Phantom Bursting Models and Complex Bursting Patterns in Pancreatic Islets

Specific Aims

(1) *Study the dynamics of, and make improvements to, a new mathematical model of the pancreatic β-cell.* This model has two novel features: a Ca^{2+} subspace compartment situated between the endoplasmic reticulum (ER) and the cell membrane, and a mechanism for bursting that is driven by two or more slow variables (Phantom Bursting).

(2) *Investigate potential mechanisms for complex bursting in pancreatic islets.* These oscillations consist of two types: fast bursts superimposed on medium oscillations, and fast and medium bursts clustered together to form slow waves of burst activity. Such oscillations are frequently observed in islets, and provide an assay for the multiple chemical and electrical pathways that interact within the β-cell.

(3) *Investigate synchronization properties of cells exhibiting complex bursting.* Whatever the mechanism for complex bursting in islets, it must be amenable to synchronization. One mechanism that we propose for complex bursting involves glycolytic oscillations. We will investigate whether synchronization of complex bursting oscillations involving glycolytic oscillations can be achieved through electrical coupling alone, and if not, whether an additional contribution from the gap-junctional diffusion of glycolytic intermediaries is sufficient to synchronize the oscillations. Both two-cell investigations and larger islet simulations (5x5x5 cube) will be performed.

(4) *Investigate the dynamic mechanism by which electrical coupling between phantom bursters attenuates noise and generates islet bursting.* In mathematical models, stochastic channel noise tends to disrupt regular bursting oscillations, possibly explaining why regular islet-like bursting is rarely observed in single cells. We have observed that electrical coupling attenuates these disruptions. Coupling also allows a second slow process to emerge, possibly by the same dynamic mechanism that attenuates noise. We will investigate the mechanism by which coupling regularizes and slows down bursting oscillations by performing a fast/slow bifurcation analysis of two coupled phantom bursting model cells with stochastic channel noise. We will also perform larger islet simulations to determine whether a collection of coupled noisy phantom bursting cells with single-cell activities distributed according to recent experimental data can generate regular islet-like bursting.

Background and Significance

A note on citations

NSF-supported papers, cited as (**Pub. #**), are listed in Results from Prior NSF Support.

Structure of the islet, major tasks, and modeling paradigm

Pancreatic β-cells secrete the hormone insulin in response to elevations in blood glucose. The cells are clustered into micro-organs called islets of Langerhans. Cells within an islet are electrically coupled in a nearest-neighbor fashion through gap junctions, resulting in a coordination of electrical behavior [1]. This coupling creates major challenges for both theoretical and experimental studies. One would like to study the properties of a single cell isolated from the islet, since the isolated cell is more amenable to biophysical techniques and the model system is simpler. However, single β-cells behave differently than β-cells in intact islets. Thus, two major tasks are involved in the study of islets. First, one must investigate how single β-cells work. Second, one must understand why the behavior of coupled cells is so different from that of a single cell.

Our ongoing mathematical modeling studies, done in conjunction with experimental studies in a collaborating lab, have focused on both tasks, since we believe that the two are interrelated. Our approach is to develop models that exhibit the proper single cell behavior, but are also endowed with mechanisms that allow the cells to exhibit islet behavior under the proper conditions, such as (but not limited to) when electrically coupled. These studies are done in collaboration with Drs. Leslie Satin, Arthur Sherman, and Keith Tornheim (see Nature of the Collaboration).

Why study islet bursting?

The earliest *in vitro* electrical recordings from mouse islets demonstrated that they burst, a behavior characterized by an active phase of spiking followed by a silent phase where the membrane is repolarized [2]. Subsequent studies show that the burst plateau fraction, the ratio of active phase duration to total period, is an increasing function of the bath glucose concentration [3]. Electrical impulses bring Ca^{2+} into the cells, which evokes the secretion of insulin, so the larger the plateau fraction the greater the rate of insulin secretion. Thus, electrical bursting is responsible for the transduction of glucose concentration to the proper level of insulin secretion. Later studies have shown that islets burst *in vivo*, demonstrating that this patterned spiking is not an artifact of islet extraction [4, 5]. Also, there is a great deal of evidence that insulin secretion is pulsatile [6-8]. Oscillatory levels of insulin appear to be the norm, and loss of this oscillatory behavior is linked to type II diabetes [9]. Thus, the electrical bursting that leads to oscillatory insulin secretion is a key physiological response to elevations in blood glucose.

Islet as a model system

Understanding the islet has clinical motivations, since defects in insulin secretion are a major factor in type II or late-onset diabetes [10]. The islet is also of interest biophysically as a model system for studying bursting oscillations, biochemical signaling, and network behavior of an electrically coupled system. Pancreatic β-cells share many properties with neurons, including commonalities in electrical properties and Ca^{2+} handling. The β-cell also has many biochemical pathways that are reflected in the electrical and Ca^{2+} dynamics, both of which are measurable. Since many of these pathways also play a role in other endocrine cells and in nerve cells, much of what is learned about β-cells has general applicability. The islet has motivated a number of mathematical studies ([11-17], **Pub. 1**) and thus has intrinsic mathematical interest.

