

PART 1

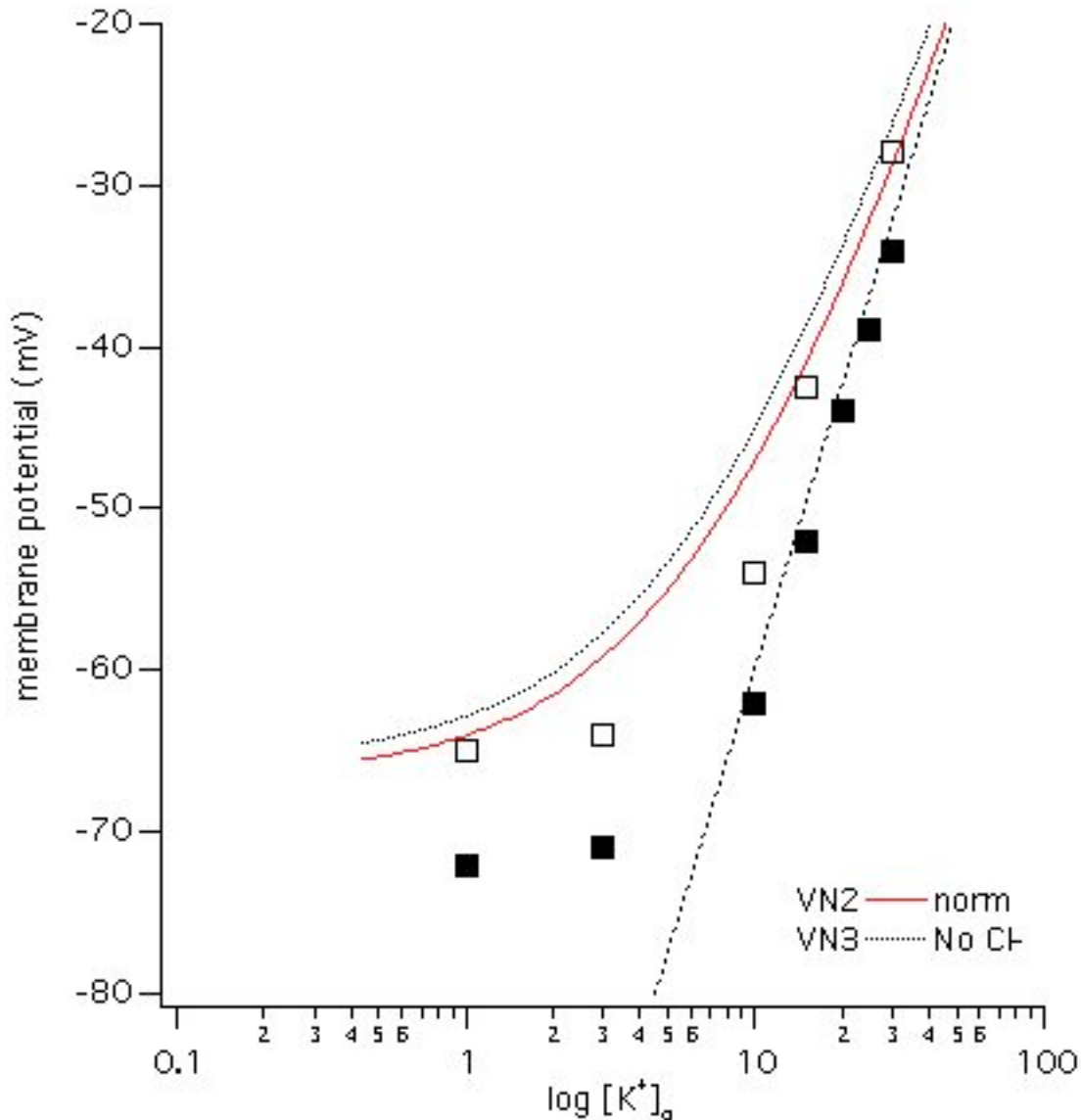
Figures 2,5,6,7 show experiments performed in a spinal cord preparation in vitro. Intracellular recordings were performed using sharp electrodes, which unlike patch electrodes in the whole-cell configuration, do not dialyze the cell and alter intracellular ion concentrations. Bath solution contained (in mM): 126 NaCl, 25 NaHCO₃, 3 KCl, 1 MgSO₄, 12 glucose, 1.2 NaH₂PO₄, and 2 CaCl₂. In order to block synaptic transmission in Figs 2,5,6 the CaCl₂ was reduced to 0 mM and replaced with equimolar MnCl₂. The resting membrane potential (dubbed "RMP") is indicated in Figures 5,6,7.

Figure 2 shows the effect of [K⁺]_o (1 to 30 mM) on baseline membrane potential (V_m). Black squares are in the presence of normal bath solution (above), white squares after replacing NaCl in the bath solution with Na-isethionate. Figure 5 shows the time course of the effect of reducing [Cl⁻]_o.

