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Implementation and analysis of neuromodulatory mechanisms in a mathematical model of neuron R15 in Aplysia

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Rice University, 1994
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Implementation and Analysis of Neuromodulatory Mechanisms in a Mathematical Model of Neuron R15 in Aplysia

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE Master of Science

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Abstract

An existing model of the bursting neuron R15 in *Aplysia* has been modified to incorporate the effects of the neuromodulatory agents serotonin and dopamine upon the conductances of the subthreshold currents $I_R$ and $I_{SI}$. The model duplicates the various forms of behavior exhibited by R15 *in vitro* in the presence of serotonin or dopamine, implying that the modeled mechanisms are sufficient to evoke the varied behavioral patterns exhibited by R15. The response of the model to extrinsic stimuli is examined. Serotonin enhances the cell's response to current stimuli of either polarity, while dopamine reduces the cell's response. The model demonstrates bistable behavior, indicating that the model's current state of behavior is dependent upon past behavior and/or stimuli. A nullcline and bifurcation analysis upon reduced models explains the mechanism of bursting and the effects of serotonin and dopamine through the existence of saddle-node and Hopf bifurcations.
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Chapter 1

Introduction

One of the most heavily studied animals in neurobiology is the aquatic “sea-hare” of the genus Aplysia. Also inappropriately referred to as “sea-slugs”, they are actually molluscs with a soft internalized shell. A picture of a typical Aplysia is shown in Fig. 1.1.

1.1 History of the Study of Aplysia

Aplysia have been studied by biologists for hundreds of years. Pliny the Elder made many references to a large marine snail, now believed to be an Aplysia, in his work Naturalis Historia, written in the first century A.D. [50]. Other early ancient scholars named it Lepus Marinus (sea-hare), since its thick tentacles and flabby posterior body resemble a rabbit when the animal is in a contracted position [13]. The sea-hare was rediscovered by French naturalists during the Renaissance, and its internal anatomy was first described in the late 17th and early 18th century. Linnaeus referred to the sea-hare by multiple names in successive editions of Systema Naturae [50], and the genus name Aplysia, meaning “that which does not wash” (a reference to its inking defense mechanism) was made by Gmelin in 1789 [31].

Detailed anatomical studies were first made of Aplysia by Cuvier in 1803, and he was the first to distinguish between multiple species of Aplysia. The first monograph on Aplysia was written by Rang in 1828, who is credited with being the first student of the natural history of Aplysia. Both Cuvier and Rang were also known for their detailed lithographs and woodcuts of Aplysia, such as those depicted in Fig. 1.1. There are now at least 35 known species of Aplysia, mostly (but not exclusively) in
Figure 1.1: *Aplysia depilans* in its relaxed and contracted states
These images are actually woodcarvings by S. Rang, who wrote the first monograph on *Aplysia*, *Histoire Naturelle des Aplysiens*, in 1823. Reproduced from [50].
coastal tropical and subtropical climates, such as the Mediterranean, the Texas and Florida gulf coasts, and California [50].

In the early 20th century, *Aplysia* started to become heavily studied by molluscan biologists. The definitive contemporary references on the genus *Aplysia* are those written by Eales [31, 32]. In the mid-1950's, *Aplysia* caught the attention of neurobiologists, and today is one of the most heavily studied animals in neurobiology.

1.2 Why Study *Aplysia*?

According to one biologist, an *Aplysia* has “a simple repertoire of behavior - it does nothing but eat, rest, and copulate - and that's what makes it important ... if you go into something complex, like a mouse or rat, it just makes life more difficult” [71]. Virtually every aspect of *Aplysia*’s central nervous system and sensory organs have been studied, including its vision system, food chemoreceptors, osmoregulation of water and salt balance, mechanoreception, circulatory regulation, sleep cycles, and circadian rhythms in the eye [50, 86]. However, the animal is most noted as a tool for the study of the cellular basis of learning and behavior [51]. To quote Dr. Eric Kandel, a pioneer in behavioral neurobiology, “Aplysia is very good for studying higher-order features, such as how synapses change as a result of experience, how they are modified in short and long-term memory, and what molecular events underly memory” [71]. For example, the *Aplysia* “gill-withdrawal reflex” exhibits many aspects of classical conditioning. The animal withdraws its gill when a stimulus is applied to another part of the body, such as the mantle-shelf or the siphon. Both the mantle-shelf and the siphon possess sensory neurons which directly contact motor neurons for the gill, initiating gill-withdrawal. These sensory neurons also synapse onto numerous excitatory and inhibitory interneurons, which in turn also synapse upon the motor neurons which control the gill. By using both the mantle-shelf and siphon as “control inputs”, classical conditioning can be demonstrated, and the mechanisms underlying
conditioning have been investigated at the subcellular level (see [51] for a general overview).

1.3 Anatomy of Aplysia

1.3.1 General Anatomy

Molluscan biologists theorize that all molluscs today are descended from one basic ancestral body plan with four basic features [50]. Since these features describe the basic anatomy of Aplysia quite well, they are repeated here for reference. Figure 1.2 is a dorsal view of Aplysia punctata, and Fig. 1.3 is a side view of the molluscan ancestral body plan. The four basic features are: 1) a head with a mouth, two eyes, and tentacles; 2) a single foot (akin to that which a snail uses to crawl around); 3) a visceral mass which contains the heart, kidneys, digestive glands, and reproductive organs; and 4) a covering for the entire body called the mantle. From my own personal experience, the visceral mass actually occupies a small portion of the internal body. The rest of the internal space is referred to as the mantle cavity, and is predominantly a respiratory space into which the gills open. This emptiness gives the animal the appearance of a “bag of water” when it is initially opened up for dissection.

1.3.2 Nervous System

The nervous system of Aplysia is especially ideal for study. The neurons are clustered into eight different identifiable nerve ganglia (some in pairs) which are anatomically outlined in Fig. 1.4. These ganglia generally have an orange and white appearance, and are connected by long thin filament-like nerve fibers which run through the animal’s body cavity. Aplysia have only about 20000 neurons, which is very small compared to vertebrate nervous systems. Some of these neurons are among the largest of any known animal [71], making them ideal for study.
Figure 1.2: Dorsal view of *Aplysia punctata*
Major external body structures are indicated. Reproduced from [50].
Figure 1.3: Generic body plan of molluscs

All molluscs possess a body plan with similar major features. The above illustration, although generic to molluscs, accurately depicts the internal organization of a typical *Aplysia*. Reproduced from [50].
Figure 1.4: Nervous system of *Aplysia californica*
All major ganglia are indicated. This is a dorsal view, with the head of the body at the top of the illustration. Compare with Fig. 1.3, which indicates the location of many of the ganglia with respect to major body organs. Reproduced from [50].
Figure 1.5: Dorsal and ventral cell maps of the abdominal ganglion
The above illustration is particular to *Aplysia californica*, but is rather homologous across all *Aplysia* species. Reproduced from [49].
The neuron which is the subject of this study, R15, is located in the abdominal ganglion, referred to as the visceral ganglion in some of the earlier literature (pre-1967). The neurons in this ganglion have been implicated in the control of numerous functions, including reproduction, respiration, circulation, excretion, defensive movement of the mantle, and neuroendocrine processes (such as egg-laying and water balance) [50]. The major cells of this ganglion were systematically identified and named in 1967 [26], although a few had been studied earlier. This ganglion is depicted in Fig. 1.5. At the same time, the intrinsic behavior of many of the neurons in the ganglion were mapped out [36], and interconnections between several of the neurons were identified. Neurons were classified as having one of three modes of electrical behavior: 1) silence, in which the cell remains in a hyperpolarized state of electrical silence; 2) beating, in which the cell fires action potentials at a (somewhat) steady tonic frequency; and 3) bursting, in which the cell alternates between a state of hyperpolarized silence and a grouped discharge of multiple action potentials. These three different modes are shown in Fig. 1.6.

1.4 Studies on R15

The bursting behavior of selected neurons in the abdominal ganglion is of great interest to neurobiologists. Although many biological neural networks are known to exhibit bursting behavior, there are fewer examples of single cells that exhibit endogenous bursting behavior. The abdominal ganglion possesses not just one, but five types of bursting neurons [4]. Three types are individual endogenous bursters: R15, L10, and the LUQ cells (L3 to L6). There are also two population bursters, the “bag-cells” and the L25/R25 network, whose bursting is a product of the synaptic interactions between the neurons of the population, and initial electrical or hormonal stimulation of these neurons are necessary to initiate bursting. The physiological function of these neurons are known to varying degrees. A recent comparison of these neurons and their known physiological functions is presented in [4].
Figure 1.6: Different behavioral patterns recorded from neuron R15. 
R15 demonstrates three different modes of behavior: beating (A), bursting (B), and silence (C). These modes can be switched between by application of appropriate neurotransmitters or a constant stimulus current. Recordings are from the Department of Neurobiology and Anatomy, University of Texas Medical School, courtesy of Drs. Doug Baxter (A and B) and Jason Goldsmith (C). Data was sampled at 3.33 kHz.
Figure 1.7: Typical bursting patterns recorded from neuron R15
Intracellular recordings were made in the Department of Neurobiology and Anatomy, University of Texas Medical School. Recording in A was made by myself, recording in B (from a second R15 cell) was made by Dr. Doug A. Baxter. Data was sampled at 1 kHz, which is why some of the action potential peaks are not adequately captured. Some noise in the tape recording unit is also evident in the trace in A. These recordings demonstrate the inherent quantitative variability in bursting between R15 cells. Specifically, note the differences between A and B regarding the length of the burst phase and the length and depth of the interburst hyperpolarization phase.
1.4.1 Experimental Studies

Neuron R15 was previously described as having an intrinsic bursting behavior. Today, this cell is the second-most studied nerve preparation (after the giant squid axon), and is often portrayed as the stereotypical bursting neuron. The bursting behavior of R15 was initially discovered in the mid-1950's independently by Arvanitaki and Chalazonitis [8] and Tauc [87]. Some of the early experimental work was also performed by Strumwasser, who coined the phrase “parabolic burster” to describe the nature of R15's bursting [86]. This phrase describes the parabolic increase and decrease of spike-frequency that occurs during bursting. For an extensive review of the known published R15 literature (through 1985), refer to [3]. Figure 1.7 shows typical recordings of endogenous bursting in R15.

During the last two decades evidence has been accumulating from a variety of sources indicating that second messenger systems can modulate neuronal excitability, and that several types of membrane ionic currents can be modulated by a single second messenger within an individual cell [16, 64, 65]. Modulation of multiple voltage and ligand-gated ion channels can provide the neuron with the capability of producing complex responses when second messenger systems are stimulated by neurotransmitters. R15 is known to be modulated by either bath or localized application of numerous neurotransmitters and second messenger analogues, including acetylcholine (ACh), dopamine (DA), Aplysia egg-laying hormone (ELH), Phe-Met-Arg-Phe-amide (FMRFamide), γ-aminobutyric acid (GABA), serotonin (5-HT), cyclic-adenosine monophosphate (cAMP), and cyclic-guanosine monophosphate (cGMP) [19, 30, 55, 60, 63]. Due to the large amount of experimental data available, this study is focused upon the effects of 5-HT [30, 11, 61, 66] and DA [39, 40, 66]. Nevertheless, the effects of 5-HT and DA are very similar to the effects of ELH [60] and FRMFamide [55], respectively. At appropriate concentrations, 5-HT is capable of modifying the endogenous behavior of R15 from its normal bursting state to silence,
elongated bursting, or tonic beating [30, 61]. Appropriate doses of DA can bias the cell to a hyperpolarized silent state [39].

1.4.2 Functional Studies

Considerable research effort has been directed toward determining the physiological function of R15. Experimentation has occurred not just on the isolated ganglion but in intact animal preparations as well. Following is a brief summary of what is known so far.

The bag cells in the abdominal ganglion of Aplysia are normally silent. However, brief (1-2 sec) electrical stimulation of these cells elicits sustained spiking activity in the bag-cells that last for over twenty minutes [59]. This spiking is accompanied by a release of multiple peptides that diffuse throughout the abdominal ganglion. Injection of the releasate from this discharge into intact Aplysia initiates egg-laying [58]. After the start of a bag-cell discharge, R15 demonstrates an increase in burst intensity and number of spikes per burst [15]. One of the peptides released during bag-cell discharge, ELH, demonstrates similar effects upon R15 when applied locally to the cell soma [14]. This observation suggests that the effects of bag-cell discharge upon R15 and other cells in the abdominal ganglion may not occur by synaptic contact, but by local diffusion of ELH (and other peptides) throughout the ganglion [15, 14, 69]. This viewpoint has been further supported by the finding that the releasate from bag-cell stimulation in an in vitro preparation produced prolonged excitation of cells in a second assay ganglion [70]. The assay ganglion was located in a separate chamber which received the perfusate/superfusate from the chamber containing the source ganglion.

R15 is believed to integrate various aspects of egg-laying behavior, acting a mediator of some of the excitatory effects of bag-cell release. These effects include the excitation of the R25/L25 network that triggers respiratory pumping [5], excitation of motoneuron L7 which mediates contraction of the pleuroabdominal connectives [6],
and activation of peristaltic movements in the segment of the large hermaphroditic duct through which eggs move during egg-laying behavior [7]. All of these effects are mediated by R15α1 peptide, a putative neurotransmitter released from the synaptic endings of R15. This peptide has also been found to cause an increase in water retention when injected into *Aplysia* [89].

1.4.3 Modeling Studies

Due to its unique bursting behavior, R15 has been the subject of modeling studies for over 20 years. Early models were used to mimic the mechanisms producing the cyclic slow-wave behavior which underlies bursting. The first known study is that of Strumwasser [86], who tried to model the slow-wave behavior of R15 on an analog computer in the late 1960’s. A systemic diagram of this model is shown in Fig. 1.8. Although the specific mechanisms considered by Strumwasser have long since been refuted, it is interesting to note that as far back as the 1960’s physiologists sought to better understand the mechanisms underlying bursting behavior using mathematical modeling techniques. Modeling studies used as an adjunct to experimental investigation can demonstrate the feasibility of different mechanistic theories, as well as simulate experiments that might be difficult or even impossible to perform *in vivo* or *in vitro*. For example, some ionic membrane currents, for various technical reasons, cannot be isolated and quantitatively characterized. Mathematical models allow one to "fill in the blanks" for such currents, and demonstrate that the behavior of the model agrees qualitatively with experimental observations. Besides studying the physiological role of R15, the multiple modes of behavior exhibited by R15 make it a useful neuron to study as a model Central Pattern Generator in neural networks.

Numerous mathematical models, based upon Hodgkin-Huxley (HH)-type membrane dynamics, have been developed to model the intrinsic activity of R15 [3, 12, 18, 22, 74, 76]. Some of these models are empirical in nature, others are adaptions of the HH membrane model for squid axon [45], while still others are based upon
Figure 1.8: Block diagram of an early quantitative model accounting for endogenous pacemaker oscillations in R15. This model was implemented on an analog computer in the late 1960's. This model assumed that the bursting mechanism was due to Na⁺ influx by an inward sodium current and Na⁺ efflux by a Na-Cl pump with asymmetrical hysteresis. Later work has repudiated this mechanism. However, this figure demonstrates that long before computing technology allowed us to make descriptive models based upon quantitative measurements, physiologists were employing computational models as an investigative tool. Reproduced from [86].
quantitative published measurements wherever possible. R15 is not the only known bursting cell. The pancreatic $\beta$-cell is another bursting cell that is involved in insulin production in higher animals, and has been modeled quite extensively [22, 24, 25, 52]. Mathematically, a bursting system can be described as one whose dynamics operate on two different time scales, in which a slow oscillation makes a passage through a region which produces rapid oscillations. Such bursting behavior is not demonstrated exclusively in electrophysiological models, and is also observed in numerous chemical and biochemical systems [27, 35, 67].

The model presented here is a modification and extension of a previously published model of the R15 neuron[18]. The objective of the current modeling study is to simulate the wide range of behavior exhibited by R15 in vitro in the presence of various bath concentrations of 5-HT or DA. This new model will be developed in Chapter 2, while the behavior of the model in the presence of varying concentrations of DA and 5-HT is presented in Chapter 3. The response of the model to depolarizing and hyperpolarizing current pulses in the presence (and absence) of 5-HT and DA is also investigated in Chapter 3, and these results are used to interpret similar experimental observations. The techniques of nonlinear analysis and bifurcation analysis [29] are employed in Chapter 4 to mathematically investigate the mechanisms which underly 1) the onset of a burst during a burst cycle, and 2) the neuromodulation of the slow-wave which underlies bursting behavior. This is achieved by examining reduced-order systems via the application of multiple scale techniques.
Chapter 2

Model Development

The original model consists of two components: a Hodgkin-Huxley (HH) type model of the membrane dynamics and a fluid compartment model that characterizes the intra- and extracellular media associated with the neuron soma. For specific details regarding the fundamental development of the model, the reader is directed to the original model [18]. Below, the membrane and fluid compartment models are briefly reviewed, and indications are given where modifications have been made, particularly in the response of certain ionic currents to the presence of neuromodulatory agents.

2.1 Membrane Model

Figure 2.1 shows the HH-type equivalent circuit model of the membrane. Under space-clamped conditions the equation for the time rate of change of membrane potential (V) is:

\[ \dot{V} = -(I_{Ca} + I_{Na} + I_{K_a} + I_{Na_C} + I_N + I_{Ca} + I_{SI} + I_{NS} + I_{K} + I_R + I_L - I_{STIM})/C_m \]  (2.1)

where the component membrane currents are as defined in the original model, \( C_m \) is the whole-cell membrane capacitance, and \( I_{STIM} \) is an applied stimulus current. Modifications to the membrane currents, as well as the development of the equations describing the effects of neuromodulation, are described below. The complete set of modeling equations for the modified R15 model is given in the Appendix.

