Locomotor Network Dynamics Governed By Feedback Control In Crayfish Posture And Walking

Bryce Chung

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Sensorimotor circuits integrate biomechanical feedback with ongoing motor activity to produce behaviors that adapt to unpredictable environments. Reflexes are critical in modulating motor output by facilitating rapid responses. During posture, resistance reflexes generate negative feedback that opposes perturbations to stabilize a body. During walking, assistance reflexes produce positive feedback that facilitates fast transitions between swing and stance of each step cycle.

Until recently, sensorimotor networks have been studied using biomechanical feedback based on external perturbations in the presence or absence of intrinsic motor activity. Experiments in which biomechanical feedback driven by intrinsic motor activity is studied in the absence of perturbation have been limited. Thus, it is unclear whether feedback plays a role in facilitating transitions between behavioral states or mediating different features of network activity independent of perturbation. These properties are important to understand because they can elucidate how a circuit coordinates with other neural networks or contributes to adaptable motor output.
Computational simulations and mathematical models have been used extensively to characterize interactions of negative and positive feedback with nonlinear oscillators. For example, neuronal action potentials are generated by positive and negative feedback of ionic currents via a membrane potential. While simulations enable manipulation of system parameters that are inaccessible through biological experiments, mathematical models ascertain mechanisms that help to generate biological hypotheses and can be translated across different systems.

Here, a three-tiered approach was employed to determine the role of sensory feedback in a crayfish locomotor circuit involved in posture and walking. *In vitro* experiments using a brain-machine interface illustrated that unperturbed motor output of the circuit was changed by closing the sensory feedback loop. Then, neuromechanical simulations of the *in vitro* experiments reproduced a similar range of network activity and showed that the balance of sensory feedback determined how the network behaved. Finally, a reduced mathematical model was designed to generate waveforms that emulated simulation results and demonstrated how sensory feedback can control the output of a sensorimotor circuit. Together, these results showed how the strengths of different approaches can complement each other to facilitate an understanding of the mechanisms that mediate sensorimotor integration.

INDEX WORDS: central pattern generator, sensory feedback, feedback control, nonlinear oscillator, bifurcation dynamics
DEDICATION

This work is dedicated to my closest family and friends who have provided me with support and direction through graduate school. I would like to thank my parents, Lisa and Harrison, and sister, Ashlee, for their guidance over the past 29 years. I would also like to thank my grandmother, Clara Lee, whose spirit continues to push me to pursue my passions and dreams.

The root of my inspiration for research in neuroscience stemmed from my cousin, Alissa, who was born with severe cerebral palsy. She continues to bring our family warmth and laughter 26 years later.

*It was a curiosity brought about by our differences that instilled in me a passion to discover what makes us the same.*
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Special thanks to Dr. Robert Clewley who was my co-advisor and mentor throughout graduate school. I attribute much of my approach to understanding complex systems to Dr. Clewley and his work with the dominant scale system reduction technique (DSSRT). His ability to communicate between biologists and mathematicians, businessmen and data scientists, as well as musicians and programmers has been a guidepost for me as I have learned to articulate my research to different scientific audiences and to make science accessible to a general audience.

I would also like to extend a special thanks to Dr. Michael Black, with whom I worked for multiple semesters to assist in teach an undergraduate neurobiology lab. In working with Dr. Black, I learned how to be a better teacher and researcher while making an impact on the education and aspirations of undergraduates at GSU.

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research and his work with data visualization methods captured my attention and inspired a small side project that was not included in my dissertation.

Finally, I would especially like to thank the staff and custodians who ensure that our department runs day in and day out, without hesitation. In particular, Tenia Wright and Fatima Adams, Rob Poh, Alisa Norvelle, Vivian Ngo-Vu, Emily Hardy, Laverne, and Chou.

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<tr>
<td>ARIN</td>
<td>Assistance reflex interneuron</td>
</tr>
<tr>
<td>CB</td>
<td>Coxopodite-basipodite</td>
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<tr>
<td>CBCO</td>
<td>Coxobasal chordotonal organ</td>
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<tr>
<td>CMA</td>
<td>Cumulative Moving Average</td>
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<tr>
<td>CPG</td>
<td>Central pattern generator</td>
</tr>
<tr>
<td>DEP</td>
<td>Depressor</td>
</tr>
<tr>
<td>EHV</td>
<td>Extended Hill-Valley</td>
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<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>ISI</td>
<td>Inter-spike Interval</td>
</tr>
<tr>
<td>LEV</td>
<td>Levator</td>
</tr>
<tr>
<td>MN</td>
<td>Motor neuron</td>
</tr>
<tr>
<td>μmol</td>
<td>Micro-molar</td>
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<tr>
<td>n</td>
<td>Nerve</td>
</tr>
<tr>
<td>nA</td>
<td>Nano-amps</td>
</tr>
<tr>
<td>OXO</td>
<td>Oxotremorine</td>
</tr>
<tr>
<td>PAD</td>
<td>Primary afferent depolarization</td>
</tr>
<tr>
<td>PADI</td>
<td>Primary afferent depolarization interneuron</td>
</tr>
<tr>
<td>PS</td>
<td>Poisson Surprise</td>
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<td>SEM</td>
<td>Standard Error of the Mean</td>
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1 General Introduction

Neural motor systems are capable of producing a range of behaviors that are adapted in unpredictable environments by sensory feedback and other inputs, such as descending motor commands [1-3]. In vivo and in vitro experiments have helped to elucidate many of the neural components of sensorimotor networks and have helped to characterize a range of functional pathways and neural ensembles that include reflexes and central pattern generators [1, 2, 4]. Computational and mathematical models have complemented biological experiments by consolidating and simplifying what is known about a system in such a way so as to allow researchers to manipulate parameters or conditions that are otherwise inaccessible [5-7]. Based on biological and computational findings, robots have been engineered based on the biological and computational principles that have been identified and can help to synthesize or elucidate shortcomings of what is currently known [8, 9].

Because sensory feedback has been largely characterized by experiments in which a system is artificially perturbed [10, 11] or the biomechanic system is irreversibly decoupled from neural motor activity [12, 13], it is not clear how sensory feedback and neural motor networks interact to produce multiple adaptable behaviors. While evidence suggests that sensory feedback can be regulated in a state-dependent fashion [11, 14], it may be the case that sensory feedback itself mediates transitions between sensorimotor network output, such as changes in gait [15].

1.1 General Approach

In the work presented here, a three-pronged approach is employed to characterize the role of sensory feedback in crayfish posture and locomotion. An in vitro brain-machine interface was used to run experiments in which biomechanical feedback could be decoupled
or re-coupled to neural motor output. Simulations of the neuromechanical system including a biomechanical leg model as well as a simplified model of the neural network were used to manipulate experimental parameters that were inaccessible through an *in vitro* approach. Finally, a phenomenological mathematical model was used to illustrate how feedback can control the output of a sensorimotor network. Together, results from *in vitro* experiments, computational simulations, and the mathematical model help to illustrate that sensory feedback can mediate its effect while conserving the dynamics of the underlying structure. These results, however, do not exclude the possibility that feedback changes the underlying dynamics of the network. Additional experiments and simulations are required to discriminate between these two possibilities.

The multi-pronged approach employed here establishes an algorithmic methodology that can be used to understand the mechanisms of sensorimotor integration across a wide range of motor control systems (Fig. 1.1). While *in vitro* experiments guide construction of computational models, those models help to characterize the effect of parameters in domains that are otherwise inaccessible biologically. The results of simulations can then be used to develop mathematical models that require a resolution of sampling that is not achieved in biological experiments. Mathematical models can then help to develop hypotheses and guide simulations of the motor system. Finally, results from both mathematical models and simulated networks can inform and help to refine hypotheses that can be tested in the biological system. In addition, the computational models and mathematical systems can be used to test whether similar principles apply in other motor systems.
Figure 1.1. Schematic of multi-pronged approach
Example advantages (first bullets) and disadvantages (second bullets) from different approaches that can complement each other to facilitate a stronger understanding of biological mechanisms that govern sensorimotor integration.
1.2 Sensorimotor Integration During Posture & Locomotion

Sensorimotor circuits integrate information from the environment with ongoing motor activity to adapt behaviors in order to accomplish a task [1, 2, 4]. During postural behaviors, resistance reflexes act to oppose perturbations and stabilize a body [16, 17]. Mediated by proprioceptors, such as chordotonal organs, biomechanical movements of a joint are transduced to neural networks via sensory neurons, called afferents [1]. On the other hand, when an animal executes a voluntary movement, activation of a resistance reflex may result in unfavorable outcomes [18]. Consequently, many motor systems have mechanisms in which resistance reflexes are blocked during voluntary movements. For example, in a crayfish locomotor control circuit resistance reflexes are blocked by primary afferent depolarization during a behavioral state that produces rhythmic bursting known as fictive locomotion [1, 19]. In crayfish, cats, and stick insects reflexes are blocked by synaptic inhibition by interneurons and motor neurons [1, 4, 20-22]. While resistance reflexes oppose movements, assistance reflexes amplify movements and are observed during behaviors such as locomotion. During locomotion, assistance reflexes are triggered at transitions between stance and swing phases of a step cycle and help to move a limb faster than if controlled by a central motor system alone [2, 23].

While these examples illustrate the functionality of resistance and assistance reflexes in different states and behaviors, less is known about their role in mediating dynamic network output or how their effect changes in different behavioral states. In addition, it remains unclear whether sensory feedback is an integral part of changing network dynamics or if it acts in concert with a network to preserve existing dynamics.
1.3 Neural Motor Control Circuit

The behavioral output of a motor network is a result of interactions between the properties of constituent neurons as well as feedback from the biomechanical system and inputs from the central nervous system, such as descending motor commands [1, 3]. A motor network activates muscles via motor neurons that move limbs around joints and sensory receptors that span joints, such as proprioceptors, are stretched or released as a limb moves. Afferents report stretch and release of proprioceptors to the motor network and can affect its output, such as triggering a reflex response [16].

1.3.1 Central Pattern Generators

In many cases, motor neurons can produce rhythmic bursting independent of inputs [24, 25]. These ensembles of neurons can also include interneurons and are called central pattern generators (CPG). Depending on the interactions between ionic currents, a motor neuron can exhibit a variety of properties that includes how they detect features of a stimulus as well as how a stimulus can alter their spiking rhythm [26-29].

One common property that has been studied is how a neuron responds to the frequency of its inputs. In some cases, a neuron will generate an action potential when it receives a train of inputs that are greater than a minimum frequency [30]. In other cases, a neuron can generate an action potential when the frequency of its input falls within a certain range [31]. Consequently, a neuron that receives inputs from a variety of sources, such as multiple afferents, can act as a filter and select certain features to which it responds [26, 27].

Another property is how the bursting or spiking rhythm of a neuron responds to perturbations via injections of current as measured by a phase response curve (PRC) [30, 32]. A Type I PRC indicates that a neuron will always delay its response to a perturbation. A Type II PRC indicates that a neuron either delays or advances a spike or burst following a
perturbation. The type of PRC that a neuron exhibits can indicate how well it will synchronize or coordinate with other neurons [30, 32]. Consequently, a motor network can respond differently to the same afferent feedback or descending commands depending on its intrinsic dynamics [33].

In the locomotor control circuit studied here, levator (Lev) and depressor (Dep) motor neurons (MN) raise and lower a crayfish walking leg around its second joint, respectively [1]. Both pools of motor neurons inhibit each other and have been studied in a quiescent state as well as a rhythmically bursting state. While descending motor commands controlling the state of the circuit have been identified [3], different states of the circuit are induced using a cholinergic agonist, oxotremorine (OXO) [25]. Thus, the Lev and Dep MNs comprise a CPG that sets up dynamic properties for the locomotor control circuit (Fig. 1.2).

1.3.2 Sensorimotor Integration

Sensory feedback can come from a variety of sources including stretch receptors, stress detectors, and force detectors and can modulate or change the behavior of a motor network [1, 15]. For example, force detectors at the point of contact of walking limbs can modulate a network by entraining a CPG and triggering transitions between the swing and stance phases of a step cycle [2]. Stress detectors, on the other hand, have been found to mediate a change in the coordination of limbs during locomotion resulting in a different gait [15]. While these two mechanisms may not be mutually exclusive, they can have vastly different effects on how a system behaves. For example, when sensory feedback modulates network output it can advance or delay the rhythm of a CPG depending on its PRC type but it does not change the type of PRC [8, 34]. On the other hand, it has been shown in neurons and nonlinear oscillators that feedback can change the type of PRC and consequently affect how it interacts with other systems [35].
Figure 1.2. Schematic of crayfish locomotor circuit
Motor neurons and interneurons comprise a neural motor central pattern generator (Motor CPG) that produces rhythmic output. Levator motor neurons (Lev MN) and depressor motor neurons (Dep MN) drive up and down movements around the second joint of a crayfish walking leg, respectively. Stretch and release of a sensory receptor that spans the joint are reported by sensory afferents. Reflex pathways are mediated via afferents and interneurons. A resistance reflex is elicited via a monosynaptic pathway and is inhibited by primary afferent depolarization interneurons (PADI). Assistance reflexes are mediated via a disynaptic pathway by assistance reflex interneurons (ARIN). Similar reflex pathways mediate Dep resistance and Dep assistance reflexes.
In the locomotor circuit studied here, a stretch receptor, called the coxabasal chordotonal organ (CBCO) reports upward and downward movements as it is released and stretched, respectively. CBCO afferents respond to both the position and rate of movement of the joint and can be separated into stretch-sensitive afferents and release-sensitive afferents [1]. When the network is in a postural state, CBCO afferents trigger resistance reflexes in response to external perturbations to the leg. When a fictive locomotion state is induced, however, external perturbations can yield assistance reflex responses [1]. While these responses are thought to facilitate behaviors that are adaptable in different environments, it remains unclear whether they change the dynamics of the network between states and in response to perturbations.

1.4 Mathematical Models

In order to understand the mechanisms through which feedback affects sensorimotor circuits, nonlinear dynamical systems can be used [17]. Implemented extensively in characterizing the mechanisms that mediate different forms of action potentials [36, 37], nonlinear systems provide a structure within which to develop the relations between a nonlinear oscillator and feedback.

1.4.1 Nonlinear Oscillators

Nonlinear oscillators are defined by a system of ordinary differential equations and describe how a system evolves over time [38]. In neuronal models, the membrane potential is represented by a state variable, $x$. Additional variables are then used to capture the dynamics of ionic currents and synaptic inputs [30]. In the Hodgkin-Huxley model [39], for example, dynamics of the membrane potential are described by a nonlinear interaction between a depolarizing sodium current and a hyperpolarizing potassium current [39]. Together, they can produce oscillations that correspond to an action potential. These mathematical models can be
derived by directly measuring biological features such as ionic currents and synaptic potentials. Alternately, the dynamics of a neuron can be approximated by measuring properties such as a frequency-stimulus curve or a phase response curve.

1.4.2 Bifurcation Analysis

The advantage of using a mathematical system to model the dynamics of an action potential is that it can be analyzed using a suite of mathematical tools. One of those tools is called a bifurcation analysis in which a system is studied to determine how its stability changes under different conditions [30, 38]. The Hodgkin-Huxley model, for example, has a single stable fixed point that corresponds to a hyperpolarized membrane potential in the absence of a stimulus. When the system is stimulated by an ionic current, however, the dynamics change and the stable fixed point becomes unstable while giving rise to a stable oscillation, called a limit cycle. This type of bifurcation is called a Hopf bifurcation and corresponds to a Type II PRC as well as a discontinuous frequency-stimulus graph [30].

Another bifurcation that is commonly encountered in neuronal dynamics is called a saddle node on invariant circle (SNIC). In this case, a stable fixed point and an unstable fixed point coalesce and disappear while leaving behind a stable limit cycle (the invariant circle). The dynamics of a SNIC yield a Type I PRC and a continuous frequency-stimulus graph [30]. As illustrated in a previous section, the differences between Hopf and SNIC dynamics can result in different coordinating capabilities and response properties of a nonlinear oscillator, such as a neuron or CPG.

1.4.3 Nonlinear Oscillators with Feedback

In a neuronal model, feedback is included in a number of ways including synaptic currents or ionic currents [30]. For example, depolarization-activated depolarizing currents generate positive feedback on a membrane potential. On the other hand, a depolarization-
activation hyperpolarizing current generates negative feedback on a membrane potential. Thus, different combinations of ionic currents can yield different bifurcation structures [26, 30].

The control of bifurcations using feedback can also be accomplished using a filter that transforms the output of a system and returns it as an input. For example, the gain of a low-pass filter can be used to control a bifurcation in the Hindmarsh-Rose neuron model [40]. Other examples also include autapses in which a neuron provides synaptic input to itself [41]. In this way, it is possible to determine whether the system undergoes a Hopf or a SNIC bifurcation, which can change how it responds to inputs. Using this approach mathematical models help to identify general mechanisms that can be used to determine how a biological system behaves. In the case of sensorimotor circuits, mathematical models can be used to reproduce the rhythmic bursting of a CPG and can be modified to take into account the effect of sensory feedback.

1.5 Applying a Multi-Pronged Approach

In the work presented here a multi-pronged approach was used in order to characterize the role of sensory feedback in a sensorimotor system. First, a novel in vitro brain-machine experiment was developed using the neuromechanical simulation software, AnimatLab [42], to determine whether sensory feedback changed the output of a locomotor circuit that controls the second joint of a crayfish walking leg. In addition to simulating movements of a virtual leg due to neural activity, AnimatLab enabled a transduction of in vitro signals to control virtual neurons that excited the virtual muscles. The software also enabled the virtual movements of the leg to be transduced to an in vitro stretch receptor. This provided a means through which the effect of opening and closing the sensory feedback loop could be tested.

Because of the inherent complexities of in vitro systems, however, isolating the effect of resistance versus assistance reflexes was not feasible. Consequently, a computational model
was employed to control variables that were biologically inaccessible. Based on in vivo and in vitro experiments largely outlined in [1] a neuromechanical model was built in AnimatLab that could reproduce the results from the in vitro brain-machine experiments as well as previously characterized resistance reflexes. Together, this multi-pronged approach connected analyses at multiple levels to determine how sensory feedback controls the behavior of a locomotor control circuit. An in vitro preparation coupled to a biomechanical simulation yielded a biologically meaningful behavior of the system and provided data that was used to validate a neuromechanical model of the system. A neuromechanical model of the hybrid in vitro experiment helped to test hypotheses of how the effect of resistance and assistance reflexes are integrated into the locomotor network. In addition, simulations provided additional data that was used to develop a mathematical model of the system. Finally, a mathematical model of the system was used to develop a hypothesis about the dynamical mechanism through which sensory feedback controls locomotor output.

While the computational model enabled system parameters to be sampled with much greater resolution and control than in vitro experiments, making a determination about the mechanisms of the underlying dynamical structure can be difficult as system dynamics are still characterized by sampling individual points in parameter space. Consequently, in order to determine how sensory feedback changes the output of a sensorimotor system, simulation results from the computational AnimatLab model were used to develop a phenomenological mathematical model that reproduced qualitative features of the in vitro system and computational model. The mathematical model can now be used to develop hypotheses about how the system responds to perturbations or coordinates with other systems and those hypotheses can be tested through simulation or in vitro experiments.
1.6 Dissertation Outline

In the Chapter 2, the results from a novel *in vitro* brain-machine interface experiment are presented. By using a virtual model of the biomechanics of a crayfish leg, experiments could be in two conditions. The first was when movements of the leg were coupled to motor neuron activity and the second was when movements of the leg were decoupled from motor neuron activity. Experiments showed that the motor network was capable of reproducing reflex responses as well as rhythmic bursting corresponding to fictive locomotion while using the *in vitro* brain-machine interface. In addition, these results indicated that closing the sensory feedback loop by coupling biomechanical feedback to motor network activity increased the frequency of bursting in the network.

In Chapter 3, a neuromechanical model of the *in vitro* brain-machine interface experiments was constructed in order to test the hypothesis that the increased burst frequency of the motor network was due to positive feedback. The model was a simplified representation of *in vitro* results [1] and was capable of reproducing reflex responses as well as rhythmic bursting. Simulation results showed that blocking assistance reflexes reduced the burst frequency of the network and was consistent with other locomotor systems in which assistance reflexes help to facilitate transitions between stance and swing during step cycles of a leg [2].

In order to determine how sensory feedback changes the output of the sensorimotor circuit, it was necessary to run a large number of simulations of the neuromechanical model and to sample the parameter space in a structured way. Consequently, it was critical to develop an efficient way of classifying network activity as quiescent, bursting, or tonically spiking. In Chapter 4, a novel spike train analysis algorithm is presented. The Extended Hill-Valley analysis method used fluctuations in a smoothed, history-dependent analysis signal that was derived from a neural spike train. Briefly, rapid fluctuations were classified as bursts and
slow, maintained fluctuations were determined to be tonic spiking. The algorithm was tested on a variety of spike trains and its performance was qualitatively as well as quantitatively compared to two other methods that are commonly used.

In Chapter 5, a database of simulations was run in order to determine how the network behaved when the motor CPG was activated separately from resistance and assistance reflexes. This was not possible to do with in vitro experiments. In addition, simulations were run in one of two biomechanical coupling conditions. The first was when biomechanical feedback was based on movements of the virtual leg that was driven by activity of the MN CPG. The second condition was when biomechanical feedback was decoupled from MN activity. Consequently, simulation results illustrated a separable contribution of the MN CPG, resistance reflexes, and assistance reflexes to changes in network activity. While the MN CPG produced two activity regimes (quiescence and rhythmic bursting), reducing the strength of resistance reflexes and increasing the strength of assistance reflex interneurons (ARINs) resulted in three activity regimes (quiescence, rhythmic bursting, and tonic spiking). Additional simulations separated the effect of sensory feedback and showed that resistance reflexes mediated short bouts of tonic spiking (different than the tonic spiking regime) in the quiescent and rhythmic bursting regimes while ARIN activation increased the burst frequency of the network and induced a tonic spiking regime.

In Chapter 6, simulations were used to determine whether the balance of resistance and assistance reflexes was able to reorganize network dynamics. Sets of simulations were run under three levels of CPG activation corresponding to quiescence, rhythmic bursting (fictive locomotion), and tonic spiking. In each set of simulations, the strength of resistance and assistance reflexes were varied independently by changing the activation of primary afferent depolarizing interneurons (PADIs) or the activation of ARINs. CPG activity states were classified by analyzing simulations that were run when biomechanical feedback was decoupled from
motor output. When the biomechanical feedback loop was closed, results showed that sensory feedback reorganized network dynamics if the CPG was in a quiescent or rhythmic bursting state. In addition, sensory feedback induced a region of short bouts of tonic spiking when there was an imbalance between resistance and assistance reflexes. There was no clear change in network dynamics when the CPG was in a tonically active state in the biomechanically decoupled condition.

Finally, in Chapter 7 a mathematical model is presented in order to determine whether sensory feedback is changing the dynamics of the underlying MN CPG. The mathematical model reproduced qualitative features of the neuromechanical model that included the three regimes induced by sensory feedback as well as the two regimes created by the CPG alone as described in Chapter 5. In addition, examples of simplified feedback dynamics were presented to illustrate how the mathematical model can account for a range of behaviors observed for different ratios of positive negative feedback mediated by assistance and resistance reflexes, respectively. A bifurcation analysis of the mathematical model showed that sensory feedback was not changing the underlying structure of the bifurcations, but, instead, was moving the operating point of the network around the bifurcations set up by the central oscillator.
2 The Effect of Sensory Feedback on Crayfish Posture and Locomotion: I.

Experimental Analysis of Closing the Loop


As primary author, my contributions to this work include:

- Set up of the hybrid experimental preparation and electrophysiology rig
- Running 4 of the 6 experiments analyzed in the results
- Analysis of all 6 experimental results
2.1 Introduction

Although real-time sensory feedback is critical for the correct performance of nearly all behavior, its role in producing motor output from the central nervous system has been difficult to study [18]. This is largely a technical problem: it is difficult to reversibly compare the effects of closed (i.e., intact) and open feedback loops on motor output in the same preparation. To address this problem in the present study, we developed a hybrid neuromechanical preparation in which an isolated crayfish ventral nerve cord drives the movements of a computational neuromechanical model of the crayfish thorax and leg. Movements of the leg then excite appropriate sensory afferents to provide real-time feedback to the nerve cord. A comparison of motor responses when the real-time feedback loop is intact to responses when it is open revealed that sensory feedback has large and immediate effects on rhythmic locomotor patterns. These results highlight the importance of sensory feedback in forming and shaping motor output.

2.1.1 Walking, Posture, and Reflexes

Walking is a behavior that results from interactions between central pattern generators (CPGs), which produce a rhythmic motor pattern and sensory feedback, which modulates the pattern [13, 43]. For example, during stepping in cats, the stance-to-swing transition is promoted by the responses of sensory afferents to flexion of the hip and decreased tension in the ankle extensor muscle [2, 21, 22]. Similar reflex mechanisms regulate the stance-to-swing transition in stick insects [44, 45] and can entrain locomotor CPGs in crayfish [10, 46].

The reflexes that entrain crayfish CPGs include both negative feedback, “resistance” reflexes that help resist leg perturbations, and positive feedback “assistance,” reflexes that enhance on-going movements [47-49]. However, it is difficult to determine experimentally in a dissected preparation how reflexes contribute to locomotion when the reflex feedback loop is
open and motor discharges do not result in sensory feedback [16]. The difficulty can be overcome by using electromechanical devices to stimulate the appropriate sensory afferents in response to the motor output and thereby close the sensorimotor feedback loop [50, 51]. Here we use a computational neuromechanical model of a crayfish leg to provide a virtual periphery for an in vitro crayfish ventral nerve cord and produce real-time sensory feedback to its motor output. With this hybrid preparation, we show how sensory feedback from elevation and depression movements of a crayfish leg modulates the levator/depressor CPG by regulating transitions between different phases of the step cycle.

2.1.2 Closing the Sensory Feedback Loop Experimentally

We studied the reflex and CPG interactions in crayfish using an electronic interface that coupled an in vitro nerve cord preparation to a computational neuromechanical model of a crayfish leg. Levator (Lev) and depressor (Dep) motor nerve activity evoked up and down model leg movements that released and stretched a model stretch receptor, the coxobasal chordotonal organ (CBCO). The interface then transmitted the model CBCO length changes to a speaker-driven probe attached to the live CBCO, an elastic strand with afferents that respond to leg depression (stretch) and elevation (release) (Fig. 2.1). Excited CBCO afferents projected their activity back through the CBCO nerve to the ventral nerve cord.

We used this hybrid preparation to measure the effects of reafference in quiescent preparations, where only resistance reflexes could be evoked, and in active preparations, where both assistance reflexes and spontaneous Lev/Dep burst pairs occurred in addition to resistance reflexes. We found that the sensory feedback loop excited a rhythmic pattern of Lev/Dep burst pairs that ended immediately upon opening the feedback loop. In addition, we found that sensory feedback changed the structure of Lev/Dep burst pairs by making them shorter and shortening the duration of the Lev burst relative to the Dep burst. Both the higher
burst pair frequencies and the restructured burst pairs appear to result from the positive feedback that mediates the assistance reflexes.

2.2 Materials and Methods

2.2.1 In vitro Preparation and Extracellular Recordings

Adult crayfish (*Procambarus clarkii*) of either sex were obtained from a commercial supplier (Atchafalaya Biological Supply, Raceland, LA) and maintained communally in laboratory aquaria until use. Experimental animals were gradually chilled to 3°C until immobile and unresponsive, when their thoracic and abdominal portions of the ventral nerve cord were prepared as before [52, 53]. The distal segments of the right or left fifth walking leg were pinned out in a Sylgard-lined dish (Dow-Corning) with the associated motor and sensory nerves (Fig. 2.1, Crayfish in vitro nerve cord). The third, fourth, and fifth thoracic ganglia were desheathed and maintained in a bath perfusion of crayfish saline [52]. Steel extracellular pin electrodes were used to record activity from the Lev and Dep motor nerves as well as the CBCO sensory nerve. Extracellular activity was amplified (A-M Systems) and digitized (Micro3 1401; Cambridge Electronic Design) for online data processing using Spike2 (Cambridge Electronic Devices). The proximal end of the CBCO (closest to the sensory cells) was fixed to the Sylgard bottom of the Petri dish, while the distal end was attached to a probe that was driven in real time by the electronic interface.

2.2.2 Electronic Interface Between the Nerve Cord and Model

A custom electronic interface transmitted voltage signals between the *in vitro* nerve cord and the AnimatLab biomechanical leg model (Fig. 2.1, Interface).¹ A Spike2 sorting

¹ To distinguish model elements from their real counterparts, all model element names are italicized.
**Figure 2.1. The in vitro hybrid preparation**

*Crayfish leg and body model (left):* an oblique frontal view of the *crayfish thorax* and *leg* model, with an inset showing an expanded view of the *thorax* and proximal portion of the 5th *leg*. *Phasic* and *tonic depressor muscles* (red and pink, respectively) and *Levator muscles* (blue and light blue, respectively) are shown with their origins on the *Thorax* or *Coxa* and their insertions on the *Basis*, below and above, respectively, the *coxopodite-basipodite joint* (CB joint; white “X”). The *coxobasal chordotonal organ* (CBCO) stretch receptor (gold) spans the *CB joint* between the *Coxa* and *Basis* in parallel with the *Levators*. The Interface (middle): stretch (brown) and release (green) of the model CBCO is transduced to the *in vitro* CBCO via a probe connected to a speaker. The Interface also transduces extracellular depressor (Dep) and levator (Lev) nerve activity as digitized impulses to virtual inputs in the *leg* model (red, pink, blue, and light blue neurons). A switch depicted in the Interface allows the model *leg* to be decoupled from extracellular activity to open the loop. *Crayfish in vitro nerve cord* (right): 3rd (T3), 4th (T4), and 5th (T5) thoracic ganglia and first abdominal (A1) ganglion are isolated along with the proximal motor nerves of the 5th walking leg. Pin electrodes record the activity in the CBCO nerve (CBCO n) and Dep and Lev nerves (Dep n and Lev n, respectively). Oxotremorine (OXO) is perfused across the nerve cord.
algorithm distinguished large phasic Dep and Lev motor neuron (MN) spikes from smaller phaso-tonic and tonic MN spikes according to amplitude and generated large or small 2-ms voltage pulses in real time for each detected spike. The large and small voltage pulses were applied to the AnimatLab leg model to excite the phasic and tonic muscles, respectively, and cause the leg to move. The resulting down or up leg movements stretched or released the model CBCO. The digital length of the CBCO model was converted to an analog output, amplified by a DC power amplifier, and applied to drive a loudspeaker attached to the probe. Movements of the probe produced in real time the same length changes in the live CBCO as occurred in the model CBCO. Tests using a light beam interrupted by movement of the probe showed that the probe could follow faithfully the fastest movements commanded by the changing length of the model CBCO.

2.2.3 Biomechanical Crayfish Leg Model

A model of a crayfish thorax and fifth walking leg was constructed in AnimatLab v1 (www.AnimatLab.com) to reproduce the biomechanical responses of a real crayfish leg. All the parameter values and structural arrangements can be seen in the model file, “Crayfish hybrid experimental model,” which is available in ModelDB (https://senselab.med.yale.edu/ModelDB/). The ModelDB accession number for this model is 150697. (A list of all parameter values is in the file Model Parameters Supplemental Material.xls in Supplemental Materials; see ENDNOTE). The biomechanical crayfish leg and thorax model was constructed from optical scans and mass measurements of a 9 cm crayfish. The animal was killed after cold, and the cephalothorax and segments of the fifth walking leg were separated and individually weighed and optically scanned (NextEngine). The wireframe mesh files were imported to AnimatLab v1 and connected by hinge joints to recreate the fifth walking leg attached to the thorax (Fig. 2.1, Crayfish leg and body model). The density of each model leg segment was assumed to be
uniform. During simulation, the thorax was fixed under water and the leg was free to move vertically around the full extent of the CB joint (coxopodite-basipodite joint) subject to muscular forces, gravity, and drag, all of which were simulated in AnimatLab v1. Other leg joints were immobile. AnimatLab simulations use two solvers that solve simultaneously the underlying equations that describe the neural responses and the biomechanical interactions as they evolve in time. The neural responses are produced by a custom-built solver that uses an exponential Euler method for numerical integration [54], while the biomechanics equations are solved with the Vortex simulator from CM-Labs (Montreal).

Active movement of the leg around the CB joint is produced in the crayfish by the levator and depressor muscles, which contain four and five muscle heads, respectively, including both phasic and tonic muscle fibers [55]. Separate muscle models were used to represent the tonic and phasic portion of each Lev and Dep muscle head (Fig. 2.1, Crayfish leg and body model). They were attached in parallel to a common origin and insertion in the model placed according to the origin and insertion of the corresponding muscle head in vivo [55].

The biomechanical properties of the muscles were represented by Hill models, each of which contains a serial spring coupled in series to the parallel combination of a spring, a dashpot, and a force actuator [56]. The length-tension curves and serial and parallel spring constants were adapted from an analysis of the crayfish leg extensor muscle [57, 58]. Phasic muscles were given spring constants and maximal actuator force values equal to five times those of tonic muscle models of the same cross-sectional area and from the same muscle head [59]. The spring constants and actuator values of each model were then set proportional to the cross-sectional area of the corresponding tonic or phasic portion of the crayfish muscle head that it represented [55]. The spring constants and forces of all the muscle heads were scaled again to enable the leg to move up and down in a fashion similar to those of live animals that are held by the thorax underwater (unpublished observations). The spring constants and
dashpot constants of a Hill model help determine the muscle force time constants [56]. Given those spring constants, the tonic and phasic muscle dashpot constants were set to produce muscle force time constants in the range of those found in other crustaceans, because they are unknown for crayfish [57]. The electrical properties of the tonic and phasic muscle models were represented by single RC electrical compartments with a resting potential of -70 mV, and electrical parameter values were based on measurements of corresponding leg muscles of related decapod crustaceans [60]. The actuator force of each muscle was set as a sigmoidal function of muscle membrane potential such that 10 to 90% of maximum force was generated by membrane potentials between -65 and -25 mV.

The large and small pulses produced by the interface in response to large and small levator and depressor motor nerve spikes were used to excite the model levator and depressor phasic and tonic muscles (Fig. 2.1). Each nerve’s pulses were applied to a levator or depressor input neuron, which transmitted only the large pulses directly to the phasic muscles to evoke large, nonfacilitating excitatory postsynaptic potentials (EPSPs). These EPSPs were modeled after similar EPSPs recorded in the phasic extensor muscle of the crayfish leg [61]. Each input neuron also transmitted both large and small pulses to a levator or depressor tonic motor neuron. To prevent the tonic neuron from firing in response to the large pulses, the phasic muscle inhibited the tonic neuron for the duration of each phasic pulse. Consequently, the tonic neuron fired only in response to small pulses triggered by tonic MN spikes. The tonic neurons excited the tonic muscles through facilitating, conductance-based synapses that produced facilitating EPSPs modeled after those recorded in the tonic extensor muscles of the crayfish leg [61].
2.2.4 Experimental Procedure

The *in vitro* preparation was bathed in continuously introduced (5.5 ml/min) chilled, aerated saline. Extracellular recording was continuous, with experimental trials separated by at least 2 min. Before the series of trials, the probe was adjusted to set the CBCO length to equal that of the model CBCO at rest, when the *leg* was horizontally extended at the beginning of a simulation. An experimental trial consisted of a simulation that began with the motor connection to the model open or closed. The model *leg* immediately dropped under its gravitational load at the beginning of a simulation; the fall of the *leg* stretched the CBCO. The CBCO stretch caused the real CBCO to be identically stretched; the stretch-sensitive CBCO afferents elicited reflex responses recorded from the motor nerves. Simulation periods typically lasted 60 or 120 s during which the model ran in real time, ±5%. Under open loop conditions, the recorded Lev and Dep motor nerve spikes had no effect on the model muscles and the *leg* remained depressed under gravity. Under closed loop conditions, Lev and Dep MN spikes evoked contractions in the corresponding model muscles, producing *leg* elevations or depressions. These produced corresponding releases and stretches of the CBCO and consequent CBCO afferent responses (Fig. 2.1, green and beige arrows, respectively). At the end of each simulation, the probe immediately returned to its starting position and the CBCO returned to its original resting length.

2.2.5 Oxotremorine Application

Isolated nerve cords from seven animals were exposed to oxotremorine (OXO; Sigma), a muscarinic cholinergic agonist, by replacing the normal saline flow into the Petri dish with 10 or 50 μM OXO in chilled, aerated saline (Fig. 2.1). The flow was maintained for ~30 min and then replaced with a saline flow to wash out the OXO.
2.2.6 Statistical Analysis of Bursting

Open and closed loop conditions were randomly assigned to each trial of the series conducted during the hybrid preparation experiment on each animal. Experimental data were selected from sequential trials that included open and closed loop runs during experiments on each of six preparations. Motor bursts were defined using the Poisson Surprise method [62] in DataView (see http://www.st-andrews.ac.uk/~wjh/dataview/) based on threshold-detected phasic units of our extracellular recordings. Levator/depressor and depressor/levator burst pairs were identified when a burst in one nerve immediately followed a burst in the other. Measurements of bursting activity from each animal were averaged and used for statistical analysis. Statistical tests were calculated and plotted with SigmaPlot 11.2.

A Mann-Whitney U-test statistic with two degrees of freedom was used to compare responses under open loop and closed loop conditions. Significance was determined using a two-tailed $P$ value. Results are reported as means ± SD.

2.3 Results

2.3.1 Resistance and Assistance Reflexes

In the isolated nerve cord, MNs driving the leg are usually tonically active at low frequencies, similar to the patterns recorded from a quiescent animal [1]. In a quiescent animal, an imposed lift or depression of the leg will evoke a resistance reflex in which release-sensitive or stretch-sensitive CBCO afferents monosynaptically excite Dep or Lev MNs, respectively, to resist the imposed movement [63]. In closed loop trials of hybrid preparation experiments, lifting the model leg shortened the CBCO, which caused the loudspeaker probe to shorten the real CBCO by the same amount (Fig. 2.1). In seven preparations, an imposed upward force was applied to the leg 120 times and produced a movement that excited release-sensitive CBCO afferents and recruited Dep MNs as part of a resistance reflex on 105 of these leg lifts.
Upon termination of the applied force, a second resistance reflex was evoked as the leg was driven downward and excited stretch-sensitive CBCO afferents that excited Lev MNs (Fig. 2.2A, brown background, and C, markers 5–10). These chained reflex responses counteracted both phases of leg movement. In open loop (not shown), similar Dep responses occurred in response to leg lift, but the leg fell more slowly than in closed loop because the Dep muscles were not excited.

