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Slow Variable Dominance in Pancreatic β-Cell Models

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SLOW VARIABLE DOMINANCE IN PANCREATIC $\beta$-CELL MODELS

By

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Like nerve and many other endocrine cells, pancreatic β-cells are electrically excitable and produce electrical impulses in response to elevations in glucose. These electrical impulses typically come in the form of bursting. One type of bursting model with two or more slow variables has been called ‘phantom bursting’ since the burst period is a blend of the time constants of the slow variables. In this dissertation, the relative contributions that slow variables make to the bursting produced by two different phantom bursting models are quantified using a measure called the ‘dominance factor’. Using this quantification, it is demonstrated that the control of different phases of the burst can be shifted from one slow variable to another by changing a model parameter. It is also demonstrated that the contributions that the slow processes make to bursting can be non-obvious. One application of the dominance factor is in making predictions about the resetting properties of the model cells. This application is demonstrated using a general phantom bursting model.
CHAPTER 1
INTRODUCTION

The pancreas, an organ that stretches across the back of the abdomen behind the stomach (Fig. 1.1), is responsible for releasing digestive enzymes into the duodenum (the first section of the small intestine) and hormones, such as insulin and glucagon, directly into the blood stream. Because of the pancreas’ dual roles, it is considered to be both a part of the digestive and endocrine systems. Located within the pancreas are clusters of endocrine cells called islets of Langerhans (Fig. 1.1). These islets can be composed of a few hundred to thousands of cells. The four major cell types that make up the islets are α, β, δ, and pp cells with a majority (50-80%) being β-cells [61]. Each of these cells produce and secrete a different hormone. These hormones are glucagon (α-cells), insulin (β-cells), somatostain (δ-cells), and pancreatic polypeptide (pp-cells).

Hormones secreted by pancreatic islets are important for regulating glucose levels in the blood stream. Insulin, produced by β-cells, is used by cells, such as muscle and fat cells, to take up and metabolize glucose into adenosine triphosphate (ATP), which the cells use for energy. Insulin is also responsible for the storage of glucose in the form of glycogen (mostly in the liver). Glucagon, produced by α-cells, has the opposite effect. Instead of lowering glucose levels, glucagon raises glucose levels in part by causing the liver to convert its stored glycogen into glucose and release it into the blood stream. Therefore, insulin and glucagon work together to maintain glucose homeostasis. However, this dissertation will focus on the insulin secreting β-cell.

Like nerve and many other endocrine cells, β-cells are electrically excitable and produce electrical impulses in response to elevations in glucose. These electrical impulses typically come in the form of bursting. Bursting oscillations consist of periodic episodes of electrical activity followed by quiescence (Fig 1.2). These bursting oscillations are accompanied by oscillations in the free cytosolic calcium, Ca^{2+}, con-
Figure 1.1: The pancreas stretches across the back of the abdomen behind the stomach. Within the pancreas, lies clusters of endocrine cells called the islets of Langerhans. β-cells are located within these islets. Source: National Diabetes Information Clearinghouse http://diabetes.niddk.nih.gov/dm/pubs/pancreaticislet (accessed on May 20, 2011).

In the presence of glucose, the β-cells in an islet burst synchronously and are coupled by gap junctions [27, 45, 48, 49, 50]. Gap junctions couple the electrical activity and allow for the flow of small molecules between cells by connecting the cytoplasm of these cells. Because of this coupling, islet behavior is different than that of single β-cells. In fact, in islets, an increase in the incidence of gap junctions was observed concentration [5, 56], which drives pulses of insulin secretion [4, 6, 30]. In other words, β-cells secrete insulin, in response to elevated blood glucose levels.

The mechanism for insulin release in β-cells can be seen in Fig. 1.3. A rise in blood glucose levels leads to the uptake of glucose into the cell. Then, the cell metabolizes the glucose converting ADP to ATP and causing the ATP/ADP ratio to rise. This rise inactivates ATP-sensitive K⁺ (K(ATP)) channels. These channels allow the flow of K⁺ out of the cell; therefore, closing these channels causes the cell’s plasma membrane potential to rise. This causes voltage activated Ca²⁺ channels to open, allowing more Ca²⁺ to enter the cell. The rise in Ca²⁺ leads to the release of insulin from storage granules. The insulin can then leave the cell and enter the bloodstream [15]. Insulin is released in a pulsatile manner, which has been shown to have a greater hypoglycemic effect than a continuous infusion of insulin [43].
Figure 1.2: Electrical bursting of a $\beta$-cell produced with a mathematical model.

during stimulation of insulin secretion [46, 47]. It has also been shown that isolated $\beta$-cells release less insulin than intact islets [14, 33, 52].

People with diabetes, a syndrome with multiple types of onset and symptoms, always have some impairment of their $\beta$-cells. This impairment can present itself in the misfunction of insulin oscillations [39]. Commonly, diabetes is categorized into two types, Type 1 and Type 2. Type 1 diabetes is an autoimmune disorder caused by the destruction or dysfunction of $\beta$-cells by the immune system. This type of diabetes usually presents in childhood and is referred to as juvenile diabetes. However, the majority ($\sim 90\%$) of those affected with diabetes are diagnosed with Type 2 diabetes. Type 2 diabetes is caused by a gradual decline of $\beta$-cells over time usually starting with what is known as insulin resistance. As previously mentioned, insulin works to maintain glucose homeostasis by allowing cells to absorb glucose from the bloodstream and by suppressing the secretion of glucose in the liver. When these target tissues fail to respond adequately to insulin, blood glucose levels rise. The physiological condition where insulin becomes less effective at lowering the amount of glucose in the blood stream, resulting in high blood sugar levels is known as insulin resistance. Insulin resistance plays a large role in Type 2 diabetes and is often a precursor to the disorder because it causes the $\beta$-cells to work harder in order to produce more insulin. This over production of insulin can help maintain normal glucose levels for many years before diabetes develops [53]; however, overworking of the $\beta$-cells can lead to the impairment in their function or their destruction [34, 51]. Therefore, since insulin is exclusively produced by $\beta$-cells, a better understanding of $\beta$-cell function can
possibly lead to improvements in the treatment of diabetes.

Even though our understanding of biology increases every day, it can still be difficult if not impossible to discover the mechanism of how certain things, like \( \beta \)-cells, function experimentally. Mathematical models can be very useful in order to gain a greater understanding of the mechanisms that cannot be studied in the lab. In particular, many mathematical models for bursting cells have been developed [24]. Not only can mathematical models help one gain a greater insight into how certain biological processes work, they can also help scientists design new experiments in order to test predictions made with the model.

The first mathematical model for pancreatic islet bursting was developed by Chay
and Keizer [20]. This and many other first generation models all contain a single slow process that groups the spikes into bursts. However, many different slow processes were used. The Chay-Keizer model has bursting driven by the slow rise and fall of cytosolic Ca\textsuperscript{2+}, acting on Ca\textsuperscript{2+}-activated K\textsuperscript{+} (K(CA)) channels [20]. Other models used slow inactivation of a voltage-dependent Ca\textsuperscript{2+} current [19, 37]; slow changes in the ratio of ATP to ADP acting on ATP-sensitive K\textsuperscript{+} (K(ATP)) channels [36, 57]; and the effects of the endoplasmic rectiulum (ER) acting either indirectly on cytosolic Ca\textsuperscript{2+} or directly through store-operated current [17, 18]. The ER is both a store and a source of Ca\textsuperscript{2+} in the cell. Emptying of intracellular Ca\textsuperscript{2+} stores like the ER can activate Ca\textsuperscript{2+} influx into the cell. This is called store-operated Ca\textsuperscript{2+} entry. All of these first generation models produce bursts with a period of 10 − 35 seconds. However, both islets and isolated β-cells have been shown to exhibit bursting oscillations with a wide range of periods. Although not universally categorized, bursting can be divided into three types (fast, medium, and slow) based upon the period of the burst. Fast bursting has a period between 2 and 5 seconds. These oscillations are often observed in single β-cells [38, 64]; however, they can also be seen in islets [12, 13, 22]. Medium bursting oscillations have a period ranging from 10-60 seconds. These oscillations are typically observed in islets. Slow bursting oscillations have a period ranging from 2 to 4 minutes and have been observed in both single cells [3] and islets [40, 60]. Although the first generation models can be made to have this range of burst periods mathematically, in order to do this the slow processes would no longer have biologically accurate parameter values. Therefore, these models are limited in their ability to produce bursting with the wide range of periods seen experimentally. In order to reproduce the wide range of burst periods, a new class of models with multiple slow variables was introduced called phantom bursting models.

Singular geometric perturbation analysis (also called fast/slow analysis) has proven to be very useful in the analysis of mathematical models of bursting with one slow variable [54, 55]. This analysis makes use of the separation of times scales between those variables that change rapidly (the fast variables) and those that change slowly (the slow variables). Although, fast/slow analysis was originally used to analyze bursting with one slow variable, it was extended to describe phantom bursting [7]. While this analysis can clarify how slow variables can work together in order to terminate the active and silent phases of bursting, the relative contributions that each slow variable makes to bursting was not determined. So, the question of how the slow
variables contribute to the burst period is left unanswered.

In this dissertation, the contributions that each slow variable makes to the termination of the active and silent phases of bursting will be described using two different phantom bursting models of pancreatic $\beta$-cells. Chapter 2 describes fast/slow analysis on a model with only one slow variable. Then, in Chapter 3, a description of the generic phantom bursting model [7] is given, along with a discussion of the fast/slow analysis of the model. Chapter 4 describes the method of quantification and gives the results of quantification of the general phantom bursting model. One important use of this quantification in interpreting the biology of model cells is making predictions about their resetting properties. This application is discussed in Chapter 5. A second, more biophysical, phantom bursting model of the $\beta$-cell [8], is introduced in Chapter 6. Chapter 7 discusses the results of quantification on this more biophysical phantom bursting model. Finally, Chapter 8 contains a brief summery of results and conclusions.
CHAPTER 2

SINGULAR PERTURBATION ANALYSIS

Singular perturbation analysis, also called fast/slow analysis, is a useful tool for studying bursting models with only one slow variable. This analysis makes use of the separation of time scales between those variables that change rapidly (the fast variables) and those that change slowly (the slow variables). This chapter introduces fast/slow analysis and uses it to analyze a general bursting model with a single slow variable.

2.1 Mathematical Model with a Single Slow Variable

In most models of pancreatic \( \beta \)-cells, the model represents a \( \beta \)-cell in an intact islet. As previously mentioned, in an islet all or most of the \( \beta \)-cells burst synchronously because they are coupled by gap junctions. The models presented in this dissertation represent one of these coupled cells. Therefore, the models describe islet behavior, which is different from that of isolated \( \beta \)-cells.

First generation models for the pancreatic \( \beta \)-cells contained only one slow variable. This slow variable is responsible for grouping spikes into bursts. The model used in this chapter is a general bursting model for \( \beta \)-cells with one slow variable. The fast subsystem consists of the cell’s plasma membrane potential \( (V) \) and the activation variable \( (n) \) for the delayed rectifier \( K^+ \) current. The slow subsystem consists of a slow negative feedback variable \( s \), the activation variable for the slowly activating \( K^+ \) current \( (I_s) \).
The model equations are:

\[
\frac{dV}{dt} = -(I_{Ca} + I_K + I_s + I_L)/C_m \quad (2.1)
\]

\[
\frac{dn}{dt} = (n_\infty(V) - n)/\tau_n(V) \quad (2.2)
\]

\[
\frac{ds}{dt} = (s_\infty(V) - s)/\tau_s, \quad (2.3)
\]

with ionic currents:

\[
I_{Ca} = g_{Ca}m_\infty(V)(V - V_{Ca}), \quad I_K = g_Kn(V - V_K) \quad (2.4)
\]

\[
I_s = g_s(V - V_K), \quad I_L = g_L(V - V_L). \quad (2.5)
\]

$I_{Ca}$ is an inward Ca\(^{2+}\) current that activates very rapidly (assumed instantaneous), $I_K$ is a rapidly activating outward K\(^+\) current, and $I_L$ is a leak current. $C_m$ is the membrane capacitance of the cell. The $g$ parameters are the maximum current conductances, and $V_{Ca}$, $V_K$, and $V_L$ are the Nernst potentials.

