Direct Parameter Fitting of Action Potentials in Skeletal Muscle Cells Which Include Longitudinal Segments

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DIRECT PARAMETER FITTING OF ACTION POTENTIALS IN SKELETAL MUSCLE CELLS WHICH INCLUDE LONGITUDINAL SEGMENTS

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

by

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ABSTRACT

Suda, Tyme. M.S., Department of Physics, Wright State University, 2023. Direct Parameter Fitting of Action Potentials in Skeletal Muscle Cells which include Longitudinal Segments

Excitation of skeletal muscle cells triggers a large voltage spike known as an action potential (AP), leading to muscle contraction. Modeling of an AP is typically done using the method developed by scientists Hodgkin and Huxley (HH). In the HH method, voltage and time gated Na$^+$ and K$^+$ ionic currents are simulated, along with a positive “Leak” ionic current and capacitive current. Due to the complexity and the computational time required for simulation, direct fitting of HH parameters to experimental APs has rarely been attempted. A previous thesis at Wright State performed direct fitting for the case of a single compartment muscle cell. This study will introduce propagation to the existing model by adding small longitudinal segments simulating the currents flowing and triggering APs in later portions of the cell. If no adjustments to the single compartment HH parameters are made, adding increasing longitudinal segments leads to a very poor simulation of the AP shape. The simulated AP is too wide, and the peak voltage is too low. However, very few clear links between the number of segments and changes in specific HH parameter values were identified. Each parameter was found to vary by at least a factor of 2 between similar data sets. It is clear that multiple parameter sets are allowable, obscuring direct links between segments and parameter values. In order to further understand the robustness of the found parameter sets and to identify what regions of parameter space is allowable in order to achieve a well fit AP a confidence interval test was completed. Scanning
through each parameter and fitting the rest revealed that each of $\alpha_h, \beta_h, k_{jh}, \bar{V}_n, k_{an}, \bar{\alpha}_n, \bar{\beta}_n$, and Na permeability hold a well-defined interval in which near perfect fits can be found. While for $k_{ah}, k_{bn},$ and $k_{bm}$ no upper-bound was identified, and for K permeability, $\bar{V}_h, k_{am}, \bar{\beta}_m, \bar{\alpha}_m, \bar{V}_m$ the patterns where unclear.
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INTRODUCTION AND BACKGROUND

**Purpose**

Electrical excitation in muscular cells drives their contraction allowing for most biological life to move. The excitation current, delivered from motor neuron or an electrode in laboratory conditions, triggers the formation of an action potential (AP) at the site of the stimulation. That then propagates through the cell releasing \( \text{Ca}^{2+} \) ions and triggering the contraction. Due to the complexity of the ion movements involved with APs, previous attempts to simulate APs used parameters pulled from experiments in which ions were studied individually, and then the parameters were combined to simulate the AP. The result is a simulated AP that does not fit the experimental AP very closely (Metzger, 2021). In previous work, we attempted direct fitting of the parameters to experimental AP shapes using the Hodgkin-Huxley (HH) method of AP simulation. However, this previous work did not account for the fact that ions, and the AP itself, move longitudinally within the cylindrically shaped muscle cells. The main purpose of this research is to correct the discrepancy by introducing longitudinal segments.

To understand the effects of longitudinal segments, three main studies were completed. The first studied the direct effects of adding more segments without changing any channel or membrane parameter. The goal here being to identify at what point adding more segments has no effect, if there is such a point, while getting an idea of what changes the AP goes through. If the segments interact with each other, the effects of this interaction must be understood. Additionally, the question of whether it is even necessary to shift the HH parameters was answered here.
The second study dealt with adding more segments while increasing cell length as to closer match conditions in the lab (~4mm long muscle segments). The goal was to understand how each of the HH parameters must shift to better match the APs observed in real cells while adding more segments to better approximate reality. To the best of our knowledge the only two studies that directly compare simulated APs with directly recorded traces are from Rich lab (Novak et al., 2015; Metzger, 2021), attempting to verify the HH parameters. Neither of these previous studies examined longer cells nor allowed for longitudinal AP propagation.

The final study attempted to gain a better understanding of the numerically allowable states for each HH parameter. From the literature, many HH parameters are found to vary by over a factor of three between studies on the same animals (Cannon et al., 1993; Filatov et al., 2005). A clear consensus on what ranges each parameter can take does not seem to exist. This study’s main focus was to find the regions of each parameter for which the HH model is still able to return a good fit of the AP. Additionally an attempt was made to understand the interconnections between each parameter as they relate to correcting for shifts in the other HH parameters.

**Background**

Electricity has been known to have a role in nervous and muscular systems since the late 18th century with the studies by Luigi Galvani (Bresadola, 1998) and inspired one of the most iconic books ever written, Frankenstein by Mary Shelley (Ruston, 2015). However, it took over a hundred years to understand this phenomenon and eventually lead to working model. The first action potential (AP) model, the Hodgkin-Huxley model (HH), was created by Hodgkin, Katz and Andrew Huxley in 1952 (Hodgkin and Huxley, 1952) in which they modeled the AP of giant
squid axon using an early voltage clamp experiment. This model has since become the basis of the current field of electrophysiology (Beeman, 2014).

In 1973 Adrian and Peachey first adopted the HH model to a muscular cell system (Adrian and Peachey, 1973), specifically for frogs. Since then, many adaptations of the model have been used to better understand disease and the cells themselves. Many of which would experimentally find new HH parameter sets for different species such as goat (Adrian and Marshall, 1976), or combination of frog and rat (Cannon et al., 1993). While others adapt previously found parameter sets to their own use (Wallinga et al., 1999; Fortune and Lowery, 2009; Filatov et al., 2005).

This study builds upon the backs of these giants attempting to further refine the model and allow for more accurate understanding and modeling of APs in muscular cells. Specifically, current is not applied to the whole surface of any muscular cell, nor are they short enough that such an application is an accurate representation of reality. Adding small longitudinal segments to the HH model can simulate propagation of the AP down the cell, more closely resembling reality and hopefully lead to better understanding and possibly contributing to combating one of the many muscular diseases.

The adding of longitudinal segments allows for simulation of the propagation of the AP by triggering one segment producing an AP that then triggers the next segment, eventually traveling through the cell. To fully approach reality these segments should have as close to zero length as possible, simulating smooth propagation. The AP also propagates into the cell via t-tubules, which was already explored in the previous work (Metzger, 2021). This model, adopted in MATLAB by Dr. Brent Foy, has since been expanded to allow for this longitudinal AP.
Additionally, this study focuses on directly fitting the AP shape while varying each HH parameter freely within a reasonable bound.

Direct fitting of the AP shape is not common practice, in most cases fitting is not done. Normally the HH parameters are either derived experimentally or values from past studies are used. In the cases when directly fitting the experimentally found AP shape from a voltage clamp experiment seems to have only happened in Rich lab (Novak et al., 2015; Metzger, 2021). In the few past fitting practices, the tendency has been to fit individual currents instead of the overall AP shape (Tomek, 2019; Fabbri, 2017; Hafner, 1981). This approach limits the number of parameters but does not necessarily produce a simulated AP with the correct shape. By fitting the direct AP shape a better study of some of the important features of APs can take place. The rising or falling slopes, half height width, and overall AP height can be better matched. All, important indicators of excitability (Novak et al., 2015; Filatov et al., 2005).

*Basis of the HH Model*

At all times there is a potential difference across the cell membrane of a cell. If only a singular ion was responsible for the membrane potential (only that ion channel open) a simple calculation using the Nernst equation \( E_{\text{ion}} = \frac{RT}{zF} \ln \left( \frac{[\text{ion}_{\text{out}}]}{[\text{ion}_{\text{in}}]} \right) \) would supply the membrane potential (assuming ionic concentrations are known). However, this potential is normally set by a combination of Na\(^+\), K\(^+\), and Cl\(^-\) ionic flows requiring the use of the Goldman-Hodgkin-Katz equation (GHK equation) written in general terms for n ions of relative conductivities \( g_i \) below.

\[
V_{\text{membrane}} = \frac{RT}{F} \ln \left( \frac{\sum_{i=1}^{n} g_i [i_{\text{out}}]}{\sum_{i=1}^{n} g_i [i_{\text{in}}]} \right)
\]
At rest Na\(^+\) channels are largely closed (Jurkat-Rott and Lehmann-Horn, 2004), while ClC-1 ion channels passively distribute Cl\(^-\) across the cell membrane (Adrian, 1961). Potassium is allowed to “Leak” through the Kir (potassium inward-rectifier) family of channels (Jurkat-Rott and Lehmann-Horn, 2004). This leads the K\(^+\) Nernst potential largely setting the resting membrane potential to approximately -85mV, while the ClC-1 channel helps to keep it stable (Jurkat-Rott and Lehmann-Horn, 2004). However, ionic currents through many other ion channels contribute slightly. In most models, including the version used in this study, all these relatively weak ionic flows including the inward rectifying K\(^+\) current are grouped together and simply modeled as the positively charged “Leak Ion,” vastly simplifying the required model.

The formation of an AP begins with the cell at its resting potential. A stimulus current then triggers depolarization, exciting the cell above its resting potential and triggering Na\(^+\) begin flowing into the cell mostly through the voltage gated Nav1.4 channels (Jurkat-Rott and Lehmann-Horn, 2004). If a threshold defined as when the inward flow of Na\(^+\) is equal to the outward flow of K\(^+\) and Cl\(^-\) is not reached cell returns back to rest and no AP is triggered (Fitzhugh, 1960). However, if the stimulus current is strong enough to excite the cell above this threshold value a sudden spike in potential, our AP, begins. The inward flowing Na\(^+\) current pushes the potential higher and higher triggering the slow activation of Kv channels (mostly the Kv1.1 channel). K\(^+\) then begins to flow out of the cell while the Na\(^+\) channels (Nav1.4) begin to inactivate. Closing and becoming unresponsive to the membrane potential for a time. At this point the potential peaks and repolarization begins, driven by a massive outflow of K\(^+\), returning the cell to the resting potential (Jurkat-Rott and Lehmann-Horn, 2004).
The HH Model

The HH model is deceptively simple, describing the membrane as if it is a capacitor and set of resistors, one for each ionic current, in parallel. This leads to a total membrane current:

\[ I_m = C_m \frac{dV}{dt} + I_i \]

Where \( C_m \) is the membrane capacitance, \( V \) the total membrane potential, and \( I_i \) the total ionic current.

\[ I_i = I_{Na} + I_K + I_L \]

Each ionic current is driven by a driving potential \( (V - E_{ion}) \), the difference between the Nernst potential of that particular ion \( (E_{ion}) \) and the membrane potential. The Leak current is simplest to describe, as there is no voltage gating. Hence why it is dubbed “leak” as it will leak with any shift in voltage. It is simply the product of the driving voltage and the leak conductance \( (g_L) \).

\[ I_L = g_L(V - E_L) \]

Both Na\(^+\) ions and K\(^+\) ions have voltage gating terms. For sodium both fast activation and slow inactivation must be modeled. The gating variable \( m \) is introduced to model the fast activation, while \( h \) is used to specify the slow inactivation. In terms of the maximal Na\(^+\) conductance \( (g_{Na}) \) the Na\(^+\) ionic current is then described as:

\[ I_{Na} = g_{Na} m^3 h(V - E_{Na}) \]

In a very similar manner, the K\(^+\) ionic current uses the gating variable \( n \) to limit the flow below the maximal K\(^+\) conductance \( (g_K) \).

\[ I_K = g_K n^4(V - E_K) \]
All three gating variables can then be described by a rate equation with two separate coefficients $\alpha$ and $\beta$:

$$\frac{di}{dt} = \alpha_i(1 - i) - \beta_i i$$

Where $i$ can be replaced by any of the three gating variables $m$, $h$, or $n$. Each set of $\alpha$ and $\beta$ can be described by a combination of five additional parameters: scaling parameters $\overline{\alpha}_i$ and $\overline{\beta}_i$, voltage dependencies $V_i$, and additional rate dependencies $k_{\alpha i}$ and $k_{\beta i}$.

$$\alpha_m = \frac{\overline{\alpha}_m(V - \overline{V}_m)}{1 - exp\left(-\frac{V - \overline{V}_m}{k_{\alpha m}}\right)}$$

$$\beta_m = \overline{\beta}_m exp\left(-\frac{V - \overline{V}_m}{k_{\beta m}}\right)$$

$$\alpha_h = \overline{\alpha}_h exp\left(\frac{V - \overline{V}_h}{k_{\alpha h}}\right)$$

$$\beta_h = \frac{\overline{\beta}_h}{1 - exp\left(-\frac{V - \overline{V}_h}{k_{\beta h}}\right)}$$

$$\alpha_n = \frac{\overline{\alpha}_n(V - \overline{V}_n)}{1 - exp\left(-\frac{V - \overline{V}_n}{k_{\alpha n}}\right)}$$

$$\beta_n = \overline{\beta}_n exp\left(-\frac{V - \overline{V}_n}{k_{\beta n}}\right)$$
METHODS
To begin and understand the effects that adding longitudinal segments have on the Hodgkin-Huxley model of skeletal action potentials a series of optimization on of the 18 parameters of interest, $\bar{V}_m$, $\bar{V}_\alpha$, $\bar{V}_\beta$, $k_{\rho} m$, $k_{\rho} h$, $k_{\rho} n$, $\alpha_{m}$, $\beta_{m}$, $\alpha_{h}$, $\beta_{h}$, $\alpha_{n}$, $\beta_{n}$, $g_{Na}$, $\bar{g}_K$, and $g_{leak}$, were ran. The action potentials are simulated in MATLAB utilizing the ode15s stiff differential equation solver. All fits would utilize the final results from optimizations found in Sabrina’s thesis (Metzger. 2021) on the same data sets for singular longitudinal segments as the initial parameter guesses.

Establishing a Metric for goodness of fit
Before any optimization could begin a definition of “goodness of fit” had to be defined. For any optimization approach an objective function must be minimized. In most cases the residual sum of squares between individual data points and the proposed model is an adequate objective function. For modeling of action potentials (APs) in muscular cells it does introduce one problem; it assumes that the timing of the firing of the AP and the model AP must be equal. For our model however small shifts in stimulus current can largely influence this timing as illustrated below in Figure 1.
Figure 1 Current Effects on Timing.

Top shows a completed fit for a data set with appropriate current. Bottom left shows the same fit with reduced current, in this case 80% of the optimal current, increasing the time delay between stimulus and AP. Bottom right shows the same fit but with increased current, 125% of the optimal current decreasing the time delay from stimulus to AP.
When using the Hodgkin-Huxley method of modeling the voltage-gated Na\(^+\) and K\(^+\) ion channels to simulate an action potential it can be noticed that small shifts in current result in large shifts in timing of the AP. It can also be seen that the overall shape of the AP has relatively minor changes due to the current shifts as compared to the timing. A point by point least squares method of fitting would completely throughout any such AP, significantly overemphasizing the timing.

Although timing is important it is only one feature of the AP that should be looked at. The peak voltage, width at half height, and maximal rising and falling slopes are all common metrics for understanding an AP. It became a goal to translate these features into our objective function. The initial idea was to simply calculate the residual sum of squares for each of the five features, this would prove to be mildly problematic due to trying to minimize 18 different parameters to so few data points. This approach slowly evolved into the current objective function.

The idea is, in order to have more data about the overall shape of the AP, they are bisected by 20 horizontal lines each being separated 5% of the resting potential to peak height from the real AP. The timing of the intersects of each of these lines with the modeled AP and real AP are recorded. In addition, the timing of the actual peak of each AP is recorded. From this the times it took for the APs to get from some % of the max height to the peak or from the peak back down to that max height is found. The residual squared between these time periods for the simulated and the actual APs is then used as the main portion of the new objective function.
Figure 2 An Example of the horizontal lines utilized.

In this case each corresponds to a shift of 10% of the AP height. The timing difference between the peak of raw pulse and the intersect is compared to the same for the simulated pulse and then be utilized in the objective function.

The horizontal lines were generated utilizing only the real APs to avoid the possibility that a taller, yet similarly wide simulated AP could return a very small residual squared or even none. This would foreseeably be the case if the simulation and the data took the same amount of time to rise to the maximum from any given voltage percentage or return from max to some given percentage. By setting the horizontal lines purely based off the data a new issue is introduced. What to do if the simulated AP never reaches one of the horizontal lines (is 5% or more shorter than the real AP). When this happens some of the time points for the simulated AP
cannot be found due to one (or more) of the horizontal lines not intersecting with it. To allow this situation to not throw errors and fail, the delta t for any such case is treated as zero. This means that any width that the raw data has there increases the objective function and reduces the “goodness of fit”.

Taking this into account, the residual sum of squares between these delta t acts as a surrogate for rising/falling slopes and the width at half height while holding some information about the peak AP voltage, all without caring about timing of the simulated AP. The residual squared between the initial resting potential, peak AP voltage, and timing of the AP are also found as part of the objective function, although each scaled to avoid over emphasizing a singular feature. Overall, the objective function used is expressed as:

\[
X = \left( \frac{\sum_{1}^{2n} \Delta t_{slopes}^2}{2n} + \frac{\Delta t_{AP}}{20} \right) \alpha \beta \quad (1)
\]

Where \( \Delta t_{slopes}^2 \) is the residual squared of any of the 2n times found from the n horizontal lines. The sum of all residuals squared is divided by the total number of the, 2n. In our cases n=20 lines were utilized although more and less were tested. Less was found to have too little information to well define the AP while more were deemed unnecessary upon visual inspection. \( \Delta t_{AP} \) is the time difference between the peak of the simulated AP and the AP modeled, this divided by 20 to largely deemphasize this but still allow for timing to be considered if the shape of the AP was already near perfectly achieved. The sum of these values is then scaled by two constants \( \alpha \) and \( \beta \). The constant alpha, defined below as equation 2, is a scaling factor based off the resting potential difference, \( \Delta V_{rest} \).

\[
\alpha = 1 + \frac{\text{floor}(\mid \Delta V_{rest} \mid)}{2} \quad (2)
\]
\(\Delta V_{\text{rest}}\) is simply the difference in initial resting potential of the simulated modeled APs. When \(\Delta V_{\text{rest}}\) is less than 1mV \(\alpha\) is simply equal to 1, not scaling the objective function at all. Any larger \(\Delta V_{\text{rest}}\) results in scaling, up to 2.5 times when \(\Delta V_{\text{rest}} < 4\text{mV}\). If \(\Delta V_{\text{rest}} \geq 4\text{mV}\) the attempt is considered a failure and the objective function is set to 50000 throwing out the attempt.

