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CONTROL AND SYNCHRONIZATION OF INTRACELLULAR CALCIUM DYNAMICS: A ROBUST SLIDING CONTROL APPROACH

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Abstract: Intracellular calcium Ca2+ is a ubiquitous second messenger used to activate various cellular processes, including muscle contraction, fertilization and development of deuterostome eggs, cell growth, neuromodulation, synaptic plasticity and sensory perception. In this work we propose a feedback control scheme for both control and synchronization of intracelullar calcium dynamics. The control scheme is based on a high-order robust sliding control approach. Numerical simulations shows the effectivity of the feedback control laws to synchronize two calcium oscillators and for regulation and tracking of a single calicum oscillator.

Keywords: Intracellular Calcium Oscillations; Biological Processes; Nonlinear Feedback; Synchronization.

1. INTRODUCTION

Ca^{2+} oscillations trigger different cellular functions, including muscle contraction, heart beat, cell death, brain processing and store information (Berridge *et al.*, 1998). To do all of this, Ca^{2+} acts as an intracellular messenger, relaying information within cells to regulate their activity (Goldbeter, 1996). To coordinate all of these functions, Ca^{2+} signals need to be flexible yet precisely regulated, thus control actions are neccesary. In this paper we introduce a feedback control law for coordination and control of intracellular calcium functions. In particualr, we introduce a versatile nonlinear feedback control scheme that can be used in the synchronization, suppression/regulation and tracking of the nonlinear behavior displayed by a intracellular Ca^{2+} model. By manipulation of an external input with a feedback control scheme, we can (i) suppress intracellular calcium oscillations, and (ii) can enforce two calcium oscillators to chaotic synchronization, via simple and complex oscillations of influx of Ca^{+2}.

For control and synchronization of simple to complex oscillations there are several control approaches that can be used in biological systems (Fradkov and Pogromosky, 1998). However, in this paper we introduce the application of a new sliding type control approach that has three nice

features for biological applications: (*i*) robustness against model uncertainties, (*ii*) simplicity in the design, and (*iii*) switched type responses.

The sliding-mode control schemes, have shown several advantages like allowing the presence of matched model uncertainties and convergence speed over others existing techniques as Lyapunov-based techniques, feedback linearization and extended linearization (Aguilar-Lopez and Alvarez-Ramirez, 2002). On the other hand, standard sliding-mode controllers has the main drawback that the closed-loop trajectory, of the designed solution, is not robust even with respect to the matched disturbances on a time interval preceding the sliding motion. Indeed, the classical sliding-mode controllers are robust in the case of matched disturbances only, so that the designed controller ensures the optimality only after the entrance point into the sliding mode. To try to avoid the above disadvantage the high-order sliding-mode technique has been proposed (Sira-Ramirez, 2000; Scarry *et al.*, 2000; Levant, 2001). This control scheme consider a fractional power of the absolute value of the tracking error, coupled with the sign function, this structure provides several advantages as simplification of the control law, higher accuracy and chattering prevention (Levant, 2001). In this paper, a second order sliding-mode controller coupled with an integral action is applied for the control and synchronization of intracellular calcium dynamics.

2. INTRACELLULAR CALCIUM MODEL

The mechanisms underlying the spatial and temporal patterns of the global Ca^{2+} response have been investigated extensively in recent years (see, for example, Goldbeter, 1996 and references there in). The mechanism of Ca^{2+} oscillations and that of associated waves rests on the regulation of Ca^{2+} levels within the cell.

A variety of models for Ca^{2+} oscillations and waves have been proposed (Schuster *et al.*, 2002 and references therein). Differing by the degree of detail with which the dynamics and control of the $InsP_3$ receptor are treated, most of these models are based on CICR as the main instability-generating mechanism. We consider the model of Houart et al. (1999), which exhibits a diversity of calcium responses, notably steady states, spiking and bursting oscillations, multirhythmic and chaotic regimes. The model contains three variables, namely the concentrations of free Ca^{2+} in the cytosol (x_1) and in the internal pool (x_2), and the IP_3 concentration (x_3).

