Resonance in the PY Cell

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Abstract

The property that describes the neuron's preferred response to inputs of injected current is called resonance. To produce resonance, a cell must have two requirements: a low-pass filter (attenuates high frequencies) and a high-pass filter (attenuates low frequencies). The neuron whose resonance frequency we wish to determine is the PY neuron of the STNS (stomatogastric nervous system) of *Cancer borealis*. The STNS consists of rhythmic neural networks that control the movement of a crustacean's foregut. Noting that all neurons exhibit a low-pass filter, we investigate the high-pass filter of the PY neuron, and its properties.

The PY neuron has an I_h (hyperpolarizing activating current) channel as its high-pass filter. Thus, for the PY neuron to exhibit resonance, it must have an I_h channel (or any other high-pass filter) in addition to the inherit low-pass filter property. To inspect if PY has resonance we used the current clamp method and injected a sinusoidal current that sweeps through several frequencies. This current, also called ZAP, produced a voltage response that we can analyze via an impedance versus frequency graph. Resonance was not seen in the preliminary experiments so we decided to use 10 mM CsCl to block I_h, to verify that I_h did not cause an unnoticeable resonant peak. However, resonance was still not seen, so we injected an artificial I_h current via dynamic clamp. Under this method, resonance was produced by increasing the conductance of I_h.

To model these results we used the Hodgkin-Huxley equation and created the same scenario. Under low conductance, resonance was not evident, but when conductance increased the PY neuron had frequency preference. This leads us to believe that while the PY neuron has an I_h current, its conductance is too low to produce resonance. The probable cause for this, is that PY cells are small in size relative to the other STG cells, resulting in less I_h channels per unit membrane.

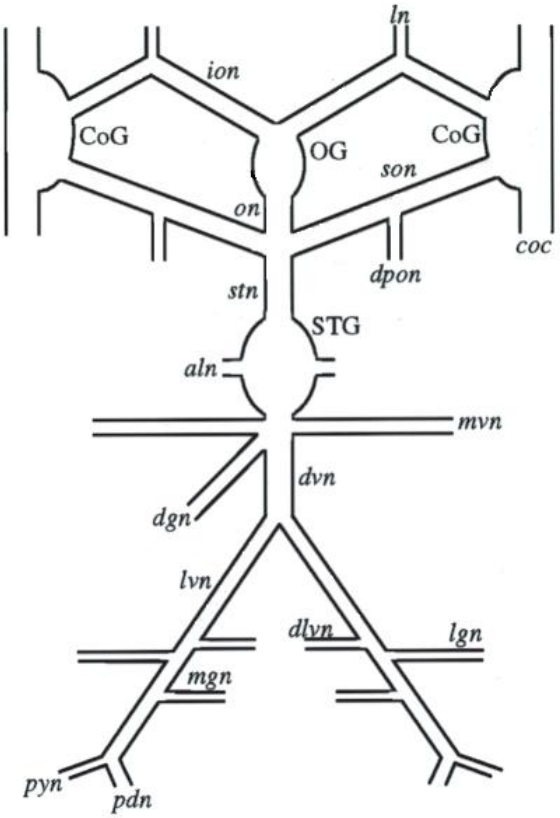
Introduction

We experiment on the stomatogastric nervous system (STNS) of crustaceans, more specifically, *Cancer borealis*. The STNS consists of rhythmic neural networks that control the movement of a crustacean's foregut. When the STNS is dissected out of the crustacean, it produces motor patterns similar to those that occur in-vivo. There are two important neural networks that produce the relatively harmonious rhythm detected: the gastric and pyloric networks. The network that is studied more extensively and the one that will be used in our experiment, is the pyloric network. This network, as well as the gastric network, cannot be expected to produce certain motor patterns (or more qualitatively, voltage responses) based on their anatomical make-up alone, since the outputs of these systems depend on the intrinsic and synaptic properties of the neurons. Furthermore, since the outcomes of the neurons are variable, depending on the modulation that is present, we will subject the neuron to different frequency ranges and observe its response.

In this system, we study the response of neurons to various frequencies. Resonance is the frequency at which neurons respond best to inputs of injected current (Hutcheon, Y Yarom, 2000). Therefore, the neurons that exhibit resonance have a preferred frequency, and will only accept current at that range of frequency. The neuron we experiment on is the PY (pyloric neurons). If the PY neuron has a resonant frequency, then it will respond best to a restricted range of frequency inputs that results in a comparable voltage response. Our hypothesis is that the neuron will show a frequency preference, and thus a higher voltage response when the preferred frequency is introduced.

The Stomatogastric Nervous System (STNS)

The STNS, seen in figure 1, consists of four ganglia that are connected, called the paired commissural ganglia (CoGs), unpaired esophageal ganglion (OG), and the stomatogastric ganglion (STG). The CoGs and the OG have modulatory neurons that control the STG's properties. The STG received these modulatory inputs through the *stn* (Harris-Warrick, Marder et al. 1992). There are about 25 neurons in the STG of *C. borealis*, 5 of which are PYs -the neuron we are interested in (Kilman, Marder, 1996). The STG itself contains numerous neurons that control the gastric mill (Huxley 1880) and pyloric areas of the stomach.



1.Schematic representation of the stomatogastric nervous system of *Cancer Borealis.* Abbreviations are: CoG, commissural ganglion; OG, oesphageal ganglion; STG, stomatogastric ganglion; *aln*, anterior lateral nerve; *coc*, circumoesophageal connective; *dlvn*, dorso-lateral ventricular nerve; *dpon*, dorsal posterior oesophageal nerve; *dvn*, dorsal ventricular nerve; *dgn*, dorsal gastric nerve; *ion*, inferior oesophageal nerve; *lgn*, lateral gastric nerve; *lvn*, lateral ventricular nerve; *mgn*, medial gastric nerve; *mvn*, medial ventricular nerve; *on*, oesophageal nerve; *pdn*, pyloric dilator nerve; *pyn*, pyloric nerve; *son*, superior oesophageal nerve; *stn*, stomatogastric nerve (Swensen, Golowasch 2000).

The pyloric rhythm is faster than the gastric rhythm and is composed of the activity of the pyloric dilator (PD), pyloric (PY), lateral pyloric (LP), ventricular dilator (VD), and inferior cardiac (IC) neurons. The gastric mill rhythm is composed of the activity of the medial gastric (MG), dorsal gastric (DG), lateral posterior gastric (LPG), gastric mill (GM), and LG neurons (Bucher, Taylor, Marder, 2006). The system we are more concerned about is the pyloric, since this is where our neuron of interest lies.

The pyloric rhythm is triphasic with a period of about 1-2 seconds as seen by figure 2. This rhythm consists of action potentials produced by the PD neurons, which are then followed by action potentials in the LP neuron, and then the PY neuron. Considering that the CoGs and the OG regulate this triphasic rhythm, to learn more about the intrinsic properties of a neuron, the impulse activity from the two ganglia must be impeded. To study the resonance properties of the PY, the CoG and OG must not have any interaction with the STG, so that the activity results from the neuron's intrinsic properties (this will be done by using tetrodotoxin, a sodium channel blocker). Moreover, the intrinsic properties of the PY neuron may also be subject to blockers, which will be taken advantage of when studying resonance.

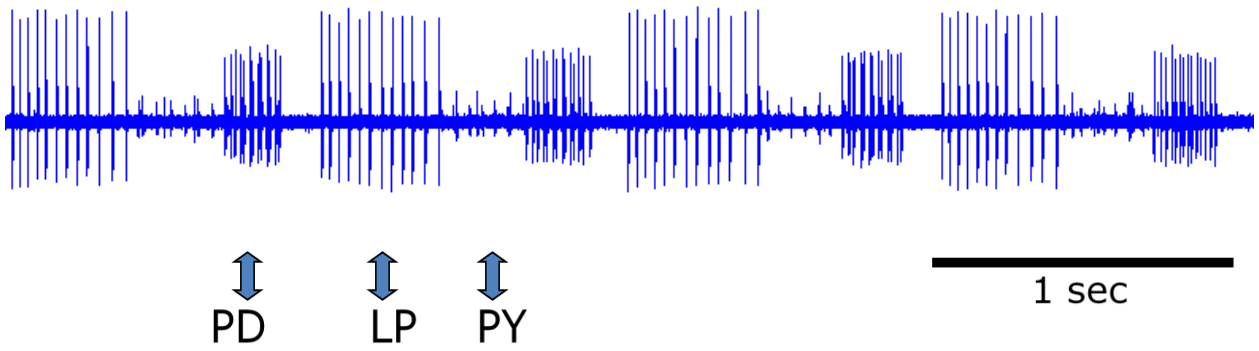


Figure 2. Extracellular recording of the *lvn*, shows a triphasic property with a period of 1 second. This triphasic pattern generally starts with the pacemaker neuron PD, and then is followed by a pause. After the pause, the follower neurons LP and PY fire respectively.

