Stochastic simulations of cell-cell signalling in hepatocytes

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ABSTRACT

We present results of a Monte Carlo simulation of stochastic effects for two models of intercellular calcium wave propagation in rat hepatocytes. Both models involve gap junction diffusion by a second messenger. In general taking into account the stochastic effects improves agreement with experiment. Both stochastic models exhibit baseline fluctuations and variations in the peak heights of Ca^{2+}. In addition, we find for one model that there is a distribution of latency times, rather than a single latency time, with a width which is comparable to the experimental observation of spike widths. We also find for the other model with low gap junction diffusion that it is possible for cell multiplets to oscillate independently initially, but to subsequently become synchronized.

Keywords: Stochastic, intercellular, calcium waves, gap junctions, hepatocytes.

1 INTRODUCTION

Cell to cell signals control the development of multicellular organisms as well as most of their functions [1]. These signals have many different manifestations and provide excellent examples of nanoscale biology. Calcium signaling plays a particularly important role in cell communication. Such intercellular communication can take different forms, including gap junction coupling, paracrine signaling and the recently discovered extracellular calcium signaling [2].

A paper by Tordjmann et al. [3] studied calcium waves induced by noradrenaline and showed that gap junction coupling is necessary for the coordination of the oscillations between the different cells. The authors also demonstrated that it is necessary to have hormone stimulation at each hepatocyte in order to have cell-cell calcium signal propagation. In a subsequent paper [4] they continued these studies, combining single-cell studies with experiments on cell populations isolated from the peripheral and central zones of the liver cell plate. They found strong evidence that the sequential pattern of calcium responses to vasopressin in these multicellular rat hepatocyte systems was due to a gradient of cell sensitivity (from cell to cell) for the hormone. Based upon these experimental studies, two models have been put forward in order to explain the observed results.

The first model is due to Dupont et al. who [5] studied a model based on junctional coupling of multiple hepatocytes which differ in their sensitivity to the hormonal stimulus. The model yielded intercellular waves that were confirmed experimentally [5]. The authors also presented experimental evidence that the degree of synchronization is greater for the first few spikes, in agreement with the prediction of their model. They also presented evidence that suggested, within the context of their model, that IP_3 diffusion through gap junctions plays the dominant role in the synchronization of intercellular spiking (rather than Ca^{2+} diffusion).

An alternative model has also been proposed by Höfer [6] to explain the experimental results obtained in the first paper by Tordjmann et al [3]. Höfer noted that this experiment revealed a rather large variability in oscillator frequency between adjacent cells, which he argued is likely to be of random nature. As a consequence he studied the possibility that this originates from random variations in the structural properties of cells (cell size, cell shape, or ER content). In addition, Ca^{2+} was assumed to be the second messenger [6]. His results were in reasonable agreement with those of [3].

Both models are deterministic, described by differential equations with boundary conditions for the cell multiplets and with diffusion between cells. Such models, however, do not incorporate stochastic effects such as fluctuations in the baseline values of calcium and variations in the amplitudes and widths of the spikes that have been seen experimentally [3], [4].

To obtain a better explanation of the experimental results, we have studied stochastic versions of the above two models. Our simulation is based on a Monte Carlo method due to Gillespie [7]. Stochastic models of intracellular Ca^{2+} spiking for a variety of cell types have been studied previously [8].

2 Ca^{2+} SYNCHRONIZATION OF HETEROGENEOUS CELLS

We first study a stochastic version of the deterministic model proposed by Höfer [6] to explain the synchronization of calcium oscillations in heterogeneous hepatocyte cells found by Tordjmann et al. [4]. He assumed that the concentration of IP_3 rapidly reaches a steady-state value (which can differ for different cells) that is treated as a parameter of the model. We will be considering in this study single cells, doublets and triplets.

Let x_j and z_j be, respectively, the cytosolic calcium concentration and the free calcium content in cell j. The latter is defined as $z_j = x_j + \beta_j y_j$, where y_j denotes the free calcium concentration in the ER.

