Functional Implications from Changes in Volume and Periaxonal Space of C-fibers

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Functional Implications from Changes in Volume and Periaxonal Space of C-fibers

Funktionella implikationer från ändringar av volym och periaxonalt utrymme i C-fiber

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Abstract

Scientists have not come up with effective drugs for neuropathic pain patients yet. Swelling in neuropathic C-fibers (slow pain fibers) has been observed, and in this study the effects of swelling on nerve pulses were studied.

This study concerns the morphological implications on action potential conduction in neuropathic C-fibers. The effect of periaxonal space and axon diameter changes has been studied to simulate a swelled C-fiber. The conduction velocity, stimulation threshold and latency shift were measured while sending in single, double pulses or pulse trains. This was done using the Hodgkin-Huxley model, which is a mathematical model for the initiation and propagation of an action potential in a neuron. To implement the model, the simulation program Neuron, run in a Matlab environment, was used.

The results show that if the periaxonal space is decreased the output pulse frequency will be higher than the input frequency, when sending pulse pair and pulse-pair trains. Also an increased diameter will give the same effect in the pulse pair case but not in the pulse pair train case. The results show that swelling in C-fibers can be a causal reason for neuropathic pain.

Sammanfattning

Vetenskapsmän har ännu inte kommit på bra läkemedel för neuropatisk smärta hos patienter. Man har funnit svullnad i neuropatiska C-fiber (långsamma smärtfiber) och i den här studien simulerar jag hur detta påverkar nervpulser med en matematisk modell.

Den här studien handlar om morfologiska implikationer på aktionspotentialfortledning i neuropatiska C-fiber. Effekten från ändringar av det periaxonala utrymmet och axon-diametern har studerats för att simulera en uppsvälld C-fiber och för att se hur detta påverkar ledningshastighet, stimulations tröskel och latensändring medan enkel-, dubbelpulser eller pulståg skickats in. Till detta användes Hodgkin-Huxley-modellen som är en matematisk modell för initieringen och propageringen av en nervcells aktionspotential. För implementeringen av modellen används simulationsprogrammet Neuron kört från Matlab.

Resultaten visar att om det periaxonala utrymmet är förminskat så kommer pulsfrekvensen ut att vara högre än frekvensen in. Det visar även att en förstorad diameter ger samma effekt i dubbelpulsfallet men inte i dubbelpulståg-fallet. Resultaten visar att svullnad i C-fiber kan vara en kausal anledning till neuropatisk smärta.

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1. Introduction

1.1 Background

The project assignment was initiated by Dr. Erik Fransén and Marcus Petersson, two scientists at the department of Computational Biology (Kungl. Tekniska Högskolan and Stockholm Brain Institute). They are currently working on a joint project with AstraZeneca Södertälje and the labs of Martin Schmelz and Richard Carr at Heidelberg University.

For a long time scientists have been trying to develop effective drugs for patients with neuropathic pain. Changes in the propagation pattern in neuropathic pain fibers were recently discovered. It has lead to the hypothesis that these changes might be related to the ion channels of these nerve fibers.[1] To find this supposed relation one must understand the physiological process of a nerve signal. It is possible to measure the signal velocity of some nerve fibers. But the C-fibers are very small, which makes it very difficult. This is where the mathematical modeling and computer simulations come into play. A model of a C-fiber was created by Marcus Petersson and Jenny Tigerholm by getting the pulse propagation to mimic experimental data from earlier studies (patients and animals). This model was used in to simulate the propagation pattern of the nerve fibers in neuropathic pain patients. In this project the cause was considered to be tissue swelling.[2] The simulation changes made to simulate this are explained in the theory chapter about swelling. Two important determinants affected by the swelling are the diameter of the axon and the periaxonal space, called theta. I assume that swelling means increased diameter and decreased theta (periaxonal space).

1.2 Objectives

These are the objectives of this project:

- **Swelling:** Simulate changes (increase and decrease) in diameter and theta and see how this affects the reversal potential, spike threshold, conduction velocity, conduction failure, pulse trains (frequency and number of pulses), activity-dependent slowing and recovery cycles.
- Analyze the graphs from the above mentioned simulations from a pharmaceutical perspective and speculatively propose what the medication must achieve in order to cure or relieve neuropathic pain.

1.3 Restrictions

This is a very wide area and there are many parameters to experiment with. This project focuses on diameter and theta changes. The Hodgkin-Huxley model was not explained in detail but detailed enough to understand how the model was used in the simulations. The conclusion regarding drugs for neuropathic pain was held on a simple level.

1.4 Method of Solution

Literature about neuropathic pain, C-fibers and the Hodgkin-Huxley model was read. Mathematical models written in Matlab and Neuron code on computers with Unix operative system were used. Most of the code was already written but an understanding was needed to make changes for the experiments. The code was written by Marcus Petersson and Jenny Tigerholm. The model used for simulations is a multi-compartment model with ion channels of Hodgkin-Huxley type (which is explained in chapter 2.4), implemented in the simulation program Neuron.[3] Depending on the number of pulses used the simulation time can vary between 1 minute and several hours. The simulations were done on KTH-computers with Ubuntu 11.00+ as operating system. Simulations were initiated from a laptop connected to the KTH-computer through SSH (Secure Shell protocol).

Running the simulations locally instead of using SSH, would have made the work process faster. But it also would have taken time to set up a computer for the simulations. There are other simulation programs than Neuron to use, e.g. Genesis. But since the code was already written with Neuron and previous studies using a similar model showed positive results, the this model was chosen.[1] When using a mathematical model with a lot of parameters to draw conclusions it is important to remember that it is a model of reality and not reality itself.

1.5 Environment

This project was done at the department of Computational biology (CB) at KTH. CB works with mathematical modeling of biological systems and they develop algorithms and software for simulating biological functions and analyses of biological data. They are also involved in joint projects with experimental biologists.

Neuron is the simulation environment used in this project. It is used to build computational models of neurons and networks of neurons. Matlab is used to analyze and visualize the data files that are created by Neuron.

2. Theory

2.1 Action potential

It is important to know what ions are located inside and outside nerve fibers to understand the action potential (AP) and the results and discussion section. The following figure illustrates some of the ions inside and outside the nerve fibers.

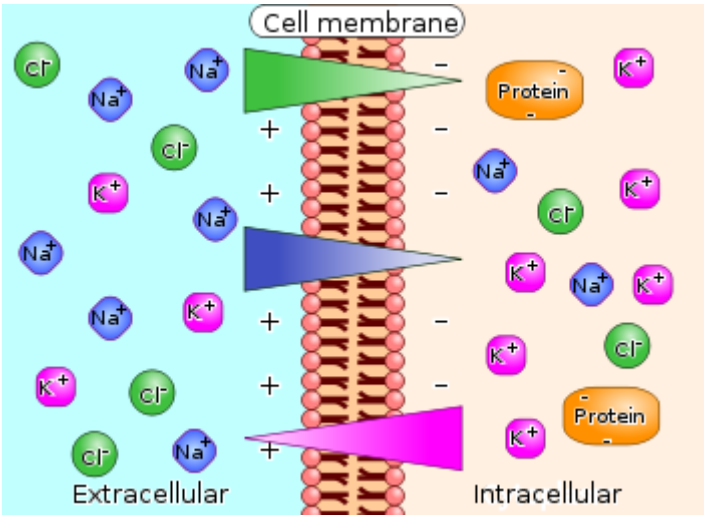

Figure 2.1 Some of the ions located inside and outside nerve fibers. Figure taken from ref 4.

