Membrane potential

| Cell membrane		
	Inside	Outside
Na⁺	12 mM	120 mM
K⁺	125 mM	5 mM
Cl⁻	5 mM	125 mM
A⁻	108 mM	----
H₂O	55,000	55,000
Total osmolarity, 250 mOsm		

Note: Values listed for typical mammalian cell.

Electrical potential difference across the membrane is called the membrane potential. The membrane potential of a cell at rest is called the resting membrane potential. Its usual range in neurons is -60 mV to -70 mV
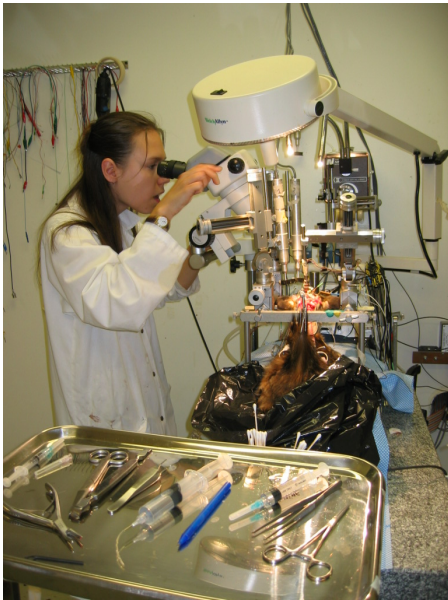
The resting membrane potential results from the separation of charges across the cell membrane. Na+ and Cl- are more concentrated outside the cell, and K+ and organic anions (A-) are more concentrated inside.

All living cells must have membrane potential.

Recording the Membrane Potential
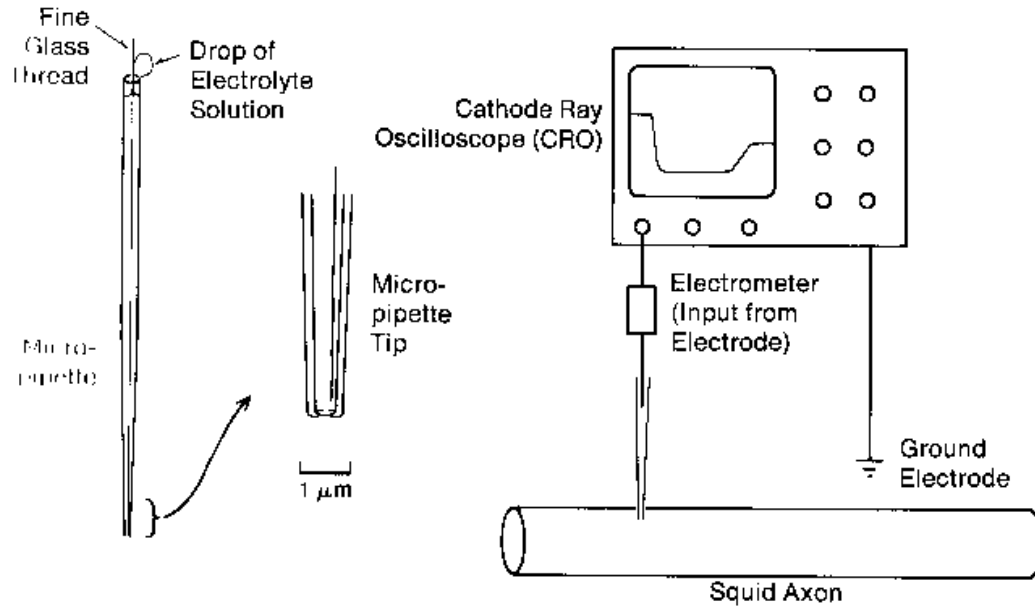
The micropipette is used for electrical recording (extracelluar, intracellular, patch), electrical stimulation or delivery of substances.

Fine Glass Thread — Drop of Electrolyte Solution — Micropipette — Micropipette Tip — 1 μm — Cathode Ray Oscilloscope (CRO) — Electrometer (Input from Electrode) — Ground Electrode — Squid Axon

Intracellular recordings in vivo. Group of prof. Amzica, Universite Laval, Quebec, Canada

Patch clamp
(developed in late 70s by E. Neher, B. Sakmann, Nobel Prize 1991)
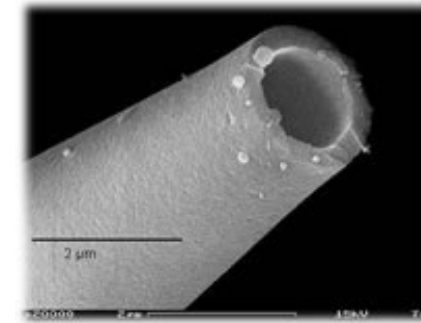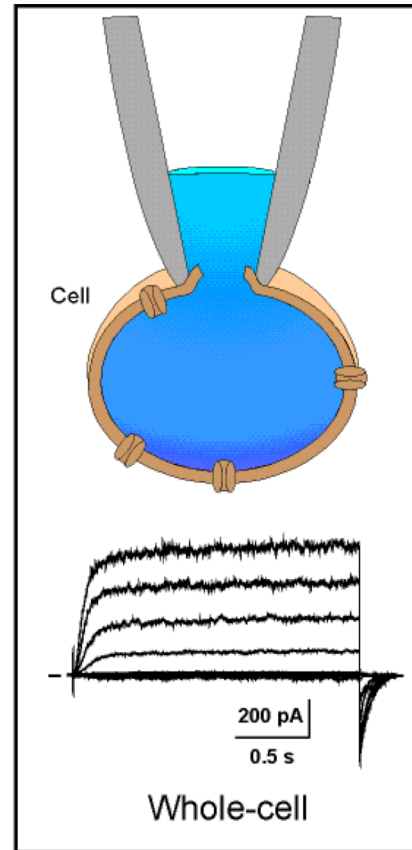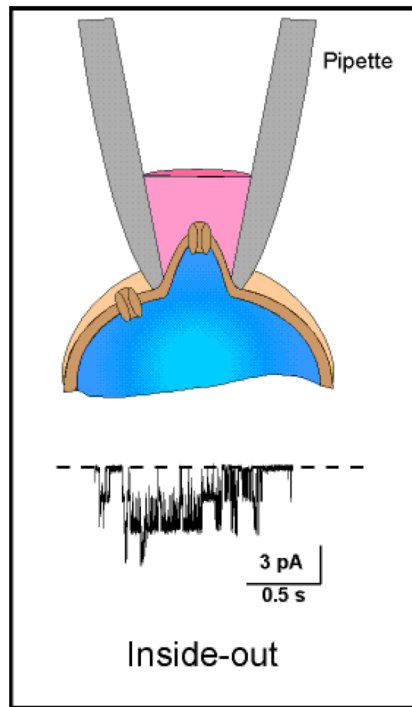
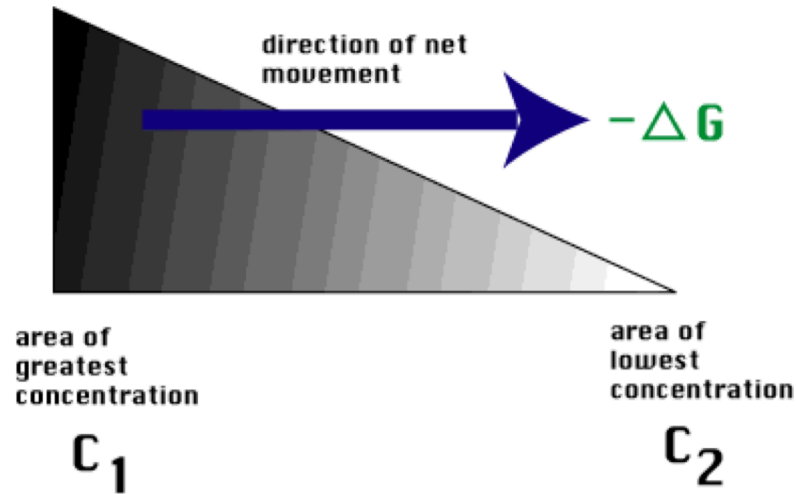A glass micropipette that has an open tip diameter of about one micrometer,

Patch clamp micropipette are prepared in the same way as normal micropipettes but have smooth surface tips that help to form a high resistance seal with the cell membrane instead of breaking through it.

Patch clamp allows recording of the currents of single ion channels (indside-out) and electrical behavior of the entire cell (whole cell).
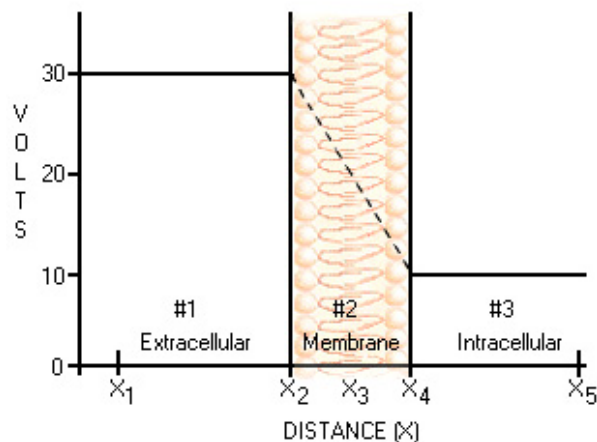

Classical patch clamp setup, with microscope, antivibration table and micro manipulators

Chemical and electrical forces

direction of net movement

$-\triangle G$

area of greatest concentration

C_1

area of lowest concentration

C_2

$$W_C = 2.3RT \log \frac{C_1}{C_2}$$

R – gas constant

T – absolute temperature

Direction of Voltage Gradient

VOLTS

30

20

10

0

#1 Extracellular

#2 Membrane

#3 Intracellular

x_1 x_2 x_3 x_4 x_5

DISTANCE (X)

$F_e = -(z)(dV/dx)$

$$W_E = qV = zFV$$

F – Faraday's constant

V – potential difference

z - valence number of the ion

Faraday's constant is the magnitude of electric charge per mole of electrons; $F = eN_A$

The Nernst Potential

At equilibrium (no net flux of ions):

$$W_E = W_C$$

$$zFV = 2.3RT \log \frac{C_1}{C_2}$$
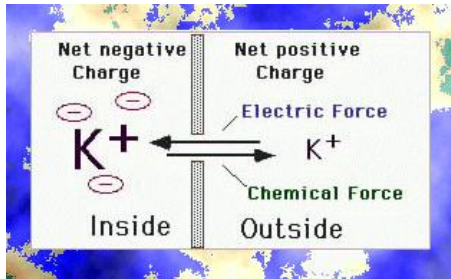
$$V = 2.3 \frac{RT}{zF} \log \frac{C_1}{C_2}$$

The Nernst equation

Walter Hermann Nernst born June 25, 1864 in Briesen (Wąbrzeźno), died November 18, 1941 in Zibelle. Received Nobel Prize in Chemistry, 1920r.