Conventional microelectrode recordings of endogenous bursting of R15 were made. The data was digitized, and a numerical fourth-order central difference scheme [46] was applied to the data to obtain an estimate of the time rate of change of membrane
**Figure 2.1:** Equivalent circuit model of the whole-cell membrane

Nonlinear conductances are indicated by rectangular boxes. Time-dependent conductances are represented by horizontal black bars. Conductances modulated by 5-HT (via cAMP) and/or DA are highlighted. In addition to the seven ionic currents, the model consists of a $Na^+/K^+$ pump ($I_{NaK}$), a $Ca^{2+}$-extrusion pump ($I_{CaP}$), and a sodium-calcium exchanger ($I_{NaCa}$).
Figure 2.2: Comparison of model-generated and experimentally-obtained instantaneous I-V plots

Data shown is for all action potentials within one burst for both model-generated and experimentally obtained data. Instantaneous current was calculated from experimental data by sampling the burst at 300 μs, then employing a fourth-order central difference method with an assumed whole-cell capacitance of 15 nF. The arrows indicate the currents that make major contributions at different phases of the action potential. The upstroke of the action potential is initiated by $I_{Na}$. $I_K$ is responsible for the initial repolarization of the action potential. $I_{Ca}$ is activated on the repolarizing phase of the action potential, delaying the rate of repolarization. Insets show the time-course of the model-generated and experimental data.
potential ($\dot{V}$) of the action potentials that occur during a burst. Equation (2.1) assumes space-clamp conditions where the total transmembrane current is zero. From eqn. (2.1), the capacitive displacement current is given by:

$$I_d = C_m \dot{V} = - \left[ \sum I_{ion} - I_{STIM} \right]$$

(2.2)

where $\sum I_{ion}$ represents the sum of the ionic membrane currents. Using the formula $I_d = C_m \dot{V}$, together with an estimate of the whole-cell capacitance (15 nF), an instantaneous I-V plot was generated (Fig. 2.2), where the current ordinate is $-I_d$ and $I_{STIM}$ is zero. These I-V plots indicate instantaneous membrane current and are particularly useful in comparing the dynamic response of experimentally-obtained and model-generated action potential data. Due to the inherent cell-to-cell variability in cell capacitance and burst characteristics, the data shown in Fig. 2.2 has not been fit rigorously. The character of the action potential is not a focus of this study; however, we wish to demonstrate that the dynamics of the currents underlying the action potential are qualitatively similar for both the model and the cell's endogenous behavior. The sequence of activation and inactivation of the fast currents underlying the action potential are seen in Fig. 2.2 as the instantaneous I-V loop is followed in a counter-clockwise direction. Specifically, upstroke is initiated by a rapid Na$^+$ current ($I_{Na}$), and the activation threshold is similar in both the model-generated and experimental data (Fig. 2.2). Repolarization occurs by activation of the delayed-rectifier K$^+$ current ($I_K$). The fast Ca$^{2+}$ current ($I_{Ca}$) is predominantly active during the repolarizing phase of the action potential. Such delayed activation of $I_{Ca}$ has also been noted in an experimental and modeling study of the squid giant presynaptic terminal [9]. The general character of the I-V plots for the model-generated and experimental data are similar, including the inward notch seen in the repolarizing phase of the I-V plot due to the delayed activation of $I_{Ca}$. Figure 2.2 contains data from all of the action potentials within a burst, and both the model-generated and experimental data indicate that the phenomena of spike-broadening occurs over the
course of the burst cycle. This is particularly evident during the spike-repolarization phase of the action potential (Fig. 2.2), and in the model it is due to the progressive activation of $I_{Ca}$ and inactivation of $I_K$ over the time-course of a burst.

When this comparison to experimentally-derived data was first made with the original model (not shown) the I-V plots differed significantly, particularly during the repolarization phase of the action potential. Specifically, we observed 1) an excessive amount of inward current during the repolarization phase of the model-generated data, and 2) a second inward notch in the character of the repolarization phase of the model-generated I-V plot. Both of these differences were attributed to an excessive amount of $Na^+$ reactivation in the original model. It was also found that a modification of $I_{Ca}$ altered the character of the "notch" produced by inward current that appears during the repolarization phase of the I-V plot (Fig. 2.2).

In the following paragraphs, we will explain the modifications made to the currents of the original model. Please refer to [18] for an explanation of the mechanisms and formulation of each of these currents.

2.1.1 Inward Currents

Fast Na$^+$ Current ($I_{Na}$). The excessive amount of inward current during repolarization of the action potential was attributed to a large degree of overlap between the steady-state activation and inactivation curves of the gating variables $m$ and $h$, respectively. This was corrected by shifting the expressions for $m_\infty(V)$ in the depolarizing direction by 7 mV and $h_\infty(V)$ in the hyperpolarizing direction by 3 mV. The time-constant $\tau_h$ was modified in the voltage range below -20 mV, which is outside the range of the experimental data upon which it is based [1]. This was achieved by modification of the constants in the expression for $A_h$. In addition, the time-constants $\tau_m$ and $\tau_h$ were scaled by a factor of 0.2 and 0.75, respectively. The value for $\tau_m$ is not critical, and the model generates similar results when the assumption $m = m_\infty(V)$ is made. These changes result in an activation threshold of approximately -20 mV,
consistent with reported \( I_{Na} \) measurements [3]. The maximum sodium conductance (\( \bar{g}_{Na} \)) was increased from 30 \( \mu S \) to 38 \( \mu S \) to fit the recorded action potential data.

**Fast Ca\(^{2+}\) Current (\( I_{Ca} \)).** In some molluscan neurons (including R15) \( I_{Ca} \) exhibits calcium-dependent inactivation [33, 88]. A \( Ca^{2+} \)-inactivation term has been added to \( I_{Ca} \), which serves to reduce \( Ca^{2+} \) influx at high intracellular \( Ca^{2+} \) concentrations. This modification partially contributes to the lengthening of the burst at high concentrations of 5-HT. The time-constant expression \( \tau_d \) associated with \( I_{Ca} \) was modified in the voltage range below 0 mV, which is outside the range of experimental data upon which it is based [1]. This was achieved by modifying the constants in the expression for \( B_d \).

**Slow-Inward Ca\(^{2+}\) Current (\( I_{SI} \)).** The slow-inward current is voltage-activated and calcium-inactivated. It is the key current responsible for inducing bursting in the model. In order to generate a typical burst, the conductance of this current (\( \bar{g}_{SI} \)) has been reduced from 0.75 \( \mu S \) to 0.65 \( \mu S \) and have changed the slope of the expression for the gating variable \( s_{\infty}(V) \). There is a lack of quantitative data describing this current in the literature, and these changes are within the qualitative criteria for this current defined in the original model.

Voltage clamp experiments have shown that \( I_{SI} \) is increased by bath application of 5-HT, and such increases are most evident at concentrations greater than 10 \( \mu M \). The effect of 5-HT upon \( I_{SI} \) is mediated via the intracellular second messenger cAMP [61, 66]. Other experiments have shown that DA, possibly acting via cGMP, decreases this current [39, 66]. In the new model, \( I_{SI} \) is modulated by both 5-HT (via cAMP) and DA, according to the following equation:

\[
I_{SI} = \bar{g}_{SI} s \left( \frac{K_{SI,Ca}}{[Ca^{2+}] + K_{SI,Ca}} \right) F_{SI,mod}(V - E_{Ca})
\]  

\[
F_{SI,mod} \equiv \left( \frac{K_{DA}}{[DA] + K_{DA}} \right) \left( 1 + \frac{K_{SI,mod}}{1 + \exp \left( \frac{-(cAMP) - K_{SI,cAMP}}{D_{SI,cAMP}} \right)} \right)
\]
where $\tilde{g}_{SI}$ is the maximal conductance ($\mu$S) for this current in the absence of cAMP, DA, and Ca$^{2+}$. $s$ is a voltage-dependent gating variable (dimensionless), $K_{SI, Ca}$ (mM) represents the half-inactivation value of Ca$^{2+}$ concentration, and $F_{SI, mod}$ is a dimensionless modulation term that represents the effects of both the second messenger cAMP and the neurotransmitter DA on $I_{SI}$. $K_{DA}$ (mM) represents the half-inactivation concentration value for DA upon $I_{SI}$, and is based on published data [40]. It should be noted that similar effects upon the I-V characteristics have been reported at much lower concentrations of DA [66]. The constants $K_{SI, mod}$ (dimensionless), $K_{SI,cAMP}$ (mM), and $D_{SI,cAMP}$ (mM$^{-1}$) determine the relative increase in $\tilde{g}_{SI}$ produced by increased cAMP concentration. These constants were determined from the anticipated range of cAMP concentration in model (1 $\mu$M $\leq$ cAMP $\leq$ 5 $\mu$M, see below). The specific parameter values used in eqns. 2.3 and 2.4 are given in the Appendix. The modulatory effects of 5-HT (acting via $I_{SI}$ and $I_{R}$) and DA (acting via $I_{SI}$) upon the steady-state I-V plot of the model are shown in Fig. 2.3.

Non-specific Inward Current ($I_{NS}$). The nonspecific inward current is analogous to the current that is sometimes reported as $I_{D}$ [2, 3]. This is a slow current that is only active during the burst phase and affects the character of the depolarizing afterpotential (DAP). We have retained the mathematical expression used to describe this current, but have modified the values of $K_{NS, Ca}$ and $\tilde{g}_{NS}$ to obtain a DAP that is consistent in character with published data, as well as experimental observations in our laboratory.

2.1.2 Outward Currents

Delayed-Rectifier Potassium Current ($I_{R}$). The delayed-rectifier K$^+$ current is implemented as in the original model.

Anomalous-Rectifier Potassium Current ($I_{R}$). The conductance of the anomalous-rectifier current has been made a function of cAMP concentration. It has been shown experimentally that $I_{R}$ is increased by bath application of 5-HT in concentrations as
Figure 2.3: I-V plots as a function of 5-HT and DA concentration. Numbers indicate concentration of 5-HT or DA in μM. The conductance $g_{Na}$ is set to zero to highlight the changes in the subthreshold currents. For each concentration of 5-HT or DA, a voltage clamp to -50 mV was simulated. The steady-state $Ca^{2+}$ concentration from that clamp step was then used, and all gating variables were set to their steady-state values, parameterized by membrane potential. These plots are similar to those found experimentally using a holding potential of -50 mV and applying voltage clamp steps of brief (1-2 sec) duration. 

A. Effects of 5-HT. At low concentrations of 5-HT (0-10 μM), $I_R$ is increased, shifting the I-V plot in the outward direction (solid lines). At higher concentrations of 5-HT, $I_{SI}$ is increased, shifting the I-V plot in the inward direction at depolarized potentials (dashed lines). An increase in $I_{SI}$ also increases the steady-state $Ca^{2+}$ concentration (not shown), which affects the calcium-dependent $I_{CaP}$ and $I_{NaCa}$. The effect of increased $Ca^{2+}$ concentration upon these currents is to shift the I-V curve in a depolarized direction. 

B. Effects of DA. Application of DA reduces the conductance of $I_{SI}$, shifting the steady-state I-V curve in an outward direction.
low as 0.1 μM, and that this action is mediated by cAMP [11, 30]. A factor has been added to the original expression for $I_R$ to account for the action of cAMP, which is believed to occur via the recruitment of additional ion channels [41]. The expression utilized for $I_R$ is given as:

$$I_R = \bar{g}_R F_{R,\text{mod}} \left( \frac{V - E_K + 5.66}{1 + \exp\left(\frac{(V - E_K - 15.3)ZF}{RT}\right)} \right)$$  \hspace{1cm} (2.5)$$

$$F_{R,\text{mod}} = 1 + \frac{K_{R,\text{mod}}}{1 + \exp\left(\frac{-[(cAMP) - K_{R,cAMP}]}{D_{R,cAMP}}\right)}$$  \hspace{1cm} (2.6)$$

where $\bar{g}_R$ is the maximal conductance (μS) in the absence of cAMP and $F_{R,\text{mod}}$ is a dimensionless modulation term that represents the effect of [cAMP] on $I_R$. The constants $K_{R,\text{mod}}$ (dimensionless), $K_{R,cAMP}$ (mM), and $D_{R,cAMP}$ (mM⁻¹) are parameters for the Boltzmann-type characterization of the binding site for cAMP. The effective value of $g_R$ ($\bar{g}_R F_{R,\text{mod}}$) in the absence of 5-HT and DA is 0.315 μS, which is close to the value of 0.32 μS used in the original model. The specific values used for the remaining parameters in Eqns. 2.5 and 2.6 are given in the Appendix.

2.1.3 Background Currents

Na⁺/K⁺ Pump ($I_{NaK}$). In the original model the Na⁺/K⁺ pump was modeled as a constant outward current source. Recently, it has been reported that $I_{NaK}$ demonstrates a voltage-dependency that reduces the magnitude of the current at hyperpolarized potentials. This form of voltage dependency has been demonstrated across a wide range of tissues, including oocytes [82], squid axon [77], and guinea pig ventricle [28, 37]. Since a material balance upon either Na⁺ or K⁺ is not implemented in the model, $I_{NaK}$ is not a critical dynamic current, and for practical purposes can be considered to be a voltage-dependent background current. This voltage-dependency is represented by an expression that is based upon quantitative data from heart muscle [37]:

$$I_{NaK} = \left(\frac{[Na^+]_i}{[Na^+]_i + K_{P,Na}}\right)^3 \left(\frac{[K^+]_o}{[K^+]_o + K_{P,K}}\right)^2 \left(\frac{1.5}{1.5 + exp\left(\frac{-V + 80}{40}\right)}\right)$$  \hspace{1cm} (2.7)$$
It is reported that application of ouabain causes an endogenously bursting cell to start beating [48]. Although not a part of this study, the model produces similar results.

**Na⁺-Ca²⁺ Exchanger** \( (I_{NaCa}) \). The sodium-calcium exchanger current is implemented as in the original model.

**Ca²⁺ Extrusion Pump** \( (I_{CaP}) \). The maximum value of the Ca²⁺ pump \( (I_{CaP}) \) has been reduced from 15 nA to 7 nA and the half-activation value \( (K_{P, Ca}) \) has been decreased from 1000 nM to 350 nM. This pump is described as having a high-affinity for Ca²⁺ but a low-capacity (see [18] for a full discussion). These modifications more adequately reflect this description, allowing the pump to account for the majority of Ca²⁺ extrusion at low Ca²⁺ concentrations, but to saturate at higher Ca²⁺ concentrations. This saturation did not occur in the original model.

**Leakage Current** \( (I_L) \). Several currents that are normally included in the measurement of the leakage current, such as \( I_R, I_{NaCa}, I_{CaP}, \) and \( I_{NaK} \), have been modeled separately. Therefore, it was necessary to set the reversal potential \( E_L \) to +10.3 mV rather than the more typical value of -45 mV [38]. This value of \( E_L \) is a more depolarized value than that used in the original model (0 mV), due to the incorporation of a voltage-dependent expression for the Na⁺/K⁺ pump. Since leakage currents are difficult to quantify, its magnitude has been kept as small as possible. The conductance \( g_L \) has been set to 0.075 µS, which is less than the value used in the original model (0.1 µS).

### 2.2 Fluid Compartment Model

#### 2.2.1 Calcium Regulation

Calcium concentration in the extracellular fluid compartment is considered constant. However, Ca²⁺ concentration in the intracellular compartment is regulated by the presence of a calmodulin-type Ca²⁺ buffer and the Ca²⁺ extrusion that is effected by \( I_{NaCa} \) and \( I_{CaP} \). The only change made in the material balance equation for \( [Ca^{2+}]_i \)
Figure 2.4: Calcium fluid compartment models and intracellular regulatory pathways

Net change in $Ca^{2+}$ concentration is determined by 1) the $Ca^{2+}$ flux generated by the $Ca^{2+}$ component of the ionic currents and pumps/exchangers and 2) the uptake and release of $Ca^{2+}$ by the calmodulin buffer. Adenylyl Cyclase (AC) catalyzes production of cAMP, and is activated in the presence of 5-HT. Degradation of cAMP occurs by cleavage of cAMP by phosphodiesterase (PDE). The conductances of $I_{SI}$ and $I_R$ are increased by cAMP, through phosphorylation of the channel proteins via protein kinases (PK). DA decreases the conductance of $I_{SI}$, acting directly on the channel through mechanisms that have not been fully elucidated.
is a correction to the term representing the $Ca^{2+}$ fraction of the nonspecific current $I_{NS}$. In [18], it was stated that the fraction of $\bar{g}_{NS}$ conducting $Ca^{2+}$ was $0.197 \mu S$. However, a reworking of the derivation presented shows it to actually be $0.197 \times \bar{g}_{NS} \mu S$. Therefore, this term has been changed from $0.197/\bar{g}_{NS}$ to $0.197$ (dimensionless).

The material balance equation for $[Ca^{2+}]_i$ is given as:

$$[Ca^{2+}]_i = \frac{I_{NaCa} - I_{SI} - I_{Ca} - 0.197 \left( I_{NS} \frac{V - E_{Ca}}{V - E_{NS}} \right)}{2V_i F} - n_B [B]_i \dot{O}_C$$  \hspace{1cm} (2.8)

where $V_i$ is the effective cell volume, $F$ is Faraday's constant, $[B]_i$ is the intracellular concentration of the cytosolic buffer, $n_B$ is the number of binding sites per molecule of the buffer, and $O_C$ is the fraction of binding sites already occupied by $Ca^{2+}$ ions (buffer occupancy). The buffer occupancy equation used in the model is the same as that given in the original model.

2.2.2 Cyclic-AMP Regulation

An equation has been added to the model to account for the production and degradation of intracellular cAMP. Production of cAMP from adenosine triphosphate (ATP) is catalyzed by adenylyl cyclase (AC). Application of 5-HT increases intracellular cAMP in R15 and this has been shown to occur via activation of AC (for review see [65]). The time rate of change of cytosolic cAMP is represented by the difference of a production term representing AC activity which is modulated by levels of neurotransmitter and a degradation term which represents both cleavage of cAMP by phosphodiesterase and diffusion away from the channel receptors at the cell membrane. This is not a rigorous treatment of cAMP activity. Many processes at the second messenger level, (e.g. channel phosphorylation) are not considered. In this study it is the intent of this study to demonstrate the effect of cAMP as a second messenger and how its concentration is affected by 5-HT.

The equation used to describe the time rate of change of cytosolic $[cAMP]$ is:

$$[cAMP] = k_{ac} \left( 1 + K_{mod} \left( \frac{[5-HT]}{[5-HT] + K_{5HT}} \right) \right) - v_{pde} \left( \frac{[cAMP]}{[cAMP] + K_{pde}} \right)$$  \hspace{1cm} (2.9)
The value for $K_{5HT}$ (mM) was selected from experimental results on *Aplysia* abdominal ganglion [20], where it is reported that a 5-HT concentration of 6 μM leads to a half-maximal increase in cAMP production. Similar results (14 μM) have been reported in *Aplysia* sensory neurons [72]. It has been reported that basal whole-cell cAMP concentrations of the larger cells in the abdominal ganglion are in the range of 1-6 μM, and that 5-HT can cause a 4-5 fold increase in cAMP [44]. Using this information, we set the basal cytosolic cAMP concentration at 1 μM, and the maximal level at 5 μM. These references were chosen since the cell is considered to be a single fluid compartment, although it should be noted that recent measurements in *Aplysia* sensory neurons have demonstrated spatial gradients in cAMP-dependent protein kinase activity [10].

