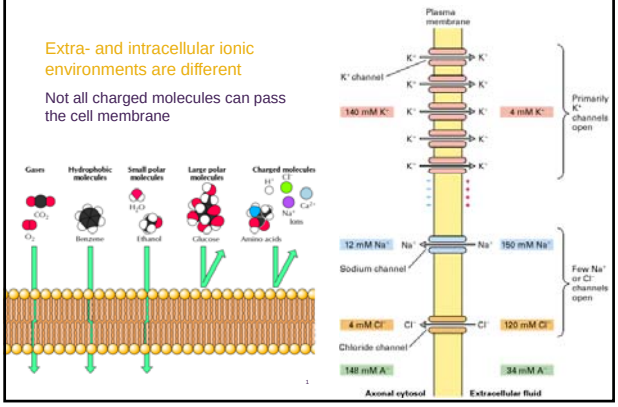
Background – membrane potential

Extra- and intracellular ionic environments are different

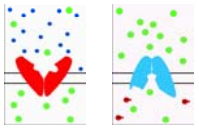
Not all charged molecules can pass the cell membrane



Background – ion movement

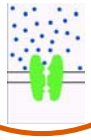
Transporters & Pumps

- slow: ~10^3 ions/sec
- active (Energy source required)



Ion-Channels

- fast: ~10^7 ion/s
- passive



Ion movement in ion channels follows the electrochemical gradient of the individual ion

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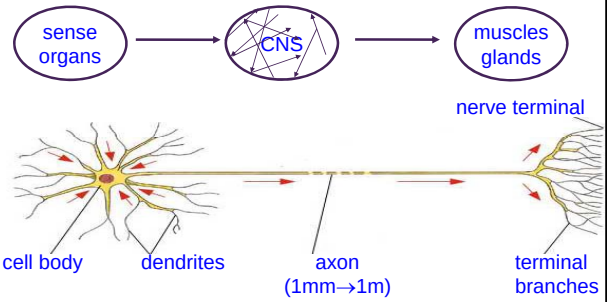
Background – bioelectricity



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Hodgkin, Huxley and the Squid

Hodgkin

Huxley

Squid

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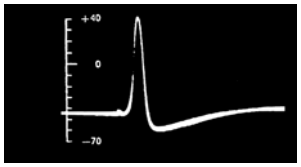
Action potential



Hodgkin and Huxley's recording of an action potential in 1939

Using technology developed by Cole and Curtis for measuring and controlling voltage and current (voltage/current clamp)

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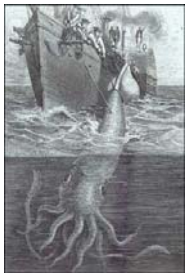
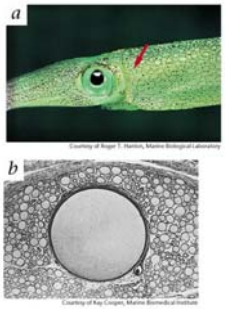
Not the Giant Squid

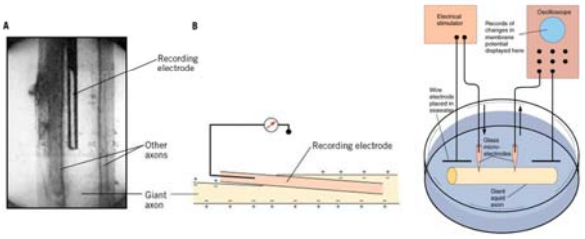


But the Squid *giant* axon

0.5 – 1 mm diameter



Experimental setup

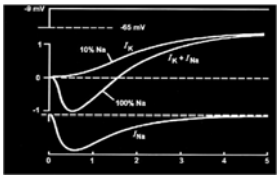


What is going on?

- Membrane capacitance was measured (Cole, Curtis with Wheatstone Bridge)
- Membrane conductance was measured (Cole, Curtis)
- Membrane potential was known (Cole, Curtis, Goldman, Hodgkin, Katz, Huxley and Nernst)
- But how can a membrane suddenly "break down" (Bernstein) in a unidirectional systematic way?
- And even generate a positive overshoot?

