



# A mathematical model of spontaneous calcium(II) oscillations in astrocytes

Maxim Lavrentovich, Sheryl Hemkin\*

*Department of Chemistry, Kenyon College, Gambier, OH 43022, USA*

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## Abstract

Astrocytes exhibit oscillations and waves of  $\text{Ca}^{2+}$  ions within their cytosol and it appears that this behavior helps facilitate the astrocyte's interaction with its environment, including its neighboring neurons. Often changes in the oscillatory behavior are initiated by an external stimulus such as glutamate, recently however, it has been observed that oscillations are also initiated spontaneously. We propose here a mathematical model of how spontaneous  $\text{Ca}^{2+}$  oscillations arise in astrocytes. This model uses the calcium-induced calcium release and inositol cross-coupling mechanisms coupled with a receptor-independent method for producing inositol (1,4,5)-trisphosphate as the heart of the model. By computationally mimicking experimental constraints we have found that this model provides results that are qualitatively similar to experiment.

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## 1. Introduction

In the past few decades, the suggested role of astrocytes in the central nervous system has increased dramatically. Initially thought of as being a passive structural element that held the neurons together, they are now found to play a much more active part in the signaling process (Charles et al., 1991; Cornell-Bell et al., 1990; Dani et al., 1992). The evidence that indicates that astrocytes interact with neurons has come from experiments *in situ* (Kang et al., 1998; Newman and Zahs, 1998; Pasti et al., 1997), and with neuron–astrocyte co-cultures (Araque et al., 1998; Nedergaard, 1994). The work has shown that stimulated changes in the astrocyte's cytosolic  $\text{Ca}^{2+}$  concentration can alter the surrounding neural activity by releasing chemicals such as glutamate and ATP. This leads to the idea that the synapse is tripartite, where the pre- and postsynaptic neural elements, as well as the astrocyte, respond to one another via neuro- and gliotransmitter release (Haak et al., 1997; Holtzclaw et al., 2002; McHugh et al., 2000; Parri et al.,

2001). These interactions can have profound results, with effects that can apparently alter long-term depression (LTD) and play a role in disease states such as epilepsy (Massey et al., 2004; Tian et al., 2005). In many of these cases where astrocytes are seen to interact with their environment, a change in the concentration or oscillatory behavior of the astrocyte's  $[\text{Ca}^{2+}]_{\text{cyt}}$  appears to be an integral step in the process.

While the changes in the astrocyte's oscillatory  $\text{Ca}^{2+}$  behavior is often found to be a result of external stimulation, these oscillations can also be formed spontaneously. Spontaneous  $\text{Ca}^{2+}$  oscillations have been observed in the astrocyte's cytosol both in culture (Charles, 1994; Fatatis and Russell, 1992; Harris-White et al., 1998) and *in situ* from cells originating in several different parts of the brain, such as the ventrobasal (VB) thalamus, hippocampus and cortex (Aguado et al., 2002; Nett et al., 2002; Parri et al., 2001). When the neural input to the astrocyte is blocked, the timing of these  $\text{Ca}^{2+}$  spikes appears to be relatively random within the astrocytic field; however, when the neurons were allowed to interact, the spontaneous behavior became more correlated between the astrocytes (Aguado et al., 2002; Parri et al., 2001).

\*Corresponding author. Tel.: +1 740 427 5093.

E-mail address: [hemkins@kenyon.edu](mailto:hemkins@kenyon.edu) (S. Hemkin).



$$\begin{aligned}
 v_{serca} &= v_{M2} \left( \frac{X^2}{X^2 + k_2^2} \right), \\
 v_{PLC} &= v_p \left( \frac{X^2}{X^2 + k_p^2} \right), \\
 v_{CICR} &= 4v_{M3} \left( \frac{k_{CaA}^n X^n}{(X^n + k_{CaA}^n)(X^n + k_{CaI}^n)} \right) \\
 &\quad \times \left( \frac{Z^m}{Z^m + k_{ip3}^m} \right) (Y - X).
 \end{aligned}$$

