NEUROMODULATION OF INHIBITORY FEEDBACK TO PACEMAKER NEURONS AND ITS CONSEQUENT ROLE IN STABILIZING THE OUTPUT OF THE NEURONAL NETWORK

by Shunbing Zhao

A dissertation submitted to the

Graduate School-Newark

Rutgers, The State University of New Jersey

in partial fulfillment of requirements

for the degree of

Doctor of Philosophy

Graduate Program in Biology

Written under the direction of

Professor Farzan Nadim

and approved by

_____

_____

_____

_____

Newark, New Jersey

October 2009

ABSTRACT OF THE DISSERTATION

Neuromodulation of Inhibitory Feedback to Pacemaker Neurons and Its Consequent Role in Stabilizing the Neuronal Network

By Shunbing Zhao

Thesis Advisor: Dr. Farzan Nadim

Stable oscillations can be important for the proper function of neuronal networks. Rhythmic movements, for example, often rely on stable input from central pattern generator (CPG) networks that generate the underlying oscillations. I used the crustacean pyloric motor network as a model oscillatory neural system. The primary goal is to characterize the effects of the neuropeptide proctolin on the LP to PD synapse and consequently to investigate functional role in shaping the network output.

First, I characterized the effects of proctolin on both the spike-mediated and graded components of the LP to PD synapse. The results showed that both components of the LP to PD synapse were enhanced by bath-applied proctolin. The results also showed that proctolin caused facilitation of the LP to PD synapse with injection of low amplitude depolarization steps. This facilitation is associated with a slow inward Ca^{2+} like current.

Second, I investigated the function of the LP to PD synapse in the pyloric network. The results showed that the LP to PD synapse reduced the variability in

the pyloric period. Also, analysis of the phase response curve (PRC) showed that the LP to PD synapse reduced the effect of perturbations. We used synaptic-PRC and its relationship with synaptic phase and synaptic duty cycle to explain how the LP to PD synapse counteracts the effect of perturbation.

Third, I examined the role of proctolin in shaping the neural network output. It was found that in the presence of proctolin the variability of pyloric period was reduced. Furthermore, using PRC analysis, I demonstrated that proctolin reduced the effect of extrinsic perturbations on the pacemaker neurons in the presence of LP to PD synapse. The results suggest that proctolin, through its enhancement on the LP to PD synapse, plays an active role in stabilizing the pyloric network oscillation.

Our findings suggest that modulations of the inhibitory feedback synapse can be a useful approach to regulate the stability of neuronal networks. Insights gained from this thesis could be applied to mammalian nervous system such as feedback or recurrent inhibitory circuits in cortex or oscillator-driven respiratory CPGs.

Acknowledgement

First and foremost, I am heartily thankful to my advisor Dr. Farzan Nadim for being the greatest advisor and mentor. Without his stimulating support, guidance and encouragement I would never have finished. I would also like to thank my committee members Dr. Jorge Golowasch, Dr. Amitabha Bose and Dr. Christina Mouser for their helpful advice and comments.

Next, I would like to thank all of the members in the Nadim and Golowasch labs. I learned a lot from them.

Last but definitely not least, I would like to give my special thanks to my family. I especially thank my wife Yangyang for her patient love I also thank my parents for their love and support throughout my life. My family, especially my sons Grant and Nathan has always been the inspiration and motivation in my life.

Table of Contents

Abstract iii

Acknowledgement v

Table of contents vi

List of figures viii

Chapter 1: General Introduction 1

Background and significance 1

Overview of the stomatogastric nervous system 5

The pyloric circuit 6

The advantages of the pyloric circuit 8

Neuromodulation in STNS and Neuropeptide proctolin 9

Questions addressed in the thesis 11

Chapter 2: Neuromodulation of a key synapse in a rhythmic network 15

Introduction 15

Materials and Methods 18

Results 21

Discussion 32

Chapter 3: Inhibitory feedback to pacemaker neurons stabilizes oscillation in the rhythmic network 54

Introduction 54

Materials and Methods 57

Results 60
Discussion 68

Chapter 4: The role of neuromodulation in promoting stability of pacemaker neurons rhythm period.....81
Introduction.....81
Materials and Methods 83
Results 84
Discussion 88

Chapter 5: General Discussion.....96

References 107

List of Figures and Tables

Figures

Chapter 1

Figure 1.1 13

Chapter 2

Figure 2.1 36

Figure 2.2 37

Figure 2.3 38

Figure 2.4 40

Figure 2.5 42

Figure 2.6 43

Figure 2.7 45

Figure 2.8 47

Figure 2.9 49

Figure 2.10 51

Figure 2.11 53

Chapter 3

Figure 3.1 71

Figure 3.2 72

Figure 3.3 74

Figure 3.4 76

Figure 3.5 78

Figure 3.6 80

Chapter 4

Figure 4.1 90

Figure 4.2 92

Figure 4.3 93

Figure 4.4 94

Chapter 1

General Introduction

Background and Significance

Oscillatory activities are widespread in the nervous system (Basar, 2008) and are involved in circadian activity (van Esseveldt et al., 2000), sleep and arousal (McCormick and Bal, 1997), learning (Lisman, 1997), and motor pattern generation (Marder and Calabrese, 1996). Oscillations are also implicated in some neurological diseases such as Parkinson's and epilepsy (Wong et al., 1986; Galvan and Wichmann, 2008). Due to the widespread significance of oscillatory activities in a variety of behaviors and pathological conditions, it is important to understand how oscillations are generated, and what regulates and maintains their output patterns. Much of our understanding of neural oscillation comes from central pattern generators (CPGs). Understanding CPG circuits has and will aid in uncovering the principle and mechanisms involved in oscillatory neuronal circuit operation.

CPGs are networks of neurons that produce rhythmic motor behavior such as flying, breathing, walking, and swimming in the absence of rhythmic sensory or central input (Hooper, 2000). The study of CPGs has provided a neuronal basis for understanding the generation and regulation of these rhythmic activities. CPGs must be able to produce stable patterns in order to maintain the rhythmic movement in response to the changes in environment. The activity pattern of

CPGs is regulated not only by the intrinsic properties of neurons, but also by the strength and dynamics of the synapse between these neurons (Marder and Calabrese, 1996). Studies have shown that synaptic dynamics can have important functional consequences at the network level, such as motor pattern selection (Combes et al., 1999), synchronization (Tsodyks et al., 2000) and bistability (Manor and Nadim, 2001) in recurrent networks, phase maintenance (Bose et al., 2004) and network stability in hippocampus (Deeg, 2009). CPG operation fundamentally depends on the neuromodulators and synapses within the CPG circuit which are subject to vast neuromodulation (Marder et al., 2005; Dickinson, 2006). Therefore, characterizing the effects of neuromodulators on the synaptic dynamic will provide insight into understanding the operation of CPGs. The main goal of this thesis is to characterize the effects of a neuropeptide on an inhibitory feedback synapse to pacemaker neurons in a well-characterized CPG, and consequently elucidate the functional role of this synapse in maintaining the stability of the CPG oscillations.

Inhibitory synapses are dominant in most known CPGs such as leech heartbeat (Calabrese and Peterson, 1983), stomatogastric ganglion (Marder and Calabrese, 1996) and mammalian locomotion (McCrea and Rybak, 2008). Several studies have shown that the inhibitory synapses are involved in maintaining the stability of neural activity (Elson et al., 1999; Mamiya and Nadim, 2004; Ozeki et al., 2009; Sieling et al., 2009). In many CPG circuits, it is well known that inhibitory synapses from rhythm-generating neurons or sub-network

of neurons are used to set the relative phases of the follower neurons within the CPG (Tryba and Ritzmann, 2000; Nusbaum and Beenhakker, 2002; Norris et al., 2006; Norris et al., 2007). However, the functional role of inhibitory feedback synapses from follower neurons back to the rhythm-generating neurons remains unclear. In this thesis, I will examine the hypothesis that the presence of such inhibitory synapses generically acts to stabilize the network oscillations in response to both fast and slow perturbations.

Neuromodulators such as monoamines and peptides play a critical role in determining the output pattern of the neural network activity by modifying the intrinsic properties of neurons or strength and dynamics of the synapse between these neurons, thereby having an important role in achieving both a stable and a flexible operation (Katz and Harris-Warrick, 1990b; Marder and Thirumalai, 2002; Nusbaum and Beenhakker, 2002). In this thesis, after exploring the effects of the neuromodulator of our interest on the inhibitory feedback synapse, I will examine the consequences of neuromodulation of this synapse in reshaping neuronal output.

It has been found that both invertebrate and vertebrate CPGs operate with a similar set of general principles (Marder and Calabrese, 1996). Thus, findings from studies of relatively smaller, more accessible invertebrate CPGs can be translated to the rather larger, vertebrate CPGs. Several invertebrate CPGs have been extensively studied such as feeding in mollusks, heartbeat in leeches and

locomotion in leeches, mollusks and crustaceans (Nusbaum and Beenhakker, 2002). The stomatogastric nervous system (STNS), with its relatively simpler nervous system, has become an invaluable model system for the study of neural network operations. More than 20 different neuromodulators have been identified in the STNS (Marder et al., 2005; Marder and Bucher, 2007). Thus, the STNS provides a very useful model for the investigation of neuromodulation of the neural network.

This thesis aims to examine the neuromodulation and functional role of the inhibitory synapse from the lateral pyloric (LP) neuron to the pyloric dilator (PD) neurons in the crustacean pyloric network. The LP to PD synapse is the only chemical synaptic feedback to the pacemaker group (AB/PD neurons) of the pyloric circuit. However, modulation of the dynamics of this synapse and its potential mechanism remains largely unknown. In chapter 2, I will examine the effects of the neuropeptide proctolin on the strength and dynamics of two different components, spike-mediated and graded, of the LP to PD synapse, as well as the mechanisms underlying the neuromodulation of this synapse. This key synaptic feedback plays a very important role in regulating pyloric rhythm. However, its role in the generation and coordination of the pyloric rhythm is unknown. In chapter 3, I will investigate the functional role of the LP to PD synapse in the pyloric network. In chapter 4, I address the role of proctolin in shaping the neural network output. The thesis concludes with a brief discussion in chapter 5.

Overview of the stomatogastric nervous system

The STNS controls the rhythmic crustacean foregut movement responsible for feeding (Johnson and Hooper, 1992). This foregut can be subdivided into four compartments by their function in the feeding process: the esophagus (swallowing), the cardiac sac (food storage), the gastric mill (chewing) and the pylorus (filtering) (Nusbaum and Beenhakker, 2002). The STNS is comprised of four ganglia: the paired commissural ganglia (CoG), the esophageal ganglion (OG) and the stomatogastric ganglion (STG) and their related nerves (Figure. 1A). The pyloric and gastric mill rhythms are generated in the stomatogastric ganglion (STG). The esophageal ganglion (OG, ~ 15 neurons) contains the motor network for the cardiac sac rhythm and some modulatory projection neurons that regulate the activities of the STG. The paired commissural ganglia (CoGs) contain about 550 neurons each. They receive and integrate sensory input from the foregut and provide neuromodulators to generate and coordinate the motor pattern of the STG. The best understood CPGs in this system are the pyloric circuit and gastric circuit, which drive the rhythmic movement of the pylorus and gastric mill, respectively, by innervating their corresponding striated muscles (Selverston, 1974; Selverston and Mulloney, 1974; Maynard and Selverston, 1975).

The pyloric circuit

In the crab *Cancer borealis*, the pyloric rhythm controls the rhythmic muscle contraction in the pylorus, which is the part of the stomach that filters food (Marder and Calabrese, 1996). The pyloric rhythm is generated by the pyloric circuit which is composed of 11 neurons: the anterior burster (AB), two pyloric dilators (PDs), the ventricular dilator (VD), the inferior cardiac (IC), the lateral pyloric (LP) and five pyloric constrictors (PYs). The pyloric rhythm is a tri-phasic rhythm that is composed of an alternate bursting of neurons. During this process, the PD and VD neurons are responsible for dilation, while LP, PY and IC neurons are responsible for constriction (Marder and Calabrese, 1996). In general, the cycling frequency of the pyloric rhythm ranges from 0.5 to 2 Hz and can maintain a similar tri-phasic rhythm over this range (Weimann et al., 1991; Weimann and Marder, 1992; Bucher et al., 2005). Figure 1 B shows a schematic drawing of the pyloric circuit. The AB neuron is the only interneuron in the pyloric network. It is electrically coupled to two PD neurons. These three neurons burst synchronously and are referred as the pyloric pacemaker group. These pacemaker neurons directly inhibit all other pyloric neurons (Eisen and Marder, 1982; Miller and Selverston, 1982a, b). Each burst of impulses in AB/PD pacemaker group is referred to as the beginning of a new cycle of the pyloric rhythm. After the pacemaker group stops the burst, the LP and IC neurons are the first to rebound from pacemaker inhibition. During the LP bursting, the PY neurons are inhibited. When PY starts bursting, it terminates the LP burst. The PY burst is then

terminated by the next pacemaker burst. The IC neuron is coactive in the same phase as the LP neuron, and VD neuron bursts are in the same phase as the PY neuron. The VD neuron does not depolarize with the AB/PD group although it is electrically coupled to pacemaker group (Figure 1B) because the AB neuron strongly inhibits it (Eisen and Marder, 1982). Action potentials from pyloric neurons can be recorded extracellularly on the lateral ventricular nerve (*lvn*), the medial ventricular nerve (*mvn*), the pyloric constrictor nerve (*pyn*), the lateral pyloric nerve (*lpn*) and pyloric dilator nerve (*pdn*). In Figure 1C, the recordings on the *lvn*, *lpn* and *pdn* are shown.

The neurons in the pyloric circuit are connected through chemical synapses and electrical synapses (coupling). Electrical synapses in the STG are realized as gap junctions between the neurons (Marder and Eisen, 1984b). It has been shown that electrical coupling plays a role in chemical synaptic efficacy (Rabbah et al., 2005). All the chemical synapses in the pyloric circuit are glutamergic except the synapses from PD and VD, which are cholinergic (Marder, 1976). These chemical connections are all inhibitory and consist of both spike-mediated and graded components (Mulloney, 1987). Spike-mediated transmission refers to the synaptic release evoked by action potentials. Graded synaptic transmission is named because the amount of transmitter release is a graded function of the presynaptic membrane potential. Although graded synapses are believed to provide the major portion of the synaptic transmission in the STG (Graubard et al., 1980), the pyloric network output is passed to the muscles and to other

ganglia by action potentials. Between the pyloric neurons, there are several chemical synaptic connections including AB/PD to LP, AB/PD to PY, PY to LP, and the LP to PD. Although we treat AB/PD as pacemaker group, AB and PD neurons exhibit distinct short-term dynamics and contribute differently to the total synaptic output (Rabbah and Nadim, 2007). The LP to PD synapse is the only chemical synaptic feedback to the pyloric pacemaker group. It has been shown that it plays an important role in regulating the pyloric rhythm (Mamiya and Nadim, 2004). In this thesis, I will focus on this feedback synapse.

The advantages of the pyloric circuit

The main goal of this thesis is to elucidate the functional significance of the feedback synapse to pacemaker and to investigate its neuromodulation in a CPG neural network. The pyloric network of the STG has been used as an ideal model system for the study of rhythmic motor pattern generation and neuromodulation (Marder and Calabrese, 1996). This is due to several reasons: First, the STNS containing pyloric network can be dissected out of the animal. The pyloric network is able to maintain the same pattern *in vitro* as it generates *in vivo* (Selverston, 1974; Selverston et al., 1976; Gutierrez and Grashow, 2009). Second, there is a relatively small number of neurons in the pyloric circuit. All the synaptic connections between the neurons and intrinsic properties of these neurons are known. Their basic functional properties such as neurotransmitter, types of transmission, reversal potentials are acknowledged (Maynard and

Selverston, 1975; Hartline and Gassie, 1979; Eisen and Marder, 1982; Marder and Eisen, 1984a, b). Third, the neurons in the STG have large diameters, which make it possible for long and simultaneous recordings from several neurons. Finally, the pyloric circuit is subject to extensive neuromodulation that can be controlled *in vitro* (Marder and Thirumalai, 2002).

Because of the characteristics listed above, it is practical for us to investigate a specific synapse in a controlled manner. We can study its dynamics, neuromodulation and consequent function in shaping the network pattern.

Neuromodulation in STNS and Neuropeptide proctolin

The stomatogastric nervous system of the crab Cancer borealis is subject to extensive neuromodulation. The pyloric network in the STG receives the neuromodulatory inputs from the two CoGs and the single OG via the *stn*. Over 20 different neuromodulators have been discovered in the STNS (Marder et al., 2005; Marder and Bucher, 2007). Neuromodulators maintain and modulate the pyloric rhythm by altering the intrinsic properties and their synapses (Marder and Thirumalai, 2002). Several of these neuromodulators were shown to elicit different patterns of the pyloric rhythm (Marder and Thirumalai, 2002). Allatostatin was found within the STNS of a crustacean species and has inhibitory actions on the pyloric network of the crab *C. borealis* (Skiebe and Schneider, 1994; Saideman et al., 2006). RPCH has little effect on pyloric cycle frequency, although it enhances the LP to PD synapse (Thirumalai et al., 2006).

In this thesis, I will focus on the neuromodulatory effects of a single, well characterized neuropeptide, proctolin. Proctolin is a pentapeptide, L-Arginiyl-L-Tyrosyl-L-Leucyl-L-Prolyl-L-Threonin (RYLPT), that was isolated and structurally characterized in 1975 from whole body extracts of the American cockroach *Periplaneta Americana* (Starratt and Brown, 1975). It was found that proctolin is widely distributed in many insects (Orchard et al., 1989). The neuropeptide proctolin was also found in the crab stamatogastric nervous system and has strong effects on neuronal networks within STNS (Nusbaum et al., 2001). Several neurons have been found to release proctolin. Three pairs of proctolin-containing interneurons send inputs to the STG via the *stn*. One of them is the modulatory projection neuron (MPN), which also contains GABA (Blitz et al., 1999). The other two pairs consist of modulatory commisural neuron 1 (MCN1) and modulatory commisural neuron 7 (MCN7) (Blitz et al., 1999).

The mechanisms of proctolin effects have been studied in insect muscle contractions. Proctolin increases intracellular Ca^{2+} concentration through the modulation of voltage-dependent or voltage-independent channels (Baines et al., 1990; Wegener and Nassel, 2000). Proctolin has been extensively studied in stomatogastric ganglion of many decapod crustaceans (Marder et al., 1986). Proctolin strongly excites the STG (Hooper and Marder, 1984; Marder et al., 1986), and the actions of different proctolinergic modulatory projection neurons have been studied at the neuronal network level (Nusbaum, 2002). The effects of proctolin on the intrinsic properties of several STG neurons have been

characterized (Golowasch and Marder, 1992; Swensen and Marder, 2000). Proctolin causes a depolarization in several STG neurons at membrane potential close to the threshold for action potential (Golowasch and Marder, 1992).

Questions addressed in the thesis

In this thesis, I focus on the LP to PD synapse of the pyloric circuit of the stomatogastric nervous system of the crab *Cancer borealis*. Using this synapse, I investigate how feedback synaptic inputs to the pacemaker affect the output of the oscillatory neuronal networks. Also, the neuromodulation of synaptic transmission plays an important role in determining synaptic dynamics. Hence, exploration of neuromodulation of synaptic dynamics and the mechanism underlying neuromodulation will contribute to understanding the effects of neuromodulators in shaping neuronal network output. In this thesis, the following specific questions are addressed:

Chapter 2:

What are the effects of proctolin on the strength and dynamics of the inhibitory synapse from the lateral pyloric (LP) neuron to the pyloric dilator (PD) neurons, and what are the mechanisms underlying the neuromodulatory effects of proctolin? The synapse from the LP to the PD neuron has both a graded and a spike-mediated component. I examined the effect of proctolin on the strength and dynamics of the two components of LP to PD synapse. I explored how proctolin changes the strength and dynamics of these two components.

Chapter 3:

What is the role of the LP to PD synapse in the pyloric network? Although this synapse has been extensively studied, its role in the generation and coordination of the pyloric rhythm is not completely known. In response to perturbation, the pyloric network must be able to produce stable patterns to maintain rhythmic movement. I tested the hypothesis that this synapse stabilizes the oscillation of the pyloric network by reducing the variability in cycle period.

Chapter 4:

How does the modulation of the feedback synapse by proctolin affect the neural network output? Based on the results from chapter 2 and chapter 3, I investigated how the feedback of inhibitory synapse shapes the network output when the properties of this synapse are altered by proctolin.

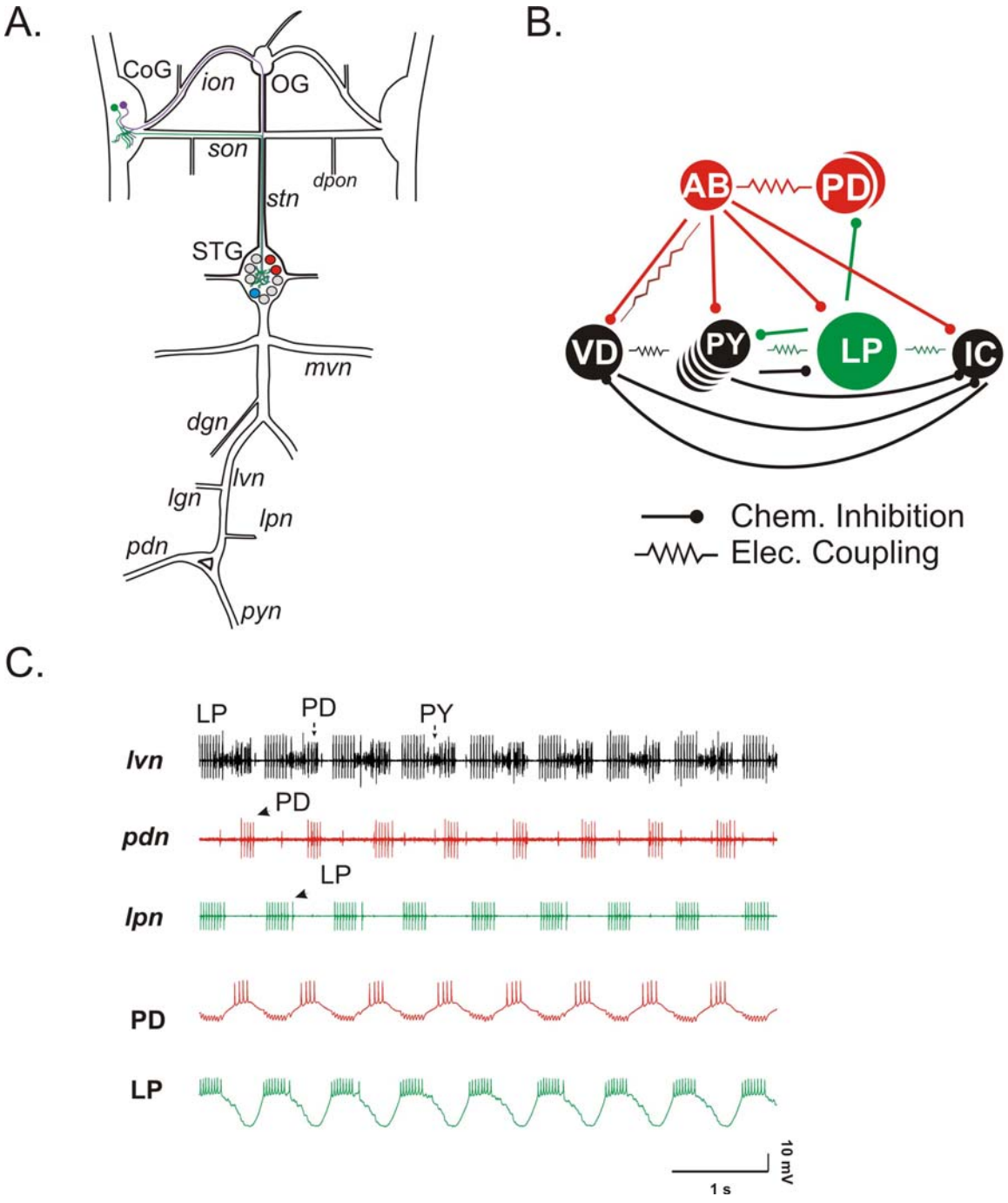


Figure 1 The pyloric network in the stomatogastric nervous system (STNS). **A.** Schematic diagram of the STNS. The paired Commissural ganglia (COGs) and esophageal ganglion (OG) contain modulatory projection neurons that send axons to the stomatogastric ganglion (STG) through stomatogastric nerve (*stn*). Most pyloric neurons

are motor neurons which project their axons through the dorsal ventricular (*dvn*) and lateral ventricular nerve (*lvn*) or through the medial ventricular nerve (*mvn*). The pyloric constrictor nerves (*pyn*) and pyloric dilator nerves (*pdn*) connect the axons to their respective muscles. **B.** Pyloric circuit in the STG. Anterior burster (AB) and a pair of pyloric dilators (PDs) neurons form the pacemaker group. Chemical inhibitory and electrical (gap junctions) synapses between the pyloric neurons are shown with different line (smooth and ragged lines, respectively). LP denotes lateral pyloric neuron and PY denotes pyloric constrictor neuron. VD and IC represent the ventricular dilator and the inferior cardiac, respectively. Note that the follower neurons, the VD, the PY and the LP neurons receive inhibitory synapse from the pacemaker neurons. **C.** Tri-phasic pyloric rhythm was recorded simultaneously both intracellularly (AB, PD, LP and PY neurons) and extracelluarly (*lvn, mvn, pyn and pdn*).

Chapter 2

Neuromodulation of a key synapse in a rhythmic network

Introduction

Synapses are responsible for the complex process of information transfer between neurons. Short-term synaptic dynamics such as facilitation and depression have been shown to play an important role in shaping the neuronal output at network level. Many studies have shown that synapses contribute greatly to network plasticity, which has been implicated in various tasks, such as motor pattern selection (Combes et al., 1999), synchronization (Tsodyks et al., 2000) and bistability (Manor and Nadim, 2001) in recurrent networks, sound localization (Cook et al., 2003) and network stability in hippocampus (Deeg, 2009).

The rhythmically-active pyloric network is a small oscillatory network in the stomatogastric nervous system and it is one of the most extensively explored systems for studying the effects of neuromodulation (Marder and Bucher, 2001). Many studies on neuromodulation of network activity have focused on effects of neuromodulators on the intrinsic properties of neurons (Marder and Thirumalai, 2002). However, the network activity is not only decided by the activity of its neurons, but also by the state of its synapses. Several studies have shown that synapses are also the targets of neuromodulation (Katz and Harris-Warrick, 1990a; Johnson et al., 1995; Ayali et al., 1998; Baimoukhametova et al., 2004). In pyloric circuit, the lateral pyloric (LP) is one of the follower neurons. It provides

the only chemical synapse feedback to the pacemaker ensemble (AB/PD). This only feedback synapse has been shown to play a vital role in regulating the pyloric activity (Manor et al., 1997; Mamiya and Nadim, 2004), In this chapter, I concentrate on the effects of the neuromodulator proctolin on this key synapse.

