# BT5051:
# TRANSPORT PHENOMENA IN BIOLOGICAL SYSTEMS

## *CHOOSE-FOCUS-ANALYZE (CFA) EXERCISE*

# Modelling And Analysis Of Hyperalgesia Condition

Work submitted by

Sahana G
BE17B038

**CONTENTS**

## INTRODUCTION TO THE WORLD OF NEURON

The human brain is the most complicated bio-machine on our planet. It is astounding that we are all made up of our memories, cognition, response and characteristics; and that, all these are controlled by one small organ. The complicated structuring of the neural network and its efficient functioning and communication is what makes it a fantastic and unique device. Neurons are different from most other cells in the body in that, they are polarized and have distinct morphological regions, each with specific functions. To understand this neural network, it is important to analyse how neurons communicate with each other via the synaptic connection. This communication between neurons is called Synaptic Transmission. Each neuron mediates the transportation of information as electrical signals within them. The propagation of signals is constituted by various intrinsic parameters.

A few interconnected neurons, a *microcircuit*, can perform multiple sophisticated functions such as provide sensory information, mediate memory and learning, etc. Multiple microcircuits constitute a *macrocircuit*. These circuits constitute the underlying biochemical machinery for mediating key neuronal properties such as learning and memory and the genesis of neuronal rhythmicity.

## MOTIVATION

The performance of any machine will always be accompanied by both pros and cons, and identifying flaws in an efficient machinery, such as the brain and its communication network, is always surprising. These conditions could be detrimental to the survival of the organism, or sometimes to the entire species community. It is therefore necessary and important to analyse such conditions. One such neuronal condition is Hyperalgesia, which is increased sensitivity to pain. This state occurs due to slight modification in the permeability of an ion channel which affects an entire neuronal circuit and thereby causes an increased response to pain. What may not hurt for a normal person might cause severe pain for a person with hyperalgesia. This large impact due to a minute change in the intrinsic parameter of an ion channel is amusing and is what motivated me to choose this project to further focus and analyze.

# OBJECTIVE

The object of this work is to characterize the effects in the condition of “Hyperalgesia” and analyze various transport phenomena involved in the system. We will also delve into various other mechanisms that make up the system.

# THEORY, PRINCIPLES AND CONCEPTS

## RESTING MEMBRANE POTENTIAL

Nerve cells generate convey and transmit information by synapses across large distances. These signals depend on changes in the resting electrical potential across the neuronal membrane. Nerve cells have various ion channels and hence permit more than one ion species and constantly maintain an electrochemical gradient across the membranes. When at rest, neuron generates a constant voltage across their membranes, termed as the **Resting Membrane potential**. During the Resting Membrane Potential, there are –

- More sodium ions (Na+) on the outside than inside.
- More Potassium ions (K+) on the inside than outside.

## HYPER/DE- POLARIZATION

Action potentials are electrical impulses that send signals around the brain, or say, even the body. When an electric current is passed across the neuronal membrane, an action potential occurs. Usually, this current is stimulated by a receptor or synaptic potentials. If input current makes the membrane potential more negative, the effect is termed as **Hyperpolarization**. This is due to the passive response in the neuron. If input current makes the membrane potential more positive than the resting membrane potential (reverse polarity current is injected), the effect is termed **Depolarization** and it could result in an Action Potential if certain minimum voltage is crossed. This voltage is termed **Threshold Potential**.

The Action Potential (AP) is an all-or-none event because they either occur fully or not at all. When input stimulus current's amplitude or duration is increased, multiple APs occur. Thus, the intensity of the stimulus corresponds to the frequency of AP and not its amplitude.

