Control of Calcium Homeostasis and Load Regulation in a Ventricular Z-Plane Model

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Abstract

Calcium is considered one of the most important ions involved in the intricate workings of the heart. The control of intracellular calcium is crucial to the process called excitation-contraction coupling, enabling the chambers of the heart to contract and relax. It is involved in some of the most common heart diseases, like cardiac dysfunction and arrhythmias, so knowing how it behaves during the calcium homeostasis cycle, the process of self-regulation which takes place in every beat, it is crucial for medical purposes. If we want to understand the basic physiology of this organ it is important to study in detail how calcium moves through the various organelles of the myocyte provoking this process. In this work, calcium fluxes will be studied in order to better understand the mechanisms of self-regulation of calcium concentrations in ventricular myocytes. It will require the comprehension of the intracellular calcium dynamics and their interaction and influence in the different microdomains of the cell. Be aware of ryanodine receptors and sarcoplasmic reticulum importance as well as the different mechanisms to increase or reduce the concentration of this ion in the myocyte, and study how these mechanisms affect cytosolic calcium concentration, which at the end is the responsible of the cardiac response. We will investigate the formation of steady state solutions and how the NCX work for getting stable responses. In this way a subsequent study has been carried out by means of simulations using a z-plane ventricular model to observe how different variables affect calcium homeostasis.
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Chapter 1

Introduction

Calcium is considered one of the most important ions involved in the intricate workings of the heart. The control of intracellular calcium is crucial to the process called excitation-contraction coupling, enabling the chambers of the heart to contract and relax. For understand the basic physiology of heart function is fundamental to know in quantitative detail how calcium moves around the different cardiac muscle cell organelles in order to bring about excitation-contraction coupling. It's also important to know how it interacts with the spatial microdomains of the cell to achieve the desired cardiac function and to stress that focusing attention on any of the individual steps that control calcium can be misleading. A full understanding of the control of contraction requires considering how the various steps are integrated to produce a global coordinated response.

1.1 Heart Physiology and Action Potential

The heart is a muscular organ in humans and other animals, which pumps blood through the blood vessels of the circulatory system. It is divided into four chambers: upper left and right atria; and lower left and right ventricles. The normal resting heart rate is called the sinus rhythm, created and sustained by the sinoatrial node, a group of pacemaking cells found in the wall of the right atrium. These cells are excited by an electric stimulus created by the movement of specific electrolytes going into and out of the cytosol. This entails the creation of a potential action that is born in the sinoatrial node and spreads to nearby cells through the cardiac tissue. (Fig. 1).

The cardiac action potential can be divided in four phases. The first consists of a rapid, positive change in voltage across the cell membrane (depolarization). The second phase is also known as the "plateau" phase due to the membrane potential remaining almost constant, as the membrane slowly begins to repolarize. During phase 3 (the "rapid repolarization" phase) of the action potential, a negative change in membrane potential is produced. And finally, phase 4 occurs when the cell is at rest, in a period known as diastole in the ventricular myocytes. The time scale of this whole process is milliseconds.

Figure 1: Cardiac action potential shape and its corresponding muscle contraction.
1.2 Cardiac Excitation–Contraction Coupling

Cardiac excitation-contraction coupling is the process that couples electrical excitation of the cardiac muscle cell (cardiomyocytes) to contraction of the heart, which propels blood out. The calcium ion Ca\(^{2+}\) is an essential messenger in cardiac electrical activity and it’s the direct activator of the myofilaments, which cause contraction. Myocyte mishandling of Ca\(^{2+}\) is a central cause of both contractile dysfunction and arrhythmias in pathophysiological conditions\(^1\).

During the cardiac action potential, Ca\(^{2+}\) enters the cell through depolarization-activated Ca\(^{2+}\) channels (L-type channels) located at the sarcolemma (cardiac cell membrane) as inward Ca\(^{2+}\) current (\(J_{Ca}\)), which contributes to the action potential plateau. Ca\(^{2+}\) entry triggers Ca\(^{2+}\) release (\(J_{rel}\)) from the sarcoplasmic reticulum (SR), where the main source of Ca\(^{2+}\) of the cell is stored. The combination of Ca\(^{2+}\) influx and release raises the free intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), allowing Ca\(^{2+}\) to bind to the myofilament protein troponin C (TnC), which then switches on the contractile machinery (Fig. 2). In summary, contraction of cardiac muscle is initiated by an increase of [Ca\(^{2+}\)]. This first phase of the cardiac cycle, when the contraction occurs, is what is called the systole and the magnitude of the cytosolic calcium rise during this phase at the cellular level is called systolic Ca\(^{2+}\) transient, and must be controlled to produce a constant cardiac output\(^2\).

During the second phase, or diastole, for relaxation to occur [Ca\(^{2+}\)]\(_i\) must decline, allowing Ca\(^{2+}\) to dissociate from TnC. This requires Ca\(^{2+}\) transport out of the cytosol by four pathways involving SR Ca\(^{2+}\)-ATPase (SERCA), sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchange (NCX), sarcolemmal Ca\(^{2+}\)-ATPase or mitochondrial Ca\(^{2+}\) uniport (Fig. 2). For the heart to be in a steady state it is essential that, during each cardiac cycle, exactly the amount of Ca\(^{2+}\) that had entered from outside the cell is pumped back out and that which is released from the SR is returned. Let us address now the key Ca\(^{2+}\) transport systems in cardiac myocytes, how they interact dynamically and how they are regulated.

![Figure 2: Mechanisms of excitation-contraction coupling in cardiomyocytes; arrows indicate Ca\(^{2+}\) shifts in systole/contraction (left) and diastole/relaxation (right)\(^3\). \(J_{Ca}\) entering the cell through the L-type sarcolemma channels triggers Ca\(^{2+}\) release from the SR. This calcium binds to the TnC to provoke contraction. During the diastole cytosolic concentration is reduced thanks to SERCA and NCX activity.](image-url)
1.2.1 The Role of Calcium in Contraction and Flux Balance

The relation between the amount of total cytosolic \([\text{Ca}^{2+}]\) that must be supplied in the activation of contraction and the increased \([\text{Ca}^{2+}]_i\) indicates that there is a powerful cytosolic \(\text{Ca}^{2+}\) buffering\(^4\). This means that exists several macromolecular complexes coexisting with calcium, with \(\text{Ca}^{2+}\) binding sites, that contributes in the regulation of calcium concentrations. The magnitude of the systolic rise of \([\text{Ca}^{2+}]_i\) depends not only on the magnitude of the fluxes but, in addition, on the \(\text{Ca}^{2+}\) buffering power of the cell. These include TnC and calmodulin, as well as membrane binding sites.

As a result of a strong myofilament cooperativity with respect to \([\text{Ca}^{2+}]_i\), the contraction force depends on \([\text{Ca}^{2+}]_i\) and \([\text{Ca}^{2+}]_{\text{Tot}}\) \(\langle [\text{Ca}^{2+}]_i \rangle\) plus bound \(\text{Ca}^{2+}\) in highly nonlinear relationships. In addition, the physiological contraction generates both ventricular pressure and rapid shortening to eject blood. Generally speaking, there are two main ways to change the strength of cardiac contraction: by altering the amplitude or duration of the \(\text{Ca}^{2+}\) transient, and by altering the sensitivity of the myofilaments to \(\text{Ca}^{2+}\).

The final stage of the calcium transient, also named relaxation, consists on the removal of \(\text{Ca}^{2+}\) from the cytosol to basal values. This is achieved by several routes, the quantitative importance of which has been shown in some studies that varies between species\(^2\). Analysis in human ventricular myocytes and \(\text{Ca}^{2+}\) flux balances are quantitatively like rabbit, where the SR \(\text{Ca}^{2+}\)-ATPase (SERCA) pump removes 70% of the activator \(\text{Ca}^{2+}\), and \(\text{Na}^+/\text{Ca}^{2+}\) exchange (NCX) removes 28%, leaving only about 1% each to be removed by the sarcolemmal \(\text{Ca}^{2+}\)-ATPase and mitochondrial \(\text{Ca}^{2+}\) uniporter, the slow systems. Furthermore, atrial and ventricular cells exhibit distinctive differences in \(\text{Ca}^{2+}\) cycling during excitation-contraction coupling. Atria myocytes have a higher SERCA activity and delayed action potential propagation between the sarcolemma and the inner cell\(^6\).

An example of the relevance of this relaxation routes, during heart failure, functional expression of SERCA is reduced and NCX is increased, such that these systems contribute more equally to the decline in \([\text{Ca}^{2+}]_i\). These changes counterbalance each other with respect to twitch relaxation and \([\text{Ca}^{2+}]_i\) decline, leaving it unaltered. But both changes tend to reduce \(\text{Ca}^{2+}\) content in the SR, limiting SR \(\text{Ca}^{2+}\) release current \(J_{\text{rel}}\), and this may be a central cause of systolic contractile deficit in heart failure\(^7\). This indicates the relevance of the homeostatic process, which returns the cell to basal levels, and how it is related with cardiac disfunction.

1.2.2 Calcium Current

As I have mentioned before, calcium enters the cell from the extracellular medium through a voltage-dependent specialized channels, when the action potential excites the membrane. Myocytes exhibit two classes of voltage-dependent \(\text{Ca}^{2+}\) channels, L-type and T-type\(^2\). As T-type \(J_{\text{Ca}}\) is negligible in most ventricular myocytes, \(J_{\text{Ca}}\) generally refers to the L-type here. \(J_{\text{Ca}}\) is activated by depolarization, but \(\text{Ca}^{2+}\)-dependent inactivation at the cytosolic side limits the amount of \(\text{Ca}^{2+}\) entry during the excitation. This \(\text{Ca}^{2+}\)-dependent inactivation is a local effect and is mediated by calmodulin bound to the carboxy terminus of the \(\text{Ca}^{2+}\) channel. L-type \(\text{Ca}^{2+}\) channels (LCC) are located primarily at sarcolemmal-SR junctions, in front of the SR \(\text{Ca}^{2+}\) release channels or ryanodine receptors (RyRs), a serie of channels stimulated by calcium concentration responsible for the SR depletion and the large \(\text{Ca}^{2+}\) concentration increase during the systole, as we’ll proceed to explain later in more detail. During excitation-contraction coupling, SR \(\text{Ca}^{2+}\) release also contributes to \(\text{Ca}^{2+}\)-dependent inactivation of \(J_{\text{Ca}}\)^8.
1.2.3 Sarcoplasmic Reticulum and Calcium Release

The main function of the SR is to store Ca\(^{2+}\) and regulate its flux for the contraction to occur during the excitation-contraction coupling. As stated before, SR complex is the responsible of the large cytosolic calcium concentration increase during the depolarization. Ca\(^{2+}\) is released from the SR through a specialized release channel, the RyR, via the process of Ca\(^{2+}\)-induced Ca\(^{2+}\)-release (CICR). The entry of a small amount of trigger Ca\(^{2+}\) through the sarcolemmal L-type Ca\(^{2+}\) current \(J_{Ca}\) produces a localized increase of \([Ca^{2+}]_i\) in the small space between the surface and SR membranes. This then increases the open probability of the RyR, resulting in the efflux of Ca\(^{2+}\) from the SR into the cytoplasm. Obviously, for the heart to function as a pump, it must relax as well as contract. Relaxation is initiated by a reduction of \([Ca^{2+}]_i\), produced either by pumping Ca\(^{2+}\) back into the SR by the SERCA or out of the cell, by the NCX.

