



Three aspects of neural signalling with focus on pain– propagation, inhibition and application

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ABSTRACT

This thesis includes three different aspects of neural signalling, a new mathematical model for the action potential and its propagation, an experiment of how general anaesthetic affect the membrane of a neuron and speculations about what mechanisms underlie pain relief of the bioelectric dressing; *Procellera*TM.

At present the mathematical models for an action potential and its propagation is based on Hodgkin & Huxley's model which is heavily founded on experimental result and consists of a very complex expression. This thesis present a new model which is based on the observed phenomenon that the axon is expanded when the action potential is transferred, this results in a much simpler expression. These equations are also visualized through simulations in finite element method based programme, *COMSOL*TM and show the same behaviour as an action potential, i.e. a pulse shape.

The essential feature of general anaesthetic is that it inhibits neural signals. How it does this is debated in the scientific community. Different field of science provides different explanations. There is a well known rule; the Meyer-Overton rule which says that the effect of general anaesthetic is proportional to its solubility in olive oil which resembles the axon membrane. This thesis presents experiments where QCM-D (Quartz Crystal Microbalance with Dissipation monitoring) is used to observe how the resonance frequency and dissipation of a membrane is change when general anaesthetic is dissolved in it. It also present an experiment monitoring the electrical properties changes of the membrane due to general anaesthetic.

The bioelectric dressing *Procellera*TM is at present claimed to reduce the pain sensation in a wound, by inhibition of the pain signal. The dressing contains zinc and silver elements and therefore this thesis speculates in how these metals can result in pain reduction. This speculation discuss different receptors inhibition, chemical reactions and other mechanisms which are possible to occur in the interaction of the dressing and the wound.

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1 INTRODUCTION

We are all familiar with pain, how it feels and what can cause it, but we might not always think about how it works. Once in a while we take a pill to reduce pain and before surgery we get anaesthetics, this is nothing strange in our world, but what do we really know about this? The nervous system is the organ responsible for pain among other senses such as: touch, temperature, smell and vision as well as motion and thoughts. This system is very important in our body because it controls a lot of the communication between body and brain. The nervous system can be divided into two parts; the afferent and the efferent system. Through the afferent system cells and organs all over the body send information to the brain and through the efferent system the brain sends throughout the body. This thesis will focus on the afferent system.

The information from the organs is translated into electrical impulses which are triggered through mechanical, temperature or chemical stimulus from the surroundings. Since this signalling system is an interdisciplinary scientific phenomenon and so important for our functioning and everyday life it is very interesting to study from different perspectives. This master thesis aims to study three aspects of neural signalling. The first aspect is the mathematical and physical perspective of neural signals propagation along the neurons, more specifically refining the existing models of the phenomena. The second aspect is to study the pain mechanism and how to inhibit it, with focus on anaesthesia. The third one is to study a product with pain relieving effect; *ProcelleraTM*, a bioelectric wound dressing and the knowledge about pain and neural signalling will be applied to understand the action of the dressing.

The most commonly used mathematical model of neural signalling, Hodgkin and Huxley, has recently started to be questioned and criticized for not including all the relevant components [1][2]. This model is heavily founded on experimental results and does not explain the phenomena in a strict physical sense [1]. For example it does not take to account the volume change in the nerve due to the signal propagation [2].

Nerve signal propagation is activated by different stimulus such as touch, temperature or pain, sensed by receptors. Of these stimulus, pain is the most difficult one to specify, and the most unpleasant one, therefore pain relief's are widely used and constantly under development and refinement. For example before and during surgery anaesthetic is commonly used to disable pain sensation and the mechanism of anaesthetic is debated. There is a known rule, the Meyer-Overton rule, which states that the effect of an anaesthetics substance is proportional to the substance solubility in the membrane. This indicates that the anaesthetic affect is achieved when the anaesthetic is absorbed in the membrane. The researchers in the physical field [1][3] have this view while the pharmaceutical field state that anaesthetics mechanism is through binding to specific receptors [4].

An example of a new development in pain relieving products is *ProcelleraTM* which is a bioelectric wound dressing that not only function as a protection for the wound and enhancing the healing of the wound; *ProcelleraTM* it is at present also claimed to reduce pain. This dressing's function will be investigated further since it is not fully known.

2 BACKGROUND

This chapter will present the different compartments in neural signalling starting with the system and signalling paths. The chain of signalling is presented as it occurs, starting in the skin where the receptors are located. The receptors are presented followed by the neuron. The neuron section includes the generation and transfer of the signal and is followed by a section on pain including pain inhibition. This chapter is ended by a presentation of how the signalling can be described electrically and mathematically.

2.1 THE NERVOUS SYSTEM

The nervous system is extremely important since it controls the whole body, enables all our senses, interprets impressions to feeling and thoughts and is responsible for the signalling between the organs and the brain. The system is divided into two parts which are responsible for different processes, the central nervous system and the peripheral nervous system. The central nervous system includes the brain and the spinal cord and is responsible of receiving information from the body's organs, processing information into action, thoughts or feeling and sending commands back to the body. The peripheral nervous system is the rest of the body and is responsible for the collection of information as well as the receiver of commands. The nervous system consists of neurons which are connected to each other and they create a complex network. The neurons collecting information from the body's organ are usually called sensory neuron, the neurons which implements the commands from the brain are called motor neurons since they are connected to muscles and the neurons in the spinal cord are called interneuron and transfer information from sensory neurons to the brain as well as signals from and the brain to the motor neurons. The signal path to the brain is called afferent and the signal path from the brain is called efferent (Figure 1). This thesis will focus on the sensory neurons. [5]

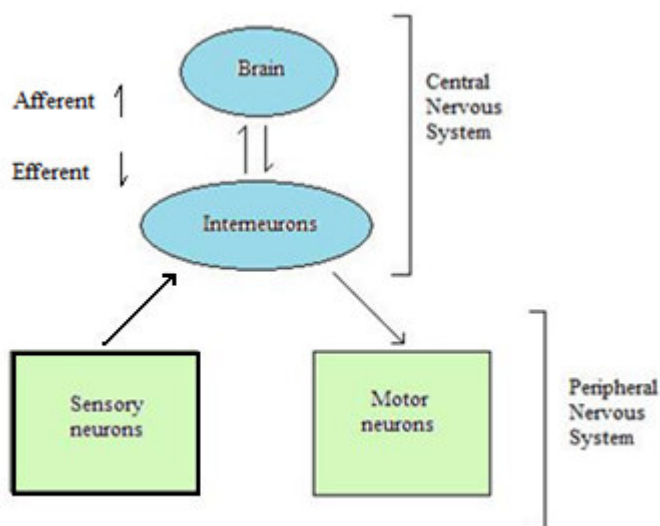


Figure 1 shows the two parts of the nervous system and the signalling in-between. The central nervous system consists of the brain and the spinal cord, the neurons in the spinal cord are called interneuron and transfer signals between the brain and the body's organs. The peripheral nervous system consists of the body excluding the spinal cord and the brain. The sensory neurons collect information from the organs and send the information to the brain through interneuron (afferent system). The motor neurons receive commands from the brain through interneuron and pass it to the muscles in the organs (efferent system). The marked box and path is the ones focused on in this thesis.

2.2 SKIN MORPHOLOGY

Skin is a very important organ covering our entire body and acts as a protecting layer of tissue as well as an insulator. The inner organs are also covered by a type of skin, layers of epithelial cells. There are two types of skin covering our body; glabrous and hairy skin. The glabrous skin is hairless skin found in our palms and under our feet. The skin is divided into three layers where the epidermis is the most superficial one followed by dermis and thereafter subcutaneous layer. [6]

Epidermis is about 0.05-0.15 mm thick and has a corrugated shape (Figure 2), this is visible in our fingerprints. This layer is made up by cells called keratinocytes which are stacked on each other. In epidermis there are also melanocytes which produce melanin, the dark pigment which contributes to the skin colour and provide UV protect. There are no blood vessels in epidermis, but the cells in the deepest part are nursed through diffusion from the blood capillaries in the upper dermis. The keratinocyte cells are formed in the basal region and then they move up through dermis. As they move up they gradually die and change shape, flatten due to the lack of nutrients. Through this, epidermis is constantly renewing itself and its top layer consists of dead cells, approximately 15-30 cell layers. It takes about four weeks for the cells to move up to the surface of epidermis. Epidermis also have cells responsible for the immune response by engulfing foreign material entering epidermis, they are called Langerhans cells. In the lowest part of epidermis there are some receptors which come from the dermis. [7]

Dermis is about 1-4 mm thick and mostly consists of connective tissue and acts as a cushion like protection of the body from stress and strain. This layer is responsible for the flexibility of the skin, body temperature regulation and also the sensation of touch, pressure, heat and pain. The sensation of the different parameters is registered by receptors located in the dermis, the interface between dermis and epidermis and some receptors project into the epidermis (Figure 2). Dermis also contains blood vessels, nerves and hair follicles.

The *subcutaneous layer* consists of loose connective tissue and about 50% body fat. It helps to insulate the body by monitoring heat gain and loss. This layer also acts as a protecting cushion and composes the transition between skin and muscles (and bones). [7]

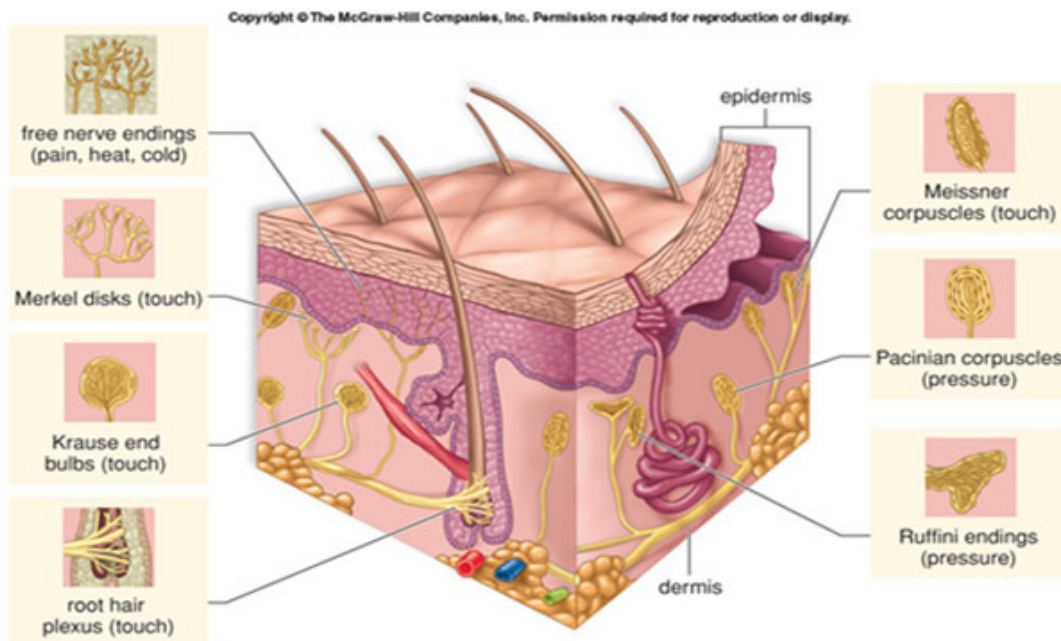


Figure 2 shows hairy skin and its' two upper layers in the skin, epidermis and dermis. It also shows the different receptors located in the skin and what stimuli they sense. Free nerve endings, upper left, are sensing painful chemical, mechanical and temperature stimuli and the rest are mechanoreceptors sensitive to mechanical changes. Merkel disks located in the basal epidermis sense tapping. Meissner corpuscles sense light touch and are located just below epidermis. Pacinian corpuscles are located in dermis and are sensitive to vibrations. Ruffini endings are sensing stretch in the skin. Krause end bulbs are not included in this thesis. (Copyright clearance from The McGraw-Hill Ryerson)

2.3 RECEPTORS

Receptors are proteins located in the cell membrane of the neurons and are the receivers of information, stimulus, in the nervous system. There are different types of stimulus from the surrounding tissue; they are touch (vibrations), pressure, temperature and chemical. These stimuli are registered by specialized receptors which are connected to the neurons. Some receptors register pure pressure and some register vibrations which will be sensed as touch. Only if the stimulus is big enough a signal will be sent out through the neuron. In the skin the receptors are low threshold mechanoreceptors, thermoreceptors and nociceptors. Receptors with the capability to sense painful (noxious) stimuli and send the pain signal are called nociceptors and include high threshold mechanoreceptors and noxious thermoreceptors. Some of the receptors are sensitive to several types of stimuli; polymodal receptors. An overview of all receptors can be seen in *Table 1* on page 10.

2.3.1 LOW THRESHOLD MECHANORECEPTORS

Mechanoreceptors are the receptors registering mechanical stimuli such as pressure and touch (vibrations) on the surrounding tissue. They can be either fast adapting or slow adapting depending on the signal response of the capsule surrounding their ends. The fast adapting respond only at the onset and offset of the stimuli while the slow adapting respond at a constant rate during the stimulation. There are four main types of mechanoreceptors in glabrous skin, two of each adapting kind called type 1, with a small receptive field, and type 2, with a large receptive field. The receptive field accounts for the area where they are able to register stimuli; hereafter this is the definition of a "field". These four types have different tissues encapsulating or connected to their ends and forming the receptor. These four are; Meissner's corpuscles, Merkel's disk, Pacinian corpuscles and Ruffini's endings (*Figure 2*). [8]

Messiner's corpuscles located just below epidermis, are fast adapting type 1 and detect light touch and soft movements in the frequency interval 20-50 Hz. Merkel's disks are found in the basal epidermis and are slow adapting type 1 and are sensitive to pressure or tapping stimuli (5-15 Hz). Pacinian corpuscles are located deep down in dermis, are a fast adapting type 2 and function as receptors for deep pressure and vibration in the intervals 60-400 Hz. Ruffini's endings are placed in the connective tissue and of the slow adapting type 2 and are sensitive to stretch in the skin. This means that the receptors found close to the surface has a smaller receptive field and only will sense stimuli from a small skin area while the receptors found deeper down in the tissue are sensitive to stimuli applied on a larger skin area. [8] [7]

The densities of the low threshold mechanoreceptors vary a lot depending on the location in the body. For example it can be very difficult to distinguish two fingers pressed to you back, a distance around 5 cm is required. While in your palm it is much easier and you can distinguish them even though they are next to each other. [9] The density of low threshold mechanoreceptors in your palm has been estimated to 58 units/cm² and at your fingertips 241 units/cm². [10]

The mechanoreceptors mentioned above are all low threshold mechanoreceptors. They are stimulated by a pressure on the capsules or surrounding tissue which mechanically stimulates the free nerve endings (dendrites) within to produce a signal. [9]. The fast adapting receptors act as filters to filter out slowly changing or steady stimuli, they are selectively sensitive to changing stimulus. In hairy skin the slowly adapting are the same as in glabrous skin but the fast adapting type 2 are located in the deep tissue instead of the deep dermis. Fast adapting type 1 does not have an exact analogue but there are free nerve endings wrapping around hair follicles, sensing mechanical change on the hair. One hair unit is connected to approximately 20 hair follicles leading to an irregularly shaped receptive field. [8][11]

2.3.2 THERMORECEPTORS

Together with mechanoreceptors (touch) the sensation of temperature helps to identify objects and materials, for example metals are perceived colder than wood. Thermoreceptors sense temperature changes in a certain interval. There are receptors that sense warmth and receptors that sense cold, but one receptor does not sense both. The normal temperature in the skin is 30-34 °C [12]. The signalling strength in the intervals are bell-shaped and range between 17 and 30 °C for cold and between 30 and 50 °C for warmth. This means that the maximum signalling for cold is around 25 °C and around 40-43 °C for warmth and outside the intervals the signalling is silent. The thermoreceptors are located in the interface between dermis and epidermis but recently some result indicate that they also project into epidermis. [13]

Both warm and cold sensing fibres have small receptive fields and the receptors are members of the transient receptors potential (TRP)¹ family. TRP ion channels² activity depends on the temperature of the surroundings. They are embedded in the terminals of afferent nerve fibres which end as free nerve endings. [8][13]

¹ Membrane protein transducing thermal changes

² Channels in the membrane which transfer ions in and out.

2.3.3 NOCICEPTORS

Nociceptors are receptors sensing chemical, mechanical or thermal stimuli in the noxious range. The afferent signalling of pain from the nociceptors is sent through two types of fibres depending on the stimuli. The noxious mechanical stimulus is sent through medium thin fibres, with medium velocity. While polymodal nociceptors which sense several types of stimulus is sent through thin fibres with a low velocity. [12] Nociceptors are generally located at free nerve endings and have the ability to be sensitized. They have a distinct sensitivity and must therefore be a result of distinct membrane receptors activation. [16] [9]

Chemical compounds released due to actual or potential tissue damage on the surrounding tissue or the neurons are caught by the receptors in the dendrites located at the free nerve endings and stimulate the pain signal. [9] A chemical called Bradykinin is released from injured cells and it activates bradykinin-sensitive channels on the neurons. A low pH (high concentration of H^+) can also cause pain through activation of proton-gated receptors and they are activated at pH values below 6.5. The normal pH in the body is around 7.2 but during damage or a wound it decreases drastically. [14] The pH in the epidermis part of the skin is normally around 4- 6 and is the reason why the pH is decreased when a wound occurs. [15]

Thermal activation of pain signal propagation are due to temperatures around 17 °C for noxious cold and around 47 °C for noxious heat. These receptors are often also sensitive to mechanic stimuli, i.e. they are polymodal. The high threshold mechanoreceptors are activated at around 15 bar and with a threshold lower than 6 bar receptors are considered insensitive to mechanic stimuli. [13] This can be compared to the atmospheric pressure of 1 bar.