The sodium (and potassium) hypothesi(s)

- The action potential is a regenerative wave of Na^+ permeability increase



- Followed by another transient K^+ permeability increase

Einstein, Faraday, Nernst, Goldman, Hodgkin and Katz

$$M_s = -D_s\frac{dc_s}{dx} - u_s c_s \frac{d\psi}{dx}$$ Diffusion + electrophoresis

Using Nernst-Einstein: $D_s = \frac{u_s RT}{z_s F}$ and Faraday's constant

$I_s = M_s z_s F$ In combination, yields the Nernst-Planck:

$$I_s = -z_s F D_s \left[\frac{dc_s}{dx} + \frac{z_s F}{RT} c_s \frac{d\psi}{dx}\right]$$

Einstein, Faraday, Nernst, Goldman, Hodgkin and Katz II

Solving for equilibrium when: $I_s = 0$

And intregrating along the x-axis (across the membrane) we get:

$$\psi_i - \psi_o = \frac{RT}{z_s F}\ln\frac{[s]_o}{[s]_i}$$ The Nernst Equation

Or as it is most often written: $E_x = \frac{RT}{zF}\ln\frac{[X]_o}{[X]_i}$

R is the gasconstant, *T* absolute temperature

Einstein, Faraday, Nernst, Goldman, Hodgkin and Katz III

The Nernst Equation is valid at *equilibrium* (ie. the *reversal potential*), in "not to high" molality solutions

Ion	Intracellular conc (mM)	Extracellular conc (mM)
Na^+	10	137
K^+	135	2
Cl^-	11	143

RT/*zF* at 20º C = 25.3 mV/*z*

The individual reversal potentials for the main charge carriers are thus:

$E_{rev(Na)}$= 25.3*ln*(137/10) = 66.2 mV

$E_{rev(K)}$= 25.3*ln*(2/135) = -106.6 mV

$E_{rev(Cl)}$= -25.3*ln*(143/11) = -64.9 mV

Einstein, Faraday, Nernst, Goldman, Hodgkin and Katz IV

But how does 66.2 –106.6 – 64.9 = -68.6 ??

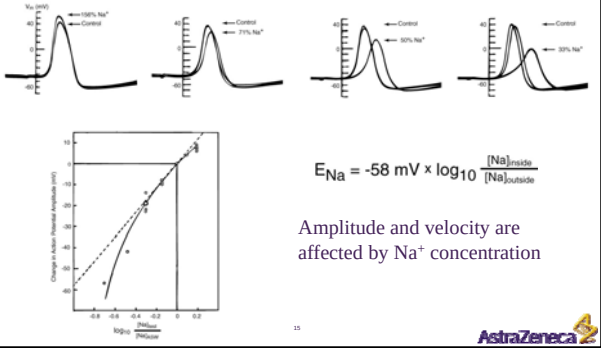
What's missing is the *relative permeabilities* of the main charge carriers. This is included in the "big" Nernst equation, conceived by Goldman, Hodgkin and Katz back in the 1950'ies:

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}$$

At rest: P_K:P_{Na}:P_{Cl} = 1:0.05:0.1 Thus with the model cell:

$$Vm = 25.3 \ln\left(\frac{1\cdot 2 + 0.05\cdot 137 + 0.1\cdot 11}{1\cdot 135 + 0.05\cdot 10 + 0.1\cdot 143}\right) mV = -68.6 mV$$

Help from Nernst



$$E_{Na} = -58 \text{ mV} \times \log_{10} \frac{[Na]_{inside}}{[Na]_{outside}}$$

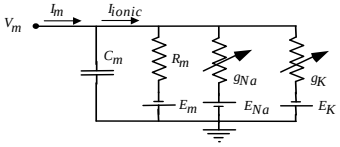
Amplitude and velocity are affected by Na^+ concentration

Assumptions

$$I_m(t) = I_{ionic}(t) + C_m \frac{dV_m}{dt}$$

$$I_{ionic} = I_{Na} + I_K + I_{leak}$$

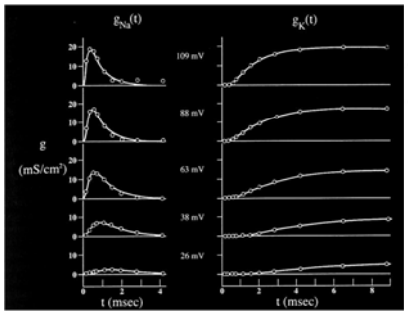
$$I_{Na}(t) = g_{Na}(V(t),t)\cdot(V(t) - E_{Na})$$