From these assumed cAMP concentrations, appropriate values were selected for $k_{ade}$ (mM/ms), $K_{mod}$ (dimensionless), and $v_{pde}$ (mM/ms). The half-maximal value scaling the rate of degradation of cAMP by PDE, $K_{pde}$, was set to 3 μM, which is within the operating range of cAMP concentration used within the model. All the values used in Eqn. 2.9 are specified in the Appendix.

### 2.3 Computational Methods

The model has 12 state variables, including membrane potential, $Ca^{2+}$ concentration, $Ca^{2+}$ buffer occupancy, cAMP concentration, and 8 gating variables. Temporal integration of this system of equations was accomplished using an implicit fifth-order Runge-Kutta method with variable step-size [42]. The steady-state I-V curves generated in this paper (Figs. 2.3, 3.5, 3.7) are not true steady-state plots. Intracellular $Ca^{2+}$ concentration is set to a desired value (typically the $Ca^{2+}$ concentration at a steady-state holding potential), and all remaining state variables are set to their steady-state values as a function of membrane potential. These I-V curves are similar to those generated in voltage-clamp experiments by applying a brief (1–2 sec) volt-
age clamp pulse to a desired command potential, assuming the $Ca^{2+}$ concentration changes minimally during the clamp step.

Two software packages were used to generate most of the data presented in Chapter 4. $V_s$, solution surfaces and calculation of the $Ca$ and $s$ nullclines lying on $V_s$, were accomplished using PITCON [78, 79], a continuation program which tracks the fixed solutions of a systems of equations as a free variable is varied. Bifurcation diagrams, the stability analysis of fixed points, and the two-parameter continuation of limit points were performed using AUTO [29], a bifurcation analysis package which solves for fixed and periodic solutions of a system of differential equations as one or two system parameters are varied.

All simulations were written in the C and FORTRAN programming languages and were implemented on a Sun Microsystems IPX computer.
Chapter 3

Results

3.1 Response to 5-HT

The response of the model to low concentrations of 5-HT (Fig. 3.1) is initially investigated. With increasing concentrations of 5-HT in the range 0-2 μM, an increase in the length and depth of the interburst hyperpolarization is observed in both the experimental data and model-generated burst pattern [30, 61]. However, little change occurs in the burst length. Electrical silence is produced at a 5-HT concentration of 10 μM [30]. This transition is due to an increase in the conductance of the outward current $I_R$ (Fig. 2.3) and is mediated by cAMP. When $I_R$ becomes sufficiently large, bursting activity ceases. In the model, most of the increase in $I_R$ occurs over the low range (0-10 μM) of 5-HT concentrations. This is in agreement with published data [61, Fig. 2], where it is reported that a significant increase in $I_R$ occurs at 5-HT concentrations between 0-10 μM, and a minimal increase thereafter at a concentration of 50 μM.

High concentrations of 5-HT are reported to increase (rather than decrease) the excitability of R15 [61]. Elevated concentrations of 5-HT increase the concentration of cytosolic cAMP, which leads to an increase in $I_{SF}$ (Fig. 2.3). Figure 3.2 shows the model-generated response to bath application of higher concentrations of 5-HT. As 5-HT is increased beyond 20 μM, the burst lengthens, which at higher doses leads to beating (5-HT > 60 μM). A tabulation of the dose-dependent effects of high concentrations of 5-HT upon the model is shown in Table 3.1. Since model-generated bursting resumes at concentrations of 5-HT greater than 20 μM, the values shown
Figure 3.1: Model-generated responses to low doses of 5-HT
An increase in 5-HT concentration at low doses (0-10 μM) causes an increase in the outward current $I_R$. This results in an increase in the length and depth of the interburst hyperpolarization (A,B,C) with a minimal change in the burst length. At 10 μM 5-HT, $I_R$ is increased sufficiently to bias the model into a hyperpolarized silent state (D). Numbers indicate bath concentration of 5-HT.
Figure 3.2: Model-generated responses to high doses of 5-HT
Responses to 5-HT concentrations of 10, 25, 40, 50, and 100 μM are shown. The model is silent at a 5-HT concentration of 10 μM (A). Increases in 5-HT above 10 μM cause an increase in the inward current $I_{SI}$, and the model resumes bursting activity at 5-HT concentrations greater than 20 μM (B). Further increases in $I_{SI}$ results in an increase in burst length with a minimal change in the length of the interburst interval (C,D). At a sufficient concentration of 5-HT (> 60 μM), the model exhibits tonic beating (E).
in Table 3.1 are normalized to the model-generated data at 25 \( \mu M \) 5-HT. There is a steady increase in cycle length and a pronounced increase in burst length with increasing concentrations of 5-HT. The increase in burst length is due to a combination of two mechanisms. First, if \( \tilde{g}_{Na} \) is set to zero, the slow-wave that underlies the bursting oscillations can be observed (not shown). As 5-HT concentration is increased, so does the period of the slow-wave, due to an increase in the conductance of \( I_{SI} \). Second, the \( Ca^{2+} \)-inactivation of \( I_{Ca} \) reduces the rate of \( Ca^{2+} \) influx during the burst. If the \( Ca^{2+} \)-inactivation term of \( I_{Ca} \) is omitted and \( \tilde{g}_{Ca} \) is scaled appropriately, the model exhibits much shorter bursts at high 5-HT concentrations.

Reduction of PDE activity by PDE inhibitors such as IBMX has been shown to elevate the concentration of cAMP, leading to the same effects as produced by 5-HT [62, Fig. 12]. This model demonstrates that the effects of 5-HT on the bursting waveform can be simulated by an appropriate reduction in the model parameter \( u_{pde} \) in Eqn. 2.9 (not shown).

3.2 Response to DA

DA inhibits \( I_{SI} \) and leads to a cessation of bursting activity [39, 66]. Figure 3.3 illustrates the model-generated response to increasing concentrations of DA. As DA concentration is increased, the conductance of \( I_{SI} \) is reduced, which results in a diminished \( Ca^{2+} \) influx during the burst and a shortened burst length. (see [18] for a discussion of the bursting mechanism). Concentrations of DA slightly greater than 100 \( \mu M \) terminate bursting completely, placing the cell into a stable hyperpolarized silence.
Table 3.1: Model-generated changes in cycle length (CL), burst length (BL), and duty cycle (DC) for high doses of 5-HT. Both BL and CL are normalized to their values at 25 μM 5HT. DC is expressed as the fraction of the burst period occupied by the burst. A value of 1.0 for DC corresponds to beating behavior (Fig. 3.2E). NA: Not Applicable.

<table>
<thead>
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<th>5HT (μM)</th>
<th>CL</th>
<th>BL</th>
<th>DC</th>
</tr>
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</tr>
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</tr>
</tbody>
</table>

Figure 3.3: Model-generated responses to DA
Responses to DA concentrations of 0, 100, and 500 μM are shown. Application of DA decreases $I_{ST}$, resulting in a weakening of the burst phase and a slight increase in the interburst interval (A, B). At concentrations larger than 100 μM, $I_{ST}$ is decreased sufficiently to no longer sustain bursting, leaving the model in a sustained hyperpolarized state (C).
A. 500 µM DA

B. 10 µM 5-HT

C. -1.2 nA bias

Figure 3.4: Response of silent model to 200 ms depolarizing current pulses. Bars under membrane potential traces represent timing and duration of applied current pulses. Numbers under bars indicate magnitude of current pulses. A. Model is silenced by 500 µM DA. Brief current pulses of large amplitude are unable to elicit a self-sustaining burst. This is due to an inability of \( I_{SI} \) to sufficiently activate in the presence of 5-HT. B. Model is silenced by 10 µM 5-HT. Current pulses of sufficient amplitude (3.5 nA) will elicit a self-sustaining burst. Stable silence is maintained by \( I_R \), which is predominant at hyperpolarized potentials. Depolarization into the region where \( I_{SI} \) activates elicits a sufficient inward current to sustain a burst after removal of the current pulse. C. Unmodulated case, in which the membrane was silenced by a 1.2 nA hyperpolarizing current. A current pulse of sufficient amplitude (5.5 nA), will elicit a self-sustaining burst. However, this burst is weaker than the one shown in B, and requires a larger current pulse to activate. This is due to the lesser magnitude of the net-inward current in the negative-slope region (see Fig. 3.5).
Figure 3.5: I-V characteristics of the silenced model
Letters correspond to equivalent panels of Fig. 3.2. \(Ca^{2+}\) concentration for each curve is set to its resting value, and all gating variables are then set to their steady-state value as a function of membrane potential. These curves represent the steady-state I-V characteristics of the model at the moment that a depolarizing current pulse is applied. The conductance \(\bar{g}_{Na}\) is set zero to highlight the changes in the subthreshold currents.
3.3 Response to Current Stimuli in the Presence of 5-HT or DA

Both DA and 5-HT can hyperpolarize R15 into silence. Experimentally, a brief depolarizing current pulse applied to a cell that has been silenced by 5-HT will elicit a burst, while a similar pulse applied to a cell silenced by DA will not [61]. These simulations demonstrate this effect (Figs. 3.2A and 3.2B) which can be explained as follows. With bath application of 10 μM 5-HT, silence is induced by an increase in the outward current $I_R$; however, a depolarizing pulse of sufficient amplitude can activate $I_{SI}$ sufficiently to elicit a single sustained burst. In contrast, DA induces electrical silence via a graded decrease in the conductance of the inward current $I_{SI}$. At DA concentrations that produce electrical silence, $g_{SI}$ has been diminished to such a degree that it is not possible to activate $I_{SI}$ sufficiently to elicit a sustained burst with a brief depolarizing current pulse, regardless of the stimulus amplitude. These results compare favorably with published experimental observations [61, Fig. 9D].

Figure 3.2C demonstrates a similar protocol applied to the unmodulated model when silenced by a hyperpolarizing bias current of 1.2 nA. This value of current was chosen so that the model resting potential was similar to that used in Figs. 3.2A and 3.2B. This simulation was performed to verify that the results shown in Figs. 3.2A and 3.2B are not a reflection of an unmodulated aspect of the membrane response. Figure 3.2C demonstrates that it is possible to elicit a sustained burst with a 200 ms depolarizing pulse, but that the magnitude of the current pulse required to do so is much larger than that used to elicit a burst in Fig. 3.2B. Furthermore, the burst elicited in Fig. 3.2C is weaker than that shown in Fig. 3.2B.

The mechanisms for these responses are explained by the I-V plot of Fig. 3.5. This plot shows the steady-state I-V plot for the three different modulatory mechanisms shown in Fig. 3.2. The conductance $g_{Na}$ is set to zero so changes in the steady-state values of the subthreshold currents can easily be observed. $Ca^{2+}$ con-
centration is not at steady-state for all values of membrane potential, but is fixed at its resting concentration when the cell is maintained in a state of hyperpolarized silence. Therefore, these I-V plots represent the steady-state I-V characteristics of the model at the moment that a depolarizing current pulse is applied.

With application of 500 μM DA, $I_{SI}$ is greatly reduced (Fig. 3.5). Calcium influx shifts the I-V plots upward (e.g. Fig. 7 of [18]). Upon application of a depolarizing current pulse, voltage activation of $I_{SI}$ causes $Ca^{2+}$ influx, which reduces $I_{SI}$. Upon removal of the current pulse, a sustained burst does not occur since the I-V plot no longer exhibits a net inward current near the action potential threshold.

In the case of 5-HT or bias-current modulation, the model demonstrates a significantly larger net inward current. Upon removal of a depolarizing current pulse, the I-V curve is still net inward in the region of the $I_{Na}$ action potential threshold. Each firing of an action potential results in additional influx of $Ca^{2+}$, which reduced $I_{SI}$. The burst stops when the entering $Ca^{2+}$ has reduced $I_{SI}$ to the point where the I-V curve indicates a net outward current and the $I_{Na}$ threshold cannot be reached again. The 5-HT case offers a longer sustained burst than the current-modulated case due to a difference in the manner in which silence is achieved. 5-HT silences the model by activating $I_{R}$, which is primarily active at hyperpolarized potentials below -40 mV. In the current modulated case, silence is achieved by shifting the I-V plot upward at all potentials. As a result, the net-inward current in the negative-slope region is less than that with 5-HT modulation. The 5-HT modulated case exhibits a longer sustained burst because it demonstrates a larger net-inward current, requiring a greater amount of $Ca^{2+}$ influx (i.e. a longer burst) in order to sufficiently inactivate $I_{SI}$.

Either hyperpolarizing or depolarizing current pulses, applied to R15 after induction of beating via bath application of 5-HT, will show a temporary cessation of beating activity that lasts several seconds [61]. When the model is first placed in a beating mode via bath application of 100 μM 5-HT and then stimulated with either
Figure 3.6: Response of beating model to depolarizing and hyperpolarizing current pulses

Model was induced into a tonic beating mode by 100 μM 5-HT (A1 and A2) or a 3 nA bias current (B1 and B2). A hyperpolarizing current pulse (2 nA, 500 msec) is applied at the arrow (A1 and B1). A depolarizing current pulse (4nA, 5 sec) is applied during the horizontal bar (A2 and B2). In the 5-HT induced beating mode, both depolarizing and hyperpolarizing current pulses elicit a self-sustaining hyperpolarization after removal of the stimulus current (A1 and B1). Such a hyperpolarization does not occur when beating is induced by a 3 nA bias current (A2 and B2).
a hyperpolarizing or depolarizing current pulse, similar effects are observed (Fig. 3.6, Panels A1 and B1). Beating with a similar beat frequency can also be induced by application of a 3 nA depolarizing bias current. However, it is not possible to interrupt the beating for a sustained period of time with similar depolarizing or hyperpolarizing current pulses (Fig. 3.6, Panels A2 and B2). The model allows us to investigate these mechanisms.

Hyperpolarizing current pulses of sufficient amplitude or duration will interrupt beating activity [61]. Figure 3.6, Panels A1 and A2, compares the results of applying a hyperpolarizing current pulse (2 nA, 500 msec) to the model placed in a beating mode produced by either 5-HT (Panel A1) or a 3 nA depolarizing bias current (Panel A2). In the 5-HT-induced beating mode, a net outward (hyperpolarizing) membrane current exists for several seconds after removal of the current pulse, resulting in a sustained hyperpolarization of over 10 seconds. In the current-induced beating mode (Panel A2), a hyperpolarizing current pulse can eliminate beating only for the duration of the pulse, plus the short amount of time that it takes for the membrane potential to rise to the action potential threshold so that beating can resume. Upon removal of the current pulse, net membrane current is immediately inward, and no self-sustaining membrane hyperpolarization occurs.

Figure 3.7 demonstrates the mechanism underlying the difference between these two responses. The total ionic current for each beating mode is shown as a function of membrane potential. Since the I-V plots are also parameterized by \( \text{Ca}^{2+} \) concentration, the mean \( \text{Ca}^{2+} \) concentration (which is constant during beating) is used for each beating mode. These plots represent the characteristic I-V plot of the model at the instant that a hyperpolarizing current is applied. In the 5-HT-induced beating mode, the I-V plot (curve A1) shows a region of outward ionic current between the membrane potentials of -65 mV and -35 mV. Therefore, a hyperpolarizing current introduced into this range of potentials will result in an outward current that will continue the hyperpolarization of the membrane after removal of the pulse. This hy-
Figure 3.7: I-V characteristic of the beating model
I-V plot labels correspond to labels in Fig. 3.6. To generate these plots, the model is "Ca^{2+}-clamped" at the mean Ca^{2+} during beating, in order to depict the character of the I-V plots at the onset of a current pulse. Hyperpolarizing current pulses cannot elicit a sustained hyperpolarization in A1 because the I-V plot shows that net inward current exist at all hyperpolarized potentials, immediately driving the cell back towards beating. A sustained hyperpolarization can occur in A2 due to the outward current which exists between approximately -65 mV to -35 mV.
perpolarization continues until \(I_{CaP}\) and \(I_{NaCa}\) (which is net-outward with respect to \(Ca^{2+}\) at hyperpolarized potentials) have extruded enough \(Ca^{2+}\) to partially remove the inactivation of \(I_{SI}\) so that \(I_{SI}\) may activate once again.

In the current-induced beating mode \(I_{STIM} = 3\ \text{nA}\), the net ionic current at membrane potentials below the action potential threshold is always inward (Fig. 3.7m, curve A2). This explains the lack of a sustained hyperpolarization, for upon removal of the hyperpolarizing pulse, regardless of its amplitude, a net-inward current will always exist to depolarize the membrane back to the action potential threshold. This was verified with additional simulations (not shown), which revealed that neither increasing the hyperpolarizing current pulse magnitude to 10 nA nor increasing the duration of the current pulse to 2.5 sec was capable of producing a sustained hyperpolarization.

The reasoning for the difference in the current stimulus response of the two beating cases discussed above is similar to the previous explanations of the membrane silencing mechanisms. In the 5-HT modulated case, beating occurs due to an increase in the conductance of \(I_{SI}\) to a point that net-inward current always exists near the \(I_{Na}\) action potential threshold. However, at more hyperpolarized potentials, net outward current exists due to the 5-HT induced increase in \(I_{R}\). It is this outward current that continues the hyperpolarization of the membrane potential well after the current stimulus has been removed. In the bias current induced beating mode, beating occurs due to an inward shift of the entire I-V curve. The bias current increases net-inward current at all membrane potentials, preventing the existence of a hyperpolarized region of outward membrane current.

