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NEURON AND ASTROCYTE COMPUTATIONAL MODELS FOR DESCRIBING THE BRAIN COMPLEXITY

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ABSTRACT. This work presents an overview of the most representative models employed for describing the behavior of biological neuronal networks. In particular, the pioneering work of A. L. Hodgkin and A. F. Huxley on the neuron physiology modeling and the developments of E. M. Izhikevich for the description of neuronal networks that are affordable from a computational point of view, will be presented. Moreover, the role played by astrocyte cells will be dealt. Astrocytes not only play a supporting role in correct neuronal function, but also show communication activities, through Ca^{2+} waves, between themselves and with neurons. Furthermore, this kind of signalling is essential for the proper functioning of the human brain neuronal activity, as, for example, in memory and learning. An astrocytes malfunctioning is, in fact, implicated in diseases such as Alzheimer's, Parkinson's, stroke and epilepsy.

1. Introduction

For decades neurons have been considered as the only cells involved in the generation and control of brain signaling, while the surrounding glia was supposed to provide structural and metabolic support to neuronal function. Neuroscience research has focused for long time on neurons and their interacting networks. However the brain also consists of a large number of other different cell types, among which glial cells that represent roughly 50% of the brain cells (Kettenmann and Verkhratsky 2008; Azevedo *et al.* 2009). In recent years it has be proven that glial cells and specifically astrocytes play several important roles in the central nervous system. Astrocytic metabolic deregulation is a hallmark of neurodegenerative diseases and damaging processes such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and traumatic brain injury (Volterra and Meldolesi 2005; Maragakis and Rothstein 2006; Hamby and Sofroniew 2010; Kimelberg and Nedergaard 2010; Parpura *et al.* 2012).

Some of the synapses of the central nervous system are in contact with astrocytes that wrap around them, thus forming a structural ensemble called the "tripartite synapse": presynaptic neuron, post-synaptic neuron and the ensheathing astrocyte (Araque *et al.* 1999). In a tripartite synapse the neurotransmitters released from neurons also bind receptors on the adjacent astrocyte process, activating signaling pathways in the astrocyte which modulate

synaptic behavior. In addition to contacting neurons, astrocytes are interconnected with each other by gap junctions, specialized channels which allow nutrients and ions to diffuse between networks of astrocytes, expanding further the range and magnitude of synaptic regulation of neurons by astrocytes.

The field of computational neuroscience has almost solely been concentrated on modeling the role of neuronal components in a variety of cellular-, network-, and system-level phenomena. The role of astrocytes, beyond their part in homeostatic and metabolic control, has been much less addressed. Instead of action potential excitability, astrocytes express Ca^{2+} based excitability which has been shown to be associated in modulation of neuronal signaling.

In this article we will consider the close interactions between synaptic terminals and surrounding astrocytic processes, through the use of biophysical models. Calcium signaling is the most commonly measured readout of astrocyte activity. These models can be used to reveal complex interactions and emergent dynamics. Moreover, we will evaluate some of the models in more detail by implementing, in a Python code, the equations of the original publications. Our work is expected to guide future computational glioscience studies and help researchers in selecting suitable models for their research questions. We additionally wish to set some criteria for a successful, reproducible model in the computational neuroscience capable of simulating a tripartite synapse.

2. Hodgkin–Huxley model

With a series of articles published in the 1950s, Hodgkin and Huxley (1952) have opened the door to a detailed understanding of how electro-physiological signals are transmitted within the nervous system. It was from these works that the description of the potential for action was born. The Hodgkin and Huxley model expresses the electrical behavior of a nerve cell. The first step in their analysis was to divide the total membrane current into a capacitance current and an ion current. like this:

$$I = C_M \frac{\mathrm{d}V}{\mathrm{d}t} + I_i \tag{1}$$

where I is the total membrane current density (inward current positive), I_i is the ionic current density (inward current positive), V is the displacement of the membrane potential from its resting value (depolarization negative), C_M is the membrane capacity per unit area (assumed constant), and t is the time.