Activation of nociceptors leads to local release of various chemical compounds which causes oedema and redness of the surrounding tissue, neurogenic inflammation. This release also activates other nociceptors which result in a higher sensitivity in the painful area. [16]

Nociceptors are mostly free nerve endings and they are the most common receptors in the skin and have a density of approximately $1.6 \cdot 10^5$ neurites³/cm². [17]

2.4 NEURONS

Neurons are the cells which constitute the nervous system. They are very important for our existence, especially if you agree with Descartes who said “I think therefore I am”⁴, since the neurons encode memories and produce thoughts and emotions. They are responsible for passing information from one location to another, and thereby also regulate body functions such as muscle contraction, sensation, body temperature, heartbeat, sleep-wake cycle and breathing. Neurons are connected to each other and create the nervous system and the neurons have a wide variety of shape and size (Figure 3) depending on their function and location. The neurons located in the brain usually are pyramidal cells and have many branching to be able to communicate with many other neurons. Motor neurons, neurons communicating with muscles are responsible for motions are multipolar and have an extensive network of branches. Sensory neurons are responsible for the sensation of touch, pressure and pain and are located in the skin and muscles; they do this through converting these stimuli to electrical signals. Interneurons are responsible for the communication between different neurons. The two last ones are the ones involved in the sensory system and the ones which will further be focused on in this thesis, since the sensory neurons receive (and transform), conduct and transmit the sensory information to the interneuron which sends the information further on to the brain for processing. [18]

³ Projection from the neurons cell body, either dendrite or axon

⁴ Quote from Descartes *Discourse on Method* (1637)

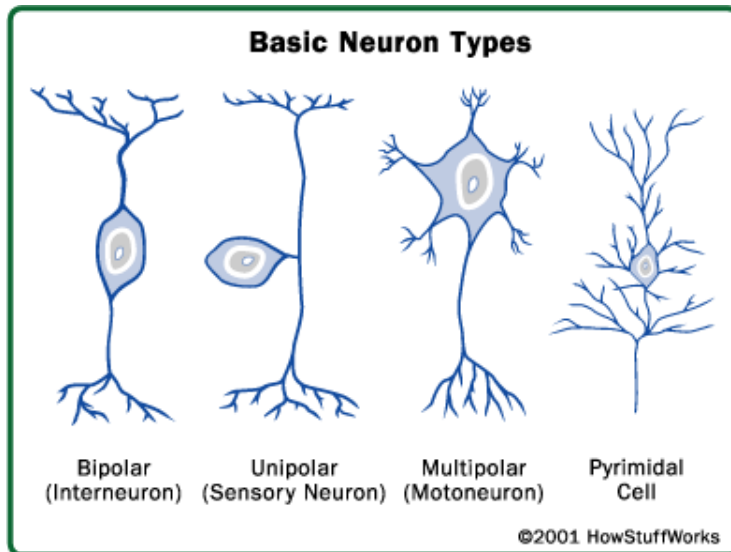


Figure 3 shows the four different types of neurons. Interneurons are responsible for the communication between neurons and sensory neurons are responsible for the sensation of touch, pressure and pain. Motor neurons are in charge of the motions (muscle contraction), while the pyramidal neurons are responsible for the communication between neurons in the brain. (Copyright clearance HowStuffWorks)

2.4.1 STRUCTURE

Since neurons are cells they have a nucleus which is located in the cell body, the soma. In sensory neurons the soma is located on the side (Figure 3) and in the other types it is located in-between the branches (Figure 3 & 4). The nucleus is responsible for manufacturing membrane constituents, synthesize enzymes and chemical substances needed for specialized nerve functions. Out from the soma there are branch-like protrusions called dendrites and there is also a single protrusion called an axon. The axon is the fibre which conducts the signals. [5] [19]

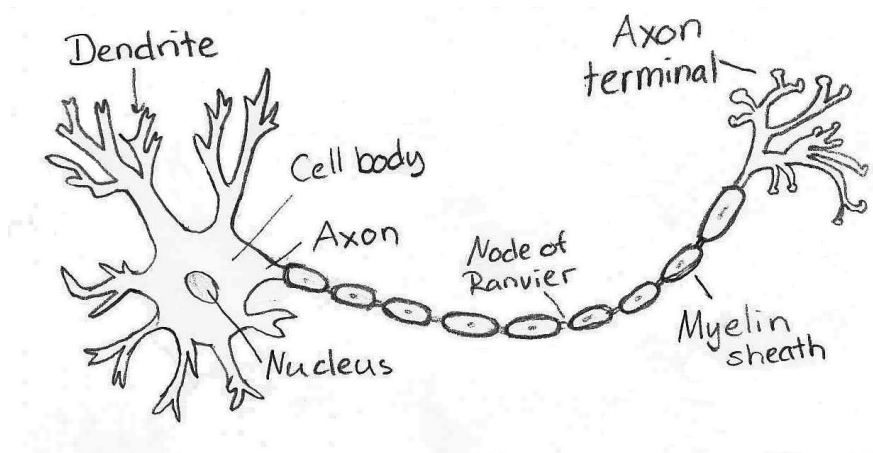


Figure 4 shows the structure of a typical myelinated neuron where you can see the dendrites branching out from the cell body, the soma. The nucleus is located in the cell body and an axon is also processed out from it. The axon can be covered by myelin sheath produced by Schwann's cells which act as an insulator. This together with the nodes of Ranvier makes the signal propagate faster and then reach the axon terminals where the signal is transmitted further.

The dendrites (*Figure 4*) are responsible for receiving information; in the sensory neurons this information is received from the surroundings through receptors (section 2.2). In interneuron this information comes from other neurons and in our case specifically from the sensory neurons. The interneuron is located in the spinal cord. Sensory neurons only have one dendrite which is processed out from the soma and then branched out into several to increase the receptive field while all the other neurons have several (*Figure 5*). The dendrites account for up to 90 % of the neuron's surface area. [5] The branching of dendrites can result in up to 100 000 inputs to a single neuron, but despite what kind of input the formed signal always is the same. The signal is changes in electrical potential over the neuron's cell membrane. [19]

The axon (*Figure 4*) is responsible for conducting the signal and can be seen as a biological wire. The axon has previously in the text been referred to as a fibre and they pass the signal to the interneuron located in the spinal cord, therefore the axon can be up to 1m long in humans. The signal is encoded information from the dendrites and propagates as electrical impulses in the nerve cell membrane, called action potentials (section 2.4.3). The signal can propagate with different speeds depending on the type of axon (*Figure 5*). The slow conducting fibres are the thin fibres (non-myelinated) and have conduction velocity of 0.5-1.0 m/s and a diameter of 0.3-1 μ m, they are called C-fibres (nociceptors). There are neurons which have medium thin (thinly myelinated) axons (diameter 1-5 μ m) that conduct an action potential with the speed of 12-30 m/s and are called A δ -fibers (nociceptors). The low threshold mechanoreceptors are connected to thick axons (heavily myelinated) and have a velocity of 40-70 m/s and a diameter of 5-15 μ m, these are called A β . [20]

Myelinated is when the axon is insulated by myelin sheaths, which prevent leakage of current across the cell membrane and increases the conducting velocity. The myelin is produced by Schwann cells by wrapping layer upon layer of their own membrane forming a tight spiral around the axon. There are gaps in the myelin at regular spaces which creates the so called Ranvier nodes which are responsible for the reactivation of the signal since the signal is passively transferred within the axon and signal strength decrease along the way. The end of the axon has branches which end as knobs and are called axon terminals and harbour the synapses. [21] [12]

The synapses are located at the end of the axon, the axon terminal and are responsible for the signal transmission to the interneuron. This signalling is transmitted through chemical substances released from one neuron to another.

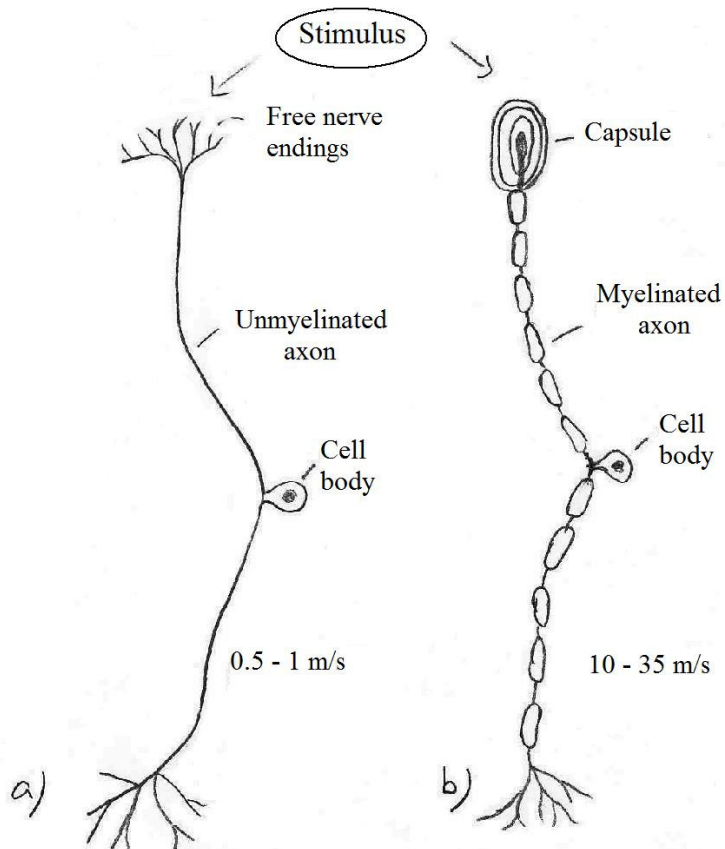


Figure 5 shows schematically how the receptors in the dendrites register stimulus and then transform it into an electrical signal which is propagated in the axon and called action potential. **(a)** Demonstrates a nociceptor while **(b)** and **(c)** are examples of neurons with low threshold mechanoreceptor. The conductance increases if the axon is myelinated as in **(b)**.

A summary of the afferent signalling in the sensory neurons and their fibres is shown below.

Stimuli	Receptor	Threshold	Adaptation rate	Fibre	Myelinated/ diameter	Conduction velocity
Light vibration	Messiner's Corpuscle	20-50 Hz	Rapid	A β	Heavily/ 5-15 μ m	40-70 m/s
Light pressure						
Pressure	Merkel disc	5-15 Hz	Slow	A β	Heavily/ 5-15 μ m	40-70 m/s
Tapping						
Deep pressure	Pacinian	60-400 Hz	Rapid	A β	Heavily/ 5-15 μ m	40-70 m/s
Vibration						
Skin stretch	Ruffini ends	-	Slow	A β	Heavily/ 5-15 μ m	40-70 m/s
Cold	Thermoreceptors (free nerve endings)	17-30 °C	Slow	A δ	Thinly/ 1-5 μ m	10-35 m/s
Warmth		30-50 °C		C	Non/ 0.3-1 μ m	0.5-1 m/s
Noxious cold	Nociceptors (free nerve endings)	\square 17 °C	Slow	Sharp pain- A δ	Thinly/ 1-5 μ m	10-35 m/s
Noxious warmth		>47°C				
Noxious pressure		15 bar (15·10 ⁵ Pa)		/	/	/
Chemical (bradykinin, serotonin, H ⁺ etc.)		<6.5 pH		Burning pain- C	Non/ 0.3-1 μ m	0.5-1 m/s

Table 1 shows which receptor the stimulus activates and what fibre type it is connected to and its' properties. The four first receptors are low threshold mechanoreceptors which are connected to neurons with myelinated axons and therefore have a high conducting velocity. Both the thermoreceptors and the nociceptors consist of free nerve endings which belong to neurons which have either thinly myelinated or non-myelinated axons. This leads to a much lower conducting velocity than the low threshold mechanoreceptors.

2.4.2 MEMBRANE POTENTIAL

At rest there is a potential over the neurons membrane of about -70 mV. This potential is created through different ion distributions between outside and inside (Table 2). This is basically concentration and charge differences which generates a chemical and an electrical gradient, together they create an electrochemical gradient. [22]

Ion	Extracellular (mM)	Intracellular (mM)
Na^+	145	12
K^+	4	155
Cl^-	123	4.2

Table 2 shows typical concentrations at rest of different ions. Sodium and chloride ions have the highest concentration outside the cell while potassium ions have the highest inside. These distributions are responsible for the concentration gradients.

The gradients are maintained through diffusion, active transport and passive transport ion pumps (Figure 6). Diffusion transport is simply the permeability of the membrane, which is not very high. Active transport ion channels transfer ions against the chemical gradient and consume energy while the passive transport ion channels transfer ions along the chemical gradient. [22]

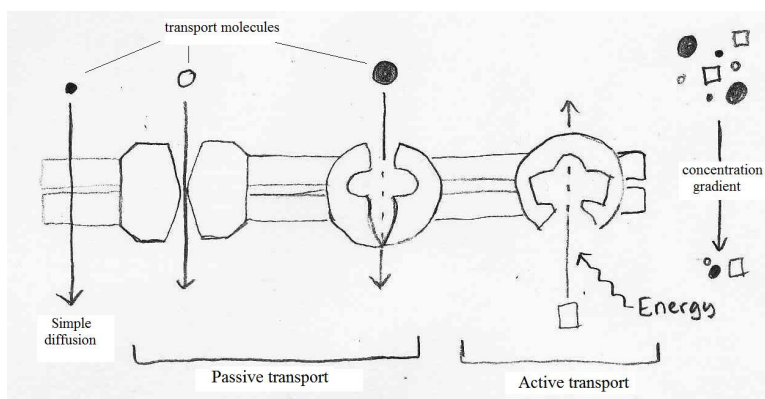


Figure 6 shows the different ion pumps in a membrane which maintain the membrane potential at rest. To the left the simple **diffusion** is presented, the permeability of the membrane. In the middle the **passive transport** is shown and they transport the ions along the ion concentration. The **active transport** transfer ions against the concentration gradient.

Na^+/K^+ pump is an active transport channel (Figure 7) which maintains the concentration difference between outside and inside concentrations of sodium and potassium ions. This pump consumes energy and transfer three Na^+ from the inside to the outside at the same time two K^+ are moved from the outside to the inside. Both ion types are transported against their chemical gradient. [23]

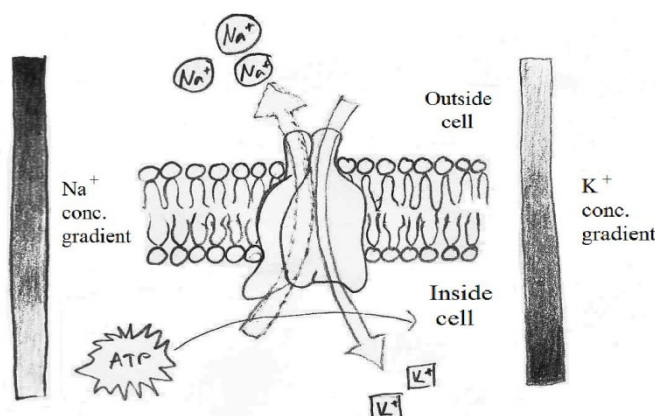


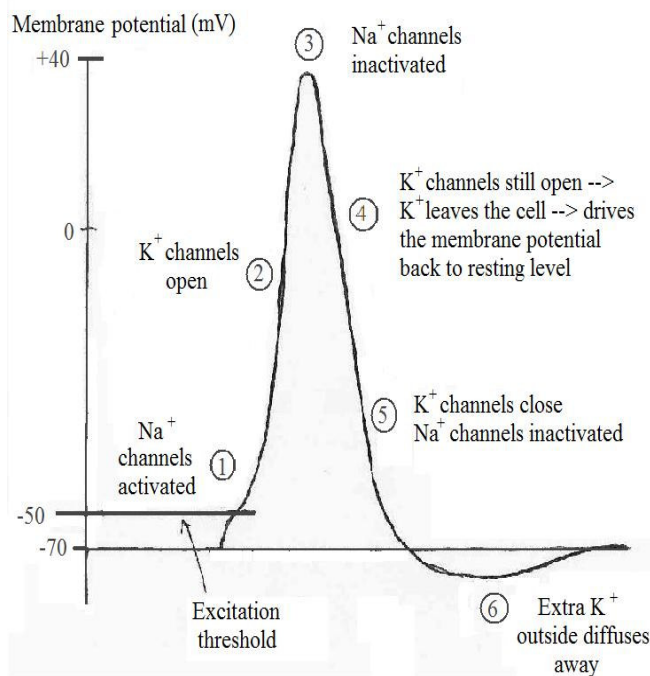
Figure 7 shows the Na^+/K^+ pump which transfer the ions against their concentration gradient. Sodium is transported out of the cell while potassium is transferred from the outside to the inside. This is an active transport channel and to enable this it requires energy in the form of ATP (Adenosine TriPhosphate). This ion pumps is very important to maintain the membrane potential at rest which is about -70 mV.

Cells are negatively charged on the inside due to large negative molecules and at rest the neuron has a potential difference of -70 mV across the membrane. The only negatively charged ion involved in the membrane potential is chloride (Cl^-).

The total resting potential is due to the sum of all the ion's concentration gradients across the membrane and with concern to their charges. (See Nernst equation, section 2.5.1) [23]

2.4.3 ACTION POTENTIAL

Action potentials are changes of the electrical potential over the axon's (or dendrite's) cell membrane; this is a result of ionic flow across the membrane. The cell membrane is nearly impermeable⁵ to ions so they need assistance to pass through; this is done by voltage gated ion channels. When the ions pass the membrane the potential difference decreases (less negative). To result in an initiation of an action potential in the axon the potential difference must reach a threshold value of approximately -50 mV. The initiation potential is created by stimulus from the surroundings stimulating receptors which activate ion channels and enable a current flow across the membrane. This flow of ions generates a potential difference across the membrane and if the sum of all the generated potentials is equal to the threshold value the voltage gated⁶ ion channels in the cell membrane will be activated and an impulse sent. If there are not enough stimuli to yield this potential difference the action potential will not be initiated. This activation can be seen as a switch with on and off since it is all or nothing. The voltage gated ion channels mainly assist potassium and sodium ions but in the steady state membrane potential chloride ions are involved as well. [24]



*Figure 8 shows the different steps of a single action potential and what state the Sodium and Potassium ions are in. The short horizontal line to the left shows the threshold (-50mV) for the generation of an action potential. The step marked as number 1 is when the Sodium channels opens and is a part of the **depolarization** along with step 2 where the Potassium channels starts to open. The third step is when the Sodium channels are inactivated and is the transition state between depolarized and repolarised. The fourth is when the Sodium channels are closed while the Potassium still are active and are a part of the **repolarisation**. The fifth also take part in the repolarisation and the Sodium channels are reset and the Potassium are closed. The sixth phase is responsible for the **hyperpolarisation** and is due to the Potassium ion channels closes slower which result in a more negative potential than the resting potential (-70 mV).*

The information is encoded through the frequency of action potentials and the location information comes from where in the spinal cord the fibre enters. This means that several action potentials are sent and the intensity and pattern of them gives information about the stimuli which caused them. To be noticed is that all action potentials have the same magnitude of around +40 mV in neurons. The action potential has different phases due to different states of the sodium and potassium ion channels shown in Figure 8. First of all at rest potassium ions have their higher ion concentration inside the cell (Table 2) while sodium ions have the highest concentration outside the cell (Table 2).

When the voltage threshold is reached in the membrane the first phase is initiated, the depolarization phase (less negative). The Na⁺ voltage gated ion channels are activated first and an influx of Na⁺ ions into the cell occurs due to the concentration gradient (and electrical attraction), which depolarizes (less negative) the membrane further. The depolarization opens more Na⁺ channels which admit more influx of Sodium ions and even more depolarization. This process is self-amplifying until the Na⁺ equilibrium is reached, which inactivate the ion channels.