Application of the muscarinic cholinergic agonist OXO to a crayfish nerve cord can change the state of the nervous system from quiescent to active, where assistance reflexes, resistance reflexes, and levator and depressor motor bursts occur (Fig. 2.3) [11, 25, 53, 64, 65]. In seven preparations, we found that application of OXO to the desheathed ganglion reversed the reflexes completely or partially: on 80 occasions an imposed leg lift elicited an assistance reflex alone or with a resistance reflex (Fig. 2.3C). In response to a leg lift, Lev MNs fired throughout the period of applied force and continued as the leg began to fall, creating chained assistance and resistance levator reflex responses (Fig. 2.3A, green background, C, markers 1–5). In a second assistance reflex response, Dep MN activity increased as the leg continued to fall and peaked as the leg reached its lower limit (Fig. 2.3A, brown background, and D, markers 6–10).

In addition to simple reflex reversals, OXO exposure also enabled leg lifts to trigger pairs of Lev and Dep MN bursts (Fig. 2.3B). In these preparations, an imposed leg lift evoked an assistance reflex (Fig. 2.3C) that triggered a strong burst of Lev MN activity (Fig. 2.3, B, green background, and C, markers 1–5), and was immediately followed by a burst of Dep MN activity (Fig. 2.3, B, brown background, and D, markers 6–10). The abrupt rise in Dep MN activity as the leg moved rapidly downward (Fig. 2.3B, marker 10) suggests that the Dep
Figure 2.2. Resistance reflex response of the quiescent hybrid experimental preparation to leg lift

A: responses to a 1-N upward force applied to the model leg for 0.4 s (vertical gray arrow). Traces are as labeled. Traces 3 (gray), 4, and 6 show neuronal activity as labeled; traces 3 (black), 5, and 7 show corresponding firing rates. The green vertical band identifies the depressor resistance reflex response; the brown vertical band identifies the levator resistance response. The numbered gold markers correspond to those in B. B: depressor resistance reflex pathway. 1: Upward force (gray arrow) raises the leg (green leg) and releases the CBCO (green arrow). 2: Probe releases the CBCO and excites the release-sensitive CBCO afferents in the CBCO n. 3: afferent activity excites Dep motor neurons (MN). 4: Recorded Dep n activity excites model Dep MNs and muscles. C: levator resistance reflex pathway. 5: Contraction of Dep muscles brings the leg down (red leg). 6: Downward leg movement (red leg) stretches the CBCO (brown arrow). 7: Probe stretches the CBCO and excites stretch sensitive afferents in the CBCO n. 8: Afferents excite Lev MN. 9: recorded Lev n activity excites Lev MNs and muscle. 10: Lev muscle contraction slows the fall of the leg.
MN burst may have been triggered by a Dep assistance reflex (Fig. 2.3D). These Lev/Dep burst pairs caused the leg to maintain its elevated posture for the duration of the Lev MN burst and then return to a fully depressed position (Fig. 2.3B, top).

### 2.3.2 Closing the Loop Speeds Bursting

In all of the preparations, exposure of the ganglion to OXO evoked low-frequency bursting in the Lev and Dep motor nerves when the leg simulation was not running, i.e., when the in vitro preparation was completely uncoupled from the leg model. During these periods, the CBCO was held at a constant length that corresponded to the leg being held in a fixed horizontal position (Fig. 2.4, N.s. = No simulation). The bursting was often irregular: Lev and Dep bursts could be long or short and occurred in a variable phase relationship, while assistance reflexes were present or absent. However, in six preparations where assistance reflexes were present, spontaneous Lev and Dep bursts occurred in pairs, usually with a Lev burst immediately followed by a Dep burst, infrequently at long, variable intervals.

In 11 trials run in 6 preparations, switching on the leg simulation in closed loop caused the initially extended model leg to fall and stretch the CBCO and thereby stretch the real CBCO (Fig. 2.4, Closed loop; see Hybrid experiment video.mp4 in Supplemental Materials; see ENDNOTE). The initial leg fall was immediately followed by rhythmic levator and depressor burst pairs. The levator burst occurred first (a Lev/Dep pair) in all but 3 of the 78 burst pairs recorded in the 6 preparations so that the leg was repeatedly raised and lowered in a pattern reminiscent of walking. The depressor burst occurred first (a Dep/Lev pair) in the remaining three burst pairs. The rhythmic burst activity stopped at the end of the simulation when the loop opened and the CBCO returned to its resting length. When the leg simulation was run in the open loop configuration in seven trials in the same six preparations, the leg also fell at the
Figure 2.3. Assistance reflex responses and triggered bursts in OXO
Numbered markers in A and B correspond to those in C and D. A: assistance reflex response to a leg lift as in Fig. 2.2A. The green band marks the levator assistance reflex response, and the brown band marks the depressor assistance reflex response. B: assistance reflex response to a leg lift and a Lev/Dep burst pair. C: levator assistance reflex. 1: The leg lift (green leg) releases the CBCO strand (green arrow). 2: Probe releases the CBCO and excites the release-sensitive afferents. 3: Afferents excite the Lev MNs, often evoking a Lev burst. 4: Recorded Lev n activity excites the Lev MNs and muscles. 5: Lev muscle contraction maintains leg elevation (blue leg) and slows the leg fall. D: depressor assistance reflex. 6: Falling leg (red leg) stretches the CBCO strand. 7: Probe stretches the CBCO and excites the stretch-sensitive afferents. 8: Afferents excite Dep MNs. 9: Recorded Dep n activity excites Dep MNs and muscles. 10: Contraction of the Dep muscles brings the leg rapidly down (red leg).
beginning and stretched the CBCO. The CBCO stretch evoked reflex motor nerve responses but no subsequent leg movements because the connection between the motor nerve and muscle model was disabled (Fig. 2.4, Open loop). While the CBCO was stretched during these Open loop trials, 21 Lev/Dep burst pairs were evoked and 0 Dep/Lev burst pairs.

To analyze the responses under open and closed loop conditions (Fig. 2.4), Lev and Dep bursts were identified separately and together as Lev/Dep burst pairs (see MATERIALS AND METHODS and Figs. 2.4, and 5A). The frequency and duration of Lev/Dep burst pairs, the interval between burst pairs, the durations of the Lev and Dep bursts in each burst pair, and the interval between bursts of a pair were measured for open and closed conditions for each animal and then compared statistically. The three Dep/Lev burst pairs were not counted. We found that burst pair frequencies recorded in closed loop were significantly greater than those in open loop (P = 0.030; Fig. 2.5B). In open loop, the average burst pair frequency across animals was 0.04 ± 0.03 Hz, whereas in closed loop, the frequency was 2.5 times greater, 0.10 ± 0.04 Hz. The interval between burst pairs was also much shorter in closed loop than in open loop (P = 0.052; Fig. 2.5C). In closed loop, these intervals averaged 7.8 ± 4.2s, whereas in open loop they averaged 33.6 ± 39.6 s. The durations of burst pairs in closed loop were shorter than those in open loop (P = 0.065; Fig. 2.5D). In closed loop, burst durations averaged 6.7 ± 3.4 s, two-thirds the average value in open loop, 9.9 ± 2.9 s.

2.3.3 Closing the Loop Changes the Structure of Burst Pairs

The structure of Lev and Dep bursts within each burst pair also differed in closed and open loop. In closed loop, the Lev burst was shorter than the Dep burst in five of the six animals, although the average Lev burst duration across animals did not differ from the average Dep burst duration (P = 0.310). In open loop, the Lev burst durations exceeded the Dep burst durations in only half the animals and the average Lev and Dep burst durations
Figure 2.4. Effect of closing the loop on spontaneous bursting after OXO exposure
Traces show leg angle and CBCO length changes (top 2 traces), CBCO n, Lev n and Dep n responses during trials when the feedback loop was closed ("Closed loop") and open ("Open loop"). The Lev, Dep, and Lev/Dep bursts are shown at bottom. The “no simulation” periods, when the simulation was not running, are marked “N.s.” and covered in gray.
Figure 2.5. Identification of Lev/Dep burst pairs and burst pair measurements and statistics

A: Lev/Dep burst pair with the bursts and durations identified. The thresholds (“T”) for identification of the large (phasic) Lev and Dep MN spikes are shown with a dashed line. Identifications of the Lev burst, Dep burst, and Lev/Dep burst pairs are shown below. The durations of burst and burst pair intervals are identified below that; burst identification procedures are explained in Methods and Materials. B–G: box whisker plots for various statistics on burst and burst pairs under closed loop (CL) and open loop (OL) conditions. The data points, which are average values from single animals, are shown for CL and OL conditions linked by line segments. Data for individual animals are represented by the same colors across the plots. Each box shows the median (middle line) and 25th and 75th percentiles (box bottom and top) of the data distribution. Each plot is labeled at top; the parameters are identified in A. *Statistically significant difference between CL and OL responses.
did not differ (P = 0.818; Fig. 2.5E). Moreover, in closed loop the Lev burst in each animal was consistently shorter than in open loop, although the average Lev bursts across animals in open and closed loop did not differ (P = 0.394). The Dep bursts did not differ in open and closed loop. Half the Dep bursts in closed loop were shorter than the Dep bursts in the same animal in open loop and half were longer, and there was no difference in their averages (P = 0.485; Fig. 2.5F). Finally, in four of the six animals, the interval between Lev and Dep bursts was shorter in closed loop than in open loop, while in the other two it was longer. However, because the variance in the closed loop intervals was so much smaller than the variance in the open loop intervals, the averages (closed loop: 0.6 ± 1.1 s; open loop: 3.5 ± 2.6 s; P = 0.041) differed significantly (Fig. 2.5G).

2.4 Discussion

The results described here highlight the importance of sensory feedback in the generation of motor patterns. The timing and selection of particular afferents by the movement itself ensure that the centrally generated motor patterns will be shaped from moment to moment by current conditions to produce well-adapted movements and behaviors. This is apparent in these experiments even though the feedback was from only one of several sensory organs that affect the leg’s motor patterns.

The effects of proprioceptive sensory inputs on rhythmic motor outputs have been studied primarily in anesthetized, restrained, and dissected nervous system preparations where limb or body movement that could produce sensory feedback is limited or absent. Spontaneous rhythmic bursting with variable frequencies and duty cycles have been seen in isolated crayfish nerve cord preparations after application of muscarinic agonists like OXO [66, 67]. Imposed proprioceptive sensory inputs can evoke rhythmic alternating resistance reflex responses from antagonist MNs in quiescent preparations [10] and can entrain rhythmic
bursting in active preparations [65, 68, 69]. In our preparations, OXO induced a variety of active rhythmic patterns; to study the effect of closing the feedback loop on motor activity, we focused on those preparations in which reflex reversal occurred and a leg lift triggered a Lev/Dep burst pair.

By artificially closing the feedback loop, the role of reafference can be studied in a stable preparation [50, 51]. The hybrid experimental preparation provides the isolated nervous system with a virtual periphery that generates reafferent responses from the CBCO in real time to movements evoked either by external perturbation or by central motor commands. The neuromechanical leg model and hybrid preparation provides opportunities for producing reafferent responses to leg movements around other joints, including the thoracic-coxa joint that mediates forward/backward leg movement, and ground contact with the distal end of the leg. In the present experiments, the nature and timing of the resulting CBCO feedback resembled the natural feedback that a freely behaving animal would experience in response to its own movements. Moreover, the ability to interrupt the feedback experimentally has helped to reveal its significance for the normal function of the nervous system in posture and locomotion.

2.4.1 **Resistance Reflex Responses**

Resistance reflexes in crayfish, stick insects, and other animals act through negative feedback loops to stabilize a postural stance [16]. In our tonically active, closed loop hybrid preparations, resistance reflexes created a chained response to a leg lift perturbation by first exciting the release-sensitive CBCO afferent-to-Dep MN pathway that countered the lift and pushed the leg down. A second resistance reflex excited the stretch-sensitive afferent to Lev MN pathway to counter the rapid downward leg movement.
2.4.2 Assistance Reflexes Trigger Bursts During Rhythmic Bursting in Closed Loop

Assistance reflexes use positive feedback to reinforce imposed movements and to promote rapid transitions between locomotor phases [14, 47, 65]. Application of OXO in the hybrid preparation enabled assistance reflexes to help the applied force to lift the leg (Fig. 2.3, A and C). Once the raised leg began to fall, however, Lev MNs continued to fire, suggesting that they were excited by a resistance reflex that opposed the fall. Activation of muscarinic receptors is known to strengthen the resistance reflex responses of MNs in a voltage-dependent fashion [11], so that both assistance and resistance reflexes may be enhanced by the OXO exposure.

Earlier work identified a disynaptic pathway to mediate the leg depression assistance reflex, in which stretch-sensitive CBCO afferents excite an assistance reflex interneuron (ARIN), and it excited Dep MNs to assist the downward movement of the leg [65, 70]. We presume that the circuit is symmetric, such that our experimental leg elevation excited release-sensitive afferents that then excited release-sensitive ARINs [1]. The ARINs would then have excited Lev MNs to assist the leg rise, while the resistance reflex is blocked or reduced by presynaptic inhibition of the monosynaptic connection between the release-sensitive afferents and Dep MNs [71].

OXO exposure helps induce an active state in which the excitability of the ARINs and the Lev and Dep CPG, which includes some MNs, is increased [1]. We found that the assistance reflex evoked by an imposed leg lift often triggered a Lev/Dep burst pair that caused the leg to remain raised during the levator burst and then rapidly depress in response to the Dep burst (Fig. 2.3B and C). Once the Lev/Dep CPG became spontaneously active, closing the loop should enable the assistance reflex to trigger a Lev/Dep burst pair in response to leg movements produced by the CPG.
This mechanism is illustrated in Fig. 2.6, which compares an open loop burst pair to a closed loop burst pair. In open loop, the Lev/Dep half-center CPG generated Lev/Dep burst pairs at a low rate. The Dep and Lev half-centers are linked through mutual inhibition [1], so that the Lev MNs were strongly inhibited by each Dep burst (Fig. 2.6A, red arrowhead and marker 1). The Lev MNs’ firing rates then gradually increased before triggering their own burst (Fig. 2.6A, marker 2). The Lev burst produced strong inhibition that silenced the Dep MNs (Fig. 2.6A, marker 3, blue arrowhead). Towards the end of the Lev burst, Dep MNs began to fire rapidly after having been inhibited (Fig. 2.6A, marker 4). They quickly formed a Dep burst that strongly inhibited the Lev MNs, leaving only the common inhibitor MN active (Fig. 2.6A, markers 5 and 1, and red arrowhead) [72]. The Dep firing rate then slowed and allowed the Lev firing rate to increase again in another cycle.

It is apparent that the open loop burst frequency depended on the intrinsic dynamics of the CPG and on the effects of mutual inhibition between half-centers. In closed loop, the effects of the intrinsic dynamics and mutual inhibition on the half-center burst frequency were supplemented by positive feedback provided by the assistance reflexes (Fig. 2.6B). As in open loop, the Lev firing rate increased from having been strongly inhibited by the Dep burst. The Lev firing rates were initially too low to raise the leg. However, as their firing rates increased, they evoked an upward leg movement (Fig. 2.6B, marker 1, light blue arrowhead) that excited release-sensitive CBCO afferents (Fig. 2.6B, marker 2, green arrowhead). The afferents are likely to have excited the release ARIN and produced a levator assistance reflex that triggered a burst in the Lev MNs (Fig. 2.6B, marker 3). As in open loop, the Lev MNs strongly inhibited the Dep MNs (Fig. 2.6B, marker 3, blue arrowhead) until some Dep MNs began to increase their firing rates (Fig. 2.6B, marker 4). They inhibited the Lev MNs while they excited the Dep muscle and caused the leg to move down (Fig. 2.6B, marker 4, pink arrowhead). This is likely to
Figure 2.6. Mechanisms of burst pairs occurring in open loop and closed loop
Traces show changes in CBCO length, CBCO n activity, Lev n activity, and Dep n activity. A: open loop. B: closed loop. The numbers, colored markers, and arrowheads are explained in the text.
have excited stretch-sensitive CBCO afferents and a depressor assistance reflex (Fig. 2.6B, marker 5, brown arrowheads). The assistance reflex excited a depressor burst that then inhibited the Lev MNs (Fig. 2.6B, marker 6, red arrowhead) and increased depressor muscle tension.

2.4.3 Assistance Reflexes Reset the CPG Early in Each Cycle to Accelerate the Lev/Dep Rhythm

The shorter closed loop interval between burst pairs (Fig. 2.5C) suggests that the levator assistance reflex is triggered early in each cycle of the closed loop rhythm relative to the cycle of the open loop rhythm. By triggering the burst, the assistance reflex should reset the closed loop rhythm, so that the rhythm would be reset early in every cycle by this proprioceptive feedback from the leg movement. It appears likely that a depressor assistance reflex is also triggered at the end of the Lev burst of each burst pair and that this triggers the Dep burst (Fig. 2.6B, marker 5). This would account for the shorter closed loop interburst duration in four of the six animals (Fig. 2.5G) and would indicate that the Dep assistance reflex resets the Dep rhythm at each cycle. Together, the early resettings shorten the burst pair periods and speed the motor rhythm.

CBCO feedback also restructured the Lev/Dep burst pairs. The burst pairs were shorter when the feedback loop was closed (Fig. 2.5D), because the Lev bursts were shorter (Fig. 2.5F), and in four of six animals, because the interval between Lev and Dep bursts was shorter (Fig. 2.5G). If the depressor assistance reflex helped to advance the onset of the Dep burst, this may account for both of these changes in the burst pair structure. The advance of the Dep burst would shorten the interval between Lev and Dep bursts and cause an earlier inhibition of the Lev MNs that would shorten their burst. CBCO input may also increase the Lev/Dep burst
pair frequency by increasing the excitation of central neurons linked to the CPG, as input from wing stretch receptors does for the flight system of locust [73].

2.4.4 Regulating the Step Cycle in the Context of Walking

A step cycle can be defined as lasting from the onset of the swing phase until the end of the stance phase. In our closed loop experiments, where sensory feedback is provided only by the CBCO, the transitions are triggered by an assistance reflex response to the moving leg, which resets the CPG each cycle and thereby accelerates the rhythm. However, in freely walking animals, the transitions must be coordinated with the overall movement of the leg and its changes in load as it supports and propels the animal.

In several animals, interjoint reflexes assist the transition to the next phase of the step cycle when the leg becomes unloaded and ready to shift from stance to swing. In the cat, a fall in the tension of ankle extensors at the end of the stance phase disinhibits leg flexors, while hip sensors that signal hip extension also help trigger the stance-to-swing transition [2, 21]. In the stick insect, leg elevation is promoted by receptors that respond to flexion at the femur-tibia joint, which occurs as the leg pulls the animal forward [44, 45]. In crustacea, several sensory pathways exist for signaling readiness for a stance-to-swing transition. The funnel canal organs on the dactyl are sensory afferents that respond to leg contact with the substrate and can reset and coordinate the walking rhythm in the crab [74, 75]. Cuticular stress detectors, which respond to stress developed in the cuticle of the proximal segments of the crayfish leg, can also entrain both promotion/remotion and elevation/depression movement rhythms of the leg around the adjacent thoracic-coxopodite and CB joints, respectively [68]. When the nervous system is in an active state, the thoracic-coxopodite muscle receptor organ reports remotion of the leg and, through reflex reversal, excites leg remotors and inhibits promotors [46, 76]. During walking, these different receptors may act to signal when the leg is unloaded and ready
to make a stance-to-swing transition. Their responses are likely to help silence the depressors and excite the levators in an interjoint reflex [47] and thereby reverse the downward torque on the leg needed to support the body. This condition will produce the upward leg movement that triggers the assistance reflex and levator burst response observed in our experiments. It seems likely that this assistance reflex mechanism, perhaps together with a similar one that excites leg promoters [48, 49, 77], produces the stance-to-swing transition during each step of normal walking.
3 The Effect of Sensory Feedback on Crayfish Posture and Locomotion: II.

Neuromechanical Simulation


As second author of this paper, my contributions included:

- Developing simulation protocol
- Data analysis
3.1 Introduction

The neural circuits for the control of movement are not well understood for any animal because they depend on the interaction of descending commands with sensory feedback and locally generated responses in lower motor centers. Here we provide a model of those circuit interactions for the crayfish, in which feedback from a leg stretch receptor organ provides reflex input to postural and locomotor circuits and substantially changes their outputs. The model is based on detailed circuit analysis [1] and on recent experimental results obtained with a hybrid neuromechanical preparation [78]. Simulations of those hybrid experiments show that the transition from a quiescent postural state to an active locomotory state depends on neuromodulatory activation of a central pattern generator (CPG) and an assistance reflex and on inhibition of an antagonistic resistance reflex. The assistance reflex resets the CPG early in each cycle and thereby accelerates the intrinsic locomotor rhythm.

The circuit mechanisms that control posture and locomotion are remarkably similar in legged vertebrates and arthropod invertebrates [14]. While an animal is standing stationary, negative feedback from resistance reflexes acts against imposed movements to stabilize and maintain a posture [16]. Resistance reflex pathways are often monosynaptic and activate antagonist muscles that oppose a movement. Once an animal is in motion, however, positive feedback from polysynaptic assistance reflexes acts to reinforce movements in coordination with central motor patterns and resistance reflexes. Together, resistance and assistance reflexes alternate during walking to help produce stance and swing movements for each leg [4, 79]. While many pathways that mediate those reflexes and central motor commands have been identified, it is not yet clear how they interact during the locomotor cycle of the active state or during the transition between the quiescent and active states.

In the companion article [78], we described how an in vitro crayfish ventral nerve cord preparation linked to a neuromechanical model of a crayfish leg produced a closed
sensorimotor feedback loop that enabled these issues to be studied. Motor activity recorded from levator (Lev) and depressor (Dep) motor nerves excited model Lev and Dep leg muscles that moved the model leg up and down and thereby released and stretched a model coxobasal chordotonal organ (CBCO). Movement of the model CBCO drove a probe attached to the real CBCO and released and stretched it identically in real time. Release- and stretch-sensitive afferents from the real CBCO were excited by the model leg movements and projected back to the ventral nerve cord to complete the feedback loop [63].

When the hybrid preparation was in the quiescent state, resistance reflexes reduced the perturbation of the leg caused by an imposed force. Exposure to oxotremorine (OXO), a muscarinic cholinergic agonist, induced an active state in which the same imposed force evoked an assistance reflex that triggered sequential opposing motor bursts [65, 79]. A brief force that lifted the leg excited levator motor neurons (Lev MNs) and triggered a burst of Lev MN activity that kept the leg elevated well after the force was removed. The Lev burst was immediately followed by a Dep motor burst that moved the leg back down. Later in the active state, Lev/Dep burst pairs occurred spontaneously at a low frequency when the feedback loop was opened (viz., the motor nerve was disconnected from the model leg muscles). When the feedback loop was closed, the frequency of Lev/Dep burst pairs immediately increased to nearly three-times greater than in open loop and drove the leg rhythmically up and down in a manner similar to walking. Our article concluded that the closed loop increase in burst pair frequency resulted from the Lev assistance reflex that cut short the interval between Lev/Dep burst pairs and triggered a new burst pair that reset the burst pair rhythm. It showed that two aspects of the transition from quiescent to active states, reflex reversal and excitation of the Lev/Dep CPG, were linked to produce a faster, feedback-dependent rhythm.

A circuit mechanism for this transition in crayfish has been proposed where resistance reflexes present in quiescent preparations are reversed in active preparations to produce
assistance reflexes [1]. In the quiescent crayfish, a leg elevation resistance reflex is excited when a vertical perturbation excites release-sensitive afferents from the CBCO. The afferents monosynaptically excite Dep MNs to resist the leg movement (El Manira et al. 1991a). In ventral nerve cord preparations exposed to the muscarinic cholinergic agonist OXO, stimulated releasesensitive afferents excite the assistance reflex interneuron (ARIN), which then excites the Lev MNs [65]. The resistance reflex is blocked by presynaptic inhibition of the afferent terminals that excite Dep MNs [80] and by postsynaptic inhibition of the Dep MNs [81]. Finally, neurons that are part of the Lev/Dep CPG are disinhibited by OXO block of a muscarine-sensitive outward current [53]. This allows a rhythmic pattern of Lev/Dep activity to occur that is thought to drive walking [47].

Here we present a computational model of the neuromechanical control of elevation and depression movements of the crayfish leg (Fig. 3.1). The neural circuit portion of the model reflects current understanding of postural and locomotor circuits that control elevation and depression movements. The model expresses the mechanisms described above and includes a CPG, monosynaptic resistance reflexes, and polysynaptic assistance reflexes. The circuit model is linked to the neuromechanical leg model that was used in the hybrid preparation experiments to allow us to simulate those experiments [78]. The motor output of the circuit model drives movements of the neuromechanical model, which then provides CBCO afferent feedback to the circuit model. Simulations of the hybrid preparation experiments with the model have shown that our current understanding of the postural and locomotor circuits for leg elevation and depression can account for our experimental results. They demonstrate how the system can shift from a quiescent state that controls posture to an active state that mediates locomotion through the interaction of a CPG and assistance reflexes.
Figure 3.1. Neuromechanical model of the crayfish thorax, leg, and thoracic circuitry for elevating and depressing the leg

Crayfish leg and body model: Thorax and leg with an inset showing the CB leg joint blown up and made transparent to show the phasic and tonic (blue and light blue) levator muscles, phasic and tonic (red and pink) depressor muscles, and the CBCO strand (yellow). Leg elevation (blue arrow) and depression (red arrow) cause CBCO release (green arrow) and stretch (brown arrow), respectively. Neural circuit model: Neurons are represented by colored circles and rounded rectangles, synaptic connections by colored lines, excitatory synapses by forks, inhibitory synapses by filled circles, and electrical synapses by resistance symbols. The 3 cells of the depressor half-center of the depressor/levator CPG are within a pink rectangle, the cells of the levator half-center are within a light blue rectangle. CBCO release (green arrow) excites release-sensitive CBCO Afferents (green rounded rectangles within a light green rectangle at top), and CBCO stretch (brown arrow) excites stretch-sensitive CBCO Afferents (brown rectangles within a yellow rectangle at bottom). Electrical stimulation of the OXO neuron is symbolized by an electrode labeled “Stim.” CBCO, coxobasal chordotonal organ; CPG, central pattern generator; Dep, depressor; Lev, levator; IN, interneuron; MN, motor neuron, OXO, oxotremorine; ARIN, assistance reflex interneuron; ARCIN, assistance reflex control interneuron; PADI, primary afferent terminal inhibitor.
3.2 Methods and Materials

3.2.1 Description of the Model

A computational neuromechanical model of the hybrid experimental preparation was built in AnimatLab v1 (www.AnimatLab.com) based on descriptions of the known reflex circuitry [1] (Fig. 3.1). To build this model, the leg and body model used in the hybrid preparation experiments was connected to a simplified neuronal network model of the CPG and reflex circuitry.¹ This neuronal network model replaced the live in vitro nerve cord preparation and hybrid interface used in the hybrid preparation experiments [78]. (A list of parameter values used in the model is available in Supplemental Materials in Model Parameters Supplemental Material.xlsx. The equations used in the network model are listed in Equations.docx; see ENDNOTE.) The leg and body model contained Hill model muscles that were driven by the motor output of the neuronal network, as well as a model CBCO that was connected directly to model sensory afferents. All the parameter values and structural arrangements can be seen in the model file, “Crayfish hybrid simulation model,” which is available in Model DB (https://senselab.med.yale.edu/ModelDB/. The ModelDB accession number for this model is 150698).

Model neurons were each represented by a single compartment integrate-and-fire model that had a resting potential of -70 mV with 0.1 mV of noise. Chemical synaptic transmission following a presynaptic spike was simulated by an exponentially decaying postsynaptic conductance triggered in a postsynaptic neuron after a synaptic delay of 0.5 ms. The postsynaptic conductance was in series with an appropriate reversal potential, so that the postsynaptic current equaled the product of the conductance and the difference between the membrane potential and the reversal potential. Electrical synapses were represented by

¹ To distinguish model elements from their real counterparts, all model element names are italicized.
electrical coupling resistances between neurons. Throughout the model, neuronal and synaptic properties were set so as to enable the network to replicate responses recorded from referenced experimental preparations.

Detection of stretch or release of the crayfish CBCO is mediated by afferents that are rate and position sensitive [82]. The CBCO has ~20 afferents that respond to stretch or the rate of stretch and ~20 afferents that respond similarly to release [63]. In our model, we included three stretch-sensitive afferents: two identical rate-of-stretch units (Stretch Rate Resist Afferent and Stretch Rate Assist Afferent) and one whose response was proportional to the amount of stretch (Stretch Resist Afferent). Three corresponding release-sensitive afferents were also included: a Release Rate Resist Afferent, a Release Rate Assist Afferent, and a one Release Resist Afferent (Fig. 3.1). Transduction for the stretch- and release-rate afferents was mediated by linear current vs velocity functions with opposing slopes: 1 nA·mm⁻¹·s⁻¹ for stretch and -1 nA·mm⁻¹·s⁻¹ for release. Similarly, transduction for stretch- and release-sensitive afferents was mediated by linear current vs. length functions with opposing slopes: 30 nA/mm for stretch and -30 nA/mm for release.

The sets of 12 depressor and 19 levator MNs that innervate the leg [63, 83] were each represented by a pair of motor neurons, one tonic Dep or Lev MN and one phasic Dep or Lev MN. The tonic and phasic MNs were connected to the muscle models by conductance-based synapses that simulated the tonic and phasic neuromuscular junctions of the crayfish leg extensor muscle [61]. A spike in a phasic MN produced a large, nonfacilitating EPSP in all the phasic muscles, while a spike in a tonic MN produced a small, strongly facilitating EPSP in all the tonic muscles. A common inhibitor MN inhibited all muscles and was itself inhibited by Phasic Lev and Dep MNs [84]. Open loop conditions were created by disabling the connections between the MNs and the muscles.
Resistance reflexes in the crayfish are mediated by monosynaptic connections between stretch- or release-sensitive afferents and the Lev or Dep MN pools, respectively [63, 65]. Similar connections in the model enable stretch-sensitive resist afferents to mediate a resistance reflex triggered by leg depression, while the release-sensitive resist afferents mediate a resistance reflex triggered by leg elevation. Resistance reflexes also include disynaptic inhibitory pathways that inhibit antagonist MNs [71]. These are represented in the circuit model by a disynaptic pathway from CBCO stretch or release rate-sensitive afferents to either a Stretch or Release X Inhib neuron, which then inhibits the antagonist Dep or Lev MNs.

Assistance reflexes in the crayfish are mediated by disynaptic pathways in which stretch- or release-sensitive afferents excite one or more ARINs, and these excite units in the Dep or Lev MN pools, respectively [65]. In the model, a leg depression assistance reflex was produced when a Stretch Rate Assist Afferent excited a Stretch Assistance Reflex Interneuron (Stretch ARIN) and that cell excited depressor MNs (Fig. 3.1). The leg elevation assistance reflex was organized in a symmetrical fashion. Sensory afferents also excite an Assistance Reflex Control Interneuron (ARCIN) that controls the gain of the assistance reflex by inhibiting the ARIN [65].

The crayfish levator/depressor CPG includes two pools of electrically coupled neurons (half-centers) that are mutually inhibitory [66, 81]. The levator pool contains interneurons (INs) and a few of the 19 tonic and phasic Lev MNs, while the depressor pool includes INs and a few of the 12 tonic and phasic Dep MNs [1, 53, 64]. The participating neurons are conditional bursters, depending on muscarinic cholinergic activation and depolarization. Our model contains a simplified Lev/Dep CPG (Fig. 3.1), in which the Lev and Dep half-centers each include three electrically coupled neurons: an IN and the phasic and tonic MNs. The Lev and Dep INs (but not the MNs) each have a slowly activating and a very slowly inactivating inward current that mediates rhythmic bursting. Mutual inhibition between the half-centers is mediated
by three sets of inhibitory synapses: 1) mutual synaptic inhibition of the Lev and Dep INs; 2) inhibition of the antagonist IN by the agonist Tonic MN; and 3) inhibition of the antagonist Phasic and Tonic MNs by the agonist Phasic MN.

Lev and Dep bursts in our experiments occurred in burst pairs, in which the Lev burst immediately preceded the Dep bursts ([78]; see below). Although the actual mechanisms of this functional asymmetry are unknown, the design of the model Lev/Dep CPG was asymmetric to favor this pattern. These asymmetries included 1) a larger Lev IN inward current; 2) stretch- and release-sensitive CBCO afferents that differed in their sensitivities to leg angle; and, 3) stronger inhibition of Dep elements by Lev elements than vice versa.

A muscarine-sensitive, persistent outward potassium current prevents bursting by Lev and Dep MNs in quiescent (tonically active) preparations [53, 64]. This current is simulated in our model as a postsynaptic conductance-mediated outward current tonically produced in these neurons by a persistently depolarized presynaptic Outward Current neuron (Fig. 3.1). Tonic activity of the Outward Current neuron, like the outward current in the live preparation, keeps the CPG INs and the ARINs hyperpolarized and prevents both rhythmic bursting and assistance reflex responses during simulations of the tonically active preparation.

In the crayfish, the outward current is blocked by activation of muscarinic acetylcholine receptors, thereby enabling the half-center cells to produce bursting activity [53]. The action of OXO in blocking the outward current was simulated in the model by synaptic inhibition of the Outward Current neuron by an OXO neuron. Application of OXO to the preparation was simulated by application of a depolarizing stimulus current to the OXO neuron, which then inhibited the Outward Current neuron and reduced or stopped its inhibition of the Lev and Dep INs and the ARINs. Their depolarization allowed the Lev and Dep INs to begin bursting and allowed the ARINs to mediate assistance reflexes.
Enabling assistance reflexes is one part of reflex reversal, which also depends on the suppression of opposing resistance reflexes. Depolarizing inhibition of the primary afferent terminals (PAD) blocks the reflex excitation of MNs by the CBCO afferents when the opposing set of MNs is active [71, 85, 86]. In the model, a pair of inhibitory interneurons, the Lev and Dep PADIs, are each excited by one set of motor neurons to inhibit the rate sensitive CBCO afferent that excites the other set.

### 3.2.2 Simulation Protocol

The duration of computational simulations for each condition was 60 s, and simulations for each condition were run 6–8 times. Noise (0.1 mV) applied separately to each neuron model made each simulation different. To simulate experimental tests of leg lift reflexes, a 1-N force was applied to the leg for 0.5 s and activity was recorded for further analysis.

To reproduce open loop conditions in the hybrid experiment [78], connections between the motor neurons and leg muscles were disabled. Consequently, motor neuron activity did not result in muscle potentials or tension and there was no movement of the leg. Whether a simulation was run in open or closed loop, all neuron activity was recorded and saved for further analysis.

Motor bursts were identified in each trial for comparison between conditions using the Poisson Surprise method in the data analysis software, DataView (see http://www.st-andrews.ac.uk/_wjh/dataview/). Once motor neuron bursts were identified separately for Lev and Dep motor neurons, Lev/Dep, or Dep/Lev burst pairs were defined when one burst occurred immediately after the other. Nonparametric statistics were used to determine whether different simulation conditions changed the system significantly. Statistics are reported as averages ± SD.
3.3 Results

3.3.1 Quiescent State is Characterized by Tonic Neuronal Activity and Resistance Reflexes

The quiescent state of the preparation is characterized by the low-level tonic activity of sensory neurons, INs, and MNs as well as resistance reflex responses to imposed leg movements. This state is maintained by a muscarine-sensitive tonic outward current that hyperpolarizes Lev and Dep MNs that, in crustaceans, are part of the CPG networks [1, 87] and so prevents spontaneous rhythmic activity. The outward current is also likely to hyperpolarize as yet unidentified CPG INs and to prevent assistance reflex responses by hyperpolarizing the ARINs. In our model, the corresponding cells are quiet and a tonic outward current was evoked in CPGs and ARINs by hyperpolarizing conductance-based synapses from an “outward current” model neuron (Fig. 3.1).

3.3.2 Resistance Reflex Response to Leg Lift: 0 nA OXO Stimulation

In the hybrid preparation experiments, reflex responses to imposed leg lifts were measured in a series of six 60-s trials in which a 0.5 s, 1-N upward force was applied to the model leg five times at 10-s intervals during each trial. The same protocol was followed in simulations of those experiments and similar responses were evoked. These are shown in 2-s poststimulus time histograms of firing frequency responses in 20-ms bins and averaged over the 30 responses to leg lifts (Fig. 3.2A).

Each simulated upward force stimulus evoked a pair of chained resistance reflex responses that first resisted the imposed upward leg movement and then resisted the later downward movement (Fig. 3.2, A and B). Each leg lift released (i.e., shortened) the CBCO (Fig. 3.2, A and B, marker 1), which transiently excited the CBCO Release Rate Resistance Afferent
**Figure 3.2. Resistance responses to leg lift**

A: time courses of responses to an upward 1-N force applied to the leg at 0.5 s for 0.5 s. The top 2 traces ("physical") display the change in the angle of the CB joint ("Leg Angle") and the CBCO length. The next 2 traces display the membrane potential change of the Release Afferent and Stretch Afferent neurons. The bottom 4 traces display the average firing rates in 25-ms bins of individual neurons, as labeled, to five leg lifts in each of 6 trials. The averages are taken from 30 responses obtained in 6 simulation trials. Each trace is color-coded according to the color of the neuron in Fig. 3.1 and in B and C. The pale green vertical stripe marks the depressor resistance reflex response to the leg lift; the broader brown vertical stripe marks the levator resistance reflex response to the fall of the leg. Numbered gold markers identify responses with events in B and C as explained in the text. B and C: circuit diagram of Fig. 3.1 is modified to show the active neurons during the depressor (B) and levator (C) resistance reflexes in full color and the inactive or inhibited neurons in dim or light colors. B: depressor resistance reflex pathway. The green arrow represents the effect of CBCO release on the release-sensitive afferents. The red arrow represents the effect of Dep MNs on Dep muscles and leg depression.

C: levator resistance reflex pathway. The brown arrow represents the effect of CBCO stretch on the stretch-sensitive afferents. The blue arrow represents the effect of the Lev MNs on Lev muscles and the leg depression.
The afferent monosynaptically excited both the Phasic and Tonic Dep MNs (Fig. 3.2, marker 3), which excited the Dep muscles, but the applied upward force prevented the leg from depressing. The Dep MNs and Release X Inhib neuron inhibited the antagonist Lev MNs, while the Dep PADI inhibited the Stretch Rate Resistance Afferent. The CBCO Release Rate Assistance Afferent was also excited by the leg lift but evoked only subthreshold responses in the Release ARIN, which was hyperpolarized by the tonic Outward Current input. As a result, no disynaptic assistance reflex response of the Lev MNs occurred.

