Methods

# 2.1 Single-compartment model

I constructed a computational model of single neuronal compartment. This model is analogous to a portion of dendrite bathed in an extracellular ionic solution.

The internal cytosolic environment of the compartment was separated from the extracellular bath cell membrane. 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).

*l*

r

The compartment was cylindrical with a default radius of 1um and length of 10um (a approximating a dendrite).

|  |  |  |
| --- | --- | --- |
| **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 |
| **Variables** |  |  |
| [Na+]o | 145e-3 | Extracellular Na Concentration |
| [Na+]i |  | Intracellular Na Concentration |
| [K+]o | ko = 3.5e-3 | Extracellular K Concentration |
| [K+]i |  | Intracellular K  Concentration |
| [Cl-]o | 119e-3 | Extracellular Cl Concentration |
| [Cl-]i |  | Intracellular Cl Concentration |
| [X-]o | 29.5e-3 | Extracellular Impermeant anion Concentration |
| [X-]i |  | Intracellular Impermeant anion Concentration |

## 2.1.1 Membrane potentials

Membrane potentials 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 Huang (REF):

In the above, the major intracellular ion concentrations (sodium, potassium, chloride, and impermeant anions) are summated. This sum is multiplied by Faraday’s constant to convert mol to coulomb. The numerator is divided by the membrane capacitance as well as the area constant (ratio of cylinder surface area to volume).

Ionic reversal potentials across the membrane (E*m* ion) are defined as the membrane potential at which there is no net flux of a particular ion between the cytosol of the compartment and the extracellular environment.

The difference between the membrane potential and the ionic reversal potential is equal to the driving force across the membrane for that ion (DF*m* ion).

## 2.1.2 Boundary potentials

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.

## 2.1.3 Na+/K+-ATPase pump

The Na+/K+-ATPase pump moves sodium into the cell and potassium out of the cell in the ratio 2:3, thus helping to establish a negative membrane potential.

Modelling this process was performed in either a static or dynamic manner. When modelled statically the flux through the pump was constant, in contrast to the dynamic Na+/K+-ATPase flux which varies on the concentration difference between sodium inside and outside, modelled as:

The flux rate (Jp) is a function of the pump constant (P) and the third power of the sodium (Na) concentration ratio between the inside and outside of the neuronal membrane.

## 2.1.4 KCC2 pump

The type 2 K-Cl cotransporter (KCC2) serves to flux chloride… Flux through the KCC2 channel was modelled using the formula suggested by (Doyon, 2016)

## 2.1.5 Impermeant anion manipulation

Impermeant anions could be manipulated in four ways:

1. Intracellular impermeant addition of new anion species.
2. Intracellular impermeant change of charge
3. Extracellular impermeant addition of new anion species
4. Extracellular impermeant change of charge.

**FIGURE**

## 2.1.6 Volume

# 2.2 Multi-compartmental model

## 2.2.1 Electrodiffusion

In addition to transmembrane motion, ionic species can move in the axial plane. 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).

|  |  |  |  |
| --- | --- | --- | --- |
| **Diffusion constants** | | | |
| **Symbol** | **Value** | **Description** | **Source:** |
| DNa | 1.33 x10-7 dm2/s | Na+ diffusion constant |  |
| DK | 1.96 x10-7 dm2/s | K+ diffusion constant |  |
| DCl | 2.03 x10-7 dm2/s | Cl- diffusion constant |  |

## 2.2.4 Synapses

Synaptic input, both GABAergic (inhibitory) and Glutamatergic (excitatory), were simulated as a pulse of either chloride or sodium influx into a compartment at a given time. The Destexhe et al. (ref.) kinetic model of receptor binding was used to computationally calculate synaptic inputs.

