CMP 610b Lecture 1
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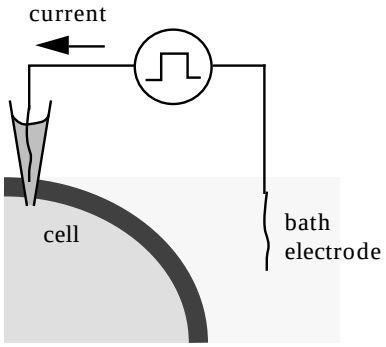
Microelectrodes

In this lecture we are concerned with two kinds of microelectrodes: 'conventional' microelectrodes which are used to impale cells, and 'patch' electrodes that are sealed against the membrane of a cell. Conventional microelectrodes are glass pipettes with very fine tips (usually < 1µm) and are typically filled with a very concentrated salt solution (e.g. 3 *M* KCl). Patch pipettes have somewhat larger tips and are typically filled with physiological solutions (e.g. Ringer's, Tyrode's solutions). They are 'electrodes' because a chlorided silver wire connects the aqueous solution to the metal conductors in the electronic devices that measure potentials, pass currents, etc.

HOW DO CURRENTS FLOW THROUGH ELECTRODES?
1. The Ag-AgCl junction

Let us consider the situation drawn below, where a stimulator causes a current to flow into the cell through a microelectrode. There is a Ag-AgCl wire in the microelectrode, and another in the bathing solution (the bath electrode).



The arrow of current says that net positive charge is moving in that direction. (In the metal wires, actually electrons are moving in the opposite direction). At the wire in the microelectrode the following reaction is taking place

$$Cl^- + Ag \rightarrow AgCl + e^- \qquad (1)$$

This reaction is reversible, but it is mainly going in this direction. At the bath electrode the opposite reaction is the main one taking place,

$$AgCl + e^- \rightarrow Cl^- + Ag \qquad (2)$$

Notice two things about reaction (1).

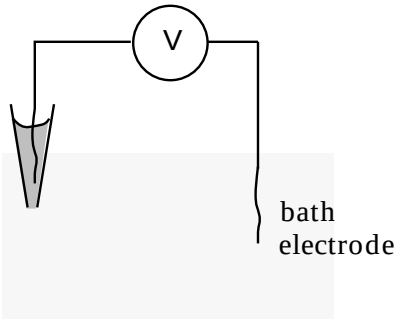
a. It will go faster when $[Cl^-]$ is large
b. It will go faster if the metal electrode has a positive potential φ_e.

Meanwhile reaction (2) is independent of [Cl] but will go slower if φ_e is positive.

We can write down the relative rates of reactions (1) and (2) at one electrode, and what interests us at the moment is the case in which the rates are equal, in which case no net current would be flowing.

$$\frac{\text{rate}_1}{\text{rate}_2} = r_0\,[\text{Cl}^-]\; e^{(\varphi_e - \varphi_{solution})e_0 / kT} \qquad (3)$$

Now what would be the potential difference between the microelectrode (me) and bath electrode (be), given as $V = \varphi_{me} - \varphi_{be}$, if no cell is present and we require that no current be flowing?



If [Cl⁻] were the same at each of the silver wires, we would expect no potential difference by the symmetry of the system. More typically, the microelectrode is filled with 3 *M* KCl while the bath electrode is immersed in physiological saline, say 150 m*M* NaCl. Setting the ratio of the rates (eqn. 3) to unity for each electrode we have

$$V = \varphi_{me} - \varphi_{be} = \frac{kT}{e_0} \ln\left(\frac{[\text{Cl}]_{be}}{[\text{Cl}]_{me}}\right) \qquad (4)$$

So for the concentrations $[\text{Cl}]_{be} = 0.1M$ and $[\text{Cl}]_{me} = 3M$ we have V≈ –90 mV. This negative potential at the microelectrode tends to repel the 'excess' Cl ions.

There would not be this electrode offset potential if a salt bridge were used to connect the bath to another pool containing the same solution as in the microelectrode.

