Multimodal Information Encoding in Astrocytes

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Abstract

Astrocytes encode information about external stimuli through complex intracellular calcium dynamics. We explored these dynamics in terms of frequency and amplitude modulations using both deterministic and stochastic astrocyte models that capture such Ca^{2+} dynamics in inherent astrocyte properties. We examined these modulations in networks of such astrocytes connected via gap junctions. Our ultimate goal is to study bidirectional communication between neurons and astrocytes and quantify the information encoded and transmitted in hybrid networks in the context of such modulating dynamics.

Introduction

While neurons are considered the first major cell type in the Central Nervous System (CNS), astrocytes come second. Astrocytes are a subtype of glial cells which are abundantly present in the CNS and outnumber neurons by a factor of 10 in certain parts of the brain.
Contrary to the long-term belief that neurons are the only cell types in the brain that underlie cognitive tasks and information processing and that astrocytes are only responsible for housekeeping and maintaining a homeostatic environment in the CNS, research has recently proved that astrocytes show very interesting nonlinear dynamics in response to neuronal excitation and modulate synaptic plasticity, thus constituting a fundamental component in information processing [1]. Figures 1 and 2 show networks of astrocytes and neurons forming spontaneous connections in a petri dish.

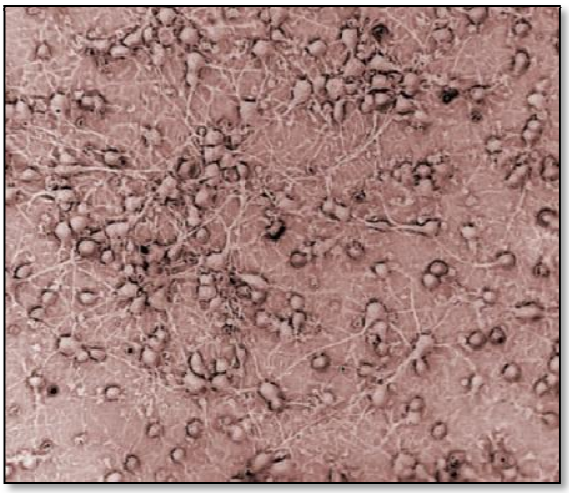

Figure 1*: Bright field view of a co-culture of primary astrocytes and neurons extracted from the frontal cortex of an 18-day old prenatal fetus of Sprague Dawley rats (courtesy of Helen Saad)*

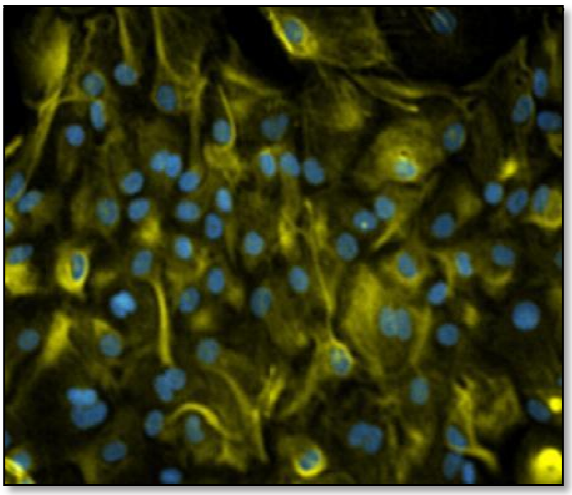

Figure 2*: Fluorescent view of a culture of primary astrocytes extracted from the frontal cortex of a 1-day old Sprague Dawley rat. Nuclei are blue colored while cell bodies and processes are shown in yellow (courtesy of Helen Saad)*

Astrocytes, similar to many other cell types such as pancreas cells and heart cells, encode and transfer information by responding to external stimuli via calcium signaling. These extracellular triggers can be neural activity or chatter to which astrocytes listen and respond via calcium signaling and by releasing gliotransmitters, such as glutamate, that in turn affect the neurons to which those astrocytes are connected [2, 5]. Moreover, astrocytic calcium signaling has been proved to cause the secretion of vasodilatory substances from perivascular astrocytic endfeet which results in improved blood flow [3, 4].

Calcium is indeed a very important messenger that takes part in cell signaling whereby calcium activity occurs not only inside the cell (intracellular calcium dynamics) but also as a form of communication between cells (intercellular calcium) [6]. Intracellular calcium is released from a cell's endoplasmic reticulum (ER) upon an increase in cytosolic inositol triphosphate (IP3) concentration. Intercellular signaling, however, occurs through the mechanism of a wave that propagates among cells in a network. It may involve diffusion of calcium and/or IP3 through gap junctions, which in turn allow diffusion between neighboring cells. The mechanisms of IP3 and calcium dynamics will be detailed in the section that follows.

Displayed in figure 3 are two snapshots showing temporal calcium dynamics in a network of co-cultured primary astrocytes and neurons in terms of the relative change in the fluorescence of these cells in a 12-second time-lapse; calcium and fluorescence are directly correlated because the cells shown were loaded with a fluorescing ester dye (Fluo4-AM) that binds calcium atoms and increases/decreases its fluorescence with the number of calcium atoms bound/unbound.

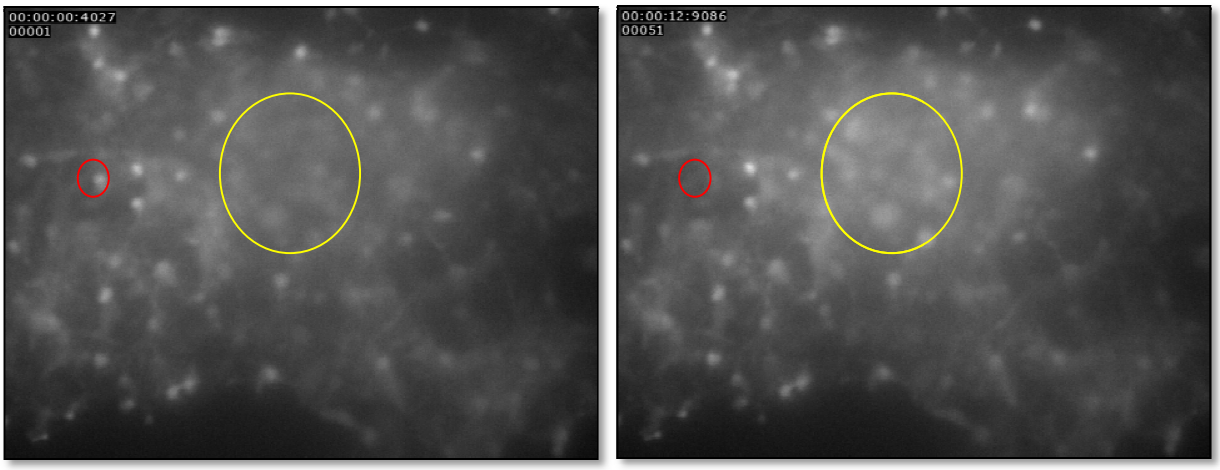

Figure 4: *Calcium dynamics in a co-culture of primary neurons and astrocytes. A sample of the change in calcium is highlighted in the red (decrease in calcium) and yellow (increase in calcium) circles (courtesy of Helen Saad)*