Similar to \(\alpha\), \(\beta\) is also a scaling factor based off the potential. In the case of \(\beta\), the scaling factor relates to the difference in AP height and defined as:

\[
\beta = 1 + \frac{\text{floor}(\Delta V_{\text{err}} \times 20)}{2}\]  

(3)

\(\Delta V_{\text{err}}\) is defined as:

\[
\Delta V_{\text{err}} = \left| \frac{\Delta V_{\text{Sim}} - \Delta V_{\text{Mod}}}{\Delta V_{\text{Mod}}} \right| 
\]

(4)

\(\Delta V_{\text{Sim}}\) is the difference in potential from peak of the simulated AP to its initial resting potential and \(\Delta V_{\text{Mod}}\) is the same value for the AP that was modeled. Flooring \(\Delta V_{\text{err}}\) when multiplied by 20 allowed for intervals of 5% error in AP height to be observed. When \(\Delta V_{\text{err}} < 5\%\) \(\beta\) is simply 1 causing no scaling of the AP. For every additional 5% the AP is scaled by 50% of the unscaled value up to a scaling factor of 3.5 when 25\% \(\leq \Delta V_{\text{err}} < 30\%\). If \(\Delta V_{\text{err}} \geq 30\%\) attempt is considered a failure and the objective function is set to 50000 throwing out the attempt.

Two additional checks are done before the value of the objective function is returned. The first being a simple check that every horizontal line has two intersects with both the simulated pulse and the one being modeled after the stimulus pulse. This is done as a very quick
check that we actually have an AP. If any failed to be found, or too many are, (that is from the top line due to the AP being a bit short) the simulation is considered a failure and the objective function returns 50000. This check is in place to prevent any strange behavior such as secondary APs being accepted.

The second check is even simpler, does the AP return to near rest? This is actually two checks, the first asking if the endpoints of the simulation and the data set are 10mV or more apart, if so return 50000 and throw the AP out. The second check was added to avoid a common phenomenon of spontaneous APs from forming for HH parameter sets near instability. It was observed that in almost all cases where the AP would have been spontaneous the simulated AP would return to rest much slower than in the data and in many cases the voltage would even start to increase again at the very tail end of the AP. Additionally when no spontaneous AP existed the simulation would almost always return to rest faster than in the data, leading to the endpoints of the simulation being lower in voltage. Considering this a check was put in place to throw out any AP where the endpoint of the simulation occurred more than 1mV above that of the data. This is likely stricter than necessary but allowed for near complete avoidance of spontaneous APs being found. Any greater voltage check would occasionally lead to spontaneous APs.

One problem with finding the residual $\Delta t_{slopes}$ arises from the initial voltage spike created by the initial current used to induce the AP. The Hodgkin-Huxley model using only the voltage-gated Na$^+$ and K$^+$ ion channels in addition to a “Leak” catch all ion channel is not good at modeling this initial voltage spike, especially for short duration high current pulses such as used in some experimental protocols. To avoid letting the fit include this and potential skew it further the base voltage for finding the locations of the 20 horizontal lines used for the rising slope is not taken as the resting potential, but as the minimum voltage that is reached between the
initial current spike and the peak of the AP. Keeping the minimum Voltage as the resting potential for the down slope allows for ignoring the initial voltage spike without deemphasizing the downslope and return to rest. An example of this for 10 horizontal lines is shown below as Figure 3.

![Membrane Potential](image)

*Figure 3 An example of the actual horizontal lines utilized.*

Each shift is still 10% of the height but the start for the upslope and down slope have been redefined. For the upslope the end of the stimulus pulse is utilized while the value of the raw data at the end of the fitting region is used for the downslope.
**Fitting Algorithms**

The MATLAB fitting algorithms surrogateopt and fminsearch were utilized to minimize the described objective function. Surrogateopt, from the global optimization toolbox, is specialized in finding the global minimum of objective functions that take a long time or a lot of resources to run. It works by first constructing a surrogate for the actual function by sampling the objective function quasi-random points repeatedly within predetermined bounds of the parameters of interest (Surrogate Optimization Algorithm, 2018). A set of radial basis functions are interpolated through these points to create the surrogate for the objective function. The minimum of this much simpler surrogate function is then identified. The value of the objective function at that location in parameter space is then identified. If this point is lower than the current minimum point found it is saved as the new guess of the global minimum. Either way the surrogate function is updated utilizing the new information about the objective function. This will repeat until either a certain number of iterations are done, a certain amount of time has passed, or when all sample points are at some certain distance away from each other.

Surrogateopt works well to find what should be the global minimum with significantly less computational time than many other algorithms for computationally complex objective functions, but it does not work well for quick fits or refining of fits after small shifts. The period of random sampling is non-optional forcing the algorithm to require a minimum number of iterations. Additionally, it was observed that for this simulation any improvement in the minimization of the objective function tended to take on the order of 150 to 200 iterations. When quick local fit on the order of 50 to 100 iterations was desired surrogateopt is not an option. For this reason, the fminsearch algorithm in MATLAB was utilized when small shifts in current are introduced.
Fminsearch is a direct search method of finding local minima that does not require any information on the gradient of the objective function. It works by utilizing Nelder-Mead simplex algorithm. Which constructs the vertexes for a simplex around the initial guess by adding 5% of one component to the initial guess to that initial guess. Then the algorithm slowly shifts the simplex, contracting and reflecting points as it slowly works its way to the local minimum until an exit condition such as number of iterations has been reached (fminsearch, 2006; Optimizing Nonlinear Functions, 2022). In practice the Nelder-Mead simplex algorithm allows for rapid improvement in the objective function, even if it will not find the global minimum unless given an exceedingly good initial guess (Lagarias et al., 1998). Not needing any information about the gradients and the relative quickness of fminsearch in improving the value of the objective function made it a desirable function to use for quick improvements to existing fits.

**General Fitting Approach**

Starting with the parameter sets described in the previous work of Sabrina (Metzger, 2021) in optimizing while treating the muscle cell as a singular longitudinal segment, optimization for up to 16 longitudinal segments where found. The general framework for fitting the action potential while introducing multiple longitudinal segments boiled down to:

1. Fitting the passive parameter to the hyperpolarizing pulse
2. Fitting the active parameters to the AP
3. Adjusting the current as needed
4. If such adjustment was done a quick fit for the active parameters is run
5. Repeating Step 3 and 4 until the AP timing matches closely
6. Rechecking the hyperpolarization and adjusting as needed
Passive Parameter fitting

The fictious leak ion is utilized to represent the non-modeled ionic channels, mostly K+ due to the inward-rectifier K+ channel and Cl-. This ionic current is largely passive in its response to voltage changes and is responsible for the cell’s hyperpolarization when introduced to a “negative” current. The permeability of the cell membrane to this leak ion is responsible for the curvature of the hyperpolarization curve of the membrane potential while the leak ion concentration is largely responsible for small shifts in the resting potential of the cell membrane.

To reflect this the first 20 milliseconds of the hyperpolarization for each cell is fit to the model by simply adjusting the intercellular Leak ion concentration, current, and leak ion permeability for a given number of longitudinal segments. The return to rest and the later duration of the hyperpolarization (post 20ms) was avoided in order to emphasize effects of slow gating ion channels not presently modeled. In addition, due to limitations of the model in that the leak current and intracellular leak ion concentration are not able to be fitted together with the leak permeability the hyperpolarization is fit by hand and eye. The focus of which is on the curvature of the initial hyperpolarization.
Figure 4 An example of Hyperpolarization Adjustment.

Top left shows the starting point of the fit before any adjustment. Top right shows the simulation after allowing $L_i$ to decrease to raise the simulated resting potential. Bottom left shows the effect of decreasing the stimulus current in order to match the depth of the hyperpolarization. Bottom right shows the final fit after the leak permeability is adjusted to fit the curvature. Additionally, $L_i$ and the stimulus current are further adjusted to account for shift in resting potential and depth as the permeability is shifted.

To do this the Leak ion concentration is first adjusted to allow for the resting potential of the simulation and the data set to match near the region where hyperpolarization occurs. The maximum hyperpolarization of the model is then matched by adjusting the hyperpolarizing current. Once the resting potential and the maximum hyperpolarization are matched closely the
leak ion concentration is adjusted to allow for the curvature of the hyperpolarization to match that of the data. The permeability of this fictitious positive ion also adjusts the maximum hyperpolarization. Due to this fitting of the hyperpolarization becomes a dance of changing the permeability and current to eventually arrive at a similar voltage response to the data.

**Active Parameter fitting**

After finding the leak permeability using the hyperpolarization the resting potential near the AP was matched to the data by again shifting the intracellular leak ion concentration. Rarely did this need to be shifted, but for certain data sets were the AP of interest and the hyperpolarization occurred with significant time delay the resting potential drifted enough between requiring the leak ion concentrations to be adjusted to match the new resting potential.

The other 17 parameters that control the cell’s active response to a stimulus current; sodium permeability, potassium permeability, the five Hodgkin-Huxley sodium activation, five inactivation, and five potassium parameters, are then fit together utilizing the before described objective function.

The process of this first involves utilizing surrogateopt to first attempt to minimize the objective function. 500 iterations were chosen to be ran with 75 initial surrogate points. 75 initial surrogate points, with one of which the initial guess was chosen as a sweet spot for allowing the function to get an idea of what happens within the parameter bounds without taking too long when ran with larger numbers of longitudinal segments in the model. Ideally a near infinite number of iterations would have been ran, but in our observations, it was found the surrogateopt tends to do the most improvement within the first 300 iterations, and when using more
longitudinal segments 500 iterations could already take well over an hour to run. More iterations were deemed not worth it.

Once the initial 500 iterations were ran the simulated AP was then observed and the input current was adjusted to allow the action potential timing to coincide with that of the data. If this required large shifts in current and changed the location significantly surrogateopt would then be ran once more with 500 iterations, but in most cases only small shifts in current were necessary. These small shifts in current had very minor effects on the overall AP shape resulting in a minor increase in the value of the objective function. To refine this fit a short fit on the order of 150 iterations was performed using fminsearch. On occasion this process of shifting the current the fitting would need to be repeated until the AP would stabilize occurring with similar timing to that of the raw data.

**Checking the Hyperpolarization**

Once the fit has been refined one final check of the hyperpolarization is run. The sodium and potassium parameters have minor effects on the cell’s response to a hyperpolarizing current. In most cases the current/intracellular leak ion concentration/leak permeability did not need shifted, but on occasion the resting potential would have drifted slightly requiring the leak ion concentration to be adjusted, or the maximum hyperpolarization would require the current to be adjusted. In no observed case did the curvature change observably and require a shift in leak permeability.
**Number of Segments**

Once the final check of the hyperpolarization was run the parameter set is saved and considered to be the final fit for whatever number of longitudinal segments for that specific data set. This parameter set is then used as the initial guess for the next set after increasing the number of longitudinal segments. The initial fit is always done with a singular segment and increased up to 16 segments. In most cases the number of segments used in each fit was doubled each iteration up to the final 16 segments, but on the occasion that the fit had trouble smaller increases in number of segments were made. In addition to better understand how the parameters change with increased longitudinal segments, one of the eight cells studied had fits done for each segment increase, for a total of 16 parameter sets.

**Robustness of fit and Uniqueness of parameter sets**

After all optimizations had been run and full sets of parameters found for each of the 8 data sets, the question of whether the true values of these parameters were found remains. Does a data set allow for only a singular parameter set or can there be a multitude of similarly well fit APs with distinctly different parameters? To attempt to answer this and to get a better idea of how robust these solutions are, three studies were done.

The first study being simply swapping the data sets and parameter sets around until each data set had been compared to the outputs of each found parameter set. The main goal being to see how much the raw data sets differ, and if the distinctly different parameter sets could produce a similar AP. An additional consideration of what happens when the initial resting potential was let vary from that of the data set the fit was found on to resting potential of each other data set. Currents were also adjusted in this case to allow the timing of the simulated and real APs to align.
The second study undertaken was a logical continuation of the previous, but instead of simply plotting the different APs from the found parameter to different data sets, fitting them. The main goal of these new fits was to vary the initial parameters and see if they reconverge to something similar to that of the parameter set found initially for that data set or diverge to distinctly different sets.

The final and arguably most important study undertaken is to understand the limits of the parameters. This was done by varying each individual parameter small amounts then running a new fit. The parameter was continuously varied up (or down) until the new fit was able to return similar value for the objective function. From this the goal was to extract the confidence interval of the parameter. Before moving on to the next parameter and doing the same. Additionally, since all other parameters were allowed to vary in the fit, and these were recorded information on how the other parameters need to change in order to maintain the features of the AP.
RESULTS

Fiber Length Investigation Using Single Segment Parameters
Long Fixed Length Fiber, Vary Number of Segments

Before any fitting can occur an understanding of the effects of adding longitudinal segments, simulating the passage of current through the cell, must be had. A total muscle length of 4mm and radius of .03mm was used for all simulations in this section. Initial guesses for channel and membrane parameters were taken from previous fits using a model with a single segment (Metzger, 2021). The number of longitudinal segments was then increased by factors of 4 up to 64 segments.

An initial observation from the simulations was that the minimum current required to elicit an action potential and achieve the correct timing decreased as the number of segments increased from 1 to 4 or 8 segments. Beyond about 8 segments, the required current was essentially unchanged. When current stimulates the cell, the current is applied to one segment. In this instant the current can then travel some distance in the cell, we will refer to this as the space constant. In simulations of only one segment the current is essentially applied to the whole cell requiring a more in order to produce the needed voltage change and trigger an AP. As more segments are added less membrane is stimulated allowing for smaller currents to induce the same potential change. However once more than one segment exists within the space constant attempting to only stimulate a singular segment becomes stimulation of the whole space constant. Essentially the stimulus current can no longer decrease when adding more segments as the total amount of membrane stimulated remains the same.
Figure 5 Segment Number investigation Central Segment APs.

Shown as the solid blue lines are the simulated APs in the central segment of the cells. Current is applied to this same segment with the same membrane and channel parameters utilized. These are overlayed with the experimental trace (dotted lines). The top left corresponds to singular long segment stimulated with 950nA. Top right shows the same cell broken into 4 equal sized longitudinal segments (.1cm each) the second segment has 266nA of stimulus current applied. Bottom left shows the same for a 16-segment cell simulation (.025cm each) with 190nA applied to the 8th segment. Bottom right corresponds to a 64-segment cell (.00625cm each), the same stimulus current as for 16-segments is applied to the 32nd segment where the AP is also measured. An anomaly of currents feeding back to cause secondary peaks (discussed below) can be observed in both the 4-segment and 16-segment simulations.
Simulations of every longitudinal segment when current is applied to the central segment of a 0.4cm long cell. Upper left shows a single segment simulation, upper right 4-segments, lower left 16, and lower right 64 segments. The left most segment (earliest AP) corresponds to the segment in which current was applied. Segments further from this stimulus pulse exhibit APs later and later. In the 4 and 16 segment models feedback between the APs can be observed, when another segment undergoes an AP while an earlier one is still happening a small secondary peek in the earlier AP can be observed at that time.

Figure 6 Segment Number investigation All Segment APs.

Currents were applied in the central segment of the muscle and the AP was also observed in that segment (Figure 5) although the APs in all other segments were also simulated and can be observed in Figure 6. For one data set ~950nA had to be applied for a singular
segment, dropping all the way to ~266nA for 4 segments, and coming to rest at ~190nA for both 16 segments and 64 segments. Additionally, as segments are added the height of the AP decreases while the width increases. Secondary peaks were also observed in the intermediary numbers of segments.

In Figure 6, one can see that a secondary peak corresponds directly to when adjacent segment is experiencing its AP peak. The segments themselves are interacting with the APs feeding back onto each other. When there are too few segments this feedback leads to secondary peaks, but as the number of segments increases, and the AP peaks from adjacent segments occur very close in time with each other this phenomenon disappears. It is believed that this feedback still exists with higher numbers of segments but no longer causes unrealistic secondary peaks.

Figure 6 also illustrates that near the edges of the cell, further away from the initial stimulus pulse, the APs increase in magnitude and seem to slowly return to a shape similar to that of the AP for a singular long segment. The direct response to the stimulus pulses also slowly decreases in magnitude as the AP propagates through the cell. Only segments close to the stimulated segment show an initial voltage spike prior to firing the AP. A welcome result, only segments within the so called “space constant” where current is applied should have a direct response to the initial stimulus current. Later segments are stimulated by the propagation of the APs themselves.

In real cells secondary AP peaks do not occur. Thus, the simulation must avoid creating such peaks. These secondary peaks only occur when there are too few segments (but more than one) in a cell causing large discrete timing differences between AP firing times of subsequent segments. To avoid this the segments themselves must be kept below a certain length in simulation. This is not a problem in real life due to currents propagating smoothly within the cell.
as if it had infinite segments. We cannot simulate infinite segments, and even going above 100 segments causes simulation times to be significant.

Given the desire to eventually arrive at a Hodgkin-Huxley (HH) parameter set that more closely resembles reality a balance between segment length, cell length, and number of segments had to be struck. To do this, secondary peaks had to be eliminated while keeping in mind simulating large numbers of segments was not feasible due to computing power and time constraints. To avoid secondary peaks a maximum segment length was found to be ~.125mm. For segment lengths longer than this very slight secondary peaks were occasionally observed, while at or below this length no secondary peaks could be seen. This is not to say that 100% of the effects of having long segments was completely removed but enough was as to get a reasonable understanding of the effects adding segments would have.

**Variable Fiber Length, Fixed Segment Length**

Given the constraints of the minimum found segment length to avoid the secondary peak anomaly yet still having a need to study realistic cell length forced the use of segments of exactly the maximum length that disallowed for the anomaly, .125mm. This allowed avoidance of the major effects of the anomaly while still allowing for fibers that reach up to 2mm long at 16 segments. This is approximately half the length of the average muscle fiber used in the Rich Lab but running full on fits for over 16 segments was judged to be too computationally intensive and time consuming to be feasible.

