Chapter 3

Extracellular Conductivity and Neuronal Activity: Consequences for Nerve Signal Transmission and Neural Development

This chapter is subdivided into two sections i.e., Section 3.1 deals with the effect of extracellular conductivity on Neuronal activity and its influence on Neuronal Growth and Section 3.2 deals with the effect of Extracellular Conductivity on Nerve Signal Propagation for a larger ECS. Section 3.1 involves examining the effect of neuronal activity and how it influences neuronal growth. Furthermore, Section 3.2 examines the effects of ECS on nerve fiber of different shapes such as tapered, flared and uniform nerve fiber.

3.1 Neuronal activity and its influence on Neuronal Growth

3.1.1 Introduction

The size of the axon and myelination are the two key factors that impact the conduction velocity of neural impulse [111][112]. The process through which signal conduction takes place in a myelinated nerve fiber is known as the saltatory conduction [34][33], [36] where the propagation of action potential occurs only at the Nodes of Ranvier, or the spaces between two adjacent myelinated segments and because of which the nerve signal travels at a much faster rate.

Various physiological processes such as ageing, neuro-diseases, injury etc can result in demyelination [113]. Here, the myelin coating weakens or occasionally disappears causing the neural signal to move considerably more slowly or even there could be a loss of the neuronal signal, which leads to axonal degeneration. Myelin capacitance and axoplasm conductance have a greater influence on conduction velocity. Increased charge storage requirements, a greater time constant, and decreased saltatory conduction efficiency are the reasons why a larger myelin capacitance results in slower nerve impulse transmission.

Remyelination is the biological process that repairs damaged myelin sheaths around nerve fibers which typically occurs after an injury or illness like multiple sclerosis. This repair process is carried out by specialized cells known as Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system do this repair [114], [115][116]. In

remyelination, the conduction characteristics of the axon gets restored enabling the smooth transmission of the neuronal signal along the length of the axon. In phenomenon such as multiple sclerosis where the protective myelin sheath gets lost completely or partially, remyelination becomes important because it repairs the damaged myelin sheath which is necessary for effective nerve signal transmission. This procedure lessens impairment by preventing irreparable damage to neurons, slowing the progression of disease, and aiding in the recovery of lost neurological functions [117]. Demyelinating factors like ageing, trauma, infection, neurotoxins, ion channel disfunction etc, can lead to decreased rate of change in membrane potential ($\frac{\mathrm{d}V_m}{\mathrm{dt}}$) [118], [119] which eventually leads to slow signal transmission, increased threshold for action potential firing, reduced excitation and conduction blocking. Ortiz et.al in their work [120], has shown that appropriate neural activity can aid in remyelination through stimulating a previously damaged nerve fiber (demyelinated axons), suggesting that neuronal activity itself can aid in remyelination through appropriate mechanisms. Moreover, similar observations have also been made by [121], [122] where they suggested that one potential approach for the therapeutic treatment of demyelinating illnesses is to regulate neuronal excitability.

3.1.2 Myelin Capacitance and thickness of myelin layer

In general, system's capacitance can be defined as its capacity to store charge per unit of voltage. For a membrane, the amount of charge that can be stored across the membrane layer gives the capacitance which can be further expressed as,

$$C = \epsilon \frac{A}{d} \tag{3.0}$$

Where, C is the capacitance, ϵ is the permittivity of the material between the plates ($\epsilon = \epsilon_0 \epsilon_r$), ϵ_0 is the permittivity of free space and ϵ_r is the relative permittivity, A is the area and d is the myelin thickness of the membrane. Thus, as per Eq.3.0, capacitance is inversely related to the thickness of the membrane. This capacitance can be equated with the myelin capacitance as a decrease in C indicates a drop in the effective capacitance of the system, which may indicate a relative rise in the myelin sheath's contribution to the system's overall capacitance. This might happen if the myelin thickens or becomes more intact, improving insulation. Furthermore, a decrease in the value of C indicates an increase in the electrical conductivity of the axonal membrane which may suggest that the myelin sheath is changing or adapting as a physiological response. The opposite effect is true for an increased value of C.

Hence, it is to be noted that decrease in the value of the capacitance can lead to increase in the myelin thickness i.e., remyelination. Moreover, various other works associated with stimulating the nerve fiber has also shown that nerve fiber can itself aid in remyelination.

3.1.3 Demyelination

The degenerative process known as demyelination is defined by the degeneration of the myelin sheath, which serves as insulation for nerve fibers [113], [123]. This syndrome is a hallmark of multiple sclerosis (MS) and other neurological disorders, including acute disseminated encephalomyelitis, Guillain-Barré syndrome, and different hereditary leukodystrophies [114], [117]**.** Therefore, it becomes essential to comprehend the mechanics, causes, and effects of demyelination in order to create therapies that work and enhance patient's conditions. There are several ways that demyelination may occur, and they frequently include inflammatory processes. The immune system misidentifies myelin as a target in autoimmune diseases like MS, which causes inflammation and damage. The vital component of the immune system such as T and B cells protect the body from infections and other harmful substances. T cells are specialized in recognizing and eliminating aberrant or contaminated cells as well as controlling the immune system. In contrast, B cells generate antibodies which are proteins that identify and attach to particular antigens in order to neutralize or eradicate them.

Apart from immune-related processes, demyelination may arise from Viral infections, for example, can cause demyelination directly or indirectly through an autoimmune reaction. For example, infections like cytomegalovirus (CMV) and Epstein-Barr have been linked to multiple sclerosis. Demyelination may also be a result of metabolic problems. For instance, shortages in important nutrients, such vitamin B12, might affect myelin formation and maintenance. The body might not be able to make enough myelin or properly repair damaged myelin if it is deficient in these substances [124], [125], [126][127], [128] Demyelination may also result from genetic predispositions. Myelin formation and maintenance can be impacted by specific genetic mutations, which can result in structural abnormalities that increase the myelin's vulnerability to harm [127], [128]. Moreover, demyelination may result from exposure to environmental pollutants. Certain medications, chemicals, and heavy metals can have neurotoxic effects that harm Schwann cells and oligodendrocytes. The loss of the myelin sheath may result from this damage's disruption of the regular myelination process [129], [130], [131].

3.1.4 Stages of Demyelination

Damage to the myelin sheath or the cells that produce it usually triggers demyelination, which then results in pathological changes. An initial trauma, which could be caused by an infectious disease, the immune system, or metabolic variables, starts the process. Immune cells are drawn to the injury site as a result of this insult, which starts an inflammatory reaction. Cytokines and chemokines are generated when the inflammatory response is triggered, which draws more immune cells to the location. Schwann cells and oligodendrocytes may be harmed by this inflammation, which will hinder their capacity to create and preserve myelin. The myelin sheath starts to degrade as the inflammatory process goes on increasing with time [129], [132], [133], [134], [135].The myelin sheath is actively being removed from the axon at this point. Patches of this may appear, resulting in the development of demyelinated lesions. The underlying axon becomes exposed due to myelin loss, leaving it open to more injury. Degeneration may occur in the exposed axons as demyelination advances. Axons may become dysfunctional in the absence of the protective myelin coating, which would hinder signal transmission. Axonal degeneration can take place under extreme circumstances, leading to an irreversible loss of nerve function [136], [137], [138]**.**

The consequences of demyelination can be range from mild to severe depending upon the extent of the damage and the location of the damaged area. Below are some of the outcomes of demyelination:

i). **Issues with Neuronal Signal Transmission:** The slowing down or obstruction of electrical impulse transmission is the most obvious effect of demyelination. Numerous neurological symptoms, such as weakness, sensory abnormalities, and cognitive deficits, may result from these abnormalities [113], [123]**.**

ii). **Higher Energy Efiiciency:** The energy efficiency of myelinated fibers is higher than that of unmyelinated fibers. The energy required to sustain signal transmission rises with demyelination, which may put additional strain on the impacted neurons [139], [140]**.**

iii). **Axonal Loss:** Axonal loss is largely caused by demyelination, especially in diseases like multiple sclerosis (MS). Here, Axons become exposed and more prone to damage when their myelin sheaths break down, which impairs signal transmission [114], [131], [138], [141].

