Modelling a Respiratory Central Pattern Generator Neuron in *Lymnaea stagnalis* *

Sharene D. Bungay † Sue Ann Campbell ‡

1 Introduction

Lymnaea stagnalis, often termed the great pond snail, is characterized in part by its ability to take in oxygen both cutaneously and aerially (via its rudimentary lung). The central nervous system of the *Lymnaea* is composed of a relatively small number of large, identifiable neurons. In 1990, Syed et al. [1] established that respiratory rhythmogenesis in the *Lymnaea* is controlled by a 3-neuron central pattern generator (CPG) as depicted in Figure 1. Syed et al. [1, 2] were able to identify and culture the three neurons that make up the CPG, both singly and together, where they reform the same connections as are present *in vivo*. Based on various experimental manipulations, they described the following characteristics of this network. One of these neurons, the RPeD1 (Right Pedal Dorsal 1) neuron, is spontaneously active and is responsible for the initiation of respiration, upon receipt of input from the respiratory orifice. The other two neurons, VD4 (Visceral Dorsal 4) and Ip3I (Input 3), are quiescent except during active respiration. The VD4 neuron (which is responsible for inspiration) is connected via reciprocal inhibitory synaptic connections to both the RPeD1 neuron and the Ip3I neuron (which is responsible for expiration). The Ip3I neuron has excitatory synaptic con-

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†Department of Computer Science, Memorial University of Newfoundland, St. John's, NL, A1B 3X5. Corresponding author.

‡Department of Applied Mathematics, University of Waterloo, Waterloo, ON, N2L 3G1.

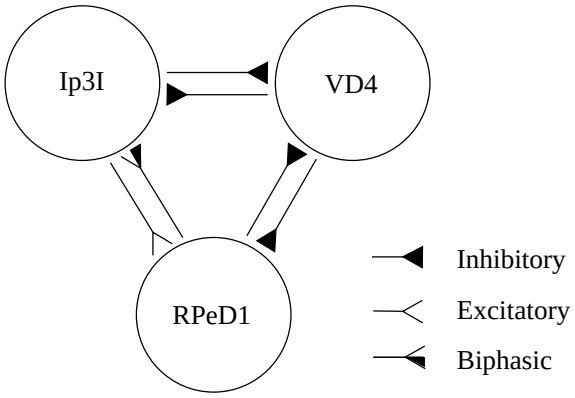

Figure 1: Central pattern generator that controls respiration in the *Lymnaea stagnalis*.

nections to RPeD1 while the return connections are biphasic, i.e., excitatory via postinhibitory rebound.

The ultimate goal of this work is to develop a mathematical model of the breathing CPG in the *Lymnaea*. The work described here focuses on the development of a Hodgkin-Huxley type mathematical model of the RPeD1 neuron which includes ionic currents for sodium, potassium, and calcium. Results from model simulations are compared to available experimental data.

2 The Model

A Hodgkin-Huxley type mathematical model is used to describe the rate of change of membrane potential (V) as

$$C_m \frac{dV}{dt} = -I_{Na} - I_K - I_{Ca} - I_L \tag{1}$$

where C_m is the membrane capacitance, and $I_{Na,K,Ca,L}$ are the total sodium (Na), potassium (K), calcium (Ca), and leak (L) currents respectively. The sodium current, I_{Na}, is a sum of two currents: a standard sodium current I_{Na_S} and a persistent sodium current I_{Na_p}. Similarly, the potassium current, I_K, is a sum of two currents: a standard potassium delayed rectifier current I_{KV} and a potassium A current, I_A. The individual currents are modelled in

the standard way:

$$
\begin{aligned}
I_{Na_s} &= g_{Na} m^3(V) h(V) (V - V_{Na}) && (2)\\
I_{Na_p} &= g_{Na_p} m_p^3(V) h_p(V) (V - V_{Na}) && (3)\\
I_A &= g_A q^2(V) b(V) (V - V_K) && (4)\\
I_{KV} &= g_{KV} n^4(V) (V - V_K) && (5)\\
I_{Ca} &= g_{Ca} r(V) s(V) (V - V_{Ca}) && (6)
\end{aligned}
$$

where $g_z, z \in \{Na, Na_p, A, KV, Ca\}$ are the conductances, and $V_x, x \in \{Na, K, Ca\}$ are the reversal potentials of the currents. The activation variables, m, m_p, q, n, and r, and inactivation variables, h, h_p, b, and s, are described by first order equations

$$\frac{dy}{dt} = \frac{y_\infty(V) - y}{\tau_y(V)}, \qquad y \in \{m, m_p, h, h_p, q, b, n, r, s\} \tag{7}$$

where $y_\infty(V)$ denotes the respective steady-state activation/inactivation functions, and $\tau_y(V)$ the time constants of activation/inactivation.

