Mechanisms of the Coregulation of Multiple Ionic Currents for the Control of Neuronal Activity

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MECHANISMS OF THE COREGULATION OF MULTIPLE IONIC CURRENTS FOR
THE CONTROL OF NEURONAL ACTIVITY

by

WILLIAM BARNETT

Under the Direction of Gennady Cymbalyuk, PhD

ABSTRACT

An open question in contemporary neuroscience is how neuromodulators coregulate
multiple conductances to maintain functional neuronal activity. Neuromodulators enact
changes to properties of biophysical characteristics, such as the maximal conductance or
voltage of half-activation of an ionic current, which determine the type and properties of
neuronal activity. We apply dynamical systems theory to study the changes to neuronal
activity that arise from neuromodulation.
Neuromulators can act on multiple targets within a cell. The coregulation of multiple ionic currents extends the scope of dynamic control on neuronal activity. Different aspects of neuronal activity can be independently controlled by different currents. The coregulation of multiple ionic currents provides precise control over the temporal characteristics of neuronal activity. Compensatory changes in multiple ionic currents could be used to avoid dangerous dynamics or maintain some aspect of neuronal activity. The coregulation of multiple ionic currents can be used as bifurcation control to ensure robust dynamics or expand the range of coexisting regimes. Multiple ionic currents could be involved in increasing the range of dynamic control over neuronal activity. The coregulation of multiple ionic currents in neuromodulation expands the range over which biophysical parameters support functional activity.

INDEX WORDS: Bursting, Central pattern generator, Neuromodulation
DEDICATION

This dissertation is dedicated to Gwendolyn Barnett and Thomas Barnett.
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This dissertation work would not have been possible without the support of many people. I want to express my gratitude to my advisor Gennady Cymbalyuk.
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Central pattern generators control the motor activity of animals (Marder and Calabrese, 1996; Marder et al., 2005; Goudling, 2009). While some central pattern generators are well studied, it is not well understood how the output of central pattern generators is regulated to produce functional motor activity in animals through neuromodulation and homeostatic mechanisms. Regulation of activity can happen at a cellular level or at a network level. It can be important for one salient feature of the activity of a cell to be maintained or scaled while another aspect of the activity changes. In the pyloric network of the spiny lobster, qualitative aspects of activity are maintained despite the upregulation of the transient potassium current by a compensatory upregulation of a hyperpolarization activated current (MacLean et al., 2003). Similarly, a central pattern generator might maintain some aspect of its activity. For example, certain networks controlling motor output are able to maintain cycle phase over a range of cycle periods: the networks controlling swim behavior in the lamprey (Wallen and Williams, 1984), the swim motor pattern in the leech (Friesen and Pearce, 1993), and the lobster pyloric rhythm (Hooper, 1997). Regulation of network and motor activity is a fundamental aspect of neuromuscular control.

Invertebrates are useful subjects to study functional neuronal networks. Their networks are smaller than mammalian networks. Individual cells perform the same specific function from animal to animal, and these cells are identifiable across preparations. Moreover, a number of model species, such as the medicinal leech, are well documented in the literature (Kristan et al., 2005). While the specific mechanisms underlying the function of these networks may not be directly comparable to those found in functionally similar networks in mammals, discoveries made in invertebrate networks can provide insight into mammalian functional circuits.
Neuromodulation allows a central pattern generator to produce a wider range of outputs than its intrinsic properties might otherwise permit (Katz, 1995). Neuromodulation in the leech heartbeat central pattern generator is a topic of contemporary study. Two modulators that are particularly well studied are FMRFamide and myomodulin. The neuropeptide FMRFamide is a critical modulator of cardiac systems that has been identified in the leech (Kuhlman et al., 1985a,b; Li and Calabrese, 1987). FMRFamide acts on heart interneurons by modulating intrinsic voltage-dependent ion channels (Simon et al., 1992; Nadim and Calabrese, 1997). Myomodulin is a neuropeptide first identified in Aplysia and later found in the leech (Cropper et al., 1987; Wang et al., 1998) Myomodulin modulates the activity of the heart interneuron by altering characteristics of the hyperpolarization-activated current and the Na⁺ / K⁺ pump current (Masino and Calabrese, 2002a; Tobin and Calabrese, 2005).

The ability to modulate multiple biophysical properties is a critical tool in an organism’s ability to adapt to its environment. The exact number of ion channels or the exact magnitude of an ionic conductance in a specific neuron cannot be directly encoded into an animal’s genes. Rather, there are mechanisms for activity-dependent and activity-independent homeostatis of functional neuronal activity (MacLean et al., 2003, 2005; Schulz et al., 2006, 2007; Khorkova and Golowasch, 2007; Abbott and LeMasson, 1993; Cao and Oertel, 2011). The correlation of ionic conductances provides a simple mechanism to maintain a neuron’s role in a given organism independent of its life history. The involvement of multiple conductances provides flexibility in a dynamical sense, as well. A conductance of a specific ionic current may allow a neuron to tune its activity within certain limits of functional activity, but the addition of one or more neuromodulatory targets could modify the limits of functional activity. The coregulation of multiple ionic conductances is a critical feature of homeostasis of functional neuronal activity.

Neuromodulators change neuronal activity by acting on biophysical characteristics of ion currents. These modifications to the neuron can change the properties of its activity, which can be small, such as a change in firing frequency, or more substantial, such as a change from a silent cell to a spiking cell. A neuron can be treated as a dynamical system.
Transitions between distinct regimes of activity are called bifurcations in this paradigm, and bifurcations provide quantitative predictions of the dependence of activity on crucial parameters.

Currents that are important to maintaining the functionality of oscillatory regimes are good targets for neuromodulation. Small changes to the kinetics of these currents can have large consequences for neuronal output. For example, the time scale of inactivation of the slow Ca\(^{2+}\) determines the duration of the burst in the leech heart interneuron and the heart interneuron half-center oscillator (Olypher et al., 2006). The conductance of hyperpolarization-activated current changes the interburst interval and therefore determines the duration of the burst in the opposing cell of the half-center oscillator (Sorensen et al., 2004; Hill et al., 2001). In individual heart interneurons, the hyperpolarization-activated current reinforces endogenous bursting activity (Tobin and Calabrese, 2005). The synaptic inhibition of the half-center oscillator motif can prevent loss of functionality despite the introduction of competing non-functional regimes (Cymbalyuk et al., 2002; Marin et al., 2013). These critical currents contribute to the robustness of the oscillatory pattern. Appropriate regulation could enhance the functional role of the central pattern generator, but inappropriate regulation could lead to the dysfunctional activity.

Rhythmic behaviors are controlled by devoted oscillatory neuronal networks: central pattern generators (Wallen and Williams, 1984; Harris-Warrick and Flamm, 1987; Hooper and Marder, 1987; Bal et al., 1988; Friesen and Pearce, 1993; Marder and Calabrese, 1996; Marder and Bucher, 2001; Norris et al., 2007; Ramirez et al., 2011). Central pattern generators adjust their patterns according to motor tasks, sensory feedback, and environmental conditions (Arbas and Calabrese, 1984; Wallen and Williams, 1984; Marder and Bucher, 2001; Smarandache et al., 2009). They comprise neurons from a spectrum of endogenous properties varying from tonic spiking through bursting to silent neurons (Harris-Warrick and Flamm, 1987; Hooper and Marder, 1987; Bal et al., 1988; Marder and Calabrese, 1996; Marder and Bucher, 2001; Ramirez et al., 2011). These neurons can be sensitive to neuromodulatory tone, descending tonic drive, and phasic sensory feedback (Harris-Warrick and
Flamm, 1987; Hooper and Marder, 1987; Bal et al., 1988; Marder and Bucher, 2001). Neurons like conditional oscillators can be found in either tonic spiking, bursting, or silent regimes, when decoupled from a circuit (Harris-Warrick and Flamm, 1987; Hooper and Marder, 1987; Bal et al., 1988; Marder and Bucher, 2001). Major open questions are concerned with the determining the key cellular properties which are characteristic for neurons in central pattern generators. Are there organizing principles of cellular dynamics which allow neurons to produce precise patterns in an orchestrated fashion? Are there mechanisms which coordinate dynamic properties of neurons to accomplish adaptation of motor behavior to continuously changing conditions? We address these questions with dynamical systems theory.

1.1 Neurons Are Dynamical Systems

Bifurcation analysis provides theoretical guidelines and a powerful analytical framework to tune the activity of neuronal models. A bifurcation is a qualitative change in the activity of a dynamical system, such as a transition from silence to spiking. Bifurcation analysis identifies general dynamical laws governing transitions between activity regimes in response to changes in controlling parameters. By using these quantitative laws, the temporal characteristics of a neuronal model can be tuned to reproduce the characteristics of experimentally recorded activity by navigating the parameter space in the vicinity of bifurcations. In most of the bifurcations described here, the general shape of the waveform—for example of the shape of action potentials in a time series—is preserved, while a specific temporal characteristic changes.

The knowledge of the bifurcations of a model neuron provides quantitative laws governing the temporal characteristics of spiking activities. Neurons can be envisaged as nonlinear dynamical systems. Bifurcation analysis has been applied to the study of spiking, bursting, and chaotic neuronal systems (Rinzel, 1987; Guckenheimer et al., 1993, 1997; Rinzel and Ermentrout, 1998; Ermentrout and Terman, 2010; Ghigliazza and Holmes, 2004; Rabinovich et al., 2008). Biophysical parameters are used as controlling parameters, and changes in regimes of activity can be identified concomitantly with bifurcations (Rinzel, 1978b; Guck-
The association of transitions between regimes of neuronal activity with specific bifurcations gives predictions about the properties of stationary and oscillatory solutions (Booth et al., 1997; Doiron et al., 2003; Shilnikov and Cymbalyuk, 2005; Shilnikov et al., 2005; Barnett and Cymbalyuk, 2014). This approach is applicable for tuning the dynamics of neuronal activity—such as bursting, spiking, and rest states—with biophysical parameters. We provide examples of four bifurcations that determine quantitative characteristics of activity: the homoclinic bifurcation, the saddle-node bifurcation on an invariant circle (for bursting and for tonic spiking), the blue sky catastrophe, and the Andronov-Hopf bifurcation (Table 1.1). We present the dynamic laws generated by these bifurcations in the context of spiking, bursting, and transient activity.

1.1.1 Spiking

Three bifurcations of closed periodic orbits are ubiquitously implicated in the control of spiking activity. A saddle-node bifurcation on invariant circle (SNIC) and a homoclinic bifurcation describe how the frequency of spiking depends on a controlling parameter $\alpha$, e.g. the injected current. A supercritical Andronov-Hopf bifurcation describes the dependence of the amplitude of oscillations on the bifurcation parameter.

A SNIC occurs when an equilibrium possessing the properties of both a saddle and a node—a saddle-node equilibrium—is born on a closed periodic orbit, and the eigenvectors associated with the half-stable direction of the saddle-node equilibrium are tangent to the periodic orbit. This bifurcation describes the transition of spiking into silence (Gutkin and Ermentrout, 1998). At the critical value for bifurcation, $\alpha^*$, the saddle-node equilibrium obstructs the spiking orbit. For values of $\alpha$ that support spiking and are close to $\alpha^*$, the frequency of spiking activity is proportional to $\sqrt{|\alpha - \alpha^*|}$. This bifurcation does not constrain the amplitude of spiking. This scenario is an example the type I spiking scenario (Gutkin and Ermentrout, 1998; Izhikevich, 2007b).

A homoclinic bifurcation of a saddle equilibrium can terminate a spiking regime (Izhike-
Bursting. Four types of bifurcations are crucial for the control of temporal characteristics in bursting activity: the SNIC for bursting, the blue sky catastrophe, cornerstone bifurcation, and the Lukyanov-Shilnikov bifurcation.

In bursting activity, the SNIC describes the transition from bursting to silence (Booth et al., 1997; Doiron et al., 2003; Barnett and Cymbalyuk, 2014). According to this bifurcation, the interburst interval grows smoothly and is proportional to $1/\sqrt{|\alpha - \alpha^*|}$ as the bifurcation parameter, $\alpha$, approaches the critical value $\alpha^*$. At the bifurcation, a saddle-node equilibrium is born on the bursting orbit.

The blue sky catastrophe occurs when a saddle-node periodic orbit is born on the manifold that governs spiking in a bursting neuron (Shilnikov and Cymbalyuk, 2005; Barnett and Cymbalyuk, 2014). This bifurcation describes the transition from bursting to tonic spiking. The burst duration grows smoothly and is proportional to $1/\sqrt{|\beta - \beta^*|}$ as the bifurcation parameter, $\beta$, approaches the critical value $\beta^*$.

The cornerstone bifurcation satisfies the conditions of both the SNIC and the blue sky catastrophe; it is a global codimension-2 bifurcation (Shilnikov and Turaev, 2000; Barnett and Cymbalyuk, 2014). This bifurcation describes the independent control of interburst interval and burst duration: one parameter, $\alpha$, controls the transition from bursting to silence as in the SNIC, and another parameter, $\beta$, controls the transition from bursting to...
to spiking as in the blue sky catastrophe. At the critical value for both parameters, \((\alpha^*, \beta^*)\), there occurs both the saddle-node equilibrium and the saddle-node periodic orbit. In this way, the duration of the interburst interval is controlled as in the SNIC, and the burst duration is controlled as in the blue sky catastrophe.

The Lukyanov-Shilnikov bifurcation is a homoclinic bifurcation of a periodic orbit (Lukyanov and Shilnikov, 1978). In bursting, this bifurcation occurs when the unstable manifold of the saddle-orbit leads into the stable manifold of the orbit (Shilnikov et al., 2005). The unstable manifold near the saddle-orbit determines the duration of each burst. Near this bifurcation, bursting is commonly chaotic and on average, the burst duration grows proportionally to \(-\ln(|\alpha - \alpha^*|)\) as the bifurcation parameter, \(\alpha\), approaches the critical value \(\alpha^*\).

**Transient Activity** Transient dynamics are critical for information processing (Rabinovich et al., 2008). The temporal characteristics of transient activity can similarly be governed by bifurcations. Typically, temporal laws are associated with global bifurcations. However, a local bifurcation can determine the timescale of transient neuronal responses to external perturbations, such as a pulse of synaptic current.

The saddle-node bifurcation for periodic orbits can impose temporal laws on transient spiking activity in an endogenously silent neuron (Roa et al., 2007). For values of the bifurcation parameter \(\alpha\) which support silence and are close to the birth of the spiking regime at \(\alpha^*\), the dynamics of perturbation-induced transient spiking activity are controlled by the proximity \(|\alpha - \alpha^*|\) to the bifurcation. Far from the bifurcation, a perturbation may trigger a single action potential, but as the quantity \(|\alpha - \alpha^*|\) becomes small, the same perturbation induces an increasing number of spikes. The duration of this transient activity—under the condition of an invariant external stimulus—is proportional to \(1/\sqrt{|\alpha - \alpha^*|}\). A similar scenario is generated by the cornerstone bifurcation (Barnett and Cymbalyuk, 2014).

In the context of the tonically spiking neurons, the cornerstone bifurcation describes a transient silent interval after inhibition according to the properties imposed by a saddle-
node bifurcation for equilibria (Barnett and Cymbalyuk, 2014). The return to the spiking state after inhibition is delayed if $\alpha$ is close in value to $\alpha^*$, and this delay is proportional to $1/\sqrt{|\alpha - \alpha^*|}$.

1.1.2 Dissertation Outline

This dissertation is organized into three main chapters. The common thread shared among these three chapters and the theme that has informed my research is that multiple ionic currents can be covaried to maintain or produce functional neuronal activity. I use methods developed in the theory of dynamical systems to study how the simultaneous regulation of multiple biophysical properties modulates neuronal activity.

The first chapter covers the description of a global codimension-2 bifurcation, its role in the control of temporal characteristics of oscillatory activity, and how it coordinates the coregulation of important membrane conductances. This so-called cornerstone bifurcation generates a family of three dynamical mechanisms. These mechanisms are all executed by the coordinated manipulation of the voltage of half-activation of a hyperpolarization-activated current ($-\theta_h$) and a non-inactivating potassium current ($-\theta_K^2$). The first mechanism provides control over temporal characteristics of endogenous bursting activity. In this case, $\theta_K^2$ and $\theta_h$ are coordinated to independently control burst duration and interburst interval. A second mechanism controls the duration of individual transient bursts in endogenously silent neurons. In this mechanism, $\theta_h$ was used to tune the type of activity of the cell to silence and $\theta_K^2$ was used to control the duration of the transient burst. A third mechanism controls the duration of the latency to spiking after inhibition in endogenously spiking neurons. In this mechanism, $\theta_K^2$ was used to set the cell into a spiking mode and $\theta_h$ was used to control the duration of latency to spiking. As proof-of-principle concepts, we designed two central pattern generators that used these mechanisms. The first example was a central pattern generator that controlled crawling in the Drosophila larva. We coregulated the hyperpolarization-activated current and the non-inactivating K$^+$ current with $\theta_h$ and $\theta_K^2$ to produce different instantations of the model with a duty cycle of 10% over a range of cycle
periods, and demonstrated that the intrinsic duty cycle of individual participant neurons could determine the phase lag in the network. The second example was a central pattern generator that controlled the timing of protraction and retraction for six-legged locomotion. The network was composed of pairs of protractor and retractor interneurons. Protractor interneurons were endogenously bursting, and retractor interneurons were endogenously silent. By controlling the transient bursts specifically in retractor interneurons, we demonstrated control of the emergent gait and smooth transitions between two types of gait.

In the second chapter, we investigated the robustness of a bursting regime against perturbations in a model of a leech heart interneuron. This activity demonstrated bistability of plateau-like bursting and silence. We developed a protocol to trigger a switch from a bursting state to a silent state. By systematically varying the start-time within the phase of the burst and the amplitude and polarity of the perturbation, we mapped the basin of attraction of the silent regime. We identified the dynamical transition crucial for the appearance of the silent regime imposed by a subcritical Andronov-Hopf bifurcation. The propensity of the neuron to bistability was defined as the difference in values of the leak conductance for which the model supports bistability. We tracked the Andronov-Hopf bifurcation and the termination of the bursting regime while co-varying the leak conductance and each of the maximal conductances of the voltage gated currents. This analysis revealed key conductances that control the propensity of the neuron to bistability. We coregulated these conductances to produce a model with a greatly increased range of bistability in the leak conductance.

In the third chapter, we investigated the dynamics of $\text{Na}^+/\text{K}^+$ pump current and how coregulation controls the functional range of neuronal activity. In the leech heart interneuron, the application of myomodulin, which inhibits the $\text{Na}^+/\text{K}^+$ pump current, decreases the period of bursting activity. The application of monensin, which enhances the $\text{Na}^+/\text{K}^+$ pump current, also decreases the period of bursting activity. We developed a new canonical model of the leech heart interneuron which included $\text{Na}^+$ dynamics and the $\text{Na}^+/\text{K}^+$. In one instantiation of the model, we reproduced changes to temporal characteristics due to treatment with monensin, $\text{Cs}^+$, and bicuculline. In this model we examined how the $\text{Na}^+/\text{K}^+$
pump current, the slow Ca\(^{2+}\) pump current, and the hyperpolarization-activated current interacted to determine burst characteristics. In another instantiation of the model, we reproduced changes to temporal characteristics due to treatment with myomodulin and Cs\(^{+}\). Finally, we investigated mechanisms wherein myomodulin coregulated the maximal conductance of the h-current and the Na\(^{+}/K^{+}\) pump current to control the range of functional activity.

Table (1.1). Dynamical laws arising from specific bifurcations. The bifurcation parameter \(\alpha\) takes its critical value for bifurcation at \(\alpha^*\).

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<td>Homoclinic</td>
<td>(\text{Period } \propto -\ln(\vert \alpha - \alpha^* \vert))</td>
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<tr>
<td>SNIC</td>
<td>(\text{Period } \propto 1/\sqrt{\vert \alpha - \alpha^* \vert})</td>
</tr>
<tr>
<td>Blue Sky Catastrophe</td>
<td>(\text{Period } \propto 1/\sqrt{\vert \alpha - \alpha^* \vert})</td>
</tr>
<tr>
<td>Andronov-Hopf</td>
<td>(\text{Amplitude } \propto \sqrt{\vert \alpha - \alpha^* \vert})</td>
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PART 2

A CODIMENSION-2 BIFURCATION CONTROLLING ENDOGENOUS BURSTING ACTIVITY AND PULSE-TRIGGERED RESPONSES OF A NEURON MODEL

2.1 Abstract

The dynamics of individual neurons are crucial for producing functional activity in neuronal networks. An open question is how temporal characteristics can be controlled in bursting activity and in transient neuronal responses to synaptic input. Bifurcation theory provides a framework to discover generic mechanisms addressing this question. We present a family of mechanisms organized around a global codimension-2 bifurcation. The cornerstone bifurcation is located at the intersection of the border between bursting and spiking and the border between bursting and silence. These borders correspond to the blue sky catastrophe bifurcation and the saddle-node bifurcation on an invariant circle (SNIC) curves, respectively. The cornerstone bifurcation satisfies the conditions for both the blue sky catastrophe and SNIC. The burst duration and interburst interval increase as the inverse of the square root of the difference between the corresponding bifurcation parameter and its bifurcation value. For a given set of burst duration and interburst interval, one can find the parameter values supporting these temporal characteristics. The cornerstone bifurcation also determines the responses of silent and spiking neurons. In a silent neuron with parameters close to the SNIC, a pulse of current triggers a single burst. In a spiking neuron with parameters close to the blue sky catastrophe, a pulse of current temporarily silences the neuron. These responses are stereotypical: the durations of the transient intervals—the duration of the burst and the duration of latency to spiking—are governed by the inverse-square-root laws. The mechanisms described here could be used to coordinate neuromuscular control in central pattern generators. As proof of principle, we construct small networks that control
metachronal-wave motor pattern exhibited in locomotion. This pattern is determined by the phase relations of bursting neurons in a simple central pattern generator modeled by a chain of oscillators.

2.2 Introduction

The control of temporal characteristics of functional neuronal activity depends on the coregulation of multiple membrane conductances. The dynamics of individual neurons are crucial for the functionality of neuronal networks. Precise timing and reliability of temporal responses are critical for memory, pattern recognition, and especially motor control (Rabinovich et al., 2008; Roa et al., 2007; Marder et al., 1996; Hooper et al., 2002; Kopp-Scheinpflug et al., 2011; Meng et al., 2011; Ramirez et al., 2011; Norris et al., 2007; Hooper, 1997; Mouser et al., 2008). Functional bursting activity, latency to spiking, and transient oscillatory activity are necessary components determining reliability and precise timing. For example, transient spiking activity is critical for information representation and memory (Roa et al., 2007; Marder et al., 1996; Doiron et al., 2003; Laing et al., 2003); precise control over delay to firing after inhibition is important for the detection of temporal patterns (Hooper et al., 2002; Kopp-Scheinpflug et al., 2011; Meng et al., 2011); and bursting activity is crucial for rhythmic motor functions including respiration, locomotion, and neurogenic cardiac systems (Ramirez et al., 2011; Norris et al., 2007).

Rhythmic motor functions are executed by precisely coordinated oscillatory patterns of contracting muscles. These functions require flexibility of rhythmic patterns to cope with environmental conditions such as temperature, load, or the demand for speed of locomotion. Accordingly, some rhythmic behaviors scale their pattern, maintaining phase relations across a wide range of periods, for example, according to different speeds of locomotion. Examples of pattern scaling behaviors include the pyloric rhythm in Crustacea, crayfish swimmeret beating, the leech heartbeat, leech swimming, lamprey swimming, and crawling of the Drosophila larvae (Kristan et al., 1974; Arbas and Calabrese, 1984; Wallen and Williams, 1984; Williams et al., 1989; Friesen and Pearce, 1993; Hooper, 1997; Skinner and
Bifurcation theory explains how dynamical systems like neurons precisely change their dynamics in response to the variation of a controlling parameter like neuromodulatory tone or descending drive (Guckenheimer et al., 1997; Rabinovich et al., 2008; Hoppensteadt and Izhikevich, 1997; Ermentrout, 1996; Teka et al., 2011; Ermentrout and Kopell, 1994a; Rinzel, 1987; Cymbalyuk et al., 2002; Malashchenko et al., 2011d,a; Rinzel, 1978b; Marder et al., 1996; Roa et al., 2007; Meng et al., 2011; Ermentrout, 2004b; Jones et al., 2003; Booth et al., 1997; Gutkin and Ermentrout, 1998; Laing et al., 2003; Doiron et al., 2003; Cymbalyuk and Calabrese, 2001; Shilnikov and Cymbalyuk, 2005; Cymbalyuk and Shilnikov, 2005b; Shilnikov et al., 2005; Ghosh et al., 2009; Izhikevich, 2000; Channell et al., 2007; Ghigliazza and Holmes, 2004). A variety of network mechanisms producing specific temporal patterns of activity have been previously studied (Kopell and Ermentrout, 1988; Cohen et al., 1992; Ermentrout and Kopell, 1994b,a; Skinner and Mulloney, 1998; Hoppensteadt and Izhikevich, 1997; Hill et al., 2001; Jones et al., 2003; Mouser et al., 2008; Rabinovich et al., 2008; Varkonyi et al., 2008; Kozlov et al., 2009; Popovych et al., 2011; Yanchuk et al., 2011; Soofi et al., 2012).

Our study emphasizes the dynamics of single neurons as an organizing principle underlying pattern formation. We present a global codimension-2 bifurcation and assert that this so-called cornerstone bifurcation can precisely control the regulation of temporal characteristics in periodic and transient neuronal activity. We suggest that this bifurcation generates a family of cellular mechanisms which can answer the aforementioned questions. These mechanisms also explain the operation of conditional oscillators in parameter space near a functional bursting regime.

In neurons, control over regimes of activity is ubiquitously executed through neuromodulation. Potassium currents and hyperpolarization-activated currents are common targets for neuromodulation (Luthi and McCormick, 1998, 1999; MacLean et al., 2003, 2005; Klop-
penburg et al., 2008; Rodgers et al., 2011b; Ikematsu et al., 2011; Amendola et al., 2012). Among the usual targets of neuromodulation are the kinetic parameters controlling the voltage dependence of conductances of ionic currents such as a non-inactivating potassium current, $I_{K2}$, and a hyperpolarization-activated current, $I_h$. $I_{K2}$ is an outward current that is activated during the burst and creates a mechanism controlling termination of the burst. To serve this role, the activation variable of this current must be significantly slower than the duration of a single spike. On the other hand, $I_h$ is an inward current that is activated during the silent phase of bursting activity, and it creates a mechanism controlling the duration of the silent phase. The voltage of half-activation of $I_{K2}$, $-\theta_{K2}$, could control burst duration, and the voltage of half-activation of $I_h$, $-\theta_h$, could control the interburst interval. Here, we present a model in which the dependence of burst duration and interburst interval on $\theta_{K2}$ and $\theta_h$ can be quantitatively described by inverse-square-root laws.

Biophysically accurate neuronal models allow the utilization of well developed techniques from bifurcation theory. In a model, bifurcations can predict the dependence of temporal characteristics of oscillatory regimes near the bifurcation (Gutkin and Ermentrout, 1998; Booth et al., 1997; Doiron et al., 2003; Laing et al., 2003; Ghosh et al., 2009). In type I spiking neuronal dynamics, a saddle-node bifurcation on an invariant circle (SNIC) describes a transition from tonic spiking into silence; the interspike interval obeys the inverse-square-root law imposed by the bifurcation. The period of spiking grows proportionally to $1/\sqrt{\alpha - \alpha^*}$ where $\alpha^*$ is the bifurcation value of the parameter $\alpha$ and $\alpha > \alpha^*$. In our case, the bifurcation parameters are either $\theta_{K2}$ or $\theta_h$. The blue sky catastrophe, a special case of the saddle-node bifurcation for orbits, imposes the same inverse-square-root law on burst duration (Shilnikov and Turaev, 2000; Shilnikov and Cymbalyuk, 2005). A saddle-node bifurcation for periodic orbits can control the transition from bursting into tonic spiking (Doiron et al., 2003; Laing et al., 2003; Shilnikov and Cymbalyuk, 2005; Shilnikov et al., 1998, 2001). When the criteria for the SNIC and the blue sky catastrophe are simultaneously satisfied, a global bifurcation of codimension-2 occurs (Shilnikov and Turaev, 2000; Shilnikov et al., 1998, 2001). By coregulating these currents, we reveal the cornerstone bifurcation and how
the bifurcation parameters control burst duration and interburst interval, latency to spiking in the response of a spiking neuron to inhibition, duration of a burst elicited by stimulation of a silent neuron, and multistability of spiking and silence. We apply these mechanisms to construct simple proof of principle models of central pattern generators (CPGs). One of these mechanisms describes the coregulation of two biophysical parameters in the vicinity of the cornerstone bifurcation such that the duty cycle of bursting in a single cell is maintained across a wide range of periods. Connected into a chain, such oscillators self-organize their activity into a metachronal wave pattern, which is preserved against variation in period. We present this model in regard to crawling behavior in *Drosophila* larvae.

2.3 Results

2.3.1 Regimes of Activity

We developed a generic low-dimensional Hodgkin-Huxley type neuronal model stemming from a model of the leech heart interneuron under certain pharmacological conditions (Cymbalyuk and Calabrese, 2001; Shilnikov and Cymbalyuk, 2005). In order to reduce the mathematical complexity of the system, we simulated the activity of the interneuron in bath with $\text{Co}^{2+}$ and 4-aminopyridine (4-AP). Application of $\text{Co}^{2+}$ blocks $\text{Ca}^{2+}$ currents and the synaptic current. Application of 4-AP blocks most of the $\text{K}^+$ currents. The remaining currents in this model are the leak current, the non-inactivating potassium current, $I_{K2}$, the fast sodium current, $I_{Na}$, and a constant polarizing current. Our model also includes the hyperpolarization-activated current, $I_h$, which is present in this pharmacological scenario but has not been previously represented in our reduced models. Intracellular recordings in these conditions show slow seizure-like oscillations with periods that are tens of seconds long (Angstadt and Friesen, 1991; Opdyke and Calabrese, 1994a). The slow variable in this system is the activation of $I_{K2}$, $m_{K2}$; its time constant was 2 s. As such, the temporal characteristics of these variables are instrumental in the dynamics of bursting activity. By systematically manipulating parameters that determine the dynamics of the slow variables,
we observed the range of bursting activity and bifurcations between qualitatively distinct regimes of neuronal activity. We investigated the effects of changes to the kinetics of $I_{K2}$ and $I_h$.

