ION CHANNEL DIVERSITY and CHARACTERIZATION

Voltage clamp techniques
K channels
Na channels
Ca channels
Ligand-gated channels
Channelopathies

OUTLINE

Voltage clamp techniques

whole cell, single channel, gating

K channels

Na channels

Ca channels

Cardiac AP

Na nerve vs cardiac

Ica: L vs T type; drugs (BayK, nitrendipine)

Ito: inactivation, subtypes Kv1.4, Kv4.2/3, accessory subunits

Ikr,Iks, Ikur (drugs dofetilide)

IK1 – rectification

Cardiac channelopathies (LQTS, SQTS, Brugada syndrome)

Ligand-gated channels (AChR)

Pancreatic beta cell channels (KATP, ICa)

Voltage clamp techniques

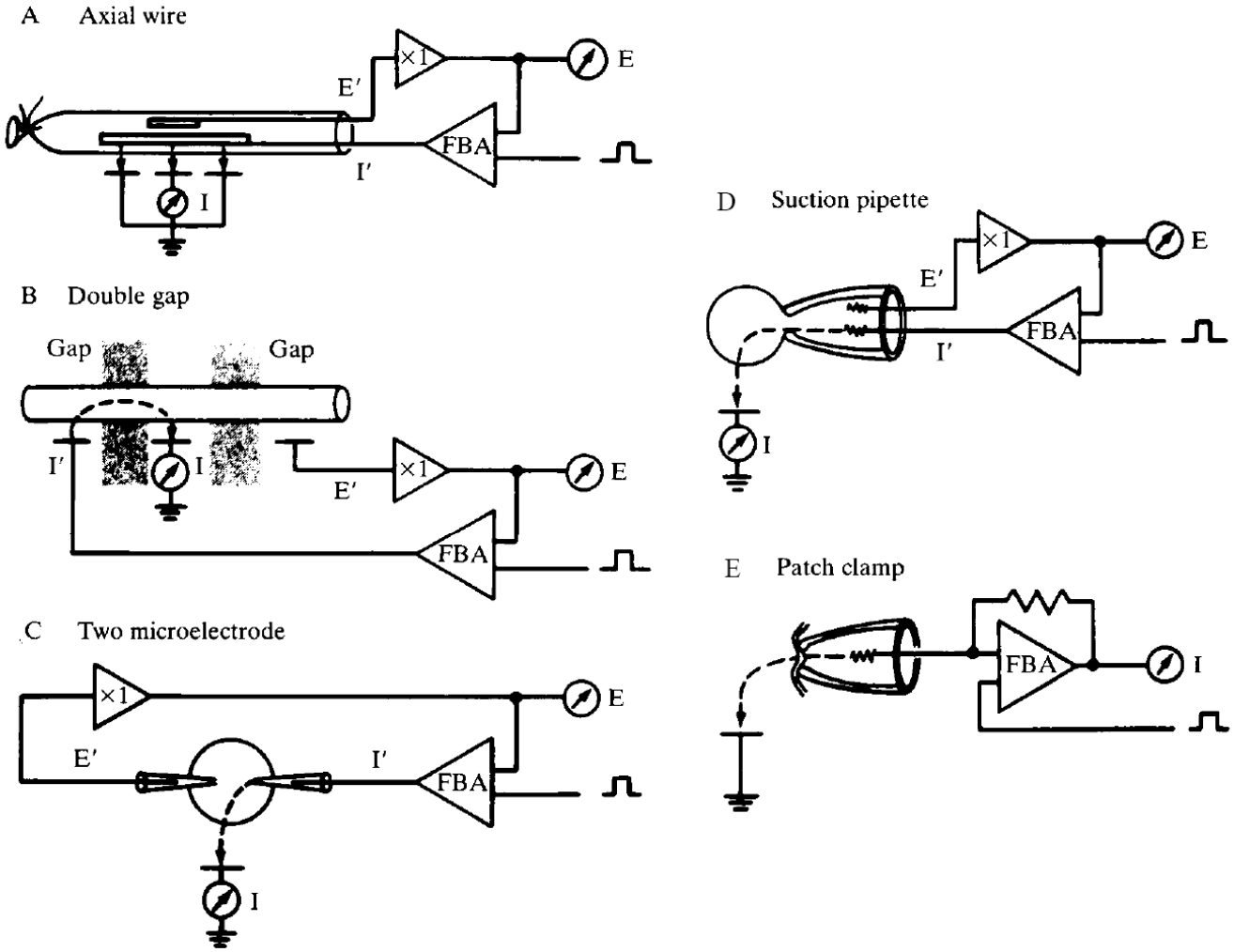
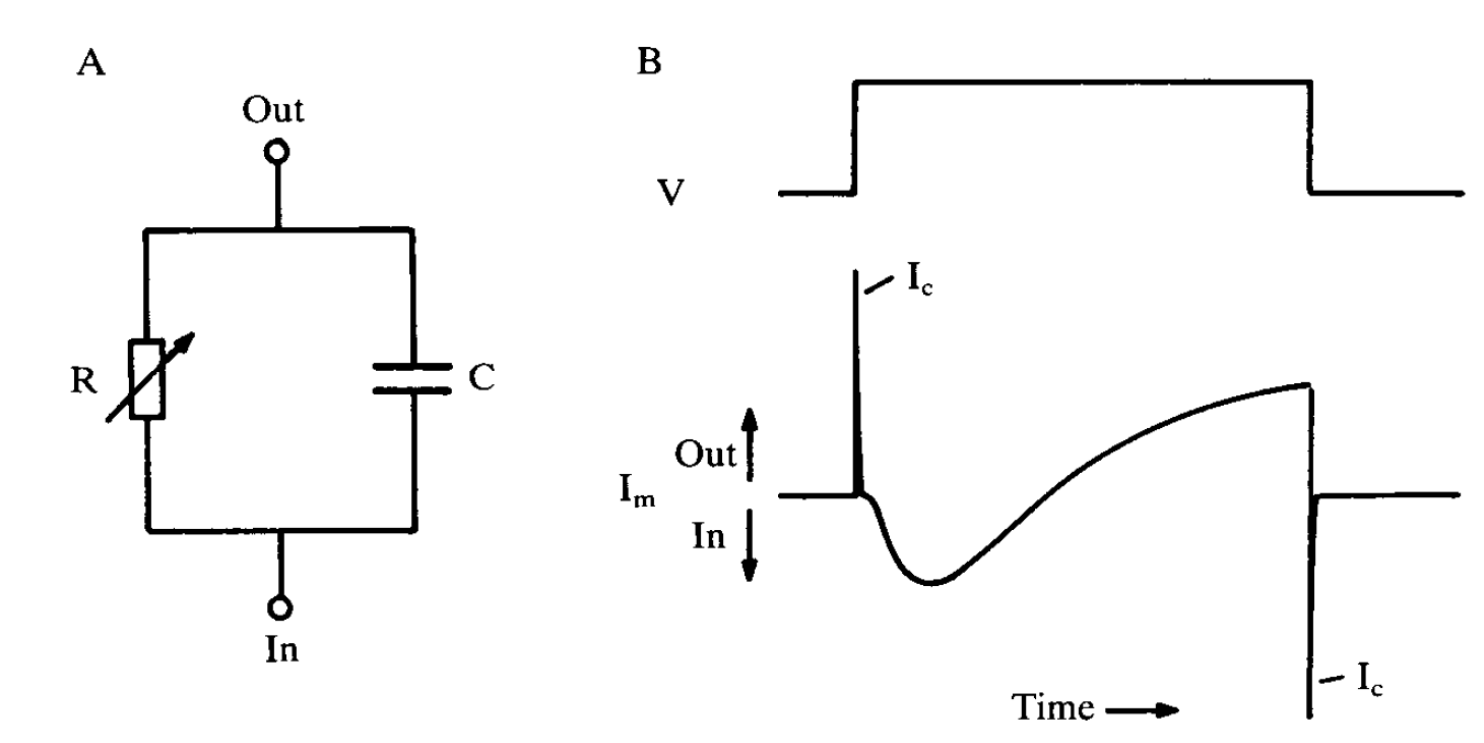


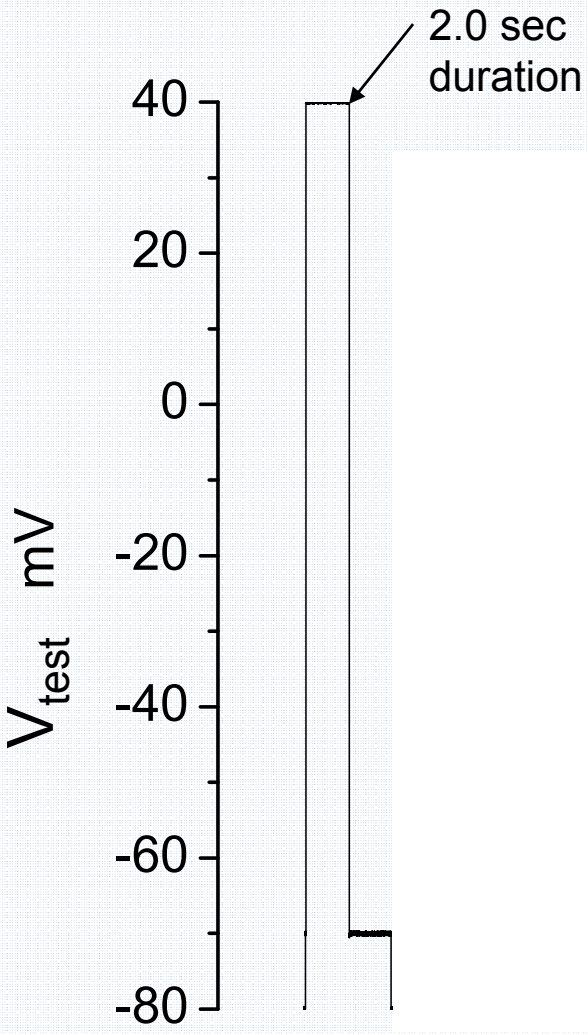
Fig. 3. Voltage clamp techniques. In each case FBA is the feedback (or clamping) amplifier, E′ is the voltage electrode and I′ is the current electrode. (Reproduced with permission from Hille, 1984).

Capacitance currents (I_c) and ionic currents (I_i) are activated by rapid changes in membrane potential using voltage clamp



$$I_m = I_i + C\frac{dV}{dt}$$

Variable V_{test} can be applied with voltage clamp



(10 sec between each pulse)

Simplified schematic of voltage clamp circuit

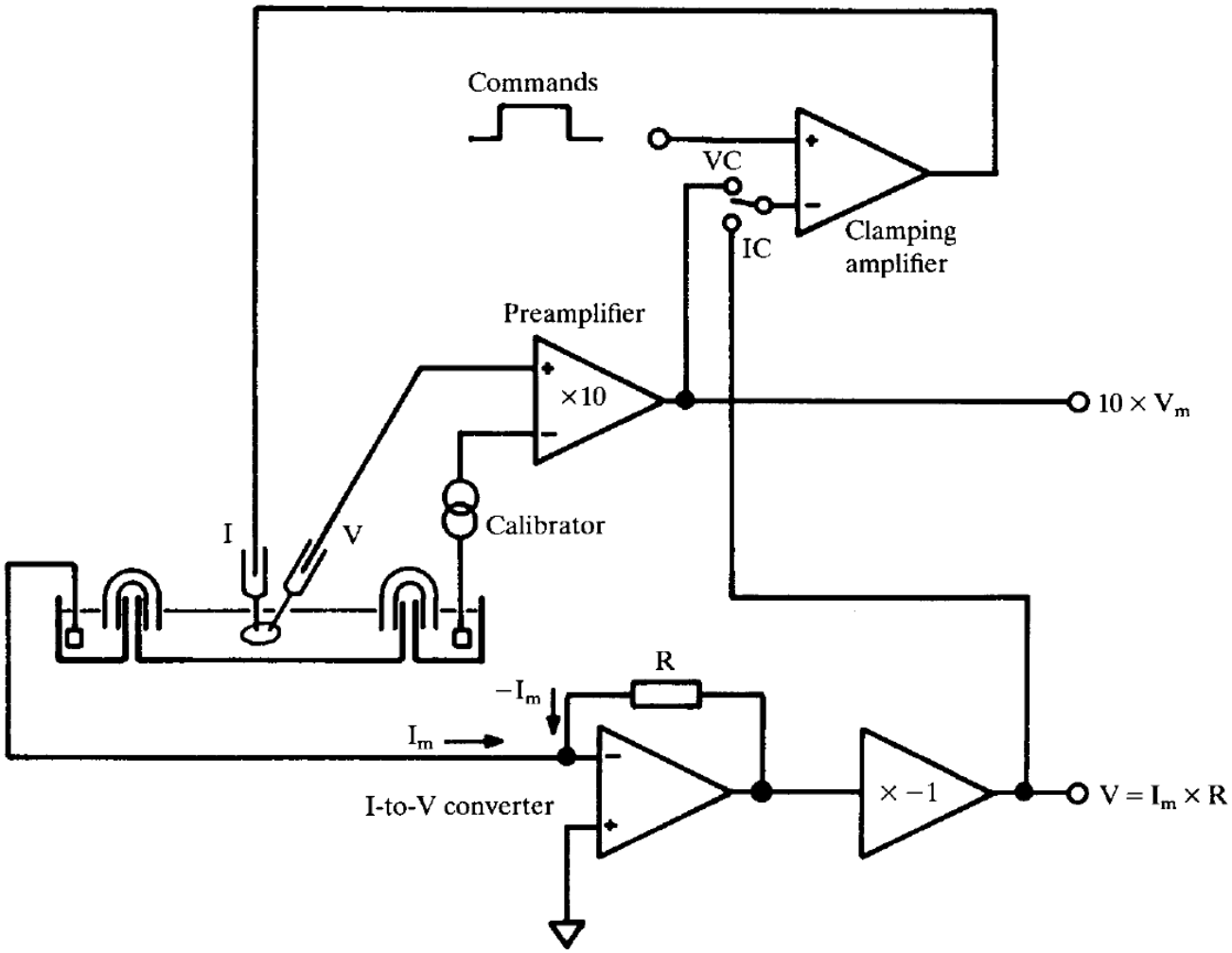
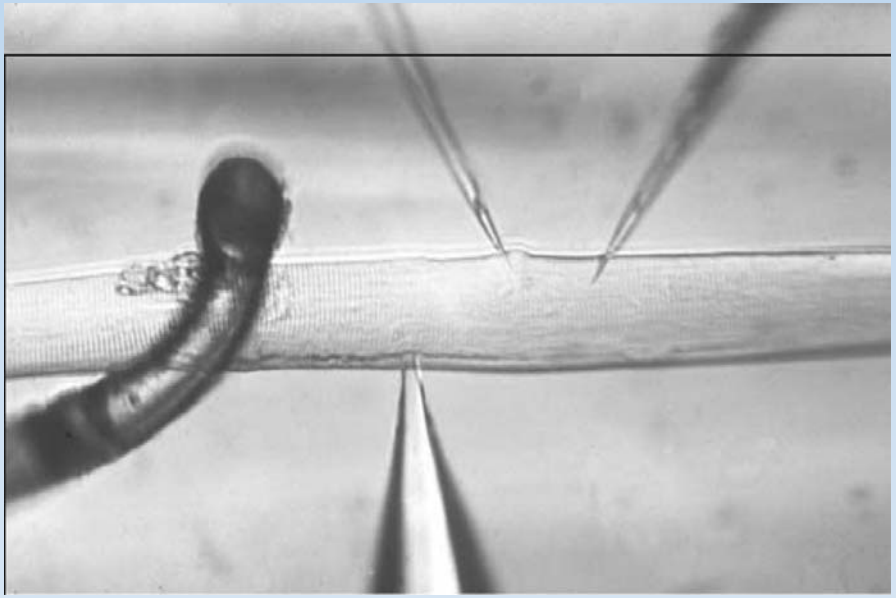


Fig. 4. Two electrode voltage clamp circuit. The circuit may be switched either to clamp voltage (VC) or current (IC).

Original patch clamp recordings (1981)



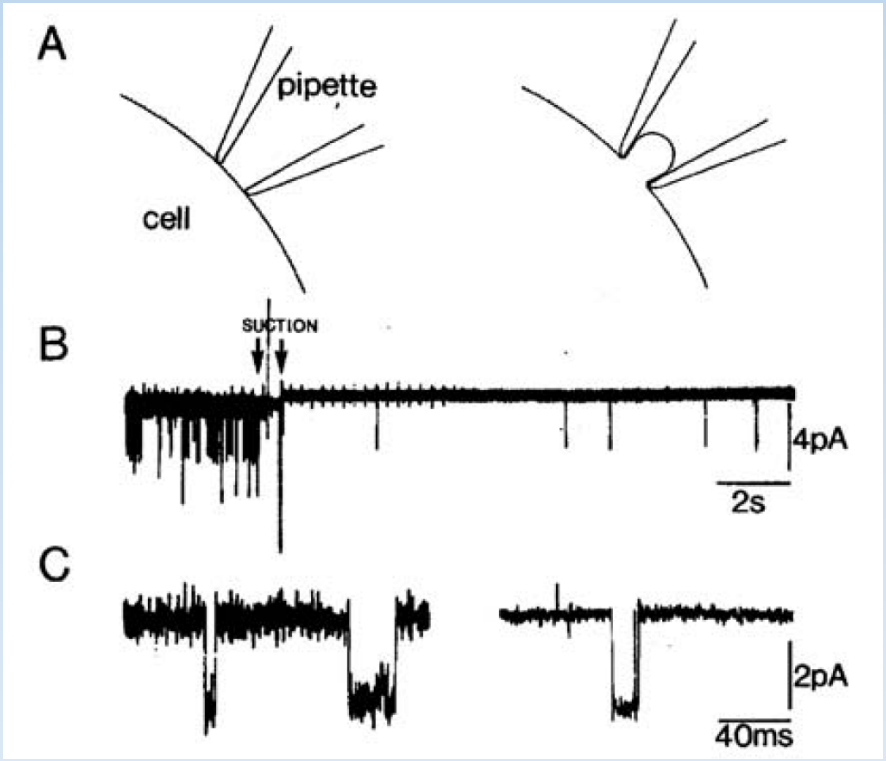
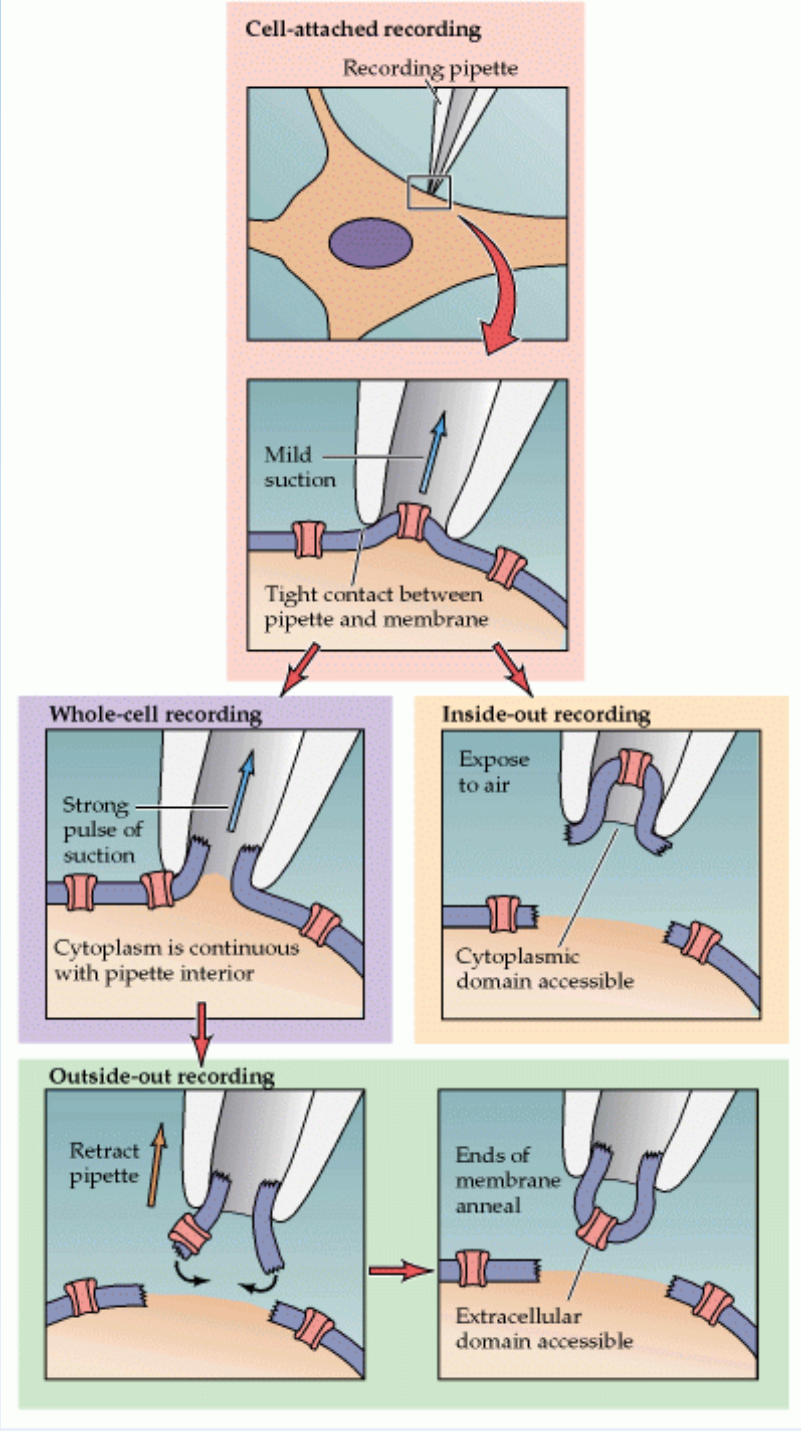


Figure 5. The Patch-clamp technique (Hamill et al, 1981) *Pflugers Arch* 391: 85-100
Upper panel shows the photograph of the preparation used for the first recordings of acetylcholine receptor single channel currents from denervated frog (Rana pipiens) cutaneous pectoris muscle (Neher & Sakmann, 1976).
Lower panel is the reproduction of Fig 6 from 1981 Pflügers Archiv paper (Hamill et al., 1981), which shows giga-seal formation between pipette tip and sarcolemma of frog muscle. (A) Schematic diagrams showing a pipette pressed against the cell membrane when the pipette-membrane seal resistance is of the order of 50-100 Megohms (left), and after formation of a gigaseal when a small patch of membrane is drawn into the pipette tip (right). (B) The upper trace

Four modes of patch clamp technique



High-throughput, automated patch clamp instruments

EVOLUTION and Ion channel diversity

Diversity of ion channels

Example: nematode C. *elegans*

73 K channels *(20 6 TM, 3 IRK, 50 TWIK)*
89 ligand-gated channels *(42 ACh, 37 inhibitory $GABA_A$ or glutamate, 10 excitatory glutamate)*
5 voltage-gated Ca channels
6 chloride channels
24 gap junction channels (connexins)
22 mechanosensitive channels
6 cyclic-nucleotide gated channels
11 TRP-related channels

Total: 236 channel subunit genes

Origin of ion channel diversity

1) gene duplication & divergence

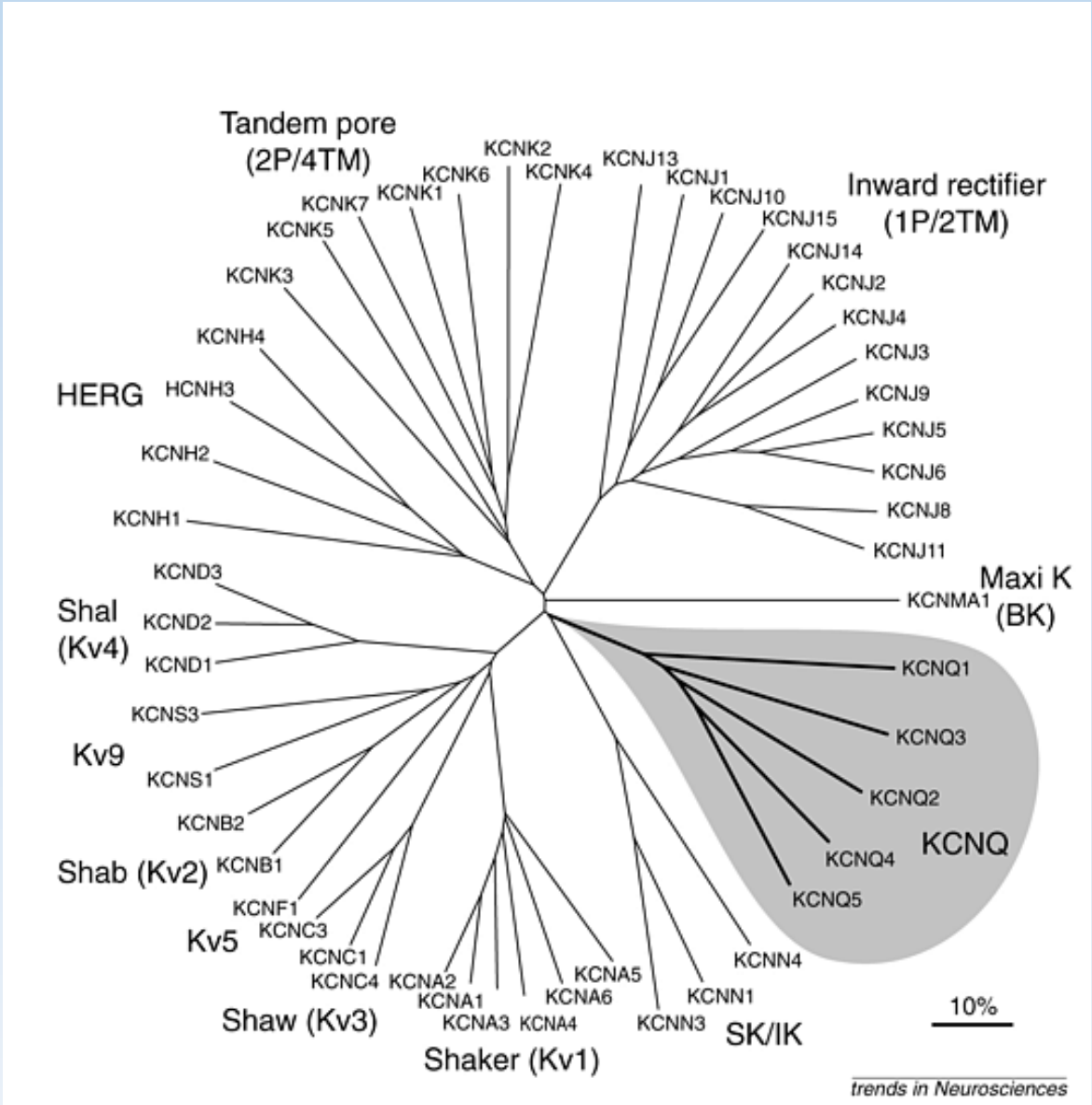
2) alternative mRNA splicing

3) heteromultimeric assembly of different pore-forming (α) subunits

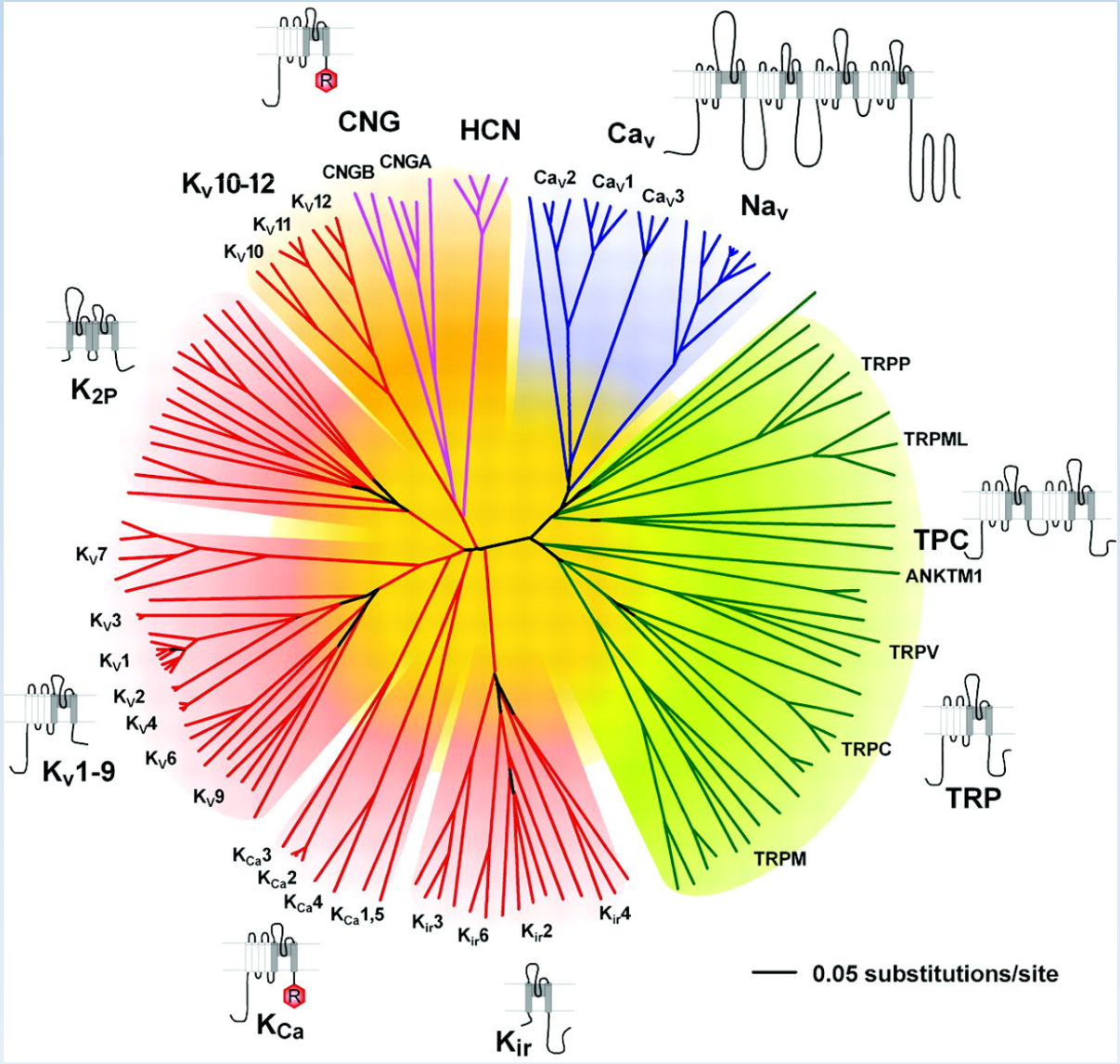
4) heteromultimeric assembly of alpha and auxillary (β,γ,δ) subunits

1) Gene duplication and divergence

(example: K channels)



