Collision Between Nerve Signals

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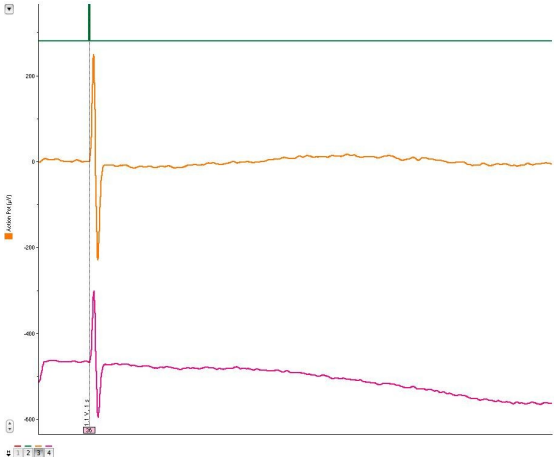

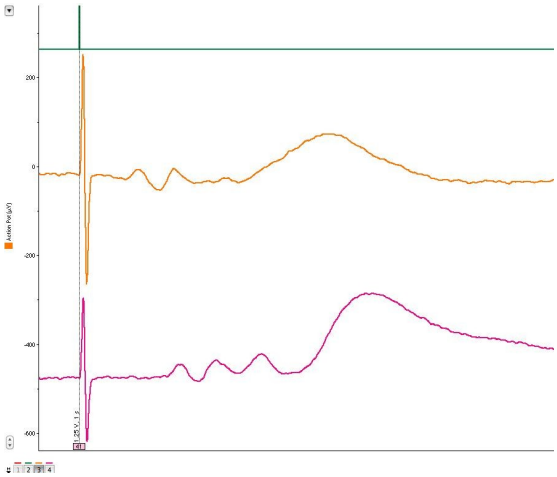

Bachelor Project
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Abstract

In this project I will investigate what happens when two nerve signals collide. The collision will be created by stimulating a nerve from the right side and the left side simultaneously. In this way two nerve signals will traverse in opposite directions along the nerve and meet in the middle. By comparing properties of a traveling signal from right and from left respectively, to the properties of two traveling signals, I will investigate the outcome of a collision between two nerve signals. The general view is that when the two signals meet they will annihilate each other so that both waves disappear after they meet. I find that this is in fact not true. My result suggest that the two signals don't affect each other so that each wave goes through the other and has the exact same properties before and after collision has taken place.

Contents

1 Introduction

1.1 Lipids and membrane proteins

A cell membrane mainly consist of lipids and proteins. Lipids are long hydrocarbon chains with a charged head group in one end(see figure 1.1). The hydrocarbon chains are similar to fatty acids and will not mix with aqueous solutions. The head group is polar and like ionic salts this part of the molecule will mix well with aqueous solutions. Therefore a lipid is said to be an amphilic molecule, which refers to the fact that one part of the lipid(the headgroup) mix well with water while the other part (the carbon chains) don't[1].

Proteins are chains made of amino acids. There are 20 different amino acids that make up proteins. A membrane protein is a protein found in a biological membrane. There are two types of membrane proteins. Integral proteins that have a strong interaction with the membrane and peripheral proteins that have a much weaker interaction with the membrane(see figure 1.2 and 1.3).

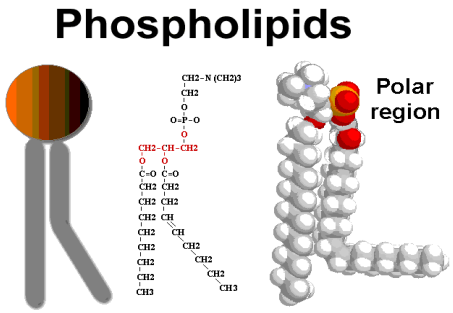

Figure 1.1 Schematic and chemical structure of a phospholipid [2]

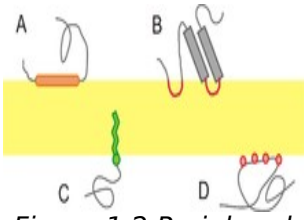

Figure 1.2 Peripheral proteins [3]

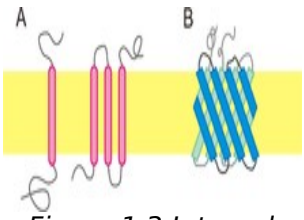

Figure 1.3 Integral proteins [3]

1.2 The cell membrane

The effect of the amphilic property of lipids is that in the cell membrane the lipids form a so called two dimensional lipid bilayer as seen on figure 1.4. Two layers of lipids are connected to each other. In each of the two layers the head groups form a wall against the water inside and outside the cell respectively. Because of this structure the hydrocarbon chains is shielded from water in the cell membrane. Proteins are embedded in the membrane. The membrane contains lumps and clusters of proteins and lipids so the proteins and lipids are not evenly distributed in the membrane.

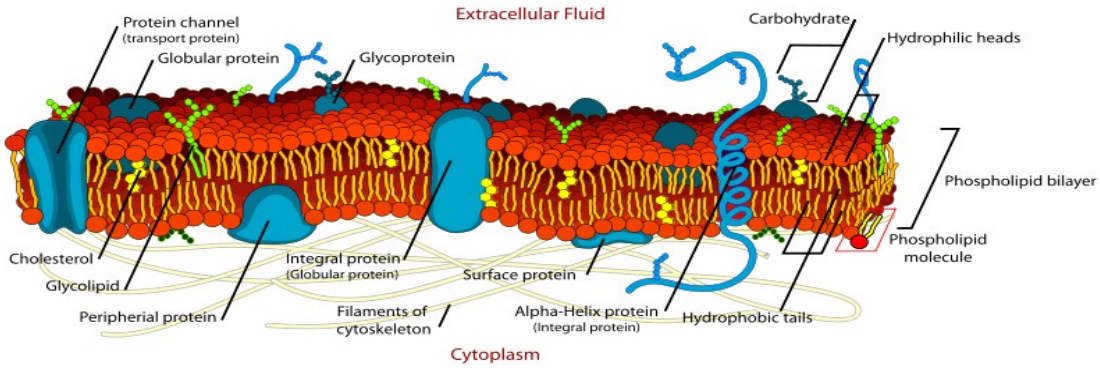

Figure 1.4 Example of a cell membrane [4]

A cell membrane keeps the interior of the cell with all the organelles together. The properties of a cell membrane allows the cell to maintain and regulate the concentration if ions and other components in the cell. A lipid bilayer is a very good electric insulator. This means that since ions are charged ions can't go through a lipid bilayer. But the protein structures embedded within the lipid bilayer form so called ion channels in the membrane. Ion channels are certain areas of the membrane where the presence of a specific protein makes it possible for a specific ion to diffuse through the membrane a that specific area. Through forming of ion channels the proteins within the membrane allows for regulation of ion concentration within the membrane.

1.3 The Nervous System

The nervous system is what allows humans and animal to react to our surroundings and gives us consciousness. The nervous system is basically a network of nerves that can send and receive nerve signals from and to one another. The nervous system of vertebrate animals, which include humans, can be divided into two parts. The central nervous system and the peripheral nervous system. The central nervous system consist of the brain and the spiral chord. The spiral chord is connected to the brain and signals to and from the brain goes through the spiral chord. The central nervous system is connected to the peripheral nervous system. The peripheral nervous system consist of sensory neuron that detects sensory inputs from the outside. The peripheral nervous system also consists of nerves connecting these sensory neurons to the central nervous system. It also consist of motor neurons and connections between motor neurons and the spinal chord. Motor neurons are neurons that can send signals to muscles to produce muscle movement. Through the dynamic between these systems the body can adjusts and react to external surroundings and input.

Since the nervous system mainly consists of nerves, the properties of nerves and the way signals are transmitted in nerves are a very important and interesting scientific

subject.[5]

1.4 Vertebrates/ and invertebrates

Vertebrates are animals with a spinal chord and a backbone. For example reptiles, mammals and birds. Invertebrates are animals without a backbone. For example insects, worms and crabs(see figure 1.5). The nervous system in vertebrates are unique to vertebrates and vertebrates are the only group of animal that have a proper brain. 3-4 % of all animal species are vertebrates. The vast majority of animal species are invertebrates[6].