After some simplification Höfer obtained the following deterministic model for the time evolution of the x_j and z_j variables:

$$\begin{aligned}\frac{dx_j}{dt} &= \rho_j \left(\gamma_0 + \gamma_c \frac{P_j}{K_0 + P_j} - \gamma_4 \frac{x_j^2}{K_4^2 + x_j^2} \right. \\ &+ \frac{\alpha k_r(x_j; P_j)}{\beta_j} (z_j - (1 + \beta_j) x_j) \\ &\left. - \alpha \gamma_3 \frac{x_j^2}{K_3^2 + x_j^2} \right) + \gamma (x_i - x_j); \qquad (1)\end{aligned}$$

$$\begin{aligned}\frac{dz_j}{dt} &= \rho_j \left(\gamma_0 + \gamma_c \frac{P_j}{K_0 + P_j} - \gamma_4 \frac{x_j^2}{K_4^2 + x_j^2} \right) \\ &+ \gamma (x_i - x_j) \qquad (2)\end{aligned}$$

The last term, proportional to γ, denotes diffusion between cells. The index pairs $(i; j)$=(1,2) and (2,1). The system can be easily generated to the case of more than two cells. In these equations P_j is the IP_3 concentration in cell j. The IP_3 R release function $k_r(x_j; P_j)$ describes the gating kinetics of the IP_3 receptor and it is given by

$$k_r(x_j; P_j) = k_1 \frac{\left(d_2 \frac{d_1 + P_j}{d_3 + P_j} P_j x_j \right)^3}{(d_p + P_j)^3 (d_a + x_j)^3 \left(d_2 \frac{d_1 + P_j}{d_3 + P_j} + x_j \right)^3} + k_2.$$

The parameters ρ_j, α and β_j define various structural characteristics of the cell and account for the heterogeneous behavior of different cells. Table 2 summarizes the values we adopt for these parameters.

The above set of equations are deterministic and do not consider at all the fluctuations that appear from the fact that the chemical reactions do not occur uniformly and continously in time. Gillespie's method considers specifically that (a) the concentration of molecular species can only vary by a discrete amount and (b) the chemical reaction itself is a stochastic process that occurs with a certain rate. In accordance with Gillespie's method, we introduce the number populations of cell j as X_j and Z_j, such that the concentrations of the reactants are obtained as: $X_j = x_j/W; Z_j = z_j/W$. Here W is the volume of cytosolic compartment of the cell, with fluctuation effects being most notable for small W.

Par.	Value	Par.	Value
P	2.0 μM	d_1	0.3 μM
γ_0	0.2 $\mu M s^{-1}$	d_2	0.4 μM
γ_c	4.0 $\mu M s^{-1}$	d_3	0.2 μM
K_0	4.0 μM	d_p	0.2 μM
γ_4	3.6 $\mu M s^{-1}$	d_a	0.4 μM
K_4	0.12 μM	k_2	0.02 s^{-1}
γ_3	9.0 $\mu M s^{-1}$	ρ	0.02 μm^{-1}
K_3	0.12 μM	α	2.0
k_1	40.0 s^{-1}	β	0.1

Table 1. Typical simulation constants for model with intercellular diffusion of Ca^{2+}.

Following [6] we consider a spherical cell with a radius of 6 μm, with a cytosolic volume of about $W = 300\ \mu m^3$.

To determine the maximum value of γ we should use in the stochastic model we simulated the experimental study of the doublet of hepatocytes, namely, first with only one of the cell stimulated with a hormonal input and then with both cells simultaneously stimulated. From the experimental results we know that local perfusion is not sufficient for coordinated oscillations. Global perfusion of both cells, on the other hand, produces a well synchronized Ca^{2+} oscillation in the two cells. In our simulations we see that the two cells respond differently, with different periods of oscillations; in neither case does the unstimulated cell show Ca^{2+} oscillations. But if we stimulate both hepatocytes they respond with well coordinated Ca^{2+} oscillations. This yields the value of $\gamma_{max} = 0.07\ s^{-1}$.

Next we study the behavior of two connected hepatocytes. To simulate the experimental situation of two slightly different cells, we follow Höfer and choose different structural parameters, with $\beta_1 = 0.15$, $\beta_2 = 0.2$, The calcium oscillations in the two cells are totally uncoordinated if the membrane permeability set to zero, as should be the case. For value of the permeability $\gamma = 0.07\ s^{-1}$ we find 1:1 locking (Fig. 1).

