Methods

# 2.1 Multicompartment Morphology

We utilized a multicompartment model to simulate a dendrite. Nine individual compartments were linearly arranged with ionic motion across compartments possible via electrodiffusion Each compartment was modelled as a cylinder with a diameter of 1µm and length of 20µm. The extracellular environment was modelled as an “infinite bath” with fixed ion concentrations, shown in **schematic 1**.

We make two assumptions regarding the morphology: that the compartment swells only in the radial dimension (therefore not affecting the electrodiffusion equations that are length dependant), and that the surface area of the compartment is fixed (as volume changes we consider the cell to wrinkle or become more turgid).

For the final set of experiments, we added a soma (length = 40µm; diameter = 2µm) onto compartment 1.

# 2.2 Transmembrane ion flux

Ionic flow between the internal and external environment were permitted via leak, sodium-potassium ATPase, and KCC2 channels. The direction of ionic flow was determined by respective osmolar and electrical gradients. The conductances of the leak channels were equal to those calculated in other studies (Table 1). **Schematic of the channels modelled in a single compartment.**

Graphical user interface

Description automatically generated with medium confidence

|  |  |  |
| --- | --- | --- |
| **Symbol** | **Value** | **Description** |
| **Constants** |  |  |
| F | 96485.33 C/mol | Faraday Constant |
| R | 8.31446 J/(K\*mol) | Universal gas constant |
| T | 310.15 K | Absolute temperature (37C) |
| **Parameters** |  |  |
| Cm | 2x10-4 F/dm2 | Membrane capacitance |
| gNa | 20 µS/cm2 | Na leak conductance |
| gK | 70 µS/cm2 | K leak conductance |
| gCl | 20 µS/cm2 | Cl leak conductance |
| gKCC2 | 20 µS/cm2 | KCC2 conductance |
| p | 0.1 C/(dm2/s) | ATPase pump rate |
| [Na+]o | 145e-3 | Extracellular Na Concentration |
| [K+]o | 3.5e-3 | Extracellular K Concentration |
| [Cl-]o | 119e-3 | Extracellular Cl Concentration |
| [X-]o | 29.5e-3 | Extracellular Impermeant anion Concentration |
| *Hodgkin-Huxley parameters* |  |  |
|  | 120 mS/cm2 | Maximal Na conductance |
|  | 36 mS/cm2 | Maximal K conductance |
| V1 | 100 mV·ms |  |
| V2 | -55 mV |  |
| V3 | 10 mV |  |
| V4 | 0.125 ms-1 |  |
| V5 | -65 mV |  |
| V6 | 80 mV |  |
| V7 | 10 mV·ms |  |
| V8 | -40 mV |  |
| V9 | 10 mV |  |
| V10 | 4 ms-1 |  |
| V11 | -65 mV |  |
| V12 | 18 mV |  |
| V13 | 0.07 ms-1 |  |
| V14 | -65 mV |  |
| V15 | 20 mV |  |
| V16 | 1 ms |  |
| V17 | -35 mV |  |
| V18 | 10 mV |  |
|  |  |  |
| **Variables** |  |  |
| [Na+]i |  | Intracellular Na Concentration |
| [K+]i |  | Intracellular K  Concentration |
| [Cl-]i |  | Intracellular Cl Concentration |
| [X-]i |  | Intracellular Impermeant anion Concentration |
| n | 0 |  |
| m | 0 |  |
| h | 0 |  |
| αn | 0 | Rate of potassium Hodgkin Huxley channel opening |
| βn | 0 | Rate of potassium Hodgkin Huxley channel closing |
| αm | 0 | Rate of fast act |
| βm | 0 |  |
| αh | 0 | Rate of potassium Hodgkin Huxley channel opening |
| βh | 0 | Rate of potassium Hodgkin Huxley channel closing |
|  |  |  |

## 2.2.1 Membrane potential

Membrane potential refer to the voltage differences between the inside of the compartment and the extracellular environment. All membrane potentials are denoted with a subscript “*m”.*

The membrane potential (Vm) was calculated using the ‘Charge Difference’ approach defined by Fraser and Huang1

This formula is a derivation of the capacitance equation (V = q/Cm). The numerator of the equation sums calculates the difference between cation and anion concentrations, which is multiplied by the volume to get a molar quantity. Multiplication by Faradays constants (F) converts the molar quantity to coulombs. The membrane capacitance (Cm) is calculated per unit surface area; thus, we multiply by surface area to get the total membrane capacitance.

