Electrophysiology

What is Electrophysiology?

"electro" - "physiology"

"is the study of the electrical properties of biological cells and tissue"
www.wikipedia.com

"is the branch of physiology that studies the relationship between electric phenomena and bodily processes"
www.thefreedictionary.com

"is the science concerned with the flow of ions in biological tissues and, in particular, the electrical recording techniques that enable the measurement of this flow."
www.brainsonline.org

Milestones of Electrophysiology

Luigi Galvani and Alessandro Volta late 18th century
- frog legs with muscles and nerves exposed “animal electricity”

  (Galvanic cell, Voltaic pile - first battery)

Carlo Matteucci 1838
- potential difference between outside and inside the muscle cell

Emil Heinrich Du Bois-Reymond 1850 (Alexander von Humboldt)
- refined methods to record muscle current (nerve galvanometer)

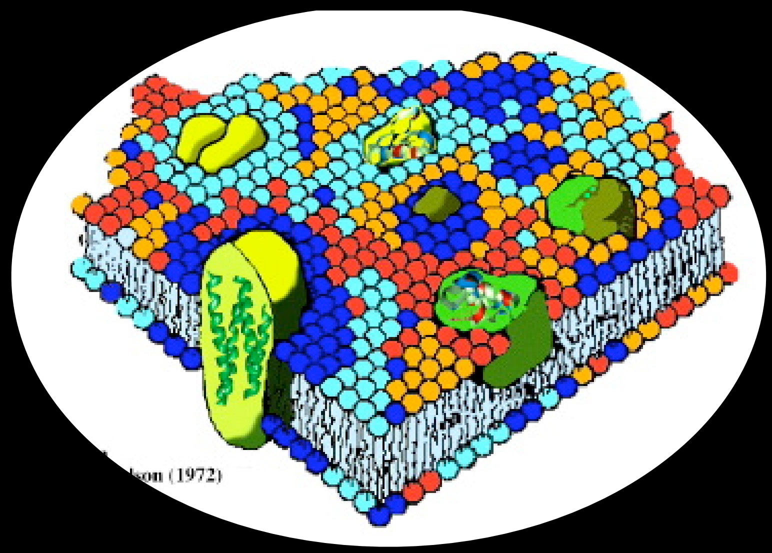
Hermann von Helmholtz 1852
- measured the speed of frog nerve impulses (10 to 100 m/s)

Karl von Nägeli, Karl Cramer and Wilhelm Pfeffer middle of 19th century
- osmolarity experiments -> membrane around cells

  (Pfeffer cell - technique to measure osmolarity)

Evert Gorter and Frank Grendel 1925
- cell membrane is a lipid bilayer

Jonathan Singer and Garth Nicolson 1972
- fluid mosaic model of the cell membrane



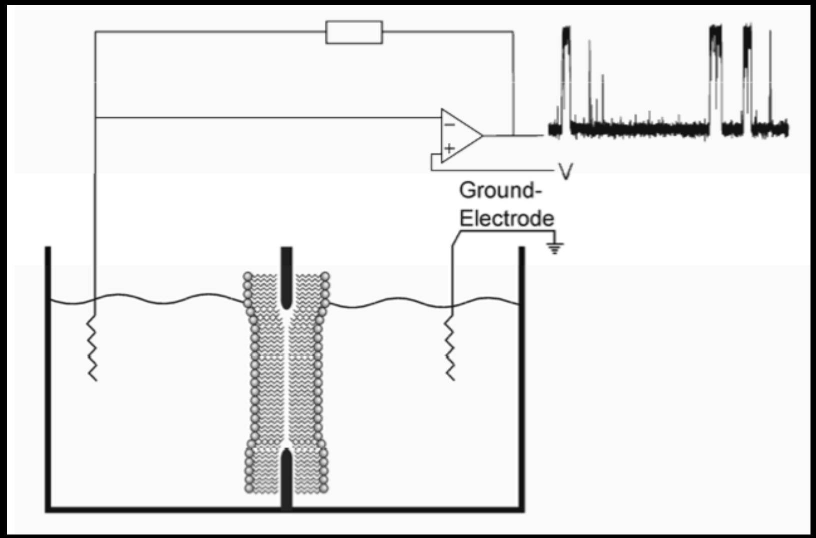
Modern Electrophysiology

John Zachary Young 1930’s
- first indication of ion selective membrane channels in squid giant axon

Alan Hodgkin and Andrew Huxley 1930’s
- first inserted electrodes in muscle to measure membrane potential in resting and activated muscle
- voltage clamp (devised by Kenneth Cole) to measure ion current fluxes through an ensemble of channels

Noble Prize 1963

Paul Müller and Donald Rudin 1960’s
- first measurements of ion flux through single pores
(BLM - Black Lipid Membranes)



Erwin Neher and Bert Sakmann 1976
- tight seal patch clamp technique enables detailed study of ion channels

Nobel Prize for Physiology and Medicine in 1991

Impact of Studying Ion Channels

Each living cell has ion channels !!

basic understandings of physiological processes in living cell
e.g. signalling processes in the nervous system

~400 genes in human genome code for channel proteins (1.5% of all)

~40 known hereditary channelopathies
(e.g. heart diseases, muscle dystrophy, cystic fibrosis renal disorders)

4 from TRP channels
TRPC6 - glomerulosclerosis (kidney disease)
TRPM6 - hypomegnesemia / hypocalcemia
TRPP2 - autosomal dominant polycystic kidney disease
TRPML1 - mucolipidosis type IV

The Biological Basics of Electrophysiology

Today, 04/21/2008

Electrophysiological Techniques

Monday, 04/28/2008

More about Patch Clamp

Monday, 05/05/2008

Calcium Imaging

Monday, 05/12/2008

Definitions

electric potential - Volt [V]

difference in electric charge between two points (voltage gradient)

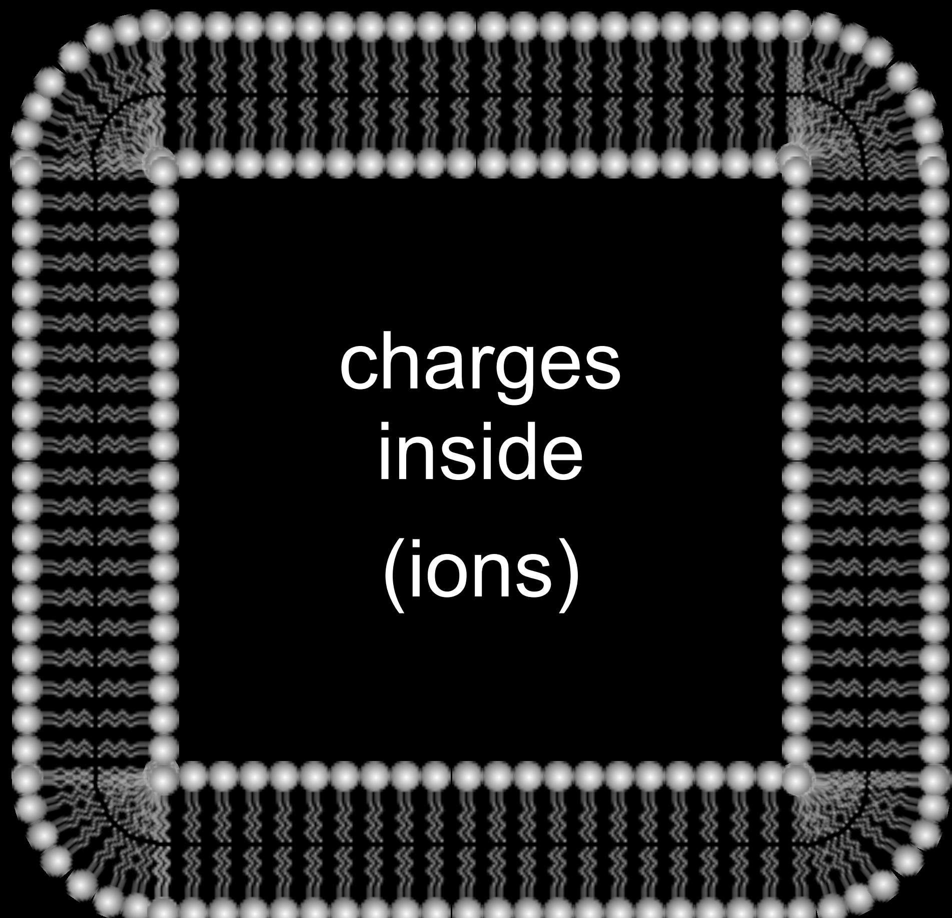
electric charge - Coulomb [C]

