Measurements of Nerves: Collision in Solution, Potassium Chloride Effect on Action Potential.

thesis in membrane biophysics
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Author
Mette Kim Kristensen
rxt153@alumni.ku.dk

Primary Supervisor
Thomas Heimburg
Professor for Biophysics
theimbu@nbi.ku.dk

Secondary Supervisor
Tian Wang
Ph.D. student
tatiana_wt@hotmail.com

research group
MEMBRANE BIOPHYSICS GROUP

Niels Bohr Institute
Faculty of Science
University of Copenhagen

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FACULTY OF SCIENCE
UNIVERSITY OF COPENHAGEN

Abstract

This thesis is composed of two parts of electrophysiological measurements on the nerves from lobster. The first part will be the collision experiment of two simultaneously generated impulses, propagating form opposite ends of the nerve. The experiments were performed on the abdominal ventral cord of lobster, Homarus americanus or Homarus gammarus in a glass capillary with saline solution inside. The two nerve pulses passed each other and stayed virtually unchanged after the collision, instead of annihilation. This is in agreement with the electromechanical soliton theory for nerve pulse propagation brought up by T. Heimburg and A.D. Jackson, in contrary to the well accepted Hodgkin-Huxley theory. The second part is to study the kinetics of external potassium chloride concentration effect on the compound action potential in axon bundles from lobster legs. High potassium chloride concentration blocks nerve conduction and the signal attenuates with time. The objective of this part is to establish more accurately kinetics in the specific conditions for blocking the signal, which is to be used in other research of magnetic signal in measurement of nerves.

Resume

Dette projekt består af to dele af elektrofysiske målinger på nerver fra hummer. Den første del er en kollision eksperiment, af to impulser der samtidig bliver genereret fra hver sin ende af nerven. Eksperimentet er udført i glaskapillær, på den abdominal ventral cord af Homarus americanus eller Homarus gammarus sammen med en saltvands opløsning. De to nerve impulser passere hinanden, samtidig med at de forbliver næsten uforandret efter kollisionen og tilintetgøre ikke hinanden. Dette er i overensstemmelse med den elektromekaniske soliton teori for udbredelsen af impulser i nerver, præsenteret af T. Heimburg og A. D. Jackson, hvilket ikke er i overensstemmelse med den generelle accepteret teori af Hodgkin-Huxley. Den anden del af projektet består af at studere den kinetiske effekt af ekstern eksponering af kaliumklorid på den samlede aktionspotentiale. Objektivet af denne del er at etablere en bedre kinetik i denne specifikke område af blokering og måling af signalet i nerver, som skal bruges i anden forskning om magnetisk signaler i nerver.

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Introduction

The action potential in nerves is a composition of transmembrane voltage pulse, which propagates along the neuronal axon. The Hodgkin-Huxley [1] (HH) model, using the model of a parallel resistor, to describe the process during a nerve impulse generation and the voltage-dependent ion gates. Ions flows along the concentrations gradient through channel proteins, the relaxation processes in the proteins, subsequently leading to a refraction period, following a pulse propagation, where the nerve is unexcitable. Therefore, in the case two nerve pulses are to travel simultaneously from opposite ends of the nerve, the pulses will be annihilated upon collision. The model also falls short of explaining many other non-electrical properties, which can be observed during experiments. Such as the well know changes in thickness and length during the pulse propagation. In accordance with the resistor model, the action potential produce and release heat. But investigations has found heat release during the first phase, followed by immediate absorption of heat during the second phase of the action potential, happens in phase with the voltage changes which can be observed. This data is an indication, that the action potential is an adiabatic (non-dissipative) sound wave, as suggested by T. Heimburg and A. D. Jackson [2] *On soliton propagation in biomembranes and nerves.* Where the action potential is an electromechanical pules.
At physiological temperatures, the elastic constant of the biomembrane depend on the non-linearity of the lateral pressure. In accordance with the electromechanical model, a collision would not lead to an annihilation of the two pulses, but to an almost non-existing depletion, upon penetration. which is in contradiction to the HH model, where two colliding pulses, would lead to complete annihilation of the two pulses. Considering the two very different outcome during a collision, this thesis will investigate whether a collision will lead to annihilation or not.

> *The transition from a paradigm in crisis to a new one from which a new tradition of normal science can emerge is far from a cumulative process, one achieved by an articulation or extension of the old paradigm. Rather it is a reconstruction of the field from new fundamentals, a reconstruction that changes some of the field's most elementary theoretical generalizations as well as many of its paradigm methods and applications. During the transition period there will be a large but never complete overlap between the problems that can be solved by the old and by the new paradigm. But there will also be a decisive difference in the modes of solution. When the transition is complete, the profession will have changed its view of the field, its methods, and its goals.*
> Thomas S. Kuhn,
> *The Structure of Scientific Revolutions*
> (1962), 84-5.

1 Theory

This section contains theory used for this project.

1.1 The paradigm of the action potential

In 1952 Hodgkin-Huxley (HH) *Membrane current in nerve* [1], conducted a series of experiment on giant nerve bundles from squids. As the nerve bundle was stimulated with a current, a pulse propagation could be measured along the nerve and they set forth to find a theory to explain the pulse propagation. The theory they came up with was; that the nerve axon worked as a parallel resistor as can be seen in figure (1.1), hereby explaining the apparent all-or-nothing reaction to the current.

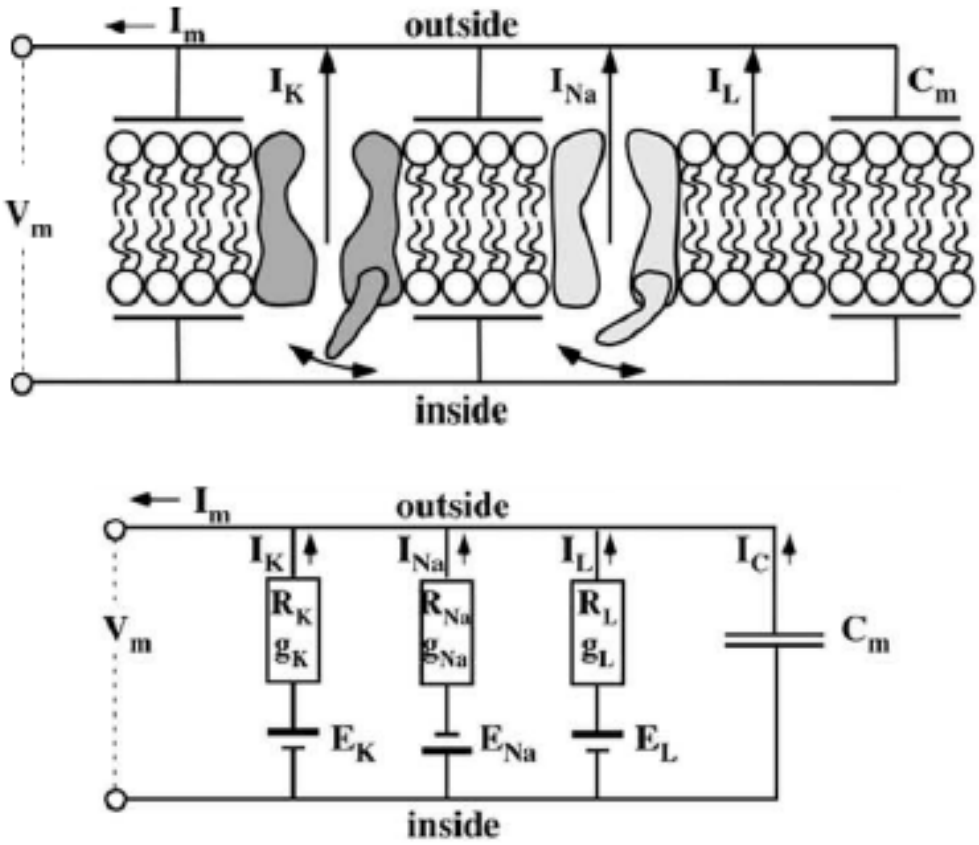


Figure 1.1: Hodgkin-Huxley parallel resistor model, with the voltage-dependent ion gates. Borrowed from [2]