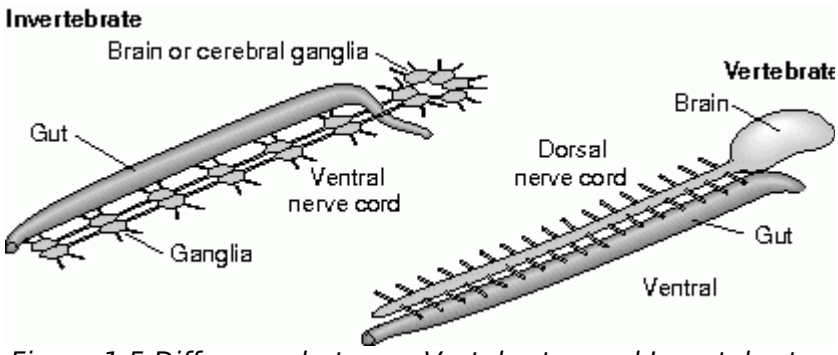

Figure 1.5 Difference between Vertebrates and Invertebrates [7]

1.5 Nerve cells/Neurons

A nerve cell or neuron can receive a nerve signal from other nerve cells and pass it on to other nerves. A nerve consist of a body called the soma, dendrites and the axon. The soma contain a nucleus and a lot of other molecules needed to keep the nerve cell alive and functioning. The dendrites can be considered as branches which reach out and connects to other nerve cell's. If a nerve cell is stimulated sufficiently a nerve signal will propagate down the axon. When the nerve signal reaches the end of the axon, dendrites from other nerve cells connected to the axon can intercept the signal and the signal can be passed on to axons of other nerve cells through the dendrites.

A nerve is a so called excitable media. If the total signal or stimulation sensed from all dendrites in a nerve exceeds a certain threshold value, the nerve will be activated and send a signal through its axon. If the total stimulation sensed from the dendrites is below the threshold value, then nothing happens. As long as the stimulation is above the threshold value the signal that the neuron is sending is independent of the strength of the stimulation received. Immediately after a neuron has send a signal the neuron

spends a certain period in refractory state where it can't send another signal. The time that the neuron spends in this state is called the refractory time.

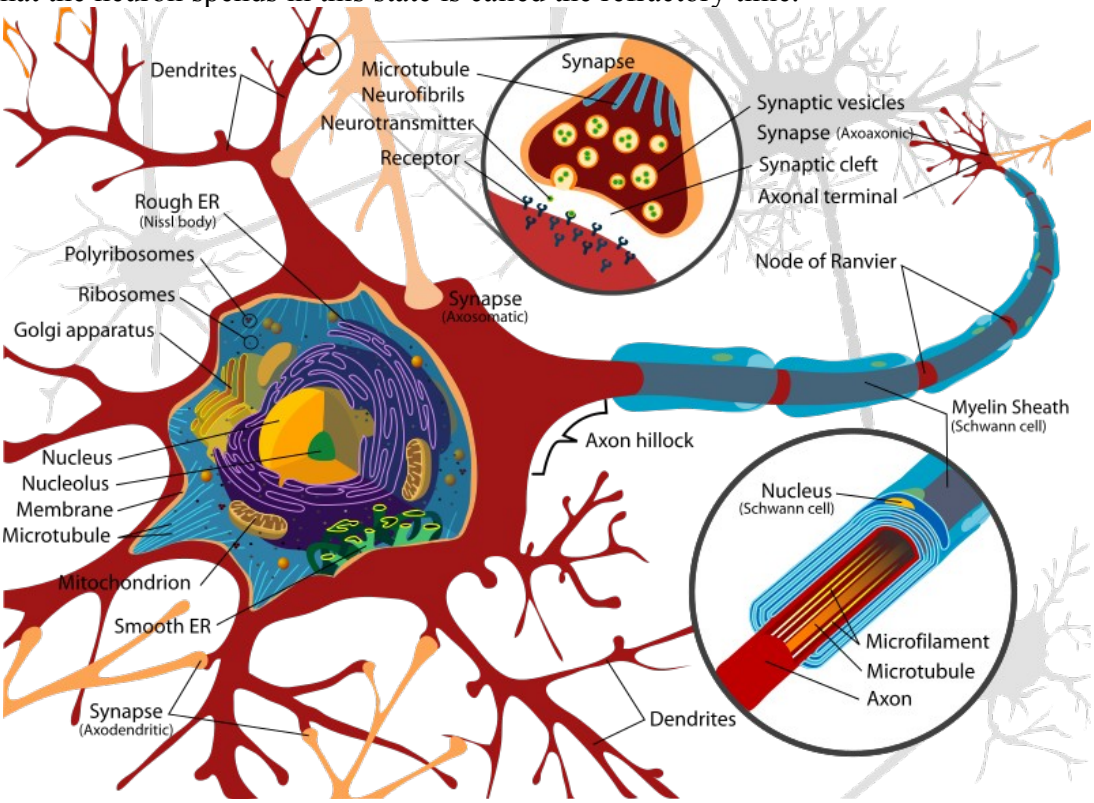

Figure 1.6 Schematic picture of a nerve cell [8]

Neurons are often classified into three main types depending on their role in the nervous system. Sensory neurons and motor neurons which was mentioned earlier and inter neurons. Inter neurons are neurons that from a connection between other neurons. Inter neurons are neither sensory or motor neurons.

But neurons can also be classified into four main categories according to their structure. Unipolar, Pseudounipolar, multipolar and bipolar(see figure 1.7). A unipolar neuron only have one projection from the soma. A pseudounipolar neuron is a sensory neuron where the axon has split into two branches where the one branch goes to the spinal chord and the other branch goes to skin muscle or joint. A multipolar neuron possesses one axon and a lot of dendrites. Multipolar neurons include motor and interneuron and the majority of neurons in the brain are multipolar neurons. A bipolar neuron is a neuron where the dendrites and the axon are on opposite sites of the soma. Bipolar neurons are specialized sensory neurons.

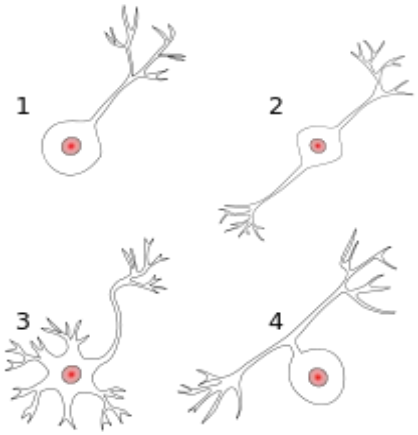

Figure 1.7 The four main structural types of nerves. 1: Unipolar neuron 2: Bipolar neuron 3: Multipolar neuron 4: Pseudoinupolar neuron [9]

Myelination:

In this project I have worked with earthworms and recordings has been made of nerve signals propagating through the so called median and giant fibers in earthworms. These fibers can, for all practical purposes, be regarded as long axons running through the entire length of the worms. An important aspect of the The median and giant fibers in relation to this experiment, is that they are covered by a myelin sheat.

A myelin sheath is one or more layers of myelin covering a neuron. Myelin is an insulating material and consist of water, lipids and proteins. The myelin sheat makes nerve signals propagate faster through the nerve. The myelin layer also increases electrical resistance and thereby helps to maintain electrical current within the neuron.

1.6 Ion channels

Ion channels are membrane proteins that mediate the transport of different ionic species between the inside and the outside of the cell [10]. A number of ions exist in one ionic concentration inside the cell and another ionic concentration outside the cell. These concentration differences give rise to a different amount of charge on each side of the membrane or an electrical gradient across the cell membrane. The membrane potential is the difference between the electric potential inside and outside the cell caused by these ions.

The lipid bilayer acts as an insulator so the ions cannot pass through the lipid part of the membrane. But proteins are embedded in the membrane and some protein can creates a channel where a given type of ion can pass through. Such channels are called

ion channels. If an ion channel is open the related type of ions can pass through the ion channel and if the ion channel is closed they can't. Some ion channels called voltage gated ion channels are open with a probability given by the membrane potential.

