Modeling the non-linear dynamics of calcium in chromaffin cells

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Abstract

In response to fear or stress, chromaffin cells secrete catecholamines like adrenaline into our bloodstream. Secretion is controlled by intracellular calcium oscillations, which can be measured experimentally.

Experimental measurements of calcium in rat chromaffin cells show that different cells from the same population display different oscillatory patterns when submitted to the same external stimulus. The origin of this heterogeneity remains an open question.

In this thesis we construct a new mathematical model that describes the non-linear dynamics of intracellular calcium in chromaffin cells, where calcium oscillations are produced by the interplay between the electrical activity of the cell membrane and the release of calcium from the endoplasmic reticulum. We do that by coupling two existing models: Gall-Susa and Li-Rinzel model. By constructing such model, we are able to reproduce both oscillatory patterns of intracellular calcium observed, and study their origin . We find that a difference in the radius of the cells provides a reasonable testable explanation for the existence of different types of oscillations observed in chromaffin cells.

1 Introduction

Calcium is an important intracellular messenger that regulates cellular processes such as gene transcription, muscle contraction and hormonal secretion. In the case of chromaffin cells, intracellular oscillations of calcium control the secretion of catecholamines like adrenaline. Because of their wide availability, their ease of isolation and preparation in primary cultures, and the fact that intracellular Ca^{2+} oscillations in chromaffin cells are produced by the same mechanisms that other secretory cells, chromaffin cells have been widely used in biochemical and electrophysiological. Plus, chromaffin cells are structurally very similar to pos-synaptic sympathetic neurons, being also used neuropharmacological studies [1, 2]. Thus, findings on catecholamine release in chromaffin cells can be extrapolated to basic mechanisms in the central and peripheral nervous system [3].

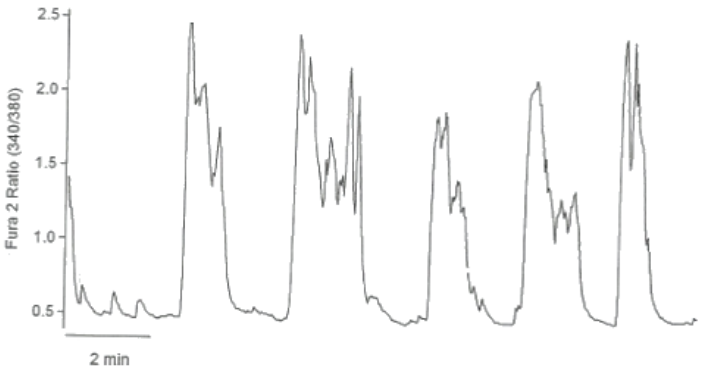
This means that the findings made in the study of chromaffin cells (namely, in the mechanisms that underlie its intracellular calcium oscillations) is not only important to better understand how these cells work, but any secretory cell.

1.1 Intracellular calcium dynamics

Variations in the cytosolic concentration of calcium, induced by an external stimulus, act as a signal to the cell. The most common pattern of calcium signaling is a temporal pattern of periodic discharges and elevations of cytosolic calcium concentrations [4].

In chromaffin cells, calcium plays an essential role in coupling chromaffin cell stimulation to the secretory response: chromaffin cells from the adrenal medulla synthesize, store and secrete catecholamines like adrenaline, contributing to the cardiovascular and metabolic adaptations of the body to stressful and/or dangerous situations.

Rat chromaffin cells in culture exhibit oscillations of cytosolic calcium concentration when 100 μM of methacholine, a synthetic drug that stimulates the production of IP_3 in the cytosol, are administrated to the cells (Figure 1).



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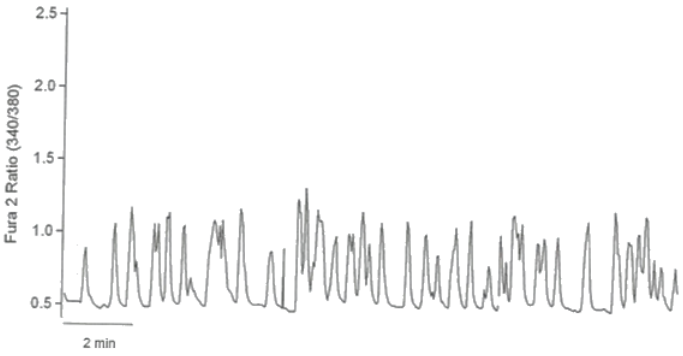


(b)

Figure 1: Experimental measurements of intracellular calcium in rat chromaffin cells provided by Dr. A. Martínez, from the Faculty of Medicine of Universidad Autónoma de Madrid. The intracellular calcium measurements were made using Fura 2, a ratiometric fluorescent dye that binds to free intracellular calcium. Two different oscillatory patterns were observed, (a) and (b).

One would like to understand why some cells display large Ca^{2+} peaks (with smaller variations superimposed) as in Figure 1 (a), while others show a faster, base-line spiking, as in Figure 1 (b). For that, we need to understand the mechanisms underlying the intracellular calcium dynamics.

Calcium flux into the cytosol can occur via two principal pathways: inflow from the extracellular medium through Ca^{2+} channels present in the cell membrane and release from internal stores, namely from the endoplasmic reticulum (ER). The calcium channels present in the cell membrane are voltage-gated channels which, as their name indicates, open in response to depolarization of the cell membrane, allowing the entry of calcium in the cell. Calcium release from internal stores such as the ER is mediated principally by the IP_3 receptor, a Ca^{2+} channel presence in the ER membrane that opens in the presence of the IP_3 molecule.