A further subdivision of the membrane current can be made by splitting the ionic current into components carried by sodium ions (I_{Na}), potassium ions (I_K) and other ions (I_l):

$$I_i = I_{Na} + I_K + I_l \tag{2}$$

The individual ionic currents expressed in terms of ionic conductances (g_{Na}, g_k and $\bar{g}_l$) are obtained by the relations:

$$I_{Na} = g_{Na}(E - E_{Na})$$
$$I_K = g_k(E - E_K)$$
$$I_l = \bar{g}_l(E - E_l)$$

where E_{Na} and E_K are the equilibrium reverse potentials for the sodium and potassium ions, whereas E_l is the potential at which the "leakage current" due to chloride and other ions is zero. For practical applications it is convenient to write these equations in the form:

$$\begin{aligned} I_{Na} &= g_{Na}(V - V_{Na}) \\ I_K &= g_k(V - V_K) \\ I_l &= \bar{g}_l(V - V_l) \end{aligned} \tag{3}$$

where

$$\begin{aligned} V &= E - E_r, \\ V_{Na} &= E_{Na} - E_r, \\ V_K &= E_K - Er, \\ V_l &= E_l - Er \end{aligned}$$

and E_r is the absolute value of the resting potential; V, V_{Na}, V_K, and V_l can then be measured directly as displacements from the resting potential.

However, the model is extremely expensive to implement. It takes 120 floating point operations to evaluate 0.1 ms of model time (assuming that each exponent takes only ten operations), hence, 1200 operations/ms. Thus, one can use the Hodgkin–Huxley formalism only to simulate a small number of neurons or when simulation time is not an issue.

Since the goal of every scientist exploring information processing in the mammalian brain is to have a computationally efficient neurons network which is biologically plausible according to the principles of known anatomy. In this regard we have chosen to use the Izhikevich model. The model combines the biologically plausibility of Hodgkin–Huxley-type dynamics with high computational efficiency.

3. Izihkevich model

The Izihkevich model (Izhikevich 2003) consists of a system of two first order non-linear differential equations. It is a quadratic integrate-and-fire type model with a recovery variable and is able to replicate several characteristics of biological neurons while remaining computationally efficient:

$$\begin{aligned} V_m' &= 0.04 \cdot V_m^2 + 0.05 \cdot V_m + 140 - u + I \\ u' &= a(bV_m - u) \end{aligned} \tag{4}$$

where u and v are dimensionless variables, I is the input to the system, and a and b are dimensionless parameters which are manipulated to describe different firing patterns. The variable v is of most interest as it represents the membrane potential of the neuron in microvolts (mV) and u represents a membrane recovery variable, which accounts for the activation of K^+ ionic currents and inactivation of Na^+ ionic currents, and provides negative feedback to v. After the spike reaches its apex (30 mV), the membrane voltage and the recovery variable are set according to the following rule: if $v \geq 30$ mV, then $V_m \to c$ AND $u \to u + d$.

By changing the parameters a, b, c and d different firing patterns can be simulated. Each parameter corresponds to different aspects of neural behaviour:

- The parameter a describes the time scale of the recovery variable u; smaller values result in slower recovery time.
- The parameter b describes the sensitivity of the recovery variable to the sub threshold fluctuations of the membrane potential v.
- The parameter c describes the after-spike reset value of the membrane potential v caused by the fast high-threshold K^+ conductances.
- The parameter d describes after-spike reset of the recovery variable u caused by slow high-threshold Na^+ and K^+ conductances.

Neocortical neurons in the mammalian brain can be classified into several types according to the pattern of spiking and bursting seen in intracellular recordings of electrical activity. For our work we consider the Regular Spiking (RS) type of neurons, which are the most typical neurons in the cortex. When presented with a prolonged stimulus the neurons fire a few spikes with short inter spike period and then the period increases, known as spike frequency adaptation. Increasing the strength of the injected dc-current increases the inter spike frequency, though it never becomes too fast because of large spike-after hyperpolarizations. Real neuron can be stimulated by injecting pulses of direct current through an electrode, with the "patch clamp" techniques. Then the membrane potential response is recorded as function of current intensity and pulse duration. When a stepped input current is used to stimulate a neuron, the cell continues to fire a sequence of spikes called spike trains, as illustrated in Fig. 1.