The parameters $-\theta_{K2}$ and $-\theta_h$ represent the voltages of half-activation of the variables $m_{K2}$ and $m_h$. We conducted an empirical investigation of the model by systematically varying $\theta_{K2}$ and $\theta_h$. The model exhibited a variety of regimes. Silence and tonic spiking corresponded to equilibria and periodic orbits. Bursting activity was either periodic or weakly chaotic. For low values of $\theta_{K2}$ and $\theta_h$ (region I), the model exhibited tonic spiking (Fig 2.1A). For high values of $\theta_{K2}$ and low values of $\theta_h$ (region II), the model exhibited bursting activity (Fig 2.1A). For high values of $\theta_{K2}$ and $\theta_h$ (region III), the model was silent (Fig 2.1A). For low values of $\theta_{K2}$ and high values of $\theta_h$ (region IV), the model exhibited bistability of tonic spiking and silence (Fig 2.1A).

We determined the boundaries between bursting and tonic spiking and between bursting and silence by integrating the system over a range of values for $\theta_h$ and $\theta_{K2}$. Samples varied from one another in the burst duration and the interburst interval (Table 2.1). The transition from bursting to tonic spiking occurred near $\theta_{K2} = -0.0105 \, V$ almost independently of $\theta_h$ (Fig 2.1A). The transition from bursting to silence occurred near $\theta_h = 0.0413 \, V$ with weak dependence on $\theta_{K2}$ (Fig 2.1A). As the parameters were changed to approach the transition from bursting to tonic spiking or from bursting to silence, the burst duration or interburst interval increased, respectively. We described the changes in the bursting wave form by duty cycle: the ratio of burst duration to cycle period. The duty cycle generally increased as $\theta_{K2}$ or $\theta_h$ decreased (Fig 2.1A).

2.3.2 The Inverse-Square-Root Laws Control Bursting Activity

We associated saddle-node bifurcations of equilibria and periodic orbits with the transitions between different regimes. The transitions between I and IV and between II and III coincided with the saddle-node bifurcation for equilibria (Fig 2.1A, black curve). The borders between regions I and II and between regions III and IV coincided with the saddle-node
bifurcation for periodic orbits (Fig 2.1A, grey curve). The location of the codimension-2 bifurcation was interpolated as the intersection of the curves representing the saddle-node bifurcation for equilibria and the saddle-node bifurcation for periodic orbits at ($\theta_{K2}^* = -0.010505 V$, $\theta_{h}^* = 0.041356538 V$).

We show that the saddle-node bifurcations along the border between $I$ and $II$ and between $II$ and $III$ are a blue sky catastrophe and a SNIC, respectively, through a series of curve fits and analysis of slow motion. We investigated temporal characteristics of bursting near these bifurcation curves (Fig 2.2). The system was directly integrated for a series of parameter values approaching the bifurcation values. As $\theta_{h}$ approached the saddle-node bifurcation for equilibria, the interburst interval grew in a fashion asymptotic to the parameter value of bifurcation (Fig 2.2A-B). The parameter $\theta_{K2}$ was similarly varied so as to approach its critical value for the saddle-node bifurcation for periodic orbits. Samples of bursting activity for values of $\theta_{K2}$ successively closer to the bifurcation value showed an asymptotic increase in the burst duration (Fig 2.2C-D).

We performed curve fits to two slightly different expressions for burst duration and interburst interval:

$$f_{K2}(\theta_{K2}) = \frac{b}{\sqrt{\theta_{K2} + d}} + c, \quad (2.1)$$

$$f_{h}(\theta_{h}) = \frac{b}{\sqrt{\theta_{0h} - \theta_{h}}} + c. \quad (2.2)$$

The coefficients $b$, $c$, and $d$ were determined with an optimization routine (see Methods for details). Curve fits for burst duration took the form $f_{K2}(\theta_{K2})$ (Eq. 2.1). Curve fits for the interburst interval took the form $f_{h}(\theta_{h})$ (Eq. 2.2). The parameter $\theta_{0h}$ was the value for the saddle-node bifurcation. We performed curve fits for sixteen data sets – eight distinct data sets for each of the two bifurcations. The dependence of the burst duration and interburst interval on $\theta_{K2}$ and $\theta_{h}$ fit well to the quantitative expressions in Eqs 2.1 and 2.2, respectively. Qualitatively, the temporal dependence of activity on each parameter corresponds to the
analytical prediction of $1/\sqrt{\alpha - \alpha^*}$. As such, these results strongly suggested that the curves depicted in the bifurcation diagram in Figure 2.1 represent a blue sky catastrophe on the border of the transition from bursting (region II) to spiking (region I) and a SNIC on the border of the transition from bursting (region II) to silence (region III). We also confirmed these results for the value of the time constant of activation of the potassium current 0.9 s used in the (Shilnikov and Cymbalyuk, 2005).

2.3.3 The Inverse-Square-Root Laws of Pulse Triggered Responses

For parameter values in regions I and III (Fig 2.1), tonic spiking and silence were attracting regimes. However, a perturbation from the tonic spiking regime in I or the rest state in III triggered transient activity that shared the inverse-square-root laws with the corresponding characteristics of periodic bursting in region II (Fig 2.3). For example, activity at parameter values in region III was quiescent, but a brief hyperpolarizing pulse of appropriate duration and amplitude triggered a single burst (Fig 2.3 A-C). These individual bursts closely resembled the characteristic waveforms of bursting activity observed in Region II. We investigated this parameter space by observing pulse-triggered bursts at parameter values such that $\theta_h$ was fixed at 0.0415 V and $\theta_{K2}$ approached the value for the saddle-node bifurcation for periodic orbits at the border between regions III and IV. As $\theta_{K2}$ approached the value of the bifurcation, the burst duration of pulse triggered bursts grew (Table 2.2). Even though there was no closed periodic orbit in region III, the phase point slowed down as it passed near the ghost of the saddle-node periodic orbit. We performed a curve fit of this data to the function $f_{K2}(\theta_{K2})$ (Eq. 2.1; Fig 2.3D).

We carried out a similar analysis for the border of the transition from regions I to IV. When tonic spiking activity was perturbed with a hyperpolarizing pulse, the system spent some time in a transient hyperpolarized silent state before returning to spiking activity. The trajectory during this time interval resembled the trajectory during the interburst interval of an endogenously bursting cell. We fixed $\theta_{K2}$ at -0.0107 V, and we sampled region I for values of $\theta_h$ close to the saddle-node bifurcation for equilibria (Fig 2.3E-G). As $\theta_h$ grew, the
latency to spiking grew (Table 2.2). As the parameter $\theta_h$ took values close to the saddle-node bifurcation for equilibria, the phase point spent more and more time near the ghost of the saddle-node equilibrium. We quantified the dependence of the latency to spiking on $\theta_h$ by performing a curve fit to the function $f_h(\theta_h)$ (Eq. 2.2a; Fig 2.3H).

2.3.4 The Manifolds of Slow Motion

This model exhibit bursting of square-wave type. The bursting phase is controlled by the activation of $I_{K2}$. Over the course of the burst, $m_{K2}$ incrementally grows with each spike. Vice versa, $m_{K2}$ controls the shape of each spike. The shape of each spike depends on the value of $m_{K2}$, and the next increment of $m_{K2}$ depends on the shape of the spike. To account for this mutual interaction and to formalize the progression of the burst towards termination, we used slow-fast decomposition. This technique allows us to describe this process in terms of the dynamics of one variable: the slow variable.

We considered the model as a slow-fast system. The variable $m_{K2}$ was the slow subsystem, and $\{V, h_{Na}, m_h\}$ composed the fast subsystem. The magnitude of $m_{K2}$ controls rest states (equilibria) and spiking (periodic orbits) of the fast subsystem. These equilibria and periodic orbits are located on manifolds that could be determined by decoupling the slow-fast system and reintroducing the slow variable as a parameter (Rinzel, 1987; Izhikevich, 2000). Trajectories of the full system stay near these manifolds of slow motion (Fenichel, 1979; Rinzel, 1987; Izhikevich, 2000). The slow motion manifold that determines periodic orbits describes the breadth of specifications of spike shapes in the fast subsystem. The slow motion manifold that determines the silent phase describes the characteristics of equilibria of the fast subsystem. We used a more accurate approach, whereby we manipulated a parameter of the slow variable ($\theta_{K2}$) to compute the manifolds of slow motion in the full system (Pontryagin and Rodygin, 1960; Cymbalyuk and Shilnikov, 2005b; Shilnikov and Cymbalyuk, 2005; Shilnikov et al., 2005). We performed slow-fast decomposition and determined the slow motion manifolds to study the structure of the codimension-2 bifurcation. We applied the Pontryagin & Rodygin averaging method (Pontryagin and Rodygin, 1960; Cymbalyuk
and Shilnikov, 2005b; Shilnikov and Cymbalyuk, 2005; Shilnikov et al., 2005). The stability of the regimes in the full system was determined by bifurcation analysis.

We identified the manifolds of slow motion by analyzing simple periodic orbits and equilibria using the parameter $\theta_{K2}$ as a continuation parameter. We produced a family of orbits, $M_o$, by varying $\theta_{K2}$. Starting at a value ($\theta_{K2} = -0.03$ V) where the system exhibited a stable periodic orbit, we continued this orbit as it went through a series of bifurcations (Fig 2.4). It folded over twice at saddle-node bifurcations for periodic orbits, first losing stability at $SNo_1$ and then regaining stability at $SNo_2$. Shortly after $SNo_2$, the orbit lost stability in a period doubling bifurcation $PD$. Also, we computed the slow manifold associated with equilibria, $M_{eq}$, in the fast subsystem (Fig 2.4). Starting at a value ($\theta_{K2} = 0.04$ V) where the system exhibited a stable equilibrium, we continued the equilibrium as it went through two saddle-node bifurcations for equilibria. It lost stability at $SNe_1$, and folded again at $SNe_2$. The saddle-node bifurcations at $SNo_1$ and $SNe_1$, the two elements of the codimension-2 bifurcation, occurred at the same parameter value. To further confirm that these bifurcations are parts of the cornerstone bifurcation, we show that $SNe_1$ and $SNo_1$ occur on the corresponding slow motion manifolds of the full system.

The orbits and equilibria portrayed in Figure 2.4 are orbits and equilibria of the full system corresponding to different values of $\theta_{K2}$. In addition to portraying these data sets in terms of the bifurcation parameter (Fig 2.4), we examined each data set in the ($m_{K2}, V$) projection of the phase space (Fig 2.5) Shilnikov and Cymbalyuk (2005); Shilnikov et al. (2005); Cymbalyuk and Shilnikov (2005b). In this projection, $M_o$ and $M_{eq}$ corresponded to orbits and equilibria in the fast subsystem. Equilibria of the full system are determined by the intersection of the nullclines of the slow variable at a given value of $\theta_{K2}$ and $M_{eq}$. At the critical value for the cornerstone bifurcation, there are two equilibria determined by this analysis: one equilibrium is of the saddle-node type and the other equilibrium is unstable (Fig 2.5A). $SNe_1$ was located where the slow manifold, $M_{eq}$, was tangent to the nullcline of the slow variable (Fig 2.5A). Orbits of the full system are determined by the intersection of the average nullclines of orbits in $M_o$ evaluated at a given value of $\theta_{K2}$ and the average
values of \( V \) and \( m_{K2} \) in \( M_o \). The family of orbits, \( M_o \), was separated into a stable manifold, \( M^s_o \), and an unstable manifold, \( M^u_o \) (Fig 2.5A). Our analysis reduced \( M_o \) to the average coordinate of each orbit in \( M_o \): \( \langle m_{K2}, \langle V \rangle \rangle \). Additionally, we computed the average value of the nullcline of \( m_{K2} \) for each orbit in \( M_o \): \( \langle m'_{K2} = 0, \langle V \rangle \rangle \) (see Methods for definitions). We compared the set \( \langle m_{K2}, \langle V \rangle \rangle \) to \( \langle m'_{K2} = 0, \langle V \rangle \rangle \) to determine the location of orbits of the full system. \( SNo_1 \) occurred on \( M^s_o \) where the average coordinates of orbits became tangent to the average nullcline of orbits on \( M_o \) (Fig 2.5A). These analyses show that \( SNo_1 \) belongs to \( M^s_o \) and \( SNe_1 \) belongs to \( M_{eq} \).

This structure of the slow motion manifolds produces the dynamics of square-wave bursting (Fig 2.5B). Our analysis describes the geometry of bursting and the structure of the bifurcation. Bursting trajectories of the full system are located near these manifolds of slow motion Femenich (1979); Cymbalyuk and Shilnikov (2005b); Shilnikov and Cymbalyuk (2005); Shilnikov et al. (2005). Let us describe the motion of the phase point on a bursting trajectory in terms of a slow-fast system. During the burst, the phase point winds fast around \( M^s_o \) with \( m_{K2} \) slowly increasing. The burst terminates on the fold where \( M^s_o \) and \( M^u_o \) coalesce. This fold corresponds to \( SNo_2 \), which is a saddle-node orbit in the fast subsystem. Following the termination of the burst, the phase point quickly drops onto the stable segment of \( M_{eq} \) and follows it with \( m_{K2} \) slowly decreasing until \( SNe_1 \), which is a saddle-node bifurcation for equilibria in the fast subsystem. The phase point quickly moves up, increasing in \( V \), as the system executes the first spike in a burst and then it is strongly attracted onto \( M^s_o \) for the duration of the burst. During the interburst interval, the system follows \( M_{eq} \), and during the burst, the system winds around \( M^s_o \). The direction of these motions is determined by the sign of the derivative of the slow variable (during the interburst interval) or the sign of the averaged derivative of the slow variable (during the burst).

These slow motion manifolds also play a role in the generation of stereotyped transient responses. In a square-wave bursting neuron, the onset of firing and the termination of the burst occur as the system experienced a fast transition from one slow manifold to another. By extracting these features, the evolution of the burst can be broken into two sets of
dynamics: those that control the onset of firing and those that control the time course of the burst. In a square-wave bursting neuron, the burst is represented as the winding of the phase point around the slow-motion manifold for oscillations in the fast subsystem. Similarly, the interburst interval is the period of quiescence that accompanies the slow passage of the phase point over the manifold for equilibria in the fast subsystem. The isolation of these features extends the significance of the slow-fast analysis in endogenously bursting neurons to encompass transient activity in an otherwise silent or spiking cell.

The parameter space that we analyzed is partitioned by two bifurcation curves: one horizontal and one vertical (Fig 2.6). The horizontal curve is a saddle-node bifurcation for equilibria ($SNe_1$ in Fig 2.4). It divides the 2-D parameter space into two subspaces (red curve in Fig 2.6A). At this bifurcation, a stable equilibrium disappears; the parameter space above this curve supports the stable equilibrium. The vertical solid blue curve represents a saddle-node bifurcation for periodic orbits (Fig 2.6A; $SNo_1$ Fig 2.4). At this bifurcation, a stable periodic orbit—representing tonic spiking—disappears; the parameter space to the left of this curve supports tonic spiking.

The cornerstone bifurcation, which is located at the intersection of the two bifurcation curves (red and blue solid curves), organizes bursting, spiking, and silent regimes (Fig 2.6A). The bifurcations defining the borders of region $II$ (SNIC and blue sky catastrophe) lead to qualitative changes in the vector field which eliminate periodic bursting by obstructing the passage of the phase point over one or both manifolds of slow motion (Fig 2.6A).

At the codimension-2 bifurcation point, the slow motion manifold determined by the equilibria of the fast subsystem is obstructed by a saddle-node equilibrium, and the slow manifold for spiking is obstructed by a saddle-node bifurcation for periodic orbits (Fig 2.6B).

Perturbations in the ($\theta_h, \theta_{K2}$) parameter space from the codimension-2 point lead to the disappearance of the saddle-node equilibrium or simple periodic orbit or cause the saddle-node orbit or equilibrium to split in two (Fig 2.6C-F).

In region $I$, a stable orbit and a saddle orbit were found on the slow manifold for spiking (Fig 2.6C). The stable periodic orbit corresponds to the tonic spiking regime, and is
the only attracting regime in this region. Perturbations can reveal characteristics of the slow manifold associated with the equilibrium of the fast subsystem. The phase point can spend a significant amount of time in a quiescent state before firing resumes after the cessation of an inhibitory perturbation (Fig 2.3E-G). This perturbation causes a fast transition of the phase point from a stable orbit onto the slow-motion manifold of equilibria for the fast subsystem. The phase point followed the slow-motion manifold, and the neuron exhibited a transient quiescence. For parameter values near the border between regions I and IV, the phase point could spend a long time near the ghost of the saddle-node equilibrium before the onset of firing.

In region II, both the manifolds of slow motion are unobstructed. This parameter region corresponds to the endogenous bursting regime (Fig 2.6D). During a burst, the phase point winds around the slow motion manifold for oscillations; during the interburst interval, the phase point follows the slow motion manifold for equilibria in the fast subsystem.

In region III, a stable equilibrium obstructs the slow manifold for equilibria of the fast subsystem (Fig 2.6E). The stable equilibrium is the only attracting regime in this region. A perturbation can trigger a train of action potentials (Fig 2.3A-C). During a transient burst, a fast transition occurred as the phase point moved from the stable equilibrium onto the slow manifold that controls oscillating activity in the fast subsystem. The model neuron executed a stereotyped burst as the phase point evolved across this manifold. Near the border between regions III and IV, the phase point could spend a long time near the ghost of the saddle-node orbit, producing a very long transient burst.

In region IV, there were stable and unstable orbits as well as stable and unstable equilibria. As a result, tonic spiking co-existed with a silent regime, and perturbations could elicit switches from one regime to another (Fig 2.6F).

The mechanisms controlling burst duration and interburst interval in region II determine a scheme of two-parameter coregulation that supports bursting with a given duty cycle across a wide range of cycle periods. This scheme could explain the maintenance of phase relations in a network of coupled oscillators.
2.3.5 Pattern-scaling in a Chain of Oscillators

We applied a mechanism maintaining the duty cycle of endogenously bursting activity to show scaling in a metachronal-wave locomotor pattern. We developed a basic model of a central pattern generator for crawling of larval *Drosophila*. *Drosophila* larvae crawl by means of peristaltic contractions of the body. Posterior-to-anterior waves of peristaltic contraction produce forward motion. The phase lag of motor activity in neighboring segments scales proportionally to the period so that the phase relations between activity in neighboring segments is maintained. This scaling occurs over a two-fold range of cycle periods (Pulver et al., 2012). Activity in the nerve cord in isolated preparations occurs on the time scale of tens of seconds (Fox et al., 2006; Hughes and Thomas, 2007). The phase delay from segment-to-segment of segmental nerve cord activity is roughly 10 % of the cycle period (Fox et al., 2006). We suggest that the scheme of coregulation controlling the duty cycle of participating neurons could present a cellular mechanism for the metachronal-wave pattern scaling. We modeled the metachronal-wave central pattern generator (CPG) as a chain of coupled oscillatory neurons.

The network was assembled from five endogenously bursting neurons from region II (Fig 2.1A). If these neurons receive an inhibitory pulse during the interburst interval, they respond with a burst, and the activity is reset. This observation suggests that if these neurons are coupled in a chain, they will trigger burst responses one after another as though in a domino effect. We expect that this effect would be robust if the neurons were connected in a chain such that the strength of inhibitory coupling is stronger in one direction than in the other. This sequential propagation of excitation along a chain is referred to as a metachronal wave which would travel in the direction of stronger coupling. With this organization of activity, the phase delay is determined by burst duration. Thus by scaling the duty cycle, one can scale the pattern of the metachronal wave. We manipulated the network by changing the values of $\theta_{K2}$ and $\theta_h$ to control the burst duration and interburst interval. We coordinated these changes to maintain a constraint of 10 % duty cycle within 0.1 % tolerance for individual oscillators over a wide range of periods from 15 s to 85 s (Table 2.3). We predicted that
a chain of coupled oscillators each with an intrinsic duty cycle of 10% would produce a metachronal wave with phase delay of 10% at the period of the individual oscillators. With this duty cycle, in order to produce rhythms faster than 68 s, we obtained parameter sets with values of $\theta_{K2}$ beyond the range depicted in Figure 2.1A. They are located so close to the SNIC that they appear to sit on the curve (Fig 2.1A). We did not investigate whether the SNIC extends beyond this range, yet we were able to obtain parameter sets that supported activity with a duty cycle of 10%.

The model is represented by a chain of identical bursting neurons with duty cycle 10% as described in Methods. To show pattern scaling, we investigated this model with parameters of individual cells varied according to our coregulation scheme defined in Table 2.3. Network activity self-organized into a metachronal-wave pattern following an interval of transient activity in all ten instantiations (Fig 2.7). After the interval of transient activity, waves of bursts propagated along the chain of oscillators from the most posterior cell (segment 7) in the anterior direction. We quantified the time it took a network to reach a periodic state by counting the number of cycles of the posterior cell before the transient activity subsided and the propagating wave fully formed. In each instantiation of the network, the formation of the metachronal wave occurred within two cycles of the bursting neuron in segment 7.

We characterized metachronal waves in the activity of each network instantiation by the period and duty cycle of activity as well as the relative phase shift of bursting from one segment to its nearest neighbor. We compared the temporal characteristics of network bursting activity to those of activity produced by a single cell with the same values of $\theta_{K2}$ and $\theta_h$ (Table 2.3). The period and duty cycle of bursting activity of a single cell and the network matched well; these periods differed by less than 0.1% and 3%, respectively. The disparity between the period of single-cell and network activity was greater for network instantiations with larger period. In each network instantiation, the phase shift of the metachronal wave exhibited some dependence on cycle period (Fig 2.8). For parameter values that produced activity with lower periods, the phase shift tended to be greater than that predicted by the duty cycle. For example, in network instantiations with parameter values that produced
activity with period 15.1 s and 21.0 s, the average relative phase between segments was 13.0 % and 13.2 %, respectively. For parameter values that produced activity with higher periods, the phase shift tended to be close to 10 %. The instantiation of the network that produced activity with period 85.1 s had an average relative phase shift of 10.5 %.

The cornerstone bifurcation controls periodicity through both burst duration and interburst interval. By coregulating $\theta_{K2}$ and $\theta_h$, we vary the period of bursting while keeping the duty cycle fixed. As instantiations of the network become closer to the critical parameter values for the cornerstone bifurcation, the period of activity increases according to the inverse-square laws governing burst duration and interburst interval. Since duty cycle is fixed over variation in period, the burst duration in this model increases via spike addition. As such, the cycle period varies linearly with spike number (Fig 2.8).

The period of a metachronal wave was well approximated by the intrinsic period of the individual oscillator on which that network instantiation was based. For parameter sets that produced activity with higher periods of bursting, the temporal characteristics of individual oscillators better predicted the temporal characteristics of network activity. The metachronal-wave pattern was maintained over a five-fold change in the period of activity.

2.3.6 Control of Motor Pattern in an Insect Walking Central Pattern Generator

Insect locomotion presents a type of pattern adaptation in which the phase relations of the pattern change with the speed of walking. Here, we apply the mechanism describing the stereotypical burst responses of a silent neuron to explain these changes. We construct a simple CPG model including silent neurons to produce appropriate adjustments to the motor pattern. The changes to the dynamics of neurons through the variation of a single parameter could be considered as response to neuromodulatory tone.

During locomotion, a cycle of leg motion is comprised of two intervals: protraction (swing) and retraction (stance). In forward walking, the protraction of legs progresses sequentially one after the other from posterior to anterior. The duration of protraction is roughly invariant, and the duration of retraction is linearly dependent on the cycle period of
each step (Wendler, 1965; Wilson, 1966; Graham, 1985). These recorded constraints determine phase relations in the metachronal wave gait over a range of speeds; the phase relations are controlled by the duration of retraction. These temporal characteristics have been reported in a fictive motor pattern induced by pilocarpine (Buschges et al., 1995; Bassler and Buschges, 1998; Buschges et al., 2008). The central pattern generator that controls insect walking has not been described (Buschges et al., 1995; Bassler and Buschges, 1998; Buschges et al., 2008; Daun-Gruhn, 2011). The burst duration of retractor motorneurons varies with cycle period while the burst duration of protractor motoneurons is roughly invariant over cycle period (Buschges et al., 1995). The neuronal mechanisms controlling the duration of the retraction and the relative phases of motion between segments are not well understood.

We attempt to answer open questions in the study of locomotor systems: how is the duration of the retraction interval controlled, and how are the relative phases of motion between segments controlled?

To answer these questions, we developed a simple model that described the central pattern generator controlling the motion of legs of a six-legged animal. One side of the network contained six interneurons that were arranged into three coupled segmental oscillators. Each oscillator described a CPG controlling a single leg in its segment. These segmental oscillators corresponded to the three thoracic segments. From anterior to posterior, these segments are called the prothoracic, mesothoracic, and metathoracic segments. The segmental oscillators were each made of two-cell mutually inhibitory networks (Fig 2.9). Here we describe a mechanism where the stereotypical response of a silent neuron controls the duration of retraction.

This network is relatively small, and the dynamics of each component neuron are well described by our mechanisms. We constructed segmental oscillators composed of individual cells with predictable activity. We controlled the duty cycle and phase of activity in single segmental oscillators by varying $\theta_{K2}$, and hence, controlling the burst duration in retractor interneurons. Given our knowledge of these systems, we intuitively designed the intersegmental connectivity to promote the propagation of a metachronal activity, and we controlled
duty cycle and phase of activity in a network composed of three segmental oscillators. In this larger network, activity progressed from posterior to anterior in a metachronal wave. Moreover, we demonstrated a smooth transition from metachronal to tripod gaits in examples of this activity as the controlling parameter was varied in retractor interneurons.

Segmental oscillators were composed of two interneurons that controlled protraction and retraction. Protractor and retractor interneurons were defined by our model (see Methods) and differed in values of \( \theta_{K2} \) and \( \theta_h \). We could choose any bursting properties from the map in Figure 2.1A. We configured these parameters to produce target endogenous activity in the different cells (Table 2.4). Protractor interneurons had values \( \theta_{K2} = -0.004 \) V and \( \theta_h = 0.041 \) V that supported endogenous bursting (region II; Fig 2.6A-D). The parameter values for protractor interneurons did not vary between trials. Retractor interneurons were given \( \theta_h = 0.0414 \) V that supported the endogenous silent regime (region III, Fig 2.6A-E). The difference between \( \theta_{K2} \) and its bifurcation value determines the burst duration of a burst fired in response to inhibition. By varying the value of \( \theta_{K2} \) from -0.004 V to -0.01 V in retractor interneurons, we controlled the duration of the retractor interneuron bursts triggered by synaptic input from the protractor interneurons. We based this experiment on the mechanism for control of pulse-triggered burst duration (Fig 2.3A-D). Each segmental oscillator was organized with mutual inhibition between protractor interneurons and retractor interneurons (Fig 2.9). Inhibition from protractor interneurons to retractor interneurons was an order of magnitude stronger than inhibition from retractor interneurons to protractor interneurons (Table 2.5). A burst in the protractor interneuron triggered a burst in the retractor interneuron to complete one cycle. The relatively weak inhibition to the protractor interneuron from the retractor interneuron prevented the protractor interneuron from firing another burst until the burst in the retractor interneuron terminated. The strength of the retractor-to-protractor synapse was balanced with the protractor-to-retractor synapse so that the protractor interneuron fired a burst immediately after cessation of inhibition from the retractor interneuron. Adjusting these synaptic conductances did not require much effort and was achieved on the second attempt.
A series of simulations showed that the activity of the segmental oscillator depended on $\theta_{K_2}$ in retractor interneurons (Fig 2.10). The parameter $\theta_{K_2}$ in retractor interneurons varied from -0.004 $V$ to -0.01 $V$. As $\theta_{K_2}$ in retractor interneurons decreased from -0.004 $V$, the burst duration of retractor interneurons increased (Fig 2.10). At $\theta_{K_2} = -0.010$ $V$, the retractor interneuron had a burst duration of 34.3 s (Fig 2.10A). When $\theta_{K_2} = -0.007$ $V$, the retractor interneuron burst duration was 8.0 s (Fig 2.10B). At $\theta_{K_2} = -0.004$ $V$, the burst duration of the retractor interneuron was 4.6 s (Fig 2.10C). In each case, the interburst interval of the retractor interneuron was controlled by the burst duration of the protractor interneuron, and the interburst interval of the protractor interneuron was controlled by the burst duration of the retractor interneuron. Changing $\theta_{K_2}$ from -0.004 $V$ to -0.01 $V$ resulted in an increase in burst duration: as $\theta_{K_2}$ decreased, the number of spikes in the burst increased. The increasing proximity of $\theta_{K_2}$ to its bifurcation value introduces a bottle-neck in the vector field nearby the location on the manifold for spikes where the saddle-node orbit will be born. Having identified the qualitative nature of the bifurcation, we made specific quantitative predictions as to how the waveform of bursting activity changes with changes to the bifurcation parameter. Over the course of the burst, $m_{K_2}$ accumulated, and the amplitude of spikes increased. The spike-averaged velocity of $m_{K_2}$ approached zero during the passage nearby the ghost of the saddle-node orbit as $\theta_{K_2}$ approaches its bifurcation value (Fig Ref Manifolds). During this time, the fast subsystem was able to complete an increasing number of spikes. Since the saddle-node orbit was born on the slow manifold for spiking and the phase point is drawn to this manifold, spikes that occurred on the portion of the manifold close to the location where the saddle-node orbit would appear had temporal characteristics increasingly similar to those of the saddle-node orbit. In the bursting waveform, the progressive growth of spike amplitude became slower as $\theta_{K_2}$ approached its bifurcation value. As predicted by this mechanism, the burst duration was determined by the inverse square-root law (Fig 2.10D):

$$f_{K_2}(\theta_{K_2}) = \frac{b}{\sqrt{\theta_{K_2} + d + c}}.$$  (2.3)
With a curve fitting routine, we show that this expression fitted the dependence of burst duration of the retractor interneurons on $\theta_{K2}$ with coefficients $b = 0.820$, $c = -5.852$, and $d = 0.010$. The parameter $\theta_{K2}$ in protractor interneurons was fixed during these simulations. The burst duration in the activity of protractor interneurons was roughly invariant as $\theta_{K2}$ in retractor interneurons changed.

