EXPERIMENTAL AND THEORETICAL ANALYSIS OF VENTRICULAR RAT CARDIOMYOCYTE

David Civantos, Joan Farre, Oriol Vidal, and Joan Villalonga

I. INTRODUCTION

The aim of this project is to approach ourselves to the research methods in biophysics. We will study the Action Potential (AP) of a cardiomyocite from the experimental and numerical point of view. Our goal is to understand a numerical model that simulates the ion currents during an AP in the rat cardiomyocyte made by S. Pandit in 2001 [11].

In the first part, we are going to explain which are the main experimental techniques used in biology in order to get information about the membrane potential, ion concentrations and ion currents in a cell. The data obtained in experiments is crucial in order to prove the model and fit some parameters.

Finally we will implement a modification in the model of S. Pandit [11]. We will change the whole part of the model that regulates the L type $Ca^{2+}(I_{Ca,L})$ current by the part of the code that regulates the $I_{Ca,L}$ from the model developed by Mahajan, et. al. in 2008 [9] to study the AP of a rabbit cardiomyocite. This two models are based in two different methods and the main goal of this modification is to prove if a channel in the code can be change independently of the rest of the model.

Our project is based in simulating the physiology of an AP with a numerical model. Therefore, in order to formulate the model we have to know what is and how occurs the AP.

The action potential is a signal caused by a change in the membrane potential (E_m), an external stimuli generates a positive peak in the E_m, see Figure 1. This raise in the membrane potential propagates cell to cell in the tissue generating an electrical wave that carries information.[1]

All this process is regulated by the flux of three ions: Ca^{2+}, K^+ and Na^+. This ions can travel through the cell membrane thanks to a particular kind of proteins called ion channels which let ions cross depending on their state, opened, closed or inactivated. The channels state can depend on external factors such as the presence of some particular ligand or the level in the membrane potential.[1]

The AP occurs in different phases:

a. Depolarization: Some external stimulation, ususally the action potential of a neighbour myocyte or neuron, raises the E_m from the Rest Membrane Potential (RMP), which normally is around -80 mV[11], to the threshold level were the Sodium channels open and allow the current of Na^+ to go inward the cell (I_{Na}). This flow is very fast and causes a rapid increasing in the membrane potential reaching a positive maximum. During this phase the called L type Calcium channel is opened, but its inward current of Ca^{2+} ($I_{Ca,L}$) is relatively slow compared with I_{Na}.

b. Early repolarization: when E_m reaches its maximum, the Sodium channels are inactivated and the Potassium channels are opened allowing the outward current of K^+ (I_K) to flow. At this membrane potential, $I_{Ca,L}$ is very weak because the driving force of the channel ($E_m - E_{Ca}$) is very low. As a consequence, the only important current is I_K, which causes a quick repolarization, a decrease in E_m. This step is very short and it doesn't decrease E_m substantially. [3]

c. Plateau phase: In this phase the $I_{Ca,L}$ gets higher and compensates the I_K. It makes the E_m remaining positive and decreases very slowly. In this step, the Calcium concentration $[Ca^{2+}]_i$ increases a lot causing the SR Ca release. The sarcoplasmatic reticulum (SR) is an organelle, divided in two parts: JSR (junctional SR) and NSR (network SR), which storages Ca^{2+}. The activation of this release is Ca-dependent. When SR Ca is released, $[Ca^{2+}]_i$ is maximum and the myocyte contraction occurs.[3]

d. Repolarization: $I_{Ca,L}$ inactivation is Calcium-dependent, therefore, after the SR Ca release, the high $[Ca^{2+}]_i$ causes the inactivation of this channel. Then $I_{Ca,L}$ stops and the only current remaining is I_K, which quickly repolarizes the E_m to the RMP.

The process explained is a simplification of what really happens during an AP in a myocyte, which is much more complex due to the presence of more channels of this kind with important functions. Some of Ca-dependent actions are still unknown. In Pandit's model, some more channels and currents are taken into account but with the previous explanation we can get a major idea about what happens during the AP, see Figure 1.