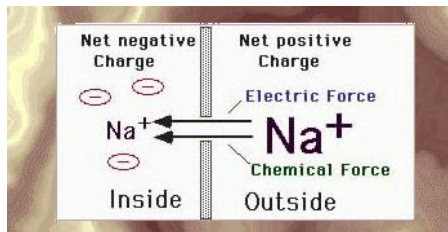V - reversal potential (also known as the Nernst potential).

The Nernst Potential

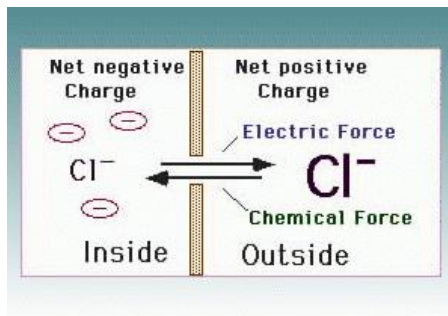$$V_K = 2.3 \frac{RT}{F} \log \frac{[K^+]_{out}}{[K^+]_{in}}$$

$$V_K = 58 \log \frac{[K^+]_{out}}{[K^+]_{in}} \text{ mV}$$

$$= 58 \log \frac{5}{125} \text{ mV} = -81 \text{mV}$$

$$V_{Na} = 58 \log \frac{[Na^+]_{out}}{[Na^+]_{in}} \text{ mV}$$

$$= 58 \log \frac{120}{12} \text{ mV} = +58 \text{mV}$$

$$V_{Cl} = 58 \log \frac{[Cl^-]_{in}}{[Cl^-]_{out}} \text{ mV}$$

$$= 58 \log \frac{5}{125} \text{ mV} = -81 \text{mV}$$

The membrane potential is the weighted average of each contributing ion's equilibrium potential.

Millman equation:

$$V_m = \frac{P_K}{P_{tot}} V_{K^+} + \frac{P_{Na}}{P_{tot}} V_{Na^+} + \frac{P_{Cl}}{P_{tot}} V_{Cl^-}$$
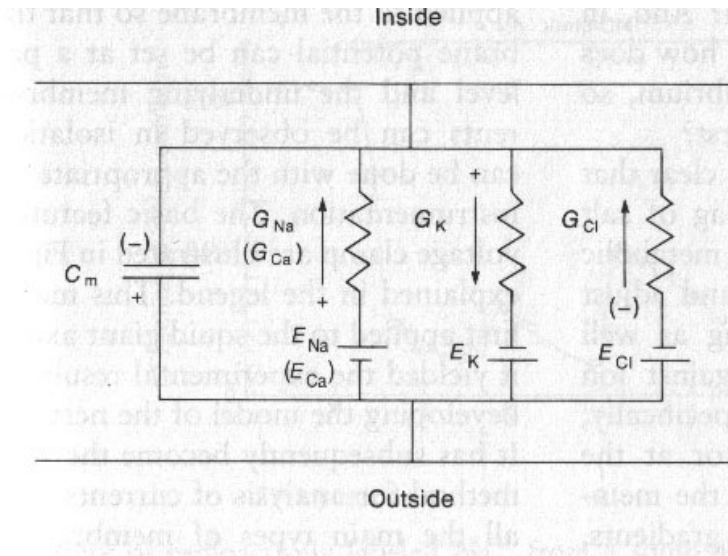
Goldman or Goldman-Hodgkin-Katz (GHK) equation:

$$V_m = 58 \log \frac{P_K [K^+]_{out} + P_{Na} [Na^+]_{out} + P_{Cl} [Cl^-]_{in}}{P_K [K^+]_{in} + P_{Na} [Na^+]_{in} + P_{Cl} [Cl^-]_{out}} \, \text{mV}$$

P – relative membrane permeability [m/s]

For $P_{Na} = 0.04 * P_K$, and neglecting Cl⁻, we get (from the Goldman equation):

$$\boxed{V_m = \text{-60 mV}}$$

Equivalent circuit

Equivalent electrical circuit for the electrical properties of the nerve membrane. Each equilibrium potential is represented by a battery across the membrane which has the appropriate polarity and voltage for that ion. In series with the battery is a resistance which is related to the membrane permeability for that ion. The reciprocal of the resistance is conductane (G). Conductance is related to the membrane permeability as follows (using K as the ion in question):

$$G_K \propto P_K \frac{[K^+]_{out}}{[K^+]_{in}}$$

The channels for each type of ion are separate and independent. In addition, the membrane is able to store electrical charges, hence it has also capacitance (C).

Sodium-potassium pump
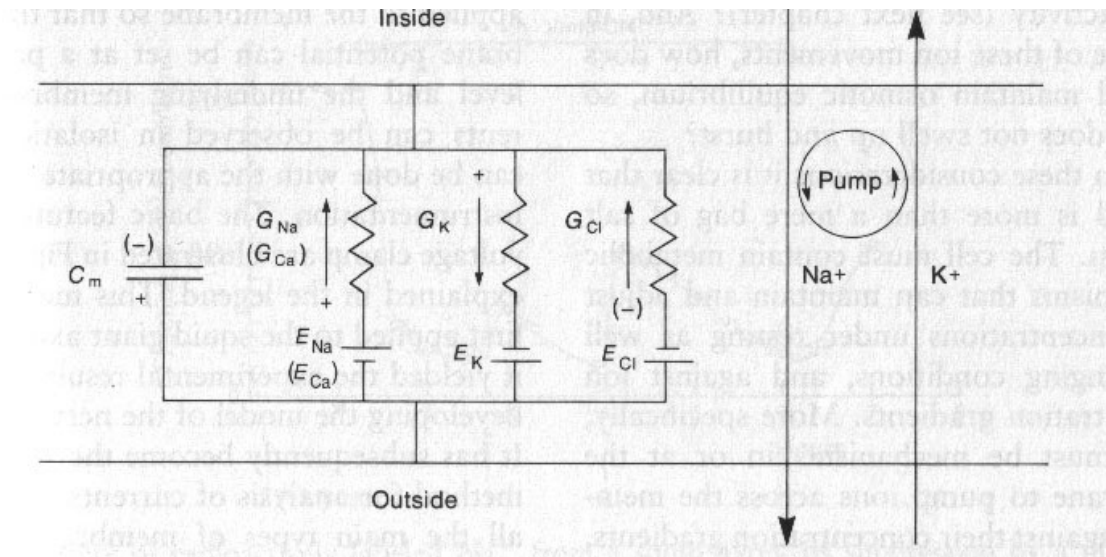
In order to maintain a resting potential, the cell cells must keep a low concentration of sodium ions and high levels of potassium ions within the cell. It requires an active transport i.e., the movement of a substance across a cell membrane against its concentration gradient (from low to high concentration). The mechanism responsible for this is the sodium-potassium pump, which pumps three sodium ions out of the cell for every two potassium ions pumped in. Energy (from hydrolysis of ATP to ADP) is required for this process. For neurons, the sodium-potassium pump can be responsible for up to 2/3 of the cell's energy expenditure.

Action potential
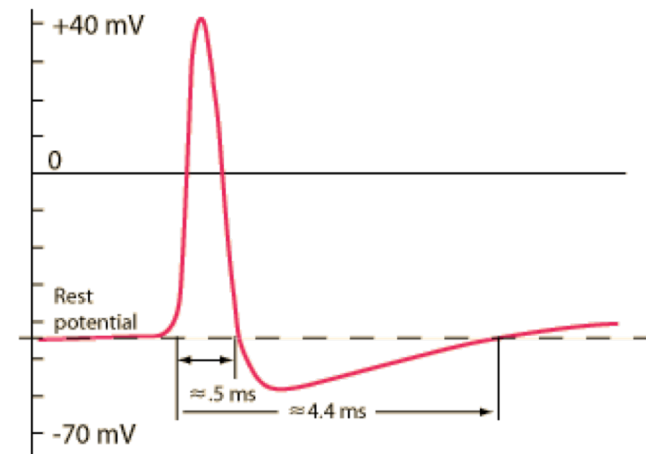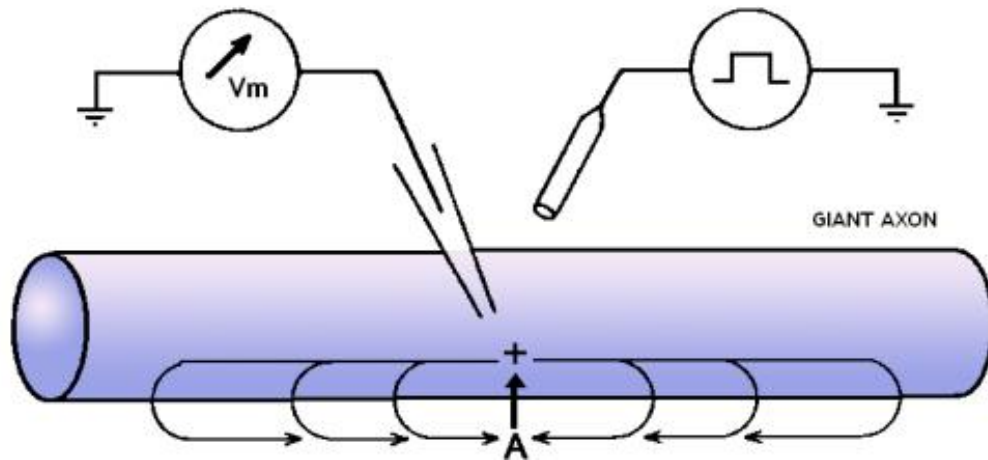
Action potential (AP) is a transient depolarizatinon of the membrane potential. Early experiments (K.C. Cole i H. J. Curtis, 1939; A. Hodgkin, A. Huxley, 1939) showed that the membrane becomes almost 50 mV positive inside at the peak of the AP. If the AP was due to transient breakdown in permeability to all ions, it would depolarize membrane to zero, but not beyond.

