# Research Proposal

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Investigating the effect of impermeant anions and electrodiffusion on the computational properties of neurons

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**Plagiarism Declaration**

I know that plagiarism is wrong. Plagiarism is to use another’s work and pretend that it is one’s own. I have used the American Medical Association (AMA) convention for citation and referencing. Each contribution to, and quotation in, this research proposal from the work(s) of other people has been attributed and has been cited and referenced.

This research proposal is my own work.

I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

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**Date: 11 November 2020**

# Background

Neuron are the fundamental units of the brain. In algorithmic terms, neurons have input, processing, and output layers. Input to neurons are conveyed via dendrites where signals received at synapses in the distal portion spread proximally to the cell body. There are several ways in which the spread of electrical signals in dendrites have been computationally modelled, most prominent are the equivalent circuit model by Hodgkin and Huxley, and the Cable model by Rall. These two models generally provide good predictions as to how signals spread along dendrites, however they are inadequate at modelling signal spread in low volume spaces with rapid ionic fluxes such as dendritic spines. Moreover, our group has recently shown that impermeant anions may play an important role in neuronal signalling, and this too is not accounted for in traditional models. I plan to construct a biophysically plausible multicompartmental electrodiffusion based model incorporating impermeant anions, and then investigate the electrical and information processing properties of such dendrites.

1. **Dendrites and dendritic spines**

Dendrites are cytoplasmic extensions which emerge from the soma**.** The proximal portion of the dendrite may have the same organelles as the soma (barring the nucleus). Interestingly, both proximal and distal portions of dendrites contain endoplasmic reticula and ribosomes1. This has implications in terms of the protein synthesis and the heterogenous distribution of impermeant anions across the dendrite which may have functional implications.

It is thought that dendritic branching structures also may have functional implications. **?thickness?** There are on average 1-9 dendrites per cell body each giving rise to a dendritic tree. The specific architecture of dendritic trees likely relates to the function characteristics of the related neuronal population. Dendritic spines are pedunculated appendages which also only occur in specific areas. ‘Spiny’ neurons are those that have a high density of dendritic spines which can be in the range of 40 000 – 100 000.

The function of dendritic spines is still not precisely known.  **Some speculated function of spines.**

1. **A neuroelectronic basis for computational dendritic modelling**

Detailing the biophysical properties of dendrites allows for the precise modelling and prediction of dendritic behaviour. Dendrites are walled by a cell membrane (lipid bilayer) that is approximately 3-4 nm thick. This membrane is impermeable to most charged molecules however and subsequently charges build up on either side of the cell membrane, thus the membrane is modelled as a capacitor. Ions can escape through various channel proteins and pumps which extend across the membrane. These channels can be modelled as resistors.

*Figure of a lipid bilayer with the membrane proteins.*

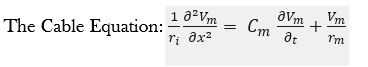
Ions move across both longitudinally along the length of the dendrite as well as across the membrane through down concentration and electrostatic gradients in order to reach a steady state where there is no net ionic movement. In most conditions the resting membrane potential is in the range of -90mV to +50mV. The primary ionic species which contributes to the overall charge are sodium (Na+), potassium (K+), and chloride (Cl-); bicarbonate (HCO3-) and hydrogen ions(H+) also play a small role. All these ionic species can move between the intracellular and extracellular compartments. Another electrical species which is often neglected are impermeant anions. These are negatively charged molecules such as proteins and nucleic acids which exist both intracellularly and extracellularly but cannot move between these compartments. Due to the vast extracellular space volume relative to the intracellular volume the concentration of the anions is much larger intracellularly and may play an important role in the electrical properties of the dendrite.

*Schematic of the various ionic species and the relative concentrations*

Cable theory aims to amalgamate these biophysical principles and

Hodgin and Huxley developed the first known computational model of a neuron by studying the giant squid axon in **1927.** Prior to this time, it had been known that nerve cells communicated via electric stimuli however this was not formalized until their depiction of the equivalent circuit.

