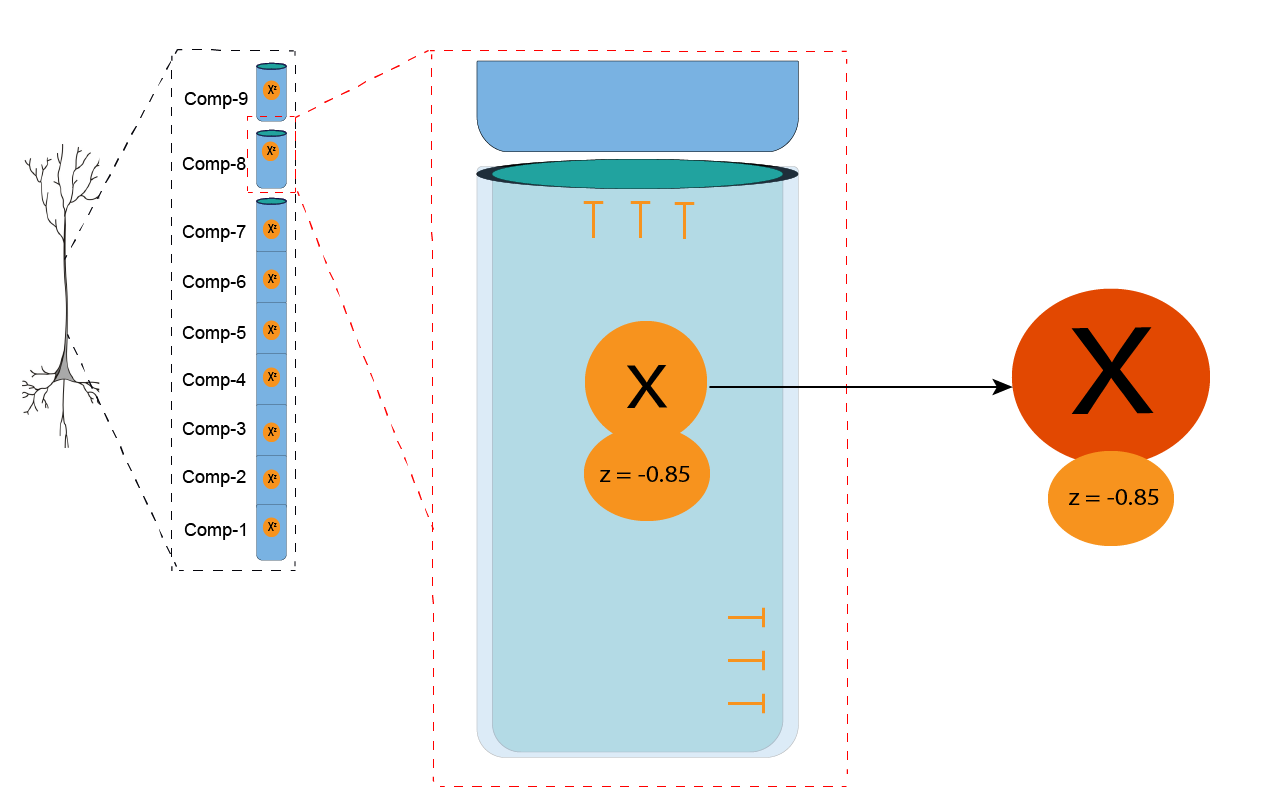
# 1 – Impermeant anion concentration changes

## 1.1 impermeant anion concentration sets local compartment volume

We simulated increases in impermeant anion quantity with our nine-compartment model. The molar quantity of impermeant anions in compartment number 8 (Comp8) was increased at a fixed rate of 3x10-16 mole/min between 20 and 40s, whilst the average charge (valence) of impermeant anions remained constant (z = - 0.85) (Schematic 1).