Experiments have proven intracellular calcium dynamics to encode information about extracellular stimuli via frequency modulation (FM) and in some cases via amplitude modulation (AM) [7]. It has also been shown that these types of modulation, in addition to amplitude and frequency modulation (AFM), are exerted in minimal models of astrocytes such as the Li-Rinzel (LR) model as well as in more detailed models that take into account IP3 formation and degradation inside an astrocyte. This work will detail three astrocytic models that have been studied in terms of astrocytic calcium modulation as being FM, AM, and AFM depending on the parameters and stimuli applied to these models. We also explored calcium dynamics in a network of modeled astrocytes connected to each other via gap junctions, the results of which are explained in the sections that follow.

Modeling the Astrocyte

Deterministic Models

Three deterministic models of intracellular calcium dynamics in astrocytes were studied in terms of the calcium dynamics exerted in response to changes in IP3 concentration as a result of applying different external stimuli such as varying glutamate concentrations. Exploration of all three models meant to emphasize the richness of intracellular Ca^{2+} dynamics hence of the encoding modes in astrocytes.
As shown in figure 6, a user interface was developed (using Matlab) in order to help explore the calcium and IP3 dynamics in different astrocytic models (deterministic and stochastic) and in response to different parameters and stimuli.

It is to be noted that the following convention is followed in the rest of the figures unless otherwise stated: stimuli are displayed in blue, intracellular Ca^{2+} concentration is displayed in red, and intracellular IP3 concentration is displayed in green.
Moreover, the models studied are described briefly and the reader is encouraged to seek a detailed explanation by referring to [8] and [9].

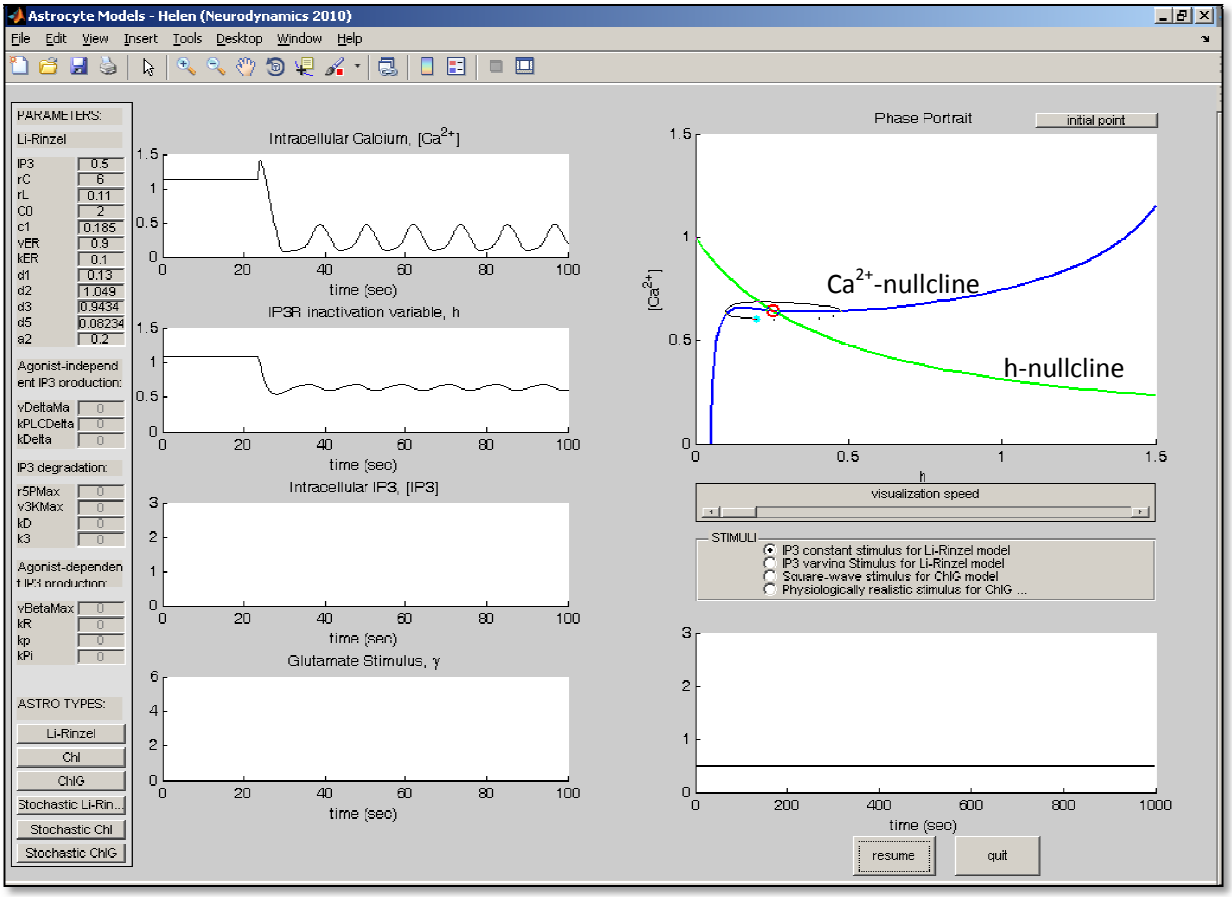

Figure 5: *User interface*

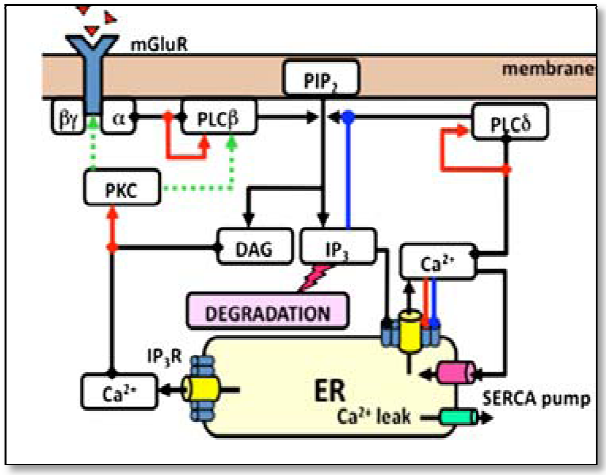

Figure 6: *Astrocytic pathways captured in the 3 models explored (courtesy of De Pitta 2009)*