1 C = 6.24×10^{-18} x charge of 1 electron
elementary charge 1 e is 1.602×10^{-19} C

Requirements to establish a Voltage Gradient

insulator

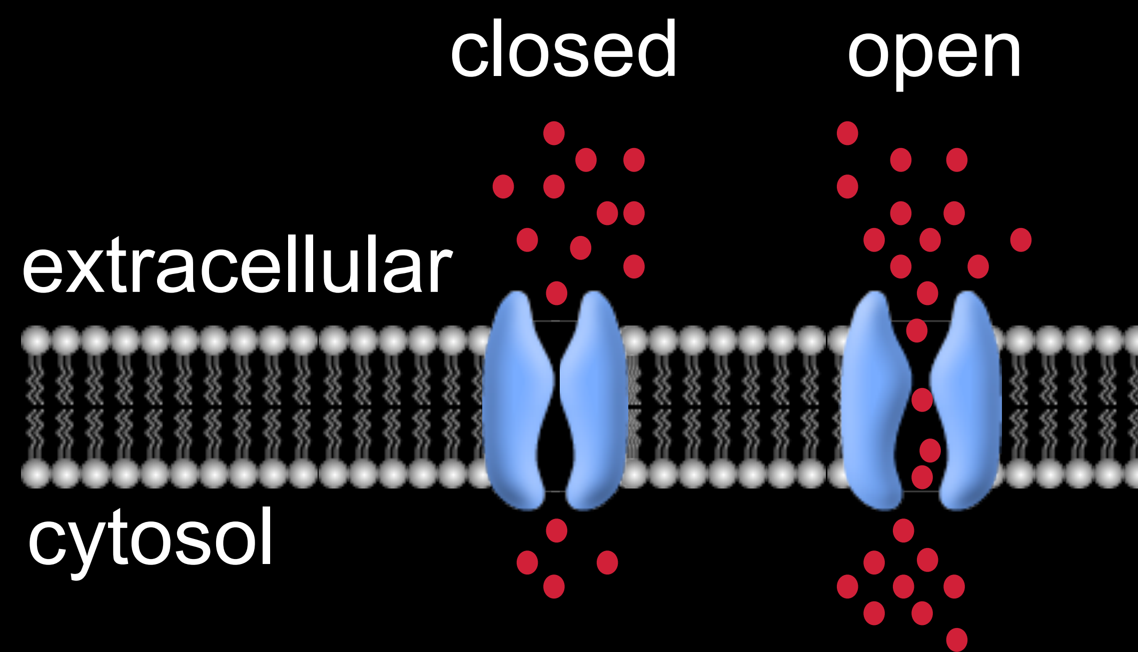
plasma membrane



charges outside
(ions)

incorporated molecular switches

ion channels



electrical properties

open ion channels
"conductance"

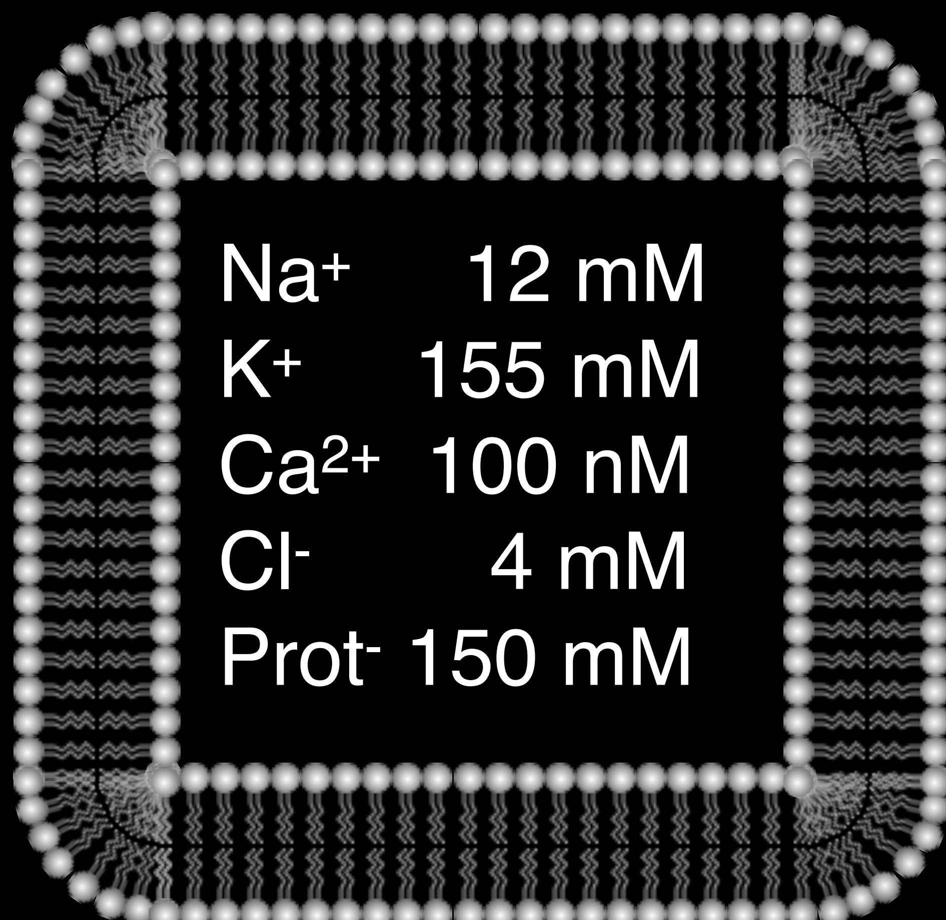
membran blocks flow of charges
ion channels in closed state
"resistance"

separation of charges
"capacitance"

Definitions

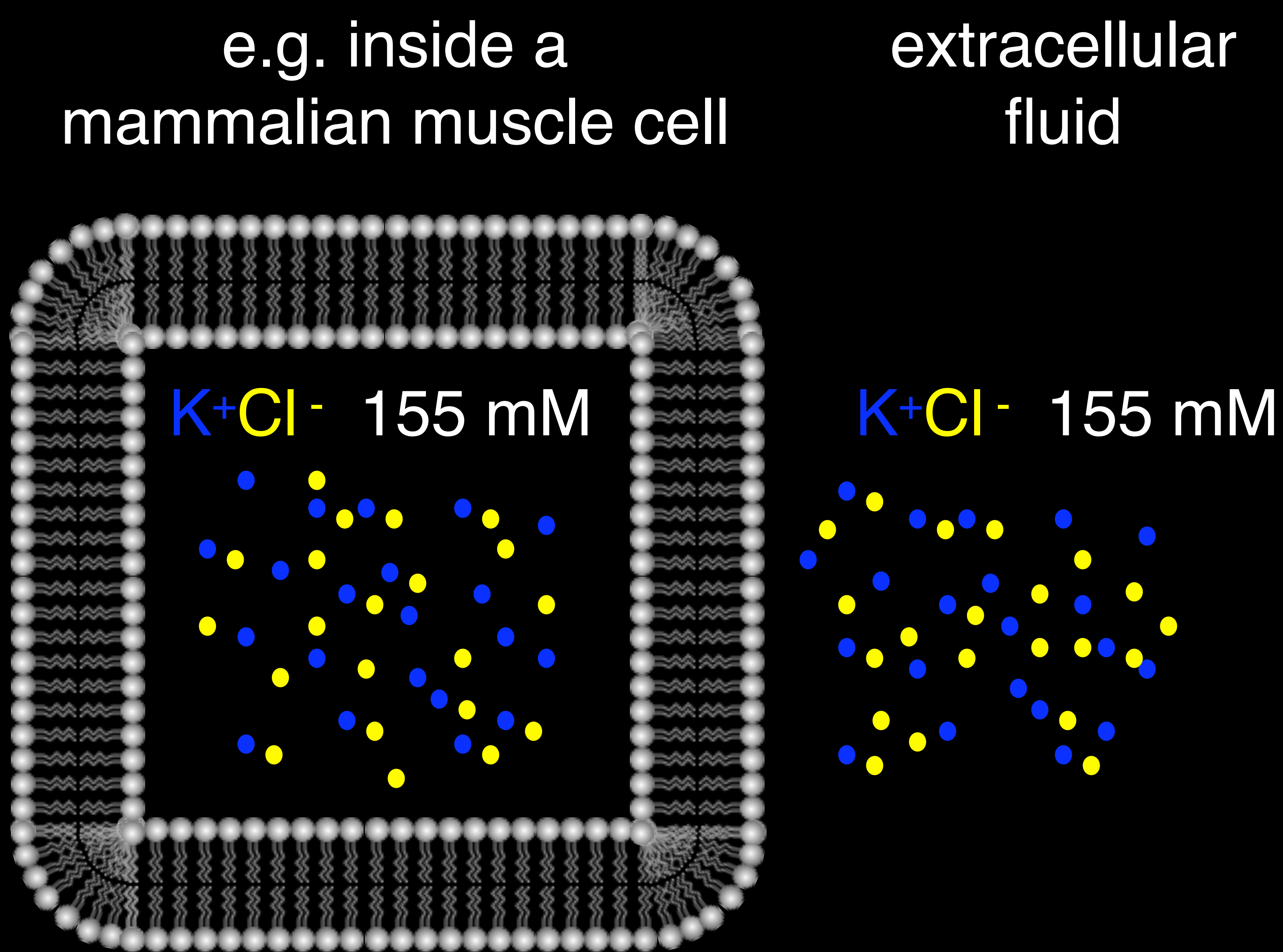
electric potential - Volt [V]	difference in electric charge between two points (voltage gradient)
electric charge - Coulomb [C]	1 C = 6.24×10^{-18} x charge of 1 electron elementary charge 1 e is 1.602×10^{-19} C
capacitance - Farad [F]	electric charge per volt (Coulombs / Volt) typ. cap. of cell lipid bilayer 0.01 pF/cm^2
current - Ampere [A]	flow of charges per s (Coulombs / Sec)
conductance - Siemens [S]	flow of charges per Volt (Amperes / Volt)
resistance - Ohm [Ω]	R = 1/Conductance Ohm's Law $V = I \times R$