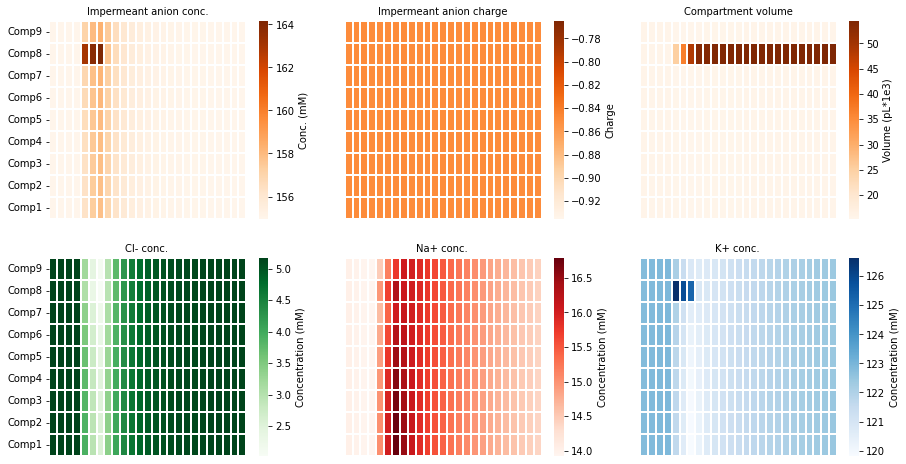
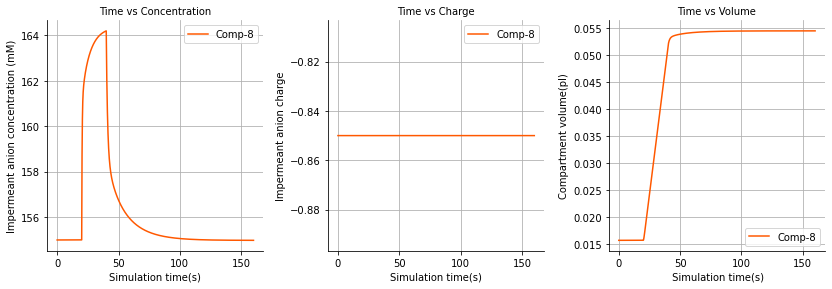


**Schematic 1:** Impermeant anion concentration increase in compartment 8, while impermeant anion charge (z) is held constant.

Approximately 1x10-16 moles of impermeant anions were added to Comp8 between 20 and 40s, resulting in a concentration increase of approximately 9mM (Figure1A). The volume of Comp8 more than tripled to accommodate the increase in impermeant anion load. Although impermeant anions were only fluxed in Comp8 there were transient changes in ionic composition and compartment volumes in all other compartments.

The ion concentrations and volumes of all other compartments returned to their original values once the flux ended in Comp8. Volume increase in Comp8 persisted beyond the flux duration.

**Figure 1A** – Increased impermeant anion (IA) concentration in compartment 8 between 120-180s results in persistent local change in compartment volume whilst permeant anion concentrations in all compartments return to steady state values following IA manipulation.



We also evaluated the electrical changes in the simulation above (Figure 1B). The membrane potential (Vm) dropped transiently in all compartments during impermeant anion flux into Comp8. This was coupled by transient decreases in sodium and potassium reversal potentials in all compartments. However, once the flux had ended and the model reached a new steady state compartment membrane potential, ionic reversal potentials and ionic driving forces returned to baseline level in all compartments.

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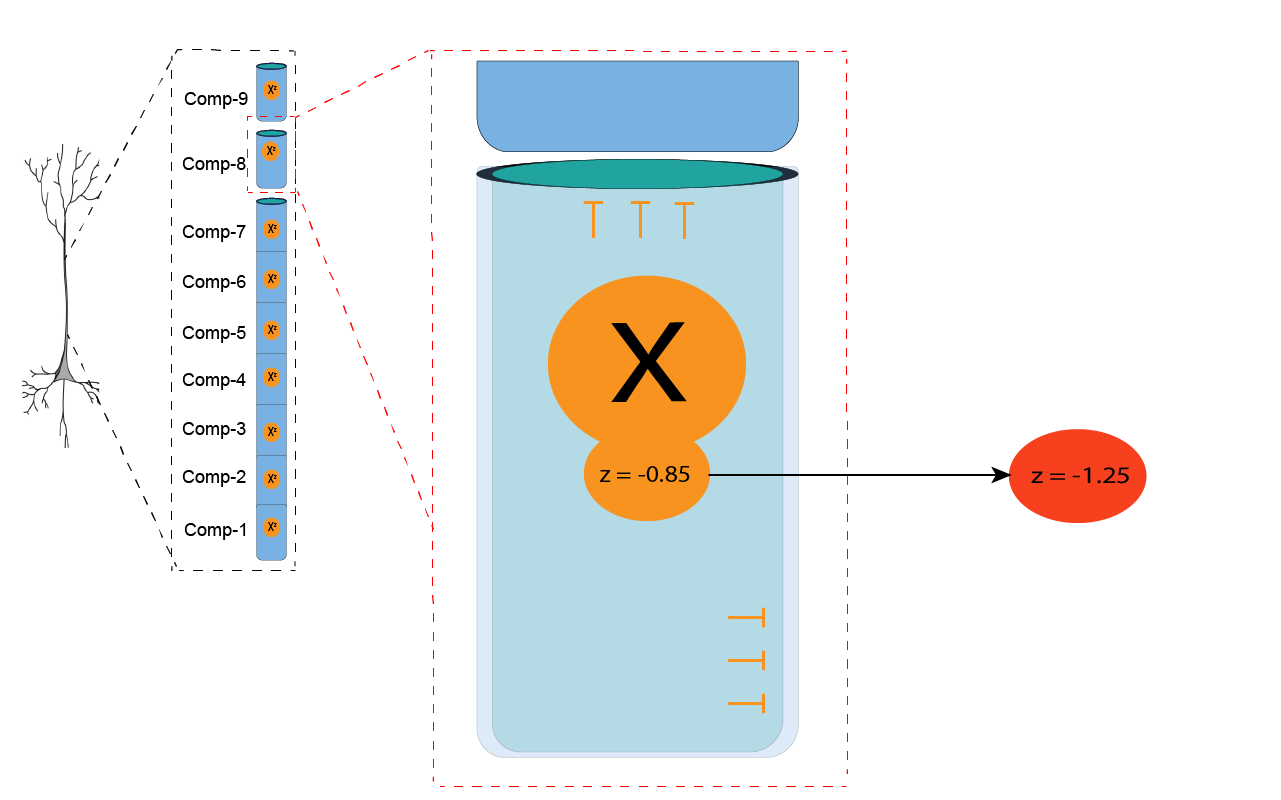
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**Figure 1B** – Increased impermeant anion concentration in compartment 8 leads to unchanged ionic driving forces and thus no change to the excitability of the dendrite.