Experiments on AP generation mechanism were performed on the squid giant axon, which is up to 1 mm in diameter. It provided a great experimental advantage as it allowed to insert voltage clamp electrodes inside the axon.
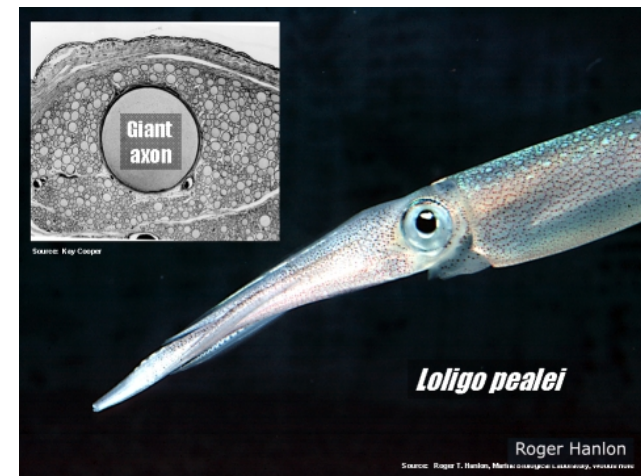
Loligo pealei

Action potential – the sodium impulse
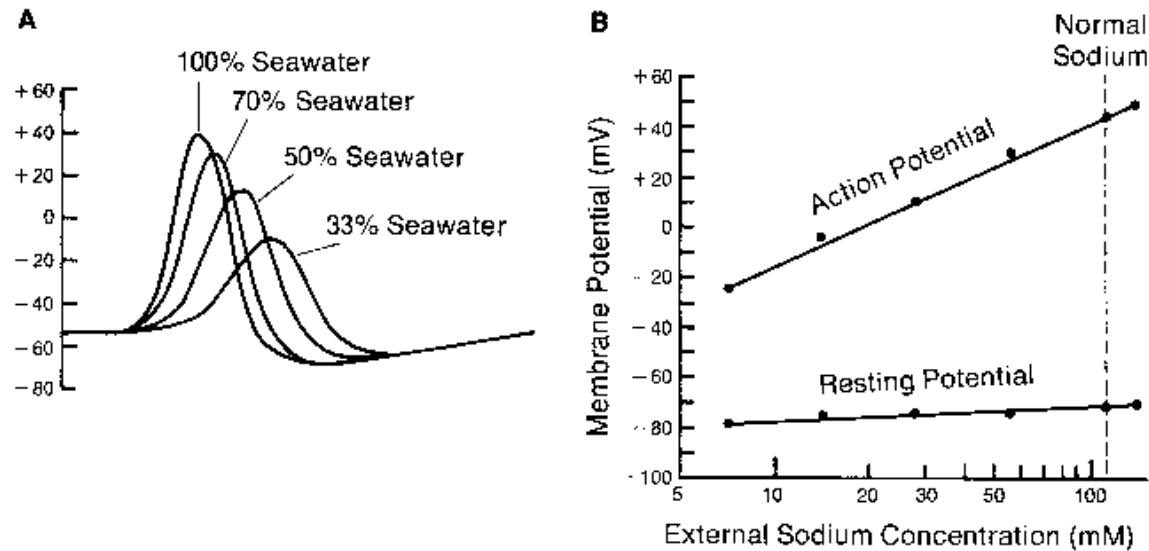
Dpependence of the action potential on Na ions.

A. The peak of the AP decreases with reducion of external sodium concentration. Strong dependence of the maximum on the Na+ concentration suggest large permeability to Na+ during an impulse.

B. Changing external sodium has very little effect on the resting membrane potential.

Alan Hodgkin and Bernard Katz discovered that AP amplitude depends on external Na+. They put forward a hypothesis that transient increase in permeability to Na+ and influx sodium ions to the cell is responsible for AP. It was confirmed by the fact that the peak of AP is near the Na+ equilibrium potential of about +55 mV. Their experiments also showed that repolarization of AP may be related to increase in permeability to K+ and efflux of potassium ions out of the cell.

Action potential - all or nothing

Membrane depolarization → Increase in g_{Na} → Na$^+$ inflow → (cycle)
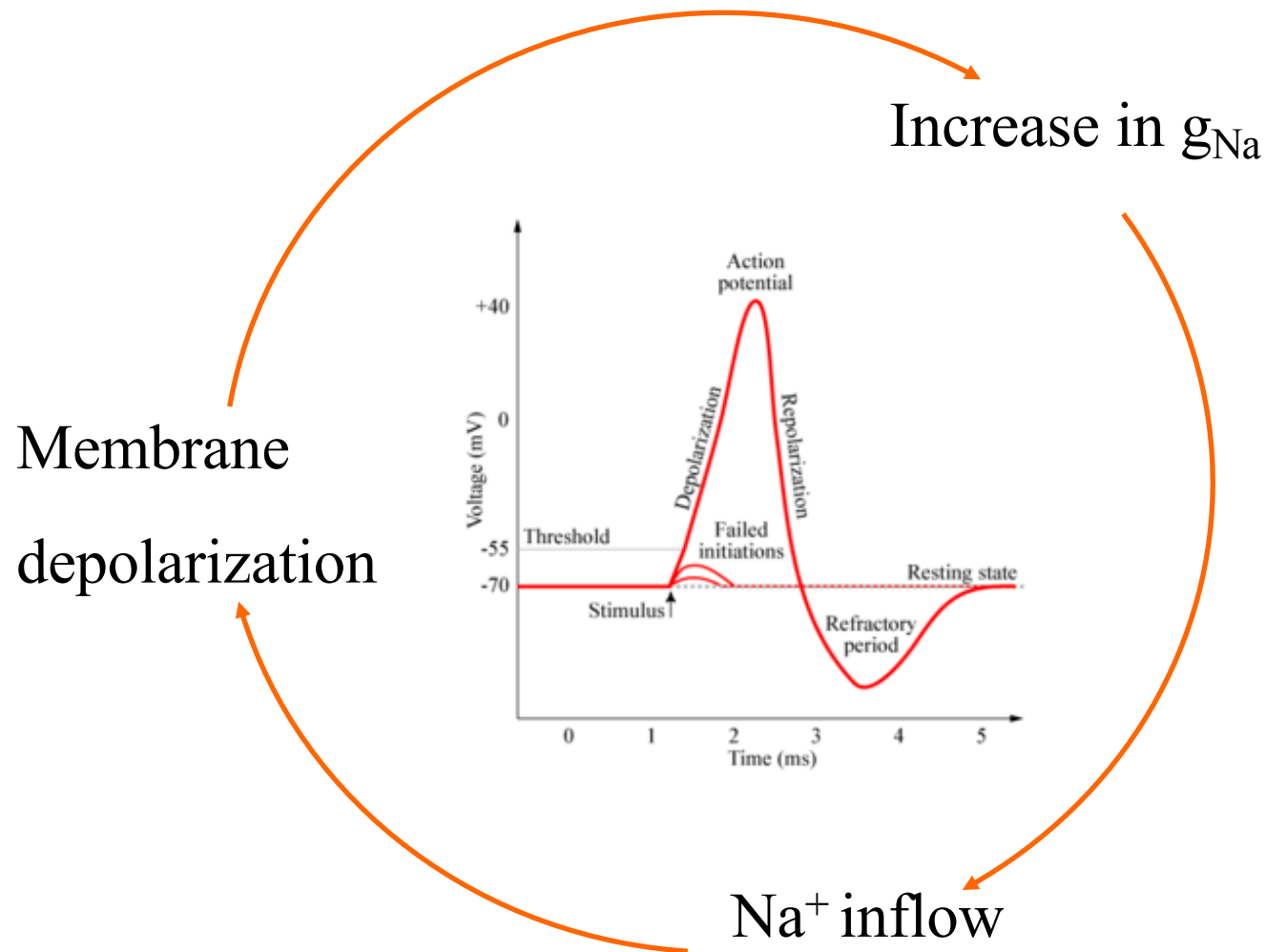
Na+ conductance is involved in a positive feedback cycle with the membrane depolarization. This is reinforcing regenerative relation similar to that between heat and chemical reaction underlying the explosion of a gunpowder. It gives the 'action' to the action potential.

AP has a threshold
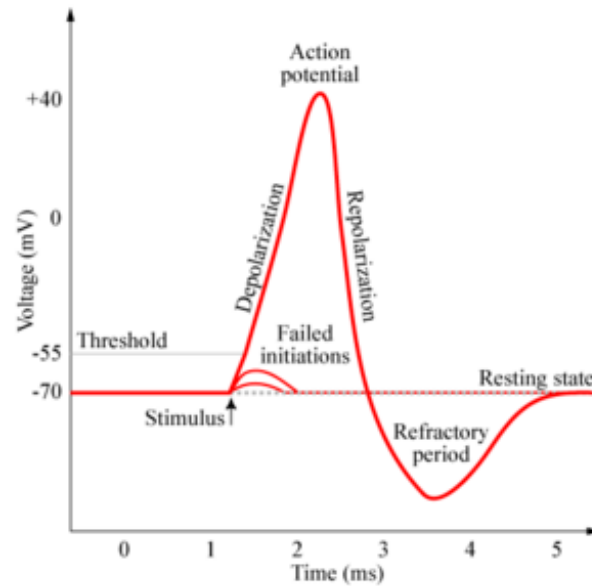
Subthreshold depolarizations are compensated by passive efflux of potassium ions out of the cell. If the efflux of potassium ions cannot compensate the active influx of sodium ions to the cell, the membrane reaches the threshold for impulse and action potential is generated.

Refractory period
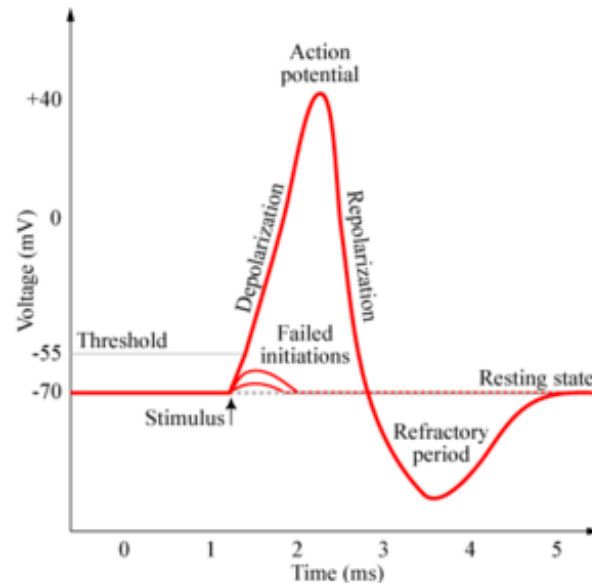
The action potential is also followed by a brief period of diminished excitability, or refractoriness, which can be divided into two phases. The *absolute refractory period* comes immediately after the peak of the action potential; during this period it is impossible to excite the cell no matter how great a stimulating current is applied. This phase is followed directly by the *relative refractory period,* during which it is possible to trigger an action potential but only by applying stimuli that are stronger than those normally required to reach threshold. These periods of refractoriness, which together last just a few milliseconds, are caused by the residual inactivation of Na+ channels and increased opening of K+ channels.

Voltage clamp

The voltage-clamp technique was developed by Kenneth Cole in 1949 to stabilize the membrane potential of neurons for experimental purposes. It was used by Alan Hodgkin and Andrew Huxley in the early 1950s in a series of experiments that revealed the ionic mechanisms underlying the action potential. This technique permits measurement of the effect of changes in membrane potential on the ionic conductances of the membrane.

1 One internal electrode measures membrane potential (V_m) and is connected to the voltage clamp amplifier

2 Voltage clamp amplifier compares membrane potential to the desired (command) potential

3 When V_m is different from the command potential, the clamp amplifier injects current into the axon through a second electrode. This feedback arrangement causes the membrane potential to become the same as the command potential

4 The current flowing back into the axon, and thus across its membrane, can be measured here

Measure V_m

Command voltage

Voltage clamp amplifier

Reference electrode

Measure current

Saline solution

Squid axon

Recording electrode

Current-passing electrode

Voltage clamp technique for studying membrane currents of a squid axon.