Single-cell and islet behaviors

Isolated β-cells typically exhibit one of four behaviors ([18], [19], [20], **Pub. 1**). Three classes of cells were described in a study by the Satin lab [18]. Class I cells spike continuously (33% of the 52 cells studied). Class II cells are fast bursters, with period < 5 sec (52%). Class III cells produce periodic depolarized plateaus, without recognizable impulses (15%). We have further characterized these three classes of cells in terms of their Ca^{2+} dynamics, and have shown that the behaviors can be reproduced by our recent Ca^{2+} Subspace Model if channel noise is included (**Pub. 9**). Single β-cells can also exhibit a very slow bursting rhythm, with corresponding slow Ca^{2+} oscillations [19, 20]. In contrast, β-cells in intact islets typically exhibit a bursting oscillation with period of 10-60 sec. Often the bursting is complex, with fast bursts superimposed on slower oscillations (**Pub. 9**), or with fast and medium bursts clustered together on a yet slower wave of activity [21, 22]. We have recently shown that blocking gap junctions in islets greatly increases the fraction of islets that exhibit fast bursting or continuous spiking (**Pub. 11**). These results are consistent with our modeling work, which predicts that islets will behave more like single cells when gap junctions are blocked.

The Phantom Bursting model

The majority of prior β-cell models were developed to describe bursting in islets. That is, the single-cell model represents the "average" cell in a synchronized islet. These models have a common dynamic structure, with two or more "fast variables" for the generation of impulses, and a single "slow variable" that is the pacemaker for bursting. The slow variable was originally thought to be free cytosolic Ca^{2+} [23, 24], but as data accumulated alternative slow variables were postulated. These included voltage-dependent inactivation of Ca^{2+} channels [25, 26], the ATP/ADP ratio acting on K(ATP) channels [27, 28], and ER Ca^{2+} acting through CRAC channels [29]. (ATP=adenosine trisphosphate; ADP=adenosine diphosphate.) However, the actual time constants for the different postulated slow variables are inconsistent with the 10-60 sec burst period of islets. The cytosolic Ca^{2+} concentration and the voltage-dependent inactivation of Ca^{2+} current both change with time constants of a few seconds [26, 30, 31], while the ATP/ADP ratio and the ER Ca^{2+} concentration (Ca_{er}) typically change with time constants of several minutes [7, 32-35].

The phantom bursting model was developed to overcome this problem and to account for the discrepancy between single-cell and islet behavior (**Pub. 1**). Here there are two (or more) slow variables, s_1 and s_2, and the time constant for s_1 is smaller (1-2 sec) than that for s_2 (1-2 min). For some parameter values, bursting is driven by s_1 alone (fast bursting, as in class II cells), for other parameter values bursting is driven by s_2 (slow bursting), and for intermediate parameter values the bursting involves oscillations in both s_1 and s_2 (medium islet-like bursting). Thus, phantom bursting models are capable of producing the entire range of burst periods observed in isolated β-cells and in islets.

Our minimal phantom bursting model consists of two fast variables, the voltage V and a K^+ channel activation variable n, and two slow variables, s_1 and s_2, which are assumed here to be activation variables for the hyperpolarizing K^+ currents I_{s1} and I_{s2}:

$$\frac{dV}{dt} = -(I_{Ca} + I_K + I_{s1} + I_{s2} + I_L)/C_m \qquad (1)$$

$$\frac{dn}{dt} = (n_\infty(V) - n)/\tau_n \tag{2}$$

$$\frac{ds_1}{dt} = (s_{1\infty}(V) - s_1)/\tau_{s1} \tag{3}$$

$$\frac{ds_2}{dt} = (s_{2\infty}(V) - s_2)/\tau_{s2}\,. \tag{4}$$

Results from prior NSF support

(Award #9981822, "Modeling and Analysis of Multimodal Bursting in Pancreatic β-Cells")

Publications supported by NSF

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4. Bertram, R. (2001) *Differential Filtering of Two Presynaptic Depression Mechanisms*, Neural Computation, 13:69-85.
5. Bertram, R., M. I. Arnot, G. W. Zamponi (2002) *Role for G Protein Gβγ Isoform Specificity in Synaptic Signal Processing: A Computational Study*, Journal of Neurophysiology, 87:2612-2623.
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12. Bertram, R., A. Sherman (2002) *A Modeling Study of Ca^{2+} Handling in Pancreatic β-cells*, Biophysical J., submitted.

13. Quine J. R., R. Bertram, M. Chapman, T. Cross (2002) *Mathematical Aspects of Protein Structure Determination with NMR Orientational Restraints*, in preparation.
14. Bertram, R., A. Sherman (2002) *Emergent Phantom Bursting Through Diffusive Coupling*, in preparation.

Note: **Pub. 3, 6, 7,** and **13** focus on computational structural biology, and are not related to the focus of prior NSF support. In all cases NSF funding was acknowledged.

Dynamics of Phantom Bursting

Figure 1 illustrates phantom medium bursting. Panels B and C show the burst trajectory superimposed on the slow manifold in the V-s_1 phase plane. (The slow manifold is the set of points where $\dot{V} = 0$ and $\dot{n} = 0$, with s_2 constant and s_1 treated as a bifurcation parameter.) Also shown is the s_1 nullcline (dotted). In a "classical" bursting model, the nullcline only intersects the middle (unstable) branch of the slow manifold, and the trajectory cycles in a clockwise direction around the bottom (stable) branch and the periodic branch [12]. The bottom branch, providing the silent phase, ends with a saddle node bifurcation; the periodic branch, providing the active phase, ends with a homoclinic bifurcation (diamonds). The period of bursting is determined by the s_1 time constant, and the bursting proceeds unaltered when s_2 is clamped at its average value. In phantom bursting, the nullcline intersects the periodic branch during the active phase (panel B). Here the phase point stalls (white ball) as s_2 slowly increases, sliding the slow manifold to the left (arrow) and eventually releasing the phase point when the homoclinic slides past the nullcline. Similarly, the nullcline intersects the bottom branch of the slow manifold during the silent phase (panel C), and the phase point stalls until s_2 decreases sufficiently to release it. Phantom bursting requires oscillations in both s_1 and s_2, so the period depends on τ_{s1} and τ_{s2}, and can range from seconds to minutes (**Pub. 1)**.