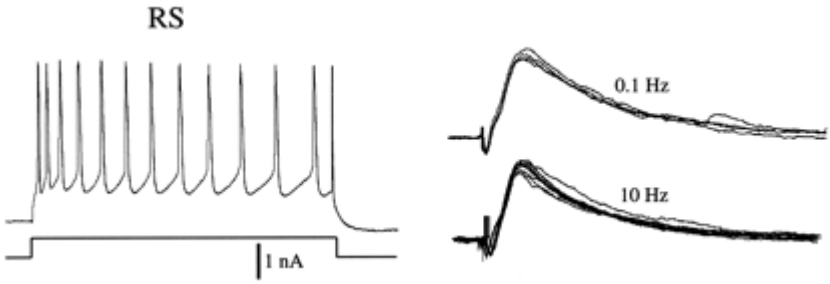

FIGURE 1. Spike discharge pattern of RS neuron of rat in layer 2/3 of area 17 during injection of depolarizing current pulse (200 msec, 0.5 nA).

The observed behavior can be as well simulated with the Izhikievich model by setting the *deep voltage reset* parameter c to 65 mV and the "after-spike reset" d to 8. The output of such a calculation, performed by using Python code, is show in Fig. 2. From the comparison with rat RS neuron patterns it is evident that the response to the neuro-computational simulation obtained with the Izhikievich model is resembling with great accuracy the experimental reference used.

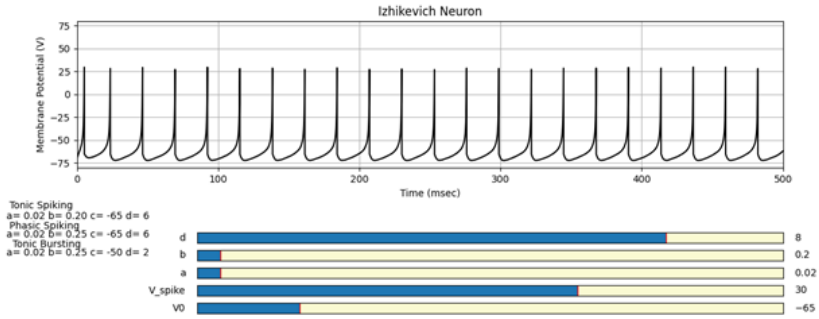

FIGURE 2. Simulation with the Python program of the Izhikevich model for RS neurons.

4. Astrocytes computational model

An increasing number of experiments confirm that astrocytes are not just passive read-out units; they are heavily involved in the modulation of neuronal synapses and their activity (Fellin *et al.* 2004; Perea *et al.* 2009; Clarke and Barres 2013). Although astrocytes are not electrically excitable cells, in particular they cannot generate action potentials, they possess a form of chemical excitability based on changes in their intracellular calcium concentration. From a physiological point of view, astrocytes regulate the current of the synaptic signal between two neurons by modulating the amount of neurotransmitters in the synaptic cleft through the dynamics of inter and intracellular calcium. The groundbreaking discovery in the 1980s that astrocytes express a wide repertoire of neurotransmitter receptors, often mirroring those present on surrounding synapses, marked a new era in glia research. It was subsequently shown that these receptors can be activated by the diffusion of neurotransmitters during synaptic activity and cause an increase in Ca^{2+} in astrocytes. This event can in turn cause the release by astrocytes of chemical transmitters called “glyotransmitters”, such as glutamate, ATP, D-serine, and lead to intercellular communication between astrocytes and neurons (Araque *et al.* 1999). The gliotransmitters released by astrocytes are capable of activating neuronal receptors and thus modify neuronal electrical excitability and synaptic transmission (Fellin *et al.* 2004; Schipke and Kettenmann 2004; Jourdain *et al.* 2007). These observations led to the concept of "tripartite synapses", according to which astrocytes are considered to be the third element of the signal integration unit (Araque *et al.* 1999; Volterra *et al.* 2002).

