to experimental data:

$$\alpha_m = 0.1 \frac{25 - V_m}{e^{\frac{25 - V_m}{10}} - 1}; \quad \beta_m = 4e^{\frac{-V_m}{18}}$$

$$\alpha_h = 0.07e^{\frac{-V_m}{20}}; \quad \beta_h = \frac{1}{e^{\frac{30 - V_m}{10}} + 1}$$

$$\alpha_n = 0.01 \frac{10 - V_m}{e^{\frac{10 - V_m}{10}} - 1}; \quad \beta_n = 0.125e^{\frac{-V_m}{80}}$$

The values of the gating variables range between 0 and 1, and their product gives the channel open probability.

Finally, the third term in the equation for I_x consists of the difference between the membrane potential V_m and the Nernst (reversal) potential E_x . It combines the effects of diffusion and the electromagnetic force on ions moving across the membrane. Diffusion acts to equalize ionic concentrations on either side of the membrane. However, if the membrane has a potential difference relative to its surroundings (i.e. it is charged), then the positively-charged ionic species will be attracted to the membrane when it is negatively charged and vice versa. The reversal potential determines the equilibrium point for a particular ion type (that is, the membrane potential at which a zero net flux of that particular ionic species happens across the membrane). It is given by the formula:

$$E_x = \frac{-RT}{zF} \ln \frac{[x]_i}{[x]_e}$$

where R , T , z and F are the gas constant, temperature, the valency of the ion x , and the Faraday constant, respectively. The terms $[x]_i$ and $[x]_e$ represent the intracellular and extracellular concentrations of the ionic species x .

In the Hodgkin-Huxley model, the terms for I_{Na} , I_K , and I_l can be added together to yield the term I_{ion} , representing the current flow through the ion channels. In the absence of applied currents, I_{tot} is set to 0 and the equation for transmembrane potential becomes:

$$\frac{dV_m}{dt} = \frac{-1}{C_m} (I_{ion})$$

In non-self-excitatory cardiac myocytes, an external stimulus current I_{stim} is applied to the cell (model) to elicit an action potential. This is included in the formula to yield:

$$\frac{dV_m}{dt} = \frac{-1}{C_m} (I_{ion} - I_{stim})$$

This equation is solved numerically to yield the action potential trace.

3.2.2 Evolution of cardiac electrophysiological models

Within the last fifty years, collaborations between experimentalists and modellers have yielded a number of biophysically-detailed models of cardiac cells (Noble, 2002) inspired by the work of Hodgkin and Huxley. Over time, the complexity of the cellular electrophysiology models increased following discoveries of new ion currents, ion pumps and exchangers responsible for ionic homeostasis in the cell, and knowledge on intracellular compartments such as the sarcoplasmic reticulum. The first model of a cardiac action potential was that of a Purkinje cell by (Noble, 1960). There, the original formulation of the potassium current by Hodgkin and Huxley was altered to yield a longer action potential that more closely resembled the cardiac AP. Shortly afterwards, the inward-rectifier potassium current (I_{K1}) was discovered and incorporated into the next model of the Purkinje cell, yielding an AP model generated by I_{Na} , I_K and I_{K1} currents (Noble, 1962). Experimental investigations soon revealed a more complicated picture, with an inward calcium current playing a role in AP generation. (Beeler and Reuter, 1977) proposed a model of a cardiac ventricular myocyte that included four ionic currents underpinning the AP: the I_{Na} and I_{K1} currents, as well as the slow inward calcium current I_s , and a time-dependent potassium current I_{X1} . The balance of the inward calcium and the time-dependent potassium currents controlled cellular repolarization.

The next significant advancement came with the model of a Purkinje myocyte by (DiFrancesco and Noble, 1985). It was the first model to incorporate the sodium-potassium pump, I_{NaK} , and the sodium-calcium exchanger, I_{NCX} , as well as the sarcoplasmic reticulum as an intracellular store of calcium ions. In this way, the model was capable of not only AP generation, but also included ion homeostasis and a description for the intracellular calcium transient generation. Following this advancement, iterations between modelling and experiments led to deeper understanding and further refinement of cellular ionic processes. For instance, (C. Luo and Rudy, 1994; C. H. Luo and Rudy, 1994) developed a formulation of the L-type calcium current (including the voltage- and calcium-mediated gating mechanisms) that was incorporated into the ventricular myocyte model. Similarly, the discovery of the rapid-delayed and slow rectifier components of the outward potassium current (Horie et al., 1990; Sanguinetti and Jurkiewicz, 1990) prompted (Zeng et al., 1995) to include I_{Kr} and I_{Ks} into the ventricular AP model of Luo and Rudy. The iterations between modelling and experiment continue to lead to refined models of single-cell electrophysiology, with models of ventricular/ atrial/ Purkinje myocytes now available for different animal species. In many cases, more than one cellular electrophysiology model of a specific cell type for a given species is available (Bueno-Orovio et al., 2016).

3.2.3 Biophysically-detailed models of human atrial electrophysiology

To date, eight biophysically-detailed models describing the electrophysiology of the human atrial myocyte have been reported in the literature. These are the models of (Courtemanche et al., 1998), (Nygren et al., 1998), (Maleckar et al., 2009), (Koivumäki et al., 2011), (Grandi et al., 2011), (Colman et al., 2013), (Voigt et al., 2013), and (Schmidt et al., 2015). For simplicity, those models are henceforth referred to by their first author names.

The Courtemanche, Nygren, Maleckar, Koivumäki and Grandi models are compared against one another in Figure 3.1. In terms of the model structure (Figure 3.1A), all five models include the major transmembrane currents responsible for AP generation, i.e. the fast Na^+ current I_{Na} , the ultra-rapid K^+ current I_{Kur} , the transient outward K^+ current I_{to} , the rapid and slow components of the delayed rectifier potassium current I_{Kr} and I_{Ks} , the L-type Ca^{2+} current I_{CaL} , the inward rectifier K^+ current I_{K1} , the sodium-potassium pump and the sodium-calcium exchanger I_{NaK} and I_{NCX} . The models also incorporate the Ca^{2+} pump PMCA, as well as the calcium and sodium background currents I_{bCa} and I_{bNa} . The models incorporate a description of intracellular Ca^{2+} handling which includes the uptake and release currents J_{up} (SERCA) and J_{rel} (RyR) of the sarcoplasmic reticulum, as well as K^+ , Na^+ and Ca^{2+} ion homeostasis regulating intracellular ionic concentrations. Inter-model differences in terms of transmembrane ion channels are as follows: the Maleckar model is the only one to include the acetylcholine-activated potassium current to model effects of vagal stimulation. The Koivumäki model includes a description of the funny current I_{f} active in phase 4 of repolarization. The Grandi model contains the plateau potassium current, as well as the calcium-activated chloride current $I_{\text{Cl(Ca)}}$ and the background chloride current I_{bCl} .

However, the five models differ in the mathematical formulations of most of the transmembrane currents, the intracellular calcium handling sub-system, and the number of model compartments. The Courtemanche model builds on the guinea pig ventricular model by (C. Luo and Rudy, 1994; C. H. Luo and Rudy, 1994), with the currents I_{Na} , I_{CaL} , I_{Kur} , I_{to} , I_{Kr} , I_{Ks} , and I_{K1} reformulated based on experimental data obtained from human atrial myocytes. While making use of the same human experimental data as the Courtemanche model, the Nygren model is nevertheless based on the rabbit atrial AP model of (Lindblad et al., 1996). The Maleckar model reformulates the repolarizing

currents I_{Kur} and I_{to} of the Nygren model based on more recent experimental data. The Grandi model is derived from an earlier model of ventricular electrophysiology from the same group (Grandi et al., 2010), which is in turn based on the rabbit ventricular model by (Shannon et al., 2004). Adaptations to the ventricular myocyte model by (Grandi et al., 2010) were performed to bring it in agreement with human atrial electrophysiology. For instance, the conductance of the inward rectifier I_{K1} current was reduced by a factor of 6 to account for a smaller level of I_{K1} -carrying channel in atrial vs. ventricular cells. The I_{to} current as well as atria-specific I_{Kur} from the Maleckar model were incorporated into the Grandi atrial model.

Differences in the intracellular calcium handling are seen between the five models (Figure 3.1B). The intracellular calcium handling sub-system in the Courtemanche model is based on the guinea pig ventricular model of (C. H. Luo and Rudy, 1994), while Grandi uses the formulation of the rabbit ventricular calcium handling from (Shannon et al., 2004). In the Nygren and Maleckar models, the calcium subsystem is based on in the rabbit atrial model of (Lindblad et al., 1996). The calcium-handling subsystem of the Koivumäki model includes a spatial dimension to account for the fact that human atrial myocytes lack transverse tubules.

Inter-model differences related to the number of cellular compartments are also seen (Figure 3.1B). The Courtemanche model is simplest in this regard, consisting of the extracellular and intracellular spaces between which the transmembrane currents flow. The Nygren and Maleckar models additionally comprise the cleft space outside of the sarcolemma, where local accumulation and depletion of ions is permitted. This cleft space acts as a buffer between the intracellular and the extracellular ionic solutions and can be thought of as an effective extracellular space. The Grandi model consists of the intracellular, subsarcolemmal and extracellular spaces, with the subsarcolemmal space bridging the intracellular and extracellular compartments and acting as an effective intra-

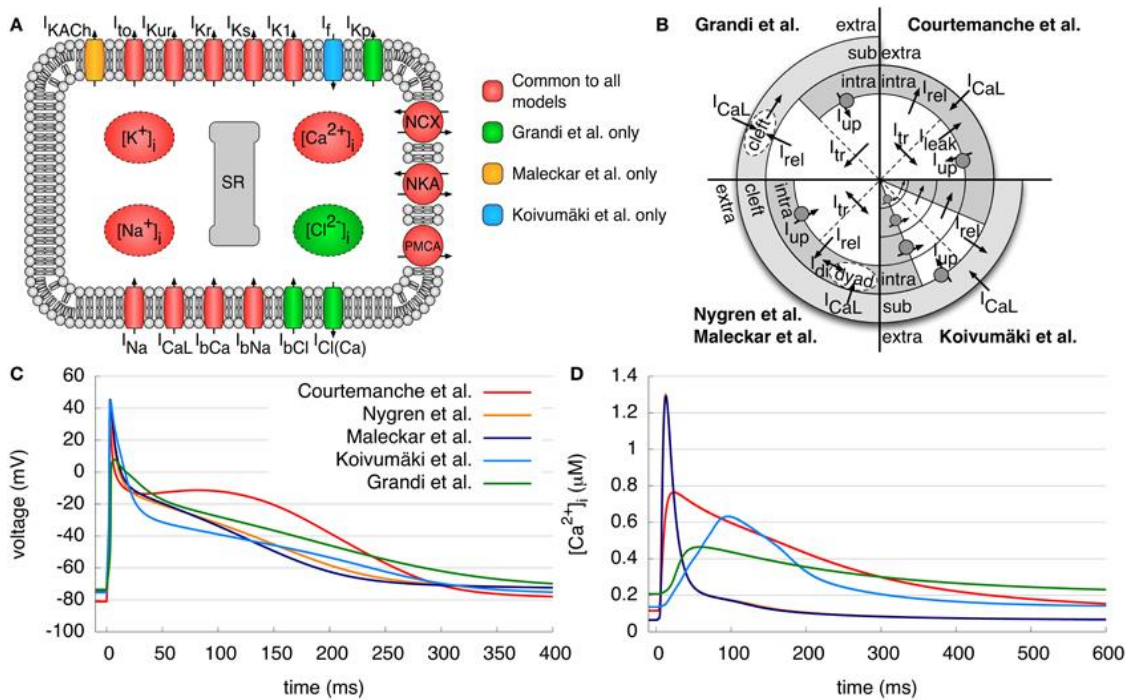


Figure 3.1 Comparison of Courtemanche, Nygren, Maleckar, Koivumäki, and Grandi human atrial models. **A** Model structure, **B** model compartments, **C** action potentials and **D** intracellular calcium transients yielded by the five models. Reproduced from (Wilhelms et al., 2012).

cellular space in terms of ionic concentrations seen by transmembrane currents. The Koivumäki model is similar to Grandi, in that it includes the intracellular, subsarcolemmal and extracellular spaces.

As a result of these differences, the models generate different action potentials and intracellular calcium transients (Figure 3.1C-D). The Courtemanche model generates a spike-and-dome AP morphology with a slowly-decaying intracellular calcium transient, while the Nygren-based models produce triangular APs. The Nygren and the Maleckar models also display a larger calcium transient amplitude and faster decay. The Grandi model yields an AP that is triangular but lower in amplitude than in the other models, and its calcium transient is slower to rise and decay. The Koivumäki model produces a calcium transient that is slowest to increase.

Biophysically-detailed models of human atrial electrophysiology building on the ones discussed above have also been proposed. For instance, (Colman et al., 2013) constructed

their own model of human atrial AP, taking the Courtemanche model as a starting point, incorporating the I_{Kur} and I_{to} formulations from the Maleckar model, and a simplified two-compartment intracellular calcium handling from the Koivumäki model. Likewise, (Voigt et al., 2012) extended the Grandi model by including Na^+ regulation of the inward potassium rectifier and the acetylcholine-activated potassium currents, while (Schmidt et al., 2015) additionally incorporated the formulation for the background potassium current known as TASK-1 into the (Voigt et al., 2012) model.

In this thesis, the Courtemanche, Maleckar, and Grandi models were selected for single-cell investigations described in subsequent chapters. Whilst the Koivumäki model includes the most up-to-date description of the intracellular calcium handling in human atrial myocytes, it was not selected for the modelling work because investigating the intracellular calcium handling processes was not part of my research question. Instead, the main focus of this thesis has been to investigate the importance of changes in transmembrane ionic current densities on the cellular AP and on the inter-atrial gradient in APD for arrhythmogenesis under the conditions of nNOS block/ depletion. For this purpose, the models of Courtemanche, Maleckar and Grandi – with a simpler calcium subsystem formulation – are thought to be sufficient.

3.3 Models of whole atria

While cellular models can provide useful insights into the AP generation in physiological and pathological conditions, arrhythmias are emergent phenomena at the tissue level. I now shift attention from single-cell models to whole-atria modelling.

3.3.1 Geometrical models of whole atria

Over the past years, a number of geometrical whole-atria models have been proposed. The simplest models consist of either a two-dimensional surface or a three-dimensional sheet (with a finite thickness) folded in the three dimensional space to mimic the general

shape of the atrium (e.g. (Dokos et al., 2007; Gray and Jalife, 1998; Vigmond et al., 2001)). Therefore, such models are not derived from any imaging data; instead, the authors use a series of assumptions during model construction. Within such models, the tissue can be constructed as purely homogeneous, or including detail on cardiac micro-structure (such as fibre orientation and conduction anisotropy, as in (Vigmond et al., 2001)). The usefulness of such simplified models is in answering fundamental questions related to e.g. atrial anatomy. For instance, (Vigmond et al., 2001) constructed models of whole atria represented by two spheres – one sphere per chamber – with holes for anatomical elements such as pulmonary veins. The authors used those models to assess the relative importance of certain anatomical structures by comparing the propagation of the electrical stimulus in the atrial model with or without that anatomical structure, and confirmed e.g. the importance of superior vena cava/pulmonary vein openings as anchor points for rotors. Nonetheless, a drawback of such simple models is lack of anatomical detail due to the models not being derived from imaging data.

An improvement over the simple geometrical models are whole-atria meshes obtained with medical imaging tools, such as MRI or CT scans (e.g. (Adeniran et al., 2015; Krueger et al., 2013; Seemann et al., 2006)). Such models include detailed/realistic anatomical elements seen in whole atria (see e.g. Table 3.1). A number of studies use the data from the Visible Human Project for this purpose (the project provides digital image datasets of complete female and male corpses in MRI, CT, and anatomical modes; see https://www.nlm.nih.gov/research/visible/visible_human.html). The images within the Visible Female dataset are of a higher resolution than in the visible man dataset, and the former is used more frequently in modelling studies (Table 1 in (Dössel et al., 2012)).

3.3.1.1 Whole-atria mesh based on the Visible Female dataset

In this thesis, I use the human whole-atria mesh developed by (Seemann et al., 2006), based on the Visible Female dataset. The whole-atria mesh consists of 266450 nodes and 1378054 elements. Each computational node has a single-cell electrophysiology model assigned to it. The nodes' boundaries are defined by the elements in the whole-atria mesh. For each element, the fibre direction as well as tissue conductivities (transverse and longitudinal to the fibre) are specified. The atrial anatomical structures within the (Seemann et al., 2006) mesh are illustrated in Figure 2.2, and include the SAN, CT, PMs, LA, RA, LAPG, RAPG, BB, IABs, FO, AVR and RAI. Table 3.1 summarizes tissue anisotropy and CV properties within these atrial structures as implemented in the mesh.

3.3.2 Electrical excitation propagation: monodomain and bidomain models

Cardiomyocytes are preferentially connected end-to-end via low-resistance gap junctions, making it easy for the excitation to spread quickly across many cells along the fibre direction. As a result, the myocardium can be thought of as an electrical continuum, albeit with distinct intracellular and extracellular spaces with anisotropic properties. Such rationale underpins the mono- and bidomain formulations of excitation propagation.

Under the bidomain model, partial differential equations describe the propagation of electrical excitation in the intracellular and the extracellular spaces. The two spaces are connected by the cellular membrane. Different electrical potentials (the intra- and extracellular electrical potentials Φ_i and Φ_e) are associated with the two spaces. However, within each space, the electrical potential is considered uniform (due to gap junction coupling). The equations underpinning the bidomain model are:

$$\nabla \cdot (\sigma_e \nabla \Phi_e) = -\beta I_m - I_{se}$$

$$\nabla \cdot (\sigma_i \nabla \Phi_i) = -\beta I_m - I_{si}$$

Table 3.1 Tissue anisotropy and heterogeneities in the 3D whole-atria model described in (Seemann et al., 2006). Explanation of terms: anisotropic ratio - transversal to longitudinal ratio of conductivity, CV – conduction velocity. Reproduced from (Sánchez et al., 2013).

Anatomical structure	Anisotropic ratio	CV (cm/s)
LA	1:2	59
RA	1:2	59
SAN	1:1	31
CT	1:10	116
PMs	1:2	98
FO	1:2	59
RAPG	1:2	59
IAB	1:2	59
BB	1:2	98
RAI	1:1	44
LAPG	1:2	59
AVR	1:2	59

with σ_i and σ_e being the intra- and extracellular conductivity tensors, I_m representing the transmembrane current density (corresponding to I_{tot} in equation 3.1), β representing membrane to cell volume ratio, and I_{si} and I_{se} being the externally applied current sources in the intra- and extracellular domains.

Under the assumption of (i) equal anisotropy ratios in the intracellular and extracellular domains, or (ii) negligible extracellular potentials (Keener and Sneyd, 1998), the bidomain equations are simplified to yield the monodomain model:

$$\nabla \cdot (\sigma \nabla V_m) = \beta \left(C_m \frac{dV_m}{dt} + I_{ion} + I_{stim} \right)$$

where I_m has been replaced by the explicit expression for the total transmembrane current, and stimulus current I_{stim} has been introduced. The advantage to using the monodomain model over the bidomain formulation is a reduced computational cost. In the absence of an external field (such as a defibrillation field), the solutions obtained with

the mono- and bidomain models are quite similar (Potse et al., 2006). I use the monodomain model to compute electrical signal propagation in whole atria in Chapter 6.

3.3.3 Modelling the impact of AF on tissue properties

Amongst others, AF remodels electrical and structural properties of the affected atrial tissue, as reviewed in the previous chapter. Traditionally, the effects of the AF-induced electrical remodelling were simulated by increasing I_{K1} by 100%, and decreasing I_{Kur} , I_{CaL} and I_{to} by 50%, 70% and 50% at the single-cell/computational node level (e.g. (Cherry and Evans, 2008; Cherry et al., 2008; Courtemanche et al., 1998)), based on experimental data obtained from patients in persistent/ chronic AF. Emergence of experimental data on the impact of persistent/ chronic AF on additional ionic currents and intracellular calcium handling motivated an inclusion of I_{Kr} , I_{Ks} , I_{NCX} , as well as SERCA and RyR into computational studies on AFER (Table 2.1; (Colman et al., 2013; Koivumäki et al., 2014)). For instance, (Colman et al., 2013) applied selective and concurrent changes in I_{CaL} , I_{Kur} , I_{K1} , I_{to} , I_{Kr} , I_{Ks} , I_{NCX} , SERCA and RyR to the model of whole atria similar to that in Table 3.1, to investigate the impact of ionic changes on inducibility and persistence of re-entry. The authors showed that AFER shortens APD and increases APD heterogeneity in atria; these factors contribute to increased maintenance and inducibility of re-entrant excitation waves (regions of APD heterogeneity facilitate re-entry by providing anchors for re-entry and facilitating wave break (Vigmond et al., 2004)).

AF-induced structural remodelling has traditionally been incorporated into whole-atria models by a uniform 30% decrease in tissue conductivities (Dössel et al., 2012), to mimic the reduced cell-to-cell coupling resulting from an increased degree of fibrosis. More recently, heterogeneous fibrosis patterns have also been investigated. (Gharaviri et al., 2012) modelled the decreased conductivity in the epicardium but not in the endocardium, and showed that this so-called ‘endo-epicardial dissociation’ stabilizes AF (a larger

number of fibrillatory waves can coexist in the atrium). Separately, (Zahid et al., 2016) constructed 20 whole-atria models with spatially-heterogeneous fibrosis patterns based on patient-specific MRI images, and showed that AF is perpetuated by rotor drivers located in the fibrosis boundary zones.

3.4 Computational approaches for studying variability

Variability manifests itself at all levels in biological systems. At the single-cell level, four different methods for studying variability in cellular cardiac electrophysiology have been proposed. Attempts have also been made to study variability in tissue and whole organ. In what follows, I review the methods used for studies of cell-to-cell variability, maintaining a focus on the experimentally-calibrated population of models approach used in subsequent chapters.

The following sections constitute a part of the review article ‘Variability in Cardiac Electrophysiology: Using Experimentally-Calibrated Populations of Models to Move beyond the Single Virtual Physiological Human Paradigm’ by (Muszkiewicz et al., 2016), published in Progress in Biophysics and Molecular Biology.

3.4.1 Description of the experimentally-calibrated population-of-models methodology

Figure 3.2 illustrates the process of developing and analysing an experimentally-calibrated population of models, described in more detail in the following sections.

3.4.1.1 The research question and the baseline model of cellular electrophysiology

The research question (and corresponding hypotheses) will inform both the choice of experimental data and the modelling process. These will be the two corner stones for the construction of the experimentally-calibrated population of models. A common

assumption is that inter-individual variability affects electrophysiology at the level of ionic current properties (such as the ionic current conductances, time constants of channels opening/closing, and other parameters characterising the currents), and not at the level of ion channel structure (which is represented in the models through equations describing each modelled channel's transitions between gating states) (Britton et al., 2013; Groenendaal et al., 2015; Sarkar et al., 2012). Therefore, at the initial stage of modelling, one selects an appropriate cardiac cell model whose equations are used as a 'scaffold', whilst the baseline model parameters are varied to represent variability in ionic current properties.

Aside from the research question, additional factors that may play a critical role in selecting the baseline model to use as the scaffold are model complexity and unique model characteristics, particularly if multiple models of a particular cell type exist. For instance, several human atrial electrophysiology models have been published. As described in Section 3.2.3, the Courtemanche and Colman models produce a spike-and-dome action potential; however, the latter model includes a different formulation of the calcium handling subsystem. The models of Nygren, Maleckar and Koivumäki generate more triangular APs. At the same time, the Maleckar model is the only one able to incorporate the effects of vagal stimulation on the AP due to the inclusion of acetylcholine-activated potassium current, while the Koivumäki model contains a much more detailed description of the intracellular calcium transient compared to the remaining models. The assumptions made in a particular study, together with key features of experimental data to be modelled, will determine the relative importance of the model-specific properties for answering the research question (for instance, do experimental APs display a spike-and-dome morphology? How finely-detailed does the calcium subsystem need to be?).

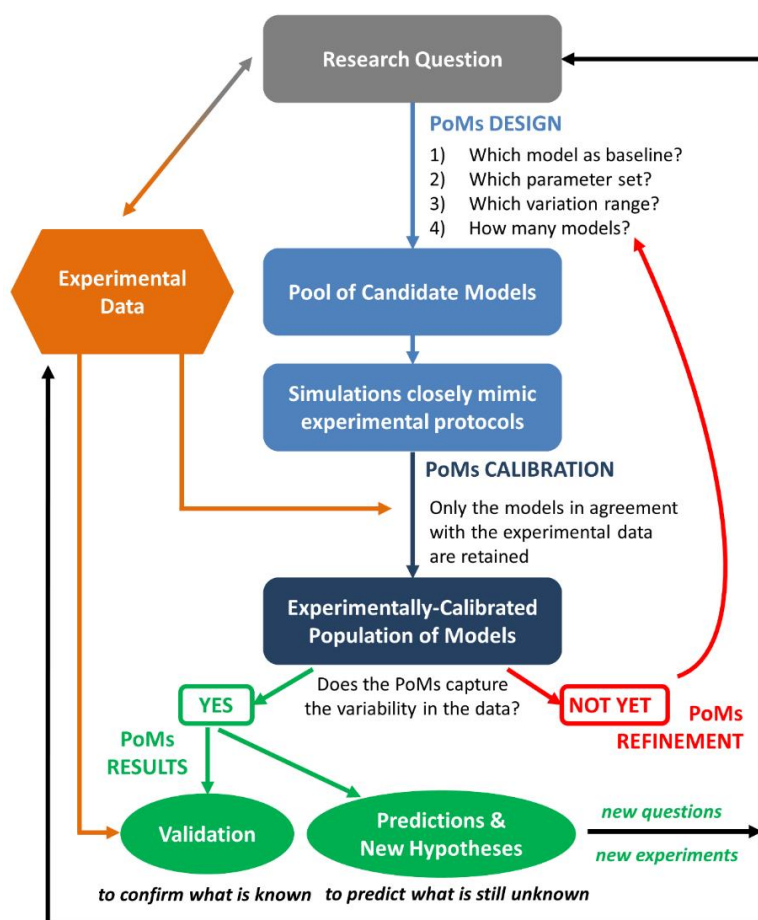


Figure 3.2 Flowchart illustrating the process behind constructing an experimentally-calibrated population of models (abbreviated as PoMs). Reproduced from (Muszkiewicz et al., 2016).

3.4.1.2 Generating the population of candidate models

Populations of models extend cardiac modelling beyond the use of a single model that represents the average or typical behaviour of that cell type and species, to an ensemble of models with different values of underlying parameters and differing behaviours of model outputs. The choice of parameters to vary is therefore a significant part of designing a population of models.

A parameter should only be varied if an argument, based on the aims of the study, can be made as to why it should be varied. While many parameters within a model of a biological system will exhibit variability in experiments, different biological parameters vary through different mechanisms. For example, both ionic current conductances and kinetics

can vary between individuals. However, conductances vary primarily due to variation in the number of ion channels in the cell membrane, while kinetics vary primarily due to changes in channel structure. Genetic mutations that alter channel kinetics and functionality are generally considered pathological, and differentiate individuals from the range of the population that is considered as healthy. Therefore, a population created by varying channel kinetics might have a different biological interpretation from a population created by varying conductances alone.

After determining the parameters that will be varied for the population of candidate models, the next choice is to determine the range over which to sample parameter values. Ranges for parameter sampling should be chosen according to the hypothesis of a particular study and the assumptions made about what the resultant population will represent. Wider parameter ranges allow for more extreme parameter values and therefore more extreme model behaviours. For ionic current conductances, expanding the range towards large values could represent increased levels or activity of that current, as might occur in compensation for block of another current (Marder and Taylor, 2011), or due to beta-adrenergic stimulation (Heijman et al., 2013). Expanding the range towards zero could represent decreased levels of an ion channel, but very low values could also represent mutations that cause loss-of-function of a channel (Rosati and McKinnon, 2004), or diseases that impair channel function (Borlak and Thum, 2003; Farid et al., 2011).

A common step in modelling studies to date is to assume that ionic current conductances vary significantly between individuals and constitute the major determinant behind physiological variability (Britton et al., 2013; Groenendaal et al., 2015; Sarkar et al., 2012). Aside from maximal current conductances, variability in time constants of (in)activation has also been considered when the underlying research question/ hypothesis motivated this (Romero et al., 2009; Sarkar and Sobie, 2011; Britton et al., 2013). For

instance, (Britton et al., 2013) varied maximal current conductances of eight transmembrane ionic currents as well as three time constants regulating channel kinetics, since those parameters were shown (Corrias et al., 2011) to have the largest impact on the cellular AP biomarkers that were at the centre of (Britton et al., 2013)'s work. Nonetheless, the majority of studies to date focus on maximal current conductances as primary determinants of cell-to-cell variability at the AP level. This stems from the following two reasons:

1. In non-diseased cellular models, experimentally-measured cell-to-cell variability in peak current densities and hence in maximal current conductances often spans a 10-fold range (see e.g. Figure 4.6B for experimental current density measurements in Chapter 4 of this thesis), while the values of time constants determining channel kinetics vary less (e.g. (Clerx, 2017) observed a variation from roughly one-half to twice the most common value for the time constants of I_{Na} inactivation). In a biological system, different parameters vary via different mechanisms, and a relatively common and wide variability in ion channel numbers in cell membrane translates into variability in maximal conductances of ionic currents in the corresponding mathematical model. At the same time, under control/ non-diseased conditions, variability in ion channel structure (translating into variability in channel kinetics) is less commonly seen.
2. Computational feasibility plays an important role in the number of parameters that are allowed to vary in the generation of a population of candidate models. Varying all model parameters is computationally intractable. In practice, a modeller's task is to select a subset of parameters that (i) are likely to vary most, and/ or (ii) are most likely to impact the cellular biomarkers of relevance to the study.

Thus, for modelling of non-diseased, healthy cardiomyocytes, the results of previous modelling studies (Corrias et al., 2011; Romero et al., 2009) suggest that a variation of approximately $\pm 30\%$ in conductance values (from the value of the parameter in the baseline model) might be a moderate estimate on the level of variability, while $\pm 50\%$ would allow substantial variability. For studies that are interested in extreme behaviours, particularly including conditions where one or more types of ion channel may be almost completely inhibited or not present in the cell, $\pm 100\%$ variation in ionic current conductances could be used (Britton et al., 2013). Once the range of parameter variation has been established, the next step in the experimentally-calibrated population-of-models method is to generate a large number of parameter sets for the pool of candidate models, sampled from a high-dimensional parameter space. Sampling every possible combination of parameter values is computationally infeasible given the complexity of most cardiac cell models. Most studies of experimentally-calibrated populations of models in cardiac electrophysiology (Britton et al., 2014, 2013; Passini et al., 2016; Zhou et al., 2013) used Latin Hypercube sampling (Burrage et al., 2015; McKay et al., 1979), a parameter sampling method that does not scale the computational cost with the number of varied parameters, allowing for an exploration of a complex parameter space.

Different techniques could be used to construct an experimentally-calibrated population of models, and an important question is how effective Latin Hypercube sampling is in yielding an ensemble of models that captures the desired features of experimental data. An argument can be made that Latin Hypercube sampling is a crude mechanism for exploring a high-dimensional parameter space and other statistical techniques may be more effective. Alternative approaches such as Approximate Bayesian Computation (ABC) could indeed be considered to perform Bayesian statistical inference for models that do not possess a computationally tractable likelihood function (Beaumont et al., 2002). ABC algorithms search the parameter space until they find a certain number of

parameters generating simulated data that are close to the observed experimental data with respect to some summary statistics. In this manner, the ABC identifies a compact region in the parameter space that produces simulated data from a statistical model that is as close as possible to the observed data. Conversely, experimentally-calibrated populations of models can cover a wide region of the parameter space with all models treated equally likely. Whereas ABC can be considered statistically stronger, it may be less effective in identifying outliers than the experimentally-calibrated population-of-models approach. For specific research questions, capturing outliers is crucial, especially when one aims to identify unlikely abnormalities in cardiac function such as cardiac arrhythmias. A comparison of LHS and ABC approaches is evaluated in Chapter 4 of this thesis.

A key aspect during the construction of the population of candidate models is the issue of covariation between two or more varied parameters. In the previous chapter, I remarked on the importance of covariation in cellular regulating networks. This issue must be considered in tandem with the complexity of cellular cardiac electrophysiology models. The models do not include every pathway from gene expression to protein phosphorylation and degradation; rather, only ionic currents and the intracellular calcium handling are typically represented. Parameters that are usually varied within the candidate populations are maximal conductances of ionic currents. At present, there is only one study by (Milstein et al., 2012) where a reciprocal modulation of I_{K1} and I_{Na} within a macromolecular complex is described. Otherwise, covariation between maximal current conductances in the heart under control/ non-diseased has not been reported (Gemmell et al., 2016). Hence, when generating populations of candidate models under control conditions, one typically assumes lack of a-priori correlations between ionic current

conductances. This assumption can be revised, should experimental evidence on covariation between ionic currents in cardiac myocytes become available.

Under both non-diseased and pathological conditions, covariation between ionic current amplitude and membrane capacitance can be expected. This is dealt with (i) by dividing ionic current amplitude by membrane capacitance, thus obtaining ionic current density, and (ii) in pathological conditions, by adjusting the membrane capacitance to its (mean) experimental value in the corresponding population of models. Amongst other changes, (Passini et al., 2016) increased membrane capacitance by +90% in the population of non-diseased cardiomyocyte models, and obtained a population mimicking the behaviour of cardiomyocytes under conditions of hypertrophic cardiomyopathy.

3.4.1.3 Simulation and calibration of the candidate population of models

The population of candidate models generated in the previous step is now simulated and calibrated, to select the models whose simulated electrophysiological properties are in range with the same properties in experimental data. This step yields the experimentally-calibrated population of models. It is crucial that the simulations mimic the experimental conditions and protocols as closely as possible, considering important aspects such as ionic concentrations in the bath, stimulation amplitude and stimulation frequency as well as any ion channel inhibitors used in the experiments.

Calibration criteria naturally differ from study to study, depending on the experimental data available. In some studies, experimental data characterising only the AP properties of cardiomyocytes are provided, while other studies use additional information on the intracellular calcium transient (Passini et al., 2016). Table 2.3 (Chapter 2) provides a summary of the AP properties and ranges used in different studies, emphasizing consistent variability in the AP across multiple species and sample/tissue types.

In the experimentally-calibrated population-of-models studies to date (Britton et al., 2014, 2013; Passini et al., 2016; Sánchez et al., 2014; Zhou et al., 2013), the experimentally-permitted range of values for model outputs are determined by calculating the minimum and maximum values obtained from experimental measurements. Extreme outliers are often discarded to exclude potential abnormalities due to damaged preparation or dislodged microelectrode impalement. For smaller datasets, this must be done using intuition and experience, as with few data points it is difficult to judge the boundary at which reasonable experimental variation ends. Larger datasets would offer a possibility of refining the way experimentally-permitted calibration criteria are determined. For example, (Prinz et al., 2004) used limits of 2 standard deviations from the mean to set ranges on their calibration criteria for constructing populations of neuronal models. Another approach for refining the calibration step may involve the simultaneous use of different calibration criteria derived from multiple experimental sources (e.g. combining AP-level information with measurements of calcium transient or individual ionic currents; the latter is explored in Chapter 4 of this thesis). Hard limits on model acceptance could be replaced as well by more flexible bounds or likelihood estimators. This would allow analysing the impact of data scarcity, or the matching of specific biomarker distributions, on the predictions generated from different calibrated populations. These aspects of course depend on the availability of sufficiently large experimental datasets, essential for the intended use of the resulting population (to capture the broadest range of possible physiological variability, and in particular outlying phenotypic variants, versus replicating specific or average cell behaviours).

In the calibration process, an important point to consider is the impact of inter- vs. intra-patient variability on the resultant experimentally-calibrated population of models. In principle, one could expect intra-patient variability to be smaller than inter-patient

variations in cellular ionic properties and AP biomarkers, given reduced genetic variation. At present, not enough experimental data points are obtained per patient to allow for discrimination between inter- and intra-patient variability effects. For instance, in the experimental dataset used in Chapter 4 of this thesis, an average of two cells per patient were utilized for cellular AP recordings. The studies on implications of differences between intra- and inter-patient variability, as well as their impact on the calibration process and hence on the resultant populations of models, are of particular interest for arrhythmia research, and will be performed once appropriate experimental data become available.

3.4.1.4 Analysis of the experimentally-calibrated population of models

Analysis of the experimentally-calibrated population presents a number of challenges that differ from those posed by analysis of a single model. Under this methodology, there is no favoured parameter set; instead, all parameter sets within the experimentally-calibrated population are equally valid as all produce output that is consistent with the range seen in experimental data. Additionally, the underlying distribution of varied parameters in the population is determined by the calibration criteria applied to the model outputs. Experimental data for the distributions or ranges of the underlying parameters are usually unavailable and so the parameter distributions underpinning the experimentally-calibrated population reflect the selectivity of the calibration criteria and experimental data used, rather than direct experimental measurements of the parameter distributions.

A first step for understanding the properties of the experimentally-calibrated population is visualisation of the underlying parameters and model outputs. Useful tools are scatter plots (Figure 3.3A), to examine correlations between pairs of variables such as ionic conductances; as well as histograms and boxplots, to summarise the distributions of parameters and model outputs without assuming a particular probability distribution for

the simulated data (Figure 3.3B). Direct visualisation of the high-dimensional parameter and model output spaces is difficult. However, machine learning techniques such as principal component analysis can be used to identify covariance between multiple variables, while other techniques such as clutter-based dimension reordering (Gemmell et al., 2014; LeBlanc et al., 1990; Peng, 2005; Peng et al., 2004; Taylor et al., 2006) are available to represent high-dimensional data in a single image.

It should be noted that the number of models in the experimentally-calibrated population is usually larger than the number of experiments used to calibrate the population, as well as the number of experiments used to validate model predictions. This is because the aim of the virtual population is to capture as much of the experimental range of variability seen across the dataset as possible, and to explore a large number of possible variant models consistent with experiment.

3.4.2 Novel insights from experimentally-calibrated population-of-models studies

Once a population's basic properties have been analysed, the population is often simulated in conditions beyond those that were used to develop it, and the results used as predictions of the population, or to generate new hypotheses. A traditional single model's prediction would either be treated as a qualitative one (e.g. whether the AP duration (APD) increases, decreases, or stays the same following the application of a drug), or tested to see whether it was close to the mean value from experiment (by comparing to the standard deviation of the experimental data). In contrast, an experimentally-calibrated population of models can be used to generate quantitative predictions of the range of effects expected in a given intervention that are directly comparable to experimental data. A prediction on the range of effects can be informative in cases where outlier responses are important (for instance in safety pharmacology).

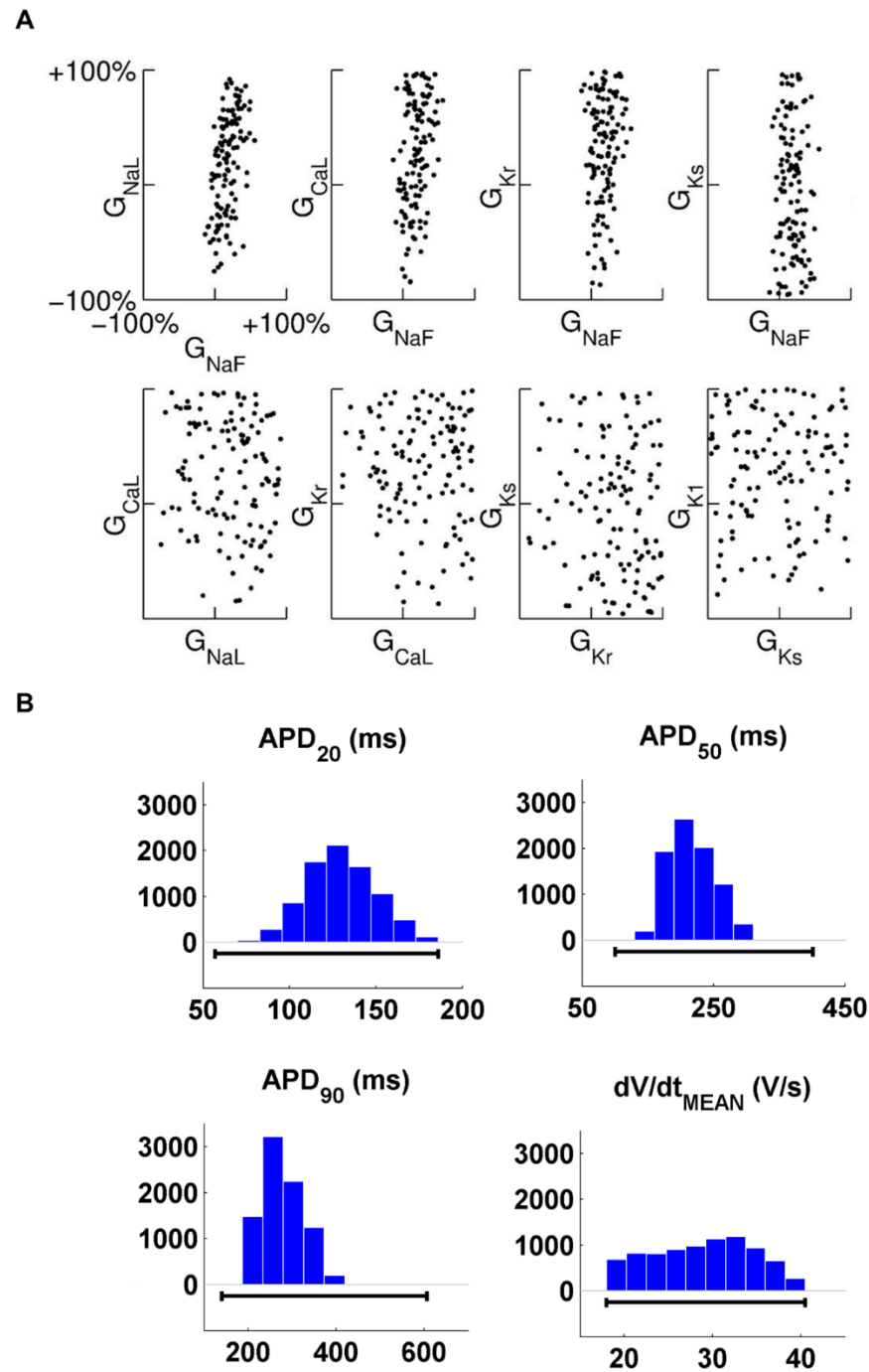


Figure 3.3 Visualizations helpful for understanding the properties of the experimentally-calibrated population of models include scatter plots and histograms summarising the population's properties without assuming a particular probability distribution for the simulated data. **A** Scatter plots of ionic properties underpinning the experimentally-calibrated population in (Britton et al., 2013), with the scale in all graphs including $\pm 100\%$ variation with respect to the baseline model value. **B** Histograms illustrating the distribution of the AP properties across the experimentally-calibrated population of non-diseased human ventricular myocytes in (Passini et al., 2016). Histograms show the number of models for each of the bins. Black lines indicate the experimental range used to calibrate the population for each AP property.