1.1.1 The HH model:

Using the parallels of the total current,

$$I(t) = I_1(t) + I_2(t) + I_3(t) \tag{1.1}$$

by differentiate Faraday's law

$$\frac{dV}{dt} = \frac{1}{C}\frac{dq}{dt} \Leftrightarrow \frac{1}{C}\left(I_1(t) + I_2(t) + I_3(t)\right) \tag{1.2}$$

giving the HH model

$$\frac{dV}{dt} = -\frac{1}{C}\left(I_{Na}(t) + I_K(t) + I_L(t)\right) \tag{1.3}$$

where

$$\begin{aligned} I_{Na} &= g_{Na}(V - V_{Na}) \;\; where\; g_{Na} \;\; is\; voltaged - dependent \\ I_k &= g_K(V - V_K) \;\; where\; g_K \;\; is\; voltaged - dependent \\ I_L &= g_L(V - V_L) \;\; and\; g_L \;\; is\; a\; constant \end{aligned} \tag{1.4}$$

where $V = E - E_r$ and V_{Na}, E_r is the absolute value of resting potential and E_{Na} and E_K is the equilibrium potential for Na^+ and K^+
There are *n* gates in the ion channel, so to find the voltage-dependence of the gate position and α_n and β_n are the transition rate constance (voltage-dependent), then α_n is the number of times per seconds a gate in the shut state opens, and β_n is the number of times per seconds a gate in the open state shuts. The fraction of gates opening and the fraction of the gates shuts, per seconds are given by

$$\begin{aligned} F_{open} &= \alpha_n(1 - n) \\ F_{shut} &= \beta_n n \end{aligned} \tag{1.5}$$

the change rate *n*

$$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n \tag{1.6}$$

and the equilibrium when $n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}$, so

$$\begin{aligned} n(t) &= \frac{\alpha_n}{\alpha_n + \beta_n} - \left(\frac{\alpha_n}{\alpha_n + \beta_n} - n_0\right) exp\left(\left(-\alpha_n + \beta_n\right)t\right) \\ &\Rightarrow n_\infty - (n_\infty - n_0)\, exp\left(-\frac{t}{\tau_n}\right) \end{aligned} \tag{1.7}$$

where τ_n is the time constant and $\tau_n = \frac{1}{\alpha_n + \beta_n}$, so if α_n or β_n is large, then τ_n will be short and $n \to n_\infty$ will happen rapidly. On the other hand, if α_n or β_n is small, then τ_n will be long and $n \to n_\infty$ will happen slowly. There is a time delay where

the nerve can not be excitable again, due to K^+ channels which are controlled by $4n$ activations gates

$$\frac{dn}{dt} = \frac{1}{\tau_n}(n_\infty - n) \Rightarrow g_K = n^4 g_K \tag{1.8}$$

where g_K is the maximum K^+ conduction and the Na^+ channels controlled by $3m$ activations gates and $1h$ inactivation gate

$$\begin{aligned} \frac{dm}{dt} &= \frac{1}{\tau_m}(m_\infty - m) \\ \frac{dh}{dt} &= \frac{1}{\tau_h}(h_\infty - h) \end{aligned} \tag{1.9}$$

So the probability of finding the activation gate open, increased with depolarization and the probability of finding the inactivation gate open, decreased with depolarization. So in the event two impulses, travelling from opposite ends of an axon, the impulses would be annihilated as they collided, due to the time delay.
For this HH became widely celebrated by the scientific community and even received a Nobel Prize for their work in 1963. Even thought there were critics, since there still was questions left unanswered, such as the well known changes in thickness and length of the nerve, during action potential, along with the heat release during the first phase of the action potential and the reabsorption of the heat during the second phase. But since a better theory did not surface, the HH theory was adapted by the rest of the scientific community and a paradigm was born.

1.1.1.1 Ion channels underlying the action potential

As the nerve is at rest, the inside of the axon has a negative potential and the outside has a positive potential. This is caused by the K^+ diffusing from the higher concentration inside the axon, to the lower concentration, outside the axon. During the initiation of an action potential, the inside of the cell will be positive at the peak of the action potential, positive in regrades to the outside of the cell. Since the neighbouring sections of the membrane still remains at rest and negatively charged, the positive charge will be attracted to the negatively charged neighbouring sections and will try to move in that direction. In the case, that the positive charge is large enough to depolarize the neighbouring sections of the membrane to the threshold, and start a voltage-dependent change in Na^+ permeability, it will initiate a new action potential in the neighbouring sections of the membrane. The process once initiated will propagate all the way to the end of the axon, causing the charge transfer along the surface. The initiation of the action potential is caused by the voltage-dependence increase in the Na^+ permeability, along with the repolarization of the action potential during the process of the Na^+ inactivation and by the slow increase in the K^+ permeability.
The hyperpolarizating afterpotential is due to the elevation of K^+ permeability, after the Na^+ has returned to it's normal levels. To predict the membrane potential by the ration of K^+ and Na^+, the Goldman-Hodgkin-Katz (GHK) equation [3] can be used

$$V_m = 60 \cdot log \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i}(mV) \tag{1.10}$$

where V_m is the membrane potential in millivolt and α is proportional to the permeabilities of Na^+ and K^+, $\alpha = (P_{Na}/P_K)$.
Is the membrane highly permeable to K^+, then $P_{Na} = 0$ and $\alpha = 0$, which reduces the GHK equation to the Nernst Bernstein's [3] equilibrium equation for K^+

$$V_m \stackrel{?}{=} E_K = 60 \cdot log\frac{[K^+]_o}{[K^+]_i}(mV) \tag{1.11}$$

Is the membrane highly permeable to Na^+ and very low K^+ permeability, then α will be very large, thereby making Na^+ very large and K^+ can therefore be neglected, reducing the GHK equation to the Nernst Bernstein's equilibrium equation for Na^+:

$$V_m \stackrel{?}{=} E_{Na} = 60 \cdot log\frac{[Na^+]_o}{[Na^+]_i}(mV) \tag{1.12}$$

There is an unequal distribution of K^+ inside and outside the nerve, and the membrane is selectively permeable to K^+, therefore can a rough estimate of the membrane potential be made by using equation (1.11). Though, the present of Na^+ in the extracellular medium is essential for the excitability of the nerve, without it the nerve axons are unable to propagate informations.

Through experiments with the toxin the toxin *terodotoxin* (TTX) which is isolated form the ovaries of a Fugo (Japanese puffer fish), blocks the voltage-dependent changes in Na^+ permeability, but has no effect on the K^+ permeability. In the event an axon has been permeate with TTX, no action potential can be propagated through the axon. Another compound capable of blocking or inhibiting the voltage-dependent permeability changes is *tetraethylammonium* (TEA), which has an effect on the voltage-dependent K^+ permeability and no effect on voltage-dependent Na^+ permeability at all. With TEA the inactivation of K^+, can be observed as the action potential becomes longer in its duration and lacking the hyperpolarization.

As any paradigm, also this paradigm when too many questions are left unanswered, there will be others to whom it will not be satisfactory, when a theory fails to explain, why within the system no net heat has been released, as would be with parallel resistors and if the resting potential indeed is unexcitable. As the years went on, the list of unanswered questions only became longer, since the HH model did not take into consideration such things as entropy, temperature and pressure.

1.2 The soliton model

The thing about a paradigm is, that a paradigm shift can happened, when someone comes along and challenge the paradigm. This has been done by many, to whom the the failure of the HH model, to provide satisfactory answers, has not been good enough. In 2005 T. Heimburg and A. D. Jackson [2] (HJ) *On soliton propagation in biomembranes and nerves* did exactly that, when they suggested that the action potential in fact was not an current govern by the laws of an electrical system, but a singular

electromechanical soliton. The HJ theory takes into consideration, that the primary component in the nerve membrane are lipids and therefore will behave as lipids and the chain-melting transitions of the lipid membrane. This theory answers some of the unanswered questions, such as the changes in thickness and length during the pulse propagation, the release and reabsorption of heat, since the soltion pulse is connected to the lipid transition phase of the membrane, dependent on the non-linearity of an elastic constant.
At physiological temperatures, the nerve membrane is in a fluid state, where changes in enthalpy and entropy are linked to the melting transition, along with changes in volume, thickness and the overall area of the nerve membrane. During a nerve pulse, heat changes throughout the whole nerve takes place, without any net heat being released to the surroundings. This will only occur if the nerve pulse is an isentropic pulse propagation, since the entropy, which is a function of before and after state, a reversible process caused by the changes in the state of the lipids in the membrane.
A paradigm is only a paradigm until a new paradigm comes along, with a better or more credible theory than the one before and a paradigm shift may occur. HH took the experimental results and fit the result into a known theory, using the instruments which science could provide at that time. HJ provided a theory regarding the electromechanical soliton and the lipid membrane, and has on numerous occasions [2][4] [5][6], been able to link these experimental findings to the theory of the soliton model and hereby finding answers previously left unanswered.