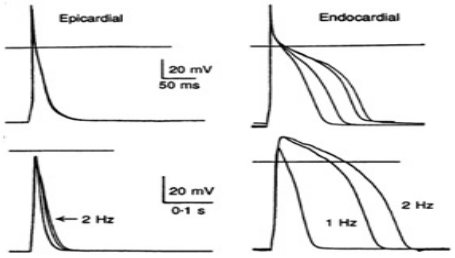


FIG. 1: Here we can see which is the shape of the AP, E_m vs time. [14]

II. EXPERIMENTAL TECHNIQUES

Cell biology and biophysics have a great variety of techniques and experiments to obtain measurements. We will comment some of the most relevant which were used in the *Pandit, 2001* model.

a. Cell isolation : This is not a measurment experiment itself but an essential technique to develop other experiments such as the Whole-Cell Patch Clamp.

The main goal is to separate cells that, for example, form solid tissues into individual ones. There are many ways to accomplish it, but we will focus in the methodology for rat ventricular myocytes. [2]

The rat's heart is perfused through the aorta with 25 ml of Ca^{2+}-free Krebs buffer, followed by 15-30 min with Krebs buffer containing 5pM Ca^{2+} and type II collagenase. The separated ventricles are chopped into small ($\sim$1 mm$\sim$) pieces and incubated in fresh enzyme solution for 5-10 min. After repeated washing by a series of centrifugations and resuspensions in enzyme-free Krebs buffer, intact cells are usually separated by low speed ($\sim$300 rpm; 10 min) centrifugation.

b. Whole-Cell Patch Clamp : These kind of techniques are divided in two main methods. Intracellular recording of transmembrane current while holding transmembrane voltage at a known value is referred to as **voltage clamp**, whereas the measurement of transmembrane voltage while holding transmembrane current at a known value is termed **current clamp**. Such recording techniques typically involve the use of a small bore pipette (microelectrode) containing a conductive salt solution that probes one side of an isolated cell membrane (the intracellular space) for comparison to a reference electrode placed on the other side of the membrane (the extracellular space). An electrical circuit is formed between the recording and reference electrode with the cell of interest in between.

There are many variations in how the microelectrode is attached to the membrane, in particular, Whole-cell recordings involve the rupture of a membrane patch, thus providing access from the interior of the pipette to the intracellular space of the cell. With this technique currents through multiple channels are measured simultaneously. [8]

c. Calcium Imaging : These procedures measure calcium concentrations by making use of what are called calcium indicators, small molecules that exhibit altered fluorescent properties when bound with Ca^{2+}.[13]

III. RECORDED DATA

A. Data used in our model:

The mathematical model we have used fits its parameters with experimental data measured by other scientists. We will focus on action potential, calcium transient concentration and calcium currents through the cell data.

First of all, the myocyte structure parameters are extracted from an aticle from 1995 . The capacitance of the left ventricular rat cardiomyocytes is measured from the integral of the current transient resulting from 5 mV depolarizing steps, averaged 998 pF, and his volume is 16 pL. [4]

The action potential behavior data is taken from an article published in 1995 [14]. This paper presents the Whole-Cell Current Clamp recorded data of the membrane potential during an AP, characteristic explained in the first section. Cells from rat left ventricle were rested for 30s and then stimulated at 0.5, 1 and 2 Hz.

The Ca^{2+} transient concentration measurments are also extracted from the first article named before [4]. We need to remark the buffering of $[Ca^{2+}]_i$ by intracellular ligands. For a cell volume of 16 pL, this Ca^{2+} influx results in an increase in intracellular Ca^{2+} of 28 mol/L. [4]

And finally, the Ca^{2+} current through the cell. Our model also uses the data from the same article. [4] The Ca^{2+} influxes during the action potential were recorded from a rat ventricular myocyte using step Whole-Cell Voltage-Clamp techniques applied from a holding potential of 60 mV.

Depolarizing steps elicit only $I_{Ca,L}$, which is activated rapidly and then inactivated during the depolarization. Ca^{2+} current dependence in front of time data, and the current-voltage relation related to it is obtained, as you can see in Figure 2.

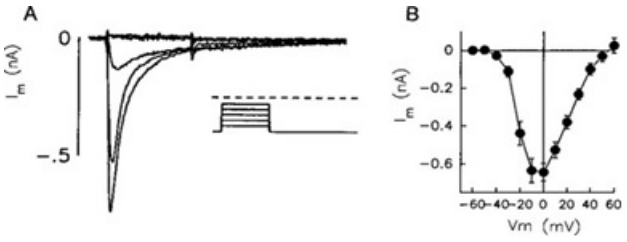


FIG. 2: The first plot shows the Ca^{2+}-dependent difference currents elicited by a train of 300-millisecond depolarizations. The second shows peak current-voltage relation averaged from five different myocytes. [4]

B. Comparison:

Once described and explained these important data used in our model, obtained from the different measurements, we are going to compare it with other experiments done in other laboratories, and we will see the similarities and differences.

