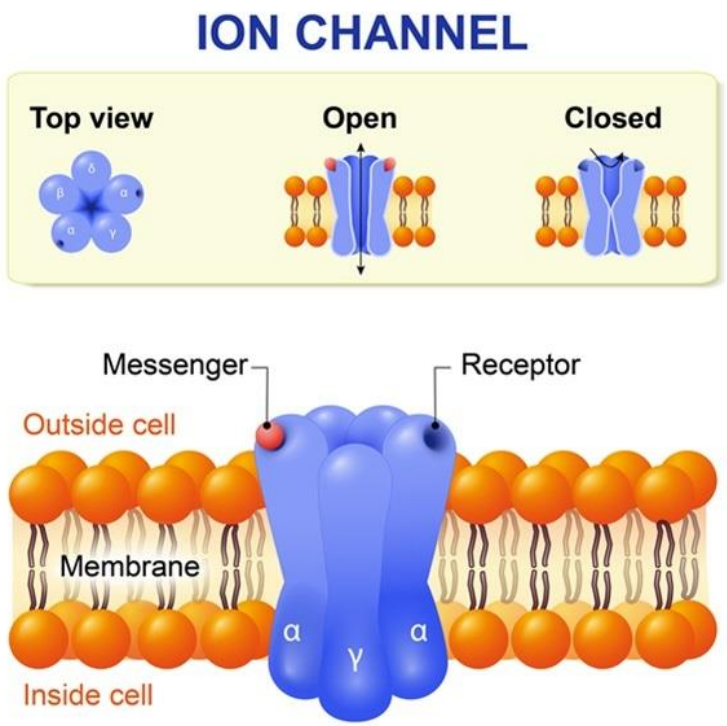
Ion Channels

2- Ligand Gated Ion Channels

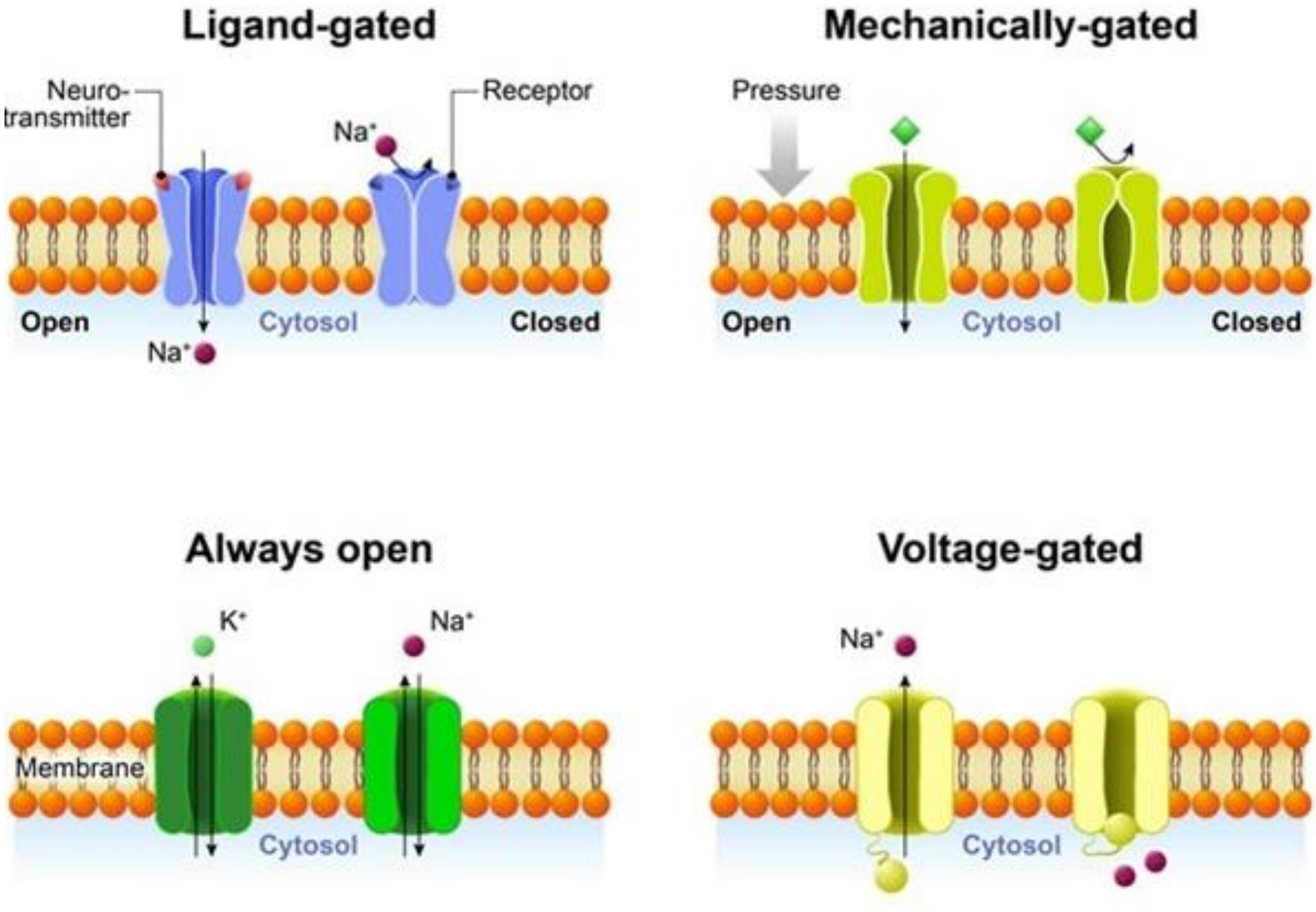
Ion Channels

Ion channels are pore-forming membrane proteins whose function is
- establishing a resting membrane potential,
- shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane,
- controlling the flow of ions across membranes,
- regulating cell volume.

Their activation translates into a rapid physiological effect

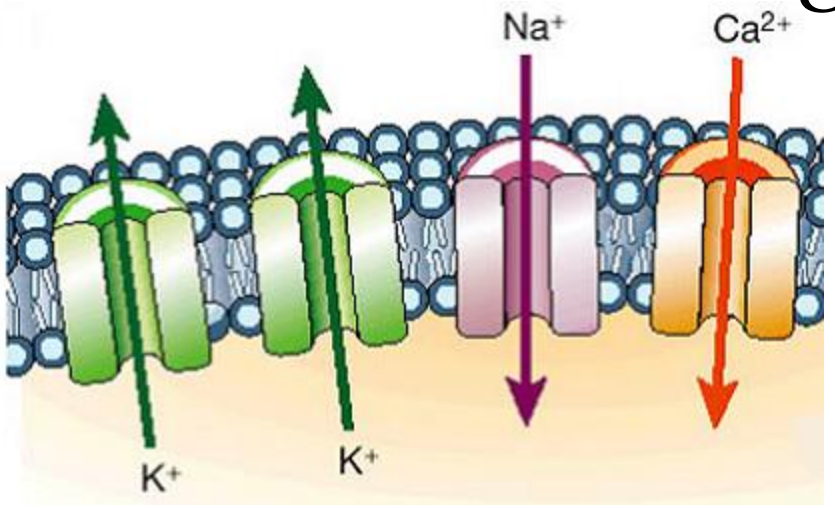


Different classes of ion channels



Which force moves the ions?

The electrochemical gradient

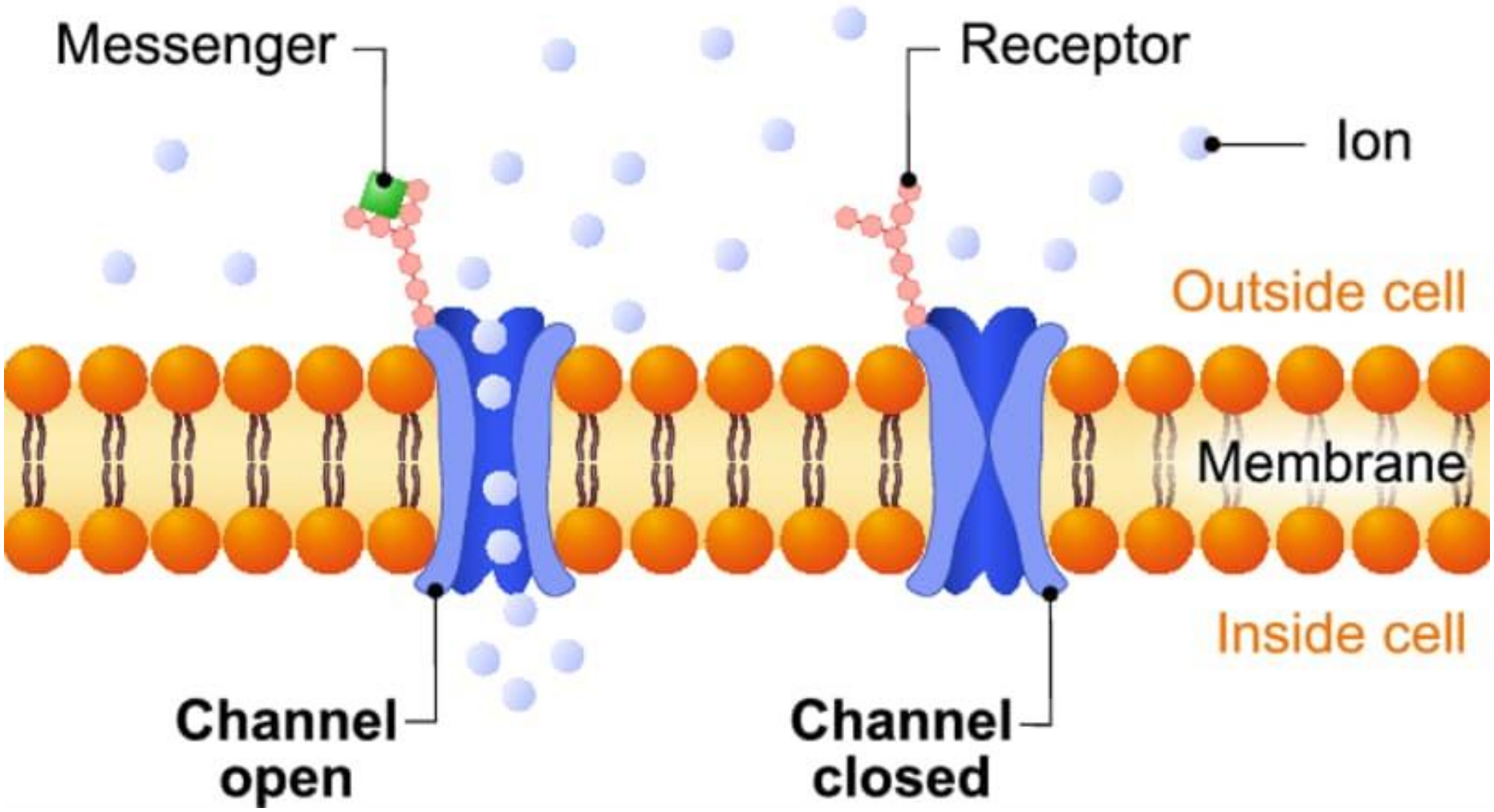


0 mV

-90 mV

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
K+	140	4
Na+	15	145
Cl-	4	110
Ca2+	0.0001	5

Ligand Gated Ion Channels



Ligand Gated Ion Channels

• **PERMEABILITY to CATIONS (Na^+, Ca^{++}, K^+)**

Ion entry produces
DEPOLARIZATION
(cell activation)

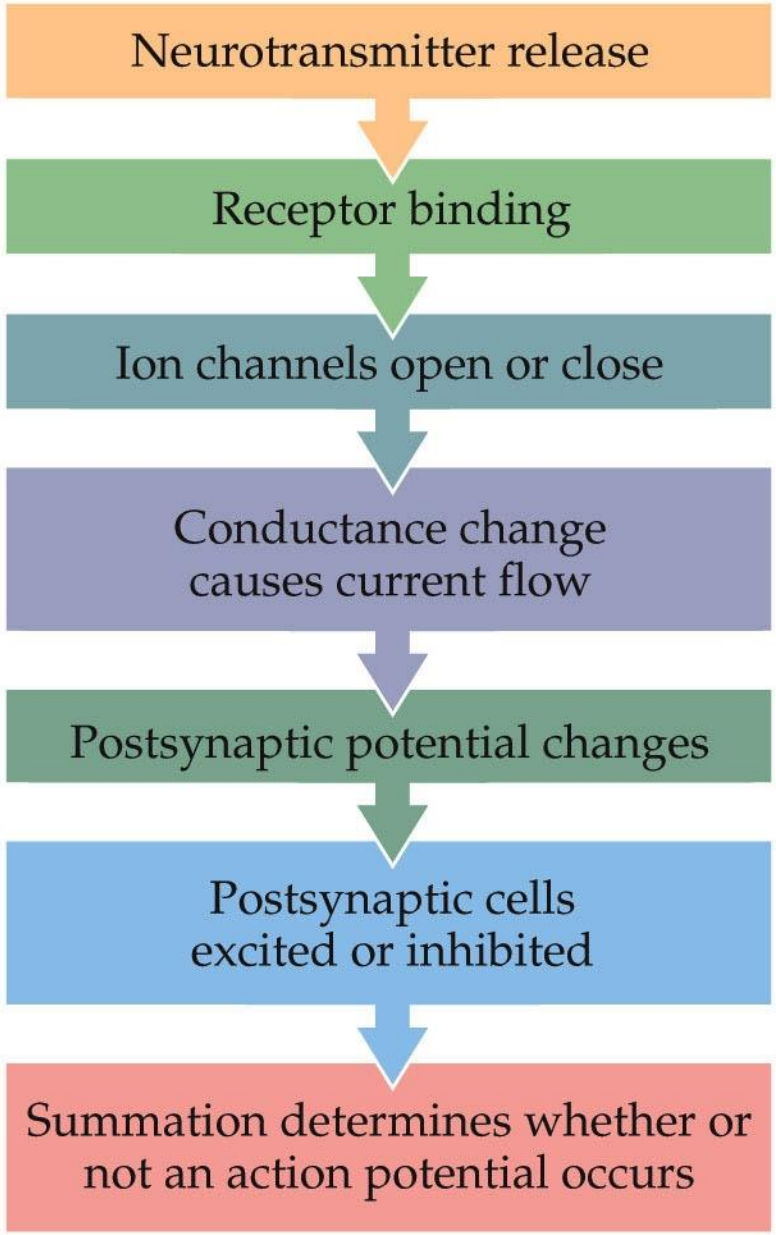
LIGAND	RECEPTOR
Acetilcholine	Nicotinic R
Glutamate and other excitatory aa	NMDA R AMPA R KAR
Serotonin	5-HT3 R
ATP and purines	P2X
Ciclic nucleotides (cAMP and cGMP)	CNG

• **PERMEABILITY to ANIONS (Cl^-)**

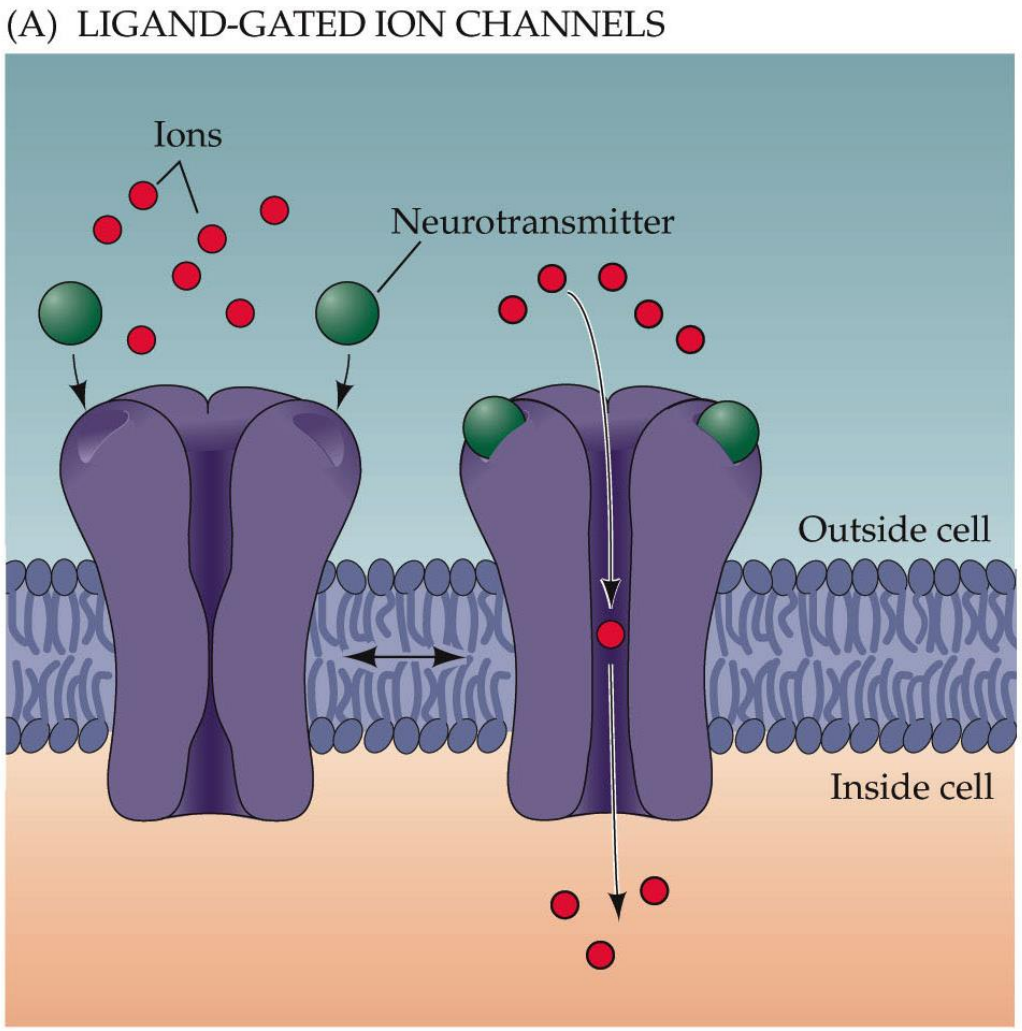
Ion entry produces
HYPERPOLARIZATION
(cell inhibition)

LIGAND	RECEPTOR
GABA	$GABA_A$
Glicine	Gly R

The sequence of events



Ligand Gated Ion Channels are also called Ionotropic Receptors

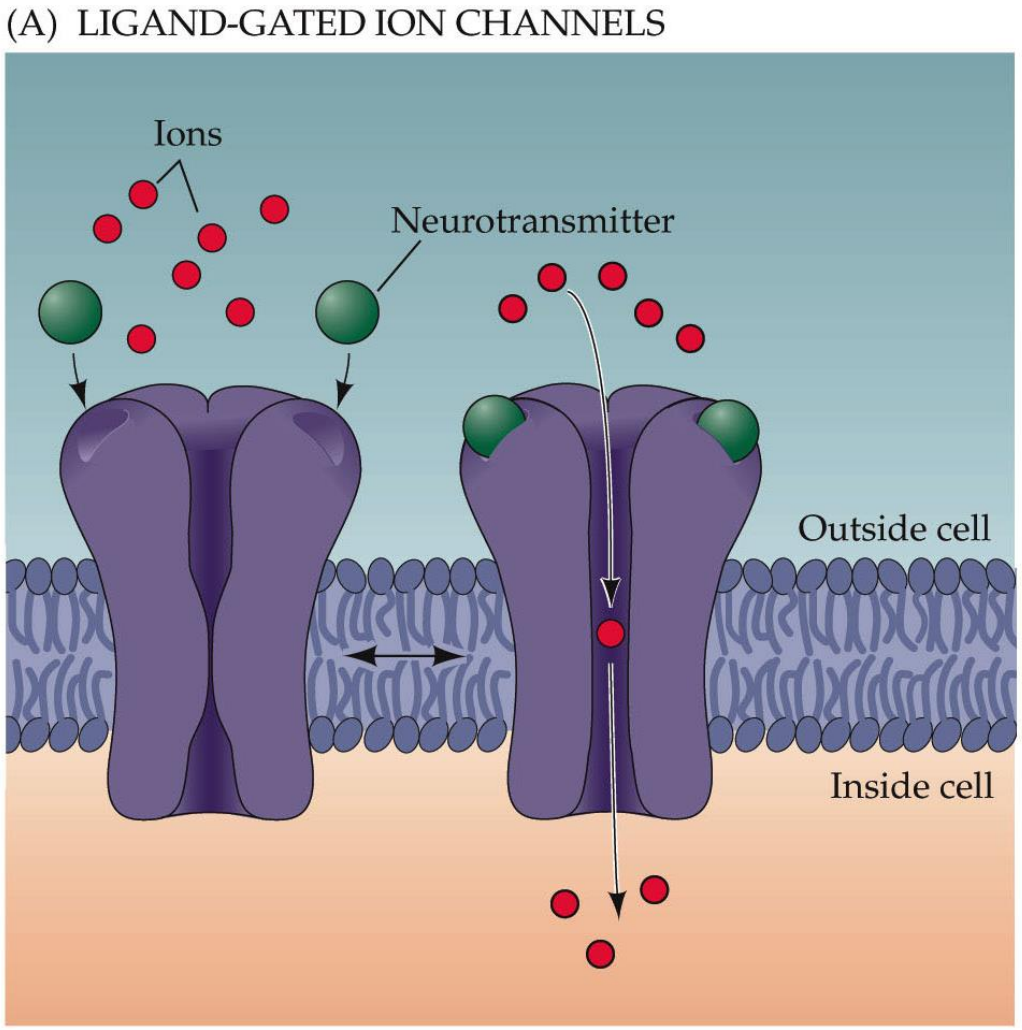


ICE, Fourth Edition, Figure 5.23 (Part 1)