We defined the segmental phase delay as the phase delay of the retractor interneuron to the protractor interneuron. This value was approximated as follows:

$$\phi_R = DC_R \approx \frac{BD_R}{BD_R + BD_P}, \quad (2.4)$$

Where $\phi_R$ was the segmental phase delay, $DC_R$ was the duty cycle of the retractor interneuron, $BD_R$ was the burst duration of the retractor interneuron and $BD_P$ is the burst duration of the protractor interneuron.

A model including three coupled segmental oscillators show the propagation of a metachronal wave (Table 2.5). The posterior-to-anterior synaptic weights were stronger than anterior-to-posterior, determining the direction of travel of the metachronal wave. Waves of bursting activity propagated from the metathoracic segment to the prothoracic segment (Fig 2.12). Since only protractor interneurons were endogenously bursting, these waves were initiated from the metathoracic protractor interneuron. Following the termination of inhibition from the metathoracic protractor interneuron, the mesothoracic protractor interneuron fired a burst. Following the termination of inhibition from the mesothoracic protractor interneuron, the prothoracic protractor interneuron fired a burst. In retractor interneurons, we varied $\theta_{K2}$, and hence, the burst duration of these cells (Table 2.6). The burst duration of the retractor interneuron determined the cycle period of the network and controlled which neurons were firing at the same time. As such, the retractor burst duration determined what type of gait was exhibited by network activity.

We performed a series of simulations to determine how network activity depended on this parameter. Variation of $\theta_{K2}$ values for retractor interneurons changed the burst duration of
retractor interneurons (Fig 2.11 A). The parameter $\theta_{K2}$ in retractor interneurons was varied from -0.004 $V$ to -0.01 $V$ in steps of 0.0001 $V$. The burst duration of retractor interneurons monotonically increased from roughly 4.6 s to 34.3 s as $\theta_{K2}$ decreased from -0.004 $V$ to -0.010, but the burst duration of the protractor interneurons remained roughly invariant, varying from roughly 4.6 to 4.8 (Table 2.6). Over this range of values for $\theta_{K2}$, the cycle period increased from roughly roughly 10.6 s to 40.4 s. The burst duration of retractor interneurons in each segment shared the same dependence on $\theta_{K2}$.

The phase progression of prothoracic and mesothoracic protractor interneuron or retractor interneuron bursts showed nonlinear dependence on $\theta_{K2}$ of retractor interneurons (Fig 2.11B). Mesothoracic protractor or retractor interneurons fired before prothoracic protractor or retractor interneurons in all simulations. For lower values of $\theta_{K2}$ in retractor interneurons, the phase progression of retractor and protractor interneurons contracted into the beginning of the network cycle. The phase of prothoracic and metathoracic retractors decreased from 0.97 and 0.48 to 0.26 and 0.13 as $\theta_{K2}$ changed from -0.01 $V$ to -0.004 $V$ in retractor interneurons. Similarly, the phase of prothoracic and metathoracic protractors decreased from 0.95 and 0.47 to 0.26 and 0.13. A burst in a protractor interneuron triggered a burst in the retractor interneuron of the same segment. The protractor interneuron was unable to fire again until the burst in the retractor interneuron terminated, and the retractor interneurons fired very long bursts. Thus, the phase of the protractor and retractor interneurons in the prothoracic and mesothoracic segments moved closer in phase to the start of the network cycle.

Changes to $\theta_{K2}$ in retractor interneurons impacted network activity by directly controlling burst duration in retractor interneurons. The burst duration of retractor interneurons was a linear function of network cycle period (Fig 2.11C).

In the full model, there are three sets of oscillators on each of two sides with mutual inhibition between protractors (Fig 2.9). The parameters $\theta_{K2}$ and $\theta_h$ have been tuned to produce activity with 50 % phase difference between protractor and retractor where $\theta_{K2}$ is -0.005 $V$. The model demonstrated a smooth transition from the metachronal gait to the
tripod gait. For the range of values of $\theta_{K2}$ used, we observed three qualitatively distinct gaits. We have given examples for each of these three cases (Fig 2.12A). In the first gait—the metachronal gait—the protractor interneuron activity in each segment did not overlap with protractor interneuron activity in any other segment. An example of the metachronal gait manifested at $\theta_{K2} = -0.010 \text{ V}$ where bursts occurred in a single segment at a time (Fig 2.12A). This pattern of activity persisted while the duty cycle of the retractor interneuron was greater than $2/3$. In the second gait, the protractor interneuron activity in the prothoracic and metathoracic segments did overlap for some time but were not synchronous. In our network, an example of a metachronal gait with larger duty cycle appeared at $\theta_{K2} = -0.0075 \text{ V}$ where the metachronal wave of bursting in protractor interneurons began in the metathoracic segment before the termination of the burst of the protractor interneuron in the prothoracic segment, but not soon enough for bursting in the prothoracic and metathoracic segments to be synchronous (Fig 2.12B). In the third gait—the tripod gait—activity in the prothoracic and metathoracic protractor interneurons was synchronous. An example of the tripod gait appeared at $\theta_{K2} = -0.005 \text{ V}$ as an alternating pattern where simultaneous bursting occurred in protractor interneurons in the prothoracic and metathoracic segments was followed by protraction in the mesothoracic segment (Fig 2.12C). The network exhibited a duty cycle of approximately 50 % during the tripod gait.

2.4 Discussion

The flexibility of the central nervous system relies on the ability of neurons to meet a breadth of temporal specifications. Oscillatory neuronal networks, such as central pattern generators, maintain functional output over a wide range of cycle periods in order to produce appropriate behavior (Kristan et al., 1974; Arbas and Calabrese, 1984; Wallen and Williams, 1984; Williams et al., 1989; Friesen and Pearce, 1993; Dicaprio et al., 1997; Hooper, 1997; Skinner and Mulloney, 1998; Mouser et al., 2008; Suster and Bate, 2002; Fox et al., 2006; Norris et al., 2007; Hughes and Thomas, 2007; Smarandache et al., 2009; Mullins et al., 2011; Pulver et al., 2012). Neuromodulation provides control over regimes of activity according
to motor tasks by coordinating adjustments to the biophysical parameters of ionic currents (Marder and Calabrese, 1996; Marder and Bucher, 2001). To understand this process, we must answer a key question in neuroscience. How do biophysical characteristics govern functional activity? This question is particularly important for understanding motor control of rhythmic movements. Among the biophysical characteristics that affect excitability, the maximal conductances and voltages of half-activation of ionic currents are the most prominent targets for neuromodulation (Hooper and Marder, 1987; Harris-Warrick and Flamm, 1987; Nadim and Calabrese, 1997; Luthi and McCormick, 1998, 1999; MacLean et al., 2003, 2005; Tobin and Calabrese, 2005; Schulz et al., 2006, 2007; Khorkova and Golowasch, 2007; Kloppenburg et al., 2008; Rodgers et al., 2011b,a; Ikematsu et al., 2011; Amendola et al., 2012).

The maximal conductances of ionic currents are coregulated to produce functional activity (MacLean et al., 2003, 2005; Schulz et al., 2006, 2007; Khorkova and Golowasch, 2007). Homeostasis of a functional pattern of activity, either activity-dependent or activity-independent, has been well documented in terms of covariation of maximal conductances. One mechanism for activity-independent homeostasis of functional activity is determined by the pattern of gene expression of ionic channels. The activity of an identified neuron is specified by the patterns of gene expression of a set of ionic channels (MacLean et al., 2003; Schulz et al., 2006, 2007; Khorkova and Golowasch, 2007). Correlations have been shown in the quantities of mRNA that code for various voltage gated ion channels in crab (Schulz et al., 2006, 2007). Modeling studies have explored the role of correlation in biophysical parameters in the maintenance of functional activity (Taylor et al., 2006, 2009; Soofi et al., 2012). The biophysical parameters of hyperpolarization-activated currents and potassium currents are often correlated (MacLean et al., 2003; Schulz et al., 2006, 2007). For example, coregulation has been revealed by the injection of the mRNA coding A-type potassium current which induced a corresponding increase in $I_h$ (MacLean et al., 2003). In most cells considered, the mRNA quantities for these currents are strongly correlated (Schulz et al., 2006, 2007). The mRNA correlations map to correlations in expression of channel protein, which in turn deter-
mines the maximal conductance of ionic currents. Correlations in the maximal conductance that support functional activity has been shown in models (Taylor et al., 2006, 2009; Soofi et al., 2012). Maximal conductances are also correlated in mechanisms of activity-dependent homeostasis (Abbott and LeMasson, 1993; Cao and Oertel, 2011).

The voltages of half-activation of ionic conductances are also subject to neuromodulation (Luthi and McCormick, 1998, 1999; Kloppenburg et al., 2008; Rodgers et al., 2011b; Ikematsu et al., 2011; Amendola et al., 2012). These parameters are shifted in neuromodulation of potassium currents and hyperpolarization-activated currents (Luthi and McCormick, 1998; ?; Kloppenburg et al., 2008; Rodgers et al., 2011b; Ikematsu et al., 2011; Amendola et al., 2012). For Kv2.1 expressed in HEK293, the voltage dependencies of activation and inactivation are shown to be shifted negatively by 30 mV and 22 mV in response to phosphorylation by AMPK (Ikematsu et al., 2011). In the pyloric network of the spiny lobster, depending on the time of exposure and concentration of dopamine, the magnitude and direction of shifts varies but in all cases the shifts appear to be small (Rodgers et al., 2011b; Kloppenburg et al., 2008). Hour-long application of dopamine induces dose-dependent shifts in the voltage dependence of the conductance of an A-type potassium current (Rodgers et al., 2011b). In LP neurons, nM and µM concentrations positively shift the activation by 4.6 mV and 1.7 mV, respectively, while the shifts of the inactivation curve have different directions: 3.3 mV and -1.2 mV, respectively. In PD neurons, both nM and µM concentrations negatively shift the activation and inactivation curves by 1.3 mV or less (Rodgers et al., 2011b); moreover, a ten minute application of a larger concentration of dopamine (100 µM) induces a larger negative shift of 7.6 mV to the activation curve (Kloppenburg et al., 2008). In ferret thalamocortical neurons, repeated negative pulses depolarize the activation of $I_h$ by 3.7 mV, and application of cAMP depolarizes the activation of $I_h$ by 12 mV (Luthi and McCormick, 1998, 1999). Neuromodulators can coregulate currents that have complementary effects on membrane dynamics—such as hyperpolarization-activated currents and potassium currents—to tune aspects of excitability (Amendola et al., 2012). These shifts appear to be small and negligible. However, our results show that precise control of these biophysical param-
eters in these reported ranges could be sufficient for effective neuromodulation. Amendola et al. (Amendola et al., 2012) show independence of neuromodulation of half-activation of h-current and half-inactivation of A-type potassium current. Such ability to independently control these parameters supports the biological feasibility of the mechanisms of independent control of burst duration and interburst interval by variation of half- activations of $I_{K2}$ and $I_h$, presented in this report.

We have demonstrated a family of cellular mechanisms that provides three different kinds of control for temporal characteristics in neuronal activity by coregulating the kinetics of activation of a non-inactivating potassium current and a hyperpolarization-activated current. These controlling mechanisms are organized by a global codimension-2 bifurcation: the cornerstone bifurcation. The cornerstone bifurcation occurs at the intersection of two codimension-1 bifurcation curves—the saddle-node bifurcation for periodic orbits and the saddle-node bifurcation for equilibria—in the two-dimensional parameter space defined by the voltages of half-activation of $I_{K2}$ and $I_h$.

In our model, the kinetics of activation of $I_{K2}$ and $I_h$ have distinctly separate, complementary roles in rhythmogenesis. $I_{K2}$, with its voltage of half-activation between 0.006 and 0.011 V (Fig 2.1), activates incrementally during each spike in the burst. For this mechanism to function, the activation variable must be significantly slower than the time scale of spiking. Over the course of the burst, this activation accumulates until the current is sufficiently activated to terminate oscillations in the fast subsystem. As the parameter $\theta_{k2}$ is changed such as to approach the critical value for bifurcation, the steady-state activation curve of $I_{K2}$ is shifted so that the current is less activated by each spike. Finally, at the critical value for bifurcation, the activation of this current no longer accumulates sufficiently over the course of spiking activity to terminate a burst. $I_h$ plays a similar role during the interburst interval. Its voltage of half-activation lies roughly between -0.038 and -0.042 V (Fig 2.1), and it is responsible for the slow depolarization leading to the first spike in the burst. As the parameter $\theta_h$ approaches its critical value for bifurcation, the steady state activation curve of $I_h$ is shifted so that the current is less involved during the interburst
interval. At the critical value for bifurcation, this current does not sufficiently activate to initiate the depolarization of the burst.

2.4.1 Bifurcation Theory and Control of Neuronal Activity

Bifurcation theory considers transitions (bifurcations)–either continuous and smooth or discontinuous and catastrophic–in response to a smooth change in a physical parameter. Applications of bifurcation theory in physics, engineering, and neuroscience have established a new interdisciplinary field: bifurcation control (Berge et al., 1987; Rinzel and Ermentrout, 1998; Rabinovich et al., 2008). Bifurcation control offers techniques to control regimes of activity using knowledge of bifurcation locations in parameter space and techniques to control the type of a bifurcation. These techniques prescribe how to precisely control the target characteristics of dynamical regimes by changing bifurcation parameters. Common targets of control include steady state characteristics of equilibria, temporal characteristics and magnitude of oscillatory regimes and transient activity, the borders of basins of attraction, and properties of and the routes to turbulence in deterministic systems (Rinzel, 1978b; Hoppensteadt and Izhikevich, 1997; Rinzel and Ermentrout, 1998; Ermentrout, 1996; Teka et al., 2011; Ermentrout and Kopell, 1994a; Rinzel, 1987; Cymbalyuk et al., 2002; Malashchenko et al., 2011d,a; Marder et al., 1996; Roa et al., 2007; Meng et al., 2011; Ermentrout, 2004b; Booth et al., 1997; Gutkin and Ermentrout, 1998; Laing et al., 2003; Doiron et al., 2003; Cymbalyuk and Calabrese, 2001; Shilnikov and Cymbalyuk, 2005; Cymbalyuk and Shilnikov, 2005b; Shilnikov et al., 2005; Ghosh et al., 2009; Izhikevich, 2000; Channell et al., 2007). For example in the control of turbulent activity, different types of intermittency have been associated with specific bifurcation types (Pomeau and Manneville, 1980; Berge et al., 1987). Intermittency of type I and type II are related to the saddle-node bifurcation and the Andronov-Hopf bifurcation, respectively (Pomeau and Manneville, 1980; Berge et al., 1987).

Bifurcation theory has been applied to describe the general laws of neuronal dynamics that govern characteristics of spiking and bursting activity as a controlling bifurcation pa-
rameter approaches a transition between qualitatively different regimes of activity (Rinzel, 1978b; Hoppensteadt and Izhikevich, 1997; Rinzel and Ermentrout, 1998; Ermentrout, 1996; Teka et al., 2011; Ermentrout and Kopell, 1994a; Rinzel, 1987; Cymbalyuk et al., 2002; Malashchenko et al., 2011d,a; Marder et al., 1996; Roa et al., 2007; Meng et al., 2011; Ermentrout, 2004b; Booth et al., 1997; Gutkin and Ermentrout, 1998; Laing et al., 2003; Doiron et al., 2003; Cymbalyuk and Calabrese, 2001; Shilnikov and Cymbalyuk, 2005; Cymbalyuk and Shilnikov, 2005b; Shilnikov et al., 2005; Ghosh et al., 2009; Izhikevich, 2000; Channell et al., 2007). Excitability in neuronal systems has been characterized by type of bifurcation. A saddle-node bifurcation on an invariant circle generates class I excitability (Ermentrout, 1996; Hoppensteadt and Izhikevich, 1997; Rinzel and Ermentrout, 1998; Gutkin and Ermentrout, 1998). At this bifurcation, a neuron makes a smooth transition from silence into tonic spiking as the bifurcation parameter smoothly changes. This bifurcation controls the interspike interval according to the inverse-square-root law. At the bifurcation point the neuron is silent and, thus, the frequency is zero. The spiking orbit appears at the bifurcation with a large, full-scale amplitude. It is well described by a canonical model: the $\theta$ neuron (Ermentrout and Kopell, 1986; Ermentrout, 1996; Hoppensteadt and Izhikevich, 1997). A supercritical Andronov-Hopf bifurcation generates class II excitability (Rinzel, 1978b; Hoppensteadt and Izhikevich, 1997; Ermentrout, 1996; Gutkin and Ermentrout, 1998). At this bifurcation, the rest state loses stability, and a spiking orbit with zero amplitude and non-zero frequency is born. The amplitude of spiking grows according to the square root of the bifurcation parameter. Depending on the class of excitability, the response of the neuron to a stimulus, in type 2, may advance or delay the next spike depending on phase or, in type 1, will advance in response to depolarizing pulses and delay in response to hyperpolarizing pulses (Ermentrout, 1996; Hoppensteadt and Izhikevich, 1997). The type of phase response describes synchronization in neuronal networks (Ermentrout, 1996; Hoppensteadt and Izhikevich, 1997; Gutkin and Ermentrout, 1998). The quantitative laws described by bifurcation theory are generic and are not uniquely associated with specific biophysical properties of ionic currents. For example, similar dynamical mechanisms may appear in neuronal
systems relying on distinct ionic currents (Prinz et al., 2004), and qualitatively distinct dynamics such as type 1 versus type 2 can be realized in a neuronal system by variation of a few biophysical parameters (Rinzel and Ermentrout, 1998). Dynamical mechanisms have also been used to explain the generation of bursting in terms of slow-fast systems (Rinzel, 1987; Izhikevich, 2000; Rinzel and Ermentrout, 1998). Bursting is usually based on bistability of spiking activity and a subthreshold regime in the fast subsystem and dynamics of a slow variable governing switches between these regimes (Rinzel, 1987; Ermentrout and Kopell, 1986; Izhikevich, 2000). The bursting activity in our model is a square-wave burster (Rinzel and Ermentrout, 1998; Shilnikov and Cymbalyuk, 2005).

Similarly to spiking activity, temporal laws have been described for bursting activity where the length of the burst duration or interburst interval is subject to control by a bifurcation parameter (Booth et al., 1997; Shilnikov and Cymbalyuk, 2005; Laing et al., 2003; Doiron et al., 2003; Ghosh et al., 2009). The control of a specific temporal characteristic has been shown by the manipulation of single bifurcation parameter. In models of the leech heart interneuron under various experimental conditions, we have extensively studied how bifurcations determine the mechanisms that support bursting activity. Where the blue sky catastrophe controls the transition from bursting to tonic spiking, it imposes the inverse-square-root law on burst duration (Shilnikov and Cymbalyuk, 2005). Where homoclinic bifurcations control the transition from bursting to the spiking regime–such as the Lukyanov-Shilnikov scenario–a logarithmic law is imposed on burst duration (Shilnikov et al., 2005). In this report, we describe independent control of burst duration and interburst interval with two parameters.

Examples of control over burst duration and interburst interval have been shown in a model of the electrosensory lateral line lobe pyramidal cell (Doiron et al., 2003). In (Doiron et al., 2003), the model shows a unique type of bursting called ghostbursting. In slow-fast decomposition, the fast subsystem of the ghostbursting model does not exhibit bistability (Doiron et al., 2003); such bistability is a common feature of bursting models (Rinzel, 1987). This lack of bistability is described as unique to the ghostbursting mechanism, and as such, it
makes a key difference between ghostbursting and our model in the topology of the manifolds that govern slow motion. In our model, bursting activity is based on bistability of spiking and a stable equilibrium in the fast subsystem, thus classified as square-wave bursting; the slow manifolds of spiking activity and equilibria are separated in the phase space. This bistability of regimes in the fast subsystem allows for the bifurcation curves obtained in the full system—the saddle-node bifurcation for periodic orbits and saddle-node bifurcation for equilibria—to cross in the two parameter space. This feature of the bifurcation curves allows the cornerstone bifurcation to generate a family of mechanisms that independently control the temporal characteristics of bursting and transient responses to external stimuli. The curves for saddle-node bifurcation of periodic orbits and saddle-node bifurcation for equilibria cross, dividing parameter space into four regions: spiking, bursting, silence, and bistability of spiking and silence. Similarly to our model, the scenario presented in (Doiron et al., 2003) involves a saddle-node bifurcation for fixed points (SNFP) and a saddle-node bifurcation for periodic orbits (SNLC). In contrast to our model, on the two-dimensional bifurcation diagram in (Doiron et al., 2003), SNLC terminates at SNFP. It is important to emphasize that the saddle-node bifurcation for periodic orbits and the saddle-node bifurcation for equilibria are local bifurcations. In our model, the control of bursting activity is governed by specific global bifurcations: the blue sky catastrophe and the saddle-node bifurcation on an invariant circle are global bifurcations, which include the saddle-node bifurcation for periodic orbits and the saddle-node bifurcation for equilibria, locally. In our model, the two mechanisms, by which burst duration and interburst interval of periodic bursting activity are controlled, are uncoupled.

Another key feature that distinguishes ghostbursting from our scenario, is that in the ghostbursting model the SNLC describes the transition from tonic spiking to chaotic bursting, and SNFP describes the transition of chaotic bursting into silence. Doiron et al. show that these transitions to chaos are intermittency type I (Doiron et al., 2003; Berge et al., 1987; Pomeau and Manneville, 1980). This bursting activity is governed by inverse-square-root laws for burst duration and interburst interval on average. In contrast, our model shows
periodic bursting; the blue-sky catastrophe describes the transition between periodic bursting and tonic spiking (Shilnikov and Cymbalyuk, 2005), and SNIC describes the transition between periodic bursting and silence. The ability to control temporal characteristics of periodic bursting activity is critical for the control of precise rhythmic movements. For the first time, a biophysically realistic neuronal model is described which combines two inverse-square-root laws and realizes independent control over burst duration and interburst interval. Moreover, this is the first model of a physical system showing the cornerstone bifurcation. By coordinating burst duration and interburst interval, the duty cycle could be preserved over a wide range of cycle periods under neuromodulatory control.

2.4.2 A Cellular Mechanism of Pattern Scaling

We suggest that these cellular mechanisms could contribute to control of various types of motor activities. For example, phase shifts of a metachronal-wave pattern could be determined by the duty cycle of each element in a chain of inhibitory coupled bursting neurons. Metachronal-wave patterns are ubiquitous in animal locomotion such as that of the leech, the crayfish, Drosophila larvae, and the lamprey (Wallen and Williams, 1984; Williams et al., 1989; Suster and Bate, 2002; Fox et al., 2006; Hughes and Thomas, 2007; Mullins et al., 2011; Smarandache et al., 2009; Pulver et al., 2012). In each case, the motor pattern is maintained across a range of periods. The lamprey can swim with one body wave cycle with period in the range from 0.13 s to 0.66 s; the leech can swim with one body wave cycle with period from 0.39 to 1.1 s; or Drosophila larvae can crawl with body contractions of cycle period from 0.6s to 1.3 s (Kristan et al., 1974; Wallen and Williams, 1984; Williams et al., 1989; Mullins et al., 2011; Pulver et al., 2012). Phase lags between spinal segments are scaled such that the lag between neighboring segments is approximately 1 % of the cycle period in lamprey, 5 % of the cycle period in leech, or 10 % of the cycle in Drosophila (Kristan et al., 1974; Wallen and Williams, 1984; Williams et al., 1989; Friesen and Pearce, 1993; Suster and Bate, 2002; Fox et al., 2006; Hughes and Thomas, 2007; Mullins et al., 2011; Pulver et al., 2012). Coupled oscillators have been commonly used in the study of central
pattern generators. Phase delay in oscillating patterns have been explained with various network mechanisms such as frequency gradient, coupling gradient, and coding phase delays through adjustment of coupling (Kopell and Ermentrout, 1988; Cohen et al., 1992; Matsushima and Grillner, 1992; Ermentrout and Kopell, 1994a,b; Skinner and Mulloney, 1998; Varkonyi et al., 2008; Yanchuk et al., 2011; Popovych et al., 2011). For example, by tuning the coupling strengths and synaptic delays in neuronal networks, complex activity patterns can be generated, stored, and retrieved (Yanchuk et al., 2011; Popovych et al., 2011).

Drosophila larvae crawl by means of peristaltic contractions of body wall muscles. Posterior-to-anterior waves of contraction propel the animal forward at varying speeds. The segmental phase delay of the propagation of these waves is proportional to the cycle period of oscillations (Pulver et al., 2012). Fictive motor patterns persist in the isolated nerve cord (Suster and Bate, 2002; Fox et al., 2006). The period of this fictive pattern occurs on a time scale of tens of seconds (Fox et al., 2006; Hughes and Thomas, 2007). The phase delay measured from segment to segment in segmental nerve cords is 10% of the period of the motor pattern (Fox et al., 2006).

We modeled this phenomenon in a chain of coupled bursting neurons. The model corresponded to segments three through seven of the larval Drosophila (see Methods). By preserving duty cycle and varying cycle period we achieved maintenance of delay in phase between neighboring neurons in the chain. Moreover, the duty cycle of a model of a single cell predicts the phase delay in the network. Our model demonstrated a five-fold scaling of the metachronal-wave pattern, which is comparable to the two-fold scaling of the motor pattern in Drosophila (Pulver et al., 2012). This is an example of a cellular mechanism which translates to network activity. Although this mechanism does not require extensive synaptic tuning, it could work in conjunction with synaptic mechanisms to produce more a more sophisticated phase delay-cycle period relation.
2.4.3 Transient Response to Stimulus

Dynamical mechanisms govern rhythmic neuronal activity in the vicinity of bifurcations. These types of mechanisms are often discussed within the context of steady state activity. Stereotyped transient responses and activity could process or represent sensory stimulation (Butera et al., 1997; Hooper et al., 2002; Rabinovich et al., 2008; Kopp-Scheinpfug et al., 2011; Meng et al., 2011; Doiron et al., 2003; Laing et al., 2003). Such transient responses can be represented by heteroclinic connections in the phase space of a neuronal model. Heteroclinic connections can reliably dominate or control transient neuronal activity by drawing trajectories from a large basin of attraction into a specific and functional response to perturbation before relaxing into steady state activity. Strong attraction to slow manifolds makes evoked responses reproducible. This control manifests as a temporally precise response to inhibition.

These parameters provide two types of control over temporal characteristics for two types of transient activity. First, we demonstrate control of the duration of pulse-triggered bursts in a silent neuron. As the voltage of half-activation of $I_{K2}$ approaches the critical value for the saddle-node bifurcation for periodic orbits, the duration of individual bursts increases. Second, we demonstrated control of the duration of latency to spiking in response to a stimulus in an a periodically spiking neuron. As the voltage of half-activation of $I_h$ approaches the critical value for the saddle-node bifurcation for equilibria, there is an increase in the latency to spiking after inhibition in periodically spiking neurons. The dynamical mechanisms underlying transient responses described here feature the inverse-square-root law shared with type-I intermittency (Pomeau and Manneville, 1980; Berge et al., 1987; Doiron et al., 2003; Laing et al., 2003). In (Laing et al., 2003), pulses of injected current induced individual transient bursts from a stable periodic spiking regime, called type I burst excitability.

**Inverse-square-root Law for Pulse-Triggered Burst** In this article, we showed that the silent model could respond to a hyperpolarizing pulse by a stereotypical burst
of spikes. We report that the burst duration of the pulse-triggered bursts scaled as $\theta K^2$ approached the critical value for the saddle node bifurcation for periodic orbits (transition from III to IV). Similarly, Roa et al. have shown the inverse-square-root law in transient spiking activity for type II excitability neuronal models with parameter values near a saddle-node bifurcation for periodic orbits (Roa et al., 2007). In Roa et al., a constant applied current is used as the controlling parameter, and an additional brief pulse of current is used to move the phase point away from a stable equilibrium (Roa et al., 2007). These models then spend significant time spiking as the phase point passes near the ghost of the saddle-node periodic orbit before relaxing back onto the stable equilibrium. The time neurons spend spiking is proportional to the inverse of the square root of the value of the constant applied current. This mechanism is similar to the inverse-square-root law we reported here for pulse-triggered burst duration with values of $\theta K^2$ and $\theta_h$ near the border between regions III and IV. The main difference between underlying mechanisms is that in our model, the phase point moves along a slow motion manifold for oscillations and slows down near the ghost of a saddle-node orbit on that manifold. In our model, the inverse-square-root law in transient activity occurred as the phase point wound around the slow motion manifold.

**Latency to Spiking After Inhibition**  
Our model produces a stereotypical latency to spiking after a brief hyperpolarizing pulse in a spiking neuron. By coordinating $\theta K^2$ and $\theta_h$, we place the system in a region of the parameter space with a stable tonic spiking regime such that it is close to the saddle-node bifurcation for equilibria (in region I close to the border with region IV). In this region after inhibition, the phase point traveled along the manifold of slow motion and slowed down as it passed the ghost of a saddle-node equilibrium. Control over latency to spiking could explain phenomena crucial for pattern coding and motor control.

In Steuber et al., pauses in Purkinje cell firing are implicated in pattern coding (Steuber et al., 2007). In their model, a potassium current plays a key role in the mechanism supporting delay to spiking. It is activated by the calcium influx which is induced by sufficient
activation of parallel fiber synapses. Pauses in the spontaneous firing of the Purkinje cell occur after parallel fiber-evoked bursts, and long-term depression of parallel fiber synapses shorten pause duration. These pauses code the pattern of synchronous activation of multiple parallel fibers.

Potassium and hyperpolarization-activated currents have been implicated in the dynamics of delay to spiking. In Meng et al., the authors show control over delay to firing by manipulating the ratio of transient potassium conductances (Meng et al., 2011). In the brainstem superior paraolivary nucleus, $I_h$ controls the timing of firing in rebound from hyperpolarization (Kopp-Scheinpflug et al., 2011). The kinetics of slow conductances may contribute to duration sensitivity after inhibition from a sensory stimulus (Hooper et al., 2002).