Neuromodulators in the stomatogastric nervous system have been shown to affect the intrinsic properties of individual neurons (Golowasch and Marder, 1992; Harris-Warrick et al., 1998; Swensen and Marder, 2000) and/or on the strength and dynamic of synapses among these neurons (Johnson and Harris-Warrick, 1997). Thus, these substances are able to reconfigure the neuronal networks to produce a variety of outputs, which are thought to be important for the proper function of the nervous system (Swensen and Marder, 2001; Marder and Thirumalai, 2002). I investigated the effect of neurally-released modulatory peptide proctolin (Nusbaum, 2002) on the synapse form lateral pyloric (LP) to the pyloric dilator (PD) neuron, which has spike-mediated and non-spike-mediated (graded) components. Previous studies mostly focus on the graded component. It has been shown that the graded synapse from LP to PD shows depression (Manor et al., 1997). However, in the presence of proctolin, low-amplitude presynaptic stimuli switch the short-term dynamic of this graded component from depression to facilitation. Such a shift in dynamics can act as a band-filter, which might contribute the role of that synapse in regulating network activity. Furthermore, I found this shift to facilitation is correlated with presynaptic inward current. As previously mentioned, the LP to PD synapse also has a spike-

mediated component. The neuromodulatory effect on this component is mostly unknown. I demonstrate that proctolin enhances this spike-mediated component. Thus, in ongoing rhythm, the LP to PD synapse is shown to be significantly strengthened. Such enhancement has important functional implications for stability of oscillatory networks.

Materials and Methods

Preparation and identification of the neurons Experiments were carried on the stomatogastric nervous system (STNS) of the crab *cancer borealis*. Animals were purchased from local markets (Newark, NJ, USA) and maintained in artificial seawater tanks at 10 -12 °C until use. They were anesthetized by cooling on ice for 30 minutes prior to each dissection. The STNS (including the STG, the esophageal and the commissural ganglia) was dissected out using standard methods (Selverston et al., 1976; Blitz and Nusbaum, 1997). The isolated complete STNS was pinned down on a Sylgard-coated Petri dish and the STG was desheathed to allow penetration of the cell bodies. All preparations were continuously superfused with chilled (11-13°C) physiological saline (containing in mM: KCl; 11, NaCl; 440, $CaCl_2$; 13, $MgCl_2$; 26, Trizma base; 11.2, Maleic Acid; 5.1, pH=7.4-7.5).

Neuromodulator Proctolin (Sigma-Aldrich, St. Louis, MO) was dissolved as stock solution in distilled water to a final concentration of 10^{-3} M, divided into aliquots and frozen at –20 °C. The final concentration of 10^{-6} M was made by dissolving the stock solution in the physiological saline immediately before use. Proctolin was bath applied by means of a switching port in a continuously flowing superfusion system. 10^{-6} M proctolin was used because it has been shown to best replicate the effects of proctolin release from endogenous modulatory neurons on the pyloric network (Blitz et al., 1999).

Electrophysiology Pyloric neurons were identified according to their activity patterns, synaptic interactions and their axonal projections in identified nerves. After identification the neuromodulaor input to the STG is removed by stn transaction in this section. Intracellular recordings were made using Axoclamp 2B amplifiers (Axon Instruments, Foster City, CA) in either single-electrode current clamp, or two-electrode voltage clamp (TEVC) modes. Microelectrodes for intracellular recording were pulled using a Flaming-Brown micropipette puller (Sutter Instruments, Novato,CA) and filled with 0.6 M K_2SO_4 and 20 mM KCl (resistance 15-25 MΩ). Pyloric neurons were identified according to their activity patterns, synaptic interactions and their axonal projections in identified nerves. After identification of neurons, the neuromodulatory input to the STG was removed by *stn* transaction.

In order to study the graded component of the LP to PD synapse, the preparation was superfused with 10^{-7} M TTX (Biotium, Hayward, CA) to block spike-mediated transmission. The LP neuron was TEVC'd with a holding potential of -60 mV and stimulated with multiple square pulses of different amplitudes. Square pulses of fixed 500 ms duration were to activate the graded component of LP to PD synapse in 10^{-7} M TTX. Short square pulses of fixed 10 ms duration were used to elicit spikes and activate the spike-mediated component. Antidromic spikes were elicited by stimulating the nerve *lpn* using a pulse stimulator (A-M system isolated pulse stimulator 2100, USA) with 0.5 msec, 3-10 V voltages pulses. Mn^{2+} saline (where Ca^{2+} in the physiological saline is substituted with 12.9 mM Mn^{2+} and 0.1

mM Ca^{2+}) was used to block Ca^{2+} currents. In order to measure presynaptic Ca^{2+} current, the experiments were performed in normal saline and Mn^{2+} saline. The difference between the presynaptic currents measured in normal saline and in Mn^{2+} saline is a putative Ca^{2+} current.

Transmitter applications were performed via focal pressure application onto the desheathed STG neuropil, using a TooheySpritzer (Toohey Company). For focal applications, a blunt-tipped micropipette was filled with Glutamate (3 mM) together with fast green indicator and positioned over the STG neuropil for the duration of whole protocol.

Analysis and Statistics of Data Data were digitized and analyzed using pClamp 9 software (Molecular Devices) or acquired using the Scope software (available at http://stg.rutgers.edu/software developed in the Nadim laboratory), sampled at 4 kHz and saved on a PC using a PCI-6070-E data acquisition board (National Instruments, Austin, TX). SigmaStat (Aspire Software International, Leesbrug, VA), Origin (OriginLab, Natick, MA), and CorelDraw software packages were used for statistical and graphical analysis.

Results

The two components of the LP to PD synapse

This inhibitory synapse consists of two different components: graded and spike-mediated. As illustrated in Figure 2.1 A, the amplitude of the graded component of the LP to PD synapse increases continuously in response to increasing amplitude of presynaptic neuron LP when the spike-mediated component is blocked by using TTX. Figure 2.1 B shows that injection of 10 ms pulse with amplitude 30 mV in the LP neuron elicits a spiked-mediated transmission in the PD neuron. Action potential in pyloric neurons is generated in the axon hillock. The LP neuron was voltage clamped at the soma, which is electrotonically distant from the axon hillock. Therefore, the injection of 10 ms pulse with the amplitude 30 mV (above the threshold for action potential) in the soma of the LP neuron will elicit a spike, which can escape voltage clamp. Preliminary experiments showed that the short pulse does not generate detectable graded IPSP.

The effects of proctolin on the strength of the spiked-mediated component of the LP to PD neuron

I first examined whether proctolin affects the strength of the spiked-mediated component of the LP to PD synapse. In order to elicit single spike-mediated IPSPs, the presynaptic LP neuron was voltage clamped at -60 mV and stimulated with short pulses of different amplitudes (V_{LP} = 20, 30, 40, 50 mV) of

fixed duration (10 ms). Figure 2.2A shows the spike-mediated IPSP evoked in the PD neuron in control (black trace) and in the presence of 10^{-6} M proctolin (red trace) indicating that bath application of proctolin significantly strengthens the spike-mediated IPSPs at various amplitudes (Figure 2.2B) (two-way ANOVA post-hoc Tukey analysis, $p<0.05$, N=8). Additionally, the amplitude of the IPSP increased as the LP neuron was progressively depolarized in both control and proctolin. What causes the increase in the IPSPs in PD neuron as we inject more depolarizing pulse into the presynaptic LP neuron? It has been reported that in the leech heart interneurons, the spike-mediated synaptic transmission is mainly regulated by high-voltage-activated calcium current, while the graded component mainly activated the low-threshold calcium channel (Ivanov and Calabrese, 2006). I hypothesized that calcium entry through the low–threshold calcium channels may be dependent on the amplitude of the presynaptic pulses. Thus, by increasing the amplitude of the presynaptic pulses, background calcium concentration would be increased, which in return resulted in more neurotransmitter release, therefore larger IPSPs.

I used two protocols to test this hypothesis. In the first protocol (spike-only), the LP neuron was voltage clamped at -60 mV and, as in Figure 2.2 it was stimulated with one short square pulse of different amplitudes (V_{LP} 20, 30, 40, 50 mV) and fixed duration (10 ms) (Figure 2.3A). In the second protocol (subthreshold), short square pulses of fixed duration (10 ms) were injected during a depolarizing step (-60 mV to -50 mV for two seconds) (Figure 2.3A). The resulting IPSPs were recorded in PD neuron in control and in the presence of proctolin (10^{-6} M). As

shown in Figure 2.3B, the IPSPs were significantly strengthened when the membrane was held at -50 mV compared to -60 mV (two-way ANOVA post-hoc Tukey analysis, $p<0.05$, $N=10$). Therefore, this suggests that further depolarization of the presynaptic neuron would increase the concentration of intracellular calcium in LP neuron. The next question would be through which type of calcium channel, this entry of calcium occurs. It has been reported that Ni^{2+} blocks the low-threshold calcium channel responsible for graded chemical synaptic transmission in the leech (Angstadt and Calabrese, 1991). In the second experiment we examined the effects of 100 μM Ni^{2+} on the amplitudes in these two sets of experiments. If Ni^{2+} blocked the low-threshold calcium channel there would be little difference between the amplitudes evoked by the short spike-only and using subthreshold. In Figure 2.3C, the IPSPs elicited by using spike-only and subthreshold were weakened by 100 μM Ni^{2+}, but the amplitude of IPSPs in subthreshold clamp was greater than the ones in spike-only step (two-way ANOVA, $p<0.05$, $N=5$). In Figure 2.4D, the difference of the IPSP amplitudes between spike-only and subthreshold was measured in the presence of Ni^{2+} (diff: black) and absence of Ni^{2+} (diff+Ni: grey). With 100 μM Ni^{2+} treatment, there is no significant change in difference of the IPSP amplitudes. These results suggest that increasing the background calcium might cause the increase of the IPSP amplitude as the membrane potential increases, but not through Ni^{2+}-sensitive low-threshold calcium channels alone.

To completely exclude the graded component of the LP to PD synapse I also elicited spikes in LP neuron, antidromically, by stimulating the LP nerve in the presence and absence of proctolin. The lateral pyloric nerve (*lpn*) was stimulated with 1 ms pulses of about 5 V. This stimulation can generate antidromic spikes which back propagate to STG where the LP neuron was voltage clamped at different levels (V_{LP}=-70, -60, -55, and -50 mV). Our results show that, as the holding level of the LP neuron was further depolarized, the amplitude of IPSPs in the PD neuron was accordingly increased in both control and 10^{-6} M proctolin (Figure 2.4A,B). As shown in Figure 2.4 B, this increase in IPSPs is more prominent in the presence of proctolin in comparison to control.

The effects of proctolin on the short-term dynamics of the spiked-mediated component of the LP to PD neuron

So far, I have shown that the neuromodulator proctolin enhances the spike-mediated component of the LP to PD synapse. Another interesting question would be if proctolin could modulate the dynamics of spike-mediated synaptic transmission. To address this question, I focused on the effects of proctolin on the short-term dynamics of the spike-mediated component of the LP to PD synapse.

The LP neuron was clamped at -50 mV and the *lpn* was stimulated by two successive antidromic spikes with different inter-spike intervals (ISI: 30, 50, 100, 250, 500 ms). The postsynaptic currents (IPSCs) in the PD neuron were

averaged over 10 repeated traces (Figure 2.5 A). As shown in Figure 2.5 B, proctolin enhances the corresponding synaptic currents regardless of ISI duration. In Figure 2.5 C, we plotted the ratio of the peaks of second to first corresponding IPSCs against ISI. The spike-mediated component of LP to PD synapse exhibited short-term depression in both control and proctolin at shorter ISIs. As the ISI increased, there was less depression (Figure 2.5C). However, there was no difference in depression between control and proctolin.

The effects of proctolin on the strength of the graded component of the LP to PD neuron

Graded synaptic transmission has been observed in both invertebrate and vertebrate chemical synapses. It is generally believed that graded synaptic transmission is the major form of synaptic communication among pyloric neurons (Raper, 1979; Graubard et al., 1983; Hartline et al., 1988). So we decided to investigate the effects of proctolin on graded synaptic transmission of LP to PD synapse. In order to study the graded component of LP to PD synapse, the preparations were bath applied with 10^{-7} M TTX to remove action potentials and therefore block spike-mediated transmission. The LP neuron was voltage clamped at a holding potential of -60 mV and a series of 500 ms depolarizing square voltage pulses with different amplitudes (20 mV, 30 mV and 40 mV) were applied in the presence and absence of proctolin. The resulting postsynaptic potentials were recorded from the PD cell (Figure 2.6A).

IPSPs in the PD neuron became larger in amplitude as more depolarizing pulses were injected in the LP neuron in both control and 10^{-6} M proctolin (Figure 2.6). For all voltage pulses the amplitude of the IPSP was larger in the presence of proctolin compared to control, indicating the strengthening effect of proctolin on the LP to PD synapse. (Figure 2.6B 2 way-ANOVA; post-hoc Tukey analysis, $P<0.05$, $N=6$).

The effects of proctolin on the dynamic of the graded component of the LP to PD synapse

The LP to PD synapse has been previously shown to display short-term depression (Manor et al., 1997). To characterize short-term plasticity of the LP to PD synapse, we injected a series of multiple low-amplitude (20 mV, V_{LP} in Figure 2.7) and high-amplitude (not shown) voltage pulses into the LP neuron and recorded the postsynaptic potential in the PD neuron. The experiment was performed with two different sets of treatment. First, recordings were done in both control saline and in the presence of 10^{-6} M proctolin (Figure 2.7 A, B). Second, the experiments were performed in normal Ca^{2+} or after blocking all Ca^{2+} currents (and therefore synaptic transmission), in both control and proctolin, by substituting the Ca^{2+} with Mn^{2+} (see Methods). In all conditions we made simultaneous measurements of the presynaptic current (Figure 2.7, I_{LP}) and the postsynaptic potential (Figure 2.7, V_{PD}). The difference between the presynaptic

currents measured in Ca^{2+} saline and in Mn^{2+} saline is a putative calcium current (Figure 2.7, $I_{LP}(Ca^{2+} – Mn^{2+})$). In the control saline (with normal Ca^{2+}), there was little synaptic response and no apparent synaptic plasticity was observed (Figure 2.7A, V_{PD}). In contrast, the synaptic response showed facilitation in proctolin (with normal Ca^{2+}) (Figure 2.7B, V_{PD}). V_{PD} is not shown in Mn^{2+} saline because there was no synaptic transmission in Mn^{2+} saline either in control or in proctolin. The putative calcium current was small in control conditions and its amplitude showed no obvious variation among the different voltage pulses (Figure 2.7A, $I_{LP}(Ca^{2+} – Mn^{2+})$). However, in the presence of proctolin, this current increased in amplitude with each subsequent pulse, indicating accumulation of Ca^{2+} currents (Figure 2.7B, $I_{LP}(Ca^{2+} – Mn^{2+})$ trace.

To reveal how proctolin causes the facilitation in response to low-amplitude presynaptic depolarization, I compared the presynaptic inward current $I_{LP}(Ca^{2+} – Mn^{2+})$ and the IPSPs to assess whether facilitation was correlated with the presynaptic inward current. Due to the variability in the peak current and IPSC and the fact that the measured presynaptic currents were at times contaminated with outward currents we compared the total charge due to $I_{LP}(Ca^{2+} – Mn^{2+})$ with the area of the IPSP during the pulse. To compare whether facilitation was due to an increase in the presynaptic current $I_{LP}(Ca^{2+} – Mn^{2+})$, the integrated presynaptic current and IPSPs were normalized as the ratio of the area difference from 1^{st} response to steady-state 5^{th}, respectively. Figure 2.8 B shows that facilitation is associated with the increase of the presynaptic inward current. Moreover, when

the integrated presynaptic inward current ($I_{LP}(Ca^{2+} - Mn^{2+})$) in response to each pulse was compared to the integrated IPSP for all pulses (not only the first and fifth), there was a modest positive correlation between the presynaptic inward current and the postsynaptic potentials (Figure 2.8C, linear fit: $R=0.65$ $P<0.001$ $N=9$).

The neuromodulator proctolin speeds up the recovery from depression

Short-term depression is present in the stomatogastric ganglion (Hartline, 1992). It has been reported that synaptic depression plays a pivotal role in shaping network function (Mamiya et al., 2003; Manor et al., 2003). So far I have shown the effects of proctolin on the short term dynamics of the graded or spike mediated components of LP to PD synapse. Now the question would be how proctolin would affect these two components in conjunction. To address this question, the LP neuron was voltage clamped at -60 mV and stimulated with two 500 ms depolarizing pulses of 30 mV with IPIs of 100 ms, 200 ms, 500 ms and 1000 ms in control and in proctolin (Figure 2.9A right and left, accordingly).

In both control and proctolin, amplitude of the 2nd IPSP was smaller than 1st IPSP indicating short-term depression. The ratio of 2nd over 1st IPSPs in the PD neuron was plotted against different inter-pulse intervals of pulses injected into LP neuron (Figure 2.9B). These results show that the level of depression was reduced by increasing inter-pulse intervals both in control and in proctolin. However, when compared to control, proctolin significantly improves the recovery

of short-term depression (two-way ANOVA, $p<0.001$, N=5). When inter-pulse interval reached 1000 ms there was no significant difference between the levels of depression in control versus proctolin.

The effects of proctolin on the LP to PD synapse during the ongoing pyloric rhythm

So far, I have demonstrated proctolin can enhance both graded and spike-mediated components of LP to PD synapse. It has been shown that neuromodulator proctolin strongly excites the pyloric rhythm (Hooper and Marder, 1984). However, none of these studies has quantified the effect of proctolin on the LP to PD synapse under ongoing pyloric rhythm. To study the effect of proctolin on the LP to PD synapse during an ongoing pyloric activity, PD neuron was voltage clamped at its resting potential during ongoing pyloric cycles. The corresponding currents in PD as a result of LP synapse onto PD were recorded, in control and in proctolin (10^{-6} M) (Figure 2.10A). Also, the traces of extracellular recordings from *lvn* was recorded as point of reference for the ongoing pyloric activity as a source of our measurements.

In the pyloric network, PD neurons and AB neuron are electrically coupled. Although one PD was voltage clamped, it was still coupled to the other PD and AB and was affected during the other PD and AB burst. Therefore, the synaptic response in the PD neuron was integrated from the end of previous PD burst to the start of the following PD burst (vertical red dot line shown in Figure 2.10A).

The synaptic area was significantly bigger in proctolin than control and wash (one-way ANOVA, $p<0.05$, N=5, Figure 2.10B). The amplitudes of the synaptic current peak during the LP burst (horizontal red dot line in Figure 2.10A) were measured. Proctolin significantly strengthens the amplitudes of synaptic response from LP to PD (one-way ANOVA, $p<0.05$, N=5, Figure 2.10C).

The effects of proctolin on the postsynaptic site of LP to PD synaptic transmission

In principle, a neuromodulator can change the strength of a synapse by modifying either the presynaptic transmitter release or the postsynaptic transmitter receptor response. So far, we have demonstrated modulation of proctolin at the presynaptic level. In order to examine the action of proctolin on the postsynaptic cell, PD was voltage-clamped at -55 mV and glutamate which is the neurotransmitter was puffed three times (puff duration 1 sec) onto the STG neuropil. The resulting current in the PD neuron was recorded in control and in the presence of proctolin (Figure 2.11 A). There is variability in the response to the glutamate puff in different preparations since the corresponding hot spots on the STG can be very different from one preparation to another. The synaptic currents were normalized to the control for each preparation in order to reduce this variability.

Figure 2.11 B shows that in the presence of proctolin, the postsynaptic response to glutamate in the PD neurons is significantly strengthened. (One-way ANOVA, $p<0.05$, $N=5$). Since I puffed glutamate three times during each experiment, the corresponding currents became smaller during the second and third puffs (Figure 2.11 A). It is evident that this can be due to desensitization of neurotransmitter receptors in response to the neurotransmitter puff.

Discussion

Our results show that proctolin can enhance both spike-mediated and graded components of LP to PD synapse. Before we investigated how spike-mediated component of LP to PD synapse was enhanced by proctolin, we examined the possible mechanism by which the spike-mediated synaptic transmission could be strengthened. In the leech heart CPG, it was found that low-threshold calcium currents activate the graded component of synaptic inhibition, whereas spike-mediated synaptic transmission is triggered by high-threshold calcium currents (Angstadt and Calabrese, 1991; Lu et al., 1997). In a previous modeling study, we proposed that there is cooperation between the mechanisms underlying the graded and spike-mediated components of the LP to PD synapse. This cooperation could explain how the spike-mediated IPSP becomes larger as the amplitude of the short voltage pulses increased. However, our results showed that Ni^{2+} didn't weaken the enhancement of the IPSP amplitude in subthreshold clamp. This finding suggests that the increasing of background calcium might cause the increasing of IPSP as the membrane potential increases, but not through low-threshold calcium channels. We propose that proctolin could increase the background calcium levels through a calcium channel or through the modulator-activated voltage-gated channel (Swensen and Marder, 2000).

Graded synaptic transmission has been observed in both invertebrate and vertebrate chemical synapses. It was generally believed that graded synaptic transmission is the major form of synaptic communication among pyloric

neurons(Raper, 1979; Graubard et al., 1983; Hartline et al., 1988). Several previous studies examined the effects of neuromodulators on the pyloric synapses by using long, single square presynaptic current pulses (Johnson and Harris-Warrick, 1990; Johnson et al., 1995). These studies showed that octopamine, 5-HT and dopamine are capable of modulating the strength of graded chemical synaptic interactions within the pyloric motor circuit. My results showed that proctolin enhanced the graded component of the LP to PD synapse. We believe that these changes of synaptic strength have functional consequence on the network output.

In principle, a neuromodulator can change the strength by modifying either presynaptic transmitter release or postsynaptic transmitter receptor response. Our findings indicated that the enhancement of the LP to PD synapse by proctolin is associated with a presynaptic inward current. However, it does not rule out modulation of postsynaptic site. Our data showed that in response to focal application of the neurotransmitter glutamate, proctolin strengthened the receptor response in the postsynaptic neuron PD. This result suggests that the enhancement of synaptic transmission may arise at least in part from effects of proctolin on the postsynaptic neuron.

Short-term synaptic dynamics such as depression and facilitation play an important role in regulating network operation. Understanding the mechanisms that control short-term plasticity will provide approaching on how neurons process incoming information. My data showed that proctolin enhanced the

spike-mediated component, but in average it does not change the dynamics of the spike-mediated component. I observed that there is more variety of synaptic size in the presence of proctolin. Our results indicated that in response to high amplitude presynaptic input the LP to PD exhibited short-term depression in both control and proctolin. Typically, there are several mechanisms underlying short-term depression. One mechanism for synaptic depression is desensitization of neurotransmitter receptors (Otis et al., 1996; Neher and Sakaba, 2001). A second mechanism is depletion of releasable vesicles (Wang and Kaczmarek, 1998; Oleskevich et al., 2000). A third mechanism is the inactivation of presynaptic Ca^{2+} ions. Our result in Figure 2.11 showed desensitization of neurotransmitter receptors in response to neurotransmitter puff. However, it does not rule out the other mechanisms. In contrast to stimulation with high amplitude pulses, we found that proctolin switches the dynamics of graded component of the LP to PD synapse from depression to facilitation when the presynaptic LP neuron was stimulated at low amplitudes. Our results showed that the switch to facilitation is correlated with the slow activation of a presynaptic Mn^{2+}-sensitive inward current, suggesting that it is a slowly accumulating Ca^{2+} current activated by proctolin. It is still not clear how proctolin causes this accumulation. Using a mechanistic model of synaptic release, we have shown that the actions of proctolin on the LP to PD synapse can be explained by the activation of a low-threshold calcium current that has slow activation and inactivation kinetics (Zhou et al., 2007). Another possibility is that non-specific channels activated by

proctolin were permeable for calcium and entry of calcium through these channels cause the accumulation.

Neuromodulation plays an essential role in the development and adaption of the nervous system, and is thought to be important for both the functions and disorders of the nervous system (Hasselmo, 1995; Cooper and Jan, 2003; Dickinson, 2006). Characterizing the neuromodulatory effects on synaptic dynamic and elucidating the mechanisms would provide insight on understanding action of the neuromodulator at network level.

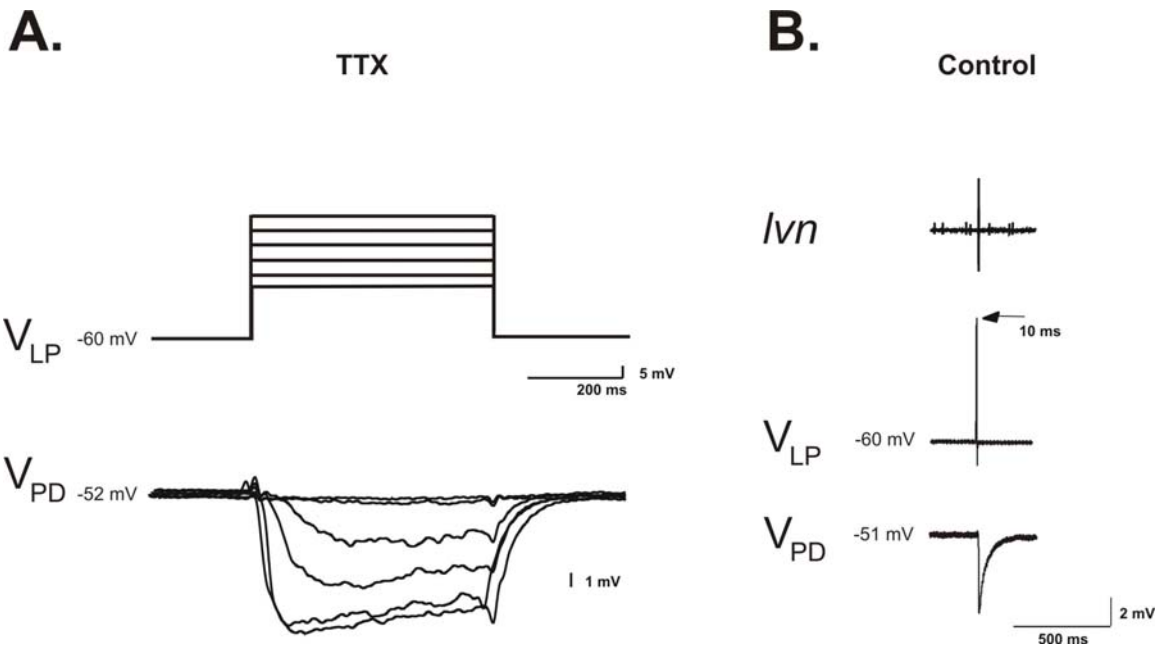


Figure 2.1. The LP to PD synapse has both a spike-mediated and a graded component **A.** After blocking action potentials by adding 10^{-7}M TTX, LP neuron was voltage-clamped at -60 mV and injected with 6 depolarizing pulses of 500 ms with increasing amplitude (from 20 to 50 mV in 5 mV increments). In response there was an IPSP in the PD neuron whose amplitude increased as a function of the presynaptic voltage, indicating the graded component of the LP to PD synapse. **B.** In control saline, injection of a 10 ms pulse with amplitude 30 mV (arrow) in the LP neuron elicits a spiked-mediated IPSP in the PD neuron. The nerve *lvn* shows the extracellular recording of the LP spike.

