SUMMER RESEARCH INTERNSHIP REPORT

On

COMPUTATIONAL CELLULAR NEUROPHYSIOLOGY

Under the supervision of

Dr Rishikesh Narayanan

Molecular Biophysics Unit

Indian Institute of Science, Bangalore

Submitted by

Sahana G

BE17B038

Department of Biotechnology

Indian Institute of Technology, Madras

Chennai 600 036

Duration- May 22, 2019 to July 22, 2019

CONTENTS

ACKNOWLEDGEMENTS

I'd like to thank all the members of the Cellular Neurophysiology Lab for their help and support throughout my stay there. Special mentions to Prof Rishikesh Narayanan for his time, the resources and knowledge imparted, and mainly for accepting my internship request.

THEORY

INTRODUCTION

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 generate 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. One way to elicit an action potential is to pass electrical current across the membrane of the neuron. Usually this current is stimulated by receptor or synaptic potentials. If the current stimulated in this fashion makes the membrane potential more negative (**hyperpolarization**), the membrane potential simply changes in proportion to the magnitude of the injected current. Such hyperpolarizing responses are a result of the passive electrical responses in the neuron. When current of the opposite polarity is delivered, so that the membrane potential of the nerve cell becomes more positive than the resting potential (**depolarization**), something different and unique occurs. The minimum level of membrane potential to elicit an AP is called the **threshold potential**. The Action Potential (AP) is an all-or-none event, because they either occur fully or not at all. With sufficient increase in the amplitude or duration of the stimulus current, multiple action potentials occur. Therefore, we can conclude that the intensity of stimulus is encoded I the frequency of action potentials rather than in their amplitude.

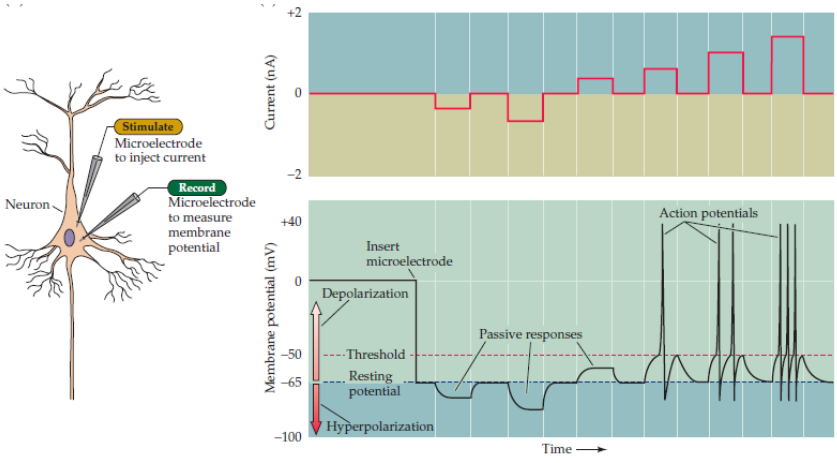


Figure explaining Action Potential – Purves, Neuroscience.

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 membrane, depends on proteins called **active transporters**, which as the name suggests, actively move specific ions in and out of the membrane. 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.

The electrical potential generated across the membrane at electrochemical equilibrium, the **equilibrium potential**, can be predicted by a simple formula called the **Nernst equation**.

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

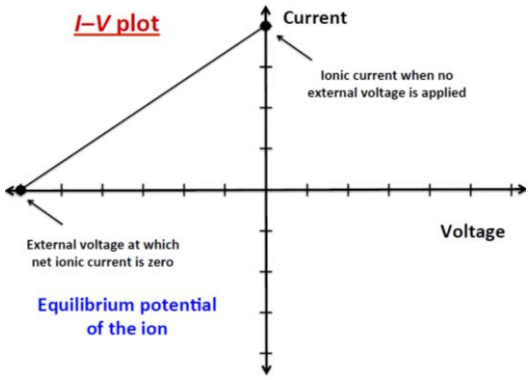
R: Gas Constant=1.98 cal/K-mol
T: Absolute temperature
z: Valence of ion
F: Faraday's constant=96480 C/mol

However, since the permeability of the ions are not taken into action, David Goldman, came up with another equation, termed the **Goldman equation** which looks like the following. For the case most relevant to neurons, in which K+, Na+, and Cl- are the primary permeable ions, 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 Ohms law and appears to take this form.



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

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

However, the relationship is not always linear and is dependent on a lot of other parameters. In such case, the following equation – **Goldman-Hodgkin-Katz (GHK) current equation**, will give us the current-voltage relationship.

$$I = \frac{Pz^2VF^2}{RT}\left(\frac{[C]_{in} - [C]_{out}\exp\left(-\frac{zVF}{RT}\right)}{1-\exp\left(-\frac{zVF}{RT}\right)}\right)$$

It is also noted that K+ leak channels are abundant on the cell membranes, causing higher permeability to K+ ions even when the cell's membrane potential is at RMP. This high K+ permeability, makes RMP close to K+ ion's equilibrium potential.

TYPES OF VOLTAGE CLAMPINGS

The **voltage clamp** is a technique used to control the voltage across the membrane of a small or iso-potential area of a nerve cell by an electronic feedback circuit. In this setup, electrode currents are injected into the cell by an intracellular electrode. Implications – Positive electrode current depolarize the cell. Negative electrode current hyperpolarizes the cell. By this technique, membrane potential is maintained. The procedure/ mechanism of Voltage clamp is explained in the figure that follows.

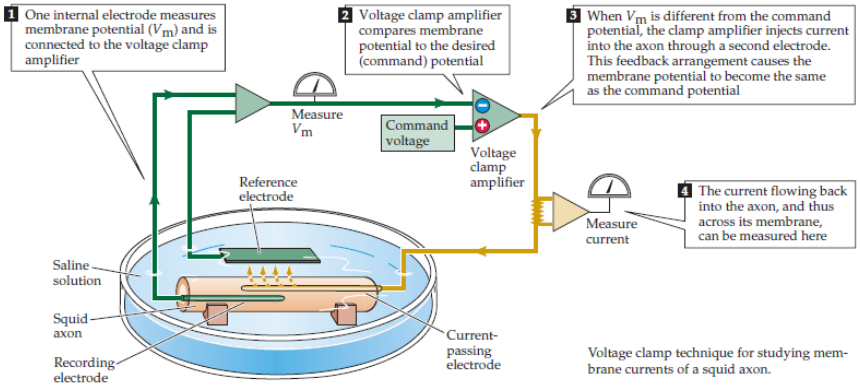


Figure explaining Voltage Clamp– Purves, Neuroscience.

A similar method with higher resolution, enough to measure electrical currents flowing through single ion channel, is called the **Patch Clamp** technique. This is a type of intracellular recording that relies on attaching a flat open tip of a glass pipette to the outer membrane of a single cell by means of suction and then recording the activities of ion channels on the membrane. There are various types of Patch clamping.

- Whole cell recording – Due to the suction applied, the membrane is ruptured and the cytoplasm is continuous with pipette interior.
- Inside-out recording – Due to the suction applied, a small section of the membrane is sucked out with the pipette. The (former) intracellular surrounding is now exposed out. This is particularly useful when studying the influence of intracellular molecules on the ion channel functions.
- Outside-out recording - Due to the suction applied, a small section of the membrane is sucked out with the pipette. The (former) extracellular surrounding is now exposed out. This is particularly useful when studying the influence of extracellular molecules on the ion channel functions.

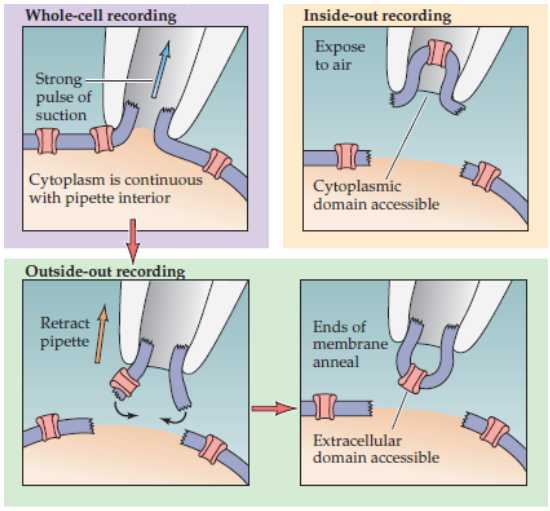


Figure explaining Patch Clamp techniques– Purves, Neuroscience.

THEORETICAL AND COMPUTATIONAL BASIS TO ELECTRPHYSIOLOGY

IONIC CURRENT AND CHARACTERISTICS

As mentioned earlier, the sodium ion concentration is higher outside the cell and potassium ion concentration is higher on the inside. When positive ions enter the cell, the cell depolarizes and when the membrane potential crosses the threshold, cell elicits action potentials. Initially the cell membrane is highly permeable to K+ ions and hence the cell is at RMP. When the permeability coefficient shifts temporarily towards Na+ ions, the membrane potential is increased to a positive value and then falls back to the resting membrane potential. This is what happens within a neuron when action potential is elicited.