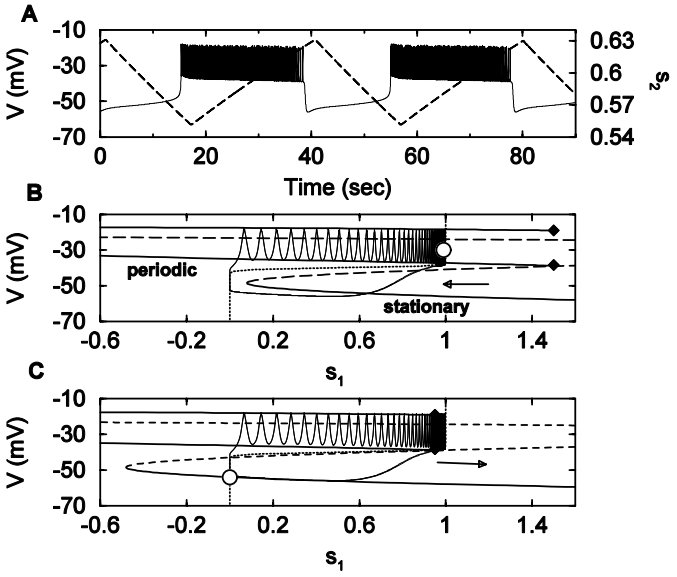

Figure 1: Phantom bursting requires oscillations of both s_1 and s_2. From **Pub. 14**.

Conversion from fast to medium bursting with D-clamp

With the phantom bursting model one can change the burst period by changing the conductance of the s_1 current, g_{s1}. With large g_{s1} the bursting is controlled by s_1, and is therefore fast. As g_{s1} is reduced s_1 must rise to larger values to shut off the spiking, and eventually it can rise no further and the phase point stalls in the active phase until a slow rise in s_2 allows it to escape. Thus, the burst period increases as g_{s1} is decreased. This is a prediction of the model that cannot be tested directly since pharmacological agents that block candidate s_1 currents affect other currents. An alternative approach is to add a current to the cell that activates with a time constant similar to that of the suspected s_1 current, but with opposite polarity. With this approach, the model predicts that one should be able to convert fast bursting in a single β-cell into a slower bursting pattern, such as one observes in intact islets (Fig. 2, top). The Satin lab tested this prediction using a dynamic clamp (D-clamp), which differs from standard current clamp in that the current injected is based on the calculated response of a hypothetical voltage-dependent conductance to cell membrane potential at each instant in time [18, 36]. This D-clamp protocol was able to convert spiking or fast bursting single cells into medium bursters (Fig. 2, bottom). Similar conversions have been made in over 40 cells. Attempts at D-clamp conversion to medium bursting prior to the development of the Phantom Bursting model were unsuccessful; the Phantom Bursting model suggested the correct form of the current to be applied under D-clamp (i.e., a depolarizing current with activation time constant of ~1 sec). These data provide the first examples of medium bursting in single β-cells, and they support the existence of a slow process in single β-cells. This work was reported in **Pub. 1,** and was also recently demonstrated with Ca^{2+} fluorescence imaging, and reproduced with the Ca^{2+} subspace model (**Pub. 9**).

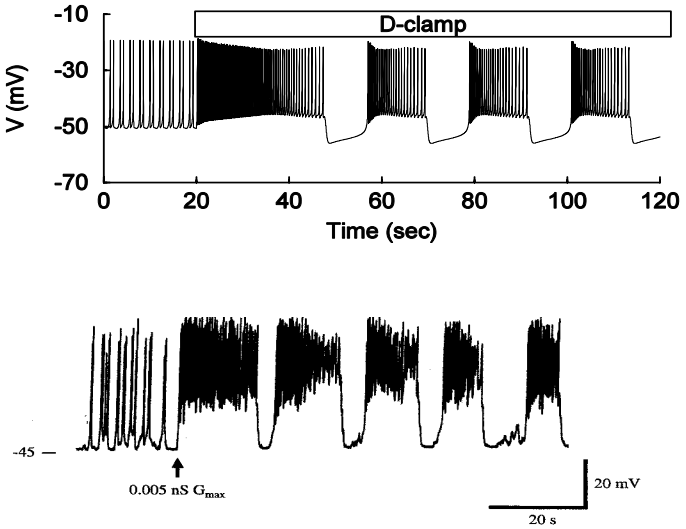

Figure 2. Conversion from fast spiking to medium bursting. (top) Prediction from a phantom bursting model. (bottom) Validation of prediction using D-clamp. From **Pub. 9**.

The Ca^{2+} subspace model

The D-clamp conversion is one example where modeling motivated a new experiment. The converse was true in the development of the *Ca^{2+} subspace model*. In an attempt to isolate the slow process driving islet bursting, the Rorsman lab developed a voltage

clamp protocol in which the cell is depolarized to –40 mV (mimicking the "voltage plateau" during a burst) and then short action potentials are simulated (depolarizations to 0 mV), followed by a return to –40 mV. Ionic current recorded during this protocol showed a slow buildup of a Ca^{2+}-dependent current carried by K^+, called $I_{K(slow)}$ [30]. A similar "pulse protocol" was used by the Satin lab to study the effects of the drug thapsigargin (Tg), which blocks Ca^{2+} pumps (SERCA pumps) in the endoplasmic reticulum (ER). The Ca^{2+} concentration is normally much higher in the ER than in the cytosol, but when the SERCA pumps are blocked the ER Ca^{2+} concentration (Ca_{er}) slowly declines. The Satin lab found that when Tg was applied the $I_{K(slow)}$ current first increased in magnitude, but after several minutes (as the ER presumably continued to drain) $I_{K(slow)}$ disappeared (Fig. 3A, B). The same behavior was found to occur when insulin was added to the bath. (Insulin reduces the ER Ca^{2+} concentration [37, 38].)