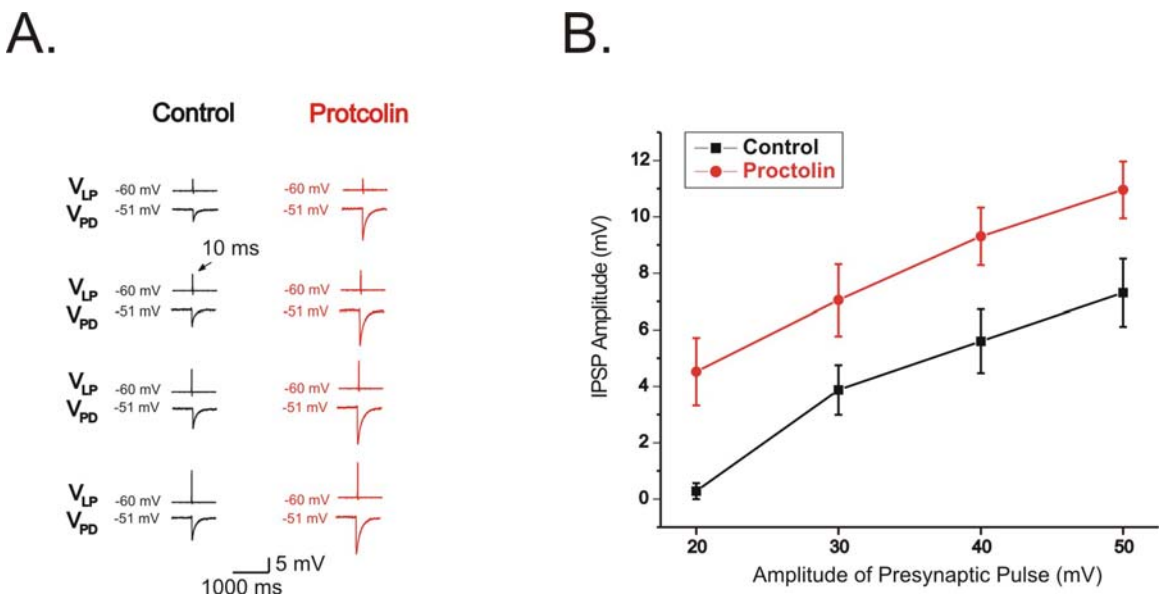


Figure 2.2 Unitary spike-mediated IPSPs are strengthened by proctolin **A.** The LP neuron was voltage clamped at -60 mV. The LP neuron was stimulated with one short square pulse of duration 10 ms and increasing amplitude (20 to 50 mV). The resulting IPSPs were recorded in PD neuron in control and in proctolin (10^{-6} M). **B.** The peak IPSPs plotted against the amplitude of the presynaptic pulse in control and in proctolin (N=8).

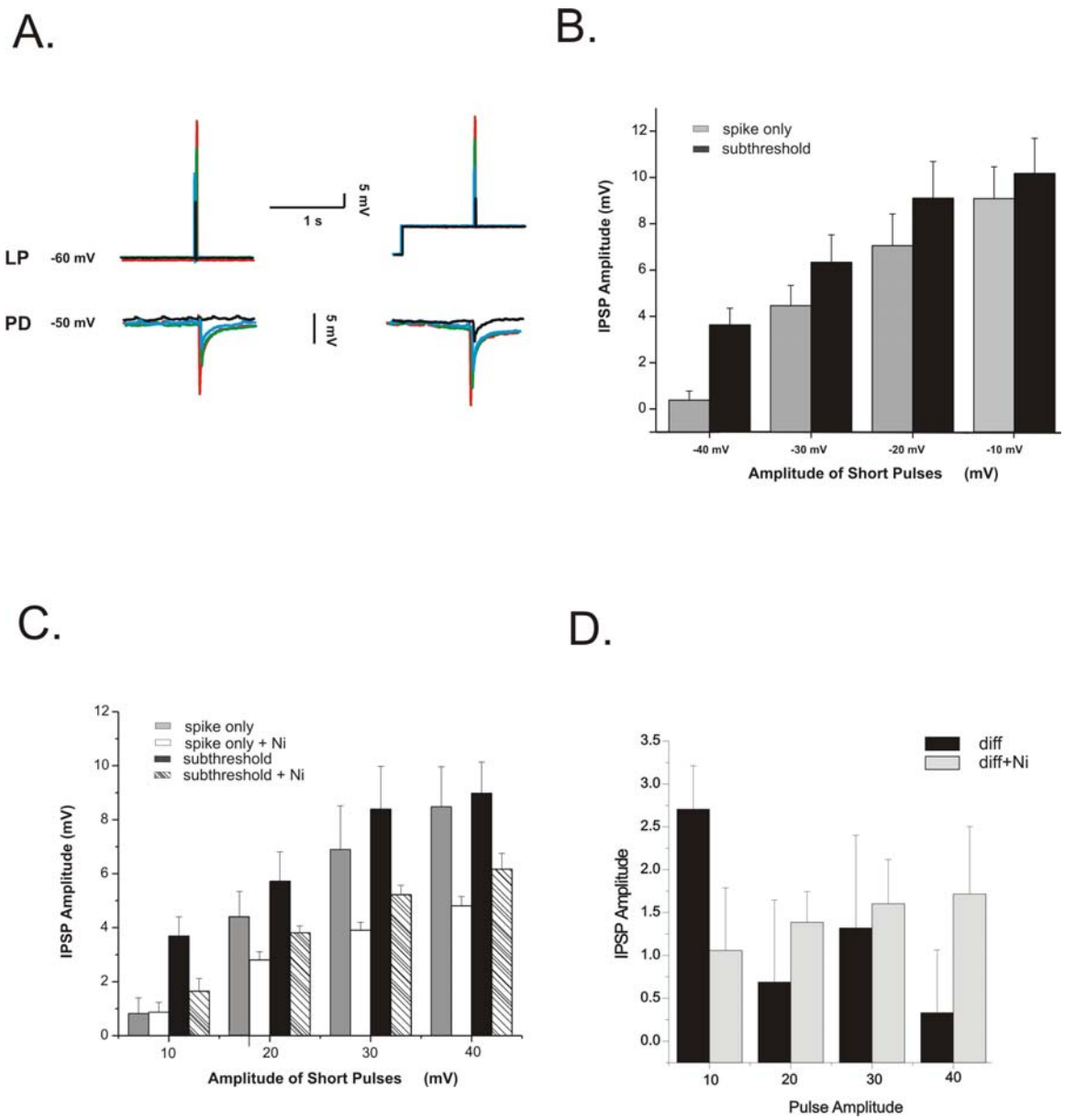
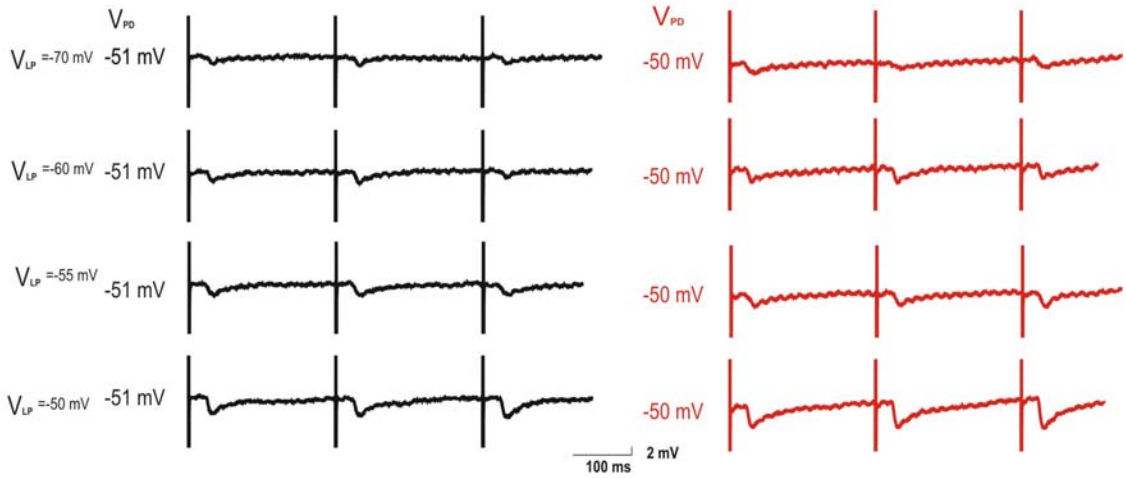


Figure 2.3 Unitary spike-mediated IPSPs are strengthened under subthreshold clamp. **A.** The LP neuron was voltage clamped at -60 mV. In one protocol (spike-only, left) the LP neuron was stimulated with one short square pulse of duration 10 ms and increasing amplitudes (20, 30, 40, 50 mV, different colors, left traces). In the second protocol (subthreshold, right), the short square pulses of fixed duration (10 ms) were injected during a subthreshold depolarizing step to -50 mV (duration 2 s; right traces). The resulting IPSPs were recorded in PD neuron in control and in proctolin (10^{-6} M). **B.** The

peak IPSPs amplitudes averaged over 10 different experiments. Data recorded in the spike-only protocol are presented in gray, data recoded in the subthreshold protocol in black. **C.** The effect of 100 μM Ni^{2+} on the IPSP amplitudes with both spike-only and subthreshold protocols. **D.** The difference of the IPSP amplitudes between spike-only and subthreshold with Ni^{2+} (diff: black) and without Ni^{2+} (diff+Ni: grey).

A.


V_PD
V_LP =-70 mV -51 mV
V_LP =-60 mV -51 mV
V_LP =-55 mV -51 mV
V_LP =-50 mV -51 mV
V_PD
-50 mV
-50 mV
-50 mV
-50 mV
100 ms
2 mV

B.

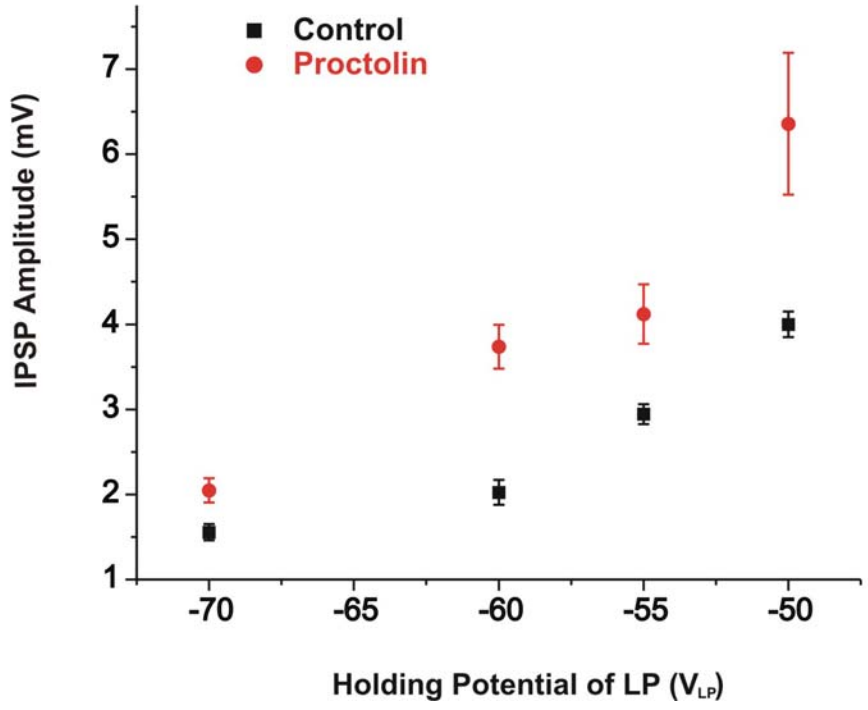

Control
Proctolin
IPSP Amplitude (mV)
1
2
3
4
5
6
7
-70
-65
-60
-55
-50
Holding Potential of LP (V_LP)

Figure 2.4 Unitary spike-mediated IPSPs evoked by antidromic spikes are enhanced by increasing the holding membrane potential of the LP neuron and proctolin also strengthens these IPSPs. **A.** The LP neuron was voltage clamped at the different levels (V_{LP}=-70, -60, -55 and -50 mV). The IPSPs were recorded in PD neuron in response to antidromic spikes in the LP neuron. in control (black) and in proctolin (red) (10^{-6} M). **B.** The peak IPSP amplitude plotted against the holding potentials of the LP neuron in control and in proctolin (N=4).

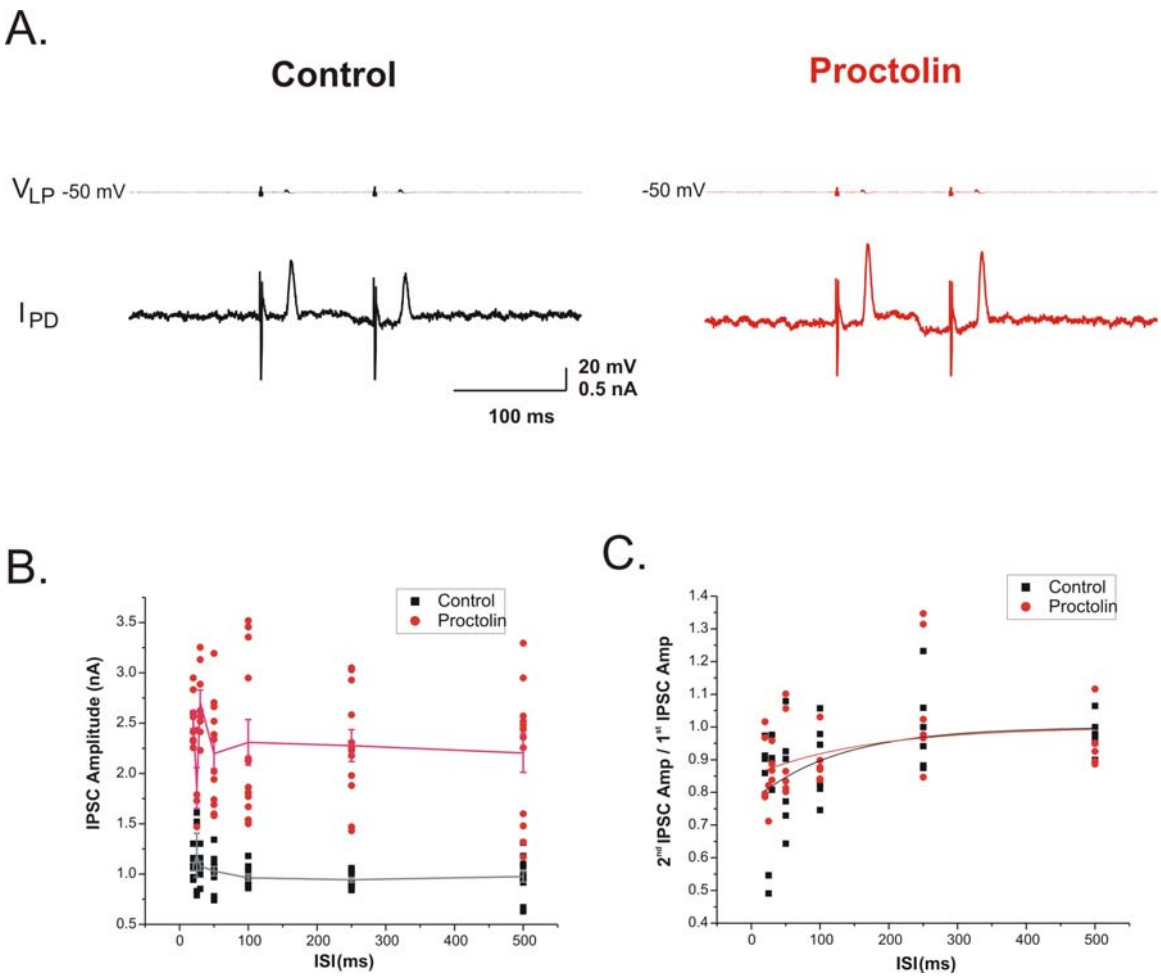
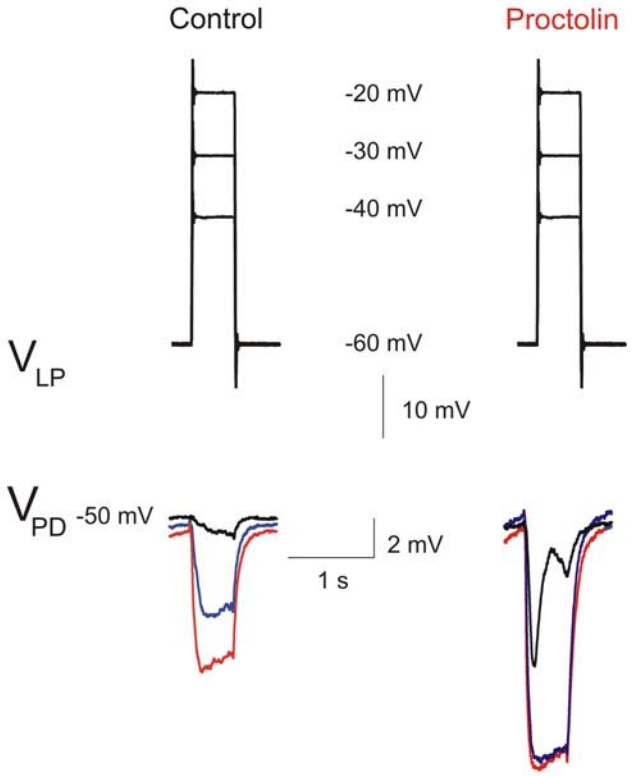


Figure 2.5 The effects of the neuromodulator proctolin on the dynamics of the LP to PD spike-mediated transmission. **A.** Spike-mediated synaptic currents in the PD neuron in response to two successive antidromic spike with a 100 ms interval. The LP neuron and PD neuron were voltage clamped at -50 mV; the resulting synaptic currents were recorded in PD neuron and repeated 10 times. The averaged traces were shown in control (black traces) and in proctolin (red traces). **B.** The IPSC amplitudes plotted against inter-spike interval in control and in proctolin (N=4 experiments). **C.** The ratio of the IPSC amplitudes in response to the first two successive antidromic spikes plotted against inter-spike interval in control and in proctolin (N=4). The exponential fit curves ($y=y_0 + Aexp(-x/\alpha)$) were plotted in control (black: $y_0=1$, $A=-0.23$, $\alpha=125$) and in proctolin (red: $y_0=1$, $A=-0.15$, $\alpha=167$).

A.


Control
Proctolin
-20 mV
-30 mV
-40 mV
-60 mV
V_{LP}
10 mV
V_{PD} -50 mV
2 mV
1 s

B.

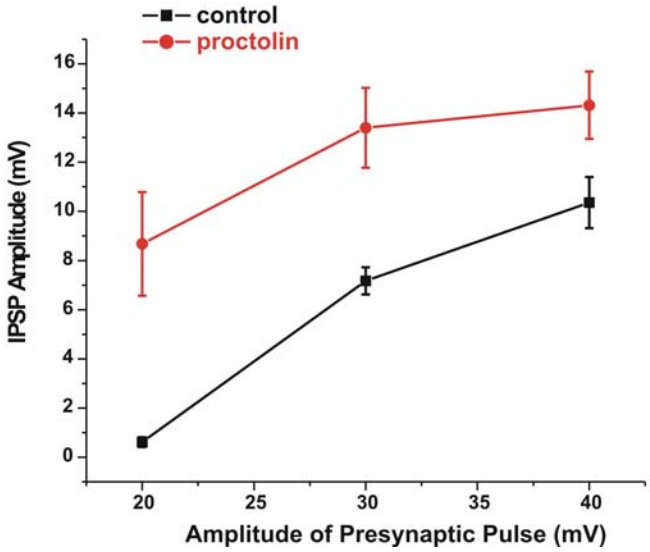

control
proctolin
IPSP Amplitude (mV)
0
2
4
6
8
10
12
14
16
20
25
30
35
40
Amplitude of Presynaptic Pulse (mV)

Figure 2.6 The neuromodulator proctolin strengthened the graded component of the LP to PD synapse. **A.** The LP neuron was voltage clamped at –60 mV and stimulated with square pulses of increasing amplitudes (20, 30, 40mV) in control and in proctolin (10^{-6}M). The resulting IPSP peak amplitudes were measured in the PD neuron in current clamp mode. **B.** The peak amplitudes of graded IPSP were plotted against the presynaptic depolarization in control and in proctolin (N=6).

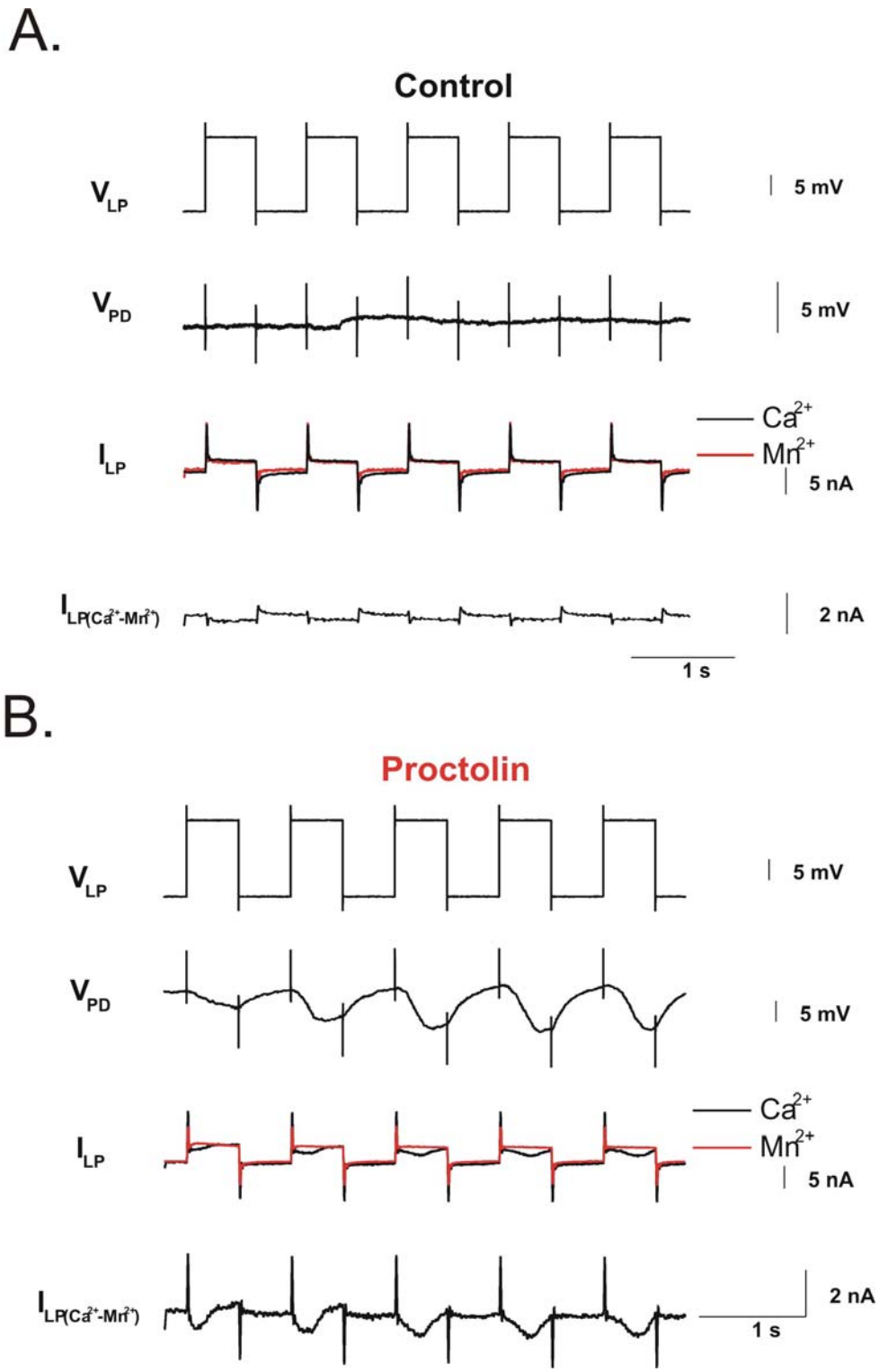
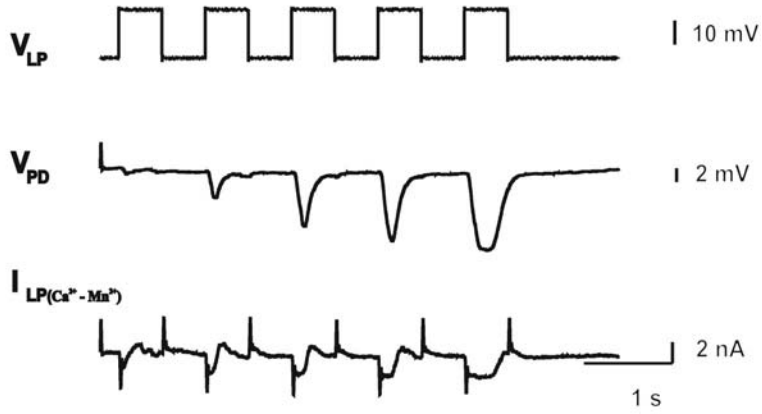
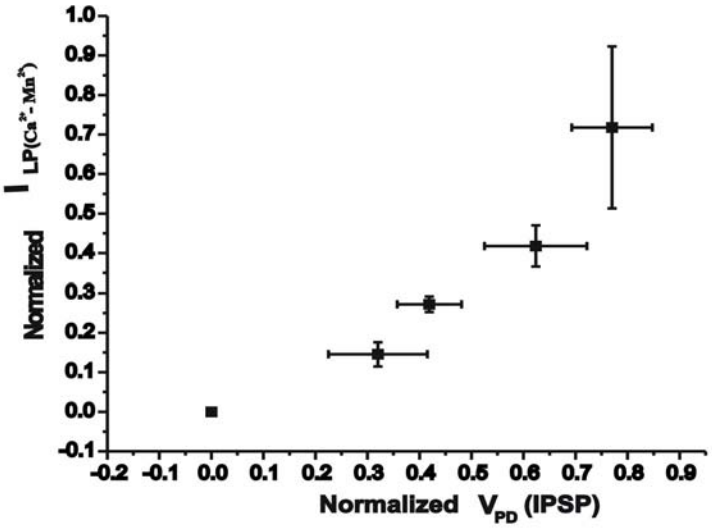

A.
Control
V_{LP}
5 mV
V_{PD}
5 mV
I_{LP}
Ca^{2+}
Mn^{2+}
5 nA
$I_{LP(Ca^{2+}-Mn^{2+})}$
2 nA
1 s
B.
Proctolin
V_{LP}
5 mV
V_{PD}
5 mV
I_{LP}
Ca^{2+}
Mn^{2+}
5 nA
$I_{LP(Ca^{2+}-Mn^{2+})}$
2 nA
1 s

Figure 2.7 Proctolin caused a facilitation of the LP to PD inhibitory postsynaptic potentials in response to low amplitude presynaptic depolarizing pulses. The presynaptic currents in the LP neuron (I_{LP}) are measured in Ca^{2+} saline (black) and in Mn^{2+} saline (red) using a train of low-amplitude presynaptic voltage pulses. The difference between the two measured currents ($I_{LP}(Ca^{2+} - Mn^{2+})$) reveals a new inward current activated in proctolin. **A**. a series of low amplitude presynaptic depolarizing pulses cause little response in postsynaptic neuron PD. **B.** Proctolin caused facilitation in PD neuron in response to low amplitude presynaptic depolarizing pulses. The amplitude of the current ($I_{LP}(Ca^{2+} - Mn^{2+})$) increases with each pulse.