Once the decision was made to slowly increase cell length while adding segments of .125mm fitting of the HH parameters could begin. Eight different APs taken using 2 differing protocols. Four having strong currents, on the order of 500-800nA, applied for ~.2ms, referred to
as the protocol 1 data sets. While the four utilizing protocol 2 have much smaller stimulus currents, on the order of 150-250nA, applied for ~1ms. These differences in stimulus currents lead to very different behavior of the stimulus pulse but overall, relatively similar AP shape post pulse. One important note on this is that AP timing never shifted significantly between data sets. This indifference to AP stimulus on timing is not observed in the simulation. Higher currents lead to significantly earlier AP firing, even firing while the Stimulus current was still occurring. While low currents would lead to significant delay in the AP firing, even being up to ~10ms of delay. An example of this is shown as Figure 7. The fact that there does not seem to be a direct link between stimulus current and AP timing in real cells suggests that one of the not yet modeled ion channels might be partially responsible for regulating this timing and would be something to investigate as the model is further refined.

![Figure 7 Current Effects on AP Timing.](image)

Examples of a low current (left) and high current (right) stimulating a 1 segment .125mm long cell. To get the timing to coincide ~36.5nA must be applied but at approximately half that (18.75nA) there as around a 10ms delay slightly less current leads to no AP forming. At double the optimal stimulus current (73nA) the AP peaks ~.75ms early.
Given this effect on AP timing stimulus current has when models, but the relative indifference to it in real cells the current was allowed to vary in order to allow the AP to peak at the same time as the dataset. The shift is almost purely for visualization, as the objective function largely ignores the exact timing of the AP. Taking this into account the 11 Sodium parameters, 6 potassium, and the leak permeability are fit to the objective function as describe in the methods section. For all data sets an initial fit for 1 segment was done, and for most the segment number was doubled each fitting iteration up to 16 segments, a total of 5 fits for each data set. In a few cases, other numbers of segments were fit, and in one case a fit was done for all 16 individual segment numbers. This was in order to understand better how the parameter set evolves as more segments are added.

Before we get into these parameter sets and how they evolved as more segments where added it should be understood what effects on the AP shape we are trying to compensate for. Given the initial investigation into this was riddled with the anomalous secondary peek by utilizing our maximal segment length we can eliminate this effect and understand what we are actually correcting for. The effects of starting with a good fit for one segment of .125mm then adding more segments of the same length while keeping membrane and channel parameters unchanged can be seen below in Figure 8 and Figure 9. While testing, segment number was doubled from 1 up to 128 segments.
Figure 8 Effects of Segment Number for constant segment length on the stimulated segment.

Shown are the AP corresponding to the central segment in which current is applied for 1 segment (top left) 4 segments (top right) 16 segments (bottom left) and 64 segments (bottom right). The currents are adjusted to allow for similar peak times.
Figure 9 Effects of Segment Number on all segments.

Shown are modeled APs for all present segments. Top left corresponds to 1 segment, top right 4, bottom left 16, bottom right 64 segments.

Similar effects to the study with fiber length of 4mm and increasing segments can be observed. With more segments the maximum voltage achieved lowers while the base of the AP widens. Additionally, the initial voltage spike from the stimulus gets more prominent for segments within the “space constant” at which current is applied. One important observation that was not present in the previous study due to the presence of the anomaly is that post 16 segments the overall shape of the AP of interest (central where current applied) has very little change. Not
shown here is the model for 32 segments or 128 segments due to their extreme similarity to that of 64 segments.

Important to note are the applied currents to the cells. For small numbers of segments, the current needed increases but at ~32 segments this value holds constant. At first glance the increases in current do not seem to match the results utilizing a set length of 4mm. It is the exact opposite relation observed for a constant cell length. But if you consider increasing the number of segments from 1 to 16 segments (or above given it held constant post this) only decreased the current by a factor of 5 while the surface area between 1 and 16 segments in the second study increases by a factor of 16 (cells simulated as cylinders, ends are not considered). Basically, surface area is increasing much faster than the current shrinks for a given area pushing the amount of stimulus current up until it hits the threshold needed for a single segment to fire an AP. This is the same relationship observed while studying a 4mm long cell and can seen below in Table 1.

*Table 1: Segment Number Effects on Current*

<table>
<thead>
<tr>
<th>Number of Segments</th>
<th>Current (nA)</th>
<th>Current decrease factor from 4mm study (multiples of 1 seg current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>114.9</td>
<td>3.57</td>
</tr>
<tr>
<td>8</td>
<td>184.4</td>
<td>4.47</td>
</tr>
<tr>
<td>16</td>
<td>210.5</td>
<td>5</td>
</tr>
<tr>
<td>32</td>
<td>227.9</td>
<td>5</td>
</tr>
<tr>
<td>64</td>
<td>227.9</td>
<td>5</td>
</tr>
</tbody>
</table>
**Short Fixed Length Fiber Behaves Like Single Segment Regardless of Number of Segments**

A series of figures were generated on a .125mm long cell to illustrate the effects of increasing segments while avoiding the anomaly produced from longer segments. With very few segments there was a shift in the AP shape but for large numbers of segments any difference from a singular segment seemed to be lost. This produced the near existential dread of, oh no was everything we did a product of the anomaly. Had we just not produced small enough segments? To test this the model was run for increasingly long cells with increasing number of segments.

![Figure 10 Segment Number Effect on a .125mm Cell.](image)

Left 1 segment, right 100 segments. No visual change is observable.

For a .5mm long cell segment (Figure 11) this phenomenon was even more obvious. There is a small but distinct difference in AP height and timing at our previously decided on minimum segment length of .125mm (4 segments). As more segments are added this shift seems to disappear. At 100 segments the AP looks almost identical to a simulation of only 1 segment. Notable however is that the initial voltage spike gained prominence with added segments.
Similar phenomena were observed for all very short cells, but as the cell lengthens the prominence of the initial voltage spike increased for many segmented cells.

Figure 11 Simulation of a .5mm cell.

Top left 1 segment, top right 4, bottom left 10, and bottom right 100 segments. At 4 segments, the previously thought non-anomalous segment length of .125mm the AP exhibits slight shortening, but by 10 or 100 segments this disappears. The only visual evidence of 10 or 100 segments not being one is a slightly exaggerated initial response to the current. There is no visual difference between 10 and 100 segments.

Once the cell started to get in to be of a more reasonable length the seeming return to acting like a singular segment starts to disappear. At 1mm (Figure 12), a many segmented cell
does not produce a similar AP to a singular segment. As more segments the AP widens and shortens as observed with constant length segments. The phenomenon of returning to the original shape does not, however, fully disappear. At 8 segments the Widening and shortening of the AP is significantly more prominent than at 50 or even 500 segments. The return to the original shape does not seem to complete, at 50 segments or 500 segments there is almost no difference in the AP. The exact same phenomenon can be observed with longer cells such as the 2mm segment modeled in Figure 13 and Figure 14.
Figure 12 Simulations of a 1mm long cell.

Top left 1 segment, top right 8, bottom left 50, and bottom right 500 segments. Significant shortening and widening of the AP are observable for all situations greater than 1 segment. At 8 segments, the “non-anomalous” length of .125mm each there is slight over exaggeration of the initial voltage spike and the AP is ever so slightly wider than for 50 or 500 segments. No noticeable difference can be observed between 50 and 500 segments.
Figure 13 Simulations of a 2mm long cell.

Top left 1 segment, top right 16, bottom left 100, and bottom right 500 segments. Significant shortening and widening of the APs are observable for all situations greater than 1 segment. At 16 segments, the “non-anomalous” length of .125mm each there is slight over exaggeration of the initial voltage spike and the AP is ever so slightly wider than for 100 or 500 segments. No noticeable difference can be observed between 100 and 500 segments.
Figure 14 A 1000 segment simulation of the 2mm long cell.

Left shows almost no difference between 100 or 500 segments observable in the previous figure. Right illustrates how the initial voltage spike is reduced, the AP height is increased, and width decreased as time passes and the AP propagates nearer to the ends of the cell.

The phenomenon of many segment cells acting like a single segment cell appears to be only present in very short cells < .5mm. Due to these observations the fits performed for 2 and 4 segments (.25mm and .5mm) might not hold much of any real information, but for the 8 segment (1mm) and 16 segment (2mm) fits preformed are much closer to reality. They however do have some portions of the artifact caused by too few segments present. Some very small shift in AP shape when further reducing segment length as seen in Figure 12 and Figure 13 that has not been taken into account. At approximately ~.02mm (1mm 50 segments, 2mm 100 segments and so on) the changes in AP shape seem to stop. This difference between that of the previously thought “non-anomalous” segment length, .125mm and using .02mm is very minimal. Ideally more segments would have been used for all fits (.02mm each) but given time and computing power constraints using .125mm segments gives a reasonable approximation of reality.
For future studies interested in very short cell segments there is no need to introduce longitudinal segments. This physically makes sense. For a very short cell attempting to apply current to any given segment is akin to simply applying a current to the whole cell, there is near zero propagation time when compared to the time it takes for an AP to fire, the space constant could be considered the whole cell. For longer cells, however, this is not true. The propagation time approaches the same magnitude or even becomes greater than the time it takes for an AP to fire. This should mean that the segments interact causing the AP to shift in shape with propagation leading to the observed smearing of the AP. This timing spread can be seen below in Figure 15. Segments closer to the current probe fire an AP much earlier than further segments. This seems to cause segments just about the AP to have slightly higher peaks and initial voltage spikes, both of which drop slightly with propagation. Near the edges of the cell the peak potential of the AP increases.
Figure 15 Simulations of all segments of a 4mm long cell.

Top left 1 segment, top right 32 segments, bottom left 100 segments, and bottom right 500 segments. Easily visible in the 32 or 100 segment simulations is the reduction of the direct stimulus response further down the cell. The AP height also begins to slightly decrease, stabilize, and finally increase when near the edges of the cell (farther in time, and further to the right in these figures). The AP width seems to do the opposite, increasing and stabilizing before decreasing.

Fitting With Multiple Segments

Graphical Results for All Eight Experiments

Now that a basis of what features must be corrected for, and what anomalies can happen, the actual effects of increasing the number of longitudinal segments on the parameters can be
established. But before I get into the results of the fits what we conceptually expect to see based off the shifting shape of the AP can be established.

From the previous section it can be seen that the up slope of the modeled AP with large numbers of segments is significantly shallower. It is understood that the initial slope and voltage spike of an AP is mostly controlled by the activation of voltage gated sodium channels, therefore one would expect slightly more responsive activation of these channels as more segments are added. Additionally, the height of the AP significantly decreases with more segments. To correct this, we can expect the inactivation of sodium channels to be less responsive allowing for them to stay open longer pushing the maximum voltage back up. Lastly the downwards slope of APs when modeled with more segments is significantly decreased. This slope is controlled mostly by slow activation of the voltage gated potassium channels. To increase this downslope we can expect more responsive potassium channel activation.

All final fits for protocol 1 data sets can be seen below as Figure 16 and protocol 2 as Figure 17. All protocol 2 data sets were able to be modeled more accurately resulting in much lower values of the objective function (order of most being >1) than for protocol 1 data sets (most minimized to the order of 10 with the lowest being ~3 and highest closer to 25). An additional note about these fits is that for most cases the fit better for higher segment numbers. Two data sets where a better fit was not found for higher numbers of segments were from protocol 1. These two had relatively constant values of the objective function for all segment numbers.
Figure 16 All Final Fits to Protocol 1 Data Sets.

Shown are every final fit to protocol 1 AP data sets each with increasing number of segments. Peak heights and down slopes where able to be fit reasonably well but the strong initial stimulus was never able to be modeled causing the rising slope of each AP to be more off than ideal. Additionally notable is that the top left data set had a significantly lower AP peak (22.75mV) as opposed to all other data sets (most peaking near 35mV).
Figure 17 All Final Fits to protocol 2 Data Sets.

Shown are every final fit to protocol 2 AP data sets each with increasing number of segments. The full 1ms stimulus pulses were able to be modeled much more accurately leading to less issues fitting the up slope than any protocol 1 data set. Notable is that the J18 AP (upper right) was able to be modeled much more accurately than any other, due to this that data set is focused on for fitting all 16 segments and the latter sections. An additional note is that the J16 (lower left) peaked significantly higher than any other studied AP at 49.5mV as opposed to the near 35mV most others peaked at.

For the protocol 2 data sets this increase in goodness of fit for increasing parameters might be explained due to the stimulus voltage spike approaching reality. As more segments are added this initial spike gets more pronounced. For the protocol 2 data sets where current was
applied for a longer duration but with significantly lower magnitude this initial current spike causes a shallow voltage to spike the closely resembles that of the modeled spikes with higher numbers of segments (8+ segments seem to closely resemble this). For few segments I was never able to induce quite as pronounced of a voltage spike that would lead to a well-timed AP. This shifting of the initial voltage spike might also be why 2/4 of the protocol 1 data sets also see this improvement with more segments, although the sheer magnitude of the voltage spike in protocol 1 data sets and near instant drop was never able to be modeled.

An additional important note is that very few parameter sets were able to closely model the slow return to resting potential after the AP present in every data set. This is, however, not unexpected, the slow return to rest involves channels and channel behavior not currently modeled in this basic Hodgkin-Huxley approach. Fitting these altered portions of the return to rest will require further additions to the model.

Effects of Segment Number on HH Parameter Values
The actual effects of increasing the number of longitudinal segments present in the muscular sections studied on the HH parameter sets is much more ambiguous than desired. No singular parameter holds a definite trend for all data sets studied. The values of each parameter as they vary with increasing segment number (with constant segment length) can be seen in Table 2 through Table 5 and Figure 18 through Figure 21.

The three modeled permeabilities, Sodium, Potassium, and the leak are shown below in Figure 18. Sodium permeability did not have an overall trend shared with all data sets. Individually each data set had semi-consistent trends, for about half the data sets seemed to increase the maximum Sodium conductance as more segments were added, while the other half
seem to suggest the opposite relation. Notable is that for one segment average value of the maximum Na permeability held near constant at 11.1±3µm/s for one segment to 11.6±5µm/s for 16 segments. Potassium permeability behaved very similarly changing from 3.31±1.5µm/s at average for one segment to 3.39±1.7µm/s at 16. The only permeability that seemed to hold a definite trend was for the leak. All data sets besides the protocol 2 data 3 set (green) purely decreased as more segments were added. The Protocol 2 data 3 set did not have as clear a hyperpolarization region for which the leak permeability was fit. If this data set is included the average for one segment decreased from 64.6±7.8nm/s to 54.1±12nm/s at 16 segments, excluding it leads to 63.4±7.6nm/s at 1 segment and 50.6±11.2nm/s at 16 segments. The average values for 1, 2, 4, 8, and 16 segments and standard deviations in such can be shown below as Table 2.

Perhaps most surprising, was that the values of each permeability varied between a factor of 2 to 5 from one experimental fit to another. This could be representation of biological variability or simply could be a sign that the model is "underdetermined", such that the experimental data did not contain sufficient information to confine any specific parameters to a narrow range. Thus, a good fit could be obtained by having some parameters compensate for variation in other parameters. This concept is partially studied in the sensitivity section later.
### Table 2: Average Maximal HH Permeabilities

<table>
<thead>
<tr>
<th>Segments</th>
<th>Na Permeability (µm/s)</th>
<th>Kdr Permeability (µm/s)</th>
<th>Leak Permeability (nm/s)</th>
<th>Leak Permeability, excluding J03 (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.1±3.0</td>
<td>3.31±1.5</td>
<td>64.6±7.8</td>
<td>63.4±7.6</td>
</tr>
<tr>
<td>2</td>
<td>11.2±2.9</td>
<td>3.29±1.5</td>
<td>64.3±8.2</td>
<td>62.7±7.4</td>
</tr>
<tr>
<td>4</td>
<td>11.2±3.2</td>
<td>3.37±1.5</td>
<td>63.8±8.7</td>
<td>61.7±6.8</td>
</tr>
<tr>
<td>8</td>
<td>11.2±4.6</td>
<td>3.24±1.6</td>
<td>61.4±8.4</td>
<td>58.9±5.2</td>
</tr>
<tr>
<td>16</td>
<td>11.2±5.0</td>
<td>3.41±1.7</td>
<td>54.1±12</td>
<td>50.6±7.4</td>
</tr>
</tbody>
</table>
Figure 18 Trends in Permeability.

Shown are values of Na, Kdr, and the “Leak Ion” permeability found from fitting to the known data sets and graphed vs the number of segments. Neither Na nor K have a consistent trend. Both seem to slightly rend upwards in value but there are very notable exceptions. Leak permeability on the other hand seems to have a definite trend downwards as more segments are added, although the protocol 2 data 3 set (green) seemed to trend slightly upward before remaining constant.

Like that of the permeabilities, the HH parameters that control the activation of the sodium channels never held a 100% consistent trend. But if looked at all together a few trends stand out. $\overline{V_m}$ decreases with increasing numbers of segments with the closet to complete consistency. $K_{\beta m}$ seems to do the same but it is much harder to tell how significant this is given
half the sets remain relatively constant and the rest decrees, although not all smoothly. It is notable that the W12 and W18 data sets (yellow and blue in Figure 19) which start high with $K\alpha m$ converge towards the rest while continuing to maintain much higher values of $\bar{\alpha_m}$ and $\bar{\beta_m}$. These two parameters both seem to increase when looking at their average values, although $\bar{\beta_m}$ has most parameter sets staying reasonably constant and only 3 having a significant increase (two of which being W12 and W18). $K\alpha m$ has the least definite trend, every data set seems to have $K\alpha m$ doing something different. Due to this chaotic relation $K\alpha m$ seems to hold with segment number it would be very unsurprising if $K\alpha m$ cannot be easily compensated by other parameters slightly shifting, or even have little to no effect on the direct shape of the AP and in turn the value of the objective function.

Similar to leak parameters large variance was observed. For the activation parameters the least variance is observed in $\bar{V_m}$, having only a factor of 1.5 difference between the highest and lowest value. The greatest variance is nearly a factor of 5 seen in $\bar{\alpha_m}$.

**Table 3: Average Na Activation HH Parameters**

<table>
<thead>
<tr>
<th>Segments</th>
<th>$\bar{V_m}$ (mV)</th>
<th>$K\alpha m$ (mV)</th>
<th>$K\beta m$ (mV)</th>
<th>$\bar{\alpha_m}$ 1/(ms * mV)</th>
<th>$\bar{\beta_m}$ (1/ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-46.7±6.6</td>
<td>8.69±1.2</td>
<td>37.2±16</td>
<td>0.238±0.13</td>
<td>1.51±1.0</td>
</tr>
<tr>
<td>2</td>
<td>-47.0±6.4</td>
<td>8.73±1.1</td>
<td>36.8±16</td>
<td>0.238±0.13</td>
<td>1.52±1.0</td>
</tr>
<tr>
<td>4</td>
<td>-47.9±5.7</td>
<td>8.87±1.3</td>
<td>37.0±18</td>
<td>0.250±0.13</td>
<td>1.52±0.99</td>
</tr>
<tr>
<td>8</td>
<td>-49.7±4.6</td>
<td>8.83±1.6</td>
<td>29.2±16</td>
<td>0.276±0.13</td>
<td>1.56±1.2</td>
</tr>
<tr>
<td>16</td>
<td>-50.7±7.4</td>
<td>8.83±2.2</td>
<td>26.8±12</td>
<td>0.291±0.13</td>
<td>1.80±1.4</td>
</tr>
</tbody>
</table>
Figure 19 Trends in Na Activation Parameters.

