Calcium mediated regulation of astrocytes response in the brain

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Until recently glia was belived to serve as structural and chemical support for the neurons. However, novel studies point towards the active role of glia in the brain. As most aboundant glial cell in the mammalian brain, astrocytes have been found to be involved in sensing and regulating synaptic activity. The aim of this project is therefore to study the modelling of calcium dynamics, a key secondary messenger in numerous pathways, related to these new active roles that astrocytes have been found to play in the CNS, and whose malfunction can lead to the appearance and evolution of several mental diseases. Along the study, different dynamics for inositol triphosphate (IP_3), the signaling molecule of the ion channels, will be implemented in order to understand the behaviour of a primitive "dressed neuron", that is, neuron-atrocyte interaction.

I. INTRODUCTION

Research among this kind of glial cell has demonstrated they listen to the neuronal chatter, respond to it and talk back to the neurons, thus modulating their functions [5]. As many other cells, astrocytes use oscillations in order to transmit messages. Ca^{2+} oscillatory transients in the cytosol have been experimentally detected after the application of neurotransmitters, and serve as a messenger containing information in it's frequency and amplitude to a large variety of cellular events [2].

Calcium oscillations

Previously mentioned oscillations in the cytosol are the result of release and uptake of Ca^{2+} mainly from the Endoplasmatic Reticulum (ER) and the cytosol, but also from outside the cell. ER acts as the main source of Ca^{2+} ions inside the cell, and liberates them to te cytosol via IP_3-sensible channels. These are reuptaken into the ER via the sarco/endoplasmic reticulum Ca^{2+}- ATPase (SERCA) channel.

IP_3R is an IP_3-gated, Ca^{2+} permeable channel located in the ER membrane. It has been determined to have a tetrameric structe with each subunite's size close to 2700 residues. Requiring both IP_3 and Ca^{2+}, when in active state allows the transport of Ca^{2+} from the ER to the cytosol. It's working principle is shown in FIG. 1.

Binding of IP_3 (black circle) to the IP_3R determines whether a a stimulatory (green) or inhibitory (red) Ca^{2+}-binding site is available. IP_3 binding causes the stimulatory site to become accessible and the inhibitory site to be concealed; binding of Ca^{2+} (blue circle) to the former then triggers opening of the channel. Multiple studies also showed how Ca^{2+} tunes the sensitivity of the channel to IP_3 [3].

On the one hand, as ER Ca^{2+} storage empties these IP3R channels lose sensitivity to the IP_3 making them prone to be closed. On the other hand, as the reservoir empties IP3R increases it's sensitivity, easing the opening of the channels and favoring the flux of Ca^{2+} towards cytosol.

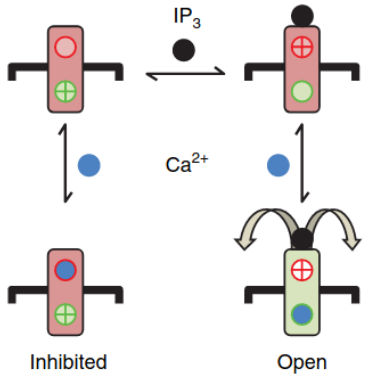

FIG. 1: Binding agents and configurations of IP3R channels. [3]

The most important mechanism used to refill it back is performed by SERCA pumps. This proteinic complexes are embedded in the sarcoplasmic reticulum and use the chemical energy produced from the conversion of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) to transport calcium ions across the membrane from the cytosol to the ER, against a concentration gradient. Upon the binding of a Ca^{2+} ion on the cytoplasmatic side the protein goes through a conformational change getting the ion to the other membrane's side [4].

Overall, the back and forth motion of ions inside the cell from the ER to the cytosol via last two mechanisms described lead can lead to calcium oscillation transients which play a key role in many neural processes. However, in order to carry information as messenger and establish effective synaptic Ca^{2+} concentration in the cytosol has to reach a low enough equilibrium level after a peak of IP_3 induced by atrocyte activation.