Ions make the Voltage Gradient



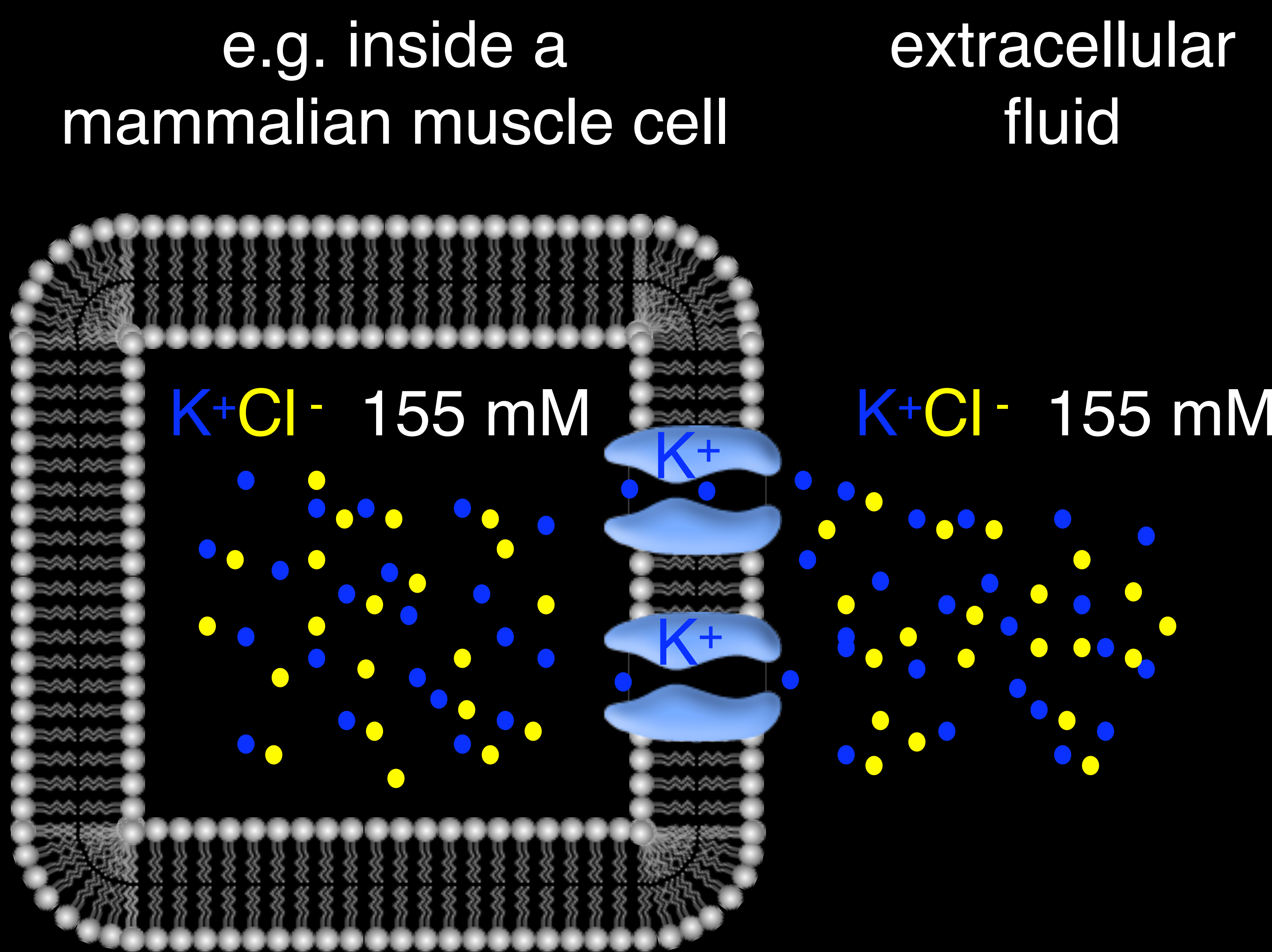
	e.g. inside a mammalian muscle cell	extracellular fluid
Na^+	12 mM	145 mM
K^+	155 mM	4 mM
Ca^{2+}	100 nM	1.5 mM
Cl^-	4 mM	123 mM
$Prot^-$	150 mM	traces