Demyelination is a complicated process that affects the nervous system profoundly. Prolonged investigation of the fundamental causes of demyelination and possible therapeutic interventions may yield favourable results for those impacted by demyelinating processes.

3.1.5 Remyelination

Restoring myelin sheaths around demyelinated axons is a critical biological process referred to as remyelination [116]. This phenomenon is especially important when considering demyelinating conditions like multiple sclerosis (MS), in which substantial neurological impairments result from myelin loss. Developing insight into the mechanisms of remyelination can help develop treatment approaches that might improve this process of healing**.** Progenitor cells, which are immature cells with the capacity to specialize need to relocate to the injured region in order to grow into glial cells capable of myelin regeneration. This predominantly affects oligodendrocyte precursor cells (OPCs), which are extensively dispersed throughout the brain and spinal cord in the central nervous system (CNS). These OPCs go to the site of injury during demyelination, multiply, and develop into oligodendrocytes, which are able to create new myelin sheaths around the axons [142], [143]. Schwann cells in the Peripheral Nervous System (PNS) causes remyelination and the PNS often has a greater capacity for repair than the CNS, where remyelination might be restricted and ineffective. After an injury, Schwann cells can multiply, regress to an immature state, and then mature once again to generate new myelin, aiding in the restoration of nerve function [144], [145].

Remyelination is a complicated process involving several cellular and molecular processes, among them are:

i). **Recruitment and Activation of OPCs**: OPCs are activated and move to the lesion site in response to demyelination. Different signalling molecules, such as growth factors, chemokines, and extracellular matrix constituents, control this process.

ii). **Differentiation of OPCs:** OPCs differentiate into adult oligodendrocytes once they reach the lesion site. Pro-differentiation and inhibitory signals work in concert to closely control this differentiation. Olig2, Sox10, and Myrf are examples of transcription factors that are important in promoting differentiation.

iii). **Formation of Myelin Sheath:** To generate new myelin sheaths, mature oligodendrocytes extend processes that encircle the axons. Axonal signals, cell adhesion molecules, and the

cytoskeletal dynamics of the oligodendrocytes all have an impact on the creation of these sheaths.

iv). **Support and Interaction of Axons:** Axons themselves play an active role in the remyelination process by providing cues that promote OPC differentiation and myelination. For example, neuronal activity can enhance remyelination by increasing the expression of molecules like brain-derived neurotrophic factor (BDNF).

After demyelination, remyelination is essential to returning nerve function to normal. To effectively treat demyelinating illnesses, a complete understanding of the basic mechanisms behind remyelination and the variables influencing it is essential.

3.1.6 Rate of Change of Membrane Potential ($\frac{dV_m}{dt}$)

It is understood that variation in the rate of change of membrane potential ($\frac{dV_m}{dt}$) can influence myelination [146], however in-depth understanding and analysis of its underlying mechanism are subject of research. Therefore, it is essential to understand how the rate of change in membrane potential affects myelination in order to design targeted therapeutics for demyelinating diseases and to optimize the properties associated with nerve conduction.

Literature have shown that the myelin layer has the potential to regrow if $\frac{dV_m}{dt}$ could be increased [147]. Several mechanisms can aid in an increase in $\frac{dV_m}{dt}$ which includes stronger excitatory neurotransmitter release (eg, glutamate) leading to larger influx of sodium ions, decrease release of inhibitory neurotransmitter (eg, GABA- gamma-aminobutyric acid), temperature especially warm temperature can aid increase biological processes including movement of ions, generally relevant for organisms that can regulate body temperature [148], [149] and physical activity i.e., through increases the density of sodium ion channel leading to faster influx of sodium ions [150].

3.1.7 Contribution

In this work, an effort has been made to understand whether the nerve fiber can itself aid in remyelination through a mathematical model incorporating the ECS dependent parameters. As per Eq.3.0, the simulation findings show that remyelination may be supported by a higher rate of change of membrane potential ($\frac{dV_m}{dt}$) since it lowers capacitance, which suggests a thicker myelin layer. As stated before, a number of investigations have demonstrated how neuronal

activity can independently promote remyelination. However, a robust mathematical framework is also needed to fully comprehend this aspect of myelination which would also incorporate the fundamental properties of the ECS and would be less mathematically and computationally complex. The results obtained from the framework seems to validate the existing findings that neuronal activities can itself aid in remyelination.

3.1.8 Proposed Methodology

For the analysis shown in Fig. 3.1, a passive electrical equivalent of the Hodgkin-Huxley (H-H) model [88] is considered. The H-H model is used for the purpose of analysis as it precisely depicts the ionic currents and voltage variations across a neuron's membrane. The H-H model is essential for signal processing and offers important insights into the excitability and transmission of nerve signals. The proposed framework can be equated to a porous pipe in which leakage occurs through pores which are perpendicular to the direction of the current and the ion channels are neglected since the emphasis is on the membrane's passive characteristics such capacitance and resistance as opposed to active mechanisms like ion channel gating. The fiber's behaviour is modelled as a simple conductor in this context, and ion flow is viewed as a passive leakage across the membrane rather than being impacted by particular ion channels, which normally actively regulate and modify the ionic currents. Without having to deal with the complexities of channel dynamics, this simplification makes it possible to analyse the fiber's fundamental electrical characteristics. The ECS related parameters are incorporated into the proposed framework which is inspired from [75]. In [22], the authors have tried to incorporate ECS dependent parameters onto the H-H model to obtain the Local Field Potential (LFP) around a stimulated cell.