Model 1: The Li-Rinzel model

The first model explored is the Li-Rinzel (LR) model [8] which is a two-variable model that describes the dynamics of calcium-induced calcium release (CICR) in an astrocyte. Even though calcium dynamics occur in a very complex fashion that involves many cellular pathways some of which being already well defined and others still subject to ongoing research, the LR model captures the main mechanism of intracellular calcium balance by considering only three fluxes described as the CICR mechanism (as shown in figure 6):

(a) A calcium release from the ER into the cytosol via an IP3 receptor (IP3R) that is mutually gated by Ca2+ and IP3 concentrations whereby an increase in cytosolic IP3 concentration activates it while an increase in cytosolic Ca^{2+} deactivates it *(the terms cytosolic and intracellular are used interchangeably in this report).*
(b) A passive leak of Ca^{2+} from the ER to the cytosol.
(c) An active uptake of Ca^{2+} into the ER via the sarcoER Ca^{2+} ATPase (SERCA) pump.

The governing differential equations are *(C denotes cytosolic Ca^{2+} concentration and h is the fraction of open IP3R subunits)*:

$$\frac{dC}{dt} = (r_C m_\infty^3 n_\infty^3 h^3 + r_L)(C_0 - (1 + c_1)C) - v_{ER}\frac{C^2}{C^2 + K_{ER}^2}$$

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

$$where\ h_\infty = \frac{Q_2}{Q_2 + C},\ \tau_h = \frac{1}{a_2(Q_2 + C)},\ and\ Q_2 = d_2\frac{I + d_1}{I + d_3}$$

The 11 parameters used in the model are listed in table 1 with their default values as stated in [8]. These values led to AM dynamics for IP3 concentrations ([IP3]) that vary between 0.35 µM and 0.65 µM as shown in the phase plot diagram and the Ca^{2+} display over time in figure 6. More precisely, different bifurcations diagrams were obtained – as expected – for different IP3 levels. The nullclines intersect at fixed stable points for [IP3] less than 0.35 µM and greater than 0.65 µM. however, for [IP3] values within the specified range, the nullclines intersect at an unstable fixed point and a limit cycles appear.

Modulation of parameters under certain conditions resulted in heterogeneous intracellular calcium dynamics which proves that encoding modes depend on inherent cell properties: for example, a decrease in the SERCA pump affinity (kER) from a value of 0.1 to 0.05 led to a switching of the intracellular Ca^{2+} signal from being AM to becoming FM. In addition, as shown in figure 7, a decrease of the parameter denoting Ca^{2+} activation of the IP3R (d5) led to switching the modulation from AM to AFM.

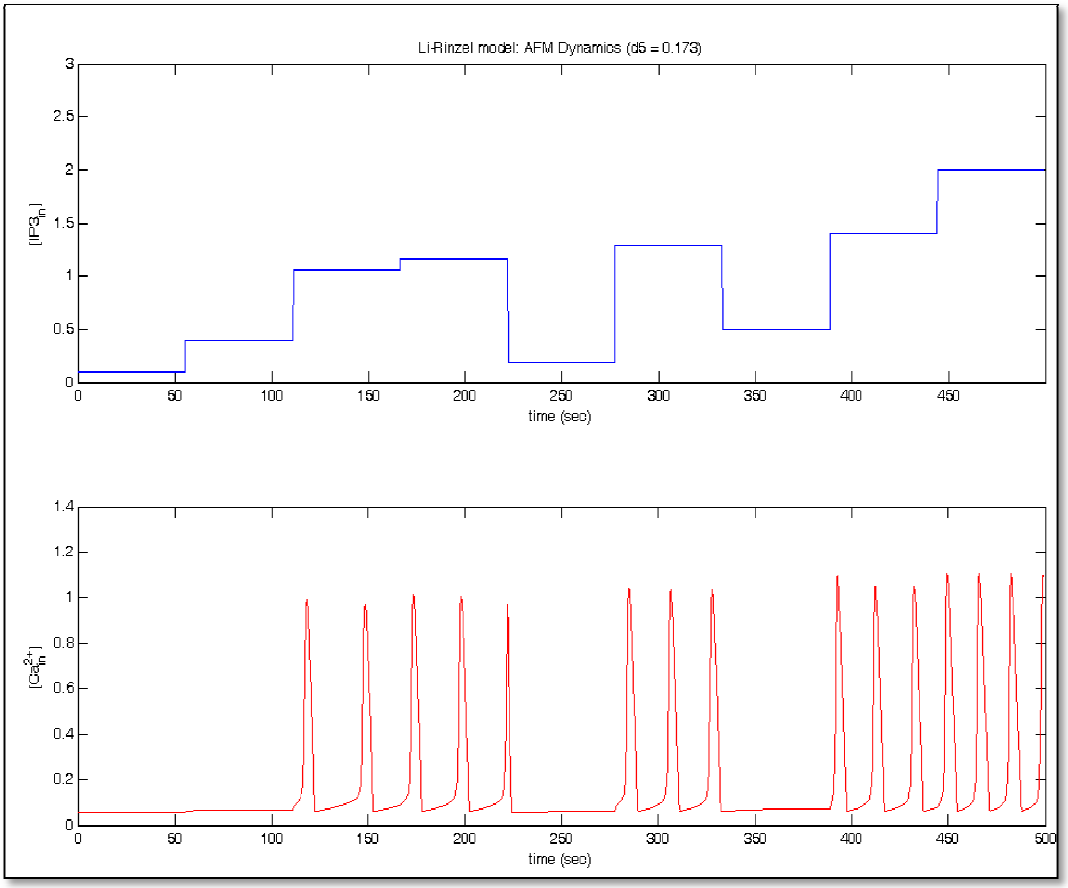

Figure 7: *Signal modulation in the LR model: d5 is decreased from 0.9434 (AM) to 0.173 (AFM)*