Resonance

The property that describes the neuron's preferred response to inputs of injected current is called resonance (Hutcheon, Y Yarom, 2000). Understanding resonance in different neurons of the stomatogastric nervous system results in an enhanced view of how the pyloric rhythm is modulated. The property of resonance has been studied since the 1980s on the neurons of guinea pigs (Gimbarzevsky 1984). Resonance has already been found in the AB, PD, and LP neurons of the stomatogastric nervous system (Tohidi 2008).

To produce resonance, a cell must have two requirements: a low-pass filter (attenuates high frequencies) and a high-pass filter (attenuates low frequencies). Fortunately, a low-pass filter is a property that exists in all passive cells and is produced by the leak conductance. To have a high-pass filter, the cell must follow two criteria: 1. it must actively opposing changes in membrane voltage and 2. It activates slowly relative to the membrane time constant (Hutcheon, Y Yarom, 2000). The high-pass filter property in the PY neuron, which follows both of these characteristics, is the I_h (hyperpolarization activating current). Thus, for the PY neuron to exhibit resonance it must have an I_h channel in addition to the inherit low-pass filter property.

We analyze resonance by injecting a range of different currents into the neuron, and recording the voltage response. The voltage response may be seen visually when analyzing the data, or quantitatively by an impedance versus frequency graph. Impedance is simply resistance that is dependent upon frequency, whose resistance is defined as voltage divided by current. To sweep through different frequencies of current, we used the ZAP (Impedance Amplitude Profile) function. The ZAP allows us to set the frequencies that the current will sweep through, and the

time frame that each frequency will last. Thus, we are able to run the ZAP protocol to analyze the voltage response dependent upon frequency. The response of the neuron is best where the impedance amplitude is greatest when plotted against frequency, or when the voltage response is greatest when plotted against time.

Methods

Dissection

All the dissections were done on the crab species *Cancer borealis*, which were kept in an artificial seawater environment until the time of dissection. After the crab is taken out of the tank, it is put in an ice bucket for 30 minutes to anesthetize it. The crude dissection is the first part of the dissection with the main purpose of dissecting the crab's stomach (visible when the carapace is removed) out of the organism. The second part of the dissection is called the fine dissection, and is done under a microscope. This process involves freeing of the stomatogastric nervous system from the crab's stomach to resemble figure 1. When the STNS is dissected, it is pinned on a Sylgard Petri dish. The STG is then desheathed, exposing the cells around the ganglion, and more importantly our cell of interest-the PY. Vaseline wells must then be placed around two nerves, the *lvn* (lateral ventricular nerve) and *mvn* (medial ventricular nerve) for proper identification of PY.

Preparation for Experiment

The STNS is placed in an in-vitro environment that allows for its survival, which includes constant saline profusion, a temperature within the range of 10-13 º C, and an almost neutral pH of 7.4-7.5. The saline that we use is composed of 11 mM KCl, 440 mM NaCl, 13

mM $CaCl_2 \cdot 2H_2O$, 26 mM $MgCl_2 \cdot 6H_2O$, 11.2 mM Trizma base, and 5.1 mM Maleic acid (Nadim).

There are two types of electrodes used for this experiment: extracellular electrodes and intracellular electrodes or microelectrodes. The extracellular electrode's main purpose, for this experiment, is to assist in the identification of neurons. One electrode is placed inside of a Vaseline well, and the other electrode is placed anywhere outside of the well. This allows the electrodes to measure the difference in voltage, which is essential to the accurate identification of the PY cell.

The microelectrodes are used to impale neurons. They differ in resistance when using them either for the recording of voltage (use higher heat, ~345), or injection of current (use lower heat, ~340). The microelectrodes are filled with a 0.6 M K_2SO_4 and 0.02 M KCL solution and are attached to manipulators that allow for the fine control of the electrodes. Axoclamp amplifiers are used for the intracellular recordings, and the extracellular recordings use a differential AC amplifier.

Identification of PY

To correctly identify PY, a well must be put on both, the *mvn* and the *lvn*. The extracellular rhythm must correspond to the intracellular PY rhythm (can be seen in figure 3).

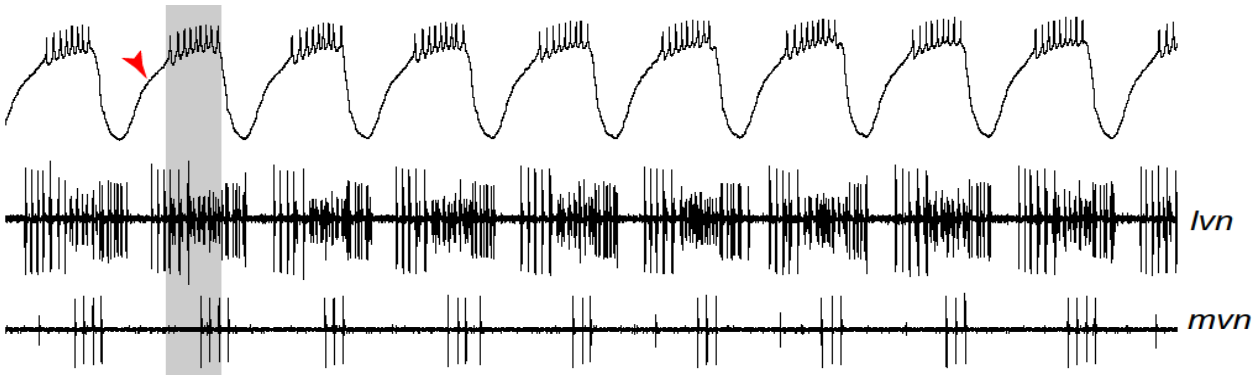


Figure 3. The intacellular rhythm of the PY is located the top. The extracellular *lvn* rhythm is in the middle, and the extracellular *mvn* rhythm is on the bottom. The shaded region shows that the intracellular spikes are in-phase with the *lvn's* PY bursts, but out-of phase with the *mvn's* VD bursts.

After seeing that the intracellular spikes match the bursts of the PY on the *lvn*, it is not enough information to conclude that the cell that is impaled is the PY. Surprisingly, another neuron (VD), also matched its spikes with the *lvn's* PY. Therefore, the extracellular rhythm of the *mvn* is also needed. The VD on the *mvn* is in-phase with the VD spikes, but out-of-phase with the PY spikes. As figure 3 shows, the PY spikes are out of phase with the VD on the *mvn*. Therefore, the PY has only two criteria: 1. it's intracellular spikes must match the bursts on the *lvn,* and 2. It's spikes must be out-of phase with the *mvn's* VD bursts.

Experiment

The experiment is done via the current clamp method. This method involves two microelectrodes, in which one measures voltage and the other injects constant current. Both electrodes impale the PY cell, and certain precautions are taken to ensure both electrodes are in the same cell. For example, when both electrodes appear to be inside the PY cell, hyperpolarize the cell through one electrode, and see if the other recording responds to the hyperpolarization (the same should be done with depolarization). If both recordings respond, and are in-phase with each other, then both electrodes are in the same cell and experimentation may begin.

In order to test the PY neuron's intrinsic resonance property without any influence from neuromodulation, the bath is superfused with 10^{-7} mM tetrodotoxin (TTX). When this Na^{+} channel blocker is applied, the PY neuron must show no rhythm to confirm that TTX has efficiently blocked the sodium channels. Now the PY neuron must show it has a high-pass filter

capability, which in this case will be represented by the hyperpolarization activating current (I_h). When current is injected into the neuron, the voltage response should have a sag that is similar to the one seen in figure 4, which shows that the I_h activated when the cell was hyperpolarizing, and is attempting to depolarize the cell.

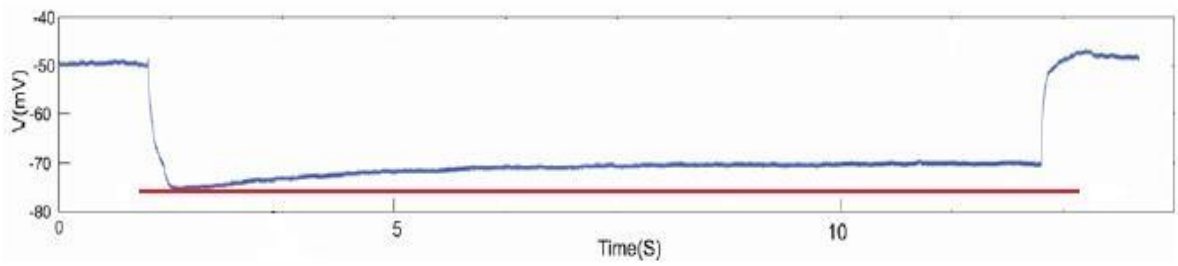


Figure 4. The sag proves the presence of I_h. The red line is added to provide a more obvious example of the sag. *Note: This voltage response is a result from an artificial I_h injection, which was shown to visualize the sag more clearly.*

The current injected to prove I_h, ranges from 1 nA to 7nA in 1 nA increments. This is done only to see under which current (amplitude) I_h is visible, and analyze how it changes as the current increases. The step current injections are performed using Scope[1], which is also the same software that will be used for the ZAP protocol.