One of the earliest model conceived predicted that the self-amplification of Ca^{2+} release from the ER into the cytoplasm lies at the basis of intracellular Ca^{2+} oscillations. This regulation is a result of a positive feedback exerted by cytosolic Ca^{2+} on its release from intracellular stores, known as Ca^{2+} -induced Ca^{2+} release (CICR)[13], coordinated by receptors in the ER membrane, the IP_3 receptors. These receptors are sensitive to both IP_3 and Ca^{2+}, having an activation site for IP_3 and for Ca^{2+} that behave as co-agonists [5].

The first theoretical model based on microscopic kinetics of IP_3 and Ca^{2+} gating on the IP_3 receptor is the De Young-Keizer model for Ca^{2+} oscillations. In this model, release of Ca^{2+} is mediated by the IP_3 receptor while Ca^{2+} uptake is accomplished through (Ca^{2+})-ATPase pumps present in the ER membrane.

One IP_3 receptor is composed by 4 subunits. The De Young-Keizer model describes the dynamics of the IP_3 receptor assuming the existence of three binding sites on each subunit of the IP_3 receptor: one for IP_3 and two for Ca^{2+} which include an activation and an inactivation site. A subunit is in an open state when it has the activation binding site of the IP_3 and of the Ca^{2+} occupied and the inactivation binding site of the Ca^{2+} empty. The model assumes that Ca^{2+} passes through the IP_3 receptor only when three subunits are in the open state.

The De Young-Keizer model is a nine-variable model that, while unique in giving detailed gating kinetics, depends on a relatively high number of variables.

On the construction of our new model that describes the non-linear dynamics of calcium in chromaffin cells, a simplified version of the De Young-Keizer model will be used, the Li-Rinzel model [6].

1.2 Electrical properties of the cell membrane

An external stimulus may provoke the opening of Ca^{2+} channels in the cell membrane, granting the entry of Ca^{2+} from the extracellular medium into the cell. This phenomenon can be better understood by studying the electrical properties of the cell membrane and the models for membrane potentials.

The cell membrane is selectively permeable, only allowing certain ions to pass through it, and it controls the flux of ions in and out of the cell, maintaining a difference of ions concentration between the extra and intracellular medium. This disparity gives rise to a potential difference across the membrane, the membrane potential.

Many cells use the membrane potential as a signal to operate different functions. In the case of chromaffin cells, the electrical behavior of the cell membrane influences Ca^{2+} entry.

The electrical activity of excitable cells has its origin in the property of proteins composing the cell membrane - which include channels and transporters - that control the passage of ions across the membrane. The activity of ion channels and transporters generates differences in ion transmembrane concentration originating an accumulation of charge from both sides of the membrane, by electrodiffusion.

The lipid bilayer may therefore be regarded as a capacitor. Moreover, one can think of the cell membrane as a capacitor in parallel with ionic currents, and can describe the cell's membrane potential by the following equation

$$C\frac{dV}{dt} + I = 0 \tag{1}$$

where the current I is given by

$$I = g(t)(V - V_i) \tag{2}$$

wand where g is the conductance of the ionic channel, V the potential difference across the cell membrane, and V_i the Nerst equilibrium potential of the ion specie i.

In terms of an electrical circuit, the different ion selective channels work in parallel with the cell membrane, as illustrated in Figure 2.

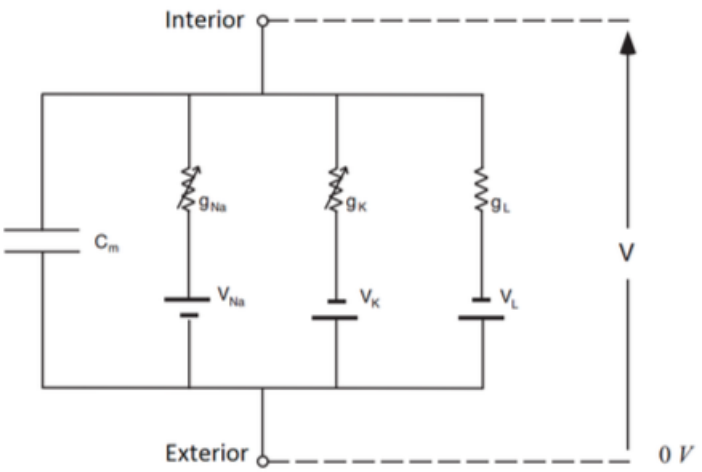


Figure 2: Equivalent electrical circuit of the cell membrane (Hodgkin and Huxley, 1952). Each branch of the electrical circuit represents the contribution to the total transmembrane current from a specific ion specie channel. In this case, three different type of ion channels are embedded in the cell membrane, Na^+, K^+ and a leakage L.

Calcium oscillations are modulated inside an electrical model of the cell membrane. In particular, the Gall-Susa model, that investigates the role of plasma membrane Na^+/Ca^{2+} in pancreatic β-cells, describes intracellular calcium oscillations having into account [7].

2 Dynamics of theoretical models

Calcium oscillations in chromaffin cells are produced by the interplay between the electrical activity of the cell membrane and the release of calcium from the endoplasmic reticulum. Thus, our model needs to have into consideration this two components. This will be accomplished by coupling two previously published models: Gall-Susa and Li-Rinzel model.

Gall-Susa model describes calcium oscillations taking into account the electrical activity of the cell membrane, but not the role of the IP_3 receptor. On the other hand, Li-Rinzel model describes calcium oscillations considering the contribution of calcium release from the ER only. Knowing that both mechanisms play a major role in the regulation of $[Ca^{2+}]_i$, we will incorporate the flux of calcium passing through the IP_3 receptors and ER pumps described in the Li-Rinzel model, into the Gall-Susa model, thus having a complete mathematical model that describes the non-linear dynamics of Ca^{2+} in chromaffin cells.

The fact that the Gall-Susa model structure resemblances the structure of Li-Rinzel encourages the incorporation of one model into the other (both are based on the Hodgkin-Huxley model [8]). Also, both Gall-Susa and Li-Rinzel are simple models, having a small number of variables, which makes it easy to incorporate one into the other.

