

**Yin and yang of leech heart central pattern generator:  
endogenously bursting neurons yoked together into a half-center oscillator**

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Experiments and dynamical system analysis were combined to study bursting and other dynamic behaviors of heart interneurons both as single cells and in the mutually inhibitory half-center oscillator configuration. This analysis has revealed general biophysical mechanisms that underlie such behaviors as equilibrium, plateau, spiking behavior, slow oscillatory behavior and bursting behavior in a single cell. This study indicates that the half-center configuration enhances robustness of oscillations making them less susceptible to changes in membrane parameters, while endogenous capability to produce bursting behavior limits the minimum period of the half-center oscillator and ensures bursting behavior if the strength of mutual inhibition is weakened.

### **1. Introduction**

Bursting behavior is an oscillatory activity consisting of intervals of repetitive spiking separated by intervals of quiescence. It has been observed in many different neurons and associated with a variety of different functions of the nervous system. Our study is focused on determining mechanisms underlying bursting. The theory of nonlinear dynamical systems provides powerful tools for this study. Here we tease apart the functional advantages of bursting properties provided by intrinsic membrane dynamics and by network interactions.

Neurons are excitable cells with the complex dynamics expressing variety of qualitatively different regimes, such as bursting, tonic spiking, subthreshold oscillations, plateaus and rest potentials. The complexity of endogenous dynamics originates from dynamical diversity of ionic currents which can be separated by different time scales and other characteristics. Analysis in terms of fast-slow dynamical systems gives insights into mechanisms of endogenous generation of bursting behavior.

The one of the oldest network mechanisms underlying the bursting behavior is called half-center oscillator introduced by Brown in 1911 [3]. A half-center oscillator consists of two mutually inhibitory neuronal units where a unit is either a single neuron or a population of neurons. This term emphasizes mutually inhibitory interactions as necessary for generation of oscillations, thus according to this mechanism the two units constitute one oscillator.

Here we use our recent results [5] obtained from modeling and experimental analysis of leech heart timing network to outline cellular and network mechanisms of generation bursting behaviors

and the functional advantages deriving from these mechanisms.

## Model

We exploit the mathematical model [6] developed and thoroughly tested in our previous studies [6,8,9 ]. The single oscillator interneuron model includes nine voltage-dependent currents: a fast  $\text{Na}^+$  current ( $I_{\text{Na}}$ ), a persistent  $\text{Na}^+$  current ( $I_P$ ), two low-threshold  $\text{Ca}^{2+}$  currents, one rapidly ( $I_{\text{CaF}}$ ) and one slowly ( $I_{\text{Cas}}$ ) inactivating, a hyperpolarization-activated cation current ( $I_h$ ), a delayed rectifier-like  $\text{K}^+$  current ( $I_K$ ), a persistent  $\text{K}^+$  current ( $I_{K2}$ ), a fast transient  $\text{K}^+$  current ( $I_{KA}$ ) and FMRFamide-activated  $\text{K}^+$  current ( $I_{KF}$ ). All currents but  $I_{\text{Na}}$  were characterized in voltage-clamp studies, and the kinetic data incorporated into the model. It is described by 16 stiff ordinary differential equations with activation and inactivation variables determining different ionic currents operating on different time scales (Table 1).