Figure 2.1 shows that there is more sodium outside the cell and more potassium inside the cell. These concentrations together with other ions create the resting membrane potential. In the following figure the basic steps of an action potential are demonstrated.[4]

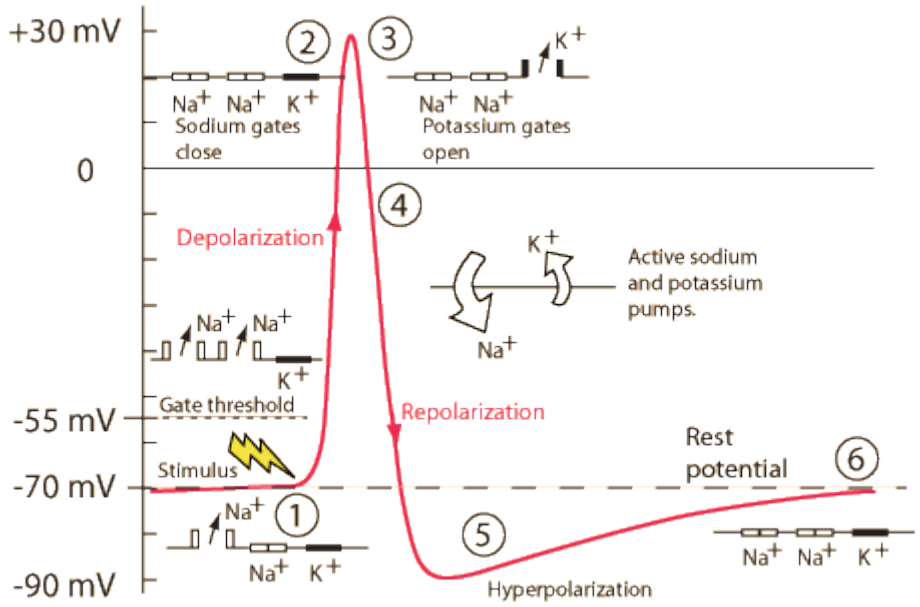

Figure 2.2. The basic steps of an action potential. Figure taken from ref 5.

Figure 2.2 shows the basic steps of an action potential, where the y-axis represent the membrane potential and the x-axis time. In the first step, the cell is stimulated up to the threshold followed by a depolarization up to step 2, where sodium gates start closing and

potassium gates start opening. The cell is repolarized in step 3 and step 4, where Na/K-pumps are also activated. In step 5, the cell becomes hyper polarized, but the Na/K-pumps slowly restore the resting potential, as seen in step 6.[5]

2.2 C-fibers

C-fibers are peripheral unmyelinated axons located in the somatic sensory system. The following figure shows an example of the pathway of a C-fiber.

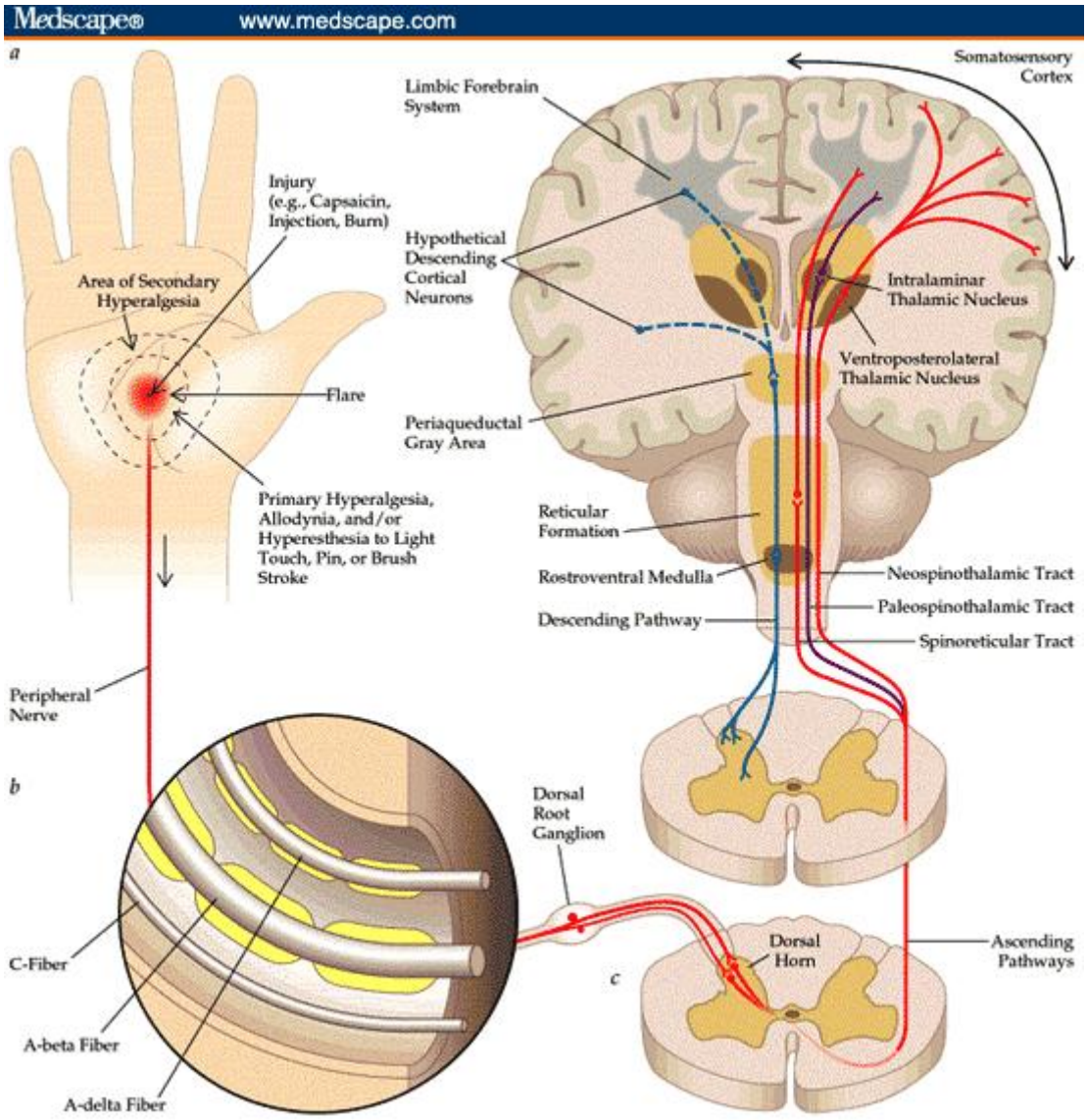

Figure 2.3. A C-fiber, other nerve fibers and how they connect to the spine and brain. C-fibers are small compared to other nerve fibers and they are not myelinated. Figure taken from Stephen et al.

C-fibers are afferent fibers that send signals with different types of information to the central nervous system. They respond to many types of stimuli since there are different kinds of receptors. The one concerned in this project is the C-fiber nociceptor axons, i.e. the ones responsible for slow pain. It is not the sharp initial pain you feel, but instead the longer-lasting slow pain. The pulses are slower, because the C-fibers are not myelinated and have a very

small diameter (~0.2-1.5µm) as seen in Figure 2.3. The conduction velocity of C-fiber pulses is about 1m/sec. [1,6,7]

2.3 Neuropathic pain

Neuropathic pain occurs when the peripheral or central nervous system is malfunctioning. Instead of signaling when there is a natural cause such as tissue damage, the pain can be initiated by a very weak stimulus or no stimulus at all. Neuropathic pain must not be confused with nociceptive pain, which is caused by the stimulation of pain receptors by algogenic substances (pain inducing substances). Neuropathic pain means having chronic hyperalgesia and Allodynia. Hyperalgesia means having an increased sensitivity to pain caused by damage to peripheral nerves or nociceptors. Allodynia means feeling pain caused by a stimulus that normally would not cause pain. Neuropathic pain can occur in both C- and A-fibers, but this report focuses on C-fibers. [8-10]

Swelling of the C-fiber axons is believed to be an effect of damage or pathological changes to the peripheral nervous system. In this study swelling is simulated by increasing the diameter and decreasing the theta space (periaxonal space) of the axon. These simulation parameters are explained in chapter 2.5.

