Simulation of a calcium release unit in a cardiomyocyte to study pulsus alternans

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Introduction

Cardiac alternans, characterized by a beat-to-beat alternation in the strength of the heart contraction, is a phenomenon that currently drives a very active body of research due to its link to life-threatening heart conditions such as arrhythmia and fibrillation. At the moment, the mechanism through which alternans arises is not well understood. It is known to be associated by an alternation in the levels of intracellular calcium, although it has also found to be related to an instability in the electrical propagation through the tissue; these two mechanisms have been observed both separately and together in several experimental setups. In particular, the role of RyR in alternans is a current topic of debate.

In cardiac cells, the contraction is driven by the release of Ca$^{2+}$ ions from the sarcoplasmic reticulum. This happens across the cell in thousands of calcium release units, which are sets of coupled protein clusters that release calcium in response to a signal in the form of an action potential, through a positive-feedback mechanism known as calcium-induced calcium release.

The objective of this work is to implement a stochastic model of a calcium release unit to compute how the probability of a calcium spark to occur depends on a variety of biophysical parameters related with the dynamics of intracellular calcium and the stochastic configuration transitions of the protein channels. Then, the obtained probability functions are used in a simplified coupled return maps model of a cardiac cell to explore the parameter ranges that allow the appearance of calcium alternans. The fact that the homeostasis is simplified in this model allows for the study of the alternans as a consequence mainly of the behavior of local units.

Physiology of the heart: Action potential and ion transients

The cardiac tissue is a kind of striated (meaning it’s made of functional units called sarcomeres) muscular tissue; it is exclusive to the heart. Most (99%) cardiac muscle cells are cardiomyocytes, or cardiac myocytes. As any other muscle cells, they contract in response to an action potential, which is a temporary change of the electrical voltage at the cell membrane. Through periodic and coordinated contractions, the cardiac muscle delivers blood to the rest of the body. The other 1% of the tissue corresponds to the pacemaker cells, which are specialized cardiomyocytes and are responsible for the initiation and propagation of the cardiac action potential. In particular, the sinoatrial node consists in a grouping of pacemaker cells that in normal conditions is responsible for the initiation of the action potential that then propagates throughout the cell. The pacemaker cells are connected to neighboring cardiomyocytes via gap junctions, which enable them to locally depolarize adjacent cells.

The resting potential of a cardiomyocyte is about $-80 \text{ mV}$. When stimulated by an action potential, voltage-gated channels on the cell membrane (which is called sarcolemma in the case of striated muscle cells) open, allowing the rapid influx of positively charged sodium ions that cause the membrane to depolarize. This is a positive-feedback mechanism that lasts 3-5 ms; at that point the membrane potential reaches about $(+30 \text{ mV})$ and the ion channels close. This is followed by a period in which the membrane potential declines relatively slowly. The long duration of this phase is largely due to the opening of a kind of voltage-gated calcium channels called L-type Calcium Channels, or LCC for short, which allows for the influx of Ca$^{2+}$ ions that sustains the depolarization while potassium K$^+$ ions exit the cell. This plateau phase lasts about 175 ms. Then, once the membrane potential reaches about 0 mV, the Ca$^{2+}$ channels close and all the K$^+$ channels open, allowing K$^+$ ions to exit and causing the membrane potential to drop to the initial resting level. This repolarization phase lasts about 75 ms, and at this point the cycle can start again. The entire event, lasting between 250 and 300 ms, is shown schematically in Figure 1.
Figure 1: Transmembrane potential during a full contraction event.

The depolarization period for a cardiac myocyte is significantly longer than that of a regular (skeletal) muscle cell. As stated before, this is due to the influx of calcium ions through the LCC. Furthermore, when the LCC open, the influx of Ca$^{2+}$ from outside the membrane triggers the release of additional Ca$^{2+}$ stored on the sarcoplasmic reticulum, or SR (the sarcoplasmic reticulum is smooth endoplasmic reticulum found in myocytes). It does so by binding to, and activating, a protein in the SR called Ryanodine Receptor 2, RyR2 for short, it belongs to the class of intracellular calcium channels that are the ryanodine receptors; they owe their name to the alkaloid ryanodine, with which they have a very high affinity. The result, therefore, is an overall significant increase in Ca$^{2+}$ in the region next to the SR within a short lapse of time, which is referred to as a calcium spark. This auto-catalytic mechanism of calcium release from the SR is referred to as calcium-induced calcium release (CICR) [37].

The Ca$^{2+}$ ions released during the calcium spark then diffuse into the bulk cytoplasm. Thousands of calcium sparks occur almost synchronously across the cell, causing an increase of the Ca$^{2+}$ concentration; the Ca$^{2+}$ ions bind then to the regulating protein troponin, allowing the binding of myosin and actin and causing the cell to contract. Finally, the sarco/endoplasmic reticulum calcium-ATPase (SERCA) actively pumps back the calcium ions into the lumen of the SR, while the sodium-calcium exchanger transports the rest of the ions to the extracellular medium, decreasing the Ca$^{2+}$ concentration and causing the cell to relax.

In cardiomyocytes, about 80% of the calcium ions required for contraction are supplied by the SR (through CICR), with the other 20% coming from the influx through the membrane during the plateau phase. In normal conditions, the Ca$^{2+}$ concentration within the SR is of the order of 1 mM, whereas the basal level in the cytoplasm is in the nM range, reaching the µM range during the calcium transient.

The sudden increase of cytosolic calcium that causes the cell contraction, and the subsequent decrease in the concentration, is referred to as a calcium transient.

**Alternans**

*Pulsus alternans,* or mechanical alternans, is a condition in which there is a beat-to-beat oscillation in the strength of cardiac muscle contraction at a constant heart rate. It has been the object of extensive study since its discovery in the 19th century[1, 2, 3]. This interest is due to the phenomenon being related with heart disease; in particular, T-wave alternans, the resulting beat-to-beat alternation in the shape of the T wave in an electrocardiogram (ECG), is associated with an increase in the risk of arrhythmia and tachycardia[4, 5].
It is known that it is possible to induce alternans by pacing human or other mammalian heart tissue by a rapid enough periodic signal. Experiments consistently show the existence of a critical (threshold) pace beyond which sustained alternans is induced; this threshold cycle length varies among mammalian species and depends on several factors\cite{6,7,8,9}. Driving paces close to but above the critical cycle length may produce transient alternans\cite{6,8}. Transient alternans can also be observed after a single premature systole\cite{10,11}.