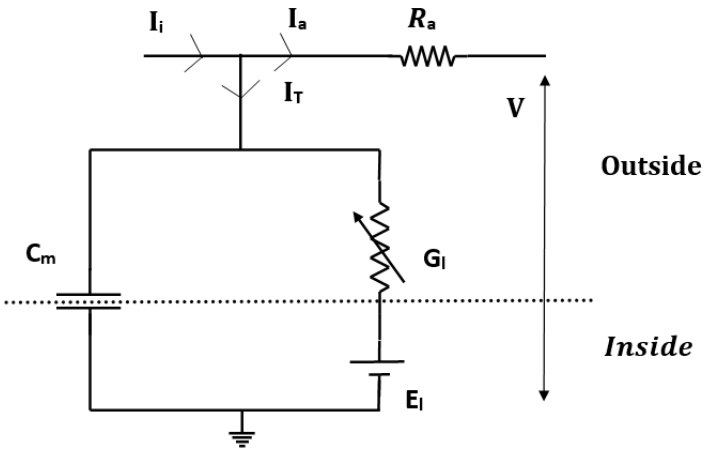

Fig.3.1: Equivalent Circuit of Passive Membrane Model

In Fig.3.1, I_i *i*s the input current, I_T is the transmembrane current and I_a is the axial current. Therefore, using KCl,

$$I_i = I_T + I_a \quad (3.1)$$

From the passive H-H model, the transmembrane current and the axial current can be further expressed as,

$$I_T = C_m \frac{dV_m}{dt} + \frac{(V_m - E_l)}{R_l} \quad (3.2)$$

And,

$$I_a = \frac{(V_m - V)}{R_a} \quad (3.3)$$

Here, V is the membrane potential at the next point. Therefore, putting Eq.3.2 and Eq.3.3 in Eq.3.1, the resultant equation obtained is,

$$I_i = C_m \frac{dV_m}{dt} + \frac{(V_m - E_l)}{R_l} + \frac{(V_m - V)}{R_a} \quad (3.4)$$

Here, C_m is the membrane capacitance, and R_l is the membrane resistance which can be expressed as $R_l = r_l \pi D_i l$ respectively. D_i is the internal diameter of the nerve fiber diameter l is the length of the fiber considered. V_m is the resting membrane potential, E_l is the equilibrium potential of leakage ions and R_a is the axial resistance respectively. Considering the linear passive characteristics of the fiber and its minimal leakage in relation to axial current, the only resistance that is significant is the axial resistance. Hence, the current flow can be computed based on the potential difference between the two places of interest. If $\frac{V_m - V}{R_a}$ shows the axial current due to the potential difference between the two points in the nerve fiber, Thus, modifying Eq.3.4, the nerve membrane capacitance can be expressed as,

$$C_m = \frac{I_i}{\frac{dV_m}{dt}} - \frac{(V_m - E_l)}{R_l \frac{dV_m}{dt}} - \frac{(V_m - V)}{R_a \frac{dV_m}{dt}} \quad (3.5)$$

Now, considering both the internal resistance of the fiber and the ECS resistance contributing to the axial resistance, the axial resistance can be expressed as,

$$R_a = R_e + R_i \quad (3.6)$$

Where, $R_e = \frac{4 r_e l}{\pi {D_e}^2}$ and $R_i = \frac{4 r_i l}{\pi {D_i}^2}$ are the volumetric resistance of the ECS and internal resistance of the nerve fiber [97]. Moreover, the characteristics internal resistance,

characteristic external resistance are r_i and r_e, and D_e is the diameter of the ECS respectively. Now, putting values of R_e and R_i in Eq.3.6, the axial resistance expression thus becomes,

$$R_a = \frac{4r_e l}{\pi {D_e}^2} + \frac{4r_i l}{\pi {D_i}^2} \tag{3.7}$$

Now, putting Eq.3.7 in Eq.3.5, the resultant membrane capacitance expression thus becomes,

$$C_m = \frac{I_i}{\frac{dV_m}{dt}} - \frac{(V_m - E_l)}{(r_l \pi D_i l)\frac{dV_m}{dt}} - \frac{(V_m - V)\pi {D_i}^2 {D_e}^2}{(4r_i l {D_e}^2 + 4r_e l {D_i}^2)\frac{dV_m}{dt}} \tag{3.8}$$

Eq.3.8 shows the relationship between the membrane capacitance (C_m) and the rate of change in membrane potential ($\frac{dV_m}{dt}$). Observing Eq.3.8, it can be inferred that the membrane capacitance C_m is inversely proportional to the rate of change of membrane potential ($\frac{dV_m}{dt}$), this suggests that larger the value of $\frac{dV_m}{dt}$, smaller will be the membrane capacitance (C_m). Thus, Eq.3.8 show that the membrane capacitance (C_m) depends on both membrane potential V_m and the rate of change of membrane potential ($\frac{dV_m}{dt}$).

3.1.9 Simulation Considerations

The length (l) of the fiber is varied from 10 µm to 100 µm respectively. The length is so considered because it strikes a balance between computing efficiency, biological realism, and accurate signal propagation dynamics modelling. Small axons, and fine neuronal branches typically fall within this range, which guarantees that the model can faithfully capture physiological behaviour while also being computationally viable [151], [152]. The maximum leakage conductance (G_l) is considered to be 0.0003 Siemens /cm^2. The resting membrane potential (v_m) is -65 mV. The equilibrium potential for leakage ions (E_l) is considered to be -55 mV. The standard parameters have been inspired from the H-H model parameters suggested by [17] and the simulation is conducted on MATLAB platform.

3.1.10 Results and Discussions

Eq. 3.8 provides the relationship between the membrane capacitance and the rate of change in membrane potential ($\frac{dV_m}{dt}$) and the results are generated by changing $\frac{dV_m}{dt}$ for each observation and the consequent effect it has on the membrane capacitance value. The primary objective of this simulation is to show how variations in $\frac{dV_m}{dt}$ with scaling factors of 0.5, 0.1, 2, and 4

