Introduction

# Cellular architecture and electrical properties of neurons

## Building blocks of the brain

Neurons are the functional units of the brain. The 1012 neurons in the human nervous system act symphonically to process information that produces the cognitive functions humans are capable of1. Neurons receive multiple inputs, integrate, and transform them, and produce an output in the form of an electrical signal directed towards another neuron. Although there are a variety of neuronal morphologies, all neurons have a shared meta-structure which allows them to act as functional units.

The soma (cell body), of the neuron contains the nucleus and surrounding cytoplasm that encases neuronal organelles. The synthesis of proteins and other macromolecules that act to provide structural and functional integrity occurs predominantly in the soma. Furthermore, the soma acts as a receptive area for synaptic inputs to the neuron.

Emerging from the soma are primary dendrites which have several distal arborizations with specific morphologies unique to certain neuronal populations (surface area, number of branches etc.)2. The proximal portion of the dendrite is a cytoplasmic extension of the soma with a similar density of endoplasmic reticula and ribosomes. As the dendrite branches further, the diameter of the dendritic branch narrows with lesser amounts of cellular organelles present. Moreover, along the length of some dendrites exist dendritic spines which are pedunculated cytoplasmic extensions which also act as regions for predominantly excitatory input1 and early signal processing.

Should the summation of incoming inputs reach a threshold, the neuron fires an action potential down its axon. Unlike the soma and dendrite, axons do not have cellular organelles capable of synthesising macromolecules. The axon is narrow with a uniform diameter along its length that acts to transmit the output from the cell body towards other neurons.

**SCHEMATIC DEMONSTRATING NEURON STRUCTURE HIGHLIGHTING RIBOSOMES ETC ALONG DENDRITE. Something similar to Johnston and Wu figure 1.2**

## Neural signal propagation

Information in the brain is propagated by electrical and chemical signals between neurons. Synapses are the interface between two communicating neurons where such integration between chemical and electrical signals occurs. An activated pre-synaptic neuron will release chemical neurotransmitters into the synaptic cleft which bind to transmembrane receptor proteins on the post-synaptic neuron. This neurotransmitter-receptor interaction results in a conformational change to the receptor protein such that it can act as a channel for rapid ion flux between the extracellular environment and the neuron.

The magnitude and direction of ionic flux determines the effect of neurotransmission on the post-synaptic membrane as they can either cause an excitatory signal by increasing the membrane potential (depolarizing) or an inhibitory signal by decreasing the membrane potential (hyperpolarizing). Excitatory and inhibitory signalling is tightly controlled to provide precise neural communication. Understanding the homeostatic mechanisms regulating excitatory and inhibitory balance is critical as dysfunction in these mechanisms provide the basis for neurological disorders.

## Ionic homeostasis in neurons and the Nernst Potential

Ionic homeostasis is the ability for of cell to have stable ionic concentrations and adjust to external changes through self-regulating control mechanisms. Uniform ionic concentrations ensures that most neurons remain at rest, while only specific neurons are activated for precise neural signalling. Ionic homeostasis is maintained by a semipermeable cell membrane that acts as a gatekeeper for most ionic fluxes.

Ionic motion across a semipermeable membrane is governed by several forces. *Diffusion* refers to the movement of ions from a region of high ionic concentration to a region of low concentration across the membrane. Water movement follows ionic flux across the membrane via *osmosis* such that the osmoneutrality is maintained. Lastly, *drift* is the flux of charged molecules that occurs due the local electrical field. These electrical forces set up electroneutrality across the membrane such that there is a balance of positive and negative charges on either side. The net flux of ions therefore is a combination of both chemical and electrical forces.

The *Nernst-Planck Equation* (NPE) (1.1) is used to calculate the net flux for a particular ion. The equation makes use of the Einstein Relation which states that fluxes of diffusion and drift are additive in the same medium. Therefore, the NPE combines Ohm’s Law for Drift and Fick’s Law of Diffusion to calculate the net flux or current.