2.1 Li-Rinzel model

The Li-Rinzel model is a two-variable model that describes the cytosolic Ca^{2+} dynamics, $\frac{d[Ca^{2+}]_i}{dt}$, taking into account the activation/deactivation dynamics of the IP_3 receptors, $\frac{dp}{dt}$.

$$\frac{d[Ca^{2+}]_i}{dt} = \underbrace{(c_1 v_1 w_\infty^3 p^3([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{receptor flux}} + \underbrace{c_1 v_2([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{leakage flux}} - \underbrace{\frac{v_3[Ca^{2+}]_i^2}{k_3^2 + [Ca^{2+}]_i^2}}_{\text{pump flux}} \tag{3}$$

$$\frac{dp}{dt} = \frac{p_\infty - p}{\tau_p} \tag{4}$$

The first equation describes the Ca^{2+} exchange between the cytosol and the ER. When the concentration of calcium in the endoplasmic reticulum, $[Ca^{2+}]_{ER}$, is greater than the concentration in the cytosol, $[Ca^{2+}]_i$, a flux of

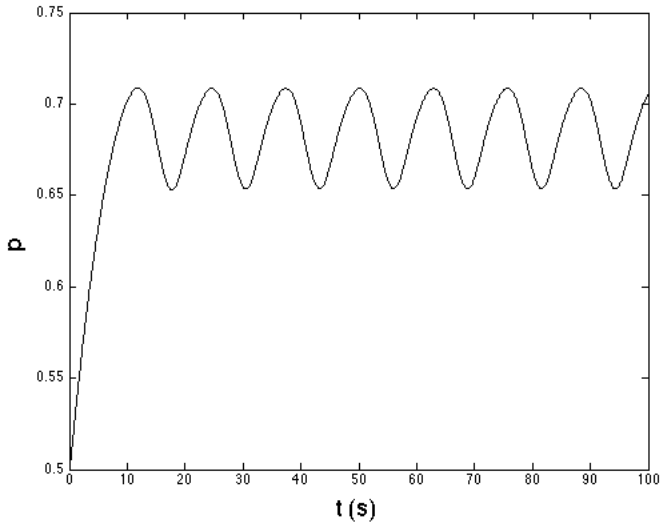
Ca^{2+} flows from the interior of the ER into the cytosol through IP_3 receptors present in the ER membrane, described by the first term of equation 3. While v_1 represents the rate/permeability of the IP_3 receptor, $w_\infty^3 p^3$ represents the probability of the IP_3 receptor being open; more precisely, w_∞^3 is the probability of the IP_3 receptor having the activation binding site of IP_3 and of Ca^{2+} occupied and p^3 is the probability of having the inactivation binding site of Ca^{2+} empty. This last one varies according to equation 4, where p_∞ is the fraction of not inactivated IP_3 receptor in the stationary state.

Besides the flux of Ca^{2+} that passes through the IP_3 receptor, there is also a leakage flux released from the ER, described by the second term of equation 3.

Finally, the last term of equation 3 reflects the activity of the ATPase pumps present in the ER membrane that takes Ca^{2+} from the cytosol into the ER, against its concentration gradient, at a rate v_3.

The functions defining the variables w_∞, p and p_∞ are defined in the original article that describes the Li-Rinzel model [6].

The computed solution obtained with XPP (http://www.math.pitt.edu/ bard/xpp/xpp.html) of the Li-Rinzel model are represented in figure 3.



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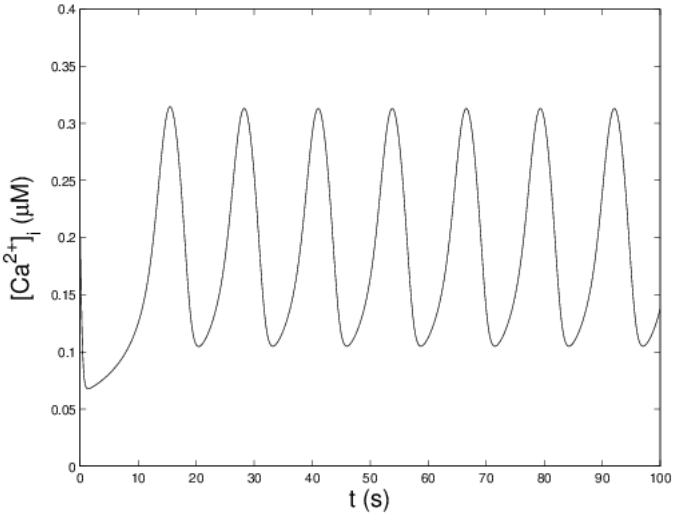


(b)

Figure 3: Reproduction of the time course of p, (a), and of $[Ca^{2+}]_i$, (b), for the Li-Rinzel model. Computed solutions of equations 3 and 4 (see [6] for parameters' values used).

2.2 Gall-Susa model

The mathematical description of Gall-Susa model is an adapted and expanded version of the Hodgkin-Huxley model. It takes into consideration the electrical activity of the cell membrane and the intracellular calcium dynamics. The model is described by the following set of equations:

$$C\frac{dV}{dt} = -I_K - I_{Ca} - I_{K(Ca)} \tag{5}$$

$$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)} \tag{6}$$

$$\frac{d[Ca]_{ER}}{dt} = \underbrace{-k_{rel}([Ca]_{ER} - [Ca]_i)}_{\text{ER flux}} + \underbrace{k_{pump}[Ca]_i}_{\text{ER pump flux}} \tag{7}$$

$$\frac{d[Ca^{2+}]_i}{dt} = \underbrace{-\alpha I_{Ca} - k_{Ca}[Ca^{2+}]_i}_{\text{membrane flux}} + \underbrace{k_{rel}([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{ER flux}} - \underbrace{k_{pump}[Ca^{2+}]_i}_{\text{ER pump flux}} \tag{8}$$

