The Effect of Morphology on the Passive Properties of Neurons

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**Abstract:**

Passive properties of neuron refer to the inherent capacitive and resistive characteristics of the cell membrane and the cytoplasm of neuron. As the morphology of neuron varies drastically from one to another, the passive properties also vary. We studied the passive properties of Pyloric Dilator (PD) neurons of *Cancer borealis*. We injected negative current pulses ($I_{\text{app}}$) into the soma of identified PD neurons and recorded the resulting voltage response. We then filled the same neuron with a fluorescent dye and imaged its anatomical structure using a confocal microscope. Using the software Neurolucida, we traced the 3D image of the neuron and then analyzed the tracing and obtained a detailed morphology with the software Neuroexplorer. Based on this detailed morphology, a detailed model neuron was developed. We determined the passive membrane properties of the PD neuron by comparing the experimental with the model voltage response for three different negative currents injected at the soma of the neuron in the Neuron simulation environment. We then used the cable equation to perform a spatial analysis of the compartments to determine the voltage along the length of each compartment at the steady state. We found that if an extension was added to the model, its response to current pulses better fit the experimental measurements. Such an extension may account for the fine morphological details that were neglected during tracing. We also reduce the complicated morphological model into a simplified model neuron based on the passive membrane properties.
**Introduction:**

- **The Cable Equation for Neuron:** A neuron can be compared to a thin tube of cell membrane that is filled with a core of electrically conducting medium. The resistance due to electric current flow across the cell membrane is much greater than the resistance due to short length increments along the cylindrical core. In neurophysiology, cable equation is a partial differential equation which is expressed as –

$$
\lambda^2 \frac{\partial^2 V}{\partial x^2} - v - \tau \frac{\partial V}{\partial t} = 0
$$

Here, $V$ is voltage, dependent on the independent variables time, $t$ and distance, $x$. $\lambda$ and $\tau$ are constants which depend on the intrinsic properties of neuron. For $X = \frac{x}{\lambda}$ and $T = \frac{t}{\tau}$, where $T$ and $X$ both are dimensionless. At the steady state, $\frac{\partial V}{\partial t} = 0$. Therefore, the cable equation would reduce to an ordinary differential equation –

$$
\lambda^2 \frac{d^2 V}{dx^2} - V = 0
$$

A general solution to this ordinary differential equation is –

$$
V = C_1 \cosh(L - X) + C_2 \sinh(L - X)
$$

Here, $\lambda$ is the length constant of cylindrical core conductor and $\tau$ is the passive membrane time constant. Here, $L$ is the electrotonic length of a branch, as if $L = \text{length}/\lambda$.

- **Passive Properties of Neuron:** Cell membrane contains different kinds of channels for different ions and molecules to pass through. All of these, except leak channels, are regulated by some variable, i.e. voltage. When positive current is injected into the neuron, the voltage across the membrane becomes more positive, this phenomenon is known as depolarization, leading to the opening of voltage gated channels. On the other hand, when
negative current is injected into the neuron, the voltage across the membrane becomes more negative, this phenomenon is known as hyperpolarization, which does not trigger the opening of any voltage-gated channel. However, current can pass through the leak channels at all times. A neuron is in passive state when all channels, except the leak channels, are closed. Since, injection of hyperpolarizing current does not trigger the opening of any voltage-gated channel; injecting hyperpolarizing current into the neuron is a way of studying the passive properties of neuron. In this study, hyperpolarizing current ($I_{app}$) was injected into the soma of PD neurons and the voltage response for the injected hyperpolarizing current ($I_{app}$) at the soma was recorded using another electrode placed at the soma. In order to assure the passive state of the neuron, the voltage-gated channels were blocked by adding a neurotoxin called tetrodotoxin (TTX).

