Neural Oscillations arising from a Linear Current with Negative Conductance

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ABSTRACT

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Abstract

Slow oscillations underlying neuronal bursting commonly involve a regenerative inward ionic current with a nonlinear inverted bell-shape *IV* curve. In the crab pyloric central pattern generator (CPGs), multiple peptidergic modulatory inputs activate the regenerative inward current I_{MI} in several pyloric neurons, which is critical for the generation of neuronal oscillations. Our recent work suggests that the contribution of such regenerative currents to the production of oscillations is limited to the region of the *IV* curve in which the current exhibits a linear negative-slope conductance (I_{NL}). When I_{NL} is introduced with dynamic clamp in the pyloric pacemaker PD neurons, it can recover oscillations, even when the neuron is isolated by TTX(Bose, Golowasch, Guan, & Nadim, 2014) . **However, it is unknown whether other pyloric neurons can produce oscillations in the presence of I_{NL} and, if not, what factors determine the ability of I_{NL} to produce them.**

We examined whether, in the presence of TTX, I_{NL} is sufficient for producing slow oscillations in synaptically-isolated pyloric neurons. We found that the pyloric dilator PD neuron can produce I_{NL}-induced oscillations in a range of g_{NL} (40-300 nS) and E_{NL} (-15

to +15 rel. to E_{rest}) values. The oscillation cycle period and amplitude decline with $|g_{NL}|$ and E_{NL}. In contrast to the pyloric dilator (PD) neuron, even when g_{NL} and E_{NL} were varied in a large range, none of the follower pyloric neuron types pyloric constrictor (PY) (0/6), lateral pyloric (LP) (1/8), inferior cardiac (IC) (0/3), ventral dilator (VD) (0/3), lateral posterior gastric (LPG) (0/3) could produce slow oscillations with I_{NL}. We explored what factors may oppose the expression of oscillations in LP neurons in the presence of I_{NL}. Our previous modeling work suggests that I_{NL}-induced oscillations depend on a balance between I_{NL} and the voltage-gated outward currents (Bose et al., 2014). We therefore compared the outward currents in the PD and LP, PD and VD neurons. We found that the LP and VD neurons has a significantly larger high-threshold K current (I_{HTK}: delayed rectifier and Ca^{2+}-dep. K^+ currents) than PD (20% larger at 0 mV), and that PD has a larger I_A than LP (45% larger at 0 mV).

We thus examined whether changing the levels of I_{HTK} and I_A would affect the ability of PD or LP and VD to oscillate with I_{NL}. We found that LP and VD can oscillate with I_{NL} by reducing I_{HTK} using TEA (N=4). However, in the presence of the 4AP we find that I_A does not contribute to PD oscillation in I_{NL} (N=3). We conclude that ***interaction of I_{NL} with a slow voltage-gated outward current, is potentially leading to the generation of neuronal oscillations: these neurons are tuned to express their currents levels in a way that fit in such an oscillatory range if they have the oscillatory properties.***

This study has very important scientific value because it: 1. Contribute to understanding the role that leak currents –I_{NL} play in the transition from non-oscillatory to oscillatory

activity. 2. Allows us to assess and define the intrinsic properties of different pyloric neurons. 3. Helps us to understand how a linear leak current I_{NL} can mimic a nonlinear regenerative current I_{MI} to recover oscillatory activity.

ACKNOWLEDGEMENTS

Wow, where to start? There is way too much to thank Dr. Farzan Nadim for, but I will try. Dr.Nadim first took me in as a rotating student back in the spring 2012. Here I was able to see that Dr.Nadim created a productive atmosphere where hard work was expected, while at the same time fun could be had. Dr. Nadim's commitment to the people in his lab was what really stood out during my brief rotation. If you worked hard for Dr. Nadim, he worked hard for you. I have had one of the best possible lab experiences that I could have ever imagined, and it is because of Dr. Nadim. I would specifically like to thank Dr. Nadim for his guidance and support (both in and out of lab) during both the good times and the not-so-good times.

To my committee members: thank you for your wisdom and suggestions to my dissertation writing. To the Biological department administrators: thank you for helping me.

How about the Dr. Nadim's lab? To all of you: thank you for bringing your great personalities to work every day. It made going to school enjoyable.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Chapter 3

LIST OF ABBREVIATIONS

STNS stomatogastric nervous system

STG stomatogastric ganglion

COGs commissural ganglia

OG esophageal ganglion

AB anterior burster (AB) neuron

PD pyloric dilator neuron

LP lateral pyloric neuron

LPG lateral posterior gastric neurons

VD ventricular dilator neuron

IC inferior cardiac neuron

PY pyloric constrictor neuron

TTX Tetrodotoxin

TEA Tetraethyl ammonium

4AP 4-aminopyridine

I_{NL} linear negative current

I_{HTK} High threshold potassium current

I_A Potassium current

Chapter 1: General Introduction

Background

Oscillations are a major feature of some neural networks in the central nervous system (CNS). Oscillatory network activity is known to underlie a variety of behaviors including circadian activity (van Esseveldt, Lehman, & Boer, 2000), sleep and arousal (McCormick & Bal, 1997), learning (Lisman, 1997), and motor pattern generation (Marder & Calabrese, 1996). Abnormalities in oscillations in the CNS can lead to pathological states such as epilepsy (Galvan & Wichmann, 2008; Wong, Traub, & Miles, 1986) . Oscillations that underlie bursting often depend on nonlinear regenerative inward ionic currents (Bose, Golowasch, Guan, & Nadim, 2014) such as the persistent sodium current I_{NaP} (Opdyke & Calabrese, 1994), calcium current I_{ca} (Liljelund, Netzeband, & Gruol, 2000), and non-specific cation currents (McCormick & Pape, 1990). The nonlinear inverted bell-shape IV relationship of these inward currents is thought to be necessary for oscillations generations (Bose et al., 2014). Recent work suggests that the contribution of such regenerative currents for neuronal oscillation generation is limited to the region of the IV curve in which the current exhibits a linear negative-slope conductance, or I_{NL}. Both experimental (Zhao, Golowasch, & Nadim, 2010) and mathematic modeling studies (Bose et al., 2014) showed that a linear current with negative conductance, representing the negative conductance region of a typical regenerative current can induce oscillatory activity in pacemaker neurons.

Pyloric network oscillations depend critically on neuromodulators and oscillations cease when neuromodulatory inputs are removed (decentralized) (Bucher & Marder, 2013) .

Many of these neuromodulatory substances can activate a regenerative *modulatory inward current,* or $I_{MI,}$ in several pyloric neurons (Swensen & Marder, 2000). I_{MI} is believed to be essential for producing pyloric oscillations (Zhao et al., 2010). The experiments of Zhao et al, performed in decentralized preparations, suggest that the effect of I_{MI} is tantamount to a decrease in total leak conductance; it enables an increase in neuronal excitability and can be approximated linearly as a leak current (I_{NL}) with negative conductance. Previous studies have shown that many other neurons in this network can also express I_{MI} (Swensen & Marder, 2000). Although Zhao et al showed that I_{NL} in pyloric pacemakers can restore the pyloric rhythm, whether other pyloric neurons can produce oscillations in the presence of I_{NL} is still unknown. In the following pages I will examine whether I_{NL} is sufficient for producing slow oscillations in other synaptically-isolated pyloric neurons.

My objective for this study is twofold: (1) to determine whether isolated pyloric follower neurons can produce oscillation in presence of I_{NL}; (2) to explore the underlying mechanism of why certain neuron and not the others are responded to I_{NL}-induced oscillation.

My hypothesis*: *Interaction of I_{NL} with a slow voltage-gated outward current, is potentially leading to the generation of neuronal oscillations: these neurons are tuned to express their currents levels in a way that fit in such an oscillatory range if they have the oscillatory properties.

Significance

It has been shown that leak currents play an important role in the regulation of neuronal excitability and oscillatory activity (Zhao et al., 2010). A sets of neuromodulators acting on metabotropic receptors have been shown to negatively regulate leak currents by decreasing their conductance; these include glutamate (Blethyn, Hughes, Toth, Cope, & Crunelli, 2006), thyrotropin-releasing hormone (TRH) (Bayliss, Viana, & Berger, 1992) and serotonin (Weber, Schmitt, Wischmeyer, & Doring, 2008). It also has been shown that when I_{NL} is introduced into the pyloric pacemaker group, the rhythmic pattern of the entire network is recovered. This recovered rhythm is identical to the ongoing pyloric rhythm prior to decentralization and to the rhythm produced by bath application of a modulatory neuropeptide in a decentralized preparation (Zhao et al., 2010). Thus, the importance of this study, firstly, it will contribute to understanding the underlying mechanism how I_{NL} produces oscillations in the PD neurons relate to the known effects of neuromodulators.

Secondly, this study is based on the general hypothesis that the voltage dependence of the inward regenerative current is not necessary for generating oscillations. Instead, a linear current with negative conductance- I_{NL} is sufficient to produce oscillatory activity. If that is the case, any kind of regenerative inward currents that is capable of eliciting oscillations at neuronal levels can be replaced by a linear current. This potential for replacement provides a simple and useful tool in understanding the dynamics of neuronal oscillations. For instance, using I_{NL} allows us to assess and define the intrinsic properties of different pyloric neurons, and discern in the combination currents that transform a non-oscillatory neuron to an oscillatory neuron.

An Overview of the Stomatogastric Nervous System

The stomatogastric nervous system (STNS) is a well-studied model system and its activity is responsible for the feeding behaviors. The STNS locate on top of the stomach and contains four ganglia, including two commissural ganglia (CoGs), one oesophageal ganglia (OG) and one stomatogastric ganglia (STG). The CoGs and OG contain the neurons whose axons project to the STG via STN and these neurons release neuromodulatory substances in which are crucial for the STG to produce the rhythmic activities.

Located in the STG, there are two CPGS, the gastric network and the pyloric network. The gastric mill activity is slow (around 0.1 Hz) and responsible for chewing, while the pyloric activity is fast (around 1 Hz) and responsible for filtering. The pyloric network and the gastric mill network are not totally independent from each other, and many evidences show the interactions between their activities via modulatory commissural neuron 1 (Bartos, Manor, Nadim, Marder, & Nusbaum, 1999; Wood, Manor, Nadim, & Nusbaum, 2004).