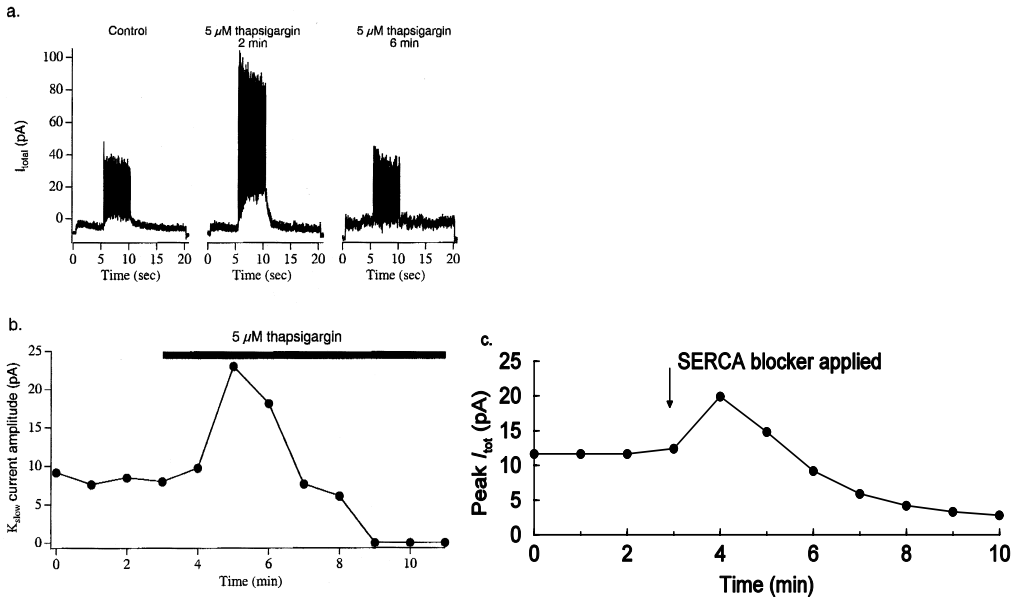

Figure 3. Thapsigargin first increases, then decreases $I_{K(slow)}$. (**A, B**) Experimental data from a single β-cell. (**C**) Modeling results using Ca^{2+} subspace model. From **Pub. 8**.

Our initial attempts to reproduce this Tg- or insulin-induced biphasic behavior of $I_{K(slow)}$ were unsuccessful. The models used here were not minimal phantom models, but models that contained the standard currents and variables known to be important in β-cells, including the cytosolic and ER Ca^{2+} concentrations, a Ca^{2+}-activated K^+ current ($I_{K(Ca)}$), and K^+ and Ca^{2+} currents. In all attempts the model $I_{K(slow)}$ (the K(Ca) current) increased following simulated Tg application, but did not subsequently decrease. This motivated a re-evaluation of Ca^{2+} handling in the β-cell, leading to a model in which Ca^{2+} released from the ER enters a "subspace" located between the ER membrane and the cell's plasma membrane (Fig. 4). Because of this preferential Ca^{2+} influx from the ER the Ca^{2+} concentration in the subspace is elevated above that of the bulk cytosol. A key assumption made is that the K(Ca) channels sense Ca^{2+} from the subspace. With this *Ca^{2+} subspace model* we were able to reproduce the biphasic response of $I_{K(slow)}$ to Tg or insulin (Fig. 3C). Further evidence for the existence of a Ca^{2+} subspace is provided by

Ca^{2+} fluorescence data showing that the Ca^{2+} concentration during prolonged depolarization (i.e., steady state) is reduced in the presence of Tg [39]. Without a subspace, the steady-state concentration would be the same with or without Tg (this is discussed in **Pub. 12**). (If draining of the ER activates a Ca^{2+}-release activated current (CRAC), then the cytosolic concentration would be *higher* following Tg application.)

Insulin feedback model

The Satin lab has demonstrated that extracellular insulin activates K(ATP) channels, hyperpolarizing β-cells [40]. With the aid of an undergraduate student, Keola Wierschem, we developed a model for this (**Pub. 10**). Data show that feedback is slow (average of 3.7 minutes required to hyperpolarize single cells), so in our model the fraction of K(ATP) channels activated by insulin acts as an s_2 variable, while the Ca^{2+} concentration acts as s_1. We demonstrated that when insulin is rapidly removed from the neighborhood of the model cell the cell can either spike continuously or produce fast bursting. When removal is slower the bursting has a "medium" period of tens of seconds. The islet structure poses a hindrance to diffusion, and if one assumes that insulin removal is slower in islets than in studies involving single cells (where the bath is rapidly exchanged), then insulin feedback may help explain the difference in burst period between single cells and islets. This model will be tested by the Satin lab using insulin receptor blockers.