With regard to the action potential, we have searched an article made in 1993. It also gives us information about calcium and potassium currents in ventricular myocytes isolated from diabetic rats. Referent to action potentials and membrane currents, they were recorded using the Whole-Cell Patch Clamp techniques in current-clamp mode for AP recordings and voltage-clamp mode

for membrane current measurements. [12]. Cell capacitance was obtained at the beginning of each experiment by measuring the time constant of the exponential decay of the capacitive transient elicited by a 2 mV depolarizing pulse, and the estimation of current density was expressed in pA/pF. We show the results of this experiments in the Appendix B.

Then, from the same article, we also have obtained some information about the calcium currents. The amplitude of the calcium current was expressed as the difference between peak inward current and steady-state level at the end of the depolarizing pulse. For each cell this amplitude was normalized by taking into account the membrane capacitance. For both normal and diabetic cells, I_{Ca} density, expressed as pA/pF, is plotted as a function of step potential. In both types of preparation, only depolarizations less negative than -50 mV elicited an inward current. The different results of the calcium current influx versus the time and the current-voltage data obtained in this paper are also shown on the Appendix B. Of course it is in our interest only the normal cells, not diabetic.

And finally, with regard to the calcium concentration, we have obtained the data from another article published in 1999. [6]. In Figure 3 we can see the measurements of indo-1 fluorescence used in this experiment versus time. We can see that follows the same shape that the characteristic of the calcium concentration transient used in Pandit's model.

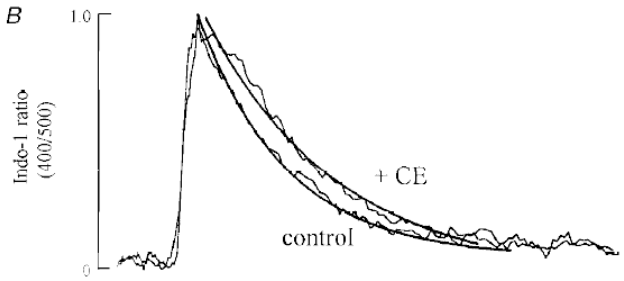


FIG. 3: The plot shows normalized transients obtained from control cells. In this case 5 transients were averaged.[6]

IV. MATHEMATICAL MODEL

As we said before, we have used a mathematical model to simulate the behaviour of the cardiomyocyte. Mathematical modeling is the art of translating problems from an application area into tractable mathematical formulations whose theoretical and numerical analysis provides insight, answers, and guidance useful for the originating application [10].

The main goal of the model proposed by Pandit was to mathematically reconstruct the action potentials from adult rat left ventricular epicardial and endocardial myocytes under normal conditions. By using the equations proposed in the model formulation, they (and also we) could get the searched action potentials, the ionic currents involved in the genesis of these action potentials, and the concentrations of this ions. The fundamental equation the model uses (Eq. 1) comes from thinking the membrane is like a capacitance; we explain in more detail where Eq. 1 comes from in Appendix C.

$$dV/dt = -(I_{Na} + I_{CaL} + I_t + I_{ss} + I_f + I_{Kl} + \\ + I_B + I_{NaK} + I_{NaCa} + I_{CaP})/C_m \quad (1)$$

The initial specific aim of the Pandit's study was to understand whether the experimentally observed differences in I_{Na} and I_t could account for the epicardial-endocardial AP disparity. By the way, we have not been interested in this part and we have focused ourselves in understand how the real experiments were made , how the mathematical model worked and compare the results between them.

This study uses a mathematical model called "Gates model". As we showed before, the AP is basically a movement of ions through some transport proteins called channels. We can imagine this channels as electrical resistances: the intensity that passes through it depends on the conductance (g) and on the driving force ($E_m - E_{ion}$). If the conductance is very low, the channel is closed and if it is very high the channel is opened. In the model the conductance of every different channel will depend on different variables and it will have different time constants with are fitted using experimental data.

