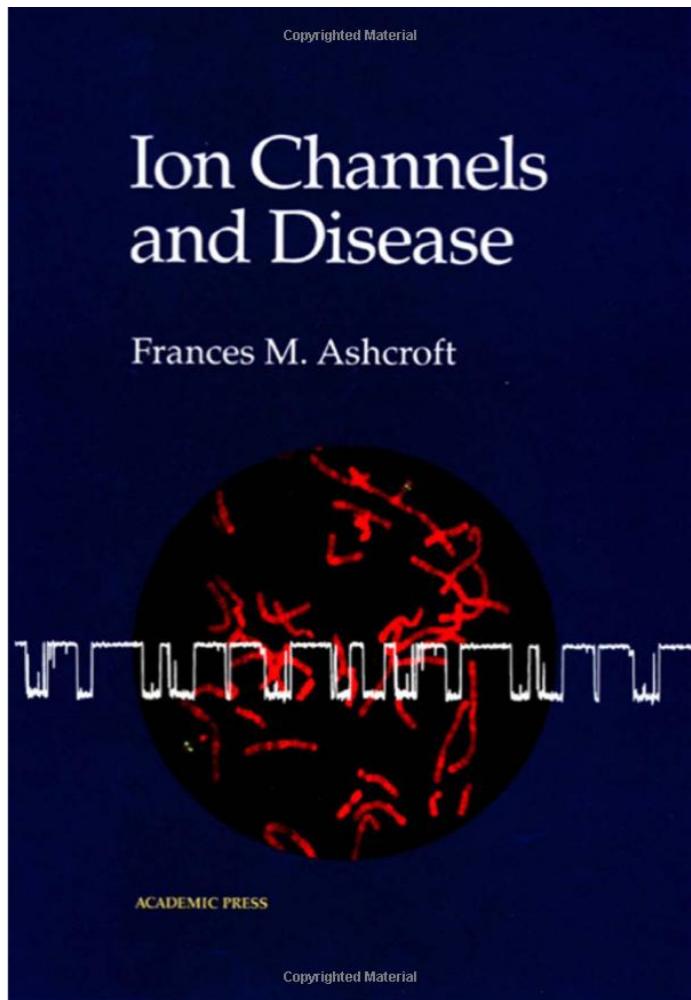
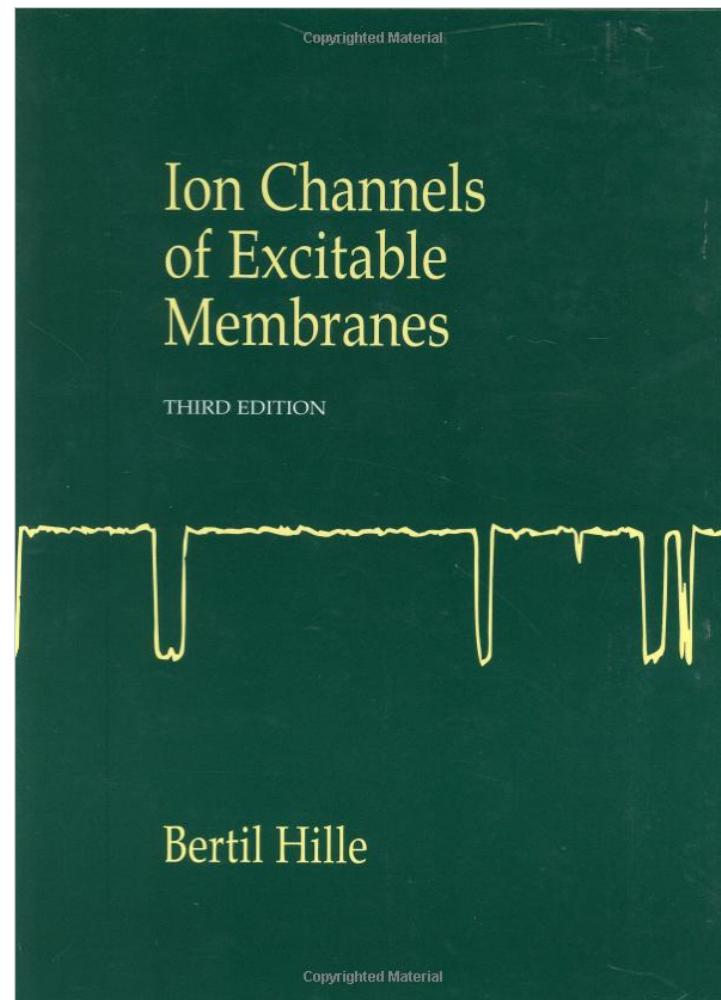


## Channelology literature

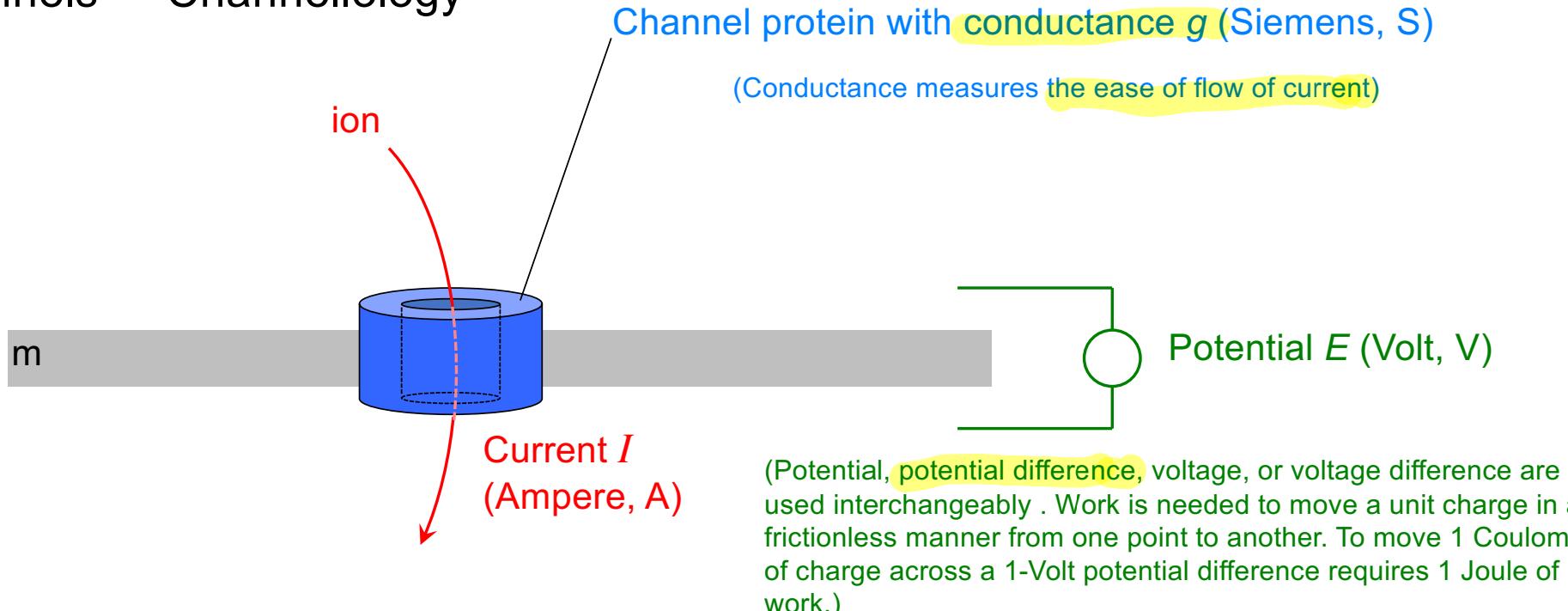


ISBN-13: 978-0120653102



ISBN-13: 978-0878933211

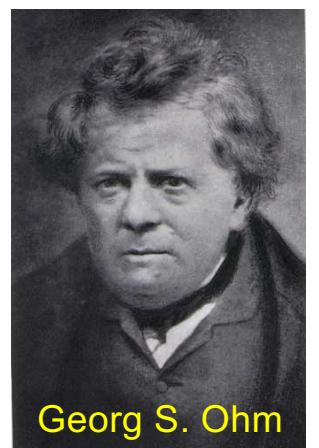
## Channels – "Channelloogy"