The intensity of pain is related to the frequency of the input stimulus. A higher frequency means more pain and a lower frequency means less pain. The conduction velocity and amplitude of the pulse does not affect the pain intensity.[11]

2.4 Hodgkin- Huxley model

The aim of this section is to give an understanding of the Hodgkin-Huxley model, reversal potential and driving force. The Hodgkin-Huxley model is a mathematical model for the action-potential process in neurons. To understand the Hodgkin-Huxley model one must first know the meaning of reversal potential. The reversal potential, also called the equilibrium potential, is the cross-membrane potential at which there is no net ionic current for a specific ion across the cell membrane. It can be calculated with the Nernst equation. If multiple ions are involved the Goldman-Hodgkin-Katz voltage equation is used instead. The following equation is the Nernst equation.

$$E_i = V_{in} - V_{out} = \frac{RT}{zF} \ln \frac{[C]_{out}}{[C]_{in}} \qquad [2.1]$$

R is the gas constant (1.98 cal/ °K-mol); T is the temperature (°K); F is Faradays constant (96,480 C/mol); z is the charge of the ion; C is the ion concentration inside or outside the cell.

In this study the reversal potential for sodium (Na) and potassium (k) are discussed. The sodium reversal potential is the force that drives the AP currents to its peak positive value (depolarization) and potassium reversal potential is the force that drives it down to its lowest value (repolarization and hyper polarization).

The reversal potential is used when membrane currents are calculated and needed to understand what the driving force concept is. To understand equation 2.1 and coming equations the following circuit is presented. It is a basic component in the Hodgkin-Huxley model.

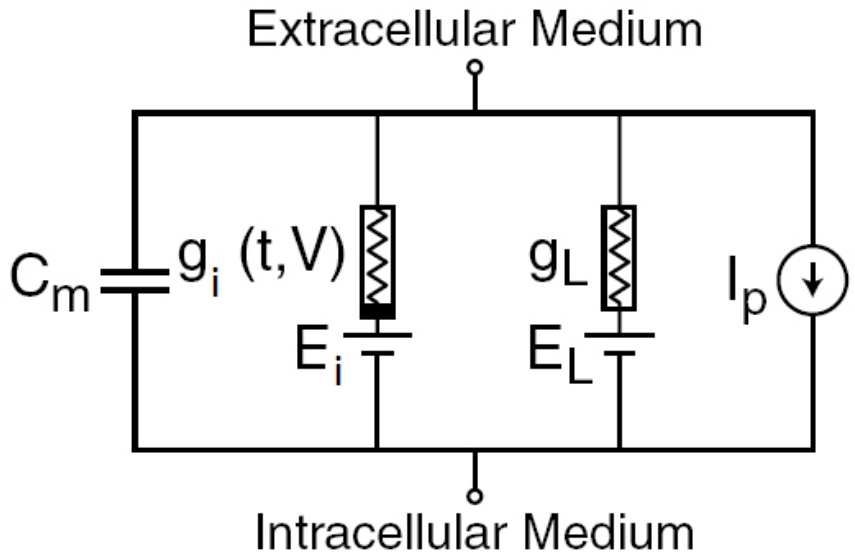

Figure 2.4. This is a basic component for a Hodgkin Huxley type cell membrane equivalent circuit. The membrane capacitance is represented by C_m. g_i and g_L are the membrane conductances (S/cm^2) (where i in g_i represents a specific ion channel). Reversal potentials are denoted by E_i and E_L. Currents caused by ion pumps and exchangers are represented by I_p.[12]

$$I_i(V_m, t) = (V_m - E_i)g_i \qquad [2.2]$$

The reversal potential for a specific ion channel is denoted by E_i. g_i represent the specific channel conductances.

(V_m-E_i) is the driving force that together with g_i gives the ion current. It is simply the difference between the present membrane potential and the reversal potential for that ion. This study focuses on sodium (Na) and potassium (K) since they are of great importance regarding the action potential (AP), unlike chloride (Cl) and calcium (Ca). To calculate the total current across the membrane, instead of a single ion channel, we have the following equation.

$$I_m = C_m \frac{dV_m}{dt} + I_k + I_{Na} + I_L + I_p \qquad [2.3]$$

If equation 2.3 is compared to Figure 2.4, one can see that all the components are parts of the total membrane current. Each ion channel current (e.g. I_k) is calculated with equation 2.2.

To completely understand the Hodgkin-Huxley model, one must look more carefully on g_i, which is nonlinear and contains the complexity of the Hodgkin-Huxley model. The aim in this section was only to give an overview and to understand the terminology used in the report.[12,13]

2.5 Neuron model

The model consists spatially of an axon divided into a branch and parent part. In the simulations, an action potential (AP) is initiated by a small square-wave current. The pulse

propagates along the branch and the parent axon. It might not propagate at all though, if the current stimulus is lower than the required threshold. Instead, it fades out at the beginning of the branch. Some parameters that one can change are stimulation current, number of stimulations, time between stimulations, the theta space and the diameter. The following figure is an illustration of the simulation model.

Figure 2.5. The long Parent model used in Neuron for simulations. The short parent model is 1 cm instead of 10 cm. Figure taken from ref 1.

Figure 2.5 shows the model used for the simulation in Neuron. Some simulations were done with a long parent (10cm) and some with a short parent (1cm). The AP starts from left in the beginning of the branch axon, propagates to "Cone" and along the parent until the end of the parent axon. The time it takes for a pulse to propagate from the beginning of branch to end of parent is called latency. The following figure shows what theta is and why area-units are not used in the coming figures. [1]

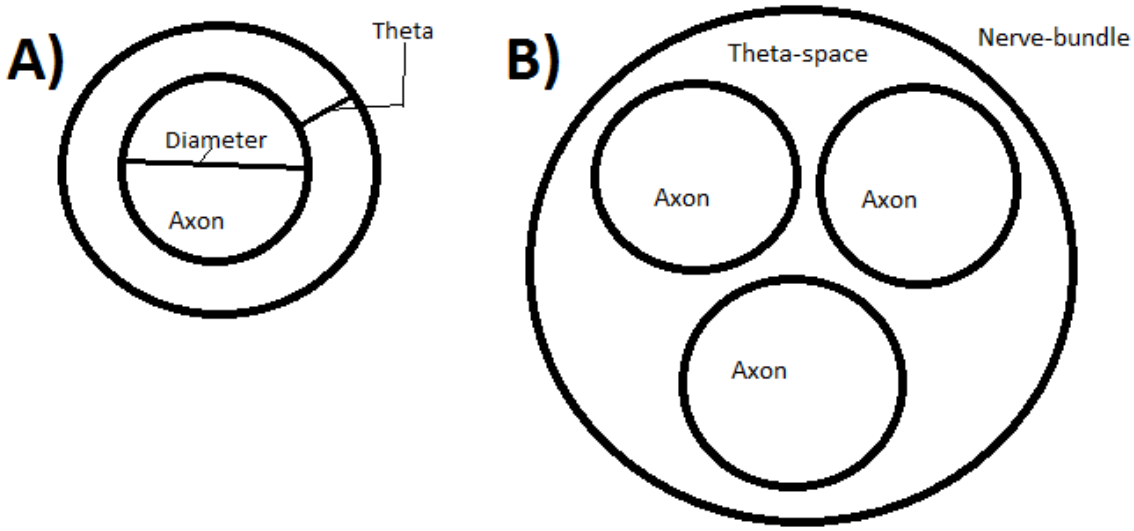

Figure 2.6 . A) Cross-sectional view of Neuron model axon. B) A bundle of nerves.

Figure 2.6 shows how theta is changed in the model. Theta is the space between axons and not a membrane. The Neuron model use this setup and that is why theta (length) is used and not theta-space (area) in the simulation results.

3. Method

First, the latency shift was measured between two consecutive pulses and then the activity dependent latency shift using 39 pulse pairs sent after each other, while changing theta and the diameter. After that, the discovered behavior was explained using reversal potential and conduction velocity with a single pulse as input. Finally, the current threshold behavior for different theta and diameter values was measured. Five simulations were conducted for measuring Latency shift, activity dependant latency shift, reversal potential, conduction velocity and current threshold.

