# Research Proposal

*Name:* **Eran Frank Shorer**

*Degree:* **MSc (Med) Neuroscience**

*Supervisor:* **Dr. Joseph Valentino Raimondo**

Investigating the effects of impermeant anions and electrodiffusion on the Electrical and computational properties of neurons

****

**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.

**Signature: E.S.**

**Date: 29 November 2020**

The brain processes information via intricate non-linear interactions between chemical and electrical signals which vary along narrow spatiotemporal scales further constrained by human physiology. The propagation of such signals can be simulated using computational software such as NEURON1,2 and GENESIS3 which are based on theoretical descriptions of neurons by Hodgkin and Huxley4 in their equivalent circuit model, and was later expanded upon by Rall5 who incorporated geometric aspects in Cable Theory. These theories provide valuable predictions of how signals propagate, however are limited in certain respects. Firstly, they are inaccurate in low volume spaces and in areas with rapid ionic fluxes (e.g. dendritic spines)6,7, and secondly fail to adequately account for impermeant anions which can influence disease processes. Herein I propose constructing an electrodiffusion based multicompartmental neuronal model which will incorporate impermeant anions and adapt to small scale changes to ionic concentrations. Once constructed I shall run simulations to elucidate the impact impermeant anions have on the biophysical and computational properties of dendrites, and further probe how this may be implicated in disease processes such as epilepsy, neurodegenerative disorders and cerebral oedema. This work will provide better theoretical descriptions of normal and pathological neuronal functioning and may provide avenues for therapeutic intervention.

**Current computational models and their limitations**

State of the art software used to model biophysical processes involved in neural signalling includes NEURON and GENESIS developed at Yale and Cambridge respectively. Such software enables neuroscientists to cross-validate experimental findings and allows for estimation of values which are not easily attainable through current experimental methods2. Moreover, NEURON and GENESIS allow for theoretical hypothesis testing which can develop theory of the biophysical mechanisms that exist in the nervous system. However, simulation software can only be as accurate and robust as their theoretical backbone. Hodgkin-Huxley principles of equivalent circuits, and Cable Theory by Rall1 are the predominant theoretical foundations for these software.

Cable Theory considers the equilibrium potential of each ion species as a constant battery (i.e. constant driving force)8. The driving force of an ion is the difference between the membrane potential and the reversal potential of the ionic species. In larger neurons the membrane potential and reversal potentials change slowly and in a relatively fixed ratio to each other, thus the assumption that each ion can be considered a constant battery is relatively sound. In smaller spaces however, such as in dendritic spines, there are rapid ionic fluxes within a compartment. Therefore, the ionic reversal potentials and membrane potentials will unlikely fluctuate with the same ratio. In such instances modelling ionic driving forces as a constant parameter is inappropriate.

Cable Theory also assumes that extracellular potential is constant (ground); local field potentials and electroencephalographic changes clearly prove this to be false. Moreover there are ephaptic coupling effects through the interactions of local electric fields of nearby neurons which are also not accounted for9.

The assumptions made in Cable Theory limits its applicability as well as the software which is founded upon it (NEURON and GENESIS). Nevertheless, these models provide similar approximations to experimental setups across large spatial scales (e.g. squid giant axons) and neural networks10. In order to account for the detailed spatiotemporal changes in smaller scales electrodiffusion based models that are founded on the Nernst-Plank Equation have been proposed by several groups9,11. In these instances, the ionic driving force is dynamic and the extracellular potentials and electric fields can be accounted for ‘. These models allow for the study of areas which cannot be adequately modelled by Cable Theory and provide a computational framework to simulate disease states with rapid ionic changes.

**Rapid ionic changes are implicated in disease processes**

Rapid ion fluxes occur in certain neurological disorders and hence modelling these dynamics are essential in the search for the mechanisms and cures of these disorders. In cortical spreading depression there is a wave of depolarizing brain activity followed by a transient wave of inhibition. This pattern of brain activity is thought to account for migraine auras and have been found to perpetuate neural damage in ischaemic and haemorrhagic strokes. Such waveforms are thought to result from interstitial K+ diffusion12 occurring at rapid time scales.

Epileptic seizures are also characterized by rapid ionic changes13. Moreover, seizures and status epilepticus, are challenging to study experimentally, thus computational models offer a more accessible means to test hypotheses. Models which aim to simulate the cellular processes occurring in epilepsy and cortical spreading depression require the capacity to account for ionic fluctuations and subsequent changes in ionic driving forces. In silico testing using electrodiffusion based models may ultimately provide novel mechanisms for the treatment of these life-threatening conditions

**Electrodiffusion based models**

Electrodiffusion based models incorporate ionic movement due to electric fields (drift), 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”*  14*.* A detailed understanding of both the electrochemical diffusive properties of ions and the respective electric fields *(“breeze”)* which surround them is therefore needed to model this phenomenon.