The combination of the insulating property of the lipid bilayer and the ion channels in the membrane means that the cell membrane is selectively permeable. This means that for some ions the membrane is impermeable and acts like a strong insulator while for other ions the membrane is permeable because of some open ion channels.

If the membrane is permeable for an ion two forces acts on the ion. One force trying to even out the concentration on each side of the cell and another stemming from the membrane potential (charge gradient) and the fact that an ion is charged. Each type of ion has its own equilibrium potential given by the nerst equation. The equilibrium potential is the membrane potential where these two forces acting on the ions are equally strong. The membrane potential will naturally go towards the existing equilibrium potential. If the membrane potential changes the permeability for each type of ion is altered. That means that the equilibrium potential changes and the membrane potential will go towards the new equilibrium potential.

1.7 Signal transmission

A sodium channel is a voltage gated ion channel that allows sodium ions to go through the membrane if they are open [11]. If the membrane potential increases locally, the probability for sodium channels to be open also increases locally. That gives rise to a local current of positive kalium ions into the cell which increases the potential which again increases the probability for sodium channels to be open. That means that a stimulation of the membrane will initiate a positive feedback loop based on sodium channels.

Potassium channels allow for transport of positive potassium ions out of the cell and thus a negative feedback loop for membrane potential is also initiated when the membrane is exposed to a stimuli. The positive sodium channel feedback loop is faster than the negative potassium channel feedback loop, so the membrane potential will first increase for a while until the negative potassium feedback process kicks in.

When an ion channel has been open for some time it will close itself or inactivate itself.

All this means that a quick shift in membrane potential will then initiate a few positive and negative feedback processes of activation, deactivation and inactivation of potassium and sodium channels. These processes of activation,deactivation and inactivation are all connected through the membrane potential in a little network of feedback loops and the interplay between these processes cause the membrane potential to first increase until a maximum is reached and then fall back to equilibrium, when exposed to a stimuli.

A cell membrane has a uniform resting potential of about -60mV. At this potential the membrane is permeable for potassium. If the influx rate of sodium is not great enough the efflux of potassiumions flowing out of the cell will offset the effects of sodium flowing into the cell. This can explain the all or nothing behavior of nerve signals.

If the depolarization sensed by nearby sodium channels is sufficiently great, a new network of processes will be initiated further down the membrane, and as a result of that a signal will propagate down the membrane. Otherwise no signal will be seen.

1.8 Motivation

In the previous chapters it is described how nerve cells transmit nerve signals according to an all or nothing response to a stimuli. It is described how a nerve signal travels through the membrane and the part ion channels play in signal transmission. But there has been no mathematical description of how a nerve-signal propagate.

There exist two mathematical models for signal propagation in nerve cells, which deserves to be taken serious. The first one is the Hodgkin-Huxley Model(HH) and the second one is the very new Soliton Model. They both have differential equations which can be used to make simulations and thereby make predictions for the propagation of a nerve signal. This experiment investigates what happens when two nerve pulses collide. The HH and the Soliton Model predict two different outcomes of this experiment. Therefore this experiment can potentially falsify one of or both of the models.

The collision between two nerve signals have been investigated previously by Tasaki [12]. He concluded in a collision experiment that the transmission of pulses is blocked. Therefore according to this reference the nerve signals annihilate each other. According to the soliton model simulations the signals should go through each other without annihilation [13]. In this bachelor project I will perform new experiments to investigate the collision between two nerve signals.

2 Theoretical Models

In this section I will introduce the two theoretical models for signal propagation in nerve cells [14].

2.1 The Hodgkin-Huxley model

In the Hodgkin-Huxley model the cell membrane is treated as a capacitor and the protein or ion channels are treated as voltage dependent resistors. Based on their experiments on nerve axons Hodgkin and Huxley concluded that the membrane contained components acting as independent voltage and time dependent conductors of k^+ and Na^+ ions [14]. They treated the membrane as an insulator that acts as a capacitor and constructed a model of the electrical circuit in a membrane. Based on this circuit they came up with a differential equation for the flow of current through the membrane. The Hodgkin-Huxley model only contains electric variables unlike the soliton model which is based on thermodynamical variables and considerations. Besides the sodium and potassium channels the circuit also contains a leak current mainly of chloride (cl^-) ions.

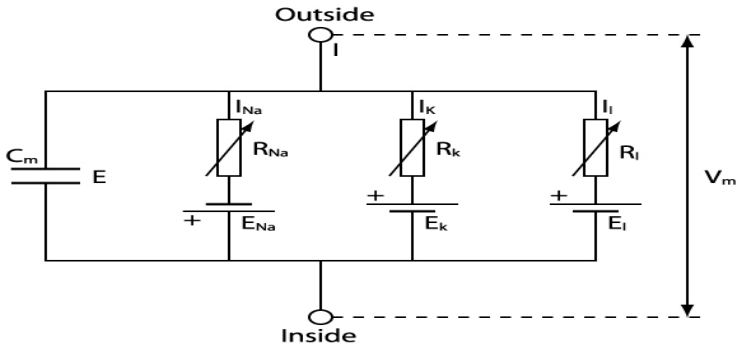

Figure 2.1 Electrical circuit of the Hodgkin-Huxley model [15]

Two currents make up the total current. An Ohmic current and a current charging up the capacitor or membrane. In normal condition where there is no stimuli or external voltage, there is still a current through the membrane because of the different concentrations of ions on each side of the membrane. This give rise to diffusion. The tendency of a molecule to travel from a high to low concentration area. According to the Hodgkin-Huxley model the total current through the membrane is given by:

$$I = C_M \frac{dV_m}{dt} + g_K(V_m - E_k) + g_{Na}(V_m - E_{Na}) + g_l(V_m - E_l) \quad \text{eq. 2.1}$$

E_K,E_{Na} and E_l are the so called nerst potentials for potassium, sodium and chloride ions. The nernst potentials is what accounts for the tendency of a molecule to go from low to high concentration. The nernst potentials are all functions of the inner and

outer concentration of their respective ion.

In the Hodgkin-Huxley model the membrane is regarded as a cable and it is assumed that the voltage follows the wave equation. The model combines eq 2.1 an equation from cable theory and the wave equation and end up with the final equation for propagation of an electric pulse:

$$\frac{a}{2R_i\theta^2} = C_M \frac{dV_m}{dt} + g_K(V_m - E_k) + g_{Na}(V_m - E_{Na}) + g_l(V_m - E_l) \quad \text{eq. 2.2}$$

g_K and g_{Na} are the conductance value of the kalium and natrium channels. Physically they represent the amount of open protein channels in the membrane. Hodgkin and Huxley suggested a very complicated method for deciding the value of g_{Na} and g_K. It involves three differential equations and over 20 fit parameters that should be fitted so that g_K and g_{Na} and the overall model fits experimental data. This is a weakness for the Hodgkin-Huxley model since a model should ideally be as simple as possible. Otherwise one could suspect that a model can only be fitted to a certain kind of data because the model can be fitted to a wide range of data in general.

The HH model can reproduce the shape and propagation of a nerve signal. However it can't explain why the signal comes with a volume or density pulse traveling down the axon under signal transmission. Also when current run through a resistor energy is lost to the release of heat in the transistor. Therefore the Hodgkin-Huxley model predicts that energy is lost and that heat will be released under the transmission of a nerve signal. This is not the case. Under signal transmission there is a release followed by a re-uptake of heat. The total heat exchange under a signal event is zero. The activation and the propagation of a nerve signal is an adiabatic process. Since the HH model can't explain these properties of the nerve signal, the Hodgkin-Huxley model cannot be regarded as a complete theory.

The HH model doesn't predict anything about thermodynamical properties such as pressure and temperature. This makes it harder to falsify. An ideal scientific theory should be very easy to falsify.

2.2 The soliton model

The carbon chains in a lipid can have different conformations, because the carbon atoms making up the chains are free to rotate around the bond between them. At low temperature the lipids chains exist in a so called trans conformation and at higher temperatures the lipid chain begin to exist in a gauche conformation. In gel state the lipid chains in the membrane mainly exist in trans conformation and in fluid state the lipid chains mainly exist in a gauche conformation. The phase transition of a cell membrane is the point where the amount of chains in trans conformation is equal to the amount of chains in gauche conformation.