Neurotransmitter Receptors

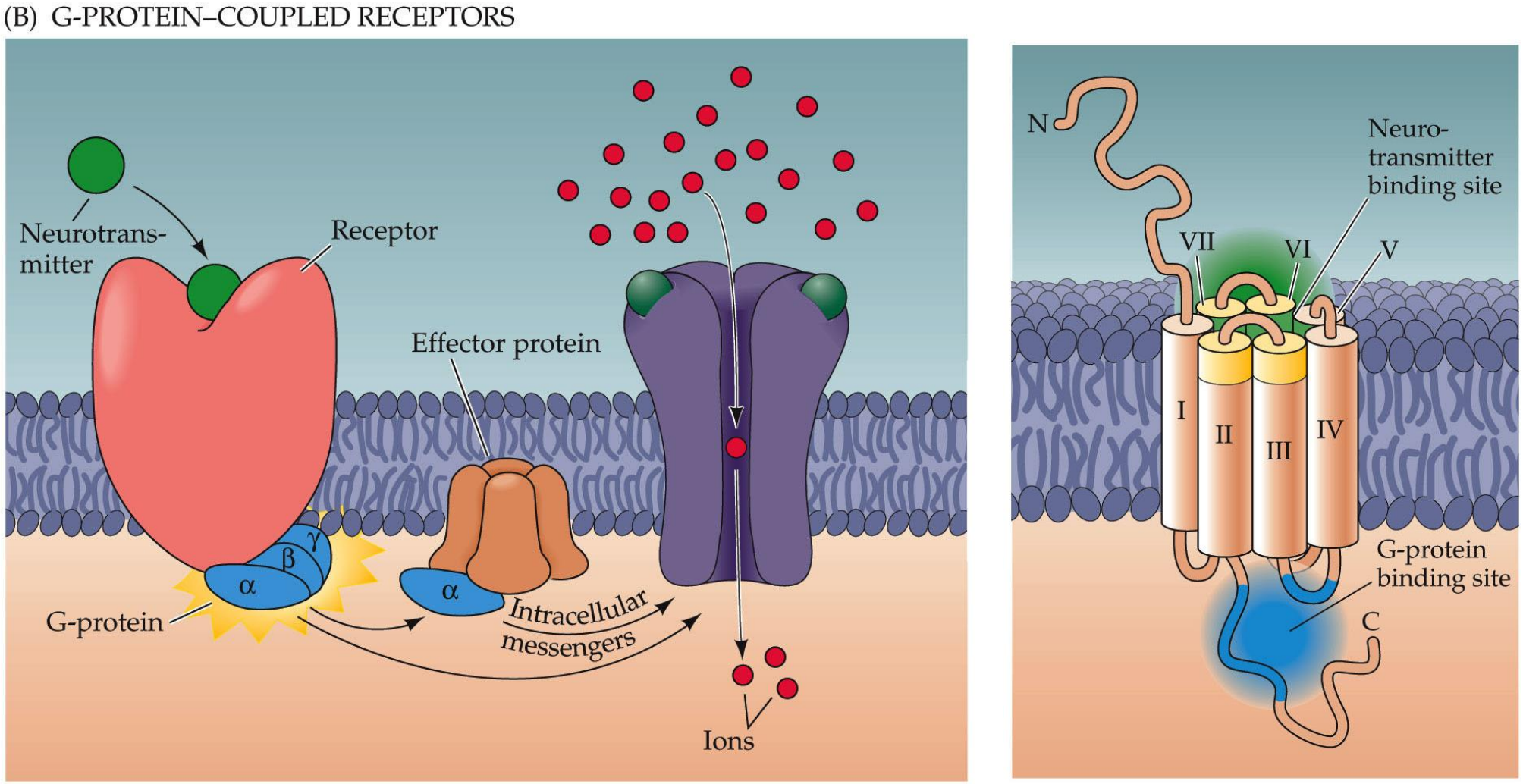
Agonist	Ionotropic ligand-gated channels (fast 0.1 - 100 ms)	Metabotropic G-protein-coupled receptors (slow 0.05 – 100 s)
	Ionotropic	**Metabotropic**
ACh	Nicotinic (Cationic)	Muscarinic
Glutamate	AMPA, NMDA, Kainate (Cationic)	mGluR
GABA	$GABA_A$ (Anionic)	$GABA_B$
Glycine	Glycine (Anionic)	—
Serotonin	5-HT_3 (Cationic)	5-$HT_{1,2,4\text{-}7}$
ATP (a purine)	P2Y (Cationic)	P2X
Histamine	—	H_1, H_2, H_3
Catecholamines	—	α_1, α_2, β, D_1, D_2
Anandamide	—	Cannabinoid R
Odorants	—	>500 odorant receptors[b]
Tastants	Some	Some[b]

Ionotropic Receptors



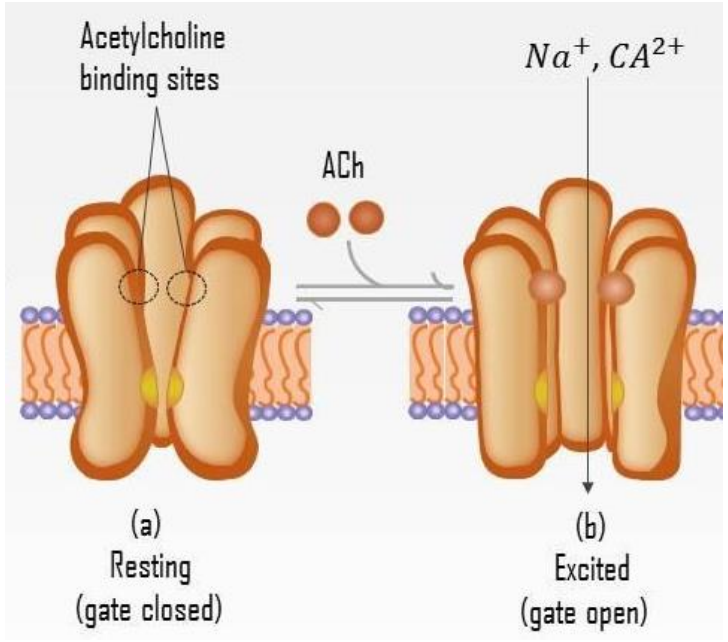
ICE, Fourth Edition, Figure 5.23 (Part 1)

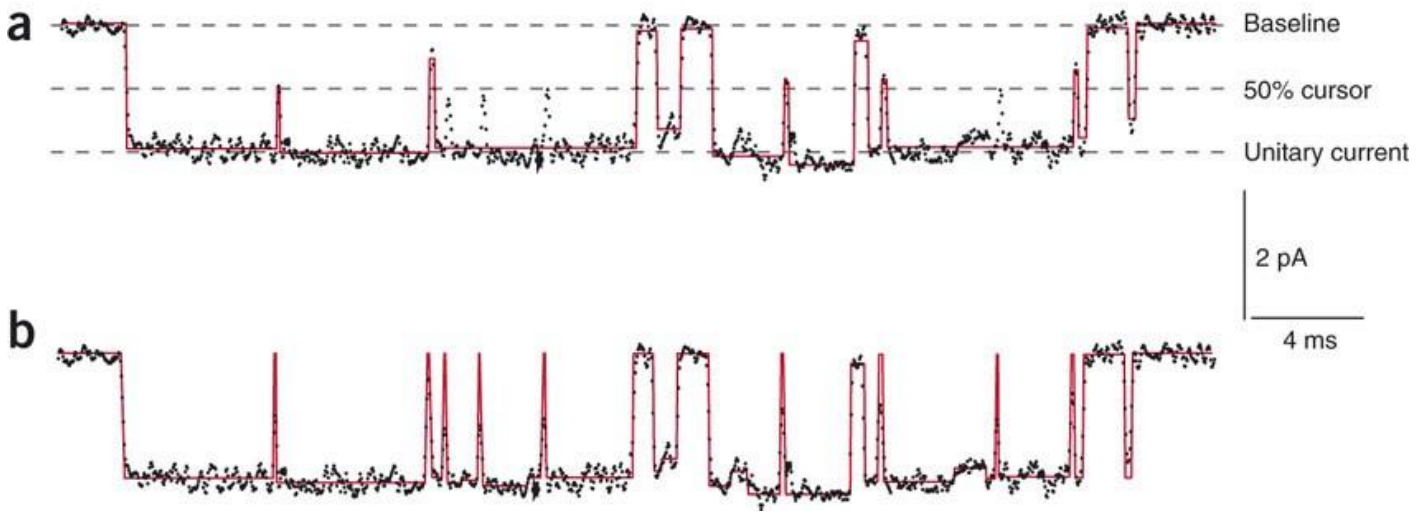
Metabotropic Receptors



***NEUROSCIENCE, Fourth Edition,* Figure 5.23 (Part 2)**

Ligand Gated Ion Channels - Gating





Ligand Gated Ion Channels - Neurotransmitters

• **PERMEABILITY to CATIONS (Na^+, Ca^{++}, K^+)**

Ion entry produces **DEPOLARIZATION (cell activation)**

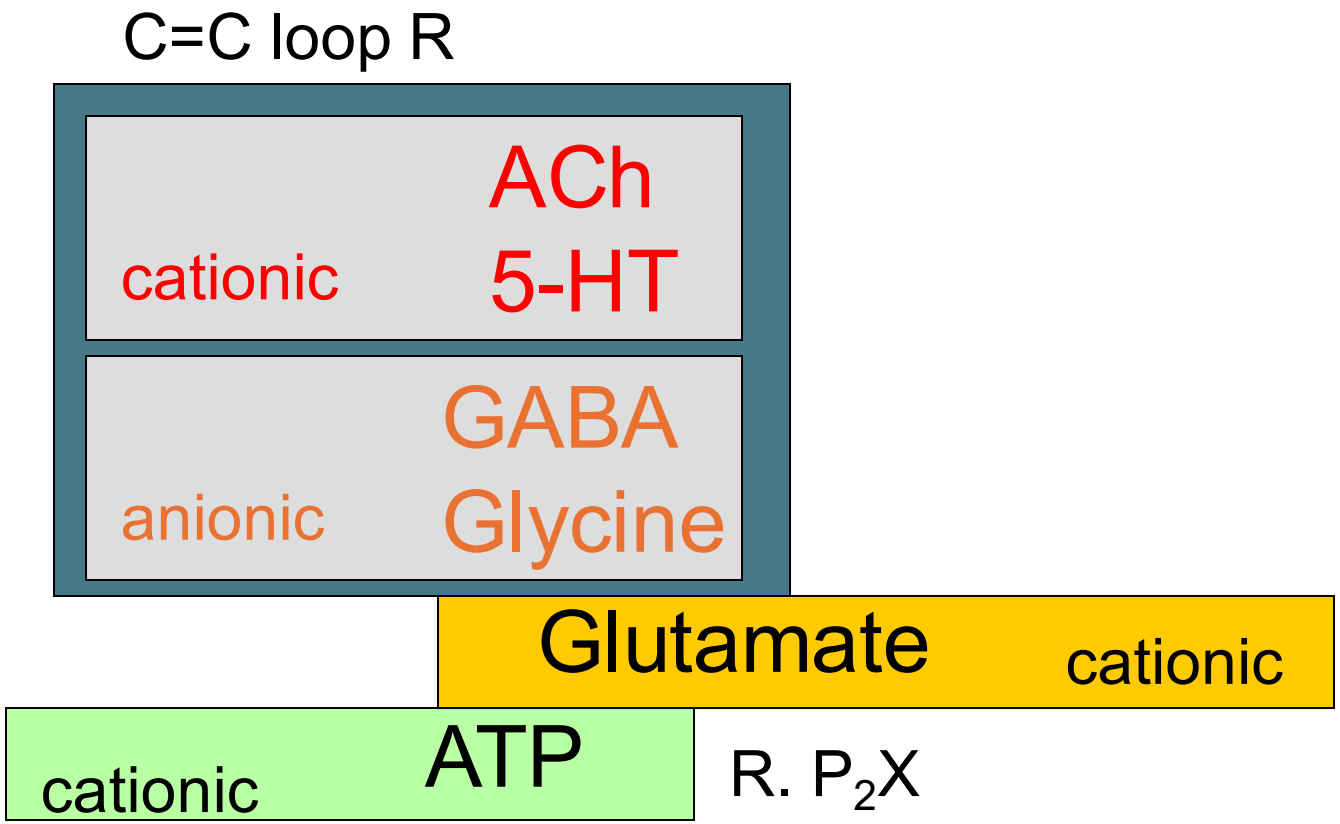
LIGAND	RECEPTOR
Acetilcholine	Nicotinic R
Glutamate and other excitatory aa	NMDA R AMPA R KAR
Serotonin	5-HT3 R
ATP and purines	P2X
Ciclic nucleotides (cAMP and cGMP)	CNG

• **PERMEABILITY to ANIONS (Cl^-)**

Ion entry produces **HYPERPOLARIZATION (cell inhibition)**

LIGAND	RECEPTOR
GABA	$GABA_A$
Glicine	Gly R

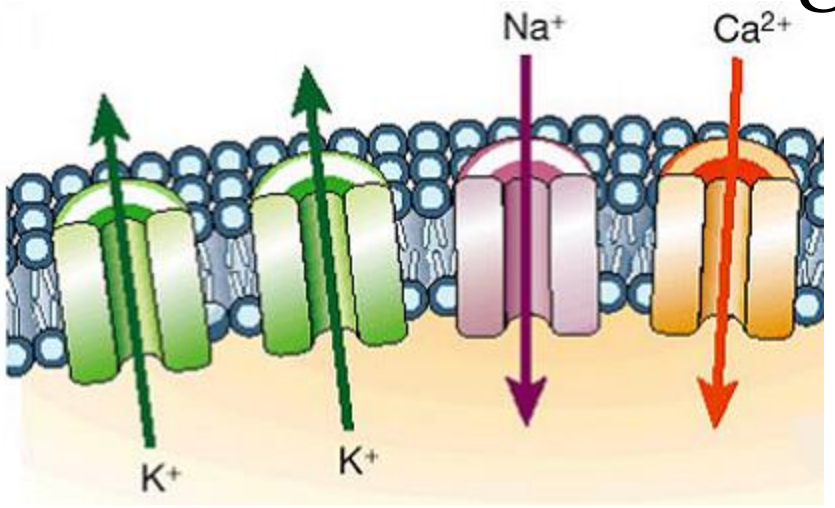
Ionotropic receptor families



What happens in the postsynaptic membrane when ligand-gated channels open?

Always remember that….

The electrochemical gradient



0 mV

-90 mV

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
K+	140	4
Na+	15	145
Cl-	4	110
Ca2+	0.0001	5

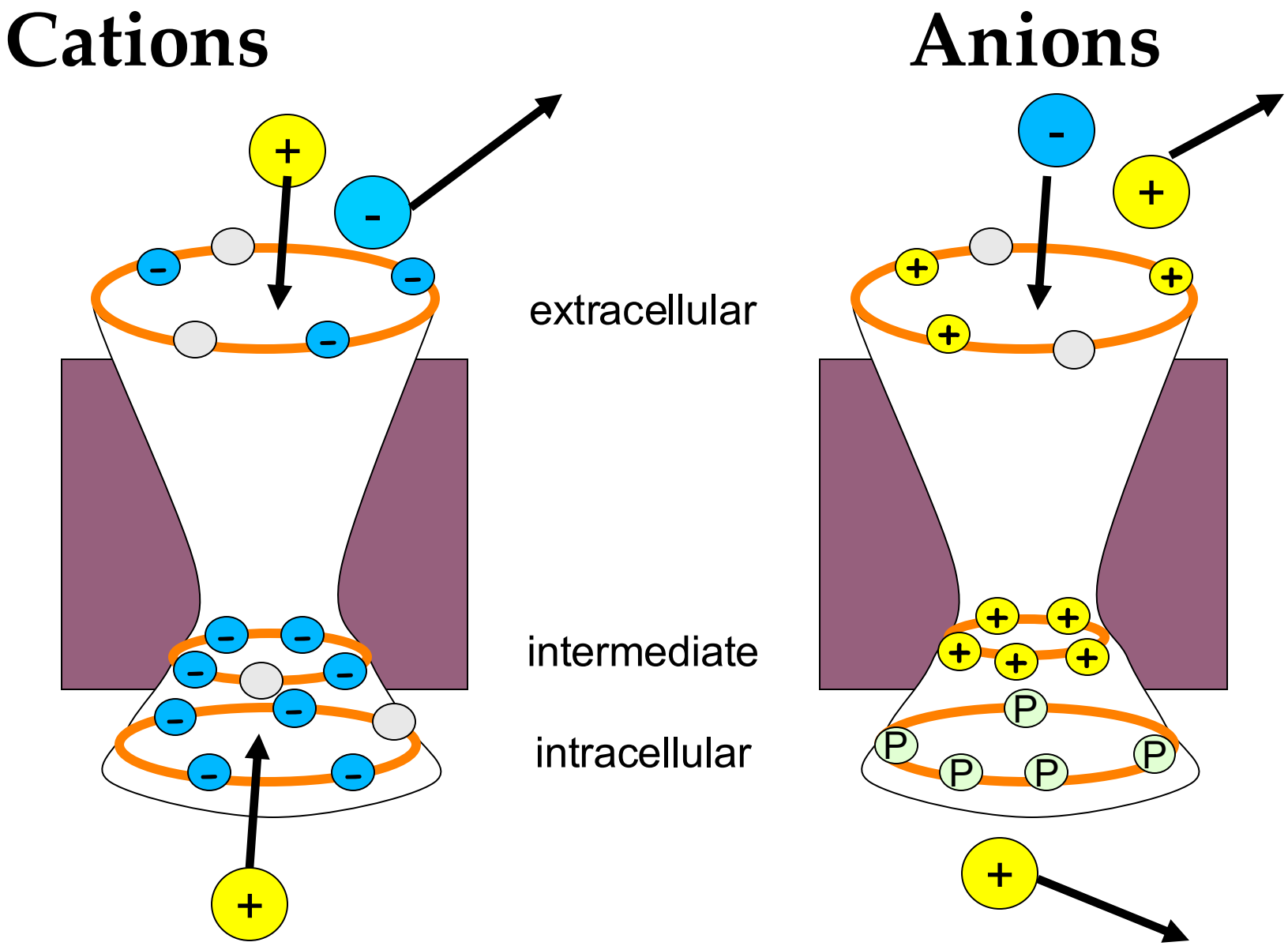
Selectivity

Ligand Gated Ion Channels :

Loose selectivity (anions/cations)

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

Loose selectivity



Ligand Gated Ion Channels – Neurotransmitters - Ions

• **PERMEABILITY to CATIONS (Na^+, Ca^{++}, K^+)**

Ion entry produces **DEPOLARIZATION (cell activation)**

LIGAND	RECEPTOR
Acetilcholine	Nicotinic R
Glutamate and other excitatory aa	NMDA R AMPA R KAR
Serotonin	5-HT3 R
ATP and purines	P2X
Ciclic nucleotides (cAMP and cGMP)	CNG

• **PERMEABILITY to ANIONS (Cl^-)**

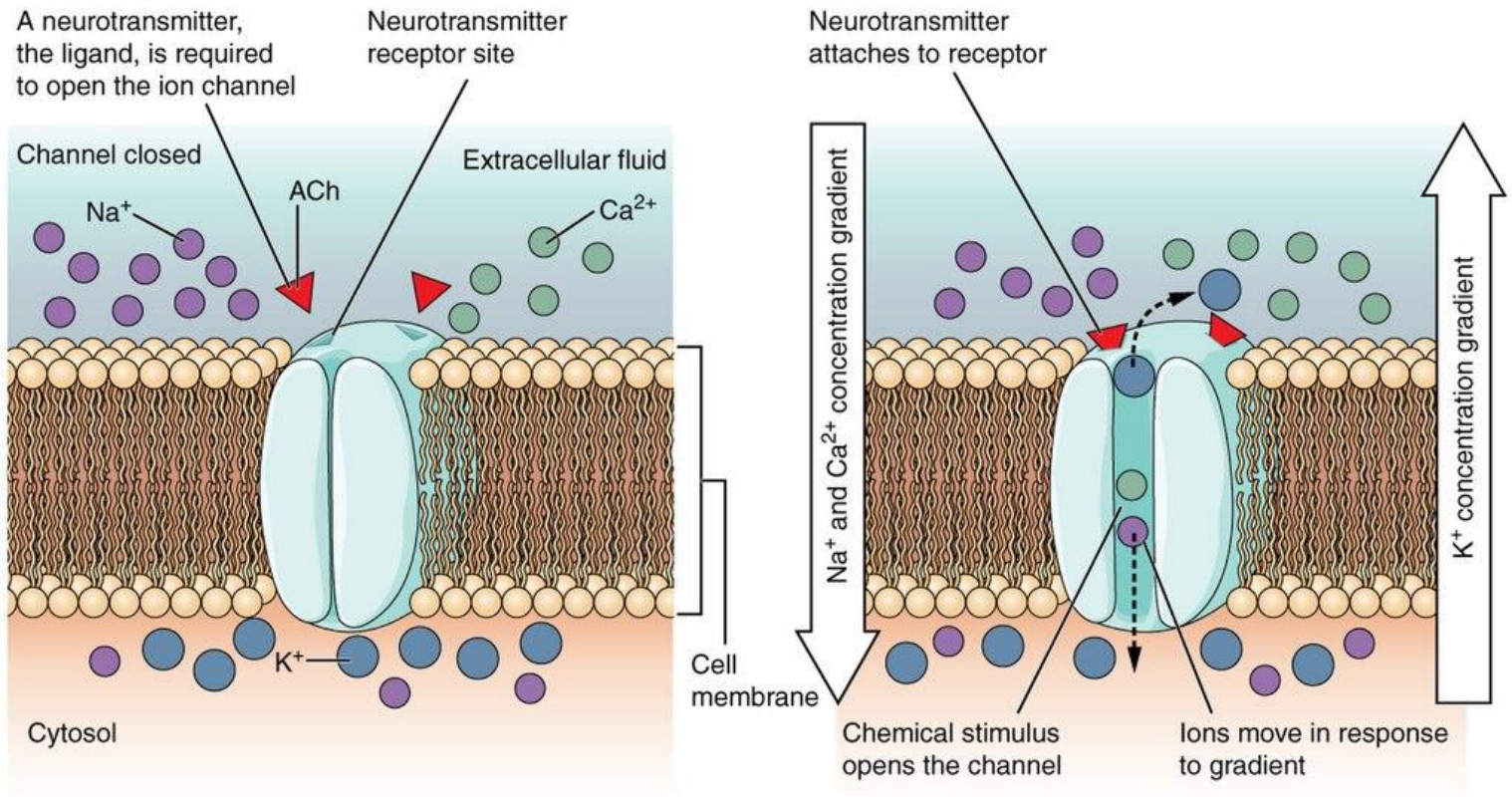
Ion entry produces **HYPERPOLARIZATION (cell inhibition)**

LIGAND	RECEPTOR
GABA	$GABA_A$
Glicine	Gly R

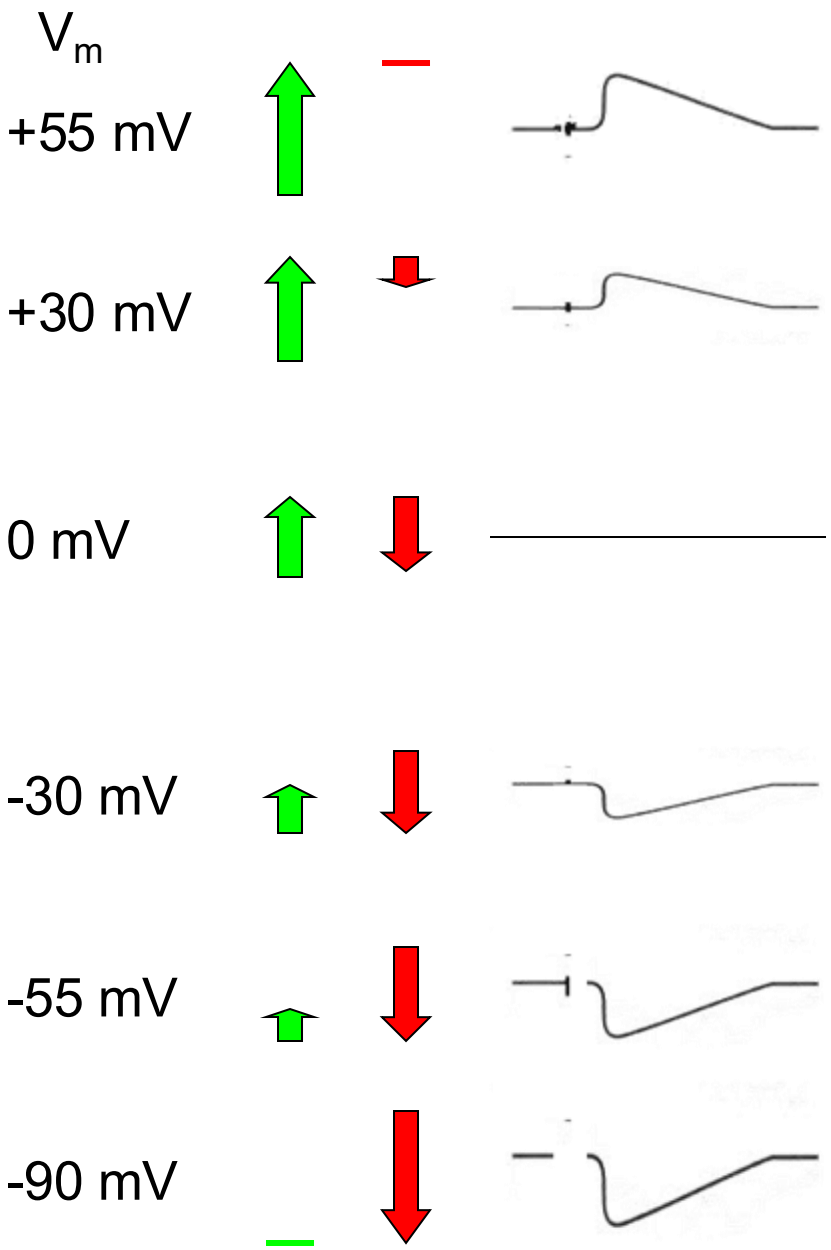
Cationic Ion Channel

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + [illegible]}{P_K[K^+]_i + P_{Na}[Na^+]_i + [illegible]}$$