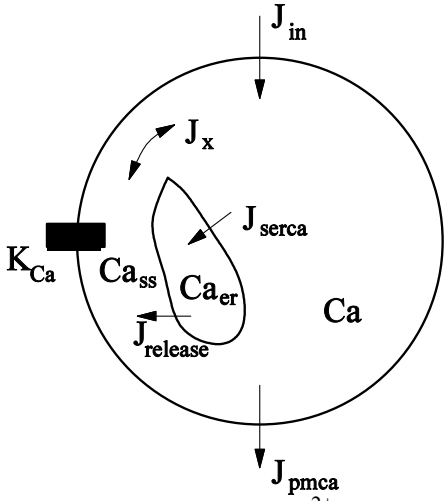

Figure 4. The Ca^{2+} subspace model. J represents Ca^{2+} flux. Differential equations describe the cytosolic (Ca), ER (Ca_{er}) and subspace Ca^{2+} concentrations (Ca_{ss}).

Broader impact of prior NSF supported work

We have made great progress over the past few years in understanding how β-cells work. This progress has been driven by the close collaboration between mathematical and experimental labs, and will benefit both mathematical and biological communities. The majority of our β-cell work conducted during the past three years is currently being written up or has recently been submitted (**Pubs. 9, 10, 11, 12, 14**). We expect that publication of this work will have a large impact on the way that the community interprets the behavior of islets and single β-cells, and will motivate new experiments and mathematical studies of bursting oscillations.

Prior support was through an RUI grant, so student assistants working directly with the PI on the project were undergraduates (and one high school student). Jessie Swanson was an undergraduate math student when she started; she recently completed her Master's thesis with the PI. Kelsey Mayo was a high school junior who spent one summer working on a project involving the effects of creatine kinase on ATP/ADP dynamics. Mandy Swann was a senior math student who spent one semester analyzing a model for glycolytic oscillations. Keola Wierschem is a physics senior whose work has focused on a potential role for insulin feedback on bursting in β-cells, and is included in **Pub. 10**. Keola is currently working on a model of glycolytic oscillations in β-cells.

Research Plan

Aim 1: Further development and analysis of the Ca^{2+} subspace model

Elements of the model

Fast ionic currents: I_{Ca}, I_K, I_{leak}
Slow ionic currents: $I_{K(Ca)}$, $I_{K(ATP)}$
State Variables: V, n, Ca, Ca_{er}, Ca_{ss}, ADP, ATP

Fig. 4 illustrates the Ca^{2+} fluxes and location of K(Ca) channels. The slow ionic currents have the forms:

$$I_{K(Ca)} = g_{K(Ca)}\left(\frac{[Ca_{ss}]^8}{[Ca_{ss}]^8 + 0.7^8}\right)(V - V_K) \tag{5}$$

$$I_{K(ATP)} = g_{K(ATP)}\psi(ADP, ATP)(V - V_K) \tag{6}$$

where $\psi(ADP, ATP)$ is a function of the ADP and ATP concentrations in the β-cell. Depending on implementation, this may be a simple function of the ratio ATP/ADP, or a more complicated function of ADP and ATP (see below). ATP and ADP may be fixed (as in the original subspace model), or may vary depending on the intracellular Ca^{2+} concentration, or may vary due to oscillations in glycolysis. We will consider each.

Mechanism of bursting and Ca^{2+} feedback on metabolism

The Ca^{2+} subspace model is an example of a phantom bursting model, where the subspace Ca^{2+} concentration (Ca_{ss}) plays the role of the faster s_1 variable, while ER Ca^{2+} (Ca_{er}) plays the role of the slower s_2 variable. The Ca_{er} affects Ca_{ss}, which affects membrane potential through the K(Ca) current. Also important in β-cells is an ATP-sensitive current, $I_{K(ATP)}$. The conductance of this current is regulated by the glucose concentration through its dependence on ATP and ADP, or more precisely, the ATP/ADP ratio [41]. In the original version of the Ca^{2+} subspace model these concentrations were constant. However, recordings of oxygen consumption, nucleotide concentrations, or mitochondrial membrane potential indicate that ATP, ADP, and the ATP/ADP ratio, vary in time with period ranging from ≈1 min to nearly 10 min [7, 42-44]. These oscillations are likely due to oscillations in the intracellular Ca^{2+} concentration, which affects the mitochondrial membrane potential and thus the production of ATP from ADP [42, 45-48], or due to oscillations in glycolysis [7]. This additional slow oscillation increases the complexity of the model, since there are now three slow processes (Ca_{ss}, Ca_{er}, ATP/ADP) that may interact to produce bursting.

We propose to extend the Ca^{2+} subspace model by including Ca^{2+}-dependent nucleotide oscillations (we refer to this as the *extended Ca^{2+} subspace model*). The mathematical model for these oscillations will be based on the work of Magnus and Keizer, who developed a detailed model of mitochondrial ATP production as a function of cytosolic and mitochondrial Ca^{2+} concentrations. Magnus and Keizer chose parameters so that ATP and ADP concentrations respond rapidly to changes in Ca^{2+} concentrations, and thus Ca^{2+}-dependent oscillations in the ATP/ADP ratio are sufficiently rapid to drive electrical bursting with a 15 sec period. However, data suggest that oscillations in ATP/ADP typically occur on a slower time scale [7, 20, 35, 42, 43, 49]. We will adjust parameters so that the mitochondrial response to changes in cytosolic Ca^{2+} is slower, making it impossible for ATP/ADP oscillations alone to drive 15 sec islet bursting. However, this slower feedback can in principle interact with a faster variable, such as Ca_{ss}, to drive medium islet-like bursting. It can also compete with the ER Ca^{2+} concentration, and one or the other (or both) may play the s_2 pacemaking role described in the phantom bursting model.