Equivalent circuit

$$E_{rest} = \frac{RT}{F} \ln \frac{P_{Na}[Na^+]_o + P_K[K^+]_o}{P_{Na}[Na^+]_i + P_K[K^+]_i}$$

Conductance is voltage dependent



$I_{Na} = g_{Na} (V - E_{Na})$

$I_K = g_K (V - E_K)$

And the "gating particles" h, m and n

$g_{Na} = \bar{g}_{Na} m^3 h$

$g_K = \bar{g}_K n^4$

Solving for time

activation

$$\frac{dm}{dt} = \frac{m_\infty - m}{\tau_m}$$

inactivation

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h}$$

non-inactivating

$$\frac{dn}{dt} = \frac{n_\infty - n}{\tau_n}$$

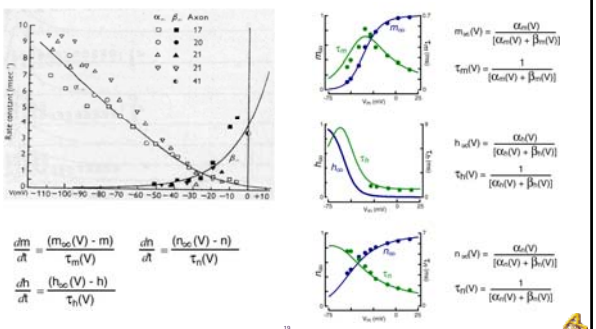
General solution

$$n(t) = n_\infty - (n_\infty - n_0)e^{\frac{-t}{\tau_n}}$$

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

With parameters α and β as a function of voltage

Then just measure…



$$\frac{dm}{dt} = \frac{(m_\infty(V) - m)}{\tau_m(V)} \qquad \frac{dh}{dt} = \frac{(h_\infty(V) - h)}{\tau_h(V)} \qquad \frac{dn}{dt} = \frac{(n_\infty(V) - n)}{\tau_n(V)}$$

And they found

$\bar{g}_{Na} = 120\ \text{mS/cm}^2$

$$\alpha_m(V) = \frac{25 - V}{10 \cdot \left(e^{(25-V)/10} - 1\right)} \qquad \alpha_h(V) = 0.07 \cdot e^{-V/20}$$

$$\beta_m(V) = 4 \cdot e^{-V/18} \qquad \beta_h(V) = \frac{1}{e^{(30-V)/10} + 1}$$

$\bar{g}_K = 36\ \text{mS/cm}^2$

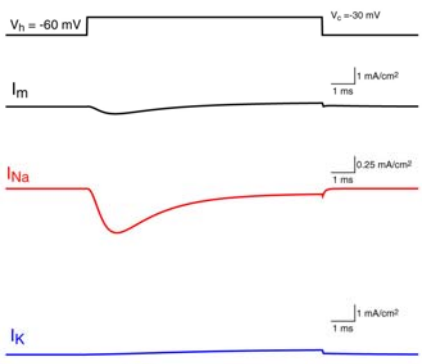
$$\alpha_n(V) = \frac{10 - V}{100 \cdot \left(e^{(10-V)/10} - 1\right)}$$

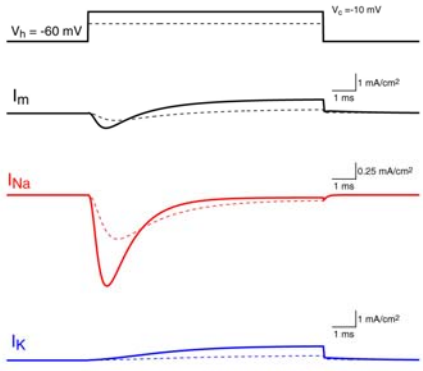
$$\beta(V) = 0.125 \cdot e^{-V/80}$$

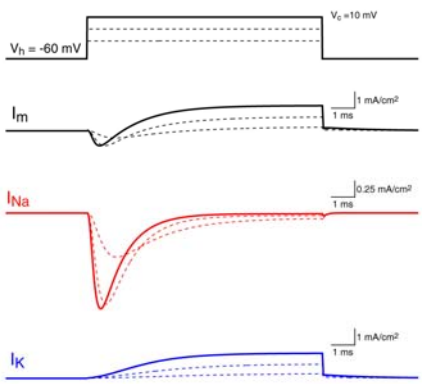
Entered in to the equations:

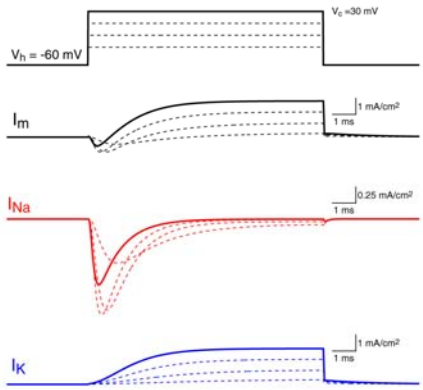
$g_{Na} = \bar{g}_{Na} m^3 h \qquad I_{Na} = g_{Na} (V - E_{Na})$

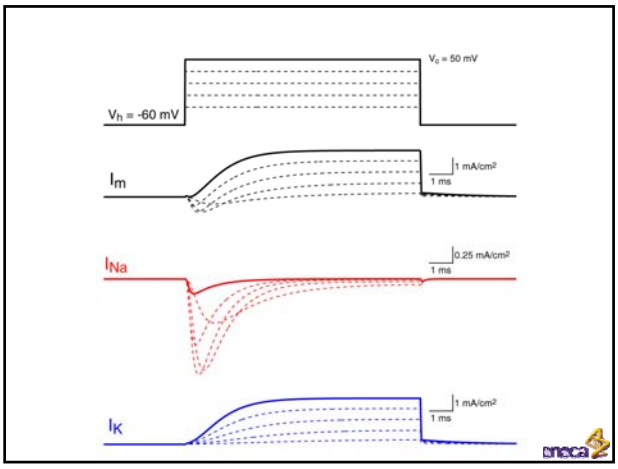
$g_K = \bar{g}_K n^4 \qquad I_K = g_K (V - E_K)$

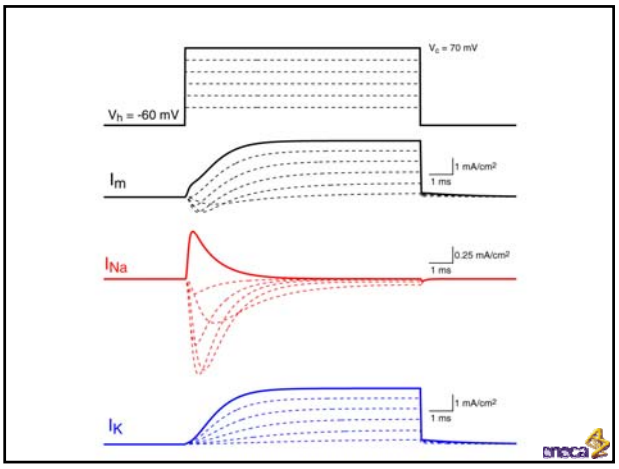






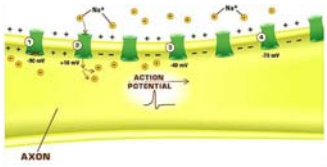






The Hodgkin-Huxley model

- Describes (and explains) the actionpotential
- Explains the threshold for spike initiation
- Explains the refractory period



- And for their efforts they got the 1963 Nobel Prize in Medicine

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The Technique

- In 1981 a paper came out in Pflügers Archive: "Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches" by Hamill, Marty, Neher, Sakmann and Sigworth...

