# Research Proposal

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

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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 is typically simulated using computational approaches that employ equivalent circuits and theoretical descriptions by Hodgkin and Huxley1 (Hodgkin Huxley model) and Rall2 (Cable Theory). These approaches provide valuable predictions of how signals propagate, however are limited in certain respects. Firstly, they are inaccurate in low volume spaces and in areas prone to rapid ionic fluxes (e.g. dendritic spines)3,4, and secondly fail to account for impermeant anions. 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. In particular, I will determine whether impermeant anions can alter the isopotential status of neurons. I will also determine whether impermeant anions can alter the propagation of synaptic input, and possibly affect neuronal output. Finally, I will probe how such alterations could play a role 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**

Neurons are fundamental computational units of the brain. Electrical signals received by dendrites undergo arithmetic and non-arithmetic transformations while propagating toward the soma. If a threshold voltage is reached the neuron will produce an output in the form of an action potential which is transmitted via its axon. A detailed understanding of this process is essential to fathoming how the brain performs its marvellous and complex functions.

Numerous theoretical models attempt to mathematically and conceptually describe this process, the most prominent of which are equivalent circuit-based models and Cable Theory5. In equivalent circuit-based models, cellular mechanisms which are involved in signal processing in the neuron are likened to electronic components in an electrical circuit. This work earned Hodgkin-Huxley the Nobel Prize for physiology in 1963 as their model could accurately predict neural outputs in the squid giant axon. Cable Theory extends equivalent circuit models by considering how the electrical signal attenuates as it propagates from the dendrite to the soma thus ensuring that dendritic diameter, dendritic length, and intracellular axial resistance are accounted for.

There are however instances where equivalent circuit models and Cable Theory do not provide good descriptions due to their inherent limitations. One major limitation of Cable Theory is that it considers the equilibrium potential of each ion species as a constant battery6. Across large spatial scales (e.g. squid giant axons) 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 and provides similar predictions as can be gathered from experiment7. 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.

A second limitation of traditional neural models is that they do not appreciate the role of impermeant anions in signal processing. Impermeant anions are negatively charged molecules which are produced inside the neuron yet have no flux through the cell membrane. Such molecules contribute to the electrical and osmotic properties of the neuron but their role in the signal propagation is under investigated and remains unknown.

When impermeant anions are added to a multicompartmental model, a third limitation may occur. That is, in Cable Theory individual compartments are considered isopotential (equal membrane potential)8. By adding impermeant anions in different concentrations in the various compartments it is likely they will become non-isopotential. If the neuron was indeed non-isopotential, this would have implications on the signal propagation properties of the neuron.

Although no approaches have yet accounted for impermeant anions, an electrodiffusion based strategy has been proposed by several groups to address the first limitation (inaccuracies with rapid ionic flux)910,10,11.

**Electrodiffusion based models**

Electrodiffusion, calculated with the Nernst-Plank equation, encompasses ionic movement resulting from electric fields (drift), as well as the movement of ions along their concentration gradients (diffusion). Incorporating these two aspects simultaneously in discrete spatiotemporal locations allows ionic reversal potentials to be dynamic and hence addresses the first limitation of *‘traditional’* neural models. Alan 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”*  12*.* 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.4 distinguishes 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 Sejnowski9 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 Sejnowski9 provides further evidence 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 provides a partial explanation to the mystery of why most synaptic input onto spines is excitatory. Savtchenko et al4 also speculate that electro-diffusive phenomena influence synaptic plasticity at dendritic spines13.

Despite the promises of electrodiffusion based models, modelling in this highly dynamic, non-linear and intricate fashion requires significant computational power. This stumbling block prevented neuroscientists from adopting electrodiffusion models, however with the computational resources now publicly available, the computations involved can be performed in a few hours as opposed to days or weeks. With rapid developments in computing power it is expected that electrodiffusion based models will become more popular and consequently the door to new insights into disorders of rapid ionic fluxes will be opened.

**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 potential avenues for therapeutic intervention. 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+ diffusion14 occurring at rapid time scales.

Epileptic seizures are also characterized by rapid ionic changes15. 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 and treatments for these life-threatening conditions.

**Impermeant anions**

The second major limitation of equivalent circuit models and Cable Theory is that they overlook the role of impermeant anions. Impermeant anions are strongly negatively charged molecules (e.g. proteins, nucleic acids, metabolites etc.) which accumulate intracellularly. Unlike sodium (Na+), potassium (K+), chloride (Cl-), and bicarbonate (HCO3-) ions which can move freely between intracellular and extracellular compartments, impermeant anions are trapped within the cell. There are also proteins and negatively charged molecules extracellularly, however due to the vast extracellular volume relative to the intracellular volume, the concentrations of impermeant anions which exist extracellularly is minimal. 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. Seeing that the cellular machinery for protein synthesis (ribosomes and endoplasmic reticula)16 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 leading to non-isopotential compartments. Exploring how electrical signals propagate along dendrites with these non-isopotential 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 membrane17,18. 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. al19 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 al19 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 occurring20.

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)21.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)22.

**Conclusion**

Equivalent circuit models and Cable Theory are means of modelling neural signal propagation but are limited in that they cannot make accurate predictions in areas of rapid ionic fluxes (e.g. dendritic spines). Moreover, they do not account for impermeant anions; molecules whose effect on signal propagation remains unknown. Both rapid ionic fluxes and impermeant anions contribute to diseases which can’t be adequately modelled with current strategies. Electrodiffusion based models allow for accurate, albeit computationally expensive, predictions in instances where traditional models are limited. In this MSc I propose constructing an electrodiffusion based model to investigate the impact impermeant anions have on the isopotential status of neurons and the implications this will have on neural signalling. Once developed this model will allow me to advance neuroscientific theory regarding the role of impermeant anions and may further provide important mechanistic explanations of disease processes.

# 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. To develop a computational tool to dynamically model ion homeostasis, volume regulation and electrical changes that occur within a neuron
   1. Create a single compartmental model
   2. Create a multicompartmental model incorporating the properties of diffusion and electrical drift
   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 effect of impermeant anions on the isopotential status of neurons.
3. Investigate how excitatory or inhibitory synaptic input is modified by the presence of impermeant anions.
4. Investigate the impermeant anions have on information processing (action potential generation).
5. Explore how any observed effects may be relevant to disease processes.

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