Table 1

Parameter		Description	AM	FM
		L-R core[1]		
r_C	s^{-1}	Maximal CICR rate	6	
r_L	s^{-1}	Maximal rate of Ca^{2+} leak from the ER	0.11	
C_0	μM	Total cell free Ca^{2+} concentration referred to the cytosol volume	2	
c_1	-	Ratio between cytosol volume and ER volume	0.185	
v_{ER}	μMs^{-1}	Maximal rate of SERCA uptake	0.9	
K_{ER}	μM	SERCA Ca^{2+} affinity	0.1	0.05
d_1	μM	IP_3 dissociation constant	0.13	
d_2	μM	Ca^{2+} inactivation dissociation constant	1.049	
d_3	μM	IP_3 dissociation constant	0.9434	
d_5	μM	Ca^{2+} activation dissociation constant	0.08234	
a_2	s^{-1}	IP_3R binding rate for Ca^{2+} inhibition	0.2	
		Agonist-independent IP_3 production[2]		
$\bar{v}_\delta$	μMs^{-1}	Maximal rate of IP_3 production by PLCδ	0.02	0.05
$K_{PLC\delta}$	μM	Ca^{2+} affinity of PLCδ	0.1	
κ_δ	μM	Inhibition constant of PLCδ activity	1.5	
		IP_3 degradation[3]		
$\bar{r}_{5P}$	s^{-1}	Maximal rate of degradation by IP-5P	0.04	0.05
$\bar{v}_{3K}$	μMs^{-1}	Maximal rate of degradation by IP_3-3K	2	
K_D	μM	Ca^{2+} affinity of IP_3-3K	0.7	
K_3	μM	IP_3 affinity of IP_3-3K	1	
		Agonist-dependent IP_3 production[4]		
$\bar{v}_\beta$	μMs^{-1}	Maximal rate of IP_3 production by PLCβ	0.2	0.5
K_R	μM	Glutamate affinity of the receptor	1.3	
K_p	μM	Ca^{2+}/PKC-dependent inhibition factor	10	
K_π	μM	Ca^{2+} affinity of PKC	0.6	

Model 2: The ChI model

Although the LR model is sufficient for predicting the different signal modulations that the Ca^{2+} dynamics can display, it neglects the fact that IP3 displays very interesting dynamics through its production and degradation. In other words, IP3 production is regulated by synaptic activity and is thus necessary for proper modeling of astrocyte dynamics in response to neuronal activity (such as the release of extracellular glutamate).

This being stated, we explored the three-variable ChI model introduced in [9]. In this model, IP3 production is brought forth by PLCδ, an isoenzyme which signaling is modulated by intracellular Ca^{2+} and is agonist independent. The governing equation of IP3 degradation and production by the PLCδ isoenzyme is *(I denotes IP3 concentration)*:

$$\frac{dI}{dt} = \frac{v_{\delta_{max}}}{\tau_h} Hill(C^2, K_{PLC\delta}) \; - \; v_{3k_{max}} Hill(C^4, K_D) Hill(I, K_3) \; - \; r_{5P_{max}} I$$

Where the generic Hill function: $Hill(x^n, K) \stackrel{\text{def}}{=} \frac{x^n}{x^n + K^n}$

The IP3 governing equation in addition to the Ca^{2+} and h equations (stated in the LR model) constitute the three governing equations of the ChI model.
Similarly to the LR model, this model also exhibited multiple encoding mechanisms of Ca^{2+} dynamics depending on the cell inherent properties represented by the model parameters that are listed in sections 1 and 2 of table 1 along with their default values (the parameters listed in table 1 belong to all three models considered and are separately stated in sections whereby section 1 lists the parameters used in the LR model). Results of AFM Ca^{2+} and IP3 dynamics are not displayed for this specific case in this report but can be provided upon request. It is important to note that IP3 dynamics are consistently AFM while Ca^{2+} dynamics are AM, FM, or AFM depending on the model parameters.

Model 3: The G-ChI model

In this third model, glutamate effect on IP3 production by the isoenzyme PLCβ is taken into account, in addition to the IP3 production by PLCδ. This new model has the same governing equations as those of the ChI model in addition to a new production term in the IP3 equation as shown below *(γ denotes extracellular Glutamate concentration)*:

$$\frac{dI}{dt} = v_{\beta_{max}} . Hill\left(\gamma^{0.7}, K_R\left(1 + \frac{K_p}{K_R} Hill(C, K_\pi)\right)\right) + \frac{v_{\delta_{max}}}{1 + \frac{I}{K_\delta}} Hill(C^2, K_{PLC\delta})$$
$$- v_{3k_{max}} Hill(C^4, K_D) Hill(I, K_3) \; - \; r_{5P_{max}} I$$

Similarly to the LR and ChI models, this model also exhibited multiple encoding mechanisms of Ca^{2+} dynamics (figure 8) depending on the cell inherent properties represented by the model parameters that are listed in table 1 along with their default values.

As shown in figure 8, both Ca^{2+} and IP3 dynamics are AFM for the specified wide range of external glutamate concentration [100 nM, 5 μM].

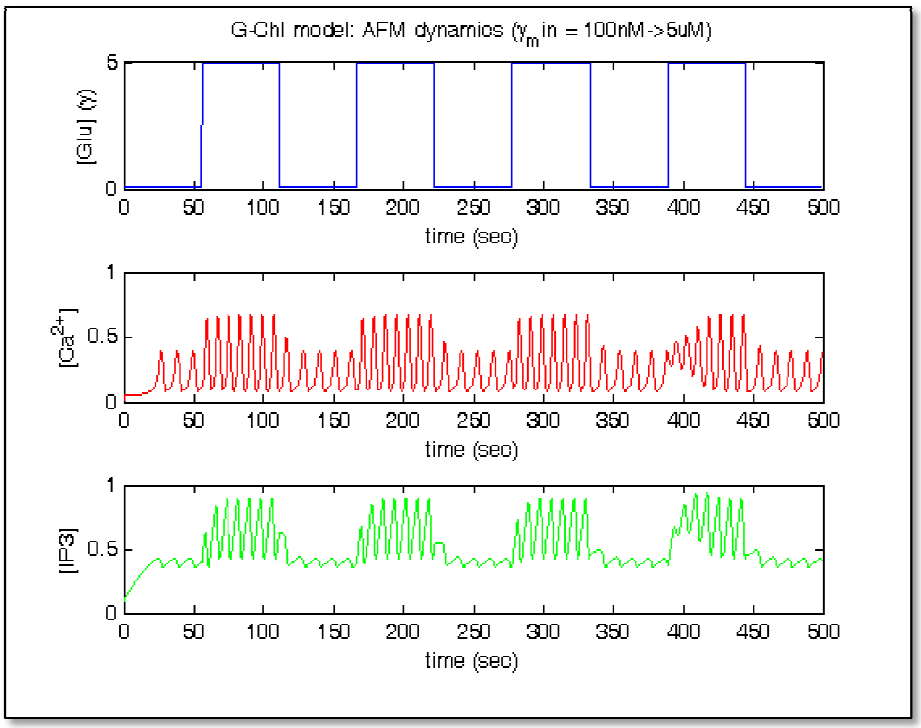

Figure 8: *Signal modulation in the G-ChI model*

We also explored Ca^{2+} and IP3 dynamics in the case of a physiological glutamate stimulus [9] and observed zigzag IP3 dynamics, contrary to the consistent AFM IP3 oscillations observed in all the other cases considered. We hold these results as important ones to further explore and quantify using Shannon information theory [16].