- **Stomatogastric Nervous System:** This study was conducted on the Pyloric Dilator (PD) neurons, located in the stomatogastric nervous system (STNS) of *Cancer borealis*. The STNS consists of four ganglia, a pair of commissural ganglia (CoGs), unpaired esophageal ganglion (OG), and the stomatogastric ganglion (STG). When the STNS is dissected out of the crustacean and stored in-vitro, its neural networks generate patterns similar to that of in-vivo. There are two important neural networks that generate pattern, gastric and pyloric networks. The STG consists of 26 neurons and their properties are very well known; thereby, the neurons are easily identifiable by comparing the extracellular pattern of the neural networks with the internal pattern of each neuron.
The Pyloric Network: The STNS contains the pyloric neural network (Figure 1.2). The anterior burster (AB) neurons are the primary pacemaker neurons in the network. PD neurons are coupled with the AB neuron. The lateral pyloric (LP) neuron and PD neuron have reciprocal synaptic interaction. These synapses are believed to be found in the fine processes of the neuronal structure. In this system, LP and PD have a mutual inhibitory relationship. By developing morphologically detailed models of these neurons, it could be possible to determine the location, shape, and number of synapses present between the two motor neurons.

Methods:

- Dissection: The crabs were stored in seawater tanks at 12° - 14°C. Before starting dissection, the crabs were numbed by submerging in ice for about 30 minutes. We isolated the STNS.
from *Cancer borealis* using the method outlined by Golowasch & Marder, 1992. The first part of the dissection is crude dissection where the stomach is isolated from the crab. In the second part of the dissection, the fine dissection, we isolated the STNS from the stomach using a dissecting microscope and then the isolated STNS was pinned down into a Petri dish as shown in Figure 1.1. The Petri dish was superfused with a saline solution composed of 11mM KCl, 440mM NaCl, 13mM CaCl₂, 26mM MgCl₂.6H₂O, 11.2mM Trizma base, and 5.1mM Maleic Acid which has a pH 7.4-7.5 at a temperature of 10° - 14°C. After pinning down the STNS, the STG was desheathed. A Vaseline well was made around one of the lateral ventricular nerves (lvn) for proper identification of the PD neurons.

- **Identification of PD Neurons:** Two types of electrodes were used in this experiment. Extracellular electrodes were used to collect the extracellular signal by placing one electrode inside the Vaseline well and another electrode outside of the well. Microelectrodes were used to collect intracellular signal. The microelectrodes were pulled using P-97 Flaming/Brown Micropipette Puller by Sutter Instrument. The resistance for the microelectrodes used for voltage recording and injecting current needs to be high (approx. 30kΩ) and low (approx. 20kΩ) respectively. In order to do so, the microelectrodes used to record voltage were pulled at a higher temperature (approx. 345°C) and the microelectrodes used to inject current were pulled at a lower temperature (approx. 340°C). The microelectrodes were filled with 0.6 M K₂SO₄, and 0.02 M KCl and attached to a manipulator to be able to precisely impale the neurons. The extracellular signal was retrieved using a differential amplifier and the intracellular signal was recorded using
Axoclamp amplifier. In order to identify the PD neurons we impaled the somas at the STG and retrieved intracellular signal. We compared this signal with the simultaneous extracellular signal and thereby, identified the PD neurons as shown in figure 2. When the spikes from the intracellular signal are in the same phase as the spikes in the extracellular signal for PD neuron, we concluded that the impaled cell was a PD neuron.

![Figure 2: Extracellular recording of the LVN. The signal pattern starts with PD, followed by LP after a small pause, and then by PY neurons. The recording from inside the PD neuron corresponds to the PD spikes in the Extracellular signal](image)

- **Voltage Recording:** After identifying the PD neurons, we impaled the soma using two microelectrodes at the same time. One microelectrode was used to record the voltage response whereas the other microelectrode was used to inject current into the cell. Before injecting current into the cell, we added Tetrodotoxin (TTX) to the saline and waited until the patterns diminished. Then current pulses of -1nA, -2nA and -5nA were injected at a period of 1 sec with a duty cycle of 50% as shown in figure 3. The voltage response was recorded using the software Scope (Figure 3).