3.1 Latency shift

Pulse latency means the time it takes for a pulse to propagate from the beginning to the end of the axon. Latency shift was demonstrated in two ways. The first is called latency shift, and is difference between the propagation times of pulse 2 and 1. The second is called relative latency shift, and is the fraction between pulse 2 and 1. These were used to get an understanding of the relationship between the propagation times of the two pulses. The conduction velocity and reversal potential were used to explain this relationship. The following figure illustrates how latency shift and relative latency shift was calculated.

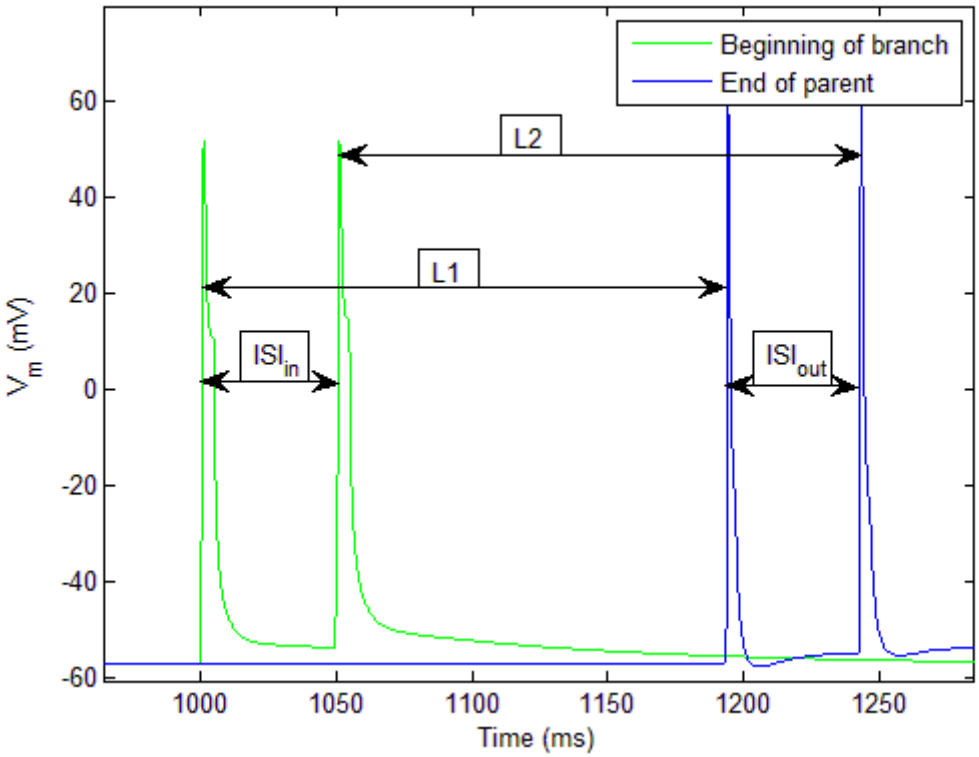

Figure 3.1. Two consecutive pulses measured at two locations, in the beginning of branch (green) and the end of parent (blue).

Figure 3.1 shows the meaning of ISI, which represents the inter spike interval for the pulses sent in and received out. It also shows the latency for pulse 1 (L1) and pulse 2 (L2). The following equation is used for calculating the latency shift.

$$Latency\ shift = L2 - L1 \quad [3.1]$$

The following equation is used for calculating the relative latency shift.

$$Relative\ latency\ shift = \frac{L2}{L1} \quad [3.2]$$

The following simulation was conducted to measure the latency shift:

Simulation 1
Measurement: Latency shift (LS)
Input: 2x1 pulses
Parameters: Theta, diameter, ISIin
Model: 10 cm parent

3.2 Activity dependant latency shift

Activity dependant latency shift (ADLS) was measured to see if the theta or diameter changes have an impact on the difference between the latency shift of the first and last pulse pair. By comparing the first and the last pulse pair, the activity dependent latency shift (ADLS) can be analyzed. As said in chapter 3.1 latency shift is the difference between the propagation time of two pulses and relative latency shift is the quotient of two pulses. The long parent model was used and measurements were taken in the end of parent. For the pulses in the following figures an ISIin of 50 ms was used. IPI means inter pair interval, and represents the time between double pulses at the beginning of the axon. 39 pulse pairs were used as input, which results in 78 pulses.

The following simulation was conducted to measure ADLS:

Simulation 2
Measurement: Activity dependant latency shift (ADLS)
Input: 2x40 pulses, ISIin = 50ms, IPI = 1000 ms
Parameters: Theta, diameter
Model: 10 cm parent

3.3 Reversal potential

In order to understand latency shift, the reversal potential for potassium and sodium was measured. The following simulation was conducted to measure the reversal potential.

Simulation 3
Measurement: Reversal potential
Input: Single pulse
Parameters: Theta & diameter
Model: 1 cm parent

3.4 Conduction velocity

In order to understand latency shift, the conduction velocity was measured in the following simulation.

Simulation 4
Measurement: Conduction velocity
Input: Single pulse
Parameters: Theta & diameter
Model: 1cm parent

3.5 Current threshold

The minimum current stimulation needed for a complete pulse to propagate was measured. The following simulation was conducted to measure the current threshold.

Simulation 5
Measurement: Current threshold
Input: Single pulse
Parameters: Theta & diameter
Model: 1 cm parent

3.6 Swelling and Pain

The following figure was made to illustrate what was tested in order to find a connection between swelling and neuropathic pain.

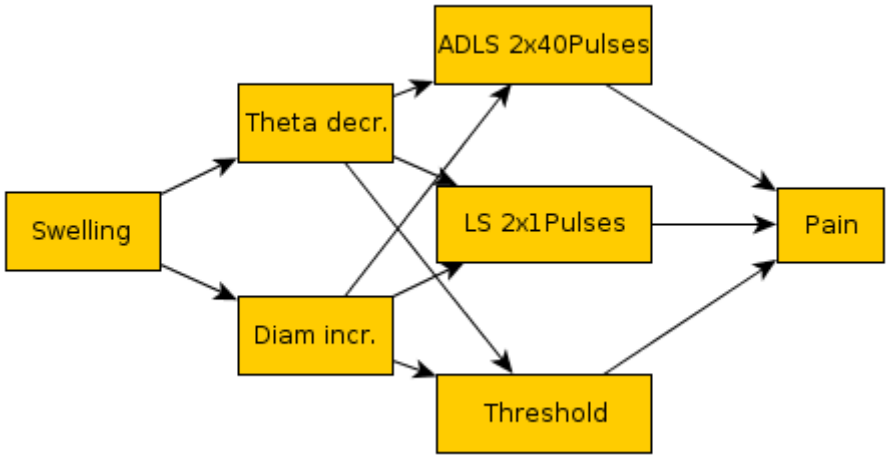

Figure 3.2. Demonstrates the method of solution for the simulation part and the parameters that were tested in order to find a connection between swelling and neuropathic pain.

Figure 3.2 starts from left with an axon in a swelled state pointing to its implications which is increased axon diameter and decreased periaxonal space (theta). The next column of arrows shows the connections that was tested, all of which would lead to pain. ADLS (activity

dependent latency shift) simulates a longer lasting input type while LS (latency shift) simulates a very short lasting input. The threshold part shows if there is any current threshold relationship with the theta space or the axon diameter. ADLS, LS and threshold connections lead to neuropathic pain. As you can see the reversal potential and conduction velocity simulation is not included. Their purpose is to achieve an understanding of the ADLS, LS and threshold behavior.

3.7 Default and interval values

3.7.1 Default

In most of the figures in the results chapter, the default value is highlighted with the label 'def' or 'default'. This is interesting, because it gives a perspective on the range of values that has been used. These are the default values;

- $th_{theta} = 2 = 29nm$. In figures where theta is used, th_{theta}-axis is multiplied by 14,5nm.
- $kd_{diam} = 1 = [p = 1\mu m, b = 0.25\mu m]$. kd_{diam}-axis in figures is multiplied by p representing the parent diameter and b representing the branch diameter.
- Current stimulation = 0.01nA. This is the default current that initiates the AP.