Shown are the values of the five Na activation parameters and their trends as the number of parameters increase. $\bar{V}_m$ and $k_{\beta m}$ tend to trend slightly downwards, $\bar{\alpha}_m$, tends slightly upwards, while $\bar{\beta}_m$, and $k_{\alpha m}$ do not seem to hold significant trends. No parameter holds a 100% consistent trend.
After the Sodium channels are opened, they quickly begin to shut down and become inactive. This inactivation is modeled with the HH approach using $V_h, K_\alpha h, K_\beta h, \overline{\alpha_h}$, and $\overline{\beta_h}$. When adding longitudinal segments only $V_h$ holds a near consistent trend. For almost every parameter set $V_h$ increases with every additional segment. The spread of values also increased slightly. Table 4 might suggest an additional trend that $K_\beta h$ seems to decrease while $\overline{\alpha_h}$ seems to increase as more segments are added. Although this does not seem to be the case for most parameter sets. Only the W12 and W18 data sets (yellow and blue) exhibit this consistent increase/decrease, all other sets stay relatively constant and be clustered together. This might suggest that there exists two (or more) stable sets of parameters, ones similar to the W12 and W18 data sets and ones more similar to the rest. For ones with elevated $K_\beta h$ and $\overline{\alpha_h}$ and reduced $V_h$ and $\overline{\beta_h}$ similar to that of the W12 or W18 data sets the trend in $K_\beta h$ and $\overline{\alpha_h}$ with increasing segments may be different. Although this is impossible to conclude with only two data sets and would be something to look into later. No other Na inactivation parameter seems to hold any particular trend with increasing segments.

All Na inactivation parameters exhibited high variance. $V_h$ held the least variance only a factor of 2 difference in the highest and lowest values. But the most variance of any other parameter is also seen in the Na inactivation parameters. $K_\beta h$ varies all the way from 2.35mV to 19.5mV, a factor of 8.3.
<table>
<thead>
<tr>
<th>Segments</th>
<th>$\bar{V}_h$ (mV)</th>
<th>$K_\alpha h$ (mV)</th>
<th>$K_\beta h$ (mV)</th>
<th>$\bar{\alpha}_h$ (1/s)</th>
<th>$\bar{\beta}_h$ (1/ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-46.0±10</td>
<td>20.8±7.7</td>
<td>7.69±6.8</td>
<td>5.54±2.8</td>
<td>7.37±3.7</td>
</tr>
<tr>
<td>2</td>
<td>-46.2±9.8</td>
<td>19.9±6.9</td>
<td>7.64±6.7</td>
<td>5.58±2.8</td>
<td>7.48±3.7</td>
</tr>
<tr>
<td>4</td>
<td>-44.3±11</td>
<td>22.1±8.3</td>
<td>7.74±6.8</td>
<td>5.75±2.8</td>
<td>7.84±4.1</td>
</tr>
<tr>
<td>8</td>
<td>-42.5±11</td>
<td>26.7±13</td>
<td>6.66±5.8</td>
<td>6.03±3.7</td>
<td>7.48±4.0</td>
</tr>
<tr>
<td>16</td>
<td>-41.2±13</td>
<td>24.1±14</td>
<td>6.46±5.0</td>
<td>5.94±4.0</td>
<td>6.84±3.7</td>
</tr>
</tbody>
</table>
Figure 20 Trends in Na Inactivation Parameters.

Shown are values in the Na inactivation parameters graphed vs number of segments. Of such parameters only $V_h$ (top) seems to hold a near consistent trend of increasing. All other parameters seem to for the most part hold reasonably stable or have mildly chaotic trends. One important note is that for the W12 and W18 data sets (yellow and blue) $V_h$ is significantly lower than for
other data sets, both $k_{\beta h}$ and $\overline{\alpha_n}$ seem to be increased while $\overline{\beta_n}$ is decreased seemingly to compensate for this.

Lastly, we can look into how the Potassium HH parameters $\overline{V_n}, K_{\alpha n}, K_{\beta n}, \overline{\alpha_n},$ and $\overline{\beta_n}$ change as more longitudinal segments are added to the muscular cell. Of these parameters $K_{\alpha n}$ has no discernible trend. $K_{\beta n}$ almost looks to be consistently increasing but the uncertainty in the average value increases nearly as fast, showing that the spread of the values is increasing with increased segments as well. Double checking this against the graph of $K_{\beta n}$ in Figure 21 confirms that this seeming trend upwards is almost completely the effect of a singular data set (W12, blue in the figures). The values of $\overline{\beta_n}$ might suggest a similar relation, only decreasing with number of segments, but again the increase uncertainty and seems to be mostly caused by the rapid drop in $\overline{\beta_n}$ present in the J16 data set. Ignoring this significant drop $\overline{\beta_n}$ seems to be largely unaffected by increasing segments.

$\overline{V_n}$ like the two other main gaiting voltage holds a reasonably consistent trend. As more segments are added in most cases the needed voltage slightly dropped. This trend is both visually present in Figure 21 and numerically in Table 5. The last Potassium parameter, $\overline{\alpha_n}$, has a similarly distinct trend, only increasing instead of decreasing. The values of $\overline{\alpha_n}$ for the J03 data set (green) should be noted as in data set all values of $\overline{\alpha_n}$ are between a half and a third that of any other data set. This seems to be compensated by significantly reduced values of $\overline{V_n}$ and elevated values of $K_{\alpha n}$. This could be evidence of a secondary stable state for those parameters that is still able to reproduce very similar APs.

The Potassium parameters were found to vary the least. Each only varies by a factor of 2 or 3 at most. This might point to less direct biological variance in the potassium parameters,
suggest a more restricted region in which good fits can be found, or simply suggest that the
goodness of fit has less reliance on these parameters, and therefore less need to shift them.

Table 5: Average Potassium HH Parameters

<table>
<thead>
<tr>
<th>Segments</th>
<th>$V_n$ (mV)</th>
<th>$K_a n$ (mV)</th>
<th>$K_\beta n$ (mV)</th>
<th>$\bar{\alpha}_n 1/(s * mV)$</th>
<th>$\bar{\beta}_n$ (1/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-38.9±9.5</td>
<td>5.22±1.1</td>
<td>24.7±14</td>
<td>15.6±4.1</td>
<td>100±27</td>
</tr>
<tr>
<td>2</td>
<td>-38.9±9.7</td>
<td>5.30±1.1</td>
<td>25.2±14</td>
<td>15.6±3.9</td>
<td>99.3±27</td>
</tr>
<tr>
<td>4</td>
<td>-38.5±9.1</td>
<td>4.99±1.3</td>
<td>25.0±14</td>
<td>15.9±4.1</td>
<td>98.5±27</td>
</tr>
<tr>
<td>8</td>
<td>-39.3±9.4</td>
<td>4.83±1.4</td>
<td>25.8±15</td>
<td>16.9±4.8</td>
<td>99.3±33</td>
</tr>
<tr>
<td>16</td>
<td>-40.9±9.4</td>
<td>5.18±1.3</td>
<td>28.8±17</td>
<td>19.4±5.7</td>
<td>95.7±37</td>
</tr>
</tbody>
</table>
Figure 21 Trends in Potassium Parameters.

Shown are all values in the K parameters extracted from each fit and plotted vs number of segments. For most data sets $\bar{V}_n$ trended downwards, but this was not true for all data sets. $\bar{\alpha}_n$ has the most consistent increasing trend while no other Potassium parameter seemed to have a significant trend. Notable though is that if $\bar{\beta}_n$ started high it tended to increase or started low decrease while in the middle it stayed relatively consistent.
Overall, about half the 18 HH parameters studied seemed to hold semi-consistent trends. While every fitted parameter had at least one observed exception to any perceived connection to number of segments. The trend down in leak ion permeability, $\bar{\alpha}_m$, $K_\beta m$, and $\bar{V}_n$ coupled with the increases in $\alpha_m$, $\bar{V}_h$, and $\alpha_n$ seem to be the main contributors in reducing the observed effects widening and shortening of the AP as segments are added. This is not to say that one (many or all) of the other 11 parameters are not tied into these changes, only that they did not seem to hold any definite trends. This semi chaotic parameter space coupled with the existence of the W12 and W18 data parameter sets (both have similarly elevated $\alpha_m$, $\beta_m$, $k_\beta h$, $\alpha_h$, Potassium permeability, $\bar{V}_n$, and $k_\beta n$ with decreased $\bar{V}_h$ and $\beta_h$) or the J03 data set (low $\bar{V}_n$ and $\alpha_n$ with elevated $k_\alpha n$) strongly seems to suggest the existence of multiple feasible parameter sets.

**Can a Single Parameter Set Work?**

Upon noticing that experimental AP shapes were very similar, yet in the fits to these experiments the membrane and channel parameter values varied by factors of 2 or more, the idea different parameter sets can produce the same AP shape was raised. One way to study this was to apply a parameter set fit to one experiment to each of the other experimental data traces and see how much the quality of the fit was affected. Before any swapping of parameters can occur, it is best to familiarize ourselves with the different idiosyncrasies of each data set. This is visualized below in Figure 22 in which end timing of all current pulses where synced and all data sets were then overlayed and Figure 23 in which the peak AP timings are matched.
**Figure 22 A Comparison of all Data Sets Modeled.**

Top shows all 8 data sets utilized in these studies, the timing of end of the initial voltage pulse is matched. Bottom left shows just the protocol 2 data sets and bottom right just those produced by protocol 1. All hold similar shape but have slight variance in resting potential, timing, and peak voltage.
Figure 23 A Comparison of all Data Sets Modeled With matching AP Peak Timing.

All Eight data sets overlayed to better illustrate differences between them when ignoring timing differences. Mainly notable is the variance in peek potential and resting potential. J03 additionally has an unusual bulge in its return to rest.

Just by observing, it is obvious that the J16 data set peaks significantly higher than any other while the W03 data set peaks significantly lower than any other. Additionally, the initial resting potentials have some variance. The slight timing differences when the stim currents are lined up as in Figure 22 are extremely prominent, but they do not seem to be related to the amount of current applied. If these timing differences are overlooked (Figure 23) the overall AP shape in each data set seems to hold little variance other than slight differences in the
resting/peak potentials. The only notable exception being J03 that does not return to rest as smoothly. Protocol 1 data sets also exhibit much more prominent initial voltage spikes, but this seems to hold little effect on the actual AP. Numerically the resting potential, maximal rising slope, maximal falling slope, half height width, AP peak potential, and peak delay form the end of the current pulse are recorded below in Table 6.

Table 6: Data set Features

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Peak Voltage (mV)</th>
<th>Peak Time Delay (ms)</th>
<th>Rest Potential (mV)</th>
<th>Maximal Rising Slope (mV/ms)</th>
<th>Maximal Falling Slope (mV/ms)</th>
<th>Half Height Width (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W07</td>
<td>33.07027</td>
<td>0.524966</td>
<td>-82.831</td>
<td>585.2143</td>
<td>-185.068</td>
<td>0.927848</td>
</tr>
<tr>
<td>W12</td>
<td>35.39792</td>
<td>0.817971</td>
<td>-85.1443</td>
<td>550.2015</td>
<td>-155.057</td>
<td>1.049933</td>
</tr>
<tr>
<td>W18</td>
<td>36.74266</td>
<td>0.817971</td>
<td>-87.5856</td>
<td>525.1923</td>
<td>-150.055</td>
<td>1.086558</td>
</tr>
<tr>
<td>W03</td>
<td>22.88454</td>
<td>0.891222</td>
<td>-82.7579</td>
<td>395.1447</td>
<td>-135.049</td>
<td>1.098767</td>
</tr>
<tr>
<td>J16</td>
<td>49.49951</td>
<td>1.056</td>
<td>-88.501</td>
<td>560.1247</td>
<td>-176.748</td>
<td>0.864</td>
</tr>
<tr>
<td>J14</td>
<td>39.12354</td>
<td>1.536</td>
<td>-84.5032</td>
<td>530.5608</td>
<td>-157.356</td>
<td>0.912</td>
</tr>
<tr>
<td>J18</td>
<td>33.08105</td>
<td>0.72</td>
<td>-86.0138</td>
<td>360.6478</td>
<td>-128.269</td>
<td>1.104</td>
</tr>
<tr>
<td>J03</td>
<td>39.00146</td>
<td>0.768</td>
<td>-86.2122</td>
<td>407.5368</td>
<td>-112.693</td>
<td>1.248</td>
</tr>
<tr>
<td>Average</td>
<td>36.1±7.5</td>
<td>.89±.3</td>
<td>-85.4±2.1</td>
<td>489.3±87</td>
<td>-150.0±24</td>
<td>1.03±.13</td>
</tr>
</tbody>
</table>

As was seen visually there is slight variance in delay, peak height, and resting potential, but variance in the maximal slopes half height width that are not prominent in the figures can also be observed. The W03 data set is one clear outlier, with significantly lower peak potential
which also seems to lower the maximal slopes. This data set also holds the highest resting potential. Even with these oddities it holds nearly average delay and half height width. The J16 data set is the second most obvious outlier. This data set has the highest peak potential and lowest resting potential with the thinnest half height width. Oddly enough it does not hold the highest maximal slopes, they are however significantly higher than any other protocol 2 data set. The extra width that was observed in J03 due to the weird downslope can also be observed due to the low maximal downslope causing a high width. Otherwise, there are a few outliers in rising slope (J18 and W07) and falling slope (W07 and J03) and semi-significant variance in timing.

Seeing that there are definite differences between the data sets it should be obvious that the well fit models will not fit any other data set anywhere near as well. But what is not clear is how these parameter sets might be able to fit another data set if the resting potentials and timing are matched to the new data set. To understand this, two short studies were completed, one simply shifting the AP timing by adjusting current and allowing the model to use the new data set for its initial guesses at the initial potential and ion flow. The second took the idea of setting the resting potential one step further by shifting the intracellular leak ion concentration to match the resting potential of the new data set.

When allowing the initial guess at the membrane potential to be set based off the new data set without adjusting the intracellular leak ion concentration while swapping all HH parameters, one thing stood out. The W12 and W18 parameter sets are unstable. This can be seen in the values of the objective function in Table 7. Rows in this table represent what channel and membrane parameters are used, while columns show what data set was used. The current was adjusted to match the AP timing visibly. The instability in W12 and W18 parameter sets are illustrated by the value of the objective function being 50002, the failure state indicating a failure
to return to rest (highlighted in orange on Table 7). When this value is obtained it means that there was not an actual AP that formed. In terms of the instability in the W12 and W18 data sets the model was not able to find a stable resting potential near the -85mV expected and instead found a much higher resting potential at approximately -35mV. When this happens in the model the cell is no longer able to form APs. An example of this coming from attempting to use the W18 parameter set on the W03 data set is shown in Figure 24.

Additionally, many swaps failed to match the initial resting potential or peak potential. Values of 50003 in Table 7 indicate one of these two features was over 30% off from the data. This mostly occurs with the W07, W03, and J16 parameter sets. The J16 data set holds a higher peak potential than normal while W03 holds a peak potential much below average, easily explaining this, but the W07 data set is close to average. It is unclear what causes the failure for the W07 parameter set.
Table 7: Objective Function Values for Swapping Parameter sets and Adjusting Current.

Columns define the data set used and the row defines what data set the parameter set was originally generated for.

<table>
<thead>
<tr>
<th>Data Set</th>
<th>W03</th>
<th>W07</th>
<th>W12</th>
<th>W18</th>
<th>J03</th>
<th>J14</th>
<th>J16</th>
<th>J18</th>
</tr>
</thead>
<tbody>
<tr>
<td>W03</td>
<td>11.975</td>
<td>135.3</td>
<td>252.3</td>
<td>50003</td>
<td>101.375</td>
<td>21.75</td>
<td>50003</td>
<td>22.3125</td>
</tr>
<tr>
<td>W07</td>
<td>173.15</td>
<td>6.45</td>
<td>196.25</td>
<td>50003</td>
<td>50003</td>
<td>20.475</td>
<td>50003</td>
<td>50003</td>
</tr>
<tr>
<td>W12</td>
<td>50002</td>
<td>50002</td>
<td>19.275</td>
<td>40.275</td>
<td>16.325</td>
<td>8.325</td>
<td>11.6</td>
<td>6.5</td>
</tr>
<tr>
<td>W18</td>
<td>50002</td>
<td>50002</td>
<td>50002</td>
<td>11.8</td>
<td>50002</td>
<td>50002</td>
<td>5.68125</td>
<td>50002</td>
</tr>
<tr>
<td>J03</td>
<td>1984.125</td>
<td>305.6</td>
<td>363.675</td>
<td>2.075</td>
<td>31.0875</td>
<td>105.3</td>
<td>7.225</td>
<td></td>
</tr>
<tr>
<td>J14</td>
<td>120.9375</td>
<td>27.925</td>
<td>51.5625</td>
<td>17.7</td>
<td>0.675</td>
<td>50003</td>
<td>4.8375</td>
<td></td>
</tr>
<tr>
<td>J16</td>
<td>50003</td>
<td>50003</td>
<td>260.375</td>
<td>89.7</td>
<td>63.975</td>
<td>50003</td>
<td>2.05</td>
<td>47.5</td>
</tr>
<tr>
<td>J18</td>
<td>498.875</td>
<td>482.4</td>
<td>83.35</td>
<td>129.6</td>
<td>5.7</td>
<td>12.15</td>
<td>36.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

63
Figure 24 W18 parameters used with the W03 initial guess at the membrane potential.

This higher guess at the resting potential leads to a secondary resting potential to be found at approximately -35mV that is not conducive the formation of APs.

As expected, in the cases where APs were able to form none had as good as a fit as the parameter set found for that specific data set. It is very notable however, that no parameter set found utilizing a protocol 2 data set was able to closely fit any of protocol 1 data sets. Even the other parameter sets found with protocol 1 data sets struggle to be considered reasonable fits on the rest of the protocol 1 data sets. The only notably good fit on a data set from protocol 1, not specifically tailored to that data set is when using semi-unstable W12 parameter set on the W18
data set which is also seen to be unstable. However, the opposite swap fails to produce an AP. This might suggest that the W18 parameter set is very susceptible to any shifts in resting potential.