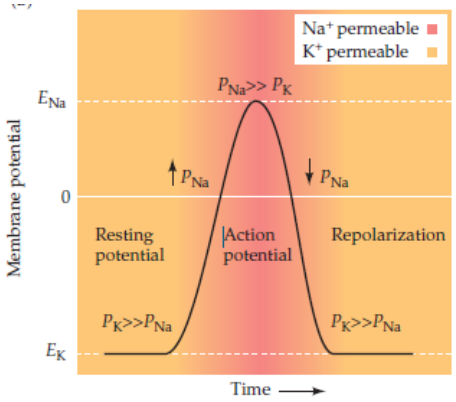
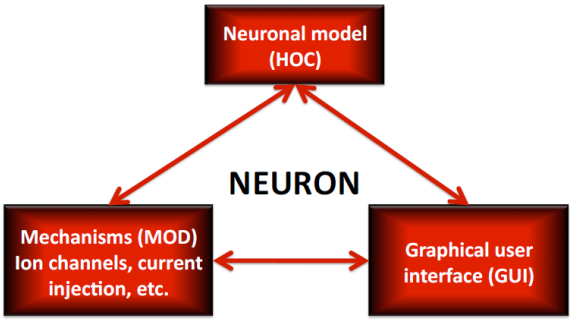


Figure explaining ionic basis of Action Potential – Purves, Neuroscience.

COMPUTATIONAL BASICS

Neuron 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. We can also specify the requisite step size and thereby choose specific integration mechanisms without worrying about how that's implemented.

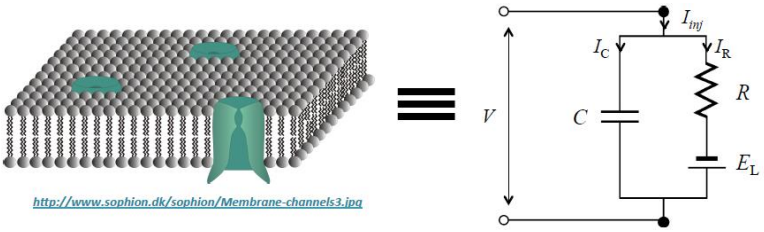


The most common methods for obtaining numerical solutions to differential equations in NEURON are by Forward Euler method, Backward Euler method and Crank-Nicholson method. In general, small the step size, better the accuracy. But if the step size is extremely small, the computation will go on forever and hence this is a trade-off between time and accuracy. Larger step sizes cause oscillations in the solution and this will constitute a form of numerical instability.

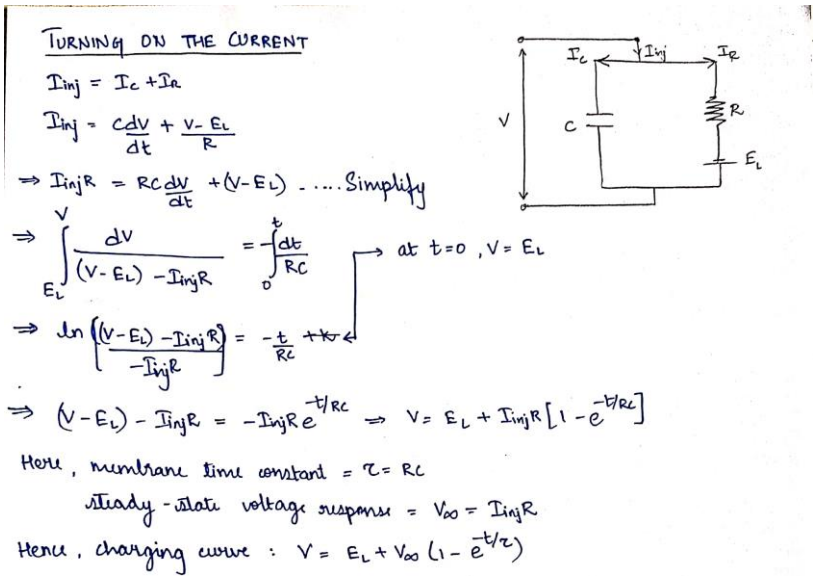
To understand the basics better, I was given a basic **assignment on Basic Programming with NEURON**. The questions primarily focused on usage of vectors and its in-built functions, reading from and writing on-to a file, sorting the elements of an array in descending order. Most of the algorithms required for-loops and other conditionals algorithms.

PASSIVE PROPERTIES OF NEURON

The membrane is considered to be a 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.



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 $t=0$, $V = (E_L + I_{inj}R)$

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

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

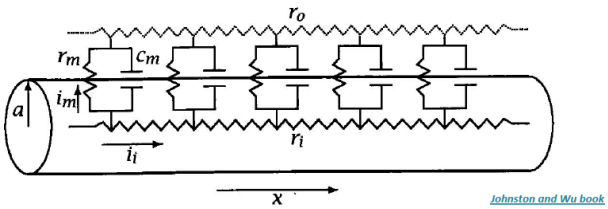
Hence, discharging curve : $V = E_L + V_\infty e^{-t/\tau}$

Initial assumptions were that the **neuron is spherical** in nature, and hence 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 constitute Rm. Important passive properties of the neuron included Rm (membrane resistance), Cm (membrane capacitance) and Rin (input resistance). Input resistance was theoretically estimated as Rm/ (Surface area of the iso-potential surface). It can also be computed as the ratio between the steady state voltage response and the injected current.

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

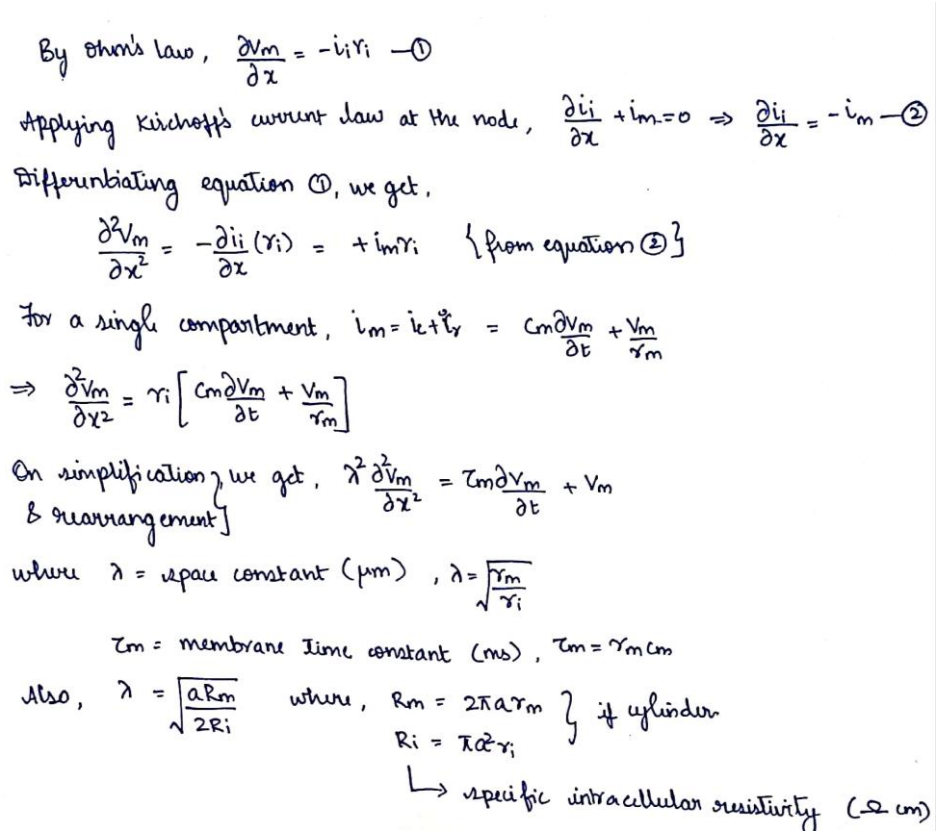
- There is no extracellular resistance.
- Membrane properties are uniform throughout the structure and is not voltage dependant, i.e., there are no voltage gated channels.
- Current flow is only through on direction and hence radial current is absent.

The propagation of the electrical signal through this cylindrical structure is given by **Cable Theory,** devised by Lord Kelvin (William Thomson) and then pioneered by Wifrid Rall, to give us a better understanding of the neuronal processes.



Johnston and Wu book

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



Hence the charging curve is no longer exponential but is now an error function. The formulae for input resistance will now depend on a few parameters and thereby formula changes. However, the charging curve and distant-dependant attenuation is same for an infinite and semi-infinite cable. But again, real dendrites are of finite length. Therefore, charging curve will be a sum of exponentials. Attenuation of voltage along the dendrite increases with frequency of the signal and it is generalised that steady state attenuation is always lesser than attenuation of time-varying signals.

To understand these properties and applications better, I was given my second **Assignment on Passive Properties.** Basic tutorial on how to create a compartment for the cell body, dendrite, assign values for various intrinsic parameters was first taught to me. The code is first written on an editor (which in my case is *Sublime – Text editor*), but is however saved as a **.hoc file,** so that when we open this file again, the GUI appears and not the code from editor.

Objective - I learnt the algorithms for Current Clamp on various compartments, Voltage clamp, how to record the Voltage attenuation along the length of the compartment and how to design a piston model and apply the above technique and analyse from that model.

Basic Algorithm –

Creating a compartment –

```
create soma // Creating a compartment
access soma // Accessing it to specify other intrinsic properties.
soma {
      diam = 100 // diameter. in micro meters
      L = 50 // length. in micro meters
      Ra = 100 // axial resistivity. In ohm.cm
      cm = 1 // membrane capacitance. in micro Farads
      insert pas // Inserting passive (leak) channels into the compartment
```

```
        e_pas = -65 // resting membrane potential. In milli volts
        g_pas = 1/30000 // leak conductance. in (ohm cm2)^-1
}
```

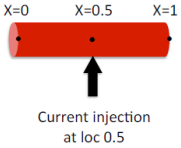
Note - Compartment in a NEURON is always a cylinder.