Since its description, two main mechanisms have been suggested to explain alternans. The first, based on the Frank-Starling law, explains alternans in terms of the heart’s stroke volume being related with the blood volume just before the beat (the end-diastolic volume): in essence, a reduced end-diastolic volume will cause a reduced force in the contraction during the next systole, which leads to a reduced end-systolic volume, which in turn causes a greater end-diastolic and thus more contraction in the next beat. This process is consistent with the effect of higher high rates causing the onset of alternans, since it would reduce the time available for diastolic filling. Experimental studies have indeed observed alternation of left ventricular end-diastolic volume in both intact and isolated working hearts\cite{12,13,14,15,16}.

The second explanation for alternans implies a beat-to-beat alternation of myocardial contractility. This is supported by experimental evidence of alternans in isolated ventricular myocytes\cite{17,18} as well as in isolated hearts with ventricular volume kept constant\cite{19,20}. Given the variety of the conditions in which alternans is observed, it is possible that, in an intact heart, both the Frank-Starling mechanism and contractility play a role in its onset.

**Intracellular calcium dynamics**

Direct measurements of the intracellular Ca$^{2+}$ in both isolated cardiac muscle preparations and isolated perfused heart have shown an alternation of the Ca$^{2+}$ transient in phase with the alternation of the strength of the contraction\cite{21,17,22,19,20} (Fig. 3). Presumably, the alternation of of the Ca$^{2+}$ transient reflects an alternation of the release of Ca$^{2+}$ from the SR (an indication for this comes from the fact that ryanodine, which specifically inactivates the RyR release channels, consistently abolishes alternans\cite{21,22,18,23,24}). Typically, an alternation of the action potential duration is observed in addition to the alternation in the Ca$^{2+}$ transient (this alternation has been observed in isolated multicellular preparations or single myocytes as well as through recordings of the action potential from the surface of intact hearts)\cite{8,25,26,17,27,23,24,28}. However, experimental studies have shown that calcium alternans can be observed without electrical alternans in isolated myocytes clamped with voltage pulses of constant duration\cite{17}. 
Figure 3: a) A cardiomyocyte. Calcium levels can be tracked using markers, in this case fluo-4 calcium indicator. b) Space-averaged calcium levels showing normal calcium transients. c) In this case, calcium alternans is clearly visible; the peaks of the calcium transients show an evident alternation between a high and a low level. (Source: Leif Hove-Madsen, CSIC-ICCC)

Alternans has been explained as a consequence of a period doubling bifurcation in the dynamics of Ca$^{2+}$ cycling in deterministic models of the cellular calcium concentration[29]. Essentially, a period doubling bifurcation arises when a system that presents a strongly non-linear response is driven at a pace shorter than its characteristic recovery time scale. In this situation, the system lacks the time necessary to recover some variable to its initial value, and this difference between them is amplified due to the mentioned non-linear response. Therefore, an alternating behavior is favored in which the system undergoes a sequence of small recovery - small response - large recovery - large response.

More recently, the space and time dynamics of cellular Ca$^{2+}$ have been studied, showing onset of calcium alternans as a consequence of rapid pacing[30]. This suggests that nonlinearities in the underlying Ca$^{2+}$ kinetics might cause calcium alternans.

However, these studies did not take into account the intrinsically stochastic nature of the ions at the channel level. Other studies that developed computational models of intracellular Ca$^{2+}$ and that did take the inherent stochasticity into account show that calcium alternans can emerge from the collective behavior of interacting stochastic units[31], in particular as an order-disorder phase transition[32].

Furthermore, the role of RyR in the onset of alternans has been studied in theoretical studies, but not in stochastic models. The relative roles of the SR load and the RyR are still a matter of debate[36].
Calcium release unit model

Introduction

A cardiomyocyte regulates its calcium concentration through thousands of similar domains, referred to as calcium release units. A calcium release unit (CaRU, or also CRU) comprises a region of the sarcolemma of about 1 μM that includes a number of voltage-gated Ca\(^{2+}\) channels (LCC), as well as a nearby cluster of ryanodine receptor (RyR) channels in the surface of a sarcoplasmic reticulum (SR). Experiments suggest that every CaRU consists of 1-10 LCC and around 50-100 RyR channels in the RyR channel cluster.

As explained, upon excitation by an action potential, the opening of the LCC can cause a calcium spark due to the auto-catalytic nature of the release of Ca\(^{2+}\) sequestered in the SR through the RyR.

![Diagram of calcium release units](image)

Figure 4: Left: Schematic showing a few calcium release units and their distribution by the cell membrane (gray); a single CaRU is marked in red. Right: A zoomed-in diagram of a single CaRU showing the different compartments that will be considered (the dyadic space and the junctional SR).

Given the low number of protein channels in each CaRU, the response response of the unit is intrinsically stochastic and has to be studied in probabilistic terms: under the same conditions (i.e. for the same model parameters), a CaRU can either present a Ca\(^{2+}\) spark or fail to do so. The goal, then, of constructing this model of a CaRU is to compute the probability of a Ca\(^{2+}\) spark to occur as a function of variables such as the initial configuration of the RyR, the Ca\(^{2+}\) concentration (or load) in the SR and various dynamical parameters of the ions and the ion channels, such as the diffusion rates of the ions and the properties of the RyR.

Homeostasis

In the absence of sparking events, a calcium release unit is characterized by steady-state levels of calcium in the SR and in the cytoplasm. This particular values are determined by a complex interaction between the SERCA, the Na-Ca exchanger (NCX), the RyR and the LCC. In particular, the cytosol level depends on a balance between the LCC and the NCX, but the properties of the NCX itself depend on the cytosol level, and that is also influenced by the RyR and the SERCA, since they determine the calcium transient and the SR uptake.

If we were to develop a complete model of a cardiomyocyte, these mechanisms would be taken into account and this steady state would be eventually reached. In this study, instead, these calcium levels are externally fixed, and it is assumed that the cell reaches them through the same mechanisms. This strict control on the homeostasis calcium levels is very hard to implement in experiments, but can be...
done in simulations and it can give insight into the appearance of alternans as a function of the SR load; assuming that in each case the homeostatic balance were to yield that particular SR load.

