

# A New Model of the Human Atrial Myocyte with Variable T-tubule Organization for the Study of Atrial Fibrillation

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## Abstract

*Atrial fibrillation is the most common arrhythmia, yet treatment strategies are sub-optimal due to incomplete understanding of the underlying mechanisms. Spatio-temporal sub-cellular calcium cycling may play a critical role in the development of alternans and spontaneous activity, which may underlie arrhythmia in the human atria.*

*In this study, we construct a novel electrophysiological model of the human atrial myocyte which incorporates new data on atrial intracellular structure and explicitly accounts for variations in T-tubule organization.*

*The model reproduces spatio-temporal calcium dynamics associated with normal cardiac excitation. In preliminary simulations, the model demonstrates that a loss of T-tubules can promote both alternans and spontaneous calcium waves.*

*The model produced in this study provides novel insight into arrhythmia mechanisms in the human atria and provides a platform for future investigation of pro-arrhythmic calcium dynamics.*

## 1. Introduction

Atrial fibrillation (AF) is characterized by an inability of the upper chambers of the heart to undergo a coordinated and regular contraction, underlain by rapid and irregular electrical activity in atrial tissue. AF is common and can have serious consequences, including a substantially increased risk of stroke, heart failure, and mortality [1]. Due to the growing epidemic of AF prevalence and the resulting strain on the healthcare system, the development of improved treatment strategies is vital; greater understanding of the disorder is an important component in achieving this goal.

Dissecting the mechanisms underlying the rapid and irregular electrical activity associated with AF is a major research challenge. Mapping studies suggest that AF excitation patterns are associated with rapid ectopic activity (abnormal pacing from regions of the atria other

than the pacemaking sino-atrial node) and re-entry (self-perpetuating excitation patterns) [2], and modelling studies have indicated a link between these two types of activity [3]. Whereas the mechanisms underlying the generation of re-entry have been studied in recent years, the mechanisms underlying the generation of ectopic activity in human AF are still unclear.

Ectopic activity in tissue results from synchronisation of triggered activity - spontaneously induced electrical action potentials (APs) - in the single cell. Spontaneous intracellular calcium ( $\text{Ca}^{2+}$ ) release events mediated by the ryanodine receptors (RyRs), such as sub-cellular  $\text{Ca}^{2+}$  waves, have been conjectured to play a role in the development of single-cell triggered activity through the activation of the inward sodium-calcium exchanger current ( $I_{\text{NaCa}}$ ). Atrial myocytes have been shown to have variable densities and organization of T-tubules (TTs), and this may play a role in pro-arrhythmic  $\text{Ca}^{2+}$  handling properties such as the development of  $\text{Ca}^{2+}$  waves and alternans.

However, currently available mathematical models of human atrial single cell electrophysiology and  $\text{Ca}^{2+}$  handling do not explicitly account for variable TT density and organisation. In this study, we develop a new model of the human atrial cell which explicitly accounts spatio-temporal stochastic  $\text{Ca}^{2+}$  dynamics and incorporates our own data on atrial TTs and cell size.

## 2. Materials and methods

Atrial tissues were obtained from 16 consenting patients in sinus rhythm undergoing cardiac surgery. Procedures and experiments involving human atrial cells were approved by the West of Scotland Research Ethics Service (REC: 99MC002). Tissues were fixed in 2% paraformaldehyde then cryoprotected overnight in 15, then 30% sucrose solution. Samples were then rapidly frozen in isopentane and kept at  $-80^\circ\text{C}$  until needed.  $10\ \mu\text{m}$  cryosections were then prepared. TTs and surface membrane were stained with Alexa 594 conjugated wheat germ agglutinin and imaged ( $\sim 25$  images/section) using confocal microscopy (voxel size:  $145\ \text{nm X,Y}$  &  $300\ \text{nm Z}$ ). Fluorescence was excited at  $535\ \text{nm}$ , and emitted light

was collected 590-617 nm. Images were deconvolved using custom a Python implementation of the Richardson-Lucy algorithm. 3-D TT density, and distances between cell membrane elements, were measured in ~45 myocytes/patient, using ImageJ software.