Table 1. Parameter values corresponding to the various types of simpe and complex oscillatory behavior of Fig. 1 (Taken from Houart et al., 1999)

Par	(*a*)	(*b*)	(*c*)	*Par*	(*a*)	(*b*)	(*c*)
β	0.6	0.46	0.65	ε	0.1	1	13
K_2	0.1	0.1	0.1	n	4	2	4
K_5	1	1	0.3194	m	2	4	2
K_{x_3}	0.2	0.1	0.1	p	2	1	1
K_d	0.4	0.6	1	V_0	2	2	2
K_{x_2}	0.2	0.2	0.3	V_1	2	2	2
K_{x_1}	0.5	0.3	0.6	V_{M2}	6	6	6
k	10	10	10	V_{M3}	20	20	30
k_f	1	1	1	V_4	2	2.5	3
				V_5	1	1	1

$$\frac{dx_1}{dt} = V_{in} - V_2 + V_3 + k_f x_2 - k x_1 \tag{1}$$

$$\frac{dx_2}{dt} = V_2 - V_3 - k_f x_2 \tag{2}$$

$$\frac{dx_3}{dt} = \beta V_4 - V_5 - \varepsilon x_3 \tag{3}$$

where

$$V_{in} = V_0 + V_1\beta, \qquad V_2 = V_{M2}\frac{x_1^2}{K_2^2 + x_1^2}$$
$$V_3 = V_{M3}\frac{x_1^m}{K_{x_1}^m + x_1^m}\frac{x_2^2}{K_{x_2}^2 + x_2^2}\frac{x_3^4}{K_{x_3}^4 + x_3^4} \tag{4}$$
$$V_5 = V_{M5}\frac{x_3^p}{K_5^p + x_3^p}\frac{x_1^n}{K_d^n + x_1^n}$$

V_0 refers to a constant input of Ca^{2+} from the extracellular medium and V_1 is the maximum rate of stimulus-induced influx of Ca^{2+} from the extracellular medium. Parameter β reflects the degree of stimulation of the cell by an agonist and thus only varies between 0 and 1. The rates V_2 and V_3 refer, respectively, to pumping of cytosolic Ca^{2+} into the internal stores and to the release of Ca^{2+} from these stores into the cytosol in a process activated by cytosolic calcium (CICR), V_{M2} and V_{M3} denote the maximum values of these rates. Parameters K_2, K_{x_2}, K_{x_1} and K_{x_3} are threshold constants for pumping, release, and activation of release by Ca^{2+} and by IP_3, k_f is a rate constant measuring the passive, linear leak of x_2 into x_1, k relates to the assumed linear transport of cytosolic Ca^{2+} into the extracellular medium, V_4 is the maximum rate of stimulus-induced synthesis of $InsP_3$. V_5 is the rate of phosphorylation of IP_3 by the 3-kinase, it is characterized by a maximum value V_{M5} and a half-saturation constant K_5 (Houart *et al.*, 1999).

2.1 Open-loop behavior

Figure 1(a) shows typical Ca^{2+} oscillations generated by the model. Although simple Ca^{2+} oscillations resembling those shown in Fig. 1 are usually observed in response to external stimulation, complex oscillations have also been reported in experiments performed with hepatocytes responding

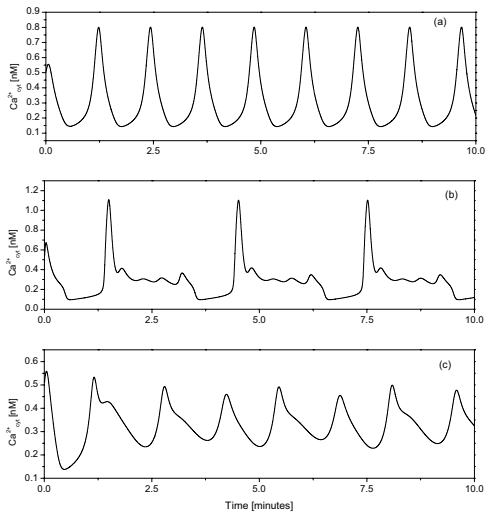

Fig. 1. Simple and complex intracellular Ca^{2+} oscillations. (a) Simple oscillations, (b) bursting oscillations and (c) chaos. Parameter values are given in Table 1.