The only parameter sets that seemed to perform reasonably on any data set are those from J14 and J18, with the J16 parameter set not preforming too much worse. However, the J16 parameter set consistently had a much sharper fall back to rest than any other parameter set, with a very thin half height width and higher maximum potential. All observations are to be expected based off the values Table 6. Similarly, the J18 parameter set struggled to reach the same peak AP potential, only exceeding the potential when matched to W03 data set which as previously discussed is anonymously short. The APs produced with the J14 parameter set seemed to be able to fit all other data sets much better than any other.
Figure 25 An example of each parameter set graphed over the W12 data set.

W18 is used as it is the closest to the average data set. Top left J03 parameter set, notably wider in all cases than any other. Top right J14 parameter set. Middle Left J16, typically overshooting by a significant margin with a sharper downslope. Middle right J18, typically slightly short with a slower downslope but still a close fit. Bottom Left W03, always appearing short with a
significantly higher resting potential. Bottom right W07, tending to be slightly small with a blockier and steep downslope.

The secondary parameter swapping study in which intracellular leak ion concentration ($L_i$) was additionally allowed to vary holds similar observations as when $L_i$ was allowed to vary. However, shifting $L_i$ did allow for the formation of APs in cases that previously lead to the secondary much higher resting potential. In these cases, however the resting potential of the data set was not able to be obtained in the model. $L_i$ had to be increased to force the resting potential lower. For certain parameter sets when increasing $L_i$ as to match the resting potential a threshold at which spontaneous APs would form would be reached before matching the resting potential. In Table 8 the cases when the resting potential was unable to be matched are highlighted in yellow or red, red indicated full failure the resting potential being over 30% off making the objective function mark it as a failure, dark yellow are the extreme cases, that still passed, the resting potential $>2$ mV from ideal, while lightly shaded yellow is for cases with difference in resting potential $<2$ mV. In all cases if the $L_i$ was reduced to be able to increase the simulated resting potential the simulation either found the secondary higher resting potential or formed spontaneous APs (not needing any additional current).

All cases in which the resting potential was not able to be matched either occurred when trying to fit the W03 or W07 data sets or when using the W18 parameter set. Both W03 and W07 have unusually high resting potential, slightly higher than $-83$ mV, as opposed to the near $-86$ mV held by all other used data sets. Most parameter sets do not allow for potentials to reach this high without beginning to activate voltage gated ion channels leading to spontaneous firing or the secondary steady state resting potential. Only the data set that did not have high resting potential
yet was able to be adjusted up to the -82.8mV seen in W03 and W07 is J03. The J03 parameter set was also noted to be the only one with elevated k_{an} and reduced V_n and \alpha_n. Perhaps these shifts in the Potassium parameters allow for more variance in resting potential to not cause problems. However, neither W03 nor W07 seemed to exhibit any obvious difference in the HH parameters from average.

Ignoring the W03 and W07, the J14 parameter set performed very well on every data set. This matches the observation found when L_i was not allowed to shift. The J16 and J18 data sets also performed similarly well producing the same defects in AP height and falling potential. However, the W12 data set also seemed to perform similarly well. The W12 parameter set produced more pointy looking APs, having more sudden changes from upslope into downslope.
Table 8: Objective Function Values for Swapping Parameter sets, Adjusting Current and Intracellular Leak Ion Concentration.

Columns define the data set used and the row defines what data set the parameter set was originally generated for.

<table>
<thead>
<tr>
<th>Data Set</th>
<th>W03</th>
<th>W07</th>
<th>W12</th>
<th>W18</th>
<th>J03</th>
<th>J14</th>
<th>J16</th>
<th>J18</th>
</tr>
</thead>
<tbody>
<tr>
<td>W03</td>
<td>11.3</td>
<td>132.5625</td>
<td>175.5</td>
<td>231.95</td>
<td>36.35</td>
<td>14.25</td>
<td>31.65</td>
<td>11.025</td>
</tr>
<tr>
<td>W07</td>
<td>164.45</td>
<td>8.625</td>
<td>69.475</td>
<td>154.875</td>
<td>30.4</td>
<td>9.375</td>
<td>7.8</td>
<td>15.3</td>
</tr>
<tr>
<td>W12</td>
<td>211.5625</td>
<td>552.75</td>
<td>19.275</td>
<td>26.65</td>
<td>15</td>
<td>10.05</td>
<td>4.4625</td>
<td>6.775</td>
</tr>
<tr>
<td>W18</td>
<td>50003</td>
<td>50003</td>
<td>103.2188</td>
<td>11.8</td>
<td>15.7125</td>
<td>50003</td>
<td>4.0125</td>
<td>7.425</td>
</tr>
<tr>
<td>J03</td>
<td>974.875</td>
<td>581.275</td>
<td>314.075</td>
<td>257.15</td>
<td>2.5</td>
<td>17.85</td>
<td>56</td>
<td>8.05</td>
</tr>
<tr>
<td>J14</td>
<td>117.4688</td>
<td>146.7</td>
<td>27.775</td>
<td>21.3</td>
<td>12.575</td>
<td>0.825</td>
<td>8.5</td>
<td>2.8</td>
</tr>
<tr>
<td>J16</td>
<td>333.725</td>
<td>67.1</td>
<td>120.75</td>
<td>90.2625</td>
<td>33.0375</td>
<td>5.5875</td>
<td>1.625</td>
<td>24.05</td>
</tr>
<tr>
<td>J18</td>
<td>305.55</td>
<td>205.35</td>
<td>74.675</td>
<td>97.575</td>
<td>5.15</td>
<td>4.225</td>
<td>20.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Figure 26 Protocol 2 parameter sets graphed vs W18 when $L_i$ is allowed to shift.

Top left J03 parameter set, notably wider in all cases than any other. Top right J14 parameter set, the best fit to most. Bottom left J16, typically overshooting by a significant margin with a sharper downslope. Bottom right J18, typically slightly short with a slower downslope but still a close fit.

Overall, each data set is unique with different slopes, maximal potentials, resting potentials, timing, and half height widths. The differences observed in the HH parameter sets can be attributed to these differences in AP shape. However, this does not rule out the possibility of multiple stable parameters sets that could achieve reasonable fits. The J14, J16, J18, and W12 parameter sets all performed reasonably with different data sets yet the HH parameters of each far from match. Additionally resting potential seems to play a heavy role in viability of a
parameter set. If cell could have its resting potential elevated to near -83mV without leading to spontaneous firing the W12 or W18 parameter sets would need to be completely scrapped, and even the J14, J16, and J18 parameter sets would start to have issues.

Figure 27 Protocol 1 data sets graphed against W18 when $L_i$ is allowed to shift.

W12 is used in the case of the W18 parameter set. Top right W03, significantly short in all cases. Top left W07, appears “small” with sharper downslope. Bottom Left W12, slightly too tall in most cases with sharper than normal transition in the down slope. Bottom right, the W18 fit.

Confidence Interval

As shown in the previous sections, although the parameter sets seem to matter, especially when near instability the other parameter sets where not terrible fits in all cases. There exists
multiple points in parameter space for which the AP of interest can be arrived at. In order to test the flexibility of parameter space, the idea is to have each parameter scanned over, and all other parameters allowed to be fit until the upper and lower limits of each parameter that still allow for good fits are found.

**Constraints and Considerations**

This study was completed utilizing the J18 data set due to it having near average features: peak height, resting potential, half height width, delay, and slopes, while having the best fit out of all data sets. This does however somewhat limit the scope of this study; it would be interesting to later continue and run the confidence interval testing on other data sets to see if the observed relations hold true. Additionally, the cell studied was chosen to be set to 2mm long and have 16 longitudinal segments, the fit for this exact cell was utilized as the starting point of all simulations in this section. This length/number of segments should allow for a realistic simulation of longer cells without being too computationally intensive as to be impossible. As discussed in the previous section some residual of the anomaly is likely still present in these fits due to the segment lengths not being infinitesimal, but this effect should be minimal.

All simulations for sections were ran on the GLUON HPC cluster at Wright State allowing for up to 16 optimizations to be run in parallel on the same node. Up to 10 nodes were utilized at a time for the purpose for this study (at maximum 160 optimizations were ran at once). Each attempt on a node took between 5 hours and 36 hours, most of which took on the order of 15 hours to run depending on how many needed optimizations runs for the parameter. The total computing time easily exceeded 500 hours on the cluster. Considering the significant time investment, it was deemed infeasible to study more than one cell.
Additionally due to the choice of utilizing surrogateopt as the optimization method, multiple runs for every simulation were deemed necessary. Surrogateopt utilizes semi-random sampling of the parameter space to construct the initial surrogate function. When the initial guess was particularly bad the algorithm would struggle to find a passable fit. To combat this, the number of initial guesses were increased to 100 in order to construct a more robust initial surrogate function. This was not enough, however, in some cases the first attempt at fitting arrived at nothing, but a secondary attempt would achieve a success. Taking this into account, every fit was ran at least 5 times, and for regions in parameter space in which the initial guess was particularly bad, fits would be ran up to 25 times. Often a secondary run with more repeats would be ran if any anomalous increases in the objective function were observed in the scan over the parameter of interest.

**Confidence Interval Methodology**

As already mentioned, all optimizations completed in order to determine the confidence interval are done on the J18 data set using 16 segments on a 2mm long cell. The HH parameters from this fit are shown below in Table 9. Starting from these parameters, one is chosen to be fixed and all others are set to be allowed to be fit. The initial value of the fixed parameter is saved before shifting its value by some percentage before allowing for all other parameters to be fit.
Table 9: All HH parameters fit from J18 16 segment fit of a 2mm long cell.

The starting point of all optimizations in this section.

<table>
<thead>
<tr>
<th>Na Activation Parameters</th>
<th>Na Inactivation Parameters</th>
<th>K Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability (µm/s)</td>
<td>$V_h$ (mV)</td>
<td>Permeability (µm/s)</td>
</tr>
<tr>
<td>0.001646</td>
<td>-39.194</td>
<td>0.000252</td>
</tr>
<tr>
<td>$V_m$ (mV)</td>
<td>$K_\alpha h$ (mV)</td>
<td>$V_n$ (mV)</td>
</tr>
<tr>
<td>-43.505</td>
<td>24.438</td>
<td>-35.973</td>
</tr>
<tr>
<td>$K_\alpha m$ (mV)</td>
<td>$K_\beta h$ (mV)</td>
<td>$K_\alpha n$ (mV)</td>
</tr>
<tr>
<td>8.952</td>
<td>3.461</td>
<td>5.3468</td>
</tr>
<tr>
<td>$K_\beta m$ (mV)</td>
<td>$\bar{\alpha}_h$ (1/s)</td>
<td>$K_\beta n$ (mV)</td>
</tr>
<tr>
<td>29.109</td>
<td>0.002923</td>
<td>23.318</td>
</tr>
<tr>
<td>$\bar{\alpha}_m$ 1/(ms mV)</td>
<td>$\bar{\beta}_h$ (1/ms)</td>
<td>$\bar{\alpha}_n$ 1/(s mV)</td>
</tr>
<tr>
<td>0.2709</td>
<td>11.629</td>
<td>0.01673</td>
</tr>
<tr>
<td>$\bar{\beta}_m$ (1/ms)</td>
<td></td>
<td>$\bar{\beta}_n$ (1/s)</td>
</tr>
<tr>
<td>1.1614</td>
<td></td>
<td>0.09550</td>
</tr>
</tbody>
</table>

In all cases the initial scan over the parameter space was done by decreasing the parameter of interest down 10% all the way to 20% of its initial value and by increasing from the initial state by 20% up to three times the initial value. For each new value of the parameter of interest five 500 iteration long optimizations using 100 initial surrogate points are ran using surrogateopt minimizing the predefined objective function. The fit with the lowest value of the objective function for each percentage of the parameter of interest is saved. These values of the objective function would then be plotted vs the %value of the parameter of interest. The region would then be expanded or refined as needed before running a new set of simulations.
This initial sweep allowed us to see the beginnings of the pattern for each parameter set. Many needed to have the region expanded until a failure to achieve an adequate fit is found. While in other cases an obvious region seems to appear, but the edges of this region needed smaller shifts to gain more information. The third situation found was that there did not appear to be a particular pattern. In these cases, significantly more iterative fits were run in order to attempt to tease out a pattern. This seeming incoherence only occurred when small shifts in the parameter would lead to a failure state using the initial values causing the surrogateopt algorithm to struggle.

**Confidence Interval Results**

After continuously running fits for each parameter, expanding the bounds and refining the edges, three main patterns emerged. The most common pattern is that of reasonably well-defined bounds. In this case, between two states of the parameter of interest a good fit was able to be found, but outside of this region the objective function was massively higher, or always resulted in a failure state. The second most common pattern found is the lack of a distinct pattern. In most cases the seemed to be multiple regions in which the parameter of interest might fall and still result in a reasonable fit, but these regions did not seem to correspond to a clear pattern. Lastly a couple of the parameters were found to be pretty much free variables, changing their value had little to no effect on the objective function after refitting. In certain cases, the value could be scaled over 100 times its stating state with no real effect.
**Na Activation Parameters**

The voltage gated activation of the sodium channels is controlled by the permeability of the membrane to Sodium and the 5 HH Na activation parameters: $\tilde{V}_m$, $k_\alpha m$, $k_\beta m$, $\bar{\alpha}_m$, and $\bar{\beta}_m$. In combination these parameters largely influence the initial response of the cell to a stimulus, mainly influencing the up-slope and maximal height of the AP. When scanning through these parameters they were in general found to have larger impact on the goodness of fit as defined in the objective function than Na inactivation of Potassium parameters. For many of the Sodium activation parameters multiple distinct regions of acceptable fit were found with small regions of failure or bad fits between them. It is unclear if these in-between regions where no good fit was found are impossible to have good fits or if with more iterations a reasonable fit might be found. An additional thing to note is that the fitting algorithm struggled to fit the sodium activation parameters after even small shifts and in most cases upwards of 15 repeated attempts at fitting were done, in many cases only one would lead to a reasonable fit.

**Na Permeability**

Sodium permeability was found to have four distinct regions where fits are possible. These can be seen below in Figure 28. Between 60% and 170% of the initial permeability, 1.646E-3 cm/s, the best fits were found. In this region the objective function varied from .2 to .45 suggesting near perfect fits. Interestingly enough, all other parameters in region stayed near that of their initial value (refer to Figure 29 and Figure 30). Nothing seemed to need to be done to compensate for these shifts in permeability.

As the permeability increases up from 170% up to 260% of the initial state a second region of reasonable fits are obtained. The fits themselves are quite a bit worse resulting in the
objective function valuing approximately 1. These fits can still be considered quite good but no longer the near perfection of the first region. Unlike in the first region a significant shift in almost every other parameter was observed. Only $\overline{V_h}$, $\overline{\alpha_m}$, and $\overline{\beta_h}$ remain consistent with their initial values while $k_o m$, $K$ permeability, and $\overline{\alpha_n}$ distinctly dropped. All other HH parameters jumped significantly, an example of this can be seen in the shift of $\overline{\beta_m}$ seen below as Figure 30. Despite the sudden shift all parameters seemed to remain relatively constant exhibiting no visible trend as the Sodium permeability continues to increase.

Once the Sodium permeability exceeded 260% chaos seemingly began to reign until 350%. In this region fits can still be found but extreme trouble was observed in arriving at good fits. This resulted in both the fit goodness and all HH parameters varying wildly. It is entirely possible that fits found in this region could be improved upon, but after running 40 iterative attempts at improving each fit in this region this was deemed infeasible.

After 350% sodium permeability no fit was obtained. In each attempt no matter how the rest of the HH parameters varied the objective function always hit a failure state. Similarly, when above 40% and below 60% and again below 25% all attempted fits failed. It is not unexpected to find hard cut offs but observing a secondary region where the Sodium Permeability is between 25% and 40% where quite reasonable fits (objective function order 1) are able to be found again was unexpected. In this tiny region particular patterns in the HH parameters are hard to observe, but most are on the very low end. Only $\overline{V_m}$, $\overline{V_h}$, and $\overline{\alpha_n}$ seemed to be elevated over their averages.

From observing the effects of shifting the Sodium permeability it would be advisable to remain between $9.87E-4$ cm/s (60%) and $2.8E-3$ cm/s (170%) but a second distinct set of parameters exists when the permeability is between $3.0e-3$ cm/s (180%) and $4.4e-3$ cm/s (270%).
Additionally small shifts in sodium permeability within either of these regions do not seem to need to be highly compensated by any other parameter. The other two regions suggest two other stable states, but neither held as distinctive of a shift in any other HH parameter or was as stable.

![Graph of objective function vs percent shift in Na permeability.](image)

**Figure 28 Values of the objective function graphed vs percent of the shift in Na Permeability.**

Four distinct regions where fits could be found can be seen. Between 60% and 170% the best fits can be found. Both between 25% to 40% and 180% to 260% result in reasonable fits (objective on order 1). Between 270% and 350% fits where possible but generally worse and harder to obtain. Outside of these regions all attempted fits failed.
Figure 29 A heatmap of each HH parameter as Sodium Permeability was scanned over.

Each are normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a fail state is set to NaN (grey), darker blue is a worse fit.
Figure 30 The values of $\bar{\beta}_m$ vs Sodium Permeability.

Each is set as a percent of their initial state. This shows the two distinct regions in which most parameter remained constant, between 60% to 170% and 180% to 270%. Outside these regions no obvious trends are observed.

Details of confidence interval results for all other parameters can be found in Appendix A.

**Confidence Interval Summary**

Scanning over each HH parameter was found to produce unique patterns in the objective function values. These fell into three main categories, a well-defined interval ($\bar{\alpha}_h, \bar{\beta}_h, k_{\beta h}, \bar{V}_n, k_{\alpha n}, \bar{\alpha}_n, \bar{\beta}_n$, and Na permeability), no upper bound ($k_{\alpha h}, k_{\beta n}, k_{\beta m}$), and multiple regions with seeming chaotic interactions between (K permeability, $\bar{V}_h, k_{\alpha m}, \bar{\beta}_m, \bar{\alpha}_m, \bar{V}_m$). In each case where
there seemed to be multiple regions one, normally containing the initial state of that parameter, resulted in better fits. The region in which the best fits were found while scanning over every parameter can be seen below in Table 10.