## 2.2.2 Ionic reversal potential and driving force

Ionic reversal potential across the membrane (Eion) is defined as the membrane potential at which there is no net flux of a particular ion between the internal and the extracellular environment. The derivation of this equation is discussed in the introduction.

At an ion’s resting potential, the electrical forces balance the osmotic forces across the membrane and hence there is no net ion flux. In neurons however the membrane potential is rarely at ionic reversal potential and thus there is a potential difference for the ionic flux – defined as the ionic driving force (DF ion).

## 2.2.3 Leak channels

Leak channels permit bidirectional motion of ions across the membrane. A cell membrane will have multiple leak channels; however, we simplify this by modelling a single leak current which can be thought of as the net ion leak. We calculated leak current using ohms law, where the channel conductance (inverse of resistance) multiplied by the ionic driving force.

Ionic conductances(gna, gk , and gcl) were set to those in Dusterwald et al.2 , which were obtained from conductances used in the similar models on ModelDB3 and ion channel genealogy4.

## 2.2.4 Na+/K+-ATPase pump

The Na+/K+-ATPase shifts two potassium ions into the cell in exchange for three sodium ions which move extracellularly. This process is critical in establishing a negative resting membrane potential and ensuring volume control. We modelled the flux through the ATPase (*Jp*) based on the formula employed by Keener and Sneyd5:

The flux rate (*Jp*) is a function of the pump constant (P) and the sodium (Na) concentration ratio between the internal and external environment. The pump constant was set to 0.1 C/(dm2.s) based on a fitted curve establishing actual values of sodium concentrations and membrane potentials6. To minimize the number of dynamic changes in the simulation we kept *Jp*  by using the initial sodium concentration (as opposed to updating it at each time step).

## 2.2.5 KCC2 pump

The type 2 K-Cl cotransporter (KCC2) is an essential regulator of chloride ion concentration. It utilizes the difference between the potassium and chloride reversal potentials to move a chloride and potassium ion bidirectionally across the cell membrane. Flux through the KCC2 channel was modelled using the formula suggested by Doyon et al7,8, where the conductance of KCC2 was also provided as a fixed value (20 µS/cm2)

## 2.2.6 Net transmembrane ion flux equations

The rates of change for each ion concentration across the membrane was based on the sum of the leak, ATPase and KCC2 fluxes for the respective ions. Surface area (SA) is multiplied in as conductances and pump rates are given per unit area. This sum is divided Faraday’s constant to convert the charge to moles and is divided by the volume to get a concentration change (*d*[Ion]) per unit time (*dt*).

# 2.3 Axial ion flux

## 2.3.1 Electrodiffusion

In addition to transmembrane motion, ionic species can move in the axial plane. **SCHEMATIC.** For simplicity this is regarded as a single dimension along the length of the axon or dendrite. Like transmembrane motion, chemical and electrical forces drive ionic movement through the cytoplasm; however, unlike movement across the membrane, there are no channels that permit ionic movement at discrete spatial points. Instead, ions are assumed to be able to move with equal probability across the entire surface area.

To model the axial flux of ions the Nernst-Planck Equation (NPE) was used. This equation calculates the flux of ions based on diffusion (chemical forces) and drift (electrical forces) and has been shown to be more accurate than either of these forces alone especially in small structures such as dendrites. The NPE calculates the flux density J for ion C as follows:

The diffusion constant (D) for each ion is depicted in the table below, z refers to the ionic charge, [C] is the ionic concentration, and x is the axial length the forces occur across. The distance between the midpoints between adjacent compartments were used as the dx term.