# 2 – Impermeant anion valence change

We next evaluated the local and dendrite-wide impact changing impermeant anion average charge in one compartment may have. An identical 9 compartment model was used (as in Figure 1). The multicompartment model was placed within an extracellular bath containing fixed ion concentrations, while electrodiffusion was employed to model ionic movement across the cell membrane as well as between compartments. Impermeant anion charge started at a baseline of z = - 0.85 in all compartments. In compartment 8 the impermeant anion charge was decreased to z = -1.25 in a linear fashion between 120s – 180s during the simulation (Schematic 2). Note that absolute osmoles of impermeant anions were not changed in any of the compartments.

**Schematic 2:** Impermeant anion charge is manipulated in compartment 8 from -0.85 gradually to -1.25.



## 2.1 Local changes to impermeant anion charge (z) alters local compartment volume and permeant ion concentrations

Impermeant anion charge reduction in compartment 8 resulted in a local increase in intracellular osmotic pressure with resultant swelling of compartment (Figure 2A, top row). The compartment volume remained persistently elevated even after the IA charge manipulation ceased. Impermeant anion concentration in compartment 8 decreased proportionally to the increase in compartment volume. There were no volume changes in adjacent compartments and hence there were changes to IA concentrations in these compartments (Figure 2A, middle row).

To compensate for the increased intracellular negative charges in compartment 8 there were respective increased concentrations of sodium and potassium ions, and decreased concentrations of chloride (Figure 2A, bottom row). Once the impermeant anion charge manipulated ended at 180s, the concentrations of these permeant ions remained fixed at a new equilibrium. In the adjacent compartments there were no such changes to permeant anion concentrations. Therefore, it appears that local impermeant anion charge sets local permeant ion concentrations.

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**Figure 2A: Impermeant anion charge alters local compartment volume with proportional changes to impermeant anion concentration**

**Left pane:** Impermeant anion charge was changed from z=-0.85 to z=-1.2 between 120s and 180s only in compartment 8.

**Middle and right pane:** Compartment 8 volume and impermeant anion concentration changes proportionally to the change in impermeant anion charge in compartment 8. No significant changes are seen in any other compartments.

## 2.2 Local changes to impermeant anion charge (z) create a non-isopotential neuron with no changes to ionic driving force

Reducing the IA charge from -0.85 to -1.25 led to a decrease in the membrane potential (Vm) of compartment 8 (Figure 2B, top row). The ionic reversal potentials for chloride, sodium and potassium also shifted downwards in direct proportion to Vm. The proportional shift of both Vm and the ionic reversal potential results in a maintenance of the same ionic driving force throughout the duration of the simulation. Notably, the driving forces in compartment 8 were also identical to the driving forces in the other compartments (Figure 2B, bottom row). The implication thereof is that even though there is a local compartment along the dendrite with an altered membrane potential the excitability of the dendrite should theoretically be uniform across compartments.

The change in membrane voltage could be a result of a) changes in ratios of the net ionic sum (Na + K – Cl +zX); b) changes in the area scaling constant (Ar) which scales Vm based on the compartment volume to area ratio; or c) a combination of the above factors.