After I_h has been identified, we inject the ZAP current using the same nanoamps that were used in the current injections. This will allow us to be sure that the voltage response of the neuron is achieved at a current that allows I_h to activate. The specifics of the ZAP protocol used via Scope are: conversion factor=1mV/V, pre=1000 and post=1000 ms, precycles =3, duration=180,000 ms, and amplitude is equal to the current that was used in the step injections.

When the voltage response of the ZAP injection is obtained, we then superfuse the bath with 10 mM CsCl, to block I_h. The same ZAP protocol is followed again. This is done to ensure

[1] Software can be downloaded via http://cancer.rutgers.edu/software/index.html.

that when we analyze our results, the resonance we achieve is the result of I_h and not an anomaly. If further proof of resonance is needed, CsCl may be washed out, and if resonance due to I_h occurred before the CsCl was added, it should occur again once CsCl is washed out.

Dynamic Clamp

The dynamic clamp software allows for the measurement of membrane potentials and the control of current that is injected via a microelectrode. In dynamic clamp, the current that is injected resembles the current that would flow through a real membrane ("artificial current"). Therefore, we can mimic I_h (or any other ionic current) and inject it into PY, since it can be represented by an equation, which is entered into dynamic clamp.

Equation used:

$$I(V,t) = \bar{g}m(V - E_{rev})$$

Where V is the membrane potential of the neuron, m is the activation gate, $\bar{g}$ is the maximum conductance, and E_{rev} is the reversal potential.

Dynamic Clamp Protocol:

V= -50 mV

K= 4.0

T_{lo}=270 ms

T_{hi}=1800 ms

Where K is slope, T_{lo} and T_{hi} are the time constants on the bottom end and the top end of the dynamic clamp graph, respectively.

XPP script using the Hodgkin-Huxley model

The Hodgkin-Huxley (HH) model is a way to describe the initiation and propagation of action potentials in neurons with nonlinear ordinary differential equations. The principle of conservation of electrical charge states that the charge of a system remains constant, regardless of the changes within the actual system. Our applied current, I_{ex} , can therefore be split into the leak current and the I_h current as a function of time. The equation for external current is as follows:

$$I_{ex}(t) = I_l(t) + I_h(t) \quad (1)$$

Also, capacitance may be defined as

$$C\frac{dv}{dt} = -\sum I(t) \quad (2)$$

where C is capacitance, v is the voltage across the capacitor, and t is time. Therefore, $\frac{dv}{dt}$ is the voltage across the PY neuron's membrane and $\sum I(t)$ is the sum of the ionic currents which pass through the cell membrane.

According to Ohm's law, voltage divided by resistance equates to current. Therefore, equation (3) may be used for all ions, c.

$$I_c = \frac{V_c}{R_c} \quad (3)$$

Conductance (g) is the reciprocal of resistance. This allows conductance to be substituted into Ohm's law, as can be seen by equation (5).

$$g_c = \frac{1}{R_c} \quad \text{so} \quad (4)$$

$$I_c = \frac{1}{R_c} V_c \qquad \text{or} \qquad I_c = g_c V_c \tag{5}$$

V , the driving force, can be expanded into the difference between the membrane potential and the ion's equilibrium potential, as seen below:

$$I_c = g_c (V - E_c) \tag{6}$$

Our model only requires us to use the I_h current and the leak current, I_l , which is defined by equation 7. The leak channels participate in the ability to allow the movement of ions across the cell membrane, thereby contributing to the membrane potential. Moreover, the leak channels are represented by a voltage independent conductance.

$$I_l = g_l (V - E_l) \tag{7}$$

The I_h current (hyperpolarization activated current) is defined by a voltage and time dependent equation (equation 8). I_h describes the voltage gated ion channels represented by a nonlinear conductance g_h.

$$I_h = g_h \, r \, (V - E_h) \tag{8}$$

Moreover, I_h will only exhibit its maximal conductance if all the channels are open. However, since this is not always the case (some of the channels are sometimes blocked), we must introduce a probability factor in our XPP model. The activation variable (*r),* will be the probability that our channels are open. This variable is time dependent and ranges from 0 to 1. The activation variable is represented by these equations:

$$\frac{dr}{dt} = \left(\frac{r_\infty - r}{T}\right) \qquad T = \frac{C_r}{1 + e^{(v - v_{kr})/S_{kr}}} \tag{9}$$

$$r_\infty = \frac{1}{1+e^{(v-v_r)/S_r}} \tag{10}$$

T is the time constant or the time needed for it approach an equilibrium value, V_r is the voltage needed to open half of the channels available, and S_r is the slope of r_∞.

Our Hodgkin Huxley differential equation for the model we used is:

$$C\frac{dv}{dt} = I_{ex} - g_l\,(V - E_l\,) - g_h\,r\,(V - E_h) \tag{11}$$

$$\frac{dr}{dt} = \frac{r_\infty - r}{\tau} \tag{12}$$

The external current applied is a sine function that sweeps through different frequencies, which representing ZAP current that we used experimentally.

The parameters used for this model are as follows:

Leak Current	**I_h Current**	**Activation Variable**	**Time Constant**
$I_l\ = g_l\,(V - E_l\,)$	$I_h = g_h\,r\,(V - E_h)$	$r_\infty = \frac{1}{1+e^{(v-v_r)/S_r}}$	$T = \frac{C_r}{1+e^{(v-v_{kr})/S_{kr}}}$
$g_l\ = 0.1\ M\boldsymbol{\Omega}^{-1}$	$g_h = 0.37\ M\Omega^{-1}$	$v_r = -70\ mV$	$V_{kr} = -110\ mV$
$E_l\ = -70\ mV$	E_h = -10 mV	$S_r = 7\ mV$	$S_{kr} = -13\ mV$

Results

Experimental

When a step current was injected into the PY neuron, the voltage response showed a sag, and a slow time constant relative to the membrane time constant, indicative of I_h. The higher the current injected into the PY neuron, the more the cell hyperpolarizes. We chose currents that are below, the same, and higher than the PY neuron's natural hyperpolarization maximum. Figure 5 shows how these changes affected the amount of sag I_h produced, thereby quantifying I_h's response in the neuron. As can be seen by the graph, a small amount of injected current is not enough to activate the I_h to a visible amount. In fact, the neuron slowly hyperpolarizes even more, resulting in a negative change of voltage. The current injected from -3 nA to -5 nA produces no change of voltage, since I_h is only activated enough to inhibit the neuron from hyperpolarizing further. When the current injected is -6 to -7 nA, the I_h clearly depolarizes the cell, producing a visible sag seen in figure 4.

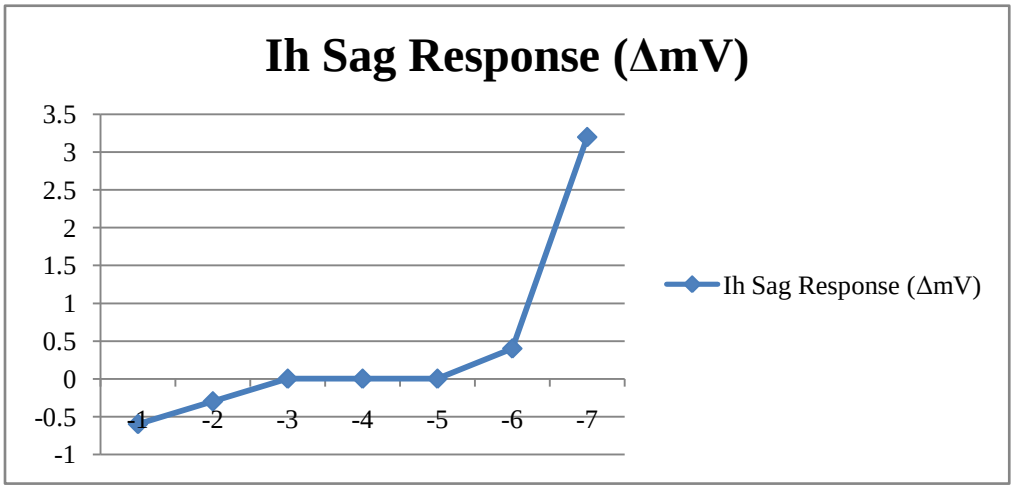


Figure 5. I_h response to changes in injected current from 1 nA to 7 nA in an experiment.

In every PY neuron, we had to determine two important facts: 1. If I_h existed in the PY neuron, and 2. what current (specific to the neuron) needs to be injected to produce a reasonable hyperpolarization value to exhibit I_h. In figure 6, the amount of current needed to produce a

significant I_h response is -7 nA. This current pulled the voltage down to -120 mV, far below the hyperpolarization value of an in-vivo neuron. Therefore, we were assured that the current was more than enough to activate I_h. The bottom graph in figure 6 shows the properties of I_h- a slow time constant, and depolarizing when activated by hyperpolarization.