In some studies, the focus is not on understanding the mechanisms behind purely quantitative changes such as APD prolongation due to drug application. Instead, different cell groups may show qualitatively different responses to the same intervention. For example, the same drug may trigger abnormal repolarizations in cardiomyocytes from one individual, while cells from another subject will repolarize normally (as in (Passini et al., 2016; Zhou et al., 2015, 2013)). In this case, the aim of the analysis is to identify sub-populations of models within the experimentally-calibrated population that display the behaviour of particular interest, and determine whether any combinations of parameters can be used to distinguish this sub-population from the remaining models. Differences identified in this manner can then be used to inform new hypotheses regarding the plausible mechanisms causing a particular behaviour to occur. Standard methods for single model analysis can be used on exemplar models within the sub-population to provide evidence for whether a proposed mechanism is responsible for the observed behaviour. The methods that could be used here include detailed analysis of state variables, to establish cause-and-effect in the model; as well as bifurcation analysis, to analyse the different dynamical states a particular model can occupy.

The first study that utilized the experimentally-calibrated population-of-models methodology was that by (Britton et al., 2013), who built a population of models capturing experimentally-observed variability in the AP properties of isolated rabbit Purkinje fibres under control conditions. The population was subsequently used to investigate the effects of dofetilide, a selective drug inhibiting I_{Kr} , and the resultant ranges of APD prolongation predicted by the population were in agreement with blinded experimental results that were not used to construct the population.

Since then, the experimentally-calibrated populations-of-models approach has been used in eight studies on variability in cardiac electrophysiology (Gemmell et al., 2014; Liberos

et al., 2016; Passini et al., 2016; Pueyo et al., 2016; Reilly et al., 2016; Sánchez et al., 2014; Zhou et al., 2015). Out of these eight studies, four deal with AF (Liberos et al., 2016; Reilly et al., 2016; Sánchez et al., 2014, 2013). The study by (Reilly et al., 2016) is concerned with a therapeutic strategy for preventing AF, and is further described in Chapter 5 of this thesis. (Liberos et al., 2016) investigated ionic currents that could destabilize rotor dynamics in chronic AF to improve anti-AF therapies. (Liberos et al., 2016) constructed a population of 173 models of human atrial tissue models that incorporated AF-induced remodelling, and found self-sustained re-entrant circuits in 126 models that had a higher levels of I_{Na} and I_{CaL} currents. Inhibition of the I_{CaL} current abolished rotors in 38 models (out of the subset of 126), and those models were characterized with a lower availability of I_{Na} . These findings may have important implications for the treatment of chronic AF.

(Sánchez et al., 2014) constructed populations of single-cell models calibrated with experimental datasets from over 450 cell samples extracted from patients in sinus rhythm and chronic AF, in order to investigate ionic determinants of variability in SR vs chronic AF. Despite differences at the AP level between the populations representing control and chronic AF scenarios, both populations showed similar mechanisms underlying variability in different stages of the AP: I_{CaL} , I_{to} and I_{Kur} were found to be key in modulating inter-subject differences in early repolarization, whereas I_{K1} and I_{NaK} determined cell-specific values of APD_{90} .

Single-cell level variability in APD_{90} in the population of chronic AF models by (Sánchez et al., 2014) spanned 140–330 ms, prompting the authors to analyse the impact of APD on the dynamics of AF at the level of whole atria. This was performed in (Sánchez et al., 2013), who found that long atrial APDs were associated with slow but very regular fibrillatory patterns, while shorter APDs were linked to high frequency re-entrant activity. All these examples illustrate the power of the experimentally-calibrated populations to

predict and explain disease phenotype, provide the means for a quantitative comparison between experimental and simulation data, and investigate potential disease therapies.

3.4.3 Comparison of the experimentally-calibrated populations of models with other approaches for studying variability

In addition to the experimentally-calibrated population-of-models approach, three other frameworks for computational studies of cell-to-cell physiological variability in cardiac electrophysiology have been devised: the sensitivity analysis method, the population or ensemble-of-models framework without experimental calibration, and cell-specific modelling approaches (Figure 3.4). Sensitivity-analysis-based methods explore the effect of variation in model parameters on model outputs such as the action potential or intracellular calcium transient. The parameters of interest are varied around set values – usually the baseline model values – one at a time. Univariate sensitivity analysis was used by Romero et al. to investigate variability by varying one parameter at a time by $\pm 15\%$ and $\pm 30\%$ from its baseline value, and to explore the effect this had on pre-clinical biomarkers of arrhythmic risk, including the APD, in a human ventricular single-cell model (Romero et al., 2009).

In the absence of experimental calibration, the population-of-models approach can be thought of as an extension of the sensitivity-analysis framework, in that it permits multiple parameters to be varied simultaneously, and in the context of cardiac electrophysiology, it was first used in a series of papers by (Sobie and Sarkar, 2011; Sobie, 2009). There, multiple parameters were varied simultaneously in models of ventricular cells, with parameter values sampled from a log-normal distribution centred on their baseline values. Then, the authors used regression analysis to create simplified models relating changes in parameters describing ionic currents to changes in cellular properties. Using similar methods, Walmsley et al. investigated mRNA expression levels

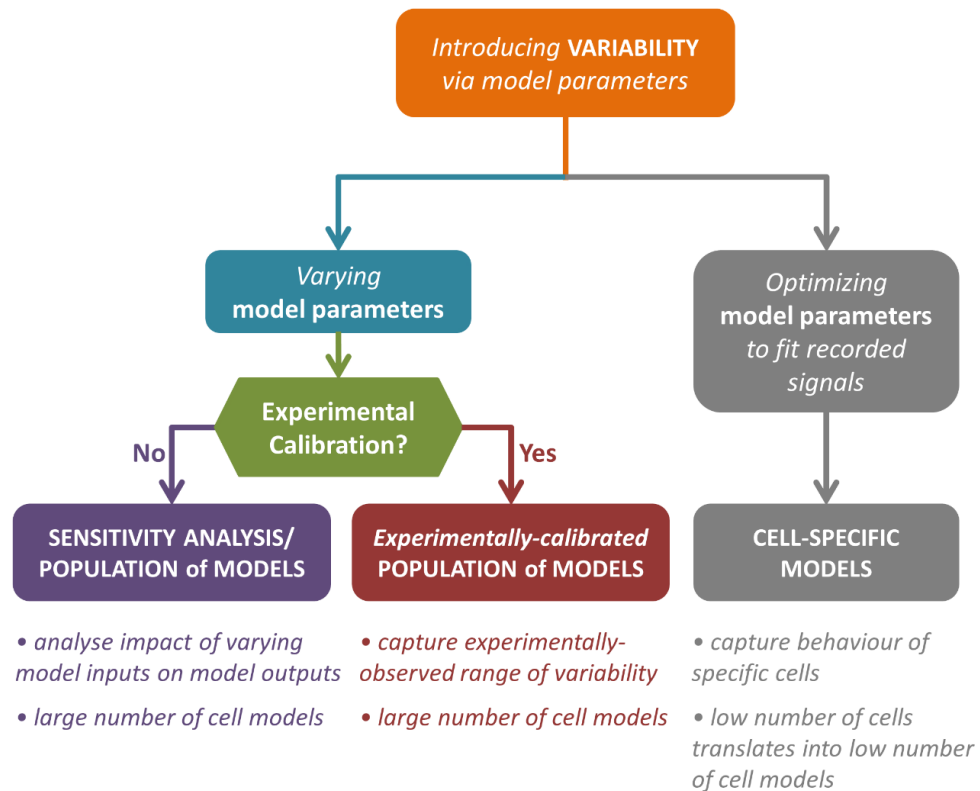


Figure 3.4 Schematic illustrating the different approaches for studying physiological variability. Reproduced from (Muszkiewicz et al., 2016).

on the cellular electrophysiological remodelling in failing human hearts (Walmsley et al., 2013). (Yang and Clancy, 2012) probed sex-based differences in human susceptibility to cardiac ventricular tachyarrhythmias; and Cummins et al. compared rate-dependent properties of 13 models of ventricular cells in the context of drug action (Cummins et al., 2014). Most recently, the methodology was used by (Devenyi and Sobie, 2015), who studied regulation of calcium transient in rat cardiomyocytes via iterations with experiments.

Both the sensitivity-analysis approach and the population-of-models framework without experimental calibration are very useful as tools to probe the relationships between ionic current properties and cellular properties such as the AP. However, unlike the experimentally-calibrated population-of-models methodology, they do not incorporate a calibration step permitting one to constrain models with experimental data. Therefore, they cannot generate models representative of datasets extracted from various patient or

subject groups. This is important, as experimentally-calibrated populations-of-models provide a framework to quantitatively (rather than qualitatively) compare simulation and experimental predictions. Additionally, narrow ranges of parameter variation combined with the absence of calibration criteria mean that the sensitivity-analysis and ensemble-of-models frameworks cannot be used to generate models whose predictions are far from the baseline model value, while at the same time mimicking the experimental recordings.

In cell-specific modelling approaches, the aim is to generate biophysically-detailed models of specific cells used in experiments. (Syed et al., 2005) were the first to illustrate this approach by using a genetic algorithm to fit a biophysically-detailed model of human atrial action potential to the AP trace generated by different cell models, as well as by experimental data. Separately, (Davies et al., 2012) constructed 19 models of 19 dog ventricular mid-myocardium myocytes. The models were fitted to the action potential recordings in the early and late stages of cellular repolarization under control conditions; a subsequent comparison of model predictions to experimental observations of drug action revealed that models had a prediction accuracy of over 80%. Hence, the approach proved useful; however, the fact that the experimental AP traces used to constrain the models were collected with standard laboratory protocols implies that the uniqueness of parameter values in the resultant cell models cannot be guaranteed. Improvements to the methodology were put forward by (Kaur et al., 2014) and (Groenendaal et al., 2015). (Kaur et al., 2014) demonstrated that incorporating membrane resistance alongside action potential measurements led to faster convergence in the estimation of cell specific model parameters. (Groenendaal et al., 2015) developed a series of complex electrophysiology protocols involving stochastic pacing of the cells in experiments, coupled with the use of automated parameter optimization techniques, to construct 4 specific models of 4 isolated guinea pig ventricular myocytes. Cell-specific modelling could be a powerful approach

to address specific research questions, also in line with a tightly interconnected model-simulation-experiment system. However, the complexity of the protocols involved limits the number of cells that can be investigated and modelled, and therefore it does not provide the wide coverage of the parameter space of the experimentally-calibrated populations of models.

3.5 Numerical techniques

Simulations of single cells yield action potentials. Action potential generation is associated with a rapid depolarization phase that takes place on the time scale of a few milliseconds, and a slower repolarization phase that can last for several hundreds of milliseconds. To solve the associated system of ordinary differential equations (ODEs), a numerical solver with an adaptive time step must be used. For this purpose, I use the solver CVODE (part of the SUNDIALS library (Hindmarsh et al., 2005)), implemented within the CHASTE open-source software package for modelling and simulations in computational biology (Pitt-Francis et al., 2009). For the purpose of simulating populations consisting of thousands of *in silico* models, the framework developed by (Walmsley et al., 2013) and embedded in CHASTE was implemented. All simulations were performed on a desktop computer utilizing Intel CORE i5 vPro processor with 4 cores at the Department of Computer Science of the University of Oxford. To conduct simulations for a population of 15,000 models where each model was paced for 100 beats at five pacing frequencies, the computational node was used for c.a. 12 to 16 hours (with the Courtemanche and Maleckar-based populations requiring less computational time than the Grandi-based population).

Whole-atria simulations were performed with Elvira (Heidenreich et al., 2010), a finite-element code that computes the numerical solution to the monodomain equation. The motivation behind using the finite element method is that it makes it easier to find

solutions to partial differential equations over complex and irregular cardiac meshes. The whole-atria mesh by (Seemann et al., 2006) with functional and anatomical detail (fibre orientation, conduction anisotropy, heterogeneity in conduction velocity) was used. Whole-atria simulations were performed on ARCUS-B, a 246-node cluster utilizing Intel E5-2640v3 Haswell 64GB CPU nodes with 16 cores per node, at the Advanced Research Computing (ARC) facilities at the University of Oxford. To simulate 10 seconds of electrical activity in whole atria using one computational node, c.a. 8 hours of computational time was required.

4 CONSTRUCTING HUMAN ELECTROPHYSIOLOGY MODELS FROM A SPECIFIC PATIENT COHORT DATASET: METHODOLOGICAL CONSIDERATIONS

This chapter constitutes a manuscript under the same title, presently under review.

4.1 Introduction

The previous chapter included a review of recent advances in cellular cardiac electrophysiology modelling, as well as the integral role that modelling has played in studies of inter-subject variability. This chapter addresses methodological considerations into the construction of experimentally-calibrated populations of models, which constitutes one of the methods that can be used for investigations into physiological variability. Unlike some of the previous studies that used the experimentally-calibrated populations-of-models approach, I have access to a rich experimental dataset of cellular action potential recordings and ionic current density measurements obtained from cells from a relatively-controlled patient cohort, permitting me to perform a critical analysis of the methodology. Separately, the experimental dataset used for construction of the populations of models was obtained from isolated human atrial myocytes in the absence

of the nNOS inhibitor SMTc. Therefore, the populations of models described in this chapter act as control populations in later chapters, where they are used for investigations into nNOS-mediated mechanisms leading to the action potential shortening and increased re-entry inducibility.

Experimental protocols described in this chapter were performed by Dr Xing Liu (Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK), who also provided the full experimental dataset. The simulations conducted with Sequential Monte-Carlo sampling were performed by Dr Brodie Lawson (ARC Centre of Excellence of Mathematical and Statistical Frontiers, Queensland University of Technology, Brisbane, Australia).

4.2 Motivation

Understanding human physiology for the purpose of understanding *in vivo* pathologies presents as key challenges the heterogeneity of the human population and the dynamic nature of properties such as ion channel densities. Whereas experiments using animal lines can be controlled to decrease heterogeneity, the human population, which is the target of investigations for clinical medicine, is highly heterogeneous, even if specific patients' cohorts are identified. Furthermore, ion channel densities are far from being constant values, particularly *in vivo*. New exciting research shows the constant modulation of ionic current properties by signalling molecules such as nitric oxide (Reilly et al., 2016), as well as hormones (Abi-Gerges et al., 2006, 2004; Tadros et al., 2014), nutrients (Zeevi et al., 2015), circadian rhythms (Jeyaraj et al., 2012) and temperature (Fenton et al., 2013; Filippi et al., 2014). Therefore, when investigating human physiology, one is dealing with a dynamically-moving target, modulated by external factors out of one's control, and therefore for which laboratory electrophysiology recordings offer a very valuable but restricted snapshot.

The reality is that a key constrain in human research is access to human tissue, which limits the recordings that are possible. For that reason, datasets often consist of standard action potential (AP) measurements for a single frequency, and sometimes, only biomarker values are available (especially when a large number of preparations are considered; see for instance, (Pueyo et al., 2016; Sánchez et al., 2014)). In addition to the AP measurements, ionic currents can sometimes be characterised in human electrophysiology studies through voltage clamp recordings, using cells that are different to the ones used in AP recordings. The isolation procedure itself affects ionic current densities (Yue et al., 1996). In addition, ionic current recordings utilise protocols that aim at isolating the contribution of a specific ionic current by means of electrical stimulation and/or pharmacology. These are rarely perfect, and can introduce significant changes in ionic current properties. For example, Cd^{2+} ions, used to block the L-type calcium current, are known to shift the steady-state activation and inactivation of potassium repolarization currents to more positive potentials by around 20 mV (Stengl et al., 1998).

Taking into consideration the points raised above, the goal of this chapter is to investigate methodological aspects for the construction of computational models to augment experimental investigations conducted in human atrial electrophysiology using human atrial cardiomyocytes. The starting point is a rich experimental dataset consisting of AP and ionic current recordings from human atrial cardiomyocytes from a patient cohort (Liu et al., 2013; Reilly et al., 2016), and three generic human atrial single cell AP models proposed in the literature (Courtemanche et al., 1998; Grandi et al., 2011; Maleckar et al., 2009). Importantly, AP properties yielded by those 3 baseline models were found to lie outside of the experimental range. In order to allow for a quantitative comparison between experiments and simulations, I target the development of populations of human atrial models with simulated electrophysiological properties in range with a specific set of

experimental recordings (Britton et al., 2013; Sánchez et al., 2014). My study expands the methodology by investigating the impact of sources of variability both internal and external to the cell, and the information embedded in several experimental calibration criteria (considering cellular or/and ionic recordings), and two different parameter sampling methods: Latin Hypercube sampling (LHS) (McKay et al., 1979), and statistically more rigorous Sequential Monte Carlo (SMC) (Drovandi et al., 2016) within the framework of Approximate Bayesian Computation (ABC).

Importantly, my aim goes beyond the construction of models as pure representation of the data (Boon and Knuuttila, 2009; Carusi et al., 2012; Muszkiewicz et al., 2016). Indeed, I consider the physiological models as useful tools to explore a wide range of possible ionic profiles (rather than trying to estimate the ionic profiles of the cells involved in the experiment), to overcome some of the limitations of the experiments outlined above. Exploring a wide range of scenarios (including outliers) with an apparently normal behaviour but with the potential to yield different pathological phenotypes under certain conditions is one of my aims, as it is of the highest interest in arrhythmia research.

4.3 Methods

4.3.1 Experimental dataset

The whole-cell voltage and current clamp experiments were used to measure action potentials (AP) and I_{to} , I_{Kur} , I_{CaL} and I_{K1} ionic current densities in isolated human atrial myocytes obtained from right atrial appendages of patients in sinus rhythm (SR) undergoing coronary artery bypass graft/ aortic valve replacement/ mitral valve replacement in Oxford, UK. The cells were obtained from patients aged 69 ± 10 years (mean $\pm$ standard deviation; (Reilly et al., 2016)). Table 9.2 in Appendix 2 provides detailed information on the patient cohort.

4.3.1.1 Action potential measurements

The patch-clamp experiments were performed with the pipette solution containing, in mM: 130 K-Aspartate, 10 HEPES, 5 Na₂ATP, 2 MgCl₂, 5 CaCl₂, 11 EGTA (pH adjusted to 7.2 using KOH), while the extracellular solution contained, in mM: 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 10 glucose, 5 HEPES (pH adjusted to 7.4 using NaOH). The temperature was held at 310.15K. APs from a total of n=29 cells from N=8 patients were measured at 5 stimulation frequencies (0.25, 0.5, 1, 2, 3 Hz). Every cell was stimulated a minimum of 25 times; the recorded APs were subsequently averaged to yield a single mean AP trace used to determine the AP properties for each cell. For each cellular AP, the resting membrane potential (RMP), action potential amplitude (APA), and action potential duration at 20%, 50%, and 90% repolarization (APD₂₀, APD₅₀, APD₉₀; Figure 4.1A) were quantified. Figure 9.1A in Appendix 2 shows all AP properties as a function of pacing frequency for each of the n=29 cells individually.

4.3.1.2 Voltage clamp data

Whole-cell voltage clamp experiments were used to determine I_{to}, I_{Kur}, I_{K1}, and I_{CaL} as previously detailed in (Liu et al., 2013; Reilly et al., 2016). Figure 9.1B in Appendix 2 shows the experimental voltage clamp curves of I_{to}, I_{Kur}, I_{K1}, and I_{CaL}.

To measure I_{to}, the outward currents were evoked by steps in the range of -70 to +50 mV for 700 ms from a holding potential of -80 mV (sampling rate: 5 kHz); a pre-step of 200 ms to -40 mV was used to inactivate the sodium current. I_{to} was measured as the steady-state current subtracted from peak outward current at the start of the depolarizing step, and normalized to membrane capacitance value. Steady-state currents were measured at the end of the depolarizing steps. I_{to} was measured in n=30 cells from N=7 patients.

To measure I_{Kur}, the outward currents were evoked by steps in the range of -35 to +45 mV for 500 ms from a holding potential of -80 mV; a pre-step of 200 ms to -35 mV was

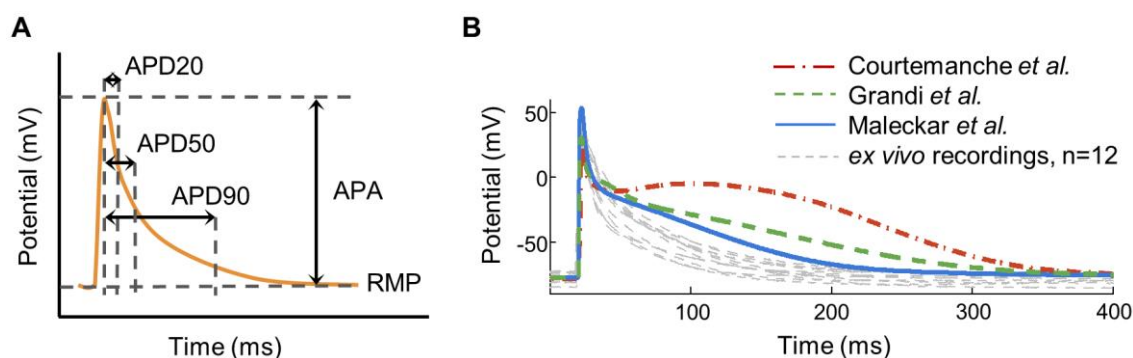


Figure 4.1 **A** Action potential (AP) biomarkers used in this study: APDxx - AP duration at xx% repolarization (ms), RMP - resting membrane potential (mV); APA - action potential amplitude (mV). **B** AP traces produced by Courtemanche, Grandi and Maleckar models (with baseline values of ionic current conductances) juxtaposed with a randomly sampled subset of experimental APs.

used to inactivate the sodium current. I_{Sus} was measured as the amplitude of the current remaining at the end of the pulse relative to the zero current level at the end of the pre-step to -35 mV. 4-AP (50 $\mu\text{mol/L}$) sensitive I_{Kur} was obtained by subtracting I_{Sus} from the remaining current after 4-AP inhibition (Wang et al., 1993). I_{Kur} was measured in $n=30$ cells from $N=7$ patients.

In voltage clamp experiments measuring I_{Kur} and I_{to} , the pipette solution contained, in mM: 120 K-Aspartate; 20 KCl, 5 Na_2ATP , 2 MgCl_2 , 5 EGTA, 10 HEPES (pH adjusted to 7.2 using KOH; pCa 7.0). Extracellular solution contained, in mM: 140 NaCl, 5.4 KCl, 1.8 CaCl_2 , 1.2 MgCl_2 , 10 glucose, 5 HEPES, 0.5 CdCl_2 (pH adjusted to 7.4 using NaOH). Cd^{2+} was used in experiments to block L-type calcium channels. However, Cd^{2+} ions are known to shift the steady-state activation and inactivation of potassium repolarization currents to more positive potentials by c.a. 20 mV (Stengl et al., 1998). I account for this by shifting experimental I-V curves of potassium currents by -20 mV.

I_{K1} was obtained by digital subtraction of current traces, recorded with a ramp pulse from -100 to +40 mV, in the presence or absence of 1 mmol/L BaCl_2 (Wang et al., 1993), as described previously (Voigt et al., 2010). The extracellular solution contained in mM:

120 NaCl, 20 KCl, 1 MgCl₂, 1.8 CaCl₂, 10 glucose, 10 HEPES, pH 7.4. High extracellular K⁺ was used to shift the reversal potential to more positive values to obtain larger inward currents (Voigt et al., 2010). The pipette solution contained in mM: 80 K-aspartate, 8 NaCl, 40 KCl, 2 CaCl₂, 5 Mg-ATP, 2 EGTA, 0.1 GTP-Tris, HEPES 10, pH 7.4. To measure I_{K1}, n=36 cells from N=7 patients were used.

I_{CaL} was elicited with step depolarization protocols, using test potentials in the range of -50 to +50 mV in 10 mV increments with a holding potential at -40 mV (Sears et al., 2003). Recordings were begun and finished after the Ca²⁺ current amplitudes and access resistance had stabilized. I_{CaL} was measured as the difference between the current at the end of the 250 ms test pulse and the peak inward current. I_{CaL} was measured in n=18 cells from N=6 patients. The cells could be split into two groups based on the value of the transmembrane voltage at which the peak inward current was detected, as the peak I_{CaL} current was registered at 10 mV and 20 mV in 8 and 10 cells, respectively. Pipette solution contained, in mM: 120 Cs-Aspartate, 10 Tea-Cl, 5 MgATP, 2 MgCl₂, 1 CaCl₂, 11 EGTA, 10 HEPES (pH 7.2 with CsOH). Extracellular solution contained, in mM: 140 Tea-Cl, 5.4 CsCl, 1.2 MgCl₂, 5 HEPES, 11 glucose, 1.8 CaCl₂ (pH 7.4 with CsOH). In the experiments where the I_{to}, I_{Kur} and I_{CaL} current densities were measured, the temperature was held at 310.15K, while the I_{K1} current density was elicited at 295.15K.

4.3.2 Constructing a population of human atrial electrophysiological models

4.3.2.1 Models of human atrial electrophysiology

Three recent models of human atrial electrophysiology by Courtemanche *et al.* (Courtemanche et al., 1998), Maleckar *et al.* (Maleckar et al., 2009), and Grandi *et al.* (Grandi et al., 2011) were used as a basis for the experimentally-calibrated atrial cell populations. Throughout the thesis, I refer to these models by their first authors' names

for simplicity. For a review of these and other models of human atrial electrophysiology, the reader is referred to the previous chapter. Figure 4.1B shows a subset of experimental AP recordings and the *in silico* APs generated by the Courtemanche, Grandi, and Maleckar models at 1 Hz pacing. It transpires that the APs of the baseline Maleckar and Grandi models are closest to the experimental AP recordings.

Slight modifications were introduced in the models to reproduce experimental I_{CaL} recordings displaying two groups of cells with peak I_{CaL} current density occurring at 10 and 20mV, respectively. In the baseline Maleckar, Grandi, and Courtemanche models, the peak I_{CaL} current density occurs at 10 mV, 0 mV and 10 mV (Figure 9.2, Appendix 2). I evaluated the impact of the half-potentials of activation (V_{act}) and inactivation (V_{inact}) of the I_{CaL} current in all three models (baseline values of V_{act} and V_{inact} are -9.0 mV, -9.0 mV, -10.0 mV, and -27.4 mV, -30.0 mV and -28.0 mV in the Maleckar, Grandi, and Courtemanche models, respectively). For each of the three models under consideration, a parameter sweep was conducted where both V_{act} and V_{inact} were scaled by factors ranging from -2.0 to +2.0 of their baseline model values in steps of 0.2. Results show that peak I_{CaL} current at 20 mV in the Maleckar, Grandi and Courtemanche models can be achieved by altering V_{act} in the d-gate of I_{CaL} to 3.6 mV, 10.8 mV, and 4.0 mV, respectively, while a change of V_{act} to 0.0 mV in the Grandi model results in the peak I_{CaL} current occurring at 10 mV (Figure 9.2, Appendix 2).

4.3.2.2 Populations of candidate models

Experimentally-calibrated population of models covering a wide range of possible ionic scenarios relevant for arrhythmia research were generated using Latin Hypercube sampling (LHS) and sequential Monte Carlo (SMC) methods. As in previous studies (reviewed in (Muszkiewicz et al., 2016)), my underlying hypothesis is that variability in ion channel densities is the main determinant of cellular AP variability in atrial

electrophysiology. Thus, within candidate populations, models share the same equations, but use a different set of ionic current conductance values.

LHS generates parameter sets over a large number of parameters efficiently and without bias (Burrage et al., 2015; McKay et al., 1979). As in previous studies (Britton et al., 2014, 2013), the maximal conductances/permeabilities of I_{to} , I_{Kur} , I_{CaL} , I_{Kr} , I_{Ks} , I_{K1} , I_{Na} , I_{NaK} , I_{NCX} , J_{up} , and J_{rel} were varied simultaneously over $\pm 100\%$ of their baseline model values. The candidate models were then simulated in conditions closely resembling experiments and only the models whose outputs fell in range with experimental data were retained for further investigations. For each of the three models used in this study, two candidate populations of $N=15,000$ models were created (corresponding to the two distinct I_{CaL} peak densities). I compared results obtained with LHS to the SMC approach, described in (Drovandi et al., 2016). SMC considers models as ‘particles’ that are initialised across the parameter space and move around it in order to satisfy the experimentally-imposed constraints on model outputs. The SMC algorithm stops when a specific number of models that fulfil the constraints are found. The same parameters over identical ranges of values were permitted to vary in SMC and LHS approaches.

4.3.2.3 Calibration of the populations with electrophysiological recordings

Calibration consisted of retaining the models yielding AP and/or ionic properties in range with the experimental recordings. AP calibration was conducted using the following biomarkers: APD_{20} , APD_{50} , APD_{90} , RMP, APA, the difference $APD_{90}-APD_{50}$ (triangulation criterion) (Table 4.1), as well as linearity between APD_{20} and APD_{50} properties (Figure 4.2). APD_{90} rate dependence was also used for calibration by requiring that the values of APD_{90} at 3 Hz and 0.25 Hz generated by each *in silico* model fulfilled the inequality $1.11 \geq APD_{90}(3Hz)/APD_{90}(0.25Hz) \geq 0.75$ (as the minimum and maximum values of the ratio $APD_{90}(3Hz)/APD_{90}(0.25Hz)$ *ex vivo* were 1.11 and 0.75, respectively).

In the case of LHS, experimental constraints were applied simultaneously. In the SMC sampling, the calibration constraints were applied sequentially for every biomarker (section 9.1.1, Appendix 2). At the level of ionic currents, I ensured that the maximum values of ionic current densities *in silico* did not exceed the maximal ionic current densities obtained in *ex vivo* measurements, if needed following AP calibration. This enabled me to investigate the information contained within the AP biomarkers vs. ionic current properties.

4.3.3 Simulation protocols

4.3.3.1 Computational action potential measurements

The models were simulated in conditions resembling experiments as closely as possible. Detailed information is provided in section 9.1.2 of Appendix 2. Briefly, the temperature was set to 310.15 K. I assumed that ionic concentrations in the pipette and extracellular solutions used in experiments corresponded to the intracellular and extracellular concentrations in the models. The intracellular Na^+ and K^+ and extracellular Na^+ , K^+ , and Ca^{2+} concentrations were held constant in time. Each model was stimulated for 100 beats at every frequency. To evaluate the effect of stimulus strength as an external source of variability, two scenarios were considered (summarized in Table 4.2). First, stimulus magnitude and duration were unchanged relative to the original formulations of the Courtemanche, Maleckar, and Grandi models. Second, to ensure that the amount of charge injected into the cell was comparable between the 3 model populations, the stimulus magnitude was reduced from 100% to 60% of its baseline value in the Maleckar-based populations, yielding a stimulus of -9.0 pA/pF over 6 ms. In the Courtemanche model, the stimulus amplitude was reduced from the baseline value of -20 pA/pF to -9 pA/pF, and stimulus duration was extended from 2 ms to 5 ms. In the Grandi model, the stimulus amplitude and duration were kept at baseline values of -9.5 pA/pF and 5 ms.

4.3.3.2 Computational voltage clamp protocols

The protocols followed in voltage clamp simulations mimicked as closely as possible the voltage clamp experiments previously described by (Liu et al., 2013; Reilly et al., 2016). Details are provided in section 9.1.3 of Appendix 2. Briefly, in the simulations of I_{to} and I_{Kur} , the inhibitory effect of Cd^{2+} on I_{CaL} was modelled by setting $G_{CaL}=0$ (Plonsey, R. and Barr, 2007). Block of I_{K1} by Ba^{2+} ions was simulated by setting $G_{K1}=0$. Block of I_{Kur} by 4-AP was simulated by turning G_{Kur} to zero. In the simulations of I_{CaL} , all conductances for potassium and sodium currents were set to zero to simulate block by caesium (Plonsey, R. and Barr, 2007) and the absence of Na^+ in solutions. Intracellular K^+ and Na^+ and extracellular K^+ , Na^+ and Ca^{2+} concentrations were held constant and to their experimental equivalents. The temperature was set to 310.15K in the simulations where the I_{to} , I_{Kur} and I_{CaL} current densities were elicited, while in the simulations of I_{K1} current density, the temperature was 295.15K. Figure 9.2 in Appendix 2 depicts the I_{to} , I_{Kur} , I_{K1} and I_{CaL} currents in the baseline Maleckar, Grandi and Courtemanche models.

Table 4.1 Minimum and maximum values of the AP properties from n=29 cells *ex vivo*.

f (Hz)	APD ₂₀ (ms)		APD ₅₀ (ms)		APD ₉₀ (ms)		RMP (mV)		APA (mV)		APD ₉₀ -APD ₅₀	
	min	max	min	max	min	max	min	max	min	max	min	max
0.25	0.9	11.9	4.7	39.0	72.8	154.7	-86.7	-66.5	79.7	136.5	56.7	121.4
0.5	0.7	11.3	4.7	34.7	69.9	147.0	-85.4	-67.6	86.2	133.1	54.2	118.9
1	0.7	11.5	4.9	34.1	68.9	142.8	-85.2	-66.1	94.6	137.5	58.3	117.6
2	0.9	11.0	5.1	34.5	64.4	135.9	-85.9	-66.2	100.6	130.7	54.9	113.5
3	0.5	11.6	5.0	37.1	60.7	131.6	-87.2	-65.6	92.8	132.1	51.5	111.4

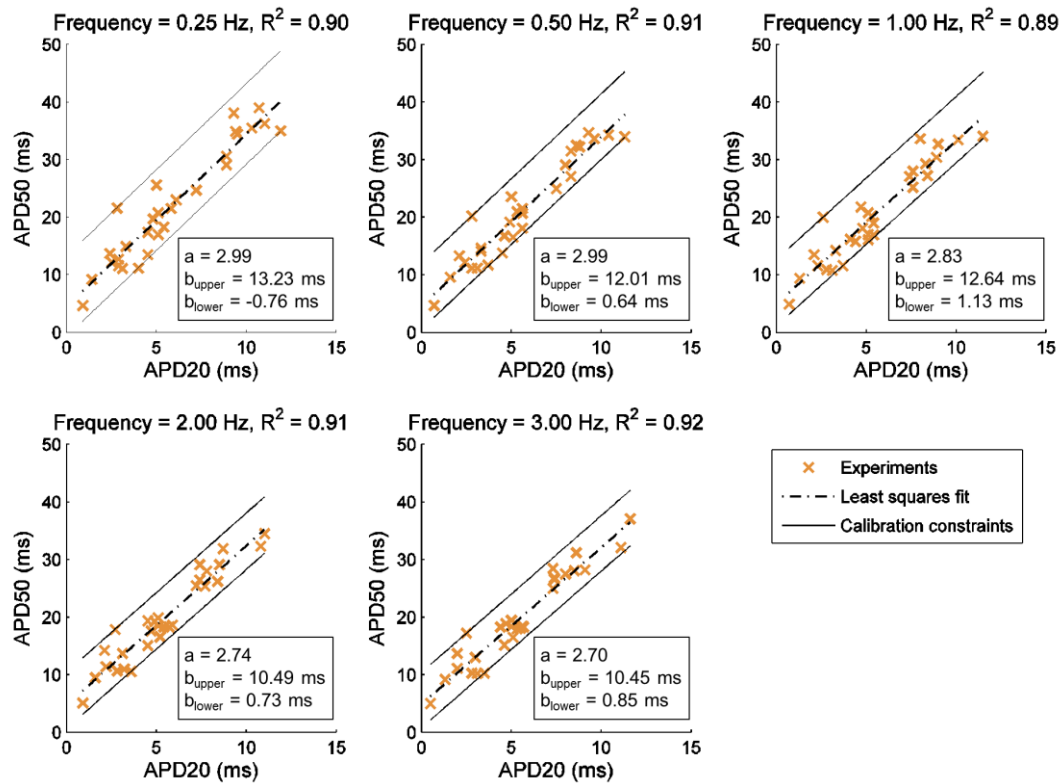


Figure 4.2 Linear relationship between APD₂₀ and APD₅₀ properties at 5 pacing frequencies *ex vivo*. Experimental data are shown in orange, dashed-dotted lines are the least-squares fits to the data (corresponding R^2 value given at the top of each plot), solid black lines represent constraints corresponding to $aAPD_{20} + b_{upper} \geq APD_{50} \geq aAPD_{20} + b_{lower}$ imposed on APD₂₀ and APD₅₀ properties generated by *in silico* models. The constant a was obtained from the least-squares fit, while b_{lower} and b_{upper} were determined from the values of the outermost experimental data points.

Table 4.2 The amplitude and duration of stimulus current applied to the candidate populations based on the Courtemanche, Maleckar, and Grandi models. Baseline stimulus scenario uses the stimulus current parameters as in the original publications.

	(i) Amplitude and duration of baseline stimulus	(ii) Amplitude and duration of altered stimulus
Courtemanche	-20.0 pA/pF, 2 ms	-9.0 pA/pF, 5 ms
Maleckar	-15.0 pA/pF, 6 ms	-9.0 pA/pF, 6 ms
Grandi	-9.5 pA/pF, 5 ms	-9.5 pA/pF, 5 ms (unchanged)

4.4 Results

4.4.1 Human atrial populations calibrated with AP data: conductances vs. other factors

Figure 4.3 shows the simulated AP biomarkers produced by the Maleckar-based population with baseline stimulus amplitude (-15 pA/pF), calibrated with experimental AP properties. Out of the $N=30,000$ candidate models generated with LHS, $n=974$ produced AP properties in range with experimental observations. The biomarkers APD_{90} and APA span the experimentally-permitted range of values fully, indicating that substantial variability in maximal ionic current conductances is sufficient to generate a wide range of those AP properties. Contrastingly, simulated APD_{20} and APD_{50} values span the lower half of their experimental ranges at all pacing frequencies, indicating that the population of models is not capable of capturing longer early repolarization times observed in experiments.

I hypothesised that the stimulus current amplitude could impact early repolarization via the modulation of the AP peak, provided the stimulus is sufficiently strong to elicit an AP. Figure 9.3 and Figure 9.4 in Appendix 2 show the impact of the stimulus current amplitude on the AP as well as on ionic currents affecting early repolarization in the Maleckar model. For the baseline model values of the stimulus amplitude and duration (-15 pA/pF and 6 ms, respectively), the membrane becomes sufficiently depolarized within ~3 ms to activate the I_{Na} current, which in turn elicits the AP. However, since the stimulus is applied for 6 ms rather than 3 ms (Table 4.2), the stimulus acts as an additional inward current for the remaining 3 ms, leading to stronger I_{to} and I_{Kur} currents and a spikier AP. Reducing the stimulus amplitude to -9 pA/pF activates I_{Na} after ~5 ms, and the stimulus acts as an additional inward current for 1 ms only. As a result, the amplitudes of I_{to} and I_{Kur} currents are diminished and the early repolarization of the AP is prolonged.

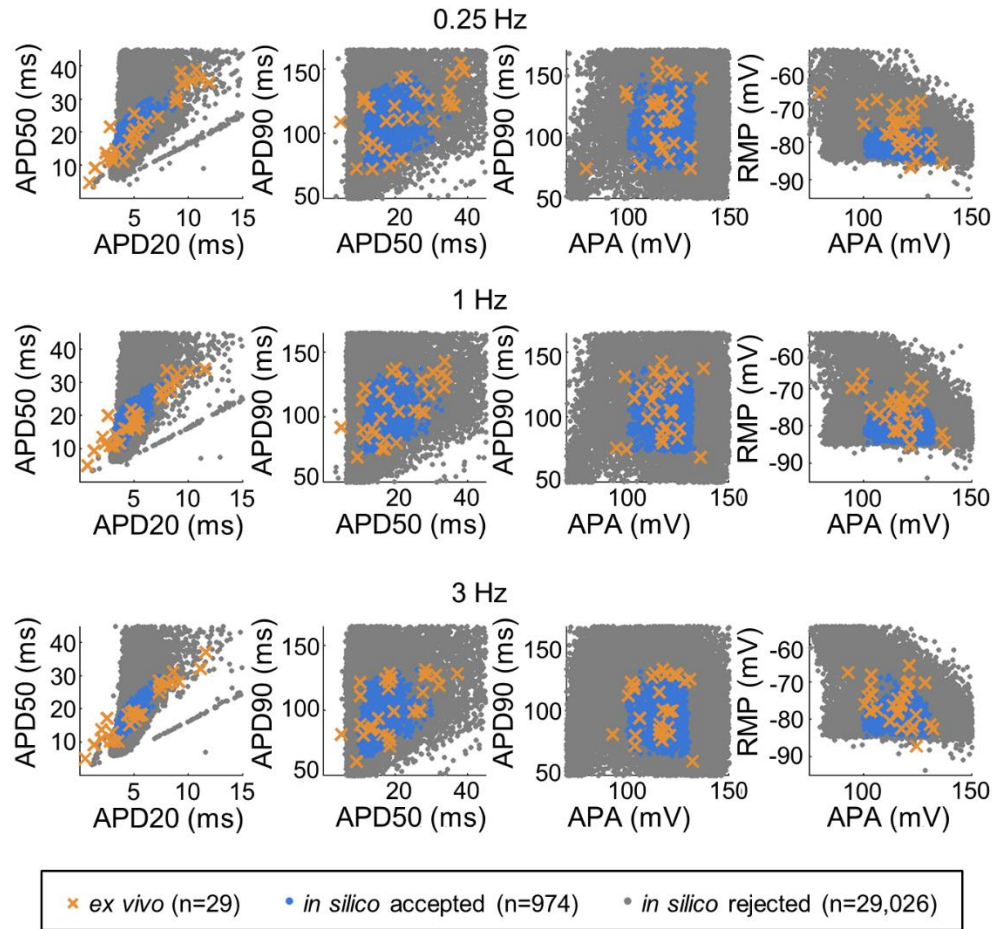


Figure 4.3 Experimentally-calibrated population of human atrial myocytes constructed with LHS, based on the Maleckar model with baseline stimulus strength (-15 pA/pF), and calibrated with experimentally-derived criteria on action potential properties. Legend: orange crosses – *ex vivo* data, blue dots – accepted *in silico* models, grey dots – rejected *in silico* models. Plots of AP properties at 0.25, 1, and 3 Hz are presented. Similar results were obtained at other stimulation frequencies.