The term  $v_{in}$  represents the flow of calcium from the extracellular space through the astrocyte's membrane and into the cytosol. This flow is essential to the creation of the spontaneous oscillations as they are virtually eliminated when the specimen is bathed in  $\text{Ca}^{2+}$ -free medium (Parri et al., 2001). (The few oscillations that occur in  $\text{Ca}^{2+}$ -free medium are due to release of the ion from intercellular stores.) Similar loss of activity occurs after the application of  $\text{Co}^{2+}$ , a non-specific voltage gated Ca channel blocker. Although the N, P, Q high-voltage-activated channels cause a decrease in the oscillations, a more significant decrease is caused by a block of the L-type channels (Aguado et al., 2002; Parri and Crunelli, 2003; Parri et al., 2001). By changing the value associated with the  $v_{in}$  term, the simulated flow of  $\text{Ca}^{2+}$  ions into the cytosol is being modified, and based on experiment, this appears to mainly correspond to an inactivation of L-type channels and to a lesser degree the high-voltage-activated channels. The range of values used in this study are of the same magnitude as those used in Hofer et al. (2002) and are representative of the relatively small flux that occurs during spontaneous oscillations in the absence of agonist stimulated  $\text{Ca}^{2+}$  ion flux.

The term  $k_{out}X$  represents the rate of calcium efflux from the cytosol into the extracellular space. The values for these constants were determined by fitting the Hofer et al. (2002) model to experimental measurements of Venance et al. (1997).

$v_{CICR}$  represents the  $\text{IP}_3\text{R}$ -mediated flux of calcium from the ER to the cytosol and is specifically built to accommodate the evidence that the  $\text{IP}_3\text{R}$ -2 is preferentially expressed in astrocytes (Holtzclaw et al., 2002; Sharp et al., 1999). As this receptor is influenced by  $\text{IP}_3$  and calcium ions from both the ER and cytosol, its dynamics will depend on 3 main terms. Fitting experimental data show the  $\text{IP}_3\text{R}$ -2 to have a sigmoidal dependence on  $\text{IP}_3$  that is slightly steeper than that seen for the other isoforms (Tu et al., 2005b). Further experiments show that all isoforms of the  $\text{IP}_3\text{R}$  display bell-shaped cytosolic  $\text{Ca}^{2+}$ -dependence in the physiological range (Bezprozvanny et al., 1991). For the type-2 isoform, the activating and inhibiting affinities,  $k_{CaA}$  and  $k_{CaI}$ , as well as the Hill coefficient,  $n$ , came from experimental evidence fitted to the modified model for the  $\text{IP}_3\text{R}$  proposed in papers by Tu et al. (2005a, b). In the third term, the receptor will depend on the gradient of the calcium concentration between the ER and cytosol ( $Y-X$ ).

Finally, the value to guide the maximum flux of calcium ions into the cytosol,  $v_{M3}$ , comes from work on hepatocytes (Hofer, 1999).

$v_{serca}$  describes the sarco(endo)plasmic reticulum ATPase that fills the ER with calcium ions from the cytosol. Based on experiment, the rate of inflow appears to follow sigmoidal dynamics and the Hill form is used for its description. The Hill coefficient is found to have a value of two (Falcke, 2004) and the maximum flux of calcium ions out of the pump,  $v_{M2}$ , is based on experimental data for SERCA pumps in chicken cerebellums (Falcke, 2004; Schatzmann, 1989). The parameter describing the half-maximal value effect is of the same order of magnitude as many experimental results for SERCA pumps and has also been used in other models (Falcke, 2004; Houart et al., 1999).

Finally,  $k_f(Y-X)$  represents the leak flux from the ER into the cytosol due to the concentration gradient. The high relative  $\text{Ca}^{2+}$  concentration in the ER makes passive diffusion through the membrane possible and there are suggestions that  $\text{Ca}^{2+}$  may be able to leak through unactivated  $\text{IP}_3\text{Rs}$  (Parri and Crunelli, 2003; Simpson and Russell, 1997). (To account for the  $\text{Ca}^{2+}$  buffering encountered in the ER, the initial concentration for the simulation is set to the low end of the range of what has been encountered experimentally.) Although many models make the assumption that the actual concentration gradient ( $[\text{Ca}^{2+}]_{ER} - [\text{Ca}^{2+}]_{cyt}$ ) can be simplified to  $[\text{Ca}^{2+}]_{ER}$  due to the negligible concentration of  $\text{Ca}^{2+}$  in the cytosol, other simulations from our lab have shown that at times the dynamical behavior is sensitive to this simplification.