V. NUMERICAL RESULTS

A. Simulation:

We have used Myokit; it is a python-based software package designed to simplify the use of numerical models in the analysis of cardiac myocytes. It was created as part of a PhD Thesis at Maastricht University, and is being developed further at the University of Oxford [7].

The code we have used it is been took from a website called "cell.ML" [5]. In this website, you can download the code directly to run it into Myokit.

We have familiarised ourselves with Myokit environment and we have obtained the simulated results of the mathematical model. As you can see in the Appendix D, we obtain the AP, the L-type Calcium current, the Calcium ion concentration and a lot of parameters we will discuss above.

B. Modification:

Since the first day we wanted (and our tutors encouraged us) to make a little variation in the original code of the rat ventricular myocyte. The proposed variation was to substitute the way the rat's code had to calculate the L-type Calcium current by another form to obtain it used in another mathematical model, proposed by Aman

Mahajan et. al [9]. This second mathematical model was purposed to modelize a rabbit ventricular action potential and, in contrast with the rat's model, this one uses a states model.

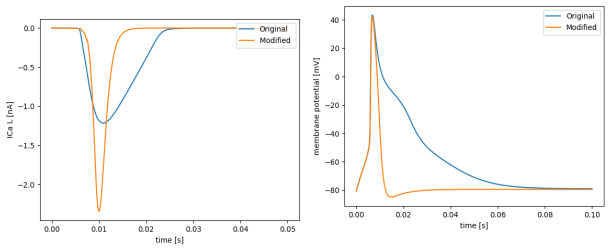
A states model works different than the gates model mentioned before. This model presents an Ca^{2+} L type channel which can be in seven different states (4 inactivated, 2 closed and 1 opened). So, instead of having a channel always opened but with changing conductivity and driving force, we have a channel with changing probability of being opened or closed.

This code's variation was not trivial at all. The currents of calcium in both models depended on the ion concentration but these concentrations (we saw before) are not homogeneous in all the cell and they are defined according to the region they are. The difficulty was that these concentrations are not defined the same way in the two models. We have had to assign and match the different concentrations.

There are four different concentrations of calcium in each model. In the rat's model there is: myoplasm concentration (Ca_i), NSR concentration (Ca_{NSR}), JSR concentration (Ca_{JSR}) and restricted subspace concentration (Ca_{ss}). On the other hand, in the rabbit's model, we have: intracellular concentration (Ca_i), NSR concentration (Ca_{NSR}), dyadic concentration (Ca_{dyad}) and submembranal concentration (Ca_{submem}). At this point, it seemed easy to match the first two concentration of each model, the NSR and the myoplasm (or intracellular) ones; and that is exacly what we did. The problem came when we wanted to match the following ones. The submembranal concentration is, as the name says, the calcium concentration right under the membrane and the dyadic concentration is the concentration in the dyadic junction; which is the space between the cell membrane and an organelle come into close contact of each other. In our case this organelles are the RyR receptors, a type of intracellular calcium channels in various forms of excitable animal tissue like muscles and neurons and the principal cellular mediator of CICR (Calcium Induced Calcium Release, explained in the introduction). After a long time spent reading and studying both articles,we decided to assign this two remaining concentrations (Ca_{dyad} and Ca_{submem}) to the restricted subspace concentration, but with a constant between them. The main reason is because the restricted subspace concentration is the calcium concentration between the JSR space and the T-tubules (extensions of the cell membrane that penetrate into the centre of skeletal and cardiac muscle cells). Since both Ca_{dyad} and Ca_{submem} are concentrations defined under the membrane and also the Ca_{ss}, the only difference (bridging the gap) is the volume occupied; and this is fixed by finding the right constant between them.

The next step was to introduce the L-type Calcium current of the rabbit to the voltage differential equation. As we have seen before, the AP is calculated as in Eq. (1). The rabbit's model uses another way to calculate the AP. When they calculates each currents, they associate a voltage/time to this current and they only sum all the associated "voltages". So, at the end, we eliminated the L-type Calcium current from the initial equation and we added this term outside the quotient.