to a variety of agonists (Houart *et al.*, 1999 and references there in). With appropriate parameter values the model can display complex Ca^{2+} oscillations, including bursting, chaos and quasiperiodicity. Two sets of parameter values corresponding to these modes of complex oscillatory behavior are listed in Table 1. The different types of oscillations are illustrated in Fig. 1.

2.2 Equilibrium points and zero-dynamics

In this section, first the equilibria points of the Ca^{2+} model are provided and the local stability is analyzed and secondly, the stability of the zero dynamic is established. The system parameters used to perform these analyzes are given in Table 1 (c).

The equilibrium points of autonomous systems satisfy the condition $\dot{x} = f(x) = 0$. It is important to realize that since the states in the Ca^{2+} model should not necessarily be zero for any realistic condition, since it involves an oscillatory behavior, the local stability of the set of equilibrium points is of interest rather than the equilibrium points itself. The equilibrium points for Ca^{2+} model are

$$x^* = [0.33\ 0.7812\ 0.1365]$$

The Jacobian matrix in x^* which is a 3×3 matrix is given by

$$J = \begin{bmatrix} 9.13 & 2.55 & 30.89 \\ -19.13 & -2.55 & -30.89 \\ -2.10 & 0 & -13.9 \end{bmatrix}$$

Eigenvalues for the above matrix are $1.6332 \pm 5.5528i, -10.5838$. Thus, the eigenvector is locally unstable at x^*.

Zero dynamics of a system are defined as the minimal order dynamics of its inverse. For nonlinear systems, such as the Ca^{2+}model, the realization of this inverse could be very complicated. However for affine control systems that are partially controlled, it is possible to asses the stability properties of the zero dynamics following the dynamics of the uncontrolled states (Maya-Yescas and Aguilar-Lopez, 2003). Following the methodology proposed in (Maya-Yescas and Aguilar-Lopez, 2003), zero dynamics stability of the Ca^{2+} model was investigated and we have found that the zero dynamics is stable.

3. NONLINEAR FEEDBACK CONTROL DESIGN

In this section we develop a nonlinear feedback control scheme for control and synchronization of intracellular calcium oscillations. A difficulty in the models of calcium signaling is the uncertainty about the values of the rate constants, kinetic values and diffusion coefficient. For control design we chose the free calcium (Ca^{2+}) concentration as the measurable dynamic variable (Schuster *et al.*, 2002), because this is the quantity most frequently measured (for instance, Ca^{2+} can be measured using fluorescent dyes). On the other hand, in the following, we consider an external influx of Ca^{2+} as the control input. Influx of Ca^{2+} from outside the cell is know to affect the frequency of Ca^{2+} oscillations and waves (Smith *et al.*, 2002).

Control objectives are (i) the regulation or tracking of Ca^{2+} dynamics to a steady state or a desired periodic or chaotic behavior, (ii) the synchronization of the output of a single Ca^{2+} oscillator to an array of Ca^{2+} oscillators by manipulation of the influx of Ca^{2+}, $u = V_{in}$ from the extracellular medium. Then,

$$\frac{dx_1}{dt} = u - V_2 + V_3 + k_f x_2 - k x_1 \tag{5}$$

$$y = h(x) = x_1 \tag{6}$$

The control input V_{in} is a plausible manipulated variable since it is more readily amenable to experimental manipulation (Marhl and Schuster, 2003; Goldbeter, 1996). The above equation can be written as,

$$\frac{dx_1}{dt} = f(x) + g(x)u \tag{7}$$

$$f(x) = V_3 - V_2 + k_f x_2 - k x_1$$

The function $f(x)$ contains model uncertainties related to kinetic parameters. In this case $g(x) =$