In the soliton model the nerve pulse is regarded as a mechanical wave (soliton) traveling down the axon. This wave is coupled to the lipid transition in membranes. The soliton pulse is a propagating density pulse in the fluid membrane. At the position of the pulse the membrane is locally in gell state.

The behavior of thermodynamical properties around phase transition means that the compressibility of the membrane is nonlinear. This means that the compressibility of the membrane changes when it is compressed. Nonlinearity is necessary for the propagation of a soliton. This suggest that the further away the membrane is from phase transition state the harder it is to create a soliton. In the body the phase transition temperature is slightly below body temperature so the cell membrane is in the fluid state in the body. But the membrane state is close enough to phase transition state to allow for propagation of a soliton. By changing the surroundings for example by increase the pressure, the membrane state can be altered to state further away from phase transition state and thereby creation of a soliton will be more unlikely and require a greater stimuli.

The soliton model is based on the wave equation for area density change:

$$\frac{\partial^2}{\partial t^2}\Delta\rho^A = \frac{\partial}{\partial z}\left(c^2\frac{\partial}{\partial z}\Delta\rho^A\right)$$

where t is time,z is the position along the nerve axon and c is the sound velocity. Close to melting transition sound is a function of density so c is expanded around its value in fluid phase.

$$c^2 = c_0^2 + \rho\Delta\rho^A + q\left(\Delta\rho^A\right) + \ldots$$

The speed of sound is frequency dependent. Therefore a term is added to the equation:

$$-h\frac{\partial}{\partial z^4}\Delta\rho^A,\ h = constant$$

Combining these equations gives the final partial differential equation:

$$\frac{\partial^2}{\partial t^2}\Delta\rho^A = \frac{\partial}{\partial z}\left[\left(c_0^2 + \rho\Delta\rho^A + q\left(\Delta\rho^A\right)^2 + \ldots\right)\frac{\partial}{\partial z}\Delta\rho^A\right] - h\frac{\partial}{\partial z^4}\Delta\rho^A$$

This equation can be simplified mathematically and used to simulate and plot the variable $u = \Delta\frac{\rho^A}{\rho_0^A}$ as a function of position z and time t.

The soliton model is based on thermodynamics while the HH model is purely electric. The soliton model is consistent with the thickening of the nerve axon and the release and re-uptake of heat under a signal event. The soliton model can explain the voltage

component by the electrostatic features of the membrane.

The soliton model predict that when two waves collide they will go through each other and have the same shape and amplitude as before collision. Only if the waves travel with a velocity very close to the minimum possible velocity the waves will change to a significant extend under collision.

With a few exceptions the HH model generally predicts that the waves will block each other and that no waves will exist after collision. The exceptions are soliton-like regimes where which are certain velocity intervals where the end result end up being the same as in the soliton model.

3 Methods and Materials

3.1 Materials

Earthworms
Nerve Chamber
ML856 PowerLab 26T
Stimulator cables
recording cables
MLA2540 5-Lead Shielded Bio Amp Cable
aluminium foil for isolation of cables
Faraday cage
Anesthetization (10% of ethanol in tap water)
A little piece of metal.

3.1.1 Earthworms

In an ideal experiment one should dissect a living animal for example a worm, take out the ventral cord and then make recordings on the pure nerve. But doing that is very difficult and takes a lot of time. Therefore I mainly made recording of nerve signals extracellular on an anesthetized earthworm. That means that the nerve signal from the nerve inside the worm was measured through the skin of the worm. That unfortunately means that the signal was very weak compared to measurements on a pure nerve. With a pure nerve from an earthworm the signal you can measure is about a factor 100 stronger than the measurements I made extracellular on an earthworm.

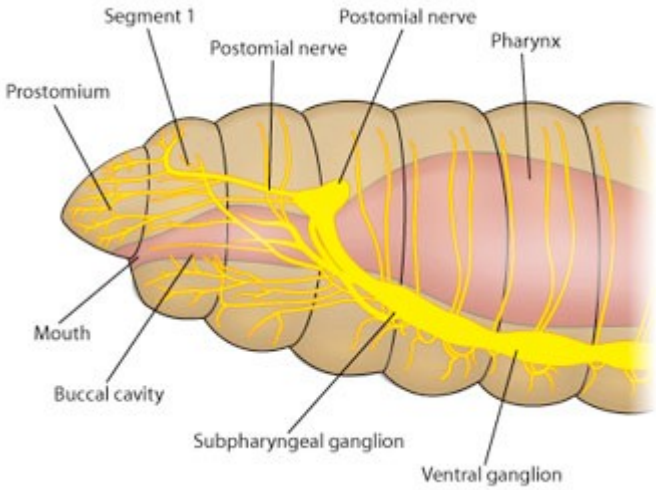

Figure 3.1 Earthworm Ventral Cord [16]

The important thing about an earthworm in this context is that an earthworm contains

a medial giant fiber and two lateral giant fibers (see figure 3.2). These fibers consist of an array of overlapping giant neurons and they behave like a long axon that runs the entire length of the worm and they can be stimulated from both ends. An earthworm also contains a bunch of very small neurons but they don't run the length of the worm and they are so small that the signal from these neurons can be disregarded when measuring the signal from the giant fibers. Normally a signal only runs in one direction through a nerve in a biological system but that is because of the way the nerve is connected to other cells. A stimuli everywhere on the fibers in an earthworm can start a signal.

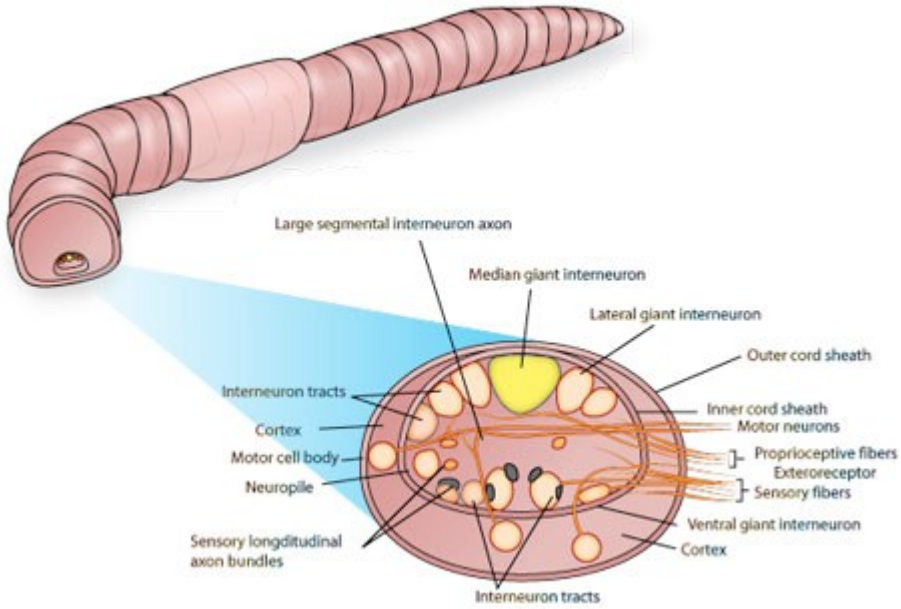

Figure 3.2 Earthworm array of neurons [16]

In our experiments we used two types of earthworms. The *Lumbricus terrestris* and the *Eisenia foetida*. These worms are anatomically equal at the level of the Ventral Cord. *Lumbricus terrestris* is generally bigger than *Eisenia foetida*(see figures 3.3 and 3.4).

Figure 3.3 Lumbricus terrestris [17]

Figure 3.4 Eisenia fetida (different scale) [18]

3.1.2 Nerve Chamber

In my experiment we used two different nerve chambers. A big one which was commercial and a small one which was home made (see figures 3.5 and 3.6).