**Description of the model**

The dynamical model of a CaRU next described is based in previously published models[32, 29, 33, 34]. This model defines two dynamical variables, corresponding to the Ca\(^{2+}\) concentrations two distinct regions, or *compartments*, of the CaRU: the Ca\(^{2+}\) concentration in the *dyadic space* or *dyadic junction* (the narrow region between the sarcolemma and the SR, i.e. between the LCC and the RyR channels), \(c_d\), and the Ca\(^{2+}\) concentration within the *junctional SR* (the volume within the SR closest to the RyR channels), \(c_{jSR}\). The two concentrations are then coupled by a number of fluxes, some of which represent the diffusion between the different compartments and some of which take into account the stochastic state transitions of the ion channels. These stochastic transitions are modeled via Markov schemes.

The time evolution of the different concentrations within the CaRU is, thus, described as follows:

\[
\frac{dc_d}{dt} = -J_{CaL} + J_{rel} - J_{ds} \quad (1)
\]

\[
\frac{dc_{jSR}}{dt} = J_{sr} - \frac{v_d}{v_{jSR}} J_{rel} \quad (2)
\]

where \(J_{ds}\) and \(J_{sr}\) correspond to diffusive currents within the different compartments, \(J_{CaL}\) accounts for the (inward) calcium current from the extracellular medium into the dyadic space through the LCC, and \(J_{rel}\) represents the release of calcium ions from the junctional SR into the dyadic space.

**Diffusive currents**

Diffusion within compartments of the CaRU is assumed to be proportional to the corresponding concentration difference:

\[
J_{ds} = \frac{1}{\tau_d} (c_d - c_{d,0}) \quad , \quad J_{sr} = \frac{1}{\tau_{sr}} (c_{SR} - c_{jSR})
\]

with their respective (constant) diffusion times. Here, \(J_{ds}\) accounts for the diffusion of the Ca\(^{2+}\) away from the dyadic space and to the rest of the cytoplasm, while \(J_{sr}\) represents the diffusion within the SR (both \(c_{d,0}\) and \(c_{SR}\) are taken as constants).

**Action potential**

The influence of the external action potential is implemented via a step voltage function (a step voltage clamp is usual in experimental studies):

\[
V(t) = \begin{cases} V_{\text{max}} & \text{if } t < APD \\ V_{\text{rest}} & \text{if } t > APD \end{cases}
\]

where \(APD\) refers to the *action potential duration*, given by \(APD = 100T/(100 + T)\), \(T\) being the pacing period (in ms).

**LCC stochastic dynamics**

The inward current of calcium from the extracellular medium into the dyadic space depends on the number of LCC channels on the open state, \(O_{LCC}\), electrical potential, and the current Ca\(^{2+}\) concentration in the dyadic space, \(c_d\):

\[
J_{CaL} = g_{CaL} O_{LCC}(4z_m c_d \frac{[Ca]_0}{e^{z} - 1})
\]

where \(z = \frac{F}{RT} V\), \(V\) being the voltage, \(F\) the Faraday constant, \(R\) the ideal gas constant and \(T\) the temperature; and \(z_m = 0.341zF\).
To compute $O_{LCC}$ we consider $N_{LCC} = 5$ LCC channels in the CaRU. Each channel has 5 possible states (Figure 5): 2 closed states, $C_1$ and $C_2$; two inactive states, $I_1$ and $I_2$; and an open state, $O$. This 5-state model of the LCC is obtained from [34].

![Figure 5: Five-state model of a LCC[34].](image)

The transitions between the states are stochastic and are modeled via Markov chains. They are implemented as usual, by obtaining a random number from a uniform probability distribution in (0,1) and then computing the next state for the LCC by comparing the random number to the probability of each transition, according to the transition rates:

$$a_{12}(V) = p_\infty$$,  
$$a_{21}(V) = 1 - p_\infty$$; with  
$$p_\infty = \frac{1}{1 + e^{-(V-15)/8}}$$  
(6)

$$a_{15}(V) = \frac{p_o}{\tau_o}$$,  
$$a_{51}(V) = \frac{1 - p_o}{\tau_o}$$; with  
$$p_o = \frac{1}{1 + e^{-(V+40)/10}}$$,  
$$\tau_o + (p_o - 450)p_{of} + 450$$,  
$$p_{of} = 10 + 4954e^{V/15.6}$$,  
(7)

$$a_{45}(V) = (1 - p_{if})/3$$ with  
$$p_{if} = \frac{1}{1 + e^{-(V+40)/3}}$$  
(8)

$$a_{24} = 0.00413 + 0.024f_{ca}$$,  
$$a_{34} = 0.00195 + 0.01826f_{ca}$$; with  
$$f_{ca} = \frac{1}{1 + (K_{LCC}/c_d)^3}$$  
(9)

And rate balance requires

$$a_{43} = a_{34}(a_{23}/a_{32})(a_{42}/a_{24})$$,  
$$a_{54} = a_{45}(a_{51}/a_{15})(a_{24}/a_{42})(a_{12}/a_{21})$$  
(10)

with $a_{23}$, $a_{32}$ and $a_{42}$ constants. Note that some rates depend only on the voltage (6 to 8), which is how the channel opening due to the membrane depolarization is implemented: The voltage jump causes the channels to transition from $C_2$ to $C_1$, at which point they stay in an equilibrium between $C_1$ and $O$. Similarly, the rates that depend on the concentration $c_d$ (9) lead to the inactivation of the LCC channels once a calcium spark happens.

**Stochastic RyR channel dynamics**

Analogously to the case of the LCC, the released calcium from the junctional SR to the dyadic space is taken to be proportional to the number of open RyR channels, $O_{RyR}$, and to the concentration difference between the compartments:

$$J_{rel} = g_{rel}O_{RyR}(c_{jSR} - c_d)$$  
(11)

Each of the $N_{RyR} = 70$ RyR in the cluster can be in one of four states (Figure 6)[35]. The transitions of each RyR channel are computed also using a Markov scheme.
The fraction of RyR in an "active" state (C and O) at the beginning of the beat will be used as a variable of the spark probability. It is defined in general as follows:

\[ f_R = \frac{C_0 + O_0}{C_0 + O_0 + I_{1,0} + I_{2,0}} \]  

(12)

In this particular work all the RyR starting in an active state will do so in the closed C state, while the rest will be assumed to be in the \( I_2 \) inactive state. Therefore, in fact, we will take

\[ f_R = \frac{C_0}{C_0 + I_{2,0}} \]  

(13)

Two of the transition rates depend on the dyadic concentration:

\[ k_{co} (c_d) = k_a c_d^2, \quad k_{i2i1} (c_d) = k_b c_d^2 \]  

(14)

Note that the probability of transitioning from the closed to open state increases with the square of \( c_d \). This reflects a cooperative binding of the RyR to Ca\(^{2+}\) that is derived from experiments.