- Leading to Neher and Sakmann receiving the Nobel Prize in 1991 for their discoveries concerning the function of single ion channels in cells

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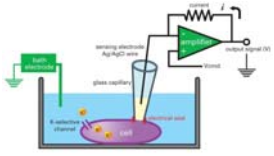
Rigs – The technique

Conventional patch clamp



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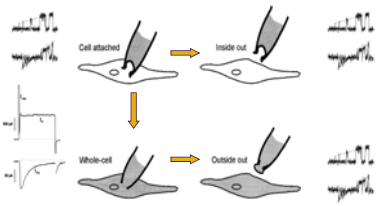
The patch clamp technique

- Small pipette (tip diameter ~1 μm) with salt solution. Gently pushed on to the cell membrane with slight positive pressure (no impalement)
- Slight negative pressure in pipette creates high-resistance seal (> GΩ) between membrane and glass (also mechanically stable)
- The giga-seal allows the measurement of very small currents (pA; i.e. single-channel level)



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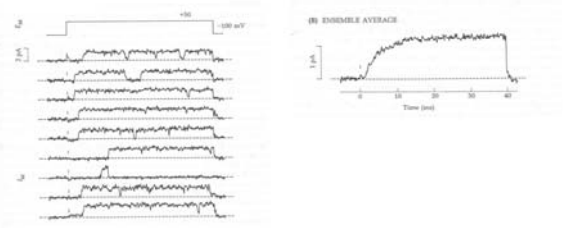
The patch clamp technique

- Enables recordings of single ion channels – or whole cell currents
- Measurement of the protein function – as it functions



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Voltage Clamping

- Three principles:
  - Injecting positive current into the cell depolarizes it, - injecting negative current hyperpolarizes it
  - When current is injected into the cell, it takes some time to hyperpolarize/depolarize the cell because the cell's capacitance must be charged/discharged
  - When there is no net flow of ions into the cell, the membrane potential doesn't change

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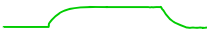
Voltage Clamping

Current injection and voltage responses:

Positive current injection (I)



Depolarised voltage response (V)

Negative current injection (I)

Hyperpolarised voltage response (V)

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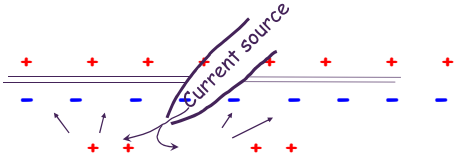
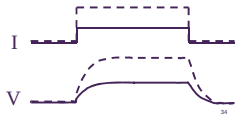
Voltage Clamping

Current injection and voltage responses:





$$V = V_0 \exp(-t/\tau)$$

$$\tau = R_s C$$

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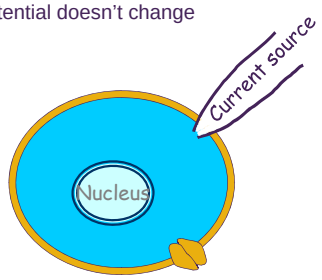
Voltage Clamping

When there is no net flow of ions into the cell, the membrane potential doesn't change



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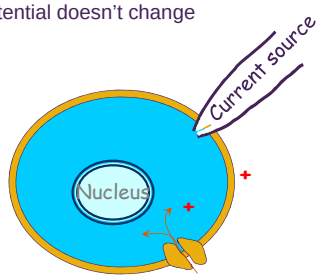
Voltage Clamping

When there is no net flow of ions into the cell, the membrane potential doesn't change



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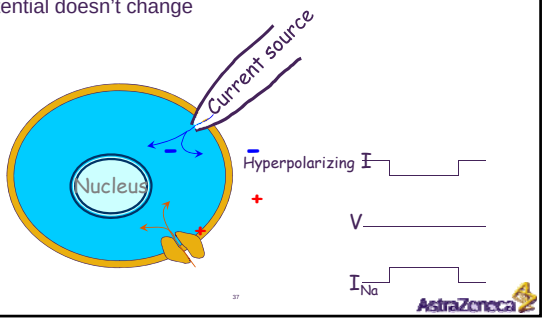
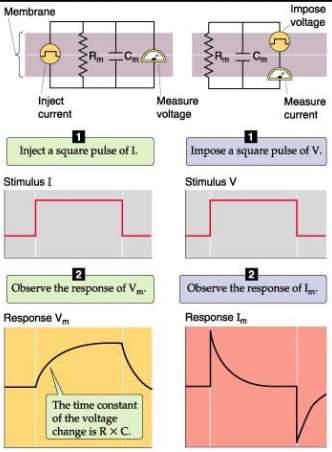
Voltage Clamping