- **Dye-filling & Fixation:** The neuron was then impaled by a microelectrode filled with
fluorescent dye Alexa Fluor 568. In order to dye-fill the neuron, a current pulse of -5nA for 3 sec following by 1nA for 0.1 sec was injected through the electrode for 500 times. Once the dye-filling was completed, the Petri dish containing the STNS was wrapped with aluminum foil wrap and was stored in 4°C temperature for 12 hours. Then the preparation was fixed. First, 4% paraformaldehyde was added to the Petri dish, and the preparation was left in dark for 5 minutes. Then the preparation was washed with 0.1% phosphate buffer 3-4 times. This process was repeated for 5 times.

![Figure 3: Voltage responses for three different applied negative currents.](image)

- **Imaging:** Once the preparation was fixed, the STG was separated from the STNS using a dissecting microscope and then it was mounted on a slide. The slide was stored at 4°C temperature. The imaging was done using Zeiss LSM 510-Meta Confocal microscope. Using ArKr laser (488, 568nm) 30mW laser module, images were taken through optical sectioning in the Z axis. Thereby, a series of images of the preparation, focused at 0.25 µm intervals throughout a depth of 43.5 µm, were taken using the microscope as shown in figure 4.
- **Tracing:** We traced the 3D image of the neuron using Neurolucida. The software needed to be calibrated using a calibration slide. In order to do so, we took an image of the calibration slide with grids of 25 µm. Then, the image was opened into Neurolucida and the software was then calibrated setting the scale according to the image of the grid. After calibrating, we defined the z-axis as 0.25 µm and chose a reference point. By browsing through the different layers we marked the branching points using different markers. After marking the bifurcating points, we traced the image of the neuron. This tracing was saved as an .asc file. The contour of the soma was traced in a separate file. Using Neuroexplorer we opened this file and using structure analysis we determined the morphology of the soma.
**Results:**

- **Experimental Data:** We injected -1nA, -2nA and -5nA current into the soma of our PD neuron. For each current, 15 pulses were injected. The voltage response of the neuron was recorded at the soma of that neuron. Each pulse was 0.5 sec long and there was a 0.5 sec interval between two pulses. The pulses were averaged using Align 2.05\(^1\).

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\(^1\) A software developed by Dr. Farzan Nadim, Dept. of Mathematical Science, NJIT
Model: We used the software, NEURON, to create a model neuron based on our experimental data. The detailed morphology traced using Neurolucida was saved into an .asc file. Using NEURON’s Import 3D command we opened this file into NEURON and this data was exported to the NEURON’s cell builder. Since, the soma was not traced with the dendritic tree, the soma was created and connected separately (Appendix “morph.hoc”). Using point process an electrode was set at the soma of the model. The experimental data was loaded into NEURON. Using NEURON’s multiple run fitter (MRF) optimum values of membrane resistance \( R_m \), capacitance \( C_m \), and core conductance \( R_i \) were determined. The process was repeated for all \(-1nA, -2nA, and -5nA\) current injection. The passive membrane parameters determined by the detailed model is presented in Table 1.

![Voltage Response](image)

Figure 7: Voltage Response for \(-1nA, -2nA, and -5nA\) current injected at the soma of the experimental and detailed model PD neurons.

| Membrane Resistance \( R_m \) (\( \mu F/cm^2 \)) | 3.5 |
| Membrane Capacitance \( C_m \) (\( \Omega cm^2 \)) | 16000 |
| Core Resistance \( R_i \) (\( \Omega cm \)) | 80 |

Table 1: Passive membrane parameters determined by comparing the experimental voltage responses with detail morphological model voltage responses for three different currents.
**Modeling in NEURON:**

- **Detailed Morphological Model:** Although using NEURON we could determine the passive membrane properties by fitting the experimental graph with the model, in order to obtain a better fit of the curve we decided to add an extension compartment to our detailed morphological model. The passive membrane properties determined from this model are listed in Table 2.

![Graph showing voltage responses for -1nA, -2nA, and -5nA currents](image)

*Figure8: Voltage Response for -1nA, -2nA, and -5nA current injected at the soma of the experimental and extended model PD neurons.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Resistance $R_m$ (µFcm²)</td>
<td>1.3</td>
</tr>
<tr>
<td>Membrane Capacitance $C_m$ (Ωcm²)</td>
<td>45100</td>
</tr>
<tr>
<td>Core Resistance $R_i$ (Ωcm)</td>
<td>85</td>
</tr>
</tbody>
</table>

- **Simplified Model:** After determining the passive membrane properties by comparing the voltage responses of the experimental and the extended model, the determined membrane capacitance, resistance, and core resistance were used to determine the morphological
details of a simplified model neuron (Figure 10). The morphological parameters for this simplified model are shown in Table 3.