The rates corresponding to inactivation, on the other hand, depend linearly on \( c_d \):

\[ k_{ci} (c_d) = k_{oi} (c_d) = K c_d \]  

(15)

The rest of the rates are constant, with \( k_{ic} = k_{co} = 1/\tau_R \). Detailed balance requires \( k_{i1i2} = k_{oc} (k_b / k_a) \).

The factor \( V_d / V_{jSR} \) that multiplies \( J_{rel} \) in equation (2) accounts for the different volumes of the dyadic space and the junctional SR, respectively; this ratio is taken to be \( V_d / V_{jSR} = 0.0168 \).

The characteristics of the inactivated states and whether they are prominent or necessary for the ending of the calcium release, or if on the other hand a 2-state model for the RyR without inactivated states would suffice to reproduce the spark behavior, is unclear at the moment.

**Characteristics and behavior of the model**

**Effect of the opening of the LCC**

Figure 7 shows how the opening of LCC relates to the calcium concentration within the cell. As an LCC opens (since the opening rates only depend on the voltage and a step function-like action potential is being used, the probability of opening is constant while the action potential lasts), \( c_d \) quickly rises until reaching a saturation concentration; this concentration is consistent among different openings of an LCC and depends essentially on \( g_{CaL} \). This concentration also proportional to \( O_{LCC} \), which means that if a second channel opens, \( c_d \) will simply approach twice the value; however, this requires the first channel not to close for a longer period and happens rarely. Once the channel closes, diffusion makes \( c_d \) drop again to \( c_{d,0} \).
Sparking and non-sparking events

As discussed before, the stochastic nature of the model (in particular, the ion channels) implies that consecutive simulations can have qualitatively different behaviors. Specifically, the model may or may not reproduce a calcium spark for a given set of parameters. This is shown in Figures 8 and 9.

On 8 we see a typical sparking event. An increase in $c_d$ (top) increases the number of open RyR channels (bottom right), since the opening rate is is proportional to $c_d^2$; this is a positive-feedback loop that results in what constitutes the calcium spark. This ends once $c_d$ is high enough, because now most of the RyR start to rapidly transition to the inactive states (for which the transition rates also increase with $c_d$, but only linearly). The maximum concentration reached during the spark depends, among other parameters, on the SR load, $c_{SR}$. The fact that the inactivated RyR transition to active states only at a constant rate guarantees the existence of a refractory period in which another spark cannot happen.

In contrast, 9 shows a case in which a calcium spark doesn’t happen, and the only changes in $c_d$ are due to the opening of LCC, as previously shown.

Figure 8: Sparking event at around $t = 17$ ms (top: dyadic calcium ($c_d$); bottom left: number of open LCC ($O_{LCC}$); bottom right: states of the RyR channels; $t \leq 30$ ms, corresponding to a constant voltage of $V_{max} = +5$ mV).
Results

Probability distribution and effective cooperativity

As previously discussed, the reason for building this model was to obtain a set of probability distributions for a calcium spark to happen as a function of the SR load, \( c_{SR} \), and the initial fraction of RyR in an active (closed) configuration, \( f_R \), and to study how it depends on other parameters of the model such as diffusion time scales and the behavior of the RyR channels.

Figure 10 shows an example of what a typical probability distribution looks like. As both the SR load and the RyR active fraction increase, the spark probability tends to 1. Furthermore, for most values of \( f_R \), the probability as a function of the load (Fig. 11a) shows a sigmoid dependence, in agreement with previous results.

Figure 10: Calcium spark probability distribution as a function of the SR load and the fraction of active RyR, shown color-coded for the two variables. Parameters: \( \tau_d = 0.04 \) ms, \( k_a = 2.1 \cdot 10^{-4} \mu\text{M}^{-2}\text{ms}^{-1} \), \( k_{oc} = 1 \text{ ms}^{-1} \), \( K = 10^{-3} \mu\text{M}^{-1}\text{ms}^{-1} \).

To extract information from the probability distribution and to be able to systematically evaluate the effect of altering various parameters, the data is fitted to a function formulated in terms of effective cooperativities. Inspired by previous works, the function used is the following:
\[ P_s(c_{SR}, f_R) = \left( \frac{1 - AC_{SR}f_R}{1 + \left( \frac{c_{SR}}{c_{SR}^*} \right) \gamma_x} + AC_{SR}f_R \right) \left( \frac{1}{1 + \left( \frac{f_R}{f_R^*} \right) \gamma_R} \right) \]  

(16)

Where \( c_{SR}^* \), \( f_R^* \), \( \gamma_x \), \( \gamma_R \), and \( A \) are free parameters that need to be adjusted. The fitting function is composed as a product of two factors, each of which is analogous to a Hill function for each of the two variables; the extra term \( AC_{SR}f_R \) in the \( c_{SR} \) factor is added upon observing that a) the probability doesn’t approach zero as \( f_R \) tends to zero, and b) the probability curves don’t behave as purely a product of two independent functions (as seen by normalizing the curves to their respective maxima). Indeed, this function is the one that consistently shows a much better fit (Fig. 11). The parameters are adjusted by minimizing the 2-norm of the difference between the two matrices that contain the probabilities (the one obtained from the simulations and the output of the fitting function).

![Figure 11: Comparison between the simulated probability curves and the fitted ones according to equation 16.](image)

(a) Probability as a function of each of the two variables (i.e. the rows and columns of the matrix in Fig. 10).

(b) The fitted curves from the same data. They correspond to the values \( c_{SR}^* = 1.52 \), \( f_R^* = 0.22 \), \( \gamma_x = 5.52 \), \( \gamma_R = 2.02 \) and \( A = 1.20 \).

Figure 11: Comparison between the simulated probability curves and the fitted ones according to equation 16.