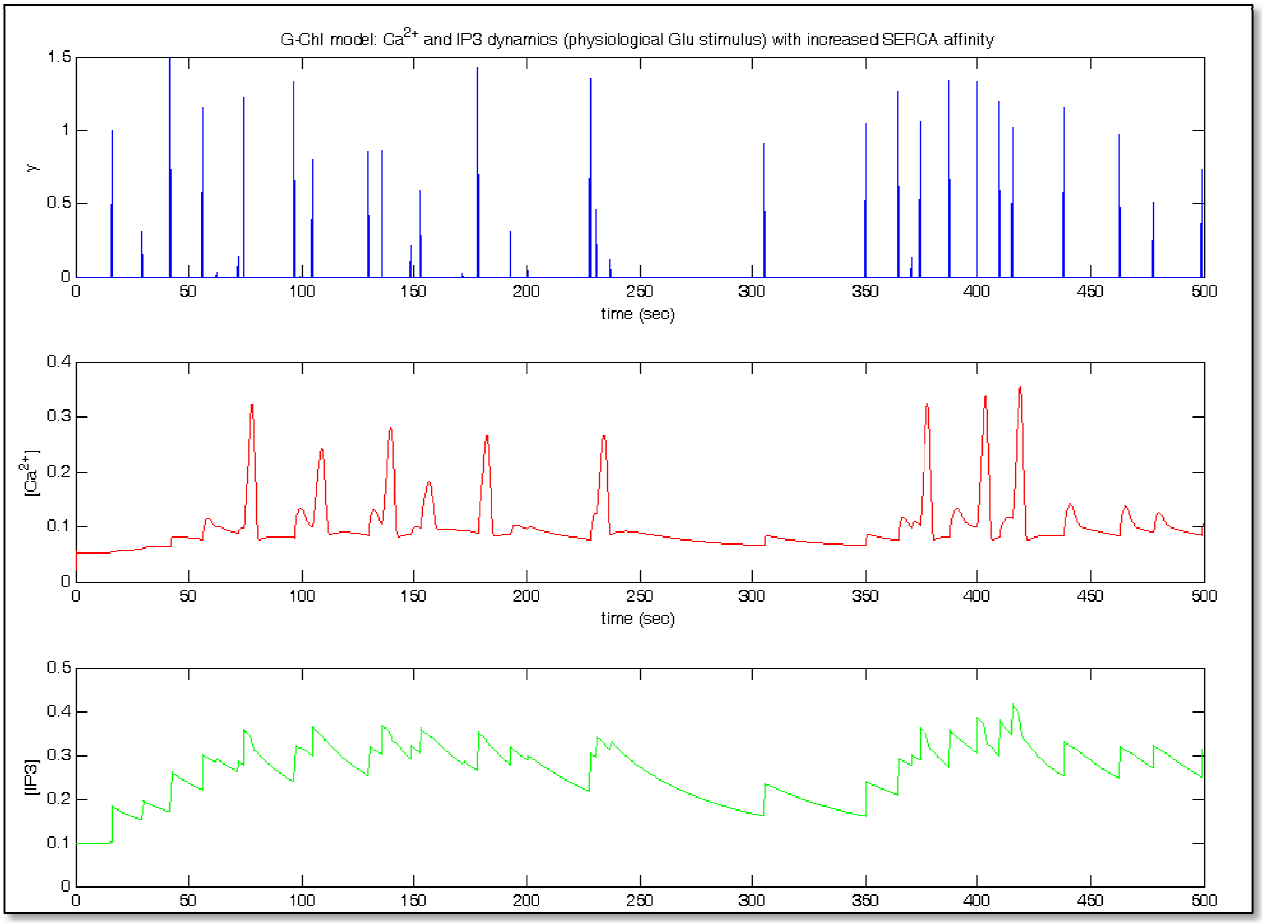

Figure 9: *Physiological Glutamate Stimulus*

Stochastic Models:

Experimental evidence has shown that IP3Rs are spatially organized in clusters on the ER membrane and the collective opening and closing of IP3Rs in a cluster results in Ca^{2+} puffs or sparks [10, 11, 12]. We modeled this stochastic behavior by adding a white Gaussian noise term to the dh/dt equation (h being the Ca^{2+} inactivation variable on the IP3R) and observed the resulting Ca^{2+} and IP3 dynamics in the case of a 20 DB signal to noise ratio.

We will only interpret the results obtained using the G-ChI model:
It was observed experimentally that the average of the inter-pulse intervals (in the case of FM Ca^{2+} dynamics) is in the same range as their standard deviation [2]. We explored this observation by applying increased levels of glutamate concentrations to a G-ChI stochastic astrocyte and obtained results that are conform to the experiments as shown in figures 10 and 11. This proves that the signal to noise ratio of 20 DB applied to dh/dt is a fair consideration in our case.