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**Figure 2B: Impermeant anion charge sets local membrane potential, ionic reversal potential and ionic concentration without changing driving force. Area scaling is not applied to the membrane potential.**

Impermeant anion charge was changed from z=-0.85 to z=-1.2 between 120s and 180s.

**Top row:** Membrane potential and ionic reversal potential shift permanently only in the compartment manipulated (compartment 8).

**Middle row:** No permanent change to the ionic driving forces as membrane and ionic potentials in manipulated compartment change in proportion to other compartments.

## 2.3 Local changes to impermeant anion charge (z) results in local changes to ionic concentrations

For a constant driving force to be maintained a proportional change to the ionic reversal potential is needed. Ionic reversal potential is determined by the ratio of intra-compartmental ionic concentration to extracellular concentration. Considering that the extracellular bath was held at constant concentration, the intracellular concentration of each ion had to change. In the top three panes of Figure 3D, it is shown that the concentrations of chloride, sodium, and potassium all shown permanent change respectively.

In the middle panes the transmembrane fluxes in compartment 8 and compartment 4 (manipulated versus non-manipulated compartments) were compared to identify if changes to the transmembrane were underpinning the concentrations changes seen for each ion. There appears to be a change in the amount being fluxed through individual channels in compartment 8, however the nett flux (the sum of the inward and outward flux) is balanced. The changes in ion flux across the membrane in compartment 8 were due to area scaling of the channel fluxes.

As there was no nett flux across the membrane changes to ion channels were not responsible for the concentration changes observed, instead changes between compartments (because of electrodiffusion) was likely causing the changes in ion concentration and ionic reversal potentials that allow for a constant ionic driving force. The lower panes of Figure 3D show how respective electrodiffusive changes during the manipulation of impermeant anions created concentration changes in compartment 8 which were not observed in other compartments. Once the charge of impermeant anions stabilized at -1.25, there was no further ionic flux between compartments and the system reached a steady state.

Ultimately, we have shown that electrodiffusion results in ionic microdomains where local impermeant anion charge is manipulated. This results in a non-isopotential neuron as the local membrane potential changes in a single compartment, while the ionic driving forces remain constant due to proportional changes to ionic reversal potentials that are established through the non-uniform ion flux between compartments.

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# 3 – Multiple impermeant anion valence changes

## 3.1 The effects of different impermeant anion valences

We next performed five simulations each with decreasing impermeant anion charge (z) in compartment 8 (range of -0.45 to -1.25) (schematic 3). An inverse relationship between impermeant anion charge and impermeant anion concentration as well as with resting membrane potential, while volume was directly related to charge. These relationships only occurred in the manipulated compartment. (Figure 3a).

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**Schematic 3. Impermeant anion charge (valence) in Comp-8 manipulated in five separate simulations**

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Figure 3a**. Local impermeant anion(IA) charge sets local IA concentration, volume, and membrane potential.** Drops in concentration and membrane potential in the compartment with charge decreases **(left & right pane)**. Compartment volume increases linearly with decreasing IA valence **(middle pane)**

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Description automatically generatedFigure 3b shows an inverse relationship between charge of impermeant anions and chloride concentration; and an opposite, direct, correlation with sodium and potassium concentration in compartment 8. There were no changes to the transmembrane driving forces for any ion, however the boundary driving forces were impacted with equal but opposing voltages pulling ions from compartment 8 to compartment 9 and compartment 7 in a “tug-of war” scenario that establishes a steady state ion concentration gradient between compartments.