Neurons are traditionally modelled using the cable theory or equivalent circuits. In such models the equilibrium potential of each ion is represented by a battery whose driving force (EMF) is given by the Nernst Potential. Changes in the membrane permeability are modelled by changes to conductances. Such changes to conductances do not change the equilibrium potentials of the neuron.



Cable theory is useful in modelling many neuronal processes, however there are limitations to its applicability. In areas of small intracellular volume (e.g. dendritic spines), the ionic concentrations can change rapidly (e.g. calcium transients). Such concentration changes are not accounted for in cable theory as ionic reversal potentials are fixed and not dependant on concentration changes with time. Thus an additional term is needed to account for this, and the equation needs to be computed for each ion in time.

1. **Electrodiffusion based models**

In the traditional computational models two main assumptions are made; firstly, that the extracellular soup is far larger than the subcellular space; and that there are no external sources of electric fields. Both assumptions used to simplify the complexity of the brain are likely invalidated in living tissue.

According to Savtchenko et al.2 there are 3 major sources of electric fields: (1) fields as a result of electric current flow also referred to local field potentials, (2) fields to the heterogeneity in the distribution of channels causing net submembrane currents, (3) fields across the synaptic cleft

Electrodiffusion based models incorporate the electrical driving forces (drift) of ions, as well as the movement of ions along their concentration gradients (diffusion). Albert Hodgkin, one of the pioneers in the field of neuronal modelling, makes the following analogy: “*diffusion is like a hopping flea… electrodiffusion is like a flea that is hopping in a breeze”*  3*.* To make this analogy more complete we need to consider different fleas moving at different speeds with different breezes. Moreover, each flea is impact the other as a function of time.

Clearly modelling this highly dynamic and complex process requires significant computational power. This factor has dissuaded computational biologists from pursuing such models, however with the computational resources publicly available in the year 2020/2021, such computations can be performed in a few hours as opposed to days or weeks.

1. **Impermeant anions in an electrodiffusion model**

The Gibbs-Donnan Effect describes the effect of impermeant anions in the cell4.

Dusterwald et. al5 showed that the addition of impermeant anions in a multicompartmental model effects the electrical and osmotic functions of dendrites.

In a single compartmental model, Dr. Dusterwald showed that altering the concentrations of impermeant anions intracellularly or extracellularly does not change the steady state concentrations of the major ionic species due to balanced osmotic changes. However, when the average charge of impermeant anions changes there are significant changes to reversal potentials of various ions, as well as changes to the membrane potential. Due to the relatively constant ratio of these changes the driving force (Vm – Reversal potential) of the various ions do not significantly change.

Similar effects were demonstrated in a multicompartmental model, however the changes to the driving force were even less due to the impact electrodiffusion had on the sodium ion concentrations thus impacting the Na\_K\_APTase.

In both the single and multicompartment simulations, impermeant anion concentrations were key determinants of cell/compartment volumes. Similarly changing the average charge of impermeant anions had a persistent impact on cell volume.

As impermeant anions had significant effect on cell volumes it was postulated that adding impermeant anions in the apical portions of the dendrites may mimic the increases in cell size of a growth cone. This too was shown via simulations thus illustrating the potential ability of impermeant anions to grow neuronal processes, and it may be possible that neurons could use the transport and tethering of impermeant anions to grow or modify the volume of neuronal compartments

Don’t know what the effect of varied Vm on these properties will be

1. **Impermeant anions in disease**

Osmotic balance in the brain is highly regulated by impermeant anions, therefore in cerebral oedema it is likely impermeant anions play a role in the pathophysiological processes. After a stroke or a traumatic brain injury (TBI) the brain swells leading to an increase in intracranial pressure (ICP). High ICPs result in the paradoxical occlusion of blood vessels leading to worsening ischaemia.

Elkin et al.6 showed that when Na-K-ATPase pumps fail (due to ATP depletion that occurs with ischaemia), cations flood into the neuron as they are drawn to the impermeant anions. The inward movement of cations driven by the Donnan osmotic pressure into ischaemic tissue causes water to follow inwards and result in brain swelling. Thus it is likely that impermeant anions are perpetuating the pathological processes which occur in cerebral oedema.