STNS has many advantages. The STG contains pyloric CPG whose cellular and synaptic properties have been well-established. Moreover, the pyloric network is under extensive neuromodulators' control capable of reconfiguring neuron and circuit properties. Neuromodulators have proven to be essential to the production of oscillations and in regulating excitability of individual neurons (Thoby-Brisson & Simmers, 2002).

Pyloric network and its tri-phase activity

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 & 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 & 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 (Bucher, Prinz, & Marder, 2005; J.M. Weimann & Marder, 1992; J. M. Weimann, Meyrand, & Marder, 1991). In 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 & Marder, 1982; Miller & Selverston, 1982a, 1982b). 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. Action potentials from pyloric neurons can be recorded extracellularly on the lateral ventricular nerve (*lvn*), and the medial ventricular nerve (*mvn*).

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 & Eisen, 1984). 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 including AB/PD to LP, AB/PD to PY, PY to LP, and the LP to PD.

Neural properties in the pyloric network

Neurons in the pyloric network possess a variety of voltage-gated ion channels (Golowasch & Marder, 1992a), including the transient potassium A-current (I_A), Ca^{2+}-sensitive K^+ channel (Ouyang, Patel, Vanderburgh, & Harris-Warrick, 2010) and hyperpolarization-activated inward current (I_H) channel (Goeritz, Ouyang, & Harris-Warrick, 2011). The high threshold potassium currents (I_{HTK}) contains Ca^{2+} dependent K current and delay recifer K current(Zhao & Golowasch, 2012).The conductance of potassium channels varies several folds among individuals (Golowasch, Abbott, & Marder, 1999; Golowasch, Goldman, Abbott, & Marder, 2002) They are not independent of each other (Zhao & Golowasch, 2012). Interactions and dynamics among various voltage-gated ion channels determine the properties of a neuron's activity.

Neuromodulators in the pyloric network

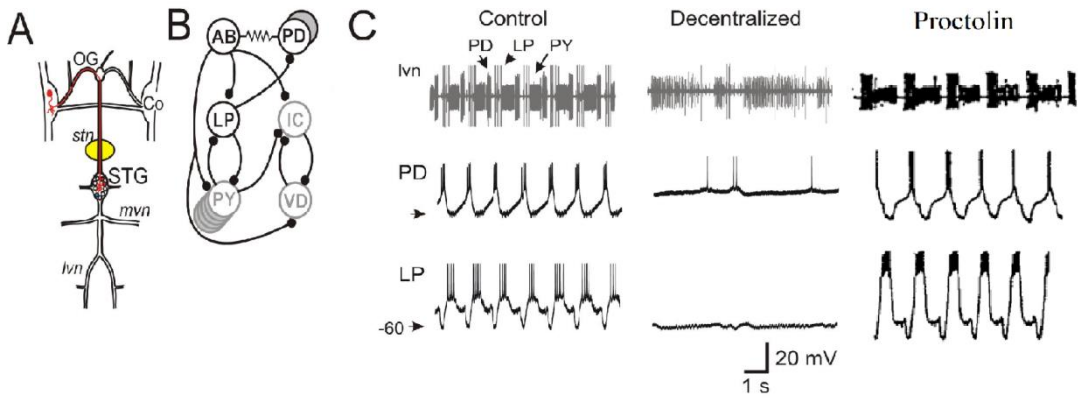


Figure 1.1. Proctolin restores oscillation in the decentralized prep. A. Diagram of the stomatogastric nervous system (STNS) preparation. B. Network connectivity of STG C. *Left*, characteristic extracellular and intracellular recordings from STG. *Middle*, neuromodulatory input has been removed. *Right* Preparation after neuromodulator proctolin is present. From (Zhao et al., 2010)

Neuromoduation has been reported in many CPGs, including leech heart (Nadim & Calabrese, 1997). The neurons and synapses that make up the pyloric network are the targets of many neuromodulators. Some neurons of the commissural ganglia and oesophageal ganglion descend through the stn and release many neuromodulators onto the STG. Neuromodulators are required for proper activity; removing neuromodulators, by applying TTX or cutting the STN causes the pyloric network to stop producing rhythmic activity (Figure 1C). Interestingly, if a neuromodulator such as proctolin, a peptide, is perfused in the saline right after decentralization, this activity can be restored

(Hooper & Marder, 1984, 1987; Thoby-Brisson & Simmers, 1998). Proctolin can restore rhythmic activity in the decentralized prep through the activation of I_{MI} (Golowasch & Marder, 1992b).

The I_{MI} Current

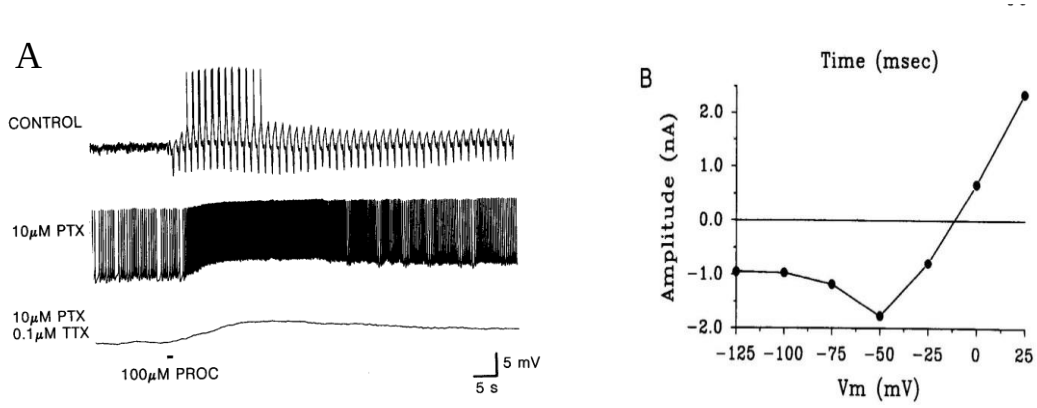


Figure 1.2 Proctolin activates I_{MI} current (Golowasch & Marder, 1992b). A. Proctolin response of an LP neuron was at different levels of pharmacological isolation. Top trace, normal saline; Middle trace, PTX. Bottom trace, PTX+TTX. B. Voltage dependence of the proctolin current I_{MI} measured in voltage clamp.

In the crustacean stomatogastric nervous system (STNS), multiple neuromodulators activates a voltage-gated nonlinear regenerative inward current-I_{MI}. I_{MI} was first shown to be activated by the neuropeptide proctolin and it is essential for neural oscillations in the pyloric network (Golowasch & Marder, 1992b). When neuromodulators are removed in an *in vitro* preparation by decentralization, pyloric oscillations are ceased and the pyloric neurons either spike tonically or become quiescent (Marder & Bucher, 2007). This indicates that the presence of the regenerative inward current I_{MI} in pyloric neurons may be necessary to produce oscillations in the pyloric pacemaker neurons as well as the

network. Also it is known that addition of an artificial version of I_{MI} by the dynamic clamp technique to the AB and PD neurons can elicit oscillations(Zhao et al., 2010).

The inverted bell-shaped IV curve of I_{MI} could be approximated by two linear components, one with negative slope-I_{NL} and the other with positive slope-I_{PL}.

The positive region can depolarize the neuron but it will reduce the neuron excitability. However, the negative region of I_{MI} current, I_{NL}, has been shown both increases excitability as well as enables pyloric pacemaker neuron to produce oscillations(Zhao et al., 2010).

The linear negative leak current (I_{NL}).

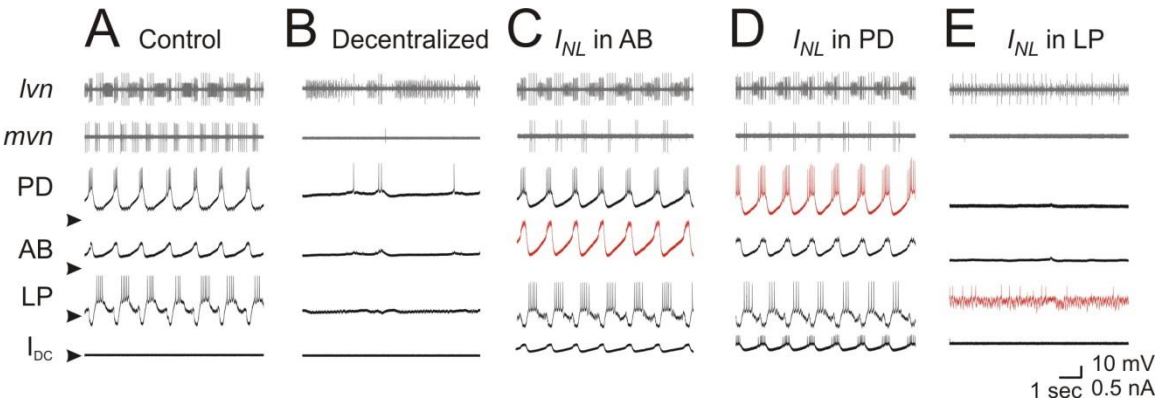


Figure 1.3. **Effects of the I_{NL} on pyloric network activity**. Simultaneous recordings of *lvn* and *mvn* (extracellular), and of PD, AB and LP neurons (intracellular) are shown in all panels. Bottom traces show the current injected with dynamic clamp (I_{DC}) in the respective neuron. **A**. Control pyloric activity before decentralization. **B**. Lack of rhythmic activity after decentralization. **C, D**. Rhythmic activity that strongly resembles

the control pyloric activity is observed when negative leak current I_{NL} (g_{NL} = -80 nS) is injected into the AB neuron (C) as well as into one of the two PD neurons (D). **E**. No rhythmic activity can be induced when negative leak current I_{NL} (g_{NL} = -80 nS) is injected into the follower LP neuron. Arrowheads point to -60 mV and 0 nA. From (Zhao et al., 2010)

I_{MI} current has been shown to be critical for neural oscillation generation. Zhao, Golowasch and Nadim (2010) proposed a mechanism by which I_{MI} can produce oscillations in decentralized prep: The authors suggest that a decrease in total leak conductance enables oscillations by increasing neuronal excitability in PD and AB neurons (Zhao et al., 2010). The authors propose that the region of the *IV* curve of I_{MI} that has negative slope-I_{NL} is the only region of the curve necessary to produce oscillations.

The author has also shown that I_{NL} (as well as I_{MI}) can recover oscillatory network activity when introduced in the pyloric pacemaker PD neurons, whereas when injected into follower neuron LP, it failed to generate the oscillations (Zhao et al., 2010).