Figure 3.5 Nerve Chamber and cables [19]

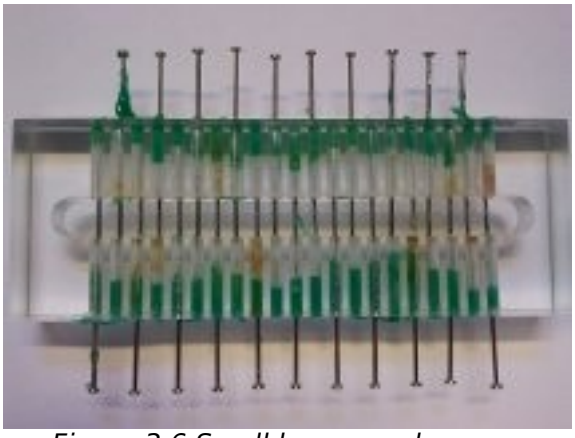

Figure 3.6 Small home made nerve chamber

The MLTO16/x nerve chamber used in this experiments consists of 17 stainless steel wire electrodes . Some electrodes are spaced 0.5 cm apart and others 1 cm apart. In this experiment cables where connected to the electrodes on the nerve chamber and to the MLA2540 5-Lead Shielded Bio Amp Cable shown below. The MLA2540 cable was connected to the Bio Amplification Channel in the ML856 PowerLab 26T described below. The Bio Amplification Channels where used because the signal had to be amplified up to the order of microvolt to be detected. In this way a voltage difference between two electrodes of our choice could be created from the cables connected to output channels and measured through the cables connected to input channels.

Figure 3.7 The MLA2540 5-Lead Shielded Bio Amp Cable [20]

3.1.3 ML856 PowerLab 26T

ML856 PowerLab 26T is a a data acquisition system used to perform measurements on biological systems. The system includes hardware with input and output channels, high and low pass filters, a bio amplifier among other things. It also includes the software program labchart and it comes with stimulator and recording cables. The hardware unit is used to stimulate the nerve,amplify and record the signal while at the same time using filters and bio amplification to remove unwanted frequencies to improve signal to noise ratio. The hardware is connected to a computer which have the software program labchart installed. The integration of hardware and software allows for hardware functions to be controlled from the software program labchart on the computer.

Figure 3.8 ML856 Powerlab 26T [21]

3.1.4 Labchart

From labchart I could initiate the stimuli and the recording of the nerve signal by pressing a button on the screen. From labchart I could control properties like the amplitude of the stimulation, type of signal filtering, recording time and number of stimulations. In Labchart the data is displayed and saved as graphs in a diagram with time on the x-axis and voltage difference on the y-axis. By comparing these graphs with measurements of distance between the stimulating points and the recording points on the worm, properties like speed and amplitude of the nerve signals can be calculated. The strength of the stimuli is present on each set of graphs/data so the threshold value of the nerve could be read from the graph which had the lowest stimulation value of the graphs showing a nerve signal. Labchart can take the average of an arbitrary number of measurements with the same stimuli. This was a very important tool for improving signal to noise ratio.

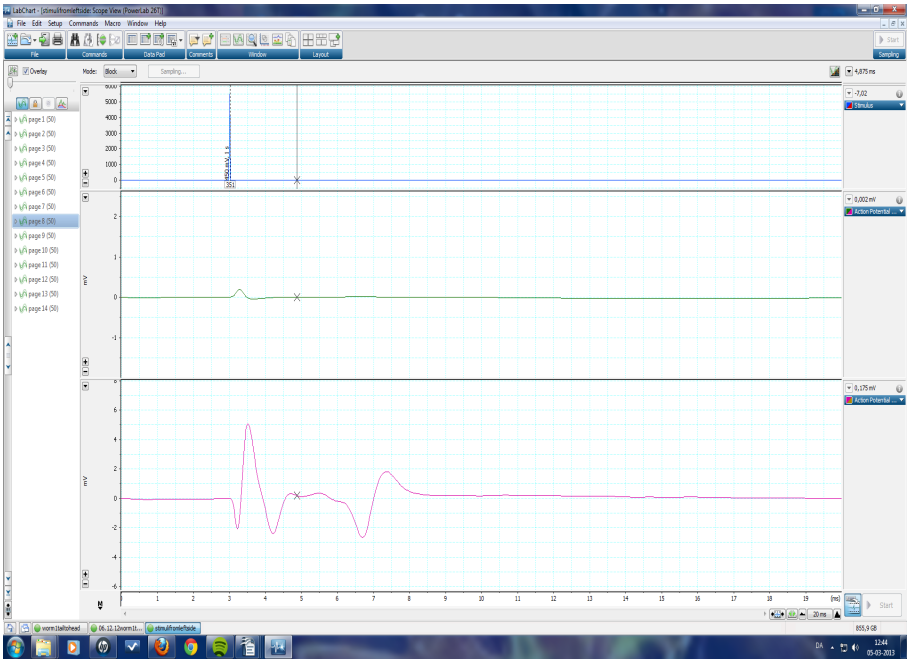

Figure 3.9 Screenshot from the software program labchart. The picture shows data from a recording of a nerve signal in a nerve extracted from an earthworm

3.2 Methods

In order to measure the nerve-signal the worm is placed on a nerve chamber. A nerve chamber is a row of electrodes that in this experiment was spaced at intervals of 0.5cm and 1cm. The worm is placed along the row of electrodes. It must be placed so that the ventral side touches the electrodes because otherwise the signal cant be seen. This is because the nerve that creates the signal lies very close to the ventral side of the worm. The worm must also be stretched and perpendicular to the electrodes, so that the distance between two electrodes is the same as the length of the section of the worm that is between the two electrodes.

Electrodes beneath the tail or head of the worm is now connected to stimulator cables and electrodes touching an arbitrary place further down the worm is connected to recording cables. The stimulation and recording cables are connected to The MLA2540 5-Lead Shielded Bio Amp Cable described earlier. This cable is connected to the Bio Amp channel in the ML856 Powerlab 26T data acquisition hardware. Finally this hardware is connected to a computer running the program lab chart.

Now the nerve in the worm can be stimulated through the stimulator cables and measured from the recording cables. The stimuli is an induced voltage difference over the electrodes connected to the stimulator cables. Since the worm is lying on the electrodes the stimuli is induced in the worm too. We used was a square signal where

the worm is exposed to a constant stimuli for a given amount of time (see figure 3.10).

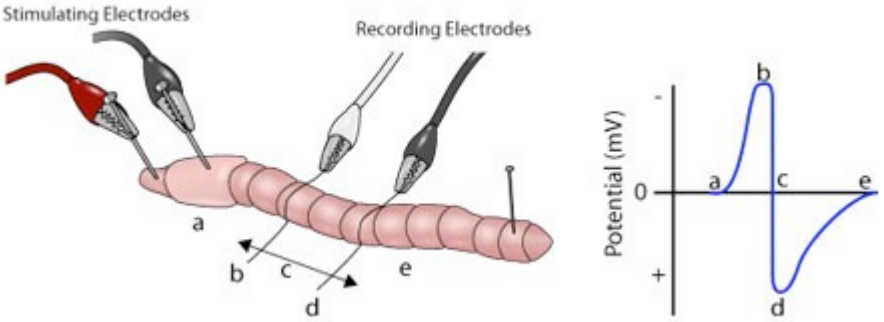

Figure 3.10 Basic principle of the experimental setup [16]

If the worm moves while recordings take place it creates a huge amount of noise. Therefore, in order to get useful data, the worm must almost lie completely still when recording the nerve-signals. Therefore the worm must be anesthetized before it is placed in the Nerve Chamber. That is done by preparing a 1:10 alcohol/water solution and place the worm in this solution for about 5 minutes. If this is not enough to anesthetize the worm the solution can be made 1:5. If the worm is left in the solution for about 10 minutes or more it is possible that the worm will die. As a general rule, when the worm stop moving, the worm is ready for experimenting and can be removed from the anesthetic solution and cleared with tap water.

First the amplitude and the width of the stimulating pulse is fixed. You then begin with a weak stimuli (low voltage) and slowly increase the amount of voltage while every time recording the voltage difference measured from the recording cables. At some point the threshold voltage is reached and a nerve signal will appear in the data acquired. Now the threshold value can be deduced from the data. According to theory a nerve signal also called spike should have a wave like shape. If the signal does not have this shape it is probably not a nerve-signal.