Fast/slow bifurcation analysis of the extended Ca^{2+} subspace model

The extended Ca^{2+} subspace model has some interesting mathematical properties. In one region of parameter space oscillations in the ATP/ADP ratio are unimportant for bursting (the ratio can be clamped without affecting bursting). In other parameter regimes ATP/ADP oscillations are absolutely essential. This postulated redundancy in slow processes can explain data showing that bursting persists when either (but not both) the K(ATP) conductance or Ca_{er} is effectively clamped through pharmacological treatments [50, 51]. We will perform a fast/slow bifurcation analysis of the extended model to understand how the role of the s_2 variable is handed from Ca_{er} to ATP/ADP as one traverses parameter space. This will help us understand how 3 slow variables share the roles of burst pacemakers, and how these roles are transferred from one slow process to the other. The importance of this analysis extends beyond the study of β-cells, since bursting in many neurons likely involves more than one slow process.

Aim 2: Investigate mechanisms for complex bursting in islets

Pancreatic islets generate at least two distinct types of complex bursting. *Type 1 complex bursting* consists of fast bursts superimposed on a slower oscillation in membrane potential (**Pub. 9**). A Ca^{2+} recording of an islet exhibiting this behavior is shown in Fig. 5. *Type 2 complex bursting* is characterized by clustering of fast or medium bursts [21, 22, 52]. Both types occur somewhat frequently, and they provide a glimpse into the interacting biochemical and electrical oscillations that take place within β-cells. Models of potential pathways can then be tested by their ability to reproduce consistent features of complex bursting.

Type 1 complex bursting through electrical coupling

We have demonstrated previously that type 1 complex bursting can be produced by electrical coupling between two phantom bursting models (**Pub. 14**). This was shown, and analyzed using fast/slow bifurcation analysis, using minimal models (Eqs. 1-4). We will investigate whether this behavior can be produced by coupling together two model cells described by the Ca^{2+} subspace model. We will also perform islet simulations with a

5x5x5 cube of model cells. The aim is to understand whether this bursting pattern can be produced by our models in a robust way. If so, it will demonstrate that the behavior can be produced as an emergent property of the network. Also, it will provide further support for the phantom bursting mechanism, since coupled "classical" bursters do not produce this pattern. If the complex pattern is not produced robustly, then we will investigate whether it can be produced via an intracellular, rather than network, mechanism.

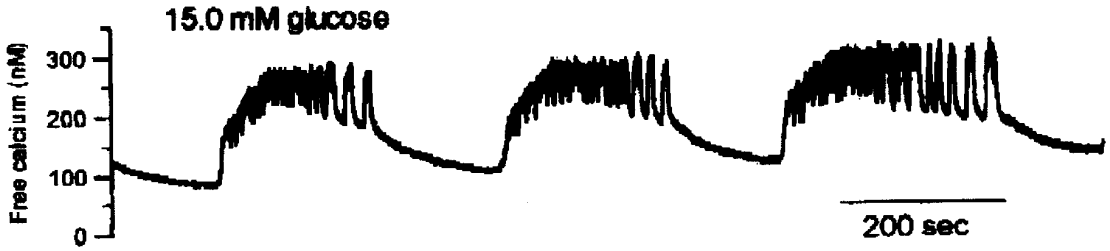

Figure 5. Islet Ca^{2+} recording showing type 1 complex bursting oscillation. From **Pub. 9**.

Type 2 complex bursting through glycolytic oscillations

Glycolysis, the first step in the metabolism of glucose, has been observed to oscillate in yeast, muscle extracts and β-cells [7, 35, 53-56]. This raises the possibility that glycolytic oscillations may drive or modulate islet bursting [57]. Indeed, clinical tests have shown that subjects deficient in phosphofructokinase, a key enzyme in glycolytic oscillations, have impaired pulsatile insulin secretion [58]. We postulate that type II complex bursting is due to slow glycolytic oscillations in ATP/ADP superimposed with Ca^{2+}- and V-dependent oscillations of ionic current. Thus, we propose that if the ATP/ADP level were fixed, a regular medium burst pattern would be produced due to oscillations in Ca_{ss} and Ca_{er}, for example. When ATP/ADP is allowed to vary in time due to Ca^{2+}- and V-independent glycolytic oscillations, this has the effect of packaging the bursts into clusters. We will investigate this hypothesis using two models of glycolytic oscillations, one detailed and the other minimal. The minimal model is the 2-variable model (ATP and ADP) of Goldbeter and Lefever, which is simple but captures qualitative features of glycolytic oscillations [59]. The detailed model is based on that of Smolen [60], and accounts for the important interactions of glycolytic intermediaries with phosphofructokinase (Fig. 6). This allosteric enzyme is crucial for glycolytic ATP production and the production of substrates for downstream metabolic processes.

The motivation for using two different models in this study is that the simple model is more amenable to mathematical analysis, but the detailed model is necessary to make testable predictions and to calibrate with β-cell data (provided by the Tornheim and Satin labs, or obtained from the literature). Thus, we propose a parallel approach. With the simple model we will investigate the dynamics of an intrinsic slow glycolytic oscillation coupled with a minimal phantom bursting model (Eqs. 1-4), applying phase plane and fast/slow bifurcation analysis. We will contrast this with a glycolytic oscillation coupled to a simple "classical" bursting model (e.g., the Chay-Keizer model [24]). Are there characteristics of type 2 complex bursting (e.g., systematic variation of burst period) that can be captured by one simple system, but not the other? For the detailed model, the Smolen glycolytic model will be coupled to the Ca^{2+} subspace model, both with and without Ca^{2+} feedback onto mitochondrial ATP production. How does the Ca^{2+} feedback

affect the resulting bursting? Which characteristics of complex bursting can be captured with one of these minimal models, and which require the more detailed model? Can the detailed model account for recent (unpublished) data from the Satin lab that removal and reapplication of glucose can convert slow bursting islets to medium bursters?