The mechanism by which a depolarizing current pulse temporarily interrupts beating is much more complex. Due to the large number of factors that potentially contribute to this effect, we found it useful to apply a theoretical "state-variable clamp" to the slow variables \(Ca^{2+}\), \(s\), and \(b\), in order to determine which changes in these variables were essential for the interruption of beating by a depolarizing current pulse.
Figure 3.8: Sequence of events underlying interruption of beating by a depolarizing current pulse in the presence of 5-HT.
Current pulse (4 nA, 5 sec) denoted by the bar shown in Panel A. Time course of membrane potential (V), Ca\(^{2+}\) concentration, s activation, b activation, and membrane currents \(I_{SI}\) and \(I_{NS}\) are shown in Panel A. I-V plots of the model were generated by setting \(Ca^{2+}\), s, and b to their values at time-points \(t_1\), \(t_2\), and \(t_3\), and setting all remaining gating variables to their steady-state values as a function of membrane potential. Application of depolarizing current at \(t_1\) increases spike frequency, leading to an increase in \(Ca^{2+}\) influx, as well as the mean values of s and b. Upon removal of the current pulse \(t_2\), s and b rapidly decay, but \(Ca^{2+}\) decays much more slowly. When s and b have decayed to approximately their prestimulus values \(t_3\), \(I_{SI}\) is reduced due to the still elevated \(Ca^{2+}\) concentration. This loss of inward current causes a further reduction in spike-frequency, allowing s and b to decay further, which further reduces inward current. Eventually there is not enough subthreshold current to maintain beating, and a subthreshold hyperpolarization occurs. Refer to text for a detailed explanation of these events.
In this technique, a state-variable is clamped to its mean value during normal tonic beating by setting its derivative to zero. All other state-variables are integrated as before, and the same depolarizing current pulse is applied to the model. If a sustained hyperpolarization does not occur after application of a current pulse, this implies that the clamped state-variable is necessary for the sustained hyperpolarization to occur. Using this method, it was found that changes in $Ca^{2+}$, s, and b were all necessary for the interruption of beating to take place. Due to the large number of $Ca^{2+}$-dependent currents, this method can be extended by clamping a specific $Ca^{2+}$-dependent term (such as the $Ca^{2+}$-dependent inactivation of $I_{SI}$) while still allowing $Ca^{2+}$ concentration to vary and affect all other $Ca^{2+}$ dependent processes. Using this method, it was determined that the $Ca^{2+}$-dependent inactivation of $I_{SI}$ was also a necessary mechanism, while the $Ca^{2+}$-dependent activation of $I_{NS}$ was not. Furthermore, clamping the $Ca^{2+}$-dependent terms of $I_{CaP}$ and $I_{NaCa}$ did not prevent the interruption of beating, although clamping $I_{CaP}$ did increase the lag time between the end of the depolarizing current pulse and the onset of the hyperpolarization by 50%.

From these results, it was determined that the necessary processes underlying the interruption of beating by a depolarizing current pulse are the voltage-dependent activation of $I_{SI}$ and $I_{NS}$ and the calcium-dependent inactivation of $I_{SI}$. With these insights, the following explanation for the sequence of events that occur during and after the application of a depolarizing current pulse is formulated. Figure 3.8A shows the time course of membrane potential, $I_{SI}$, $I_{NS}$, and the "slow" state variables ($Ca^{2+}$, s, b) during and shortly after application of a depolarizing current pulse (4 nA, 5 sec). Figure 3.8B shows the steady-state I-V relationships of the model parameterized by the $Ca^{2+}$, s, and b values at time $t_1$, $t_2$, and $t_3$.

Initially, when the model is in a tonic beating state, the slow variables $Ca^{2+}$, s, and b are relatively constant. Application of a depolarizing current pulse increases spike frequency, increasing the mean values of s and b. The variables s and b increase due to the nature of the voltage-dependency of their time-constant and steady-state
expressions. The increased spike-frequency also causes an increase in $Ca^{2+}$ influx. As a result of these increases, both $I_{SI}$ and $I_{NS}$ increase in magnitude, with the increase in $s$ having a more pronounced effect on $I_{SI}$ than the $Ca^{2+}$-dependent inactivation. Upon removal of the current pulse at time $t_2$, the increased spike frequency continues due to a net increase in the subthreshold inward currents $I_{SI}$ and $I_{NS}$. However, the spike frequency is now less than that during application of the 4 nA current pulse, and therefore $s$ and $b$ start to decay. Correspondingly, $I_{SI}$ and $I_{NS}$ decay, which causes a further reduction in spike frequency. At time $t_3$, $s$ and $b$ have decayed to their pre-stimulus values at $t_1$. However, the $Ca^{2+}$ concentration is still elevated, being a much slower process. Consequently, $I_{SI}$ is thus less inward than it was at time $t_1$. This reduction in a subthreshold (i.e. slow) current results in a continued decay in spike frequency, causing a concomitant decrease in both $s$ and $b$. Eventually $I_{SI}$ and $I_{NS}$ decay to a point that beating cannot be sustained. This loss of inward current results in a sustained hyperpolarization occurring by a mechanism identical to that described previously for the effects of a hyperpolarizing current pulse.

To summarize, this effect is due to the difference in time scales between the voltage-dependent activation of $s$ and $b$ and the $Ca^{2+}$-dependent inactivation of $I_{SI}$. $Ca^{2+}$ concentration is still elevated when $s$ and $b$ have decayed to their pre-stimulus levels, effectively reducing $I_{SI}$ so that a tonic beating state cannot be maintained. A sustained hyperpolarization ensues, during which a significant degree of $Ca^{2+}$ efflux occurs (similar to the hyperpolarizing phase of the normal burst cycle). When spiking resumes, $Ca^{2+}$ concentration returns to its pre-stimulus level.

3.4 Bistability

The model also exhibits a bistable region of behavior, in which bursting and beating activity coexist. One region is located in the range of concentrations of 5-HT where the transition from bursting to beating occurs, from approximately 58 µM to 63 µM. Both the bursting and beating modes demonstrate stable long-term electrical behavior
Figure 3.9: Bistable behavior exhibited by the model
In the presence of 60 μM 5-HT, a hyperpolarizing current pulse (2 nA, 500 msec) causes a transition from beating to bursting. 300 seconds later, bursting activity continues. A depolarizing current (0.1 nA, 30 sec) is then applied to the model. Upon removal of the current pulse, beating activity continues. After an additional 300 seconds, the model remains in a beating mode, demonstrating the robustness of the mode shift.

and are not transient phenomena. The behavioral mode is dependent upon the initial conditions, and it is possible to switch between modes by application of DC current pulses. This is shown in Fig. 3.9, where the model is initially started in a beating state, with a 5-HT concentration of 60 μM. In Panel A, a hyperpolarizing current pulse (2 nA, 500 msec) is applied, and the model makes the transition to a bursting mode, which persists for at least 300 seconds, demonstrating the long-term stability of the mode shift. A depolarizing current pulse (0.1 nA, 30 sec) is then applied to the beating mode. When the pulse is removed, the model continues to beat 300 seconds later, demonstrating the long-term stability of the mode shift. Similar bistable modes are also exhibited for values of $I_{STIM}$ from 1.05 nA to 1.62 nA.
Chapter 4

Model Analysis

In this section a reduced-system analysis of the model will be performed. First, the appropriate model reductions are derived. Then the reduced models are utilized to explain the mechanisms of bursting and neuromodulation.

4.1 Model Reduction

In this analysis the model developed in Chapter 2 will be referred to as the FULL system. For our analysis, the FULL system is identical to the model previously described, except that a steady-state assumption is made for the cAMP concentration ([cAMP] = 0), and cAMP concentration is given as:

\[
[cAMP] = \frac{K_{pde}K_{ad}c(1 + K_{mod}\frac{[5-HT]}{K_{SHT}+[5-HT]})}{v_{pde} - K_{ad}(1 + K_{mod}\frac{[5-HT]}{K_{SHT}+[5-HT]})}
\]  \hspace{1cm} (4.1)

Our analysis of bursting and neuromodulatory mechanisms is accomplished by subdividing the model into FAST and SLOW subsystems, an approach taking by many others in the analysis of bursting mechanisms [54, 73, 76, 80, 81, 84]. Our approach is most similar to that presented in [81]. We assume that the variables Ca and s are much slower than all other variables of the system. The FAST subsystem is defined as:

\[
\dot{V} = -(I_{CaP} + I_{NaK} + I_{NaCa} + I_{Na} + I_{Ca} + I_{SI} + I_{NS} + I_{K} + I_{R} + I_{L} - I_{STIM})/C \hspace{1cm} (4.2)
\]

\[
\dot{m} = (m_{\infty}(V) - m)/\tau_{m}(V) \hspace{1cm} (4.3)
\]
\[ \dot{h} = (h_{\infty}(V) - h)/\tau_h(V) \]  
\[ \dot{d} = (d_{\infty}(V) - d)/\tau_d(V) \]  
\[ \dot{j} = (j_{\infty}(V) - j)/\tau_j(V) \]  
\[ \dot{b} = (b_{\infty}(V) - b)/\tau_b(V) \]  
\[ \dot{n} = (n_{\infty}(V) - n)/\tau_n(V) \]  
\[ \dot{i} = (i_{\infty}(V) - i)/\tau_l(V) \]  

where \( Ca \) and \( s \) are treated as parameters of the FAST subsystem. The SLOW subsystem represents the slow-wave that underlies bursting behavior in R15, and the slow-wave can be seen in the FULL system by setting \( \tilde{g}_{Na} \) to zero. We begin by developing a reduced set of equations which describe the SLOW system. Initially, we consider the SLOW system as a system of three variables, \( s, Ca, \) and \( OC \), defined as follows:

\[ \dot{s} = (s_{\infty}(V_{ss}) - s)/\tau_s(V_{ss}) \]  
\[ \dot{Ca} = \left( \frac{I_{N\alpha Ca} - I_{SI} - I_{CaP} - 0.197(V_{ss} - E_{Ca})}{2Vol_iF} \right) - n_B [B]_i \dot{OC} \]  
\[ \dot{OC} = Ca k_u (1 - OC) - k_R OC \]  

where \( s \) is the activation variable of the slow-inward calcium current \( I_{SI} \), \( Ca \) is intracellular \( Ca^{2+} \) concentration, \( OC \) is the occupancy of the calcium buffer, and \( V_{ss} \) is the steady-state solution of the FAST system when \( \tilde{g}_{Na} = 0 \). This definition of \( V_{ss} \) differs from that presented by Rinzel and Lee [81], and will be treated in the discussion. The currents \( I_{Ca} \) and \( I_{NS} \) are not active during oscillations of the SLOW system and are solely included for completeness. The occupancy variable \( OC \) can be eliminated from the SLOW system since it changes rapidly with respect to \( Ca^{2+} \) concentration during slow-wave behavior. Thus, \( Ca^{2+} \) binding is assumed to be instantaneous during the slow-wave and \( OC \) is approximated by a first order Michaelis-Menten relationship:

\[ \dot{OC} = \frac{Ca}{Ca + K_{OC}} \]
where $K_{OC}$ is given by $k_R/k_U$. Differentiating eqn (4.13) in time yields the following expression:

$$\dot{O}_c = \frac{d}{dt} \left( \frac{Ca}{Ca + K_{OC}} \right) = \frac{K_{OC}}{(Ca + K_{OC})^2} \frac{dCa}{dt} \quad (4.14)$$

Equation (4.14) may be substituted into eqn. 4.11, and a new expression for $\dot{C}_a$ developed. These manipulations reduce the SLOW system to the following set of nonlinear first-order differential equations:

$$\dot{s} = \frac{(s_{oo}(V_{ss}) - s)/\tau_s(V_{ss})}{(I_{NaCa} - I_{SI} - I_{Ca} - I_{CaP} - 0.197(I_{NS}V_{ss} - E_{Ca}))} \quad (4.15)$$

$$\dot{C}_a = \frac{(I_{NaCa} - I_{SI} - I_{Ca} - I_{CaP} - 0.197(I_{NS}V_{ss} - E_{Ca}))}{2V_{ol}F S_{OC}} \quad (4.16)$$

where

$$S_{OC} \equiv 1 + n_B[B]_i \frac{K_{OC}}{(Ca + K_{OC})^2}.$$  

The steady-state membrane potential ($V_{ss}$) in eqn. 4.16 cannot be determined analytically in terms of $Ca$ and $s$, and therefore must be solved implicitly. This is achieved by performing temporal integration of equations (4.15) and (4.16) along with eqn. (4.2) with $\bar{g}_{Na}$ set to zero. The validity of this steady-state assumption can be demonstrated numerically. For example, if $V$ is always at its steady-state value as parameterized by $(Ca, s)$, then we should be able to “speed up” the dynamics of $V$ (by reducing $C_M$) and achieve identical results. If our steady-state assumption is invalid, the model should produce significantly different results if $C_M$ is reduced.

Figure 4.1 compares the response of the FULL system with $\bar{g}_{Na}$ set to zero, the SLOW system, and the SLOW system with $C_M$ reduced 1000-fold. The temporal change in membrane potential and the $(Ca, s)$ phase plane response are shown (panels A and B). The SLOW system yields results virtually identical to that of the FULL system with $\bar{g}_{Na}$ set to zero, verifying the validity of the model reductions from the FULL system to the SLOW system. Furthermore, reducing $C_M$ by a factor of 1000 in the SLOW system yields near-identical results. The membrane potential waveform shows a slightly shorter period, but its dynamic similarity to the unmodulated SLOW
**Figure 4.1:** Comparison of FULL and SLOW systems with $\tilde{g}_{Na}$ set to zero

The responses of the FULL system with $\tilde{g}_{Na}$ set to zero (solid), the SLOW system (dashed), and the SLOW system with $C_M$ reduced 1000-fold (dotted) are shown. The temporal membrane response at 5-HT concentrations of 0 $\mu$M 5-HT (A) and 60 $\mu$M 5-HT (C) are shown, as well as ($Ca, s$) phase plane responses at similar concentrations (B and D, respectively). These plots demonstrate that the model reductions of the SLOW system accurately replicate the response of the slow-wave of the FULL system across a wide range of parameter values (the dashed and solid lines are indistinguishable). The SLOW system with 1000-fold reduction of $C_M$ demonstrates that membrane potential is near steady-state for all ($Ca, s$) values, since large increases in the dynamics of $V$ result in minimal changes of behavior of the SLOW system. The period in both cases has changed by approximately one second, while the phase-plane trajectories are virtually identical.
system in light of a 1000-fold change in $C_M$ verifies that $V$ is virtually at steady-state and that the SLOW system is essentially of second-order. This is even more obvious when this same comparison is made in the $(Ca, s)$ plane (the two state variables of the SLOW system), where the results are nearly identical.

In future sections, we will demonstrate how neuromodulatory agents or an externally applied stimulus current can change the character of the slow-wave. Due to these changes in the model dynamics, we have verified that the model reductions in this section are valid across the range of possible values for $I_{STIM}$, 5-HT, and DA. Panels C and D of Fig. 4.1 compare the response of the three slow models at a 5-HT concentration of 60 $\mu$M, where the character of the slow-wave is markedly different.

### 4.2 The Bursting Mechanism

Our analysis of the model's bursting mechanism utilizes multiple scale methods, similar to those employed in other studies of bursting cell models [54, 80, 81, 84]. The FULL system is subdivided into FAST and SLOW systems, as described in the previous section. A bifurcation analysis of the FAST system parameterized by the SLOW variables $Ca$ and $s$ is performed, and the results are used to explain how bursting occurs in the FULL system. In this section we are solely concerned with the response of the FAST system as $Ca$ and $s$ are varied. The response of the SLOW system as external parameters are varied is treated in subsequent sections. The focus of this section is highlighted in Fig. 4.2. It is not our intent to perform a detailed analysis of the bursting mechanism, however, we wish to demonstrate that it is conceptually similar to the analysis already presented by Rinzel and Lee [81].

#### 4.2.1 A Topological View of the Slow-Wave

Prior to discussing how $Ca$ and $s$ parameterize the response of the FAST system, it is necessary to consider the intrinsic behavior of the SLOW system and the topology of
its state space. Throughout this section, we consider only the unmodulated response of the SLOW system (i.e. $I_{STIM}$, DA, and 5-HT = 0).

When $\bar{g}_{Na}$ is set to zero in the FULL system, the model displays the underlying slow-wave (Fig. 4.3). The membrane currents governing this slow wave are discussed fully in [18], and such slow-wave behavior is found in other bursting cellular models [18, 22, 74, 75, 76, 81]. The character of the slow-wave can vary as system parameters change, and some modeling studies [18, 74, 75, 76] have chosen to study the slow-wave behavior as a predictive explanation of the response of the full system when parameters are changed. This will be considered in the following section.

In the Model Reduction section, we demonstrated numerically that the SLOW system is of second order in $(Ca, s)$. Intuitively, we can think of $V_{ss}$ as a two-dimensional surface in $(Ca, s, V)$ space, and all periodic and fixed point solutions of the SLOW system lie upon the $V_{ss}$ surface. This is shown in Fig. 4.4, along with the $Ca$ and $s$ nullclines of the SLOW system upon the $V_{ss}$ surface. The nullclines are the set of state variables for which the derivative function of one of the state variables equals zero, and these nullclines govern the direction of flow of periodic solutions of the SLOW system. The shape of the $V_{ss}$ surface determines the character of the mem-
brane potential oscillations as $C_a$ and $s$ change. Figures 4.3 and 4.4 compare the response of the SLOW system with that of the FULL system. It is clear that for the interburst portion of the FULL system, the solution is similar to that of the SLOW system. Both trajectories lie upon $V_{ss}$ and obey the nullclines. However, at a point during the burst cycle, the $I_{Na}$ current activates and the FULL system starts to fire tonic action potentials. $V$ becomes an active state variable, and the periodic solution trajectory leaves the $V_{ss}$ surface.

4.2.2 The Onset of a Burst Occurs via a Saddle-Node Bifurcation of the FAST System

The behavior of the SLOW and FULL system, and a bifurcation analysis of the FAST system in terms of the SLOW variables, is more easily viewed by considering the two-
Figure 4.4: FULL and SLOW system behavior in \((C_a, s, V)\) space
Axes are \(C_a\), \(s\), and \(V\). Mesh lines indicate the \(V_{ss}\) surface, which is the solution set to the FAST system when \(\tilde{g}_{Na}\) = 0. The trajectory of the SLOW system (solid) lies upon the \(V_{ss}\) surface. The trajectory of the FULL system is also shown and lies upon the \(V_{ss}\) surface during the interburst phase. The intersections of the \(C_a\) and \(s\) nullclines (dotted) with the \(V_{ss}\) surface are also indicated. The line labeled SB (dashed) marks a saddle-point bifurcation of the FAST system. This line marks the region of the \((C_a, s)\) phase plane in which the firing of action potentials occurs. Mesh discretization on \(V_{ss}\): \(\Delta C_a = 50\) nM, \(\Delta s = 0.1\).
Figure 4.5: Projection of SLOW and FULL systems onto $(Ca, s)$ phase plane Projection of Fig. 4.4 onto the $(Ca, s)$ phase plane. Solid lines denote the trajectories of the FULL and SLOW subsystems. Dotted lines represent the $Ca$ and $s$ nullclines of the SLOW system. Dashed line SB represents the values of $(Ca, s)$ for which a saddle-node bifurcation of the FAST system occurs. In general, when the trajectory of the FULL system is "above" this line in this phase space, bursting occurs, while when the trajectory of the FULL system is "below" this line, the trajectory lies on the $V_{ss}$ surface and "obeys" the nullclines of the SLOW system. This gray line labeled $\alpha$ represents the values of $(Ca, s)$ used to parameterize FAST system in the bifurcation diagram of Fig. 4.6. Points P1 and P2 demonstrate the homoclinic nature of the saddle-node bifurcation. These panels show ten seconds of simulated activity of the FAST system at the indicated $(Ca, s)$ parameter values. For $(Ca, s)$ values close to SB, the period of oscillation (spiking) of the FAST system becomes very large.
dimensional \((Ca, s)\) phase plane, shown in Fig. 4.5. This figure is a projection of the trajectories and nullclines of Fig. 4.4 upon the \((Ca, s)\) plane.