A.


V_LP
10 mV
V_PD
2 mV
I_LP(Ca2+ - Mn2+)
2 nA
1 s

B.


Normalized I_LP(Ca2+ - Mn2+)
Normalized V_PD (IPSP)

C.

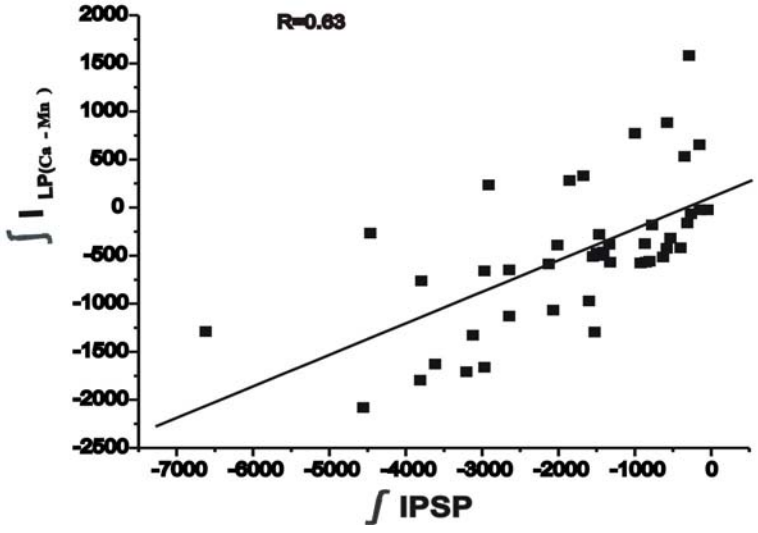
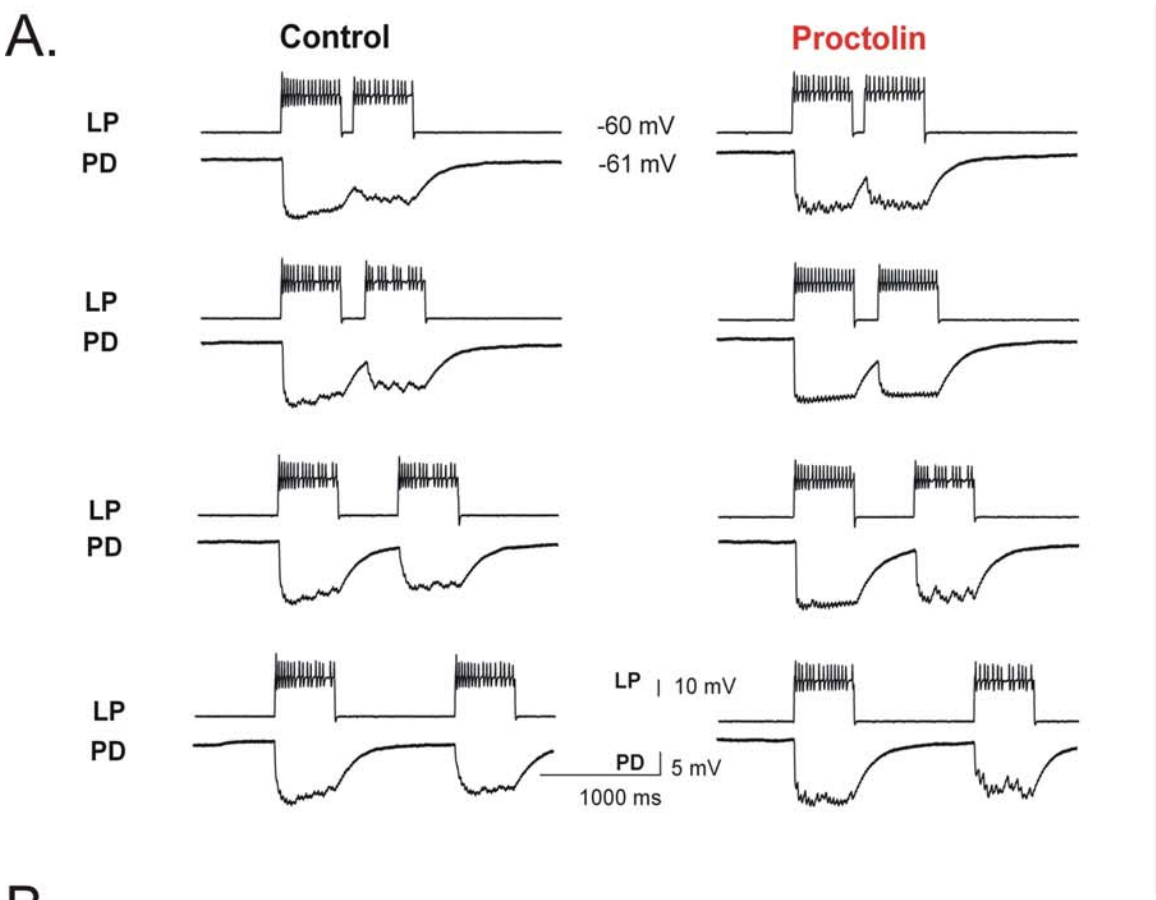

R=0.63
∫ I_LP(Ca - Mn)
∫ IPSP

Figure 2.8 Facilitation of the LP to PD inhibitory postsynaptic potentials in proctolin is associated with the activation of a Ca^{2+}-like inward current. **A.** The LP neuron was voltage clamped at -60 mV. The LP neuron was stimulated with 5 square pulses of fixed duration (500 ms). The resulting IPSPs (V_{PD} trace) were recorded in PD neuron in proctolin (10^{-6} M). The presynaptic currents in the LP neuron (I_{LP}) are measured in Ca^{2+} saline and in Mn2+ saline. The difference between the two measured currents (I_{LP}(Ca2+ - Mn^{2+})) reveals a new inward current activated in proctolin. **B.** The integrated presynaptic inward current (I_{LP}(Ca2+ - Mn2+)) and postsynaptic potentials (V_{PD}) in response to each pulse are normalized as (Area-1st Area)/(5th Area), respectively showing positive association between these two factors. Traces were integrated for the duration of each pulse. **C.** The value of the integrated presynaptic inward current (I_{LP} (Ca^{2+} - Mn^{2+})) in response to each pulse plotted against the value of the integrated IPSP (V_{PD}). Linear regression fit shows a correlation between the presynaptic inward current and the postsynaptic potentials ($R=0.65$, $P<0.001$).


A.
Control
Proctolin
LP
PD
-60 mV
-61 mV
LP
PD
LP
PD
LP
PD
LP
PD
LP
10 mV
PD
5 mV
1000 ms

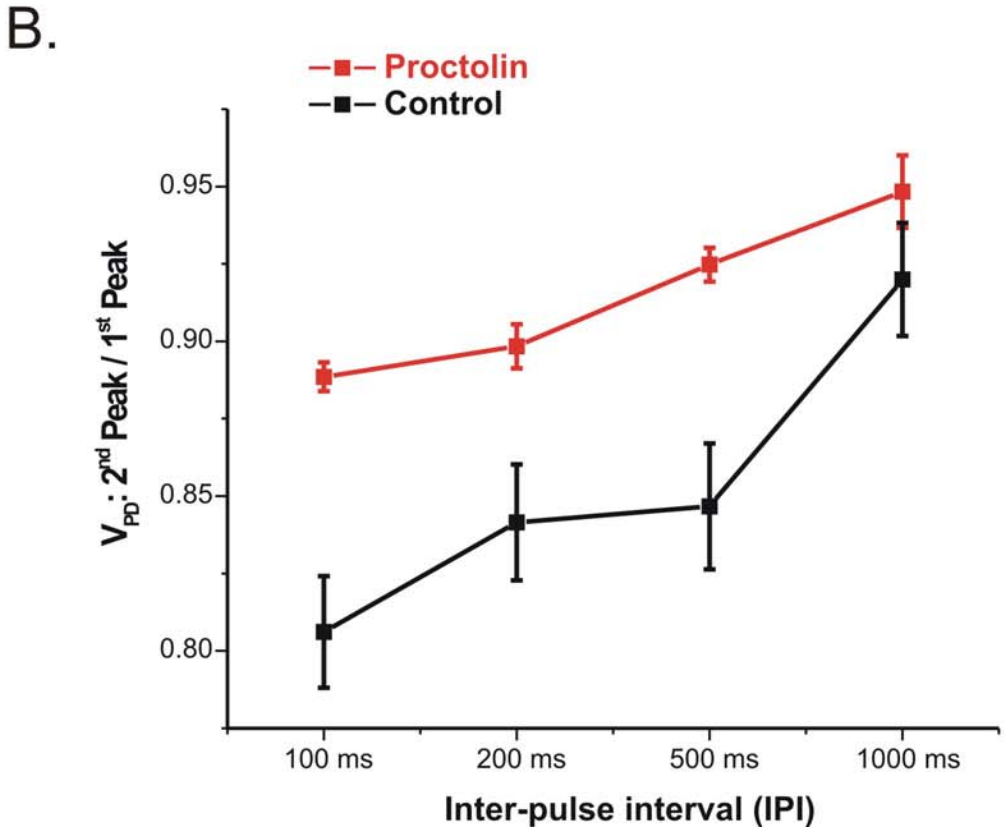

B.
Proctolin
Control
0.95
0.90
0.85
0.80
V_{PD}: 2nd Peak / 1st Peak
100 ms
200 ms
500 ms
1000 ms
Inter-pulse interval (IPI)

Figure 2.9 Proctolin speeds up recovery from the synaptic depression in response to presynaptic square pulses. **A.** The LP neuron was stimulated with two 500 ms pulses of 30 mV amplitude from -60 mV with IPI 100 ms, 200 ms, 500 ms and 1000 ms in control (left panel) and in proctolin (right panel). The IPSP elicited by LP neuron was recorded in PD neuron. **B.** In both control (black) and proctolin (red), the 2nd IPSP was smaller in amplitude than the 1st IPSP, indicating short-term depression. Depression (2nd peak over 1st peak) of the PD IPSP was significantly less in proctolin compared to control (two-way ANOVA, $p<0.001$, $N=5$).

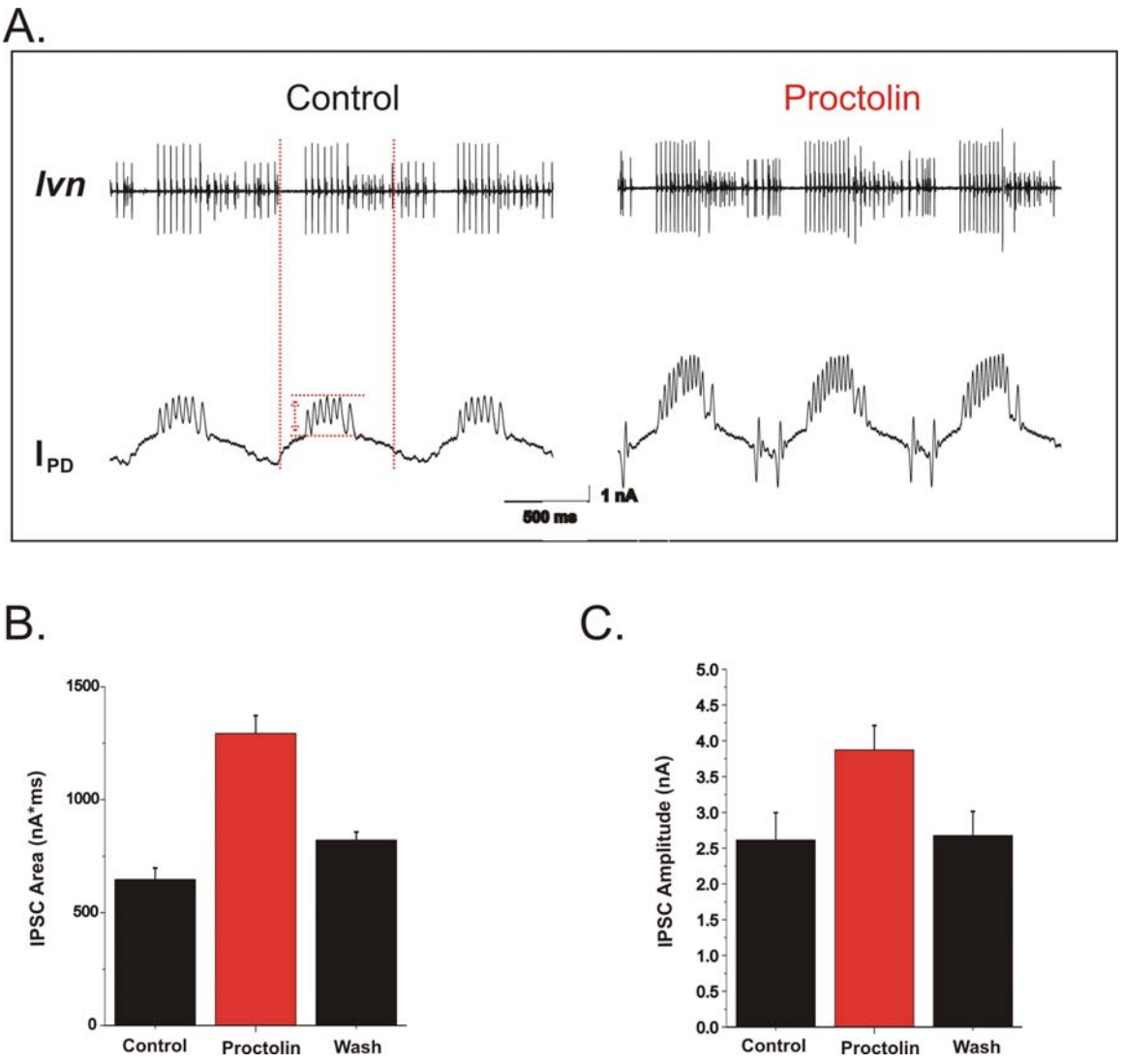


Figure 2.10 Proctolin enhances the LP to PD synapse during the ongoing pyloric rhythm. **A.** The traces of extracellular recordings from *lvn* show the ongoing rhythm activity of the pyloric network in control and in proctolin (10^{-6} M). One of the two PD neurons was voltage clamped at -55 mV; the resulting synaptic currents were recorded in PD neuron in control (left) and in proctolin (right). **B.** The synaptic response in PD neuron was integrated from the end of previous PD burst to the start of the following PD burst (vertical red dot line shown in A). The synaptic area was significantly larger in proctolin than control and wash (one-way ANOVA, $p<0.05$, N=5). **C.** The IPSC peak

amplitude during the LP burst (distance between horizontal red dotted lines in A) was measured. Proctolin significantly strengthened the amplitudes of synaptic response from LP to PD (One-way ANOVA, $p<0.05$, $N=5$).

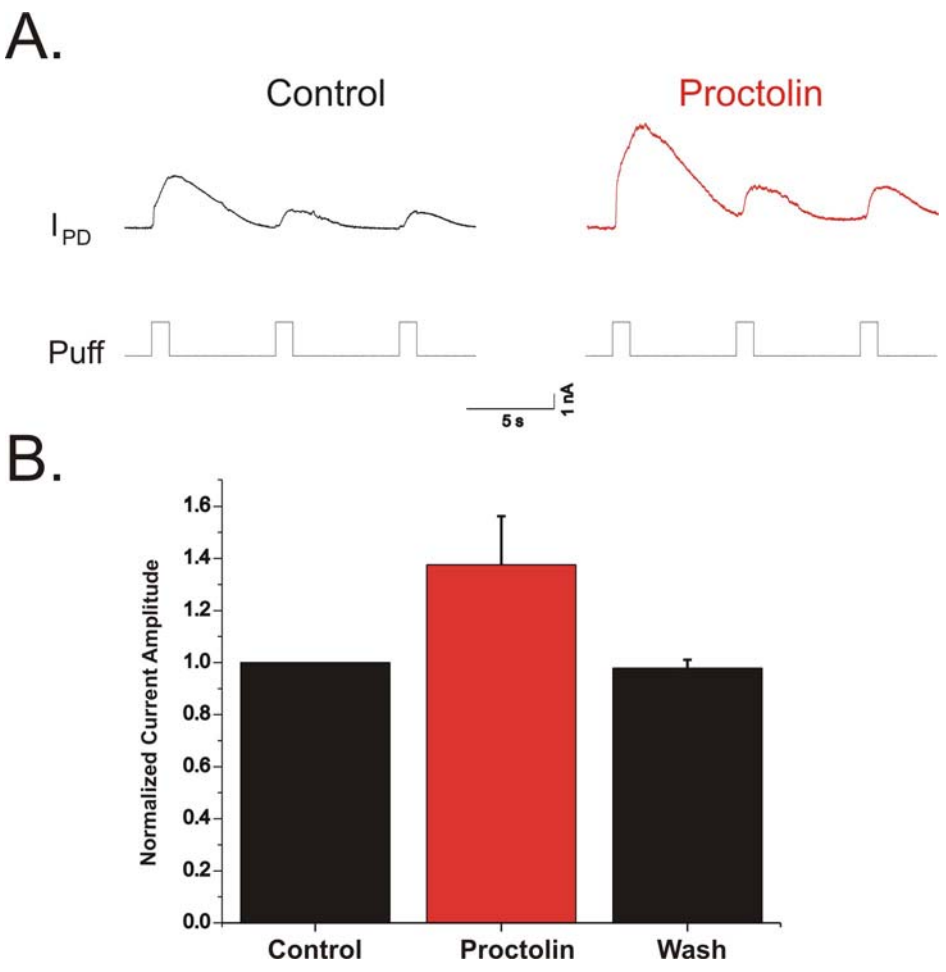


Figure 2.11 Proctolin enhances the current in the PD neuron in response to glutamate puff. **A.** One of the PD neurons was voltage-clamped at -55 mV. Glutamate (3 mM) was puffed on the STG neuropil (puff trace) and the resulting current was recorded in the PD neuron (I_{PD}) in control (left) and in proctolin (right). **B.** The I_{PD} was normalized to control for each preparation. The normalized amplitude of response current was significantly bigger in proctolin than control and wash (one-way ANOVA, $p<0.05$, N=5).

Chapter 3

Inhibitory feedback to pacemaker neurons stabilizes oscillation in the rhythmic network

Introduction

Stability and variability of neuronal activities are important for proper functions of the neuronal network circuits. Neuronal circuits must be flexible so they can adjust their outputs in response to a variety of environmental changes. On the other hand, it must be stable in order to generate appropriate output. Oscillatory activity generated by neuronal circuits in the nervous system has been extensively studied and is involved in circadian activity (van Esseveldt et al., 2000), sleep and arousal (McCormick and Bal, 1997), learning (Lisman, 1997), and motor pattern generation (Marder and Calabrese, 1996). The stable output patterns of the neuronal network are essential for the proper function of rhythmic networks. For example, a reliable, rhythmic heartbeat is critically important for survival. The crustacean cardiac ganglion is able to generate and maintain stable oscillatory activity in dealing with a variety of disturbances and perturbations (Cooke, 2002).

Output patterns of the neuronal network are controlled by the intrinsic properties of the element neurons and the synaptic connections among them (Marder and Calabrese, 1996; Nusbaum and Beenhakker, 2002). Various combinations of ion channels and synapse strengths can converge to produce similar neuronal

network outputs (Marder and Prinz, 2002). Individual neurons in the neuronal network can maintain a stable pattern of activity via changes in intrinsic properties (Marder and Prinz, 2002; Schulz, 2006). Also, synaptic plasticity in homeostasis plays an important role in maintaining network stability in the hippocampus in response to fluctuations of their inputs (Deeg, 2009). Perturbation of the oscillations in the sleep cycle by waking experience contributes to memory and learning (Buzsaki, 2006).

The central pattern generator circuits can be isolated for *in vitro* study. However, *in vivo* they are exposed to the entire system. They must receive various inputs and interact with other neuronal networks. In the face of the external perturbations, how does the CPG maintain its stability? In this chapter, I will address this http://www.popyard.org/question using the pyloric network of the crab *Cancer borealis*. The pyloric rhythm of the crab *Cancer borealis* is generated by a pacemaker group (one AB and two PD neurons). They give all followers pyloric neurons inhibitory synapse and receive the only chemical feedback synapse from LP neuron. Some computational and biological studies have shown that reciprocally inhibitory synapses affect the cycle period and the relative phasing between neurons (Nadim et al., 1999; Manor and Nadim, 2001; Mamiya and Nadim, 2004). Although this only feedback synapse has been extensively studied, in the ongoing pyloric rhythmic network, the functional advantage of the feedback synapse from LP neuron remains for further investigation.

A recent study has reported that the changes in the strength of the LP to PD synapse has nearly no effects on the average pyloric cycle period (Thirumalai et al., 2006). As shown in Figure 3.1, when the LP to PD synapse is blocked by picrotoxin, a glutamate receptor antagonist, we observed that the pyloric period became unstable. This phenomenon lead to our hypothesis that the LP to PD feedback synapse acts to stabilize the oscillation by reducing the variability in the cycle period. The pyloric variability in the cycle period can be measured by the coefficient of variance of the period. The inputs of perturbation to oscillators at different phases can cause changes in the oscillation period, which can be described as phase response curve (PRC) (Pinsker, 1977). The PRC is a useful tool for capturing the functional significance of an input to an oscillator. We found that the LP to PD synapse increases the reliability of the pyloric rhythm period and attenuates the perturbation response. The insight obtained from our findings will contribute to understanding the underlying mechanisms of the neuronal oscillation's initiation, break up and stability.

Materials and Methods

Preparation

Adult male crabs *Cancer Borealis* were obtained from local fish market (Newark, NJ) and maintained in saltwater aquaria at 12-14 ºC. The isolated stomatogastric nervous system (STNS) was pinned on a silicone elastomer (Sylgard)-coated Petri dish and superfused with standard *Cancer* saline solution (concentrations all in mM) containing 440.0 NaCl, 11.0 KCl, 13.0 $CaCl_2$, 26.0 $MgCl_2$, 5.0 Maleic acid, and 11.2 Trizma base, pH 7.4–7.5.

Electrophysiological recordings

Pyloric neurons were identified according to their activity patterns, synaptic interactions and their axonal projections in identified nerves. Microelectrodes for intracellular recording were pulled using a Flaming-Brown micropipette puller (Sutter Instruments, Novato,CA) and filled with 0.6 M K_2SO_4 and 20 mM KCl (resistance 15-25 MΩ). Intracellular recordings were made from the soma of the cells using Axoclamp 2B amplifiers (Axon Instruments, Foster City, CA). Extracellular recordings from nerves were made using stainless steel wire electrodes from identified nerves. The signals were amplified using a Differential AC amplifier model 1700 (A-M systems, Carlsborg, WA).

The gastric mill rhythm was elicited by stimulation of dorsal posterior oesophageal nerves (*dpon*) according to the protocol (Bartos and Nusbaum, 1997b; Blitz and Nusbaum, 1997). The biological LP to PD synapse was removed by hyperpolarizing the LP neuron. Data were digitized and analyzed

using pClamp 9.0 software (Molecular Devices) or acquired using the Scope software (available at http://stg.rutgers.edu/software and developed in the Nadim laboratory).

Phase response curve.

Brief perturbation (50 ms wide square with amplitude 2 nA) was injected at different phases (0.1-0.9) using Phase Response software (written in National Instruments Measurement Studio by Nadim). Intracellular recordings were made from the LP and one of the two PD neurons during the ongoing pyloric rhythm and the LP to PD synapse was removed by hyperpolarizing the LP neuron to compare with control. The phase of the perturbation injection was calculated according to the period of the previous cycle of the PD neuron oscillation.

Alternatively, a brief perturbation (50 ms wide square with amplitude 2 nA) was injected every 10 seconds for 15 minutes. In this way, the perturbation appears at all different phases randomly.

Analysis

The coefficient of variance was calculated using the mean period and standard deviation from ~50 pyloric cycles in each preparation. Spike2 (Cambridge Electornic Design) was used to acquire phase response curve with random perturbation. SigmaStat (Aspire Software International, Leesbrug, VA), Origin (OriginLab, Natick, MA), and CoreDraw software packages were used for statistical and graphical analysis. A standard ANOVA or Two Way Repeated

Measures ANOVA (Two Factor Repetition) tests were performed. If the p value was smaller than $\alpha = 0.05$ results were considered significant.

Results

The LP to PD synapse reduces the variability in the pyloric oscillations in the absence of gastric mill rhythm.