The flux for each ion was calculated between adjacent compartments *i* to *i* + 1, with flux units of mol/(s.dm2). To determine the molar concentration of ions the flux was multiplied by the shared surface area and divided by the compartment volume to determine the flux in terms of molar concentration (M/s).

## 2.3.2 Boundary potentials and axial driving force

Boundary potentials refers to the voltage changes that occur across the boundary between compartments such that the boundary acts as a theoretical membrane. These potentials assess the axial driving forces at discrete spatial points in the neuron. All boundary potentials will be denoted with a subscript “*b*”.

The boundary potential (V*b*) between two compartments (compartment A and B) is defined as the difference between the membrane potentials of the 2 compartments.

Boundary reversal potentials for a particular ion (E*b* ion) is calculated in a similar way to ionic reversal potentials across the membrane however the ionic concentrations between the two compartments, rather than the internal and external environments, are used in the calculation.

The axial driving force across the boundary for an ion (DF*b* ion) is therefore defined as the difference between the boundary potential and the boundary ionic reversal potential for that ion.

# 2.4 Impermeant anion flux

## 2.4.1 Changing impermeant anion concentraiton

In figure 1 we changed the concentration of impermeant anions in compartment 8. We used a flux rate of 5mM/s for 60 seconds. As our time step was 10-6s, we increased the concentration of impermeant anions by 5nM per time step.

## 2.4.2 Changing impermeant anion charge

In figures 2-5 we changed the valence of impermeant anions away from the default (z = -0.85). In figure 2 we calculate the difference between the desired final valency and the initial default valency. This value is divided by the time step to get the incremental value by which to change the valency at each iteration. Instead of starting at default, in figures 3-5 we start the simulation with alternate impermeant anion valency in compartment 8 and allow the simulation to reach a steady state.

# 2.5 Compartment volume and surface area

We updated the volume of each compartment at every time step to ensure constant osmoneutrality. Each compartment was modelled as a cylinder with volume (*w*) calculated as:

Radius is denoted by *r*, and cylinder length by *l.* We imagined the cylinder to have open ends (allowing for diffusion) so surface area (*SA*) was calculated as:

Surface area was assumed to be constant for the duration of the simulation, approximating a compartment that wrinkles and becomes more turgid as the volume swells. The implication of this assumption is that ion channel and pump conductances are not constantly rescaled by fluctuating surface areas.

Compartment volume was updated at each time point as this has significant implications for the voltage equations. Volume updates were modelled based on the work of Hernandez and Cristina9 using the equation below:

In this equation, *dw* is the volume change which is equal to the product of the partial molar volume of water (*vw*), the osmotic permeability of a biological membrane(*pw*), and the surface area (*SA*). This factor is then multiplied by the difference between the internal and external osmolarities (Π).

We do not model the volume changes that result from cytoplasmic matrix constituents moving between compartments.

## Volume Normalization

Volume changes occur because of the difference between internal and external osmotic composition. Changes to IA charge and concentration affect compartment volumes. To understand the impact of IAs on passive and active dendritic signalling properties effects of volume changes on the compartment need to be excluded.

Fixing the internal osmolarity of the compartment achieves this aim. As the charge of IA is changed there are changes to the ionic composition of the compartment. At each time step we update the IA concentration to ensure that the osmolarity of the compartment is the same as the previous time step.

# 2.6 Passive cable properties

We validated our electrodiffusion based model with other models in the field, as well as with an equivalent circuit model in NEURON, by comparing membrane time and space constants after a current was injected.

In NEURON we created a model with 9 compartments (length: 20µm; diameter: 1µm) linearly arranged. We set the leak membrane conductance to 0.0011S/cm2 , which is the sum of the leak conductances and the KCC2 conductance in our electrodiffusion model. Membrane capacitance was set at 2uF/cm with resting membrane potential set to -72.6mV.

To simulate excitatory current in the biophysical model we determined the amount of charge (coulombs) would be required for a specific duration of time using the formula (I = Q/t). This value was converted to moles (though division by Faraday’s constant).