The secondary goal of this experiment, to identify links between parameters, was much less successful. Surprisingly few scans reviled any clear visual connections between the parameters themselves and many relations observed in one scan where not matched by scan of the linked parameter. The only reciprocal relation observed is that when $V_n$ increases $\alpha_n$ seems to decrease. Eight other links where observed, each is recorded below in Table 10, but without observing the same relation in the paired parameter leaves some doubt on if they are truly linked. To improve the identification of interlinked parameters these are also investigated mathematically via the correlations.

Table 10: Summary table of each confidence region and Links between parameters.

Positive links suggest that both parameters increase together while negative suggest increasing one reduces the other to achieve comparable fits.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suggested Interval</th>
<th>Interval %</th>
<th>Suggested Positive Links</th>
<th>Suggested Negative Links</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na permeability</td>
<td>9.87E-4 to 2.8E-3cm/s</td>
<td>-40%, +70%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_m$</td>
<td>-42.42 to -58.70mV</td>
<td>-2.5%, +35%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_m$</td>
<td>0.2167 to 0.3251 1/(ms*mV)</td>
<td>-20%, +20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{\beta}_m )</td>
<td>0.5227 to 1.568 1/( ms )</td>
<td>-55%, +35%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>( k_{\alpha m} )</td>
<td>2.24 to 25.06mV</td>
<td>-75%, +10%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( k_{\beta m} )</td>
<td>5.822mV or above</td>
<td>-80% or above</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( \bar{V}_h )</td>
<td>-19.60 to -54.87mV</td>
<td>-50%, +40%</td>
<td>( k_{\beta h} )</td>
<td></td>
</tr>
<tr>
<td>( \bar{\alpha}_h )</td>
<td>0 to .0088 1/( ms )</td>
<td>-100%, +200%</td>
<td>Na Permeability</td>
<td></td>
</tr>
<tr>
<td>( \bar{\beta}_h )</td>
<td>2.326 to 13.955 1/( ms )</td>
<td>-80%, +20%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( K_{\alpha h} )</td>
<td>9.77 mV or above</td>
<td>-60% or above</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( K_{\beta h} )</td>
<td>0.0087 to 6.75 mV</td>
<td>-99.75%, +95%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>K permeability</td>
<td>1.26e-4 to .0022 cm/s or above</td>
<td>-80%, +775%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( \bar{V}_n )</td>
<td>-19.79 to -88.14mV</td>
<td>-45%, +145%</td>
<td>( \bar{V}_m )</td>
<td></td>
</tr>
<tr>
<td>( \bar{\alpha}_n )</td>
<td>0.0134 to 0.0335 1/(( ms \times mV ))</td>
<td>-20%, +100%</td>
<td>( \bar{V}_n )</td>
<td></td>
</tr>
</tbody>
</table>

| \( \alpha \) | --- | --- | --- |
|---|---|---|
| \( \beta \) | --- | --- | --- |
| \( V \) | --- | --- | --- |
Parameter Correlations

After identifying the intervals of best fit, the interconnection between parameters could be further studied. Each confidence interval test generated a data set which describes how the other 16 parameters must shift to still result in good fits. Using this data within the ranges described by the identified confidence intervals (as described in Table 10) a table of correlation coefficients between the best fit values of each parameter was generated. This can be seen below as Figure 31 Heatmap of inter-parameter correlations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Interval</th>
<th>Correlation 1</th>
<th>Correlation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{\beta}_n$</td>
<td>1e-3 to 0.2319 1/ms</td>
<td>-90%, +155%</td>
<td>$k_{am}$ and $k_{bn}$</td>
</tr>
<tr>
<td>$k_{an}$</td>
<td>0 to 42.78 mV</td>
<td>-100%, +700%</td>
<td></td>
</tr>
<tr>
<td>$k_{bn}$</td>
<td>10.49mV or above</td>
<td>-55% or above</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 31 Heatmap of inter-parameter correlations**

The darker green a cell the higher the correlation coefficient suggesting positive linkage. Similarly, the darker red a cell the more negative the correlation coefficient suggesting negative linkage between parameters. Rows signify that parameter was scanned over in the confidence interval test while columns are what parameter from the given data set is looked at.
To make a claim that two parameters are linked the same impact must be seen when scanning over both. If a parameter seems to be linked to the scanned parameter before linkage can be claimed the same observation would have to be made when scanning through its pair. For example, when scanning over $\alpha_m$ a strong positive correlation to $k_{\beta m}$ can be seen ($p = .814$) while if instead scanning over $k_{\beta m}$ and looking for a correlation with $\alpha_m$ only a very weak negative correlation ($p = -.134$) can be seen. These do not agree and therefore a linkage between the two parameters cannot be claimed. It could however be worth future investigation with more parameters determined through other methods. To reflect this only relatively strong, paired correlations ($|p| >= .5$) have any claims made about them. The choice of $|p| >= .5$ as the cutoff was made to avoid the situation in which a single outlying data point would suggest a correlation. In future studies using larger samples of data point within the bounds of the confidence intervals this problem should disappear and allow for a smaller cutoff correlation to be chosen.

A total of 8 paired correlations can be observed through Figure 31. They are as follows:

1. Na Permeability is inversely linked with $\alpha_m$
2. $\bar{V}_n$ is positively linked with $\alpha_n$
3. Na Permeability is positively linked with $\bar{\beta}_h$
4. $k_{a_m}$ is inversely linked with $k_{\beta m}$
5. $k_{\beta h}$ is inversely linked with $\bar{\beta}_n$
6. K Permeability is inversely linked with $\alpha_n$
7. $\bar{V}_n$ is inversely linked with $\bar{\beta}_n$ (positively in terms of %)
8. $k_{a n}$ is inversely linked with $\alpha_n$
Further study will need to be done to confirm these relations and the usage of them but as of now these 8 pairs have the highest likelihood of compensating each other. Interestingly, $\alpha_n$ is found to be correlated with three separate parameters. If this is confirmed it would be worth it to determine the value of $\alpha_n$ via separate methods and avoid fitting it. However, it was also found to have a relatively clear confidence interval and will still be used in our further studies.

In addition to the pairwise correlations the very strong but only single directionally observed correlations should be mentioned. In this case the cutoff coefficient is defined as $|p|>=.75$. These situations have extremely clear links on one scan but are not observed in a scan of the paired parameter. This could be a real phenomenon that compensating for shifts in one parameter can be partially done with a second, but when trying to do the opposite issues due to the rest of the parameter’s behavior prevent easy compensation. It could also be a correlation to a non-scanned parameter or simply be an artifact of the fitting process. Whatever the case any future study looking into the interconnectivity of each parameter should focus on the interlink between $\alpha_m$ with $k_\beta m$, $\beta_m$ with $k_\beta m$, $k_n$ with $\beta_m$, and Na permeability with K permeability in addition to the 8 reciprocal relations.

*Fitting Post Confidence Intervals*

Through the confidence interval testing strong evidence of what the bounds of each parameter is was found. Even more importantly some of the parameters lacked hard bounds or had extremely large intervals. Including these parameters is likely detrimental to identifying any trends in the more tightly bound parameters. The lack of any clear trends in parameter spaces can be linked to the parameter space being overdetermined. Considering this a second round of fitting while increasing the number of longitudinal segments was completed. The goal being the
same, to identify how each parameter must shift from the single segment model into the more realistic multi-segmented model. This time however the problem of AP shape should be much less over defined.

From the confidence interval testing $k_{\beta m}$, $k_{\alpha h}$, and $k_{\beta n}$ leaving them the obvious candidates to exclude. Additionally, $k_{\beta h}$, $K_{\alpha n}$, $K$ permeability, and $\bar{\alpha}_K$ each have very large confidence intervals and were deemed unnecessary to fit. This cut the total number of fit parameters down from 18 to 11, significantly limiting parameter space. Each of these seven parameters were chosen to be set from the previous best fit values in the 16-segment fit of the J18 data set (as used in the confidence interval testing). The initial states of all other parameters were set from the same data set.

\textit{Table 11 Trends in Permeabilities within confidence interval bounds}

<table>
<thead>
<tr>
<th>Segments</th>
<th>Nav permeability (cm/s)</th>
<th>Standard deviation (cm/s)</th>
<th>Leak Permeability (cm/s)</th>
<th>Standard deviation (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.001425</td>
<td>0.000264</td>
<td>6.19E-06</td>
<td>4.71E-07</td>
</tr>
<tr>
<td>4</td>
<td>0.001431</td>
<td>0.000265</td>
<td>6.06E-06</td>
<td>4.78E-07</td>
</tr>
<tr>
<td>8</td>
<td>0.001384</td>
<td>0.000307</td>
<td>5.78E-06</td>
<td>5.26E-07</td>
</tr>
<tr>
<td>12</td>
<td>0.00136</td>
<td>0.000387</td>
<td>5.54E-06</td>
<td>5.98E-07</td>
</tr>
<tr>
<td>16</td>
<td>0.001365</td>
<td>0.000373</td>
<td>5.21E-06</td>
<td>5.93E-07</td>
</tr>
<tr>
<td>24</td>
<td>0.001358</td>
<td>0.000356</td>
<td>4.86E-06</td>
<td>6.78E-07</td>
</tr>
<tr>
<td>32</td>
<td>0.001427</td>
<td>0.000371</td>
<td>4.38E-06</td>
<td>6.08E-07</td>
</tr>
</tbody>
</table>
Figure 32 Graphed trends in permeabilities within confidence interval bounds

Leak permeability (bottom) is shown to decrease with added segments while Na Permeability (top) holds no clear trend.

As in the pervious fitting study the Leak permeability was fit to the hyperpolarizing pulse. This led to the same observed trend in leak permeability, as more segments are added the leak permeability decreases. Interestingly even though the visual changes in the AP shape reduce when adding segments above 16 the leak permeability seemed to steadily decrease, seemingly irrespective of number of segments, in order to obtain the same curvature in the hyperpolarizing pulse. At some point this would likely slow down, most likely around 64 segments where previous testing of the effects of adding segments seemed to stop affecting the AP shape. This
should be tested but was deemed largely infeasible due to time constraints on fitting making even a 32-segment fit take the better part of a day to achieve.

Sodium permeability showed no such trend. It varies more than in the earlier testing but in both cases remained semi-constant and lacked any upward/downward trends. This lack of any clear trend is matched by all four of the fitted sodium activation parameters. If looking at the average values of the four activation parameters (shown in Table 12) there is a possible slight decreasing trend in $V_m$ which would match the slight trend previously observed. This however seems to be the product of three of the data sets with significant downward trends in $V_m$.

*Table 12 Trends in Na Activation parameters within confidence interval bounds*

<table>
<thead>
<tr>
<th>Segments</th>
<th>$V_m$ (mV)</th>
<th>$k_a m$ (mV)</th>
<th>$\alpha_m$ (1/(ms*mV))</th>
<th>$\beta_m$ (1/ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-38.97 ± 3.2</td>
<td>10.15 ± 1.1</td>
<td>0.268 ± 0.013</td>
<td>1.075 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>-38.67 ± 3.0</td>
<td>10.20 ± 1.1</td>
<td>0.259 ± 0.013</td>
<td>1.075 ± 0.10</td>
</tr>
<tr>
<td>8</td>
<td>-39.05 ± 3.1</td>
<td>10.05 ± 1.2</td>
<td>0.262 ± 0.013</td>
<td>1.097 ± 0.07</td>
</tr>
<tr>
<td>12</td>
<td>-39.90 ± 4.4</td>
<td>9.91 ± 1.1</td>
<td>0.267 ± 0.015</td>
<td>1.124 ± 0.17</td>
</tr>
<tr>
<td>16</td>
<td>-39.67 ± 5.0</td>
<td>9.65 ± 1.3</td>
<td>0.250 ± 0.020</td>
<td>1.096 ± 0.17</td>
</tr>
<tr>
<td>24</td>
<td>-40.13 ± 4.7</td>
<td>9.50 ± 1.3</td>
<td>0.250 ± 0.021</td>
<td>1.085 ± 0.20</td>
</tr>
<tr>
<td>32</td>
<td>-40.22 ± 6.0</td>
<td>9.67 ± 1.3</td>
<td>0.252 ± 0.022</td>
<td>1.111 ± 0.23</td>
</tr>
</tbody>
</table>
Figure 33 Graphed trends in Na Activation parameters within confidence interval bounds

Shown are the four fitted Na activation parameters, shown are $V_m$, $k_m$, $\beta_m$, and $\alpha_m$ in clockwise order from top left. No clear trends can be observed in any of the four activation parameters.

The sodium inactivation parameters were much better behaved. In fact, the changes in $V_h$ seems to match with how the AP shape changes almost perfectly. $V_h$ increases but it does not increase ceaselessly, somewhere between 12 and 16 segments the shifts in $V_h$ almost completely stop. $V_h$ has strong effects on the AP peak potential, reducing it is observed to increase AP peak potential. Given the observed trend of the AP shape to have a reduced peak potential with increased segments and the near stopping of this height change when passing 16 segments the observed changes in $V_h$ match perfectly. $\beta_h$ on the other hand, seems to remain close to constant
with a possible slight downward trend. The slight downward trend also matches expectations and would slightly increase the AP peak potential but the relative consistency of $\bar{\beta}_h$ suggests $V_h$ has a much more important role in this shift.

Table 13 Trends in Na Inactivation parameters within confidence interval bounds

<table>
<thead>
<tr>
<th>Segments</th>
<th>$\bar{V}_h$ (mV)</th>
<th>Standard deviation</th>
<th>$\bar{\beta}_h$ (1/ms)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-46.2833</td>
<td>3.085554</td>
<td>11.41381</td>
<td>2.336943</td>
</tr>
<tr>
<td>4</td>
<td>-45.9438</td>
<td>3.783249</td>
<td>11.29215</td>
<td>2.250352</td>
</tr>
<tr>
<td>8</td>
<td>-43.9923</td>
<td>2.266697</td>
<td>10.76228</td>
<td>1.762353</td>
</tr>
<tr>
<td>12</td>
<td>-35.8231</td>
<td>6.392385</td>
<td>10.14234</td>
<td>1.74182</td>
</tr>
<tr>
<td>16</td>
<td>-31.1712</td>
<td>8.843014</td>
<td>9.782153</td>
<td>2.267941</td>
</tr>
<tr>
<td>24</td>
<td>-30.6487</td>
<td>9.650665</td>
<td>9.393304</td>
<td>2.462574</td>
</tr>
<tr>
<td>32</td>
<td>-32.5454</td>
<td>10.78278</td>
<td>9.431613</td>
<td>2.686469</td>
</tr>
</tbody>
</table>
**Figure 34 Graphed trends in Na Inactivation parameters within confidence interval bounds**

\( \overline{V_h} \) (top) increases until around 16 longitudinal segments are included before leveling out while \( \overline{\beta_h} \) (bottom) remains close to constant with a possible slight downward trend.

Lastly the Potassium parameters also show slight trends but nothing as distinct as with the Leak permeability or \( \overline{V_h} \). The clearest trend is for \( \overline{V_n} \) which seem trends downward in five of the seven tested data sets. Both \( \overline{\alpha_n} \), and \( \overline{\beta_n} \) have less clear trends. \( \overline{\alpha_n} \) seems to decrease before increasing, a result completely unexpected in most likely an artifact of the AP still being over determined. On the other hand, \( \overline{\beta_n} \) stays near constant for half the fits and increases for the other half. More interestingly the shifts in all three fitted potassium parameters, \( \overline{V_n}, \overline{\alpha_n}, \) and \( \overline{\beta_n} \) all nearly stop changing somewhere between 12 and 16 segments. This encourages the thought that
the ever so slight trends that do exist for the three might be directly related to the shifts in AP shape that slow to a crawl near that point. They should largely be affecting the downward slope, but the lack of consistent trends suggest that the downward slope is still over determined with these parameters.

*Table 14 Trends in K parameters within confidence interval bounds*

<table>
<thead>
<tr>
<th>Segments</th>
<th>( V_n ) (mV)</th>
<th>Standard deviation</th>
<th>( \alpha_n ) (1/ms*mV)</th>
<th>Standard deviation</th>
<th>( \beta_n ) (1/ms)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-35.5689</td>
<td>2.286187</td>
<td>0.015594</td>
<td>0.00147</td>
<td>0.067325</td>
<td>0.03241</td>
</tr>
<tr>
<td>4</td>
<td>-36.0459</td>
<td>2.301975</td>
<td>0.015463</td>
<td>0.001355</td>
<td>0.074126</td>
<td>0.026416</td>
</tr>
<tr>
<td>8</td>
<td>-34.0596</td>
<td>3.511959</td>
<td>0.014941</td>
<td>0.001181</td>
<td>0.067021</td>
<td>0.031608</td>
</tr>
<tr>
<td>12</td>
<td>-35.6417</td>
<td>6.352987</td>
<td>0.016591</td>
<td>0.002724</td>
<td>0.106587</td>
<td>0.051723</td>
</tr>
<tr>
<td>16</td>
<td>-39.0003</td>
<td>7.192011</td>
<td>0.017159</td>
<td>0.002262</td>
<td>0.11348</td>
<td>0.045649</td>
</tr>
<tr>
<td>24</td>
<td>-41.9158</td>
<td>7.859677</td>
<td>0.017375</td>
<td>0.002406</td>
<td>0.106598</td>
<td>0.047971</td>
</tr>
<tr>
<td>32</td>
<td>-41.1389</td>
<td>8.184428</td>
<td>0.017239</td>
<td>0.002438</td>
<td>0.100922</td>
<td>0.053313</td>
</tr>
</tbody>
</table>
Figure 35 Graphed trends in K parameters within confidence interval bounds

\( \bar{V}_n \) (top) is shown to have a slight downward trend while neither \( \bar{\alpha}_n \) (middle) or \( \bar{\beta}_n \) (bottom) have clear trends. Both, however, level off around 16 segments.
CONCLUSIONS AND FUTURE WORK

Introducing longitudinal segments has an important impact on the AP shape. The HH parameters from a well fit AP simulation with a singular segment is no longer acceptable as more segments are introduced and realistic propagation of currents is approached. As segment lengths shrink and more are introduced the AP begins to smear, reaching a lower max potential and existing for longer. The HH parameters must be adjusted.