The steady-state activation and inactivation functions are of the form

$$y_\infty(V) = \frac{1}{1 + \exp\left(\frac{V - V_y^{1/2}}{K_y}\right)}, \qquad y \in \{m, m_p, h, h_p, q, b, n, r, s\} \tag{8}$$

where $V_y^{1/2}$ is the half-activation voltage, and K_y is the rate or "slope factor".

The activation time constants are described by the following functions

$$
\begin{aligned}
\tau_y(V) &= \frac{\tau_{0,y} \exp\left(\frac{\delta_y\left(V - V_y^{1/2}\right)}{K_y}\right)}{1 + \exp\left(\frac{V - V_y^{1/2}}{K_y}\right)}, \qquad y \in \{m, q, n\} && (9)\\
\tau_{m_p}(V) &= 11.7 + 0.004 \exp\left(\frac{-V}{7.6}\right) && (10)\\
\tau_r(V) &= t_r, && (11)
\end{aligned}
$$

where t_r is a constant. The inactivation time constants were taken to be voltage independent, i.e., constant:

$$\tau_y(V) = t_y, \qquad y \in \{h, h_p, b, s\}.$$

Values for all other parameters of the model were obtained either directly from the literature or via fitting of experimental data found in the literature. The values so obtained and the sources are given Table 1. The membrane capacitance was taken to be $C_m = 0.333\ \mu F$ in accordance with data from [3].

Table 1: Parameter values used.

z	y	g_z μS	V_z mV	$V_y^{1/2}$ mV	K_y mV	$\tau_{0,y}$ ms	δ_y	t_y ms
Na[1]	m	0.5	22	-34.74	-9.32	6.98	0.16	
	h			-59.95	9.4			3.44
Na_p[2]	m_p	0.25	22	-18	-16.4			
	h_p			-46	7.43			300
Ca[3]	r	0.05	80	-18.08	-7.2			10.5
	s			-24	8.7			90
KV[4]	n	0.2	-70	-42.5	-24.5	62.56	0.83	
A[4]	q	0.01	-70	-62.3	-8.3	16.1	0.087	
	b			-69.1	8.8			200
L[5]		0.00025	-12.2					

[1] Parameter values computed using data from [4] and [5].
[2] Parameter values computed using data from [6].
[3] Parameter values computed using data from [7] and [8].
[4] Parameter values computed using data from [9].
[5] Parameter values set so that the resting potential would be in the physiological range given in [3].

3 Results

Numerical simulations of the model were carried out using XPPAUT, a differential equation simulation tool developed by B. Ermentrout. See [10] for details. A Runge-Kutta fourth order solver with stepsize 0.1 ms was used. Figure 2 illustrates the spontaneous spiking behaviour of the model with the parameter values listed in Table 1. While the frequency of the spiking, $\approx$ 0.65 Hz, is consistent with experimental observations [3], the spike amplitude is smaller than that seen in experiments. Examination of the individual currents indicate that sodium initiates the spiking behaviour, after which