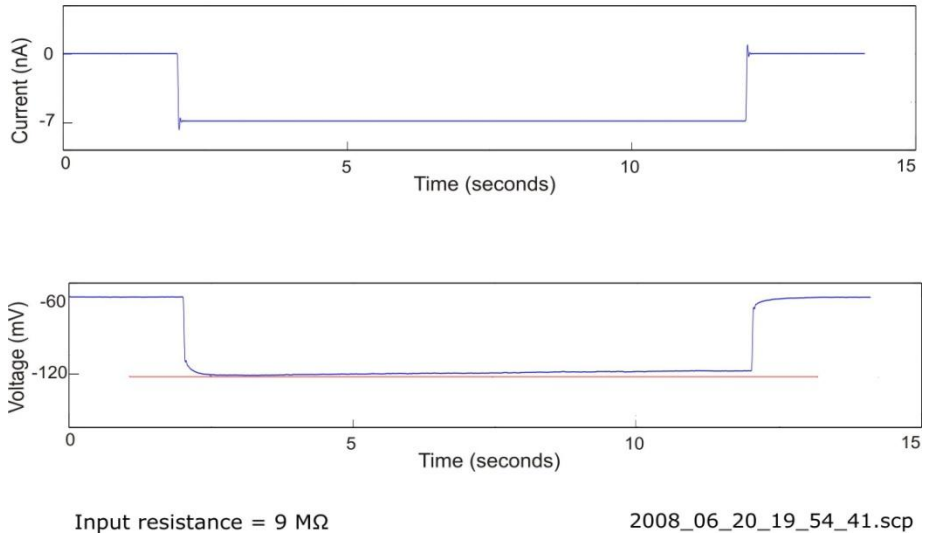


Figure 6. A current of -7 nA was injected (top graph). This current brought the membrane voltage down to -120 mV, below the normal membrane potential of a cell. The voltage response graph (on the bottom) shows the effect of a typical I_h current, with the red line added for obvious proof.

We then introduced a ZAP current into the PY neuron with an amplitude of 1 nA to 7 nA, but in this paper we will look at 7 nA, since this is the current that produces the most visible I_h sag. The ZAP in figure 7 and in all our experiments ran for 180 seconds with 3 precycles. The ZAP frequency ranged from 0.1 Hz to 20 Hz, which we found to be a sufficient range for the purpose of our experiment. The ZAP produced under this protocol is seen on the top in figure 7. Using the current clamp technique, we recorded the response of the neuron to the ZAP current, seen on the bottom of figure 7. The voltage response ranged from -20 mV to -120 mV, and this range continued to decrease as the frequency increased.

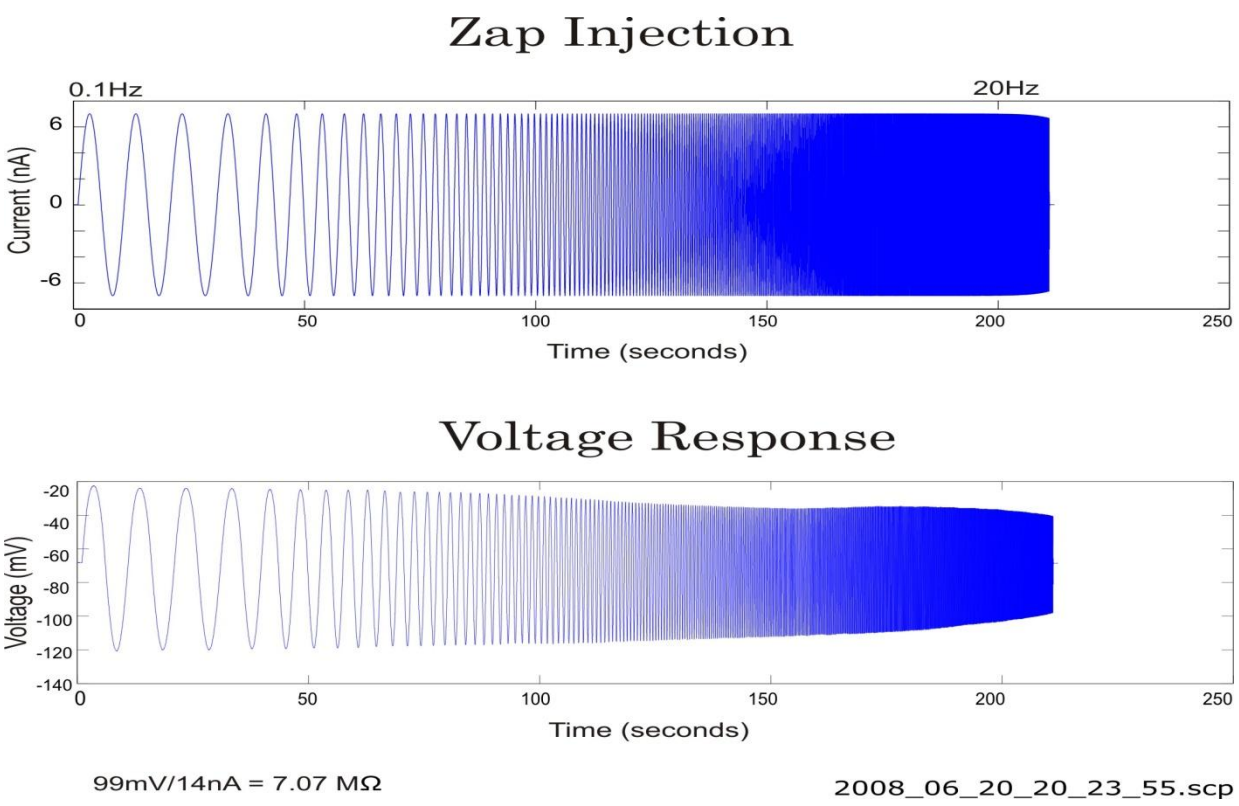


Figure 7. The ZAP injection (top) and the voltage response (bottom) using current clamp.

To analyze this data, we used two methods to increase accuracy. Method 1 (figure 8) uses a Matlab script that creates an artificial line through the middle (approximately) of the voltage response and the ZAP injection. This line allows Matlab to determine when the ZAP current or voltage response crossed the line in order to analyze each cycle for both graphs. One cycle in the script, is from crest to trough and repeats again from crest to trough. The current or voltage (depending on which graph) can then be quantified by dividing the voltage of each cycle by the current of the corresponding cycle, which results in resistance. The graph we then plot is impedance versus frequency, which consists of various points representing each cycle calculated. The problem with this method is that the voltage response might not cross the artificial line (depending on the data) and results in a misleading analysis. However, we correct this issue by using a second method where no line is needed for affirmation. Method 2 (also a Matlab script) consists of using fast Fourier transform (FFT) to determine impedance. The equation for

impedance using FFT is

$$Z = \frac{FFT(V)}{FFT(I)} = \frac{FFT(output)}{FFT(input)}$$

where Z is impedance, V is voltage, and I is current. Therefore, FFT allows a change in domain from time to frequency.

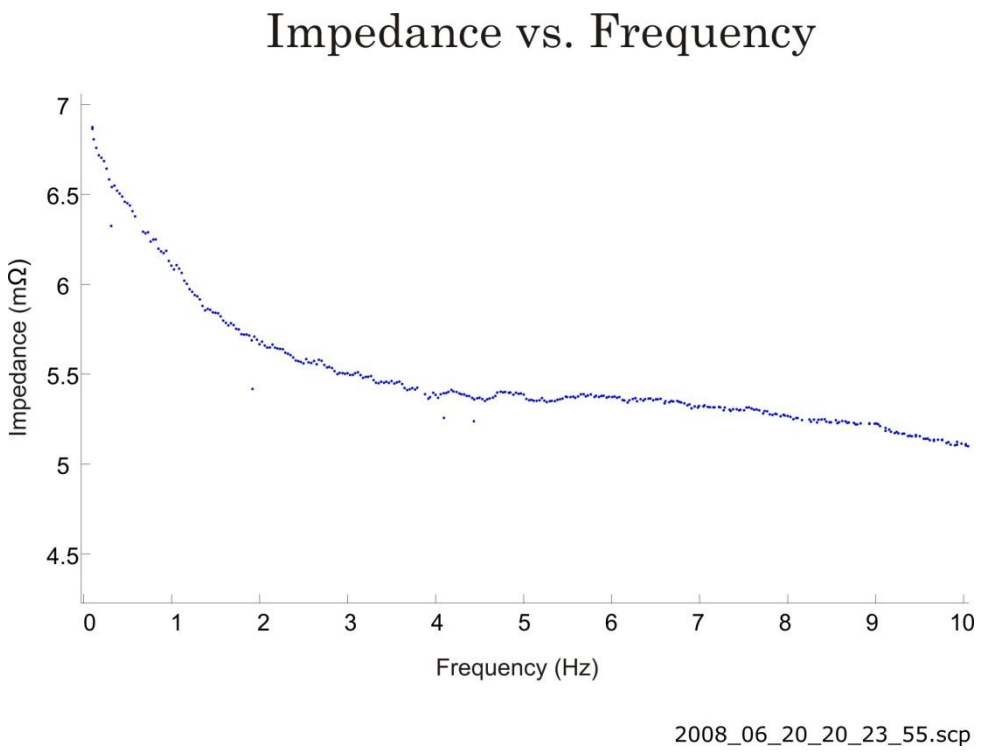


Figure 8. Method 1. There is no apparent resonance, which would be seen by a high pass filter and a low pass filter creating a visible peak in the graph.

