Abstract

In this letter we present the results of a simple model for intercellular communication via calcium oscillations, motivated in part by a recent experimental study. The model describes two cells (a "donor" and "sensor") whose intracellular dynamics involve a calcium-induced, calcium release process. The cells are coupled by assuming that the input of the sensor cell is proportional to the output of the donor cell. As one varies the frequency of calcium oscillations of the donor cell, the sensor cell passes through a sequence of N:M phase locked regimes and exhibits a "Devil’s staircase" behavior. Such a phase locked response has been seen experimentally in pulsatile stimulation of single cells. We also study a stochastic version of the coupled two cell model. We find that phase locking holds for realistic choices for the cell volume.
Oscillatory increases in the intracellular concentration of calcium control a variety of important, diverse biological functions, including muscle contraction, metabolism and gene expression [1, 2, 3]. In the latter case, for example, calcium oscillations lead to the expression of genes that are essential for dendritic development and neuronal survival. A recent review of the versatility and universality of calcium signalling has been given by Berridge et al [1]. Typically cells have a Ca\(^{2+}\) rest concentration of about 100 nM, but when activated rise to concentrations of roughly ten times this. Such increases can be produced by ligands (agonists) binding to receptors located on the plasma membrane, through a process involving the second messenger inositol-1,4,5-trisphosphate (IP\(_3\)). These can be receptor-specific, as shown in a recent study of the relationship between the production of IP\(_3\) and the calcium response [4]. An important characteristic of the spike-like Ca\(^{2+}\) oscillations is that they are frequency, rather than amplitude, encoded. That is, an increase in the agonist concentration increases the frequency of oscillation, but has little effect on its amplitude. Another significant characteristic is that calcium signals can be propagated between cells, providing an important means of cell communication. Such intercellular communication can take different forms, including diffusion of calcium or IP\(_3\) through gap junctions and paracrine signaling. Recently deterministic models have been developed for signalling through gap-junction diffusion, via a second messenger such as calcium or IP\(_3\) [5, 6, 7]. Important stochastic effects have also been included [8] for gap-junction signalling, as well as for other aspects of calcium dynamics [9, 10, 11]. In one type of paracrine signalling, a calcium spike in one cell causes the release of a secondary agonist, such as ATP, to the extracellular space, followed by stimulation of purinergic receptors on nearby cells [12, 13]. Recently a new paracrine mechanism for intercellular communication has been proposed [14], based on the fact that the calcium liberated as a consequence of intracellular calcium spiking is often extruded to the extracellular neighborhood of the cell. The recent experimental study showed if this space is limited such that the local extracellular calcium fluctuations are sufficiently large, calcium-sensing receptors (CaR) [15] on the surfaces of adjacent cells can be activated, producing secondary spikes in these cells. Thus calcium receptors may
mediate a new form of intercellular communication in which cells are informed of the intracellular signaling of their neighbors via extracellular calcium fluctuations. However, the experimental results yield only qualitative information about the response of the sensor cell to the donor cell.

In this letter we present the results of a simple model for paracrine intercellular communication via calcium oscillations, motivated in part by this recent experimental study. As one still does not understand in detail the complex biochemistry involved in the CaR coupling, we limit ourselves to studying a simplified model that might capture the qualitative features of this new form of signaling. There are two aspects to describing the intercellular communication: the intracellular dynamics and the coupling between cells. A number of theoretical models have been developed to explain intracellular Ca oscillations [16, 17, 18, 19]. The basis for most of these is that after an agonist (hormone) binds to the extracellular side of a receptor bound to the membrane, the Gα subunit at the intracellular side of the receptor-coupled G-protein is activated. This activated G-protein then stimulates a phospholipase C (PLC) which helps form a second messenger IP₃ and diacylglycerol. IP₃ then binds to specific receptors in the membrane of an internal store of calcium (such as the endoplasmic reticulum). The binding helps open calcium channels, which leads to a large flux of calcium ions from the internal store into the cytosol, which then stimulates the release of additional calcium ions. Some details of this complex progress, however, remain unknown.

As there are many different models for the intracellular calcium oscillations, we choose the simplest to illustrate how the results of communication between cells might differ depending on the internal cell dynamics. This is the so-called minimal model, involving two dynamical variables, the cytosolic calcium and an internal store of calcium (such as the endoplasmic reticulum) respectively [16], in which an agonist induces calcium oscillations in a single cell. We couple two such cells, the donor cell and the sensor cell, by assuming that the stimulus of the target cell is proportional to the cytosolic calcium content of the first cell. Since some of the cytosolic Ca²⁺ produced
in the donor cell is extruded into a small space near a CaR receptor, this seems to be a reasonable assumption. This avoids modeling the extracellular diffusion of Ca\(^{2+}\) as well as the complex receptor dynamics that is presumably involved in the calcium-sensing receptor mechanism proposed by Höfer et al. [14]. However, our model is consistent with the spirit of the single cell minimal model in that it provides a minimal two cell coupling that yields interesting intercellular communication. We should also note that under in vivo conditions, hormones are not released steadily, but are released in a pulsatile fashion. Thus our results for the sensor cell responding to an input signal are in principle relevant to the physiologically interesting question of how the intracellular cytosolic calcium responds to a pulsatile application of agonists.