Impermeant anions such as intracellular proteins play an important role in several disease processes. The more salient of these are the Tauopathies which hallmark neurodegenerative disorders. In Alzheimer’s Disease the Tau protein builds up intracellularly and starts off as a soluble protein but as it becomes phosphorylated and bundled together with microtubules these proteins become insoluble and thus are trapped within the intracellular compartment. The progression of Alzheimer’s Disease is described by Braak’s staging which is based on the spread of Tau inclusions from the entorhinal cortex (Braak stage I and II), to the hippocampus (stage III), then to the neocortex (stage IV and V). The spread of intracellular Tau proteins also reflects the symptom progression in Alzheimer’s Disease from asymptomatic to deficits in memory and ultimately higher cortical functioning.7

What is the average intracellular charge of Tau proteins?

Several other neurodegenerative disorders are also characterised by subcellular protein build up. In Parkinson’s Disease intracellular alpha-synuclein deposition and is also correlated to disease progression and symptomatology. Similarly, in Pick’s Disease, Pick bodies can be found inside neurons. Interestingly, Tau proteins can also accumulate in glial cells and play a role in Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD). Increased Tau proteins in astrocytes have also been linked to aging.

Extracellular protein deposition also occurs in Huntington’s disease, Multiple Sclerosis, Spinocerebellar Ataxia and Transmissible Spongiform Encephalopathy (the most common being Jakob-Creutzfeldt disease)8

A common theme throughout the disease spectrum is neurodegenerative change associated with subcellular build up of protein. Perhaps such intracellular protein deposition plays a role in the electrical and computational properties of dendrites and thus may contribute disease processes.

**Most popular models:**

**Limitations of current models:**

**Dendritic computation**

These models make use of the Cable Theory by Rall which assumes that the ionic reversal potentials remain constant along the length of the cable9. Recent work by our group has suggested that variable activity of chloride channels along dendrites may result in local changes to chloride reversal potentials resulting in non-isopotential dendritic compartments– thus violating a core assumption in Cable Theory. Investigating what the implications of this violation are on the electrical and information processing properties of the neuron may shed further light on how the brain is able to function and may better depict how neurons behave in certain disease states.

Status quo did not include impermeant anions and electrodiffusion.

Why haven’t they been done before

Computational expensive – but now feasible?

Don’t go into the mathsy part of it

**Model**

The Cable Theory of Rall was devised to model the attenuation of electrical signal across a dendrite. The full derivation of the Cable equation and its steady state solution are made in Appendix A and B respectively.

The Cable Equation:

With this equation we can integrate and solve for Vm. The steady-state solution to the cable equation is:where,

According to this solution the membrane voltage is dependent on the initial voltage multiplied by an exponential function reflecting the decay of the signal based on the distance along the cable and inherent cable properties10.

In this equation there is no consideration for the dynamic effects of the reversal potential based on rapid changes in ions. Moreover, as the reversal potentials of the ions are considered constant, the equation fails to consider the fact that impermeant anions may be distributed in a spatially heterogenous manner.

**Spatial aspects of synaptic integration**

* In pyramidal cells, distal inputs are amplified via dendritic spikes or plateau potential supporting local coincidence detection and gain modulation
* Dendritic inward currents play a major role in control of spiking or response to synchronous inputs (plasticity)
* Dendritic branching structures and axonal geometries

**Disease**

* Detailed spatial representations help predict the effects of extracellular electrical stimulation. This is essential for deep brain stimulation used in the treatment of Parkinsons Disease.
* CCCs in disease (Kalia paper)

One of the fundamental assumptions of the cable theory is that the voltage across the dendritic compartment is uniform. This assumption is critical as it provides the basis for the cable equation which calculates the voltage across the dendrite at any point in time.

# Aims and objectives

The overall aim of my thesis is to use a biophysical computational neuronal model to investigate the implications of non-isopotential dendrites on the electrical and information processing properties of neurons.