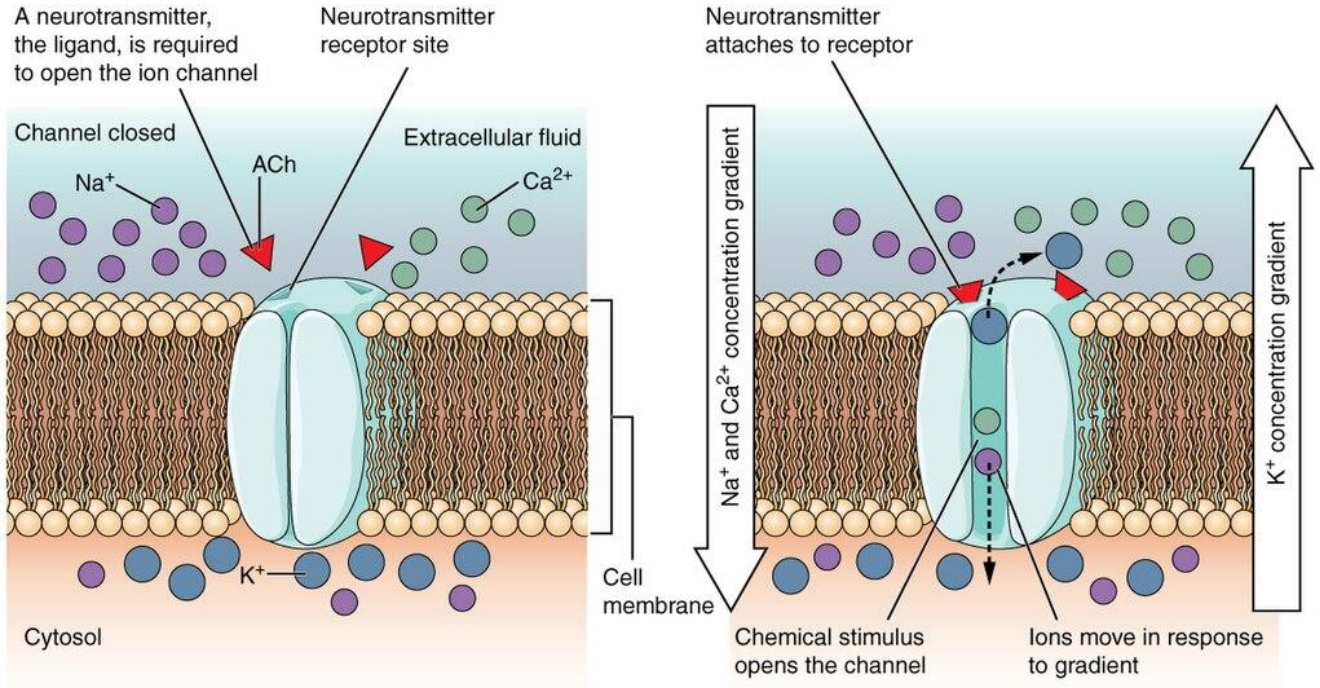
- Na^+ (E_{Na}= + 63 mV)
- K^+ (E_K = - 80 mV)



Cationic Ion Channel



- Na^+ (E_{Na}= + 63 mV)
- K^+ (E_K = - 80 mV)

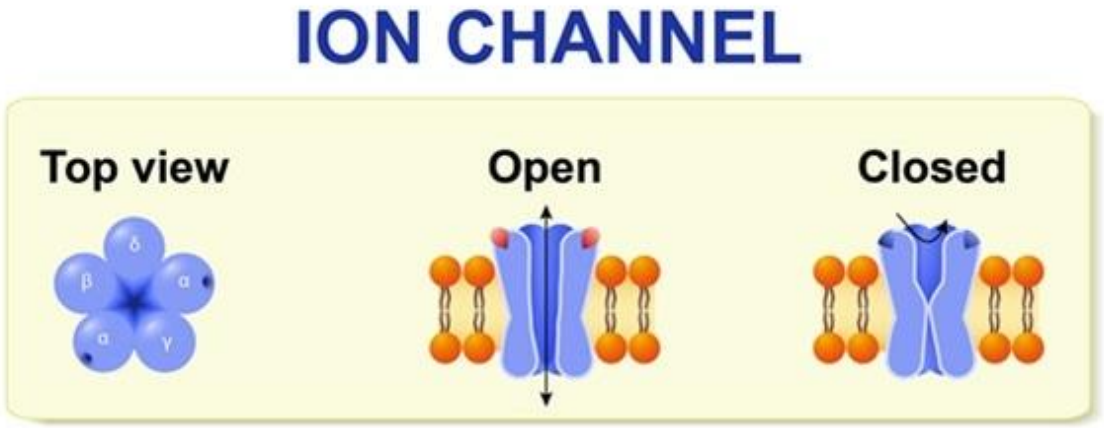


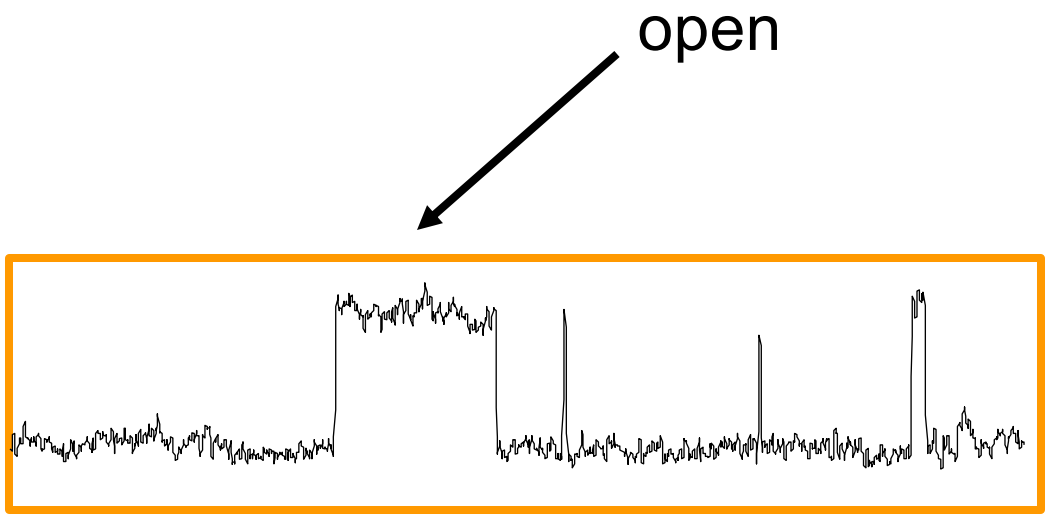
Anionic

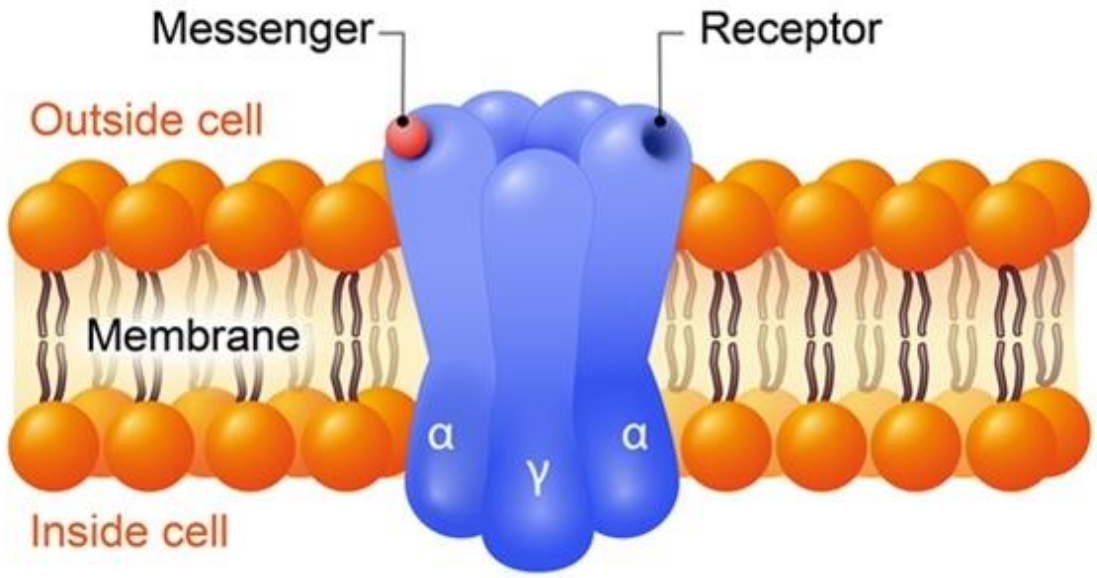
$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_i + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_e + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e}$$

$V_m \rightarrow E_{Cl}$

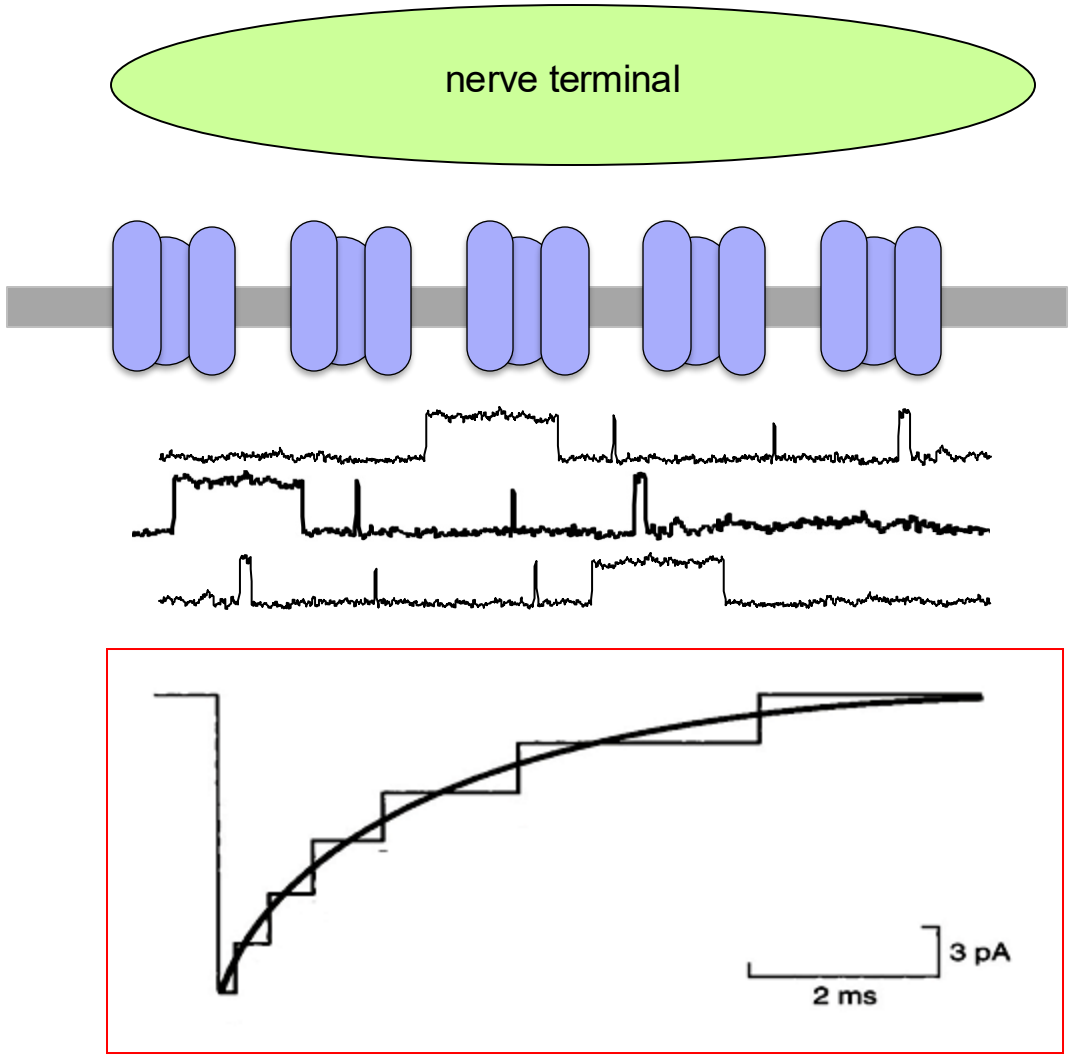
Functional features





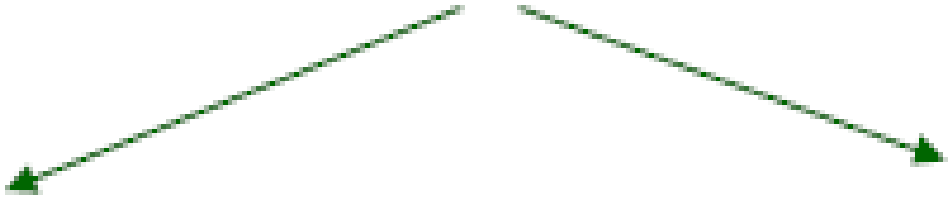


Functional features



Total Current

Ligand Gated Ion Channels in Vertebrates



Mixed Anionic

Cl^-
HCO_3^-

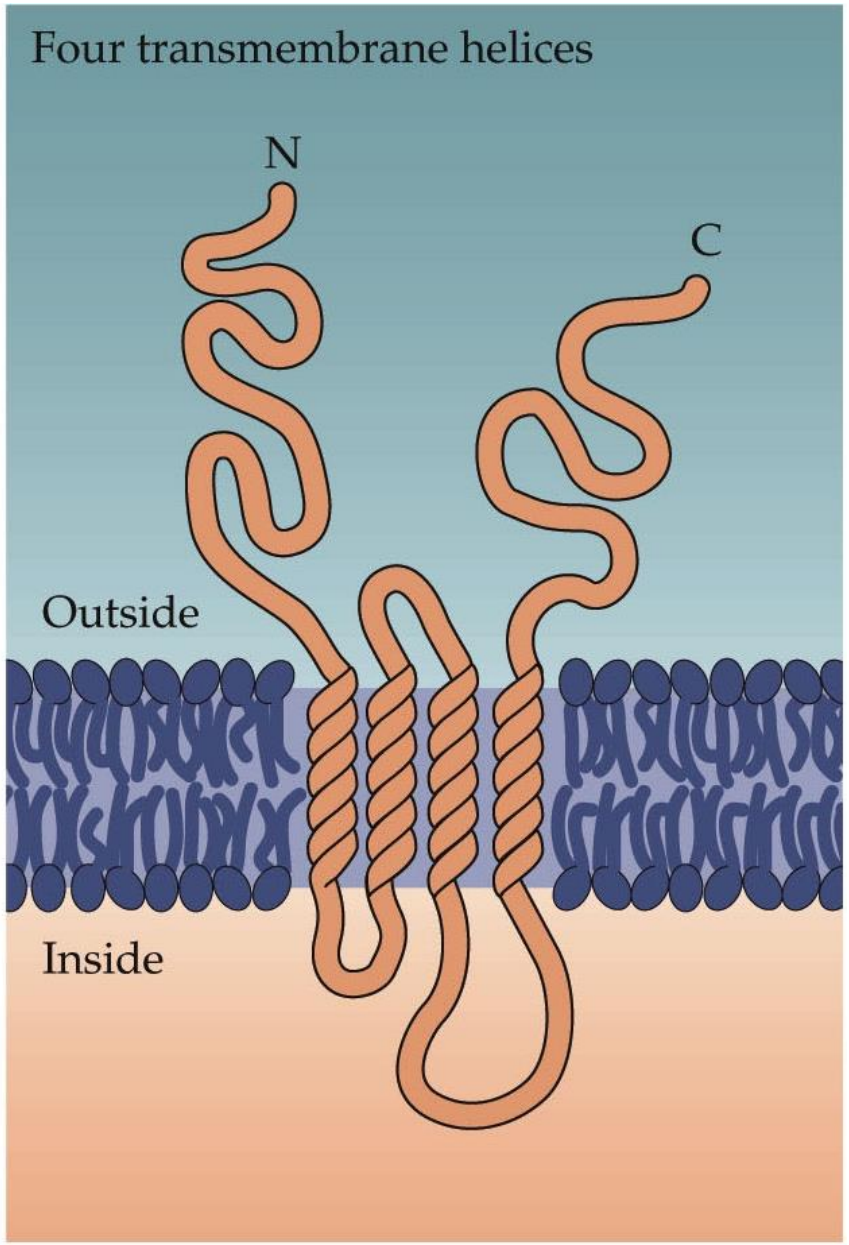
$GABA_AR$
GlycineR

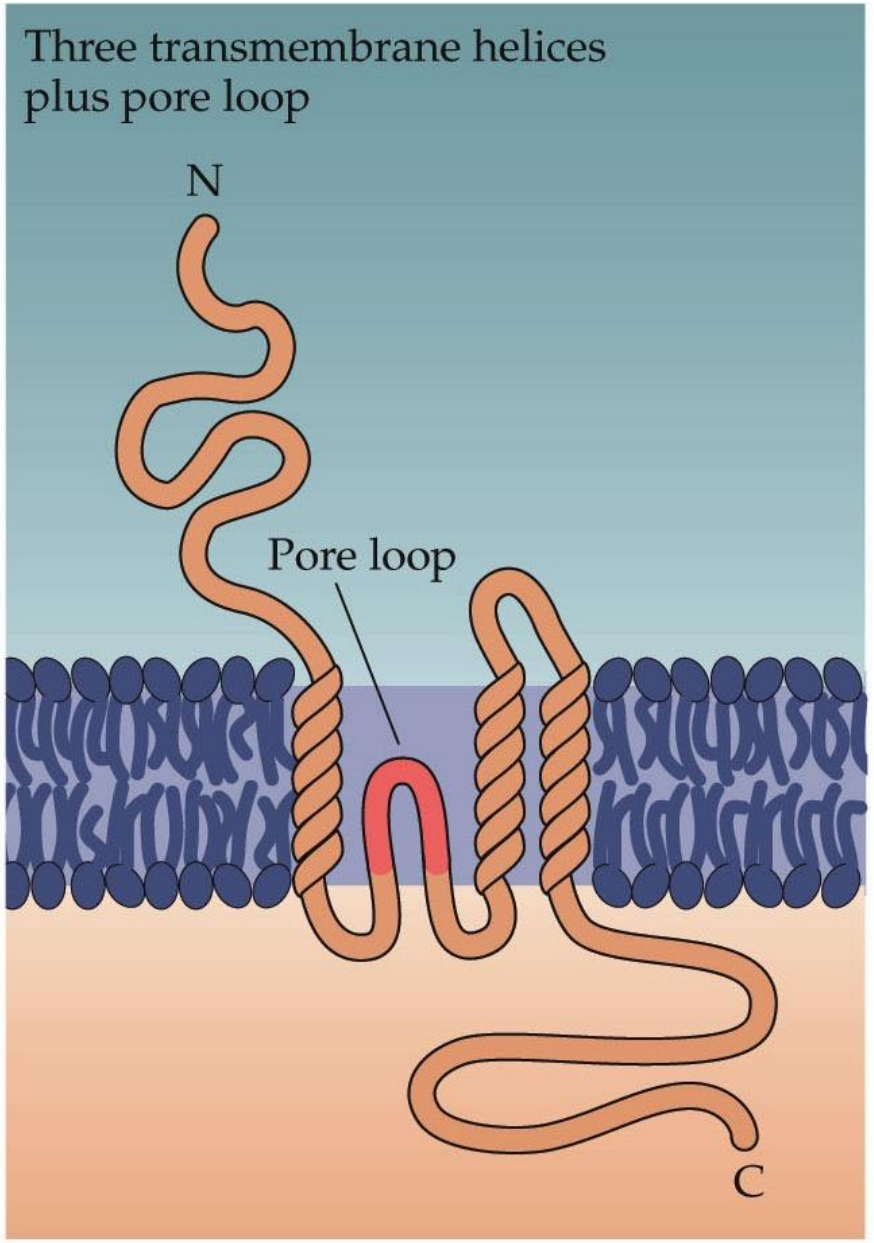
Mixed Cationic

Na^+
K^+
(Some also Ca^{2+})