The fluctuations in the  $\text{IP}_3$  concentration is modeled by a production and degradation term. Although  $\text{IP}_3$  is typically formed through a path initiated by an external signal, in the case of spontaneously generated oscillations, production must be initiated by a mechanism internal to the cell. It has been shown that these oscillations are not dependent on receptor-mediated PLC activity, but PLC activity is nonetheless indicated (Parri and Crunelli, 2003), and for that reason, our work focuses on the delta-isoform of PLC ( $\text{PLC}\delta 1$ ). Studies indicate that this isoform is not as closely situated to the stimulating receptor sites as the beta and gamma forms, and consequently does not appear to be activated via the normal external signals (Rebecchi and Pentyala, 2000). It does, however, appear to be activated by changes in the physiological levels of cytosolic  $\text{Ca}^{2+}$ , which suggests that the  $\text{IP}_3$  formation in astrocytes undergoing spontaneous activity is the result of the internal stimulation of  $\text{PLC}\delta 1$  by cytosolic calcium ions (Allen et al., 1997; Pandey et al., 1996; Parri and Crunelli, 2003). (This process is described in our  $\text{IP}_3$  production term,  $v_{PLC}$ .) Assuming sigmoidal dynamics for this process, we followed the  $\text{PLC}\delta 1$  kinetics found in the Hofer model (Hofer et al., 2002; Parri and Crunelli, 2003), which uses parameters fit from experiment (Pawelczyk and Matecki, 1997). The degradation of  $\text{IP}_3$  ( $k_{deg}Z$ ) is based on the linear

dependence of  $\text{IP}_3$  degradation by 5-phosphatase and is also seen in models (Hofer et al., 2002; Houart et al., 1999).

Although PKC action is primarily thought to be driven by external signals, it can also respond to internal forces. To that end, experimental evidence shows that the inhibition of PKC results in the spontaneously generated behavior changing from a spiking regime to one that results in sustained, elevated calcium ion concentration (relative to baseline) (Allen et al., 1997; Pawelczyk and Matecki, 1997). This indicates that PKC does have a role in the formation of this behavior in astrocytes and appears to at least indirectly interact with the  $\text{IP}_3\text{R}$ . Although that work did not directly connect the behavior to a particular cellular structure, studies with PKC on various cell types show that it can influence L-type channels in a variety of ways depending on what isoform is present, the expression level, etc. (Kang and Walker, 2005; McHugh et al., 2000). However, in contrast to the astrocyte work noted above, several studies have indicated that it can inhibit L-type calcium channels and thus decrease the rate of  $\text{Ca}^{2+}$  influx from the extracellular matrix in both astrocytes and cardiac cells (Haak et al., 1997; Kang and Walker, 2005; McHugh et al., 2000). Although the experimental work is still inconclusive, our simulations suggest that PKC is intimately involved in generating the spontaneous behavior.

#### 4. Results and discussion

This model supports the idea that spontaneous  $\text{Ca}^{2+}$  oscillations can be generated without the aid of external stimulation (Parri and Crunelli, 2003). Small changes in the rate of  $\text{Ca}^{2+}$  entry through the plasma membrane cause enough change in the local  $[\text{Ca}^{2+}]_{\text{cyt}}$  to activate the ICC–CICR mechanisms, which are key to the development and propagation of oscillatory behavior. This agrees with experimental observations that more cells exhibit spontaneous behavior after an increase in the cytosolic concentration of  $\text{Ca}^{2+}$ , thus giving experimental indications that the concentration of the calcium ions in the cytosol is closely linked to the ability of a cell to undergo this type of behavior (Parri and Crunelli, 2003). In our model, the cytosolic  $\text{Ca}^{2+}$  concentration can be most easily manipulated by  $v_{\text{in}}$ , the parameter representing the influx of the ions from the extracellular matrix. As  $v_{\text{in}}$  is raised, and consequently the concentration of cytosolic  $\text{Ca}^{2+}$  is elevated, it is found that the model system moves from a steady state to period-1 behavior (Fig. 3) then back to an elevated steady state. (The bifurcation diagram is represented by the black curve in Fig. 2.) Experimentally the dependence of the spontaneous oscillations on  $[\text{Ca}^{2+}]_{\text{cyt}}$  is noted in Parri and Crunelli (Parri et al., 2001).