Since I_{NL} can recover the rhythmic activity when it has been introduced into the pacemaker neuron (PD & AB), whether other pyloric neurons can produce oscillations in the presence of I_{NL} it is still unknown.

Questions addressed

We have showed that after decentralization and the resultant loss of I_{MI}, the pyloric rhythm could be restored by dynamic-clamp injection of I_{NL} into the PD neuron; yet, a similar injection into the follower LP neuron could not restore oscillations in that cell or the network (Zhao et al., 2010). This observation leads to a fundamental question. Given that PD and LP possess the same ionic currents at comparable levels(Liu, Golowasch, Marder, & Abbott, 1998) , why does PD, and not LP, oscillate in the presence of I_{NL}?

In this thesis, I will attempt to determine whether I_{NL} is sufficient for eliciting bursting oscillations in isolated pyloric neurons. I will also explore the mechanism in producing oscillation with I_{NL} in certain neurons but not others. The following specific questions are addressed: (1) which isolated pyloric neurons can produce oscillations in the presence of I_{NL}? And which combination of I_{NL} injections can induce neuron oscillations? (3) What distinguishes a pacemaker from a non-pacemaker neuron?

Chapter 2 Material and Methods

General Methods:

Preparation and identification of the neurons

Experiments were conducted 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 ganglion and the paired commissural ganglia) were dissected out using standard methods (Blitz & Nusbaum, 1997; Selverston, Russell, & Miller, 1976). 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 super fused 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).

Electrophysiology

Pyloric neurons were identified according to their activity patterns, synaptic interactions and their axonal projections in identified motor nerves. After identification the neuromodulaory input to the STG is removed by superperfusing (0.1uM) TTX. 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).

Ionic currents

The following currents were recorded from pyloric STG neurons:

Transient A-type current (I_A), high threshold potassium current (I_{HTK}), all currents have been characterized using two electrode voltage clamp (TEVC) and isolated as described below.

Transient A-type current (I_A)

I_A in the *Cancer borealis,* LP neuron was described by Golowasch and Marder (1992). I_A deinactivated at holding potentials below -60 mV. I_A activation steeply increased during pulses to voltages between -40mV and +20 mV, after which it levels off. I_A reached its peak amplitude more rapidly as the voltage increased. I_A in crab was partially and irreversibly blocked by 4-aminopyridine (4-AP) but is not affected by Ca^{2+}. I_A was measured by subtracting I_{HTK} from I_{Ktotal} from a holding potential of -80mV.

High threshold potassium current (I_{HTK})

High threshold potassium current (I_{HTK}) was comprised of I_{Kd} and I_{KCa}(Zhao & Golowasch, 2012). I_{HTK} was characterized using voltage steps (from -60 to +40mV) induced from a holding potential of -40mV where I_A was inactivated (Golowasch & Marder, 1992a) .Current amplitudes were determined at the peak (which we defined as 30 ms after the start of the pulse) and at steady state (790 ms).

The Dynamic Clamp Technique

We used the dynamic clamp technique to introduce or subtract from the biological neurons. In dynamic clamp, the ionic currents were injected into biological neuron through a current-clamp amplifier.

The Netclamp software (Gotham Scientific) was used for current injection in dynamic clamp experiments. Data acquisition was performed using the Digidata 1332A data acquisition board and pClamp 10.3 software (Molecular Devices).

In dynamic clamp, ionic currents are calculated using Hodgkin-Huxley-type equations as described below and continuously updated by recording the membrane potential (V) of the neurons in real time (4 kHz sampling frequency) to calculate an ionic current that can then be injected into the neuron (Sharp, O'Neil, Abbott, & Marder, 1993) I_{MI} was described by:

$$I_{MI}(V) = \bar{g}_{MI}\, m(V)(V - E_{MI})$$

$$m_{\infty}(V) = \frac{1}{1 + \exp((V + 55)/-5)}$$

$$\frac{dm}{dt} = \frac{m_{\infty}(V) - m}{\tau_m}$$

where $E_{MI} = -10$ mV and $\tau m = 4$ ms. The values for g_{MI} varied depending on the experiment as described in the Results. The standard leak current is described by $I_{NL}=g_{NL}*(V-E_{NL})$ where g_{NL} is the leak conductance. When the value of leak conductance was set to be negative in the dynamic clamp software it resulted in a reduction of the total neuronal conductance.

In dynamic clamp, the characteristic voltage and kinetic parameters of the currents can be fitted by a manual fitting program to modify the intrinsic current levels. For modeling

I_{HTK}, we first measured the I_{HTK}, then we used a manual fitting program that calculated currents using Hodgkin-Huxley-type equations to match voltage clamp measured currents: the followings are the equations we use:

$$I_{HTK}(V) = g_1 m_1(V) h_1(V)(V - E_{rev}) + g_2 m_2(V) h_2(V)(V - E_{rev})$$

$$m_\infty(V) = \frac{1}{1 + \exp(-(V - V_m)/K_m)}$$

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)}$$

$$\tau_m(V) = T_m\_low + \frac{T_m\_hi - T_m\_low}{1 + \exp(-(V - V_m)/K_m)}$$

Where *m (V)* represented the activation term, and *h (V)* represented the inactivation term. For I_{HTK}, two independent inactivation conductance terms were needed. The activation and inactivation terms have the same form (The last equation indicated only for *m(V)*).

Table 1 Hodgkin-Huxley equation parameters used in fits I_{HTK}, and equations in text

	I_{HTK} (1)	I_{HTK} (2)
V_m	-10	20
K_m	-10	-20
V_h	-25	25
K_h	1	20
T_m_low	1	1
T_m_hi	50	1
T_h_low	5	50
T_h_hi	5	50
G_{max} (nS)	1000	1200
E_{rev} (mV)	-80	-80

Analysis and Statistics of Data. Data were digitized and analyzed using pClamp 10.3 software (Molecular Devices), sampled at 4 kHz and saved on a PC using a PCI-6070-E data acquisition board (National Instruments, Austin, TX). CorelDraw software was used to graphical analysis. Graphpad Prism 6.0, Origin8.0 (OriginLab, Natick, MA) software packages were used for statistical analysis.

Chapter 3 Results

3.1.I_{NL} enables oscillation in PD neuron in presence of TTX.

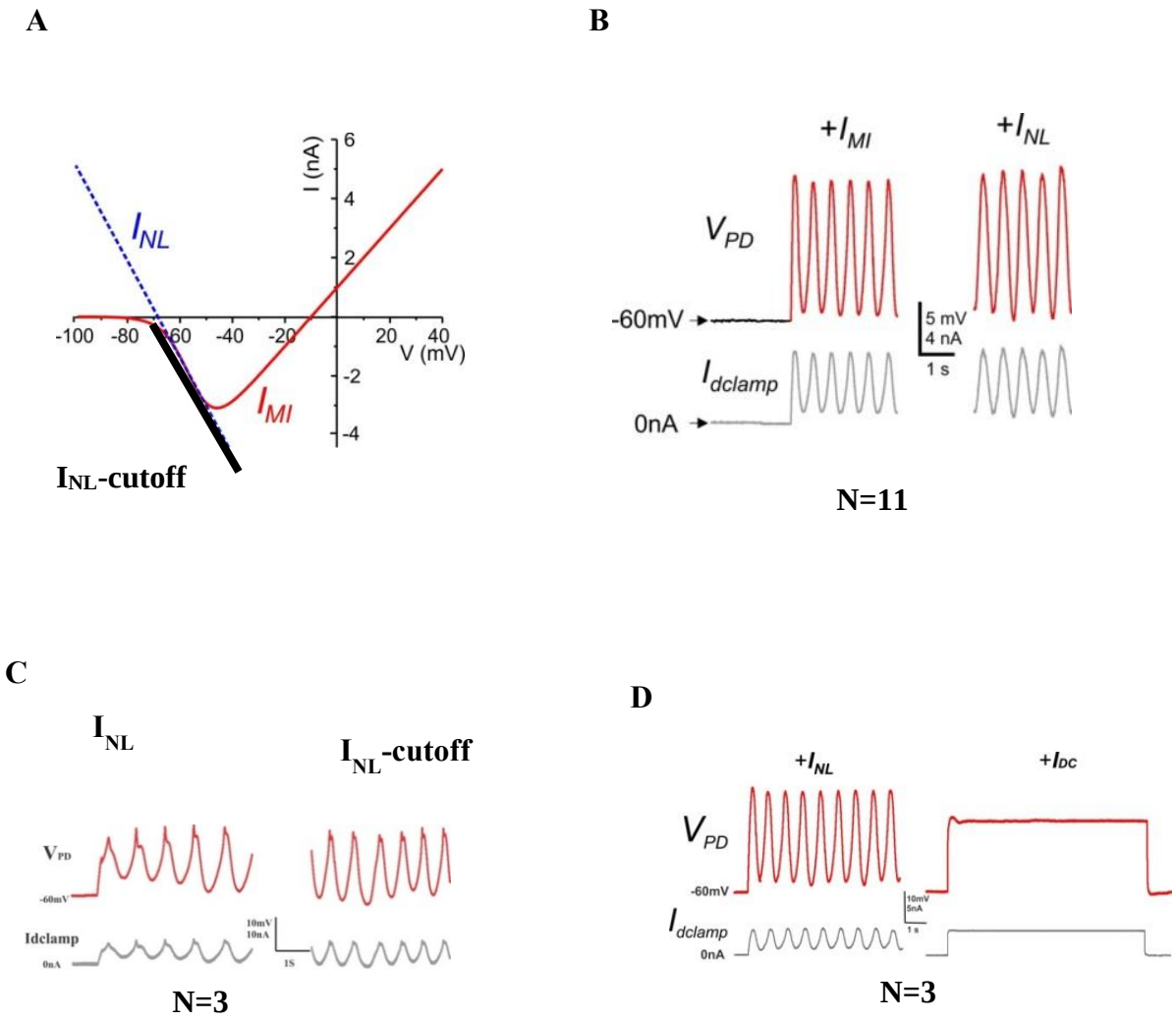


Figure 3.1: I_{NL} enables oscillation in PD neuron in presence of TTX. (A) IV curve of I_{MI} and its linear approximations. Full I_{MI} is the red trace, I_{NL} is the dashed blue line that extends across the entire voltage range. I_{NL} –cutoff is a negative part of I_{NL}. (B) I_{NL} can produce oscillations in the isolated PD in TTX (N=11). (C) I_{NL} –cutoff can produce oscillations in the isolated PD in TTX (N=3). (D) Equivalent DC current in PD does not produce oscillations (N=3).