The amount of Ca\(^{2+}\) released from the SR for a given \(J_{Ca}\) and the amplitude of the Ca\(^{2+}\) transient depends on at least 2 factors: (1) the properties of the RyR, in particular the relationship between \([Ca^{2+}]_i\) and the open probability of the RyR, (2) and the Ca\(^{2+}\) content of the SR and hence the Ca\(^{2+}\) release flux when a channel opens. The amplitude of the transient, in turn, controls Ca\(^{2+}\) efflux by the NCX and SERCA pumps, so it controls the SR recovery. This autoregulation of SR load influences the response to maneuvers that modify, for example, the properties of the RyRs. A high load of Ca\(^{2+}\) in the SR directly increases the amount of Ca\(^{2+}\) available for release, but also greatly enhances the fraction of SR Ca\(^{2+}\) that is released for a given \(J_{Ca}\) trigger. This is due, at least in part, to a stimulatory effect of high intra-SR free \([Ca^{2+}]_i\) \([Ca^{2+}]_{SR}\) on the open probability of RyRs\(^9\). This increased RyR sensitivity to \([Ca^{2+}]_i\) at high \([Ca^{2+}]_{SR}\) means that what is often referred to as ‘spontaneous release’ at high cellular SR Ca\(^{2+}\) content might be considered mechanistically to be triggered by high \([Ca^{2+}]_{SR}\) (sometimes in synergy with high \([Ca^{2+}]_i\)). This is the basis of aftercontractions, ‘spontaneous’ release causing delayed after-depolarizations that can trigger arrhythmias\(^10\).

At moderately low SR Ca\(^{2+}\) content, \(J_{Ca}\) can fail to induce release from the SR. This may help the SR to reload if it becomes relatively depleted. Indeed, low release allows more Ca\(^{2+}\) influx through \(J_{Ca}\) (less inactivation) and NCX (less shift towards Ca\(^{2+}\) extrusion). A decline in SR Ca\(^{2+}\) load, even locally, may contribute dynamically to the turn-off of Ca\(^{2+}\) release from the SR during excitation-contraction coupling SR Ca\(^{2+}\) load can be raised by increasing Ca\(^{2+}\) influx, decreasing Ca\(^{2+}\) efflux, or enhancing Ca\(^{2+}\) uptake into the SR \(J_{up}\), for example, increasing the stimulation frequency, action potential duration, \(J_{Ca}\) or \([Na^+]_i\), in order to raise the level of cytoplasmic Ca\(^{2+}\).

The higher the \([Ca^{2+}]_i\), the greater the rate of Ca\(^{2+}\) pumped into the SR by the SERCA. Much attention has been focused on the effects of directly modulating the activity of SERCA and, in particular, its interaction with the inhibitory accessory protein phospholamban\(^11\). Phosphorylation of phospholamban relieves the inhibition of SERCA, thereby stimulating its activity, allowing faster twitch relaxation and decline of \([Ca^{2+}]_i\). Because the SERCA competes better with NCX, phosphorylation of phospholamban also enhances Ca\(^{2+}\) content in the SR.
1.2.4 RyR Channels and CICR

The RyR protein functions as the major component of a calcium channel located in the SR that supplies ions to the cardiac muscle during systole. To enable cardiac muscle contraction, calcium influx through voltage-gated LCC in the plasma membrane allows calcium ions to bind to RyR located on the SR. This binding causes the release of calcium through RyR from the SR into the cytosol, where it binds to troponin C, enabling cardiac muscle contraction. So the RyR is the mechanisms through the SR is able to release its $\text{Ca}^{2+}$ load. As stated before, the open probability of this channel is stimulated by $[\text{Ca}^{2+}]_{\text{SR}}$, which means that releases for a given triggering calcium current increases with the SR load. This can produce induced ‘spontaneous’ releases at high concentrations.

The RyR is both the SR $\text{Ca}^{2+}$ release channel and a scaffolding protein that localizes numerous key regulatory proteins to the junctional complex. These include calmodulin (which can exert as $\text{Ca}^{2+}$-dependent modulator of RyR function), and others which can alter RyR and $J_{\text{Ca}}$ gating. RyRs are also coupled to other proteins at the inner SR surface which participate in both intra-SR $\text{Ca}^{2+}$ buffering and modulation of the $\text{Ca}^{2+}$ release process. RyRs are arranged in large organized arrays (up to 200 nm in diameter with more than 100 RyRs) at the junctions between the SR and sarcolemma (couplon), on the surface and in t-tubules. These arrays constitute a large functional $\text{Ca}^{2+}$ release complex at the couplon (10–20 LCC/100 RyR). $\text{Ca}^{2+}$ sparks reflect the nearly synchronous activation of a cluster of about 6-20 RyRs at a single junction. $\text{Ca}^{2+}$ sparks are the fundamental units of SR $\text{Ca}^{2+}$ release both at rest (rare, stochastic events) and during excitation-contraction coupling, where several thousand $\text{Ca}^{2+}$ sparks in each cell are synchronized in time by the action potential, such that the local rises in $[\text{Ca}^{2+}]_{i}$ are completely overlapping in time and space, making the $\text{Ca}^{2+}$ transient appear spatially uniform. Resting $\text{Ca}^{2+}$ sparks are normally rare and isolated by the space between couplons, but when cellular and SR $\text{Ca}^{2+}$ load rise, the exclusively local stochastic cluster behavior is overcome and $\text{Ca}^{2+}$ released at one junction can activate a neighbouring junction due to the stimulatory effect of high $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{i}$, on the open probability of RyRs, creating a global coordinated response.

The mechanisms underlying both activation and termination of $\text{Ca}^{2+}$ release from the SR are controversial, but by far the most widely accepted mechanism is $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$-release (CICR), in particular CICR mediated by the LCC current. It seems that the opening of one local LCC is sufficient to activate fully the release process at that couplon, because neighbouring RyRs are activated either by high local $\text{Ca}^{2+}$ or coupled gating between RyRs. Notably, only a fraction of the LCC and RyRs in a cell or couplon needs to open to produce the regular fluxes. Having more than one $\text{Ca}^{2+}$ channel per couplon creates a safety margin to assure that each couplon will normally fire under the action potential. Overwhelming data support this general model as the main excitation-contraction coupling mechanism in heart, but contributions from other pathways have been also been proposed.

CICR is inherently a positive-feedback mechanism, but its turn-off is essential for diastolic refilling of the heart. Local depletion of SR $\text{Ca}^{2+}$ cannot explain completely the turn-off of release, because very long lasting $\text{Ca}^{2+}$ sparks are observed that do not decline with time. Thus, diffusion from other regions of the SR can limit local $\text{Ca}^{2+}$ depletion in the SR. During a global $\text{Ca}^{2+}$ transient, however, the whole $[\text{Ca}^{2+}]_{\text{SR}}$ declines. Because $[\text{Ca}^{2+}]_{\text{SR}}$ modulates RyR gating, $[\text{Ca}^{2+}]_{\text{SR}}$ depletion might contribute to shutting-off global SR $\text{Ca}^{2+}$ release during a twitch. But, as stated above, this cannot explain fully why $\text{Ca}^{2+}$ sparks turn off or why SR $\text{Ca}^{2+}$ release terminates. Two types of RyR inactivation have been reported (and both depend on $[\text{Ca}^{2+}]_{i}$). One is an absorbing inactivation, in which the RyR is unavailable for reopening until it recovers. The second type is called adaptation, in which the RyR after activating relaxes to a lower open probability, but can still be reactivated by a
higher [Ca\(^{2+}\)]. Inactivation of RyRs may be important in minimizing inappropriate SR Ca\(^{2+}\) release events between heartbeats. In summary, it seems that both RyR inactivation and partial inner depletion of SR Ca\(^{2+}\) (to reduce RyR opening) both contribute to the turn-off of release.

### 1.2.5 Na\(^+\)/Ca\(^{2+}\) Exchange

Na\(^+\)/Ca\(^{2+}\) exchange is reversible, with a stoichiometry of three Na\(^+\) ions to one Ca\(^{2+}\) ion, and produce the ionic current \(J_{\text{NaCa}}\). NCX can extrude Ca\(^{2+}\) (as an inward \(J_{\text{NaCa}}\)) or bring Ca\(^{2+}\) into the cell (as outward \(J_{\text{NaCa}}\))\(^{18}\). High [Ca\(^{2+}\)], favors Ca\(^{2+}\) efflux, whereas positive membrane potential \(V_m\) and high [Na\(^+\)], favors outward \(J_{\text{NaCa}}\). Early in the action potential \(V_m\) exceeds \(V_{\text{NaCa}}\) (\(V_{\text{NaCa}} = 3V_Na - V_{Ca}\), where \(V_Na\) and \(V_{Ca}\) are equilibrium potentials for Na\(^+\) and Ca\(^{2+}\)), which tends to drive Ca\(^{2+}\) entry by outward \(J_{\text{NaCa}}\), until \(V_m = V_{\text{NaCa}}\) during repolarization. Note that \(V_{\text{NaCa}}\) changes because [Ca\(^{2+}\)] (and thus \(V_{Ca}\)) changes. On repolarization of the action potential (diastole), the negative \(V_m\) and high [Ca\(^{2+}\)], drive a large inward \(J_{\text{NaCa}}\) and this reflects Ca\(^{2+}\) extrusion from the cell. However, due to \(J_{\text{Ca}}\) and \(J_{\text{rel}}\), local submembrane [Ca\(^{2+}\)] raises very fast, and even if it may not get as high as [Ca\(^{2+}\)], it still causes \(J_{\text{NaCa}}\) to become inward very early in the rise of the action potential, such that very little Ca\(^{2+}\) enters through \(J_{\text{NaCa}}\). Thus, under physiological conditions NCX works mainly in the Ca\(^{2+}\) extrusion mode, driven mostly by the Ca\(^{2+}\) transient. The positive \(V_m\) during the action potential plateau can, however, limit Ca\(^{2+}\) extrusion. This emphasizes the importance of considering local versus bulk ion concentration. And although NCX may normally work mainly in the Ca\(^{2+}\) efflux mode, the amount of Ca\(^{2+}\) influx through \(J_{\text{NaCa}}\) can be increased greatly if [Na\(^+\)] is elevated, if SR Ca\(^{2+}\) release and/or \(J_{\text{Ca}}\) is inhibited, or if action potential duration is prolonged\(^{19}\). We want to place special emphasis on this last part. The NCX activity is not only dependent on its conductivity, but also on all the other factors. Especially on the levels of calcium release of the SR, being more effective for high transient amplitudes, which promote the change to extrusion mode faster.

