A fire-diffuse-fire framework for the functional organisation of cellular calcium signals

Background

Ca^{2+} is critically important for a large number of cellular functions, such as muscle contraction, cardiac electrophysiology, bursting oscillations and secretion (in response to hormonal agonists or neurotransmitters), synaptic plasticity, sensory perception and adaptation in photoreceptors [1]. Mechanisms by which a cell controls its Ca^{2+} concentration are of central interest in cell physiology. The recent use of Ca^{2+} specific fluorescent reporter dyes and digital videomicroscopy has begun to reveal the complexity of Ca^{2+} dynamics in spatially extended cellular systems. Ca^{2+} signalling in a wide diversity of cell types frequently occurs as repetitive, but transient, increases in $[Ca^{2+}]$ or Ca^{2+} oscillations. The changes in $[Ca^{2+}]$ associated with Ca^{2+} oscillations generally do not occur uniformly throughout the cell but are initiated at a specific site and spread in the form of waves. The functional significance of these spatiotemporal patterns of $[Ca^{2+}]$ is a subject of increasing activity within the cellular signalling community. For example, in the spatial domain the spreading of a Ca^{2+} wave provides a mechanism by which a regulatory signal can be distributed throughout the cell [2]. Two classes of oscillations or waves are readily distinguished: those that depend primarily on the influx of Ca^{2+} through voltage-operated Ca^{2+} channels (VOCCs) from the extracellular space, and those that depend primarily on Ca^{2+} release from internal stores. In this latter class, distinctions can be made on the basis of whether the release of Ca^{2+} is dominated by the ryanodine receptor (RyR), the inositol (1,4,5)-trisphosphate (IP_3) receptor or a combination of both. When activated, both Ca^{2+} entry and Ca^{2+} release channels can give rise to brief pulses of Ca^{2+} that form a small plume around the mouth of the channel before diffusing into the cytoplasm. Such signals can remain localised and activate effectors within the immediate vicinity of the channels, they can recruit effectors or they can be summated to yield global increases that propagate throughout and between cells. Local Ca^{2+} signals therefore serve to activate specific targets before diffusion or regenerative mechanisms spread the Ca^{2+} across a cell.

A diverse array of local Ca^{2+} signals have been visualised in electrically non-excitable cells. One type of local Ca^{2+} signal that appears to operate in many, if not all, electrically non-excitable cells is Ca^{2+} *puffs*. These elementary events have amplitudes typically ranging from $\sim 50-600$nM, a spatial spread of $\sim 6\mu$m and a total duration of ~ 1 second.

Such events were first observed in Xenopus oocytes [3] but have subsequently been observed in HeLa cells, neurites and endothelial cells (reviewed by Bootman *et al*. in [4]). It is now well established that cell-specific recruitment of a generic elementary signal underlies different global signals. In HeLa cells [5] and Xenopus oocytes [6], Ca^{2+} puff sites that have a higher sensitivity to IP_3 consistently trigger Ca^{2+} waves.

In heart and skeletal muscle, release of Ca^{2+} from the sarcoplasmic reticulum (SR) by RyRs is the key event linking membrane depolarisation and mechanical activity during excitation-contraction coupling. RyRs occur in clusters that give rise to localised Ca^{2+} release events denoted Ca^{2+} *sparks*. These events are analogous to the Ca^{2+} puffs described above, although they are usually faster in onset and decline, and have a more restricted spread ($\sim 1-3\mu$m). Spatiotemporal recruitment of Ca^{2+} sparks underlies the global Ca^{2+} signals that subsequently activate myocyte contraction.

The fluorescent imaging of localised Ca^{2+} sparks or puffs has now made it clear that Ca^{2+} release is a stochastic process that occurs at spatially discrete sites that are clusters of RyRs or IP_3 receptors in the SR or endoplasmic reticulum (ER). Moreover, it has recently been recognised that spatial nonuniformity in the parameters that control Ca^{2+} exchange between the cytoplasm and these membranes plays an important role in Ca^{2+} signalling [1]. Experiments on various types of cell also show effects that are best accounted for by a heterogeneous distribution of Ca^{2+} channels [7].

Although theoretical work on Ca^{2+} dynamics has increased in recent years (reviewed in [8]), the spatially extended nature of the cell combined with the stochastic nature of localised calcium release and the heterogeneous distribution of Ca^{2+} stores has received far less attention (with the notable exception of the work of Falcke [9]). Recently, however, Coombes and Timofeeva have developed a mathematical framework to address these issues [10, 11]. The first prototype model developed in this framework is equivalent to a stochastic generalisation of the deterministic Fire-Diffuse-Fire (FDF) model of Keizer *et al*. [12]. The latter is a model of cardiac myocytes in which calcium release occurs via RyRs located in a regular array in the SR. Motivated by a reduction of the biophysically detailed De Young-Keizer model we have

also been able to formulate a version of the FDF model that describes IP_3 receptors [13]. Importantly our initial model is both analytically tractable and computationally cheap. Preliminary studies have already shown behaviour consistent with more detailed models of calcium release [10, 13].