1.2.1 The soliton model theory on collision

Alfredo Golzalez-Perez *et al.* [7], conducted a series of collision experiments, using ventral cord from earthworms and lobster. Using the theory of the soliton model from T. Heimburg and A. D. Jackson [2] *On solition propagation in biomembranes and nerves.* Where the equation of sound propagating soliton in a membrane cylinder, is given by equation (1.13), from [2].

$$\frac{\partial^2}{\partial t^2}\Delta\rho = \left[\left(c_0^2 + p\Delta\rho + q(\Delta\rho)^2\right)\frac{\partial}{\partial x}\Delta\rho\right] - h\frac{\partial^4}{\partial x^4}\Delta\rho \tag{1.13}$$

the density variation is given by $\Delta\rho = \rho - \rho_0$, ρ_0 is the empirical equilibrium value, c_0 is the velocity of small amplitude sound and given by $c_0 = 1/\sqrt{\rho_0\kappa_s}$ where κ_s is the lateral compressibility. A more thorough review of the equation can be found in the Appendix A.1. According to the soliton model, in the event of a collision the two soliton pulses, will pass through each other, without noticeable change in shape, as can be seen in figure (1.2). The recording chamber used by Gonzales-Perez *et al.* [7], can be seen in figure (1.3)

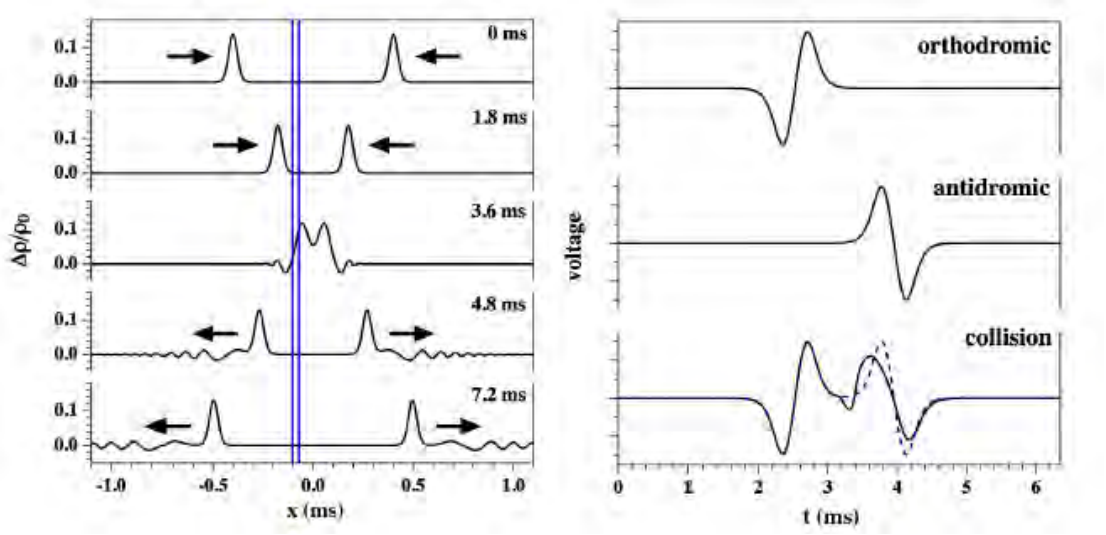


Figure 1.2: Left: The collision of two pulses in the soliton theory model. After the collision the shape of the two pulses should remain virtually unchanged. The two blue lines outlines the position of the hypothetical recording electrodes. Right: The calculated voltage difference between the two electrodes are shown in the top panel, where the orthodromic and the antidromic trace can be seen. The bottom solid trace, is from the recording of the colliding pulses and the dashed line, is from the sum of the two pulses individually. The figure is borrowed from [7].

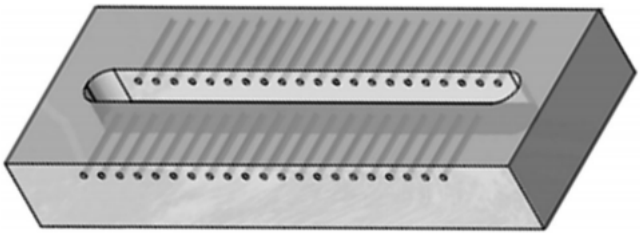


Figure 1.3: The recording chamber used by A. Gonzales-Perez *et al.* and borrowed from [7].

1.2.2 The extracellular recording from a nerve bundle

A nerve bundle is made up from groups of individual axons. Here places two stimulating electrodes near the end of the nerve bundle, thereby depolarize the the axons to the threshold, to initiate the action potential. Two recording electrode are then placed near the opposite end of the nerve bundle, to record the extracellular changes in the potential, which is being produced as the action potential is propagating along the nerve bundle. As the nerve is being stimulated with a low voltage, a stimulus artifact will be picked up by the recording electrodes, a small initial deflection. By increasing the intensity, bringing the nerve bundle past its threshold, it produces a large artifact. The time delay between the stimulus artifact and the diphasic action potential, is the

time it has taken for the action potential, to propagate, from the initiation site to the site of recording,

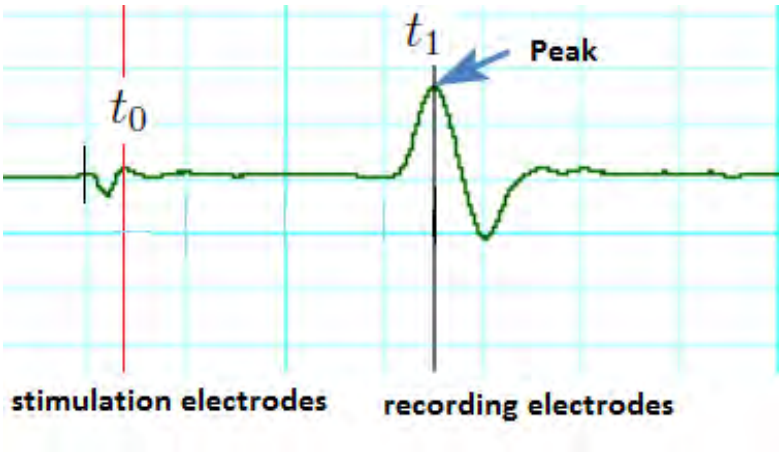


Figure 1.4: Actual recording of the action potential during experiment, with the time of the stimulus artifact as t_0 and the time of the action potential peak as t_1

To calclate the velocity from the recording of the action potential

$$velocity = \frac{d}{(t_1 - t_0)} \tag{1.14}$$

Where d is the distance between the stimulation electrodes and the recording electrodes in meter, t_0 is the time of the stimulus artifact, and t_1 is the time the action potential peak is recorded by the recording electrodes.
If the voltage is increased further, a point will be reached where the stimulus artifact will continue to increase, but do not produce a larger action potential. This occurs since the nerves are in a all-or-nothing fashion, within the nerve bundles, the axon with the lowest threshold pick-up the action potential first. The action potential is then recorded by the recording electrodes, to be displayed on the recording device. By increasing the intensity, more axons are brought to the threshold, giving multiple action potentials propagating along the different axons in the nerve bundle, which will be picked-up by the recording device. Eventually all the axons in the nerve bundle will have reached their threshold, thereby causing no further action potentials, by increasing the stimulation intensity. This is also known as a *compound action potential*. It may also happened that the recording device will yield multiple peaks, caused by the nerve bundle being made up from many different axons, some which are myelinated, and some which are not, this gives the different axons different propagations velocities, which will reach the recording device at different times.

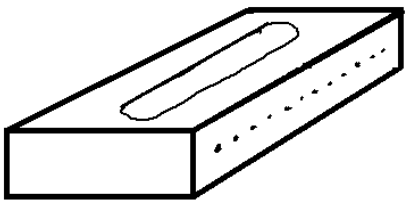
2 Materials and methods

2.1 Hardware and software

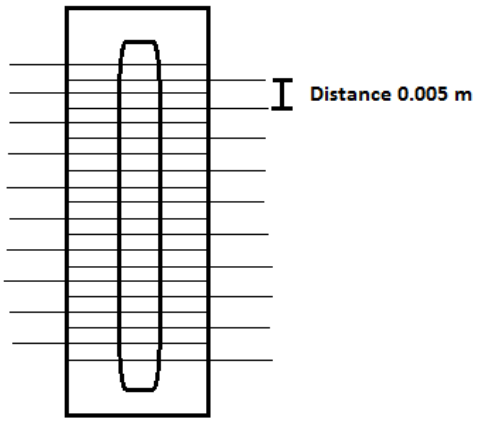
The PowerLab 26T data acquisition hardware, purchased from AD Instrument Europe (Oxford, UK). The instrument has an internal bio-amplifier, which allows the recording electrical potential in the scale of microvolt and has two recording channels. The LabChart software also from AD Instrument was used to control the PowerLab 26T, by sending the signal and recording the signal (further information, regarding the PowerLab 26T and LabChart, can be found on the company website [8].

2.2 Recording chambers

Two different chambers are used to the experiments, the dry chamber, which is made of plexiglass and measure 7×2.5 cm, with a height of 1 cm, there is a groove running through the middle, as can be seen in figure (2.1a) and stainless steal pins, inserted in through the sides, so that the nerve bundle will be resting onto the pins, which can be seen in figure (2.1b), the recording electrodes attaches to the pins in one end and the stimulation electrodes are placed in the other end. By counting the pins, from the stimulation electrodes to the recording electrodes (disregarding the ground wire), the distance can be calculated, since there is 0.25 cm between each pin, if counted on both sides.