A recent review of Electrodiffusion by Savtchenko et al.7 classify three major sources of electric fields. Firstly, fields as a result of electric current flow also referred to local field potentials or extracellular currents. Such currents are not accounted for in Cable Theory. Secondly, fields occurring due to the heterogeneity in the distribution of membrane ion channels causing net submembrane currents, and thirdly, fields across the synaptic cleft. In electrodiffusion ionic currents affect the field, and likewise, the electric field affects ionic currents.

Calculating the detailed interaction between the field and current allows for the simultaneous and precise determination of ionic concentrations at discrete moments in space and time11; such dynamic values are not accessible in Cable Theory. Qian and Sejnowski15 developed one of the first electrodiffusion based models and compared it to Cable Theory. They found that in settings of rapid ionic flux and thin dendritic processes (<0.1 um) significant errors were made in the predictions of membrane potentials and concentrations when the Cable Theory was used relative to their one-dimensional electrodiffusion based model.

Another finding by Qian and Sejnowski15 reflects that electrodiffusion based models can help advance neuroscientific theory. They showed that due to electro-diffusive properties inhibitory inputs which synapse on dendritic spines are ineffective. This may provide partial explanation for the mystery of why most synaptic input onto spines is excitatory. Savtchenko et al7 also speculate that it is the electro-diffusive phenomena which influence synaptic plasticity at dendritic spines16.

Modelling this highly dynamic, non-linear and complex process requires significant computational power. This factor has dissuaded computational biologists from pursuing such models, however with the computational resources now publicly available, such computations can be performed in a few hours as opposed to days or weeks. Incorporating electrodiffusion into computational models of neurons is a necessary step in making accurate models which can deal with rapid ionic fluxes. A further step forward would be to include impermeant anions in electrodiffusion based models.

**Impermeant anions**

Impermeant anions are negatively charged molecules (e.g. proteins, nucleic acids, metabolites etc.) which are often neglected in the traditional computational models of neurons. Unlike sodium (Na+), potassium (K+), chloride (Cl-), and bicarbonate (HCO3-) ions which can move freely between intracellular and extracellular compartments, impermeant anions are trapped on either side of the membrane as there are no specific channels or pumps for them in human tissues. Due to the vast extracellular volume relative to the intracellular volume, the concentrations of impermeant anions are much larger intracellularly. Computational models often assume a fixed charge and concentration for impermeant anions in both the intra and extracellular environments, however in reality these parameters may vary.

If we consider dendrites to be consisting of multiple compartments, there can be even further complexity regarding impermeant anions. Considering that the cellular machinery for protein synthesis (ribosomes and endoplasmic reticula)17 occurs throughout the dendrite and soma in a non-uniform manner, it is likely proteins are heterogeneously distributed in the neuron. These proteins contribute to the milieu of impermeant anions. Variations in the in valence and concentration of local impermeant anions may result in difference in the electrical and osmotic properties in individual dendritic compartments. Exploring how electrical signals propagate along dendrites with these heterogenous compartments may lead to novel insights into neural functioning.

The Gibbs-Donnan effect describes the broad implications impermeant anions have on cellular compartments enclosed by a semi-permeable membrane18,19. As impermeant anions are trapped intracellular they require cations of equal net ionic charge to move intracellularly to ensure electroneutrality. This will bring water into the cell via osmosis and subsequently dilute the intracellular compartment. The concentration gradient of permeant anions will then also be driven inwards. This repetitive cycle would ultimately lead to uncontrolled cell swelling and bursting if not for active sodium extrusion via Na-K-ATPases. Another possible cellular strategy could be to pump water out of the cell however there is no evidence of aquaporins or similar structures in neurons.

Computational simulations developed by Dusterwald et. al20 tested the above hypotheses by adding impermeant anions in single and multicompartment neuronal models and explored their effects on the electrical and osmotic properties of dendrites. In a single compartmental model, altering the concentrations of impermeant anions intracellularly and/or extracellularly did not change the steady state concentrations of the major ionic species due to balanced osmotic changes. However, when the average charge of impermeant anions changed, there were significant deviations in the reversal potentials of various ions, as well as changes to the membrane potential. Although, due to the relatively constant ratio of changes in membrane and reversal potentials, the driving force of the various ions do not significantly change.