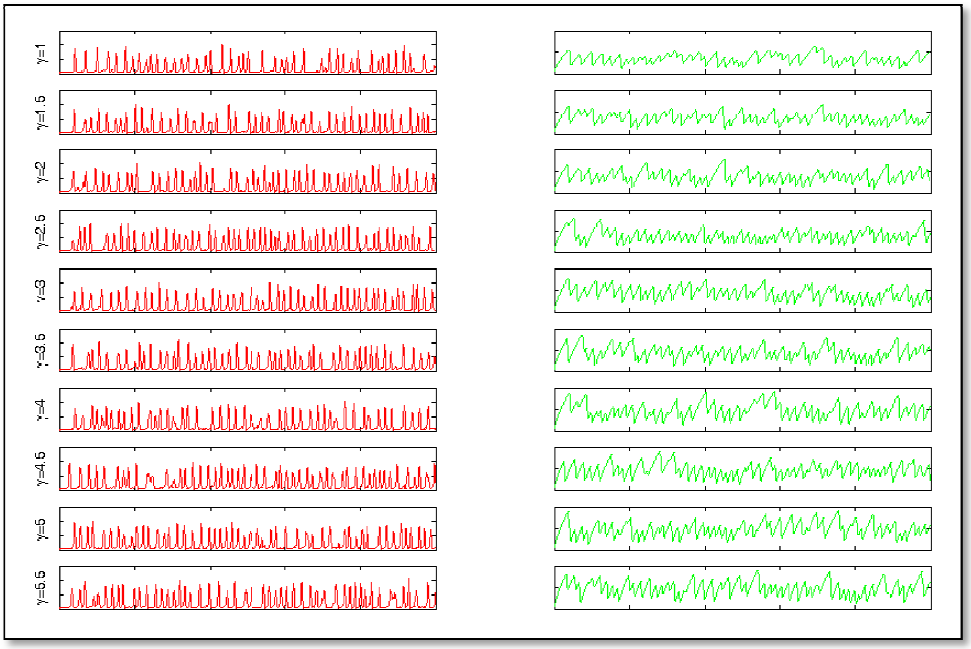

Figure 10: *Increasing γ = [Glu] with SNR = 20 DB (stochastic G-ChI model)*

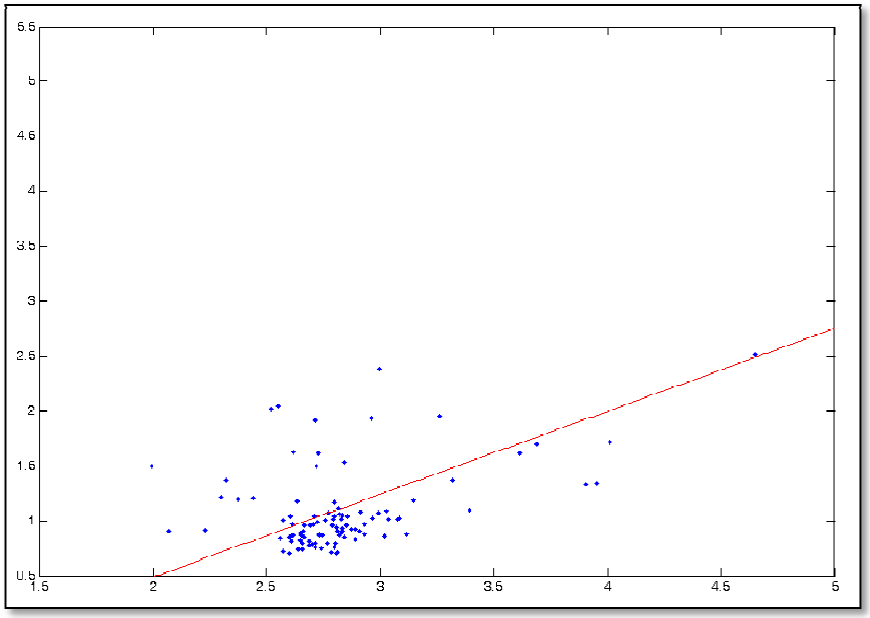

Figure 11: *Standard deviation of the IPI as a function of the IPI mean (SNR = 20 DB)*

Coupling of Astrocytes via Gap Junctions

Astrocytes communicate with each other via intracellular Ca^{2+} oscillations which lead in turn to intercellular communication among adjacent astrocytes and neurons. This communication occurs either via gap junctions (figure 12) or via release of gliotransmitters such as ATP and glutamate which eventually affect adjacent astrocytes and neurons. This is specifically the case of the neurotransmitter glutamate that gets released from adjacent neurons and stimulates the nearby astrocytes by affecting the intracellular Ca^{2+} dynamics. The excited cells will in turn signal to adjacent astrocytes and neurons through gap junctional communication and mainly through the release of the gliotransmitter glutamate [13, 14, 15].

In this study, we explored the coupling dynamics of astrocytes connected via gap junctions in an aligned geometry as shown in figure 12. IP3 and Ca^{2+} are transmitted between these astrocytes through these gap junctions with the permeability of IP3 being larger than that of Ca^{2+} for the reason that most intracellular Ca^{2+} is buffered and thus is harder to be transported via the gap junctions openings [5].

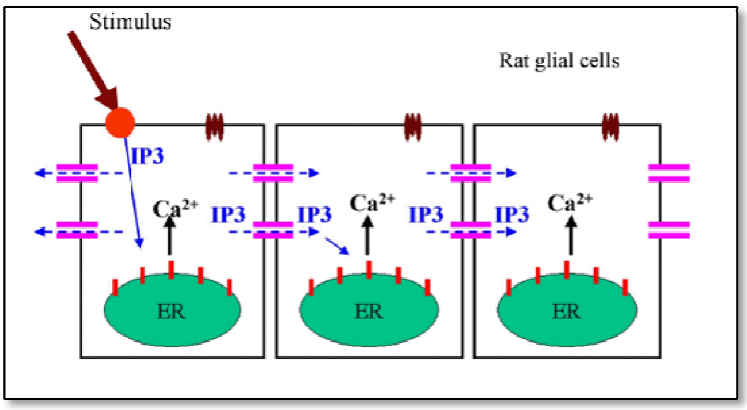

Figure 12: *Gap junctional communication between adjacent astrocytes (Courtesy of Jianwei Shuai)*

Networks of 2 to 100 G-ChI astrocytes were modeled and glutamate was applied to one or more cells in order to explore the Ca^{2+} and IP3 dynamics in the cells forming those networks. Astrocytes were modeled in Matlab using object oriented programming which provides transparency and ease of implementation of very large cellular networks. Moreover, a 0.1 to 0.5% change in the parameter values was applied in order to account for a physiologically more realistic model because, in reality, no two cells are exactly similar. Individual cells were modeled as a homogeneous reservoir and consequently, Ca^{2+} and IP3 were assumed to be uniform everywhere in the same cell. This limitation was set as a first step in exploring the dynamics of coupled astrocytes and will be waived in the future enhancements that will be

applied to the current model in order to include diffusion of Ca^{2+} and IP3 through the cell in order for a certain amount to reach the gap junctions and get transported to adjacent cells.