We divided the total amount of moles by the time step (10-6s) and divided by the compartment volume to achieve the desired concentration to be added to the compartment at every iteration of the simulation for the duration of the current pulse. We added this value to intracellular sodium (for excitatory currents) or intracellular chloride concentration (for inhibitory currents).

To calculate the membrane time constant (τ), we traced the voltage decay in the compartment that received the current injection. The difference between the peak/trough and baseline membrane potential (Vm) was multiplied by 0.63 (equivalent to 1-*e*). The membrane time constant was set to the time difference between this value and the peak/trough.

The length constant (λ) was calculated in a more indirect manner. Firstly, the peak membrane potential deflections in each compartment were plotted against the compartment length (using the compartment midpoint as the compartment estimate). Secondly, a exponential decay line of best fit was plotted against this curve and lastly, the lambda was taken as the point on the curve 63% of the way between the maximum deflection and minimum deflection.

We reviewed mammalian neuron-based computational models on ModelDb to compare the time and length constants in our multicompartmental neuron. We specifically examined the mean diameter and length of terminal apical dendrites as well as their passive electrical properties. A summary of the dendritic properties is shown in the table below:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Neuron type** | **Mean diameter (µm)** | **Mean length (µm)** | **Time constant (ms)** | **Length constant (µm)** | **Ri (Ωcm)** | **Rm**  **(Ωcm2)** | **Cm (µF/cm2)** | **Mean threshold current for spike generation (pA)** | **Source** |
| HL2/3 pyramidal neurons in temporal cortex | 0.78 | 162.31 | 12.03 | 163.94 | 200 | 2756 | 0.5 | 267.20 | Deitcher et al10 |
| HL2/3 in neocortex | 1 | 6x103 | 16.5 | 692.21 | 203 | 38907 | 0.45 | Not performed | Eyal et al11,12 |
| HL5 temporal lobe | ?? | ?? | 27.53 | ?? | 495.73 | 5.71x1013 | 1.6 | Not performed | Rich et al13 |
| Subfornical organ neuron | 10 |  |  |  |  | 3142 | 1.59 | Not performed | Medlock et al14 |
| Layer 5 pyramidal cell | 600 |  |  |  |  |  |  |  | Van Dijck et al15 |
| Dorsal Root Ganglion |  |  |  |  |  |  |  | 100 | Verma et al.16 |
| Primary visual cortex pyramidal cells | 1 | 250-500 |  |  |  |  |  |  | Lee17 |
| Bipolar cells in Medial Superior olive | 3 | 200 | 0.5 | 137 | 200 | 43MΩ | 1 | 1000 | Zhou et al18 |
| Interneurons in the dorsolateral geniculate nucleus | 0.5 | 673 | 24 |  | 113 | 22 000 | 1.1 | 40-70 | Halnes et al.19 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| OUR Model | 1 | 90 |  |  | 200 | 87.5 | 2 |  |  |

## Comparison with NEURON simulation envrionment

In NEURON we assembled nine compartments (lengths: 20µm; diameter: 1µm) linearly with identical membrane capacitances (2µF/cm2) and total passive/leak conductances (0.00011 S/cm2). An axial resistance of 200 Ω-cm was chosen based on values used in similar dendritic models found on ModelDB.3–6 Diffusion constants for the electrodiffusion model were set to the default values to compare to the NEURON model. Data from NEURON was extracted and plotted using Matplotlib in an identical manner to the data arising from the electrodiffusion model. In both models we pulsed a +0.1nA current into compartment 9.

## Membrane time and space constants

Tau(𝝉), the membrane time constant, is defined as the time for the membrane voltage to reach 66% of the final membrane voltage. Tau is considered as the product between the membrane resistance (Rm), and the membrane capacitance (Cm). Tau is therefore solely predicted based on membrane specific determinants; consequently, one would not predict that impermeant anions fluxes alter Tau.