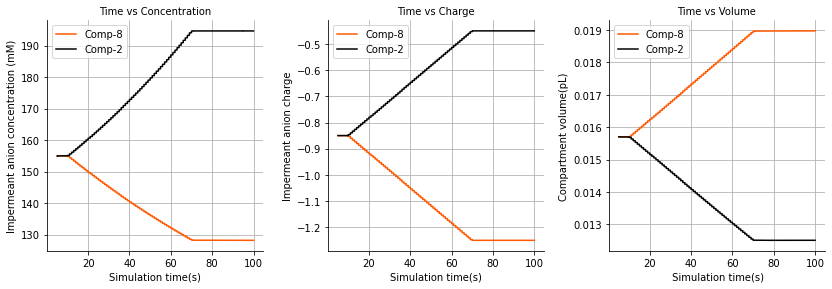
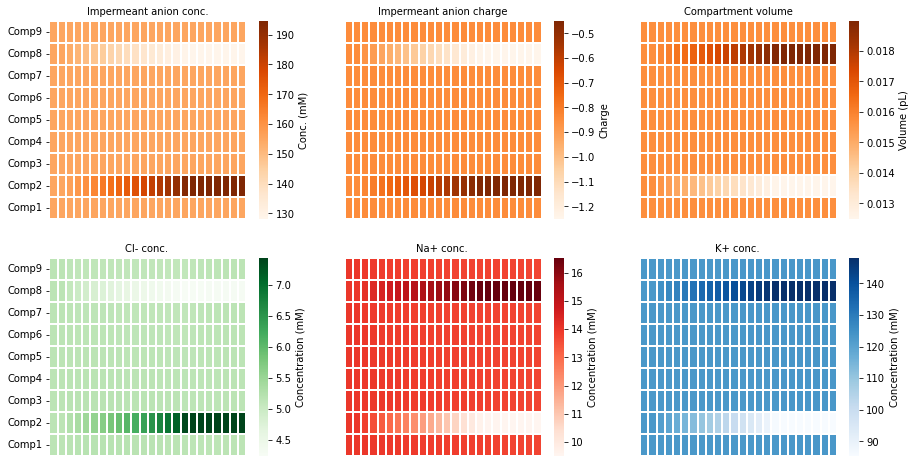
Figure 3b. **Impermeant anion charge sets up non-uniform concentration gradients between compartments maintained by competing axial driving forces.** Five simulations were conducted where the impermeant anion charge in Comp 8 differed (z = -0.45; -0.65; -0.85;-1.05; -1.25). *Top row:* The steady state permeant ion concentrations varied linearly in relation to decreasing IA charge resulting in a concentration gradient between Comp8 and its neighbouring compartments. *Middle row:* There was no change to steady state transmembrane driving forces. *Bottom row:* Boundary(axial) driving forces increase in amplitude in relation to the absolute difference between the IA charge and the default charge of -0.85. The direction of the force is determined whether the IA charge is greater or lesser than -0.85.

## 3.2 Different impermeant anion valences within the same dendrite

We next simulated a multicompartmental model with opposing valence changes in compartment 8 (z=-0.45) and compartment (-1.25) (fig3c). The concentration of anion and volume either compartment changed in opposite directions to each other with none of the non-manipulated compartments being affects. We also showed that in this multicompartment model there can be microdomains of all anions relative to the valence of impermeants.

In the compartment 8 and compartment 2 there were opposing fluctuations in the membrane potential of the compartment (fig3d). Equal changes to the reversal potential for each ion created constant driving forces irrespective of the ion concentrations.

Figure 3c**. Multiple ion microdomains occur in compartments with varied impermeant anion charge (IA).** *Top row:*We altered impermeant anion average charge in Comp8 (z=-0.45) and Comp2 (z=-1.25) between 120 and 180s. This resulted in respective changes to IA concentration and compartment volume in Comp8 and Comp2. *Middle row:* No changes demonstrated in IA concentrations and cell volumes in non-manipulated compartments. *Bottom row:* Distinct microdomains of local ion concentration differences occurring due to the change in IA average charge, persisting beyond the manipulation period.



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Figure 3d**. Constant ion driving forces despite varied membrane potentials across the multicompartment dendrite with altered impermeant anion valences.** We altered impermeant anion average charge in Comp8 (z=-0.45) and Comp2 (z=-1.25) between 120 and 180s. *Top row:* Membrane potential increase in Comp8 and decrease in Comp2. *Middle row:* Ionic reversal potential increases and decreases in Comp8 and Comp2 respectively. *Bottom row:* No changes to steady state driving forces at the end of the simulation.