The two equations describing gap junctional coupling are:

$$\frac{\Delta C_1}{\Delta t} = -\frac{\Delta C_2}{\Delta t} = -P_{Ca}(C_1 - C_2)$$

$$\frac{\Delta I_1}{\Delta t} = -\frac{\Delta I_2}{\Delta t} = -P_{IP3}(I_1 - I_2)$$

where P_{Ca} and P_{IP3} are the respective values for Ca^{2+} and IP3 permeability at the gap junctions. In the case of infinitesimal Δt, the equations become partial differential equations since the Δ symbol will be replaced by the differential operator d.

Sample results of the astrocyte gap junction coupling are shown in figures 13 and 14: two AFM encoding G-ChI astrocytes were coupled via gap junctions and glutamate was applied merely to astrocyte 1. As shown in figure 13, astrocyte 2 responded indirectly – as a result of gap junctional communication - to the glutamate stimulus applied to astrocyte 1 and also encodes information via AFM pulsations. Figure 14 details the AFM modulation in both astrocytes by displaying - in green - the differences in frequencies which, in addition to the obvious alterations in amplitude, constitute AFM encoding of the glutamate stimulus applied to cell 1.

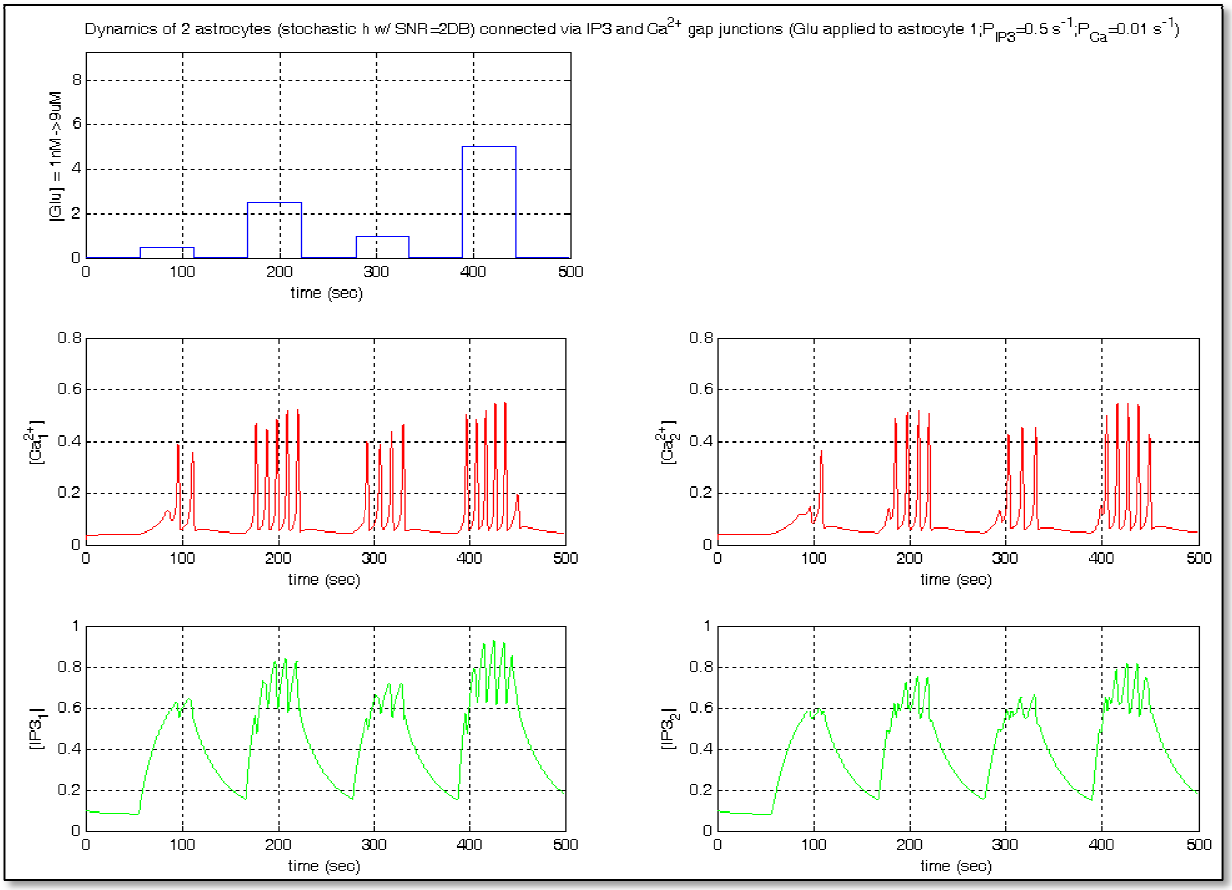

Figure 13: *Ca^{2+} and IP3 dynamics of a two 'deterministic G-CHI' astrocyte Network (glutamate stimulus applied to astrocyte 1)*