![Figure 2.1: Steady state activation curves for $m$, $n$, and $s$. These are sigmoidal Boltzman functions, which increase with membrane potential ($V$) and saturate.](image)
Table 2.1: Parameter values for the generic bursting model with only one slow variable

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$</td>
<td>4524 fF</td>
</tr>
<tr>
<td>$g_{Ca}$</td>
<td>280 pS</td>
</tr>
<tr>
<td>$g_K$</td>
<td>1300 pS</td>
</tr>
<tr>
<td>$g_L$</td>
<td>25 pS</td>
</tr>
<tr>
<td>$g_s$</td>
<td>40 pS</td>
</tr>
<tr>
<td>$V_{Ca}$</td>
<td>100 mV</td>
</tr>
<tr>
<td>$V_K$</td>
<td>$-80$ mV</td>
</tr>
<tr>
<td>$V_L$</td>
<td>$-40$ mV</td>
</tr>
<tr>
<td>$\tau_s$</td>
<td>20 s</td>
</tr>
</tbody>
</table>

The steady state activation curves for $m$, $n$, and $s$ are sigmoidal Boltzmann functions, which increase with membrane potential and saturate (Fig 2.1):

$$m_\infty(V) = \frac{1}{1 + \exp[(-22 - V)/7.5]} \quad n_\infty(V) = \frac{1}{1 + \exp[(-9 - V)/10]} \quad (2.6)$$

$$s_\infty(V) = \frac{1}{1 + \exp[(-40 - V)/0.5]} \quad (2.7)$$

The only voltage-dependent time constant is $\tau_n$:

$$\tau_n(V) = \frac{9.09}{1 + \exp[(V + 9)/10]} \quad (2.8)$$

The other parameter values are listed in Table 2.1.

The fast subsystem governs spiking during the active phase of a burst, while the slow subsystem controls when the spiking is turned on and off. The spiking activity causes $s$ to slowly increase. When $s$ is sufficiently large, $I_s$ suppresses the action potentials and the cell returns to a hyperpolarized silent state. Model equations were solved numerically using the CVODE algorithm implemented in the XPPAUT software package [28].

Since bursting is driven by $s$ and $\tau_s = 20$ seconds, the burst period is approximately 20 seconds (Fig. 2.2A). The fast variable $n$ changes significantly over the duration of a single spike (Fig. 2.2B), while $s$ oscillates with a sawtooth pattern over
the duration of the burst (Fig. 2.2C). During a burst, Ca$^{2+}$ enters the cell and the Ca$^{2+}$ current is negative by convention (Fig. 2.2D), while K$^+$ exits the cell and the K$^+$ current is positive (Fig. 2.2E). The slowly activating K$^+$ current, $I_s$ and the leak current, $I_L$ are also depicted (Fig. 2.2F,G).

Figure 2.2: (A) Bursting with one slow variable, s. (B) The activation variable (n) for the delayed rectifier K$^+$ current. (C) The slow variable, s, increases during the active phase of a burst and decreases during the silent phase. (D) The inward Ca$^{2+}$ current, $I_{Ca}$, which activates rapidly. (E) The rapidly activating outward K$^+$ current, $I_K$. (F) The slowly activating K$^+$ current, $I_s$. (G) The leak current, $I_L$.

### 2.2 Fast/Slow Analysis

Bursting is achieved by the slow variable, s, switching the fast dynamics between a steady state and a periodic solution. A greater understanding of the system can
be achieved by treating $s$ as a parameter and looking at the solution structure of the fast subsystem. When $s$ is set to a constant value, the solution structure of the fast subsystem can be seen in the V-n plane. This structure depends on the curves along which the derivatives of $V$ and $n$ are zero, called nullclines. The z-shaped V-nullcline (green) in Figs. 2.3-2.8 is obtained by setting the right-hand side of Eq. (2.1) equal to zero. Although this equation cannot be solved for $V$, it can be solved for $n$:

$$n = -\frac{I_{Ca} + I_L}{g_k(V - V_K)} - \frac{g_s}{g_K} s. \quad (2.9)$$

The n-nullcline (brown) is found by setting $dn/dt = 0$ and is given by:

$$n = n_{\infty}(V). \quad (2.10)$$

When $s = 0.2$, the V-nullcline (green curve) intersects the n-nullcline (brown curve) once (Fig. 2.3). This intersection occurs when $dV/dt = dn/dt = 0$ and is an equilibrium solution of the system. In this case, the equilibrium is a stable focus (black circle). A sample trajectory is also depicted (black curve). The actual trajectory of the system depends upon the initial conditions chosen; however, all trajectories will
end at the equilibrium point. For this value of $s$, the system is silent (not oscillating).

Figure 2.4: When $s$ is increased to 0.4, the V-nullcline still intersects the n-nullcline only once. However, the system has gone through a Hopf bifurcation. There is now an unstable focus (open circle) and a stable limit cycle (bold black curve) solution. For this value of $s$, the system oscillates. A sample trajectory is also drawn (black curve).

Increasing $s$ causes the V-nullcline to shift to the left. This can be seen from Eq. (2.9), which shows that for each value of $V$, $n$ becomes smaller. When $s = 0.4$, the system has undergone a Hopf bifurcation. A Hopf bifurcation occurs when a periodic orbit appears or disappears through a local change in the stability of a steady state solution. In this case, the equilibrium solution is now unstable (open circle) and a stable limit cycle appears (bold black curve) (Fig. 2.4). This limit cycle represents a periodic solution, which means that for this value of $s$ the system oscillates.

Increasing $s$ further causes the V-nullcline to intersect with the n-nullcline in three locations (Fig. 2.5). The addition of two additional solutions means that the system has undergone a saddle node bifurcation. Saddle node bifurcations occur when the system goes from having no equilibrium solutions to one to two or vice-versa. The stable limit cycle (bold black curve) and unstable focus (open circle) are still present. However, there is now a saddle point (triangle) and a stable node (black circle) solution. The system is now bistable, since there are two stable solutions for the system. The stable manifold (solid blue curve) of the saddle point acts as a
Figure 2.5: When $s$ is increased to 0.6, the V-nullcline intersects the n-nullcline in three places. There is still an unstable focus (open circle) and a stable limit cycle (bold black curve). However, there is now a saddle point (triangle) and a stable node (black circle). The stable manifold (solid blue curve) of the saddle point creates a basin of attraction to either the limit cycle or stable node. The initial conditions chosen will determine if the system will oscillate or be silent. The unstable manifold (dashed blue curve) of the saddle point is also pictured.

separatrix that separtates the basins of attraction of the stable node and the limit cycle. The unstable manifold (dashed blue curve) is also pictured. Depending upon the initial conditions chosen, the system will either oscillate or be silent.

The system remains bistable until after a homoclinic bifurcation. When $s \approx 0.67$, one branch of the stable manifold connects with a branch of the unstable manifold creating an infinite period orbit (solid blue curve) (Fig. 2.6). This is the point where the homoclinic bifurcation occurs. Increasing $s$ further results in the loss of bistability. There now exists only one stable equilibrium solution (Fig. 2.7).

With $s = 0.8$, there are still three equilibrium solutions to the system (Fig. 2.7). There is an unstable focus (open circle), a saddle point (triangle), and a stable node (black circle). However, there is no longer a periodic solution. Therefore, the system is now at rest. Increasing $s$ further to 1, moves the V-nullcline far enough to the left such that the nullclines intersect only in one place. The system has now undergone another saddle node bifurcation, and the system is still silent.
Figure 2.6: When $s$ is approximately 0.67, one branch of the stable manifold connects with a branch of the unstable manifold, creating an infinite period homoclinic orbit (solid blue curve).

Figure 2.7: When $s = 0.8$, there are still three intersections of the nullclines; however, there is now only one stable node (black circle) and no periodic solution. There is an unstable focus (open circle) and a saddle point (triangle). For this value of $s$, the system is silent. A sample trajectory is also pictured (black curve).
Figure 2.8: When $s = 1$, there is only one intersection of the $V$-nullcline and the $n$-nullcline, a stable node (black circle). For this value of $s$, the system is silent. A sample trajectory is also pictured (black curve).

### 2.3 Bifurcation Diagram

All of this information can be summarized in a bifurcation diagram (Fig 2.9). The stationary solutions (where both $dV/dt$ and $dn/dt = 0$) form the slow manifold or $z$-curve. The solid part of the curve represents the stable solutions and the dashed part represents unstable solutions. There are two saddle node bifurcations (triangle) where the curve folds. A branch of periodic solutions (bold black curve) emerges from a Hopf bifurcation (circle) and represents action potentials (both minimum and maximum voltage are indicated). The periodic branch terminates at an infinite-period homoclinic bifurcation (square); this homoclinic bifurcation takes place where the periodic branch touches the $z$-curve. Between the lower saddle node and the homoclinic, the system is bistable. In other words, the system has two stable solutions, one periodic and one stationary. It is this region of bistability that is necessary for bursting to occur.

The slow dynamics of $s$ are now introduced, treating $s$ as a variable rather than a bifurcation parameter. The $z$-curve now plays the role of a generalized $V$-nullcline (where $dV/dt = 0$), and the $s$-nullcline is added to the figure (dotted curve). The $s$-nullcline is found by setting $ds/dt = 0$ and is given by:
Figure 2.9: Fast/slow analysis of bursting with a single slow variable. The slow variable $s$ is treated as the bifurcation parameter. The $s$-nullcline (dotted curve) and burst trajectory are superimposed on the bifurcation diagram. The circle represents a Hopf bifurcation, the square represents a homoclinic bifurcation, and the triangles represent saddle node bifurcations.

$$s = s_\infty(V).$$

(2.11)

The burst trajectory is now superimposed over the z-curve showing the system dynamics.

During the silent phase, the burst trajectory follows the bottom of the z-curve, moving leftward, since it is to the right of the $s$-nullcline. Once the lower knee is reached, the phase point moves to the only attractor, the periodic branch that represents the spiking phase of the burst. Since it is now to the left of the $s$-nullcline, it moves rightward until the homoclinic bifurcation is reached, at which time the phase point returns to the bottom branch to restart the silent phase.
CHAPTER 3

GENERAL PHANTOM BURSTING MODEL

The previous chapter described a bursting model with one slow variable and used fast/slow analysis to analyze the bursting produced with that model. Although a model with a generic slow variable, $s$, was used for simplicity, this model is analogous to the first generation $\beta$-cell models containing one slow variable. In these models, the burst period is similar to the time constant of the slow process, since it is the slow process that is responsible for the beginning and ending of a burst. Other bursting models contain more than one slow variable. One type of bursting model with two slow variables produces phantom bursting, so named because the burst period can be a blend of the slow variables [7]. This chapter introduces the general phantom bursting model described in Bertram et. al. [7] and extends fast/slow analysis to phantom bursting. The general phantom bursting model was first described in order to explain how a model could exhibit a wide range of burst periods, not for biological accuracy. In the next chapter, the general phantom bursting model will be used for its simplicity to illustrate the method of quantification.

3.1 Mathematical Model with Two Slow Variables

Like the bursting model described in the previous chapter, the general phantom bursting model for pancreatic $\beta$-cells is composed of fast and slow subsystems [7]. The fast subsystem consists of the cell’s plasma membrane potential ($V$) and the activation variable ($n$) for the delayed rectifier $K^+$ current. The slow subsystem consists of two
distinct slow negative feedback variables, $s_1$ and $s_2$. These are activation variables for slowly activating K$^+$ currents $I_{s1}$ and $I_{s2}$, respectively. Both $s_1$ and $s_2$ are slow in relation to $V$ and $n$, which operate on a time scale of tens of milliseconds. However, the $s_1$ variable, with time constant $\tau_{s1} = 1 \text{ sec}$, is considerably faster than $s_2$ with $\tau_{s2} = 2 \text{ min}$. The model equations are:

$$\frac{dV}{dt} = -(I_{Ca} + I_K + I_{s1} + I_{s2} + I_L)/C_m$$ \hspace{1cm} (3.1)

$$\frac{dn}{dt} = (n_\infty(V) - n)/\tau_n(V)$$ \hspace{1cm} (3.2)

$$\frac{ds_1}{dt} = (s_{1\infty}(V) - s_1)/\tau_{s1}$$ \hspace{1cm} (3.3)

$$\frac{ds_2}{dt} = (s_{2\infty}(V) - s_2)/\tau_{s2},$$ \hspace{1cm} (3.4)

with ionic currents:

$$I_{Ca} = g_{Ca}m_\infty(V)(V - V_{Ca}), \quad I_K = g_Kn(V - V_K)$$ \hspace{1cm} (3.5)

$$I_{s1} = g_{s1}s_1(V - V_K), \quad I_{s2} = g_{s2}s_2(V - V_K)$$ \hspace{1cm} (3.6)

$$I_L = g_L(V - V_L).$$ \hspace{1cm} (3.7)

$I_{Ca}$ is an inward Ca$^{2+}$ current that activates very rapidly (assumed instantaneous), $I_K$ is a rapidly activating outward K$^+$ current, and $I_L$ is a leak current. $C_m$ is the membrane capacitance of the cell. The $g$ parameters are the maximum current conductances, and $V_{Ca}$, $V_K$, and $V_L$ are the Nernst potentials. The steady state activation curves for $m$, $n$, $s_1$, and $s_2$ are sigmoidal Boltzman functions, which increase with membrane potential and saturate (Fig. 3.1):

$$m_\infty(V) = \frac{1}{1 + \exp[(-22 - V)/7.5]}, \quad n_\infty(V) = \frac{1}{1 + \exp[(-9 - V)/10]}$$ \hspace{1cm} (3.8)
\[ s_{1\infty}(V) = \frac{1}{1 + \exp\left[\frac{-40 - V}{0.5}\right]}, \quad s_{2\infty}(V) = \frac{1}{1 + \exp\left[\frac{-42 - V}{0.4}\right]} \]  

The only voltage-dependent time constant is \( \tau_n \):

\[ \tau_n(V) = \frac{9.09}{1 + \exp\left[\frac{V + 9}{10}\right]} \]  

Figure 3.1: Steady state activation curves for \( m, n, s_1 \), and \( s_2 \). These are sigmoidal Boltzmann functions, which increase with membrane potential \( V \) and saturate.