2.4.4 Bistability of Spiking and Silence

Another important feature of the dynamics of our model is that it shows bistability of spiking and silence (region IV, Fig 2.6). Perturbations can trigger a switch from one regime to the other (not shown). This phenomenon has been described in the squid giant axon model (Rinzel, 1978b). In the Hodgkin-Huxley model of the squid giant axon, the stable equilibrium and the oscillating regime are separated by the stable manifold of an unstable orbit (Rinzel, 1978b). This mechanism was also described in a simplified model of the leech heart interneuron (Malashchenko et al., 2011a). This model supports a number of types of multistability including a case where a stable equilibrium and a spiking regime co-exist and are separated by the manifold of a saddle equilibrium (Malashchenko et al., 2011a). Similar mechanisms are described for co-existence of spiking and silence and of bursting and silence in the canonical model of the leech heart interneuron (Cymbalyuk et al., 2002; Malashchenko et al., 2011d). In the example presented here, the stable periodic orbit sits on the strongly attracting slow manifold for oscillations which is separated from the stable equilibrium by the manifolds of a saddle equilibrium and saddle orbit.
2.4.5 Designing Artificial Neurons

In the example presented here, a codimension-2 bifurcation that satisfies the criteria for the SNIC and the blue sky catastrophe controls burst duration, interburst interval, pulse triggered bursting, and latency to spiking. In conclusion, we suggest that the control over the temporal characteristics of neuronal activity presented here is critical for designing functional artificial neurons in biomedical and neuroengineering fields (Simoni et al., 2004). The parameter $\theta_{K^2}$ asserts control of burst duration in an endogenously bursting neuron and of duration of pulse-triggered bursts in a silent neuron. The parameter $\theta_h$ asserts control of interburst interval in an endogenously bursting neuron and latency to spiking in a spiking neuron. By coordinating these parameters, one could tune an artificial neuron to produce bursting activity with any burst duration and interburst interval over a large range of values for the cycle period. Moreover, the transitions between different regimes of activity are smooth and safe over the range of $\theta_{K^2}$ and $\theta_h$ addressed here, so parameters can be tuned without fear of the onset of multistability or catastrophe.

2.5 Methods

We developed a Hodgkin-Huxley style neuronal model. It contains three voltage gated currents: a fast $\text{Na}^+$ current, $I_{Na}$; a non-inactivating $\text{K}^+$ current, $I_{K^2}$; and a hyperpolarization-activated current, $I_h$. From our previous models, it inherited the blue sky catastrophe (Cymbalyuk and Calabrese, 2001; Shilnikov and Cymbalyuk, 2005; Cymbalyuk and Shilnikov, 2005b; Shilnikov et al., 2005). The model is as follows:
\[ C \frac{dV}{dt} = -[\bar{g}_{\text{Na}}m_{\text{Na},\infty}(V)^3h_{\text{Na}}[V - E_{\text{Na}}] + \bar{g}_{\text{K}2}m_{\text{K}2}^2[V - E_{\text{K}}] + \bar{g}_{h}m_{h}^2[V - E_{h}] + \bar{g}_{\text{leak}}[V - E_{\text{leak}}] + 0.006], \]

\[ \frac{dh_{\text{Na}}}{dt} = \left[ \frac{1}{1 + \exp(500(V + 0.0325))} - h_{\text{Na}} \right] / 0.0405, \]

\[ \frac{dm_{h}}{dt} = \left[ \frac{1}{1 + 2 \exp(180(V + \theta_{\text{h}})) + \exp(500(V + \theta_{\text{h}}))} - m_{h} \right] / 0.1, \]

\[ \frac{dm_{\text{K}2}}{dt} = \left[ m_{\text{K}2,\infty}(V, \theta_{\text{K}2}) - m_{\text{K}2} \right] / 2, \]

where

\[ m_{\text{Na},\infty}(V) = \frac{1}{1 + \exp(-150(V + 0.0305))}, \]

and

\[ m_{\text{K}2,\infty}(V, \theta_{\text{K}2}) = \frac{1}{1 + \exp(-83(V + \theta_{\text{K}2}))}. \]

The activation of \( I_{\text{Na}} \) is instantaneous and is denoted as \( m_{\text{Na},\infty}(V) \). The inactivation of \( I_{\text{Na}} \) and the activations of \( I_{\text{K}2} \) and \( I_{h} \) are \( h_{\text{Na}}, m_{\text{K}2}, \) and \( m_{h} \). The maximal conductances of ionic currents are \( \bar{g}_{\text{Na}} = 105 \ nS, \bar{g}_{\text{K}2} = 30 \ nS, \bar{g}_{h} = 4 \ nS, \) and \( \bar{g}_{\text{leak}} = 8 \ nS \). The reversal potentials are \( E_{\text{Na}} = 0.045 \ V, E_{\text{K}} = -0.07 \ V, E_{h} = -0.021 \ V, \) and \( E_{\text{leak}} = -0.046 \ V. \) The regulating parameters \( -\theta_{\text{K}2} \) and \( -\theta_{h} \) represent the voltages of half-activation of the variables \( m_{\text{K}2} \) and \( m_{h} \). The function \( m_{\text{K}2,\infty}(V, \theta_{\text{K}2}) \) is the steady state activation function of \( I_{\text{K}2} \). The capacitance, \( C \), is 2 \( nF \). Similar to (Shilnikov and Cymbalyuk, 2005; Cymbalyuk and Shilnikov, 2005b; Shilnikov et al., 2005), \( m_{\text{K}2} \) is the slow variable.

The majority of the parameters of this model were pulled directly from our previous model (Shilnikov and Cymbalyuk, 2005). These models stem from the model explaining the dynamics of slow plateau-like bursting activity in the leech heart interneurons under the conditions when \( \text{Ca}^{2+} \) currents are blocked with divalent ions and most of the \( \text{K}^+ \) currents...
are blocked with 4-aminopyridine (Angstadt and Friesen, 1991; Opdyke and Calabrese, 1994a; Cymbalyuk and Calabrese, 2001). Here, we added a hyperpolarization activated current as was described in Hill et al. (Hill et al., 2001). We adjusted $g_{Na}$ and the time constant of $I_h$ during the search for the cornerstone bifurcation. We also adjusted the time constant of $m_{K2}$, the slow variable, to make the variable slower in order to emphasize the separation of time scales.

We built a model representing the CPG that produces a metachronal-wave pattern of locomotion in *Drosophila* larvae. It follows the generic motif of a chain of coupled oscillators (Kopell and Ermentrout, 1988; Cohen et al., 1992; Matsushima and Grillner, 1992; Ermentrout and Kopell, 1994a,b; Skinner and Mulloney, 1998; Varkonyi et al., 2008). This network was composed of a sequence of endogenously bursting model neurons (parameters taken from region II in Fig 2.1). We connected these cells into a chain of coupled oscillators, where each node in the chain was an endogenously bursting neuron. We numbered these cells from anterior to posterior according to the segments in the *Drosophila*, such that the most anterior neuron corresponded to the third segment, and the most posterior neuron corresponded to the seventh segment. Coupling was accomplished through inhibitory synapses. The nearest of the posterior cells provided the strongest synaptic input. The synaptic input of the anterior cell was weaker than any of those from posterior cells. The total synaptic current onto cell $j$ was aggregated into the term $I_{syn,j}$, and the current balance equation became:

$$C \frac{dV_j}{dt} = -[I_{Na,j} + I_{K2,j} + I_{h,j} + I_{leak,j} + 0.006 + I_{syn,j}]$$

We defined $I_{syn,j}$ as the sum of the synaptic currents from each presynaptic cell $i \in [3, 7]$:

$$I_{syn,j} = \sum_{i=3}^{7} g_{syn,i,j} s_{i,j} [V_j - E_{syn}]$$
where $g_{\text{syn},i,j} =$
\[
\begin{cases} 
0.03 & i - j = -1 \\
3 & i - j = 1 \\
0.3 & i - j = 2 \\
0.3 & i - j = 3 \\
0 & \text{otherwise}
\end{cases}
\]

and \[ \frac{ds_{i,j}}{dt} = \left[ \frac{1}{1 + \exp(-5000(V_i - 0.02))} - s_{i,j} \right] / 0.01. \]

For Figure 2.7, the synaptic reversal potential was $E_{\text{syn}} = -0.0625$ V. Initial conditions for each cell in the network were almost synchronous. The coordinates were taken from the minimum between the second and third spike in periodic bursting activity. To slightly disturb the synchronous initial conditions, perturbations of $10^{-8}$ were added to $V$ in odd cells. The parameters used to control the cycle period and duty cycle were $\theta_K^2$ and $\theta_h$ (Table 2.3).

We performed numerical integration using the 8-9 order Prince-Dormand method from the GNU Scientific Library (http://www.gnu.org/software/gsl/). The continuation of stationary states, periodic orbits, and most bifurcations was performed using CONTENT (Khibnik et al., 1993b). The continuation of the saddle-node bifurcation for periodic orbits was performed using XPPAUT (Ermentrout, 2004b).

Figure 2.1 A describes the temporal characteristics of bursting activity at 4294 different parameter values. We sampled the activity on a grid for values of $\theta_K^2$ from $-0.01054$ V to $-0.00602$ V in steps of $0.00004$ V and for values of $\theta_h$ from $0.0375$ V to $0.0413$ V in steps of $0.0001$ V.

We analyzed the activity of all trajectories with custom-made scripts in MATLAB (The Mathworks, Inc.). We computed burst duration, interburst interval, cycle period, and duty cycle. Burst duration is the time from the first spike in a burst to the last spike in a burst. Interburst interval is the time from the last spike in a burst to the first spike in the next burst.
burst. Cycle period is the time from the first spike in a burst to the first spike in the next burst. Duty cycle is burst duration divided by cycle period.

In Figure 2.2 and Figure 2.3, to obtain curve fits to a set of data, we used a Trust-Region optimization routine available in MATLAB. While fitting the expression Eq. 2.1, the coefficients $b$, $c$, and $d$ were varied by the optimization routine. While fitting the expression Eq. 2.2, the coefficients $b$ and $c$ were varied by the optimization routine. The value for $\theta_0^0$ determined in CONTENT and was fixed during optimization. The parameters TolFun and TolX were each $10^{-9}$.

In Figure 2.5 and Figure 2.6, we depict manifolds of slow motion for simple periodic orbits. To identify the blue sky catastrophe, we compared the average coordinate of each orbit to the average value of the nullcline of $m_{K2}$ for each orbit on $M_o$. The average coordinate of each orbit was $(\langle m_{K2} \rangle, \langle V \rangle)$ where $\langle V \rangle = \frac{1}{T} \int_0^T V \, dt$ and $\langle m_{K2} \rangle = \frac{1}{T} \int_0^T m_{K2} \, dt$. The average value of the nullcline was $(\langle V \rangle, \langle m'_{K2} \rangle = 0)$ where $\langle m'_{K2} \rangle = 0$ is defined by $\langle m_{K2,\infty}(\theta^*_{K2}) \rangle = \frac{1}{T} \int_0^T m_{K2,\infty}(V, \theta^*_{K2}) \, dt$. These integrals were computed numerically using the trapezoidal technique with custom-made scripts in MATLAB.

2.6 Figure Legends
Figure (2.1). **Tonic spiking, bursting, and silence are mapped onto the \((\theta_{K2}, \theta_h)\) bifurcation diagram.** (A) Tonic spiking, bursting, silence, and multistability of tonic spiking and silence are supported in the corresponding parameter regions labeled I, II, III, and IV. Bursting is described by duty cycle, which is the ratio of burst duration to cycle period. Duty cycle is represented as a color map from 0 to 100 %. The three empty red circles mark sample parameters sets with duty cycle 10 %. The grey curve indicates the position of the saddle-node bifurcation for periodic orbits. The black curve indicates the position of the saddle-node bifurcation for equilibria. Examples of waveforms of bursting activity at four different parameter sets \((\theta_{K2}, \theta_h)\): (B) \((-0.0105 \text{ V}, 0.0413564925 \text{ V})\), (C) \((-0.0075 \text{ V}, 0.041326 \text{ V})\), (D) \((-0.0075 \text{ V}, 0.038 \text{ V})\), and (E) \((-0.0105 \text{ V}, 0.038 \text{ V})\). The point at (B) is near but not on the codimension-2 bifurcation point.
Interburst interval and burst duration are scaled according to saddle-node bifurcations. Graphs are plotted in the log-log scale. The interburst interval and burst duration are depicted as blue dots. The curves fitted to these data are depicted as red curves. Curve fits for the interburst interval took the form \( b/\sqrt{\theta^0_h - \theta_h} + c \). (A-B) The two examples provided here were computed at fixed values for \( \theta_{K2} \) of -0.010 V (A) and -0.009 V (B) in order to demonstrate that these inverse-square-root laws were general rather than local properties. (A) Coefficients \( b = 0.00687614 \) and \( c = 15.85933790 \). The parameter \( \theta^0_h \) was 0.0413523801025906. (B) Coefficients \( b = 0.00652804 \) and \( c = 15.10833261 \). The parameter \( \theta^0_h \) was 0.0413430845706376. (C-D) These examples were provided at values for \( \theta_h \) of 0.040 V (C) and 0.039 V (D). Curve fits for burst duration took the form \( b/\sqrt{\theta_{K2} + d} + c \). (C) Coefficients \( b = 0.97192591 \), \( c = -11.80755281 \), and \( d = 0.01050511 \). (D) Coefficients \( b = 0.97039764 \), \( c = -15.81396058 \), and \( d = 0.01050462 \).
Figure (2.3). The inverse-square-root laws in transient responses triggered by a pulse of current for parameter values that support silent (region III) and tonic spiking (region I) regimes. (A-D) An individual burst was triggered by a hyperpolarizing pulse of injected current. Pulses of current were 0.03 s in duration and 0.1 nA in amplitude. The duration of individual bursts were (A) \((\theta_{K2} = -0.0077 V, \theta_h = 0.0415 V)\) 10.327403 s, (B) \((\theta_{K2} = -0.01043 V, \theta_h = 0.0415 V)\) 103.48097 s, and (C) \((\theta_{K2} = -0.010496 V, \theta_h = 0.0415 V)\) 309.27622 s. (D) The log-log graph of burst duration of individual bursts plotted against \(\theta_{K2}\). The blue dots correspond to the burst duration measured at the respective values of \(\theta_{K2}\). The red curve is the graph of the curve fitted in the form \(b/\sqrt{\theta_{K2}} + d + c\). Coefficients of the curve fit are \(b = 0.97239439\), \(c = -8.48809474\), and \(d = 0.01050536\). (E-H) Latency to spiking was shown by administering an individual pulse of injected current. Pulses were 0.03 s in duration and 0.2 nA in amplitude. The delays shown here are (E) \((\theta_{K2} = -0.0107 V, \theta_h = 0.04134 V)\) 10.287 s, (F) \((\theta_{K2} = -0.0107 V, \theta_h = 0.041358041 V)\) 103.378 s, and (G) \((\theta_{K2} = -0.0107 V, \theta_h = 0.0413580468 V)\) 317.679 s. (H) Latency to spiking was by administering an individual pulse of injected current. Pulses were 0.03 s in duration and 0.2 nA in amplitude. The delays shown here are (E) \((\theta_{K2} = -0.0107 V, \theta_h = 0.04134 V)\) 10.287 s, (F) \((\theta_{K2} = -0.0107 V, \theta_h = 0.041358041 V)\) 103.378 s, and (G) \((\theta_{K2} = -0.0107 V, \theta_h = 0.0413580468 V)\) 317.679 s. (H) Latency to spiking was plotted in log-log scale against the sampled values of \(\theta_{K2}\). Coefficients of the fitted curve were \(b = 0.00709613\) and \(c = 14.47354286\). The parameter \(\theta_h^0\) was 0.04135804734566 V.
Figure (2.4). The dependence of equilibria and periodic orbits on the parameter $\theta_{K2}$. For each orbit, we plot the maximum, minimum, and average voltage. The green curves represent the evolution of a stable orbit as $\theta_{K2}$ is varied. This stable orbit coalesced with a saddle orbit at a saddle-node bifurcation for periodic orbits ($SNo_1$). We back-traced this saddle orbit (dashed light blue curves) between $SNo_1$ at $\theta_{K2} = -0.010500$ V and a second saddle-node bifurcation for periodic orbits ($SNo_2$) at $\theta_{K2} = -0.013027$ V where it coalesced with a stable orbit (solid orange curves). This orbit lost stability in a period doubling bifurcation ($PD$) at $\theta_{K2} = -0.011948$ V. The saddle orbit (dashed dark blue) terminated in a homoclinic bifurcation (Hom). The purple curve represents the equilibria states of the system. The solid purple component indicates a stable equilibrium. The stable equilibria coalesced with the saddle equilibrium ($SNe_1$) in a saddle-node bifurcation at $\theta_{K2} = -0.010506$ V, and this saddle equilibrium coalesces with another saddle equilibrium in a saddle-saddle bifurcation at the point labeled $SNe_2$ at $\theta_{K2} = 0.029936$ V.
Figure (2.5). **Structure of the manifolds of slow motion.** (A) The slow motion manifolds for parameter values of both the SNIC and the blue sky catastrophe calculated at $\theta_h^*$. The stable and unstable portions of the slow motion manifold for oscillations are represented by $M^s_o$ and $M^u_o$, respectively in green and blue. The manifold is composed of many orbits calculated for different values of $\theta_{K2}$ (see Fig 2.4). The average voltage is plotted against the average slow variable for each orbit in dark green ($\langle V \rangle$). The average nullcline of the slow variable is plotted in orange ($\langle m'_{K2} \rangle = 0$). The nullcline for the slow variable is represented by the grey curve $m'_{K2} = 0$, and the equilibrium state for the fast subsystem is the purple curve $M_{eq}$. The saddle-node orbit is the closed orange curve labeled as $SNo_1$. The saddle-node equilibrium is the green dot labeled as $SNc_1$. (B) $M_o$ and $M_{eq}$ are calculated at $\theta_h = 0.038 \, V$. The closed orange curve is a sample periodic burst computed at $\theta_{K2} = -0.0105 \, V$ and $\theta_h = 0.038 \, V$. The trajectory of bursting closely follows the manifolds of slow motion.
Figure (2.6). **Diagram of the cornerstone bifurcation (CS).** Caption continued on next page.
Figure (2.6). **Diagram of the cornerstone bifurcation (CS).** (A) The CS is located at the intersection of the saddle-node bifurcation for equilibria ($SNe_1$; red curve) and the saddle-node bifurcation for periodic orbits ($SNo_1$; solid blue curve). The dashed blue curve is $SNo_2$, where a large amplitude stable orbit is born. The solid green curve is a period doubling bifurcation. A series of period doubling bifurcations occur between this curve and the dashed green curve, where the large amplitude regime terminates. For values of $\theta_{K2}$ larger than where this regime terminates, we consider four adjacent regions of the parameter space. In the region marked $M$, we observe only a small amplitude orbit, which corresponds to tonic spiking. In $B1$, a large amplitude orbit co-exists with the small amplitude orbit. In $C1$, the large amplitude orbit becomes chaotic and vanishes in a period doubling cascade. In $B2$, the tonic spiking regime co-exists with a stable equilibrium. In $T$, the small amplitude orbit and the stable equilibrium co-exist with a large amplitude orbit. In $C2$, the large amplitude orbit becomes chaotic and vanishes in a period doubling cascade. (B-F) Representations of the dynamics of the system at different points in the parameter space. The orange curves represent trajectories, and the black arrows indicate the direction of motion of the phase point. The two sets of light green and blue curves represent the maximum and minimum of orbits on the slow motion manifolds for oscillations. The green and blue portions indicate the attracting and repelling segments of this manifold, respectively. The solid and dashed purple curves correspond to stable and unstable equilibria in the fast subsystem, respectively. Filled red dots represent stable equilibria, and unfilled red dots represent unstable equilibria. Solid and dashed vertical dark green lines represent stable and unstable simple periodic orbits, respectively. (B) The structure of the state space at the CS point. A saddle-node periodic orbit exists on the slow motion manifold for oscillations, and a saddle-node equilibrium exists on the slow motion manifold corresponding to the equilibria of the fast subsystem. (C) A stable periodic orbit and a saddle periodic orbit exist on the slow motion manifold for oscillations. (D) Periodic bursting is observed. The phase point moves as indicated by the black arrows in a clockwise fashion. (E) A stable equilibrium of the full system obstructs the stable segment of the equilibria of the fast subsystem. (F) Spiking co-exists with the silent regime.
Figure (2.7). Scaling of the metachronal-wave pattern in chains of coupled endogenously bursting neurons. Neurons connected through inhibitory coupling with strongest connections to the immediate anterior neighbor. Metachronal Waves produced by three examples of the network. The parameter values for \((\theta_{K_2}, \theta_h)\) used to create these trajectories were as follows: (A) \((-0.0069999 V, 0.041319316864014 V)\), (B) \((-0.0040999 V, 0.041268055725098 V)\), and (C) \((0.005905 V, 0.04073603515625 V)\).
Figure (2.8). Temporal characteristics of metachronal wave pattern across a range of cycle periods. The phase relations of a metachronal-wave pattern are determined by the duty cycle of each element in a chain of inhibitory coupled bursting neurons. The parameters are changed in a coordinated fashion to support different cycle periods while the duty cycle is kept constant. (A) The phases of oscillators are portrayed relative to the oscillator in the seventh segment. (B) The average number of spikes per burst varies linearly with cycle period as $\theta_{K2}$ and $\theta_h$ are changed. The black markers indicated average spike number with error bars. The grey line was the linear function for spike number fitted to our data: $0.49(cycle\, period) + 0.93$. 
Figure (2.9). Connectivity of insect walking central pattern generator. Abbreviations indicate the identity of individual interneurons: PP, PR, MsP, MsR, MtP, and MtR are short for prothoracic protraction, prothoracic retraction, mesothoracic protraction, mesothoracic retraction, metathoracic protraction, and metathoracic retraction. Interneurons responsible for protraction are colored orange, and interneurons responsible for retraction are colored purple. The synaptic weights are indicated by the size of the circle on the synaptic connection; from small to large, synaptic conductances are 0.1 nS, 0.15 nS, and 3 nS (Table 2.5).
Figure (2.10). **Activity of segmental oscillator** Interneurons responsible for protraction are colored orange, and interneurons responsible for retraction are colored purple. The parameter $\theta_{K2}$ in the retractor interneurons took values (A) -0.01 V, (B) -0.007 V, and (C) -0.004 V. The parameter $\theta_h$ in protractor and retractor interneurons took values 0.041 V and 0.0414 V, respectively.
Figure (2.11). **Temporal characteristics of CPG interneurons as function of retrac-tor $\theta_{K2}$** Abbreviations indicate the identity of individual interneurons: PP, PR, MsP, MsR, MtP, and MtR are short for prothoracic protraction, prothoracic retraction, mesothoracic protraction, mesothoracic retraction, metathoracic protraction, and metathoracic retraction. The activity of interneurons controlling protraction are colored orange, and the activity of interneurons controlling retraction is colored purple. Vertical dashed grey lines correspond to simulations depicted in Figure 2.12. (A) Burst duration changes linearly against cycle period in protractor interneurons, but is invariant against cycle period in retracor inter (B) Burst duration of retractor interneurons in the network is determined by $\theta_{K2}$. (C) Phase of protractor or retractor interneurons in mesothoracic and prothoracic segments to interneuron in metathoracic segment. The order of $\theta_{K2}$ parameter values for vertical dashed grey lines in (C) are as follows from left to right: -0.004 V, -0.007 V, and -0.010 V. Note that the characteristics among protractor and retractor interneurons for different segments are very similar or the same, so the markers for different segments are on top of one another.
Figure (2.12). **Activity of three coupled segmental oscillators.** Progression from metachronal to tripod gate is shown from (A) to (C). Abbreviations indicate the identity of individual interneurons: PP, MsP, and MtP are short for prothoracic protraction, mesothoracic protraction, and metathoracic protraction. The parameter $\theta_{K2}$ in the retractor interneurons took values (A) -0.01 V, (B) -0.0075 V, and (C) -0.005 V.
Table (2.1). Temporal characteristics of bursting for different parameter values in region II

<table>
<thead>
<tr>
<th>Figure 2.1:</th>
<th>(B)</th>
<th>(C)</th>
<th>(D)</th>
<th>(E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_{K2}$ ($V$):</td>
<td>-0.0105</td>
<td>-0.0075</td>
<td>-0.0075</td>
<td>-0.0105</td>
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<tr>
<td>$\theta_h$ ($V$):</td>
<td>0.0413564925</td>
<td>0.041326</td>
<td>0.038</td>
<td>0.038</td>
</tr>
<tr>
<td>burst duration (s):</td>
<td>412.0</td>
<td>9.8</td>
<td>5.4</td>
<td>488.3</td>
</tr>
<tr>
<td>interburst interval (s):</td>
<td>281.6</td>
<td>217.5</td>
<td>2.0</td>
<td>1.9</td>
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</table>

Table (2.2). Measure of burst duration and latency to spiking in response to inhibition from Figure 2.3 (A-C,E-G).

<table>
<thead>
<tr>
<th>Figure 2.3</th>
<th>$\theta_h$ ($V$)</th>
<th>$\theta_{K2}$ ($V$)</th>
<th>burst duration (s)</th>
<th>latency to spiking (s)</th>
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</thead>
<tbody>
<tr>
<td>(A)</td>
<td>0.0415</td>
<td>-0.0077</td>
<td>10.327403</td>
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</tr>
<tr>
<td>(B)</td>
<td>0.0415</td>
<td>-0.01043</td>
<td>103.48097</td>
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<tr>
<td>(C)</td>
<td>0.0415</td>
<td>-0.010496</td>
<td>309.27622</td>
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<tr>
<td>(E)</td>
<td>0.04134</td>
<td>-0.0107</td>
<td>NA</td>
<td>10.287</td>
</tr>
<tr>
<td>(F)</td>
<td>0.041358041</td>
<td>-0.0107</td>
<td>NA</td>
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<tr>
<td>(G)</td>
<td>0.0413580468</td>
<td>-0.0107</td>
<td>NA</td>
<td>317.679</td>
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</table>

Table (2.3). Cycle period, duty cycle, and values for $\theta_{K2}$ and $\theta_h$. Labeled parameter sets were used to produce activity found in Figure 2.7. All parameter sets were used to produce activity used in the analysis found in Figure 2.8. Average network cycle period and average network duty cycle each had coefficients of variation less than $2 \times 10^{-4}$ % and 4 %, respectively.

<table>
<thead>
<tr>
<th>$\theta_{K2}$ ($V$)</th>
<th>$\theta_h$ ($V$)</th>
<th>Period (s): Cell</th>
<th>Network</th>
<th>Duty Cycle (%): Cell</th>
<th>Network</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0059</td>
<td>0.040736</td>
<td>15.1</td>
<td>15.1</td>
<td>10.0</td>
<td>9.9</td>
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<td>0.0019</td>
<td>0.041048</td>
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<td>21.0</td>
<td>10.0</td>
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<tr>
<td>-0.0001</td>
<td>0.041145</td>
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<td>27.4</td>
<td>9.9</td>
<td>9.9</td>
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<td>-0.0011</td>
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<td>9.8</td>
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<tr>
<td>-0.0031</td>
<td>0.041244</td>
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<td>39.5</td>
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<td>10.4</td>
</tr>
<tr>
<td>-0.0041</td>
<td>0.041268</td>
<td>48.0</td>
<td>48.0</td>
<td>10.0</td>
<td>9.9</td>
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<tr>
<td>-0.0057</td>
<td>0.041298</td>
<td>61.8</td>
<td>61.8</td>
<td>10.0</td>
<td>10.3</td>
</tr>
<tr>
<td>-0.0061</td>
<td>0.041306</td>
<td>68.2</td>
<td>68.1</td>
<td>10.0</td>
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<tr>
<td>-0.0069</td>
<td>0.041318</td>
<td>82.3</td>
<td>82.1</td>
<td>10.0</td>
<td>10.0</td>
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<td>-0.0070</td>
<td>0.041319</td>
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<td>85.1</td>
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<td>9.9</td>
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Table (2.4). Parameter values for walking CPG.

<table>
<thead>
<tr>
<th>Interneuron Type</th>
<th>$\theta_{K2}$ ($V$)</th>
<th>$\theta_h$ ($V$)</th>
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<tr>
<td>Protractor</td>
<td>-0.004</td>
<td>0.041</td>
</tr>
<tr>
<td>Retractor</td>
<td>-0.004 to -0.01</td>
<td>0.0414</td>
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Table (2.5). Synaptic connectivity of central pattern generator for insect walking.