Figure 4.5 compares the \((Ca, s)\) trajectories of the SLOW and FULL model. In the SLOW model, the primary mechanism for \(Ca\) influx is the slow-inward calcium current \(I_{SI}\). However, in the FULL system, \(Ca\) influx is due primarily to the fast calcium current, \(I_{Ca}\). Each occurrence of an action potential during the burst activates \(I_{Ca}\) on its repolarizing phase, causing a rapid influx of \(Ca^{2+}\) into the cell (see also Fig. 4.3). The \(Ca^{2+}\) influx due to \(I_{Ca}\) causes the FULL system trajectory in the \((Ca, s)\) plane to move in the horizontal \((Ca)\) direction more rapidly than that of the SLOW system. When the trajectory of the FULL system lies "below" SB, the solution of the FULL system lies upon \(V_{ss}\) and obeys the SLOW nullclines. This corresponds to the silent phase of the burst cycle. When the trajectory of the FULL system lies "above" SB, the stability of the FAST system changes, and spiking (oscillatory) activity occurs.

The stability of the FAST system was determined by performing a bifurcation analysis of the FAST system using the software package AUTO [29]. Our analysis shows that there is a set of \((Ca, s)\) values for which the FAST system undergoes a saddle-node bifurcation. A saddle-node bifurcation occurs when two fixed points coalesce and disappear as a parameter of the system is altered in a continuous manner [43]. The set of \((Ca, s)\) values for which this bifurcation occurs is shown by the line marked SB in Figs. 4.4 and 4.5. This definition, in the context of a fast-slow subdivided system, is consistent with earlier mathematical treatments of the bursting mechanism in a cellular model [54, 81]. As expected, SB is virtually isopotential in the \(V\) plane, consistent with the usual definition of an action potential threshold.

Figure 4.6 shows a bifurcation diagram of the FAST system when \(Ca\) is fixed at 124.3 nM and \(s\) is varied from 0 to 1, corresponding to the \((Ca, s)\) values along the line marked A in Fig. 4.5. Before continuing, it is necessary to explain how to interpret the bifurcation diagrams presented in this study.
Bifurcation diagrams (Figs. 4.6, 4.10, 4.12, and 4.14) display the solution structure of a system as a single parameter of the system is varied. Both fixed and periodic solutions are displayed, and the stability of both types of solutions are indicated. In these diagrams, the following notation is utilized. All solutions are displayed in terms of membrane potential, since $V$ is the dominant state variable of the FAST system, and in the SLOW system is parameterized by $(Ca, s)$, making it a convenient one dimensional indice for representational purposes. Stable fixed points are indicated by solid lines, and unstable fixed points by dashed lines. Periodic solutions are represented by showing both the minimum and maximum of the oscillation ($V_{\text{min}}$ and $V_{\text{max}}$, respectively) and (for some diagrams) the period of the oscillation. $V_{\text{min}}$ and $V_{\text{max}}$ of stable periodic solutions are indicated by dotted lines, and those of unstable periodic solutions by dash-dotted lines. Hopf bifurcations are represented by open circles. A Hopf bifurcation is a parameter set where a fixed point of a solution changes stability, i.e. when the eigenvalues for the linearized system at a fixed point cross the imaginary axis. Furthermore, a Hopf bifurcation corresponds to an intersection between the fixed and periodic solution sets, and is the manner in which all of the periodic solution studied in this paper arise. For an introductory treatment to the terminology and techniques discussed in this section refer to the texts by Hale and Koçak [43] and Wiggins [91].

Consider an initial starting point $(124.3, 0.0)$ on $V_d$. This point can be located on Figs. 4.4 and 4.5, and is located on the bifurcation diagram of Fig. 4.6 at the intersection of the lowest solid line (stable fixed point) and the left border of the gray box marked “physiological range of $s$.” As $s$ is increased from zero, steady-state membrane potential becomes more depolarized. At a critical value of $s$ $(s_B)$, the two lower fixed points coalesce and disappear. This is a saddle-node bifurcation, and corresponds to the intersection of the A and SB lines in Fig. 4.5. At this point, the stable attracting solution no longer exists, and the solution is attracted to the closest periodic attractor, resulting in spiking activity. This saddle-node bifurcation
is also a homoclinic orbit of the FAST system, i.e. for \((Ca, s)\) values infinitely close to (and above) SB, the FAST system has a period approaching infinity [91]. This is demonstrated at points P1 and P2 of Fig. 4.5, where a simulated 10 second response of the FAST system is shown for the specified values of \((Ca, s)\). At P1, the period of the FAST system is approximately 200 msec, while at P2, where \((Ca, s)\) lies close to SB, the period is approximately 3 seconds.

Figure 4.6 is parameterized by \(s\) from -1 to 2. This is outside the physiological range of \(s\), which by definition is a unitless quantity that only varies between 0 to 1. However, if \(s\) is considered as a numerical parameter of the FAST system, it is necessary to use nonphysiological values of \(s\) in order to study the full solution structure of the FAST system. Consider the origin and growth of the family of periodic solutions in Fig. 4.6. An unstable periodic solution arises out of the Hopf bifurcation around \(s = -0.30\). This periodic solution doubles back on itself to become a stable periodic solution and then doubles back on itself again. Therefore, this diagram indicates that there exist not one, but two possible periodic solutions to the FAST system at certain values of \((Ca, s)\), which are labeled AP1 and AP2. A trajectory which passes through the saddle-node bifurcation at SB will always fall within the basin of attraction of AP1, which corresponds to the "correct" action potential seen during bursting. The second periodic solution (AP2) predicted by this analysis was verified to exist through direct numerical integration with appropriate initial conditions. The insets in Fig. 4.6 show 500 milliseconds of periodic behavior for AP1 and AP2 when \((Ca, s) = (124.3, 0.3)\). While this second solution is most likely a mathematical artifact of the model, it is worth noting since we have never seen a membrane model capable of producing two qualitatively different action potentials for an identical set of parameter values. This high degree of complexity is due to the fact that our FAST system has 8 independent variables, while most systems analyzed in this manner have only two or three.
Figure 4.6: Bifurcation diagram of FAST system as $s$ is varied. $Ca$ is fixed to 124.3 nM. Values of $(Ca, s)$ parameterizing this diagram correspond to the vertical gray line labeled $\alpha$ in Fig. 4.5. Saddle-node bifurcation (SB) corresponds to the intersection of $\alpha$ and SB in Fig. 4.5. Stable fixed points are indicated by solid lines, and unstable fixed points by dashed lines. Maximum ($V_{max}$) and minimum ($V_{min}$), of periodic solutions are also indicated. Stable periodic solutions are indicated by dotted lines, and unstable periodic solutions by dash-dotted lines. Hopf bifurcation is represented by open circles. The two stable periodic solution branches are labeled AP1 and AP2, and insets display 500 msec of periodic behavior for each branch when $s = 0.3$. AP1 corresponds to the action potential seen during bursting, while AP2 is an anomalous action potential.
Figure 4.7: Comparison of $V_{ss}$ and $V_f$ surfaces

$V_{ss}$ is the solution to the FAST system for $\bar{g}_{Na} = 0$, while $V_f$ is the solution to the FAST system for $\bar{g}_{Na} = 38$. $V_{ss}$ is represented in gray, and is a monotonic solution in $(Ca, s)$. $V_f$, however, has up to three solutions for a given value of $(Ca, s)$, and is seen to fold away from $V_{ss}$ at potentials where $I_{Na}$ is active (approximately -30 mV to -15 mV). The saddle point bifurcation of FAST, which represents the limit point set of the lower fold of $V_f$, is indicated by a dashed line. This is the same as the dashed line labeled SB in the two dimensional phase plane of Fig. 4.5.
4.2.3 \( V_{ss} \) is a region of the FAST steady-state manifold

In Fig. 4.6 we demonstrated the bifurcation of the fixed points of the FAST system as a function of \( s \) with a fixed \( Ca \) value. It is important to note that the saddle-node bifurcation is a function of both \( Ca \) and \( s \). As \( Ca \) is varied, the location of the saddle-node bifurcation and the regions of attraction for the periodic solutions will also change.

Recall that \( V_{ss} \) is the steady-state solution of the FAST system as a function of \((Ca, s)\) with \( g_{Na} = 0 \). Let \( V_f \) represent the fixed points of the FAST system as a function of \((Ca, s)\) with \( g_{Na} = 38 \). This is similar to the definition of \( V_{ss} \) presented in [81]. A comparison of the two surfaces is shown in Fig. 4.7. The lowest (most hyperpolarized) surface of \( V_f \) is virtually identical to \( V_{ss} \), since at hyperpolarized potentials there is no activation of \( I_{Na} \). The solutions to the SLOW system lie upon this surface, while the FULL solution relaxes onto this surface during the interburst interval when bursting, and remains on this surface during electrical silence. Figure 4.6 is actually a slice of \( V_f \) in the \((V, s)\) plane with \( Ca \) fixed at 124.3 nM, and the three different fixed point solutions at low values of \( s \) can be thought of as a cross section of the three surfaces in Fig. 4.7. If viewed from above (i.e. looking down at the \((Ca, s)\) plane), the saddle-node bifurcation of the FAST system corresponds those \((Ca, s)\) values that denote the limit set of the existence of the lower two surfaces which make up the lower fold of \( V_f \). The choice of representing the bursting solution on \( V_{ss} \), rather than \( V_f \), in Fig. 4.4 is purely representational. Both surfaces are virtually identical in the region where the bursting solution relaxes to the steady-state manifold.

4.3 Modulatory Mechanisms

The model developed in Chapter 2 incorporates modulatory mechanisms to model the effects of 5-HT and DA upon the cell’s endogenous behavior. In Chapter 3, we studied these modulatory effects qualitatively, in terms of the effects of the neuromodulatory
agents upon the conductances of key currents in the model. In addition, the effects of an externally applied stimulus current \( I_{STIM} \) upon the behavior of the model have also been considered [18].

It is difficult to assess the role of neurotransmitters upon the behavior of the model simply by examining how they change the conductances of ion channels. Although the conductance of \( I_{SI} \) is changed by DA and 5-HT, changes in this current modify the operating concentration of \( Ca \), which in turn affects other \( Ca^{2+} \) sensitive currents \( (I_{NS}, I_{Ca}, I_{CaP}, I_{NaCa}) \). Due to the indirect effects of neurotransmitters upon the other currents of the model, another analytical approach is to study the effects of neuromodulatory agents upon the nullclines of the model.

Many modeling studies have employed the techniques of bifurcation analysis to study how the behavior and stability of a system changes as a parameter is varied. In the previous section, we performed such an analysis, examining the stability of the FAST system as parameterized by \( Ca \) and \( s \). In this section, we shall examine how DA, 5-HT, and \( I_{STIM} \) modify the stability of the SLOW system, which underlies bursting behavior. Figure 4.8 shows the focal point of the analysis presented in this section. This analysis treats the SLOW system as independent of the FAST system, and the validity of this assumption will be discussed. Under this assumption, there is an implied relationship between the behavior of the SLOW system and the expected behavior of the FULL system. Oscillatory behavior in the SLOW system corresponds to bursting behavior in the FULL system. Hyperpolarized silence in the SLOW system corresponds to hyperpolarized silence in the FULL system, and depolarized silence in the SLOW system corresponds to tonic beating in the FULL system, since \( V \) is above the action potential threshold for \( I_{Na} \). Equivalently, referring back to Fig. 4.5, we can think of beating as a stable fixed point in the \( (Ca, s) \) plane which lies "above" SB.
Figure 4.8: Block diagram of idealized FAST and SLOW subsystems
In this section, the behavior of the SLOW subsystem (highlighted) is studied as $I_{STIM}$, DA, or 5-HT are varied.

In the following sections we will be examining the solution structure of the SLOW system as $I_{STIM}$, DA, or 5-HT are varied. This will be done via the use of bifurcation diagrams and nullcline plots.

4.3.1 Nullcline Plots

The bifurcation diagrams provide us with information on the overall solution structure. However, they do not provide us with insight into the mechanisms by which a change in a parameter alters the model’s behavior. Insight into the modulatory mechanisms (from a mathematical perspective) of $I_{STIM}$, DA, and 5-HT can be gained by examining the effects of parameter changes upon the nullclines of the SLOW system.

Figure 4.9 shows a nullcline plot of the SLOW system, along with its trajectory in phase space when all external control parameters ($I_{STIM}$, DA, 5-HT) are set to zero. The shape of the nullclines, and thus the location and stability of the fixed points, changes as parameters are varied. Before proceeding with our analysis, it is necessary to derive some general rules relating the location of the fixed points in $(Ca, s)$ to the stability of the SLOW system.
Figure 4.9: Phase plane behavior of SLOW system during normal bursting
The $\dot{s}$ (dotted) and $Ca$ (dashed) nullclines are indicated. The phase trajectory is shown (solid) and is traversed in a clockwise direction. The fixed point of the system is located at intersection of the two nullclines.
We are assuming that the system is of second order, as demonstrated in the Model Reduction section. The eigenvalues of the linearized system at a fixed point determine the stability of the fixed point. The eigenvalues are the solution of:

$$\det(\lambda I - M) = 0$$

where

$$M = \begin{vmatrix} a & b \\ c & d \end{vmatrix} = \begin{vmatrix} \frac{\partial C_a}{\partial \lambda} & \frac{\partial C_a}{\partial \lambda} \\ \frac{\partial C_a}{\partial h} & \frac{\partial C_a}{\partial h} \end{vmatrix}$$

(4.18)

which results in the following polynomial:

$$\lambda^2 - (a + d)\lambda + (ad - bc) = 0$$

(4.19)

Due to the implicit nature of $V_{ss}$, we cannot compute the elements of $M$ directly. However, the signs of $C_a$ and $\dot{s}$ (i.e. the direction of flow) are known, and this information can be used to determine the signs of the elements of $M$ at any point lying upon both nullclines (i.e. a fixed point). These signs are indicated by the + and − signs on each side of the $C_a$ and $\dot{s}$ nullclines in Figs. 4.11, 4.13, 4.15, and 4.16. For any fixed point in $(C_a, s)$, we find that:

$$a < 0$$

$$b > 0$$

$$c < 0$$

The sign of $d$ depends upon the location of the fixed point along the $\dot{s}$ nullcline. Consider the $\dot{s}$ nullcline to be a letter Z. For those fixed points along the diagonal of the Z, $d > 0$, and for those fixed points on the upper or lower branch of the Z, $d < 0$. For the fixed point to be stable, we require that the eigenvalue solutions of eqn. (4.19) have a negative real part. The following conditions satisfy this requirement:

$$ad - bc > 0$$

$$a + d < 0$$
Both of these conditions are satisfied when \( d < 0 \). From our knowledge of the signs of the elements of \( M \), we make the following claims:

1. A fixed point lying on the upper or lower branch of the \( \dot{s} \) nullcline is a sufficient condition for the fixed point to be stable.

2. A fixed point lying on the diagonal of the \( \dot{s} \) nullcline is a necessary condition for the fixed point to be unstable.

We will see in the following bifurcation diagrams that the Hopf bifurcations determine the boundaries between oscillatory and stable behavior. From this observation, we extend the above claims to aid in the analysis of the nullcline plots:

1. A fixed point on the lower branch of the \( \dot{s} \) nullcline corresponds to a stable state of hyperpolarized electrical silence in the SLOW system, which corresponds to a similar state in the FULL system.

2. A fixed point on the upper branch of the \( \dot{s} \) nullcline corresponds to a stable state of depolarized electrical silence in the SLOW system, which corresponds to a beating state in the FULL system.

3. A fixed point on the diagonal of the \( \dot{s} \) nullcline is a necessary (but not sufficient) condition for slow-wave behavior in the SLOW system, corresponding to bursting in the FULL system.

Thus, we can obtain a general idea of when silence in the SLOW system (silence or beating in the FULL system) must occur and when slow-wave activity (bursting in the FULL system) can occur.

### 4.3.2 Modulatory Effects of \( I_{STIM} \)

\( I_{STIM} \) is capable of biasing R15 into silent, bursting, or beating modes. A bifurcation diagram of the effects of \( I_{STIM} \) upon the SLOW system is shown in Fig. 4.10. At
Figure 4.10: Bifurcation diagram of SLOW system as \( I_{STIM} \) is varied. Stable fixed points are indicated by solid lines, and unstable fixed points by dashed lines. Maximum (\( V_{max} \)), minimum (\( V_{min} \)), and period of periodic solutions are also indicated. Stable periodic solutions are indicated by dotted lines, and unstable periodic solutions by dash-dotted lines. Hopf bifurcations are represented by open circles. The labels I1 through I3 on the horizontal axis are for reference with Fig. 4.11.
large hyperpolarizing (negative) values of $I_{STIM}$, the SLOW system is in a state of hyperpolarized silence. As this current becomes less negative, the resting membrane potential becomes less hyperpolarized, a trend exhibited by both the FULL model and experimental preparations. Periodic solutions arise via a Hopf bifurcation as $I_{STIM}$ is further increased and the fixed point loses stability. In this periodic regime of behavior, $V_{m}$ becomes less hyperpolarized as $I_{STIM}$ is increased. This trend is exhibited by both the FULL model and in experimental preparations, where the depth of the interburst hyperpolarization decreases as an increasing depolarizing stimulus current is increased. Finally, at higher values of $I_{STIM}$, bursting terminates via another Hopf bifurcation, and the fixed point becomes stable. The stable fixed point becomes even more depolarized with increasing $I_{STIM}$. This indicates a state of tonic beating in the FULL model, and an increase in the depolarization of the fixed point suggests an increase in spike frequency. Both the FULL model and experimental preparations exhibit such behavior.