When the upward force ended, gravity and the tension in the Dep muscles moved the leg downward. The downward leg movement (marker 4 in Fig. 3.2, A and C) stretched the CBCO (Fig. 3.2, marker 5) and strongly excited the Stretch Rate Assistance and Resistance Afferents (Fig. 3.2, marker 6). The Stretch Rate Resistance Afferent excited the Tonic and Phasic Lev MNs but failed to excite the Lev IN and the Lev ARIN, which were both hyperpolarized by the Outward Current. The Lev MN firing increased tension in the Lev muscles (Fig. 3.2, marker 7), which slowed the downward movement of the leg (Fig. 3.2, marker 8). The Lev MNs inhibited the Dep MNs and the Lev ARIN, and they excited the Lev PADI, which inhibited the Release Rate Resistance Afferent.

### 3.3.3 OXO Stimulation Blocked the Outward Current Neuron

The outward current that hyperpolarizes elements of the levator/depressor CPG is blocked by application of a muscarinic agonist, OXO, which promotes transition of the thoracic circuitry from the quiescent state to an active state [25]. We simulated the effect of OXO application in the model by stimulation of “OXO,” a model neuron that synaptically inhibits the Outward Current neuron (Fig. 3.3B, marker 1). To simulate other differences between the circuit in the active state and the circuit in the quiescent state, OXO also excites other cells,
including the Dep and Lev PADI, the Release and Stretch ARIN and ARCIN, and the Common Inhibitor MN (Fig. 3.1).

3.3.4 Assistance Reflex Response to Leg Lift: 4.0 nA OXO Stimulation

Upon depolarizing OXO with 4 nA of current (Fig. 3.3B, marker 1), the Outward Current was weakly inhibited and the ARIN and PADI were weakly excited. The same leg lift evoked a weak resistance reflex and a strong assistance reflex in which a small burst of activity in Tonic Lev MNs helped to raise the leg (Fig. 3.3A, green band). This occurred in response to 26 of the 30 leg lifts over the six trials; the four other leg lifts triggered full levator motor bursts. Figure 3.3A shows the average response of each of the circuit elements to these 26 leg lifts. The leg lift (Fig. 3.3B, marker 2) released the CBCO and excited Release-sensitive CBCO Afferent (Fig. 3.3B, marker 3). Depolarization by OXO enabled the Release ARIN (Fig. 3.3B, marker 4) to respond to the Release Rate Assistance Afferent (Fig. 3.3A) by inhibiting the Tonic Dep MN and exciting the Lev MNs (Fig. 3.3A, marker 5). The levator muscle contraction assisted the leg lift (Fig. 3.3A, marker 6).

The initial leg lift also excited the Release Rate Resistance Afferent that evoked a resistance reflex by monosynaptically exciting the Dep MNs. Both Tonic and Phasic Dep MNs fired a brief burst of spikes before being inhibited by the Release ARIN. The Tonic Lev MN also excited the Lev PADI, which then inhibited the Release Rate Resistance Afferent to prevent further resistance reflex responses.

When the leg fell at the end of the upward force (Fig. 3.3A, brown band; Fig. 3.3, A and C, marker 7), the CBCO stretch excited stretch-sensitive afferents (Fig. 3.3, A and C, marker 8). They continued to excite the Lev MNs (Fig. 3.3, A and C, marker 9) in a resistance reflex that helped to slow the fall of the leg (Fig. 3.3, A and C, marker 10).
Figure 3.3. Assistance and resistance response to leg lift
The same figure layout as in Fig. 3.2, with the same 1-N upward leg stimulus applied during 4-nA OXO neuron stimulation. A: responses to leg lift. The average neuron firing rates are calculated from 25 responses obtained in 6 trials. B and C: circuit diagram of Fig. 3.1 is modified to show the active neurons during the levator assistance (B) and levator resistance (C) reflexes in full color and the inactive or inhibited neurons in dim or light colors. The numbered gold circles mark the responses associated with the sequence of steps in B and C and are explained in the text. B: Levator assistance reflex pathway. The green arrow marks the effect of CBCO release on release-sensitive CBCO afferents; the blue arrow marks the effect of Lev MNs on levator muscles and leg lift. C: Levator resistance reflex pathway. The brown arrow marks the effect of CBCO stretch on stretch-sensitive CBCO afferents; the blue arrow marks the effect of Lev MNs on levator muscles and leg lift.
3.3.5 Reflex Reversal and a Lev/Dep Burst Response to Leg Lift: 5.2 nA OXO

Stimulation

As the experimentally applied OXO took effect, the levator assistance reflex response to an imposed leg lift often triggered a Lev/Dep MN burst pair (Fig. 3.3B in [78]). The model network produced similar Lev/Dep burst pair responses (Fig. 3.4A) to leg lifts after the OXO stimulation was increased to 5.2 nA (Fig. 3.4B, marker 1). Eight of 30 leg lifts imposed over 6 trials evoked Lev/Dep MN burst pairs; the other 22 leg lifts evoked resistance or assistance reflexes (Figs. 3.2A and 3.3A) or they occurred during or shortly after a spontaneous burst pair.

The OXO stimulation during each trial (Fig. 3.4B, marker 1) tonically excited the Lev and Dep PADs (Fig. 3.4A), which largely suppressed the resistance reflexes by inhibiting the two CBCO resistance afferents, the Release Rate Resistance Afferent and the Stretch Rate Resistance Afferent. However, a leg lift (Fig. 3.4A, marker 2) evoked a robust assistance reflex through a pathway containing the Release Rate Assistance Afferent (Fig. 3.4A, marker 3), the Release ARIN (Fig. 3.4A, marker 4), and the Tonic Lev MN. This assistance reflex response helped to trigger bursts in the Lev IN and the Tonic and Phasic Lev MNs (Fig. 3.4A, marker 5) that maintained leg elevation long after the upward force stopped (Fig. 3.4A, marker 6). The leg began to fall immediately after the Lev burst stopped (Fig. 3.4, A and C, marker 7). As before, the leg fall excited the Stretch Rate Assistance Afferent (Fig. 3.4, A and C, marker 8), which then excited the Stretch ARIN (Fig. 3.4, A and C, marker 9). Stretch ARIN excited the Dep MNs (Fig. 3.4, A and C, marker 10) to fire a burst and both it and the Dep MNs inhibited the Lev MNs. The Dep MN burst drove the leg down (Fig. 3.4, A and C, marker 11).
Figure 3.4. Assistance reflexes trigger bursts
The same figure layout as in Fig. 3.2, with the same 1-N upward leg stimulus applied during 5.2-nA OXO neuron stimulation. A: responses to leg lift. B and C: circuit diagram of Fig. 3.1 is modified to show the active neurons during the levator (B) and depressor (C) assistance reflexes in full color and the inactive or inhibited neurons in dim or light colors. The numbered gold circles mark the sequence of steps and their responses and are explained in the text. B: Levator assistance reflex pathway. The green arrow marks the effect of CBCO release on release-sensitive CBCO afferents; the blue arrow marks the effect of Lev MNs on levator muscles and leg lift. C: Depressor assistance reflex pathway. The brown arrow marks the effect of CBCO stretch on stretch-sensitive CBCO afferents; the red arrow marks the effect of Dep MNs on depressor muscles and leg fall.
3.3.6 Spontaneous Lev/Dep and Dep/Lev Burst Pairs: 5.7 nA OXO Stimulation

Several minutes’ exposure of an isolated crayfish nervous system to 50 μM OXO induced spontaneous bursts in levator and depressor MNs [53]. In the 6 hybrid preparation experiments, the bursting was organized as a low frequency (~1/25 s) series of 21 levator/depressor (Lev/Dep) and 0 depressor/levator (Dep/Lev) burst pairs when frequency (~1/10 s) series of 75 Lev/Dep burst pairs and 3 Dep/Lev burst pairs when the feedback loop was closed (Fig. 3.5A, here and in [78]). The model network produced the same set of phenomena when the OXO neuron was stimulated with 5.7 nA (Fig. 3.5B). (A video of one closed loop simulation trial, Hybrid simulation video.mpr, is available in online Supplemental Materials; see ENDNOTE.)

As in the hybrid preparation experiments (Fig. 1 in [78]), the feedback loop was opened in our simulated experiments by disconnecting the model MNs from the model muscles (Fig. 3.1), so the model was effectively paralyzed but could still respond to CBCO responses evoked by imposed model leg movements. The model leg was kept in an initially stationary horizontal position for 30 s at the beginning of each simulation trial to allow the effects of OXO stimulation to reach a steady state before beginning the 60-s experimental period (Fig. 3.5B). At that time, the leg was allowed to fall and stretch the CBCO, which excited the Stretch Rate Afferent and the Tonic and Phasic Lev MNs in a resistance reflex. In this open loop configuration, the leg fall triggered a Lev MN burst followed immediately by a Dep MN burst. Subsequent burst pairs occurred at a frequency of 0.04 ± 0.003 Hz (Fig. 3.6B) and were nearly equally likely to be a Dep/Lev pair (6 in six 60 s trials) as a Lev/Dep pair (8 in six 60-s trials). The interval between burst pairs was 23.1 ± 2.5 s (Fig. 3.6), and the Lev/Dep burst pair duration was 3.6 ± 0.1 s (Fig. 3.6D).

When the loop was closed, the frequency of burst pairs increased significantly by a factor of 2.5 to 0.10 ± 0.01 Hz (P = 0.001); they were exclusively Lev/Dep pairs (Figs. 3.5B and
Figure 3.5. Effect of closing the feedback loop on burst pair frequency
A: open and closed loop responses of a hybrid experimental preparation under exposure to 50 mM OXO, copied from Fig. 3.4 in Chung et al. 2014. B: responses of the neuromechanical model during stimulation of the OXO neuron with 5.7 nA when the CBCO feedback loop was open (left), when it was closed (middle), and when it was closed and the ARINs were disabled (right). Colors of the traces match the neuron colors in Fig. 3.1.
shorter than in open loop (Fig. 3.6C), as was the burst pair duration, which was 3.2 ± 0.1 s (P = 0.001) (Fig. 3.6D). In both open loop and closed loop simulations, the durations of the Lev bursts were longer than the durations of the Dep bursts (OL: P = 0.004, CL: P = 0.001; Fig. 3.6E). In addition, Lev bursts in closed loop were shorter than those in open loop (P = 0.051), whereas Dep bursts in closed loop were similar to those in open loop (P = 0.181). Finally, the interval between Lev and Dep bursts in the burst pairs was similar but less variable in closed loop than in open loop (Fig. 3.6F).

Burst pairs in closed loop differed significantly from burst pairs in open loop, largely because of the role of the assistance reflex responses in triggering the closed loop bursts. These differences are apparent in Fig. 3.7, which shows the membrane potential changes of the circuit elements during Lev/Dep bursts in closed loop and open loop, and Fig. 3.8, which shows the changes in their average firing rates. Under both conditions, OXO stimulation depolarized the Lev and Dep INs, the Stretch and Release ARINs, and the Lev and Dep PADIs, which fired tonically near 50 Hz (Figs. 3.7 and 3.8). In closed loop, the interval between Lev/Dep burst pairs ended when the Tonic Lev MN began to fire more rapidly because of subthreshold input from the Lev IN (Fig. 3.7, Closed Loop). The Lev IN and Tonic and Phasic Lev MNs are electrically coupled to each other (Fig. 3.1), so that changes in the membrane potential of one affects the firing of the others. The Lev IN is an endogenous burster because of a slowly activating, voltage-sensitive depolarizing conductance, which gradually depolarized the Lev IN after it was inhibited by the previous Dep IN burst. Electrical coupling allowed the depolarizing current to be driven from Lev IN into the Lev Tonic MN, which has a low spiking threshold and so began to fire increasingly rapidly. This Tonic Lev MN activity excited the Tonic Lev muscles and their contraction began to raise the leg. (Fig. 3.7, Closed Loop; Fig. 3.8,
Figure 3.6. Identification and statistics of burst pair responses obtained from open and closed loop trials under 5.7 nA OXO neuron stimulation

A: top 4 traces show the Tonic Lev, Phasic Lev, Tonic Dep, and Phasic Dep neuron responses during 2 Lev/Dep burst pairs. Dep/Lev burst pairs were analyzed similarly. The pale blue vertical stripes mark the Lev phase; the pink vertical stripes mark the Dep phase. Middle 3 traces show Dataview event markers for each of the top traces, identifying the Lev burst (top), Dep burst (middle), and Lev/Dep burst pair (bottom). The bottom 5 lines mark those bursts and burst pairs as well as the interval between burst pairs and the interval between bursts. B–F: simulation results of Lev/Dep burst pair responses from 7 simulation trials run in closed loop and Lev/Dep and Dep/Lev burst pairs from 6 simulation trials run in open loop, with the averages of individual simulation trials presented as single data points, and box plots showing the median and 25th and 75th percentiles for each set of simulation trials. Those found to be significantly different (see text) are identified with a double-end bar and asterisk.
Closed Loop). This movement released the CBCO and excited the Release Rate Assist and Resist Afferents. The Assist Afferent excited the Release ARIN, which then excited all three elements of the Lev half-center, the Lev IN, Lev Phasic and Lev Tonic MNs, to complete a positive feedback loop (Fig. 3.1 and Figs. 3.7 and 3.8, Closed Loop). This triggered a Lev burst that raised the leg and inhibited the Dep half-center. The Lev MNs also excited the Lev PADI, which inhibited the Release Rate Resistance Afferent to prevent resistance reflex excitation of the Dep MNs.

Because the open loop simulation lacked the positive feedback mediated by the ARINs, the initial Lev burst was triggered by the endogenous bursting Lev IN and its electrical coupling with the Tonic and Phasic Lev MNs (Figs. 3.1, 3.7, and 3.8, Open Loop). Instead of the abrupt onset characteristic of closed loop bursts, the open loop bursts began more gradually (Fig. 3.7, Open Loop Lev/Dep, Fig. 3.8, Open Loop). The Dep/Lev burst was triggered similarly, with the endogenously bursting Dep IN interacting with the Tonic and Phasic Dep MNs while inhibiting the Lev half-center (Fig. 3.7, Open Loop Dep/Lev).

Mutual inhibition between Lev and Dep half-centers (Fig. 3.1) ensured that the Dep IN and MNs were inhibited during the Lev burst (Fig. 3.7). When the Lev MN burst ended, the Dep IN began to depolarize, which excited the Tonic Dep MN. In closed loop, this caused the leg to begin to depress, which stretched the CBCO and strongly excited the Stretch Rate Assist Afferent (Fig. 3.8). This Assist Afferent excited the Stretch ARIN, which then excited the Dep half-center. This completed the positive feedback loop and excited the Dep burst and the Dep PADI, which prevented interference from Lev resistance reflexes.

In open loop, the end of the Lev half-center burst removed inhibition of the Dep half-center, which then produced a strong Dep burst (Fig. 3.8, Open Loop). The absence of feedback prevented the initial increase in the Dep MN firing rates that the assistance reflex evoked in closed loop.
Figure 3.7. Single burst pairs under closed and open loop taken from 1 simulation trial
The top 2 traces show the CB angle and CBCO length as in Figs. 2–5. The other traces display the membrane potential changes of different model neurons in colors according to Fig. 3.1. *Left*: Lev/Dep burst pair in closed loop; *middle*: Lev/Dep burst pair in open loop; *right*: Dep/Lev burst pair in open loop.
3.3.7 Blocking the ARINs

Both the hybrid experimental result [78] and our simulations suggest that assistance reflexes played a critical role in mediating the higher frequency of Lev/Dep bursting in closed loop. To test this, closed loop simulations were repeated when ARINs were disabled. Under these conditions, only one Lev/Dep burst occurred in six 60s trials (Fig. 3.5B, No ARINs). With the ARINs disabled and a high level of activity in the PADIs evoked by OXO neuron stimulation, there was no sensory input to the MNs or to the Dep or Lev INs. The Tonic Lev and Dep MNs, however, remained near their spiking thresholds and the Lev IN and Dep IN were depolarized as a result of OXO stimulation. Consequently, a combination of electrical coupling between each IN and its MNs and mutual inhibition between Lev and Dep half centers resulted in irregular alternating activity between Lev and Dep MNs that triggered the occasional burst pair when one half center happened to exceed burst threshold.

3.4 Discussion

The circuit model is a greatly simplified (see MATERIALS AND METHODS) description of the crayfish thoracic circuitry that mediates leg elevation and depression movements during posture and walking [1]. We joined the circuit and leg models to create a model of the hybrid experimental preparation and used that model to simulate the experiments with the hybrid preparation in both quiescent and active states and under open and closed loop conditions. We found that our simulations could reproduce and account for the results of the hybrid preparation experiments. In the absence of OXO stimulation, leg lifts evoked chained resistance reflexes (Fig. 3.2) similar to those evoked in the quiescent hybrid preparations (Fig. 3.2 in [78]). Low levels of OXO stimulation reduced the resistance reflex and created an assistance reflex (Fig. 3.3A), similar to the reflex changes experienced by the hybrid preparation.
Figure 3.8. Average post-stimulus time histograms (PSTH)
Average post-stimulus time histograms (PSTH) of 39 Lev/Dep burst pairs from 7 closed loop trials (left) and 8 Lev/Dep burst pairs from 6 open loop trials, with OXO neuron depolarized by 5.7 nA in both. The 6 Dep/Lev burst pairs recorded in those 6 open loop trials are not included. Separate average PSTHs were plotted for the Lev and Dep burst portions of the burst pairs. The onset of the Phasic Lev MN burst marked time 0 for the Lev portions of the average PSTHs, while time 0 for the Dep portions of those plots was the onset of the Phasic Dep MN burst.
soon after exposure to OXO (Fig. 3.3A in [78]). Higher levels of OXO stimulation enabled the assistance reflexes to trigger a Lev/Dep burst pair (Fig. 3.4) that was similar to the burst pair evoked in the hybrid preparation (Fig. 3.3B in [78]). A still higher level of OXO stimulation induced spontaneous, low-frequency burst pairs in open loop and higher frequency burst pairs in closed loop (Fig. 3.5B). These were similar to the burst patterns produced by the hybrid preparation under open and closed loop conditions (Fig. 3.5A).

The higher frequency of burst pairs in closed loop resulted from decreases in the interval between the pairs, in the duration of the pairs, and in the interval between bursts in each pair (Fig. 3.6, C, D, and F). These results agree with our hybrid experiment results (Fig. 3.5, C, D, and G in [78]). Table 1 provides a comparison of the average values of the model and experimental results taken from Fig. 3.6 in the present article and from Fig. 3.5 [78]. In both simulations and experiments, the largest difference between bursting in closed and open loop was the interval between burst pairs, which was 15 s, or three times, longer in open loop simulations and nearly 26 s, or more than four times, longer in the open loop hybrid experiments. The other open and closed loop differences were much smaller in absolute terms and somewhat smaller in percentage terms, and they were of the same sign in both the experiments and the simulations. The only exception to this is the relative durations of the Lev and Dep bursts in a pair. In simulation, the Lev bursts were shorter than the Dep bursts, whereas with one exception, the reverse was true of our experimental preparations.
3.4.1 Hypothesized Mechanism of Increased Bursting Frequency

During walking, many of the leg reflexes present in the quiescent state that promote postural stability are modulated in the active state to assist walking [63, 79]. Results from the hybrid preparation experiments showed that in the active state the assistance reflex responses to leg lift could trigger Lev/Dep burst pairs. This capability appeared to account for the increased burst pair frequency in closed loop. Small upward movements early in each interval between Lev/Dep burst pairs evoked a levator assistance reflex that triggered an early Lev/Dep burst pair and reset the Lev/Dep CPG.

Our simulations showed that leg elevation assistance reflexes mediated by the Release ARIN can have this effect and that the burst frequency fell to low levels when the ARINs were disabled. The timing of ARIN excitations in each burst pair enabled them to produce positive feedback at the transitions from swing to stance and stance to swing. Early upward movements of a depressed leg excited the Release ARIN that, in turn, excited Lev MNs. Their responses increased upward movement of the leg and helped to trigger the burst. The Stretch ARIN played a similar role in downward movements of the raised leg.
3.4.2 A Similar Mechanism for Leg Promotion and Remotion?

Both resistance and assistance reflexes are also active at the most proximal leg joint, the thoracic-coxa (TC) joint [69], where they likely contribute to the locomotor rhythm. The assistance reflexes are initiated by the rate-sensitive afferents of the thoracic-coxa muscle receptor organ (TCMRO) and the thoracic-coxa chordotonal organ (TCCO), and they may help remote the leg during the stance phase of forward walking and promote the leg during the swing phase [77].

Their similarity to the levator and depressor assistance reflexes suggests that the promotor and remotor assistance reflexes may also increase the frequency of the walking rhythm when their feedback loop is closed. During forward walking, leg elevation is coincident with leg promotion, and leg depression is coincident with leg remotion [1]. The leg promotor MNs are active at about the same time as the Lev MNs and would excite a promotor assistance reflex similar to the levator assistance reflex. Indeed, in both instances, rate-sensitive afferents and the lower threshold MNs are the ones that participate in assistance reflexes [77]. Just as the levator assistance reflex helps excite a levator burst to begin the swing phase of leg movement, the promotor assistance reflex may help trigger a promotor burst at the same time. At the end of the swing phase, the fall of the leg would excite the depressor assistance reflex while the first remotor spikes move the leg backwards and excite the remotor assistance reflex. These assistance reflexes would trigger the depressor and remotor bursts to produce the stance phase of the walking rhythm.

3.4.3 Reflex Reversal in Other Animals

The assistance reflexes described here serve similar functions to those described in insects and in mammals that promote locomotor phase transitions and load compensation during locomotion [14]. A positive feedback reflex between stress-sensitive afferents in the leg
cuticle of cockroach legs and a leg depressor MN increased the power stroke output during walking when the animal was placed under load [88]. The femoral chorodotonal organ (FCO) in the stick insect mediates resistive reflexes of the femoral-tibia joint to stabilize posture when the animal is resting and assistive reflexes during locomotion to aid in movement [89, 90]. In cats, a decrease in ankle extensor tension that occurs as the weight shifts off the leg helps to promote the stance-to-swing transition [21].

These individual reflex responses play definite roles within the context of specific movements, where their timing and coordination with both the central commands and other reflexes are critical. For example, the levator assistance reflex described here is both synergistic and coactive with reflexes mediated by the TCCO and TCMRO and by the funnel canal organ on the dactyl and the cuticular stress detectors near the CB joint [10, 68, 91]. However, which reflexes are synergistic and coactive will depend on the movement and behavior; movements that are part of forward walking will require different reflex synergies from those that contribute to backward walking or turning [10, 79, 92]. In the cockroach and stick insect, sets of campaniform sensilla in different leg segments of the leg encode forces during walking to produce combinations of reflexes that are appropriate for distinct phases of movement [93, 94]. In the cat, proprioceptive signals from the hip join those from the ankle extensor tendon to help determine the timing of the stance to swing transition [2].

3.4.4 Understanding Complex Closed Loop Sensorimotor Systems With the Aid Of Computational Neuromechanical Models

The complexity, variability, and state dependence of these reflexes and their interactions with CPGs makes the underlying mechanisms difficult to understand with unaided intuition. Moreover, much of the experimental data come from experiments on open loop systems, where the dynamic relationship among sensory feedback, motor output, and behavior
is broken, and emergent properties are hidden. Neuromechanical models like the one described here can help reveal those closed loop dynamic relationships and explore their consequences for motor control [95]. The neuromechanical model includes explicit descriptions of the components of the system, including the different reflex pathways, the CPG, the physical movement of the limbs under gravity, and the stimulation of proprioceptors and exteroceptors by those movements. The model then can be asked how the components might interact to reproduce the experimentally observed behavior of the animal, and which components and processes should have the largest effects on that behavior. The model’s failures will help to identify faults in our understanding of the system or its components, while its successes can provide a platform for extending our analysis to still more complex aspects of the system.

3.5 Endnote

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at the institutional website of one of the authors, which at the time of publication they indicate is https://www.dropbox.com/sh/kfbs96wmak2mlty/AACdqTm1hF5KRBajvcCW8mrva?dl_0. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.
4 Discrimination of Bursts and Tonic Activity in Neural Spike Trains Using an Extended Hill-Valley Analysis Method

4.1 Introduction

Understanding the mechanisms that govern the output of a neural network requires consistent classification of different types of activity across experimental conditions. Biological experiments and computational models, for example, show that external cues or internal dynamics can cause a single neuron to exhibit a variety of activity patterns including quiescence, tonic spiking, and bursting [1, 7, 96, 97]. As the analysis of both neurons and networks grows more complex it is imperative that tools are developed to allow for a qualitative categorization of activity in an objective way.

4.1.1 Reliably Measuring Fluctuations of Spike Train Features

In some cases, visual inspection of a spike train is sufficient to classify activity. It is often the case, however, that activity is ambiguous or changes over time and visual classification can be impractical if there are large amounts of data to analyze. While automated algorithms provide fast and objective categorization of activity, most focus only on detecting bursts [62, 98-101], can require significant recalibration when used on different data sets, and are limited in their ability to concurrently discriminate tonic spiking from bursting.

The novel Extended Hill-Valley (EHV) method presented here consistently detected both bursting and tonic spiking activity based on a smoothed, history-dependent signal derived from a spike train. Several contexts were used to illustrate its performance in comparison to two other burst detection algorithms. Using visual inspection as the basis for measuring algorithm performance, EHV consistently outperformed the other methods with no recalibration and successfully classified different types of neural activity.
One of the challenges in automated classification of neural activity is finding a metric that accurately and reliably captures meaningful properties of a spike train. This is accomplished visually by looking at relative changes in spike frequency often with the aid of an instantaneous frequency plot. Due to changes in baseline firing frequency and the variability of inter-spike intervals (ISI), however, consistently determining the start and termination of an activity pattern can be difficult and is often subjective. In some cases, for example, the onset and termination of a burst can be readily discerned by eye based on clear breaks in a spike train, such as the simulated bursts in Fig. 4.1A (See Materials and Methods). In other cases breaks are not clearly defined and determining whether a low frequency spike should be included in a burst can be ambiguous as illustrated in the simulated activity in Fig. 4.1A. Burst detection algorithms determine whether a series of spikes are part of a meaningful activity pattern by employing metrics based on local or global ISI distributions. For example, the Poisson Surprise (PS) method detects bursts by finding sequences of spikes whose ISIs do not likely occur by chance [62]. Another algorithm, called the Cumulative Moving Average (CMA) method, uses an ISI histogram of the entire spike train to determine an absolute ISI threshold that is used to identify the start and termination of meaningful activity patterns [99].

4.1.2 Approaches Using Inter-spike Interval Distributions

The ISI histogram of a spike train can have very different properties that can affect the performance of detection algorithms (Fig. 4.2). In the case of a bursting neuron that is rhythmic (Fig. 4.2A) the ISI histogram has a group of short ISIs that correspond to spikes that occur during bursts and a group of long ISIs that occur at the onset of a burst. When long episodes of tonic spiking occur (Fig. 4.2B) or as activity patterns become less distinct (Fig. 4.2C), however, the location of a meaningful ISI threshold becomes ambiguous. In the case of a
Figure 4.1. Defining burst onset and termination can be ambiguous
Raster plots of two example spike trains illustrate how burst detection can vary across methods. Clearly (A) and ambiguously (B) defined burst onset and termination can occur across a data set. (i) Raster plots (top trace) show analyzed neural activity and instantaneous frequency is shown below. Colored bars above raster plots indicate bursts detected by visual inspection (black), the Extended Hill-Valley method (magenta), the Cumulative Moving Average method (green), or the Poisson Surprise method (blue). Barbed colored lines above raster plots indicate detection of a tonic spiking event. (ii) Zoomed in views of the dashed outline in (i) show the spikes raster plots (top trace) and instantaneous frequency for a burst event. The Convolved Signal (third trace) shows the first step of signal conditioning for the Extended Hill-Valley analysis method. The Smoothed Signal (bottom trace) shows the final analysis signal used in the Extended Hill-Valley analysis method. Peaks and troughs of the smoothed signal are indicated by black dots.
A. Rhythmic Bursting

B. Tonic Spiking & Bursting

C. Ambiguous Activity

D. Transitioning States

Figure 4.2. Raster plots and inter-spike interval histograms for spike trains with distinct neural activity
Spike raster plots (top panels), instantaneous frequency graphs (second panels) and inter-spike interval histograms (bottom panels) are shown for four different activity patterns: (A) rhythmically bursting spike train; (B) tonic spiking and bursting spike train; (C) ambiguous activity; and, (D) activity that is transitioning through quiescence, bursting, and tonic spiking. Black diamonds indicate the primary threshold used to determine spikes that were in a burst and the white diamonds indicate the secondary threshold used to determine burst-related spikes.
neuron that exhibits quiescence, bursting, and tonic spiking in the same spike train (Fig. 4.2D) the ISI histogram is severely skewed by spikes that occur spontaneously during quiescence and have very large ISI values.

The EHV classification method is not derived from an ISI distribution, but, instead, classifies neural activity based on fluctuations of a smoothed analysis signal derived from a spike train (Fig. 4.1.ii). Whereas the original Hill-Valley burst detection method [100] identified activity patterns based on a rectified and smoothed analysis signal, the EHV method added a history-dependent factor to the signal for analysis. Consequently, differences in signal fluctuations were accentuated between bursts and episodes of tonic spiking, which allowed the EHV method to concurrently identify both types of neural activity with consistency.

4.2 Materials and Methods

4.2.1 Data Set

Spike train data were taken from the activity of motor neurons in a locomotor circuit of crayfish [1, 23]. Briefly, the circuit is comprised of mutually inhibitory leg levator and depressor motor neuron pools that are modulated by sensory feedback from afferents as well as pharmacologically using a muscarinic agonist, oxotremorine (OXO) [1, 23]. In the absence of OXO, the network is in a quiescent state that acts to maintain static posture. In this state, leg perturbations trigger phasic reflex responses from motor neurons that resemble a burst. Shortly after applying OXO via bath perfusion, the network transitions to an excited state where external perturbations trigger bursts. Finally, when OXO reaches sufficient levels the mutually inhibitory motor neurons burst rhythmically, corresponding to a locomotive behavioral state. The variety of behavioral states of the circuit motivated the development of the Extended Hill-Valley algorithm.
In vitro spike trains were from experiments in which extracellular activity was recorded from motor and sensory nerves of an in vitro nerve cord [23]. A biomechanical model was used to simulate movements of a crayfish leg and was used to control the stretch and release of an in vitro stretch receptor connected to the nerve cord. Experiments that used different configurations of sensory feedback and pharmacological excitation resulted in a wide range of activity patterns, only a few of which are illustrated here. Analyzed recordings were 940 s long.

Simulated spike trains were generated by a model of the locomotor circuit in AnimatLab [42, 102] and included motor neurons, sensory neurons, excitatory and inhibitory interneurons, muscles, and a biomechanical model of the leg [23]. The model used here had 31 neurons each with 0.1 mV of noise, which generated variability in simulation results. The network was a simplified version of the biologically characterized network [1] and was modified from Bacque-Cazenave, et al. [23] in order to allow for separate control of the effect of pharmacological excitation to the network. Simulations were run for 300 s with 40 s relaxation period at the beginning of each run. Some simulations included an external perturbation at 160 s where the leg was elevated for 1.5 s and then released. Spike trains were taken from the tonic levator motor neuron that excites levator muscles to lift a leg during postural movements and walking.

### 4.2.2 Visual Inspection

Bursts and tonic activity were first characterized by visual inspection using raster plots of each spike train and graphs of their instantaneous firing frequency (Appendix A, Fig. A.3). Bursts were identified by looking for clusters of spikes that had a rapid increase in firing frequency. Tonic spiking was characterized by looking for sequences of spikes whose frequency curve rose and maintained an elevated level for a period of time before returning to a baseline firing rate.
4.2.3 Extended Hill-Valley Algorithm

Based on the Hill-Valley method of burst detection [100], the Extended Hill-Valley analysis (EHV) is comprised of two major analysis phases: 1) signal conditioning, and 2) signal analysis. Signal conditioning is accomplished in the original method by smoothing the squared voltage values of extracellular recordings [100]. In EHV, signal conditioning begins by convolving the spike train with a decaying exponential function. A spike train can be calculated by using a simple threshold to identify the onset time of spikes. Because a convolution is equivalent to aligning the convolution function at each of the spike events and summing overlapping values, this step produces a continuous signal in which spikes have a lasting effect through a certain time period. The convolution of spike events resulted in a signal with peaks aligned at each spike, so the final signal was smoothed using a Gaussian kernel. Three parameters can be set for signal conditioning: 1) convolution function width, 2) convolution function magnitude, and 3) Gaussian width. The first two set the time length of effect for each spike and the magnitude of that effect, respectively. The third parameter determines the duration of a time window used to smooth the signal.

The last step of signal conditioning is trough and peak detection, which was accomplished using a continuous wavelet transform. If a trough was detected in the middle of a flat, zero amplitude region of the analysis signal it was adjusted to help increase accuracy by avoiding a skewed width of neighboring peaks. Set by default at 0.1 (10%), the trough was adjusted to the point when the analysis signal reached 10% of the peak height of the preceding hill, if one existed. The same trough adjustment was calculated for the immediately following peak height if one existed. Consequently, troughs that were detected in a flat region of the final analysis signal were replaced with two trough points that more accurately reflected the actual width of each surrounding hill.
In the original Hill-Valley method, the signal analysis step detects bursts by setting a threshold and finding points where the signal rises above and falls below the threshold to indicate the onset and offset of an event, respectively. Signal analysis of the Extended Hill-Valley method was a recursive algorithm that detected bursts and bouts of tonic activity based on the height and width of successive peaks. Bursts were identified by measuring the height-to-width ratio of the rising and falling slopes of each hill. The rising height-to-width ratio of a hill was calculated as the height from the preceding trough to the peak of a hill and the width was measured as the time from the preceding trough to the immediately following trough. The falling height-to-width ratio was calculated in a similar fashion. In this way, hills whose rising and falling ratios exceeded the Minimum Peak Ratio for bursts were considered to be a burst.

In addition, the falling peak height needed to exceed the Exclusion Ratio of the rising peak height in order to ensure that the spike activity rose and fell sufficiently. In the case of multi-peak bursts, the rising and falling ratios were calculated from the highest peak in the hill-valley sequence to the trough preceding the first peak and the trough following the last peak of the sequence, respectively.

Tonic activity was classified by measuring the variability of successive peaks and troughs. The onset of tonic activity was marked by a peak whose rising height-to-width ratio exceeded a Minimum Peak Ratio for tonic activity. Then, the algorithm iterated through successive peaks and troughs to measure their cumulative standard deviation. If the standard deviation remained less than the Maximum Hill-Valley STD, additional peaks and troughs were included. If the standard deviation exceeded that maximum, then the end of tonic spiking was marked. Tonic spiking events were also subject to a second condition that ensured the activity remained elevated above the Minimum Tonic Level, which was a percentage of the initial rising peak height. Thus, tonic activity was defined by a series of spikes that occurred with an elevated firing rate that was within a prescribed range of variability. Because tonic spiking can
occur at lower rates as well as much higher rates, a scaling factor was introduced for peaks and troughs whose absolute amplitudes were greater than 100. When this condition was true, the rising (falling) height-to-width ratios were scaled by the ratio of the rising (falling) peak-to-trough height divided by the absolute peak height measured from 0. Thus, variability around larger amplitudes of the analysis signal that reflected a very elevated and sustained firing rate was classified as tonic activity.

When burst or tonic spiking events were detected in the analysis signal, the onset and offset of each event were calculated as a certain percent of the trough to peak height. For burst events, the onset was determined by finding the first spike that occurred after the rising analysis signal reached 0.2 (20%) of its trough-to-peak height. Termination of a burst occurred at the last spike before the analysis signal fell below 0.2 (20%) of its trough-to-peak height. Similar calculations were used with a threshold of 0.3 (30%) to determine the onset and termination of tonic spiking events.

4.2.4 Cumulative Moving Average Method

The cumulative moving average (CMA) method determines the onset and termination of a burst by using an adaptive approach to calculating an inter-spike interval (ISI) threshold value [99]. It started by calculating an ISI histogram for the entire spike train. A cumulative sum of the binned ISI values was then calculated and used to determine a cumulative moving average curve. The CMA was calculated by dividing the cumulative sum by the number of bins included in the summation. Then, the skew of the histogram was calculated and used to determine a scaling factor (see [99] for details). Two factors were calculated, one that marked ISIs that belonged to spikes within a burst, called $\alpha_1$, and one that took into account the variability of ISIs at the beginning and end of a burst, called $\alpha_2$. Each factor was used to calculate a CMA value along the tail (increasing values of ISI) of the CMA curve that was a certain percentage of
the peak CMA value and corresponded to an ISI threshold. The ISI threshold determined by the first factor, called the “burst spike threshold,” was defined to be larger than the latter and captured spikes with shorter ISI values, called “burst spikes.” The second factor was used to calculate the “burst-related spike threshold,” which identified spikes that might be part of a burst sequence, called “burst-related spikes.” After sequences of burst spikes that included at least 3 spikes were identified, burst-related spikes that immediately preceded or followed each burst sequence were added to extend the bursts. Finally, bursts were merged into one event if they were separated by no more than the duration set by the burst-related spike threshold.

4.2.5 Poisson Surprise Method

The Poisson Surprise method operated on the assumption that an ISI histogram of an entire spike train should be Poisson-distributed [62]. Consequently, a burst of spikes with very short ISIs is surprising because their distribution is not Poisson-like. Implementation of the algorithm started by calculating the average firing rate for the entire spike train. Then, detection of bursts started by iterating through the spike train and identifying short sequences of at least 7 spikes whose ISIs were no greater than 0.5 (50%) of the average ISI of the entire spike train. Once a short sequence was identified, spikes were added on to the end of the burst and the surprise of the sequence was calculated. The surprise was calculated as \(-\log(P)\), where \(P\) is the probability that the ISI values are Poisson-distributed. Consequently, large surprise values mean that it is very unlikely that the ISIs of the spike sequence are Poisson-distributed. If the surprise was high, then additional spikes were added to further maximize the surprise value. If addition of over 5 spikes did not maximize the surprise, then no further spikes were added to the burst. After addition of spikes to the end of the burst, spikes were removed from the beginning of the burst to again maximize the surprise of the sequence. Spikes were removed from the beginning of the burst until the surprise was no longer increased. Only spike
sequences whose surprise was larger than 0.3 were considered to be bursts. It should be noted that in the original publication of the Poisson Surprise method, a minimum surprise value of 10 was used. In this dataset, however, a surprise value of 10 yielded no bursts for the simulated rhythmic bursting data and detected very few events in the other sample data sets.