The voltage clamp is based on the negative feedback mechanism. Membrane potential is measured by Membrane potential amplifier connected to an intracellular electrode and to an extracellular electrode in the bath. The membrane potential signal is displayed on an oscilloscope and is also fed into one terminal of the Voltage clamp amplifier. This amplifier has two inputs, one for membrane potential (Vm) and the other for the Command voltage. The command potential, which comes from a signal generator, is selected by the experimenter. The Voltage clamp amplifier subtracts the membrane potential from the command potential. Any difference between these two signals is amplified and sent to a current electrode, a thin wire that runs the length of the axon. The clamp circuit produces a current equal and opposite to the ionic current flowing across the membrane.
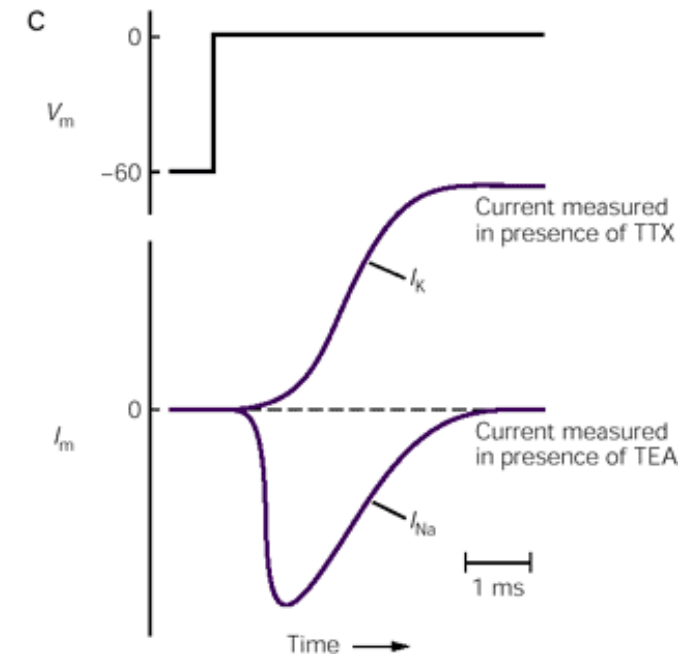
Hodgkin and Huxley experiment - results

A
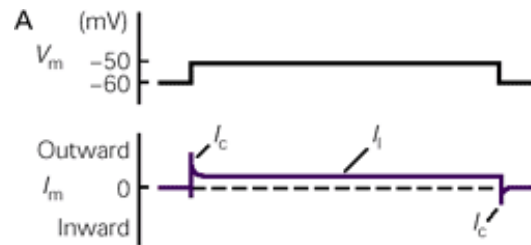
A small depolarization is accompanied by capacitive and leakage currents (I_c and I_l, respectively).

B
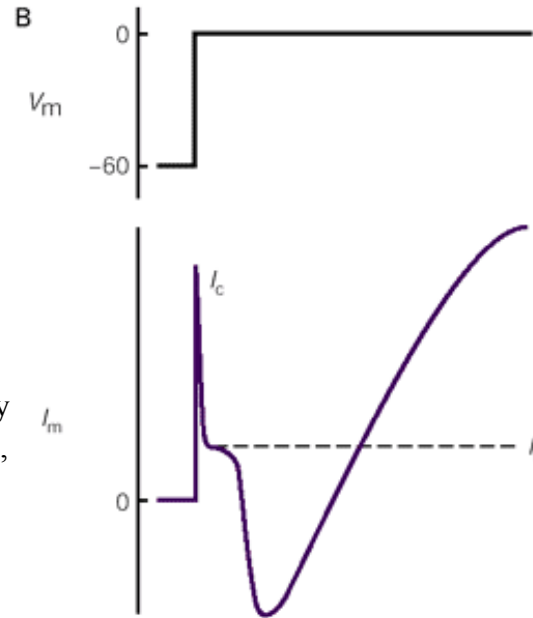
A larger depolarization results in larger capacitive and leakage currents, plus an inward current followed by an outward current.

C

Current measured in presence of TTX

Current measured in presence of TEA

1 ms

Time
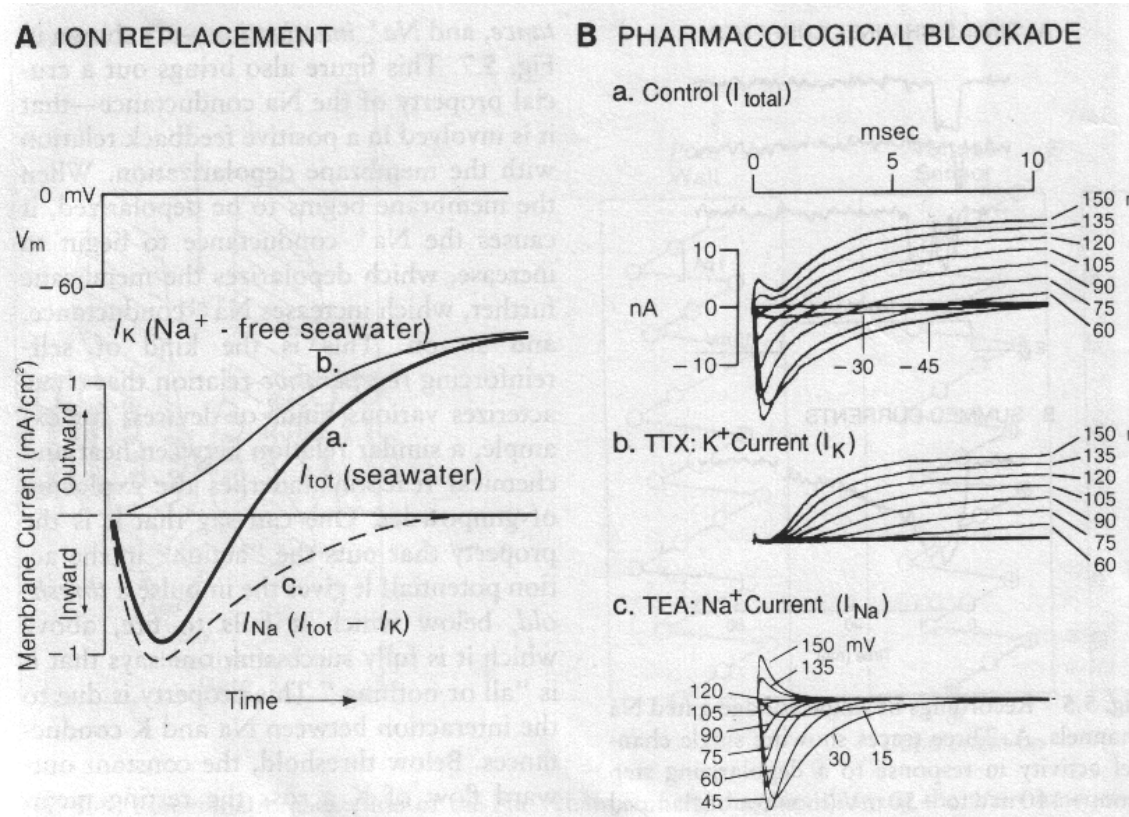
Depolarizing the cell in the presence of tetrodotoxin (which blocks the Na+ current) and again in the presence of tetraethylammonium (which blocks the K+ current), reveals the pure K+ and Na+ currents (I_K and I_{Na}, respectively) after subtracting I_c and I_l.

• Fugu (puffer fish) is a sushi dish prepared from the meat containing TTX

•Training for fugu chef takes about 3 years, 35 % pass the exam.

•In Japam 5-10 persons per year die from fugu poisoning.

•Fugu is the only dish that the Japanese Emperor is not allowed to eat

Hodgkin and Huxley experiment - results

A ION REPLACEMENT

B PHARMACOLOGICAL BLOCKADE

a. Control (I total)

b. TTX: K$^+$ Current (I$_K$)

c. TEA: Na$^+$ Current (I$_{Na}$)

Linear *I-V* relation (Ohm's Law $V = IR$)

$$g_K(V,t) = \frac{I_K(V,t)}{(V - V_K)}$$

$$g_{Na}(V,t) = \frac{I_{Na}(V,t)}{(V - V_{Na})}$$

Knowing IK, INa, VK, VNa, and V one may calculate gK i gNa. IK, INa may be taken from voltage clamp, VK, VNa are constants, V is set by the experimenter.