The spikes occur with a regular frequency, and the period of oscillation varies from approximately 150 to 700 s, which is characteristic of the relatively slow dynamics seen in most spontaneous activity (Fig. 3). In many of the astrocytes that display spontaneous activity, the time between spikes tends to be longer than 100 s, although in

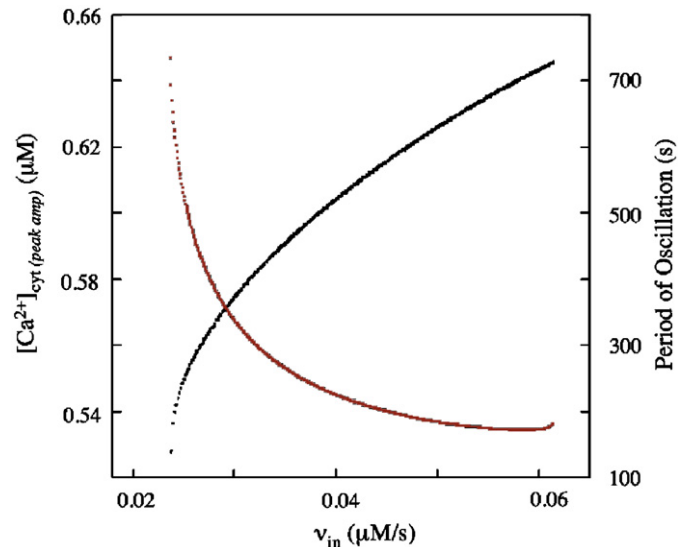


Fig. 2. Bifurcation diagram of  $\text{Ca}^{2+}$  overlaid with the periodicity of oscillations (black curve). The single curve for the bifurcation diagram indicates that this model displays period-1 behavior in  $[\text{Ca}^{2+}]_{\text{cyt}}$  throughout the oscillatory window of  $v_{\text{in}}$ . At smaller  $v_{\text{in}}$  values the behavior is steady state, at larger  $v_{\text{in}}$  values the behavior is also steady state, but at a higher concentration (red curve). In the period-1 region, as the  $[\text{Ca}^{2+}]_{\text{cyt}}$  is increased by raising  $v_{\text{in}}$ , the oscillatory period decreases as seen in experiment (Parri and Crunelli, 2003). Parameter values for all figures unless noted:  $v_{M2} = 15.0 \mu\text{M/s}$ ,  $v_{M3} = 40.0 \text{ s}^{-1}$ ,  $v_{\text{in}} = 0.05 \mu\text{M/s}$ ,  $v_p = 0.05 \mu\text{M/s}$ ,  $k_2 = 0.1 \mu\text{M}$ ,  $k_{\text{CaA}} = 0.15 \mu\text{M}$ ,  $k_{\text{CaI}} = 0.15 \mu\text{M}$ ,  $k_{\text{IP3}} = 0.1 \mu\text{M}$ ,  $k_p = 0.3 \mu\text{M}$ ,  $k_{\text{deg}} = 0.08 \text{ s}^{-1}$ ,  $k_{\text{out}} = 0.5 \text{ s}^{-1}$ ,  $k_f = 0.5 \text{ s}^{-1}$ ,  $n = 2.02$ ,  $m = 2.2$ . Initial conditions:  $\text{Ca}_{\text{cyt}}^{2+} = 0.1 \mu\text{M}$ ,  $\text{Ca}_{\text{ER}}^{2+} = 1.5 \mu\text{M}$ ,  $\text{IP}_3 = 0.1 \mu\text{M}$ .

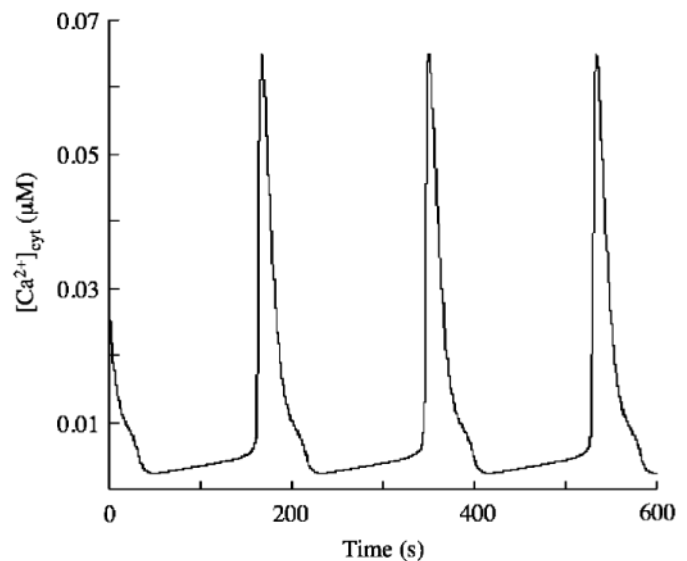


Fig. 3. Typical structure of the simulated oscillations.