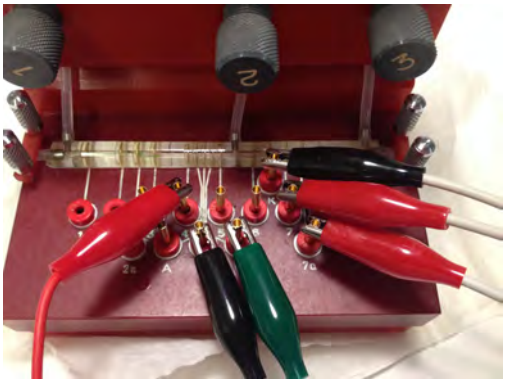


(a) Sketch of the dry chamber.

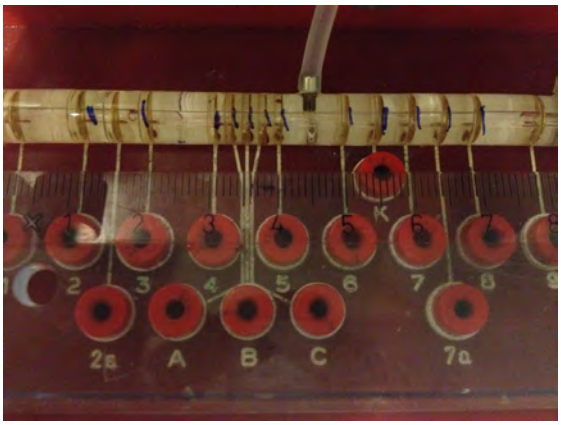


(b) Sketch of the dry chamber viewed from the above.

Figure 2.1: Sketch of the dry recording chamber, not up to scale.



(a) The liquid chambers glass capillaries, with the three injections sites, the recording electrodes and the stimulation electrodes.



(b) The liquid chamber, finding the distance between the stimulation and the recording electrodes.

Figure 2.2: The liquid glass capillary recording chamber.

The liquid recording chamber is a long glass capillary, as can be seen in figure (2.2a) which has wires placed on the inside of the capillary. There are three sites for injection of liquid, making it possible to change the liquid while the nerve is still resting inside the capillary. The three injections sites and both ends of the capillary can be sealed off, to avoid any loss of liquid or air to enter the capillary. Each wire inside the capillary are connected to an outlet, where the stimulation and recording electrodes can be placed as can be seen in figure (2.2a) and the distance between the wires can seen in figure (2.2b).

2.3 Central nervous system of the lobster

The lobster belongs to the Invertebrates, animals without a spinal column. The anterior cerebral ganglion (the superesophageal ganglion), lies above the esophagus[2], a pair of circumesophageal, one of two strands of nerve tissue surrounding esophagus and connecting dorsal supraesophageal ganglia with posterior subesophageal ganglia. The the superesophageal ganglion or brain, contains three regions.

- The Protocerebrum:
  The most anterior region, receives input from the eyes and the frontal organs.
- The deuterocerebrum:
  The middel region, receives input from the antennae and assosiated with the olfaction.
- The tritocerebrum:
  The most posterior, is connected to the nerves which runs to the labrum[3], the

[2]The part of the alimentary canal which connects the throat to the stomach

[3]A structure corresponding to a lip, especially the upper border of the mouthparts of a crustacean or insect.

alimentary canal, along with the circumsophageal commissure, the part that connect the brain to the ventral nerve cord

There are two pairs of giant fibers, the lateral and the medial, which runs down the lenght of the abdomen in the ventral nerve cord. The medial starts in the protocerebrum, and the lateral starts posterior to the brain. These fibers conducts nerve impulses very fast, since they are well myelinated [9].
In each of the walking legs, there are a lateral and a medial big bundle nerve, which runs along the ventral side. These are also being used in the following experiment.

2.3.1 Nerve preparation

The lobsters used in this series of experiments are the humaus americanus (American lobster) or the Homarus gammarus which is the native lobster of the oceans surrounding Denmark, both are part of the Nephropidae lobster family [9]. The lobsters were purchased live form the local fishmonger, there is a detailed description of the dissection and removal of the different nerves in the Appendix A.2.3. The ventral cord contains four giant axons, two median giant axons which runs as a single neuron through the ventral cord and two lateral giant axons, which are formed by six neurons connected at each ganglia and can be seen in figure (2.3) before it's removed and in figure (2.4) after is has been removed. The lateral giant axons in the abdominal region (tail), has a larger diameter than the median giant axons. After removal, the nerves has been kept in petri-dishes containing lobster saline solution adapted from [10], the exact composition can be seen in table A.1. The big bundle nerve from the walking legs are not removed from the legs, until right before use, to keep them as fresh as possible.

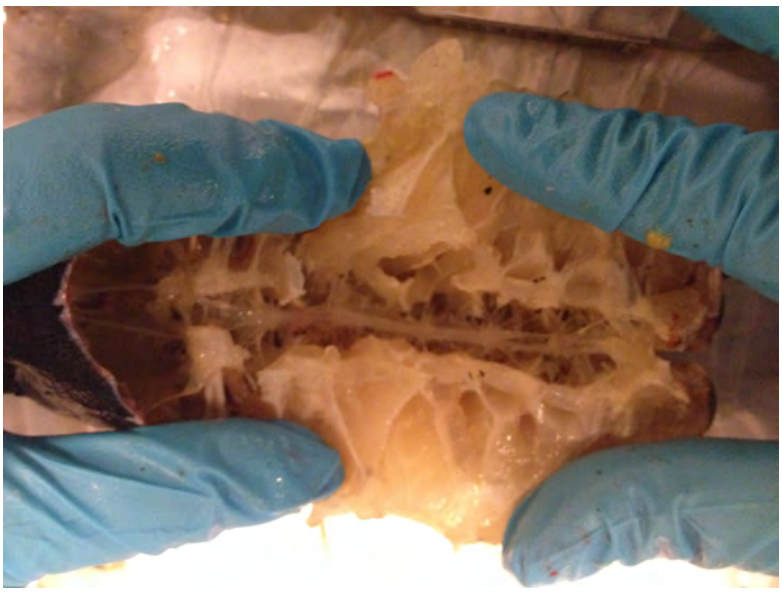


Figure 2.3: The cephalothorax with the thoracic nerve exposed, during the dissection of the lobster.

Figure 2.4: The ventral nerve cord from lobser. Left: Thoracic nerve cord. Right: Abdominal nerve cord.

2.4 Collision

Using the abdominal ventral cord from lobster. The nerve is first tested in the dry recording chamber to ensure that the nerve is undamaged from the extraction. After an adequate signal has been confirmed, the nerve bundle is placed back into a petri-dish, where sewing thread carefully is tied to both ends of the nerve bundle. The other ends of the sewing thread is tied to a thin metal wire. The liquid recording chamber is then filled up with lobster saline, the exact composition can be found in table (A.1) and the nerve bundle is carefully pulled into the glass capillary, by using the metal wire as a guiding needle. More lobster saline is added, from the three injections sites, until the glass capillary is free of air bubbles and sealed off, so no liquid can escape the capillary. The stimulations electrodes are placed in the orthodromic direction and the recording electrodes are placed in the antidromic direction and the signal is recorded, the stimulation electrodes are then placed in the antidromic direction and the and the recording electrodes in the orthodromic direction, once again recording the signal, to ensure that the full length of the abdominal ventral cord is undamaged and a good signal can be recorded from the orthodromic and the antidromic direction.
Two pairs of stimulation electrodes are placed by each ends of the nerve bundle and the recording electrodes are placed $1/3$ of the total length of the axon in the orthodromic direction. The recording will then be the difference in potential between the two electrodes and the resulting signal will then be approximately the first derivative of the true pulse shape. When two pulses are generates simultaneously, from opposite ends of the nerve, the orthodromic pulse will be recorded before colliding with the antidromic pulse and the antidromic pulse will then be recorded, after the collision with the orthodromic pulse. In the case of the annihilation of the two pulses during colli-

sion, only the orthodromic pulse should be recorded. Should the collision of the two pulses penetrate each other, there should be the recording of the orthodromic pulse followed by the recording of the antidromic pulse.

2.5 Potassium chloride

Using the big nerve bundle from the walking legs of the lobster. The nerve bundle is first tested, in the dry recording chamber, to ensure it's undamaged form the extraction, by carefully placing the nerve bundle across the metal pins in the groove. Then attaching the stimulation electrodes in the orthodromic direction and the recording electrodes in the antidromic direction. By adjusting the voltage in the LabChart software, a good signal can be archived.
After ensuring a good signal, the nerve bundle is moved into a petri-dish containing lobster saline, the composition of the saline can been seen in table (A.1) along with the desired concentrations of KCl, the exact amount of KCl added to the saline to make the different concentrations, can be found in the Appendix A.2.2, table (A.2). A timer is started and following the appropriate time interval, the nerve bundle is placed upon the dry recording chamber and 3V stimulation to the nerve bundle is recorded. The nerve bundle is placed into the KCl solution again, for another duration of time and the process is repeated until the signal has disappeared. When the signal is gone, the nerve bundle is once again placed into a petri-dish with the normal lobster solution, so that the signal may be recovered again.