We consider a coupled cell version of the minimal model, coupling two cells through a term proportional to the calcium output of the first (donor) cell. This term will serve as an external stimulus for the second (sensor) cell. The donor cell dynamics is described by two differential equations for its cytosolic Ca\(^{2+}\) concentration, \(y_1\) and its internal store of Ca\(^{2+}\), \(y_2\):

\[
\frac{dy_1}{dt} = V_0 + \beta_1 V_1 - V_2 + V_3 + k_f y_2 - ky_1 \tag{1}
\]
\[
\frac{dy_2}{dt} = V_2 - V_3 - k_f y_2 \tag{2}
\]

where \(V_2 = \frac{V_{m2} y_2^2}{(k_2^2 + y_2^2)}\) and \(V_3 = \frac{V_{m3} y_1 y_2^2}{(k_3^2 + y_1^2)(k_2^2 + y_2^2)}\). This model has been studied extensively [16, 17]. It is known that for a given set of the parameter values Ca\(^{2+}\) oscillations will occur when the parameter \(\beta_1\), which increases with the concentration of the external hormonal stimulus, lies in a range \(\beta_{\text{min}} < \beta_1 < \beta_{\text{max}}\). The minimum and maximum values depend mainly on the parameters \(V_0\) and \(V_1\).

The sensor cell is modeled using the same equations for its cytosolic and internal calcium concentrations \(y_1'\) and \(y_2'\) as given in Eqs(1,2). However, instead of a term \(\beta_2 V_1'\) representing a constant stimulus, we use the term \(\beta_2 y_1 V_1'\), which provides the
coupling between the cells. This assumes that the stimulus to the donor cell from the extruded calcium from the donor cell is proportional to the the latter’s cytosolic calcium concentration. In general, the structural parameters $V_0$, $V_1$, $V_2$, $V_3$, $k_f$, $k$ of the first cell and $V'_0$, $V'_1$, $V'_2$, $V'_3$, $k'_f$, $k'$ of the second cell can be different, but for the sake of simplicity we take them to be the same. We find in general that oscillations in the donor cell due to a constant hormonal input produce oscillations in the sensor cell. This is in qualitative agreement with the experimental observation [14], but the detailed predictions of our model require further experimental study.

We have calculated the N:M rhythms predicted for this coupled minimal model as a function of $\beta_1$ for fixed $\beta_2$, where N denotes the number of stimuli arising from the donor cell and M the number of responses of the sensor cell in a given time interval. For example, the frequency of response can be the same as the frequency of the stimulus, i.e. N:M is 1:1. However, in general the Ca$^{2+}$ response in the sensor cell is blocked when the frequency of pulses of the donor cell is increased. In Fig. 1 we show a 3:2 response. This phenomenon of blocking is also seen in heart patients, where it is known as Wenckebach periodicity. As one varies $\beta_1$ the sensor cell passes through a sequence of N:M phase locked regimes (in response to the oscillatory stimuli from the donor cell) and exhibits a ”Devil’s staircase” behavior [20], as shown in Fig. 2. That is, between any two steps there is a countless number of staircases. This behavior has been found earlier in a study of a finite difference model of cardiac arrhythmias [21] as well as in a model of intracellular calcium oscillations [18] in which the hormonal stimulus was modeled by a sequence of square well pulses. However, this is the first prediction of such behavior in coupled, nonexcitable cells. We find, for example, that with $k = 6s^{-1}$, $k' = 6s^{-1}$, $\beta_2 = 0.4$ and all other parameters as in Table 1 the variation of $\beta_1$ from 0.3 to 0.415, i.e. increase in the concentration of the external stimulus which increases the frequency output of the first cell, leads to the ratio of the stimulus/response from 1:1 rhythm ($\beta_1 = 0.3$), through 5:4 ($\beta_1 = 0.4$), 4:3 ($\beta_1 = 0.405$), 3:2 ($\beta_1 = 0.41$), 5:3 ($\beta_1 = 0.412$) to the 2:1 rhythm ($\beta_1 = 0.415$). On the other hand, various rhythms also can be obtained by fixing, for example, $\beta_1 = 0.3$ and varying $\beta_2$ from 0.38 to 0.345 (all other parameters as described above). In this
case we find the following sequence of the stimulus/response rhythms: 1:1 rhythm ($\beta_2 = 0.38$), 5:4 ($\beta_2 = 0.37$), 4:3 ($\beta_2 = 0.365$), 3:2 ($\beta_2 = 0.36$) and finally a 2:1 rhythm ($\beta_2=0.345$). Some examples of the Devil’s staircase are shown in Fig. 2 and Fig. 3. This response of the sensor cell is similar to experimental results of Schöfl et al. [22] who applied square wave pulses to phenylephrine to liver cells every 30 seconds. They found stimulus/response rhythms such as 2:1, but with less regularity than shown here [23]. A subsequent stochastic model based on a deterministic model of intracellular dynamics due to Chay et al [18] yielded results qualitatively similar to the experiment [23].