Hodgkin and Huxley experiment
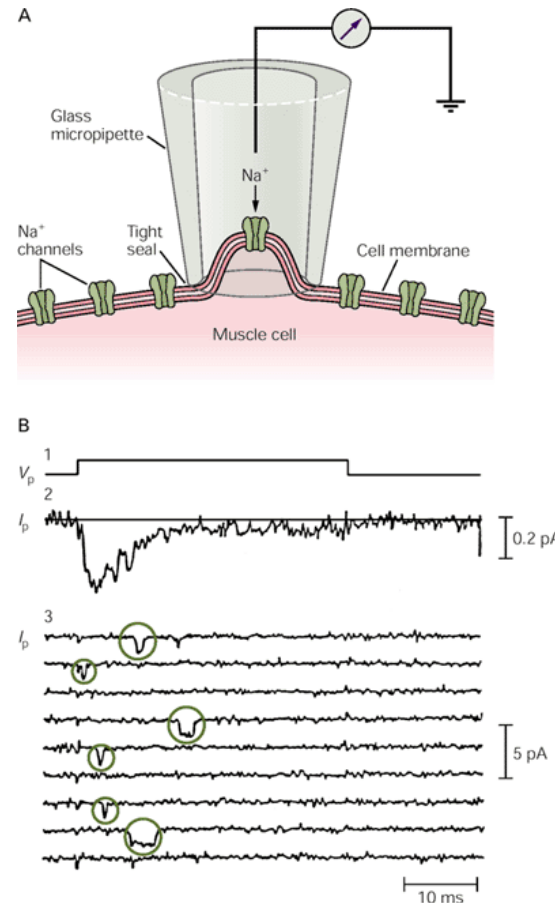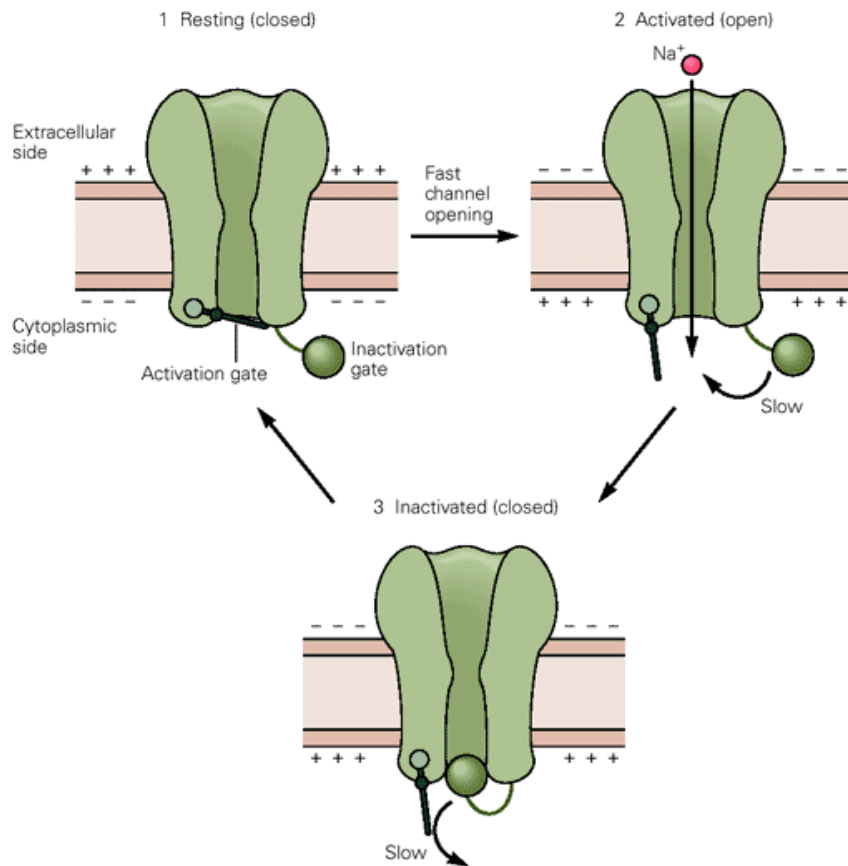
Hodgkin-Huxley model
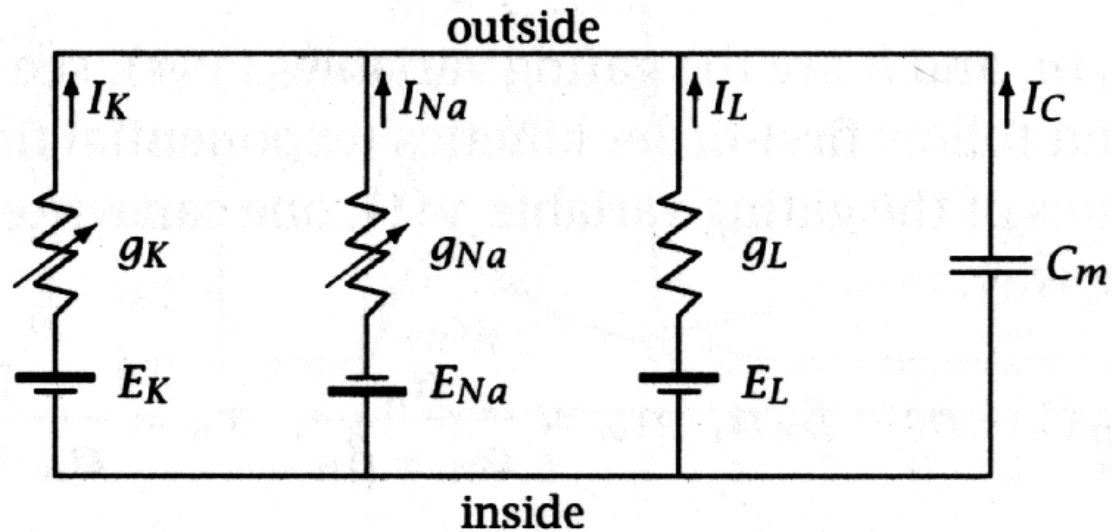
The cover of the 1963 Nobel Prize Programme with Andrew Huxley and Alan Hodgkin (Nobel Prize in Physiology or Medicine)

$$I_m = C_m \frac{dV}{dt} + I_K + I_{Na} + I_L$$

$$I_m = C_m \frac{dV}{dt} + g_K(V,t)(V - V_K) + g_{Na}(V,t)(V - V_{Na}) + g_L(V - V_L)$$

Hodgkin and Huxley model - gates

Voltage clamp experiments for different values of V allowed to suggest that voltage-gated Na+ channels have two gates, which respond in opposite ways to depolarization. In the resting (closed) state the activation gate is closed and the inactivation gate is open (1). Upon depolarization a rapid opening of the activation gate allows Na+ to flow through the channel (2). As the inactivation gates close, the Na+ channels enter the inactivated (closed) state (3). Upon repolarization, first the activation gate closes, then the inactivation gate opens as the channel returns to the resting state (1). K+ channels have only activation gate which opens slowly upon depolarization.

Individual voltage-gated channels may be recorded by patch clamp. They open and close in an all-or-none fashion. Their sum gives a smooth time course of the total transmembrane current.

Gate model – Hodgkin and Huxley (1952)

Closed $\qquad$ Open

$\qquad\qquad\alpha$

$1 - y \quad \xrightleftharpoons{} \quad y$

$\qquad\qquad\beta$
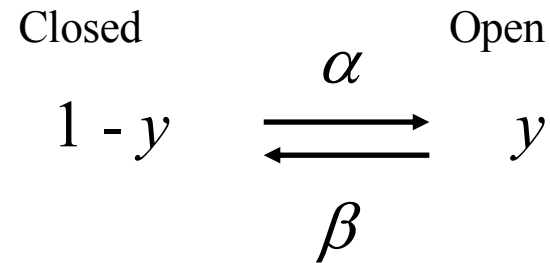
y - the probability of the gate in the open state,

1-y – the probability of the gate to be in the closed state

α, β – rate coefficients

First order kinetics yields:

$$\frac{dy}{dt} = \alpha(1-y) - \beta y$$

At steady state:

$$\frac{dy}{dt} = 0 = \alpha(1-y_\infty) - \beta y_\infty$$

Therefore:

$$y_\infty = \frac{\alpha}{\alpha + \beta}$$

Substituting this into equation:

$$\frac{dy}{dt} = \alpha(1-y) - \beta y = \alpha - (\alpha + \beta)y = y_\infty(\alpha + \beta) - (\alpha + \beta)y$$

$$= (\alpha + \beta)(y_\infty - y)$$

Gate model – Hodgkin and Huxley (1952)

Integration yields:

$$\int \frac{dy(-1)}{(y_\infty - y)} = (-1)\int (\alpha + \beta)dt$$
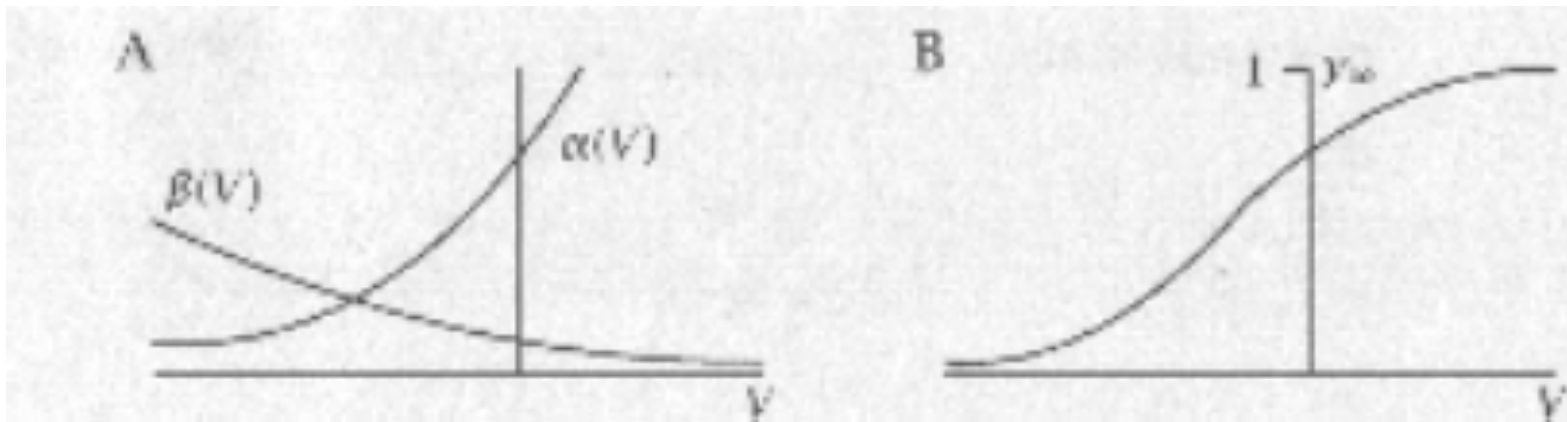
$$\ln(y_\infty - y) = -(\alpha + \beta)t + C$$

$$y = y_\infty - Ae^{-(\alpha+\beta)t}$$

$y_\infty(V)$ Steady state

$$\tau(V) = \frac{1}{\alpha(V) + \beta(V)} \qquad \text{Time constant}$$

The voltage dependence of the rates and the steady state of the HH model.

Hodgkin and Huxley model

From the time course of measured gK i gNa Hodgkin and Huxley found that gK i gNa do not follow simply exp(-t/τ) but rather power functions of exp(-t/τ). They proposed:

$$g_K(V,t) = Y_K(V,t)\overline{g}_K = n^4\overline{g}_K$$

$$g_{Na}(V,t) = Y_{Na}(V,t)\overline{g}_{Na} = m^3h\overline{g}_{Na}$$

Recalling the gate model:

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n, \quad n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}, \quad \tau_n = \frac{1}{\alpha_n + \beta_n}$$

$$\frac{dm}{dt} = \alpha_m(1-m) - \beta_m m, \quad m_\infty = \frac{\alpha_m}{\alpha_m + \beta_m}, \quad \tau_m = \frac{1}{\alpha_m + \beta_m}$$

$$\frac{dh}{dt} = \alpha_h(1-h) - \beta_h h, \quad h_\infty = \frac{\alpha_h}{\alpha_h + \beta_h}, \quad \tau_h = \frac{1}{\alpha_h + \beta_h}$$

Hodgkin and Huxley model

These equations yield the following solutions for n, m and h:

$$n(t) = n_0 - [(n_0 - n_\infty)(1 - e^{-t/\tau_n})]$$

$$m(t) = m_0 - [(m_0 - m_\infty)(1 - e^{-t/\tau_m})]$$

$$h(t) = h_0 - [(h_0 - h_\infty)(1 - e^{-t/\tau_h})]$$

Substituting n, m, h into gNa i gK:

$$g_K(t) = \overline{g_K} n^4 = \overline{g_K}[n_0 - (n_0 - n_\infty)(1 - e^{-t/\tau_n})]^4$$

$$g_{Na}(t) = \overline{g_{Na}} m^3 h$$

$$= \overline{g_{Na}}[m_0 - (m_0 - m_\infty)(1 - e^{-t/\tau_m})]^3 [h_\infty - (h_0 - h_\infty)(1 - e^{-t/\tau_h})]$$

$$= \overline{g_{Na}} m_\infty h_0 (1 - e^{-t/\tau_m})^3 e^{-t/\tau_h}$$

because m_0 i h_{inf} are neglectably small.