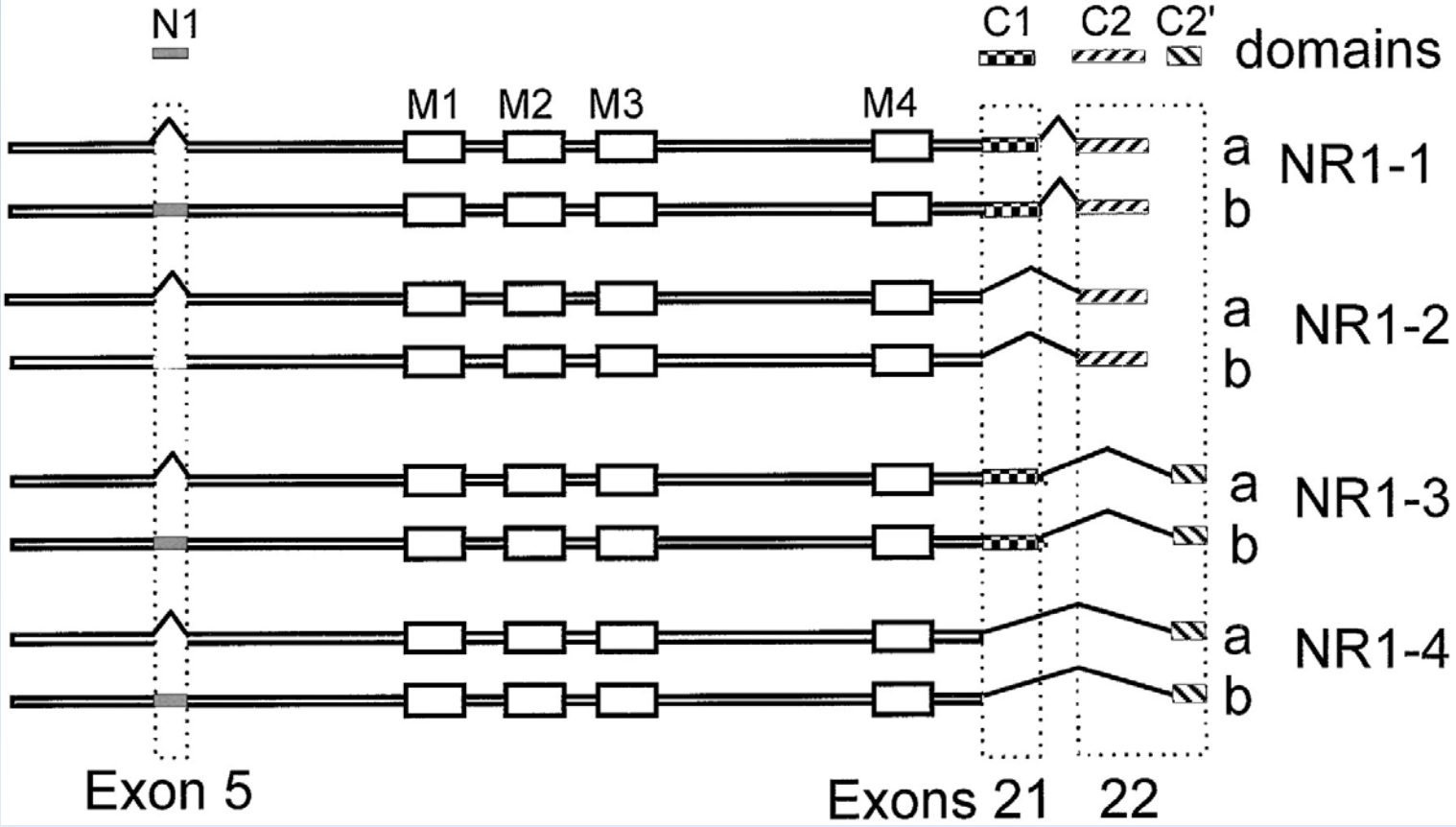
Amino acid relationships of the minimal pore regions of the voltage-gated ion channel superfamily (143 types)



Pharmacol Rev 57:387-395, 2005

2) Alternative mRNA splicing

(NMDA subtype of Glutamate receptor channels)

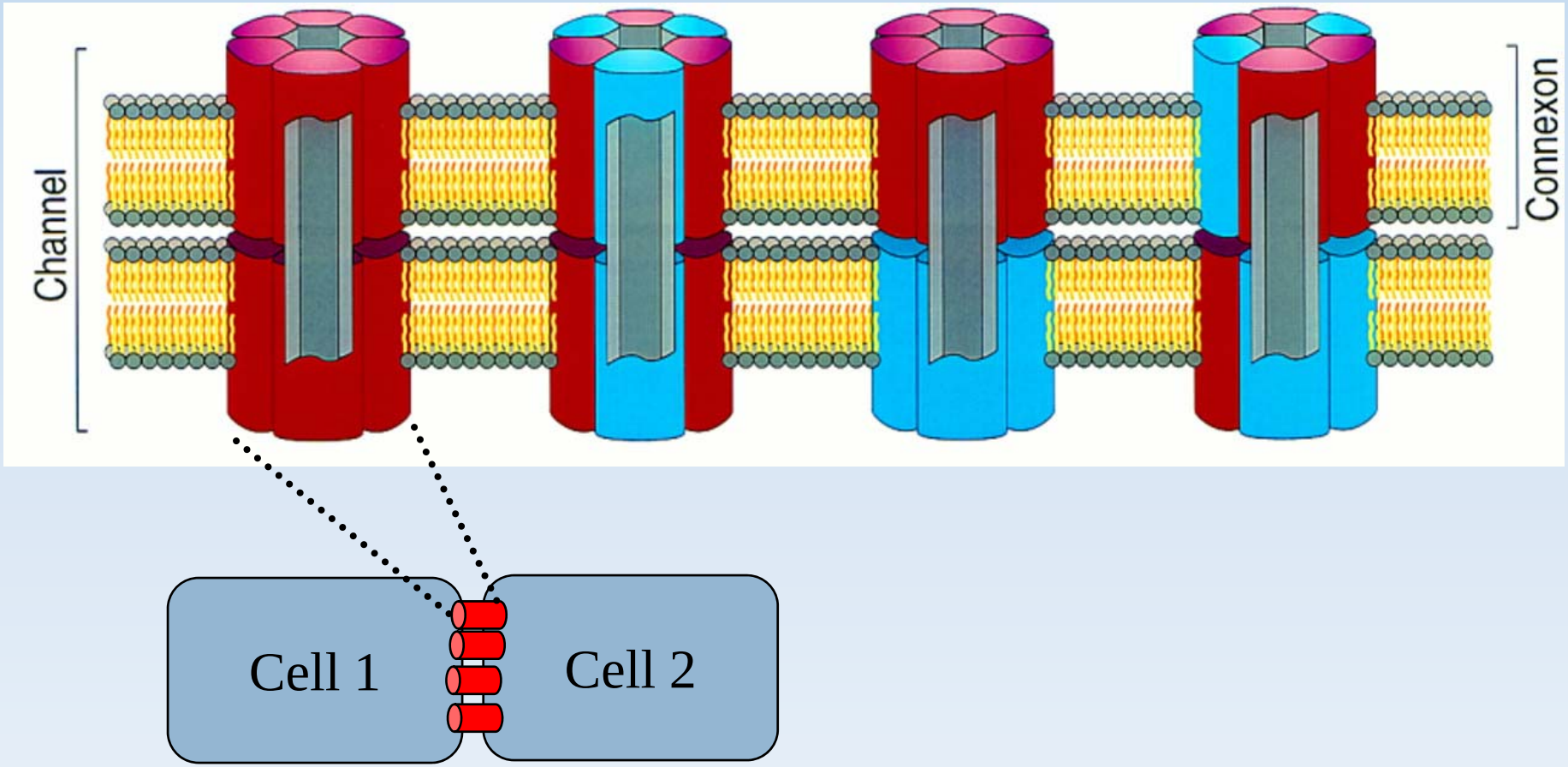


The different NR1 subunit splice variants arise from alternative splicing of the exons 5, 21, and 22, giving rise to the cassettes N1, C1, C2, and C2'.

3) Heteromultimeric assembly of different α-subunits

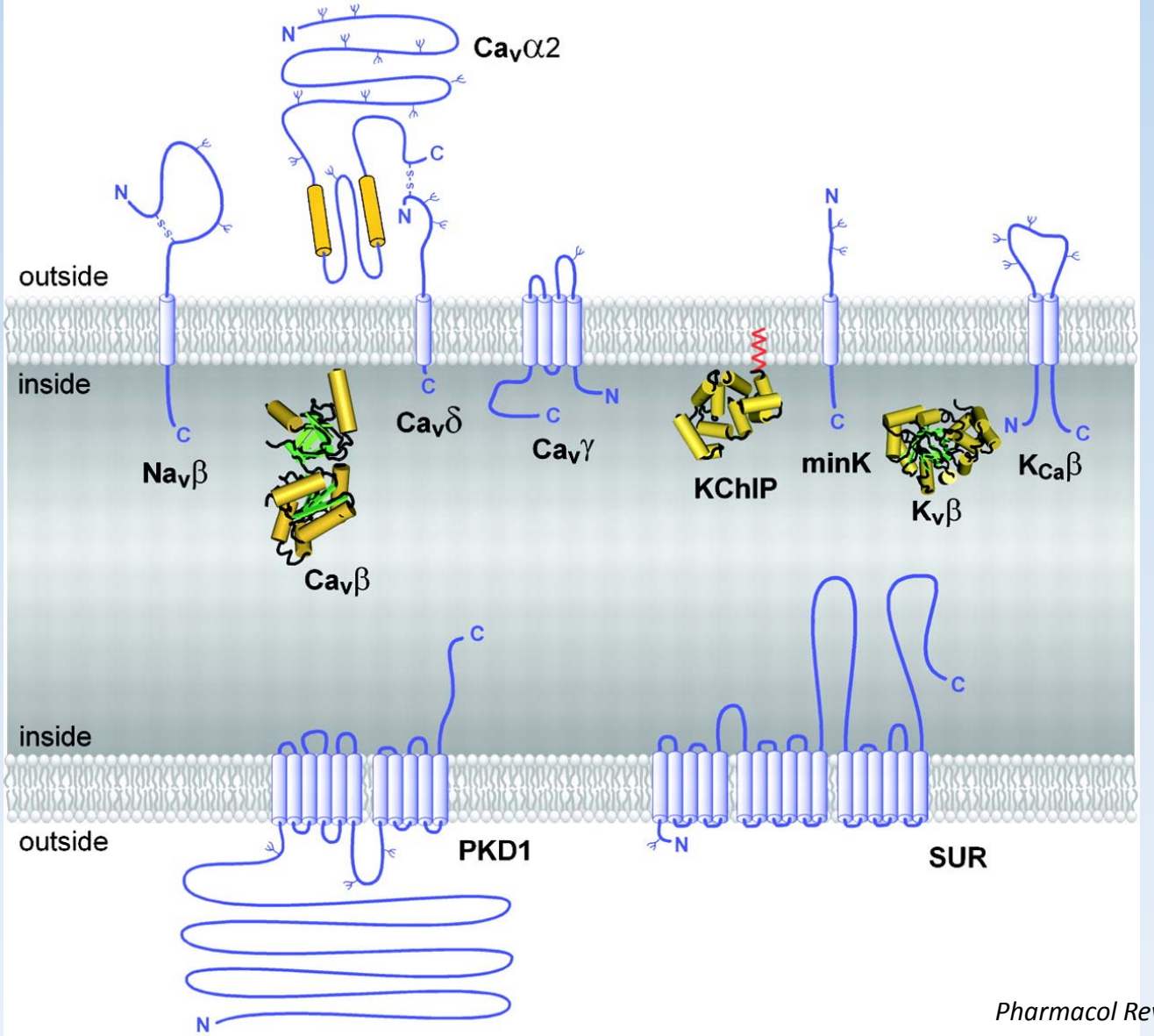
example: six Connexins combine to form a connexon

two connexons combine to form a gap junction channel



Kumar and Gilula. Cell 84, 1996

4) Auxiliary subunits of the voltage-gated ion channel superfamily

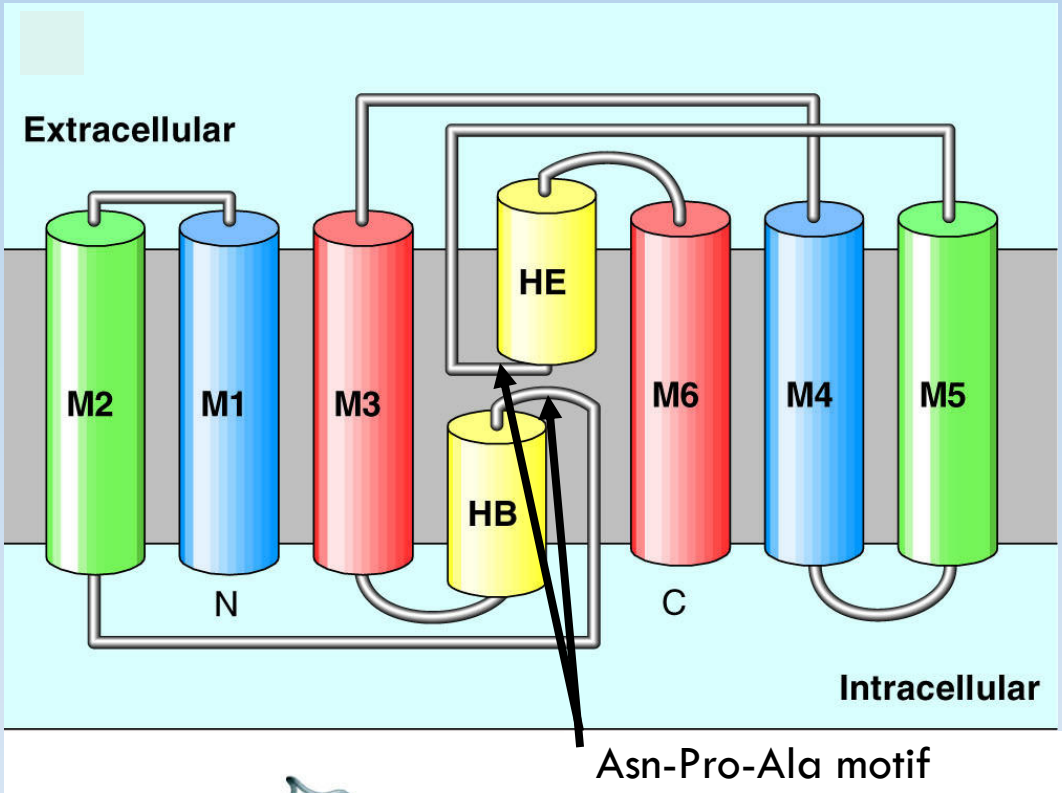


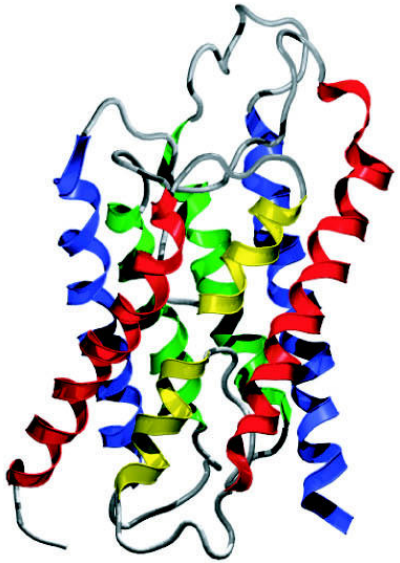
Pharmacol Rev 57:387-395, 2005

Aquaporins (water channels)

Movement of water across cell membrane is often faster than predicted for a purely passive process.

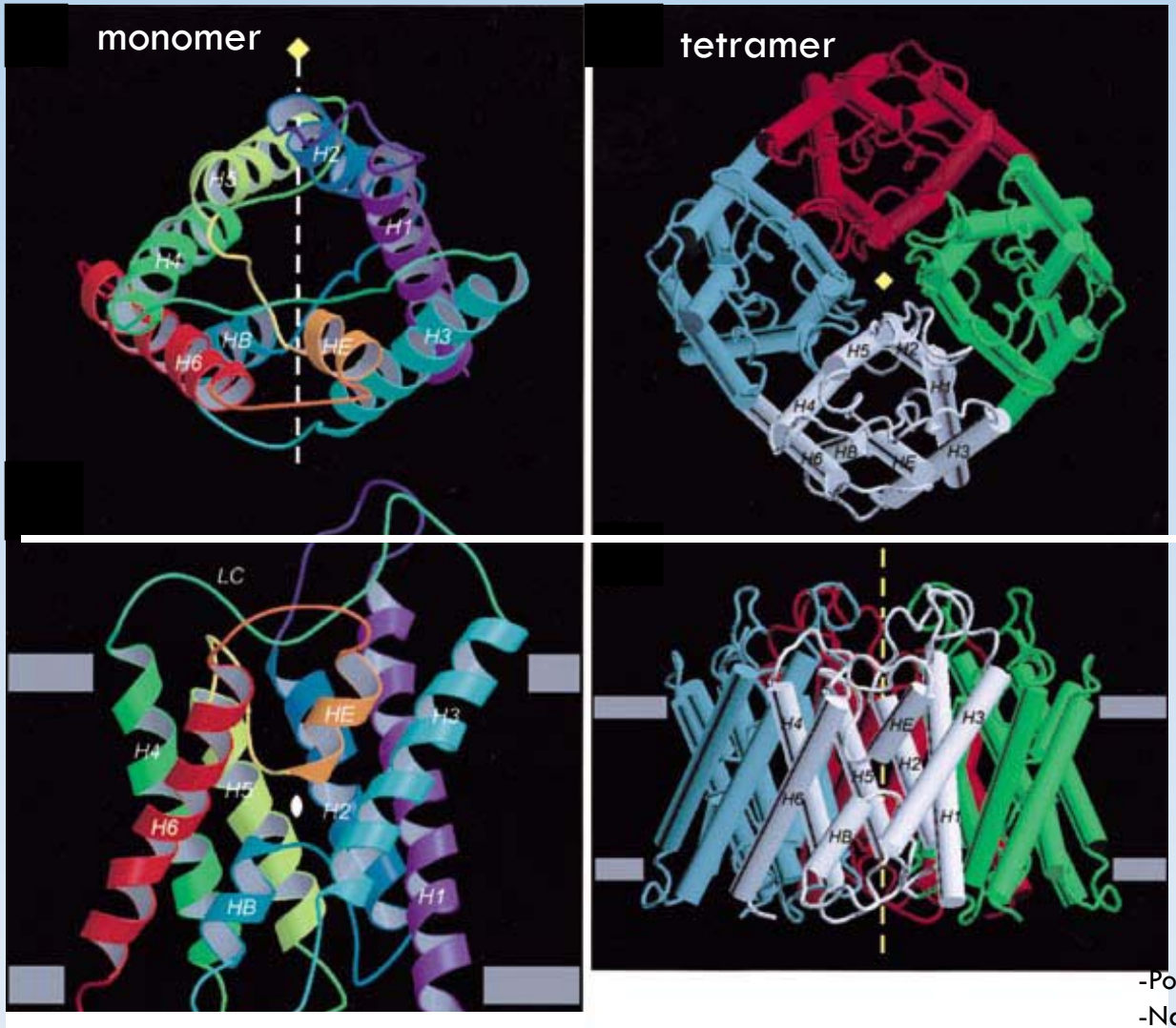
AQPs (6 types) are widely expressed
- erythrocytes (200,000/RBC),
- specialized regions of the kidney, iris, lens epithelia,
- lung alveolar capillaries and endothelium
- epithelium of colon,
- capillary and lymphatic endothelium of all muscle tissue.





Aquaporin monomer

(water channel)



End-on view from the extracellular surface

side view

AQP1

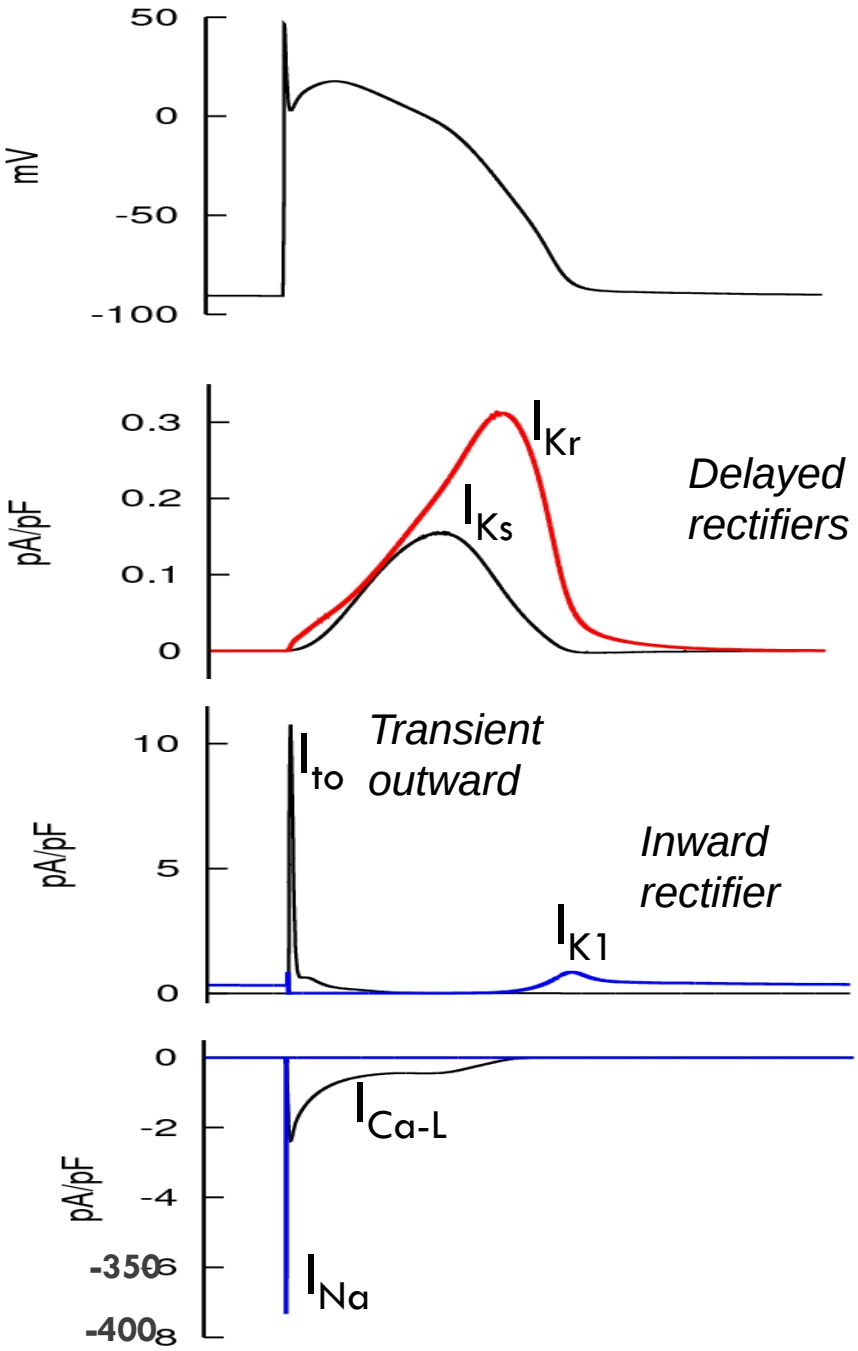
-Pore radius ~ 5 Å
-Narrowest part of pore (1 amino acid): 3 Å
(diameter of water molecule: 2.8 Å)
-Exclude all ions, including H^+

Each Monomer: six membrane-spanning helices, two pore helices
4 water channels/tetrameric structure

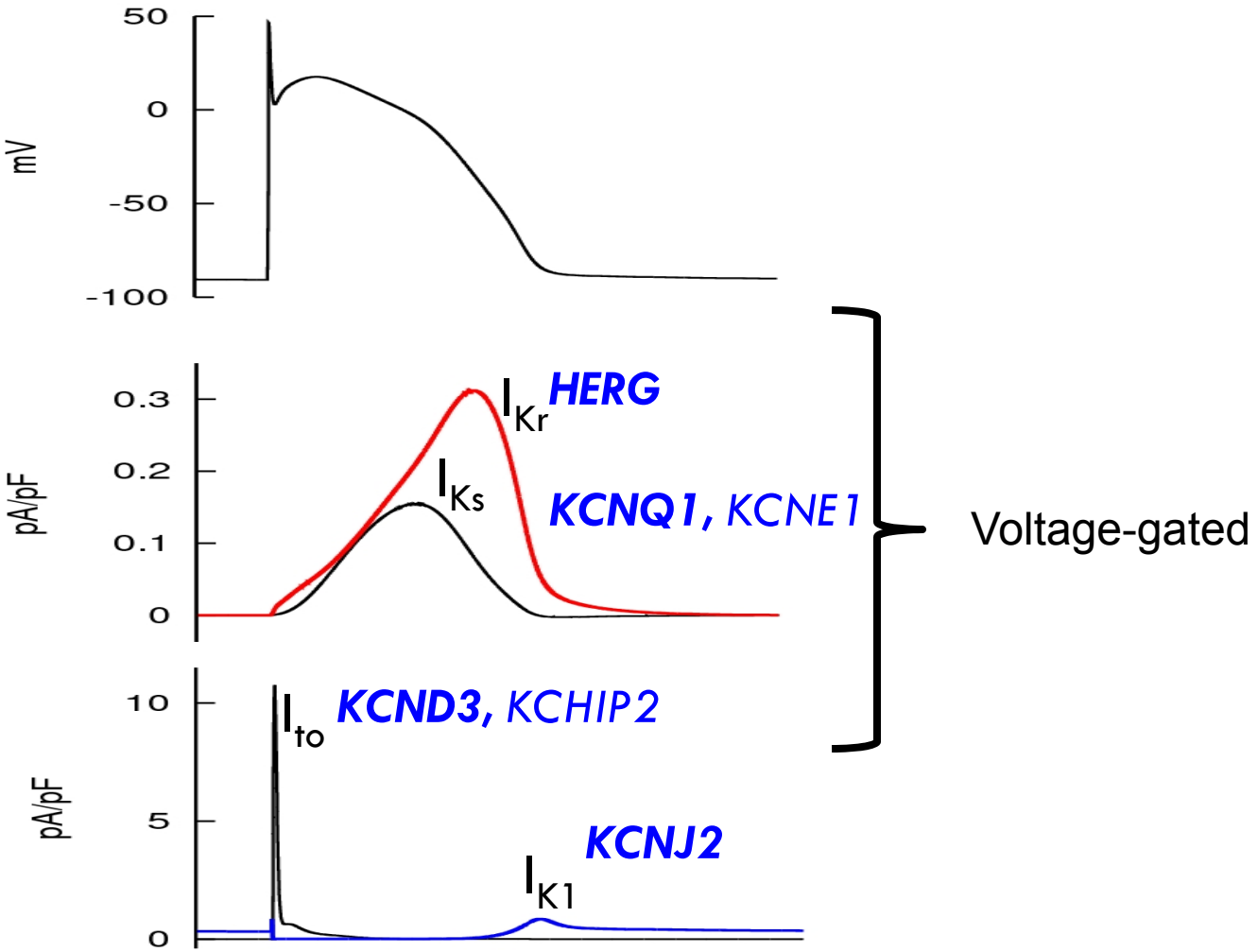
MURATA et al (2000)
Nature **407**: 599 - 605

Ion channels and cardiac excitability

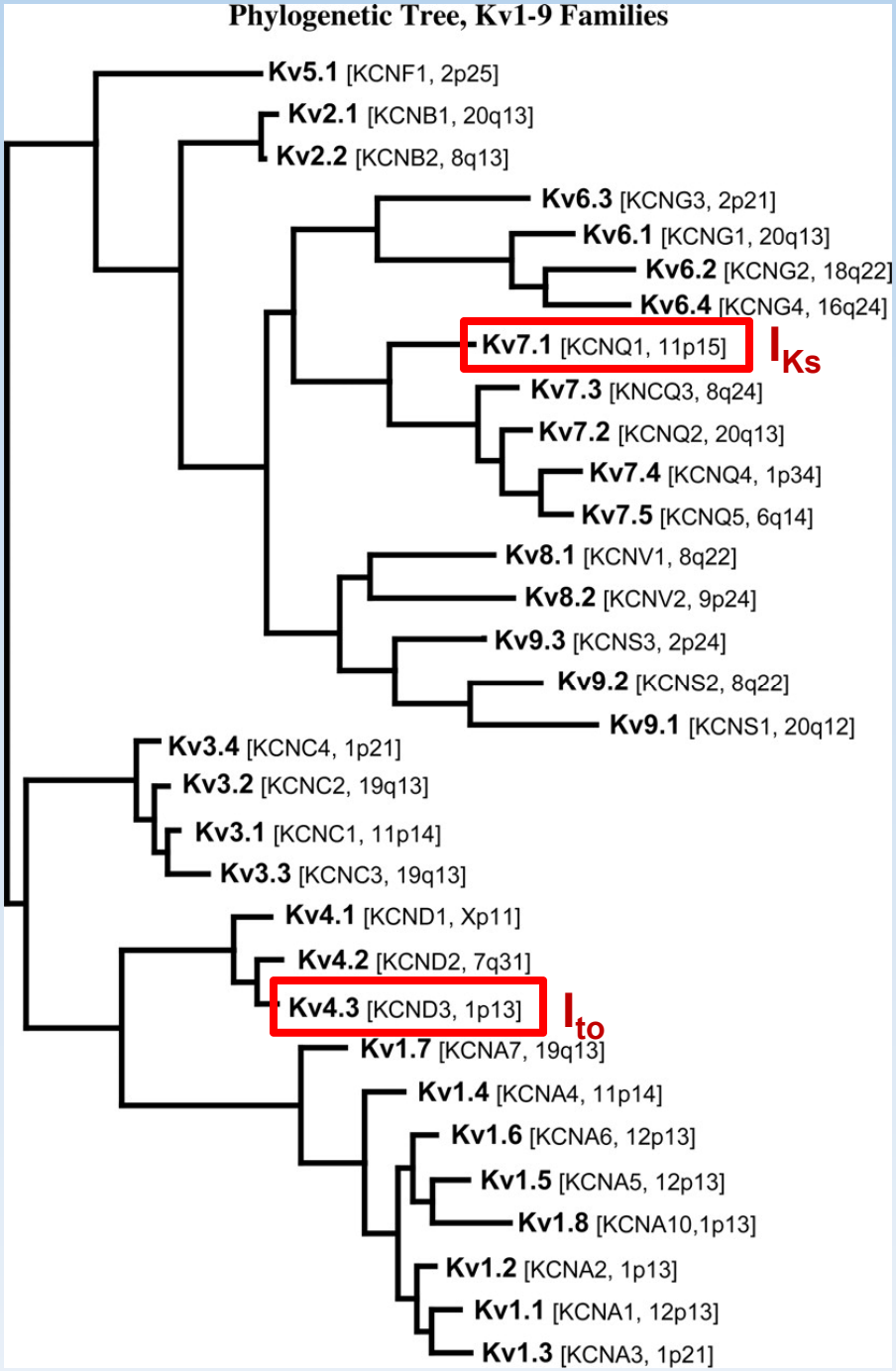
Ionic currents in human ventricular myocyte

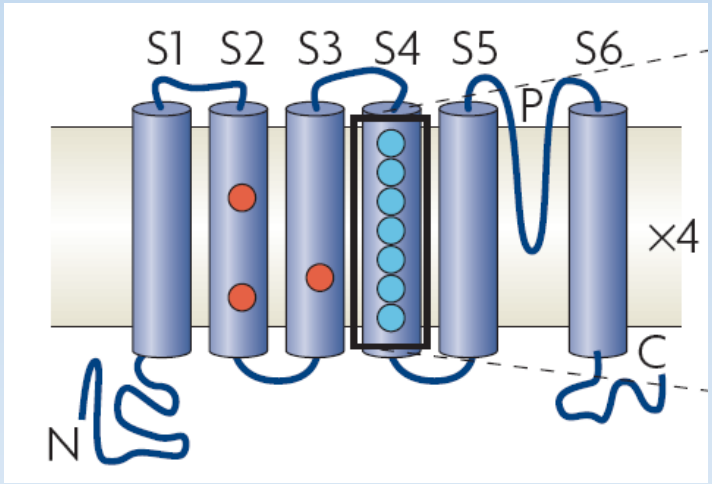


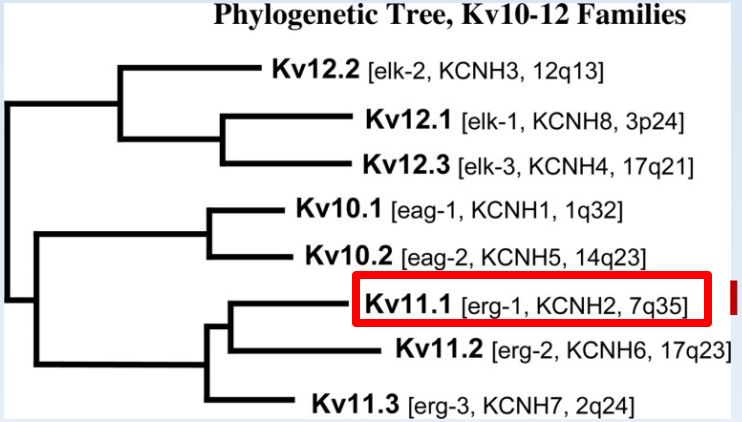
Molecular basis of human cardiac potassium channels