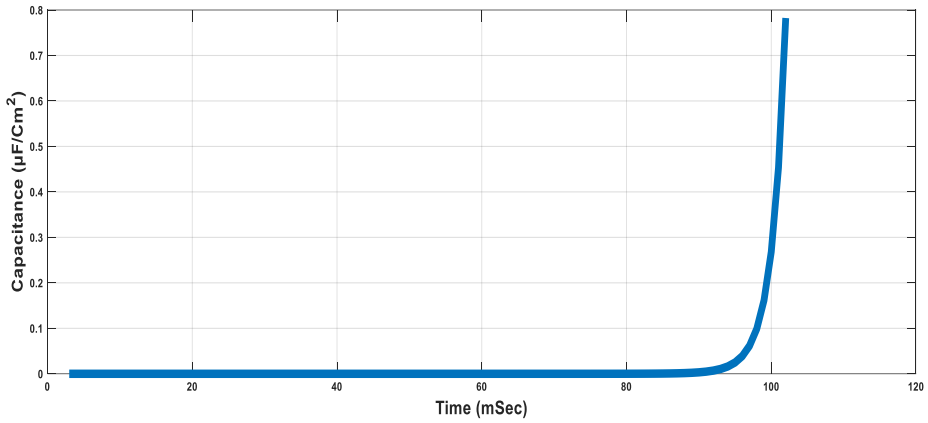

Fig.3.1.1 Membrane capacitance when $\frac{dV_m}{dt}$ is unchanged

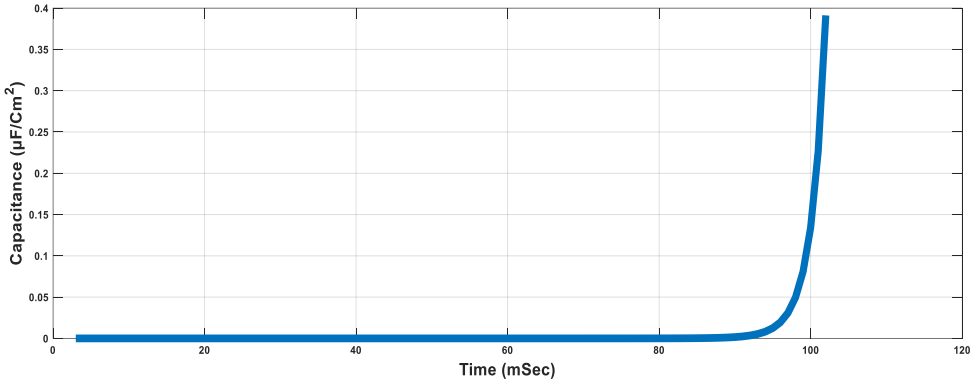

Fig.3.1.2 Membrane capacitance when $\frac{dV_m}{dt}$ is increased by 2 times

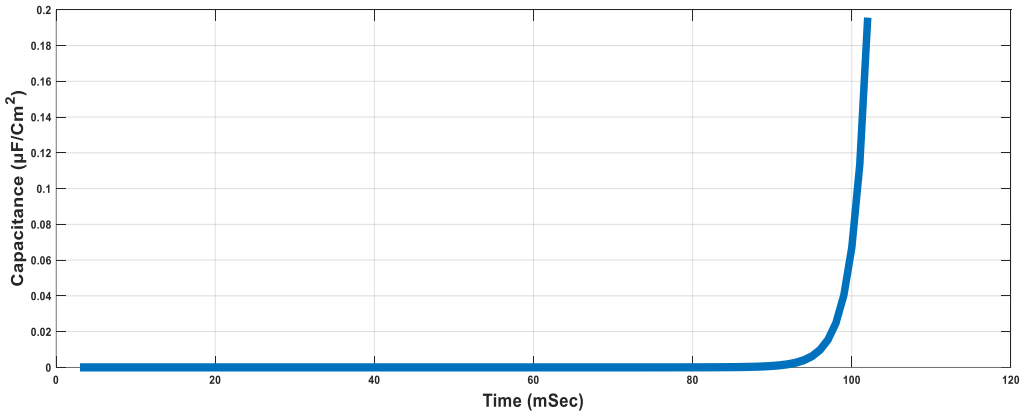

Fig.3.1.3 Membrane capacitance when $\frac{dV_m}{dt}$ is increased by 4 times

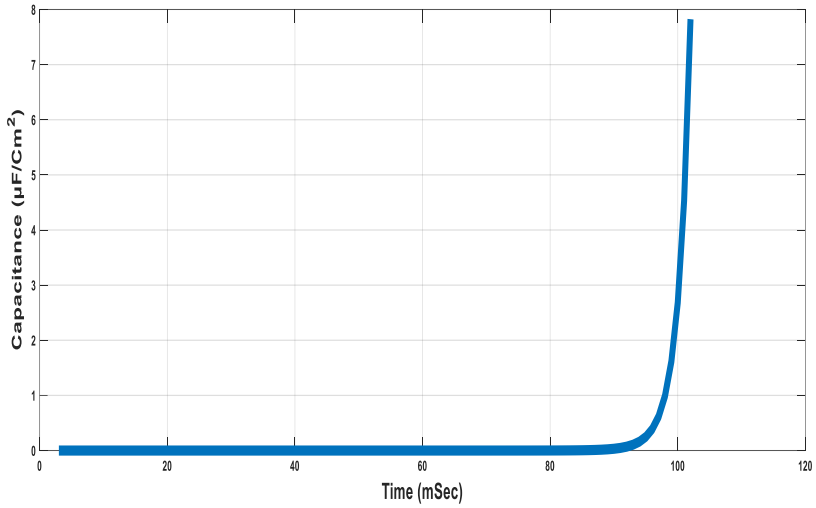

Fig.3.1.4 Membrane capacitance when $\frac{dV_m}{dt}$ is decreased by 0.1 times

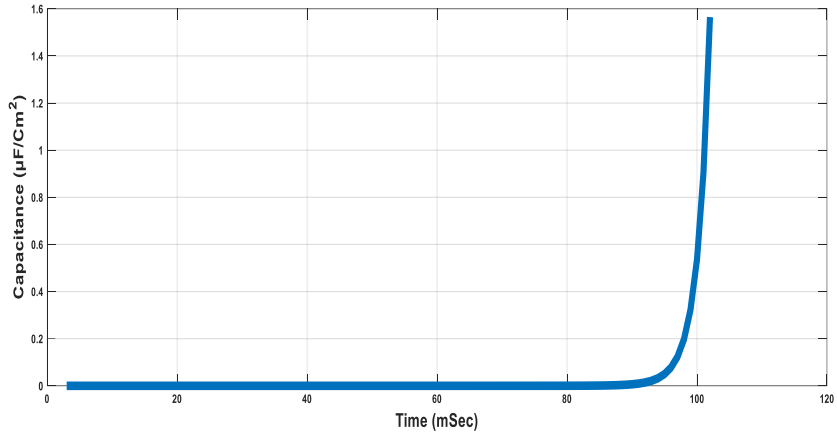

Fig.3.1.5 Membrane capacitance when $\frac{dV_m}{dt}$ is decreased by 0.5 times

influences the nerve membrane capacitance value. Neuronal function is significantly impacted by the variations in $\frac{dV_m}{dt}$; a neuron might get closer to firing an action potential with a quicker $\frac{dV_m}{dt}$, and may fire less frequently at a slower $\frac{dV_m}{dt}$. Communication and information processing in the nervous system depend on the accurate and dynamic variations in the rate of change of the membrane potential ($\frac{dV_m}{dt}$) across time. Neurons may relay information and coordinate complicated activities because of this modulation which also controls how rapidly and efficiently electrical signals like action potentials are generated and propagated along neurons. From Fig.3.1.2 and Fig.3.1.3 it can be seen that when $\frac{dV_m}{dt}$ increases by 2 times and 4 times respectively, the membrane capacitance decreases accordingly which suggests that there is an

increase in myelin thickness. The insulating myelin layer reduces the membrane's capacitance or capacity to hold charge as its thickness increases. In accordance with the characteristics of thicker myelin, a lower capacitance enables the nerve to transmit impulses more rapidly and effectively.

Similarly in Fig.3.1.4 and Fig.3.1.5 it is also seen that when $\frac{dV_m}{dt}$ decreases by scaling factor of 0.5 and 0.1, the membrane capacitance increases correspondingly suggesting that there is a decrease in myelin thickness. It is seen from Fig.3.1.2, Fig.3.1.3, Fig.3.1.4 and Fig.3.1.5, that an increase in the value of $\frac{dV_m}{dt}$ results in a decreased C_m value which implies that for membrane potential to change more rapidly for a given input current, membrane capacitance must be smaller. This is because C_m represents the ability of the nerve membrane to store charges, lower value of C_m indicates that less amount of charge is needed to alter the membrane potential by some amount; this accounts for faster voltage responses i.e. higher $\frac{dV_m}{dt}$. Thus, neurons with lower membrane capacitance such as those with myelination, exhibit quicker responses to electrical stimuli. The changes in membrane thickness with varying $\frac{dV_m}{dt}$ is shown in Table 3.1.

Table 3.1: Change in the Rate of change of membrane Potential with corresponding Maximum Capacitance value.