Perturbations to the pyloric rhythm, such as the intrinsic noise in the pyloric neuron and the excitory inputs from the descending projections neurons doesn't affect the pyloric cycle period on average, but results in more variability of the pyloric rhythm. I examined whether the inhibitory synaptic feedback can counteract these perturbations and reduce the variability of the pyloric rhythm. I first obtained simultaneous recordings of the *lvn*, *pdn*, *dgn*, *lgn* nerves and LP neuron (Figure 3.2A). The recording of the *lvn* shows the characteristic bursting pattern of the pyloric rhythm. The recording of the *pdn* was used to measure the pyloric period. The recordings of the *dgn* and *lgn* indicated that the gastric mill was silent. The LP to PD synapse was removed by hyperpolarizing the LP neuron (Figure 3.2A, HyperLP) to compare to control (Figure 3.2A, Control). To compare the variability of the pyloric period under control condition and in HyperLP, within each preparation, 40-60 period cycles were measured. Then I calculated coefficient of variances (CV), which is relative ratio of standard deviation to mean pyloric period. The mean period in control was 736.92 ± 68.82 ms and in HyperLP was 717.80 ± 64.20 ms, respectively (mean ± SEM; n=12; Figure 3.2B). It showed that there was no significant difference between the mean periods of control and HyperLP (one-way ANOVA $p>0.05$). However, the

coefficient of variance under control condition was significantly smaller than that with removal of LP to PD synapse (one-way ANOVA $p<0.05$). The mean coefficient of variance in control recordings was 0.0168 ± 0.00179 and in HyperLP recordings was 0.0358 ± 0.00451, respectively (mean ± SEM; n=12; Figure 3.2B). These results suggest that the stability of the pyloric network cycle period is significantly increased in the presence of the LP to PD synapse.

The LP to PD synapse reduces the variability in the pyloric oscillations in the presence of gastric mill rhythm

Two rhythmically active motor circuits, the gastric mill and pyloric, cooperate and interact in the STG. The gastric mill rhythm produces another natural perturbation of the pyloric rhythm (Marder and Bucher, 2007). The gastric mill rhythm is driven by the reciprocal inhibition between interneuron 1 (INT1) and the lateral gastric (LG). During its burst phase, the LG neuron inhibits the modulatory projection neuron MCN1 in the STG and thereby removes the local chemical excitation from MCN1 to the pyloric pacemaker neurons. Consequently, the pyloric rhythm is slowed down during the LG burst phase. However, during the burst phase of INT1, the LG neuron is silent and MCN1 excites the pyloric pacemaker neurons (Bartos and Nusbaum, 1997b; Marder and Bucher, 2007).

To examine the effect of the gastric mill rhythm on the pyloric period, I elicited the gastric mill rhythm by stimulating the descending projection neurons (see

Methods). As shown in Figure 3.3A, the recordings traces from the *dgn* and *lgn* indicated the activity of gastric mill. I found that there was still no significant difference between the mean periods of control and HyperLP with gastric mill rhythm (one-way ANOVA $p>0.05$). The mean period in control recordings was 718.76 ± 69.23 ms and in HyperLP recordings was 724.59 ± 72.03 ms, respectively (mean ± SEM; n=12; Figure 3.3B). In the presence of gastric mill activity, the mean coefficient of variance in control recordings was 0.0305± 0.00235 and in HyperLP recordings was 0.0535 ± 0.00541, respectively (mean ± SEM; n=12; Figure 3.3C). The variability of the pyloric period was greater in the presence of the gastric mill rhythm than in its absence (one-way ANOVA $p<0.05$). Also, the coefficient of variance without the LP to PD synapse was significantly larger than under control condition (one-way ANOVA $p<0.05$).

The effects of the LP to PD synapse on phase response curve

The transient excitatory inputs can be observed as little spikes in the intracellular realistic waveform of the pacemaker neuron AB (Marder et al., 1998). These excitatory inputs result in variability to the pyloric rhythm since they are not homogenous. The inputs of perturbation to oscillators at different phases can cause the different changes in oscillator period.

In order to investigate the effect of the LP to PD synapse on PRC shapes, we constructed and compared the PRCs of pacemaker group neuron PD with the

synapse or after removing the synapse by hyperpolarizing the LP neuron. Using the Phase Response software, I injected artificial inhibitory or excitatory perturbation into PD neuron. Figure 3.4A shows example traces with excitatory perturbation injected at phase 0.6. An excitatory perturbation (a square pulse of 2 nA amplitude and 50 ms duration) was injected into the PD neuron at phase 0.6, which was calculated with $\Delta t / P_0$ under control and HyperLP conditions. Δt is the delay of the perturbation onset after the first spike in PD burst. P_0 is defined as the free run period and P is defined as the perturbed period. In Figure 3.4B and C, the phase reset $\Delta\varphi_{PD}$ $((P_0 – P) / P_0)$ against the perturbation phase φ_{pert} $(\Delta t / P_0)$ were plotted, with the green bar illustrating the LP to PD synapse phase. The phase onset and offset of the LP to PD synapse were 0.39 ± 0.02 and 0.070 ± 0.025, respectively (mean ± SEM N=10). If the period is shortened, the value of $\Delta\varphi_{PD}$ is positive and if the perturbation increases the period, the value of $\Delta\varphi_{PD}$ is negative. When the excitatory perturbation was injected (Figure 3.4B), the period was prolonged at earlier phase and was advanced in both control and HyperLP conditions. More importantly, without the LP to PD synapse the PRC (red) shift away from zero (dot line) compared with the PRC in control (black). At phase 0.1, 0.6, 0.7 and 0.8 they were significantly different (Two Way Repeated Measures ANOVA $p<0.05$ N=10). When the inhibitory perturbation was used (Figure 3.4C), the period was shortened at earlier phase and was prolonged at late phase in both control and HyperLP conditions. However, when the LP to PD synapse was kept (black line), the PRC was closer to zero (dot line) compared to the PRC with removal of the LP to PD synapse (HyperLP, red). At phase 0.1, 0.2, 0.6 and 0.7

they are significantly different (Two Way Repeated Measures ANOVA $p<0.05$). These results suggest that the LP to PD synapse stabilized the pacemaker's oscillation and reduced the effect of perturbations.

The effects of the LP to PD synapse on phase response curve in response to random perturbation

I next examined the effects of the LP to PD synapse on phase response curve in response to random perturbation. In order to input the perturbations randomly, a brief perturbing current (50 ms wide square with amplitude 2 nA) was injected into one PD neuron every 10 seconds for 15 minutes. Thus, the delay of the perturbation onset after the PD burst kept changing and the perturbation could be injected at all different phases randomly. After the excitatory or inhibitory perturbation was randomly injected, the phase reset ($\Delta\varphi_{PD}$ $((P_0 - P) / P_0)$) was plotted against the perturbation phase φ_{pert} ($\Delta t / P_0$) (Figure 3.5A, B). As shown in Figure 3.5A, when the excitatory perturbation was injected, the phase reset ($\Delta\varphi_{PD}$) was delayed at earlier phase and was advanced at late phase both in control and HyperLP conditions. When the inhibitory perturbation was used (Figure 3.5B), it had opposite effects on the phase reset. The phase reset was advanced at early phase and was delayed at late phase in both control and HyperLP conditions. Furthermore, when the LP to PD synapse was removed in both excitatory and inhibitory perturbation cases, the PRCs were bounded away from zero compared to control.

In order to characterize the overall effect of random perturbation on the oscillatory stability, we analyzed the absolute value of the phase reset. I found that when the LP to PD synapse was removed (HyperLP) the mean of the absolute value of the phase reset ($|(P_0 – P) / P_0)|$) was significantly larger compared to control in response to excitatory perturbation (Figure 3.5C) or inhibitory perturbation (Figure 3.5D) (t-test, $p<0.05$ $n=4$). These results indicate that the LP to PD synapse attenuates the disturbing influence of extrinsic perturbation on the stability of the pacemaker's oscillation.

The mechanism underlying the reduction of variability of oscillation by the feedback synapse

Our results showed that the LP to PD synapse stabilized the pacemaker's oscillation and reduced the effects of perturbation. Our modeling study found that the underlying mechanism could be explained by the model neuron's synaptic phase response curve (Synaptic-PRC) and its relationship with synaptic phase and synaptic duty cycle. Synaptic phase points to the phase of the onset of the synapse. Synaptic duty cycle (DC_{syn}**)** was measured as the fraction of the cycle period during which the synapse was active.

In order to construct Synaptic-PRC, we removed the LP to PD synapse by hyperpolarizing LP neuron and used dynamic clamp technique to inject synaptic

input (300 nS, E_{rev}=-80) to the PD neuron. This allows us to change the values of synaptic duty cycle (DC_{syn}**).** Figue 3.6A displayed phase reset ($\Delta\varphi_{PD}$) changes in response to three different DC_{syn} (Control-DC_{syn} = 0.3, short-DC_{syn} = 0.2 and long-DC_{syn} = 0.45). We examined the effect of both synaptic phase and synaptic duty cycle on cycle period. We found that if the synapse arrived at an early phase of the cycle, it shortened the period and resulted in positive phase reset ($\Delta\varphi_{PD} > 0$). If the synapse onset occurred during the middle phase of the cycle, the synapse had little effect on the cycle period. In contrast, if the synapse onset occurred at a late phase of the cycle, it lengthened the cycle period and resulted in a negative phase reset ($\Delta\varphi_{PD} < 0$). For the different synaptic duty cycles, Figure 3.6A showed that the long-DC_{syn} caused a downward shift of the synatic-PRC and the short-DC_{syn} lead to an upward shift of the synaptic-PRC. Note that at early phase, there was a convergence of the Synaptic-PRC curves and a divergence of the Synaptic-PRC curves occurs at late phase.

So far, we have shown how the two factors, synaptic phase and synaptic duty cycle of the feedback synapse affect the cycle period. The flow chart in Figure 3.6B represented the sequential events in explaining how the feedback synapse counteracted the perturbation. For example, if perturbation shortens the cycle period, on one hand the synaptic phase is shifted to later phase by the perturbation, on the other hand the synaptic duty cycle becomes longer and the Synaptic-PRC is shifted down. The changes of both synaptic phase and synaptic duty cycle result in a negative phase reset and lengthen the period (Figure 3.6A).

Similarly, if perturbation lengthens the cycle period, it will results in a positive phase reset. In this way, the feedback synapse counteracts the perturbation effect.

Discussion

Inhibitory feedback synapse plays an important role in neural oscillation and rhythmic activity in brain (Pelletier and Lacaille, 2008; Saraga et al., 2008). We used the crab pyloric network to investigate the effects of inhibitory feedback synapse on pacemaker's oscillation. The LP to PD synapse is the only inhibitory feedback synapse to AB/PD pacemaker group in the pyloric network. Several studies have shown that the pyloric cycle period can be regulated through the LP to PD synapse (Nadim et al., 1999; Mamiya and Nadim, 2004, 2005). Manor and Nadim (Manor and Nadim, 2001) demonstrated that the network consisting of one pacemaker and one follower neuron showed robust bistability of the oscillation. In one state, cycle is long and controlled by synapse. In the other mode, depressed synapse contributes little or nothing to the cycle period. Our results showed there was little or no change in the pyloric period when the LP to PD was removed (Figure 3.2B and 3.3B). When the cycle period was long under certain condition, we observed the removal of the LP to PD synapse shortened the cycle period. In this study, we examined the effects of the LP to PD synapse on the pyloric period at the steady state. In some cases, the cycle period was transiently shortened after the removal of the LP to PD synapse. However, the pacemaker could adjust itself back to the steady state. In this mode, the LP to PD synapse reduces the variability in the pyloric period (Figure 3.2C and 3.3C). The average period is determined by the pacemaker and the feedback synapse acts as stabilizer and maintains the stability of the pyloric rhythm.

Mamiya and Nadim's work (Mamiya and Nadim, 2004) also suggested that the LP to PD synapse acts to stabilize the pyloric period at a relative long-term level. Changing the cycle period affects the waveform of the LP neuron. Then, the amplitude and peak time LP to PD synapse are altered. These changes in synaptic amplitude and peak time in turn oppose the change in cycle period thus stabilizing the rhythm. The short-term dynamic of the LP to PD synapse is required in this stabilizing process. Our modeling and experimental studies showed that the LP to PD synapse can counteract the influence of transient perturbations within one cycle period. This stabilizing effect is dependent on the intrinsic dynamics of the oscillatory neuron itself.

PRC is a useful tool to quantify and elucidate the stability of oscillations under noisy perturbations (Tateno and Robinson, 2007). In this chapter, we demonstrate two concepts of PRC. PRC in Figure 3.4 and 3.5 was used to measure the variability in response to short noisy perturbation. Synaptic-PRC is calculated in response to a stimulus consisting of a long-lasting and possibly strong synaptic input. It has been shown that under control conditions the phase of the LP to PD synapse is kept relatively constant (Hooper, 1997; Bucher et al., 2005). Without changing the synaptic phase and duty cycle, the change in LP to PD synapse has relatively little effect on the pyloric period (Thirumalai et al., 2006). We used synaptic-PRC and its relationship with synaptic phase and synaptic duty cycle to explain how the LP to PD synapse counteracts the effect of perturbation. Our modeling results showed that when a perturbation changed the

cycle period it caused change of the synaptic phase and duty cycle. Changing the synaptic phase and duty cycle resulted in a compensatory change in cycle period. Thus, it counteracted the effects of the perturbation.

Our result showed that the inhibitory feedback synapse reduces the variability in pacemaker's cycle period and acts to reduce the sensitivity of the pacemaker to external perturbation. These findings are quite general and could apply to other feedback synapses in the oscillatory network.

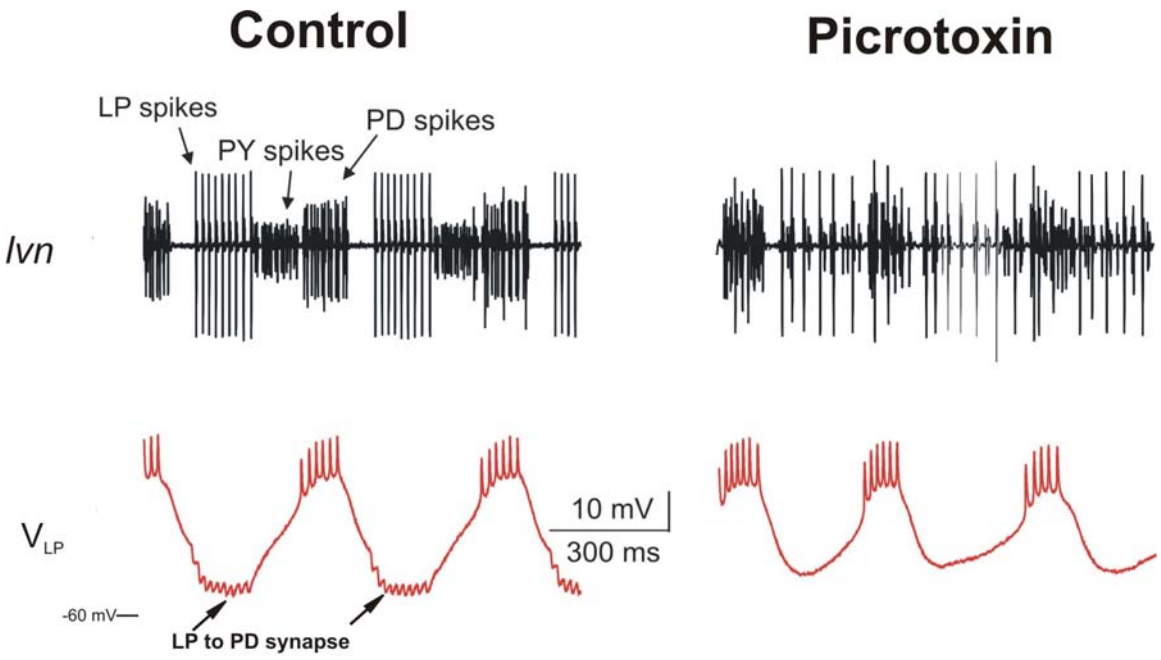


Figure 3.1 Extracellular (*lvn*) and intracellular (V_{LP}) traces recorded in the ongoing pyloric rhythm in control (left) and in 10^{-5} M picrotoxin. Arrows on *lvn* point out the LP, PY, PD spikes, respectively. Arrows on V_{LP} trace indicate the LP to PD synapse in control. In picrotoxin the LP to PD synapse is blocked.

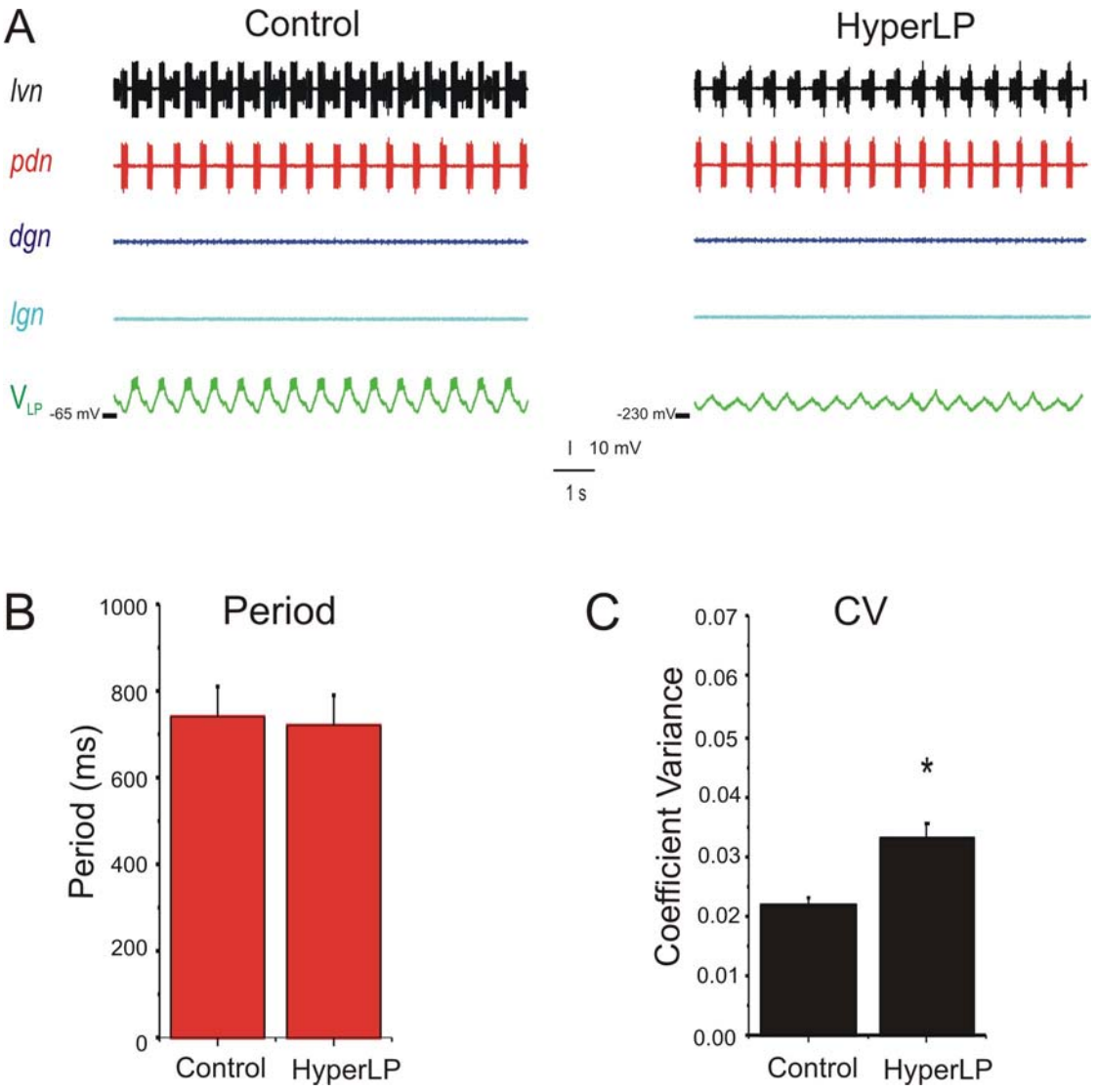


Figure 3.2 The pacemaker neuron's oscillating variability was reduced in the presence of the LP to PD synapse. **A.** Extracellular recording traces from latral ventricular nerve (*lvn*), pyloric dilator nerve (*pdn*), and intracellular voltage traces from the lateral pyloric neuron (LP) indicate the activity of pyloric circuit neurons. Extracellular recording traces from dorsal gastric nerve (*dgn*) and lateral gastric nerve (*lgn*) show the activity of the gastric mill. Recordings were taken when 0 nA (control) or 5 nA (HyperLP: LP was hyperpolarized to remove the LP to PD synapse.) currents were injected. **B.** The pyloric period was averaged over 40 to 60 cycles from the *pdn* for each preparation. There was no significant change in the mean pyloric period between control and HyperLP conditions (N=12)**. C.** The coefficient of variance was bigger in HyperLP compared to

control. The variability of the pyloric rhythm period was significantly higher when the LP to PD synapse was removed (one-way ANOVA, $P<0.05$, $N=12$).

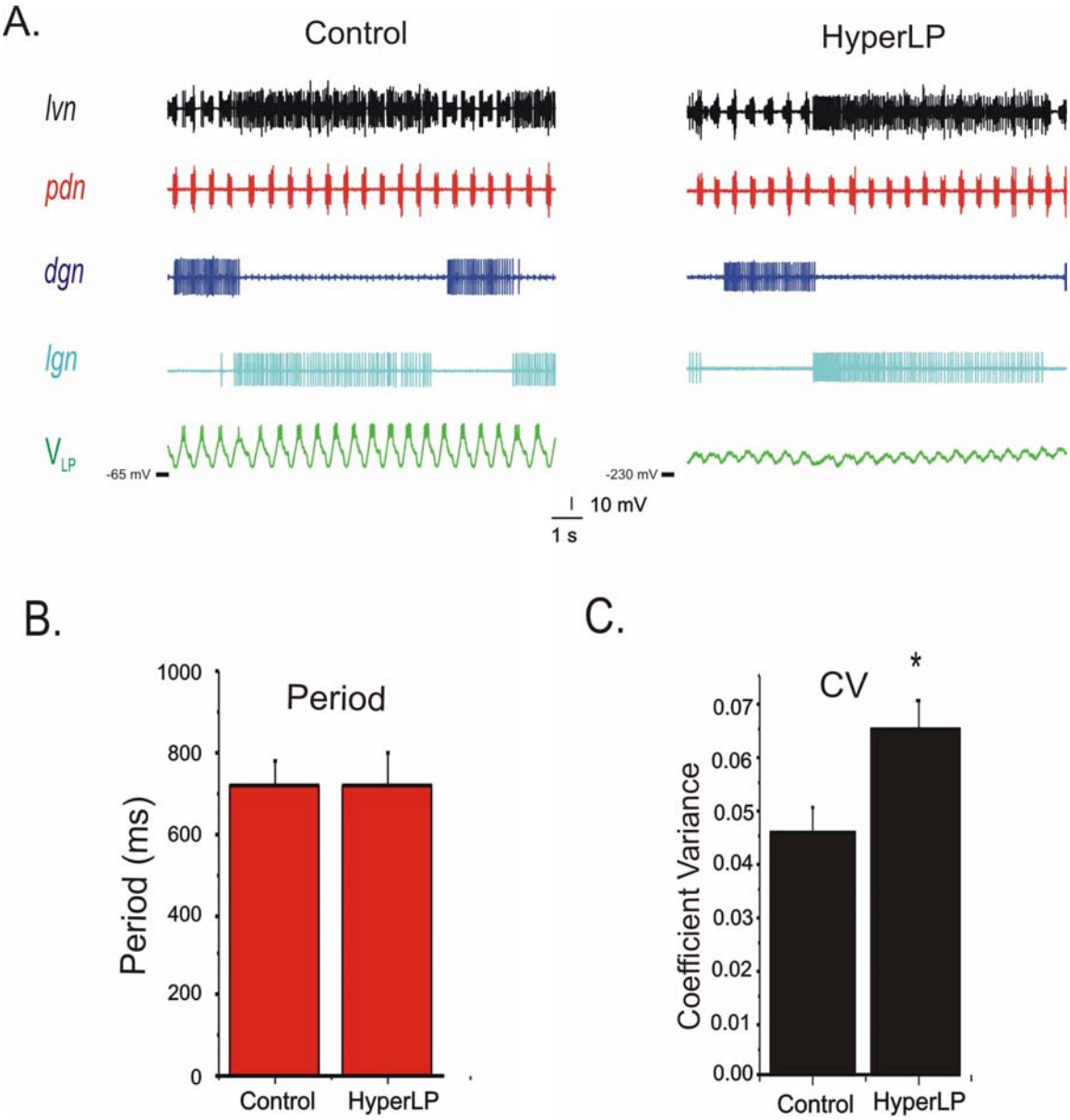


Figure 3.3 The coefficient of variance of the pyloric period with the removal of the LP to PD synapse increased in the presence of the gastric mill rhythm. **A.** Extracellular recording traces from latral ventricular nerve (*lvn*), pyloric dilator nerve (*pdn*), and intracellular voltage traces from the lateral pyloric neuron (LP) indicate the activity of pyloric circuit neurons. Extracellular recording traces from dorsal gastric nerve (*dgn*) and lateral gastric nerve (*lgn*) show the activity of gastric mill. The LP neuron was hyperpolarized to remove the LP to PD synapse in HyperLP. **B.** The pyloric period was averaged over 40 to 60 cycles from the *pdn* for each preparation. There was no

significant change in the mean pyloric period between control and removal of LP to PD synapse (left)**. C.** The coefficient of variance is significantly higher in HyperLP compared to control when the gastric mill rhythm is active (one-way ANOVA, $P<0.05$, $N=12$).