In recent years, numerous studies have been carried out on the release mechanism of chemical transmitters from astrocytes, among all the gliotransmitters, glutamate is undoubtedly the one that has received the most attention. Recently, through electron microscopy, it has been shown that astrocytes possess specific receptors for glutamate on the outer surface of the plasma membrane. The binding of glutamate to its membrane receptor initiates a series of reactions: the receptor interacts with the G protein which triggers IP_3 signaling. The formed IP_3 molecules diffuse into the cytosol and bind to a specific receptor for IP3 located on the surface of the smooth endoplasmic reticulum (ER) triggering a mechanism called “calcium-induced calcium release” (CICR).

In 1994 Yue-Xian Li and John Rinzel deduced a two-variable system, analogous in form to the classic Hodgkin-Huxley model, to describe calcium dynamics in astrocytes. Their work consists in the reduction to just two variables of a more complicated model developed by De Young and Keizer (1992), with nine variables, describing Ca^{2+} oscillations mediated by IP_3 receptors in endoplasmatic reticulum. The original model is based on experimental measurements of the open probability of Ca^{2+} channel and IP_3 binding constant. The subunits of the IP_3 receptor channel have one IP_3 binding site and two Ca^{2+} binding site, one for the activation and one for inhibition at high Ca^{2+} concentration. The dynamic variables of the LR model are only the concentration C of free cytosol and the fraction h of the open inositol triphosphate receptor subunits. With the assumption that IP_3 binding is not related to Ca^{2+} occupancy at the inactivation site, Li and Rinzel (1994) obtain a "minimal" model which still retains the ability to reproduce experimental observations:

$$\frac{dC}{dt} = J_{chan}(C,I) + J_{leak} - J_{pump}(C) \tag{5}$$

and

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \tag{6}$$

The dynamics of C is controlled by three fluxes, corresponding to:

J_{chan}**:** a release of Ca^{2+}, mutually controlled by Ca^{2+} and by IP_3 concentration;
J_{leak}**:** a passive loss of Ca^{2+} from the endoplasmic reticulum (ER) to the cytosol;
J_{pump}**:** an active absorption of Ca^{2+} in ER due to the action of the pumps;

$$\begin{aligned} J_{chan}(C) &= \Omega_c m_\infty^3 n_\infty^3 h^3 [C_T - (1+\rho_A)C]\,, \\ J_{leak}(C) &= \Omega_L [C_T - (1+\rho_A)C]\,, \\ J_{pump}(C) &= \frac{O_p C^2}{{K_p}^2 + C}\,, \end{aligned}$$

along with the gating variables:

$$m_\infty = \frac{I}{I+d_1},\ n_\infty = \frac{C}{C+d_5}, \quad h_\infty = \frac{Q_2}{Q_2+C},\ \tau_h = \frac{1}{a_2(Q_2+C)}, \quad Q_2 = \frac{I+d_1}{I+d_3}d_2\,.$$

In the above sets of equations the symbols denote:

Ω_L**:** calcium loss rate;
C_T**:** total cell free Ca^{2+} concentration in ER, ρ_A;
O_p**:** max. SERCA pump absorption rate (Sarco-Endoplasmic-Reticulum Ca^{2+}-ATPase);
K_p**:** activation constant of the SERCA pump;
Ω_C**:** maximal IP_3 flux;
a_2**:** receptor binding constant for Ca^{2+} at the inhibition site when IP_3 is occupied;
:: ratio of receptor dissociation/binding constants for:
d_1**:** IP_3 when the Ca^{2+} inhibition site is empty;
d_3**:** IP_3 when the Ca^{2+} inhibition site is occupied;
d_2**:** Ca^{2+} at inhibition site when IP_3 is occupied;
d_5**:** Ca^{2+} at the activation site.

TABLE 1. Biophysical parameters for the modulation of Ca^{2+} oscillations in AM; parameters for FM are in square brackets.