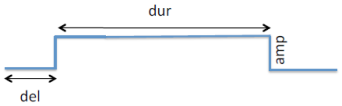
Current Clamp –

The current clamp technique is a point process, i.e., located only at a point.

```
objectvar stim
soma stim = new IClamp(0.5)

stim.del = 10
stim.dur = 700
stim.amp = 0.1
```





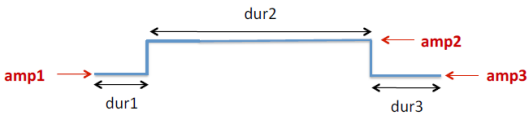
Voltage Clamp –

The voltage clamp is also a point process.

```
objref vc

vc=new SEClamp(0.5)
vc.dur1=2
vc.amp1=v_init
vc.dur2=10
vc.amp2=Vhold
vc.dur3=8
vc.amp3=v_init
```

SEClamp is a point process



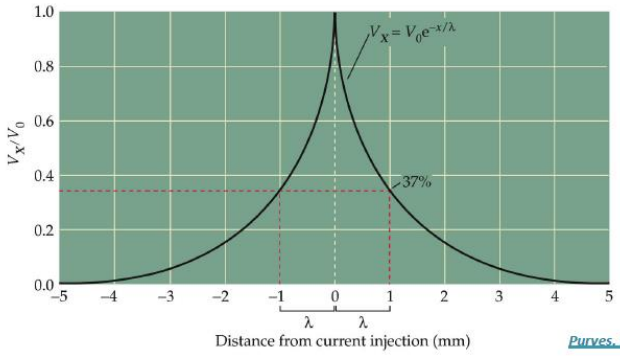
Creating a dendrite –

It is also considered as a compartment and is created the same way a soma is created. However, the dimensions would change drastically. Diameter and length of a dendrite are around 10-20 micro meters and 1000-2000 micro meters respectively. Due to its larger length, a dendrite is usually segmented by the **d-Lambda rule** and thence Cable theory.

```
dend {
    nseg=int((L/(0.1*lambda_f(100))+0.9)/2)*2+1
}
```

Recording voltage attenuation –

Due to passive properties and the presence of leak channels, voltage attenuates along the length of the compartment. Voltage attenuation is a function of space constant. When steady state voltage is plotted as a function of distance, in an infinite cable, the following graph is obtained.



Purves, Neuroscience Book

An easier approach to calculate the steady state response is by using the *minmax.mod* file. It is some sort of a pre-written function that helps us access the following variables from a voltage trace - *tmax_minmax()* {Time at which voltage is maximum}, *tmin_minmax()* {Time at which voltage is minimum} , *vmax_minmax()* {Value of maximum voltage}, *vmin_minmax()* {Value of minimum voltage}. We must include `<insert minmax>` within the compartment whose voltage is analysed.

Designing a piston model –

When a soma and a dendrite/axon is connected, we get a piston model. One can also specify the location of desired connection within the curved brackets.


```
create soma, dend

connect dend(0), soma(1)
```

Tasks from the Assignment –

Question 1

To a single passive compartment of specified dimensions, upon injection of current pulse at the centre of the compartment, I was asked to record the voltage from the same location and observe the changes in charging curve with variation in dt.

Observation

As dt increases, steady state potential decreases and the overall charging and discharging curve slows down. Also, as dt increases, time taken for completing the simulation decreases. The differential equations from the charging/discharging curve is plotted on NEURON based on Backward Euler method. The stability readings can be inferred from the theory above.

Question 2

To a single passive compartment of specified dimensions, upon injection of current pulse at the centre of the compartment, I was asked to record the voltage response and hence compute Input resistance. Variations in input resistance upon changing various intrinsic parameters like Rm, Cm, diameter and length of the compartment were also analysed.

Observation

As membrane resistance increases, for the same injected current, by Ohms Law Voltage increases. Hence the voltage deflection increases, thereby increasing input resistance. Cm affects only the membrane time

constant. The only effect due to membrane time constant is the delay in reaching the steady state voltage. The voltage itself, remains unaffected. With increase in diameter, the injected current should traverse a larger surface area. Hence Voltage decreases and subsequently Input resistance decreases. With increase in length, surface area increases, and hence input resistance decreases (as $Rin = Rm/\pi dL$).

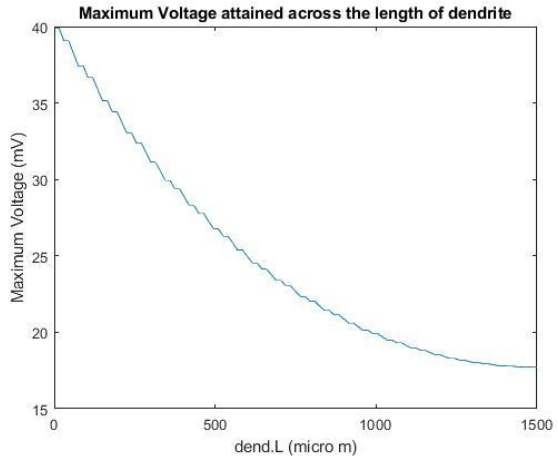
When Input resistance was computed theoretically assuming the compartment to be an iso-potential surface, Rin = Rm/Surface area. The theoretical and experimental value of Rin were comparable and followed the same trend.

Question 3

To a single passive dendrite of specified dimensions and appropriate compartmentalization, upon injection of current pulse at one end of the dendrite, I was asked to record voltage response and plot the stead state voltage response as a function of length of dendrite. Variations in the steady state voltage attenuation upon changing various intrinsic parameter like Rm, Ra, Cm, length and diameter of the compartment were analysed.

Observation

Steady state voltage attenuation was observed. For explanation, refer theory above. Since, voltage attenuation is solely responsible by space constant and that in turn depends on the Rm (direct), Ra (inverse), diameter (direct), and not on Cm, voltage attenuation is affected correspondingly by the above parameters. Increasing the length would mean that the current will now have to traverse a larger distance. Hence it attenuates more. Also, as Electronic length (L = l/space constant) increases, meaning lesser segmentation, potential decreases less with distance, meaning lesser attenuation.



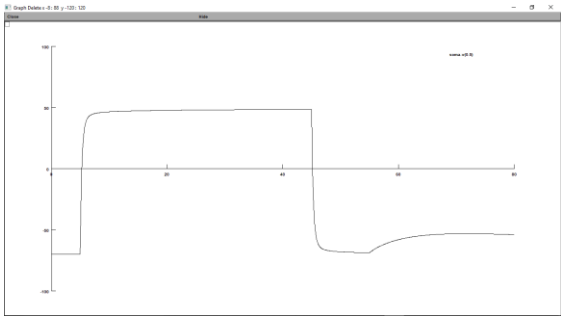
Question 4

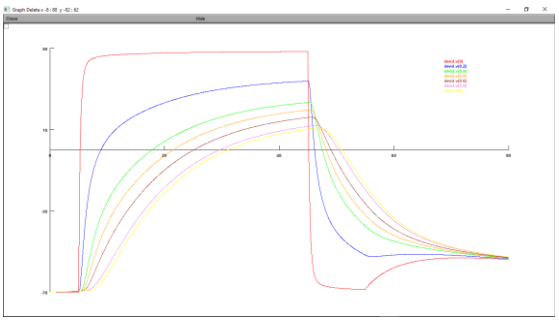
To a single passive somatic compartment, a dendrite was connected and compartmentalised using d-Lambda rule. Dimensions and Parameters of the compartments were specified. The somatic compartment was then voltage clamped. I was asked to analyse the voltage traces at soma, and various points along the dendrite and then comment on the maximum voltage attained across the length of the dendrite. Also, upon variations in Rm, Ra, Cm, diameter and Length, the distance-dependence curve was plotted and analysed.

Observation -

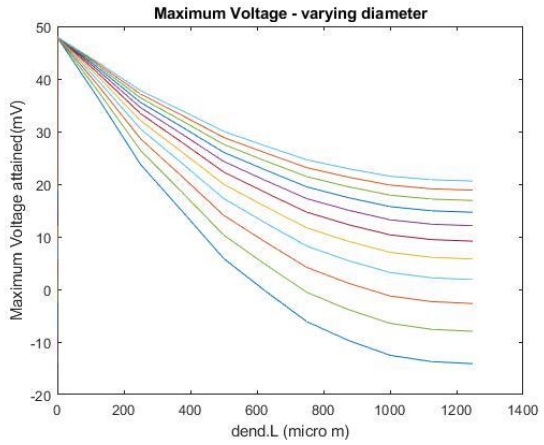
Voltage trace at the soma was expected to be a sharp step pulse, but instead turned out to be a trace with sudden changes at the start and end of the pulse. This is possibly due to the capacitive current that induces and increase in voltage across the membrane.

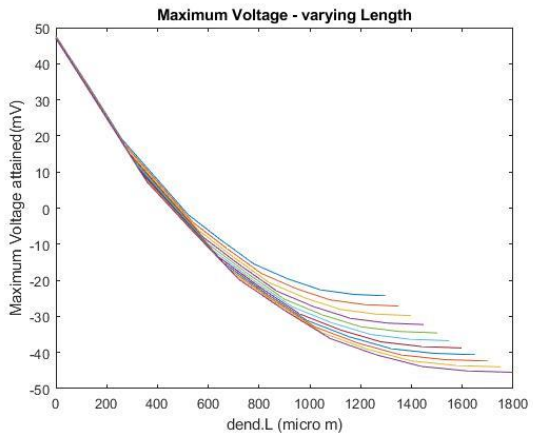
The voltage clamping did not cover the entire compartment; in fact voltage at dend(0) was not equal to the holding voltage. This is due to attenuation along the compartments, also referred to as poor space clamping.





Change in passive properties (Rm/Ra/Cm) did not vary the plot distinctively. As diameter increases, surface area that the current should traverse increases leading to lesser current reaching distant parts. Hence, maximum voltage recorded drops. Increasing the length would mean that the current will now have to traverse a larger distance. Hence it attenuates more.