3.7.2 Intervals

This section will prepare the reader to understand how the intervals were chosen. A phenomenon called AP bursting often appeared when high or low parameter values were used. AP bursting means that APs initiate spontaneously close to another AP without any current stimulation. Other values were chosen when APs did not propagate at all, or when it seemed high or low enough.

4. Results and Discussion

First, the latency shift results from simulation 1 are presented and discussed, followed by the ADLS results from simulation 2. Their behavior is explained by the results in the following two sections about the reversal potential and conduction velocity from simulation 3 and 4. Finally the results of the current threshold from simulation 5 are presented and discussed.

4.1 Latency shift

In the following figures the data from simulation 1 is analyzed. Two consectutive pulses were used as input while changes were made to the diameter, theta and ISIin.

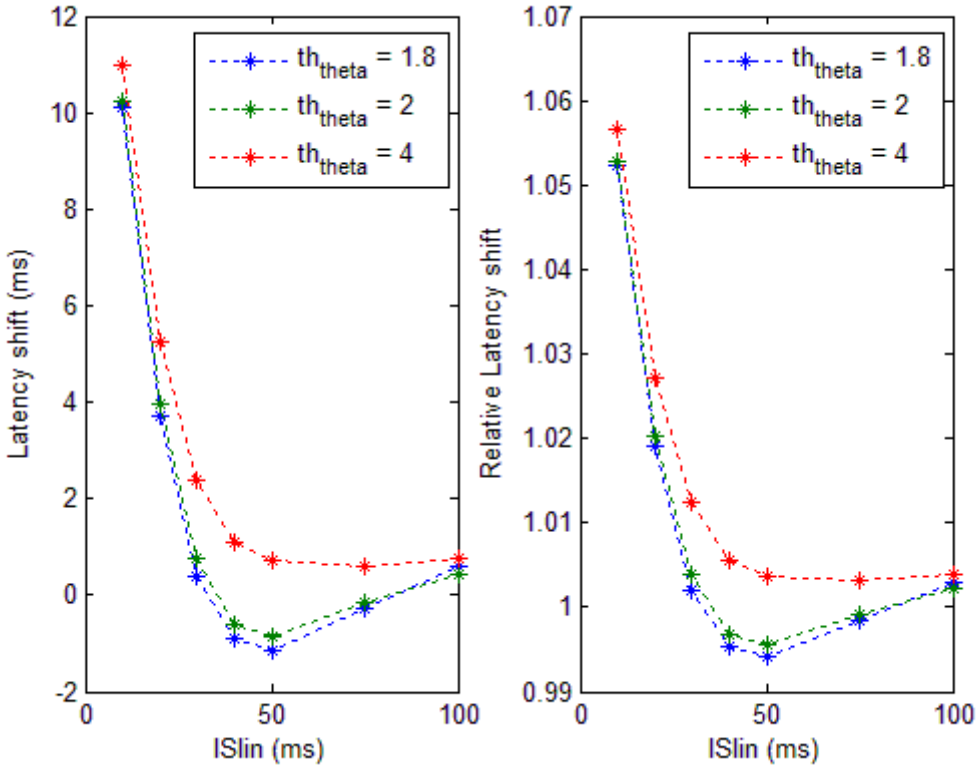

Figure 4.1. Latency shift and relative latency shift of two pulses for different theta and ISIin values. (ISI = inter spike interval)

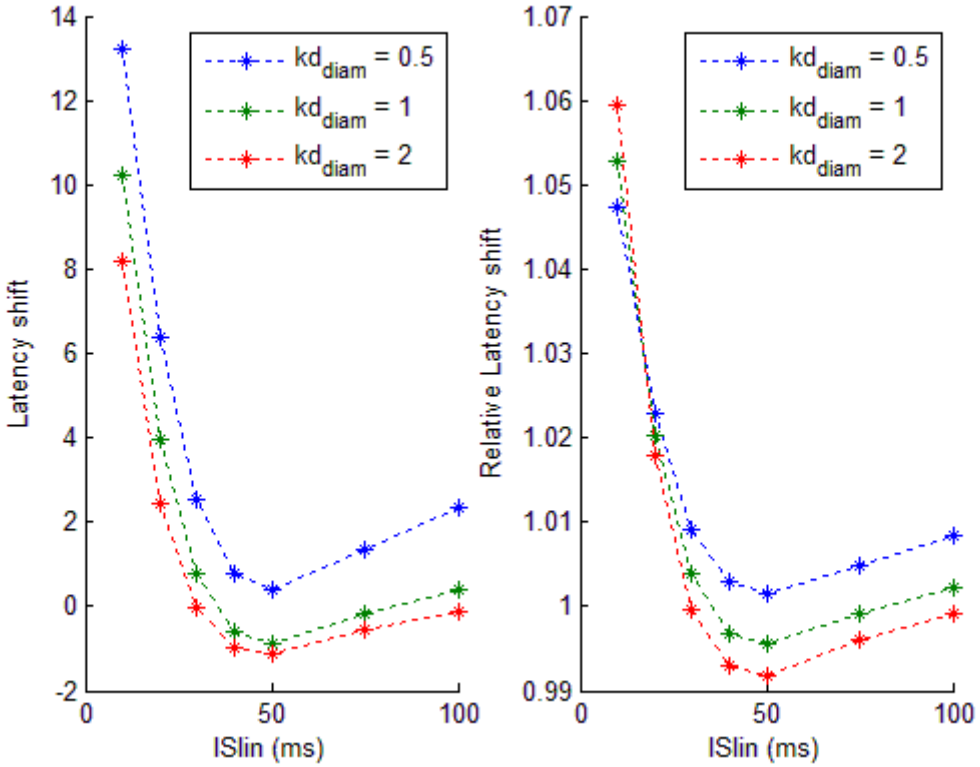

Figure 4.2. Latency shift and relative latency shift for different diameter and ISIin values. (ISI = inter spike interval)

As seen in Figure 4.1 and Figure 4.2 swelling, i.e. increased diameter and decreased theta, makes the relative latency shift lower. A lower relative latency shift means having a higher output frequency at the end of the axon. The relative latency shift is lower at ISIin-values between about 20 and 60 ms. In the right graph of Figure 4.1 and Figure 4.2 some values of the y-axis are equal to or go below 1. This is interesting because it means that the second pulse is faster than the first pulse. This means that the output frequency of pulses can be bigger than the input frequency and perhaps cause a non painful touch to be interpreted as painful. This is explained more in the reversal potential part, chapter 4.3.

4.2 Activity dependent latency shift

The following figures show an analysis of data from simulation 2, where 39 double pulses were used as input. The first and last pulse pairs were compared.