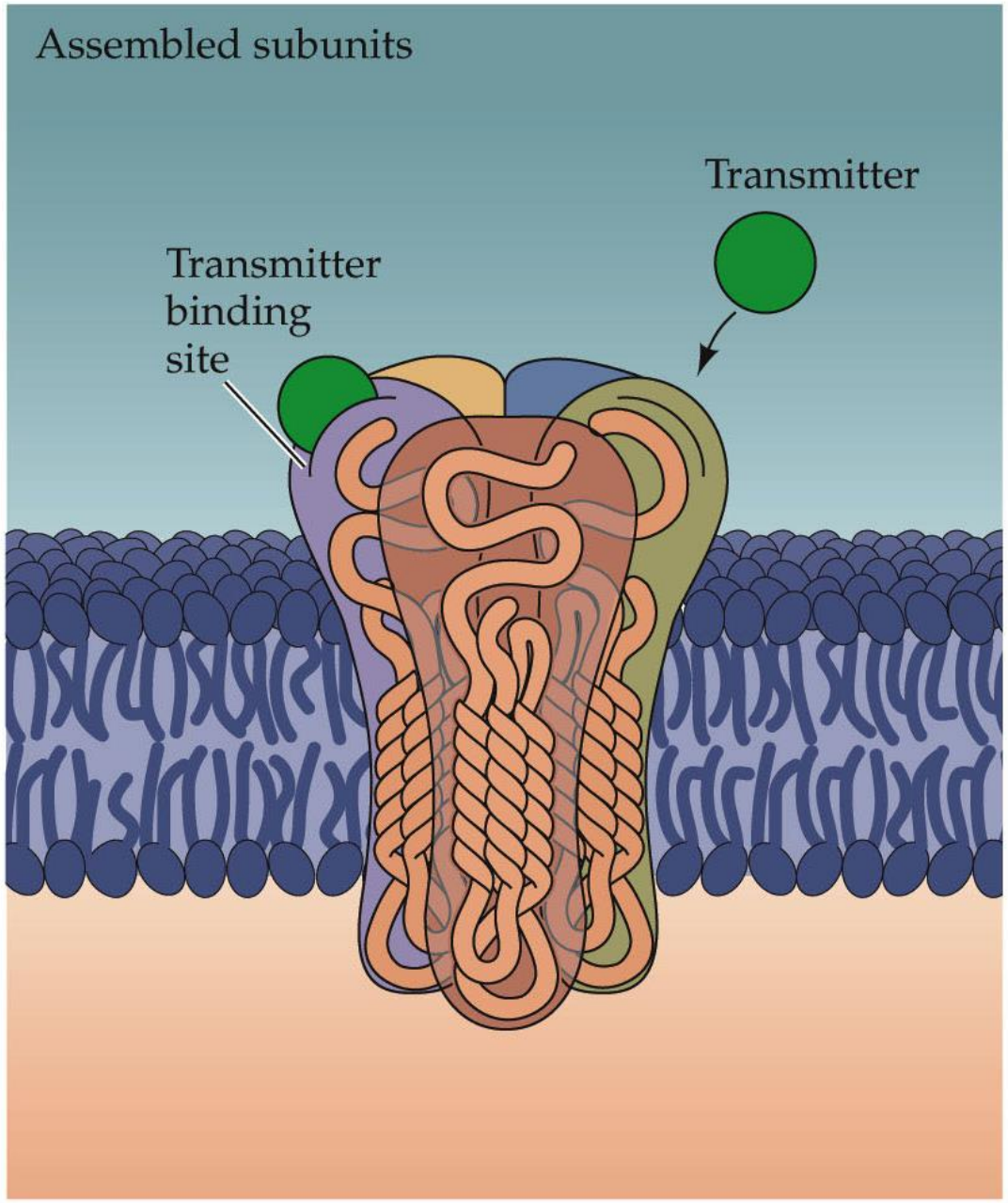
nAchR
GluR
$5\text{-}HT_3R$
PurineR

General structure





General structure



General structure

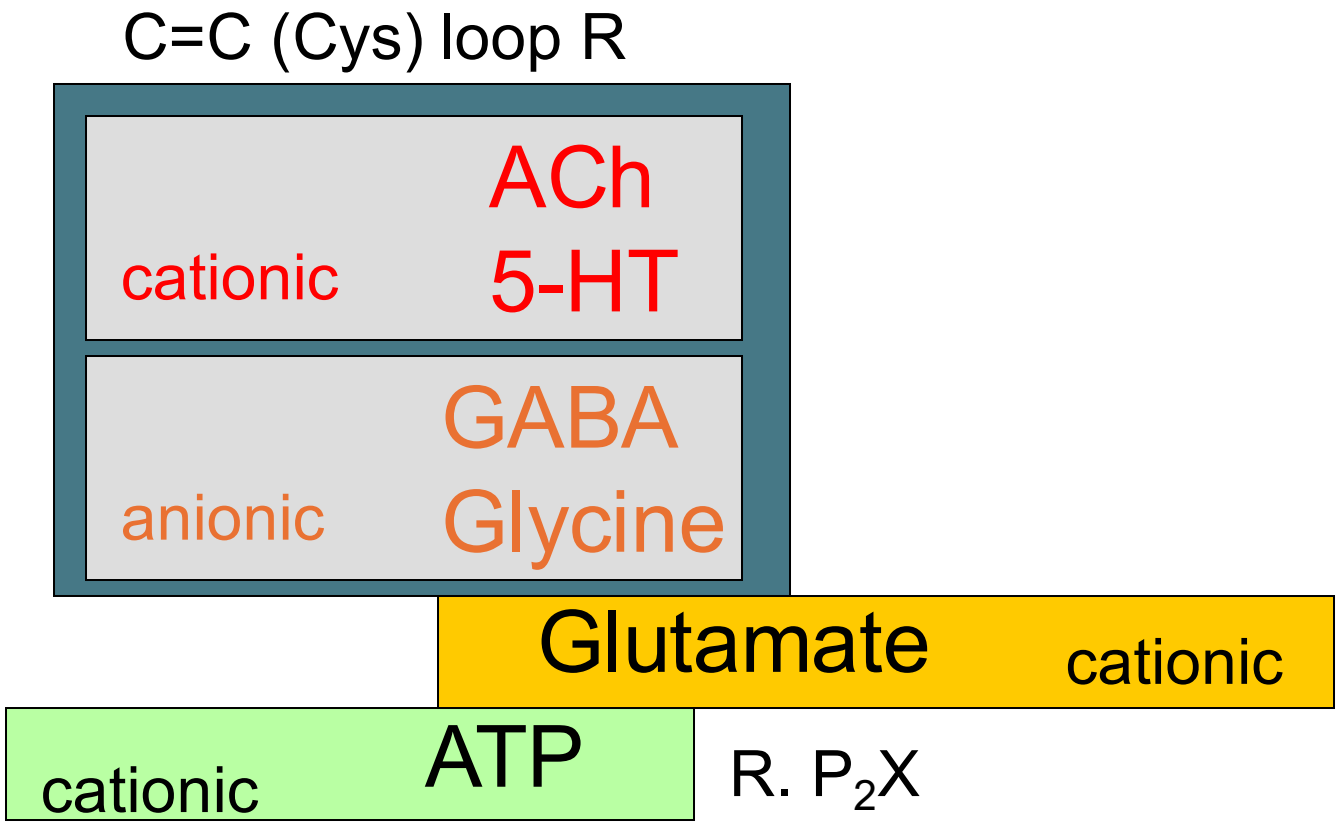
Except for rare exceptions, ionotropic receptors are heteropolymers, consisting of four or five subunits arranged in a circle, which can be present in many variations.

The structural analysis of the ionotropic receptors molecules suggests to classify them in two large families, derived from two distinct ancestral genes:

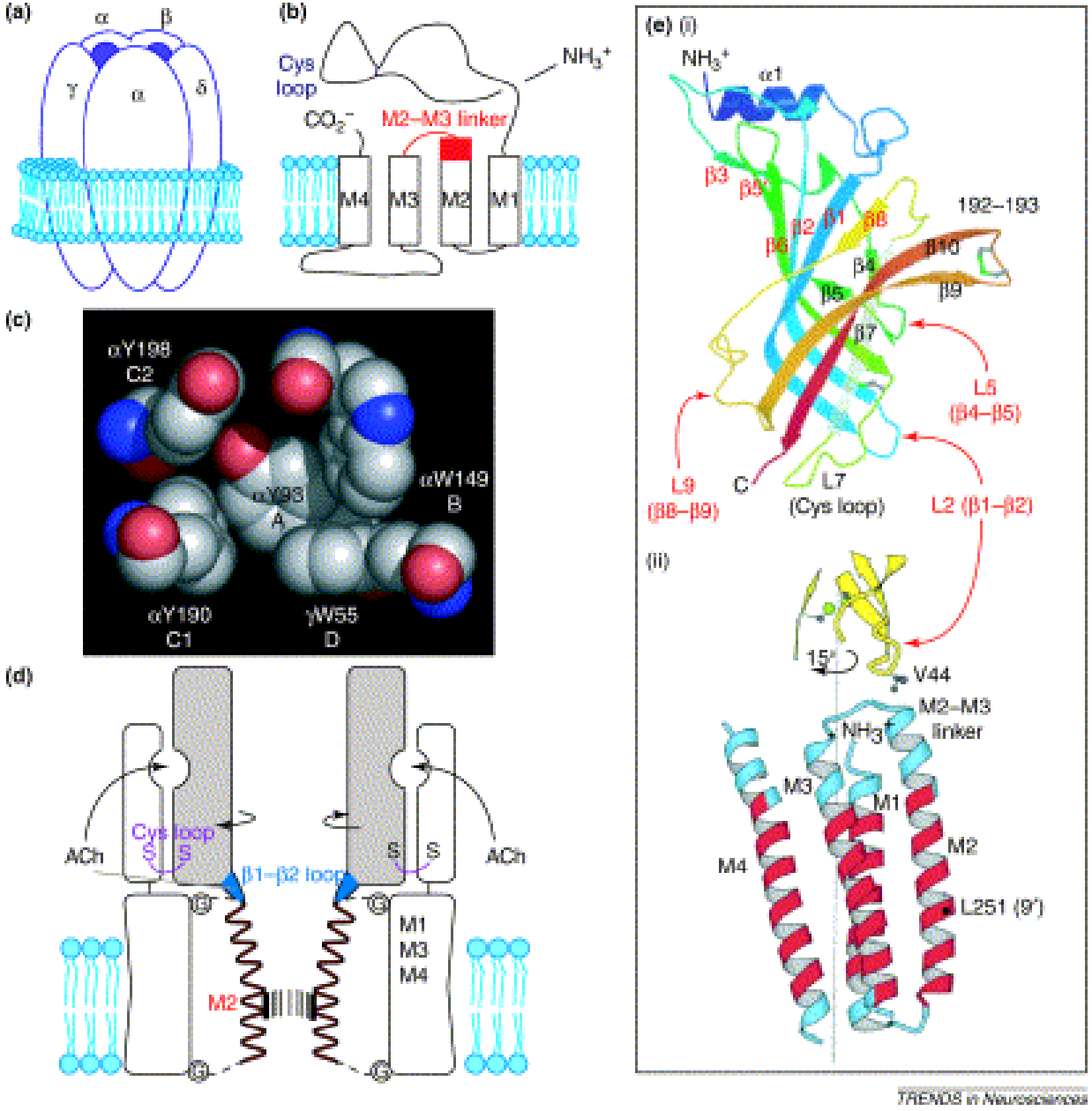
a)the family that has as its protoype the nicotinic acetylcholine receptor (nAchR) and its like; - nAchR represents the reference model for the whole class of ionotropic receptors;

b)the family which has the ionotropic receptor for ac. glutamic (iGluR).

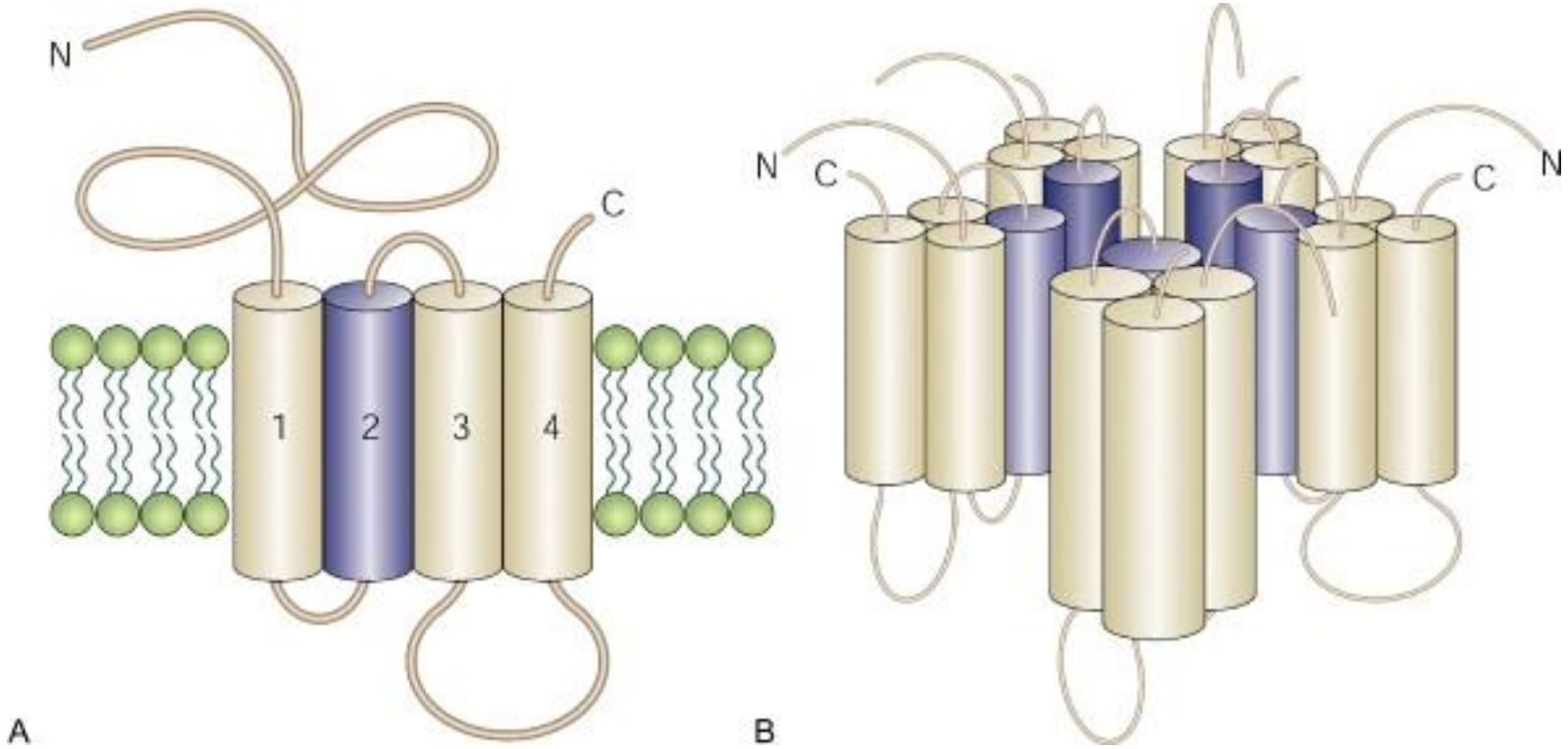
Ionotropic receptor families



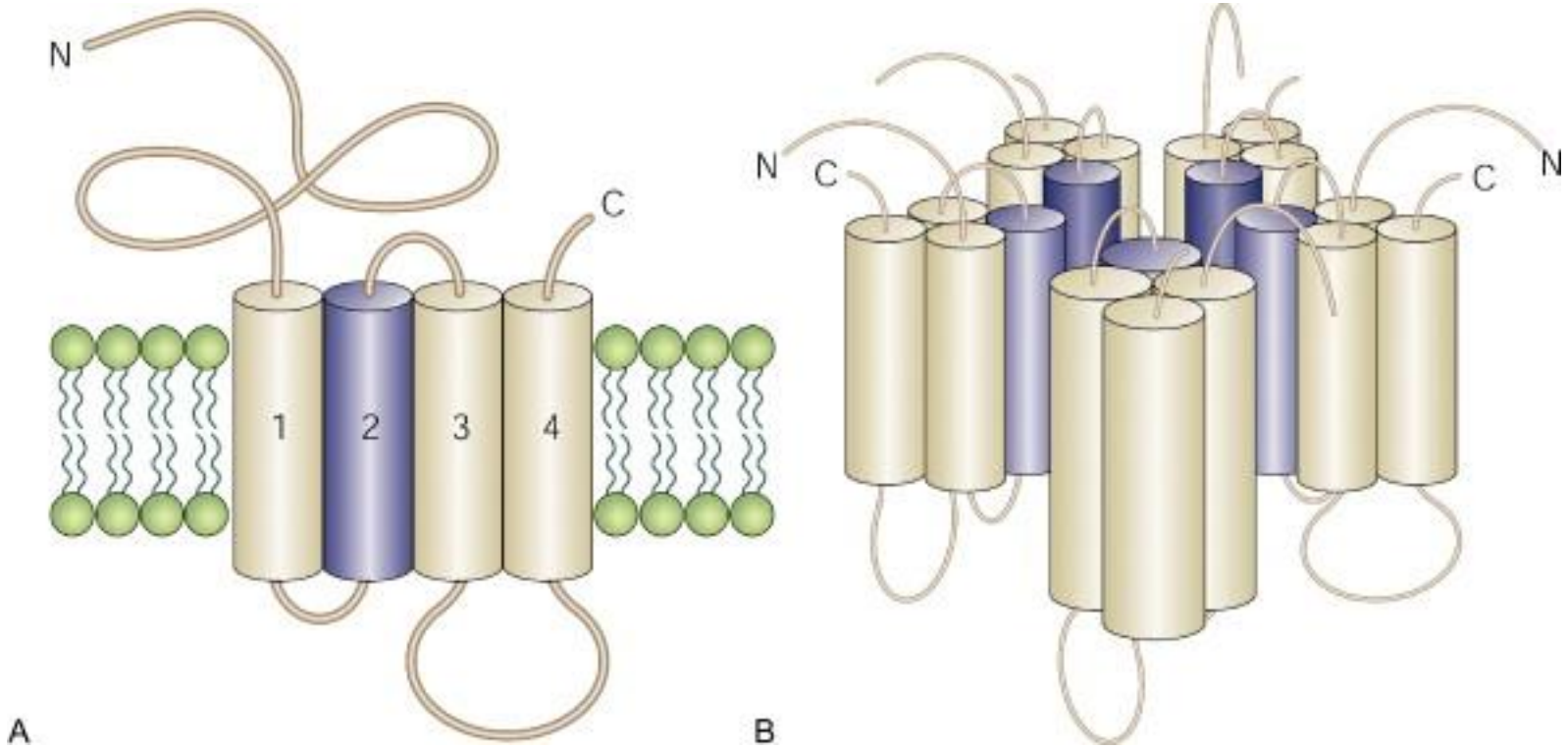
Cys-loop receptors



Cys-loop receptors

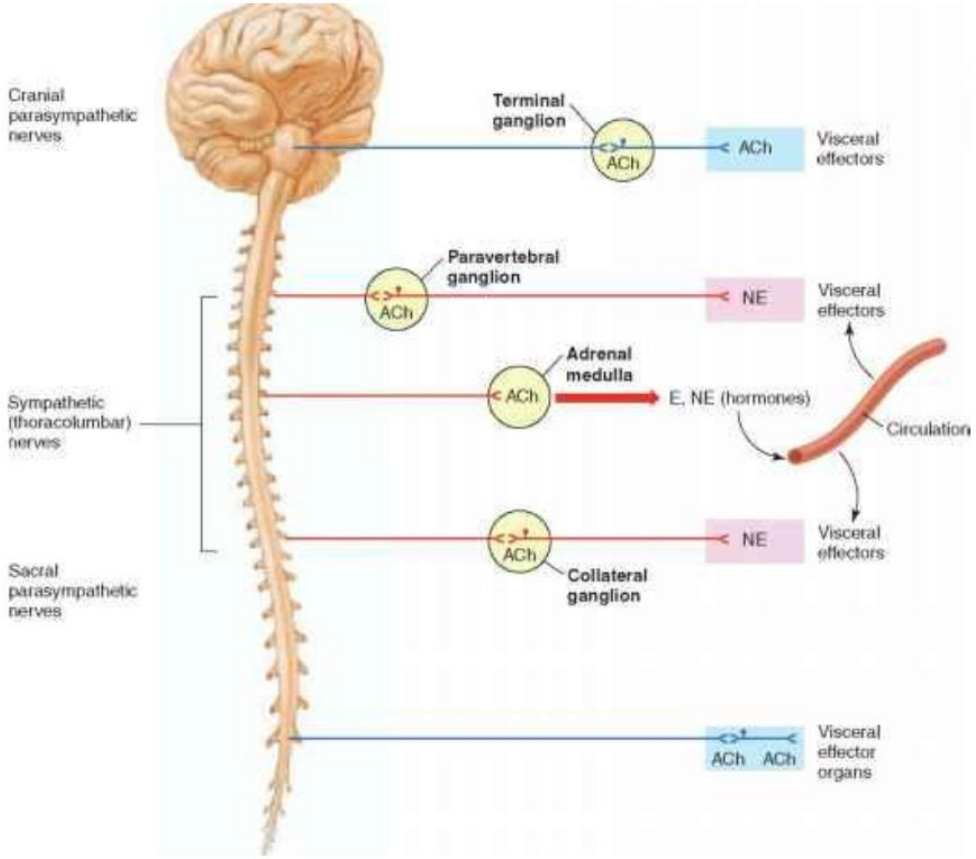


Cys-loop receptors: Ionotropic Acetylcholine Receptors

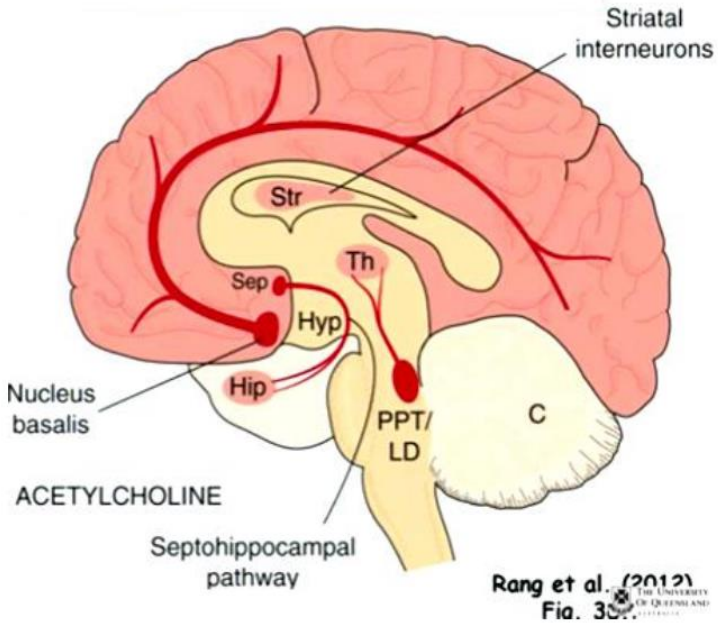


Acetylcholine

Acetylcholine (ACh) is the neurotransmitter of cholinergic system.
These nerve cells are activated by or contain and release acetylcholine



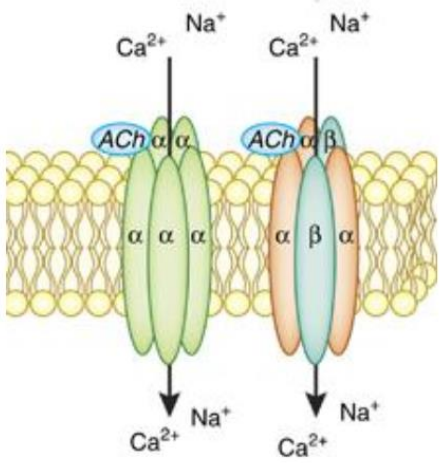
ACh is the **preganglionic** neurotransmitter for both the sympathetic and parasympathetic nervous system, and the **postganglionic** neurotransmitter in the parasympathetic nervous system



The brain **cholinergic** system has been associated with a number of cognitive functions, including memory, selective attention, and emotional processing.

Acetylcholine Receptors

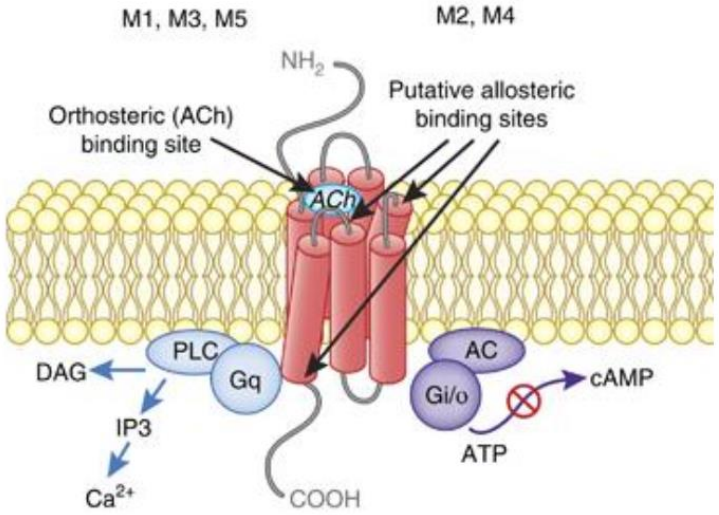
Acetylcholine binds to both muscarinic and nicotinic receptors.



Key effectors (examples)
↑ $[Ca^{2+}]i$
↑ VDCC
↑ PKC

NICOTINIC R

N_N, N_M



Key effectors (examples)
↑ PLCβ
↑ $[Ca^{2+}]i$
↑ MAPK

Key effectors (examples)
↑ AC
↑ MAPK
↑ GIRK ch.