ACTIVE PROPERTIES OF NEURON

The action potential is basically an output of consequence 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 outward direction (positive current).

Closed state $\underset{\beta_n(V)}{\overset{\alpha_n(V)}{\rightleftharpoons}}$ Open state

$\frac{dn}{dt} = (1-n)\alpha_n(V) - n\beta_n(V)$; or

$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)}$ where $n_\infty = \frac{\alpha_n(V)}{\alpha_n(V)+\beta_n(V)}$; $\tau_n(V) = \frac{1}{\alpha_n(V)+\beta_n(V)}$

$\Rightarrow n(t) = n_\infty(V) + \left[(n_0(V) - n_\infty(V))\, e^{-t/\tau_n(V)}\right]$

Hence we expect : $g_K(t) = \bar{g}_K \cdot n(t)$

But this does not fit the traces !!! , However , $g_K(t) = \bar{g}_K \cdot n^4(t)$ does !!

Hence , $I_K(t) = \bar{g}_K n^4(t) \cdot (V - E_K)$

where $n \rightarrow$ activation Term.

Sodium channel is inactivating in nature. Hence, we model one gate t 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 , $g_{Na}(t) = \bar{g}_{Na} \cdot m^3(t) \cdot h(t)$

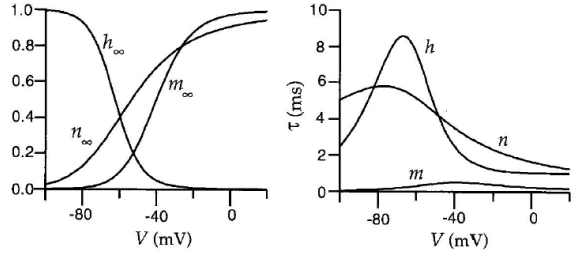
Hence , $I_{Na}(t) = \bar{g}_{Na}\, m^3(t) \cdot h(t) \cdot (V - E_{Na})$

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 basic of an action potential.

- Depolarisation is attained when the Sodium activation gate is opened.
- Influx of Na+ ions depolarize the membrane further and the action potential reaches close to the reversal of Na.
- Asvoltage increases, sodium channels inactivate and potassium channels open, hyperpolarising the mebrane.
- Loss of sodium influx accompanied by potassium efflux brings the voltage down.

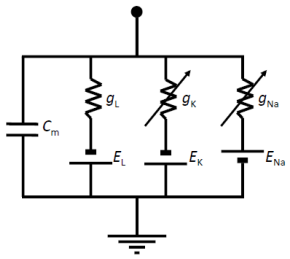
For modelling a compartment with this parallel conductance model, when Kirchhoff law is applied, we get,

$$C_m \frac{dV}{dt} = -g_L(V - E_L) - \bar{g}_{Na} m^3 h(V - E_{Na}) - \bar{g}_K n^4 (V - E_K)$$

$$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)}$$

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)}$$

$$\frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)}$$



Arrows indicate voltage-dependence

To understand these properties and applications better, I was given my third **Assignment on Active Properties.**

Objective - I learnt the algorithms for inserting Hodgkin Huxley (HH) channel, Current Clamp and voltage clamp in a compartment with active properties and how to design a multicompartmental model.

Basic Algorithm –

Inserting HH channels –

```
create soma // Creating a compartment
access soma // Accessing it to specify other intrinsic properties.
soma {
      diam = 100 // diameter. in micro meters
      L = 50 // length. in micro meters
      Ra = 100 // axial resistivity. In ohm.cm
      cm = 1 // membrane capacitance. in micro Farads
      insert hh // Inserting HH channels. Inbuilt in NEURON. And specify
various parameters that can be accessed globally.
      gnabar_hh = 0.12
      gkbar_hh = 0.036
      ena_hh = 50
      ek_hh = -90
}
```

The algorithms for IClamp and SEClamp are the same.

Designing a multicompartmental model –

Suppose we want to connect multiple dendrites to the soma at various locations, we ought to include the following code. Rest of the code remains the same.

```
create dend[ndend]
connect dend[0](0), soma(0)
connect dend[1](0), soma(1) //Connecting location (0) of dend[1] to location
(1) of soma.
```

Tasks from the assignment –

Question 1

To insert HH channels in a single compartment and plot the f-I curve and analyse the trace by varying gnabar_hh, gkbar_hh, Rm, Cm, Ena, Ek, diameter and temperature of the compartment and temperature at which the simulations ran.

Observation

Since injected current is depolarising in nature, as current increases, firing frequency increases.

As gnabar_hh increases, Na+ conductance increases, and hence more depolarisation and higher firing rate. As gkbar_hh increases, K+ conductance increases and hence more hyperpolarisation and lower firing rate.

As Rm increases, subthreshold voltage increases and hence faster and more spiking. As Cm increases, conduction velocity is decreased and hence lesser firing. Slower charging of the analogous capacitor implies sodium channels are inactivated before reaching threshold thereby making the system to take more time to reach threshold.

Increase in the reversal potentials of both the ion channels increase firing rate. Increasing Ena means increase in driving force for Na+ ions, and that leads to higher depolarization. when you set Ek to be a certain value, your RMP will move towards that value. As you hyperpolarize Ek, you are hyperpolarizing the membrane, and therefore it becomes difficult to fire for the same current injection. Whereas, when you depolarize it, you are spontaneously activating Na channels and therefore you fire spontaneously.

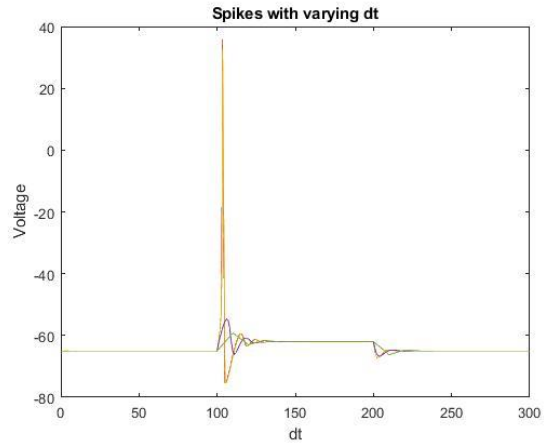
As diameter increases, input resistance decreases ($Rin=Rm/\pi dL$), thereby reducing firing rate. The kinetics of channels become faster with increase in temperature. So, with initial increase in temperatures you have the AP becoming thinner. At very higher temperatures, the reason for no action potentials is that the inactivation time constant also gets faster (that, along with faster potassium channel kinetics, is the reason the AP width becomes shorter in the first place), and at higher temperatures, the inactivation is faster than the activation, allowing no action potential to fire.

Question 2

I was asked to record the voltage from a neuronal compartment and observe the changes in charging curve with variation in dt (increase and decrease) and also compare the observations from this question with observations from Question 1 in the first assignment.

Observation

As dt increases, by equation, m (activation term – Na+), h (inactivation tern – Na+), n (activation term – K+) decreases. Sodium ion conductance is related by m^3*h. Hence, as m decreases, m^3 decreases even further. Hence the activation level drastically decreases over the effects of inactivation. This leads to reduce in AP amplitude. But there is always a trade-off between speed and accuracy of our results with change in dt and steps_per_ms. In the active case, the dt needs to be much smaller than the passive case in the previous assignment. Because, there the fastest time constant was ~30-40 ms, and here the fastest time constant (sodium channel activation) is < 1 ms. So, you need better resolution here.



Question 3

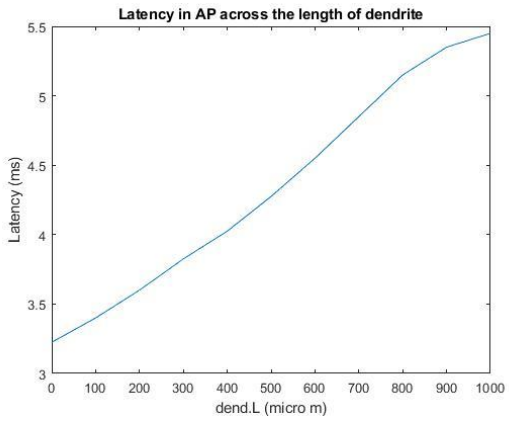
The neuronal model has 2 compartments – soma with HH channels and a long dendrite. There are two segments to this question – I was asked to initiate a single AP in the compartment and analyse (a) the latency of AP and (b) The amplitude of AP as distance from some increases with Passive and Active Propagation in the dendrite. The above procedures were repeated with change in intrinsic parameters of both the compartments. (Ra/Rm/Cm)

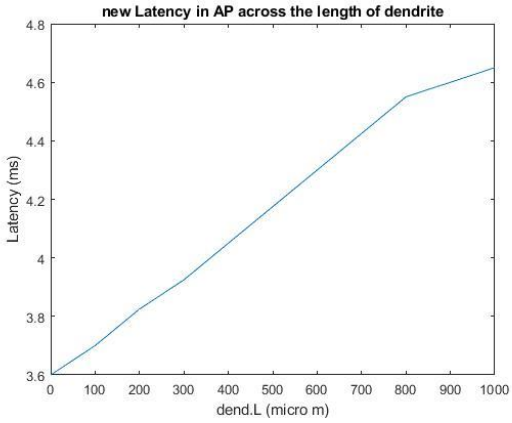
Observation – Comparative study between Passive and Active Propagation

Latency is defined as the time taken by the voltage to cross the threshold and reach its maximum from the point of injection of current. Latency is also an inverse index of neural conduction velocity.