Aims and Objectives. At its heart our proposal is based around developing a novel FDF framework that includes components of the calcium signalling toolkit relevant to understanding current experiments being performed in the molecular signalling laboratory of the Babraham Institute. Models within this framework will be used to investigate mechanisms of calcium signalling in spatially extended cell models in two and three dimensions. In particular we will address issues of enhanced sensitivity, mechanisms of pacemaker activity, the onset of wave failure and the enhancement of rhythmicity in rat atrial myocytes. Through an increased understanding of how this cell type gives rise to both natural and pathological calcium signals we will pursue experiments for the illustration of non-equilibrium phase transitions. This may provide the first experimental realisation of the critical exponents for the intensely studied directed percolation universality class in statistical physics. Essentially, we are attempting to marry diverse disciplines to provide a novel analysis of cellular calcium signals. Since this work is in its infancy, we have deliberately chosen to highlight achievable goals. Rather fortuitously the Nottingham Center for Mathematical Medicine currently (and for the next two years) will play host to Drs Timofeeva and Lemon. Both have made significant contributions to the body of work on FDF models [10, 14] and are keen to have an input on this project. This will promote the likely success of the early stages of this proposal and help to build a platform for more adventurous experiments and analysis, based around a significant dialogue between an RA at Nottingham and the experimental group at Cambridge. With this critical mass of active calcium researchers we feel that this project has the capacity to develop novel insights into cellular signalling.

Programme and Methodology. The methodology we propose to employ draws upon a number of established principles from different theoretical and experimental disciplines, but is predominantly from those of nonlinear dynamics, numerical analysis of stochastic and deterministic systems, computational cell biology, statistical physics and molecular signalling. We now provide more details of the methods of our proposed programme of research.

Development of the framework

The need to make links to experiments forces one to look for cell models that incorporate both the discrete nature of calcium stores and the stochastic nature of calcium release. Work by Coombes [15] on calcium waves in FDF models has focused on the former aspect and has recently been extended to cover the stochastic nature of calcium release [10]. FDF models use a threshold process to mimic the nonlinear properties of Ca^{2+} channels. The stochastic nature of release is incorporated via the introduction of threshold noise. This leads to a model with simple probabilistic update rules for the release of calcium from internal stores. By avoiding a Markov process description of channel gating we side-step the need for computationally expensive Monte Carlo type simulations. Moreover, the simplicity of the underlying deterministic FDF model can lead to further computational improvements. When considering a discrete set of release sites and calcium puffs that have a simple on/off temporal structure the calcium profile can be solved for in closed form. This obviates the need to numerically evolve a partial differential equation to obtain calcium profiles (at least for diffusive transport). The simplest model within this framework may be written in the form

$$u(\underline{r},t)=\sum_{n\in\Gamma}a_n(p)H(\underline{r}-\underline{r}_n,t-p\tau)+(G\otimes u_p)(\underline{r},t),\qquad p\tau<t<(p+1)\tau,\quad p\in\mathbb{Z}. \tag{1}$$

Here $u(\underline{r},t)$ represents the concentration of Ca^{2+} at a point $\underline{r}\in\mathbb{R}^l$ at time $t\in\mathbb{R}^+$ (and l is the physical dimension of the cell). The discrete set Γ indexes the stores located at (experimentally determined) positions $\underline{r}_n$ and τ is the temporal duration of a puff or spark. The $a_n(p)\in\{0,1\}$ are release indicator functions that act as coefficients in the expansion of the solution over a fixed set of basis functions $H_n(\underline{r},t)$. These basis functions have been obtained in closed form for the case that transport is via diffusion (in the presence of low affinity calcium buffers) in terms of the Green's function $G(\underline{r},t)$ of the diffusion operator. The second term on the right hand side of (1) represents a spatial convolution of the propagator $G(\underline{r},t-p\tau)$ with initial data $u_p(\underline{r})\equiv u(\underline{r},p\tau)$. Hence, the dynamics is naturally separated into a part that keeps track of release from internal stores and another that describes the spread of Ca^{2+} by diffusion. Note that (1) only has to be sampled in discrete time to fully specify cell behaviour since the basis coefficients remain unchanged over the duration of release. The probability that $a_n(p)=1$ is given in terms of the probability that $u(\underline{r}_n,p\tau)$ is bigger than