### 1.2.6 Homeostasis

Calcium homeostasis refers to the self-regulation of the concentration of calcium ions during the excitation-contraction process, when all the mechanisms previously explained work together in order to achieve an stable cardiac response. All the different calcium regulators are integrated in order to achieve an steady state response for all the myocyte microdomains, so the calcium concentration follows a periodic behavior synchronous with the action potential, returning to its basal values in each beat. The main requirement that must be fulfilled to obtain this type of response is that calcium entering from the extracellular medium must be pumped out, and the calcium released from the SR, must be pumped back, in each cycle. In this way we achieve a neutral calcium balance within the whole cell, avoiding SR depletion or overloading, which can induce cardiac pathologies.
1.3 General Structure of Ventricle Myocytes

1.3.1 T-Tubules

The transverse tubules (t-tubules) are invaginations of the external membrane of cardiac muscle rich in LCC and other proteins devoted to the critical task of excitation-contraction coupling (Fig. 3b). They are thought to promote the synchronous activation of the whole depth of the cell despite the fact that the signal to contract is relayed across the external membrane. However, recent work has shown that t-tubule structure and function are complex and tightly regulated in healthy myocytes.

The process of CICR is most effective in ventricular cells where the t-tubules are more abundant than in atrial cells. The t-tubule network is responsible for just one-third of the capacitance of the membrane, but most of the influx of Ca^{2+} that triggers the release of intracellular SR Ca^{2+} enters across the t-tubular fraction. This is because there is a different complement of ion channels in the surface and t-tubular fractions, with a threefold to ninefold higher number of LCCs in the t-tubule fraction than in the sarcolemma surface.

1.3.2 Z-Planes

The basic myocyte (also called muscle fiber) structure, are the sarcomeres, the contractile units of the cell. The sarcomeres are composed of thin and thick filaments. Thin filaments are made of actin and thick filaments are made of myosin. This proteins generate the contraction when the calcium is binded to the TnC. The sarcomeres are arranged forming long chains in what are called the myofilaments or myofibrils, which are grouped within the surface sarcolemma, forming a symmetric transverse structure. A cut of this transverse structure is what we call the z-plane (Fig. 3a).

The myofilaments represent 45 to 60 per cent of the cardiomyocytes’ volume. These contractile complexes are organized in such way that a minority of these units are close to the surface sarcolemma. In cells without t-tubules, the wave of Ca^{2+} propagates from the periphery of the cell into the centre either by simple diffusion or by a wave of propagated CICR. Such a system would first activate the peripheral sarcomeres and then the deeper sarcomeres, resulting in sub-maximal force production. A system where current is simultaneously relayed to the core of the cell would mean a larger instantaneous force is produced, which is more equally shared between sarcomeres. The t-tubules make this possible by triggering SR Ca^{2+} release near to all sarcomeres simultaneously, regardless of how deep they lie within the cell. Atria cells have a lack or poor development and irregular organization of t-tubules. Due to this, appears a delay between the calcium release in the sarcolemma and the inner cell, not present in ventricular myocytes.

![Figure 3: a) Z-plane muscle fiber structure. b) Three-dimensional structure of the t-tubular system in a rat ventricular myocyte.](image-url)
1.4 Objectives

Understanding the functioning of the cardiac cycle is crucial for the prevention of cardiac dysfunction and arrhythmias. To this end, the first objective was to study the physiology of the heart and the theory after the excitation-contraction process, to acquire a high degree of knowledge that allowed us to cover the work in more detail and professionalism, which has been shown with the previous highly technical introduction.

In order to continue in our study of the functioning if the heart, Ca$^{2+}$ fluxes during the excitation-contraction coupling process and the role of the main regulators that control the dynamics of this ion in the different microdomains of myocytes are investigated in a mathematical model of a z-plane of a ventricular cell. We tried to understand calcium homeostasis by playing with a series of parameters that have a direct impact on the response and self-regulation of this process, in order to understand who controls the Ca$^{2+}$ transient, that in the end is the responsible of the heart contraction. Since there are a lot of mechanisms integrated working in the homeostasis cycle, trying to control one feature is not easy because it affects the behavior of the others. The parameters studied were: currents conductivities ($g$) of the currents $J_{rel}$, $J_{up}$, and $J_{NaCa}$, corresponding to SR Ca$^{2+}$ release during the action potential and activity of the SERCA pump and the NCX to decrease $[Ca^{2+}]_i$ during repolarization; the SR Ca$^{2+}$ equilibrium constant ($K_{SR}$); and finally the transition rates $k_{on}$, $k_{off}$ and $k_{in}$, that define the stochastic behavior in the changes of state of the RyR, which makes a total of seven variables to regulate the homeostasis.

Given that the first goals were reached quickly, an objective not present on the initial schemes was proposed. It consisted in analyze the homeostasis from an original point of view. Since we are looking for stationary states, where the calcium that leaves the SR must return during the relaxation, avoiding SR depletion. Therefore after a beat the SR concentration must be restored, within a few margins. We will apply an SR recovery constraint protocol to be able to select sets of realistic parameters that can produce regular homeostasis, and study the transient and SR depletion after one beat simulations, with no NCX activity. In this way we will study how to control the transient amplitude in order to achieve an steady state solution for a given SR load, which within all the selected parameters, is the normally the one that can be measured experimentally.

Finally, we will prove whether is possible to stabilize the SR load with the NCX through its conductivity, $J_{NaCa}$, in the event that the others are fixed to a certain values, obtained from the previous constraint analysis. We want to know how the transient affects to the NCX activity and see how far the exchanger is able to stabilize the homeostatic response, to demonstrate that control of homeostasis is possible thanks to the exchanger in cases of cardiac abnormality in which the SR load does not stabilize at the initial value, causing the SR load to increase with each beat and produce irregular [Ca$^{2+}$] transients.
Chapter 2

Model

The experimental and clinical possibilities for studying cardiac activity in human ventricular myocardium are very limited. Therefore, the use of alternative methods such as computer simulations is of great importance. In this study we use a mathematical model that reproduces detailed properties of a Z-plane of a single ventricular cell, such as the major ionic currents, calcium transients, and action potential duration and restitution. (Model based on ref. 21)

Figure 4: Model of z-plane CaRUs structure with the different calcium domains. One Z-plane is made of multiple Calcium Release Units or CaRUs. Each CaRU has an LCC-RyR junction and five microdomains where the calcium concentration evolution is determined following a differential equation where the main fluxes are represented.
2.1 Description of the Model

The cardiomyocyte is considered as a two-dimensional array of calcium release units (CaRUs), which represents the z-plane of the cardiomyocyte. Each CaRU is formed by a cluster of RyRs opposite to a group of LCCs, situated at the t-tubules of the sarcolemma, simulating a couplon. CaRUs have internal dynamics of calcium ions and diffusion of calcium among neighboring CaRUs. They are divided into five different sub-compartments where the corresponding calcium concentration is defined (Fig. 4). The dyadic concentration \( c_d \), which is the concentration in the space between the cluster of RyRs and the LCCs. The cytosol concentration \( c_s \). The network SR \( c_{SR} \) and junctional SR \( c_{jSR} \) concentrations, of the SR interior and the junction of SR \( jSR \) with the RyRs cluster. And finally the subsarcolemma concentration \( c_{sl} \), concentration of the transition zone between the dyadic space and the cytosol, consisting on a few nm depth layer below the membrane.

2.1. Deterministic Equations

The dynamics of all fluxes is deterministic except for the transition between the different states of the RyR and LCC, which is modeled stochastically. The dynamic equations defining the evolution of calcium concentrations of the different compartments in each CaRU are the following:

\[
\frac{dc_d}{dt} = -J_{Ca} + J_{rel} - J_{ds} \tag{1}
\]

\[
\frac{dc_s}{dt} = \beta(c_s)[\frac{v_d}{v_s}J_{ds} - J_{si} + J_{NaCa} - J_{buff}^{TiCs} + D_s \nabla^2 c_s] \tag{2}
\]

\[
\frac{dc_i}{dt} = \beta(c_i)[v_s J_{si} - J_{buff}^{TICl} - J_{up} + D_i \nabla^2 c_i] \tag{3}
\]

\[
\frac{dc_{SR}}{dt} = \frac{v_i}{v_{sr}}J_{up} - J_{tr} + D_{SR} \nabla^2 c_{SR} \tag{4}
\]

\[
\frac{dc_{jSR}}{dt} = J_{tr} - \frac{v_d}{v_{jSR}}J_{rel} \tag{5}
\]

Diffusion among CaRUs is implemented using a five-point laplacian that considers the four nearest neighbors of a particular CaRU. It is only present on adjacent compartments of different CaRUs, and these are the subsarcolemma space, the cytosol and the SR. \( D_s \), \( D_i \) and \( D_{SR} \) are the diffusion coefficients. There are also diffusive currents among compartments within the same CaRU. From the dyadic to subsarcolemma and then to the cytosol \( J_{ds} \) and \( J_{si} \), and from network SR to jSR \( J_{tr} \). They are taken to be proportional to the concentration difference, with constant relaxation times.

The presence of buffers is neglected in SR and only considered in cytosolic and subsarcolemma spaces. Buffering dynamics is treated differently depending on whether calcium attaches to TnC or to other buffers like calmodulin. \( J_{buff}^{TiCs} \) and \( J_{buff}^{TICl} \) are the currents due to dynamic attachment to TnC. They are given by the binding and unbinding rates, and the concentration of TnC in each compartment. The other buffers are treated in the rapid equilibrium approximation using the functions \( \beta(c_i) \) and \( \beta(c_i)^{22} \).
Relations between volume compartments also play a role on the magnitude of currents among compartments. The volume of the cytosol \(v_i\) in a CaRU is \(\approx 0.5\ \mu m^3\), while the volume of network SR \(v_{SR}\) and jSR \(v_{jSR}\) is 20 times smaller. The subsarcolemmal space is considered to be 100 times smaller than the cytosolic volume, and the dyadic space is roughly 1200 times smaller.