3 Experimental results

3.1 Collision results

The results of the collision experiment, conducted in the liquid recording chamber, using the abdominal ventral cord from the lobster, where the first lateral giant axon will be stimulate first, at the lowest stimulation voltage and the medial giant axon will need higher voltage to be stimulated, which will be peak number two. The small fibres in the ventral cord, will appear as small signals, they require a higher stimulation voltage. Therefore, during low stimulations only one peak will be recorded, as the stimulation voltage increases, multiple peaks will be recorded.
All experiment has been preformed in the regular lobster solution table A.1. The nerve bundle is first stimulated from both ends independently, prior to preforming the collision experiment. The stimulation voltage was slowly increased, until a good signal had been reached from both ends.
The recording electrodes are placed near the orthodromic end, making it necessary for the antidromic pulse to pass through the orthodromic pulse to reach the recording electrodes. Therefore, in the event that the orthodromic pulse and the antidromic pulse both are being recorded, the two pulses will have to pass each other. On the

other hand, is only the orthodromic puls recorded, the two pulses has been annihilated upon collision.

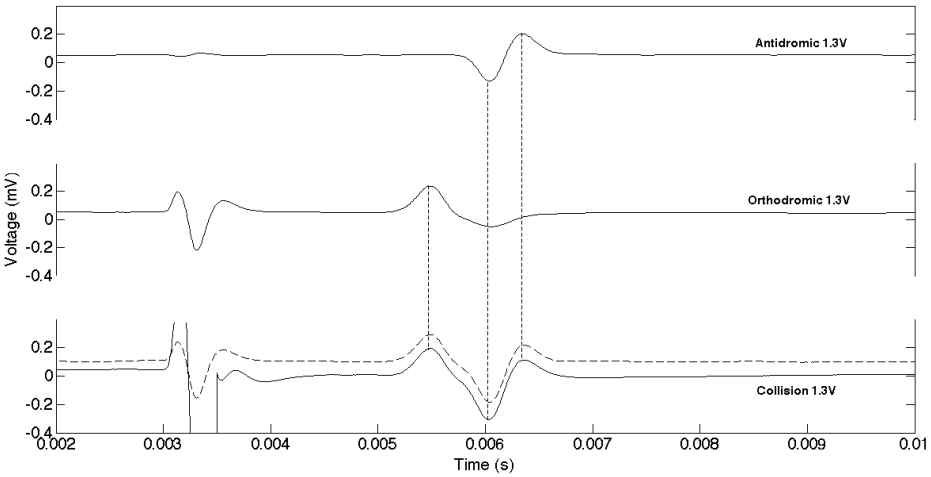
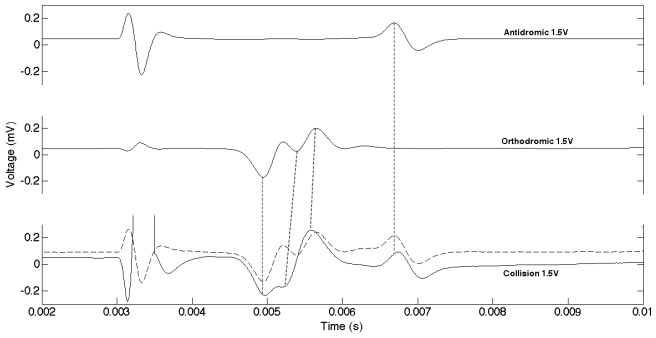
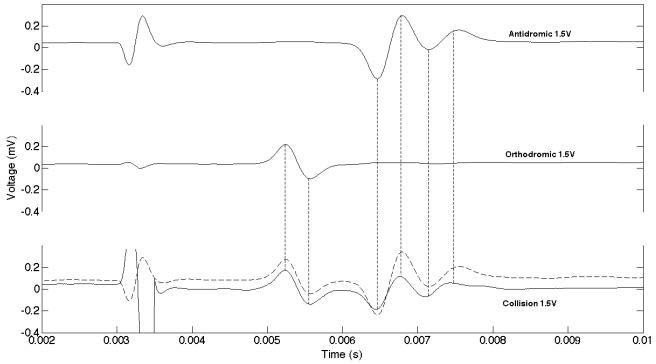


Figure 3.1: Collision experiment in the liquid chamber, on the ventral abdominal nerve from the lobster, at the stimulation voltage of 1.3V. Top: Only stimulation in the antidromic direction. Center: Only stimulation in the orthodromic direction. Bottom: The collision experiment (solid line), compared with the sum the top (antidromic) and the center (orthodromic) trace. The two traces are virtually superimposable, which are an indication that non of the signals has been annihilated, during the collision.

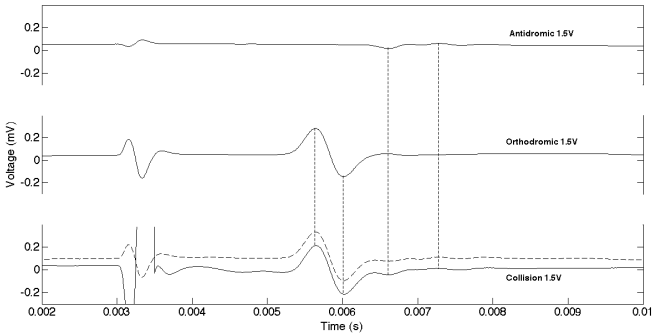
The recorded results from the collision experiments, using different stimulation voltage: In figure (3.1) the stimulation has been made with 1.3V. At the low voltage, only the lateral giant axon is stimulated, in both the antidromic and the orthodromic direction. As the two pulses collide, which can be seen in the bottom panel as the solid line, since the recording electrodes are placed closest to the orthodromic end, it will also be the first signal recorded. The antidromic signal will therefore not be recorded until after the collision and should be recorded after the orthodromic pulse, unless the signals are annihilated during collision. The dashed lines in the collision experiment, shows all signals are conserved compared to the sum of the signal of the antidormic and the orthodromic stimulation.



(a) Collision in the liquid recording chamber at 1.5V

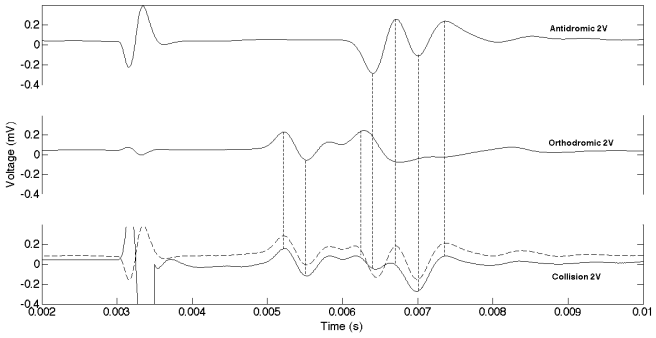


(b) Collision in the liquid recording chamber at 1.5V

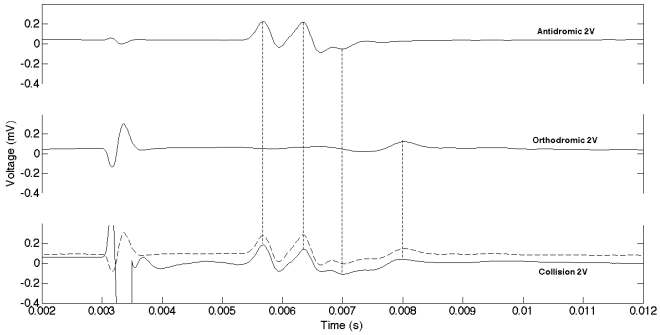


(c) Collision in the liquid recording chamber at 1.5V

Figure 3.2: Collision experiments in the the liquid recording chamber, using the ventral abdominal cord of the lobster and 1.5V stimulation. Top panel: the action potential propagating antidromic after the stimulation artifact. Center panel: The action potential propagating orthodromic after stimulation. Bottom panel: The recording of the two colliding pulses (solid line). The dashed line is the sum of the individual pulses.



(a) Collision in the liquid recording chamber at 2V



(b) Collision in the liquid recording chamber at 2V

Figure 3.3: Collision experiments in the the liquid recording chamber, using the ventral abdominal cord of the lobster and 2V stimulation. On the top panel: the action potential propagating antidromic after the stimulation artifact. Center panel: The action potential propagating orthodromic after stimulation. Bottom panel: The recording of the two colliding pulses (solid line). The dashed line is the sum of the individual pulses.