some threshold u_c. The inclusion of threshold noise allows us to write

$$P(u > u_c) = \int \rho(\xi)\Theta(u - u_c - \xi)d\xi = f(u - u_c). \tag{2}$$

where $\rho(\xi) = f'(\xi)$ describes the distribution of threshold noise (and Θ is a Heaviside function). Functional forms for the distribution of this threshold noise can be inferred from recent experiments and suggest that the probability of release per unit time has a sigmoidal form [16]. This leads to a discrete-time model with simple probabilistic update rules for the release of calcium from internal stores, whose main computational overhead (in evaluating $(G \otimes u_p)(\underline{r}, p\tau)$) can be alleviated with the use of Fast Fourier Transforms.

Here we identify some outstanding mathematical challenges associated with this approach. *Threshold determination*: By re-formulating the dynamics of detailed biophysical models of calcium release using the language of excitable systems we propose to identify a state-dependent barrier between active and passive states. This will allow the reduction of high dimensional receptor models to simple state and parameter dependent FDF thresholds (as we have previously done for the eight-state IP_3 receptor model [13]). *FDF waves in confined spaces*: It remains an open question to analyse FDF waves in fully three dimensions with lateral barriers to diffusion. This is especially relevant to the modelling of subsarcolemmal waves in atrial myocytes, where release sites are arranged along one-dimensional lines in a three-dimensional cell. By generalising the one-dimensional analysis in [15] and incorporating the appropriate inhomogeneous mixed boundary conditions this problem may be tackled using Fourier techniques along the lines described in [14]. *Nonlinear SERCA pumps*: Under normal physiological conditions pumps that are embedded in the membrane consume nucleotide trisphosphates and pump Ca^{2+} up the gradient from the cytosol to sequester it back into internal stores. As it stands the FDF framework incorporates only a linear model of this process (and is incorporated in the propagator $G(\underline{r}, t)$). The main difference compared to SERCA pumps is that the latter saturate. A common model of such a process is to consider a sink of the form $u^n/(K^n + u^n)$. A piecewise constant version of such a function (valid as $n \to \infty$) will be studied within the FDF framework, using appropriate matching conditions for dynamics on either side of a discontinuity. *Threshold noise distribution*: As well as using experimental data to determine the distribution of effective threshold noise it should be possible to derive these distributions from models of stochastically gated receptor channels. Using Fokker-Planck techniques recently presented in [17] and extending work by Coombes *et al.* [11], will allow us to re-formulate cluster dynamics in terms of stochastically forced excitable systems. We will then be in a position to consider how this stochastic forcing is transformed into threshold noise. *Distribution of release events*: The stochastic phase plane analysis used for addressing threshold noise is also ideally suited for establishing the distribution of release event duration. Once this is determined the discrete time update rule will be replaced by an iterated function system where τ is treated as a random variable (with known distribution). *Array enhanced coherence resonance*: Interestingly in a homogeneous FDF model with stores arranged on a regular lattice coherent motion in the form of simultaneous and periodic release of calcium from all stores can be induced purely by noise [10]. We shall use recent ideas of Naundorf *et al.* [18], on the dynamics of stochastic oscillator networks, to analyse this phenomena and probe its robustness in the presence of both heterogeneous and disordered distributions of stores. *Intercellular waves*: In layered cellular systems it is common to find intercellular waves that move via the sequential propagation of intracellular waves [8]. The spread of such waves is made possible by both direct cell-cell coupling through gap junctions which can allow the passage of both IP_3 and Ca^{2+} as well as the transport of extracellular messengers (and in particular ATP). The FDF framework will be extended to cover cell networks with the inclusion of internal boundary conditions that determine fluxes arising from gap junction coupling and diffusing extracellular messengers. Gap junctions may be mathematically described with terms that are linearly proportional to concentration gradients across cell boundaries, whilst extracellular signals will be modelled using the diffusion equation. The theory of intercellular wave propagation will be developed based around an extension of that previously developed for intracellular waves in [15] (that incorporates the new boundary conditions). Preliminary work in this direction may be found in [19].