I first need to determine whether I_{NL} is sufficient to elicit bursting oscillations in the isolated pyloric neurons. Experiment was conducted on superperfusing 0.1uM TTX, all the neurons have been synaptic isolated, bursting oscillations in different neurons ceased and the slow wave oscillation of their membrane potentials were suppressed and replaced with almost completely quiescent membrane potential of -60 to -50mV. Using the dynamic clamp technique, we reduced the magnitude of the I_{NL} in isolated neurons in a way that the resting membrane potential was not significantly changed.

This was done by adding a negative linear conductance with an E_{NL} near the E_{rest} of the neuron. A small value of g_{NL} in any of the neurons had no effect. When a sufficiently large g_{NL} value was added to either one of the two PD neurons, it produces slow oscillations. When the regenerative current I_{MI} was injected into the same neuron, the oscillatory activity was resulted (Figure 3.1 B). To further verify only the negative region of the I_{NL} , instead of full I_{NL}, is responsible for oscillation generation in PD, I_{NL}-cutoff was introduced into PD neuron and it made PD oscillate (Figure 3.2 C).

There is another possibility that PD produced oscillation in the presence of I_{NL} is due to the depolarization of the PD neuron itself. To eliminate that reason, equivalent amounts of current pulse were introduced into the PD neuron. The PD neuron cannot produce oscillation (Figure 3.1D), which indicates that PD oscillation by I_{NL} is not due to the cell depolarize.

3.2.I_{NL}-induced oscillation in PD is limited to certain combination of g_{NL} and E_{NL}

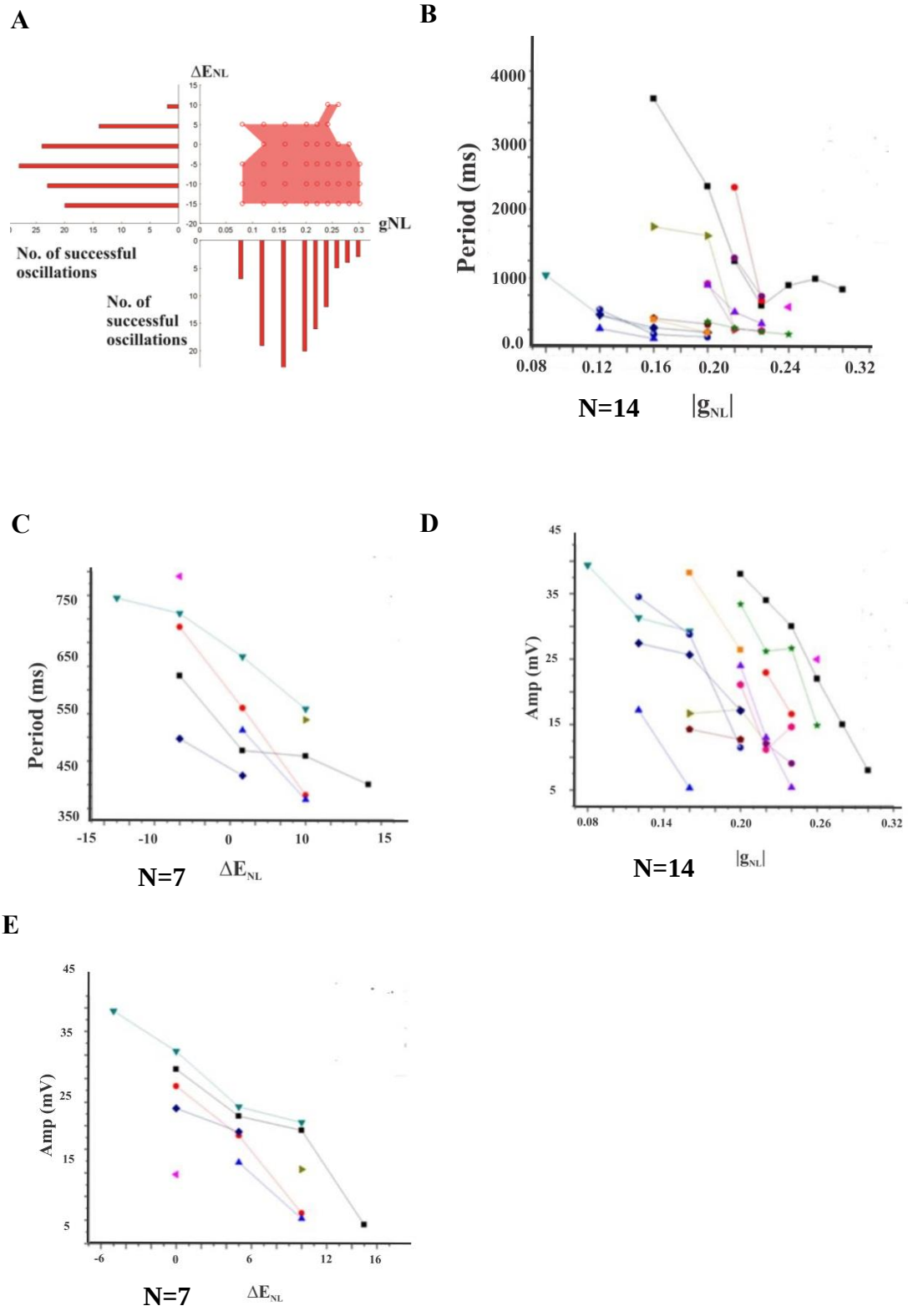


Figure 3.2. I_{NL}-induced oscillation in PD is limited to certain combination of g_{NL} and E_{NL} (A) PD neuron can produce I_{NL}-induced oscillations in a range of $|g_{NL}|$(40-300 nS) and E_{NL} (-15 to +15 rel. to E_{rest}) parameter values. (B-E) Regression analysis shows that period and amplitude of oscillations decrease with $|g_{NL}|$ and ΔE_{NL} .

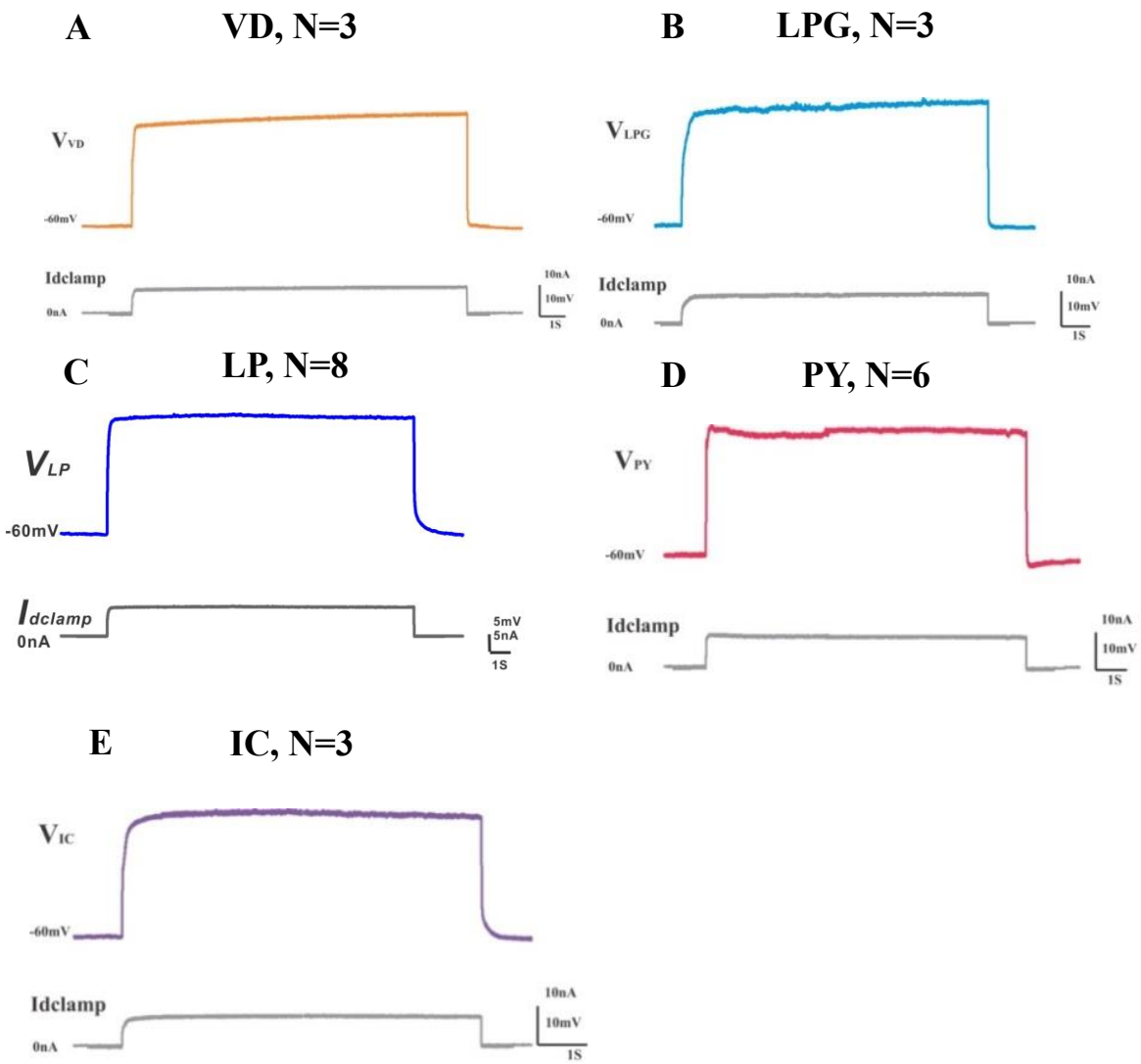
In the pyloric network, one type of neuron might response differently to external stimuli across preparations. To eliminate such variability, different amount of I_{NL} were introduced into the PD neuron to see in which combination of $|g_{NL}|$ and E_{NL} can make PD oscillate. We found that I_{NL} –induced oscillation in the PD neuron is limited to certain region (Figure 3.2 A). When the g_{NL} is close to certain level (g_{NL}=-160nS) or E_{NL} is close to the rest membrane potential of that neuron, PD had more possibility to produce oscillation, whereas in other combinations, there was less chance for PD to oscillate.