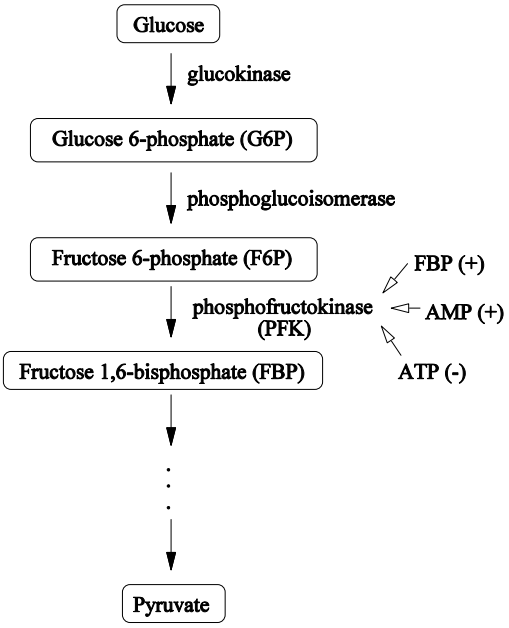

Figure 6. The first few steps of glycolysis, focusing on positive and negative regulators of PFK. The Smolen model contains equations for G6P, FBP, and ATP.

Aim 3: Study the synchronization properties of cells exhibiting complex bursts

Ca^{2+} fluorescence data of islets and electrical recordings from a cell within an islet provide different information about islet activity. The fluorescence measurements are indicators of the spatial average of the Ca^{2+} concentration, while the electrical recording is a local indicator of the membrane potential in a single cell (that is coupled to other cells). Thus, it is possible that an electrical recording may not reflect the dynamics of the islet. However, simultaneous Ca^{2+} and membrane potential measurements have shown a great deal of synchrony between the two ([31], **Pub. 9**), suggesting that the islet operates as a single synchronized unit. Data also show that complex bursting patterns of both types are associated with complex Ca^{2+} oscillations ([52], **Pub. 9**). This indicates that complex bursts are (in these cases) synchronized throughout the islet; otherwise the complex behavior would be smeared out of the Ca^{2+} recording.

Synchronization of type 1 complex bursting

If our hypothesis is correct, that type 1 complex bursting is an emergent property of coupling between phantom bursters, then one would naturally expect a complex Ca^{2+} pattern. It is not clear, however, that the model Ca^{2+} traces will have features similar to the Ca^{2+} data (e.g., Fig. 5). The Satin lab has a great deal of data of this type, and from this we will establish characteristics that are shared by the majority of the cells in the sample. We will compare this data set with simulated Ca^{2+} traces from our coupled

system of Ca^{2+} subspace models (see Aim 2). Requiring that both V and Ca^{2+} match experimental recordings will greatly constrain the model and further test the hypothesis.

If the hypothesis is incorrect, and electrical coupling between Ca^{2+} subspace models does not robustly produce type 1 complex bursting (see Aim 2), then we will investigate whether a process intrinsic to the individual cells can be synchronized through electrical coupling. A discussion of this is below, in the context of type 2 complex bursting.

Synchronization of type 2 complex bursting

It is our hypothesis that type 2 complex bursting involves a glycolytic oscillation, in which case it follows that oscillations produced by this mechanism must be amenable to synchronization. However, it is not clear that this will be possible. The glycolytic oscillation is thought to be independent of membrane potential and Ca^{2+}, so electrical coupling will not directly influence the oscillation. How will oscillations in different cells (with potentially different phases and/or periods) become synchronized? Is synchronization of the glycolytic oscillations even necessary, or can coherent V and Ca^{2+} oscillations be produced even though glycolytic oscillations are unsynchronized? We will investigate these questions first using electrical coupling alone, applied to both minimal and detailed β-cell models. If we find that electrical coupling alone is insufficient to synchronize the electrical and Ca^{2+} oscillations, then we will investigate whether diffusion of glycolytic intermediaries such as G6P, FBP, or ATP (Fig. 4) through gap junctions can provide, along with electrical coupling, the required synchronizing factor. This study will provide a test of the plausibility of glucolytic oscillations as a mechanism for type 2 complex bursting, and thus address the question of whether glycolytic oscillations (when they occur) play a functional role in the islet.

Aim 4: Investigate coupling of noisy phantom bursters

Emergent medium bursting

We have recently completed a mathematical study of the effects of electrical coupling between two phantom bursters, using minimal models (**Pub. 14**). This study revealed the great degree of dynamic flexibility that exists in this system, much greater than had previously been reported for coupled "classical" bursters with a single slow process [14, 16, 61]. In **Pub. 14** we showed that two identical phantom bursters in the fast bursting mode can produce medium islet-like bursting when coupled, and if a fast burster is coupled to a slow burster or a continuous spiker than medium bursting or type 1 complex bursts can be produced, depending on the coupling conductance. In both cases, the coupling allowed the influence of the second slow process (s_2) to emerge, while the faster s_1 process dominated when the cells were uncoupled. We find these results very exciting, since these transformations from single-cell-like behavior to islet-like behavior could not be achieved with prior classical bursting models.