VOLTAGE-GATED K CHANNELS

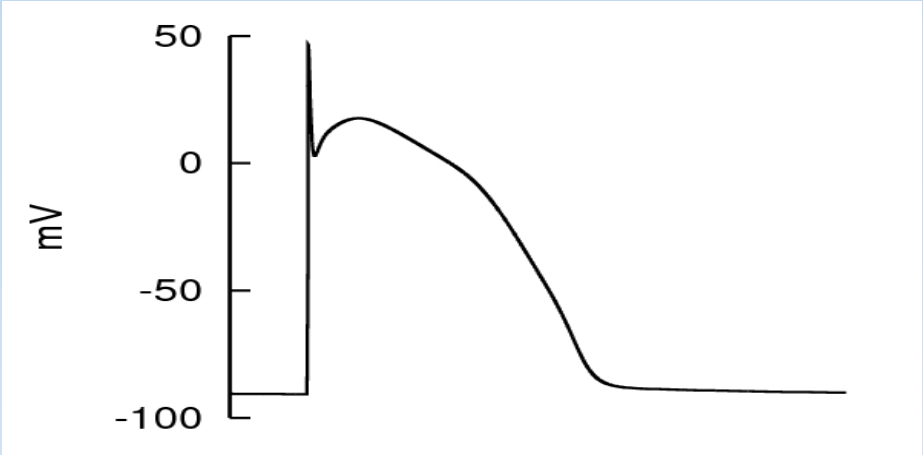


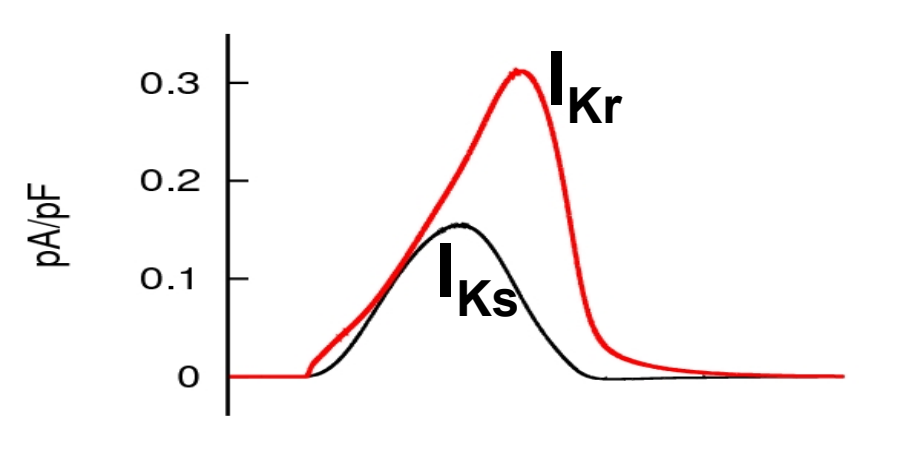




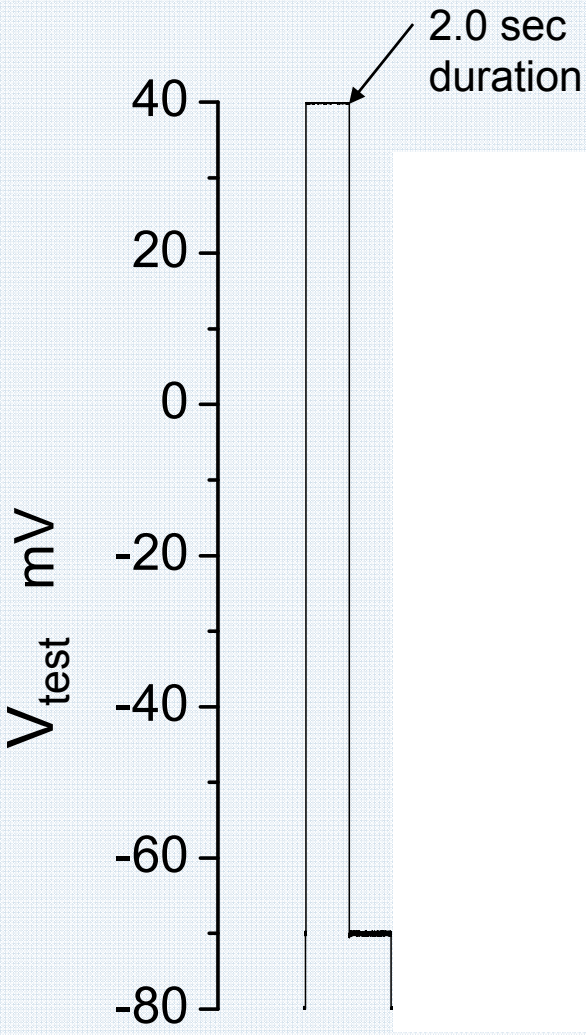
Pharmacol Rev 57:473-508, 2005

Delayed rectifier K currents: I_{Kr} and I_{Ks}



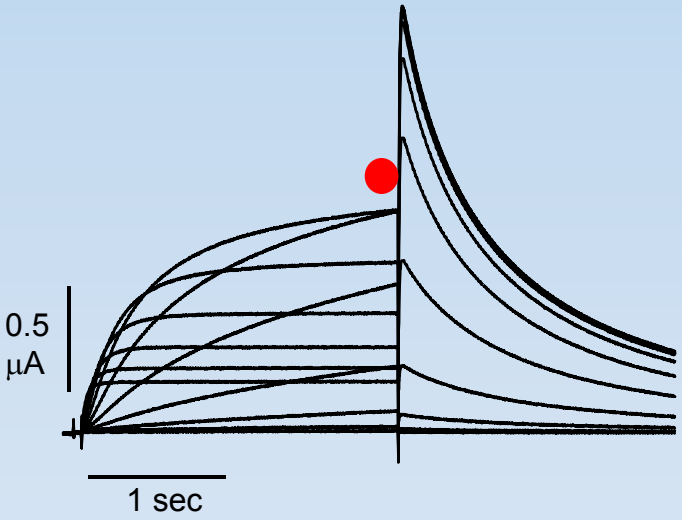


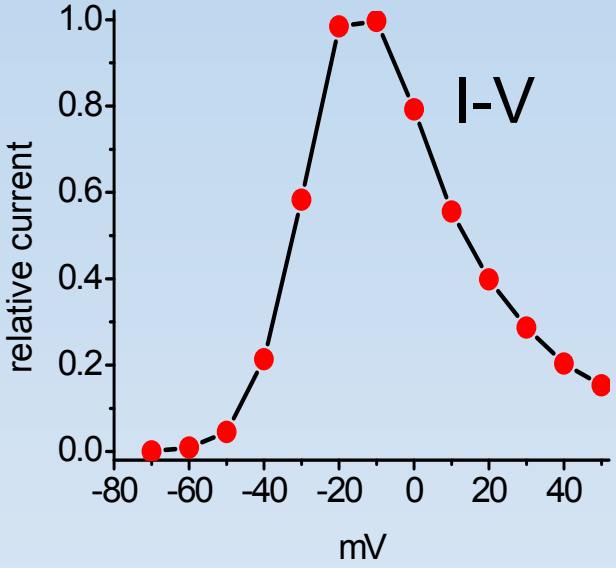
Variable V_{test} can be applied with voltage clamp



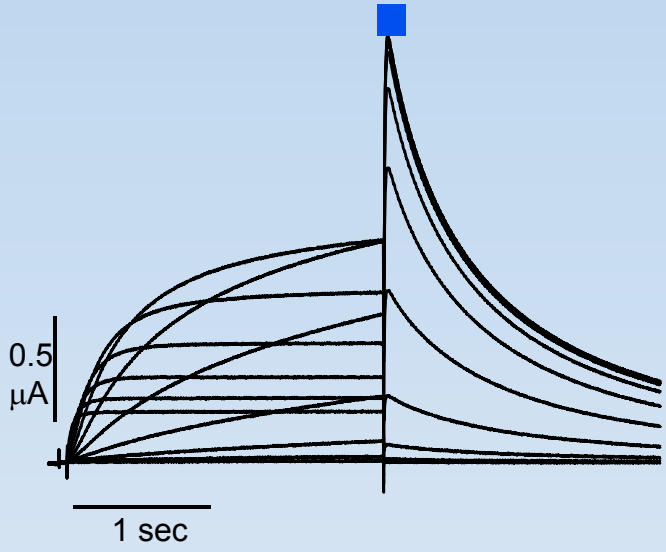
(10 sec between each pulse)

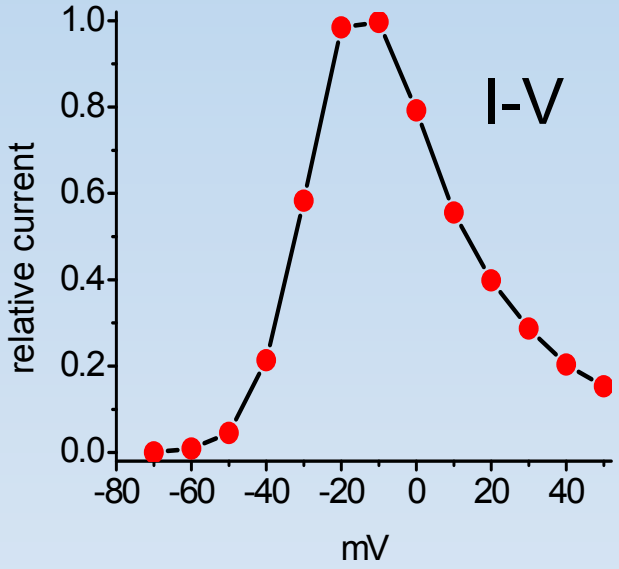
I_{Kr}: hERG subunits

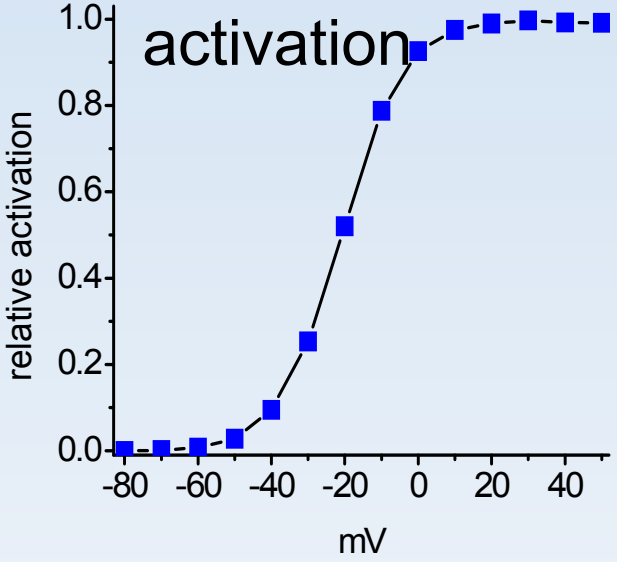




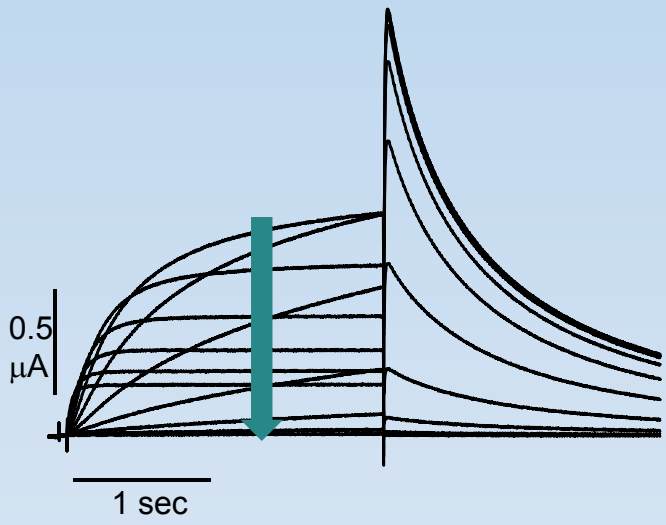
I_{Kr} (hERG)

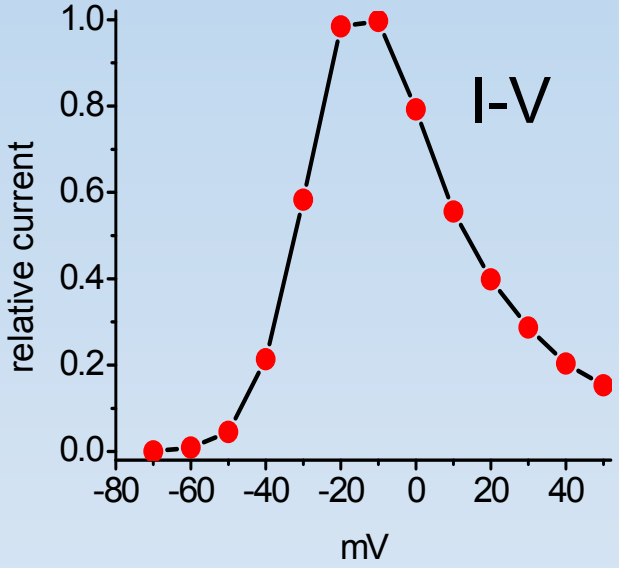

0.5
µA
1 sec

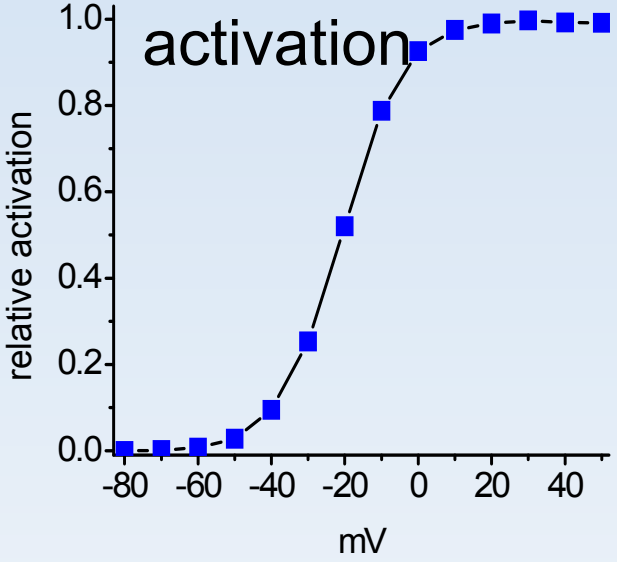

I-V
relative current
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
0
20
40
mV

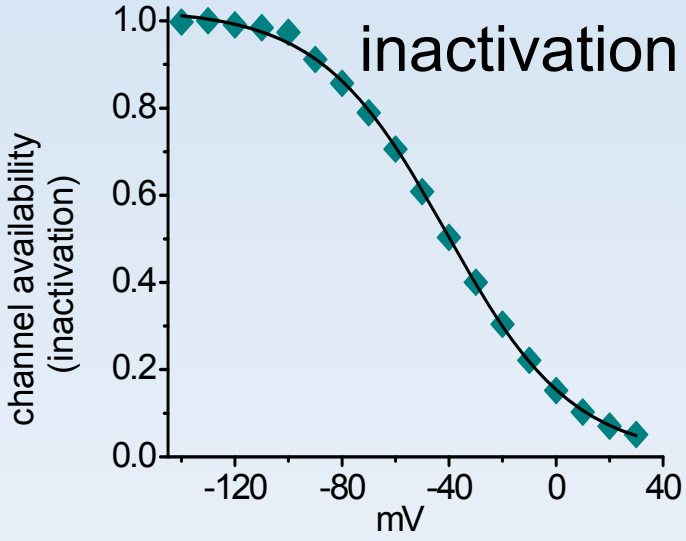

activation
relative activation
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
0
20
40
mV

I_{Kr} (hERG)


0.5
μA
1 sec

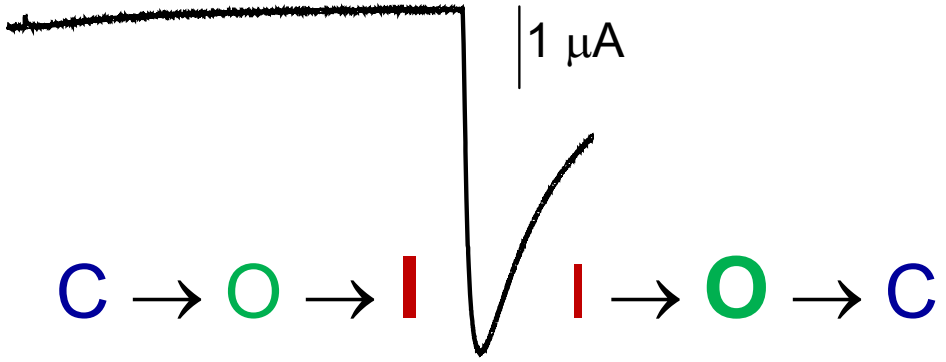

I-V
relative current
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
0
20
40
mV


activation
relative activation
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
0
20
40
mV

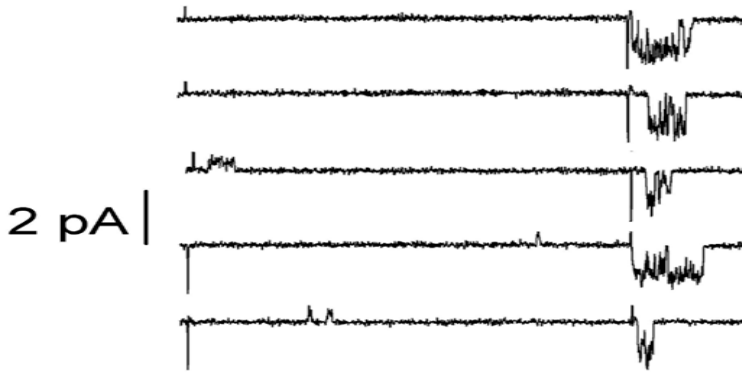

inactivation
channel availability
(inactivation)
1.0
0.8
0.6
0.4
0.2
0.0
-120
-80
-40
0
40
mV

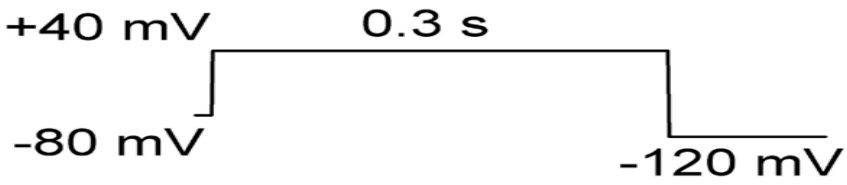
hERG single channel currents

Whole cell current

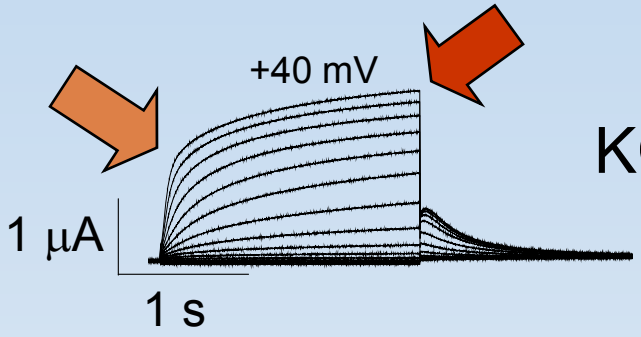


Single channel current

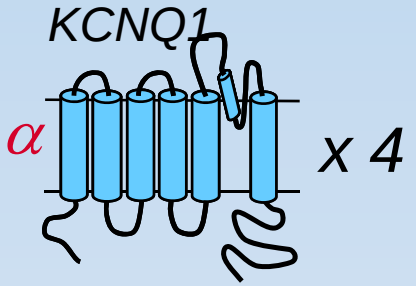


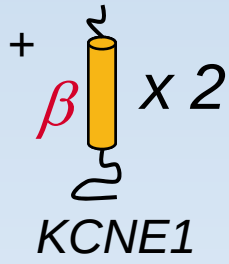


I_{Ks}: KCNQ1 + KCNE1 subunits



KCNQ1 alone

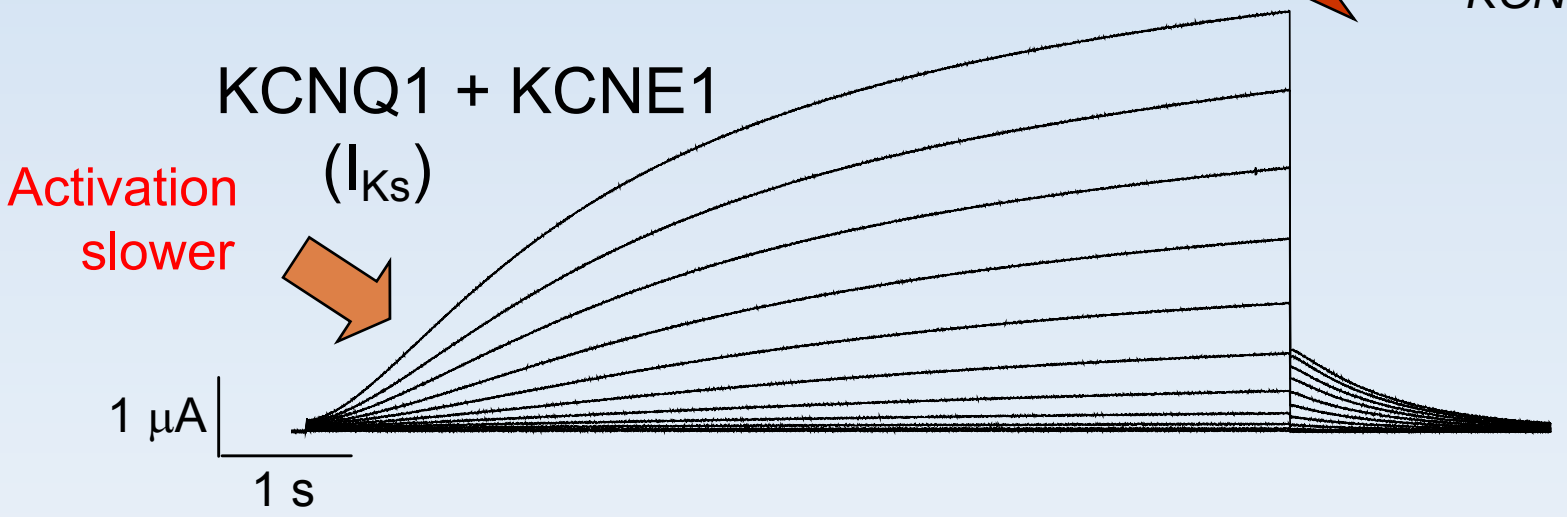




Amplitude increased

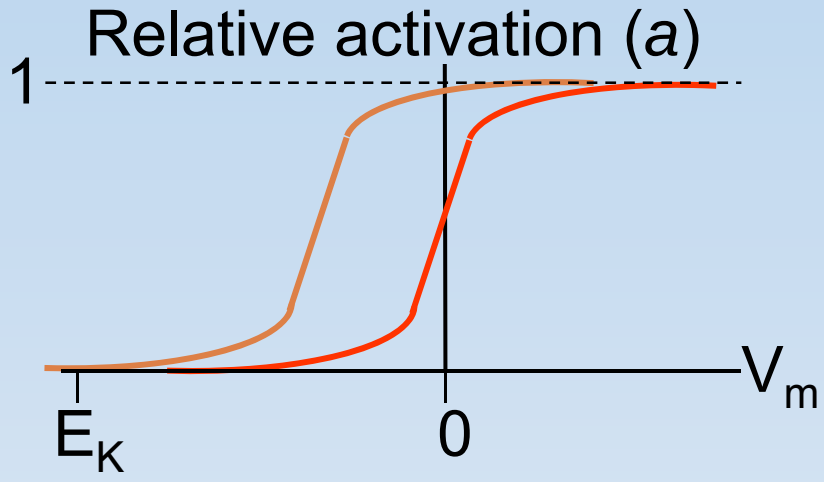
KCNQ1 + KCNE1
(I_{Ks})

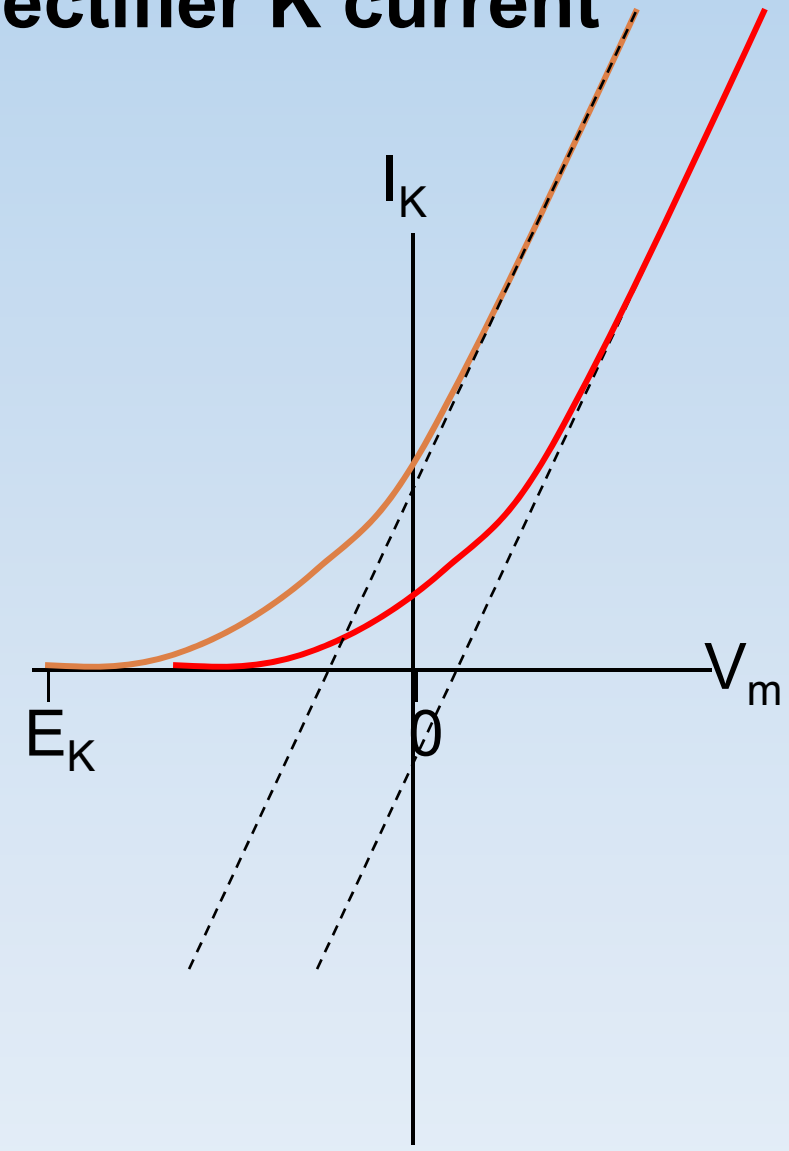
Activation slower

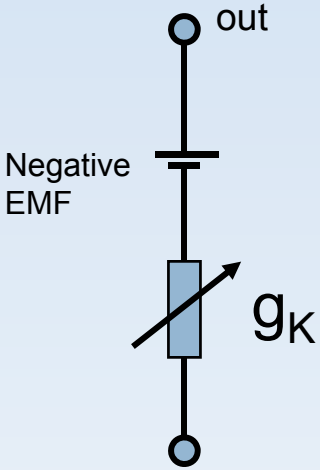


Sanguinetti et al (1996) Nature

I-V relationship: delayed rectifier K current



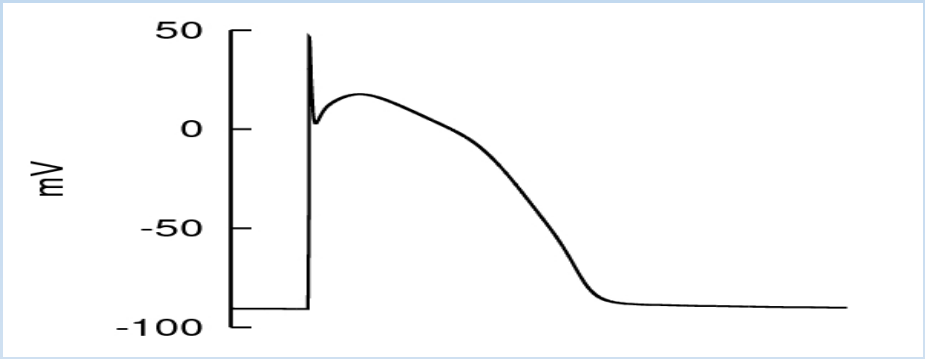


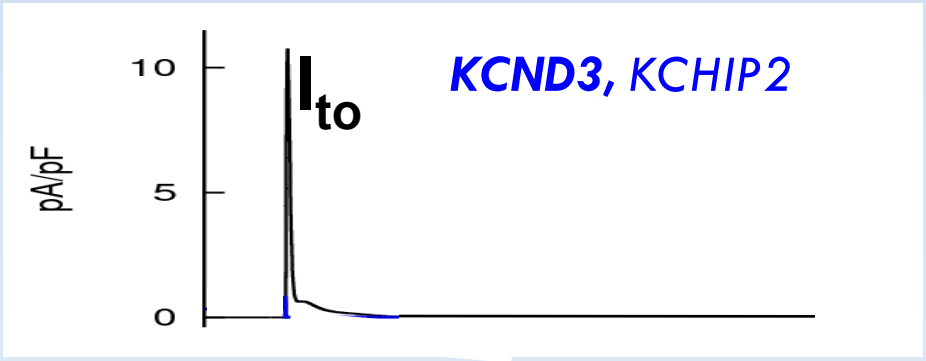


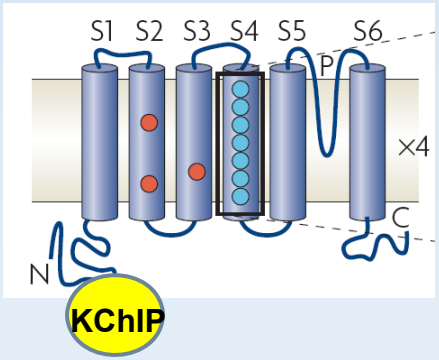
K channel pore

$$I_{K(peak)} = (g_K)_{max} \times a \times (V_m - E_K)$$

Transient outward K current: I_{to}

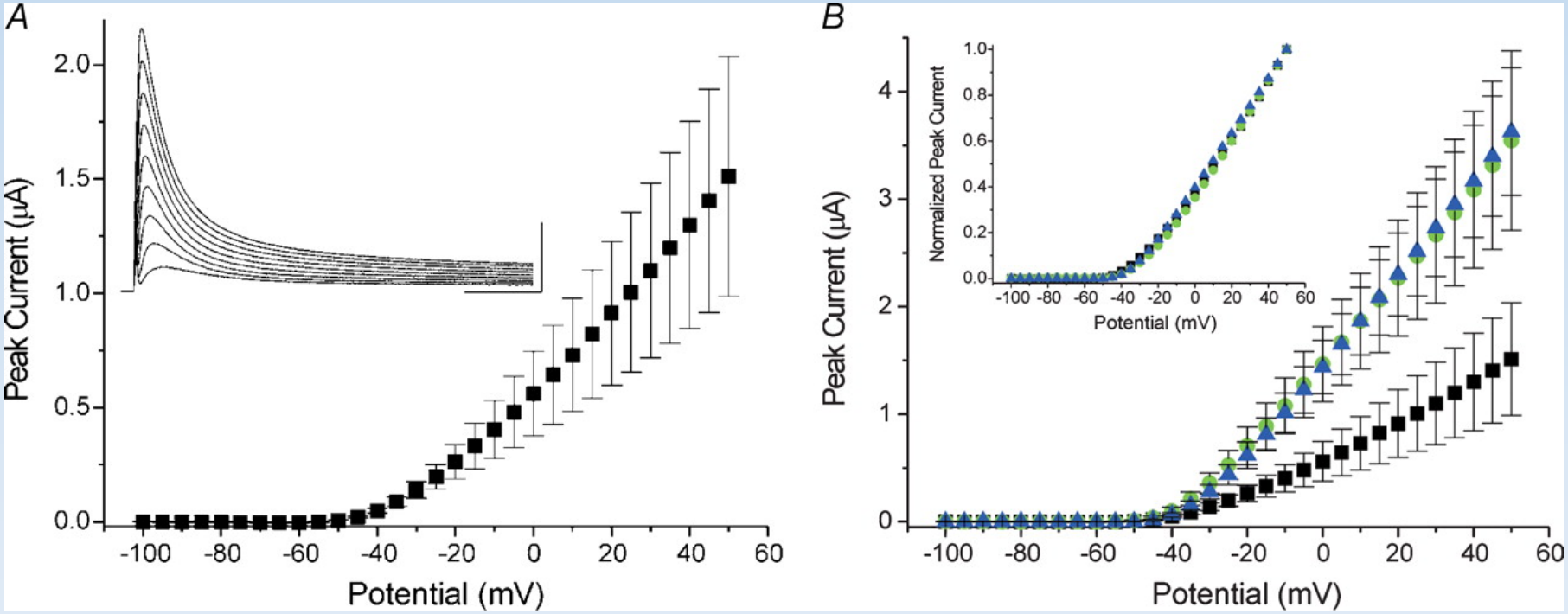






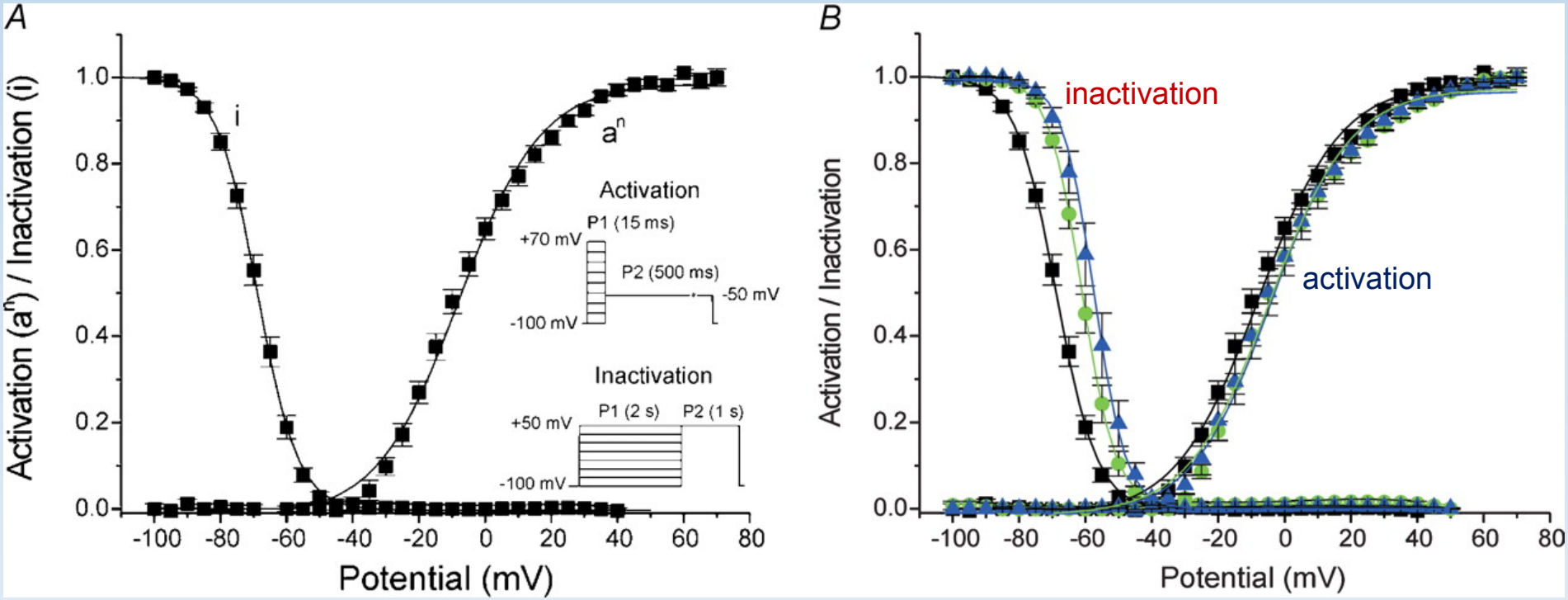
KChIP2 = K Channel Interacting Protein #2