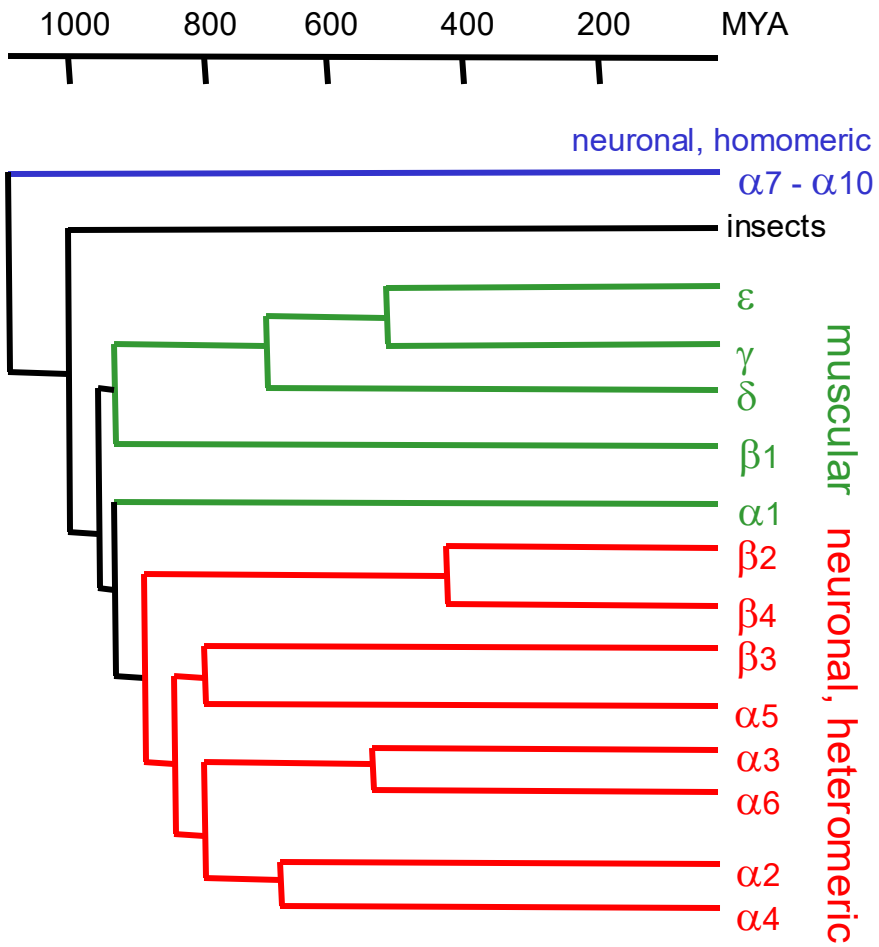
MUSCARINIC R

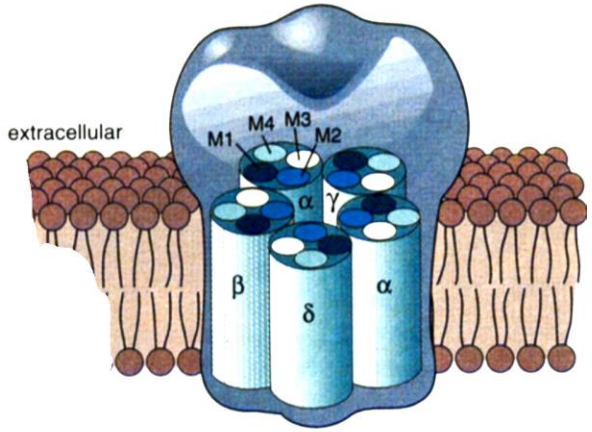
M_1, M_2, M_3, M_4, M_5

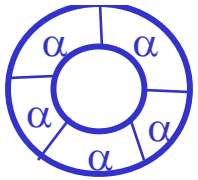
Nicotinic receptors are **Receptor-operated channel receptors** and get their name from nicotine, which selectively binds to the nicotinic receptor.

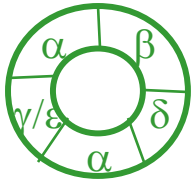
Muscarinic receptors are **G-protein coupled receptors** and get their name from a chemical that selectively attaches to that receptor, called muscarine.

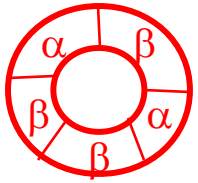
Ionotropic Nicotinic Receptors





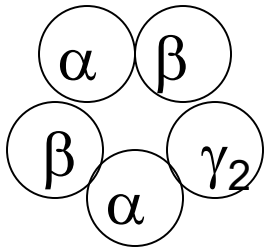






nAChRs are hetero- o omo-pentamers

Ionotropic $GABA_A$ Receptors

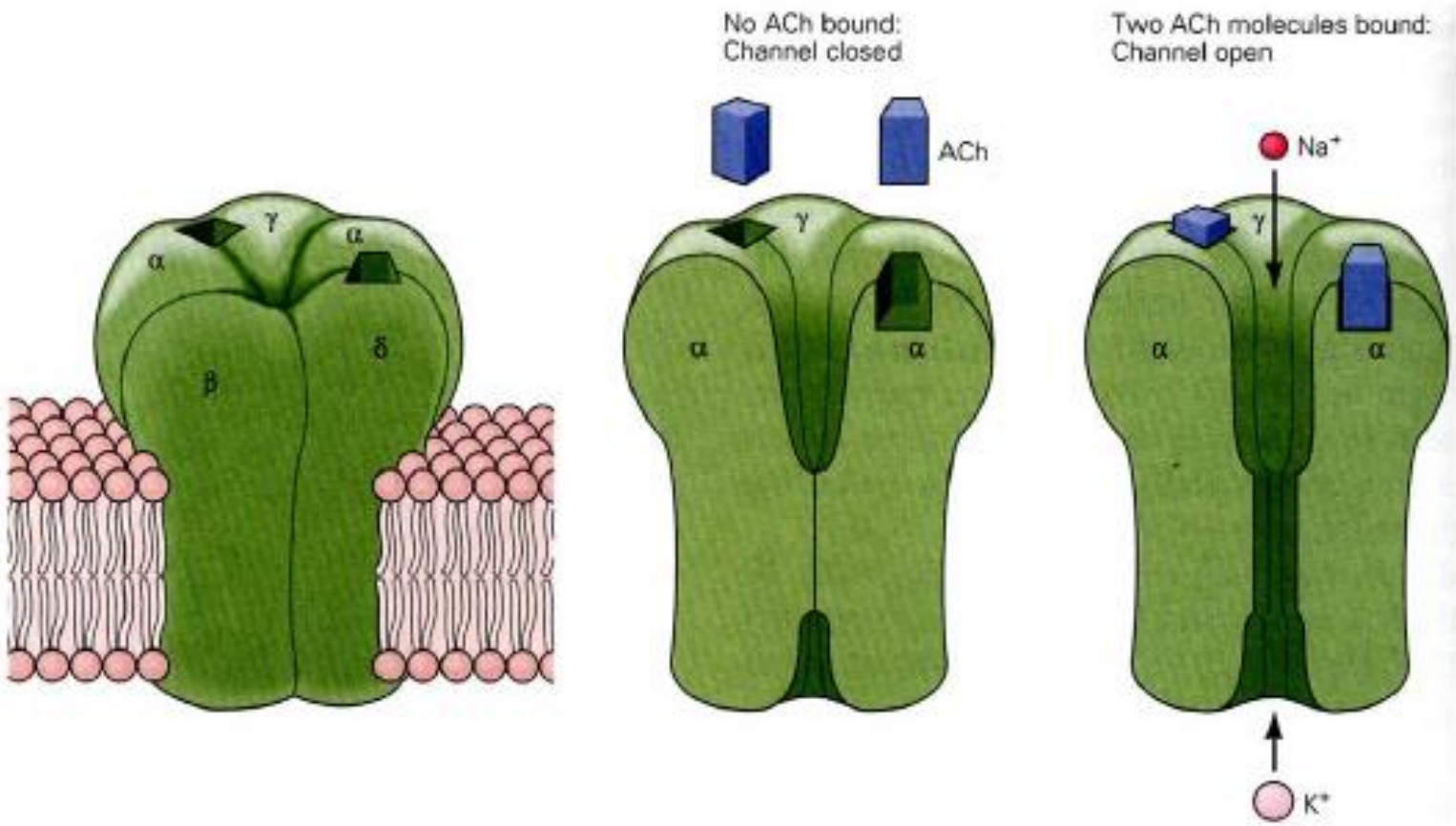


Opening Probability and duration of the openings are conditioned by:

benzodiazepines
barbiturates
anesthetics
Alcohol
steroid hormones

Gene	Protein	Chromosome location (human)
GABRA1	α1	5q31.1–33.2
GABRA2	α2	4p12–p13
GABRA3	α3	Xq28
GABRA4	α4	4p14–q12
GABRA5	α5	15q11–q13
GABRA6	α6	5q31.1–33.2
GABRB1	β1	4p12–p13
GABRB2	β2	5q31.1–33.2
GABRB3	β3	15q11–q13
GABRG1	γ1	4p14–q21.1
GABRG2	γ2	5q31.1–33.2
GABRG3	γ3	15q11–q13
GABRD1	δ1	1p
GABRE1	ε	Xq28
	ρ1	6q14–q21
	ρ2	6q14–q21

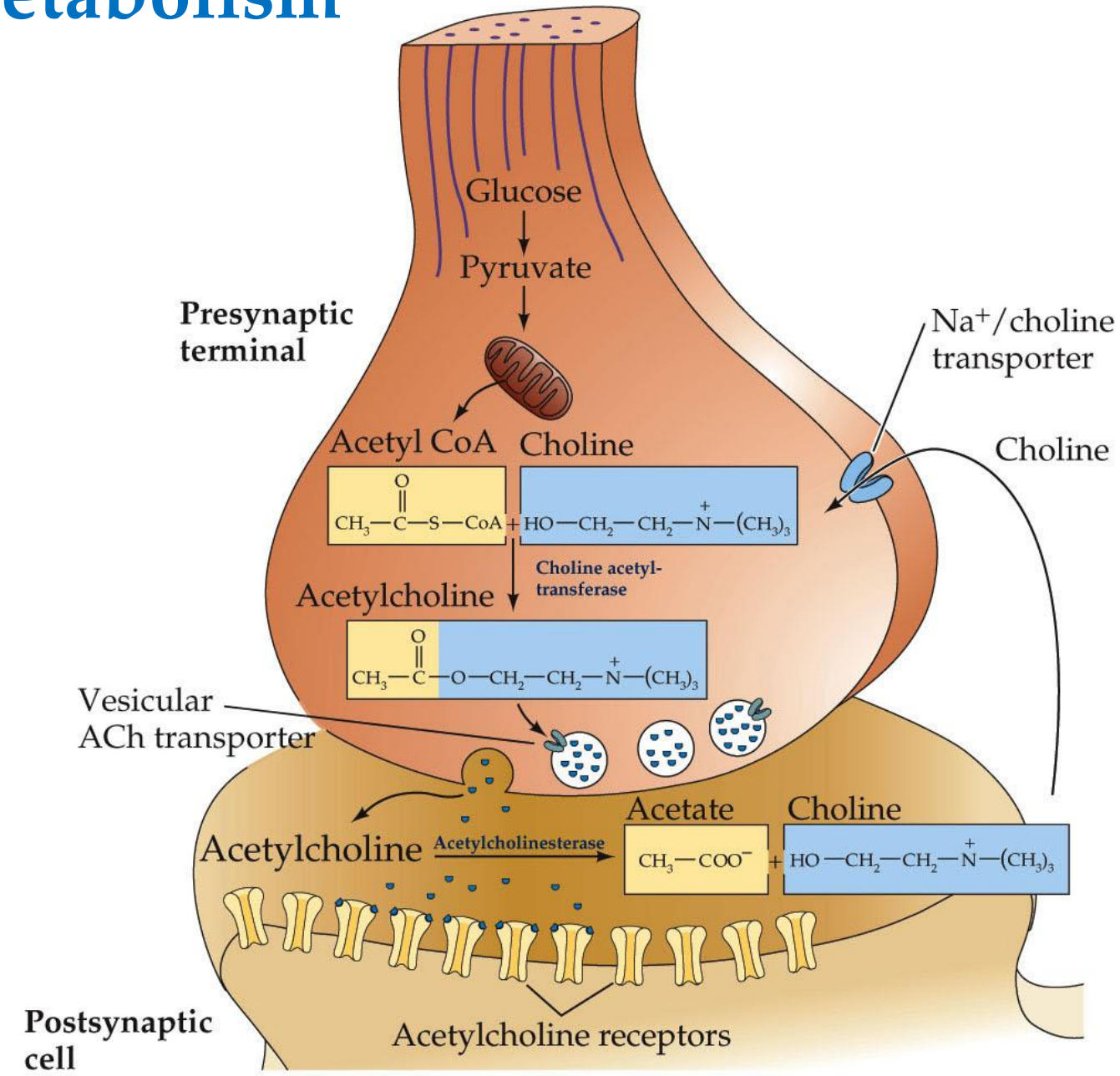
Nicotinic Acethylcholine Receptors nAChR



$$R \underset{k_{-1}}{\overset{A,\ k_1}{\rightleftharpoons}} AR \underset{k_{-2}}{\overset{A,\ k_2}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

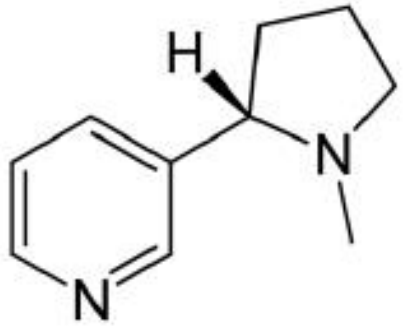
(closed) (closed) (closed) (open)

ACh metabolism



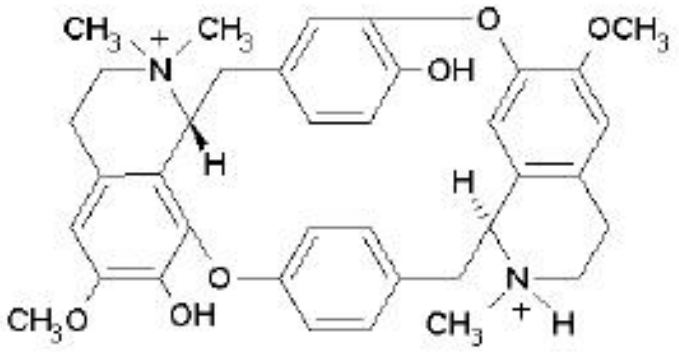
Substances that interact with nicotinic transmission

Nicotine



Nicotina tabacum

Curare

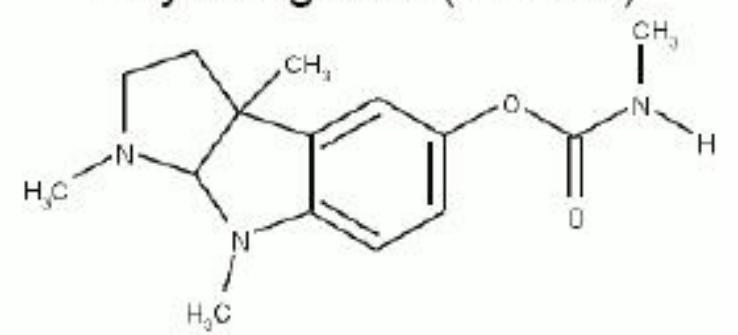


· 5H2O 2Cl⁻

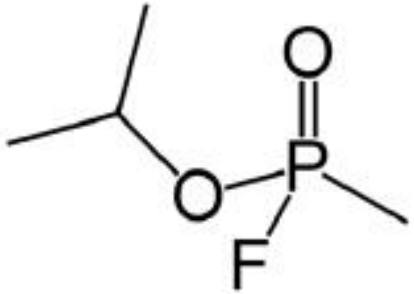
Strychnos Toxifera

Substances that interact with nicotinic transmission

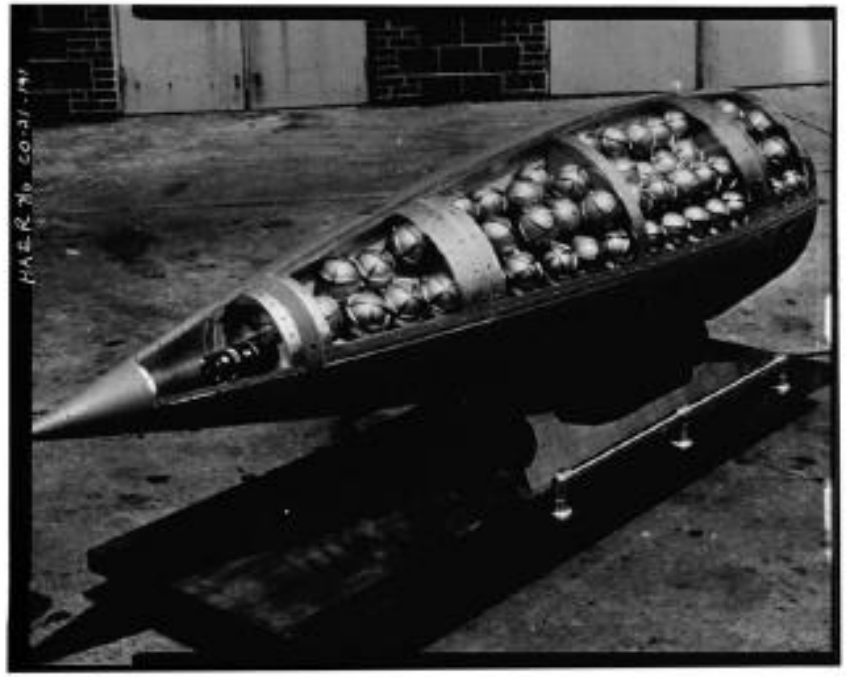
Physostigmine (eserine)



Nerve Gas (Sarin)

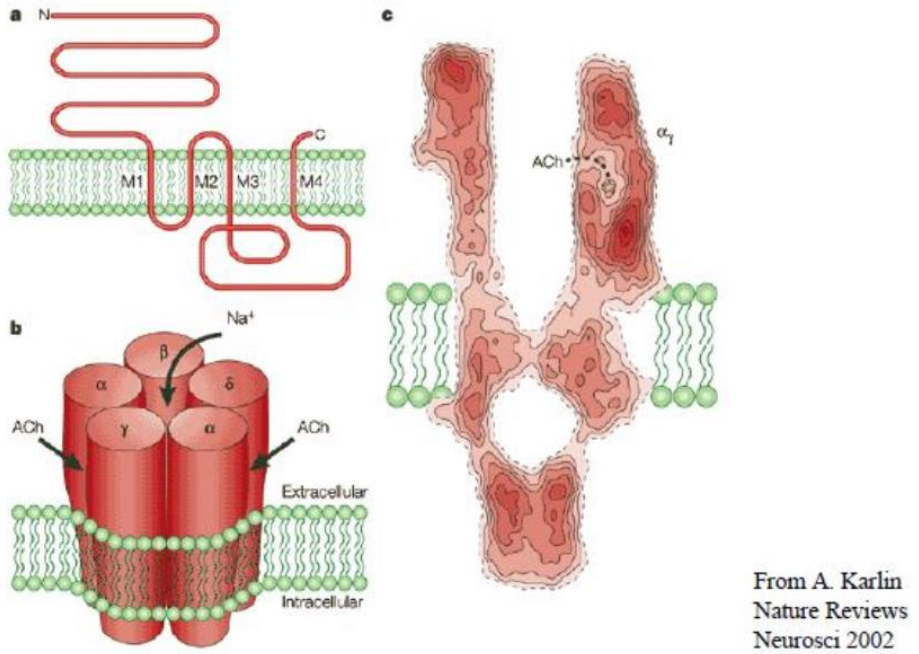


Physiostigma Venenosum
(Calabar bean)

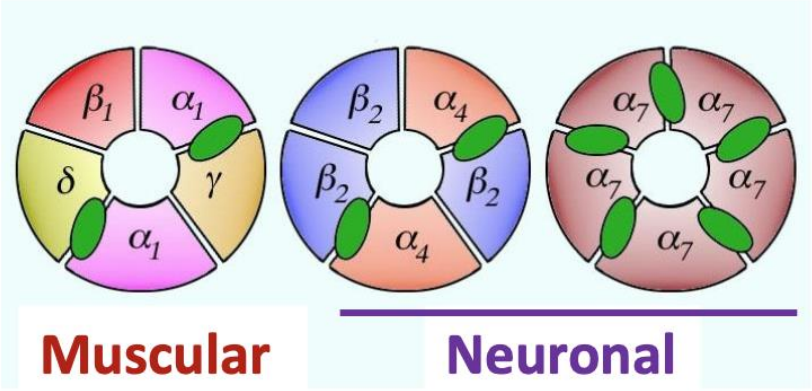


Ionotropic Nicotinic Receptors

- *5 transmembrane subunits*:
  α (2), β, γ, δ or ε
- Each subunit possesses 4 TSM
- They form a pentameric structure, with a **γ** subunit interposing the **2 α** subunits