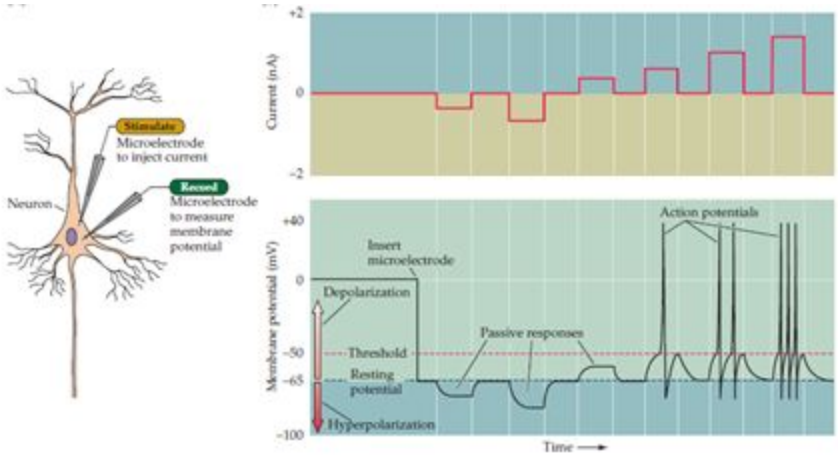


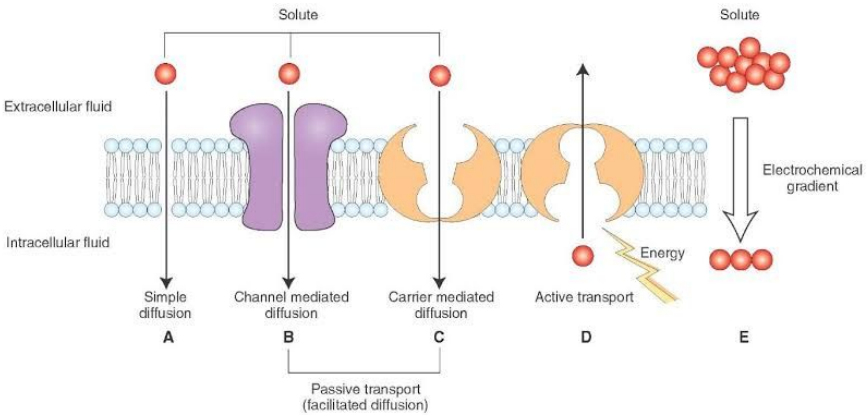

***Fig 1:*** *Figure explaining Action Potential – Purves, Neuroscience.*

## IONIC MOVEMENT AND THEIR DEPENDENCIES

The Electrical potential that's generated across the membrane is dependent on the following.

- Difference in concentration gradient for specific ions across the nerve cell membrane.
- The permeability of those specific ions.

The ionic concentration across a membrane depends on proteins called **active transporters**, which actively move specific ions in and out of the membrane in a process called Diffusion. **Diffusion** is the force on molecules to move from areas of high concentration to areas of low concentration. They are situated on the membrane and are hence responsible for the concentration gradient that exists. However, **ion channels** are proteins that selectively allow only certain kinds of ions through the membrane to set up the concentration gradient.




***Source*** *- http://what-when-how.com/neuroscience/electrophysiology-of-neurons-the-neuron-part-1/*

***Fig 2****: Figure explaining the movement of ions in an ion channel.*

At electrochemical equilibrium, the electrical potential generated can be predicted by the **Nernst equation**. This is termed as the **equilibrium potential**.

$$V = \frac{RT}{zF} \ln \frac{[C]_{out}}{[C]_{in}}$$

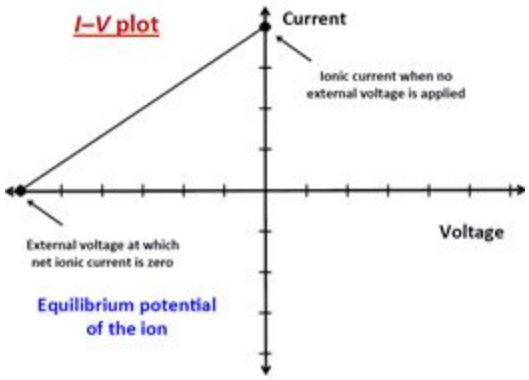
*R*: Gas Constant=1.98 cal/K-mol
*T*: Absolute temperature
*z*: Valence of ion
*F*: Faraday's constant=96480 C/mol

However, since the permeability of the ions is not taken into action, David Goldman, came up with another equation, termed the **Goldman equation** which looks like the following. In neurons, K+, Na+, and Cl- are the basic permeable ions. Hence the equation is given by -

$$V = \frac{RT}{F} \ln \frac{P_K[K^+]_{out} + P_{Na}[Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{out}}$$

Where P(K), P(Na) and P(Cl) are permeability of K+, Na+ and Cl- ions respectively.

For ions travelling in and out of the membrane, the current-voltage relationship is given by Ohm's law and appears to take this form.




$$\boldsymbol{I = g \times (V - E\text{rev})}$$

I – current across the membrane.
g – conductance of that ion.
V – membrane voltage.
Erev – Reversal potential of that ion calculated by the Nernst equation.

## PASSIVE PROPERTIES OF NEURON

The membrane is considered to be an RC circuit. The electrical resistance, in other words, the conductance, is due to the leak channels and the ions that move through them. Electrical capacitance is due to the bilipid layer that separates ions across the membrane and in a way stores charge.