⁵ Also impermeable to proteins and other molecules which are not soluble in fat.

⁶ Open and closed due to voltage

The K^+ voltage gated ion channels are also opened by a voltage in the membrane but due to a slower kinetics than Na^+ ion channels it takes longer time. When the K^+ opens the flow of cation⁷ will be in the opposite direction than Na^+ since the concentration gradient is the opposite, the Potassium ions will leave the cell. This leads to a repolarisation (more negative) of the membrane, therefore this phase second phase is called repolarisation phase. The efflux of K^+ ions overwhelms the influx of Na^+ and the membrane potential goes back to the resting potential.

The third and last phase is called hyperpolarisation and is caused by the fact that Potassium ion channels do not close immediately upon repolarisation due to the slow kinetics. This result in that too many cations are released and a dip in the voltage can be observed, the membrane potential is lower than at rest. K^+ ion channels are closed due to negative potential and not due to concentration equilibrium as in the Na^+ channels.

A single action potential has duration of 2 ms and with a speed of 2 m/s (C-fibre) versus 30 m/s (A δ -fibre) the spread would be 4 mm versus 60 mm. When the action potential has propagated through the axon and reaches the synapse they trigger the synaptic transmission which is described in the next subsection. [25] [26] [27]

2.4.4 SYNAPTIC TRANSMISSION

While action potentials are the communication within the neuron the synaptic transmission is the one between neurons, more specific between synapse (axon terminal) of the transmitting neuron and the dendrite in the receiving neuron. They are not in physical contact with each other; there is a cleft of about 20 μm (*Figure 9*). The signal can either be inhibitory (inactivate) or excitatory (activate) and is done chemically, therefore it is much slower than an action potential. The chemical substances are called neurotransmitters and are produced in the neuron's nucleus. They leave the nucleus in a vesicle⁸ and then they are transported through the axon to the synapse. In the synapse they are released into the cleft to the dendrite of the other neuron as a consequence of the membrane voltage caused by the action potential. There are several different neurotransmitters such as; Acetylcholine, glutamate, serotonin, γ -amino butyric (GABA) and glycine. The three first ones are used to excite the interneuron and the two last are inhibitory. There are also gaseous molecules which work as neurotransmitters but their mechanism is a little different, they diffuse out of the synapse and diffuse into the next neuron and are not limited to the synaptic cleft. Nitric oxygen and carbon monoxide are example of these gaseous neurotransmitters. [28] [29] [30]

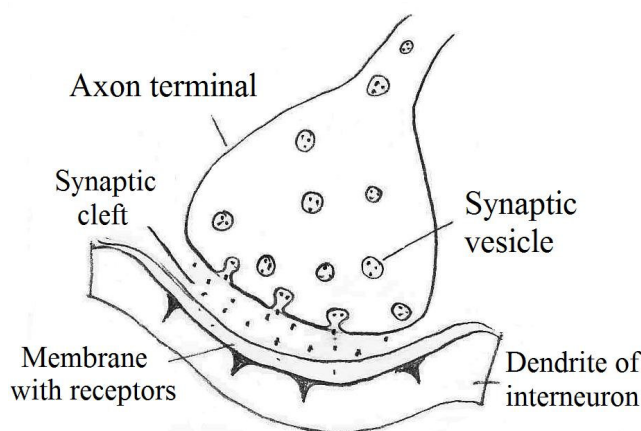


Figure 9 shows a synapse containing the vesicles with neurotransmitters and the dendrite of the interneuron. The space in-between, 20 μm , is the synaptic cleft which the neurotransmitters pass to send the signal to the next neuron. The release of neurotransmitters is caused by the action potential. The neurotransmitters get attached to the receptors in the dendrites which generate an action potential and the signal has been passed on.

⁷ Positively charged ion

⁸ A part of the bilipid membrane forming a capsule

2.5 PAIN

Pain is a very important warning signal which signalizes that something is wrong in our body and the brain interprets it, but pain is also very individual. Pain is not a measurable parameter since it is a feeling and as nurse McCaffrey said “Pain is what the patient says it is”⁹. The pain signal is sent due to tissue damage or potential tissue damage for all the different tissues in our bodies. Depending on the tissue damage there are different kinds of pains; nociceptive, neuropathic, idiopathic and psychic. Nociceptive pain is the most common one and is what we would call normal pain, it is pain sent in an intact nerve system. Neuropathic pain is due to a damaged nerve system and idiopathic pain is pain where the cause is unknown while psychic pain is due to a psychological disease. [31]

Nociceptive pain is caused by noxious mechanic, chemical or thermal stimuli due to wound, tumour, inflammation or ischemia¹⁰. For pain due to tissue damage in the skin there is a close relationship between stimuli dose and nerve impulse, high stimuli result in more intensive pain. A wound can evoke two of the pain types, nociceptive and neuropathic pain.

2.5.1 PAIN DUE TO WOUND

When a wound appears, tissue damage is a fact, the cells in the area break. This releases a lot of substances into the surrounding tissue and some of these substances have the ability to evoke nociceptive pain through chemical stimuli of the neurons. The cell membranes¹¹ break down to phospholipids. Phospholipids are thereafter broken down to arachidonic acid, a polyunsaturated omega-6 fatty acid. From arachidonic acid several important substances in inflammation, pain and wound healing are derived such as prostaglandins. Prostaglandins can activate the pain signalling through attaching to the free nerve endings as chemical stimuli, but they can activate even stronger by being broken down to bradykinin [32]. Bradykinin [33] also acts as chemical stimuli to the free nerve endings activating the pain signalling. (*Figure 10*)

Other substances released and activating the pain signal chemically are histamine and serotonin [34]. Damaged cells leak potassium ions which yield an increased extracellular concentration. There is also another mechanism that results in increased extracellular potassium concentration and it is lack of oxygen in the tissue due to damage which leads to inhibition of the Na^+/K^+ pump (section 2.3.X). The increased extracellular concentration depolarizes pain receptors which generate an impulse and therefore yield pain. [32]

The neuropathic pain is due to damage of the nerves which increases the release of substance P. [35] Substance P is a neuropeptide, a short chain of amino acids and it stimulates the mast cells to release histamine which starts the inflammation process; note that histamine also generates pain. Hereby the nerves also contribute to the inflammation process. The release of substance P can also be triggered by stimuli from bradykinin. [32] Nerve damage also increases the sensitivity in the area which means that nociceptors threshold is decreased and the low threshold mechanoreceptors can result in pain. [36] The sensitization of the wound area is also increased by the chemicals released from the damage cells and tissue mentioned above. [37]

The wound has a pH value of about 5.5-6.5 in the acute phase which is in the range of pain inducing pH (table 1 p. 10). [15] This means that there is a lot of H^+ in the fluid. The pH varies during the different phases of wound healing. An infection in a wound also generates pain through bacteria which produces toxins which injures the tissue even more. [37]

⁹ Margo McCaffrey is famous for this quote dated back to 1972

¹⁰ Insufficient blood supply.

¹¹ Cell membranes are built up by biphospholipid layer.

2.5.2 PAIN INHIBITION

There are many different ways to inhibit the sensation of pain and the body itself also have mechanisms and ways of doing this. For example everybody probably had someone blowing on their wound as a child saying it is alright. This actually has effect on the pain sensation in two ways. The first is called “gate control theory” and state that fine touch has a suppressive effect on pain signals in the interneuron connection. This means when you blow or touch the wound area this reduces pain. The second way is by saying it is alright, this gives a soothing affect which has a pain relieving effect in the brain. [38]

As indicated above the pain can be reduced at different levels in the signalling system, the levels are brain, brainstem, spinal cord (interneuron) and peripheral¹². Pain suppression in the brain consists of feelings such as calm, comfort, joy, positive expectations and also hypnosis. The positive expectation effect is more known as the placebo effect. Pain suppression in the brainstem can be acupuncture, opioids¹³ and antidepressives.[39]

Pain suppression in the spinal cord is mostly based on the “gate control theory” and is triggered by vibration, heat, cold, pressure and touch. This mechanism only works if the stimulus is performed in the same spinal cord segment, near the painful area. The impulses in touch- and pressure fibres are according to the “gate control theory” activating nerve cells in the bone marrow which inhibit the signal transfer from the primary to the secondary neuron (*Figure 10*). These interneuron connections are inhibited through reduced release of glutamate from the primary neuron and decreased sensitivity for glutamate in the secondary neuron (section 2.3.2) [40]. This mechanism can be triggered externally through transcutaneous electrical nerve stimulation (TENS) where electrodes are attached to the skin and an alternating current (10-20 mA, 80-100 Hz) is sent which triggers the touch- and pressure fibres. [41]. It can also be artificially mimicked through morphine injection in the bone marrow (epidural anaesthesia).

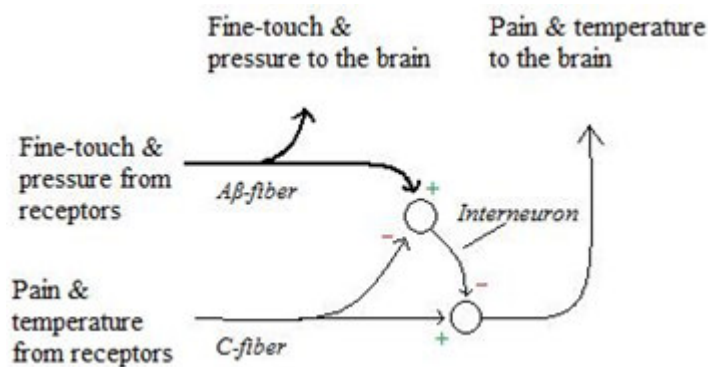


Figure 10 shows the gate control theory. The Aβ-fibre with fine-touch and pressure signals has an excitatory input (green plus sign) on the interneuron while the C-fibre has an inhibitory input. The relative activity on the interneuron determines whether the pain signal will be sent or not.

Peripheral pain suppression is done through blocking the signal of pain close to the source, preventing the nociceptors to sense or send the signal. This is usually achieved through pharmaceutical substances such as; acetyl salicylic acid (ASA), non steroid anti inflammatory drug (NSAID) and paracetamol. Both ASA and NSAID¹⁴ inhibit pain by blocking cyclo oxygenas, the enzyme breaking down arachidonic acid to prostaglandins, and without the peptides attaching to the free nerve endings there will be no pain (*Figure 11*). These two are also, through their mechanism, inhibiting the inflammation and therefore the healing processes. Paracetamol is not anti-inflammatory but it's full mechanism is not known. The breakdown of phospholipids to arachidonic acid can be inhibited through injection of cortisone; this is therefore anti-inflammatory just as ASA and NSAID. Local anaesthetic also has a peripheral pain suppression effect through reduction of Na⁺ permeability in the cell membrane. [42]

¹² The body excluding brain and brainstem..

¹³ Morphine like substances produced by the body itself.

¹⁴ Examples of NSAID are aspirin, ibuprofen and naproxen

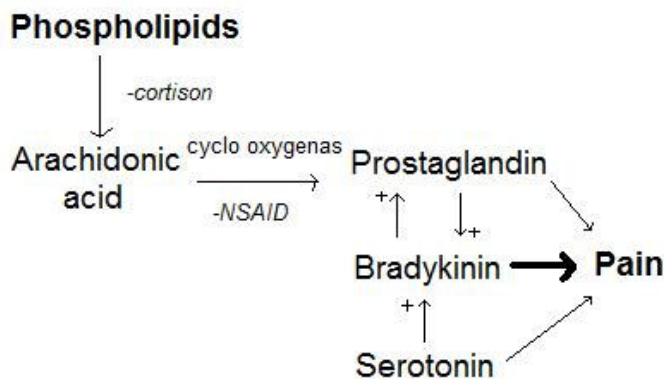


Figure 11 shows how phospholipids from the cell membrane are broken down to arachidonic acid and then the enzyme, cyclo oxygenas breaks it down to prostaglandin and further on to bradykinin. Serotonin stimulates production of bradykinin and all these three stimulate the pain signalling chemically. In *italic* different pain relief's are shown, cortisone inhibits the formation of arachidonic acid while NSAID (and ASA) inhibit cyclooxygenas.

2.6 MATHEMATICAL AND PYSICAL MODELS

The neurons can be described in a mathematical and physical manner since there are different forces which generate the ion flow across the membrane, the electrical charge and the concentration gradient. The cell membrane can be described as an electrical circuit which is shown in *Figure 12*. The different phenomena in the neuron can be described through different theories. The rest potential is usually described and calculated through Nernst equation and the action potential is described with or based on the Hodgkin & Huxley model.

The electrical circuit describing the cell membrane consists of capacitances and resistances and the base of the circuit is the biphospholipid layer. The membrane has an ability to transfer ions through it which creates a current and in a neuron these ions usually are potassium, sodium and chloride ions at rest and potassium and sodium for the action potential. A capacitance has the ability to store charges and consists of two conducting regions, in this case outside and inside the cell, separated by an insulator which is constituted by the membrane. For the neuron the charge difference between inside (negative) and outside (positive) generates the capacitance and this can induce a current. A current can also be induced by the concentration difference for a specific ion, when the ion channels are open the ion moves through diffusion, trying to even out the ion distribution. This ion flow is enabled through ion channels and this is represented as resistors. The voltage gated ion channels are represented by voltage dependent resistors which have a corresponding battery driving the current. The simple diffusion over the membrane is represented by a regular resistor where the current is driven by the capacitance (*Figure 12*). [1]

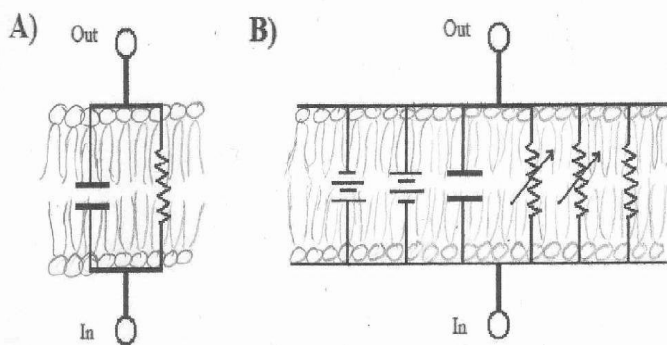


Figure 12 shows the cell membrane illustrated as an electrical circuit. **A** shown a simple circuit over the membrane at rest with a capacitance and a resistance, for the total ion current. In **B** a more specific circuit is shown where there is a resistance for each ion channel. The resistance with an arrow through them is the voltage dependent resistors (Na^+ and K^+ voltage gated ion channels) and they have a corresponding battery. **Out** represent extracellular and **In** intracellular for both **A** and **B** and the voltage is between these.

2.6.1 NERNST EQUATION

This section will present the mathematical equation for the membrane potential at rest; Nernst equation. Nernst equation is derived from Gibbs free energy equation which describes the available energy in a system to do non-mechanical work and the non-mechanical work performed on it, ΔG . In the neuron case the equation consists of a chemical (diffusion and active transport) and an electrical (voltage change caused by ion flow) compound: [43]

$$\begin{aligned} G_x(i) &= G_x^0 + RT \ln[X]_i + z_x F V_i \\ G_x(e) &= G_x^0 + RT \ln[X]_e + z_x F V_e \end{aligned} \quad (\text{Gibbs free energy}) \quad (1)$$

x = ion type, i = intracellular, e = extracellular, G^0 = free energy at some reference state
 R = universal gase constant, T = absolute temperature in Kelvin, $[X]$ = concentration of ion x
 z = valence (charge) of ion x , F = Faraday's constant, V = electrical potential

Usually only electrical and chemical forces are considered in this equation but more compounds can be added if needed. The difference in Gibbs free energy (the non-mechanical work done on the system) across the membrane is expressed as follows:

$$\Delta G_x = G_x(i) - G_x(e) = RT \ln \frac{[X]_i}{[X]_e} + z_x F (V_i - V_e) \quad (2)$$

The difference in Gibbs free energy between the intracellular and extracellular space is zero ($\Delta G_x = 0$) at equilibrium. At that point the concentration gradient is equal to the voltage gradient. This makes it possible to rewrite the equation and it yields the potential difference across the membrane, this equation is the Nernst equation: [43]

$$V_i - V_e = \frac{RT}{z_x F} \ln \frac{[X]_e}{[X]_i} = V_{mx} \quad (\text{Nernst Equation}) \quad (3)$$

Nernst equation can be further specified by including all the ions instead of only one, this yields the total membrane potential at rest. This is called Goldman-Hodgkin-Katz equation and includes the permeability (p_x) for each ion.

$$V_m = \frac{RT}{F} \ln \frac{p_K [K]_e + p_{Na} [Na]_e + p_{Cl} [Cl]_i}{p_K [K]_i + p_{Na} [Na]_i + p_{Cl} [Cl]_e} \quad (\text{Goldman-Hodgkin-Katz Equation}) \quad (4)$$

Notice that chloride has its intracellular concentration in the numerator and extracellular in the denominator while all the other has the opposite; this is because chloride's valence charge (z) is negative. All the ions have a valence of the value one therefore it can be taken away. p_x is the permeability of the ions.

2.6.2 HODGKIN & HUXLEY

The Hodgkin & Huxley model is a set of equations which was created in 1952 to match Alan Lloyd Hodgkin's and Andrew Huxley's experimental result from an axonal membrane and represent the membrane as an electrical circuit. They assumed that the capacitance of the membrane was constant and that the trans-membrane voltage changed with the total current (ions: K^+ , Na^+ and other ions) across the membrane. This is illustrated in *Figure 13* and gives us [44]:

$$I_m = C_m \frac{dV}{dt} + I_{ion} \quad (\text{Hodgkin \& Huxley Equation}) \quad (5)$$

I_m is the total current over the membrane and consists of the capacitance induced and the ion channel (I_{ion}) generated current.

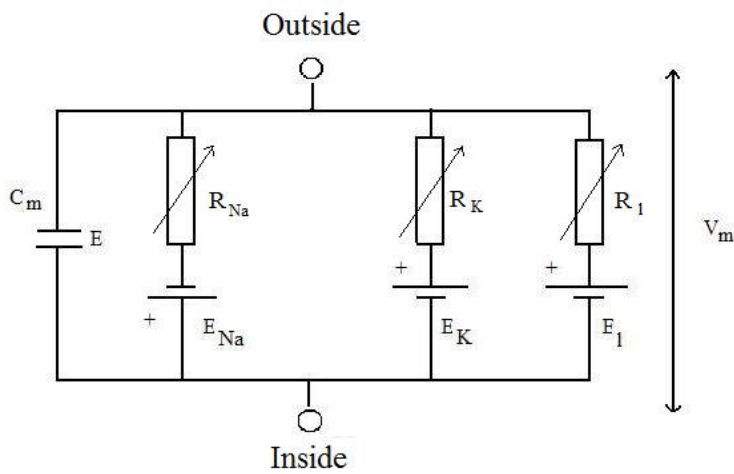


Figure 13 shows an illustration of the Hodgkin & Huxley model. The V_m represent the V in the equation. This figure shows the direction of the currents, all out from the cell except Na^+ which enters the cell. R_l is the resistance for the leak current which is the simple diffusion over the membrane. Each ion current has a resistance (inverse resistance=conductance) and if we compare this picture with the one of the cell above this is more developed and also include a battery for each ion current. The membrane has a capacitance (C_m) as the one above.