# 4 – Current addition

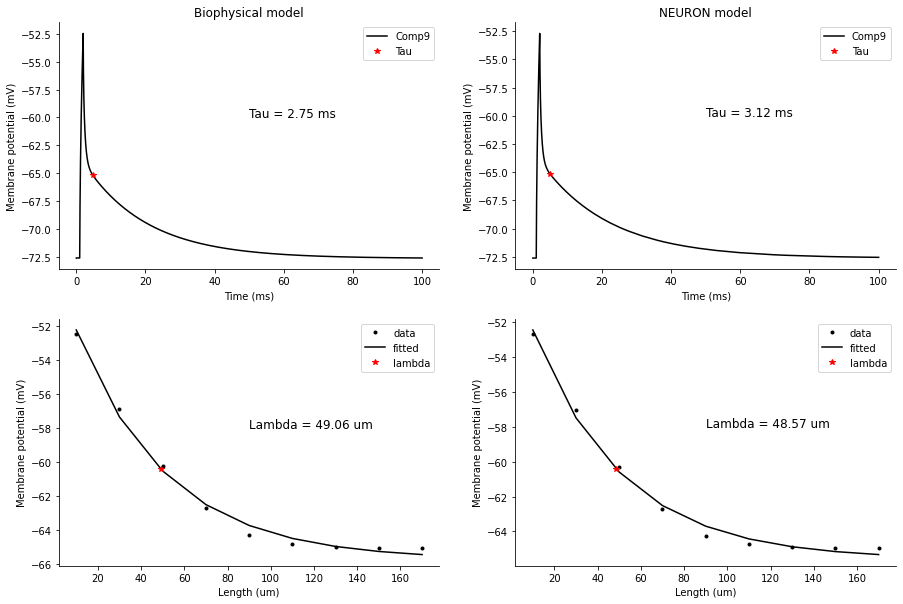
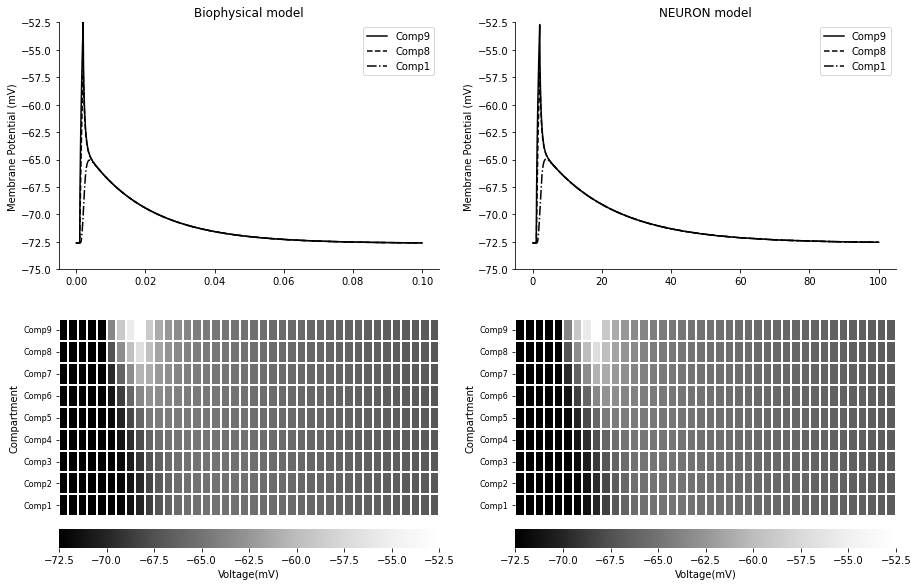
## 4.1 Comparison between the electrodiffusion based model and equivalent circuit model

We next compared the passive electrical properties of our electrodiffusion based model to an equivalent circuit model constructed in NEURON1,2. Nine compartments (lengths: 20µm; diameter: 1µm) were linearly assembled with identical membrane capacitances (2µF/cm2) and total passive/leak conductances (0.00011 S/cm2). For the NEURON model 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.

We pulsed a step current of +0.1nA with a 1ms duration onto compartment 9 and calculated the time(τ) and length(λ) constants in each model (**Figure 4a**). The maximum amplitude of depolarization in Comp9 was similar for the NEURON model (-52.78mV) compared to the electrodiffusion based model (-52.63mV). There were also similar degrees attenuation of current in the NEURON model (τ = 3.12ms; λ = 48.57µm) relative to the electrodiffusion-based biophysical model (τ = 2.75ms; λ = 49.06µm).

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**Figure 4a.** **Cable properties in an equivalent circuit model (NEURON) versus biophysical electrodiffusion based models.** A 0.1nA current for 1ms was pulsed into compartment 9 (Comp9) of a 180µm long, 9 compartment, dendritic model. Heat maps shown between times 0-5ms.

*Left column:* Electrodiffusion-based biophysical model; Max voltage = -52.63mV; τ = 2.75ms; λ = 49.06µm; Electrodiffusion constants at default values.

*Right column:* Equivalent circuit-based NEURON model (raw data graphed with matplotlib); Max voltage = -52.78mV; τ = 3.12ms; λ = 48.57µm; Ra = 200Ω-cm.

## 4.2 Cable properties with varied Impermeant anion valency in all compartments

We then used the same experimental setup to determine the effect changing impermeant anion valency may have on passive neuron properties after excitatory and inhibitory current pulses. Separate simulations were performed with impermeant anion valency set to z = -0.45, z = -0.85, and z = -1.25 in all 9 compartments. A +0.1nA current was pulsed onto compartment 9 for 1ms. In **Figure 4b,** excitatory currents showed greater amplitudes in the simulations with more positive impermeant anion valency.

