Modelling chloride dynamics and impermeant anions in a single neuronal model

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1 Single compartment model with volume and chloride dynamics

We calculate time series for a single compartment including the following variables: intracellular concentrations of sodium, potassium, chloride and impermeable anions (Na_i, K_i, Cl_i and X_i, with charge z); membrane voltage (V); cellular volume. The steady state should occur in the presence of both a pump leak mechanism (sodium-potassium ATPase) and chloride-potassium extrusion (type 2 potassium-chloride co-transporter, KCC2, subject to some constant conductance g_{KCC} and the reversal potentials of potassium and chloride, while taking into account the usual passive forces across the membrane on each ion.

1.1 Initialisation

The compartment is initialised as a cylindrical compartment with a fixed radius. Extracellular ionic concentrations, which are shown with subscript e where relevant, are set (and remain constant), as are the conductance parameters for the ionic flux equations, and the scaling constant A_m (surface area per unit volume). The initial intracellular concentrations are calculated assuming charge neutrality and osmotic equilibrium, i.e. they satisfy (1) and (2) below. The charge z represents the average intracellular charge of the impermeant anions, here represented by an X_i.

$$0 = K_i + Na_i - Cl_i + zX_i \tag{1}$$

$$ose = osi = K_i + Na_i + Cl_i + X_i \tag{2}$$

1.2 Loop over time

At each time step, we calculate first the membrane potential V_m, then the changes in ionic concentrations, and finally an update of the cellular volume.

V_m is calculated using Equation 3, the "Charge Difference" approach introduced by Fraser and Huang (2004). Roughly, it assumes that voltage over the membrane is equivalent to difference in charge over the membrane as a ratio of the capacitance C_m across the membrane — of course, the small accumulation of charge near the membrane magnified by the capacitance accounts for the membrane potential in traditional terms, too. This approach is favourable because the initial voltage can be calculated without assuming a steady state and then consistently updated using the same equation. It also allows for some analytical solutions. Note that the formulation below incorporates the scaling constant A_m, which will be explained later, for the first time.

$$V_m = \frac{F(Na_i + K_i - Cl_i + zX_i)}{C_m} \cdot \frac{volume}{s.area}$$

$$V_m = \frac{F(Na_i + K_i - Cl_i + zX_i)}{A_m C_m} \tag{3}$$

From the voltage equation, ionic flux is calculated and added to each ion's intracellular concentration. This is done using the standard equivalent circuit model, which behaves very similarly to the constant field equations, and includes the influence of KCC2 and the sodium-potassium ATPase.

The ATPase transports two potassium ions inside the cell for three sodium ions moved outside the cell. The pump rate J_p (here in units of flux per square cm) is a function of the sodium gradient, as then it has less activity when the intracellular sodium concentration depletes. A cubic function has been shown to approximate more accurate kinetic models reliant on ATP concentration. Thus

$$J_p = P\big(Na_i/Na_e\big)^3 \tag{4}$$

where P is some initial pump rate (a constant).

KCC2 is calculated using Fraser and Huang's formulation in Equation 5 (but can easily be changed to another model — as may be useful when comparing the model to the parametric solution in the single compartment).

$$J_{KCC2} = g_{KCC2} \cdot g_K(Cl_e \cdot K_e - Cl_i \cdot K_i) \tag{5}$$

Thus for each ion:

$$\frac{dI_{Na}}{dt} = g_{Na}(V - E_{Na}) - 3p \tag{6}$$

$$\frac{dI_K}{dt} = g_K(V - E_{Na}) + 2p - J_{KCC2} \tag{7}$$

$$\frac{dI_{Cl}}{dt} = g_{Cl}(V - E_{Cl}) - J_{KCC} \tag{8}$$

In practical terms, to calculate the flux for the entire cell at a time step each flux equation is multiplied by A_m and dt, the interval of time between each time step (usually 1 ms). Each ionic concentration is then updated by adding the relevant flux to the current intracellular concentration.

Next, volume is updated. Change in volume is assumed to be instantaneous and expressed by changing the previous volume by a driving force given by the ratio of intracellular to extracellular osmolarity (Equation 9). Of note here is the role of A_m, since a changed volume ought to change the surface area and hence cellular capacitance. It is assumed that changes in volume are only represented by changes in the length of the cell, but not its radius. Thus we have $\frac{s.area}{volume} = \frac{2\pi rad \cdot len}{\pi rad^2 \cdot len} = \frac{2}{r} = A_m$, which gives us the constant scaling ratio in (3).

$$volume = volume\frac{osmo_i}{osmo_e} \tag{9}$$

Finally, intracellular concentrations are updated in the new volume. Either here or at the beginning of the time loop, concentrations, V_m and volume (relative to the initial volume) are appended to arrays for storage.

2 Multi-compartment model generalisation

The generalisation to a multi-compartment model theoretically involves stitching together many single compartments obeying the model outlined in section 1, perhaps with different properties or parameters in cases. The most important aspect in considering the series of new compartments created is how ions diffuse from one compartment and into the next. For this we propose a schema including both pure charge and pure concentration fluxes, which are combined in the Nernst-Planck Equation.

3 A two-compartment model of passive forces: diffusion and drift

In a simple system, the movement of a collection of points — uncharged molecules for now — across a barrier that is permeable to these points could be considered as molecular flux. If each side of the barrier (or membrane)

represents a compartment filled with water and the molecules are soluble, then one simulates osmotic diffusion: the molecules should move across the membrane until there is an equal concentration in each compartment. If the compartments have the same volume, this means there are an equal number of molecules in each (Fig. 1).