Ions make the Voltage Gradient



no diffusion
no current

Ions make the Voltage Gradient



only K+ diffusion

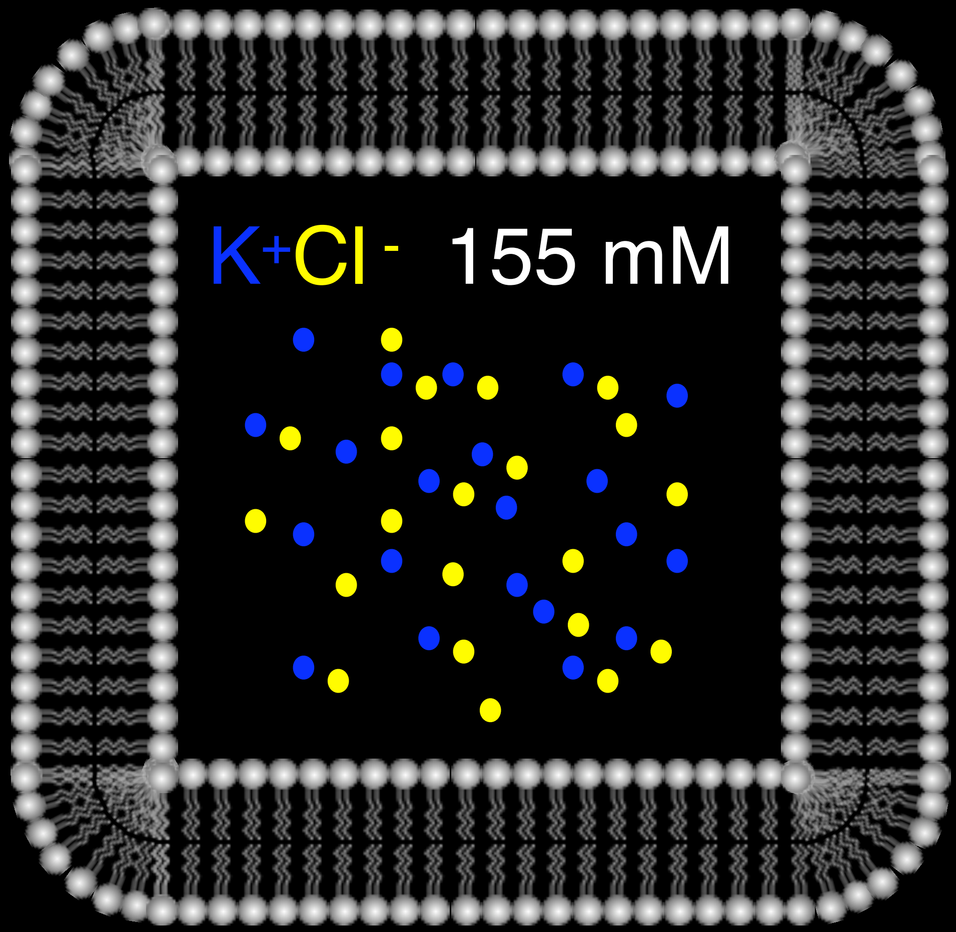
no current

positive charges go equally both ways

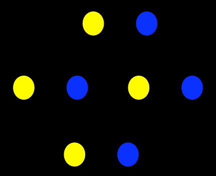
Ions make the Voltage Gradient

e.g. inside a mammalian muscle cell

extracellular fluid



K^+Cl^- 4 mM

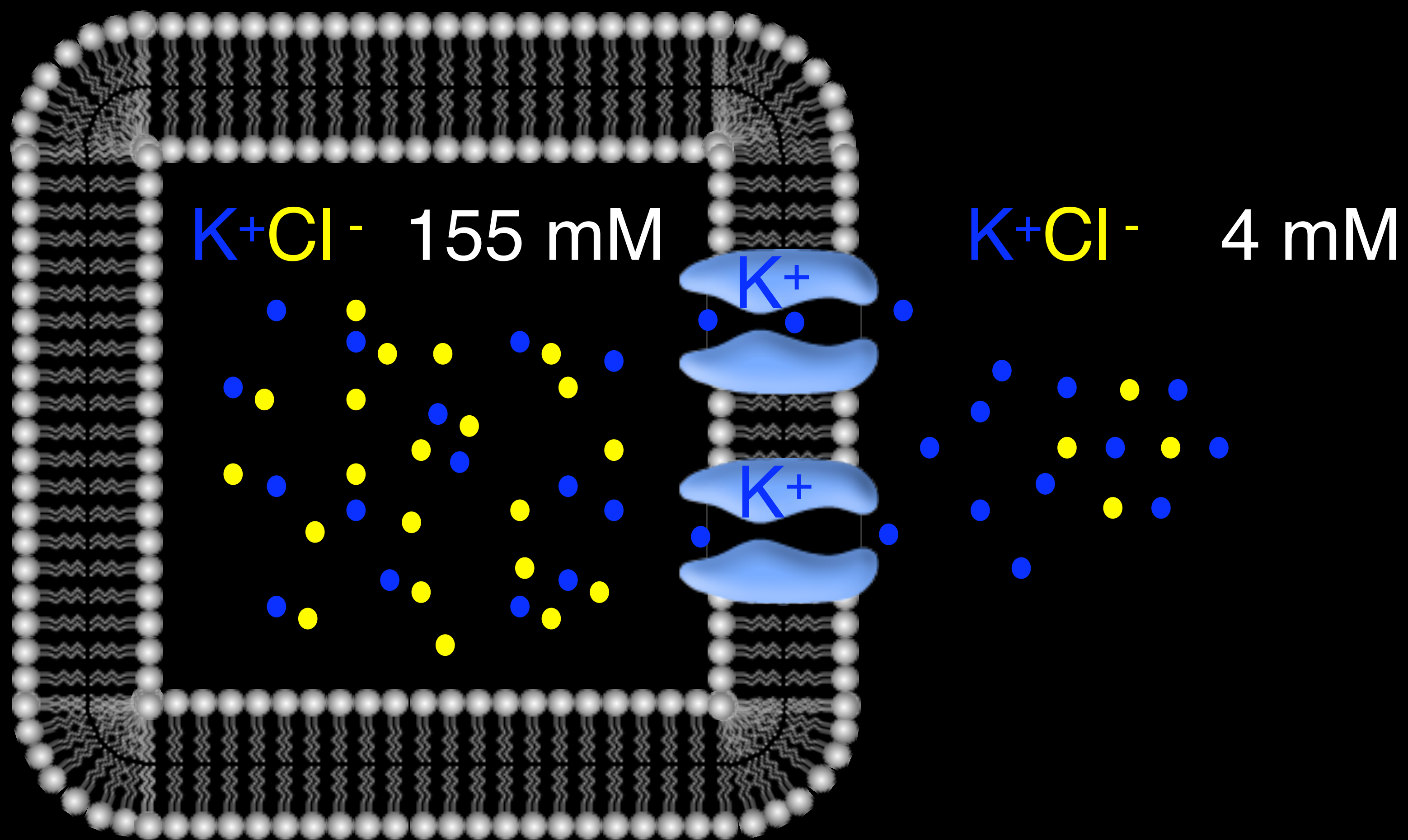


no diffusion
no current

Ions make the Voltage Gradient

e.g. inside a mammalian muscle cell

extracellular fluid



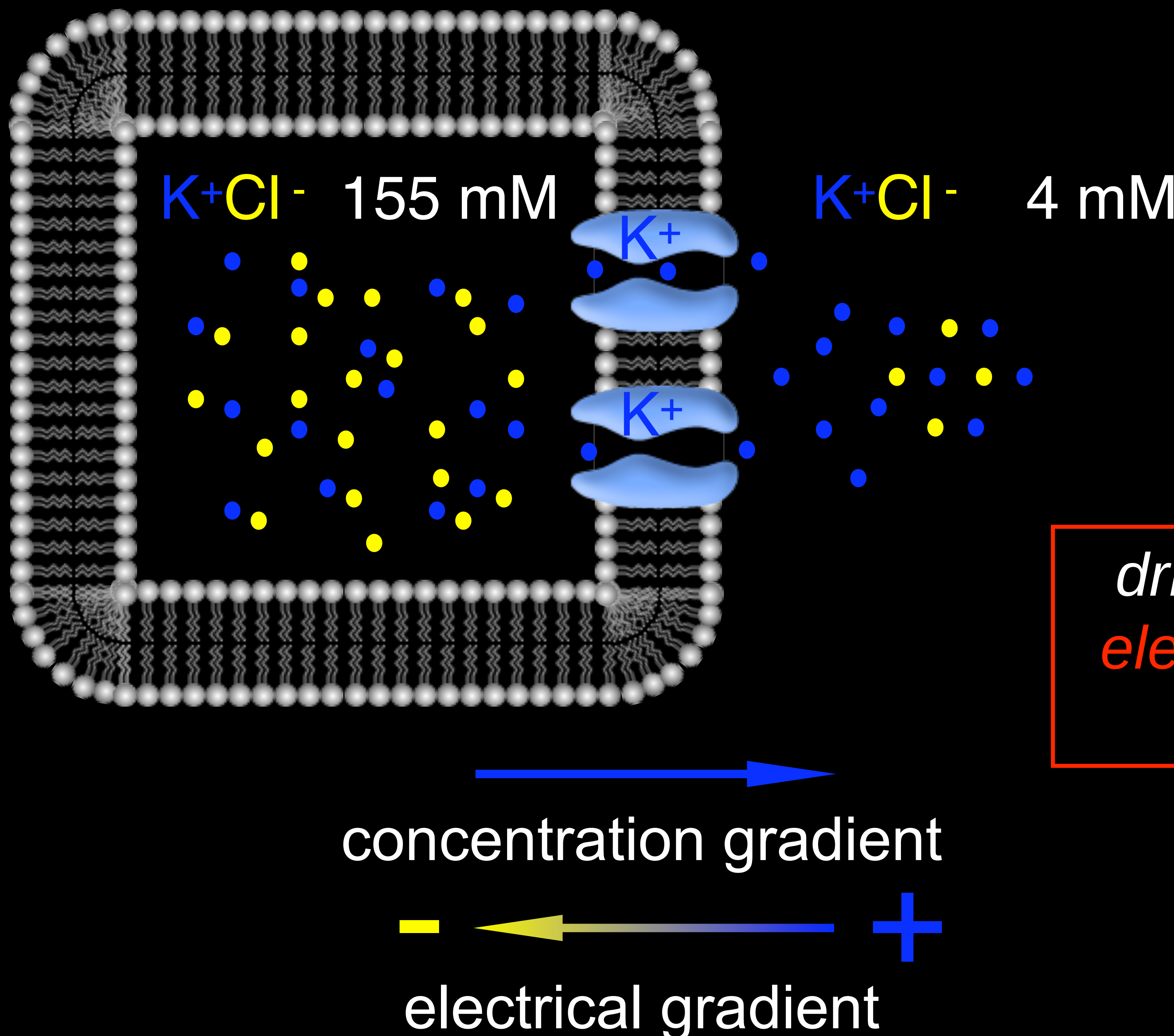
only K^+ diffusion

electric current

Ions make the Voltage Gradient

e.g. inside a mammalian muscle cell

extracellular fluid



driving force is electrochemical gradient

electrical potential is forming and ends in an equilibrium potential for K^+: E_K

Equilibrium Potential single Ions

The equilibrium potential for a single ion can be calculated using the

NERNST EQUATION

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_{out}}{[ion]_{in}}$$

R = gas constant (8.315 J/mol x K)
T = absolute temperature (310 K)
z = valence of the ion
F = Faraday constant (9.65×10^4 C/mol)
ln = natural logarithm

ion	e.g. inside a mammalian muscle cell	extracellular fluid
Na^+	12 mM	145 mM
K^+	155 mM	4 mM
Ca^{2+}	100 nM	1.5 mM
Cl^-	4 mM	123 mM

Calculation E_K by Nernst

Equilibrium Potential single Ions

The equilibrium potential for a single ion can be calculated using the

NERNST EQUATION

Walther Nernst
Noble Prize
1920

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_{out}}{[ion]_{in}}$$

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ion	e.g. inside a mammalian muscle cell	extracellular fluid	equilibrium potential at 310 K (37°C)
Na^+	12 mM	145 mM	+ 67 mV
K^+	155 mM	4 mM	- 98 mV
Ca^{2+}	100 nM	1.5 mM	+ 129 mV
Cl^-	4 mM	123 mM	- 90 mV