The individual ionic currents are expressed through the permeability of the membrane for the specific ion. The permeability can be expressed in terms of the inverse resistances, the conductance (g), which yields [44]:

$$I_{ion} = I_{Na} + I_K + I_{Leak} \quad (6)$$

$$I_{Na} = g_{Na}(V - V_{Na})$$

$$I_K = g_K(V - V_K)$$

$$I_{leak} = \bar{g}_{leak}(V - V_{leak})$$

V_{Na} , V_K and V_{leak} are the membrane potential of the individual ion at rest and it is yield through the Nernst Equation. The conductance of potassium and sodium is both time and voltage dependent while the conductance of the “leak” ions is a constant which is indicated by the line over it (\bar{g}_{leak}). H&H further assumed that the ion channels are either open or closed, like a switch. This means the conductance is either zero or a value g . [44].

Since there is a delay in the ion channels, the time and voltage dependence of the conductance Hodgkin & Huxley defined the conductance as follows:

$$\begin{aligned} g_K &= n^4 \bar{g}_K \\ g_{Na} &= m^3 h \bar{g}_{Na} \end{aligned} \quad (7)$$

The \bar{g} s are constants with the dimension conductance/cm² and n , m and h are dependent on the rate constants, α and β , as follows:

$$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n \quad (8)$$

$$\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h$$

The rate constants α and β vary with voltage but not with time and have the dimension time⁻¹ while n , m and h are dimensionless and vary between 0 and 1. In the case of potassium the α -term represents the opening and β represent the closing. The sodium channel has two stages, activated and inactivated which corresponds to m resp. h . The activation has the reaction rate α_m and reverse reaction rate is denoted β_m while the inactivation rate is denoted α_h and the reverse reaction rate is β_h . [44]. The different configurations of the Sodium and the Potassium channels are illustrated in *Figure 14*.

Potassium channel open and close:	$1 - n \rightleftharpoons n$	α_n corresponds to the top arrow and opening while β_n corresponds to the closing and the bottom arrow.
Sodium channel activation:	$1 - m \rightleftharpoons m$	α_m rate constant to the right and β_m to the left.
and inactivation:	$1 - h \rightleftharpoons h$	α_h rate constant to the right and β_h to the left.

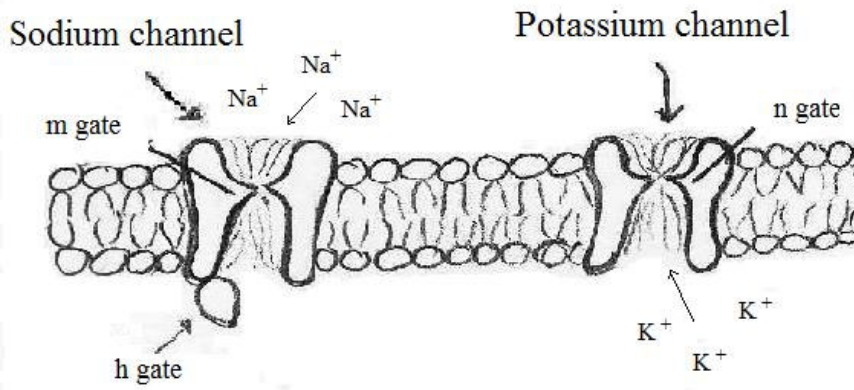


Figure 14 shows the configuration of the Sodium and the Potassium channel. The Sodium channel has three states, active, inactive and closed, which is because of the two gates, m and h showed in the figure. The Potassium channel only has two states, open and close; this is because it only has one gate, one parameter affecting which states it is in, n .

The rate constants are as mentioned dependent on the voltage and H&H described this relationship based on their experimental data. Their expressions for the rate constants are:

$$\begin{aligned}\alpha_n &= \frac{0.01(V+10)}{\exp\left(\frac{V+10}{10}\right)+1} \quad \text{and} \quad \beta_n = 0.125\exp\left(\frac{V}{80}\right) \\ \alpha_m &= \frac{0.1(V+25)}{\exp\left(\frac{V+25}{10}\right)-1} \quad \text{and} \quad \beta_m = 4\exp\left(\frac{V}{18}\right) \\ \alpha_h &= 0.07\exp\left(\frac{V}{20}\right) \quad \text{and} \quad \beta_h = \frac{1}{\exp\left(\frac{V+30}{10}\right)+1}\end{aligned}\tag{9}$$

Remember that n is for potassium channels while m and h are for the sodium channels. V is expressed in mV.

When summering this section different compartments into one complete equation, the Hodgkin & Huxley equation will be:

$$I = C_m \frac{dV}{dt} + \bar{g}_K n^4 (V - V_{mK}) + \bar{g}_{Na} m^3 h (V - V_{mNa}) + \bar{g}_{leak} (V - V_{mleak})\tag{10}$$

Where n, m and h is voltage and time dependent as described earlier.

This expression is the base for all the equations and expression within the neural signalling field. This thesis will further on propose a new way of viewing this expression, which include a new mechanism for the opening and closing of the voltage gated ion channels and it also generates a simpler expression.

3 METHOD

First of all a vast literature study is performed to generate a deep understanding for the scientific area and different aspects. Secondly the hypotheses are established and the methods to prove or decline them are determined.

This thesis investigates three different aspects of neural signalling: propagation, inhibition and applied to a product. This is done using three different methods, one for each aspect: simulation, experiment and theoretical.

The membrane current expression presented by H&H is a very complex one and does not take into account the propagation or the volume expansion when an axon conducts a signal. The propagation has been described before but is usually based on H&H. Through simulations in the Finite Element Method based program COMSOLTM different equations describing the propagation along the axon is investigated and visualized. This thesis suggests that the membrane thickness varies with voltage and this influence on the ion flow and the propagation of an action potential is examined.

The signalling can be inhibited in different ways for example through pain killers and anaesthetics. These mechanisms are not fully known therefore an experiment on anaesthetics affect on a membrane is performed in this thesis work. The experiment is performed with POPC¹⁵ lipids and 2,6 diisopropylphenol (propofol) using QCM-D¹⁶, manufactured by Q-sense. QCM-D is a device which collects and monitors the resonance frequencies and dissipation of a quartz crystal.

*Procellera*TM is a bioelectric wound dressing which has been claimed to also have a pain relieving effect. Possible mechanism which can result in pain relief is theoretically investigated through literature studies and chemical knowledge.

¹⁵ Palmitoyl oleoyl phosphatidyl choline

¹⁶ Quartz Crystal Microbalance with Dissipation monitoring

4 SIMULATION

This chapter will provide a new expression for the action potential and its propagation which will be visualized through simulations. To enable a simulation over the propagation along the axon, the Hodgkin & Huxley need to be further developed (the cable equation) and thereafter this equation will be compared to the diffusion equation and known equations with wave solutions. The simulations are executed in COMSOLTM.

4.1 BACKGROUND

This section will provide equations describing the propagation of an action potential along the axon starting with the cable equation. The cable equation is a model developed specifically in the field of neuroscience. Thereafter other equations from the literature which are similar to the cable equation will be described; specifically they all have the same base but with one term varying. Most of the equations are visualized through simulation and presented in Section 4.5.

4.1.1 THE CABLE EQUATION

Hodgkin & Huxley's original model was further developed to describe the propagation of the impulse along the axon; this was done by using the cable theory [45] The axon is divided into small unites where each unit is represented by a circuit, the cable circuit consists of several circuits connected together (Figure 15) and this yields an expression describing the nerve impulse propagation is yield.

First the Hodgkin & Huxley expression for the current over the membrane, I , must be converted from per area unit to length unit because instead of looking in the trans-membrane direction the propagation is viewed along the axon. This is done by using the surface-to-volume ratio of the axon approximated to a cylindrical model:

$$A_m = \frac{2\pi al}{\pi a^2 l} = \frac{2}{a} \quad (11)$$

A_m =surface-to-volume ratio, a = fibre radius, l = fibre unit length

Since this model describes the propagation along the axon the longitudinal intracellular resistance needs to be included, it is expressed as:

$$r_l = \frac{R_l}{\pi a^2} \quad (12)$$

r_l = longitudinal intracellular resistance per length unit [Ω/m]

R_l =longitudinal intracellular resistivity [Ωm], a = radius [m]

The longitudinal extracellular resistance is negligible since the area outside is indefinite which leads to a resistance which closes zero. These parameters and the cable circuit are illustrated in Figure 15.

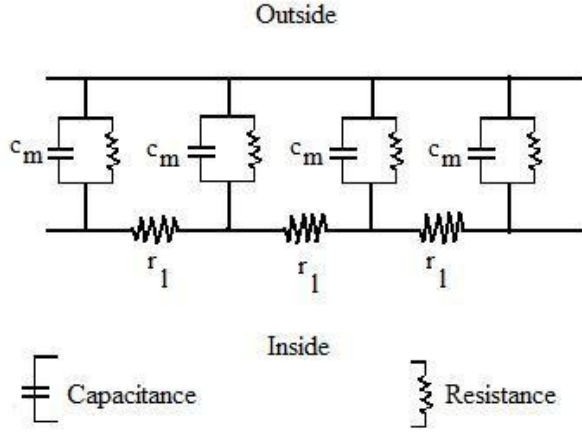


Figure 15 illustrate the cable theory circuit where several electrical compartments are connected. The axon is assumed to be a cylinder and the resistance in the extracellular space is considered zero. This model includes both membrane and intracellular resistance. The membrane resistance is included in I_{ion} as described in Hodgkin & Huxley while the intracellular resistance (denoted r_l in the image and R_l in the equation) is included directly in the equation above. The longitudinal extracellular resistance is neglected.

This gives us the following expression for the Hodgkin & Huxley membrane current:

$$I_m = A_m \left(C_m \frac{dV}{dt} + \bar{g}_K n^4 (V - V_{mK}) + \bar{g}_{Na} m^3 h (V - V_{mNa}) + \bar{g}_{leak} (V - V_{mleak}) \right)$$

$$= \frac{2\gamma}{a} \left(C_m \frac{dV}{dt} + \bar{g}_K n^4 (V - V_{mK}) + \bar{g}_{Na} m^3 h (V - V_{mNa}) + \bar{g}_{leak} (V - V_{mleak}) \right) \quad (13)$$

The membrane current can also be expressed by combining Ohm's law and Kirchhoff's current law. The potential fields, intracellular (ϕ_i) and extracellular (ϕ_e) changes over a small longitudinal distance Δx and are expressed through Ohm's law as follows (this is in the longitudinal direction):

$$\Delta \phi_i = -I_i (r_i \Delta x) \rightarrow \frac{\Delta \phi_i}{\Delta x} = -I_i r_i \quad (14)$$

$$\Delta \phi_e = -I_e (r_e \Delta x) \rightarrow \frac{\Delta \phi_e}{\Delta x} = -I_e r_e \quad (15)$$

Where r_i and r_e is the resistance per unit length in the longitudinal direction and is converted to fibre unit length as follows:

$$r_i = \frac{R_l A_i}{l} \quad \text{and} \quad r_e = \frac{R_e A_e}{l} \quad (16)$$

R = resistance of the axon per unit area, A = cross-sectional-area, l = length per unit fibre

When the distance $\Delta x \rightarrow 0$ equations 14 and 15 can be written as:

$$\frac{\partial \phi_i}{\partial x} = -I_i r_i \quad \text{and} \quad \frac{\partial \phi_e}{\partial x} = -I_e r_e$$

Kirchhoff's current law:

$$\Delta I_i = -I_m \Delta x \rightarrow \frac{\Delta I_i}{\Delta x} = -I_m \quad \text{when } \Delta x \rightarrow 0 \rightarrow \frac{\partial I_i}{\partial x} = -I_m$$

$$\Delta I_e = I_m \Delta x \rightarrow \frac{\Delta I_e}{\Delta x} = I_m \quad \text{when } \Delta x \rightarrow 0 \rightarrow \frac{\partial I_e}{\partial x} = I_m$$

Combining Ohm's law and Kirchhoff's current law:

$$\begin{aligned}\frac{1}{r_i} \frac{\partial}{\partial x} \left(-\frac{\partial \phi_i}{\partial x} \right) &= -I_m \quad \rightarrow \quad \frac{\partial^2 \phi_i}{\partial x^2} = r_i I_m \\ \frac{1}{r_e} \frac{\partial}{\partial x} \left(-\frac{\partial \phi_e}{\partial x} \right) &= I_m \quad \rightarrow \quad \frac{\partial^2 \phi_e}{\partial x^2} = -r_e I_m\end{aligned}$$

Since the membrane potential is expressed as the potential difference between intracellular and extracellular these two expressions can be written together as follow:

$$V = \phi_i - \phi_e \quad \rightarrow \quad \frac{\partial^2 V}{\partial x^2} = \frac{\partial^2 \phi_i}{\partial x^2} - \frac{\partial^2 \phi_e}{\partial x^2} = r_i I_m - (-r_e I_m) = I_m (r_i + r_e) \quad (17)$$

Rewriting this equation and remembering that the extracellular longitudinal resistance can be neglected yields this expression for the membrane current:

$$I_m = \frac{1}{r_i} \frac{\partial^2 V}{\partial x^2} \quad (18)$$

Combining this equation with the one from Hodgkin & Huxley (10) yields:

$$\frac{1}{r_i} \frac{\partial^2 V}{\partial x^2} = \frac{2\gamma}{a} \left(C_m \frac{\partial V}{\partial t} + \bar{g}_K n^4 (V - V_{mK}) + \bar{g}_{Na} m^3 h (V - V_{mNa}) + \bar{g}_{leak} (V - V_{mleak}) \right) \quad (19)$$

We simplify by averaging the ionic current parameters and writing the expression: $g_m = \bar{g}_m f(V)$ Where $f(V)$ indicates a function of the voltage, here $V - V_m$. We also set $\bar{g}_m = I/R_m$ (and $n = m = h = 1$) where R_m is a constant and this yield the following:

$$\begin{aligned}\frac{1}{r_i} \frac{\partial^2 V}{\partial x^2} &= \frac{2}{a} \left(C_m \frac{\partial V}{\partial t} + \frac{1}{R_m} f(V) \right) \quad \rightarrow \\ \frac{a R_m}{2 r_i} \frac{\partial^2 V}{\partial x^2} &= C_m R_m \frac{\partial V}{\partial t} + f(V)\end{aligned} \quad (20)$$

This easily can be compared with the cable equation:

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau \frac{\partial V}{\partial t} + V \quad (21)$$

Where λ is the length constant ($\sqrt{\frac{a R_m}{2 r_i}}$) and τ is the time constant ($C_m R_m$), while the function of voltage simply is V . The $f(V)$ term is the one which will be varied in the following study and the equation which is the foundation for the following expressions is therefore:

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau \frac{\partial V}{\partial t} + f(V) \quad (22)$$

4.1.2 EQUATIONS

This section will present related and well-known equations with different expressions of $f(V)$.

Pure diffusion equation:

If we replace the voltage with concentration we get the diffusion equation and if we set; $f(V)=0$ we describe pure diffusion. This is the simplest case of all and the equation is:

$$D \frac{\partial^2 c}{\partial t^2} = \frac{\partial c}{\partial t} \quad (23)$$

Where D is the diffusion coefficient and c is the concentration. [46] The concentration can be compared with the voltage in our phenomenon; hence D represents λ^2/τ and is a constant.

The cable theory:

This equation is presented above and is shown here to visualize the equations development. For the cable equation $f(V) = V$

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau \frac{\partial V}{\partial t} + V \quad (21)$$

Where λ is the length constant, τ is the time constant and V is the voltage

Fisher equation:

Fisher equation appears in heat and mass transfer, biology and ecology. It has a known solution in the form of travelling waves which indicates that it travels in space with time. [47]

Here $f(w) = aw(I-w)$ where a is an arbitrary constant.

$$\frac{\partial w}{\partial t} = \frac{\partial^2 w}{\partial x^2} + aw(1 - w) \quad (24)$$

Its travelling-wave solutions are:

$$w(x, t) = \frac{1}{\left[1 + C \exp\left(-\frac{5}{6}at \pm \frac{1}{6}\sqrt{6ax}\right)\right]^2}$$
$$w(x, t) = \frac{1 + 2C \exp\left(-\frac{5}{6}at \pm \frac{1}{6}\sqrt{-6ax}\right)}{\left[1 + C \exp\left(-\frac{5}{6}at \pm \frac{1}{6}\sqrt{-6ax}\right)\right]^2}$$

Where C is an arbitrary constant.

FitzHugh-Nagumo equation:

FitzHugh-Nagumo equation is an equation which can be found in the field of genetics, biology and heat and mass transfer. It has a known solution in the form of travelling waves which indicates that it travels in space with time. [48]

Here $f(w) = -w(1-w)(a-w)$ where a is a arbitrary constant.

$$\frac{\partial w}{\partial t} = \frac{\partial^2 w}{\partial x^2} - w(1-w)(a-w) \quad (25)$$

The solution is:

$$w(x, t) = \frac{A \exp(z_1) + aB \exp(z_2)}{A \exp(z_1) + B \exp(z_2) + C}$$

Where A, B and C are constants and z_1 and z_2 are defined as below:

$$z_1 = \pm \frac{\sqrt{2}}{2}x + \left(\frac{1}{2} - a\right)t, \quad z_2 = \pm \frac{\sqrt{2}}{2}ax + a\left(\frac{1}{2}a - 1\right)t$$

Simplified nonlinear cable equation:

Philip Nelson [49] presents a simplified way of writing the cable equation in the book *Biological Physics- energy information, life* (2008).

Here $f(V) = \frac{V(V-V_1)(V-V_2)}{V_1V_2}$

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau \frac{\partial V}{\partial t} + \frac{V(V-V_1)(V-V_2)}{V_1V_2} \quad (26)$$

Where V_1 and V_2 are constants, λ is the length constant and τ is the time constant.