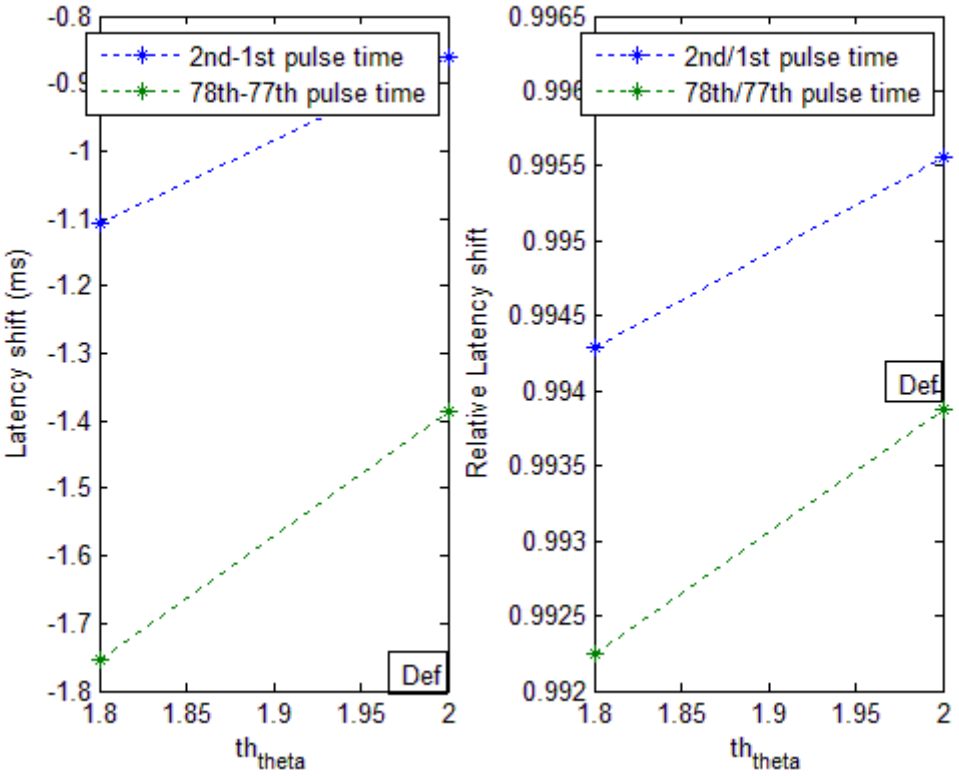

Figure 4.3. Activity dependent latency shift against theta. Measurements were taken at end of parent.

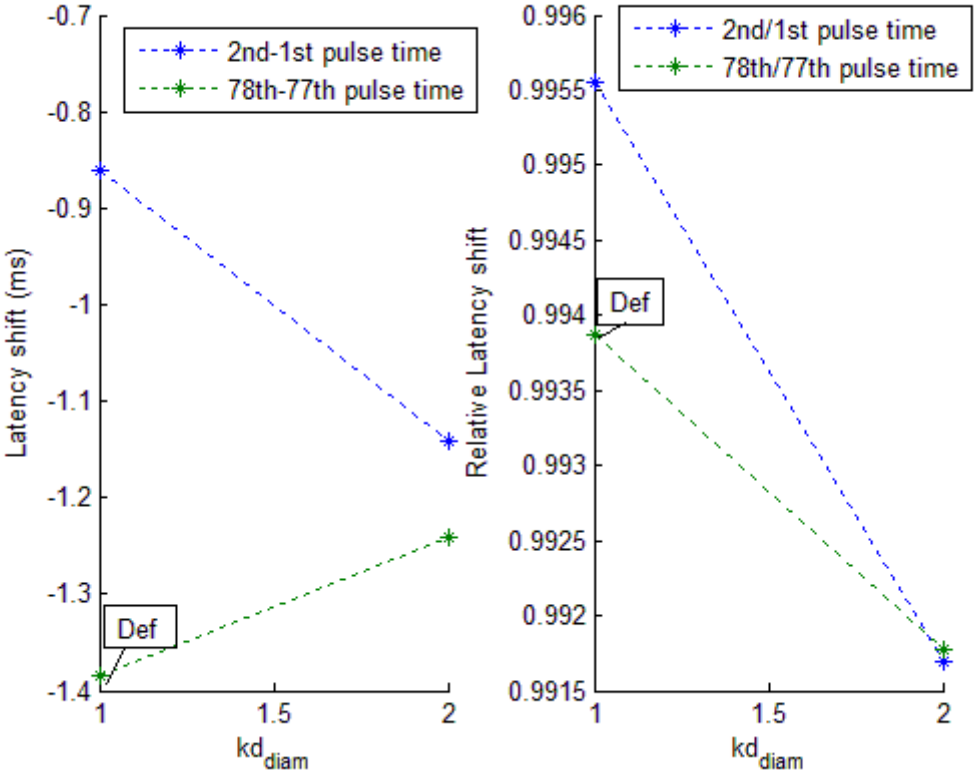

Figure 4.4. Activity dependent latency shift against diameter.

In Figure 4.3 we see that the latency shift increases in the time between the first and the last pulse pair coherent with the theta change. The ADLS increases as theta decreases. In Figure 4.4, where the diameter is changed, the same effect is not present. The increase of diameter (swelling) does not create a bigger ADLS, in fact it has the opposite effect but to a very low degree. The behavior is not explained since it involves various ion-channel currents, which has not been studied sufficiently during this project. If there is ADLS, it will indicate that longer lasting inputs also cause pain. Therefore we can conclude that a swelling can be a causal reason for longer lasting pain inputs, but not because of the diameter increase.

4.3 Reversal potential

The following figures show raw data and analyses of data from simulation 3 where the reversal potential for potassium (K-rev) was measured while changing theta and diameter.

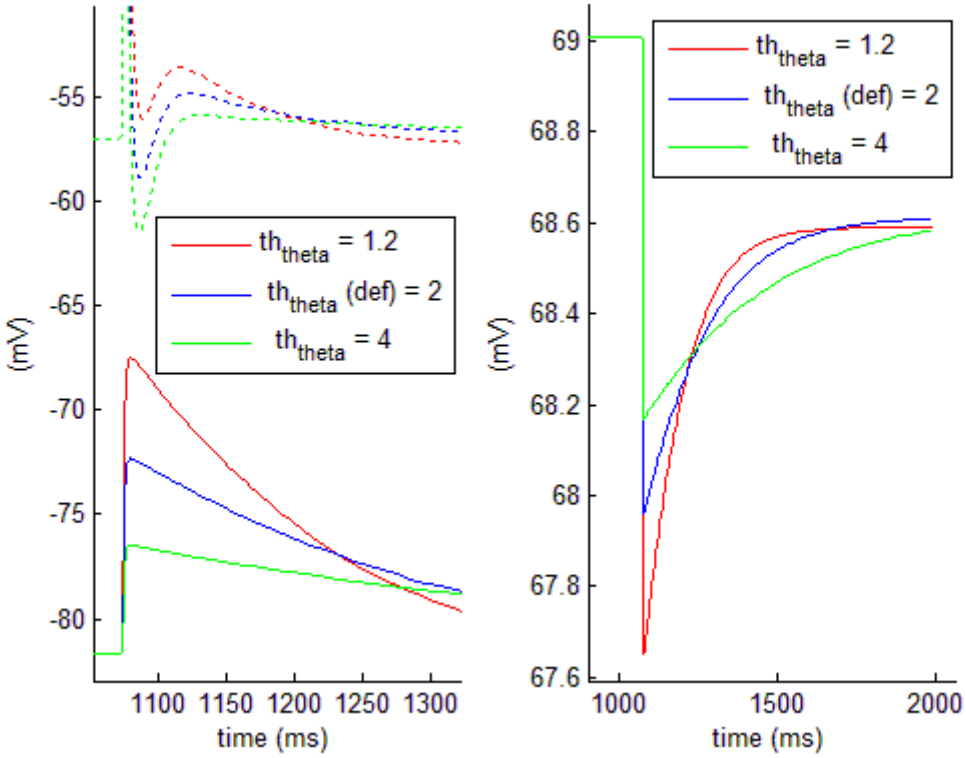

Figure 4.5 Left graph: Raw data of potassium reversal potentials (parent) in the lower half and AP in the upper half for theta changes. Right graph: Sodium reversal potentials (parent) for theta changes.