To make sure I_h did not have an effect that was not readably noticeable in figure 8, we used cesium chloride to block I_h. As a control, we recorded the ZAP injection (top) and voltage response (bottom) before and after the addition of CsCl, as seen in figure 9. In this experiment, the neuron was able to hyperpolarize to more than -140 mV by only injecting -6 nA. The voltage response before and after the addition of CsCl is relatively the same in both graphs in figure 9, ranging from -20 mV to -140 mV.

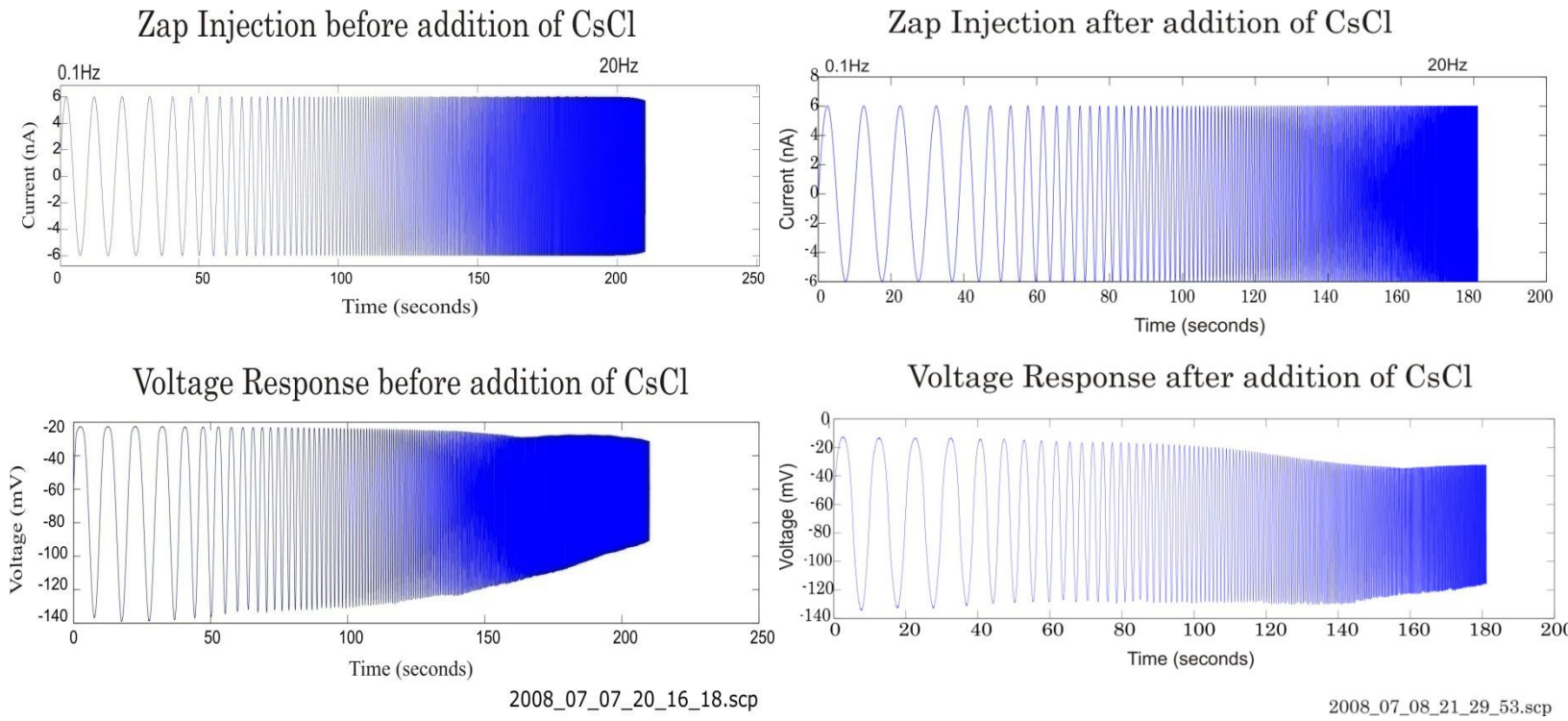


Figure 9. ZAP injection and voltage response before the addition of 10 mM CsCl on the left, and after the addition of CsCl on the right.

We then analyzed the data before and after the addition of CsCl. There seems to be no difference of whether or not I_h is blocked using both methods in figure 10. In all the graphs, the impedance decreases as frequency increases at a relatively steady rate. We then decided to inject an artificial I_h current into our PY neuron via dynamic clamp. As always, we had to prove I_h existed in our PY neuron (left panel in figure 11). In this experiment, only a -1 nA current injection was needed to observe I_h which hyperpolarized the neuron to -90 mV (still more hyperpolarized than its natural state). Using dynamic clamp, we injected an artificial I_h current with a conductance of 30 nanoSiemens (right panel in figure 11). In the artificial current, the sag I_h produces is clearly seen. Moreover, the PY neuron was hyperpolarized to -70 mV, which is relatively

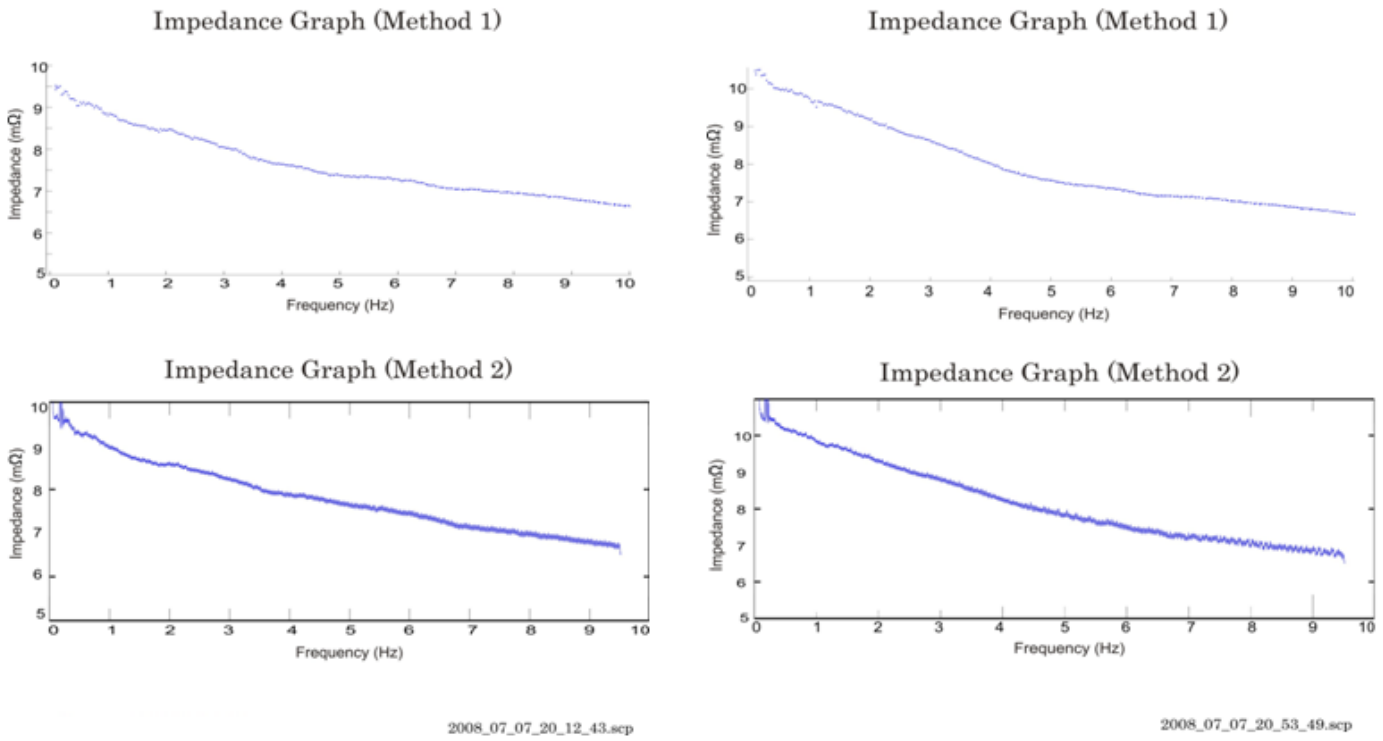


Figure 10. Left Panel: before the addition of CsCl, right panel: during the addition of CsCl. On the top of both panels is method 1 and on the bottom is method 2.