(1.1)

In (1.1), *I* is the current, *µ* the ionic mobility constant for a particular ion, *z* the valence, *NA* is Avogadro’s number, *F* is Faraday’s constant, [*C*] is the ionic concentration, *V* is the voltage, *x* is the distance between the potentials, *R* is the gas constant and *T* is the temperature. The negative sign in both terms indicate that the direction of current flow is opposite to the concentration/voltage gradients.

In steady state (resting) conditions, the net flux (*I*)for an ion is equal to zero. Therefore, equation (1.1) can be rearranged and solved for V, referred to as the *Nernst Equation* (1.2). The Nernst potential, also known as the equilibrium potential or reversal potential for a particular ion, is the voltage across the membrane (V*m*) at which the electrical forces balance the osmotic forces3.

(1.2)

In (1.2), *Eion* is the reversal potential for a particular ion, [*ion*]*o*is the concentration of the ion outside the membrane (extracellularly) and [*ion*]*i* is the intracellular ion concentration.

## Membrane potential and ionic driving forces

Two traditional ways have been used to calculate the resting membrane potential (V*m*), the “charge-sum” and “charge-difference” approach. The “charge-sum” approach entails setting the summation of the currents to equal zero (which is becomes a differential equation that is solvable4. The solution however requires the initialization values to be known. The “charge-difference” method5 provides the same solution for Vm, without requiring initialization values, by utilizing the formula for capacitance (. Charge (*q*) is the difference between the extracellular and intracellular charges. A neuron existing in a neutral extracellular environment will have an extracellular charge of zero while the sum of the internal concentration and valences are required to calculate the intracellular charges by multiplying the net charge by Faraday’s constant (F) to convert mol to coulombs.

(1.3)

In the charge-difference equation, [*Na*+]*i* represents sodium concentration, [*K*+]*i* is the potassium concentration, [*Cl*-]*i* is the chloride concentration. [*X*-]*i* refers to the concentration of impermeant anions, with a valency of *z.*

The membrane potential (*Vm*)of a neuron at rest lies within the range of - 62 to - 75mV, indicating that the internal environment of the neuron contains more negative charges than the external environment. Concentration of ions also differ across the membrane as shown in table 1. The driving force (DF) of an ion is equal to the difference between the membrane potential and its equilibrium potential (*Vm* -*Eion*) and is the net force acting on the ion at rest.

To calculate the ionic current across the membrane due to the driving force Ohm’s law can be used (I = V/R). The inverse of resistance (1/R), conductance (*gion*), is typically preferred in neuroscience as it intuitively relates to how easily an ion can cross the membrane. This is predominantly determined by the selective ion channels open at a given time. Ohm’s Law with conductance and ionic driving force is shown in equation (1.4).

(1.4)

|  |  |  |  |
| --- | --- | --- | --- |
| Ion | Intracellular  concentration (mM) | Extracellular concentration  (mM) | Equilibrium  potential (Eion)  (mV) |
| Sodium (Na+) | 5-15 | 145 | 90.7 - 61.1 |
| Potassium (K+) | 140 | 5 | - 89.7 |
| Chloride (Cl-) | 4 | 110 | - 89 |

## The Na-K ATPase establishes the membrane potential and cell volume

# Modelling dendrites

* ? The need to model neurons computationally

## Modelling dendrites

Several mathematical/theoretical models have been proposed to predict neural functioning, each of which balance detail with abstraction. On one extreme black-box models focus solely on the input, processing, and output (functional) aspects of neurons while ignoring the role structural morphology plays. These models are useful trying to predict neuronal spiking frequencies based on varied inputs for example.

Contrastingly, detailed morphological models most accurately account for neuronal morphology such as dendritic branching patterns and axonal geometries. Unusual dendritic morphologies are present in disorders such as Down’s syndrome, senile dementias, and epilepsy1 , therefore when attempting to study such conditions these more detailed models may be more appropriate. However, not all aspects of morphology are necessary for understanding the dynamics of a single neuron and efforts to incorporate detailed structure may be redundant6. Equivalent circuit multi-compartmental models strike a balance between detail and abstraction.