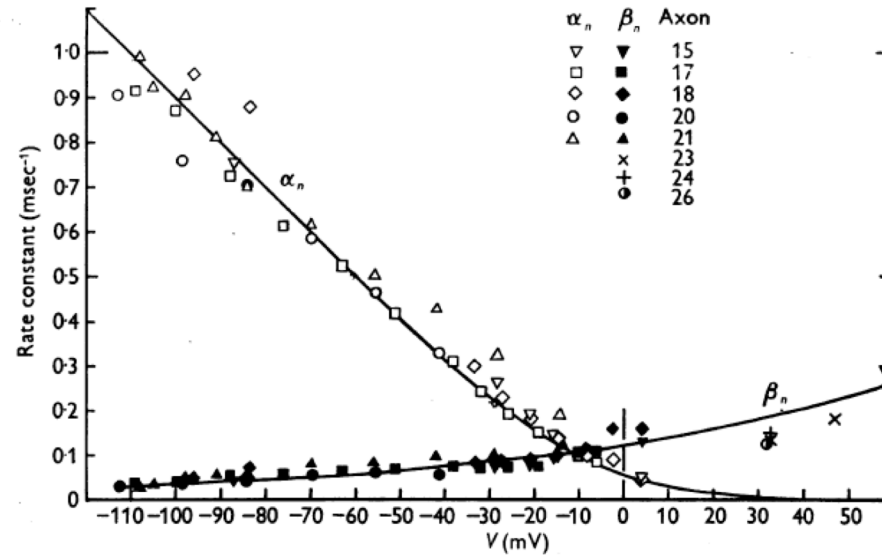# Hodgkin and Huxley model

By fitting the equations for gNa, gK to the time records of gNa, gK at various voltages, HH measured $\tau_n, \tau_m, \tau_h, n_\infty, m_\infty, h_\infty$ and calculated $\alpha_n, \beta_n, \alpha_m, \beta_m, \alpha_h, \beta_h$ as follows:

$$\alpha_n = \frac{n_\infty}{\tau_n}, \quad \beta_n = \frac{1-n_\infty}{\tau_n}$$

$$\alpha_m = \frac{m_\infty}{\tau_m}, \quad \beta_m = \frac{1-m_\infty}{\tau_m}$$

$$\alpha_h = \frac{n_h}{\tau_h}, \quad \beta_h = \frac{1-n_h}{\tau_h}$$

To find relationship between α, β and membrane voltage, they collected all measurements and plotted them against V. Next, they fitted theoretical functions to the experimental points. These expressions gave formulas for steady state conductances at any voltage.

Hodgkin and Huxley model

Finally, the model was described by the following set of equations:

$$I_m = C_m \frac{dV}{dt} + \bar{g}_K n^4 (V - V_K) + \bar{g}_{Na} m^3 h (V - V_{Na}) + \bar{g}_l (V - V_l)$$

where

$$\frac{dn}{dt} = \alpha_n (1 - n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h$$

and

$$\alpha_n = 0.01(V + 10) / \left(\exp \frac{V + 10}{10} - 1 \right)$$

$$\beta_n = 0.125 \exp(V/80)$$

$$\alpha_m = 0.1(V + 25) / \left(\exp \frac{V + 25}{10} - 1 \right)$$

$$\beta_m = 4 \exp(V/18)$$

$$\alpha_h = 0.07 \exp(V/20)$$

$$\beta_n = 1 / \left(\exp \frac{V + 30}{10} - 1 \right).$$

Hodgkin and Huxley model

Using numerical methods Hodgkin and Huxley solved HH model equations and obtained remarkable fits between the recorded and calculated action potentials. HH model is considered a greatest achievement in quantitative brain modeling or even in all biological sciences. HH theory also accounts for the conduction of action potential along nerve fibres.
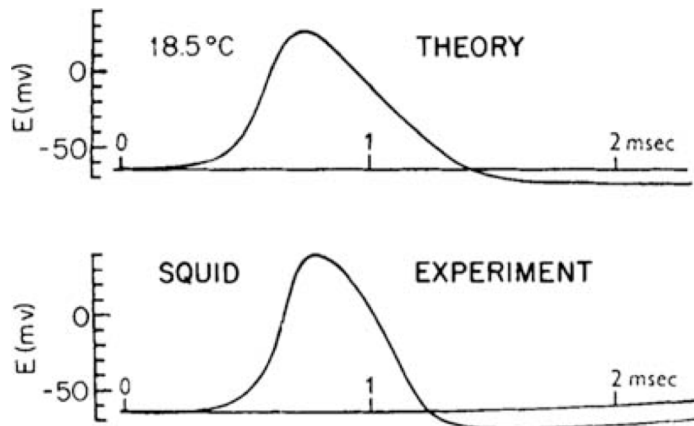
From: Hodgkin, A. L., and A. F. Huxley. A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. London 117: 500–544, 1952.
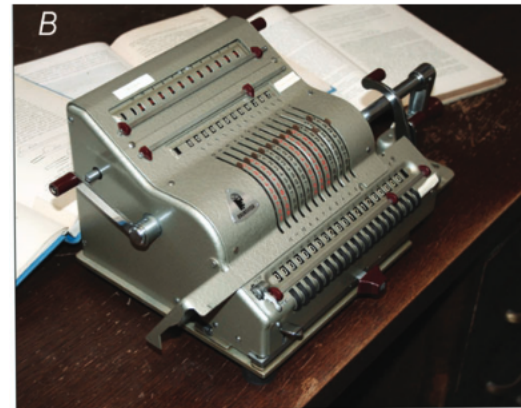

The Brunsviga 20 hand-operated mechanical calculator used by Andrew Huxley to solve the differential equations numerically. Calculations of the propagated action potential took about three weeks to complete.
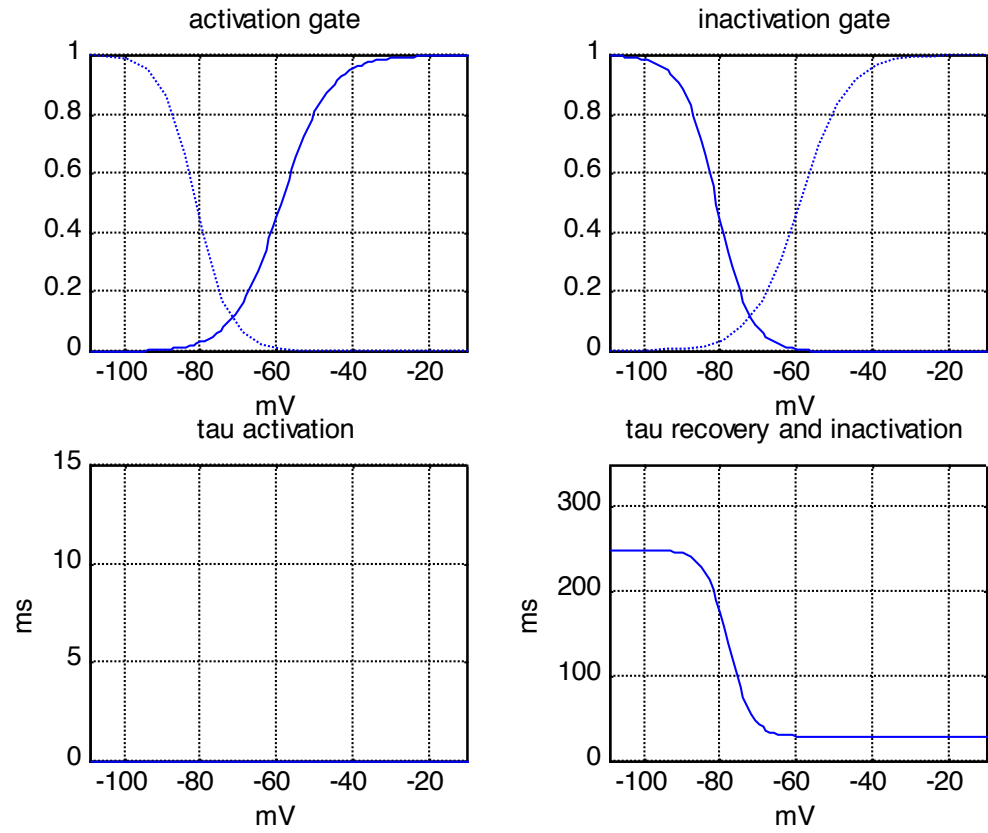
HH model has also some limitations. It describes macroscopic currents but not currents at the level of single channels. Also, it assumes constant ion concentrations.

Action potential generation - summary

The Function of Voltage Gated Channels
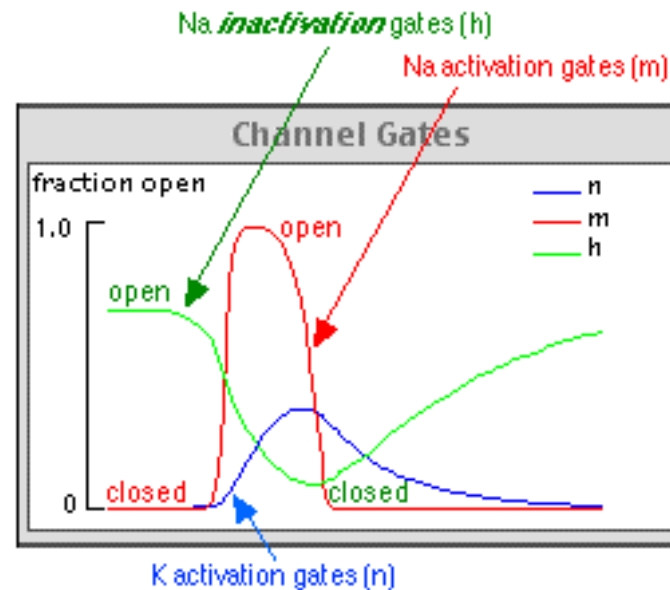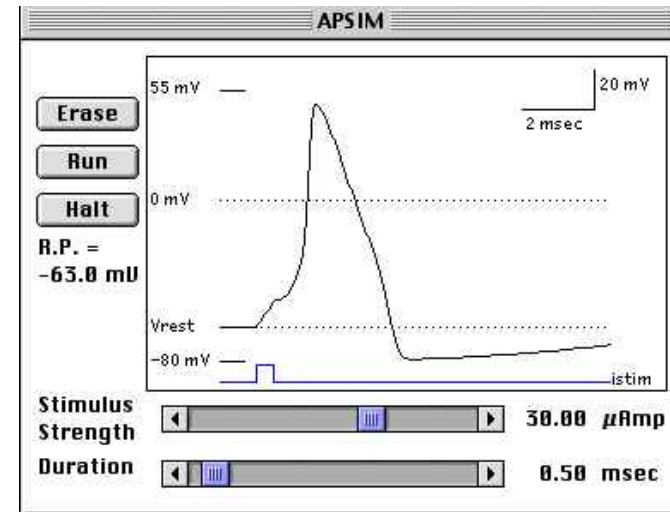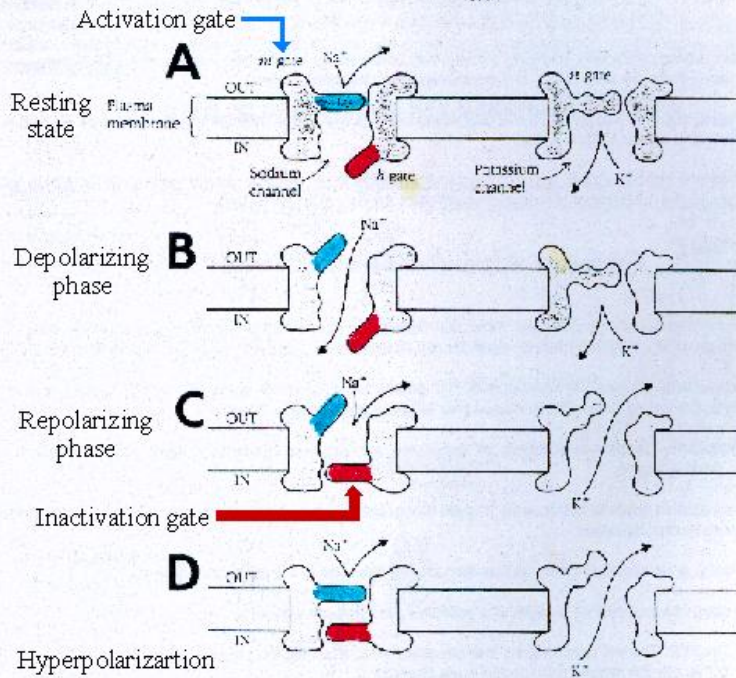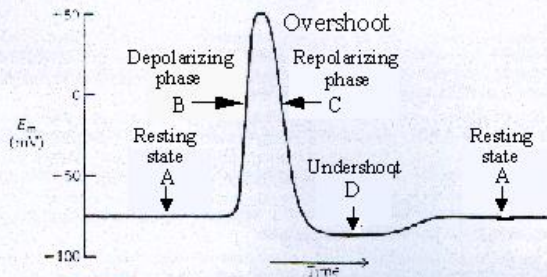
# Typical ionic channel characteristics