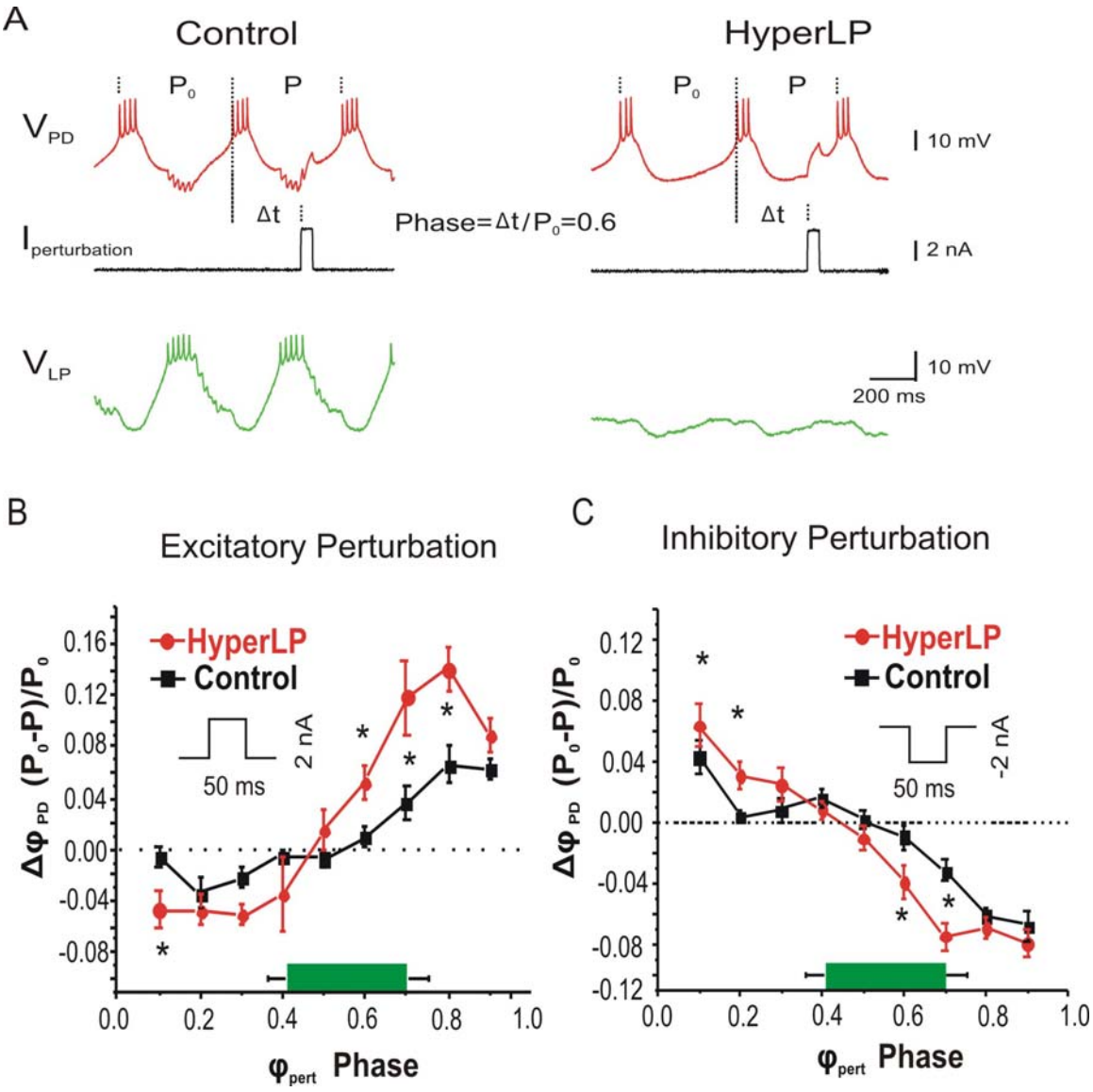


Figure 3.4 The LP to PD synapse reduced the effect of artificial perturbation. **A.** An example of the voltage trace of the PD neuron in response to the artificial perturbation (2 nA positive current injection) at phase 0.6 when LP to PD synapse was kept (left) and removed by injecting ~-5 nA current (right). The phase of the perturbation injection was calculated according to the previous period (P_0). Phase = $\Delta t/ P_0$=0.6 indicated that when the perturbation phase was set at 0.6, the perturbation current was injected at Δt (0.6 P_0) after the first action potential of the PD neuron (long vertical dotted line). **B.** The current injection of amplitude 2 nA and duration 50 ms was used as the excitatory perturbation. The green bar indicates the LP synaptic phase. Without the LP to PD synapse (HyperLP), the phase reset $\Delta\varphi_{PD}$ ((P_0 – P) / P_0) (Red) was more negative at early

phase and more positive at late phase compared to control (Black). (* P<0.05, N=10) **C.** Using the inhibitory perturbation (-2 nA), with the LP to PD synapse the phase reset $\Delta\varphi_{PD}$ lies closer to zero (dotted line) compared to HyperLP (Red). (* P<0.05, N=10**)**

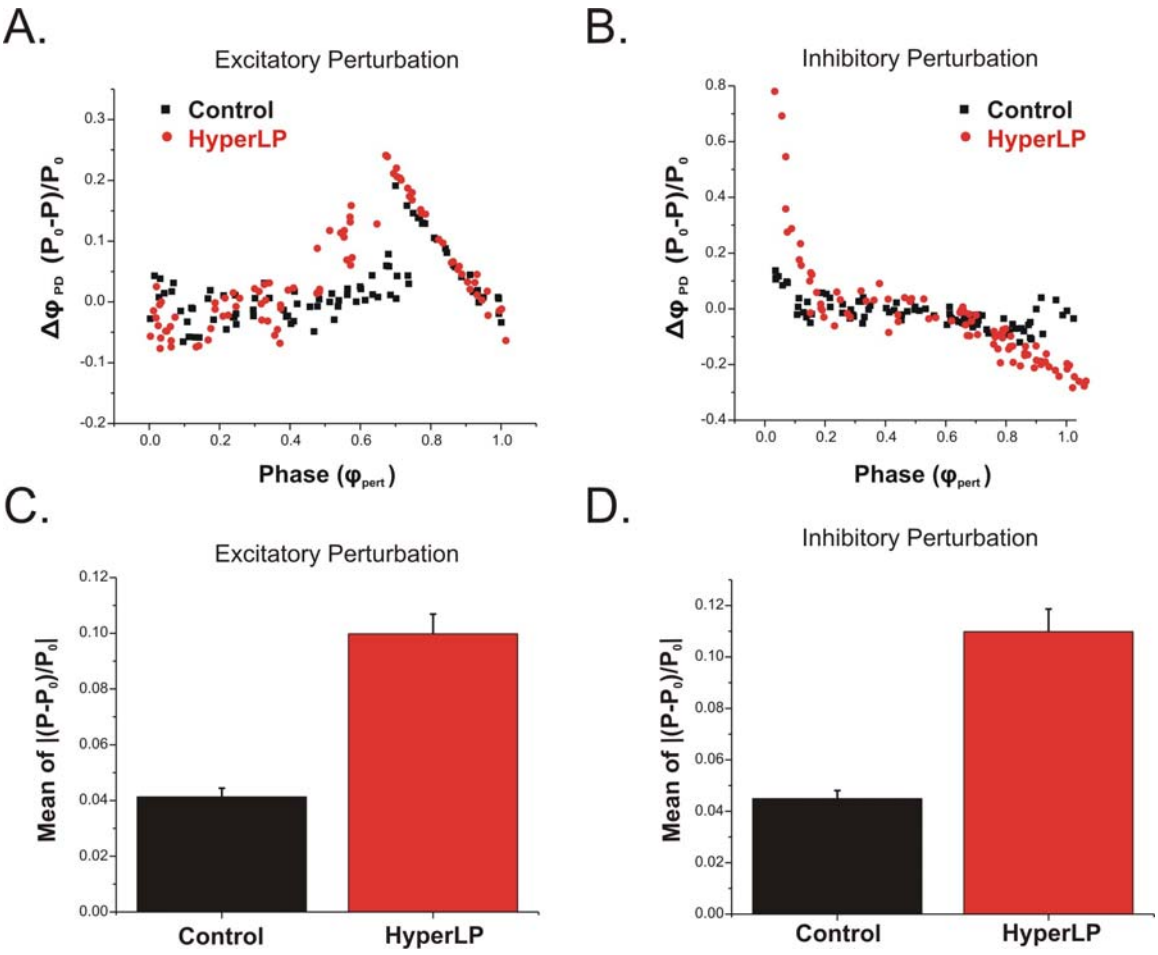
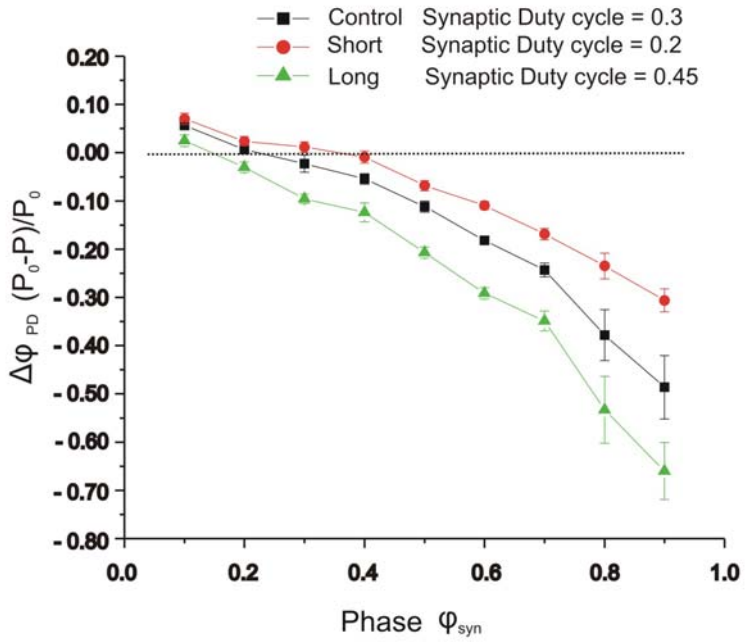


Figure 3.5 The LP to PD synapse attenuated the disturbing influence of extrinsic random perturbations **A.** The current injection of amplitude 2 nA and duration 50 ms was used as the excitatory perturbation. The phase reset ($\Delta\varphi_{PD}$ ((P_0 – P) / P_0)) was plotted against the perturbation phase φ_{pert} (Δt / P_0). Excitatory perturbation at early phase lengthened the period ($P>P_0$) and thus resulted in a negative phase response. At the late phase it shortened the period ($P<P_0$) and resulted in a positive phase response. Without the LP to PD synapse (HyperLP), the phase reset $\Delta\varphi_{PD}$ ((P_0 – P) / P_0) (Red) was more negative at early phase and more positive at late phase compared to control (Black). **B.** -2 nA current injection for 50 ms was used as the inhibitory perturbation. Inhibitory perturbation did the opposite as the excitatory perturbation. It shortened the

period at the early phase and prolonged the period at late phase. In the presence of the LP to PD synapse the phase reset $\Delta\varphi_{PD}$ lies closer to zero compared to HyperLP (Red). **C, D.** Comparison of the phase reset between control and removal of the LP to PD synapse. When the LP to PD synapse was removed (HyperLP) the mean of the absolute value of the phase reset ($|(P_0 – P) / P_0)|$) was significantly larger compared to control in response to excitatory perturbation (Figure 3.5C) or inhibitory perturbation (Figure 3.5D) (t-test, $p<0.05$ $n=4$).

A.



B.

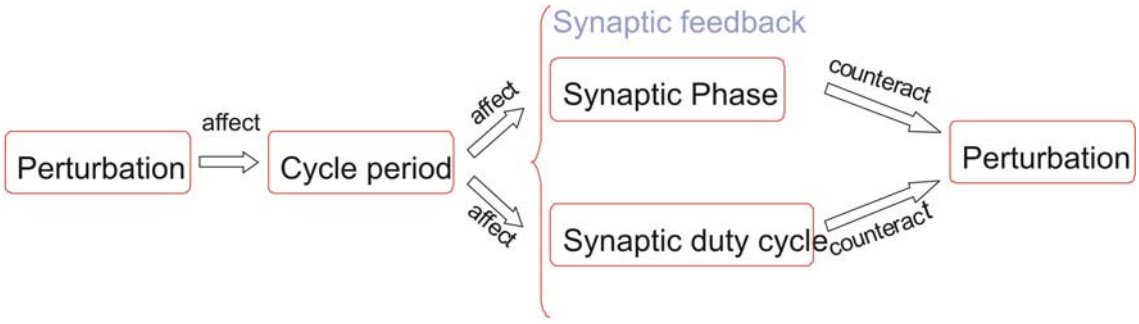


Figure 3.6 A. Synaptic-PRC with different synaptic duty cycles. The different synaptic durations (300 nS, E_{rev}=-80, shown as different DC_{syn}. control: black 0.3; short: red 0.2; long: green 0.45) were injected into pacemaker neuron PD when LP-to-PD synapse is removed by injecting ~-5 nA current. In three cases, the synapse at an early phase of the cycle shortened the period and resulted in positive phase reset ($\Delta\varphi_{PD}$ > 0). The synapse at a late phase of the cycle, it lengthened the cycle. A longer DC_{syn} caused a downward shift of the synaptic-PRC and A shorter DC_{syn} lead to an upward shift of the Synaptic-PRC. (N=6) **B.** The flow chart represented the sequential events in explaining how the feedback synapse counteracts the perturbation.

Chapter 4

The role of neuromodulation in promoting stability of pacemaker neurons rhythm period

Introduction

CPGs are subject to widespread regulation by neuromodulators. Endogenously released neuromodulators are necessary for the proper operation of CPGs in mammalian systems (Pena and Ramirez, 2002; Kiehn, 2006). Neuromodulation is also critically important in reconfiguring a network to produce a multitude of outputs and coordinating interactions between related CPGs (Selverston, 2005; Dickinson, 2006; Saideman et al., 2007). Neuromodulators exert their effects on the rhythmic activity of CPGs on individual neurons or on the synapses between them (Selverston, 2005; Marder and Bucher, 2007). In the STNS of the crab *Cancer borealis*, the well-defined pyloric neuronal circuit is subject to neuromodulation by over 20 neuromodulators (Marder et al., 2005; Marder and Bucher, 2007). In the pyloric network, neuromodulators influence every neuron and every synapse (Marder and Bucher, 2007). Consequently, the same network can be reconfigured into different outputs.

Rhythmic motor movements often rely on stable input from CPG networks that generate the underlying oscillations. The CPG is generally a robust system (Selverston and Ayers, 2006). After it has been disturbed, the CPG can return to its ongoing trajectory. One important parameter of the neuronal output in the

rhythmic activity is its frequency (or equivalently period). Several monoamines have been shown to change the cycle frequency in the pyloric circuit by targeting pacemaker neurons or synaptic connections (Ayali and Harris-Warrick, 1999). Not only the cycle period itself is important, but the stability is also required for proper function in rhythmic activity. Inhibitory synapses play a critical role in maintaining the dynamic stability of neural activity (Elson et al., 1999; Mamiya and Nadim, 2004; Sieling et al., 2009). The LP to PD synapse gives the only inhibitory feedback to the pacemaker group. It has been shown that the LP to PD synapse is involved in controlling the pyloric period (Manor and Nadim, 2001; Mamiya and Nadim, 2004).

In this chapter, we examine the hypothesis that neuromodulator proctolin promotes stability of network oscillations but has little effects on the oscillation frequency. In chapter 2, we showed that proctolin can enhance the LP to PD synapse and changes the short-term dynamics of this synapse. Also, we have previously demonstrated that the LP to PD synapse acts to stabilize oscillation by reducing the variability in cycle period (chapter 3). Based on these results, we explored the role of the modulatory neuropeptide proctolin on the stability of oscillations in the pyloric network. Our results suggest that proctolin, through its influence on the LP to PD synapse, plays an active role in stabilizing the pyloric network oscillation. Although the actual role of neuromodulation in the intact animal remains unknown, our findings would provide insight into understanding the functional effects of specific synaptic changes in network output.

Materials and Methods

See materials and methods in chapter 3.

Additional methods:

Random noise currents (0.5 nA 20 ms 5Hz with Poisson distribution) were injected into the pacemaker neuron PD using the Scope software when the coefficient of variation of the pyloric period was compared in control and proctolin.

Phase-plot analysis.

Phase-plots were plotted using DataMaster 2.1 software. It was used to demonstrate the average percentage of any given neuron's burst duration in a cycle (Hooper and Marder, 1987). The first action potential of the PD neuron burst was set as phase 0 and the first action potential of the next PD neuron burst as phase 1.

Results

The effects of proctolin on coefficient of variation of the pyloric period

We have characterized the effects of proctolin on the LP to PD synapse in chapter 2. The results showed that proctolin strongly enhances the LP to PD synapse and modulates its dynamics. In chapter 3, we showed that the LP to PD synapse stabilizes the pyloric rhythm. As a consequence of its modulation, what is the functional role of proctolin in maintaining the network output during the pyloric ongoing rhythm? To address this question, we first compared the variability of the pyloric period in control condition and in the presence of 10^{-6} M proctolin when the LP to PD synapse was kept or removed. Under all conditions, random noise currents (0.5 nA 20 ms 5Hz with Poisson distribution) were injected into the pacemaker neuron PD (Figure 4.1A, Noise traces) in order to increase the variability of the pyloric rhythm. The simultaneous recordings of the *pdn*, LP and PD neurons are shown in Figure 4.1A. The recording of the *pdn* was used to measure the pyloric period. The LP to PD synapse was removed by hyperpolarizing the LP neuron (Figure 4.1A, bottom panel, without-synapse) or kept (Figure 4.1A, top panel, with-synapse) in control and proctolin treated condition. To compare the variability of the pyloric period under all conditions, 40-60 period cycles were measured within each preparation. We then calculated coefficient of variations (CV). Figure 3.2B shows that there was no significant change in the mean period under various conditions (two-way Repeated Measures ANOVA $p>0.05$). When the LP to PD synapse was removed (without-

synapse), the mean coefficient of variation was significantly larger compared to with-synapse under control, proctolin and wash conditions (two-way Repeated Measures ANOVA $p<0.05$). The mean coefficient of variation was significantly smaller in proctolin compare to control, but not when the LP neuron was hyperpolarized. This suggested that proctolin reduced the variability of the pyloric period in the presence of the LP to PD synapse.

The effects of proctolin on the activity of the LP neuron

Synaptic transmission is dependent upon the activities of the presynaptic neuron. Modulation of the presynaptic neuron can result in a change in the functional strength and timing of synaptic transmission. Therefore, to study the significance of the LP to PD synapse modulation in the ongoing rhythm, we examined activities of the LP neuron with and without proctolin treatment. Figure 4.2 is a phase-angle plot (see Material and Methods) and shows the phase relations of the PD and LP neurons firing patterns during control condition, in the presence of proctolin and after washout. The data were pooled from six experiments. The LP neuron started firing a little earlier and ended slightly later in the cycle under proctolin treated condition compared to control (Figure 4.2), but the onset and offset of the LP burst were not significant (one-way ANOVA, $P>0.05$, $N=6$). The relative duration of the LP burst increased from 0.225 ± 0.0283 in control to 0.278 ± 0.0156 in proctolin (mean ± SEM). Proctolin significantly prolonged the relative duration of the LP burst (one-way ANOVA, $P<0.05$, $N=6$). However, there was no significant change in the phase duration of the PD burst.

Figure 4.3A shows simultaneous intracellular recordings from PD and LP neurons during one LP neuron burst in control and in 10^{-6}M proctolin. The amplitude of the LP to PD synapse is larger in the presence of proctolin compared to control. Figure 4.3B demonstrates that proctolin caused a significant increase in the number of LP neuron spikes per burst (one-way ANOVA, $P<0.05$, N=6). The mean number of LP spikes per burst in control condition was 5.580 ± 1.06 and in proctolin treated condition was 9.150 ± 0.754, respectively (mean ± SEM; n=6; Figure 4.2B). Since the increase in LP burst length was associated with the increase in the number of LP spikes per bursts, we examined the spike density of the LP burst. Figure 4.2 demonstrates that proctolin significantly increased the spike density of the LP burst compared to control (one-way ANOVA, $P<0.05$, N=6). Since the onset and offset of the LP burst were not apparently changed by proctolin, it suggested that the main effect of proctolin on the LP to PD synapse was the increase in spike density of the LP burst.

The effects of proctolin on phase response curve

Given the strong effect of proctolin on the LP to PD synapse, we investigated how proctolin reshaped the phase response curve (PRC) of the PD neuron via the LP to PD synapse. To address this question, we constructed and compared the PRCs under different conditions (Figure 4.4A): in control with the LP to PD synapse active (control with Syn, black) or inactive (control without Syn, gray);

and with proctolin treatment with the LP to PD synapse active (proctolin with Syn, red) or inactive (proctolin without Syn, pink). A short hyperpolarizing current pulse (-2 nA and 50 ms duration) was injected as an inhibitory perturbation (Figure 4.4A, left panel) and a depolarizing pulse (2 nA and 50 ms duration) as an excitatory perturbation (Figure 4.4A, right panel). In response to removing the LP to PD synapse, the pyloric period became longer when perturbed with hyperpolarizing pulses and became shorter with depolarizing pulses at late phase in both control and proctolin. At early phases, the changes were not apparent. In order to evaluate the effect of proctolin on the PRCs, we compared the mean absolute value of the phase reset ($|(P_0 - P) / P_0|$) over all the phases, which indicates the perturbation effect (Figure 4.4B). It showed that the LP to PD synapse significantly reduced the effect of perturbations in both control and proctolin (one-way ANOVA, * $P<0.05$, $N=7$). There was no significant change in perturbation effect between control and proctolin in the absence of the LP to PD synapse. However, proctolin significantly reduced the absolute value of the phase reset in the presence of the LP to PD synapse (Figure 4.4B; one-way ANOVA, *$P<0.05$, $N=7$). This suggests that proctolin promoted the stability of the cycle period of the rhythm through its enhancement of the LP to PD synapse.

Discussion

We showed proctolin did not change the cycle period of the pyloric rhythm over six preparations (Figure 4.1B). Previous studies showed that when the anterior inputs (*stn*) were blocked, proctolin increased the cycle frequency (Hooper and Marder, 1987). It was also reported that with the *stn* intact, proctolin does not cause significant changes in cycle period (Dickinson et al., 2008). It is believed that the effects of many neuromodulators on the STNS are state-dependent (Nusbaum and Marder, 1989a, b). In our case, proctolin has different effects on the cycle period under various preparations. Proctolin increased the cycle period when the rhythm was slow. In some cases, it had no effect. The effects of proctolin could be state-dependent.

Figure 4.1C, demonstrated that proctolin reduced the variability of the cycle period of the pyloric rhythm. We showed that this effect is due to the modulations of proctolin on the LP to PD synapse. This synapse gives inhibitory input to the pacemaker group. In Figure 4.3A, also in chapter 3, we showed that proctolin strongly enhanced the LP to PD synapse. Thus, this synapse decreases the input resistance of the pacemaker group further, resulting in preventing other input noises from changing the membrane potential of the pacemaker. The decrease in input resistance is due to the strengthened synaptic current by proctolin, which occurred for a fraction of the cycle. Therefore, this partially explains that the LP to PD synapse acts to reduce the sensitivity of the

pacemaker to external noise and maintains the stability of the cycle period of the pyloric rhythm. When the LP to PD synapse was removed, proctolin didn't cause significant change in the coefficient of variation. However, the p-value was roughly small. Proctolin might also stabilize the rhythm by modulating the intrinsic properties of the pacemaker neurons.

In chapter 2, we showed that proctolin strengthened the LP to PD synapse in response to the same presynaptic input. Figure 4.3C demonstrated that proctolin increased the spike density of the LP burst, which then evoked more spike-mediated component of the LP to PD synapse. Therefore, the LP to PD synapse can be enhanced more in the ongoing rhythm. Previous studies of synaptic PRC showed that stronger inhibitory inputs produce more phase advances at early phase and caused more phase delays at late phase, although this effect would saturate as the input strength increased (Prinz et al., 2003; Thirumalai et al., 2006). In chapter 3, we used the synaptic PRC to explain the stabilizing effect of the LP to PD synapse on the pyloric period. When a perturbation caused a change in the cycle period, it changed the synaptic phase and duty cycle and resulted in a compensatory change in the cycle period. This compensatory change was more effective when the enhanced LP to PD synapse altered the shape of the synaptic PRC. Overall, these results revealed a previously unknown function of proctolin that it plays an active role in stabilizing the pyloric network oscillation through its influence on the LP to PD synapse.

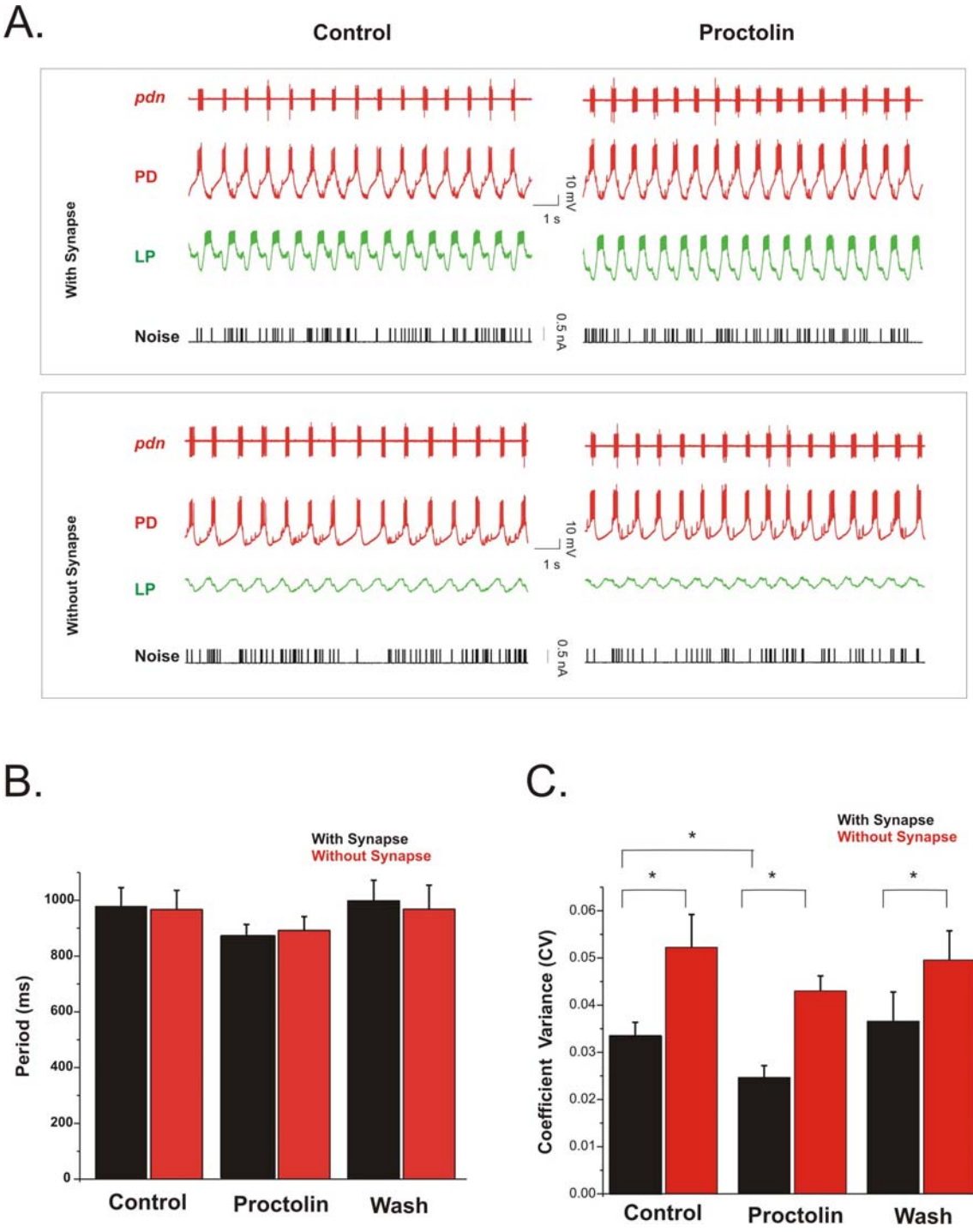


Figure 4.1 The pacemaker neuron's oscillating variability was reduced in the presence of 10^{-6} M proctolin. **A.** Extracellular recording traces from pyloric dilator nerve (*pdn*), and intracellular voltage traces from the PD and LP neurons indicate the activity of pyloric circuit neurons. Recordings were taken when 0 nA (With Synapse) or 5 nA (Without

Synapse; LP was hyperpolarized to remove the LP to PD synapse). Currents were injected in both control (left panel) and in the presence of 10^{-6} M proctolin (left panel). Under all conditions, random noise currents (0.5 nA 20 ms 5Hz with Poisson distribution) were injected into the pacemaker neuron PD (Noise traces) **B.** The pyloric period was averaged over 40 to 60 cycles from the *pdn* for each preparation. There was no significant change in the mean pyloric period under any conditions (N=6)**. C.** The coefficient of variation was bigger without the LP to PD synapse (Without-Synapse) compared to With-Synapse under control, proctolin and wash conditions. The variability of the pyloric rhythm period was significantly in proctolin condition when the LP to PD synapse was kept (two-way ANOVA, $P<0.05$, N=6).