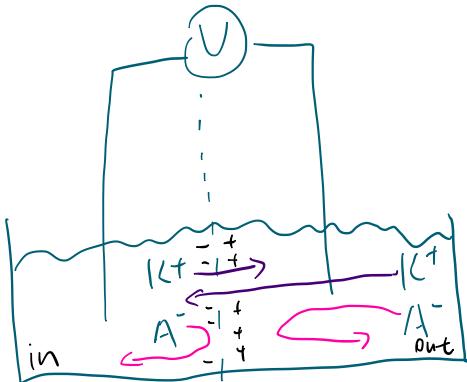
$$\text{Ohm's law: } I = gE \\ ( E = IR )$$

Analogy: Water pipes

Resistance  $R$  (Ohm,  $\Omega$ )  
(Resistance: inverse of conductance)



## Blackboard black lipid bilayer setup and Nernst equation



$\rightarrow H_2O$ -selective  $\rightarrow \partial V$

$\downarrow$  for  $K^+$   $\rightarrow \bar{E} = \bar{E}_{K^+}$

At first ① is (charge) ② is 0

At equilibrium  $\textcircled{1} = -\textcircled{2} \Rightarrow RT \ln \frac{[K^+]_{out}}{[K^+]_{in}} = -ZF \bar{E}_{K^+}$

$$\bar{E}_{K^+} = \frac{RT}{-ZF} \ln \frac{[K^+]_{out}}{[K^+]_{in}}$$

out  $\rightarrow$  in  $Z=1$

in  $\rightarrow$  out  $Z=-1$

Driving force:

①  $\Delta$  Concentration

$$\text{free energy } \Delta G_{\text{chem}} = R \cdot T \ln \frac{[K^+]_{out}}{[K^+]_{in}}$$

② Work against electric field

$$\Delta G_{\text{elec}} = Z \cdot F \cdot \bar{E}_{K^+}$$

Number  
of charge  
with  
one  
particle

if out < in,  $Z=1$   $\bar{E}_{K^+} > 0$

it is not possible,

# Nernst potentials:

*Conventions:* Side 1: inside (intracellular)  
Side 2: outside (extracellular)

All membrane potentials are measured inside minus outside.

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}$$

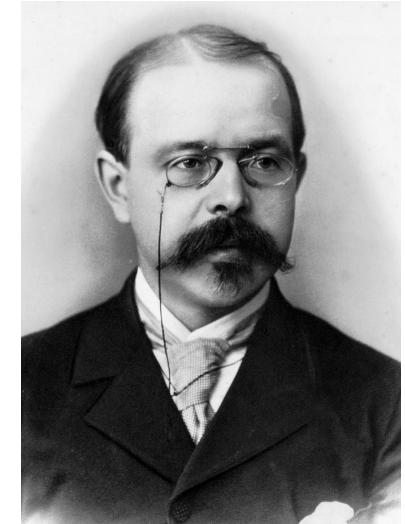
$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i}$$

$$E_{Ca} = \frac{RT}{2F} \ln \frac{[Ca]_o}{[Ca]_i}$$

$$E_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_i}{[Cl]_o}$$

↓  
negative  
charged

o: outside; i: inside



Walther H. Nernst

**TABLE 1.3 Free Ion Concentrations and Equilibrium Potentials for Mammalian Skeletal Muscle**

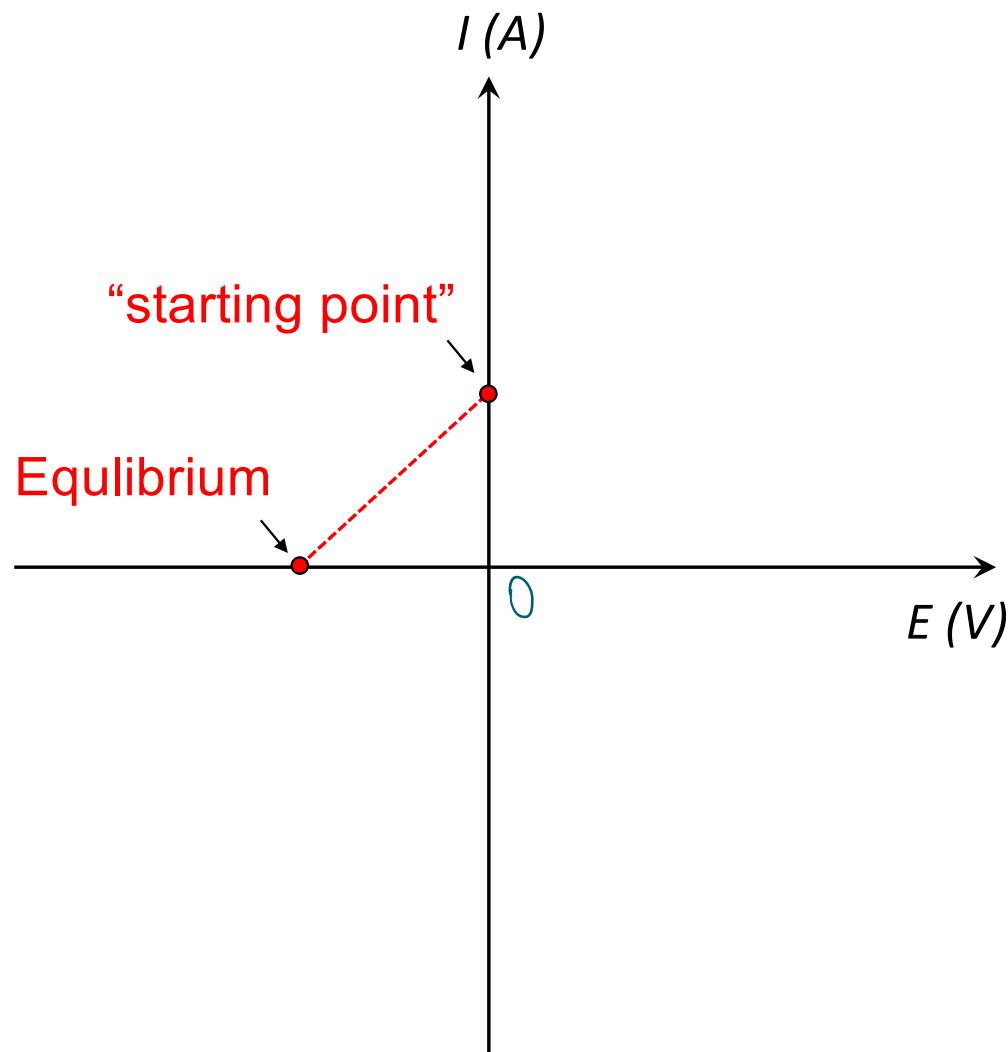
Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	$\frac{[Ion]_o}{[Ion]_i}$	Equilibrium potential <sup>a</sup> (mV) → if open channel when the initial conditions are the first three columns.
Na <sup>+</sup>	145	12	12	+67 → inside charged positively
K <sup>+</sup>	4	155	0.026	-98
Ca <sup>2+</sup>	1.5	100 nM	15,000	+129
Cl <sup>-</sup>	123	4.2 <sup>b</sup>	29 <sup>b</sup>	-90 <sup>b</sup>

<sup>a</sup> Calculated from Equation 1.11 at 37°C.

<sup>b</sup> Calculated assuming a -90-mV resting potential for the muscle membrane and that Cl<sup>-</sup> ions are at equilibrium at rest.

Bertil Hille: "Ion Channels of Excitable Membranes."  
Sinauer Associates, Inc., Sunderland USA, 2001.

## *IV* curve

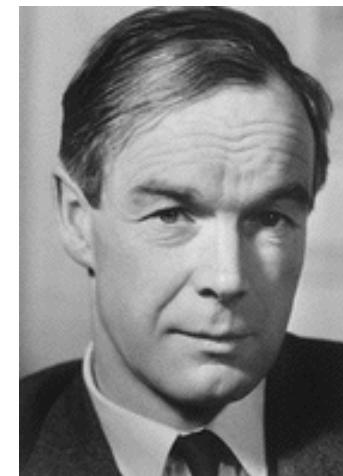


For our Gedankenexperiment, the hypothetical “*IV plot*” would not go through zero.