The framework that we will develop is a general one that can handle many cell types. However, to capitalise on recent experimental progress in determining the precise spatiotemporal recruitment pattern of sparks in rat atrial myocytes [20] we shall focus on developing a model of this particular cell type. An important aspect of this cell that can naturally

be accommodated within our FDF framework is the separation of stores into subsarcolemmal junctional SR (j-SR) and central nonjunctional SR (nj-SR) classes. It is known that Ca^{2+} rise in atrial myocytes occurs at so-called *eager-sites* in the subsarcolemmal region followed by calcium-induced-calcium release (CICR) wave propagation into the deeper layers of the cell. It would appear that enhanced excitability of the eager-sites leads to a predetermined microscopic activation sequence of Ca^{2+} sparks whereby single cells produce reproducible inhomogeneous Ca^{2+} release upon depolarisation. Models of the VOCC channels (that mediate the entry of the electrical signal into the cell) will be developed using FDF voltage (rather than Ca^{2+}) dependent threshold functions (that once again we plan to derive from detailed biophysical models). Since eager-sites display the highest frequency of spontaneous Ca^{2+} sparks in resting cells we shall model the functional distinction between j-SR and nj-SR stores via both threshold noise and reduced thresholds. Both of these serve to enhance cell sensitivity. In particular we shall investigate how the geometry of release sites gives rise to nucleation phenomena. On the flip-side of this work we be able to probe the way in which the failure to recruit Ca^{2+} sparks appropriately can lead to defective excitation-contraction coupling in cardiac cells [21].

In addition to forming the global Ca^{2+} transient underlying contraction, Ca^{2+} sparks can also cause depolarisation of cardiac cells and thereby enhance or corrupt the rhythm of the heart. Incubation of electrically-paced atrial myocytes with the hormone endothelin-1 causes the appearance of spontaneous subsarcolemmal Ca^{2+} sparks [22], which are probably due to the activation of IP_3 receptors that co-localise with RyRs in these cells. The progressive increase in cytoplasmic Ca^{2+} caused by the summation of infrequent subsarcolemmal Ca^{2+} sparks promotes electrogenic forwardmode Na^+/Ca^{2+} exchange. Because of the strategic firing of subsarcolemmal Ca^{2+} spark sites, only a few such events may be necessary to create enough inward current to drive a cell to the threshold for depolarisation [23].

The ability of a few Ca^{2+} sparks to enhance cardiac automaticity (increase the frequency of spontaneous action potentials) has potentially serious implications for the generation of cardiac arrhythmias and sudden heart failure. Once again the FDF framework is ideally suited to probing the issue of spontaneous release from the SR from a theoretical perspective. In particular, we will consider the role of the dual presence of both RyR and IP_3 receptors and their spatial distribution in generating delayed after-depolarising (DAD) currents [24]. Moreover, a theoretical study of wave initiation and propagation will be compared to experiments where each receptor class is pharmacologically knocked out. One specific aim is to determine the conditions that separate subthreshold DADs (associated with a non-regenerative ring of Ca^{2+} around the cell) from suprathreshold DADs (associated with a ring of sparks that propagates to the deep cell layers).

Finally we propose to address the issue of directed percolation and its experimental realisation. Recent developments in non-equilibrium statistical physics have led to the upsurge of interest in phase transitions from fluctuating phases into absorbing states; the so-called universality class of directed percolation (DP) [25]. DP is the new testing ground of non-equilibrium statistical mechanics, much as the Ising model is for equilibrium statistical physics. Rather bizarrely values of the DP critical exponents have yet to be confirmed experimentally. According to the Janssen-Grassberger DP conjecture, any spatiotemporal stochastic process with short range interactions, fluctuating active phase and unique non-fluctuating (absorbing) state, single order parameter and no additional symmetries, should belong to the DP class. Since moderate changes in, say, external $[Ca^{2+}]$ can switch a cell from a saltatory wave propagating regime to a wave-blocking one [7] this suggests the exciting possibility that intracellular calcium dynamics could provide an experimental realization of the DP process [26]. In further support of this claim preliminary numerical simulations of a one dimensional stochastic FDF model (with a periodic distribution of release sites) has already illustrated that stochastic calcium release leads to the spontaneous production of calcium sparks that may merge to form saltatory waves. In the parameter regime where deterministic waves exist it has been possible to identify a critical level of noise defining a non-equilibrium phase-transition between propagating and abortive structures. A statistical analysis shows that this transition is the same as for models in the DP universality class [10]. We propose that the analysis of a cell model for calcium release and transport is a natural way in which to determine the critical levels of extracellular Ca^{2+}, and values of other controllable variables, necessary for an experiment to exhibit the types of abortive waves that would signal the onset of a DP phase transition. Cellular calcium signals will be visualized by loading cells with fluorescent calcium-sensitive indicators (e.g. Fluo4) using established methods. Images of cells loaded with fluorescent indicators will be acquired using a Noran Oz laser scanning confocal microscope (30-120 frames/s). The Laboratory of Molecular Signalling has considerable expertise in recording