The other time constants and parameter values are listed in Table 3.1. As \( V \) is varied over the interval \([-55,-20]\) mV, which is the normal range of activity, \( \tau_n \) ranges from 6.2 to 9.0 msec. The fast subsystem governs spiking during the active phase of a burst, while the slow subsystem controls when the spiking is turned on and off. The spiking activity causes \( s_1 \) and \( s_2 \) to slowly increase. When these variables are sufficiently large, \( I_{s1} \) and \( I_{s2} \) suppress the action potentials, and the cell returns to a hyperpolarized silent state. Figure 3.2A shows one example of bursting with this model. The slow variables, \( s_1 \) and \( s_2 \) oscillate over the duration of the burst (Fig. 3.2B,C). Figures 3.2D,E show the slow currents \( I_{s1} \) and \( I_{s2} \). Figures 3.2F,G
show the inward Ca$^{2+}$ current and rapidly activating outward K$^+$ current.

Table 3.1: Parameter values for the general phantom bursting model

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$</td>
<td>4524 fF</td>
</tr>
<tr>
<td>$g_{Ca}$</td>
<td>280 pS</td>
</tr>
<tr>
<td>$g_K$</td>
<td>1300 pS</td>
</tr>
<tr>
<td>$g_L$</td>
<td>25 pS</td>
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<tr>
<td>$g_{s1}$</td>
<td>2-40 ps</td>
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<td>$g_{s2}$</td>
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<td>$V_{Ca}$</td>
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</tr>
<tr>
<td>$V_K$</td>
<td>−80 mV</td>
</tr>
<tr>
<td>$V_L$</td>
<td>−40 mV</td>
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<tr>
<td>$\tau_{s1}$</td>
<td>1 s</td>
</tr>
<tr>
<td>$\tau_{s2}$</td>
<td>2 min</td>
</tr>
</tbody>
</table>

When the $I_{s1}$ conductance ($g_{s1}$) is large, bursting is driven by $s_1$. Since $\tau_{s1} = 1$ sec, the burst period is only a few seconds (Fig. 3.3A). For this fast bursting, $s_2$ is almost constant, while $s_1$ varies with a sawtooth pattern (Fig. 3.3B). In fact, if $s_2$ is clamped at its average value, the bursting continues almost unaltered. To analyze the bursting, a fast/slow analysis was performed. The $s_1$ variable is treated as the bifurcation parameter for the fast subsystem with $s_2$ held constant at its average value. Therefore, the system only has one slow variable. The analysis is the same as the model with one slow variable in the previous chapter. The fast subsystem bifurcation diagram, or $z$-curve, is shown in Fig. 3.4A. The burst trajectory is superimposed over the $z$-curve showing the system dynamics, with $s_1$ no longer treated as a parameter. The $z$-curve now plays the role of a generalized $V$-nullcline (where $dV/dt = 0$), and the $s_1$-nullcline is added to the figure. The $s_1$-nullcline is found by setting $ds_1/dt = 0$ and is given by:

$$s_1 = s_1(\infty)(V).$$ (3.11)

During the silent phase, the burst trajectory follows the bottom of the $z$-curve, moving leftward. Once the lower knee is reached, the phase point moves to the only attractor, the periodic branch. It now moves rightward until the homoclinic bifurcation is reached, at which time the phase point returns to the bottom branch to restart the silent phase.

When $g_{s1}$ is lowered, variations in $I_{s1}$ are insufficient to move the system between
active and silent phases. This requires a contribution from $I_{s2}$. During the active phase, $s_2$ slowly increases, and it slowly decreases during the silent phase (Fig. 3.2C and 3.3D), increasing and decreasing the current, respectively. Medium bursting is produced (Fig. 3.3C) with period influenced by both $s_1$ and $s_2$. If $s_2$ is clamped, the bursting is replaced by a steady-state solution or continuous spiking. If $s_1$ is clamped, the burst period greatly increases. Fig. 3.4B shows two $z$-curves for medium bursting. The curve on the left has $s_2$ fixed at its maximum value achieved during a burst, while the curve on the right has $s_2$ fixed at its minimum value. During the active phase of a burst, the phase point gets caught at the intersection of the periodic branch with the $s_1$-nullcline. If $s_2$ remained constant, then the system would exhibit continuous spiking. However, $s_2$ increases due to the spiking, moving the $z$-curve leftward. Eventually, the $z$-curve shifts far enough that the homoclinic bifurcation terminating the periodic branch moves past the nullcline. The trajectory then enters the silent phase. While in the silent phase, the phase point gets caught at the intersection of
the bottom branch of the z-curve with the $s_1$-nullcline. Now, if $s_2$ remains constant the system would exhibit a steady-state solution. However, $s_2$ decreases, shifting the z-curve to the right. Eventually, the phase point is able to return to the active phase.

The burst period is determined both by the time required for the phase point to move along the z-curve (controlled by the $s_1$ dynamics) and the time required to translate the z-curve and periodic branch back and forth (controlled by the $s_2$ dynamics). The analysis in the next chapter quantifies the contributions of these motions to the burst period.

Further reduction in $g_{s_1}$ leads to a further increase in the burst period. The trajectory still gets stuck at the intersection of the z-curve and $s_1$-nullcline as in Fig 3.4B for medium bursting. However, it now takes longer for $s_2$ to shift the z-curve far enough to the left for the homoclinic bifurcation to move past the nullcline, since reducing $g_{s_1}$ stretches the z-curve. Bursting is now solely driven by $s_2$ (Fig. 3.3E). Since $\tau_{s_2} = 2$ min, the burst period is nearly 2 min. The $s_1$ time course is a square
wave, characteristic of the fast variable in a relaxation oscillation. In fact, \( s_1 \) is part of the fast subsystem. While \( s_1 \) plateaus at its highest value during the active phase, \( s_2 \) varies with a sawtooth pattern (Fig. 3.3F). In the extreme cases where \( g_{s1} \) is very big or very small, we can say that bursting is fast or slow based on the period of oscillations. However, it is difficult to define precisely where the transition occurs from fast to medium and from medium to slow bursting. Using the method of quantification described later, these transitions will be defined.

Figure 3.4: (A) Fast subsystem bifurcation diagram of fast bursting (\( g_{s1} = 20 \) pS) with \( s_1 \) as the bifurcation parameter and \( s_2 = 0.436 \). The \( s_1 \)-nullcline and burst trajectory are superimposed on the bifurcation diagram. The circle represents a Hopf bifurcation, the square represents a homoclinic bifurcation, and the triangles represent saddle node bifurcations. (B) Fast/slow analysis of medium bursting (\( g_{s1} = 7 \) pS). There are two bifurcation diagrams with \( s_1 \) as the bifurcation parameter. The curve on the left has \( s_2 \) fixed at its maximum value (0.633) achieved during the bursting, while the curve on the right has \( s_2 \) fixed at its minimum value (0.600). The burst trajectory is superimposed on the diagram. Arrows indicate direction of movement of the \( z \)-curve driven by variations in \( s_2 \).
3.2 Phantom Relaxation Oscillator

The generic phantom bursting model can be reduced to a phantom relaxation oscillator by making the activation kinetics of the delayed rectifier current instantaneous. That is, setting \( n \) to \( n_\infty(V) \) in Eq. (3.5) and removing the differential equation for \( n \). This replaces the spikes of an active phase of bursting with a depolarized voltage plateau (Fig. 3.5C,D). When \( g_{s1} \) is large, a fast relaxation oscillation is produced, which is driven by \( s_1 \). This can be analyzed in the \( s_1-V \) plane, with \( s_2 \) held constant at its average value. In Fig. 3.5A, the solid z-shaped curve is the \( V \)-nullcline, given by:

\[
s_1 = -\frac{I_{Ca}(V) + I_K(V) + I_L(V)}{g_{s1}(V-V_K)} - \frac{g_{s2}}{g_{s1}} s_2. \tag{3.12}
\]

The \( s_1 \)-nullcline is the dotted curve.

As in Fig. 3.4A the \( s_1 \)-nullcline intersects the z-shaped curve, now the \( V \)-nullcline, on the middle branch, and the full-system equilibrium is unstable. The phase point travels along the bottom branch during the silent phase and the top branch during the depolarized phase. This is a standard relaxation oscillation (Fig. 3.5C). This relaxation oscillation is driven by \( s_1 \) (Fig. 3.5E), while \( s_2 \) is nearly constant (Fig. 3.5G). When \( g_{s1} \) is reduced the relaxation oscillation is driven by both \( s_1 \) and \( s_2 \) (Fig. 3.5D). This is referred to as a phantom relaxation oscillation. Now, both \( s_1 \) and \( s_2 \) oscillate (Fig. 3.5F,H). As in Fig. 3.4B, in the \( V-s_1 \) plane, the \( V \)-nullcline moves with changes in \( s_2 \) to end the active and silent phases (Fig. 3.5B). In fact, Eq. (3.12) makes it evident that increasing \( s_2 \) translates the \( V \)-nullcline leftward.
Figure 3.5: Phase plane analysis of fast and medium relaxation oscillations. (A) The V-nullcline (z-shaped curve) and $s_1$-nullcline (dotted curve) for fast oscillations with $g_{s1} = 40$ pS and $s_2$ fixed at 0.436. The trajectory (heavy solid curve) follows the upper and lower branches of the V-nullcline. (B) The V-nullcline and $s_1$-nullcline for medium oscillations with $g_{s1} = 20$ pS. The V-nullcline on the left has $s_2$ at it maximum value (0.619), while the V-nullcline on the right has $s_2$ at its minimum value (0.591). (C) Fast relaxation oscillations driven by $s_1$. (D) Phantom relaxation oscillations driven by $s_1$ and $s_2$. (E-H) $s_1$ and $s_2$ oscillate over the duration of an oscillation. When, $s_1$ is driving bursting, $s_2$ is nearly constant (E,G).
CHAPTER 4

QUANTIFICATION OF PHANTOM RELAXATION OSCILLATIONS AND PHANTOM BURSTING

The generic phantom bursting model has been analyzed using fast/slow analysis (Chapter 3) to understand the mechanism of bursting and the wide range of burst periods that can be produced. While this analysis clarified why the different slow variables control fast or slow bursting and how the two work together to produce medium bursting, the relative contributions of the two slow variables to the generation of the medium bursting was not determined. That is, for a given medium bursting pattern, it was never determined quantitatively how much $s_1$ contributed to the burst period and how much $s_2$ contributed. In this chapter, a measure called the dominance factor will be described. This measure gives the relative contributions of the two slow variables to bursting. Then, the results of quantification for the phantom relaxation oscillator and the general phantom burster will be discussed. This work was published in Watts et al. (2011) [63].

4.1 Method of Quantification

A method for quantifying the contribution that each slow variable makes to the active and silent phases of the oscillation will now be developed, starting with the phantom relaxation oscillation and relying on the fact that activity is terminated and restarted as the slow variables increase during the active phase and decrease during the silent phase. The method is illustrated in Fig. 4.1. At the beginning of the active phase (AP) of a relaxation oscillation the time constant, $\tau$, for one of the slow
Figure 4.1: Measuring the effect of a slow variable on the duration of the active phase. The time constant of the slow variable, $\tau$, is increased by $\delta \tau$ at the beginning of the active phase (arrow). This causes the slow variable to slow down and the active phase duration to increase by $\delta AP$ (bold curve).

variables is increased by $\delta \tau$. This slows down the slow variable, so if slow variation of this variable contributes to the termination of the active phase, the active phase should increase by $\delta AP$. The larger the slow variable’s contribution to the active phase duration, the larger $\delta AP$. If the variable has no influence on the active phase duration, then slowing it down will give $\delta AP = 0$. The procedure is repeated for the second slow variable and the silent phase (SP). Note that only one variable’s time constant is perturbed at a time, at the very beginning of a phase, and the only thing measured is how this perturbation affects that phase. The system is not allowed to equilibrate after a time constant is changed, because then both variables will vary over a slightly different range than before the perturbation. This change in the range of variations of the slow variables may also lead to a change in the AP and SP durations compounding the effect of the original perturbation.