**Influence of \( g_{rel} \)**

The first parameter tested is the constant \( g_{rel} \), which scales the \( \text{Ca}^{2+} \) flux when the RyR channels open in a calcium spark. Computing the probabilities while this parameter reveals that, while it does affect the spark probability, it does not change the slopes significantly (i.e., the fitting yields the same effective cooperativities). The effect of \( g_{rel} \), then, can be seen as essentially “shifting” the probability distributions; a higher value will displace the distribution towards lower SR loads, and vice versa. This is consistent by the fact that the associated flux, \( J_{rel} \), depends on the product \( g_{rel}c_{SR} \) but not on both variables independently, so increasing one while decreasing the other shouldn’t significantly vary the overall behavior.

This “shifting” of the probabilities by changing \( g_{rel} \) is implicitly used for the rest of the analysis, since it allows for the comparison of the slopes within the same SR load for different values of other significant parameters.
Influence of the diffusion

The model includes two diffusive currents, \( J_{ds} \) and \( J_{sr} \) (equation 3). These account for the diffusion from the dyadic space into the cytoplasm and the diffusion within the SR. In other words, these cause \( c_d(t) \) to drop to \( c_{d,0} \) and \( c_{jSR} \) to reach \( c_{SR} \) in absence of other fluxes. Each of these diffusive currents is taken to be simply proportional to the concentration difference and inversely proportional to their respective time scales, \( \tau_d \) and \( \tau_{sr} \).

On one hand, simulations were performed using the values \( \tau_{sr} = \{1, 5, 20, 100\} \) without observing any noticeable change on the probabilities and the cooperativities. On the other hand, the effect of \( \tau_d \) is clearly significant. As figure 12 shows, larger values drastically reduce the slopes of the curves (as the fitted values of \( \gamma_x \), \( \gamma_R \) reflect, table 1), practically eliminating any cooperative behavior.

![Figure 12: Effect of varying \( \tau_d \) from 0.1 to 4 ms. Fitted effective cooperativities in parentheses.](image)

(a) \( \tau_d = 0.1 \) ms (\( \gamma_x = 5.9 \), \( \gamma_R = 3.0 \))  (b) \( \tau_d = 1 \) ms (\( \gamma_x = 5.0 \), \( \gamma_R = 2.5 \))  (c) \( \tau_d = 4 \) ms (\( \gamma_x = 2.4 \), \( \gamma_R = 1.5 \))

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Table 1: The cooperativities change significantly under changes of \( \tau_d \). Values shown for \( k_{oc} = 1 \) ms\(^{-1} \), \( k_a = 2.1 \cdot 10^{-4} \mu M^{-2} \text{ms}^{-1} \) and \( K = 10^{-3} \mu M^{-1} \text{ms}^{-1} \) (\( \tau_d \) in units of ms).

Influence of the RyR opening and closing rates

As explained previously, the model assumes 4 states for the RyR with a constant closing rate \( k_{oc} \) and an opening rate \( k_{co} = k_a c_d^2 \), reflecting a cooperative binding of the calcium ions to the RyR.

After computing and fitting the probability distributions for \( k_{oc} = \{0.05,0.1,0.5,1\} \) ms\(^{-1} \) and \( k_a = \{0.2, 0.4, 0.8, 2.1, 3.0\} \cdot 10^{-4} \mu M^{-2} \text{ms}^{-1} \), the first result is that larger values of \( k_a \) yield larger effective cooperativities, while smaller values cause both smaller cooperativities and a saturation of the probability for values as low as \( P = 0.6 \) (Fig. 13 and Table 2).
Figure 13: Effect of $k_a$ (expressed in units of $10^{-4}\,\mu M^{-2}\,ms^{-1}$). Plots with $\tau_d = 0.1$ ms. Fitted effective cooperativities in parentheses.

$$\begin{array}{ccc}
  k_a & \gamma_x & \gamma_R \\
  0.2 & 2.3 & 0.3 \\
  0.4 & 2.5 & 0.4 \\
  0.8 & 3.3 & 0.8 \\
  2.1 & 5.5 & 2.0 \\
\end{array}$$

Table 2: The cooperativities change significantly under changes of $k_a$. Values shown for $k_{oc} = 1$ ms$^{-1}$, $\tau_d = 0.04$ ms and $K = 10^{-3}\,\mu M^{-1}\,ms^{-1}$ ($k_a$ in units of $10^{-4}\,\mu M^{-2}\,ms^{-1}$)

An important point is that for the largest value tested ($k_a = 3.0$, in the same units as before) the fitting no longer adjusts as well to the data. The curves seem to no longer have a sigmoidal shape but rather to show a switch-like behavior (Fig. 14).

Figure 14: $k_a = 3.0$. Notice how the curves are qualitatively different from, for example, fig. 11a, in that (especially the ones with the lower maximum) show a probability of zero for a significant range of both variables.

In contrast to $k_{co}$, $k_{oc}$ doesn’t seem to have an effect nearly as important. This is visible qualitatively from the probability distributions, and furthermore the fitting yields similar exponents for all the $k_{oc}$ values used (around $\gamma_c = 7.5$ and $\gamma_R = 4.0$). However, as noted above, the fitting no longer works as well for $k_a = 3.0$, which is the value for which these $k_{oc}$ were tested; the fitting errors are significantly larger in this case and so the exponents are not as reliable.

It is useful to keep in mind that the state transition rates reflect the expected value for the mean time of staying at a given configuration; in particular, the expected time corresponds to the inverse of the rate. Thus, for instance, a larger value for the opening rate $k_{co}$ reflects a smaller expected time for the RyR to stay in the closed configuration, and vice versa; and the same goes for $k_{co}$. This gives an intuition of larger $k_a$ increasing the probability of a spark, as it implies that for a given $c_d$ there is a larger probability of a transition to the open state for a given RyR.
Role of RyR inactivation

Upon computing the probabilities for different values of the constant in the inactivation rates, $K$, it shows that its value has no significant effect on the cooperativities, to the point of similar results being obtained for $K = 0$, i.e. for no inactivation in the RyR at all (that does not, however, make the system equivalent to that with only 2 states for the RyR, since a fraction $1 - f_R$ of the RyR channels are inactive at $t = 0$ and recover at a constant rate). The value of $K$ still affects, however, the value of $g_{rel}$, meaning the inactivation does play a role; only that the slopes are not affected.

Figure 15: Effect of the inactivation. It shows little effect on the slopes of the probability curves. Shown for $k_a = 3.0 \cdot 10^{-4} \mu M^{-1}\text{ms}^{-1}$, $k_{oc} = 0.5\text{ms}^{-1}$, $\tau_d = 0.1\text{ms}$.