![Graph showing voltage response for -1nA, -2nA, and -5nA current injected at the soma of the experimental and simplified model PD neurons.](image)

**Table 3:** Morphological parameters of the simplified model neuron based on the passive membrane properties determined by the extended model neuron.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Parameter</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma Diameter (µm)</td>
<td>135</td>
<td>B Length, l_b (µm)</td>
<td>1300</td>
</tr>
<tr>
<td>Soma Length (µm)</td>
<td>135</td>
<td>C Diameter, d_c (µm)</td>
<td>45</td>
</tr>
<tr>
<td>A Diameter, d_A (µm)</td>
<td>45</td>
<td>C Length, l_c (µm)</td>
<td>300</td>
</tr>
<tr>
<td>A Length, l_A (µm)</td>
<td>750</td>
<td>D Diameter, d_d (µm)</td>
<td>12</td>
</tr>
<tr>
<td>B Diameter, d_B (µm)</td>
<td>31</td>
<td>D Length, l_d (µm)</td>
<td>1500</td>
</tr>
</tbody>
</table>
Discussion:

- **Spatial Analysis of Simplified Model**: We used the cable equation to perform a spatial analysis along the length of the compartments of the simplified model neuron. The lengths and diameters of the compartments were determined using NEURON.

Table 4: List of the relevant voltages and boundary conditions used in the spatial analysis.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Description</th>
<th>Boundary Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>Voltage at the beginning of A</td>
<td>$B_{Ae}$ Boundary condition at the end of A</td>
</tr>
<tr>
<td>$V_A$</td>
<td>Voltage along the length of A</td>
<td>$B_B$ Boundary condition at the beginning of B</td>
</tr>
<tr>
<td>$V_{Ae}$</td>
<td>Voltage at the end of A</td>
<td>$B_{Be}$ Boundary condition at the end of B</td>
</tr>
<tr>
<td>$V_B$</td>
<td>Voltage along the length of B</td>
<td>$B_C$ Boundary condition at the beginning of C</td>
</tr>
<tr>
<td>$V_C$</td>
<td>Voltage along the length of C</td>
<td>$B_{Ce}$ Boundary condition at the end of C</td>
</tr>
<tr>
<td>$V_{Ce}$</td>
<td>Voltage at the end of C</td>
<td>$B_D$ Boundary condition at the beginning of D</td>
</tr>
<tr>
<td>$V_D$</td>
<td>Voltage along the length of D</td>
<td>$B_{De}$ Boundary condition at the end of D</td>
</tr>
</tbody>
</table>

Figure 10: Schematic representation of the simplified model
Here, we assume that the end compartments have sealed ends. Therefore, the boundary conditions at the end of the compartments, B and D are,

\[ B_{De} = 0 \]
\[ B_{Be} = 0 \]

According to Rall’s cable theory, the other boundary condition at the beginning of the compartment can be defined by the boundary condition at the end of the compartment.

Now the boundary conditions at the beginning of the compartments B and D are,

\[ B_B = \frac{B_{Be} + \tanh(L_B)}{1 + B_{Be} \tanh(L_B)} \]
\[ B_D = \frac{B_{De} + \tanh(L_D)}{1 + B_{De} \tanh(L_D)} \]

Here \( L_B \) and \( L_D \) are the electrotonic lengths of the compartments B and D respectively.