Resting Membrane Potential - V_M

only one ion: equilibrium potential $_{ion}$ = resting membrane potential

the sum of the equilibrium potentials of all ions species determines V_M

equilibrium potentials don't count equal: permeability for each ion is different

=> relative conductance (membrane permeability) of each ion is important

GOLDMAN (-HODGKIN-KATZ) EQUATION

$$V_M = \frac{RT}{F} \ln \frac{[Na]_o \times P_{Na} + [K]_o \times P_K + [Cl]_i \times P_{Cl}}{[Na]_i \times P_{Na} + [K]_i \times P_K + [Cl]_o \times P_{Cl}}$$

relative conductance at rest

P_K	:	P_{Na}	:	P_{Cl}
1	:	0.01	:	0.1

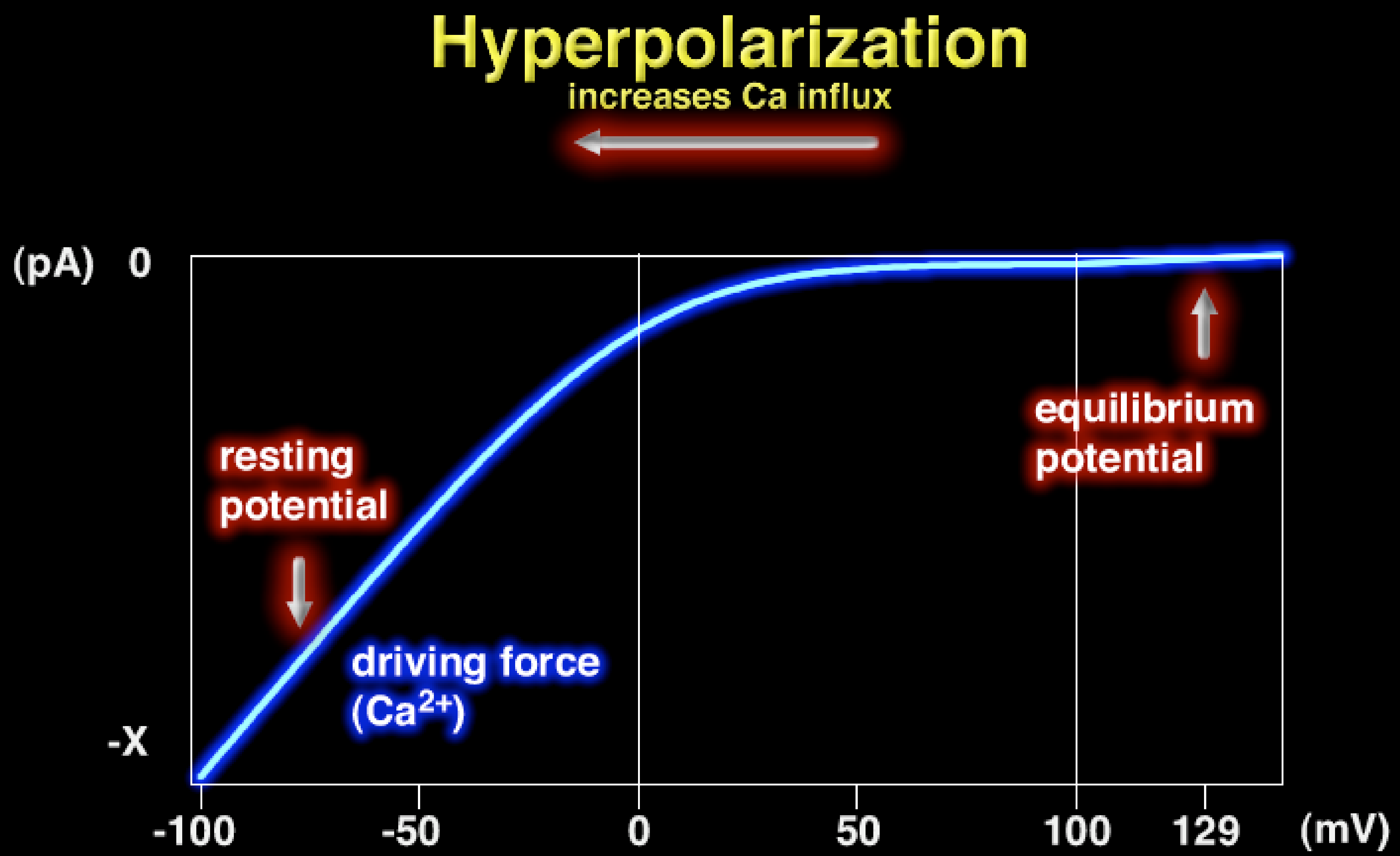
V_M close to E_K

Ionic Driving Force

the electrochemical gradient determines the driving force for an ion species

the further away the resting potential from the equilibrium potential, the stronger the electrical driving force for that ion species

driving force = $V_M - E_{ion}$



open Ca^{2+} channel

Ionic Driving Forces at Resting V_M

ion	e.g. inside a mammalian muscle cell	extracellular fluid	equilibrium potential at 310 K (37°C)
Na^+	12 mM	145 mM	+ 67 mV
K^+	155 mM	4 mM	- 98 mV
Ca^{2+}	100 nM	1.5 mM	+ 129 mV
Cl^-	4 mM	123 mM	- 90 mV