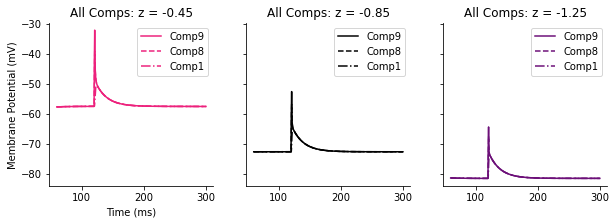
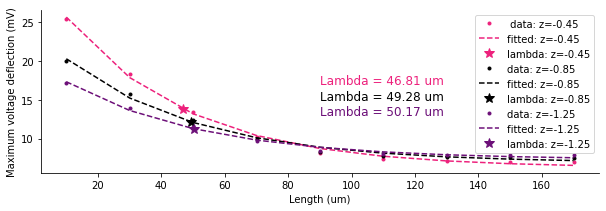
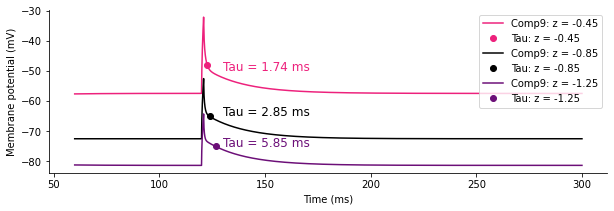


Figure 4b. **Cable properties in the multicompartment biophysical model with varied impermeant anion valency in all compartments.** A 1ms +0.1nA current was pulsed into compartment 9 (Comp9) of a 180µm long, 9 compartment, dendritic model where all compartments had an impermeant anion valency (z) of -0.45; z = -0.85; z = -1.25 in three separate simulations.

*Top row:* Voltage tracing for Comp9,Comp8 and Comp1 in each simulation.

*Middle row:* Time constants (Tau/τ). z = -0.45: τ = 1.74ms. z = -0.85: τ = 2.85ms; z=-1.25: τ = 5.85ms

*Bottom row:* Length constants (Lambda/ λ). z = -0.45: λ = 46.81µm; z = -0.85: λ = 49.28µm; z=-1.25: λ = 50.17µm

## 4.3 Cable properties when Impermeant anion valency is changed just in comp 8

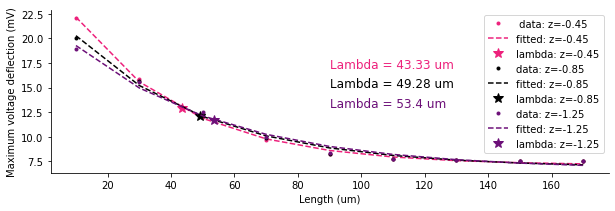
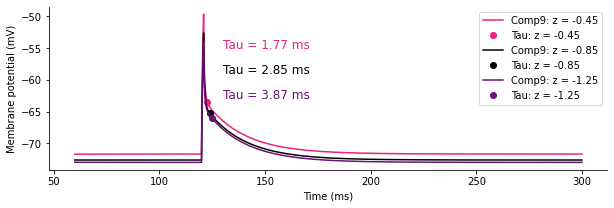
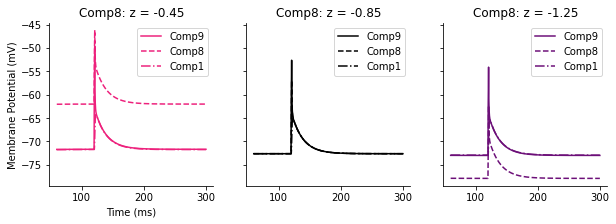
We examined the influence a local change in impermeant anion valency may have on excitatory and inhibitory signalling. As above, a nine-compartment model was linearly arranged and linked via electrodiffusion (electrodiffusion constants set to half the default).

Figure 4c. **Cable properties in the multicompartment biophysical model with varied impermeant anion valency in a nearby compartment.** A 1ms +0.1nA current was pulsed into compartment 9 (Comp9) of a 180µm long, 9 compartment, dendritic model. Valency (z) of impermeant anions in Comp8 were set at: -0.45; -0.85, -1.25. Remaining compartments had a default valency of z = -0.85.

*Top row:* Voltage tracing for Comp9,Comp8 and Comp1 in each simulation.

*Middle row:* Time constants (Tau/τ). z = -0.45: τ = 1.77ms. z = -0.85: τ = 2.85ms; z=-1.25: τ = 3.87ms

*Bottom row:* Length constants (Lambda/ λ). z = -0.45: λ = 43.33µm; z = -0.85: λ = 49.28µm; z=-1.25: λ = 53.4µm



# 5 – impact of impermeant anions on synaptic transmission

We extended our electrodiffusion based biophysical model to understand the impact impermeant anions had on synaptic integration. A soma(length: 40µm; diameter 2µm) was joined onto compartment 1 to make and synaptic inputs were placed on compartment 8. An alpha function was used to model synaptic excitatory (“Glutamatergic”) and inhibitory (“GABAergic”) inputs. To model the impact of synaptic integration Hodgkin-Huxley type channels were modelled in the somatic compartment, with an action potential threshold set at -55mV.