Simulation results in Figure 4.4 show that indeed reducing the stimulus magnitude to -9 pA/pF alters the shape of the AP peak (Figure 4.4A), leading to a wider range of APD₂₀ and APD₅₀ properties in the simulations when conductances are varied (Figure 4.4B-D). The overall number of accepted models also increases to n=1,559, indicating that more parameter combinations now yield AP biomarkers consistent with experimental recordings.

Figure 4.5 shows the population of $n=593$ human atrial myocytes based on the Grandi model with baseline stimulus amplitude (-9.5 pA/pF), calibrated with experimental AP properties. Overall, in the Grandi-based population, the simulated APs and biomarkers span the experimental ranges of variability in all AP properties (Figure 4.5B-C). Further analysis of the population reveals that variability in I_{CaL} peak density location plays a crucial role in the early repolarization phase of the AP. The Grandi-based population consists of $n=360$ and $n=233$ models with I_{CaL} maximized at 10 and 20 mV, respectively, and the models exhibiting I_{CaL} peak density at 20 mV produce APs with longer early repolarization compared to those that maximize I_{CaL} at 10 mV (Figure 4.5A). This is in contrast to the Maleckar-based population, where early repolarization is controlled not by variability in I_{CaL} peak density location, but stimulus strength. These findings further emphasise the importance of sources of cell-to-cell variability beyond maximal ionic current conductances for a good coverage of the biomarker space.

Not all models succeeded in reproducing the experimental variability in the experimental AP recordings. Despite the wide variability in ionic current conductances, I_{CaL} peak density location, and reduction in stimulus amplitude, the Courtemanche-based population of $n=1,559$ accepted models did not succeed in covering the experimental AP ranges, particularly in the early repolarization phase (Figure 9.5, Appendix 2). Instead of the triangular AP morphology captured by the Maleckar- and Grandi-based populations, the Courtemanche models produced spike-and-dome APs characterized by short APD_{20} and APD_{50} values.

To investigate the causes of this inter-model difference in AP morphology, I performed an action potential clamp simulation, whereby the transmembrane potential was controlled to follow a triangular AP morphology in the three generic models. This allowed for a direct comparison of the transmembrane ionic currents across the models while eli-

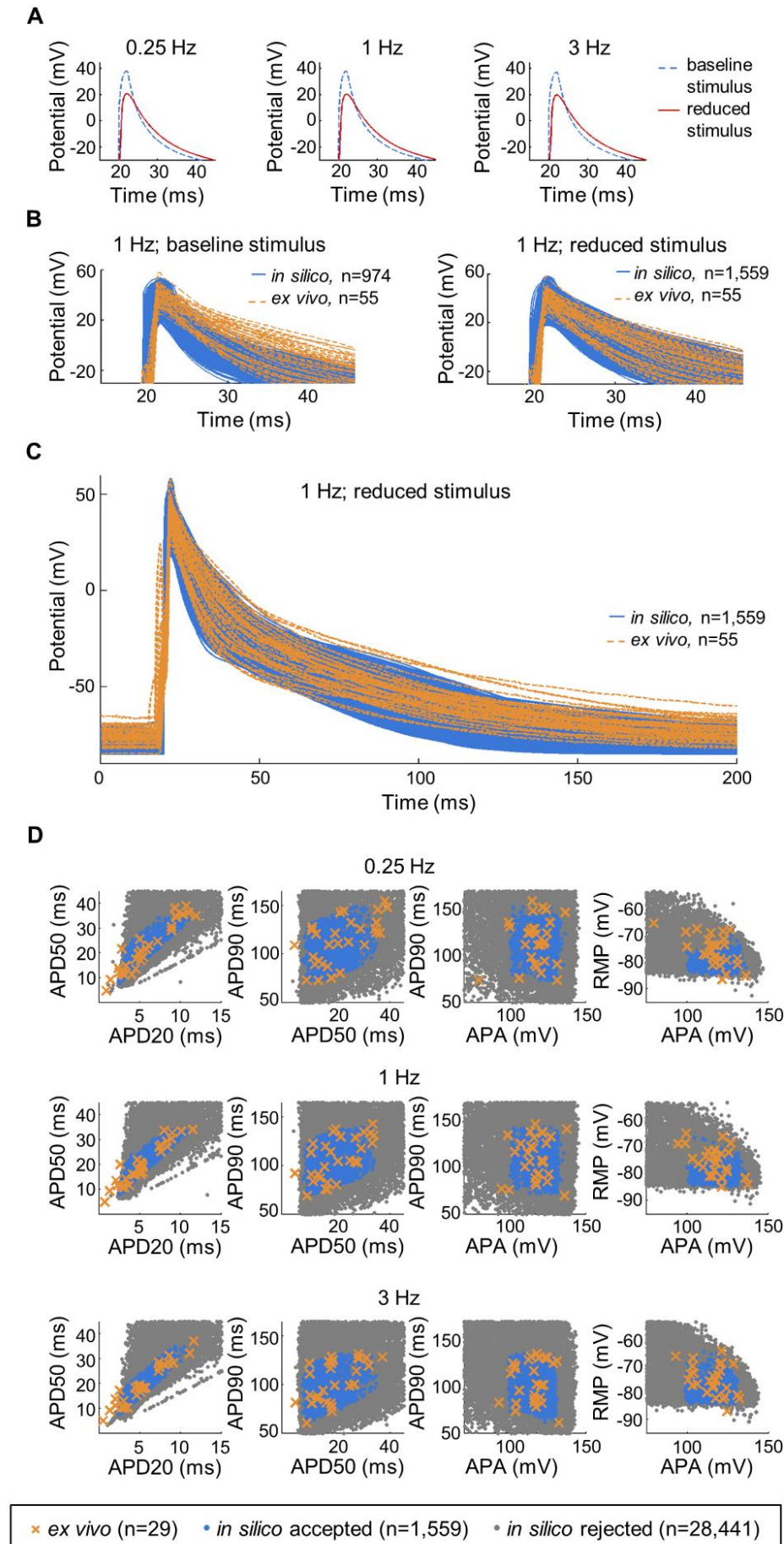


Figure 4.4 Importance of the stimulus strength as an external factor determining AP variability in the experimentally-calibrated population based on the Maleckar model, constructed with LHS, and calibrated with experimental AP properties. **A** Reducing stimulus strength from -15 pA/pF (red continuous line) to -9 pA/pF (blue dashed line) alters AP peak morphology at 0.25, 1, and 3 Hz. **B** Comparing AP peak morphology in *ex vivo* data (orange dashed lines) to modelling results (blue continuous lines) at 1 Hz with baseline stimulus strength and reduced stimulus in the experimentally-calibrated populations. **C-D** Experimentally-calibrated population of human atrial myocytes with reduced stimulus strength (-9 pA/pF). **C** AP traces at 1 Hz in *ex vivo* recordings (orange dashed lines) and *in silico* population (blue continuous lines). **D** Selected plots of AP properties at 0.25, 1, and 3 Hz. Legend: orange crosses – *ex vivo* data, blue dots – accepted *in silico* models, grey dots – rejected *in silico* models. Similar results were obtained at other stimulation frequencies.

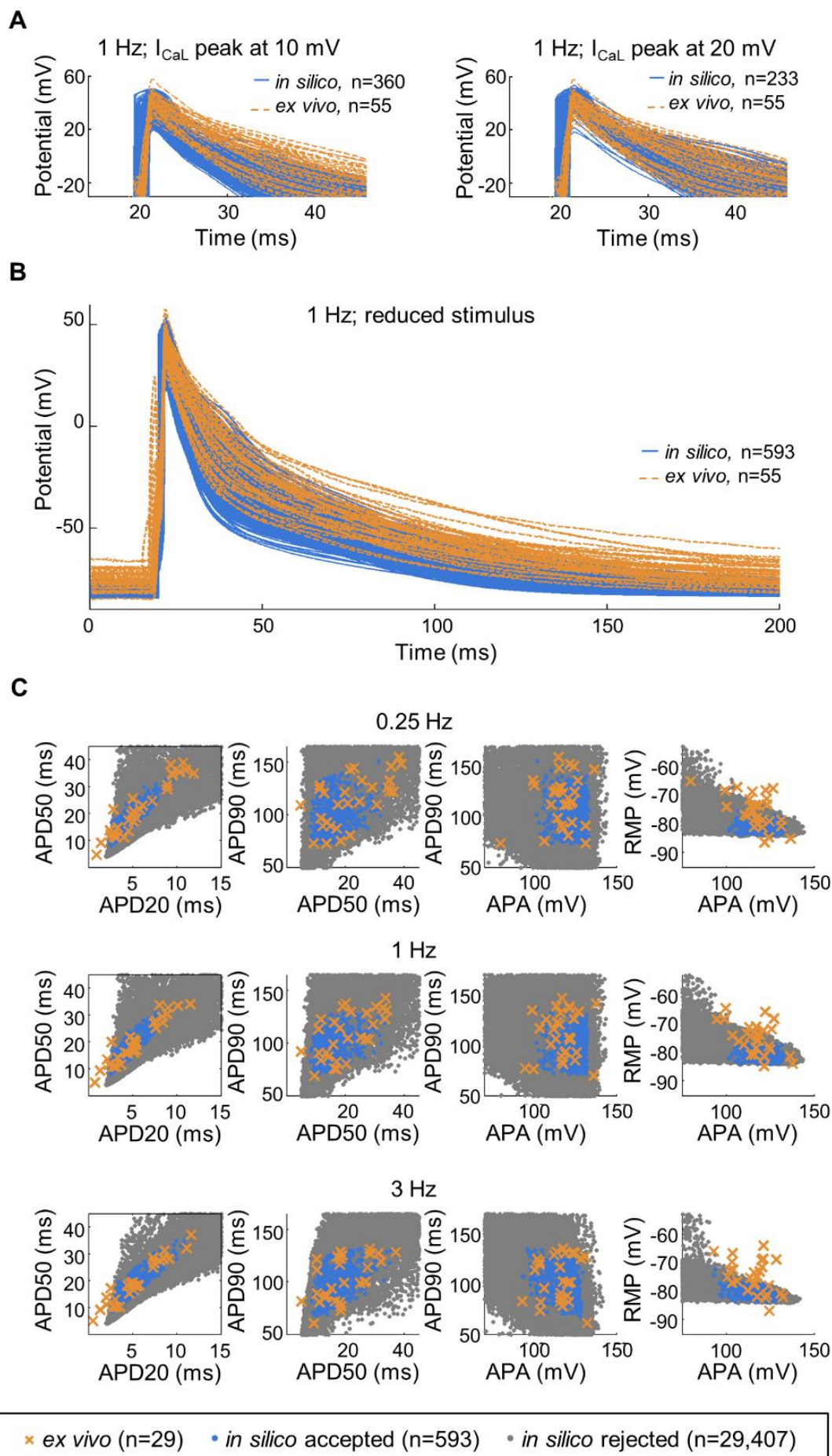


Figure 4.5 Intrinsic determinants of variability in the Grandi population of models constructed with LHS and calibrated with experimental AP data. **A** Comparing the AP peak morphology in *ex vivo* data (orange dashed lines) to modelling results (blue continuous lines) at 1 Hz with I_{CaL} peak density at 10 mV and at 20 mV. **B** AP traces at 1 Hz in *ex vivo* recordings (orange dashed lines) and *in silico* population (blue continuous lines). **C** Selected plots of AP properties at 0.25, 1, and 3 Hz. Legend: orange crosses – *ex vivo* data, blue dots – accepted *in silico* models, grey dots – rejected *in silico* models. Similar results were obtained at other stimulation frequencies.

minating any non-linear interactions between the currents. Out of I_{Kur} , I_{to} and I_{CaL} transmembrane currents impacting early repolarization (within $\sim 2-3$ ms of the AP peak), differences were observed in I_{to} and I_{Kur} in the Courtemanche model relative to Maleckar and Grandi, suggesting that I_{Kur} and I_{to} are responsible for the spike in the early depolarization in the Courtemanche model (Figure 9.6, Appendix 2). In the following section, I focus on the Maleckar and Grandi-based populations, as they best capture experimental ranges of variability in the AP.

4.4.2 Ionic properties underlying human atrial populations calibrated with experimental AP data

Figure 4.6A illustrates scatter plots of key ionic conductances underpinning the Maleckar- and Grandi-based populations calibrated with experimental AP data. Consistent with previous studies (Britton et al., 2013; Groenendaal et al., 2015; Sobie, 2009), most conductances span their initial range of values ($\pm 100\%$ of baseline) almost entirely. The only parameters whose ranges of values are noticeably constrained are conductances/permeabilities of I_{Na} , I_{NaK} and I_{K1} , consistent in both populations. In the Maleckar-based population, models with G_{Na} below -59% of baseline are absent from the population; this is due to I_{Na} 's critical role in the generation of the AP amplitude as shown in the correlation analysis in Figure 9.7, Appendix 2. In the Grandi-based population, APA is additionally modulated by I_{NaK} (Figure 9.7, Appendix 2), explaining a stronger constraint on G_{NaK} relative to Maleckar-based models, and a weaker constraint on G_{Na} ranges (Figure 4.6A). Lastly, models with both low G_{NaK} and low G_{K1} are absent from both AP-calibrated populations because of I_{NaK} and I_{K1} 's roles in late repolarization and their impact on RMP (Figure 9.7). Overall, most conductances underpinning the two calibrated populations span wide ranges of values, emphasising the robustness of the atrial AP to variability in ionic conductances.

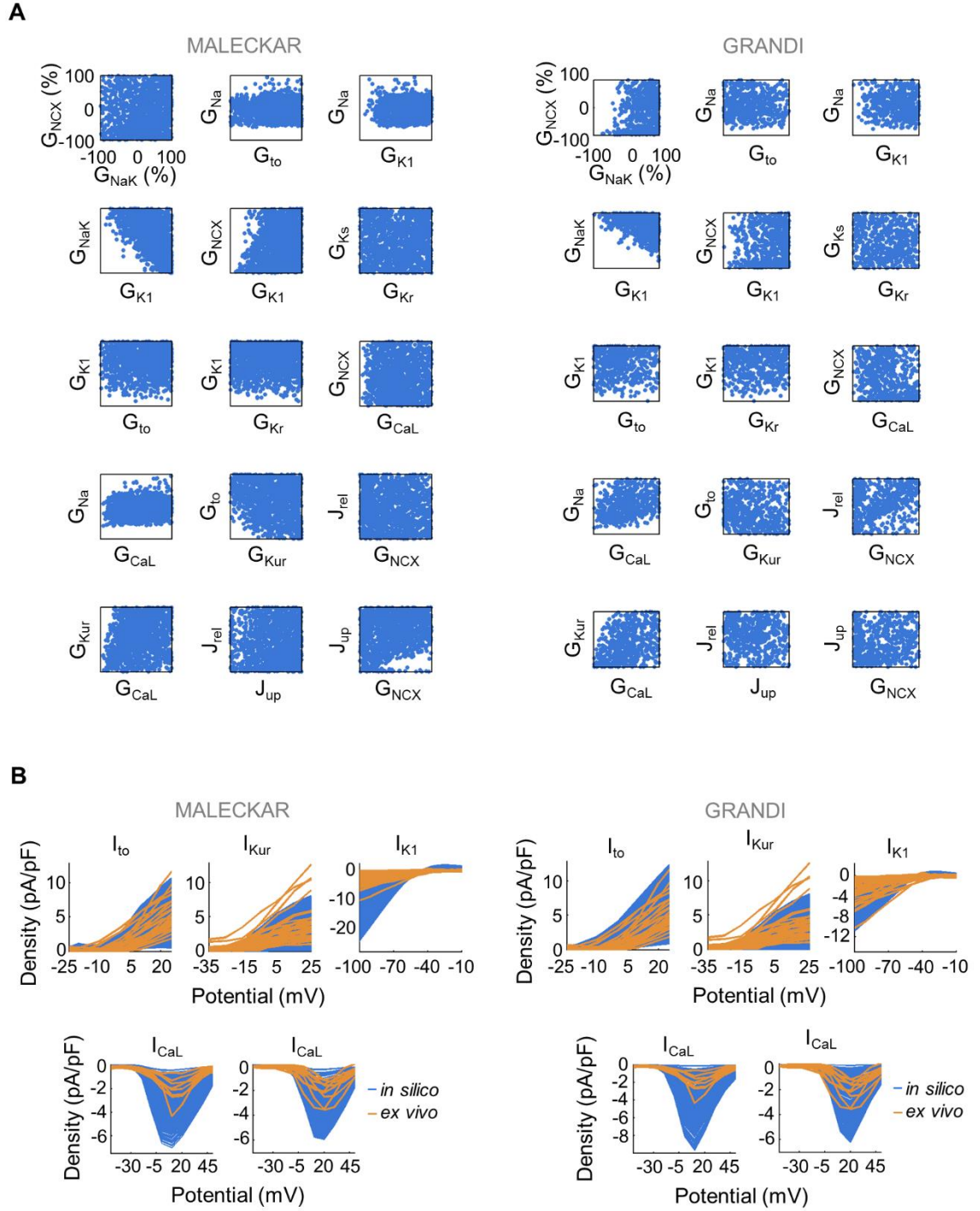


Figure 4.6 Impact of experimental calibration with AP properties on maximal conductances and ionic current densities underpinning the Maleckar- and Grandi-based populations of models constructed with LHS. **A** Selected pairwise plots of maximal current conductances in the populations of $n=1,559$ and $n=593$ *in silico* human atrial myocytes based on the Maleckar and Grandi models, respectively. **B** Simulated I-V curves of I_{to} , I_{Kur} , I_{K1} , and I_{CaL} with peaks at 10 and 20 mV in the Maleckar- and Grandi-derived populations (blue) compared with experimental results (orange).

Several previous studies with experimentally-calibrated populations of models only had access to cellular AP data, and would conclude with analyses such as those shown in Figure 4.6A. However, the experimental dataset used here consists of cellular AP recordings as well as I_{to} , I_{Kur} , I_{K1} and I_{CaL} current density measurements. Therefore, unlike in previous studies, I can further investigate the simulated current densities in the AP-calibrated populations relative to experimental recordings. This permits me to assess the impact of the calibration with AP properties on the populations of models. It is important to note that the experimental measurements of a specific ionic current and cellular AP cannot be obtained simultaneously from the same cell, and therefore the experimental recordings of I_{to} , I_{Kur} , I_{K1} and I_{CaL} do not underpin the experimental APs (furthermore, the cells used for measurements of one current are different to those used for recordings of other currents). However, ionic currents and cellular APs were measured in cells from the same patient cohort, so the comparison of simulated and experimental ionic current densities is still of interest.

In Figure 4.6B, simulated current-voltage relationships for I_{to} , I_{Kur} , I_{K1} and I_{CaL} obtained using both populations calibrated with AP biomarkers are compared to experimental recordings. This is further illustrated in Table 4.3, which shows minimum and maximum values of ionic current densities measured in experiments and the corresponding minimum and maximum values of ionic current conductances necessary to capture the experimentally-observed ranges of variability. The comparison highlights large variability in peak current densities observed in experiments and simulations. The peak densities of I_{to} and I_{Kur} in both Maleckar-and Grandi-based populations span $\pm 100\%$ of their baseline values and cover the bulk of the experimental range. In the case of I_{Kur} , even larger variations of maximal current conductances (beyond $+100\%$ of baseline) could be justified experimentally. These findings illustrate that a set of AP measurements

obtained from a relatively-controlled patient cohort can be recreated/simulated with a wide variability in underpinning ionic current conductances, consistent with large heterogeneity in the human population.

Differences are observed in the ranges of I_{K1} density between Maleckar- and Grandi-based populations calibrated with experimental data on AP biomarkers. In the Maleckar-based population, the models display maximal I_{K1} density of up to -24.62 pA/pF, exceeding the experimental limit of -10.33 pA/pF. In contrast, the Grandi-based population produces I_{K1} density in range with the experimental recordings. In both calibrated populations, the maximal current conductances of I_{K1} span $\pm 100\%$ of baseline model values almost entirely (Figure 4.6A), indicating that the inter-population difference is a manifestation of distinct experimental datasets used in construction of the generic Maleckar and Grandi models. Indeed, the baseline Maleckar model yields I_{K1} density of -12.31 pA/pF (at -100 mV) and is in part based on experimental data of (Bosch et al., 1999) who reported I_{K1} density of -9.83 ± 0.96 pA/pF (at -90 mV). The Grandi model, derived from a model of the human ventricular myocyte, takes into account the c.a. 6-fold smaller density of I_{K1} in atria relative to ventricles (Grandi et al., 2011; Schram et al., 2002), and yields baseline I_{K1} density of -5.49 pA/pF, closer to experimentally-measured value of -3.5 ± 0.3 (at -120 mV) reported by (Workman et al., 2001). The three-fold difference in experimental measurements of I_{K1} density reported by (Bosch et al., 1999; Workman et al., 2001) provides another illustration of the wide heterogeneity in human atrial myocytes.

As with potassium repolarization currents, both Maleckar- and Grandi-based AP-calibrated populations contain models with L-type calcium current density spanning $\pm 100\%$ of baseline. However, the +100% limit translates into I_{CaL} density of over -7 pA/pF and -10 pA/pF in the Maleckar- and Grandi-based populations, respectively. These

Table 4.3 Minimum and maximum values of ionic current densities measured in experiments *ex vivo*, and the corresponding minimum and maximum values of ionic current conductances (relative to baseline model values) required to mimic the experimentally-observed ranges of variability.

	Experiment		baseline Maleckar model			baseline Grandi model		
Current	Min density (pA/pF)	Max density (pA/pF)	Baseline density (pA/pF)	Conductance (% change from baseline)		Baseline density (pA/pF)	Conductance (% change from baseline)	
				Min	Max		Min	Max
I_{to} (at 30 mV)	2.28	13.52	5.32	-57%	+154%	7.65	-70%	+77%
I_{Kur} (at 25 mV)	0.99	12.68	4.02	-75%	+215%	4.02	-75%	215%
I_{K1} (at -100 mV)	-0.20	-10.33	-12.31	-98%	-16%	-5.49	-97%	+88%
I_{CaL} (10 mV peak)	-1.99	-3.56	-3.82	-69%	-7%	-5.36	-63%	-33%
I_{CaL} (20 mV peak)	-0.63	-4.35	-3.17	-80%	+37%	-3.51	-82%	+24%

values exceed the experimentally-measured peak current of -4.35 and -3.56 pA/pF (for I_{CaL} density maximized at 10 mV and 20 mV) seen in the experimental data, indicating that I_{CaL} in the generic Maleckar and Grandi models is large compared to experimental data. However, cells used in the experiments were obtained from a cohort of elderly patients (Table 9.2, Appendix 2), and I_{CaL} density is known to decrease with age (Herraiz-Martínez et al., 2015). Therefore, the generic Maleckar and Grandi models may constitute a better match for cardiomyocytes from younger patients.

4.4.3 Human atrial populations calibrated with AP and ionic current datasets

We proceeded to further calibrate the two model populations using voltage-clamp I_{CaL} and I_{K1} measurements by selecting models whose peak currents did not exceed the maximal experimental values. Histograms of each current density are shown in Figure 4.7A for the Maleckar-based population. Constrains on maximal I_{CaL} and I_{K1} densities result in a sharp cut-off on maximal ionic current densities of these currents, but appear to have little effect on I_{Kur} and I_{to} beyond uniform model attrition (Figure 4.7A). At the level of the AP properties, the constraint on I_{CaL} does not affect the variability of AP properties, as the majority of models still span the experimentally-permitted ranges of AP biomarkers (Figure 4.7B). However, the constraint on I_{K1} current appears to visibly reduce models with short APD_{90} , probably due to I_{K1} being the main determinant of APD_{90} in the AP-calibrated population (Figure 9.7, Appendix 2). Figure 4.7C-D shows the Maleckar-based population of models calibrated with AP properties as well as I_{K1} and I_{CaL} . Simulated AP traces capture the majority of experimental AP space, and the range of simulated ionic current densities of I_{CaL} and I_{K1} now offers a better match with experimental recordings.

Figure 4.8 illustrates the effect of additional calibration criteria on I_{CaL} and I_{K1} on the Grandi-based population of models. Since I_{K1} density matched the experimentally-observed range in the AP-calibrated population, the additional constraint with this current does not have an appreciable impact on the population. The major effect is of I_{CaL} calibration, which results in a uniform model attrition in the AP biomarker space, indicating that large I_{CaL} density does not appreciably alter the AP properties. The resultant Grandi-based population of models, calibrated with both AP and ionic current densities, provides a very good coverage of the experimental AP biomarkers and ionic currents.

Multi-scale Modelling and Simulations into the Mechanisms Linking Neuronal Nitric Oxide Synthase and Atrial Fibrillation

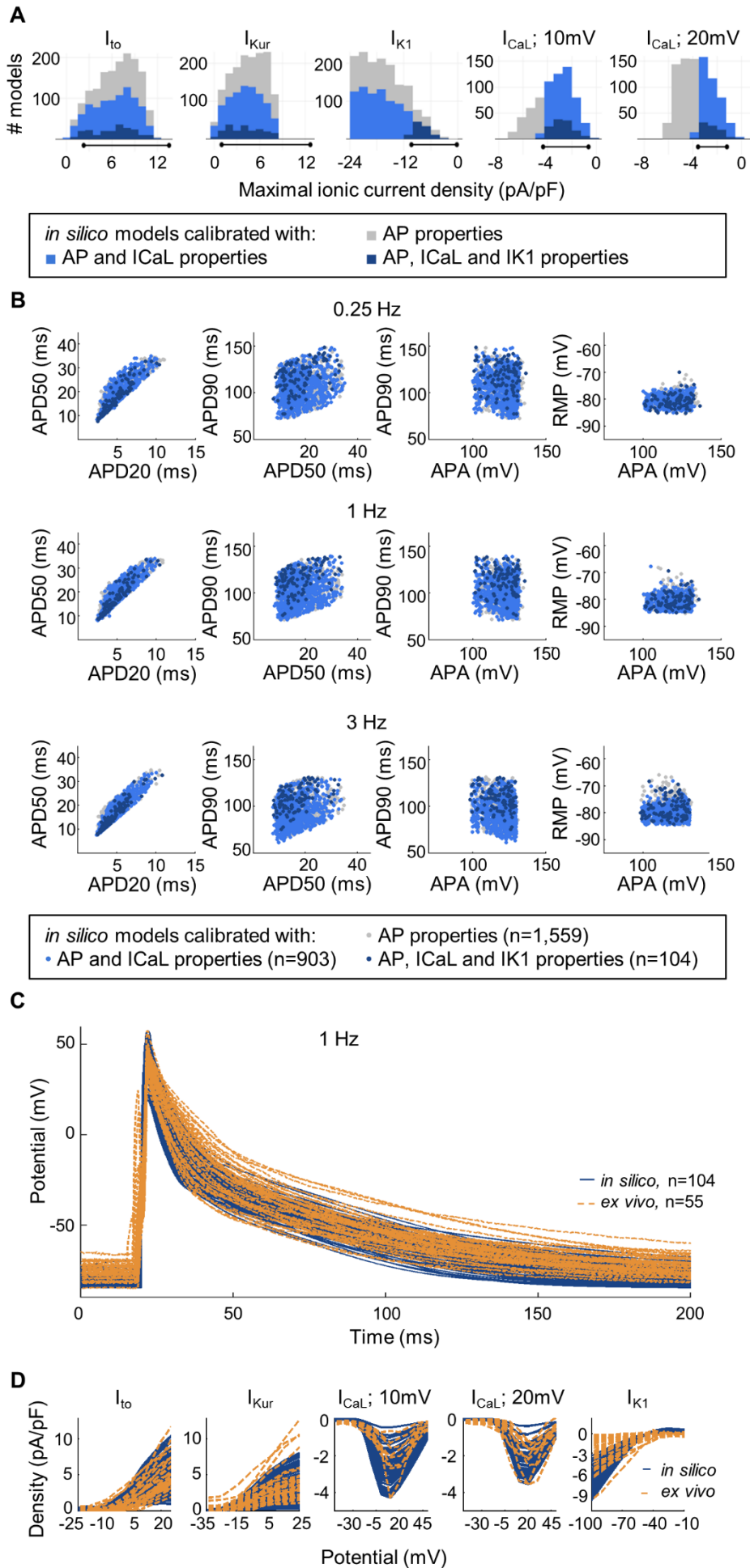


Figure 4.7 Impact of additional calibration criteria at the level of maximal I_{CaL} and I_{K1} ionic current densities on the Maleckar-based population derived from LHS. **A** Histograms of maximal ionic current densities, and **B** AP biomarker plots. Grey, light blue and dark blue correspond to calibration with AP criteria alone; calibration with AP and I_{CaL} criteria; as well as calibration with AP, I_{CaL} and I_{K1} criteria. In **A**, experimental ranges are indicated by black bars. **C** Action potential traces and **D** I-V curves produced by the Maleckar-based population of models calibrated with AP biomarkers and I_{CaL} , I_{K1} current magnitudes (dark blue) compared to experimental measurements (orange).

Multi-scale Modelling and Simulations into the Mechanisms Linking Neuronal Nitric Oxide Synthase and Atrial Fibrillation

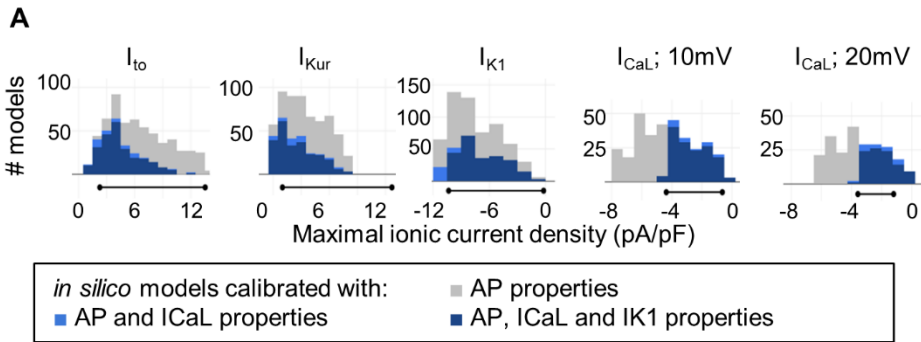
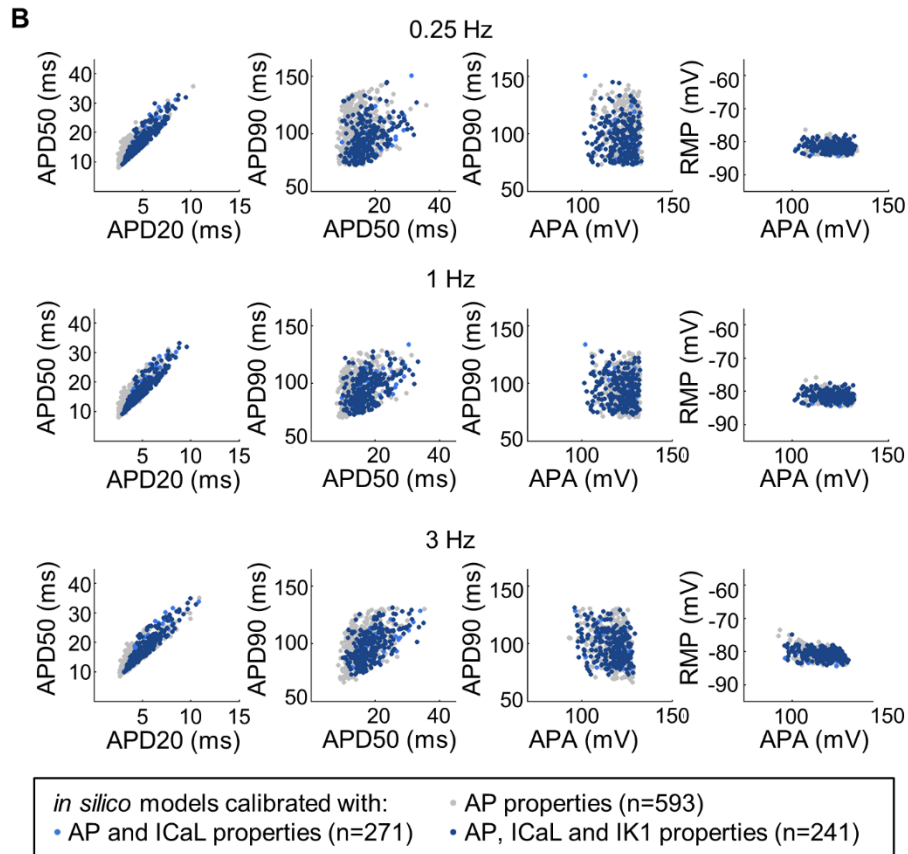
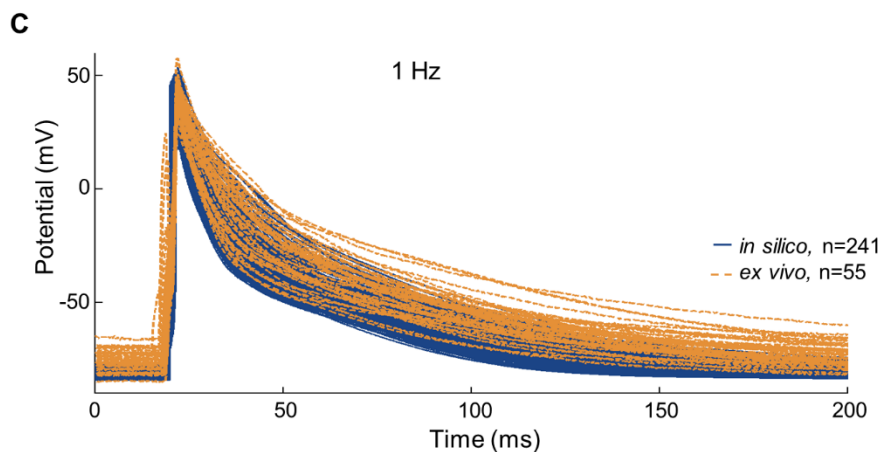
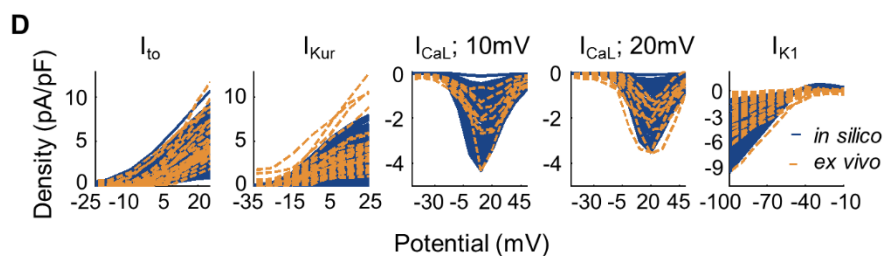


Figure 4.8 Impact of additional calibration criteria at the level of maximal I_{CaL} and I_{K1} ionic current densities on the Grandi-based population derived from LHS. **A** Histograms of maximal ionic current densities, and **B** AP biomarker plots. Grey, light blue and dark blue correspond to calibration with AP criteria alone; calibration with AP and I_{CaL} criteria; as well as calibration with AP, I_{CaL} and I_{K1} criteria. In **A**, experimental ranges are indicated by black bars. **C** Action potential traces and **D** I-V curves produced by the Grandi-based population of models calibrated with AP biomarkers and I_{CaL} , I_{K1} current magnitudes (dark blue) compared to experimental measurements (orange).

4.4.4 Distributions of conductances in the accepted models

Box plots depicting the distribution of conductances in the Maleckar- and Grandi-based populations, both with and without the additional calibration with I_{CaL} and I_{K1} ionic current densities, are shown in Figure 4.99. In the Maleckar-based population calibrated with AP properties, the majority of the conductance values underpinning the 1,559 accepted models are spread within $\pm 35\%$ of the median, indicating that most of the unusual parameter values were rejected in the calibration process. Medians of 8 out of 11 varied parameters differ by more than $\pm 10\%$ relative to the baseline model values (only the medians of G_{Kr} , G_{Ks} and J_{rel} – the parameters that do not impact AP biomarkers (Figure 9.7) – are close to their baseline model values). This is to be expected in view of the significant differences between the AP biomarkers produced by the baseline Maleckar model and the cells in experiments.

Amongst others, additional calibration of the Maleckar-based population with I_{CaL} and I_{K1} current densities has the effect of lowering the median of G_{Kur} to the level seen in the baseline Maleckar model (additional calibration enforces lower G_{CaL} and implies a weaker I_{CaL} current, hence a weaker I_{Kur} is required to counteract I_{CaL} 's effects on the AP). Nonetheless, the medians of 7 out of 11 of maximal current conductances remain far from the baseline Maleckar model values, underscoring the differences between the biomarkers produced by the *in silico* baseline model and the experimental measurements.

In the Grandi-based population calibrated with AP biomarkers, the medians of 7 out of 11 varied parameters underpinning the 593 accepted models deviate by more than $\pm 10\%$ from baseline. Following additional calibration with I_{K1} and I_{CaL} densities, this is true for 6 out of 11 parameters (including for G_{Kr} , G_{Ks} , J_{up} and J_{rel} that do not significantly impact the AP; Figure 9.7). This is another manifestation of the differences between the baseline models of human atrial electrophysiology and experiment (Reilly et al., 2016).

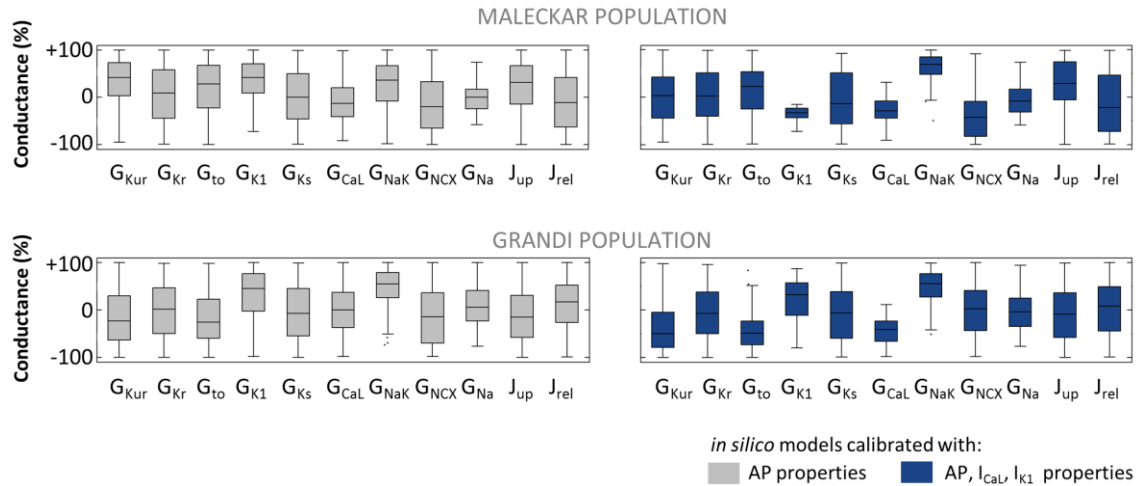


Figure 4.9 Box plots of maximal current conductances in the Maleckar and Grandi-based populations, illustrating the effect of calibration with AP properties alone (grey) or together with I_{CaL} and I_{K1} current densities (dark blue). Outliers are shown as dots.

4.4.5 Comparison of the human atrial populations obtained with Latin Hypercube and Sequential Monte Carlo sampling methods

In order to investigate the potential impact of using a different sampling method in the construction of the populations of models, the process of populations-of-models construction was repeated using SMC sampling. For all three baseline models of human electrophysiology, the populations constructed with SMC were found to be essentially equivalent to those generated by LHS. For example, the Courtemanche-based population produced APD_{20} and APD_{50} at the lower end of experimentally observed values despite the wide range of variability in conductances, reduced stimulus amplitude and variability in peak I_{CaL} density (Figure 9.8, Appendix 2). The Grandi- and Maleckar-based populations captured the full range of AP biomarkers, both in the absence and presence of additional calibration criteria on I_{CaL} and I_{K1} current magnitudes (Figure 9.9, Figure 9.10, Figure 9.12, and Figure 9.13 in Appendix 2). Parameter ranges underpinning the populations constructed with SMC and simulated ionic current densities were similar to LHS results as well, with the Grandi-based population providing a slightly better coverage of the experimental AP biomarker space and ionic current densities (Figure 9.11

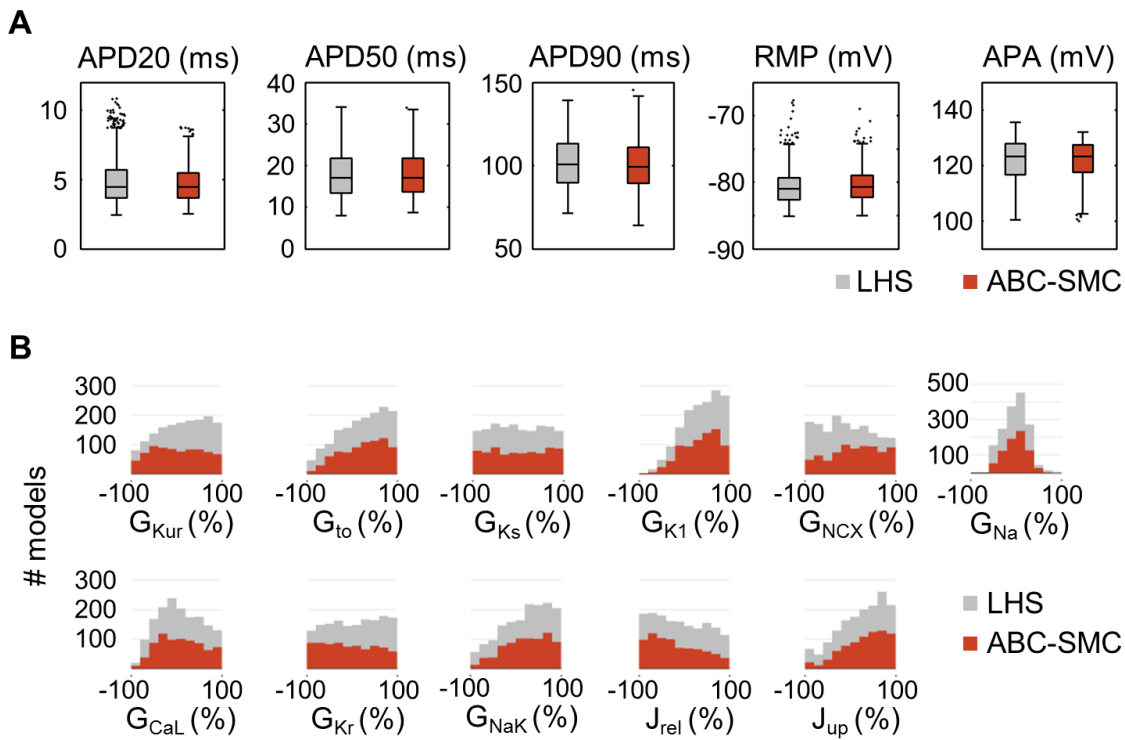


Figure 4.10 Comparison between experimentally-calibrated populations derived from the Maleckar model and obtained with LHS (grey) and SMC within the framework of Approximate Bayesian Computation (ABC; red) following calibration with experimental AP properties. **A** Box plots of AP biomarkers (with outliers shown as dots) and **B** histograms of the parameters underpinning the populations.