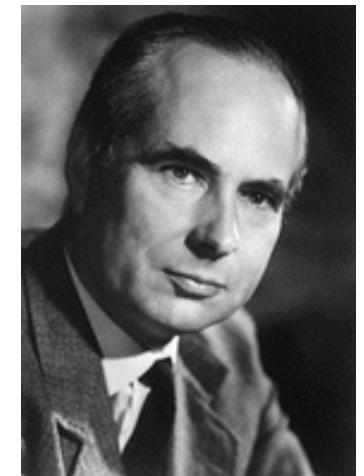
The modified current-voltage law (Ohm's) then becomes

$$I_K = g_K(E - E_K)$$

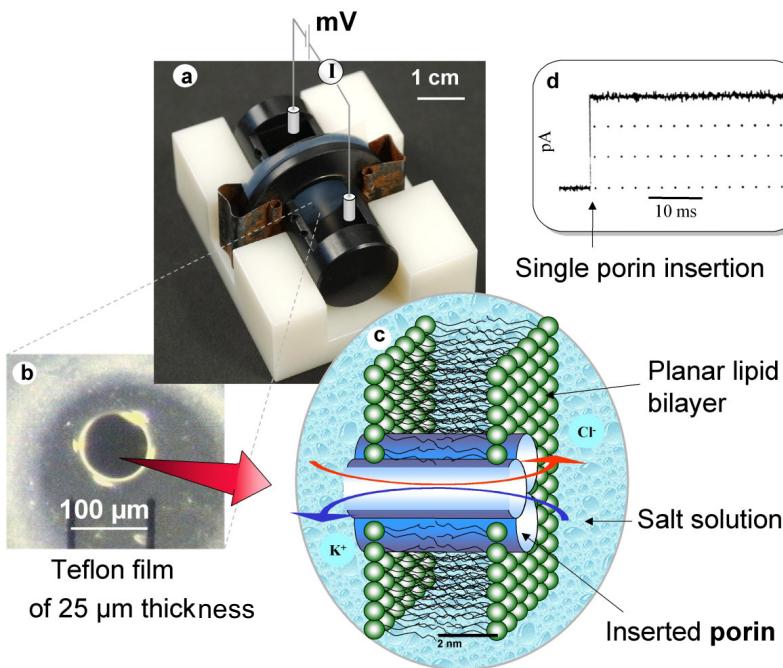
The “electromotive force” in the pore is  $E_K$  and the net driving force on  $K^+$  ions is now  $E - E_K$  and not  $E$ . This modification was introduced by Hodgkin and Huxley.



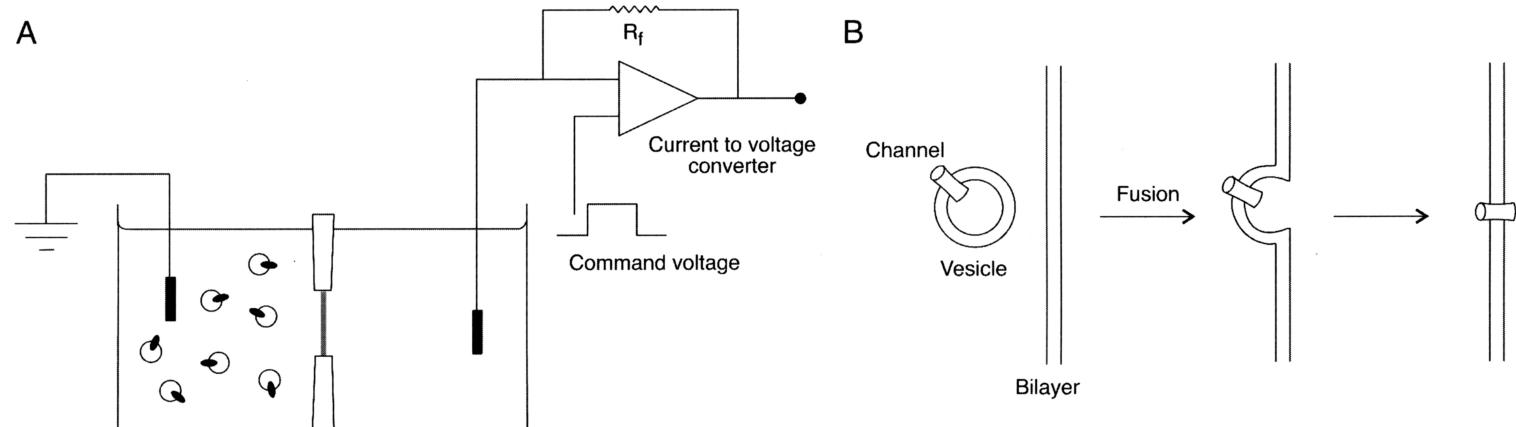
Alan L. Hodgkin      Andrew F. Huxley  
Nobel Prize Physiology/Medicine 1963



# Black lipid bilayer setup



Berkane E et al., J Nanobiotechnology 3: 3 (2005)

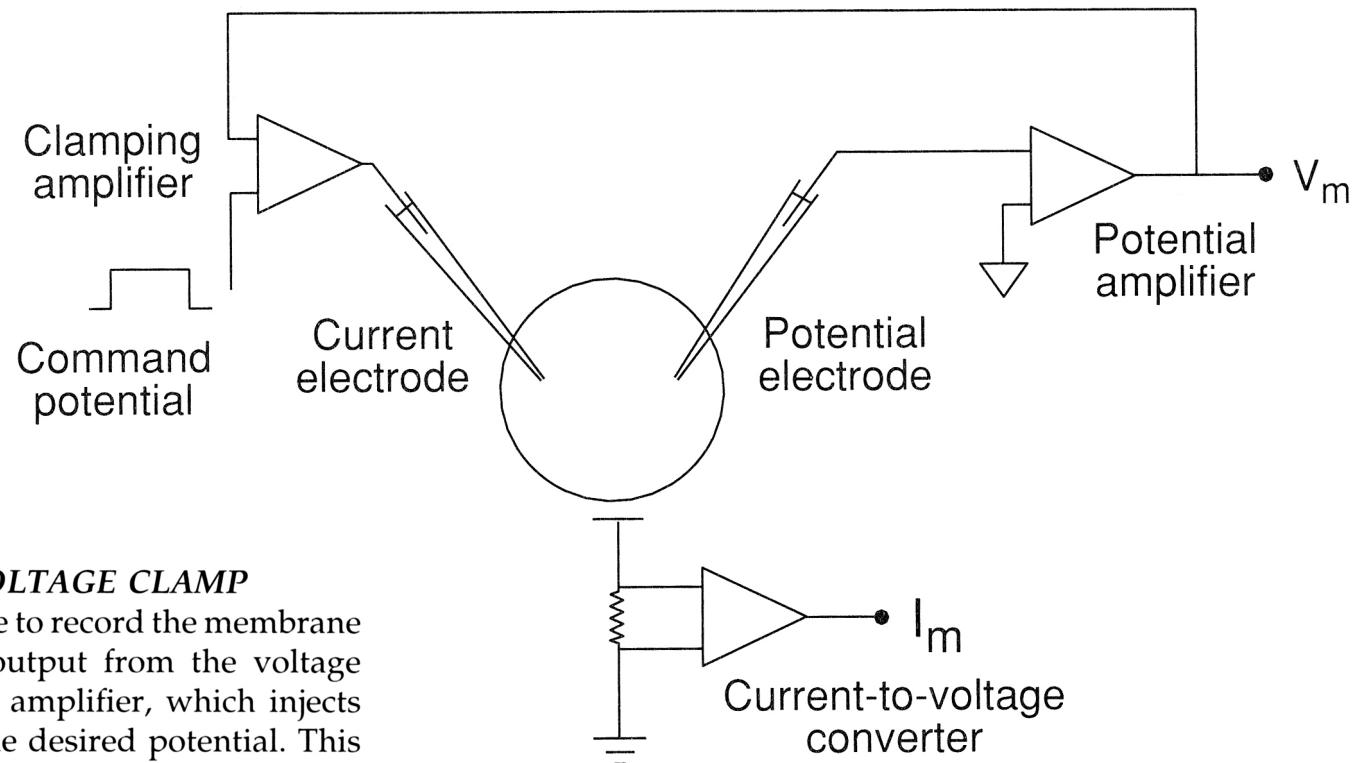


**FIGURE 4.6 BILAYER RECORDING METHOD**

A bilayer is formed by painting lipid across a small hole that connects two aqueous compartments and allowing it to thin to a bilayer (A). Membrane vesicles are then added to one side of the bilayer, which is known as the *cis* side (the opposite side is known as the *trans* side). When some of these vesicles fuse with the bilayer, the channels they contain are incorporated into the bilayer (B). The bilayer may be voltage clamped in order to record currents flowing through these channels. The orientation of the channels in the membrane depends on whether the vesicles from which they derive were “inside-out” or “outside-out”; because all vesicles do not necessarily share the same orientation, the same may also be true of channels in the bilayer.