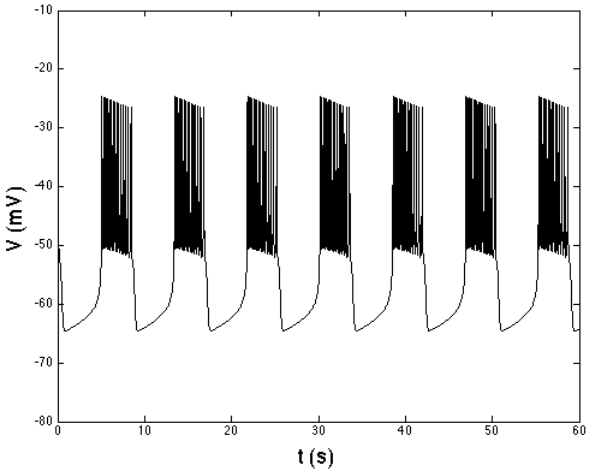
Equation 5 describes how the potential of the cell membrane varies as different ion currents pass through it, and

equation 6 describes the dynamics of the open probability n of the voltage-gated K^+ channels.

Equation 8 describes how the concentration of free cytosolic calcium varies as Ca^{2+} passes through the cell membrane and through the ER membrane. Through the cell membrane, we have the calcium current passing through voltage-gated Ca^{2+} channels that it is going to reflect in an increase of $[Ca^{2+}]_i$ (αI_{Ca}); on the other hand, we have Ca^{2+} being pumped out of the cell by ATPase pumps present in the membrane that work against its concentration gradient ($k_{Ca}[Ca^{2+}]_i$), contributing to the decrease of $[Ca^{2+}]_i$. Besides this, there are Ca^{2+} exchanges between the cytosol and the ER - last two terms of equation 8 and equation7. When $[Ca^{2+}]_{ER}$ is bigger than $[Ca^{2+}]_i$, a leakage of Ca^{2+} starts to flow according to its concentration gradient, $k_{rel}([Ca^{2+}]_{ER} - [Ca^{2+}]_i)$, while there is Ca^{2+} constantly being pumped from the cytosol into the ER by ATPases present in the ER membrane, $k_{pump}[Ca^{2+}]_i$.

The currents I_K, I_{Ca} and $I_{K(Ca)}$ are defined in the original article that describes the Gall-Susa model [7].

Using XPP to numerically integrate equations 5- 8, we reproduced the results obtain by Gall and Susa (model III of [7]) in terms of cytosolic calcium dynamics. The results are illustrated in Figure 4.



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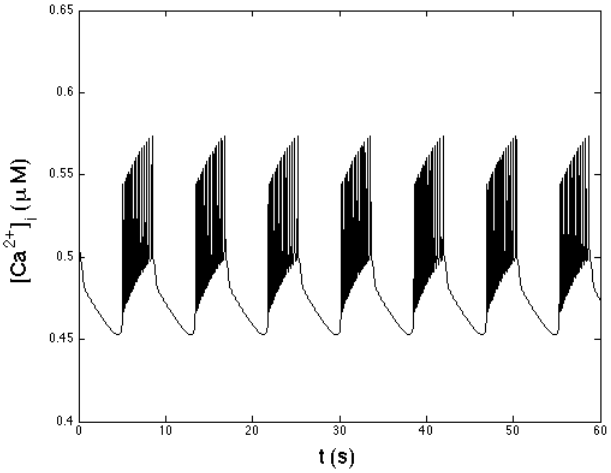

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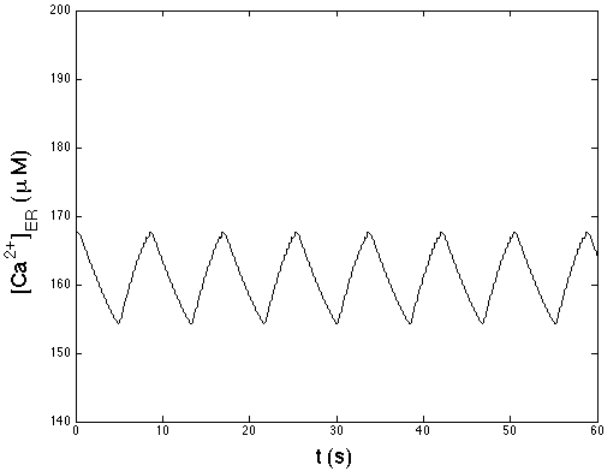


(e)

Figure 4: Reproduction of the time courses of the electrical activity, V, and calcium concentrations in the cytosol, $[Ca^{2+}]_i$, and in the ER, $[Ca^{2+}]_{ER}$, for the Gall-Susa model (see model III of [7] for parameters' values).

3 A new model for calcium oscillations in chromaffin cells

As mentioned before, our goal is to construct a new model for the intracellular calcium dynamics in chromaffin cells, by coupling the Gall-Susa and the Li-Rinzel models. This requires an adaptation of the models as they are out together, to ensure that their dynamics remain approximately the same as new variables are added.

Unlike Gall-Susa, Li-Rinzel model describes the exchange of Ca^{2+} between the cytosol and the ER having into account the flux of Ca^{2+} that passes through the IP_3 receptors. Thus, in order to have a model that fully describes the intracellular Ca^{2+} dynamics of chromaffin cells we need to have a model that describes not only the electrical activity of the cell membrane and the flux of Ca^{2+} passing through it but also the flux of Ca^{2+} passing through the IP_3 receptors present in the ER membrane.

Such model can be built by incorporating the flux of Ca^{2+} passing through the IP_3 receptors of Li-Rinzel model and the evolution equation describing its dynamics into Gall-Susa model.