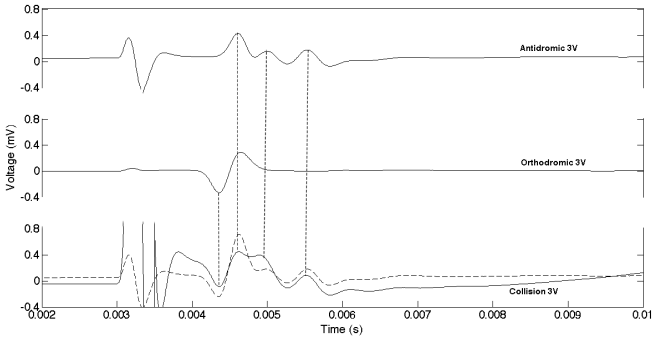


Figure 3.4: Collision in the liquid recording chamber at 3V, the panels are in the same as in figure (3.3). The lateral, medial and the small fibres has been stimulated in both the antidromic and the orthodromic ends.

In figure (3.2), the abdominal ventral cord has been stimulated by 1.5V. The medial ventral axon is becoming stimulated in the orthodromic end in figure (3.2a) and

the antidromic end in figure (3.2b), once again the pulse was not annihilated upon collision, in any of the experiments. the dashed line shows that all the signals are conserved. Figure (3.3) with 2V, the medial ventral cord has been stimulated both antidromic and orthodromic in figure (3.3a) in figure (3.3b) the medial ventral cord along with some small fibres has been recorded from the antidromic and only a very small signal from the orthodromic end. Using 3V of stimulation renders clear signals from both the antidromic direction and the orthodromic direction as can be seen in figure (3.4) and (3.5).

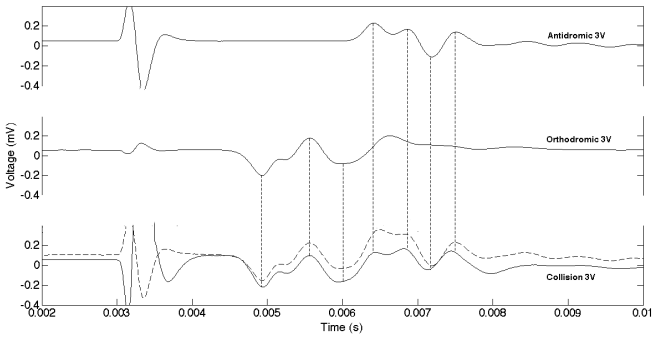


Figure 3.5: Collision in the liquid recording chamber at 3V, the panels are in the same as in figure (3.3). The lateral, medial and the small fibres has been stimulated in both the antidromic and the orthodromic ends.

In none of the collision experiment using the abdominal ventral cord, did the signal in the antidromic direction become annihilated doing the collision. All resulted in the recording of the orthodromic pulse and the antidromic pulse consecutively, with almost no alteration in the antidromic signal, as this is the signal recorded after the collision of the two simultaneously produced pulses.

3.2 Potassium chloride results

Six different big bundle nerves from the walking leg of the lobster, has each been exposed to 40 mM, 60 mM, 80 mM, 120 mM and 240 mM potassium chloride, until the signal was gone. Since every nerve is different in it's ability to propagate the signal and the time it takes for the depolarization at the different concentrations, it is not surprising to find variation in the time for the signal to be gone at certain low concentrations.
When placed in a higher concentration of KCl over a longer duration of time, the nerve bundle gains KCl since the P_K and P_{Cl} both are high, as sufficient KCl has entered, the nerve bundle as expected from the Nernst Bernstein's equilibrium equation (1.11) for potassium, the nerve will not be able to repolarize as $[K^+]_i = [K^+]_o$. There is a correlation between the concentration and the time spend in the solution. At lower concentration such as 40 mM, the nerve bundle has to spend more time in the solution, before the signal is gone. At concentrations as 120 mM it is between 30 ± 10 to 10 ± 10 seconds and for 240 mM 4 ± 2 to 1 ± 2 seconds. The repolarization when

returned to a solution with a lower concentration KCl is therefore dependent on the concentration of KCl and the time spend in the solution. The time collected for the signal to return, is the the time it has taken for the signal to rebound back to it's original strength.
The collected data can be seen in table 3.1 and the resulting plot can be seen in figure (3.6), where each line in the plot represent one nerve bundle as it has been exposed to different concentrations of KCl. Afterwards the signal was recovered again in the usual lobster saline, which contains 16 mM KCl, or in lobster saline containing only 8 mM KCl. A test was preformed on one nerve, exposed to 40 mM KCl and recovered in 16 mM, where the recovery time was 25 minutes. The nerve was once again exposed to 40 mM KCl and recovered in 8 mM KCl, which only took 5 minutes, so the recovery time in 8 mM is 5 times faster, which has been taken into consideration for the recovery time in table 3.2 and the resulting plot in figure (3.7).

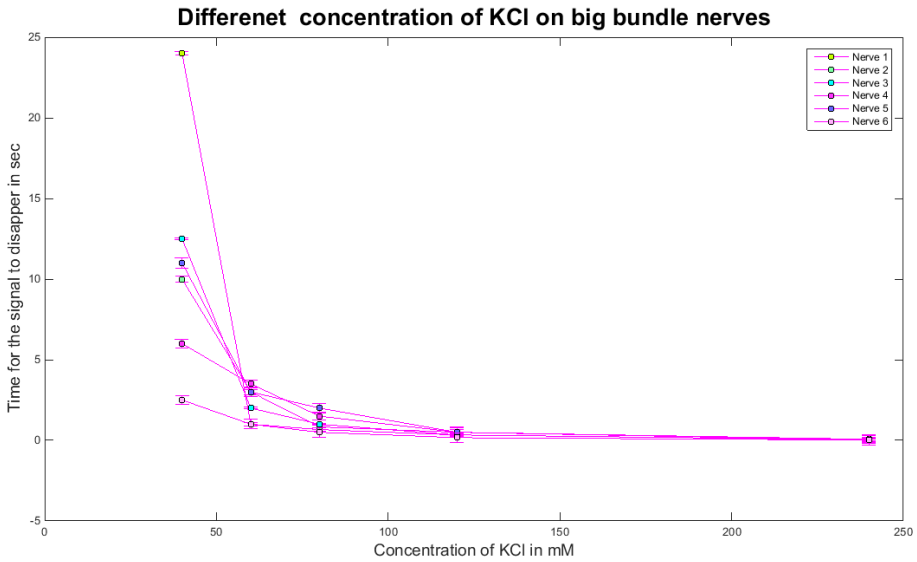


Figure 3.6: The time it has taken for the signal to disappear, when a big bundle nerve from the walking leg of a lobster, is exposed to different concentrations of KCl. Each line represent a different nerve bundle, all the nerve bundles has been exposed to 40 mM, 60 mM, 80 mM, 120 mM and 240 mM KCl. The time it has taken for the signal to be gone has been plotted

KCl in mM	Time for the signal to be gone in seconds					
40	1440 ± 10	600 ± 10	750 ± 10	360 ± 10	660 ± 10	150 ± 10
60	60 ± 10	180 ± 10	120 ± 10	210 ± 10	180 ± 10	60 ± 10
80	40 ± 10	50 ± 10	60 ± 10	90 ± 10	120 ± 10	30 ± 10
120	20 ± 10	30 ± 10	20 ± 10	30 ± 10	30 ± 10	10 ± 10
240	2 ± 2	1 ± 2	2 ± 2	4 ± 2	1 ± 2	1 ± 2

Table 3.1: The time it takes for the signal to gone in different concentrations of KCl

KCl in mM	Time for the signal to return in seconds					
40	1500 ∓ 10	2400 ± 10	810 ± 10	4950 ± 10	3500 ± 10	1920 ± 10
60	2100 ± 10	720 ± 10	1012 ± 10	2100 ± 10	4500 ± 10	240 ± 10
80	2400 ± 10	4020 ± 10	1200 ± 10	3000 ± 10	6000 ± 10	900 ± 10
120	3000 ± 10	3900 ± 10	660 ± 10	6000 ± 10	10800 ± 10	780 ± 10
240	3000 ± 10	6000 ± 10	1520 ± 10	9000 ± 10	6000 ± 10	1200 ± 10

Table 3.2: The time it takes for the signal to return, after being exposed to different concentration of KCl