<table>
<thead>
<tr>
<th>Synaptic Connection</th>
<th>Synaptic Conductance (nS)</th>
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<tr>
<td>Intrasegment:</td>
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</tr>
<tr>
<td>Retractor to Protractor</td>
<td>0.15</td>
</tr>
<tr>
<td>Protractor to Retractor</td>
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<tr>
<td>Intersegment:</td>
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<tr>
<td>Prothoracic Protractor to Mesothoracic Protractor</td>
<td>0.1</td>
</tr>
<tr>
<td>Mesothoracic Protractor to Prothoracic Protractor</td>
<td>3</td>
</tr>
<tr>
<td>Mesothoracic Protractor to Metathoracic Protractor</td>
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</tr>
<tr>
<td>Metathoracic Protractor to Mesothoracic Protractor</td>
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Table (2.6). Summary of network activity.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Retractor $\theta_{K2}$ (V)</th>
<th>Period (s)</th>
<th>Burst Duration:</th>
<th>Protractor (s)</th>
<th>Retractor (s)</th>
</tr>
</thead>
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<tr>
<td>Prothoracic</td>
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<td>40.4</td>
<td></td>
<td>4.8</td>
<td>34.3</td>
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<tr>
<td>Mesothoracic</td>
<td>-0.01</td>
<td>40.4</td>
<td></td>
<td>4.8</td>
<td>34.3</td>
</tr>
<tr>
<td>Metathoracic</td>
<td>-0.01</td>
<td>40.4</td>
<td></td>
<td>4.8</td>
<td>34.3</td>
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<tr>
<td>Prothoracic</td>
<td>-0.007</td>
<td>13.9</td>
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<td>8.0</td>
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<td>Mesothoracic</td>
<td>-0.007</td>
<td>13.9</td>
<td></td>
<td>4.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Metathoracic</td>
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<td>13.9</td>
<td></td>
<td>4.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Prothoracic</td>
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<td>10.6</td>
<td></td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Mesothoracic</td>
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<td>10.6</td>
<td></td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Metathoracic</td>
<td>-0.004</td>
<td>10.6</td>
<td></td>
<td>4.4</td>
<td>4.7</td>
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</table>
PART 3

BISTABILITY OF SILENCE AND SEIZURE-LIKE BURSTING

3.1 Introduction

Robustness and multistability in oscillatory neuronal activity are controlled by the coregulation of multiple ionic currents. There is growing evidence that neuronal circuits exhibiting seizure episodes are also prone to multistability (Hahn and Durand, 2001; Foss and Milton, 2003; Suffczynski et al., 2004, 2005; Frohlich and Bazhenov, 2006; Frohlich et al., 2010; Vincent et al., 2011; Wu and Shuai, 2012; Koppert et al., 2013). Multistability is a commonplace feature of nonlinear dynamical systems. Multiple distinct regimes of activity can coexist, and short transient signals could produce long lasting changes to the activity of the system. For example, a neuron could toggle between different regimes when an appropriate perturbation is applied. State dependence or hysteresis are hallmarks of multistability (Nadim et al., 2008). Cardiac and neuronal systems have been shown to exhibit multistability (Rinzel, 1978a; Best, 1979; Guttman et al., 1980; Jalife and Antzelevitch, 1980; Lechner et al., 1996; Turrigiano et al., 1996; Cymbalyuk et al., 2002; Loewenstein et al., 2005; Le et al., 2006; Paydarfar et al., 2006; Briggman and Kristan, 2008; Newman and Butera, 2010; Malashchenko et al., 2011b,c; Dovzhenok and Kuznetsov, 2012; Wu and Shuai, 2012).

Multistability could explain why seizures suddenly start and stop (Frohlich et al., 2010). This concept gives a basis for the development of closed loop stimulation treatments (Durand and Bikson, 2001; Foss and Milton, 2003; Berenyi et al., 2012). Seizure activity can be stopped with the application of a perturbation (Durand and Bikson, 2001; Drinkenburg et al., 2003; Foss and Milton, 2003; Berenyi et al., 2012). The characteristic dynamics of seizure activity manifest on the level of individual cells, and investigation at this level is critical for understanding the cellular mechanisms of epilepsy (Bikson et al., 2003; Cressman et al., 2009; Barreto and Cressman, 2011; Pathak et al., 2010; Fransen and Tigerholm, 2010;
Krishnan and Bazhenov, 2011; Wu and Shuai, 2012; Tigerholm et al., 2012). Here we apply dynamical systems theory to address two open questions about single-cell seizure dynamics: how to determine properties of perturbations leading to switches among coexistent regimes and how to assess changes in the propensity of a neuron to multistability.

We focus on the dynamics of identified leech neurons, the leech heart interneurons (HN). They are part of the central pattern generator that controls the heart beat of the medicinal leech. The advantage of studying these neurons is the availability of a well developed biophysically realistic model (Hill et al., 2001). The HNs have been extensively studied using a combination of experimental and theoretical techniques (Hill et al., 2001; Cymbalyuk et al., 2002; Sorensen et al., 2004; Cymbalyuk and Shilnikov, 2005a; Olypher et al., 2006; Norris et al., 2007; Malashchenko et al., 2011b,c). A number of predictions of the HN model have been confirmed experimentally (Hill et al., 2001; Cymbalyuk et al., 2002; Sorensen et al., 2004; Olypher et al., 2006; Norris et al., 2007; Wright and Calabrese, 2011). Models of the HN exhibit a diverse repertoire of activity, including bistability of bursting and silence, bistability of tonic spiking and silence, bistability of bursting and tonic spiking, and bistability of different tonic spiking regimes (Cymbalyuk et al., 2002; Cymbalyuk and Shilnikov, 2005a; Malashchenko et al., 2011b,c). Here, we consider a model of the HN under pharmacological treatment with $Co^{2+}$ and 4-aminopyridine (4-AP). This pathological activity is captured in a previous model of the reduced HN (Cymbalyuk and Calabrese, 2001). Our reduced model exhibits endogenous slow-wave plateau bursts on a time course of tens of seconds. It also exhibits coexistence of this seizure-like bursting and silence. We investigated the dynamics of these activities.

We described three methods for characterizing the dynamics underlying multistability. We performed a bifurcation analysis to reveal the range of parameters in which bistability is observed, and to identify the transitions that bound those ranges. For parameter values that supported bistability, we tested properties of electrical perturbations that switched the model from bursting to silence. Finally, we demonstrated a novel technique to elucidate the roles of specific ion currents in the model’s propensity for bistability. The propensity measure
is achieved by varying the maximal conductance of each maximal current, and determining the the range of values of the conductance of the leak current for which bursting and silence coexisted.

3.2 Model

The present model is derived from a model describing the HN under the application of Co\(^{2+}\) and 4-AP (Cymbalyuk and Calabrese, 2001). We have included the hyperpolarization activated current (Angstadt and Calabrese, 1989). Our model of the HN contains five ionic currents: a fast sodium current, \(I_{Na}\), a potassium current, \(I_{K2}\), a hyperpolarization-activated current, \(I_h\), a persistent sodium current, \(I_P\), and a leak current, \(I_{leak}\). The five state variables are the membrane potential, \(V\), the inactivation of \(I_{Na}\), \(h_{Na}\), the activation of \(I_{K2}\), \(m_{K2}\), the activation of \(I_h\), \(m_h\), and the activation of \(I_P\), \(m_P\). We consider the activation of \(I_{Na}\) to be instantaneous; it is expressed as a function of \(V\). The cell is modeled as follows:

\[
CV' = -\left[\bar{g}_{Na} m_{Na,\infty}(V)^3 h_{Na} [V - E_{Na}] + \bar{g}_{P} m_{P} [V - E_{Na}] + \bar{g}_{K2} m_{K2}^2 [V - E_{K}] + \bar{g}_{h} m_{h}^2 [V - E_{h}] + g_{leak} [V - E_{leak}] + I_{inj}\right]
\]

\[
h_{Na}' = \left[\frac{1}{1+\exp(500(V+0.026))} - h_{Na}\right] / \tau_{h_{Na}}(V),
\]

\[
m_{P}' = \left[\frac{1}{1+\exp(-192(V+0.039))} - m_{P}\right] / \left[0.01 + \frac{0.2}{1+\exp(400(V+0.057))}\right],
\]

\[
m_{K2}' = \left[\frac{1}{1+\exp(-80(V+0.018))}\right] - m_{K2} / 0.25,
\]

\[
m_{h}' = \left[\frac{1}{1+2 \exp(180(V+0.047))\exp(500(V+0.047))} - m_{h}\right] / 2.1,
\]

where \(m_{Na,\infty}(V) = \frac{1}{1+\exp(-150(V+0.027))}\)

and \(\tau_{h_{Na}}(V) = 0.004 + \frac{0.006}{1+\exp(500(V+0.028))} + \frac{0.01}{\cosh(300(V+0.027))}\).

The maximal conductances of \(I_{Na}\), \(I_{K2}\), \(I_h\), and \(I_P\) were \(\bar{g}_{Na}\), \(\bar{g}_{K2}\), \(\bar{g}_{h}\), and \(\bar{g}_{P}\) respectively. The conductance of the \(I_{leak}\) was \(g_{leak}\). The reversal potentials of sodium, potassium, the hyperpolarization-activated current, and the leak current were \(E_{Na}\), \(E_K\), \(E_h\), and \(E_{leak}\). We considered the values for these parameters taken from Cymbalyuk and Calabrese (2001)
(parameters for $I_{Na}$, $I_{K2}$, $I_P$, and $I_{leak}$) and Angstadt and Calabrese (1989) (parameters for $I_h$) to be canonical: $\bar{g}_{Na} = 200$ nS, $\bar{g}_P = 6.156$ nS, $\bar{g}_{K2} = 97.1$ nS, $\bar{g}_h = 4$ nS, $g_{leak} = 6.5$ nS, $E_{Na} = 0.045$ V, $E_{K2} = -0.07$ V, $E_h = -0.021$ V, $E_{leak} = -0.058$ V, and $C = 0.5$ nF. The parameter $I_{inj}$ was 0 nA except for those times at which current was injected into the system.

3.3 Methods

We used two approaches to investigate multistability of bursting and silence in a neuronal model. First, we investigated the properties of perturbations that led to a switch from seizure-like bursting to a hyperpolarized silent state by applying square pulses of current to the neuron. This choice of perturbation was inspired by experiments demonstrating bistability in the squid giant axon (Guttman et al., 1980). We systematically varied both the phase and the amplitude of the pulse to obtain sets of pulse parameters that produce switches. We analyzed the evolution of these sets in response to changes in $g_{leak}$ and pulse duration. Second, we used a novel technique to assess the influence of each ionic current on propensity for multistability. We computed the range of $g_{leak}$ supporting bistability of seizure-like bursting and silence: the index of propensity for multistability. We computed this index over a range of values for the maximal conductance of each voltage-gated current one at a time.

We studied pulse perturbations in a system where the exhibited bursting activity was periodic, and thus, the phase of a perturbation could be strictly defined. Bursting activity was quantified by its temporal characteristics: the burst duration, the interburst interval, and the period. The burst duration was the time interval between the first and last spikes of the burst. The interburst interval was the time interval between the last spike in a burst and the first spike of the next burst. The period was the sum of these two characteristics. We used the coefficient of variation of these three characteristics to establish that bursting activity was periodic; we chose parameter sets such that the coefficient of variation of each characteristic was less than 2%. Analysis was performed on a set of 100 bursts.

Each pulse was characterized by its amplitude, duration, and the time of the onset.
Depending on these three characteristics, a pulse of injected current could switch the activity from bursting to silence. The phase was defined as the time of the pulse onset, expressed as a percentage of the period of bursting activity. The time of the first spike of the burst was the reference time (0% phase). We conducted simulations using the same initial conditions, which were obtained by integrating the system for 5000 seconds; after this time, we considered the model neuron to be settled onto the periodic bursting orbit (Fig. 3.1). From simulation to simulation, we varied the amplitude ($I_{\text{pulse}}$) and phase of the pulse. Initially, we varied $I_{\text{pulse}}$ in increments of $10^{-3}$ nA from -100 nA to 100 nA and the phase in increments of 0.1% from 0.1% to 100%. There were two observed outcomes of the stimulation protocol.

We implemented the square pulse of current using three intervals of time. The coordinates at the end of each interval were used as the initial conditions at the beginning of the following interval. First, the system was numerically integrated from initial conditions up to the time of the application of the pulse; during this time, $I_{\text{inj}}$ was 0 nA. The second interval represented the time course of the pulse, and $I_{\text{inj}}$ was equal to $I_{\text{pulse}}$. The pulse duration was 30 ms except for simulations where it was a controlling parameter. Finally, the third interval represented the post-stimulus interval; $I_{\text{inj}}$ was 0 nA, and the system was integrated for 2000 seconds.

We identified two possible outcomes based on the analysis of the activity during the final interval of time:

**Case 1:** The system settled onto the stable equilibrium. Following the perturbation, there were sometimes one or more subthreshold oscillations before the system settled onto the rest state.

**Case 2:** The system returned to the bursting regime. Following the perturbation, one or more subthreshold oscillations were observed before the system returned to bursting.

As a result, we obtained sets of phase and $I_{\text{pulse}}$ for which we recorded a switch from bursting to silence. These sets of contiguous pulse parameters were called windows (Fig. 3.2). A window was defined in terms of phase as an interval within which a pulse could produce a
switch. For a given phase in a window, pulses with amplitudes falling between two threshold amplitudes would trigger a switch. We characterised the shape of the windows in terms of the amplitude of switch-triggering pulses by the maximal window span. We defined the window span as the difference between the two threshold values for a given phase in a window.

We investigated how the shape of the windows evolved in response to changes in either \( g_{\text{leak}} \) or pulse duration. For certain of these parameter values, the maximal span of the windows diminished, and a better resolution of the simulation grid was required. After initial screening, the detected windows were visually evaluated, and if the increment of \( I_{\text{pulse}} \) was 10\% or more of the window’s height, we decreased the increments of \( I_{\text{pulse}} \) to \( 10^{-4} \) nA for that window (Figs. 3.3,3.4). Moreover, for Figure 3.4B, we used increments of \( 10^{-5} \) nA for pulse durations greater than 300 ms.

We used custom written codes in the C programming language using the GNU Scientific Library (http://www.gnu.org/software/gsl/). Numerical simulations were performed with a variable step Runge-Kutta integrator with absolute and relative tolerances set to \( 10^{-9} \). Trajectories were generated and analyzed on the OCTANS research computing cluster at Georgia State University.

To characterize the roles of different ionic currents, we introduce a new measure of the propensity of a neuron to multistability. The index of propensity for multistability is the range of \( g_{\text{leak}} \) where multistability was observed. We determined this range by comparing the range where the model exhibited bursting to the range where the model exhibited a hyperpolarized rest state. Generally, the model was silent for large \( g_{\text{leak}} \). We used single parameter (\( g_{\text{leak}} \)) bifurcation analysis to determine the value at which the equilibrium lost stability. We tested for all codimension-1 bifurcations of equilibria. For some range of \( g_{\text{leak}} \), the model exhibited bursting. Typically, the maximal \( g_{\text{leak}} \) for which the system exhibited bursting was larger than the minimal \( g_{\text{leak}} \) for which the system exhibited silence. This overlap in the values of \( g_{\text{leak}} \) determines the range of \( g_{\text{leak}} \) for which the model supports bistability. This one-parameter analysis was extended to two-parameters to determine the dependence of the propensity index on the maximal conductance of each voltage-gated ionic
current one at a time. We repeated this two-parameter analysis for the maximal conductance of each voltage-gated current.

The minimal $g_{\text{leak}}$ supporting the stable equilibrium was computed by continuation of equilibria in CONTENT (Khibnik et al., 1993a). The first point on the bifurcation curve was obtained by setting $g_{\text{leak}}$ to 40 nS, where the system was silent. This rest state was continued until it lost stability in an Andronov-Hopf bifurcation. The Andronov-Hopf bifurcation was then continued in two parameters: $g_{\text{leak}}$ and the maximal conductance of a voltage gated ionic current. We produced a two-dimensional bifurcation diagram for the maximal conductance of each voltage gated current.

The critical value of $g_{\text{leak}}$ where the bursting regime terminated was found using an iterative routine with numerical simulations of the model done in CONTENT. At each iteration, we updated two estimates for this critical value of $g_{\text{leak}}$. The lower estimate, $g_{\text{leak}}^-$, was initially given a value where bursting was observed. The upper estimate, $g_{\text{leak}}^+$, was assigned a value where no bursting regime was observed. A trial value of $g_{\text{leak}}$, $g_{\text{leak}}^0$, was selected on the interval between $g_{\text{leak}}^-$ and $g_{\text{leak}}^+$. We integrated the system at $g_{\text{leak}}^0$ for 500 s. If bursting was observed over this interval, $g_{\text{leak}}^-$ was updated to $g_{\text{leak}}^0$, and the coordinates at the end of the trajectory were saved to be used as the initial conditions for the following iteration. If bursting was not observed or did not persist over the entire interval, $g_{\text{leak}}^+$ was updated to $g_{\text{leak}}^0$, and the initial conditions were not updated. We iterated this procedure while the difference between $g_{\text{leak}}^-$ and $g_{\text{leak}}^+$ was greater than $10^{-3}$. The value reported as the border of the transition from bursting to silence was the final value of $g_{\text{leak}}$. The entire procedure was repeated over a range of values for each of the maximal conductances of each voltage gated current.

One- and two-parameter bifurcation analyses of equilibria and oscillatory regimes were done either in XPPAUT (Ermentrout, 2004a) or CONTENT where the tolerance parameters for continuation were $10^{-8}$. We used MATLAB (The MathWorks, Inc.) to make figures for this manuscript. The trajectories generated for these figures were produced with the ode15s integrator with an absolute tolerance of $10^{-11}$ and a relative tolerance of $10^{-10}$. 
Figure (3.1). Bursting activity of the model for $g_{\text{leak}} = 8.79$ nS. (A, B) Activity is projected on to the hyperplane $(V, m_{K2}, m_h)$. (A) The stable equilibrium and one of the saddle equilibria are depicted as solid green and empty red points respectively. (B) A close up of the saddle orbit and the stable equilibria. (C) Bursting activity plotted as $V$ versus time. The dashed line is the voltage of the stable equilibria ($V_{\text{st}} = -0.0494$ V).

3.4 Results

3.4.1 Pulse-Triggered Switch

We investigated a model which exhibited coexistence of a periodic bursting regime and a hyperpolarized equilibrium (Fig. 3.1). At $g_{\text{leak}} = 8.79$ nS, the average period of activity was 5.7 s ($\sigma = 7.2 \times 10^{-4}$ s), the average burst duration was 1.8 s ($\sigma = 7.2 \times 10^{-4}$ s), and the average interburst interval was 3.9 s ($\sigma = 1.6 \times 10^{-3}$ s) (Fig. 3.1C). The hyperpolarized equilibrium had a resting potential of -0.0494 V (Fig. 3.1A). A numerical analysis of equilibria and simple oscillatory regimes revealed saddle equilibria at -0.0449 V and -0.0229 V and a saddle orbit nearby the stable equilibrium (Fig. 3.1B). We proceeded with the analysis of this system using techniques developed by Malashchenko et al. (2011b).

A single parameter bifurcation analysis (not shown) indicated that the stable rest state
associated with bistability gained stability at a subcritical Andronov-Hopf bifurcation ($g_{\text{leak}} = 8.778 \text{nS}$). At this bifurcation, an unstable equilibrium became stable, and a saddle orbit was born with frequency $2.34 \text{ rad/s}$. In the vicinity of the bifurcation, the amplitude of the orbit grew sharply. The bursting regime disappeared at $g_{\text{leak}} = 8.797 \text{nS}$. The bursting and silent regimes coexisted between these values. By perturbing regular bursting activity with square pulses of current (Methods), we found contiguous sets of pulse parameters that switch the neuron from bursting to silence, referred to here as windows.

We investigated two bistable systems: one relatively close to the Andronov-Hopf bifurcation ($g_{\text{leak}} = 8.78 \text{nS}$) and the other relatively close to the termination of the bursting regime ($g_{\text{leak}} = 8.79 \text{nS}$). At $g_{\text{leak}} = 8.78 \text{nS}$, the windows computed in the (phase, $I_{\text{pulse}}$) parameter space were smaller compared to those obtained at $g_{\text{leak}} = 8.79 \text{nS}$ (Fig. 3.2). For each of these two values of $g_{\text{leak}}$, we found that there were two windows: an earlier window that was a set of depolarizing current pulses and a later window that was a set of hyperpolarizing current pulses. For $g_{\text{leak}} = 8.78 \text{nS}$, the ranges of the phase and amplitude of depolarizing pulses were from 59.1% to 63.0% and from 0.022 nA to 0.043 nA (Fig. 3.2A). The ranges of the phase and amplitude for hyperpolarizing pulses were from 81.6% to 85.9% and from -0.014 nA to -0.025 nA, respectively. For $g_{\text{leak}} = 8.79 \text{nS}$, the two windows were larger (Fig. 3.2B). The ranges of the phase and amplitude for depolarizing pulses were from 48.7% to 61.4% and from 0.006 nA to 0.076 nA, respectively. The ranges of the phase and amplitude for hyperpolarizing pulses were from 70.2% to 84.3% and from -0.004 nA to -0.035 nA. Both the amplitude of the saddle orbit in the state space and the size of the windows in the (phase, $I_{\text{pulse}}$) parameter space grew as $g_{\text{leak}}$ increased from the Andronov-Hopf bifurcation. These results support the notion that the stable manifold of the saddle orbit creates a separatrix between the two basins of attraction.

To further investigate the relation of the windows in the (phase, $I_{\text{pulse}}$) parameter space to the saddle orbit, we examined the geometry of each window as $g_{\text{leak}}$ changed (Fig. 3.3). Each window was roughly oval in shape. Close to the Andronov-Hopf bifurcation, each of the two windows were quite small, and as the value of $g_{\text{leak}}$ increased, the size of each of the
Figure (3.2). The shaded regions (windows) represent sets of parameters (phase and amplitude) for a single current pulse able to switch bursting activity to silence. All pulses were square in shape and 0.03 s in duration. The pair of windows found for $g_{\text{leak}} = 8.78 \text{ nS}$ (A) were smaller than those found for $g_{\text{leak}} = 8.79 \text{ nS}$ (B).

two windows increased (Fig. 3.3A). Moreover, the span of each window in phase increased as $g_{\text{leak}}$ increased.

At the occurrence of an Andronov-Hopf bifurcation, two complex-conjugate eigenvalues cross the imaginary axis. An orbit is born at this critical value of $g_{\text{leak}}$. The eigenvalues take the form $1/\tau \pm i\omega$, where $\tau$ characterizes the time constant of relaxation nearby the equilibrium and $\omega$ characterizes the frequency of oscillations of the nascent orbit. The amplitude of this orbit is zero at the bifurcation, and it grows proportionally to $\sqrt{g_{\text{leak}} - b}$, where $b$ is the bifurcation value (Izhikevich, 2007a). The window of depolarizing pulses and the window of hyperpolarizing pulses represent maps of the basin of attraction of the stable equilibrium into the (phase, $I_{\text{pulse}}$) parameter space.
We quantified the size of each window by finding the maximal span. Our analysis shows that the amplitude of the saddle orbit and the maximal span of both windows scaled proportionally to $\sqrt{g_{\text{leak}} - b}$. The span of each window was compared to the amplitude of the saddle orbit. We used a curve-fitting MATLAB routine and found that each of these three curves was of form $a \times \sqrt{g_{\text{leak}} - b}$ (Fig. 3.4A). The coefficients $a$ and $b$ were determined with a Trust-Region algorithm for each of the three data sets. In all three cases, the coefficient $b$ was approximately 8.7787 nS. We denote different subscripts for the window of depolarizing pulses, the window of hyperpolarizing pulses, and the amplitude of the saddle orbit: $a_+$, $a_o$, and $a_x$. The value of the coefficients for these three data sets were as follows: $a_+ = 0.4860$ nA, $a_o = 0.2785$ nA, and $a_x = 0.0180$ V. The span of each window and the amplitude of the saddle orbit were normalized to their values at $g_{\text{leak}} = 8.79$ nS for comparison (Fig. 3.4A). These windows allowed us to probe the unstable orbit in the vicinity of the bursting trajectory. Note that the dependence of the maximal span for hyperpolarizing pulses and the dependence of the amplitude of the saddle orbit were well matched. However, the dependence of maximal span for depolarizing pulses underestimated that of the amplitude of the saddle orbit. We speculate that the window of hyperpolarizing pulses represented a better map because the phase interval of the periodic bursting trajectory corresponding to this window appeared closer in proximity to the saddle orbit than did the phase interval corresponding to the window of depolarizing pulses (Fig. 3.5). Since all three curves are well fitted by the same approximation as the theoretical dependence imposed by subcritical Andronov-Hopf bifurcation, we conclude that the saddle periodic orbit generates the mechanism separating the attraction basins for the bursting regime and hyperpolarized stable equilibrium. The most parsimonious explanation for the mechanism of this bistability is that the stable manifold of this orbit is the separatrix between the two attracting regimes.

To explore the effect of pulse duration on window shape, we fixed $g_{\text{leak}}$ to 8.79 and repeated our pulse protocol for different pulse durations. We varied the pulse duration from 0.01 s to 1 s (Fig. 3.4B). Generally, windows computed with shorter pulses of current were larger, and windows computed with longer pulses of current were smaller and slightly shifted.
in phase towards the beginning of the burst. For a pulse duration of 0.01 s, the window of depolarizing pulses spanned from 0.018 nA to 0.219 nA and from 48.8% to 61.6%, and the window of hyperpolarizing pulses spanned from -0.012 nA to -0.109 nA and from 70.4% to 84.5%. For a pulse duration of 0.4 s, the window of depolarizing pulses spanned from 0.00051 nA to 0.00699 nA and from 44.6% to 57.9%, and the window of hyperpolarizing pulses spanned from -0.00029 nA to -0.00238 nA and from 67% to 81.3%. In general, longer pulses of injected current require smaller amplitude to trigger a switch. However, shorter pulses of injected current are effective over a greater range of pulse amplitudes.

The minimal and maximal threshold amplitudes evolved as the duration of the pulse changed (Fig. 3.4B). These thresholds decreased dramatically as pulse duration increased from 0.01 s to 1 s. The minimal and maximal amplitude thresholds of the window of hyperpolarizing pulses decreased from 0.1090 nA to 0.0010 nA and from 0.0120 nA to 0.0001 nA, respectively (Fig. 3.4B). The minimal and maximal amplitude thresholds of the window of depolarizing pulses decreased from 0.0180 nA to 0.0003 nA and from 0.2190 nA to 0.0057 nA, respectively (Fig. 3.4B).

We projected these windows (for $g_{\text{leak}} = 8.79$ nS) onto two time series: first was a case where a pulse triggered a switch (Fig. 3.5A) and second was a case where a pulse did not (Fig. 3.5B). If a pulse was applied with the appropriate timing and amplitude to trigger a switch, its onset was inside a window. For example, the onset of a depolarizing pulse that was of appropriate phase and amplitude was inside the upper window (Fig. 3.5A). In contrast, a hyperpolarizing pulse that missed a window did not produce a switch (Fig. 3.5B).

The windows of pulse parameters that switch from bursting to silence could be examined through perturbations to the bursting trajectory (Fig. 3.5C, 3.5D) We projected these two trajectories on the $(V, m_K^2, m_h)$ hyperplane. Bursting trajectories passed nearby the saddle orbit (Fig. 3.1B, 3.5C, 3.5D). Pulses of current that did produce a switch were often followed by damped oscillations as the system settled onto the stable rest state (Fig. 3.5C). These damped oscillations manifested in the $(V, m_K^2, m_h)$ hyperplane as a spiral onto a stable focus (Fig. 3.5C). Pulses of current that did not produce a switch often produced some
Figure (3.3). The evolution of window shape as a result of change in $g_{\text{leak}}$ (A) and pulse duration (B). The shaded regions represent sets of pulse parameters (phase and amplitude) for a single current pulse able to switch bursting activity to silence. The color maps indicate (A) the value of $g_{\text{leak}}$ and (B) the duration of the pulse ($t_{\text{dur}}$) for which each window was computed.
Figure (3.4). The dependencies of the maximal span (A) and the window thresholds (B) on $g_{\text{leak}}$ and pulse duration, respectively. (A) The maximal span and the amplitude of the saddle orbit grew proportionally to $a * \sqrt{g_{\text{leak}} - b}$. The maximal span for depolarizing pulses and hyperpolarizing pulses are marked by green + and blue ◦ symbols, respectively. The amplitude of the saddle orbit is the set of red × symbols. The solid green, blue, and red curve are curve fits of the maximal span for the window of depolarizing pulses, the maximal span for the window of hyperpolarizing pulses, and the amplitude of the saddle orbit each to $a * \sqrt{g_{\text{leak}} - b}$. The size of each window and the amplitude of the saddle orbit was normalized to their respective values at $g_{\text{leak}} = 8.79$. (B) The threshold amplitudes—the minimum and maximum threshold of pulses that triggered a switch—decreased as pulse duration increased. The thresholds are plotted in the log scale. The green and blue curves correspond to depolarizing and hyperpolarizing pulses, respectively. Solid curves and dashed curves indicate maximal and minimal thresholds, respectively.
Figure (3.5). Examples of perturbation of bursting activity with mapped windows. The shaded areas (windows, Fig. 3.2B) indicate properties of pulses which produced a switch from bursting to silence. (A) A pulse of current with amplitude 0.02 nA switched the activity to silence. The rest potential ($V_{st} = -0.0494$ V) represents the stable equilibrium (black dashed line). (B) A pulse of current with amplitude -0.015 did not switch the activity to silence. (C, D) A pulse triggered switch from bursting to silence can only be achieved if it was delivered within the appropriate range of phases (red between the X or the + symbols). The dark blue curve is a trajectory of the bursting orbit. The duration of the pulse of $I_{inj}$ is indicated by the pink. The light blue curve indicates the trajectory after the pulse. The purple curve is a periodic saddle orbit, and the stable equilibrium is represented by the black point. (C) and (D) correspond to (A) and (B) respectively.
transient activity before the system settled onto the bursting attractor. For example, the membrane potential would sometimes make one or more subthreshold oscillations before returning to bursting activity (Fig. 3.5B, 3.5D). Subthreshold oscillations appeared as loops around or nearby the saddle orbit in the \((V, m_K, m_h)\) hyperplane.

### 3.4.2 Range of Bistability

To assess the influences of each current on the propensity of the neuron to multistability, we systematically varied the maximal conductances of each of the voltage gated currents \((\bar{g}_h, \bar{g}_{K2}, \bar{g}_{Na}, \text{and} \bar{g}_P)\), and for each sampled value of each of these conductances, we identified the values of \(g_{\text{leak}}\) over which the system exhibited bistability. The index of propensity for multistability was defined as the difference between the value in \(g_{\text{leak}}\) for a subcritical Andronov-Hopf bifurcation (AH) and the critical value at which bursting activity disappears.

The curves indicating the AH and the transition from bursting to silence (BtS) are depicted in Figure 3.6. Consider, for example, Figure 3.6A. We observed bursting activity for \(g_{\text{leak}}\) to the left of the BtS curve, and a rest state for \(g_{\text{leak}}\) to the right of the AH curve. The range of observed bistability occupies the region on the diagram between the two curves.