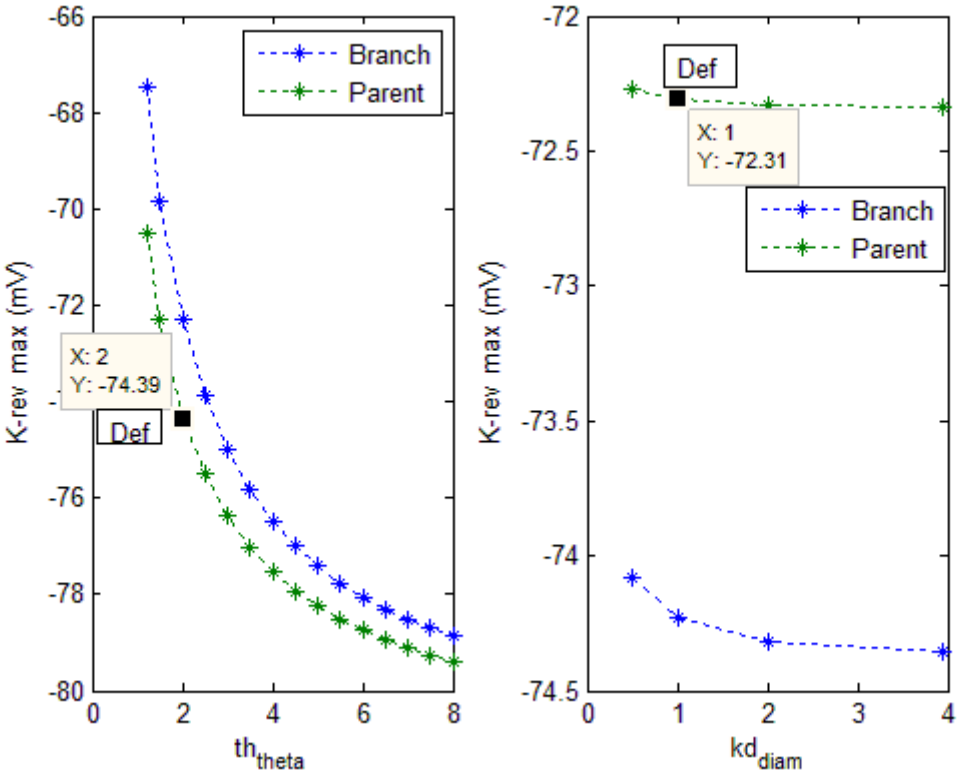

Figure 4.6. Left graph: Maximum reversal potential for potassium (K-rev) against changes in theta. Right graph: Maximum potassium reversal potential against diameter.

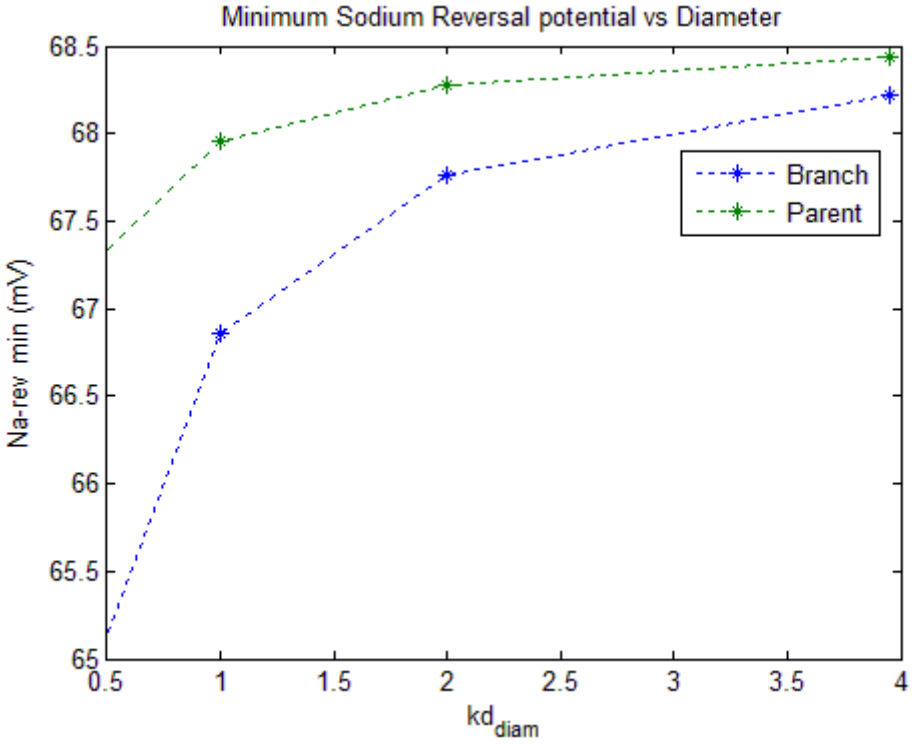

Figure 4.7. Reversal potential for sodium (Na-rev) against changes in the diameter.

When the theta space is increased K-rev becomes more negative. Since theta is the space outside of the axon, a higher theta would give a lower extracellular potassium concentration, because of the space being increased but not the number of potassium ions. When swelling occur (decreased theta) the extracellular potassium concentration is increased and a weaker reversal potential acquired as seen in the left graph of Figure 4.6.

The present membrane potential minus the reversal potential is the driving force of the AP currents. Sodium currents drive the potential up and depolarize the potential. Potassium currents drive the potential down and hyper-polarize potential. A higher extracellular potassium concentration means a less negative reversal potential, as seen in equation 2.1. A weaker potassium reversal potential means less hyper polarization.

Changing the diameter seems to have no effect on the maximum potassium reversal potential. Looking at the minimum sodium reversal potential in Figure 4.7 while changing the diameter instead, we see that a higher diameter gives higher reversal potential. The change in reversal potential seems to be too low to make a difference though (~3mV).

When theta is low we get a weaker (less negative) K reversal potential. A weaker reversal potential means that the AP is hyper polarized less and is closer to the threshold, see Figure 4.5. When the second pulse is generated it needs less time to reach the AP threshold than the first pulse, and gets a shorter propagation time, hence a higher conduction velocity.

4.4 Conduction velocity

In the following figure the conduction velocity data from simulation 4 is analyzed.

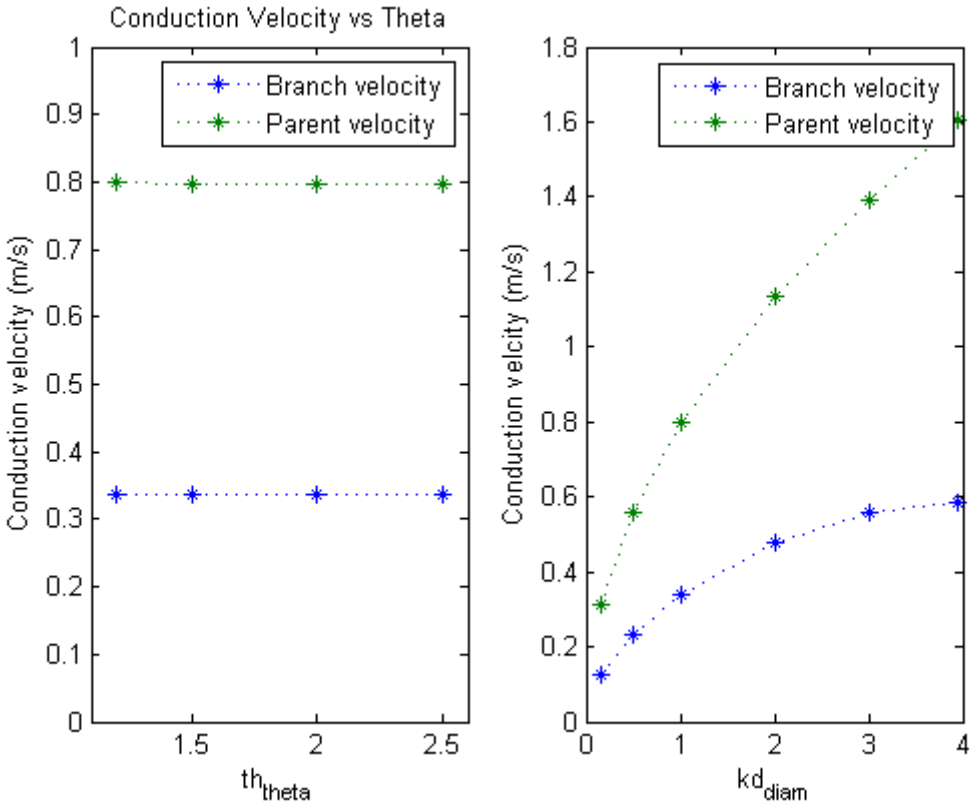

Figure 4.8. Left graph: Conduction velocity along the axon was measured for branch and parent, while changing theta and diameter.