4.2.6 Calculating the Jaccard Index to Compare Algorithm Performance

Using visual inspection as the basis for comparison, the quality of algorithm performance was measured using the Jaccard Index. The Jaccard Index measures the similarity between two sequences of events. Classification results were transformed to a Boolean sequence by sampling the burst and tonic spiking events at time steps of $\Delta t$. The resulting Boolean sequence was 0 when no event occurred and 1 when an event was detected. The Jaccard Index was calculated as,

$$Jaccard \ Index = \frac{A \cap B}{A \cup B}$$

where A and B corresponded to the two Boolean sequences being compared and the symbols, $\cap$ and $\cup$, are the intersection and union of the two sets, respectively. The intersection of two sets is the number of times that an event occurs in both sequences. The union of two sets is the number of times that an event occurs in one or both sequences. Thus, the intersection of two sequences of events that do not occur at the same time will be small in comparison to the union of the two sequences in which one or both sequences have an event and the Jaccard Index will be close to 0. When two sets have events that occur at the same time, however, the intersection when both sequences have an event is similar to the union when one or both sequences have an event. In this case the Jaccard Index is close to 1.


4.2.7 **Parameter Optimization**

Parameters were optimized in two stages using the Jaccard Index. Optimization started with all parameters set to their originally published values. Each parameter was sampled individually over a large range of values (Table A.1). A Jaccard Index curve was generated for each parameter by calculating the Index at each value in the range. The parameter value corresponding to the maximum peak of the curve was selected as the basis for the second stage of optimization. The second stage of optimization was a higher resolution of sampling for each parameter across a narrower range of values (Fig. A.2). This step was used to determine the best parameter value and to measure the sensitivity of each method to parameter changes. Parameter values were finally selected by comparing the Jaccard Index curves across data files and identifying the value that worked best for most activity types.

4.2.8 **Online Resources**

The model used to generate the simulate spike trains is available online at ModelDB. The scripts used to analyze simulated and *in vitro* spike trains are available online at GitHub.

4.3 **Results**

The Extended Hill-Valley (EHV) algorithm detects bursts and bouts of tonic spiking in neural activity. Performance of the EHV method was tested on a range of *in vitro* and simulated spike train data sets (Appendix A, Fig. A.3). Analysis results were then compared to two other burst detection methods that use inter-spike interval distributions to detect features in a spike train. The quality of analysis for all three algorithms was compared based on results from a visual classification of spike train activity.
4.3.1 Dataset

In order to illustrate the results of the EHV algorithm and to compare its performance, a set of in vitro and simulated spike trains were selected for analysis. In vitro spike trains were taken from extracellular recordings of motor neurons of a crayfish locomotor circuit [1, 23]. The analysis was run on spike trains from experiments that were 950 s long and samples were selected based on a visual classification of activity. Simulated spike trains were from motor neuron activity that was generated by a model of the same locomotor circuit [23]. The network model consisted of neurons that each had 0.1 mV of noise, which introduced variability during simulations. Depending on the state of the network, motor neurons exhibited quiescence, bursting, or tonic spiking activity patterns [1, 23]. Simulated spike trains were 300 s long and were selected from a database of simulation results based on visual classification of activity.

4.3.2 Extended Hill-Valley Algorithm

The EHV analysis used a recursive algorithm with classification loops that identified burst events and bouts of tonic activity. Whereas the original Hill-Valley method smooths the squared voltage values of an extracellular recording for burst detection [100], EHV smooths a convolved spike train for classification in order to accentuate differences between bursts and bouts of tonic spiking. In addition, the original Hill-Valley method uses a threshold to determine the beginning and end of burst events [100] while the Extended Hill-Valley analysis recursively measures the height-to-width ratio of successive peaks in the analysis signal. For a full, detailed schematic of its implementation see Supplemental Figure 1. The EHV analysis occurred in two main stages: 1) signal conditioning, and 2) signal analysis. Signal conditioning was accomplished by first convolving the spike train with a decaying exponential function (Fig. 4.1.ii, Convolved Signal). The rate of change of the resulting analysis signal reflected the rate at which the firing frequency changed and the signal amplitude was proportional to the firing rate.
To eliminate sharp peaks at the time of each spike, the analysis signal was also smoothed (Fig 4.1.ii, Smoothed Signal). Finally, peaks and troughs (i.e., “hills” and “valleys,” respectively) were identified using a peak detection algorithm (Fig 4.1.ii). Three parameters were set for signal conditioning: 1) convolution width, 2) convolution amplitude, and 3) smoothing width. The convolution width determined how long it would take for the effect of each spike to diminish over time. The amplitude defined the maximum effect of each spike at the time of its occurrence. The smoothing width dictated how many neighboring data points were used to calculate a weighted average for the final analysis signal.

The second stage of signal analysis was based on the principle that the amplitude and slope of the analysis signal captured local history-dependent changes in the activity of a spike train. A burst, for example, is defined as a sequence of spikes with very short inter-spike intervals (ISI) that are preceded and followed by spikes with longer ISIs. This was reflected in the EHV analysis signal as a hill with sufficiently steep rising and falling slopes (Fig 4.3). Tonic spiking is defined as a series of spikes with an elevated frequency and sustained ISIs. In the EHV analysis signal this was captured as a series of hills and valleys that rose above a minimum amplitude and whose peaks and troughs stayed within a prescribed range of variability (Fig 4.4).

4.3.3 Algorithm Parameter Selection

Three algorithms were used to identify bursts in a spike train and these were compared to bursts identified by visual inspection. Each of the three algorithms (Extended Hill-Valley, EHV; Cumulative Moving Average, CMA; and Poisson Surprise, PS) had parameters that could be changed to optimize its performance for different parts of the spike train. Because data sets can be large and consist of thousands of spike trains, however, it is not practical to recalibrate an algorithm for each part of the spike train. To illustrate the capability of each algorithm to
**A. Single-Peak Burst Detection**

![Single-Peak Burst Detection Diagram](image)

**B. Multi-Peak Burst Detection**

![Multi-Peak Burst Detection Diagram](image)

**C. Burst Detection Algorithm**

![Burst Detection Algorithm Diagram](image)

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**Figure 4.3. Extended Hill-Valley signal analysis and burst detection flow chart**

The Extended-Hill Valley (EHV) analysis signal is used to detect bursts in spike trains. Raster plots (top traces), instantaneous frequency graphs (middle traces), and EHV analysis signal (bottom traces) illustrate bursting activity of a neuron. Bursts are marked above each raster plot as thick bars and are color-coded based on the detection algorithm that was used. (A) A single-peak burst is characterized by its height-to-width ratio (h/w) of its corresponding hill in the EHV analysis signal (see text for explanation). (B) Classification of a multi-peak burst occurs by using the highest peak (dashed blue line) to measure its height and the full width to calculate the height-to-width ratio. (C) A reduced flow diagram of the EHV algorithm highlights the main burst detection steps in thick outlines and arrows (see text). A full schematic of the EHV algorithm can be found in Appendix A, Fig. A.1.
classify activity across a large dataset with no recalibration, the same parameters were used to analyze the entire spike train. Consequently it was important to identify parameters that performed well across a variety of activity patterns (Appendix A, Table A.1).

Parameter selection for EHV and PS was accomplished through a two-stage optimization process that consisted of a coarse sampling of values followed by a local sampling. Algorithm performance was measured by comparing results to a visual analysis using the Jaccard Index, which quantifies the similarity between two sequences of events (See Materials and Methods). In the first stage, initial parameter values were taken from the published descriptions of the algorithms. The Jaccard Index was calculated by comparing the visually identified bursts to those identified by the algorithm. Each parameter value was varied individually across a broad range, and the value that yielded the highest Jaccard Index was selected as the starting point for the next stage. In the second stage, the parameters were set based on the results from the first stage and a smaller range was sampled at a higher resolution for each parameter (Appendix A, Table A.1). A Jaccard Index curve was calculated again and the best parameter value was chosen for the final analysis (See Appendix A, Fig. A.2). Parameters for the CMA method were not optimized and were taken directly from their original publication because of the complexity of their selection [99].

4.3.4 Detecting Bursts

The simplest classification was a burst that occurred in a single hill of the EHV analysis signal (Fig. 4.3A). The rising height, $h_1$, of the hill was measured from the leading trough to the peak. Similarly, the falling height, $h_2$, of the hill was calculated from the peak to the lagging trough. The width of the hill, $w$, was calculated from the leading trough to the lagging trough. If both the rising and falling height-to-width ratios, $\frac{h_1}{w}$, exceeded a user-defined value, called the “Burst Threshold,” then the hill was considered a burst. In the case that a burst occurred across
**Figure 4.4. Extended Hill-Valley classification of tonic activity**

Spike raster plots and instantaneous frequency graphs illustrate tonic spiking activity. The EHV analysis detects features in the EHV analysis signal. Burst events are indicated above each raster plot by thick bars while bouts of tonic activity are marked by lines with barbs on either end. Results of visual inspection, CMA, and PS algorithms are shown for comparison. (A) Onset of tonic activity is identified when the height-to-width ratio ($h/w$, red arrows) of a hill exceeds the user-defined “tonic threshold.” Termination of tonic activity is determined by either a trough that falls below a minimum activity level, $MIN$, or by an excursion of a peak or trough that exceeds the Hill-Valley STD, $+STD$. (B) The EHV algorithm is capable of discriminating bursts and tonic spiking that are immediately adjacent. The instantaneous firing frequency is shown below the raster plot to assist in visual identification of bursts. (C) A series of criteria are used to identify tonic activity as highlighted by the thick outlines and arrows in a reduced flow diagram of the Extended Hill-Valley analysis algorithm. A full flow chart of the EHV algorithm can be found in Appendix A, Fig. A.1.
multiple hills (Fig. 4.3B), the height-to-width ratios were measured from the highest peak in the sequence to the leading and lagging troughs of the entire sequence. Because heights were measured from trough to peak rather than the absolute height of the peak, bursts were detected independent of different baseline firing rates.

Visual inspection of an in vitro spike train that was spontaneously bursting (Fig. 4.5A) identified 25 bursts and no tonic activity. The EHV and CMA algorithms detected 15 and 14 bursts, respectively, while the PS algorithm detected 61 bursts. Full analysis results for sample data are shown in Fig. A.3 of Appendix A and short examples were selected in Fig. 4.5 to highlight the differences of algorithm performance. Analysis of simulated rhythmic bursting (Fig. 4.1A), identified 36 bursts with an average duration of 4.88 +/- 0.15 s by visual inspection. EHV detected 35 bursts that had a similar average duration of 4.62 +/- 0.12 s and CMA detected 44 bursts with an average duration of 3.81 +/- 0.27 s. PS detected 239 burst events with an average duration of 0.24 +/- 0.01 s.

EHV detected fewer bursts in the in vitro spike train because some events that were visually identified had a lower spike frequency and the corresponding hills of the EHV analysis signal did not meet the burst threshold criteria.

The shape of the ISI distribution used by CMA analysis resulted in an unfavorable selection of ISI thresholds for simulated rhythmic bursting. CMA uses two ISI thresholds to identify the onset and termination of bursts. In the case of simulated rhythmic bursting (Fig. 4.2A, black and white diamonds), CMA detected spikes at the beginning or end of a burst that had an ISI greater than the threshold value but were still visually identified to be part of a burst. The burst threshold ISI for in vitro bursting was 5.8 s and was sufficient to classify most of the bursts in the spike train. A secondary threshold was used to add spikes to the beginning or end of a burst in case they had a lower ISI but were still part of an event. Because of the shape
of the ISI histogram the secondary threshold was 22.0 s and resulted in several of the bursts being joined into single events.

A larger number of bursts was detected by PS than by the other two algorithms. Because PS identifies bursts based on the likelihood that a sequence of spikes occurs by chance, spike trains with low ISI variability resulted in poor performance. For spontaneous *in vitro* bursting, the average firing frequency of spikes in visually identified bursts was 42.56 +/- 1.63 Hz while the average population firing frequency was 42.35 +/- 0.71 Hz. In the case of simulated rhythmic bursting the visually identified events had an average spike frequency of 55.07 +/- 1.07 Hz while the population average of the entire spike train was 54.53 +/- 0.39 Hz.

4.3.5 Detecting Tonic Activity

Tonic activity can occur in either isolated bouts or in conjunction with a burst event (Fig. 4.4). In isolation, the onset of tonic activity was identified by a hill whose height-to-width ratio exceeded a user-defined “Tonic Threshold,” which was less than the burst threshold. The duration of tonic activity was not constrained by a minimum or maximum time period but, instead, was dictated by fluctuations of the analysis signal. Thus the longest duration of a tonic event was the length of a recording. Termination of a bout of tonic activity can occur in one of two ways. Starting from the first hill, the algorithm added successive peaks and troughs so long as they occurred within a user-defined limit of variability, called the “Hill-Valley STD” (Fig. 4.4B). The algorithm also checked that each lagging trough remained above a user-defined fraction of the rising peak of the first hill, called the “Minimum Signal Value.” If either of these conditions was not met the algorithm terminated the tonic event. Because of the linked recursive loops for burst and tonic spiking detection, the algorithm could also detect when a bout of tonic activity was terminated by a burst and when a burst was immediately followed by tonic activity (Fig. 4.4C).
A. *In vitro* Bursting

B. *In vitro* Tonic Spiking

C. Simulated Tonic Spiking

D. Simulated Tonic Spiking & Bursting

E. Simulated Transitioning Activity

F. Simulated Multi-Stability

G. *In vitro* Ambiguous Activity

- **Visual Inspection**
- **Extended Hill-Valley**
- **Cumulative Moving Average**
- **Poisson Surprise**
Figure 4.5. Algorithm results and comparison of methods
Spike raster plots and instantaneous frequency graphs illustrate a wide range of activity patterns from *in vitro* and simulated spike trains. Full spike analysis of spike train data can be found in Supplemental Figure 3. The smoothed analysis signal used by EHV is shown to illustrate the accentuated fluctuations during bursts and tonic spiking. (A) *In vitro* bursting. (B) *In vitro* tonic spiking. (C) Simulated tonic spiking. (D) Simulated tonic spiking and bursting. (E) Simulated transition from quiescence (not shown) to bursting to tonic spiking. The transition from bursting to tonic spiking occurs at 211 s (F) Simulated multi-stability. A neuron is perturbed at 160 s and switches from tonic spiking to bursting. (G) Different analysis methods resulted in a range of results for *in vitro* ambiguous activity. Burst events are indicated above each raster plot by thick bars while bouts of tonic activity are marked by lines with barbs on either end. Bar and line color indicates which detection algorithm was used.
In the case of *in vitro* tonic activity (Fig. 4.5b), 33 tonic spiking events were identified by visual inspection. EHV and CMA performed similarly and yielded 33 and 31 events, respectively. PS detected 181 bursts in the spike train. Two simulated spike trains were selected because their activity was either exclusively or predominantly tonic spiking. In the first example, visual inspection identified one tonic spiking event that lasted 249.76 s, the duration of the simulation (Fig. 4.5C). EHV yielded a single event of tonic activity lasting 249.78 s whereas CMA detected 49 bursts with an average duration of 5.08 +/- 0.87 s. PS detected 1,959 bursts with an average duration of 0.08 +/- 0.0 s. In the second example that was predominantly tonic spiking with some bursts, visual inspection identified 7 bursts and 3 bouts of tonic spiking activity (Fig. 4.5D). EHV detected 6 bursts and 3 bouts of tonic activity. CMA detected 16 bursts while PS detected a total of 51 bursts. While EHV yielded results that were similar to visual inspection in all three cases, PS performed poorly for reasons similar to those outlined in burst detection.

CMA performed moderately well across samples but was only able to detect short or long duration bursts rather than discriminating between bursts and tonic spiking, respectively. When the neuron exhibited only tonic spiking, CMA yielded multiple burst events because small fluctuations in single ISIs resulted in termination of a burst according to the CMA threshold criteria. For the simulated dataset that was predominantly tonic spiking, removal of all 3 events with durations longer than the average burst duration left 13 bursts that more closely resembled events that were identified by visual inspection.

### 4.3.6 Classifying Complex Activity

Because neural activity was not always exclusively bursting or tonic spiking the algorithms were also used to analyze spike trains that exhibited a mixture of activity. In a simulated spike train that transitioned across activity states from quiescence to bursting to
tonic spiking, visual inspection identified 15 burst events and 1 bout of tonic activity (Fig 4.5E). EHV detected 12 bursts and 1 bout of tonic activity. CMA detected 7 bursts that matched visually verified events, but also detected one long-duration burst spanning several events that were otherwise classified as bursts and tonic activity. PS detected 426 short bursts, most of which occurred during events that were also identified by visual inspection. Upon closer inspection, the average firing rate of spikes during quiescence and bursting was 131.36 +/- 2.07 Hz and the average firing rate during tonic activity was 210.67 +/- 0.56 Hz. Because the average firing rate of the population was 200.73 +/- 0.60 Hz the PS method performed well to detect events during the quiescence and bursting activity states when the average firing rates were different, but did not perform well during tonic spiking when the average firing rates were similar.

The spike train in Fig. 4.4B, C illustrated a simulated complex spiking pattern that consisted of a high frequency burst immediately followed by tonic spiking, which has been referred to as phaso-tonic bursting [85]. In addition, there are a few bouts of tonic activity that did not occur in conjunction with a burst. With the help of a plot of the instantaneous firing frequency, visual inspection identified 12 phasotonic [85] bursts and 15 bouts of tonic activity. EHV detected 13 bursts and 11 tonic spiking bouts, 6 pairs of which corresponded to episodes of phasotonic bursting. CMA detected 13 events, many of which spanned both bursting and tonic spiking activity that occurred during phasotonic bursts. PS detected 44 bursts with much shorter durations than EHV and CMA.

A neuron can be multistable if it exhibits more than one stable pattern of activity [96, 103, 104]. In the spike train shown in Fig. 4.5F, a perturbation was applied to a motor neuron at 160 s and triggered a switch from tonic spiking to bursting. With the aid of an instantaneous firing frequency plot, visual inspection identified 18 bursts and 2 bouts of tonic activity. EHV detected 12 bursts and 8 bouts of tonic activity. The discrepancy between visual analysis and
EHV was due to 5 bouts of tonic spiking that were detected by EHV and corresponded to bursts that were identified by eye. During these events, the firing rate was lower and the height-to-width ratio for EHV did not meet the minimum criteria for bursts. CMA yielded 84 bursts with varying durations, many of which matched visually characterized bursts. PS returned 82 events that occurred mainly during the bursting regime of the multi-stable spike train as visually identified burst events were broken into several short-duration bursts by the PS algorithm.

### 4.3.7 Classifying Ambiguous Activity

In an analysis of *in vitro* data where it was not clear whether activity was bursting, tonic spiking, or spontaneous it was difficult to determine whether one algorithm performed better than the others. Visual inspection of ambiguous *in vitro* data (Fig. 4.5G) identified 29 bursts ranging from 0.61 s to 7.62 s and 13 tonic spiking events with durations ranging from 5.69 s to 48.3 s. EHV identified 5 bursts with durations ranging from 2.70 s to 4.28 s as well as 35 tonic spiking events that had durations ranging from 2.36 s to 32.80 s. While CMA detected 48 bursts with durations ranging from 0.62 s to 41.87 s, PS detected 67 bursts ranging in duration from 0.26 s to 2.87 s.

A simulated spike train with ambiguous activity was also analyzed (Fig. 4.1B). Visual classification identified 25 bursts and 4 episodes of tonic spiking activity. EHV detected 20 bursts and 7 bouts of tonic activity. CMA detected 53 burst events and PS detected 111 bursts. There were no long duration bursts that clearly corresponded to tonic activity detected by either PS or CMA. Visual comparison of the results from both *in vitro* and simulation analyses suggested that CMA had a tendency to merge bursts and pick up brief sequences of short ISI spikes while PS divided bursts into several shorter events. While the bursts detected
by EHV were supported by visual verification corresponding to large increases in the instantaneous firing frequency, there appeared to be an overestimation of tonic activity.

4.3.8 Quality of Algorithm Performance

In order to assess the quality of performance of the three algorithms, a Jaccard Index was used to compare algorithm results to a visual analysis. Briefly, the Jaccard Index measures the similarity between two sequences of events (see Materials and Methods). When an algorithm detected events that closely matched those identified by visual inspection its Jaccard Index was close to 1 (100%) and it was considered to have performed well. When the events detected by an algorithm did not match those identified by visual inspection the Jaccard Index was close to 0 (0%) and it was considered to have performed poorly.

The performance of the three algorithms was measured across a variety of neural spiking activity including rhythmic bursting, tonic spiking, and mixtures of bursting and tonic spiking. Because it would be difficult and time consuming to recalibrate algorithm parameters for every spike train, the parameters were optimized and the same values were used across data sets (see Materials and Methods). Briefly, parameter optimization was accomplished using a two-step search process. The first step was a coarse sampling of algorithm performance for a wide range of values based on the originally published algorithm parameters. The second step was to sample a smaller range of parameter values at a higher resolution based on the best algorithm performance from the first step. The final parameter value was selected by looking at the highest Jaccard Index across the different spiking activity.

In general, bursts detected by the PS method were up to 50% similar to burst events identified by visual inspection (Fig. 4.6, blue bars). The algorithm performed best on in vitro data that were either bursting or spontaneously active and performed poorly (0 – 15% similarity) for both simulation and in vitro data that were predominantly tonic spiking or
transitioned between activity types. For simulation data that were either bursting or a combination of bursting and tonic or spontaneous activity PS detected bursts that were 25 – 40% similar to visual inspection.

Performance of the CMA method varied across test data (Fig. 4.6, green bars). The best performance was 95% similarity for a rhythmically bursting spike train. Moderate performance (30 – 60% similarity) was achieved for ambiguous activity for both in vitro and simulated data as well as for a spike train with spontaneous bursting or both bursting and tonic spiking activity. CMA performed poorly (0 – 30% similarity) on spike train data that included large amounts of tonic spiking activity.

The Jaccard Index for EHV classification showed that the algorithm detected burst events that were up to 90% similar to visually identified events (Fig. 4.6, pink bars) and tonic spiking events that were up to 100% similar. EHV detected bursts that were more than 80% similar to visually identified events in data that were rhythmically bursting, mostly tonic spiking, transitioning, and phasotonically bursting. Moderate burst detection (40 – 80% similarity) was achieved by EHV for data that were ambiguous (not clearly bursting nor tonic spiking), in vitro bursting, and a mixture of bursting and tonic spiking. For in vitro data that were not clearly bursting or tonically spiking EHV detected bursts that were about 20% similar to visual inspection.

Because the algorithm distinguishes between bursting and tonic spiking events, a Jaccard Index was evaluated for EHV separately for tonic spiking (Fig. 4.6, magenta bars). For simulation data that were visually classified as exclusively tonic, transitioning, or mostly tonic as well as exclusively tonic in vitro data, EHV identified events that were over 80% similar. Phasotonic and mixed activity were between 70 to 80% similar while the algorithm detected tonic activity that was 0 – 20% similar in ambiguous simulation data and spontaneous in vitro data.
Figure 4.6. Quantitative comparison of algorithm performance

The Jaccard Index compares two sequences of binary events. A Jaccard Index close to 0 indicates poor performance whereas a value close to 1 indicates good performance. A Jaccard Index was calculated for all three algorithms to measure their performance in comparison to visual analysis for each spike train. The Extended Hill-Valley method generally outperformed the other two methods for both burst (pink) and tonic spiking (magenta) detection. The Cumulative Moving Average method (green) performed best on rhythmically bursting data and poorly on spike trains with tonic activity. The Poisson Surprise method (blue) performed moderately well for ambiguous in vitro data.
4.4 Discussion

Defining bursts based on the distribution of inter-spike intervals (ISI) has proven to be fast and powerful and has resulted in a fractured approach to classifying neural activity. Bursts that are defined by adaptive ISI threshold methods (e.g., Cumulative Moving Average, CMA) are not necessarily comparable to those that are detected by probability-based methods (e.g., Poisson Surprise, PS). In the results presented here, the bursts detected by different algorithms did not always correspond to each other or to events identified by eye. In addition, because different types of neural activity have different ISI distributions, it is not clear how to find a parameter range for either an adaptive ISI threshold or probability-based method that translates well across data sets and is resilient to changes in baseline spike frequency. Finally, these ISI-based burst detection algorithms, by definition, do not yield results that discriminate between bursting and tonic activity.

The Extended Hill-Valley (EHV) analysis method uses a smoothed, history-dependent analysis signal to classify neural activity and can detect bursts and bouts of tonic spiking across data sets without changing the parameters. EHV, however, has a larger number of parameters that require calibration to determine how features of bursts and tonic spiking are defined. Once calibrated, classification of neural activity by EHV outperformed both PS and CMA on a range of spike train patterns. For spike trains that were selected because of their ambiguous activity, however, all three classification algorithms yielded results that were different (similarity of ~50% or less) than events classified by visual inspection. The classification results for ambiguous activity underscored the difficulties and nuances of determining how to objectively define a burst or tonic spiking.
4.4.1 Comparing Performance of Classification Algorithms

The ability of the PS algorithm to detect bursts varied across test data and yielded poor results based on visual classification. PS performed best on an in vitro spike train that exhibited irregular bursting or spike trains with ambiguous activity. In most cases, PS overestimated the number of bursts and yielded events that had much shorter durations than those identified visually. Because the basic tenet of PS is to detect bursts based on identifying sequences of spikes with short ISIs that are not likely due to chance the algorithm was dependent on fluctuations in firing frequency. Consequently, PS performed poorly when the frequency of spikes during burst events was not sufficiently different from the average frequency of spikes calculated during periods of bursting and outside of bursting together.

The CMA method was sensitive to the shape of a spike train’s ISI histogram. Using the skew of the ISI histogram to select a threshold, bursts were defined as a sequence of spikes with ISIs that were less than the ISI threshold. In addition, a secondary threshold was used to extend a burst if neighboring spikes had an ISI that was slightly longer than the burst threshold and shorter than the secondary threshold. CMA performed well in analyzing spike trains with predominantly bursting activity, but when spike trains exhibited a mixture of activity patterns the ISI histogram yielded thresholds that had a tendency to join or split events that were identified visually. In the case of spike trains that exhibited visually recognizable tonic activity, CMA was able to detect long-duration events that had an elevated firing frequency, but was not able to discriminate long duration bursts as tonic spiking events in its output.

The EHV method generally outperformed both CMA and PS based on a comparison to a visual analysis of spike trains. The improvement in performance was partially due to its ability to discriminate bursting from tonic spiking events. A large part of its success, however, was because the analysis signal was not sensitive to small deviations of ISI and the use of ratios to detect features eliminated its dependence on an ISI distribution or histogram. Furthermore, the
algorithm detected events based on local fluctuations in the analysis signal rather than a comparison to global activity allowing it to adapt to changing baseline firing rates.

When the three activity classification algorithms were compared using spike trains that were ambiguous (i.e., not clearly bursting or tonic spiking), they yielded results that were different (similarity of ~50% or less) than events identified by visual inspection. Because visual analysis can often be subjective, it was not clear whether the differences in performance were due to limitations of the classification capabilities of the three algorithms or if it was because the visual analysis was an inaccurate basis for comparison. In the first case, the discrepancies in burst detection results for ambiguous activity underscored the subjectivity of classifying spike trains that did not have clear patterns of activity. In addition, the strong performance of the EHV algorithm on a range of well-defined spike train activity patterns suggested that it was able to appropriately classify neural activity in an objective manner. Thus, the difference between visual inspection and EHV might be used to capture the degree of ambiguity in a spike train. In the latter case, performance of the three algorithms may have differed from visual inspection because of the subjectivity of classifying neural spike train patterns by eye. Consequently, differences in baseline firing rate and variability of determining the onset and offset of bursts or tonic spiking may have contributed to inconsistencies during visual event identification.

4.4.2 Advantages to Detecting Local Features of Spike Train Fluctuations

Neurons can exhibit complex activity patterns that include transitions between or mixtures of quiescence, bursting, and tonic spiking [1, 78, 103, 104]. Triggered by internal or external cues, characterizing transitions between activity states requires an algorithm that is not sensitive to changes in baseline firing rates and can analyze a spike train independent of changing ISI distributions. EHV accomplishes this by using ratios to quantify fluctuations in a
smoothed, history-dependent analysis signal that accentuates differences between bursts and tonic spiking regardless of baseline firing rates. An added advantage to the analysis signal is that it can be used to quantify the intensity of an event by quantifying the area under the analysis curve because the spike frequency is reflected in the amplitude of the signal [100]. Finally, EHV results can be used as the basis to characterize complex activity patterns. Using a hierarchical decision tree, for example, the output from EHV can be used to classify at least five different types of activity patterns (Fig. 4.7). When EHV detects no bursting or tonic spiking events, for example, the presence of spikes indicates that a neuron is spontaneously firing and a lack of spikes indicates that the neuron is quiescent. Alternatively, when EHV detects bursting and tonic spiking in a spike train, complex firing patterns such as phasotonic bursting can also be characterized. Phasotonic bursting occurs when a neuron fires at a high frequency (a “phasic” burst) followed immediately by a lower, maintained frequency (tonic activity) [1, 36]. By detecting bursting and tonic spiking that occur close in time the EHV method can be used to identify a variety of neural activity patterns. Beyond these examples, the output of EHV can be used as the basis to further analyze spike trains for specific activity patterns during spontaneous spiking as well as other complex activity such as spike frequency adaptation.

The EHV analysis method was developed to detect bursts and bouts of tonic activity in spike trains based on a smoothed, history-dependent signal that reflects local changes in spike frequency. The key features that are detected by EHV are, for bursts, a sequence of spikes where the frequency quickly rises and falls and, for tonic spiking, when spiking is maintained at a certain frequency for a minimum duration of time. The metrics used by EHV to measure these features are ratios rather than absolute values or probabilities and allow EHV to analyze spike trains independent of their ISI distribution or baseline firing rate. Thus, the results of EHV provide an objective way to define and quantify bursting and tonic spiking activity.
Figure 4.7. Hierarchical feature detection for complex activity patterns
Complex activity patterns can be decomposed into features that can be detected by algorithms. If bursting and tonic spiking are both detected in a spike train and events occur close in time a hierarchical feature can be derived to also detect phasotonic spiking events. During intervals where no events are detected the presence of spikes indicates that a neuron is spontaneously spiking. The Extended-Hill Valley method can discriminate between bursts and tonic spiking further enabling detection of more complex activity patterns.
5 Sensory Feedback Changes Network Dynamics in Simulations of Locomotor Circuit

5.1 Introduction

Neural circuits that control limbs, such as legs and arms, integrate sensory information with motor output in order to stabilize a limb during posture or generate rhythmic movements during locomotion [1, 17, 105, 106]. A number of hypotheses have been proposed to explain how sensory feedback compensates for perturbations during movement [107, 108]. Understanding how sensory feedback and motor neurons interact, however, has been difficult because of the complexity of the neural networks and their biomechanical coupling [12, 13, 106, 109]. While reflexes have largely been characterized by perturbing a system [1, 17, 109, 110] the mechanisms through which their effects are integrated by locomotor circuits and contribute to multifunctional output during posture and locomotion remain unclear. Using in vitro brain-machine interface experiments and neuromechanical computational modeling a better understanding of how sensory feedback can affect the dynamics of coupled motor neurons is being developed [23, 50, 51, 78, 111]. In particular, computational models allow biologically inaccessible variables to be manipulated and studied. Consequently, we are able to dissect how different aspects of sensory feedback, such as the type of reflex (i.e., resistance or assistance) as well as the timing or strength of feedback, contribute to the activity of the network in different behavioral states.

5.1.1 Reflexes in Sensorimotor Systems

During postural behaviors, resistance reflexes act to stabilize a limb by opposing external perturbations [17]. If an assistance reflex is triggered during posture, however, it may act to amplify a small perturbation and cause a limb to move rapidly, which, in turn, can
destabilize an animal’s posture. During locomotion, on the other hand, assistance reflexes can help to rapidly move a leg from swing to stance or stance to swing [23, 112]. If a resistance reflex is triggered at a stance or swing transition, however, opposing motor neurons would be activated and the leg may not move. In a crayfish locomotor control circuit, both assistance and resistance reflexes are modulated by interneurons [1, 16]. The likelihood of triggering an assistance reflex, for example, increases when assistance reflex interneurons (ARINs) are tonically excited. Tonic excitation of primary afferent depolarization interneurons (PADIs), however, increases the response of PADIs to MN activation resulting in a phase-dependent reduction of the strength of resistance reflexes. Here, we present evidence that supports the hypothesis that sensory feedback acts to shape the output of a locomotor circuit and must, itself, be modulated in order to facilitate desirable output.

**5.1.2 Characterizing the Role of Sensory Feedback Using Neuromechanical Simulations**

In a locomotor circuit that controls the second joint in a crayfish walking leg [1] two pools of mutually inhibitory motor neurons burst rhythmically when activated by a cholinergic agonist called, oxotremorine (OXO) [53]. When the network is in a quiescent state and motor neurons are not bursting, resistance reflexes act to oppose external perturbations [1, 23, 78]. When the network is moderately activated by application of OXO, assistance reflexes can be elicited that act to amplify ongoing movements of a leg [23]. When the network is further activated, motor neurons burst rhythmically and sensory feedback can modulate the output of the circuit [1]. While *in vitro* brain-machine interface experiments [78] and simulations of the locomotor circuit [23] show that sensory feedback modulates network output the implications for sensorimotor integration remain unclear.

Here, we used neuromechanical simulations of a sensorimotor network to test the hypothesis that resistance and assistance reflexes change the behavior of the motor network.
First, simulations were run to emulate the effect of bath application of OXO by increasing the activation of the CPG OXO, PADI OXO, and ARIN OXO neurons together. Then, simulations were run in which only the CPG OXO activation was increased. Finally, simulations were run to test the hypothesis that resistance and assistance reflexes have different effects on network dynamics. These results showed that phase-dependent blocking of resistance reflexes inhibited bouts of tonic spiking. In addition, activation of assistance reflexes increased the frequency of bursting of the CPG and assistance reflex interneurons induced prolonged tonic spiking. These results showed that that resistance and assistance reflexes changed the behavior of the network in different ways.

5.2 Materials and Methods

5.2.1 Neuromechanical Computational Model of Locomotor System

A computational model of a locomotor control circuit in crayfish was built using the software, AnimatLab [42]. Based on the biological network that controls a joint in a crayfish leg as described by Cattaert [1] the model consisted of 31 neurons that included motor neurons, afferents, and excitatory and inhibitory interneurons (Fig. 5.1) [23]. The output of the neural network was coupled to a biomechanical leg model that moved around a single joint. Movements of the virtual leg were integrated back into the neural motor circuit via stretch- and release-sensitive afferents of a sensory receptor that spanned the leg joint. This arrangement of coupling and feedback between the neural network and biomechanical model allowed the feedback loop to be decoupled. Consequently, simulations could be run in which movements of the leg were not drive by motor activity, called Open Loop (No Feedback), to characterize the behavior of the CPG alone. In addition, simulations could be run in which movements of the leg were driven by motor neurons, called Closed Loop (Feedback), to characterize how the system behaved as a whole.
Figure 5.1. Schematic of neuromechanical locomotor circuit

Movements of a biomechanical leg model are transduced to afferents via a stretch receptor. Muscles driven by levator (Lev) and depressor (Dep) motor neuron (MN) pools move the model leg up and down, respectively. Monosynaptic Lev resistance reflexes are mediated by tonic and phasic stretch-sensitive afferents. Resistance reflexes are inhibited by interneurons called, primary afferent depolarizing interneurons (PADI). Lev assistance reflexes are mediated by phasic release-sensitive afferents and an assistance reflex interneuron (ARIN). Dep resistance and assistance reflexes are mediated via similar pathways. Tonic excitation to the motor central pattern generator (CPG) is controlled by a neuron called, the OXO CPG neuron (not shown). The strength of resistance (assistance) reflexes is controlled by tonic excitation to PADIs (ARINs) by the OXO PADI (OXO ARIN) neurons (not shown). Increasing activation of the OXO PADI neuron reduces the strength of resistance reflexes. Increasing activation of the OXO ARIN neuron increases the strength of assistance reflexes. For a full diagram of the network see Appendix B, Figure 1.
The main features of the neural network included resistance reflexes, assistance reflexes, and a central pattern generator (CPG). Modified from its original publication [23] the network model used here allowed for separate control of CPG excitation, resistance reflex strength, and assistance reflex strength. The CPG consisted of two pools of mutually inhibitory motor neurons, which activated muscles that raised and lowered the leg. When the leg was raised by levator (Lev) motor neurons a stretch receptor was shortened, called release. When the leg was lowered by depressor (Dep) motor neurons a stretch receptor was lengthened, called stretch. Reflex pathways were organized around the CPG and the excitability of the entire system was controlled by a tonic stimulus (Fig. 5.1). The membrane potential of neurons and afferents included 0.1 mV of noise that introduced variability within and across simulations.

Monosynaptic resistance reflexes were triggered by release- and stretch-sensitive afferents that recruited motor neurons to oppose external perturbations (Fig. 5.2A). Tonic resistance reflex afferents were sensitive to changes in the position of the leg and phasic resistance reflex afferents were sensitive to the rate of change of position of the leg. The strength of resistance reflexes was controlled by an interneuron called, the Primary Afferent Depolarization Interneuron (PADI). During the resting state of the network the PADIs were quiescent and resistance reflexes were prominent (Fig. 5.2A). When the network was active, however, the PADIs inhibited the polysynaptic resistance reflex and weakened the network’s resistance reflex response to perturbations (Fig. 5.2A).

Polysynaptic assistance reflexes were triggered by stretch- and release-sensitive afferents that recruited motor neurons to amplify external perturbations (Fig. 5.2B). Phasic assistance reflex afferents responded to the rate of change of leg position and excited interneurons, called assistance reflex interneurons (ARINs). The strength of assistance reflexes increased as ARINs became more active (Fig. 5.2B). When the network was in a resting state
Figure 5.2. Modulation of reflex responses by interneurons

a) Resistance reflexes act to oppose external perturbations. (a.i) Upward movements of the leg model (green trace) excite release-sensitive afferents (arrow 1) and elicit a resistance reflex from depressor (Dep, red) motor neurons (MN) that act against the imposed movement (arrow 2). As the leg falls, stretch-sensitive afferents (arrow 3) excite levator (Lev, blue) MNs and trigger a resistance reflex response (arrow 4). (a.ii) When primary afferent depolarizing interneurons (PADI) are activated and fire tonically, Dep (*) and Lev (**) resistance reflexes are attenuated.

b) Assistance reflexes act to amplify ongoing movements. (b.i) When assistance reflex interneuron (ARIN) activation is low, leg lifts still excite release-sensitive afferents (arrow 1) and can elicit a Dep resistance reflex (arrow 2). Stretch-sensitive afferents are excited as the leg falls (arrow 3) and can elicit a Lev resistance reflex (arrow 4). When ARIN activation is increased, a leg lift triggers a Lev assistance reflex response (*), which excites PADI (***) that block the Dep resistance reflex response. A Dep burst (****) follows a Lev burst when ARIN activation is high.
assistance reflexes were weak as ARINs were inactive. During active states of the network assistance reflexes were stronger and could elicit bursts from motor neurons [23].