To seek a travelling wave solution he assumed: $v(x, t) = \tilde{v}\left(t - \frac{x}{v}\right)$ where v is the speed of which the travelling wave moves in space. This means:

$$\left(\frac{\lambda}{v}\right)^2 \frac{\partial^2 \tilde{v}}{\partial t^2} = \tau \frac{\partial \tilde{v}}{\partial t} + \frac{\tilde{v}(\tilde{v}-\tilde{v}_1)(\tilde{v}-\tilde{v}_2)}{\tilde{v}_1\tilde{v}_2} \quad (27)$$

This equation is rewritten through the following dimensionless quantities:

$$u \equiv \frac{\tilde{v}}{v_2}, \quad y \equiv \frac{-v t}{\lambda}, \quad s \equiv \frac{v_2}{v_1}, \quad \text{and} \quad Q \equiv \frac{\tau v}{\lambda}$$

It only has solutions for:

$$v = \pm \frac{\lambda}{\tau} \sqrt{\frac{2}{s} \left(\frac{s}{2} - 1\right)}$$

In order to keep the solution stable s must be bigger than 2 since otherwise we will have the square root of a negative number yielding a complex solution.

$$s \equiv \frac{v_2}{v_1} > 2$$

Finally the equation is:

$$\frac{\partial^2 u}{\partial y^2} = -Q \frac{\partial u}{\partial y} + su^3 - (1+s)u^2 + u \quad (28)$$

4.2 HYPOTHESIS

This thesis will investigate the developed H&H model, the cable equation in a new way which has not been presented before. My hypothesis is that through expressing the voltage dependence of the conductance as a voltage dependence of the membrane thickness a simpler and different expression is obtained to describe the action potentials pulse form. This would lead to a dependence which is anchored in the observed physical reality, volume expansion of the axon [2], and not as complicated as the one H&H described.

Since membrane thickness variations affect both the capacitance and conductance they should not be held constant as in Hodgkin & Huxley, therefore the new situation describes the voltage dependence of the membrane thickness.

4.3 THEORY

A study has shown that there is a volume change of the nerve fibre due to the impulse propagation [2] and this thesis suggests that the volume change is in the axon membrane. Since the capacitance and the conductance (resistance) are both dependent on the membrane thickness this can be an interesting way of describing the propagation. The membrane thickness is assumed voltage dependent since a voltage over the lipids would affect the compactness. Therefore the cable equation will be rewritten and the capacitance and conductance will be broken down to the smallest compartments. First the equation will be transformed into a nicer expression

Recall Equation 19 with $f(V) = V - V_m$:

$$\frac{aR_m}{2r_i} \frac{\partial^2 V}{\partial x^2} = C_m R_m \frac{\partial V}{\partial t} + V - V_m \quad (29)$$

Assume the potential is measured in terms of V_m then we can replace V with u where $u = (V - V_m)/V_m$ and u is dimensionless. This yields the following expression:

$$\frac{aR_m}{2r_i} \frac{\partial^2 u}{\partial x^2} = C_m R_m \frac{\partial u}{\partial t} + u \quad (30)$$

Recall also that $C_m R_m = \tau$ (time constant) and $\frac{aR_m}{2r_i} = \lambda^2$ (λ -length constant).

The capacitance and the conductance (resistance) per area unit is broken down the smaller compartment and yields:

$$C_m = \frac{\varepsilon_0 \varepsilon}{d} \quad \text{and} \quad g_m = \frac{1}{R_m} = \frac{1}{d \rho_m} \quad (31)$$

ε_0 = permittivity in free space [F/m], ε = dielectric constant of the membrane, d = membrane thickness[m], ρ =resistivity [Ω m]

This gives us:

$$\lambda^2 = \frac{a R_m}{2 r_i} = \frac{a d \rho_m}{2 r_i} \quad \text{and} \quad \tau = C_m R_m = \frac{\varepsilon_0 \varepsilon}{d} \cdot d \rho_m = \varepsilon_0 \varepsilon \cdot \rho_m \quad (32)$$

This means that the length constant varies with the membrane thickness while the time constant does not. Since the membrane thickness, d , according to this thesis is voltage dependent it will be expressed as $d(u)$. This also means that the length constant is voltage dependent but at rest it has a constant value which corresponds to the membrane thickness at rest, d_0 . Therefore the expression is elongated with d_0 in order to break out the voltage dependence of the membrane thickness from the length constant:

$$\lambda^2(u) = \frac{a d}{2 r_i \varepsilon_0 \varepsilon} \cdot \frac{d_0}{d_0} = \lambda_0^2 \cdot \frac{d(u)}{d_0} = \lambda_0^2 \cdot \sigma(u) \quad (33)$$

$\sigma(u) = \frac{d(u)}{d_0}$ is an expression for the change in membrane thickness, the ratio between the thickness during an action potential and at rest. This will hereafter be called the expansion factor and is denoted as $\sigma(u)$.

In order to convert the equation into a dimensionless expression the time constant and the length constant are included in the time and space derivatives as follows:

$$\sigma(u) \cdot \lambda_0^2 \frac{\partial^2 u}{\partial x^2} = \tau \frac{\partial u}{\partial t} + u \rightarrow \sigma(u) \frac{\partial^2 u}{\partial \tilde{x}^2} = \frac{\partial u}{\partial \tilde{t}} + u \quad (34)$$

Where $\tilde{x} = \frac{x}{\lambda}$ and $\tilde{t} = \frac{t}{\tau}$.

The space dependent can also be rewritten in order to yield a strictly time dependent equation, an ordinary differential equation (ODE).

$$\frac{\partial^2 u}{\partial \tilde{x}^2} = \frac{\partial^2 u}{\partial \tilde{t}^2} \cdot \frac{\partial \tilde{t}^2}{\partial x^2} \cdot \frac{\lambda_0^2}{\tau^2} = \frac{\lambda^2}{\tau^2 v^2} \frac{\partial^2 u}{\partial \tilde{t}^2} \quad (35)$$

Where v is the velocity of the travelling wave in space, provided that we find a solution.

This gives us the following equation:

$$\sigma(u) \cdot \frac{\lambda_0^2}{\tau^2 v^2} \frac{\partial^2 u}{\partial \tilde{t}^2} = \frac{\partial u}{\partial \tilde{t}} + u \quad (36)$$

This equation is the one to be compared with the existing equation (21)(26). We investigate $\sigma(u)$ expressed in different ways; linear, nonlinear and strictly nonlinear dependence of voltage.

(37)

- 1) Linear dependence: $\sigma(u) = \frac{(\alpha-u)}{\alpha}$
- 2) Nonlinear dependence: $\sigma(u) = \frac{(\alpha-u)(\beta-u)}{\alpha\beta}$
- 3) Strictly nonlinear dependence: $\sigma(u) = \frac{(\alpha-u)(\alpha+u)}{\alpha^2}$

4.4 PROCEDURE

In order to perform the simulation a computational programme called COMSOLTM is used. It is a FEM (Finite Element Method) - programme which is used to calculate and visualize equations.

The diffusion equation and the equations containing the space dependence are calculated using the 1D transport of diluted species equations in COMSOLTM. As we have seen above the concentration which transport diluted is based on can be compared with the voltage in our phenomenon.

The equations only containing the time dependence are calculated using 1D ordinary differential equations (ODE) in COMSOLTM.

4.5 RESULTS

This section visualizes the progress of the equations, starting with basic diffusion and the cable equation. The voltage term in the cable equation is thereafter developed to a nonlinear dependence of voltage through FitzHugh-Nagumo equation. The expression is also transformed into an ordinary differential equation (ODE) showed in the simplified nonlinear cable equation. Lastly this thesis suggestion for the equation and with different parameters for σ 's voltage dependence is presented and visualized in the ODE form. In appendix 3 the thesis equation with the space dependence is presented. All the equations have dimensionless parameters.

4.5.1 THE DIFFUSION EQUATION

$$D \frac{\partial^2 c}{\partial t^2} = \frac{\partial c}{\partial t} \quad (24)$$

Where $D = 0.1$ and the initial value is set to a Gaussian pulse. $V_0 = V_{00} \exp\left(-\frac{(x-x_0)^2}{\sigma^2}\right)$

This simulation shows how the voltage profile is decreased and smeared out as time goes. Diffusion is phenomenon which strives to even out the distribution which means that this is a suspected behaviour.

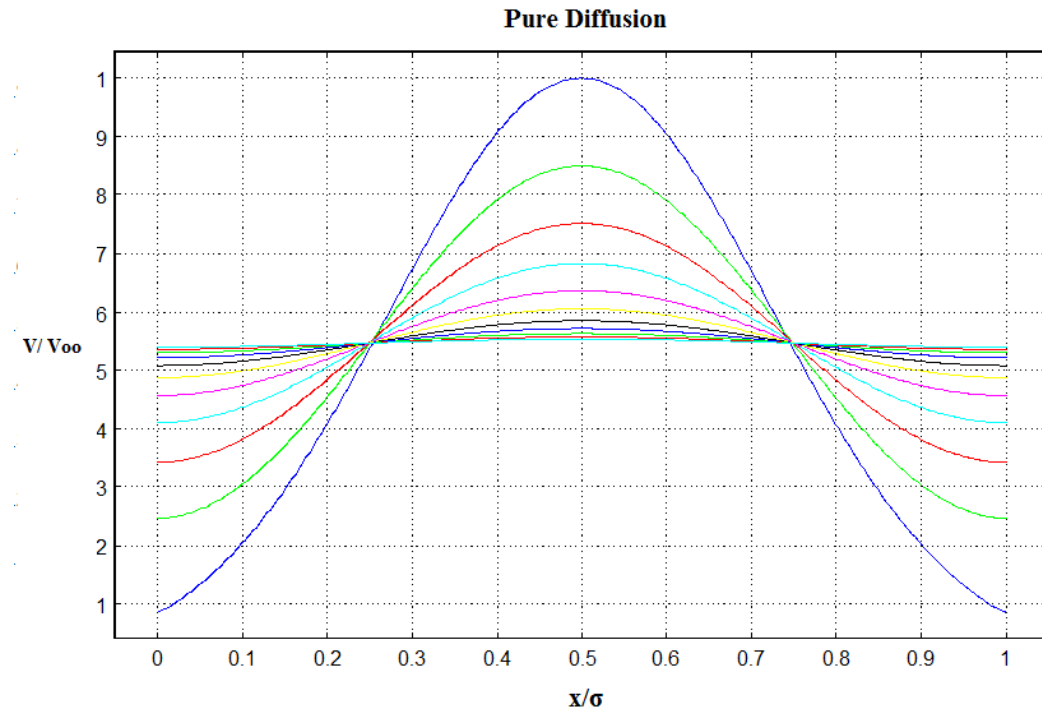


Figure 16 shows the diffusion equation and the case of pure diffusion. $D=0.1$ and the initial value is set to a Gaussian pulse. Both axes are dimensionless and the x -axis shows the location on the axon length unit in relation to the length of the unit. The y -axis shows the voltage in relation to the initial voltage. The different lines represent different times and the one with highest magnitude is the initial voltage profile. The voltage profile is smeared out as times passes and the magnitude is evened out.

4.5.2 THE CABLE EQUATION

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau \frac{\partial V}{\partial t} + V$$

Where λ^2 is set to 0.1 and τ to 1 which yields; $D = \frac{\lambda^2}{\tau} = 0.1$, as above and this is in order to visualize the difference the V term makes on the behaviour in comparison to the pure diffusion equation above.

The initial value is set to a Gaussian pulse: $V_0 = V_{00} \exp\left(-\frac{(x-x_0)^2}{\sigma^2}\right)$

This simulation shows that as time goes by the voltage profile is smeared out and there is no movement in the space direction only a decrease in magnitude. The decrease in magnitude goes faster than in the case of pure diffusion.

Notice that in the cable equation the conductance voltage dependence from the H&H model is not included.

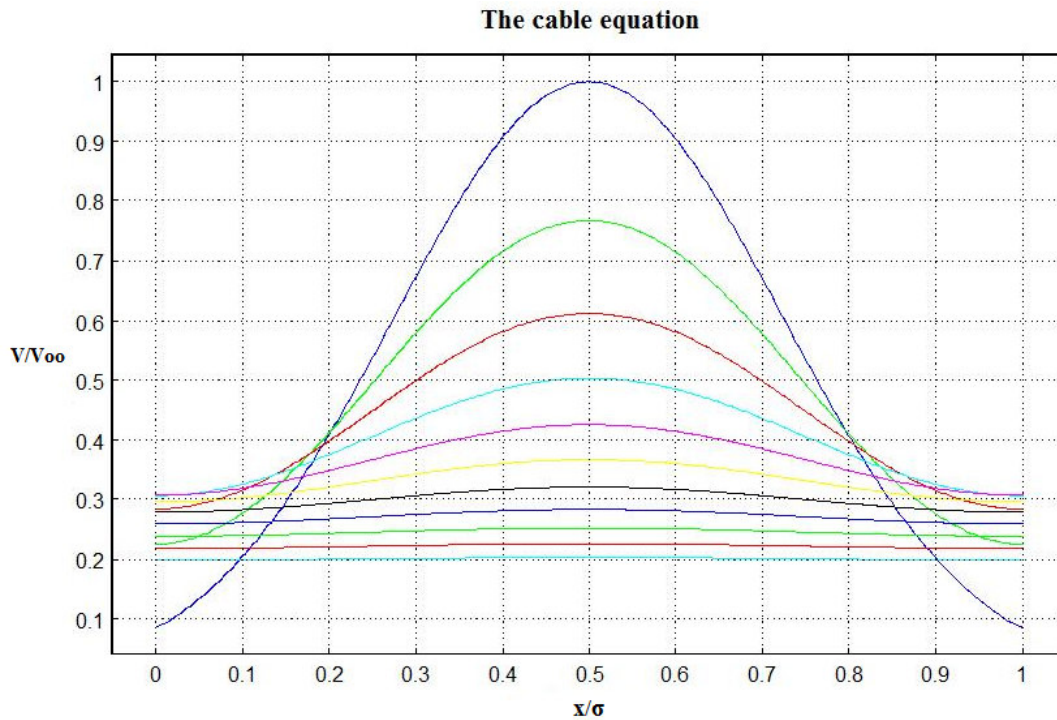


Figure 17 shows the cable equation. $\lambda^2 = 0.1$, $\tau = 1$ and the initial value is set to a Gaussian pulse. Both axes are dimensionless and the x-axis shows the location on the axon length unit in relation to the length of the unit. The y-axis shows the voltage in relation to the initial voltage. The different lines represent different times and the one with highest magnitude corresponds to the initial voltage profile. The voltage profile is smeared out as times goes and the magnitude is decreases, this happens faster than in the pure diffusion case.

4.5.3 FITZHUGH-NAGUMO EQUATION

$$\frac{\partial w}{\partial t} = \frac{\partial^2 w}{\partial x^2} - w(1-w)(a-w)$$

The initial value is set to:

$$w(x, 0) = \frac{A \exp(z_1) + a B \exp(z_2)}{A \exp(z_1) + B \exp(z_2) + C}, \text{ where } z_1 = -\frac{\sqrt{2}}{2}x \text{ and } z_2 = -\frac{\sqrt{2}}{2}ax \text{ since } t = 0$$

The values of a, A, B and C are set to:

$$a = 10, A = 2, B = 20 \text{ and } C = 10$$

This simulation visualizes how the wave moves as time goes, which also is the case in the propagation of an action potential along the axon. However, this does not show a pulse profile which is needed for the form of an action potential.

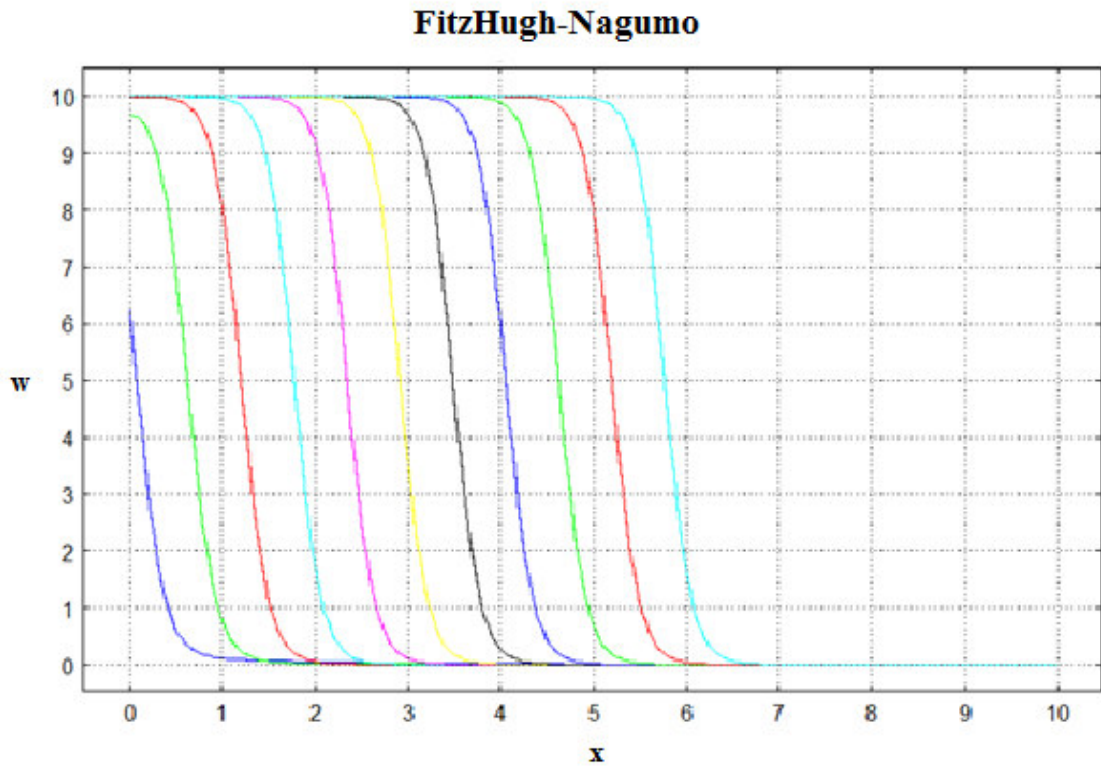


Figure 18 shows Fitzhugh- Nagumo equation. The initial value is set as above and each curve represents a time step of 0.1 seconds. This shows how the wave travels along the axon since the curve is moved in the x-direction.

4.5.4 SIMPLIFIED NONLINEAR CABLE EQUATION

$$\frac{\partial^2 u}{\partial y^2} = -Q \frac{\partial u}{\partial y} + su^3 - (1+s)u^2 + u$$

Where $Q = 1$ and $s = 2.1$ in order to fulfil the demands on the parameter since otherwise there will not be any solution.

This simulation shows that the simplified nonlinear cable equation in the ODE form is in a wave form and shows that there are wave solutions to the equation. This wave travels as time goes with the velocity v . This equation does not show the pulse form which is desirable.