Ω_C	$6\ s^{-1}$	d_1	$0.13\ \mu M$
Ω_L	$0.11\ s^{-1}$	d_2	$1.49\ \mu M$
O_p	$0.9\ s^{-1}$	d_3	$0.9434\ \mu M$
ρ_A	0.185	O_2	$0.2\ \mu M^{-1}s^{-1}$
C_T	$2.0\ \mu M - [4.0]$	d_5	$0.08234\ \mu M$
K_p	$0.1 - [0.051]$	-	-
IP_3 production and degradation			
O_β	$0.8\ \mu Ms^{-1}$	O_δ	$0.025\ \mu Ms^{-1}$
O_{3k}	$0.86\ \mu Ms^{-1}$	K_δ	$0.5\ \mu M$
K_{3k}	$1.0\ \mu M$	O_{5p}	$0.86\ s^{-1}$

The IP_3-induced calcium release channels (IICR) and CICR are represented by m_∞ and n_∞, respectively. Therefore, the level of IP_3 is directly controlled by the signals affecting the cell from its external environment. In turn, the level of IP_3 determines the dynamic behavior of the LR model. The calcium signal can therefore be considered as coded information relating to the level of IP_3.

It has been shown experimentally that Ca^{2+} signals in response to external stimuli encode information either via frequency modulation (FM) (Parpura *et al.* 2004) or amplitude modulation (AM) (Carmignoto 2000). In the following we have explored the range of biophysical parameters for which the LR model system can exhibit a frequency modulation (FM) and/or an amplitude modulation (AM) with the level of IP_3 (I) as a control parameter. As an example we report in Figs. 3 and 4 the Ca^{2+} concentration *vs* time, in presence of a step ramp of IP_3 levels. The values of used parameters, which give rise to the expected amplitude and frequency modulation, are illustrated in Table 1.

From the above analysis we found that K_p, d_5 (the receptor affinity for IP_3), and C_T can all regulate the transition between AM and FM coding dynamics.

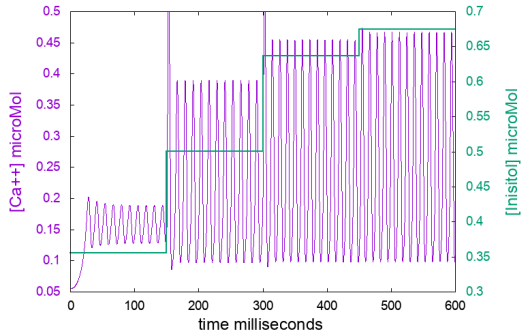

FIGURE 3. The original LR parameters (Table I) provide amplitude variability of oscillations that occur at almost fixed frequency, IP_3 = 0.355, 0.5, 0.637, 0.675.

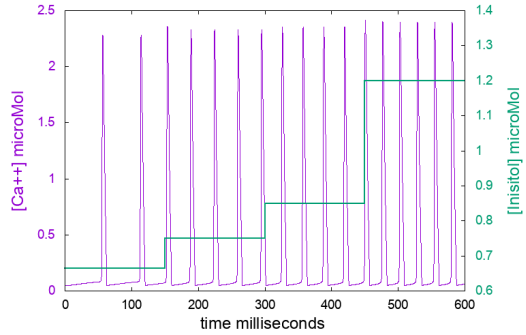

FIGURE 4. A higher SERCA pump Ca^{2+} affinity ($K_p = 0.051\ \mu$M) is responsible for oscillations with variable frequency but nearly constant amplitude, IP_3 = 0.6634, 0.75, 0.85, 1.2.