Our main goal was getting with the modified model the same AP simulation gotten with the original code. We wanted to change some of the parameters with are fitted with experimental data in order to fit the original simulation. In the development of the project we can state:



(a) $I_{Ca,L}$ vs time

(b) Action potential, E_m vs time

FIG. 4: Plots obtained running the original code (blue) and the modified code without changing any parameter (yellow)

a. The first code that we used had a mistake, which we did not found. The consequences of this mistake was a very low $[Ca]_i$ (the results obtained can be seen in the Appendix 4). In the following results we have used code of Pandit's model for a diabetic myocite, which gave us the expected $[Ca]_i$ that matches with experimental data seen in figure 3.

b. The new model triggers the $I_{Ca,L}$ too late and its duration is too short, as we can see in Fig. 4. Therefore, in order to fit our modified model to the original model we should change the parameters which elongate the duration and that trigger faster the $I_{Ca,L}$.

c. After a lot of proves changing parameters we have not get any important progress, the action potential we get has not a plateau phase due to the short and late $I_{Ca,L}$ activation. It is not trivial to fit these parameters because the two models are very different and they take into account different Ca concentrations. In appendix D we explain some of the efforts we have done.

VI. CONCLUSIONS

In conclusion, we have learned a lot about cardiomyocytes ion dynamics and the techniques used to measure them. The physiology behind the action potential is very complex and can be modelled in a lot of different ways, taking more into account some channels than others. For this reason is hard to accomplish a modification like the one we have tried, the channels cannot be changed from one model into another that easily because there are lots of important parameters for every kind of current.

REFERENCES

[1] Bruce Alberts et al. *Molecular Biology of the cell.* Garland Science, 2015. ISBN: 978-0-8153-4432-2.

[2] Michael Apkon and Jeanne M. Nerbonne. "Characterization of Two Distinct Depolarization-activated K + Currents in Isolated Adult Rat Ventricular Myocytes". In: *The Rockefeller University Press* 97 (1991).

[3] Donald M. Bers. "Calcium Cycling and Signaling in Cardiac Myocytes". In: *Annual Review of Physiology* 70 (2008), pp. 23–49. DOI: 10.1146/annurev.physiol.70.113006.100455.

[4] R. B. Clark Bouchard R. A. and W. R. Giles. "Effects of action potential duration on excitation-contraction coupling in rat ventricular myocytes. Action potential voltage-clamp measurements." In: *Circ. Res* 76 (1995), pp. 790–801.

[5] *cell.ML.* URL: http://models.cellml.org/exposure/ea62c9c8a502afe364350d353ebf4dd5/pandit_clark_giles_demir_2001_epicardial_cell.cellml/view.

[6] Ho Sook Choi and D. A. Eisner. "The role of sarcolemmal Ca2+-ATPase in the regulation of resting calcium concentration in rat ventricular myocytes". In: *Journal of Physiology* 515 (1999), pp. 109–118.

[7] Michael Clerk. *Myokit.* URL: http://myokit.org/.

[8] Bruce G.Kornreich. "The patch clamp technique: Principles and technical considerations". In: *Journal of Veterinary Cardiology* (May 2007).

[9] Aman Mahajan et al. "A Rabbit Ventricular Action Potential Model Replicating Cardiac Dynamics at Rapid Heart Rates". In: *Biophysical Journal* 94 (2008), pp. 392–410.

[10] Arnold Neumaier. *Mathematical Modeling.* URL: https://www.mat.univie.ac.at/~neum/model.html. (accessed: 19.05.2019).

[11] Sandeep V. Pandit et al. "A Rabbit Ventricular Action Potential Model Replicating Cardiac Dynamics at Rapid Heart Rates". In: *Biophysical Journal* 81 (2001).

[12] Danielle Feuvray Philippe Jourdon. "Calcium and Potassium currents in ventricular myocytes isolated from diabetic rats." In: *Journal of Physiology* 470 (1993), pp. 411–429.

[13] Lora Talley Watts R. Madelaine Paredes Julie C. Etzler and James D. Lechleiter. "Chemical calcium indicators". In: *Methods* (2008).

[14] D. Severson Shimoni Y. and W. R. Giles. "Thyroid status and diabetes modulate regional differences in potassium currents in rat ventricle." In: *J. Physiol* 488 (1995), pp. 673–688.

Appendix A: Calcium Indicators

Ca^{2+}indicators bind and interact only with freely difusible Ca^{2+} ions. The majority of Ca^{2+} within cells is not free to diffuse but tightly bound to various cellular buffers. The ratio of bound to free Ca^{2+} varies from cell to cell as well as within the various compartments of the cell. Chemical Ca^{2+} indicators themselves also act as Ca^{2+} buffers and can therefore impact both the levels and most noticeably, the kinetics of Ca^{2+} signaling within cells. For these reasons, users must carefully consider not only the spectral characteristics of a chemical indicator, but also pay close attention to its binding properties.