Frances Ashcroft: “Ion Channels and Disease.” Academic Press, 2000.

## Two-electrode cell clamping

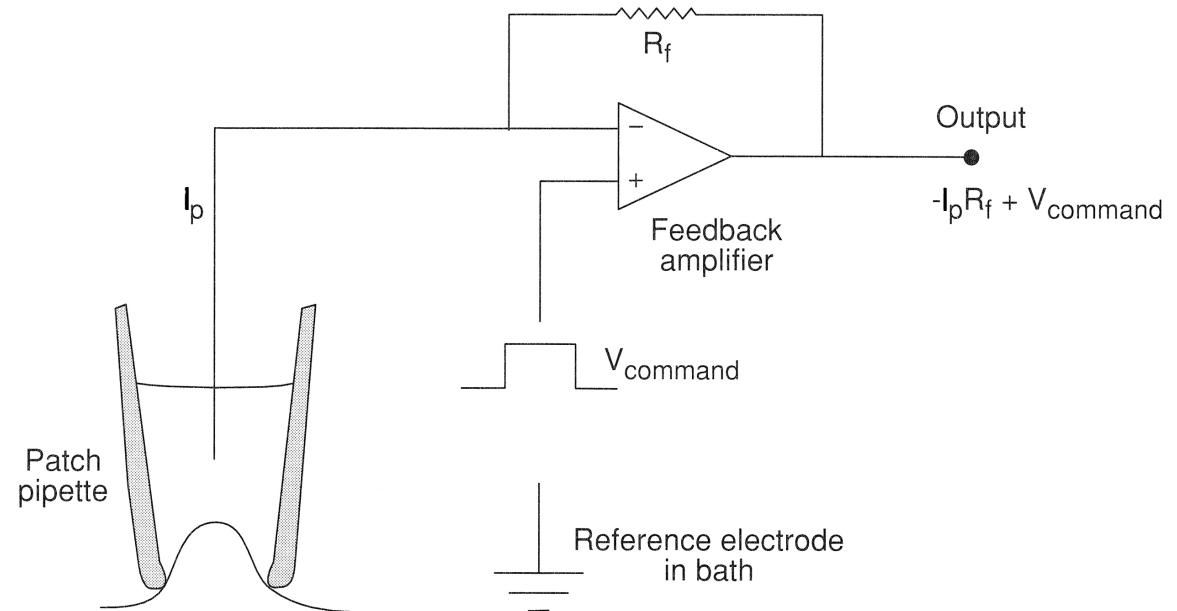
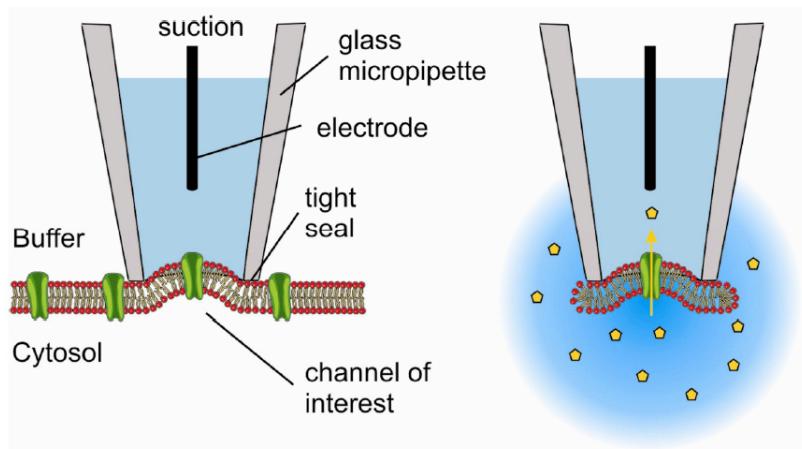


**FIGURE 4.3 A SIMPLE TWO-ELECTRODE VOLTAGE CLAMP**

Two microelectrodes are inserted into the cell, one to record the membrane potential and the other to pass current. The output from the voltage electrode is fed into one input of the clamping amplifier, which injects current into the cell to hold the membrane at the desired potential. This potential (the command potential) is applied to the other input of the clamping amplifier. A feature of operational amplifiers is that they act to maintain the same voltage at their inputs, thus the clamping amplifier injects current into the cell to keep the membrane potential and the command potential at the same value. The current injected is equal in amplitude, but opposite in sign, to that flowing across the plasma membrane. The current ( $I_m$ ) can be measured using a current-to-voltage converter placed either in the injection circuit or in the ground circuit, as shown.

Frances Ashcroft: "Ion Channels and Disease."  
Academic Press, 2000.

# Patch clamping



**FIGURE 4.5 PATCH CLAMP CIRCUIT**

The patch pipette is connected to the inverting input of a feedback amplifier. Because the input resistance of this amplifier is essentially infinite, all the current recorded by the pipette flows through the feedback resistor ( $R_f$ ). This has a very high value, typically  $10\text{ G}\Omega$ . Its high resistance enables the tiny single-channel currents ( $\sim 1\text{ pA}$ ) to be measured, since the patch current ( $I_p$ ) is given by the voltage drop across  $R_f$ , i.e.,  $V = I_p R_f$ . The command potential ( $V_{command}$ ) is applied to the other input of the feedback amplifier and the amplifier passes current through the feedback resistor to keep the voltage at the inverting input the same as the command voltage. This means that the desired potential is also applied to the pipette and thus to the patch membrane.