Mathematical model of Ca^{2+}

From now on, we will genereally refere to $[Ca^{2+}]_{CYT}$ as Ca^{2+} for seek of simplicity. The former scheme is displayed in order to set the general outline of calcium ions inside the cell.

The general behaviour of an astrocyte is the follow-

ing: when a neuron fires, it releases quantal amounts of neurotransmitters into the synaptic cleft. As neurotransmitters (agonist) bind to the metabolic glutamate receptor on the astrocytes (astrocyte activation), triggering the release of IP_3 intracellularly which peaks and then decays because diffussion or degradation [2].

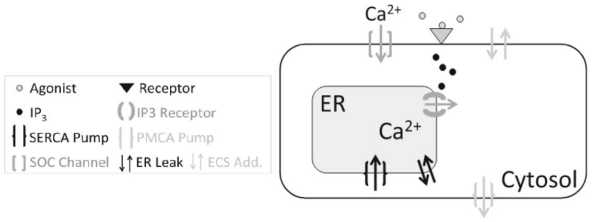

FIG. 2: Simplified schematic of an astrocyte and its major calcium components. Arrows show the direction of calcium flux. [2]

In this project we have considered a slightly simplified version of last scheme. We are assuming the cellule as a closed environment in terms of Ca^{2+}, that is, we will consider a constant $[Ca^{2+}]_{TOT}$ inside the cell. Consequently we will only consider the SERCA pump flux from the cytosol to the ER, the IP_3R channels in the opposite direction and a leak flux that accounts for the diffusion in the ER membrane.

From FIG.2 follows the differential equation for the concentration of calcium ions in the intracellular space:

$$\frac{d[Ca^{2+}]_{CYT}}{dt} = J_{IP3} - J_{SERCA} + J_{leak}$$

with:

$$J_{IP3} = f_v v_1 m(p)^3 n(c)^3 q(c,p)^3([Ca^{2+}]_{ER} - c)$$

$$J_{leak} = f_v v_2 \left([Ca^{2+}]_{ER} - c\right)$$

$$J_{SERCA} = \frac{v_3 c^2}{K_s^2 + c^2}$$

Where p is the concentration of IP_3, c is the concentration of Ca^{2+} in the cytosol and f_v ts the ratio of volumes between the ER and the cytosol, which will allow us to define $[Ca^{2+}]_{TOT} = c + f_v[Ca^{2+}]_{ER}$. From now on, we'll refer to concentration of Ca^{2+} in the cytosol as Ca^{2+}.

IP3R channels working principle depends on three variables, m and n, which we'll assume to be in the fast manifold slave them to:

$$m = \frac{p}{p + K_p} \qquad n = \frac{c}{c + K_n}$$

and q, which represents the fraction of activated IP_3Rs and therefore the gate dynamics previously explained, that has the following temporal evolution:

$$\frac{dq}{dt} = \alpha_q(1 - q) - \beta_q q$$

$$\alpha_q = a d_1 \frac{p + K_p}{p + d_2} \qquad \beta_q = ac$$

being a, d_1, d_2, K_s, K_p and K_n constants.

This simplified model resembles the Hodgkin-Huxley model for electrically excitable membranes.

Generaly, many models can successfully reproduce the oscillating behaviour of calcium in the cytosol. These models can be classified into 'minimal' models containing two variables and 'extended' models of three and more variables . They are mostly based on calcium-induced calcium release (CICR), and only differ in the different mechanisms limiting this release. Extended models include other kind of interactions, such as the dependence of the IP 3R sensitivity on the calcium concentration in ER [6].

Astrocytes modeling

We will use Euler's integration method for seek of simplicity to integrate the differential equations. Along this project we will consider different constant values for key parameters such as $[Ca^{+2}]_{TOT}$ but most importantly different dynamics scaling in complexity in IP_3, transitioning from decoupled to system-coupled, with the aim of exploring Ca^{2+} dynamic changes.