A model of 3D spatio-temporal  $\text{Ca}^{2+}$  handling was developed for the human atria, in a similar manner to previous ventricular models [4] – Figure 1. The model comprises of a description membrane ion current electrophysiology coupled to a 3D diffusion model describing intracellular  $\text{Ca}^{2+}$  dynamics. The ionic model is updated from previous models to include lab-specific data on the major currents.

### 3. Results

TT density was examined in cryosections from human atrial tissue samples. TT density and TT  $\frac{1}{2}$  distance were measured as described previously [5]. The data indicate that TT density in human atrial myocytes is highly variable and correlates with cell size; larger cells had a greater expression of TTs; small cells contained very few or no TTs.

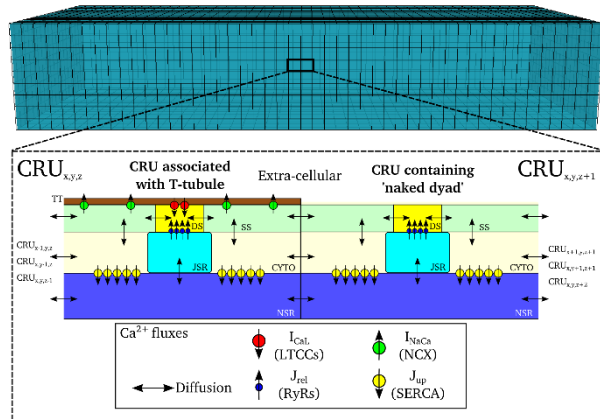


Figure 1 Schematic structure of the spatio-temporal calcium model, comprised of a number of CRUs. Each CRU contains multiple compartments (cytosolic compartments: dyadic cleft space (DS) – the restricted region within which  $\text{Ca}^{2+}$ -induced- $\text{Ca}^{2+}$ -release occurs; sub-space (SS) and bulk cytosolic space (CYTO); SR compartments: junctional SR (jSR) – the portion of the SR which interacts with the DS; the network SR (nSR)). Diffusion between compartments occurs for the SS, CYTO and nSR spaces; the jSR and DS are restricted to each compartment. A schematic for a CRU associated with a T-tubule (left) and a naked dyad (right) is shown: the difference is the presence or absence of membrane  $\text{Ca}^{2+}$  currents,  $I_{\text{CaL}}$  and  $I_{\text{NaCa}}$ .

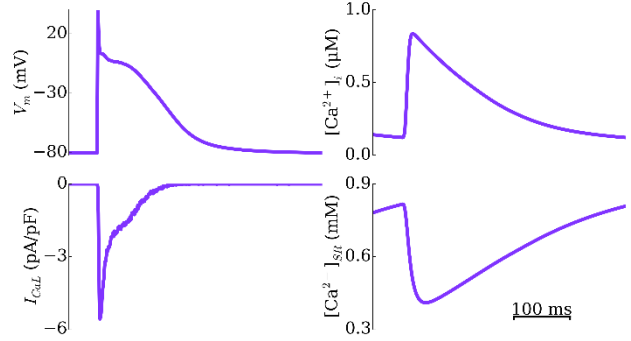


Figure 2 Whole-cell average behaviour of the control model, showing the AP (top left), L-type  $\text{Ca}^{2+}$  current ( $I_{\text{CaL}}$ ) trace (bottom left), cytosolic intracellular  $\text{Ca}^{2+}$  transient (top right) and SR  $\text{Ca}^{2+}$  concentration (bottom right).

In control conditions, the mathematical model reproduces whole-cell characteristics as observed experimentally (Figure 2). Action potential (AP) morphology is similar to those recorded in our lab [6], with average  $\text{Ca}^{2+}$  transients also within experimental ranges.

TT organisation had a significant influence on spatio-temporal calcium cycling (Figure 3). In the large cell with high TT density, an almost uniform rise and decay in  $\text{Ca}^{2+}$  is observed throughout the cell during the  $\text{Ca}^{2+}$  transient. In the small cell with no TTs, propagation of  $\text{Ca}^{2+}$  from the surface sarcolemma to the cell interior is clear. In the intermediate cell with patchy TTs, significant gradients in  $\text{Ca}^{2+}$  are observed in the cytosolic and SR spaces.