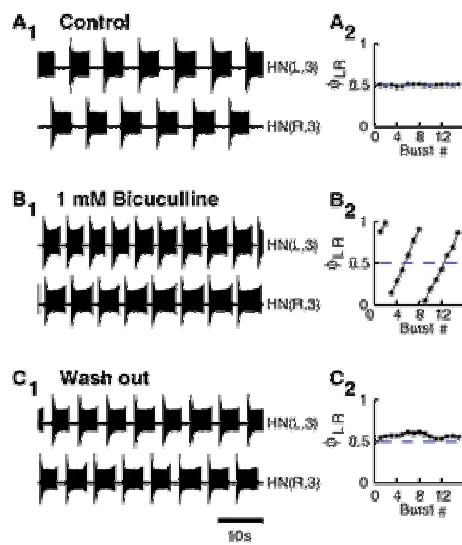
	from -70 mV to -50 mV	from -50 mV to -10 mV
Ultra fast (0.1 msec)	$m_{\text{Na}}$	$m_{\text{Na}}$
Fast (~2-10 msec)	$h_{\text{Na}}, m_{\text{K1}}, m_{\text{CaF}}, m_{\text{Cas}}$	$m_p, m_{\text{KA}}, h_{\text{Na}}, m_{\text{K1}}, m_{\text{CaF}}$
Moderate (~100 msec)	$h_{\text{KA}}, m_{\text{K2}}, m_p, m_{\text{KA}}$	$m_{\text{Cas}}, h_{\text{CaF}}, h_{\text{KA}}, m_{\text{K2}}$
Slow (~ 0.5 sec)	$h_{\text{K1}}, h_{\text{Cas}}, h_{\text{CaF}}$	$h_{\text{K1}}$
Very Slow (~ 2-6 sec)	$m_h$	$h_{\text{Cas}}, m_h$

Table 1. State variables operate on more than two different time scales. Some of state variables have different time scale depending on the membrane potential (marked by red in the table).

A model of a half-center oscillator is made up of two identical oscillator interneuron models linked by mutually inhibitory connections [5,6].

## Results

Rhythmic bursting within the heartbeat pattern generator of the medicinal leech is based on the activity of the two mutually inhibitory pairs of oscillator heart interneurons located in ganglia 3 and 4. Bicuculline methiodide (0.1 mM) has been shown to block mutual inhibition between these interneurons [11]. Moreover, simultaneous intracellular recording with sharp microelectrode showed that the oscillator interneurons, which burst in alternation in normal saline, spike tonically when pharmacologically isolated by bicuculline (Fig. 1). Using extracellular recording techniques, we have shown that oscillator heart interneurons continue to burst when pharmacologically isolated with bicuculline.



cycle period.

**Fig 1.** Oscillator heart interneurons recorded extracellularly burst independently when pharmacologically isolated with bicuculline (1 mM). A<sub>1</sub>, Oscillator interneurons burst rhythmically in alternation in normal saline. A<sub>2</sub>, Instantaneous phase between the neurons' activity plotted against burst number stays close to 0.5. B<sub>1</sub>, The oscillator interneurons burst independently in bicuculline methiodide (1 mM). B<sub>2</sub>, The instantaneous phase drifts gradually from 0 to 1, demonstrating independent bursting with different cycle periods. C<sub>1</sub>, The oscillator interneurons burst in alternation after wash out with normal saline. C<sub>2</sub>, The instantaneous phase stays near to 0.5, although with larger deviations. The instantaneous phase was defined as the delay of the HN(R,3) burst median spike relative to the HN(L,3) burst median spike divided by the current HN(L,3)

### The difference in temporal characteristics between endogenous bursting and bursting in a half-center oscillator, observed experimentally, suggests strong coupling.

Temporal characteristics of the bursting regime in bicuculline are significantly different to those observed in a half-center configuration: period, spike frequency and spike number per burst were smaller by ~25%, 30% and 35%, respectively; duty cycle was longer by ~25%. Sharp microelectrode penetration prevents the endogenous bursting most likely by shifts in  $E_{leak}$  and  $g_{leak}$ . To study the effects of this microelectrode-induced change in leak current parameters on model activity, we constructed a two-parameter bifurcation diagram ( $E_{leak}$  vs  $g_{leak}$ ) of model activities.

### Bursting behaviors in the models of a single neuron and a half-center oscillator

Analysis of a single neuron model reveals that bursting behavior is sensitive to variation of the parameters  $E_{leak}$  and  $g_{leak}$ . The bifurcation diagram ( $E_{leak}$  vs  $g_{leak}$ ) shows a narrow stripe of parameter values where bursting behavior occurs, separating large zones of tonic spiking and silent behaviors (Fig. 2A). In contrast to Fig. 2A, similar analysis performed for a half-center oscillator outlines a large area of robust bursting (Fig. 2B).