I_{to}: KChip2b and 2d increases membrane expression of Kv4.3 channels



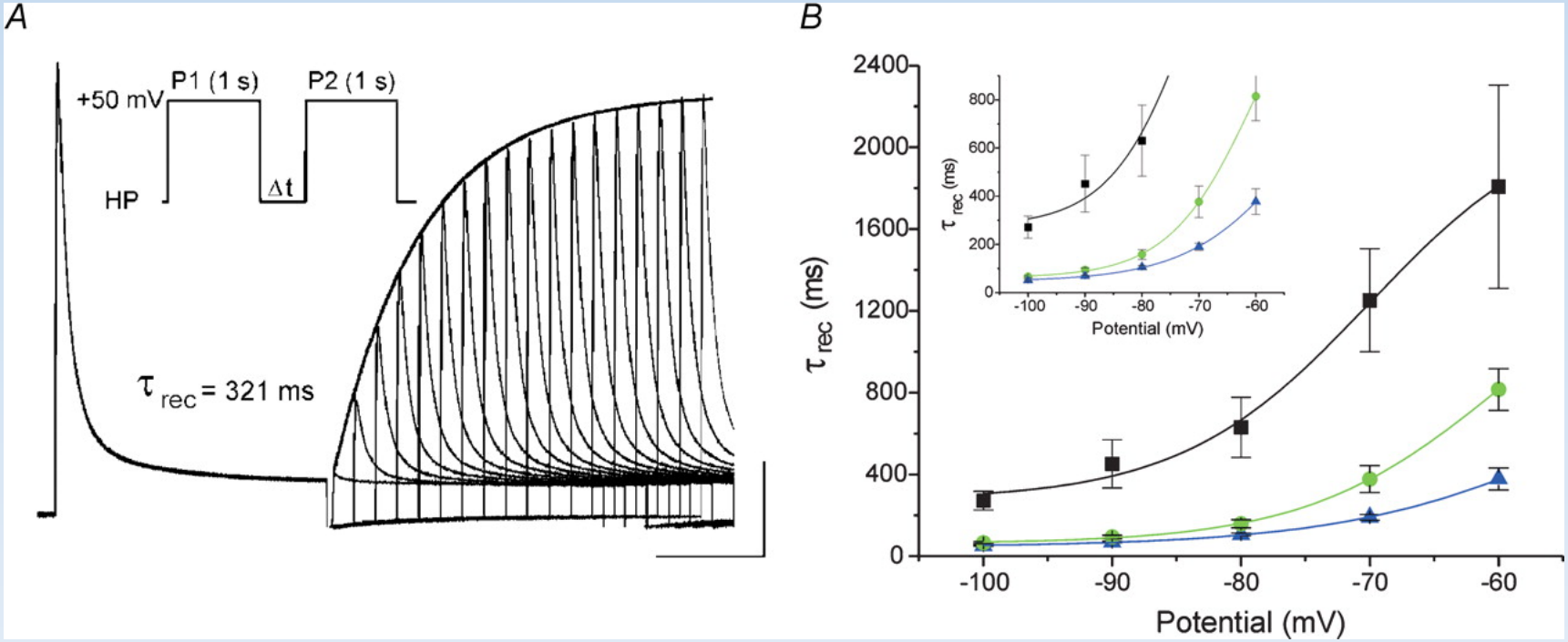
Patel et al (2004)
J Physiol 557:19

I_{to}: KChip2's alters Kv4.3 channel inactivation

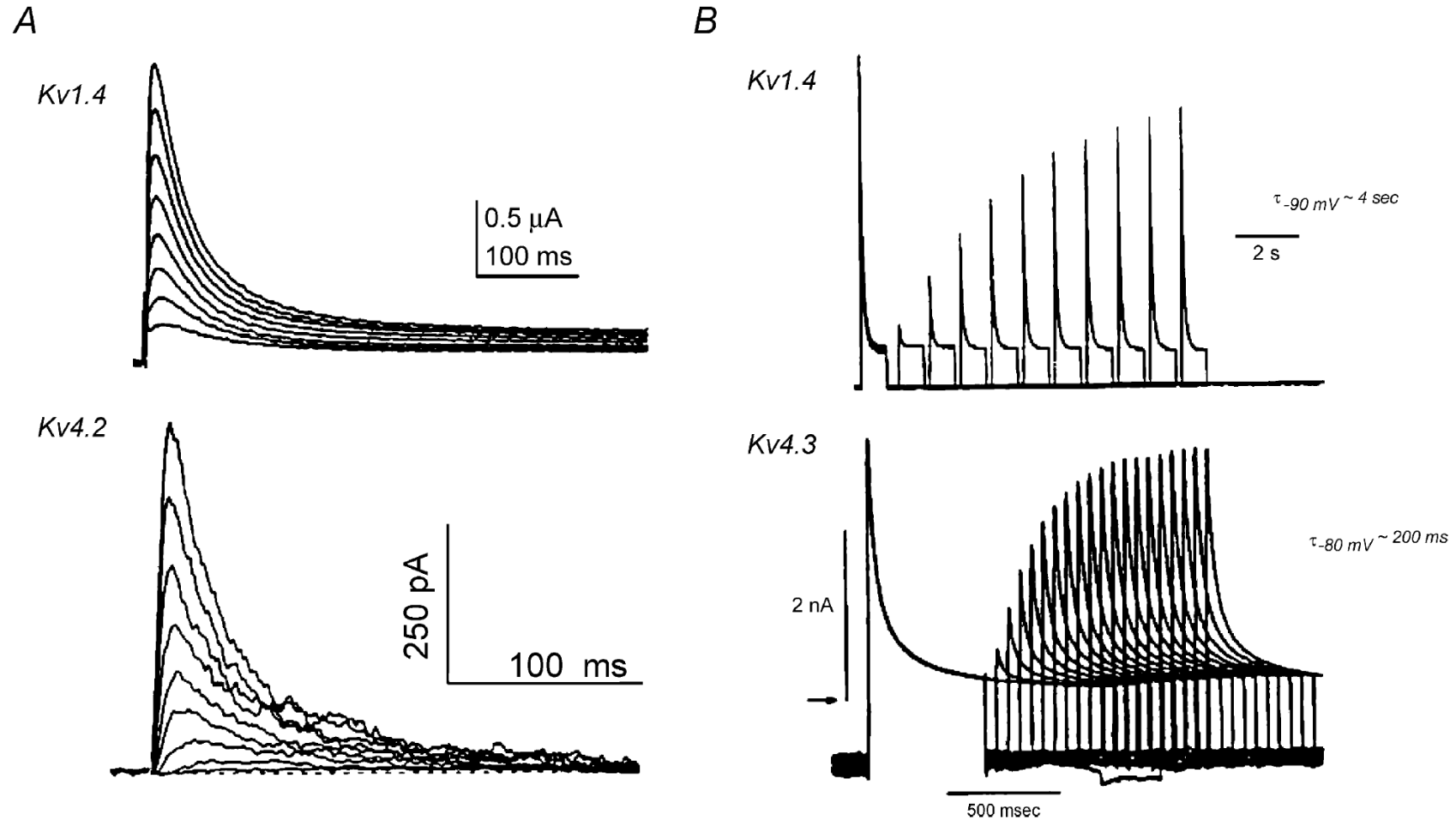


Patel et al (2004)
J Physiol 557:19

I_{to}: KChip2 accelerates recovery from inactivation of Kv4.3 channel current

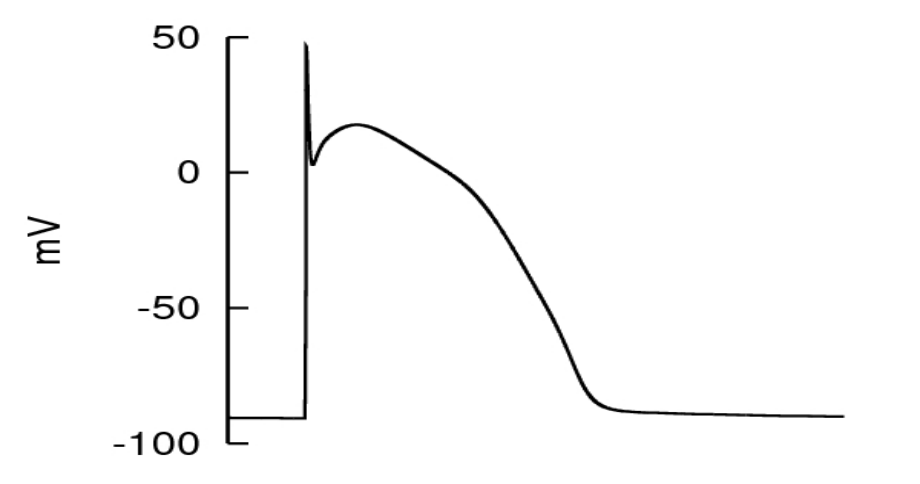


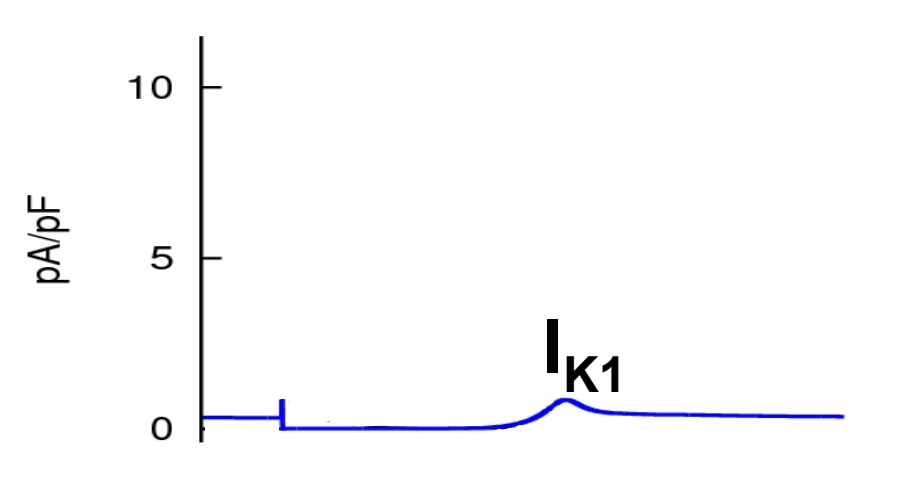
Two components of I_{to}: Kv1.4 (slow) and Kv4.2/3 (fast)

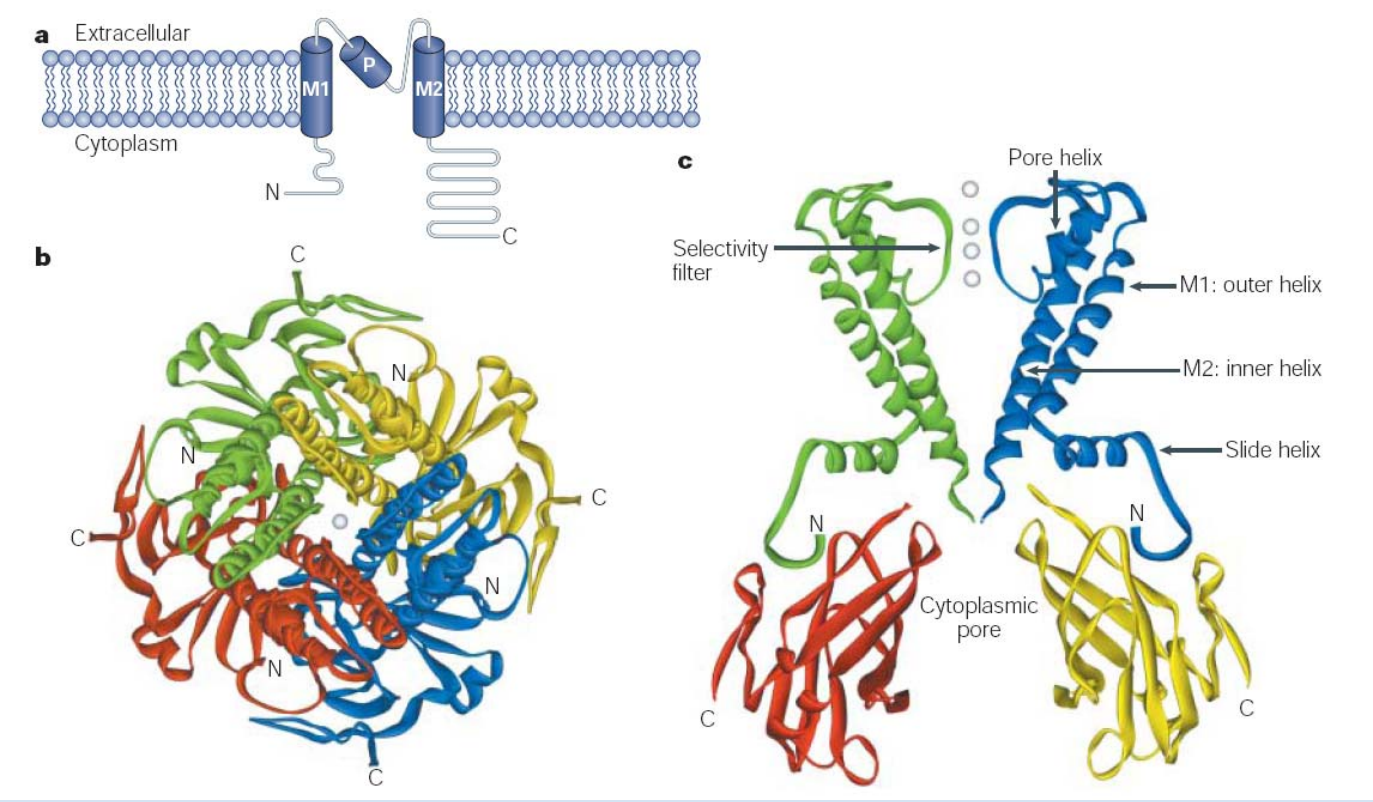


Patel et al (2004)
J Physiol 557:19

Inward rectifier K current: I_{K1}

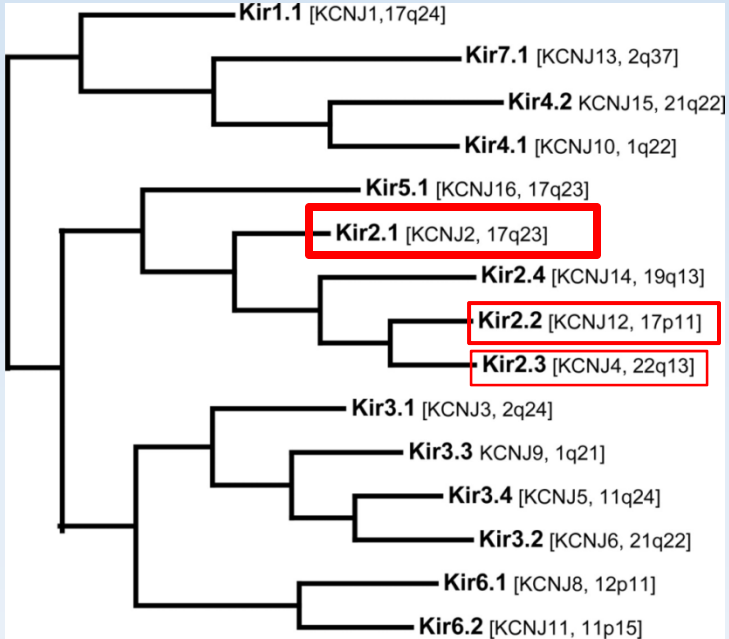






Bichet et al (2003) Nature Reviews Neurosci. 4:957

Inward rectifier K channels



Pharmacol Rev 57: 509-526, 2005

I_{K1}: I-V relationship determined from single channel recordings

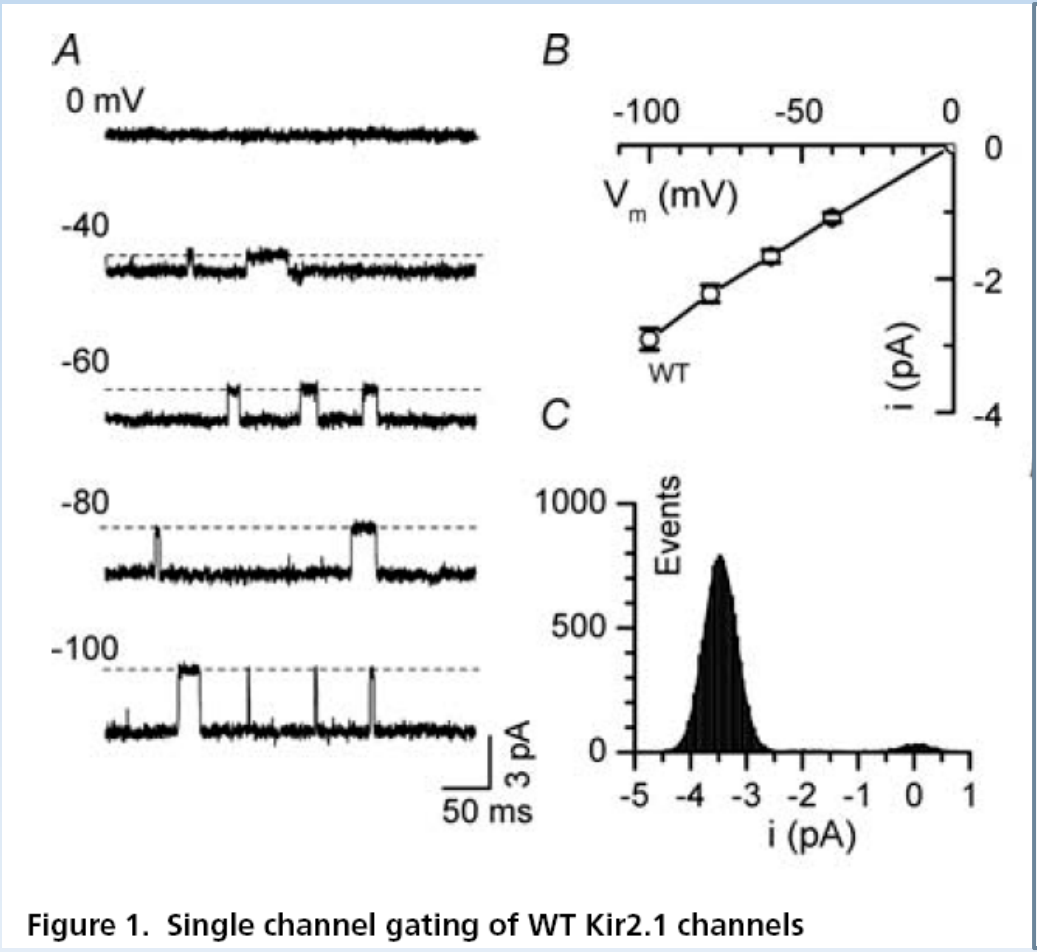
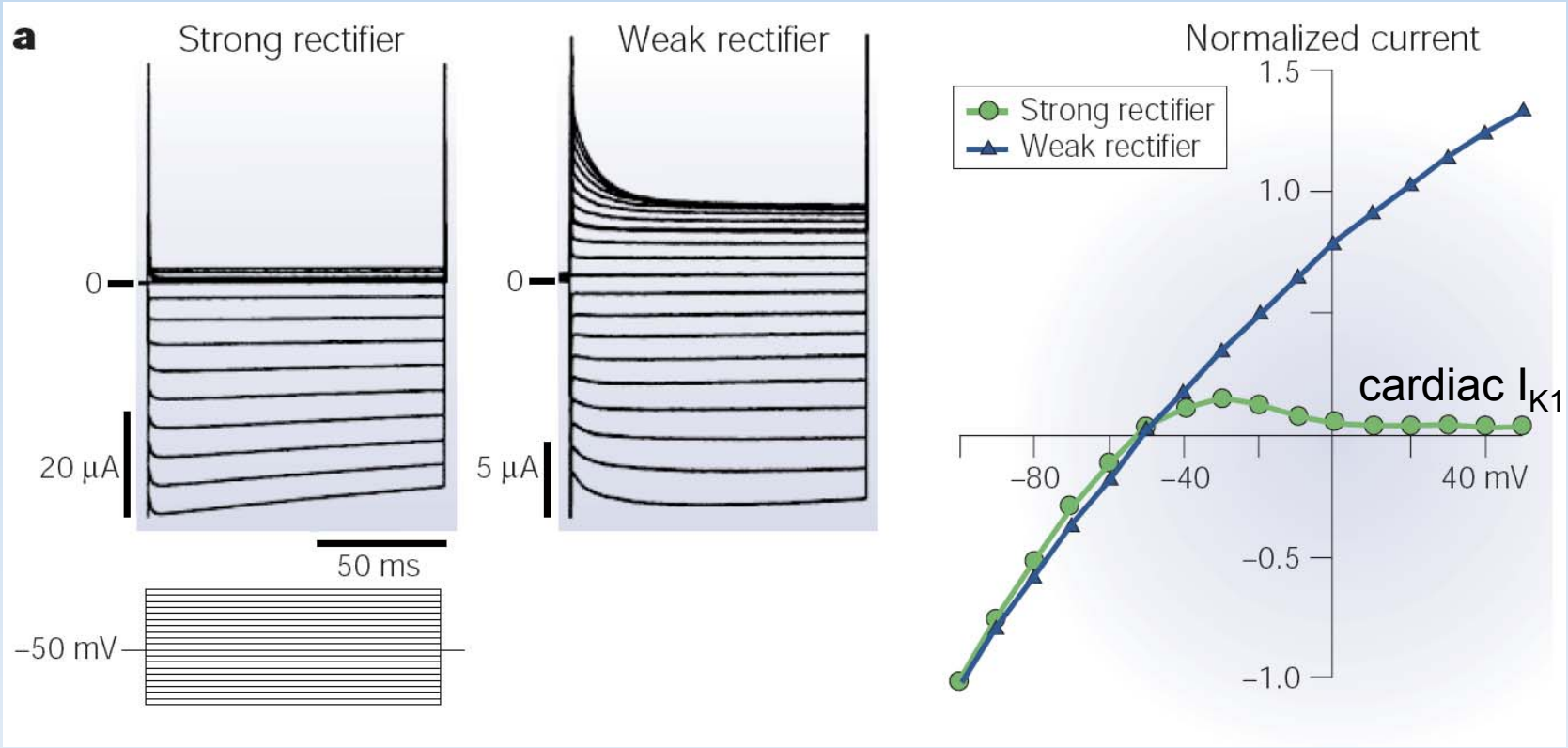


Figure 1. Single channel gating of WT Kir2.1 channels

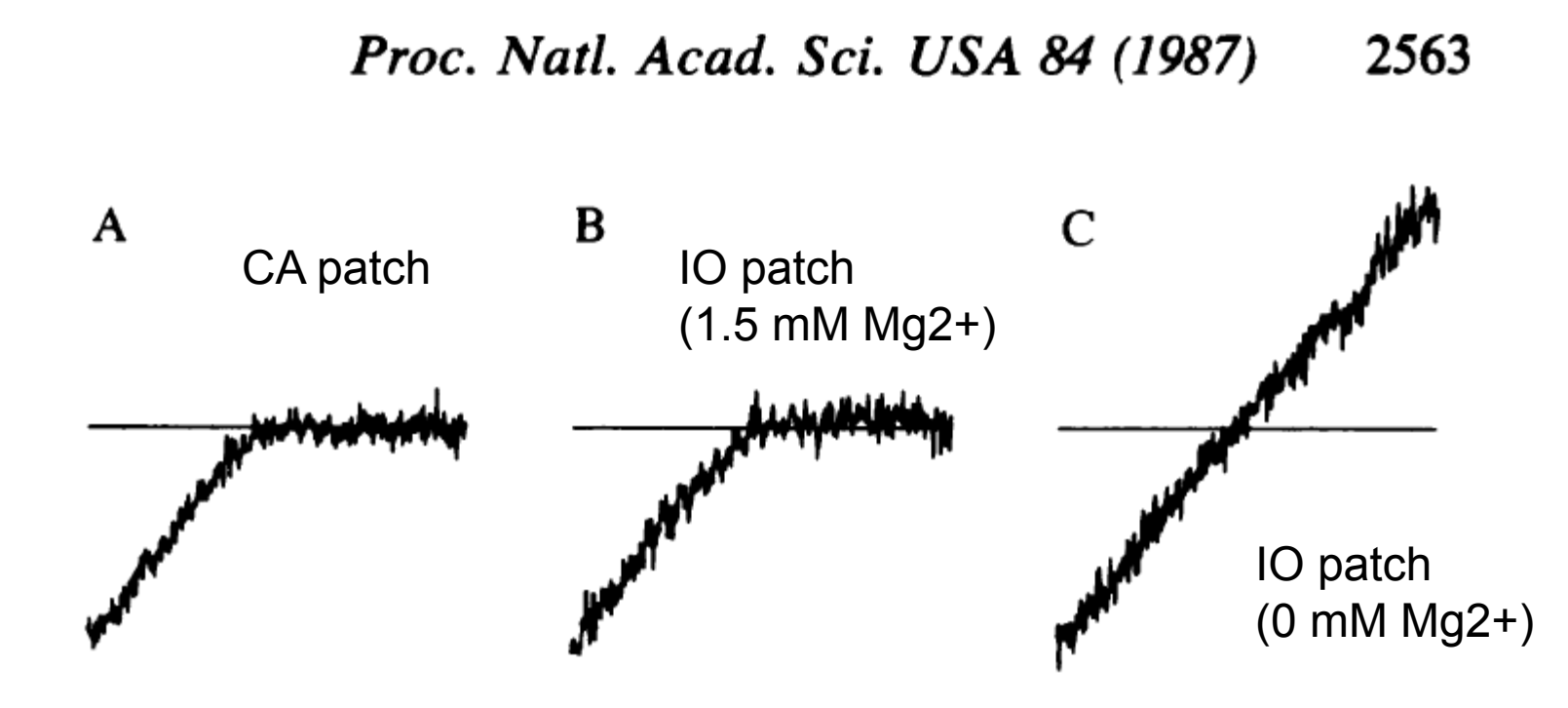
High $[K^+]_o$

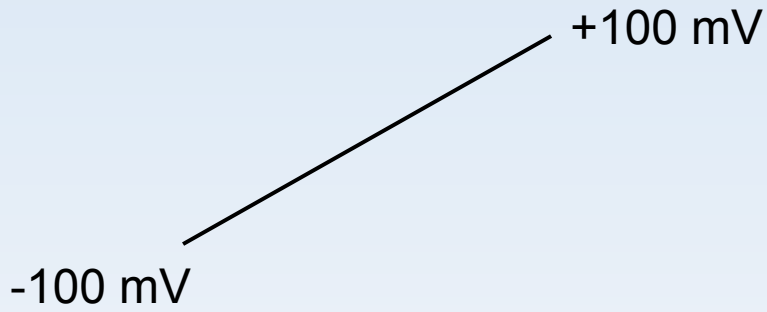


Normal $[K^+]_o$ (4 mM)