Deterministic models such as the one used above neglect potentially important stochastic effects such as fluctuations in the baseline values of $\text{Ca}^{2+}$ and variations in the amplitudes and widths of the spikes. Since the number densities of the intracellular signaling molecules are typically low (of the order of $1 \times 10^2 \mu\text{m}^{-3}$, stochastic effects could be important. To see whether such effects are significant here, we have also studied a stochastic version of our model, using a Monte Carlo method due to Gillespie [24]. We have studied the stochastic model for different values of the cell volume $\Omega$ (assumed to be the same for both cells). For very small $\Omega$ fluctuations destroy the phase locking, while in the limit of large $\Omega$ one recovers the deterministic limit. Both results are what one would expect. For intermediate values of $\Omega$, however, such as $\Omega = 2000$, which is the approximate volume of hepatocyte cells, we find that phase locking persists, although with occasional lapses. Some typical results for this case are shown in Fig. 4. Thus we find a stochastic version of the Devil’s staircase for values of the cell volume that are realistic. We also found that cells can switch between frequencies in the stochastic model if we choose $\beta_1$ and $\beta_2$ such that the deterministic model would give a frequency locking of the cells on the edge of one of the steps of the Devil’s staircase.

In conclusion, we have shown that a coupled minimalist model can account for a variety of calcium oscillations that have been seen experimentally in hepatocytes stimulated with time-dependent pulses of hormone [22]. This simple model can also describe intercellular communication between cells via calcium-sensing receptors, with
results that are at least qualitatively consistent with a recent experimental study [14]. We have found in addition that the deterministic version of the model yields a Devil’s staircase type of phase locking between the donor and sensor cell. We have also found that this phase locking is present in a stochastic version of this model, which is a novel feature of the study. The stochastic model is more realistic than the deterministic model and yields baseline fluctuations and variations in the amplitude of the spikes in $Ca^{2+}$, as seen in experimental studies of calcium oscillations. Whether or not paracrine communication in real biological systems will exhibit a Devil’s staircase behavior is an open question, however, as there are many ways in which one should improve our model to make it more realistic. For example, we are currently extending this study to include the plasma membrane receptor dynamics [25], and oscillations in $IP_3$ that have been seen in some recent studies [26, 27, 4]. Our preliminary results from a study of two cells whose internal dynamics is given by a model of Kummer et al [19] with coupling through receptor dynamics similar to that of Riccobene et al [25] show that bursting behavior, in addition to the type of behavior reported here, is also possible for this form of paracrine communication. The fundamental problem of paracrine cell communication would seem to be a rich field for further experimental and theoretical investigation.

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Figure Captions

Fig.1 Calcium oscillations of two connected cells ($\beta_1=0.3$, $\beta_2=0.36$). Frequencies of cells are locked in a sequence of 3:2. Deterministic model.

Fig.2 Devil’s staircase, a ratio $N/M$ (where $N$ is the number of spikes of the donor cell and $M$ is the number of spikes of the sensor cell) as a function of $\beta_1$ at fixed $\beta_2=0.3$.

Fig.3 Devil’s staircase, a ratio $N/M$ as function of $\beta_1$ at fixed $\beta_2=0.2$.

Fig.4 Calcium oscillations of two connected cells ($\beta_1=0.17$, $\beta_2=0.3$). Frequencies of cells are locked in a sequence of 1:1 with occasional fluctuations. Stochastic model with $\Omega = 2000$. 
References


[15] The CaR receptors were initially cloned from the parathyroid gland and possess the characteristic seven transmembrane segments of the G-protein coupled receptor family.


Table 1: Parameters for the minimal two variable model.

<table>
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<th>Parameter</th>
<th>Value</th>
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<td>$k$</td>
<td>$6 s^{-1}$</td>
</tr>
<tr>
<td>$k_f$</td>
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<tr>
<td>$k_2$</td>
<td>$1.0 \mu M$</td>
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<td>$k_a$</td>
<td>$0.9 \mu M$</td>
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<td>$k_r$</td>
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<td>$V_{m3}$</td>
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