The system is considered to be in the active phase when $V > -40$ mV and to be in the silent phase when $V < -40$ mV. Now, a measure of the contribution of $s_1$ to the duration of the active phase is given by $\delta AP_{s_1}/\delta \tau_{s_1}$, an approximation to the derivative of the AP duration with respect to $\tau_{s_1}$. Then, the normalized contribution of $s_1$ to the AP duration ($C_{AP}^{s_1}$) is given by

$$C_{AP}^{s_1} = (\delta AP_{s_1}/\delta \tau_{s_1})(\tau_{s_1}/AP).$$
Figure 4.2: Interpretation of the dominance factor, $DF = \cos \theta - \sin \theta$. When $\theta = 0$, $DF = 1$ and the oscillation is fast. When $\theta = \frac{\pi}{2}$, $DF = -1$ and the oscillation is slow. Medium frequency oscillations occur when $\theta \in (0, \frac{\pi}{2})$ and $DF \in (-1, 1)$.

With $C_{s1}^{AP}$ defined in this way, if $s_1$ is the only slow variable contributing to the duration of the AP, an increase in $\tau_{s1}$ of 5% so that $\delta\tau_{s1}/\tau_{s1} = 0.05$ would result in an increase in AP of 5% so that $\delta AP_{s1}/AP = 0.05$, and therefore $C_{s1}^{AP} = 1$. If $s_1$ has no effect on the active phase duration, then $C_{s1}^{AP} = 0$. In most cases both $s_1$ and $s_2$ will contribute, so $0 < C_{s1}^{AP} < 1$. Similarly, the effect that $s_1$ has on the silent phase duration can be quantified by increasing the time constant at the beginning of the SP and measuring the effect that it has on the SP. Thus,

$$C_{s1}^{SP} = (\delta SP_{s1}/\delta \tau_{s1})(\tau_{s1}/SP).$$  \hspace{1cm} (4.2)

Likewise, the same technique is used on the $s_2$ variable to obtain:

$$C_{s2}^{AP} = (\delta AP_{s2}/\delta \tau_{s2})(\tau_{s2}/AP)$$ \hspace{1cm} (4.3)

$$C_{s2}^{SP} = (\delta SP_{s2}/\delta \tau_{s2})(\tau_{s2}/SP).$$ \hspace{1cm} (4.4)
By comparing $C_{s1}^{AP}$ to $C_{s2}^{AP}$ and $C_{s1}^{SP}$ to $C_{s2}^{SP}$, we can evaluate the respective contributions of $s_1$ and $s_2$ to AP and SP durations. This is facilitated by using a measure called the dominance factor (DF) for each phase:

$$DF_{AP} = \frac{C_{s1}^{AP} - C_{s2}^{AP}}{\sqrt{(C_{s1}^{AP})^2 + (C_{s2}^{AP})^2}}, \quad DF_{SP} = \frac{C_{s1}^{SP} - C_{s2}^{SP}}{\sqrt{(C_{s1}^{SP})^2 + (C_{s2}^{SP})^2}}. \tag{4.5}$$

Defined in this way, the dominance factor has a trigonometric interpretation in the $C_{s1} - C_{s2}$ plane (Fig. 4.2). The length of the vector $\vec{C} = (C_{s1}, C_{s2})$ is $|\vec{C}| = \sqrt{(C_{s1})^2 + (C_{s2})^2}$. Then, $C_{s1} = |\vec{C}| \cos \theta$, $C_{s2} = |\vec{C}| \sin \theta$, and from Eq. (4.5), $DF = \cos \theta - \sin \theta$. When $s_1$ dominates $\theta = 0$ and $DF = 1$, and when $s_2$ dominates $\theta = \frac{\pi}{2}$ and $DF = -1$. For all $\theta$ between 0 and $\frac{\pi}{2}$, $DF$ is between these two extremes. The DF can go outside of this range if either $C_{s1}$ or $C_{s2}$ is negative (as discussed later).

### 4.2 Dominance Curves for the Phantom Relaxation Oscillator

Figure 4.3 shows the results of applying this method of quantification to the phantom relaxation oscillator for various values of $g_{s1}$. Here and in other figures, $\delta \tau/\tau = 1$ is used. The rationale for using this somewhat large value is discussed later for the case of phantom bursting. Fast oscillations occur with high values of $g_{s1}$, while slow oscillations occur with low values of $g_{s1}$ (Fig. 4.3A). The C values for various values of $g_{s1}$ are shown in Fig. 4.3B. $C_{s1}^{AP}$ (open circle) and $C_{s1}^{SP}$ (closed circle) start near 0 for small values of $g_{s1}$, then increase to 1 as $g_{s1}$ is increased, while $C_{s2}^{AP}$ (open triangle) and $C_{s2}^{SP}$ (closed triangle) start at 1 and decrease to 0. Figure 4.3C shows $DF_{AP}$ (open circles) and $DF_{SP}$ (closed circles). For low values of $g_{s1}$, DF is close to $-1$ indicating that $s_2$ is the variable driving the oscillations, which therefore have a large period (Fig. 4.3A). For high values of $g_{s1}$, DF is close to 1 indicating that $s_1$ is the variable driving the oscillations, which have a short period since $\tau_{s1}$ is small. It also shows that the switch between $s_1$-driven oscillations and $s_2$-driven oscillations occurs near $g_{s1} = 20$ pS. However, the switch of control does not occur simultaneously for the AP and the SP. When $g_{s1} = 20$ pS the AP is driven primarily by $s_2$, while the SP is primarily driven by $s_1$. This difference in contribution to the AP and SP between $s_1$ and $s_2$ is not simply due to the difference in their time constants. The
Figure 4.3: Results of the quantification method on the phantom relaxation oscillator. $\delta \tau = \tau$ here and in other figures that follow. The results obtained using $\delta \tau = 0.05\tau$ are similar for the relaxation case. (A) Oscillation period decreases with $g_{s_1}$. (B) C values for active and silent phases and for $s_1$ and $s_2$. (C) For low values of $g_{s_1}$ the DF is close to $-1$ indicating that $s_2$ is the variable driving slow oscillations, while for high values of $g_{s_1}$ DF is close to 1 indicating that $s_1$ is the variable driving slow oscillations.

difference in their time constants leads to $s_1$’s dominance of fast bursting and $s_2$’s dominance of slow bursting. Rather, it is due to the difference in their activation. Figure 3.5B illustrates that the phase point gets stuck in the AP shown by the vertical trajectory as $s_2$ moves the $V$-nullcline leftward, ending the AP. The slow increase in $s_2$ moves the $V$-nullcline leftward, while the phase point is at the upper intersection of this nullcline with the $s_1$-nullcline. Once this intersection disappears, the phase point moves vertically downward, and then to the left. While it is not as clear for the SP, $s_2$ moves the lower knee before the phase point reaches it. Therefore, the phase point does not get stuck. So, the contribution of $s_2$ to the termination of the SP is
minimal, while \( s_2 \) controls the termination of the AP.

![Figure 4.4: The difference between the trajectory and the corresponding point on the \( s_1 \)-nullcline for the phantom relaxation oscillator (bold dot-dashed curve)](image)

### 4.3 Difference Curves

Another way to visualize the shift in control of \( s_1 \) and \( s_2 \), is to plot the difference between the trajectory and the corresponding point on the \( s_1 \)-nullcline (Fig. 4.4) for the active phase and silent phase of an oscillation. These ‘difference curves’ offer a test of the validity of the dominance factor results. Figure 4.5A gives the difference between the trajectory and the corresponding point on the \( s_1 \)-nullcline for the active phase of a phantom relaxation oscillation. The x-axis indicates how far into the active phase the trajectory is, with 0.5 being half-way. The difference curves for four different values of \( g_{s1} \) have been plotted. When \( g_{s1} = 40 \), the difference is always greater than zero, so the phase point never gets stuck. Thus, it is expected that the dominance factor would be approximately 1, which is what was computed (Fig. 4.3C). When \( g_{s1} \) is lowered to 21 pS, the trajectory starts to stall towards the end of the active phase. This means that \( s_2 \) is gaining some control during the active phase. Lowering \( g_{s1} \) further (20 pS), the trajectory stalls earlier in the active phase. Therefore, it is expected that \( s_2 \) has more control and the dominance factor should be closer to \(-1\) than in the previous case. Again, this is what is seen when calculating the dominance factor (Fig. 4.3C). When \( g_{s1} = 21 \) pS, the \( DF_{AP} = 0.15 \). This indicates that \( s_1 \) and \( s_2 \)
share control almost equally. However, $g_s = 20$ shows that $s_2$ is largely responsible for the termination of the AP ($DF_{AP} = -0.71$). Finally, when $g_s = 10$ pS, the trajectory is stalled almost immediately. Now, it is expected that the dominance factor would be approximately $-1$, which is what was computed (Fig. 4.3C).

Likewise, Fig. 4.5B shows the difference between the trajectory and the corresponding point on the $s_1$-nullcline for the silent phase of a phantom relaxation oscillation. Again for $g_s = 40$, the difference between the trajectory and nullcline never equals zero. Therefore, it is expected that the dominance factor would be approximately 1, which is what was computed (Fig. 4.3C). When $g_s$ is lowered to 21 pS, the difference again is never zero; however, the trajectory gets closer to the nullcline than in the previous case. Lowering $g_s$ further to 20 pS, the trajectory and nullcline still do not intersect; but, the trajectory gets close to stalling indicating that $s_2$ is gaining more control over the silent phase. Thus, it is expected that the dominance factor is getting closer to $-1$, which is what was computed (Fig. 4.3C). For $g_s = 21$ pS, $DF_{SP} = 0.84$, while for $g_s = 20$ pS, $DF_{SP} = 0.38$. Finally, for $g_s = 10$, the trajectory stalls almost immediately. Thus, it is expected that the dominance factor is approximately $-1$, which is what was computed (Fig. 4.3C).
The difference curves show that as $g_{s_1}$ is decreased, the trajectory gets stuck earlier in the phase. This indicates that $s_2$ is gaining control and that the dominance factor decreases. These curves also show that the trajectory gets stalled in the active phase for some values of $g_{s_1}$, but not in the silent phase. Therefore, the shift in control happens earlier for the silent phase than the active phase. Therefore, it is expected that for some values of $g_{s_1}$, $s_1$ would control the silent phase, while $s_2$ controlled the active phase. In fact, the dominance factor curves gives these results (Fig. 4.3). Therefore, the difference curves validate the results obtained using the method of quantification.

Figure 4.6: Results for the general phantom bursting. (A) Burst period decreases with $g_{s_1}$. (B) C values for active and silent phases and for $s_1$ and $s_2$. (C) For low values of $g_{s_1}$, DF is close to $-1$ indicating that $s_2$ is the variable driving slow bursting, while for high values of $g_{s_1}$, DF is close to 1 indicating that $s_1$ is the variable driving fast bursting. The type of bursting can be defined in terms of the dominance factors.
4.4 Dominance Curves for the General Phantom Burster

Figure 4.6 shows the results of applying the method of quantification to the phantom bursting model for various values of $g_{s1}$. These results are similar to those obtained for the relaxation oscillator (Fig. 4.3). Thus, a similar transition between $s_1$-dominated and $s_2$-dominated dynamics is seen, except that now the switch of control between $s_1$ and $s_2$ occurs at a lower value of $g_{s1}$. At $g_{s1} = 8$ pS, the control is mixed; $s_1$ controls the duration of the SP, while $s_2$ controls the duration of the AP. While a small $\delta\tau$ works well with the relaxation oscillator, it can produce very jagged contribution (C) curves when used with bursting. This is because a small $\delta\tau$ can lead to the addition of an extra spike in some cases, but not others. The curves are smoother with a larger $\delta\tau$ ($\delta\tau = \tau$). In the case of the phantom relaxation oscillator, the dominance factor curves are similar for $\delta\tau = 0.05\tau$ and $\delta\tau = \tau$ (used in Figs. 4.3, 4.6, 4.8).

![Figure 4.7: As $s_1$ rises during an active phase, $I_{s1}$ increases, which promotes the termination of the AP. However, $I_{s1}$ starts to decline toward the end of the burst, leading to burst prolongation. Therefore, an increase in the time constant for $s_2$ ($\tau_{s2}$), leads to a longer decline in $s_1$ (bold part of curve), which acts to increase AP duration.](image)

During the active phase of a fast burst $s_1$ increases monotonically (Fig. 3.3B). During the active phase of a medium burst $s_1$ first increases, but then decreases (Fig. 3.3D and Fig. 4.7). This decrease occurs when the trajectory is “stuck” near
the end of the periodic branch. The value of $s_1$ averaged over a spike declines as $s_2$ rises and shifts the z-curve leftward, since now the spike spends a longer period of time at its nadir, underneath (and to the right of) the $s_1$-nullcline. In other words, $s_1$ declines due to the decrease in spike frequency near the end of the active phase. If $\tau_s$ is now increased, the duration of the decreasing phase of $s_1$ will be extended. This extra decrease in $s_1$ provides an extra increase in the AP duration. Figure 4.7 is an exaggerated picture of the decrease in $s_1$ leading to burst prolongation. As $s_1$ declines, the hyperpolarizing current $I_{s1}$ also declines, tending to increase the AP duration. As a result, $C_{AP}^{s_1} > 1$, as seen in Fig. 4.6B for $g_{s1}$ near 8 pS. This does not occur in the relaxation oscillator since there are no spikes to bring the trajectory to the right of the $s_1$-nullcline.