Ionic currents in cortical neurons

**Table 7.1** Voltage-gated ionic currents in cortical neurons

Current	Symbol	Ion	V_{th}	Inactivation	Blocked by	Modulation	Function
1. Voltage-gated (depolarization)							
Na^+ currents							
Fast	$I_{Na(fast)}$	Na^+	−50	Fast	TTX		spike
Slow	$I_{Na(slow)}$	Na^+	−65	Slow	TTX		prepotential
Ca^{2+} currents							
High-threshold	$I_{Ca(L)}$	Ca^{2+}	−15	Slow Ca^{2+}-dep	Cd^{2+} DHP	NE (+) ACh (−)	spike
Low-threshold	$I_{Ca(T)}$	Ca^{2+}	−40	Fast V-dep	Ni^{2+}	ACh (+)	burst firing
High-threshold	$I_{Ca(N)}$	Ca^{2+}	−25	Medium V & Ca^{2+}-dep	Cd^{2+} ωCTX-GVIA	NE (+,−) Aden. (−) Others (−)	spike (?) presyn. (?)
High-threshold	$I_{Ca(P)}$	Ca^{2+}	−20	Slow	ωAga-IVA		presyn. (?)
K^+ currents							
Delayed rectifier	$I_{K(DR)}$	K^+	−40	Slow	TEA (10 mM)		
Transient	$I_{K(A)}$	K^+	−60	Fast	4-AP (> 0.1 mM)	ACh (−)	spike repolar.
Delay current	$I_{K(D)}$	K^+	−75	Slow	4-AP (< 0.1 mM)	DTX	delayed firing, spike repolar.
M current	$I_{K(M)}$	K^+	−65	None	Ba^{2+}	ACh (−) 5-HT (−) Somato. (+)	spike train accommod. mAHP
2. Voltage-gated (hyperpolarization)							
Slow inward rectifier	I_Q, I_h, I_f	Na + K	−60	None	Cs^+, THA		rest V_m
Fast inward rectifier	$I_{K(IR)}$	K^+	−80	Slow	Cs^+, Ba^{2+}	G_o (+)	
Time-depend. Cl^- currents	$I_{Cl(V)}$	Cl^-	−20	None	Cd^{2+}	PBs	dendrites (?)
		Cl^-	−60	None	Cd^{2+}		
3. Ca^{2+}-gated							
Fast K^+ current	$I_{K(C)}$	K^+	−40	None	TEA (1 mM)		spike repolar. f&mAHP
Slow K^+ current	$I_{K(AHP)}$	K^+	None	None	Ba^{2+}	ACh (−) NE (−) 5-HT (−) Hist. (−)	spike train accommod. sAHP
Cl^- current	$I_{Cl(Ca)}$	Cl^-					
Cation current		Na + K				ACh (+)	AHP (?)
4. Other currents							
Leak (?)	$I_{K(L)}$	K^+	None	None	Ba^{2+}	ACh (−)	rest V_m
Cl^-	I_{Cl}	Cl^-					
Anoxic	$I_{K(ATP)}$	K^+					hyperpol.
Na^+ Act. K^+	$I_{K(Na)}$	K^+					
Stretch		Na + K					mechanorec.

Ca$^+$ currents

A T-Type Calcium Currents
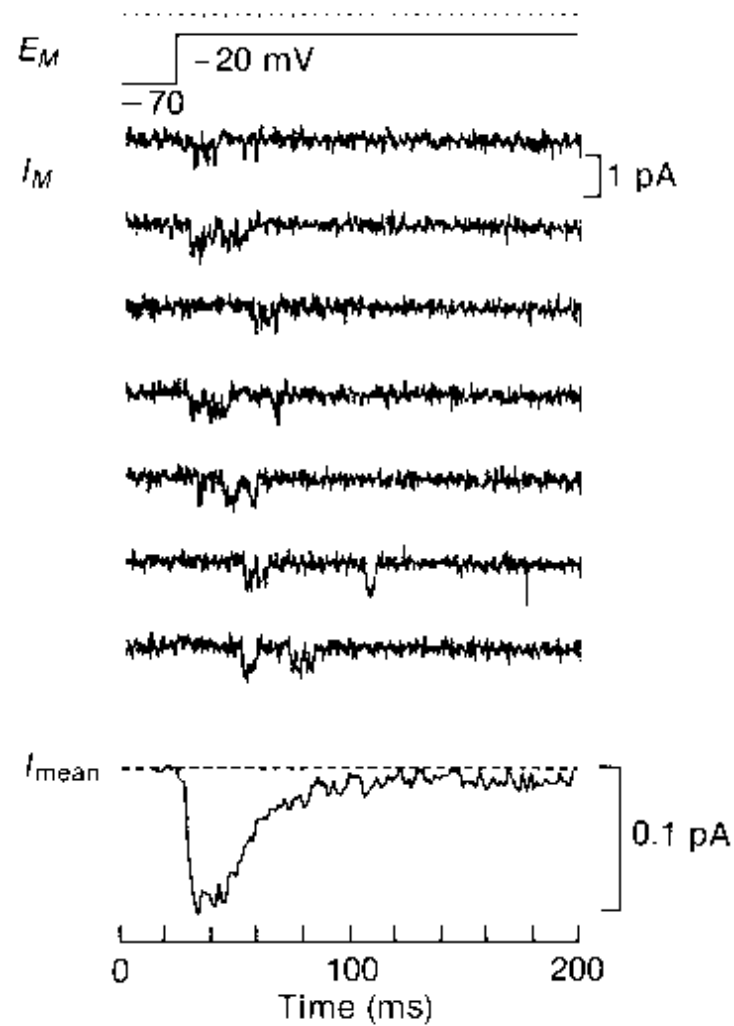
B L-Type Calcium Currents
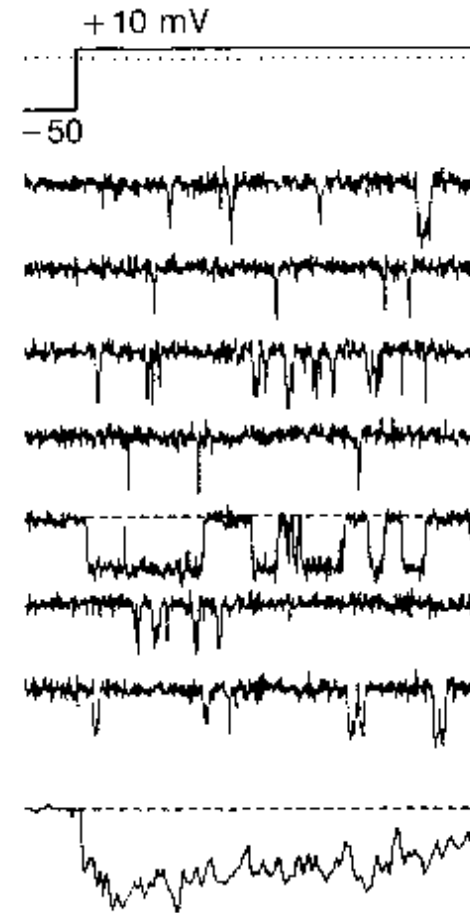
Two types of calcium channels recorded using patch clamp. A. T-type (transient lub LVA – low voltage activated channel). B. L-Type (long lasting lub HVA – high voltage activated channel).

K$^+$ currents

$$I_{K(DR)} + I_{K(A)}$$

The greatest diveristy exists among K+ channels. Potassium channels are the main mechanisms for maintaining the equilibrium of the cell and for controlling membrane excitability. Since the potassium equilibrium potential is near the resting membrane potential, activation of K+ channels tend to return the membrane potential to the resting level.

$$I_{K(C)}$$
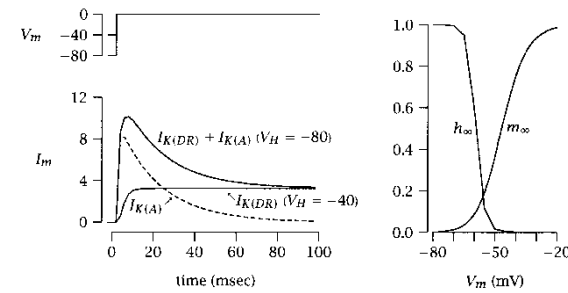
Delayed rectifier $I_{K(DR)}$