Again, using Rall’s cable theory, the boundary condition at the end of a continuous compartment, depends on the boundary conditions of the branch compartments and their diameters. Therefore, the boundary condition at the end of the compartment C,

\[ B_{Ce} = \left( \frac{d_D}{d_C} \right)^{3/2} B_D \]

Now, the boundary condition at the beginning of the compartment B,

\[ B_C = \frac{B_{Ce} + \tanh(L_C)}{1 + B_{Ce} \tanh(L_C)} \]

Here, \( L_C \) is the electrotonic length of the compartment C. Based on the boundary conditions at the beginning of the compartments B, and C, the boundary condition at the end of the compartment A, is,

\[ B_{Ae} = 2 \left( \frac{d_B}{d_A} \right)^{3/2} B_B + \left( \frac{d_C}{d_A} \right)^{3/2} B_C \]
Now, using a solution the cable equation at the steady state, the voltage along each of the four compartments can be defined by the following equations,

\[
\frac{V_A}{V_o} = \frac{\cosh(L_A - X) + B_{Ae}\sinh(L_A - X)}{\cosh(L_A) + B_{Ae}\sinh(L_A)} \quad \text{for} \quad 0 \leq X \leq L_A
\]

\[
\frac{V_B}{V_o} = \frac{\cosh(L_B - X) + B_{Be}\sinh(L_B - X)}{\cosh(L_B) + B_{Be}\sinh(L_B)} \quad \text{for} \quad L_A \leq X \leq L_A + L_B
\]

\[
\frac{V_C}{V_o} = \frac{\cosh(L_C - X) + B_{Ce}\sinh(L_C - X)}{\cosh(L_C) + B_{Ce}\sinh(L_C)} \quad \text{for} \quad L_A \leq X \leq L_A + L_C
\]

\[
\frac{V_D}{V_o} = \frac{\cosh(L_D - X) + B_{De}\sinh(L_D - X)}{\cosh(L_D) + B_{De}\sinh(L_D)} \quad \text{for} \quad L_A + L_C \leq X \leq L_A + L_B + L_C
\]

Using these equations, the voltage along the length of each compartment, at the steady state after current injection, is shown in Figure 11.
Determining Passive Membrane Properties: In our experiment, at first we used a morphology that was obtained from tracing the 3D image of neuron. However, when we created a model using NEURON, the model response corresponded to the experimental response for very high passive membrane capacitance. Several studies have shown that the extreme values of membrane capacitance are 0.5 and 2 \( \mu \text{F/cm}^2 \). However, the membrane capacitance determined from this model was 3.5\( \mu \text{F/cm}^2 \) (Table 1). Also, several studies have pointed the values of membrane capacitance towards the order of 1\( \mu \text{F/cm}^2 \) (Rall). Based on these studies, we decided to keep the capacitance of the model in the order of 1\( \mu \text{F/cm}^2 \) and add an extension to the existing model to account for the missing fine processes that could have been missed during tracing. We varied the size of this extension to match our experimental results. The response from this extended model was fitted better with the experimental response.

Conclusion:

From this project we have reached several conclusions. We have found out that, while creating a detailed morphological model, if the fine details are not taken into account, the model may differ greatly from biological data. The ignored fine details can sum up to a very large surface area which could particularly affect the membrane capacitance determined from
the model. Such error can be reduced by adding additional surface area to the model and thus accounting for the ignored details. A complicated morphological model can be reduced to a simpler model that still reproduces the biological data.

Through this project we have also learned to build model neuron based on its morphology. We look forward to use this knowledge in building a complex synaptic model for the LP-PD mutually inhibitory relationship, which could be used to determine the size, shape, location, and number of synapses present between these two neurons.
References:


Appendix:

- **Codes for NEURON**: (The code was created using following the tutorial found at the www.neuron.yale.edu/neuron website)

  o **Morph.hoc**
    
    create soma, ext
    soma.L=135
    soma.diam=135
    ext.L=350
    ext.diam=350

    connect axon[0](0), soma(1)
    connect ext(0), axon[13](1)

    forall{
      insert pas
      Ra = 80
      cm = 1.2
      g_pas = 1/15900
      e_pas= 0
    }

  o **Params.hoc**

    Rp = 80
    Cp = 1
    Rm = 1000

    proc init() {
      forall {
        Ra = Rp
        cm = Cp
        g_pas = 1/Rm
      }
      finitialize(v_init)
      if (cvode.active()) {
        cvode.re_init()
      } else {
        fcurrent()
      }
      frecord_init()
    }