The dissociation constant or its inverse, the association constant (K_a), describes how tightly an indicator dye binds Ca^{2+} ions. The K_d has molar units and corresponds to the Ca^{2+} concentration of at which half the indicator molecules are bound with Ca^{2+} at equilibrium. The user must frequently balance the desire to increase the strength of indicator signal with the problems associated with increasing an indicator's concentration. On occasion, it is possibly to work with an indicator with a lower Ca^{2+} affinity. That can reduce the impact of buffering but frequently is done so at the cost of limiting the signal strength.

In Referring to its spectral properties, there exist two kinds of indicators: Single wavelength indicators, which exhibit significant Ca^{2+} dependent changes in fluorescence intensity without shifting their excitation or emission wavelengths, and Ratiometric indicators, that shift the peak wavelength of either their excitation or emission curve upon binding Ca^{2+}. For instance, the Indo-1 indicator, shifts its main emission peak from 475 nm (calcium-free) to 400 nm in the presence of calcium.[13]

Appendix B: Recorded data

a. Calcium concentration transient of Pandit's model As we have explained before, the calcium concentration in the myocyte can be measured with a calcium imaging fluorescent. Now we are going to see in this figure below how it depends versus the time.

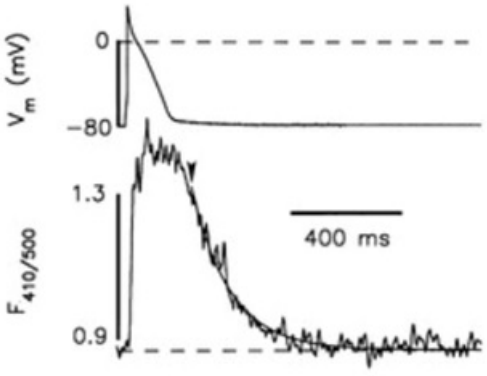


FIG. 5: The first one shows long action potential voltage-clamp waveform, and the second shows indo 1 fluorescence transient.[4]

It is also important to say that the time course of

decay of $[Ca^{2+}]$i and I_{ex} during diastole were both fit to single exponential functions, starting at the time indicated by vertical arrowhead.

b. Comparison of the action potential Here we can observe the recording of an AP in another laboratory as we stated in the recorded data section, see figure 6. It follows the same dynamics as the AP data used in Pandit's.

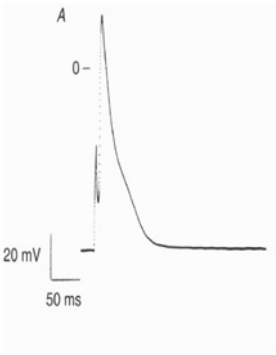


FIG. 6: AP measurment from cell isolated rat myocyte using whole-cell voltage clamp technique.[12]

c. Comparison of the Calcium currents As we said before, in the Figure 7 you are going to see the different dynamics of the calcium current used in another paper, not Pandit's. We can verify that Calcium current follows the same dynamics as in Figure 2.

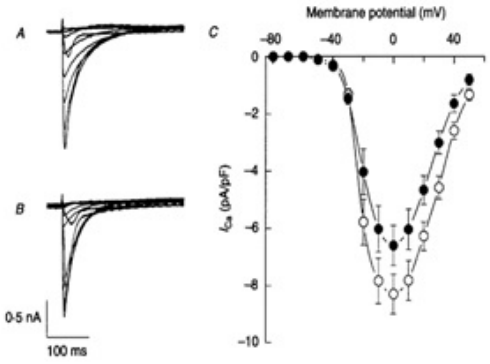


FIG. 7: Calcium currents recorded in single ventricular myocytes isolated from a normal (A) and from a diabetic rat heart (B). Currents were elicited by 250 ms depolarizing voltage steps applied at 0 1 Hz from a holding potential of -80 mV, in 10 mV increments between -50 and + 50 mV. And in the right one is represented the current density voltage relationships for calcium current, expressed in pA/pF, recorded in ventricular myocytes isolated from normal(•) from diabetic (◦) rat hearts.[12]