The density of TTs inversely correlated with the susceptibility to  $\text{Ca}^{2+}$  alternans (not shown):  $\text{Ca}^{2+}$  diffusion into regions of no TTs is significantly affected at rapid pacing rates, leading to alternating behaviour of success and failure to propagate, underlying  $\text{Ca}^{2+}$  transient alternans.

In preliminary investigations, intracellular  $\text{Ca}^{2+}$  gradients promoted the development of  $\text{Ca}^{2+}$  waves - the site of origination is localised to patches of naked dyads and then proceeds to propagate throughout the cell (Figure 4). The incidence of  $\text{Ca}^{2+}$  waves was increased under an increase in  $I_{\text{CaL}}$  and intracellular uptake ( $I_{\text{up}}$ ) activity, similar to that observed under beta-stimulation. In such conditions,  $\text{Ca}^{2+}$  waves could result in triggered activity in the single cell (Figure 4).

Figure 3 (next page) Spatio-temporal  $\text{Ca}^{2+}$  dynamics during cardiac excitation in cells of three different sizes and T-tubule organisation. Timings are relative to the stimulus, shown for the 3D cell and a 2D slice through the cell.

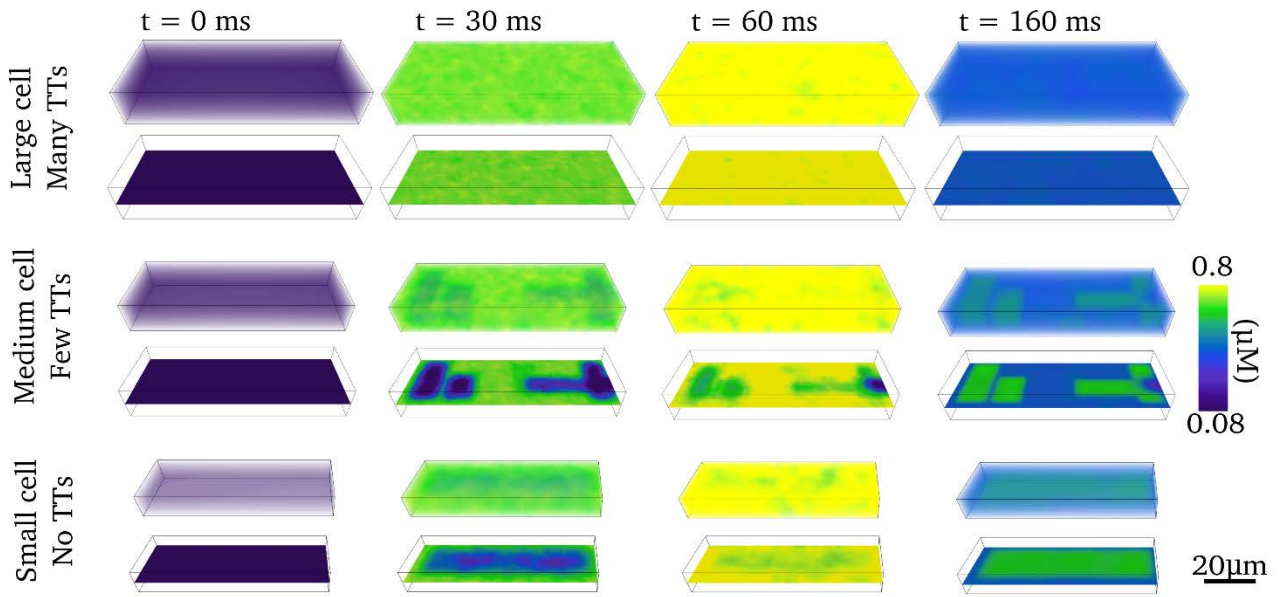


Figure 3

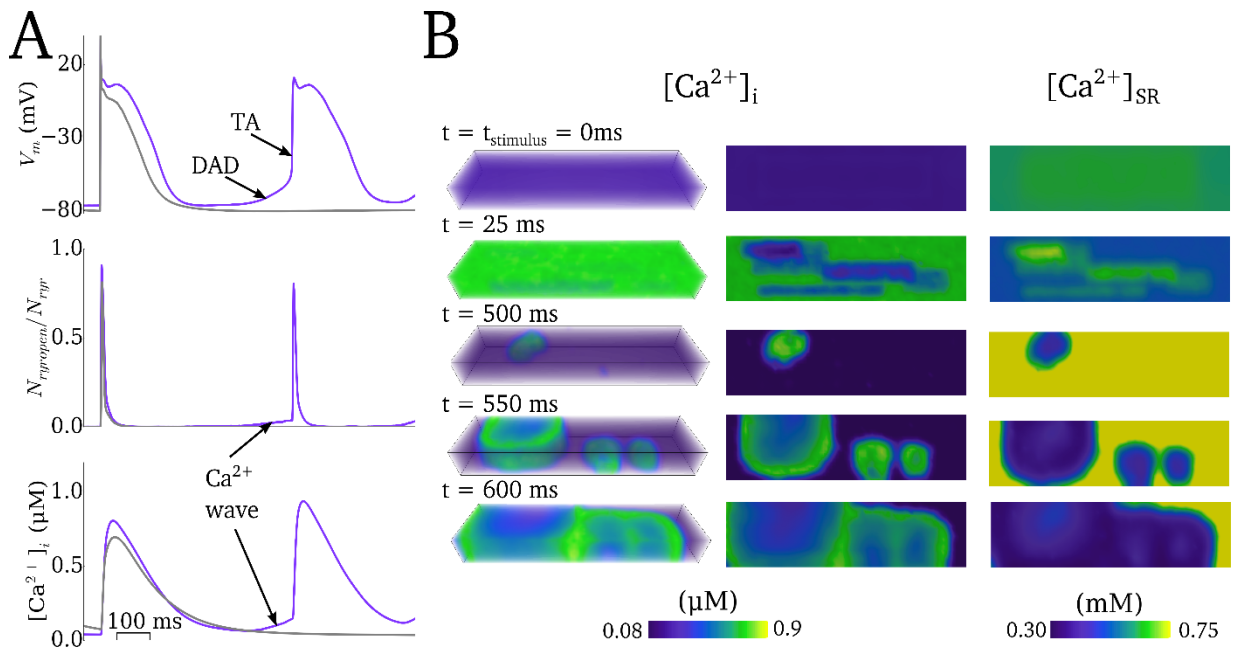


Figure 4

Figure 4 (previous page) Triggered activity in the single cell (A) and spontaneous intracellular  $\text{Ca}^{2+}$  waves (B). In A, APs (top), proportion of open RyR (middle) and  $\text{Ca}^{2+}$  transients (bottom) are shown for a