We created a series of two-parameter diagrams with \(g_{\text{leak}}\) and each of the maximal conductance of the voltage gated currents (Fig. 3.6A). In each two-parameter analysis, we compared the dependence on \(g_{\text{leak}}\) of the BtS transition and the AH bifurcation to compute the index of propensity over a range of values of each maximal conductance of voltage gated currents. In most cases, we varied each of these conductances such that the index of propensity increased to roughly 0.3 to 0.5 nS (Table 3.1). The range of bistability for the canonical values of these conductances was 0.016 nS. We varied \(\bar{g}_h\) from 0.5 to 20 nS (Fig. 3.6A). The index of propensity increased as \(\bar{g}_h\) increased, and its maximum value was 0.43 nS. The parameter \(\bar{g}_{K2}\) was varied from 90 to 200 nS (Fig. 3.6B). The index of propensity generally increased as \(g_{K2}\) increased; its maximal value was 0.49 nS. Under variation of \(\bar{g}_{Na}\), the index of propensity did not greatly increase; its maximal value was 0.04 nS (Fig. 3.6C). The range of bistability increased as \(\bar{g}_P\) diminished in magnitude (Fig. 3.6D). We varied this
Figure (3.6). Bifurcation diagrams of equilibria and bursting determining index of propensity for multistability. The blue curve indicates an Andronov Hopf bifurcation. For parameter values to the right of this curve there exists a stable rest state. The red curve indicates the disappearance of the bursting regime. To the left of this curve we observed bursting activity. The bifurcation parameters are (A) $\bar{g}_h$, (B) $\bar{g}_{K2}$, (C) $\bar{g}_{Na}$, and (D) $\bar{g}_P$ on the vertical axes and $g_{leak}$ on each of the horizontal axes. The dotted black lines indicate the canonical value of the parameter. The range of bistability is the difference in $g_{leak}$ between the red curve and the blue curve for a particular value on the vertical axis. As either $\bar{g}_h$ (A) or $\bar{g}_{K2}$ (B) increases the range of bistability increases. (C) $\bar{g}_{Na}$ does not influence the range of bistability. (D) The range of bistability increases as $\bar{g}_P$ decreases.
Table (3.1). The index of propensity for multistability increases with changes in the maximal conductances of $I_h$, $I_{K_2}$, $I_{Na}$, and $I_P$. The maximum range of bistability corresponds to the underlined conductance values in each column.

<table>
<thead>
<tr>
<th>maximal conductance:</th>
<th>$g_h$ (nS)</th>
<th>$g_{K2}$ (nS)</th>
<th>$g_{Na}$ (nS)</th>
<th>$g_P$ (nS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>canonical value:</td>
<td>4</td>
<td>97.1</td>
<td>200</td>
<td>6.156</td>
</tr>
<tr>
<td>maximal value:</td>
<td>20</td>
<td>200</td>
<td>400</td>
<td>8</td>
</tr>
<tr>
<td>minimal value:</td>
<td>0.5</td>
<td>90</td>
<td>140</td>
<td>2</td>
</tr>
<tr>
<td>max. index of propensity:</td>
<td>0.43</td>
<td>0.49</td>
<td>0.04</td>
<td>0.33</td>
</tr>
</tbody>
</table>

parameter from 2 to 8 nS, and the maximal value of the index for propensity was 0.33 nS.

Following our previous analysis (Cymbalyuk et al., 2002; Malashchenko et al., 2011b,c), we varied $E_{leak}$ from -0.07 V to -0.04 V and $g_{leak}$ from 1 to 16 nS. We observed simple oscillating regimes, bursting activity, and a depolarized and a hyperpolarized equilibria. The parameter values for which bursting existed was bounded on two sides (Fig. 3.7A). At these boundaries, the bursting regime transitioned to a qualitatively different regimes of activity.

Consider a bifurcation of the depolarized stable equilibrium. As $E_{leak}$ decreased, this equilibrium lost stability in a supercritical Andronov-Hopf bifurcation, and a stable orbit was born ($AH_2$). This orbit corresponds to the small amplitude tonic spiking regime. A saddle-node bifurcation for periodic orbits occurs at the parameter value for which this stable orbit and a saddle orbit coalesce and vanish ($SNo_1$). The XPPAUT bifurcation analysis software suite allowed us to continue the saddle orbit.

We tracked this orbit with increasing values of $E_{leak}$ until it vanished in a second saddle-node bifurcation ($SNo_2$). The stable large amplitude orbit originating at this saddle-node bifurcation continued with decreasing values of $E_{leak}$. This orbit lost stability in a period doubling cascade (initial period-doubling bifurcation at $PD$).

Consider the vertical dashed line in Figure 3.7A located at $g_{leak} = 8.79$ nS. Activities along this line make a good example of the order in which we observed different regimes of activity for values of $g_{leak}$ below 10.6 nS. On this line, bursting activity transitioned into the regime of stable oscillations at $E_{leak} = -0.0580$ V ($SNo_1$). As $E_{leak}$ continued to
increase, the stable orbit vanished at the supercritical Andronov-Hopf bifurcation at $E_{\text{leak}} = -0.0490\, V$ ($AH_2$). For values of $E_{\text{leak}}$ above that of $AH_2$, we observed the depolarized stable equilibrium. The large amplitude tonic spiking regime coexisted with depolarized quiescence between the second fold orbit at $E_{\text{leak}} = -0.0442\, V$ ($SN_{o2}$) and $E_{\text{leak}} = -0.0480\, V$ where spiking regime becomes unstable in a period doubling cascade.

The lower boundary of bursting activity was characterized by bistability of bursting and silence as described in the previous section. As $E_{\text{leak}}$ decreased, a saddle equilibrium gained stability in a subcritical Andronov-Hopf bifurcation giving rise to a saddle orbit. The upper boundary of bistability coincided with the Andronov-Hopf bifurcation, and the lower boundary of bistability was marked by the disappearance of the bursting regime.

We further investigate the range of bistability in the $(g_{\text{leak}}, E_{\text{leak}})$ parameter space by coordinating the maximal conductances the voltage gated current. We selected values for three of the maximal conductances that increased the propensity of the system to bistability: $\bar{g}_h = 20\, \text{nS}$, $\bar{g}_{K2} = 200\, \text{nS}$, $\bar{g}_p = 2\, \text{nS}$ (Table 3.1; Fig. 3.6). For these parameter values, we perform a two parameter bifurcation analysis in the $(g_{\text{leak}}, E_{\text{leak}})$ parameter space (Fig. 3.7B).

Over the range of parameter values examined in Fig. 3.7B, we observed three qualitatively different regimes of activity: monostable bursting, monostable silence, and coexistence of bursting and silence. A subcritical Andronov-Hopf bifurcation ($AH$) separates the region where bursting is the only observed regime from the region of bistability of bursting and silence. Bistability is observed for values of $g_{\text{leak}}$ greater than that of $AH$ and less than the value at which the bursting regime disappears.

The activities produced by the model for the coordinated parameter set is sparse compared to the variety of regimes produced by the model at canonical values of $\bar{g}_h$, $\bar{g}_{K2}$, and $\bar{g}_p$. The range of bistability of bursting and silence for canonical parameters (excluding $g_{\text{leak}}$ and $E_{\text{leak}}$) has minimum of 0.02 nS and a maximum of 0.45 nS. The range of bistability for the parameter set of coordinated maximal conductances increased overall, ranging from 0.75 nS to 4.02 nS.
Figure (3.7). Two parameter bifurcation diagrams of oscillatory regimes and equilibria in the $E_{\text{leak}}, g_{\text{leak}}$ plane. Caption continued on next page.
Figure (3.7). Two parameter bifurcation diagrams of oscillatory regimes and equilibria in the $E_{\text{leak}}, g_{\text{leak}}$ plane. (A) The white region on the bifurcation diagram represents parameter values where bursting was the only observed attracting regime of activity. The region where hyperpolarized silence was the only observed regime is shaded yellow, and the region where both hyperpolarized silence and bursting were observed is shaded light green. The Andronov-Hopf bifurcation that terminated the hyperpolarized equilibrium ($AH_1$) is on the border between light green region the where only bursting is observed. The salmon colored region represents depolarized silence. The Andronov-Hopf bifurcation that terminated the depolarized equilibrium ($AH_2$) is the purple curve. In the light blue region, the only observed regime was a depolarized small amplitude spiking, and this regime terminated at the saddle-node bifurcation for periodic orbits labeled $SNo_1$. We observed large amplitude spiking that coexisted with depolarized silence (grey hash-marks on salmon background). The upper boundary of this regime was a saddle-node bifurcation for periodic orbits (red curve) labeled $SNo_2$. This spiking regime vanished into a period-doubling cascade: the first period double bifurcation is the dashed-white curve, and the regime terminated approximately at the black curve. (B) The yellow region indicates parameter values for which we observed only the hyperpolarized equilibrium. We choose parameter values for $\bar{g}_h$, $\bar{g}_{K_2}$, and $\bar{g}_P$ according to the trends in Figure 3.7 so that the range of bistability (green) is greatly increased when compared to (A). The hyperpolarized equilibrium gained stability at an Andronov-Hopf bifurcation (dark green line) labeled $AH$.

3.5 Discussion

Seizure dynamics are particularly challenging to study because ictal paroxysm is difficult to predict. If detected in a timely fashion, such seizure activity could be stopped with the application of perturbations (Durand and Bikson, 2001; Drinkenburg et al., 2003; Foss and Milton, 2003; Jahangiri and Durand, 2011). Recently, significant progress has been made in the development of epileptic treatment devices using these principles (Berenyi et al., 2012). The notions that epileptic tissue can switch suddenly from normal to pathological activity and then from pathological to normal activity with the application of an artificial perturbation suggests that the two regimes could coexist. Multistability is prevalent in a number of computational models of seizure dynamics (Hahn and Durand, 2001; Suffczynski et al., 2004, 2005; Frohlich and Bazhenov, 2006; Frohlich et al., 2010; Wu and Shuai, 2012). The thorough understanding of seizures and the development of treatments for epilepsy requires interdisciplinary approaches combining experimental and theoretical techniques. The
roles of ionic currents and concentrations in the dynamics of seizures have been thoroughly investigated utilizing the theory of dynamical systems (Hahn and Durand, 2001; Foss and Milton, 2003; Suffczynski et al., 2004, 2005; Frohlich and Bazhenov, 2006; Frohlich et al., 2010; Filatov et al., 2011; Krishnan and Bazhenov, 2011; Barreto and Cressman, 2011; Tigerholm et al., 2012; Wu and Shuai, 2012). A wealth of theoretical results highlights the role of intrinsic cellular properties of individual cells in epileptogenesis (Hahn and Durand, 2001; Frohlich and Bazhenov, 2006; Kager et al., 2007; Xie et al., 2008; Cressman et al., 2009; Tigerholm et al., 2012). Despite significant progress, there are compelling questions that are not understood: (1) how to switch an ongoing seizure to a normal regime and (2) how to assess roles of different ionic currents in the propensity for multistability.

We developed a biophysically accurate Hodgkin-Huxley style neuronal model of seizure-like dynamics in an identified leech neuron. This model exhibits coexistence of seizure-like bursting and stable hyperpolarized silence. We investigated properties of pulses of injected current that produced a switch of activity from bursting to silence. Perturbations were characterized by amplitude and phase in the fashion of previous research (Hahn and Durand, 2001; Suffczynski et al., 2004, 2005; Jahangiri and Durand, 2011). Tests revealed two prominent sets of perturbations. These windows of pulse parameters produced switches during the interburst interval; depolarizing pulses were effective early in the phase and hyperpolarizing pulses were effective later in the phase. Assuming generality in topology of seizure bursting activity across phyla, these results suggest that seizure activity could be susceptible to perturbations during interburst intervals, and moreover, either depolarizing or hyperpolarizing pulses could be effective. In the past, results obtained by studying the dynamics of the squid giant axon have been informative in the context of multistability of dynamic disease (Hahn and Durand, 2001; Paydarfar et al., 2006; Xie et al., 2008; Dovzhenok and Kuznetsov, 2012).

The studies of spiking dynamics in the squid giant axon described a mechanism supporting bistability in terms of dynamical systems theory. It identified the unstable regime—a saddle periodic orbit—which creates the separatrix between periodic spiking and the sta-
ble equilibrium. An important feature of this approach is the identification of bifurcations through which the unstable regime appears and dissappears in response to variation in controlling parameter. In this system, an unstable periodic orbit appears at an subcritical Andronov-Hopf bifurcation and dissappears at a saddle-node bifurcation for periodic orbits. This bifurcation scenario is essential for the description of the mechanism of bistability. This approach allowed Guttmen et al. (Guttman et al., 1980) to experimentally demonstrate bistability. One of the effective techniques employed by Guttman et al. (Guttman et al., 1980) was the application of precisely-timed pulses of injected current. A single pulse of current created a perturbation to the stable regime, which could trigger a switch from one basin of attraction to the other. Following this approach, pulse stimulation protocols applied to the Hodgkin-Huxley model identified properties of perturbations that would produce a switch from spiking to silence (Hahn and Durand, 2001; Suffczynski et al., 2005). This analysis is informative in the context of seizure-dynamics (Hahn and Durand, 2001; Suffczynski et al., 2005).

This mechanism of multistability of spiking and silence is similar the mechanisms supporting bursting and silence in the canonical model of the leech heart interneuron (Cymbalyuk et al., 2002; Malashchenko et al., 2011b). The combination of stimulation protocols with the variation of a bifurcation parameter creates a new powerful tool to evaluate the structural stability and the evolution of basins of attraction. This report connected the geometry of the unstable periodic orbit with the properties of the pulse parameters used to probe the basin of attraction of the hyperpolarized stable equilibrium. We showed that the maximal span of window of pulse parameters follows the square-root law imposed on the amplitude of the saddle orbit by the Andronov-Hopf bifurcation. The most parsimonious explanation for the mechanism of this bistability is that the stable manifold of this orbit is the separatrix between the two attracting regimes.

In this report, we present an analysis of the roles of ionic currents in multistability of bursting and silence. We singled out $g_{\text{leak}}$ as the controlling biophysical parameter for a number of reasons. The leak current is present in any biophysically accurate neuronal model.
Biological experiments demonstrate that certain leak currents are modulated by neurotransmitters such as serotonin and noradrenaline (Perrier et al., 2004; Sirois et al., 2002) and extracellular pH (Larkman and Perkins, 2005; Koizumi et al., 2010). Computational models of the HN, an Aplysia snail neuron, a thalamic relay neuron and a respiratory control neuron exhibit a wealth of dynamical regimes in response to variation of the leak current (Cymbalyuk et al., 2002; Cymbalyuk and Shilnikov, 2005a; Guckenheimer et al., 2005; Malashchenko et al., 2011b). The influence of the leak current suggested by computational models is consistent with observations from biological preparations. For example, when the leech heart interneuron is pierced with a sharp micro-electrode for intracellular recording—a procedure that introduces an additional leak current—the cell exhibits tonic spiking (Schmidt and Calabrese, 1992). When extracellular recordings are taken with a suction electrode, though, the cell exhibits endogenous bursting (Cymbalyuk et al., 2002). The contrast in these results suggests that the addition of a leak current qualitatively changes the HN’s regime of activity. A similar phenomenon was reported in Xenopus frog neurons (Aiken et al., 2003).

To characterize the roles of different ionic currents, we introduce a new measure of the propensity of a neuron to multistability. The index of propensity for multistability is the range in $g_{\text{leak}}$ where multistability was observed. The range was determined by the parameter values of two boundaries: the border where the rest state gained stability at an Andronov-Hopf bifurcation and the border where the transition from bursting to silence occurs. We showed that the index of propensity for multistability was sensitive to the maximal conductances of certain ionic currents. Starting with the canonical set of parameters, we varied $g_{\text{leak}}$, and determined the index of propensity for multistability was initially small: 0.016 nS. We showed that this index could be increased 200 fold following coordinated changes to the maximal conductances of the voltage-gated currents: $I_h$, $I_{K2}$, and $I_P$. We accomplished this by increasing $\bar{g}_h$, increasing $\bar{g}_{K2}$, and decreasing $\bar{g}_p$. These results suggest that propensity for multistability in a neuron could be increased or eliminated in a predictable fashion by pharmacological manipulation of intrinsic ionic conductances. This analysis is generic and could be used in the development of pharmacological treatments designed to produce either
multi- or mono-stable dynamics.

Invertebrate systems are ideal for the investigation of cellular mechanisms (Kristan et al., 2005; Marder et al., 2005; Clarac and Pearlstein, 2007). The number of neurons in invertebrates is relatively small, individual neurons are identifiable from preparation to preparation, and there is a wealth of literature detailing biophysical phenomena and properties. Mechanisms supporting multi-stability have been described in experimental and theoretical studies in invertebrate neurons (Rinzel, 1978a; Best, 1979; Guttman et al., 1980; Canavier et al., 1994; Lechner et al., 1996; Cymbalyuk et al., 2002; Le et al., 2006; Paydarfar et al., 2006; Newman and Butera, 2010; Malashchenko et al., 2011a,c). Usually these mechanisms involve an unstable saddle-type periodic orbit.

The leech heart interneuron (HN) is an attractive model in which to study the cellular mechanisms underlying seizure dynamics. The leech heart interneurons have ionic currents sharing dynamic properties with those strongly implicated in epilepsy. Under pharmacological conditions inducing seizure-like dynamics such as application of the calcium and potassium channel blockers $Ca^{2+}$ and 4-AP, the currents remaining in these cells are a fast sodium current, hyperpolarization-activated current, a non-inactivating potassium current, and a persistent sodium current. These currents correspond to hyperpolarization-activated currents, M-currents, and persistent sodium currents in the mammalian brain. The dysfunction of these currents play key roles in epileptic dynamics (Poolos, 2012; Strauss et al., 2004; Wierschke et al., 2010; Brenner and Wilcox, 2012; Biervert et al., 1998; Peters et al., 2005; Miceli et al., 2013; Mantegazza and Catterall, 2012; Holland et al., 2007; Chen et al., 2011). A mutation in KV7.2 is associated with human epileptogenesis (Biervert et al., 1998). Genetic experiments have tied dysfunction of KV7.2 to epileptic episodes in rat and mouse models (Peters et al., 2005; Miceli et al., 2013). In human epilepsy and a rat genetic model, $I_h$ is diminished in cortical epileptic tissue (Strauss et al., 2004; Wierschke et al., 2010). Mutation in SCN3a, which codes for persistent sodium channels has been shown in human pediatric partial epilepsy (Holland et al., 2007). The magnitude of persistent sodium current is increased after epileptic experience in pilocarpine model-epilepsy rats (Chen et al., 2011).
The dynamics of the HN is well studied: the ionic currents have been experimentally characterized following the Hodgkin-Huxley formalism, and a canonical mathematical model has been informative for the design of new experiments (Hill et al., 2001; Cymbalyuk et al., 2002; Sorensen et al., 2004; Olypher et al., 2006; Norris et al., 2007; Wright and Calabrese, 2011). Here, we consider a model of the HN under pharmacological treatment with $Co^{2+}$ as a model for seizure-like bursting. Under this treatment, the ganglia of the medicinal leech exhibits pathological activity, where the membrane potential of many cells oscillate in synchronous slow bursting (Angstadt and Friesen, 1991). When accompanied by a potassium channel blocker, application of $Co^{2+}$ elicits slow plateau-like bursting activity in the HN (Opdyke and Calabrese, 1994b). A plateau-like burst is characterized by an initial spike and then a silent interval of depolarized potential. This depolarization block is a characteristic of activity in epileptic tissue. This scenario falls within the same framework as other models of chemically-induced seizures. Low extracellular $Ca^{2+}$ is associated with pathological oscillations and epilepsy (Pumain et al., 1985; Jefferys and Haas, 1982). The application of 4-AP is a common method for seizure initiation (Szente and Baranyi, 1998; Galvan et al., 1982; Yang et al., 2002; Takeshita and Bahar, 2011).

For canonical parameter values, our new model describes this particular pharmacological scenario: treatment with $Co^{2+}$ and 4-AP. Moreover, by taking into account the pharmacological blockade of ionic currents our model is relatively small in dimension, which makes the model readily accessible to thorough mathematical analysis. These results are based on a biophysically accurate model; these results make clear falsifiable predictions. Based on our previous experience with the canonical model of the HN (Cymbalyuk et al., 2002; Sorensen et al., 2004; Olypher et al., 2006), we expect to demonstrate multistability of seizure-like bursting and silence under these conditions.

In this report, we determined how the manipulation of individual ionic currents or coordinated groups of ionic currents can qualitative change the dynamics of a neuronal system by increasing the range of parameter values where bistability is observed. This sort of pharmacological manipulation could be used to increase the prevalence of a normal regime
in a pathologically oscillating system.

The results predict how to reveal multistability of bursting and silence in the leech heart interneurons. Pharmacological and electrophysiological techniques have been used to manipulate endogenous conductances in this preparation. Here we report that an increase in $\bar{g}_h$ increases the index of propensity of multistability and, hence, the range of parameter values where bistability could be observed. Application of the endogenous peptide myomodulin to the HN increases the conductance of the hyperpolarization-activated current (Tobin and Calabrese, 2005). The described predictions concerning coordinated changes of $\bar{g}_h$, $\bar{g}_{K2}$, and $\bar{g}_P$ could be implemented in artificial conductances using dynamic clamp. In this fashion, a biohybrid system was used to inject a Ca$^{2+}$ current and a synaptic current with arbitrary dynamics in the HN (Olypher et al., 2006). Once multistability is exhibited, we will use our pulse protocol to elicit pulse-triggered switches and confirm the structure of the windows of pulse parameters.

The methods and results presented here could be useful for developing new treatments for seizures involving closed-loop control. Recent studies of closed-loop transcranial electrical stimulation suggest that transient applications of current pulses to cortical neurons can effectively shorten seizure episodes (Berényi et al., 2012). This is a promising result for the notion that switches between coexisting healthy and pathological regimes may terminate some seizures shortly after the onset. On the other hand, analysis of the propensity for multistability could allow for more effective design of pharmacological treatments.

### 3.6 Acknowledgements

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PART 4

THE COREGULATION OF THE NA/K PUMP AND THE H-CURRENT AS A MECHANISM FOR ROBUST NEUROMODULATION

Central pattern generators (CPGs) are oscillatory neuronal circuits that control rhythmic motor functions such as swimming, walking, breathing, and heartbeat (Marder and Calabrese, 1996). In this chapter, I emphasize that this type of mission-critical rhythmic behaviors must operate over a large range of motor characteristics in order to cope with variable environmental demands (Tang et al., 2010, 2012). Control over regimes of activity in neurons is executed through neuromodulation, which enacts changes to the biophysical properties of ion channels or the Na\(^+/\)K\(^+\) pump to ensure robustness Marder et al. (2014); Tobin and Calabrese (2005). The change from one regime to another can be expressed as a bifurcation, where the changes to crucial biophysical parameters reach a threshold value to induce a qualitative transition in neuronal activity (for example, a change from bursting to tonic spiking). Neuromodulators coordinate changes to biophysical characteristics in a functional range bounded by these bifurcations. An open question in contemporary neuroscience is how neuromodulators coregulate multiple conductances to maintain or modify functional neuronal activity.

The heartbeat CPG of the medicinal leech provides an experimental platform to study the mechanisms of neuromodulation in a rhythmic motor function. Neuromodulation in the leech heartbeat central pattern generator is a topic of contemporary study. For example, the neuropeptide FMRFamide is a critical modulator of cardiac systems that has been identified in the leech (Kuhlman et al., 1985a,b; Li and Calabrese, 1987). FMRFamide acts on heart interneurons by modulating intrinsic voltage-dependent ion channels(Nadim and Calabrese, 1997; Simon et al., 1992). Myomodulin is a neuropeptide first identified in Aplysia (Cropper et al., 1987) and later found in the leech (Wang et al., 1998). Myomodulin modulates the
activity of the heart interneuron by altering characteristics of a hyperpolarization-activated current and the Na\(^+\)/K\(^+\) pump current (Masino and Calabrese, 2002b; Tobin and Calabrese, 2005). The Na\(^+\)/K\(^+\) pump current is of particular interest for the present study.

The role of the pump can be experimentally assessed by pharmacological manipulation which could be either technically or biologically derived. In a neuronal model, we study the action of the neuropeptide myomodulin and the antibiotic monensin. Previously, it has been shown that the leech heart interneuron is a neuromodulatory target of myomodulin, which inhibits the pump (Tobin and Calabrese, 2005). The Na\(^+\)/H\(^+\) antiporter monensin can be used to directly manipulate the intracellular sodium concentration, and through this action can be used to increase the activity of the Na\(^+\)/K\(^+\) pump (Hill and Licis, 1982).

In leech heart interneurons, the application of myomodulin decreases the cycle period of the half-center oscillator (Tobin and Calabrese, 2005). It does so by increasing the hyperpolarization-activated current and inhibiting the pump current. After the application of Cs\(^+\), which blocks the h-current, the co-administration of myomodulin and Cs\(^+\) still induces a decrease in cycle period. The h-current is not necessary for myomodulin to induce a decrease in the cycle period of the half-center oscillator.

The application of monensin decreases the cycle period of the half-center oscillator as well (courtesy of Daniel Kueh; Kueh et al., 2015). By introducing an electroneutral leak of Na\(^+\) in the direction of the electrochemical gradient, it increases the intracellular Na\(^+\) concentration, which increases the Na\(^+\)/K\(^+\) pump current. After the application of Cs\(^+\), the co-administration of Cs\(^+\) and monensin does not significantly change the cycle period. The h-current is necessary for monensin to decrease the cycle period of the half-center oscillator.

These results appear to be counterintuitive. How can either stimulation or inhibition of the Na\(^+\)/K\(^+\) pump current decrease the period of a central pattern generator and what role does h-current play in this phenomenon? We explore the hypothesis that the h-current and the pump-current interact in order to produce functional output in the half-center oscillator in a wide range of periods and parameter values. Below, I will investigate the role of the Na\(^+\)/K\(^+\) pump in the dynamics of half-center oscillator activity, with specific regards to its
interaction with the h-current. I will examine mechanisms that support bursting activity that are directly or indirectly related to the Na\(^+\)/K\(^+\) pump current, the h-current, and the slow Ca\(^{2+}\) current. I will study how neuromodulation could coregulate the biophysical parameters of the Na\(^+\)/K\(^+\) pump current and the h-current.

4.1 Methods

We developed a single-compartment model of a heart interneuron using Hodgkin-Huxley style equations. Our model has a leak (\(I_{\text{leak}}\)) current and a Na\(^+\)/K\(^+\) pump (\(I_{\text{pump}}\)) current, with the leak current having a Na\(^+\) (\(I_{\text{leak,Na}}\)) and a K\(^+\) (\(I_{\text{leak,K}}\)) component. The model also has eight voltage-gated currents: a fast Na\(^+\) current (\(I_{Na}\)), a persistent Na\(^+\) current (\(I_P\)), a rapidly inactivating low-threshold Ca\(^{2+}\) current (\(I_{CaF}\)), a slowly inactivating low-threshold Ca\(^{2+}\) current (\(I_{CaS}\)), a hyperpolarization-activated inward current (h-current, \(I_h\)), a delayed rectifier-like K\(^+\) current (\(I_{K1}\)), a persistent K\(^+\) current (\(I_{K2}\)), and a fast transient K\(^+\) current (\(I_{KA}\)). The model of a single heart interneuron can be converted into a half-center oscillator by including a spike-mediated synaptic current (\(I_{\text{SynS}}\)) and a graded synaptic current (\(I_{\text{SynG}}\)), as follows:

\[
C \frac{dV}{dt} = -(I_{Na} + I_P + I_{K1} + I_{K2} + I_{KA} + I_{h,Na} + I_{h,K} + I_{CaF} + I_{CaS} +
I_{\text{Leak,Na}} + I_{\text{Leak,K}} + I_{\text{Pump}} + I_{\text{SynS}} + I_{\text{SynG}}) \tag{4.1}
\]

where \(C\) is the membrane capacitance (in nF), \(V\) is the membrane potential (in V), and \(t\) is time (in s). Our half-center oscillator model differs from previous models (Cymbalyuk et al., 2002; Hill et al., 2001) because it includes a Na\(^+\)/K\(^+\) pump current and it describes changes in intracellular Na\(^+\) concentrations that occur as a result of the Na\(^+\) fluxes carried by ionic currents, Na\(^+\)/K\(^+\) pumps, and monensin-facilitated diffusion:

\[
\frac{d[Na]_i}{dt} = M ([Na]_o - [Na]_i) - \frac{I_{Na} + I_P + I_{h,Na} + I_{\text{leak,Na}} + 3I_{\text{pump}}}{nF}
\]

[Na]_i is the changing intracellular Na\(^+\) concentration, [Na]_o is the extracellular Na\(^+\)
concentration that is kept constant, \( v \) is the volume (\( \sim 6.7 \) pL) of the intracellular Na\(^+\) reservoir, \( F \) is Faraday’s constant, and \( M \) is the exchange rate (in s\(^{-1}\)) of Na\(^+\) and H\(^+\) by monensin, which is based on Fick’s law of diffusion. Because the Na\(^+\)/K\(^+\) pump exchanges two K\(^+\) ions for three Na\(^+\) ions, the contribution of the pump current to intracellular Na\(^+\) concentrations was multiplied by a factor of 3. The Na\(^+\)/K\(^+\) pump current has a sigmoidal dependence on intracellular Na\(^+\) concentrations, which is expressed as follows:

\[
I_{pump} = \frac{I_{max_pump}}{1 + \exp \left( \frac{[Na]_{ih} - [Na]_{is}}{[Na]_{ih} - [Na]_{is}} \right)}
\]

where \( I_{max_pump} \) is the maximum Na\(^+\)/K\(^+\) pump current, \([Na]_{ih}\) is the intracellular Na\(^+\) concentration for the half-activation of the Na\(^+\)/K\(^+\) pump, and \([Na]_{is}\) is the sensitivity of the Na\(^+\)/K\(^+\) pump to \([Na]_{i}\).