# Patch clamping

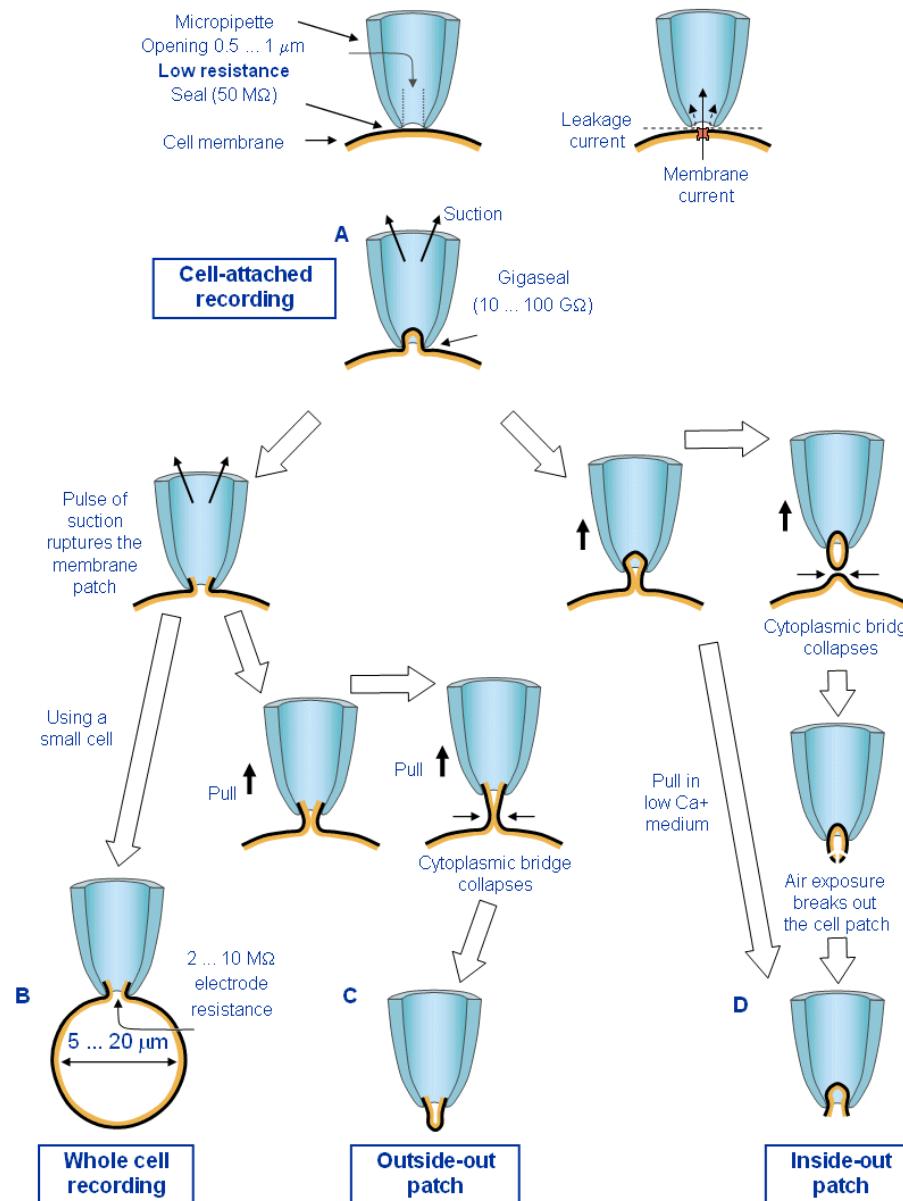
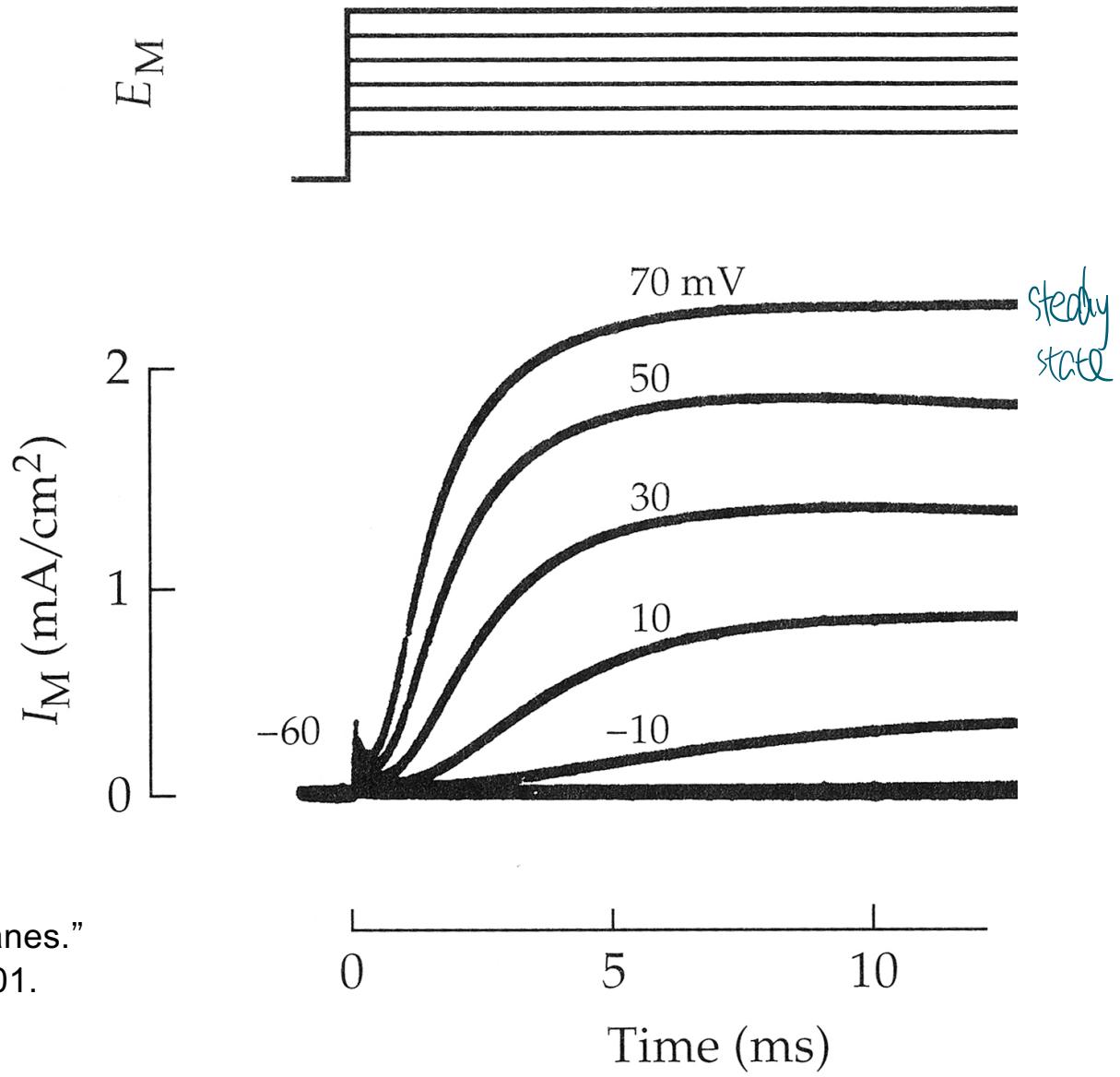


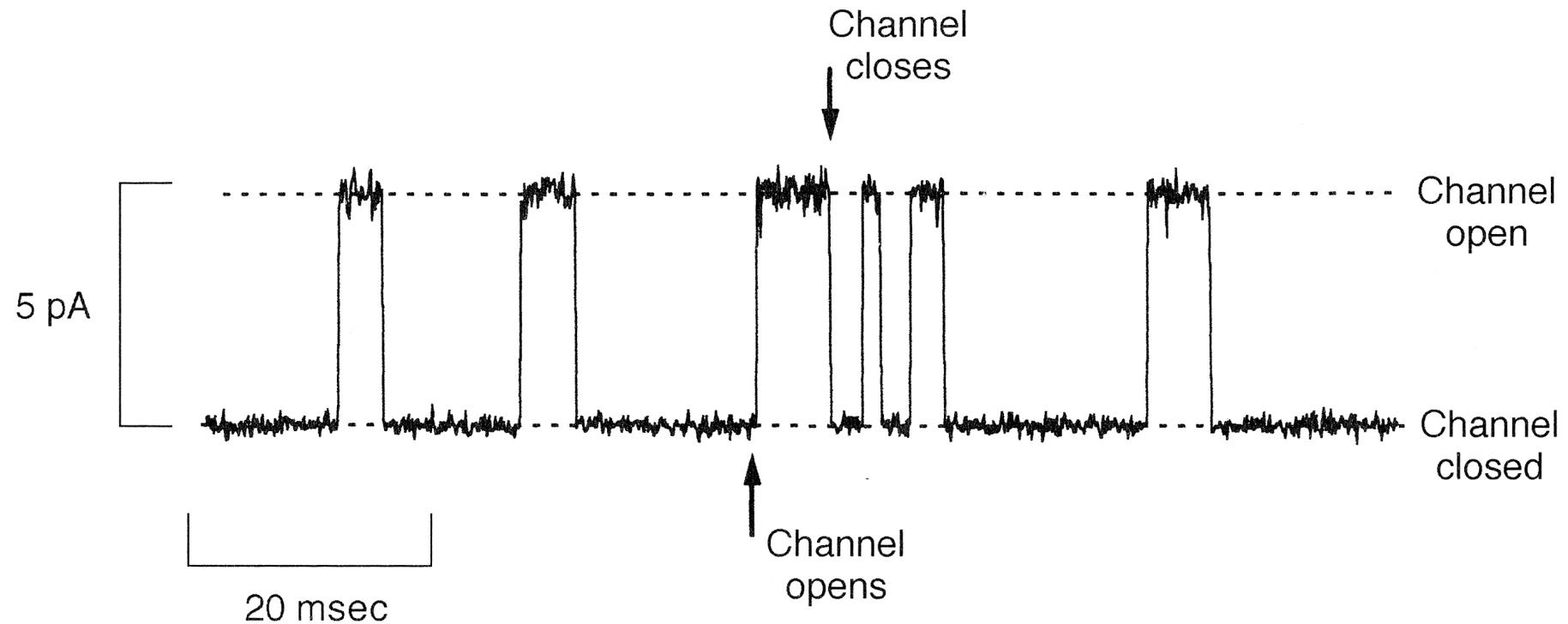
Fig. 4.27 from Jaakko Malmivuo & Robert Plonsey:  
Bioelectromagnetism - Principles and Applications  
of Bioelectric and Biomagnetic Fields, Oxford  
University Press, New York, 1995.  
(<http://www.bem.fi/book/>)  
(modified from Hamill OP et al., *Pflugers Arch* **391**:  
85-100 (1981))

## Ensemble measurements



Bertil Hille: "Ion Channels of Excitable Membranes."  
Sinauer Associates, Inc., Sunderland USA, 2001.