lets assume $V_M = -80$ mV

relative conductance at rest

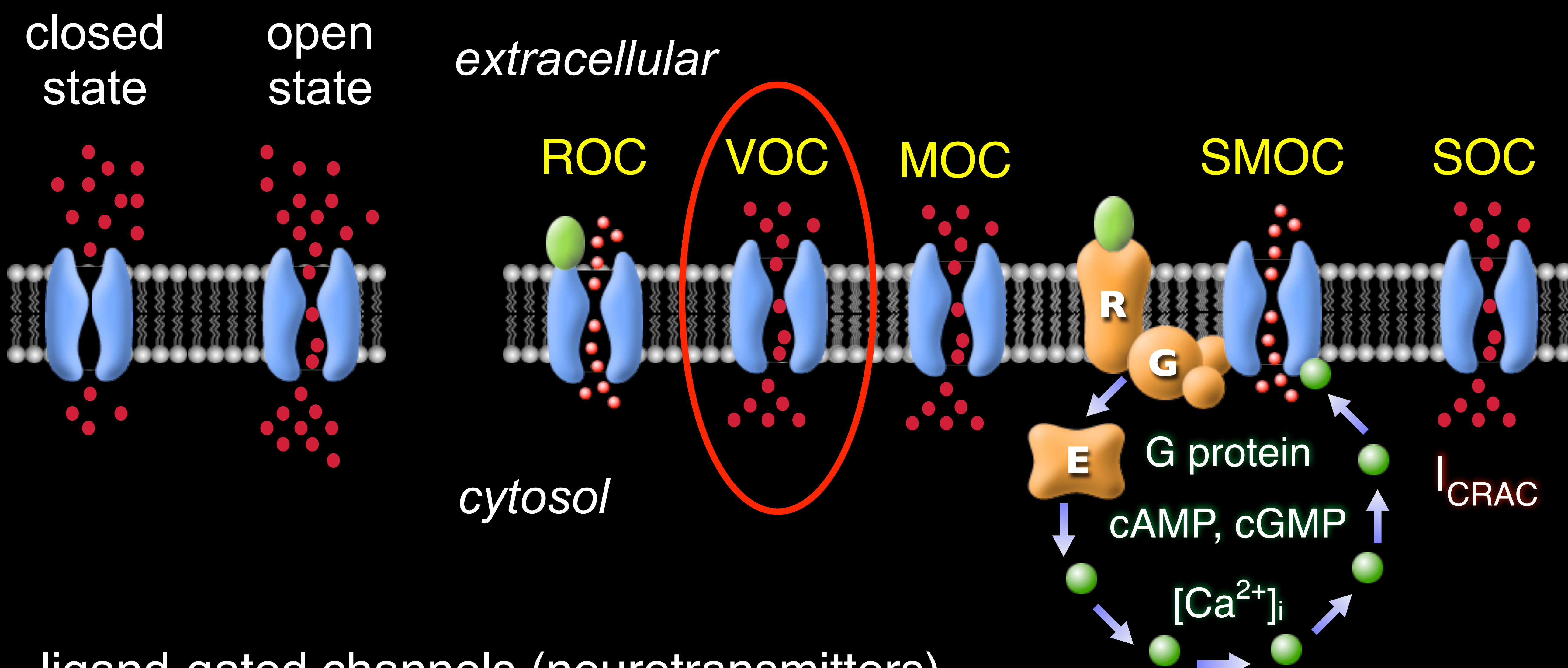
$P_K : P_{Na} : P_{Cl}$

$1 : 0.01 : 0.1$

high permeability for K^+ but low driving force

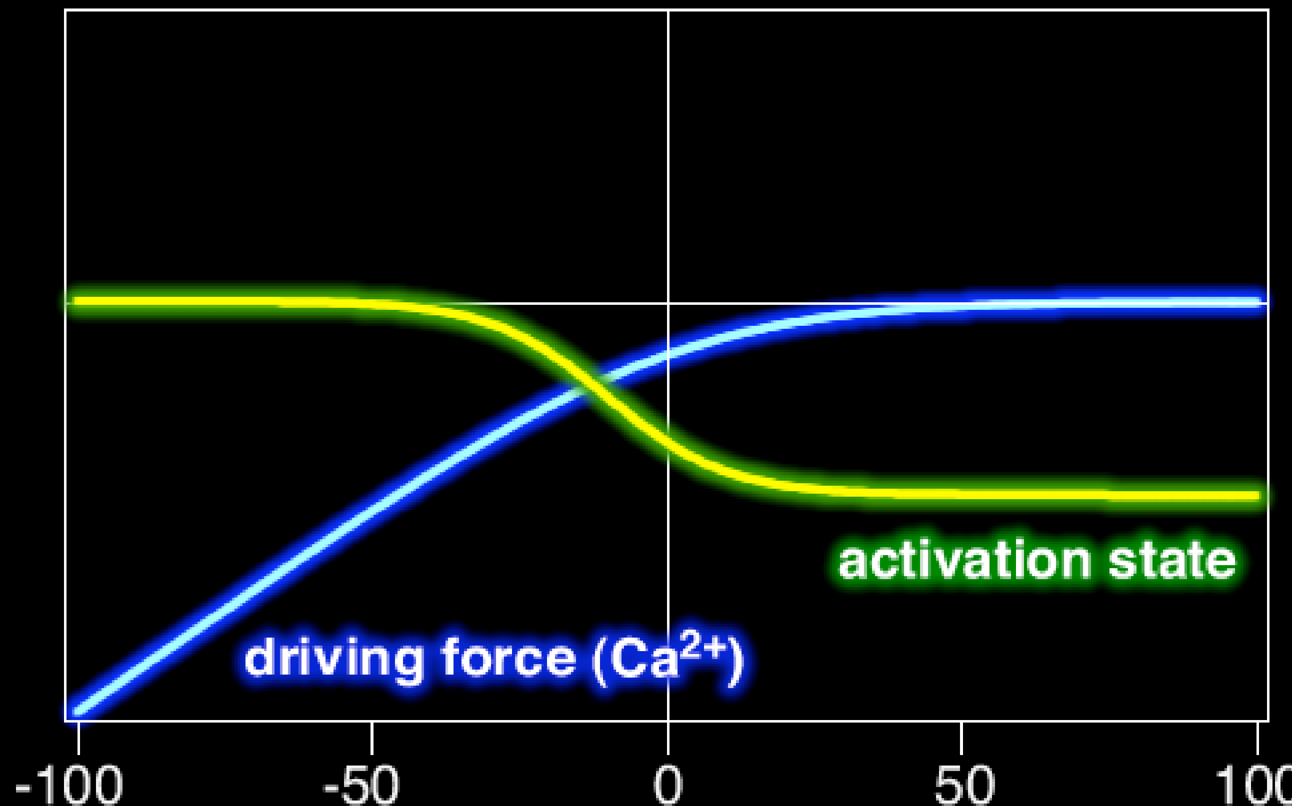
low permeability for Na^+ but high driving force

Activation of Ion Channels

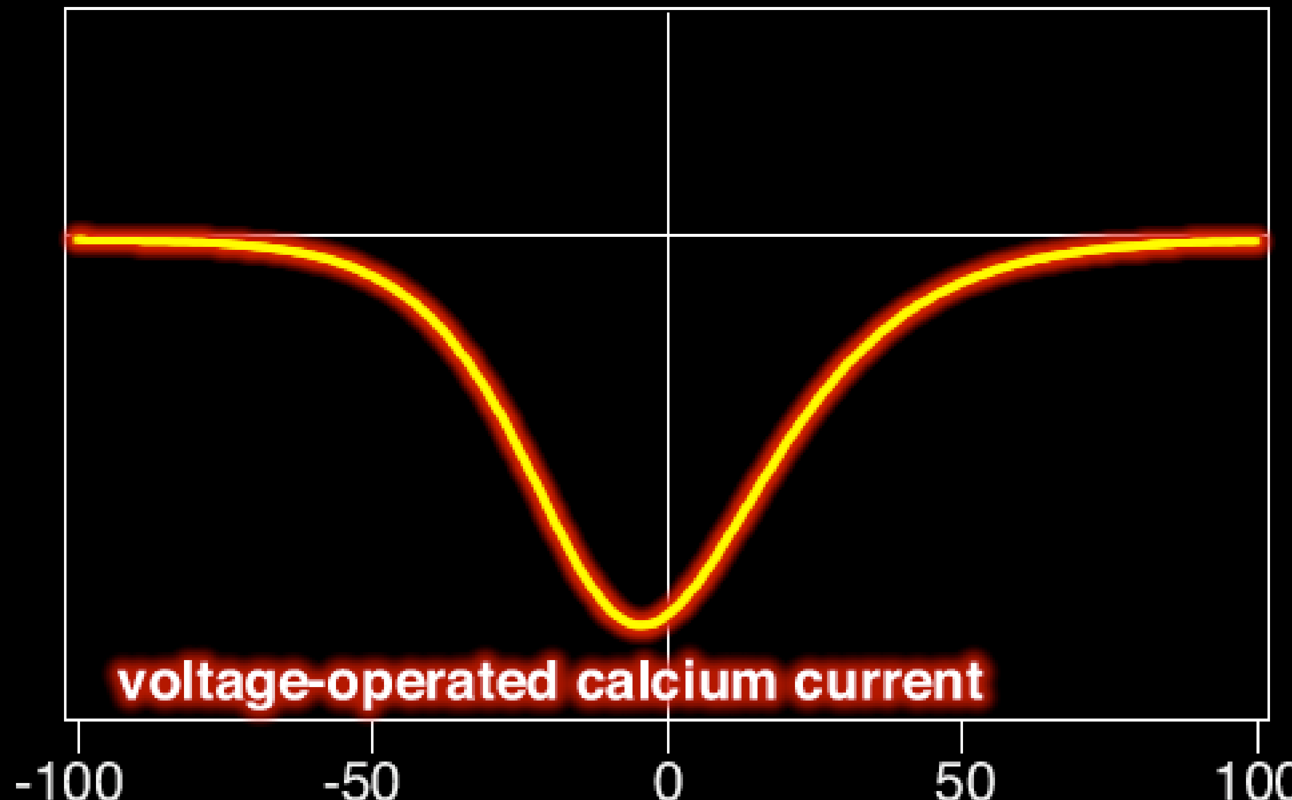


- ligand-gated channels (neurotransmitters)
- voltage-gated channels (transmembrane potential)
- mechanosensitive channels (osmotic pressure, stretching)
- second messenger-gated channels (nucleotides, G-proteins, Ca^{2+})
- store-operated (Ca^{2+}) channels
(- gap junctions (porins not gated))