The other fluxes correspond to the inward calcium current from the extracellular medium to the dyadic space through the LCCs \(J_{Ca}\), the outward flux from the subsarcolemma to the extracellular medium due to the NCX \(J_{NaCa}\), the uptake of calcium from the cytosolic medium into the SR due to the SERCA pump \(J_{up}\), and the release of calcium from the junctional SR into dyadic space \(J_{rel}\).

For the calcium concentrations on the dyadic and subsarcolemma spaces, a rapid equilibrium approximation is also considered, and their time derivatives are approximated to zero. Due to their reduced volume they arrive to their corresponding stability values very fast.

\[
\frac{dc_d}{dt} \approx 0 \quad \frac{dc_s}{dt} \approx 0
\]

Global concentration in the cytosol, subsarcolemma or SR are obtained using spatial averages of the concentration in the compartments of each CaRU.

The NCX, LCC states and the inward calcium current \(J_{Ca}\) are explicitly dependent on transmembrane potential, which is introduced by stimulating the cell at a pacing period \(T\) with a clamped action potential given by:

\[
V_m(t) = \begin{cases} \frac{(V_{max} - V_{rest})}{V_{rest}}(t - \frac{A PD}{t})^2 & \text{if } \tilde{t} < A PD \\ V_{rest} & \text{if } \tilde{t} > A PD \end{cases}
\]

where \(\tilde{t} = t - nT\), with \(n = 1, 2, \ldots\), and where the action potential duration (APD) is a function of the period (in ms) given by \(A PD = 100T/(100 + T)\). In our model transmembrane potential is clamped because otherwise it will not only depends on the calcium concentrations but would also be affected by the sodium and potassium fluxes, which are not integrated in our simulation.

### 2.1.2 NCX and SERCA Pump

In our model, sarcolemmal Ca\(^{2+}\)-ATPase and mitochondrial Ca\(^{2+}\) uniport are not integrated since they only represent the 1% of the total Ca\(^{2+}\) extracted during the cytosol during repolarization.

On the other hand, the crucial NaCa exchanger current is given by:

\[
J_{NaCa} = \frac{g_{NaCa} e^{\eta \tilde{t}}[Na]^3[Ca]_o - e^{(\eta-1)\tilde{t}}[Na]_o^3c_s}{1 + (K_{da}/c_s)^3} \frac{e^{\eta \tilde{t}}[Na]^3[Ca]_o - e^{(\eta-1)\tilde{t}}[Na]_o^3c_s}{S(c_s)(1 + k_{stat}e^{(\eta-1)\tilde{t}})}
\]
with \( z = V_m F / (RT) \), where \( V_m \) is the membrane potential, \( F \) the Faraday constant, \( R \) the constant of gases and \( T \) the temperature. It depends on extracellular calcium and sodium concentrations \([Na_o]\), \([Ca_o]\), and on intracellular sodium \([Na_i]\). \( S(c_s) \) is a function of sarcolemmal concentration which depends also on calcium and sodium concentrations and its equilibrium constants.

The SERCA pump is considered to be thermodynamically limited and the associated current is a function of the cytosol and SR calcium concentrations and their corresponding equilibrium constants:

\[
J_{up} = g_{up} \frac{(c_i/K_i)^2 - (c_{SR}/K_{SR})^2}{1 + (c_i/K_i)^2 + (c_{SR}/K_{SR})^2}
\]

The associated conductivities \( g_{NaCa} \) and \( g_{up} \) are two of the seven key variables we will work later on with the study of the homeostasis. Modifying these parameters will give or extract power to their corresponding fluxes. The SR equilibrium constant \( K_{SR} \) will be another studied variable. It gives the concentration of \( Ca^{2+} \) inside the SR reached at steady state and an idea of the capacity of the SR to store \( Ca^{2+} \). It will have special importance in which will be the corresponding value of \( Ca^{2+} \) recovery that must be accomplished in the recovery constraint.

### 2.1.3 Stochastic Dynamics

Calcium release and L-type calcium current do not follow an strict deterministic equation since they depend on the number of RyRs or LCCs in the open state. Both kind of channels change between its different possible states following an stochastic law based on a serie of transition rates.

The inward calcium current from the extracellular medium towards each CaRU is dependent on the number of LCC channels in the open state \( O_{LCC} \) (Fig. 5), voltage, and the average value of calcium close to the membrane \( c_s \), according to:

\[
J_{Ca} = g_{CaLO_{LCC}} 4z_m \frac{e^{2z} c_s - [Ca_o]}{e^{2z} - 1}
\]

where \( z \) is defined as in the previous subsection and \( z_m = 0.341 F \). To compute \( O_{LCC} \) we consider the presence of 5 LCC channels in each CaRU with five possible states (Fig. 5): two closed states \( (C_1 \) and \( C_2) \), two inactivated states \( (I_1 \) and \( I_2) \) and one open state \( (O) \). The stochastic dynamics of the transitions is implemented for each of the LCC channels. At each time step a RANMAR algorithm to select a random number is used for each LCC and change its state depending on this number according to the transition rates. Some rates are directly dependent on voltage, leading to the opening of LCC when the membrane depolarizes, and similarly, some rates dependent on calcium concentration lead to the inactivation of the LCC channel.
Figure 5: Five-state model of an LCC, with their corresponding transition rates. Some rates are dependent on voltage (open) and others on calcium concentration (inactivation).

In each CaRU there is a cluster of 70 RyRs. As before, we consider each of them can be in four different states (Fig. 6): Close (C), open (O), and two inactivated states ($I_1$ and $I_2$). Calcium release from the jSR to the dyadic space is taken to be proportional to the concentration difference and the number of RyR in the open state:

$$J_{rel} = g_{rel} O_{RyR} (c_{jSR} - c_d)$$  \hspace{1cm} (11)

The transition dynamics between each state is performed again using a RANMAR algorithm at every time step for each RyR channel, changing its states in function of that random number and the transition rates. For RyRs some transition rates are dependent on the dyadic concentration, in order to simulate the $[\text{Ca}^{2+}]_{i}$ stimulatory effect on the open probability.

Figure 6: Four-state model of a RyR. In our model $k_{on}$ and $k_{in}$ are divided by a factor $10^4$ and $10^2$ respectively. \hspace{1cm} (23)

In order to control the homeostasis in the following simulations some of these parameters will be taken as variables in the study. The release conductivity $g_{rel}$ to control SR $\text{Ca}^{2+}$ release, and three of the transition rates: the responsible to open the closed channels ($k_{on}$); the responsible to close the open channels ($k_{off}$); and finally the inactivation rate, responsible to its inactivation ($k_{in}$). \hspace{1cm} (23)
2.2 Model Implementation

The model is implemented using fortran, a high level programming language that is mainly used for scientific and mathematical purposes. With it, the CaRUs matrix is created simulating the z-plane of a ventricular cell. The differential equations that govern the evolution of the calcium flow in the different CaRU domains are programmed and solved in each element of the matrix by an Euler mathematic method, for a given period of time, which is fixed by the number of pulsations desired. The main code uses a second auxiliary code, the RANMAR, which is a random number generator. It is used to represent the stochastic part that the LCC and RyR channels introduce into the system. Depending on the number generated, it is decided whether a given channel changes state. The result of using the main code is the evolution of the calcium concentrations, as well as the main currents and the states of the RyR.
Chapter 3
Study of the Homeostasis

3.1 General Homeostasis and Steady State

A steady state solution is reached whenever in each cycle or beat the concentration of calcium in each of the sub-compartments of the myocyte recovers its original value to start a new cycle from the same starting point. In this way a stable homeostasis is achieved, which results in a stable cardiac response. To achieve this type of behavior, all the systems participating in the regulation of homeostasis have to work at the same time, compensating each other and with well-established parameters.

As described in the introduction, various mechanisms are involved during Ca\(^{2+}\) homeostasis in order to regulate the calcium fluxes and reach this steady state, providing an adequate cardiac response. These mechanisms are integrated with each other so that myocytes are able to self-regulate calcium levels in the different microdomains of the cell through a positive feedback process. In the model described previously, each of the minimum cell structures that make this possible, the calcium release units (CaRUs), is simulated. In each of them, the dynamic equations that determine the calcium concentrations are executed taking into account all these regulatory mechanisms, and the total concentration of calcium in the different microdomains is extracted by spatial averages. In order to understand this process we have studied how a series of variables associated with the control of homeostasis affect the regulation of calcium. The parameters studied are a total of seven and directly control the SERCA pump, the NCX, the SR Ca\(^{2+}\) release, the SR load and the state changes in the RyRs (Tab. 1). To this end, it has been observed how small variations of each of these parameters modify independently a steady state solution (Fig. 7 and Fig. 8).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{SR})</td>
<td>SR equilibrium constant</td>
<td>Regulates SR Ca(^{2+}) load</td>
</tr>
<tr>
<td>(g_{rel})</td>
<td>SR release conductivity</td>
<td>Regulates (J_{rel})</td>
</tr>
<tr>
<td>(g_{NaCa})</td>
<td>NCX conductivity</td>
<td>Regulates NCX power (J_{NaCa})</td>
</tr>
<tr>
<td>(g_{ap})</td>
<td>SERCA conductivity</td>
<td>Regulates SERCA power (J_{ap})</td>
</tr>
<tr>
<td>(k_{on})</td>
<td>RyR open rate</td>
<td>Controls number of open/close RyR</td>
</tr>
<tr>
<td>(k_{off})</td>
<td>RyR close rate</td>
<td>Controls number of open/close RyR</td>
</tr>
<tr>
<td>(k_{in})</td>
<td>RyR inactivation rate</td>
<td>Controls number of inactivated RyR</td>
</tr>
</tbody>
</table>

Table 1: Controlled parameters in the study of the homeostasis.
Figure 7: Average $[\text{Ca}^{2+}]_{\text{SR}}$ (a) and $[\text{Ca}^{2+}]_i$ (b) in a steady state homeostasis response with $K_{\text{SR}} = 1250$, $g_{\text{NaCa}} = 45$, $g_{\text{up}} = 0.5$, $g_{\text{rel}} = 2$, $k_{\text{on}} = 0.5$, $k_{\text{off}} = 0.2$ and $k_{\text{in}} = 0.3$. (Excitation frequency of 500 ms). \(^{23}\)

Figure 8: Average currents (a) and number of RyR channels at closed and open state (b) in a steady state homeostasis response with $K_{\text{SR}} = 1250$, $g_{\text{NaCa}} = 45$, $g_{\text{up}} = 0.5$, $g_{\text{rel}} = 2$, $k_{\text{on}} = 0.5$, $k_{\text{off}} = 0.2$ and $k_{\text{in}} = 0.3$. (Excitation frequency of 500 ms). \(^{23}\)

In Fig. 7 and Fig. 8, we illustrate what was explained previously in the theoretical basis about the dynamics of homeostasis during the cardiac cycle. At the beginning of the action potential the RyR channels are opened due to the triggering $J_{\text{Ca}}$ that crosses the membrane through the LCC (Fig. 8b). This causes the calcium concentration inside the SR to decrease (Fig. 7a) while the calcium concentration in the cytosol increases (Fig. 7b). This calcium will be attached to the TnC to produce the contraction. The high levels of $[\text{Ca}^{2+}]_i$ activate the operation of the SERCA pump and the NCX, which causes the transient to decrease and the start of SR recovery, resulting in the relaxation phase.