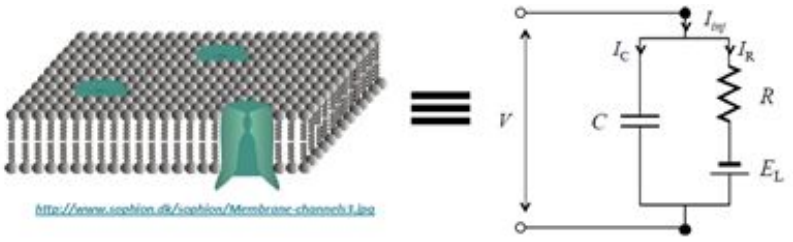
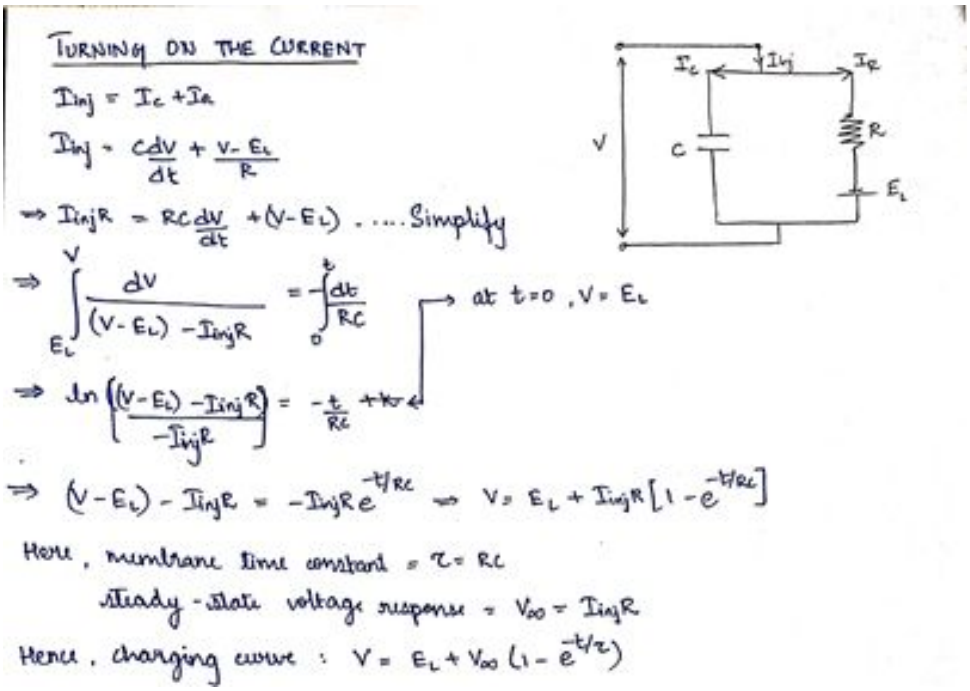



***Fig 3**: Image comparing the neuronal membrane to an electric circuit - Dales, Purves*

By Kirchhoff's current law and Ohm's law,



TURNING OFF THE CURRENT → $I_{inj} = 0$

$I_{inj} = I_C + I_R = 0$

$\Rightarrow 0 = C\frac{dV}{dt} + (V - E_L)$ .... Simplify

$\Rightarrow \int_{E_L + V_\infty}^{V} \frac{dV}{(V - E_L)} = \int_0^t \frac{dt}{RC}$ at $t=0$, $V = (E_L + I_{inj}R)$

$\Rightarrow \ln(V - E_L) - \ln V_\infty = -t/RC$

$\Rightarrow \frac{V - E_L}{V_\infty} = e^{-t/RC} \Rightarrow V = E_L + V_\infty e^{-t/RC}$

Hence, discharging curve : $V = E_L + V_\infty e^{-t/\tau}$

Initial assumptions were that the **neuron** forms an iso-potential surface. The membrane resistance was assumed to be constant throughout the compartment and that there was no voltage dependant conductance that constitutes Rm. Important passive properties of the neuron included Rm (membrane resistance), Cm (membrane capacitance) and Rin (input resistance).

However, a neuron is not an iso-potential surface and that the voltage is variable with the location on the compartment. There are a few assumptions regarding this consideration as well. They are –

- There is no extracellular resistance.
- Membrane properties are uniform throughout the structure and are not voltage-dependent, i.e., there are no voltage-gated channels.
- Current flow is only through on direction and hence radial current is absent.

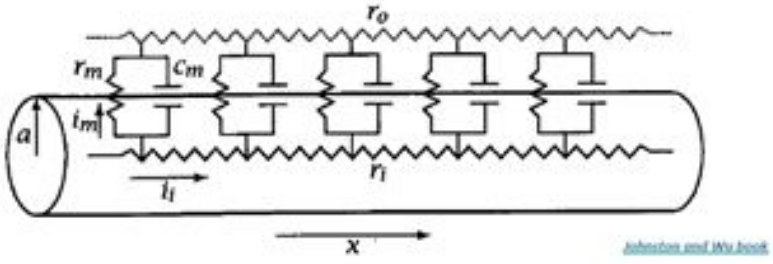
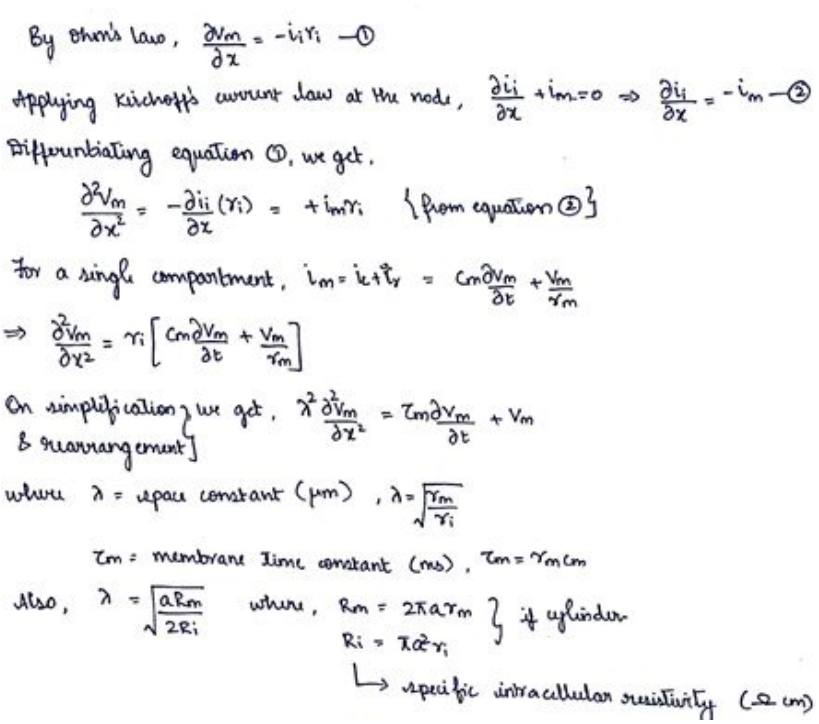