You now continue to increase the voltage and check if you almost get the exact same signal every time. As mentioned earlier the signal transmitted through a nerve is independent of the strength of the stimulation. If the signal is noise coming from an electrical device the signal often change with time and changes when the stimuli changes. Therefore if the same signal is recorded for a long time over a large range of stimulation and if the signal has the right shape it is very likely that the signal comes from a transmitting nerve.

In this way data is now recorded with many different worms and for each worm nerve signals are measured with respect to two different configurations. One setup where the stimulation comes from the tail and another where stimulation comes from the head. From this data the properties of nerve-signals from the tail and nerve-signals from head are determined.

Things like temperature, the dryness of the worm and how much the worm is stretched influences the signal a lot. Therefore, as much as it is possible, the worm must be in the same state at all time when recording is being done. For example if the worm starts moving after data recording for one setup I finished you have anesthetize it again. After that the properties of the worm has changed which means that you have to start all over again with the specific worm.

Now for a couple of worms the same is done but where a third configuration is added where the worm is stimulated from both the tail and the head.

The distance between the point or points of stimulation and the point or points of recording on the worm is measured and noted. This information together with the data required can be used to describe certain properties of nerve-signals like speed and amplitude of the signal. [22].

By comparing data from setups where the worm is stimulated from either right or left or both right and left the experiment can give information about what happens when two nerve-signals collide in a nerve.

Noise minimization:

In most rooms a sensitive instrument measuring electrical signals can easily measure electrical signals or noise coming from computers, lab instruments, phones and other electrical devises. Electric signals of 50 Hz is coming from electric cables. This frequency can be removed by insulating the nerve chamber with a Faraday cage. Another possibility is to remove the frequency digitally after data is recorded. In order to get perfect data all external electric signals should be eliminated from the measurements taken under the course of the experiment. Because the signal measured through the skin is in the order of microvolt, the noise coming from the outside had to be minimized a lot, if we wanted to get just slightly useful data.

Figure 3.11 The Faraday Cage, the Nerve Chamber and the Bio Amp Cable

One very important method used to minimize noise, was to put the nerve chamber in a Faraday cage. A Faraday cage is a box or a cage that shields everything inside the cage from all external electrical signals. In this particular case the Faraday Cage was a metallic cookie box. A screwdriver was used to make a little hole, so a grounded cable could be connected to the cookie box. In this way every electric signal that hit the cookie box would run to the grounding cable and from the grounding cable to the ground and away from the nerve chamber. A bigger hole unfortunately also had to be made because there had to be a hole for the cables connecting the nerve chamber and the recording hardware. Therefore the the setup was not totally shielded from external electric signals.

Another thing that had to be done was that all cables which was a part of the experimental setup was covered with aluminum foil and all this aluminum foil covering the cables was also connected to a grounded cable.

4 Results and discussion

4.1 Experiment with a living earthworm

I managed to collect a high amount of data showing that a nerve signal can be initiated from both ends of the giant fibers running along the entire length of an earthworm. I started with a low stimuli and increased it slowly until a spike suddenly started to appear on the screen. The lowest stimuli able to create a spike then had to be the threshold value. To reassure myself further that the spike seen was in fact a nerve signal, and not just something that accidentally looked like a nerve signal, I checked that the spike wasn't there when the worm was taken out of the setup and that the spike was always there when the worm was there and the stimuli was above the threshold value. If this was the case I knew the spike had to come from the worm. Also I knew from theory that the worm had to have a threshold value because of the fibers in the worm. When we continued to increase the stimuli we didn't get any new spikes. The fact that no other spike was seen when stimuli was increased strongly indicated that the spike had to come from the nerve inside the worm.

Another good indication that the spike was a nerve signal, was that the width and the amplitude of the spike didn't change when stimuli was changed. This is because a nerve signal propagating down a nerve is independent of the strength of the stimuli initiating the signal. This is not the case with spikes stemming from lab equipment. spikes stemming from activity of the lab equipment changes significantly when stimuli is changed.

Figure 4.1 show one set of data where the stimuli on the worm was 1.2 volt and no

signal appeared. Figure 4.2 is data from the same worm. When the stimuli was increased to 1.25 volt a nerve signal appeared in the data. This means that for the this particular worm the threshold voltage was between 1.2 v and 1.25v. The big spike after about 3.5 ms comes from the lab equipment itself and is called the stimulus artifact.

The results show that a nerve signal can be created everywhere on the worm. The threshold value of the worms varied from worm to worm but always lied in the interval from 0.5 v to 3.2 v. The variation of threshold values are due to differences in the thickness of the worms and their nerves. Differences in temperature between experiments and the dryness and anesthetization of the worm also affect the threshold value.

On one worm of the type *Eisenia foetida* the nerve signal speed was 5.6 m/s when stimuli came from one side and 6.1 m/s when stimuli came from the opposite side. On one worm of the type Lumbricus terrestris I got a nerve signal speed of 2.18 m/s. The signal speed and amplitude vary according to the size of the worm, how dry the worm is, the shape of the worm and how anesthetized it is.

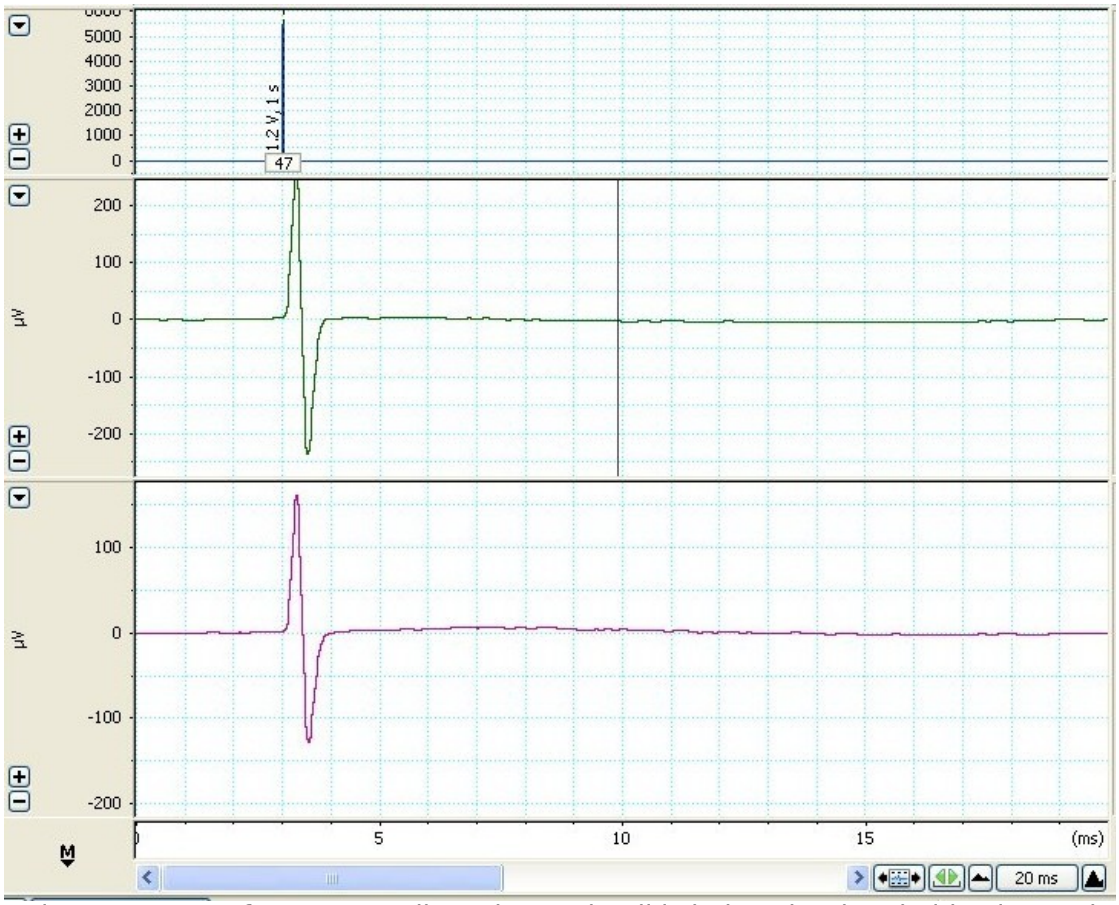

Figure 4.1 Data from a recording where stimuli is below the threshold value and therefore no nerve signal appear in the data. The big spike after 3.5 ms is created by the lab equipment itself and is called the stimulus artifact.