terms of frequency, it is often not a regularized process (Aguado et al., 2002; Nett et al., 2002). The regular frequencies seen in our model is a product of its minimalist roots. However, it should be noted that the simple oscillatory behavior seen in Fig. 3 agrees with experimental results from epileptiform tissues, where the calcium oscillations seen after seizure have regular oscillatory



patterns with periods of averaging approximately 100 s (Manning and Sontheimer, 1997; Parri and Crunelli, 2003; Tashiro et al., 2002). While the number of experiments carried out on epileptiform tissue is small, it may be suggested that the regular period of oscillation in these astrocytes may be the result of an oscillatory mechanism that is less complex than that in normal cells.

In addition, the cytosolic  $\text{Ca}^{2+}$  concentration of the simulations oscillates within physiological levels and the spike shape and peak amplitude is similar to that seen in experiment, especially in the studies done on epileptic tissue (Manning and Sontheimer, 1997). Simulations also show a link between the increase in cytosolic  $\text{Ca}^{2+}$  and the decrease in the oscillatory period, which have also been seen experimentally (Parri and Crunelli, 2003) (red curve in Fig. 2).

Although PKC is not explicitly modeled within our system, it is possible to mimic this inhibition by changing the term  $v_{in}$ . By following the idea that the influx of external  $\text{Ca}^{2+}$  is indirectly related to the concentration of PKC, PKC inhibition can be modeled by increasing the value of  $v_{in}$  (Fig. 4). Our elevated steady-state concentrations do qualitatively represent the physiological results seen in Parri and Crunelli (2003) and thus suggest that PKC does have a role in this process.

With its ability to quickly store and release a large amount of  $\text{Ca}^{2+}$ , the ER has long been thought to play a key role in the propagation of  $\text{Ca}^{2+}$  oscillations and waves. Thus, the understanding of the pathways used by  $\text{Ca}^{2+}$  for entry and exit to the ER, and how these paths affect the dynamical behavior of  $\text{Ca}^{2+}$  are of importance. The

activation and inhibition of the sarco(endo)plasmic reticulum ATPase (SERCA) pump, a primary route by which  $\text{Ca}^{2+}$  is returned to the ER, has profound effects on the behavior of the calcium ions. By decreasing the value of  $v_{M2}$ , the parameter controlling the maximum flow rate through the SERCA pump, the flow of  $\text{Ca}^{2+}$  ions into the ER is lowered and the inhibition of the pump is mimicked. Simulations show that when this flow rate is lowered, the ability to form multiple high amplitude spikes is reduced (Fig. 5). Similarly, experimental inhibition of the SERCA pump by cyclopiazonic acid leads to large reduction in the number of cells undergoing the spontaneous behavior, and for those cells that did exhibit concentration changes the typical result was a short initial increase in cytoplasmic calcium, and then a slow return to baseline levels after a few minutes (Nett et al., 2002; Parri and Crunelli, 2003; Parri et al., 2001). Further observations of the inhibition of the SERCA pump by thapsigargin found that there was a decrease in both the amplitude and frequency of the  $[\text{Ca}^{2+}]_{\text{cyt}}$  oscillations (Aguado et al., 2002; Tashiro et al., 2002). In the simulations, the frequency of the oscillations increases in contrast to experiment. However, similar to experiment, by reducing the flow rate into the ER, our simulations show that the oscillatory amplitude was reduced and would eventually go to steady state (Fig. 6). This is brought about by the increase in the baseline concentration of cytosolic  $\text{Ca}^{2+}$  that feeds into increasing the rate of  $\text{IP}_3$  production and degradation; ultimately this increases the frequency, but shortens the duration of the open phase of the  $\text{IP}_3\text{R}$ .

In terms of the release of  $\text{Ca}^{2+}$  from the ER, the  $\text{IP}_3$  receptor is a central player. To simulate the inhibition of this receptor, the value of  $v_{M3}$  was lowered. As the flow of calcium ions through the  $\text{IP}_3\text{R}$  was reduced the results (Fig. 7) show that oscillations cease. Similar dynamics are seen experimentally when heparin or 2-aminoethoxydiphenylborate (2-APB) interact with this receptor (Nett et al., 2002; Parri and Crunelli, 2003).