The Ca^{2+} flux passing through the IP_3 receptors is given by

$$f = c_1 v_1 w_\infty^3 p^3 ([Ca^{2+}]_{ER} - [Ca^{2+}]_i) \tag{9}$$

while the dynamics of the IP_3 receptors is described by the following equation

$$\frac{dp}{dt} = \frac{p_\infty - p}{\tau_p} \tag{10}$$

Adding this two components of the Li-Rinzel model to the Gall-Susa model, we obtain a model described by the following set of equations

$$\frac{dV}{dt} = -\frac{1}{C}\left(I_K + I_{Ca} + I_{K(Ca)}\right) \tag{11}$$

$$\frac{dn}{dt} = \frac{n_\infty - n}{\tau} \tag{12}$$

$$\frac{dp}{dt} = \frac{p_\infty - p}{\tau_p} \tag{13}$$

$$\begin{aligned}\frac{d[Ca^{2+}]_i}{dt} = &- \underbrace{\alpha I_{Ca} - k_{Ca}[Ca^{2+}]_i}_{\text{membrane flux}} \\ &+ \underbrace{k_{rel}([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{leakage flux}} - \underbrace{c_1 k_{pump}[Ca^{2+}]_i}_{\text{ER pump flux}} \\ &+ \underbrace{c_1 v_1 w_\infty^3 p^3 ([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{flux trhough the IP3 receptors}}\end{aligned} \tag{14}$$

$$\begin{aligned}\frac{d[Ca^{2+}]_{ER}}{dt} = &- \underbrace{\frac{1}{c_1} k_{rel}([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{leakage flux}} \\ &+ \underbrace{k_{pump}[Ca^{2+}]_i}_{\text{ER pump flux}} - \underbrace{c_1 * v_1 w_\infty^3 p^3 ([Ca^{2+}]_{ER} - [Ca^{2+}]_i)}_{\text{flux through the IP3 receptors}}\end{aligned} \tag{15}$$

Because this set of equations describes variations of Ca^{2+} concentrations, they must depend on the volume of the ER and the cytosol. Gall-Susa model assumes that the volume of the ER and the volume of the cytosol are approximately the same, which is not accurate. The ratio between the ER and the cytosol is $\approx 20\%$. Because of this, we also had to add a variable $c_1 (= \frac{Vol_{ER}}{Vol_{cytosol}})$ to the terms of the model corresponding to the Gall-Susa model.

Also, the values of some parameters had to be adjusted so that the order of magnitude of the fluxes and the dynamics of the channels involved in the exchange of Ca^{2+} remain roughly the same in our new model as in the Gall-Susa and Li-Rinzel models.

In particular, we had to change the threshold value of activation of the IP_3R by IP_3 d_1 (from $d_1 = 0.13$ to $d_1 = 1$), the threshold value for activation of the IP_3R by Ca^{2+} d_5 (from $d_5 = 0.08234$ to $d_5 = 0.2$), the maximum IP_3-gated permeability v_1 (from $v_1 = 6s^{-1}$ to $v_1 = 0.45s^{-1}$). Although these values are very different from the ones of the original model (Li-Rinzel model), there is no agreement on the realistic values for this parameters and so, there is no reason to exclude the values chosen by us and to not consider them valid.

The value for the rate of Ca^{2+} pumped out of the cell, k_{pump}, originally used in the Gall-Susa model ($k_{pump} = 0.2ms^{-1}$) has been modified in agreement with experimental data. We had to decrease the value of this parameter conserving the behavior of the original model. For that, the change of k_{pump} (from $k_{pump} = 0.2ms^{-1}$ to $k_{pump} = 0.0589$) led to a change of the rate of Ca^{2+} release from the ER k_{rel} (from $k_{rel} = 0.0006ms^{-1}$ to $k_{rel} = 0.0002ms^{-1}$), of the maximal conductance of the Ca^{2+} -activated K^+ channels $g_{K(Ca)}$ ($g_{K(Ca)} = 30000pS$ to $g_{K(Ca)} = 29000pS$), and the maximal conductance of the K^+ channels g_K (from $g_K = 2700pS$ to $g_K = 1600pS$). The conductance depends on the number of channels existing on the cell membrane. Considering the order of magnitude of the conductances in study, the decreases performed to their original values do not reflect on a dramatic change of the number of channels, and thus remain valid. However, it is important to note that this is merely an estimate having into consideration the values for the conductances of the ion channels found in literature [9], since there is no way to measure the conductance of one single channel.

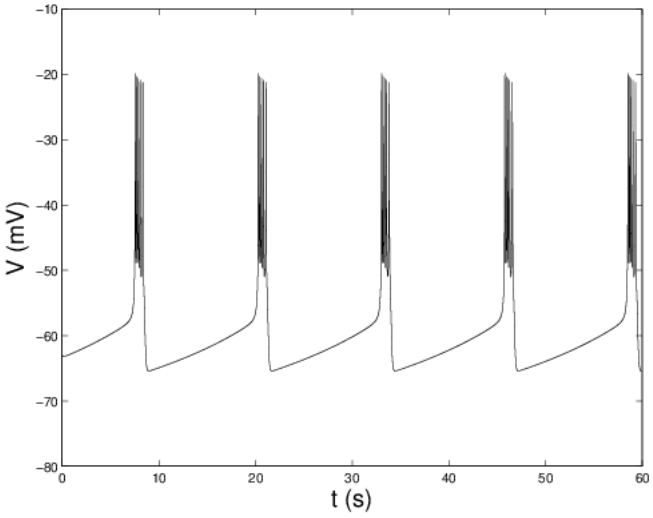
All the other values remain the same and were already validated for the publication of the respective original models, [6] and [7].