Variation in the Rate of change of Membrane Potential ($\frac{dV_m}{dt}$)	**Maximum Capacitance Value (μF/Cm²)**
No change in $\frac{dV_m}{dt}$	0.7824
Increased by 2 times	0.3912
Increased by 4 times	0.1956
Decreased by 0.5 times	1.5648
Decreased by 0.1 times	7.8240

Action potential appears to play an essential role in neuronal growth or remyelination, as the values shown in Table 3.1 obtained from the simulation results demonstrates that increasing the rate of change of membrane potential can also increase myelin thickness and

contribute to neuronal growth according to the relation given in Eq.3.0. It is seen from Fig.3.1.1 that when there is no change to $\frac{dV_m}{dt}$, the value of membrane capacitance obtained is 0.7824 $\mu F/C_m^2$ which is very close to the actual value i.e., 0.9 $\mu F/C_m^2$ [153]. This suggests that the proposed framework in which the ECS dependent parameters are included in the passive nerve model manages to replicate results close to an actual nerve fiber.

In Fig.3.1.2, it is seen that when $\frac{dV_m}{dt}$ is increased by 2 times, the value of membrane capacitance obtained is 0.3912 $\mu F/C_m^2$ and from Fig.3.1.3 it is seen that when $\frac{dV_m}{dt}$ is further increased by 4 times, the value of membrane potential obtained is 0.1956 $\mu F/C_m^2$. These observations suggests that as $\frac{dV_m}{dt}$ is increased the value of membrane capacitance reduces suggesting increase in thickness of myelin layer. Moreover, from Fig.3.1.4 and Fig.3.1.5 when $\frac{dV_m}{dt}$ is decreased by 0.5 times and 0.1 times respectively, the values of membrane capacitance obtained are 1.5648 $\mu F/ C_m^2$ and 7.8240 $\mu F/ C_m^2$ respectively which also suggests that decrease in $\frac{dV_m}{dt}$ can lead to decrease in myelin thickness.

In cases like ageing, diseases, injuries etc., the myelin layer wrapping around the axon has the potential to regrow if $\frac{dV_m}{dt}$ could be increased. As discussed above, there are various ways through which $\frac{dV_m}{dt}$ can be increased which would lead to myelin layer to be regenerated on its own which includes stronger excitatory neurotransmitter release (eg, glutamate) leading to larger influx of sodium ions, decrease release of inhibitory neurotransmitter (eg, GABA-gamma-aminobutyric acid), warm temperature, [148], [149] and physical activity [150]. The comparison table for the work with standard literature is shown in Table 3.2.

Comparison Table:

Table 3.2: Comparison Table for the current finding with the existing literatures

Work	Methods Used	Results	Remarks
Ortiz, F. C., Habermacher, C., Graciarena, M., Houry, P. Y., Nishiyama, A., Oumesmar, B. N., & Angulo, M. C. (2019).	In- Vivo methods, particularly photostimulation and electrophysiological recordings in mice to investigate how myelination is affected by neural activity.	In vivo, moderate and regulated neuronal activity improves remyelination, restores action potential conduction in	ECS parameters are not considered.

Neuronal activity in vivo enhances functional myelin repair. *JCI insight*, *4*(9).		demyelinated lesions, and expands the pool of mature oligodendrocytes.	
Bergles, D. E., & Richardson, W. D. (2016). Oligodendrocyte development and plasticity. *Cold Spring Harbor perspectives in biology*, *8*(2), a020453.	Oligodendrocyte precursor cell behaviour is studied using laser ablation, and its molecular properties in the central nervous system (CNS) are examined using genetic tools.	In response to damage or illness, adult oligodendrocyte precursor cells speed up their cell cycle and produce more myelinating oligodendrocytes (OLs) to replace damaged myelin. They create synapses with unmyelinated axons and produce additional OLs and myelin locally in response to electrical activity in those axons.	ECS parameters are not considered.
Demerens, C., Stankoff, B., Logak, M., Anglade, P., Allinquant, B., Couraud, F., ... & Lubetzki, C. (1996). Induction of myelination in the central nervous system by electrical activity. *Proceedings of the National Academy of Sciences*, *93*(18), 9887-9892.	In vitro culturing of mouse embryonic brain hemispheres to investigate how myelination is affected by electrical stimulation.	The process of myelination depends heavily on electrical activity, and disturbances in this activity may have important ramifications for demyelinating disorders like multiple sclerosis.	ECS parameters are not considered.
Current work	**Modeling and Simulation method is used to understand that for a passive fiber, whether neuronal activity itself can aid in remyelination. ECS parameters are considered**	**Nerve fiber can itself aid in remyelination if the rate of change of membrane parameters can be increased.**	**Effect of the Periaxonal fluid is not considered**

The results obtained from the proposed framework is aligned with the existing literatures suggesting that adequate neuronal activities itself can aid in remyelination as a lower value of membrane capacitance could be equated with an increased myelin thickness due to the reasons mentioned in section 3.1.2. Moreover, the proposed framework which incorporates the ECS dependent parameters into the H-H model provides a robust approach in understanding of neuronal activity and its role in remyelination and the model manages to replicate results that are consistent with existing literature. Furthermore, the model is mathematically and computationally less complex and is useful for rapid prototyping. However, the model is a simplified representation and for a more detailed analysis, the effect of periaxonal fluid should be considered which is to be explored further.

3.2 Extracellular Conductivity and Nerve Signal Propagation for a larger Extracellular Space

This section simulates an environment which focuses on understanding the effect of the ECS on nerve fiber of different shapes such as tapered, flared and uniform nerve fiber. Moreover, this chapter also analyses how an ECS with size larger than the size of the fiber diameter, a situation which mimics the development phase of the nerve fiber and during brain edema affects neuronal signal.

3.2.1 Introduction

The general understanding of the ECS is that a larger ECS leads to more signal attenuation in comparison to a smaller ECS [64]. Moreover, it is also understood that the size of ECS gets enlarged during brain edema (swelling) through enhanced permeability of the blood-brain barrier (BBB), which results in pressure gradients between vascular and extracellular compartments [99]. In addition to altering the tissue composition and increasing the intercapillary distances for medication and oxygen perfusion, fluid retention inside the larger ECS adds to the edema. This enlargement can further contribute to the occurrence of hypoxia like activities in which the brain is devoid of sufficient oxygen levels.

During the development stage, it is seen that the size of the ECS is larger than fiber diameter [65]. The extracellular matrix (ECM) and interstitial fluid within the ECS function as a dynamic environment that can expand or contract in response to cellular activity and developmental requirements [61], [71], [154]**.** The ECS has to make space for the growing axons and dendrites that result from the proliferation of neurons. The larger ECS makes it easier for ions and signalling molecules to travel, which is necessary for proper neuronal function and

communication. Effective volume transmission is made possible by this spatial configuration, allowing chemical signals to pass through the ECS and reach target cells.

Tønnesen et.al in their work, has observed that the size of the ECS can be well above 1 µm (minimum = 50 nm, maximum = 3.2 µm, median = 0.27 µm) [62]. However, the microscope involved in their work failed to resolve structure which are less than 50 nm. This is significant as various other research findings has shown that the size of the ECS ranges in the nanometer scale [59], [60].