To further describe how $|g_{NL}|$ and E_{NL} affect PD oscillations, we plot the relationship between $|g_{NL}|$, E_{NL}, cycle period and amplitude. We found that as g_{NL} increases, both cycle period (4000ms-500ms) and amplitude (40mV -5mV) decline (Figure 3.2B and 3.2D). As for E_{NL}, the cycle period (800ms-300ms) and amplitude (50mV-5mV) reduces as the E_{NL} move further from Erest (Figure 3.2C and 3.2E).

3.3. I_{NL} cannot produce oscillations in other pyloric neurons in the presence of TTX.

Next, we attempted to determine whether I_{NL} can produce oscillation in the pyloric follower neurons (LPG, LP, IC, PY, and VD). I applied the same protocol to these follower neurons. We found that I_{NL} cannot produce oscillation in none of these pyloric follower neurons (Figure 3.3 A-F). Thus, we can conclude that only PD neurons but not

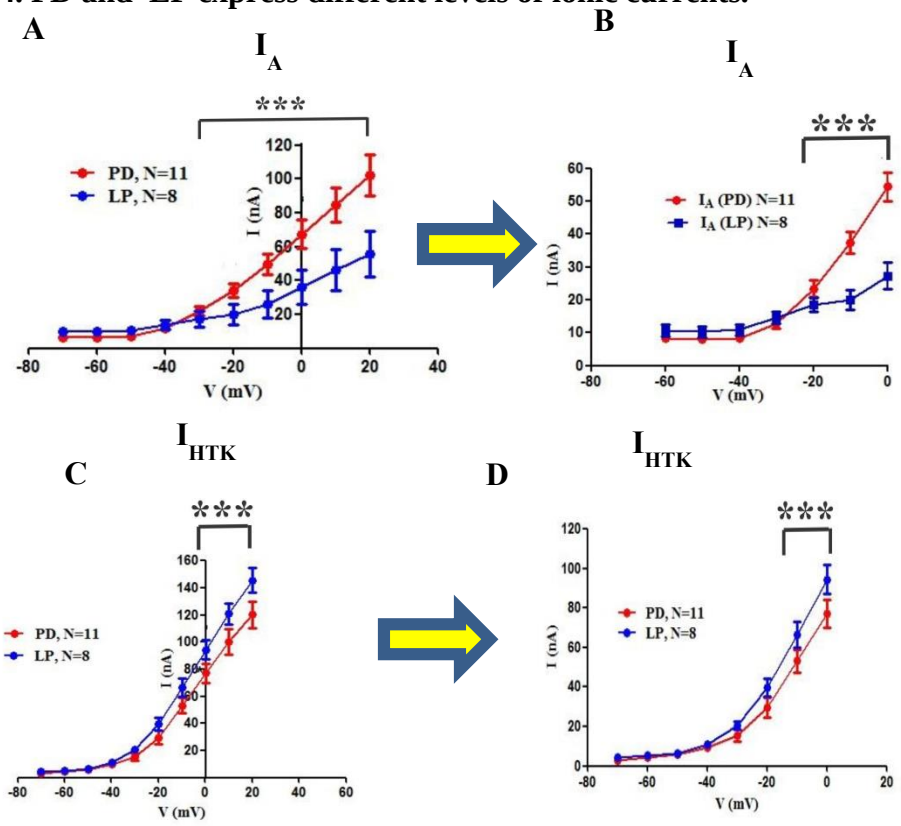
the follower neuron (LPG, LP, IC, PY, and VD) can produce oscillation with I_{NL} with the exception of one LP neuron can produce oscillation with I_{NL}.



	PD	LPG	LP	IC	PY	VD
TTX # oscillation / # tried	16/16	0/3	1/8	0/3	0/6	0/3

Figure 3.3: I_{NL} cannot produce oscillations in other pyloric neurons in TTX. (A-F) I_{NL} cannot produce oscillations in other pyloric neurons in TTX. Table 2 shows that only PD but not the other pyloric neurons (LPG, LP, IC, PY, and VD) can elicit oscillation with I_{NL}.

3.4. PD and LP express different levels of ionic currents.



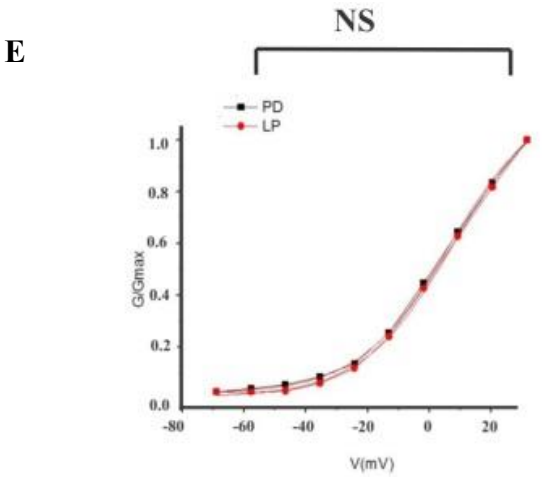


Figure 3.4. ***PD and LP express different levels of I_{HTK} and I_A.*** (A, B) PD and LP express different levels of I_A (PD (N=11) vs LP (N=8): from -20mV to 20mV, Two-way ANOVA, *** p<0.001) (C, D) PD and LP express different levels of I_{HTK}.(PD (N=11) vs LP (N=8): from -20mV to 20mV, Two-way ANOVA, *** p<0.001). (E) PD and LP show identical kinetics of I_{HTK} (PD (N=11) vs LP (N=8): from -70mV to 20mV, Two-way ANOVA, NS (Not Significant)).

In order to explore the underlying mechanisms in which only PD neurons produce oscillation in the presence of I_{NL} and other pyloric follower neurons do not. I checked the outward current expression levels of different neurons. Neurons in the pyloric network carry a wide range of voltage-gated ion channels (Golowasch & Marder, 1992a), in particular, transient potassium A-current (I_A) and high threshold potassium current I_{HTK} determine the properties of the activity of neuron(Zhao & Golowasch, 2012).

Previous studies have been showed that PD would produce oscillation by I_{NL} whereas LP can not oscillate by I_{NL} in the decentralized crabs (Zhao et al., 2010). We found the similar effects under TTX (Figure 3.3 C). As we compared ionic current expression level

in PD and LP, we found that PD and LP express different levels of $I_{A,}$ PD causes the production of larger levels of I_A than the LP neuron does (At -20mV: PD (N=11) vs LP (N=8):102.17+/-12.24nA vs 55.77+/-17.71nA; Two-way ANOVA, ***p<0.001) (Figure 3.4 A and B). The LP neuron, however, has a higher level of I_{HTK} than the PD neuron does (At -20mV: PD vs. LP:120.05+/-18.53nA (N=11):145.59+/-9.06nA (N=8); Two-way ANOVA,*** p<0.001) (Figure 3.4 C.D). I_{HTK} activation curve shows that there are similar kinetics of I_{HTK} between the LP and PD neurons I_{HTK} (PD (N=11) vs LP (N=8): from -70mV to 20mV, Two-way ANOVA, NS) (Figure 3.4 E).

3.5. LP can produce oscillations with I_{NL}, if I_{HTK} is reduced.

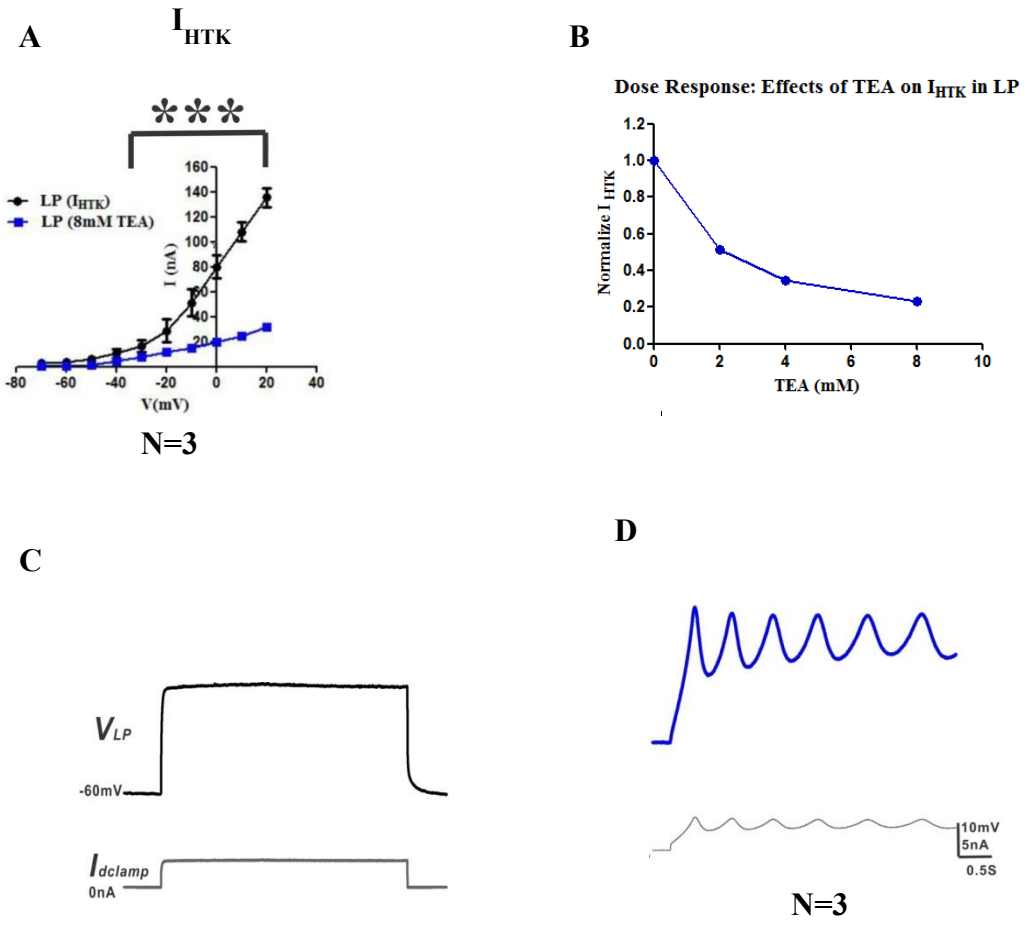


Figure 3.5. ***LP can produce oscillations with I_{NL}, if I_{HTK} is reduced***. (A) IV relationship of I_{HTK} level in the LP neuron before and after TEA (LP (N=3), from -20mV to 20mV: Two-way ANOVA, ***p<0.001). (B) Dose response of TEA on $I_{HTK.}$(N=3). (C, D) LP can produce oscillations with I_{NL} when I_{HTK} is reduced (N=3).