Due to the fact that dyadic concentration is treated on the fast equilibrium approximation, the dyadic calcium when $J_{\text{Ca}}$ enters the cell diffuse to neighboring compartments really fast, which makes the RyR to close or inactivate quickly. It’s important to remark that in this case we have an optimum response because the values of key parameters have been properly selected. The $[\text{Ca}^{2+}]_{\text{SR}}$ at this precise level of SR calcium load (1250 μM) is in steady state because NCX works on getting out of the cell what enters trough $J_{\text{Ca}}$ in each beat. So SERCA pump returns into SR the same amount of $\text{Ca}^{2+}$ released trough the RyRs in each cycle, letting the recovery to be regular and stable.
3.1.1 SR Variations

The amount of Ca\(^{2+}\) that can be stored by the SR is influenced by its equilibrium constant \(K_{SR}\), and the quantity of Ca\(^{2+}\) released through the RyR its determined by number of channels in the open state, which is given by their transition rates (they will be treated separately) and its conductivity \(g_{rel}\). In order to see how modifying these parameters impacts on the homeostasis we proceed to study the variations of each parameter independently, with all the other variables fixed, using the Fig. 7 steady state response.

Increasing the SR capacity (\(K_{SR} = 1800 \mu M\)) means biggest release due to the \([Ca^{2+}]_{SR}\) stimulatory effect on RyR open state, which translates in bigger transient amplitudes (Fig. 9c). But maintaining the same SERCA and NCX activity causes the total SR charge to decrease with time (Fig. 9a), since we are increasing SR depletion without increasing SR recovery. Consequently, transient decreases in each beat (Fig. 9c). The higher \([Ca^{2+}]\), values due to bigger releases makes NCX switch to extrusion mode before, which as stated previously works mainly in Ca\(^{2+}\) extrusion mode, but only after the action potential when stimulated by high cytosolic calcium concentrations. Changing sooner NCX to extrusion mode makes it compete with the SERCA pump more time. On the other hand, decreasing SR capacity (\(K_{SR} = 900 \mu M\)) results on the opposite effect. Less charge means less release and smaller transients (Fig. 9c). The SR Ca\(^{2+}\) load increases with time (Fig. 9a) because the exchanger works less time on the extrusion mode competing less with SERCA. Also \([Ca^{2+}]\) grows in time, but this is more visible with conductivity variations, where similar responses occur.

*Figure 9: Average \([Ca^{2+}]_{SR}\) and \([Ca^{2+}]_i\) for different values of \(K_{SR}\) (left) and \(g_{rel}\) (right). On the left we see how high loads translate into high transients and low loads into small releases. On the right we see that for high release conductivities the transient amplitudes are larger, and that there is small or even no release for low conductivities. The overall effect is that for large transients the SR total calcium concentration decreases with time, and increases for small transients.*
We proceed to check what happens when the release conductivity changes with the other parameters fixed. Low conductivities \((g_{rel} = 0.5)\) lead to low or even negligible \(Ca^{2+}\) transients (Fig. 9d), and consequently the same effect as before in NCX activity. It competes less time with SERCA, so the SR overloads with time (Fig. 9a). The same occurs with high values \((g_{rel} = 80)\) where high peak transients change the exchanger activity to extrusion mode sooner decreasing the amount of \(Ca^{2+}\) pumped back into the SR, lowering the load in time and decreasing \([Ca^{2+}]_i\) (Fig. 9d).

With SR conductivity is more easy to see what happens to the total calcium balance within the cell. High releases, which causes hight transients (Fig. 9d), favors the inactivation of the LCC, which means less \(Ca^{2+}\) is entering the cell. This added to the fact that in this cases NCX works more time pumping \(Ca^{2+}\) outside the cell, decreasing the charge of SR in each beat, gives us a total negative balance of calcium within the cell. This can be extrapolated to the opposite case, where positive balance is achieved when LCC channels are not inactivated due to low transients and the exchanger works in influx mode more time, so a gain on \([Ca^{2+}]\), and \([Ca^{2+}]_{SR}\) appears at every cycle.

### 3.1.2 SERCA and NCX Variations

As we have seen before, during repolarization SERCA and NCX compete with each other to reduce cytosolic calcium concentration, in order to restore the intracellular calcium level. Modifying its activity leads to an imbalance between the flow that enters and exits both through the sarcolemma and SR, causing the charge of the cell and the SR to increase or decrease with each beat. In this sense enhancing the activity of one is equivalent to reduce that of the other. Repeating the same process as before, we are going to modify independently SERCA and NCX conductivities, in order to see how they change the steady state homeostatic response.

Low SERCA conductivity \((g_{up})\) or high NCX conductivity \((g_{NaCa})\) carry a negative total balance of calcium, reducing the SR load (Fig. 10a and 10b) and, in turn, reducing the transient in each cycle (Fig. 10c and 10d). The opposite situation carry a positive total calcium balance that increases SR load and transient peak amplitude. Notice that SERCA activity has a more relevant role in the speed of recovery, higher values in \(g_{up}\) result in more pronounced transient decay and much faster recovery of \([Ca^{2+}]_{SR}\) (Fig. 10a). Notice also that, for no exchanger activity \((g_{NaCa} = 0)\), there is no calcium going out the cell, so all calcium crossing the sarcolemma through LCC stays within the cell. For no SERCA activity \((g_{up} = 0)\), there is no recovery and all the SR \(Ca^{2+}\) release remains at the cytosol escaping slowly through the exchanger instead of being pumped back to the SR (Fig. 10a).
Figure 10: Average $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{i}$ for different values of $g_{\text{up}}$ (left) and $g_{\text{NaCa}}$ (right). For high NCX or low SERCA activity there is a negative calcium flux balance (c and d), and the opposite for large SERCA and low NCX. No SERCA activity implies no SR recovery (a) and a big transient decreasing with time (c).

3.1.3 Effects of RyR Transition Rates on Homeostatic Levels

We proceed now to investigate how the RyR transition rates affects the homeostatic response, modifying each rate independently while maintaining the other parameters fixed, in order to break the steady state achieved in Fig 7. The state changes of the RyRs are simulated following a Markovian stochastic model based on a series of transition rates, such as $k_{\text{on}}$, $k_{\text{off}}$ and $k_{\text{in}}$, that determine the probability of moving from one state to another, being the probabilities of changing from close to open, from open to close and to inactivate the channel, respectively. Here, closing and inactivation rates play similar roles, and are completely opposite to the open rate. Increasing or decreasing those values translate in the RyR state population distribution.

As the transition rate of open channels is decreased, or the other two increased, SR $\text{Ca}^{2+}$ release falls causing low transients (Fig 11b, 11d and 11f), since the population of open channels drops. Low cytosolic calcium concentration means less LCC inactivation and a reduced exchanger activity on extrusion mode, followed by the corresponding increase on $[\text{Ca}^{2+}]_{i}$ and $[\text{Ca}^{2+}]_{\text{SR}}$ in each beat, due to the extrusions systems competition (Fig 11a, 11c and 11e).

In the limit where all channels are closed or inactivated ($k_{\text{on}} = 0$), there is no release from SR (Fig. 11a), and the total calcium concentration increases as $J_{Ca}$ enters the myocyte in each excitation. The other limit is when all the channels are open regardless of the $J_{Ca}$ triggering effect, which causes the SR to be emptied instantaneously until it reaches equilibrium with $[\text{Ca}^{2+}]_{i}$, since what is pumped back by the SERCA is immediately ejected again (Fig 11c). This is translated into a extremely large
transient (Fig. 11d) at the first beat which leads to a high NCX activity during a short period of time. Then the NCX exchanger keeps slowly emptying the cell.

Notice that for high open probabilities, some RyR channels open even during repolarization, as it is observed on Fig. 11a for $k_{on} = 5^{23}$. This illustrates what has been already explained about the SR load stimulatory effect on the RyR open probability. For high open rates the channels are more sensitive to $[\text{Ca}^{2+}]_{\text{SR}}$ stimulation for a given $[\text{Ca}^{2+}]_{i}$.

Figure 11: Average $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{i}$ for different values of $k_{on}$ (up), $k_{off}$ (middle) and $k_{in}$ (down).$^{23}$ Increasing $k_{on}$ has the same effect that decreasing $k_{off}$ or $k_{in}$. High $k_{on}$ leads to high SR releases (a) and high transients (b). High transients evolve in a loss of load with time. The opposite effect occurs for low $k_{on}$. In the limit $k_{on} = 0$ there is no release (a), and in the limit $k_{off} = 0$, SR is constantly depleted.
3.1.4 Summary

What guides the evolution of homeostasis is mainly the behavior of the transient, which in turn depends on all the parameters that regulates the calcium fluxes within the cell (Tab. 2). These parameters are the SR load capacity, the main flux conductivities and the RyR transition rates. From the previous analysis, we can conclude that the different parameters affect in distinct ways to the transient peak, but at the end, for a fixed SR load, large transients always decrease with time, and small transients increase. In this way, the evolution of the transient is already defined at the first beat, since its behavior its always related with the transient amplitude on the previous cycle. This is what we refer when we speak of a system of self-regulation through feedback.