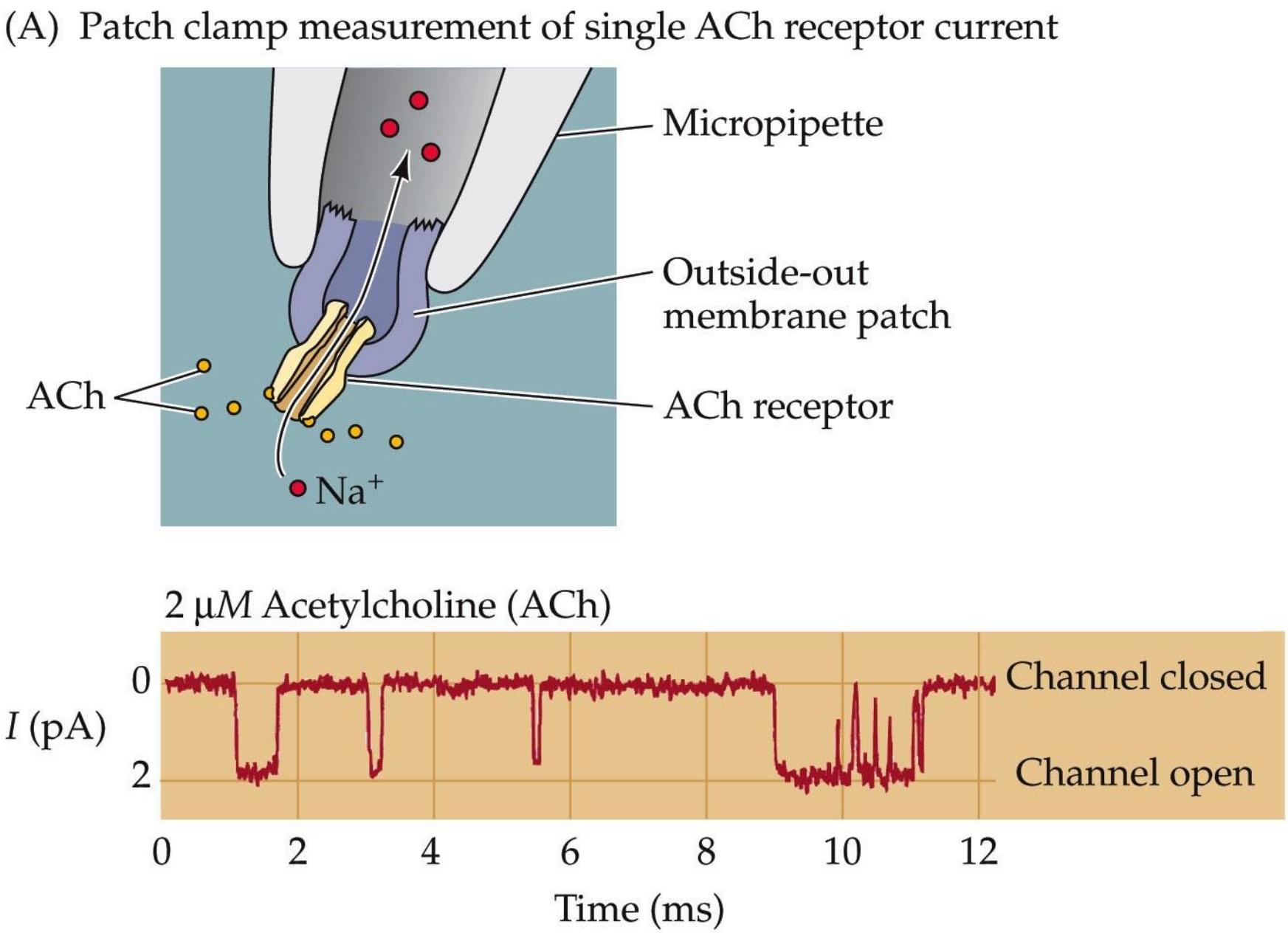


From A. Karlin
Nature Reviews
Neurosci 2002

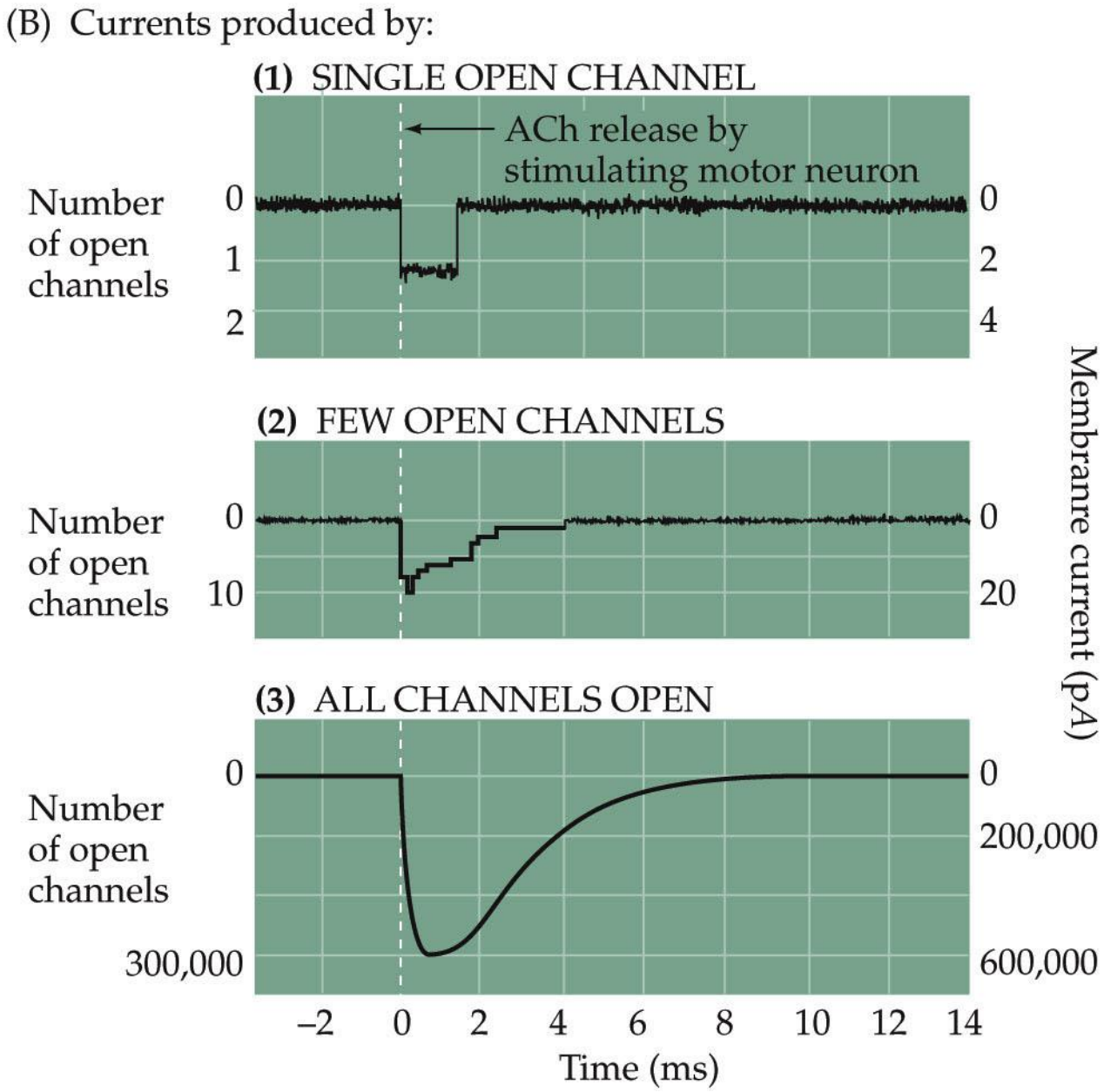


- **MUSCULAR** nicotinic receptor is a cation channel allowing cell entrance of NA, and to a lesser extent , of K and Ca
- **NEURONAL** nicotinic receptor preferentially permits Ca entry

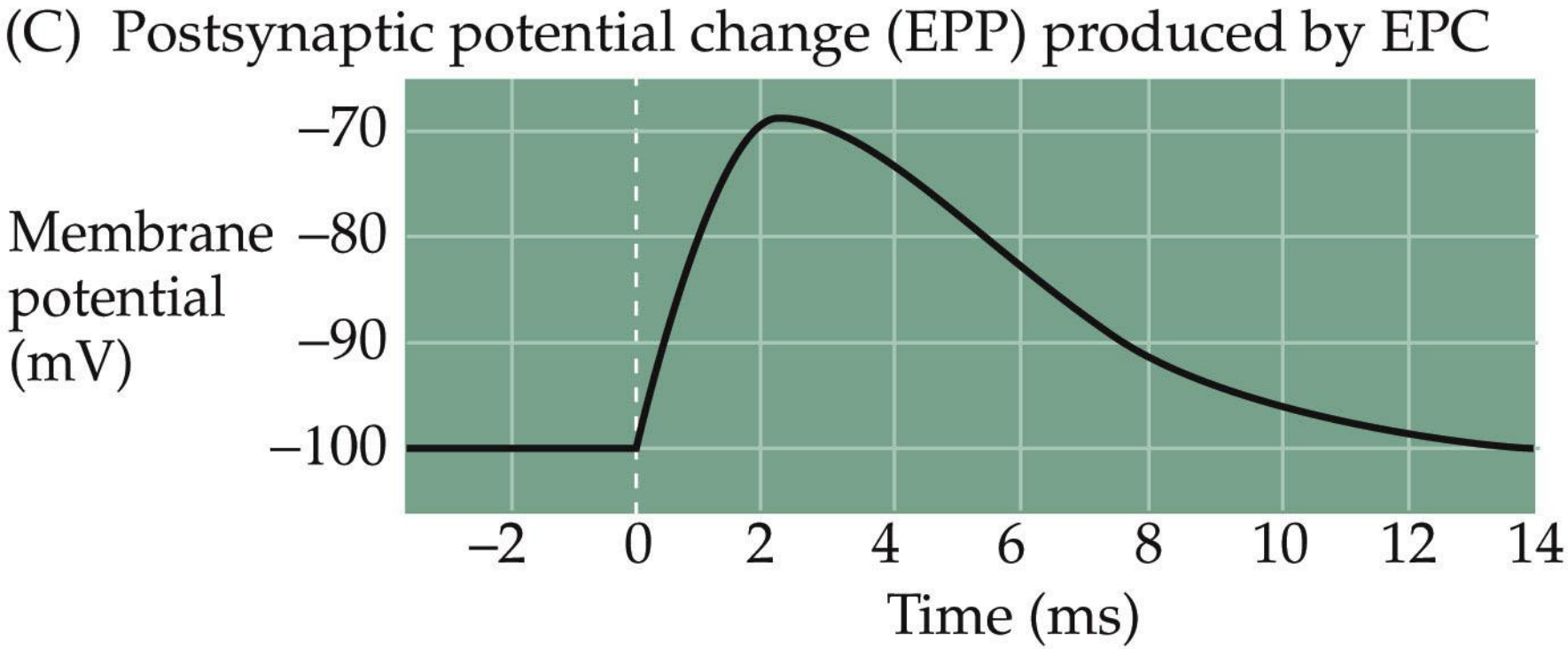
nAChR at NMJ


(A) Patch clamp measurement of single ACh receptor current
Micropipette
Outside-out membrane patch
ACh receptor
ACh
Na^+
2 μM Acetylcholine (ACh)
I (pA)
0
2
Channel closed
Channel open
0
2
4
6
8
10
12
Time (ms)

nAChR at NMJ

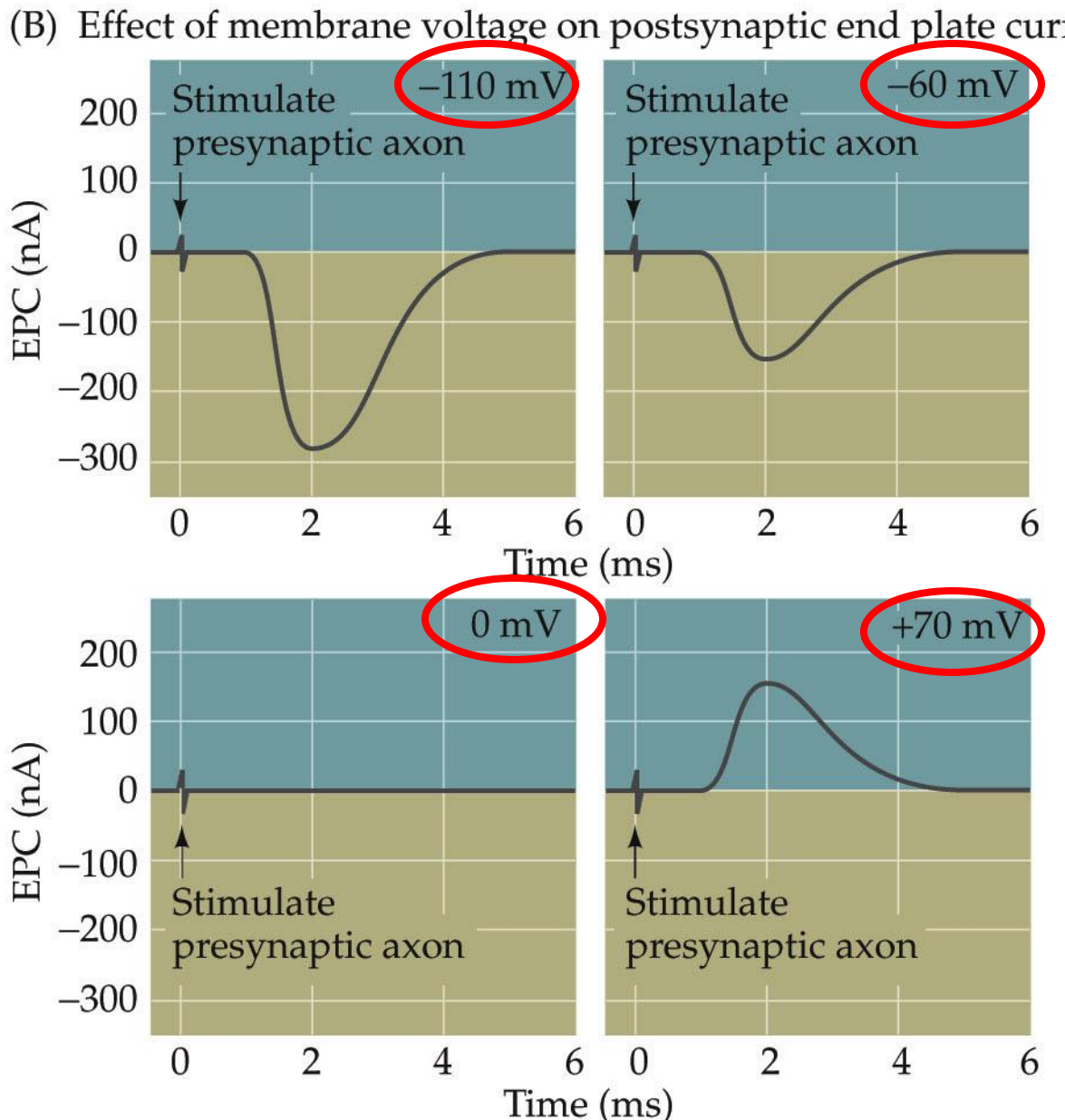

(B) Currents produced by:
(1) SINGLE OPEN CHANNEL
ACh release by stimulating motor neuron
Number of open channels
0
1
2
0
2
4
(2) FEW OPEN CHANNELS
Number of open channels
0
10
0
20
(3) ALL CHANNELS OPEN
Number of open channels
0
300,000
0
200,000
600,000
–2
0
2
4
6
8
10
12
14
Time (ms)
Membranre current (pA)

nAChR at NMJ



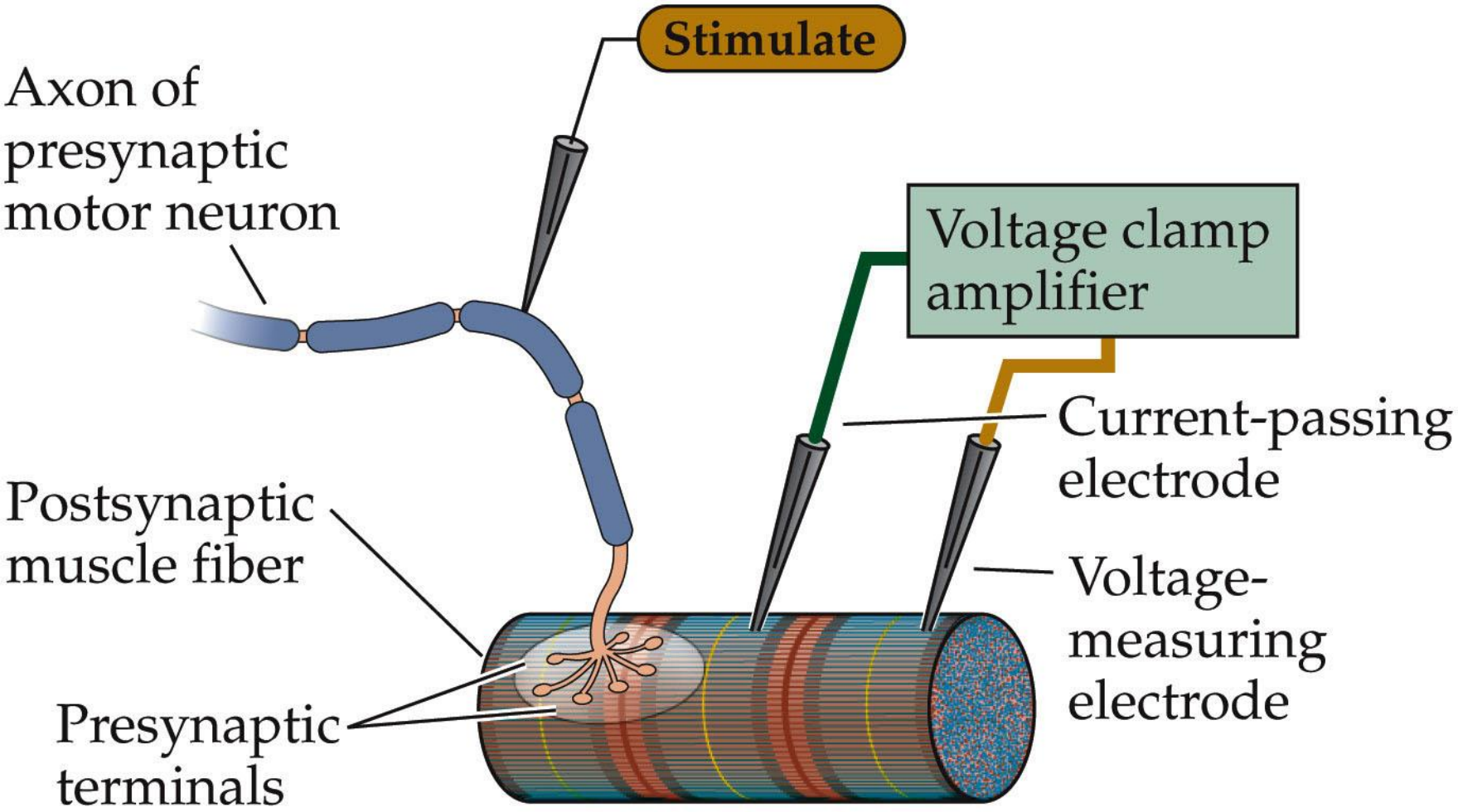
***NEUROSCIENCE, Fourth Edition,* Figure 5.16 (Part 3)**

Influence of the postsynaptic membrane potential on the nicotinic (muscular) currents

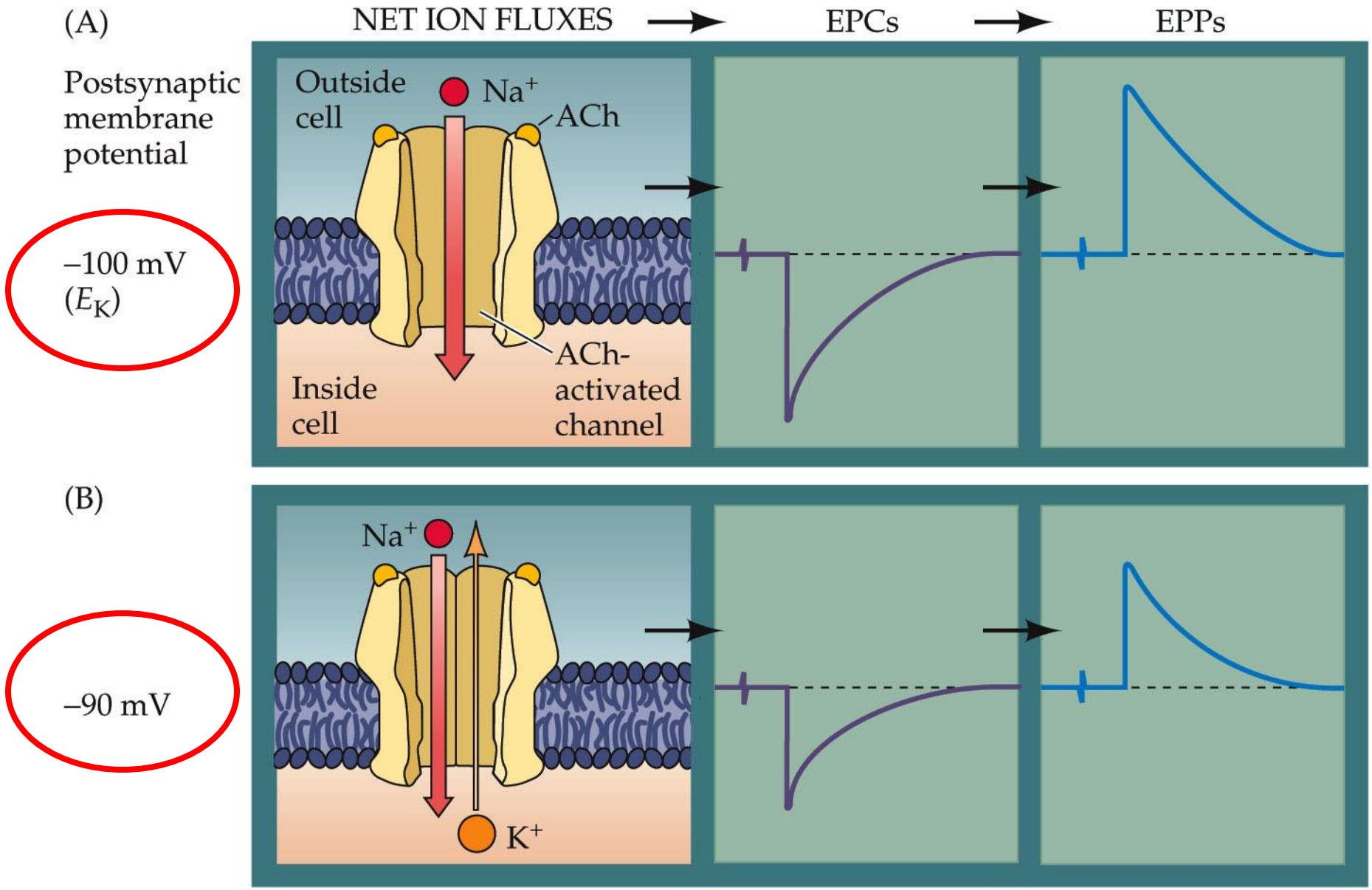


Influence of the postsynaptic membrane potential on the endplate currents

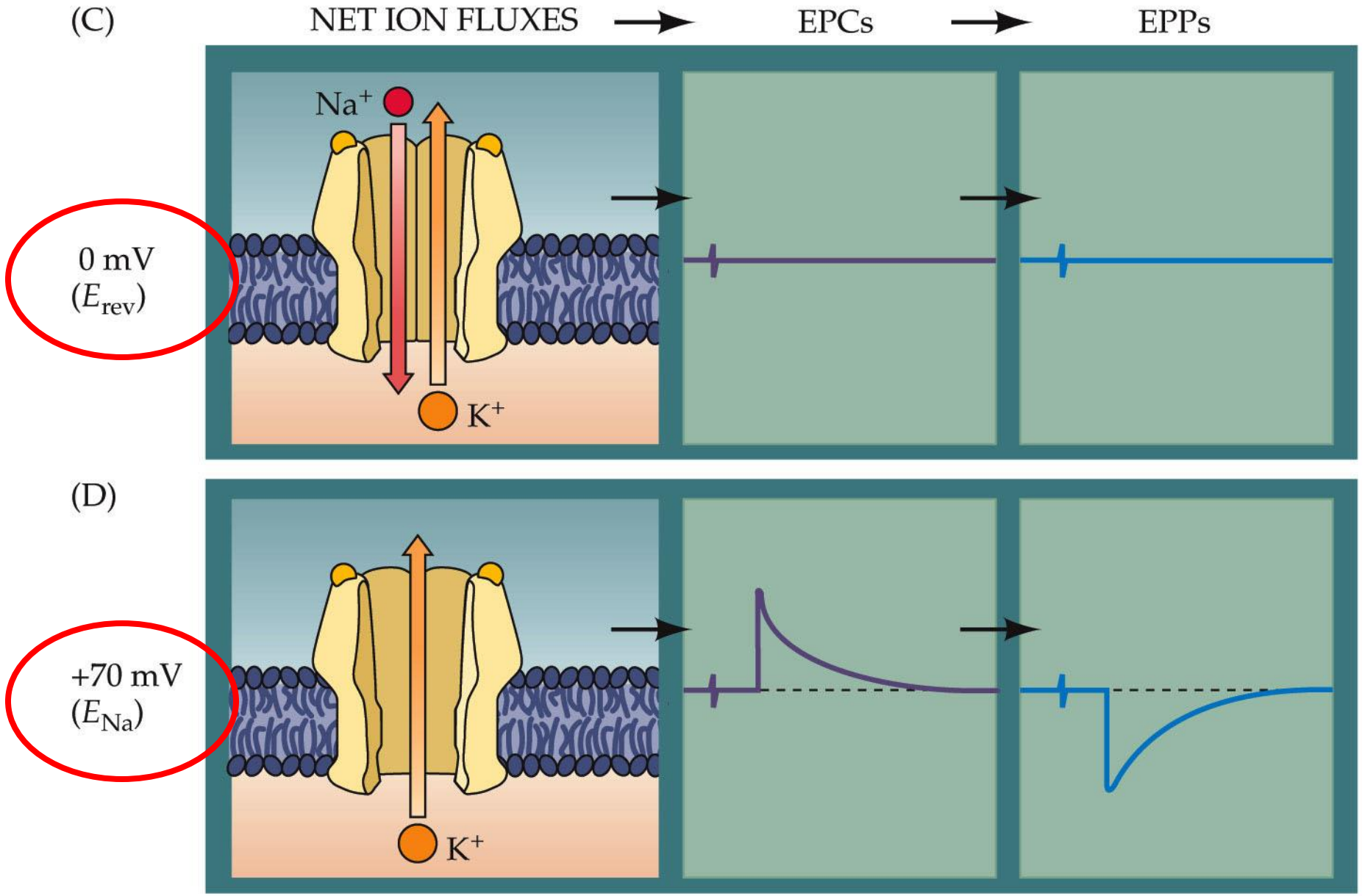
(A) Scheme for voltage clamping postsynaptic muscle fiber



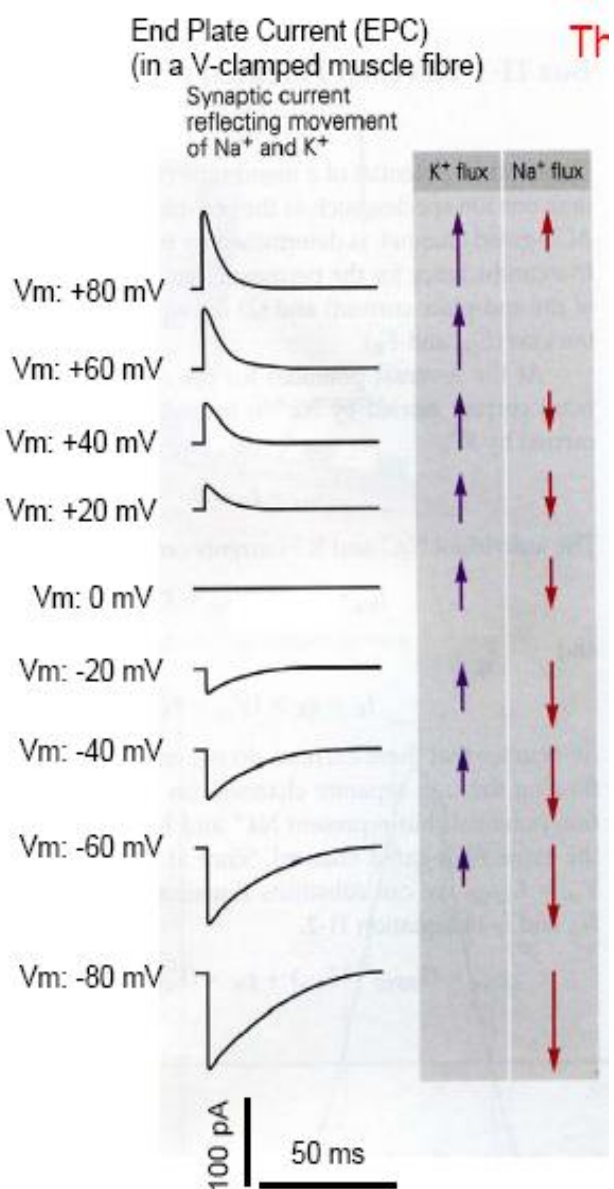
Na^+ & K^+ contribute to the current generated by the activation of nACh .... How do they move?