The bifurcation diagrams can be used in conjunction with nullcline plots in order to gain some insight into the mechanism by which the transition between behavioral modes occurs as $I_{STIM}$ is varied. Figure 4.11 shows the $Ca$ (dashed) and $\dot{s}$ (dotted) nullclines for the $I_{STIM}$ values I1, I2, and I3 indicated on the bifurcation diagram of Fig. 4.10. Points I1 and I3 correspond to the $I_{STIM}$ values which bound the region of stable oscillations, and I2 is an intermediate $I_{STIM}$ value of 0.0 nA. Varying $I_{STIM}$ shifts the $\dot{s}$ nullcline horizontally to the left (decreasing $I_{STIM}$) or right (increasing $I_{STIM}$). The $Ca$ nullcline shows little dependency upon $I_{STIM}$. The parameter values at which transitions between behavioral modes take place (I1 and I3) place the fixed point close to the transition regions between the diagonal branch of the $\dot{s}$ nullcline and the upper or lower branch of the $\dot{s}$ nullcline. Therefore, the mechanism for changing behavior in the SLOW system by $I_{STIM}$ is predominantly a horizontal shift in the $\dot{s}$ nullcline. This nullcline shifts the location and stability of the fixed point of the SLOW system.
Figure 4.11: Effects of $I_{STIM}$ upon SLOW system nullclines
$\dot{Ca}$ nullclines are dashed, and $\dot{s}$ nullclines are dotted. Labels on fixed points correspond to $I_{STIM}$ values labeled on central axis of Fig. 4.10.
Figure 4.12: Bifurcation diagram of SLOW system as DA is varied
Stable fixed points are indicated by solid lines, and unstable fixed points by dashed lines. Maximum ($V_{max}$), minimum ($V_{min}$), and period of periodic solutions are also indicated. Stable periodic solutions are indicated by dotted lines, and unstable periodic solutions by dash-dotted lines. Hopf bifurcations are represented by open circles.
4.3.3 Modulatory Effects of DA

DA is capable of eliminating bursting at high concentrations in both the FULL model and experimental preparations. This is also indicated by the bifurcation diagram of the SLOW system shown in Fig. 4.12. The period of the SLOW system is relatively invariant (19-20 seconds) until bursting terminates. As DA is increased towards the Hopf bifurcation, $V_{\text{max}}$ becomes more hyperpolarized, suggesting a reduction in spike frequency and burst length in the FULL model, since the slow-wave would be above the action potential threshold for a shorter amount of time. Such effects are observed in the FULL model. As DA is further increased, a Hopf bifurcation occurs and the only stable solution of the system is a state of hyperpolarized electrical silence. Further increases in DA hyperpolarize the stable fixed point, a trend also exhibited in the FULL model. This hyperpolarization is in agreement with experimental work which indicates the reduction of an inward current as DA concentration is increased [40, 66].

Figure 4.13 shows the $\dot{C}a$ (dashed) and $\dot{s}$ nullclines (dotted) for the DA values D1, D2, and D3 indicated on the bifurcation diagram of Fig. 4.12. The region of stable oscillations is from a DA concentration of 0 $\mu$M (D1) to 101.0 $\mu$M (D2). Increasing DA shifts the $\dot{s}$ nullcline to the left. This is due to the presence of $I_{\text{SI}}$ in the mathematical expression for $\dot{V}$, which determines $V_{ss}$ and shapes the $\dot{s}$ nullcline. Varying DA also changes the slope of the $\dot{C}a$ nullcline, due to the significant role that $I_{\text{SI}}$ plays in the equation for $\dot{C}a$. As DA is increased past D2, the fixed point becomes stable as it moves from the diagonal branch to the lower branch of the $\dot{s}$ nullcline. This is similar to the mechanism by which silence is obtained by decreasing $I_{\text{STIM}}$, only it occurs by parameter-dependent shifts in both the $\dot{C}a$ and $\dot{s}$ nullclines.

4.3.4 Modulatory Effects of 5-HT

The neurotransmitter 5-HT has a multitude of effects upon the behavior and burst pattern of R15, and these effects are well-mimicked in the FULL model. Many of these
Figure 4.13: Effects of DA upon SLOW system nullclines. Ca nullclines are dashed, and s nullclines are dotted. Labels on fixed points correspond to DA values labeled on central axis of Fig. 4.12.
Figure 4.14: Bifurcation diagram of SLOW system as 5-HT is varied.

Stable fixed points are indicated by solid lines, and unstable fixed points by dashed lines. Maximum ($V_{\text{max}}$), minimum ($V_{\text{min}}$), and period of periodic solutions are also indicated. Stable periodic solutions are indicated by dotted lines, and unstable periodic solutions by dash-dotted lines. Hopf bifurcations are represented by open circles.
changes are exhibited or implied by the response of the SLOW model. A complete bifurcation diagram of the effects of 5-HT upon the SLOW system is shown in Fig. 4.14.

As 5-HT concentration is increased from 0.0 $\mu$M, the SLOW system indicates a gradual hyperpolarization of $V_{\text{min}}$ and an increase in period. Similar responses to low concentrations of 5-HT are seen in the FULL model and experimental preparations. At low 5-HT concentrations, R15 exhibits an increase in the depth and duration of the interburst hyperpolarization phase [30]. A transition to electrical silence, accomplished via a Hopf bifurcation of the fixed point, occurs at a 5-HT concentration of approximately 1.09 $\mu$M. This silence is maintained until 5-HT concentration is further increased to 20.7 $\mu$M. A similar region of silence is also exhibited by the FULL model, and some R15 preparations exhibit a silent mode of behavior at a low concentration of 5-HT [30]. At higher concentrations of 5-HT, bursting is resumed via passage through a second Hopf bifurcation point. In this region, the SLOW model exhibits a steady-increase in period, as well as depolarization of both $V_{\text{min}}$ and $V_{\text{max}}$. The FULL model exhibits a steady increase in burst length and spike frequency, results which are consistent with the depolarization of $V_{\text{max}}$ in the SLOW system. At even higher concentrations of 5-HT, the SLOW model exhibits a third Hopf bifurcation to a stable depolarized fixed point. This should correspond to a state of tonic beating in the FULL system, and such behavior is demonstrated by both the FULL system and experimental preparations [61]. The SLOW model also exhibits a bistable region of behavior from approximately 69.3 to 87.5 $\mu$M, where a stable periodic and fixed point solution coexist. A bistable region where bursting and beating co-exist is exhibited by the FULL model at a slightly lower range of 5-HT concentrations (approximately 60 $\mu$M).

Due to the existence of two distinct regions of bursting behavior, we have separated our nullcline analysis of the effects of 5-HT upon the SLOW system into two
Figure 4.15: Effects of low doses of 5-HT upon SLOW system nullclines. Ca nullclines are dashed, and 5 nullclines are dotted. Labels on fixed points correspond to 5-HT values labeled on central axis of Fig. 4.14.
concentration ranges of 5-HT: low (0-10 μM) and high (10-100 μM). We will first
discuss the changes in the nullclines at low concentrations of 5-HT.

Figure 4.15 shows the \( \dot{Ca} \) (dashed) and \( \dot{s} \) (dotted) nullclines for the 5-HT values
S1, S2, and S3 labeled on the bifurcation digram of Fig. 4.14. The region of stable os-
cillations is from a 5-HT concentration of 0 μM (S1) to 1.16 μM (S2). Concentrations
of 5-HT greater than S2 (such as S3) results in a stable hyperpolarized silence in the
SLOW model. Starting at 0.0 μM, increasing 5-HT concentration shifts the lower
portion of the \( \dot{s} \) nullcline horizontally to the left, with minimal changes in the \( \dot{Ca} \)
nullcline. Low 5-HT concentrations primarily affect \( I_R \), which does not appear in
the \( \dot{Ca} \) equation. The \( \dot{s} \) nullcline is changed mostly along its lower portion because
\( I_R \) is active at hyperpolarized potentials, so it is only in the region of \((Ca,s)\) space
where \( V_{ss} \) is hyperpolarized (see Fig. 4.4) that changes in \( I_R \) will exhibit an effect.
Therefore, the transition to silence is achieved at low 5-HT concentrations by a shift
of the lower portion of the \( \dot{s} \) nullcline to the left. Once the shift in the \( \dot{s} \) nullcline is
sufficient to place the fixed point on the lower branch of the \( \dot{s} \) nullcline (as it passes
S2), the fixed point becomes stable.

Figure 4.16 shows the \( \dot{Ca} \) (dashed) and \( \dot{s} \) (dotted) nullclines for the 5-HT values
S4, S5, and S6, as labeled on the bifurcation diagram of Fig. 4.14. The region of
stable oscillations is from a 5-HT concentration of 20.2 μM (S4) to 87.5 μM (S6).
S5 represents an intermediate concentration of 40 μM. The effects of 5-HT at high
concentrations are opposite of the effects of DA, since high concentrations of 5-HT
increase the conductance of \( I_{St} \), as opposed to DA, which decreases the conductance
of \( I_{St} \). Therefore, the mechanisms by which the nullclines shift are similar (but in an
opposite direction) to those described previously for the effects of DA section.

As 5-HT is increased from within the region of hyperpolarized silence (such as at
S3 in Fig. 4.15), the \( \dot{s} \) nullcline shifts to the right, and the \( \dot{Ca} \) nullcline decreases
in slope. Eventually, this shift in both nullclines places the fixed point back on the
diagonal of the \( \dot{s} \) nullcline, where it is possible for the fixed point to become unstable,
Figure 4.16: Effects of low doses of 5-HT upon SLOW system nullclines. $C_a$ nullclines are dashed, and $\dot{s}$ nullclines are dotted. Labels on fixed points correspond to 5-HT values labeled on central axis of Fig. 4.14.
allowing oscillatory behavior to occur. This happens as 5-HT is increased past S4, which is approximately at the boundary between the diagonal and lower branch of the \( \hat{s} \) nullcline. Oscillatory activity in the SLOW system continues at S5, and a transition to depolarized silence occurs as 5-HT is increased past S6, which lies approximately at the boundary between the diagonal and upper branch of the \( \hat{s} \) nullcline.

To conclude this section, we have demonstrated that the effects of varying external parameters upon the behavior of the SLOW system can be visualized with the use of bifurcation diagrams. Changes in behavior occur by shifting the nullclines of the system, which alter the location of the fixed point and place it in regions where its stability can be either suggested or determined. Previously, we claimed that locating a fixed point on the diagonal of the \( \hat{s} \) is a necessary (but not sufficient) for a fixed point to become unstable. In theory, the transition between bursting and beating or silence could occur anywhere on the diagonal \( \hat{s} \) nullcline. However, our analysis indicates that the parameter values for which transitions in behavior of the SLOW system occur are nearby to those parameters for which the fixed point crosses from the diagonal nullcline to the upper or lower branch of the \( \hat{s} \) nullcline, increasing the utility of the nullcline plots in explaining behavior transitions.

### 4.3.5 Role of Unstable Orbits

All of the bifurcation diagrams shown demonstrate that the periodic solutions emanating from a Hopf bifurcation point are initially unstable, and that the regions of bistability all have an unstable and stable periodic orbit. In fact, all of the Hopf bifurcations of the SLOW system described in this study are subcritical, and this is the manner in which periodic solutions arise from a subcritical Hopf bifurcation, as outlined in several texts (e.g. [43], pages 344-355).

The FULL model exhibits bistable (i.e. bursting and beating) behavior when 5-HT is in a transition region between bursting and beating behavior. Figure 4.14 shows that there is a wide region of 5-HT concentrations, from 69.3 to 87.5 \( \mu \text{M} \), where
Figure 4.17: Bistable solutions in the SLOW system
Stable (thick solid) and unstable (dashed) periodic solutions of the SLOW system at a 5-HT concentration of approximately 82.3 μM are indicated. Trajectories for two similar initial conditions, one on each side of the unstable orbit are indicated. Trajectory A converges to the periodic solution, while trajectory B converges to the stable fixed point.
the SLOW system has a periodic stable solution, a periodic unstable solution, and a stable fixed point which corresponds to a state of depolarized silence. As 5-HT is increased, the regions of attraction for the two stable solutions change, and it is the unstable orbit that delineates the boundary between these two stable attractors. This is demonstrated in Fig. 4.17, which shows the periodic and fixed solutions of the SLOW system at a 5-HT concentration of 82.3 μM. The stable periodic solution is indicated by the thick black line, and the unstable solution by the dotted line. Two trajectories are shown for similar initial conditions located on each side of the unstable orbit. The trajectory on the outside of the unstable orbit (A) converges to the periodic solution, while the trajectory on the inside of the unstable orbit (B) converges to the stable fixed point. In an earlier version of the FULL model [18], it was demonstrated that certain values of $I_{\text{stim}}$ at the bursting/beating interface for which multiple periodic solutions exist [17]. Here we have demonstrated that even in a second-order reduced system, the regions surrounding the Hopf bifurcations as $I_{\text{stim}}$, DA, or 5-HT demonstrate bistable behavior.
Chapter 5

Discussion

5.1 Comparison with Experimental Results

Serotonin has diverse and widespread effects on R15. At low concentrations of 5-HT (0-10 $\mu$M), an increase in both the depth and duration of the interburst hyperpolarization is observed, which often leads to electrical silence as 5-HT concentration is increased [30]. This is due to a cAMP-mediated increase in $I_R$ [11]. The model adequately mimics this effect (Fig. 3.1).

High concentrations of 5-HT (> 20 $\mu$M) increase the burst length and the fraction of the burst cycle in which bursting takes place. High concentrations also often result in tonic beating [61]. In the model, the effect of higher 5-HT concentrations is primarily mediated by an increase in $I_{SI}$, which leads to both a lengthening of the slow-wave that underlies bursting and an increase in the mean $Ca^{2+}$ concentration of the model. This increase in $Ca^{2+}$ concentration inhibits $I_{Ca}$ and slows $Ca^{2+}$ influx, further lengthening the burst. The model shows an increase in burst length for 5-HT concentrations from 20 to 60 $\mu$M. When $I_{SI}$ is sufficiently increased by 5-HT, tonic beating occurs. Unlike the effects of 5-HT at low concentrations, the effects of 5-HT at high concentrations are not as well understood. These effects are observed experimentally, but no consistent dose-response data exists. For example, in Levitan and Levitan, 1988, burst lengthening is shown for a 5-HT concentration of 50 $\mu$M (Fig. 1D), but the same cell later demonstrates tonic beating at a 5-HT concentration of 25 $\mu$M (Fig. 1F).
Both DA and 5-HT can silence R15, but this occurs via different mechanisms. DA silences R15 through a decrease in $I_{SI}$, while 5-HT silences neuron R15 through an increase in $I_R$. Fig. 3.2 shows that self-sustained bursts cannot be triggered by current pulses applied to a cell silenced by DA [39], but can be triggered by current pulses to a cell that has been silenced by 5-HT [61].

Just as the effects of current pulses upon R15 silenced by either DA or 5-HT differ, the effects of current pulses upon R15 in a tonic beating state differ as well. Figure 3.6 demonstrates that tonic beating induced by high 5-HT concentrations can be interrupted by either hyperpolarizing or depolarizing current pulses, and this interruption results in a sustained hyperpolarization. These effects are also seen experimentally [61, Figs. 9C and 9E]. The model also indicates that a long depolarizing pulse (on the order of seconds) is required to interrupt bursting, and that there is a delay of a few seconds between the end of the current pulse and termination of bursting. Both of these effects have been demonstrated experimentally [61, Fig. 9E]. The interruption of beating by a depolarizing pulse, and the delay before this happens, is a result of a difference in the decay rates of $s$ and $b$ compared to $Ca^{2+}$. A cell induced into tonic beating by a bias current, however, cannot be interrupted by a current pulse for any sustained length of time.

The effects of current pulses upon silent and beating R15 cells in a bath containing 5-HT demonstrate that the effects of 5-HT cannot be described as simply excitatory or inhibitory [61]. The results show that 5-HT application appears to sensitize the cell's response to brief current pulses, and that this sensitivity is due to an increase in two opposing currents, $I_R$ and $I_{SI}$ (see Figs. 3.2 and 3.6).

The model exhibits bistable behavior at certain parameter values of $I_{STIM}$ and 5-HT where both bursting and beating modes co-exist. It is possible, through application of an appropriate stimulus current, to switch between these behavior modes. This may have physiological significance, although no plausible role for the existence
of bistable mechanisms has been postulated. These regions of bistability motivated some of the work performed in the analysis section.

5.2 Analytical Insights

The present model exhibits bistable regions of parameter space in $I_{STIM}$ and 5-HT where bursting and beating modes co-exist, and an earlier version of this model exhibits multiple stable bursting solutions at certain values of $I_{STIM}$ [17]. It is possible that such bistable behavior could have physiological implications. What is the nature of the state space that allows multiple periodic solutions to coexist? The aforementioned example of bistability and multiple bursting solutions were found by trial and error. Rather than simply trying numerous combinations of parameter values and initial conditions, we wish to analytically examine the character of the solution space of periodic and equilibrium solutions. This study is a first step in this process.

The primary focus of this study is to examine the effects of neuromodulatory agents upon the reduced SLOW system, and to relate these results to the behavior of the complete (FULL) system. This offers two distinct advantages. First, AUTO can easily analyze the SLOW system’s periodic solution structure, due to its low order and the lack of any fast elements necessary for generation of action potentials. Second, the reduced model provides, in a theoretical sense, an identifiable measure for the transition between bursting and beating activity. Consider the system properties which differentiate bursting solutions from beating solutions. Both types of solutions are periodic, and there is no change in intrinsic system properties defining the transition between bursting and beating. However, when using the assumptions of our reduced system, this transition is easily defined. Bursting solutions corresponds to periodic solutions of the SLOW system while beating solutions correspond to a stable depolarized equilibrium solution of the SLOW system. The change in stability corresponds to a Hopf bifurcation, which is easily detected by AUTO. If the bifurcation
diagrams of the fixed points of the FULL system (not shown) are examined, no such bifurcation at the bursting/beating interface is observed.

5.2.1 Comparison of SLOW System Predictions with the FULL System

How accurately can our results from the analysis of the SLOW system be extended to the FULL system? Figure 5.1 compares the predicted and actual behavior of the FULL system as $I_{STIM}$, DA, or 5-HT are varied. Results from the SLOW system are obtained from the bifurcation diagrams (Figs. 4.10, 4.12, and 4.14), while results from the FULL system are obtained through direct numerical integration. The behavioral properties (i.e. bursting, beating, or silence) are preserved in both models, although the range of control parameters ($I_{STIM}$, DA, or 5-HT) defining the transition regions between modes may differ. The two models exhibit a close degree of agreement in parameter space for the locations of the bursting/silence and silence/bursting interfaces.

The two systems differ in the parameter ranges that define the bursting/beating interface. Both systems exhibit a transition from bursting to beating at high values of $I_{STIM}$ or 5-HT, and both systems show an overlap (bistability) between these two behavioral modes. However, the models differ in the location and extent of these regions. This is due to our use of a reduced order model and a breakdown in some of the idealizations used in our analysis, which will be described in the following section.