I managed to collect one set of data where the same worm was first stimulated from right, then from left and then finally from both sides and where the recordings of the nerve signal wasn't spoiled by noise or movements of the worm etc. With this worm recordings were only done on one point on the worm.

This dataset shows that the worm had a threshold value on 1v when the worm was stimulated from the left side only. This signal appeared after 8ms of measuring.

When the worm was stimulated from the right side only there was a nerve signal with a threshold value of 1.6v. The signal appeared after 11.5 ms of measuring.

When the worm was exposed to stimuli from both sides one nerve signal appeared after the stimuli was increased from 1 to 1.05 volt. This signal appeared after 8,45 ms of measuring. When the stimuli increased from 1.20v to 1.25v another signal appeared after about 12 seconds of measuring.

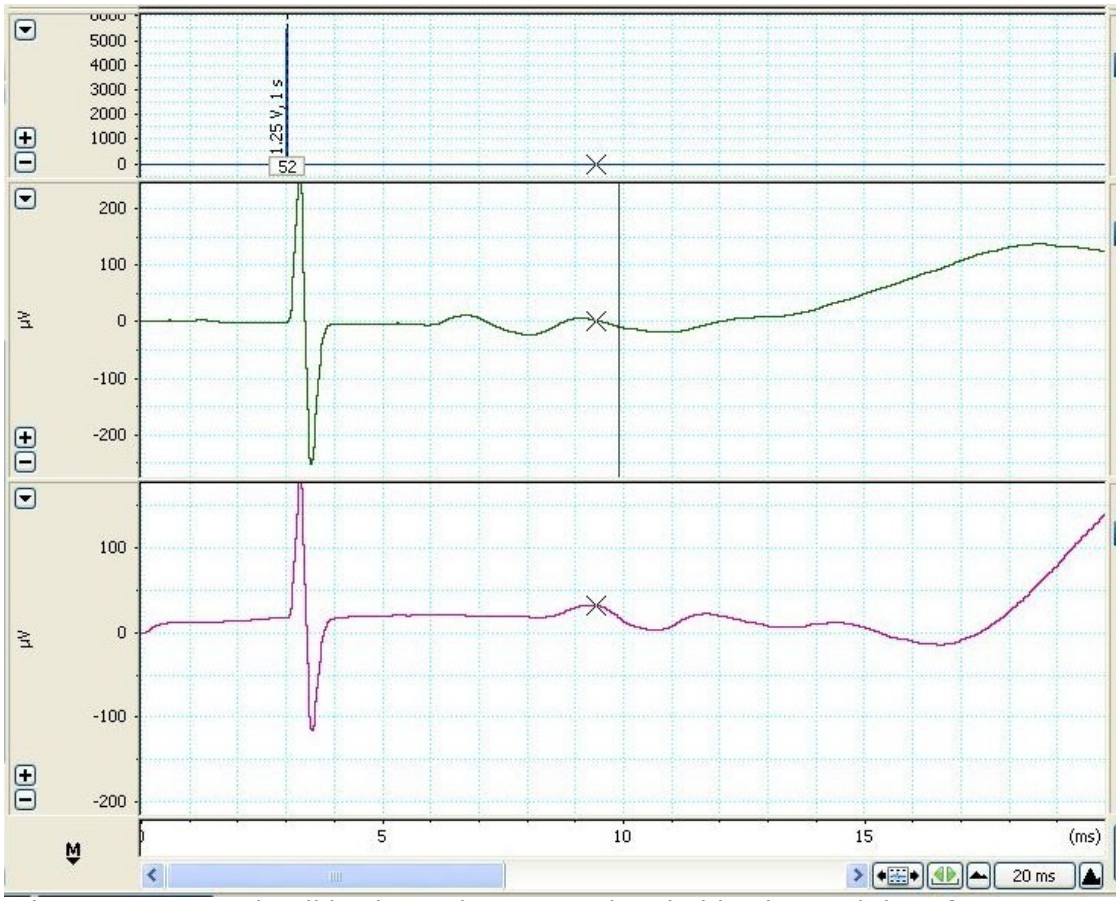

Figure 4.2 Here stimuli is above the worms threshold value and therefore a nerve signal is recorded

An earthworm has one median giant fiber that can be regarded as an axon and two lateral giant fibers that together can be regarded as an axon. Sometimes a nerve signal from the median giant fiber is followed by a nerve signal from the lateral giant fibers. When two nerve signal are seen the one can be from the median giant fiber and the other can be from the lateral giant fibers. But in that case the shape of the two spikes are the same. An example of that can be seen on figure 4.2. The spike from the median giant fiber first goes up and then down. The same is the case for the spike from the lateral giant fibers. In figure 4.3 the spikes have different shapes. This indicates that the spikes has traveled in opposite directions and thus comes from opposite sides of the worm.

The fact that two different nerve signals can be measured at the same point at two different times is an indicator that the signals doesn't annihilate each other when they collide. If they did, the signal reaching the point of measuring on the worm as the first one of the two would annihilate the other signal before that signal could reach the measuring point. If they reached the point at the same time and annihilated each other at the point of measurement one wouldn't get two full signals either.
Therefore my results strongly indicate that the nerve signals has gone through each other and that nothing significant has happened to the nerve signals under the collision. Except for maybe a decrease in their amplitude.

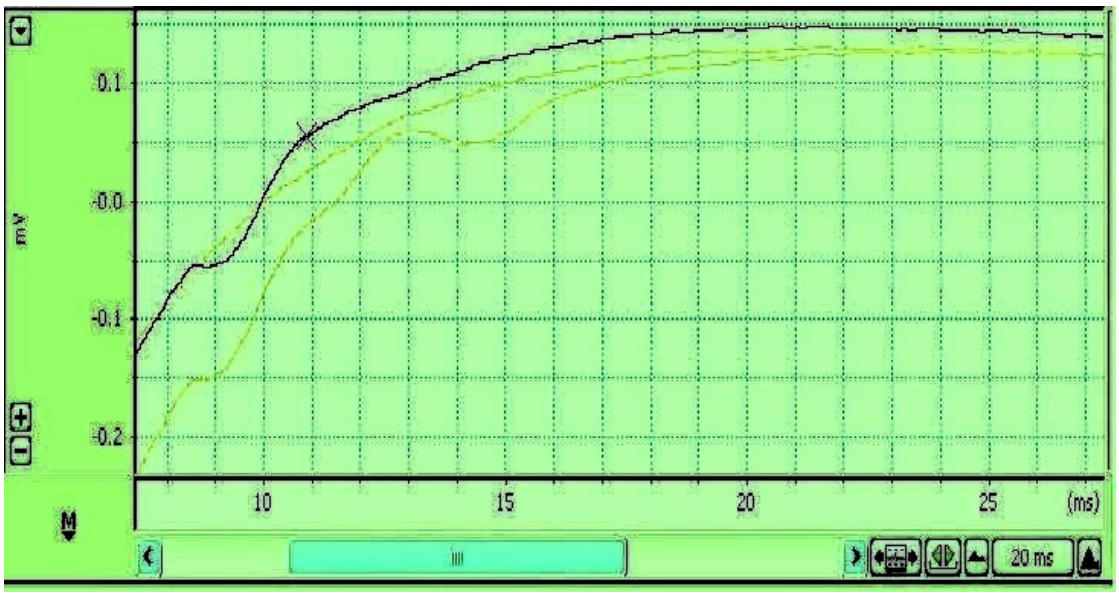

Figure 4.3 When stimuli comes from both sides there are 0,1 or 2 nerve signals depending on the strength of the stimuli

When the worm was stimulated from both sides the signal with the biggest threshold

value also appeared latest. This is was what one would expect if the signal go through each other. This is because when the worm was stimulated from right side only both the threshold value and the time until the signal appeared was greater than when stimuli was from left side only.