The hyperpolarization-activated inward current and the leak current have Na\(^+\) and K\(^+\) components. In the case of the hyperpolarization-activated inward current, we computed the Na\(^+\) component (\( I_{h,Na} \)) using the equilibrium potential of Na\(^+\) and we computed the K\(^+\) component (\( I_{h,K} \)) using the equilibrium potential of K\(^+\):

\[
I_{h,Na} = \frac{37g_h}{m_h^2} \left( V_m - E_{Na} \right)
\]

\[
I_{h,K} = \frac{47g_h}{m_h^2} \left( V_m - E_K \right)
\]

In both equations, \( g_h \) is the maximum conductance, \( m_h \) is the activation variable, \( V_m \) is the membrane potential, and \( E_{ion} \) is the equilibrium potential at 20C. Unlike the equilibrium potential of K\(^+\), the equilibrium potential of Na\(^+\) was computed at each time step as a function of a constant extracellular Na\(^+\) concentration and a changing intracellular Na\(^+\) concentration: \( E_{Na} = 0.02526 \ln \frac{[Na]_{i}}{[Na]_{o}} \)

We also computed two components (\( I_{leak,Na} \) and \( I_{leak,K} \)) of the leak current:

\[
I_{leak,Na} = g_{leak,Na} \left( V_m - E_{Na} \right)
\]

\[
I_{leak,K} = g_{leak,K} \left( V_m - E_K \right)
\]

We fixed the ratio of Na\(^+\) to K\(^+\) conducted by the leak current and used the equilibrium potentials of K\(^+\) and Na\(^+\) Hill et al. (2001) to compute this ratio. The equations for \( g_{leak,Na} \) and \( g_{leak,K} \) are:

\[
g_{leak,Na} = g_{leak} \frac{E_{leak,Ref} - E_K}{E_{Na,Ref} - E_K}
\]

\[
g_{leak,K} = g_{leak} \frac{E_{leak,Ref} - E_{Na,Ref}}{E_K - E_{Na,Ref}}
\]
The parameter $E_{Na,Ref}$ was fixed at 0.045 V. The Na$^+$ and K$^+$ components of the leak conductance, $g_{\text{leak,Na}}$ and $g_{\text{leak,K}}$ were computed ahead of time and were fixed for the duration of each simulation.

We computed the average value function of the Na$^+$/K$^+$ pump current and the h-current over a bounded integral as follows:

$$\langle I \rangle = \frac{1}{t_2-t_1} \int_{t_1}^{t_2} I dt$$

Both average currents were computed over the burst duration and interburst interval. When the average currents were computed over a burst duration, $t_1$ represented the time of the first spike in a burst whereas $t_2$ represented the time of the last spike in the same burst. When the average currents were computed during the interburst interval, in which the membrane potential was below -50 mV, $t_1$ represented the time at which the membrane potential crossed -50 mV after the last spike in the preceding burst whereas $t_2$ represented the time at which the membrane potential crossed -50 mV immediately before the first spike of the next burst. We used the trapezoid method for numerical integration (the trapz function in MATLAB).

A complete list of Hodgkin-Huxley style equations that describe ionic and synaptic currents can be found in the Appendix. We computed numerical solutions to these ordinary differential equations using the 8-9 order Prince-Dormand method from the GNU Scientific Library (http://www.gnu.org/software/gsl). All variables were computed with an absolute tolerance of $1 \times 10^{-9}$, a relative tolerance of $1 \times 10^{-10}$, and a maximum time step of $1 \times 10^{-3}$ s.

We analyzed the burst characteristics of current and voltage trajectories from our model with custom-made routines written in the C programming language. We measured the burst period, burst duration, and interburst interval. The burst period was determined as the interval between the median action potential of a given burst and the median action potential of the following burst. The burst duration was defined as the interval between the first action potential and the last action potential of a given burst. The interburst interval was defined as the interval between the last action potential of a given burst and the first action potential
of the following burst.

4.2 Modeling the effects of stimulating the Na\textsuperscript{+}/K\textsuperscript{+} pump current in eight experimental treatments

We introduced the Na\textsuperscript{+}/K\textsuperscript{+} pump current and intracellular Na\textsuperscript{+} dynamics into the biophysical model of Hill et al. (2001) to reproduce the bursting activity of the oscillator heart interneurons and account for the present experimental findings. We investigate the role of the Na\textsuperscript{+}/K\textsuperscript{+} pump current in the dynamics of bursting activity by modeling the application of monensin. We consider activity of the half-center oscillator and isolated neuron (bicuculline treatment) with the h-current intact and h-current blocked (Cs\textsuperscript{+} treatment). The effects of the application of monensin to all of these cases are investigated. To determine whether the bursting activity of our model captures the bursting activity observed in our different experiments, we compared the modeled data to the experimental data based on four burst characteristics (burst period, burst duration, interburst interval, and duty cycle) under eight parameter regimes that mimic our experimental treatments: (1) control; (2) monensin; (3) bicuculline; (4) bicuculline plus monensin; (5) Cs\textsuperscript{+}; (6) Cs\textsuperscript{+} plus monensin; (7) bicuculline plus Cs\textsuperscript{+}; and (8) bicuculline and Cs\textsuperscript{+} plus monensin. These different treatments were modeled through corresponding changes in biophysical parameters. (1) In control simulations, the model included all voltage-gated and synaptic currents and the monensin rate parameter was 0 s\textsuperscript{-1}. For comparison, we used the control data of all extracellular experiments involving subsequent monensin application. (2) We modeled the monensin-mediated diffusion of Na\textsuperscript{+} across the membrane as a non-electrogenic process controlled by the monensin rate constant, M (See Methods). (3) To represent the effects of bicuculline, the model was changed by including only the expressions representing a single oscillator heart interneuron. The bicuculline data that we used for comparison were taken from all extracellular experiments involving subsequent applications of bicuculline and monensin. (4) To represent the bicuculline plus monensin treatment, the model described the dynamics of a single cell and included a term for monensin-mediated diffusion. (5) The effects of blocking the h-current
with Cs\textsuperscript{+} were represented by reducing the maximal conductance of the h-current ($\bar{g}_h = 0.1 \text{nS}$) in our model. (6) To represent the Cs\textsuperscript{+} plus monensin treatment, the model was changed by reducing the maximal conductance of the h-current ($\bar{g}_h = 0.1 \text{nS}$) and including a term for monensin in intracellular Na\textsuperscript{+} dynamics. (7) To represent the bicuculline plus Cs\textsuperscript{+} treatment, the model included expressions for a single cell and a reduced maximal conductance of the h-current ($\bar{g}_h = 0.1 \text{nS}$). (8) To represent the bicuculline and Cs\textsuperscript{+} plus monensin treatment, the model was changed by including only expressions for a single cell, reducing the maximal conductance of the h-current ($\bar{g}_h = 0.1 \text{nS}$), and including a term for monensin in the intracellular Na\textsuperscript{+} dynamics.

The monensin rate constants, M, differ in orders of magnitude between the four treatments that include monensin application because the models had different sensitivities to this term with the half-center oscillator being the least sensitive and the single heart interneuron in Cs\textsuperscript{+} being the most sensitive: in the simulation of monensin treatment, M was $2.2 \times 10^{-3} \text{s}^{-1}$; in the simulation of the bicuculline plus monensin treatment, M was $1.5 \times 10^{-4} \text{s}^{-1}$; in the simulation of the Cs\textsuperscript{+} plus monensin treatment, M was $1.9 \times 10^{-4} \text{s}^{-1}$; and in the simulation of the bicuculline and Cs\textsuperscript{+} plus monensin treatment, M was $3.8 \times 10^{-5} \text{s}^{-1}$. Higher values of M in the models other than half-center oscillator caused dysfunctional activity such as silence.

Simulations of the control treatment exhibited the antiphase bursting activity typical of the oscillator heart interneurons that comprise a half-center oscillator (Figures 4.1A1 and 4.2A1). The burst characteristics of this model were a period of 8.0 s, a burst duration of 3.7 s, an interburst interval of 4.2 s, and a duty cycle of 47.0 % (Table S1-S4). Each of these burst characteristics fell within the experimentally observed ranges: a burst period of 4.3 s to 12.3 s, a burst duration of 2.6 s to 7.3 s, an interburst interval of 1.5 s to 6.0 s, and a duty cycle of 46.8% to 64.7% (Table S1-S4). The isolated oscillator heart interneuron model also exhibited endogenous bursting activity (Figures 4.1B1 and 4.2B1). The burst characteristics for these simulations were a period of 4.0 s, a burst duration of 2.3 s, an interburst interval of 1.7 s, and a duty cycle of 57.3% (Table S1-S4). These burst characteristics were each within
the experimentally observed ranges: a burst period of 3.2 s to 4.3 s, a burst duration of 2.0 s to 2.6 s, an interburst interval of 1.1 s to 1.9 s, and a duty cycle of 57.3% to 77.5% (Table S1-S4).

The Na\(^+\)/K\(^+\) pump current and Na\(^+\) dynamics contributed to the dynamics of bursting in this model (Figure 4.1). The Na\(^+\)/K\(^+\) pump current and the intracellular Na\(^+\) concentration oscillated on the timescale of bursting activity. The Na\(^+\)/K\(^+\) pump current had a minimum value (or baseline magnitude) of about 0.1 nA at the end of an interburst interval. During the beginning of a burst, intracellular Na\(^+\) concentration accumulated with each action potential. As intracellular Na\(^+\) increased, the pump current also increased. Over the course of a burst, the spike frequency decreased and the outward Na\(^+\) flux generated by the pump began to balance—at a maximum Na\(^+\)/K\(^+\) pump current of 0.2 nA in the control simulations and 0.19 nA in the bicuculline simulations—and exceed the inward Na\(^+\) flux produced by the voltage gated Na\(^+\) currents (Figure 4.1A1). At the termination of a burst, the intracellular Na\(^+\) concentration and Na\(^+\)/K\(^+\) pump current relaxed to a baseline of about 14 mM and 0.1 nA, respectively, over the duration of an interburst interval. In models of a single cell and the half-center oscillator, we observed a tonic and phasic component to the outward Na\(^+\)/K\(^+\) pump current.

Simulations of the control treatment and the monensin treatment exhibited antiphase bursting activity typical of the half-center oscillator (Figures 4.1A1-2 and 4.2A1-2). To model the effects of monensin, we set the monensin rate constant to $2.2 \times 10^{-3}$ s\(^{-1}\) (these rate constants have been rounded; see Appendix for exact values used in simulations). The model produced bursting activity for values of M up to $2.2 \times 10^{-3}$ s\(^{-1}\) at which value, the activity transitioned to silence. We recorded a decrease in the burst period of bursting activity compared to the control simulations. In control simulations, the burst period was 8.0 s, the burst duration was 3.7 s, the interburst interval was 4.2 s, and the duty cycle was 47.0%. In monensin simulations, the burst period was 4.4 s, the burst duration was 1.6 s, the interburst interval was 2.8 s, and the duty cycle was 35.5% (Table S1-S4). The ranges of experimentally observed burst characteristics were a burst period of 3.0 s to 4.1 s, a burst
duration of 1.4 s to 2.3 s, an interburst interval of 1.3 s to 1.8 s, and a duty cycle of 44.6% to 59.8% (Table S1-S4). Only the burst duration was within the range whereas the other three burst characteristics (burst period, the interburst interval, and duty cycle) were out of range of the experimentally observed burst characteristics. Nevertheless, these simulations do qualitatively capture the trends observed in the experiments whereby the burst period, burst duration, and interburst interval decreased from the control simulation to the monensin simulation.

When the model described heart interneurons under the bicuculline treatment and the bicuculline plus monensin treatment, simulations exhibited endogenous bursting activity (Figures 4.1B1-2 and 4.2B1-2). To model the effects of bicuculline plus monensin, the value of the monensin rate constant M was set to $1.5 \times 10^{-4}$ s$^{-1}$. The maximum value for M which produced bursting activity was $1.3 \times 10^{-3}$ s$^{-1}$; the activity for greater values of M was silent. The burst period decreased from the bicuculline simulation to the bicuculline plus monensin simulation. In the bicuculline simulation, the burst period was 4 s, the burst duration was 2.3 s, the interburst interval was 1.7 s, and the duty cycle was 57.3% (Table S1-S4). These burst characteristics were within the experimentally observed range. In the bicuculline plus monensin simulation, the burst period was 3.8 s, the burst duration was 2.0 s, the interburst interval was 1.8 s, and the duty cycle was 53.4% (Table S1-S4). Two of the four burst characteristics burst period and burst duration were within the experimentally observed range. The experimentally observed ranges were a period of 3.1 s to 3.9 s, a burst duration of 1.7 s to 2.3 s, an interburst interval of 1.0 s to 1.6 s, and a duty cycle of 53.5% to 71.1% (Table S1-S4). When compared to the bicuculline simulation, we observed a decrease in the period of bursting activity. The interburst interval in the simulated activity (1.8 s) was relatively large for the range of experimentally observed values which was 1.0 s to 1.6 s, and the duty cycle in simulated activity (53.4%) was just on the low side of the experimental range which was 53.5% to 71.1% (Table S3-S4). In simulations representing the bicuculline treatment and the bicuculline plus monensin treatment, the burst period and burst duration decreased from the bicuculline simulation to the monensin simulation whereas the interburst
interval increased from the bicuculline simulation to the monensin simulation.

The model of the Cs\(^+\) treatment and the Cs\(^+\) plus monensin treatment exhibited half-center oscillator activity (Figures 4.1C1-2 and 4.2C1-2). To model the effect of Cs\(^+\) plus monensin, the monensin rate constant was set to \(1.9 \times 10^{-4} \text{ s}^{-1}\). This was the largest value of \(M\) for which the model produced bursting activity. The activity was silent for larger values of \(M\). Similar to experimental results, we observed a decrease in the burst period in the presence of monensin. In both treatments, the model burst characteristics were within the experimentally observed range. In the Cs\(^+\) simulation, the burst characteristics were a burst period of 8.9 s, a burst duration of 3.7 s, an interburst interval of 5.1 s, and a duty cycle of 42.2\% (Table S1-S4). The ranges of experimentally observed characteristics in the Cs\(^+\) treatment were a burst period of 5.9 s to 11.6 s, a burst duration of 2.3 s to 5.9 s, an interburst interval of 3.3 s to 5.8 s, and a duty cycle of 38.6\% to 53.4 \%. In the Cs\(^+\) plus monensin simulation, the burst period was 6.6 s, the burst duration was 2.0 s, the interburst interval was 4.5 s, and the duty cycle was 30.9\% (Table S1-S4). The experimentally observed range for this treatment was a burst period of 5.9 s to 8.6 s, a burst duration of 1.7 s to 4.1 s, an interburst interval of 3.3 s to 5.5 s, and a duty cycle of 26.6\% to 48.0\% (Table S1-S4). In summary, the burst period, burst duration, and interburst interval decreased from the Cs\(^+\) simulation to the Cs\(^+\) plus monensin simulation.

The model of the bicuculline plus Cs\(^+\) treatment and the bicuculline and Cs\(^+\) plus monensin treatment (Figure 4.1D1-7D2; Figure 4.2D1-8D2) exhibited endogenous bursting with a relatively small duty cycle. To simulate the effects of bicuculline and Cs\(^+\) plus monensin, the monensin rate constant \(M\) was set to \(3.8 \times 10^{-5} \text{ s}^{-1}\). For values of \(M\) greater than \(4.6 \times 10^{-5} \text{ s}^{-1}\), the model produced silent activity. This model was the most sensitive to the monensin treatment. It is consistent with our previous notion of the sensitivity of a single cell bursting dynamics to perturbations of membrane currents and importance of h-current (Cymbalyuk et al., 2002). These modeling results reflected the increase in burst period observed in experiments from the bicuculline plus Cs\(^+\) treatment 106 to the bicuculline and Cs\(^+\) plus monensin treatment. In both simulations, the model burst characteristics were
within the range of experimentally observed burst characteristics. In the bicuculline plus Cs⁺ simulation, the burst period was 6.5 s, the burst duration was 1.2 s, the interburst interval was 5.3 s, and the duty cycle was 18.7%. The ranges of burst characteristics in our experiments for the bicuculline plus Cs⁺ treatment were a burst period of 4.3 s to 9.0 s, a burst duration of 1.0 s to 1.8 s, an interburst interval of 2.7 s to 8.0 s, and a duty cycle of 11.1% to 34.3% (Table S1-S4). In the bicuculline and Cs⁺ plus monensin simulation, the burst period was 7.9 s, the burst duration was 1.2 s, the interburst interval was 6.6 s, and the duty cycle was 15.6% (Table S1-S4). In experiments, the ranges of burst characteristics for the bicuculline and Cs⁺ plus monensin treatment were a burst period of 4.7 s to 9.5 s, a burst duration of 1.1 s to 1.9 s, an interburst interval of 3.2 s to 8.1 s, and a duty cycle of 13.7% to 32.4% (Table S1-S4). In summary, the burst period and interburst interval increased from the bicuculline plus Cs⁺ simulation to the bicuculline and Cs⁺ plus monensin simulation.

4.3 The interaction of Na⁺/K⁺ pump current with h-current and slow Ca²⁺ current explains the effects induced by monensin

To explain the results of our extracellular experiments that involved monensin, we analyzed the interactions of the Na⁺/K⁺ pump current with the h-current and the slowly inactivating Ca²⁺ current. The results were obtained through simulation in the new canonical model of the heart interneuron and the heart interneuron half-center oscillator under different experimental treatments. In the latter, the h-current and the slowly inactivating Ca²⁺ current have been shown to play key roles in determining the properties of bursting activity of a half-center oscillator (Hill et al., 2001; Nadim et al., 1995; Olypher et al., 2006; Sorensen et al., 2004). Previous canonical models of the heart half-center oscillator had a duty cycle of about 50% and were governed by a balance between an escape mechanism, promoted by h-current activation that determines the interburst interval, and a release mechanisms, promoted by inactivation of the slow Ca⁺ current that determines the burst duration. The slow Ca⁺ current controls the decay of the spike frequency within the burst of an active neuron in the half-center oscillator. In turn, the spike frequency determines the
efficiency of the synaptic inhibition of the other cell in the half-center oscillator. The more
the slow Ca$^{2+}$ current is deinactivated during the preceding inhibited phase, the larger the
current is at the beginning of a burst and the longer is the burst. Activation of the h-current
determines the duration of the interburst interval. Because the h-current is activated by
hyperpolarization, it is the primary inward current when the cell is inhibited and therefore
controls the moment at which the cell escapes from the inhibition.

The role of the slowly inactivating Ca$^{2+}$ current in control of the burst duration is well
established (Nadim et al., 1995; Hill et al., 2001; Olypher et al., 2006). The degree of inac-
tivation of the slow Ca$^{2+}$ current at the beginning of a burst determines the burst duration
(Olypher et al., 2006). The slow Ca$^{2+}$ current is gated by a relatively fast activation variable
($m_{\text{Cas}}$) and a relatively slow inactivation variable ($h_{\text{Cas}}$): $I_{\text{Cas}} = \bar{g}_{\text{Cas}} m_{\text{Cas}}^2 h_{\text{Cas}} \left[ V_m - E_{\text{Ca}} \right]$. The slow Ca$^{2+}$ current is deinactivated by the hyperpolarization during the interburst
interval (Figure 4.2). After the depolarization at the onset of a burst, the slow Ca$^{2+}$ current
slowly inactivates. For example, in the simulation of the control treatment, $h_{\text{Cas}}$ was at a
peak of 0.7 during the interburst interval, $h_{\text{Cas}}$ was 0.3 at the time of the first spike in a
burst, and it was at a minimum of 0.1 immediately after the last spike of the burst (Figure
4.2A1). The depolarization of the first spike activates the slow Ca$^{2+}$ current: $m_{\text{Cas}}$ grows to
0.9. It is only during the burst—when the slow Ca$^{2+}$ current is simultaneously activated and
deinactivated—that this current contributed to the dynamics of the cell. In this example, the
slow Ca$^{2+}$ current strongly deinactivated as the cell hyperpolarized after the termination of
the burst. Over the course of interburst interval, the membrane potential slowly depolarized.
The duration of this rise and the delay between the cessation of inhibition and the time of
the beginning of the burst determined the value of $h_{\text{Cas}}$ at the beginning of the burst. Over
the course of the burst, the slow Ca$^{2+}$ current gradually inactivated.

Our experiments with monensin reveal the role of Na$^+/K^+$ pump current in the dynam-
ics of bursting activity. The Na$^+/K^+$ pump current plays a unique role as an outward current
that is active during the inhibited and spiking phases. Changes in the Na$^+/K^+$ pump current
occur as a function of intracellular Na$^+$ concentration. Both of them are substantially larger
during the burst than during the interburst interval. The Na\(^+\)/K\(^+\) pump current effects the properties of bursting activity directly and by interacting with the h-current and the slow Ca\(^{2+}\) current.

Below, we show that monensin generally enhances the Na\(^+\)/K\(^+\) pump current. This enhancement of an outward current can directly shorten the burst duration and make the cell more hyperpolarized during interburst interval. Without the h-current, the enhanced outward current expands the interburst interval. Stronger hyperpolarization causes stronger activation of the h-current, which in turn determines the interburst interval. Also, the hyperpolarization removes inactivation of the slow Ca\(^{2+}\) current. The latter effect depends on the membrane potential and the duration of hyperpolarization and is influenced by the amount of h-current.

We investigated the role of the Na\(^+\)/K\(^+\) pump current in bursting activity by comparing the change in burst duration and strength of the Na\(^+\)/K\(^+\) pump current in each of the four simulations that do not account for monensin (e.g., Cs\(^+\) simulation) with the respective simulations that do account for monensin (e.g., Cs\(^+\) plus monensin simulation). We also examined the Na\(^+\)/K\(^+\) pump current, h-current, and membrane potential during the interburst interval.

In simulations of half-center oscillators, we observe distinct mechanisms that control burst characteristics. We considered the change in activity from the control treatment to the monensin treatment. The application of monensin dramatically decreased the burst duration from 3.7 s to 1.6 s (Figure 4.1A1-2). We attribute this change to the increase of the average Na\(^+\)/K\(^+\) pump current during the burst, which increased by 18% from the control treatment to the monensin treatment (184 pA to 222 pA). Similarly during the interburst interval, the average Na\(^+\)/K\(^+\) pump current increased by 54% (108 pA to 167 pA), and the average h-current increased by 18% (-70 pA to -83 pA) (Figure 4.1A1-2). This is an example illustrating two mechanisms such that the burst duration and the interburst interval are directly controlled by the Na\(^+\)/K\(^+\) pump current. To obtain these results with burst characteristics within the experimentally measured bounds, the value of the monensin
rate parameter used in the monensin simulations was $2.2 \times 10^{-3} \text{ s}^{-1}$. Here and below, we only considered parts of the trajectory for which the membrane potential was below $-50 \text{ mV}$ when computing the average $\text{Na}^+$/K$^+$ pump current or the average h-current during the interburst interval.

In the Cs$^+$ treatment and Cs$^+$ and monensin treatment, the simulations exhibit different mechanisms governing burst duration and interburst interval. In contrast to the previous treatments, the decrease of the burst duration and period could not be attributed to the enhancement of the pump. The burst duration decreased from 3.7 s in the simulation of the Cs$^+$ treatment to 2.0 s in the Cs$^+$ plus monensin treatment while the average pump current during the burst decreased by 7% between these treatments (165 pA to 153 pA) (Figure 4.1C1-2). We attribute the change in the burst duration to the relative inactivation of the slow $\text{Ca}^{2+}$ current. The gating variable, $h_{\text{CaS}}$, at the beginning of the burst changed from 0.34 in the Cs$^+$ treatment to 0.24 in the Cs$^+$ and monensin treatment (Figure 4.2C1-2). Further description of these mechanisms is provided below.

Although it is not apparent at first glance, the interburst interval was directly affected by the $\text{Na}^+$/K$^+$ pump current so that the enhanced pump current effectively prolonged the interburst interval. In this treatment, the half-center oscillator generates bursting through the release mechanism in both experiments and the model. The interburst interval decreased by 12% from the simulation of the Cs$^+$ treatment (5.1 s) to the simulation of the Cs$^+$ plus monensin treatment (4.5 s), while the average $\text{Na}^+$/K$^+$ pump current increased from 85 pA to 92 pA (a changed of 8%) between these treatments (Figure 4.1C1-2). This is a cumulative result of two effects. Although the silent phase of neurons is expanded due to the change in the pump, it shortens even more due to shortening of the burst duration of the opposite cell as described above. The direct effect of the $\text{Na}^+$/K$^+$ pump current can be assessed by the duration of the interburst interval when there was no synaptic inhibition (interburst interval of silent cell minus the burst duration of the active cell). This interval increases from 1.4 s in the Cs$^+$ treatment to 2.5 s in the Cs$^+$ plus monensin treatment in accordance with the increase of the pump current. This mechanism leads to the corresponding changes of
the duty cycle. In the model, the duty cycle decreased from 42.2% in the Cs+ treatment to 30.9% in the Cs+ plus monensin treatment. To obtain these results in the Cs+ and monensin treatment, we set the monensin rate parameter to $1.9 \times 10^{-4}$ s$^{-1}$. This value is an order of magnitude smaller than that used in the monensin alone treatment. It shows that the h-current enhances robustness of the half-center oscillator.

In the bicuculline treatment and in the bicuculline and monensin treatment, the effects of Na$^+/K^+$ pump current and the h-current directly affected the burst duration and interburst interval. The burst duration decreased from 2.3 s in the bicuculline treatment to 2.0 s in the bicuculline plus monensin treatment. The average Na$^+/K^+$ pump current during the burst increased by 1% from the bicuculline treatment to the bicuculline plus monensin treatment (173 pA to 175 pA) (Figure 4.1B1-2). The interburst interval increased slightly from the bicuculline treatment (1.7 s) to the bicuculline plus monensin treatment (1.8 s). The average Na$^+/K^+$ pump current increased by 2% from the bicuculline condition (127 pA) to the bicuculline and monensin condition (129 pA). Although, the average h-current increased by 8% from the bicuculline condition (-31 pA) to the bicuculline and monensin condition (-33 pA), its effects were apparently balanced by the change in the Na$^+/K^+$ pump current (Figure 4.1B1-2). To obtain these results in the bicuculline and monensin treatment, we set the monensin rate parameter to $1.5 \times 10^{-4}$ s$^{-1}$. This value is an order of magnitude smaller than that used in the monensin treatment. It confirms that the half-center oscillator motif brings robustness to the dynamics of bursting activity (Cymbalyuk et al., 2002; Marin et al., 2013). The smaller value of the monensin rate parameter used here can explain lesser effect of the monensin on the bursting characteristics.

The switch from the bicuculline and Cs+ treatment to the bicuculline, Cs+, and monensin treatment, shows an example of the direct effect of the pump onto the interburst interval. In the model, the burst duration did not change between the treatments (1.2 s) which agreed with the less than 1% change of the average Na$^+/K^+$ pump (133 pA to 134 pA) between the treatments (Figure 4.1D1-2). This change in average Na$^+/K^+$ pump current was not sufficient to elicit a change in burst duration. Between these treatments the change
in the average Na\textsuperscript{+}/K\textsuperscript{+} pump current did not explain the change of the interburst interval, but we found that the Na\textsuperscript{+}/K\textsuperscript{+} pump current at the beginning of the interburst interval did. The interburst interval increased from 5.3 s in the bicuculline and Cs\textsuperscript{+} treatment to 6.6 s in the bicuculline, Cs\textsuperscript{+}, and monensin treatment, but the average Na\textsuperscript{+}/K\textsuperscript{+} pump current actually decreased from 86 pA to 84 pA between treatments (Figure 4.1D1-2). This long interburst interval is explained by the near balance of inward and outward currents. The primary outward current during the interburst interval was the Na\textsuperscript{+}/K\textsuperscript{+} pump current. In this treatment, with the h-current removed, the only inward current during the interburst interval was the leak current. In all simulations, the reversal potential of the leak current varied about a tenth of a millivolt above and below -54 mV. As the membrane potential approaches the reversal potential of the leak current, it enters the range where the persistent Na\textsuperscript{+} current activates, which initiates the next burst. The steady state activation of the persistent Na\textsuperscript{+} current is 0.32 when the membrane potential is -54 mV. So the initial membrane potential and pump current at the beginning of the interburst interval are critical for determining the passage time and, thus, the duration of interburst interval. It is counterintuitive that the interburst interval would increase, when an outward current was decreased. We examined the trajectory of the pump current at specific points: at the time when the membrane potential crossed -50 mV after the last spike of the preceding burst, at the time when the membrane potential crossed -55 mV after the last spike of the preceding burst, and the time when the membrane potential was at a minimum. At all three of these time points, the Na\textsuperscript{+}/K\textsuperscript{+} pump current was greater in the bicuculline, Cs\textsuperscript{+}, and monensin treatment than in the bicuculline and Cs\textsuperscript{+} treatment. As such, we conclude that in these two simulations, the Na\textsuperscript{+}/K\textsuperscript{+} pump current at the beginning of the interburst interval is critical for determining the duration of the interburst interval. The monensin rate parameter in the bicuculline, Cs\textsuperscript{+}, and monensin treatment was $3.8 \times 10^{-5}$ s\textsuperscript{-1}.
### 4.4 Coregulation of the maximal conductance of the h-current and the maximal pump current by myomodulin

In the previous section, we stimulated the pump by increasing the intracellular Na\(^+\) concentration by application of monensin. The neuropeptide myomodulin acts on the heart interneuron by enhancing the h-current and inhibiting the pump. Application of myomodulin decreases the period of the half-center oscillator. It is counterintuitive that monensin—an agent that enhances the pump current—and myomodulin—an agent that inhibits the pump current—both decrease the period of the leech heart half-center oscillator. The new model of the leech heart interneuron was extended to account for experimental treatments that include myomodulin. We investigated the coregulation of the h-current and the Na\(^+\)/K\(^+\) pump current to expand the range of functional variation of parameters and bursting activity.