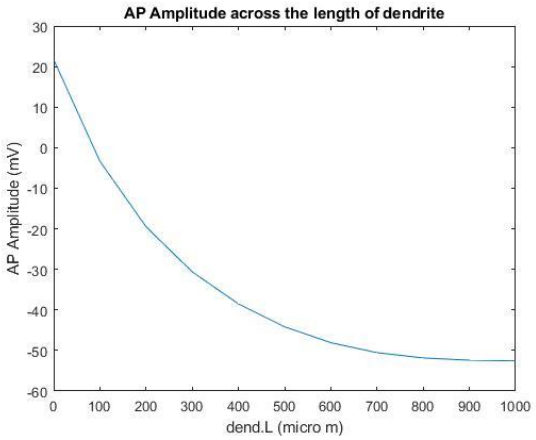
$$Velocity \quad \alpha \quad \frac{1}{C_m}\sqrt{\frac{d}{4R_mR_i}}$$

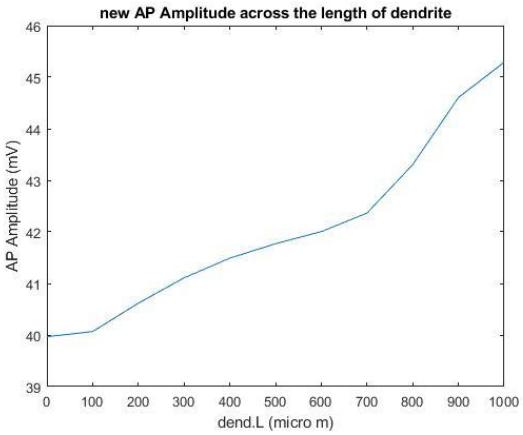
AP takes time to traverse along the dendrite and that causes latency. As, distance increases, latency increases in case of both Active and Passive propagation.





The Action potential due to current injected at the centre of the soma, gets attenuated along the dendrite as it moves forward due to presence of leak channels and hence amplitude decreases in case of Passive Propagation. However, AP Amplitude also increases due to backpropagation of signal. Due to the presence of ion channels, the action potential, now, not only undergoes passive conduction, but also propagates in both directions. Hence, when we record AP Amplitude at any instant, along the dendrite, it is the summation of forward-propagating AP and backpropagating AP.





Axial Resistance

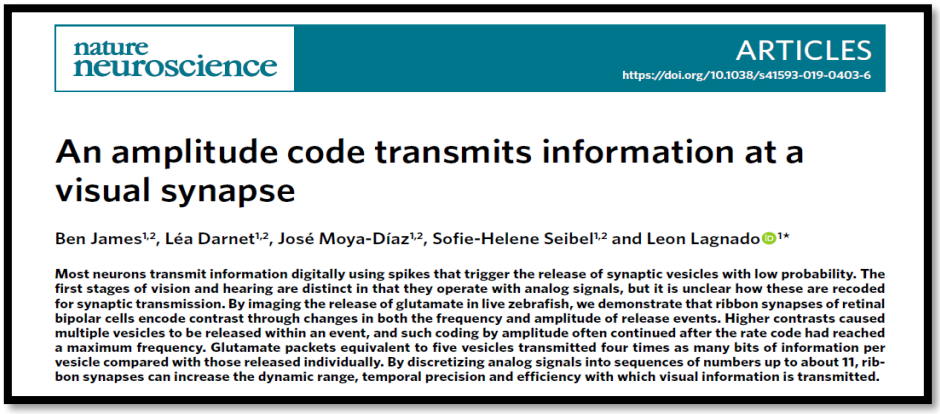
Ra of soma does not have any dependence on the above traces, possibly due to the shorter size of the compartment (diam = L = 18.8 um) in both the Passive and Active propagation case.

Passive Propagation - As Ra of dendrite increases, latency increases and then decreases due to the dynamics of voltage and attenuation accordingly. AP Amplitude increases as Ra increases due to inverse relation with length constant. *Active Propagation* - As Ra increases, space constant increases and hence attenuation increases. Therefore, latency also increases, since conduction velocity is slowed down. Also space constant also increases, which means lesser attenuation. Hence, voltage attenuation decreases, implying that AP Amplitude increases.

Similar analyses were carried out for Membrane resistance and capacitance under Passive and Active backpropagation.

LITERATURE PRESENTATION

On the second weekend of my internship period, I was asked to give a literature presentation and I chose a paper from Nature Neuroscience on **Amplitude coding at the visual synapse.**


nature neuroscience

ARTICLES

https://doi.org/10.1038/s41593-019-0403-6

An amplitude code transmits information at a visual synapse

Ben James[1,2], Léa Darnet[1,2], José Moya-Díaz[1,2], Sofie-Helene Seibel[1,2] and Leon Lagnado[1]*

Most neurons transmit information digitally using spikes that trigger the release of synaptic vesicles with low probability. The first stages of vision and hearing are distinct in that they operate with analog signals, but it is unclear how these are recoded for synaptic transmission. By imaging the release of glutamate in live zebrafish, we demonstrate that ribbon synapses of retinal bipolar cells encode contrast through changes in both the frequency and amplitude of release events. Higher contrasts caused multiple vesicles to be released within an event, and such coding by amplitude often continued after the rate code had reached a maximum frequency. Glutamate packets equivalent to five vesicles transmitted four times as many bits of information per vesicle compared with those released individually. By discretizing analog signals into sequences of numbers up to about 11, ribbon synapses can increase the dynamic range, temporal precision and efficiency with which visual information is transmitted.

The **main question** that were addressed in this paper is the means by which sensory signals re-code for transmission across a synapse.

a. A Ribbon synapse holds 10-15 vesicles together and releases them into the cleft when Ca2+ binds to it and these vesicles are transmitted to the active zone. Ribbon synapses are assumed to denote the strength of stimulus through rate code. Rate code is a result of change

in frequency of neurotransmitter/ vesicle release. Recordings show that the rate of release was 50–100 vesicles per second per active zone.

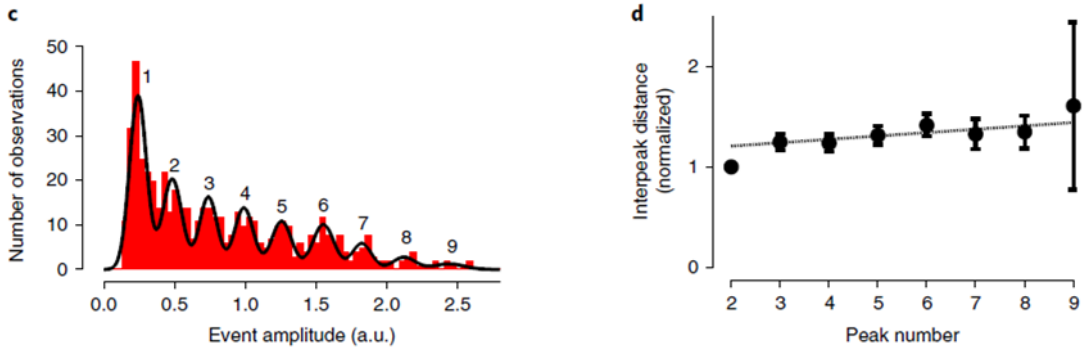
b. When these synapses are strongly activated, several vesicles are released almost simultaneously – **Multivesicular release (MVR)**. It is observed in large parts of the brain.
c. It has been observed that these releases are not binary (0/1) but instead constitute a code relating its Amplitude.

Throughout the paper, these terms were used interchangeably -
Event site = Active zone.
Number of quanta per event = Amplitude code = Number of vesicular releases.

RESULTS

1. **How do bipolar cells transmit this information across the synapse?**
   - **a.** Optical detection of MVR in vivo was first done and it was observed that a periodic stimulus (5 Hz) triggered glutamate release.
   - **b.** Line scan was run at 1 kHz, and amplitude/fluorescence was observed in active zones.
   - **c.** The time course of iGluSnFR transients were similar for events with different amplitudes.
   - d. A delF/F trace was plotted and threshold was set corresponding to 1 quanta release and the amplitude was observed. They, then plot quanta vs time and observed the frequency of the number of vesicles released from a particular active zone in a span of specified time.

2. **Does the iGluSnFR reporter provide a linear read-out of glutamate release?**
   - a. Results indicate that the iGluSnFR signal provided a linear read-out of MVR up to at least nine vesicles, and that these estimates were not skewed by saturation of the reporter.
   - b. Glutamate release events of different amplitude can therefore be considered as different synaptic symbols.



3. **Do synaptic events of varying amplitude play a role in encoding a visual stimulus?**
   - a. The Transmitter Triggered Average method of analysing (TTA) revealed that the more quanta within an event, the higher, on average, the temporal contrast driving it. Thus, MVR encodes one of the most fundamental properties of a visual stimulus, temporal contrast, in a direct way.
   - b. Second distinction between events of different amplitudes. A period of lower light intensity immediately preceding higher intensity favoured larger synaptic events through the On channel. Synchronizing the release of multiple vesicles is therefore expected to amplify the output of a bandpass filter to enhance the signalling of positive temporal contrast.

4. **Given that bipolar cells can transmit visual information as changes in both the frequency and amplitude of release events, what are the relative contributions of these two coding strategies?**
    a. First method - Quanta per cycle vs Contrast (stimulus) was plotted and observed to be a sigmoid function.
    b. Second method - Observation - Higher contrasts tended to increase both the frequency and amplitude of glutamatergic events. For n=55 synapses, Ec (Event per cycle) > Qe (Quanta per cycle) (rate dominant) and remaining n=17 synapses, Qe>Ec (amplitude dominant). They say that this change is due to each bipolar cell's individual intrinsic electrophysiological properties as well as changes in their retinal microcircuits. MVR therefore extends the range of contrasts that can be signalled beyond those allowed by the rate code alone.