When there is no net flow of ions into the cell, the membrane potential doesn't change



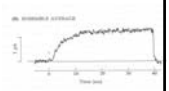


Command the voltage, record the current = Voltage clamp

Command the current, record the voltage = Current clamp

Whole-cell current responses & underlying parameters

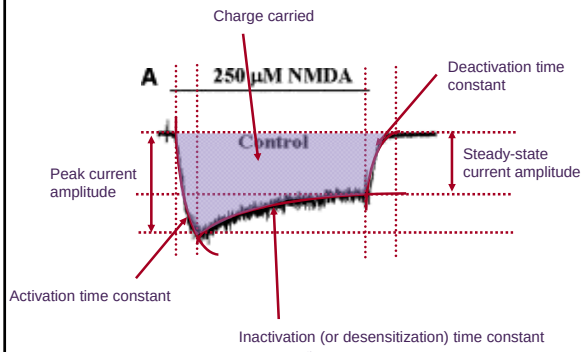
$I_{whole\text{-}cell} = n * i_{single\text{-}channel} * P_o * MOT$



$I_{whole\text{-}cell}$: total whole-cell current
n: Number of available channels
MOT: mean open time
average time a single channel stays open after opening
$i_{single\text{-}channel}$: single-channel current amplitude
Po: open probability
likelyhood that a channel will open
(intrinsic (after agonist binding) and extrinsic (agonist concentration) parameter)

- All of these parameters can be dynamic during recording
- All of these parameters could be changed by compounds

Some of the things one can measure on a whole-cell response

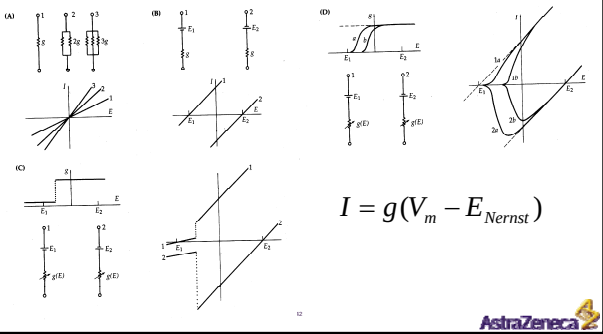


I-V curves

- Aka Current-Voltage curves
- Prerequisites: Ohm's law (and Nernst (GHK-eq possibly))
- The "fingerprint" of an ion channel
- Of high value for both voltage- and ligand gated channels

I-V curves II

Hille:

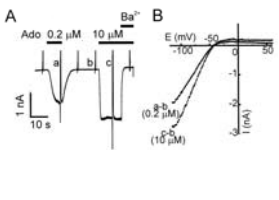


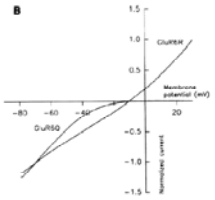
$$I = g(V_m - E_{Nernst})$$

I-V curves III

GIRK (Kir2.x)

Glutamate receptor





Inward rectification

Inward/no rectification

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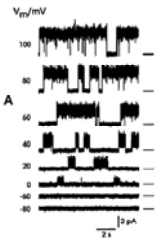
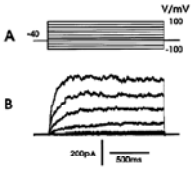
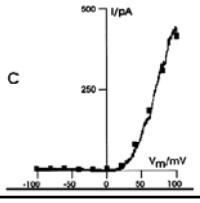
I-V Curves IV



Single channel



WCR



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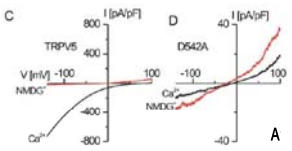
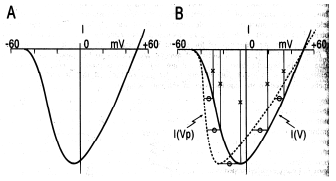
I-V Curves V