Similar effects were demonstrated in a multicompartmental model, however the changes to the driving force were further diminished due to the impact on the sodium ion concentrations (and therefore the Na-K-APTase pump rate). 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 in simulations by Dusterwald et al20 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. Speculatively, the interaction between impermeant anions and electrical fields may also contributes to plasticity through the development of dendritic spines.

Although some work has been done to explore the osmotic and electrical effects of impermeant anions there are still many unanswered questions. It remains unknown whether spatial inhomogeneities in the distribution of impermeant anions plays a role in neural function. Moreover, the impact of impermeant anions in an electrodiffusion based model has not yet been adequately explored. In my thesis I will investigate this unknown territory whilst also considering the effect of impermeant anions in disease processes.

**Impermeant anions in disease**

As physiological osmotic balance in the brain is highly regulated by impermeant anions, in cerebral oedema it is likely that impermeant anions play a role in the pathophysiological processes as well. 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. Ischaemia leads to further swelling which compresses the brain leading to more cell death in a self-perpetuating and destructive manner.

The transition from ischaemia to swelling can be partially attributed to the impact of impermeant ions. When ATP (adenosine triphosphate) is depleted in ischaemia, the sodium-potassium pumps fail leading to an inability to pump cations out of the cell. Impermeant anions drive the inward movement of cations via the Donnan osmotic pressure. This flow causes water to enter the cell and result in cell swelling. Although there are other hypotheses to explain the swelling in ischaemia, it is likely that impermeant anions are perpetuating the pathological processes that are occurring21.

Impermeant anions also contribute to the pathology seen in several neurodegenerative disorders, most prominently are the Tauopathies where Tau protein is one of the hallmarks of several diseases including Alzheimer’s Disease. The Tau protein begins as a soluble intracellular protein but as it becomes phosphorylated and bundled together with microtubules these proteins become insoluble and thus are trapped within the intracellular compartment. The clinical progression from short term memory loss to executive dysfunction in Alzheimer’s Disease closely mirrors the accumulation and spread of Tau proteins through specific brain regions (described by Braak’s staging)22.Tau proteins can also accumulate in glial cells and play a role in Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD), while increased Tau proteins in astrocytes have been linked to aging.

Several other neurodegenerative disorders are also characterised by subcellular protein build up. In Parkinson’s Disease intracellular alpha-synuclein deposition and is correlated to disease progression and symptomatology. Similarly, in Pick’s Disease, Pick bodies can be found inside neurons. Extracellular protein deposition also occurs in Huntington’s disease, Multiple Sclerosis, Spinocerebellar Ataxia and Transmissible Spongiform Encephalopathy (the most common being Jakob-Creutzfeldt disease)23.

**Conclusion**

In summary, software such as NEURON and GENESIS utilize Cable Theory to provide neuronal simulations; however, such models of neuronal signal propagation are imprecise in certain conditions, and thus electrodiffusion based model may be more suitable. Moreover, traditional models may not adequately appreciate the influence of impermeant anions on the electrical properties of the dendrite. Impermeant anions play a role in several disease processes such as that occurring in cerebral oedema and neurodegenerative disorders. Modelling the role impermeant anions play in normal and pathological states may help lead to a better pathophysiological understanding of the conditions and ultimately to better therapies.

# Aims and objectives

The overall aim of my thesis is to develope a biophysically accurate computational neuronal model incorporating electrodiffusion to investigate the influence of impermeant anions 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 on each of the dendritic compartments as well as at the soma.
3. Investigate the effect of impermeant anions may have on the osmotic regulation of neurons.
4. Investigate the effect impermeant anions may have on neuronal plasticity and information processing
5. Explore how any observed effects may be relevant to disease processes.

**THINGS I COULD INCLUDE BUT HAVE NOT HERE:**

* Details about dendrites and dendritic spines
* Computational aspects of dendritic spines and how they can be used to modulate signals and play a role in plasticity.
* 2D vs. 3D electrodiffusion models
* Variations in electrodiffusion models used in the literature
* Could impermeant anion average charge/concentration change be a substrate for longer term plasticity/updating of weights in an ML framework? What is the impact of phosphorylation on electrical and computational properties?
* Influence of dendritic branching structures on electrical properties and in epilepsy/ downs syndrome

# References

1. Hines ML. The NEURON Simulation Environment. 1997;1209:1179-1209.

2. Hines ML, Carnevale NT. NEURON : A Tool for Neuroscientists. 2001:123-135.

3. Bower J, Beeman D. *The Book of GENESIS: Exploring Realistic Neural Models with GEneral NEural Simulation System*. Springer; 1998.