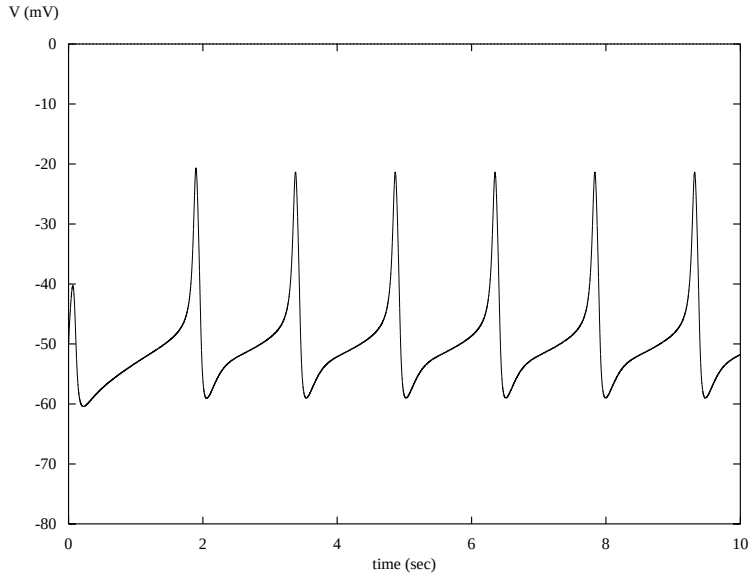

Figure 2: Spontaneous spiking behaviour exhibited by a Hodgkin-Huxley like model of the RPeD1 neuron. Initial conditions: $V = -50$, $m = m_p = n = q = r = 0$, $h = h_p = b = s = 1$.

calcium takes over. The potassium current is involved in the repolarization phase, with I_A causing the shoulder of the action potential.

To determine the robustness of spiking in the model with respect to the conductances, we carried out numerical bifurcation studies varying each conductance separately. These studies were done using the AUTO numerical continuation package [11] within the XPPAUT package. One such study, using g_{Na} as the bifurcation parameter, is show in Figure 3. In this example, stable oscillations exist between two limit points at $g_{Na} = 0.4946$ and 1.004. The unstable oscillations created by the limit points are lost in subcritical Hopf bifurcations. A similar sequence of bifurcations occurred for all the conductances. A summary of all the numerical bifurcation studies is given in Table 2.

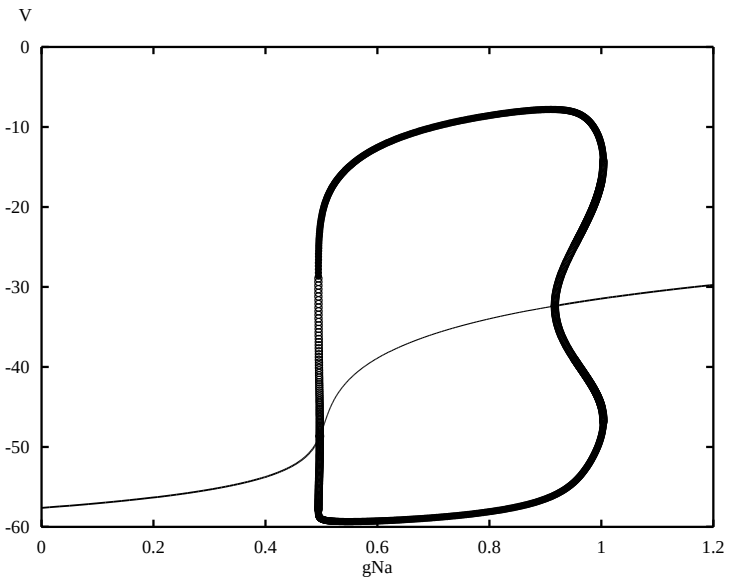

Figure 3: Numerical bifurcation curves for g_{Na}. Thick/thin lines correspond to stable/unstable equilibrium points, filled/open circles correspond to stable/unstable periodic orbits. Stable oscillations occur for $g_{Na} \in [0.4946, 1.004]$.

4 Discussion and Conclusions

We have created a Hodgkin-Huxley type model for the RPeD1 neuron of the freshwater pond snail, including 5 different ionic currents. Model results demonstrate spontaneous realistic spiking behaviour with a frequency that matches experimental results. Bifurcation analysis provides bounds on the values of the conductances for the model to exhibit periodic behaviour.

References

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Table 2: Location and type of bifurcation points for each conductance.

Parameter	Bifurcation type	Value	Period	Stable Oscillations
g_{Na}	subcritical Hopf	0.4972	1450	[0.4946, 1.004]
	limit point	0.4946	2103	
	limit point	1.004	238	
	subcritical Hopf	0.9171	175	
g_{Na_p}	subcritical Hopf	0.2471	1477	[0, 0.2446]
	limit point	0.2446	2175	
g_{Ca}	subcritical Hopf	0.0498	1468	[0.04967, 0.1339]
	limit point	0.04967	2146	
	supercritical Hopf	0.1339	129	
g_A	subcritical Hopf	0.01022	1541	[0, 0.01042]
	limit point	0.01042	2345	
g_{KV}	subcritical Hopf	0.1351	190	[0.1347, 0.2007]
	limit point	0.1347	193	
	limit point	0.2007	2128	
	subcritical Hopf	0.2004	1458	

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