3.2.2 Swelling of the Extracellular Space

An excessive buildup of fluid in the ECS or intracellular compartments of the brain is known as brain edema or swelling of the brain. It is essential to comprehend the mechanisms of brain edema, such as the function of the blood-brain barrier (BBB) and the pressure gradients that follow, in order to design therapies that effectively address disorders linked to cerebral edema. Cytotoxic edema and vasogenic edema are the two main categories of brain edema [83], [84], [155]. A breakdown of the cellular ion pumps results in an inflow of sodium (Na^+) and chloride (Cl^-) ions into the cells, causes cytotoxic edema and thus water follows these osmolytes osmotically, resulting in cellular swelling. In situations like ischaemia, where cellular metabolism is impacted by energy shortage, cytotoxic edema is frequently observed. The BBB's disintegration causes vascular edema, which increases permeability. In this case, edema occurs from the vascular compartment's fluid and protein leaks into the ECS. Vasogenic edema is frequently seen in diseases such as infections, tumours, and traumatic brain injuries.

The blood-brain barrier (BBB) is a selective barrier that controls the flow of chemicals from the bloodstream to the brain. It is made up of endothelial cells joined by tight junctions that obstruct the free movement of big molecules and ions [85]. Fluid and solutes can enter the ECS when the BBB is breached, as in vasogenic edema, because of its increased permeability. The blood-brain barrier (BBB) can be disrupted by a number of circumstances, which can have a major effect on the barrier's integrity and functionality. The involvement of inflammation is significant because inflammatory mediators have the ability to weaken the BBB's tight junctions and increase its permeability by releasing cytokines that are generated during immune responses. Hypoxia, which is defined as low oxygen levels, also causes cellular stress and damage, which exacerbates BBB failure [86], [156]. The growth of tumours is especially dangerous because they can release substances that damage the blood-brain barrier and cause localized edema around the tumour mass. When considered as a whole, these elements

emphasize the BBB's susceptibility and vital function in preserving the health of the central nervous system. Thus, the ECS enlarges as edema worsens because more fluid builds up inside it.

3.2.3 Extracellular Space during development stage

One important component of neurodevelopment is the enlarged ECS during the development stage of neurons. Many elements, including as the extracellular matrix (ECM), cellular migration, differentiation, and proliferation, all have an impact on this phenomenon [65]. The ECS is enlarged during neuron development as a result of multiple interconnected mechanisms. The accumulation of fluid in the ECS is facilitated by the blood-brain barrier's (BBB) greater permeability throughout development. This permeability can lead to swelling even while it permits the passage of vital nutrients and signalling molecules. Furthermore, signalling molecules that encourage the enlargement of the extracellular matrix (ECM) can be secreted as a result of interactions between neurons and the ECM. For example, the release of glycoproteins and proteoglycans can alter the ECM's composition and viscosity, promoting fluid buildup and cell migration.

During neuron development, the ECS is enlarged, which has several important consequences. Greater space for axonal growth and dendritic branching, which are necessary for the establishment of functional neural circuits, is provided by a larger ECS, which promotes neuronal connection. In addition, the ECS is essential for controlling ion homeostasis, which preserves the ionic conditions required for neuronal excitability. The distribution of ions like potassium and sodium, which are essential for producing and propagating action potentials, can be enhanced by an enlarged ECS. However, neurological disorders could result from disturbances in the mechanisms controlling ECS growth. Irregularities in the composition of the ECM or in the migration of neurons can lead to disorders like polymicrogyria or lissencephaly, which are marked by incorrect cortical development. Comprehending these dynamics is crucial for clarifying the processes that underlie normal neurodevelopment as well as the numerous neurological diseases [157], [158]. Extracellular matrix, cellular dynamics, and environmental factors all play a role in the complex process of ECS enlargement during the developmental stages of nerve formation which is essential for neuronal migration, connection, and general brain function.

3.2.4 Contribution

The work done in this section has undertaken the scenario in which the size of the ECS is larger i.e., in the range of μm's and the effect it has on neuronal signal for a flared, tapered and a fiber with fixed diameter. The well-known cable model of nerve is used for simulation and the parameters pertaining to the ECS have been incorporated into the model to provide a robust framework that can be used for a holistic study of nerve signal transmission under the effect of the ECS. Furthermore, an attempt has been made to understand whether the phenomenon of larger ECS leading to more signal attenuation and vice-versa holds true for a scenario in which the ECS is larger than the internal diameter of the fiber.

3.2.5 Proposed Methodology

For this work, the conventional cable equation [91] is used and the ECS related parameters are incorporated into the cable model which is inspired from [75]. The internal and extracellular potentials can be shown as,

$$\frac{\partial V_i}{\partial x} = -R_i I_i(x) \text{ and } \frac{\partial V_e}{\partial x} = R_e I_e(x) \tag{3.9}$$

Where, $R_i = \frac{4r_i}{\pi {D_i}^2}\Delta x$ and $R_e = \frac{4r_e}{\pi {D_e}^2}\Delta x$ are the volumetric internal resistance and extracellular resistances respectively [97]. The transmembrane voltage (V_m) can be expressed by subtracting $\frac{\partial V_e}{\partial x}$ from $\frac{\partial V_i}{\partial x}$ which results in,

$$\frac{\partial V_m}{\partial x} = -I_a(R_e + R_i) \tag{3.10}$$

Here, $(R_e + R_i)$ is the total axial resistance (R_a) and I_a is the axial current. Considering the mobility of current within and beyond the membrane to be identical, then, the transmembrane current is equal to both the internal and exterior currents I_T, therefore,

$$\frac{\partial I_e}{\partial x} = \frac{\partial I_i}{\partial x} = \frac{\partial I}{\partial x} = I_T \tag{3.11}$$

By differentiating Eq.3.10 with respect to x and replacing the values given in Eq.3.11, the resulting wave equation is,

$$\frac{d^2 V_m}{dx^2} + C_m(R_e + R_i)\frac{dV_m}{dt} + \frac{(R_e+R_i)}{R_m}(V_m + E_l) = 0 \tag{3.12}$$