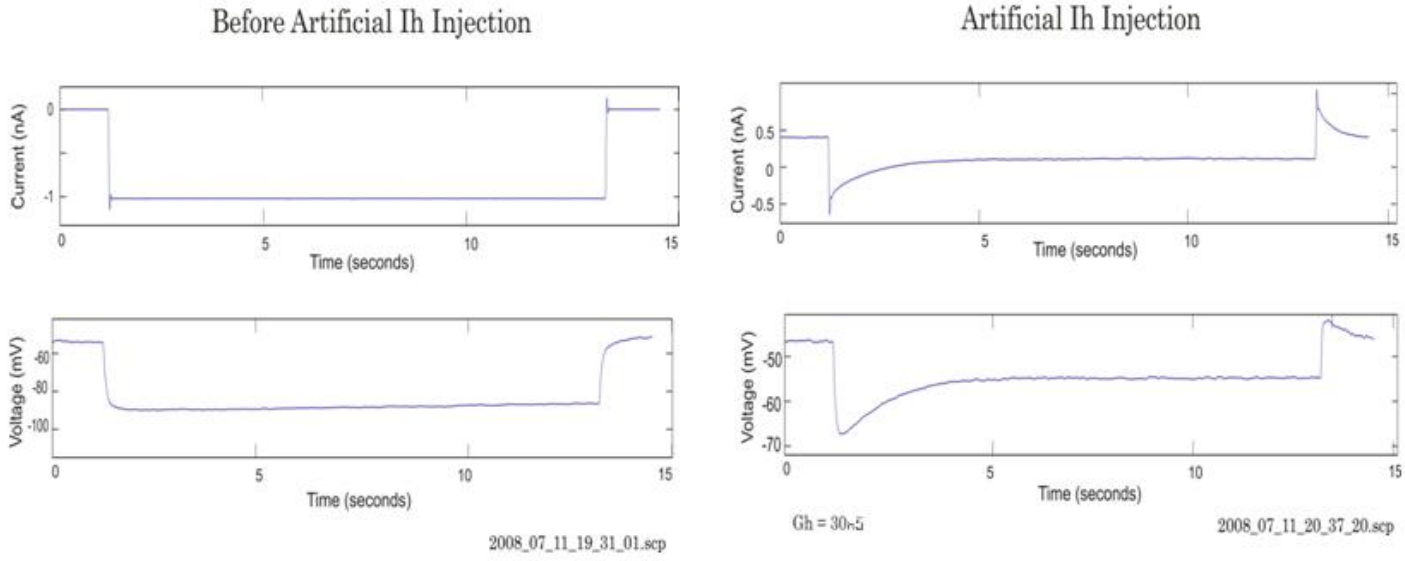


Figure 11. Step current injection and voltage response of PY neuron before an artificial I_h injection on the left panel, and during an artificial I_h injection on the right panel.

small compared to the hyperpolarization our cell underwent in previous experiments, when we injected the current needed to exhibit I_h.

We also injected the ZAP current before the artificial I_h current and after (figure 12). The ZAP injection and voltage response results before using dynamic clamp is similar to the previous experiments-not showing any apparent resonance. However, there are clear differences when recording the ZAP injection and voltage response of the artificial I_h current. In the voltage response, there is a sag between 0 seconds to 90 seconds. Also, the voltage response does not constantly decrease in amplitude as time increases. There is a slight peak formed by the crests of the cycles that range from 50 to 100 seconds. Visually, the voltage response graph appears to have resonance properties, but it must be analyzed by one of the methods to further justify this observation.

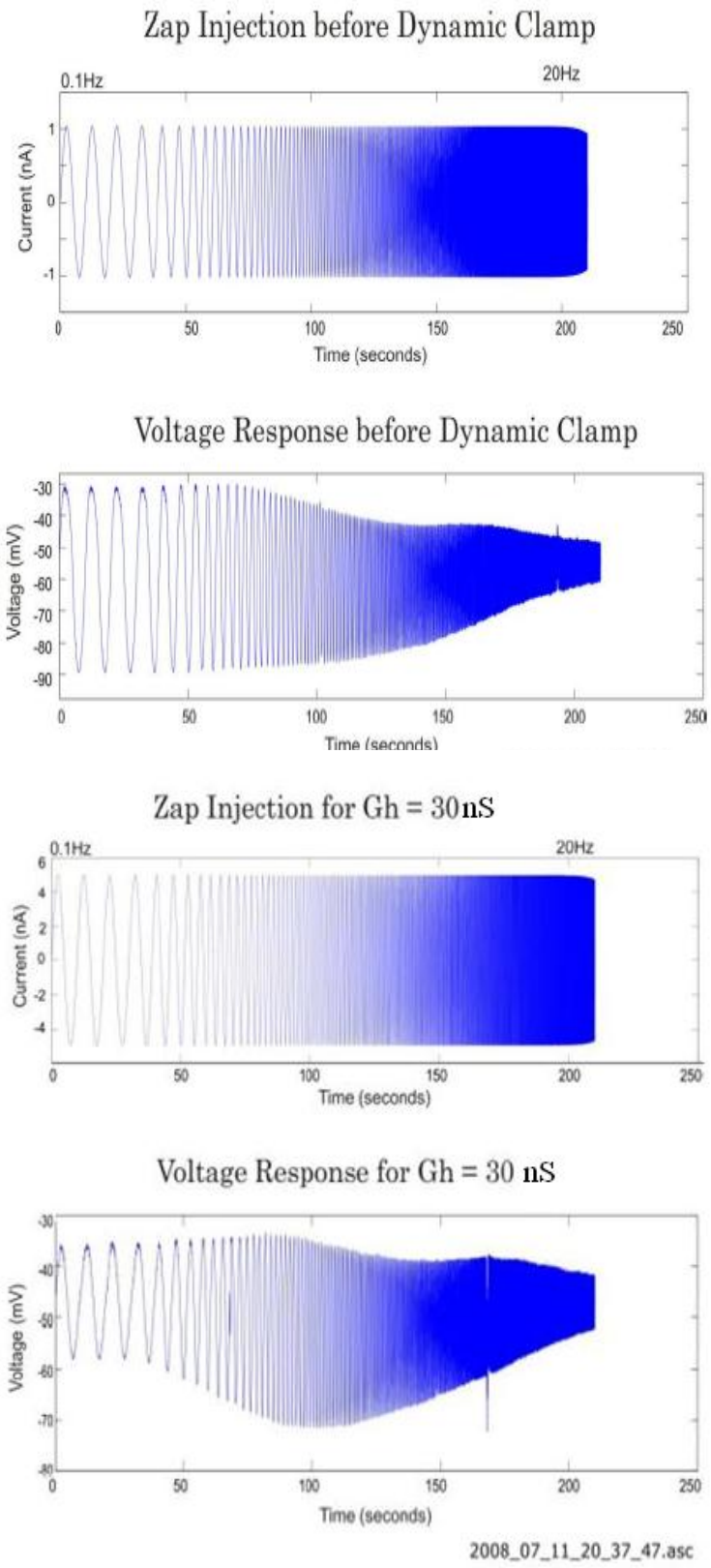


Figure 12. The top two graphs are the ZAP injection and the voltage response before using dynamic clamp. The bottom two graphs are the ZAP injection and voltage response while injecting the PY neuron with an artificial Ih current.

Analyzing the results using method 1, resonance was evident. Figure 13 shows a resonance peak at about a frequency of 0.3 Hz in both methods. When using dynamic clamp, we injected different conductances of I_h (10, 15 and 30 nanoSiemens). Each conductance produced a peak on the impedance versus frequency graph which is evident of resonance, and as the conductance increased, the impedance at small frequencies decreased. Moreover, as the frequency increased, the impedance decreased for every conductance.