The set of equations 11 - 15 forms a new mathematical model that describes the non-linear dynamics of intracellular Ca^{2+} in chromaffin cells. Equation 11 describes the

electrical activity of the cell membrane having into account the contribution of the potassium current K^+ from voltage-gated potassium channels I_K and from Ca^{2+} -activated potassium channels $I_{K(Ca)}$, and the contribution of the Ca^{2+} current, I_{Ca}. Equation 12 and 13 describe the dynamics of the gating variable of the voltage-gated K^+ channels and the dynamics of the IP_3 receptors, respectively. Equation 14 and 15 describe the concentration variations of Ca^{2+} in the cytosol and in the ER, where all the terms of the evolution equations retain their original significance.

A numerically computed solution was developed for the final cell model, which is shown in figure 5.

It is clear that the calcium concentration in the ER $[Ca^{2+}]_{ER}$ has a much slower dynamics than the voltage V or the calcium concentration in the cytosol $[Ca^{2+}]_i$. Exploiting this time scale difference led us to conclude that the slow dynamics of $[Ca^{2+}]_{ER}$ determines the length of the burst.



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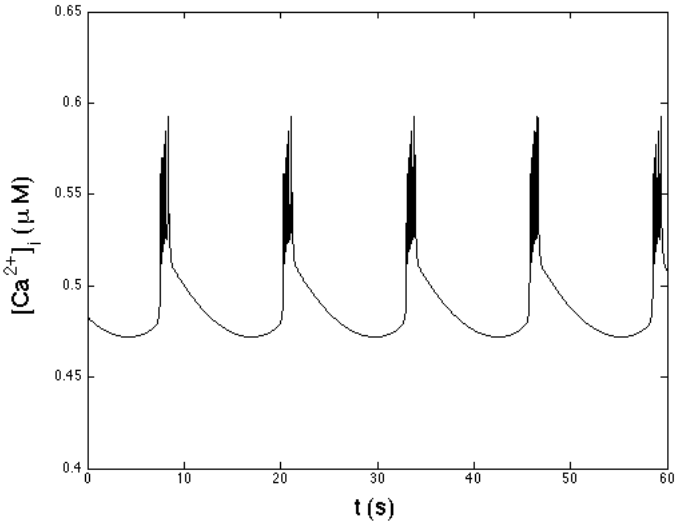

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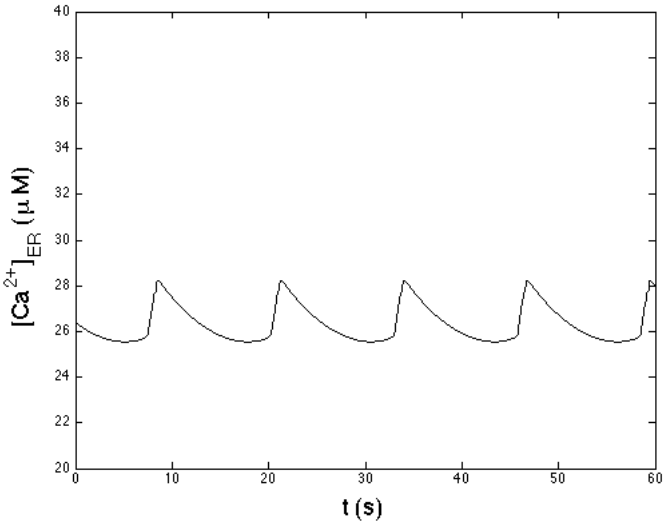
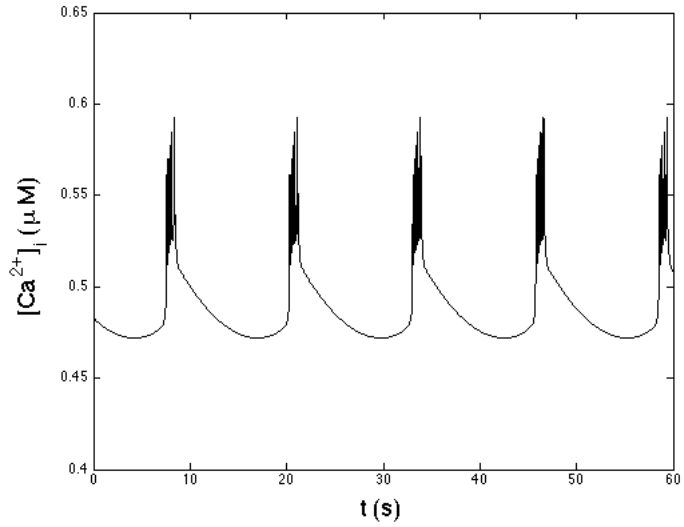


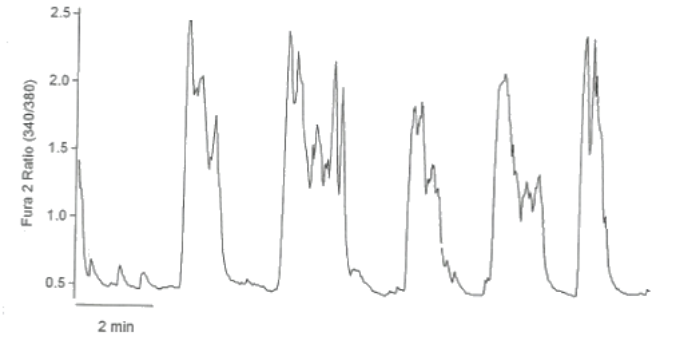
(h)

Figure 5: Reproduction of the time courses of the variables of the final model. (f) Membrane's potential of the cell. (g) Cytosolic calcium concentration. (h) Calcium concentration in the ER. Computed solutions of equations 11 - 15.

As figure 5(g) shows us, our new model exhibits bursts that bear a qualitative resemblance to one of the pattern of oscillations obtained experimentally. Comparing figures 6(i) and 6(j) we see that in both cases we have fast spikes during the active phase followed by a gradual decrease in $[Ca^{2+}]_i$.