However, not all parameters have the same impact, small changes in some may affect the behavior of intracellular calcium more than in others. In Figs. 9-11 we see that the changes that affect the release are those that alter the most the steady evolution of our base system, namely the changes in $g_{rel}$ and the transition rates, especially $k_{on}$. It makes sense given that these parameters are those that directly controls the evolution of the transient. A major release translates into a higher transient peak.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INCREASE</th>
<th>DECREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{SR}$</td>
<td>Transient decrease</td>
<td>Transient increase</td>
</tr>
<tr>
<td>$g_{rel}$</td>
<td>Transient decrease</td>
<td>Transient increase</td>
</tr>
<tr>
<td>$g_{NaCa}$</td>
<td>Recovery decrease - SR load decrease</td>
<td>Recovery increase - SR load increase</td>
</tr>
<tr>
<td>$g_{up}$</td>
<td>Recovery increase - SR load increase</td>
<td>Recovery decrease - SR load decrease</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>Transient decrease</td>
<td>Transient increase</td>
</tr>
<tr>
<td>$k_{off}$</td>
<td>Transient increase</td>
<td>Transient decrease</td>
</tr>
<tr>
<td>$k_{in}$</td>
<td>Transient increase</td>
<td>Transient decrease</td>
</tr>
</tbody>
</table>

Table 2: Summary of how changes in each parameter mainly affect the homeostatic response. Transient decrease usually is preceded by an initial transient increase, and transient increases by an initial transient decrease.
3.2 General Regulation

To better understand how the process of regulation works, we have tried to stabilize two different possible homeostasis starting only with the load capacity value, which is generally the only one within all the studied parameters that can be measured experimentally. Starting from one real and known value we can estimate the magnitude of the others if we achieve to simulate a realistic homeostasis. We want to generate steady state solutions maintaining the SR load on a desired value. It is not straightforward getting an homeostatic response at a determined SR load, since all the parameters are integrated and must be properly selected. A full understanding of its relations and effects must be required to generate a homeostasis control. Two realistic values of $K_{SR}$ have been chosen, 900 and 1800μM, and with the help of our first steady state homeostasis simulation for $K_{SR} = 1250$, we have tried to adjust the response with small variations of the parameters, using the knowledge acquired in the homeostasis study. For that we have recycled the simulations from Fig. 9a and 9c, where we have the all the parameters for the 1250μM homeostasis, but with the SR load changed. This will be the starting point from where our regulation will start.

For high loads ($K_{SR} = 1800μM$), as stated in SR variations section, is necessary to compensate the lost of $Ca^{2+}$ in time in order to put the recovery and $[Ca^{2+}]_i$ in steady state (Fig 12a). Due to the high concentrations of intracellular calcium the two extraction systems will have to work at higher power, but the increase in SERCA activity necessarily has to be bigger if we want it to compete better with NCX, since this is the main cause of the $[Ca^{2+}]_i$ loss. Reducing the release conductivity leads to a lower transient peak, less inactivation of the LCC and consequently less NCX work. This can also be done by acting on the probability of the different states of the RyR, balancing the probability to the closed state. The union of all these relationships allows us to find a set of values that are balanced with each other and provide stable homeostasis (Fig. 12b).

![Figure 12: Average $[Ca^{2+}]_SR$ in the non controlled homeostasis (a) and in the regulated homeostasis response (b) with $K_{SR} = 1800$. In a) $g_{NaCa} = 45$, $g_{up} = 0.5$, $g_{rel} = 2$, $k_{on} = 0.5$, $k_{off} = 0.2$ and $k_{in} = 0.3$. In b) $g_{NaCa} = 50$, $g_{up} = 0.7$, $g_{rel} = 1.5$, $k_{on} = 0.4$, $k_{off} = 0.2$ and $k_{in} = 0.3$. In order to compensate the SR load loss SERCA and NCX have to work higher, but SERCA has to compete better with NCX. Decreasing the release conductivity and the open state probability, the NCX works less time in efflux mode.

In the same way it is possible to do the inverse analysis, when we are in the case of SR with low loads ($K_{SR} = 900μM$). Now the NCX has to work more in comparison to the SERCA pump, although both decrease the power by the low concentrations of $[Ca^{2+}]_i$. The transient increase for the inactivation of $J_{Ca}$ and the exchanger start is achieved by the modification of the release conductivity and the transition rates (Fig. 13).
Figure 13: Average $[\text{Ca}^{2+}]_{\text{SR}}$ in the non controlled homeostasis (a) an in the regulated homeostasis response (b) with $K_{\text{SR}} = 900$. In (a) $g_{\text{NaCa}} = 45$, $g_{\text{ap}} = 0.5$, $g_{\text{rel}} = 2$, $k_{\text{on}} = 0.5$, $k_{\text{off}} = 0.2$ and $k_{\text{in}} = 0.3$. In (b) $g_{\text{NaCa}} = 32$, $g_{\text{ap}} = 0.3$, $g_{\text{rel}} = 5$, $k_{\text{on}} = 0.6$, $k_{\text{off}} = 0.2$ and $k_{\text{in}} = 0.3$. 

In order to compensate the SR overload, SERCA and NCX have to work less, but NCX has to compete better with SERCA. Increasing the release conductivity and the closed state probability, the NCX works more time in efflux mode.

3.2.1 Summary

Since homeostasis involves many related events happening in chain, modifying one single variable affects all the system. We have demonstrated that not all the chosen parameters have the same level of impact on the response. After some observations, we know that release conductivity and the transition rates, which have direct impact on the transient amplitude, are the principle responsables of the transient variations given a fixed SR load. In this section the excitation-contraction coupling process it was already understood, and the necessary modifications to achieve an steady solution known. But finding the magnitude of this variations has been a trial and error process. A more specific study is necessary to understand in witch magnitude they affect to the homeostasis response, because is possible to get an stable behavior at the sarcoplasmic reticulum but not a regular cardiac activity due to instabilities at the Ca$^{2+}$ transient. Not every transient can be stabilized into a desired SR load, and not every set of parameters can generate an steady state response. In order to find this kind of solutions, in chapter 4 we will impose a constraint that narrows the parameter search field, based on the SR recovery.
Chapter 4
Constraint Analysis

Up to now, we have learn how the different homeostasis regulatory mechanisms are related and how they affect the homeostatic response. We have even been able to regulate in our model an homeostasis stabilizing the SR load charge to a given value. But we have seen that not all the transients can be stabilized in order to achieve an steady state response and, obviously, not every set parameter can generate an homeostatic response. In our homeostasis control, although we know how the parameters were related, giving with the proper values has been a trial and error process. Is not straightforward which transients will be controllable.

In this sense, we have been able to go even further than expected and develop a novel control technique, which allows us to asses the relevance of the different parameters on the calcium transient regime. We will impose a requirement that must be accomplished by our possible homeostasis candidates. As stated before, in a steady state homeostasis concentration of calcium in each of the sub-compartments of the myocyte must recover its original value to start a new cycle from the same starting point. This means that Ca\(^{2+}\) released from the SR must be pumped back, and \([\text{Ca}^{2+}]_{\text{SR}}\) must recover to its basal concentration during the diastole. Using this constraint during the first cardiac beat, within a reasonable margins of the 10\%, will let us to avoid most of the SR depleted or SR overloaded systems.

The constraint analysis will let us to study in more detail how the release current and the RyR controls the transient amplitude, which transients can be stabilized by the NCX and how the transient amplitude affects the NCX regulatory effect, when the desired SR load is fixed.

4.1 Constraint Maps

The constraint maps are two-dimensional analysis from one interesting homeostasis feature, as could be the transient amplitude or the SR recovery. But not the entire map belongs to the area of interest, only those points that satisfy the imposed constraint (Fig. 14). Here we will study how the main transient regulators, the RyR and the release conductivity, affects the maximum transient value, or transient peak, when is imposed that the SR concentration must return to its original value after one beat and with no NCX activity. This means SERCA must pump back calcium into SR to recover \([\text{Ca}^{2+}]_{\text{SR}}\) to its starting value, avoiding SR depletion and a positive or negative balance of \text{Ca}^{2+}\ within the whole cell, which is an essential condition for achieving an steady state solution.
The map is created sweeping over two parameters and saving not only the transient peak but also the SR calcium concentration before the next beat starts, which is the achieved concentration after the SR recovery. In order to accomplish our constraint this value has to be near (within margins) to the initial load concentration (Fig. 14). If it satisfies the constraint, the corresponding transient will be a candidate for an steady state response, with the SR calcium load being stable an equal to its initial value.

Figure 14: Constrain map in the $g_{rel}$ - $k_{on}$ plane of the SR recovery after one beat without NCX activity. $K_{SR} = 1250$, $k_{in} = 0.5$, $g_{NaCa} = 0$, $g_{up} = 0.2$ and $k_{off} = 0.2$. The points that satisfy the constraint are those that fall within the load margins, which are illustrated with the black curves. 

a) There is no release, overloaded response. 

b) There is release and the recovery is near 1250μM. 

c) The SR is not refilled, depleted response.
4.1.1. $g_{rel} - k_{on}$ Recovery Constraint Maps

As we could expect, in the SR recovery $g_{rel} - k_{on}$ constraint map (Fig. 14) is illustrated how increasing both parameters provoke smaller recoveries or SR depletion. As we have already seen, increasing the release conductivity means bigger SR releases and large transients. This makes the NCX change to efflux mode sooner and compete better with SERCA. The result is the SR depletion (Fig 14c). When the conductivity is decreased, the opposite happens. Transients are smaller, and SERCA competes better with the NCX. In the limit, there is no release and the SR overloads (Fig. 14a). Changing the open transition rate has the same response, since bigger open rates means more open RyR and larger releases, and low open rates means low releases, and no release in the all-channel-closed limit.

But the real point here is the study of the region satisfying the constraint. If we measure the average transient peak for each open rate, inside the constraint region (an average over all the points with the same $k_{on}$ that falls within margins), we observe that it is not constant (Fig. 15). It exists an optimum open rate that causes the maximum transient, which must be associated to an optimum release conductivity. This could not be deduced in the previous analysis and is the result of imposing the recovery constraint. Since now not all the sets of parameters are allowed, the homeostasis search for all those combinations that makes her to remain within the constraint region. One of those combinations has the ability to generate the maximum transient peak response. This means that small changes in the open rate can lead to important changes into the homeostatic response.

![Image](image.png)

Figure 15: $\text{Ca}^{2+}$ transient peak mean for $K_{SR} = 1250$, $k_{in} = 0.5$ and $g_{NaCa} = 0$, $g_{up} = 0.2$ and $k_{off} = 0.2$. When the constraint protocol is imposed we can find an optimum open rate with an associated conductivity, which generate the largest release for a given set of parameters.

We have repeated this process for different fixed values in order to see how changing other parameters modify the behavior of this optimum open rate (Fig. 16). The range of values falling inside the constraint region is given by the fixed parameters. Modify one of them makes the open rate or the conductivity change in order to satisfy again the recovery constraint. This is the physics behind the movement of the key region among the constraint map. Increasing the SERCA activity (Fig 16a) or the closed-state probability (Fig.16b) means an increase on the $[\text{Ca}^{2+}]_{SR}$. In order to compensate it, the open rates and releases necessary to fulfill the constraint are now larger. This is illustrated in the displacement of the constraint region to the top right corner of the map (Fig 16a and 16b).
Let’s proceed to see what happens with the optimum open rate. We observe that the transient peak mean is not constant, so the optimum rate still exists. There must be an optimum pair of \( g_{\text{rel}} \) and \( k_{\text{on}} \) in each set of fixed parameters, that causes the maximum transient. In Fig. 16a, 16b and 16c we can observe how the optimum open rate increase as both the close rate and SERCA conductivity increase (comparing with Fig. 15). The explanation is the same as for the constraint region movement. Since the range of values falling inside the constraint region change in order to satisfy again the recovery constraint, with the new parameters, the optimum values changes correspondingly.