As seen in Figure 4.8, there is a connection between the diameter and the velocity of the pulse along the axon. An increased diameter means decreased axial membrane resistance. A lower axial resistance should indeed give a higher conduction velocity. Changes of theta instead of diameter generated approximately the same value. Knowing these relations is important to understand latency shift completely. The fact that the distance of the relative latency shift-curves (diameter change) is less than in latency shift-curves (ms) can be explained by the conduction velocity-diameter relationship. By dividing pulse 2 latency with pulse 1 latency we compare the pulse latency regardless of entities. Therefore this should apply for different axon lengths.

4.5 Threshold current

In simulation 5 the minimum current stimulation needed for a complete pulse to propagate was measured for different diameter values. The model did not show any pulses that had faded in the middle of the axon. They either faded instantly or propagated to end of the axon. Values for the current were tested with 0.001 nA as lowest step size (def=0.1nA).

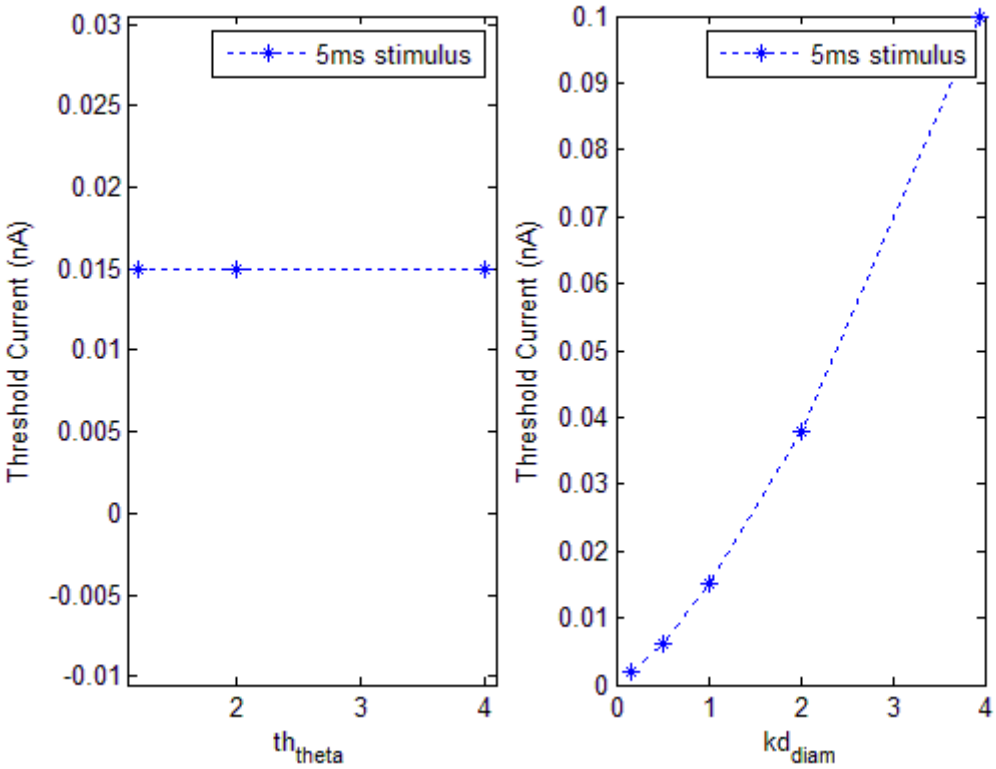

Figure 4.9. Minimum current stimulation needed for a pulse to propagate for a set of diameter values. Threshold current values were tested with 0.001 nA as lowest step size. Default was 0.1 nA.

Figure 4.9 shows that a larger diameter creates a greater space for the current to spread. Hence more current is needed to initiate an AP. We can conclude that the threshold current is not a causal reason for the pain. The threshold even increases when diameter is increased, which makes one wonder if there really is swelling at beginning of the axon where current stimulation takes place. Theta changes have no effect on the current threshold.

5. Conclusion

This section presents a summary of the conclusions and then presents some possible future hypothesis. It also gives some feedback on the code used for simulations and finally a cautious suggestion to what a medicine for this kind of pain-state would need to achieve biologically to reduce pain.

5.1 Swelling

If the following figure is compared with Figure 3.2, the reader can see that some arrows have been removed and some have been highlighted. The following figure is a demonstration of the new relationships between swelling and pain that were found.

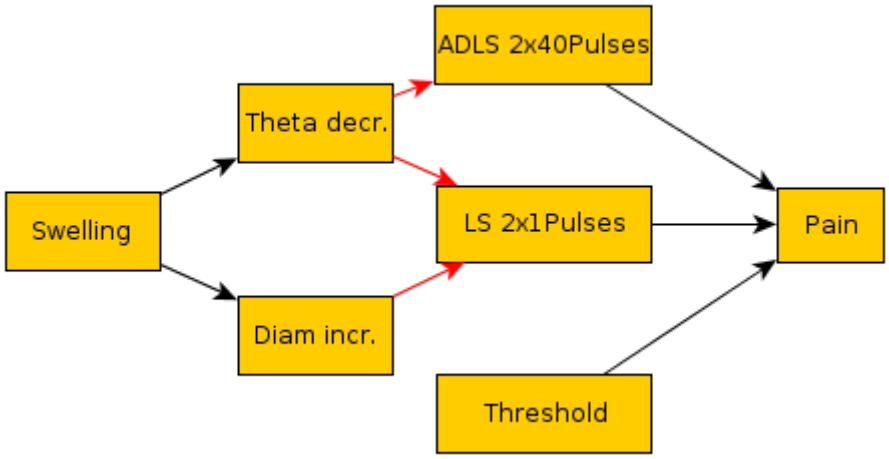

Figure 5.1. The resulting connections between swelling and pain that were found.

As seen in Figure 5.1 there are some connections that were confirmed. They can also be found in the following list which is a summary of the conclusions from chapter 4:

- When sending two pulses (LS 2x1 Pulses) and having a low theta and high diameter (swelling) the out frequency will be higher than in frequency and cause short lasting non-painful inputs to feel painful.
- When sending long pulse pair trains (ADLS 40x2 pulses) decreasing theta will cause a higher output frequency than input frequency. Diameter changes give the opposite effect and reduce out frequency slightly. We conclude that swelling can cause longer lasting non-painful inputs to feel painful, but not because of the diameter increase.
- A weaker potassium driving potential leads to less hyper polarization, which explains why the second pulse propagate faster than the first pulse. The membrane potential is closer to the AP threshold.
- Conduction velocity is affected by diameter changes, but not by theta changes.

- A lower threshold current is not a causal reason for pain. The threshold is in fact increased in the swelled state.
- We found that swelling can be a reason for pain, but not because of a lowered threshold.

When using a mathematical model with a lot of parameters to draw conclusions it is important to remember that it is a model of reality and not reality itself.

5.2 New hypotheses

During the project the following possible new hypotheses were formulated.

- There is less swelling in the most peripheral parts of neuropathic axons
- The AP bursting phenomenon can take place in real axons in swelled state

5.3 Improvements

This section presents improvements that could be made to the simulation code. AP bursts, which was discussed in chapter 3.7.2, occur when APs initiate spontaneously close to another AP without any current stimulation. Visualization of AP burst at low/high interval values was something that the code had not been implemented to do. It could be interesting to see. I did not experience any bugs in the code.

5.4 Medicine

I suggest that a future drugs for this kind of pain state aim to achieve the following changes:

- Decrease diameter or increase theta (perhaps with some anti inflammatory agent)
- Increase potassium reversal potential so that latency shift is prevented (perhaps by injecting potassium somehow)

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