Temporal characteristics of bursting behavior in the single-cell model appear to be dominated by two time constants. The slow inactivation of  $I_{Cas}$  controls the burst duration (Fig. 3A). The slow activation of  $I_h$  determines the interburst interval. Note that  $I_h$  is maximal just at the transition to the burst phase (Fig. 3A). Our previous analysis of the half-center model [6] demonstrated that bursting in the half-center model is controlled only by the slow inactivation of  $I_{Cas}$ . Because  $I_h$  activates more quickly than  $I_{Cas}$  inactivates (the time constants differ by a factor larger than two

in the voltage ranges of interest (Table 1)), the strong inhibition fully activates  $I_h$  but it is insufficient to overcome the inhibition until  $I_{Cas}$  inactivates sufficiently reducing the spike frequency and associated inhibition. Thus the maximal conductance, but not the activation time constant of  $I_h$ , controls the burst duration of the opposite cell by determining the level of  $I_{Cas}$  at which an escape can be effected and the opposite cell's burst terminated by inhibition.

Fig. 2. Bifurcation diagram of the single neuron model activities (A), and bifurcation diagram of the bursting activity in a half-center oscillator model (B). A, Pink, white, and yellow areas mark the parameter regimes where tonic spiking, bursting, and silence are stable, respectively. Green areas mark parameter regimes of multistability. Multistability (A) points to the area where bursting coexists with silence; multistability (B) points to the area where bursting co-exists with tonic spiking; multistability (C) points to the area where tonic spiking co-exists with silence. B, The blue area corresponds to the parameter region where stationary bursting activity was observed and the white area to the region where it was not. The pink patch delimits the single cell model parameters that produced bursting temporal characteristics within the ranges measured experimentally.

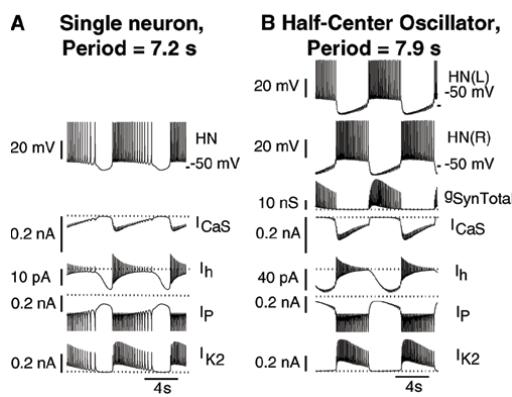
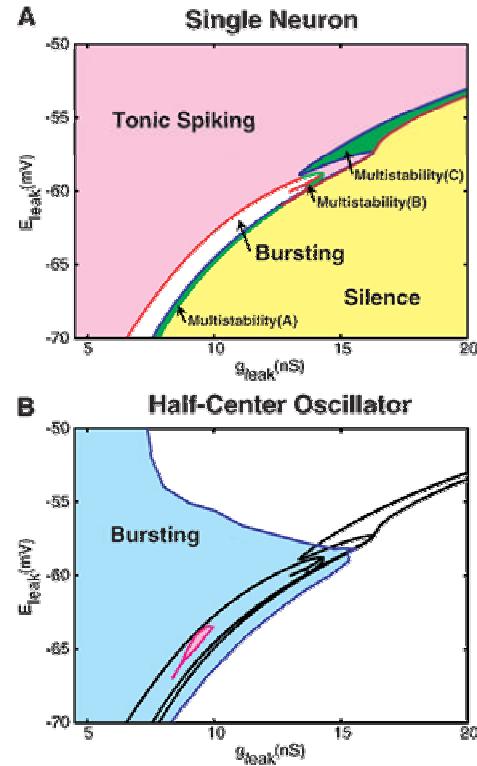


Fig.3 Voltage and current traces produced by A, the single cell and B, the half-center oscillator models. Parameters  $E_{leak}=-63.5$  mV and  $g_{leak}=9.9$  nS were chosen to comply with the experimental data on firing characteristics, and all other parameters were canonical [6]. Major inward and outward currents contributing to the slow wave of oscillation in each model are illustrated. Dotted lines mark 0 nA.