cellular and subcellular calcium transients, and have previously characterized calcium puffs/sparks as well as event-to-wave transitions using this technology. For the purposes of this study, we will use cultured cells such as HeLa cells or human umbilical vein endothelial cells, since they have robust calcium signals that can be triggered by IP_3 generating agonists. In addition, we intend to examine calcium puffs in Xenopus oocytes. These cells have an advantage over smaller mammalian cells in displaying many calcium release sites in one focal plane. Furthermore, Xenopus oocytes are amenable to injection to allow precise control over cellular IP_3 levels and alteration of cellular buffering. Calcium sparks, calcium waves and arrhythmias will be studied using primary rat ventricular and atrial myocytes. These cells display spontaneous calcium sparks when bathed in millimolar extracellular calcium, due to a process of *post-rest potentiation*, which increases the calcium loading of the sarcoplasmic reticulum, thus sensitizing ryanodine receptors and triggering random spark firing. Action potential-evoked calcium transients will be generated using field electrodes. Analysis of calcium signals will be performed off-line with customized packages (ImageJ, NIH Image).

Timeliness and novelty. This work is timely because recent studies have demonstrated the importance of local Ca^{2+} signals in defining the specificity of the interaction of Ca^{2+} with its targets. Furthermore, sophisticated optical imaging techniques used in the Bootman lab have shown that local Ca^{2+} signals are the triggers and building blocks for larger global signals that propagate through cells. Importantly we are in a position to build upon early theoretical insights gained from the study of FDF models to understand how noise and heterogeneity can contribute to calcium signalling in spatially extended cells with discrete release sites. The novelty of this work is in the use of techniques not traditionally used in the mathematical biology community, such as non-equilibrium statistical mechanics. Moreover, preliminary studies have already suggested that natural sources of cellular noise can play a significant role in generating coherent intracellular signals [10]. As far as we are aware the issue of noise induced coherence and its role in cell signalling has yet to be seriously considered experimentally.

Management and milestones. Activity will be coordinated by Dr Coombes.
The RA will spend three years at Nottingham with three extended trips per year to the Babraham Institute. Initial work will focus on extending preliminary theoretical investigations to incorporate aspects of the calcium signalling toolkit relevant to experiments being performed in the Bootman lab. There will then follow a period of analysis and numerical exploration, focusing on a model of the rat atrial myocyte. Through an increased understanding of how this cell type gives rise to both natural and pathological calcium signals we will formulate experimental designs for the illustration of non-equilibrium phase transitions (of directed percolation type). Subsequent experiments will be performed in the Bootman lab. See the Gantt chart for the specific breakdown of tasks.

Relevance to beneficiaries. This work will establish a theoretical underpinning for how cells shape calcium signals in space, time and amplitude using components from a universal signalling toolkit. By considering the combined role of space, noise and heterogeneity in generating the variety of observed calcium signals we will be able to explore the mechanisms which allow a simple ion such as Ca^{2+} to play such a pivotal role in cell biology. Since Ca^{2+} is a ubiquitous intracellular messenger this will have impact on a diverse range of subjects including gene transcription, muscle contraction and cell proliferation. One specific application of this work will be to understanding the pathological behaviours of atrial myocytes that give rise to atrial arrhthymias. These are the most common form of long lasting cardiac arrhythmia, with a seven fold increased risk of stroke.
This work will also encourage a cross-fertilisation of ideas between the fields of computational cell biology and non-equilibrium statistical physics. The design of experiments to study the statistics of calcium waves in single cells may provide the first real world realisation of the critical exponents for directed percolation. Since directed percolation is the testing ground for many ideas about non-equilibrium phase transitions this is a potentially explosive subject area.

Dissemination and exploitation. We intend to raise the awareness of the benefits of this UK based multi-disciplinary collaboration with conference presentations at the annual UK Calcium Signalling Conference, the Annual Society for Mathematical Biology meeting, the SIAM conference on Life Sciences and the Gordon conference on Theoretical Biology (which Dr Coombes is co-organising in 2006). Papers will be submitted to multi-disciplinary journals such as the Biophysical Journal, Physica D, Journal of Physiology and the Journal of Mathematical Biology. Code will

be made freely available on the internet, and notices posted through UK Nonlinear News, the Society for Mathematical Biology and the Biophysical Society.

Justification of resources. Funding is requested for a 3 year RA1A (with a background in applied mathematics), for attendance at international and European conferences/workshops and for extended collaborative visits (several weeks per year) between the groups at Babraham and Nottingham. A high-spec dual 2.5GHz Power-PC has also been requested to perform the numerical simulations of models developed within the FDF framework.

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