The data below are taken from Figure 2:

K _o	V _m	V _m (low Cl ⁻)
1	-72	-65
3	-71	-64
10	-62	-54
15	-52	-42.5
20	-44	
25	-39	
30	-34	-28

- (1) Does V_m depend predominantly on K⁺ as predicted by the Nernst equation? Give evidence and summarize your reasoning. **V_m depends predominantly on K⁺ as predicted by the Nernst equation until [K⁺]_o < 8 mM, and then the membrane shows distinct non-Nernstian behavior. This is apparent because the plot of V_m vs. log[K⁺]_o is linear for [K⁺]_o > 8 mM, which it should be if the Nernst equation is obeyed, but is nonlinear for [K⁺]_o < 8 mM.**
- (2) What two aspects of the data in Figure 2 suggest that ions other than K⁺ may influence V_m? Describe the biophysical mechanisms by which these ions influence V_m in the context of the data shown. **(1) The nonlinear portion of the curve for [K⁺]_o < 8 mM suggests that some other ion may be permeable (other than K⁺), and (2) when chloride (Cl⁻) was lowered, the whole curve shifted leftward ([K⁺]_o > 8 mM) and upward to more depolarized V_m at ([K⁺]_o < 8 mM), which suggesting that a Cl⁻ ions have a relatively constant influence on baseline V_m.**
- (3) Use Microsoft Excel, Wavemetrics IgorPro, Matlab, or any other software to plot the data (above) and illustrate your points in #1 and #2 using appropriate equations. You do not have to fit the equations to the data, just make reasonable estimates of parameters and use the theory (equations) to make your points. **Plot V_m=58*log(K/106) to show the majority of the curve for [K⁺]_o > 8 mM is linear, following Nernst equation. I also plotted the GHK voltage equation (V_m = 58*log[(αNa_o+βCl_i+K_o)/(αNa_i+βCl_o+K_i))] for reasonable assumptions: α=0.05, β=0.1, where α=P_{Na}/P_K, β=P_{Cl}/P_K. I plotted the GHK voltage equation both with and without the chloride terms. Both plots show that non-Nernstian behavior occurs for [K⁺]_o < 8 mM as the experiment showed. Also the GHK plot without chloride shows that without the chloride contribution you would expect a left-upward shift, as the data shows.**



In Figure 6 assume that the V_m trajectory at the nadir of the action potential (i.e., the after-hyperpolarization, AHP) is dominated by three membrane currents selective for K^+ : (i) delayed-rectifier K^+ current (I_{K-DR}), (ii) calcium-activated K^+ current (I_{K-Ca}), and (iii) passive "leakage" K^+ current (I_{K-leak}). Figure 6 (inset) shows spikes evoked from different baseline membrane potentials (baseline V_m on the x-axis). The y-axis plots the difference between the voltage measured at 45 ms (during the AHP) and the baseline V_m prior to evoking the spike (i.e., $\Delta V_{AHP} = V_{AHP} - V_m$). Note that the x-axis is reversed and negative V_m are plotted to the right of the origin.

- (4) What is $[K^+]_i$? Briefly explain your solution. **Since the membrane potential at the nadir of the action potential is dominated by K^+ currents, we must assume that if the AHP is depolarizing, then the AHP current is inward and ($V_m < E_K$), whereas if the AHP is hyperpolarizing, then the AHP current is outward and ($V_m > E_K$). Therefore, if we use bias current to regulate V_m the AHP should reverse when $V_m = E_K$, and $-78.5 \text{ mV} = 25.7 \cdot \ln(K_o/K_i)$. Rearranging: $K_i = 5 / \exp(-78.5/25.7) = 106 \text{ mM}$.**

In Figure 7 the in vitro preparation was given electrical stimuli applied to the ventral root, which activates Renshaw cells projecting monosynaptically to spinal motoneurons that generate glycine-mediated inhibitory post-synaptic potentials (IPSPs). Figure 7 shows ventral root-evoked IPSPs from baseline V_m from -93 to -58 mV (x-axis plots baseline V_m). The y-axis plots the difference between the voltage during the peak of the IPSP and the baseline V_m (i.e., $\Delta V_{IPSP} = V_{IPSP} - V_m$). Note that the x-axis is reversed and negative V_m are plotted to the right of the origin.