(a) $K = 5 \cdot 10^{-3} \mu M^{-1}\text{ms}^{-1}$

(b) $K = 0$ (no inactivation)
Stochastic coupled maps system

Description

The following model is an extension of the coupled return maps model in [32]. A return map, in general, is used in the context of continuous or discrete periodic processes and tracks the changes between one cycle and the next. This model to present the key features of a complete calcium cycling model while being as simple as possible. It will use the release (spark) probabilities computed using the previous CaRU model in order to study the onset of calcium alternans.

The model represents a CaRU network using an $L \times L$ array in which each cell represents a single CaRU. This means that the geometry is vastly simplified into a planar square network. The homeostasis mechanisms are also simplified.

Each node $(i,j)$ is associated with two time-discrete variables that account for the pre-systolic Ca$^{2+}$ load and the pre-systolic recovered (active) RyR fraction at beat $n$; respectively, $x_{ij}(n)$ and $R_{ij}(n)$ (here, $x_{ij}(n)$ takes values between 0 and 1; it is a normalized variable corresponding to the variable $c_{SR}$ in the previous section).

At each beat $n$, the model goes through three intermediate phases, described next, by which $x_{ij}(n+1)$ and $R_{ij}(n+1)$ are obtained from $x_{ij}(n)$ and $R_{ij}(n)$:

**Release** In the release phase, each CaRU (node $ij$) undergoes a Ca$^{2+}$ release with a probability $P_s(x_{ij}(n),R_{ij}(n))$, with the same functional form as in the previous chapter:

$$P_s(x,R) = \left( \frac{1 - AxR}{1 + (\frac{x}{Ax})^\gamma} + AxR \right) \left( \frac{1}{1 + (\frac{R}{R^*})^\gamma R} \right)$$

Thus, the variables change as follows:

$$\begin{align*}
    x_{ij}(n) &\rightarrow \tilde{x}_{ij}(n) = x_{ij}(n) \left[ 1 - r_x \eta_{ij}(n) \right] \\
    R_{ij}(n) &\rightarrow \tilde{R}_{ij}(n) = R_{ij}(n) \left[ 1 - r_{R,ij}(n) \eta_{ij}(n) \right]
\end{align*}$$

Where $\eta_{ij}(n)$ is a random variable picked by the probability function. This is where the information from the previous model is used. Specifically, is is computed at each node in he following way:

$$\eta_{ij}(n) = \begin{cases} 
    1 & \text{with probability } P_s(x_{ij}(n),R_{ij}(n)) \\
    0 & \text{with probability } 1 - P_s(x_{ij}(n),R_{ij}(n))
\end{cases}$$

Note that, while $r_x$ is a constant, $r_{R,ij}(n)$ depends on $x_{ij}(n)$; particularly, the dependence is taken as linear so that a larger $x_{ij}(n)$ means a larger $r_{R,ij}$ and thus the amount by which $R_{ij}(n)$ is also larger. This is meant to reflect that a larger SR load will, in principle, cause a larger inactivation of the RyR.

**Diffusion** In this stage, $\tilde{x}_{ij}(n)$ is averaged with its near neighbors in the network to mimic the effects of diffusion. Of course, this step doesn’t affect $\tilde{R}_{ij}(n)$, since the RyR don’t diffuse. Formally, then,

$$\begin{align*}
    \tilde{x}_{ij}(n) &\rightarrow \bar{x}_{ij}(n) = \frac{1}{(2l+1)^2} \sum_{r \in [i-l,i+l]} \sum_{s \in [j-l,j+l]} \tilde{x}_{rs}(n) \\
    \bar{R}_{ij}(n) &\rightarrow \bar{R}_{ij}(n) = \tilde{R}_{ij}(n)
\end{align*}$$
Where \( l \) represents the range of the diffusion; i.e., \( l = 1 \) means averaging over the 9 contiguous neighbors, etc.

**Uptake** (\( x \)) Finally, the model needs to mimic the pumping back of the calcium into the SR by the SERCA pump.

The SERCA pumps are able to balance the SR load under changing conditions through the action of regulating proteins; specifically, phospholamban (PLB) acts as an inhibitor of SERCA. PLB, at its turn, can be phosphorylated independently by two other proteins, protein kinase A (PKA) and calcium/calmodulin-dependent kinase type II (CaMKII)[40]. This last one, in addition to being activated by calcium, depends on the beating frequency. In particular, [41] shows a nearly linear dependence between CaMKII phosphorylation and pacing frequency. Since phosphorylation of PLB abolishes its inhibitory effect, then the calcium pumped is enhanced as a consequence of increasing pacing frequency.

In addition to that, the pump strength is consistently found to decrease as the SR load increases. This makes intuitive sense, since it has to translocate the ions against a larger concentration gradient.

In a significant simplification of the behavior of the SERCA pump, in this model the uptake is assumed to be directly proportional to a variable reflecting the pacing period, \( b \). This variable represents the time available for the calcium uptake into the SR. The last step is, then,

\[
\bar{x}_{ij}(n) \rightarrow x_{ij}(n + 1) = \bar{x}_{ij}(n) + b \left( 1 - \bar{x}_{ij}(n) \right)
\]

**Recovery** (\( R \)) On the other hand, it can be proved that, if the two inactive states recover at the same constant rate, as is the case, then the recovery is exponential with time. Therefore:

\[
\bar{R}_{ij}(n) \rightarrow R_{ij}(n + 1) = \left( 1 - \bar{R}_{ij}(n) \right) \left( 1 - e^{-T/\tau_R} \right) + \bar{R}_{ij}(n)
\]

Where \( \tau_R \) is the characteristic timescale for RyR recovery, which is inversely related to the recovery rate, \( k_{io} = k_{ic} = 1/\tau_R \).

**Behavior of the model. Onset of calcium alternans**

**Intermediate steps**

Figure 16 shows an example of the intermediate steps just described:

- The nodes which randomly undergo a release show a drop of both \( x \) and \( R \); they correspond to the ones for which \( \eta_{ij} = 1 \).
- The diffusion only affects \( x_{ij} \); it causes the values to average out, as the color coding shows, therefore leaving only the larger scale fluctuations.
- The recovery of both variables in this case yields values very similar to those of the beginning.
Appearance of global alternans

Despite its simplicity, this model is capable of showing features of more complex models such as a global calcium alternans. This can be seen, for instance, by increasing the effective cooperativities $\gamma_x$ and $\gamma_R$. An example of this behavior is shown in Figure 17.