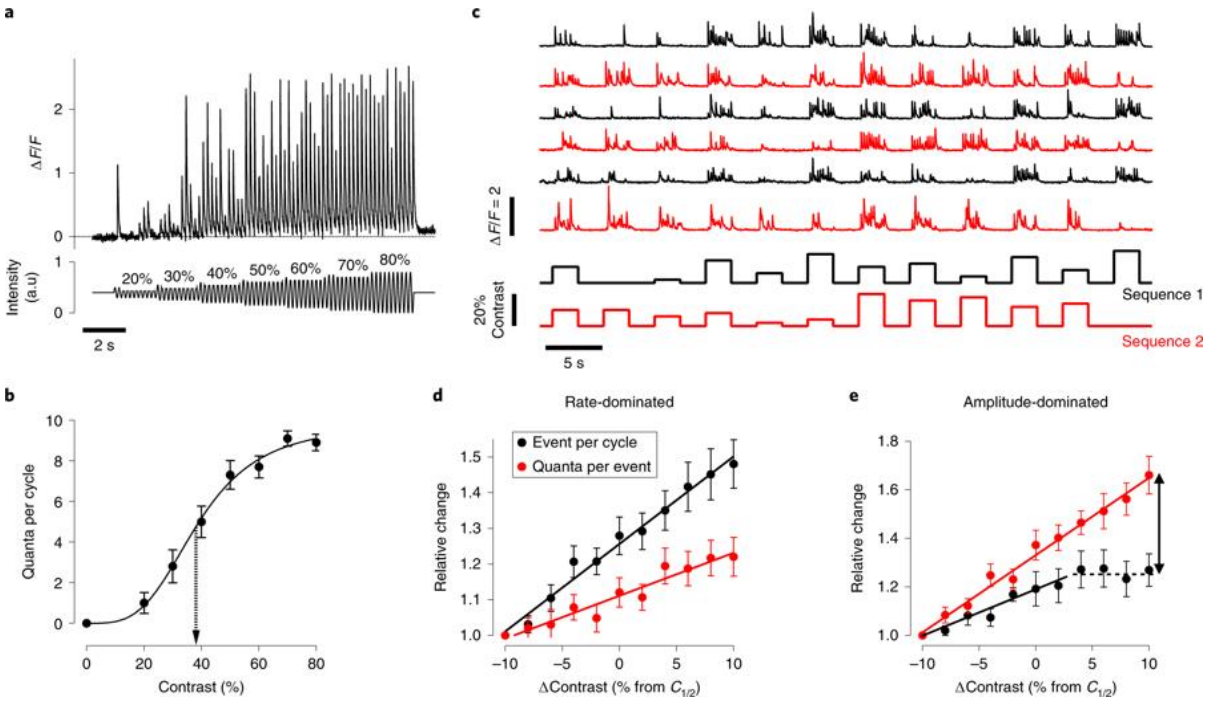
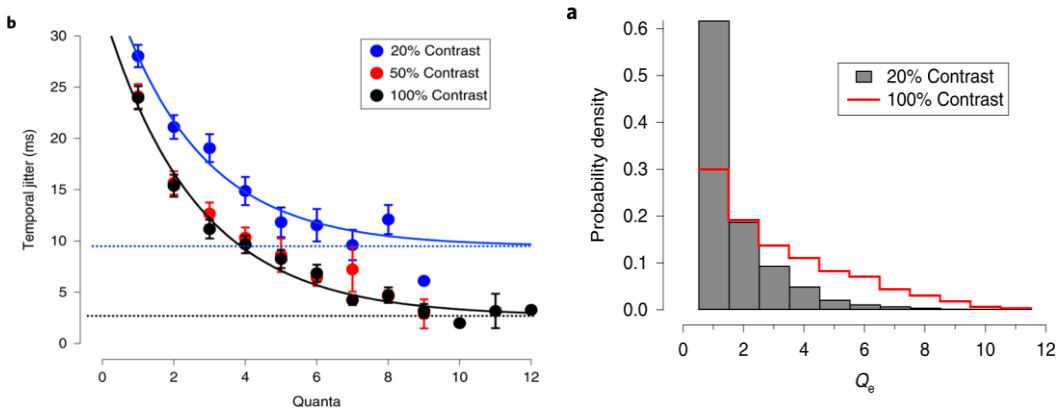


Fig – Relative contributions from Rate and Amplitude coding

5. **How does Amplitude code improve the temporal resolution of synaptic transmission?**
    a. Synaptic events of larger amplitude improved the temporal precision with which the visual signal was transmitted.
    b. Uniquantal events displayed a standard deviation ('temporal jitter') of 24–28 ms over a range of contrasts, while events composed of 7 or more quanta jittered by as little as 2.5 ms.
    c. 20% contrast contained 8 quanta with a jitter of 12.1 ± 1.4 ms, while 8 quantal events at 100% contrast jittered by just 4.6 ± 0.6 ms.



6. **Multivesicular events transmit more information per vesicle**.
    a. We measured the change in the distribution of synaptic event amplitudes at high and low contrasts. (20% and 100%) Preference of higher contrast indicated larger/higher quanta.
    b. Vesicles released individually carried an average of 0.125 bits of information, which is, as expected, significantly less than the 1–3.6 bits transmitted per spike in postsynaptic ganglion cells. Larger events transmitted progressively more information because they were rarer overall and correlated with higher contrasts rather than occurring randomly.
    c. Larger synaptic events transmitted more information per vesicle. Synaptic events composed of five vesicles carried, on average, four times as much information per vesicle compared with unitary events. Vesicle efficiency was increased.

DISCUSSIONS

This in vivo approach demonstrated that the release of two or more vesicles within a time window of 10 ms or less is a fundamental aspect of the strategy by which ribbon synapses of bipolar cells recode an analog signal for transmission across the synapse. The amplitude code complements the rate code by increasing the temporal accuracy (Fig. 5) and operating range (Fig. 4) of synapses transmitting the visual signal to retinal ganglion cells, as well as the efficiency of information transmission measured as bits per vesicle.

1. **Amplitude code at ribbon synapse** –
    a. In this context, MVR can be viewed as a mechanism by which glutamatergic vesicles are summed pre-synaptically and at one site over a time window of one time constant or less, causing them to act more effectively on the postsynaptic neuron.
    b. The efficiency of presynaptic summation depends whether postsynaptic receptors saturate or not. Example - Saturation does not appear to occur at all amacrine cells postsynaptic to rod-driven bipolar cells or auditory nerve fibres postsynaptic to hair cells, but may occur at AMPA receptors on retinal ganglion cells.

2. **Cellular Mechanisms underlying MVR**
    a. Synaptic terminals of bipolar cells in zebrafish and mice can convert the analog signal arriving down the axon into regenerative calcium spikes that cause large increases in presynaptic calcium. It is also known that strong activation of presynaptic calcium channels can release a large fraction of the readily releasable pool (RRP) of vesicles within a few milliseconds.
    b. The ionic mechanisms that generate neural signals and the synaptic processes that transmit them constitute a major energetic cost to the brain. It would be interesting to carry out an energy budget for MVR at synapses of bipolar cells where the efficiency of transmission

could be quantified as bits per unit energy, but this will require a better understanding of the underlying electrical events.

c. Variable amounts of glutamate are released from a single vesicle because of the dynamics of a fusion pore.

3. Amplitude and Rate coding at output of retina

a. For vision to be useful, large info in short time. This is possible through MVR (Also, high contrast). Moreover, MVR encoded timing of stimulus more precisely, larger glutamatergic events jittered only by a few ms.

b. To understand why a coding strategy based on amplitude might have arisen, it is useful to think about the temporal requirements of a simple rate code.

c. If at an active zone, 8 quanta are released, you'd always need a contrast >20%. Since this is not always the case, we rather introduce another symbol - Amplitude!

PROJECT

ABSTRACT

Most neurons transmit information digitally using spikes that trigger the release of synaptic vesicles with low probability. The analog calcium current, which causes the release of neurotransmitters, was discretized into stochastic synaptic release by generating Poisson spike trains. The functioning of the Poisson distribution was verified separately. We ensured that transmitter concentration equalled the summed spikes from all the active zones at each discrete time step and connecting the presynaptic and postsynaptic neuron with an AMPA synapse indicated that information was transmitted through vesicle coding. We recorded and analysed the postsynaptic voltage and firing frequency, and observed that firing frequency of the postsynaptic compartment increased as a function of the amplitude of the current stimulus given at the soma of the presynaptic neuron. We plotted the instantaneous firing rate as a function of time and observed that it resembled the input current stimulus and hence, amplitude coding was confirmed.

OBJECTIVE

To observe and verify that Amplitude coding of information is another method of neural encoding.

THEORY

The basis of the project is from the research article that I presented at the Literature presentation. Hence, the basic theory is explained above. I will briefly explain other mechanisms that have been used in the project.

L- Type Calcium Channels.

They are high-voltage activated family of voltage gated ion channels. L stands for Long-lasting, referring to the length of activation. Hence the channel stays open/activated for a longer period of time, and its conductance reaches maximum mostly at supra-threshold potentials only. When given a voltage clamp, the current is negative, as it is inward in nature.

Poisson Spike Train Generation.

For any time-varying function, r(t), with time step = dt, the Probability density function can be written as –

$$P\{1\,\text{spike during}\,\delta t\} \approx r\,\delta t$$

To include stochasticity, if this probability is lesser than a random number generated between (0,1), a spike is generated. When repeated over discrete time steps, we get a Poisson Spike Train. If the same procedure is repeated over multiple trials, and summing them up together, gives us stochastic synaptic release, considering r(t) as the Calcium current that instigates Multivesicular release.
Note – The refractory part of the procedure is ignored, assuming that the ribbon synapses are very quick in carrying out the multivesicular release.
General characteristics of a Poisson distribution are –

- The mean and variance of the Spike Count distribution should exactly be the same.
- The mean and standard deviation of the Inter-Spike-Interval's distribution should exactly be the same.