- (5) What is $[Cl^-]_i$? Briefly explain your solution. **For the same reasons as above, chloride current (I_{Cl}) dominates the IPSP. Thus when adjusting baseline V_m , the IPSP vanishes when it's driving force is zero (i.e., $V_m = E_{IPSP} = E_{Cl}$). This occurs at -80 mV, so, $Cl_i = Cl_o \cdot \exp(E_{IPSP}/25.7) = 133 \cdot \exp(-80/25.7) = 5.92$ mM.**
- (6) What is the predicted $[Cl^-]_i$, if chloride were passively distributed? Note: this system is not in electrochemical equilibrium. **If chloride is passively distributed, then Cl_o and Cl_i are not equal, but in fact will distribute themselves so that $E_{Cl} = V_m(\text{rest})$. Thus, $Cl_i = Cl_o \cdot \exp(V_m(\text{rest})/25.7) = 133 \cdot \exp(-75/25.7) = 7.185$ mM.**
- (7) What is the Nernst potential for chloride (E_{Cl}) and what is chloride's *driving force* at the resting potential? **E_{Cl} is less depolarized, i.e., more negative at -80 mV (and Cl_i is lower than expected), thus driving force is $V_m - E_{Cl} = +5$ mV.**
- (8) If a chloride conductance opens at resting membrane potential, then in which direction will chloride ions tend to flow, and what would be the sign of the current? Briefly explain. **If g_{Cl} opens at rest, then $I_{Cl} = g_{Cl} \cdot (V_m - E_{Cl})$, since $(V_m - E_{Cl}) = -75 + 80 = +5$, the current is outward (positive) and chloride ions flow in, i.e., the inward flow of chloride anions is positive (outward) current.**
- (9) If the membrane were somewhat chloride permeable at rest, then what effect would this chloride permeability have on V_m ? Briefly explain your reasoning based on the data in Figures 2, 7. **Based on the above, chloride permeability at rest will hyperpolarize the membrane. This is consistent with Figure 2, which shows that when you remove chloride ions from the bath, V_m depolarizes (for any given $[K^+]_o$).**
- (10) Propose a mechanism that results in the chloride concentration disparity (difference between $[Cl^-]_i$ predicted and measured). **A metabolic chloride pump (e.g., KCC2 in Szabadics *et al.* Science 311(5758): 233, 2006), which extrudes Cl^- and results in lower intracellular chloride concentration than predicted by passive properties and a net hyperpolarizing contribution to V_m at rest.**

The four-panel figure labeled A-D shows a novel Ca^{2+} channel that was recently characterized (Kirichok *et al.* 2004). This channel is expressed in the membrane of mitochondria. The authors studied this channel by isolating the mitochondria (dubbed "mitoplasts") in vitro. They made patch-clamp recordings from mitoplasts just as one normally records from neurons or myocytes in vitro. They studied calcium currents in mitoplasts, defined in the usual way where inward flux of cations is negative current. Here, the "bath" or external calcium concentration $[Ca^{2+}]_o$ was called cytoplasmic $[Ca^{2+}]_c$ and the internal $[Ca^{2+}]_i$ was called mitoplasmic $[Ca^{2+}]_m$.

In the whole-mitoplast patch-clamp configuration (equivalent to “whole-cell”), voltage-ramp commands generate the following family of I-V curves from -160 to $+80$ mV (below). Each trace differs only in the $[Ca^{2+}]_c$ which is varied from 20 μ M to 105 mM. Mitochondrial calcium current (I_{MiCa}) is displayed as either raw current (pA) (B) or as current normalized to mitochondrial capacitance (pA/pF) (C). Assume that $[Ca^{++}]_m$ is about 10 nM.

- (11) Do these I-V curves exhibit inward or outward rectification? Justify your answer in one sentence. Inward rectification, negative (inward) current flows more readily. **Inward rectification, negative (inward) current flows more readily.**
- (12) Can it be due to Goldman rectification (i.e., constant field theory)? Justify your answer. **Yes, Goldman rectification is conceivable in this case since $[Ca^{2+}]_c > [Ca^{2+}]_m$. In panel C you can see that $E_{rev} > 0$ for the highest $[Ca^{2+}]_c$.**
- (13) In a subsequent experiment (shown in D) the authors set $[Ca^{2+}]_c = [Ca^{2+}]_m = 105$ mM and applied the same voltage-ramp protocol. The resulting I-V curve is shown in black (D). Does the I-V curve exhibit inward or outward rectification? And, can it be due to Goldman rectification? Justify your answer. **Inward rectification, however, now $[Ca^{2+}]_c = [Ca^{2+}]_m$ so Goldman rectification is not possible.**
- (14) Based on these two sets experiments, what would you conclude about the voltage- and time-dependence of I_{MiCa} channels? **I_{MiCa} exhibits voltage-dependent rectification, preferentially opening at hyperpolarized V_m . Time dependent properties are not really explored here because the slowness of the voltage ramp command is used to approximate steady state at each point in the protocol. Thus, we are forced to assume that time-dependent properties are precluded in this analysis.**
- (15) The red trace (below) shows the current evoked by the same protocol when Ca^{++} in the mitoplasmic solution is replaced by Mg^{2+} , thus $[Mg^{2+}]_m = 105$ mM. Why does the current (red trace) flow inward but not outward under these conditions? Explain in no more than two sentences. **Inward current at negative voltages is Ca^{2+} current, but at positive potentials the current that could flow out is NOT Ca^{++} , since all has been replaced by Mg^{2+} . Since there is no outward Mg^{2+} current it must be plugging the channels from the inside.**