Voltage-operated Calcium Channel

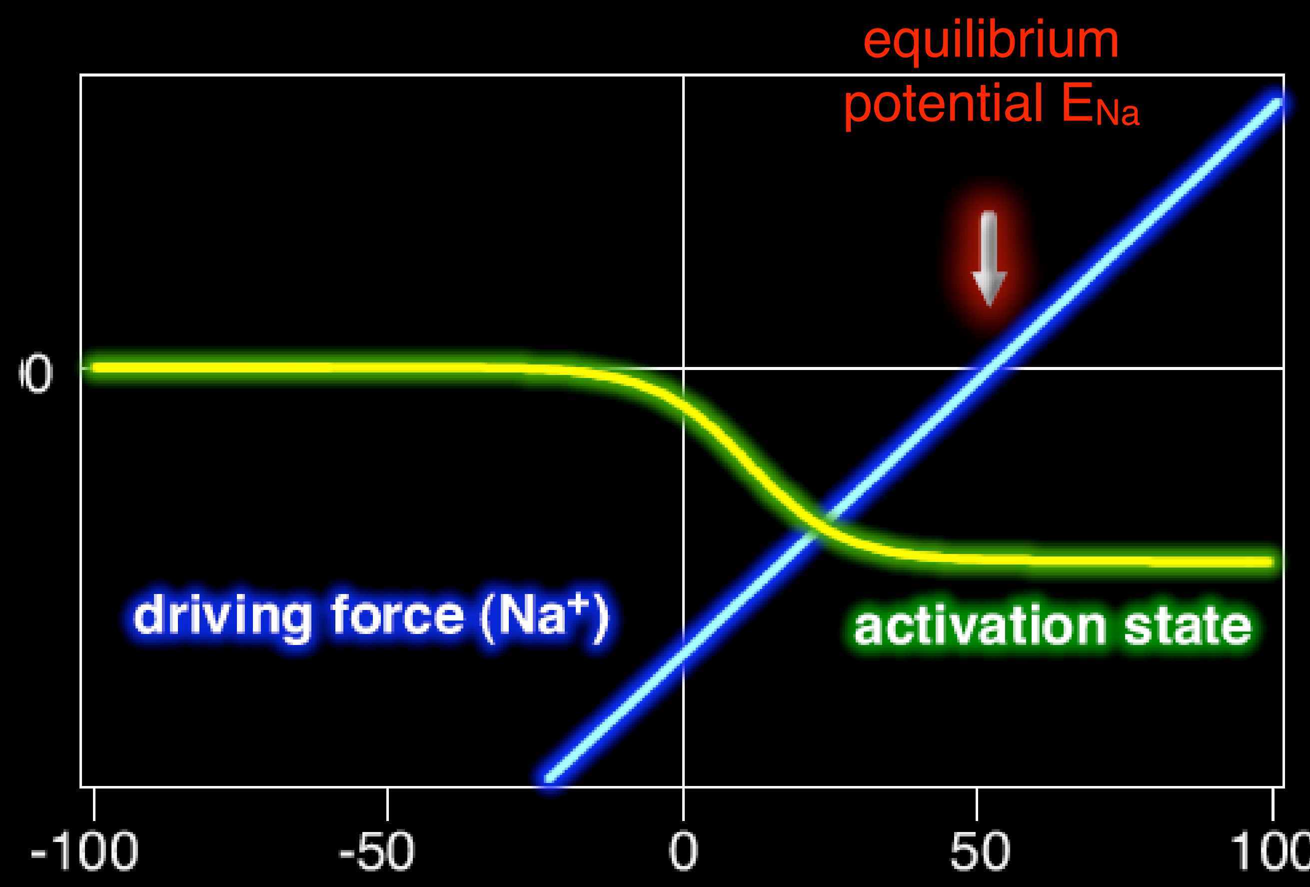


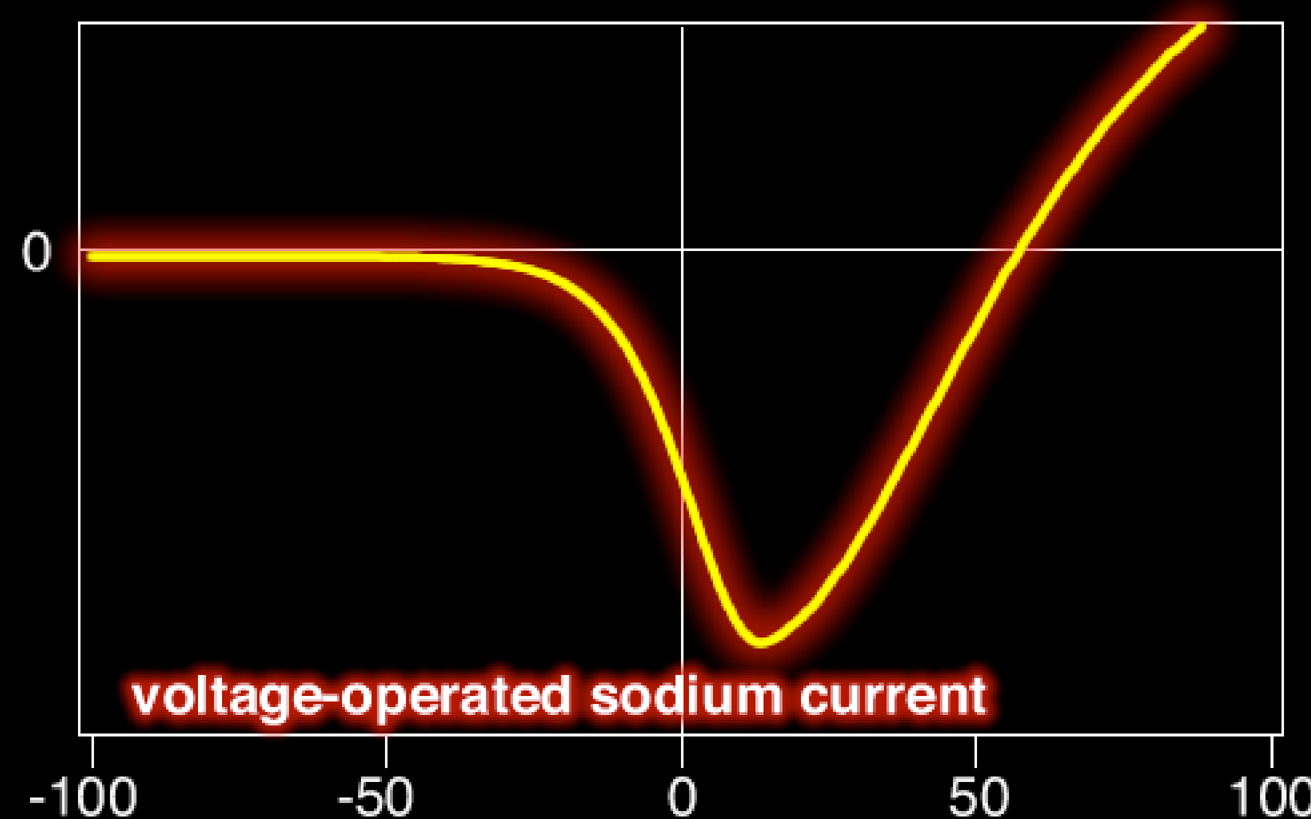
equilibrium potential E_{Ca}



typical current/voltage relationship (I/V) for a VOCC

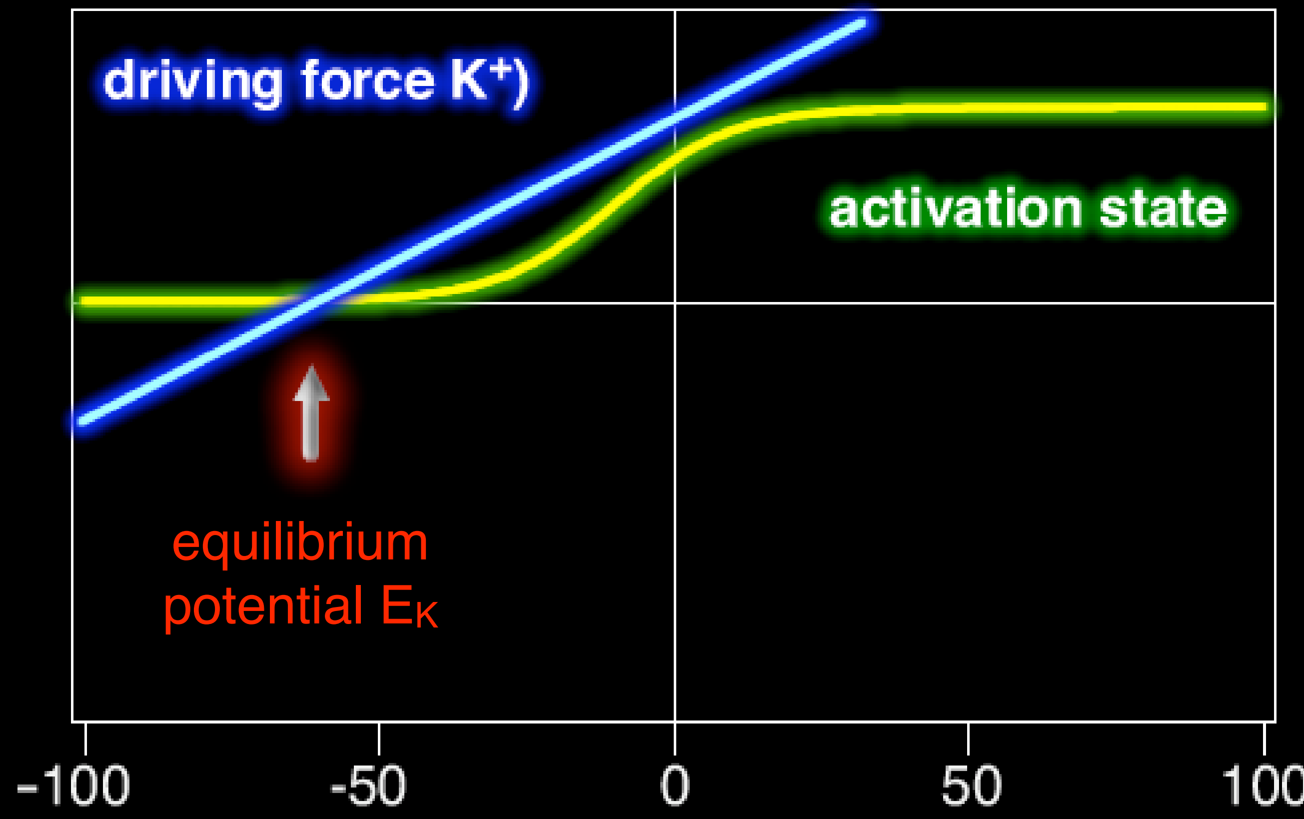
Voltage-operated Sodium channel

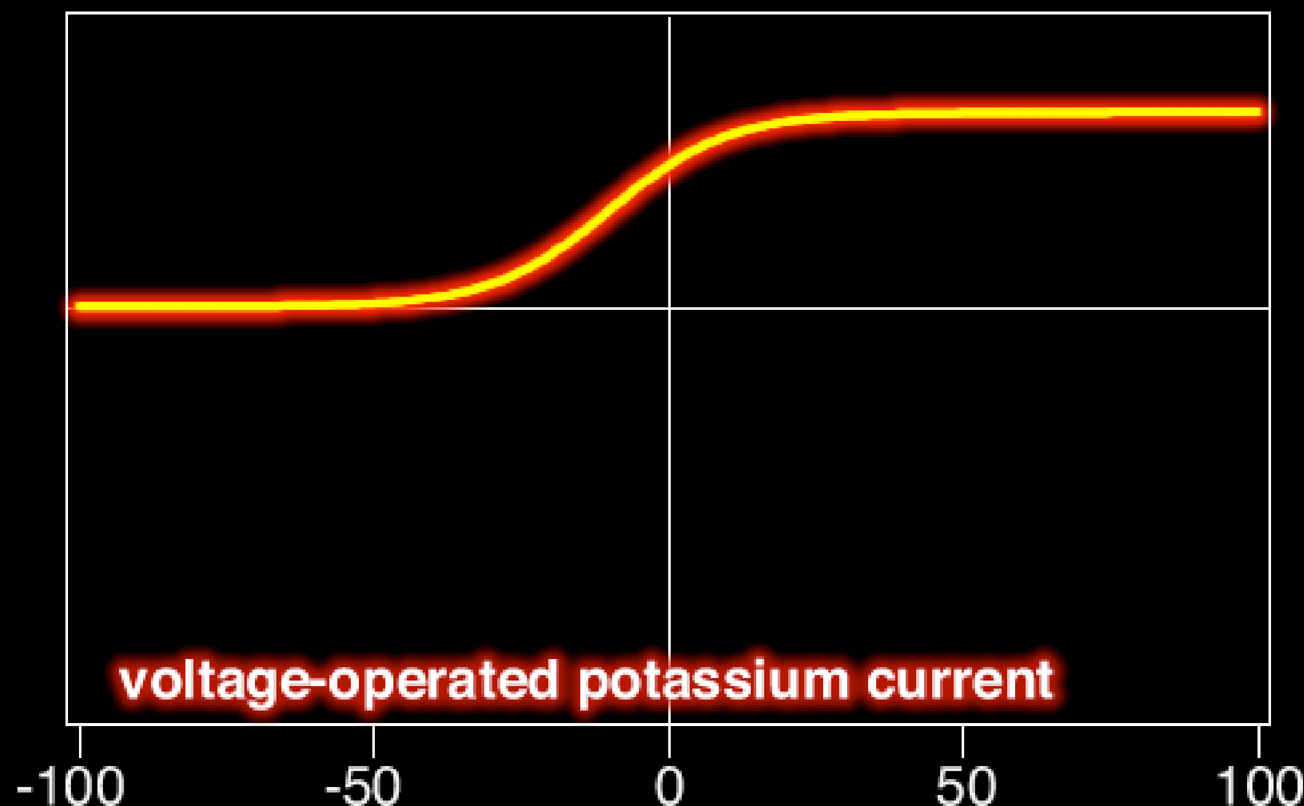




typical current/voltage relationship (I/V) for a VONaC

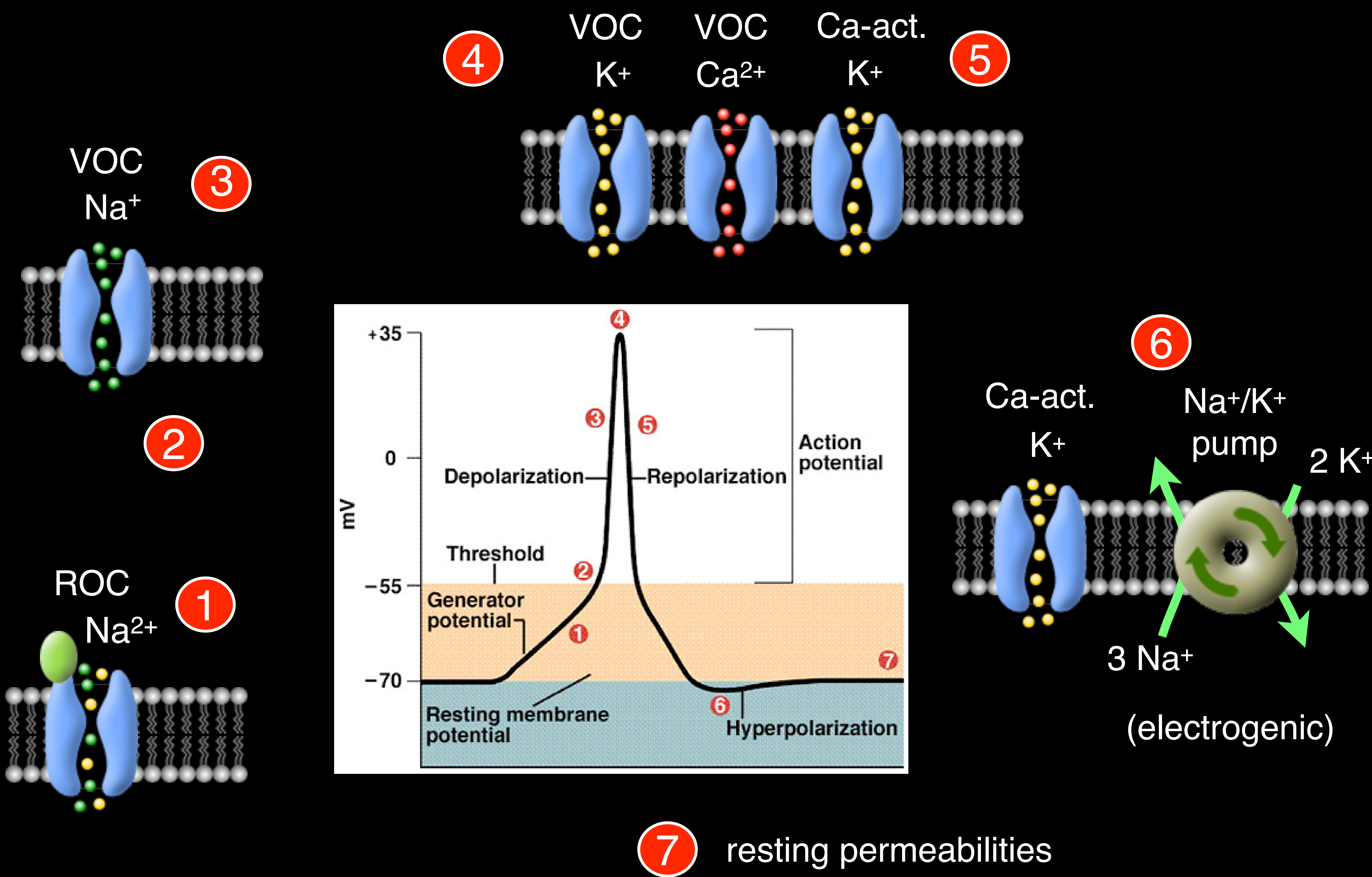
Voltage-operated Potassium Channel





typical current/voltage relationship (I/V) for a outward VOKC

Conductance Changes during an Action Potential



action potentials are ALL OR NOTHING signals

Ionic Gradient Maintenance

The active transport of ions by ion pumps against ion gradient consumes energy (ATP) or uses the chemical potential of other ions.

Na^+/K^+ pump	ATP'ase pumps 3 Na^+ out and 2 K^+ in (electrogenic) maintains Na^+ and K^+ gradients
Na^+/Ca^{2+} exchanger	shuffles 1 Ca^{2+} out in exchange of 3 Na^+ in uses Na^+ gradient keeps cytosolic $[Ca^{2+}]$ low
Ca^{2+} pump	ATP'ase, requires Mg^{2+} as cofactor drives Ca^{2+} into the endoplasmic reticulum keeps cytosolic $[Ca^{2+}]$ low
Bicarbonate$^-$/Cl^- exchanger	pumps HCO_3^- in and Cl^- out driven by Na^+ influx keeps cytosolic Cl^- low and internal pH high
$Cl^-/Na^+/K^+$ cotransporter	transports 1 Na^+, 1 K^+ and 2 Cl^- into the cell driven by Na^+ influx

Jens Skou
Noble Prize 1997

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