There are also cases during medium bursting where $C_{AP}^{s_1} < 0$, so that increasing $\tau_s$ decreases the active phase duration. This is again due to the decline in $s_1$ during the latter part of the active phase in medium bursting. If $\tau_s$ is increased, $s_1$ rises more slowly during the active phase and enters its declining phase much later in the burst. Once it enters the declining phase it declines more slowly. Together, the active phase prolongation due to the $s_1$ decline during the active phase is reduced. The end result is that slowing down $s_1$ makes the active phase shorter, so $C_{AP}^{s_1} < 0$.

The dominance curves can be used to provide, for the first time, a quantitative distinction between the types of phantom bursting. For some small $\epsilon > 0$ (here $\epsilon = 0.15$ was chosen), bursting can be defined as “fast” if $DF_{SP}, DF_{AP} > 1 - \epsilon$. Bursting is “slow” if $DF_{SP}, DF_{AP} < -(1 - \epsilon)$. Bursting is “medium” if $-(1 - \epsilon) < DF_{SP}, DF_{AP} < 1 - \epsilon$. From Fig. 4.6, slow bursting occurs for $g_{s1} < 6.75$ pS; medium bursting occurs for $6.75 \text{ pS} < g_{s1} < 11$ pS; and fast bursting occurs for $g_{s1} > 11$ pS.

In computing the dominance curves in Fig. 4.6, $g_{s1}$ was varied to produce the different types of bursting. For fast bursting, both $DF_{AP}$ and $DF_{SP}$ were close to 1, while for slow bursting both $DF_{AP}$ and $DF_{SP}$ were close to $-1$. This indicated that either $s_1$ or $s_2$ controlled both phases of the burst. On the other hand, for medium bursting ($g_{s1} \approx 8$ pS), we can have $DF_{AP} < 0$ and $DF_{SP} > 0$, showing that each slow variable controls one phase (Fig. 4.6C). If the dominance curves are computed by varying $g_{s2}$ and keeping $g_{s1}$ constant at 8.5 pS, one slow variable controls the active phase while the other controls the silent phase over most of the range (Fig. 4.8). As $g_{s2}$ is increased, the burst period decreases (Fig. 4.8A). Figure 4.8B shows the DF values for a range of values of $g_{s2}$. For low values of $g_{s2}$, $DF_{AP}$ is close to $-1$ and
Figure 4.8: Results for phantom bursting with $g_{s2}$ as the varying parameter and $g_{s1} = 8.5 \text{ pS}$. (A) Burst period decreases with $g_{s2}$. For $g_{s2} < 19 \text{ pS}$, the system spikes continuously. (B) $C$ values for active and silent phases and for $s_1$ and $s_2$. (C) For low values of $g_{s2}$, $DF_{AP}$ is close to $-1$ and $DF_{SP}$ is close to $1$ indicating that $s_2$ drives the active phase, while $s_1$ drives the silent phase. However, for high values of $g_{s2}$ $DF_{AP}$ is close to $1$ and $DF_{SP}$ is close to $-1$ indicating that $s_1$ drives the AP, while $s_2$ drives the SP.

$DF_{SP}$ is close to $1$ indicating that $s_2$ drives the active phase, while $s_1$ drives the silent phase. As $g_{s2}$ is varied, the $z$-curve shifts from left to right, changing which phase the trajectory gets stuck in. Therefore, the DF curves intersect. Figure 4.9C shows the fast subsystem bifurcation diagram for $g_{s2} = 20 \text{ pS}$. Here the phase point gets stuck in the AP and has to wait for $s_2$ to move the $z$-curve to the left, terminating the AP. The phase point does not get stuck in the SP. However, for high values of $g_{s2}$ the $DF_{AP}$ is close to $1$ and $DF_{SP}$ is close to $-1$ indicating that $s_1$ is the variable driving the AP, while $s_2$ is driving the SP. Figure 4.9A shows the fast subsystem bifurcation diagram for $g_{s2} = 100 \text{ pS}$. Now, the phase point gets stuck in the SP and has to wait for $s_2$ to terminate the SP. Figure 4.9B shows the fast subsystem bifurcation diagram for $g_{s2} = 40 \text{ pS}$. Now, the phase point never gets stuck; $s_1$ is in control of both the AP and SP. Thus, at extreme values of $g_{s2}$ each slow variable contributes to a phase of the burst, while with $g_{s2}$ held constant and $g_{s1}$ varied, a single variable controls both phases at the extreme $g_{s1}$ values. By applying the new definition of medium bursting, as $g_{s2}$ varies, the bursting goes from medium to fast and then back to medium.
Figure 4.9: Bifurcation diagrams with $g_{s1} = 8.5$ pS. The two dashed curves are the bifurcation diagrams for the extreme values of $s_2$. (A) For $g_{s2} = 100$ pS, the phase point gets stuck in the SP. (B) For $g_{s2} = 40$ pS, the phase point does not get stuck. (C) For $g_{s2} = 20$ pS, the phase point gets stuck in the AP.

### 4.5 Summary

In this chapter, a method was developed to quantify the contributions that each slow variable makes to the active and silent phases of a burst. The contribution of the variable is determined by increasing the time constant for one of the slow variables at the beginning of the phase. This increase in the time constant slows down the variable, and if the activity-dependent change in this variable contributes to the termination of the phase, then the phase duration increases. The dominance factor was used to evaluate the respective contributions that two slow variables made to the active phase and silent phase durations. The method was first used on a phantom relaxation oscillation, and the results validated using difference curves. The phantom relaxation oscillation was used first because it is the simplest model. Then, the method was
used on the general phantom bursting model. It was shown that the control over the phases of bursting depends on the parameters $g_{s1}$ and $g_{s2}$ (Figs. 4.3, 4.6, 4.8). It was also shown that the active and silent phases of a burst do not have to be controlled by the same variable (Figs. 4.8, 4.9).
CHAPTER 5

RESETTING

The previous chapter discussed the method of quantification and results of quantification on the general phantom bursting model and phantom relaxation oscillator. The dominance factor curves obtained as a parameter is varied can be useful for making predictions about the resetting properties of the model cells. One feature of bursting driven by a single slow variable is that it is possible to reset the oscillation from the silent to active phase, or vice versa, with a sufficiently large perturbation. The phase that follows the reset should be shorter than normal, since the slow variable has not had time to reach its typical starting point for that phase. Figure 5.1 shows the typical resetting behavior of a relaxation oscillator with a single slow variable ($s_1$). Figure 5.1A shows a normal oscillation with a full active phase (blue curve), while Fig. 5.1B shows that when the trajectory is reset to the active phase during the silent phase (bold black arrow) the resulting active phase is shorter (green curve).

Resetting experiments were performed on intact pancreatic islets by Cook and associates [23]. They found that resetting was indeed possible, but that the phase following the reset was often of full length. That is, for most silent-active resets the following active phase was no shorter than normal, and for most active-silent resets the following silent phase was of full duration. These are referred to as **full-length resets**. Full-length resets in both directions (bidirectional full-length resets) were shown in the same islet in one case (Figs. 3 and 4 in [23]). A later study showed full-length silent-active resets, but short active-silent resets [65]. The existence of a full-length reset in either direction indicates that the bursting is driven by more than one slow variable. But how? This chapter demonstrates that a full-length reset can be produced if one slow variable determines the silent phase duration while the other slow variable determines the active phase duration. This explanation was postulated
earlier [58] and demonstrated with a β-cell model in which the time constants were adjusted so that one slow variable changes rapidly during the silent phase (so that the other slow variable controls the silent phase duration), and vice-versa for the active phase. However, unidirectional full-length resets can be accounted for with the general phantom bursting model by simply adjusting a system parameter so that the dominance curves for the active and silent phases are well separated. The work in this chapter was published in Watts et al. (2011) [63].

5.1 Active-Silent Full-length Resetting

When a relaxation oscillator is perturbed from the silent (active) to the active (silent) phase half way through the silent phase, the immediately following active (silent) phase is reduced (Fig. 5.1). This is also true for a bursting oscillation driven by a single slow variable. When there is more than one slow variable, the resetting properties can be different. In fact, if the dominance curves are appropriate, full-
length resets may be achieved for both the phantom relaxation oscillator (not shown) and the general phantom burster (shown below).

Figure 5.2: Resetting with $g_{s2} = 27$ pS and $g_{s1} = 8.5$ pS. In this case, $s_1$ is in control of the SP (DF = 0.85), while $s_2$ is in control of the AP (DF = −0.99). (A) Half-way through the AP the system was reset to the SP (arrow), which has full length. $s_1$ has reached its maximum at the time of resetting (bottom curve). The V and $s_1$ time courses have been scaled to facilitate comparison. (B) Half-way through the SP the system was reset to the AP (arrow), which is reduced. $s_2$ is in the middle of decreasing to its minimum value at the time of resetting (bottom curve). (C) The duration of the induced AP is phase dependent. (D) The duration of the induced SP is close to the duration of the unperturbed SP if the resetting occurs after $s_1$ reaches its maximum value.

The condition required for a full-length reset is that one slow variable, $s_1$, controls one phase of the oscillation while the second slow variable, $s_2$, controls the other phase. This can be achieved by adjusting $g_{s2}$ so that the dominance curves become separated as in Fig. 4.8B. In Fig. 4.8B, for low values of $g_{s2}$, $DF_{AP} \approx −1$, while $DF_{SP} \approx 1$. This means that $s_1$ is in control of the SP, while $s_2$ controls the AP. For high values of $g_{s2}$, $DF_{AP} \approx 1$ and $DF_{SP} \approx −1$, so $s_1$ is in control of the AP and $s_2$
controls the SP.

When $s_2$ is in control of the active phase, $s_1$ reaches its maximum value very early in the AP, while $s_2$ increases monotonically. If the model cell is reset before the end of the active phase, and if $s_1$ controls the silent phase duration (as in Fig. 4.8B, for $g_{s2} = 27$ pS), then the silent phase will have a full duration. That is, $s_1$ will be starting at the same value almost regardless of when the reset occurs in the active phase. This is shown in Fig. 5.2A. However, when resetting half way through the silent phase, the induced active phase is reduced (Fig. 5.2B). This occurs because $s_2$ is in control of the active phase; $s_2$ is between its minimum and maximum values when the reset occurs, so during the subsequent AP it need only travel a portion of the distance required to terminate the AP. In this case, silent-active resetting is phase dependent (Fig. 5.2C); the duration of the induced AP is closer to the unperturbed AP duration the longer the system is in the SP before the reset. On the other hand, active-silent resetting is approximately phase independent, if resetting occurs after $s_1$ reaches its maximum value (Fig. 5.2D). That is, a reset very early in the active phase does not result in a full-length silent phase, but resets applied at most points during the AP do produce nearly full-length silent phases as in Fig. 5.2D.

### 5.2 Silent-Active Full-length Resetting

When $s_2$ is in control of the silent phase, and $s_1$ controls the active phase (e.g., for $g_{s2} = 97$ pS, Fig. 4.8B), $s_1$ reaches its minimum value early in the silent phase, while $s_2$ decreases monotonically. If the model cell is reset before the end of the silent phase, then the active phase will have a full duration. That is, $s_1$ will be starting at the same value almost regardless of when the reset occurs in the silent phase. This is shown in Fig. 5.3B. However, when resetting half-way through the active phase, the induced silent phase is reduced (Fig. 5.3A). This occurs because $s_2$ is in control of the silent phase; $s_2$ is between its minimum and maximum values when the reset occurs, so during the subsequent SP it need only travel part of the distance required to terminate the SP. In this case, silent-active resetting is nearly phase independent (Fig. 5.3C) and the active-silent resetting is phase dependent (Fig. 5.3D).
Figure 5.3: Resetting with $g_{s2} = 97$ pS and $g_{s1} = 8.5$ pS. In this case, $s_1$ is in control of the AP (DF = 0.79), while $s_2$ is in control of the SP (DF = −0.68). (A) Half-way through the AP the system was reset to the SP (arrow), which is reduced. $s_2$ is midway to its maximum value at the time of resetting (bottom curve). (B) Half-way through the SP the system was reset to the AP (arrow), which has full length. $s_1$ has reached its minimum value at the time of resetting (bottom curve). (C) The duration of the induced AP is close to the width of the unperturbed AP if the resetting occurs after $s_1$ reaches its minimum value. (D) The width of the induced SP is phase dependent.
CHAPTER 6

PHANTOM BURSTING MODEL WITH THREE SLOW VARIABLES

The single slow variable model introduced in Chapter 2 was used to explain fast/slow analysis. Then, Chapter 3 introduced the general phantom bursting model with two slow variables. The general phantom bursting model was used in order to explain and test the method of quantification described in Chapter 4. In pancreatic β-cells, slow processes such as cytosolic Ca\(^{2+}\) concentration, Ca\(^{2+}\) in the endoplasmic reticulum (ER), the ADP/ATP ratio, and slow inactivation of Ca\(^{2+}\) currents have all been postulated to contribute to bursting. Instead of using generic slow variables, the model described in this chapter contains three slow variables that correspond to biological quantities in the pancreatic β-cell: cytosolic free Ca\(^{2+}\) concentration \((c)\), endoplasmic reticulum Ca\(^{2+}\) concentration \((c_{er})\), and the ADP/ATP ratio \((a)\).