In order to explore either I_{HTK} or I_A is the main factor to determine why PD neuron produces oscillation, whereas LP cannot produce oscillation, we first looked into current expression level of I_{HTK}, as indicated in Figure 3.4 C and D, the LP neuron has much larger I_{HTK} current than PD (PD (N=11) vs LP (N=8): from -20mV to 20mV, Two-way ANOVA, *** p<0.001), if we reduce I_{HTK} level, will the LP neuron become oscillatory? To answer this question, I introduced I_{NL} into LP neuron when bath applying TEA, the specific blocker of I_{HTK}. We found that at 8mM TEA, the LP neuron becomes oscillatory in the presence of I_{NL}, with the I_{HTK} level reduced by 60% (Figure 3.4 C and D). We can conclude that I_{HTK} is the factor to determine whether the neuron can produce oscillation by I_{NL}.

3.6. The dynamic Clamp experiment shows that the LP neuron can produce oscillation with I_{NL} when I_{HTK} has been reduced.

|g|	NO I_{HTK} reduction	10% I_{HTK} reduction	20% I_{HTK} reduction	40% I_{HTK} reduction	60% I_{HTK} reduction	80% I_{HTK} reduction	100% I_{HTK} reduction
0.01	o	o	o	o	o	o	o
0.02	o	o	o	o	o	o	o
0.04	o	o	o	o	o	o	o
0.08	o	o	o	o	o	o	o
0.12	o	o	o	o	o	o	o
0.16	o	o	o	o	1	1	o
0.2	o	o	1	1	o	o	o
0.22	o	o	1	o	o	o	o
0.24	o	o	o	o	o	o	o
0.26	o	o	o	o	o	o	o
0.28	o	o	o	o	o	o	o
0.3	o	o	o	o	o	o	o

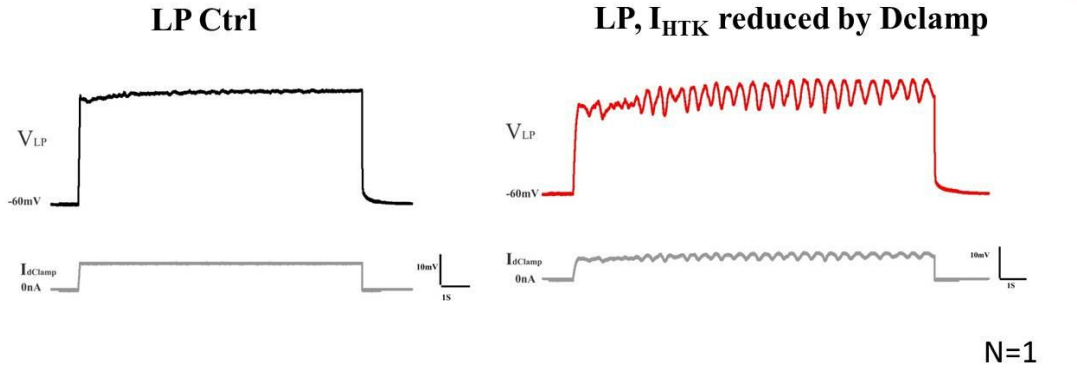


Figure 3.6. LP can produce oscillation with I_{NL} when I_{HTK} has been reduced by Dynamic Clamp. (A) Table 3 demonstrates different amount of percentage reduction from endogenous I_{HTK} by dynamic clamp. (B, C) LP can produce oscillation when the I_{HTK} level has been reduced using dynamic clamp (LP (N=1), $|g_{NL}|$(nS)=200, E_{NL}(mV)=-65; I_{HTK} (1): V_m=-10, K_m=-10, V_h=-25, K_h=1, T_m_low=1, T_m_hi=50, T_h_low=5, T_h_hi=5, $|g_{HTK}|$ (nS)=800, E_{rev}(mV)=-80; I_{HTK} (2): V_m=20, K_m=-20, V_h=25, K_h=20, T_m_low=1, T_m_hi=1, T_h_low=50, T_h_hi=50, $|g_{HTK}|$ (nS)=960, E_{rev}(mV)=-80).

LP neurons express larger I_{HTK} currents than PD neurons (Figure 3.3 C, D). By reducing I_{HTK} levels, LP neurons can become oscillatory in the presence of I_{NL} (LP (N=1), $|g_{NL}|$(nS)=200, E_{NL}(mV)=-65; I_{HTK} (1): V_m=-10, K_m=-10, V_h=-25, K_h=1, T_m_low=1, T_m_hi=50, T_h_low=5, T_h_hi=5, $|g_{HTK}|$ (nS)=800, E_{rev}(mV)=-80; I_{HTK} (2): V_m=20, K_m=-20, V_h=25, K_h=20, T_m_low=1, T_m_hi=1, T_h_low=50, T_h_hi=50, $|g_{HTK}|$ (nS)=960, E_{rev}(mV)=-

80) (Figure 3.4 C, D). We attempted to make LP neurons oscillate by using dynamic clamp to reduce I_{HTK} levels. One set of data shows that LP can become oscillatory by decreasing I_{HTK} levels with dynamic clamp. Our findings further confirm that I_{NL} in conjunction with slow outward K current enable neural oscillation to take place.

3.7. PD and VD express different levels of ionic currents.

To further confirm that I_{HTK} is the factor in which determine whether the neuron can produce oscillation in the presence of I_{NL}, we took another follower neuron VD. We compare I_{HTK} level in VD and PD neuron. We found that PD and VD do express different levels of $I_{HTK,}$ VD has a significantly larger I_{HTK} level than PD (At 20mV: VD (N=6) vs. PD (N=11):196.27+/-47.92nA:120.05+/-18.53nA, Two-way ANOVA, ***$p<0.001$) (Figure 3.7 A, B). PD has a relative larger I_A current level than VD, although the difference is not significant (At -20mV: VD (N=6) vs PD (N=11):88.39+/-19.26nA :102.17+/-12.24nA, Two-way ANOVA, NS) (Figure 3.7 C, D)

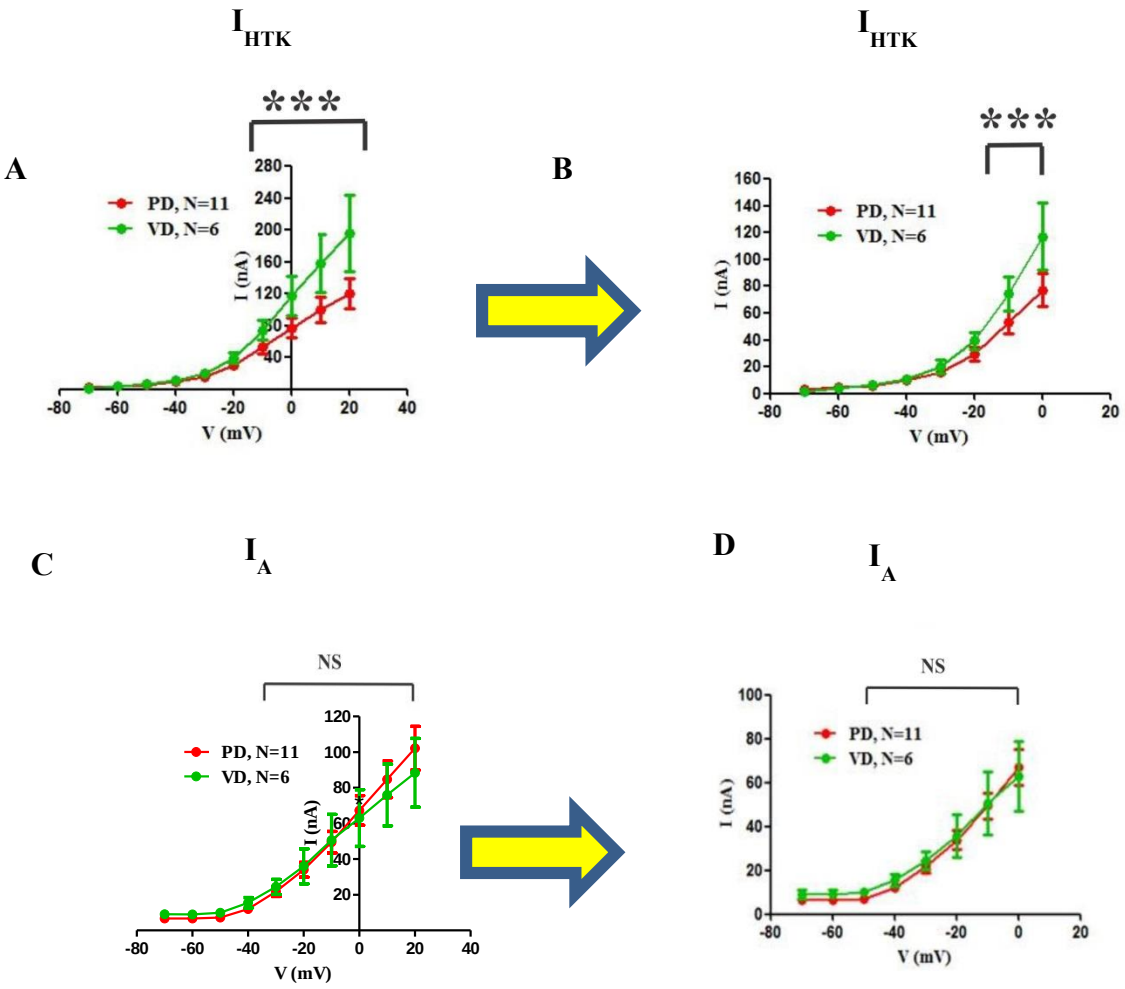


Figure 3.7. ***PD and VD express different levels of I_{HTK} and I_A.*** (A, B) PD and VD express different levels of I_{HTK} (From-10mV to 20mV: VD (N=6) vs. PD (N=11), Two-way ANOVA, ***p<0.001). (C, D) PD and VD express similar levels of I_A (From-20mV to 20mV: VD (N=6) vs. PD (N=11), Two-way ANOVA, NS).