![Figure 16](image)

Figure 16: Recovery \( g_{\text{rel}} \cdot k_{\text{on}} \) constraint maps (up) and Ca\(^{2+}\) transient peak mean (down) for \( K_{\text{SR}} = 1250, k_{\text{in}} = 0.5 \) and \( g_{\text{Ca}^{2+}} = 0 \), where: a) \( g_{\text{up}} = 2 \) and \( k_{\text{off}} = 0.2 \); b) \( g_{\text{up}} = 0.2 \) and \( k_{\text{off}} = 1 \); c) \( g_{\text{up}} = 2 \) and \( k_{\text{off}} = 1 \). The range of values falling inside the constraint region is given by the fixed parameters. Modify one of them makes the open rate or the conductivity change in order to satisfy again the recovery constraint (up). The optimum values change in accord (down). (Notice the difference on the scales)

### 4.1.2. \( g_{\text{rel}} \cdot k_{\text{on}} \) Transient Constraint Maps

In a transient constraint map we just do exactly the same as before, the constraint still being the same. After the recovery, \([\text{Ca}^{2+}]_{\text{SR}}\) must return to its initial value in order to achieve possible steady state homeostasis. In this case we just move the key region to the transient map. This allow us to see which is the transient of those points that satisfies the recovery constrain. This is illustrated in Fig. 17 were the constraint region from the recovery map (Fig. 17a) is overlapped with the transient map (Fig. 17b). Now we can verify the existence of optimum values which generate the largest transients just by looking at the key region on the transient map. In the case of Fig. 17b it is located at the bottom-right corner. It also agrees with the maximum transient peak shown in Fig. 17c.

![Figure 17](image)

We can proceed as before and look for how changes on the fixed parameters are reflected on the constraint maps and the optimum open rate (comparing with Fig. 17). On Fig. 18 we can see how increasing SERCA pump makes the transient levels to decrease both in the constraint map (Fig. 18b) and consequently the maximum transient peak (Fig. 18c). But the interest relies in that the optimal open rate is practically the same that before. We can conclude that since SERCA does not participate in a direct way in the control of the release, modifying its activity does not affect so much
in the first cardiac beat, leaving the transition rates and the release conductivity as the main regulators of the transient.

Figure 17: a) Recovery $g_{rel}k_{on}$ constraint maps. b) Transient $g_{rel}k_{on}$ constraint maps. c) Ca$^{2+}$ transient peak mean. For $K_{SR} = 1250$, $g_{up} = 0.5$, $g_{NaCa} = 0$, $k_{in} = 0.2$ and $k_{off} = 0.1$. The constraint region from the recovery map (a) is overlapped with the transient map (b). Looking at the bottom-right corner we can distinguish the optimum point (b), which agrees with the

Figure 18: Recovery $g_{rel}k_{on}$ constraint maps (left). Transient $g_{rel}k_{on}$ constraint maps (middle). Ca$^{2+}$ transient peak mean (right). For $K_{SR} = 1250$, and $g_{NaCa} = 0$, where: abc) $k_{in} = 0.2$, $k_{off} = 0.1$ and $g_{up} = 2$; def) $k_{in} = 2$, $k_{off} = 1$ and $g_{up} = 0.5$. 23

We can continue exploring how the different combinations of transition rates affect to the global transient changing the RyR open population. In Fig 18d we can see how big are the values of open rate and release conductivity in order to accomplish the constraint when the open RyR population is reduced when both, the close and inactivation rates, increase. But although the magnitudes of that values, this rates can reduce drastically the transient amplitude (Fig. 18c), showing their powerful influence over the SR Ca$^{2+}$ release. Since the constraint region has moved towards largest open rates, the optimal open rate has also increase.
4.1.3. $k_{in} - k_{off}$ Constraint Map

We have seen that small variations in the open rate can generate important changes on the transient amplitude and in the heart contraction strength. But it is also important to see how the entire RyR system controls the Ca$^{2+}$ release, not only the open probability. The $k_{off} - k_{in}$ map for a fixed open rate will give us additional information. In Fig. 19a it’s represented the transient amplitude for the maximum release conductivity that satisfies the SR recovery constraint. It is possible to observe that as the transition rates increase, a drastic change appears in the conductivity behavior (Fig. 19b). At high rates, the system approaches the limit where there are no open RyRs, preventing the release and overloading the SR. In the colormap this translates as $g_{rel}$ saturation. On the other hand, we can observe an area in the transient map (Fig. 19a) with a somewhat peculiar behavior. It would be expected that decreasing the probability of open channels would translate into a smaller SR calcium release, and into a smaller transient. But contrarily, we see that for certain values of the closed rate, increasing the inactivation rate leads into a larger transients. The explanation for this phenomenon is found in the restriction of SR recovery. For lower closed rates, when the RyR open state is less persistent, remaining open a smaller amount of time, the conductivity needed to get a proper recovery is not as high as for high closed rates, when the RyR remains more time in the open state. Since small modifications in the conductivity results in notable changes in the transient, as we have already seen, at the end, what we get is a greater transient amplitude.

Figure 19: a) Ca$^{2+}$ transient amplitude for the biggest $g_{rel}$ that satisfies the SR recovery constraint and the corresponding $g_{rel}$. $K_{SR} = 1250$, $k_{on} = 0.3$ $g_{up} = 0.5$ and $g_{NaCa} = 0.25$. Due to the recovery constraint, in some cases increasing the closing rate leads to larger calcium transients. Since for lower closed rates, the conductivity needed to get a proper recovery is not as high as for high closed rates, the transients obtained for high RyR open probabilities are smaller.
4.2 NCX as Homeostasis Regulator

The bases of the constraint analysis were finding sets of parameters capable of giving rise to a stable homeostatic response, imposing in our model a restriction on the SR recovery. In order to find homeostasis capable of maintaining a constant load from the beginning, we have imposed that after the first beat, during the relaxation, SR calcium concentration must be recovered to its basal values, within a reasonable margins. Now, we want to check if the NCX regulatory effect is able to stabilize the small differences allowed in the constraint analysis, by eliminating the excess (or defect) of the calcium introduced by the LCC channels. We also want to know if the NCX is governed by any other parameter and which are the criteria that determine which transients can be stabilized and which ones not.

In order to achieve a favorable NCX activity, we will chose those systems advantageous for the exchanger to work into the efflux mode. We have already seen that the stimulus which makes the NCX work extracting calcium from the cytosol is the cytosolic calcium concentration. So that, high transient homeostatic responses are the best if we’re going to study the regulatory power of the NCX. We can benefit from one of the previous results to achieve this kind of homeostasis in our constraint analysis.

We have shown the existence of optimal parameters which generate the homeostatic response with the major possible transient when a constraint is imposed. On the $g_{rel} - k_{on}$ constraint maps we have demonstrate that whenever a set of fixed values are given, we can find a pair of values for the release conductivity and the open rate that optimizes the SR calcium release. We are going to use these optimal parameters, obtained from a one beat homeostatic simulation, with no NCX activity and with a recovery constraint imposed, in order to see if the NCX is able to stabilize these homeostatic solutions into the initial load value, when we increase the simulation time.

In Fig. 20 we found an homeostasis response were the cytosol overloads (Fig. 20b) and the SR depletes (Fig. 20a), when no regulation is applied. We can appreciate releases non synchronous with the action potential in the SR load evolution (Fig. 20a), which can be associated to calcium stimulation on the open RyR probability. Since it has a great release conductivity and a high open state RyR population, the transient is considerably large (Fig. 20b). Using the NCX in a relatively low power we can stabilize this response. The high transient contributes on the activation of the NCX and reduce the required conductivity to achieved an steady state. Even so, the stabilization could be better, as we will see in next figures.

![Figure 20: a) Average $[\text{Ca}^{2+}]_{SR}$ and b) $[\text{Ca}^{2+}]_i$ after and before the action of NCX for $K_{SR} = 1250$ and $g_{up} = 0.5$, $k_{in} = 0.2$ and $k_{off} = 0.1$. $k_{on} = 0.25$ and $g_{rel} = 10$ are the optimum. 23 An homeostatic response controlled to a fixed SR load by the NCX.](image)
Figure 21: Average \([\text{Ca}^{2+}]_{\text{SR}}\) (left) and \([\text{Ca}^{2+}]_{\text{i}}\) (right) after and before the action of NCX for \(K_{SR} = 1250\) and \(g_{up} = 0.5\), where: \(ab)k_{in} = 0.2\) and \(k_{off} = 1; cd) k_{in} = 2\) and \(k_{off} = 0.1. k_{on}\) and \(g_{rel}\) are the critical in each case. NCX stabilize the homeostatic response for high close rates (a b) and for high inactivation rates (c d).

Generally, when there is no NCX activity, we will find homeostasis more similar to what we observe in Fig. 21. Since there is no flux of calcium going out the cell, both the SR load (Fig. 21a o 21c) and the calcium transient (Fig. 21b 21d) overload with time. In Fig. 21a can be more clearly observed the non synchronous calcium release. Regarding the NCX functioning, it requires higher conductivities when it has to control systems with lower transients, which follows what has been stated before about the relation between the transient amplitude and NCX “working time”. As more large the transient, the exchanger change to efflux mode before. But in this case, the constant release from the SR of Fig. 21a (high close rate) requires a higher exchanger control.

In the Fig. 22 we show how an increase in the close and inactivation rates affects to the homeostatic response. The well inactivated rate increase counterbalances the stimulated emission from the close rate. Since it is the lowest transient example and it’s accompanied by spontaneous release, it has the strongest NCX.

Figure 22: a) Average \([\text{Ca}^{2+}]_{\text{SR}}\) and b) \([\text{Ca}^{2+}]_{\text{i}}\) after and before the action of NCX for \(K_{SR} = 1250\) and \(g_{up} = 0.5, k_{in} = 2\) and \(k_{off} = 0.1. k_{on} = 2.7\) and \(g_{rel} = 20\) are the optimum. NCX stabilizes the homeostatic response for high close rates (a b) and for high inactivation rates (c d).
response controlled into a fixed SR load able to regulate an homeostasis with low b) by the NCX. High NCX conductivity is transients an spontaneous release.