Bichet et al (2003)
Nature Reviews Neurosci. 4:957

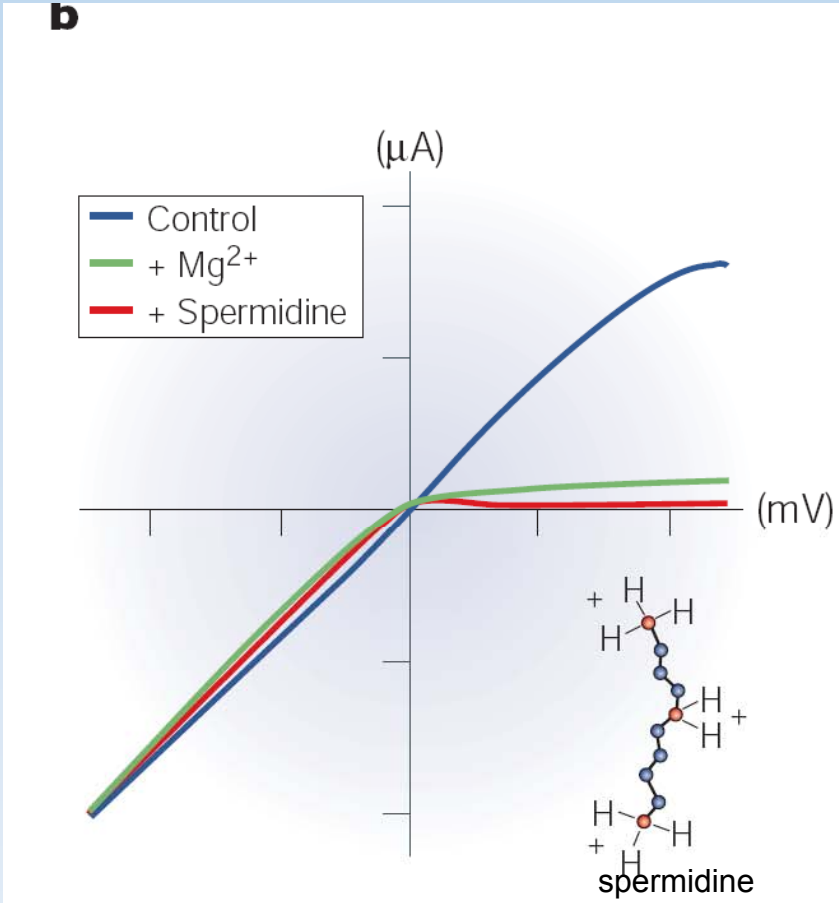
Current-voltage relationship of cardiomyocyte I_{K1}: Rectification caused by internal Mg^{2+} block

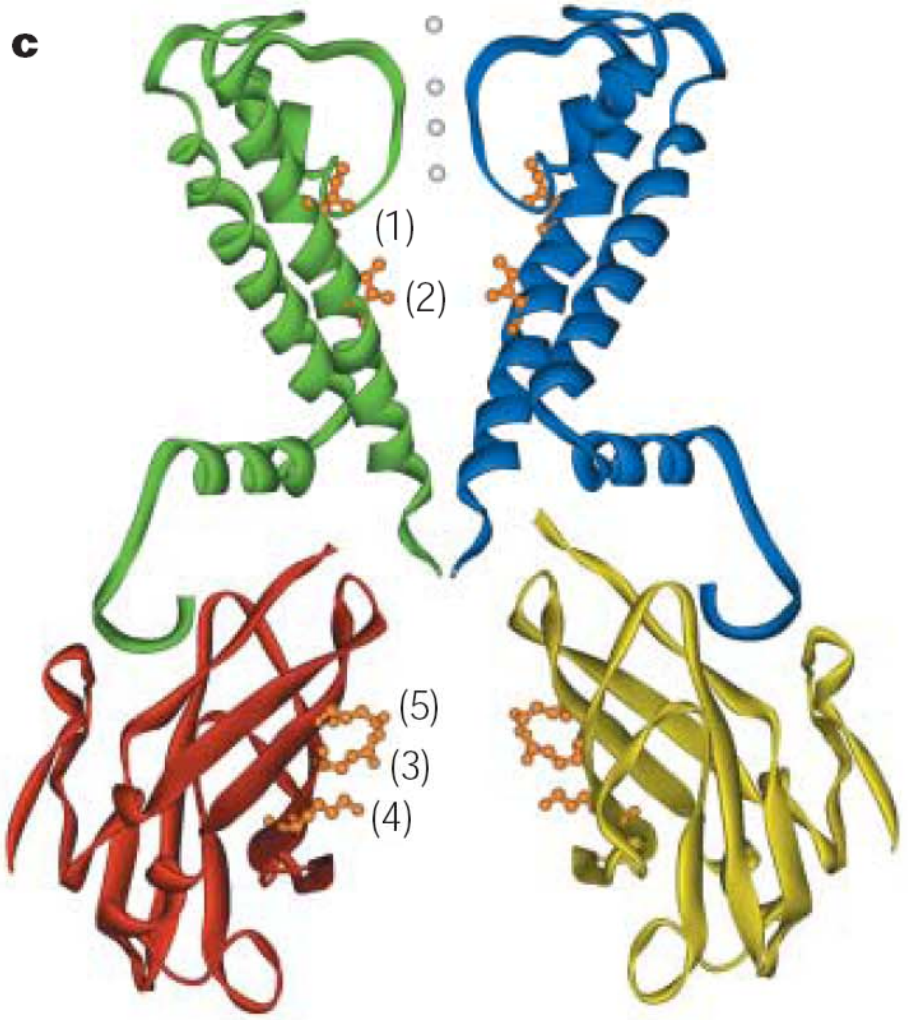




C. Vandenberg

Kir2.1 channel rectification: caused by blocking of pore by internal polyamines and Mg^{2+} ions

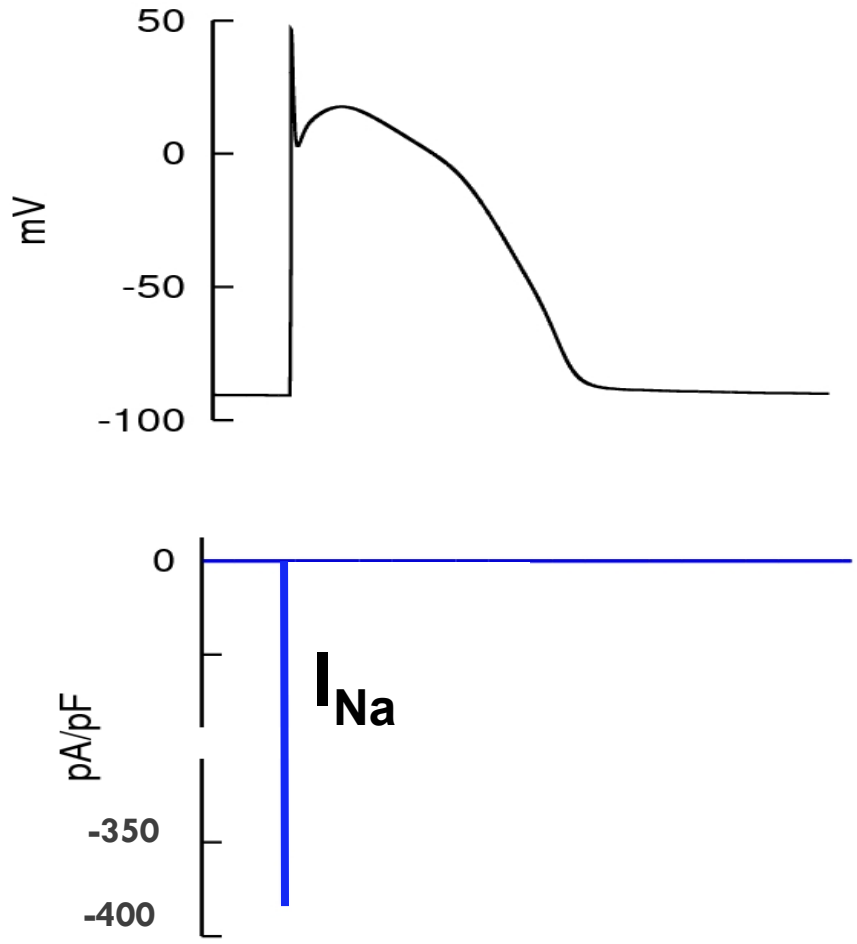




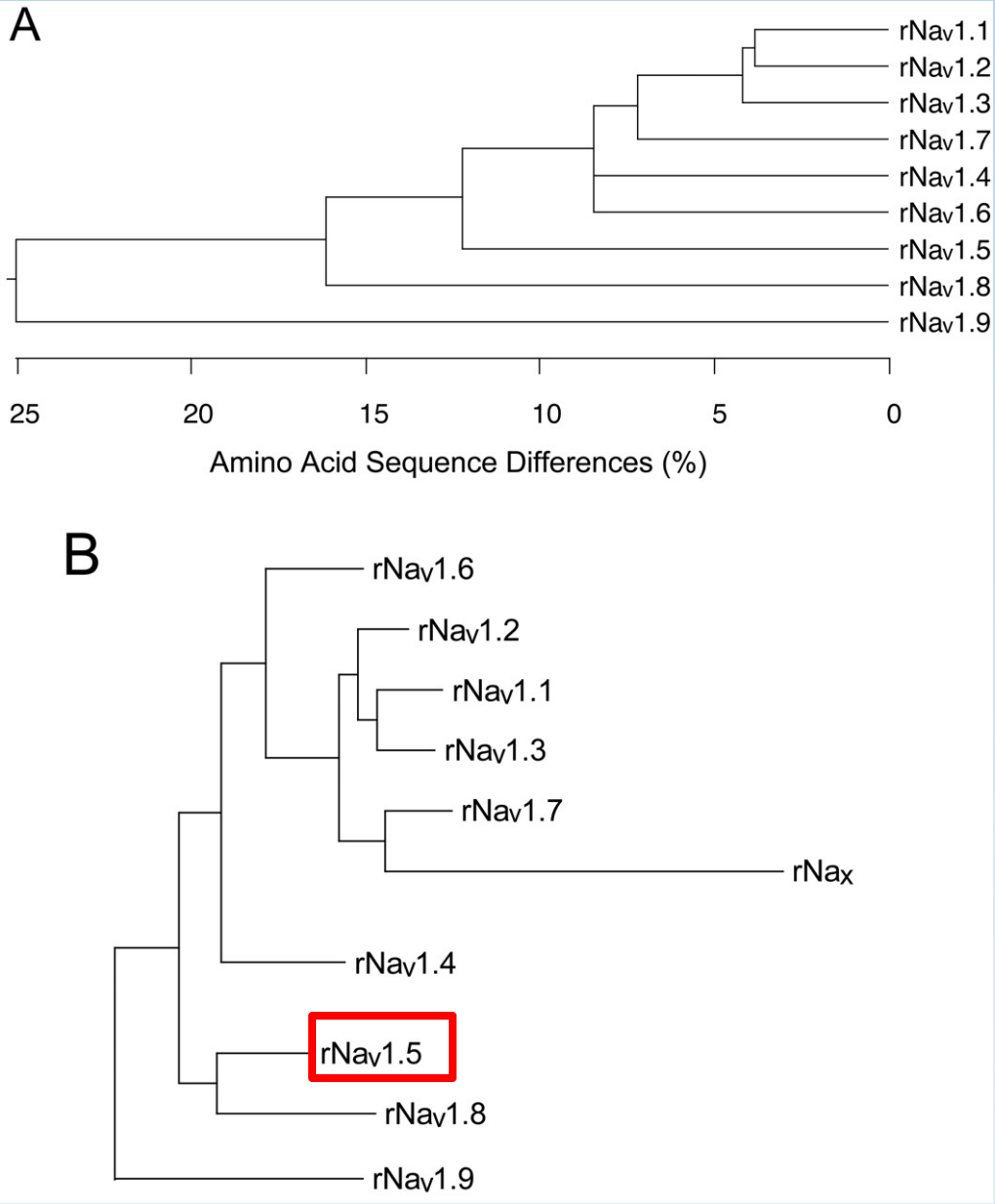
Bichet et al (2003)
Nature Reviews Neurosci. 4:957

1 – 5: residues that form binding sites for polyamines and Mg^{2+}

Inward sodium current: I_{Na}

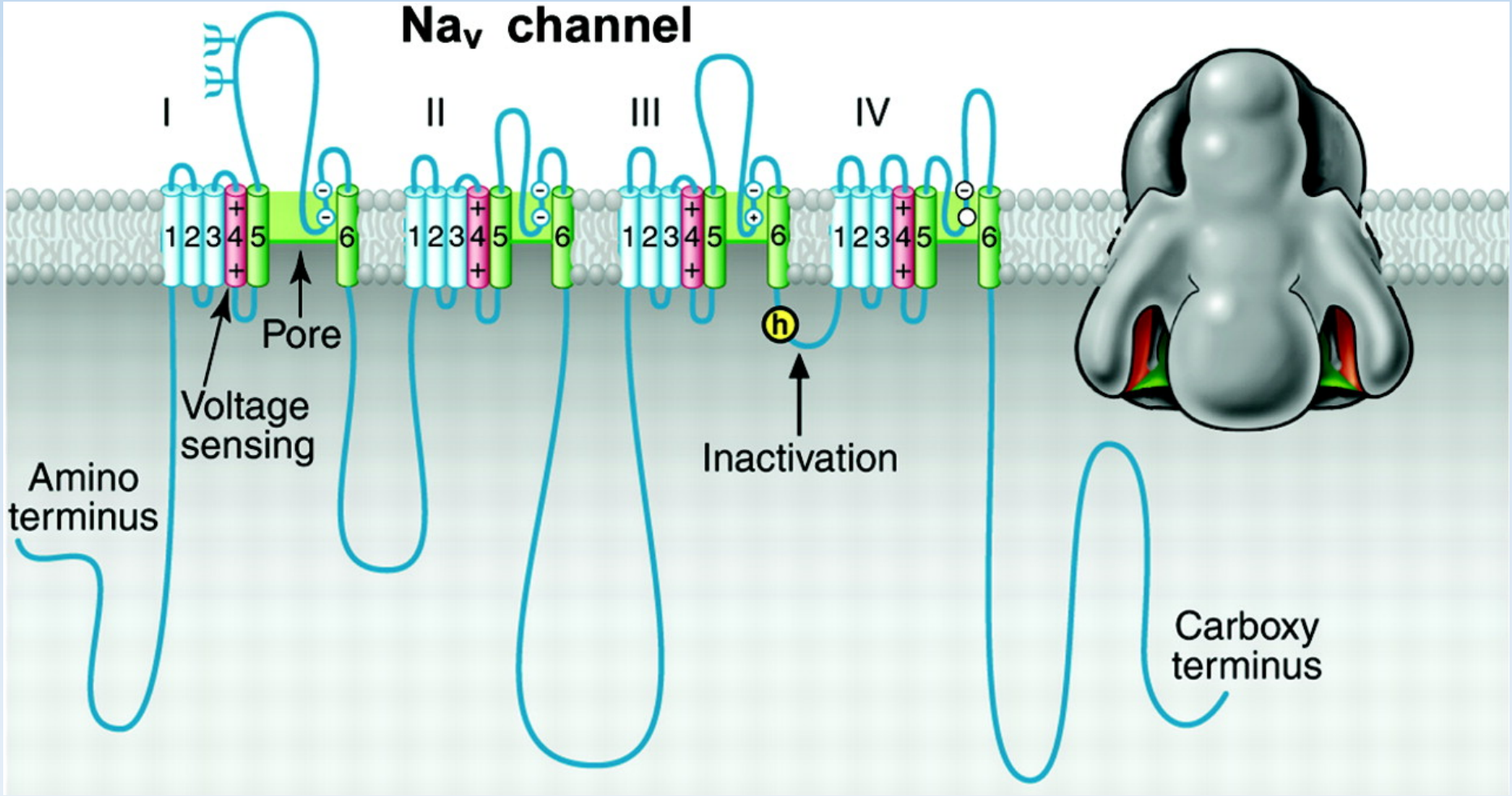


Na_V channels



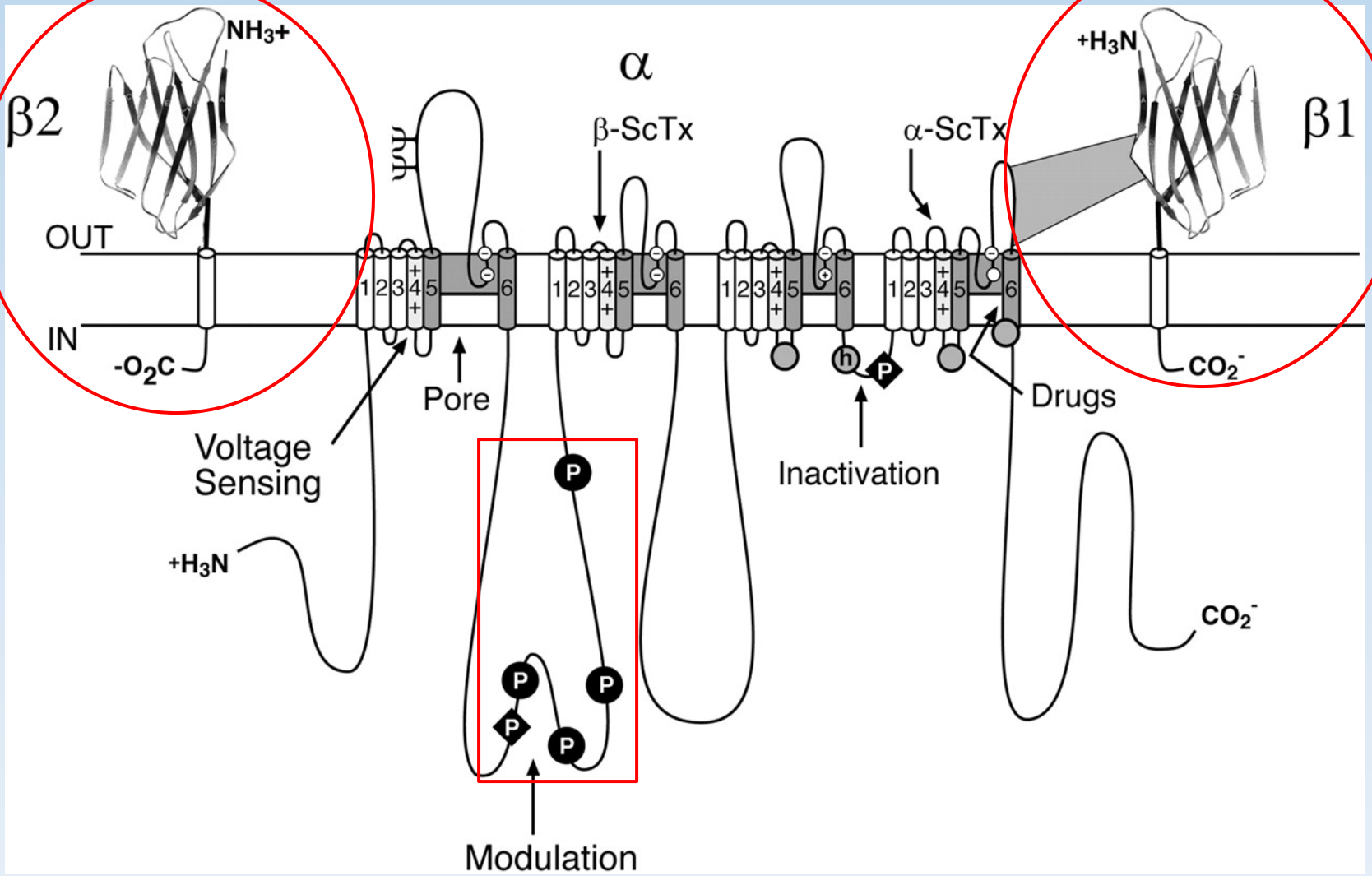
Pharmacol Rev 57:397-409, 2005

Structure of voltage-gated sodium channel



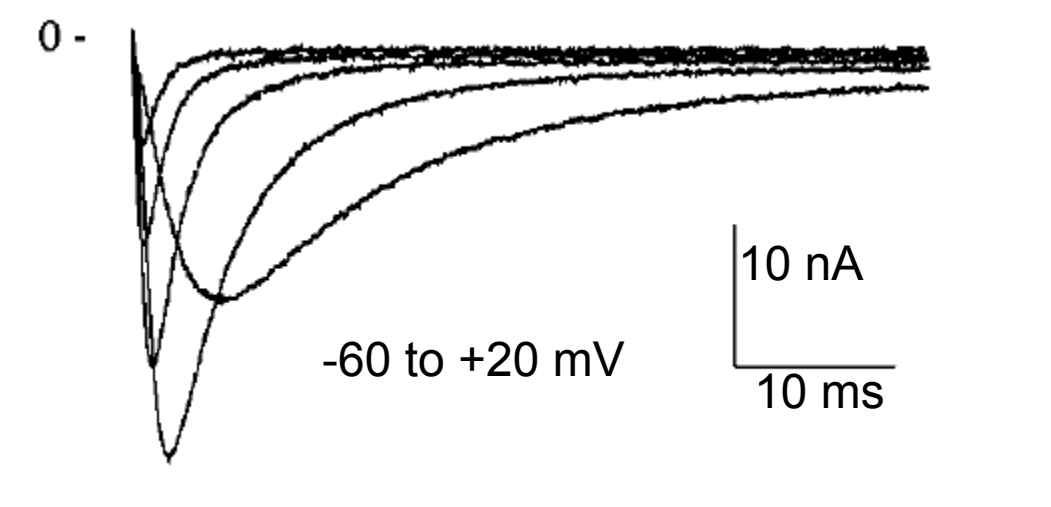
Pharmacol Rev 57:387-395, 2005

Na channel beta subunits and phosphorylation sites

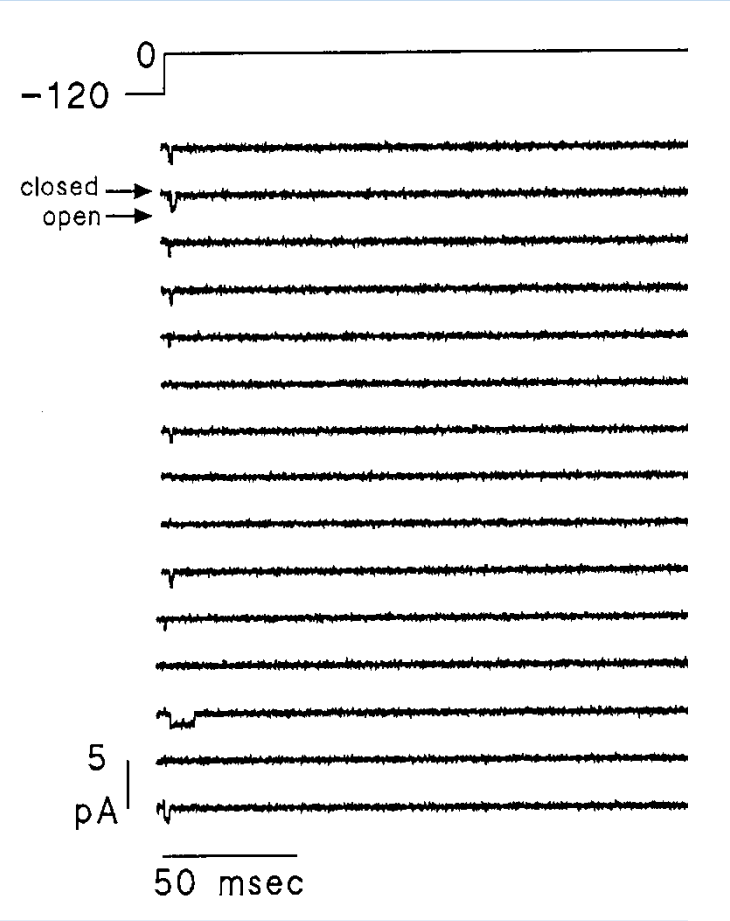


Pharmacol Rev 57:397-409, 2005

$Na_V1.5$ whole cell and single channel currents

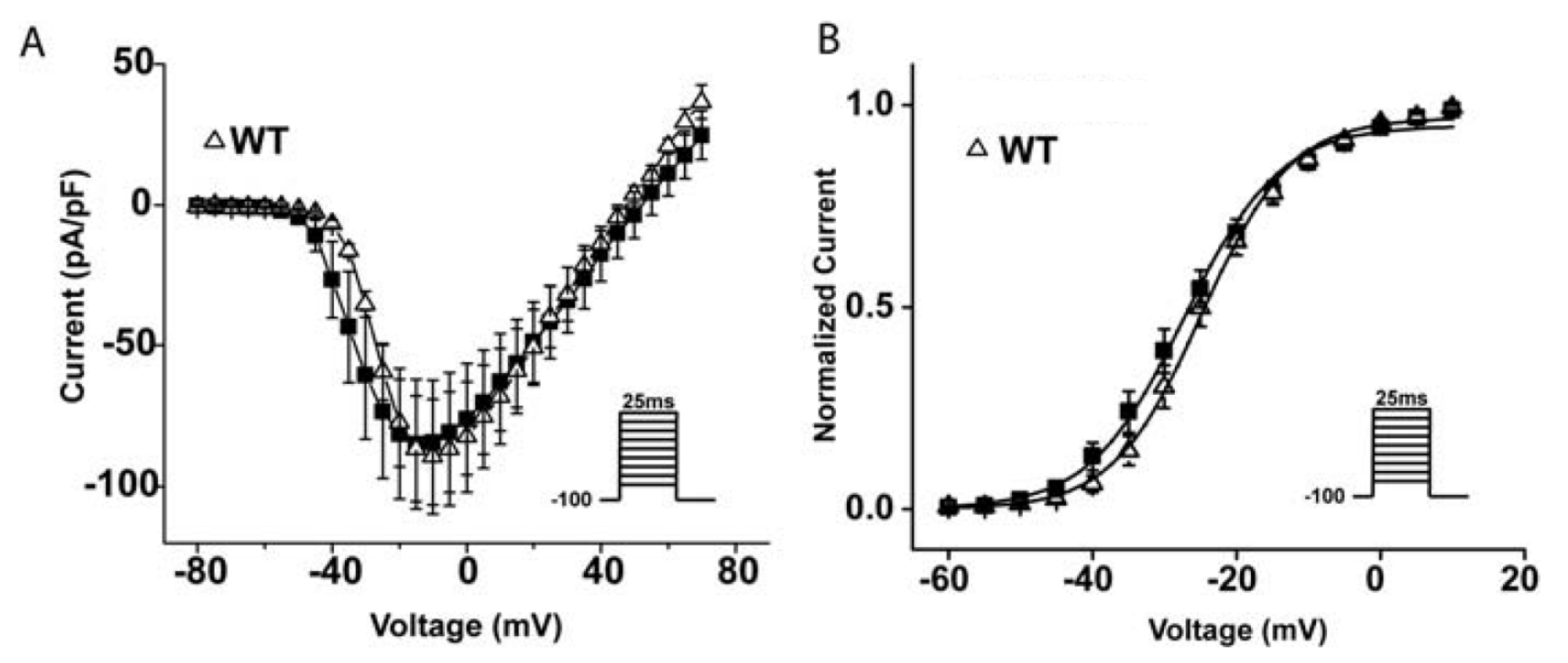


Chen and Sheets (2002)
Am J Physiol



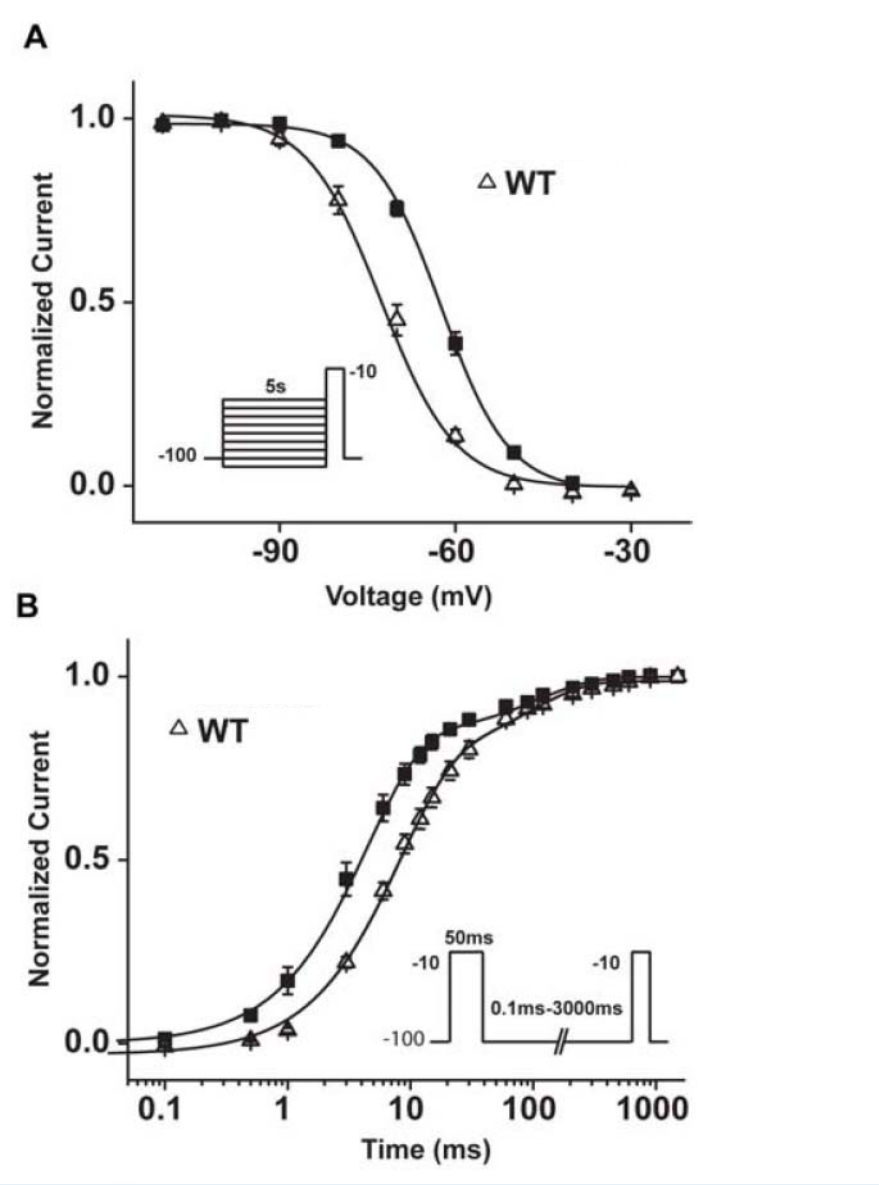
Bennett et al Nature

$Na_V1.5$: I-V and voltage dependence of activation



Bankston et al (2008)
Plos One 12:e1258

$Na_V1.5$: Steady-state voltage dependence and recovery from inactivation

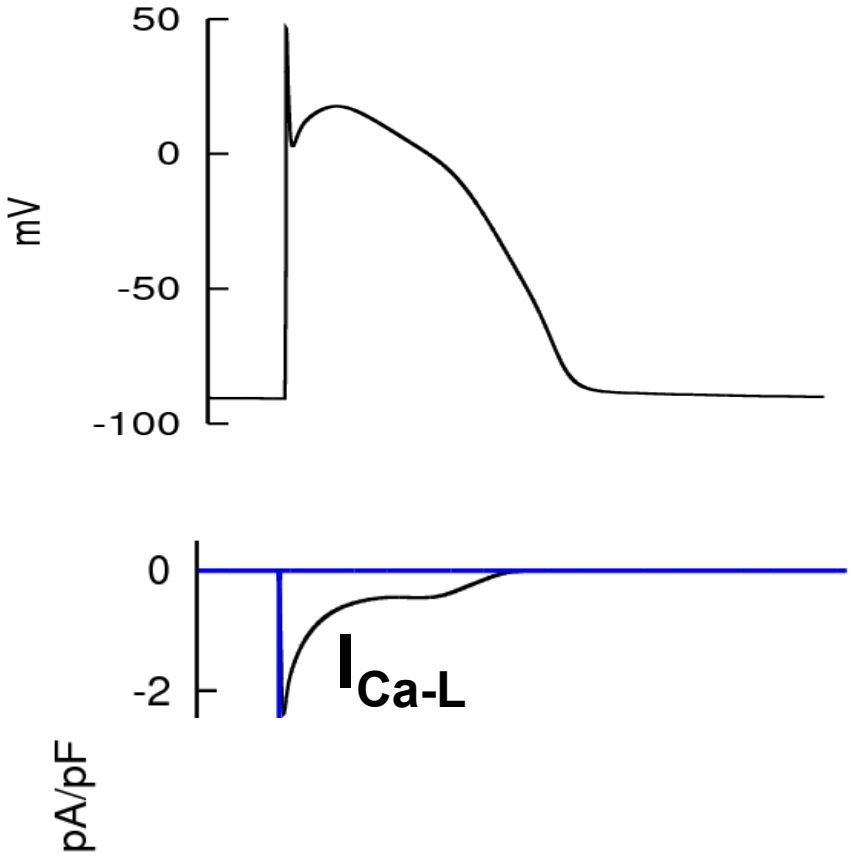


$V_{1/2}$ inact = -75 mV

recovery from inactivation

Bankston et al (2008)
Plos One 12:e1258

Inward L-type calcium current: $I_{Ca\text{-}L}$



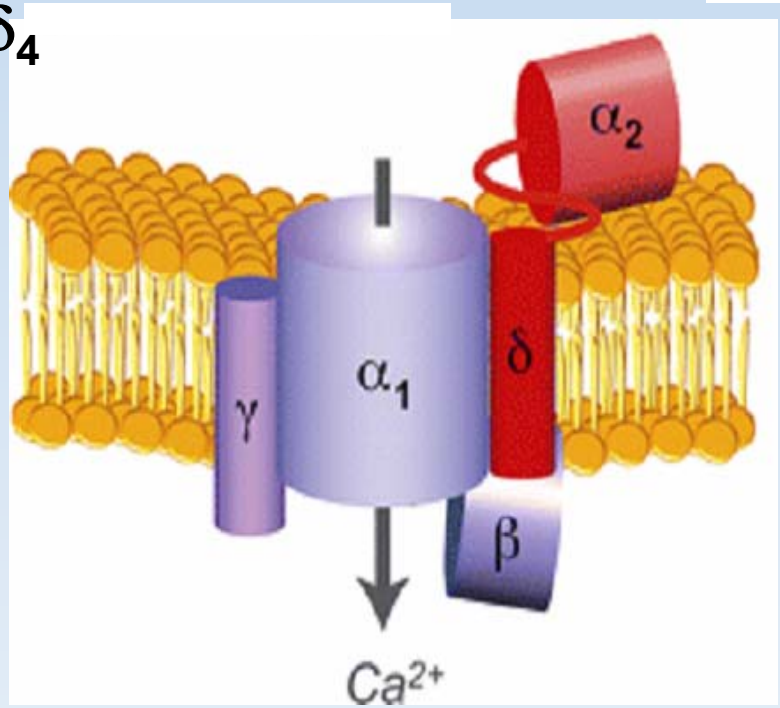
Voltage-gated calcium channels

Ancillary subunits

β_1 - β_4

γ_1 - γ_8

$\alpha_2\delta_1$ - $\alpha_2\delta_4$



α_1 subunits:

- $Ca_V1.1$
- $Ca_V1.2$
- $Ca_V1.3$
- $Ca_V1.4$

} L-type

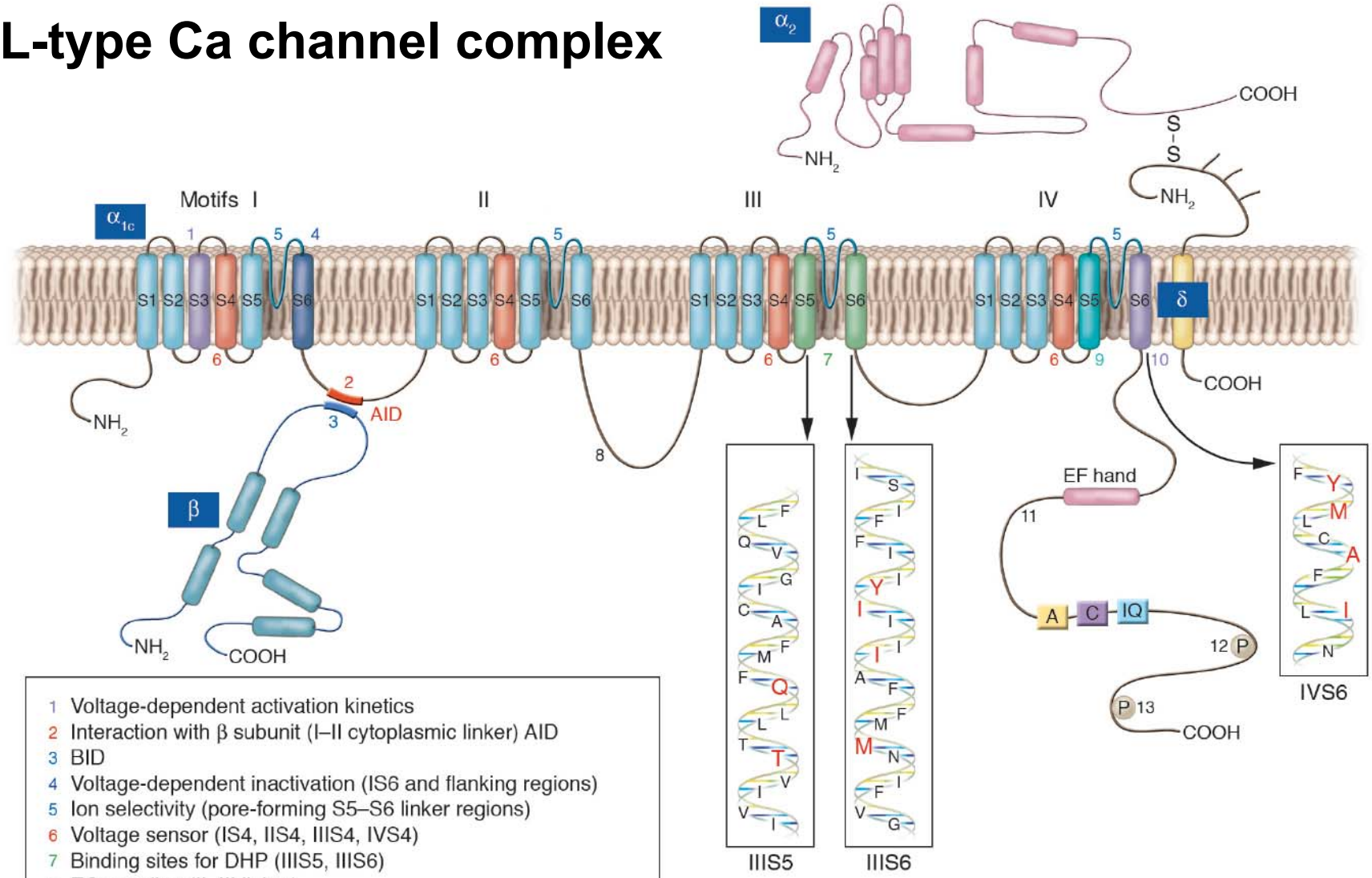
- $Ca_V2.1$ P/Q-type
- $Ca_V2.2$ N-type
- $Ca_V2.3$ R-type

- $Ca_V3.1$
- $Ca_V3.2$
- $Ca_V3.3$

} T-type

Heart L-type: $Ca_V1.2$, β_{2b}, $\alpha_2\delta_1$, (no

L-type Ca channel complex



1 Voltage-dependent activation kinetics
2 Interaction with β subunit (I–II cytoplasmic linker) AID
3 BID
4 Voltage-dependent inactivation (IS6 and flanking regions)
5 Ion selectivity (pore-forming S5–S6 linker regions)
6 Voltage sensor (IS4, IIS4, IIIS4, IVS4)
7 Binding sites for DHP (IIIS5, IIIS6)
8 EC coupling (II–III linker)
9 Role of use-dependent block (IIIS6, IVS5, IVS6)
10 Binding sites for BTZ, DHP, and PAA (IVS6)
11 Ca^{2+}-dependent inactivation (C terminal)
12 Phosphorylation site for caMKII (determines Ca^{2+} channel current facilitation and channel open probability)
13 Phosphorylation site for PKA (S1928: AKAP; A-kinase–anchoring protein)

Bodi et al (2005) *J Clin Invest*

Cardiomyocytes have two types of voltage-dependent Ca channels (T and L)

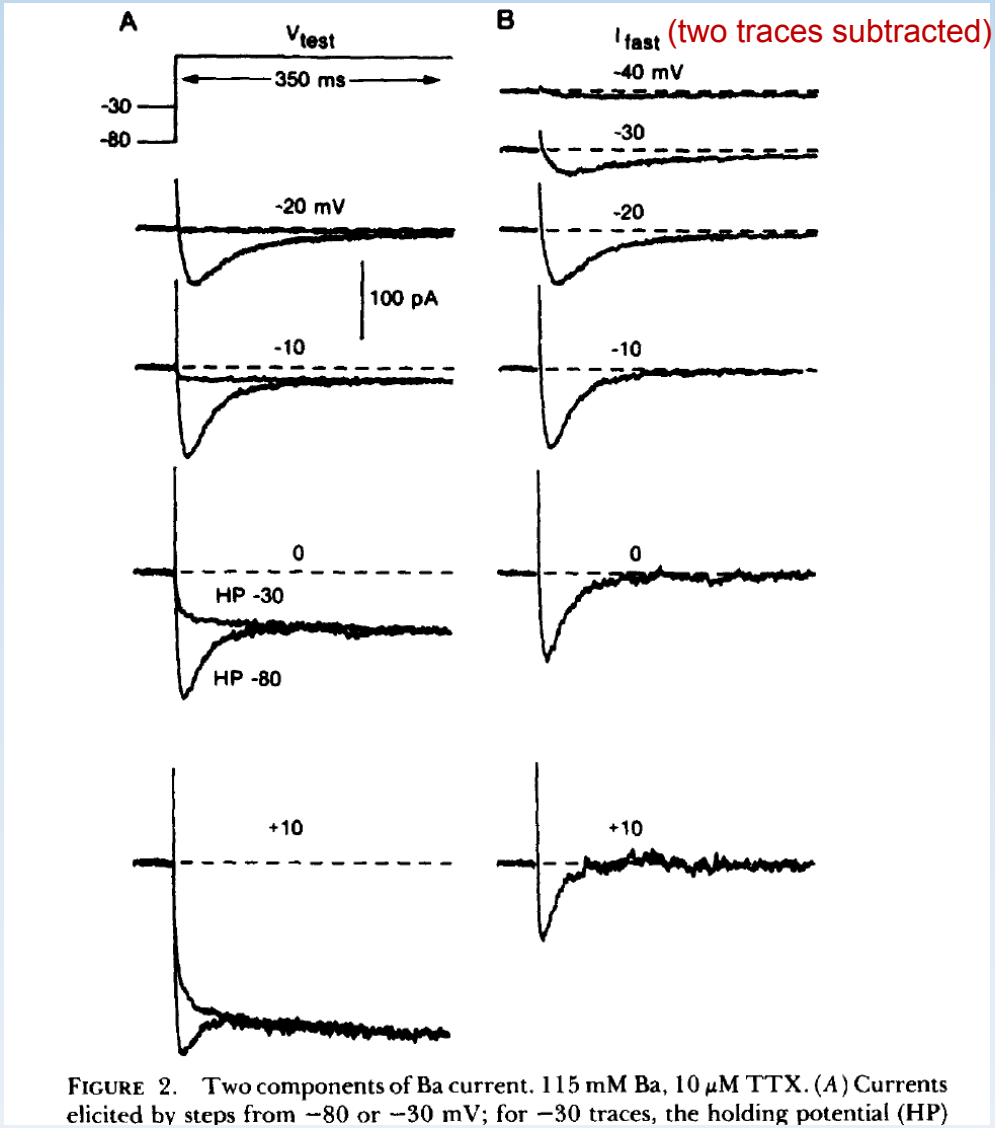
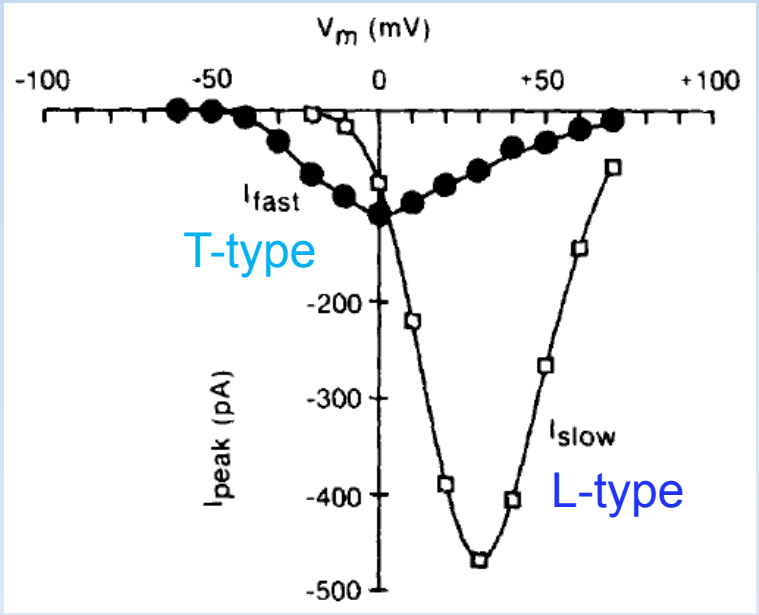


FIGURE 2. Two components of Ba current. 115 mM Ba, 10 μM TTX. (*A*) Currents elicited by steps from −80 or −30 mV; for −30 traces, the holding potential (HP)



canine atrial myocytes

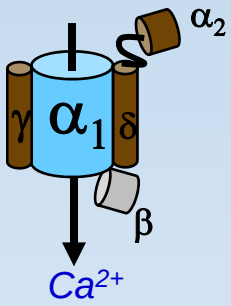
Bean (1985) *J Gen Physiol* 86:1

Physiological function and pharmacology of calcium channels

Channel	Current	Localization	Specific Antagonists	Cellular Functions
$Ca_V1.1$	L	Skeletal muscle; transverse tubules	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling
$Ca_V1.2$	**L**	Cardiac myocytes; smooth muscle myocytes; endocrine cells; neuronal cell bodies; proximal dendrites	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; hormone release; regulation of transcription; synaptic integration
$Ca_V1.3$	L	Endocrine cells; neuronal cell bodies and dendrites; cardiac atrial myocytes and pacemaker cells; cochlear hair cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Hormone release; regulation of transcription; synaptic regulation; cardiac pacemaking; hearing; neurotransmitter release from sensory cells
$Ca_V1.4$	L	Retinal rod and bipolar cells; spinal cord; adrenal gland; mast cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Neurotransmitter release from photoreceptors
$Ca_V2.1$	P/Q		-Agatoxin IVA	Neurotransmitter release; dendritic Ca^{2+} transients; hormone release
$Ca_V2.2$	N		-Conotoxin-GVIA	Neurotransmitter release; dendritic Ca^{2+} ; hormone release
$Ca_V2.3$	R			firing; dendritic calcium transients
$Ca_V3.1$	**T**			ng; repetitive firing
$Ca_V3.2$	**T**	and smooth muscle myocytes		ng; repetitive firing
Cav3.3	T	Neuronal cell bodies and dendrites		ng; repetitive firing

Pharmacol Rev 57:411-425, 2005

Calcium channelopathies (human)



- $Ca_V1.1$ (α_{1S} subunit; L-type)
  - Hypokalemic periodic paralysis type-1
  - Malignant hyperthermia
- $Ca_V1.4$ (α_{1F} subunit; L-type)
  - Stationary night blindness type-2
- $Ca_V2.1$ (α_{1A} subunit; P/Q-type)
  - Familial hemiplegic migraine
  - Episodic ataxia type-2
  - Spinocerebellar ataxia type-6
  - Episodic/progressive ataxia
- $Ca_V3.2$ (α_{1H} subunit, T-type)
  - Idiopathic generalized epilepsy
- CaV1.2 (α_{1C} subunit, L-type)
  - Timothy syndrome (arrhythmia, etc)

Long QT syndrome: 9 genes, many mutations

(incidence: ~1/2000 in general population)

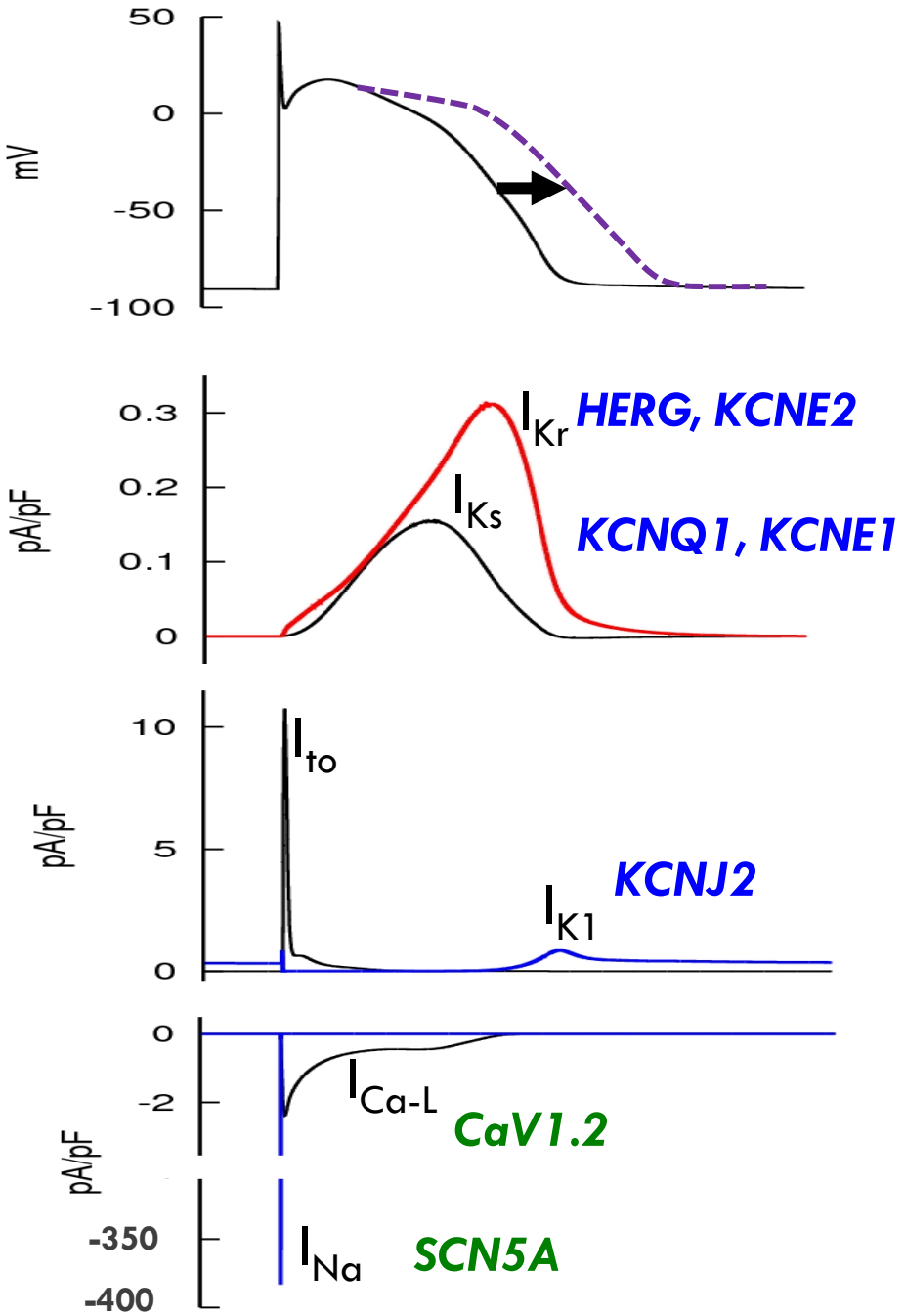
locus	Gene	channel protein	# of mutations	
LQT2	***HERG***	I_{Kr} α-subunit	291	**Romano-Ward syndrome** (dominant)
LQT6	***KCNE2***	β-subunit	11	
LQT1	***KCNQ1***	I_{Ks} α-subunit	246	**Jervell and Lange-Nielsen syndrome** (recessive)
LQT5	***KCNE1***	β-subunit	30	
LQT3	***SCN5A***	I_{Na} α-subunit	77	
LQT7	***KCNJ2***	I_{K1} α-subunit	29	
LQT8	***CACNA1C***	I_{CaL} α-subunit	3	
LQT4	***ANKB***	ankyrin-B	11	
LQT9	***CAV3***	caveolin-3	5	

Gene Connection for the Heart website (Carlo Napolitano) – Nov 2007

Long QT syndrome

Loss of function mutations in 5 different K channel genes

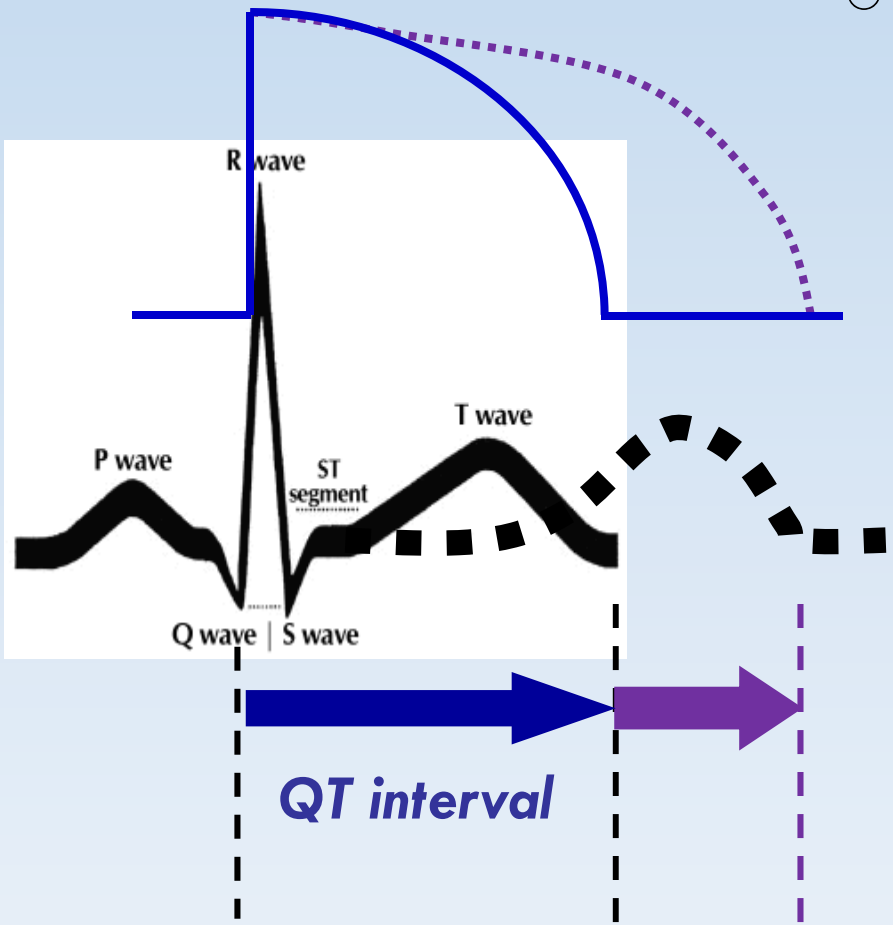
Gain of function mutations in Na or Ca channel genes

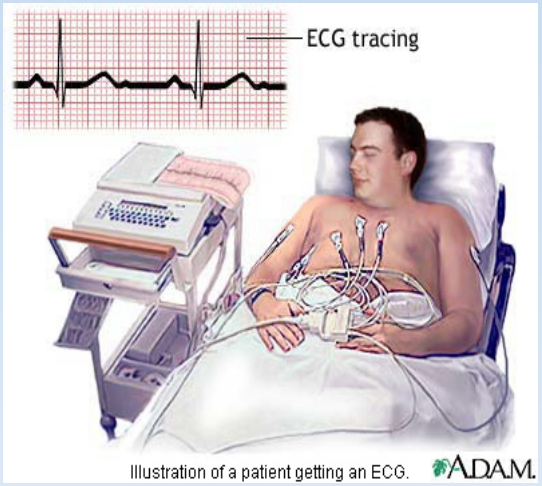


Long QT syndrome: prolonged ventricular repolarization

"long": QT_c > 440 - 460 ms

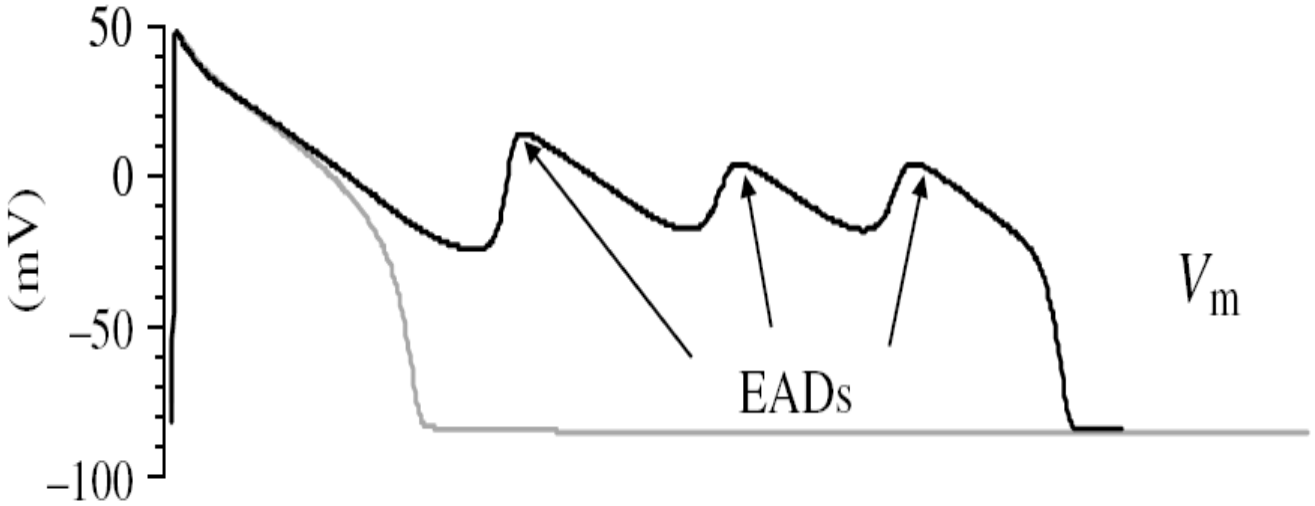
♂ ♀





Long QT syndrome:

early afterdepolarizations (EADs) trigger arrhythmia

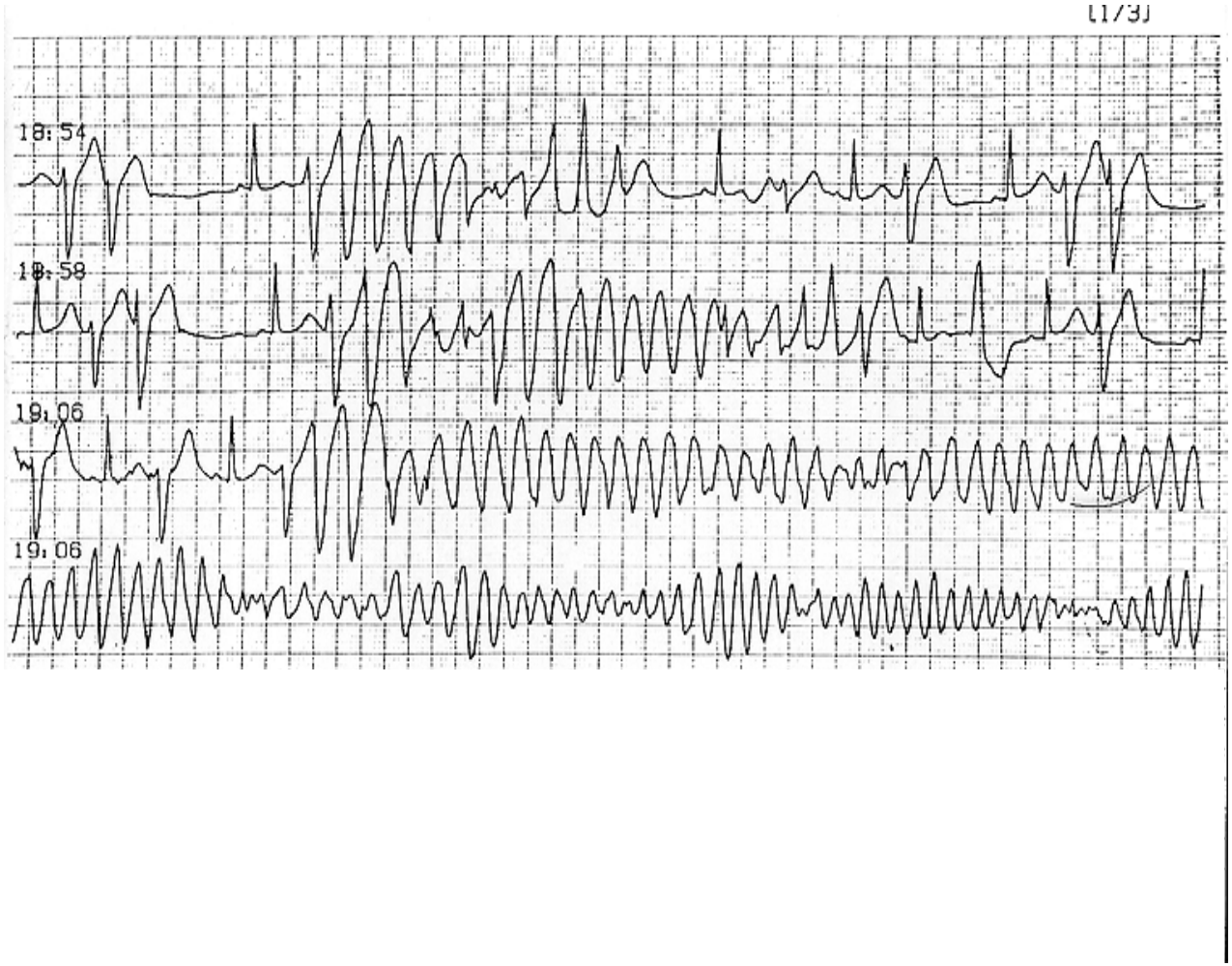


Rudy and Silva (2006)
Quarterly Rev Biophys

Torsades de pointes

(signature arrhythmia of long QT syndrome)

Continuous ECG tracing



TdP

ventricular fibrillation → *sudden death*

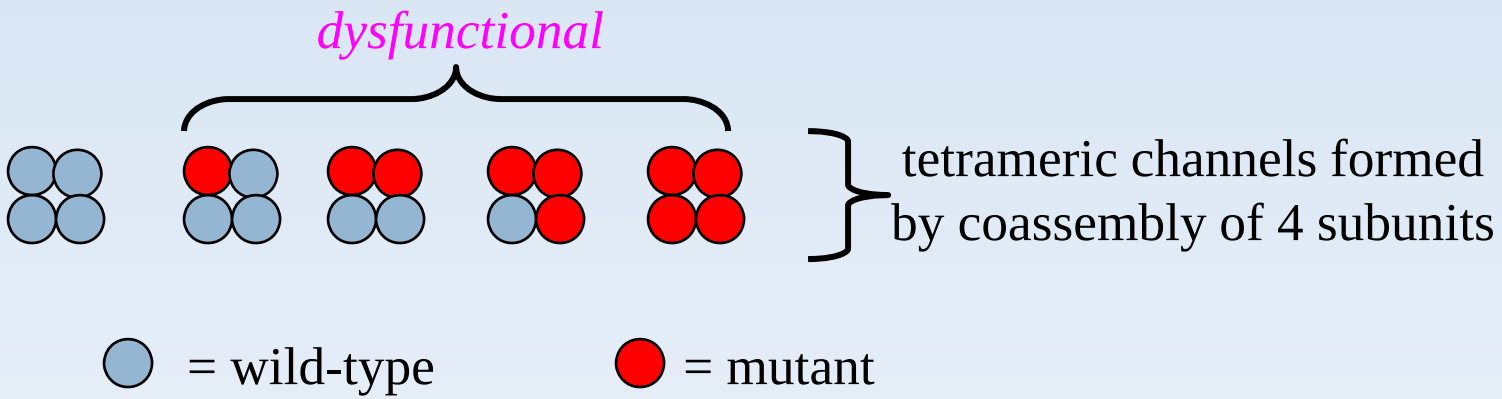
K channel mutations in LQTS

Mechanisms:

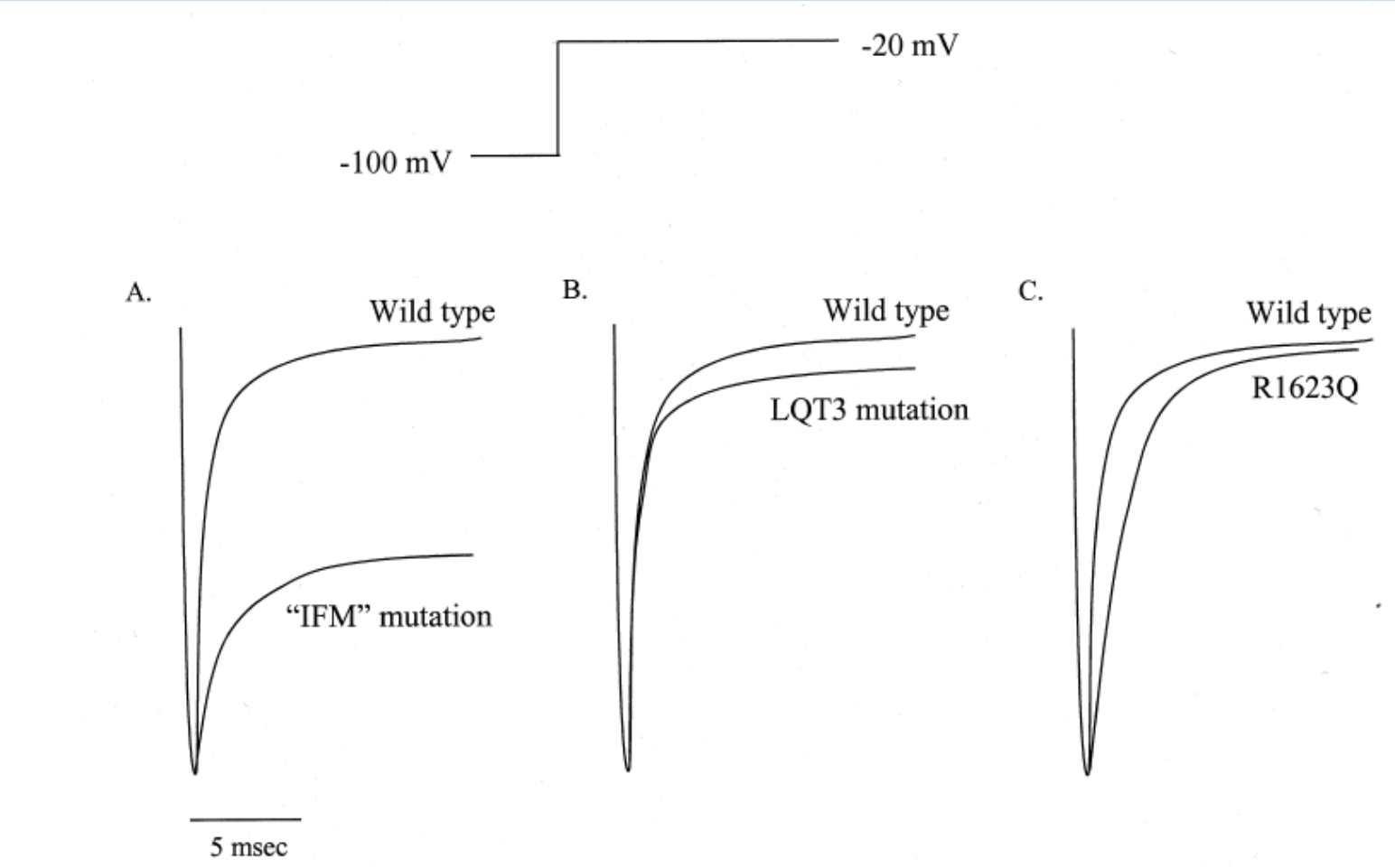
1. Loss of function (biophysical, or protein misfolding)

2. Altered function

3. Dominant-negative effect
   - coassembly of mutant with normal subunits alter, or destroy heteromultimeric channel function



Mutations can alter Na channel inactivation



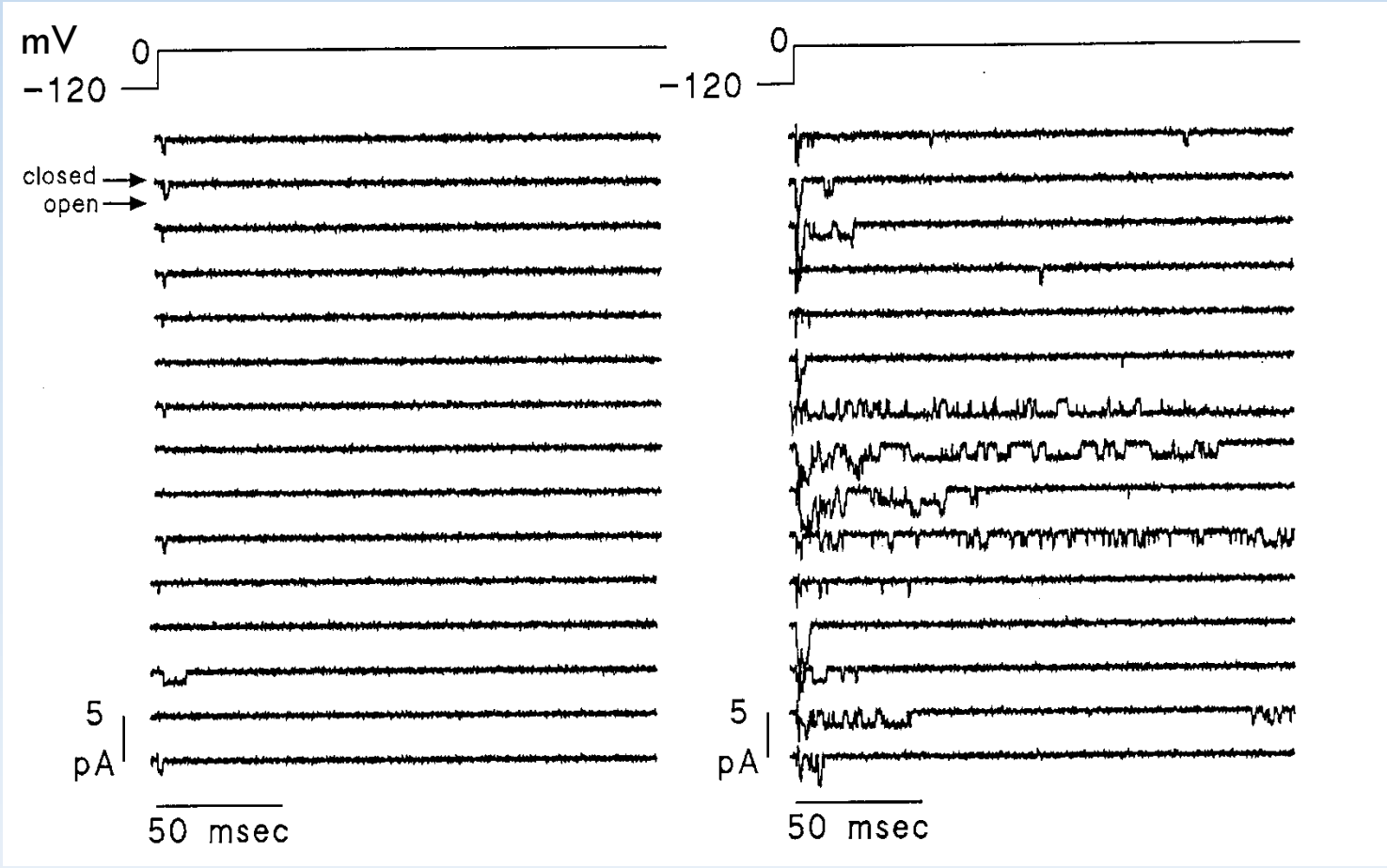
Balser (1999)
Cardiovasc Res 42:327

Long QT syndrome:
Gain of function mutation in $Na_V1.5$ channel

Wild-type Na channel
single channel currents

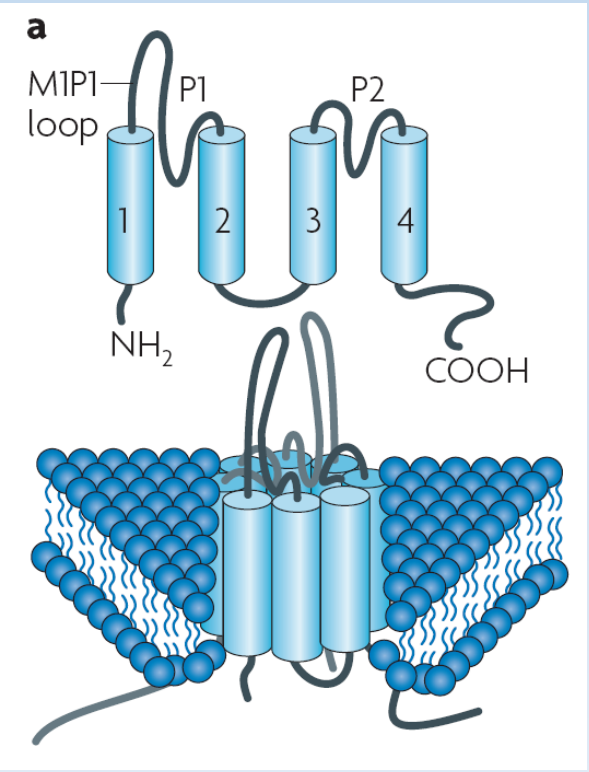
mutant Na channel
(impaired inactivation)

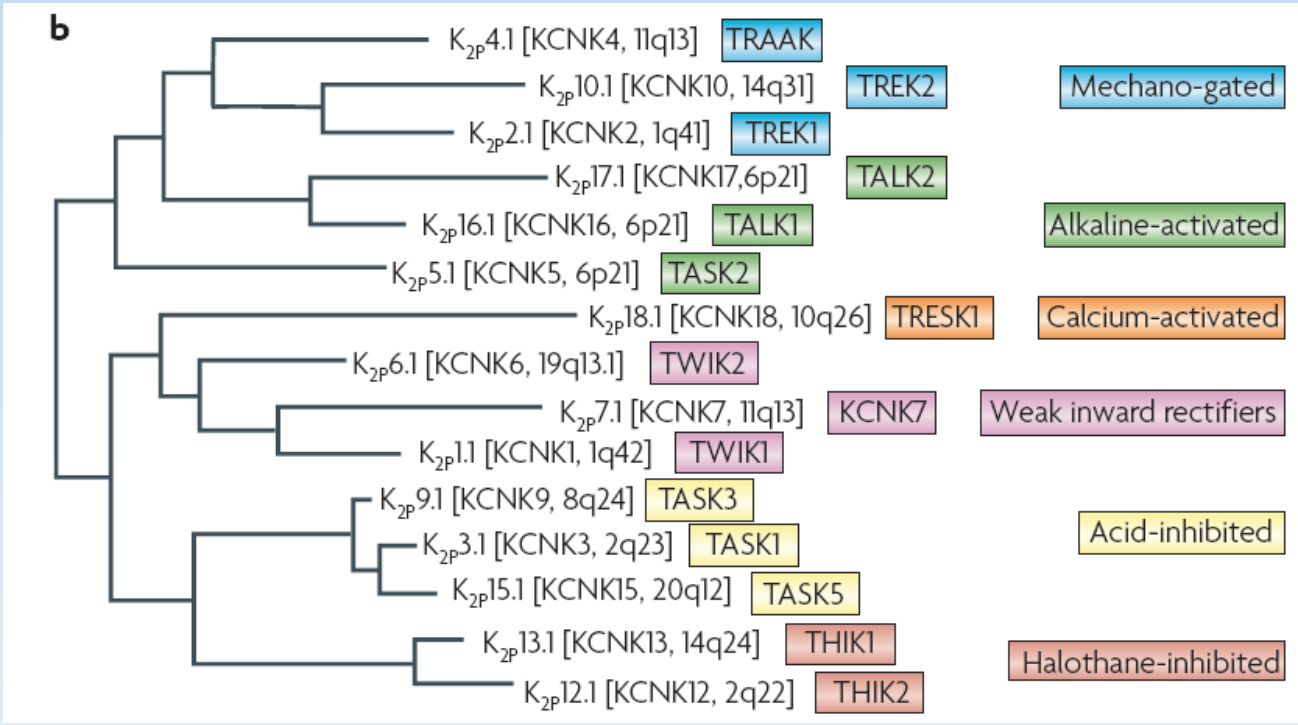
ΔKPQ



Other channels

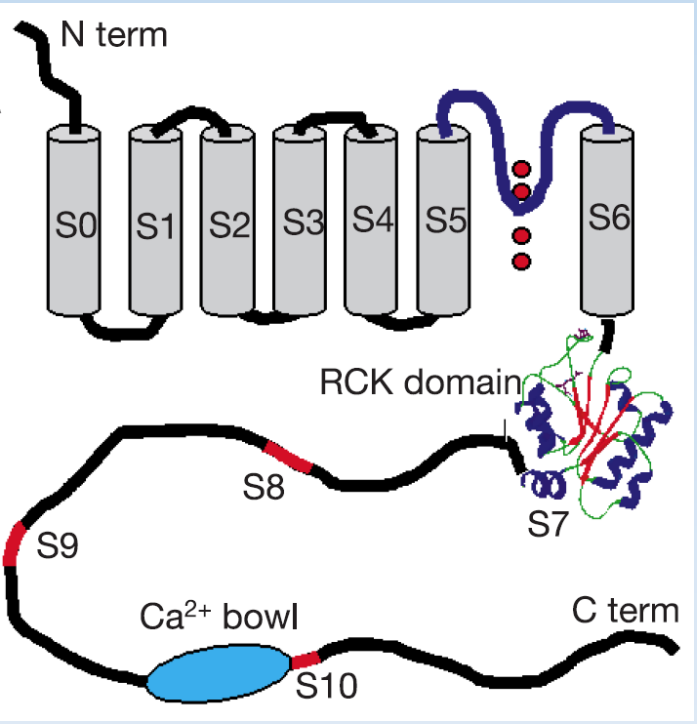
Two-pore K channels: “leak” channels





Honore (2007) *Nature Rev Neurosci* 8:251

Calcium- activated K channels



A

KCa2.2 [SKCa2, KCNN2, 5q2.2]

KCa2.3 [SKCa3, KCNN3, 1q21]

KCa2.1 [SKCa1, KCNN1, 19p13]

KCa3.1 [IKCa1, KCNN4, 19q13]

B

KCa1.1 [slo, BK, KCNMA1, 10q22]

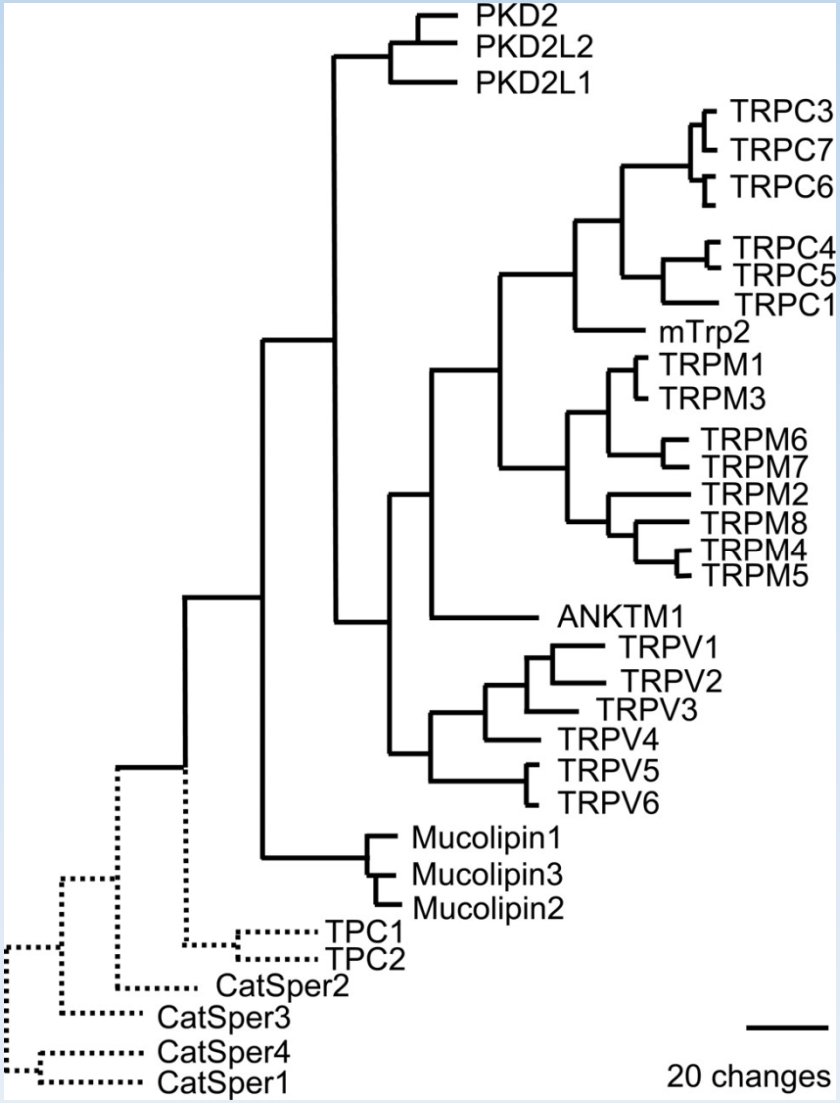
KCa5.1 [slo3, KCNU1, 8p11]

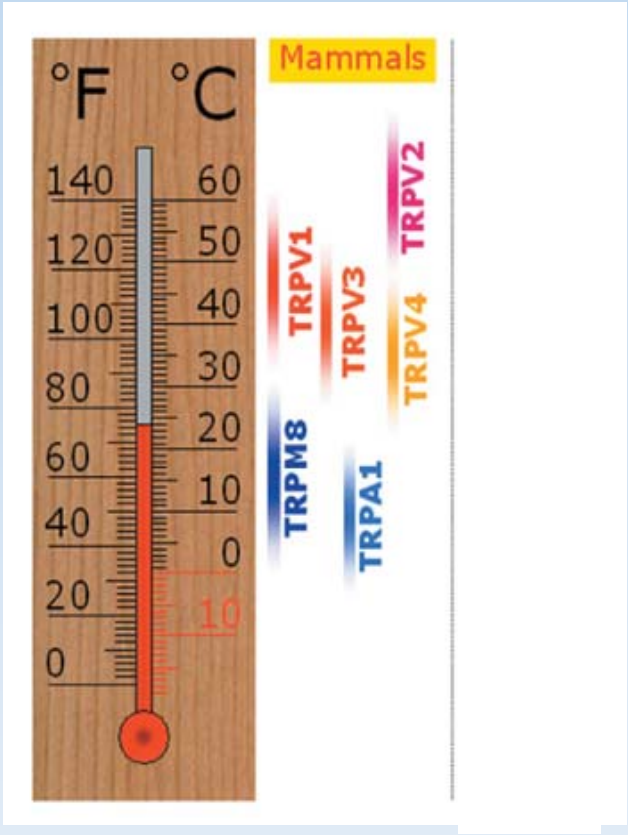
KCa4.1 [slack, slo2.2, KCNT1, 9q34]

KCa4.2 [slick, slo2.1, KCNT2, 1q31]

Na-activated

TRP channels and CatSper channels

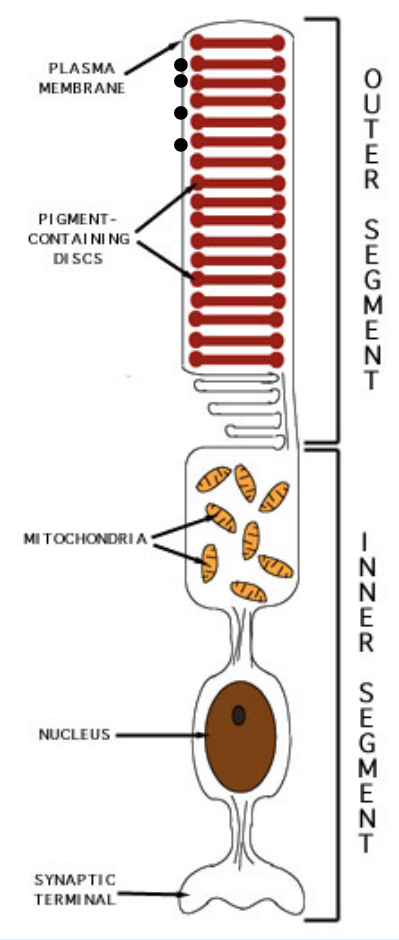




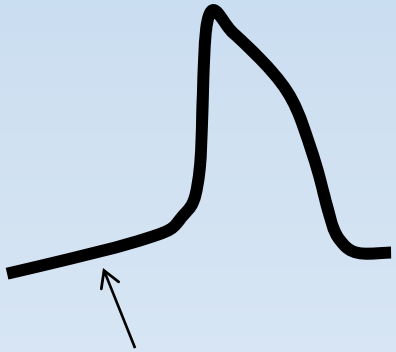
Pharmacol Rev 57:427-450, 2005

Cyclic-nucleotide gated channels

- CNGA1
- CNGA2
- CNGA3
- CNGA4
- CNGB1
- CNGB3
- HCN1
- HCN2
- HCN3
- HCN4



CNG channels ●
in photoreceptors (shown)
and olfactory neurons



HCN channels
pacemaking

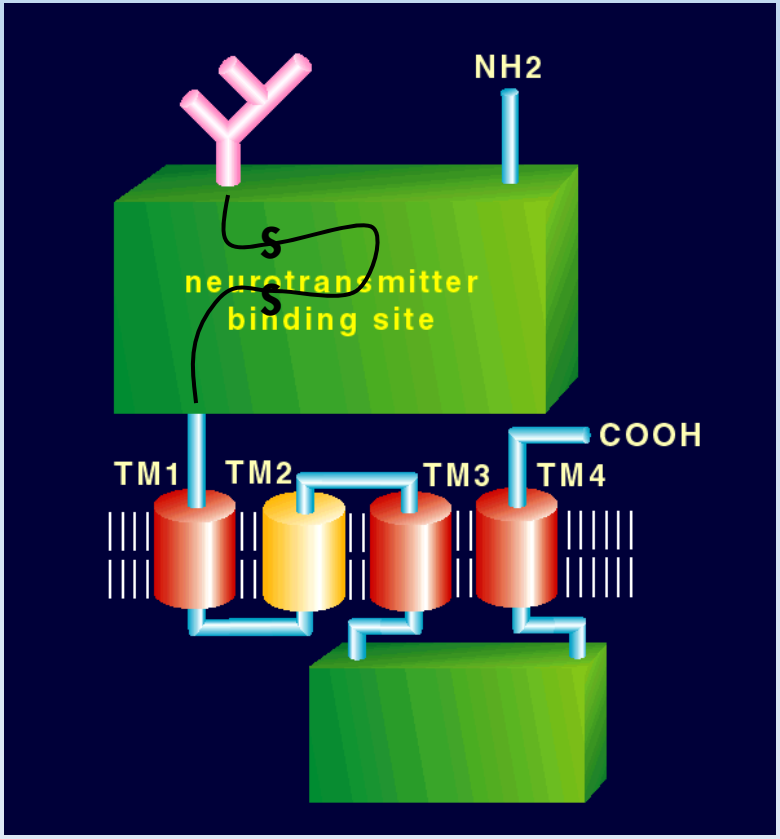
Ligand-gated ion channels

Classification of ligand-gated ion channels

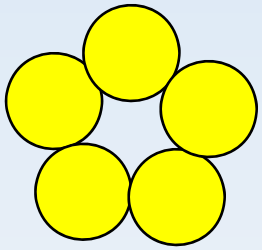
Receptor type	***Ligands***
1. Purinergic	ATP
(excitatory, depolarizing effect in neurons; cation selective)	
2. Cys-loop	acetylcholine GABA serotonin glycine
3. Glutamate	glutamate
4. Intracellular receptors	ATP cAMP cGMP

extracellular binding site

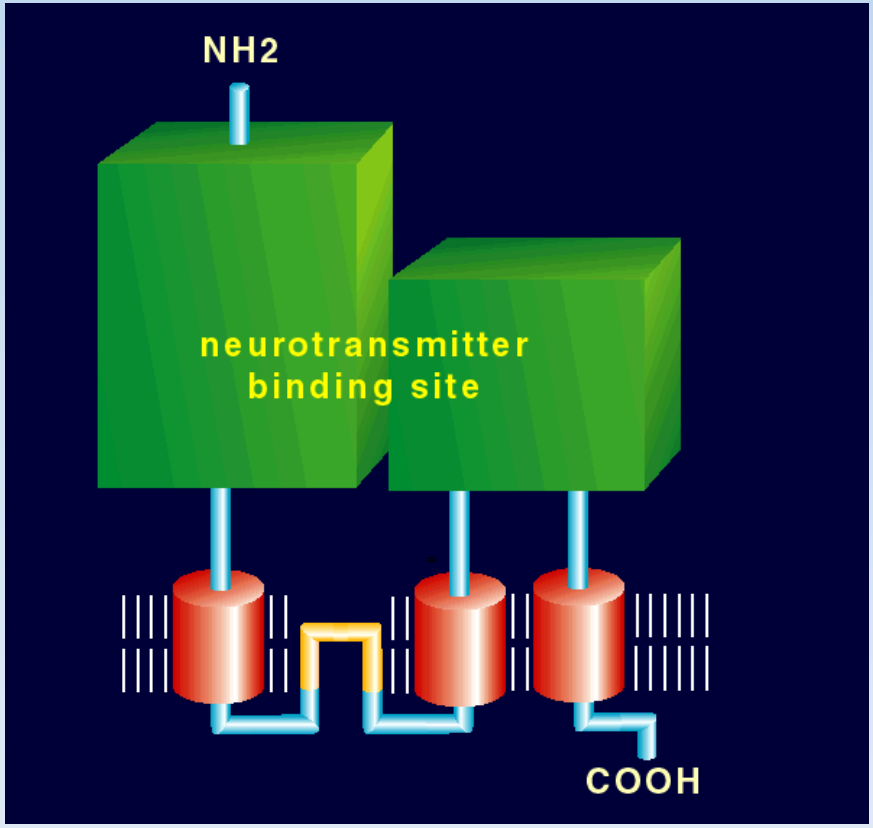
GABA, glycine, ACh, serotonin receptor channel subunit



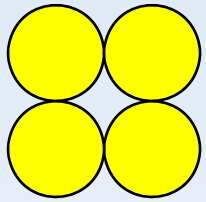
Channel (5 subunits):



Glutamate receptor channel subunit



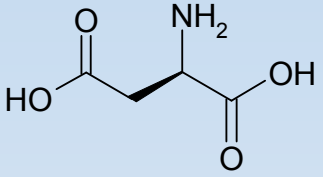
Channel (4 subunits):



Ionotropic Glutamate Receptors

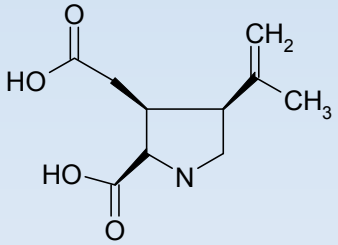
Classified according to preferred synthetic acidic ligand
(glutamate is the only natural ligand)

1. **NMDA** (***N-M****ethyl-****D-A****spartate*)

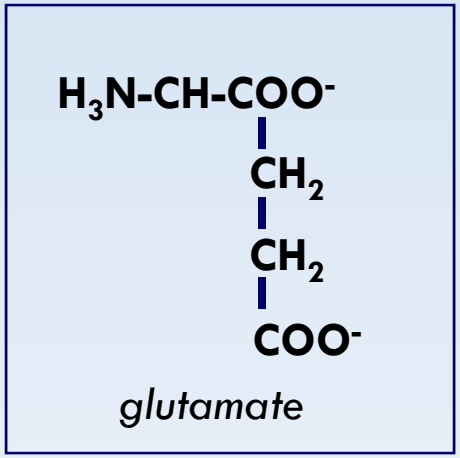


2. **AMPA** (*α-**A**mino-3-hydroxy-5-**M**ethyl-4-isoxazole**P**ropionic **A**cid*)

3. **Kainate**



metabotropic receptors:
-not ion channels
-activate 2nd messenger cascade

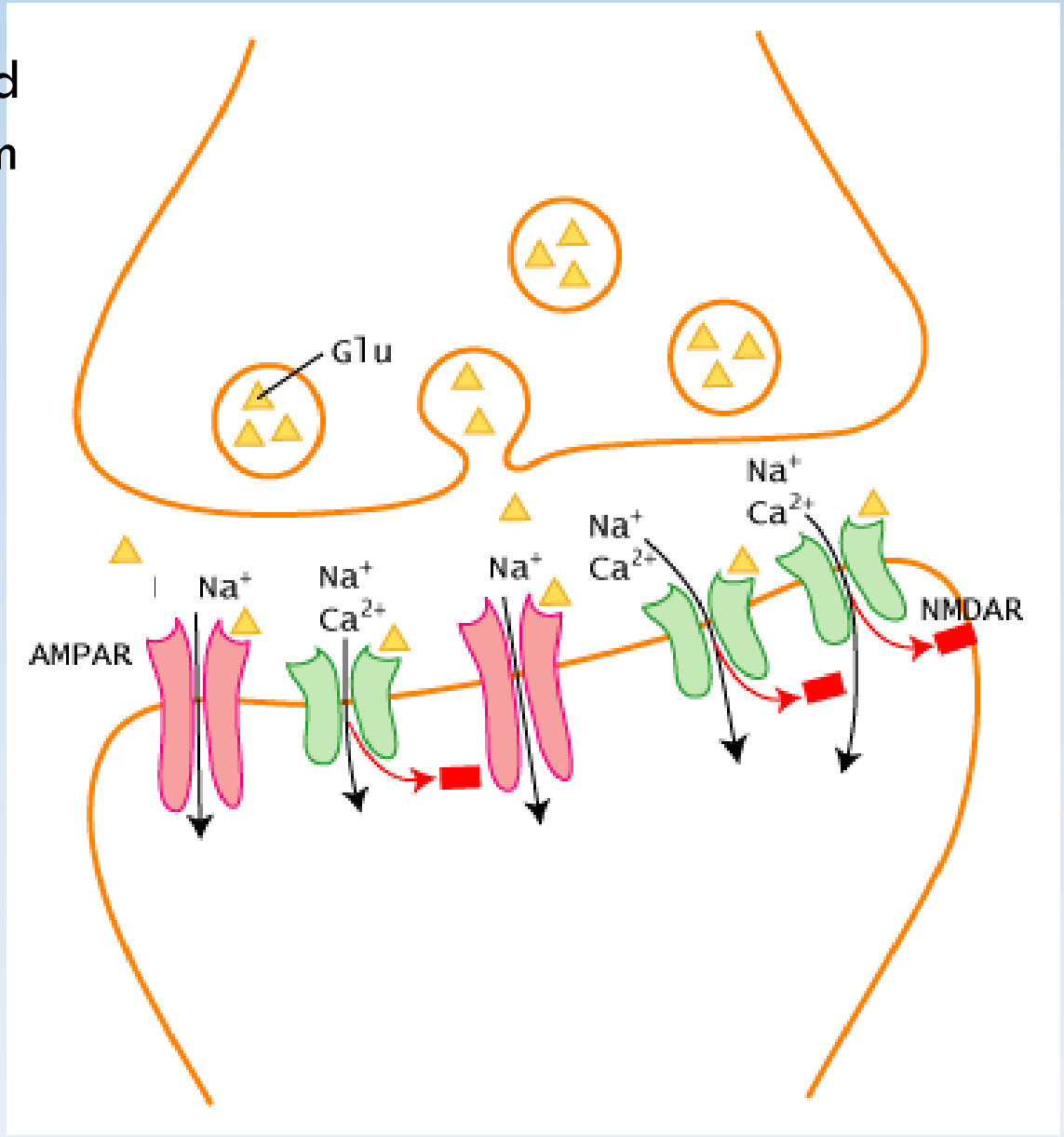


Subtypes of GluR channels

GluR channels are activated by glutamate released from presynaptic terminals

NMDA receptors are much more permeable to Ca^{2+} than AMPA or Kainate receptors

NMDA receptors are blocked by Mg^{2+} unless cell is depolarized



Mg^{2+}

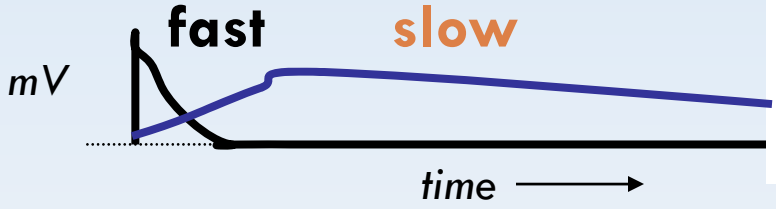
EPSPs and GluR channels

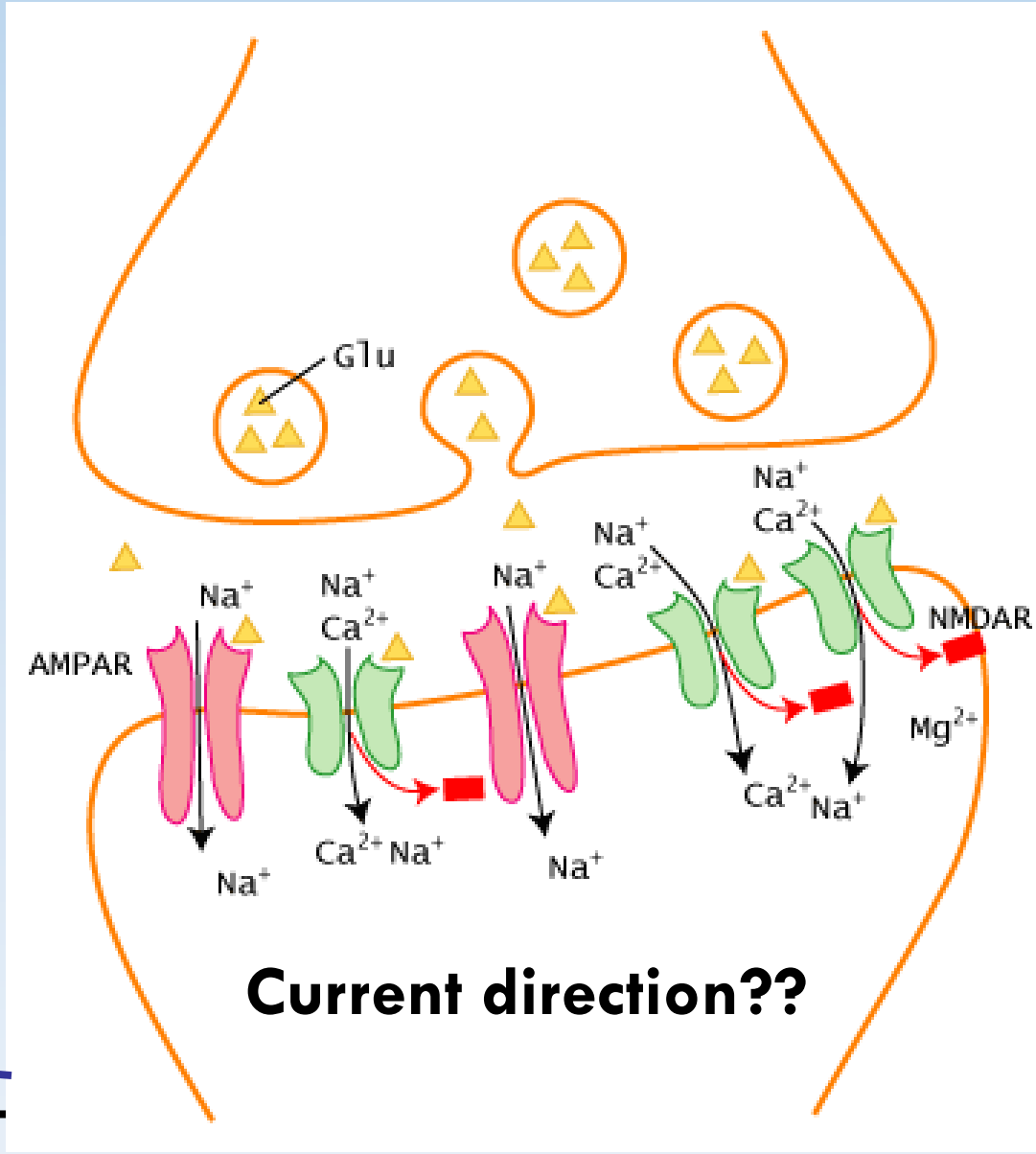
AMPA and Kainate Receptor activation mediates ***fast* EPSP**