Lamba (λ), the membrane space constant, is defined as the distance for the membrane voltage to reach 2/3 of its initial value. Lambda is determined by the membrane resistance (Rm), and internal resistance (Ri). Here one may predict that impermeant anion fluxes might change the internal (axial) resistance.

# 2.7 Synaptic modelling

We simulated synapses on our biophysical multicompartment model to assess how impermeant anions affect synaptic integration. Excitatory (NMDA/Glutamatergic) and inhibitory (GABA/GABAergic) synapses were modelled using the kinetic binding model described by Destexhe et al. 20,21:

A synaptic input begins with the release of a neurotransmitter ([N*T*]) which attach to unbound receptors (R), forming a transmitter-receptor complexes (R·[NT]). The rate of the neurotransmitter bindings is given as α, and the rate of unbinding as β. The α and β values vary according to neurotransmitter-receptor type. The strength of the current due to the synapse is dependent on the amount of neurotransmitter binding ( - calculated as a ratio of bound to unbound receptors).

Before the synaptic event it is assumed that the ratio is equal to zero as there are no bound receptors. During the synaptic event can be calculated with the formula below:

In ( …) , the bound receptor ratio change is the difference between the number of unbound receptors that are being bound minus the number of bound receptors moving to the unbound state. Using this formula, we can calculate the ratio of bound neurotransmitter at a given time point which is required to determine the current moving through the channel.

Current through the glutamatergic channels ( is equal to the conductance of the channel () multiplied by the amount of receptor bound at a particular time point () and the driving force for sodium. The negative sign indicates the inward current due to positive ions moving into the cell. Current through GABAergic channels ( consists of Cl- flux (80%) and HCO3- flux (20%). As only Cl- is modelled in our simulations we multiply by 80% and use *ECl* instead of *EGABA* to derive the proportion of the GABAergic current due to Cl-.

Using and , one can calculate the molar quantity of Na+ or Cl- as current is charge divided by time. Charge in Coulombs is converted to mole by dividing through by Faraday’s constant. We then divide by the compartment volume to determine the concentration of Na+ or Cl- that is entering the compartment at a particular time step.

# 2.8 Action potential modelling

In experiment 5 we assessed how impermeant anions may affect synaptic integration and action potential generation. To model action potentials, we used Hodgkin-Huxley equations22 with default parameter values obtained from NeuroWiki.23 Hodgkin & Huxley (HH) accurately model action potentials by plotting the dynamics of voltage gated Na+ and K+ channels that are activated once a threshold membrane potential is reached.

Current going through HH K+ channels is given by:

The potassium conductance through the Hodgkin Huxley channel (is modelled by:

The maximum K+ conductance is represented by , while *n* can be thought of as the proportion of voltage gated K+ channels open. The rate of change of *n* is a function of time, voltage, and intrinsic channel properties given by the equation below:

In (…), the *n* change is the difference between the rate of closed channels (1-*n*) that are opening and the rate () of open channels that are closing(*n*) Both and are dependant on the membrane potential and are given by the equations:

In the above equations V1-6 are constants.

Current through the Na+ - HH channel (is calculated with a similar formula to that of K+ :

Unlike the voltage gated K+ channel, the conductance of the Na+ channeldepends on the opening of two types of gates (the probability of each being open is symbolised by *m* and *h*). The *m* gate is closed at resting membrane potential and opens rapidly when the threshold potential is reached, conversely, the *h* gate is open at resting potential and is triggered to close slowly once the threshold is reached. The interaction between the two gates scales the maximal Na conductance ( to give the Hodgkin-Huxley Na+ conductance (

Both the *m* and *h* gate are modelled in the same way the K+ channel was modelled by determining the forward () and reverse rate of channel opening which is a function of both time and membrane potential. The *m* gate is modelled with the three equations below where V7-12  are constants:

The *h* gate is modelled in a similarly with V13-18 constants reflecting the internal properties of the gate.

# 2.9 Flow chart of operations

# 2.10 Class structure and class functions

# 2.11 Systematic review

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