Although most experimental work shows spike sequences similar to period-1 behavior, there are often small variations in frequency. For the parameter values tested no variation such as this were encountered, but it should be noted that this model is deterministic and treats the leak of  $\text{Ca}^{2+}$  from the extracellular matrix to the cytosol as a constant. Under normal cellular conditions it would be expected that the inward flux varies stochastically and that could result in small frequency variation. Other studies show more complex behavior (Aguado et al., 2002; Nett et al., 2002; Tashiro et al., 2002). This model, with small changes in parameter values, can also be shown to go through more complex oscillations such as a period adding regime to chaos (Fig. 8). These simulations result from a higher value for  $k_{\text{CaA}}$  and  $k_{\text{CaI}}$ , and a lower  $k_p$  value than given in Fig. 2, and an example of the oscillatory profile is shown in Fig. 9. In comparison to the simulations above, physically these changes would mean that the  $\text{PLC}\delta 1$  dynamics have a faster response to  $\text{Ca}^{2+}$ , the rate of release

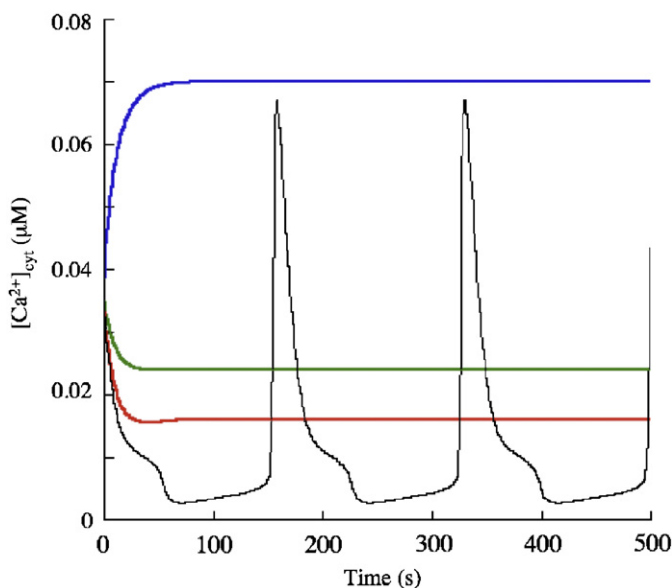


Fig. 4. With an increase in  $v_{in}$ , which mimics the increasing inhibition of PKC, the behavior changes from oscillatory to steady state. As  $v_{in}$  continues to increase, the steady-state concentration also increases relative to the baseline concentration seen in the oscillatory state. Oscillation: (black)  $v_{in}$  is equal to  $0.06 \mu\text{m/s}$ . Steady states in increasing concentration order: (red)  $v_{in}$  is equal to  $0.08 \mu\text{m/s}$ , (green)  $v_{in}$  is equal to  $0.12 \mu\text{m/s}$ , and (blue)  $v_{in}$  is equal to  $0.35 \mu\text{m/s}$ .

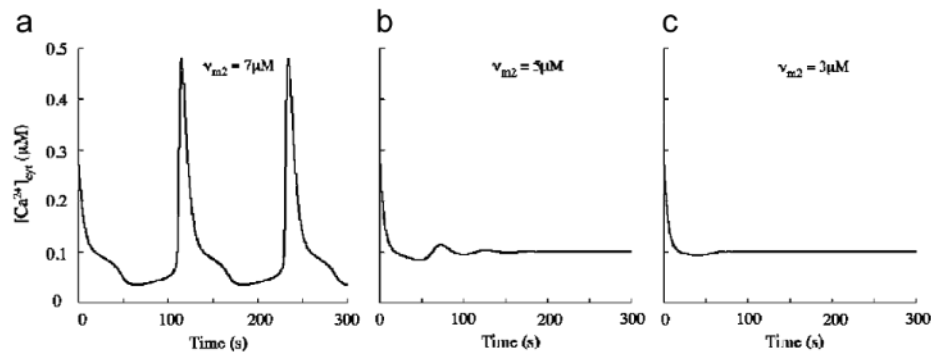


Fig. 5. Decreased flow rate through the SERCA pump leads to a damping of oscillatory behavior. The increasing inhibition of the SERCA pump is modeled by decreasing  $v_{M2}$ , the maximum flow rate through the SERCA pump. As  $v_{M2}$  is lowered (a–c) the ability to form sustained high-amplitude oscillations is diminished.