The threshold values and the appearance time of the signals recorded when stimuli came from both sides are different than when stimuli came from only one side. This was expected because it was inevitable that the properties of the worm changed during the time recordings took place. These were properties like dryness and how much the worm was anesthetized. These changes meant that the properties of the nerve signals changed during the time the recordings took place.

My results thereby supports the predictions of the soliton model but they don't fit with the predictions of the Hodgkin-Huxley model.

Figure 4.3 shows three graphs plotted in the same picture. One graph shows data from when the stimuli were below the threshold value for both the tail and the head. This graph shows no sign of a nerve signal. The other graph shows data from when stimuli was below the threshold voltage of the right side but above the threshold voltage of the left side. One nerve signal appear in this graph. The last graph shows data from where the stimuli was above both threshold voltages and two nerve signals appear in this graph. The two nerve signals starts and ends with a decrease or a valley in the last graph. Therefore they must have different shapes.

4.2 Experiment with an extracted Ventral Cord

I also made measurement on a pure nerve where recordings were done on configurations where stimuli came from right side, left side and both left and right side respectively. The results from these measurement shows that a nerve signal can be initiated from both sides. Both the signal seen when stimuli comes from right side and the signal seen when stimuli comes from left side appear in the data when the stimuli comes from both sides. There fore the data from the pure nerve also indicate that the signals go through each other without annihilation when the stimulation comes from both sides.

Figure 4.4 shows that a nerve signal appear when the nerve is stimulated from one side. Figure 4.5 shows that another nerve signal appears when the nerve is stimulated from the other side. This signal is a lot weaker and a bit further away from the stimulus artifact. In Figure 4.6 the stimuli came from both sides and both the signal from figure 4.4 and figure 4.5 appear in the data.

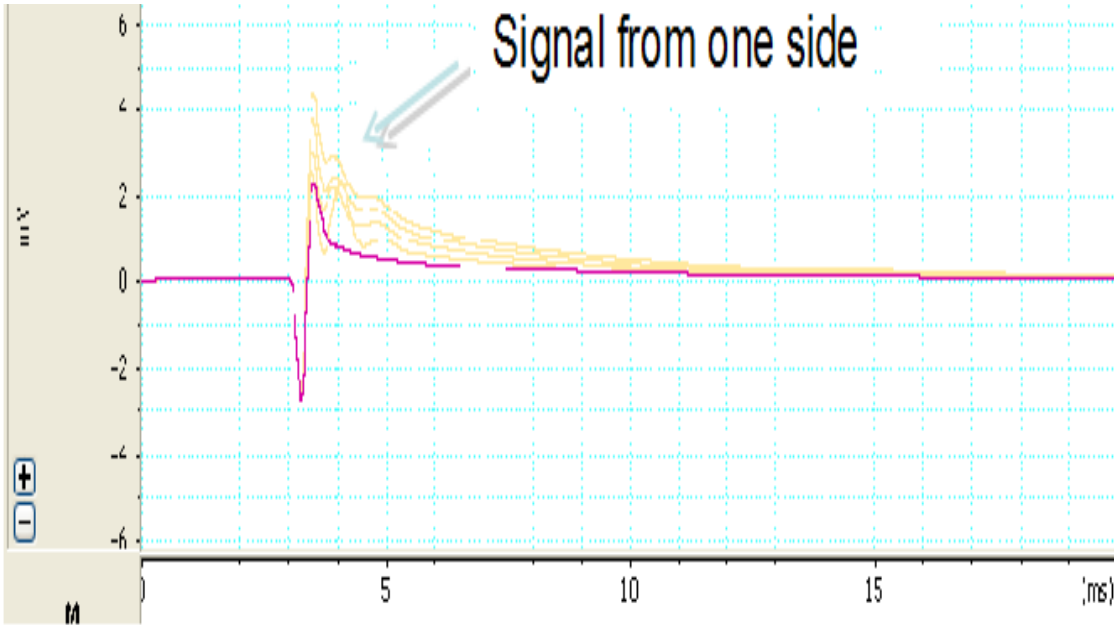

Figure 4.4 Stimulation from one side initiates a signal that appear in the data.

Figure 4.5 Stimulation from the other side initiates a small signal that appear in the data.

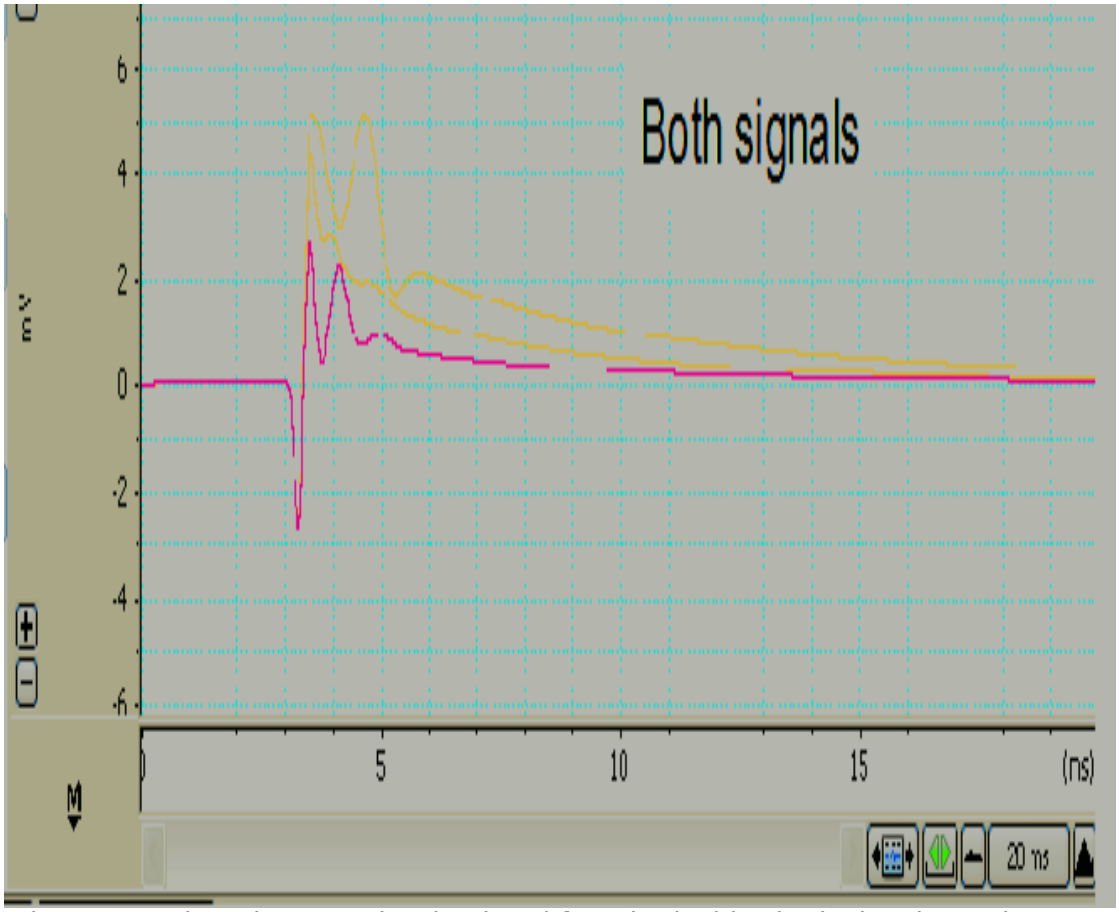

Figure 4.6 When the worm is stimulated from both sides both signals can be seen in the data

5 Conclusion

The purpose of this project was to investigate what happens when two nerve signals in an earthworm propagates in opposite directions then reach the same point in the nerve and collide with each other. There was two realistic scenarios. The first scenario was the prediction of the soliton model and the other scenario was the prediction of the Hodgkin-Huxley model. The soliton model predict that the signals pass through each other unaltered and the Hodgkin-Huxley model predict that the signal annihilate each other when they collide. The results of this experiment strongly supports the idea that the two nerve signals go through each other unaltered by the collision except for maybe for a decrease in amplitude. Thereby the results of this experiment is in line with the soliton model.

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