3.8. VD can produce oscillations with I_{NL}, if I_{HTK} is reduced

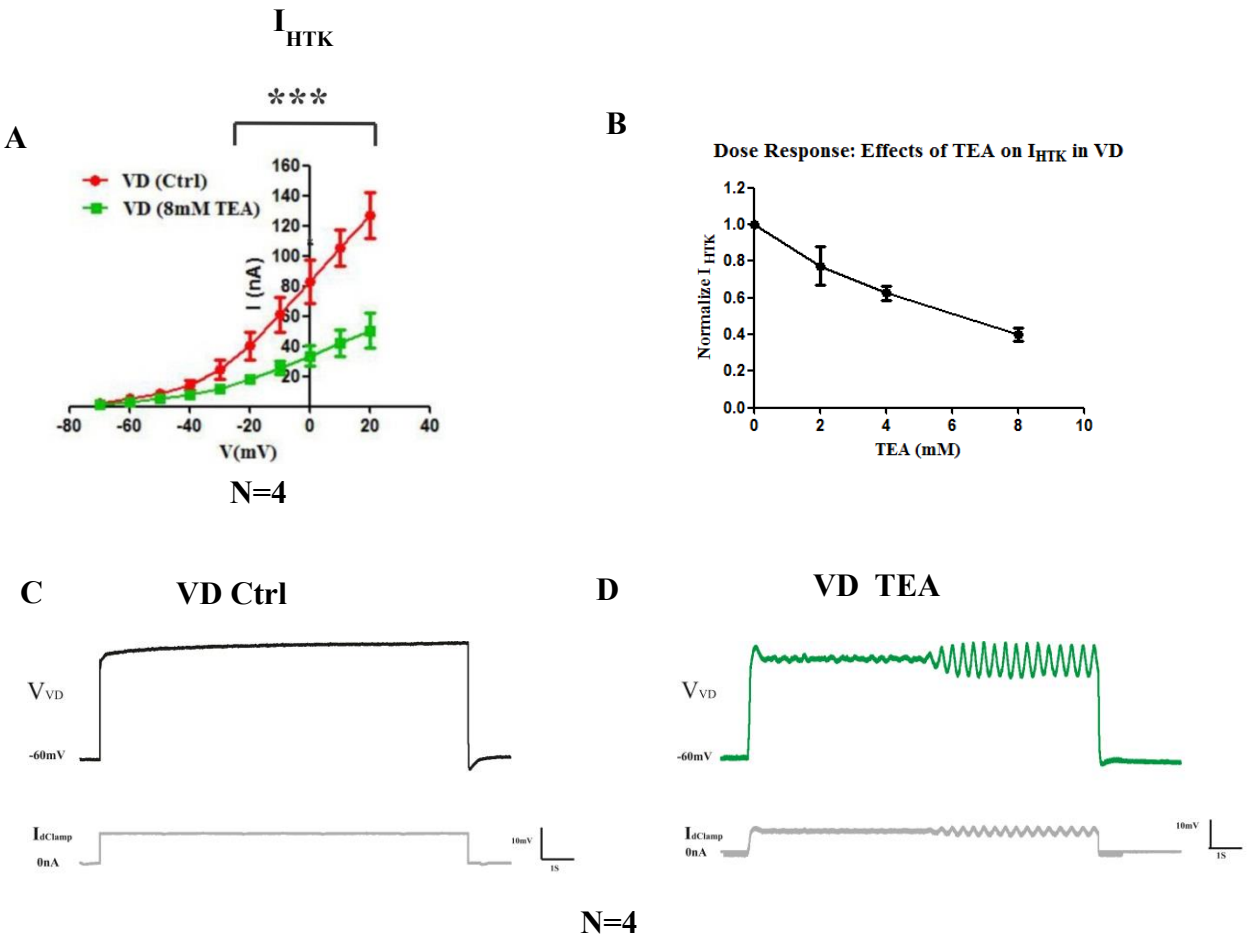


Figure 3.8. VD *can produce oscillations with I_{NL}, if I_{HTK} is reduced*. (A) IV relationship of I_{HTK} level before and after TEA (VD (N=4), Two-way ANOVA, *** $p<0.001$). (B) Dose response of TEA on I_{HTK}. (C, D) VD can produce oscillations with I_{NL} when I_{HTK} is blocked by TEA.

We repeat the similar protocol as before: Inject I_{NL} into the VD neuron while its I_{HTK} level has been reduced by its blocker TEA (Figure 3.8 A, B). At 8mM TEA, we found

that the VD neuron becomes oscillatory in the presence of I_{NL}, if the I_{HTK} level was reduced by 60%. (Figure 3.8 C, D). Thus, both LP and VD became oscillatory in presence of I_{NL} when I_{HTK} level has been reduced.

3.9. I_A does not contribute to PD oscillation in I_{NL}

In addition to I_{HTK}, I_A might be a factor to determine neural oscillations generation since we did find significant difference in I_A level between PD and LP: the PD neuron has higher I_A levels than the LP neuron (Figure 3.3 A and B).

To assess the possibility that whether I_A contribute to neuronal oscillation in the PD neuron, 4AP, a blocker of I_A(1mM), was super fused while I_{NL} was introduced into the PD neuron. It showed the IV curve demonstrating that levels of I_A are reduced in the presence of 1mM 4AP, although it is not significant difference (PD (N=3), Two-way ANOVA, NS) (Figure 3.9 A). However, I_{NL} can still produce oscillation in the presence of 1mM 4AP (Figure 3.9 B, C), which illustrated that I_A does not contribute to PD oscillations.

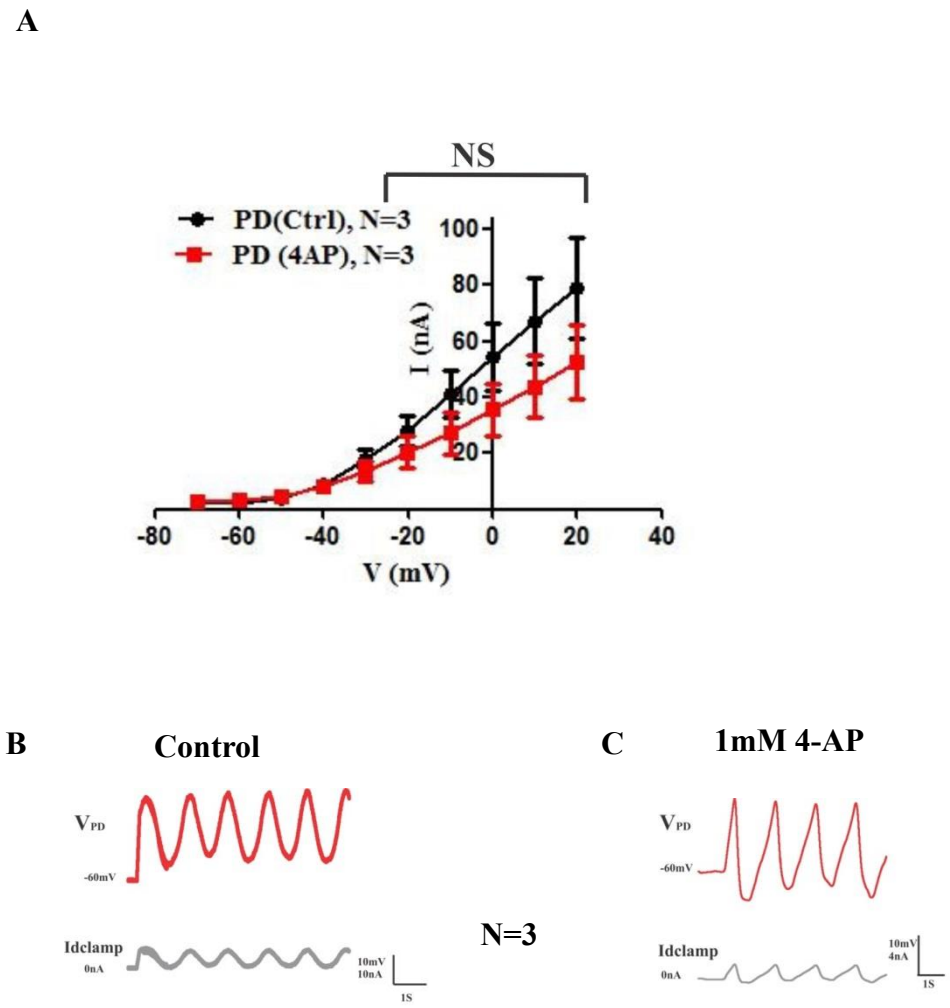


Figure 3.9. ***I_A does not contribute to PD oscillation.*** (A) IV relationship of I_A level before and after 1mM 4AP (N=3, Two-way ANOVA, NS). (B, C) PD can produce oscillations with I_{NL} when I_A is blocked by TEA.

Chapter 4 Discussion

In this study, we examined whether I_{NL} is sufficient for producing oscillations in different types of pyloric neurons. By introducing I_{NL} into distinct pyloric neuron types with dynamic clamp, we found that only PD, but not the other follower neurons (LP, PY, VD, IC, and LPG) can produce oscillation in the presence of I_{NL}. By injecting different sets of parameters of g_{NL} and E_{NL}, we found that I_{NL} –induced oscillation in the PD neuron is only limited to certain combination of g_{NL} and E_{NL}. The cycle period as well as the amplitude of oscillations in the PD neuron in the presence of I_{NL} is decreased with $|g_{NL}|$ and ΔE_{NL}.

We observed that the linear current with negative conductance I_{NL} effectively activates the PD neuron oscillations in the limited regions: certain levels of I_{NL} either inadequate or excessive for PD neuron to elicit oscillations. Modeling studies have shown that the interaction I_{NL} with the voltage-gate I_K can determine neuronal oscillations (Bose et al., 2014): when $E_{NL} > E_K$ (E_K= -80 mV), the fixed point is either unstable or no longer globally attracting, it provides the opportunity for the neuronal oscillations. When $E_K > E_{NL}$, there exists a second fixed point that is stable and globally attracting, in this case, the PD neurons cannot elicit oscillations by I_{NL}. It has been shown that I_{NL} is inadequate for PD neuron to produce oscillations when $|g_{NL}|$ is small. As $|g_{NL}|$ increased, the PD neurons oscillations would emerge through either a supercritical Hopf bifurcation or through a saddle-node of periodic orbits (Bose et al., 2014).