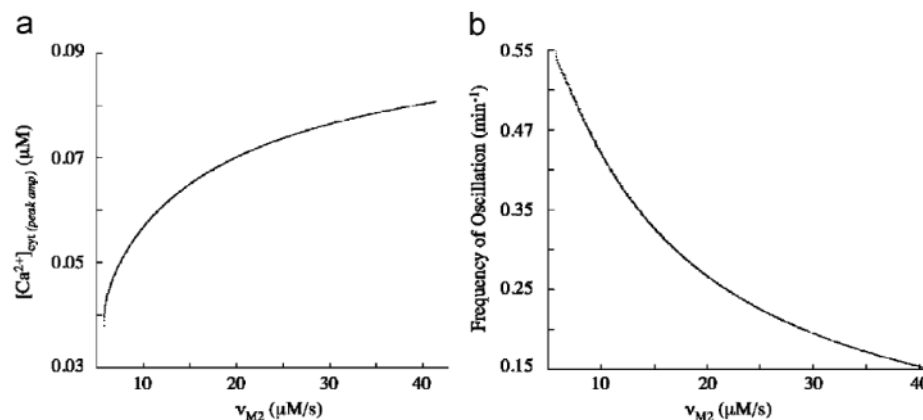


Fig. 6. Changes in SERCA pump flow rate cause changes in oscillatory amplitude and frequency: (a) within the oscillatory range, as  $v_{M2}$  is decreased (indicating greater inhibition of the SERCA pump) the peak amplitude decreases; (b) however, the periodicity increases.

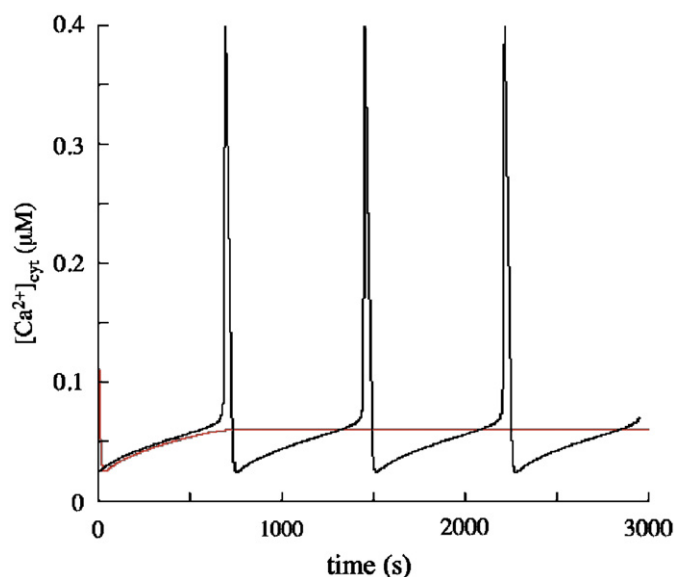


Fig. 7. Decreased flow rate through IP<sub>3</sub>R leads to loss of oscillatory behavior. The parameter  $v_{M3}$ , which guides the maximal flow rate through the IP<sub>3</sub>R, was decreased from  $40 \text{ s}^{-1}$  (black line) to  $10 \text{ s}^{-1}$  (red line) the behavior changes from period-1 oscillations to steady state.

of  $\text{Ca}^{2+}$  via the IP<sub>3</sub>R occurs at a higher concentration of cytosolic  $\text{Ca}^{2+}$  and this rate would not drop off as quickly. Within the experimental work, no linkage was made as why the different modes of behavior arose; however, based on our work it could be hypothesized that small changes in the efficiency of the mechanistic steps due to developmental stage, injury, etc., could cause a significant change in the oscillatory response of the cytosolic  $\text{Ca}^{2+}$ .

The connection between the glia and neurological health has been postulated for the better part of a century; however, there was little evidence supporting this interaction until recently. From as early as 1927 it has been suspected that glia could be involved in neurological disease. The early studies by Penfield looked at the connection between glial scarring and post-traumatic epilepsy (Penfield, 1927). Recent studies have focused on the chemical interplay between neurons and glia and have more solidly implicated astrocytes, and the changes in their cytosolic  $\text{Ca}^{2+}$  concentration, as having an influence on some neurological and epileptic events (Fellin et al., 2006; Kang and Walker, 2005; Tian et al., 2005). Although their role has not been well defined, these observations