How exactly they must be adjusted is not completely clear for just this study. The first study into the HH parameters suggested that maximal leak ion permeability, $\overline{V_m}$, $k_\beta m$, and $\overline{V_n}$ all seem to decrease while $\overline{a_m}$, $\overline{V_h}$, and $\overline{a_n}$ are increased, but no trend is completely consistent. Additionally, for different data sets optimization yielded vast variance in each HH parameter. $\overline{V_m}$ has the least variance, only a factor of 1.5 difference between highest and lowest values but every other parameter varied by at least a factor of 2. The larges seen is exhibited by $K_\beta h$ with a factor 8.3. Differences in data sets have some influence in these differences in the parameter sets but cannot fully explain the variance.

The confidence interval tests help to reveal the bounds of each parameter, showing that large regions in parameter space can result in good fits. Furthermore, the way shifting certain parameters reveal secondary regions in which good fits can found or would cause sudden shifts in most other parameters strongly suggests that there are multiple distinct regions in parameter space in which very well-fit APs can be found. Secondary studies must be done to try to limit this.

The intervals themselves can be used to limit parameter space. When done to test how the HH parameter shifts with added longitudinal segments a second time few clear trends are observed. However, $\overline{V_h}$ and the leak permeability shift significantly and distinctly. The reduced
leak permeability compensates for the change in curvature observed in the hyperpolarizing pulse while $\overline{V}_h$ corrects the height of the AP. It is however unclear how the increased AP width or reduced slopes introduced with longitudinal segments are fully compensated for.

Simply using more data sets and differing initial states, perhaps utilizing each final fit found in this study as a separate starting state, would help to better identify trends and if multiple stable regions do in fact exist, but this would not be enough. One proposed study would be to fit voltage clamp data in addition to AP data, attempting to nail down these not well identified parameters. $K_a h$, $k_{\beta n}$, and $k_{\beta m}$ are especially important to determine through a secondary method as they were not found to have an upper bound while performing the confidence interval tests, they cannot be determined while fitting only the AP.
REFERENCES


APPENDIX A: Confidence interval Details

**Na Activation Parameters**

The voltage gated activation of the sodium channels is controlled by the permeability of the membrane to Sodium and the 5 HH Na activation parameters: $\bar{V}_m$, $k_\alpha m$, $k_\beta m$, $\bar{a}_m$, and $\bar{\beta}_m$. In combination these parameters largely influence the initial response of the cell to a stimulus, mainly influencing the up-slope and maximal height of the AP. When scanning through these parameters they were in general found to have larger impact on the goodness of fit as defined in the objective function than Na inactivation of Potassium parameters. For many of the Sodium activation parameters multiple distinct regions of acceptable fit were found with small regions of failure or bad fits between them. It is unclear if these in-between regions where no good fit was found are impossible to have good fits or if with more iterations a reasonable fit might be found. An additional thing to note is that the fitting algorithm struggled to fit the sodium activation parameters after even small shifts and in most cases upwards of 15 repeated attempts at fitting were done, in many cases only one would lead to a reasonable fit.

**Na Permeability**

Sodium permeability was found to have four distinct regions where fits are possible. These can be seen below in Figure 36. Between 60% and 170% of the initial permeability, 1.646E-3 cm/s, the best fits were found. In this region the objective function varied from .2 to .45 suggesting near perfect fits. Interestingly enough, all other parameters in region stayed near that of their initial value (refer to Figure 37 and Figure 38). Nothing seemed to need to be done to compensate for these shifts in permeability.
As the permeability increases up from 170% up to 260% of the initial state, a second region of reasonable fits are obtained. The fits themselves are quite a bit worse resulting in the objective function valuing approximately 1. These fits can still be considered quite good but no longer the near perfection of the first region. Unlike in the first region, a significant shift in almost every other parameter was observed. Only $\overline{V_h}$, $\overline{\alpha_m}$, and $\overline{\beta_h}$ remain consistent with their initial values while $k_{a,m}$, $K$ permeability, and $\overline{\alpha_n}$ distinctly dropped. All other HH parameters jumped significantly, an example of this can be seen in the shift of $\overline{\beta_m}$ seen below as Figure 38. Despite the sudden shift, all parameters seemed to remain relatively constant exhibiting no visible trend as the Sodium permeability continues to increase.

Once the Sodium permeability exceeded 260% chaos seemingly began to reign until 350%. In this region fits can still be found but extreme trouble was observed in arriving at good fits. This resulted in both the fit goodness and all HH parameters varying wildly. It is entirely possible that fits found in this region could be improved upon, but after running 40 iterative attempts at improving each fit in this region, this was deemed infeasible.

After 350% sodium permeability, no fit was obtained. In each attempt, no matter how the rest of the HH parameters varied, the objective function always hit a failure state. Similarly, when above 40% and below 60% and again below 25%, all attempted fits failed. It is not unexpected to find hard cut-offs but observing a secondary region where the Sodium Permeability is between 25% and 40% where quite reasonable fits (objective function order 1) are able to be found again was unexpected. In this tiny region, particular patterns in the HH parameters are hard to observe, but most are on the very low end. Only $\overline{V_m}$, $\overline{V_h}$, and $\overline{\alpha_n}$ seemed to be elevated over their averages.
From observing the effects of shifting the Sodium permeability it would be advisable to remain between 9.87E-4 cm/s (60%) and 2.8E-3 cm/s (170%) but a second distinct set of parameters exists when the permeability is between 3.0e-3 cm/s (180%) and 4.4e-3 cm/s (270%). Additionally small shifts in sodium permeability within either of these regions do not seem to need to be highly compensated by any other parameter. The other two regions suggest two other stable states, but neither held as distinctive of a shift in any other HH parameter or was as stable.

![Graph of Objective Function vs Percent of Shift](image)

**Figure 36** Values of the objective function graphed vs percent of the shift in Na Permeability.

Four distinct regions where fits could be found can be seen. Between 60% and 170% the best fits can be found. Both between 25% to 40% and 180% to 260% result in reasonable fits (objective on order 1). Between 270% and 350% fits where possible but generally worse and harder to obtain. Outside of these regions all attempted fits failed.
Figure 37 A heatmap of each HH parameter as Sodium Permeability was scanned over.

Each are normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a fail state is set to NaN (grey), darker blue is a worse fit.
Figure 38 The values of $\overline{\beta_m}$ vs Sodium Permeability.

Each are set as a percent of their initial state. This shows the two distinct regions in which most parameter remained constant, between 60% to 170% and 180% to 270%. Outside these regions no obvious trends are observed.

$\overline{V_m}$

$\overline{V_m}$ like the sodium permeability exhibits multiple distinctive regions, but they are much less clear and much more restricted. Only between 97.5% (-42.42mV) and 105% (-45.68mV) of the initial value of $\overline{V_m}$ (-43.50mV) where good fits able to be found (see Figure 39). In this region all parameters remain relatively constant near their initial state, none seem to compensate the shift in $\overline{V_m}$ (see Figure 40). Between 80% and 135% semi reasonable fits where obtainable
(objective <10) with a seemingly random point at 112.5% where an extremely bad fit (objective = 51.9) was found. Over 50 iterative attempts at fitting this value were made, none improved this value. Below the 80% value rapid reduction in goodness of fit can be observed. Between 115% and 135% a second region of good fits (objective ~1.5) can be observed but no distinct trend in the rest of the HH parameters can be observed, most shift significantly up and down within this region.

When exceeding 135% (-58.7mV) sudden failure occurs, no fit is found above this value. Similarly, below 20% (-8.7mV) all fits failed and at 40% (-17.4mV) no fit could be found. It is possible and would seem likely that a bad fit could be found for 40% due to finding one further down at 30% although after 35 iterations of complete failure it was not deemed worth it to find. Additionally, the fit at 30% has an objective function value of 25.125, nearly 83 times worse than the initial fit.

Overall shifting $V_m$ seems to have extreme effects and only a tiny region (-42.42mV to -45.68mV) can be considered for good fits. However, it is distinctly possible that with enough shifts in the rest of the HH parameters good fits might be obtainable between -43.5mV (80%) and -58.7mV (135%) but what parameters must be shifted is unclear. Much more data would need to be gathered, and likely more parameters would need fixed to understand these relations fully.
Figure 39 Values of the objective function graphed vs percent of the shift in $V_m$.

At 112.5% there seems to be a border between two distinctive regions of fits. Between 97.5% (-42.42mV) and 105% (-45.68mV) where the best fits can be found and above 135% (-58.7mV) all attempts failed.
Figure 40 A heatmap of each HH parameter as $V_m$ was scanned over.

Each parameter is normalized independently. Darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.

$\bar{\alpha}_m$

$\bar{\alpha}_m$ has the least clear pattern of any Sodium activation parameter. Even going all the way too 4500% of its initial value (.271 1/(ms*mV) to 12.19 1/(ms*mV)) a very good fit was still obtainable (objective =.55), but before then many very bad fits where found. Additionally, no upper limit was found above 220% all fits succeeded. A lower limit does exist, however, at 20%.
Additional failure states exist at 50%, 160%-180% and 220%, it is unknown if no fit can be found for these states, but 25 attempts were made and failed.

Between 80% (.2167 1/(ms*mV)) and 120% (.3251 1/(ms*mV)) the best fits were found (objective ~.3) but between 240% and 380% and at many seemingly random points nearly as good of fits (objective < 1). Within the region containing the initial state all HH parameters remained very close to that of their start and no clear patterns of compensation can be observed in Figure 41. Outside of this small region chaos seems to reign no clear pattern in any HH parameter can be observed leading to the lack of pattern observed in the objective function. This seeming chaos suggests that for large values of $\overline{\alpha_m}$ there is significant challenge to arrive at good fits, but no upper limit (at least up to 5000%) exists. It is highly likely that most of the bad fits found could be improved, but it would take a very long time or a much better understanding of the direct linkage between HH parameters.

**Figure 41** Values of the objective function graphed vs percent of the shift in $\overline{\alpha_m}$.

Left shows the whole region scanned while right restricts the maximum value of $\overline{\alpha_m}$ to 550% (1.49 1/(ms*mV)). Between 80% (.2167 1/(ms*mV)) and 120% (.3251 1/(ms*mV)) the best fits
can be found, although many instances of good fits can be found intermittently throughout even all the way to 4500% of the initial state.

![Heatmap of HH parameters](image)

**Figure 42** A heatmap of each HH parameter as $\bar{\alpha}_m$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.

$\bar{\beta}_m$

$\bar{\beta}_m$ like $\bar{\alpha}_m$ and $\bar{V}_m$ does not hold a singular clear pattern. Between 45% (.5227 l/ms) and 125% (1.568 l/ms) of $\bar{\beta}_m$ very good fits were found (objective ~.3). Above this rapid worsening, and even failure occurs until 240%. Another failure state exists at 270%. Between
360% and 520% relatively good fits can still be found \((1.3 < \text{objective} < 5)\). Exceeding 560% or reducing below 20% seems to result in failure.

Also similar to the previous sets no clear patterns in the HH parameters outside of the 45% to 125% region of good fits can be observed. In this region all parameters remain near constant about their initial states. Some adjustments are made, but no clear patterns. Outside, only at failure states are there any recognizable patterns.

Figure 43 Values of the objective function graphed vs percent of the shift in \(\bar{\beta}_m\).

Between 45% \((.5227 \text{ l/ms})\) and 125% \((1.568 \text{ l/ms})\) the best fits are found (objective \(\sim .3\)). Outside this region no fit achieves an objective function value below 1.3. Rapid worsening and
failure bounds this region but after exceeding 240% occasional reasonable fits can be found until
560%.

**Figure 44** A heatmap of each HH parameter as $\beta_m$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.

**$K_{\alpha m}$**

Unlike any other Sodium activation parameter $k_{\alpha m}$ does not have a region of stability bounding the initial state. Any shift in $k_{\alpha m}$ results in shifts in nearly every HH parameter. The best fit is still the initial state (8.95mV), but between 120% to 130% and 25% to 70% very good
fits (objective < 1) can be found and even at 280% a reasonable fit (objective = 1.175) can still be found. Above this, however all attempts to fit failed. Below 25% also resulted in only very bad fits or complete failure. Internally too these bounds the only true failure state occurred at 200%.

Out of all the HH parameters only $V_m$ seemed to hold a near consistent trend in response to the shifting of $k_a m$ (refer to Figure 47). Within the region devoid of failure states (>200%) $V_m$ seems to decrease as $k_a m$ increases. This is not true below 60% of $k_a m$ where $V_m$ seems to hold constant at 168% (-73.0876mV). Additionally, this value of $V_m$ exceeds that of the maximum previously observed when scanning $V_m$, this might suggest that more incremental shifts of $V_m$ or differing starting points might allow for further fits to be made, possibly up to 168% $V_m$. The convers of this downward trend in $V_m$ with increase in $k_a m$ is not observable in the $V_m$ data. This casts some doubt about if these two parameters can compensate each other, more testing to prove this would be needed.
There is not a stable region about the initial state. Between 120% to 130% and 25% to 70% very good fits (objective < 1) can be found in addition to the initial state (100%, 8.95 mv). After 280% all attempted fits returned failures.

*Figure 45 Values of the objective function graphed vs percent of the shift in $k_{am}$.***
Figure 46 A heatmap of each HH parameter as \( k_{\alpha m} \) was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.
Figure 47 The values of $V_m$ vs. $K_{\alpha m}$.

Each is set as a percent of their initial state. Below 60% $K_{\alpha m}$ $V_m$ seems to max out at 168% (-73.0876mV). As $K_{\alpha m}$ increases $V_m$ appears to decrease until the first failure state occurs at 200%. No other HH parameter seems to hold a similarly constant trend with $K_{\alpha m}$.

$K_{\beta m}$

No failure states or truly bad fits were found for $K_{\beta m}$ when scanned between 20% and 6000% of its initial value (5.822mV to 1.75V). Below 20% however resulted in model failure. Many instances of relatively mediocre fits (objective ~5) were found but after almost everyone a great fit was found (objective ~.5). There is a small region of stability about the initial state between 90% and 120% this region also holds the best fits (Objective <= .3).
Due to the ability to get good fits throughout the full scan it can be conjectured that $k_{\beta m}$ is easily compensated and overall does not matter much in terms of goodness of AP fit. It would need to be determined with a different experimental method and can be left out of fits in which the HH are allowed to vary. One other important observation that was made while varying $k_{\beta m}$ is that $V_m$ and $k_{\alpha m}$ both seem to have a steady state that also seems to work as a maximum or minimum that is vastly different from the initial state found from the fit to 16 segments (Figure 50). $V_m$ seems to want to sit at and never exceed 168% (-73.0876mV) of its initial value, the same max value found when looking at the $k_{\alpha m}$ scan. $K_{\alpha m}$ refused to drop below 25% (2.24mV) and seemed to like existing near there. Backing up this hard limit is the fact that when scanning over $K_{\alpha m}$ dropping below 25% resulted in very bad fits or failure.
Figure 48 Values of the objective function graphed vs percent of the shift in $k_{\beta m}$.

Throughout the whole scan good fits can be observed (objective $\sim .5$) and no bad (objective $> 10$) its where found.
Figure 49 A heatmap of each HH parameter as $k_{\beta m}$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective $>$20) state is set to NaN (grey), darker blue is a worse fit.
Figure 50 The values of $k_\alpha m$ (left) and $V_m$ (right) vs. $K_\beta m$.

Each is set as a percent of their initial state. Both seem to hold a maximum or minimum that the surrogateopt algorithm desires to fit it to in order to minimize the objective function.

**Na Inactivation Parameters**

The inactivation of the voltage gated sodium channels is controlled by the HH parameters: $V_h$, $\alpha_h$, $\beta_h$, $k_{ah}$, and $k_{bh}$. These largely should have the most influence on the maximal AP height and width with some minor influence on both up/down slopes. On average it will be shown that Sodium Inactivation parameters when scanned over in seem to have more distinct patterns in the goodness of fit than the activation parameters. Most have clear cutoffs allowing for a distinctive region in which each parameter can vary. Only one has multiple regions in which good fits can be found.

$V_h$

The influence of $V_h$ on the goodness of fit, as defined in the objective function, is by far the least clear of any Na inactivation parameter. Like many of the activation parameters, multiple regions in which a reasonable fit can be found exist. The best fits exist between the 50% (-}
19.60mV) to 140% (-54.87mV). Inside this region almost every HH parameter remains relatively stable, only \( k_{\beta m} \) holds noticeable trend in this region (see Figure 52 and Figure 53). Figure 53 shows \( k_{\beta m} \) decreasing steadily as \( V_h \) increases while between 55% and 130% of its initial value. This suggests a link between these two parameters. However, outside this region when the fits are generally worse no such linkage between \( V_h \) and \( k_{\beta m} \) can be observed.

The bad fits (objective > 20) at 45% and 150% of \( V_h \) bound this region of sable good fits and were unable to be improved even after 50 iterative attempts at fitting these two points. Between 160% and 180% the last region of relatively good fits (objective < 2), or more accurately any successful fits. Above 180% of \( V_h \) all attempts at fitting failed giving a hard cut off on \( V_h \) at -70.55mV. Below the bad fit at 45% a less stable region of acceptable fits (.9 <= objective <= 5.125) exists when between -30% (11.75mV) and 35% (-13.72mV). Directly following this comes the first fail state, -40%. Below this the final region of successful fits, albeit the worst set, exists between -70% and -50% before continuously failing below -70%.

Clearly there exists multiple regions of \( V_h \) in which successful APs can form. However only between 50% (-19.60mV) to 140% (-54.87mV) did any sort of pattern in the rest of the HH parameters emerge. It would be advisable to keep the bounds of \( V_h \) within this region for future studies, although if the need arises any value between -30% (11.75mV) and 180% (-70.55mV) could be used without leading to undesirable AP behavior.
Figure 51 Values of the objective function graphed vs percent of the shift in $V_h$.

Left is zoomed such that only reasonable fits are displayed, while right is zoomed out to show that at 45% and 150% the fits were successful but bad. Between 50% (-19.60mV) to 140% (-54.87mV) the best fits can be found but anywhere between -30% (11.75mV) and 180% (-70.55mV) lead to successful runs. -70% (27.43mV) to -50% (19.60mV) is a secondary region in which reasonable fits could be found before. Below -70% (27.43mV) and above 180% (-70.55mV) all fits failed.
Figure 52 A heatmap of each HH parameter as $V_h$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective $>$ 20) state is set to NaN (grey), darker blue is a worse fit.
Each are set as a percent of their initial state. Between 55% and 130% of $V_h$, also the region of best fit, the value of $k_\beta m$ decreases as $V_h$ increases. The other regions of reasonable fit hold no clear pattern.

\[ \alpha_h \]

$\alpha_h$ cannot be reduced below zero, but any value up to 300% (.0088 l/ms) allows for a very good fit (objective < .6). Above 300% of $\alpha_h$ the fit rapidly deteriorates until continual failure occurs at 350% and above. While below 300% no singular HH parameter seems to very consistently as to compensate for the increase in $\alpha_h$, each seem to fluctuate around their initial values. However as soon 300% of $\alpha_h$ is exceeded most of the HH parameters shoots up.