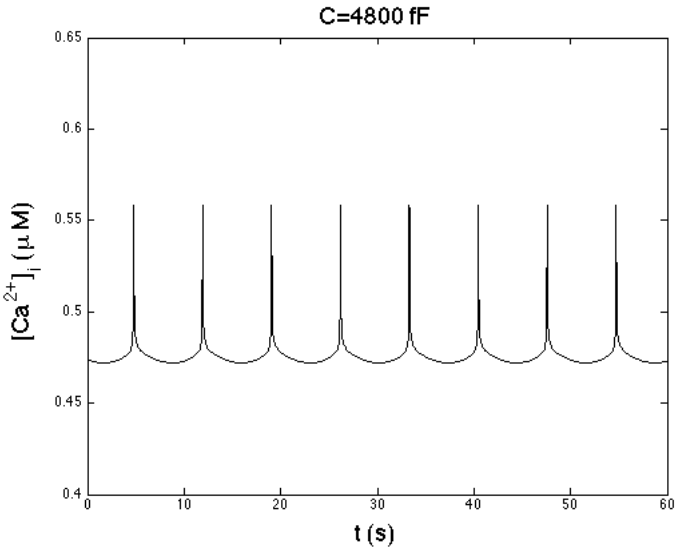


(i)

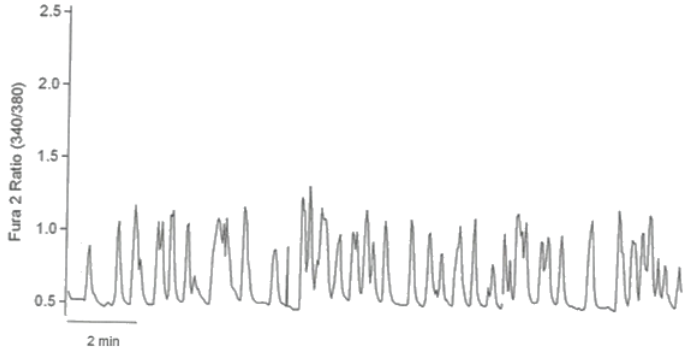


(j)

Figure 6: Comparison of predict results with experimental results. (i) Intracellular calcium concentrations, $[Ca^{2+}]_i$, for the final model. (j) Experimental measurements of calcium in rat chromaffin cells provided by Dr. A. Martínez, Faculty of Medicine of Universidad Autónoma de Madrid



(a)



(b)

Figure 7: Comparison of predict results with experimental results. (a) Time course of the intracellular calcium concentrations for the new model with $C = 4800$. (b) Experimental measurements of calcium in rat chromaffin cells provided by Dr. A. Martínez, Faculty of Medicine of Universidad Autónoma de Madrid

The main differences between the two oscillation patterns are the frequency and the amplitude of the oscillations. Comparing the two patterns obtained experimentally we see the second pattern (Figure 1 (b)) has a higher frequency and a smaller amplitude relative to the first (Figure 1 (a)). In an attempt to reproduce the second pattern of oscillations, we changed the values of parameters that could be the cause for the existence of the two different types of oscillations; namely the capacitance of the cell membrane (from $C = 5310fF$ to $C = 4800fF$).

By decreasing the value of the capacitance, the new model exhibits single spikes with a higher frequency and smaller amplitude than the pattern previously obtained. Thus, it retains the main characteristics of the second pattern obtained experimentally.

A decrease of the cell capacity from $C = 5310fF$ to $C = 4800fF$ translates in a decrease of the cell radius from $r = 6.5\mu m$ to $r = 6.18\mu m$ ($\frac{C}{A} = c$ where A is the area of the cell $4\pi r^2$ and c the specific capacitance that is

$1 \times 10^{-6}F$). This presents a possible explanation for the different patterns of oscillations observed: the sample of chromaffin cells analyzes may have been composed of cells of different sizes.

4 Conclusion and Perspectives

The motivation behind the construct of this new model came from the experimental observations of two distinct oscillatory patterns of intracellular calcium in chromaffin cells from the same population, submitted to the same conditions, and the non-existence of a good explanation for this phenomenon. Our goal was to build a model able to provide such an explanation by constructing a model able to reproduce both types of intracellular calcium oscillations observed. Not only were we able to do that, but we were also able to reproduce some other experimental results concerning chromaffin cells and their intracellular calcium concentrations.

Our new model results from the coupling of two existing models: one that describes calcium oscillations cells taking into account the electrical activity of the cell membrane - Gall-Susa model - and other based on IP_3 receptor-mediated calcium oscillations - Li-Rinzel model. With this model, we were able to reproduce both oscillatory patterns of intracellular calcium observed experimentally by decreasing the value of the cell capacitance. The decrease of a cell capacitance reflects in a decrease of its radius. Then, we can predict that the reason why cells of a same population of chromaffin cells presents two different types of oscillations is because it is composed by cells of different sizes.

There are models that describe the intracellular calcium oscillations in neurons taking into account the electrical activity of the cell membrane and the dynamics of the IP_3 receptors [10,11,12]. The parameters of these models could be adapted to describe the situation of chromaffin cells. However, these models are very complex, having a high number of variables, which makes it hard to study them and make any modifications. Plus, they fail to reproduce both patterns of oscillations observed by Dr. Martínez in chromaffin cells and the intracellular calcium dynamics that they describe does not correspond to the one observed in chromaffin cells. For example, they describe a chaotic bursting patterns of intracellular calcium and they describe the concentration of calcium in the cytosol as a slow variable.

Even though we have constructed a new model able to reproduce important experimental observations and that provides a reasonable testable explanation for the existence of the different types of oscillations observed, this model is only a first attempt to reproduce this experimental results and it has some limitations.