The objectives are as follows:

1. Develop a computational tool to dynamically model ion homeostasis, volume regulation, and electrical changes that occur within a neuron.
   1. Create a single compartment model.
   2. Create a multicompartment model incorporating the properties of electrical drift and diffusion.
   3. Create a tool to visualize the changes to the ionic concentrations, electrical properties and cell volume within each compartment as these properties vary with time.
2. Investigate the effects of adding excitatory or inhibitory synaptic input to the non-isopotential dendrite on each of the dendritic compartments as well as at the soma.
3. Investigate the effect non-isopotential neurons have on information processing and dendritic computation
4. Explore how any observed effects may be relevant to disease processes.
   1. How do impermeant anion concentrations change in diseases. Can this be modelled.

Computational models describing the dynamic biophysical properties of neurons have significantly aided modern understanding of how the brain processes information. Most detailed computational models focus on how dendrites modulate synaptic inputs and convey their electrical signals towards the soma11. Two of the most popular models, the Equivalent Circuit Model and Cable Theory, fail to couple the simultaneous electrical and osmotic changes which drive ionic currents. An Electrodiffusion based model been proposed to overcome the limitations of previous models but requires each variable in the model to be calculated for every time step. Due to the computational complexity this entails, electrodiffusion based models have been out of favour, however with the speed of modern electronic systems this challenge can be overcome.

Recent work by our group using a multicompartment model incorporating electrodiffusion found that impermeant anions may play a role in neural function. Few studies have looked at the role impermeant anions and even fewer have examined the role spatial inhomogeneities of these impermeant anions may have on the electrical and computational properties of dendrites. Herein we propose to probe the implications and possible functions spatial inhomogeneity of impermeant anions may play by using an electrodiffusion based multi-compartment neuronal model.

Recent work by our group has manipulated the properties of impermeant anions in both single and multicompartmental models. It has been shown that changing the intracellular, or extracellular concentrations of impermeant anions has no effect on the steady state membrane potential or reversal potentials of sodium, chloride and potassium. Dynamically increasing the amount of impermeant anion inside the cell does however lead to a transient hyperpolarization of the membrane while the dynamic addition is take place, but similarly returning to steady state once the addition of impermeant stops.

Although there were very minimal effects on the electrical properties of the neuron there were significant changes in the volume of the cell. There appears to be a linear relationship between the initial intracellular concentrations of impermeant anions and the final volume of the neuron.

Changing the average charge of impermeant anions did change the absolute values of the various ionic reversal potentials and the membrane potential, but in such a ratio to keep the changes to the ionic driving forces negligible.

* 1) To develop a ^^\*\*computational biophysical model\*\*^^ of a neuron
  + 1.1 - Develope a single compartment neuronal model similar to Dr. Dusterwald and Dr. Currin in the python programming language
  + 1.2 - Ensure that the model is bio realistic by comparing to laboratory data
  + 1.3 - Develope a multicompartmental model similar to Dr. Dusterwald and Dr. Chris Currin
  + 1.4 - Create a tool to visualize what is occuring in each compartment
    - Visualize the voltage, ion concentration, and volume of each compartment
* 2) To explore the^^ \*\*feasibility of non-isopotential dendrites and spatial inhomogeneities in dendritic voltages\*\*^^
  + 2.1 - Are there fundamental assumptions that are being broken when we making the neurons non-isopotential
  + 2.2- Why does NEURON assume that dendrites are isopotential
* 3) To explore how impermeant anions and non-isopotential dendrites affect ^^\*\*inhibitory and excitatory processing\*\*^^
  + 3.1 - Add excitatory stimulation to the neuron and identify the changes that occur in the various compartments
  + 3.2 - Same as the above but with inhibitory stimuli
* 4) Determining the^^ \*\*information processing \*\*^^effect at neural outputs and local dendritic processing
  + 4.1 - Summarize the work on [[dendritic processing]] by Dayan and Abbot
  + 4.2 - Think about how non-isopotential neurons may affect dendritic processing
* 5) Exploring the relevance to^^ \*\*disease processes\*\*^^
  + 5.1 - Do a mini-literature review on how various diseases may change the impermeant intracellular anions
  + 5.2 - Incorporate these findings into the model and identify how the system then functions

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