6.1 Biology of the β-cell

Figure 6.1 depicts a model β-cell. Like the previous two models, the cell has a fast inward calcium current \(I_{Ca}\) and and a fast outward potassium current \(I_K\). However, this model incorporates a Ca\(^{2+}\)-dependent K\(^+\) current \(I_{K(Ca)}\) and a nucleotide-sensitive K\(^+\) current \(I_{K(ATP)}\). Both of these currents are outward currents. These slow currents correspond to Ca\(^{2+}\)-activated K channels \((K(Ca))\) and ATP-sensitive K\(^+\) channels \((K(ATP))\), respectively. K\(\text{(ATP)}\) channels have been found in islets, cardiac myocytes, cardiac and smooth muscle cells, and various brain regions [2]. In β-cells, K\(\text{(ATP)}\) channels couple blood glucose levels to insulin secretion. These channels are often found in cells that, like β-cells, use ATP as a signaling molecule. K\(\text{(Ca)}\) channels are activated by intracellular Ca\(^{2+}\). After Ca\(^{2+}\) builds inside of the cell, the
K(Ca) channels open adding more hyperpolarizing current to help bring the membrane potential back to the resting state.

The β-cell also contains plasma membrane Ca$^{2+}$ ATPase (PMCA) pumps which remove Ca$^{2+}$ from the cell and sarco-endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) pumps which transport Ca$^{2+}$ from the cytosol to the ER. There is also Ca$^{2+}$ leak out of the ER. While ion channels allow ions of a specific type to move through the cell membrane powered by the concentration gradient, ion pumps hydrolyze ATP to provide the energy to pump ions upstream (against the gradient). In the cell, these pumps affect the cytosolic and ER Ca$^{2+}$ concentrations.

Figure 6.1: Diagram of a model β-cell. The cell has inward current $I_{Ca}$ and outward currents $I_K$, $I_{K(Ca)}$, and $I_{K(ATP)}$. There are also plasma membrane Ca$^{2+}$ ATPase (PMCA) pumps which remove Ca$^{2+}$ from the cell and sarco-endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) pumps which transport Ca$^{2+}$ from the cytosol to the ER. There is also Ca$^{2+}$ leak out of the ER.
6.2 Mathematical Model with Three Slow Variables

The model for pancreatic β-cells with 3 slow variables [8], consists of a Ca$^{2+}$ current, $I_{Ca}$, a delayed rectifier K$^+$ current, $I_K$, a Ca$^{2+}$-dependent K$^+$ current, $I_{K(Ca)}$, and a nucleotide-sensitive K$^+$ current, $I_{K(ATP)}$. The model equations for membrane potential, $V$, delayed rectifier activation, $n$, cytosolic free Ca$^{2+}$ concentration, $c$, ER Ca$^{2+}$ concentration, $c_{er}$, and the ADP/ATP ratio, $a$, are as follows:

$$\frac{dV}{dt} = -(I_{Ca} + I_K + I_{K(Ca)} + I_{K(ATP)})/C_m \quad (6.1)$$

$$\frac{dn}{dt} = (n_\infty(V) - n)/\tau_n \quad (6.2)$$

$$\frac{dc}{dt} = (c_\infty - c)/\tau_c \quad (6.3)$$

$$\frac{dc_{er}}{dt} = (c_{er\infty} - c_{er})/\tau_{cer} \quad (6.4)$$

$$\frac{da}{dt} = (a_\infty(c) - a)/\tau_a \quad (6.5)$$

with ionic currents:

$$I_{Ca} = g_C a m_\infty(V)(V - V_{Ca}), \quad I_K = g_K n(V - V_K) \quad (6.6)$$

$$I_{K(Ca)} = g_{K(Ca)} \omega (V - V_K), \quad I_{K(ATP)} = g_{K(ATP)} a(V - V_K). \quad (6.7)$$

$C_m$ is the membrane capacitance of the cell, the $g$ parameters are the maximum current conductances, the $\tau$ parameters are time constants, and $V_{Ca}$ and $V_K$ are the Nernst potentials. The variable $\omega$ is the fraction of $K(Ca)$ channels activated by cytosolic Ca$^{2+}$:

$$\omega = \frac{c^5}{c^5 + k_D^5} \quad (6.8)$$

where $k_D$ is the dissociation constant for Ca$^{2+}$ binding to the channel.
Figure 6.2: (A) Phantom bursting with three slow variables \((g_{K(Ca)} = 500 \text{ pS})\). (B) The fast variable, \(n\), changes significantly over the duration of a single spike. (C) \(c\) quickly reaches a plateau during the active phase of a burst and has a component that quickly declines during the silent phase. (D, E) \(c_{er}\) (D) and \(a\) (E) slowly increase during the active phase and decline during the silent phase. (F-I) The currents of the system, an inward Ca\(^{2+}\) current, \(I_{Ca}\), a delayed rectifier K\(^{+}\) current, \(I_K\), a Ca\(^{2+}\)-dependent K\(^{+}\) current, \(I_{K(Ca)}\), and a nucleotide-sensitive K\(^{+}\) current, \(I_{K(\text{ATP})}\).

The steady state activation functions for \(m\) and \(n\) depend on voltage:

\[
m_\infty(V) = \frac{1}{1 + \exp[(v_m - V)/s_m]}, \quad n_\infty(V) = \frac{1}{1 + \exp[(v_n - V)/s_n]} \tag{6.9}
\]

The equilibrium function, \(a_\infty(c)\), depends on the cytosolic Ca\(^{2+}\) concentration since Ca\(^{2+}\) affects ATP through its utilization by ion pumps, among other things [21, 25, 41, 42, 44]:

\[
a_\infty(c) = \frac{1}{1 + \exp[(r - c)/s_a]}. \tag{6.10}
\]
The equilibrium functions $c_\infty$ and $c_{er\infty}$ are given by:

$$c_\infty = \frac{p_{\text{leak}} c_{er} - \alpha I_{Ca}}{k_{PMCA} + p_{\text{leak}} + k_{SERCA}}$$  \hspace{1cm} (6.11)$$

$$c_{er\infty} = c(1 + \frac{k_{SERCA}}{p_{\text{leak}}})$$ \hspace{1cm} (6.12)$$

where $\alpha$ converts units of current to units of flux, $k_{PMCA}$ is the pump rate of the plasma membrane Ca$^{2+}$ ATPase pumps, $k_{SERCA}$ is the pump rate for SERCA pumps, and $p_{\text{leak}}$ is the rate of Ca$^{2+}$ leak out of the ER. Lastly, the time constants for cytosolic and ER Ca$^{2+}$ concentrations are:

$$\tau_c = \frac{1}{f_{\text{cyt}}(k_{PMCA} + p_{\text{leak}} + k_{SERCA})}$$ \hspace{1cm} (6.13)$$

$$\tau_{er} = \frac{V_{er}}{f_{er} V_{\text{cyt}} p_{\text{leak}}},$$ \hspace{1cm} (6.14)$$

where $f_{\text{cyt}}$ and $f_{er}$ are the fractions of free Ca$^{2+}$ in the cytosol and ER, respectively, and $V_{\text{cyt}}$ and $V_{er}$ are the volumes of the cytoplasmic and ER compartments. The parameter values are listed in Table 6.1. Figure 6.2 shows an example of bursting produced with this model. All variables and currents are plotted.

### Table 6.1: Parameter values for the three slow variable model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Ca}$</td>
<td>1200 pS</td>
<td>$g_K$</td>
<td>3000 pS</td>
</tr>
<tr>
<td>$V_{Ca}$</td>
<td>25 mV</td>
<td>$V_K$</td>
<td>−75 mV</td>
</tr>
<tr>
<td>$C_m$</td>
<td>5300 fF</td>
<td>$\alpha$</td>
<td>4.5 $\times 10^{-6}$ fA$^{-1}$ $\mu$M ms$^{-1}$</td>
</tr>
<tr>
<td>$\tau_n$</td>
<td>16 ms</td>
<td>$\tau_a$</td>
<td>120000 ms</td>
</tr>
<tr>
<td>$f_{\text{cyt}}$</td>
<td>0.01</td>
<td>$f_{er}$</td>
<td>0.01</td>
</tr>
<tr>
<td>$k_{PMCA}$</td>
<td>0.2 ms$^{-1}$</td>
<td>$k_D$</td>
<td>0.3 $\mu$M</td>
</tr>
<tr>
<td>$k_{SERCA}$</td>
<td>0.4 ms$^{-1}$</td>
<td>$p_{\text{leak}}$</td>
<td>0.0005 ms$^{-1}$</td>
</tr>
<tr>
<td>$v_n$</td>
<td>−16 mV</td>
<td>$s_n$</td>
<td>5 mV</td>
</tr>
<tr>
<td>$v_m$</td>
<td>−20 mV</td>
<td>$s_m$</td>
<td>12 mV</td>
</tr>
<tr>
<td>$V_{\text{cyt}}/V_{er}$</td>
<td>5</td>
<td>$s_a$</td>
<td>0.1 $\mu$M</td>
</tr>
<tr>
<td>$r$</td>
<td>0.14 $\mu$M</td>
<td>$g_{K(\text{ATP})}$</td>
<td>500 pS</td>
</tr>
</tbody>
</table>

Similar to the general phantom bursting model, bursting oscillations with different periods are produced for different values of a parameter. Here, the maximal
Figure 6.3: Bursting with c in control, $g_{K(Ca)} = 1500$ pS (A-D). c is increasing during the active phase and decreasing during the silent phase (B), while $c_{er}$ (C) and $a$ (D) remain constant. Bursting with $c_{er}$ and $a$ sharing control, $g_{K(Ca)} = 500$ pS (E-H). c quickly reaches its maximum during the active phase and its minimum during the silent phase (F), but $c_{er}$ (G) and $a$ (H) slowly increase during the active phase and decrease during the silent phase. Notice the much longer time scale for this latter form of bursting. In all cases, $g_{K(ATP)} = 500$ pS.
Fig. 6.4A with $\omega$ (Eq. (6.8)) as the bifurcation parameter. The variable $c$ could be used as the bifurcation parameter, but the dynamics are clearer with $\omega$ because total $K(Ca)$ conductance is linear in $\omega$. The stationary solutions form the $z$-curve. The solid part of the curve represents the stable solutions, and the dashed part represents unstable solutions. There are two saddle node bifurcations (triangle) where the curve folds. A branch of periodic solutions emerges from a Hopf bifurcation (circle). The periodic branch terminates at an infinite-period homoclinic bifurcation (square). The burst trajectory is superimposed over the $z$-curve showing the system dynamics, with $\omega$ no longer treated as a parameter. The $z$-curve now plays the role of a generalized $V$-nullcline, and the $\omega$-nullcline is added to the figure. Setting $dc/dt = 0$, gives $c = c_\infty$, which is then transformed by Eq. (6.8) to yield the $\omega$-nullcline (orange). During the silent phase, the burst trajectory follows the bottom of the $z$-curve, moving leftward, since it is to the right of the $\omega$-nullcline. Once the lower knee is reached, the phase point moves to the only attractor, the periodic branch that represents the spiking phase of the burst. Since it is now to the left of the $\omega$-nullcline, it moves rightward until the phase point returns to the bottom branch to restart the silent phase.