Figure 17: Obtained for $L = 100, b = 0.3, \gamma_x = \gamma_R = 15, l = 7$. 

(a) Arrays corresponding to a few consecutive beats in which the global alternans is visible both in $x(n)$ and $R(n)$.

(b) Continuous-time evolution of the SR load from a complete calcium cycling model [36].

(c) Cell-averaged SR load, showing visible calcium alternans.
In particular, in both figures 17b and 17c we can visualize calcium alternans; in both plots, the
SR load alternates between a high (red) and low (green) value. The continuous-time plot, furthermore,
shows the intermediate stages which this coupled maps model tries to mimic (Fig. 16); namely the release
(pink/green), diffusion (not necessarily visible in the cell-average of the load) and the uptake/recovery.

Order parameters
To characterize systematically the appearance of global alternans, an order parameter $m(n)$ is computed.
Inspired by previous works, it is defined as follows:

$$m(n) = \frac{1}{L^2} \sum_{i,j} m_{ij}(n)$$  \hspace{1cm} (23)

with

$$m_{ij}(n) = (-1)^n \left[ x_{ij}(n) - x_{ij}(n-1) \right]$$  \hspace{1cm} (24)

The $(-1)^n$ term is what makes $m(n)$ useful as an indicative of the presence of global alternans: indeed,
if most units are alternating in phase between two values from one beat to the next (in a small-large-small
fashion) then $m(n)$ will approach to a value equal to the magnitude of that alternation.

An alternative order parameter that is also explored uses beat-to-beat changes in $\eta_{ij}(n)$ instead:

$$\mu(n) = \frac{1}{L^2} \sum_{i,j} \mu_{ij}(n) = \frac{1}{L^2} \sum_{i,j} \left\{ (-1)^n \left[ \eta_{ij}(n) - \eta_{ij}(n-1) \right] \right\}$$  \hspace{1cm} (25)

This has the advantage that, since $\eta_{ij}(n)$ only takes the values 1 and 0, then $\mu(n)$ gives directly a
measure between 0 and 1 of how much the units are alternating in phase, independently of the magnitude
of the alternation.

As stated, order parameters allow for the systematic analysis of the onset of alternans. In particular,
the influence of the cooperativities and the recovery parameter $b$ is observed. If the dependence of
$\langle \mu \rangle = 1/N \sum_n \mu(n)$ on $b$ is computed for some sets of $\gamma_x$ and $\gamma_R$, global alternans is found to appear
consistently around $b = 0.44$ for values of $\gamma_i$ large enough. This is in agreement with the results of
previous works[32]. Figure 18 shows this, where $\gamma_x = \gamma_R = \gamma$ is assumed for simplicity.

![Order parameter $\mu$](image)

Figure 18: Onset of alternans around $b = 0.44$ for different values of $\gamma_x = \gamma_R = \gamma$.

Results and discussion
As pointed out in previous works[32], the probabilistic all-or-nothing nature of the release favors
alternating sequences of release and non-release for certain parameter values, such as a low $b$ (which is
analogous to a rapid external pacing). Of course, this is affected by the stochasticity of the system
causing this alternating behavior to be unstable. However, for probability distributions steep enough
(meaning large enough values of $\gamma_i$), these alternating sequences can last a longer number of beats, at
which point diffusion can be enough to synchronize neighboring units. This is what allows for cell-wide
alternans to last indefinitely.

The main results obtained from the simulations are the following:
The lowest values of the exponents $\gamma_i$ for which the model shows alternans are approximately $\gamma_x = \gamma_R = 12$ (Figure 19). Alternans does not appear for lower values of $\gamma_R$ even for the same or larger values of $\gamma_x$. This means that the alternation in active RyR2 plays a prominent role in the onset of calcium alternans. This agrees with previous works that found also that both an alternation in recovered RyR and SR load contribute to inducing alternans [36].

![Figure 19: Appearance of global alternans above effective cooperativities around 12.](image)

Furthermore (as pointed before in Fig. 18, and shown again below, Fig. 20), alternans appears only within a certain range of values of $b$ for given $\gamma_x, \gamma_R$. As explained previously, this is consistent with the interpretation of $b$ as the pace cycle length, since the recovery of $x$ is proportional to it. This, in turn, is consistent with the experimental evidence of alternans being generated by increasing the frequency of the external pacing [30].

![Figure 20: A larger value for $\gamma_x = \gamma_R = \gamma$ yields both a wider range of values of $b$ for which alternans is observed and a higher value of the order parameter in those in which it does, reflecting a better cell-wide coordination of the units for larger effective cooperativities, as expected. Notice that for $\gamma = 8$, which is above the largest effective found in the CaRU stochastic model, there is no alternans for any value of $b$.](image)

The system is highly sensitive to the values of $x^*$ and $R^*$, which need to be within a fairly tight range for alternans to appear even with high enough cooperativities. At this point it’s interesting to look again at figure 19a to notice that both variables are alternating between a value below and a value above the dashed lines, which correspond to $x^*$ and $R^*$, respectively. This is observed consistently when there is alternans and it’s no coincidence: given the functional form of the probability distribution, around these values is precisely where the slope of the probability is the largest, i.e. where the probability changes the most between values close to each other. As explained, this kind of strong non-linearity is what favors alternating behavior in the proper conditions. And since $b$ affects the actual values of the variables by affecting the $x(n)$ recovery, then the fact that alternans only happens for a range of $b$ can be explained as it being the range of $b$ for which the variables are close to their respective $x^*_i$ value. The plots in figure 21 illustrate exactly that.
Global alternans is not observed without a mechanism for calcium diffusion. This is to be expected, since this diffusion is the only mechanism by which neighboring units can stay synchronized alternating in phase.

Switch-like probability function

As seen in the previous chapter, the probability function used for the random release in this coupled maps system is a very good fit for the empirical probability distributions that the calcium release unit model yields for most of the parameter values explored. However, for the larger RyR opening rates tested (corresponding to \(k_a = 3.0\), in the units used), the obtained probability curves no longer were of sigmoidal shape, but rather showed a “switch-like” behavior, meaning they took nonzero values only above certain value in both the load and \(f_R\).