5. IP_3 regulation: the ChI model

It has been shown that Li-Rinzel model is able to describe Ca^{2+} oscillations in astrocytes with an affordable computational cost. As consequences this allows to extend the model by including further biochemical processes that are relevant for a more realistic description of astrocytes activity. Those extensions have to include IP_3 production and degradation within the astrocyte cell, which are mediated by two membrane-associated enzymes PLCβ and PLCδ. In astrocytes, IP_3 together with diacylglycerol (DAG) is produced by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by two phosphoinositide-specific phospholipase C (PLC) isoenzymes, PLCβ and PLCδ (Rebecchi and Pentyala 2000). PLCβ is primarily controlled by cell surface receptors; hence, its activity is linked to the level of external stimulation (*i.e.*, the extracellular glutamate) and as such, it pertains to the glutamate-dependent IP_3 metabolism. PLCδ is the enzyme responsible of endogenous IP_3 production in astrocytes, it is essentially activated by increased intracellular Ca^{2+} levels (Rhee and Bae 1997). Here we will analize a three-variable model for glutamate-induced intracellular calcium dynamics caused by the synaptic activity (De Pittà *et al.* 2009).

The model proposed for PLCδ-mediated IP_3 production was derived from structural and mutational studies (Pawelczyk and Matecki 1997; Höfer *et al.* 2002), and describe the flux of IP_3 as given by

$$J_\delta = O_\delta \left(1 - \frac{I}{I + K_\delta}\right)\left(\frac{C^2}{C^2 + K_\delta}\right) \tag{7}$$

where O_δ is the maximal rate of IP_3 production by PLCδ and K_δ is the inhibition constant of PLCδ activity. Experiments revealed that high IP_3 concentrations – *i.e.*, larger than 1μM – inhibit PLCδ activity by competing with PIP_2 to bind with the enzyme (Allen and Barres 2009)

The degradation of IP_3 in astrocytes is realized by two pathways: the dephosphorylation of IP_3 by inositol polyphosphate 5-phosphatase (IP-5P), and the phosphorylation of IP_3 by the $IP_3$3-kinase (IP_3-3K). For the description of the two IP_3 degradation dynamics the

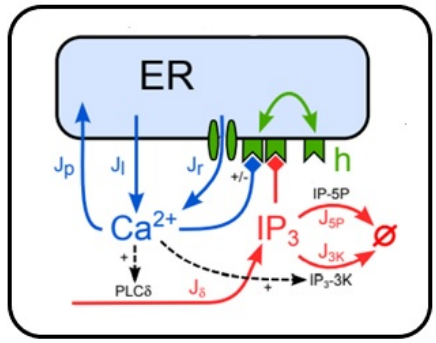

FIGURE 5. Schematic representation of the biophysical network model.

following relation has been used:

$$J_{5p} = O_{5p}\left(\frac{I}{I+K_{5p}}\right) \tag{8}$$

where O_{5p} is the maximal rate of IP-5P mediated IP_3 degradation in the linear approximation. For IP_3-3K degradation we can write:

$$J_{3k} = O_{3k}\left(\frac{C_4}{C_4+K_{3k}^4}\right)\left(\frac{I}{I+K_{3k}}\right) \tag{9}$$

where O_{3k} is the maximal rate of IP_3 degradation by IP_3-3K.

In summary, the ChI model of Ca^{2+} dynamics with endogenous IP_3 metabolism is based on the two Li-Rinzel equations (5) and (6) but the IP_3 concentration (I) is now provided by the differential equations (7), (8), (9):

$$\begin{aligned}\frac{\mathrm{d}I}{\mathrm{d}t} &= J_\delta - J_{3k} - J_{5p}\\ &= O_\delta\left(1-\frac{I}{I+K_\delta}\right)\left(\frac{C^2}{C^2+K_\delta}\right) - O_{3k}\left(\frac{C_4}{C_4+K_{3k}^4}\right)\left(\frac{I}{I+K_{3k}}\right) - O_{5p}\left(\frac{I}{I+K_{5p}}\right)\end{aligned} \tag{10}$$