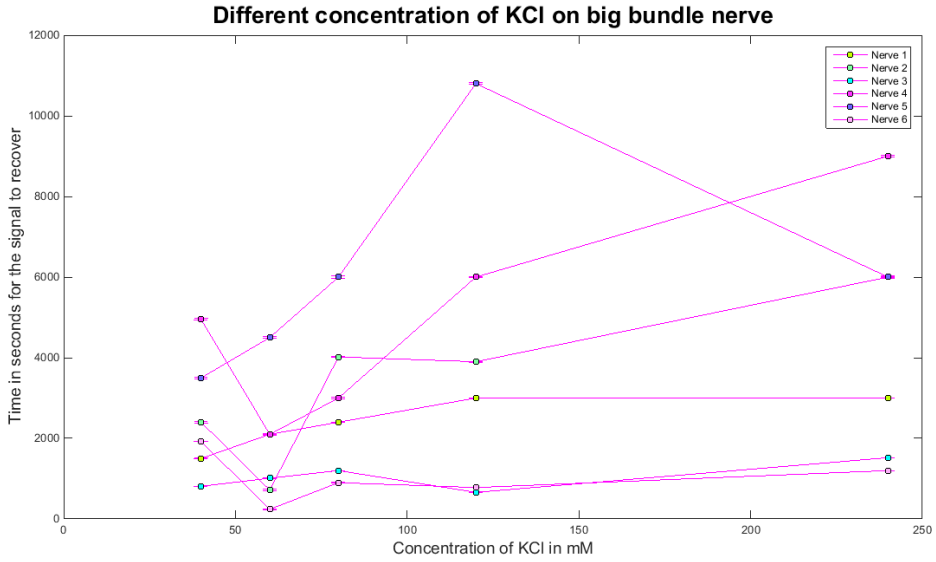


Figure 3.7: The time it has taken for the different nerves bundles from the walking leg of a lobster to recover the signal after being exposed to to different concentrations of KCl. The signal was recovered in the usual lobster saline (16 mM KCl) or in 8 mM KCl, the time difference of recovering the signal in a lower concentration, has been taken into consideration, by multiplying by 5 the time in 8 mM KCl.

3.3 Future work

More experimenting with collision in the liquid recording chamber is necessary, as very little experimenting on this part has been preformed, to ensure the consistency of the results.
The potassium chloride experiment was to establish more accurately kinetics in the specific conditions for blocking the signal, which is to be used in other research of magnetic signal in measurement of nerves, by *Rima Budvytyte postdoc.* and *Tian Wang Ph.D. student.*

4 Conclusion

The collision experiments, in the liquid recording chamber, on the abdominal ventral cord of the lobster; where stimulation electrode are place at each end of the nerve and recording electrodes are placed $1/3$ the distance from the orthodromic direction. So the pulse travelling in the orthodromic direction would have to pass the recording electrodes, prior to a collision and the pulse travelling in the antidromic direction, will not be recorded until after the collision. In the HH model, the collision would cause the two pulses to be annihilated, due to the voltage-dependent activation and inactivation gates, leaving the nerve unexcitable directly following an action potential. In the case of a collision of the two pulses, the results found during the experiments in the liquid recording chamber, is in accordance to the soliton theory model. Using different intensities, the recording of the two pulses was virtually unaltered by the collision and in non of the experiments, did the collision result in annihilation. Further studies on this matter, would be necessary, but so far the findings in this thesis, confirms the findings of A. Gonzalez-Perez *et. al* [7].
The experiments with the high concentrations of external potassium chloride effect, on the compound action potential, in axon bundles from the walking legs of lobster. The result gave a good picture of the correlation between the concentration of KCl and the time until the nerve no longer was able to repolarize. Also showing a difference in the time of each nerve bundles at the lower concentrations. The recovery in the 16 mM and 8 mM KCl solution will need more testing, to find an average for the time difference of the recovery time in the different concentration, to decrease the margin of error.

> *Scientific development depends in part on a process of non-incremental or revolutionary change. Some revolutions are large, like those associated with the names of Copernicus, Newton, or Darwin, but most are much smaller, like the discovery of oxygen or the planet Uranus. The usual prelude to changes of this sort is, I believed, the awareness of anomaly, of an occurrence or set of occurrences that does not fit existing ways of ordering phenomena. The changes that result therefore require 'putting on a different kind of thinking-cap', one that renders the anomalous lawlike but that, in the process, also transforms the order exhibited by some other phenomena, previously unproblematic.*
> Thomas S. Kuhn *The Essential Tension*, (1977), xvii.

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A Appendix

A.1 Electromechanical soliton model theory

Here is the extended theory for the soliton model.

A.1.1 Heat capacity and compressibilityin the soliton model

Finding the elastic constant from the heat capacity, by following relations,

$$c_p = \frac{\langle H^2\rangle - \langle H\rangle^2}{RT^2},\ \kappa_T^V = \frac{\langle V^2\rangle - \langle V\rangle^2}{\langle V\rangle RT},\ \ \kappa_T^A = \frac{\langle A^2\rangle - \langle A\rangle^2}{\langle A\rangle RT} \tag{A.1}$$

where c_p is the heat capacity, κ_T^V is the isothermal volume compressibility and κ_T^A the lateral compressibility, and

$$\begin{aligned}\kappa_T^V = \kappa_{T,0}^V + \Delta\kappa_T^V \Leftrightarrow \kappa_{T,0}^V + \frac{\gamma_v^2 T}{\langle V\rangle}\Delta c_p \kappa_T^A \\ \Leftrightarrow \kappa_{T,0}^A + \Delta\kappa_T^A\end{aligned} \tag{A.2}$$

$$\kappa_T^V = \kappa_{T,0}^A + \frac{\gamma_A^2 T}{\langle A\rangle}\Delta c_p$$

The sound propagation velocity in an elastic media; $c_0 = \sqrt{\rho\kappa_S}$ is a function of the isentropic compressibility, $\gamma = exp(error)$ and is a constant, κ_S^V and κ_S^A, and by using Maxwell's relations, then

$$\kappa_S^V = \kappa_T^V - \frac{T}{\langle V\rangle c_p}\left(\frac{dV}{dT}\right)_p^2,\ \ \kappa_S^A = \kappa_T^A - \frac{T}{\langle A\rangle c_p}\left(\frac{dA}{dT}\right)_p^2 \tag{A.3}$$

[4]

A.1.2 Thermal melting transition

Consider a 1D sound propagation along a cylinder shaped membrane, moving in the x-direction, then

$$\frac{\partial^2}{\partial t^2}\Delta\rho^A = \frac{\partial}{\partial x}\left(\frac{1}{\kappa_S^A\rho^A}\left(\frac{\partial}{\partial x}\Delta\rho^A\right)\right) \tag{A.4}$$

where $\Delta\rho^A = \rho^A - \rho_0^A$, then if $\Delta\rho^A \ll \rho_0^A$ it can be reduced to,

$$\frac{\partial^2}{\partial t^2}\Delta\rho^A = c_0^2\frac{\partial^2}{\partial x^2}\left(\Delta\rho^A\right) \tag{A.5}$$

where the velocity small amplitude sounds are $c_0 = 1/\sqrt{\rho_0^A \kappa_S^A}$ and since κ_S^A is dependable upon $\Delta\rho^A$ the Taylor expansion on

$$c^2 = \frac{1}{\rho^A \kappa_S^A} = c_0^2 + p\Delta\rho^A + q\left(\Delta\rho^A\right)^2 + ... \tag{A.6}$$

then by approximate the dispersive effect, by adding the dispersive term $-h\frac{\partial^4}{\partial z^4}\Delta\rho^A$ for $h > 0$ then

$$\frac{\partial^2}{\partial t^2}\Delta\rho = \left[\left(c_0^2 + p\Delta\rho + q(\Delta\rho)^2\right)\frac{\partial}{\partial x}\Delta\rho\right] - h\frac{\partial^4}{\partial x^4}\Delta\rho \tag{A.7}$$

The sound velocity is then given by

$$v^2\frac{\partial}{\partial z^2}\Delta\rho^A = \frac{\partial}{\partial z}\left[\left(c_0^2 + p\Delta\rho^A + q(\Delta\rho^A)^2\right)\frac{\partial}{\partial z}\Delta\rho^A\right] - h\frac{\partial^4}{\partial z^4}\Delta\rho^A \tag{A.8}$$

consider a solitons being localized and become extinct as $|z| \to \infty$ then

$$h\frac{\partial^2}{\partial z^2}\Delta\rho^A = \left(c_0^2 - v^2\right)\Delta\rho + \frac{1}{2}p\left(\Delta\rho^A\right)^2 + \frac{1}{3}\left(\Delta\rho^A\right)^3 \tag{A.9}$$

The soliton profile will have a maxima $\frac{\partial}{\partial z}\left(\Delta\rho^A\right) = 0$ and symmetry will occur around the maxima.
Is a membrane slightly above the melting temperature, it will then respond to compression by first lowering the elastic modulus, then followed by a steep increase [4].