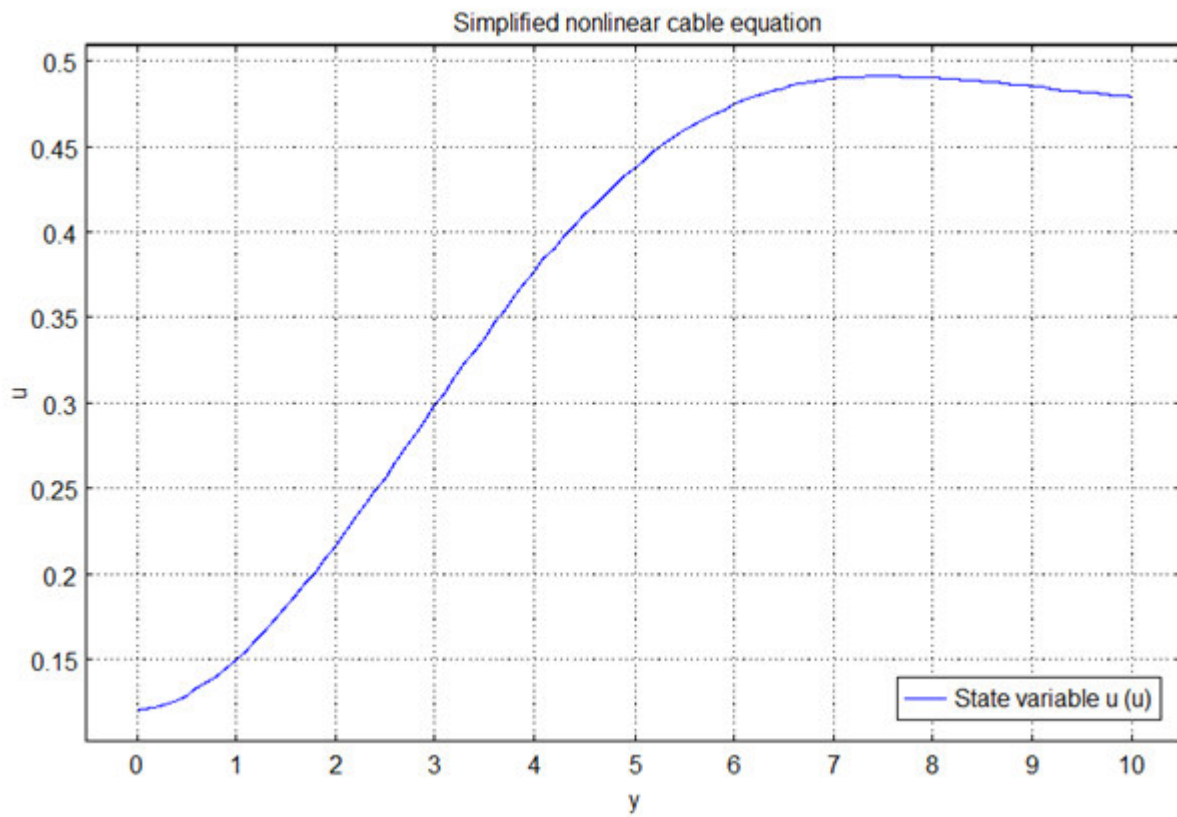


Figure 19 shows the simplified nonlinear cable equation visualized in the ODE form. The x-axis shows the dimensionless time relationship while the y-axis is the dimensionless voltage as defined earlier. The solution has the form of a wave.

4.5.5 LINEAR EXPANSION FACTOR

Now we turn to our model.

$$\frac{(\alpha - u)}{\alpha} \cdot \frac{\lambda_0^2}{\tau^2 v^2} \frac{\partial^2 u}{\partial \tilde{t}^2} = \frac{\partial u}{\partial \tilde{t}} + u \quad (38)$$

Where $\frac{\lambda_0^2}{\tau^2 v^2} = 0.1$ and α is varied in order to see for what values there is a desirable behaviour.

The simulation show that for $\alpha > 1$ a pulse-behaviour with a rapid increase first and then a decrease back to the start occurs, as below. This is just like the behaviour of an action potential, which is desirable. The figure below shows the result for $\alpha = 1.1$ and the shape of the curve is the same for all values but the magnitude varies a lot, it increases with increasing α . For examples for $\alpha = 10$ the magnitude is about 80.

Variation of $\frac{\lambda_0^2}{\tau^2 v^2}$ showed that if it decrease it no longer gives a pulse. If it is increased the peak is wider, the magnitude higher and it is moved further away from zero.

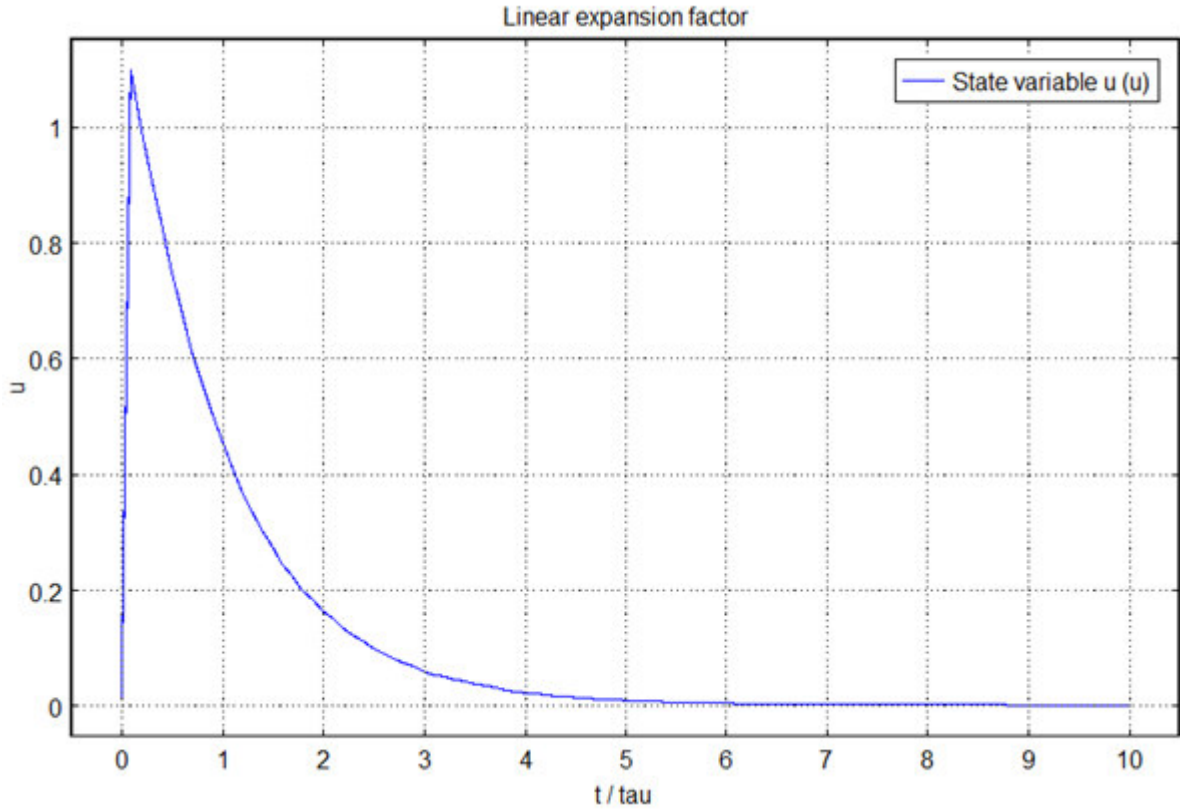


Figure 20 shows the results for the linear expression of the expansion factor of the membrane thickness in the ODE form. Both axis are dimensionless and the y-axis represent the voltage and the x-axis the time, $t / \tau = \tilde{t}$.

4.5.6 NONLINEAR EXPANSION FACTOR

$$\frac{(\alpha - u)(\beta - u)}{\alpha\beta} \cdot \frac{\lambda_0^2}{\tau^2 v^2} \frac{\partial^2 u}{\partial \tilde{t}^2} = \frac{\partial u}{\partial \tilde{t}} + u \quad (39)$$

Where $\frac{\lambda_0^2}{\tau^2 v^2} = 0.1$ and α and β are varied in order to see for what values there is a desirable behaviour.

The simulation show that for $\beta/\alpha > 1.1$ a pulse behaviour with a rapid increase first and then a decrease back to the start occurs, as below. This is just like the behaviour of an action potential. The figure below shows the result for $\beta/\alpha = 1.2$ and the shape of the curve is the same for all ratios above 1.1 but the magnitude varies, it increases with an increasing ratio. For examples for $\beta/\alpha = 10$ the magnitude is about 7.

Variation of $\frac{\lambda_0^2}{\tau^2 v^2}$ shows that if it decreases, no longer a pulse is formed. If it is increased the peak has almost the same shape and magnitude but it is moved further away from zero.

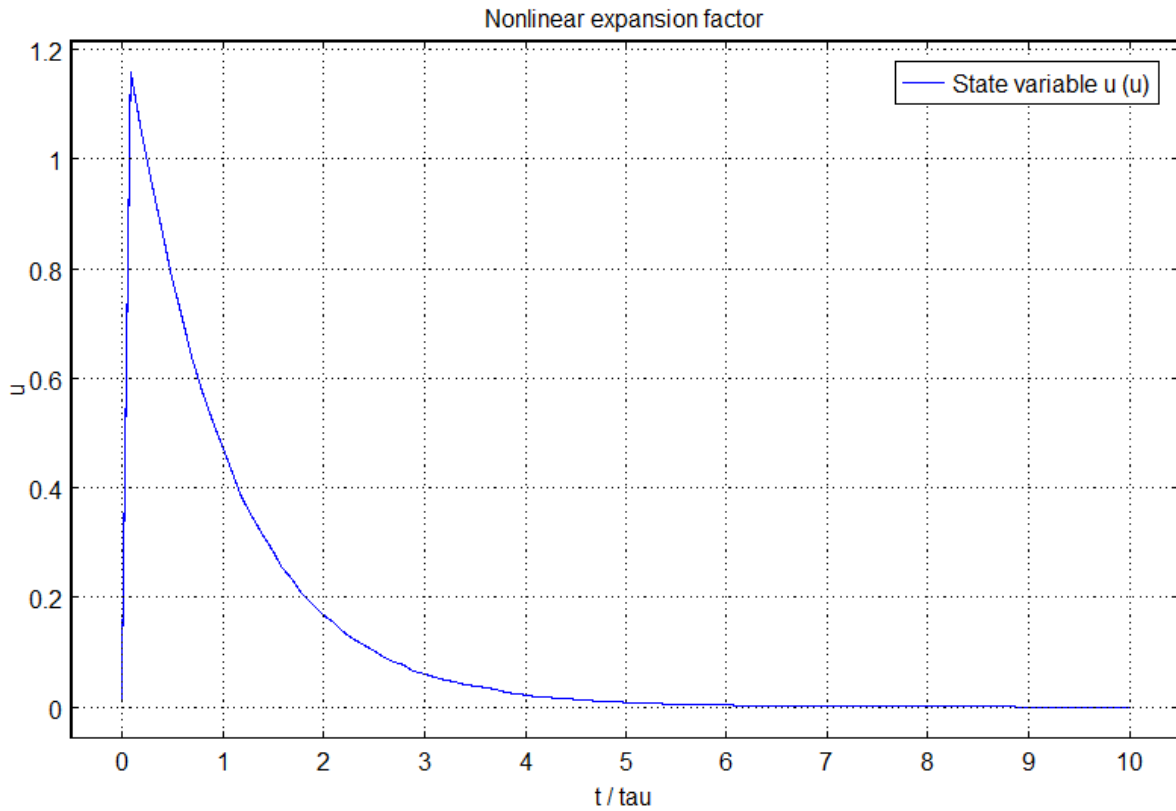


Figure 21 shows the results for the nonlinear expression of the expansion factor of the membrane thickness in the ODE form. The y-axis represents the voltage and the x-axis is dimensionless and represents the time, $t / \tau = \tilde{t}$. This shape of the curve is shown for all values of α above one.

4.5.7 STRICTLY NONLINEAR EXPANSION FACTOR

$$\frac{(\alpha - u)(\alpha + u)}{\alpha^2} \cdot \frac{\lambda_0^2}{\tau^2 v^2} \frac{\partial^2 u}{\partial \tilde{t}^2} = \frac{\partial u}{\partial \tilde{t}} + u \quad (40)$$

Where $\frac{\lambda_0^2}{\tau^2 v^2} = 0.1$ and α is varied in order to see for what values there is a desirable behaviour. $u(0)$ is set to 0.012 to have a starting point which is not zero in order to yield any result.

The simulation show that for $3 \leq \alpha \leq 5$ a pulse-behaviour with a rapid increase first and then a decrease back to the start occurs, as below. This is just like the behaviour of an action potential. The figure below shows the result for $\alpha = 4$ and the shape of the curve is the same for all values but the magnitude varies between 10 and 14.

Variation of $\frac{\lambda_0^2}{\tau^2 v^2}$ showed that if it is decreased it no longer gives a pulse. If it is increased the peak is wider, it is moved further away from zero and the magnitude is basically the same.

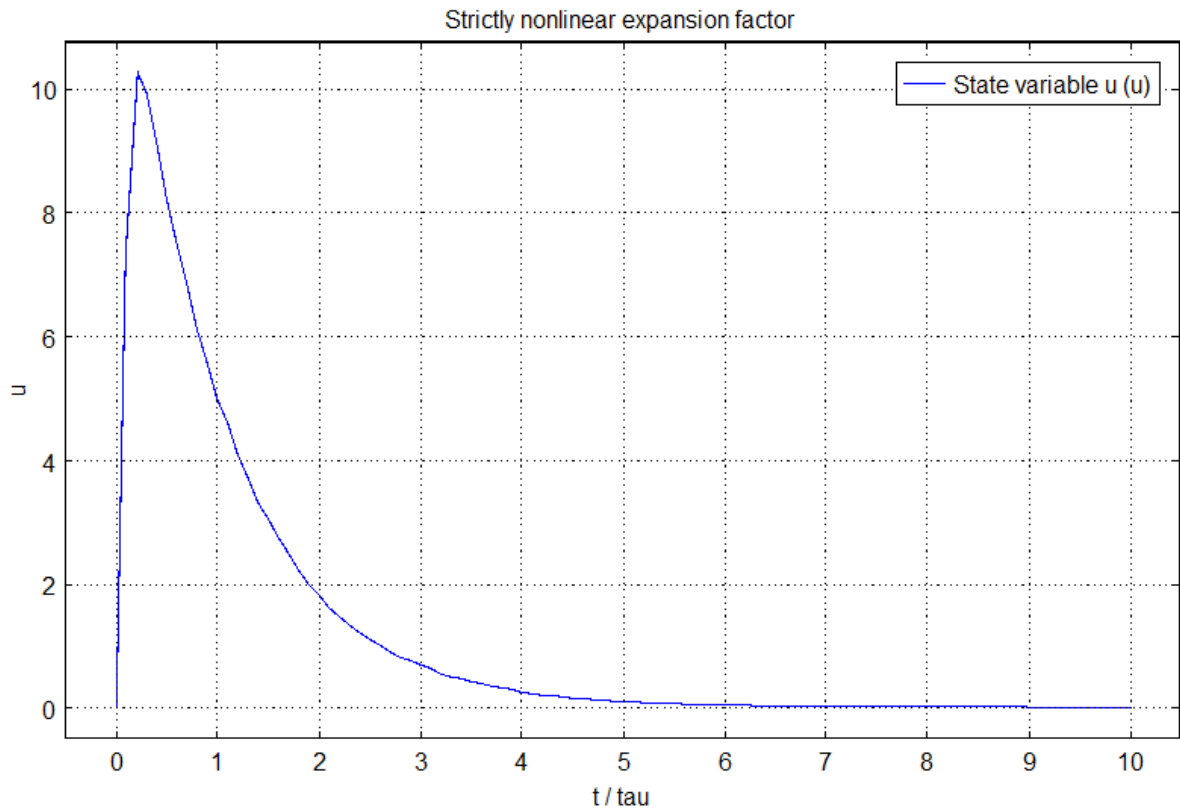


Figure 22 shows the results for the strictly nonlinear expression of the expansion factor of the membrane thickness in the ODE form. Both axis are dimensionless and the y-axis represent the voltage and the x-axis the time, $t / \tau = \tilde{t}$. This shape of the curve is shown for all values of α above one.

4.6 ANALYSIS

The equations presented in the literature show travelling waves but do not yield the pulse formation which the action potential has. This thesis presents a model for the action potential which yields the desired pulse formation in all the different expression of the membrane thickness voltage dependence. It also is anchored in a physical phenomenon for the membrane. These results indicate that this expression definitely is an interesting one and especially considering how much simpler it is compared to the H&H model. This explanation also provides a fundamentally new way of looking at the ion channels activation and the membrane behaviour through treating the conductance and the capacitance as dependent on the membrane thickness and as keys to the phenomenon. In reality this purpose that the membrane thickness varies with the voltage gated ion channels states, opened or closed.

The nonlinear expression for the membrane expansion factor yields the most stable expression since it does not vary as much as the others when the parameters are changed. The linear expression yields the same shape for a bigger interval of α than the strictly nonlinear one. While the strictly nonlinear does not change the magnitude as much as the linear when $\frac{\lambda_0^2}{\tau^2 v^2}$ is increased. Both of them show a widening of the pulse as $\frac{\lambda_0^2}{\tau^2 v^2}$ is increased in contrast to the nonlinear expansion factor. All the expression result in a movement of the peak further away from zero when $\frac{\lambda_0^2}{\tau^2 v^2}$ is increased and when it is smaller than 0.1 there is no pulse behaviour. Comparison between these different parameters and experiments can give information about $\sigma(u)$.

5 EXPERIMENT

This chapter will investigate how general anaesthetic affects the membrane in order to discuss its mechanism. An idea is that the general anaesthetic is dissolved in the membrane which occupies the membrane and results in difficulties to physically open the ion channels.

5.1 BACKGROUND

5.1.1 GENERAL ANAESTHETIC

General anaesthetic is an umbrella term for pharmaceuticals used for narcoses, reversible loss of consciousness and pain during surgery. These chemical compounds can be inhaled or injected intravenously and affect the central nervous system. The electrical signals are inhibited but the mechanism how it works is still unknown. Examples of inhalation anaesthetics are isoflurane, desflurane, sevoflurane, and nitrous oxide. Examples of intravenous anaesthetics are propofol¹⁷, ketamine, thiopental and lorazepam [4] [31]. The mechanism of general anaesthesia is debated and two different theories are presented below. The first theory states that the anaesthetic is resolved in the membrane and affects its properties. The second one says that the general anaesthetic binds to the GABA_A- receptor and thereby inhibits neural signalling.