## Equivalent circuit models and Cable Theory

Equivalent circuit models liken the core electrical properties of neurons to electronic components in a circuit, with each component in the circuit representing an electric property of the neuron.

The semipermeable plasma membrane is modelled as a capacitor based on the dielectric properties of the membrane with ion accumulating on either side of it. The capacitance of most neuronal membranes is approximately 1µF/cm2.1

Ion channels both permit and prevent ions crossing the membrane thus are modelled as resistors with variable conductances for specific ions. The conductances of these channels can vary with time and voltage, for instance at a synaptic junction when neurotransmitters bind causing post-synaptic ion channels allow ionic flux.

Internal resistance (*ri*) is the resistance to the flow of ions due to the cytoplasm (and intraneuronal structures) along the length of the dendrite. The dendrite length (*x*) and cross-sectional area (*A*) are the determinants of the internal resistance (thin and long neurons having the greatest the internal resistance). An internal resistivity constant (*rL*) is used as a scaling factor and is usually in the range of 1-3kΩ mm. Dendrites that are long and thin are termed electronically compact, and it is assumed that they have relatively constant internal resistance and thus a constant membrane potential along the length of the dendrite (isopotential)7.The formula below is used to calculate the internal resistance of a length of dendrite.

(1.5)

Lastly, the driving force of a particular ion is modelled as a battery as this provides the potential difference that sets up ion flow.

A picture containing dark

Description automatically generated

A screenshot of a computer screen

Description automatically generated with low confidence

Cable Theory is a model which compares dendritic (or axonal) segments to electrical cables that can carry current along their length.

Four assumptions of resting neurons are made in Cable Theory:1

1. The membrane resistance and capacitance (*rm* and *Cm*) are constant at all points along the dendrite.
2. Internal resistance (*ri*) is constant throughout the dendrite.
3. Current flows only in the longitudinal direction along the length of the cable (*x*). Radial current flow is therefore assumed to be zero.
4. Extracellular resistance (*ro*) is assumed to be negligible.

## Non-isopotential neurons and other limitations of Cable Theory

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 ionic species as being constant 8. Across large spatial scales the reversal potentials are relatively stable, thus the assumption that the transmembrane concentration gradient of each ion can be considered constant is relatively sound and provides similar predictions as can be gathered from experiment9. In smaller spaces however, such as in dendritic spines, there are rapid ionic fluxes within a compartment. Therefore, the ionic reversal potentials can fluctuate. In such instances modelling ionic reversal potentials 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 (e.g. proteins, nucleic acids, metabolites etc.) existing inside or outside of cells, but which cannot traverse 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)10. By adding impermeant anions with different average charge in the various compartments it is likely they will become non-isopotential. If the neuron was indeed non-isopotential, this might have implications on the signal propagation properties of the neuron, although this is not yet known.

## Electrodiffusion based models

An electrodiffusion based approach is necessary to evaluate the influence of impermeant anions on neural signal processing. 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) 1112,12,13. 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”*  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.15 distinguishes three major sources of electric fields. Firstly, fields because 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 time13; such dynamic values are not accessible in Cable Theory. Qian and Sejnowski11 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 Sejnowski11 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 al15 also speculate that electro-diffusive phenomena influence synaptic plasticity at dendritic spines16.

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. The rapid development in computing power which enables electrodiffusion based modelling has opened the door for neuroscientists to properly explore the influences of impermeant anions on neural signalling

# Impermeant anions

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 valence and concentration of local impermeant anions may result in difference in the electrical field leading to non-isopotential compartments. There are also proteins and negatively charged molecules existing extracellularly which may contribute to the electric field, 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.

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.

## Types of impermeant anions

## Gibbs-Donnan effect

## Impermeant anion homeostasis

* Mechanisms of protein degradation and turnover
* Kinesins and Dynein
* Rely heavily on Fraser and Huang 2004

## 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.

# Rationale

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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