***Fig 4**: The cylindrical model of a neuron which compartmentalized electrical circuit.*

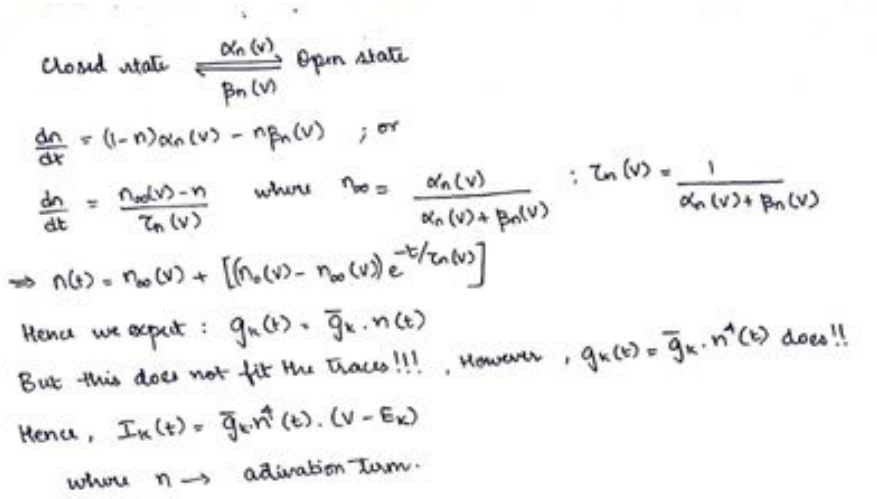
By Kirchhoff's current law, Ohm's law and the cable equation,




**ACTIVE PROPERTIES OF NEURON**

The action potential is basically an output of consequence of the motion of two ionic currents primarily -Delayed Rectifier Potassium and Sodium channels.

The delayed potassium channel is non-activating in nature and hence it either opens/closes. It is delayed with reference to the sodium current for identical voltage pulses and acts as a rectifier by allowing currents preferentially in an outward direction (positive current).



The sodium channel is inactivating in nature. Hence, we model one gate to activate the channel and the other to inactivate it, unlike potassium, where there is only one gate and that is either activated or non-activated.

Model: Two gates! One activates, and another inactivates!

Closed $\underset{\beta_m(V)}{\overset{\alpha_m(V)}{\rightleftharpoons}}$ Open  Closed $\underset{\beta_h(V)}{\overset{\alpha_h(V)}{\rightleftharpoons}}$ Open

Similarly, $\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)}$  $\frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)}$

$\Rightarrow m(t) = m_\infty(V) + \left[(m_0(V) - m_\infty(V))\, e^{-t/\tau_m(V)}\right]$ ; and

$h(t) = h_\infty(V) + \left[(h_0(V) - h_\infty(V))\, e^{-t/\tau_h(V)}\right]$

Hence we expect : $g_{Na}(t) = \bar{g}_{Na}\, m(t) \cdot h(t)$

But this does not fit the traces !!! However, $g_{Na}(t) = \bar{g}_{Na} \cdot m^3(t) \cdot h(t)$

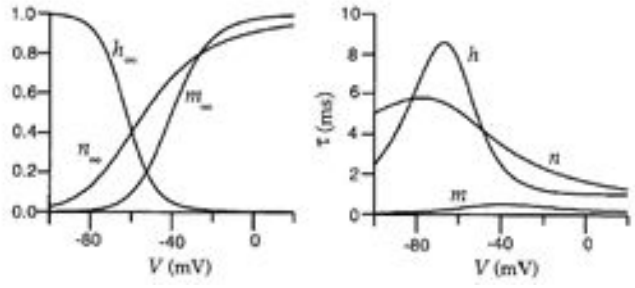
Hence, $I_{Na}(t) = \bar{g}_{Na}\, m^3(t) \cdot h(t) \cdot (V - E_{Na})$

where $m \rightarrow$ activation

$h \rightarrow$ inactivation

When all these three parameters are estimated based on appropriate voltage-clamp recordings, we get,

$$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)} \qquad \frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)} \qquad \frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)}$$




,

Therefore, modelling Na+ and K+ currents will help us analyse the ionic basis of an action potential.