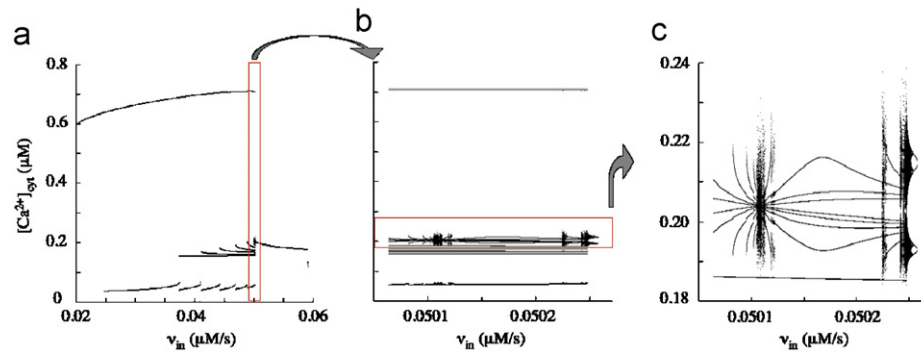


Fig. 8. Bifurcation diagrams showing more complex behavior can be generated by parameter changes. The period-doubling behavior displayed above is seen when  $k_p$  is lowered,  $k_{CaA}$  and  $k_{CaI}$  are raised. In this case,  $k_p = 0.164$ ,  $k_{CaA} = 0.27$ , and  $k_{CaI} = 0.27$ ; all other parameters values are as noted in Fig. 2. (b) Enlargement of the bifurcation pattern found within the red box in (a) and (c) is an enlargement of the pattern found in the red box of (b).

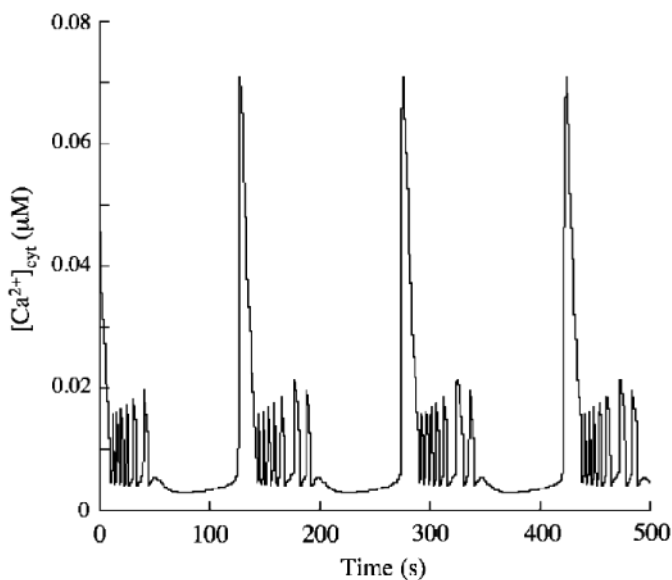


Fig. 9. Time series showing period-doubling behavior with the simulation conditions noted in Fig. 8 and  $v_{in} = 0.05 \mu\text{M/s}$ .

underscore the importance of elucidating the mechanism behind the oscillatory behavior, as it appears to be not just a physiological curiosity, but also potentially an important link in neurological health.

## 5. Conclusion

Our model shows that spontaneous  $\text{Ca}^{2+}$  oscillations in the astrocyte can arise from a mechanism that involves interactions between  $\text{IP}_3$  and pools of  $\text{Ca}^{2+}$  ions in the ER, cytosol and extracellular medium. Within the model, small changes in the rate in which  $\text{Ca}^{2+}$  enters the cell from the extracellular matrix can allow for the formation of  $\text{IP}_3$  without an external signal and ultimately trigger a behavior change that is independent of the traditional signaling molecules. Using experimentally derived rate constants our model produces results that are qualitatively similar to that observed in the laboratory. Furthermore, as these constants are changed to mimic experimentally applied

stresses, with few exceptions our model's response is comparable to what is observed. These studies indicate that spontaneous oscillations can arise by a mechanism that does not require an extracellular signal, thus the astrocyte itself can initiate a process that could lead to neural signaling. At the same time, the formation and continuation of the oscillations is via the CICR and ICC mechanisms, elements common to models of receptor-mediated behavior. This may signify that the oscillatory regime has trigger points both internal and external to the cell; thus it is potentially able to process a variety of signal types and significantly influence its neighboring cells, including neurons. It may also mean that chemical networks that are, but slight deviations from normally established networks may influence certain pathological conditions.

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