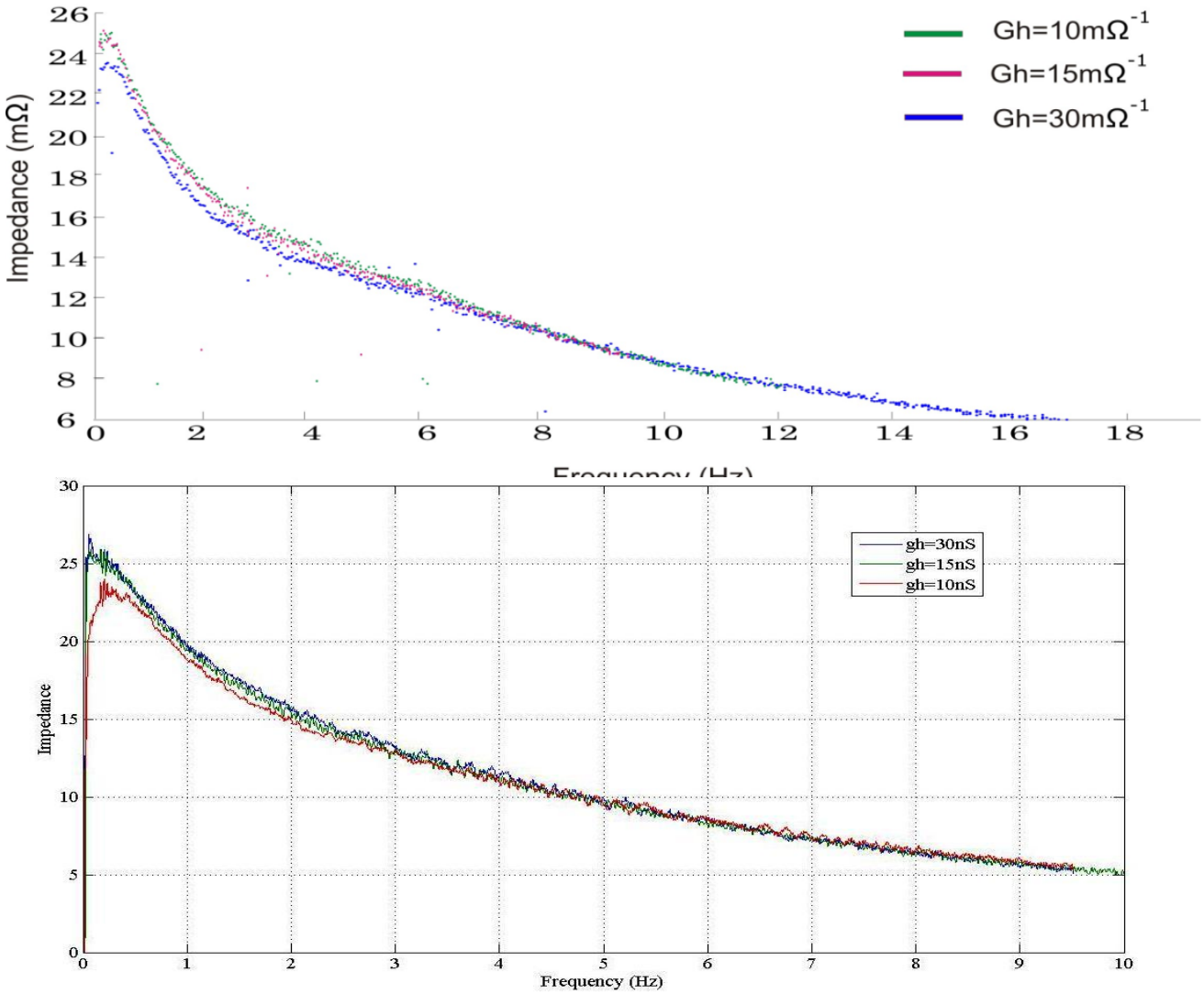


Figure 13. Method 1 (top), method 2 (bottom). These graphs show that resonance is present when an artificial I_h is injected into the PY neuron.

Model

Equation 7 allows us to deduce that if conductance is increased, the I_h current will become stronger. Therefore, we analyzed the impedance versus frequency graphs of the I_h current at a conductance of 0.0 μS, 0.01 μS,0.2 μS, and 0.4 μS .

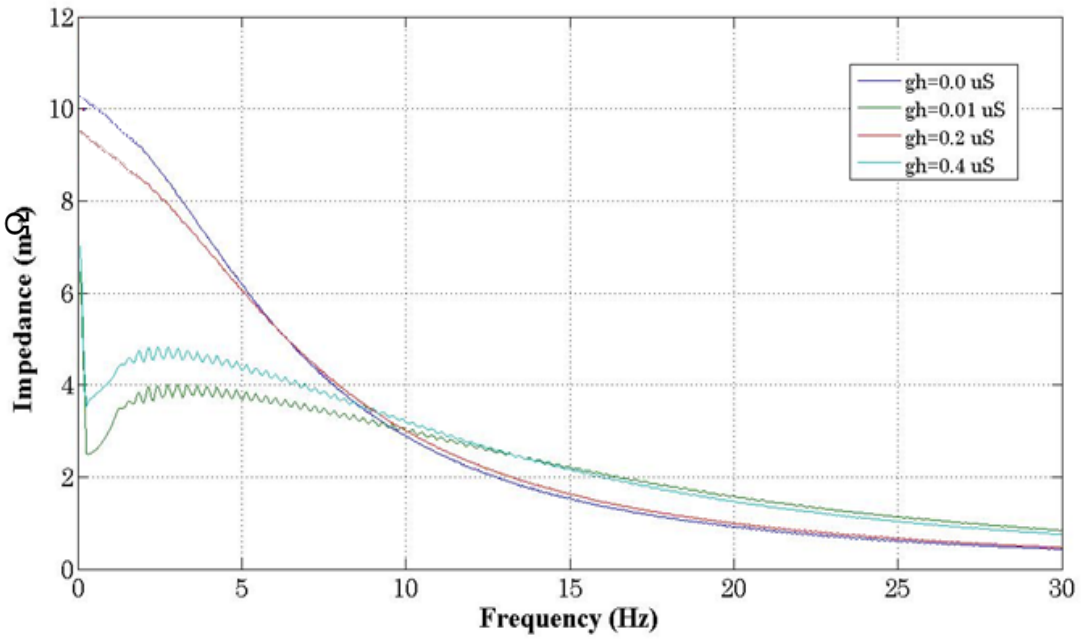


Figure 14. Increasing I_h conductance creates more visible resonance, as can be seen by the model results in this graph.

In Figure 14, a conductance of 0.0 μS obviously shows no resonant peak, however, so does a conductance of 0.01 μS. As the conductance increases in the graph, so does the visibility of the resonant peak. Moreover, the starting points of the lines in figure 14 are different, decreasing in impedance as the values of conductance increase.

To determine other factors that might affect resonance, we looked into the time constant of I_h, and capacitance (a property of the cell membrane). To increase the time constant (tau) in

the model, the rate constant (C_r) is increased, as can be seen in the equation on the graph of figure 15. When tau is decreased in value, the I_h current is better able to attenuate the voltage response at low frequencies. Consequently, this allows tau to filter the low frequencies before the resonant peak, to make it more visible and identifiable.

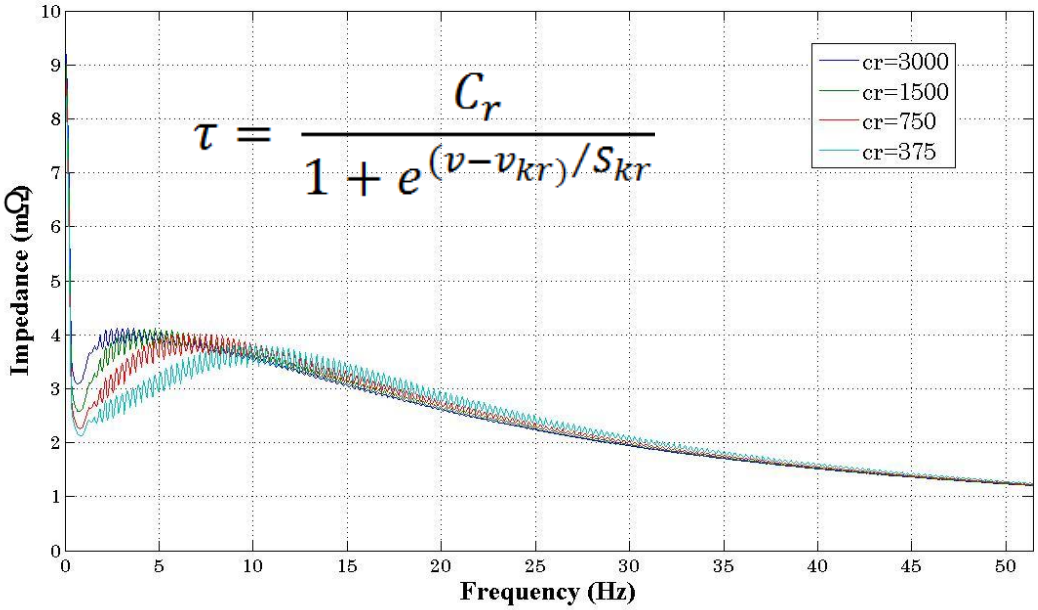


Figure 15. Lower C_r values decrease tau and thus create a filter on the left side of the resonant peak that increases its visibility.

When changing the capacitance of the cell in the model (figure 16), the impedance versus frequency graph shows that increasing capacitance provides a more visible resonant peak. This "filter" occurs on the right side of the resonant peak, and is the equivalent of the outcome of increasing cell size.

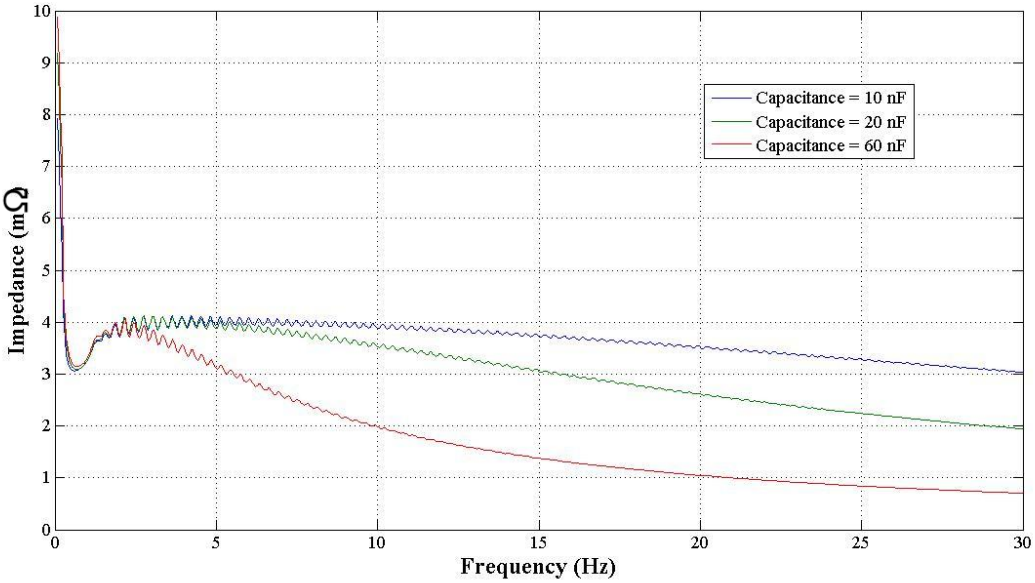


Figure 16. As capacitance increases, the resonant peak appears to be more visible because of the "filteration" that occurs on its right.