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## 5.1 Excitatory and inhibitory synapse demonstration with different impermeant anion valency

We tested subthreshold excitatory and inhibitory synaptic inputs on our multicompartment model. In figure 5.1 synaptic input was directed at compartment 8. The same simulation was repeated for 3 different impermeant anion valences in compartment 8 only (z = -0.45; z = -0.85; z = -1.25) which were at steady state before the synaptic input. The resting membrane potential in compartment 8 was different in each simulation, as shown in previous experiments. The amplitude of voltage deflection due to synaptic excitation and inhibition was not affected despite the different impermeant anion valences. Moreover, there were no differences in the somatic voltage deflections in either the excitatory or inhibitory simulations.

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## 5.2 Action potential generation with impermeant anion change at the synapse

5mM NT; 1 x10-9 conductance; z=-0.85 🡪 no AP (soma to -67.5mV)

5mM NT; 2 x10-9 conductance; z=-0.85 🡪 no AP (soma to -63mV)

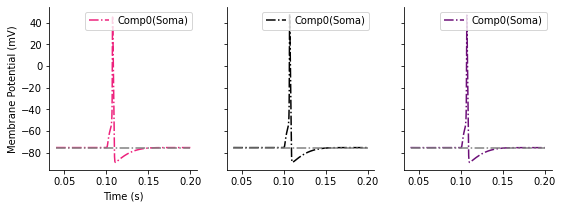
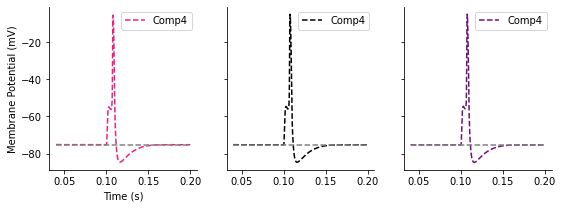
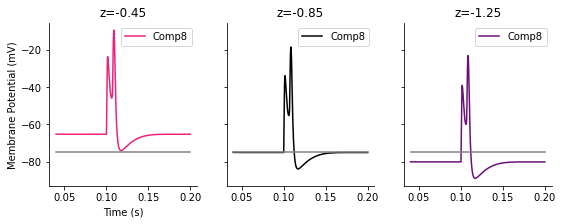
1mM NT; 3 x10-9 conductance; z=-0.85 🡪 no AP (soma to -62.5mV)

2mM NT; 3 x10-9 conductance; z=-0.85 🡪 triggers AP

3mM NT; 3 x10-9 conductance; z=-0.85 🡪 triggers AP

5mM NT; 3 x10-9 conductance; z=-0.85 🡪 triggers AP

5mM NT; 5 x10-9 conductance; z=-0.85 🡪 triggers AP

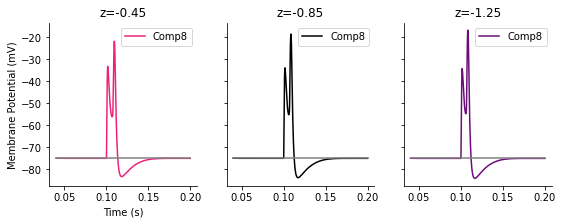
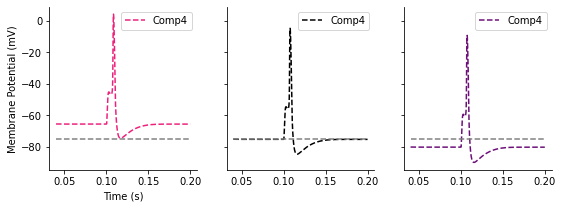
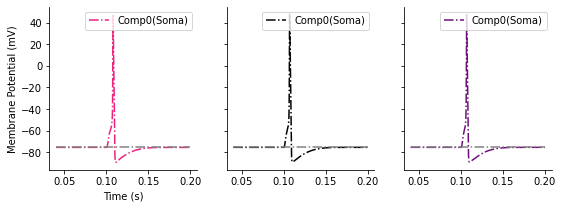


## 5.3 Action potential generation with impermeant anion change in the middle of the dendrite



T9;T10

Figure uses Exp5-4; Exp5-7;Exp5-8



## 5.4 Action potential generation with impermeant anion change at the soma

T11;T12

Exp5-4;Exp5-9;Exp5-10

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## 5.5 Inhibitory synapses and impermeant anions

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