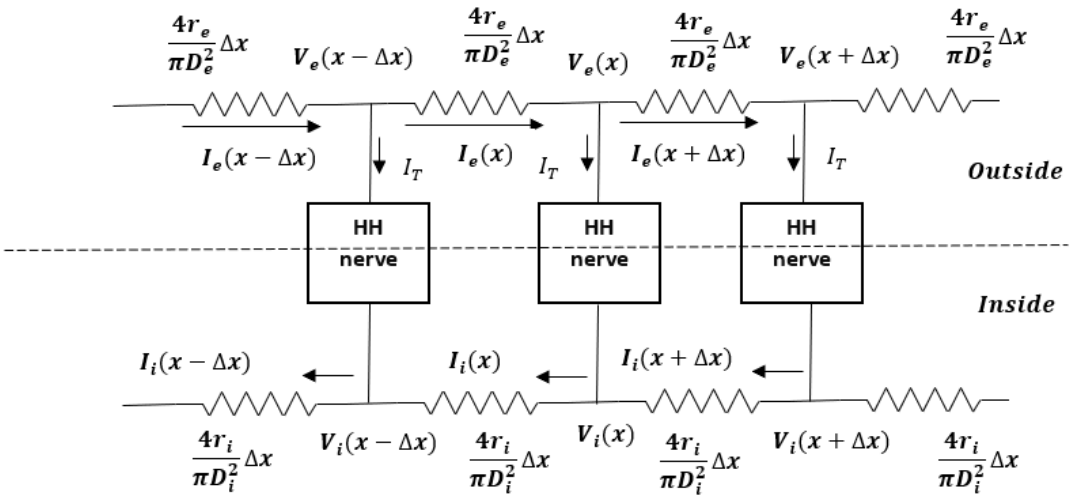

Fig.3.2 Cable model incorporating ECS parameters

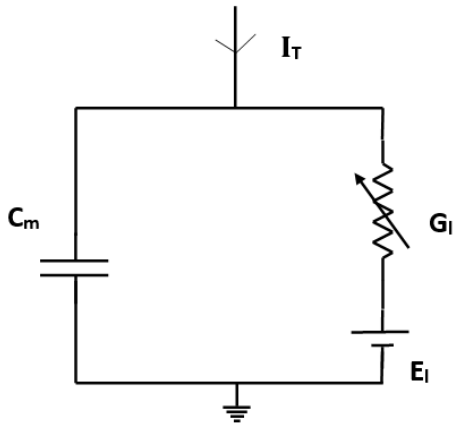

Fig.3.2.1 Individual passive H-H circuit of the cable model

The volumetric membrane capacitance and volumetric membrane resistance is given as, $C_m = c_m \pi D_i l$ and $R_m = r_m \pi D_i l$ where c_m and r_m are the characteristic membrane capacitance and membrane resistance and l is the length of the fiber and D_i is the internal diameter of the fiber. Now, considering the fiber to be equipotential cylinder then, $\frac{dV_m}{dx} = 0$. Hence, the Eq.3.12 becomes,

$$\frac{dV_m}{dt} = \left[\frac{V_{in} - V_m}{C_m(R_e + R_i)^2} - \frac{(V_m - E_l)}{R_m C_m}\right] \tag{3.13}$$

Now, further expanding the term $C_m(r_e + r_i)^2$ gives,

$$C_m(R_e + R_i)^2 = \frac{16 c_m l^2}{\pi}\left(\frac{r_i^2 l}{D_i^3} + \frac{r_e^2 l D_i}{D_e^4} + \frac{2 r_e r_i}{D_e^2 D_i}\right) \quad (3.14)$$

3.2.6 Simulation Considerations

For a constant ECS, its diameter is considered to be of 10 μm and for a uniform fiber, the diameter of the fiber is considered to be of 2 μm. The characteristic membrane capacitance is considered to be of 1 $\mu F/C_m^2$ and the length of the fiber is varied from 5 to 200 μm respectively. The length is so considered in order to have a balance between computing efficiency, biological realism, and accurate signal propagation dynamics modelling as small axons, and fine neuronal branches typically fall within this range, [151], [152]. The resting membrane potential is considered to be -65 mV and equilibrium leakage potential E_l is considered as -10.6 mV respectively; the simulation is conducted in PYTHON simulator.

3.2.7 Results and Discussions

The simulation is carried out by considering two broad scenarios. Firstly, the nerve fiber is considered to be varying and the ECS is considered to be constant and secondly, the nerve fiber is considered to be constant and the ECS is varied. Through these approaches, different scenarios are tried to be replicated in order to have a wider understanding of the ECS and its role in neuronal signal propagation.

3.2.7.1 Fiber with Constant Extracellular Space and Varying Diameter

In this section, the simulation is undertaken by considering the ECS to be constant in nature i.e. of 10 μm and the diameter of the nerve fiber to be varying for each observation. Initially, a tapered fiber is considered which is surrounded by a constant ECS of 10 μm and the tapering condition is applied by considering the fiber diameter to diminish linearly with a slope factor of 0.002 from 2 μm, the resultant plot obtained is shown in Fig.3.2.2. Furthermore, Fig.3.2.3 shows the scenario in which the fiber is considered to be flared and surrounded by an ECS of constant diameter. The flaring condition is applied by considering the fiber diameter to increase linearly with slope factor of 0.002 from 2 μm.

In both Fig.3.2.2 and Fig.3.2.3, $V_1(t)$ is the initial action potential and $V_{out1}(t)$, $V_{out2}(t)$, $V_{out3}(t)$, $V_{out4}(t)$, $V_{out5}(t)$ are considered at a distance of 5 μm, 37.5 μm, 78.125 μm, 151.25 μm, 183.75 μm respectively along the fiber and the ECS is considered to be constant i.e. of 10 μm. It is observed from Fig.3.2.2 and Fig.3.2.3 that irrespective of whether the fiber is flared or tapered, the attenuation of the signal at a constant ECS is almost the same.

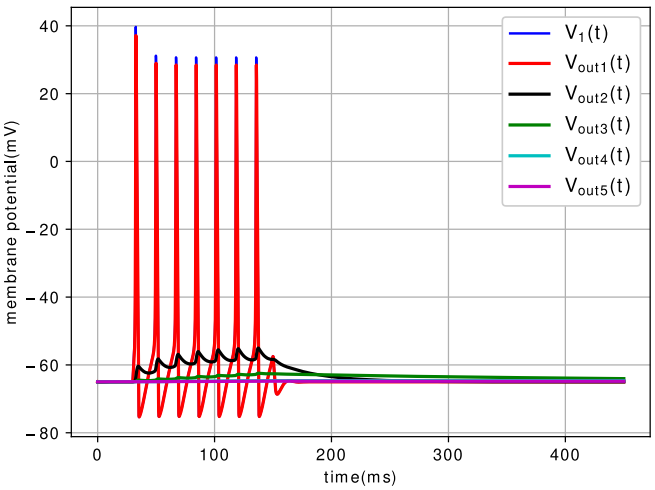

Fig.3.2.2 Tapered fiber with constant ECS

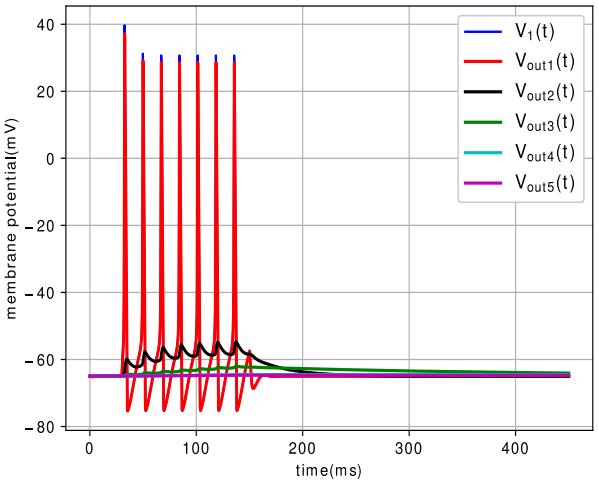

Fig.3.2.3 Flared fiber with constant ECS

3.2.7.2 Fiber with Constant Diameter and Varying Extracellular Space

This section deals with the scenario in which the diameter of the fiber is considered to be constant i.e. of 2 µm and the ECS is varied for each observation.