Figure 53 The values of $k_\beta m$ vs $V_h$.
significantly only Na Permeability and \( \overline{V_h} \) seem to remain constant. While \( \overline{V_n}, \overline{V_m}, k_{\beta m}, \) and \( k_{\beta h} \) seem to decrease significantly in this region of rapidly worsening fits.

\[ \text{Figure 54} \quad \text{Values of the objective function graphed vs percent of the shift in} \quad \overline{\alpha_h}. \]

Between 0\% and 300\% (.0088 1/ms) only good fits (objective < .6) are found. Above this the fits rapidly worsen until failure occurs.
Figure 55 A heatmap of each HH parameter as $\alpha_h$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective $>$20) state is set to NaN (grey), darker blue is a worse fit.

$\beta_h$

When the value of the objective function is graphed against the scan of $\beta_h$ there appears to be a well-defined region in which good fits can be found (see Figure 56). When between 20% (2.326 m/s) and 120% (13.955 m/s) of the initial state very good fits (objective $<$ .9) are observed. If $\beta_h$ is further reduced rapid worsening of the fit occurs until continual failure to fit is observed when below 10% (1.163 m/s) of $\beta_h$. When increasing above 120% of the initial state
the value of the objective function seems to exponentially worsen, all fits beyond 180% (20.932 1/ms) can be considered bad (objective > 10). However, none in the region tested, up to 300% (34.887 1/ms) failed.

When observing the patterns in the rest of the HH parameters as $\beta_h$ is scanned over (Figure 57) only Sodium permeability can be considered to have a semi-consistent trend. All other parameters seem to remain relatively consistent near the initial state before changing seemingly at random. Sodium permeability however seems to increase as $\beta_h$ does. This can be further seen in Figure 58. The same relation was not observed in the sodium permeability scan in which no direct linkage between these two parameters were observed. This suggests that if there is a direct link between shifts in $\beta_h$ and Sodium permeability it is a loose connection tied to too many other parameters to be sure of, more testing in which only these two parameters are shifted would be needed.
Figure 56 Values of the objective function graphed vs percent of the shift in $\beta_h$.

Between 20% (2.326 l/ms) and 120% (13.955 l/ms) of the initial $\beta_h$ state the best fits are observed. Below this rapid worsening, then failure exists while above slow decay into bad fits is exhibited.
Figure 57 A heatmap of each HH parameter as $\beta_h$ was scanned over.

Each parameter is normalized independently. Darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective > 20) state is set to NaN (grey), darker blue is a worse fit.
Figure 58 The values of Sodium permeability vs $\beta_h$.

Each is set as a percent of their initial state. A slow increase in sodium permeability seems to follow the increase in $\beta_h$ however the large jump at 180% does not seem to follow this trend nor was this observed when scanning over sodium permeability instead.

$K_{ah}$

Increasing $k_{ah}$ has no meaningful effect on the fit of the simulated AP. During the scanning process up to 10000 times the initial state of $K_{ah}$ (initially 24.4mV up to 244V) was tested to no detriment to the goodness of fit (objective < .5 for each). Decreasing $K_{ah}$ does however cause a failure state. Below 40% of $k_{ah}$ (9.77 mV) the best values found of the
objective function begin to worsen and below 20% of $K_{\alpha h}$ (4.89mV) full failure to obtain passable APs begins.

Additionally shifting $K_{\alpha h}$ does not seem to have any noticeable effect on any other HH parameter when $K_{\alpha h}$ is above 40%. Only slight variance off the initial states of each HH parameter is exhibited, none showing a continuous trend. Below 40% as the goodness of fit worsens and until failure large shifts in every other HH parameter are observed as the surrogateopt algorithm tries and fails to correct for the shifts in $k_{\alpha h}$. It would be advisable for future studies to determine $k_{\alpha h}$ through a separate experiment or simply choose a value above 9.77mV and stick with it, fitting $K_{\alpha h}$ to the AP does not seem to yield any meaningful results.
Figure 59 Values of the objective function graphed vs percent of the shift in $k_{ah}$.

While above 40% of $k_{ah}$ (9.77mV) no meaningful shift in the goodness of fit is observed for any change in $k_{ah}$. 
Figure 60 A heatmap of each HH parameter as $k_{\alpha h}$ was scanned over.

Each parameter is normalized independently. Darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function; any fit resulting in a failure or bad fit (objective $> 20$) state is set to NaN (grey), darker blue is a worse fit.

$K_{\beta h}$

The reduction of $k_{\beta h}$ has the most sudden cut off observed of any parameter. At .25% of $k_{\beta h}$ (.0087 mV) a very good fit (objective = .2) is obtainable but any reduction below this value resulted in failure to produce an acceptable AP. Above this value the fit goodness remains nearly unchanged (objective $< .4$) until exceeding 195% (6.75 mV) after this point surrogateopt
struggles to find good fits leading to rapidly deteriorating values of the objective function, until 260% (9.00 mV) the first full failure when increasing $K_{\beta h}$. After which no successful fit is found.

The behavior of the other HH parameters closely matches that of the value of the objective function. Between .25% and 195% each parameter floats around their initial state (see Figure 62). No significant changes are observable, and none seem to be compensating for the shift in $k_{\beta h}$. Outside this region, however, each parameter is rapidly shifted but changes in $k_{\beta h}$ do not seem to be able to be compensated for as the fit rapidly goes towards failure.

![K_beta_h](image)

**Figure 61** Values of the objective function graphed vs percent of the shift in $k_{\beta h}$.

Between .25% of $K_{\beta h}$ (.0087 mV) and 195% (6.75 mV) near perfect fits are achievable. Below this only failure awaits and above a quick ascent to failure can be observed.
Figure 62 A heatmap of each HH parameter as $k_{\beta h}$ was scanned over.

Each parameter is normalized independently; darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.

**Potassium Parameters**

The potassium HH parameters: Potassium permeability, $V_m$, $\alpha_m$, $\beta_m$, $k_{\alpha m}$, and $k_{\beta m}$ are responsible for the slow activation of the voltage gated potassium channels. In an AP these are largely responsible for the return to rest and have a strong influence on the maximum height, width, and down slope of the AP. Scanning over each tended to show small regions of very good
fit but had much larger regions in which surrogateopt struggled to arrive at a good fit but would not lead to failure than the sodium parameters.

**Potassium Permeability**

The potassium permeability exhibits very different behavior than other parameters studied. When increasing no failure state or truly bad fits (objective > 10) were found, instead we seem to observe repetitious beats. There would be a region good fits (objective < 1) followed by a barrier of much worse fits (1 < objective < 5) this behavior seems to repeat as the permeability increases. It is notable that the step size was increased with increasing permeability a much finer mesh of searches would be very useful in order to confirm if this beat like behavior truly continues. It was deemed infeasible due to time constraints and would be something to look into in a latter study.

The first region of very good fits runs from 50% of the initial permeability (1.26e-4 cm/s) to 860% (.0022 cm/s). The next region of comparably good fits exists between 1160% (.0029 cm/s) to 1600% (.0040 cm/s). The last well-defined region of very good fits exists between 2000% (.0047 cm/s) and 2300% (.0058 cm/s). After this region intermittent very good fits are found, but the resolution between fit attempts is too large to judge if any clusters of good fits exist about them.

It is notable that these objective function value < 1 regions seem to shrink as the potassium permeability is increased. If this is more than an artifact of semi-random nature of surrogateopt than it could mean that somewhere up above 50 times the initial value of the potassium permeability no more very good fit will be able to be found. However, increasing the permeability so high would be a bit unrealistic biologically.
It should also be noted that like every other HH parameter the linkage between other parameters is unclear. Only $\alpha_n$ seemed to have any direct link with the increase in potassium permeability. $\alpha_n$ exponentially decreases until coming near asymptotically to rest near 25% of its initial value as the permeability is increased. This can be seen in Figure 65.

![Kdr_permeability](image)

**Figure 63** Values of the objective function graphed vs percent of the shift in K permeability.

Between 50% to 860%, 1160% to 1600%, and 2000% to 2300% very good fits (objective < 1) where found. Above these points intermittent successful pulls were found.
Figure 64 A heatmap of each HH parameter as maximal $K^+$ Permeability was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.
Figure 65 The values of \( \bar{\alpha}_n \) vs Potassium permeability.

Each is set as a percent of their initial state. Exponential decay seems to be present asymptotic at 0% potassium permeability and at 25% \( \bar{\alpha}_n \).

\[ \bar{V}_n \]

\( \bar{V}_n \) Seems to have a very well-defined region in which good fits can be achieved. Below 55% (-19.79mV) rapid worsening of the fit occurs and all successful attempts below this result in objective function values > 10. Once \( \bar{V}_n \) is reduced below 10% of the initial state failure begins. Similarly, as \( \bar{V}_n \) is increased only once 245% (-88.14mV) is reached do the fits fail, before this
point all the objective function value seems to be slightly worsening as \( V_n \) increased and accelerates at 210%.

Within this region in which good fits can be found (55% to 245% of \( V_n \)) three of the HH parameters seem to hold explicit trends, seen in Figure 67 and Figure 68. \( V_m \) increases as \( V_n \) does although only by about 20% within this region. Both \( \alpha_n \) and \( k_m \) respond similarly decreasing as \( V_n \) is increased. Each seems to drop to around half that of their initial state in this region. All other HH parameters seem to fluctuate with no apparent pattern suggesting no direct links between them and \( V_n \).
Figure 66 Values of the objective function graphed vs percent of the shift in $V_n$.

Between 55% to 245% of $V_n$ very good fits can be found. The best fits are available between 65% and 120% of $V_n$ but no significant worsening occurs until beyond 210%.
Figure 67 A heatmap of each HH parameter as $\overline{V}_n$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.
Figure 68 The values of $\alpha_n$ (top left), $k_m$ (top right), and $V_m$ (bottom) vs $V_n$.

Each is set as a percent of their initial state. Within the region in which the best fits could be found (80% to 210% of $V_n$) both $\alpha_n$ and $k_m$ decayed while $V_n$ increases. $V_m$ however, becomes elevated with increasing $V_n$.

$\alpha_n$

While scanning through different values of $\alpha_n$ four distinct regions can be seen. While below 60% of the initial $\alpha_n$ state (.0134 1/(ms*mV)) rapid worsening of the fits occurs. When above this and below 200% (.0335 1/(ms*mV)) the best fits can be found all with objective function values below 1. As $\alpha_n$ continues to increase a strange region in which the surrogate fitting algorithm seemed to struggle in exists. All the way till 500% all fits were attempted 25 or more times. Despite this in many instances there was a failure to achieve good fits. Between
210% and 360% very good fits (objective $< 1$) could be achieved, but half the time the best fit would not drop the objective function value below 2.5, significantly worse fits. The final region after $\alpha_n$ exceeds 360% holds no great fits only occasionally achieving objective function values below 3.

When looking at the shift in $\alpha_n$’s effects on the rest of the HH parameters only $V_n$ stands out and seems to hold a consistent trend (Figure 71). As $\alpha_n$ is increased $V_n$ decreases this is almost strictly true in the region of best fit between 60% and 200% of $\alpha_n$. When exceeding 200% and entering the region in which surrogateopt struggled to arrive at good fits the decrease does not seem to hold true. This decrease of $V_n$ as $\alpha_n$ is increased matches the same observation made when scanning through $V_n$. These two parameters are very likely linked and small shifts in one can be compensated for by the opposite in the other. Overall, it would be advisable to keep $\alpha_n$ between .0134 1/(ms*mV) (60%) and .0335 1/(ms*mV) (200%) while keeping an eye on $V_n$, reducing it if and elevated $\alpha_n$ is needed.
Figure 69 Values of the objective function graphed vs percent of the shift in $\alpha_n$. Between 60% and 200% the best fits can be found. Below this region rapid worsening of fits occurs. Above this region surrogateopt struggled to achieve reasonable fits but they do seem to exist.
Figure 70 A heatmap of each HH parameter as $\alpha_n$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.
Figure 71 The values of $\bar{V}_n$ vs $\bar{\alpha}_n$. Each is set as a percent of their initial state.

Between 50% and 200% of $\bar{\alpha}_n$, the region of best fit, $\bar{V}_n$ seems to drop rapidly with increasing values of $\bar{\alpha}_n$. With further increase in $\bar{\alpha}_n$ into the region of much more challenging fits the same trend seems to hold true but much less confidence.

$\bar{\beta}_n$

$\bar{\beta}_n$ can be dropped near zero, but not to zero with no relevant effect on the fit goodness of the AP. However, if 0% is attempted the model fails. While above this failure state and below 255% of $\bar{\beta}_n$'s initial state (.2319 1/ms) near perfect fits (objective < .6) can be achieved. At 260% the fits suddenly worsen and continue to worsen, albeit jaggedly, until some hope of a secondary between 650% (.5912 1/ms) and 700% (.6366 1/ms). Between these values one fit
(675%) is nearly able to return the objective function below 1. But after this secondary region of acceptable fits all later attempts are met with failure or extremely bad fits. At the latest 700% (.6366 1/ms) should be the cutoff for $\beta_n$ and 255% (.2319 1/ms) would be a more advisable cutoff.

Before this cutoff (255%) almost every HH parameter stays stable at or near their initial state as found in the 16-segment fit (see Figure 73). Then 260% is hit, the sudden spike in objective function value, almost every HH parameter suddenly shifts to a new state. $K_a$, $k_\beta n$, and $\alpha_n$ all suddenly jump before remaining relatively constant. Sodium permeability, $V_m$, $k_\beta h$, $\alpha_h$, $\beta_h$, and potassium permeability each drop significantly. While all other parameters become more chaotic but do not seem to hold significant trends. This suggests that there are at least two distinct sets of parameters that can still achieve moderate or better AP fits.

The sudden shift in HH parameters corresponding to the sudden shift in fit goodness is not the only observable trend as $\beta_n$ is increased. Within the region of best fit (before 255%) when most parameters seem to hold constant, $k_a$ and $k_\beta n$ both are increasing (Figure 74). For this first distinct parameter set it seems that increasing $k_a$ and $k_\beta n$ can at least to an extent offset the shifts in $\beta_n$ at least until 255% (.2319 1/ms).
Figure 72 Values of the objective function graphed vs percent of the shift in $\beta_n$.

While between 1% and 255% of $\beta_n$’s initial state near perfect fits can be found. At 260% there is a sudden leap in the objective function value before gradual worsening begins. Between 650% and 700% a secondary region of better fits is suggested. Any value above 700% leads to a very bad fit or complete failure.
Figure 73 A heatmap of each HH parameter as $\beta_n$ was scanned over.

Each parameter is normalized independently; darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function; any fit resulting in a failure or bad fit (objective $>20$) state is set to NaN (grey), darker blue is a worse fit.
Figure 74 The values of $k_\alpha n$ (left) and $k_\beta n$ (right) vs $\bar{\beta}_n$.

Each is set as a percent of their initial state. Within the region of best fit (5% to 255% of $\bar{\beta}_n$) both seem to increase with as $\bar{\beta}_n$ is elevated.

$K_\alpha n$

Increasing $k_\alpha n$ leads to almost the same trend if objective function value as $\bar{\beta}_n$. It can be reduced to near zero with no detrimental effect on the AP fit while increasing $k_\alpha n$ only has minor effects until 1200% of the initial state (64.16 mV). Above this value sudden worsening and eventual failure to fit is observed. Unlike $\bar{\beta}_n$, increasing $k_\alpha n$ seems to steadily worsen the fit. The last achieved fit with an objective function value less than 1 is at 925% (49.46 mV).

When reducing $\bar{\beta}_n$ below its initial state no other HH parameter experiences any observable effect. Each float at (or very near) their initial state. As $\bar{\beta}_n$ is increased slight fluctuations in each parameter is clear (see Figure 76). And once 800% of $\bar{\beta}_n$ (42.78 mV) is hit these fluctuations massively increase each parameter seems to need to compensate for this shift in $\bar{\beta}_n$ massively, although there is no clear trend in how they needed to react. Due to this obvious
struggle to achieve good fits when $\bar{\beta}_n$ exceeds 800% (42.78 mV) it would be advisable to keep $\bar{\beta}_n$ between 0.0535mV (1%) and 42.78mV (800%).

Figure 75 Values of the objective function graphed vs percent of the shift in $k_{\alpha n}$.

A gradual increase in objective function can be observed as $k_{\alpha n}$ is increased up to 1200% of its initial state. Above this sudden worsening and eventual failure to fit is observed.
Figure 76 A heatmap of each HH parameter as $k_{\alpha n}$ was scanned over.

Each parameter is normalized independently. Darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective $>20$) state is set to NaN (grey), darker blue is a worse fit.

$K_{\beta n}$

$K_{\beta n}$ behaves near identically to $k_{\alpha h}$ in the fact that there does not seem to be any upper limit. $K_{\beta n}$ was tested all the way to $3e5\%$ and almost no effect on the fit was observed. No attempted value of $k_{\beta n}$ above $45\%$ of its initial state ($10.49mV$) resulted in the objective function value after fitting exceeding $1.5$ with the vast majority being below $1$. The fact the not every fit
was near perfect (objective ~.3) suggests that $k_{\beta n}$ does affect the AP but minimally. Reducing $k_{\beta n}$ to 45% also holds no significant effect but attempting any value below that lead to surrogateopt struggling and in almost all cases a very bad fit. Also similar to $k_{\alpha h}$, each HH parameter did not seem to hold any clear trends. Each fluctuated about their initial states with no clear patterns (see Figure 78).

![Graph of Objective Function vs Percent Shift in $K_{\beta n}$](image_url)

*Figure 77 Values of the objective function graphed vs percent of the shift in $k_{\beta n}$. For all values of $k_{\beta n}$ above 45% of the initial state a good fit (objective < 1) could be found. Below 45% only and fits and failure to fit can be found.*
Figure 78 A heatmap of each HH parameter as $k_{\beta n}$ was scanned over.

Each parameter is normalized independently darker blue suggests a higher percent of the initial value of that parameter. The top row shows the value of the objective function, any fit resulting in a failure or bad fit (objective >20) state is set to NaN (grey), darker blue is a worse fit.