A set of three tonic stimulus neurons were used to emulate an in vitro effect of a cholinergic agonist, oxotremorine (OXO) [1, 78]. One of the tonic stimuli, called CPG OXO, excited the CPG by blocking a hyperpolarizing current on intrinsically bursting CPG interneurons. Another tonic stimulus, called PADI OXO, controlled the state of resistance reflexes by disinhibiting interneurons that blocked the resistance reflex. The third tonic stimulus, called ARIN OXO, controlled the state of assistance reflexes by disinhibiting interneurons that excited CPG motor neurons and interneurons. When OXO stimulus levels were low the network was considered to be in a “resting” state. For elevated levels of OXO the network was referred to as “active” because reflex responses could elicit Lev and Dep burst pairs from the CPG (Fig. 5.2B).

5.2.2 Running Simulations of Neuromechanical System

Simulations were run using a custom Python program that generated neural network configuration files and then ran the simulations. A set of network configuration files was created with simulation parameters that were determined by different combinations of sensory feedback conditions and tonic stimulus to OXO neurons. Biomechanical feedback generated by the model itself was toggled either on or off. Simulations in which the biomechanics of the model were driven by output of the motor neurons and afferents received inputs from those movements were called, “Closed Loop.” When the biomechanics of the model were not driven by motor neuron activity and, instead, the leg was only displaced under its own weight due to gravity the condition was called, “Open Loop.” In addition, a range of tonic stimulus values between 0.0 nA and 15.0 nA was also sampled for different combinations of one, two, or three of the OXO stimulus neurons. These values were not directly biologically meaningful, but
activity from the full range of computationally sampled parameter values were observed from *in vitro* experiments (unpublished results).

Simulations were run from a Python program using the AnimatLab API (www.animatlab.com). The Python program used standard multiprocessing libraries to generate sets of 6,000 – 11,000 configuration files and then ran those simulations across multiple CPUs. Simulation results were analyzed by an automated neural activity classification program.

### 5.2.3 Classification of Neural Activity

Bursting and tonic spiking detection was accomplished using an automated algorithm called, the Extended Hill-Valley (EHV) method. Briefly, the algorithm uses a smoothed, history-dependent analysis signal to identify meaningful fluctuations in the firing rate of a spike train. Bursts were detected by a rapid rise followed closely by a rapid fall in the analysis signal, which corresponded to a short sequence of spikes that occurred with a high frequency. Tonic spiking events were detected by a moderate rise followed by a maintained signal level. These features reflected an elevated firing rate that was maintained for a minimum duration. Plots of frequency and duration of tonic spiking events were calculated for activity classification of tonic Lev MN spike trains.

### 5.3 Results

In order to understand the role of sensory feedback in a crayfish locomotor network it was necessary to characterize the behavior of the circuit with and without biomechanical feedback as well as when reflexes were weak and strong. Simulations run when the biomechanics of the model were not driven by motor neuron output were called, Open Loop, and acted as a control to characterize the behavior of the network when afferents were active but received no feedback. Simulations in which the biomechanics of the model were driven by
motor neuron output were called, Closed Loop, and were used to determine how sensory feedback changed the network dynamics. In addition, simulations were run for increasing levels of CPG OXO, PADI OXO, and ARIN OXO activation together or independently in order to determine whether they changed the behavior of the network.

5.3.1 Locomotor Network Exhibits Three Regimes

Determining the role of sensory feedback in controlling network output started by characterizing the behavior of the circuit for increasing values of tonic stimulus. First, we asked what the range of network activity was when the motor neuron (MN) central pattern generator (CPG) and afferents were activated together. Increasing tonic excitation to the network during in vitro experiments was accomplished via bath application of a cholinergic agonist, oxotremorine (OXO) [78]. In order to emulate this effect the tonic stimulus to all three OXO neurons was varied together from 0.0 nA to 15.0 nA. Increasing activation of the CPG OXO neuron equivalently controlled the excitation to both pools of mutually inhibitory motor neurons. Increasing activation of the PADI OXO neuron resulted in decreasing the strength of resistance reflexes because PADIs inhibited the phasic resistance reflex afferents. ARIN OXO activation resulted in increasing the strength of assistance reflexes by increasing excitation to assistance reflex interneurons (ARINs).

Activation of the three OXO neurons was varied together and separately in one of two biomechanical coupling conditions. The first condition, called Open Loop (OL or No Feedback), was used as a control condition to determine the behavior of the network when the virtual leg was not driven by motor activity and only moved under its own weight due to gravity. Consequently, the results indicated how the dynamics of the network were organized in the absence of sensory feedback. The second condition was when movements of the virtual leg were driven by motor neurons in addition to its weight due to gravity. Consequently, with the
biomechanical feedback loop closed, called Closed Loop (CL or Feedback), simulation results were indicative of how sensory feedback changed or reorganized the dynamiccis of the network. The network exhibited three distinct states of activity as the level of tonic stimulus was increased. For levels of tonic stimulus between 0.0 nA and 4.0 nA to all three OXO neurons the network exhibited quiescence in both OL and CL conditions. In the quiescent regime when tonic stimulus of the network was 3.0 nA (Fig. 5.3, Low), motor neurons fired spontaneously and short bouts of tonic spiking were detected. Tonic spiking in the quiescent regime was not considered to be a significant part of network dynamics. At moderate levels of tonic stimulus (7.5 nA, Fig. 5.3A, Moderate), rhythmic bursting was produced and corresponded to fictive locomotion of the in vitro system. In addition, short bouts of tonic spiking were observed in OL while only rhythmic bursting was seen in CL. For high levels of tonic stimulus (12.5 nA, Fig. 5.3B, High) only one of the two mutually inhibitory motor neuron pools was tonically active while the other was strongly inhibited by the constant spiking. It was not clear, however, whether the exclusive tonic spiking regime was biologically significant.

When the CPG OXO, PADI OXO, and ARIN OXO neurons were activated with a stimulus between 0.0 nA and 4.0 nA the network was quiescent and did not produce bursting or significant bouts of tonic spiking (Fig. 5.3B). As the stimulus for the entire network was increased between 4.0 nA and 11.5 nA the network produced rhythmic bursting and had a higher burst frequency in CL than in OL conditions. When stimulus was elevated between 11.5 nA and 15.0 nA the network activity was classified as exclusively tonic spiking by one motor pool, in this case the Lev MNs, and no bursts were observed. These results showed that the network exhibits quiescence, rhythmic bursting, and tonic spiking as the activation of the MN CPG and afferents was increased together.
A. Examples of Increasing CPG OXO, PADI OXO, and ARIN OXO Activation

B. Effect of CPG Activation and Feedback Gain on Bursting and Tonic Spiking
**Figure 5.3. Effect of CPG activation and sensory feedback on rhythmic bursting frequency and duration of tonic spiking**

The burst frequency of motor neurons was measured as activation of the CPG and sensory interneurons was increased. 

a) The average burst frequency (top graph) of tonic levator motor neurons (Lev MN) increases in the presence (Feedback, cyan) and absence (No Feedback, magenta) of sensory feedback. For elevated levels of activation the network does not burst. The average duration of tonic spiking (lower graph) lasted the duration of the simulation for elevated levels of CPG and feedback activation. Shaded regions indicate the standard error of the mean.

b) Spike raster plots of tonic Lev MN (blue) activity and tonic depressor motor neuron (Dep MN, red) activity show the bursting and tonic spiking characteristics for increasing CPG & feedback activation corresponding to the levels indicated in (a). Colored bars and barbed lines above spike raster plots indicate burst events and bouts of tonic spiking that were detected by the Extended Hill-Valley algorithm. The instantaneous frequency is also shown above each raster plot to help with visual classification of spike train activity. Movements of the virtual leg are shown above the spike raster and instantaneous frequency plots (green trace). The time bar indicates 10 s.
5.3.2 Central Pattern Generator Has Two Regimes

In order to determine how the dynamics of the network were organized by sensory feedback, the next question that was addressed was whether increasing the activation of the MN CPG alone also resulted in three activity regimes. Simulations were run to characterize the behavior of the network when only the locomotor CPG was stimulated. At the core of the network are two mutually inhibitory pools of motor neurons, which make up the CPG that controls joint movements of a crayfish walking leg [1, 23, 78]. Simulations were run with no activation of PADI OXO and ARIN OXO neurons (0.0 nA stimulation) while varying the activation of the CPG OXO neuron from 0.0 nA to 15.0 nA.

In OL conditions when the biomechanics model was not driven by the output of the network the CPG exhibited two activity regimes, called quiescence and bursting (Fig. 5.4A, magenta). Mediated by the CPG OXO neuron, when tonic stimulus to the CPG was between 0.0 nA and 4.5 nA the network was quiescent and exhibited spontaneous spiking. When the level of tonic stimulus to the CPG OXO neuron was 3.0 nA (Fig. 5.4B, Low) some tonic spiking activity was detected and was not considered to be a significant feature of the network dynamics. For tonic stimulus values between 4.5 nA to 15.0 nA, the CPG exhibited rhythmic bursting with a frequency that increased as the stimulus intensity increased. When tonic stimulus to the CPG OXO neuron was 7.5 nA (Fig. 5.4B, Moderate) rhythmic bursting was detected as well as short bouts of tonic spiking activity. At 12.5 nA of tonic stimulus to the CPG OXO neuron the network exhibited rhythmic bursting at a higher frequency and very few instances of tonic spiking were observed (Fig. 5.4B, High).

Closing the sensory feedback loop by driving the biomechanics of the model by output from motor neurons resulted in an increased occurrence of tonic spiking activity at the transition from quiescence to bursting (4.0 – 6.0 nA) (Fig. 5.4A, cyan). The tonic spiking that occurred at the transition between regimes was due to coactivation of a monosynaptic
a. Effect of CPG Activation Alone on Bursting and Tonic Spiking

b. Examples of Network Corresponding Network Activity
Figure 5.4. Effect of CPG activation alone on rhythmic bursting frequency and duration of tonic spiking
The burst frequency of motor neurons was measured as activation of the CPG was increased.
a) The frequency of tonic levator motor neuron (Lev MN) bursting (top plot) increased as the tonic excitation of the OXO CPG neuron was increased in the presence (Feedback, cyan) and absence (No Feedback, magenta) of sensory feedback. Dashed lines indicate the average frequency of bursting and the shaded regions show the standard error of the mean. The duration of tonic spiking of the tonic Lev MN is shown in the lower plot. b) Raster plots of tonic Lev MN (blue) activity and tonic depressor motor neuron (Dep MN, red) activity illustrate the range of network behavior with and without feedback at varying levels of CPG activation corresponding to the values indicated in (a). Colored bars barbed lines above raster plots indicate bursts events and bouts of tonic spiking detected by the Extended Hill-Valley analysis method, respectively. The instantaneous frequency of MN firing is shown above each raster plot to help with visual characterization of neural activity. Movement of the virtual leg (green trace) is shown for each simulation. The time bar is 10 s.
resistance reflex and was confirmed by simulations in which the afferent that mediated the
effect was disabled (Appendix B, Fig. B.2). In addition, the stimulus level at which the transition
occurred as well as the rate at which the burst frequency increased, however, was not
significantly affected by sensory feedback. These results showed that, while the MN CPG
alone exhibited quiescence and bursting, activation of afferents induced a tonic spiking regime,
as well.

5.3.3 Sensory Feedback Changes Network Dynamics

A comparison of network behavior when the CPG and reflexes were activated together
versus activation of the CPG alone illustrated that sensory feedback changed network
dynamics. Here, we identify the differences between activation of the MN CPG and afferents
together versus the MN CPG alone. One difference was that sensory feedback promoted
bursting at the transition from quiescence. At 5.5 nA of stimulation to only the CPG OXO
neuron the network exhibited bouts of coactivated tonic spiking when sensory feedback was
present in CL simulations (Fig. 5.5, Feedback, CPG Activation Only). This activity pattern was
caused by an alternation of a resistance reflex in one MN pool triggering a resistance reflex in
the opposing MN pool and did not clearly correspond to a behavioral state of the in vitro
system. Chained activation of resistance reflexes, for example, would result in causing the leg
to jitter or would increase the biomechanical stiffness of the limb. When tonic stimulation was
5.5 nA and included the CPG OXO, PADI OXO, and ARIN OXO neurons the network no longer
exhibited bouts of tonic spiking. Instead, the network produced rhythmic bursting with a
frequency higher than OL (Fig. 5.5, Feedback, CPG & Feedback Activation). This effect was
consistent with previous findings [23, 78] and corresponded to fictive locomotion. When the
sensory feedback loop was open (OL, No Feedback) and the CPG stimulus alone and stimulus
of the entire network was 5.5 nA, the network produced rhythmic bursting at similar
Figure 5.5. Effect of sensory feedback on rhythmic bursting frequency
Raster plots of tonic levator motor neuron (Lev MN) activity and tonic depressor motor neuron (Dep MN) activity show how sensory feedback changes the behavior of the network. The top plots illustrate network activity when 5.5 nA of stimulus was used to activate the CPG. The bottom plots show simulation results when 5.5 nA of stimulus was used to activate the CPG as well as sensory feedback interneurons. Simulations were run when the biomechanical leg was decoupled from network activity (No Feedback) and when movements of the leg were driven by network activity (Feedback). Colored bars and barbed lines above each raster plot indicate burst events and bouts of tonic spiking that were detected by the Extended Hill-Valley analysis method. Instantaneous frequency plots help to visually characterize spike train activity.
frequencies. Together these results illustrated that sensory feedback facilitated rhythmic bursting at a higher frequency and reduced the occurrence of tonic spiking when the entire network was activated together.

The second difference was that increasing activation of sensory feedback in addition to the CPG induced an exclusive tonic spiking regime for elevated stimulus levels. Whereas activation of the CPG alone resulted in two activity regimes, activation of the CPG together with sensory feedback yielded three activity regimes. Consequently, these results suggested that sensory feedback changed the qualitative dynamics of the CPG circuit. It was not clear, however, which factors of resistance reflexes and assistance reflexes contributed to the effect of facilitating the bursting regime of network dynamics. Namely, we tested the contribution of phase-dependence inhibition of resistance reflexes and the strength (independent of phase) of assistance reflexes.

5.3.4 Differential Effect of Resistance and Assistance Afferents

In order to understand how activation of resistance and assistance reflex afferents were contributing to the change in activity regimes, we asked whether the afferents mediated different aspects of the overall effect. Because activation of the entire network included both resistance and assistance reflexes it was necessary to activate them separately in order to understand whether there was a partial contribution to the change in network dynamics. Three simulation conditions were run for both OL and CL feedback: 1) CPG OXO only, 2) CPG OXO + PADI OXO, and 3) CPG OXO + ARIN OXO. The first simulation condition characterized a basic network behavior that was produced by activation of the CPG only. Stimulating the CPG OXO and PADI OXO neurons together showed how increasing CPG excitation while increasing the strength of phase-dependent inhibition of resistance reflexes via PADI activation changed network dynamics without increasing the strength of assistance reflexes. Finally, network
dynamics were also changed without modulating resistance reflexes by increasing the strength of assistance reflexes via stimulation of the ARIN OXO neuron while also increasing CPG activation via increased ARIN activity and stimulation of the CPG OXO neuron. Consequently, it was possible to dissect the contribution of resistance and assistance reflexes to changes in network dynamics.

When the CPG OXO neuron was activated together with the PADI OXO neuron and no feedback was present (OL) there was no difference in burst frequency as compared to CPG activation alone and the network only exhibited two regimes of activity (Fig. 5.6A). This indicated that resistance reflexes were not contributing to the change in network dynamics mediated by sensory feedback. Increasing the tonic stimulus to both the CPG OXO and ARIN OXO neurons together in OL, however, resulted in a rapid increase of burst frequency around 10.5 nA and an exclusive tonic spiking regime. In comparison to the frequency curve of CPG activation alone, this indicated that assistance reflexes contributed to the change in network dynamics. Because the simulation was in OL and afferents were receiving no feedback no difference was expected in comparison to CPG OXO activation only. Upon closer inspection, it was not activation of assistance reflexes that caused the network to exhibit tonic spiking. Instead, the tonic spiking was caused by increased excitation of motor neuron pools from ARINs due to ARIN OXO activation. Consequently, the rapid increase of burst frequency was caused by tonic excitation of the motor neurons by both the CPG OXO neuron and the ARINs. This effect was eliminated in simulations where the ARINs were disabled (Appendix B, Fig. B.3).

In CL simulations when sensory feedback was present, increasing the tonic stimulus of the PADI OXO and CPG OXO neurons together resulted in a higher burst frequency compared to increasing CPG OXO alone (Fig. 5.6B, green). While bouts of tonic spiking were present for
Figure 5.6. Separable effect of positive and negative feedback
Plots of the burst frequency and duration of tonic spiking events illustrate the effect of increasing activation of the CPG only (red); increasing activation of the CPG and decreasing activation of resistance reflexes by PADI activation (green); and, increasing activation of both the CPG and assistance reflexes by ARIN activation (blue). Network activity was quantified by average burst frequency (top plot) and average duration of tonic spiking events (lower plot). Shaded regions indicate the standard error of the mean. Light gray vertical dashed lines indicate the low, medium, and high values of activation sampled in previous figures.
both tonic stimulus conditions the transition from quiescence to bursting was shifted to lower levels of stimulus when PADI OXO and CPG OXO neurons were stimulated together compared to activation of CPG OXO alone. This effect occurred because activation of the PADI’s partially blocked activation of resistance reflexes, thus reducing the propensity for coactivation of opposing resistance reflexes. There was, however, a slight increasing in burst frequency for elevated levels of PADI OXO and CPG OXO activation. Increasing the tonic stimulus of CPG OXO and ARIN OXO neurons together with sensory feedback in CL induced a tonic spiking regime and the burst frequency was higher than either CPG OXO alone or CPG OXO and PADI OXO. Together, the simulation results in OL and CL indicate that the reorganization of network dynamics was caused by activation of ARINs, which resulted in additional excitation of the MN CPG beyond the stimulus of the CPG OXO neuron. In addition, these results showed that the increased burst frequency in CL was mainly due to activation of ARINs and some part of the effect may have been due to PADI activation that removed the resistance reflexes.

5.3.5 Effect of Resistance Reflexes on Network Dynamics

To further dissect the role of reflexes in controlling network behavior, simulations were run in which tonic stimulus to the CPG OXO neuron was held fixed at 3.0 nA, 7.5 nA, or 12.5 nA while tonic stimulus to either the PADI OXO or ARIN OXO neurons was varied. In addition, the non-variable reflex interneurons were either held constant at 0.0 nA stimulus or activated together with the CPG OXO neuron.

First, we asked whether PADI activation changed the behavior of the network when activation of the CPG was increased alone. Based on prior results, increasing activation of PADIs was expected to reduce the occurrence of tonic spiking bouts and slightly increase the frequency of bursting in CL. When the ARIN OXO stimulus was held at 0.0 nA and stimulus to
the CPG OXO neuron was low (3.0 nA) increasing the PADI OXO stimulus did not have an effect in either OL or CL (Fig. 5.7A, CPG Activation Only). In both sensory feedback conditions the network exhibited short bouts of tonic activity and no bursts across the entire range of PADI OXO stimulus values. Increasing PADI OXO stimulus while holding CPG OXO stimulus at a moderate level (7.5 nA) resulted in both bursts and bouts of tonic spiking (Fig. 5.7A, CPG Activation Only). For higher values of PADI OXO stimulus (6.0 nA – 15.0 nA) the CL burst frequency remained higher than OL. When resistance reflexes were strong at low values of PADI OXO stimulus (0.0 nA – 6.0 nA) the variability in the duration of tonic spiking bouts was greater than that for higher values of PADI OXO and the burst frequency was similar in OL and CL. This difference was mediated by resistance reflexes because the occurrence of bouts of tonic spiking decreased when tonic and phasic resistance reflex afferents were disabled (Fig. 5.7B, compare dashed red and green lines to solid colored lines). Finally, increasing CPG OXO activation to 12.5 nA resulted in a higher burst frequency with no difference between OL and CL and the incidence of tonic spiking was reduced in OL simulations (Appendix B, Fig. B.4A). These results suggest that the strength of resistance reflexes do not have a significant effect in controlling the unperturbed output of the network.

Next, we asked whether PADI activation changed the behavior of the network when activation of the CPG and ARINs were increased together. Activation of both the CPG and ARINs showed that the effect of assistance reflexes were stronger than resistance reflexes. In simulations where tonic stimulus of CPG OXO and ARIN OXO neurons were held constant at the same level increasing PADI OXO did not have a discernable effect. When activation of CPG OXO and ARIN OXO neurons was held low (3.0 nA) there was also no difference between OL and CL conditions (Fig. 5.7A, CPG & ARIN Activation). In addition, increasing PADI OXO while activation of CPG and ARIN OXO neurons was held at a moderate level (7.5 nA) had no effect
A. Effect of Increasing PADI Activation in Different CPG and ARIN Levels

B. Effect of Blocking Tonic and Phasic Resistance Reflex Afferents
**Figure 5.7. Effect of negative feedback on network dynamics**
A) Plots of the average burst frequency and the average duration of tonic spiking events illustrate the effect of increasing PADI OXO activation when CPG OXO activation was held constant at a low and moderate level as well as when CPG OXO and ARIN OXO activation were held low and moderate. Simulations were run when movements of the leg were driven by motor activity (Feedback, cyan) and when the leg was decoupled from motor activity (No Feedback, magenta). Dashed lines indicate the average value and shaded regions are the standard error of the mean (SEM). (B) The burst frequency (top plot) and duration of tonic spiking events (bottom plot) for increasing levels of CPG OXO activation (dashed red line) and increasing levels of CPG OXO and PADI OXO activation (dashed green line) were compared to increasing levels of CPG OXO activation with different afferents disabled (solid colored lines). Shaded regions indicate the SEM.
and the differences in burst frequency and tonic spiking were due to the activation of assistance reflexes (Fig. 5.7, CPG & ARIN Activation). When CPG and ARIN OXO were increased to 12.5 nA the network exhibited only tonic spiking activity and changing the strength of resistance reflexes had no effect (Appendix B, Fig. B.4B). Overall, when resistance reflexes were strong there was evidence that higher variability of tonic spiking duration was due to coactivation of opposing resistance reflexes. In addition, when assistance reflexes were not strong, reducing the strength of resistance reflexes led to a slight increase in burst frequency.

Finally, we asked how the effect of PADI activation was mediated by rate-sensitive afferents of the resistance reflex pathway. The resistance reflex pathway included both rate-sensitive afferents and position-sensitive afferents. Whereas rate-sensitive afferents were inhibited by PADIIs and synapsed to both tonic and phasic MNs, position-sensitive afferents were not inhibited by PADIIs and synapsed onto motor interneurons. Motor interneurons and MNs were electrically coupled. In simulations where the rate-sensitive afferents were disabled the burst frequency was increased in comparison to activation of the CPG and PADIIs together (Fig. 5.7B, magenta). This showed that rate-sensitive afferents were slowing the frequency of bursts. When the position-sensitive resistance reflex afferents were blocked, however, the burst frequency was reduced to rates similar to activation of the CPG OXO neuron alone (Fig. 5.7B, cyan). This showed that position-sensitive afferents were increasing the frequency of bursts. When both the phasic and tonic resistance reflex afferents were blocked at the same time, the burst frequency of the network was restored to rates similar to activation of the CPG OXO and PADI OXO together (Fig. 5.7B, blue). Finally, when all conditions were run without sensory feedback (OL) the burst frequency of the network was similar (Appendix B, Fig. B.4C). These results showed that the two resistance reflex pathways represented in our model were
modulating CPG dynamics in opposing ways. In addition, these results showed that modulation of the resistance reflex pathway was sufficient to change the output of the network.

5.3.6 **Effect of Assistance Reflexes on Network Dynamics**

Based on earlier results, increasing activation of ARINs was expected to facilitate bursting at the transition from quiescence to bursting as well as increase the frequency of bursting in CL. In addition, ARIN activation was expected to induce a third regime of exclusive tonic spiking of one MN pool. For low activation values of CPG OXO (3.0 nA) and no PADI OXO stimulus increasing the strength of assistance reflexes resulted in sporadic bursting and a slight increase in the duration of tonic spiking events in CL at higher levels (Fig. 5.8A, CPG Activation Only). The increased occurrence of tonic spiking at for elevated activation of ARINs was due to rapid onset of bursts that triggered alternating resistance in one MN pool, which, in turn, triggered a resistance reflex in the opposing MN pool (Fig. 5.9A). Consequently, high levels of ARIN activation coupled with low CPG activation and strong resistance reflexes (no PADI activation) resulted in sporadic bursts that were followed by bouts of tonic spiking.

First we asked whether ARIN activation changed the behavior of the network when activation of the CPG alone was increased. When CPG OXO was stimulated at 7.5 nA increasing ARIN OXO activation resulted in an increase of CL burst frequency that reached a plateau around 8.0 nA as expected from earlier results (Fig. 5.8B, CPG Activation Only). For increasing values of ARIN OXO stimulus between 0.0 nA – 6.0 nA the duration and variability of tonic spiking became smaller until activity was mainly bursting. This was also predicted from prior results as assistance reflexes started to trigger bursts and overpowered tonic spiking caused by chained resistance reflexes. At high levels of CPG OXO activation (12.5 nA) increasing ARIN OXO stimulus resulted in a transition from bursting to tonic spiking (Appendix B, Fig. B.5A). In addition, there was no difference between OL and CL burst frequency for
A. Effect of Increasing ARIN Activation with Different CPG and PADI Activation Levels

B. Effect of Blocking Phasic Assistance Reflex Afferents
**Figure 5.8. Effect of ARINs on network dynamics**

A) Plots of average burst frequency and average duration of tonic spiking for increasing activation of ARIN OXO neurons under different conditions: i) CPG OXO activation was held at a low level; ii) CPG OXO activation was held at a moderate level; iii) CPG OXO and PADI OXO activation was low; and, iv) CPG OXO and PADI OXO activation was moderate. Simulations were run when movements of the leg were driven by motor neuron (MN) activity (Feedback, cyan) and when movements of the leg were decoupled from MN output (No Feedback, magenta). Shaded regions indicate the standard error of the mean (SEM). B) Average burst frequency and average duration of tonic spiking when only CPG OXO activation was increased (dashed red), when CPG OXO and ARIN OXO activation were increased together (dashed blue), and when CPG OXO and ARIN OXO activation were increased together while assistance reflex interneurons (ARINs) were disabled (solid blue). Shaded regions indicate the SEM.
values of ARIN OXO between 0.0 nA – 4.0 nA but the CL burst frequency for moderate levels of ARIN OXO stimulus (4.0 nA – 9.0 nA) was higher than OL.

Next we asked whether ARIN activation changed the behavior of the network when activation of the CPG and PADIs was increased together. When CPG OXO and PADI OXO were activated together at 3.0 nA, tonic activity occurred for the full range of ARIN OXO activation for both OL and CL simulations (Fig. 5.8A, CPG & PADI Activation, Low). In addition, bursting occurred for ARIN OXO activation greater than 10.0 nA. In CL simulations, the combination of bursting and tonic spiking occurred because assistance reflexes triggered bursts, which, in turn, triggered opposing resistance reflexes in a bout of coactivated tonic activity by antagonist motor neurons (Fig. 5.9A).

At 7.5 nA of CPG OXO and PADI OXO activation, increasing ARIN OXO activation resulted in a slight increase of burst frequency for CL simulations (Fig. 5.8A, CPG & PADI Activation, Moderate). While tonic spiking activity occurred for all levels of ARIN OXO stimulation in OL, simulations in CL only exhibited tonic spiking for ARIN OXO activation levels between 0.0 nA and 4.0 nA. One possible explanation was that the increased PADI OXO activation reduced the strength of resistance reflexes, which reduced the coactivation of antagonist motor neurons. When tonic stimulus was elevated to 12.5 nA for the CPG OXO and PADI OXO neurons increasing ARIN OXO activation resulted in a transition from a bursting regime to a tonic spiking regime (Appendix B, Fig. B.5B). In addition, the CL burst frequency was also higher than OL. Together, these results indicated that ARIN activation resulted in higher burst frequencies and facilitated bursting as assistance reflexes overpowered resistance reflexes. When resistance and assistance reflexes were imbalanced, there were also episodes of bursting that were immediately followed by alternating resistance reflex activation by opposing MN pools.
Figure 5.9. Examples of effect of positive feedback
Neuromechanical simulation results for different levels of CPG OXO, PADI OXO, and ARIN OXO activation. Tonic levator motor neuron (Lev MN, blue raster plots) and tonic depressor motor neuron (Dep MN, red raster plots) are shown with leg movements (green traces) above. Colored bars and barbed lines above raster plots indicate burst events and bouts of tonic spiking as detected by the Extended Hill-Valley analysis method, respectively. The instantaneous firing frequency of the MNs are shown above the raster plots (black graphs). Simulations were run in which movements of the leg were decoupled from MN activity (No Feedback) as well as when movements of the leg were driven by MN activity (Feedback). A) CPG OXO and PADI OXO activation were low (3.0 nA) and ARIN OXO activation was high (12.5 nA). B) CPG OXO and PADI OXO activation were high (12.5 nA) and ARIN OXO activation was moderate (7.5 nA). C) CPG OXO and PADI OXO activation were high (12.5 nA) and ARIN OXO was moderate (6.0 nA). Time bars indicate 10 s.
In the case that activation of the CPG OXO neuron or both the CPG OXO and PADI OXO neurons was 12.5 nA the high burst frequency had sharp dips to lower frequencies. Closer inspection of the simulation results for these simulations showed that the leg did not traverse the full range of the joint and reflex responses may not have been triggered. For example, when ARIN OXO activation was 7.5 nA the leg did not traverse the full range of the joint and the burst frequency was lower (Fig. 5.9B). When ARIN OXO activation was 6.0 nA, however, the leg moved through its full range of motion for each burst and the burst frequency was higher (Fig. 5.9C). Thus, the sharp dips in burst frequency were attributed to an artifact of the biomechanics of the system and not to the dynamics of the neural network because the leg joint was unable to rotate through its full range of motion.

Taken together these results suggested that the tonic spiking regime that was exclusively led by Lev MNs as well as the increase in burst frequency were mediated by activation of ARINs. In simulations where the ARINs were disabled (Fig. 5.8B, solid blue), these effects were eliminated suggesting that positive feedback from assistance reflexes were the main contributor to changing the dynamics of the network. In addition, these results showed that an imbalance between resistance and assistance reflexes led to episodes of bursts that are followed by bouts of tonic spiking.

5.4 Discussion

It is difficult to separate the in vitro effect of resistance and assistance reflexes on locomotor control circuits because of their complexity [12, 13, 106, 109] as well as the coupling between biomechanical and neural network dynamics [17]. It is already established that central pattern generators (CPGs) can be entrained by external inputs [1, 113]. In addition, reflexes are thought to control limbs to accommodate for environmental uncertainty [17, 107, 108]. The mechanisms through which sensory feedback is integrated into a neural network so that both
postural and locomotor control can be achieved, however, remain unclear. Neuromechanical simulations using AnimatLab have enabled specific control over network parameters and sensory feedback conditions that has allowed us to dissect the effect of sensory feedback on locomotor output.

5.4.1 Resistance Reflexes

Resistance reflexes act to oppose external perturbations during postural behaviors [16]. If activated during voluntary movements, however, resistance reflexes may lead to coactivation of antagonist motor pools and result in undesirable limb behavior [18]. In the circuit studied here, primary afferent depolarization is known to presynaptically block motor neuron inputs to afferents in a phase-dependent manner [114]. Reproduced in our model by primary afferent interneurons (PADI), resistance reflexes were gated by motor neurons during rhythmic CPG bursting so as to prevent phase-dependent coactivation of antagonist motor neuron pools. While the effect of resistance reflexes on network dynamics was subtle there were two main features that were observed.

The first effect of resistance reflexes was that when they were strong and not gated their activation resulted in bouts of tonic spiking as motor pools were coactivated in chained resistance reflexes. This effect was confirmed by blocking the tonic resistance reflex afferents during activation of the CPG alone, which eliminated the bouts of tonic spiking. In addition, a similar effect was observed when PADI activation was low for moderate activation of CPG OXO and ARIN OXO neurons. In this case, CPG activity and ARIN activation promoted bursting that were immediately followed by bouts of coactivation of motor pools by chained resistance reflexes.

A second effect of resistance reflexes was a slight increase in burst frequency as their phase-dependent strength was reduced. Namely, when CPG OXO and PADI OXO activation
were increased together the burst frequency of the network was slightly higher than activation of the CPG OXO neuron alone. One hypothesis that would explain this effect was that inhibition from PADIs to rate-sensitive afferents resulted in greater deinactivation of sodium channels, which would result in greater excitability of the afferents. In simulations where rate-sensitive afferents were disabled, however, the burst frequency was increased further above both CPG OXO alone and CPG OXO + PADI OXO conditions. In contrast, when the tonic resistance reflex afferents were disabled alone the burst frequency was reduced to values similar to activation of CPG OXO alone. When all resistance reflex pathways were blocked, the burst frequency was slightly higher than activation of the CPG OXO neuron alone and the values were similar to activation of the CPG OXO and PADI OXO neurons together. Thus, it remains unclear as to how each pathway contributes to the overall effect of resistance reflexes on changes in network behavior.

5.4.2 Assistance Reflexes

Assistance reflexes act to amplify movements such as external perturbations and have been found to promote transitions between swing and stance phases during locomotion [23, 112]. If activated during postural behaviors, though, assistance reflexes could destabilize a stance. Gated by motor neurons, assistance reflex interneurons (ARINs), mediated the disynaptic response and were tonically excited by ARIN OXO neurons. The effect of assistance reflexes on network dynamics was an induced exclusive tonic spiking regime and an increased burst frequency.

The tonic spiking regime that was induced by increasing activation of ARIN OXO neurons was a result of increased excitation from interneurons rather than by eliciting assistance reflexes. This effect was illustrated in simulations when ARINs were disabled
resulting in the elimination of the tonic spiking regime and a reduction in burst frequency to values similar to activation of the CPG OXO neuron alone.

Increasing activation of assistance reflexes also resulted in an increased burst frequency of the network. While simulations in which ARINs were disabled resulted in a lower burst frequency than simulations in which the CPG OXO and ARIN OXO neurons were increased together, the frequency was lower than simulations in which the CPG OXO neuron was activated alone. In addition, tonic spiking was more prevalent when ARINs were disabled. Together, these results suggest that assistance reflexes not only act to increase the burst frequency of the network, but that background activity from ARINs may help to promote bursting.

5.4.3 Significance of Sensorimotor Integration

The biological significance of resistance reflexes is to stabilize a limb during behaviors such as posture [16, 17]. The dynamic effect of resistance reflexes on circuit dynamics, however, can result in bouts of tonic activity as opposing pools of MNs are activated. On the one hand, reducing the strength of resistance reflexes by activation of PADIs can help to prevent alternating activation of motor pools by chained resistance reflexes. On the other hand, the differences in burst frequency mediated by the alternative reflex pathways were not conclusive in this analysis. Therefore, it may be the case that the role of resistance reflexes is better illustrated through simulations in which the network is perturbed. In this way, it would be possible to measure the response of the network to external factors and to determine whether resistance reflexes help to produce a more desirable outcome.

In the case of assistance reflexes, it may be that they help to stabilize rhythmic movements, such as locomotion, by promoting transitions between swing and stance phases at specific times. On the other hand, it is not clear whether the role of background ARIN activity
is important in controlling locomotor circuit output. In addition, the tonic spiking regime dominated by Lev MNs that was induced by increasing activation of ARIN OXO neurons has not been attributed to a biologically meaningful mode of network dynamics, but there are recordings from in vitro experiments that correspond to such activity (unpublished results). Together, these results support the hypothesis presented in [78] and [23] that assistance reflexes mediate the increase in burst frequency observed during in vitro experiments. Further simulations and experiments are necessary to understand specific mechanisms through which assistance reflexes contribute to controlling limb movements.

The results and analysis presented here begin to draw a picture of how sensorimotor integration accomplishes multifunctional behavior of a locomotor control circuit. Through the use of neuromechanical simulations we were able to discern the effect of resistance and assistance reflexes by decoupling and coupling biomechanical feedback while controlling a number of parameters. This has otherwise been unrealized in vitro. Limited to simulations in which the network was unperturbed, these results illustrate that resistance reflexes can result in excessive tonic spiking by alternating activation antagonist motor pools. In addition, these results showed that assistance reflexes increase the burst frequency of the network and that they may also mediate an effect that qualitatively changes the dynamics of the network by inducing a tonic spiking regime. Sensory feedback can clearly have a significant effect on the unperturbed behavior of a locomotor control circuit. The complexity of these circuits, however, has made it difficult to dissect the role of reflexes and CPGs. Simulations provide a tool through which we can develop biological hypotheses to characterize the role of sensory feedback as well as the mechanisms through which they contribute to controlling network output.
6 Network Dynamics are Reorganized by Positive and Negative Feedback in Simulations of Locomotor Circuit

6.1 Introduction

Sensorimotor circuits integrate feedback from biomechanical systems with ongoing motor activity to produce behaviors such as posture and locomotion and to adapt them in unpredictable environments [17, 115]. For example, afferents can mediate negative or positive feedback in response to perturbations via reflexes, such as resistance reflexes or assistance reflexes, respectively. Resistance reflexes act to oppose perturbations and are observed during behaviors such as posture maintenance [1]. Assistance reflexes act to amplify ongoing movements and can occur during certain phases of a step cycle during locomotion [1, 23, 116]. In the absence of perturbations, however, sensory feedback still has an effect on a motor system as movements during voluntary actions are still reported by afferents and can result in changing the behavior of a network, such as changing the frequency of spiking or rhythmic bursting [50, 78, 111]. While in vitro studies have used perturbations to characterize the role of sensory feedback in different states, such as posture or locomotion, the mechanisms through which sensory feedback controls sensorimotor output and how its effect is modulated in different behavioral states remain unclear.

6.1.1 Sensory Feedback Adapts Centrally Generated Rhythms

Based on in vitro studies and simulated results, roboticists have engineered machines that can reproduce locomotion in a variety of environments based on simplified circuits that include a central pattern generator (CPG) and sensory feedback [9, 117-119]. These reports show that sensory feedback can play a critical role in adapting a gait to an environment, such as an inclined plane. The dynamics of a CPG, however, are also critical to behavioral success and can change due to internal factors [32, 120, 121]. The change in CPG dynamics, for
example, can change how a network responds to perturbations or how it coordinates with other motor systems [33, 34, 122].

Because of the complexity of sensorimotor circuits and their coupling to biomechanical systems, it can be difficult to carry out specific manipulations of intrinsic or extrinsic factors to test hypotheses [17, 123]. The use of computational models, however, has enabled researchers to develop and test hypotheses through simulations that can help to refine hypotheses that can be tested biologically [6, 23, 106, 109, 124]. The success of such an approach is exemplified in our current understanding of neuronal action potential generation where simulations have been used extensively to understand nonlinear interactions between ionic currents [30, 125, 126]. By allowing a sampling of parameters that are biologically inaccessible, for example, simulations of computational neuronal models have brought insight into how neurons produce different action potential shapes [127, 128]. Consequently, the effect of individual currents can be tested or new currents can be hypothesized based on what a model does or does not account for in comparison to in vitro results.