Appendix C: Mathematical models: Supplementary Material

To understand better how the model behaves, we have added some pictures and equations in this appendix. As we said, we can see the cell as an electrical circuit, and here we have a more illustrative way to understand it (Fig. 8). Pandit's model uses different types of con-

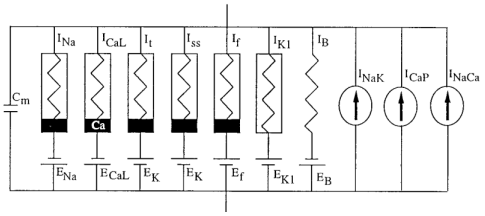


FIG. 8: Electrical equivalent circuit of the adult rat epicardial/endocardial ventricular cell.

centrations depending on the atoms which compose the different ions and on the origin of this ions. This is because the ion concentrations are not homogeneous in all the myoplasm; depending on the region, the ion concentration changes. In Fig. 9 we can see the different regions and the currents inside the cell [14]. We are not going

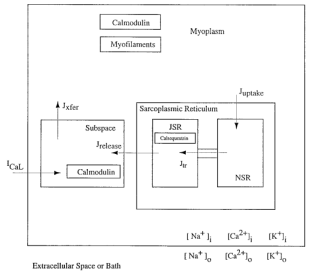


FIG. 9: Fluid compartment model of the rat cardiac cell (epi and endocardial). The expressions for the intracellular Ca^{+2} dynamics are based on the work of Winslow et al., 1999.

to make a full specific explanation about the origin of Eq. (1), but we want to say a little about this. The sarcolemma, the cell membrane of a striated muscle fiber cell, behaves as an electric circuit as we can see in Fig. 8. We can imagine the cell membrane as a capacitor with an specific capacitance and we use its definition Eq. (C1) to calculate the voltage between the inside and the outside of the cell. If we also use the definition of the intensity (in our case will be the current of the different types of ions throgh the channels) (Eq. (C2)). By using Eqs. (C1) & (C2) and calculating the differential equation of this difference of voltage we obtain directly the AP we were looking for Eq. (1).

$$C = dQ/dV \tag{C1}$$

$$I = dQ/dt \tag{C2}$$

On the other hand, we wanted to say something about the Mahajan model. This model reproduces the dynamics of the cardiac action potential and intracellular calcium cycling at rapid heart ratesrelevant to ventricular tachycardia and fibrillation. By considering this, it has been a mighty tool for illuminating various aspects of cardiac function, such as cardiac arrhytmias. So, the aim of the study was to develop a model including the calcium cycling formulations (based on experimental patch-clamp data obtained in isolated rabbit ventricular myocytes) and including a modification, called Markovian formulation, of the L-type Calcium current (this is the same formulation we have used to modify the initial Pandit's model). We can see in Fig. 10 a schematic representation of Markovian model.

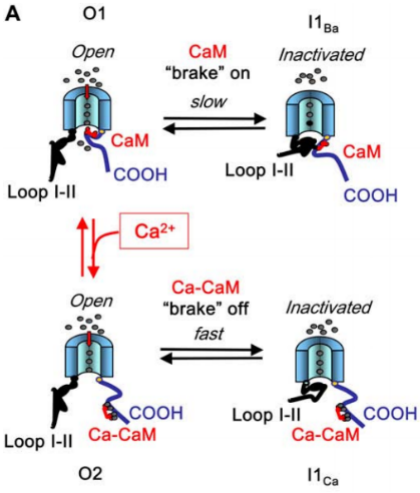
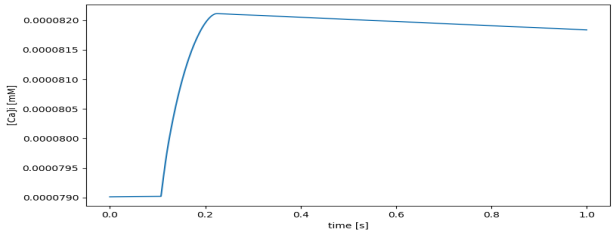
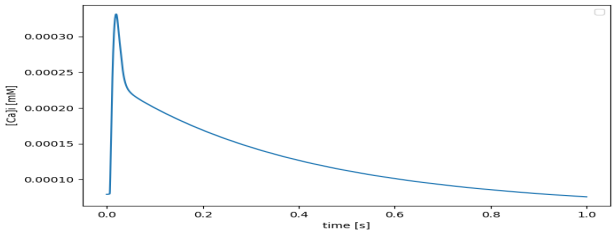


FIG. 10: Schematic representation of Markovian: Seven-state Markovian model of the L-type Ca channel. The red lines denote Ca-dependent transitions to inactivated states. [9]



(a) $[Ca]_i$ vs time



(b) $[Ca]_i$ vs time

FIG. 11: (a) is the result of running the Pandit's model code which have a mistake. (b) is the result of running the Pandit's model for diabetic myocite.