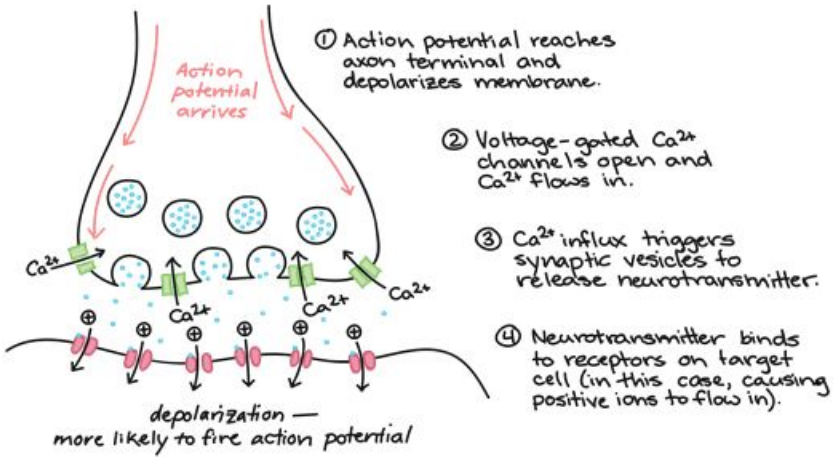
- Depolarisation is attained when the sodium activation gate is opened.
- The influx of Na+ ions depolarize the membrane further and the action potential reaches close to the reversal of Na.

- As voltage increases, sodium channels inactivate and potassium channels open, which hyperpolarize the membrane.
- Loss of sodium influx accompanied by potassium efflux brings the voltage down.

## TRANSPORT OF MESSAGE BETWEEN NEURONS

Now that we have established how ion diffusion helps transport information within a neuron, it is important to also understand how messages are passed from one neuron to another. Transport of messages between neurons primarily occurs in two ways.

- Electrical Synapses
- Chemical Synapses




*Source - Khan academy*

***Fig 5**: Image depicting the synaptic cleft with chemical synaptic transmission*

We will focus on Chemical Synapses in this project. The chemical messengers involved as broadly called as **Neurotransmitters** and these evoke postsynaptic electrical responses by binding to members of a diverse group of proteins called neurotransmitter receptors. The neurotransmitters are withheld inside the presynaptic neuron in small packets called vesicles. These vesicles release the messengers inside the synaptic cleft when they receive a signal ($Ca^{2+}$ ion binding to vesicles). Based on neurotransmitters and their receptors, the postsynaptic response could be excitatory or inhibitory.

## EXCITATORY AND INHIBITORY SYNAPSE

Excitatory synapses increase the possibility of postsynaptic firing. ***AMPA Synapse*** that we use in this project is an excitatory synapse and hence its synaptic reversal potential = 0. It is not ion-specific and also transmitter-gated. The model file for the AMPA synapse was

imported in the project's GUI. At each discrete time interval, the Transmitter concentration is assigned to the summed-up vesicle release.

$$I(t,V) = \bar{g}\, s(t)\, (V - V_{syn})$$

$$\frac{ds}{dt} = \alpha[T](1-s) - \beta s$$

- $[T]$: Transmitter concentration;
- $V_{syn}$: Synaptic reversal potential

Inhibitory synapses decrease the possibility of postsynaptic firing. ***GABA Synapse*** is a commonly used inhibitory synaptic receptor, which opens ion channels that are selectively permeable to Cl- and causes Cl- to flow across the postsynaptic membrane. Cl- induces hyperpolarizing effect and increases the postsynaptic potential of the cell and hence is inhibitory in nature. Hence, the reversal potentials of GABA receptor and Cl- ion are comparable.

## HYPERALGESIA - THE CONDITION AND ITS CAUSES

Hyperalgesia is a condition where a person develops an **increased sensitivity to pain**. Both peripheral and central mechanisms play a role in the processing of primary afferent input that makes the transition from the normal signalling of unpleasant sensory experience to a hyperalgesic state. The two general classifications of hyperalgesia as an intensification of pain sensation that is associated with tissue damage (1) occurring at the site of injury (primary hyperalgesia) and (2) occurring in undamaged tissue adjacent to and extending some distance from the site of injury (secondary hyperalgesia). We will be focussing on the primary condition in this project.