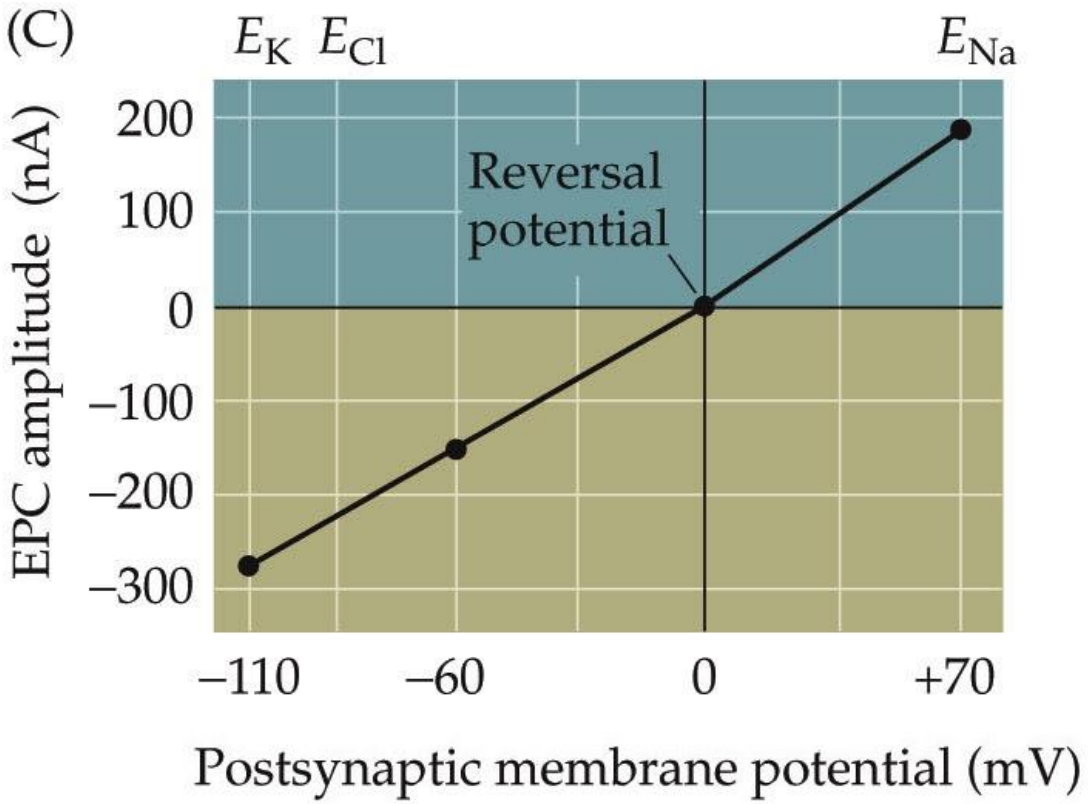
Na^+ & K^+ contribute to the current generated by the activation of nACh .... How do they move?



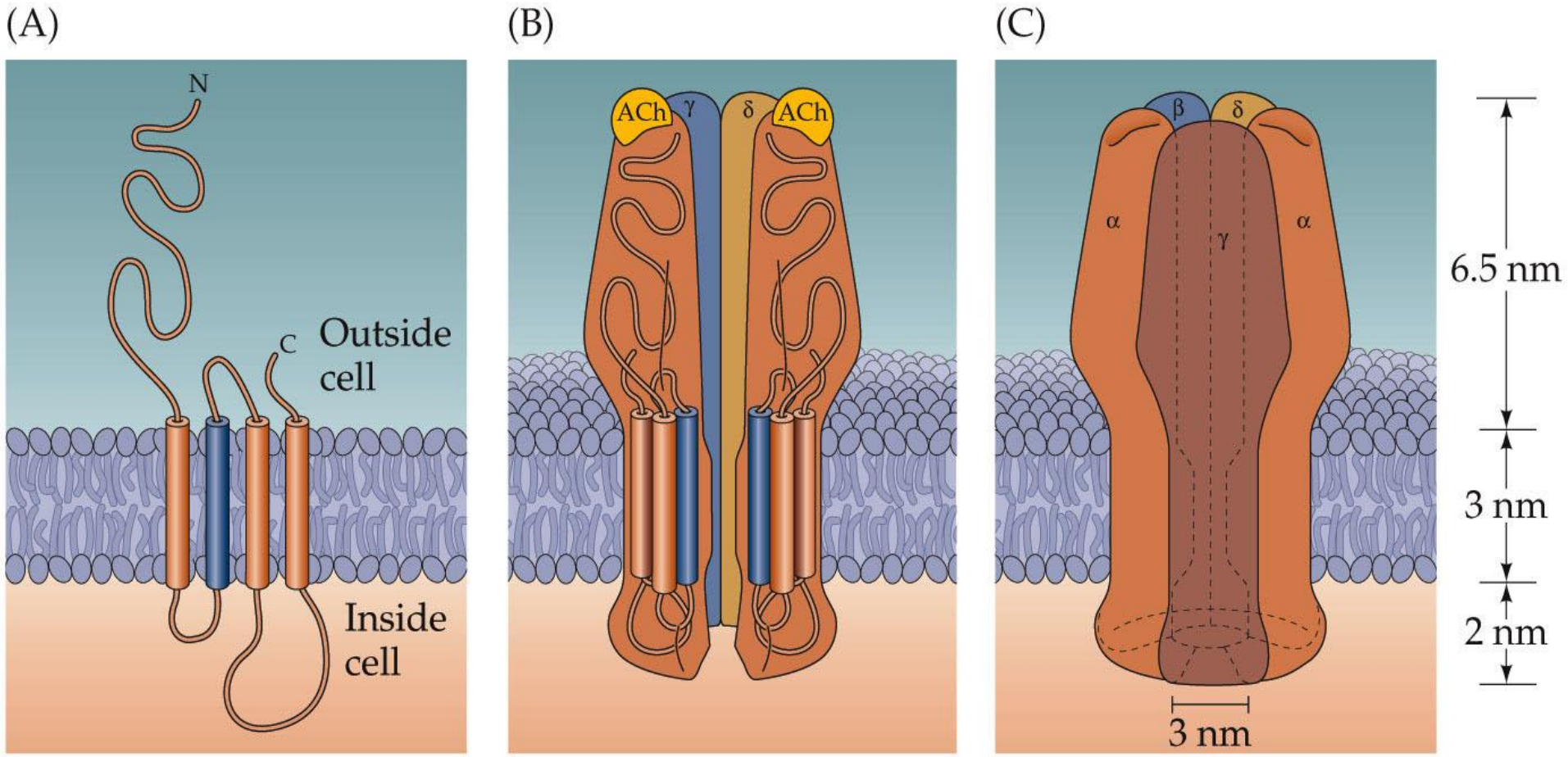
Influence of the postsynaptic membrane potential on the nicotinic (muscular) currents



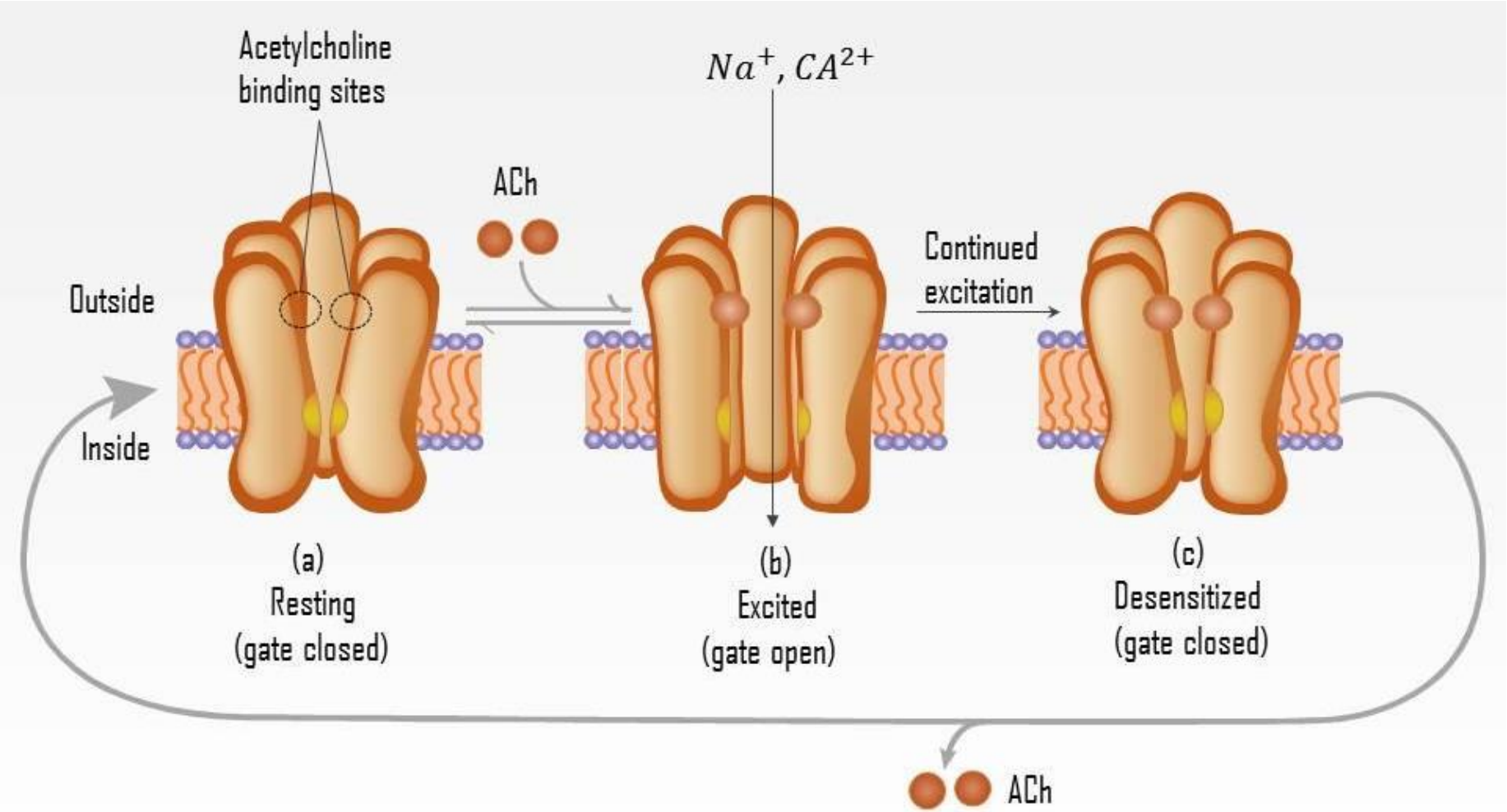
I-V curve



nAChR structure

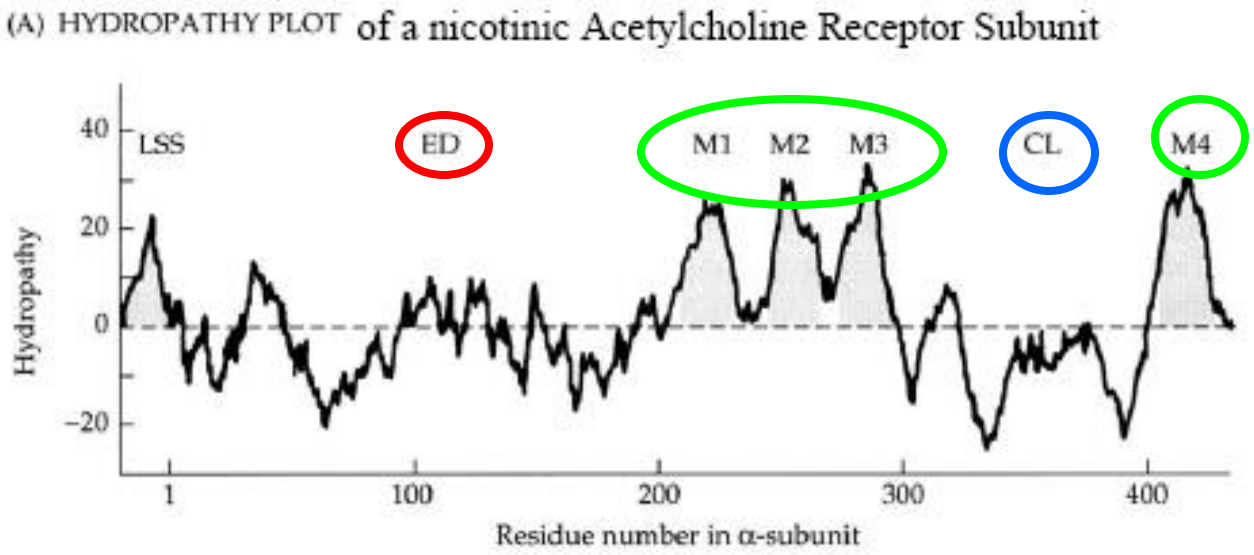


nAChR binding and states



Acetylcholine binding--> Either Na+ or Ca+2 pass --> initiate membrane depolarization --> Normally acetylcholine is lowered by acetylcholinesterase --> if abnormally remain high --> conformation change -->desensitization.

nAChR structure



LSS: Leading signal sequence (cleved in mature AchR)

ED: Extracellular domain

M1-4 Membrane spanning segment

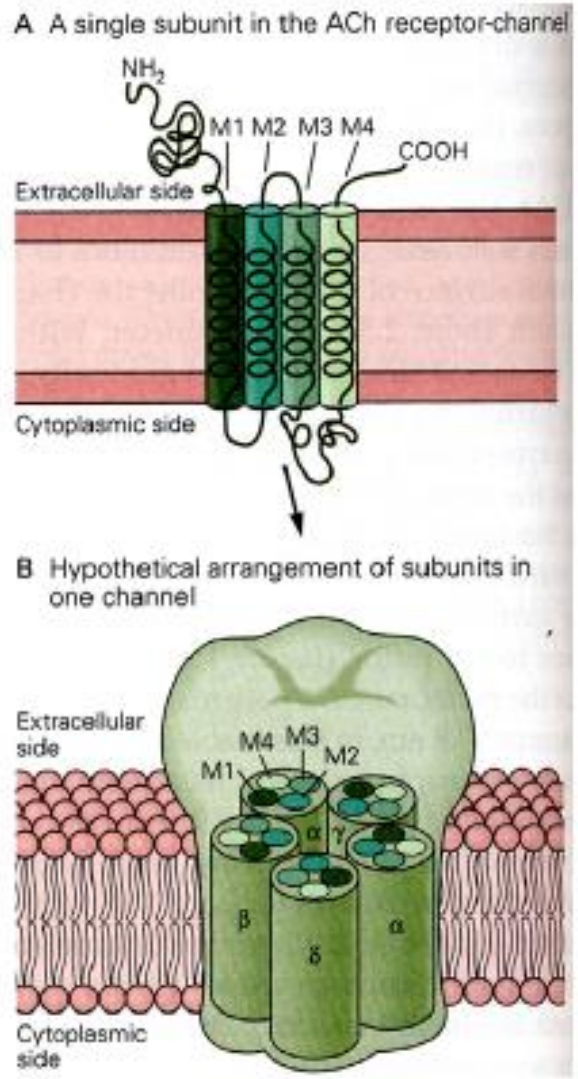
CL: Cytoplasmatic Loop

Distribution of polar and nonpolar AA provides info on how the AA sequence spans the membrane

5 subunits each with 4TM domains (hydrophobic regions)

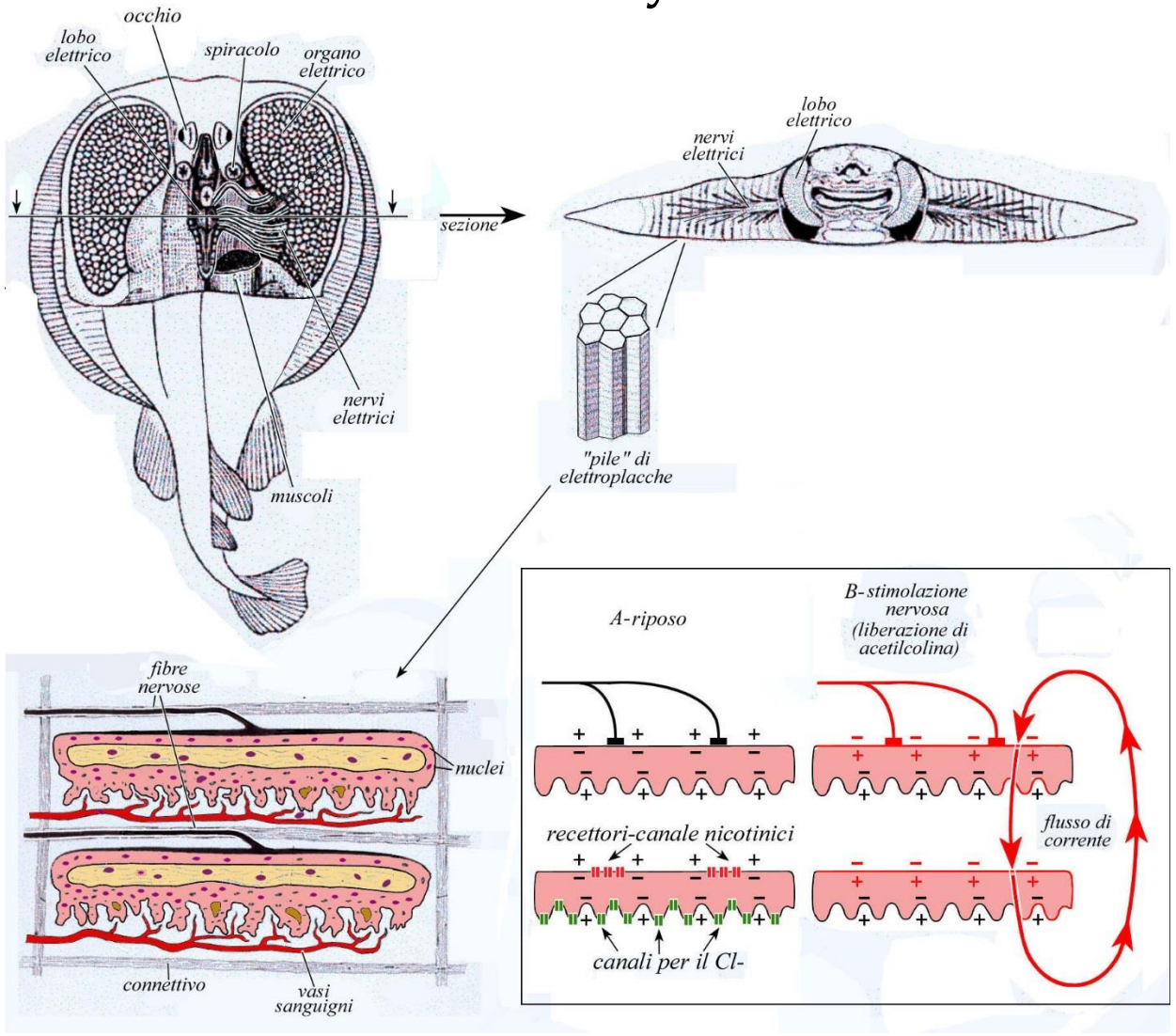
External end Wide mouth 2.5 nm

Narrowest diameter ~0.8 nm



nAChR structure

the molecules of the best known specimen: (nAchR) T, are present in the membranes of the electric organ of the Torpedo (Torpedo) in such a large quantity as to constitute "almost crystalline" structures.