Transient $I_{K(A)}$

Delay current $I_{K(D)}$

Calcium-Dependent $I_{K(C)}$, I_{AHP}

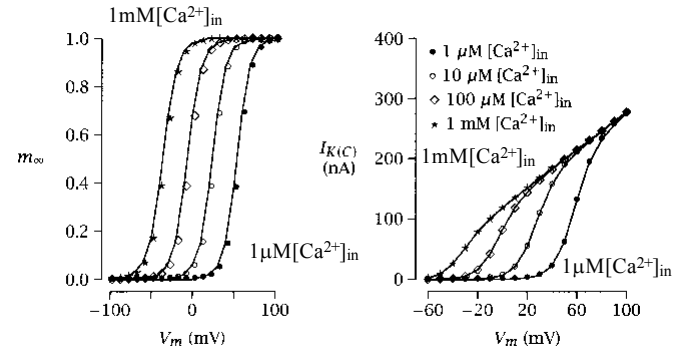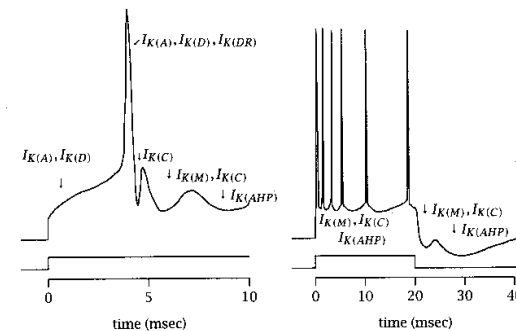Anomalous rectifier I_{AR}; I_Q; I_h

$$I_{K(DR)} + I_{K(A)} + I_{K(D)} + I_{K(C)} + I_{AHP} + I_M$$

M current I_M

Leak $I_{K,\,leak}$

Ionic currents - summary
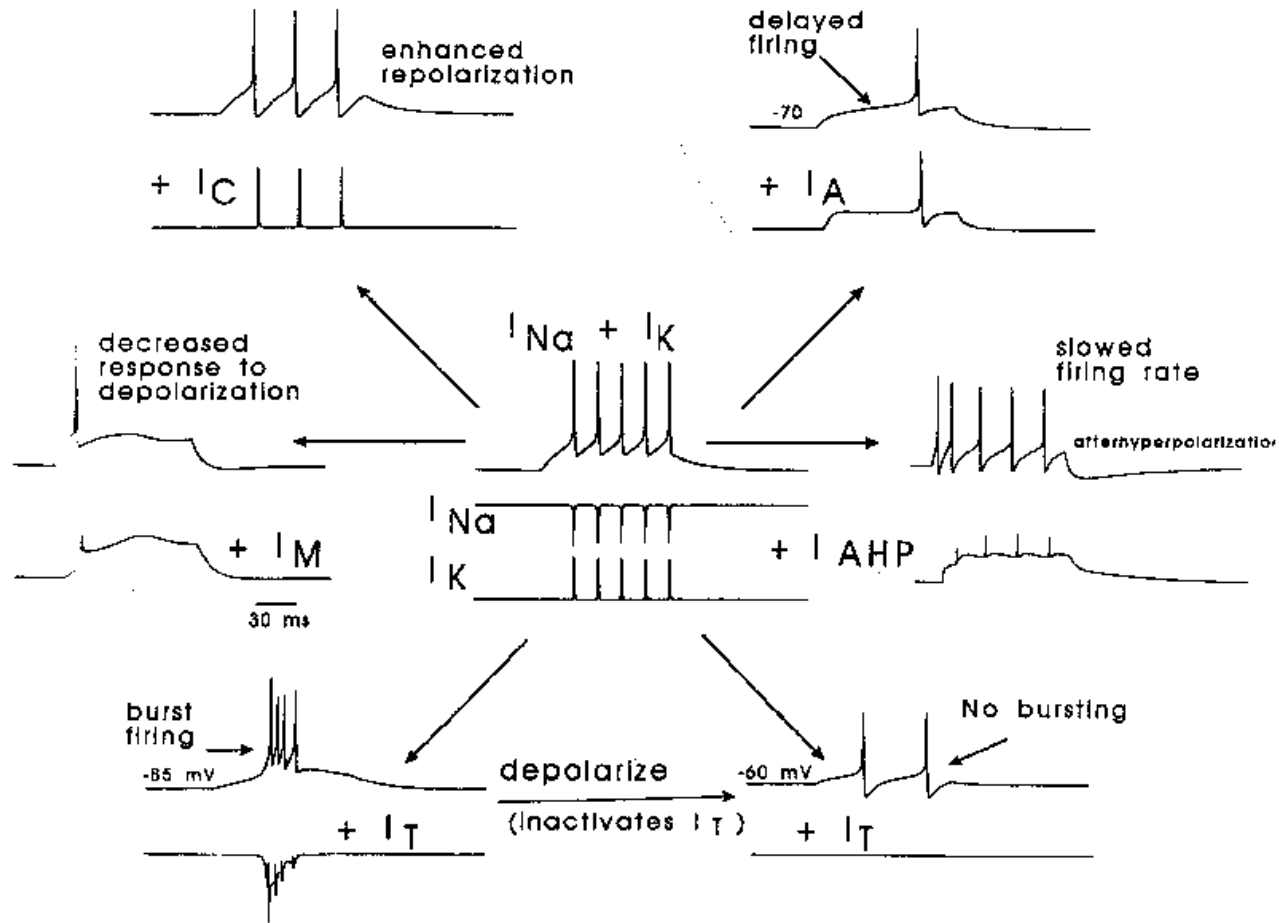
Expanded version of the equivalent circuit of the cell membrane
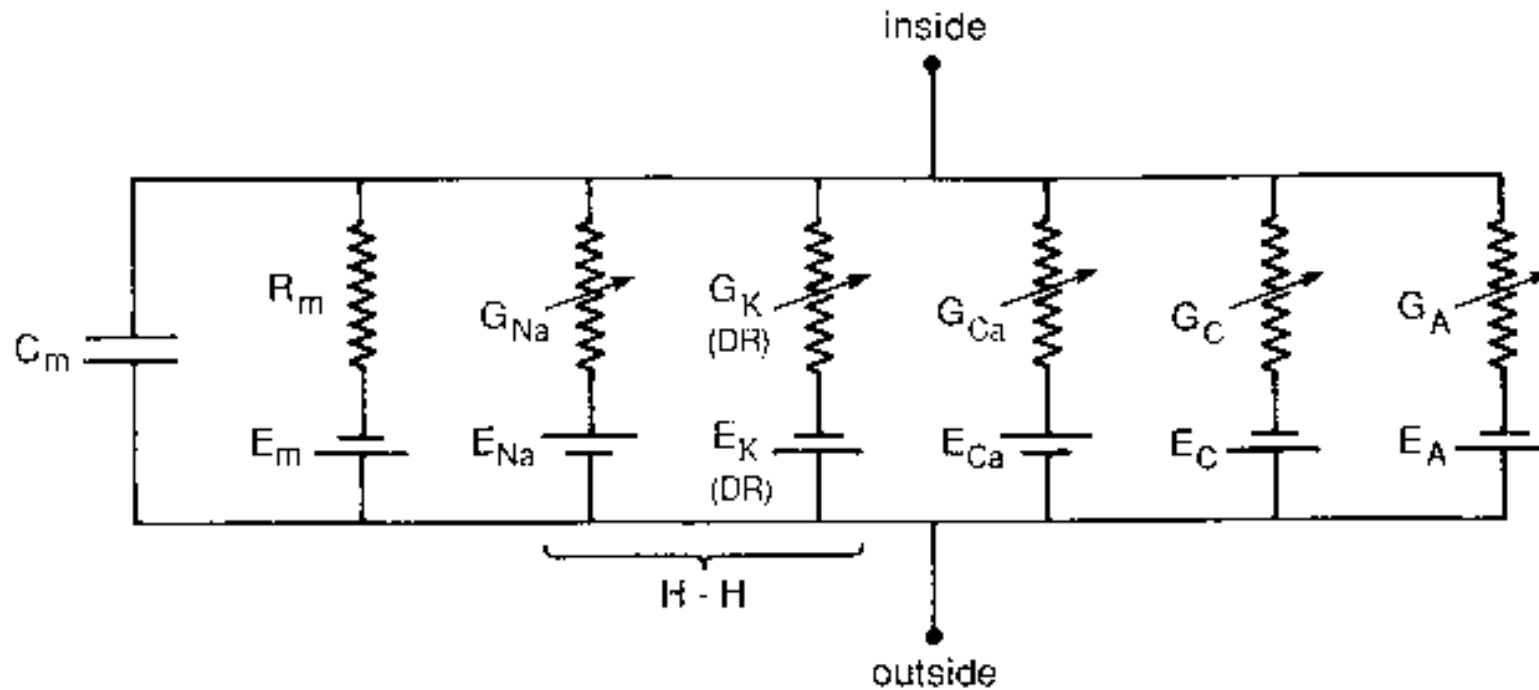
Four types of coritcal neurons?

Traditionally, there are four types of behavior of cortical neurons and they are assigned to different types of cells: RS – regular spiking, FRB – fast rhythmic bursting, FS – fast spiking, IB – intrinsically bursting.

Intracelluar recordings *in vivo* showed that firing patterns can be transformed from one type into another by slight changes in the membrane potential.
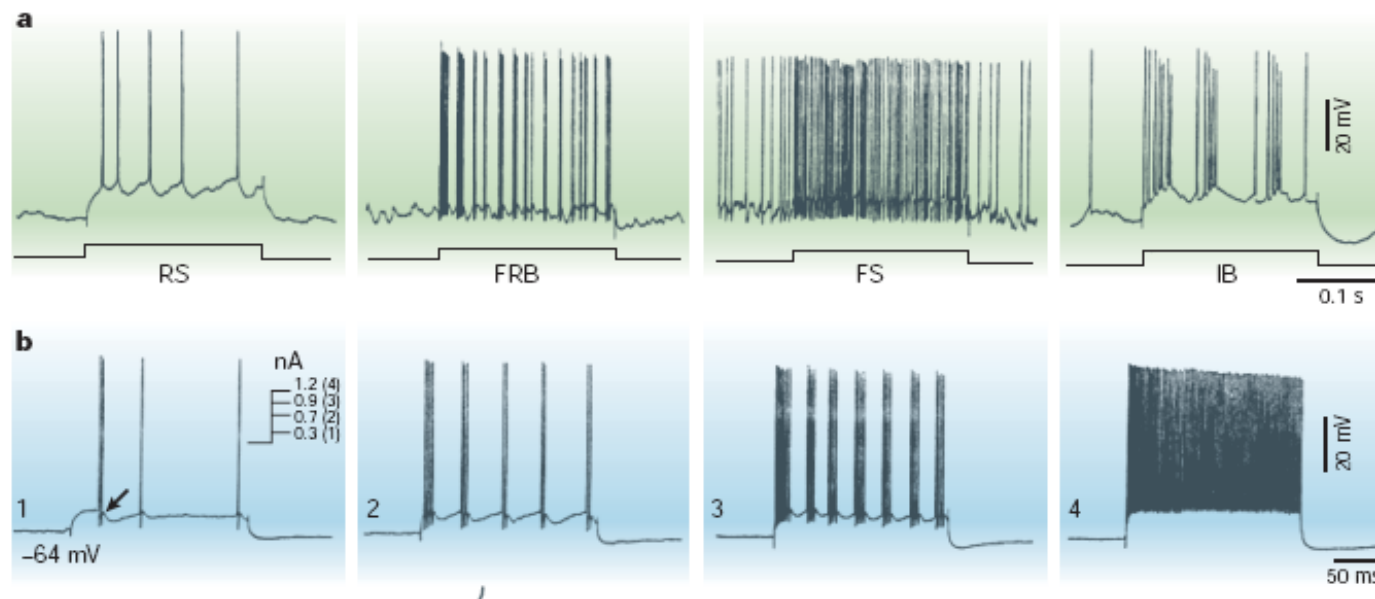
a) Intracellular recordings in awake and sleeping cats. b) intracellular recording from cortical neuron in cat under anasthesia. Current injection (b1 – inset) changes the firing pattern. Mircea Steriade, Neocortical Cell Classes Are Flexible Entities. NATURE REVIEWS | NEUROSCIENCE, VOL. 5, pp. 121-134, 2004.