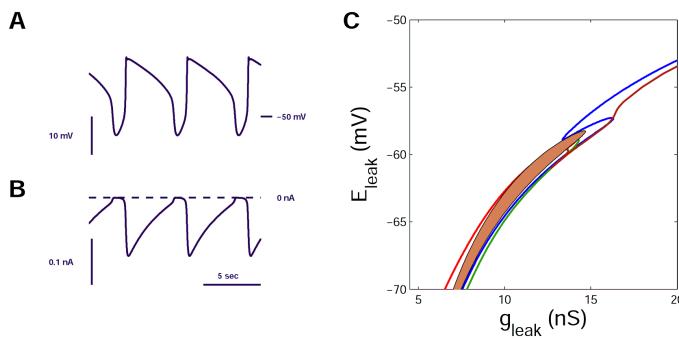
The half-center oscillator model is considerably less sensitive to the variation of the maximal conductances of the voltage-gated currents as it is with leak current parameters. One maximal conductance was varied at a time, while all others were set to their canonical values.

The single neuron model is very sensitive to the variation of  $g_{leak}$  and  $\bar{g}_P$ , each of which can only be varied in a range of  $\sim 1$  nS. The single neuron model is a bit less sensitive to the variation of  $\bar{g}_{Cas}$  and  $\bar{g}_h$ ; these conductances can be varied over a range of  $\sim 5$  nS and  $\sim 8$  nS, respectively. Other maximal conductances can be varied on the order of tens to hundreds of nS. In the half-center model, these ranges considerably expand, ranging from factor 1.7 for  $\bar{g}_{Cas}$  up to factor of 9.7 for  $\bar{g}_h$ .

### Bursting in a single cell

Principal mechanisms underlying different dynamical regimes like bursting, tonic spiking and stationary states can be understood by using the methods of the qualitative theory of slow-fast dynamical systems [1,2,4,7,10]. These methods can be supported by experiments, e.g. blockade of certain groups of the currents can simplify the neuron dynamics, and elicit characteristic behaviors of neurons. Under such conditions we can get deeper insights into the intrinsic dynamics of the neuron. One of the experimental tests commonly used in studies endogenous bursting regimes is to test whether a neuron produces oscillatory activity under a blockade of fast sodium current. Unfortunately, we do not have specific blocker for fast sodium current in the leech neurons. So, a *gedanken* experiment performed in the single neuron model shows that if the fast sodium current is blocked, slow oscillations with the period (Fig. 4A) close to the control bursting period (Fig. 3A). They are observed in the area of parameters close to the area where bursting regime is observed (Fig. 4C). This bursting regime area has borders with areas supporting stationary states so that the state with the depolarized rest potential roughly corresponds to tonic spiking area in the original system and the state with the hyperpolarized rest potential corresponds to the stationary state.

Fig. 4. Slow oscillations in the single neuron model with blocked  $I_{Na}$ . Oscillations of the membrane potential (A) and  $I_{Cas}$  (B) have period close to the one of bursting in the complete model (Fig. 3A). All model parameters are the same as in Fig. 3A. C. Bifurcation diagram of this reduced single neuron model. The orange region corresponds to the slow oscillations.



### Conclusions

The heartbeat motor pattern in leeches is based on the activity of two mutually inhibitory pairs of heart interneurons located in ganglia 3 and 4, which generate alternating bursting activity.

Experiments and dynamical system analysis were combined to study bursting and other dynamic behaviors of heart interneurons both as single cells and in the mutually inhibitory half-center oscillator configuration. This analysis has revealed general biophysical mechanisms that underlie such behaviors as equilibrium, plateau, spiking behavior, slow oscillatory behavior and bursting behavior. A two-parameter bifurcation diagram ( $E_{leak}$  vs  $g_{leak}$ ) of a model of a single heart interneuron shows a narrow stripe of parameter values where bursting occurs, separating large zones of tonic spiking and silence. Similar analysis of a half-center oscillator model outlined a much larger area of bursting. Bursting in the half-center oscillator model is also less sensitive to variation in the maximal conductances of voltage-gated currents than in the single neuron model.

This study indicates that the half-center configuration enhances robustness of oscillations thereby making them less susceptible with respect to changes in membrane parameters, while endogenous capability to produce bursting behavior limits the minimum period of the half-center oscillator and ensures bursting behavior if the strength of mutual inhibition is weakened.

### Acknowledgements

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