6.1.2 Determining How Feedback Changes Network Dynamics

Drawing on the rationale applied to understand interactions of ionic currents during action potential generation, we characterized the dynamics of a crayfish locomotor circuit by testing the effect of resistance and assistance reflexes on a motor neuron (MN) CPG. First, a subset of in vitro results was compared to simulation results in order to establish a range of activity patterns that corresponded between both systems. Then, sets of simulations were run in three different states of the MN CPG under two biomechanical feedback conditions. The first condition was when movements of the leg were only due to its weight under gravity and the sensory feedback loop was “open.” This condition was referred to as “No Feedback.” The second condition was when the sensory feedback loop was “closed” and movements of the
leg were due to its weight under gravity as well as motor neuron activity. This condition was labeled as “Feedback.” Simulations that were run in No Feedback conditions were used as a control in order to determine how the network behaved without biomechanical coupling. Finally, the strength of resistance and assistance reflexes was varied separately within each set of simulations. Comparisons between simulations with and without feedback showed that the dynamics of the locomotor circuit were controlled by sensory feedback and the ratio of positive and negative feedback.

6.2 Materials and Methods

6.2.1 Extracellular Recordings

In vitro experiments were conducted in which a brain-machine interface provided sensory feedback to an in vitro nervous system based on the movements of a biomechanical model as previously published in [78]. Briefly, the thoracic and abdominal sections of a nerve cord were dissected from a crayfish with the muscles and stretch receptor of the second joint of a walking leg intact. In vitro recordings were made from motor nerves as well as sensory nerves using stainless steel pin electrodes and action potentials were detected in real time by Spike2 (CED) and used to control movements of a virtual crayfish leg model.

A virtual biomechanical model of a crayfish leg was constructed in the neuromechanical simulation software, AnimatLab (www.animatlab.com) [42]. The model consisted of a single leg joint with levator and depressor muscles that moved the leg up and down around the joint, respectively. Movements of the leg model were produced by activating the virtual muscles based on extracellular activity from the in vitro nerve cord. As the muscles contracted to raise or lower the virtual leg a virtual stretch receptor was shortened or lengthened, respectively. Changes in length of the virtual stretch receptor were transduced to the in vitro stretch receptor using a linear actuator. With this configuration, experiments were run in which the
biomechanical feedback to the *in vitro* nervous system was based on its own motor neuron output or using artificially imposed stimuli designed in AnimatLab.

The behavioral state of the *in vitro* nerve cord could be modulated by oxotremorine (OXO), a cholinergic agonist. Experiments were conducted with varying concentrations of OXO and results were analyzed for changes in activity [1, 78]. Experiments lasted between 60 – 90 s and data were passively collected between simulations, as well. No stimuli were used during simulations. When an experimental simulation was not active, the *in vitro* stretch receptor was held in a neutral position and the recorded virtual leg position was about 0.0. At the onset of simulation, the virtual leg was allowed to move in response to motor neuron (MN) activity. Activity in the levator (Lev) motor nerve raised the virtual leg while activity in the depressor (Dep) motor nerve lowered the leg. Phasic and tonic motor neuron spikes were detected and sorted for analysis using a threshold method in the analysis software, DataView [129]. The threshold between phasic and tonic action potentials was determined by visual inspection. Phasic neurons were characterized to fire in short bouts and at high frequency while tonic neurons fired action potentials for extended periods of time and generally had smaller peak amplitudes.

### 6.2.2 Computational Model of Locomotor Circuit

A computational model of the *in vitro* experiment was built in AnimatLab using the biomechanical leg model in addition to a neural network model of the locomotor control circuit (Fig. 6.1). The same biomechanical model that was used in brain-machine experiments was also used for neuromechanical simulations. Output from a neural network model of the locomotor control circuit was used to drive virtual muscles of the leg model and the stretch and release of a virtual stretch receptor were relayed to afferents in the neural network model as
Levator (Lev) and depressor (Dep) muscles move a virtual leg up and down, respectively. Two pools of tonic and phasic Lev and Dep motor neurons in addition to an intrinsically bursting interneuron constitute a central pattern generator (Motor CPG). Afferents provide sensory feedback based on stretch and release of a sensory receptor. Primary afferent depolarizing interneurons (PADI) block tonic afferents that trigger resistance reflexes, which oppose movements. Assistance reflex interneurons (ARIN) mediate a disynaptic assistance reflex that amplifies ongoing movements. Dep resistance and assistance reflex pathways are similar to those illustrated here for Lev reflexes. Activation of the motor CPG as well as resistance or assistance reflexes was controlled separately by CPG OXO, PADI OXO, and ARIN OXO neurons (not shown). For a schematic of the full network, see Appendix C, Fig. C.1.
previously published [23]. Neurons and afferents had 0.1 mV of membrane potential noise that resulted in variability of simulation results.

The neural network consisted of motor neurons, afferents, and interneurons characterized experimentally [1]. Lev and Dep MN pools were mutually inhibitory and made up a central pattern generator (CPG) that raised and lowered the leg, respectively. Upward (downward) movements of the leg resulted in release (stretch) of a stretch receptor, respectively, and were encoded via afferents that excited MNs. Two afferents mediated the resistance reflex responses. Tonic resistance reflex afferents, called the Release Afferent and Stretch Afferent (Fig. 6.1), encoded the position of the leg and could elicit a monosynaptic resistance reflex that was not inhibited by primary afferent depolarization interneurons (PADI). Phasic resistance reflex afferents, called the Stretch Rate Afferent and Release Rate Afferent, excited motor neurons in a monosynaptic resistance reflex in response to changes in the rate of movement of the leg and were inhibited by PADIs. Assistance reflexes were mediated by phasic assistance reflex afferents called the Release Rate Assist Afferent and Stretch Rate Assist Afferent. These afferents elicited a disynaptic assistance reflex response to changes in the rate of movement of the leg by exciting assistance reflex interneurons (ARINs), which, in turn, excited motor neurons.

Interneurons controlled the strength of reflex responses and were used to emulate the in vitro effects of OXO. Simulations were run for three levels of Lev and Dep CPG activation that was controlled by the CPG OXO interneuron. The strength of reflexes was controlled by the PADI OXO and ARIN OXO neurons and their activation ranged from 0.0 nA to 15.0 nA independently. The range of stimuli did not have direct biological meaning and was determined by sampling a larger range of parameters and identifying a domain that captured interesting network behaviors. The first set of simulations was run with CPG OXO activation at 3.0 nA to study the effect of gain ratio on a quiescent network. The second set was run at 7.5 nA of CPG
OXO activation to study the effect on a bursting network. The third set was run with a CPG OXO activation of 12.5 nA to study the effect of gain ratio on a network that exhibited tonic spiking.

### 6.2.3 Locomotor Circuit Activity Maps

Activity map axes for tonic spiking duration, tonic spiking frequency, and burst frequency were scaled by percentages in order to make their interpretation more intuitive. Because PADIs reduced the strength of resistance reflexes, low values of PADI OXO activation corresponded to high negative feedback gains while high values of PADI OXO activation corresponded to low negative feedback gains. Accordingly, the x-axis of gain ratio plots ranged from 0% Negative Feedback Gain (15.0 nA PADI OXO activation) to 100% Negative Feedback Gain (0.0 nA PADI OXO activation). When ARIN OXO activation was low (0.0 nA) the strength of assistance reflexes was also low and the gain of positive feedback was considered to be low. When the activation of ARIN OXO neurons was high (15.0 nA) the strength of assistance reflexes was high and the gain of positive feedback was considered to be high.

Simulations for all three levels of CPG OXO activation as well as the full range of both PADI OXO and ARIN OXO parameter values were run in two biomechanical coupling conditions. The first condition was called, Closed Loop (Feedback), and movements of the biomechanical leg were due to motor activity as well as the weight of the leg due to gravity. The second condition was called, Open Loop (No Feedback), and the biomechanical leg model was decoupled from motor activity, thus, it only moved due to its own weight under gravity. The “No Feedback” condition was used as a control to determine how the networked behaved while afferents were activated but the biomechanical system was decoupled from network output.
6.2.4 **Python Interface for Simulations**

Neuromechanical simulations were run using AnimatLab and a custom library written in the programming language, Python. First, configuration files were generated to control simulation settings and parameter values. Separate simulation files were generated for each set of parameters. Simulations were run simultaneously on multiple CPUs and separate result files were compressed using the hd5 file format. Results were analyzed automatically using the Extended Hill-Valley classification algorithm.

6.2.5 **Burst Detection and Tonic Spiking Classification**

Simulation results were analyzed using the Extended Hill-Valley (EHV) analysis method. Briefly, the EHV analysis algorithm detects features of neural activity based on fluctuations in a smoothed, history-dependent analysis signal derived from the instantaneous frequency of a spike train. Bursts were detected in the analysis signal by identifying regions with steep rising and falling slopes. These features of the analysis signal reflected the rapid increase and decrease in spike frequency that occurs when a neuron fires a burst of action potentials. Tonic spiking activity was classified by detecting regions of the analysis signal that rose moderately fast and was sustained for a minimum period of time. In addition, the height of peaks in the analysis signal during tonic spiking activity was not as high as the peaks that occurred during bursts. The features detected in the analysis signal during tonic spiking reflected the increased and sustained firing frequency that occurs during a burst.

6.3 **Results**

In addition to illustrating behaviors that corresponded to postural and fictive locomotion states [23, 78], *in vitro* results illustrated that the locomotor control circuit was capable of producing a variety of behaviors that were not immediately explained by those states. Here, we show that a neuromechanical model of the locomotor control circuit, which produces postural
states and fictive locomotion [23], is also capable of reproducing a larger range of outputs. In order to characterize the range of behaviors exhibited by the network, simulations were run in sets of three conditions: a quiescent regime that corresponded to a postural state, a rhythmically bursting regime that corresponded to fictive locomotion, and a tonically spiking state that has not been characterized before. For each of these activity regimes simulations were also run in which different afferents were disabled in order to determine whether certain components of resistance and assistance reflex pathways mediated different aspects of network behaviors.

6.3.1 In vitro Results Illustrate Range of Behaviors

In a crayfish locomotor circuit that controls the second joint of each walking leg, application of a cholinergic agonist, oxotremorine (OXO), induces rhythmic bursting associated with fictive locomotion. In addition, application of OXO reduces the strength of resistance reflexes while increasing the strength of assistance reflexes [1, 78]. In vitro results were determined to be fictive locomotion by identifying Lev MN bursts that corresponded to upward movements of the leg during the swing phase of a step cycle and Dep MN bursts that corresponded to the stance phase. In order to understand the dynamics of the network, however, it can be advantageous to characterize a larger range of behaviors that the circuit is capable of producing [96, 130]. Thus, we asked whether increasing the OXO concentration resulted in locomotor circuit activity that was not fictive locomotion. Experiments were conducted in a bath application of varying concentrations of OXO, which, in many instances, resulted in rhythmic bursting of levator (Lev) and depressor (Dep) motor neurons (MN) (Fig. 6.2A, 50.0 umol OXO).
A. *In vitro* Resistance Reflexes

B. *In vitro* Resistance and Assistance Reflexes

C. *In vitro* Reflexes and CPG Activation

Figure 6.2. *In vitro* brain-machine interface results

Spike rasters and waveforms show a range of pharmacologically induced states of a locomotor control circuit. Mutually inhibitory levator (Lev, blue) and depressor (Dep, red) motor pools consist of phasic and tonic motor neurons (MN) as well as interneurons. Spike activity from Lev and Dep MNs are transduced to move a virtual biomechanical system up and down, respectively. Following bath application of a cholinergic agonist, oxotremorine (O XO), the activity of the locomotor network can exhibit a range of states. A) Shortly after bath application of 15 umol O XO while the bath concentration was increasing. B) After the bath concentration of 15 umol O XO had time to equilibrate. C) Following bath application of 50 umol O XO.
As the concentration of OXO was increasing shortly after applying 15.0 umol OXO (~7 min after starting bath perfusion), resistance reflexes were strong in response to an artificial sinusoidal stimulus (Appendix C, Fig. C.2). In this state, when simulations were run without a stimulus and the feedback loop was closed (~9 min after starting bath perfusion), the network exhibited spontaneous tonic activity that was present in both Lev and Dep MN channels (Fig. 6.2A.ii). Activation of both MN pools occurred because movements imposed by one MN pool triggered resistance reflexes in the opposing MN pool, which, in turn, triggered another resistance reflex in the starting MN pool, and so on. Consequently, the leg jittered around a local position. Under the same experimental conditions and after the 15.0 umol OXO bath perfusion had been sustained for over 17 mins, the network produced bursts that were immediately followed by bouts of spiking in both Lev and Dep MNs (Fig. 6.2B). These coactivated spiking patterns resulted in the virtual leg rapidly rising during a Lev MN burst and then jittering around an elevated position before falling to a lowered position. In an in vivo scenario, motor neuron activity like the two examples described here could result in the leg jittering around a local position or the biomechanical stiffness would increase making the system less responsive to perturbations. This is likely not an adaptive behavior of the neuromechanical system.

When 50.0 umol OXO was applied via bath perfusion (~120 min after starting high concentration bath perfusion), the network produced low frequency tonic spiking of Dep MNs in addition to low firing rate bursts by the Lev MNs (Fig. 6.2C). While it was difficult to identify specific reflex mechanisms, the strength of resistance reflexes was expected to be low and the strength of assistance reflexes as well as activation of the CPG was expected to be high. In this case, the increased Dep MN activity could have been due to Dep assistance reflexes as the leg position was lowered or because the high concentration of OXO resulted in a strong activation of Dep MNs that subdued the Lev MN response. Thus, the low frequency and low
firing rate bursts of Lev MNs may have been due to inhibition of the Dep MNs that was competing against the intrinsic rhythmicity of the MN CPG. It was not clear from the data, however, what mechanism caused this abnormal behavior.

These in vitro results illustrated that the network was not only capable of producing postural reflex responses and rhythmic bursting during fictive locomotion, but it can also generate MN activity patterns that may not represent in vivo behaviors as inaccessible or uncontrolled variables fluctuate. For example, the strength of resistance reflexes and assistance reflexes may be controlled by factors other than bath application of OXO resulting in fluctuations of network activity due to other “hidden variables.” Characterizing the mechanisms that govern these “pathological” states, however, can help to understand how the system is organized. While it may be the case that these examples are instances of undesirable circuit activity, they may help to determine a set of constraints on the system that define a biologically meaningful operating space in which posture and locomotion are produced by the same network.

6.3.2 Ratio of Negative and Positive Feedback Affects Network Dynamics

In order to determine how resistance and assistance reflexes contribute to the organization of network behavior, we asked whether changing the ratio of activation of PADI’s and ARIN’s had an effect on the circuit output. Because it was not possible to control the strength of resistance and assistance reflexes in vitro, simulations were run in which the strengths were varied independently. The strength of resistance and assistance reflexes in the neuromechanical model were varied independently by controlling tonic stimulation to PADI OXO and ARIN OXO neurons, respectively. PADI OXO activation emulated the effect of OXO on in vitro resistance reflex pathways by activating primary afferent depolarization interneurons (PADI), which acted to reduce the strength of resistance reflexes (Fig. 6.1). Consequently,
increasing PADI OXO activation reduced the gain of negative feedback. ARIN OXO activation emulated the effect of OXO on *in vitro* assistance reflex pathways by activating assistance reflex interneurons (ARIN) that responded to rate-sensitive afferents and excited motor neurons to amplify ongoing movements (Fig. 6.1). Consequently, when ARIN OXO activation was increased the gain of positive feedback was increased.

When simulations were run with different ratios of resistance reflex strength and assistance reflex strength, the network exhibited similar results in the absence of sensory feedback (No Feedback) and a range of activity when sensory feedback was present (Feedback) (Fig. 6.3). When no sensory feedback was present with moderate CPG OXO activation (7.5 nA) and weak resistance reflexes, the burst frequency of the network was only slightly affected by the strength of assistance reflexes (Fig. 6.3A, No Feedback). When assistance reflexes were weak, closing the sensory feedback loop resulted in an increased burst frequency and the onset of each burst was slow (Fig. 6.3A, Feedback). Increasing the strength of assistance reflexes with sensory feedback resulted in further increasing the burst frequency and the shortening the time of burst onset (Fig. 6.3A, Feedback). These results were most similar to fictive locomotion like the results reported in [78] because the *in vitro* bath application of OXO increased CPG activation, weakened resistance reflexes (negative feedback), and strengthened assistance reflexes (positive feedback), which corresponds to the general effect of OXO [1].

In the case of strong resistance reflexes, simulation results were different from the recordings reported by [78] and [23] but resembled unpublished recordings, such as those shown in Fig. 6.2. When no sensory feedback was present and CPG OXO activation was 5.0 nA with strong resistance reflexes, the network produced bursts with a very low frequency (Fig. 6.3B, No Feedback). In addition, the burst frequency in OL was higher for stronger assistance reflexes. Closing the sensory feedback loop produced consistent tonic spiking activity in both
A. Low Negative Feedback Gain

B. High Negative Feedback Gain

Figure 6.3. An imbalance of resistance and assistance feedback can result in a range of simulated network behaviors

A) Network activity when the gain of negative feedback was low (PADI OXO, 14.0 nA; CPG OXO, 7.5 nA) and the gain of positive feedback was either low (ARIN OXO, 2.0 nA) or high (ARIN OXO, 12.0 nA). B) Network activity when the gain of negative feedback was high (PADI OXO, 2.0 nA; CPG OXO, 5.0 nA) and the gain of positive feedback was either low (ARIN OXO, 1.0 nA) or high (12.0 nA). Levator motor neuron (Lev MN, blue raster plots) and depressor motor neuron (Dep MN, red raster plots) are shown below the movements of a virtual leg (green traces). Simulations were run in which leg movements were decoupled from MN activity (No Feedback) as well as when leg movements were driven by MN activity (Feedback). Colored bars and barbed lines above MN raster plots indicate bursts and bouts of tonic spiking as classified by the Extended Hill-Valley analysis. Instantaneous frequency plots are shown above MN raster plots and time bars indicate 10 s.
Lev and Dep MN channels when assistance reflexes were weak (Fig. 6.3B, Feedback). Tonic spiking activity occurred because strong resistance reflexes resulted in activation of opposing motor neuron pools. Consequently, the leg jittered in a lowered position with an occasional excursion to moderately elevated positions. When assistance reflexes were strong, the network produced bursts with a higher frequency that was often followed by bouts of tonic spiking of both Lev and Dep MNs (Fig. 6.3B, Feedback).

The results from simulations with feedback and weak as well as strong assistance reflexes resembled the *in vitro* results that were recorded early and late after applying 15 umol OXO, respectively. In order to determine the impact of sensory feedback in controlling undesirable motor activity, a database of simulations was generated for a range of resistance and assistance reflex strengths at three levels of CPG activation. The resulting figures illustrated how a balance or imbalance of reflexes created regions of desirable or undesirable behaviors.

### 6.3.3 Quiescent CPG Network

The first series of simulations were used to determine whether the ratio of PADI and ARIN activation affected network dynamics when the circuit was in a quiescent state. In order to characterize how changing the gain ratio of positive and negative feedback affected network dynamics, simulations were run with varying levels of positive and negative feedback while holding the CPG activation constant. As determined by activity in the absence of sensory feedback, the quiescent network with CPG OXO activation at 3.0 nA yielded short bouts of low frequency tonic spiking and no bursting (Fig. 6.4A, No Feedback). The duration of tonic spiking was heterogenous and there was no gain-dependent pattern (Fig 6.4B, No Feedback).

Closing the sensory feedback loop resulted in organizing the network dynamics into three regions (Fig. 6.4A, Feedback). The first region was defined for low to moderate values of
A. TONIC SPIKING

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B. BURSTING

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B. (+) Gain  (-) Gain

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C. Low  Mod.

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Figure 6.4. Activity maps and sample raster plot for quiescent CPG state
Network dynamics depend on the ratio of positive and negative feedback when the network is in a quiescent state. A) Activity maps show how the duration of tonic spiking episodes, the frequency of tonic spiking, and the burst frequency of the network change for different levels of positive feedback (y-axis) and negative feedback (x-axis). B) Simulations were run in one of two conditions: 1) No Feedback: movements of the leg were only due to its weight and not by motor neuron activity; and, 2) Feedback: movements of the leg were due to its weight under gravity as well as motor neuron activity. Axes are scaled from 0% to 100% of the maximum value sampled as described in Methods. B-D) Raster plots of levator (blue) and depressor (red) motor neuron (MN) activity when: 1) positive feedback was low and negative feedback was moderate (B); positive feedback was high and negative feedback was moderate (C); and, positive feedback was high and negative feedback was low (D). Lev MN activity moved the leg up and Dep MN activity moved the leg down as indicated by the waveform of the joint position (green trace). Arrows on the activity maps indicate the corresponding points of positive and negative feedback that are shown in the raster plots.
positive feedback gain (0 – 50%) and the dependence on negative feedback gain was subtle. In this region, there was no bursting and bouts of tonic spiking had short durations that increased slightly towards higher levels of negative feedback gain (Fig. 6.4B). For elevated levels of positive feedback, the network exhibited bursting irrespective of the gain of negative feedback. For high levels of negative feedback gain (70 – 100%) the network exhibited a mixture of bursts and bouts of tonic spiking with the longest durations occurring around 70% of negative feedback gain (Fig. 6.4C). When negative feedback was low to moderate (0 – 70%) the network exhibited short bouts of tonic spiking with rapid low frequency bursts (Fig. 6.4D). These results illustrate that sensory feedback reorganizes network behavior and depends on the balance of positive and negative feedback. For example, when both assistance and resistance reflexes are strong the system yields bursting in addition to bouts of tonic activity that would cause a leg to jitter. Because this behavior may be undesirable, lowering the strength of resistance reflexes while maintaining strong assistance reflexes resulted in more favorable rhythmic bursting with few bouts of tonic spiking.

In order to test whether the three regions were organized by afferent feedback, simulations were run in which parts of the resistance or assistance reflex pathways were blocked. The short bouts of tonic spiking, for example, were caused by tonic afferents that triggered chained resistance reflexes. This was verified by simulations in which the Release Afferent and Stretch Afferent neurons were disabled yielding results where tonic spiking was less prevalent (Appendix C, Fig. C.3A). In simulations where part of the resistance reflexes were blocked by disabling phasic afferents, the Release Rate Resist Afferent and the Stretch Rate Resist Afferent, the short bouts of tonic spiking were still present (Appendix C, Fig. C.3B). The episodes of higher frequency tonic spiking for elevated positive feedback gain, however, were eliminated while the system continued to exhibit bursting. When phasic assistance reflex afferents, the Release Rate Assist Afferent and Stretch Rate Assist Afferent neurons, were
disabled the network did not burst and only short bouts of tonic spiking were observed (Appendix C, Fig. C.3C). These results show that sensory feedback can induce different network behavior, such as bursting and varying durations or intensities of tonic spiking, in a network that is otherwise quiescent. This switch of network dynamics induced by sensory feedback may be advantageous during posture if a perturbation occurred and taking steps forward or backward would help to prevent an animal from falling.

6.3.4 Bursting CPG Network

The next series of simulations were used to determine whether the ratio of PADI and ARIN activation changed the network dynamics when the circuit was in a bursting state. In the absence of sensory feedback when CPG OXO activation was 7.5 nA the network exhibited rhythmic bursting at moderate frequency independent of the ratio of positive and negative feedback gain (Fig. 6.5A, No Feedback). In addition, short bouts of tonic activity were detected (Fig. 6.5B-D, No Feedback). Consequently, this activity regime was classified as rhythmic bursting.

When the sensory feedback loop was closed the burst frequency increased depending on the gain of positive feedback (Fig. 6.5A, Feedback). When the positive feedback gain was 0% - 30% the burst frequency increased from 0.05 Hz to 0.12 Hz (Fig. 6.5B, Feedback) and then remained between 0.12 to 0.14 Hz through 100% of positive feedback gain (Fig. 6.5c, Feedback). There were also dispersed results when the sensory feedback loop was closed that had an increased burst frequency (corresponding to red values on the color bar) and did not appear to depend on a specific ratio of positive and negative feedback gain (Fig. 6.5A, Feedback, Burst frequency). Tonic spiking occurred for low levels of positive feedback (0 – 30%) and was dependent on the gain of negative feedback. For low values of negative feedback gain, tonic spiking occurred in short bouts. When the gain of negative feedback was
A. TONIC SPIKING

Duration (s)

Spike Frequency (Hz)

BURSTING

Frequency (Hz)

B. (+) Gain  (-) Gain

No Feedback

Feedback

B.

Low  Mod.

C.

High  Mod.

D.

High  Low

No Feedback

Feedback
Figure 6.5. Activity maps and sample rasters for bursting CPG state
Activity of the network in a rhythmically bursting state is organized by sensory feedback and the ratio of positive and negative feedback. A) Activity maps show how the duration of tonic spiking episodes, the frequency of tonic spiking, and the frequency of bursts depend on positive and negative feedback. Simulations were run in one of two conditions: 1) No Feedback: movements of the leg were due to its weight under gravity and did not depend on motor neuron activity; and, 2) Feedback: movements of the leg were due to gravity and motor neuron activity. Axes were scaled from 0% to 100% based on the maximum gain value that was sampled as described in the Methods sections. B-D) Raster plots show the behavior of the network for different levels of positive and negative feedback (indicated by arrows in activity maps). Levator (blue rasters) motor neuron (MN) activity raises the virtual leg and depressor (red rasters) MN activity lowers the leg as indicated by the joint position (green trace). B) Low positive feedback gain and moderate negative feedback gain. C) Moderate positive feedback gain and low negative feedback gain. D) Moderate positive feedback gain and moderate negative feedback gain.
high (80 – 100%) the duration of tonic spiking was slightly longer. In addition, when the gain of positive feedback was between 20% to 50% and the gain of negative feedback was between 75% to 100% the network exhibited bouts of high frequency tonic spiking (Fig. 6.5D, Feedback). While bursting and short bouts of tonic spiking were homogenous across the range of sampled feedback gains without sensory feedback, closing the feedback loop resulted in two major effects. The first effect was a positive feedback-dependent increase in burst frequency. The second effect was an island of high frequency tonic spiking when negative and positive feedback was unbalanced.

Simulations in which afferents were disabled further supported that the organization of dynamics in the bursting network was structured by the interaction of negative and positive feedback in closed loop. When tonic resistance reflex afferents were blocked the region of high frequency tonic spiking was dispersed and the network exhibited a heterogeneous mixture of high and low frequency tonic spiking for positive feedback over 20% (Appendix C, Fig. C.4A). This showed that the island of frequency tonic spiking was organized by tonic afferents and that tonic spiking was not completely mediated by tonic resistance reflex afferents. On the other hand, the frequency of tonic spiking was lower when the phasic resistance reflex afferents were disabled suggesting that fast movements of the leg were responsible for triggering bouts of tonic activity (Appendix C, Fig. C.4B). Disabling the phasic assistance reflex afferents resulted in much lower burst frequencies and the network sporadically exhibited long bouts of low frequency tonic spiking when the gain of negative feedback was high (Appendix C, Fig. C.4C). This showed that assistance reflexes were responsible for increasing the burst frequency. In addition, the presence of long bouts of tonic spiking suggested that assistance reflexes facilitated bursting by overcoming other network activity.

Together these results illustrated that positive and negative feedback interact to reorganize the dynamics of a network that intrinsically bursts without feedback. While the
combined effect of feedback induced potentially unfavorable bouts of high frequency tonic spiking, the contribution of tonic and phasic afferents may play a role in mediating specific compensation mechanisms during locomotion. For example, it may be advantageous to extend the stance or swing phase of a step cycle depending on the timing of a perturbation relative to the stepping rhythm.

6.3.5 Tonic Spiking CPG Network

The last series of simulations was run to determine whether the ratio of PADI and ARIN activation affected the network dynamics when the circuit was a tonic spiking state. For elevated levels of activation to the CPG network (12.5 nA) the circuit exhibited bursting and tonic spiking depending on the value of positive feedback gain (Fig. 6.6A, No Feedback). When the gain of positive feedback was low to moderate (0 – 50%) the network was bursting at a frequency around 0.15 Hz (Fig. 6.6B, No Feedback). At elevated levels of positive feedback gain (70 – 100%) the network exhibited tonic spiking (Fig. 6.6C, No Feedback). Separating the two regions was a band of higher frequency bursting (0.18 Hz) around 55% of positive feedback gain (Fig. 6.6D, No Feedback).

When the sensory feedback loop was closed the frequency of bursting increased and the spike frequency during tonic activity increased (Fig 6.6A, Feedback). Whereas the burst frequency was heterogeneous in the absence of feedback the frequency of bursts increased as the gain of positive feedback increased in the presence of feedback. For low values of positive feedback gain (0 – 15%) the frequency of bursts was about 0.15 Hz. When the gain of positive feedback ranged between 15% to 60% the frequency of bursting was about 0.22 Hz (Fig. 6.6B, Feedback). For high values of positive feedback gain (60 – 100%) the network exhibited tonic spiking and closing the sensory feedback loop resulted in a higher frequency of spiking than in open loop (Fig. 6.6C, Feedback). In the band where the frequency of bursting was higher
A. TONIC SPIKING

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<td>Tonic High MN</td>
<td>Tonic High MN</td>
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C. BURSTING

<table>
<thead>
<tr>
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<th>Frequency (Hz)</th>
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<tr>
<td>No Feedback</td>
<td>Negative Feedback Gain (% Max)</td>
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<tr>
<td>Feedback</td>
<td>Negative Feedback Gain (% Max)</td>
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D. (+) Gain  (-) Gain

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<thead>
<tr>
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<th>No Feedback</th>
<th>Feedback</th>
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<td>Low Mod.</td>
<td>Tonic Low MN</td>
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<td>Tonic High MN</td>
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<td>High Mod.</td>
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Figure 6.6. Activity maps and sample rasters for tonic spiking CPG state

The ratio of positive and negative feedback levels change how the network behaves in a tonically active state. A) Activity maps show how the duration of tonic spiking episodes, frequency of tonic spiking, and frequency of bursting vary depending on feedback conditions. Simulations were run in one of two conditions: 1) No Feedback: movements of the leg were only due to its weight under gravity and were not affected by motor neuron activity; and, 2) Feedback: movements of the leg were due to its weight under gravity and motor neuron activity. Axes are scaled from 0% to 100% based on the maximum value sampled as described in Methods. B-D) Raster plots illustrate examples of network activity for select levels of positive and negative feedback. Levator (blue rasters) motor neuron (MN) activity raises the leg and depressor (red rasters) MN activity lowers the leg as indicated by the joint position (green trace). B) Positive feedback gain was low and negative feedback gain was moderate. C) Positive feedback gain was higher and negative feedback gain was moderate. D) Positive feedback gain was high and negative feedback gain was moderate.
without feedback (55% of positive feedback gain) closing the sensory feedback loop resulted in bursting and bouts of tonic spiking that had higher spike frequencies than in the absence of feedback.

While the tonic spiking regime was largely unaffected by blocking afferent feedback, the properties of bursting were modulated by negative and positive feedback when sensory feedback was present. When the tonic resistance reflex afferents were disabled the burst frequency was reduced and short duration tonic spiking activity remained unchanged (Appendix C, Fig. C.5A). Disabling the phasic resistance reflex afferents, however, resulted in higher burst frequencies and short duration tonic spiking was eliminated (Appendix C, Fig. C.5B). When the phasic assistance reflex afferents were disabled the burst frequency was reduced when the sensory feedback loop was closed and short duration tonic spiking was also eliminated (Appendix C, Fig. C.5C). While the tonic afferents mediated a subtle effect, the phasic afferents mediated the bouts of tonic spiking when the sensory feedback loop was closed and also acted to slow the burst frequency. Together these results indicated that sensory feedback was not effective when the CPG was tonically active, but could still modulate network activity when CPG activation was high. While it was not clear whether the tonic spiking regime was biologically significant, the interesting result here was that sensory feedback slowed the bursting rhythm, which may correspond to biomechanically favorable frequencies.

6.4 Discussion

The results presented here illustrated that the balance of positive and negative feedback can have large implications for the range of behaviors that a sensorimotor circuit exhibited. When the network was in a quiescent state that corresponded to postural behaviors, for example, simulations in which sensory feedback was absent only yielded one state in which short bouts of low frequency tonic spiking activity occurred. Coupling biomechanical feedback
to motor output in closed loop simulations restructured network dynamics into three regimes that depended on the ratio of positive and negative feedback and included bursting, low frequency tonic spiking, and high frequency tonic spiking. In addition, simulations in which individual afferents were disabled showed that the changes induced by feedback were largely mediated by phasic afferents. More specifically, episodes of tonic spiking were mediated by phasic resistance reflex afferents in response to bursts that were mediated by phasic assistance reflex afferents. This sensorimotor integration effect suggests that sensory feedback might be organizing the network while it is in a quiescent, postural state so as to produce a step cycle or a stronger activation of muscles in response perturbations.

6.4.1 Sensory Feedback Reorganizes Network Dynamics in State-Dependent Manner

When the network produced rhythmic bursting without sensory feedback corresponding to a fictive locomotion state, simulations showed that closing the feedback loop increased the burst frequency and also induced a region of high frequency tonic spiking. Similar to the quiescent network, the high frequency tonic spiking region was mediated by an interaction between phasic afferents where bursts mediated by assistance reflexes triggered tonic spiking mediated by resistance reflexes. Thus, it may be that this feature was indicative of a regime of sensory feedback in which perturbations trigger an extended swing or stance phase so as to stabilize an animal’s body in motion. On the other hand, the regions of high frequency tonic spiking, in both the quiescent and bursting states, might increase the biomechanical stiffness of the joint making the leg more resilient to perturbation.

While sensory feedback introduced a region of high frequency tonic spiking in both a quiescent network and a bursting network, the influence of afferents was different depending on the state of the motor neuron (MN) central pattern generator (CPG). In the case of a bursting network, blocking tonic resistance reflex afferents resulted in dispersing the region of high
frequency tonic spiking across the full range of negative feedback gain and yielded heterogeneous high frequency and low frequency tonic spiking for moderate to high values of positive feedback gain. In a quiescent network, blocking the same afferents reduced the frequency of tonic spiking but did not change the region in which tonic spiking was detected. In addition, blocking the phasic assistance reflex afferents when the network was bursting resulted in sporadic bouts of low frequency, long duration tonic spiking when the gain of negative feedback was high. This did not occur in a quiescent network and may have been due to consistent activation of opposing resistance reflexes when the spontaneous activity of the network was higher. In both of these comparisons it was not clear whether the change in tonic spiking duration or frequency had a biological relevance. It may be the case, for example, that the neural network was missing elements, such as neurons or neuromodulating factors, that would have prevented these effects.

Our results also showed that the tonic spiking regime was largely unaltered by sensory feedback. It was not clear, however, whether this regime was biologically meaningful. It may be the case that tonic MN activity occurs during postural behaviors, but, in this state, MNs were unaffected by afferent feedback. In addition, it would be expected that the MNs in a postural state would be activated by a central command, such as the CPG OXO neuron and not from ongoing excitation from reflex interneurons. In this case, however, the MNs received excitation from both the CPG OXO neuron as well as the ARINs, thus it was likely that the state was unfavorable. Perturbation experiments, however, might be useful to illustrate a more biologically meaningful context for the tonic spiking state of the network. On the other hand, when the network produced rhythmic bursting in this regime, blocking resistance reflexes resulted in a higher burst frequency suggesting that sensory feedback may be slowing the rhythmic frequency of the network to coordinate it with the biomechanical properties of the joint it controls.
6.4.2 Significance and Implications of Range of Network Dynamics

While *in vitro* studies may focus on specific states of a network that are relevant to specific behaviors [2, 4], these results show how it is important and useful to characterize and understand a broader range of features of a coupled neural and biomechanical system. Here, for example, we used recordings from *in vitro* brain-machine interface experiments to validate the range of simulated neural activity produced by a model of a locomotor circuit. The locomotor circuit model was then used to characterize the range of behaviors the system can exhibit. Simulation results have led to questions and hypotheses that can then be further explored through additional simulations as well as *in vitro* experiments.

The current construction of the neuromechanical model includes and excludes certain features of what is already known about the locomotor control circuit. For example, both Lev and Dep MN pools were modeled by one tonic MN, one phasic MN, and an intrinsically bursting interneuron (IN). *In vitro* experiments, however, have shown that the MN pools consist of over a dozen neurons each [1]. In addition, afferent feedback from the stretch receptor in simulations was filtered through three neurons and experimental evidence shows that there are many afferents that provide feedback to MNs [131]. Thus, it may be the case that the divergence of sensory information through multiple afferents and their convergence onto MNs allows the network to filter proprioceptive feedback so as to generate redundancies and reduce unfavorable system behaviors. The results presented here, for example, showed that a network in which a few neurons were used to encode and filter sensory information resulted in regions of network activity that were unfavorable. These regions may be eliminated in a network where sensorimotor integration occurs across many afferents and many motor neurons.

The simulation results presented here illustrate network activity that was classified based on unperturbed output. Simulations in which the network is perturbed, however, can
help to elucidate whether and how the network compensates for unanticipated changes in an environment. For example, it is possible that a perturbation delays or advances the onset of the next step cycle [34]. On the other hand, some networks only advance the next step cycle and can result in unstable coordination of CPGs [33]. Consequently, it may be useful to further characterize the differences in unperturbed network dynamics by using perturbations in order to help to identify whether sensory feedback plays a role in limb and joint coordination.