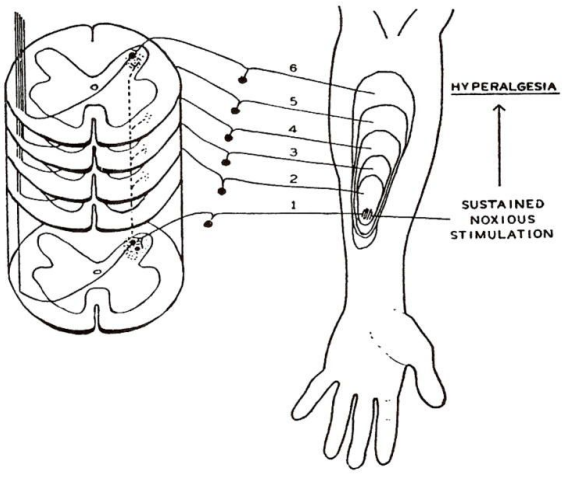



***Fig 6****: Pictorial Depiction of Hyperalgesia. This shows the nerve connection from the affected region (hand) to the spinal cord.*

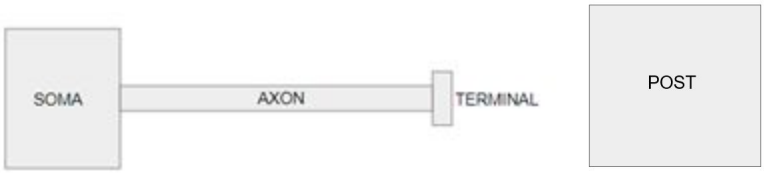
Not only are a greater number of neurons participating in the state of increased excitation, but the primary input intensifies the subsequent output to higher brain sites through prolonged, sustained activation of the spinal neurons involved through cellular mechanisms that we are only beginning to define.

In this condition, higher levels of Ca2+ are uptaken by the presynaptic terminal and they in turn bind to larger number of synaptic clefts thereby releasing a higher number of neurotransmitters (in this case, glutamate) into the synaptic cleft. The concentration of glutamate messenger which is involved in the Glutamate-Glutamine synthesis cycle (happens near the synaptic cleft) is increased. Hence, for the same number of AMPA/NMDA receptors in the postsynaptic cell, the amount of neurotransmitters present is higher. This results in higher or a prolonged stimulus to the second neuron. This neuron, in turn, communicates to the brain and identifies this area of excess stimulus as higher levels of pain. This results in hypersensitivity to a given injury, even though its response should have been much lesser than what is interpreted by the brain.

In the following sections, we will model this condition using the **NEURON** application which is a flexible and powerful simulator of neurons and networks with an easy GUI. We can specify the model of the neuron with various passive properties, active properties, ion channels, calcium channels and mechanisms, excitatory and inhibitory synapses, networks with multiple neurons, etc.

# MODELLING OF HYPERALGESIA

This is a simple model which consists of a presynaptic neuron with a soma, an axon and a presynaptic terminal, and a postsynaptic neuron. These parts are compartmentalised (cylindrical) in the model and the passive properties of each compartment are specified.




**Presynaptic neuron -**

1. **Soma** - (to avoid complexity, we have assumed that this compartment supports the passive transmission of signals only)
    a. Diameter = 100 um
    b. Length = 50 um
    c. Membrane Capacitance = 1.5 uF
    d. Axial Resistance = 100 ohm
    e. Resting Membrane potential of the compartment = -65 V
    f. Passive conductance = 1/25000 S
2. **Axon** - (to avoid complexity, we have assumed that this compartment supports the passive transmission of signals only)
    a. Diameter = 5 um
    b. Length = 2500 um
    c. Membrane Capacitance = 1 uF
    d. Axial Resistance = 100 ohm
    e. Resting Membrane potential of the compartment = -65 V
    f. Passive conductance = 1/30000 S
    g. Since the length of the axon is large, it needs to be segmented so that the signal that is being transported does not attenuate much.
3. **Presynaptic terminal** - (to avoid complexity, we have assumed that this compartment supports only calcium ion channel which operates based on the amount of signal that reaches the terminal.)
    a. Diameter = 10 um
    b. Length = 5 um
    c. Membrane Capacitance = 1 uF
    d. Axial Resistance = 100 ohm
    e. Calcium ion channel has been inserted

**Postsynaptic neuron -**

1. There is only 1 compartment for the sake of simplicity. The dimensions and properties of this compartment are enlisted below.
    a. Diameter = 100 um
    b. Length = 50 um
    c. Membrane Capacitance = 1 uF
    d. Axial Resistance = 100 ohm
    e. Resting Membrane potential of the compartment = -65 V
    f. Passive conductance = 1/30000 S
    g. Sodium ion channel is present to support Action Potential Propagation
        i. Reversal Potential = 50 V
        ii. Conductance = 0.05 S
    h. Potassium ion channel is present to support Action Potential Propagation
        i. Reversal Potential = -90 V
        ii. Conductance = 0.06 S

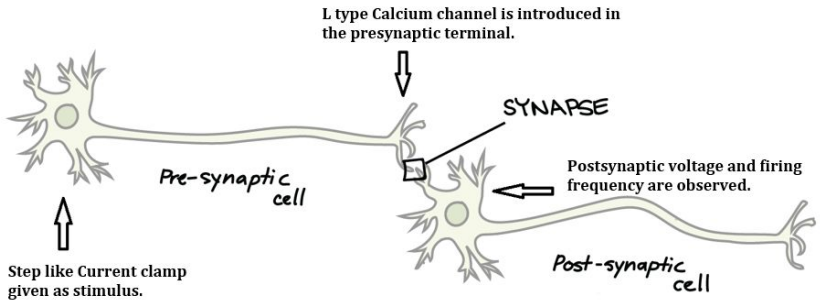



***Fig 7:*** *Structure of the model of this project indicating the input – Current clamp at presynaptic soma, output – Postsynaptic voltage, Instantaneous firing frequency of the postsynaptic cell. The figure also mentions that the L-type calcium channel was introduced at the terminal of the presynaptic neuron.*