II. 2D MODEL

The first approach we take is considering IP_3 to have a constant value. Although unrealistic, this will allow us to grasp the gist of our system behaviour. In the first place, we make a parameter study with the values of p and $[Ca^{+2}]_{TOT}$ in order to determine for which values we have oscillations. Thus, we run our 2D system (c(t),q(t)) simulation for a high t_{end} value to ensure we have reached steady state. As a general comment, initial conditions for the system dynamics don't affect it's overall behaviour.

A stability study is carried out by computing the Jacobian's eigenvalues of the system as a function of p only, finding the exact $p_{threshold}$ (yellow points in FIG.3(a)) values numerically. We thus identify a Hopf bifurcation and overall a change in dynamics from a stable node, to a repulsive focus, where a limit cycle takes place as steady state and finally into a stable focus. Usually this the p values which lead to develop a limit cycle steady state in our system for the constants values we have used are of the order of 0.345-0.945 μM.

Steady analytical states are displayed in a dashed black line, points stand for c(t_{end}) when there are no oscillations and oscillations are represented with a filled grey area which contour it determined by the values of the maximum and minimum of last oscillation computed.

The following phase phase space $q(t), c(t)$ together with the constant p value is represented in order to get

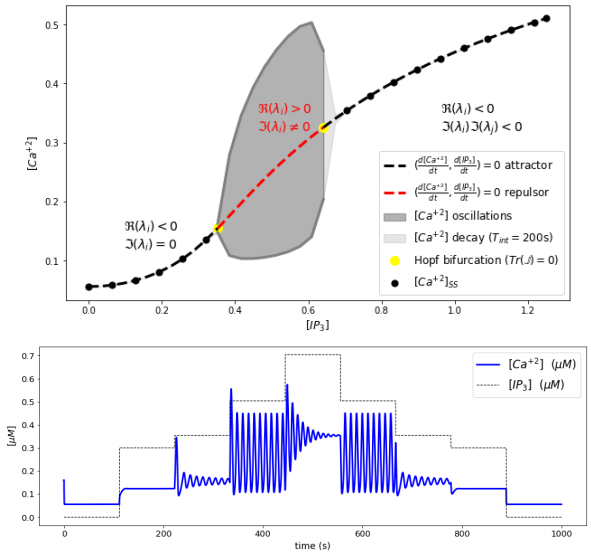

FIG. 3: (a) Bifurcation diagram of c as a function of p. (b) Calcium behaviour in the presence of step increases in IP_3.

a more accurate view of the phase space diagram. In FIG.4(a) 3D diagram also we try to outline a generalization of the oscillation regime as a surface (which should is shown partially in grey for seek of clarity). Some trajectories are plotted too for different IP_3 constant values. Initial points of the trajectories are plotted in green, therefore we check the stability we determined experimentally. Accurate description of each line style is shown in the legend.

With the intention of wrapping the study of this simple model up, we have plotted the bifurcation diagram of c taking into account $[Ca^{+2}]_{TOT}$ and p variation. We get surfaces because of the constant values we are using for two out of three variables displayed. Thereby, the low robustness degree of our system is stated when making small changes in the the value of some of our model constants, being plotted in grey and green the cycle limit regime as well as steady state analytical solutions with the initial constants and making a rounding to first digit, respectively.

III. PRESET IP_3 DYNAMICS

Once the main behaviour of the system has been studied with a fixed value of IP_3, we set a fixed time-evolution for IP_3 mimicking the real role astrocytes play when they react to neural impulses.