Regularization due to noise attenuation

The analysis mentioned above may explain how fast bursters and continuous spikers produce medium bursting when coupled, but it does not explain two other aspects of the data. First, why is medium bursting almost never observed in single cells? With phantom bursting model cells, one predicts that there is a parameter regime where this behavior should be observed in single cells. Second, why is the single-cell activity noisier than

islet activity? This noisy behavior is apparent in several experimental studies ([18], **Pubs. 9, 11**). We postulate that both of these features can be explained by channel noise, and the attenuating influence of coupling on channel noise. We will investigate this hypothesis using the Ca^{2+} subspace model with stochastic K_{ATP} channels.

Preliminary work with the Ca^{2+} subspace model indicates that medium islet-like bursting is not achieved since stochastic channel openings rapidly reset the oscillation, yielding an irregular fast bursting pattern. Thus, even though the deterministic model may be in the correct parameter regime for medium bursting, the magnitude of the channel noise is too great to allow the full oscillation to be achieved. Prior mathematical studies have shown that electrical coupling attenuates noise by increasing the total membrane area [15, 62]. However, these studies used classical bursting models. The dynamics of phantom bursting models, as exemplified by the Ca^{2+} subspace model, are much richer. As a consequence, the mechanism by with coupling attenuates noise may be different with these models. Preliminary studies indicate that coupling stretches the slow manifold of the system. Some stretching of the slow manifold is allowed in classical bursting models, but too much stretching leads to continuous spiking or a hyperpolarized steady state solution. In a phantom bursting model, stretching of the slow manifold allows oscillations in the slower s_2 variable to emerge. At the same time, with a stretched slow manifold the system is more resistant to resetting via channel noise, and will be more regular. Mathematical studies of this behavior are preliminary, and we propose to conduct a complete fast/slow bifurcation analysis using two coupled cells. We also propose to perform islet simulations using a 5x5x5 cube of stochastic cells, to investigate whether a collection of cells with electrical activity distributed as described in experimental studies from the Satin lab ([18], **Pub. 9**) can generate medium islet-like bursts.

Computer Resources

The PI uses laptop and desktop PCs, and a Sun workstation for all computations. Computers are equipped with MAPLE, MATLAB, and XPPAUT mathematical software. The PI also has access to an IBM SP3 supercomputer, which may be used in coupling studies involving 5x5x5 systems of model cells. Students have access to 12 Sun workstations. Funds are requested for a linux-based PC for graduate student use.

Nature of the Collaboration

The PI collaborates with Dr. Arthur Sherman at the Mathematical Research Branch of the National Institutes of Health and Dr. Leslie Satin at the Medical College of Virginia. This collaboration, now entering its fourth year, combines mathematical modeling and analysis (conducted by the PI and Dr. Sherman) with experiments (conducted by the Satin lab). The collaborators discuss issues relating to the project on a weekly basis by e-mail and telephone. In addition, there are biannual meetings at Dr. Satin's lab to discuss the modeling and examine data, and to plan new experiments and modeling efforts. The group also meets and gives presentations at the annual American Diabetes Association conference. Dr. Keith Tornheim, of the Boston University School of Medicine has agreed to collaborate on issues involving glycolytic oscillations. Dr. Tornheim will consult with the PI, contribute data, and will perform experimental tests of model predictions.

Broader impact of the proposed work

Impact of research on basic science

The work described in this proposal will be of interest to two target audiences. Experimental labs in the US and elsewhere are interested in many of the questions raised in this proposal, so our choice of topics along with the close collaboration with experimental labs will get the attention of the experimental islet community. The mathematical analysis of phantom bursting and the effects of electrical coupling will be of interest to those who study bursting oscillations in endocrine cells and neurons, since phantom bursting is a general mechanism that may apply to cells other than β-cells. Likewise, the concept of a Ca^{2+} subspace may apply to other cells, and indeed has been proposed in ventricular myocytes [63]. Finally, our studies of diffusively coupled bursting oscillators complement mathematical studies of coupled oscillators.

Dissemination of results

Results from this study will be disseminated at conferences and workshops. The PI typically presents scientific results at annual meetings of the Biophysical Society, the American Diabetes Association, and the Society for Mathematical Biology, and at SIAM meetings. In addition, the PI has presented work at workshops held at the Institute for Mathematics and its Applications in Minnesota and the Pacific Institute of Mathematical Sciences, and will present work this year at the Mathematical Biosciences Institute and at a symposium on mathematical neuroscience at the southeast AMS meeting (the PI is a co-organizer of the symposium). Results are also published in peer-reviewed scientific journals, and free computer codes are distributed to the scientific community.

Training opportunities

Prior NSF support was through an RUI grant, awarded while the PI was at Penn State Erie, a 4-year college. After the PI moved to Florida State University (a Research I university), funds from the grant were used to support undergraduate research (see Prior NSF Support). With this proposal, the PI is asking for financial support for a graduate student and an undergraduate student. The PI is an assistant professor in the Biomedical Mathematics program and is a member of the Molecular Biophysics (MOB) program at FSU. It is anticipated that a graduate student participating in this project will be enrolled in either program. The PI currently has a Ph. D. student from the MOB program (Tom Asbury) who is working on a project in computational structural biology. The PI has just begun working with a Ph. D. student in the Biomedical Mathematics program (Jennifer Specht) who will likely participate in the β-cell project described herein. Undergraduate researchers may come from mathematics, biology, chemistry, or physics. The interdisciplinary nature of the proposed research provides entry points for students with a wide range of backgrounds. Students working on this project will learn about mathematical modeling, techniques in the analysis of nonlinear dynamical systems, computer simulation, and biology/biochemistry. Students will also be encouraged to present their work at scientific meetings, and participate in biannual meetings with the Sherman and Satin labs.

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