A.1.3 Effects of anaesthetics on the lipid melting points

There is a correlation between the linear dependence of the melting point of the lipid membrane and the concentration of the anaesthetic. From the freezing point depression effect

$$\Delta T_m = \left(\frac{RT_m^2}{\Delta H}\right)x_A \tag{A.10}$$

Where ΔH is the enthalpy, T_m the melting temperature of the lipid membrane and x_A the molecular fraction of the anaesthetic molecules, then

$$\frac{\Delta T_m}{\Delta c_{anaesthetic}} = \frac{\beta}{ED_{50}} \tag{A.11}$$

where $\beta = exp(\alpha) = -0.534K$ and ED_{50} is the critical anaesthetic dose, if $c_{anaesthetic} = ED_{50}$ there is an empirical shift of T_m at ED_{50}, which makes $\Delta T_m = -0.53$. The relocation of the melting point of the lipid membrane during the critical anaesthetic dose, is always the same and is independent of the type of drug being used.
The critical molecular fraction of anaesthetic in the membrane $x_A = P \cdot ED_{50} \cdot V_L$, where V_L is the molar volume of the lipids and P is the partition coefficient between oil and water giving $x_A = 0.026$ which is independent of drug, hereby giving $T_m = -0.6K$ [4].

A.1.3.1 Lateral pressure profile

The lateral pressure induced in the membrane by anaesthetics induces a lowering of the solid-liquid transition temperature and is given by

$$\Pi = -\frac{dF}{dA} \Leftrightarrow -\frac{dF}{A_f dF} \Leftrightarrow -kT\frac{n}{fA_f} \Leftrightarrow /kTx_A^f \tag{A.12}$$

where $F = F_0 + nkTlnx_A^f$ is the concentration changes due to the molar fraction of fluid phase f changes and $x_A^f = \frac{n}{fA_f}$ and the total area of the fluid membrane fA_f. The pressure in the fluid phase is then

$$\Pi = -kT\frac{n}{A_f} \tag{A.13}$$

if $f \to 0$ then $p \to \infty$, causing the system to change to gel phase.
Fining the melting temperature for the lipid membrane in the presence of anaesthetics,

$$T_m^A = \frac{\Delta H + \Pi A_f}{\Delta S} \Leftrightarrow T_m + \frac{\Pi A_f}{\Delta H} T_m \tag{A.14}$$

with $x_A^0 = 0.026$ and taking the parameters of a DPPC membrane [4], where $T_m = 314.2K$ and $\Delta H = 35kJ/mol$ $\Delta T_m = -0.6K$ which is the same as the melting temperature found from the effect of anaesthetics. leaving it possible, that the effect of anaesthetics is due to the lowering of the solid-liquid transition temperature in lipid membranes, by only being soluble in the liquid phase of the membrane.

A.2 Experimental part

A.2.1 Lobster saline solution

Adapted from Add the exact compounds into 1 litre of millipore water, and finish of with ajusting the pH 7.40 with 10% HCl

Compound	Concentration (mM)	Mass in g.
NaCl	462	26.99
KCl	16	1.18
$CaCl_2$	26	3.82
$MgCl_2$	8	0.76
Glucose	11	2.178
Tris	10	1.21
MaleicAcid	10	––

Table A.1: Lobster saline solution

A.2.2 KCl solutions

The different concentrations of KCl, has been made using the lobster saline solution from table (A.1) in the appendix. To adjust the different concentration of KCl in the lobster solution, using following formula For 8 mM KCl into 100 ml saline lobster solution without KCl

$$m = M \times c \times V \tag{A.15}$$

Where m is the mass of KCl to be added, M is the molar mass and V is the volume, for 8 mM KCl in 100 ml lobster saline solution

$$74.55g \cdot mol^{-1} \times 0.008mol \cdot l^{-1} \times 0.1l = 0.0564gKCl \tag{A.16}$$

KCl in $g \cdot mol^{-1}$	Concentration in mM	KCl in g
74.55	8	0.05964
74.55	40	0.2982
74.55	60	0.4473
74.55	80	0.5964
74.55	120	0.8946
74.55	240	1.7892

Table A.2: Different concentrations of KCl

A.2.3 Dissection of the lobster

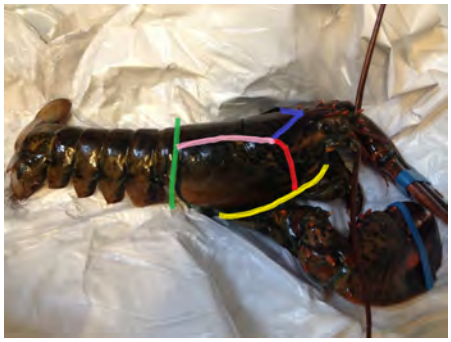
The lobster used in this series of experiments are the humaus americanus (American lobster) or the Homarus gammarus which is the native lobster of the oceans surrounding Denmark, which are both part of the Nephropidae lobster family. They feature 5 pairs of legs, the first pair have the large claws and the four other pairs are the walking legs also featuring smaller claws. The Homarus gammarus (Danish lobster) can be seen in figure(A.1a) and the Homarus americanus (American Lobster) can be seen in figure (A.1b).

(a) The Homarus gammarus (Danish lobster)

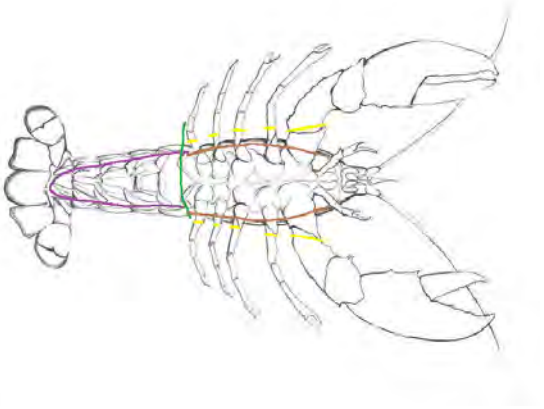
(b) The Homarus americanus (American Lobster)

Figure A.1: The lobsters use for the experiments

The live lobster is kept in the refrigerator, for approximately 30 minutes, this is to pacify the lobster. After removing the lobster from the frigs, it is necessary to hold the lobster ventral side up (turning it on it's back), this will have a sedative effect, the abdomen (tail) relaxes and become uncurled. When properly sedated, lay the lobster flat on the table dorsal side up, be sure to keep the abdomen (tail) stretched out and flat, since all the strong muscles are located in that region and can be used to push-off the table. Maintain one hand on the lobster at all times, place the walking legs spread out, away from the cephalothorax (torso).
Swiftly remove the heavy clawed legs and the walking legs, as seen in figure (A.2a) as the yellow line, using a heave sharp knife and immediately place the walking leg into a container filled with lobster saline solution (recipe can be found in table (A.1).
Turn over the lobster, to expose the ventral side, place the knife at the anterior end of the abdomen (tail) and in one quick motion (using both hands) separate the abdomen from the cephalothorax, the green line in figure (A.2a).
Turn the abdomen ventral side up, make sure to maintain a firm grip on the whole length of the dorsal side of the abdomen, since the neuromuscular reflexes are still intact and the contractions can be quit strong. Remove the swimmerets (small fins on the ventral side), then using a pair of scissors to cut along each side, anterior to posterior, close to the left lateral and the right lateral, in the softer shell of the ventral sides of the abdomen, shown as the purple line figure (A.2b). With a swift hard pull, pull the softer shell off, and the ventral nerve cord of the abdomen, should be attached to the inside, carefully detach completely and lay into a petri dish with lobster saline solution.
Take the cephalothorax, cut using scissors from posterior to anterior on the ventral side next to the harder lateral shells on each side, as shown in figure (A.2b) as the brown line. When reaching the insertion site of the first walking leg, extend the cut in the dorsal direction, until it reaches the dorsal side as seen in figure (A.2a) as the red line, repeat on the other lateral side. Turn the dorsal side up, make the incision posterior to anterior, right where the lateral begins, as can be seen in figure (A.2a) as the pink line. Finish up with a *V* incision as the blue line in figure (A.2a).



(a) The four major incisions, during dissection of the lobster. 1st is yellow, 2nd-green, 3rd-red, 4th-pink and 5th-blue.



(b) The ventral side of the lobster, showing the incisions to the ventral abdomen as purple and the incisions to the cephalothorax as brown.

Figure A.2: The major incisions during the dissection.

Now the hard outer shell can be removed, exposing the intestines, the heart and the stomach which are located most anterior, all which can be removed, along with the underlying muscle tissue and the cerebral ganglia can be seen most anterior. Carefully cut through the cartilage in the center line and expose the thoracic nerve.
Using surgical scissors, carefully detach each thoracic ganglion, by separating all the branches on each side and underneath. Finish off with making a circular incision, around the cerebral ganglia insertion point into the brain, detaching the nerve completely, so it may be put into a petri-dish with lobster saline solution.

A.3 Miscellaneous

A.3.1 Propagation of uncertainty

The experimental uncertainty, is calculated from

$$\delta f = \sqrt{\left(\frac{\partial f}{\partial x_1}\right)^2 \delta x_1^2 + ... + \left(\frac{\partial f}{\partial x_n}\right)^2 \delta x_n^2} \tag{A.17}$$