Experiments also show the absence of coordination among the calcium signals in connected hepatocytes at low concentrations of stimuli. To simulate this situation we applied a low stimulation level $P = 1\ \mu M$ to two cells, with different structural properties. We found that calcium oscillations become synchronized with time. Although this effect has not been seen experimentally, it would be interesting to have experimental observations of calcium oscillations over long time intervals for medium stimulation levels. It is possible that even cells that are initially unsynchronized may become synchronized later on.

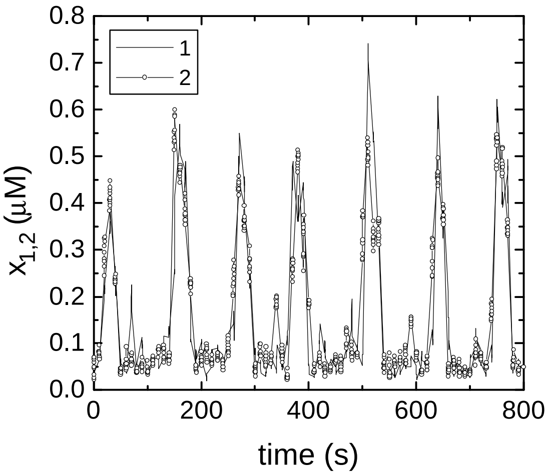

Fig. 1. Calcium oscillations for a doublet of cells with the permeability constant $\gamma = 0.07 s^{-1}$.

3 IP_3 SYNCHRONIZATION VIA HORMONAL SENSITIVITY GRADIENT

The second model we study is due to Dupont et al. [5] and considers IP_3 as the second messenger responsible for coordination of Ca^{2+} signaling in connected hepatocytes. This model is based on the experimental observation that the number of external receptors on a hepatocyte membrane depends on its location in the liver cell plate [4]. Thus the authors consider a model of a multiplet of gap junction connected cells, with a small variation in the individual cell frequencies. The dynamics of each cell j is described by a set of three dynamical variables R_j^{des}, x_j and y_j. These are the fraction of inactive IP_3 receptors, the concentration of cytosolic Ca^{2+} and the concentration of IP_3, respectively. There is intracellular di®usion of calcium and intercellular di®usion of IP_3, with the latter providing the coupling between

Par.	Value	Par.	Value
k_+	$25.0 s^{-1} \mu M^{-4}$	Ca_{tot}	$60.0 \mu M$
k_-	$2.5 \times 10^{-3} s^{-1}$	K_{IP}	$1 \mu M$
K_{act}	$0.34 \mu M$	V_K	$7.5 \times 10^{-3} \mu M/s$
k_1	$42.0 s^{-1} \mu M^{-1}$	V_{PH}	$7.5 \times 10^{-2} \mu M/s$
b	10^{-4}	K_K	$1 \mu M$
K_{PH}	$10 \mu M$	α	0.1
V_{MP}	$8.0 \mu M/s$	K_d	$0.5 \mu M$
K_p	$0.4 \mu M$	F_{IP}	$0.35 \mu m/s$

Table 2. Simulation constants for model with intercellular di®usion of IP_3.

adjacent cells. The equations of motion are taken to be

$$\frac{dR_j^{des}}{dt} = k_+ x_j^4 \frac{1 - R_j^{des}}{1 + (x_j/K_{act})^3} - k_- R_{des}; \quad (3)$$

$$\frac{dx_j}{dt} = k_1(b + IR_a)[Ca_{tot} - x_j(\alpha + 1)] - V_{MP}\frac{x_j^2}{x_j^2 + K_P^2}; \quad (4)$$

$$\frac{dy_j}{dt} = V_{plc;j} - V_K \frac{y_j x_j^2}{(K_K + y_j)(x_j^2 + K_d^2)} - V_{PH}\frac{y_j}{K_{PH} + y_j}; \quad (5)$$

$$IR_a = \frac{1 - R_j^{des}}{1 + (K_{act}/x_j)^3} \frac{y_j^3}{K_{IP}^3 + y_j^3}. \quad (6)$$

At each boundary between two cells:

$$D_{IP}\frac{\partial y^-}{\partial x} = D_{IP}\frac{\partial y^+}{\partial x} = F_{IP}(y^+ - y^-). \quad (7)$$

where the superscripts + and - indicate the IP_3 concentration at the right and left limits of the border, respectively. All parameters are given in Table 3 We consider cells $20 \mu m$ long, each containing 20 grid points.