In our model we only take into account the Ca^{2+} being pumped into the ER and a flux of Ca^{2+} that it is released into the cytosol through IP_3 receptors (besides a small leakage flux). However, there is an extra Ca^{2+} flux that we did not take into account: the flux of Ca^{2+} being released into the cytosol through the ryanodine receptors present in the ER membrane [9]. We did not take this extra flux into account in our model because it did not reveal to be essential to reproduce the two oscillatory patterns, and indeed we manage to reproduce qualitatively both types of intracellular Ca^{2+} oscillations and provide a reasonable explanation for their co-existence, which was our main purpose. Moreover, this model is based on simple existing models for calcium dynamics and thus, does not take into account the presence of Na^{+} channels that play a role in the variation of membrane's potential in chromaffin cells [12] by allowing the passage of a sodium current through the cell's membrane. But once again, we did not yet take this current into consideration because we wanted to construct a simple model able to describe our observations concerning the intracellular calcium oscillations so that now it is easier to improve it and manipulate. This way this model serves not only for the study of intracellular Ca^{2+} oscillations but may be eventually used, and after making the necessary adaptations, to study other related phenomenon in chromaffin cells. For example, to study the secretion of catecholamines in chromaffin cells and how our sympathetic nervous system responds to it. Chromaffin cells are neuroendocrine cells that are structurally and functionally in close proximity to post-synaptic sympathetic neurons, which are a part of the sympathetic nervous system. Thus, a better understanding the mechanisms underlying calcium oscillations in chromaffin cells and how different oscillatory patterns (and their origin) of intracellular calcium influence catecholamines secretion can contribute to the study of the sympathetic nervous system and it may even give some enlightenment about the source of nervous system related diseases.

For the cell population studied 80% of the cells analyzed presented the second type of oscillations, simple fast spikes with small amplitude, while only 20% presented bursting. For a future work, it would be interesting to study what is the balance between the different types of oscillations in a population of chromaffin cells of different individuals and, in case it is not the same for all individuals, study how differently their system responds (change of the hearth beat and of blood pressure, for example) to a same external stimulus.

References

[1] Morgan A., Burgoyne RD. (1997). *Common mechanisms for regulated exocytosis in the chromaffin cell and the synapse*. Semin. Cell Dev. Biol. 8(2): 141-9.

[2] Shtukmaster S., Schier M., Huber K., Krispin S., Kalcheim C. and Unsicker K. (2013). *Sympathetic neurons and chromaffin cells share a common progenitor in the neural crest in vivo*. Neural Development 8: 12.

[3] García A., García-De-Diego A., Gandía L., Borges R. and García-Sancho J. (2006). *Calcium Signaling and Exocytosis in Adrenal Chromaffin Cells*. Physiological Reviews vol. 86 nÂž. 4: 1093-1131.

[4] Dupont G., Combettes L., Bird G., Putney J. (2011). *Calcium oscillations*. Cold Spring Harb Perspect Biol; 3:a004226

[5] Dupont G., Goldbeter A. (1993) *One-pool model for Ca^{2+} oscillations involving Ca^{2+} and inositol 1,4,5-trisphosphate as co-agonists for Ca^{2+} release*. Cell Calcium. 14: 311-322

[6] Li Y., Rinzel J. (1994). *Equations for $InsP_3$ receptor-mediated $[Ca^{2+}]_i$ oscillations derived from a detailed kinetic model: a Hodgkin-Huxley like formalism*. J. theor. Biol. 166: 461-473

[7] Gall D., Susa I. (1999).*Effect of Na/Ca exchange on plateau fraction and $[Ca^{2+}]_i$ in models for bursting in pancreatic β-cells*. Biophysical Journal 77: 45-53

[8] Hodgkin A. and Huxley A. (1952). *Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo*. J. Physiol. 116: 449-472

[9] Ruiz A., Matute C. and Alberdi E. (2009). *Endoplasmic reticulum Ca^{2+} release through ryanodine and IP_3 receptors contributes to neuronal excitotoxicity*.Cell Calcium 46: 273-281.

[10] Falcke M., Huerta R., Rabinovich M. I., Abarbanel H., Elson R., Selverston A. (2000).*Modeling observed chaotic oscillations in bursting neurons: the role of calcium dynamics and IP_3*. Biol. Cybern. 82: 517-527.

[11] Ye J., Rozdeba P., Morone U. (2014). *Estimating the biophysical properties of neurons with intracellular calcium dynamics*. Physical Review E 89: 062714.

[12] Vandael D., Ottaviani M., Legros C., Lefort C., Guérineau N., Allio A., Carabelli V. and Carbone E. (2015). *Reduced availability of voltage-gated sodium channels by depolarization or blockade by tetrodotoxin boosts burst firing and catecholamine release in mouse chromaffin cells*. J. Physiol. 593.4: 905-927.

[13] Goldbeter A. Dupont G. Berridge MJ. (1990). *Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation*. Proc. Natl. Acad. Sci. USA. 87: 1461-1465

[14] Lodish H., Berk A., Zipursky S., Matsudaira P., Baltimore D., Darnell J. (2000). *Molecular Cell Biology*. W. H. Freeman, New York

[15] Rinzel J. (1985). *Bursting oscillations in an excitable membrane model* . In B.D. Sleeman and R.J. Jarvis, editors, Ordinary and partial differential equations, volume 1151 of *Lecture Notes in Mathematics*, pages 304-316. Springer, New York.

[16] Gall D. (2014). *Eléments de Biophysique et Physiologie des Cellules Excitables*.

[17] Woods NK, Padmanabhan J. (2012). *Neuronal Calcium Signaling and Alzheimer's Disease*. Adv. Exp. Med. Biol. 740: 1193-217.

[18] Mattson MP., Gary D.S., Chan S.L. and Duan W. (2001). *Perturbed endoplasmic reticulum function, synaptic apoptosis and the pathogenesis of Alzheimer's disease*. Biochem. Soc. Symp. 67: 151-62.