Table 4.4 Comparison of method efficiency, defined as number of valid models found divided by the total number of model runs, for Sequential Monte Carlo and Latin Hypercube sampling following AP calibration.

	Latin Hypercube Sampling		Sequential Monte Carlo Sampling	
	Model (Models Accepted)	Runs Efficiency	Model (Models Accepted)	Runs Efficiency
Maleckar	30000 (1559)	5.2%	8131 (759)	9.3%
Grandi	30000 (593)	2.0%	9604 (501)	5.2%
Courtemanche	30000 (1559)	5.2%	8221 (820)	10.0%

vs Figure 4.6). Figure 4.10 illustrates similarities in distributions of AP biomarkers and parameters between the Maleckar-based populations constructed with LHS and SMC.

Table 4.4 compares the efficiency of LHS and SMC methods, defined as the ratio of models that fulfil experimental AP constraints to the total number of models generated under each methodology. Under LHS, the total number of generated models corresponds to the size of the candidate population, while in SMC sampling it refers to the number of models trialled before a satisfactory number of models fulfilling calibration criteria was found. It was found that SMC sampling was 1.8 to 2.6 times more efficient than LHS, implying that it required appreciably fewer trials to locate models fulfilling AP calibration criteria.

4.5 Discussion

This study investigates important methodological aspects for the construction of populations of electrophysiology models calibrated to reproduce the experimental range of variability in AP and ionic current recordings from human atrial cardiomyocytes from a patient cohort (Liu et al., 2013; Reilly et al., 2016) based on three human atrial models from the literature (Courtemanche et al., 1998; Grandi et al., 2011; Maleckar et al., 2009). Two methods of parameter space sampling are evaluated (Drovandi et al., 2016; McKay et al., 1979) and are shown to yield similar results. The main findings are:

1. In populations calibrated with experimental AP properties, variability in ionic current conductances of $\pm 100\%$ of baseline captures experimental variability in most AP biomarkers but stimulus strength and I_{CaL} peak current density location can be important determinants of the AP early repolarization phase (quantified via APD_{20} and APD_{50}).
2. Experimentally-measured peak ionic current densities span large ranges in atrial cells from a relatively-controlled patient cohort, and can in turn translate into

ranges of variation close to (and sometimes exceeding) +100% of baseline ionic conductance values in computational models.

3. Calibration with experimental AP properties does not necessarily constrain the ranges of the underlying ionic current conductances (and, by extension, ionic currents) even if the conductances correlate strongly with AP biomarkers used for calibration.
4. Ionic current and AP biomarker space coverage in experimentally-calibrated populations of atrial models produced with two distinct parameter sampling methods – LHS and SMC – are essentially equivalent, and SMC shows improved efficiency compared to LHS.
5. Out of the populations based on the three generic models of human atrial electrophysiology, the Maleckar- and Grandi-based populations capture the experimentally-observed ranges of variability in the AP biomarkers – including APD_{20} and APD_{50} – as well as ionic currents. Due to the spike-and-dome morphology of their APs, the Courtemanche-based populations do not reproduce experimental ranges of variability in the AP recordings, with a poor coverage of APD_{20} and APD_{50} biomarker values. Since a good coverage of experimentally-measured AP properties – particularly of APD_{50} and APD_{90} – is critical for the testing of clinically-relevant hypotheses in the subsequent chapters of this thesis, the Maleckar- and Grandi-based populations are taken forward for that reason.

4.5.1 Uniqueness of experimental dataset and suitability of populations of models for the study

In this study, I used a rich experimental dataset containing both cellular AP recordings and measurements of several ionic currents obtained from human atrial myocytes from a patient cohort (Reilly et al., 2016). Such comprehensive datasets describing human electrophysiology are not commonly found in the literature due to the limited access to

human tissue. Recordings of ionic current densities necessitate experiments on isolated cells (in contrast, APs can also be measured in tissue) and can dramatically increase the overall number of cells required for experiments. For example, the total number of cells used in the experiments by (Reilly et al., 2016) exceeds 140, which is an order of magnitude larger than in typical studies concerned only with AP recordings.

The experimentally-calibrated population-of-models methodology constitutes one of several approaches that are being developed to investigate mechanisms underlying physiological variability, recently reviewed in (Muszkiewicz et al., 2016; Sarkar et al., 2012). Whereas in some studies, sophisticated protocols are designed to construct cell-specific models (as in (Groenendaal et al., 2015)), the population-of-models approach utilizes existing electrophysiological datasets obtained with a widely-used experimental set-up. Furthermore, cell-specific modelling approaches usually yield low model numbers (e.g. $n=4$ in (Groenendaal et al., 2015)) as they are limited by the number of experiments that can be performed. In contrast, the aim of the populations-of-models approach is to generate ensembles containing hundreds of models with wide ionic current profiles to investigate plausible causes and modulators of inter-subject variability.

4.5.2 Narrow cellular profiles can result from wide variations in ionic currents

The experimental data used in this study came from a relatively restricted patient cohort (elderly patients in Oxfordshire, UK) and yielded a fairly narrow range of APD biomarker values (APD₉₀ between 69 and 143 ms at 1 Hz; Table 4.1). This is in line with (Workman et al., 2012) who used isolated atrial myocytes from a similar patient cohort. Contrastingly, other measurements of APD₉₀ obtained in isolated human atrial cells were reported around 220 ms (Schmidt et al., 2015), while experiments on human atrial trabecular preparations at 1 Hz yielded APD₉₀ between 190-440 ms (Sánchez et al., 2014).

This study shows that even such a narrow range of APD behaviours as reported in the experimental data can nonetheless translate into wide ranges of variability in ionic current densities, both *ex vivo* and *in silico*. In fact, in the cases of I_{to} and I_{Kur} , the maximal ionic current conductances *in silico* would need to exceed the upper limit of +100% of baseline model values in order to capture experimental ranges of variation (Figure 4.6 and Table 4.3). This illustrates that ranges of variation within $\pm 30\%$ of baseline model values considered in earlier studies (Romero et al., 2009; Walmsley et al., 2013) may be insufficient to capture the wide heterogeneity in human preparations, and carries important implications for future studies of inter-subject variability in the human population. The wide range of plausible ionic profiles underpinning the populations of models supports the hypothesis that biological systems are generally ‘sloppy’ (Gutenkunst et al., 2007; Weiss et al., 2012), i.e. tolerant to significant variations of many parameter combinations. This is in line with the robustness of cardiac activity to changes produced by disease, mutations, and environmental stimuli.

4.5.3 Impact of calibration criteria on the populations of models and the role of the populations to span a wide range of ionic profiles

Most previous studies utilizing the experimentally-calibrated population-of-models approach applied to human had access to cellular APs but not ionic current measurements, and could not assess the impact of AP calibration on the underlying ionic currents. Therefore, a significant part of this study has been to explore the impact of experimental calibration with AP properties on the maximal current conductances underlying the populations of models, and to understand to what extent the AP-level calibration is capable of restricting the ranges of parameters in the accepted models. While the maximal current conductances were permitted to span $\pm 100\%$ of baseline at the stage of parameter sampling, the calibration with experimentally-measured AP properties could in principle lead to rejection of models whose ionic currents would be outside of physiologically-

relevant ranges, especially when a particular current's conductance correlated strongly with AP biomarkers used for calibration. However, this is only the case for I_{Na} in the Maleckar-based population: the values of G_{Na} below -59% of baseline are absent from the calibrated population since I_{Na} uniquely determines APA, i.e. one of the biomarkers used for experimental calibration (Figure 4.6; Figure 9.7). Otherwise, a number of ionic current conductances that correlated strongly with AP biomarkers, such as G_{to} or G_{K1} (impacting APD_{20} and RMP, respectively; Figure 9.7) did not have their ranges constrained by AP calibration, and could still assume values of almost $\pm 100\%$ of baseline. The potential explanations for this fall under the following categories:

- the physiologically-relevant ranges of most ionic current conductances/permeabilities can indeed span $\pm 100\%$ of baseline model values. This is supported by the experimentally-measured range of variation in I_{Kur} and I_{to} current densities, with *in silico* models capturing the majority of cell-to-cell variability in these currents seen *ex vivo*;
- the extreme (either large or small) values of particular ionic current conductances do not translate into significant changes in AP morphology or biomarkers due to multiple ionic currents regulating one AP biomarker. This is illustrated with the case of I_{CaL} current density in both Maleckar- and Grandi-based populations, where large I_{CaL} density does not appreciably alter the AP morphology or biomarkers. The implication is that the AP biomarkers alone carry insufficient information to infer and hence constrain the range of this ionic current conductance, and additional datasets – such as calcium transient measurements – are likely to be required to calibrate for G_{CaL} in human atrial myocytes;
- in the absence of additional experimental datasets capable of restricting ionic currents underpinning the populations, some scenarios found in the AP-calibrated

populations may not be present in the specific cell cohort under investigation. This could in fact be an advantage of the *in silico* populations, as they allow for exploration of scenarios that are not investigated in experiments but may nonetheless be relevant for physiological investigations. For example, this could potentially be the case with I_{CaL} density in both *in silico* populations. Whereas experiments consider atrial cardiomyocytes from elderly patients, and therefore with low I_{CaL} density, the *in silico* populations explore the implications of larger I_{CaL} densities, representing younger patients.

The latter point deserves further discussion in light of the wider heterogeneity in the human population compared to that assessed in limited number of experimental recordings in human and in controlled animal lines, the dynamic nature of ion channel densities *in vivo*, as well as the inherent limitations of experimental techniques. Experimental studies face resource constraints caused by limited access to human tissue; as a result, a limited number of cells and an even smaller number of underlying ionic processes can usually be investigated. This is a non-trivial constraint, as the wide heterogeneity in the human population necessitates investigations with large numbers of cells/cell models for studies of inter-subject variability.

Importantly, ionic current densities are not parameters but dynamically changing quantities *in vivo*, and experimental investigations *ex vivo* offer very valuable but ultimately restricted insights. In light of these complexities, the goal of the experimentally-calibrated populations of models is to explore many ionic profiles yielding viable APs within observable ranges of AP biomarkers by generating a much larger pool of cellular models than the number of cells that is experimentally feasible to investigate, to overcome some of the experimental limitations.

The specific balance of ionic currents underpinning the experimental AP recordings used to constrain the populations of models is unknown, and cannot be estimated without assumptions. One such assumption could be that the densities of I_{to} , I_{Kur} , I_{K1} and I_{CaL} ionic currents underpinning the cellular APs are similar to the densities measured in the experimental I-V recordings, given that the currents and APs were recorded in cells from the same patient cohort. Then, the additional constraints on I_{CaL} and I_{K1} current densities applied following the AP-level calibration become a way of retaining models which span the known ranges of variation in the narrow group of cells used in the experiments. This, however, has the disadvantage of discarding clinically-relevant scenarios such as age-dependent differences in the I_{CaL} current density (Herraiz-Martínez et al., 2015).

4.5.4 Parameter sampling methods

To construct experimentally-calibrated populations of models, LHS was initially proposed by (Britton et al., 2013) and subsequently used in further studies (Britton et al., 2014; Muszkiewicz et al., 2014; Passini et al., 2016; Reilly et al., 2016; Zhou et al., 2015, 2013), as it allows parameter sampling over a large number of parameters efficiently and without bias (Burrage et al., 2015; McKay et al., 1979). The results in this chapter show that both LHS and SMC produced very similar coverage of AP properties and underlying ionic current conductances; however, SMC was approximately two times more efficient than LHS in searching viable models. Single-cell AP simulations are computationally tractable and the populations allow for straightforward parallelization, and therefore computational efficiency is perhaps not necessarily a challenge.

4.5.5 Limitations and future work

The patient cohort from which human atrial myocytes were obtained is described in Table 9.2, Appendix 2; (Reilly et al., 2016). Only experimental data obtained from cells extracted from patients in SR were used in this chapter. It must be noted that many SR

patients suffer from some heart condition and/ or take specific drugs likely to affect cardiac electrophysiology. Thus, variability measured in these patients' cells is not representative of variability in the general population of healthy humans.

As remarked in Chapter 3, inter-patient cell-to-cell variability may be larger than intra-patient variability. Experimental dataset used to construct the populations of models in this chapter included on average $n=2$ atrial myocytes from the same patient, prohibiting an evaluation of inter- vs. intra-patient variability effects. A future study could explore this question, provided that appropriate experimental data become available.

Sources of variability intrinsic to the cell (model) that were considered in my study included ionic current conductances and the location of I_{CaL} peak density. Other intrinsic sources of variability could include intercellular differences in cell volume and membrane capacitance values as shown by (Polak and Fijorek, 2012), and time-dependence of ion channel activity. Similarly, external sources of variability separate from stimulus strength could include ionic concentration fluctuations in the bath. These factors could be further investigated in future studies when appropriate experimental recordings become available.

As mentioned in Chapter 3, some degree of co-variation between membrane capacitance values and the magnitudes of ionic currents elicited in voltage clamp experiments can be expected. More ion channels will fit into the membranes of larger cells, yielding larger currents; additionally, variability in e.g. t-tubule density can impact the cellular surface-to-volume ratio, altering membrane capacitance. Figure 9.14 summarizes experimental data on membrane capacitance measured in experiments in which I_{Kur} was elucidated. The mean $\pm$ standard deviation of membrane capacitance measured in $n=30$ cells from patients in SR was 119 ± 48 pF, indicating a wide range of variability in this parameter. To control for this additional source of variability, current magnitudes are often normalized

by membrane capacitance to yield current densities, as in (Reilly et al., 2016). Likewise, this study focused on current densities, not whole-cell currents.

This study focused on investigating sources and determinants of variability in AP repolarization biomarkers. The time resolution of the AP traces was not fine enough to calculate the upstroke velocity. Future work could involve incorporating depolarization biomarkers into the construction of the populations of models. Likewise, at the tissue level, a related question that could be investigated is variability in gap-junctional coupling in the patient cohort (Reilly et al., 2016). This, together with information about the cellular depolarization biomarkers as well as experimental conduction velocity measurements in tissue slabs, would shed light on the determinants of conduction velocity and on inter-patient variability at this level.

4.6 Conclusions

In this chapter, I have investigated methodological aspects for the construction of populations of electrophysiology models calibrated to reproduce the experimentally-observed ranges of variability in action potential and ionic current recordings in human atrial cardiomyocytes from a patient cohort. My results indicate that cell-to-cell variability – particularly in the early stages of cellular repolarization – can stem from factors such as variability in stimulus strength and I_{CaL} peak density location in addition to the traditionally-considered maximal ionic current conductances. I show that a narrow range of cellular behaviours can be replicated with a wide range of possible ionic profiles characterized both experimentally and computationally, and can exceed the ranges of variability on ionic current conductances used in previous studies. I also evaluate the impact of the experimentally-motivated calibration criteria on the populations of models, and discuss the meaning and usefulness of models obtained as a result. Finally, the experimentally-calibrated populations of models constructed in this chapter provide a tool

for testing of clinically-relevant hypotheses. In particular, I use the populations of models in the next chapter to probe the mechanisms underpinning nNOS-mediated effects on cellular action potential in isolated human atrial myocytes.

5 INVESTIGATING THE ROLE OF nNOS-MEDIATED IONIC CURRENT CHANGES ON HUMAN ATRIAL ACTION POTENTIAL

This chapter forms a part of the paper entitled 'Up-regulation of miR-31 in human Atrial Fibrillation begets the arrhythmia by depleting dystrophin and neuronal Nitric Oxide Synthase' by (Reilly et al., 2016), published in Science Translational Medicine.

5.1 Introduction

In the previous chapter, I developed two experimentally-calibrated populations of models capturing variability in electrophysiological properties in human right atrial myocytes under control conditions. In this chapter, I use those populations of models to probe the ionic mechanisms linking the effect of the neuronal isoform of Nitric Oxide Synthase (nNOS) on ionic currents and the atrial action potential in human right atrial myocytes. Experimental work described in this chapter has been performed by Dr Xing Liu (Radcliffe Department of Medicine, University of Oxford, Oxford, UK).

5.2 Motivation

As described in Section 2.3, AF is the most common cardiac arrhythmia worldwide. AF changes the properties of the affected atrial tissue, thereby promoting future occurrences

of itself as well as increasing resistance to treatment. Mechanisms underlying this tissue remodelling process are poorly understood. Recently, (Liu et al., 2013; Reilly et al., 2016) have shown that nNOS levels are significantly reduced in atrial myocytes from humans and goats suffering from persistent AF (Figure 5.1A). Furthermore, nNOS inhibition in isolated human and murine right atrial cardiomyocytes reduces action potential duration (APD) (Figure 5.1B, (Reilly et al., 2016)), which is a hallmark of AF-induced electrical remodelling (Allessie et al., 2002).

Investigations into mechanisms leading to AP shortening under nNOS block revealed increases in I_{to} , I_{Kur} , I_{CaL} , and I_{K1} ionic currents (Figure 5.1C). However, it is unclear which of the measured currents is most important for nNOS-mediated effects on the AP, and whether changes in ionic currents that were not investigated in experiments are necessary to explain the AP shortening. Answering these questions would be challenging with solely experimental investigations; however, computational modelling in synergy with experimental data can provide the necessary insights.

The aim of this chapter is therefore to investigate the role of the changes in each ionic current induced by nNOS depletion as measured in experiments on the human right atrial action potential. This challenge is addressed by means of experimentally-calibrated populations of human atrial myocyte models developed in the previous chapter. I probe the effect of nNOS inhibition on human atrial AP both under control conditions (i.e. in sinus rhythm (SR)) and following AF-induced electrical remodelling.

5.3 Methods

5.3.1 Experimental protocols

Action potentials (APs) were recorded using the conventional current-clamp recording setup described in the previous chapter. For each human atrial cardiomyocyte, at least 25

steady-state APs were recorded at each frequency (0.25, 0.5, 1, 2, 3 Hz). The AP amplitude and duration (at 50 and 90% of repolarization, APD_{50} and APD_{90}) were measured from the averaged traces. nNOS inhibition was achieved with S-methylthiocitrulline (SMTC). Figure 5.1B illustrates the effect of nNOS inhibition on the human AP from right atrial myocytes from patients in normal sinus rhythm (SR) and persistent AF. Under the action of SMTC, APD_{90} shortening is observed in SR cells but not in persistent AF.

Ionic currents I_{Kur} , I_{to} , I_{CaL} and I_{K1} were measured with conventional voltage-clamp setup described in the previous chapter. Figure 5.1C illustrates the mean ionic current density of I_{to} , I_{Kur} , I_{K1} , and I_{CaL} measured in human atrial myocytes obtained from patients in SR, in the presence and absence of SMTC. nNOS inhibition with SMTC in SR cells resulted in (n=number of cells, N=number of patients; (Reilly et al., 2016)):

- 24% increase in the mean I_{to} current density at 30 mV ($P<0.01$; n=30/N=5 SR cells vs. n=35/N=6 SR+SMTC cells);
- 60% increase in the mean of the 50 μ M 4-AP-sensitive I_{Kur} at 25 mV ($P=0.003$, n=30/N=7 SR cells vs. n=35/N=7 SR+SMTC cells);
- 31% increase in the mean I_{K1} current at -100 mV ($P=0.005$; n=36/N=7 SR cells vs. n=20/N=7 SR+SMTC cells); and
- a trend towards increase in I_{CaL} , with an 18% increase in the mean current at 20 mV ($P=0.12$, n=20/N=7 SR cells vs. n=18/N=5 SR+SMTC cells).

Human atrial myocytes utilized in single-cell patch-clamp experiments for AP and ionic current density measurements were obtained from a cohort of patients aged 69 ± 10 years (data reported as mean $\pm$ standard deviation; (Reilly et al., 2016)).

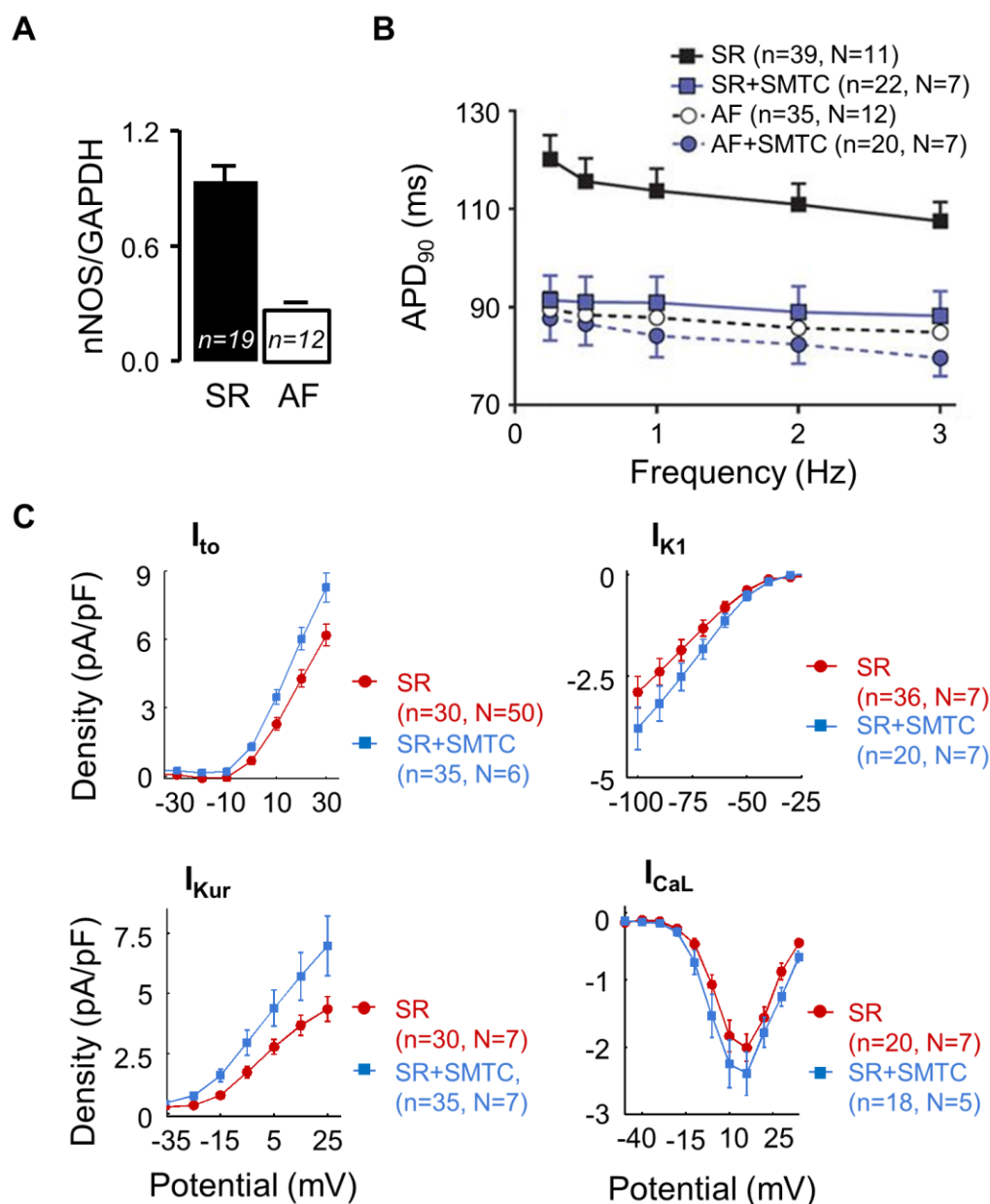


Figure 5.1 A subset of experimental results linking nNOS and persistent AF, reproduced with permission from (Svetlana N. Reilly and Xing Liu et al., ‘Up-regulation of miR-31 in human atrial fibrillation begets the arrhythmia by depleting dystrophin and neuronal nitric oxide synthase’, Science Translational Medicine, 8(340), 340ra74, 2016. Website URL: <http://stm.sciencemag.org/content/8/340/340ra74.short>). **A** Average nNOS protein levels in human right atrial samples from patients in SR (n=19) or persistent AF (n=12). GAPDH – glyceraldehyde-3-phosphate dehydrogenase. **B** Mean APD₉₀ as a function of pacing frequency in right atrial myocytes from patients in SR or persistent AF, in the presence of absence of the nNOS inhibitor SMTC. n/N denotes number of cells/patients, respectively. **C** Average densities of ionic currents I_{to}, I_{K1}, I_{Kur} and I_{CaL} measured in human right atrial myocytes from patients in SR, in the absence or presence of SMTC. n/N denotes number of cells/patients, respectively. In **A-C**, error bars denote S.E.M.

5.3.2 Populations of human atrial action potential models

Simulation studies were conducted using populations of *in silico* human atrial myocytes based on the Maleckar and Grandi models (Grandi et al., 2011; Maleckar et al., 2009) constructed in the previous chapter. Briefly, two initial populations of 30,000 human atrial models were generated as in (Britton et al., 2013). For every model within the initial cell population, simulations were conducted using voltage clamp protocols and stimulation trains of 100 beats at five pacing frequencies (0.25, 0.5, 1, 2, and 3 Hz) using identical protocols as in the patch clamp experiments. Electrophysiological properties were calculated for each model at all pacing frequencies, including the magnitude of each ionic current as measured in voltage clamp, AP amplitude, APD₅₀ and AD₉₀, AP triangulation and resting membrane potential. From the initial 30,000 sampled models, the experimentally-calibrated populations of human atrial models were constructed by retaining only the models that yielded cellular and/or ionic property values within the range obtained experimentally in SR. The resultant SR populations calibrated with AP-level information only consisted of n=1559 and n=593 human atrial models for the Maleckar and the Grandi-based populations, respectively; while the Maleckar- and Grandi-based populations calibrated with both the AP and ionic current densities contained n=104 and n=271 models. The SR populations were used to investigate the role of SMTC-induced changes in I_{to} , I_{Kur} , I_{CaL} and I_{K1} in recovering APD changes measured experimentally.

To model the effects of SMTC on persistent AF cells, populations of *in silico* AF models were first constructed by incorporating AF-induced electrical remodelling at the cellular level into the Maleckar and Grandi-based SR populations. Thus, in each SR model, I_{Kur} , I_{to} , I_{CaL} , and I_{Kr} were downregulated by 45%, 62%, 58%, 38%, respectively, while I_{K1} , I_{Ks} , I_{NCX} , J_{up} , J_{rel} were upregulated by 73%, 50%, 69%, 50%, 300%, respectively (average values from Table 2.1; (Colman et al., 2013; Koivumäki et al., 2014)), to obtain the

corresponding AF model following AF-induced electrical remodelling. Further, the effect of SMTC on I_{to} , I_{Kur} , I_{CaL} and I_{K1} ionic currents in AF was modelled taking into account the 75% decrease in nNOS levels in AF relative to SR (Figure 5.1A), which implied increases of 6%, 15%, 7.75% and 4.5% in I_{to} , I_{Kur} , I_{K1} and I_{CaL} , respectively.

5.4 Results

5.4.1 Experimentally-measured changes in I_{Kur} and I_{K1} currents play a key role in action potential shortening seen under nNOS block

Figure 5.2 shows the effect of nNOS block on APD₅₀ and APD₉₀ in human right atrial myocytes *in silico* at 1 Hz, investigated by incorporating isolated and concurrent ionic current changes elicited with SMTC into the Maleckar- and Grandi-based populations of models calibrated with AP and ionic current density datasets from SR cells *ex vivo* (Figure 4.7, Figure 4.8). Incorporating all ionic current changes elicited *ex vivo* with SMTC into both populations of SR models recovers the experimentally-observed APD decrease, suggesting that the experimental dataset includes the SMTC-induced ionic current changes that are most important for atrial AP shortening. Indeed, in both experimentally-calibrated populations, modelling the increase of I_{Kur} induced by SMTC reproduces most of the reduction in APD₅₀ observed experimentally, while the increase in I_{K1} captures a part of the APD₉₀ shortening. Simulating the concurrent increase of I_{Kur} and I_{K1} fully recovers the reduction in APD₉₀. Modelling the experimentally-observed changes in I_{to} and I_{CaL} show little effect on APD (Figure 5.2; Figure 9.15 in Appendix 3). The similarity of predictions between the two *in silico* populations supports model-independence of these results.

The critical role of I_{Kur} and I_{K1} changes in recovering the AP shortening due to nNOS block is further emphasised in Figure 5.3, which shows APD₅₀ and APD₉₀ rate dependence *in silico* and *ex vivo*. At all pacing frequencies, the mean values of APD₅₀

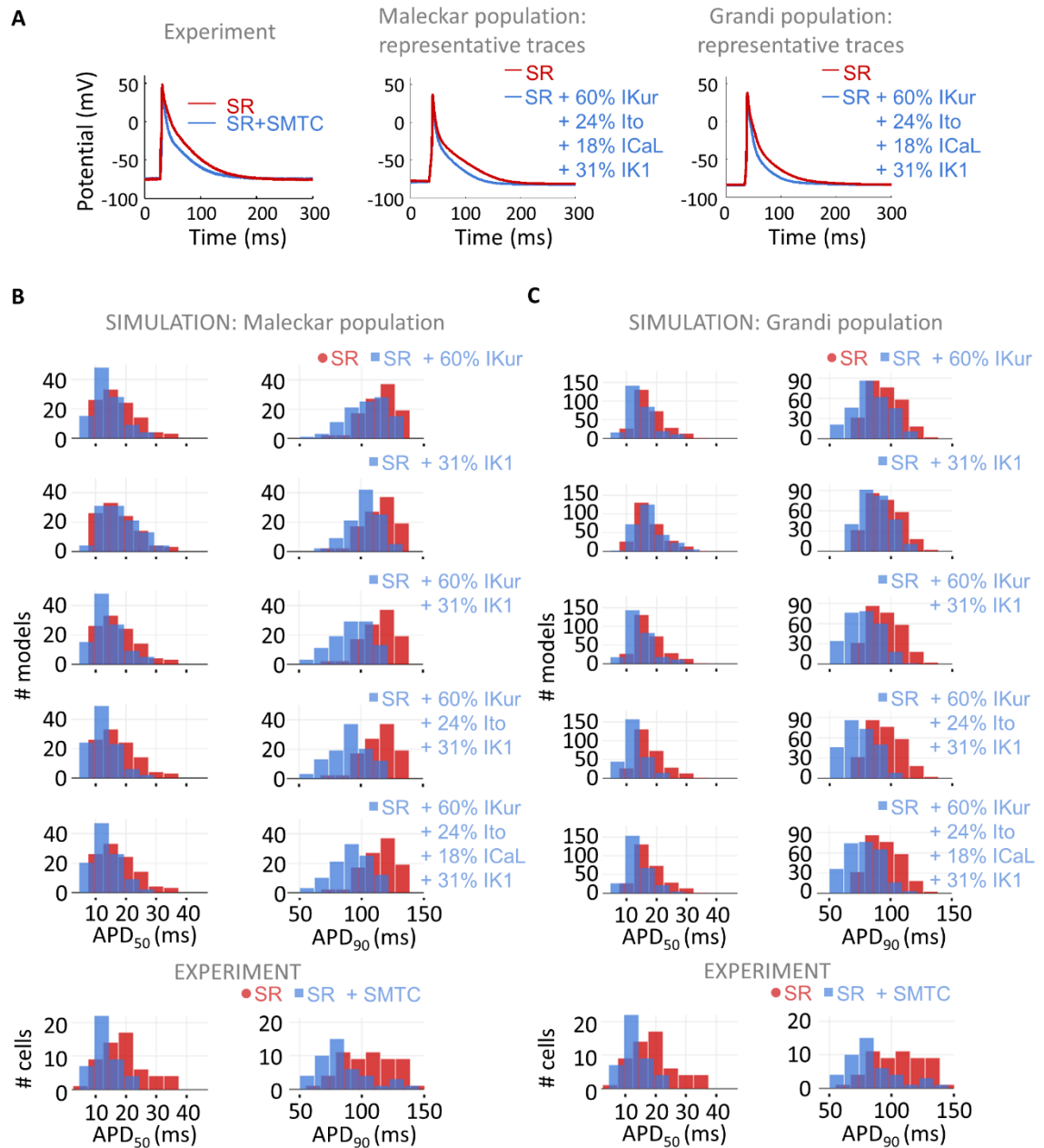


Figure 5.2 Effect of nNOS block with SMTC on human right atrial myocytes *ex vivo* and *in silico* at 1 Hz. **A** Representative AP traces elicited experimentally from human right atrial myocytes in the absence/presence of SMTC, and simulated APs produced by representative models from the Maleckar- and Grandi-based populations. **B-C** *In silico* evaluation of the impact of the increases in ion current densities elicited experimentally by nNOS inhibition on the APD₅₀ and APD₉₀ of human atrial myocytes. Experimental data are shown below for comparison. Maleckar- and Grandi-based control populations consisted of n=104 and n=271 models and were generated by experimental calibration at the level of AP and ionic current densities. For *in silico* data, red and blue bars indicate the number of cellular models in SR and with various ionic interventions, respectively. In the case of *ex vivo* data, these colours refer to the number of cells in control conditions (SR) and under nNOS block.

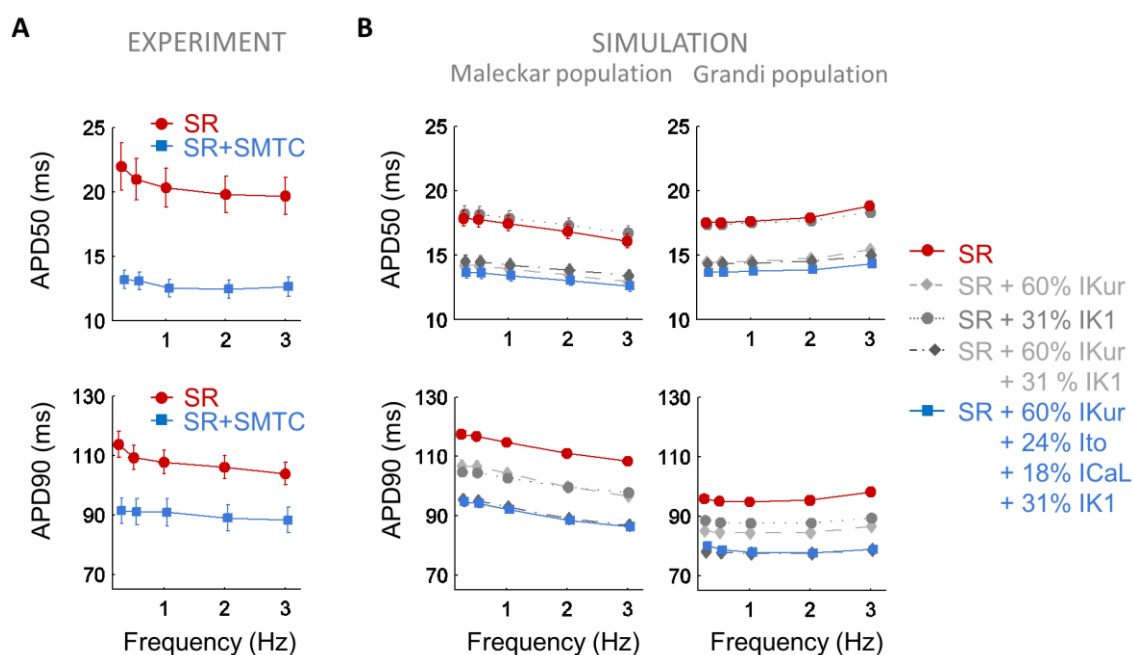


Figure 5.3 Rate dependence of APD₅₀ and APD₉₀ *ex vivo* and *in silico*, in the absence or presence of SMTC-induced changes on the human right atrial action potential. **A** Experimental recordings from human right atrial myocytes in SR (n=29, N=11) and in the presence of SMTC (n=22, N=9). n/N denotes number of cells/patients, respectively. **B** Mean APD₅₀ and APD₉₀ values produced by the Maleckar- and Grandi-based populations of models. The effects of isolated and concurrent ionic current interventions elicited experimentally by SMTC on control populations of models are shown. Maleckar- and Grandi-based SR populations consisted of n=104 and n=271 models and were generated by experimental calibration at the level of AP and ionic current densities. In **A-B** data are reported as average $\pm$ S.E.M. In **B**, error bars are too small to be visible.

and APD₉₀ in the populations incorporating the I_{Kur} and I_{K1} increases produce similar results to those yielded by the populations that include changes in all four ionic currents affected by SMTC. The models display only a weak rate dependence in APD₅₀ and APD₉₀, in agreement with experimental results in the absence and presence of SMTC.

5.4.2 Reduced nNOS levels explain the absence of SMTC-mediated action potential shortening in AF

Next, I probe the likely impact of SMTC-induced ionic current changes on atrial action potential following AF-induced electrical remodelling. Experimental measurements of ionic currents in cells from AF patients in the presence/absence of SMTC are unavailable.

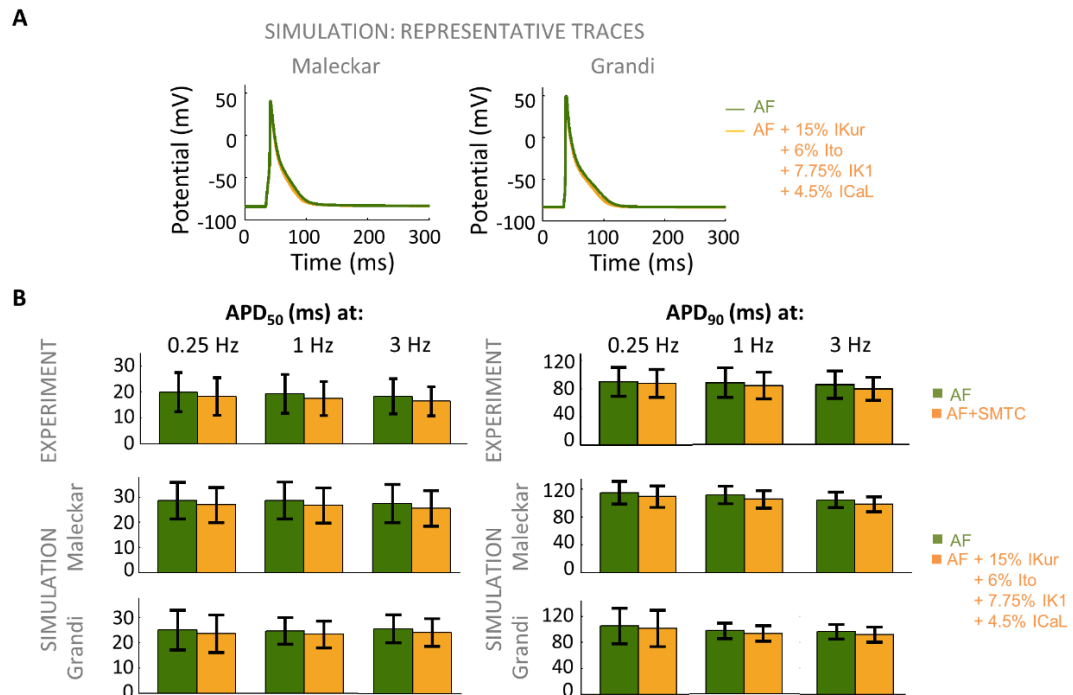


Figure 5.4 Effect of nNOS block with SMTC on human right atrial myocyte models incorporating AF-induced electrical remodelling. **A** Representative AP traces at 1 Hz pacing, and **B** Bar plots of mean APD₅₀ and APD₉₀ at 0.25, 1, and 3 Hz in the Malekar- and Grandi-based populations of $n=104$ and $n=271$ models incorporating AF-induced electrical remodelling, in the absence/presence of SMTC-induced changes on ionic currents. Inhibition of nNOS in AF is modelled with reduced levels of nNOS taken into account. In **B**, experimental results are shown for comparison ($n=35$ vs. $n=19$ cells in persistent AF vs. persistent AF+SMTC). Data are reported as mean $\pm$ standard deviation.

I hypothesise that nNOS block in AF cells would result in an increase in I_{to} , I_{Kur} , I_{K1} and I_{CaL} currents; however, the magnitude of such changes would be smaller than in SR, in line with reduced levels of nNOS in tissue from patients in AF (Figure 5.1A). Figure 5.4 shows the simulated effect of nNOS block on APD₅₀ and APD₉₀ in human atrial myocyte models of AF incorporating the well-established effects of AF-induced electrical remodelling at the cellular level (Table 2.1, (Colman et al., 2013; Koivumäki et al., 2014)). Simulations reveal that, with nNOS levels reduced by 75% and the magnitudes of resultant SMTC-related effects decreased accordingly, no changes are observed at the AP level in cellular models of AF in the presence/absence of SMTC. This is in similarity with experimental AP measurements in AF cells (Reilly et al., 2016).

5.4.3 nNOS-mediated ionic regulation of human atrial AP holds in a wider range of cellular ionic profiles than experimentally investigated

So far, I probed ionic mechanisms underpinning nNOS-mediated effects on human atrial action potential using two populations of SR models that were calibrated with experimental AP and ionic current density measurements. Those populations of models captured the range of variability observed experimentally in cells obtained from the relatively-controlled patient cohort used in (Reilly et al., 2016). It is of interest to investigate whether the nNOS-mediated effects elucidated with the narrow cell group in question would hold in cells/cell models with ionic profiles beyond those that were experimentally accessible. The Maleckar- and Grandi-based SR populations that were calibrated with experimental data on AP properties only (i.e. in the absence of additional calibration with ionic current densities; shown in Figure 4.4, Figure 4.5) offer one possibility of achieving this goal. Both AP-calibrated populations included models with maximal I_{CaL} densities larger than those recorded in experiments on cells from elderly patients (Reilly et al., 2016), and those models could be thought to correspond to, for example, ionic current profiles in cells from younger patients (Herraiz-Martínez et al., 2015). The AP-calibrated Maleckar-based population additionally includes models with a much wider range of maximal I_{K1} density than recorded in experiments by (Reilly et al., 2016), but in line with measurements reported in the literature (Bosch et al., 1999).