While these results have generated several questions and hypotheses about the mechanisms of sensorimotor integration, this approach has employed an algorithmic parameter-sampling approach [127, 130, 132] through which sensorimotor systems can be better understood. The first step was to identify a selection of in vitro results that illustrate a range of behaviors exhibited by the system [78]. Then, a simplified model of the system was constructed and simulations were run to verify that it can account for in vitro behaviors [23]. Finally, simulations were run under a variety of conditions and analyzed to determine whether sensory feedback mediated changes in network dynamics. In addition, simulation results and analysis helped to elucidate a broad range of network behaviors that were and were not observed in vitro but can be used to develop and test hypotheses about how certain effects of sensorimotor integration are mediated in a biological system.
7 Neuromechanical Simulation Results Are Reproduced by Nonlinear Oscillator Model with Sensory Feedback

7.1 Introduction

Sensorimotor networks integrate ongoing neural motor activity with sensory feedback during posture and locomotion to resist perturbations and achieve robust behaviors in unpredictable environments [1, 14, 17, 115, 133]. Output from neural motor networks that drive movements, called central pattern generators (CPG), can produce rhythmic output during behaviors such as locomotion or single-event output during behaviors such as reaching [134]. As a limb moves driven by motor outputs, biomechanical sensory feedback modulates ongoing motor activity or produces reflexes when triggered by external perturbations [1, 15, 24, 115, 135]. It is unclear, however, how afferents interact with CPG dynamics to accommodate for changes in an environment during posture or locomotion. One way in which reflexes might compensate for perturbations during locomotion, for example, is to modulate a network rhythm to advance or delay the next step of a leg’s cycle without changing the dynamics of the system [2, 8, 34, 136]. Alternatively, feedback might act to alter the network dynamics resulting in a change of its coordination pattern, such as a transition from walking to galloping [122, 137-139]. In neuronal dynamics, the interactions between feedback, external factors, and intrinsic dynamics have been studied extensively [26-28, 35, 140-142] and provide a basis through which we can understand how sensory feedback is integrated in locomotor networks. Here we used a simplified mathematical model that reproduced the oscillatory behavior of a locomotor CPG and added sensory feedback in order to determine how the output of a sensorimotor network is organized by feedback.
7.1.1 Mathematical Nonlinear Oscillator Models in Neuroscience

Mathematical models can be employed in order to describe a set of generalizable principles that dictate how a system works. When studying neurons, nonlinear interactions between ionic currents are mediated via a membrane potential and can be modeled by mathematical models [143]. For example, in the classic Hodgkin-Huxley model [39] the sodium current, $I_{Na}$, is activated when a neuron’s membrane is depolarized and results in further depolarization of the cell, thereby providing positive feedback. On the other hand, the delayed rectifying potassium current, $I_{K}$, is also activated when a cell is depolarized but acts slower than $I_{Na}$ and hyperpolarizes a cell to return a membrane to its resting potential and so provides negative feedback. Together, these two currents generate a nonlinear oscillation that corresponds to action potentials generated by neurons. Modifications have been made to the original Hodgkin-Huxley nonlinear oscillator by adding feedback via other experimentally identified ionic currents [144-146]. The addition of these currents can result in changing the intrinsic dynamics of a neuron. Depending on how the effects of positive and negative feedback from ionic currents interact, a neuron can respond and transmit different features of an input signal, such as coincident inputs or fluctuations of input firing rate [26-28, 31, 141].

7.1.2 Mathematical Characteristics Imply Biological Features

The properties of signal transmission of a neuron arise from the underlying dynamics of ionic currents that responds to the sum of its inputs [37, 125, 126, 147]. The dynamics of a neuron that integrates its inputs and fires a series of action potentials, for example, can be mathematically represented by a bifurcation called, a saddle-node on invariant circle (SNIC) [37, 141, 142]. A SNIC bifurcation occurs when a stable fixed point and an unstable fixed point coalesce and annihilate each other giving rise to an oscillation, called a stable limit cycle (in this case, also called an invariant circle) [37]. In this scenario, the stable fixed point corresponds to
the hyperpolarized state of the neuron and the limit cycle represents an action potential. Thus, when a neuron receives inputs that occur with a frequency above a certain threshold the stable and unstable fixed points are pushed towards each other and give rise to action potentials along the stable limit cycle. Another example is when a neuron fires an action potential during inputs that occur at a specific frequency and is mathematically represented by a bifurcation called, a super-critical Andronov-Hopf (Hopf) bifurcation [37, 141, 142, 148]. A Hopf bifurcation occurs when a stable fixed point becomes unstable and gives rise to a stable oscillation, called a limit cycle. Because of the interactions of ionic currents, inputs with a frequency that resonates with subthreshold oscillatory behavior around the stable fixed point can excite a neuron to fire an action potential along the limit cycle.

In some cases a set of differential equations cannot be defined to describe a system’s dynamics. When this is the case, there are two ways that a SNIC can be distinguished from a Hopf bifurcation. Because a fixed point is a point in a phase plane at which the velocity of variables in a system approaches 0, a SNIC bifurcation causes the dynamics of a system to slow. For stimulus values below the bifurcation point, the stable fixed point yields a system with no oscillations and the frequency is 0 Hz. For stimulus values above the bifurcation point the system oscillates along the stable limit cycle that occurred when the stable and unstable fixed points coalesced. When the stimulus value is greater than the bifurcation point but remains in the vicinity of bifurcation, the system dynamics are slowed because of the “ghost” of the saddle-node [30]. Consequently, the frequency-stimulus curve of a SNIC bifurcation is continuous and has a finite slope at the bifurcation. In the case of a Hopf bifurcation, the stable fixed point loses stability and gives rise to a stable limit cycle. Consequently, the frequency-stimulus curve is zero for values lower than the bifurcation and non-zero for values above the bifurcation. The slope of the frequency-stimulus curve at the bifurcation is undefined (discontinuous) [30].
Another consequence of the different phase plane geometry of a SNIC and Hopf bifurcation is how they respond to discrete perturbations. The dynamics of a system can be measured by perturbing a system at different phases of oscillation and determining whether the next oscillation is advanced or delayed and by how long. These measurements can be plotted to produce a “phase response curve” (PRC). When dynamics are organized around a SNIC bifurcation, the PRC is positive and the onset of an oscillation following a perturbation is delayed [30]. In the case of a Hopf bifurcation, the response depends on the phase of perturbation and can either advance or delay the next oscillation. Because SNICs delay their response, coordinated activity in a network of positive PRCs is unstable [33]. On the other hand, Hopf oscillators that can advance or delay an oscillation depending on the phase of an input can be organized into networks that coordinate and synchronize the oscillators [33, 136].

The SNIC and Hopf bifurcations described here illustrate how mathematical features can elucidate how a neuron responds to different properties of a stimulus [30, 37]. In addition, the underlying bifurcation structure of a neuron is determined by the nonlinear interactions of positive and negative feedback from ionic currents. Consequently, it is useful to develop mathematical models of nonlinear interactions in order to understand how a neuron responds to perturbations or coordinates with other systems [27, 35, 141].

7.1.3 Constructing a Nonlinear Oscillator Model with Sensory Feedback

Sensorimotor systems display non-linear dynamics that are similar to neuronal dynamics. In the case of sensorimotor systems, CPGs can be represented by nonlinear oscillators while afferents produce negative and positive feedback [8, 117]. Depending on the bifurcation structure of an oscillator and how it interacts with negative or positive feedback a system can contribute to a stable locomotor rhythm or lead to an unstable gait [136]. In addition to characterizing the dynamics of a locomotor CPG, it is also important to determine
how sensory feedback preserves or changes a system’s bifurcation structure as it may dictate how the system responds to perturbations or coordinates with other networks.

The principles used to describe the nonlinear dynamics of a neuron are applied here to characterize how sensory feedback controls the output of a sensorimotor network. Simulations using a neuromechanical model of a locomotor circuit that controls levation and depression of a crayfish leg around a single joint provided target responses to reproduce with a phenomenological model of the circuit and leg. This model is a simplified mathematical model of a neuromechanical system with independent control of CPG excitability, positive feedback, and negative feedback. The simplified model’s trajectories are integrated to illustrate that it can produce similar results to the neuromechanical model. Finally, a bifurcation analysis shows how sensory feedback controls the behavior of the model. These results suggest how sensory feedback changes network output without changing the underlying bifurcation.

7.2 Methods

7.2.1 Locomotor Network Simulations

Simulations were generated from a model of a crayfish locomotor control circuit constructed in the neuromechanical simulation software, AnimatLab (www.animatlab.com) [42] (Fig. 7.1). Briefly, the model consisted of two pools of mutually inhibitory motor neurons, called levator (Lev) and depressor (Dep) motor neurons, and afferents that mediated resistance and assistance reflexes [23]. Motor neurons, afferents, and interneurons as well as the nature of their synaptic connections were based on in vitro results described by Cattaert, et al. [1]. The locomotor control circuit was coupled to a biomechanical model of a crayfish leg with a single axis of rotation around a joint such that muscles activated by Lev and Dep MNs activated muscles that raised and lowered the leg, respectively. Movements of the leg were transduced
Figure 7.1. Schematic of Locomotor Network Model
The sensorimotor network that controls the second joint of crayfish walking legs consists of motor neurons (MN), interneurons, and afferents. A biomechanical leg model (not shown here) is raised and lowered by levator (Lev) and depressor (Dep) muscles, respectively. Muscles are driven by Lev (blue) and Dep (red) MNs that make up a central pattern generator (CPG). Monosynaptic resistance reflexes generate stabilizing biomechanical negative feedback and act to oppose leg perturbations are mediated by tonic and phasic afferents. The strength of resistance reflexes is controlled by primary afferent depolarization interneurons (PADI). Disynaptic assistance reflexes generate potentially destabilizing positive feedback and act to reinforce ongoing leg movements. The strength of assistance reflexes is controlled by tonic activation of assistance reflex interneurons (ARIN). The activity state of the network is controlled in vitro by a cholinergic agonist, called oxotremorine (OXO), and in simulations by three OXO neurons (not shown). The CPG OXO neuron controls the level of activation to the MN CPG. The PADI OXO and ARIN OXO neurons control the level of activation to the reflex interneurons. For a full schematic see Appendix D, Figure D.1.
by a stretch receptor to afferents that synaptically excited MNs and interneurons. Simulations were run in one of two conditions. When movements of the crayfish leg were based on the output of motor neurons the simulations were called, Closed Loop. In simulations where movements of the leg were decoupled from MN output the simulations were called Open Loop.

The locomotor circuit included three neurons that emulated an in vitro effect of a cholinergic agonist called, oxotremorine (OXO) [1, 53, 78]. OXO acts to change the strength of reflexes and to change the state of the locomotor circuit from quiescent to active by exciting rhythmic motor output [1]. The strength of resistance reflexes (negative feedback) and assistance reflexes (positive feedback) were controlled by the PADI OXO and ARIN OXO neurons, respectively. Activation of the PADI OXO neuron reduced the strength of resistance reflexes and was described as reducing the gain of negative feedback in the mathematical model. Activation of the ARIN OXO neuron increased the strength of assistance reflexes and is described as increasing the gain of positive feedback in the mathematical model. The CPG OXO neuron activated the CPG via tonic excitation to the two motor pools. In addition to running simulations with and without sensory feedback, simulations were run with varying levels of gain to the CPG as well as negative and positive feedback all together or independently.

Neurons and afferents were integrate-and-fire models with afterhyperpolarization currents and accommodation. The Lev interneuron (Lev IN) and Dep interneuron (Dep IN) also included a calcium current that produced intrinsic bursting properties. Within each motor pool, motor neurons and the bursting interneuron were electrically coupled. Neurons and afferents also included 0.1 mV of membrane potential noise.
7.2.2 Simulation Data Analysis

Neural spike trains from simulations were analyzed using the Extended Hill-Valley (EHV) analysis method. Briefly, a smoothed, history-dependent analysis signal was derived from each spike train and bursting as well as tonic spiking events were detected based on features of the analysis signal. A burst was identified by a region of the analysis signal where the slope rose rapidly and then fell rapidly after reaching a minimum peak height. This feature of the analysis signal reflected a short bout of high frequency spikes that corresponded to a neural burst. Bouts of tonic spiking were characterized by regions of the analysis signal that increased moderately fast to a height that was then maintained for a minimum duration of time. The corresponding spike train reflected a series of spikes with a maintained frequency within a certain range of variability.

7.2.3 Sampling Methodology

Spike trains were selected to guide construction of a simplified mathematical model, called a phenomenological model, by sampling the database of simulation results based on different regions of activity that were classified by the EHV analysis algorithm. These regions were identified and described in the previous two sections. Briefly, spike trains were used to constrain the behavior of the phenomenological model based on a qualitative comparison of the movement of the leg and the resulting trajectory of the dynamical system. In addition, the trajectory could be used as an approximation for the firing frequency of Lev and Dep motor neuron pools.

7.2.4 Numerical Integration & Continuation

The phenomenological model consisted of a system of ordinary differential equations (dynamical system). Integration and continuation of the system was accomplished using the dynamical systems Python library, PyDSTool [149].
7.3 Results

In order to determine how sensory feedback affects sensorimotor output, a nonlinear dynamical system with feedback was developed to reproduce a set of qualitative features that were characterized from a neuromechanical model. Simulations of the neuromechanical model were run to characterize the qualitative dynamics of the circuit. Three parameters were used to control the dynamics of the model including: 1) activation of the CPG, resistance reflex (negative feedback) strength, and assistance reflex (positive feedback) strength. First, simulations were run in which only the CPG activation was varied to characterize the features of a central oscillator. Then, a mathematical oscillator model was proposed to reproduce network activity states and burst frequency behavior. Simulations then were run in which the strength of resistance and assistance reflexes were varied independently for different levels of CPG activation. The results from these simulations were the basis for the final mathematical formulation of the effect of sensory feedback on the nonlinear oscillator. Finally, a bifurcation analysis of the system was presented to illustrate that sensory feedback changes the behavior of the locomotor control circuit without changing the bifurcation structure of the system.

7.3.1 Sensory Feedback Changes Qualitative Dynamics of Locomotor Control Circuit

The locomotor control circuit can produce a range of activity patterns that were quantified by measuring the frequency of tonic levator (Lev) motor neuron (MN) bursts and the duration of tonic spiking events. The variety of activity was dependent on the level of activation to the MN CPG as well as the balance of resistance and assistance reflexes. Therefore it was important to separate the effect of sensory feedback from the dynamics of the CPG alone in order to develop a phenomenological model of the system dynamics. We first asked what the effect of sensory feedback was on the dynamics of the MN CPG. When activation of the MN CPG was increased by stimulating the CPG OXO neuron the network transitioned from
quiescence to rhythmic bursting with (cyan) and without (magenta) sensory feedback (Fig. 7.2A). In addition, the frequency of bursting increased as the activation level increased. When sensory feedback was present the transition between quiescence and rhythmic bursting went through a region of tonic spiking between 4.0 nA and 6.0 nA. The tonic spiking was a result of a resistance reflex in one MN pool triggering a resistance reflex in the opposing MN pool in an alternating pattern. Dynamically these results showed that the network exhibited two distinct states when the strength of resistance reflexes was high and the strength of assistance reflexes was low.

As the strength of resistance reflexes decreased while the strength of assistance reflexes and activation of the CPG increased, the network exhibited three regimes of activity (Fig. 7.2B). The first regime was a quiescent regime in which no bursts were observed and tonic spiking occurred sporadically but did not contribute significantly to the dynamics of the network. Between 4.0 nA and 11.5 nA of activation to the three OXO neurons the network exhibited bursting with a higher frequency when sensory feedback was present (cyan) than when it was absent (magenta). When activation was elevated to between 12.0 nA and 15.0 nA the network entered a tonic spiking regime that was dominated by activity exclusively from one of the MN pools and not the other. These results illustrated that adding feedback reorganized the dynamics of the network into three regimes. In comparison to increasing activation of the CPG alone, changing the strength of resistance and assistance reflexes showed that the qualitative dynamics of the network were changed from two states to three states by sensory feedback.

7.3.2 Relaxation Oscillator Reproduces Central Pattern Generator Dynamics

Phenomenological models use a minimal number of variables to reproduce a set of qualitative features of a dynamic system [30, 36, 37]. The advantage in using simplified models
is that they can be used to identify generalizable principles that describe how a system works. On the other hand, a disadvantage to using simplified models is that they can overlook certain features of the original system. Understanding why a model does not account for certain features, however, can be informative in developing meaningful hypotheses and in understanding their significance. Here, we asked whether a simplified, phenomenological nonlinear oscillator model could reproduce the rhythmic output of a sensorimotor circuit involved in posture and locomotion.

In order to reproduce the dynamics of the locomotor network using a simplified dynamical system, simulation results were reduced to a single state variable and a nonlinear relaxation oscillator was used as the basis for rhythmic behavior. Because the locomotor circuit controls the movement of a single joint, the joint position was used as a single measure of system behavior. The advantage to using the joint position as a measure of system performance was that it captured the dynamics of both the neural network and the biomechanics of the musculoskeletal system. Because the goal of the reduced system was to approximate the qualitative behavior of the neuromechanical system, joint movements were mapped onto a 2-dimensional plane, called a phase plane (Fig. 7.3A). The positive and negative directions along the x-axis were used to represent joint position. Positive x values represented elevated positions (leg was above the horizontal plane) produced by Lev MN excitation. Negative values represented depressed leg positions (leg was below the horizontal plane) produced by depressor (Dep) MN excitation. The positive and negative directions along the y-axis were used to reflect the swing and stance phases of a step cycle, respectively.

Based on this arrangement, when the leg was in a horizontal position the x-coordinate was 0. Activation of Lev MNs moved the x-coordinate in the positive direction while activation of Dep MNs moved the x-coordinate in the negative direction. An alternative measure was also
Figure 7.2. Sensory feedback changes network dynamics and is phase-dependent
A) Graphs of burst frequency and duration of tonic spiking events show the effect of increasing the level of CPG activation via CPG OXO stimulus level. Simulations were run with sensory feedback intact (cyan) as well as in the absence of sensory feedback (magenta). Dashed graphs show the average frequency or duration and shaded boxes are the standard error of the mean. B) Graphs of burst frequency and duration of tonic spiking events show the effect of increasing the stimulus level of the CPG OXO, PADI OXO, and ARIN OXO neurons. This was caused the activation level of the CPG and assistance reflexes to increase while decreasing the strength of resistance reflexes. Simulations were run with (cyan) and without (magenta) sensory feedback. Dashed graphs indicate the average frequency or duration and shaded boxes are the standard error of the mean.
considered that reflected the firing rates of Lev and Dep MNs (Appendix D, Fig. D.2). While the latter metric more closely resembled the trajectories from the mathematical model, the joint position had a clearer biological interpretation. In addition to simplifying neuromechanical dynamics into a 2-dimensional state variable the reduction allowed for sensory feedback to be gated in a phase-dependent manner.

The FitzHugh-Nagumo (FHN) oscillator is a 2-dimensional nonlinear system of ordinary differentiation equations (ODE) that is commonly used in neuroscience to capture the dynamics of spiking neurons [147]. Here, we used the FHN oscillator to reproduce the oscillatory behavior of the locomotor CPG. The FHN dynamics of the FHN oscillator are organized by a cubic $x$-nullcline and a linear $y$-nullcline (Fig. 7.3A),

$$\frac{dx}{dt} = c \left( y + x - \frac{x^3}{3} + z \right) \quad \text{(1)}$$

$$\frac{dy}{dt} = \frac{-(x-a+by)}{c}. \quad \text{(2)}$$

The variable, $x$, reflected the vertical position of the leg produced by activation of Lev and Dep MNs, which would cause upward and downward movements of the leg as seen in the CB joint position traces, respectively. The variable, $y$, did not have a biological meaning, but could be thought of as being indicative of the swing or stance phases of each step cycle. The parameter, $z$, was used to translate the $x$-nullcline vertically in the phase plane. The parameter, $a$, translated the $y$-nullcline vertically in the phase plane and the parameter, $b$, changed the slope of the nullcline. The parameter, $c$, separated the time scales of the $x$- and $y$-nullclines. The separation of time scales parameter, $c$, was used as a bifurcation parameter to generate two regimes of system activity: quiescence and bursting. When $c$ was 0.563 trajectories of the FHN model did not oscillate and relaxed to the origin (Fig. 7.4A). This behavior corresponded to the quiescent state of the neuromechanical model (CPG Stim = 3.0 nA in Fig. 7.4B). When $c$
A. Fitzhugh-Nagumo Oscillator

B. Reflex Gating via Activation Functions

**Figure 7.3. Schematic of a phase oscillator and sensory feedback variables**

A) A nonlinear oscillator is used to reproduce the rhythmic activity of an activated locomotor central pattern generator (CPG). Based on the Fitzhugh-Nagumo nonlinear oscillator, a cubic $x$-nullcline (cyan) and a linear $y$-nullcline (magenta) organize system dynamics. Positive (negative) values along the $x$-axis represent elevated (depressed) leg positions produced by Lev (Dep) motor neuron (MN) activation. Positive (negative) values along the $y$-axis represent the swing (stance) phase of a step cycle. The labels a-e identify points along the nonlinear oscillation that correspond to the phase-dependent reflex activation illustrated in Fig. 7.5. Sigmoidal activation curves are used to produce phase-dependent effects of reflexes. Dep assistance reflexes, for example, are active when the leg transitions from swing to stance. Therefore, the $x$-activation function is 1 when $x > 0$ and the $y$-activation is 1 when $y < 0$. The product of the two activation functions is multiplied by a constant that determines the maximum reflex effect and a driving force that is the difference between the leg position and a steady-state reflex leg position.
was increased to 1.385 or 2.299, the FHN model oscillated between positive and negative $x$-values (Fig. 7.4A), which corresponded to upward and downward movements of the neuromechanical leg model driven by alternating Lev and Dep MN activity (CPG Stim = 7.5 nA, 12.5 nA in Fig. 7.4B). As $c$ was increased from 1.385 to 2.299, the frequency of oscillations of the FHN model decreased slightly from 0.13 Hz to 0.12 Hz, unlike the neuromechanical model in which the frequency of bursting increased slightly with a maximum around 0.15 Hz. In addition, the tonic spiking effect at the transition between quiescence to bursting was not specifically reproduced by the simplified mathematical model. The qualitative feature reproduced here, however, was the transition from quiescence to bursting (Fig. 7.2).

Comparison of neuromechanical simulation results and the trajectories from the FHN model illustrate that the nonlinear FHN oscillator model can be used to reproduce the cyclical behavior of the neuromechanical system.

### 7.3.3 Sensory Feedback Changes System Dynamics

Because the network was capable of producing a variety of output depending on the balance of resistance and assistance reflexes, we next asked whether the effect of sensory feedback could be accounted for by the FHN oscillator model with the addition of a set of feedback variables. Because of the inherent nonlinearities of the modified FHN oscillator with feedback, however, it was not feasible to optimize the dynamics of the feedback variables. Consequently, the approach was simplified by asking whether the effect of feedback could be reproduced using simplified translations of the FHN nullclines in a phase plane.

Resistance and assistance reflexes in the neuromechanical model were, by construction, phase-dependent (Fig. 7.5) [23]. Resistance reflexes, for example, are blocked by primary afferent depolarization (PAD) in which afferent inputs to motor neurons are inhibited by
A. Modified FitzHugh-Nagumo Model

\[ c = 0.563, \, z = 0.0 \]
\[ c = 1.385, \, z = 0.0 \]
\[ c = 2.299, \, z = 0.0 \]

B. Neuromechanical Simulation

**Figure 7.4. System dynamics reproduce increasing burst frequency of CPG**

Three points of the mathematical system and the neuromechanical system were sampled to illustrate corresponding activity patterns. The quiescent system and network are shown in the orange box. The bursting system and network are shown in the blue and green boxes. A) Traces of the system trajectory and phase planes of the FitzHugh-Nagumo (FHN) nonlinear oscillator model. Blue regions indicate levator motor neuron (Lev MN) activity and red regions indicate depressor motor neuron (Dep MN) activity. The blue curve is the cubic x-nullcline defined by the FHN system. The magenta line is the y-nullcline of the FHN model. Red traces on the phase plane show the trajectory of the system in the x-y plane. B) Simulation results from the neuromechanical network model. The CB joint (green trace) is raised with Lev MN activity (blue rasters) and lowered with Dep MN activity (red rasters).
neurons of the antagonist motor neuron pool [114]. Consequently, when one motor pool was active it prevented reflex excitation of the other motor pool. For example, when the Dep MNs were active during rhythmic bursting and drove the leg down, they inhibited stretch-sensitive afferents via a PAD interneuron (PADI) and blocked the resistance reflex that would excite the Lev MNs (Figs. 7.1; 7.5, a). This allows the CPG-driven leg movements to occur unopposed by resistance reflexes. Increasing the activation of PADIs via the PADI OXO neuron reduced the strength of resistance reflexes (i.e., reduced negative feedback). Activation of assistance reflexes was also phase-dependent because the interneurons that mediated the polysynaptic reflex response, called assistance reflex interneurons (ARINs), were inhibited by the opposing motor neuron pool. For example, when Lev MNs were active during rhythmic bursting they inhibited the ARIN that otherwise triggered a Dep assistance reflex (Fig. 7.5, c). Consequently, increasing the activation of ARINs via the ARIN OXO neuron increased the strength of assistance reflexes (positive feedback).

The mathematical model was modified to include variables that reproduced the effect of sensory feedback. Four reflex terms, $F_i$, were included in the modified model to represent Lev resistance reflexes, Lev assistance reflexes, Dep resistance reflexes, and Dep assistance reflexes (Appendix D, Supplementary Equations). The expression representing the dynamics for each reflex was,

$$F_i(x, y, t) = K_{max,i} \cdot \phi_i(x, t) \cdot \omega_i(y, t) \cdot (x - \theta_i). \quad (3)$$

The reflex strength for each reflex, $K_{max,i}$ was a constant that represented the maximum effect of the reflex. Each reflex had an equilibrium position, $\theta_i$, that acted to attract the state variable of the mathematical model towards an elevated or depressed leg position. The activation variable, $\phi_i(x, t)$, was used to gate reflexes by Lev and Dep activation as represented by the
Figure 7.5. Phase-dependent reflexes in neuromechanical simulations
Interneurons mediate a phase-dependency of resistance (-) and assistance (+) reflexes. “o” indicates no reflex is active. A trace of the leg joint angle (green trace) shows two rhythmic leg movements. Lev activity raises the leg and dep activity lowers the leg. Lev resistance reflexes occur when the leg is raised (d) and Lev assistance reflexes occur as the leg transitions from stance (leg is down) to swing (leg is up) (c). Dep resistance reflexes occur when the leg is lowered (a) and Dep assistance reflexes occur as the leg transitions from swing (leg moves from down to up; e) to stance (leg is down) (a). +, -, o indicate the sign of reflex during each phase of rhythmic leg movements for Lev and Dep MNs.
variable, $x$. The activation variable, $\omega_i(y, t)$, was used to gate reflexes based on the swing and stance phase of a step cycle as represented by the variable, $y$. For example, Dep assistance reflexes were active during the middle of the swing phase when $x$ is positive and $y$ is positive (Figs. 7.2C, e; 7.3A, e). Thus $\varphi_i(x, t)$ was an increasing sigmoid that was 1 when $x$ was positive and 0 when $x$ was negative (Fig. 7.3B). $\omega_i(y, t)$ was a decreasing sigmoid that was 1 when $y$ was negative and 0 when $y$ was positive. Because the equilibrium position of the Dep assistance reflex was positive, the effect of the reflex was to translate the cubic nullcline up (positive) in the phase plane. Consequently, a stable fixed point tracked along the negative branch of the cubic nullcline and attracted the state of the system toward negative $x$-values corresponding to Dep MN activity. All four reflex terms were constructed using similar logic.

In order to incorporate the effect of the four reflexes into the FHN oscillator model, their effect was summed and added to the expression for $x$. Taking into account that each reflex was phase-dependent, their effect on the state variable, $x$, was calculated by,

$$\frac{dx}{dt} = c \left( y + x - \frac{x^3}{3} + z + \sum F_i(x, y, t) \right)$$

(4)

Where $F_i$ was the effect of each reflex on the state variable, $x$. This effect was mediated via a feedback loop where changes in the state of the system resulted in changes of sensory feedback. Differences in sensory feedback, then, resulted in translations of the $x$-nullcline and the state of the system changed, in turn. Thus, the net effect of sensory feedback was to vertically translate the $x$-nullcline depending on the state point, $(x, y)$. By treating the effect of sensory feedback to be constant, however, its affect could be studied as this effectively decoupled the phase-dependency of feedback from the dynamics of the oscillator. This was mathematically equivalent to changing the value of the parameter, $z$. The results illustrated here were integrated using different sets of parameters with $c$ representing activation of the MN CPG and $z$ representing a decoupled effect of sensory feedback. Because the system of ODEs
had 8 sensory feedback variables and two CPG variables, it was not practical to use brute-force methods to find a set of parameters that generated system dynamics similar to the behavior observed in neuromechanical simulations. Thus, these results should be viewed as a proof of concept and can be used to guide a refined parameter search for a continuous dynamical system (Appendix D, Fig. D.3).

Using the same values of c that generated the quiescent and bursting regimes of the FHN system, the effect of sensory feedback was tested by sampling the mathematical model for different sensory feedback parameters. When both c and z were low (0.563 and 0.073, respectively) the modified FHN system monotonically relaxed to 0.0 (Fig. 7.6A) corresponding to a neutral leg position in the neuromechanical model in a quiescent state (CPG Stim = 3.0 nA in Fig. 7.6B). Increasing c to a moderate value of 1.385 and z to a value of 0.407 resulted in rhythmic oscillations (Fig. 7.6A) similar to rhythmic bursting of the neuromechanical network (CPG Stim = 7.5 nA in Fig. 7.6B). When c was increased further to 2.299 and z was increased to 0.779 the modified FHN system relaxed to a positive, non-zero value (Fig. 7.6A) that corresponded to a constant, elevated firing frequency of Lev MNs that was considered to be equivalent to tonic Lev MN activity (CPG Stim = 12.5 nA in Fig. 7.6B). A relevant domain of c and z is suggested later when a bifurcation analysis of the system is presented. These results illustrate that this formulation of CPG dynamics with sensory feedback reproduces the behavior of the locomotor control circuit. Further parameter optimization is necessary, however, to identify a set of parameters that generate continuous dynamics.

In order to further test whether the system of ODEs could reproduce neuromechanical simulation results, system dynamics were sampled and compared to simulation results at four different ratios of positive and negative feedback. These examples are instances in which the
A. Modified FitzHugh-Nagumo Model

\[ c = 0.563, \quad z = 0.073 \]

\[ c = 1.385, \quad z = 0.407 \]

\[ c = 2.299, \quad z = 0.779 \]

B. Neuromechanical Simulation

**Figure 7.6. Sensory feedback changes system dynamics**

Introducing sensory feedback produces three activity regimes in the neuromechanical model: quiescence (orange box), rhythmic bursting (blue box), and tonic spiking (green box). Using the same values as in Fig. 7.4 for the bifurcation parameter, \( c \), the parameter \( z \) can be changed to reproduce simulation results. A) Traces of the system trajectory and phase planes of the FitzHugh-Nagumo nonlinear oscillator model. Blue regions indicate levator motor neuron (Lev MN) activity and red regions indicate depressor motor neuron (Dep MN) activity. The \( x \)-nullcline is a cubic (blue curve) and the \( y \)-nullcline is linear (magenta line). Red traces in the phase planes show the trajectory of the system in the \( x-y \) plane. B) Simulation results of the neuromechanical system. The CB joint (green trace) is raised as by Lev MN activity (blue rasters) and lowered by Dep MN activity (red rasters).
effect of different ratios of feedback were reproduced by integrating the ODE system with constant values of feedback, $z$, in order to illustrate that the system can yield certain features. The effect of feedback, however, changes depending on the phase of oscillation and can be better described by treating $z$ as a dynamic variable that is a summation of the four feedback terms, $F_i$. As described earlier, feedback is decoupled from the oscillator dynamics in order to simplify this analysis.

When the strength of both resistance and assistance reflexes was weak and the CPG was moderately activated, the neuromechanical simulation produced low frequency bursts with slow onsets that consisted of prolonged tonic Lev MN activity (Fig. 7.7A, i). This effect was emulated when $c$ was 1.385 and $z$ was -0.350. When the strength of resistance reflexes was increased without changing the strength of assistance reflexes or CPG activation, the neuromechanical simulation produced bouts of coactivated tonic spiking where Lev and Dep MNs were recruited in chained resistance reflexes (Fig. 7.7B). This effect was reproduced when $c$ was 1.385 and $z$ was -0.530. For lower levels of CPG activation and weak resistance reflexes with strong assistance reflexes the neuromechanical simulations produced low frequency rapid bursts (Fig. 7.7C). This was reproduced by the modified FHN model when $c$ was 1.385 and $z$ was -0.8.

Because the magnitude of sensory feedback depended on the phase of oscillation and controlled the vertical position of the $x$-nullcline, the reflexes mediated a nonlinear feedback loop. In other words, the effect of reflexes changed depending on the phase of oscillation, which, in turn, moved the $x$-nullcline and caused the reflexes to change. This effect was of particular interest when assistance reflexes were increased while holding resistance reflexes strong and with lower CPG activation. In this case, the neuromechanical system produced bursts that were immediately followed by bouts of coactivated tonic spiking triggered by
A. (+) Low Gain & (-) Low Gain
i. Neuromechanical Simulation Results

B. (+) Low Gain & (-) High Gain
i. Neuromechanical Simulation Results

ii. Mathematical Model

\[ c = 1.385, \ z = -0.350 \]

ii. Mathematical Model

\[ c = 1.385, \ z = -0.530 \]
C. (+) High Gain & (-) Low Gain
i. Neuromechanical Simulation Results

D. (+) High Gain & (-) High Gain
i. Neuromechanical Simulation Results

Figure 7.7. Modified FitzHugh-Nagumo model reproduce simulation results
Neuromechanical simulations with different ratios of static positive and negative feedback gain produce a range of behaviors. Traces of the mathematical model are shown in addition to their corresponding phase planes. A) Low gain of both resistance (negative feedback) and assistance reflexes (positive feedback). B) Low gain of assistance reflexes and high gain of resistance reflexes. C) Low gain of resistance reflexes and high gain of assistance reflexes. D) High gain of both resistance and assistance reflexes. In neuromechanical simulations, the CB joint (green trace) is raised by levator motor neuron (Lev MN, blue rasters) activity and lowered by depressor motor neuron (Dep MN, red rasters) activity. Blue and red regions of the mathematical trajectories and phase planes indicate Lev MN and Dep MN activity, respectively. Black traces are a graph of $x$ versus time and indicate how the mathematical model evolves through time. Phase planes show the $x$-nullcline (blue curve) and the $y$-nullcline (magenta line) as well as the trajectory of the system (red trace).
resistance reflexes (Fig. 7.7D). This neuromechanical movement of the leg was not reproduced by static nullclines, but could be reproduced if the feedback loop moved the x-nullcline so as to reproduce an attracting fixed point on an external branch of the cubic nullcline and then a spiral point near a knee of the nullcline. While these examples were integrated with static nullclines, they illustrated that the FHN oscillator with feedback could reproduce the generic effects of closing the sensory feedback loop. Whereas the dynamics of the FHN oscillator with feedback were integrated and presented with different sets of parameters, additional system optimization and trajectory calculations are required to determine a set of parameters that can reproduce a continuous range of dynamics similar to the network (Appendix D, Fig. D.3).

7.3.4 Bifurcations Organize System Dynamics

Depending on the types of bifurcations that organize the dynamics of a system, its response to perturbations or other inputs can be different [27, 35, 141]. For example, sensory feedback mediated via reflex can exploit the intrinsic dynamics of a system without changing the underlying qualitative bifurcation structure. This type of feedback has been exploited in robotics where feedback loops reset CPG rhythms to stabilize locomotion [8]. On the other hand, feedback can change the type of underlying bifurcation of a system [40], which can result in different network output as well as changes in response to perturbations or other inputs [122, 138, 141]. Thus, we asked whether sensory feedback changes the qualitative structure of system dynamics in the modified FHN oscillator model.

At the core of the mathematical model, oscillations were organized by the bifurcation parameter, c, which controls a super-critical Andronov-Hopf (Hopf) bifurcation (Fig. 7.8A). A Hopf bifurcation occurs when a stable fixed point yields an unstable fixed point and a stable limit cycle (Fig. 7.4A). As c was increased, the Hopf bifurcation progressed from a stable fixed point (Fig. 7.8A, solid black line at x = 0) to a stable limit cycle (Fig. 7.8A, magenta curve
indicates max and min x values of limit cycle). In order to reproduce the neuromechanical network dynamics, the domain of $c$ was scaled so that the location of the Hopf bifurcation, $c=0.5757$, occurred at a value that was equivalent to 4.5 nA. Thus, the domain of $c$ was [0, 2.0]. When sensory feedback was introduced by changing the parameter, $z$ (Fig. 7.8B), the system produced three activity regimes. When $c$ was 0.563 the nullclines intersected at a stable fixed point and changing the value of $z$ traced the fixed point along the cubic nullcline (Fig. 7.8B, orange box, black curve). For the purpose of illustration a value of $z = 0.073$ was selected so that the stable fixed point was near the origin (Fig. 7.8B, orange arrow). When $c$ was 1.385 the system exhibited oscillations for values of $z$ between -0.5 and 0.5 (Fig. 7.8b, blue box, magenta curve). Using a value of $z = 0.407$ resulted in oscillatory system trajectories (Fig. 7.8B, blue arrow). Finally, when $c$ was 2.299 the system still exhibited an oscillatory regime and selecting a $z$ value outside of the regime resulted in a stable fixed point along an exterior branch of the cubic nullcline (Fig. 7.8B, green arrow). A domain for $z$ was calculated by scaling $z$ so that the bifurcation from a stable limit cycle to a stable fixed point along the exterior branch of the cubic nullcline corresponded to the transition from bursting to tonic spiking of the neuromechanical model. In order to maintain moderate values of $z$ within the oscillatory domain when $c = 1.385$, the domain was also shifted to the left. This resulted in a domain for $z$ of [-1.25, 1.0].

These results showed that the dynamics of the CPG were reproduced by increasing the activation parameter, $c$, and were described by a Hopf bifurcation (Fig. 7.8). Increasing the feedback parameter, $z$, resulted in changing the point of intersection and shifted the operating regime of the system from a stable fixed point near the origin to a limit cycle to a stable
A. $c$-$x$ Bifurcation Diagram for CPG Model

![Bifurcation Diagram for CPG Model](image)

B. $z$-$x$ Bifurcation Diagrams for CPG Model with Feedback

![Bifurcation Diagrams for CPG Model with Feedback](image)

**Figure 7.8. Sensory feedback preserves bifurcations and moves operating point of system**

Bifurcation diagrams show how the output of the mathematical behavior changes as a function of $c$ or $z$. A) Stable (unstable) fixed points are indicated by black solid (dashed) curves. A stable limit cycle is drawn by plotting the maximum and minimum value of oscillation for each value of the bifurcation parameter, $c$ (magenta). Arrows indicate the value of $c$ when the system is quiescent (orange) or bursting (blue and green). The Hopf bifurcation occurs at the point indicated by the red arrow. B) Traces show the trajectory of the system in time and blue (red) regions correspond to Lev (Dep) MN activity. Stable (unstable) fixed points are shown in the bifurcation diagrams as solid (dashed) black curves. The stable limit cycle is shown by the solid magenta curve and illustrates the maximum and minimum $x$-value of oscillations. Arrows indicate the value of $z$ that was used for integration of the quiescent (orange), rhythmically bursting (blue), and tonically spiking (green) states.
fixed point on the upper branch of the cubic nullcline. Together, these results suggest that sensory feedback does not change the dynamics of the network, but, instead, moves the operating point of around bifurcations to produce different behaviors.