1. In the worst case, the function $f(x)$ is assumed to be unknown. The output systems dynamics can be obtained from (5) and (6) as:

$$\frac{dy}{dt} = \frac{\partial h}{dx}(f(x) + g(x)u) \tag{8}$$

Let $y_{ref} = x_{1,ref}$ be a desired trajectory and $\dot{y}_{ref}$be the time-derivative of the desired trajectory signal. A desired sliding trajectory is proposed as

$$\dot{y}_{ref} = \delta_1(y - y_{ref}) + \delta_2 \int_0^t \left\{ \begin{array}{c} sign(y - y_{ref}) \\ |y - y_{ref}|^{1/p} \end{array} \right\} d\tau \tag{9}$$

where δ_1 and δ_2 are control design parameters.

Defining $e = y - y_{ref}$ as the trajectory error, we have that the error dynamics is governed by

$$\dot{e} = \frac{\partial h}{dx}(f(x) + g(x)u) - \delta_1(y - y_{ref}) - \tag{10}$$
$$\delta_2 \int_0^t \left\{ sign(y - y_{ref}) \, |y - y_{ref}|^{1/p} \right\} d\tau$$

then, a ideal control law is given by

$$u = -\left(\frac{\partial h}{dx} g(x)\right)^{-1} \left(\frac{\partial h}{dx} f(x) + \omega\right) \tag{11}$$
$$\omega = -\delta_1(y - y_{ref}) -$$
$$\delta_2 \int_0^t \left\{ sign(y - y_{ref}) \, |y - y_{ref}|^{1/p} \right\} d\tau$$

The above control leads to $e \to 0$, as $t \to \infty$, i.e., $y \to y_{ref}$. The synthesis of the ideal control law requires accurate knowledge of the term $f(x)$ to be realizable, which, however is uncertain. By exploiting the properties of the sliding part of the sliding-mode type controllers to compensates uncertain nonlinear terms, the knowledge of the nonlinear term $f(x)$ can be avoided. Thus, the following practical controller can be introduced as,

$$u = -\left(\frac{\partial h}{dx} g(x)\right)^{-1} (\omega) \tag{12}$$
$$\omega = -\delta_1(y - y_{ref}) - \delta_2 \int_0^t \left\{ \begin{array}{c} sign(y - y_{ref}) \\ |y - y_{ref}|^{1/p} \end{array} \right\} d\tau$$

Summarizing, our feedback control scheme (12) is composed by a proportional action, which has stabilizing effects on the control performance, and a high order sliding superfice, which compensates the uncertain nonlinear terms to provide robustness to the closed-loop system. This behavior is exhibited because, once on the sliding surface, system trajectories remain on that surface, so the sliding condition is taken and make the surface and invariant set. This implies that some disturbances or dynamic uncertainties can be compensated while still keeping the surface an invariant set.

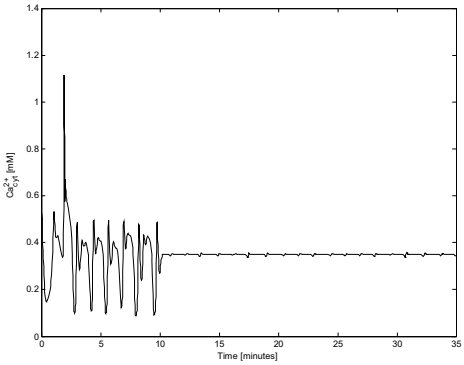

Fig. 2. Regulation of intracellular Ca^{2+} oscillations to reference $Ca^{2+} = 0.35$.

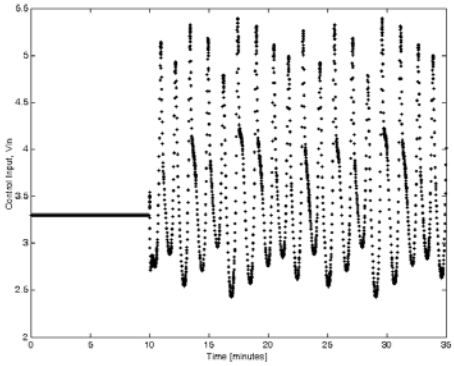

Fig. 3. Influx of Ca^{2+} for the regulation of intracellular Ca^{2+} oscillations to a constant reference.