The findings obtained with the two populations of models calibrated with AP-level data are essentially equivalent to the results yielded by the populations that included the additional constraint on I_{CaL} and I_{K1} ionic current densities. For instance, I find that incorporating the ionic current changes in I_{to} , I_{Kur} , I_{K1} and I_{CaL} into the SR populations calibrated with AP properties is sufficient to explain the shortening of the atrial AP, with I_{Kur} and I_{K1} currents still playing a key role in SMTc-mediated APD_{50} and APD_{90} decrease (Figure 5.5, Figure 9.16). Likewise, incorporating various interventions due to

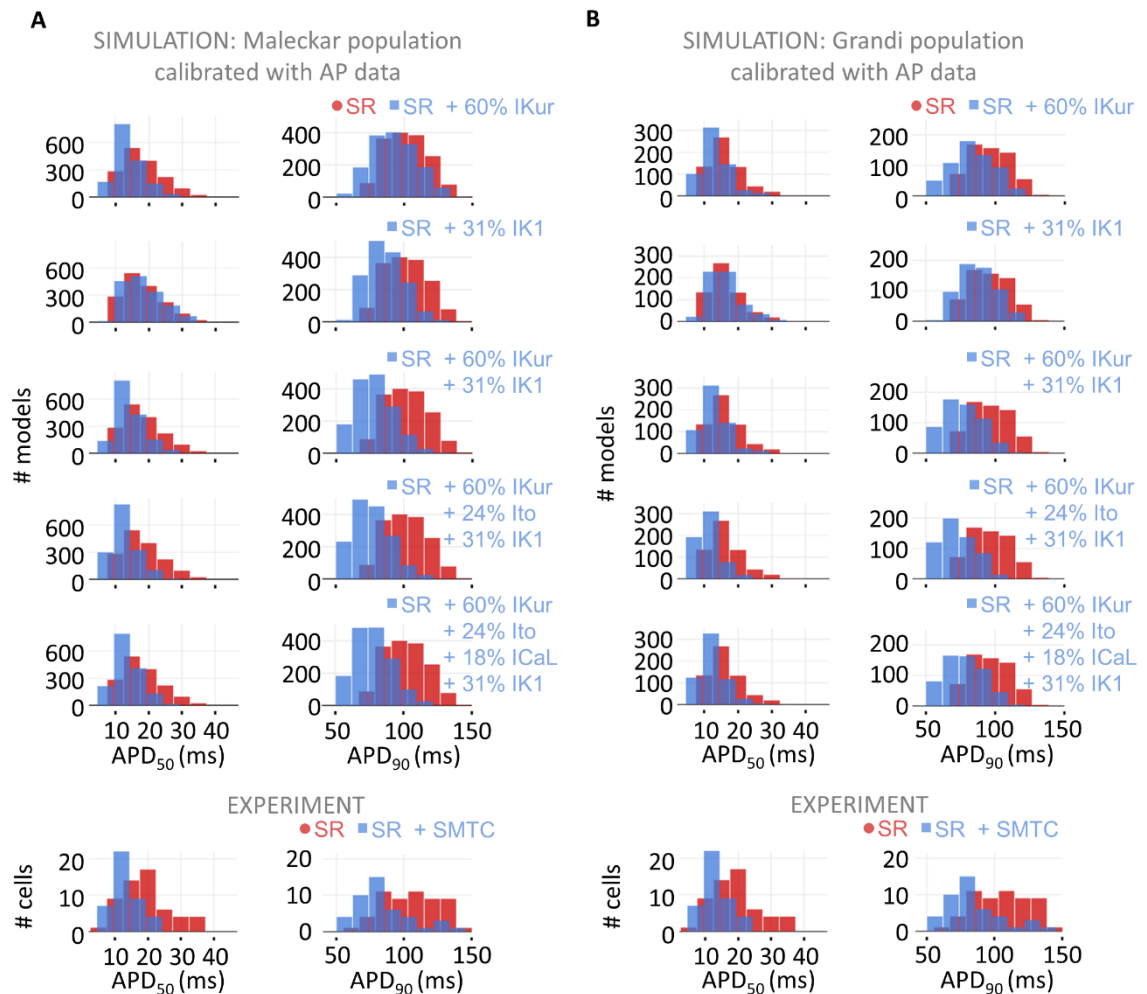


Figure 5.5 Effect of nNOS block with SMTC on APD₅₀ and APD₉₀ in human right atrial myocytes *in silico* at 1 Hz, investigated by incorporating isolated and concurrent ionic current changes elicited experimentally with SMTC to the control Maleckar- and Grandi-based populations of models calibrated with AP data only. Experimental data are shown below for comparison. Maleckar- and Grandi-based control populations consisted of n=1559 and n=593 models. For *in silico* data, red and blue bars indicate the number of cellular models in SR and with various ionic interventions, respectively. In the case of *ex vivo* data, these colours refer to the number of cells in control conditions (SR) and under nNOS block.

SMTC into the AP-calibrated SR populations produces similar APD rate dependence properties (Figure 9.17), while the absence of AP shortening in models incorporating AF-induced electrical remodelling can be explained by the decreased levels of nNOS in AF (Figure 9.18). These findings suggest that the nNOS-mediated effects on the human atrial AP extend beyond the narrow cell group that was experimentally accessible.

5.5 Discussion

In this chapter, I use experimentally-calibrated populations of models to investigate nNOS-mediated ionic mechanisms regulating human atrial action potential in sinus rhythm and following AF-induced electrical remodelling. I show that experimentally-measured changes in I_{to} , I_{Kur} , I_{CaL} and I_{K1} ionic currents elicited with the nNOS inhibitor SMTC in SR myocytes explain the shortening of cellular AP, and that I_{Kur} and I_{K1} play a critical role in the SMTC-induced APD decrease. I also show that reduced levels of nNOS in AF, modelled via decreased magnitudes of SMTC-induced effects on ionic currents, explain the lack of AP shortening in the presence of SMTC in populations of models that incorporate AF-induced electrical remodelling. Finally, I show that SMTC-induced effects on the human right atrial action potential hold in models with ionic profiles beyond those used in (Reilly et al., 2016)'s experimental investigations, indicating that the nNOS-mediated mechanisms regulating human atrial AP are robust and apply to a wider range of scenarios that could correspond to patient cohorts beyond those that were accessible to us in experiments. These results were obtained with *in silico* populations based on two distinct human atrial action potential models (Grandi et al., 2011; Maleckar et al., 2009), which additionally supports model independence of my findings.

I investigated the impact of SMTC-induced changes at the level of transmembrane ionic currents on the human atrial action potential. Several previous studies were concerned with analysing the importance of nNOS inhibition in human ventricular myocytes, focusing primarily on the calcium subsystem. In ventricular myocytes, nNOS block has been reported to cause an increase in I_{CaL} current (Sears et al., 2003), a decrease in SERCA activity (Zhang et al., 2008), and inconsistent changes in RyR activity (Gonzalez et al., 2007; Wang et al., 2008); a decrease in I_{NaK} has also been shown (Zhou et al., 2002). Changes in SERCA and RyR (and, to a lesser extent, I_{CaL}) can be expected to manifest

themselves in the intracellular calcium transient and not in the AP properties (Walmsley et al., 2013). Indeed, the correlation analysis of the experimentally-calibrated populations of models in the previous chapter showed that SERCA and RyR have little impact on the AP (Figure 9.7). Future studies could extend the experimentally-calibrated populations of models used in this chapter to include additional experimental data pertaining to the intracellular calcium transient, should such datasets become available.

In Figure 5.3, the APD_{50} and APD_{90} in the Grandi-based population of models appears to increase with increasing frequency. This is a feature of the Grandi electrophysiological model, reported previously by (Wilhelms et al., 2012). As the stimulation frequency increases, the Grandi model responds with an elevated resting membrane potential and a decreased peak membrane potential. This leads to a decrease in the action potential amplitude and weaker repolarization currents, resulting in APD prolongation.

The results in this chapter indicate that, out of the changes in the four ionic currents measured in the experiments, the increase in I_{CaL} is not crucial for nNOS-mediated APD shortening. This can be rationalised in light of the correlation analysis in Figure 9.7 in Appendix 2. Across both the Maleckar- and Grandi-based *in silico* populations, G_{CaL} is only weakly correlated with AP biomarkers, hence a relatively small 18% increase in G_{CaL} has a minor effect on the model APs. In contrast, both G_{K1} and G_{Kur} correlate strongly with AP repolarization biomarkers, and relatively large 30-60% increases in those parameters substantially impact model APs. More importantly, increase in I_{CaL} would cause APD prolongation rather than shortening, i.e. an opposite effect to the one investigated here.

The effect of nNOS inhibition on transmembrane ionic currents in atrial myocytes was to increase I_{Kur} , I_{to} , I_{K1} and I_{CaL} . This is distinct from well-documented ionic current changes following AF-induced electrical remodelling, where I_{Kur} is reduced or unchanged, while

I_{to} and I_{CaL} are significantly decreased (Table 2.1, (Colman et al., 2013; Koivumäki et al., 2014)). However, *in silico* modelling indicated that I_{Kur} and I_{K1} SMTC-induced changes accounted for the majority of the experimentally-observed APD_{50} and APD_{90} decrease under nNOS block, with I_{CaL} and I_{to} effects on human atrial AP being negligible. Separately, changes in transmembrane ionic current density resulting from nNOS inhibition take place on the scale of minutes, while AF-induced electrical remodelling requires days to complete and is associated with changes in ion channel levels (Allessie et al., 2002). If nNOS depletion were to take place in the early stages of AF, it would help to stabilize the arrhythmia by decreasing atrial refractoriness before changes in ion channel levels occur.

This is indeed the central finding in (Reilly et al., 2016), who identified a small non-coding RNA, called miR-31, ultimately responsible for tissue remodelling associated with AF. (Reilly et al., 2016)'s first goal was to investigate the mechanisms leading to nNOS depletion in persistent AF. As first reported by (Brenman et al., 1995) in skeletal muscle, nNOS forms a protein complex with a protein called dystrophin; disruption of dystrophin causes loss of nNOS from the sarcolemma and a profound decrease in nNOS activity. In line with this, (Reilly et al., 2016) observed that nNOS and dystrophin co-localize in the sarcolemma of atrial myocytes from patients in SR but not in persistent AF. Further experiments revealed that nNOS mRNA levels are decreased but dystrophin mRNA is unaltered in patients in persistent AF relative to SR or paroxysmal AF; this correlated with a reduction in both nNOS and dystrophin protein levels in persistent AF. Those findings hinted that a post-transcriptional regulation mechanism may be involved. Thus, (Reilly et al., 2016) proceeded to search for micro-RNAs that could target both nNOS and dystrophin transcripts in humans, identifying miR-31 as a potential culprit. (Reilly et

al., 2016) showed that levels of miR-31 are significantly increased in atrial myocytes from patients in persistent AF compared to SR or paroxysmal AF.

Then, (Reilly et al., 2016)'s goal became to understand how miR-31 regulates nNOS and dystrophin gene expression. They showed that in atrial myocytes from patients in SR in which transcription was inhibited, addition of miR-31 mimic led to increased nNOS mRNA decay and no change in dystrophin mRNA. In line with this, in atrial myocytes from patients in persistent AF, preventing miR-31 binding to nNOS mRNA increased nNOS mRNA levels and partially restored nNOS protein levels, but had no impact on dystrophin mRNA or protein levels. On the other hand, preventing miR-31 binding to dystrophin mRNA did not alter dystrophin or nNOS mRNA, but increased dystrophin protein levels and partially recovered nNOS protein content, indicating that dystrophin affects nNOS protein stability. Taken together, these findings show that increased miR-31 levels in human atrial myocytes accelerate nNOS mRNA decay and inhibit translation of dystrophin mRNA.

Finally, (Reilly et al., 2016) probed the impact of miR-31 levels on human atrial electrophysiology. The authors showed that in atrial myocytes from patients in SR, inhibition of miR-31 had no significant impact on miR-31 expression, dystrophin or nNOS levels; however, *increasing* intracellular miR-31 reduced nNOS mRNA levels, decreased nNOS and dystrophin protein content, and shortened APD. In atrial myocytes from patients in persistent AF, inhibition of miR-31 increased nNOS and dystrophin protein levels, and restored APD. Additional inhibition of nNOS in these cells reversed this effect, implying that miR-31 effects on atrial electrophysiology are mediated by nNOS-derived NO.

Thus, (Reilly et al., 2016) showed that increased levels of miR-31 (i) accelerate the degradation of mRNA that codes for nNOS, leading to reduction in nNOS protein content,

and (ii) decrease nNOS stability by inhibiting translation of dystrophin. The resultant decrease in nNOS levels leads to AP shortening reminiscent of AF-induced electrical remodelling, promoting the progression of AF.

This study focused on investigating nNOS-mediated impact on cellular repolarization. Aside from repolarization abnormalities, pathological changes in cell depolarization can impact electrical wavefront propagation in tissue, contributing to pro-arrhythmia. As mentioned in Section 2.4.1, (Ueda et al., 2008) noted that I_{Na} is modulated by nNOS-derived NO in ventricular myocytes of patients with a mutation in the SCN5A gene suffering from long QT syndrome. At present, it is unknown whether nNOS inhibition/depletion would impact I_{Na} in atrial myocytes of patients without this mutation. Additional simulations could investigate likely nNOS-mediated effects on AP depolarization biomarkers, such as the upstroke velocity; however, this is subject to availability of experimental data with a sufficient time resolution of the AP traces. Potential effects of nNOS inhibition/depletion on inter-cellular electrical communication via gap junctions should be at the centre of a future study focusing on alterations in conduction velocity as a mechanism of pro-arrhythmia.

It is unclear whether nNOS-mediated effect on the human atrial AP could explain the amiodarone therapeutic strategy. Amiodarone represents almost half of annual prescriptions for anti-AF treatment in the USA (Zimetbaum, 2012). Amiodarone and its active metabolite, N-desethylamiodarone, block a variety of ion channels, reducing I_{Kur} , I_{to} , I_{CaL} , as well as I_{Na} , I_f , I_{Kr} , and I_{KACh} currents (Kramer et al., 2013; Zimetbaum, 2012). Amiodarone-induced inhibition of I_{Kur} , I_{to} , and I_{CaL} currents could indeed counteract the effect of nNOS block/depletion; however, the effect of nNOS-derived NO on the remaining currents affected by amiodarone is unknown. Furthermore, amiodarone has been reported to be effective against both paroxysmal and persistent AF (Doyle and Ho,

2009; Tuset et al., 2005), i.e. both prior to and following the AF-induced remodelling at the cellular and tissue levels. In contrast, loss of nNOS precedes the remodelling process induced by long-standing AF (Reilly et al., 2016).

5.6 Conclusions

In this chapter, I used experimentally-calibrated populations of models to investigate ionic current changes triggered by nNOS inhibition on the human atrial action potential in sinus rhythm and following AF-induced electrical remodelling. Simulations reveal a key role of I_{Kur} and I_{K1} ionic currents in AP shortening observed experimentally upon nNOS block in SR, and show that the lack of nNOS-mediated AP shortening following AF-induced electrical remodelling at the cellular level is explained by the decrease in nNOS protein content. In the following chapter, I investigate the importance of nNOS inhibition on inducibility and persistence of atrial arrhythmias in human with the aid of whole-atria simulations.

6 WHOLE ATRIA MECHANISMS OF INDUCIBILITY AND PERSISTENCE OF ATRIAL ARRHYTHMIAS BY DEPLETION OF nNOS IN HUMAN

6.1 Introduction

In the previous chapter, I studied the impact of the nNOS-mediated ionic current changes on the human atrial action potential in isolated myocytes. However, disturbances of the heart rhythm are emergent phenomena occurring at the level of tissue/whole organ. Therefore, in order to investigate possible links between nNOS inhibition/depletion and AF, I now construct models of human whole atria. This chapter uses whole-atria modelling to assess possible mechanisms of pro-arrhythmia identified in experiments under nNOS block/depletion. Experimental work described in this chapter has been performed by Dr Xing Liu (Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK).

6.2 Motivation

As described in the previous chapter, recent experimental evidence suggests that decreased levels of nNOS precede the remodelling process induced by AF (Reilly et al., 2016). Two potential pro-arrhythmic mechanisms due to nNOS depletion/inhibition have been identified experimentally. First, the shortening of action potential duration at 90% repolarization (APD₉₀) in right atrial myocytes has been reported (Chapter 5; (Reilly et al., 2016)). A shorter APD₉₀ leads to a faster recovery from the previous excitation, limiting the cardio-protective effect of the cardiac refractory period (Nattel, 2002). Novel experimental findings also indicate that nNOS inhibition leads to an abolishment of the RA/LA gradient in APD₉₀ (due to the shortening of APD₉₀ in RA) in mice, with a similar trend seen in humans (Liu et al., 2016). In healthy atria, the depolarization of the left atrium (LA) with respect to the right atrium (RA) is delayed due to a time difference in propagation of electrical excitation from the sino-atrial node (SAN) (see Section 2.2.7). In spite of this difference in depolarization onset, both atrial chambers repolarize at similar times due to shorter APD₉₀ in LA relative to RA. This RA/LA gradient in APD₉₀ has been reported across multiple animal species (Lee et al., 1999; Li et al., 2001; Lomax et al., 2003a; Nygren et al., 2004; Qi et al., 1994; Ravelli and Allessie, 1997; Spach et al., 1989; M. Wijffels et al., 1995; Wirth et al., 2003) and is thought to be cardio-protective by minimizing dispersion of repolarization across atria (Ridler et al., 2011; Spach et al., 1989). Consequently, loss of this gradient may be pro-arrhythmic.

In line with these findings, nNOS gene deletion doubles the *in vivo* inducibility of AF and prolongs the duration of resultant AF episodes in mice (Reilly et al., 2016). However, whether APD₉₀ shortening or decreased cardio-protective RA/LA gradient in APD₉₀ is the main mechanism behind nNOS-mediated inducibility of arrhythmias remains unknown. The problem is additionally complicated by a differential effect of nNOS

inhibition on selected ionic currents in right and left atrial chambers: studies in mice show that nNOS inhibition yields an increase I_{to} in RA only, while increases in I_{Kur} and I_{CaL} are similar in both chambers (Liu et al., 2016). The effect of nNOS inhibition on other transmembrane currents in left and right atrial murine myocytes is an open question, with similar experiments on human RA and LA myocytes not yet performed due to limited access to human tissue.

In light of these challenges, the aim of this chapter is to investigate the impact of nNOS inhibition on inducibility and dynamics of re-entrant electrical activity in human whole atria via computer simulations. To assess whether the main mechanism of pro-arrhythmia under nNOS inhibition/depletion is due to APD_{90} shortening or the loss of RA/LA gradient in APD_{90} , I construct phenotypic whole-atria models that differ in APD_{90} and the magnitude of RA/LA APD_{90} gradient. I then analyse the mechanisms of re-entry patterns elicited with burst pacing mimicking spontaneous ectopic activity in paroxysmal AF. The whole-atria models incorporate potential differential effects of nNOS block on ionic currents in right and left atrial chambers. My findings shed light on likely mechanisms linking nNOS inhibition/depletion to re-entrant electrical activity and arrhythmias in human atria.

6.3 Methods

6.3.1 Single-cell models of human electrophysiology

In Chapter 4, I constructed populations of models that were calibrated with experimental data from human right atrial myocytes under control conditions. To study the main mechanisms modulating inducibility and sustainability of re-entry at the whole-atria level, I now select the model within the Maleckar-based population that most closely captures the average experimental results in terms of AP duration at 50 and 90% repolarization (APD_{50} and APD_{90}) in the absence/presence of simulated effects of nNOS

inhibitor SMTC. I refer to this model as the reference model or RA myocyte model. Relative to the generic Maleckar model, the maximal conductances/permeabilities of I_{Kur} , I_{Kr} , I_{to} , I_{K1} , I_{Ks} , I_{CaL} , I_{NaK} , I_{NCX} , I_{Na} , J_{up} and J_{rel} in the reference model are scaled by factors of 0.7577, 1.9844, 0.9584, 1.1006, 0.1347, 0.3630, 1.5417, 1.3254, 0.9280, 1.7434, and 1.8633, respectively.

The physiological RA/LA gradient in APD_{90} arises from inter-chamber differences in ionic currents; as a result, I use the RA myocyte model to construct a cellular model of the LA myocyte by applying appropriate RA/LA ionic current gradients to the RA myocyte model. In the absence of detailed studies into RA/LA ionic current gradients in healthy human subjects, the imposition of RA/LA ionic gradients was based on the experimental murine data from (Liu et al., 2016). Those data showed a 32% increase in I_{K1} and a 32% decrease in I_{CaL} in LA compared to RA. This is consistent with higher levels levels of Kir2.3 (a subunit of I_{K1}) in human LA tissue compared to RA (Swartz et al., 2009)). Additionally, lower density of I_{CaL} in LA relative to RA has been reported in a study by (Gong et al., 2008) in human. Thus, the changes in I_{CaL} and I_{K1} were incorporated into the maximal conductances of the RA myocyte model. The resultant model of the left atrial myocyte produced a shorter AP relative to the RA myocyte (Figure 6.1A). As in the preceding chapter, the effects of nNOS inhibition with SMTC were implemented in the RA myocyte model as increases of I_{Kur} , I_{K1} , I_{CaL} and I_{to} maximal current conductances by 60%, 31%, 18%, and 24%, respectively. Figure 6.1A illustrates the resultant RA and LA myocyte models in the absence/presence of SMTC.

6.3.2 Constructing whole-atria models

I used the human whole-atria mesh developed in (Seemann et al., 2006) and broadly used in studies of re-entrant patterns in human (Krueger et al., 2011; Sánchez et al., 2013; Seemann et al., 2006; Tobón et al., 2013). The mesh incorporates functional and

anatomical detail including fibre orientation, conduction anisotropy, and heterogeneities in conduction velocity. The anatomical structures in the whole-atria model, visualized in Figure 2.2 in Chapter 2, include right and left atrium (RA, LA), right and left atrial appendage (RAPG, LAPG), sino-atrial node (SAN), crista terminalis (CT), pectinate muscles (PMs), Bachmann bundle (BB), inter-atrial bridges (IABs), fossa ovalis (FO), atrio-ventricular ring (AVR), and right atrial isthmus (RAI). For the purposes of incorporation of cellular atrial electrophysiology into the whole-atria model, the anatomical structures in the human whole-atria mesh were classed as belonging to either RA or LA. Structures belonging to RA included the anatomical structures of RA, RAPG, SAN, CT, PMs, BB (right atrial and free running part), IABs (both left and right atrial parts), FO, tricuspid valve ring of AVR, and RAI. Structures classed as LA included the anatomical structures of LA, LAPG, BB (left atrial part), and mitral valve ring of AVR.

To probe the importance of APD_{90} as well as RA/LA gradient in APD_{90} for re-entry inducibility, I created five models of human whole-atria, incorporating different AP models (Figure 6.1B). The homogeneous SR whole-atria model incorporated the single-cell AP formulation of the RA myocyte in both atrial chambers. This whole-atria model was subsequently modified to simulate known nNOS block effects on human atrial myocytes, with the outcome referred to as the homogeneous SR + global SMTC whole-atria model. Thus, both homogeneous SR and homogeneous SR + global SMTC whole-atria models lacked the physiological RA/LA gradient in APD_{90} , but differed in APD_{90} (Figure 6.1B). In contrast, the heterogeneous SR whole-atria model included the RA/LA gradient in APD_{90} , achieved by populating the right atrial chamber with the RA myocyte electrophysiology model, while the left chamber was populated with the LA myocyte model. This whole-atria model was modified by incorporating simulated effects of nNOS inhibition on right atrial chamber only, yielding the heterogeneous SR + SMTC in RA

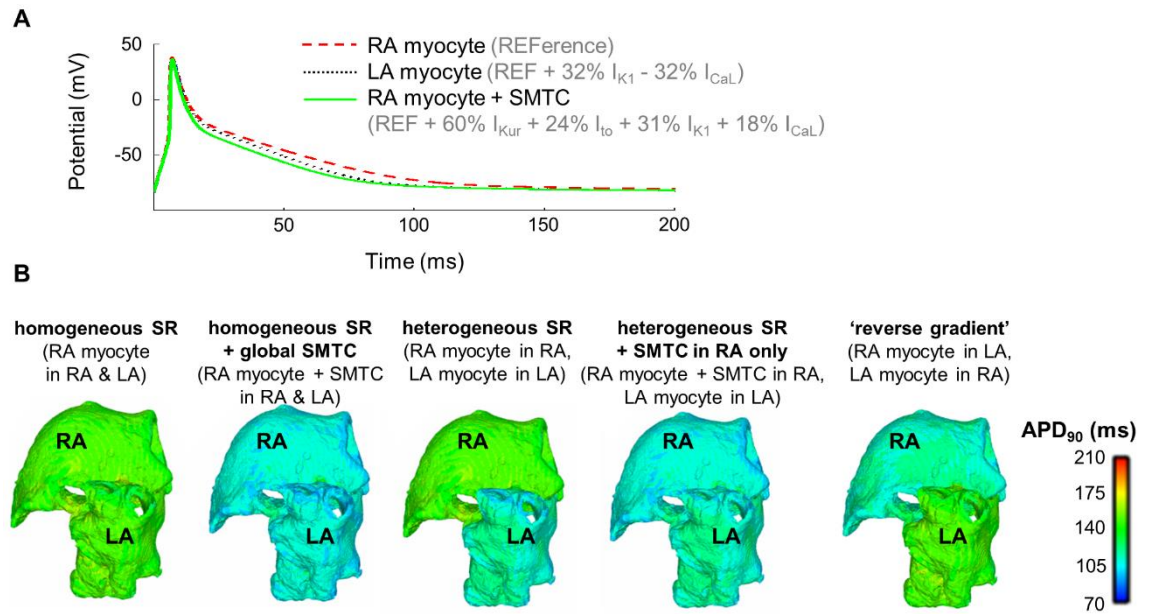


Figure 6.1 Single-cell and whole-atria models used in the study. **A** Action potentials (APs) in computer models of isolated human atrial myocytes. The model of the isolated RA myocyte (reference model) was selected from the experimentally-calibrated population of human RA myocyte models and captured average experimental results. The model of the isolated LA myocyte was constructed by applying ionic current gradients in I_{K1} and I_{CaL} conductances to the RA myocyte model. The effect of SMTC was modelled by increasing I_{Kur} , I_{to} , I_{K1} , and I_{CaL} in isolated RA myocyte model. **B** Five models of human whole atria used in the study, coloured by APD₉₀. The models of AP electrophysiology incorporated into the right and left atrial chambers are given above each whole-atria model.

whole-atria model that lacked the RA/LA gradient in APD₉₀ (Figure 6.1B). Finally, a 'reverse gradient' whole-atria model was generated by populating the right atrial chamber with the LA myocyte model of cellular electrophysiology, while the left atrial chamber was populated with the cellular RA myocyte model.

6.3.3 Stimulation protocol

Forty stimuli at 2 Hz were applied at the location of the SAN in RA to reach the steady state. Burst pacing in the form of six ectopic stimuli was applied at a fixed interval around right pulmonary veins (PVs) in LA, to mimic spontaneous ectopic activity in paroxysmal AF. Figure 9.19 indicates the sites of the SAN and burst pacing. In paroxysmal AF, 94–96% of ectopic foci have been reported in and around pulmonary veins in LA (Chen et

al., 1999b, 2001; Haisaguerre et al., 1998; Jaïs et al., 1997; Lu et al., 2001), with intervals ranging between 110 – 270 ms, with a mean $\pm$ standard deviation of 175 ± 30 ms (Haisaguerre et al., 1998). In line with this, I tested different intervals for the six ectopic stimuli from 150 to 250 ms, in 5 ms increments. All externally-applied stimuli were 2 ms in duration and twice the diastolic threshold. Total simulation time measured from the last pre-conditioning stimulus at the SAN was 4000 ms. To compute electrical wavefront propagation, the monodomain formulation (with a time step of 0.02 ms) within the Elvira software framework was used (Heidenreich et al., 2010).

6.3.4 Effective refractory period vs. conduction velocity

Two main factors impact the dynamics of electrical wavefront propagation in tissue: how quickly the tissue recovers from previous excitation (quantified via effective refractory period; related to APD_{90}), and how quickly the excitation can spread through the tissue (quantified via conduction velocity). With the exception of the fast inter-atrial conduction system, both LA and RA have the same tissue conductivities and sodium current levels, yielding the same conduction velocities (this is evident from equally-spaced isochrones in RA/LA in Figure 9.20 in Appendix 4). As a result, differences in re-entry inducibility and dynamics between the five whole-atria models will be due to APD_{90} and RA/LA APD_{90} gradient, rather than conduction velocity effects.

6.4 Results

6.4.1 The primary determinant of inducibility and dynamics of sustained re-entries is APD_{90} in LA and not inter-atrial APD_{90} gradient

Figure 6.2 summarizes inducibility and dynamics of sustained re-entries (i.e. persisting for more than 1 s) in the five models of human whole atria at different burst pacing intervals, while Table 6.1 summarizes re-entrant patterns' frequencies. The first two models considered – the homogeneous SR and homogeneous SR + global SMTC whole-

atria models – lack the RA/LA gradient in APD_{90} , and yield APD_{90} values of 139 ms and 99 ms, respectively. In the homogeneous SR and homogeneous SR + global SMTC whole-atria models, stable re-entries are induced at four and seven different burst pacing intervals, implying that reduction of APD_{90} in the absence of the RA/LA APD_{90} gradient increases re-entry inducibility by 75%. APD_{90} shortening also alters the dynamics of re-entries that arise (Figure 6.2).

Next, I analyse the heterogeneous SR vs. heterogeneous SR + SMTC in RA whole-atria models. The heterogeneous SR model contains the RA/LA gradient in APD_{90} obtained by incorporating ionic current gradients across RA/LA; by contrast, the heterogeneous SR + SMTC in RA model lacks the RA/LA gradient, as applying SMTC on RA effectively abolishes the difference in APD_{90} across the atrial chambers. Comparison of the results for these two whole-atria models reveals that loss of RA/LA APD_{90} gradient does not significantly alter re-entry inducibility or dynamics.

Analysis of Figure 6.2 also reveals that inducibility and dynamics of re-entry are very similar across homogeneous SR + global SMTC, heterogeneous SR, and heterogeneous SR + SMTC in RA whole-atria models. A common feature of these three models is APD_{90} in LA (equal to 99 ms; Figure 6.1, Figure 6.2). The main difference between the three whole-atria models is the RA/LA APD_{90} gradient, which is present in the heterogeneous SR whole-atria model, but essentially absent in the two other models (Figure 6.2). Therefore, it transpires that re-entry inducibility and dynamics are crucially dependent on the shorter APD_{90} (and therefore smaller refractory period) in LA and not on (i) APD_{90} in RA or (ii) the ionic mechanisms by which APD_{90} in LA is obtained (i.e. by simulating SMTC effects or implementation of RA/LA ionic current gradients).

The last case considered in Figure 6.2 is the ‘reverse gradient’ whole-atria model, with APD_{90} in RA and LA being 99 and 139 ms, respectively (the name of the model stems

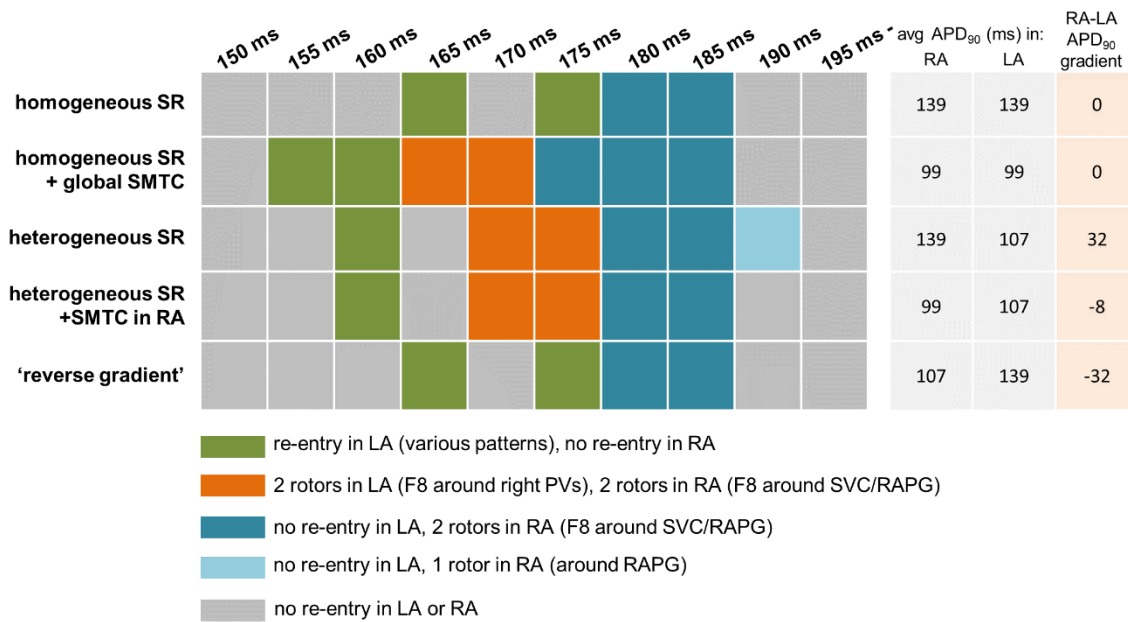


Figure 6.2 Inducibility and dynamics of sustained re-entries (persisting for more than 1 s) in the five models of human whole atria at different burst pacing intervals. Mean values of APD₉₀ in RA and LA, as well as the RA/LA APD₉₀ gradient, are shown on the right. Acronyms: RA – right atrium, LA – left atrium, F8 – figure-of-eight re-entry; PVs – pulmonary veins; SVC – superior vena cava; RAPG – RA appendage.

Table 6.1 Frequency of primary rotors in the five whole-atria models following burst pacing at different intervals, reported in Hz.

Model	155 ms	160 ms	165 ms	170 ms	175 ms	180 ms	185 ms	190 ms
Hom. SR	N/A	N/A	5.0±0.1	N/A	5.0±0.1	4.6±0.1	4.6±0.1	N/A
Hom. SR +global SMTC	5.3±0.1	5.3±0.1	5.3±0.1	5.3±0.1	5.0±0.1	5.0±0.1	5.0±0.1	N/A
Het. SR	N/A	5.3±0.1	N/A	5.3±0.1	5.3±0.1	4.8±0.1	4.6±0.1	4.6±0.1
Het. SR +SMTC in RA	N/A	4.8±0.1	N/A	5.3±0.1	5.3±0.1	5.3±0.1	5.3±0.1	N/A
'reverse gradient'	N/A	N/A	5.0±0.1	N/A	5.0±0.1	4.6±0.1	4.6±0.1	N/A

from the reversed RA/LA gradient in APD_{90} relative to the physiological scenario captured by the heterogeneous SR model). Increasing left atrial APD_{90} to 139 ms lowers re-entry inducibility to the levels seen in the homogeneous SR model. Further, re-entry dynamics in the ‘reverse gradient’ and the homogeneous SR model are identical, further underscoring the importance of left atrial APD_{90} for inducibility and dynamics of sustained re-entries.

6.4.2 Mechanisms of re-entry inducibility and dynamics

Across models, short burst pacing intervals lead to re-entry patterns in LA in the absence of rotors in RA (albeit with secondary activation of RA; indicated in green in Figure 6.2). Medium pacing intervals yield two figure-of-eight (F8) re-entries, one per atrial chamber. Relatively long intervals between ectopic stimuli result in re-entries in the right atrial chamber, with secondary activation of LA (blue; Figure 6.2). I now move to understand the mechanisms leading to the generation of the different re-entry patterns observed in the simulations.

6.4.2.1 Figure-of-eight re-entries in LA and RA

A robust pattern of re-entry, seen across three out of five whole-atria models and indicated in orange in Figure 6.2, consists of one F8 re-entry per atrial chamber. In the left atrium, the F8 re-entry circumvents the right pulmonary veins, while in RA it is formed around the right atrial appendage and the ostium of superior vena cava (Figure 6.3). Figure 9.21 illustrates the mechanism by which this pattern becomes established. One of the six ectopic stimuli encounters a uni-directional block that forces the electrical wavefront to circumvent the right pulmonary veins, initiating the F8 re-entry in LA. This LA re-entry generates an electrical wavefront that propagates via Bachmann bundle into the RA, where it sees a uni-directional block around the ostium of the superior vena cava – this event gives rise to the F8 re-entry in RA. In this re-entrant activity pattern, the wavefronts

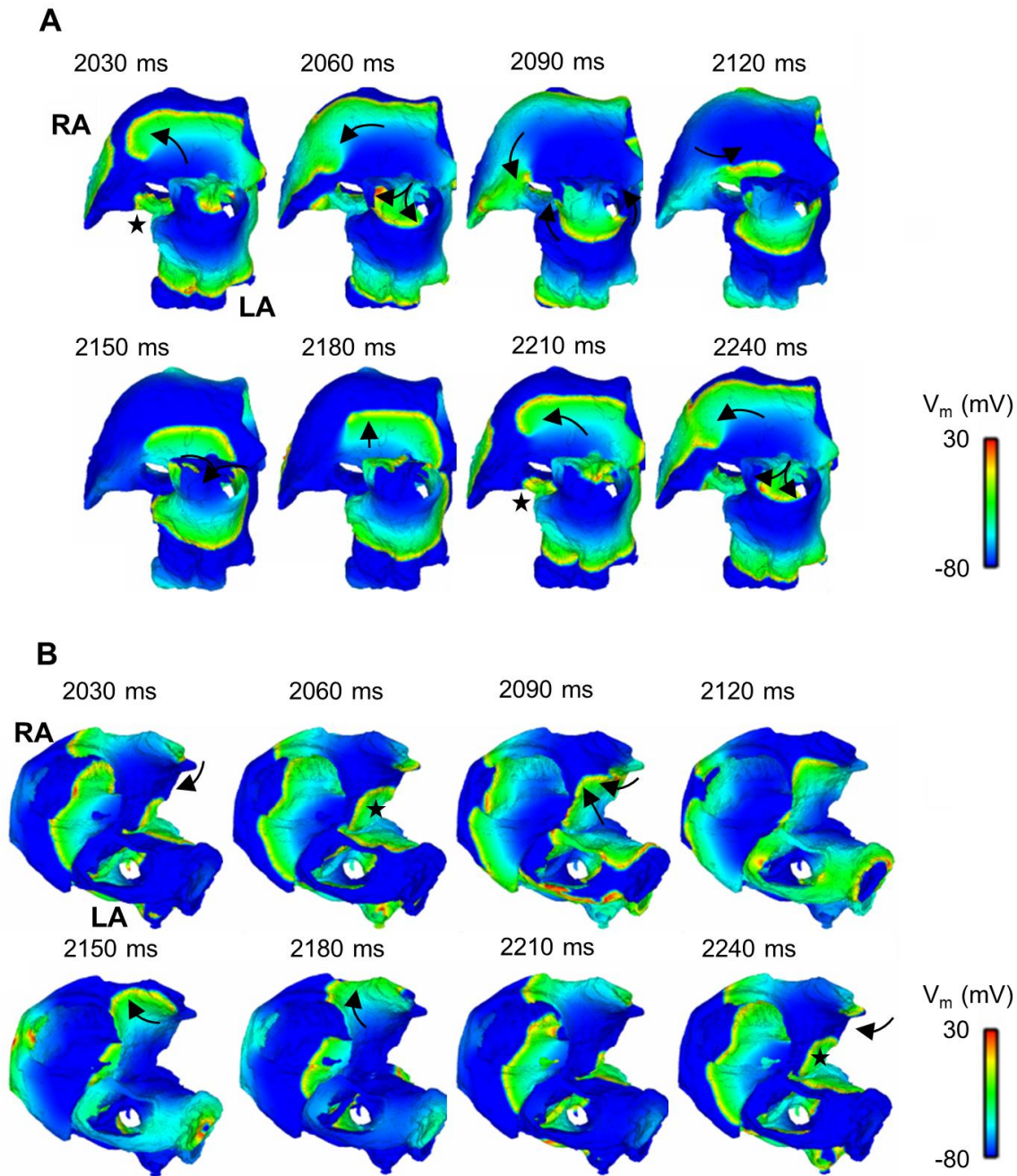


Figure 6.3 Two figure-of-eight (F8) re-entries in the homogeneous SR + global SMTc model following burst pacing at 165 ms interval. **A** Top view of atria, showing the F8 re-entry around right pulmonary veins (PVs) in LA and the rotor around the superior vena cava in RA. The electrical wavefront generated by F8 around right PVs depolarizes LA and travels into RA via Bachmann bundle (black star) to collide with RA rotors. This wavefront collision sustains F8 re-entry in RA. **B** View through valves, showing the rotor around RA appendage (RAPG). The depolarization wavefront travelling from LA into RA via Bachmann bundle reinforces the rotor around RAPG (black star). Time is measured from the start of the last pre-conditioning stimulus at the SAN.

generated in LA and RA collide around the superior vena cava (an example of this is indicated with a black star in Figure 6.3). Such wavefront collision could annihilate the re-entrant activity; however, due to the two wavefronts colliding at an appropriate time point, the rotors in RA are sustained by the wavefront travelling from LA. The time of collision is dependent on multiple factors, including conduction velocities in LA and RA and frequencies of F8 re-entries, as well as sufficiently short APD₉₀ values. All these factors are similar across the models in which the re-entry pattern in question is seen (Table 6.1).

Out of the five whole-atria models considered, two models – the homogeneous SR and the reverse gradient – do not exhibit the re-entry pattern with one F8 re-entry per atrial chamber. This is due to the long left atrial APD₉₀ in these models, which is c.a. 40 ms larger than in the remaining whole-atria models. A longer APD₉₀ in LA would make the tissue less pro-arrhythmic and decrease the chances of establishing a re-entry in left atrial chamber, consistent with my findings.

6.4.2.2 Figure-of-eight re-entry in RA in the absence of rotors in LA

A different robust pattern of re-entry, observed in four models across a range of pacing intervals (Figure 6.2), is an F8 re-entry around the right atrial appendage and the ostium of superior vena cava in RA. This is similar to the right atrial re-entrant pattern in Figure 6.3; however, it occurs in the absence of LA re-entries. The mechanism by which the F8 re-entry in RA is established is similar to that in Figure 9.21: one of the ectopic stimuli applied in LA initiates a wavefront that propagates from LA via Bachmann bundle into RA, where it encounters a uni-directional block at the superior vena cava. However, unlike in simulations with one F8 re-entry per atrial chamber, no ectopics encounter a uni-directional block between the right pulmonary veins in LA; instead, they all propagate radially outwards in the left atrial chamber. As a result, no LA re-entries are formed.

6.4.2.3 Re-entries in LA in the absence of rotors in RA

The final class of re-entry patterns established across the five whole-atria models are re-entries in LA with secondary activation of RA. Unlike in the scenarios discussed previously, the re-entry patterns in LA differ with burst pacing intervals and between models (Table 6.2). For instance, a 5-10 ms change in the burst pacing interval results in different LA re-entry patterns within the same whole-atria model (e.g. homogeneous SR + global SMTC; Table 6.2), and the same pacing protocol elicits different re-entry patterns across models with similar APD_{90} in LA (e.g. 160 ms burst pacing in homogeneous SR + global SMTC, heterogeneous SR, heterogeneous SR + SMTC in RA models; Table 6.2). This is due to the formation of re-entry by curvature of the ectopic wavefronts in the LA before reaching the inter-atrial bridge. Figure 6.4 contains selected examples of LA re-entry patterns.