control cell (grey) and a partially detubulated cell (purple) under an increase in  $I_{\text{CaL}}$  and  $I_{\text{up}}$ . The slow increase in open RyR and  $\text{Ca}^{2+}$  transient underlies a delayed after depolarisation (DAD) which crosses the excitation threshold and results in triggered activity (TA) – a spontaneous AP. In B, snapshots of spatio-temporal  $\text{Ca}^{2+}$  dynamics are shown for a partially detubulated cell undergoing a  $\text{Ca}^{2+}$  wave. At 25 ms, the patches of regions with no T-tubules are clear; these regions correspond to the location of origination of  $\text{Ca}^{2+}$  waves observed from 500 ms. Note that the simulation in B is for a cell in which the DAD did not result in TA, therefore showing the entire  $\text{Ca}^{2+}$  wave without the effect of  $I_{\text{CaL}}$  and increased RyR release associated with an initiated AP.

#### 4. Discussion and conclusions

We have developed a novel model of the human atrial single cell which accounts for 3D spatio-temporal intracellular  $\text{Ca}^{2+}$  dynamics and variable T-tubule organisation, informed by experimental analysis of atrial myocytes. Such a model allows for the investigation of the role of sub-cellular  $\text{Ca}^{2+}$  activity on the development of triggered activity in single cell, and its potential role in the development of ectopic activity and degeneration of fibrillatory conduction patterns in tissue.

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