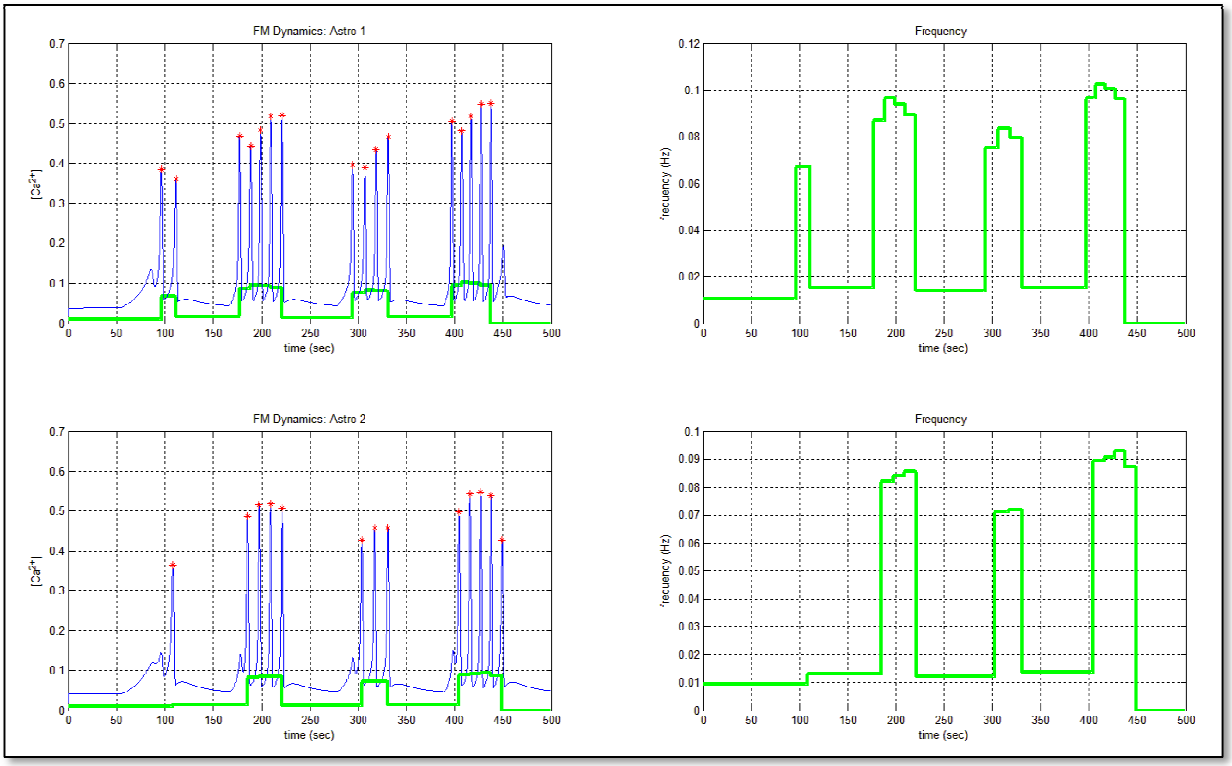

Figure 14: *AFM of a two G-CHI astrocyte Network*

Coupled astrocytes were also studied in the case of stochastic G-ChI models and results (figure 15) also show similarity in the dynamics of these astrocytes in terms of modulation of both Ca^{2+} and IP3 intracellular concentrations. IP3 dynamics, in this case, show more uniformity between the two astrocytes than do Ca^{2+} dynamics. This is result is expected in our case since the permeability of gap junctions to IP3 is much higher than the Ca^{2+} permeability and consequently, IP3 diffusion between astrocytes will lead to more uniform dynamics among connected cells. This observation is not very obvious in the case of the deterministic astrocyte models as noted earlier.

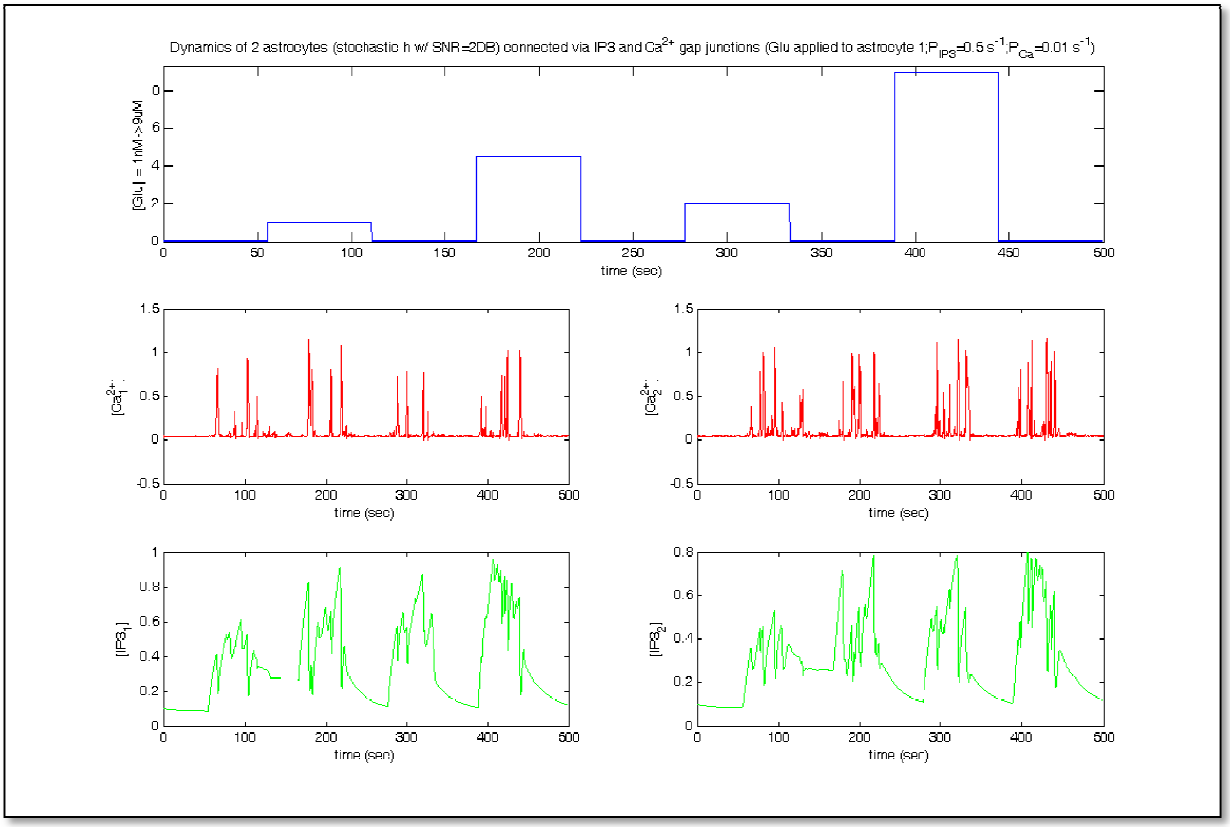

Figure 15: *Ca^{2+} and IP3 dynamics of a two 'stochastic G-CHI' astrocyte Network (glutamate stimulus applied to astrocyte 1)*

Conclusion

Ca^{2+} is the most important messenger in astrocytes, the predominant type of CNS cells that are recently recognized to play a crucial role in neuronal activity and information processing in the brain. Ca^{2+} is a messenger that takes part of both intracellular dynamics and intercellular communication.

We explored, computationally and using existing astrocyte models, how Ca^{2+} dynamics are regulated by extracellular signals such as glutamate and by the biochemical network of Ca^{2+} related signal transduction pathways in an astrocyte. We showed that information encoding in astrocytes can occur by amplitude modulation of Ca2+ oscillations, frequency modulation, or by both modes.

We also considered stochastic Ca2+ dynamics and the effect of gap junction coupling between astrocytes.

We will extend our computations to include the quantification of information encoded in Ca^{2+} oscillations or Ca^{2+} puffs using Shannon information theory [16]. The aim of this work is to explore the coupling dynamics of neurons and astrocytes and quantify the astrocytic encoding of neuronal information as a new way of proving how astrocytes participate actively in information processing that occurs in the brain.

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