6.4.2.4 Classification of atrial arrhythmias observed in the simulations

An important question to address is whether the re-entrant activity observed in the simulations is representative of AF or of a different type of atrial tachyarrhythmia, i.e. atrial tachycardia or atrial flutter. Atrial tachycardia arises due to an ectopic focus, while the mechanism underpinning atrial flutter involves macro re-entries around an anatomical obstacle such as SVC in RA, PVs in LA, or scars (the core of the anatomical re-entry is unexcitable) (Guillem et al., 2016; Jaïs et al., 2000). Depending on the re-entrant circuit taken by the electrical excitation, different types of atrial flutter can be distinguished (for instance, a circuit around SVC would be referred to as SVC flutter) (Doiny and Merino, 2013). By contrast, functional re-entries (with an excitable but unexcited core, often with a certain instability) or micro-anatomical re-entries around small blood vessels or along micro-anatomic tracks formed by e.g. atrial musculature, have been associated with AF (Cuculich et al., 2010; Guillem et al., 2016; Hansen et al., 2015; Narayan et al., 2013). As a guideline, atrial tachycardia, flutter, and fibrillation can be associated with the atrial

Table 6.2 Patterns of re-entry induced in LA in the absence of RA re-entrant activity in the five models of human whole atria. Burst pacing intervals are provided. Acronyms: F8 – figure-of-eight re-entry, S – single rotor, PVs – pulmonary veins, BB – Bachmann bundle.

Model	Patterns of re-entry seen in LA depending on burst pacing interval
Homogeneous SR	165 ms: F8 to the side of one of right PVs (Figure 6.4A) 175 ms: F8 around right PVs; S around one of left PVs
Homogeneous SR + global SMTC	155 ms: S around one of right PVs 160 ms: S around one of left PVs (Figure 6.4C)
Heterogeneous SR	160 ms: S around one of right PVs
Heterogeneous SR + SMTC in RA	160 ms: F8 re-entry around end of BB/ one of right PVs (Figure 6.4B)
'reverse gradient'	165 ms: F8 to the side of one of right PVs 175 ms: F8 around right PVs; S around one of left PVs

rate of 2.5 – 4.17 Hz, c.a. 5 Hz, and 5.83 – 10 Hz, respectively (Goodacre and Irons, 2002). However, (Sanders et al., 2005) reported LA sites activating at frequencies as low as 5 Hz in paroxysmal AF, while (Jaïs et al., 2000) described a case of atypical atrial flutter with the re-entrant circuit around the mitral valve at 2.5 Hz. Thus, the frequency criterion must be used in tandem with the underlying mechanisms for correct assignment of arrhythmia type.

The frequency of all re-entrant patterns induced in this study was 4.6 – 5.3 Hz (Table 6.1) and the abnormal electrical activity was sustained by rotors rather than ectopic discharges, ruling out atrial tachycardia (Goodacre and Irons, 2002) but not atrial flutter or fibrillation. One re-entry pattern – elicited in the simulations of heterogeneous SR model at 190 ms burst pacing interval – involved a single rotor in the RA around SVC with a secondary activation of LA (indicated in light blue in Figure 6.2), and could be clearly

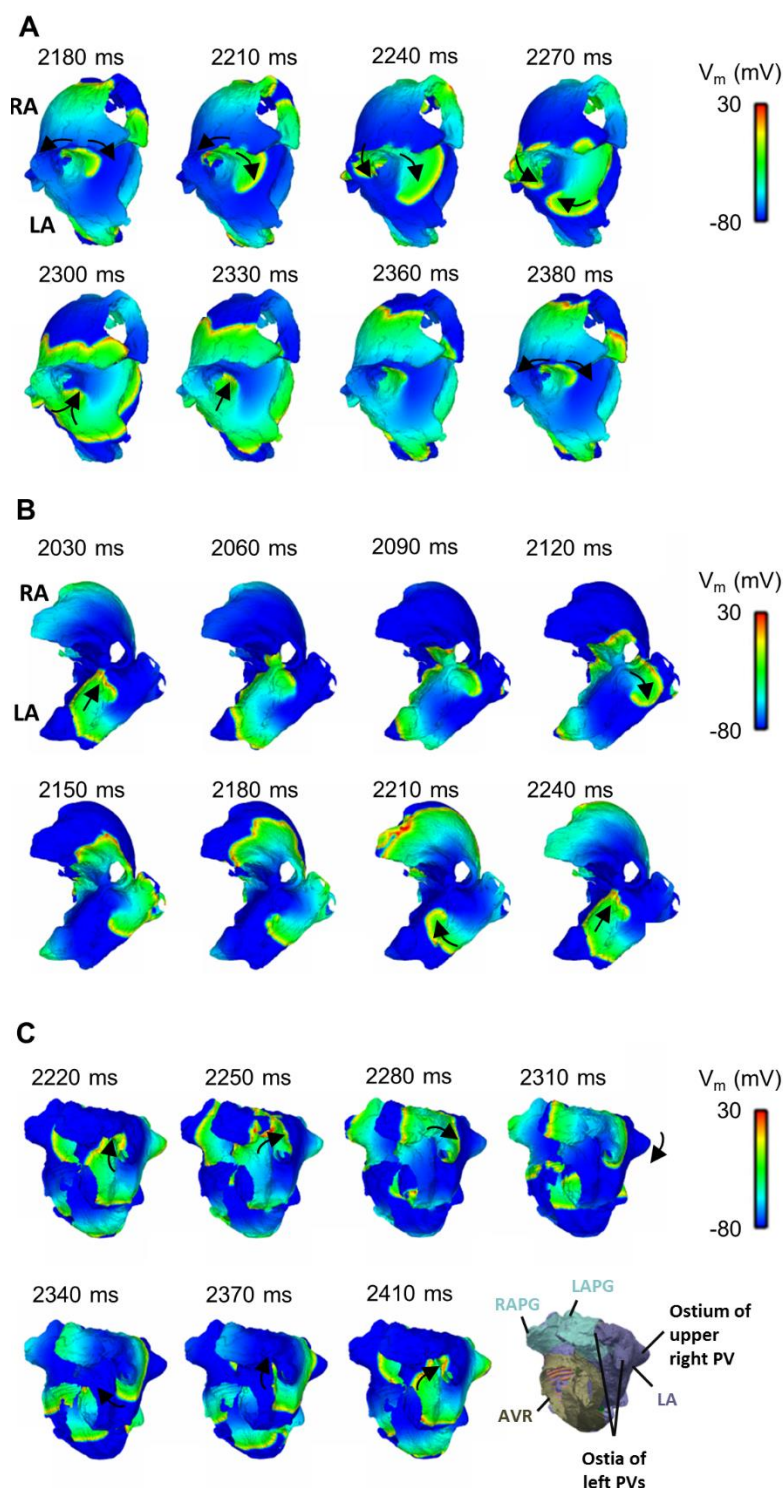


Figure 6.4 Examples of re-entrant patterns formed in LA. **A** Figure-of-eight re-entry formed to the side of one of right pulmonary veins following burst pacing at 165 ms intervals in the homogeneous SR model. **B** Rotor around the end of Bachmann bundle in LA elicited via burst pacing at 160 ms intervals in the heterogeneous SR + SMTC in RA model. **C** Rotor around one of the left pulmonary veins following burst pacing at 160 ms intervals in the homogeneous SR + global SMTC model. In **C**, a pictorial representation of atria with annotated atrial structures is included. In **A-C**, time is measured from the start of the last pre-conditioning stimulus at the SAN.

identified as an atypical form of atrial flutter, namely SVC flutter. Out of the remaining 25 observed re-entry patterns, 11 consisted of a figure-of-eight re-entry around SVC and RAPG in the right atrial chamber (shown in dark blue in Figure 6.2), and a secondary activation of the LA. In those cases, the anatomical macro re-entry around SVC could be identified as the SVC flutter; however, the rotor pivoting in RAPG tissue is an example of a functional re-entry that could be attributed to AF. Thus, this re-entry pattern appears to be a mixture of the two arrhythmia types. Interestingly, the pattern involving the F8 re-entry around SVC/ RAPG in RA is seen for all five whole-atria models at the same burst-pacing intervals of 185-190 ms and regardless of APD_{90} or its gradient between atrial chambers, suggesting that the critical factor in establishing this re-entry circuit is conduction velocity rather than tissue repolarization.

The next most common re-entry pattern – seen in 6 out of 26 of simulations (23% prevalence; shown in orange in Figure 6.2) – involves the F8 re-entry around SVC/ RAPG in RA with one anatomical and one functional rotor as described above, as well as an anatomical macro F8 re-entry around right PVs in LA. Overall, 4 rotors coexist in this case – 2 per atrial chamber – and 75% of them are anatomical rather than functional. Thus, this arrhythmia pattern is a mixture of a complicated atypical atrial flutter and AF. Finally, co-existence of multiple rotors in both atrial chambers is dependent on conduction velocities in LA and RA and frequencies of F8 re-entries, as well as sufficiently short APD_{90} values. All these factors are similar across the three models for which this re-entrant pattern is seen (Table 6.1; Figure 6.1).

The final class of arrhythmia patterns observed in the simulations are re-entries in LA with secondary activation of RA, seen in 8 out of 26 simulations (shown in green in Figure 6.2; detailed description in Table 6.2). Here, the precise re-entry circuits differ between models and burst pacing intervals, due to the formation of re-entry by curvature of the

ectopic wavefronts in the LA (Figure 6.4). In 3 out of 8 cases, namely for the homogeneous SR model paced at 165 ms, the ‘reverse gradient’ model with burst pacing at 165 ms, and the heterogeneous SR + SMTC in RA model at 160 ms pacing, one anatomical and one functional re-entry is observed. Thus, this is another example of a mixture of atrial flutter and fibrillation. In the remaining 5 out of 8 cases, a varying number (one to three) anatomical re-entries around PVs are observed, motivating the assignment of a complicated form of atypical left atrial flutter.

An interesting example of a functional re-entry pivoting around the end of BB in LA is seen in the heterogeneous SR + SMTC in RA model at 160 ms burst pacing interval (Figure 6.4B). In this setting, the average APD₉₀ in the right and left atrial chambers is 99 and 107 ms, respectively. The RA-LA APD₉₀ gradient at the junction of BB and LA is essentially abolished, and the functional re-entry forms at the boundary between the two regions with distinct APDs. This is consistent with previous findings of (Liu et al., 2013), who reported that in simulations of two-dimensional tissue slabs, the removal of the RA-LA APD₉₀ gradient stabilizes re-entries at the RA/LA boundary.

6.5 Discussion

In this chapter, I investigate the impact of nNOS inhibition/ depletion on inducibility and dynamics of re-entrant electrical activity in several functional *in silico* models of human whole atria, with the aim of assessing the relative pro-arrhythmic effects of APD₉₀ shortening and loss of the RA/LA gradient in APD₉₀. The main findings are:

1. Reduction of APD₉₀ in the absence of RA/LA APD₉₀ gradient increases re-entry inducibility and alters the dynamics of re-entries that arise;
2. Abolishment of RA/LA gradient in APD₉₀ does not alter re-entry inducibility or dynamics;

3. Re-entry inducibility is crucially dependent on APD₉₀ in LA and not on APD₉₀ in RA or the RA-LA APD₉₀ gradient;
4. Re-entrant circuits in the form of F8 re-entries around SVC/ RAPG in RA with a secondary activation of LA are exhibited across all models at identical pacing intervals regardless of APD₉₀ or its inter-chamber gradient, are due to cross-model similarities in conduction velocity. Those re-entries have been classified as a mixture of atrial flutter and fibrillation;
5. Re-entrant circuits in the form of two F8 re-entries – one per atrial chamber – are seen only in models with short left atrial APD₉₀. They have been classified as a mixture of atrial flutter and fibrillation;
6. Re-entrant activity in LA in the absence of rotors in RA is more chaotic, with small changes in pacing intervals and left atrial APD₉₀ leading to different re-entry patterns. This is due to re-entry formation by wavefront curvature in the LA. These re-entry patterns have been classified either as atrial flutter or a mixture of flutter and fibrillation.

In the simulations conducted in this study, the APD₉₀ shortening – and not the loss of RA/LA APD₉₀ gradient – is the main mechanism responsible for inducibility of re-entrant activity due to nNOS inhibition. Indeed, reduction of APD₉₀ is a well-accepted mechanism of pro-arrhythmia (Nattel, 2002).

The RA/LA gradient in APD₉₀ has been reported in several animal species under physiological conditions, and the most recent experiments on human myocytes corroborate these findings (Liu et al., 2016). Several studies on mouse and dog atrial tissue reported decreasing APD₉₀ with increasing distance from the SAN, and linked this with regional heterogeneities in ionic currents (Table 9.1; Appendix 1). Intra-atrial differences in I_{to} , I_{Kr} and I_{CaL} have been reported in canine atria, while I_{K1} and I_{CaL}

contribute to the intra-atrial gradients in APD_{90} in mouse atria. By contrast, the intra-atrial gradients in ionic currents in human tissue (particularly in control conditions) remain largely unknown. As a result, simulation studies on human whole atria tend to use ionic current heterogeneities measured in other animal species, typically settling on dog (e.g. (Adeniran et al., 2015; Sánchez et al., 2013)). However, since the main focus of this study has been on achieving the RA/LA gradient in APD_{90} for the purpose of investigating the pro-arrhythmia mechanisms due to nNOS, I implemented the intra-atrial gradients in I_{K1} and I_{CaL} as measured in mouse atrial myocytes (Liu et al., 2016). This can be revised in future studies, should the appropriate experimental data on ionic current gradients within human atria become available.

Pro-arrhythmic effects due to nNOS inhibition were evaluated by assessing re-entry inducibility across several whole-atria models. In the 26 simulations where re-entries were induced, 85% cases were classified as a mixture of atrial flutter and fibrillation, while 15% were representative of atypical forms of atrial flutter. All rotors in my simulations were located within the so-called ‘driver regions’ where the majority of abnormal atrial electrical activity was described (Haissaguerre et al., 2014). I did not observe multiple fractionated wavelets representative of chronic AF (Allessie et al., 2002; Verheule et al., 2014), as the models do not include structural remodelling such as decreased cell-to-cell coupling and endocardial fibrosis. The goal of the study was to investigate electrical effects due to nNOS inhibition, and these are thought to precede AF-induced remodelling of atria (Reilly et al., 2016).

In this study, the RA-LA APD_{90} gradient was modelled by assigning appropriate single-cell electrophysiology models to either the left or the right atrial chamber (Section 6.3.2). Thus, a discontinuity in APD_{90} at the RA/LA junctions was present in the models with a non-zero APD_{90} gradient. This is a simplification of the situation seen *in vivo*, where

APD₉₀ gradually decreases with an increasing distance from the sino-atrial node. Decision to model the RA-LA APD₉₀ gradient in this simplified manner was due to the lack of detailed experimental data on regional intra-atrial variation of (i) APD₉₀ in humans under control conditions, and (ii) the modulation thereof by nNOS inhibition. Should such data become available, whole-atria models will be appropriately refined.

In this study, nNOS inhibition was modelled by incorporating known effects of SMTC into either both atrial chambers or right atrium only. However, the changes in ionic currents due to nNOS block in human have so far only been investigated in right atrial myocytes (Reilly et al., 2016). Most recent experimental data on murine samples reveals that nNOS inhibition acts differentially on selected ionic currents in the two atrial chambers, leading to an increase in I_{to} in RA only. By contrast, nNOS block increases I_{K1} and I_{CaL} by a similar degree in both RA and LA in mouse. Similar differential effects of nNOS block could also occur in human and will be incorporated into the whole-atria models, should the experimental evidence become available.

The modelled effect of SMTC was to decrease cellular repolarization biomarkers. As mentioned in the previous chapter, in addition to abnormal repolarization, changes in cell depolarization and gap-junctional coupling can impact electrical wavefront propagation in tissue, contributing to pro-arrhythmia. (Ueda et al., 2008) reported a positive modulation of I_{Na} by nNOS-derived NO in ventricular myocytes of patients with a particular genetic background leading to the long QT syndrome. If a similar mechanism were to hold more generally, nNOS inhibition would lead to a decrease in I_{Na} and conduction velocity, further enhancing the pro-arrhythmic effects studied here. Slowed conduction velocity would also be expected if nNOS inhibition/ depletion were found to negatively impact inter-cellular conduction via gap junctions. Additional experimental studies into the potential nNOS-mediated effects on conduction velocity are thus of an

urgent importance for establishing a complete picture of nNOS-related pro-arrhythmia effects.

One of my findings is that left atrial APD₉₀ plays a crucial role both for inducibility and dynamics of re-entry patterns that arise. However, I elicited re-entrant activity by burst pacing around right pulmonary veins in LA to mimic spontaneous ectopic activity in AF (over 90% of triggered activity in paroxysmal AF occurs around PVs; (Haïssaguerre et al., 1998; Jaïs et al., 1997)). If ectopic activity was to occur at a different location (e.g. in RA), re-entry inducibility and dynamics may change. To explore this, I may apply burst pacing in the right atrial chamber in future studies. However, only 4-6% of ectopic activity in human atria has been reported to arise from the RA (Haisaguerre et al., 1998).

This computational study emphasises the importance of APD₉₀ vs. the presence/ absence of the RA-LA APD₉₀ gradient for inducibility of sustained re-entries in human whole atria. The next step is to test these findings experimentally. To do this, one requires an animal model where (i) the atrial APD₉₀ can be easily tuned, so that the scenarios with and without the RA-LA APD₉₀ gradient can be tested, and (ii) an imaging modality permitting one to visualise atrial electrical activity. One way of controlling APD₉₀ in a chamber-specific manner could be by epicardial gene painting, whereby the LA and RA would be treated with solutions containing different genes that would cause a differential change in APD₉₀ in the chambers. Epicardial gene painting was used by (Igarashi et al., 2012) in the context of connexin gene transfer as a possible therapy for AF. A different strategy could involve pharmacological intervention to prolong/ shorten APD₉₀ differentially in the atrial chambers, exploiting heterogeneities in the ionic currents underlying the physiological APD₉₀ gradient within the atria (Knobloch et al., 2002). The limitation of the latter approach is the fact that inter- and intra-atrial heterogeneities in

ionic currents are species-dependent (Table 9.1) and have been investigated only in selected animal models.

The second part of the experimental set-up to verify the results of this study would involve selecting an imaging modality. Optical mapping of whole atria infused with a voltage-sensitive dye are one possibility. This imaging modality has typically been applied to dissected atria/ whole hearts removed from animal models (Nygren et al., 2004) or to explanted human hearts (Hansen et al., 2015). Invasive and non-invasive methods for mapping of atrial electrical activity in humans *in vivo* have also been devised. An example of an invasive imaging technique is the focal impulse rotor modulation (FIRM), whereby two basket-type 64-pole catheters are inserted into both atrial chambers and a detailed map of endocardial electrical activity is recorded (Narayan et al., 2013). By contrast, electrocardiographic imaging (ECGI) is a non-invasive technique in which the electrical activity on the human torso is measured via 256 electrodes and, together with patient-specific atrial geometry obtained with MRI, is used compute the corresponding epicardial electrical activity (Cuculich et al., 2010; Haissaguerre et al., 2014, 2013). Given the level of control over atrial APD₉₀ and hence the necessity to use animal models for experimental verification of the *in silico* findings, experimental studies are unlikely to require methods more sophisticated than optical mapping.

We selected one cellular electrophysiology model from the population of Maleckar-based models developed in Chapter 4; the selected model captured average *ex vivo* experimental results on APD₅₀ and APD₉₀ in the absence/presence of SMTC. This could be extended to incorporating a different cellular electrophysiology model into the whole-atria structure, for instance to investigate outlier behaviour. An extension of the study to span atrial cellular electrophysiology models based on the Grandi formulation could also be of

interest, although the similarities in response between the two different populations of models in the previous chapter provide confidence on model independence of the results.

In this chapter, the (Maleckar et al., 2009) model was used to describe human atrial cellular electrophysiology. In a recent study, (Liberos et al., 2016) constructed a population of three-dimensional tissue models based on the more recent (Koivumäki et al., 2011) description of human atrial electrophysiology, and observed a higher prevalence of re-entries in the models with larger G_{CaL} . As described in the previous chapter, one of the effects of nNOS inhibition on the right atrial myocytes was to increase I_{CaL} current by 18%. Detailed investigations into the potential role of I_{CaL} increase as a mechanism of pro-arrhythmia, particularly in the context of utilizing a more recent description of intracellular calcium handling in human atrial electrophysiology (e.g. (Koivumäki et al., 2011)), may be explored in future studies.

6.6 Conclusions

In this chapter, I investigated the relative effect of two likely pro-arrhythmia mechanisms linked to nNOS inhibition on re-entry inducibility and dynamics using *in silico* models of human whole atria. Simulations show that the main mechanism of increased re-entry inducibility under nNOS block conditions is APD₉₀ shortening, rather than loss of the right-to-left atrial APD₉₀ gradient. In the next and final chapter, I summarize the central findings of the thesis, discuss their implications and suggest possible future lines of investigation into variability in cardiac electrophysiology as well as nNOS-mediated effects underpinning AF.

7 CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Summary

AF is the most common disturbance of the heart rhythm. It manifests itself as disorganized electrical activity in the upper chambers of the heart. The incidence of AF is forecast to rise due to population ageing and increased prevalence of associated risk factors. AF remodels the electrical and structural properties of the atrial tissue. As a result, episodes of AF become more frequent and prolonged with time, increasing resistance to treatment. A deeper understanding of the mechanisms causing AF is one of the priorities in arrhythmia research. Recent experimental data suggest that depletion of the neuronal isoform of Nitric Oxide Synthase (nNOS) is linked to AF, but the precise mechanistic links remain unknown.

Mechanisms underlying disease span multiple biological time- and length-scales; however, experiments spanning multiple scales are hard to design and conduct. Clinical studies are further complicated by restricted access to human tissue and large heterogeneity, or variability, within the human population. As a result, mechanisms preceding AF-induced remodelling in humans are hard to study with experimental techniques alone. In contrast, computer models can be used to cross multiple spatio-temporal scales; furthermore, they can be constructed with physiological variability taken into account to augment experimental investigations.

In this thesis, I have investigated the mechanisms linking nNOS and AF with the aid of multi-scale computational models that were built in synergy with experiments. First, I

constructed populations of single-cell models capturing experimentally-observed ranges of variability in human right atrial myocytes under control conditions. Then, I used those experimentally-calibrated populations of control models to investigate likely ionic mechanisms leading to cellular action potential shortening under nNOS inhibition/depletion. Finally, I probed two likely mechanisms of pro-arrhythmia due to nNOS inhibition at the level of human whole atria.

I began in Chapter 2, where I introduced important concepts in cardiac electrophysiology, describing the mechanisms by which ionic currents in the cellular membrane give rise to action potentials, and how cellular signals combine to yield the emergent electrical wavefronts in the cardiac tissue. Following that, I reviewed the literature on AF, including disease progression, as well as the mechanisms of pro-arrhythmia due to AF. I discussed known findings on the role of nitric oxide and nNOS in maintaining healthy cardiac function and changes that take place under conditions of nNOS depletion/inhibition and increased nNOS levels in ventricular tissue. Finally, I described experimental evidence for inter-subject variability in cardiac electrophysiology at multiple spatio-temporal levels, and discussed implications of variability for arrhythmia research.

In Chapter 3, I reviewed computational modelling in cardiac electrophysiology. I began at the cellular level, where I described the Hodgkin-Huxley model. I then subsequently reviewed selected cellular cardiac electrophysiology models, finishing with a description of the biophysically-detailed models of human atrial electrophysiology that are presently used in research. Then, I described whole-atria modelling, starting with the geometrical models of human atria, followed by the electrical propagation model used in thesis, as well as approaches for investigating effects of AF-induced remodelling *in silico*. Following this, I described approaches designed to study variability in cardiac

electrophysiology, including a detailed description of the population-of-models approach used in subsequent chapters of this thesis.

In Chapter 4, I investigated methodological aspects behind the construction of the experimentally-calibrated populations of models for human research. In particular, I showed that, to build populations of models consistent with variability in a narrow experimental dataset, one needs to consider sources and modulators of variability beyond the traditionally-considered maximal current conductances. I discussed the meaning of models with ionic current densities exceeding experimentally-observed ranges in light of the populations-of-models' role to explore multiple ionic profiles beyond those seen in experiments, but still consistent with experimental cellular AP recordings. Ultimately, I constructed populations of models that captured wide variability in experimental recordings from human right atrial myocytes under control conditions.

The control populations of models were subsequently used in Chapter 5 to probe the role of nNOS-mediated changes in ionic currents on the human atrial action potential. I showed that the experimentally-measured changes in I_{Kur} , I_{to} , I_{CaL} , and I_{K1} explained the shortening of the cellular AP as recorded experimentally in the presence of nNOS inhibitor SMTC. Then, I demonstrated that I_{Kur} and I_{K1} played a key role in SMTC-induced AP shortening, a finding that would have been very difficult to obtain using experimental methods alone. I further showed that the lack of nNOS-mediated AP shortening following AF-induced electrical remodelling at the cellular level can be explained by decreased nNOS protein content. All those findings were obtained with *in silico* populations of models whose ionic profiles included and extended beyond those used in experiments, and were based on two distinct human action potential models (Grandi et al., 2011; Maleckar et al., 2009). This emphasises the robustness of the nNOS-mediated mechanisms regulating human atrial AP.

In Chapter 6, I investigated the importance of nNOS inhibition on inducibility and persistence of atrial arrhythmias in human with the aid of whole-atria simulations, testing two possible mechanisms of pro-arrhythmia identified in experiments: the APD₉₀ shortening and the loss of RA/LA gradient in APD₉₀. The simulations revealed that APD₉₀ shortening leads to increased inducibility of re-entry and alters the dynamics of re-entrant waves that arise. Further, I showed that the loss of the RA/LA APD₉₀ gradient did not impact either re-entry inducibility or dynamics. Thus, the simulations demonstrated the shortening of the AP to be the dominant mechanism of pro-arrhythmia due to nNOS inhibition at the whole-atria level. Overall, this thesis has investigated the nNOS-mediated mechanisms impacting pro-arrhythmia from the ionic current level through to whole atria.

7.1.1 Key contributions and main findings

The first main contribution of this thesis is the methodological work exploring the experimentally-calibrated population-of-models methodology with the applications for human research. First, I showed that factors not usually considered in studies of cell-to-cell variability – including the magnitude of the stimulus current and the potential at which I_{CaL} density is maximized – can be important for capturing the full range of experimentally-observed variability in the cellular AP properties. Second, I demonstrated that a narrow range of AP recordings – obtained from a relatively restricted patient cohort – can be caused by a wide variability in the underlying ionic currents (in some cases exceeding the +100% range of variation on the maximal current conductances). This is a manifestation of a wide heterogeneity of the human population. Given that variability in controlled animal lines is likely to be smaller, my findings have important implications for studies of variability in humans and for arrhythmia research. Third, I showed that experimentally-calibrated populations obtained with the simple Latin Hypercube

sampling are essentially equivalent to those generated with the statistically more rigorous Sequential Monte Carlo sampling within the Approximate Bayesian Computation, demonstrating that there is not necessarily a need for complicated approaches for the population-of-models generation. Fourth, I discussed the meaning of models whose ionic current densities exceeded the experimentally-observed range of variation in light of human data scarcity and experimental limitations, highlighting the role of the populations to yield viable AP profiles within experimentally-observable ranges of cellular biomarkers. Finally, I generated two populations – one based on the Maleckar model, and another one on the Grandi model – that captured experimentally-observed variability at the level of AP properties and/or ionic currents in human atrial myocytes under control conditions (i.e. in the absence of nNOS blocker SMTC). Those experimentally-calibrated populations provided a tool for testing clinically-relevant hypotheses on nNOS inhibition effects in isolated myocytes.

The second major contribution of this thesis has been to elucidate the ionic mechanisms underpinning the AP shortening observed in isolated human myocytes under nNOS block. Experimental evidence showed that I_{Kur} , I_{to} , I_{CaL} and I_{K1} were all impacted by SMTC. However, it was unclear which current played the primary role in the AP shortening observed at the AP level, or whether those mechanisms were sufficient to explain the reported AP shortening. Furthermore, it would have been very hard to design and conduct experiments to confirm the importance of one current over any of the other ones. In contrast, investigating likely ionic mechanisms and their contributions to the AP shortening *in silico* is comparatively feasible. Thus, I used the experimentally-calibrated populations of control models developed previously to investigate the relative importance of the changes in the four ionic currents for the AP shortening. In this way, I demonstrated the primary role of I_{Kur} and I_{K1} currents in APD decrease observed experimentally in atrial myocytes from patients in normal sinus rhythm. Subsequently, I constructed populations

of models that included the effects of electrical remodelling due to AF, and showed that the lack of AP shortening in the atrial myocytes from patients with AF was likely due to reduced levels of nNOS. This is in line with nNOS depletion playing an important role in AF onset. The ionic mechanisms of nNOS action governing the cellular electrical phenotype were the same in (i) the populations based on the Maleckar and the Grandi models, and (ii) the populations with the AP calibration only, as well as with the additional calibration on ionic current densities. This highlights the robustness of nNOS-mediated mechanisms and model-independence of my results.

The third and final major contribution of this thesis is the investigation into likely mechanisms of pro-arrhythmia at the whole-organ level. Similarly to the single-cell investigations described above, experimental evidence highlighted two likely mechanisms of pro-arrhythmia due to nNOS inhibition; however, it would have been difficult to design and conduct experiments to prove the critical contribution of one mechanism over another. The likely pro-arrhythmic mechanisms identified in experiments were APD decrease and the loss of the RA/LA gradient in APD. Thus, I built phenotypic models of human whole atria – with or without the RA/LA gradient in APD, and/or with long or short cellular APDs (AP shortening was achieved by simulating effects of nNOS inhibition). I then showed that the shortening of the AP in the absence of the RA/LA gradient in APD increases inducibility of re-entrant electrical waves and changes rotor dynamics. Separately, I demonstrated that the loss of the RA/LA gradient in APD does not alter neither the inducibility of re-entry, nor the dynamics of the re-entrant patterns that arise. Thus, the main mechanism of pro-arrhythmia due to nNOS inhibition appears to be AP shortening rather than the loss of the RA/LA APD gradient.

7.2 Future directions

In what follows, I discuss possible extensions to the work in this thesis. While beyond the scope of the present studies, the proposed future investigations could lead to fruitful discoveries.

7.2.1 Intra-patient vs. inter-patient variability

A significant part of this thesis focuses on modulators and determinants of cell-to-cell variability in isolated atrial myocytes obtained from a restricted patient cohort. On average, two to three cells per patient were used for the experimental investigations. The experimentally-calibrated populations of models were built using the minimum and maximum values of experimental biomarkers derived from all available cells for which a particular experiment was performed. Thus, I did not discriminate between inter-patient and intra-patient variability. Nonetheless, a future study could investigate the causes and modulators of cell-to-cell variability within one patient (i.e. the ‘intra-patient variability’), and compare them to the variability observed between different patients (the ‘inter-patient’ or ‘inter-subject’ variability). For the study on the intra-patient variability, the focus should be on one selected region of the atrial tissue, so as to avoid confounding effects due to e.g. ionic gradients and APD differences within and between atrial chambers.

7.2.2 Going beyond the cell-to-cell variability

Variability studies in this thesis focused on the single-cell level. Moving beyond the level of isolated cells and towards tissue and whole-organ variability is of tremendous interest, as it could ultimately pave the way for personalized treatment of patients in the clinic. To date, two studies utilising the experimentally-calibrated populations-of-models methodology moved beyond the single-cell level to consider three-dimensional tissue effects (Liberos et al., 2016; Sánchez et al., 2013). Both studies focused on how variability

in cellular electrical properties impacts inducibility and dynamics of the resultant re-entrant electrical waves in tissue. For this purpose, (Liberos et al., 2016) first generated a population of 173 single-cell models calibrated with experimental data, and then created 173 three-dimensional spherical tissue models (one three-dimensional model per each single-cell model). In contrast, (Sánchez et al., 2013) built six whole-atria models, each of which was populated with several hundred distinct single-cell electrophysiology models. Future studies could focus on investigating which of the two approaches is most suitable for studies of variability at the tissue and whole-organ level.

An important extension is to investigate sources of variability beyond electrical properties of the tissue, such as fibrosis and atrial dimensions. Some researchers are already exploring the impact of patient-specific fibrosis patterns on re-entrant electrical activity in human atria with the aid of computer modelling coupled to information obtained via magnetic resonance imaging (Zahid et al., 2016). Establishing a pipeline that would enable studies of combined effects of variability in electrical properties (cellular and/or stemming from tissue effects) together with structural differences between the hearts of different patients would be needed for such investigations. Nonetheless, the prospect of such a development is very exciting and would almost certainly lead to fruitful discoveries in the field of personalized medicine and computational medicine.

7.2.3 Investigations of electro-mechanical effects

In this thesis, I studied the impact of nNOS-mediated effects on electrical properties of human atrial myocytes and whole atria. However, the cellular action potential (and, by extension, the electrical excitation of the tissue) is an upstream mechanism whose role is to trigger calcium-mediated cardiac contraction. In ventricles, nNOS has been shown to modulate the intracellular calcium transient and cellular contraction. Future studies may focus on exploring the effect of nNOS on atrial contractile properties, and any link this

may have with AF. At the single-cell level, it may be sufficient to incorporate information on intracellular calcium transient into the populations of single-cell models constructed in Chapters 4 and 5 for this purpose. However, the investigations at the level of whole-atria will likely require an extension of the present computational methodology from purely electrophysiological to electro-mechanical techniques. Studies demonstrating the capability of *in silico* modelling and simulation to investigate both electrophysiological and mechanical properties of the atrial tissue have been published recently (Adeniran et al., 2015). A major challenge in such studies is the lack of human-specific experimental data on atrial contractile properties, and hence the parametrization of the models may pose additional challenges. Nonetheless, this is a promising avenue for future work.

7.3 Conclusions

AF is a major health burden. Mechanisms underlying this disease are not well-understood and are likely to be complex. Recent experimental evidence suggests that the neuronal isoform of Nitric Oxide Synthase may play a crucial role in AF onset; however, designing and conducting experiments proving specific mechanisms linking nNOS and AF is difficult. Furthermore, experiments are additionally complicated by biological variability, which is particularly important in human studies. In this thesis, I utilize multi-scale modelling and simulation in synergy with experimental information to investigate mechanistic links between AF and nNOS-mediated effects at the level of ionic currents/cellular action potentials/whole organ in human. First, I build populations of models spanning biological variability in experimental data from isolated human atrial myocytes under control conditions. Subsequently, I use those populations of control models to identify key ionic mechanisms underpinning nNOS-mediated regulation of the cellular action potential in human atrial myocytes. I demonstrate that two out of four experimentally-investigated ionic currents – I_{Kur} and I_{K1} – play a key role in recovering

the nNOS inhibition phenotype at the AP level. Following this, I construct models of human whole-atria, and identify the AP shortening as the main mechanism of pro-arrhythmia due to nNOS inhibition at this level. In this way, I provide a picture of nNOS-mediated mechanisms impacting AF from the level of ionic currents to the whole organ.

8 BIBLIOGRAPHY

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9 APPENDICES

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APPENDIX 1 – CARDIAC ELECTROPHYSIOLOGY: FROM CELLS TO WHOLE ORGAN

Table 9.1 Intra-atrial heterogeneities in ionic currents in cells/tissue from animals/humans in normal sinus rhythm. Unless indicated otherwise, heterogeneities were determined from current density information obtained in voltage-clamp recordings of isolated atrial myocytes. Atrial structures investigated in reviewed studies include RA, LA, RAPG, LAPG, CT, PMs, AVR, right/left atrial free wall (RAFW/LAFW), right/left atrial posterior wall (RAPW/LAPW), PVs, and left atrial septum (LAS). For clarity, LA/RA and LAPG/RAPG heterogeneities in ionic currents are highlighted in pink. N/n denotes number of patients/cells. In mouse studies, it was only possible to differentiate between LA and RA. The human study by (Gong et al., 2008), marked with an asterisk (*), investigated patients with rheumatic heart disease only; this is distinct from the rest of studies on human samples. For patient cohort details of the remaining human studies, the reader is referred to original publications. For relationships between ionic currents and ion channels, see Table 1 in (Grant, 2009).

Current	Species	Sample type	Heterogeneity	Reference
I_{Kur}	Human	LAPG (n>10), RAPG (n>10) myocytes	smaller in LAPG than RAPG by 34% at 50 mV [current amplitude (pA), not density (pA/pF), taken into account]	(Caballero et al., 2010)
	Human	Levels of Kv1.5 in LA (n=44) and RA (n=42) tissue samples via RT-PCR	Kv1.5 larger in LA than in RA	(Swartz et al., 2009)
	Human	RAPG (n=18), LAS (n=16) myocytes	smaller in AS than RAPG by 37% at 30 mV	(Gong et al., 2008)*
	Mouse	LA (n=13), RA (n=12) myocytes	larger in LA than RA by 200% at 45 mV (100- μ M 4AP-sensitive current)	(Lomax et al., 2003a)
	Mouse	whole atria (n=9); (investigated: LAPG, RAPG, LAPW, RAPW)	no regional difference (determined via effect of 100- μ M 4AP block on APD70)	(Nygren et al., 2004)
	Mouse	LA (n=12, N=5), RA (n=11, N=5) myocytes	no difference (50- μ M 4AP)	Xing Liu's data
	Mouse	LA (n=5), RA (n=5) myocytes	larger in LA than RA by 100% at 50 mV (50- μ M 4AP)	(Nakamura et al., 2010)

	Dog	LA free wall (n=12), RA free wall (n=12) myocytes	no difference	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	no difference	(Feng et al., 1998)
	Pig	whole heart; measuring effect of IKur blocker on left and right atrial ERP	effects of IKur blockers were significantly stronger on the left atrial ERP than on the right atrial ERP	(Knobloch et al., 2002)
I_{to}	Human	LAPG (n>24), RAPG (n>24) myocytes	no difference at 30 mV	(Caballero et al., 2010)
	Human	LAPG (n=14, N=9), RAPG (n=41, N=21) myocytes	no difference at 50 mV	(Van Wagoner et al., 1997)
	Human	RAPG (n=16), LAPG (n=16) myocytes	no difference at 50 mV	(González de la Fuente et al., 2013)
	Human	Levels of Kv4.3 in LA (n=44) and RA (n=42) tissue samples via RT- PCR	no difference	(Swartz et al., 2009)
	Human	RAPG (n=16), LAS (n=15) myocytes	no difference	(Gong et al., 2008)*
	Mouse	LA (n=20), RA (n=25) myocytes	no difference	(Lomax et al., 2003a)
	Dog	LA free wall (n=25), RA free wall (n=25) myocytes	no difference	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	smallest in RAPG (5.7 pA/pF); larger in remaining structures by 84% (equal to 10.5 pA/pF); at 50 mV	(Feng et al., 1998)
	Dog	LA free wall (n=43), PV (n=37) myocytes	smaller in PV than in LA free wall by 25%	(Ehrlich et al., 2003)
	Dog	LA (n=25), PV (n=17) isolated myocytes	smaller in PV by 25% than in LA at 50 mV	(Cha et al., 2005)
	Rabbit	CT (n=10), PM (n=10) myocytes (all from RA)	larger in PM by 75% relative to CT at 20 mV	(Yamashita et al., 1995)
I_{Kr}	Human	LAPG, RAPG myocytes	in both cases, current was extremely small	(Caballero et al., 2010)

	Human	Levels of HERG in LA (n=44) and RA (n=42) tissue samples via RT-PCR	no difference	(Swartz et al., 2009)
	Mouse	LA (n=6), RA (n=7) myocytes	no differences (no current elicited in either case)	(Lomax et al., 2003a)
	Mouse	LA (n=7), RA (n=5) myocytes	no differences (current was measurable)	(Nakamura et al., 2010)
	Dog	LA free wall (n=16), RA free wall (n=16) myocytes	60% larger in LA than in RA (at 10mV, step current density)	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	RAPG, CT, PM: 1.4 pA/pF. In AVR, current density is larger by 71% (equal to 2.4pA/pF); at 0 mV (step current density)	(Feng et al., 1998)
	Dog	LA free wall (n=10), PV (n=10) myocytes	larger in PV than in LA free wall by 50%	(Ehrlich et al., 2003)
	Dog	LA (n=10), PV (n=11) isolated myocytes	larger in PV by 70% than in LA at 20-50 mV	(Cha et al., 2005)
	Dog	PV and LA levels of α -subunit ERG	larger in PV than in LA by 28%	(Melnik et al., 2005)
	Pig	whole heart; measuring effect of IKr blockers on ERP	effects of IKr blockers were significantly stronger on the right atrial ERP than on the left atrial ERP	(Knobloch et al., 2002)
I_{Ks}	Human	LAPG (n>10), RAPG (n>10) myocytes	no difference at +30 mV	(Caballero et al., 2010)
	Human	LAPG (n=16), RAPG (n=16) myocytes	no difference at +30 mV	(González de la Fuente et al., 2013)
	Dog	LA free wall (n=31), RA free wall (n=31) myocytes	no differences	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	no differences	(Feng et al., 1998)
	Dog	LA free wall (n=12), PV (n=12) myocytes	larger in PV than in LA free wall by 60%	(Ehrlich et al., 2003)
	Dog	LA (n=14), PV (n=16) isolated myocytes	larger in PV by 100% than in LA at 50 mV	(Cha et al., 2005)

	Dog	PV and LA levels of α -subunit KvLQT1	larger in PV than in LA by 34%	(Melnyk et al., 2005)
I_{K1}	Human	LAPG (n=14, N=9), RAPG (n=41, N=21) myocytes	no difference	(Van Wagoner et al., 1997)
	Human	LAPG (n=9, N=4), RAPG (n=40, N=26) myocytes	no difference at -100 mV	(Voigt et al., 2010)
	Human	Levels of Kir2.1, Kir2.2 and Kir2.3 in LA (n=44) and RA (n=42) tissue samples via RT-PCR	Kir2.3 larger in LA than in RA	(Swartz et al., 2009)
	Human	RAPG (n=16), LAS (n=16) myocytes	no difference at -100 mV, outward current is smaller in AS than in RAPG (voltages -60 to -30 mV)	(Gong et al., 2008)*
	Mouse	LA (n=17), RA (n=15) myocytes	larger in LA than in RA by 75% at -100mV	(Lomax et al., 2003a)
	Mouse	LA (n=7, N=2), RA (n=14, N=7) myocytes	larger in LA than in RA by 32% at -100mV	Xing Liu's data
	Dog	LA free wall (n=25), RA free wall (n=25) myocytes	no differences	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	no differences	(Feng et al., 1998)
	Dog	LA free wall (n=8), PV (n=8) myocytes	smaller in PV than in LA wall by 42% [inward (-100mV) and outward (-50 to -20 mV) components]	(Ehrlich et al., 2003)
	Dog	LA (n=12), PV (n=11) isolated myocytes	smaller in PV by 33% than in LA at -120 mV	(Cha et al., 2005)
	Dog	PV and LA levels of Kir2.1 and Kir2.3	Kir2.3 smaller in PV than in LA by 19%; Kir2.1 unchanged	(Melnyk et al., 2005)
	Rabbit	CT (n=10), PM (n=10) myocytes (all from RA)	no difference at -120 mV	(Yamashita et al., 1995)
	Sheep	mRNA concentrations of Kir2.3 from LA (n=5, N=5) and RA (n=5, N=5) via	no difference	(Sarmast et al., 2003)

		ribonuclease protection assay		
I_{CaL}	Human	Levels of CaV1.2 in LA (n=44) and RA (n=42) tissue samples via RT-PCR	CaV1.2 larger in LA than in RA	(Swartz et al., 2009)
	Human	RAPG (n=12), LAS (n=12) myocytes	smaller in AS than in RAA by 71% (peak at 0mV)	(Gong et al., 2008)*
	Mouse	LA (n=11), RA (n=10) myocytes	no differences (at 0 mV)	(Lomax et al., 2003a)
	Mouse	LA (n=19, N=8), RA (n=7, N=4) myocytes	smaller in LA than in RA by 32% at 10 mV	Xing Liu's data
	Dog	LA/RA free wall myocytes	no differences	(Li et al., 2001)
	Dog	RAPG (n=25), CT (n=25), PM (n=25), AVR (n=25) myocytes (all from RA)	I _{CaL} largest in CT (-19.5pA/pF), smallest in AVR (-8.4pA/pF), intermediate in RAPG (-13pA/pF) and PM (-12pA/pF) [the last two are not statistically different]; at 10 mV	(Feng et al., 1998)
	Dog	LA free wall (n=10), PV (n=10) myocytes	smaller in PV than in LA free wall by 35% (peak current at 10mV)	(Ehrlich et al., 2003)
	Dog	LA (n=13), PV (n=15) isolated myocytes	smaller in PV by 27% than in LA at 10 mV	(Cha et al., 2005)
	Dog	PV and LA levels of Cav1.2	no difference	(Melnyk et al., 2005)
	Rabbit	CT (n=10), PM (n=10) myocytes (from RA)	no difference at 10 mV	(Yamashita et al., 1995)
I_{Na}	Human	RAPG (n=16), LAS (n=15) myocytes	larger in AS than in RAPG by 50% (peak at -30 mV)	(Gong et al., 2008)*
	Dog	LA free wall (n=10), PV (n=10) myocytes	no difference	(Ehrlich et al., 2003)
I_{NCX}	Dog	LA free wall (n=8), PV (n=8) myocytes	no difference	(Ehrlich et al., 2003)
	Dog	PV and LA levels of NCX	no difference	(Melnyk et al., 2005)
I_{KAch}	Human	Levels of Kir3.1 and Kir3.4 in LA (n=44)	Kir3.4 smaller in LA than in RA. No difference for Kir3.1.	(Swartz et al., 2009)

		and RA (n=42) tissue samples via RT-PCR		
	Human	Carbachol-sensitive current increase in RAPG (n=40, N=26) and LAPG (n=9, N=4) myocytes	smaller in LAPG than in RAPG	(Voigt et al., 2010)
	Human	Levels of Kir3.1 and Kir3.4 in RAPG (n=8) and LAPG (n=7) tissue samples	Kir3.1 and Kir3.4 smaller in LAPG than in RAPG	(Voigt et al., 2010)
	Mouse	Charbachol-, Acetylcholine-, and Adenosine-sensitive currents in RA and LA	smaller in LA than in RA	(Lomax et al., 2003b)
	Sheep	mRNA concentrations of Kir3.1 and Kir3.4 from LA (n=5, N=5) and RA (n=5, N=5) via ribonuclease protection assay	Kir3.1 and Kir3.4 larger in LA than in RA	(Sarmast et al., 2003)

APPENDIX 2 – CONSTRUCTING HUMAN ELECTROPHYSIOLOGY MODELS FROM A SPECIFIC PATIENT COHORT DATASET: METHODOLOGICAL CONSIDERATIONS

9.1.1 Sequential Monte Carlo sampling for experimentally-calibrated populations of models

The Sequential Monte Carlo (SMC) approach imposes the experimentally-motivated calibration constraints in distinct stages (this is in contrast to Latin Hypercube sampling, where all experimentally-motivated constraints can be applied simultaneously to all models within the candidate population). At each stage, SMC requires that the values of a specific AP biomarker produced by all models in the population fall within the experimentally-permitted ranges for all pacing frequencies. Introduction of a new set of constraints can potentially result in sharp reductions in the regions of the parameter space that are viable. To ‘soften’ this effect, constraints are not introduced in a strict fashion. Expressed in simplified form, the algorithm proceeds as follows:

1. Initialise particles randomly throughout the search region of the parameter space (base values of model parameters $\pm 100\%$).
2. Test each particle for how close it falls to the acceptable range of values for the first biomarker at all pacing frequencies. This distance is the 2-norm of the vector of distances away from the acceptable range for each biomarker at each pacing frequency.
3. Take all particles that fully satisfy the constraint for all pacing frequencies (distance of zero), and then additional particles selected according to increasing distance until at least half of the particles have been chosen. The distance from the acceptable range of values of the furthest particle becomes the new acceptable limit.