Although there are differences in the ranges of behavioral modes between the two models, the SLOW system provides information about the behavior of the FULL system which otherwise could not be obtained or would be difficult to quantify. Most importantly, it demonstrates the underlying rate-limiting processes, the activation and deactivation of $s$ and the material balance of $Ca$, which underly the slow-wave activity regulating bursting behavior. In addition, we demonstrated in Chapter 4 how the SLOW bifurcation diagrams depict many of the observed effects of $I_{STIM}$, DA, and 5-HT upon the endogenous behavior of the FULL model and experimental prepa-
Figure 5.1: Comparison of behavioral modes of the SLOW and FULL Model
The bar graph above shows the regions of parameter space for different behavioral modes in the SLOW (gray) and FULL (black) model. Analytical results for the SLOW model were obtained from the bifurcation diagrams shown previously (Figs. 4.10, 4.12, and 4.14). A state of hyperpolarized silence in the SLOW model corresponds to silence in the FULL model, periodic behavior in the SLOW model corresponds to bursting in the FULL model, and depolarized silence in the SLOW model corresponds to beating in the FULL model. Parameter ranges for the FULL model were determined by temporal integration for numerous parameter values and sets of initial conditions. Since the FULL system is not treated analytically, these behavioral ranges are approximate, and could possibly span wider ranges of parameter values. All data shown above is for a variation in a single stimulus parameter (A) \( I_{\text{STIM}} \), (B) DA, or (C) 5-HT.
rations, and the mathematical mechanisms of these behavioral changes are elucidated through the use of nullcline plots. Also, the subcritical nature of Hopf bifurcations of the SLOW model provide us with a logical approach for determining the extent of the behavioral modes of the FULL model. Observe in Figs. 4.10 and 4.14 the nature of the transition between the periodic and depolarized stable solutions. As \( I_{STIM} \) or 5-HT is increased, the region of bistability is entered into in a hysteresis-like manner. If the SLOW model is at a stable depolarized equilibrium solution and \( I_{STIM} \) is reduced into the bistable region, the model will remain at a stable depolarized equilibrium. Likewise, if the model exhibits oscillatory behavior and \( I_{STIM} \) is increased into the bistable region, the model will continue exhibiting oscillatory behavior. If this type of solution structure exists in the FULL system, we can easily determine the bistable regions of FULL system. For example, suppose the FULL model is in a beating mode induced by a high value of \( I_{STIM} \). \( I_{STIM} \) is decreased a small amount, and the model is run until transient effects decay. This process is repeated until it is no longer possible to maintain beating for even a small reduction in \( I_{STIM} \). Using this technique we determined the lower \( I_{STIM} \) bound of the beating region. This same concept is employed to determine the upper bound of the bursting region, and we ultimately determined a large bistable region in the FULL model, from \( I_{STIM} = 1.05 \) nA to \( I_{STIM} = 1.62 \) nA. Considering the complexity of the FAST bifurcation diagram of Fig. 4.6 and this demonstration of bistable phenomenon in the SLOW system, it is possible that the FULL system also exhibits more complex modes of behavior, such as the region of multiple nested periodic solutions shown in [17].

To summarize, we have demonstrated that the general effects of altering the control parameters (\( I_{STIM} \), DA, and 5-HT) can be demonstrated by a reduced 2nd order model. The dynamics of the system are governed by two rate-limiting independent variables, the intracellular calcium concentration, \( Ca \), and the voltage-dependent activation of \( I_{SI} \), represented by the gating variable \( s \). This reduced system is unique in that membrane potential is not an independent variable, similar to an analysis pre-
sented in [81]. The fixed and periodic solutions to our reduced system can be pictured as lying upon a two dimensional surface \( V_{ss} \), which is the steady-state membrane potential as a function of \( Ca \) and \( s \). Physiologically, DA and 5-HT affect the model's behavior by altering the conductances of \( I_R \) and \( I_{SI} \), while \( I_{STIM} \) alters the model's membrane impedance. In this reduced analysis, the control parameters exert their actions in two ways. First, they alter the shape of the \( V_{ss} \) surface, which subsequently affects the location of the system nullclines. This is because the SLOW system nullclines are simply the intersection of the \( V_{ss} \) surface with \( \dot{Ca} \) and \( \dot{s} \) nullsurfaces in \( (Ca, s, V) \) space, so altering \( V_{ss} \) changes the location of the SLOW nullclines. We also note that \( \dot{s} = 0 \) is solely a function of membrane potential, so its shape is more affected by changes in \( V_{ss} \) than is the \( \dot{Ca} \) nullcline, which exhibits a strong calcium dependency. Second, changes in \( I_{SI} \) significantly affect the shape of the \( \dot{Ca} \) nullcline, since \( I_{SI} \) is a significant term in the equation for \( \dot{Ca} \).

5.2.2 Validity of Model Reductions

The analysis presented in this paper is essentially an extension of an earlier analysis presented by Rinzel and Lee [81] on an earlier model of R15 developed by Plant and Kim [76]. The scope of their analysis is shown in Fig. 5.2A. Rinzel and Lee partitioned their model into FAST and SLOW subsystems, and examined in detail how variations in the SLOW variables, \( x \) and \( Ca \), parameterized the behavior of the FAST system. We performed a similar analysis in section 4.2, and demonstrated that the onset and termination of a burst occurs via a saddle-point bifurcation, in a manner treated in more detail by Rinzel and Lee [81]. Given this demonstration of the basic mechanism of bursting, we extended our analysis to determine how external control parameters, \( I_{STIM} \), DA, and 5-HT, affect the behavior of the slow-wave underlying bursting behavior. This approach is shown in Fig. 5.2B.

Both of these approaches, however, are idealizations. They assume that control of the system is essentially one-directional, whereby the control parameters determine
Figure 5.2: Comparison of Reduced Model Approaches

Earlier analyses idealized the system as consisting of a SLOW and FAST subsystem, with the variables of the SLOW system parameterizing the FAST subsystem [80, 81] (A). The analysis described in this paper extends this approach, analyzing how external control parameters alter behavior of the SLOW system (B). Both (A) and (B) are idealizations, and the actual system is much more complicated (C). See text for details.
the behavior of the SLOW system, and the output of the SLOW system \((Ca\) and \(s\)) parameterizes the behavior of the FAST system. These idealizations have proven useful in accurately describing the onset of a burst, performing a reduced analysis on our system, and representing the dominant control pathways affecting the FAST and SLOW subsystems. However, the FULL system is much more complex, and the interactions between the FAST and SLOW system are not one-directional. A detailed depiction of the interactions between the FAST and SLOW systems is shown in Fig. 5.2C.

Interactions between the two systems which are not considered in this analysis include:

1. Feedback from the FAST system onto the SLOW system. This is the most obvious interaction which is not considered. In the isolated SLOW system, calcium entry into the cell occurs via \(I_{SI}\). However, in the FULL system, the dominant mode of calcium entry is via \(I_{Ca}\) (and to a lesser extent, \(I_{NS}\)), which causes an influx of calcium during the occurrence of each action potential. This is readily seen during the time-course of bursting in Fig. 4.3A in the step-like character of the calcium waveform, and also accounts for the rapid traversing of the \((Ca, s)\) phase plane in the horizontal direction in Fig. 4.5. Also, the oscillations in \(V\) due to the firing of action potentials during bursting perturb the time-course of the \(s\) gating variable, although not to a significant extent.

2. Feedback of the FAST system onto itself via activation of additional slow processes. Two of the state variables, \(b\) and \(I\), are slow variables on time-scales similar to \(s\) and \(Ca\). However, they are not studied as part of the SLOW system since they are normally at a constant value during slow-wave activity, and only change their values during the occurrence of action potentials. Possibilities for future analysis include defining these variables as dependent upon the frequency
of the FAST system, or treating them as additional bifurcation parameters when analyzing the solution space of the FAST system.

3. Effect of the control parameters upon the solution space of the FAST system. Our analysis has only concentrated upon the effects of control parameters upon the behavior of the SLOW system. They also affect the character of the solution space of the FAST system, although in a predictable manner. The features of the saddle-node bifurcation in Fig. 4.5 are preserved, but its location is shifted appropriately as the region of SLOW system activity in \((Ca, s)\) space changes.

These discrepancies account for the difference in response between the SLOW and FULL models at high values of 5-HT and \(I_{STIM}\). For example, during beating activity in the FULL system, our idealized analysis predicts that the mean \(Ca\) and \(s\) values are indicated by the location of the stable fixed point of the SLOW system. In fact, due to the frequency-dependent interactions described in (1) above, the mean \(s\) and \(Ca\) values during beating behavior are larger than the SLOW system would predict. The unresolved issues are these: when the FAST components of the system are active and perturb the SLOW trajectory, how does one predict the solution in \((Ca, s)\) phase space? In a related manner, how does one study the transition from bursting to beating behavior in an analytical manner?

One possible method for future analysis is to modify the FULL system so that it looks like the system depicted in Fig. 5.2B. This could be achieved by removing certain currents from the equations for \(\dot{V}\) and \(\dot{Ca}\), and utilizing two separate variables for membrane potential, \(V_{fast}\) and \(V_{slow}\). Specific control pathways could then be selectively “turned-on”, and the overall system behavior and solution structure could be examined. A recent publication by Smolen and colleagues [84] provides an innovative approach to this problem, which will be discussed shortly.
5.2.3 Comparison to Earlier Analytical Approaches

Many other researchers have applied analytical techniques to the analysis of bursting cellular models, most often studying models of the pancreatic \( \beta \)-cell [80, 52, 84], neuron R15 in *Aplysia* [75, 76, 81], or a generic bursting model consisting of coupled fast and slow oscillatory systems [54].

One of the first mathematical treatments of the mechanism of a bursting cell was developed by Plant and Kim [75]. Using the published data of Mathieu and Roberge [68], who recorded slow-wave oscillations in neuron R15 by blocking \( I_{Na} \) with TTX, they developed a simple oscillatory membrane model of the slow-wave. The model is essentially a relaxation oscillator, and was analyzed using nullcline techniques. Shortly thereafter, they developed another model which incorporated a spiking mechanism [76], which is the first known bursting cellular model. The slow mechanisms of this model were studied by setting \( g_{Na} \) to zero, and a nullcline analysis was performed. The model was useful for its time, given the scant amount of qualitative data on R15 which had been published at the time.

Kopell and Ermentrout [54] developed a generalized treatment of bursting, linking a subcellular oscillator with a spiking mechanism. They postulated a general mechanism of bursting consistent with what we have described here: spiking does not arise via a Hopf bifurcation, as it does in the Hodgkin-Huxley model as \( I_{STIM} \) is varied, but occurs through a homoclinic transition, where two critical points coalesce and disappear as a parameter(s) is varied, forcing the solution onto a periodic attractor.

The approach taken in this paper was performed on a \( \beta \)-cell model by Rinzel [80], and later applied by Rinzel and Lee [81] to the model of Plant and Kim [76]. They separated their model into two separate subsystems, FAST and SLOW, and analyzed how the SLOW system traversed stable and oscillatory regions of the FAST parameter space. They analyzed the mechanism of bursting in detail. We have chosen not to duplicate their treatment, and demonstrate a mechanism in our model which consistent with theirs.
Pernarowski [73] developed a model analysis approach utilizing matched asymptotic methods, and applied it to the three-variable Sherman-Rinzel-Keizer model of the $\beta$-cell [83]. Singular perturbation analysis is performed on three different phases of the burst cycle (silent, transition, and active), and the elegance of this approach is that it is mostly an analytical (as opposed to numerical) treatment. For example, analytical expressions are developed for the location of bifurcation points and the duration of the burst, properties typically determined using computational techniques.

All of the models mentioned so far are quite simple. This makes life easy for mathematicians, but it is often difficult to apply these techniques towards more complex models based upon quantitative measurements, such as the model which this paper is based upon [18]. The fact that our model incorporates numerous currents with $\text{Ca}^{2+}$ dependencies, non-HH-type expressions for the ion pumps and exchangers (ignored by many models), and a true material balance on $\text{Ca}^{2+}$, makes our analysis much more difficult. For example, many analysis and model-reduction techniques exploit the fact that all currents employ HH-type gating mechanisms and can be related solely to membrane potential, such as a recent methodology published by Kepler and colleagues [53]. Unfortunately, we cannot apply this method due to the numerous $\text{Ca}^{2+}$ dependencies on our model, and the fact that not all of the currents are related to solely membrane potential and intrinsic gating variables.

Our analysis also would be much simpler if the FAST and SLOW system did not share some system parameters, as does the model studied by Rinzel and Lee [81]. For example, every current which carries $\text{Ca}^{2+}$ appears in the equations for $\dot{V}$ (FAST) and $\dot{C}_a$ (SLOW). As a result, for any given values of $I_{STIM}$, DA, or 5-HT, an analysis of both the SLOW system and the FAST system has to be performed. It is possible to reparameterize our model-reduction approach so that the FAST system is independent of the control parameters of the SLOW system, but the unfortunately complication is that it results in a SLOW system of a higher order, destroying our compact second-order treatment.
A recently published paper by Smolen and colleagues [84] presents a numerical analysis method that may prove useful for future analysis of our model. Smolen studied a bursting $\beta$-cell model which has two slow inhibitory variables. Using a modification of AUTO [29], he employed a technique known as the “method of averaging” to analyze his system. Nullclines for the SLOW system are generated, which govern the model’s behavior in its silent (nonbursting) phase. In the active regions where action potentials occur, AUTO is used to average the FAST system’s response over the time course of one period and numerically calculate nullclines for the SLOW system.

5.3 Physiological Implications

The modeling efforts presented here have focused upon the effects of 5-HT on the activity of R15 due to the wide availability of published data. R15 is also activated by a peptide (ELH) released from the bag cells of the abdominal ganglion [14]. ELH has been found to have similar effects as 5-HT upon the cell’s bursting behavior, and the effects of both 5-HT and ELH are mediated by an increase in the production of intracellular cAMP [60]. Therefore, for the purpose of discussion the modeled 5-HT responses will be considered to be similar to those of ELH.

The simulation results presented previously demonstrate a mechanism by which application of high concentrations of 5-HT cause an increase in the length and intensity of bursting on R15 (Fig. 3.2), ultimately leading to beating at sufficiently high concentrations. After considering the physiological evidence presented above, one might simply assume that bag cell stimulation enhances the bursting of R15, leading to an increase in production of R15$\alpha_1$ peptide, which has numerous physiological effects [5, 6, 7, 89]. However, additional physiological evidence argues against such a scenario.

Although R15 exhibits endogenous bursting in vitro, it is believed to be normally silent in vivo, acting as a “conditional burster” [5]. Furthermore, R15 demonstrates
activity-dependent suppression of its response to 5-HT and ELH [57], suggesting that ELH has a more potent effect upon R15 if it is normally silent. However, egg-laying did not take place during in vivo chronic recording [5], so this concept of a "conditional burster" has not been observed and tested.

The model exhibits several mechanisms by which neuromodulatory agents can modify the activity of R15. We have demonstrated how both DA and 5-HT can silence the cell through different mechanisms, and that a 5-HT silenced cell will still elicit a sustained burst when perturbed by a brief current pulse (Fig. 3.2). Perhaps this could be considered a "conditional bursting" mechanism. Furthermore, these simulations demonstrate the effects of both hyperpolarizing and depolarizing current pulses on silent and beating cells, and using the model we offer an explanation for how depolarizing current input can temporarily terminate beating activity. A comparison of the responses of the 5-HT and bias current modulated cells to brief current pulses (Figs. 3.2 and 3.6) demonstrate that 5-HT appears to sensitize the cell to both depolarizing and hyperpolarizing synaptic input.

5.4 Comparison with Previous Models

5.4.1 Calcium Balance

Many mathematical models of bursting cells utilize a calcium balance similar to the following equation [12, 24, 52, 74]:

\[
[Ca^{2+}]_{i} = -k_{i}(I_{Ca,fast} + I_{Ca,slow}) - k_{e}[Ca^{2+}]_{i}
\]  

(5.1)

where \(k_{i}\) and \(k_{e}\) are set based upon assumptions of free:bound calcium ratios and resting calcium levels, and \(I_{Ca,fast}\) and \(I_{Ca,slow}\) represent fast and slow (if present in the model) calcium currents. The degradation term is often attributed to "diffusion" or "mitochondrial uptake." However, such balances are not true material balances. Such a balance ignores 1) extrusion of calcium by pumps or exchangers; and 2) buffer release (i.e. free:bound calcium ratios are not necessarily static during endogenous
activity). The present model considers mechanisms for both a calcium extrusion pump and sodium-calcium exchanger (see [18] for details). A calmodulin buffer is also explicitly modeled, and demonstrates that although a large amount (greater than 99%) of calcium influx is rapidly buffered, the buffer is responsible for both calcium uptake and release. While these are not modeled specifically from R15-based data, this study demonstrates that the inclusion of such mechanisms has an effect on the calcium dependency of the background currents and the shape of I-V plots. In Fig. 2.3, the hyperpolarized branch of the I-V curve shifts in the depolarizing direction with increased 5-HT concentration. This is due to the $Ca^{2+}$ dependency of $I_{CaP}$ and $I_{NaCa}$, since at increased 5-HT concentrations the $Ca^{2+}$ concentration at the membrane holding potential (-50 mV) is also increased.

5.4.2 Modulatory Mechanisms

Numerous cellular models have demonstrated the effect of single-parameter variations upon overall behavior [21, 22, 47, 52]. However, few models have formulated continuous expressions modeling the effect of modulatory agents upon one or more channel conductances. One such model is that of Keizer and Mangus [52], who developed a model with formulations for the effect of ATP upon a potassium channel.

While this work was in progress, a computational study of the effects of 5-HT upon R15 appeared in the literature [12]. This model formulated expressions for the effect of a normalized concentration of 5-HT upon an inward-rectifying potassium current and a slow-inward calcium current. Due to its similar nature, a comparison is made between the behavioral aspects of the model of Bertram and the one presented here.

Both models demonstrate bursting, beating and silent behavior. In the model of Bertram, there are three regions of activity. At low concentrations, the model bursts, and an increase in 5-HT increases the burst length, burst period, and interburst hyperpolarization. The model is silent at higher concentrations, and at even
higher concentrations operates in a bistable mode, where the model is either beating or in a state of hyperpolarized silence. This hyperpolarized state at very high concentrations does not agree with published steady-state I-V plots generated in the presence of 5-HT [11, 60, 61], and may prove to be nonphysiologic. Experimental voltage-clamp data indicates that at steady-state there exists a net inward membrane current at hyperpolarized potentials, so a stable hyperpolarized state should not exist. The model presented here does not demonstrate such a state at its highest 5-HT concentrations, and this difference is attributed to the more detailed treatment given to the calcium balance. This model also demonstrates a more physiologically diverse array of responses to 5-HT. Rather than demonstrating a simultaneous increase in burst length, burst period, and interburst hyperpolarization, our model presented here demonstrates an increase in the burst period and interburst hyperpolarization at low concentrations of 5-HT, followed by a region of silence, followed by a region of bursting accompanied by an increase in burst length and spike frequency. The model's response to low concentrations of 5-HT is consistent with the data in Fig. 1 of [30]. A general comparison of the response of this model and that of Bertram is shown in Figure 5.3.
5.5 Model Limitations

The purpose of this study was to demonstrate the effects of neuromodulatory agents on an existing model of a bursting neuron. Although the model can demonstrate many experimentally observed phenomena in the presence of 5-HT or DA, like any model, it has its limitations.