Excitatory and Inhibitory synapses

Excitatory synapses increase the possibility of post synaptic 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.

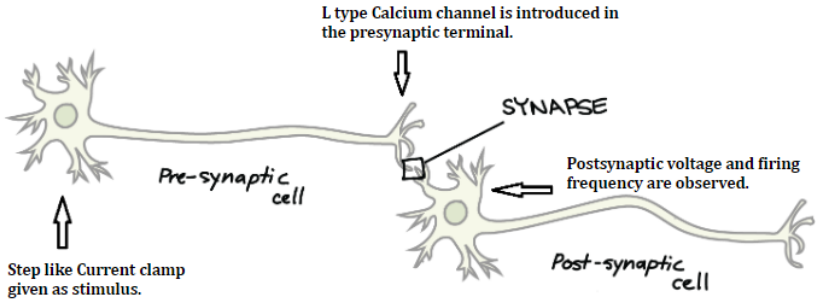
$$I(t,V) = \bar{g}\, s(t)\, (V - V_{syn})$$

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

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

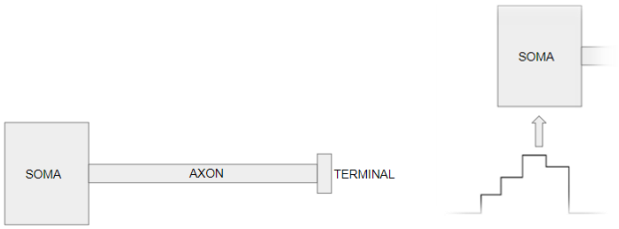
Inhibitory synapses decrease the possibility of post synaptic firing. ***GABA Synapse*** is a commonly used inhibitory synaptic receptor, which opens ion channels that are selectively permeable to Cl- and causes Cl- to flow across the postsynaptic membrane. Cl- induces hyperpolarising effect and increases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

METHOD



Step 1 – Building the presynaptic neuron.

- The presynaptic neuron has 3 compartments- Soma, Axon and a terminal.



- A step-like current clamp was given at the soma of the presynaptic neuron. This was simulated by giving multiple current clamps at different time instances.
- The voltage recordings from soma(0.5), axon(0.5) and terminal(0.5) were observed, and voltage attenuation was clearly noticed.

Step 2 – Inserting L-Type Calcium channel at the presynaptic terminal.

- Post insertion of L-type calcium channel, the Calcium current from the terminal-compartment was recorded.

Step 3 – Converting Calcium current to stochastic synaptic release.

- Ca2+ current is inward, and hence is negative in polarity. Also, the levels of Calcium current are very minimal (in orders of 10^-10). Hence to generate Poisson spikes, i.e., to have r(t) = PRE.ica(0.5)*dt, the polarity of the calcium current was first reversed and the levels were scaled to range between (0,10).
- Using Poisson Spike Train generation, the Ca2+ current (modified version) was converted into synaptic releases, incorporating stochasticity.
- We assumed that there are 10 active zones/release sites in the presynaptic terminal and hence summing up all the spike trains from each active zone indicates the overall synaptic release.

Step 4 – Building the postsynaptic neuron

- For simplicity, the postsynaptic neuron is considered as a single compartment.
- Na+ and KDR (Potassium – Delayed Rectifier) ion channels are inserted into this compartment.
- To characterize this block, a single current clamp was given (separately) at the centre of it, and the input resistance and firing frequency were observed.

Note – Characterization is not a part of the Amplitude code experiment.

Step 5 – Introduce a synaptic connection between the pre and post-synaptic neurons.

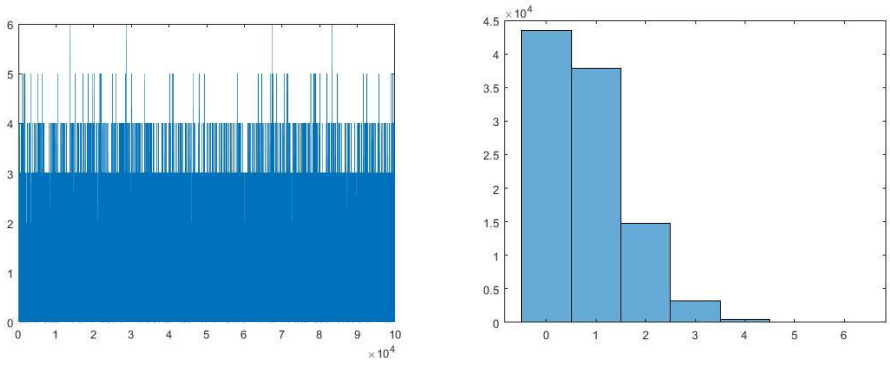
- AMPA receptors were attached to the postsynaptic compartment. It is not an ion-specific channel and hence allows the motion of both Na+ and KDR ions.
- It is also a transmitter gated receptor.
- At each discrete time step, the transmitter concentration is assigned to the summed-up vesicle release.
- The Synaptic current (syn.i) and Postsynaptic voltage (POST.v) were recorded and plotted on MATLAB.
- To ensure Amplitude coding, firing rate at the postsynaptic compartment was observed as a function of increasing current stimuli at soma of presynaptic neuron.

VERIFICATION

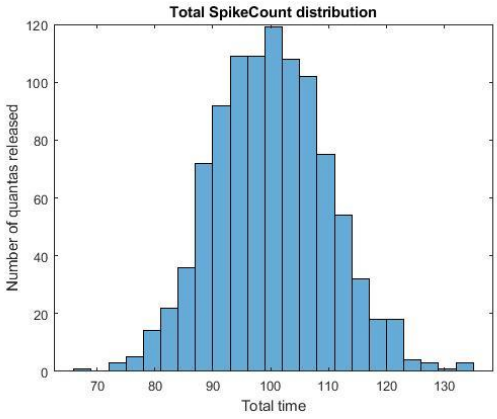
The formulae/method used for Poisson spike train generation was verified separately on MATLAB in the following ways.

1. For lower frequencies of r(t), the spike count distribution should be an exponential trace. The mean and variance of this distribution should approximately be the same.
2. For higher frequencies of r(t), the peak shifts towards the right and forms a gaussian. The mean and variance of this distribution was found to be approximately the same.
3. The trace for Inter-Spike-Interval (ISI) was an exponential in both the cases, and the mean and standard deviation of this distribution was approximately the same.

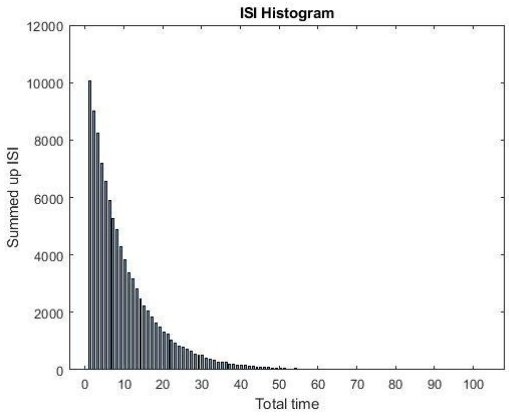
For example, consider r(t) = 80, total time = 100 seconds, and discrete time interval = 1 millisecond.



As r(t) increases, spike count histogram becomes a gaussian as you will see in the figure below.



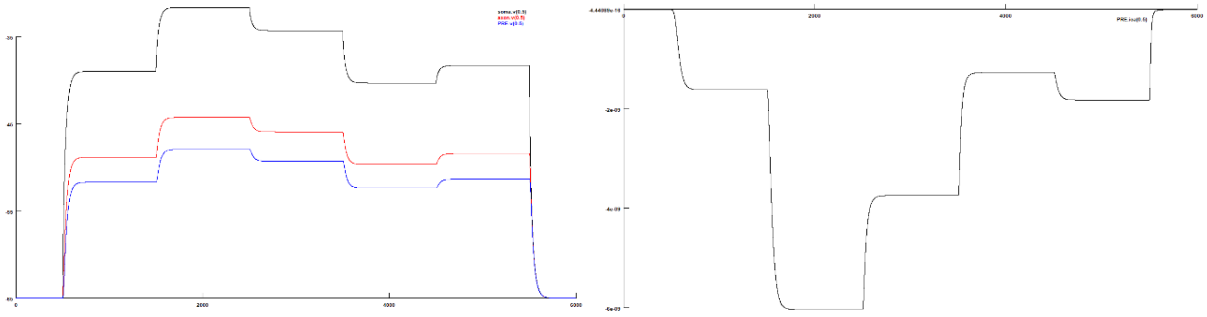
The ISI Histogram is plotted below and it has an exponential trace.