![Figure 23](image)

Figure 23: a) Average $[Ca^{2+}]_{SR}$ and b) $[Ca^{2+}]_i$ after and before the action of NCX for $K_{SR} = 1250$ and $s_{up} = 2$, where: ab) $k_{in} = 0.2$ and $k_{off} = 0.1$; bc) $k_{in} = 2$ and $k_{off} = 1$. $k_{in}$ and $s_{rel}$ are the critical in each case. The presence of SERCA helps in the homeostatic control, waiving the required NCX conductivity.

Finally we are gonna study how the SERCA influences the activity of the NCX, which also works during the repolarization decreasing the cytosolic calcium concentration. During homeostasis these two mechanisms are always competing each other and must work synchronously to get a neutral calcium flux within the whole cell. In Fig. 23b increasing the SERCA respect to Fig. 20 produces a minor transient, but the regulation is in disagree with what has been stated before. The required exchanger conductivity is smaller since the SERCA empties part of the cytosol, reducing the NCX work. We have exactly the same situation in Fig. 23d. In Fig. 23a we can see also a weaker regulation, since the homeostasis is not strictly stabilized, a phenomena also present in Fig. 20a. This could mean that for low NCX conductivities, even for high transients, the control can be complicated.

4.2.1 Summary

Through the constraint analysis method, where the SR recovery was imposed during the first beat, we have been able to find parameters that generate homeostasis which can be later adjusted thanks to the NCX activity. We have seen how the exchanger works extracting the calcium overload, which appears due to the constraint recover margins, and can conclude that low transients require more powerful NCX regulation than high transients. But also that as smaller is the NCX conductivity, more weaker is the control. Also point out the important coordination between the SERCA and the NCX, which must work together if the steady state wants to be reached.
4.3 NCX as Regulator of Frequency Variations

Finally I would like to address how the exchanger can also be used as a stabilizer in front of changes in the cardiac excitation frequency. These alterations mainly modify the relaxation time and can lead to destabilization in homeostasis by reducing the time of SERCA and NCX activity. In the case of rapid heart rates it is necessary that the mechanisms in charge of emptying the calcium cytosol work at higher powers to give way to the next beat with the concentration values already recovered. As we reduce the frequency we can see how the recovery after the first beat is cut, which entails a considerably high reduction of SR loading and an increase of the intracellular calcium for the higher rates. The second beat starts from a smaller [Ca^{2+}]_{SR}, which without proper rectification of the release conductivity or transition rates means a higher SR depletion. In this way a small transition time begins which thanks to a suitable NCX activity ends in a new steady state, with values of [Ca^{2+}]_{SR} a little lower and [Ca^{2+}]_{i} slightly higher. We can see how the relationship between NCX conductivity and the frequency is non-linear, but the more we reduce the period, the variation of the NCX must be bigger to compensate.

Figure 15: Average [Ca^{2+}]_{SR} for different frequencies regulated with $g_{NaCa}$, with all the other parameters fixed (left) and relation between de excitation frequency and the NCX necessary to stabilize the homeostasis (right)
Chapter 5
Conclusions

The heart is a complex organ that needs the participation of a large number of components for its correct functioning. Calcium is one of the most important ions in the process of excitation-contraction coupling, which is the mechanism responsible for the contraction of the heart to propel the blood into the circulatory system. This process is possible thanks to the flow of calcium through the cardiac muscle cells forming the heart. The variation of calcium concentrations in the cytosol of these cells is what ultimately causes muscle contraction, and the process that regulates calcium fluxes to achieve a stable behavior is what is called calcium homeostasis. In order to be able to study this process thoroughly, the first objective that I had to overcome was to introduce myself in the terminology of this field and to acquire a high degree of knowledge of cardiovascular physiology. With this knowledge we have been able to analyze homeostasis in depth.

During the homeostasis, Ca$^{2+}$ enters the cell through the L-type channels located at the sarcolemma as an inward Ca$^{2+}$ current, triggering the Ca$^{2+}$ release from the sarcoplasmic reticulum, where the main source of Ca$^{2+}$ of the cell is stored. The combination of Ca$^{2+}$ influx and release raises the free intracellular Ca$^{2+}$ concentration, allowing Ca$^{2+}$ to bind to the myofilament protein troponin C, generating the contraction. For the relaxation to occur, the SERCA and the NCX work reducing the calcium cytosolic concentration, pumping it back to the SR or out the cell, respectively.

Since this is a complex process where several mechanisms are involved, we have studied the homeostasis using a mathematical model, working on some key parameters and their impact when they are modified within physiologically realistic values. These parameters were the RyR transition rates, the main fluxes conductivities and the SR load capacity. We have seen that in order to achieve an steady state response the calcium entering the cell through the LCC must be pumped out by the NCX and the calcium released from the SR must be pumped back by the SERCA, achieving a stable load level. Modifying the parameters of an steady state response we have seen how they affect to the homeostasis and have begun to understand how the homeostatic control works. This knowledge has been summarized on the Tab. 2.

During this analysis we have seen that not all the parameters have the same relevance on the transient amplitude, the responsible of the heart contraction strength. Those directly related with the SR calcium release generate the biggest variations. These parameters are the RyR transition rates and the release conductivity. This was an expected result since calcium released from the SR translates into an increase of the cytosolic calcium concentration. Using the previous results we have been able to control homeostatic responses stabilized in a desired SR load level. With the relations of the Tab. 2, we have find an appropriate sets of values that generate an steady state responses.
Since we have seen which are the main regulators of the transient and we have been able to understand the homeostasis regulation, and even even get a good intuition about what is the relative relevance of each of the different parameters, the second objective have been also achieved. Let me notice that finding sets of parameters that generate homeostatic responses is not straightforward. We have seen that not all the transients can be stabilized into a given SR load, and that not all the homeostatic responses can remain constant in order to produce a regular cardiac output. In this sense, we have been able to go even further than expected and develop a novel control technique, never done before, which allows us to assess the relevance of the different parameters on the calcium transient regime. Since we want to find steady state solutions that remain on the initial load level we have impose that the SR recovery must reach its initial load value, within a margins, after the first beat. Applying this constraint we avoid most of the SR depleted or SR overloaded systems.

We have performed a constraint analysis in order to study how this new point of view affects the homeostasis. How the transient behaves and which are now the main transient regulators. Since we have a general idea of which are the main transient controllers from the previous analysis, we have studied how the transient amplitude evolves as we modify the release conductivity and the open probability rate. Aside from the previous results, which have been obtained again, we have seen that an optimum open rate exists, which generates the maximum transient, over all the combinations of $g_{rel}$ and $k_{on}$ that satisfy the recovery constraint. This means that variations on the open probability does not have the same impact over all the release conductivities that fall within the constraint margins. In other words, imposing recovery constraint generates and optimum pair of values that generate the maximum transient for the fixed parameters.

To pursue the investigation further, we have studied how the close and inactivation rate behaves for a given open probability, looking for the amplitude of the transient for the maximum conductivity that satisfies the constraint. Contrary to what one could expect, we have seen that there are some combinations of parameters where increasing the close transition rate provokes larger transient amplitudes. This is because for larger $k_{off}$, the necessary SR release to accomplish the constraint is bigger, and the result is the homeostasis stabilizes in a larger transient response. This only occurs when the constraint is imposed.

Finally we wanted to check that the NCX regulatory effect was able to stabilize the small differences allowed in the constraint analysis by eliminating in excess (or defect) the calcium introduced by the LCC channels. We also wanted to know how the transient is affected by the NCX. Since the NCX performs better in large transients this study was done using the optimal release and open rate parameters, which generate the biggest transient amplitudes. We have shown that, generally, largest transients require less NCX power, because they change to efflux mode sooner, but also we have seen that the NCX regulatory effect is highly influenced by the SERCA pump. As an additional study, we have investigated how the NCX can stabilize an homeostatic response in front of excitation frequency variations. Reducing the action potential period implies less SERCA and NCX activity, and an overload of cytosolic calcium. We have seen that increasing the NCX efflux power we can regulate the load and stabilize the homeostasis, but into a smaller SR load levels, due to the reduced SERCA activity.
Future Work

The above discussion has described systems whereby an increase of SR Ca\(^{2+}\) content leads to an increase of \(\text{Ca}^{2+}\) efflux and decrease of \(\text{Ca}^{2+}\) influx that, in turn, is compensated for the increased \(\text{Ca}^{2+}\) release. This autoregulation is a classic feedback system. It does, however, involve a delay, as the change of SR Ca\(^{2+}\) content on one beat only influences \(\text{Ca}^{2+}\) fluxes on the next beat. It is well known that delays can cause instability in this kind of feedback systems. The potential effect of this can be seen qualitatively as follows. Imagine that there is a very steep relationship between SR content and \(\text{Ca}^{2+}\) efflux. If the cell begins with a large SR content, then the \(\text{Ca}^{2+}\) transient will result in a large loss of \(\text{Ca}^{2+}\) from the cell. This will decrease the SR content. The next beat will therefore arise from a depleted SR, resulting in a smaller \(\text{Ca}^{2+}\) transient and efflux and therefore a net gain of \(\text{Ca}^{2+}\) by the cell and thence, on the next beat, a large \(\text{Ca}^{2+}\) transient. If this continues, alternating small and large \(\text{Ca}^{2+}\) transients will be produced.

Alternans is a risk factor for cardiac arrhythmia. At the cellular level cardiac alternans are defined as cyclic, beat-to-beat variations, that can be in contraction amplitude (mechanical alternans), in action potential duration (APD or electrical alternans) and cytosolic \(\text{Ca}^{2+}\) transient (CaT) amplitude at constant stimulation frequency. Electromechanical and \(\text{Ca}^{2+}\) transient alternans are highly correlated, however it has remained controversial whether the primary cause of alternans is a disturbance of cellular \(\text{Ca}^{2+}\) signaling or electrical membrane properties.

Finally, it will be really interesting to continue our study in order to understand how homeostatic feedbacks may influence the presence of alternans. Our novel method opens the possibility to study in detail, and under great control, whether changes in the different parameters are pro (facilitate the presence of alternans) or anti-arrhythmic (prevent the appearance of alternans). I think that looking for mechanisms that could stabilize the presence of alternans for any given fixed SR Ca load through changes in NCX behavior is now perfectly feasible. Similarly, the generation of protocols which would allow for the appearance of alternans at different desired frequencies, compatible with those measured in experiments, is also possible thanks to this project.
Bibliography


23. \(g_{rel}\) and \(k_{in}\) are divided by a factor \(10^2\). \(k_{on}\) is divided by a factor \(10^4\).