We adapted our model to investigate key experiments from Tobin and Calabrese (2005). A number of biophysical parameters were changed so that the temporal characteristics of the activity produced by the model reproduced the trend in temporal characteristics observed in four sets of parameter regimes that mimic experimental treatment with myomodulin and Cs\(^+\): (1) control; (2) myomodulin; (3) Cs\(^+\); and (4) myomodulin and Cs\(^+\). For each treatment, we adjusted appropriate biophysical parameters that correspond to a specific experimental treatment. (1) The simulation of the control treatment provided a canonical example of unmanipulated activity. The two key parameters, the maximal Na\(^+\)/K\(^+\) pump current ($I_{\text{pump}}^{\text{max}}$) and the maximal conductance of the h-current ($\bar{g}_h$) took values 0.456 nA and 2 nS, respectively. (2) In the simulation of the myomodulin treatment, we modeled the inhibition of the Na\(^+\)/K\(^+\) pump current by decreasing $I_{\text{pump}}^{\text{max}}$ to 0.41 nA. We modeled the increase in the h-current by increasing $\bar{g}_h$ to 4 nS. (3) In the simulation of the Cs\(^+\) treatment, we modeled the blockade of the h-current by setting $\bar{g}_h$ to 0 nS. (4) In the simulation of the Cs\(^+\) and myomodulin treatment, $\bar{g}_h$ was set to 0 nS and $I_{\text{pump}}^{\text{max}}$ was set to 0.41 nA in order to account for the simultaneous blockade of the h-current and the inhibition of the Na\(^+\)/K\(^+\) pump current. For each simulation, we measured period, burst duration, and
interburst interval. However, the period of bursting activity was the primary constraint used in preliminary optimization in order to determine whether the model reproduced the qualitative change in activity between different experimental treatments. We used three key experiments from Tobin and Calabrese (2005) to perform three critical comparisons of the temporal characteristics within our four simulations: the difference in cycle period between the control treatment and the myomodulin treatment; the difference in cycle period between the control treatment and the Cs$^+$ treatment; and the difference in period between the Cs$^+$ treatment and the myomodulin plus Cs$^+$ treatment.

The simulation of the control treatment produced characteristic antiphase half-center oscillator activity. The burst characteristics of this activity (a burst period of 8.7 s; a burst duration of 4.0 s; and an interburst interval of 4.7 s) were within the range observed in experiments (Courtesy of Daniel Kueh; Kueh et al., 2015). In the simulation of the myomodulin treatment, the burst period was shorter than that in the control simulation. The burst characteristics were a burst period of 6.6 s, a burst duration of 3.0 s, and an interburst interval of 3.5 s. In Tobin and Calabrese (2005), the burst period decreased by 17% in the half-center oscillator upon application of myomodulin. In the model, the burst period decreased by 24% (8.7 s to 6.6 s) from the control simulation to the myomodulin simulation.

In the simulation of the Cs$^+$ treatment, the burst period was long compared to that of the control simulation. The burst characteristics were a burst period of 10.2 s, a burst duration of 4.5 s, and an interburst interval of 5.7 s. In Tobin and Calabrese (2005), the burst period increased by 24% in the half-center oscillator upon application of Cs$^+$. In our model, the burst period increased 17% (8.7 s to 10.2 s) from the control simulation to the Cs$^+$ simulation.

In the Cs$^+$ and myomodulin simulation, the burst period was short compared to that of the Cs$^+$ simulation. The burst characteristics were a burst period of 9.0 s, a burst duration of 3.8 s, and an interburst interval of 5.1 s. In Tobin and Calabrese (2005), the burst period decreased by 12% in the half center oscillator in bath with Cs$^+$ upon application of
myomodulin in addition to Cs\(^{+}\). In our model, the burst period decreased by 12\% (10.2 s to 9.0 s) from the Cs\(^{+}\) simulation to the Cs\(^{+}\) and myomodulin simulation. To show how myomodulin could coregulate the h-current and the Na\(^{+}/K\(^{+}\) pump current to maintain functional activity, we covaried the maximal conductance of the h-current and the maximal pump current over wide ranges. The simulation grid was in steps of 0.1 nA for \(\bar{g}_h\) and steps of 0.001 nA for \(I_{\text{pump}}^{\text{max}}\); \(\bar{g}_h\) was varied from 0 nS to 10 nS, and \(I_{\text{pump}}^{\text{max}}\) was varied from 0.3 nA to 0.5 nA (Figure 4.3).

Within this range, we observed periodic half-center oscillator bursting activity, asymmetric half-center oscillator bursting activity, plateau bursting, and silence. For values of \(I_{\text{pump}}^{\text{max}}\) roughly above 0.42 nA, the period of bursting generally monotonically decreased as \(\bar{g}_h\) increased up approximately 6 nS, where half-center oscillator bursting activity became asymmetric (one cell fired substantially longer bursts than the other). For values of \(I_{\text{pump}}^{\text{max}}\) below 0.42 nA and values of \(\bar{g}_h\) below 4 nS, we observed a nonlinearity in the dependence of period on \(\bar{g}_h\). For example, where \(\bar{g}_h\) is zero, the period of half-center oscillator bursting activity drops from 9.0 s where \(I_{\text{pump}}^{\text{max}}\) is 0.41 nA (notice that these are the parameters for the simulation of the myomodulin treatment described in a previous passage) to 7.0 s where \(I_{\text{pump}}^{\text{max}}\) is 0.398 nA. Then the burst period increased to 8.1 s where the periodic bursting regime terminated with \(I_{\text{pump}}^{\text{max}}\) equal to 0.369 nA. For values of \(\bar{g}_h\) greater than 4 nS, the period of activity generally decreased as \(\bar{g}_h\) increased and \(I_{\text{pump}}^{\text{max}}\) decreased.

We described a scheme for the coregulation of \(\bar{g}_h\) and \(I_{\text{pump}}^{\text{max}}\) based on the relation between the canonical condition and the myomodulin condition (Figure 4.3). By coordinating \(\bar{g}_h\) and \(I_{\text{pump}}^{\text{max}}\) on this line, we predicted the action of coregulation of \(\bar{g}_h\) and \(I_{\text{pump}}^{\text{max}}\) beyond the range described by experiments with myomodulin. For \(\bar{g}_h\) nS and \(I_{\text{pump}}^{\text{max}}=0.500\) nA, the period of activity increased from 8.7 s in the canonical condition to 10.3 s. For \(\bar{g}_h=6\) nS and \(I_{\text{pump}}^{\text{max}}\) =0.364 nA, the period of activity is 6.3 s. The model predicts that \(I_{\text{pump}}^{\text{max}}\) has a relatively narrow range for functional modulation, but \(\bar{g}_h\) is available for a wide range of variation. By continuing to follow this trend and increasing \(\bar{g}_h\), the range of bursting activity in increased to more than 6 nS in \(\bar{g}_h\) and more than 0.38 nA in \(I_{\text{pump}}^{\text{max}}\). For values of \(\bar{g}_h\) above 6 nS, we
fixed $I_{\text{pump}}^{\text{max}}$ at 0.364, and varied only $g_h$. The period decreased to 5.7 s when $g_h$ was 8 nS and then 5.4 s when $g_h$ was 10 nS.

### 4.5 Discussion

Biophysically accurate models are proven tools in neuroscience. They reproduce experimental results and provide strong predictive power (Bicanski et al., 2013; Cymbalyuk and Calabrese, 2001; Cymbalyuk et al., 2002; Negro et al., 2001; Doloc-Mihu and Calabrese, 2014; Grillner et al., 1995; Hill et al., 2001; Jasinski et al., 2013; Marder et al., 2014; Nadim et al., 1995; O’Leary et al., 2013, 2014; Olypher et al., 2006; Prinz et al., 2004; Weaver et al., 2010; Rybak et al., 2004; Ryczko et al., 2015; Sorenson et al., 2004). Models that account for multiple pharmacological treatments are expected to better represent the living cell. Different treatments are accounted for in the model by changes to appropriate biophysical parameters (for example, a Cs$^+$ treatment is modeled by a change in the conductance of the h-current). These treatments can include removal of subsets of certain currents eliciting characteristic oscillatory behaviors such as seizure-like activities or slow subthreshold oscillations (Barnett et al., 2013; Bicanski et al., 2013; Cymbalyuk and Calabrese, 2000; Cymbalyuk et al., 2002; Doloc-Mihu and Calabrese, 2014; Grillner et al., 1995; Jasinski et al., 2013; Krishnan and Bazhenov, 2011; Krishnan et al., 2014). In the sections focusing on monensin, our model captured and explained the changes that occurred for each of the burst characteristics (burst period, burst duration, interburst interval, and duty cycle) in each of the eight pharmacological conditions. We expressed these constraints as a thirty-two criteria (four burst characteristics multiplied by the eight pharmacological conditions) that provided a set of measures to compare modeled activity to experimental activity. Our model quantitatively reproduced twenty-seven of the thirty-two conditions. Although five modeled burst characteristics did not fall within the experimentally observed range, they still faithfully reproduced the observed trends of the experimental data. In the section focusing on myomodulin, we successfully reproduced the trends in cycle period reported in experiments (Tobin and Calabrese, 2005).
Analysis of our model explains how interplay between the h-current, the slow Ca\(^{2+}\) current, and the Na\(^+\)/K\(^+\) pump current produces changes in bursting activity in different pharmacological conditions. It allows us to elucidate the roles of these currents under normal and modulatory conditions. Previous studies of canonical models of the heart interneurons showed special roles of the h-current and the slow Ca\(^{2+}\) current (Doloc-Mihu and Calabrese, 2011; Hill et al., 2001; Olypher et al., 2006; Olypher and Calabrese, 2009; Sorensen et al., 2004). The inactivation of the slow Ca\(^{2+}\) current strongly controls the decay of spike frequency during a burst, which causes the decay in efficiency of synaptic inhibition delivered to the opposite neuron in a half-center oscillator and determines the moment of escape of the opposite cell. The moment of the escape of the opposite cell determines the termination of the burst. The inactivation of the slow Ca\(^{2+}\) current controls the burst duration in the cell in the spiking phase (Hill et al., 2001; Nadim et al., 1995; Olypher et al., 2006). In a canonical model of an isolated heart interneuron, the slow Ca\(^{2+}\) current underlies its bursting activity and the inactivation of this current plays the key role in burst termination. In a single heart interneuron and in a half-center oscillator, the removal of inactivation of the slow Ca\(^{2+}\) current is determined by the hyperpolarization and duration of the interburst interval. Through this indirect means of action, subthreshold currents can play a crucial role in determining the burst duration as well as the interburst interval. The h-current controls the escape of the heart interneuron by terminating its interburst interval in the isolated interneuron and the half-center oscillator. We show that the Na\(^+\)/K\(^+\) pump current is active over the entire cycle of bursting activity. In our study, the manipulations of the Na\(^+\)/K\(^+\) pump current and the h-current affected the burst duration and interburst interval either directly or through a mechanism involving the inactivation of the slow Ca\(^{2+}\) current. For example, our model revealed that the burst duration is directly affected by an increase in the Na\(^+\)/K\(^+\) pump current from the control treatment to the monensin treatment. These mechanisms are consistent with the experimental data and it strongly suggests that Na\(^+\)/K\(^+\) pump current could determine the burst duration in rhythm generating circuits.

Previous analysis of the canonical model of the heart interneuron demonstrated that
the h-current provides robustness to isolated bursters and to a half-center oscillator motif. The h-current is crucial in endogenously bursting dynamics (Cymbalyuk et al., 2002; Doloc-Mihu and Calabrese, 2011, 2014; Hill et al., 2001; Marin et al., 2013; Olypher et al., 2006; Olypher and Calabrese, 2009; Sorensen et al., 2004). The half-center oscillator motif provides robustness to perturbation of cellular dynamics (Cymbalyuk et al., 2002; Doloc-Mihu and Calabrese, 2014; Hill et al., 2001; Marin et al., 2013; Olypher et al., 2006; Olypher and Calabrese, 2009; Sorensen et al., 2004). Our modelling study confirmed these conclusions: bursting activity can tolerate a larger monensin rate parameter in the half center oscillator than in the single cell. Moreover, our results predicted that bursting activity is more robust against monensin treatment in the presence of the h-current. More specifically, the models tolerate the monensin rate parameter in the following order: half-center oscillator with an h-current ($\sim 10^{-3}$ s$^{-1}$), half-center oscillator without an h-current ($\sim 10^{-4}$ s$^{-1}$), isolated heart interneuron with an h-current ($\sim 10^{-4}$ s$^{-1}$), and isolated heart interneuron without an h-current ($\sim 10^{-5}$ s$^{-1}$). A higher value for the monensin rate constant would disrupt functional activity. The values used for the different experimental treatments still caused changes in modeled activity that capture the experimental data. The robustness of the half-center oscillator model with an h-current allowed us to use higher values of the monensin rate constant ($\sim 10^{-3}$ s$^{-1}$), which caused a dramatic change in the burst duration and the burst period. In the isolated heart interneuron model, the h-current played a protective role against changes in the interburst interval. The interburst interval did not change substantially from the bicuculline condition to the bicuculline and monensin condition. However, when the h-current was blocked, the application of monensin increased the interburst interval by more than a second. Similarly, when the h-current of isolated heart interneurons were blocked in our experiments, we observed that stimulation of the Na$^+$/K$^+$ pump with monensin led to an eventual transition from bursting activity in the bicuculline and Cs$^+$ condition to a silent (non-bursting) activity in the bicuculline, Cs$^+$, and monensin saline. This observation is consistent with our model’s prediction that the h-current provides a protective role against considerable influx of Na$^+$, such as that induced by monensins, that
could disrupt functional activity.

Recently, the Na\(^+\)/K\(^+\) pump current has been implicated in normal and pathological neuronal dynamics (Barreto and Cressman, 2011; Bazhenov et al., 2004; Canavier, 1999; Cressman et al., 2009; Forrest et al., 2012; Forrest, 2014; Krishnan and Bazhenov, 2011; Krishnan et al., 2014; Li et al., 1996). The Na\(^+\)/K\(^+\) pump current determines the mode of activity exhibited by Purkinje cells (Forrest et al., 2012; Forrest, 2014). It has also been implicated with critical roles in single neuron dynamics associated with seizures. Increasing intracellular Na\(^+\) could enhance the activation of the Na\(^+\)/K\(^+\) which then determines seizure termination (Krishnan and Bazhenov, 2011; Krishnan et al., 2014). Yu et al. (2012) modeled increased influx of Na\(^+\) through leaky Na\(^+\) channels due to membrane injury. They report that an increase in the Na\(^+\)/K\(^+\) pump current increases the frequency of bursting. In our model, the contribution of Na\(^+\)/K\(^+\) pump to the dynamics could be similar to the role of the leak current, but through interaction with h-current and slow Ca\(^{2+}\) currents, it brings direct and indirect nonlinear effects on burst characteristics.
**Figure (4.1).** Simulated activity of the model including h-current, Na\(^+\)/K\(^+\) pump current, and intracellular Na\(^+\) concentration. Caption continued on next page.
Figure (4.1). Simulated activity of the model including h-current, Na$^+$/K$^+$ pump current, and intracellular Na$^+$ concentration  (A) Simulated half-center oscillator activity under a control treatment with parameters $\bar{g}_h=4.9$ nS, $M=0$ s$^{-1}$ (A1). Simulated half-center oscillator activity under a monensin treatment with parameters $\bar{g}_h=4.9$ nS, $M=2.2 \times 10^{-3}$ s$^{-1}$ (A2). Sample traces represent membrane potentials (Vm) of both left (L) and right (R) oscillator heart interneurons as well as h-current, Na$^+$/K$^+$ pump current, and intracellular Na$^+$ concentration [Na]$^i$ of the right heart interneuron. (B) Simulated activity of one isolated oscillator heart interneuron under a bicuculline treatment with parameters $\bar{g}_h=4.9$ nS, $M=0$ s$^{-1}$ (B1). Simulated activity of the bicuculline plus monensin treatment with parameters $\bar{g}_h=4.9$ nS, $M=1.5 \times 10^{-4}$ s$^{-1}$ (B2). (C) Simulated half-center oscillator activity of the Cs$^+$ treatment with parameters $\bar{g}_h=0.1$ nS, $M=0$ s$^{-1}$ (C1). Simulated half-center oscillator activity of the Cs$^+$ plus monensin treatment with parameters $\bar{g}_h=0.1$ nS, $M=1.9 \times 10^{-4}$ s$^{-1}$ (C2). (D) Simulated activity of one isolated oscillator heart interneuron under a bicuculline and Cs$^+$ treatment with parameters $\bar{g}_h=0.1$ nS, $M=0$ s$^{-1}$ (D1). Simulated activity of the bicuculline and Cs$^+$ plus monensin treatment with parameters $\bar{g}_h=0.1$ nS, $M=3.8 \times 10^{-5}$ s$^{-1}$ (D2).
Figure (4.2). Simulated activity of the model including $h_{CaS}$, $m_{CaS}$, and $I_{CaS}$.

Caption continued on next page.
Figure (4.2). **Simulated activity of the model including** $h_{CaS}$, $m_{CaS}$, and $I_{CaS}$ (A) Simulated half-center oscillator activity of the control treatment with parameters $\bar{g}_h = 4.9$ nS, $M = 0 \text{ s}^{-1}$ (A1). Simulated half-center oscillator activity of the monensin treatment with parameters $\bar{g}_h = 4.9$ nS, $M = 2.2 \times 10^{-3} \text{ s}^{-1}$ (A2). Sample traces represent membrane potentials of both left (L) and right (R) oscillator heart interneurons as well as corresponding changes in the slow inactivation variable and the fast activation variable that gate the slow Ca$^{2+}$ current of the right heart interneuron. (B) Simulated activity of one isolated oscillator heart interneuron under bicuculline treatment with parameters $\bar{g}_h = 4.9$ nS, $M = 0 \text{ s}^{-1}$ (B1). Simulated activity of the bicuculline plus monensin treatment with parameters $\bar{g}_h = 4.89$ nS, $M = 1.5 \times 10^{-4} \text{ s}^{-1}$ (B2). (C) Simulated half-center oscillator activity of the Cs$^+$ treatment with parameters $\bar{g}_h = 0.1$ nS, $M = 0 \text{ s}^{-1}$ (C1). Simulated half-center oscillator activity of the Cs$^+$ plus monensin treatment with parameters $\bar{g}_h = 0.1$ nS, $M = 1.9 \times 10^{-4} \text{ s}^{-1}$ (C2). (D) Simulated activity of one isolated oscillator heart interneuron under a bicuculline and Cs$^+$ treatment with parameters $\bar{g}_h = 0.1$ nS, $M = 0 \text{ s}^{-1}$ (D1). Simulated activity of the bicuculline and Cs$^+$ plus monensin treatment with parameters $\bar{g}_h = 0.1$ nS, $M = 3.8 \times 10^{-5} \text{ s}^{-1}$ (D2).
Figure (4.3). Map of coregulation of $I_{\text{pump}}^{\text{max}}$ and $\bar{g}_h$ in the half-center oscillator. Colored points on the map indicate symmetric half-center oscillator activity. Color indicates the burst period from 4 s (dark purple) to 10 s (yellow). The labeled markers on the map correspond to experiments from Tobin and Calabrese (2005). The coordinates labeled Canonical correspond to the simulation of the control treatment. Similarly, the coordinates labeled My, Cs, and Cs$^+$My correspond to the simulation of the myomodulin treatment, the simulation of the Cs$^+$ treatment, and the simulation of the Cs$^+$ and myomodulin treatment. The arrows indicate the change in $I_{\text{pump}}^{\text{max}}$ and $\bar{g}_h$ from the control simulation to the myomodulin simulation and the change from the the Cs$^+$ simulation to the Cs$^+$ and myomodulin simulation. The dotted line indicates the coregulation of $I_{\text{pump}}^{\text{max}}$ and $\bar{g}_h$ extrapolated from the change in coordinates from the control simulation to the myomodulin simulation.
Rhythmic movements are controlled by oscillatory neuronal networks called central pattern generators (Marder and Bucher, 2001). A central pattern generator is a neuronal network that produces functional rhythmic output with or without feedback. The flexibility of the central nervous system relies on the ability of neurons to meet a breadth of temporal specifications. This ability is limited by transitions into dysfunctional regimes, such as seizure-like activity or depolarization block. Oscillatory neuronal networks, such as central pattern generators, maintain functional output over a wide range of cycle periods in order to produce appropriate behavior. The pattern of neuronal activity can change in different ways to accommodate changes to the environment or changes to behavioral goals. Pattern scaling occurs when a coordinated pattern is preserved while the cycle period changes. For example, the pyloric network maintains the relative phase of neuronal activity within the triphasic cycle in a fashion independent of cycle period (Hooper, 1997). Pattern adaptation occurs when a feature of the pattern is changed as the cycle period changes. An example would be control of gait in six-legged locomotion (Wilson, 1966; Graham, 1985; Daun-Gruhn and Toth, 2011).

These changes of patterns are commonly governed by neuromodulation. Neuromodulation provides control over regimes of neuronal activity by coordinating adjustments to the biophysical parameters of ionic currents. The maximal conductances and voltages of half-activation are prominent targets for neuromodulation (Luthi and McCormick, 1999; Rodgers et al., 2011b; Amendola et al., 2012). The exact number of ion channels or the exact magnitude of ionic conductances of specific neurons cannot be directly encoded into an animal’s genes. The pattern of expression of ionic channels is regulated for homeostasis of neuronal activity. Generically, mechanisms of homeostasis can be activity-independent or
activity-dependent. Activity-independent homeostasis relies on the pattern of gene expression to control cellular activity. The correlation of expression of ionic channels maintains functional neuronal activity (MacLean et al., 2003, 2005). The relative corellation of channel expression is controlled by ratios in the amount mRNA (MacLean et al., 2003; Schulz et al., 2006, 2007). Neuronal models have reproduced these results by showing critical correlations in maximal conductances (Taylor et al., 2006, 2009; Doloc-Mihu and Calabrese, 2011; Soofi et al., 2012; Doloc-Mihu and Calabrese, 2014). Using neuronal models, I have answered some key questions involving neuromodulation of neuronal activity: (1) what mechanisms determine the quantitative dependence of activity patterns on biophysical characteristics; (2) what mechanisms determine the range of functional activity, and (3) what mechanisms determine how multiple ionic currents are coregulated to ensure robust dynamics?

5.1 A codimension-2 bifurcation determines activity in a neuron model

Precise timing and reliability in neuronal activity are critical for functional behavior. Rhythmic motor functions are executed by precisely coordinated patterns of contracting muscles. Some rhythmic behaviors scale their pattern, maintaining phase relations across a wide range of periods. Among such behaviors, examples are the pyloric rhythm in Crustacea, crayfish swimmeret beating, the leech heartbeat, leech swimming, lamprey swimming, and crawling in the Drosophila larvae. We asked, how could a cell-autonomous process control pattern scaling or pattern adaptaion of neuronal activity within a network. We suggest that the biophysical properties of a neuron could navigate relative to two bifurcations; the neuron could coregulate the half-activation potentials of two intrinsic currents. This hypothesis is informed by two dynamical transitions described in bifurcation theory that are applicable to neuronal dynamics: the transition between bursting and tonic spiking is defined by the blue sky catastrophe and the transition between bursting and silence is defined by the saddle-node bifurcation on invariant circle (SNIC). In the blue sky catastrophe, the burst duration grows proportionally to the inverse of the square root of the bifurcation parameter as the bifurcation parameter approaches its critical value for bifurcation (Laing et al., 2003; Shilnikov and
In the SNIC, the interburst interval grows proportionally to the inverse of the square root of the bifurcation parameter as the bifurcation parameter approaches its critical value for bifurcation (Doiron et al., 2003; Shilnikov et al., 2008). The cornerstone bifurcation is a global codimension-2 bifurcation that satisfies the criteria for both the blue sky catastrophe and the SNIC. It provides a family of mechanisms that controls the temporal characteristics of neuronal activity. It determines independent control of the burst duration and interburst interval in endogenously bursting activity, the burst duration of individual evoked bursts in an endogenously silent neuron, and the delay before spiking after inhibition in an endogenously spiking neuron. These mechanisms could explain coregulation schemes that produce precise patterns according to the demands of the environment and behavioral choices of the animal.

5.2 Bistability of seizure-like bursting and silence

The action of neuromodulation is limited by the boundaries between different types of regimes. The modification of biophysical parameters can bring about dysfunctional activity. The onset of multistability is an example of a dangerous dynamics. A multistable neuron could act in a functional mode, but be vulnerable perturbations. For example, an endogenously silent neuron could switch to a periodically firing neuron with certain electrical stimulation. The notions that epileptic tissue can switch suddenly from normal to pathological activity and then from pathological to normal activity with the application of an artificial perturbation suggests that the two regimes could coexist. Neuronal circuits that exhibit seizure episodes have been shown to be prone to multistability (Foss and Milton, 2003). Coexistence of a stable oscillating seizure state and stable non-seizure state is a manifestation of bistability in the dynamics of neuronal networks. What determines the boundaries of functional or pathological activity? Coregulation of multiple ionic currents could be used to control the onset of multistability. The dynamical transitions, or bifurcations, that control the transition between regimes could be controlled or moved in order to ensure robust dynamics or to encourage multistability.
Multistability is a prevalent feature in a number of computational models of seizure dynamics (Hahn and Durand, 2001; Suffczynski et al., 2005; Frohlich et al., 2010; Krishnan and Bazhenov, 2011; Krishnan et al., 2014). Multistable dynamics are vulnerable to perturbations of the state and provide a potential opportunity for the treatment of epilepsy. Proposed treatments use electrical stimulation to terminate seizure episodes (Berenyi et al., 2012). Bifurcation control over the boundaries of pathological regimes could improve treatment efficacy by expanding the ranges of functional regimes.

We developed an experimentally relevant protocol to assess the vulnerability of single-cell seizure-like bursting to electrical perturbations. Single square pulses of current were applied to a periodically bursting model neuron. These simulations were tested to determine whether the pulse of current triggered a switch from bursting to silence. The time in the phase of the period, the amplitude, and the polarity of the pulse were systematically varied. For values of the conductance of the leak current close to the Andronov-Hopf bifurcation, which controlled the onset of bistability, there was a relatively small family of perturbations that could trigger a switch from bursting to silence. However, for values of the leak conductance that were far from this bifurcation, there was a larger family of perturbations that could trigger a switch from bursting to silence. In other words, as the leak conductance moved away from the Andronov-Hopf bifurcation, the cell became more vulnerable to external electrical stimulation.

5.3 The coregulation of multiple ionic currents in neuromodulation expands the range of functional activity

Neuromodulators act on a multitude of disparate biophysical target parameters. We investigated how neuromodulation coordinates the biophysical characteristics of individual cells to maintain or modify functional neuronal activity. Tobin and Calabrese (2005) showed that the application of the neuropeptide myomodulin acts on two biophysical characteristics in the leech heart interneuron: it increases the hyperpolarization-activated current and it inhibits the Na\(^+\)/K\(^+\) pump. Why are two currents targeted at the same time? We suggest
that myomodulin coregulates the hyperpolarization-activated current and the Na\(^+\)/K\(^+\) pump in order to modify the activity of the leech heart interneuron. We investigated how the pump interacts with other intrinsic currents to determine the temporal characteristics of bursting activity, and then studied the coregulation of the hyperpolarization-activated current and the Na\(^+\)/K\(^+\) pump current.

The Na\(^+\)/K\(^+\) pump is often thought to play a house-keeping role in neuronal dynamics; it maintains the Na\(^+\) and K\(^+\) gradients. However, there is growing evidence that the Na\(^+\)/K\(^+\) pump plays a critical role in neuronal dynamics. For example, the pump controls duration of swim episodes in the tadpole (Zhang and Sillar, 2012). It is implicated in the termination of seizure episodes (Bazhenov et al., 2004; Cressman et al., 2009; Krishnan and Bazhenov, 2011; Krishnan et al., 2014). The role of the pump could be experimentally assessed by pharmacological manipulations that are either biologically relevant or technically derived.

The Na\(^+\)/H\(^+\) antiporter monensin can be used to directly manipulate the intracellular Na\(^+\) concentration (Hill and Licis, 1982; Kueh et al., 2015). By increasing the intracellular Na\(^+\) concentration, monensin activates the pump. Application of monensin decreases the period of bursting in the leech heart half-center oscillator (Kueh et al., 2015). However, it only does so when the hyperpolarization-activated current is present. We introduced the Na\(^+\)/K\(^+\) pump current and Na\(^+\) dynamics to the canonical model of the leech heart interneuron and reproduced the results of experimental treatments with monensin, bicuculline, and Cs\(^+\). The application of myomodulin also decreases the period of bursting in half-center oscillator. This result is counterintuitive. The application of monensin and myomodulin both decrease the period of activity. But myomodulin inhibits the Na\(^+\)/K\(^+\) pump current where monensins activates it. In the model, we investigated how the hyperpolarization-activated current and the pump current could be covaried to reproduce the experimental results. Our analysis indicated that by coregulating the maximal pump current and the maximal conductance of the hyperpolarization-activated current, myomodulin could increase the range of biophysical parameters where the neuron exhibits functional half-center oscillator activity.
5.4 General Conclusions

The coregulation of multiple ionic currents provides precise control over the temporal characteristics of neuronal activity in the vicinity of bifurcations. The cornerstone bifurcation provides a family of mechanisms that control the temporal characteristics of bursting activity and transient neuronal responses with the coregulation of two complimentary ionic conductances. These mechanisms are applicable to pattern scaling and pattern adaptation in central pattern generators that control locomotion. We predict that the voltage of half-activation of a hyperpolarization-activated current could control interburst interval in accordance with the inverse-squre-root law, and we predict that the voltage of half-activation of a non-inactivating $K^+$ current could control burst duration in accordance with the inverse-squre-root law.

The coregulation of multiple ionic currents can be used as bifurcation control to ensure robust dynamics or expand the range of coexisting regimes. Properties of neuronal activity are determined by the proximity to transitions to different regimes. By covarying pairs of maximal conductances, the location of a transition can be changed. This type of bifurcation control could ensure functional neuronal activity or help eliminate pathological neuronal activity. We predict that enhancing the hyperpolarization-activated current and the non-inactivating $K^+$ or decreasing the persistent $Na^+$ current could increase the propensity of the leech heart interneuron to multistability.

The coregulation of multiple ionic currents in neuromodulation expands the range over which biophysical parameters support functional activity. Neuromodulators can target biophysical characteristics on different currents within a cell. By modulating complimentary pairs of currents, such as the hyperpolarization-activated and the $Na^+/K^+$ pump current, functional characteristics of neuronal activity could be preserved over a large range of parameter values.
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