4. NUMERICAL SIMULATIONS

We have taken the following three cases in order to illustrate the control performance: (i) regulation or suppression of periodic Ca^{2+} oscillations to a constant reference value, (ii) enforcing of periodic behavior to a different periodic Ca^{2+} oscillations, and (iii) synchronization of two calcium oscillators. In all cases the control action is activated at $t = 10.0$ and $p = 2$.

4.1 Regulation of Intracellular Ca^{2+}

Let the desired controlled behavior be a constant reference value, *i.e.,* $Ca_r^{2+} = 0.35$. In this case we have set the control design parameters δ_1 and δ_2 as 2.5 and 0.25 respectively. The simulation results are shown in Figures 2 and 3. Note that the proposed controller maintains the Ca^{2+} concentration around a small neighborhood of the reference value via the complex oscillations of the influx of Ca^{2+} shown in Figure 3. By increasing of control parameters, we can successfully perform the complete suppression of the intracellular Ca^{2+} nonlinear dynamics with larger amplitude oscillations of the influx of Ca^{2+}. It should be noted

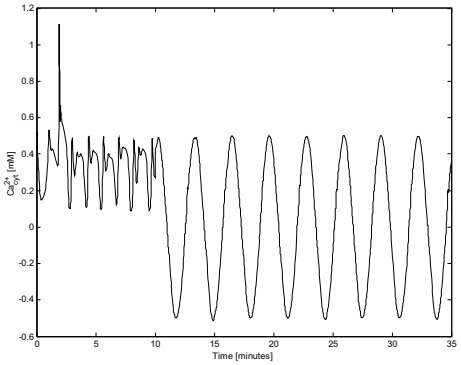

Fig. 4. Enforcing of intracellular Ca^{2+} to a periodic reference $Ca_r^{2+} = 0.35 + \sin(0.5t)$.

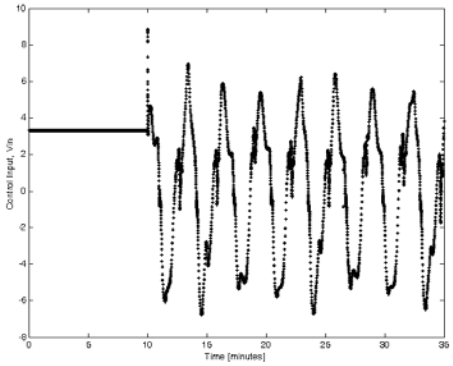

Fig. 5. Corresponding control input (influx of Ca^{2+}) for Figure 4.

that interestingly, complex oscillations of influx of Ca^{2+} signal leads to a a stationary output of the intracellular Ca^{2+} concentration.

4.2 Enforcing of Intracellular Ca^{2+}

Let the desired controlled behavior be a constant reference value, *i.e.,* $Ca_r^{2+} = 0.35$ a sinusoidal signal $Ca_r^{2+} = 0.35 + \sin(0.5t)$. Control design parameters are given as $\delta_1 = 7.5$ and $\delta_2 = 0.35$. The simulation results are shown in Figures 4 and 5. It can be seen from Figure 4 that the control inputs is periodic influx of Ca^{2+}. Such results indicate that oscillatory Ca^{2+} signals evoked by external stimuli require the periodic variation of the influx of Ca^{2+}. This conclusion can be related to the observation made in numerous experimental and theoretical studies that forcing an oscillatory system by a periodic input can readily produce simple and complex periodic behavior (Marhl and Schuster, 2003).