With this in mind, the behavior of the coupled maps system with a switch-like probability function was briefly explored. The probability function chosen is

\[
\begin{align*}
  P_s(c_{SR}, f_R) &\propto (c_{SR}^{1/\alpha}, f_R^{1/\alpha}) & &\text{for } c_{SR} > c_{SR}^* \text{ and } f_R > f_R^* \\
  P_s(c_{SR}, f_R) &= 0 & &\text{otherwise}
\end{align*}
\]

i.e. zero below a certain value for each variable and with a polynomial dependence with exponent \(1/\alpha\) from there (the same exponent is assumed for both variables for simplicity). This function is not fitted to data from the calcium release unit model; it is just used as an example of alternans being able to appear using such a release probability.

Indeed, alternans does appear starting at an exponent of around \(\alpha = 6\); just like with the previous probability function, alternans shows within a range of values for \(b\) (Fig. 22).
Conclusions

In this work, I have studied the effect of two mechanisms that can cause the onset of calcium alternans in cardiac cells. This has been done through the development and study of two related models, with different purposes. On one hand, I reproduced, and studied, a stochastic model of a calcium release unit in which the homeostatic level of the SR calcium load and the recovered RyR fraction were fixed. On the other hand, I developed a stochastic coupled maps system which represents a simplified model of a calcium release unit network; in it, a basic homeostasis mechanism can be implemented to study effect of the locally stochastic nature of the calcium release on the onset of whole-cell alternans.

The detailed model model of a calcium release unit considers a set of calcium channels (LCC) in the membrane and a cluster of ryanodine receptor (RyR) channels on the surface of the sarcoplasmic reticulum (SR). It takes into account two main regions, or compartments: the dyadic space, between the SR and the membrane, and the junctional SR, which is the region of the SR closest to the RyR channels. These two compartments are linked by a set of fluxes which account for the action of calcium diffusion as well as for the flow of ions across protein channels due to changes in their configuration. These configuration changes are intrinsically stochastic, which is built into the model using Markov chains to model the transitions of each channel between a set of configurational states. Given the intrinsic stochasticity of the model, which is due to the low number of protein channels, it is studied from a statistical standpoint by computing the probability for a calcium spark to occur as a function of the initial SR load and the initial configurational state of the RyR channels. These depend on the calcium balance maintained by the inflow of calcium ions through the LCC, on one hand, and the removal by the calcium-sodium exchanger, on the other. Then, the dependence of this probability distribution on several model parameters is systematically tested. These parameters include diffusion time scales and state transition rates of the RyR. Furthermore, the resultant probability distributions are fitted to functions expressed in terms of effective cooperativities to extract qualitative and quantitative information from them.

Once this information is obtained, the local probability release function is introduced in a very simple mechanism of homeostasis with the goal of exploring the appearance of cell-wide calcium alternans.

The second main block of this work consists on the implementation of a simplified, stochastic system of coupled return maps. Each return map aims to represent the stochastic behavior of a calcium release unit through the use of random variables and probability functions that draw from the results of the first block. In it, two discrete-time variables account for the beat-to-beat change in presystole SR load and presystole fraction of recovered RyR. The geometry and the homeostasis mechanisms are extremely simplified. Particularly, the effect of the calcium uptake into the SR by the SERCA pump is represented by a proportionality constant that accounts for the relationship between the uptake characteristic time and the pacing rate, while the calcium balance between the inward flow through the LCC and the outward current due to the Na-Ca exchanger is externally fixed. With this, the model can reproduce behaviors observed in much more complex models, including the onset of alternans, which can be studied in terms of the effect of local cooperativity. The conditions under which alternans develops are delimited. Namely, for the onset of alternans, a steep enough spark probability distribution together with smaller load recovery, which emulates the effect of a rapid external pacing, are required.

The main conclusions drawn from this work are the following:

**Calcium release unit model**

- The largest slopes in the calcium spark probability distribution are found for fast dyadic space Ca$^{2+}$ diffusion together with a high RyR opening rate. The fitting function used to characterize the probability curves yields for this case effective cooperativities of the order of 7-8 for the SR load and 3-4 for the recovered fraction of RyR.
• The inactivation of the RyR doesn’t appear to have a prominent role in the calcium spark probability. Neither does the diffusion timescale within the SR.

• A high value of the dyadic space diffusion time scale, i.e. a slow Ca$^{2+}$ diffusion, dramatically decreases the slopes in the probability curves.

• The probability curves are characterized by a sigmoidal shape for most of the range for the $k_a$ opening rate constant, but seem to transition to a switch-like behavior for high $k_a$ values, in which the spark probability is close to zero up to a certain load and recovered RyR fraction.

Simplified stochastic coupled maps system

• Despite the significant simplifications assumed for the model, the system is capable of showing key features of more complex calcium cycling models, such as the emergence of global calcium alternans.

• The comparable dependence of the onset of alternans on both effective cooperativities reveals that, in this model, alternans is sustained by an alternation in both SR load and RyR recovered fraction. This is in agreement with the results of more complex models as well.

• The model does not show calcium alternans when implementing the probability function used in the fitting of the calcium spark probability from the stochastic CaRU model, with the largest effective cooperativities obtained from it. However, it does show alternans for larger values of the cooperativities.

• On the other hand, using an ad hoc simple probability function with the same switch-like behavior shown by the simulated probability distributions at higher RyR opening rates, alternans appears in the coupled return maps system. This points at this switch-like behavior as a relevant feature of the probability curves in order to have onset of alternans. We hypothesize that fitting a probability function with these characteristics to the empirical data from the calcium release unit simulations could show that alternans can appear in the coupled maps model with the fitted parameters.

Future work

• As pointed out in the conclusions, an immediate follow-up of this work could consist on finding a probability function that fits the switch-like character of the obtained probabilities from the calcium release unit simulations to test if the consequent parameters can yield alternans when implemented in the coupled maps model.

• Furthermore, the parameter ranges in which the CaRU model shows a switch-like probability distribution and a sigmoidal ones could be analyzed in a CaRU model with the complete simulation of the homeostasis mechanisms.

• In the stochastic coupled maps system, diffusion is the mechanism through which neighboring units couple and are able to coordinate. Notably, at the $l \to \infty$ limit (long-range diffusion), all the nodes in the network are coordinated and therefore they behave, to all effects, as one single unit. At this point, then, the behavior of the cell becomes deterministic, since the total amount of stochastic channels is large enough for the system to be accurately described through rate equations. This means that, as a future work, the onset of cell-wide calcium alternans could be studied analytically, which might enable further insight into the mechanisms by which it appears.
Bibliography