Upon the initial binding of agonists in the membrane of the astrocyte, which denote as $t = t^*$, IP_3 is released in the cytosol and after a given time d_{rise} it starts to degradete/diffuse away untill finally there is no more IP_3. Preset equations for IP_3 remind of the RC circuit equations and are the following:

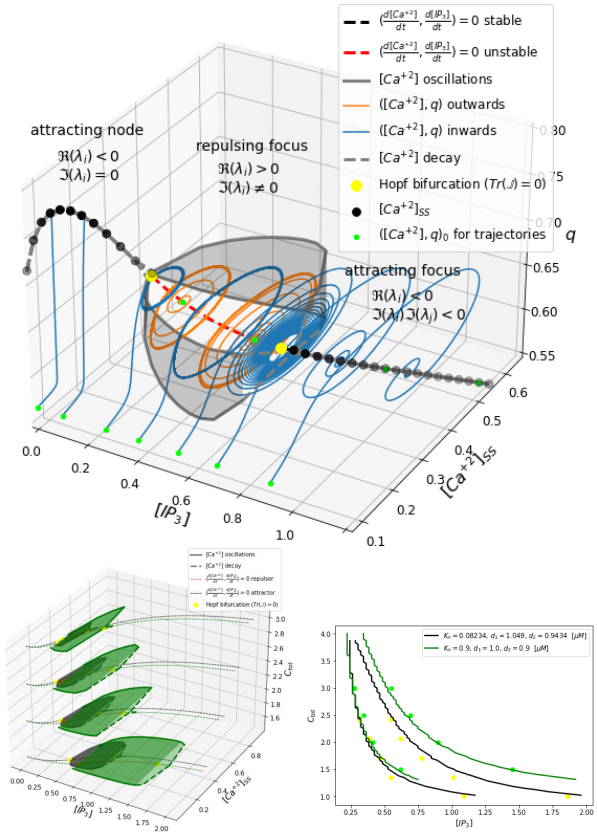

FIG. 4: (a) Extended phase space with constant p values. (b) Bifurcation diagram of Ca^{+2}. (c) Limit cycle regime for different model's constants values.

$$p(t) = \begin{cases} 0 & t < t^* \\ s_\infty \cdot \left(1 - e^{-r_{\text{rise}}(t-t^*)}\right) & t^* \leq t < t^* + d_{\text{rise}} \\ A \cdot e^{-r_{\text{dec}} \cdot (t - [t^* + d_{\text{rise}}])} & t^* + d_{\text{rise}} \leq t \end{cases}$$

$$s_\infty = \frac{A}{1 - e^{-r_{\text{rise}} \cdot d_{\text{rise}}}}, \quad r_{\text{dec}} = -\frac{1}{d_{\text{decay}}} \log\left(\frac{0.005}{A}\right)$$

By only varying key IP_3 parameters 6, the mathematical model is capable of reproducing four different transient types in Ca^{+2}. Lasts ones are the response of the astrocyte after being activated and as secondary messenger are able to significantly change the firing patterns of nearby neurons.

We fixed $t^* = 20s$ since Ca^{+2} dynamics has already reached an equilibrium state and represented the results of different combinations of parameters. IP_3 dynamics and Ca^{+2} transients are plotted in a dashed black line response and in a blue solid line, respectively. By an appropriate tuning of this parameters, despite not being the same as in 6, we have been able to obtain very similar results to the ones shown in the referenced article. The tuning of parameters has done in view of the fact that their astrocyte modelling doesn't keep $[Ca^{+2}]_{TOT}$ constant , but overall, transient's shape distinction is achieved.

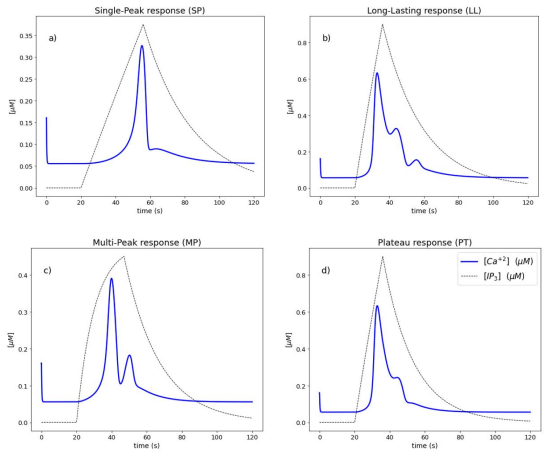

FIG. 5: Variability of calcium response types and underlying bifurcation structure. (a) Single-Peak response. (b) Long-Lasting response. (c) Multi-Peak response. (d) Plateau response.