NMDA Receptor activation mediates ***slow* EPSP**

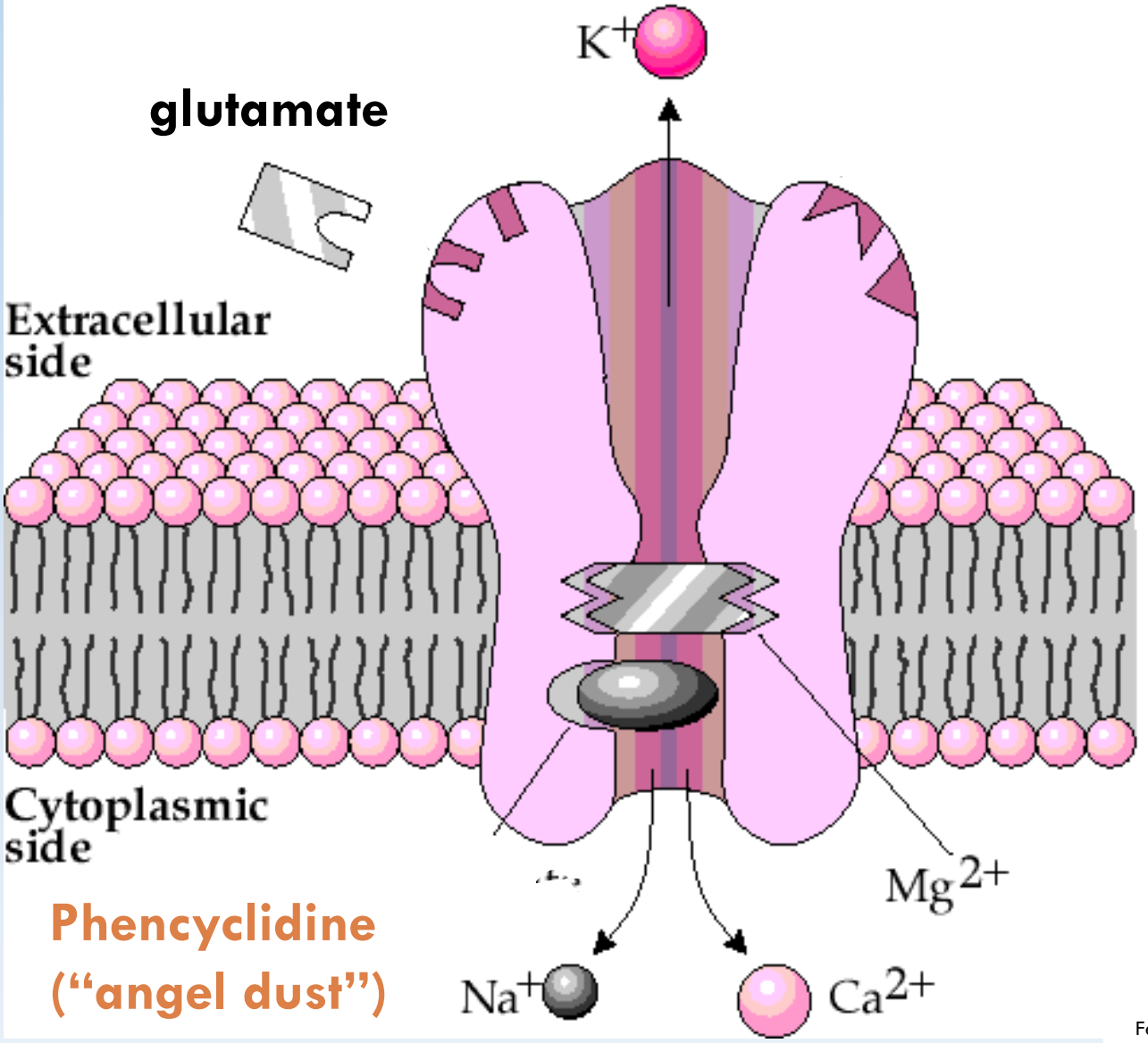
important for "long-term potentiation", a component of the learning/memory process

EPSP: **E**xcitatory **P**ost**S**ynaptic **P**otential





NMDA receptor channel regulation



Phencyclidine ("angel dust")

Feldman, Fundamentals of Neuropharmacology

Acetylcholine (ACh) receptors

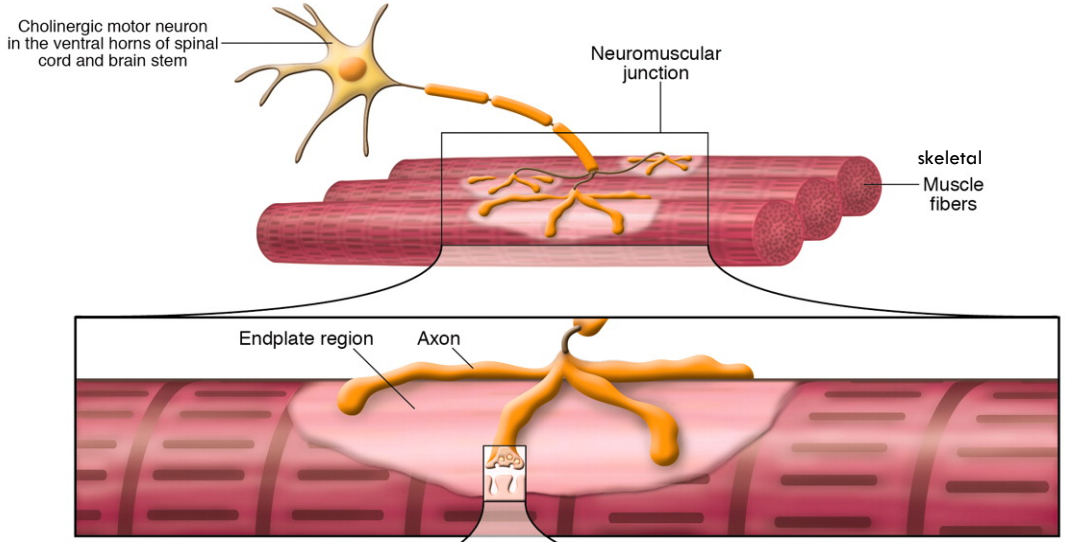
Two types:

muscarinic *metabotropic* receptors (activated by muscarine)
- ACh binding activates a 2nd messenger cascade
- blocked by atropine (from nightshade, *Atropa belladona*)
- no ion channel activity

nicotinic *ionotropic* receptors (activated by nicotine)
- ion channels: located at postsynaptic membrane of neuromuscular junction, and in some neurons

ACh is the natural ligand for both receptors

nACh receptors in neuromuscular junction



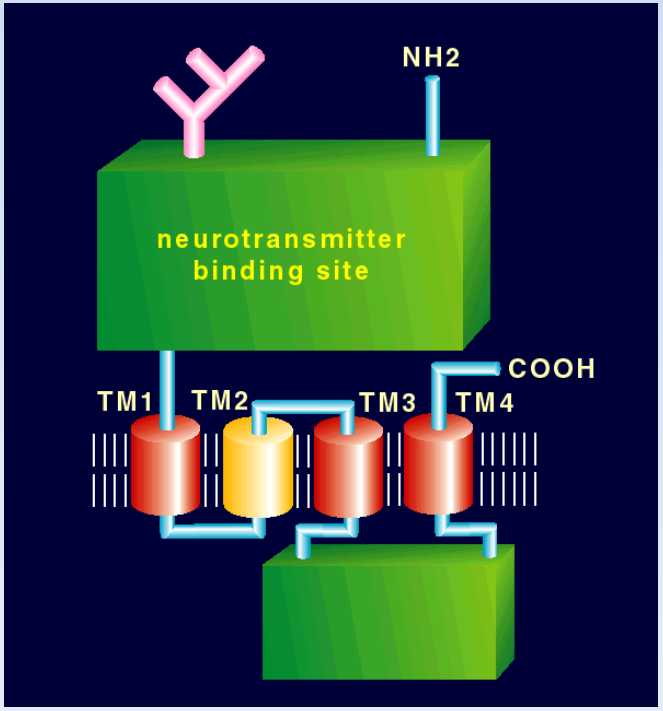
ACh degraded rapidly by ACh esterase

If End Plate Potential (EPP) is large, then action potential is initiated, muscle contracts

J. Clin. Invest. 2006;116:2843-2854

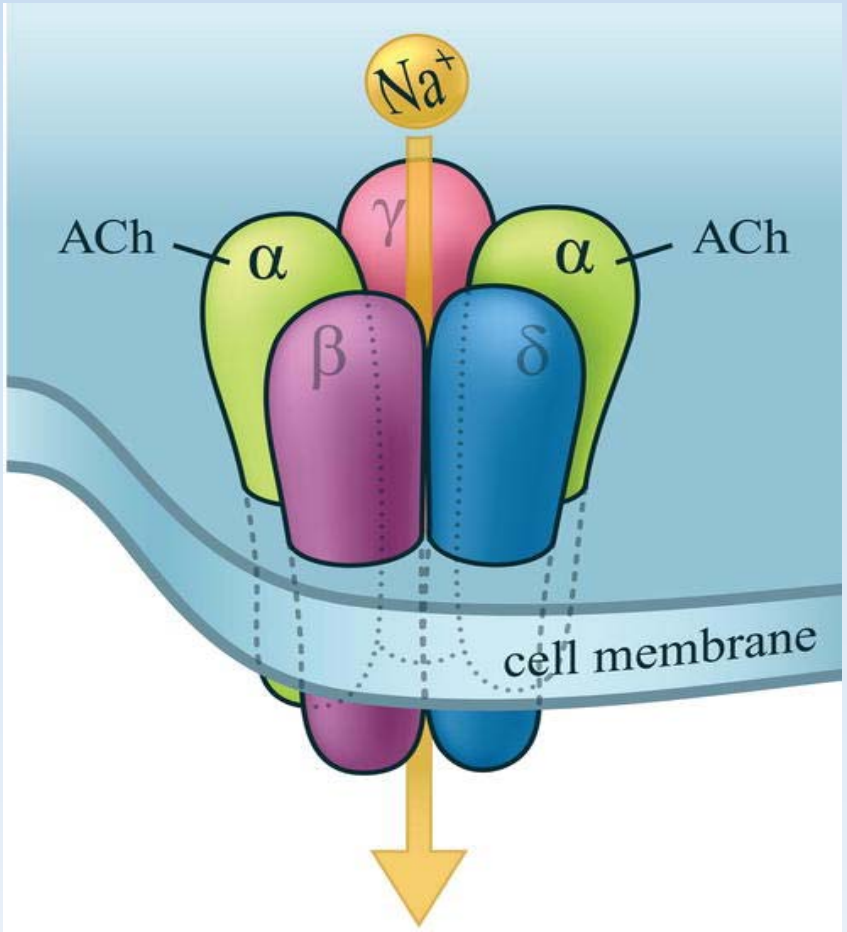
nAChR are heteromultimeric channels

Skeletal muscle: 2 α1, β1, γ, δ subunits/channel in adult
Neurons: 2 α, 3 β subunits/channel



single subunit

x 5 =



Pharmacology of nAChR channels:

Agonists:

acetylcholine, nicotine (activators)

succinylcholine: depolarizing muscle relaxant

(not rapidly hydrolyzed by ACh esterase)

Antagonists:

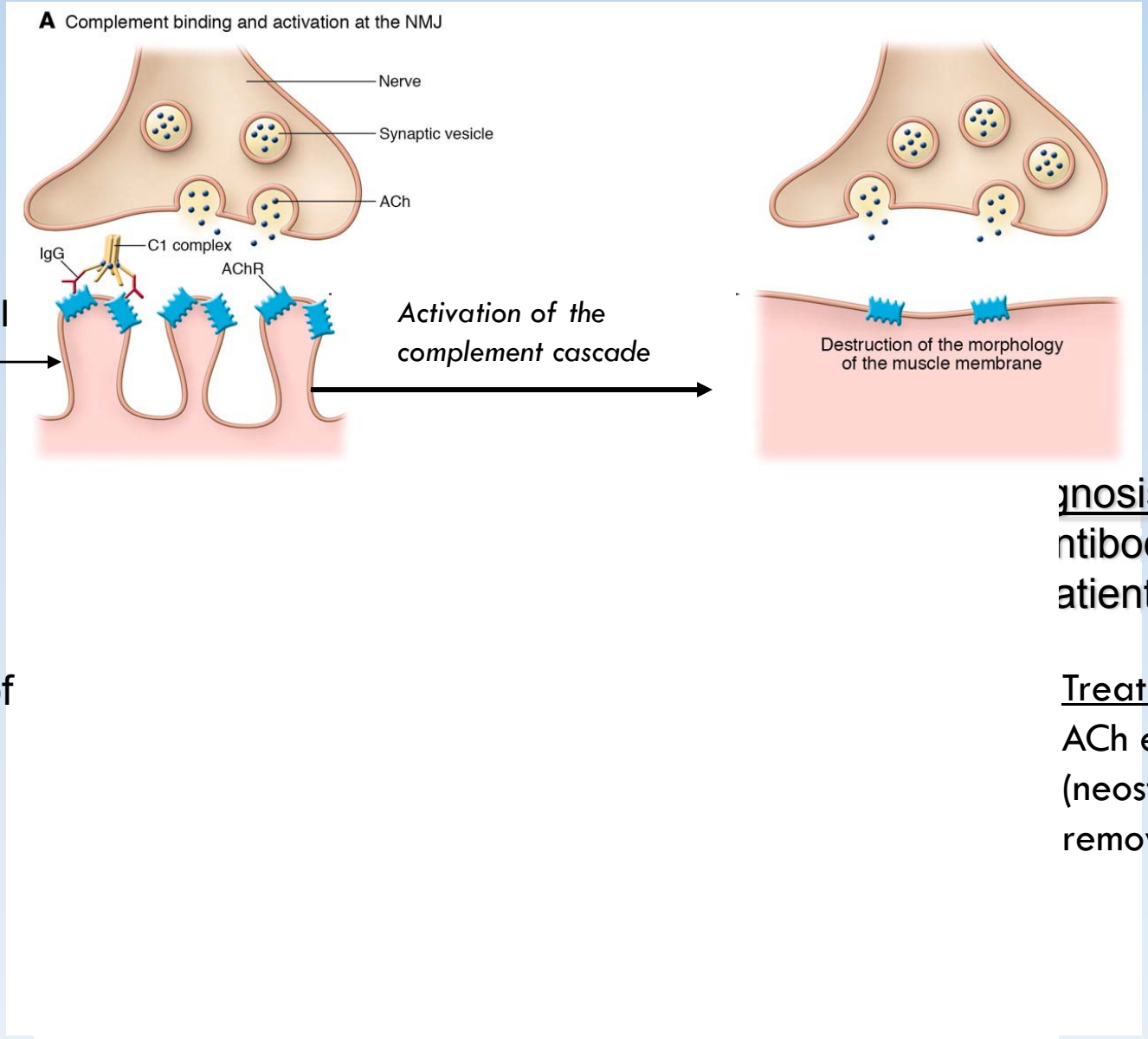
hexamethonium (ganglionic blocker)

curare (*Strychnos* vine extract; arrow/dart poison)

α-bungarotoxin (snake venom)

(cause muscle paralysis)

Myasthenia gravis: effector mechanisms of anti-AChR antibodies



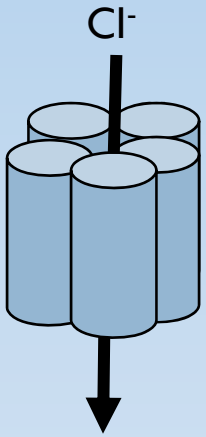
Result:
Decreased amplitude of EPP

gnosis: Detection
ntibodies in blood
atient

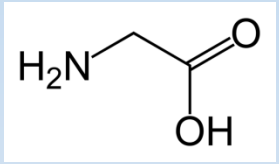
Treatment: inhibit ACh esterase (neostigmine); remove thymus

J. Clin. Invest. 2006;116:2843-2854

Glycine and $GABA_A$ receptor channels

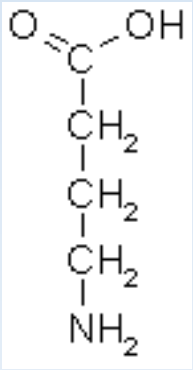


ANION (Cl^-) channels



glycine

Activated by glycine (GlyR channel)
or γ-aminobutyric acid ($GABA_AR$ channel)



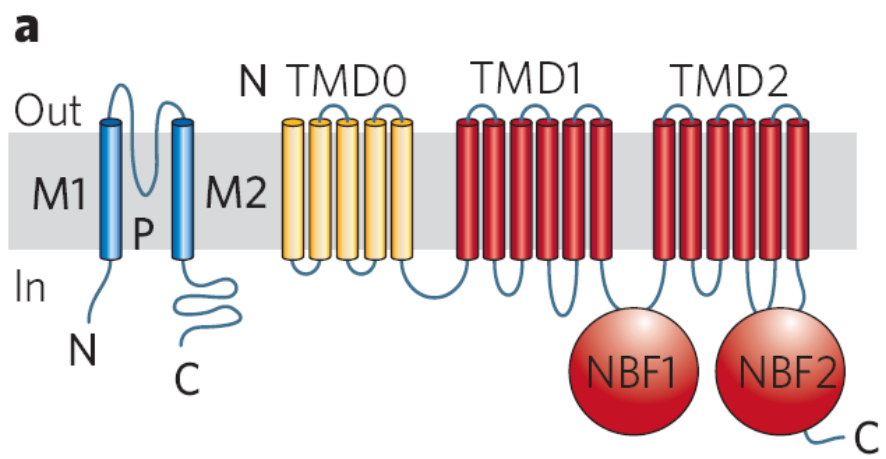
GABA

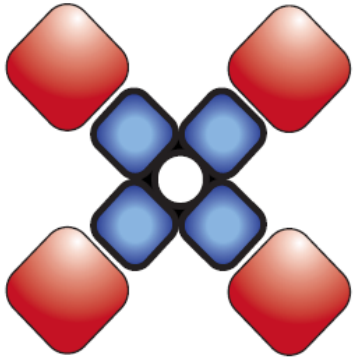
Location: neurons

GlyR: spinal cord and brain stem

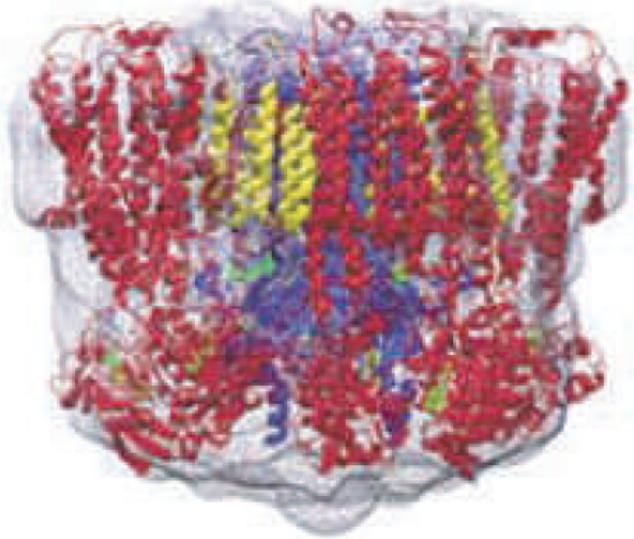
$GABA_AR$: throughout brain

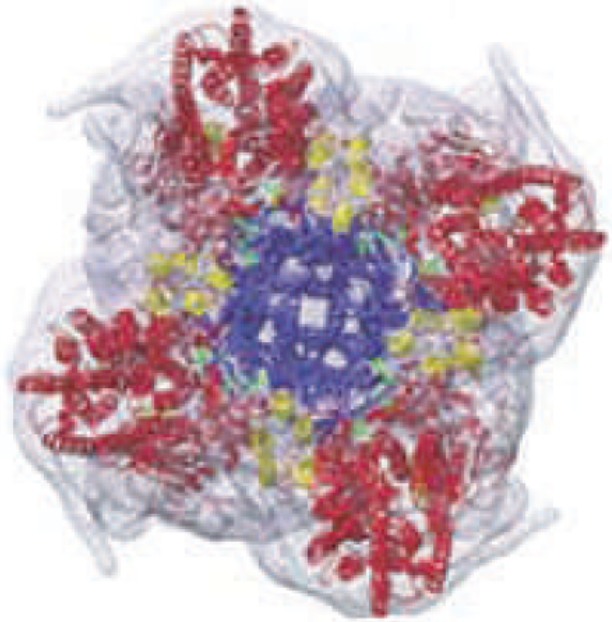
KATP channel is an octomer, formed by coassembly of Kir6 and SUR subunits





Kir6 **SUR**
(SulphonylUrea Receptor)

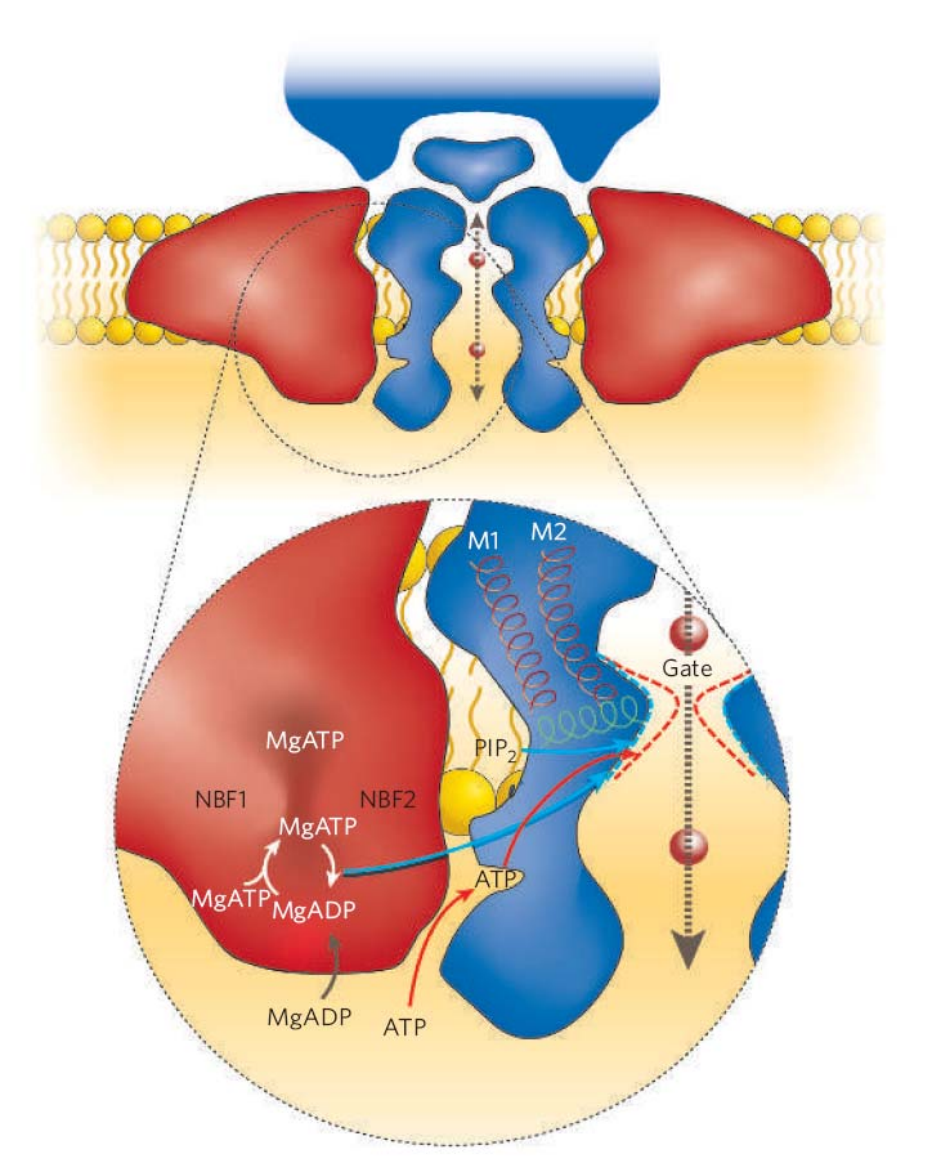




Nichols (2006)
Nature 440:470

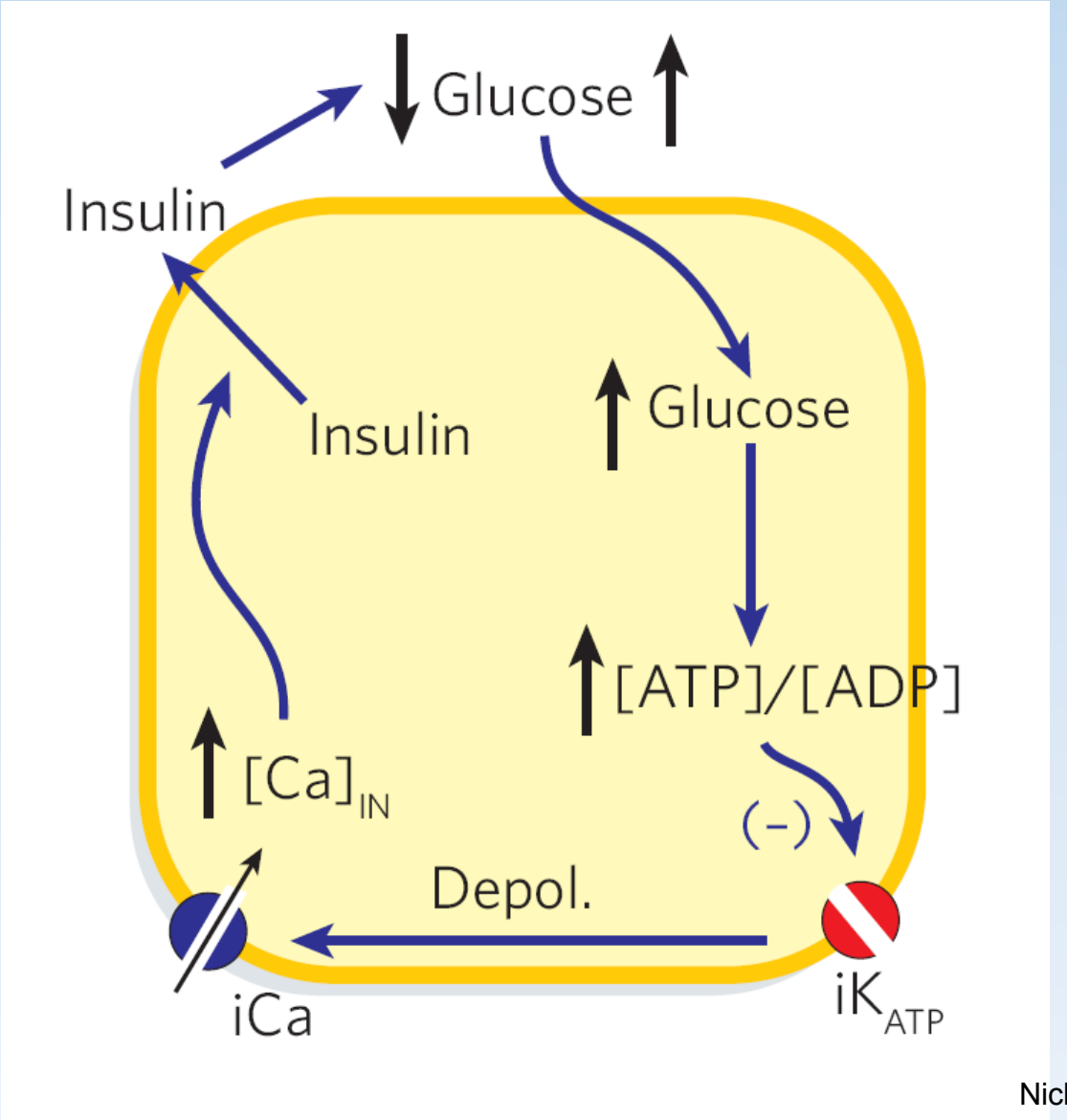
Nucleotide regulation of KATP channel

ATP closes channel; PIP2 or ATP hydrolysis activates channel



Nichols (2006) Nature 440:470

KATP channels and regulation of insulin secretion in pancreatic beta cells



Nichols (2006) Nature 440:470