Meyer-Overton rule

For general anaesthetic there is a famous rule; the Meyer-Overton rule [50] [51] which states that the anaesthetic effect is proportional to their solubility in olive oil. This indicates that the target site is the lipid bilayer in nerve cell membranes and so alters the properties of the lipids in the membrane. Therefore general anaesthetics are said to be a nonspecific agent, but this has been doubted by some. There are three target sites for changing the ion channel properties; the channel protein itself, the protein regulating the channel activity or the surrounding lipid bilayer (as in Meyer-Overton). [3] The Meyer-Overton rule cannot be explained by the well-known Hodgkin & Huxley model since the signalling in Hodgkin & Huxley is not dependent on the membrane, it only treats the pure electrical phenomena. [1] [4]

GABA_A receptor binding

General anaesthetics have been suggested to bind to the GABA_A receptors and in the pharmaceutical industry it is considered a fact. GABA_A receptors are located in the synapses and bind GABA¹⁸ which is a neurotransmitter, a chemical substance that is sent between neurons. GABA is an inhibitory neurotransmitter and when it is released, it prevents other neurons to fire an action potential due to activation of the channel which allows chloride to enter. This increases the intracellular concentration of chloride and the membrane is hyperpolarised, just as it is right after an action potential has passed. During the hyperpolarisation phase the potential is decreased (more negative) in relation to the resting state. Then it is much more difficult to excite the membrane and send a new signal, the capability to send a signal is inhibited and this is what is caused by GABA. General anaesthesia is suggested to work in the same manner as GABA. [52] [53] [4]

5.2 HYPOTHESIS

This hypothesis is about the mechanism of anaesthetics, that it dissolves in the membrane as in the Meyer-Overton rule and therefore affects the membranes electrical properties and mechanical flexibility, which hinders the voltage gated ion channels to open.

¹⁷ 2,6 diisopropylphenol, Michael Jackson died from an overdose of this substance.

¹⁸ Gamma-aminobutyric acid

5.3 THEORY

To investigate if the anaesthetic substance, propofol (2,6 diisopropylphenol), is soluble in a lipid membrane the method of QCM-D is used. Further the effect of propofol on the membranes properties such as the impedance, is examined through QCM-D/Echem

5.3.1 QCM-D AND QCM-D/ECEM

QCM-D (Quartz Crystal Microbalance with Dissipation monitoring) is a device used for collecting and monitoring resonance frequencies and dissipation of a quartz crystal. This enables characterization of a thin film (nm) formation on the crystal, for example a bilayer of lipids similar to a cell membrane. The device monitors the freely oscillating crystals' response to different frequencies and the damping of the frequencies. The damping of the frequency corresponds to the dissipation and can be interpreted as an indication of the thin film mass; therefore a difference in damping is a measure of the mass uptake or loss. This means that this device can observe the kinetics of both structural and mass changes simultaneously. [54]

The device consists of a thin quartz disc in-between two gold electrodes and since the quartz has piezoelectric properties it can be excited by applying an AC voltage across the electrodes. The crystal can be excited until it oscillates. The QCM has four cells which contain one crystal each and this makes it possible to perform four measurements simultaneously. Each cell has an inflow and outflow where the liquids are pumped through to enable a continuous flow at a set speed. When a liquid containing lipids in the form of vesicles flow over the crystal the film of lipids bilayer can be formed. The resonance frequencies of the crystal depend on the oscillating mass and if a film is formed on it the mass increases and therefore the resonance frequency is decreased. The damping of the frequency reveals the viscoelasticity of the film. [54]

The QCM-D can also be connected to a device measuring the impedance of the cell. This method is called QCM-D/Echem, where Echem stands for electrochemistry and additionally to the resonance frequency and dissipation also monitors the impedance. The impedance gives information about electrical properties of the membrane and using the software accompanied with the device the electrical circuit of the system can be obtained. This set up only allows one cell to be used at the time. [54]

The QCM-D has a crystal with gold in the bottom and a thick layer of silicon oxide on the top while the QCM-D/Echem is performed with a crystal covered with a thin layer of silicon oxide layer (5-7 nm). The reason why the layer of silicon oxide is thinner for the QCM-D/Echem is because the gold electrode easier should connect to the conductive fluid and enable a current flow.

5.3.2 PROPOFOL

Propofol or 2,6 diisopropylphenol, which is the chemical name, is an anaesthetic substance which is well used in the clinics. It is injected in the patient who thereafter becomes unconscious, will not feel any pain and is ready for surgery. The chemical structure of propofol is shown below and contains carbon, hydrogen and oxygen. It consists of a phenol group with two propyl groups.

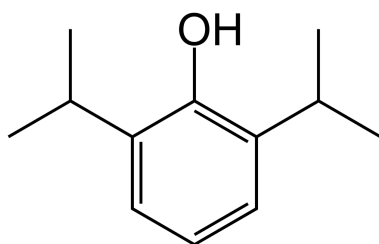


Figure 23 shows the chemical structure of 2,6 diisopropylphenol (propofol).

Since propofol is non-polar it is insoluble in water and to make it water-soluble help substances are used. The pharmaceutical form of propofol is a water-soluble complex of propofol, where propofol is in an emulsion which can include for example egg white. [50] This formulation is not optimal since it has some undesirable properties such as; induce pain when injected and can cause allergic reactions. Therefore other options are being investigated and one of them is cyclodextrin. [55]

5.3.3 CYCLODEXTRIN

Cyclodextrin and more exact methyl- β -cyclodextrin have been investigated if it can be used as formulation, a help substance to enable propofol to be injected and resolved in the body. The cyclodextrin molecule is formed like a hollow cylinder where the outside is water soluble while the inside is fat soluble. This enables the propofol to be attached inside and the complex can thereafter be contained in a water solution, which enables the injection. The injection fluid must be water-based since otherwise it cannot be transferred to and with the blood. [55]

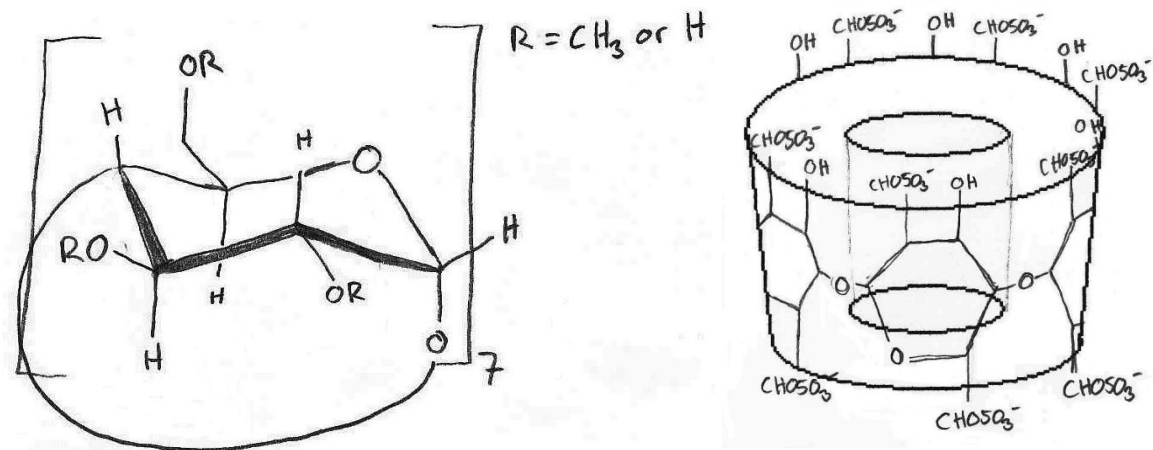


Figure 24 and 25 shows the chemical structure of methyl- β -cyclodextrin. The left figure shows a simplified structure and where R can be either a methyl group (CH_3) or a single hydrogen. The right figure visualizes the cylinder shape of cyclodextrin. The inside is fat soluble while the outside is water soluble. This means that a non polar molecule can bind to the inside and thereafter be contained in a polar environment, water based.

5.4 PROCEDURE

Two different methods for dissolving 2,6 diisopropylphenol in POPC¹⁹-lipids is used. The first one uses methyl- β -cyclodextrin as a transporter and hereafter it is called experiment 1. The second one is simply resolving 2,6 diisopropylphenol in the POPC-lipids before the bilipid layer is formed. To investigate how cyclodextrin affect the lipid bilayer an experiment only containing cyclodextrin is performed as well.

5.4.1 EXPERIMENT 1

A solution of methyl- β -cyclodextrin and 2,6 diisopropylphenol (95%) is dissolved in PBS²⁰ where the mol relationship between methyl- β -cyclodextrin and 2,6 diisopropylphenol is 2:1. The concentrations are based on cyclodextrin and the different concentrations to be examined are; 20, 10, 1 and 0.2 mg/ml. The solutions are vortexed to mix them properly and are thereafter filtered through a 0.2 μ m filter in order to get rid of dust.

Preparation of the POPC solution is done by adding concentrated POPC to PBS yielding a concentration of 0.1 mg/ml

The QCM-D measurements are started by flowing PBS into the system and then yielding a stable baseline. The bilayer lipid film is formed by adding the POPC solution to the inflow of the QCM-D and letting it flow over the crystal. After a while the solution of different concentrations are placed in the inflow to enable the cyclodextrin to transfer the propofol to the lipid bilayer. Lastly PBS is flown over to get rid of all the fluid and substances which are not attached or bonded to the crystal or the lipids.

This solution of concentration 20 mg/ml was also monitored in the QCM-D/Echem which is performed with the same procedure.

5.4.2 EXPERIMENT 2

This solution is generated by dissolving 2,6 diisopropylphenol directly in the POPC lipids with an amount of 2,6 diisopropylphenol of about 20 weight% of the POPC. This is dissolved in chloroform which is non polar and therefore the propofol and POPC lipids which are fat soluble (non-polar) are soluble in chloroform. The chloroform is thereafter evaporated using nitrogen gas (N₂). This results in a lipid layer film on the bottom of the beaker. A vacuum suction is used to get rid of the last air and then the film is dissolved in 1.2 ml PBS. The solution is extruded through first a 100 nm filter and then a 30 nm filter in order to generate vesicles. The solution has a concentration of POPC of about 0.1 mg/ml.

The solution is flown over the crystal in the QCM-D and since the propofol already is dissolved in the lipids this step is followed by the last washing step of PBS.

¹⁹ Palmitoyl oleoyl phosphatidyl choline, lipids which resembles lipids in biological membranes.

²⁰ A water-based salt solution containing sodium chloride, sodium phosphate and potassium phosphate used as a buffer in biological research.

5.4.3 EXPERIMENT 3

Methyl- β -cyclodextrin is dissolved in PBS generating solutions of different concentrations; 40, 20, 10 and 5 mg/ml. POPC lipid solution of 0.1 mg/ml are used to create the lipid bilayer and the same procedure as in experiment 1 is performed only that the solution does not contain 2,6 diisopropylphenol.

5.5 RESULTS

Results from the three experiments are presented below.

5.5.1 EXPERIMENT 1

This experiment is intended to investigate how 2,6- diisopropylphenol and methyl- β -cyclodextrin affect dissipation and resonance frequency of POPC lipid bilayer. Part one is QCM-D and part two is QCM-D/Echem. Part one includes the concentrations 20, 10, 2 and 0.2 mg/ml cyclodextrin and the relationship between cyclodextrin and propofol is about 2:1. Part two is only performed for 20 mg/ml.

PART I.

This experiment could not show that the cyclodextrin is dissolved in or attached to the lipid bilayer.

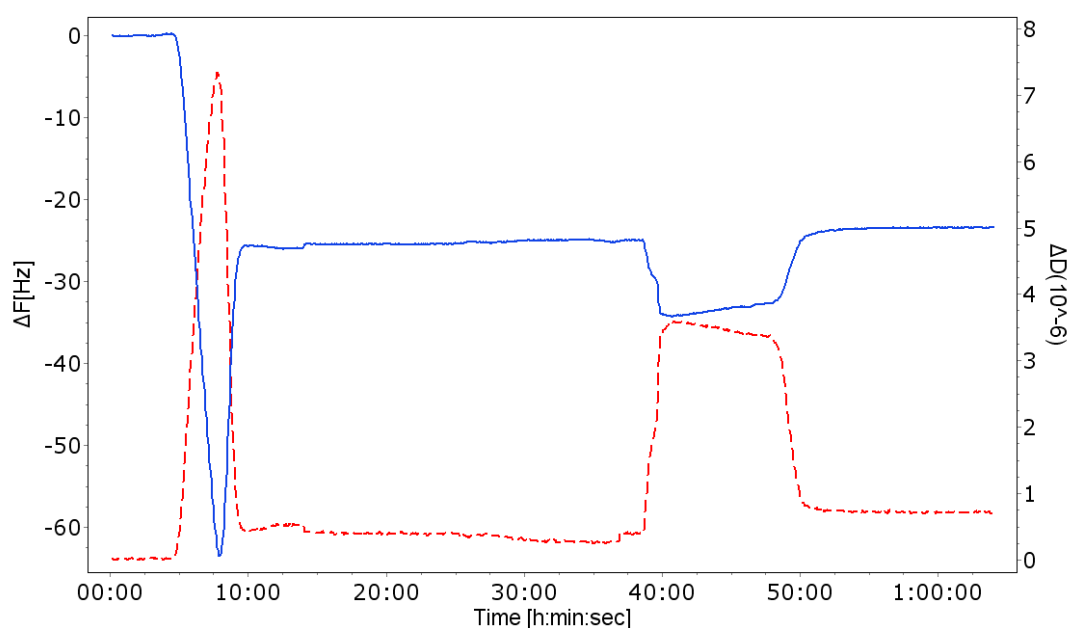


Figure 26 shows cyclodextrin+ propofol with the concentration **10 mg/ml**, the other concentration showed the same results.. The top line corresponds to the frequency and belongs to the y-axis to the left. The bottom line, dashed line is the dissipation and belongs to the y-axis to the right. The first part is a clean surface in PBS, the baseline. The extreme behaviour corresponds to a bulk change and the stable section after is the lipid bilayer. The small change is when cyclodextrin and propofol is added. When the PBS is added in the end the frequency and the dissipation goes back to the same as for the lipid bilayer alone.

PART II.

The QCM-D/Echem result shows a small change in the membranes impedance and its phase, but not enough to yield any specific information about it.

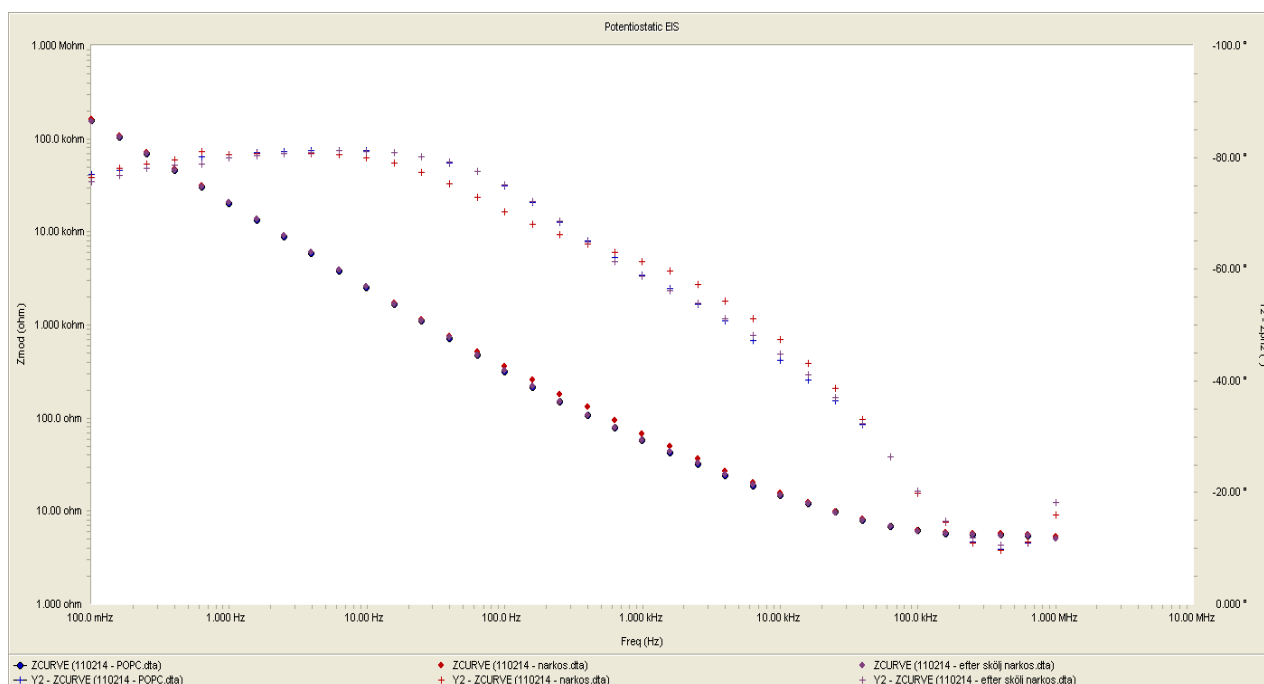


Figure 27 shows a Bode diagram over a comparison of the impedance before, during and after propofol is added to the lipid bilayer. The values are logarithmic and the dots show the impedance and belong to the left y-axis while the cross corresponds to the phase of the impedance and belong to the right y-axis. There is practically no difference between the different stages, before, during and after propofol is added which indicates that no propofol is dissolved in the membrane.

5.5.2 EXPERIMENT 2

This experiment includes 2,6- diisopropylphenol dissolved directly in the POPC lipids and monitors the effect on the formation of lipid bilayer; dissipation and resonance frequency.

This experiment resulted in that no lipid bilayer was formed. The vesicles did not fuse out on the crystal.

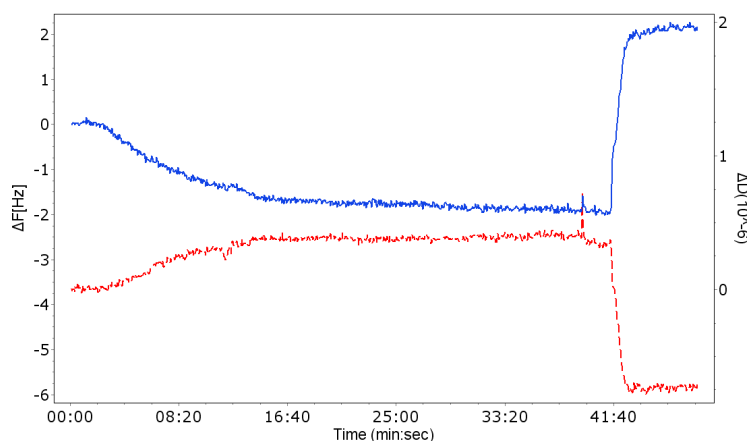


Figure 28 shows the QCM-D results of the experiment where propofol is dissolved in the POPC lipids before the lipid bilayer is formed. The first section shows the PBS baseline. The second part shows when the POPC + propofol is flown over the crystal and no lipid bilayer is formed.