The equation describing the cAMP balance is undoubtedly an oversimplification of the actual mechanisms. The role of Ca$^{2+}$ upon cAMP concentration is not considered, although it has been found to affect both adenylyl cyclase and phosphodiesterase activity in *Aplysia* [34, 56, 90]. Several papers in the literature appear to demonstrate a slow time-dependency (on the order of minutes) of the effects of neurotransmitters upon neuron R15. A detailed kinetic model is necessary to adequately describe the complex biochemical activity at the subcellular level. Preliminary attempts have been made to develop such a model [85].

One experimental observation which the model fails to duplicate is that of plateau bursting at high concentrations of 5-HT [61, Fig. 1D]. In plateau bursting in R15, two important features are evident: (a) the burst phase is significantly longer than the interburst phase and (b) the spike frequency within the burst oscillates, giving the appearance of multiple burst cycles within a single burst phase. No model exists which can explain this second feature, although models with significant burst phases are known [23]. It is possible that some insight to this mechanism can be provided with further mathematical analysis and a more detailed biochemical model of R15.
Chapter 6

Conclusions

A mathematical model of R15 has been developed which modifies and extends a previously developed R15 model by our group [18]. The model consists of two major components: a Hodgkin-Huxley-type equivalent circuit model of the membrane, a fluid compartment model accounting for the intracellular concentrations of $Ca^{2+}$ and cAMP. In the present study, the effects of neuromodulatory agents (5-HT and DA) upon the activity of R15 have been considered. 5-HT acts by increasing the intracellular [cAMP], which in turn affects the conductances of $I_R$ and $I_{SI}$. DA acts directly upon $I_{SI}$ by decreasing its conductance. The effects of these neurotransmitters upon the endogenous behavior of the model were considered, the response of the model to current stimuli in the presence of DA and 5-HT was also investigated. Finally, a reduced-system analysis was performed upon the model to investigate the mechanisms of endogenous bursting and neuromodulation. The conclusions of this study can be summarized as follows:

1. Many characteristics of the concentration-dependent response of neuron R15 to 5-HT can be simulated by the model, including the increase in the length and depth of the interburst hyperpolarization observed at low doses ($< 2 \, \mu M$), electrical silence at doses in the range 2-20 $\mu M$, and an increase in burst length at high concentrations ($> 20 \, \mu M$). These responses can also be seen as modulations of steady-state I-V plots, in a manner consistent with published experimental data.
2. The concentration dependent effect of DA on the activity of R15 is mimicked as well. Bath application of DA dampens the slow-wave underlying the burst cycle and reduces the number of spikes per burst. Concentrations of DA > 100 μM lead to electrical silence at a stable hyperpolarized potential. These responses can also be seen as modulations of steady-state I-V plots, in a manner consistent with published experimental data.

3. These model-demonstrated responses support the viewpoint that the changes in the dynamic behavior of R15 in vitro in the presence of 5-HT or DA occur by scalings of the conductances of subthreshold currents.

4. The model also mimics the response of neuron R15 to hyperpolarizing and depolarizing current pulses in the presence of 5-HT and DA. These responses are quite different than those observed in the absence of these neurotransmitters. Depolarizing current pulses will elicit a sustained burst in a cell that has been silenced by 5-HT application, but not in a cell silenced by DA. Brief depolarizing and hyperpolarizing current pulses will produce a sustained (8-10 sec) hyperpolarization in a cell induced into tonic beating by 5-HT, but not in a cell induced into tonic beating by a depolarizing bias current. Our model offers explanations as to how both inward and outward current pulses effect these responses.

5. The fact that both depolarizing and hyperpolarizing current pulses have a more pronounced effect upon the model in the presence of 5-HT suggests that 5-HT may serve to sensitize R15 to synaptic input. It is also demonstrated that while increasing R15's responsiveness, an appropriate concentration of 5-HT will maintain the neuron in a state of hyperpolarized silence, demonstrating a possible mechanism of "conditional bursting" that is believed to be characteristic of R15's in vivo behavior.
6. Dopamine has the opposite effect of 5-HT in the model, serving to dampen a cell's response to external current stimuli.

7. Our model exhibits a bistable region of behavior, where at certain concentrations of 5-HT bursting and beating may coexist. Both modes exhibit long-term stability, and our simulations show that it is possible to shift between these modes by application of a brief pulse of current. The existence of multiple modes of behavior for a given set of parameters demonstrates that at some parameter ranges the intrinsic behavior of the model is dependent upon previous behavior and/or inputs.

8. The model can be reduced into a SLOW system of two state variables and a FAST system of eight state variables. The model reductions reveal that bursting is governed by two slow rate-limiting processes: the voltage-dependent activation of $I_{SI}$, represented by the gating variable $s$, and the material balance concentration upon intracellular Ca$^{2+}$ concentration.

9. The onset of a burst is demonstrated to occur via a saddle-node bifurcation of the FAST system, consistent with a previously published analysis of bursting models [81].

10. Altering the control parameters ($I_{STIM}$, DA, and 5-HT) changes the oscillatory behavior of a reduced 2-variable model in a manner consistent with changes observed in the FULL system. These changes are characterized through the use of nullcline plots and bifurcation diagrams, and the results provide an interpretive framework for explaining many observed behaviors of the FULL system, such as bursting/beating bistability. This analysis also serves as an ideal framework for explaining the results of ongoing modeling and experimental studies.

11. The bifurcation diagrams predicted the existence of a previously unobserved second type of action potential. This action potential coexists in a region of
parameter space with the "typical" action potential observed during normal bursting behavior. This may not be of physiological significance, but is of mathematical interest because we know of no model which exhibits bistable activity on the time scale of an action potential.

12. The behavior of R15 is treated as a slowly oscillating system driving the response of another system on a much faster time-scale. This view, while representing the dominant mode of interaction between the FAST and SLOW subsystems, is incomplete. A more realistic viewpoint is to consider R15's behavior to be the result of mutual coupling between fast and slow oscillating processes, i.e. a single-cell coupled oscillator. Future frameworks for more in-depth analysis of the model were proposed, such as modeling the SLOW and FAST systems as truly independent, and then selectively "turning on" different feedback pathways.
Appendix A

Model Equations

This appendix contains the equations and values of parameters for the model. In these equations, time is in msec, current is in nA, potential is in mV, and concentrations are in mM. Some of the time-constant expressions approach zero at certain values of membrane potential, making numerical integration difficult. This problem was alleviated by adding a small constant (0.1 ms) to some of the time-constant expressions.

Inward Currents

<table>
<thead>
<tr>
<th>Table A.1: Fast Sodium Current ($I_{Na}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na} = g_{Na} m^3 h (V - E_{Na})$</td>
</tr>
<tr>
<td>$\dot{m} = (m_\infty - m) / \tau_m$</td>
</tr>
<tr>
<td>$m_\infty = \frac{1}{1 + \exp((-10.23 - V) / 10.0)}$</td>
</tr>
<tr>
<td>$\tau_m = 1 / (A_m + B_m) + 0.1$</td>
</tr>
<tr>
<td>$A_m = \frac{0.40 (V + 5.0)}{1 - \exp((-V - 6.0) / 4.69)}$</td>
</tr>
<tr>
<td>$B_m = 10.75 \exp((-28.0 - V) / 4.01)$</td>
</tr>
<tr>
<td>$\dot{h} = (h_\infty - h) / \tau_h$</td>
</tr>
<tr>
<td>$h_\infty = \frac{1}{1 + \exp((V + 25.0) / 3.0)}$</td>
</tr>
<tr>
<td>$\tau_h = 1 / (A_h + B_h) + 0.1$</td>
</tr>
<tr>
<td>$A_h = 0.067 \exp((-43.0 - V) / 25.0)$</td>
</tr>
<tr>
<td>$B_h = \frac{0.307}{1 + \exp((12.65 - V) / 23.9)}$</td>
</tr>
</tbody>
</table>
Table A.2: Fast Calcium Current ($I_{Ca}$)

\[ I_{Ca} = \bar{g}_{Ca} \left( \frac{1 - \exp\left( \frac{V - E_{Ca}}{f_{Ca}} \right)}{1 + \exp\left( \frac{V - E_{Ca}}{f_{Ca}} \right)} \right) d^2 f(V - E_{Ca}) \]

\[ \dot{d} = (d_{\infty} - d)/\tau_d \]
\[ d_{\infty} = \frac{1}{1 + \exp((10.0 - V)/3.8)} \]
\[ \tau_d = 1/(A_d + B_d) + 0.1 \]
\[ A_d = \frac{0.0062(V+10.81)}{1 - \exp((-V-10.81)/5.03)} \]
\[ B_d = 0.01 \exp((25.0 - V)/10.0) \]

\[ \dot{f} = (f_{\infty} - f)/\tau_f \]
\[ f_{\infty} = \frac{1}{1 + \exp((V+20.0)/4.0)} \]
\[ \tau_f = 1/(A_f + B_f) + 0.1 \]
\[ A_f = 0.00325 \exp((10.0 - V)/7.57) \]
\[ B_f = \frac{0.029}{1 + \exp((20.29 - V)/5.4)} \]

Table A.3: Slow Inward Calcium Current ($I_{SI}$)

\[ I_{SI} = \bar{g}_{SI} \left( \frac{K_{SI, Ca}}{K_{SI, Ca} + [Ca]_r} \right) sF_{SI, mod}(V - E_{Ca}) \]

\[ F_{SI, mod} = \left( \frac{K_{DA}}{K_{DA} + K_{DA}} \right) \left( 1 + \exp\left( \frac{K_{SI, mod}}{E_{SI, CaMnP}} \right) \right) \]

\[ \dot{s} = (s_{\infty} - s)/\tau_s \]
\[ s_{\infty} = \frac{1}{1 + \exp((-40.0 - V)/11.5)} \]
\[ \tau_s = 1/(A_s + B_s) + 0.1 \]

\[ A_s = \frac{0.0014(V-54.0)}{1 - \exp((-V+54.0)/12.63)} \]
\[ B_s = 0.00013 \exp((-11.32 - V)/16.8) \]
Table A.4: Non-Specific Cation Current ($I_{NS}$)

\[
I_{NS} = \bar{g}_{NS} \left( \frac{[Ca]}{[Ca]_{NS, C_{Ca}}} \right) b(V - E_{NS})
\]

\[
b = (b_{\infty} - b) / \tau_b \quad b_{\infty} = \frac{1}{1+\exp((-15.0-V)/3.0)}
\]

\[
\tau_b = 500 \left( \frac{0.80}{1+\exp((10.0+V)/3.0)} + 0.20 \right)
\]

Table A.5: Leakage Current ($I_L$)

\[
I_L = \bar{g}_L(V - E_L)
\]

Table A.6: Delayed Rectifier ($I_K$)

\[
I_K = \bar{g}_K n^4 l(V - E_K)
\]

\[
\dot{n} = (n_{\infty} - n) / \tau_n \quad \dot{l} = (l_{\infty} - l) / \tau_l
\]

\[
n_{\infty} = \frac{1}{1+\exp((3.05-V)/14.46)} \quad l_{\infty} = \frac{1}{1+\exp((32.5+V)/12.7)}
\]

\[
\tau_n = 1/(A_n + B_n) \quad \tau_l = 2000 \left( \frac{0.90}{1+\exp((28.0+V)/3.0)} + 0.10 \right)
\]

\[
A_n = \frac{0.0035(V+17.0)}{1-\exp((-V-17.0)/3.0)}
\]

\[
B_n = 0.04 \exp((-28.0 - V)/10.0)
\]
Table A.7: Anomalous Rectifier ($I_R$)

\[
I_R = \bar{g}_R F_{R,\text{mod}} \frac{(V - E_K + 5.65)}{1 + \exp\left(\frac{(V - E_K - 13.3)2F}{RT}\right)}
\]

\[
F_{R,\text{mod}} \equiv 1 + \frac{K_{R,\text{mod}}}{1 + \exp\left(\frac{-(I_{\text{AMP}} - I_{R,E\text{AMP}})}{D_{R,E\text{AMP}}}\right)}
\]

Table A.8: Pumps and Exchangers

\[
I_{CaP} = I_{CaP} \left(\frac{[Ca]_i}{[Ca]_o + K_{P,Ca}}\right)
\]

\[
I_{NaK} = I_{NaK} \left(\frac{[Na]_o}{[Na]_o + K_{P,Na}}\right)^3 \left(\frac{[K]_o}{[K]_o + K_{P,K}}\right)^2 \left(\frac{1.5 + \exp\left(\frac{-V - 60}{40}\right)}{1}\right)
\]

\[
I_{NaCa} = K_{NaCa}(D_{F,\text{in}} - D_{F,\text{out}})/S
\]

\[
S = 1 + D_{NaCa}([Ca]_i[Na]_o + [Ca]_o[Na]_i)
\]

\[
D_{F,\text{in}} = [Na]_i[Ca]_o \exp\left(\frac{(r-3)\gammaVF}{RT}\right)
\]

\[
D_{F,\text{out}} = [Na]_o[Ca]_i \exp\left(\frac{(r-2)\gamma-1)VF}{RT}\right)
\]

Table A.9: Internal Calcium Concentration

\[
[Ca]_i = \frac{I_{NaCa} - I_{GI} - I_{Ca} - I_{CaP} - 0.197(I_{NS} \cdot \frac{V-E_{Ca}}{V-E_{NS}})}{2V_{ol,F}} - n_B [B]_i \dot{O}_C
\]

\[
\dot{O}_C = k_U [Ca]_i (1 - O_C) - k_R O_C
\]
Table A.10: Internal cAMP Concentration

\[ [c\text{AMP}] = k_{a d e} \left( 1 + K_{m o d} \left( \frac{[\text{SHT}]}{[\text{SHT}]+K_{s u t}} \right) \right) - v_{p d e} \left( \frac{[c\text{AMP}]}{[c\text{AMP}]+K_{p d e}} \right) \]
Table A.11: Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_M$</td>
<td>17.5 nF</td>
</tr>
<tr>
<td>$I_{CaP}$</td>
<td>7.0 nA</td>
</tr>
<tr>
<td>$K_{P,Na}$</td>
<td>5.46 mM</td>
</tr>
<tr>
<td>$g_{Na}$</td>
<td>38 $\mu$S</td>
</tr>
<tr>
<td>$I_{NaK}$</td>
<td>7.7 nA</td>
</tr>
<tr>
<td>$K_{P,K}$</td>
<td>0.621 mM</td>
</tr>
<tr>
<td>$g_{Ca}$</td>
<td>17 $\mu$S</td>
</tr>
<tr>
<td>$K_{NaCa}$</td>
<td>0.01</td>
</tr>
<tr>
<td>$K_{Ca}$</td>
<td>$5 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$g_{K}$</td>
<td>70 $\mu$S</td>
</tr>
<tr>
<td>$D_{NaCa}$</td>
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</tr>
<tr>
<td>$D_{Ca}$</td>
<td>$15 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$g_{NS}$</td>
<td>0.2 $\mu$S</td>
</tr>
<tr>
<td>$\gamma$</td>
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<tr>
<td>$K_{DA}$</td>
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<tr>
<td>$g_{SI}$</td>
<td>0.65 $\mu$S</td>
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<tr>
<td>$r$</td>
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<tr>
<td>$K_{SI,AMP}$</td>
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</tr>
<tr>
<td>$g_{R}$</td>
<td>0.18 $\mu$S</td>
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<tr>
<td>$R$</td>
<td>8,314 J/kg mol °K</td>
</tr>
<tr>
<td>$D_{SI,AMP}$</td>
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<tr>
<td>$g_{L}$</td>
<td>0.075 $\mu$S</td>
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<tr>
<td>$F$</td>
<td>96,500 C/mol</td>
</tr>
<tr>
<td>$K_{SI,mod}$</td>
<td>5.5</td>
</tr>
<tr>
<td>$E_{Na}$</td>
<td>54 mV</td>
</tr>
<tr>
<td>$T$</td>
<td>295 °K</td>
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<tr>
<td>$K_{R,K}$</td>
<td>30 mM</td>
</tr>
<tr>
<td>$E_{Ca}$</td>
<td>65 mV</td>
</tr>
<tr>
<td>$Z$</td>
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<tr>
<td>$K_{R,AMP}$</td>
<td>$1 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$E_{K}$</td>
<td>$-77$ mV</td>
</tr>
<tr>
<td>$Vol_i$</td>
<td>4.0 nl</td>
</tr>
<tr>
<td>$D_{R,AMP}$</td>
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</tr>
<tr>
<td>$E_{NS}$</td>
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</tr>
<tr>
<td>$k_U$</td>
<td>100 mM$^{-1}$ msec$^{-1}$</td>
</tr>
<tr>
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</tr>
<tr>
<td>$E_L$</td>
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<tr>
<td>$k_R$</td>
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<tr>
<td>$K_{adc}$</td>
<td>$6 \times 10^{-6}$ mM/msec</td>
</tr>
<tr>
<td>$[Na]_o$</td>
<td>500 mM</td>
</tr>
<tr>
<td>$n_B$</td>
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</tr>
<tr>
<td>$v_{pde}$</td>
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</tr>
<tr>
<td>$[Na]_i$</td>
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</tr>
<tr>
<td>$K_{SI,Ca}$</td>
<td>$25 \times 10^{-6}$ mM</td>
</tr>
<tr>
<td>$K_{HT}$</td>
<td>$6 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$[K]_o$</td>
<td>10 mM</td>
</tr>
<tr>
<td>$K_{NS,Ca}$</td>
<td>$150 \times 10^{-6}$ mM</td>
</tr>
<tr>
<td>$K_{pde}$</td>
<td>$3 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$[Ca]_o$</td>
<td>10 mM</td>
</tr>
<tr>
<td>$K_{P,Ca}$</td>
<td>$350 \times 10^{-6}$ mM</td>
</tr>
<tr>
<td>$K_{mod}$</td>
<td>1.5</td>
</tr>
<tr>
<td>$[B]_i$</td>
<td>$112.5 \times 10^{-3}$ mM</td>
</tr>
</tbody>
</table>
Bibliography