6. G-ChI: a model for glutamate regulation of IP3 production

Glutamate is by far the most present neurotransmitter in the brain and is the main agent of excitatory neurotransmission. Recently, through electron microscopy, it has been shown that astrocytes possess specific receptors for glutamate on the outer surface of the plasma membrane. The bond of glutamate to its membrane receptor initiates a series of reactions. The receptor interacts with the heterotrimeric G-protein, so called because it is composed of three different polypeptide subunits called α, β and γ, forming a receptor-protein complex, on the inner surface of the membrane. The interaction with the receptor

induces a conformational change of the α subunit which causes the release of bounded GDP and its substitution with GTP which in turn activates the phospholipase effector $C\beta$ phosphatidyl-inositol- specific (PI-PLCβ)$_2$; PI-PLCβ is also localized on the inner surface of the membrane, bounded by the interaction between its PH domain (P leckstrin-h omology) and a PIP_2 molecule immersed in the bilayer. The PI-PLCβ enzyme catalyzes a reaction that splits PIP_2 into two molecules, inositol 1,4,5-triphosphate (IP_3) and diaglycerol (DAG).

Several are the available models for G protein-coupled receptors, and the choice of what model to use rather than another depends on the level of biological detail and the questions one is interested in. Here our focus is on the rate of IP_3 production. The contribution of glutamate signals to IP_3 production can be taken into account as an additional production term in the IP_3 equation of the above three-variable ChI model. The resulting new model is referred to as the G-ChI model (De Pittà *et al.* 2009).

The IP_3 production by PLCβ (J_β) can be taken proportional to the fraction of bound receptors, defined as $\Gamma_A = [R^*]/[R]_T$ where $[R^*]$ is the activation of PLCβ and $[R]_T$ the total number of receptors that is constant:

$$J_\beta = O_\beta . \Gamma_A \tag{11}$$

where O_β is the maximal rate of IP_3 production by PLCβ and lumps information on receptor surface density as well as on the size of the PIP_2 reservoir. In conclusion the G-ChI model for IP_3 / Ca^{2+} signaling is constituted by three ODEs: for the intracellular Ca^{2+} (C), for the IP_3R gating (h) and for the mass balance equation for intracellular IP_3 lumping terms, respectively:

$$\frac{dC}{dt} = \Omega_c m_\infty^3 h^3 [C_T - (1+\rho_A)C] + \Omega_L [C_T - (1+\rho_A)C] - \frac{O_p C^2}{{K_p}^2 + C} \tag{12}$$

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \tag{13}$$

$$\begin{aligned} \frac{dI}{dt} = {} & O_\beta [\theta(t) - \theta(T-t)] + O_\delta \left(1 - \frac{I}{I+K_\delta}\right)\left(\frac{C^2}{C^2+K_\delta}\right) \\ & - O_{3k}\left(\frac{C_4}{C_4+K_4}\right)\left(\frac{I}{I+K_3}\right) O_{5p}\left(\frac{I}{I+K_{5p}}\right) \end{aligned} \tag{14}$$

7. Conclusions

In this work we have presented, through computational models and simulations, the complex dynamics of Ca^{2+} in astrocytes in response to the extracellular signals of the neurotransmitter glutamate. A prerequisite for unraveling the response of astrocytes to such signals is an in-depth understanding of the complex IP_3-related metabolic pathways that regulate the intracellular dynamics of Ca^{2+}. In response to agonists, like hormones or neurotransmitters, and spontaneously as well, the majority of astrocytes exhibit oscillations of intracellular Ca^{2+}. There are two major types for these oscillations: those that are dependent on periodic fluctuations of the cell membrane potential and are associated with periodic entry of Ca^{2+} through voltage-gated Ca^{2+} channels, and those that occur in the presence of a voltage clamp. Our focus here is on the latter type and, in particular, on the so called process of Ca^{2+}-induced Ca^{2+} release (CICR) from the astrocyte's endoplasmic

reticulum stores, which depends on cytosolic concentration of the second messenger inositol 1,4,5-trisphosphate. Two main types of IP_3-mediated CICR are observed in astrocytes: (i) transient Ca^{2+} oscillations that are confined to their (primary) processes and (ii) Ca^{2+} elevations propagating along these processes as regenerative Ca^{2+} waves, often reaching the cell soma and triggering whole-cell Ca^{2+} signaling (Pasti *et al.* 1997; Sul *et al.* 2004).

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