5.5.3 EXPERIMENT 3

This experiment monitors methyl- β -cyclodextrin affect on dissipation and resonance frequency of POPC lipid bilayer. The concentrations are 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml. 40 mg/ml showed a behaviour to destroy the bilayer while 10 and 5 mg/ml did not affect the bilayer. 20 mg/ml is displayed below and showed an ability to generate a monolayer for a time period before the lipid layer was destroyed.

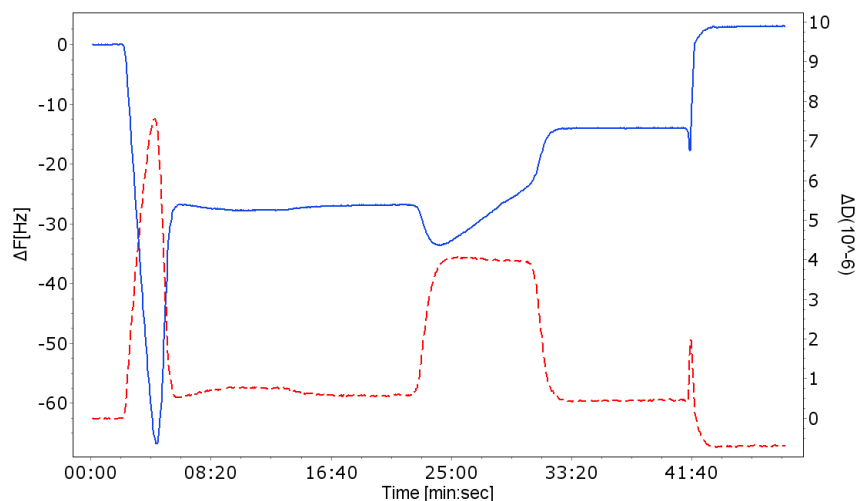


Figure 29 shows 20 mg/ml cyclodextrin. The dashed line shows the dissipation and belongs to the right y-axis while the other line corresponds to the left y-axis. The section between 8 and 22 minutes corresponds to the lipid bilayer ($\Delta F = -26$) and after that cyclodextrin is added. At the time 30 minutes PBS is added to wash out the cyclodextrin which is not attached to the bilayer, but at around 32 minutes there is a phase where the resonance frequency is stable at -13 Hz. This corresponds to a monolayer. After a while the lipid layer is destroyed completely since both the resonance frequency and the dissipation is back to the levels of the PBS baseline.

5.6 ANALYSIS

The experiment with propofol dissolved in cyclodextrin shows no effect on the lipid bilayer. This can for example be due to one of the following reasons; that propofol is insoluble in the membrane, propofol is a small molecule which may not be observed in a small amount in the membrane or cyclodextrin may also bind propofol so much that it does not attach to the membrane. The QCM-D/Echem measurement shows a too small difference in impedance in order to determine any difference in the electrical properties of the membrane.

When propofol was dissolved directly in the POPC-lipids they did not form a lipid bilayer on the crystal. This can be because propofol changes the vesicles ability to fuse over the crystal, the vesicles different compartments are tightly bond. This behaviour, that the bilayer is not formed is usually seen for negatively charged lipids which mean that the propofol might generate a negative complex together with the lipids and therefore not forming the bilayer. It can also be due to the fact that the experimental procedure showed some problems in the preparation and it could not be guaranteed that the vesicles had been formed.

Since cyclodextrin alone showed some interesting characteristic during the first experiment a whole set of experiment with only cyclodextrin was performed to monitor its affect on the lipid bilayer. This experiment showed some interesting result because cyclodextrin at a concentration of 20 mg/ml resulted in a monolayer instead of the original bilayer. This is a quite mysterious phenomenon since the lipids do not prefer to be in a monolayer since they have one hydrophobic tail and hydrophilic head and in the bilayer the hydrophilic head faces the fluid and the crystal and the tail faces the inside of the bilayer. In a monolayer this means that the hydrophobic tail faces the fluid which usually would lead to the formation of a vesicle. The cyclodextrin might place itself on top of the monolayer as a protective shield. This experiment was not easily reproduced which might indicate that this is an instable phenomenon but if it can be generated it is a very interesting behaviour.

In summary the experiment resulted in an indication that the propofol might affect the properties of the membrane but the specific way or mechanism could not be established, it needs further investigations. The experiment also showed some interesting properties concerning cyclodextrin, that it has an ability to generate a monolayer in a bilayer situation.

6 PROCELLERATM

This chapter will treat the bioelectric dressing *Procellera*TM and speculate in different mechanisms underlying the claimed pain relief.

*Procellera*TM is the first high-technology wound dressing which is electrically active, cut-to fit, comfortable and self-contained, according to the fabricator, VomarisTM. According to their clinical trials the dressing enhances the healing process and the patients say that their pain was reduced. Studies also show that it has an antimicrobial effect. [56]

6.1 BACKGROUND

In order to investigate and discuss the dressing's pain relieving mechanisms we need some background knowledge about *Procellera*TM.

6.1.1 PROCELLERA

*Procellera*TM consists of a woven polyester fabric surface with two-element microcells of silver (Ag) and zinc (Zn). The dressing needs presence of a conductive fluid to be active and function. The fluid can be exudates from the wound or simply exogenous fluids. This means that this dressing does not need any external power source or fluids added, it is self-contained. [56] The two elements, silver and zinc can yield a voltage about 0.88 V in the wound and the dressing also generates an antimicrobial environment. [56]

The company providing *Procellera*TM has done clinical trials where the hospitals report not only an enhanced healing of the wound but also pain relief.

6.2 HYPOTHESIS

My hypothesis about the bioelectric wound dressing *Procellera*TM is that there is a chemical reaction which causes pain reduction. Secondly an idea is that the antimicrobial effect of the dressing is also responsible for the pain reduction.

Lastly a hypothesis is also that the pain reduction is caused by the electric currents in the dressing.

6.3 THEORY

Since the dressing contains zinc and silver microcells, these metals existence and possible effect in the body will be studied. The zinc and silver are in contact with conducting fluids in the wound there will be several possible chemical reactions. This section will present possible redox reaction, oxidation, hydrolyse and acid reactions.

6.3.1 SILVER AND ZINC IN THE BODY

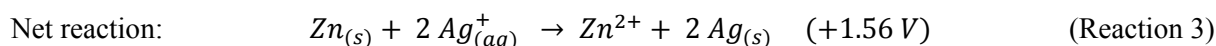
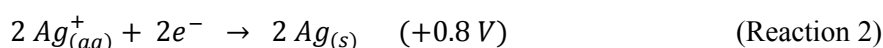
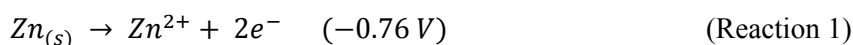
Silver is not naturally occurring in the body and it is toxic. It has an ability to damage the DNA and therefore breaking the cell cycle if it enters the cell. Cells can recover from this if the damage is not too big. Silver has a known effect to cause a fatal environment for bacteria and is therefore quite commonly used in dressings. [57] [58]

Zinc can be found throughout the body during normal conditions and is only toxic when existing in high concentrations of the free form. It is a compound in enzymes and other proteins. In the brain it has been found to be an ionic signal in specific types of synapses. Zinc seems to modulate the brains overall excitability through its effect on glutamate and GABA receptors. The GABA_A receptor can bind to Zn²⁺ which enables chloride ion flow into the cell which hyperpolarizes it and therefore inhibits the neurons ability to send a pain signal. Zinc also has an effect on other receptors in the brain, such as serotonin and proton receptors. The serotonin and the proton receptor are inhibited by the binding of Zn²⁺. In an acid environment, low pH, the proton receptor is more inhibited by zinc. Zinc can also facilitate the influx of chloride ions which brings the membrane into the hyperpolarizing state and disable the ability to send a pain signal. [58] [59]

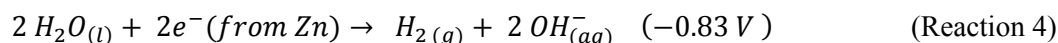
6.3.1 REDOX REACTION

Redox reaction include oxidation (increase oxidation number) and reduction (reduce oxidation number) of different metals or molecules. These reactions require or generate a voltage. [60]

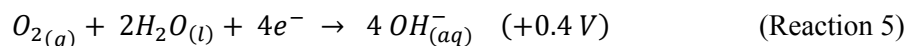
Zinc is oxidized while silver is reduced in this redox reaction (galvanic cell).



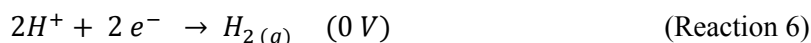
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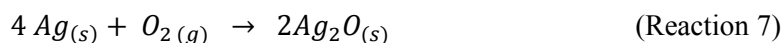


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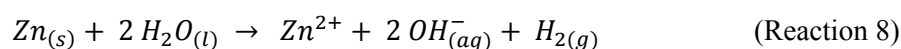
6.3.2 OXIDATION (WITH O₂)

Since the wound and dressing is in contact with air, oxidation of the metals is a possible reaction. Zinc is not particularly likely to undergo oxidation with O₂ since it is highly reducible and especially in the presence of silver, which is more likely to be oxidized. Therefore only silver oxidation will be considered. [61]

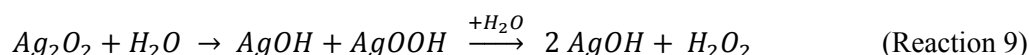


6.3.3 HYDROLYSE

The hydrolyse reaction is when water is decomposed. When solid zinc is dissolved in water it reacts and forms zinc ions, hydroxide ions and hydrogen gas as follows: [61]



Silver in a pure solid form does not have the possibility to undergo hydrolysis but as an oxide complex it does. The silver oxide complex consists of silver and oxygen where each unit has two silver atoms which are bonded to one oxygen atom each. The two oxygen atoms are also bonded to each other. [61]

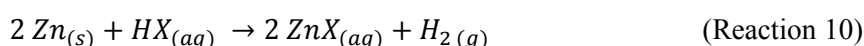


This reaction generates hydrogen peroxide which is used to purify water because it has an antibacterial effect. [62]

6.3.4 ACID REACTION

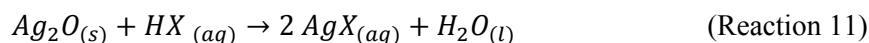
Since the milieu in the acute stage in the wound is acetic (low pH) acid reactions are presented.

When zinc reacts with acid, hydrogen is released. [63]



Where X can be chloride (Cl), fluoride (F), bromide (Br) or iodide (I).

Silver as a pure solid is almost insoluble in water but as an oxide it is soluble in water in the presence of acids, this reaction can be expressed as:



Where X can be chloride (Cl), fluoride (F), bromide (Br) or iodide (I).

6.4 ANALYTICAL RESULT

This section will present the different mechanisms which possibly can explain the claimed pain reduction of *Procellera*TM.

Reaction 4, 5, 6, 8, 10 and 11 all result in an increased pH either by consuming hydrogen or producing hydrogen oxide. By increasing the pH out of the pain sensation range (< 6.5 pH) the pain can be reduced. The voltage measured in the dressing was about ± 0.88 V which could correspond to both the normal potential of zinc and silver. It is also close to the redox reaction of water.

When a wound occurs, the extracellular potassium increases and this induces pain. If there are OH⁻ molecules in the wound fluid they might join each other and form KOH, potassium hydroxide, which would decrease the pain since there would be less potassium ions.

As mentioned, zinc has the ability to bind to the proton receptors and block these in the brain a possible explanation to the pain reduction could be that this mechanism also is present in the peripheral nervous system. When zinc binds to the pH sensitive receptors in the dendrite they are no longer able to send a signal which would result in pain, the dressing has therefore caused a pain reduction.

Zinc can block the serotonin receptors in the brain and if this mechanism would exist in the wound as well this would lead to pain reduction. The damaged cells releases serotonin into the surrounding tissue and the serotonin stimulate the nociceptors which generates pain. This means that through Zn⁺ binding of the nociceptors they are no longer able to be stimulated and therefore they won't induce pain.

The ability of Zinc to increase the chloride channels activity by facilitating the influx of chloride ions leads to hyperpolarisation. This means that the membrane potential is more negative than at resting state, due to more negative charges inside the cell. When the potential is more negative than at resting state it cannot transfer an action potential and therefore the action potential is inhibited and the pain reduced.

Silver and hydrogen peroxide (Reaction 9) has an antibacterial affect and if the bacteria are extinguished they no longer are able to produce toxins. These toxins damage cells and tissues which then releases pain inducing substances and if this does not occur the pain will be reduced.

The electrical currents could generate a flow in the wound fluid which increases the chemical reaction rates and also enables the metals to dissolve easier and to get in contact with the nerves, cells and tissue in the wound. Basically the current would enable the other pain reliving mechanisms presented in this section.

7 DISCUSSION AND CONCLUSION

All the three aspects involve changes or modifications in the neuron's membrane. The simulation suggests an expansion of the membrane as an important factor in the propagation of an action potential. The experiment suggests membrane changes due to anaesthetic and the theoretical investigation about *Procellera*TM suggest inhibition of nociceptors located in the membrane.

The voltage dependent membrane expansion is suggested to change the conductance and the capacitance of the membrane and therefore enable ion flow, this is an approach which has not been investigated or suggested before. This is an interesting and fundamentally new way of approaching this phenomenon. Since the desired pulse shape, which the action potential has is yield through the simulation it also shows that it has the desired behaviour which the alternative expressions found in the literature does not show. The presented expression is also much simpler than Hodgkin & Huxley's model but still it anchored in observed behaviour, axon expansion.

The model shows the same behaviour for all three expansion factors expression, linear, nonlinear and strictly nonlinear dependence of voltage. The expressions are not completely stable but the most stable one is the nonlinear dependence. The expressions do lack a known solution in the form of a function so the next step in this challenge should be to describe the membrane thickness as dependent on time instead of voltage. This would lead to an equation where there are known solutions [64].

Since this is a completely new aspect of the description of the action potential which actually can describe the pulse formation of the action potential it is definitely a model to investigate further. An indication that this proposed mechanism definitely is of great interest is an article which recently was published which found that the voltage gated ion channels require a voltage of 50 mV in order to physically open up. This article also did calculations on how the membrane require more voltage to open up the ion channels if the membrane is crowded, occupied by other molecules [65]. This can be transferred into our idea of the general anaesthetics mechanism, that the molecules disable the membrane thickness to change.

The experiment could not yield any definite answer to the question if the anaesthetics changed the properties of the membrane since it could not be guaranteed that the anaesthetic was dissolved in the lipids (Experiment 2) when they were not able to form the lipid bilayer. The inability to form bilayer is often seen in the case where the lipids are negatively charge which means that the propofol might affect the lipids into changing their charge to negative. This is an aspect which needs further investigations. A way of controlling if propofol is dissolved in the lipids would be to use some kind of microscopic method to look at the sample.

Since this is an area where the scientists do not agree, some say it is fused into the membrane while other say it binds to the GABA_A receptors in the central nervous system it is an interesting phenomenon to study. The scientists' promoting GABA_A receptors have not suggested an explanation to the Meyer-Overton rule.

The first experiment showed that the propofol did not dissolve in the bilayer which could be because cyclodextrin bind the molecule to tight. This experiment could be performed with another type of transferring molecule. The experiment also resulted in some interesting observations concerning cyclodextrin, it might not be optimal to use as a transport molecule and it has an ability to break down the bilayer to a monolayer. The fact that it can generate a monolayer is interesting in the understanding of how membranes function. The QCM-D cannot establish whether the membrane is entirely monolayer or partly bilayer covering half of the surface of the crystal since the QCM-D cannot yield information about the mass distribution, only the total mass through the resonance frequency. This could lead an illusion of the partly bilayer to be a monolayer. This could be further investigated using QCM-D/Echem where the distribution of the lipids also can be found. The fact that the formation of monolayer was not easily reproducible can be an indication that it actually is partially bilayer partially nothing.

The investigation concerning *Procellera*TM opens up to interesting aspects of pain relief. It suggests zinc as a possible inhibitor to serotonin receptor and facilitator of the chloride ion flow inside the cell. The dressing's chemical reactions are suggested to change the pH milieu in the wound and therefore reduce pain. This is something which should be examined further by measuring the pH in a wound with and without *Procellera*TM at different times. There should also be measurements performed on the dressing to see if hydrogen gas (H₂) is released in order to establish if the pH changing reactions actually take place. The influence of pH on wound healing has just recently started to be investigated [15] and is therefore extra interesting that it might also influence the pain sensation. Since zincs' importance and mechanism only has been studied in the brain it should also be examined in the peripheral nervous system, which according to this thesis could result in very interesting conclusions. All these suggested mechanisms can in a simple way help a lot of people with painful wounds, provided of course that they are correct.

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APPENDIX 1 –SUMMARY OF PARAMETERS

i = Intracellular, inside

e = Extracellular, outside

R = Universal gas constant, 8.3143 J/K

T = Temperature in K

F = Faraday constant, $0.9648 \cdot 10^5$ Coulomb

G = Gibbs free energy [J]

z = Valence charge

V_m = Membrane potential at equilibrium, Nernst potential, [V]

I = Membrane current per unit area [A/m^2]

I_m = Membrane current per unit length [A/m]

C_m = Capacitance per unit area [F/m^2]

c_m = Capacitance per unit length [F/m]

g_x = Conductance per unit area [S/m^2]

g_{mx} = Conductance per unit length [S/m]

n = Probability that the Potassium channel is open

m = Probability that the Sodium channel is activated

h = Probability that the Sodium channel is inactivated

β = Rate constant from open to closed

α = Rate constant from closed to open

R_m = Membrane resistance per unit area, axial direction [Ωm^2]

r_m = Membrane resistance per unit length, axial direction [Ωm]

A_m = Surface-to-volume ratio [1/m]

l = fibre length [m]

a = fibre radius [m]

ϕ = potential [J]

R_i = Intracellular resistance per unit area, longitudinal direction [Ωm^2]

r_i = Intracellular resistance per unit length, longitudinal direction [Ωm]

R_e = Extracellular resistance per unit area, longitudinal direction [Ωm^2]

r_e = Extracellular resistance per unit length, longitudinal direction [Ωm]

λ = length constant [m]

$\tau = R_m C_m$ = time constant [s]

c = concentration [mM]

ϵ_0 = permittivity in free space [As/Vm]

ϵ = relative dielectric constant of the membrane

$\sigma(u)$ = membrane expansion factor, ratio of voltage dependent membrane thickness and the membrane thickness at rest [V]

u = membrane voltage in relation to membrane voltage at rest and normalised with Nernst membrane potential

D = diffusion coefficient [m^2/s]

V_{00} = magnitude of membrane voltage at rest [V]