When the uncharged molecules in Figure 1 are substituted for permeable ions (assume that they are anions, negatively charged ions, for example Cl^-), the only other charges in the environment with which the ions can interact are repulsive. If other ions were added, they would collectively set up an electric field across the membrane. Consider that the field affects the anions by giving them an electrical potential. An electrical potential E is defined as the difference between the electrical potential differences (voltages) at two points ($V_1 - V_2 = E$). It is assumed that the positive direction of the electric field is from inside the cell to outside. Flux because of the electrical potential is called "drift."

When both electrical and chemical forces affect an ion's flux, equilibrium no longer occurs when each compartment has equal concentrations of the ion but rather when the electrochemical potential of the ion in each compartment is equal. To calculate the electrochemical potential, or Nernst potential, the fluxes because of drift and diffusion can be added together.

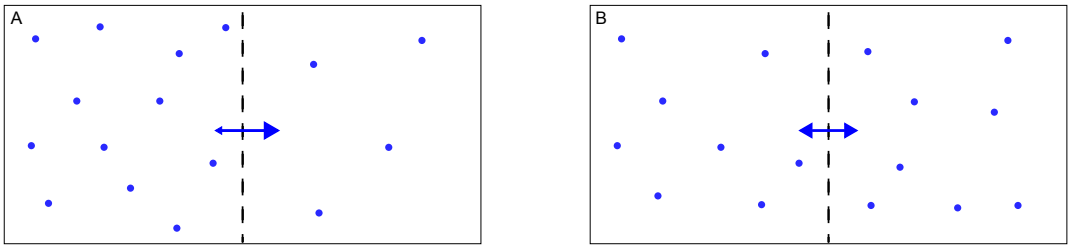

Figure 1: An illustrative two compartment model. In A molecules are distributed at random so that there are more molecules in the leftmost compartment and net flux is to the right compartment. In B the compartments have reached equilibrium and net flux is zero.

3.1 Nernst-Planck Equation

Fick's Law for diffusion says that the flux because of diffusion J_{diff} is given by $-\frac{\mu RT}{F}\frac{\mathrm{d}[C]}{\mathrm{d}x}$. Ohm's Law for drift states that for the flux because of drift, $J_{drift} = -\mu z[C]\frac{\mathrm{d}V}{\mathrm{d}x}$ (variables' meanings are listed in the footnote[1]).

[1] μ, ion mobility; $[C]$, ionic concentration; z, valence; V, voltage; x, position (compartment); k, Boltzmann's constant; T, temperature in degrees Kelvin; q, charge; R, the gas constant; F, the Faraday constant; I, current

The Einstein Relation states that J_{diff} and J_{drift} are additive in the same medium, and allows an initial expression for total flux to be obtained.[2]

$$\begin{aligned} J &= J_{drift} + J_{diff} \\ &= -\mu z[C]\frac{\mathrm{d}V}{\mathrm{d}x} - \frac{\mu RT}{F}\frac{\mathrm{d}[C]}{\mathrm{d}x} \end{aligned}$$

Dividing by N_A, Avogadro's number, and multiplying by z, ion valence, an expression for $I = JzF$, current, known as the *Nernst-Planck Equation* (10), or NPE is obtained.

$$\frac{J}{N_A} = -\frac{\mu z}{N_A}[C]\frac{\mathrm{d}V}{\mathrm{d}x} - \frac{\mu}{N_A}\frac{RT}{F}\frac{\mathrm{d}[C]}{\mathrm{d}x}$$

$$J = -\mu zF[C]\frac{\mathrm{d}V}{\mathrm{d}x} - \mu RT\frac{\mathrm{d}[C]}{\mathrm{d}x}$$

From $\mu = \frac{DF}{RT}$, where D is the diffusion coefficient of ion C,

$$J = -D\frac{zF^2}{RT}[C]\frac{\mathrm{d}V}{\mathrm{d}x} - DF\frac{\mathrm{d}[C]}{\mathrm{d}x} \tag{10}$$

If we wanted to calculate the *Nernst Equation*, which occurs at equilibrium conditions, we would set net flux equal to zero ($I = 0$) and then integrate with respect to x (derivation not shown here). Thus the Nernst Equation necessarily assumes more constraints than the NPE.

3.2 Implementation of electrochemical diffusion

The modelling programme NEURON uses only J_{diff} between compartments, with the assumption that J_{drift} has little effect. However, in addressing questions of the influence of impermeant anions, which ought to still exert some electrical influence on the ions in the cell but cannot themselves diffuse, it seems important to include charge, hence the use of the NPE.

The implementation of equation 10 is in terms of numerical derivatives over distance between compartments. This can be done using a forward Euler (or otherwise) approach, assuming instantaneous diffusion over the distance at every timestep, where the distance δx might be calculated from say the distance between the midpoints of adjacent compartments. Here,

[2] In reality these derivations apply to flux *density* (and later current density), because we do not take into account surface area. However, to simplify the derivations, and because density is different only by a scalar factor (for a constant surface area), "flux" and not flux density will be spoken about.

dV should represent the difference in voltage between adjacent compartments, and $d[C]$ the difference in concentration of ion C, while $[C]$ is the concentration in the compartment for which flux is being calculated.

The diffusion coefficient D of each ion is a constant given in the below table from Hille (1984).

Ion	Diffusion constant (10^{-5} cm^2s^{-1})
Na	1.33
K	1.96
Cl	2.03

Therefore electrodiffusion can be implemented at each time step for each pair of adjacent compartments, with net flux calculated as shifts in concentration between the compartments.