This model derives a ratio of bound to unbound neurotransmitter receptors at time points during and after the synaptic pulse. Before the synaptic pulse it is assumed that the ratio is equal to zero as there are no bound receptors. A synaptic input begins with the release of a concentration of neurotransmitter ([N*T*]) which attach to unbound receptors (R), forming a transmitter-receptor complexes (R[NT]). The kinetics can be written as:

Here α = 0.5 ms-1mM-1 and β = 0.1 ms-1, reflecting the forward and reverse rate constant respectively. The proportion of bound to total receptors, R / ([NT]R +R), is denoted by small .During a synaptic event it is assumed all the neurotransmitter is bound thus that ratio of bound to unbound receptor is equal to 1. When the pulse begins it is assumed that all the neurotransmitter is bound to the receptor, such that is equal to 1. The steady state proportion is denoted , and can be thought of as the proportion of bound receptors once the synaptic pulse is over. This proportion can be calculated with the following formula:

An exponential decay model is used to calculate the proportion of bound receptors at any given time (), equation xxx below. This model makes use of a time constant (𝝉) which is derived from the kinetic rate constants and the maximum neurotransmitter concentration.

The proportion of bound receptors is used to calculate the incoming current by multiplying by the synaptic conductance and driving force for chloride across the membrane. As chloride is the dominant anion flowing through the GABA channel at a ratio of 4/5 with bicarbonate, the incoming GABAergic current was multiplied by this ratio to calculate the intracellular chloride changes.

Similarly, excitatory synapses through the NMDA channel were calculated by multiplying the ratio of bound receptor to the synaptic conductance and the sodium driving force.

At the end of the pulse the proportion of bound receptors were recalculated using the following formula

Synapse duration

## 2.2.5. 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.

## 2.2.6 Current injection

* In our multicompartment model we have a setup of 9 compartments each with a length of 10µm, thus the total length is 90µm.
* Should we want an incoming current to decay to 2/3 of its value at 30µm we need a length constant of 60µm
* The membrane resistance is the inverse of the sum of the leak channel conductances. In our setup this gives us an Rm of 13000 Ω.cm-2 .
* Substituting into the space constant formula we arrive at an Ri of 30555.56 Ω.cm
* We can alter our electrodiffusion diffusion constants to achieve this situation.
* Alternatively, we could use the default intracellular resistivity in neuron of 100 Ω.cm. This makes the length constant 130µm.
* We can change the length of our compartments to fit this and then mess with the electrodiffusion constants and try and match it.
* Is the membrane resistance the sum of the leak conductances?

We reviewed mammalian neuron based Hodgkin-Huxley 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 al1 |
| HL2/3 in neocortex | 1 | 6x103 | 16.5 | 692.21 | 203 | 38907 | 0.45 | Not performed | Eyal et al2,3 |
| HL5 temporal lobe | ?? | ?? | 27.53 | ?? | 495.73 | 5.71x1013 | 1.6 | Not performed | Rich et al4 |
| Subfornical organ neuron | 10 |  |  |  |  | 3142 | 1.59 | Not performed | Medlock et al5 |
| Layer 5 pyramidal cell | 600 |  |  |  |  |  |  |  | Van Dijck et al6 |
| Dorsal Root Ganglion |  |  |  |  |  |  |  | 100 | Verma et al.7 |
| Primary visual cortex pyramidal cells | 1 | 250-500 |  |  |  |  |  |  | Lee8 |
| Bipolar cells in Medial Superior olive | 3 | 200 | 0.5 | 137 | 200 | 43MΩ | 1 | 1000 | Zhou et al9 |
| Interneurons in the dorsolateral geniculate nucleus | 0.5 | 673 | 24 |  | 113 | 22 000 | 1.1 | 40-70 | Halnes et al.10 |
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|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| OUR Model | 1 | 90 |  |  | 200 | 87.5 | 2 |  |  |

# 2.3 Flow chart of operations

# 2.4 Class structure and class functions

# 2.5 Systematic review

# 2.6 NEURON multicompartment model

Reviewing the charge and concentration of impermeant anions in various disease states