For a low value of $g_{K(Ca)}$ (500 pS), the burst period is $\sim 70$ sec (Fig. 6.3E). $c$ quickly reaches a plateau during the active phase and has a component that quickly declines during the silent phase (Fig. 6.3F). A second component reflects the dynamics of ER $Ca^{2+}$ handling ([1, 9, 29]). The other two slow variables, $c_{er}$ and $a$, exhibit saw tooth-like oscillations that are characteristic of variables that drive bursting [10]. Thus, $c_{er}$ and $a$ likely play large roles in the bursting oscillation. Figure 6.4B shows the bifurcation diagram with $c_{er}$ and $a$ working together to achieve the burst. Now, there are two $\omega$-nullclines shown (orange) because $c_{er}$ slowly increases during the active phase and slowly decreases during the silent phase, causing the $\omega$-nullcline to move to the right and left, respectively. The effect that $c_{er}$ has on the $\omega$-nullcline can be seen from Eq. (6.11). The curve on the right has $c_{er}$ fixed at its maximum value achieved during bursting, while the curve on the left has $c_{er}$ fixed at its minimum value. There are also two $z$-curves shown. The $z$-curve on the left has $a$ fixed at its maximum value achieved during bursting, while the curve on the right has $a$ fixed at its minimum value. Setting $dV/dt = 0$ in Eq. (6.1) and solving for $\omega$ gives:

$$\omega = -\frac{I_{Ca} + I_K}{g_{K(Ca)}(V - V_K)} - \frac{g_{K(\text{ATP})}a}{g_{K(Ca)}}$$  \hspace{1cm} (6.15)

Therefore, as $a$ increases and decreases, it causes the $z$-curve to shift to the left
Figure 6.4: (A) Fast subsystem bifurcation diagram of bursting with $c$ in control ($g_{K(Ca)} = 1500$ pS), $\omega$ as the bifurcation parameter, $c_{er} = 94.7$, and $a = 0.446$. The $\omega$-nullcline (orange curve) and burst trajectory are superimposed on the bifurcation diagram. The circle represents a Hopf bifurcation, the square represents a homoclinic bifurcation, and the triangles represent a saddle node bifurcation. (B) Fast/slow analysis of bursting with control shared by $c_{er}$ and $a$ ($g_{K(Ca)} = 500$ pS). There are two $\omega$-nullclines (orange curves) shown. The curve on the left has $c_{er}$ fixed at its minimum value (118.1) achieved during the bursting, while the curve on the right has $c_{er}$ fixed at its maximum value (129.8). There are also two z-curves shown. The curve on the right has $a$ fixed at its minimum value (0.46), while the curve on the left has $a$ fixed at its maximum value (0.48). The single headed arrow indicates the direction of movement of the appropriate nullcline when the slow variable increases.

and right, respectively. During the active phase of a burst, the trajectory stalls and would spike continuously without changes in $a$ and $c_{er}$. Due to the spiking, both variables increase, causing the $\omega$-nullcline to shift to the right and z-curve to shift to the left. Once they are shifted far enough, the trajectory escapes the active phase and enters into the silent phase since the $\omega$-nullcline no longer intersects so deeply into the periodic branch. Again the phase point stalls, now in the silent phase. Since $V$ is low, $c_{er}$ and $a$ decrease causing the $\omega$-nullcline to shift to the left and z-curve to shift to the right. Once they are shifted far enough, the trajectory enters into the active phase since the $\omega$-nullcline no longer intersects the z-curve. The arrows under
the slow variable in Fig. 6.4B indicates the direction of movement of the appropriate nullcline when the slow variable increases.

For low values of $g_{K(Ca)}$, $c$ now changes rapidly during the course of a single spike, while $c_{er}$ and $a$ oscillate with a sawtooth pattern over the duration of a burst (Fig. 6.3). Therefore, $\omega$ can now be considered to be part of the fast subsystem. Now, the system can be treated as a phantom burster with two slow variables. This is analogous to the general phantom bursting model with $c_{er}$ playing the role of $s_1$ and $a$ being treated as $s_2$. Figure 6.5 shows the bifurcation diagram with $\omega$ as part of the fast subsystem and $c_{er}$ treated as the bifurcation parameter. The nucleotide ratio $a$ enters the system through the voltage equation; therefore, changes in $a$ affect the $z$-curve. There are two $z$-curves shown. The $z$-curve on the left has $a$ fixed at its maximum value, while the $z$-curve on the right has $a$ fixed at its minimum value. The $c_{er}$-nullcline is also depicted (dotted curve). The $c_{er}$-nullcline is found by setting $dc_{er}/dt = 0$ and replacing $c$ with $c_{\infty}$, since $c$ is now part of the fast subsystem. This gives:

$$c_{er} = -\alpha I_C a (p_{\text{leak}} + k_{\text{SERCA}}).$$  \hspace{1cm} (6.16)

During the active phase of a burst, $a$ increases and shifts the $z$-curve to the left. Eventually, $a$ shifts the $z$-curve far enough that the trajectory can enter into the silent phase. The periodic branch loses stability (open circles) at a period doubling bifurcation, which is why the trajectory falls down before the end of the periodic branch. Now during the silent phase, $a$ decreases, shifting the $z$-curve to the right. The trajectory can then enter into the active phase.
Figure 6.5: Bifurcation diagram with $c_{er}$ as the bifurcation parameter and $\omega$ in the fast subsystem ($g_{K(Ca)} = 700$ pS). There are two z-curves shown. The left z-curve has $a$ fixed at its maximum value (0.468), while the z-curve on the right has $a$ fixed at its minimum value (0.459). The $c_{er}$-nullcline is also shown (dotted curve) along with the burst trajectory. Arrows indicate the direction of movement of the z-curve driven by variations in $a$. 
CHAPTER 7

QUANTIFICATION OF BURSTING WITH THREE SLOW VARIABLES

It has been shown in the previous chapter that bursting oscillations with different periods are produced for different values of $g_{K(Ca)}$. Figures 6.3 and 6.4 showed that variations in $c$ control the termination of both the active and silent phases of bursting with a high value of $g_{K(Ca)}$. However, for low values of $g_{K(Ca)}$ the other two slow variables, $c_{er}$ and $a$, exhibit saw tooth-like oscillations that are characteristic of variables that drive bursting [10]. Thus, $c_{er}$ and $a$ likely play large roles in the bursting oscillation. How large? Do they contribute differentially to the active and silent phases? These questions are discussed in this chapter as the method of quantification is applied to the more biophysical phantom bursting model with three slow variables. This work is in press in the journal Islets [62].

7.1 Background

As stated earlier, pancreatic islets exhibit bursting oscillations. These oscillations are accompanied by oscillations in the free cytosolic $Ca^{2+}$ concentration, which drives pulses of insulin secretion from $\beta$-cells [4, 6, 30]. Voltage clamp studies have shown that islet $\beta$-cells exhibit a slowly activating $K^+$ current, $K_{slow}$, that develops during simulated electrical activity and is $Ca^{2+}$ dependent [31, 32]. Later studies showed that roughly half of the $K_{slow}$ current is mediated by a slow rise in the ATP-sensitive $K^+$ ($K(\text{ATP})$) current [35]. Most recently, it was demonstrated that the other significant component of $K_{slow}$ is mediated by SK4 $Ca^{2+}$-activated $K^+$ ($K(Ca)$) channels [26]. Thus, it appears that $Ca^{2+}$ acts indirectly (through $K(\text{ATP})$) and directly (through SK4) to produce the $K_{slow}$ current. Since this hyperpolarizing current builds up dur-
ing repetitive burst-like spiking, it is likely the key current driving medium bursting in islets [11].

Using the mathematical model of pancreatic \( \beta \)-cells described in the previous chapter, how the factors that comprise \( K_{\text{slow}} \) contribute to bursting can be analyzed. The model contains three variables that change on slow time scales: the cytosolic \( \text{Ca}^{2+} \) concentration \( (c) \), endoplasmic reticulum \( \text{Ca}^{2+} \) concentration \( (c_{er}) \), and the ratio of \( \text{ADP} \) to \( \text{ATP} \) \( (a) \). The \( \text{ADP/ATP} \) ratio determines the number of open \( K(\text{ATP}) \) channels; a larger ratio yields a larger \( K(\text{ATP}) \) conductance. This ratio is determined by the glucose concentration and, in part, by the cytosolic \( \text{Ca}^{2+} \) concentration [21, 25, 41, 42, 44]. Thus, the contribution that the \( K(\text{ATP}) \) current makes to bursting is determined by the contribution that changes in the variable \( a \) make. \( \text{Ca}^{2+} \) acts directly to activate the SK4 channels, so SK4 conductance reflects the \( \text{Ca}^{2+} \) concentration in the cytosol, which in part reflects slow changes in the ER \( \text{Ca}^{2+} \) concentration [1, 8, 29]. Thus, the contribution that the SK4 current makes to bursting is determined by the contributions that changes in the variables \( c \) and \( c_{er} \) make. In this chapter, how the two components of \( K_{\text{slow}} \) can contribute to bursting is examined. It is also demonstrated with the model that their contributions can vary in non-obvious ways depending on the maximal conductances of the \( K(\text{ATP}) \) and SK4 currents, as well as the time scales of the slow variables.

### 7.2 Conductances Reveal Little about the Control of Bursting

In order to understand how the components of \( K_{\text{slow}} \) contribute to bursting, one might think that looking at the conductances of the currents will show which component starts and stops each burst. Figure 7.1 shows the model conductances for the \( K(\text{ATP}) \) \( (g_{K(\text{ATP})} \times a) \) (Fig. 7.1B) and SK4 \( (g_{K(Ca)} \times \omega) \) (Fig. 7.1C) currents, where \( g_{K(\text{ATP})} \) and \( g_{K(Ca)} \) represent the maximal conductance of the channels. The \( K(\text{ATP}) \) conductance is always larger than the SK4 conductance, so one might conclude that the \( K(\text{ATP}) \) current controls both phases of bursting. Certainly if this current were blocked, the model cell would not burst, but would spike continuously. However, this does not mean that the time-dependent variation in the \( K(\text{ATP}) \) conductance plays a role in starting and stopping the bursts. If instead, one looked only at the amplitude of the conductance variations, one would reach the opposite conclusion, since
7.3 Contribution Factors and Dominance Factors

Using the method of quantification described in Chapter 4, the contributions that the three slow variables make to bursting for different values of $g_{K(Ca)}$ were determined. The system is considered to be in the active phase when $V > -55$ mV.
and to be in the silent phase when $V < -55$ mV. For this model, $\delta \tau = \tau$ for the active phase, while $\delta \tau = 0.1 \tau$ for the silent phase. As explained in Chapter 4, when in the active phase of a burst a large $\delta \tau$ is used because a smaller one can lead to the addition of an extra spike in some cases, but not others. Since there are no spikes in the silent phase, a smaller $\delta \tau$ may be used.

Figure 7.2 shows the contribution that each slow variable makes to the termination of the active and silent phases of bursting. As $g_{K(Ca)}$ is increased, the period of bursting decreases (Fig. 7.2A). Figure 7.2B shows the contribution factors of the slow
variables for the termination of the active phase. For low values of $g_{K(Ca)}$, $c_{er}$ (grey bar) and $a$ (black bar) both contribute to the termination of the active phase. As $g_{K(Ca)}$ is increased, the contribution of $a$ declines (the black bar gets smaller). For even larger values of $g_{K(Ca)}$, $c$ is the sole variable contributing to active phase termination (while bar). This shift in control from $c_{er}$ and $a$ to $c$ is expected since increasing $g_{K(Ca)}$ accentuates the K(Ca) current. Similarly, Fig. 7.2C shows the contribution factors for the termination of the silent phase. The contributions of $a$ and $c_{er}$ start out equal for low values of $g_{K(Ca)}$, then $a$ gains control as $g_{K(Ca)}$ is increased. This is unexpected; increasing $g_{K(Ca)}$ actually leads to an increase in the role played by the ADP/ATP ratio to bursting. Only when $g_{K(Ca)} > 1100$ pS does $c$ gain control of the silent phase, as it does for the active phase. Therefore, for low values of $g_{K(Ca)}$, both phases of bursting are controlled by $c_{er}$ and $a$, while for high values of $g_{K(Ca)}$, the direct feedback of $c$ onto SK4 channels is the only component of $K_{slow}$ contributing to bursting in the model.
To summarize the relative contributions to bursting of two slow variables, such as $c_{er}$ and $a$ for small values of $g_{K(Ca)}$, the dominance factor can be calculated for each phase. In this case, a dominance factor of 1 indicates that $c_{er}$ dominates, while a dominance factor of $-1$ indicates that $a$ dominates. The results of quantification using the dominance factor are shown in Fig. 7.3. As $g_{K(Ca)}$ is increased, the period of bursting decreases (Fig. 7.3A). Also, control of the termination of the active phase switches from mostly $a$ to mostly $c_{er}$. Control of the silent phase shows the opposite trend, from equal contributions at low $g_{K(Ca)}$ to mostly controlled by $a$ for higher values of $g_{K(Ca)}$ (Fig. 7.3B). It appears that the control of the active and silent phases is not linked; one slow variable can primarily control the active phase while another can primarily control the silent phase. The distribution of control varies with SK4 channel conductance ($g_{K(Ca)}$), and thus burst period.