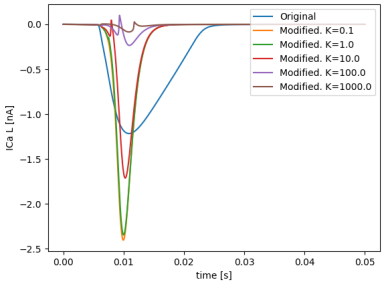


FIG. 12: We have run the code changing K, the constant of conversion between Ca_{ss} and Ca_{dyad} (Ca_{dyad}=K·Ca_{dyad})

Appendix D: Myokit simulations

First of all we proved a mistake in the code that we had downloaded directly from [5] by plotting the $[Ca]_i$, see figure 11.

We can see clearly that the shape of the data obtained in figure 11a isn't correct by comparing it with the experimental data in figure 3. However, the figure 11b is more similar to the experimental date, so we consider that the code for a diabetic cell works correctly.

Then once we had the modified code we have tried some different modifications on the parameters in order to fit the modifications with the original code plots. We can see two examples on figure 12 and 13. It can be seen in figure 12 and 13 that changing K we can control the amplitude of $I_{Ca,L}$ and changing τ_{po} its duration. We have tried to change some more parameters, the conductivity of the channel, the rate of opening, the inactivation constant, etc. and the combination of some of them but without accomplishing any significant result.

Finally, we want to plot the dyadic Ca^{2+} concentration and the submembranal Ca^{2+} concentration of the Mahajan model for the rabbit myocyte and compare it with the restricted subspace concentration of our modified code.

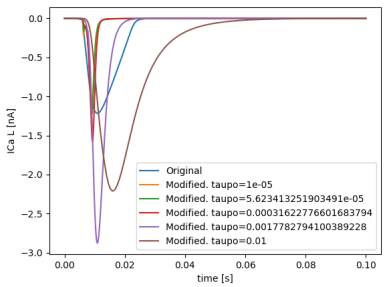
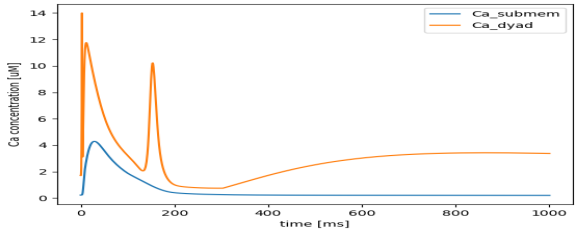
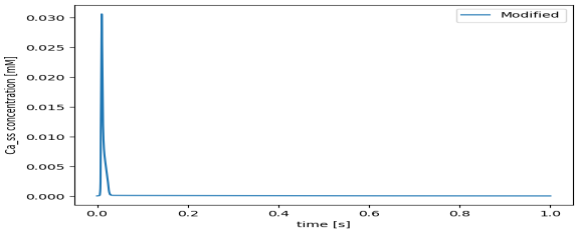


FIG. 13: We have run the code changing τ_{po}, the time constant of activation of $I_{Ca,L}$



(a) Ca_{dyad} (yellow) and Ca_{submem} (blue), of Mahajan model, evolution in time



(b) Ca_{ss}, of our modified model, evolution in time

FIG. 14: We can see that the shape of the curve are very different. The Ca_{ss} is really thinner and the maximum is 6 order of magnitude below the amplitude of the concentrations obtained for the original rabbit model

This big differences, seen in figure 14 in the Ca^{2+} concentrations near the cell membrane, with are the one that inactivate $I_{Ca,L}$ are the major reason that have stopped us of obtain the correct result for our new model. The dynamic of this concentrations isn't taken into account in the $I_{Ca,L}$ part of the code so is very hard to adapt them in the new code.