Discussion

To produce resonance, neurons are required to have a high pass filter and a low pass filter. Fortunately, a low pass filter (attenuates high frequency) exists in every neuron, since the cell membrane is the cause of this property. Therefore, the general idea is that if a cell has a high pass filter, it will exhibit resonance. The high pass filter (attenuates low frequency) in the PY neuron is due to the I_h channel, which was proved in these experiments. However, there are other types of high pass filters, depending on which neuron is looked at.

The sag response curve in this paper (figure 5) clearly shows that I_h activation is extremely dependent upon current. However, there is a point where all the I_h channels are open, and increasing the amount of current will not result in a greater sag. In other words saturating the I_h channels will result in the amount of I_h channels activated reaching a plateau. In this experiment we only used up to 7 nA, even if all the I_h channels have not yet been saturated. The reason for this, is that 7 nA usually hyperpolarized the cell far beyond what it would have in-vivo, in once case hyperpolarizing the cell beyond -120 mV. For 7 nA to cause such a high hyperpolarization value is not a surprise due to the relatively small size of the cell.

When a reasonable step current (about 7 nA) is injected into the PY neuron, the voltage response allows us to see if the PY exhibited effects produced by I_h. In fact, the first part of these sets of experiments was determining if PY even had an I_h current. To do this we determined two things about the voltage response if it has a sag, and if the sag slowly activates relative to the membrane. I_h produces a sag, since it is a hyperpolarization activating current. When the cell hyperpolarizes due to the injected current, I_h becomes activated and attempts to depolarize the cell back to I_h's reversal potential (-10 mV). However, I_h activates over a rather long period of time, since it has a slow time constant compared to the membrane time constant.

The ZAP injection allows us to see how the PY neuron responds to a stable current at several frequencies. The frequencies we found to be a good range for the PY neuron (or any other neuron) is from 0.1 Hz to 20 Hz. This wide range should ensure that resonance occurs at one of these values, since cells are not introduced to frequencies outside this range. These frequencies were set to run in one protocol that lasted for 3 minutes. This large amount of time permitted us to increase the number of sample points in our data.

In a normal experiment (no external influences), the voltage response to the ZAP injection showed no sign of resonance. In other words, only a low-pass filter was evident. As the frequency increased in the voltage response, the voltage range decreased. This is due to the low-pass filter attenuating high frequencies. If resonance was present, low frequencies would have also been attenuated, producing a bulge in the middle of where both filters are active. Moreover, the voltage response showed that the cell was reaching a voltage of -120 mV, which is enough for I_h to be active, and thus resonance to be produced.

To analyze the ZAP injection and voltage response graphs, we used two methods discussed earlier. In the impedance versus frequency graph of the "normal" experiment, impedance generally decreased as frequency increased, without a large peak one would expect if resonance was present. Considering impedance is resistance that is dependent upon frequency, we look to Ohm's law for the reason of this trend. Current is constant in the equation $R = \frac{V}{I}$, but voltage is variable. Therefore for resistance to decrease, voltage must decrease as well. The reason for the voltage decreasing as frequency increases, and therefore impedance decreasing as frequency increasing, is attributed to the low-pass filter again. However, there is a slight bump at ~6.5 Hz, which may either be due to a very small resonance peak, or more likely an artifact in the data.

To test the cause of the bump, we use 10 mM CsCl to block the I_h current. We record the same PY neuron before it is profused with CsCl, and after it has been profused with CsCl. If a bump appears again and then disappears after the addition of CsCl, then it may be attributed to I_h, but if it remains, then it is an artifact of the data. Analyzing the ZAP injection and voltage response graphs with both methods, there was no difference between the graphs, and thus the bump that was present is not a result of resonance.

However, an interesting question has yet to be answered: Why is it that I_h can be seen in the voltage response of the step current, but its influence is not seen in the voltage response of the ZAP injection? To answer this question, we use a program called dynamic clamp. This program allows us to inject an artificial I_h current into the PY neuron. The voltage response in the dynamic clamp experiment showed a very prominent sag under a conductance of 30 nanoSiemens. Moreover, the current that produced this large sag was only -0.5 mV, unlike the -7 mV that were injected and produced a minor sag.

When injected with a ZAP current, the maximum voltage the cell was brought down to was ~70 mV. However, the voltage response of the ZAP injection showed a sag from 0-100 seconds, which is a result of the I_h current depolarizing the PY neuron. The reason this effect only happened until 100 seconds, is that I_h is a high pass filter. Therefore, it attenuated the low frequencies until 100 seconds when the frequencies entered a range that the I_h does not "consider" low. Moreover the bump in between the effects of both filters is seen in the voltage response graph. When analyzing these results using method 1 and method 2, a peak is seen in the impedance versus frequency graphs.

Resonance under dynamic clamp in the PY neuron was seen at a frequency of about 0.5 Hz. When varying the conductance of the artificial I_h current in dynamic clamp, a trend started to occur. When the conductance increased, the impedance decreased. This trend goes back to

Ohm's law, since conductance is the inverse of resistance. Therefore, it is expected that conductance will vary the impedance, since impedance is resistance dependent upon frequency.

The reason that I_h was not producing resonance is that the I_h conductance was not sufficient enough to create resonance the same way the artificial I_h current was. This is probably due to the size of the PY cell. Considering the PY cell is small in size compared to other cells in the ganglion, it will have less I_h channels per unit membrane. Therefore, it did not have enough "strength" to attenuate low frequencies and depolarize the neuron.

When analyzing this situation in XPP, we noticed similar results. At low conductance values, the model showed no resonance, but as the conductance increased, so did the peak in the impedance versus frequency graph. Therefore, the PY cell does not have a preferred frequency, and technically only contains a low pass filter, since the high pass filter is too weak to be accounted for. PY is a follower neuron, and therefore may not need to have a preferred frequency in an evolutionarily sense. The cell may use the energy allotted for other metabolic tasks rather than wasting it on having a preferred resonant frequency it does not need.

Considering the relatively slow time constant of the I_h current, we decided to change C_r, thereby changing tau. At a decreased value of tau, resonance became more visible since the left side of the graph became increasingly filtered. Therefore, if the high pass filter of the PY neuron has a channel with a smaller tau than I_h , resonance might have been visible. Furthermore, considering that the PY neuron is a small cell compared to other cells that have resonance, we decided to alter capacitance. Capacitance is the ability of the cell membrane to hold charge, which is increased with a larger cell. Figure 16 shows that resonance is more visible with increased capacitance, since filtration occurs to the right of the resonant peak. This occurs since $\tau=rc$, where τ is tau, r is resistance, and c is capacitance. Increasing capacitance will increase tau,

which in turn provides us with decreasing impedance values as frequency increases. Therefore, if the PY neuron was a larger cell, resonance might have been visible.

An issue that might affect the experiments conducted is that the STNS contains 5 PY's. When we experiment on a PY neuron, we do not know which one of the 5 we have. Therefore, if one or more PY cells usually do not have resonance the others still might. However, we conducted numerous experiments on the PY neuron that proved the result of no visible resonance, and thus it is highly unlikely that one of the PY neurons exhibit resonance.

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XPP model:

```
par A=-10 gl=0.1 El=-70 gh=0.37 Eh=-10 vr=-70 Sr=7
par c=20 cr=3000 vkr=-110 skr=-13
par fmax=.01 fmin=0.0001 dur=180000


logval=log(fmax/fmin)/dur
Iex=A*sin(2*pi*(fmin/logval)*(exp(logval*t)-1))-5
aux Ix=Iex


# define currents
#Iex=A*heav(t>10000)*heav(t<140000)
#aux Ix=Iex

Il=gl*(v-El)
Ih=gh*r*(v-Eh)
aux ihx=ih


# define activation fraction of the h current

rinf=1/(1+exp((v-vr)/Sr))
taur=cr/(1+exp((v-vkr)/Skr))

aux rx=r
aux rinfx=rinf


# ODEs
v'=(Iex-Il-Ih)/c
r'=(rinf-r)/taur

@ total=60000 dt=1,xlo=0,xhi=180000,ylo=-70,yhi=-40

@ total=180000 dt=1,xlo=0,xhi=180000,ylo=-90,yhi=-30
@ bounds=10000000

init v=-60 r=0.3280024747199
```

done