### 7.4 Effect of Parameters on the Dominance Factor

The contribution that the slow variables make to bursting depends upon parameter values. Figure 7.3 demonstrated how control over the termination of the active and silent phases changed as $g_{K(Ca)}$ was varied. In Fig. 7.4, the effect of varying $g_{K(ATP)}$ on the dominance factors is shown (while fixing $g_{K(Ca)}$). As $g_{K(ATP)}$ is increased, the duration of the silent phase also increases (black dots), while the duration of the active phase decreases (white dots) slightly (Fig. 7.4A). Likewise, the dominance factor for the silent phase increases with $g_{K(ATP)}$, but the dominance factor for the active phase decreases (Fig. 7.4C). This is surprising, since one might expect that increasing the maximal conductance for the K(ATP) current would give $a$ more control over both the silent phase and the active phase, but it partially loses control of the silent phase. It is interesting to note that the silent phase also acted in a non-intuitive way when $g_{K(Ca)}$ was increased (Fig. 7.3B). With $g_{K(Ca)}$ set to a higher value (700 pS) so that the bursting was much faster, increasing $g_{K(ATP)}$ had a similar effect on the dominance factors (Fig. 7.4B,D). The duration of the silent phase rises significantly with the increases of $g_{K(ATP)}$, as does the dominance factor. The duration of the active phase changes little, yet there is a substantial shift of control from mostly $c_{er}$ to equal contributions of $c_{er}$ and $a$.

Other parameters that affect the contribution and dominance factors are the time...
Figure 7.4: Changes in durations and the dominance factors while changing $g_{K(ATP)}$ and keeping $g_{K(Ca)}$ constant. (A,B) As $g_{K(ATP)}$ is increased, the silent phase duration increases, while the active phase duration decreases. (C,D) As $g_{K(ATP)}$ is increased, $DF_{AP}$ decreases, while $DF_{SP}$ increases. ($g_{K(Ca)} = 25$) pS for A and C, while $g_{K(Ca)} = 700$ pS for B and D.

constants of the slow variables. These parameters determine how rapidly the variables change (a large time constant $\tau$ means a slow rate of change). Figure 7.5A shows the effect on the dominance factor of increasing the time constant for $a$ ($\tau_a$) with $g_{K(Ca)} = 600$ pS and $g_{K(ATP)} = 500$ pS. As $\tau_a$ is increased $a$ becomes slower and contributes less to the termination of the active phase (white bars) and the silent phase (grey bars). It is interesting to note that $a$ always has substantial influence over the silent phase, even when $\tau_a$ is very large. Figure 7.5B shows the effect of
Figure 7.5: (A) Effect of changing the time constant $\tau_a$ on the dominance factor. As $\tau_a$ increases, the dominance factor increases for both the active phase and the silent phase, indicating that $a$ loses control. The default value for $\tau_a$ is 2 min. (B) Effect of changing $\tau_{cr}$ on the dominance factor. As $\tau_{cr}$ increases, the dominance factor decreases for both the active and silent phases, indicating that $a$ is gaining control. The default value of $\tau_{cr}$ is 0.67 min. In both panels, $g_{K(c_a)} = 600 \text{pS}$ and $g_{K(\text{ATP})} = 500 \text{pS}$.

Increasing $\tau_{cr}$. As $\tau_{cr}$ is increased, $c_{cr}$ gets slower and the dominance factor decreases. Therefore, $a$ gains control over both the active phase (white bars) and silent phase (grey bars). Therefore, it has been shown that the dominance of a slow variable depends in part on the rate of change of that variable. Slowing down a variable leads to a decline in the contribution it makes to burst initiation and termination.

7.5 Summary

Using the mathematical model of the pancreatic $\beta$-cell described in Chapter 6, the question of how the two known components of the $K_{\text{slow}}$ current can contribute to bursting oscillations in islets was addressed. First, it was shown that the time-dependent conductances of the currents provide ambiguous information about the control of bursting. A better approach is to examine the variables that underlie the conductances. Using the method of quantification introduced in Chapter 4, it was shown that the control of the phases of bursting is adjustable. In particular,
it depends on the values of maximal conductances and the rate of change of the variables underlying the currents. For low values of $g_{K(Ca)}$, $c_{er}$ and $a$ shared control of the bursting, while for larger values $c$ had control (Fig. 7.2). It was also shown that the active and silent phases of bursting do not have to be controlled by the same variable (Fig. 7.3). Finally it was shown that as parameters are varied, the contributions of $a$ and $c_{er}$ change in non-intuitive ways.
CHAPTER 8

CONCLUSION

Using two different mathematical models of the pancreatic β-cell [7, 8], this dissertation determined the contributions that slow variables make to the termination of the active and silent phases of bursting and relaxation oscillations. While techniques to analyze bursting, such as singular perturbation analysis (fast/slow analysis), are useful in understanding the bursting mechanism and how the slow variables work together to produce bursting, they do not give a quantitative measurement for how much each variable contributes to the termination of the active and silent phase of a burst. Chapter 2 of this dissertation introduced fast/slow analysis and demonstrated the analysis on a model with a single slow variable. This analysis was then expanded to the general phantom bursting model (Chapter 3) and the more biophysical phantom bursting model with three slow variables (Chapter 6). Even though this analysis is a useful tool in understanding the bursting produced by a model, being able to quantify the contributions of the slow variables to bursting can tell us more about the model cells.

Using the method of quantification described in Chapter 4, it was shown that control of the phases of bursting is adjustable. In particular, it depends on the values of maximal conductances (\(g_{s1}\) and \(g_{s2}\) for the general phantom bursting model and \(g_K(Ca)\) and \(g_K(ATP)\) for the biophysical phantom bursting model). For the biophysical phantom bursting model, it was also shown to depend on the rate of change of the variables underlying the currents (\(\tau_{cer}\) and \(\tau_a\)). The results of quantification of the general phantom bursting model with two slow variables was shown in Chapter 4. For low values of \(g_{s1}\), \(s_2\) controlled bursting, while for larger values \(s_1\) had control (Fig. 4.6C). The more interesting case was for values of \(g_{s1}\), such as \(g_{s1} = 8\) pS, where \(s_1\) and \(s_2\) shared control (Fig. 4.6C). While one slow variable controlled
the active phase, the other controlled the silent phase. This control could shift by changing the parameter $g_{s2}$ (Fig. 4.8B). The results for the more biophysical phantom bursting model with three slow variables was given in Chapter 7. For low values of $g_{K(Ca)}$, $c_{er}$ and $a$ shared control of the bursting, while for larger values $c$ had control (Fig. 7.2B,C). Like in the general phantom bursting case, it was also shown that the active and silent phases of bursting do not have to be controlled by the same variable (Fig. 7.3B). For example, when $g_{K(Ca)} = 700$ pS and $g_{K(ATP)} = 500$ pS, the active phase is mostly controlled by $c_{er}$, while the silent phase is mostly controlled by $a$. This situation is altered when $g_{K(Ca)}$ is lowered to 25 pS. Now, the active phase is mostly controlled by $a$ and both $a$ and $c_{er}$ control the silent phase (Fig. 7.3B). It was also shown that as parameters are varied, the contributions of $a$ and $c_{er}$ change in non-intuitive ways. For example, as $g_{K(Ca)}$ is increased, $a$ gains more control over the silent phase (Fig. 7.3B), while as $g_{K(ATP)}$ is increased, $c_{er}$ gains more control over the silent phase (Fig. 7.4C,D).

The method of quantification assumes that the slow negative feedback variables are the only variables responsible for burst termination and that they vary monotonically during each phase of the burst. In that case, all the contribution factors $C$ should be positive and below 1. Also, for the general phantom bursting model, $C_{s1} + C_{s2} = 1$. There is good agreement to this rule in the phantom relaxation case (Fig. 4.3B), but not in the general phantom bursting case (Fig. 4.6). This is because during medium bursting $s_1$ does not vary monotonically. Instead, it quickly reaches a high value during the active phase, then slowly decreases since the spikes can push the $V-s_1$ trajectory below the $s_1$-nullcline. This decrease of $s_1$ slows down the termination of the active phase, instead of $s_1$ steadily contributing to its termination. This is why for some values of $g_{s1}$, $C_{AP}^{s1} < 0$ or $C_{SP}^{s2} > 1$. Nevertheless, the results are qualitatively similar to those obtained in the phantom relaxation case. Finally, the method should in principle be used with small $\delta \tau$. Unfortunately, the spikes make the active phase duration discontinuous, so the $C_{AP}$ values become vary variable when $\delta \tau$ is small. To avoid this problem, $\delta \tau = \tau$ was used after having checked that in the phantom relaxation case the results were quantitatively similar with $\delta \tau = 0.05 \tau$ and $\delta \tau = \tau$. Thus, the method that was originally developed for a relaxation oscillation [59] can be extended to bursting. For phantom bursting with three slow variables, it would follow that $C^c + C^{c_{er}} + C^a = 1$. For low values of $g_{K(Ca)}$, the sum of the contribution factors is close to or less than 1. However, for high values of $g_{K(Ca)}$, $C^c$
could be greater than 1. To avoid the same problem found in the general phantom bursting case, \( \delta \tau = \tau \) was used for the AP, but \( \delta \tau = 0.1 \tau \) was used for the SP.

It is difficult or impossible to do this type of analysis experimentally, since it requires an acute increase in the time constants that are typically not controllable. This highlights one advantage of mathematical models, where all parameters are controllable. It may be known from experiments that two or more slow processes are involved in the burst generation, but without a model it is difficult or impossible to know how much each variable contributes to bursting. This is the case with pancreatic \( \beta \)-cells, where slow variables such as the cytosolic Ca\(^{2+} \) concentration, the Ca\(^{2+} \) in the endoplasmic reticulum, the ATP/ADP ratio, and slow inactivation of Ca\(^{2+} \) currents have all been postulated to contribute to bursting. With the development of models containing some or all of these slow processes, and the technique described here, it becomes possible to identify the key processes driving the bursting. Also, parameters that can be modified in the lab through pharmacological means, such as the ionic conductances, may change the burst mechanism. However, one can not tell just by looking at the voltage trace. For example, Fig. 7.3A shows a quantitative change in the durations of the active and silent phases of bursting as \( g_{K(Ca)} \) is increased. This can be seen experimentally. However, the dominance factor also changes (Fig. 7.3B), reflecting a modification of the burst mechanism. As another example, Fig. 7.4B shows little change in the active phase duration as \( g_{K(ATP)} \) is varied, yet control of the active phase shifted from mostly \( c_\text{er} \) to equal contributions from \( c_\text{er} \) and \( a \). These changes would be hard to see without the aid of a mathematical model. The technique applied here could be applied to any \( \beta \)-cell model with more than one slow variable. The quantitative relationship of the processes to burst production varies with parameter values and undoubtedly varies between models. However, the point of adjustable collaboration to burst production should be valid for models that are more complex than the ones used here.

Finally, one application of the dominance factor described in Chapter 5 is in determination of parameter values that allow phase-independent resetting in the general phantom bursting model. Such resetting was documented in islets nearly 30 years ago [23]. An earlier model [58] was able to account for this. However, that model was not a phantom bursting model and thus the burst period was constrained to a relatively narrow range of values. The general phantom bursting model possesses both the desired full-length reset properties (Fig. 5.2 and 5.3), and can produce the
wide range of burst periods that is characteristic of pancreatic β-cells. The analysis predicts unidirectional full-length resets for medium bursting islets, but not for fast or slow bursting islets. Active-silent full-length resets should be found in islets with a long active phase and a short silent phase (Fig. 5.2), while silent-active full-length resets should be found in islets with a short active phase, but a long silent phase (Fig. 5.3). For fast and slow bursting only one variable controls the duration of both phases, so full-length resets should not occur.

It should also be mentioned that the analysis described in this dissertation is the only method available to quantify the contributions that slow variables make to bursting. A potential alternative, called lead potential analysis [16], has been developed to quantify the contribution of each cellular component to changes in the membrane potential in cardiac physiology. This method makes use of a theoretical equilibrium potential (the lead potential, $V_L$), which is calculated while leaving the time course of $V$ intact. The problem with this type of analysis is that the results describe which currents are the largest. Each current is given a relative contribution and the sum of the relative contributions of all the currents equal 1. If this method is applied to the general phantom bursting model, $I_K$ and $I_{Ca}$ always have greater contributions than $I_{s1}$ and $I_{s2}$ because the currents are larger (Fig. 3.2). Therefore, this method does not help determine which of the slow variables is in control of the bursting, which is the question addressed here.
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BIOGRAPHICAL SKETCH

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Margaret Watts was born on January 19, 1985 in Baton Rouge, LA. After graduating as Valedictorian of her high school class, Margaret wanted to study at Spring Hill College in Mobile, AL where she graduated summa cum laude and received her B.S. degree in Mathematics and B.A. degree in Philosophy in 2007. The same year, she also received the President’s award in both Mathematics and Philosophy. During her senior year at SHC, she discovered the field of Mathematical Biology and became interested in pursuing a doctorate degree at Florida State University. While at FSU, she has worked as a research assistant and teaching assistant. Under the advisement of Richard Bertram, she received her Master’s Degree in 2009 and doctorate in 2011. As of 2011, she has published two peer-reviewed papers and presented her work at the Frontiers in Applied and Computational Mathematics Conference at the New Jersey Institute of Technology (May 2010).