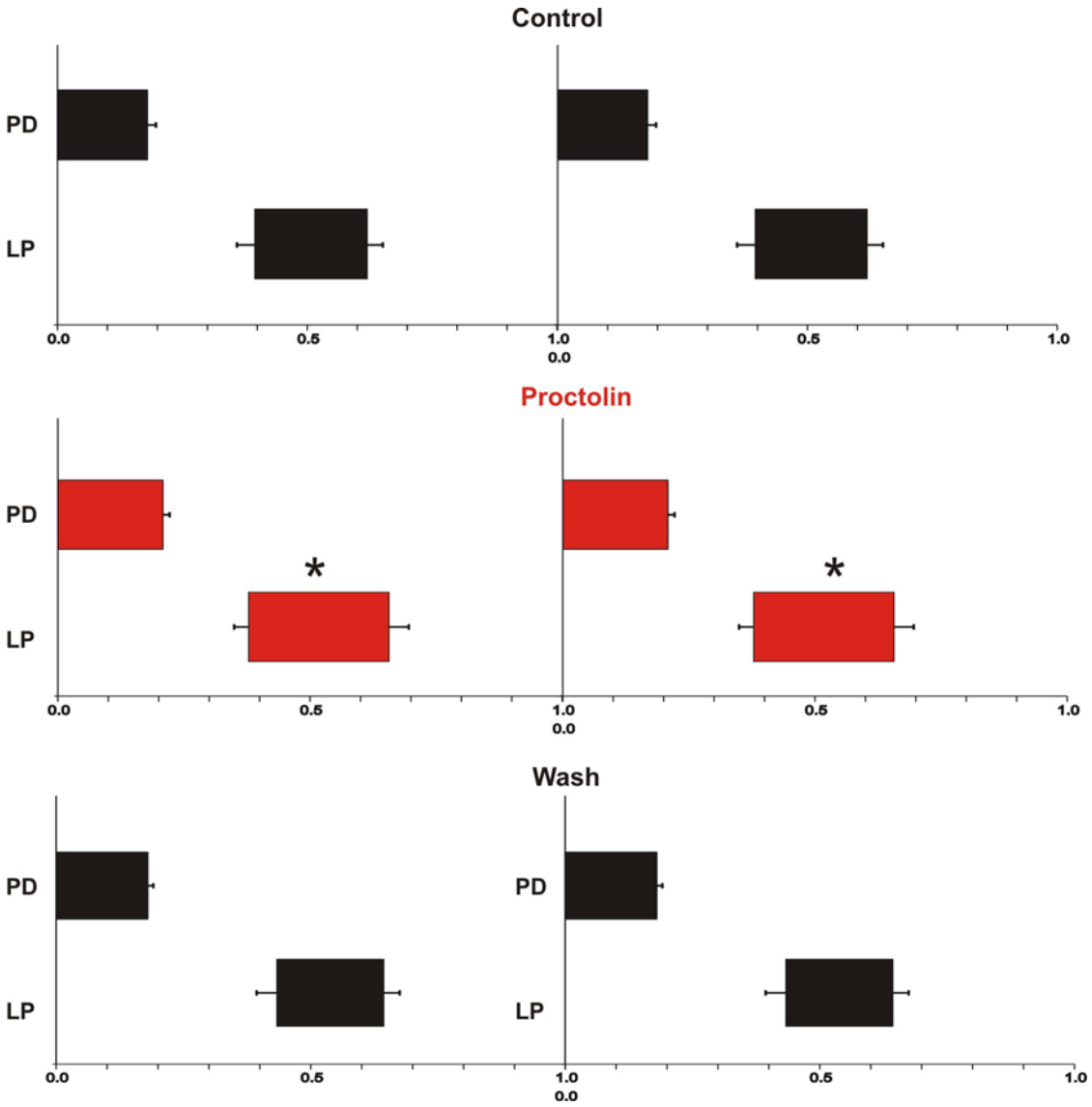


Figure 4.2 The effects of proctolin on the phase of the LP neuron. Panels show average phase plots of the activity of the LP and PD neurons in two cycles. Control (first panel); 10^{-6} M proctolin (second panel); wash (third panel). The relative duration of LP neuron burst in proctolin was significantly different from control and wash conditions (one-way ANOVA, $P<0.05$, $N=6$).

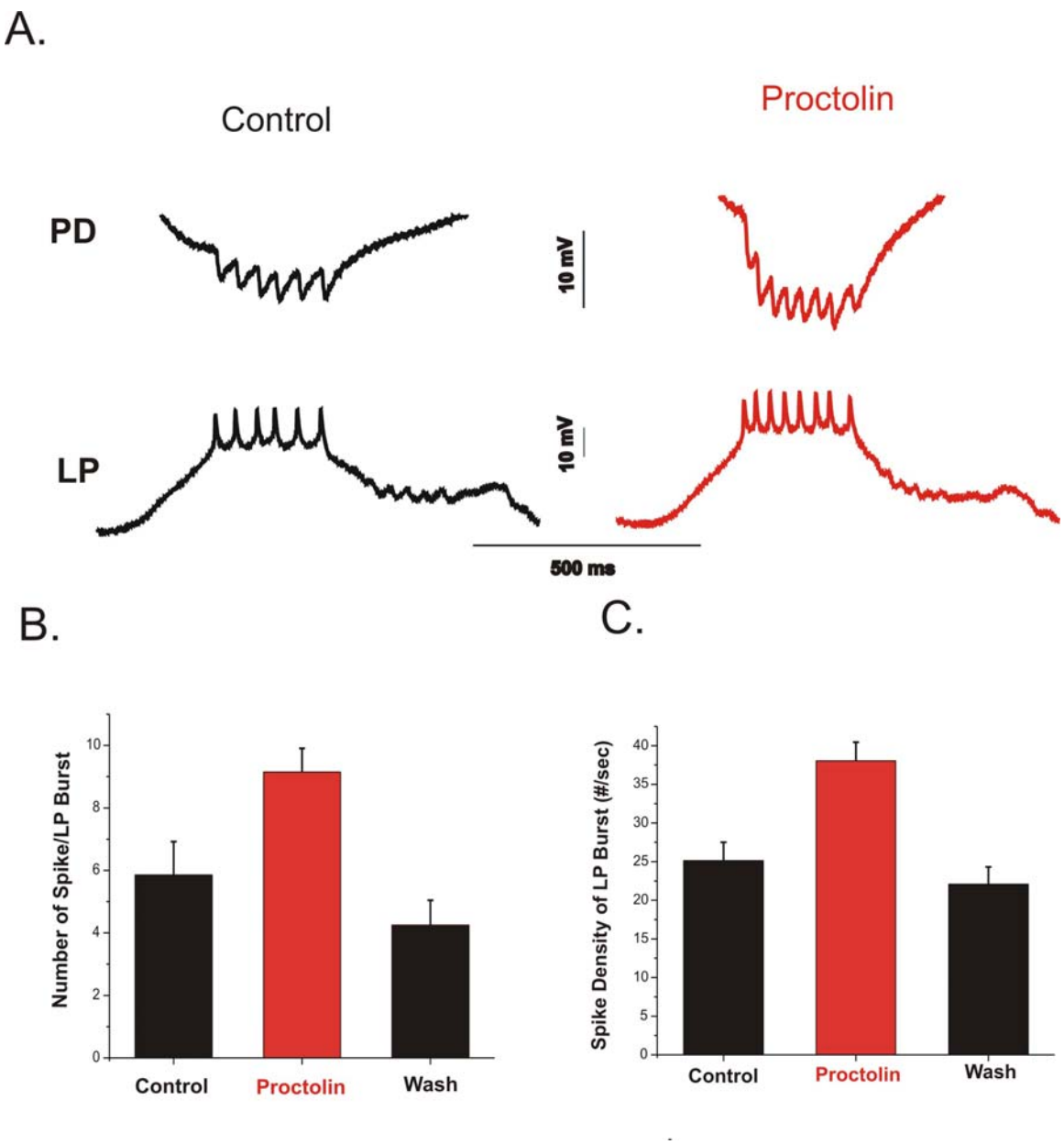


Figure 4.3 The effects of proctolin on the activities of the LP neuron and the LP to PD synapse. **A.** Intracellular recordings from the LP and PD neurons in control and in the presence of 10^{-6}M proctolin. The LP to PD synapse was larger in proctolin. **B.** The mean number of spikes per LP burst in proctolin was significantly larger compared to control and wash (one-way ANOVA, $P<0.05$, $N=6$). **C.** Spike density was set by the ratio of the spike number over burst duration. Proctolin significantly increased the mean spike density compared to control and wash conditions (one-way ANOVA, $P<0.05$, $N=6$).

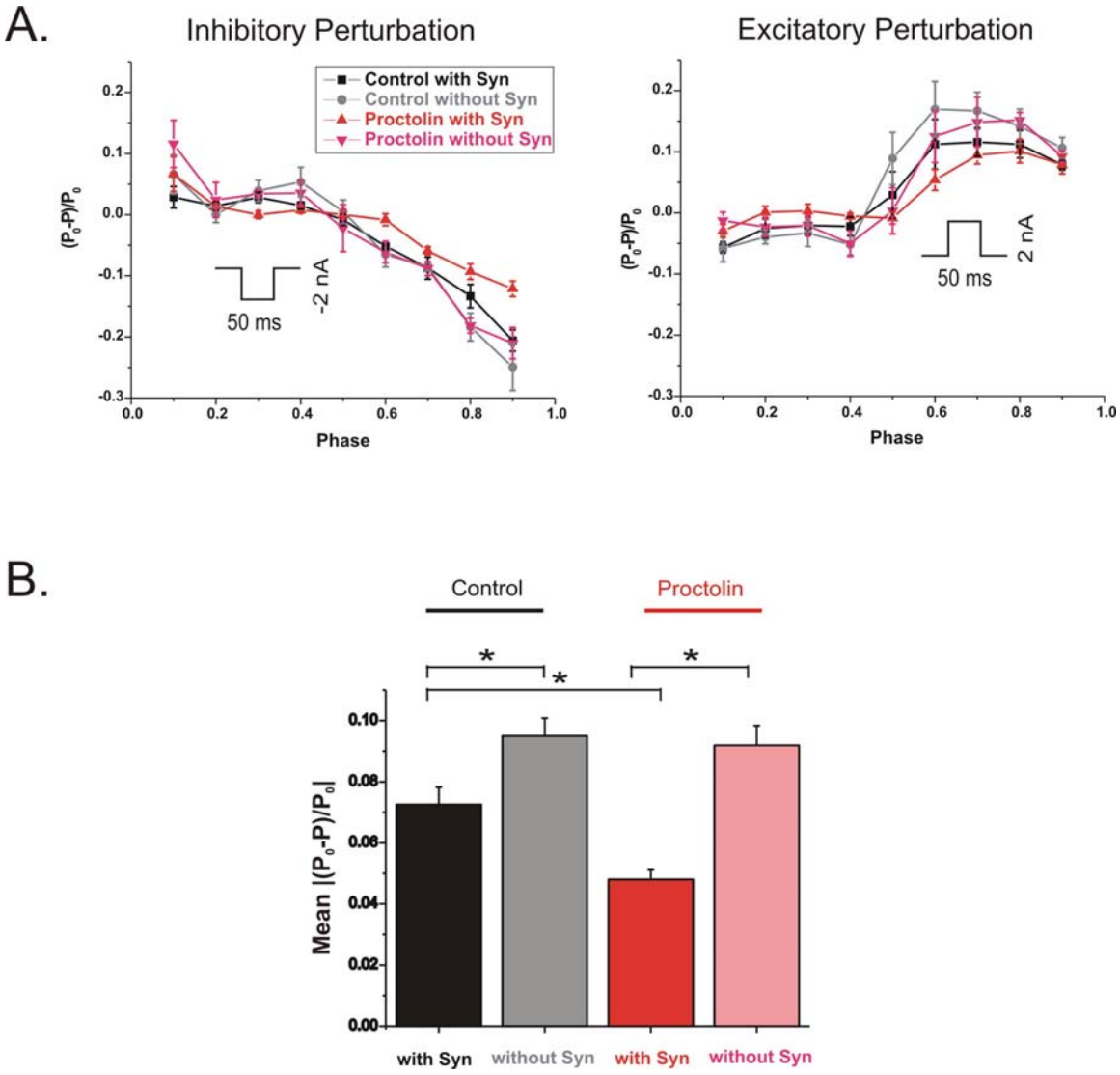


Figure 4.4 Proctolin reduced the effect of extrinsic fast perturbations via its enhancement of the LP to PD synapse. **A.** The current injection of -2 nA or +2 nA with a duration of 50 ms was used as the inhibitory (left panel) or excitatory perturbation, respectively. The PRCs were constructed under various conditions: in control with the LP to PD synapse active (control with Syn, black) or inactive (control without Syn, gray); in proctolin with the LP to PD synapse active (proctolin with Syn, red) or inactive (proctolin without Syn, pink). In response to removing the LP to PD synapse the pyloric period lengthened when perturbed with hyperpolarizing pulses and shortened with

depolarizing pulses at late phase in both control and proctolin. **B.** Comparison of the absolute value of the reset phase ($|(P_0 – P) / P_0|$) over all stimulus phases. Color coding is the same as in **A**. The LP to PD synapse significantly reduced the effect of perturbations in both control and in the presence of proctolin (one-way ANOVA, * $P<0.05$, $N=7$). There was no significant change in the perturbation effect between control and proctolin treated conditions in the absence of the LP to PD synapse. Proctolin significantly reduce the perturbation effect in the presence of the LP to PD synapse (one-way ANOVA, *$P<0.05$, $N=7$).

Chapter 5

General Discussion

Summary and Overview

The primary goal of this thesis was to characterize the effects of the neuropeptide proctolin on a key synapse in an oscillatory neuronal network and the functional role of this synapse and its modulation in shaping network output. To achieve this goal, I used the pyloric circuit of the stomatogastric nervous system of the crab *Cancer borealis* as a model oscillatory neural system. First, I examined the effects of proctolin on the strength and dynamics of the lateral pyloric (LP) neuron to the pyloric dilator (PD) neurons. I also looked for the mechanisms underlying the neuromodulatory effects of proctolin. Second, I determined the roles of the LP to PD synapse in the context of ongoing pyloric rhythm and possible mechanisms of these roles. Third, I investigated how the inhibitory feedback synapse shapes the network output when the properties of this synapse are altered by proctolin.

The main conclusions of this thesis were the following:

Chapter 2:

1. The results showed that the spike-mediated component of the LP to PD synapse was enhanced by bath-applied proctolin using two different experimental protocols.

2. Increasing the background calcium might cause the increase of the spike-mediated IPSP amplitude as the membrane potential increases, but not necessarily through Ni^{2+}-sensitive low-threshold calcium channels.
3. The spike-mediated component of the LP to PD synapse exhibited short-term depression in both control and proctolin. However, there was no difference in depression between control and proctolin treated conditions.
4. Proctolin strengthened the graded component of the LP to PD synapse. Application of proctolin caused facilitation of the LP to PD synapse with an injection of low amplitude depolarization steps. This facilitation is associated with a slow inward Ca^{2+}-like current.
5. Proctolin enhanced the LP to PD synapse in the context of ongoing pyloric rhythm.
6. In the presence of proctolin, the postsynaptic response to glutamate puff in the PD neurons is strengthened.

Chapter 3

1. The LP to PD synapse had no apparent effect on the mean period of the pyloric rhythm. However, the coefficient of variance increased when the LP to PD synapse was removed.
2. In the presence of the LP to PD synapse, the phase response curve (PRC) lay closer to zero. The LP to PD synapse is required to stabilize the pacemaker's oscillation and to reduce the effect of perturbations.

3. We used synaptic-PRC and its relationship with the synaptic phase and synaptic duty cycle to explain how the LP to PD synapse counteracts the effect of perturbation.

Chapter 4

1. The coefficient of variation of the pyloric cycle period is significantly smaller in proctolin treated than in control conditions. When the LP to PD synapse is functionally removed, the coefficient of variance increased in both control and proctolin treated conditions.
2. PRC analysis demonstrated that proctolin reduced the effects of extrinsic perturbation on the pacemaker neurons.
3. Proctolin played an active role in stabilizing the pyloric network oscillation through its influence on the LP to PD synapse.

Neuromodulation of short-term synaptic dynamics

We studied two forms of short-term synaptic dynamics: short-term depression, a decrease in synaptic strength and facilitation, an increase in synaptic strength. Both forms of synaptic dynamics affect the synaptic strength rapidly in a use-dependent manner. A variety of synapses show both short-term facilitation and depression. Short-term depression and facilitation have been shown to play roles in behavioral habituation and sensitization (Stopfer and Carew, 1996; Zucker, 1999). Recently, filtering functions have also been suggested (Lisman, 1997; Zador and Dobrunz, 1997; Dittman et al., 2000; Lindner et al., 2009). Several studies have demonstrated that short-term dynamics are involved in neural information processing and in the control of network activity (Bertram, 1997; Buonomano, 2000; Matveev and Wang, 2000; Nadim and Manor, 2000; Hanson and Jaeger, 2002; Lewis and Maler, 2002). Neuromodulation is critically important in complex neuronal processing. Many studies such as those in the mammalian cortex (Gil et al., 1997; Giocomo and Hasselmo, 2007), and the lamprey spinal cord (Parker and Gilbey, 2007) have showed that synaptic short-term dynamics are subject to neuromodulation.

In the case of the crab *Cancer borealis*, the well-defined pyloric neuronal circuit is subject to neuromodulation by over 20 neuromodulators (Marder et al., 2005). We focused on the neuromodulatory effects of a well-characterized neuropeptide, proctolin. Our results showed that in the presence of proctolin,

graded component of an inhibitory feedback synapse exhibit two different forms of short-term dynamics, which are dependent on the presynaptic input amplitude. In contrast, proctolin did not cause significant changes in short-term dynamics of spike-mediated component compared to control, although it exhibited short-term depression in both cases. Graded synaptic transmission is the major form of synaptic communication among pyloric neurons (Raper, 1979; Graubard et al., 1983; Hartline et al., 1988). This might explain why neuromodulation of short-term dynamics targets the graded component of the key synapse. Not only does the crustacean use graded synapse, but other areas of the mammalian CNS, such as retina and other sensory networks (Burrone and Lagnado, 2000; Prescott and Zenisek, 2005) and circuits of the cortex and hippocampus (Alle and Geiger, 2006; Shu et al., 2006) also manifest the mechanisms underlying graded synaptic transmission. Therefore, insights gained from the crustacean could be applied to these mammalian nervous systems.

The effects of proctolin on the short-term dynamics of the LP to PD synapse are dependent on the presynaptic amplitude. A switch from depression to facilitation might allow the pyloric network to maintain network stability and subsequently, its rhythmic motor output. We suggest that depression caused by high presynaptic amplitudes and facilitation by low presynaptic amplitudes can be used to buffer synaptic strength in response to changes in the amplitude of oscillations of the presynaptic neuron. Thus, large variations in the LP neuron do not produce large amplitude changes. Facilitation at low presynaptic amplitudes and depression at

high presynaptic amplitudes act to stabilize the effect of the feedback synapse from the LP neuron to the pacemaker neurons.

Mechanisms underlying the facilitation caused by proctolin

The crustacean pyloric network has been studied for more than four decades. The pyloric network of crustacean has proved to be a very useful model system for understanding the mechanism underlying rhythmic oscillation in neural circuits and their neuromodulation (Marder and Bucher, 2007). Using the pyloric circuit, elucidating the mechanisms that control short-term synaptic plasticity will provide information on how neurons process inputs. We found that proctolin switches the dynamics of the graded component of the LP to PD synapse from depression to facilitation when the presynaptic LP neuron was stimulated at low amplitudes. Unlike long-term potentiation, which has been shown to use both presynaptic and postsynaptic mechanisms (Lev-Ram et al., 2002; Sugiyama et al., 2008), short-term facilitation is generally governed by the presynaptic neuron (Zucker and Regehr, 2002). This conclusion is supported by our experimental results, which showed that the facilitation of the LP to PD synapse is correlated with the slow activation of a presynaptic Mn^{2+}-sensitive inward current. This suggests that it is a slowly accumulating Ca^{2+} current activated by proctolin. It is still not clear how proctolin causes this accumulation. One of the goals of our laboratory is to build a working model using experimental data. Using a mechanistic model of synaptic release, we have shown that the actions of proctolin on the LP to PD synapse

can be explained by the activation of low-threshold calcium current that has slow activation and inactivation kinetics (Zhou et al., 2007). There might be other possible mechanisms underlying the actions of proctolin. One possible mechanism is that non-specific channels activated by proctolin were permeable for calcium, and entry of calcium through these channels caused the accumulation. To clarify these two mechanisms, we might be able to examine through which channels proctolin causes the accumulation of calcium using Cd^{2+}, since Cd^{2+} can block calcium channels while exhibiting no effect on non-specific channels activated by proctolin (Golowasch and Marder, 1992).

Proctolin exerts its effects through binding to G-protein coupled receptors and subsequently activating downstream signaling pathways (Johnson et al., 2003). Studies in insect and crayfish muscle contractions showed that using second messengers IP_3 and cAMP, proctolin can increase intracellular Ca^{2+} concentrations through the modulation of voltage-dependent or voltage-independent channels (Baines et al., 1990; Bishop et al., 1991; Wegener and Nassel, 2000). However, in the STNS, much less is known about the second messenger pathway underlying the modulation of proctolin. Several studies have shown that proctolin does not cause apparent increase of cAMP levels in STG neurons (Flamm et al., 1987; Hempel et al., 1996). Also, our preliminary data showed that application of cAMP did not mimic the facilitation caused by proctolin. Recent studies have revealed that the second messenger cGMP is involved in the potentiation of crustacean muscle contraction induced by proctolin

(Philipp et al., 2006). The signal transduction pathways activated by proctolin still remain for further investigation.

Convergent and divergent actions of proctolin at network levels

There are only 26 neurons in the STG of the crab *Cancer borealis*, but they are subject to modulation from over 20 neuromodulatory inputs (Marder and Thirumalai, 2002). Swensen et al. showed that proctolin along with five other neuromodulators, converge to activate the same voltage-dependent current (inward non-specific cation current) (Swensen and Marder, 2001). Our results showed that proctolin reduced the variability of pyloric oscillations through strengthening of the LP to PD synapse. A recent study of the lobster *H. americanus* showed that RPCH, a neuromodulator that increase the strength of the LP to PD synapse, has little effect on the overall pyloric period (Thirumalai et al., 2006). It is possible that RPCH also provides a stabilizing effect on the pyloric cycle period indicating a converging action of these two neuromodulators on a functional level.

Several neurons have been found to release proctolin. Three pairs of proctolin-containing interneurons send inputs to the STG via the *stn*. One of them is the modulatory projection neuron (MPN), which also contains GABA (Coleman et al., 1992; Blitz et al., 1999). The other two pairs consist of modulatory commisural neuron 1 (MCN1) and modulatory commisural neuron 7 (MCN7). These proctolin

containing neurons can have divergent effects on network outputs through distinct co-modulator complements or via the activation of different projection neurons. Different motor patterns can be elicited from the STG networks by stimulating these proctolin containing neurons (Blitz et al., 1999; Wood et al., 2000). For example, MCN1 stimulation elicits the gastric mill rhythm, and MCN7 stimulation elicits a slower pyloric rhythm dominated by IC bursts (Bartos and Nusbaum, 1997a; Blitz et al., 1999). In either case, the pyloric rhythm is disturbed in some manner. Thus, it might be important to stabilize the pyloric rhythm, which could result from the enhancement of the LP to PD synapse by proctolin. In addition to acting as a neuromodulator released from the projection neurons, proctolin can be released hormonally through the hemolymph (Christie et al., 1995). One of functional roles of hormonal proctolin may also be to stabilize the pyloric rhythm against drastic variability.

Implication of our results for other oscillatory systems

Invertebrate and vertebrate oscillatory networks operate with a similar set of general principles (Marder and Calabrese, 1996). Thus, findings from studies of relatively smaller, more accessible invertebrate CPGs can be translated to the rather larger, vertebrate networks. Our findings underline that modulations of the inhibitory feedback synapse to an oscillator can promote the stability of the neuronal network using the pyloric network of the crab *Cancer borealis* as a model system. In the face of noise resulting from channel gating fluctuations,

noisy synaptic transmission, and background network activity (Tateno and Robinson, 2007), the oscillatory network must maintain its reliability. For example, if the respiratory oscillator fails to recover its rhythm, this can cause apnea and aspiration in response to artificial perturbation (Paydarfar and Buerkel, 1995). Many principles underlying network activities in the crustacean STNS have been found to be similar to those in the mammalian respiratory CPG (Marder, 2000; Ramirez et al., 2004). While synaptic inhibition is not essential for rhythm generation in the isolated respiratory network (Brockhaus and Ballanyi, 1998), our finding might shed light on exploration of its role in stabilizing the network.