2. The liquid junction

How does electric current flow from the micropipette into a cell? This question becomes interesting because the concentrations of ions in the microelectrode are often quite different from the concentrations in the bath or inside the cells we are measuring from. For example, consider a pipette containing 1 *M* KAcetate inserted into a cell. In the absence of potential differences, both K^+ and acetate⁻ will be diffusing into the cell; much smaller fluxes of K^+ and Cl^- will be diffusing in the opposite direction. Since K^+ diffuses about twice as fast as acetate⁻ (diffusion constants $D = 2 \times 10^{-5}$ *vs.* 1.1×10^{-5} cm²/sec) the main result will be a net positive current flow into the cell even with no potential difference across the pipette-cell interface. To have no current flow the bath must have a positive potential (about +15 mV) in order to equalize the rates of diffusion of cations and anions. This liquid junction potential, defined to be the bath potential minus the potential inside the pipette, can be estimated in various ways (all of which are approximate), using for example the Goldman-Hodgkin-Katz equation or the Henderson equation. Taking the GHK equation, and assuming that the main diffusible ions inside the cell are K^+ and Cl⁻, we obtain the liquid junction potential as

$$\varphi_{LJ} = \frac{kT}{q} \ln \frac{D_K\,[K]_e + D_{Cl}\,[Cl]_{cell}}{D_K\,[K]_{cell} + D_{Ac}\,[Ac]_e}\,. \qquad (5)$$

Differences in LJ potentials at a microelectrode can be measured by immersing the same electrode into various solutions. However, to do this measurement you must also know what the various

solutions are also doing to the potential offset of the reference electrode! See Neher, (1992)[1] for a pithy introduction to liquid junction effects and measurements.

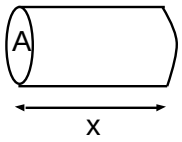
A high concentration of KCl is ideal as a microelectrode solution if you want to avoid LJ potentials. This is because K^+ and Cl^- have almost identical diffusion constants, and if the concentration in the pipette is high, the efflux of K^+ and Cl^- from the pipette will be much larger, and will effectively swamp out, any influx of the ions that are outside.

ELECTRODE RESISTANCE

In order to pass a current *I* through a microelectrode, you have to apply a voltage *V* to force the ions to move in the pipette. The resistance is the ratio of the voltage applied across the pipette to the current *I* that is elicited,

$$R = \frac{V}{I}.$$

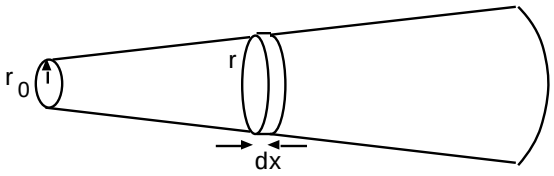
The resistance of a cylinder of conducting substance like this



is given by

$$R = \frac{\rho\, l}{A}$$

where *l* is the length, *A* the area, and ρ is the restivity of the substance. Now consider the resistance of the conical interior of a microelectrode:



The resistivity of a slab of length *dx* is

$$dR = \frac{\rho\, dx}{\pi r^2}.$$

Notice that the incremental resistance at the tip is <u>much</u> larger than up the shank where *r* is greater, because of the $1/r^2$ dependence. The total resistance is the integral of *dR* over all *x* values. To do this, first we must have a relationship between r and the x coordinate. Let

$$r = kx$$

so that *k* represents the steepness of the taper of the pipette. Then the total resistance *R* (letting the cone be infinite in length, since it makes little difference to the resistance) is

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[1] E. Neher, Correction for liquid junction potentials in patch clamp experiments. *Methods in Enzymology* **207**, 123-131, 1992.

$$R = \int_{x_0}^{\infty} \frac{\rho}{\pi r^2}\, dx$$

or by the substitution $dx = dr/k$,

$$R = \int_{r_0}^{\infty} \frac{\rho}{\pi r^2 k}\, dr$$

which yields

$$R = \frac{\rho}{\pi r_0 k}. \qquad (6)$$

Notice that the resistance is inversely proportional to the radius r_0 at the tip, and is also inversely proportional to the steepness of the taper k. Let us take some typical values for a patch pipette. For physiological salines $\rho \approx 100$ ohm-cm, and $k \approx 0.2$. For a tip radius $r_0 = 0.5$ µm (5×10^{-5} cm) one computes $R \approx 3$ megohms, which is a typical value. For pipettes with much smaller tips (such as for microelectrodes that penetrate cells) the lower ρ values afforded by more concentrated salt solutions (e.g. 3*M* KCl) help to keep the resistance from being too large.