IV. NEURON INTERACTING IP_3

Finally, a nueuron-astrocyte system is going to be simulated in order to mimic the real behaviour of the astrocyte in the NCS. We'll initially stimulate a neuron following the Hodgkin-Hauxley model and set IP_3 evolution to increment from it's equilibrium value, $[IP_3]^* = 0.16\mu M$ only when the membrane potential of the neuron is larger than 50 mV via the Heaviside function. This is done by:

$$C_m \frac{dv}{dt} = -g_K n^4 (v - v_K) - g_{\mathrm{Na}} m^3 h (v - v_{\mathrm{Na}}) - g_l (v - v_l) + I_{\mathrm{ext}} + I_{\mathrm{astro}}$$

$$\frac{d[Ip_3]}{d\iota} = \frac{1}{\tau_{IP_3}} \left([Ip_3]^* - [Ip_3]\right) + r_{IP_3} \Theta(v - 50\mathrm{mV})$$

Neurons follow dynamics which are three orders of magnitude faster than the astrocyte. To overcome this numerical challenge a study has been done where the interaction term I_{astro} was set to zero. Means of the $\Theta(v - 50\mathrm{mV})$ have been taken every 10^3 neuron unit times, resulting in a value around 0.098 for I_{ext} used and plugged that in to the astrocyte equation as a constant value for every dt of the astrocyte. This way we have analysed the astrocyte feedbackless dynamics efficiently, which we have reduced to a preset dynamics for IP_3 and a 2D study. Our conclusions included relating $\Theta(v - 50\mathrm{mV})_{mean}$ at stationary state such that oscillations took place and r_{IP_3}, which were carried out via Newton's method and used to determine a suitable r_{IP_3} to achieve the desired results. Feedbackless simulation was run for 175s applying $I_{ext} = 15\mu A/cm^2$ for 125s.

Once we explored the dynamics of the astrocyte reacting to a externally stimulated neuron we implemented the feedback I_{astro} into the neuron dynamics, which has the following experimentally fitted expression [5]:

$$I_{\mathrm{astro}} = 2.11\Theta(\ln y) \ln y, \quad y = \left[\mathrm{Ca}^{2+}\right]/\mathrm{nM} - 196.69$$

The intention of this last part of our project was to reproduce the figures presented in [5]. We were working towards an explanation of epileptic behaviour, that is, neurons remaining in their excited state without having any external stimulation.

Despite being computationally costly due to the difference speed of the dynamics and the coupling between atrocyte and neuron we achieved the reproduction of the results we pursued. We ran our simulations, now for a shorter time with $I_{ext} = 15\mu A/cm^2$ for 50s.

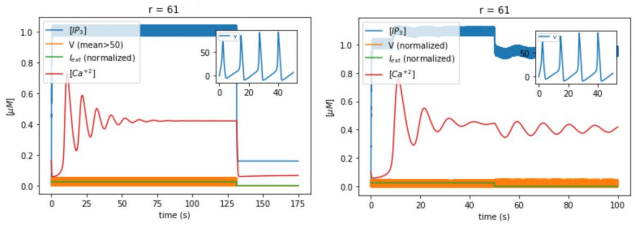

FIG. 6: (a)Feedbackless implemented results for r=61 $\mu M/s$. (b)Feedback implemented results for r=61 $\mu M/s$.

It is clearly observed how this results differ, observing how when the dc stimulation of the neuron has terminated (in blue) the feedback from the astrocyte to the neuron via Ca^{+2} is strong enough to maintain the neuronal oscillations indefinitely, ending up with the same results as [5].

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