We study, using Gillespie's method, a stochastic version of this model for di®erent cell volumes and for a range of values of the cell-cell permeability. We consider $W = 400 \mu m^3$. Figure 2 shows our results as well as those for the deterministic limit $W = 50{,}000 \mu m^3$. The results in the deterministic limit are consistent with [5], as to be expected. In contrast to the deterministic model where the induction time (latency of cell) depends only on the stimulus strength, we ¯nd a distribution of induction times in the stochastic model, due to °uctuations in the calcium concentration. Fig. 3 shows the distribution of induction times for one stimulated cell with $V_{plc} = 2 \times 10^{-3} \mu M/s$. As there does not appear to be any systematic experimental study of such a distribution, we have no data to compare our results with. It is also the case that the calcium spikes in these experiments have a width of 20 – 30 s, which means that would be di±cult to see °uctuations in the central position of the spikes.

For two connected cells we determine the cell-cell permeability following reference [5], such that a doublet of cells, with only one cell doped with stimulant, exhibits calcium oscillations only in the stimulated cell (as has been shown experimentally). We have to use a smaller value for the permeability than in the deterministic study because noise in the baseline produce spikes in the second, non-stimulated cell if the permeability is

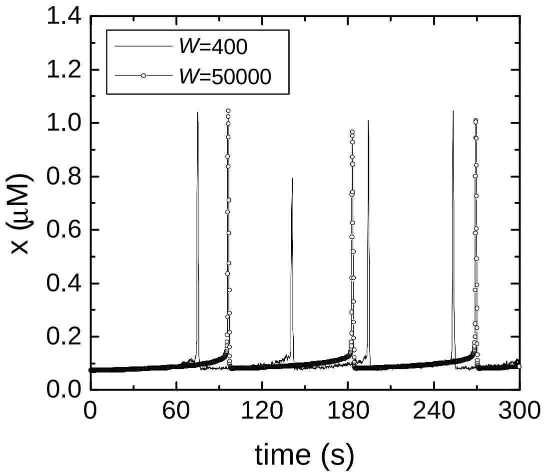

Fig. 2. Results of calcium oscillations in one cell for the stochastic version of Dupont et al. model for values of $W = 400; 50000$. Notice that, as expected, °uctuations decrease with increasing W and that the deterministic limit is already well reproduced by $W = 50000$. Initial conditions are resting states corresponding to $V_{plc} = 6{:}5 \pounds 10^{i41}$M/s.

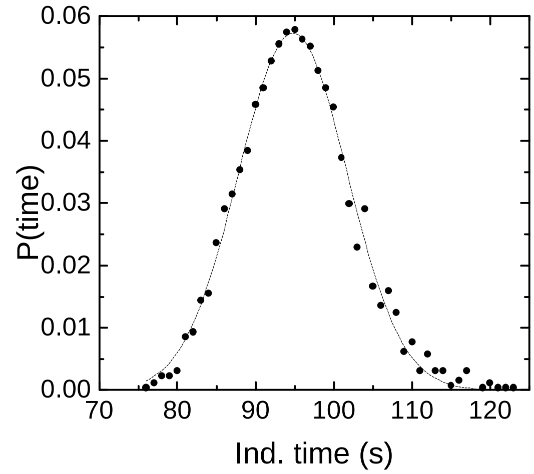

Fig. 3. Distribution of induction times for one cell with $W = 400$, $V_{plc} = 2 \pounds 10^{i31}$M/s.

larger then 0:35 ¹m/s. Another distinguishing feature from the deterministic model is that stochastic e®ects produce a variation in the spike amplitudes.

We ¯nd that two stimulated cells don't go out of phase as rapidly as in the deterministic model. The experimental results exhibit more synchronization between cells than in this stochastic model. However, the stochastic model yields better agreement with experiment in terms of the variation in amplitudes and period variations.

4 CONCLUSION

We have studied calcium oscillations in connected hepatocytes for two di®erent stochastic models of calcium dynamics. We have solved these two models using a Monte Carlo approach, considering each term in a model as a speci¯c reaction occurring with a certain reaction rate. Our models are in better agreement with experiment than are the deterministic models. Both stochastic models exhibit baseline °uctuations and variations in peak heights. All the results of both deterministic models have been reproduced for their stochastic versions in the limit of large volume, as should be the case. We conclude that it is important to take into account stochastic e®ects in modeling calcium oscillations in connected hepatocytes.

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