Although in different preparations different amounts of g_{NL} and E_{NL} led to the PD neurons oscillations, increasingly negative g_{NL} always led to a faster oscillation

frequency. That is because as $|g_{NL}|$ was increased, more current was introduced into the PD neurons, leading to a faster oscillation frequency. We also found that the oscillation decreases in amplitude when E_{NL} grows: increasing E_{NL} lead to a decline of the amount of I_{NL} introduce into PD neuron. As such, the amplitude of the oscillations reduced.

Furthermore, we explored the underlying mechanism that explained why only pacemaker neurons but not the follower neurons can elicit oscillations in the presence of I_{NL}. We hypothesized that a linear current with negative conductance- I_{NL} in conjunction with a slow outward K current could be the reason for making a non-oscillatory neuron to become oscillatory. We confirm our hypothesis by comparing PD and LP's potassium current expression levels, and we found that LP has a larger I_{HTK} than PD, whereas PD has larger I_A level than LP. We first ruled out I_A is because PD can still elicit oscillations even when I_A level is reduced by 4AP. Then we found LP can produce oscillations in the presence of I_{NL} on the condition of sufficient reduction of I_{HTK} level by TEA. To further confirm our finding, we studied another pyloric follower neuron VD, which initially cannot produce oscillations with I_{NL}, and it eventually became oscillatory in the presence of I_{NL} when its I_{HTK} levels are reduced.

The characteristic voltage and kinetics of I_K are the factors that determine whether the neuron can elicit oscillations by I_{NL}. Modeling study has been indicated that changing the time constant and the slope of its half-activation of I_K will influence the effect of I_K on neuron's oscillations by I_{NL} (Bose et al., 2014). In our experiment, we have shown that I_{NL} cannot elicit oscillation in the LP neuron by altering the amplitude of I_A. It indicated that I_A did not subsidize oscillations might be due to its fast kinetics.

We found that I_{NL} can only make the LP neurons, the VD neurons became oscillatory through reducing I_{HTK} levels, it failed to drive these neurons oscillate by increasing $|g_{NL}|$ level to coordinate with I_{HTK} levels. Our finding indicated that these oscillatory neurons (like the PD neurons) are adjusted to express their currents levels in a way that fit in such an oscillatory range, whereas the non-oscillatory neurons (like the LP neurons, the VD neurons), their currents levels exceed the oscillatory range. As such, I_{NL} can only elicit oscillations in those neurons when reducing their I_{HTK} levels.

Linear currents have been shown to be crucial for the regulation of neural oscillatory activity in the other systems: In neurons in the pre-Bo zinger complex in mammals that control the inspiratory activity (Del Negro, Koshiya, Butera, & Smith, 2002) as well as the glutamatergic interneurons within lamina I of the rat spinal cord, which exhibit oscillatory burst firing during early life (Li & Baccei, 2011), it has been shown that one of the ways to differentiate bursting from non-bursting neurons, aside from capacitance, was to measure the ratio of the conductance of persistent sodium current to the conductance of the leak current ($g_{Na,P}/g_{leak}$). Higher ratio of $g_{Na,P}/g_{leak}$ is typically observed in the bursting neurons whereas low ratio is the nonbursting neurons (Li & Baccei, 2011).

Our results indicated that the important coordination between linear current with negative conductance I_{NL} and slow voltage gated outward current is potentially eliciting neuronal oscillatory activity: Thus, we can make a general conclusion that neurons are tuned to express their currents levels in a way that fit in such a narrow range if they have the oscillatory properties.

Future Direction

We still need more data for the results of reducing I_{HTK} level by using dynamic clamp in LP and VD neurons. Those results would further confirm whether I_{HTK} is the determinant factors why certain neuron can produce oscillation whereas others cannot.

There are significant differences in I_A levels among PD and LP neurons. It would be interesting for us to see if the oscillation could be diminished by altering I_A levels with dynamic clamp.

Previous studies indicated that oscillations can be recovered when I_{NL} is added to the AB or PD neuron in the decentralized STNS (Zhao et al., 2010). These results address the questions that we could explore: Either I_{NL} allows the PD neuron to become an oscillator, independent of the AB neuron, or the production of oscillations in PD neurons is dependent on AB neurons via gap junctions. It would be interesting for us to inject different amounts of g_{NL} and E_{NL} into the PD neuron when photo-ablating the AB neuron to see if the PD neuron can become a bursting oscillator independent of AB.

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Research Technique

Electrophysiology: Whole-cell patch clamp, Ionic current recording (Ca current, Na/Ca exchanger current), Action Potential measurement, Calcium imaging. Sharp Electrode recording, synaptic current measurement, two-electrode voltage-clamp.
Molecular Biology: RNA/DNA Extraction, RT-PCR, Protein Extraction, Western blot, Immunoprecipitation.
Immunology: Immunofluorescence, ELISA.
Biomedical Literature Retrieval: skillful in utilizing the internet and database to search medical information.

Education

Department of Biology, Northeast Agricultural University 09/2004 to 05/2008

Bachelor of science

Harbin, Heilongjiang, China
Major:Animal Physiology & Biochemistry

Cardiovascular Research Center, Temple University School of Medicine 09/2008 to 05/2011

Master of Science

Philadephia, Pennsylvania, USA
Major:Physiology

Biological Science Department, Rutgers University 09/2011 to 08/2014

Graduate Student in Biological Science (Expected Master: 01/2015)

Newark, New Jersey, USA
Major:Biology

Research Experience

Department of Biology, Northeast Agricultural University 05/2006 to 06/2008

Undergraduate Student

Bachelor Thesis: Phylogenetic study of goats in Heilongjiang Province of China

Collected more than 600 goat blood samples
Extracted genomic DNA from each blood sample
Amplified PCR using 80 random primers
Obtained the phylogenetic map and tree of goats by bioinformatics and biostatistics.

Cardiovascular Research Center, Temple University School of Medicine 09/2008 to 01/2011

Graduate Student

Master Thesis: Blebbistatin protects rodent myocytes from death in primary culture via inhibiting Na/Ca exchanger and L-type calcium channel

Adult mouse cardiac myocytes
isolation. Cell culture
with/without treatment

Cell contraction measurement. Calcium Imaging:calcium transient and sarcoplasmic reticulum calcium content measurement. Electrophysiology: Myocytes Na/Ca exchanger current, Myocytes L-type calcium current, Myocytes action potential measurement, Statistics Analysis

Biological Science Department, Rutgers University 09/2011 to 08/2014

Graduate Student

Master Thesis: Slow oscillations require a balance between the linear negative slope conductance region of a regenerative inward current and slow voltage-gated outward currents.

Sharp Electrode, two-electrode voltage-clamp , and dynamic Clamp to explore the mechanism of the individual neuronal oscillation properties, Statistics

Teaching Experience

Teaching Assistant Instructor, Biological Science Department, Rutgers Newark 09/2011-05/2014

General Biology 101&102

1. Met with professor and other TAs weekly to plan student discussion sessions
2. Guided small student group discussions on scientific papers.
3. Answering students' questions, helping them complete their lab exercises.

Foundation of Molecular and Cellular Biology

1. Running laboratory session, assembling materials and supplies for laboratory session for 20 students
2. Guiding student to do experiment, answering students' questions, helping them complete their labwork.

Writing lab midterm and lab final exams.

Poster Presentations

1.Biophysical Society Meeting. March 2011

Poster: Blebbistatin protects rodent myocytes from death in primary culture via inhibiting Na/Ca exchanger and L-type calcium channel.

Yinzheng Guan, Xiaoying Zhang, Yingxin Li, Chris Szeto, Xiajie Ai, Xiongwen Chen Biophysical Meeting, Baltimore Convention Center, Baltimore, Maryland, March 5-9, 2011.

2. Society for Neuroscience Meeting. November 2013
Poster: Slow oscillations in crab pyloric neurons induce by a negative-conductance linear current.
Yinzheng Guan, Amitabha Bose, Jorge Golowasch and Farzan Nadim. Society for Neuroscience Meeting, Nov 9-13, San Diego, California

Research Publication

1. Enhanced basal contractility but reduced excitation-contraction coupling efficiency and beta-adrenergic reserve of hearts with increased Cav1.2 activity.

Mingxin Tang, Xiaoying Zhang, Yingxin Li, **Yinzheng Guan**, Xiaojie Ai, Christopher Szeto,Hiroyuki Nakayama,Hongyu Zhang, Shuping Ge, Jeffery D. Molkentin, Steven R. Houser and Xiongwen Chen.Am J Physiol Heart Circ Physiol. 2010 Aug;299(2):H519-28. Epub 2010 Jun 11.

2. Phosphodiesterases coordinate cAMP propagation induced by two stimulatory G protein-coupled receptors in hearts.

Shubai Liu, Ying Li, Sungjin Kim, Qin Fu, Dippal Parikh, Bharat Sridhar, Qian Shi, Xiaoying Zhang, **Yinzheng Guan**, Xiongwen Chen, Yang K Xiang. Proc Natl Acad Sci USA, 2012.109 (17):P.6578-83

3. Cdc42 and Rab8a are critical for intestinal stem cell division, survival, and differentiation in mice.

Ryotaro Sakamori, Soumyashree Das, Shiyan Yu, Shanshan Feng, Ewa Stypulkowski, **Yinzheng Guan**, Veronique Douard, Waixing Tang , Ronaldo P. Ferraris, Akihiro Harada, Cord Brakebusch, Wei Guo and Nan Gao. J Clin Invest, 2012.(2213): P.1052-65

4. The role of linear and voltage-dependent ionic currents in the generation of slow wave oscillations.

Amitabha Bose, Jorge Golowasch, **Yinzheng Guan**, Farzan Nadim
J Comput Neurosci 2014 Mar 27. Epub 2014 Mar 27.

5. Blebbistatin protects rodent myocytes from death in primary culture via inhibiting Na/Ca exchanger and L-type calcium channel (In Preparation)

Yinzheng Guan, Xiaoying Zhang,Xiongwen Chen

Honors

1.Take part in countrywide competition of mathematics modeling and won the second award in Heilongjiang province .2005.
2.Best undergraduate students, 2005
3.Fellowship for Gifted Students, Northeast Agriculture University., 2006-2007
4.Teaching Assistantship at Rutgers University, Department of Biological Science. 2011.9-2014.5.

Affiliations

1.American Heart Association(AHA)
2.Biophysical Society.(BS)
3.Society for Neuroscience(SFN)