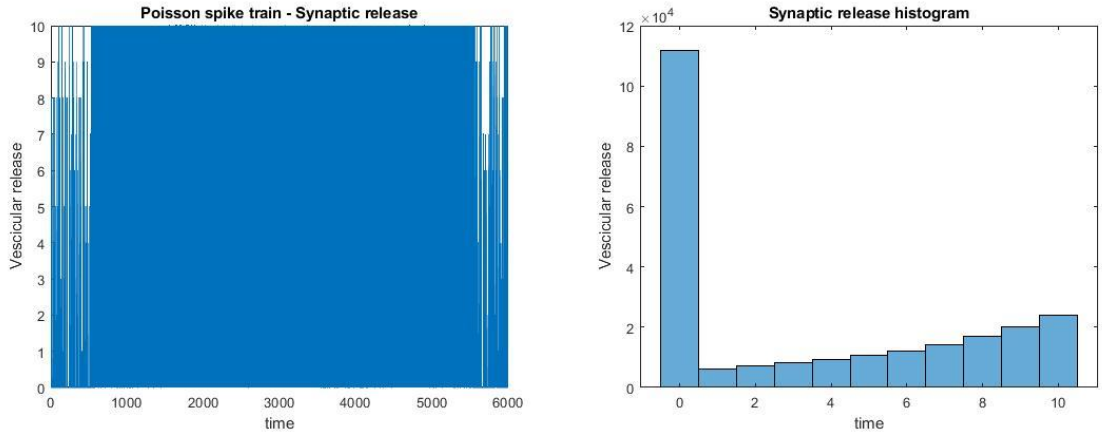
Note – Due to the stochasticity involved with the distribution, mean & variance of spike count histogram and mean & standard deviation of ISI histogram were not exactly, but approximately equal.

OBSERVATION

In correspondence to the current clamp at soma, the observed voltage traces (figure 1) and calcium current trace (figure 2) are as follows.

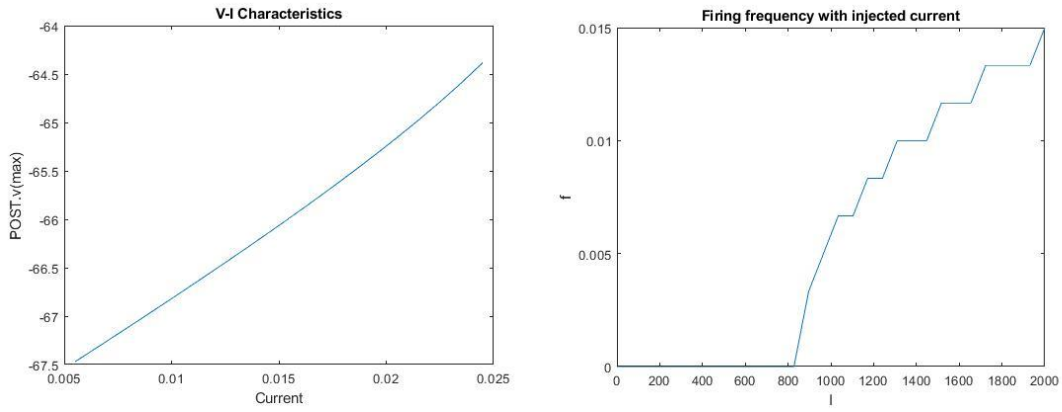


The Calcium current is then converted to stochastic synaptic release by generating Poisson Spike trains.



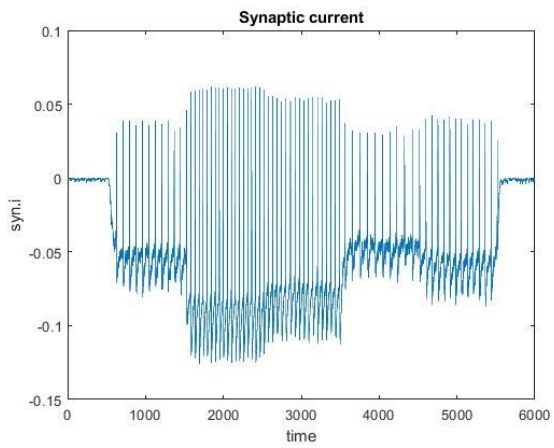
The histogram of synaptic release shows that the probability of 10 quantas being release simultaneously is greater than the release of 1 quanta. This is because of the non-homogeneous nature of Calcium current and the varying amplitude of current stimulus that affects the calcium current. Higher the calcium current, more the synaptic release.

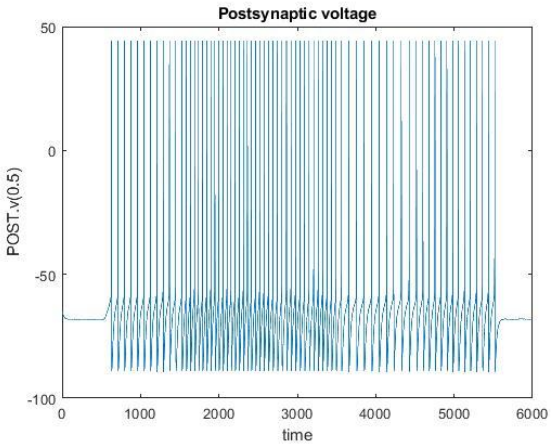
The Postsynaptic compartment was characterized separately by plotting V-I characteristics, computing Input Resistance, and the f-I curve.



The slope of the V-I characteristics = Input resistance of the compartment = 150.1856 M ohms.

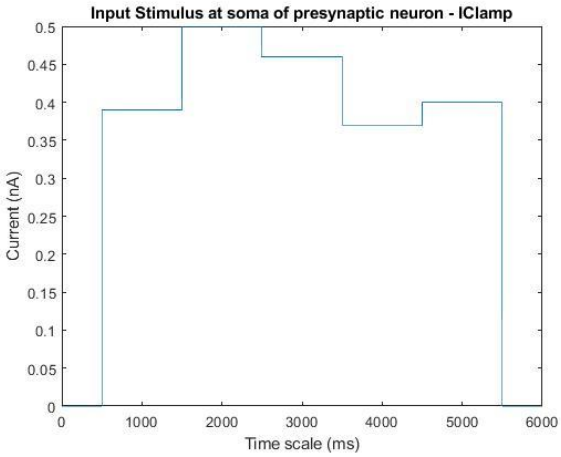
By connecting the presynaptic and postsynaptic neuron with AMPA Synapse, the synaptic current and Postsynaptic voltages were observed.

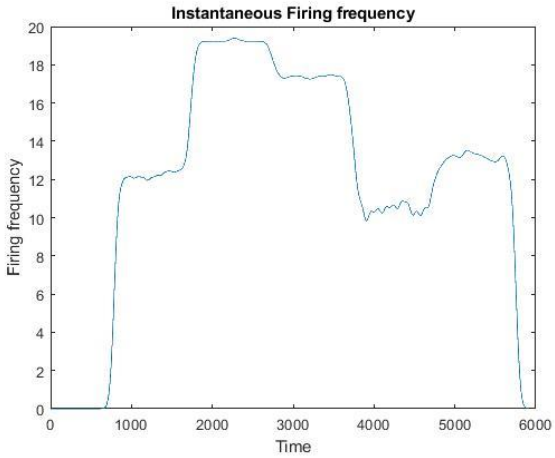




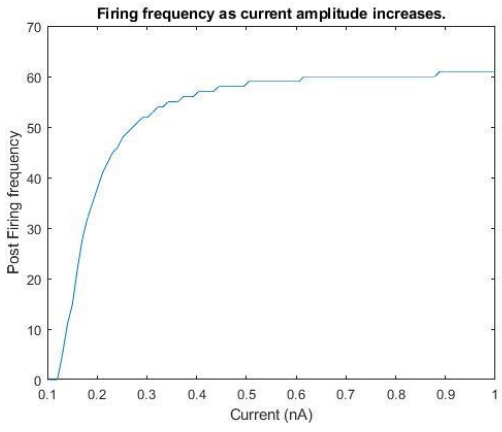
Confirmation plots –

To confirm successful Amplitude coding, the spike timings from the postsynaptic voltage plot was recorded. This was basically a binary vector of length = total time (tstop), where 1 represents the existance of a spike and 0 represents no spike. This vector was then convolved with a gaussian (smoothens the plot) to get the ***Instantaneous firing rate***. This trace was plotted alongside the input stimulus and we observed that the traces resembles each other and hence confirmed **Amplitude coding**.





We then plotted Firing frequency as a function of current stimulus (the one given at soma). The current was changed to a single pulse stimulus was increased step wise. The firing frequency of the postsynaptic cell was recorded in each run. The plot was a sigmoid function resembling the conductance plot of Calcium ion.



RESULT

The analog calcium current, which causes the release of neurotransmitters, was discretized into stochastic synaptic release by generating Poisson spike trains successfully. Amplitude coding of information was thus confirmed and verified by plotting Instantaneous firing rate and the Firing frequency of the postsynaptic cell as a function of current stimulus.

REFERENCES

1. Neuroscience – Dale Purves, George J. Augustine, David Fitzpatrick, William C. Hall, Anthony-Samuel La Mantia, James O. McNamara, S. Mark Williams.
2. Foundation of Cellular Neurophysiology – Daniel Johnston and Samuel Miao Sin Wu
3. Theoretical Neuroscience – Peter Dayan, L. F. Abbott.
4. The NEURON Guide - https://www.neuron.yale.edu/neuron/
5. An Amplitude code transmits information at the visual synapse - https://www.nature.com/articles/s41593-019-0403-6
6. Ion Channels are from - https://senselab.med.yale.edu/ModelDB/ModelList.cshtml?id=3540
7. KDR channel from CA1 pyramidal neurons for postsynaptic compartment - https://senselab.med.yale.edu/ModelDB/showModel.cshtml?model=150551&file=/AshhadNarayanan2013/na3.mod#tabs-2
8. Na3s channel from CA1 pyramidal neurons for postsynaptic compartment – https://senselab.med.yale.edu/ModelDB/showModel.cshtml?model=147538&file=/NarayananJohnston2010/na3s.mod#tabs-2
9. AMPA Receptor model file – Cellular Neurophysiology Lab, IISc.
10. L type Ca2+ channel for presynaptic terminal – Cellular Neurophysiology lab, IISc.
11. The conclusions for the assignment problems were from various websites.
    a. http://www.jneurosci.org/content/18/10/3501
    b. https://www.sciencedirect.com/science/article/pii/S0006349503750863