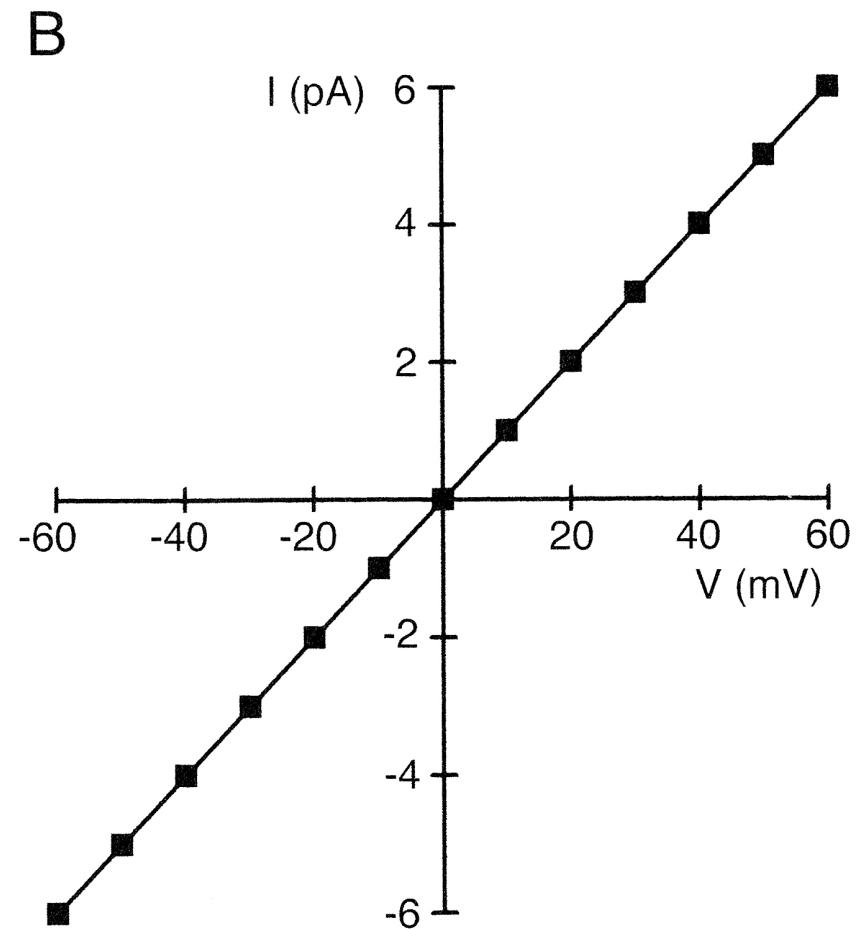
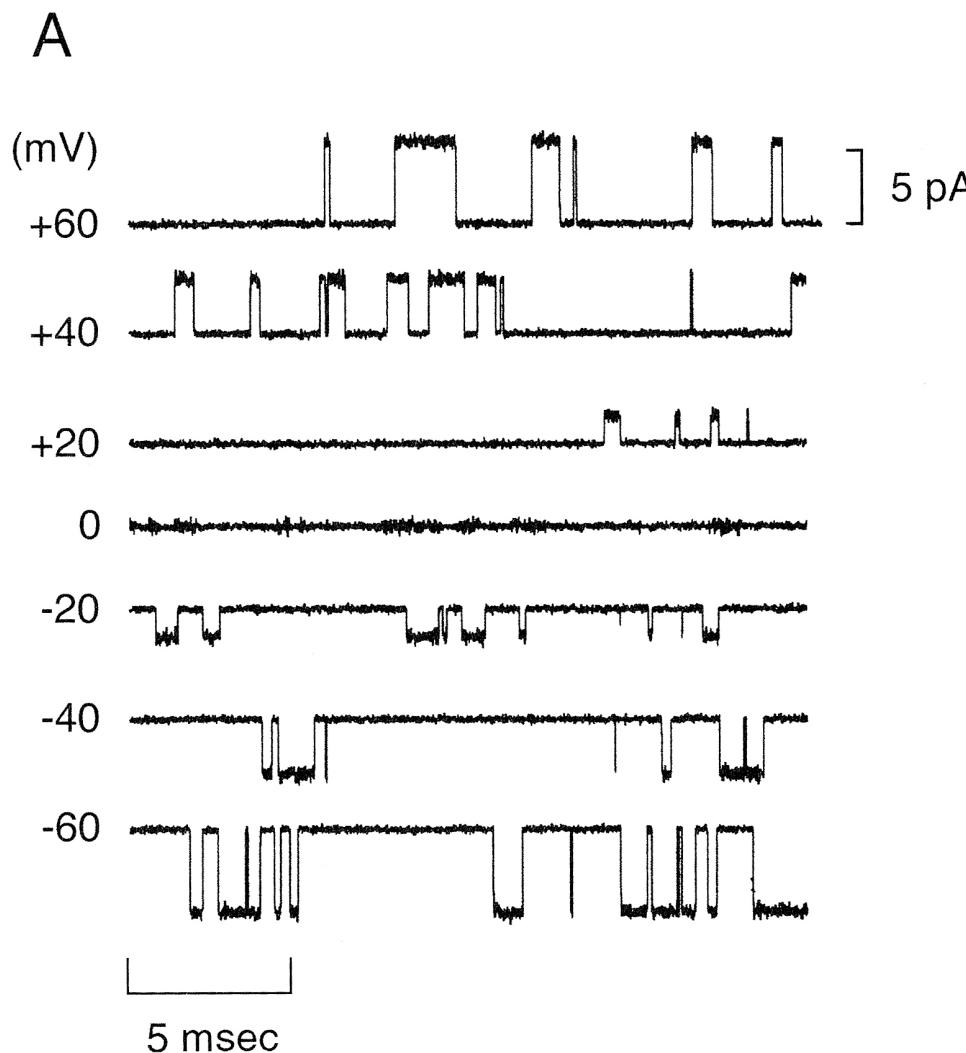
## Single channel measurements



Frances Ashcroft: "Ion Channels and Disease."  
Academic Press, 2000.

from measure  $\rightarrow$  IV plt

## Generating IV curves from single channel recordings



Frances Ashcroft: "Ion Channels and Disease."  
Academic Press, 2000.