4. Hodgkin, Huxley. A Quantitative Description of Membrane Current and its Application to Conduction and Excitation in Nerve. *J Ph*. 1952;117:500-544.

5. Rall W. Wilfrid Rall: A short autobiography.

6. Pods J, Schönke J, Bastian P. Electrodiffusion models of neurons and extracellular space using the poisson-nernst-planck equations - Numerical simulation of the intra- and extracellular potential for an axon model. *Biophys J*. 2013;105(1):242-254. doi:10.1016/j.bpj.2013.05.041

7. Savtchenko LP, Poo MM, Rusakov DA. Electrodiffusion phenomena in neuroscience: A neglected companion. *Nat Rev Neurosci*. 2017;18(10):598-612. doi:10.1038/nrn.2017.101

8. Lopreore CL, Bartol TM, Coggan JS, et al. Computational modeling of three-dimensional electrodiffusion in biological systems: Application to the node of Ranvier. *Biophys J*. 2008;95(6):2624-2635. doi:10.1529/biophysj.108.132167

9. Ellingsrud AJ, Solbrå A, Einevoll GT, Halnes G, Rognes ME. Finite Element Simulation of Ionic Electrodiffusion in Cellular Geometries. *Front Neuroinform*. 2020;14(March):1-18. doi:10.3389/fninf.2020.00011

10. Markram H, Muller E, Ramaswamy S, et al. Reconstruction and Simulation of Neocortical Microcircuitry. *Cell*. 2015;163(2):456-492. doi:10.1016/j.cell.2015.09.029

11. Solbrå A, Bergersen AW, van den Brink J, Malthe-Sørenssen A, Einevoll GT, Halnes G. A Kirchhoff-Nernst-Planck framework for modeling large scale extracellular electrodiffusion surrounding morphologically detailed neurons. *PLoS Comput Biol*. 2018;14(10):1-26. doi:10.1371/journal.pcbi.1006510

12. Enger R, Tang W, Vindedal GF, et al. Dynamics of Ionic Shifts in Cortical Spreading Depression. 2015;(November):4469-4476. doi:10.1093/cercor/bhv054

13. Raimondo J V, Burman RJ, Katz AA, Akerman CJ, Raimondo J V. Ion dynamics during seizures. 2015;9(October):1-14. doi:10.3389/fncel.2015.00419

14. Koch C. *Biophysics of Computation Information Processing in Single Neuron*. Vol 11.; 2004. doi:10.3902/jnns.11.157\_2

15. Qian N, Sejnowski TJ. An electro-diffusion model for computing membrane potentials and ionic concentrations in branching dendrites, spines and axons. *Biol Cybern*. 1989;62(1):1-15. doi:10.1007/BF00217656

16. Trappenberg T. *Fundamentals of Computational Neuroscience*.

17. Hammond C. *Cellular Neurophysiology*. Vol 480.; 1994. doi:10.1113/jphysiol.1994.sp020392

18. Kay AR. How cells can control their size by pumping ions. *Front Cell Dev Biol*. 2017;5(MAY):1-14. doi:10.3389/fcell.2017.00041

19. Blaustein M, Kao J, Matteson D. *Cellular Physiology and Neurophysiology*. 2nd ed. Philadelphia; 2012.

20. Dusterwald KM, Currin CB, Burman RJ, Akerman CJ, Kay AR, Raimondo J V. Biophysical models reveal the relative importance of transporter proteins and impermeant anions in chloride homeostasis. 2018:1-30.

21. Elkin BS, Shaik MA, Morrison B. *Fixed Negative Charge and the Donnan Effect: A Description of the Driving Forces Associated with Brain Tissue Swelling and Oedema*. Vol 368.; 2010. doi:10.1098/rsta.2009.0223

22. Goedert M, Eisenberg DS, Crowther RA. Propagation of Tau Aggregates and Neurodegeneration. *Annu Rev Neurosci*. 2017;40:189-210. doi:10.1146/annurev-neuro-072116-031153

23. Schaffert LN, Carter WG. Do post-translational modifications influence protein aggregation in neurodegenerative diseases: A systematic review. *Brain Sci*. 2020;10(4):1-37. doi:10.3390/brainsci10040232

24. Wu J and. *Foundations of Cellular Neuroscience*.

25. Dayan P, Abbot L. *Theoretical Neuroscience*. Vol 4.; 2014. doi:10.1080/2153599X.2014.951911

26. Johnston, Wu. *Foundations of Cellular Neurophysiology*.; 1995. doi:10.1097/00004691-199703000-00009