TRPV5, normalized to capacitance…



NaV with series resistance error…

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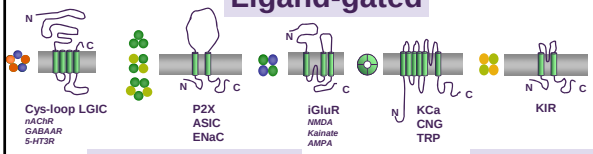
Families of ion channels

Ligand-gated



Cys-loop LGIC
nAChR
GABAAR
5-HT3R

P2X
ASIC
ENaC

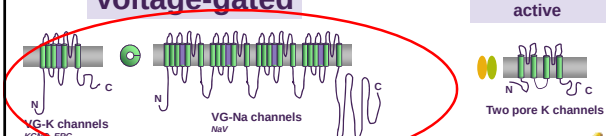
iGluR
NMDA
Kainate
AMPA

KCa
CNG
TRP

KIR

Voltage-gated

Constitutively active



VG-K channels
KCNQ, ERG

VG-Na channels
NaV

VG-Ca channels
CaV

Two pore K channels

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VDPC's

Delayed rectifier

slowly inactivating or non-inactivating

- K_vα1.x - Shaker-related: K_v1.1 (KCNA1), K_v1.2 (KCNA2), K_v1.3 (KCNA3), K_v1.5 (KCNA5), K_v1.6 (KCNA6), K_v1.7 (KCNA7), K_v1.8 (KCNA10)
- K_vα2.x - Shab-related: K_v2.1 (KCNB1), K_v2.2 (KCNB2)
- K_vα3.x - Shaw-related: K_v3.1 (KCNC1), K_v3.2 (KCNC2)
- K_vα7.x: K_v7.1 (KCNQ1) - KvLQT1, K_v7.2 (KCNQ2), K_v7.3 (KCNQ3), K_v7.4 (KCNQ4), K_v7.5 (KCNQ5)
- K_vα10.x: K_v10.1 (KCNH1)

A-type potassium channel

rapidly inactivating

- K_vα1.x - Shaker-related: K_v1.4 (KCNA4)
- K_vα3.x - Shaw-related: K_v3.3 (KCNC3), K_v3.4 (KCNC4)
- K_vα4.x - Shal-related: K_v4.1 (KCND1), K_v4.2 (KCND2), K_v4.3 (KCND3)

Outward-rectifying

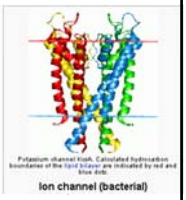
- K_vα10.x: K_v10.2 (KCNH5)

Inward-rectifying

- K_vα11.x - ether-a-go-go potassium channels: K_v11.1 (KCNH2) - hERG, K_v11.2 (KCNH6), K_v11.3 (KCNH7)

Slowly activating

- K_vα12.x: K_v12.1 (KCNH8), K_v12.2 (KCNH3), K_v12.3 (KCNH4)



ion channel (bacterial)

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VDCC's

Current Type	1,4-dihydropyridine sensitivity (DHP)	ω-conotoxin sensitivity (ω-CTX)	ω-agatoxin sensitivity (ω-AGA)
L-type	blocks	resistant	resistant
N-type	resistant	blocks	resistant
P/Q-type	resistant	resistant	blocks
R-type	resistant	resistant	resistant

Type	Voltage	α_1 subunit (gene name)	Associated subunits	Most often found in
L-type calcium channel ("Long-Lasting" AKA "DHP Receptor")	HVA (high voltage activated)	$Ca_v1.1$ (CACNA1S), $Ca_v1.2$ (CACNA1C), $Ca_v1.3$ (CACNA1D), $Ca_v1.4$ (CACNA1F)	$\alpha_2\delta$, β, γ	Skeletal muscle, bone (osteoblasts), ventricular myocytes** (responsible for prolonged action potential in cardiac cell; also termed DHP receptors), dendrites and dendritic spines of cortical neurones
P-type calcium channel ("Purkinje") /Q-type calcium channel	HVA (high voltage activated)	$Ca_v2.1$ (CACNA1A)	$\alpha_2\delta$, β, possibly γ	Purkinje neurons in the cerebellum / Cerebellar granule cells
N-type calcium channel ("Neural")	HVA (high-voltage-activated)	$Ca_v2.2$ (CACNA1B)	$\alpha_2\delta$, β_1, β_2, β_4, possibly γ	Throughout the brain and peripheral nervous system
R-type calcium channel ("Residual")	intermediate-voltage-activated	$Ca_v2.3$ (CACNA1E)	$\alpha_2\delta$, β, possibly γ	Cerebellar granule cells, other neurons
T-type calcium channel ("Transient")	low-voltage-activated	$Ca_v3.1$ (CACNA1G), $Ca_v3.2$ (CACNA1H), $Ca_v3.3$ (CACNA1I)		neurons, cells that have pacemaker activity, bone (osteocytes)