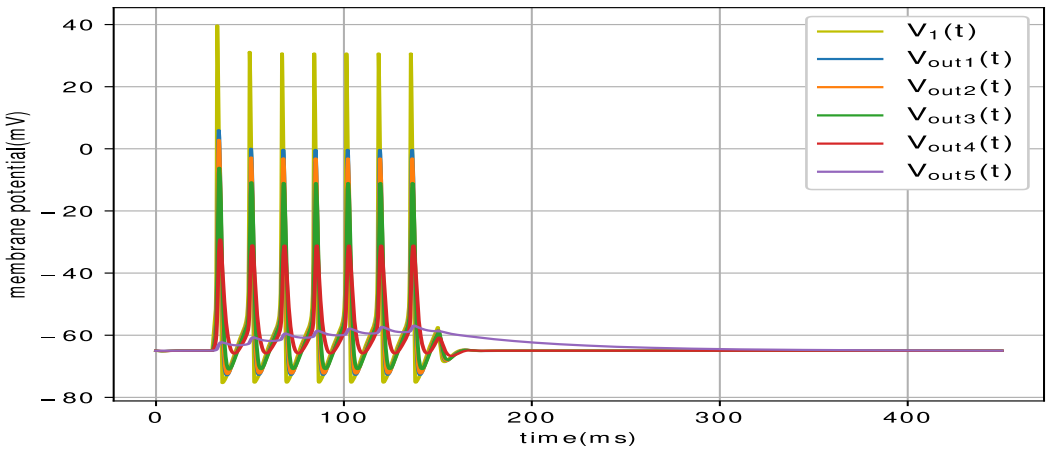

Fig.3.2.4 Uniform Fiber with increasing ECS for each observation

Here, $V_1(t)$ is the initial action potential and the corresponding action potential spikes i.e. $V_{out1}(t)$, $V_{out2}(t)$, $V_{out3}(t)$, $V_{out4}(t)$, and $V_{out5}(t)$ are considered at ECS of 0.1510 µm, 0.9510 µm, 1.9610 µm, 2.9710 µm, and 4.19 µm respectively; the length of fiber is considered to be of 70 µm. It is seen from Fig.3.2.4 that a fiber of uniform diameter which surrounded by a larger ECS attenuates the signal more than the one with decreasing ECS.

A nerve fiber of uniform diameter enclosed within a larger ECS gives the intracellular ions more advantages in terms of mobility to move towards the external media via the leakage channels due to the decreased resistivity of the ECS resulting in a higher attenuation of the neuronal signal. If the ECS is smaller, mobility of ions to dissipate towards the external media reduces significantly due to higher resistivity of the ECS causing the signal to get less attenuated. This observation is consistent with the general understanding that a larger ECS attenuates signal more than a smaller ECS. The comparison table for the work with standard literature is shown in Table 3.3.

Comparison Table:

Table 3.3: Comparison Table for the current finding with the existing literatures

Work	Methods Used	Results	Remarks
Bédard, C., Kröger, H., & Destexhe, A. (2004). Modeling extracellular field potentials and the frequency-filtering properties of extracellular space. *Biophysical journal*, *86*(3), 1829-1842.	A ball-and-stick model is used for interpreting the effects of intracellular and extracellular media on the spatial and frequency profile of the membrane potential.	Larger the ECS, larger is the attenuation of signal for an open circuit (FO) model.	Employs complex mathematical analysis.
Bruehlmeier, M., Roelcke, U., Bläuenstein, P., Missimer, J., Schubiger, P. A., Locher, J. T., ... & Ametamey, S. M. (2003). Measurement of the extracellular space in brain tumors using 76Br-bromide and PET. *Journal of Nuclear Medicine*, *44*(8), 1210-1218.	The distribution volume of the radiotracer 76Br-bromide in brain tissues is estimated using PET imaging and compartment modelling, primarily to evaluate the extracellular space in brain tumours.	Size of ECS can get enlarged during brain edema.	The effect of ECS on membrane potential is not analysed.
Hrabetova, S., Cognet, L., Rusakov, D. A., & Nägerl, U. V. (2018). Unveiling the extracellular space of the brain: from super-	To visualise the nanoscale structure of the ECS, sophisticated optical methods such as super-resolution	The ECS can be greater than the fiber diameter during developmental phases as evidenced by the fact that	There is no explicit mathematical relationship between membrane potential and the extracellular space (ECS).

resolved microstructure to in vivo function. *Journal of Neuroscience*, *38*(44), 9355-9363.	microscopy are employed in conjunction with biophysical investigations and numerical modelling.	components such as hyaluronan can affect the ECS volume and assist maintain and regulate it.	
Tønnesen, J., Inavalli, V. K., & Nägerl, U. V. (2018). Super-resolution imaging of the extracellular space in living brain tissue. *Cell*, *172*(5), 1108-1121.	Optimised surgery and anaesthesia techniques are employed to minimise motion artefacts, and non-parametric statistical tests are applied for data processing. Additionally, the work presents SUSHI, a novel fluorescent imaging approach designed to improve live-cell super-resolution imaging of biological tissues.	The size of the ECS can be well above 1 µm (minimum = 50 nm, maximum = 3.2 µm, median = 0.27 µm).	The microscope involved in this work failed to resolve structure which are less than 50 nm.
Current Work	**Modeling and Simulation method is used to understand the effect of a larger ECS on neuronal signal**	**Larger the ECS, larger is the signal attenuation and vice-versa for a passive nerve fiber. For a uniform ECS, the attenuation of neuronal signal is same for both a tapered and flared fiber.**	-

3.3 Summary and Future Remarks

This chapter was divided into two sections; the first section i.e., Section 3.1 deals with how neuronal signal itself can aid in remyelination and the second section i.e., section 3.2 deals with the effects of varying ECS on neuronal signal transmission. To summarize the chapter, it can be inferred that the variation in the rate of change in membrane potential ($\frac{dV_m}{dt}$) can have an effect on myelination such as increase in rate of change of membrane potential ($\frac{dV_m}{dt}$) causes the capacitance value to reduce, signifying remyelination according to the capacitance formula

given in Eq.3.0. $\frac{dV_m}{dt}$ can be incresead by a stronger Excitatory Neurotransmitter release like glutamate, decrease release of Inhibitory Neurotransmitter like GABA, temperature and physical activities. However, the model is a simplified representation of a nerve fiber and for a more detailed model, the effect of periaxonal fluid should be considered which is to be explored further.

Moreover, it is well defined that a larger ECS leads to a larger signal attenuation than a smaller ECS, results obtained from this chapter also found to correlate to this observations. A larger ECS offeres less resistance thus, offering less hindrence to the mobile ions to get dissipated towards the external media via the leak channels resulting in a higher signal attenuation. But, a smaller ECS offeres higher resistance to the mobile ions to dissipate towards the external media resulting in a smaller attemuation of the signal. Additionally, if the nerve fiber is surrounded by an uniform ECS, the attenuation of neuronal signal is approximately same whether the fiber is a tapered one or a flared one.

The work done in this chapter involves mathematical modeling of a nerve fiber which is mathematically and computationally less complex. Moreover, the models have incorporated the fundamental parameters pertaining to the ECS to provide a robust framework to simulate a real nerve fiber.

Publications:

Journal:

1. **Das, B.,** Baruah, S. M. B., Hazarika, U., Roy, S., "Neuronal Activity and its impact on Neuronal Growth", Journal name (Science and Technology Journal), Vol. 9, Issue. 1, pp. 21-25, 2021, DOI: 10.22232/stj.2021.09.01.04 (UGC CARE (ISSN: 2321-3388)).

Book chapter:

1. Baruah, S. M. B., **Das, B.**, & Roy, S., "Extracellular conductivity and nerve signal propagation: an analytical study", Book name (Lecture Notes in Electrical Engineering), Vol. 728, pp. 399-406, 2021, Springer Singapore, DOI: https://doi.org/10.1007/978-981-33-4866-0_49 (ISBN: 978-981-33-4866-0).