Feedback inhibition plays an important role in regulating and stabilizing the neuronal network in the brain (Tepper et al., 2004; Gonzalez-Burgos and Lewis, 2008). In the cortex, inhibitory interneurons can be activated by associational pyramidal neurons activity and results in sending feedback inhibition to pyramidal neurons (Patil and Hasselmo, 1999; Giocomo and Hasselmo, 2007). For example, an increase in the amplitude of GABA mediated inhibitory postsynaptic currents (IPSCs) promotes the stability of gamma oscillations in the rat hippocampus (Stenkamp et al., 2001). Our findings also suggest that modulating the inhibitory feedback synapse can be a useful approach in regulating the stability of neuronal networks. These mechanisms could be applied to the mammalian nervous system such as the feedback or recurrent inhibitory circuits in the cortex or oscillator-driven respiratory CPGs.

Future directions

To further clarify the underlying mechanisms for the short-term synaptic plasticity in the LP to PD synapse, we need to image the dynamic change of intracellular calcium ion concentration. These experiments would determine whether modulatory effects of proctolin on LP and PD synapses are correlated with changes in calcium concentration dynamics.

We have shown that the pyloric rhythm is disturbed by activation of the gastric mill rhythm. The gastric mill rhythm could be elicited without activation of proctolin neurons. Then preparation is treated with proctolin. We can examine whether proctolin reduces variability of the pyloric period.

Despite the current findings of neuromodulatory effects of proctolin, much remains to be investigated. Identified descending projection neurons (such as MCN1, MPN) that release proctolin are also known to release other co-modulators. The current findings could be stepping stones for investigating the actions of other neuromodulators, either individually or in an orchestrated manner.

References

Alle H, Geiger JR (2006) Combined analog and action potential coding in hippocampal mossy fibers. Science 311:1290-1293.

Angstadt JD, Calabrese RL (1991) Calcium currents and graded synaptic transmission between heart interneurons of the leech. J Neurosci 11:746-759.

Ayali A, Harris-Warrick RM (1999) Monoamine control of the pacemaker kernel and cycle frequency in the lobster pyloric network. J Neurosci 19:6712-6722.

Ayali A, Johnson BR, Harris-Warrick RM (1998) Dopamine modulates graded and spike-evoked synaptic inhibition independently at single synapses in pyloric network of lobster. J Neurophysiol 79:2063-2069.

Baimoukhametova DV, Hewitt SA, Sank CA, Bains JS (2004) Dopamine modulates use-dependent plasticity of inhibitory synapses. J Neurosci 24:5162-5171.

Baines RA, Lange AB, Downer RG (1990) Proctolin in the innervation of the locust mandibular closer muscle modulates contractions through the elevation of inositol trisphosphate. J Comp Neurol 297:479-486.

Bartos M, Nusbaum MP (1997a) Intercircuit control of motor pattern modulation by presynaptic inhibition. J Neurosci 17:2247-2256.

Bartos M, Nusbaum MP (1997b) Intercircuit control of motor pattern modulation by presynaptic inhibition. J Neurosci 17:2247-2256.

Basar E (2008) Oscillations in "brain-body-mind"--a holistic view including the autonomous system. Brain Res 1235:2-11.

Bertram R (1997) A simple model of transmitter release and facilitation. Neural Comput 9:515-523.

Bishop CA, Krouse ME, Wine JJ (1991) Peptide cotransmitter potentiates calcium channel activity in crayfish skeletal muscle. J Neurosci 11:269-276.

Blitz DM, Nusbaum MP (1997) Motor pattern selection via inhibition of parallel pathways. J Neurosci 17:4965-4975.

Blitz DM, Christie AE, Coleman MJ, Norris BJ, Marder E, Nusbaum MP (1999) Different proctolin neurons elicit distinct motor patterns from a multifunctional neuronal network. J Neurosci 19:5449-5463.

Bose A, Manor Y, Nadim F (2004) The activity phase of postsynaptic neurons in a simplified rhythmic network. J Comput Neurosci 17:245-261.

Brockhaus J, Ballanyi K (1998) Synaptic inhibition in the isolated respiratory network of neonatal rats. Eur J Neurosci 10:3823-3839.

Bucher D, Prinz AA, Marder E (2005) Animal-to-animal variability in motor pattern production in adults and during growth. J Neurosci 25:1611-1619.

Buonomano DV (2000) Decoding temporal information: A model based on short-term synaptic plasticity. J Neurosci 20:1129-1141.

Burrone J, Lagnado L (2000) Synaptic depression and the kinetics of exocytosis in retinal bipolar cells. J Neurosci 20:568-578.

Buzsaki G (2006) Rhythms of the Brain.207-230.

Calabrese RL, Peterson E (1983) Neural control of heartbeat in the leech, Hirudo medicinalis. Symp Soc Exp Biol 37:195-221.

Christie AE, Skiebe P, Marder E (1995) Matrix of neuromodulators in neurosecretory structures of the crab Cancer borealis. J Exp Biol 198:2431-2439.

Coleman MJ, Nusbaum MP, Cournil I, Claiborne BJ (1992) Distribution of modulatory inputs to the stomatogastric ganglion of the crab, Cancer borealis. J Comp Neurol 325:581-594.

Combes D, Meyrand P, Simmers J (1999) Dynamic restructuring of a rhythmic motor program by a single mechanoreceptor neuron in lobster. J Neurosci 19:3620-3628.

Cook DL, Schwindt PC, Grande LA, Spain WJ (2003) Synaptic depression in the localization of sound. Nature 421:66-70.

Cooke IM (2002) Reliable, responsive pacemaking and pattern generation with minimal cell numbers: the crustacean cardiac ganglion. Biol Bull 202:108-136.

Cooper EC, Jan LY (2003) M-channels: neurological diseases, neuromodulation, and drug development. Arch Neurol 60:496-500.

Deeg KE (2009) Synapse-specific homeostatic mechanisms in the hippocampus. J Neurophysiol 101:503-506.

Dickinson PS (2006) Neuromodulation of central pattern generators in invertebrates and vertebrates. Curr Opin Neurobiol 16:604-614.

Dickinson PS, Stemmler EA, Christie AE (2008) The pyloric neural circuit of the herbivorous crab Pugettia producta shows limited sensitivity to several neuromodulators that elicit robust effects in more opportunistically feeding decapods. J Exp Biol 211:1434-1447.

Dittman JS, Kreitzer AC, Regehr WG (2000) Interplay between facilitation, depression, and residual calcium at three presynaptic terminals. J Neurosci 20:1374-1385.

Eisen JS, Marder E (1982) Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. III. Synaptic connections of electrically coupled pyloric neurons. J Neurophysiol 48:1392-1415.

Elson RC, Huerta R, Abarbanel HD, Rabinovich MI, Selverston AI (1999) Dynamic control of irregular bursting in an identified neuron of an oscillatory circuit. J Neurophysiol 82:115-122.

Flamm RE, Fickbohm D, Harris-Warrick RM (1987) cAMP elevation modulates physiological activity of pyloric neurons in the lobster stomatogastric ganglion. J Neurophysiol 58:1370-1386.

Galvan A, Wichmann T (2008) Pathophysiology of parkinsonism. Clin Neurophysiol 119:1459-1474.

Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19:679-686.

Giocomo LM, Hasselmo ME (2007) Neuromodulation by glutamate and acetylcholine can change circuit dynamics by regulating the relative influence of afferent input and excitatory feedback. Mol Neurobiol 36:184-200.

Golowasch J, Marder E (1992) Proctolin activates an inward current whose voltage dependence is modified by extracellular Ca2+. J Neurosci 12:810-817.

Gonzalez-Burgos G, Lewis DA (2008) GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. Schizophr Bull 34:944-961.

Graubard K, Raper JA, Hartline DK (1980) Graded synaptic transmission between spiking neurons. Proc Natl Acad Sci U S A 77:3733-3735.

Graubard K, Raper JA, Hartline DK (1983) Graded synaptic transmission between identified spiking neurons. J Neurophysiol 50:508-521.

Gutierrez GJ, Grashow RG (2009) Cancer borealis stomatogastric nervous system dissection. J Vis Exp.

Hanson JE, Jaeger D (2002) Short-term plasticity shapes the response to simulated normal and parkinsonian input patterns in the globus pallidus. J Neurosci 22:5164-5172.

Harris-Warrick RM, Johnson BR, Peck JH, Kloppenburg P, Ayali A, Skarbinski J (1998) Distributed effects of dopamine modulation in the crustacean pyloric network. Ann N Y Acad Sci 860:155-167.

Hartline DK, Gassie DV, Jr. (1979) Pattern generation in the lobster (Panulirus) stomatogastric ganglion. I. Pyloric neuron kinetics and synaptic interactions. Biol Cybern 33:209-222.

Hartline DK, Russell DF, Raper JA, Graubard K (1988) Special cellular and synaptic mechanisms in motor pattern generation. Comp Biochem Physiol C 91:115-131.

Hartline DKG, K. (1992) Cellular and synaptic properties in the crustacean stomatogastric nervous system. Dymanic Biological Networks: The Stomatogastric Nervous System:31-86.

Hasselmo ME (1995) Neuromodulation and cortical function: modeling the physiological basis of behavior. Behav Brain Res 67:1-27.

Hempel CM, Vincent P, Adams SR, Tsien RY, Selverston AI (1996) Spatio-temporal dynamics of cyclic AMP signals in an intact neural circuitm. Nature 384:166-169.

Hooper SL (1997) Phase maintenance in the pyloric pattern of the lobster (Panulirus interruptus) stomatogastric ganglion. J Comput Neurosci 4:191-205.

Hooper SL (2000) Central pattern generators. Curr Biol 10:R176.

Hooper SL, Marder E (1984) Modulation of a central pattern generator by two neuropeptides, proctolin and FMRFamide. Brain Res 305:186-191.

Hooper SL, Marder E (1987) Modulation of the Lobster Pyloric Rhythm by the Peptide Proctolin. J Neurosci 7:2097-2112.

Ivanov AI, Calabrese RL (2006) Spike-mediated and graded inhibitory synaptic transmission between leech interneurons: evidence for shared release sites. J Neurophysiol 96:235-251.

Johnson BR, Harris-Warrick RM (1990) Aminergic modulation of graded synaptic transmission in the lobster stomatogastric ganglion. J Neurosci 10:2066-2076.

Johnson BR, Hooper SL (1992) Overview of thestomatogastric nervous system. In: Dynamic Biological Networks: The Stomatogastric Nervous System (Harris-Warrick RM, Marder E, Selverston AI, Moulins M, eds), pp 1-30. Cambridge, MA: MIT Press.

Johnson BR, Harris-Warrick RM (1997) Amine modulation of glutamate responses from pyloric motor neurons in lobster stomatogastric ganglion. J Neurophysiol 78:3210-3221.

Johnson BR, Peck JH, Harris-Warrick RM (1995) Distributed amine modulation of graded chemical transmission in the pyloric network of the lobster stomatogastric ganglion. J Neurophysiol 74:437-452.

Johnson EC, Bohn LM, Barak LS, Birse RT, Nassel DR, Caron MG, Taghert PH (2003) Identification of Drosophila neuropeptide receptors by G protein-coupled receptors-beta-arrestin2 interactions. J Biol Chem 278:52172-52178.

Katz PS, Harris-Warrick RM (1990a) Neuromodulation of the crab pyloric central pattern generator by serotonergic/cholinergic proprioceptive afferents. J Neurosci 10:1495-1512.

Katz PS, Harris-Warrick RM (1990b) Actions of identified neuromodulatory neurons in a simple motor system. Trends Neurosci 13:367-373.

Kiehn O (2006) Locomotor circuits in the mammalian spinal cord. Annu Rev Neurosci 29:279-306.

Lev-Ram V, Wong ST, Storm DR, Tsien RY (2002) A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. Proc Natl Acad Sci U S A 99:8389-8393.

Lewis JE, Maler L (2002) Dynamics of electrosensory feedback: short-term plasticity and inhibition in a parallel fiber pathway. J Neurophysiol 88:1695-1706.

Lindner B, Gangloff D, Longtin A, Lewis JE (2009) Broadband coding with dynamic synapses. J Neurosci 29:2076-2088.

Lisman JE (1997) Bursts as a unit of neural information: making unreliable synapses reliable. Trends Neurosci 20:38-43.

Lu J, Dalton JFt, Stokes DR, Calabrese RL (1997) Functional role of Ca2+ currents in graded and spike-mediated synaptic transmission between leech heart interneurons. J Neurophysiol 77:1779-1794.

Mamiya A, Nadim F (2004) Dynamic interaction of oscillatory neurons coupled with reciprocally inhibitory synapses acts to stabilize the rhythm period. J Neurosci 24:5140-5150.

Mamiya A, Nadim F (2005) Target-specific short-term dynamics are important for the function of synapses in an oscillatory neural network. J Neurophysiol 94:2590-2602.

Mamiya A, Manor Y, Nadim F (2003) Short-term dynamics of a mixed chemical and electrical synapse in a rhythmic network. J Neurosci 23:9557-9564.

Manor Y, Nadim F (2001) Synaptic depression mediates bistability in neuronal networks with recurrent inhibitory connectivity. J Neurosci 21:9460-9470.

Manor Y, Nadim F, Abbott LF, Marder E (1997) Temporal dynamics of graded synaptic transmission in the lobster stomatogastric ganglion. J Neurosci 17:5610-5621.

Manor Y, Bose A, Booth V, Nadim F (2003) Contribution of synaptic depression to phase maintenance in a model rhythmic network. J Neurophysiol 90:3513-3528.

Marder E (1976) Cholinergic motor neurones in the stomatogastric system of the lobster. J Physiol 257:63-86.

Marder E (2000) Motor pattern generation. Curr Opin Neurobiol 10:691-698.

Marder E, Eisen JS (1984a) Transmitter identification of pyloric neurons: electrically coupled neurons use different transmitters. J Neurophysiol 51:1345-1361.

Marder E, Eisen JS (1984b) Electrically coupled pacemaker neurons respond differently to same physiological inputs and neurotransmitters. J Neurophysiol 51:1362-1374.

Marder E, Calabrese RL (1996) Principles of rhythmic motor pattern generation. Physiol Rev 76:687-717.

Marder E, Bucher D (2001) Central pattern generators and the control of rhythmic movements. Curr Biol 11:R986-996.

Marder E, Thirumalai V (2002) Cellular, synaptic and network effects of neuromodulation. Neural Netw 15:479-493.

Marder E, Prinz AA (2002) Modeling stability in neuron and network function: the role of activity in homeostasis. Bioessays 24:1145-1154.

Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu Rev Physiol 69:291-316.

Marder E, Hooper SL, Siwicki KK (1986) Modulatory action and distribution of the neuropeptide proctolin in the crustacean stomatogastric nervous system. J Comp Neurol 243:454-467.

Marder E, Bucher D, Schulz DJ, Taylor AL (2005) Invertebrate central pattern generation moves along. Curr Biol 15:R685-699.

Marder E, Manor Y, Nadim F, Bartos M, Nusbaum MP (1998) Frequency control of a slow oscillatory network by a fast rhythmic input: pyloric to gastric mill interactions in the crab stomatogastric nervous system. Ann N Y Acad Sci 860:226-238.

Matveev V, Wang XJ (2000) Differential short-term synaptic plasticity and transmission of complex spike trains: to depress or to facilitate? Cereb Cortex 10:1143-1153.

Maynard DM, Selverston AI (1975) Organization of the stomatogastric ganglion of the spiny lobster. IV. The pyloric system. J Comp Physiol 100.

McCormick DA, Bal T (1997) Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci 20:185-215.

McCrea DA, Rybak IA (2008) Organization of mammalian locomotor rhythm and pattern generation. Brain Res Rev 57:134-146.

Miller JP, Selverston AI (1982a) Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. II. Oscillatory properties of pyloric neurons. J Neurophysiol 48:1378-1391.

Miller JP, Selverston AI (1982b) Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. IV. Network properties of pyloric system. J Neurophysiol 48:1416-1432.

Mulloney B (1987) Neural circuits. In: The Crustacean Stomatogastric System: Springer-Verlag.

Nadim F, Manor Y (2000) The role of short-term synaptic dynamics in motor control. Curr Opin Neurobiol 10:683-690.

Nadim F, Manor Y, Kopell N, Marder E (1999) Synaptic depression creates a switch that controls the frequency of an oscillatory circuit. Proc Natl Acad Sci U S A 96:8206-8211.

Neher E, Sakaba T (2001) Combining deconvolution and noise analysis for the estimation of transmitter release rates at the calyx of held. J Neurosci 21:444-461.

Norris BJ, Weaver AL, Wenning A, Garcia PS, Calabrese RL (2007) A central pattern generator producing alternative outputs: phase relations of leech heart motor neurons with respect to premotor synaptic input. J Neurophysiol 98:2983-2991.

Norris BJ, Weaver AL, Morris LG, Wenning A, Garcia PA, Calabrese RL (2006) A central pattern generator producing alternative outputs: temporal pattern of premotor activity. J Neurophysiol 96:309-326.

Nusbaum MP (2002) Regulating peptidergic modulation of rhythmically active neural circuits. Brain Behav Evol 60:378-387.

Nusbaum MP, Marder E (1989a) A modulatory proctolin-containing neuron (MPN). II. State-dependent modulation of rhythmic motor activity. J Neurosci 9:1600-1607.

Nusbaum MP, Marder E (1989b) A modulatory proctolin-containing neuron (MPN). I. Identification and characterization. J Neurosci 9:1591-1599.

Nusbaum MP, Beenhakker MP (2002) A small-systems approach to motor pattern generation. Nature 417:343-350.

Nusbaum MP, Blitz DM, Swensen AM, Wood D, Marder E (2001) The roles of co-transmission in neural network modulation. Trends Neurosci 24:146-154.

Oleskevich S, Clements J, Walmsley B (2000) Release probability modulates short-term plasticity at a rat giant terminal. J Physiol 524 Pt 2:513-523.

Orchard I, Belanger JH, Lange AB (1989) Proctolin: a review with emphasis on insects. J Neurobiol 20:470-496.

Otis T, Zhang S, Trussell LO (1996) Direct measurement of AMPA receptor desensitization induced by glutamatergic synaptic transmission. J Neurosci 16:7496-7504.

Ozeki H, Finn IM, Schaffer ES, Miller KD, Ferster D (2009) Inhibitory stabilization of the cortical network underlies visual surround suppression. Neuron 62:578-592.

Parker D, Gilbey T (2007) Developmental differences in neuromodulation and synaptic properties in the lamprey spinal cord. Neuroscience 145:142-152.

Patil MM, Hasselmo ME (1999) Modulation of inhibitory synaptic potentials in the piriform cortex. J Neurophysiol 81:2103-2118.

Paydarfar D, Buerkel DM (1995) Dysrhythmias of the respiratory oscillator. Chaos 5:18-29.

Pelletier JG, Lacaille JC (2008) Long-term synaptic plasticity in hippocampal feedback inhibitory networks. Prog Brain Res 169:241-250.

Pena F, Ramirez JM (2002) Endogenous activation of serotonin-2A receptors is required for respiratory rhythm generation in vitro. J Neurosci 22:11055-11064.

Philipp B, Rogalla N, Kreissl S (2006) The neuropeptide proctolin potentiates contractions and reduces cGMP concentration via a PKC-dependent pathway. J Exp Biol 209:531-540.

Pinsker HM (1977) Aplysia bursting neurons as endogenous oscillators. I. Phase-response curves for pulsed inhibitory synaptic input. J Neurophysiol 40:527-543.

Prescott ED, Zenisek D (2005) Recent progress towards understanding the synaptic ribbon. Curr Opin Neurobiol 15:431-436.

Prinz AA, Thirumalai V, Marder E (2003) The functional consequences of changes in the strength and duration of synaptic inputs to oscillatory neurons. J Neurosci 23:943-954.

Rabbah P, Nadim F (2007) Distinct synaptic dynamics of heterogeneous pacemaker neurons in an oscillatory network. J Neurophysiol 97:2239-2253.

Rabbah P, Golowasch J, Nadim F (2005) Effect of electrical coupling on ionic current and synaptic potential measurements. J Neurophysiol 94:519-530.

Ramirez JM, Tryba AK, Pena F (2004) Pacemaker neurons and neuronal networks: an integrative view. Curr Opin Neurobiol 14:665-674.

Raper JA (1979) Nonimpulse-mediated synaptic transmission during the generation of a cyclic motor program. Science 205:304-306.

Saideman SR, Blitz DM, Nusbaum MP (2007) Convergent motor patterns from divergent circuits. J Neurosci 27:6664-6674.

Saideman SR, Christie AE, Torfs P, Huybrechts J, Schoofs L, Nusbaum MP (2006) Actions of kinin peptides in the stomatogastric ganglion of the crab Cancer borealis. J Exp Biol 209:3664-3676.

Saraga F, Balena T, Wolansky T, Dickson CT, Woodin MA (2008) Inhibitory synaptic plasticity regulates pyramidal neuron spiking in the rodent hippocampus. Neuroscience 155:64-75.

Schulz DJ (2006) Plasticity and stability in neuronal output via changes in intrinsic excitability: it's what's inside that counts. J Exp Biol 209:4821-4827.

Selverston AI (1974) Structural and functional basis of motor pattern generation in stomatogastric ganglion of the lobster. Am Zool 74:957-972.

Selverston AI (2005) A neural infrastructure for rhythmic motor patterns. Cell Mol Neurobiol 25:223-244.

Selverston AI, Mulloney B (1974) Organization of the stomatogastric ganglion of spiny lobster. II. Neurons driving the medial tooth. J Comp Physiol 91:33-51.

Selverston AI, Ayers J (2006) Oscillations and oscillatory behavior in small neural circuits. Biol Cybern 95:537-554.

Selverston AI, Russell DF, Miller JP (1976) The stomatogastric nervous system: structure and function of a small neural network. Prog Neurobiol 7:215-290.

Shu Y, Hasenstaub A, Duque A, Yu Y, McCormick DA (2006) Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. Nature.

Sieling FH, Canavier CC, Prinz AA (2009) Predictions of phase-locking in excitatory hybrid networks: Excitation does not promote phase-locking in pattern generating networks as reliably as inhibition. J Neurophysiol.

Skiebe P, Schneider H (1994) Allatostatin peptides in the crab stomatogastric nervous system: inhibition of the pyloric motor pattern and distribution of allatostatin-like immunoreactivity. J Exp Biol 194:195-208.

Starratt AN, Brown BE (1975) Structure of the pentapeptide proctolin, a proposed neurotransmitter in insects. Life Sci 17:1253-1256.

Stenkamp K, Palva JM, Uusisaari M, Schuchmann S, Schmitz D, Heinemann U, Kaila K (2001) Enhanced temporal stability of cholinergic hippocampal gamma oscillations following respiratory alkalosis in vitro. J Neurophysiol 85:2063-2069.

Stopfer M, Carew TJ (1996) Heterosynaptic facilitation of tail sensory neuron synaptic transmission during habituation in tail-induced tail and siphon withdrawal reflexes of Aplysia. J Neurosci 16:4933-4948.

Sugiyama Y, Kawaguchi SY, Hirano T (2008) mGluR1-mediated facilitation of long-term potentiation at inhibitory synapses on a cerebellar Purkinje neuron. Eur J Neurosci 27:884-896.

Swensen AM, Marder E (2000) Multiple peptides converge to activate the same voltage-dependent current in a central pattern-generating circuit. J Neurosci 20:6752-6759.
Swensen AM, Marder E (2001) Modulators with convergent cellular actions elicit distinct circuit outputs. J Neurosci 21:4050-4058.
Tateno T, Robinson HP (2007) Phase resetting curves and oscillatory stability in interneurons of rat somatosensory cortex. Biophys J 92:683-695.
Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662-669.
Thirumalai V, Prinz AA, Johnson CD, Marder E (2006) Red pigment concentrating hormone strongly enhances the strength of the feedback to the pyloric rhythm oscillator but has little effect on pyloric rhythm period. J Neurophysiol 95:1762-1770.
Tryba AK, Ritzmann RE (2000) Multi-joint coordination during walking and foothold searching in the Blaberus cockroach. II. Extensor motor neuron pattern. J Neurophysiol 83:3337-3350.
Tsodyks M, Uziel A, Markram H (2000) Synchrony generation in recurrent networks with frequency-dependent synapses. J Neurosci 20:RC50.
van Esseveldt KE, Lehman MN, Boer GJ (2000) The suprachiasmatic nucleus and the circadian time-keeping system revisited. Brain Res Brain Res Rev 33:34-77.
Wang LY, Kaczmarek LK (1998) High-frequency firing helps replenish the readily releasable pool of synaptic vesicles. Nature 394:384-388.
Wegener C, Nassel DR (2000) Peptide-induced Ca(2+) movements in a tonic insect muscle: effects of proctolin and periviscerokinin-2. J Neurophysiol 84:3056-3066.
Weimann JM, Marder E (1992) Multiple task processing in neuronal networks: Numerous central pattern generators in the stomatogastric nervous system of *Cancer borealis.* In: Ph.D. Thesis Brandeis University.
Weimann JM, Meyrand P, Marder E (1991) Neurons that form multiple pattern generators: identification and multiple activity patterns of gastric/pyloric neurons in the crab stomatogastric system. J Neurophysiol 65:111-122.
Wong RK, Traub RD, Miles R (1986) Cellular basis of neuronal synchrony in epilepsy. Adv Neurol 44:583-592.
Wood DE, Stein W, Nusbaum MP (2000) Projection neurons with shared cotransmitters elicit different motor patterns from the same neural circuit. J Neurosci 20:8943-8953.
Zador AM, Dobrunz LE (1997) Dynamic synapses in the cortex. Neuron 19:1-4.
Zhou L, Zhao S, Nadim F (2007) Neuromodulation of short-term synaptic dynamics examined in a mechanistic model based on kinetics of calcium currents. Neurocomputing 70:2050-2054.
Zucker RS (1999) Calcium- and activity-dependent synaptic plasticity. Curr Opin Neurobiol 9:305-313.
Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355-405.

VITA

Shunbing Zhao

1996-2000	Nankai University, Tianjin, P. R. China, B.S. Major *Biochemistry*
2000-2002	Analyst Tianjin Institute for Drug Control
2002-2008	Teaching Assistant for *General Biology*, *Foundations in Biology* and *Mammalian Physiology*, Rutgers University, Newark, NJ
2005	M.S. Biology, Rutgers, the State University, Newark, NJ
2005-2008	Presenter at the Society for Neuroscience Annual Meetings
2007	Publication: Zhou, Zhao S, Nadim F. Neurocomputing. 2007 Jun;70(10-12):2050-2054
2009	Ph.D. Biology, Rutgers, the State University, Newark, NJ