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VDSC's

Protein name	Gene	Expression profile	Associated human channelopathies
$Na_v1.1$	SCN1A	Central neurons, [Peripheral Neurons] and cardiac myocytes	Inherited febrile epilepsy, GEFS and myoclonic epilepsy
$Na_v1.2$	SCN2A	Central neurons, peripheral neurons	inherited febrile seizures and epilepsy
$Na_v1.3$	SCN3A	Central neurons, peripheral neurons and cardiac myocytes	none known
$Na_v1.4$	SCN4A	Skeletal muscle	hyperkalemic periodic paralysis, paramyotonia congenita, and potassium-aggravated myotonia
$Na_v1.5$	SCN5A	Cardiac myocytes, uninnervated skeletal muscle, central neurons	Long QT syndrome, Brugada syndrome, and idiopathic ventricular fibrillation
$Na_v1.6$	SCN8A	Central neurons, dorsal root ganglia, peripheral neurons, heart	none known
$Na_v1.7$	SCN9A	Dorsal root ganglia, sympathetic neurons, Schwann cells, and neuroendocrine cells	erythromelalgia, PEPD and channelopathy-associated insensitivity to pain
$Na_v1.8$	SCN10A	Dorsal root ganglia	none known
$Na_v1.9$	SCN11A	Dorsal root ganglia	none known
Nax	SCN6A,SCN7A	heart, uterus, skeletal muscle, astrocytes, dorsal root ganglion cells	none known

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Calcium Channels

- Voltage Dependent
  - Slow, fast
- Intracellular Ca2+ channel

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Receptor-operated Ca^{2+} channels

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Ca^{2+} Channels

- Receptor-operated Ca^{2+} channels
  - Nicotinic cholinergic receptor
  - NMDA receptor ion channel
  - Purinergic receptor P2X

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Voltage-gated Ca^{2+} channels

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Gene Superfamily of Voltage-Gated Ion Channels

- Voltage-gated Na^+ channels
- Voltage-gated K^+ channels
- Voltage-gated Ca^{2+} channels

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Distinct classes of Ca^{2+} curents

- L-type
  - High activation voltage
  - **L**arge conductance
  - **L**ong lasting
  - Blocked by dihydropyridine, phenylalkylamine, benzothiazepine

Other high-voltage-activated Ca^{2+} channels

- N-type
  - **N**euronal
- P/Q-type
- R-type

Not blocked by DHPs, blocked by polypeptide toxins

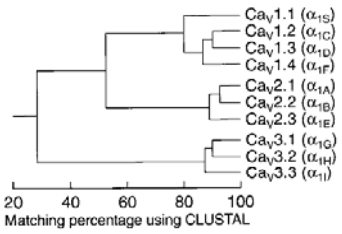
Low-voltage activated Ca^{2+} current

- T-type
  - **T**iny conductance
  - **T**ransient current
  -

Nomenclature

- Ion conducted
- Main regularor
- Alpha-1 subunit gene family

Cav1.1



EA Ertel *Neuron* 25:533, 2000.

Molecules and curents

- Cav1.1
- Cav1.2
- Cav1.3
- Cav1.4
- Cav2.1
- Cav2.2
- Cav2.3
- Cav3.1
- Cav3.2
- Cav3.3

- L-type
- P/Q-type
- N-type
- R-type
- T-type

Blockers

Cav1.1	DHP
Cav1.2	DHP
Cav1.3	DHP
Cav1.4	Not known
Cav2.1	ω-Agatoxin IVA
Cav2.2	Conotoxin
Cav2.3	None
Cav3.1	None
Cav3.2	None
Cav3.3	None

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