CONTROLLING MEMBRANE POTENTIAL

Now let us consider the effect of injecting current through a microelectrode to change the membrane potential of a cell. We start with what I call the "fundamental equation of electrophysiology",

$$I_{ionic} + C\frac{dE}{dt} = 0$$

The amount of electrical charge deposited on the cell membrane is C, the cell capacitance, times E, the membrane potential. $C\, dE/dt$ is then the rate of change of the stored charge; I_{ionic} is the ionic current through channels (or other pathways) through the membrane. Clearly changes in the stored charge must be accounted for by ionic currents through the membrane, and this equation is simply a statement of the conservation of charge.

If a microelectrode injects current into the cell, the equation becomes

$$I_{ionic} + C\frac{dE}{dt} = I_{injected} \qquad (7)$$

Note that this says that in the steady state (when dE/dt is zero) the <u>outward</u> ionic current through the membrane is just equal to the current injected <u>into</u> the cell through the microelectrode.

We would like to solve this equation for E. To do this we must know how I_{ionic} and $I_{injected}$ depend on E. The membrane current I_{ionic} is zero at the resting potential E_r and depends on the membrane conductance g_m according to

$$I_{ionic} = (E - E_r)\, g_m$$

Let V be the voltage applied to the microelectrode. Then

$$I_{injected} = \frac{V - E}{R_e}$$

or,

$$I_{\text{injected}} = (V–E)\, g_e$$

Substituting these expressions into (7) one obtains

$$(E–E_r)\, g_m + C\frac{dE}{dt} = (V–E)\, g_e$$

or, by rearranging,

$$(g_m + g_e)\, E + C\frac{dE}{dt} = g_m E_r + g_e V$$

or,

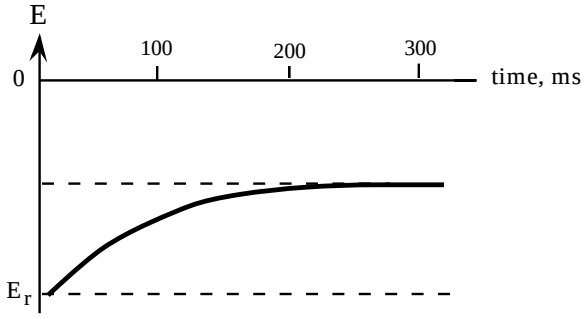
$$E + \frac{C}{g_m+g_e}\frac{dE}{dt} = \frac{g_m E_r + g_e V}{g_m+g_e}. \qquad (8)$$

This can be written in a standard form,

$$E + \tau\frac{dE}{dt} = E_\infty,$$

where τ is the time constant of changes in E, and E_∞ is the final value that E takes: it is the weighted average of E_r and V, weighted by the relative sizes of g_m and g_e.

As an example, consider trying to control the membrane potential of a *Xenopus* oocyte. For that cell $g_m = 10^{-6}$ siemens (i.e. the membrane resistance is about 1 megohm). A typical electrode has $g_e = 10^{-6}$ siemens also (1 MΩ resistance). The membrane capacitance is about 0.2×10^{-6} farads (0.2 μF). This results in E_∞ halfway between E_r and V, and τ = 0.1 seconds. For example, if V were suddenly changed fromE_r to 0 mV the membrane potential would follow a time course like this:



Thus if you wanted to control the membrane potential by applying the commanded potential V to the microelectrode, you would not be very successful: E would only follow half the magnitude of the changes you impose, and will follow the changes very slowly, with a time constant of 100 milliseconds. To get around these severe limitations one uses the voltage-clamp techniques that we will consider in the next two lectures.