4. Copy the remaining particles (those outside the new acceptable limit) onto random members of the set of particles that were chosen.
5. Perform a number of iterations (here 5) of Markov Chain Monte Carlo on the copied particles in an attempt to move them to unique locations in the parameter space that are still within the currently acceptable distance away from the constraint.
6. Repeat steps two to five until all particles correspond to models which produce values for the current biomarker which fall within the desired ranges for each pacing frequency.
7. Repeat steps two to six for each biomarker, ensuring that during trialled updates no particles move to locations that would break any of the previously satisfied constraints.

The method is explained in full mathematical detail in (Drovandi et al., 2016). Following that approach, the Markov Chain Monte Carlo steps are performed using a jumping distribution constructed by fitting a three-component Gaussian mixture model to the set of particles selected before each copy step.

9.1.2 Computational action potential measurements

In the simulations with the candidate population based on the Maleckar model, the intracellular K^+ , Na^+ , Mg^{2+} and effective extracellular K^+ , Na^+ and Ca^{2+} ion concentrations were set to 130, 10, 2, 5.4, 140, and 1.8 mM. In the simulations with the populations based on the Grandi model, the effective intracellular K^+ , Na^+ , Cl^- , Mg^{2+} and extracellular K^+ , Na^+ , Ca^{2+} , Cl^- ion concentrations were set to 130, 10, 14, 2, 5.4, 140, 1.8, and 151.4 mM, respectively. For the candidate populations derived from the Courtemanche model, the intracellular K^+ , Na^+ and extracellular K^+ , Na^+ and Ca^{2+} concentrations were set to 130, 10, 5.4, 140, 1.8 mM, respectively. In an attempt to better

mimic experimental conditions *ex vivo*, the (effective) intracellular Na^+ and K^+ and (effective) extracellular Na^+ , K^+ , and Ca^{2+} concentrations were held constant in time. In the candidate populations based on the Courtemanche model, this meant clamping the intracellular K^+ and Na^+ (the model lacks the effective extracellular space of the Maleckar model or the effective intracellular space of the Grandi model). In the populations based on the Maleckar model, the effective extracellular concentrations of K^+ , Na^+ and Ca^{2+} as well as the intracellular concentrations K^+ and Na^+ were clamped. In the candidate populations based on the Grandi model, the Na^+ concentrations in the intracellular, effective intracellular, and cleft spaces (the latter being a part of the calcium-handling subsystem) were clamped. The temperature was set to 310.15 K.

9.1.3 Computational voltage clamp protocols

In the voltage clamp simulation protocol designed to elucidate I_{to} , the holding potential of -80 mV was maintained for 200 ms prior to the pre-step to -40 mV for another 200 ms. After this, the transmembrane potential was set to the desired value (between -70 mV and 50 mV in 10 mV increments, with the exception of the increment between -10 mV and 10 mV which was set to 0.0001 mV) for 700 ms, followed by the voltage step to -40 mV for 200 ms and then to -80 mV for another 200 ms.

In the voltage clamp simulation protocol designed to elucidate I_{Kur} , the holding potential was set to -80 mV for 200 ms, followed by a pre-step to -35 mV for another 200 ms and then by the desired voltage step (between -35 mV and 45 mV in 10 mV increments) for 500 ms. Following this, the membrane potential was set to -35 mV and then to -80 mV for 200 ms each. As in experiments, I_{sus} was measured as the amplitude of the current remaining at the end of the pulse relative to the zero current level at the end of the pre-step to -35 mV. To mimic the block of I_{Kur} by 4-AP, the values of G_{Kur} were set to zero in the relevant simulations, and I_{Kur} was determined as the difference in the total

transmembrane current I_{Sus} elucidated in the absence or presence of the simulated I_{Kur} block by 4-AP.

In the simulations of I_{to} and I_{Kur} , the inhibitory effect of Cd^{2+} on I_{CaL} was modelled by setting $G_{\text{CaL}}=0$ (Plonsey, R. and Barr, 2007). The (effective) intracellular K^+ , Na^+ , Mg^{2+} and Cl^- concentrations were set to 140, 10, 2, and 24 mM, while the (effective) extracellular K^+ , Na^+ , Ca^{2+} , Cl^- concentrations were set to 5.4, 140, 1.8, 152.4 mM where applicable. The temperature was set to 310.15 K.

In the protocols for measuring I_{CaL} , the holding potential was set at -40 mV for 125 ms, followed by a voltage step to the desired voltage (between -50 mV and 50 mV in 10 mV increments, with the exception of the increment between -10 mV and 10 mV which was set to 0.0001 mV) for 250 ms. Following this, the transmembrane potential was held at -40 mV for another 125 ms. The temperature was set to 310.15 K.

In the simulations of I_{CaL} , all transmembrane potassium currents (including the background potassium current in the Courtemanche model, and the junctional component of I_{Ks} current in the Grandi model) were switched off by setting their conductances to zero to mimic experimental inhibition of potassium channels by caesium ions (Plonsey, R. and Barr, 2007); sodium currents (including the background currents) were also switched off due to the absence of Na^+ in the pipette and extracellular solutions in I_{CaL} voltage clamp experiments. The absence of Na^+ and K^+ ions in experimental solutions was additionally mimicked by setting the (effective) intracellular and extracellular Na^+ and K^+ concentrations to 0.0001 mM. The (effective) extracellular Ca^{2+} and Cl^- were set to 1.8 and 151.4 mM, while the (effective) intracellular Mg^{2+} and Cl^- were set to 7 mM and 16 mM. An exception here were the Grandi-based models, where intracellular Mg^{2+} was set to 5.5 mM as higher values of this concentration resulted in lack of numerical stability. The temperature was set to 310.15 K.

In the voltage clamp simulation protocol designed to elucidate I_{K1} , the holding potential was set to -80 mV for 50 ms, followed by a pre-step to -100 mV for another 50 ms and then by a voltage ramp from -100 mV to +40 mV over the next 1200 ms in fixed steps of 0.1167 mV/ms. Following this, the membrane potential was set to -50 mV for 100 ms and then to -80 mV for 550 ms. The inhibitory effect of Ba^{2+} on I_{K1} was mimicked by setting $G_{K1}=0$ in the relevant simulations, and I_{K1} was determined by subtraction of the total transmembrane current traces in the presence or absence of this simulated block. Temperature was set to 295.15 K. The (effective) extracellular concentrations of K^+ , Na^+ , Ca^{2+} , Cl^- were set to 20, 120, 1.8, 145.6 mM where applicable. The (effective) intracellular K^+ , Na^+ , Mg^{2+} and Cl^- concentrations were set to 120, 8, 5, and 52 mM.

In all voltage clamp simulations, the (effective) intracellular K^+ and Na^+ and (effective) extracellular K^+ , Na^+ and Ca^{2+} ion concentrations were held constant in time. The transmembrane potential was also held constant in time (i.e. $dV/dt=0$) with the exception of changes corresponding to the desired voltage steps. The stimulus current amplitude was set to zero in all simulations.

Figure 9.2 depicts the I_{to} , I_{Kur} , I_{K1} and I_{CaL} currents in the Maleckar, Grandi and Courtemanche models determined from the differences between the total transmembrane currents as measured in experimental protocols, at different points in time/ under conditions of ion channel block.

9.1.4 Numerical methods and data analysis

As described in Section 3.5, simulations were performed using Chaste (Pitt-Francis et al., 2009), with the numerical solver CVODE. Electrophysiological properties were outputted every 1 ms. Postprocessing of AP traces to yield AP properties was performed in Chaste. Data analysis was performed with Matlab. Graphs were plotted using Matlab and plot.ly (<https://plot.ly/>).

Table 9.2 Clinical and demographic characteristics of patients in SR and those with persistent AF. Acronyms: ACEI – angiotensin-converting enzyme inhibitor, ARB – angiotensin II receptor blocker, AVR – aortic valve replacement, CABG – coronary artery bypass surgery, COPD – chronic obstructive pulmonary disease, MI – myocardial infarction, MVR – mitral valve replacement. The χ^2 test was used to compare gender, smoking status, and medical history between groups. The unpaired t test was used to compare age, and the Fisher's exact test was used for surgical procedures. Reproduced with permission from (Svetlana N. Reilly and Xing Liu et al., 'Up-regulation of miR-31 in human atrial fibrillation begets the arrhythmia by depleting dystrophin and neuronal nitric oxide synthase', Science Translational Medicine, 8(340), 340ra74, 2016. Website URL: <http://stm.sciencemag.org/content/8/340/340ra74.short>).

Groups of comparison	SR	Persistent AF	P value
Total number patients, n	176	51	
Mean age, years ($\pm$ SD)	69 $\pm$ 10	73 $\pm$ 10	0.0009
Men, n (%)	123 (70)	33 (65)	0.76
Surgical procedure, n (%)			
CABG $\pm$ AVR/MVR	125 (71)	17 (33)	0.01
AVR/MVR	51 (29)	27 (53)	0.05
Maze procedure	0 (0)	7 (14)	<0.0001
Medical history, n (%)			
Smoker/ex-smoker	99 (56)	21 (41)	0.27
Hypertension	130 (74)	31 (61)	0.44
Diabetes mellitus	39 (22)	6 (12)	0.17
Heart failure	42 (24)	18 (35)	0.22
Previous MI	55 (31)	2 (4)	0.001
COPD/asthma	12 (7)	6 (12)	0.38

Smoker/ex-smoker	99 (56)	21 (41)	0.27
Medications, n (%)			
Anticoagulants	16 (9)	33 (64)	<0.0001
Antiplatelets	127 (72)	11 (22)	0.0003
β-Blockers	100 (57)	23 (45)	0.68
Statins	128 (73)	25 (49)	0.14
Calcium channel blockers	35 (20)	6 (12)	0.26
ACEIs and ARBs	97 (55)	22 (43)	0.39
Diuretics	48 (27)	22 (43)	0.13

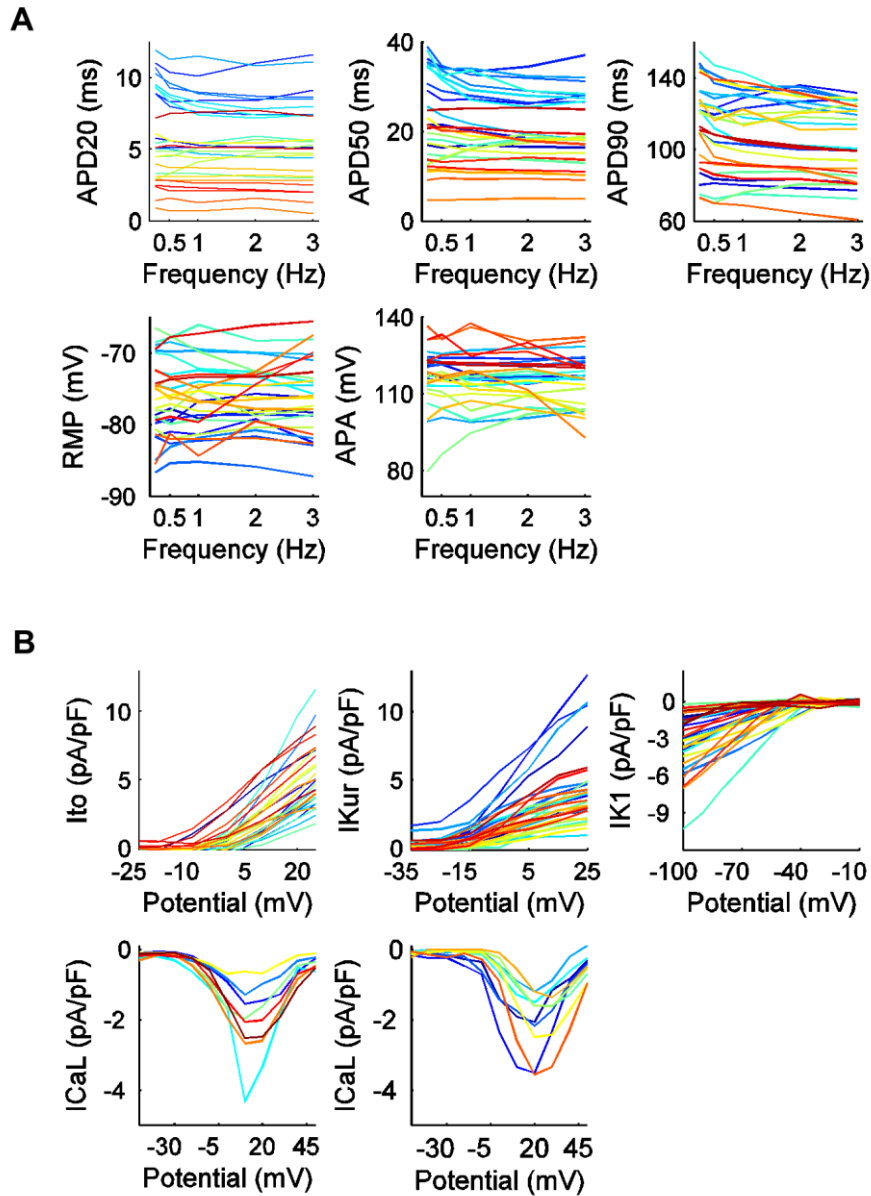
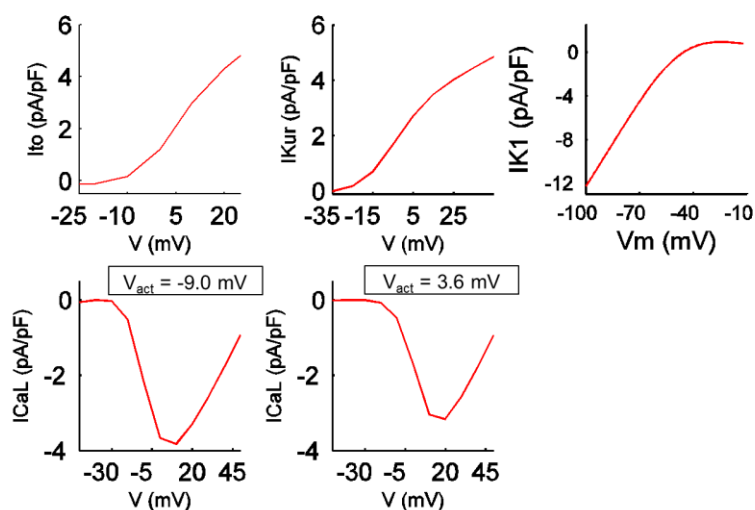
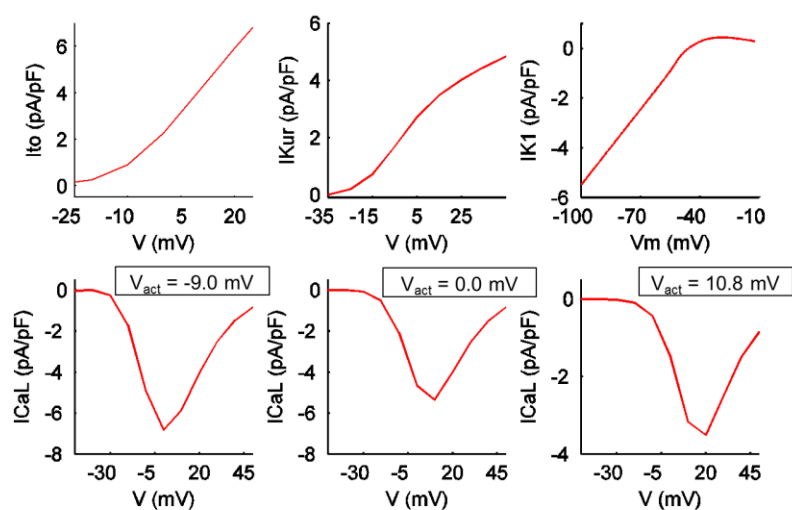


Figure 9.1 Experimental dataset used in the study. Each line represents a measurement from one isolated human atrial myocyte. **A** AP properties as a function of pacing frequency for each of $n=29$ isolated human atrial myocytes *ex vivo*. **B** Voltage clamp curves of I_{to} ($n=30$), I_{Kur} ($n=30$), I_{K1} ($n=36$), I_{CaL} with peak density at 10mV ($n=8$) and at 20 mV ($n=10$).

A - Maleckar



B - Grandi



C - Courtemanche

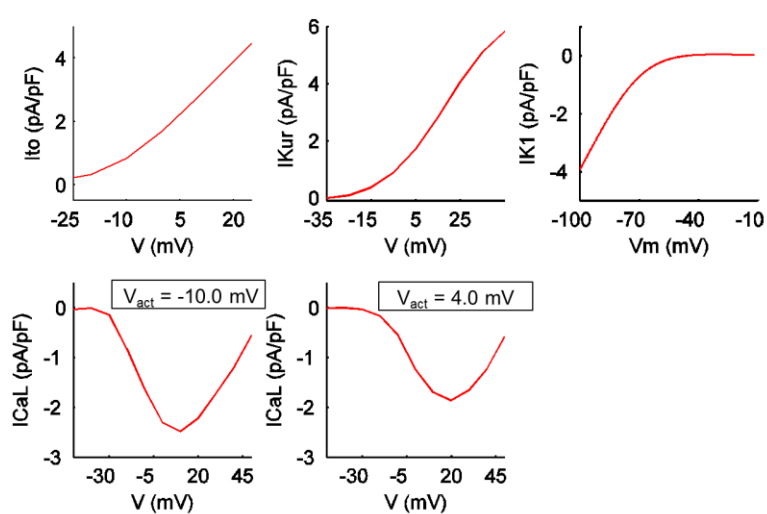


Figure 9.2 Densities of I_{to} , I_{Kur} , I_{K1} and I_{CaL} ionic currents in the baseline Maleckar, Grandi and Courtemanche models. Values of V_{act} are specified for each I_{CaL} plot.

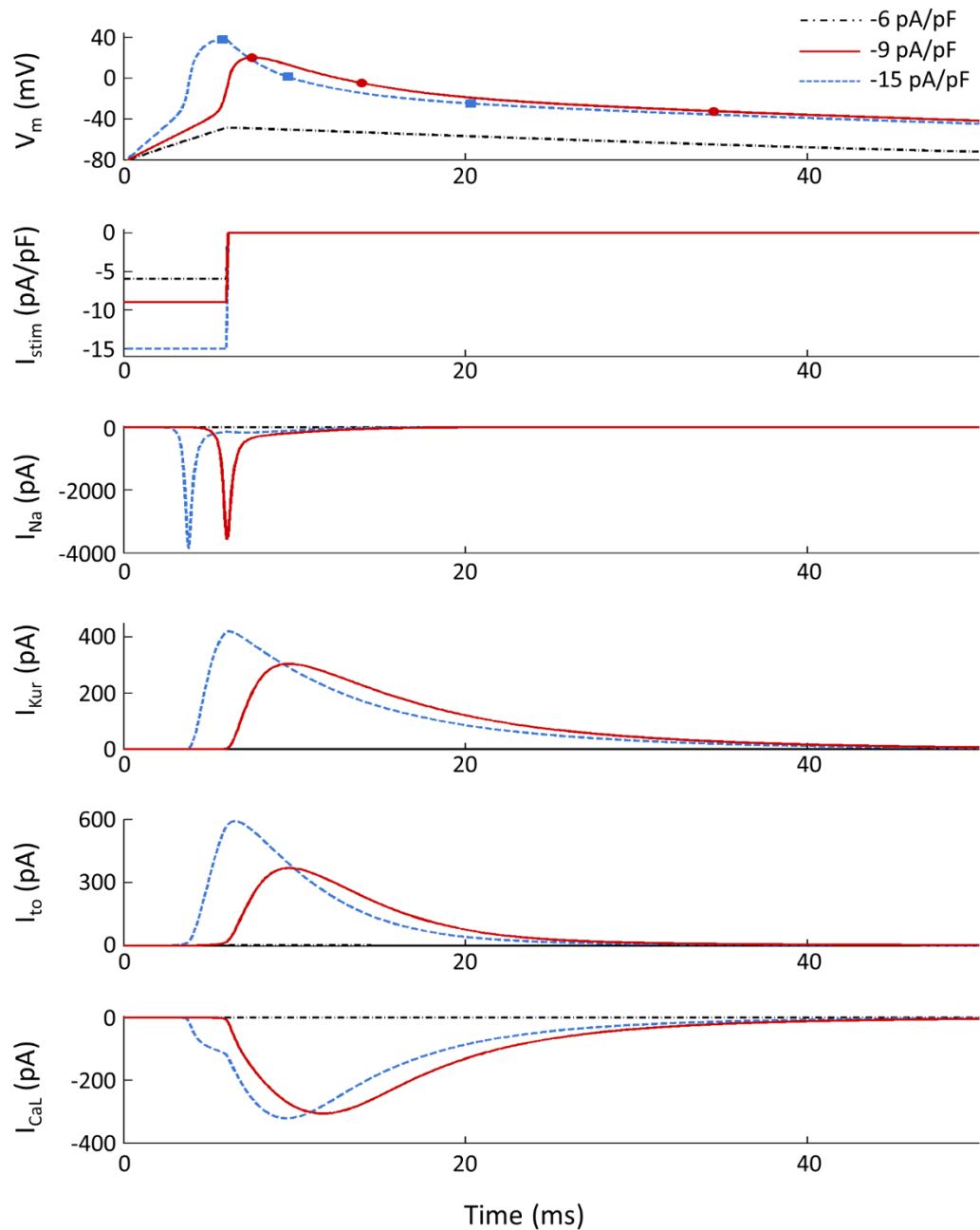


Figure 9.3 Impact of variation in the stimulus current amplitude on the atrial AP as well as on the ionic currents affecting the early repolarization stage in the Maleckar model of human atrial electrophysiology. Three values of the stimulus current amplitude are shown: -6 pA/pF in dashed-dotted black line (no action potential elicited), -9 pA/pF in continuous red line (the scenario with reduced stimulus amplitude as described in Chapter 4), and -15 pA/pF in dashed blue line (the baseline stimulus amplitude as used in the original publication by (Maleckar et al., 2009)); in every case, stimulus duration is kept at 6 ms. For the -15 and -9 pA/pF cases, blue squares and red circles indicate (from left to right) the time point at which the APA, the APD_{20} and the APD_{50} biomarkers are achieved.

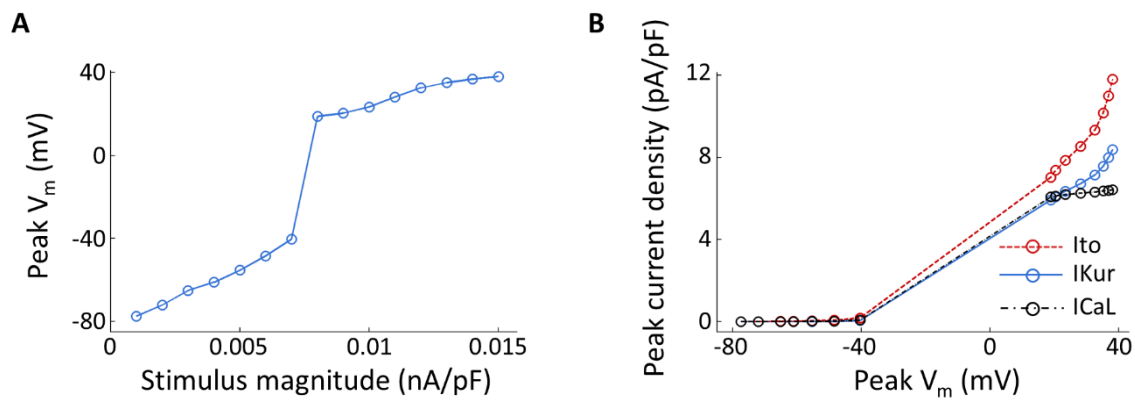


Figure 9.4 Modulation of peak current density of I_{to} , I_{Kur} and I_{CaL} by the stimulus current magnitude mediated via the membrane potential in the Maleckar model. **A** Impact of variation in stimulus current amplitude on the peak transmembrane potential, and **B** Relation between the peak transmembrane potential and peak current density of I_{to} , I_{Kur} and I_{CaL} .

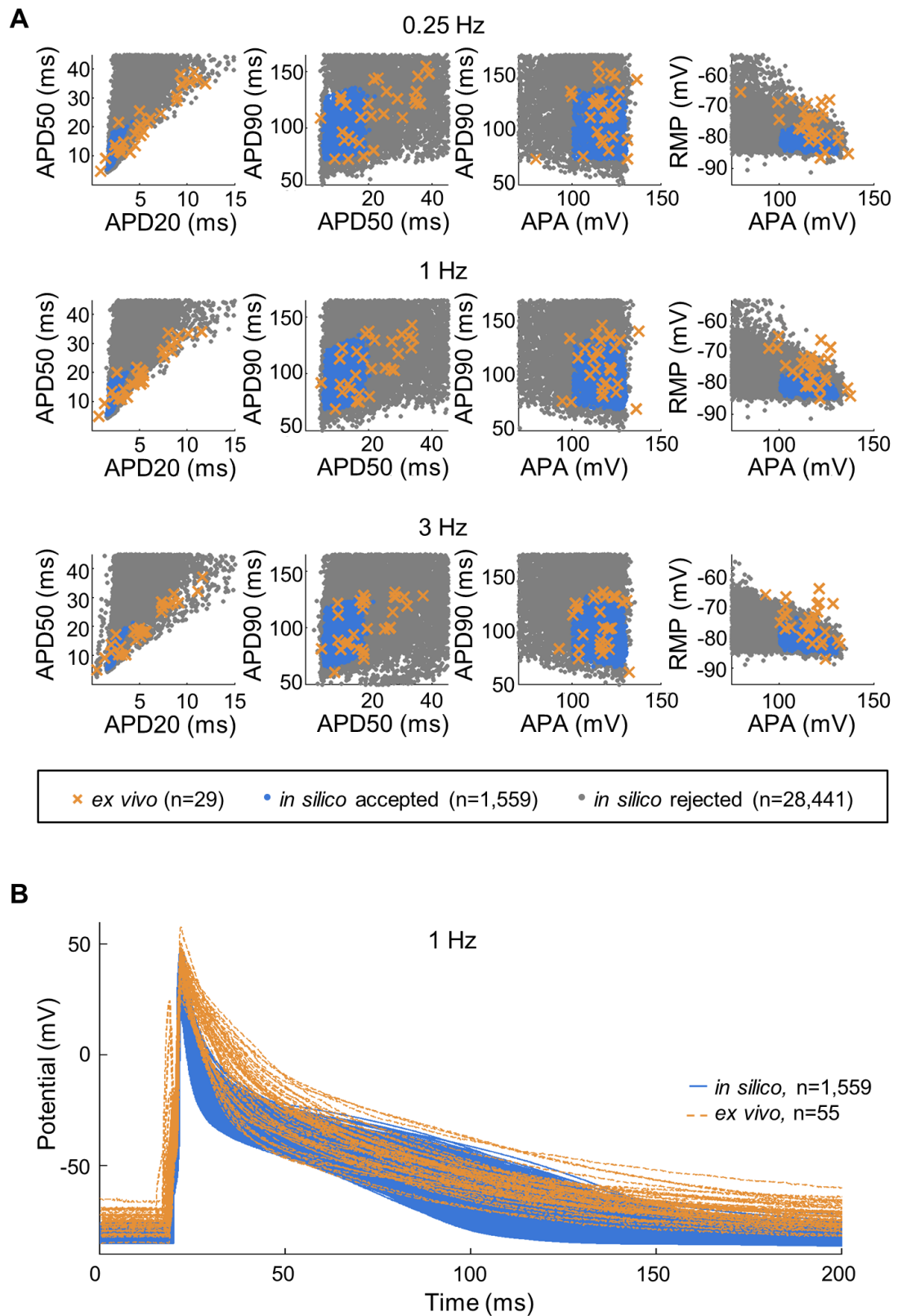


Figure 9.5 Experimentally-calibrated population based on the Courtemanche model, generated with LHS and calibrated with experimental data on AP properties. **A** AP biomarker plots at 0.25, 1, and 3 Hz and **B** AP traces at 1 Hz pacing are shown, with *in silico* accepted models in blue, and experimental data in orange. In **A**, rejected models are shown in grey.

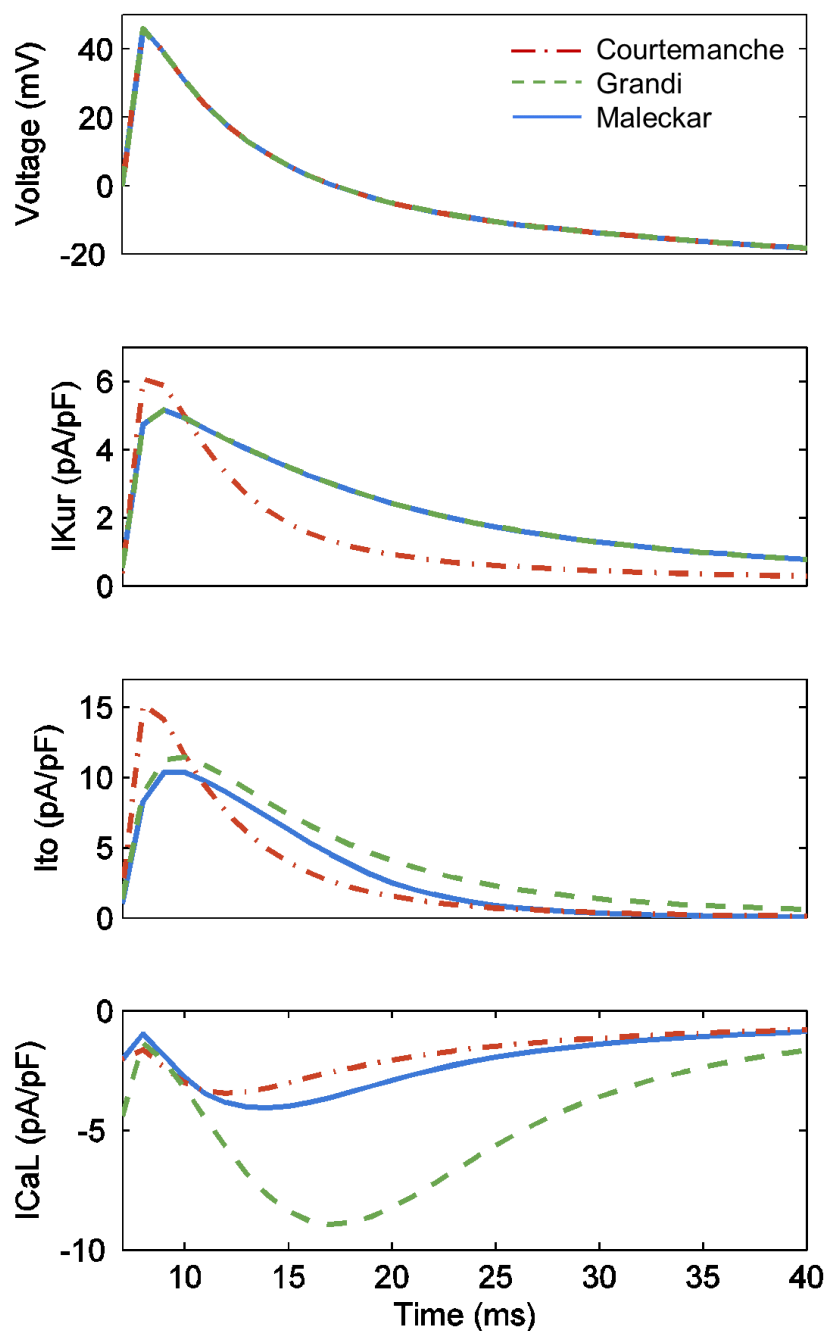


Figure 9.6 Action potential clamp simulation of the three generic models of human atrial electrophysiology, in which the transmembrane potential was forced to follow the triangular morphology of the Maleckar model. Transmembrane potential as well as I_{Kur} , I_{to} and I_{CaL} ionic currents are plotted.

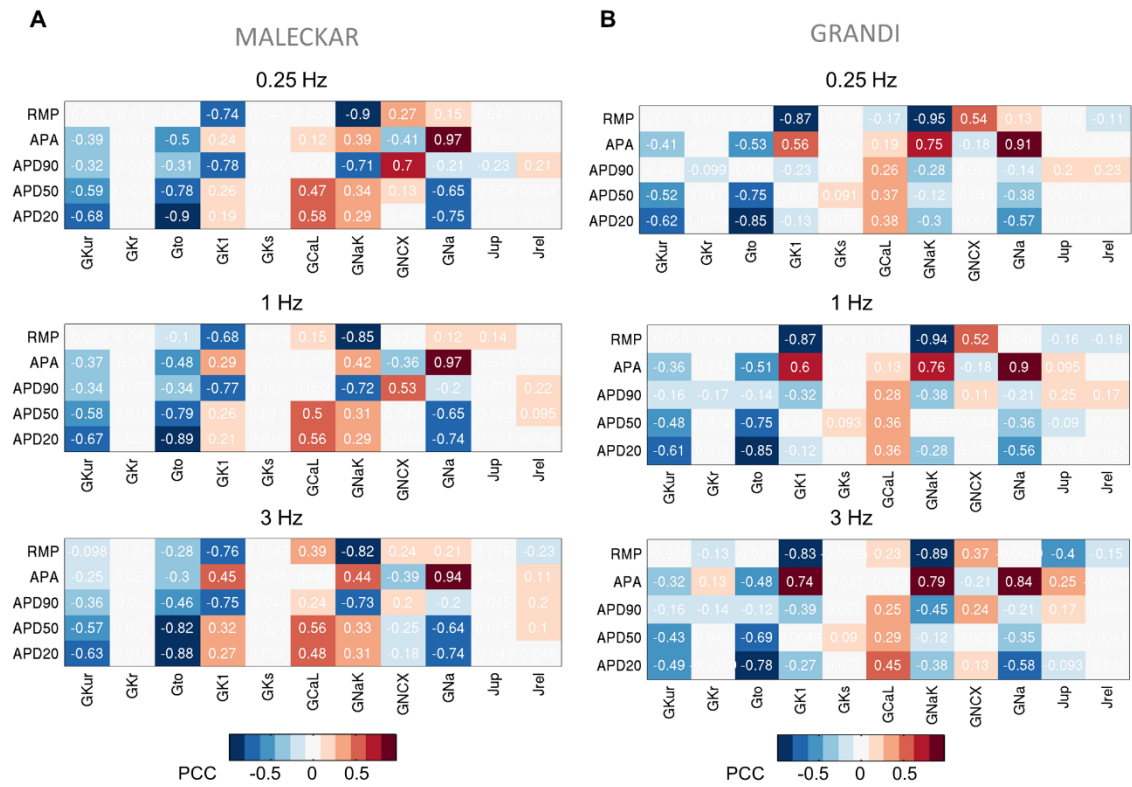


Figure 9.7 Partial correlation coefficients between varied maximal conductances and *in silico* AP properties at 0.25, 1, and 3 Hz in the Maleckar and Grandi-based populations obtained with LHS and calibrated with AP properties. Negative (-) and positive (+) correlation is highlighted by blue and red colour codes, respectively.

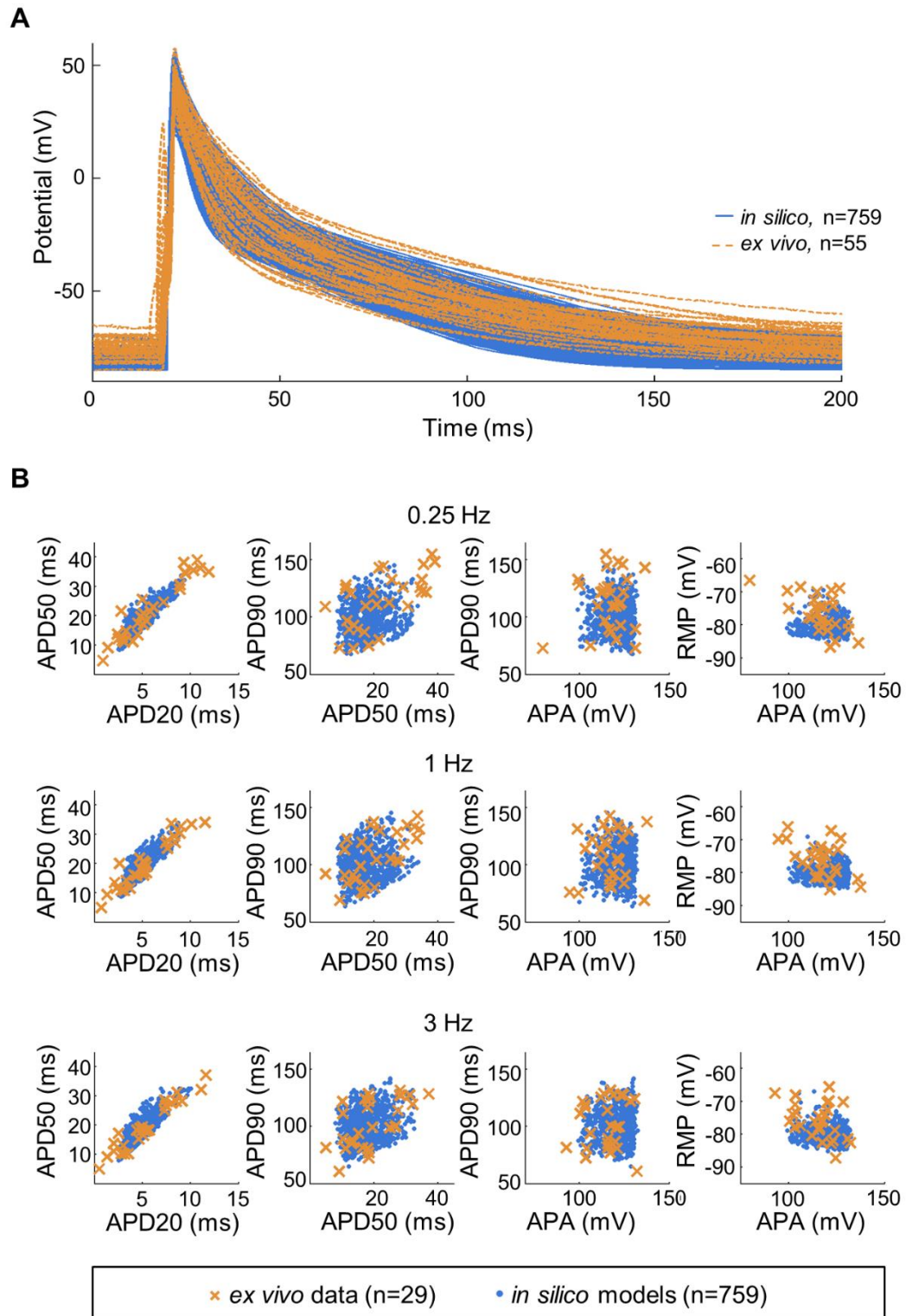


Figure 9.9 Experimentally-calibrated population based on the Maleckar model with reduced stimulus amplitude (-9 pA/pF), generated with SMC and calibrated with experimental data on AP properties. **A** AP traces at 1 Hz pacing and **B** AP biomarker plots at 0.25, 1, and 3 Hz are shown, with *in silico* results in blue and experimental data in orange.