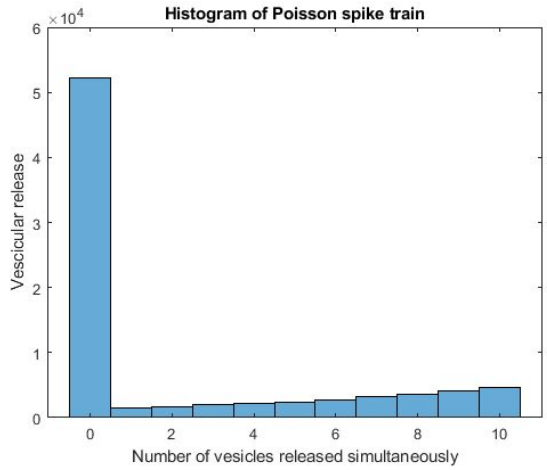
The release of vesicles, though supported by the influx of Calcium ions are pretty random in nature. To account for this stochasticity, the release of vesicles is modelled through the **Poisson generation** for vesicular release.

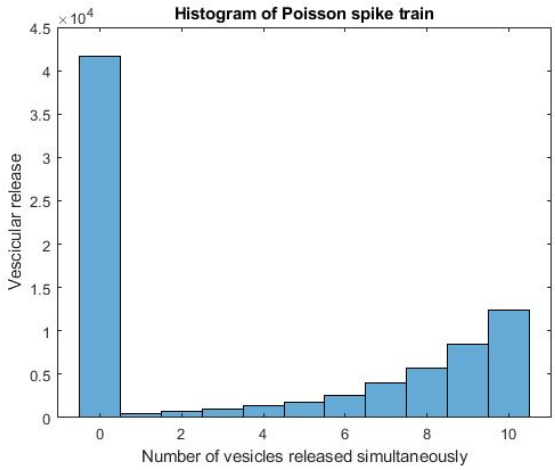
There are model files for all ion channels and synapse encoding is taken from Sense Lab, Yale University. These files have kinetic parameters, the equations for underlying transport phenomena such as the Nernst equation, the activation and inactivation of channels, etc.

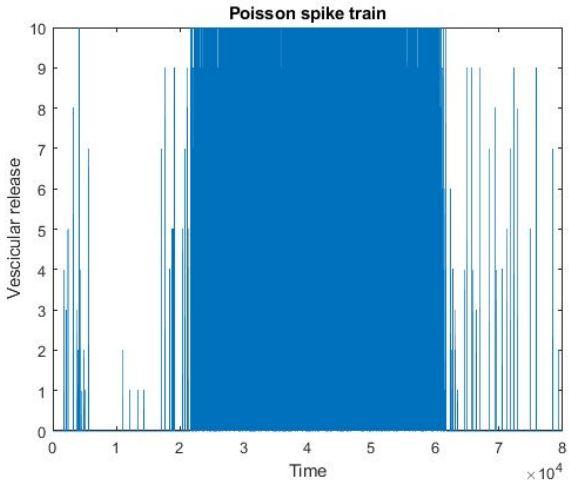
The input stimulus to the presynaptic cell is given by a current clamp at its soma. This will give sufficient input for our modelling system.

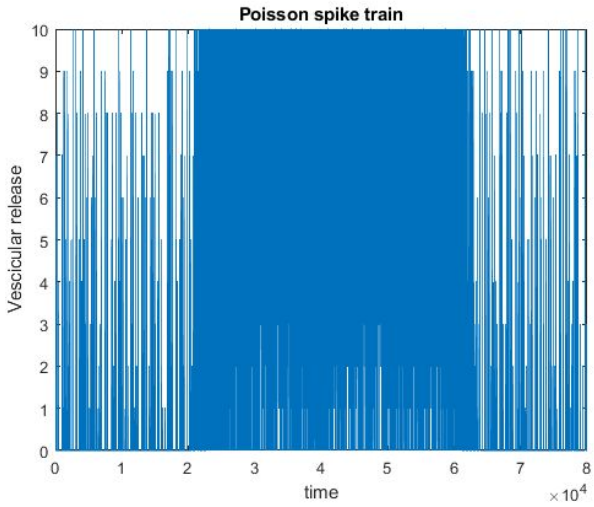
## RESULTS AND INFERENCES

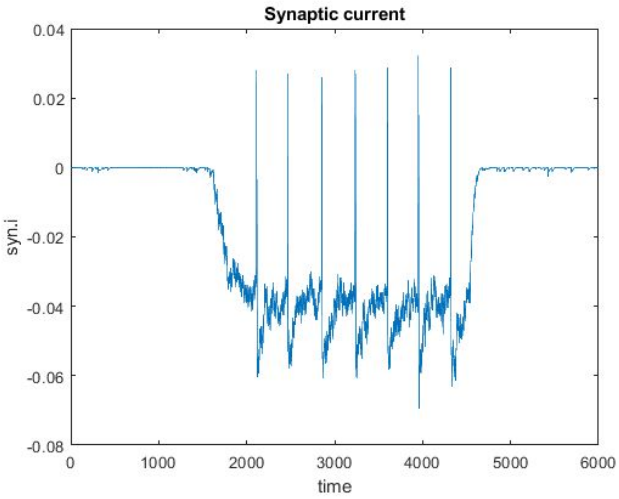
The following plots will illustrate the difference in normal and hyperalgesic conditions.
(Left: **Normal**, Right: **Hyperalgesia**)