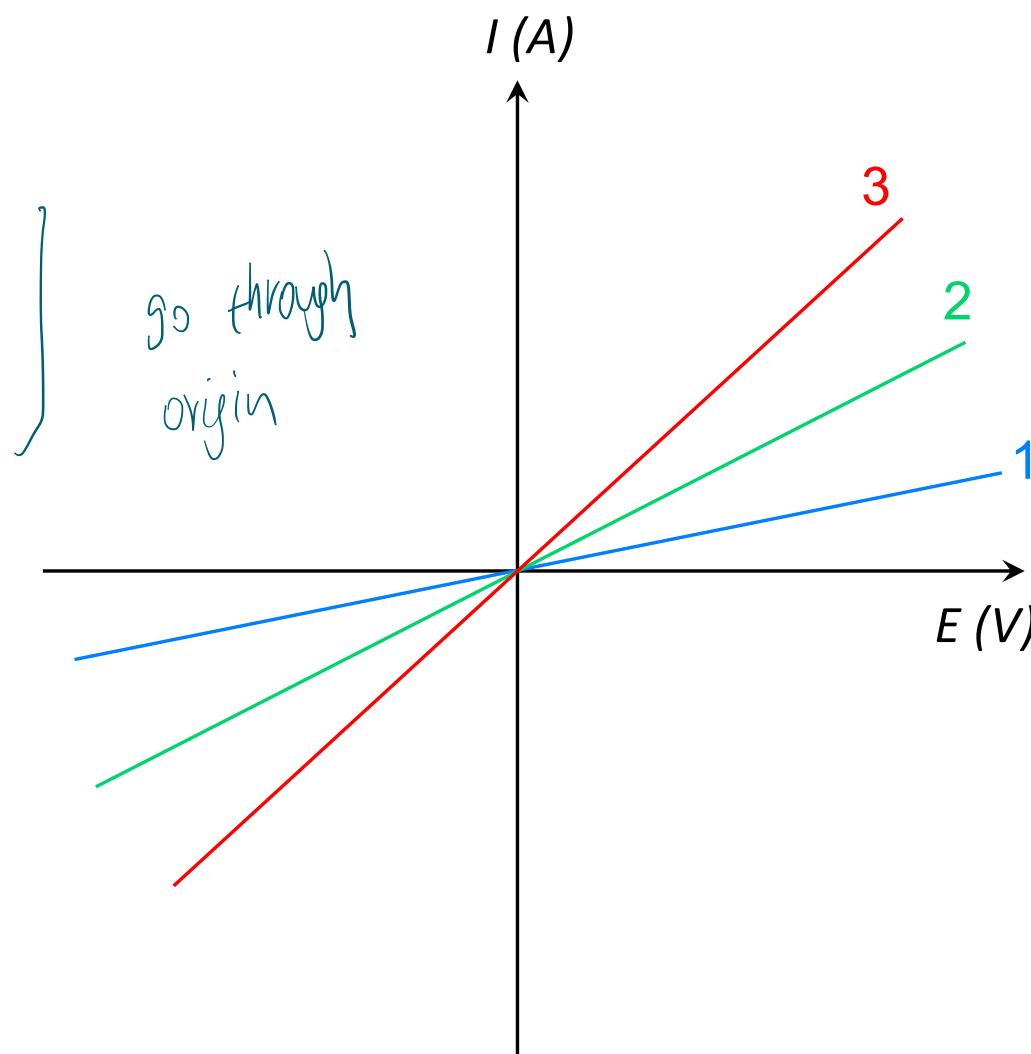
## IV plots: Example 1

$$I = g_L(E - E_h)$$

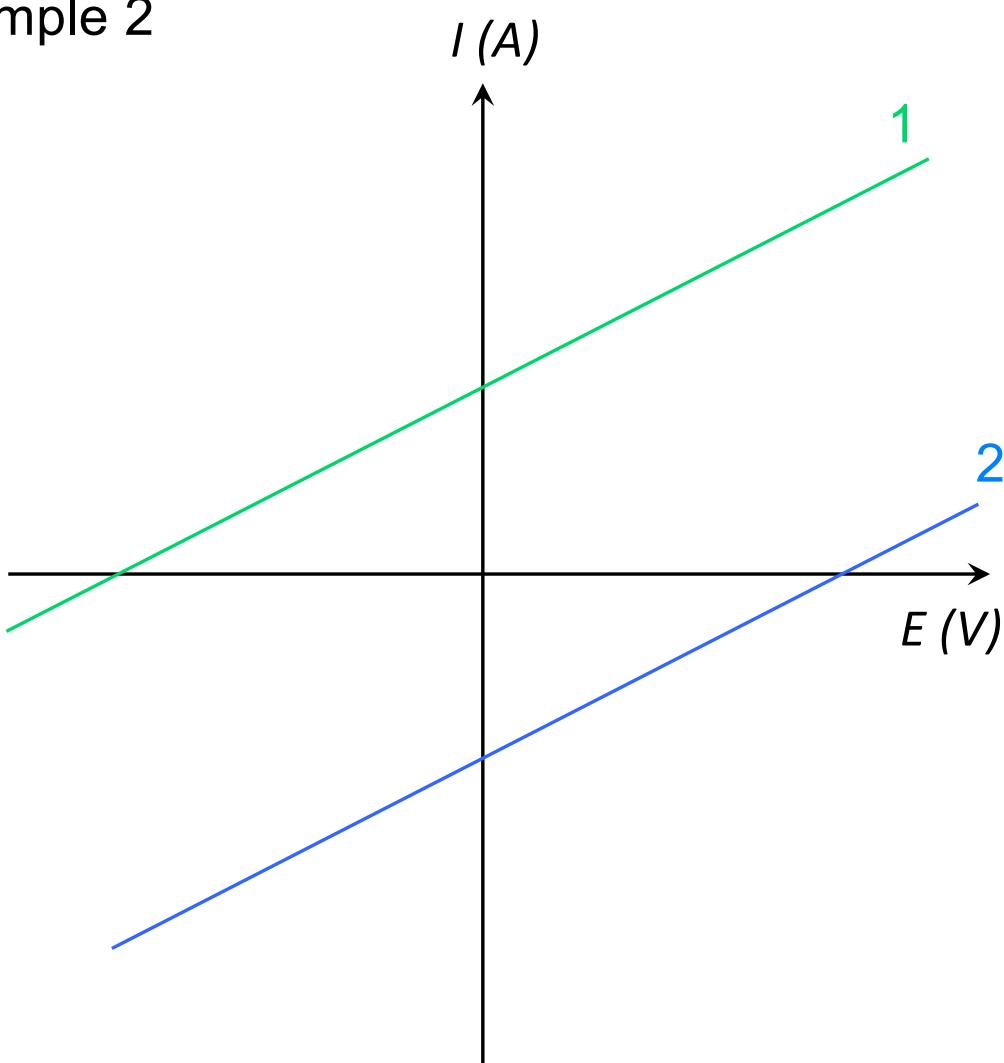
① No ion gradient

② No selective

③  $g$  is different  
 $g(3) > g(2) > g(1)$



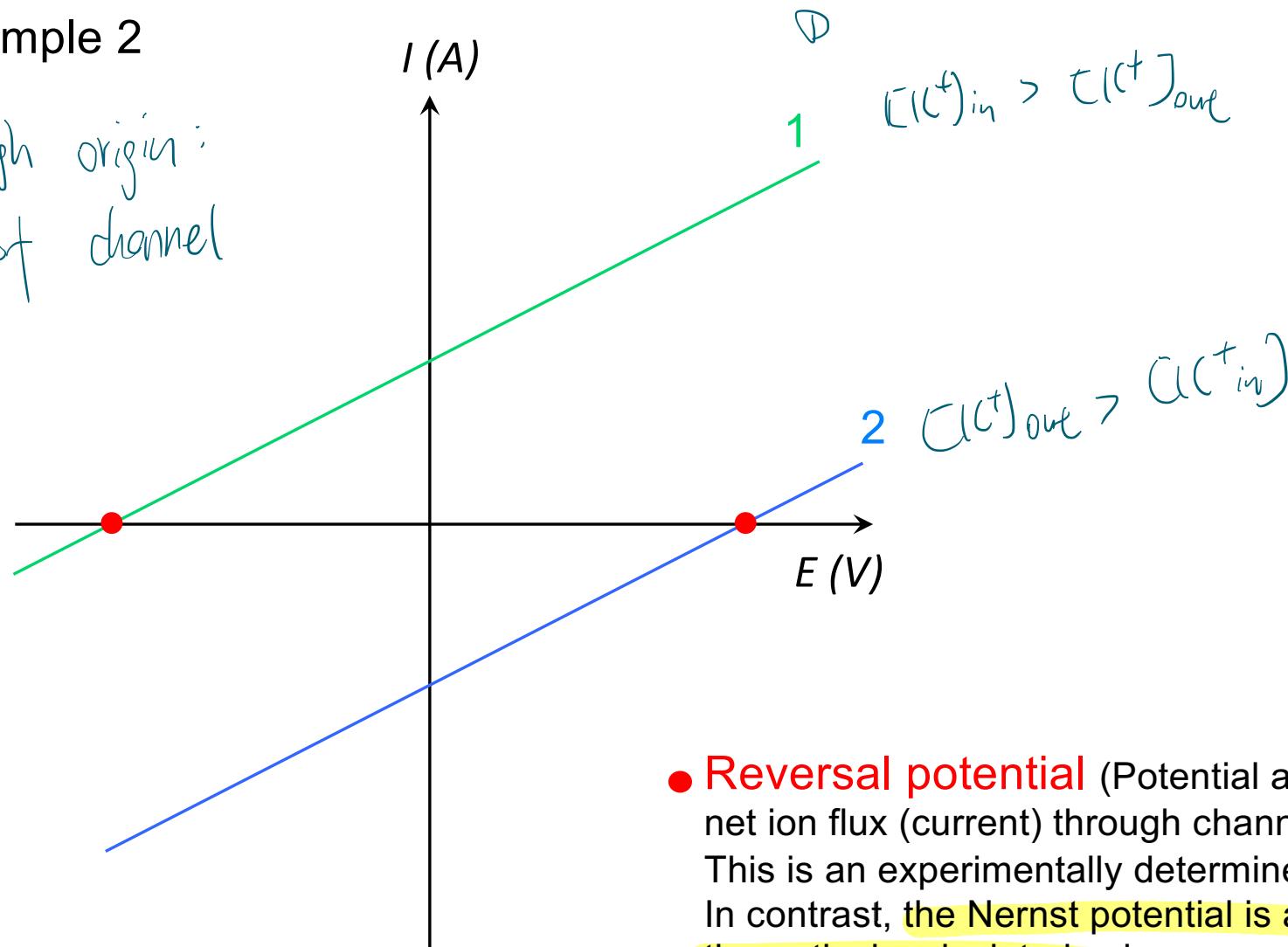
## IV plots: Example 2



Don't go through origin  $(0, 0)$   
⇒ Must have non zero gradient

## IV plots: Example 2

Don't go through origin:  
selectivity of channel

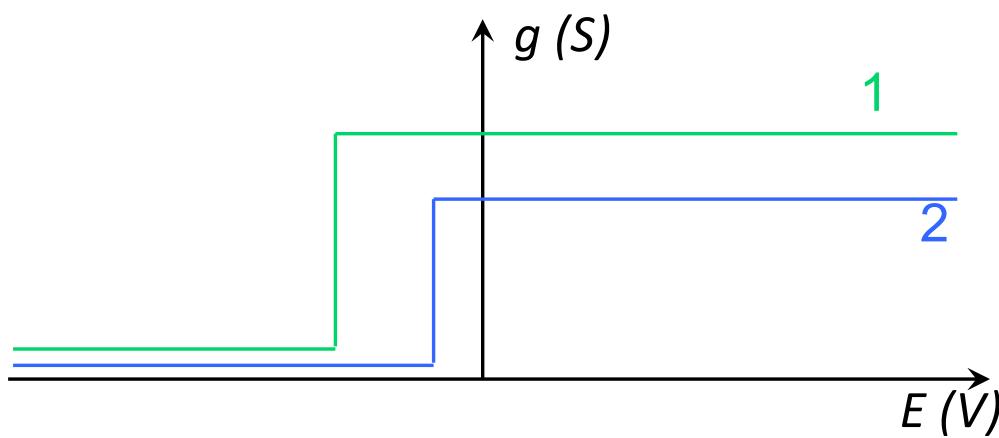
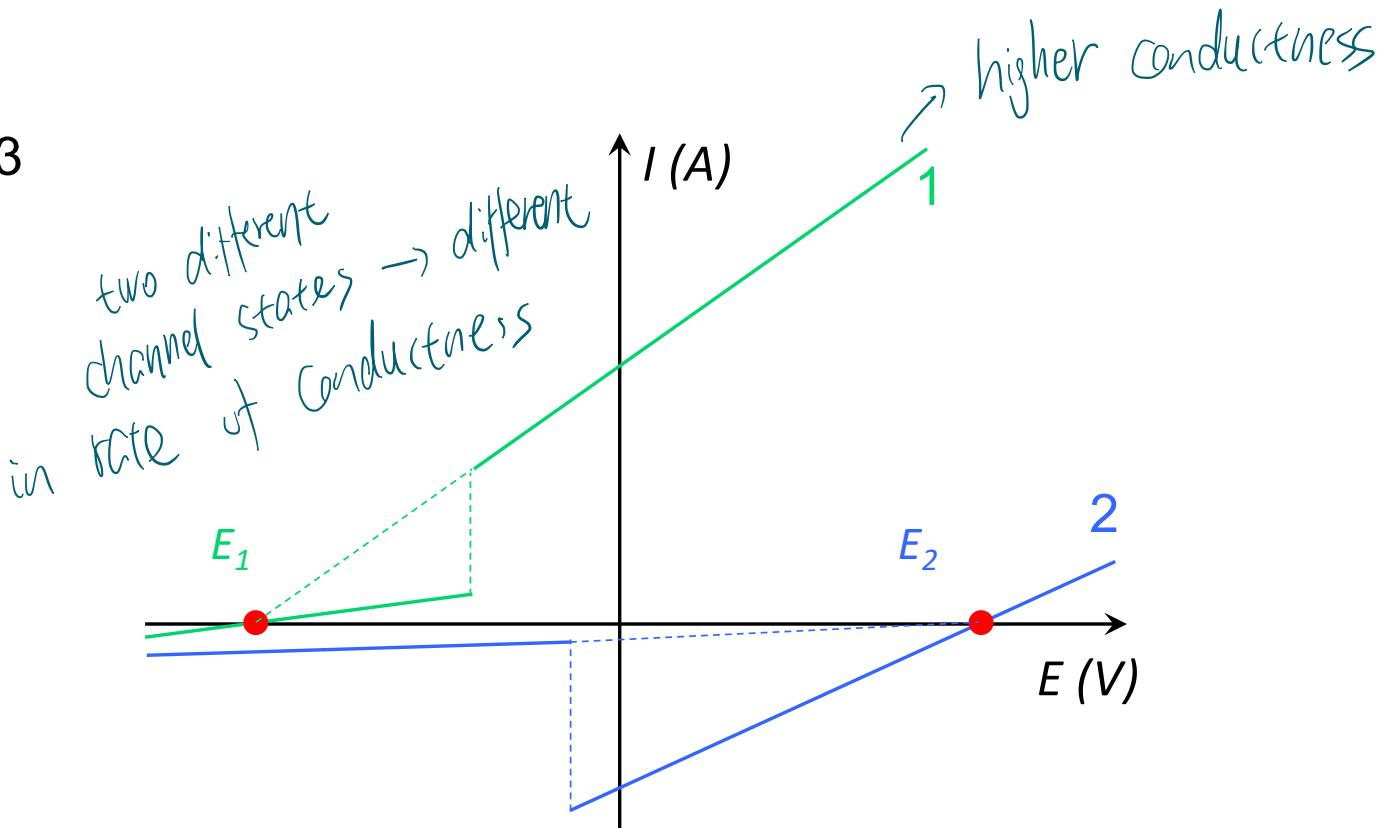


② exchange of  
best  
solutions  
(solution  
location,  
pot. location)

- **Reversal potential** (Potential at which the net ion flux (current) through channel = zero. This is an experimentally determined value! In contrast, the **Nernst potential** is a **theoretical, calculated value**.

What is condition under which reversal potential is identical with Nernst potential?

## IV plots: Example 3



$$E_K \frac{K_o}{K_i} = 0.1 \Rightarrow -59 \text{ mV}$$

$$E_{Na} \frac{Na_o}{Na_i} - 2.5 \Rightarrow 23.5 \text{ mV}$$

## Channels and IV curves problem

A. The figure to the right shows the setup of a black lipid bilayer experiment (ensemble measurements), conducted under standard conditions and using Hepes as a buffer.

Draw an *IV* diagram and indicate the expected data points at equilibrium assuming the lipid bilayer contains:

1. Permanently open  $K^+$  channels
2. Permanently open  $Na^+$  channels.

B. Consider the  $g-E$  graph below. Assuming the conditions shown on the right, draw the expected *IV* curves.