The mathematical model with feedback also reproduced more complicated dynamics that were observed when the ratio of the strength of resistance and assistance reflexes was varied independently. For example, the effect when the strength of both resistance and assistance reflexes were low was a prolonged inter-burst duration (Fig. 7.7A, i). This effect was reproduced using the mathematical model by shifting the $x$-nullcline so that the intersection of both nullclines was closer to one of its knee points (Fig. 7.7A, ii) and causing the system dynamics to slow in the region of the knee. The system reproduced the condition of strong resistance reflexes with weak assistance reflexes (Fig. 7.7B, i) when the nullclines intersected near the inflection of a knee along the cubic nullcline. Consequently, the system exhibited sub-threshold oscillations that indicated that the point of intersection of the two nullclines was a spiral fixed point (Fig. 7.7B, iii). When assistance reflexes were strong and resistance reflexes were weak the moved rapidly during Lev MN bursts (Fig. 7.7C, i). This effect was achieved by shifting the $x$-nullcline so that the point of intersection occurred along the negative (Dep) branch. In this case, the system was rapidly drawn cross the y-axis towards the stable fixed point (Fig. 7.7C, ii). Finally, when bursts were followed immediately by tonic spiking generated by strong resistance and assistance reflexes (Fig. 7.7D), network dynamics could be reproduced by chaining the effect of an assistance reflex with the effect of a resistance reflex using dynamic feedback variables.

Whereas the neuromechanical model consisted of sensory feedback that activated reflexes around a CPG of motor neurons, the mathematical model presented here incorporates feedback in a nonlinear oscillator. Together, these results showed that the behavior of a locomotor control circuit could be reproduced by a simplified system of nonlinear ODEs. In
addition, the trajectories shown here suggest that feedback modulates the output of the network by moving the network’s operating point around existing bifurcations rather than by changing the bifurcation structure.

7.4 Discussion

Understanding how sensory feedback controls motor output and the mechanisms through which it may contribute to producing a more robust sensorimotor system are unclear. Using mathematical models and a rationale similar to that applied to nonlinear dynamics of neuronal systems, we showed that a simplified mathematical model has the capability to reproduce the complex behaviors that were observed from simulation results of a locomotor control circuit. The advantage of a simplified mathematical model is two-fold. The first is that a geometric view of system dynamics in a phase plane can help to generate biological hypotheses about how a system is organized. The second advantage is that it establishes a generalizable model that can be used as a starting point to explain phenomenology of other systems.

7.4.1 Reproducing Locomotor Circuit Dynamics

In the circuit studied here, a motor neuron (MN) central pattern generator (CPG) received afferent feedback via resistance and assistance reflex pathways in a neuromechanical model [1, 23]. When activated alone, the CPG transitioned from a quiescent state to a bursting state. When sensory feedback was activated the network exhibited three states: quiescence, rhythmic bursting, and tonic spiking. Varying the strengths of reflexes also produced a range of network behavior that was dependent on the balance between resistance and assistance reflexes. The results from neuromechanical simulations were qualitatively reproduced using the FitzHugh-Nagumo (FHN) [147] nonlinear oscillator with feedback. While there are other models of nonlinear systems that exhibit rhythmic activity (i.e., [39, 145, 150]), the FHN nonlinear
oscillator provided a set of algebraic parameters that could intuitively translate the nullclines in the phase plane and could be interpreted in a biologically meaningful way. Other models simplify dynamics by using sigmoidal or trigonometric nullclines to define an expression for the state variable, which results in non-intuitive translations in the phase plane and their biological interpretation can become convoluted.

A qualitative comparison between the FHN nonlinear oscillator model and simulated results of the locomotor control circuit showed that the simplified mathematical model was capable of reproducing similar dynamics. When the CPG activation parameter, $c$, was activated without sensory feedback the system yielded two states: a stable fixed point and a stable limit cycle that produced oscillations. The stable fixed point occurred near the origin of the phase plane and corresponded to the quiescent regime of the neural network. Oscillations were generated by an Andronov-Hopf bifurcation and corresponded to the rhythmic bursting regime of the locomotor circuit. Reproducing the effect of reflexes by changing the sensory feedback parameter, $z$, resulted in three activity states: stable neutral fixed point, oscillation, and stable non-zero fixed point. In this case, the stable non-zero fixed point corresponded to the tonic spiking regime where the firing rate of Lev MNs is maintained at an elevated level.

While the results shown here were integrated with constant values of feedback, $z$, to illustrate that the system is capable of producing the qualitative features observed from neuromechanical simulations, mathematical model parameters can be further optimized to more accurately reproduce the dynamic phase-dependency of feedback.

The effect of changing the strength of resistance and assistance reflexes was also illustrated. For specific values of the sensory feedback parameter, $z$, certain effects of strong or weak assistance and resistance reflexes were also reproduced (Fig. 7.7). In the reduction of the neuromechanical feedback circuit studied here we illustrated that the system’s behavior was explained by shifting the operating point of a nonlinear oscillator (Fig. 7.8). These results do
not, however, exclude the alternative that the behaviors were produced by changing the nature of the organizing bifurcations. For example, sensory feedback can be used to control the nature of a bifurcation by changing a SNIC to a Hopf [151]. Here, the Hopf bifurcation was conserved when sensory feedback was introduced. Additional integrated trajectories are needed, however, to determine whether feedback control of a bifurcation is also capable of reproducing neuromechanical simulation results. If not, then the results would indicate that sensory feedback acts to change the operating point of the system and does not change the underlying bifurcation structure. If both mechanisms are able to reproduce simulation results, then simulations in which the system is perturbed would be needed to distinguish between the two mechanisms. This approach would require construction of a phase response curve of the neuromechanical system with the CPG alone as well as with different strengths of resistance and assistance reflexes.

7.4.2 Biological Implications and Significance of Mathematical Model

Depending on the bifurcation(s) around which a system is organized the neural network may exhibit very different properties of entrainment or responses to perturbation. A CPG that is organized around a Hopf bifurcation, for example, can respond to a perturbation by either advancing or delaying the next burst [30]. On the other hand, a system organized around a SNIC bifurcation will always delay the next burst [30]. When considering how a CPG is coordinated with other CPGs its organizing dynamics will dictate how readily its bursting rhythm synchronizes or destabilizes relative to other CPGs [33, 122]. Similarly, the organizing bifurcation(s) will determine how a CPG responds to afferent inputs from other proprioceptive sources or generated by external perturbations [33, 122]. Here we showed that the CPG of a locomotor control circuit is organized around a Hopf bifurcation and that sensory feedback shifts the operating point of the system between an oscillatory mode and stable fixed points.
along the cubic nullcline. This would mean that the network is capable of advancing or delaying its output to coordinate with other CPGs or in response to perturbations.

The approach presented here was used to elucidate a mechanism through which sensory feedback changes the behavior of a sensorimotor system. A simplified mathematical model was developed by using results from simulations of a neuromechanical model as benchmarks for qualitative features of a biological system. While the neuromechanical simulations allowed variables to be manipulated that were biologically inaccessible, the mathematical model provided a means through which the dynamics of the system could be described and analyzed. The mechanism of sensorimotor integration identified here using the mathematical model can help to generate hypotheses of how the neuromechanical model are organized, which, in turn, helps to develop testable hypotheses about biological mechanisms. In addition, this approach develops three levels of analysis in parallel in such a way so as to enable similar hypotheses to be developed and tested in other sensorimotor systems.
8 Conclusions

8.1 Difficulties of Understanding Sensorimotor Integration

Sensorimotor networks integrate neural motor activity with biomechanical feedback to produce flexible behaviors that can adapt to changing environments [1, 2, 5]. Due to the intimate relation between the ongoing activity of neural motor networks and biomechanical feedback from sensory receptors it has been difficult to study the role of sensory feedback in modulating or changing network output in posture and behavior. Most studies, for example have focused on understanding how a neuromechanical system responds to perturbations [5, 79]. Experiments in which an in vitro nervous system is coupled to a simulated body, however, have started to provide insights about how a network behaves when sensory feedback is produced by a biomechanical system that is coupled to motor output versus a biomechanical system that is not [50, 78, 111]. The advantage of such an in vitro approach is that the biomechanical feedback loop can be reversibly decoupled and artificial perturbations can still be imposed.

Here, an in vitro brain-machine interface set up was used to run experiments through which the effect of biomechanical feedback from a sensory receptor affected the ongoing activity of a neural motor network. In vitro results were used to build a computational model that provided a means to sample the behavior of the network by varying parameters that were inaccessible biologically. Finally, results from computational simulations were reproduced by a 2-dimensional nonlinear oscillator with feedback and the mathematical model illustrated how sensory feedback can change the output of a sensorimotor circuit without changing the underlying bifurcation structure.
8.2 Results Summary

*In vitro* brain-machine interface experiments showed that biomechanical feedback from sensory receptors caused an increase in the burst frequency of a neural locomotor control circuit. Governing up and down movements of the second joint of a crayfish walking leg, an *in vitro* nerve cord was coupled to a virtual model of a crayfish leg to determine the effect of sensory feedback. The virtual leg was moved up and down around a single joint in real time by extracellularly detected action potentials of levator (Lev) and depressor (Dep) motor neurons (MNs), respectively. Because Lev and Dep MNs can produce rhythmic bursting independently they are considered to be a central pattern generator (CPG). As the virtual leg moved up and down in simulation, a sensory receptor spanning the joint was released and stretched, respectively. One set of experiments was run in which movements of the biomechanical leg model were driven by *in vitro* motor neuron activity and were transduced to the *in vitro* stretch receptor via a probe. In this condition, called “Closed Loop,” sensory feedback to the *in vitro* circuit was derived from ongoing motor activity. In a condition called, “Open Loop,” movements of the virtual leg were not transduced to the *in vitro* stretch receptor and motor activity occurred independent of biomechanical feedback.

8.2.1 *In vitro* Brain Machine Interface Results

Results from *in vitro* brain-machine interface experiments showed that the hybrid set up was capable of reproducing resistance and assistance reflex responses to external perturbations (leg lifts) as well as chained Lev/Dep bursts. The nature of reflexes that the network produced in response to perturbations depended on the behavioral state of the network that could be induced by bath application of a cholinergic agonist, oxotremorine (OXO) [1, 25]. Briefly, application of OXO increased excitation of the MN CPG and increased the strength of assistance reflexes by exciting assistance reflex interneurons (ARINs). The strength
of resistance reflexes, however, decreased as the concentration of OXO increased. As the concentration of OXO increased, primary afferent depolarization interneurons (PADIs) were excited, which presynaptically inhibited resistance reflex pathways [1]. When simulations were run with the MN CPG in a rhythmically bursting state, results indicated that closing the sensory feedback loop by coupling biomechanical feedback to motor output caused the locomotor circuit to produce rhythmic bursting at a higher frequency. This corresponded to an increased rate of levation and depression of the virtual leg and a faster rate of fictive locomotion.

8.2.2 Neuromechanical Model Reproduces In vitro Results

In order to determine how sensory feedback mediated the effect of increasing the burst frequency of the network, a computational model of the circuit was constructed in the neuromechanical simulation software called, AnimatLab [42]. Briefly, the model was a simplified representation of the known biology of the locomotor circuit that governs the second joint of each walking leg [1, 23]. The neuromechanical model included the same biomechanical leg model that was used for in vitro brain-machine interface experiments and also had a neural network comprised of motor neurons, sensory afferents, and interneurons. Lev and Dep MN pools were each composed of one tonic MN, one phasic MN, and one bursting interneuron that represented a larger number of in vitro MNs and interneurons. The in vitro effect of OXO was reproduced by a CPG OXO neuron that excited MNs by inhibiting an outward current that hyperpolarized MNs in a quiescent state.

Resistance reflexes were mediated via tonic and phasic resistance reflex afferents that were sensitive to changes in the position and velocity of joint movements. The phasic resistance reflex afferent was inhibited by a primary afferent depolarization interneuron (PADI), which mediated a presynaptic inhibition of resistance reflex pathways. PADIs were activated by stimulation of PADI OXO neurons, which blocked an outward current that hyperpolarized
PADIs in a quiescent state. Assistance reflexes were mediated by a phasic assistance reflex afferent that excited an assistance reflex interneuron (ARIN). Stimulation of the ARIN OXO neuron excited ARINs by blocking an outward current that kept them hyperpolarized in a quiescent state. Neuromechanical simulations with artificial perturbations to the leg (leg lifts) yielded state-dependent reflex responses that corresponded to in vitro results. Simulation results also reproduced the increased burst frequency when the sensory feedback loop was closed. Finally, the neuromechanical model provided a means through which it was possible to test the hypothesis that the increase in burst frequency was mediated by assistance reflexes. When ARINs were disabled, thus eliminating assistance reflexes, the burst frequency was lower than when ARINs were enabled. These results supported the hypothesis that assistance reflexes mediated the in vitro effect of increasing the network burst frequency.

In addition to a postural state and a fictive locomotion state, results from in vitro brain-machine interface experiments also produced a broad range of behavioral states of chained resistance reflexes as well as bursts that triggered resistance reflex responses. These behavioral states were reproduced by the neuromechanical model and indicated that the network activity was due to different levels of activation of resistance reflexes, assistance reflexes, and the MN CPG. In order to characterize the mechanisms that mediated these effects, it was necessary to run simulations of the neuromechanical network for a large number of parameter combinations. Consequently, it was important to have an analysis method to classify neural activity reliably across different conditions.

8.2.3 Novel Neural Activity Classification Algorithm: Extended Hill-Valley Analysis

Method

Most existing neural activity classification algorithms focus on burst detection and cannot detect bouts of tonic spiking. While burst detection algorithms generally use a distribution
of inter-spike intervals to determine a series of spikes that constitute a burst, it was advantageous to, instead, derive an analysis signal from a spike train for further analysis. The method developed in this dissertation is called the Extended Hill-Valley (EHV) algorithm and used an analysis signal that was a smoothed, history-dependent signal that reflected fluctuations in spike frequency and accentuated differences between firing rates associated with bursting versus those associated with tonic spiking. Events were detected in a spike train using a height-to-width ratio of the analysis signal and the algorithm simultaneously yielded results that included bursts and bouts of tonic spiking. The algorithm was tested on a variety of simulated and in vitro spike trains and qualitatively as well as quantitatively outperformed two other burst detection methods that are commonly used.

8.2.4 Network Behavior is Organized by Sensory Feedback

The EHV method was used to analyze a database of simulation results that helped to elucidate the mechanisms through which sensory feedback changes the output of a crayfish locomotor control circuit. For example, simulation results showed that activation of the MN CPG alone yielded only two activity regimes: quiescence (postural) and rhythmic bursting (fictive locomotion). When the MN CPG, PADIs, and ARINs were activated together, however, a third activity regime was induced for elevated levels of OXO neuron stimulation: exclusive tonic spiking by one MN pool. In order to further dissect the mechanism through which sensory feedback mediated changes to neural motor activity, simulations were run in which the activation of PADIs and ARINs were increased separately with increasing activation of the MN CPG. These results showed that increasing PADI activation with the MN CPG did not significantly contribute to the increased burst frequency, but there were subtle differences in the variability of tonic spiking episodes. This meant that reducing resistance reflexes did not have an effect on network dynamics in the conditions studied here. Simulations in which ARIN
activation was increased with the MN CPG showed that the increased burst frequency and exclusive tonic spiking regime were mediated by ARINs. This was confirmed in simulations where ARINs were disabled and eliminated the effects of interest. In addition, these results showed that the regime of exclusive tonic spiking was mediated by the increased activation of ARINs, which resulted in a higher spontaneous firing rate of ARINs that synapsed directly onto CPG MNs.

8.2.5 *Network Dynamics are Organized by Relative Strengths of Resistance and Assistance Reflexes*

The neuromechanical model and newly developed EHV classification algorithm enabled an analysis of a large database of simulations, which was critical to understanding how the relative strengths of resistance and assistance reflexes contributed to organizing network dynamics. The strength of resistance and assistance reflexes were varied independently under three MN CPG conditions: quiescent CPG, rhythmically bursting CPG, and an exclusively tonic spiking CPG. Simulations in which the MN CPG was quiescent when biomechanical feedback was decoupled from neural motor activity showed that the balance between resistance and assistance reflexes reorganized the dynamics of the network into three activity regimes. Whereas uncoupled simulations produced only spontaneous spiking with short bouts of tonic spiking, closing the biomechanical feedback loop induced bursting when assistance reflexes were strong (elevated activation of ARINs). In addition, a region of tonic spiking coexisted with bursting when the strength of resistance reflexes was also high. The bouts of tonic spiking and bursting were blocked by disabling phasic resistance reflex afferents and bursting was eliminated when phasic assistance reflex afferents were disabled. These results indicated that sensory feedback could induce bursting in a quiescent network that might correspond behaviorally to an animal taking a step to stabilize itself in the case of a strong perturbation.
When the MN CPG produced rhythmic bursting with an open biomechanical feedback loop, the network dynamics were reorganized by sensory feedback in a similar fashion to the quiescent network. In this case, however, the region of bursting and tonic spiking occurred for lower levels of ARIN activation. In this state, the coexistence of bursting and tonic spiking was a result of a combined effect of tonic and phasic resistance reflex afferents and phasic assistance reflex afferents. While the main effect of closing the biomechanical feedback loop with a rhythmically active MN CPG was to increase the burst frequency, which was consistent with in vitro results, it was not clear whether the region of bursting and tonic spiking had biological implications. It may be the case that the region is an artifact of a simplified network and that its presence in a biological system is prevented by factors that are not included in the neuromechanical model [18]. Another explanation may be that these results are based on unperturbed simulations and the biological importance of the region with bursting and tonic spiking may be better understood through perturbation experiments [34]. In either case, the increased frequency of bursting that was mediated by activation of ARINs was consistent with in vitro results and facilitates a rapid transition between stance and swing of a leg during step cycles [2, 23].

Finally, the result from simulations in which the network produced tonic spiking exclusively in one MN pool when the biomechanical feedback loop was open showed that this state was resilient to changes induced by sensory feedback. When the feedback loop was closed few changes occurred to the burst frequency or duration of tonic spiking episodes. This may be the result of a saturation effect in that the burst frequency of the network is at a maximum or that the CPG is activated so strongly that sensory feedback does not have an effect. It was not clear whether this activity state was biologically meaningful.
8.2.6 Nonlinear Oscillator Model with Feedback Reproduces Network Activity

In order to determine how the relative strengths of resistance and assistance reflexes were reorganizing network dynamics, a mathematical model was designed to reproduce simulation results. Based on the FitzHugh-Nagumo (FHN) nonlinear oscillator model, a 2-dimensional phase oscillator was used to reproduce network dynamics so that positive and negative values of the state variable for the mathematical model represented raised and lowered positions of the leg. Because activation of Lev and Dep resistance and assistance reflexes depends on the phase of the step cycle [1, 23] four feedback terms with phase-dependent activation variables were used to reproduce the effect of sensory feedback. Because of the complexities involved with optimizing the system’s parameters for all four feedback terms and the central oscillator, the effect of sensory feedback was, instead, reproduced by moving the x-nullcline of the FHN oscillator up or down in the phase plane using the FHN parameter, $F$ (called $z$ in the original publication [147]). This approach showed that static nullclines could reproduce the range of network activity observed in neuromechanical simulations in a piece-wise manner and was similar to running simulations with biomechanical feedback decoupled from motor activity.

A selection of simulated network activity was used to direct development of the mathematical model. When the feedback in the mathematical model was absent the 2-dimensional oscillator reproduced the quiescent (postural) and oscillating (fictive locomotion) states of the neuromechanical model and in vitro system. Phase planes illustrated how the intersection of $x$- and $y$-nullclines organized the dynamics of how the system behaved and also elucidated a mechanism through which sensory feedback can control network output. When the feedback parameter was low and the activation of the oscillator was low the mathematical model relaxed to the origin in a fashion similar to a quiescent neuromechanical system. When both the feedback parameter and activation of the oscillator were moderate, the mathematical
model produced oscillations. For elevated values of the feedback parameter and oscillator activation, the mathematical model was attracted to a stable fixed point along an exterior branch of the cubic nullcline that corresponded to a raised leg position. As the value of the feedback parameter was increased with the activation of the oscillator, the system was moved around existing bifurcations to produce the observed results. This indicated that the effect of sensory feedback can be mediated by controlling the operating point of the system while leaving the underlying bifurcation structure intact.

8.3 Future Directions

The research presented here shows how a multi-pronged approach was used to determine how sensory feedback changes the output of a neural network that controls movements of a walking leg in crayfish. Future experiments would focus on taking the findings and conclusions from the mathematical model and using them to develop hypotheses that can be tested in a neuromechanical model. Predictions based on the results from both the mathematical model as well as the neuromechanical model can then be used to design and test hypotheses in the in vitro system.

The mathematical model, for example, presents a hypothesis about how sensory feedback organizes network activity around existing bifurcations and can be further developed to capture phase-dependent dynamics of sensory feedback. Namely, the results presented in the Chapter 7 illustrate that it is possible for sensory feedback to change network output without affecting the underlying bifurcations of the locomotor CPG. These results, however, do not exclude the hypothesis that sensory feedback controls network output by changing the underlying bifurcations, such as illustrated with the effect of feedback in [40]. Because the nature of bifurcations in the mathematical model can be used to predict how the neuromechanical model would respond to perturbations [27, 122] (as outlined in Chapter 1,
Introduction), the mathematical models can be validated through computational simulations of the perturbed network. In this case, a change in the underlying bifurcations of the network, such as from a Hopf to a SNIC, would result in a change in how the system responds to perturbations, such as a Type II phase response curve (PRC) to a Type I PRC, respectively. Perturbation simulation results could then be verified through *in vitro* perturbation experiments, as well. Together, this multi-pronged approach provides a structured algorithm that can be used to determine how sensory feedback controls network output. In addition, understanding the underlying dynamical structure of the network also provides insight into how the system would coordinate with other neural or biomechanical systems [34].

Results from the neuromechanical simulation currently focus on an unperturbed system and additional insight may come from a perturbation analysis of the network. Depending on the response of the neuromechanical network to phase-dependent timing of perturbations, simulation results can inform further refinement of the mathematical model. The advantage to measuring the neuromechanical model’s response to perturbations is that the *in vitro* system can also be interrogated using perturbations. By comparing how the *in vitro* preparation and neuromechanical network model respond to perturbations it may become apparent that the network requires additional elements or features to reproduce *in vitro* behaviors. In addition, the perturbation analyses will help to map different states of the *in vitro* preparation on to different levels of CPG activation as well as resistance and assistance reflex activation in the neuromechanical network model. Together, the results of the experiments and simulations presented here have identified a mathematical description of network dynamics that help to elucidate the mechanism through which sensorimotor circuits integrate biomechanical feedback to produce behaviors that can adapt in unpredictable environments.
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10 APPENDICES

10.1 Appendix A

Discrimination of Bursts and Tonic Activity in Neural Spike Trains

Using an Extended Hill-Valley Analysis Method

**Figure A.1. Extended Hill-Valley analysis flow chart detail**

Preliminary signal conditioning steps transform a spike train to a smooth, history-dependent analysis signal. The Extended Hill-Valley method uses a nested, recursive algorithm that classifies activity based on changes in the analysis signal by comparing peaks and troughs. The main criteria used to detect for burst events and tonic spiking events are highlighted by thick outlines and arrows. Because it is not always possible to discriminate between bursts and tonic spiking within a single hill or valley internal flags are used to track which conditions are satisfied for burst (blue) or tonic spiking (orange) criteria. Hexagonal boxes indicate a calculation. Diamonds indicate a Boolean evaluation. Rounded rectangles filled with blue or orange indicate when internal flags for bursts or tonic spiking are set, respectively. Circles indicate when internal flags are evaluated. Rounded rectangles filled with green or red indicate when the criteria are met for either bursting or tonic spiking or when a series of peaks and troughs fail to meet any activity-defining criteria, respectively.
**Figure A.2. Algorithm parameters were optimized using a Jaccard Index**

The Jaccard Index measures the similarity between two sequences of events. The Jaccard Index was calculated for each data set as each parameter was varied individually. Parameter optimization started by sampling each parameter across a large range of values and selecting the best value by finding the highest average peak of Jaccard Indexes. A second optimization step sampled each parameter in a smaller domain around the chosen value from the first stage and with a smaller step size (examples shown here). Asterisks and dashed vertical black line indicate final value for specified parameters.

(a) Changing the value of the Poisson Surprise threshold did not have an effect on algorithm performance and a final value of 0.3 was selected. (b) The convolution width for the Extended Hill-Valley method was 1500 for the final analysis and lower values resulted in reduced algorithm performance for burst detection. (c) Detection of tonic spiking was not as sensitive to the convolution width parameter.
Figure A.3. Performance of classification algorithms on simulated spike trains with a variety of activity patterns

Spike raster plots of simulated neural activity illustrate the range of results from different analysis methods. Burst events are indicated by thick bars above each raster and tonic spiking events are indicated by lines with barbs on either end. The color of bars and lines indicate which classification algorithm was used: Extended Hill-Valley (magenta), Cumulative Moving Average (green), or Poisson Surprise (blue). Each spike train illustrates a different type of neural activity and is labeled according to activity classification by visual analysis. (a) Spontaneous *in vitro* bursting. (b) *In vitro* tonic spiking. (c) *In vitro* ambiguous activity. (d) Simulated rhythmic bursting. (e) Simulated tonic spiking. (f) Simulated tonic spiking and bursting. (g) Simulated transition from quiescence to bursting to tonic spiking. (h) Simulated phasotonic bursting. (i) Simulated multistability with switch from tonic spiking to bursting after perturbation at 160 s. (j) Simulated ambiguous activity.
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Table A.1. Parameter optimization range and step size for burst detection algorithms
10.2 Appendix B

Sensory Feedback Changes Network Dynamics in Simulations of Locomotor Circuit

![Full schematic of locomotor control circuit](image)

**Figure B.1. Full schematic of locomotor control circuit**

A simplified neuromechanical model of a crayfish locomotor circuit that controls the second joint of a walking leg was constructed in AnimatLab (www.animatlab.com). A central pattern generator (CPG) consisted of mutually inhibitory tonic and phasic levator (Lev) and depressor (Dep) motor neurons (MN) as well as interneurons. Lev and Dep MNs raised and lowered a biomechanical leg, respectively. Up and down movements of the virtual leg released and stretched a sensory receptor that activated release- and stretch-sensitive afferents, respectively. Afferents mediated resistance and assistance reflex responses to leg perturbations. Assistance reflexes were mediated by assistance reflex interneurons (ARINs). Resistance reflexes were blocked by primary afferent depolarization interneurons (PADIs). The strength of resistance and assistance reflexes were controlled by PADI OXO and ARIN OXO neurons via disinhibition. The behavioral state of the MN CPG was controlled by a CPG OXO neuron.
Figure B.2. Effect of increasing tonic stimulus to CPG alone without monosynaptic resistance reflexes

The average burst frequency and the duration of bouts of tonic spiking were calculated for the network as the value of CPG OXO stimulus was increased while PADI OXO and ARIN OXO was 0.0 nA. Simulations were run when movements of the leg were not coupled to motor output (dashed magenta line) and when movements of the leg were driven by motor output (dashed cyan line). Simulations were also run in which movements of the virtual leg were driven by motor output and tonic resistance reflexes were disabled (solid cyan line). Shaded regions indicate the standard error of the mean.
Figure B.3. Differential effect of sensory feedback when ARINs are disabled in open loop
The average burst frequency and duration of tonic spiking events were calculated for the network under different simulation conditions. All simulations were run where movements of the virtual leg were decoupled from motor output and moved only due to its own weight under gravity. Simulations were run in three conditions: 1) activation of the CPG OXO neuron only (dashed red line); 2) activation of the CPG OXO and ARIN OXO neurons together (dashed blue line); and, 3) activation of the CPG OXO and ARIN OXO neurons with assistance reflexes blocked by disabling assistance reflex interneurons (ARINs). Shaded regions indicate the standard error of the mean.
A. High CPG OXO Activation

B. High CPG OXO and ARIN OXO Activation

C. Activation of PADI OXO neuron with No Feedback and Disabled Afferents

Figure B.4. Effective of negative feedback and resistance reflex afferents

The average burst frequency and duration of bouts of tonic spiking were calculated for increasing activation of PADIs, which decreases the strength of resistance reflexes. A) CPG OXO activation was high (12.5 nA). B) CPG OXO and ARIN OXO activation was high (12.5 nA). Simulations were run when movements of the leg were driven by motor output (Feedback, cyan) and when movements were decoupled from motor activity (No Feedback, magenta). C) Simulations were run in several conditions: when only the CPG activation was increased (dashed red line); when CPG and PADI activation was increased (dashed green line); when the CPG and PADIs were activated while tonic resistance reflexes were disabled (solid cyan line); when the CPG and PADIs were activated while phasic resistance reflexes were disabled (solid magenta line); and, when CPG and PADIs were activated while both tonic and phasic afferents were disabled. Shaded regions indicate the standard error of the mean.
A. High CPG OXO Activation Only

B. High CPG OXO and ARIN OXO Activation

Figure B.5. Effect of positive feedback on network dynamics
Average burst frequency and average duration of tonic spiking events as activation of ARINs was increased. A) Only activation of the CPG was high (12.5 nA). B) Activation of the CPG and ARINs were both high (12.5 nA). Simulations were run when the biomechanical leg model was driven by motor output (Feedback, cyan) and when movements were decoupled from motor activity (No Feedback, magenta). Shaded regions indicate the standard error of the mean.
Network Dynamics are Reorganized by Positive and Negative Feedback in Simulations of Locomotor Circuit

Figure C.1. Detailed network schematic
The crayfish locomotor control circuit consists of a motor neuron central pattern generator, sensory afferents, interneurons, and a biomechanical leg model. Levator motor neurons (Lev MN, blue) raise the leg and depressor motor neurons (Dep MN, red) lower the leg. Stretch-sensitive afferents (gold) can trigger Lev resistance reflexes or Dep assistance reflexes. Release-sensitive afferents (green) trigger Dep resistance reflexes or Lev assistance reflexes. Interneurons mediate phase-dependent reflex gating and reversal. Pharmacological effects of a cholinergic agonist, oxotremorine (OXO), are emulated using three pairs of interneurons (black and white). The OXO CPG neuron controls the level of activation of the motor neuron CPG elements. The OXO PADI neuron controls the strength of resistance reflexes and the OXO ARIN neuron controls the strength of assistance reflexes.
a. Fictive Locomotion

b. Resistance Reflexes

Figure C.2. Raster plots of *in vitro* locomotor circuit activity

*In vitro* recordings of motor neurons show how a locomotor circuit behaves during fictive locomotion (a) and in response to an artificial sinusoidal stimulus (b). Levator motor neurons (blue rasters) raise the leg and depressor motor neurons (red rasters) lower the leg as indicated by the joint position (green trace). a) When sensory feedback depended on the weight of the virtual leg as well as *in vitro* motor neuron activity (black bar) the network produced rhythmic bursting that corresponded to fictive locomotion. b) When movements of the leg were controlled artificially, a sinusoidal stimulus triggered strong resistance reflexes. As the leg was lowered phasic levator motor neurons (blue rasters) fired and as the leg was raised phasic depressor motor neurons (red rasters) fired.
a. Tonic Resistance Reflex Afferents Disabled

b. Phasic Resistance Reflex Afferents Disabled
c. Phasic Assistance Reflex Afferents Disabled

Figure C.3. Effect of afferents on quiescent network dynamics
Simulations were run when the network was in a quiescent state and when part of the resistance or assistance reflex pathways was blocked. Plots show how the duration of tonic spiking, frequency of tonic spiking, and frequency of bursting depended on the levels of positive and negative feedback. Simulations were run in one of two sensory feedback conditions: 1) No Feedback: movements of the leg were only due to its weight under gravity and motor neuron activity did not have an effect; and, 2) Feedback: movements of the leg were due to its weight under gravity as well as motor neuron activity. Axes are scaled from 0% to 100% based on the maximum feedback gain values as described in Methods. a) Part of the resistance reflex pathway was blocked by disabling tonic afferents (Stretch Afferent and Release Afferent) during simulations. b) Part of the resistance reflex pathway was blocked during simulations by disabling phasic afferents (Stretch Rate Resist Afferent and Release Rate Resist Afferent). c) The assistance reflex pathway was blocked during simulations by disabling phasic afferents (Stretch Rate Assist Afferent and Release Rate Assist Afferent).
a. Tonic Resistance Reflex Afferents Disabled

b. Phasic Resistance Reflex Afferents Disabled
c. Phasic Assistance Reflex Afferents Disabled

**Figure C.4. Effect of afferents on bursting network**

When the network was in a rhythmically bursting state blocking part of the resistance or assistance reflex pathways changed how the dynamics of the network were organized. Activity maps indicate the duration of tonic spiking episodes, the frequency of tonic spiking, and the frequency of bursting in one of two conditions: 1) No Feedback: movements of the leg were only due to its weight under gravity and motor neurons did not have an effect; and, 2) Feedback: movements of the leg were due to its weight under gravity as well as motor neuron activity. Axes were scaled from 0% to 100% based on the maximum level of feedback gain that was sampled as described in Methods. a) Part of the resistance reflex pathway was blocked by disabling tonic afferents (Stretch Afferent and Release Afferent). b) Part of the resistance reflex pathway was blocked by disabling phasic afferents (Stretch Rate Resist Afferent and Release Rate Resist Afferent). c) The assistance reflex pathway was blocked by disabling phasic afferents (Stretch Rate Assist Afferent and Release Rate Assist Afferent).
a. Tonic Resistance Reflex Afferents Disabled

b. Phasic Resistance Reflex Afferents Disabled
c. Phasic Assistance Reflex Afferents Disabled

Figure C.5. Effect of afferents on tonic spiking network
Activity maps show how the network behaved differently depending on the ratio of positive and negative feedback gain. Plots show the duration of tonic spiking episodes, the frequency of tonic spiking, and the frequency of bursting during simulations under different feedback conditions. In addition to different ratios of positive and negative feedback, simulations were run in two conditions: 1) No Feedback: movements of the leg were due to gravity only and did not depend on motor neuron activity; and, 2) Feedback: movements of the leg were driven by motor neuron activity and gravity. Axes are scaled from 0% to 100% based on the minimum and maximum value of sampled gain levels as described in Methods. a) Tonic afferents (Stretch Afferent and Release Afferent) were disabled, which blocked part of the resistance reflex pathway. b) Phasic afferents (Stretch Rate Resist Afferent and Release Rate Resist Afferent) were disabled and blocked part of the resistance reflex pathway. c) The assistance reflex pathway was blocked by disabling phasic afferents (Stretch Rate Assist Afferent and Release Rate Assist Afferent).
10.4 Appendix D

Neuromechanical Simulation Results Are Reproduced by Nonlinear Oscillator Model with Sensory Feedback

Figure D.1. Measuring network performance in a single state variable
A) Using joint position (green trace) the activity of a locomotor control circuit is reflected in a single variable. Levator (Lev, blue) motor neurons (MN) and depressor (Dep, red) MNs activate muscles that move the leg up and down, respectively. System dynamics including both neural sensorimotor integration and biomechanical movement and feedback are captured by the leg position (green trace) B) Using a motor neuron firing rate approximation, activity of the
a. Joint position

b. Firing rate convolution

Figure D.2. Measuring network performance in a single state variable
A) Using joint position (green trace) the activity of a locomotor control circuit is reflected in a single variable. Levator (Lev, blue) motor neurons (MN) and depressor (Dep, red) MNs activate muscles that move the leg up and down, respectively. System dynamics including both neural sensorimotor integration and biomechanical movement and feedback are captured by the leg position (green trace) B) Using a motor neuron firing rate approximation, activity of the locomotor control circuit can be tracked. Lev MN spikes are convolved with an exponential decay function and the resulting continuous signal from the Lev IN, Lev Tonic MNs, and Lev Phasic MNs are summed into a single trace (blue trace). A similar convolution and summation is calculated for the Dep MN pool (red trace). The Dep signal is then subtracted from the Lev signal to produce a final trace that is positive when Lev MNs are active and negative when Dep MNs are active (Thick green trace, second plot).
a. FitzHugh-Nagumo Model with Feedback Gain Coefficient of 0.0

b. FitzHugh-Nagumo Model with Feedback Gain Coefficient of 1.0
Figure D.3. Use of visualization of system dynamics and gated reflexes to optimize system parameters
A graphical user interface was developed to visualize the system trajectory in the FitzHugh-Nagumo phase plane as well as the activation of reflexes and their effect. Reflex activation curves (green sigmoids) are shown in the top eight plots to the right (left to right: Lev assistance reflex, Lev resistance reflex, Dep assistance reflex, Dep resistance reflex). The net activation (green bar) along with the net effect (blue bar, not visible) of each reflex is shown in the four bar plots below. The phase plane (left) illustrates where the $x$-nullcline (cyan curve) and $y$-nullcline (magenta curve) intersect and plots the state point (black point). A trace of the state point of the system is plotted in the phase plane (red trajectory) as well as on a time axis (bottom, green trace) to illustrate how the system behaves. a) When the feedback gain coefficient is 0, there is no effect of feedback on the nullclines in the phase plane. Dynamics are integrated with effectively “static” nullclines. b) When the feedback gain coefficient is 1, the effect of feedback causes the cubic $x$-nullcline to shift vertically, which changes how the system behaves. Note that the cubic nullcline (cyan) is translated vertically by the effect of sensory feedback in this case and the limit cycle trajectory (red) does not trace the nullcline.
### 10.4.1 FitzHugh-Nagumo Relaxation oscillator

\[
\frac{dx}{dt} = c \left( y + x - \frac{x^3}{3} + z \right)
\]
\[
\frac{dy}{dt} = -\frac{(x - a + by)}{c}
\]

- \(x\) State variable, Lev and Dep activity
- \(y\) Variable, swing and stance phase
- \(a\) Constant, vertical translation of linear \(y\)-nullcline
- \(b\) Constant, slope of linear \(y\)-nullcline
- \(c\) Constant, time scale separation of slow- and fast-subsystems (bifurcation parameter)
- \(z\) Constant, vertical translation of cubic \(x\)-nullcline (bifurcation parameter)

### 10.4.2 Sigmoidal Reflex Activation Functions

\[
F_i(x, y) = K_{\text{max},i} \cdot \varphi_i(x) \cdot \omega_i(y) \cdot (x - \theta_i)
\]

\[
\phi_i = \frac{\varphi_{i,\infty}(x) - \varphi_i}{\tau_{\varphi,i}}
\]

\[
\varphi_{i,\infty} = \frac{1}{1 + e^{-A(x-B)}}
\]

\[
\omega_i = \frac{\omega_{i,\infty}(y) - \omega_i}{\tau_{\omega,i}}
\]

\[
\omega_{i,\infty} = \frac{1}{1 + e^{-A(x-B)}}
\]

- \(K_{\text{max},i}\) Maximum reflex effect
- \(\theta_i\) Equilibrium position of reflex
- \(\varphi_i\) \(x\)-activation function
- \(\omega_i\) \(y\)-activation function