Numerical results for regulation and enforcing tasks via the manipulation of the external influx of Ca^{2+}, *i.e.*, the sum $v_0 + v_1\beta$, via our feedback control scheme, are in accordance with experimental and theoretical studies. Indeed, it can be seen

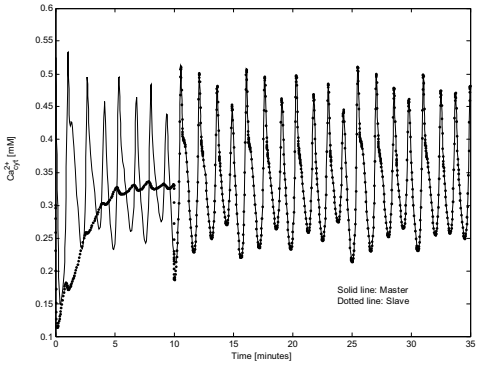

Fig. 6. Synchornization of two intracellular Ca^{2+} models.

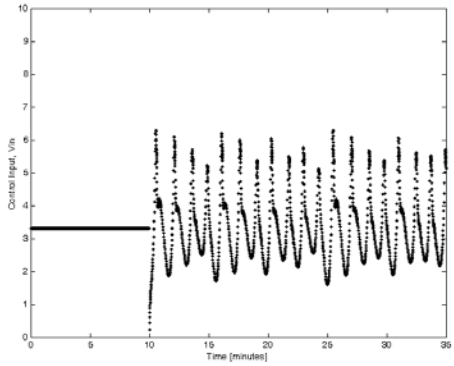

Fig. 7. Corresponding influx of Ca^{2+} for Figure 6.

that oscillations can be triggered by an increase in β due to stimulation by an external signal, or simply by an increase in v_0 originating from an increase in extracellular Ca^{2+}. On the other hand, bringing the value of v_0 down to zero eventually suppresses the oscillations and the hypothesis of a constant input $v_1\beta$ from the IP_3-sensitive Ca^{2+} store also ceases to hold in these conditions, as this store can no more be replenished (Marhl and Schuster, 2003; Goldbeter, 1996).

4.3 Synchronization of two intracellular Ca^{2+} models

Let us consider the chaotic synchronization of two models of intracellular Ca^{2+}. Synchronization of intracellular Ca^{+2} is known to occur in a large number of cells, which is often associated with waves spreading within the intracellular environment (Perc and Marhl, 2004). In the standard terminology of chaos synchronization, the first oscillator is considered as the master subsystem, and the second oscillator is considered as the slave subsystem. The synchronization objective is that the slave oscillator follows the dynamics displayed by the master oscillator. Control design parameters are given as $\delta_1 = 6.0$ and $\delta_2 = 0.01$. Simulation results are shown in Figures 6 and 7. Synchro-

nization is obtained with forcing oscillatory influx of Ca^{2+} that is very similar to the basic Ca^{2+} oscillation. The forcing signal could be seen as a signal from an adjacent cell, which stimulates the synchronized oscillations, *i.e.*, stimulates the cell to oscillate with the same temporal pattern as its neighbors (Marhl and Schuster, 2003).

5. CONCLUSIONS

In this work, we have presented a nonlinear feedback control approach for control and synchronization of the intracellular calcium nonlinear dynamic. The significance of Ca^{2+} oscillations stem from the crucial importance of this ion in the control and coordination of many key cellular processes. In both cases, i.e., control and synchronization, the free Ca^{2+} is manipulated as a function of external influx of Ca^{2+}. Our control approach is composed by an proportional stabilizing action and an high order sliding-mode contribution. The key feature of this control approach is that a simple design with good robustness and performance capabilities is obtained, which exploits the robustness properties of the high order sliding-mode contribution to deal with model uncertainties. We have shown via numerical simulations the satisfactory performance of the proposed control scheme for both control and synchronization tasks. In spite that our results have been obtained for a simple intracellular Ca^{2+} model, we expect that the control approach presented here could be used in more detailed models of intracellular Ca^{2+} models where an external input can be manipulated, since the proposed high-order sliding mode approach is robust against uncertain, unknown or poorly known parameters.

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