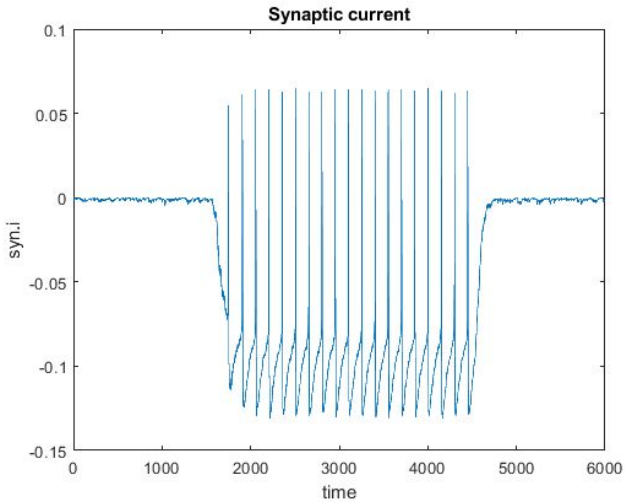


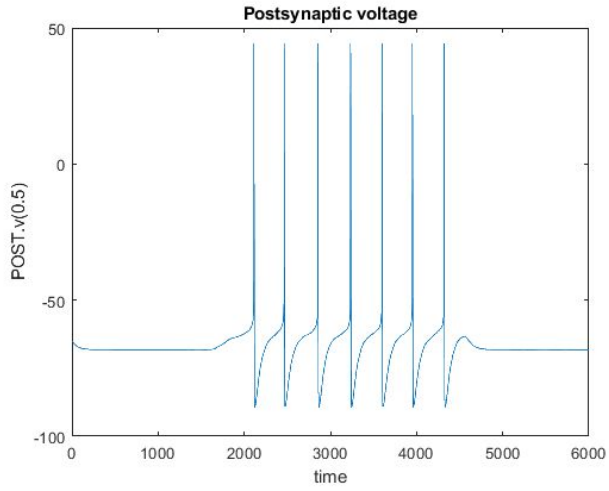



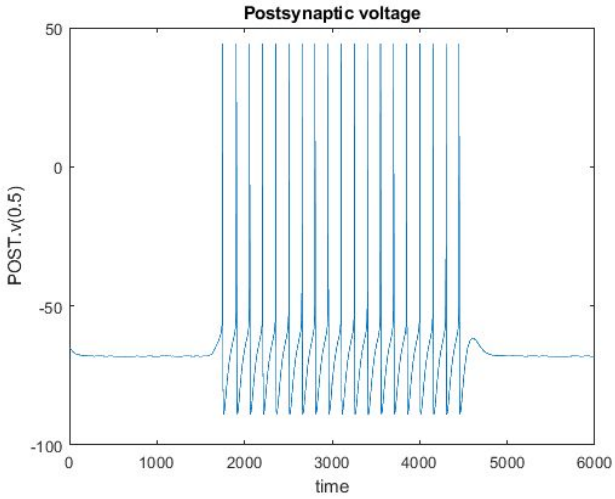






It is quite evident from the above images that postsynaptic response is amplified in the case of Hyperalgesia which results in increased sensitivity to pain.

**Treatment for Hyperalgesia:**

- Drugs that can block the receptors in the brain and spinal cord. For example -
  - Buprenorphine
  - Ketamine.
- A muscle or nerve block, which uses a local anaesthetic to numb or delay painful nerve impulses.

Most of the treatment procedures are based on a trial-and-error approach with frequent adjustments based on patient's response.

## FUTURE DIRECTIONS

It is very important to understand that the model we've built is a very basic one. It could further encompass a lot of details such as involving exact parameters from the respective positions of the neuron and not a generalised model file. We could as well collect data from multiple people and test their correspondence with our model. We could further build a network that captures all the individual parts involved - inflamed/injured region -> spinal cord -> section of the brain.

It is important to also analyse how the abnormality in Ca2+ ion channel arises.

## ACKNOWLEDGEMENTS

I would like to thank Prof. G K Suraishkumar for giving me the opportunity to perform this exercise as part of his Transport Phenomena in Biological Systems course. This exercise has helped me understand and analyse how ions travel within our body and how very small changes could have huge impact factors. I would also like to thank Prof. Rishikesh Narayanan who helped me learn the basics of the NEURON program.

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All simulations were done using the software NEURON.
Graphs from the Result section was plotted using MATLAB.