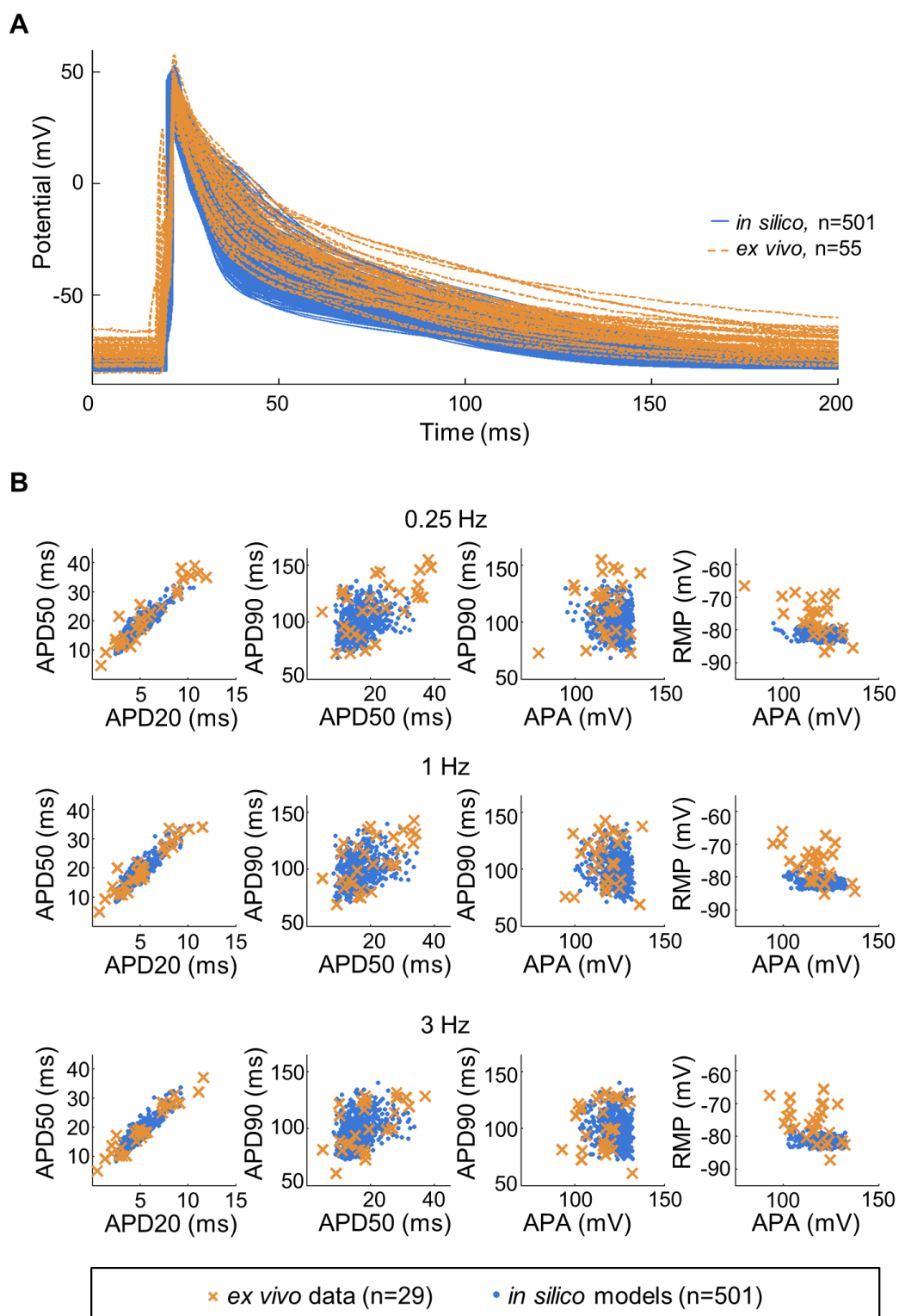


Figure 9.10 Experimentally-calibrated population based on the Grandi model, generated with SMC and calibrated with experimental data on AP properties. **A** AP traces at 1 Hz pacing and **B** AP biomarker plots at 0.25, 1, and 3 Hz are shown, with *in silico* results in blue and experimental data in orange.

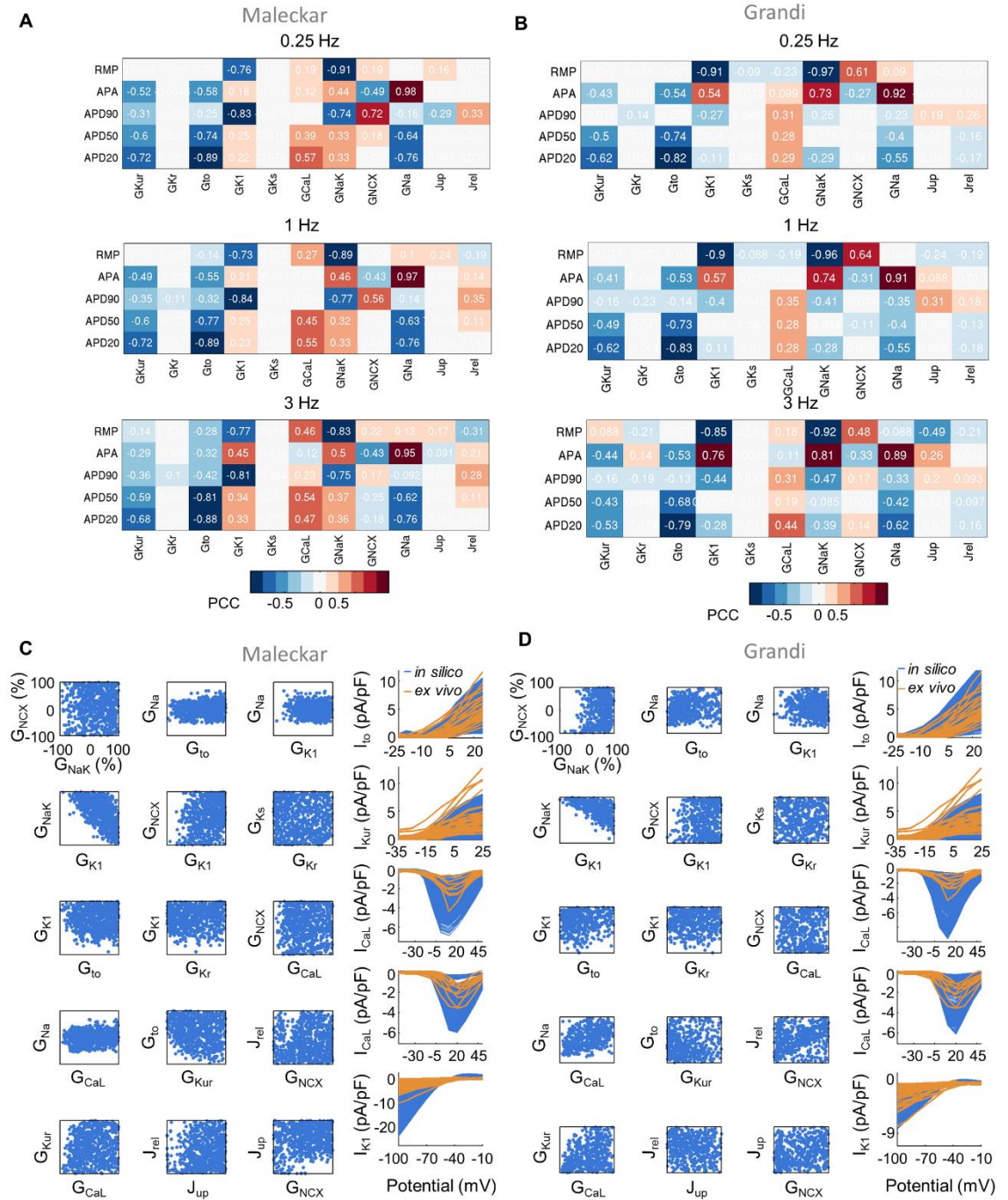


Figure 9.11 Impact of experimental calibration with AP properties on the Malekar- and Grandi-based populations of models obtained with SMC. **A-B** Partial correlation coefficients between varied maximal conductances and *in silico* AP properties at 0.25, 1, and 3 Hz in populations. Negative (-) and positive (+) correlation is highlighted by blue and red colour codes, respectively. **C-D** Selected pairwise plots of maximal current conductances and simulated I-V curves of I_{to}, I_{Kur}, I_{K1}, and I_{CaL} with peaks at 10 and 20 mV in the Malekar- and Grandi- derived populations (blue) compared with experimental results (orange).

Multi-scale Modelling and Simulations into the Mechanisms Linking Neuronal Nitric Oxide Synthase and Atrial Fibrillation

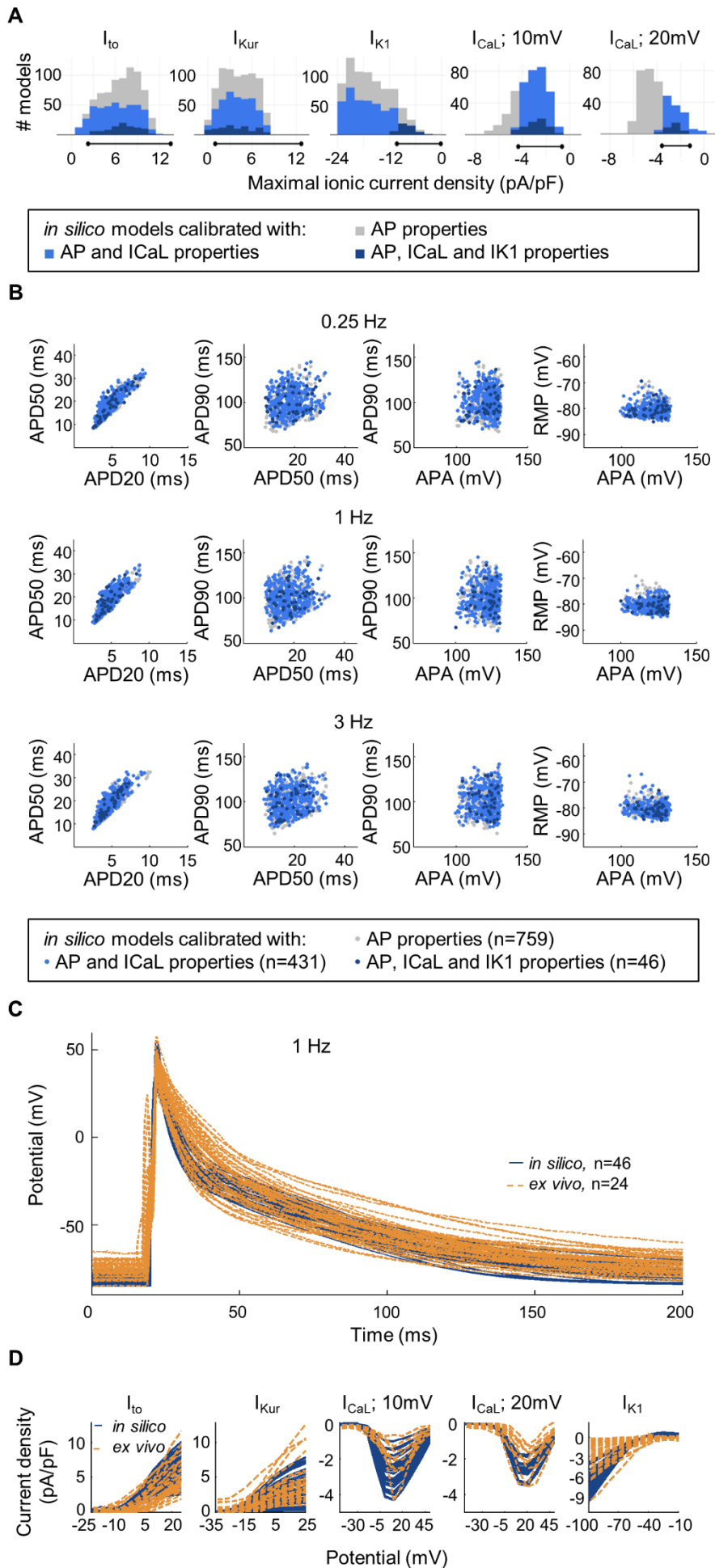


Figure 9.12 Impact of different calibration criteria at the level of maximal I_{CaL} and I_{K1} ionic current densities of the Maleckar-based population derived from SMC. **A** Histograms of maximal ionic current densities, and **B** AP biomarker plots. Grey, light blue and dark blue correspond to calibration with AP criteria alone; calibration with AP and I_{CaL} criteria; as well as calibration with AP, I_{CaL} and I_{K1} criteria. In **A**, experimental ranges are indicated by black bars. **C** Action potential traces and **D** I-V curves produced by experiments (orange) and the *in silico* population of Maleckar models calibrated with AP criteria as well as I_{CaL} and I_{K1} current magnitudes (dark blue).

Multi-scale Modelling and Simulations into the Mechanisms Linking Neuronal Nitric Oxide Synthase and Atrial Fibrillation

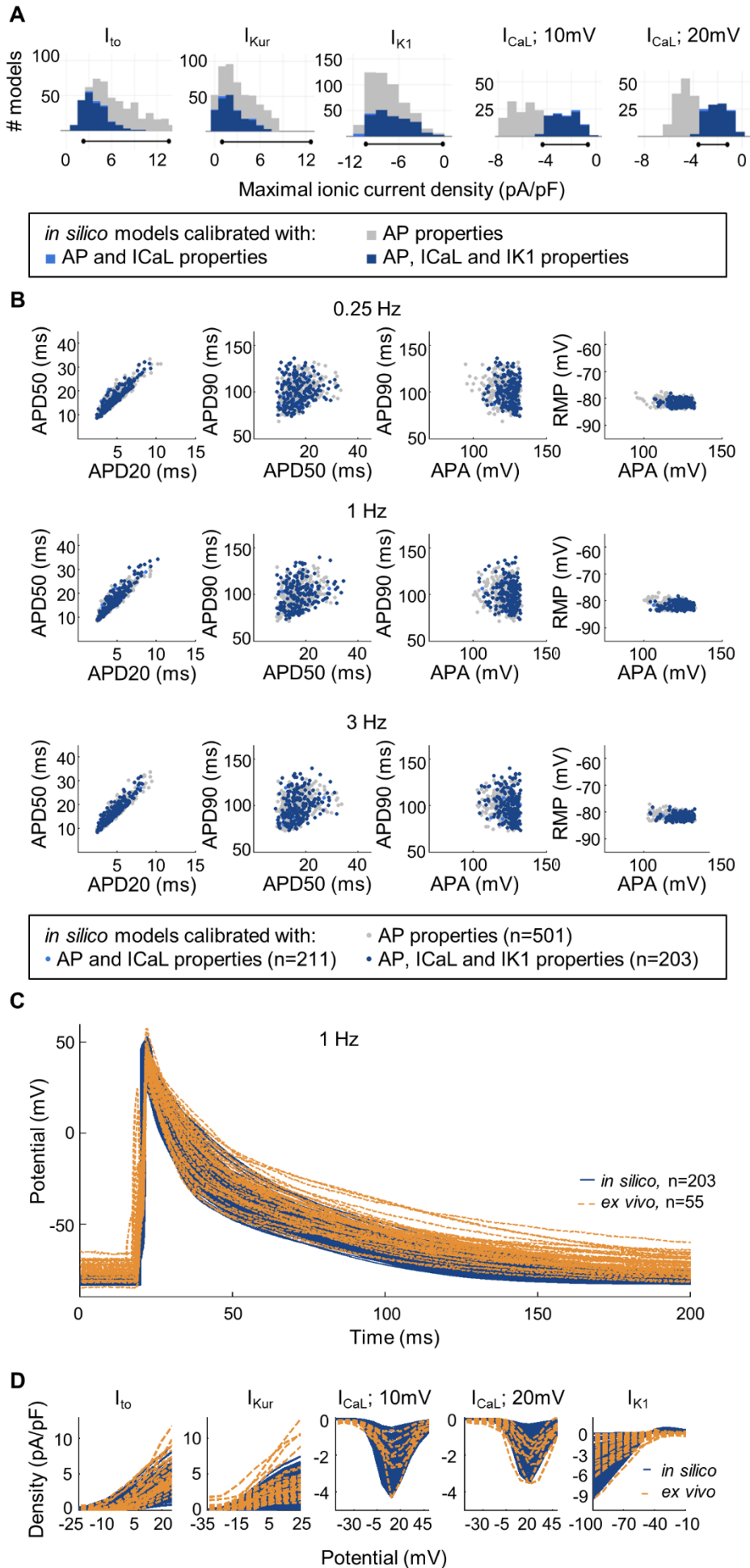


Figure 9.13 Impact of different calibration criteria at the level of maximal I_{CaL} and I_{K1} ionic current densities of the Grandi-based population derived from SMC. **A** Histograms of maximal ionic current densities, and **B** AP biomarker plots. Grey, light blue and dark blue correspond to calibration with AP criteria alone; calibration with AP and I_{CaL} criteria; as well as calibration with AP, I_{CaL} and I_{K1} criteria. In **A**, experimental ranges are indicated by black bars. **C** Action potential traces and **D** I-V curves produced by experiments (orange) and by the *in silico* population of Grandi models calibrated with AP criteria as well as I_{CaL} and I_{K1} current magnitudes (dark blue).

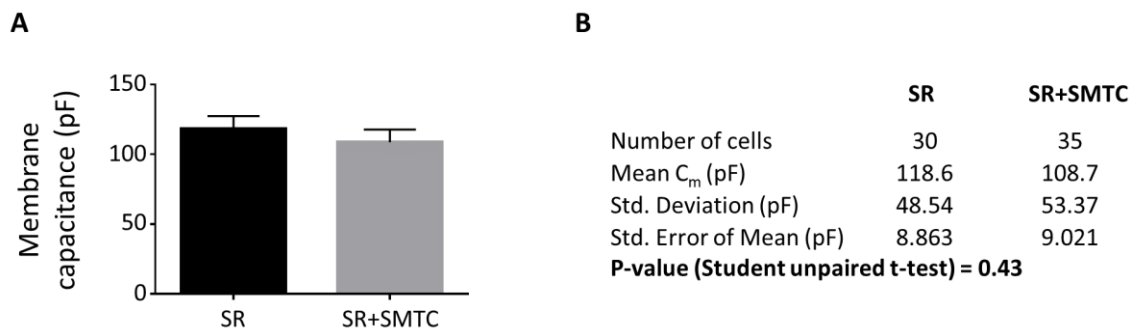


Figure 9.14 **A** Membrane capacitance as measured in experiments in which I_{Kur} was elucidated, in the absence and presence of nNOS inhibitor SMTC. **B** Statistical information, including the number of cells in which the measurements were conducted, the mean membrane capacitance (C_m), standard deviation, the standard error of mean, as well as a statistical significance test between the SR and SR+SMTC groups.

APPENDIX 3 – INVESTIGATING THE ROLE OF nNOS-MEDIATED CHANGES IN IONIC CURRENTS ON HUMAN ATRIAL ACTION POTENTIAL

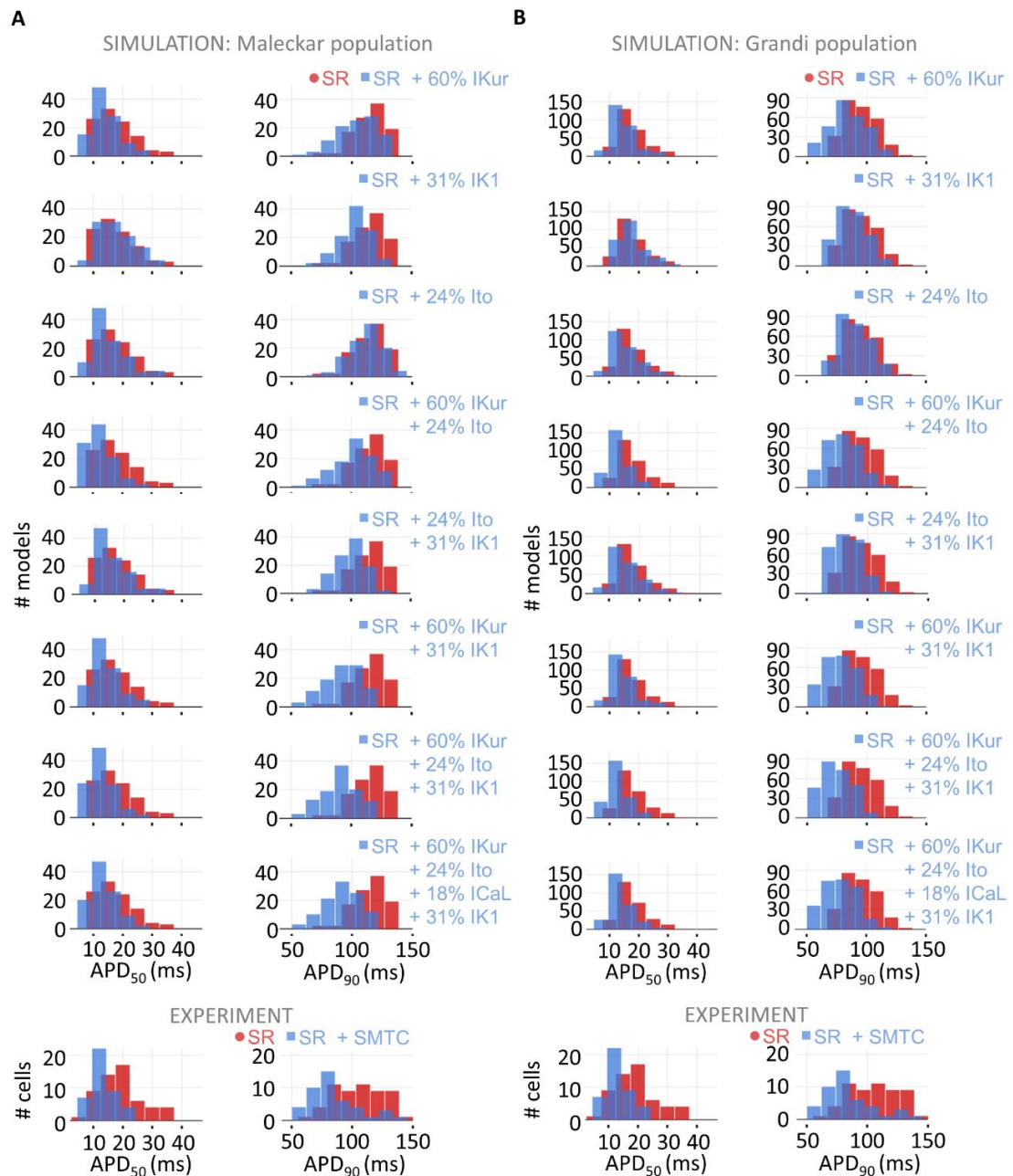


Figure 9.15 *In silico* evaluation of the impact of the increases in ion current density elicited experimentally by nNOS inhibition on the APD₅₀ and APD₉₀ of human atrial myocytes. Experimental data are shown below for comparison. Maleckar- and Grandi-based control populations consisted of n=104 and n=271 models and were generated by experimental calibration at the level of AP and ionic current densities.

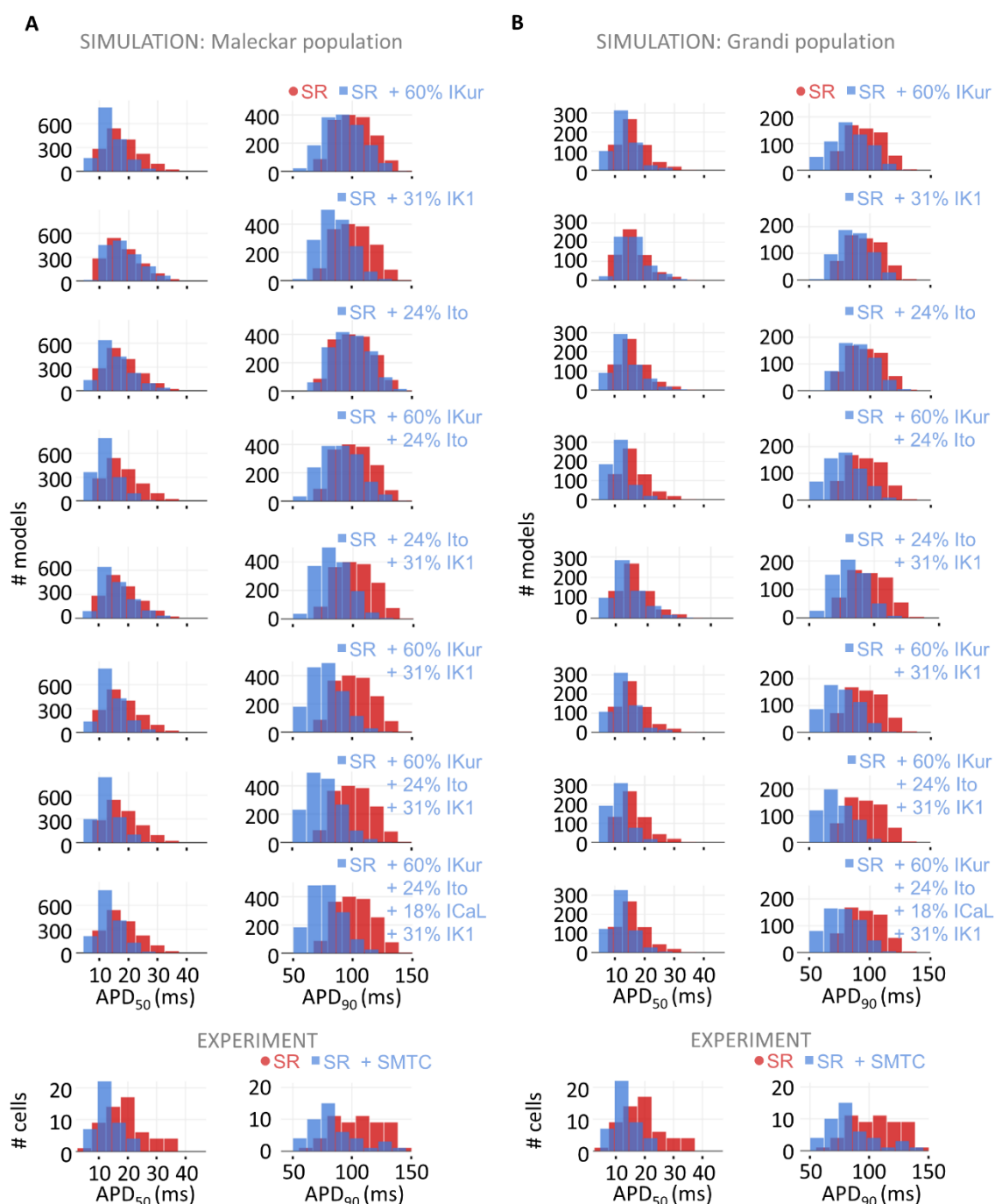


Figure 9.16 *In silico* evaluation of the impact of the increases in ion current density elicited experimentally by nNOS inhibition on the APD₅₀ and APD₉₀ of human atrial myocytes. Experimental data are shown below for comparison. Malekar- and Grandi-based control populations consisted of n=1559 and n=593 models and were generated by experimental calibration at the level of AP only.

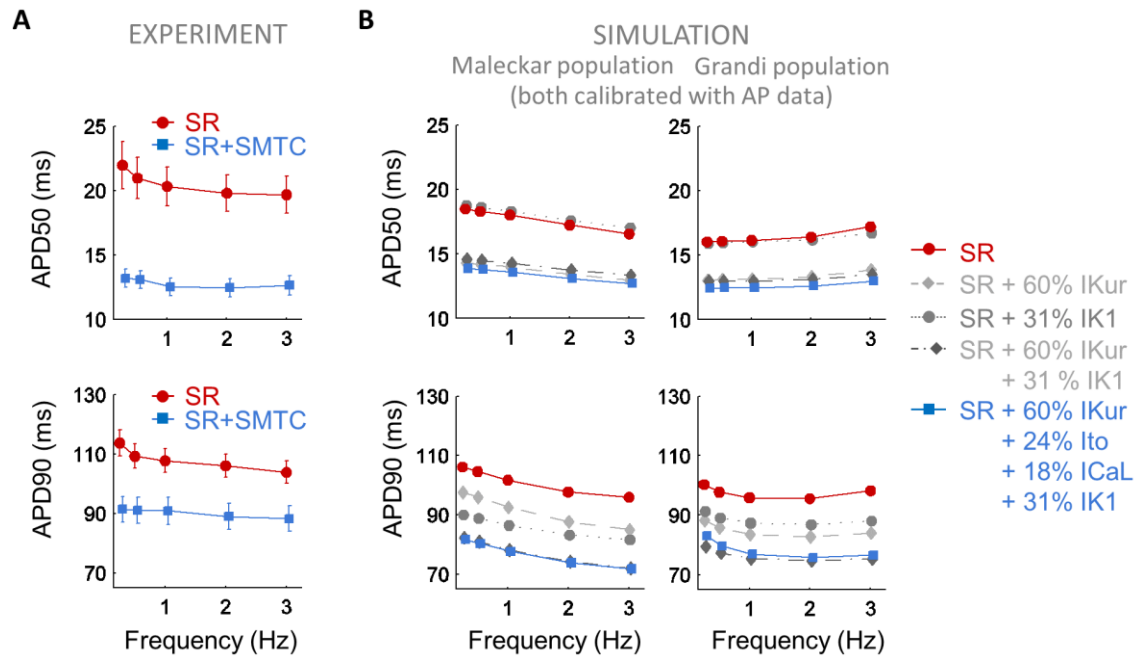


Figure 9.17 Effect of nNOS block with SMTC on the rate dependence of APD₅₀ and APD₉₀ in human right atrial myocytes *in silico*, investigated by incorporating isolated and concurrent ionic current changes elicited experimentally with SMTC to the control Maleckar- and Grandi-based SR populations calibrated with AP data only, with experimental data included for comparison. **A** Experimental recordings from human right atrial myocytes in SR (n=29, N=11) and in the presence of SMTC (n=22, N=9). n/N denotes number of cells/patients, respectively. **B** APD₅₀ and APD₉₀ values produced by the Maleckar- and Grandi-based populations consisting of n=1559 and n=593 models, respectively. The effect of isolated and concurrent ionic current interventions elicited experimentally by SMTC on control populations of models are shown. In **A-B** data are reported as average $\pm$ S.E.M. In **B**, error bars are too small to be visible.

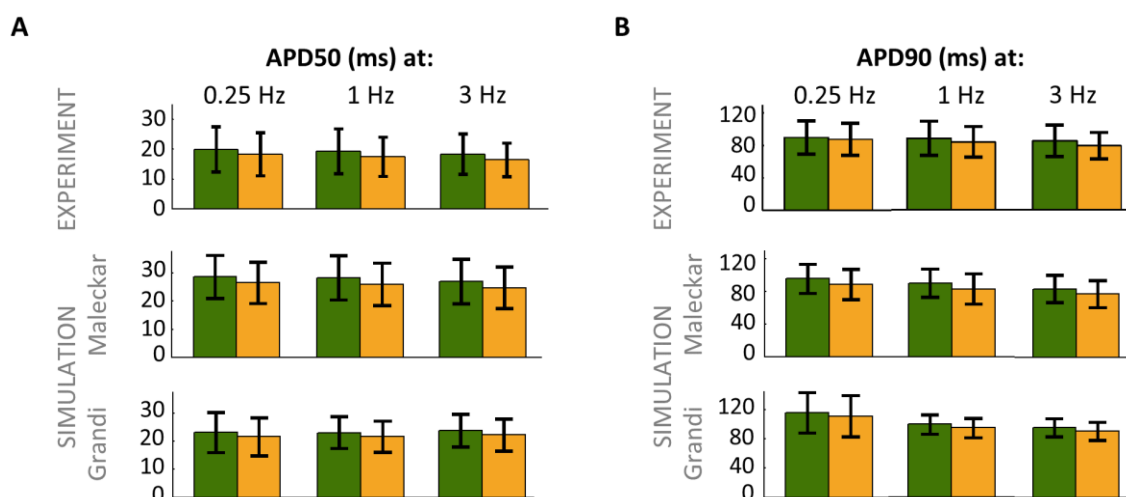


Figure 9.18 Effect of nNOS block with SMTC on human right atrial myocyte models incorporating AF-induced electrical remodelling. Mean APD₅₀ and APD₉₀ at 0.25, 1, and 3 Hz in the Maleckar- and Grandi-based populations of n=1559 and n=593 models incorporating AF-induced electrical remodelling and/or SMTC-induced changes on ionic currents considering reduced levels of nNOS in AF. Experimental results are shown for comparison (n=35 vs. n=19 cells in persistent AF vs. persistent AF+SMTC). Data are reported as mean $\pm$ standard deviation.

APPENDIX 4 – WHOLE ATRIA MECHANISMS OF INDUCIBILITY AND PERSISTENCE OF ATRIAL ARRHYTHMIAS BY DEPLETION OF nNOS IN HUMAN

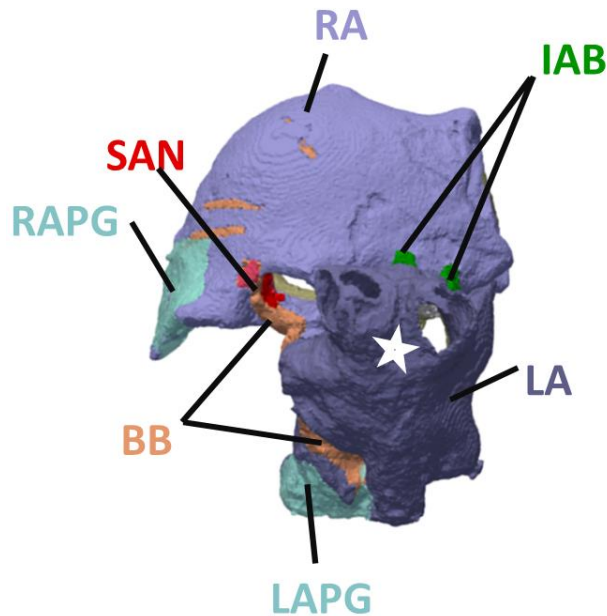


Figure 9.19 Top view of atria with the location of burst pacing indicated with a star. Acronyms: SAN – sino-atrial node, RA – right atrium, IABs – inter-atrial bridges, RAPG – right atrial appendage, BB – Bachmann bundle, LA – left atrial appendage, LA – left atrium.

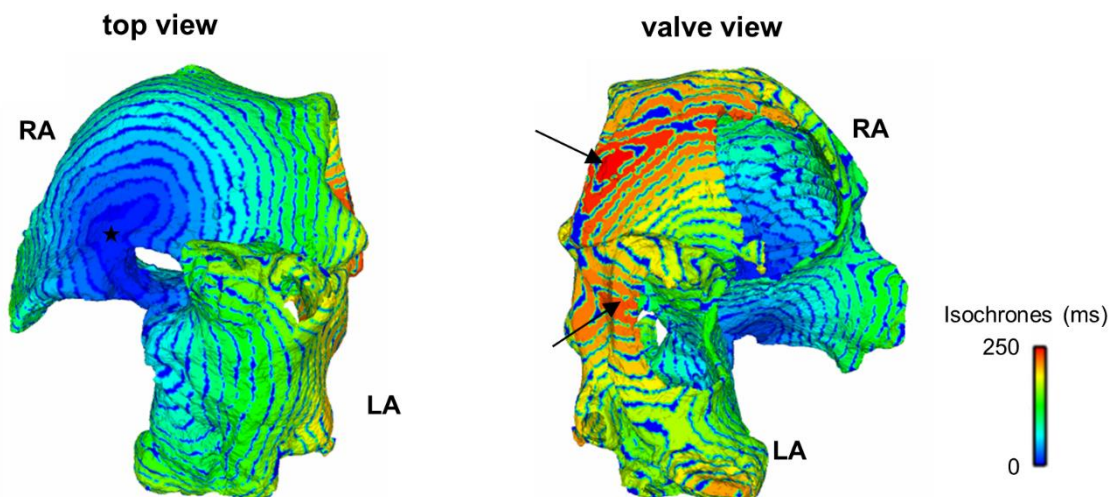


Figure 9.20 Activation time maps in the homogeneous SR whole-atria model following the last pre-conditioning stimulus at the SAN. The black star in top view of atria indicates the location of the SAN; arrows in the valve view indicate the regions of RA and LA that are excited last.

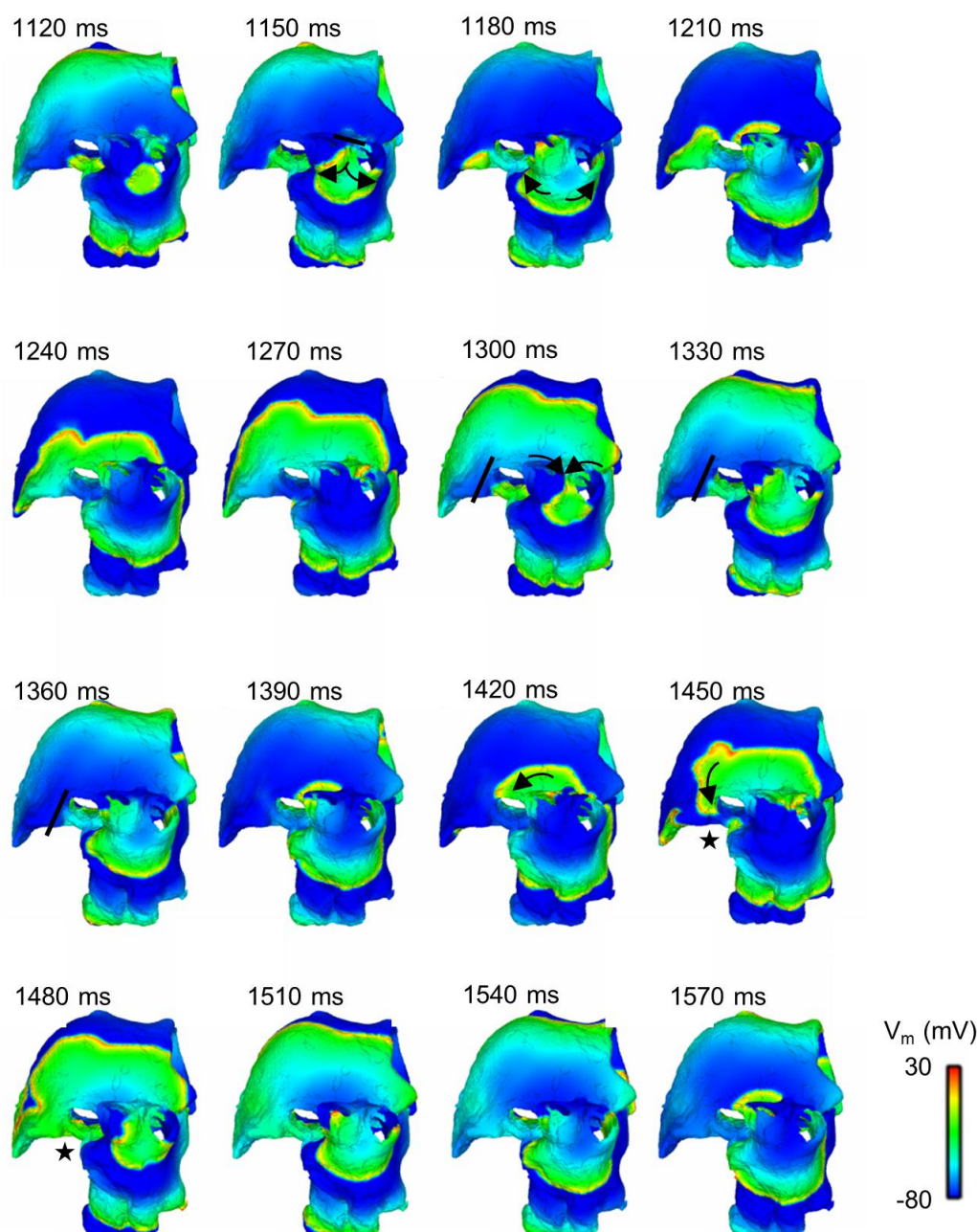


Figure 9.21 Mechanism by which the two figure-of-eight (F8) re-entries form in the homogeneous SR + global SMTc whole-atria model with burst pacing at 165 ms. Top 8 images show that one of the ectopic stimuli applied between the right pulmonary veins (PVs) encounters a uni-directional block, forcing the wavefront to circumvent the PVs and yielding the F8 in LA. This re-entry is reinforced by subsequent ectopics (one instance of this is shown at 1300 ms) prior to cessation of pacing. Bottom 8 images illustrate how the wavefront propagating from LA into RA via Bachmann bundle (BB) encounters a uni-directional block at the superior vena cava (SVC). As a result, two rotors in RA form. The collision of RA wavefronts with the excitation entering RA through the BB sustains RA rotors (black star). Time is measured from the start of the last pre-conditioning stimulus at the sino-atrial node.