α-bungarotoxin (Cobra) binds to nAchRs with very high affinity, facilitating protein extraction and purification

Steve Irwin

nAChR structure

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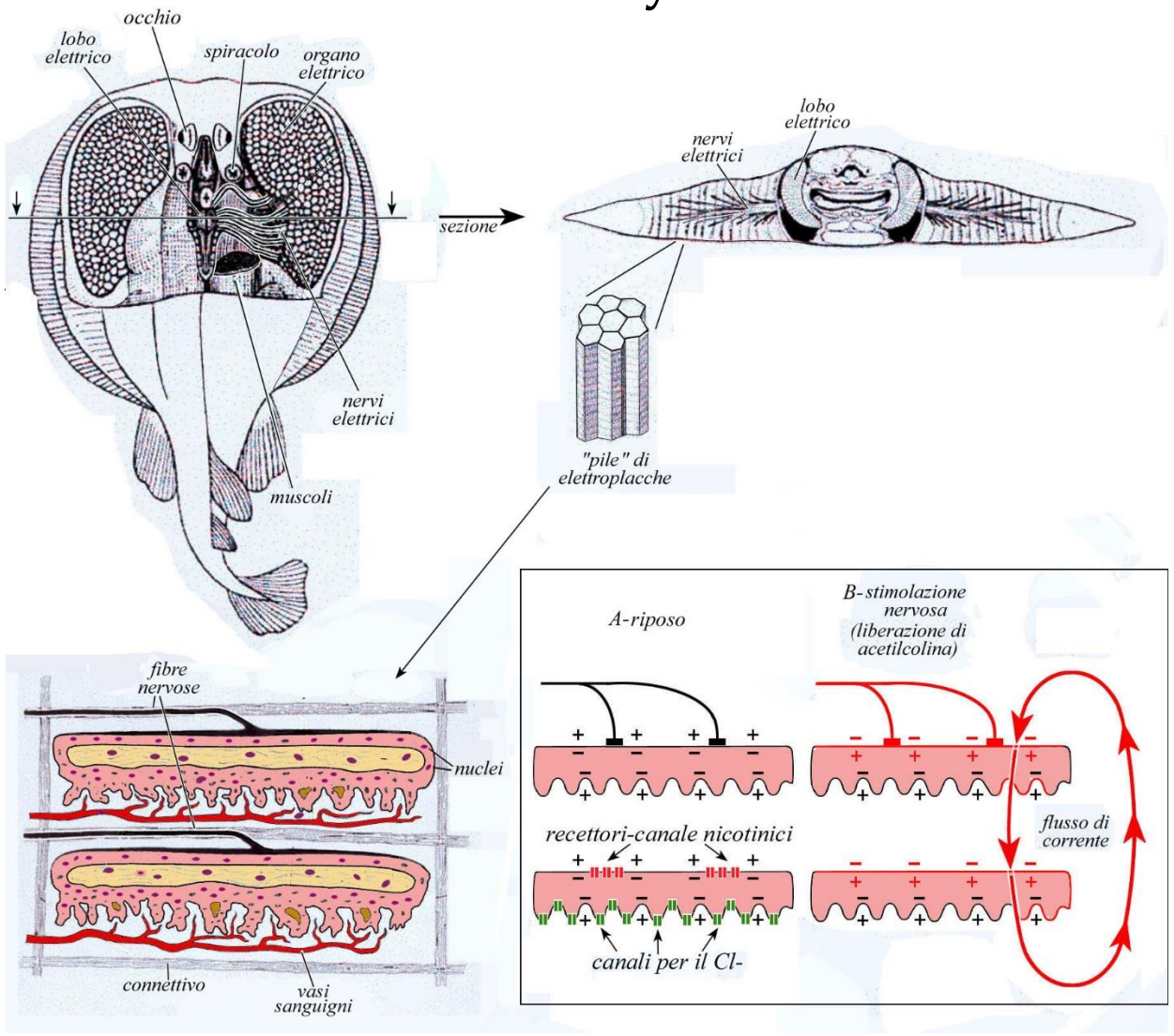
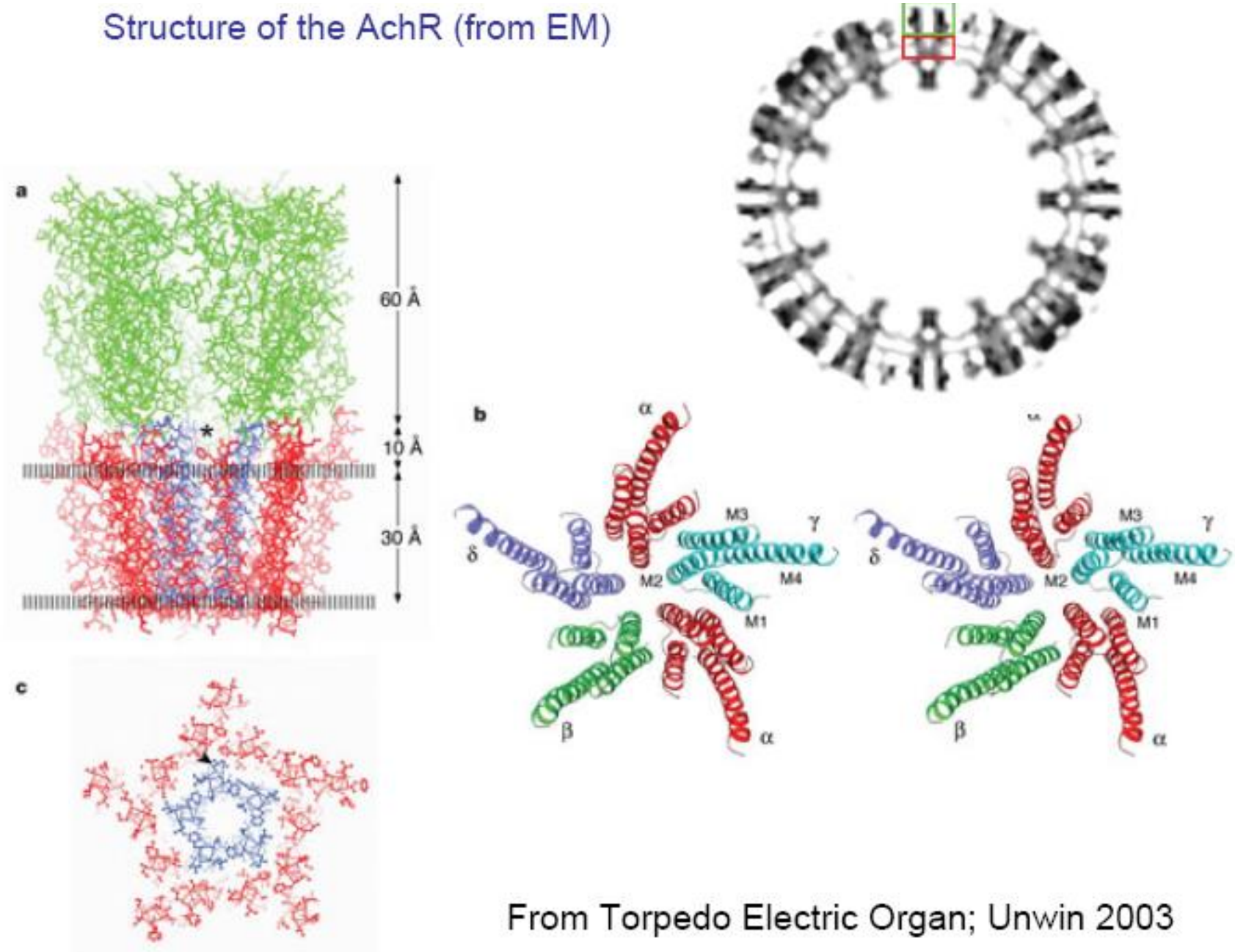


α-bungarotoxin (Cobra) binds to nAchRs with very high affinity, facilitating protein extraction and purification

the structure of the (nAchR) T molecule is resolved to the atomic level (with X-ray diffraction).

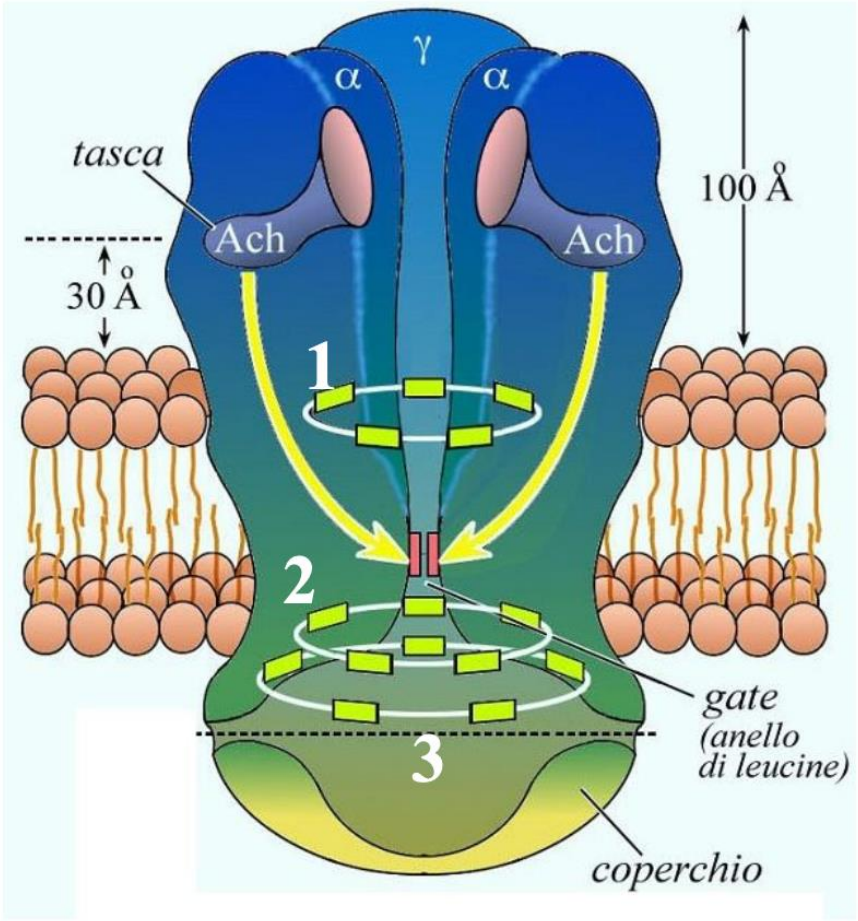
nAChR structure

Structure of the AchR (from EM)



From Torpedo Electric Organ; Unwin 2003

nAChR structure

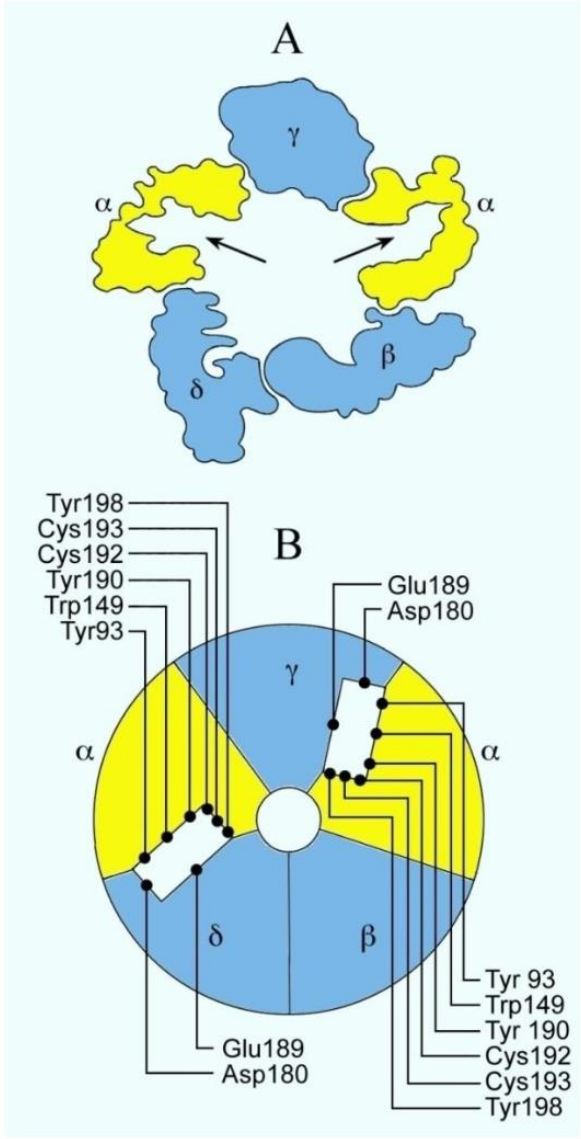


- The M2 sequence of each TSM is a segment enriched in Ser or Thr (negatively charged) aminoacids, forming three rings.

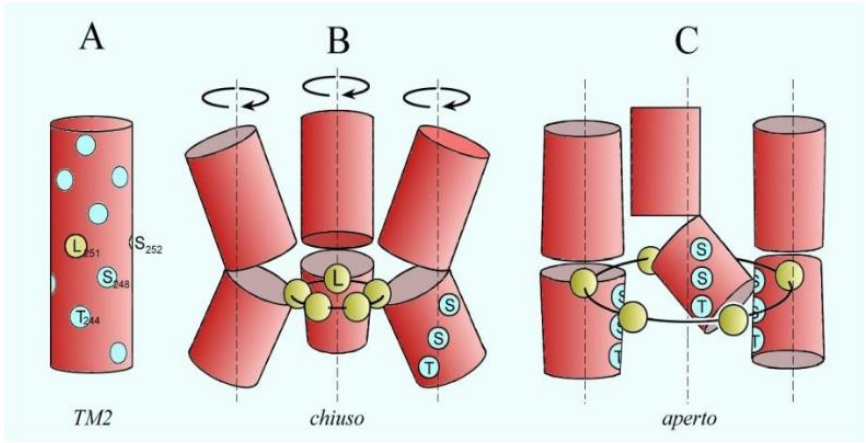
- These three consecutive rings represent the **SELECTIVITY FILTER** facilitating the entry of cations and excluding anions.

- The cytoplasmic region of the receptor includes a **REGULATORY P SITE** that can be modulated by phosphorylation.
- At this level additional regulatory sites have the role to anchor the receptor to specific regions of the cell membrane

nAChR structure

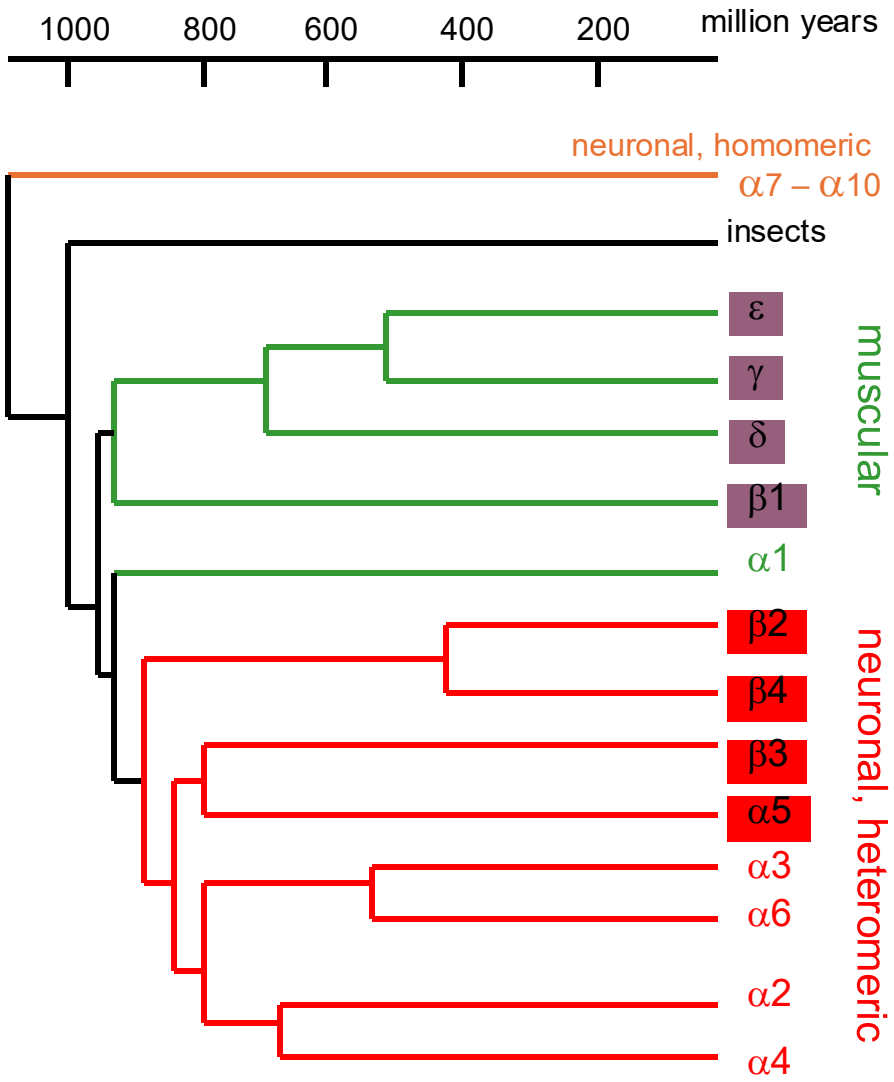


- The **2 α** subunits represent the **BINDING SITE** for the ligand (Ach). Both subunits must be occupied by Ach to allow receptor activation. The first binding facilitates the second (**cooperation**)



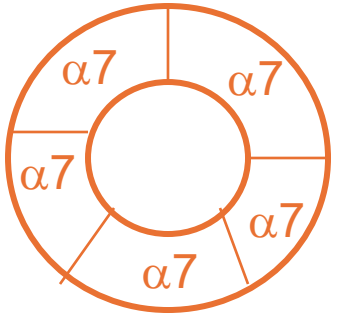
- **GATING:** Binding of the 2 Ach molecules induces a conformational change that opens the channel.

Nicotinic Receptor Subunits



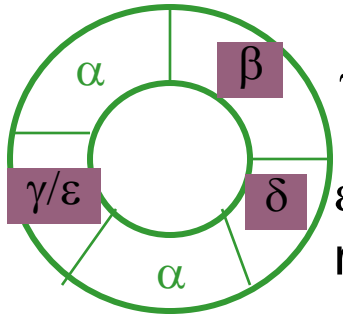
17 genes in vertebrates

nAChRs are homo- or hetero-pentamers



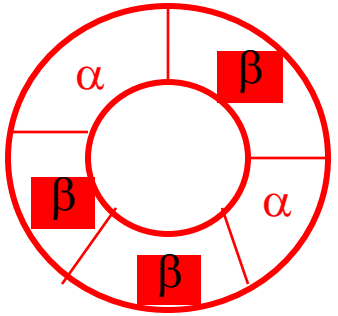
Subunit expression: regional & developmental regulation

Subunit composition: nAChR-channel functional properties

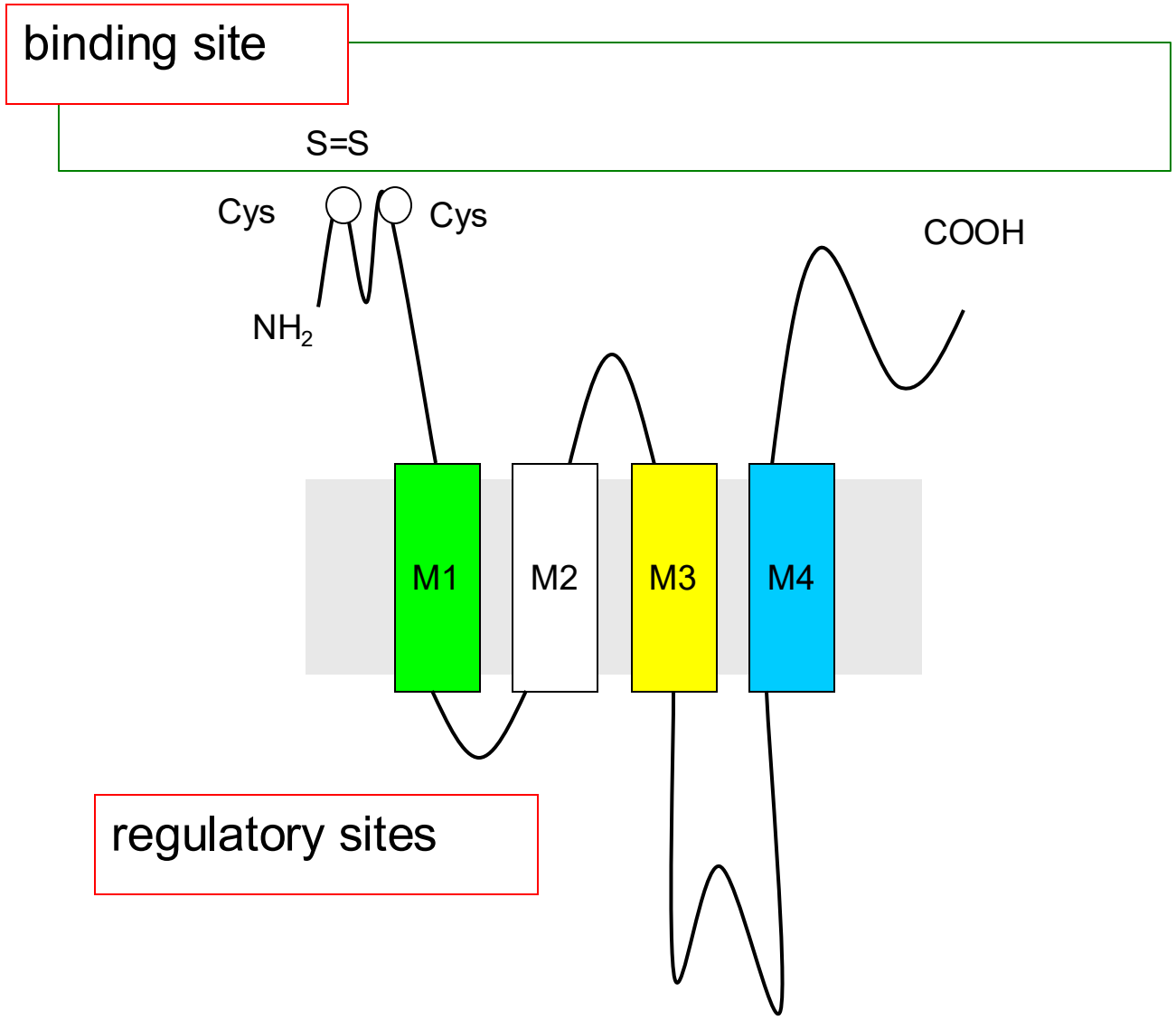


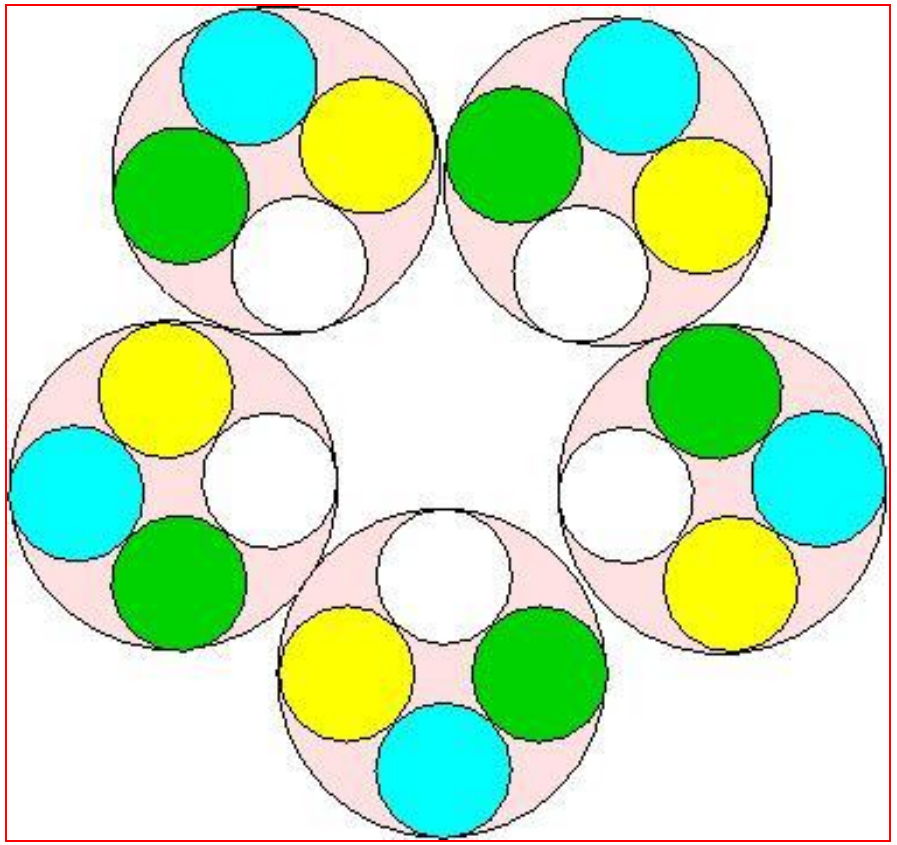
γ-nAChR (30 pS; 5 ms)

ε-nAChR (50 pS; 1 ms)
regulated by innervation

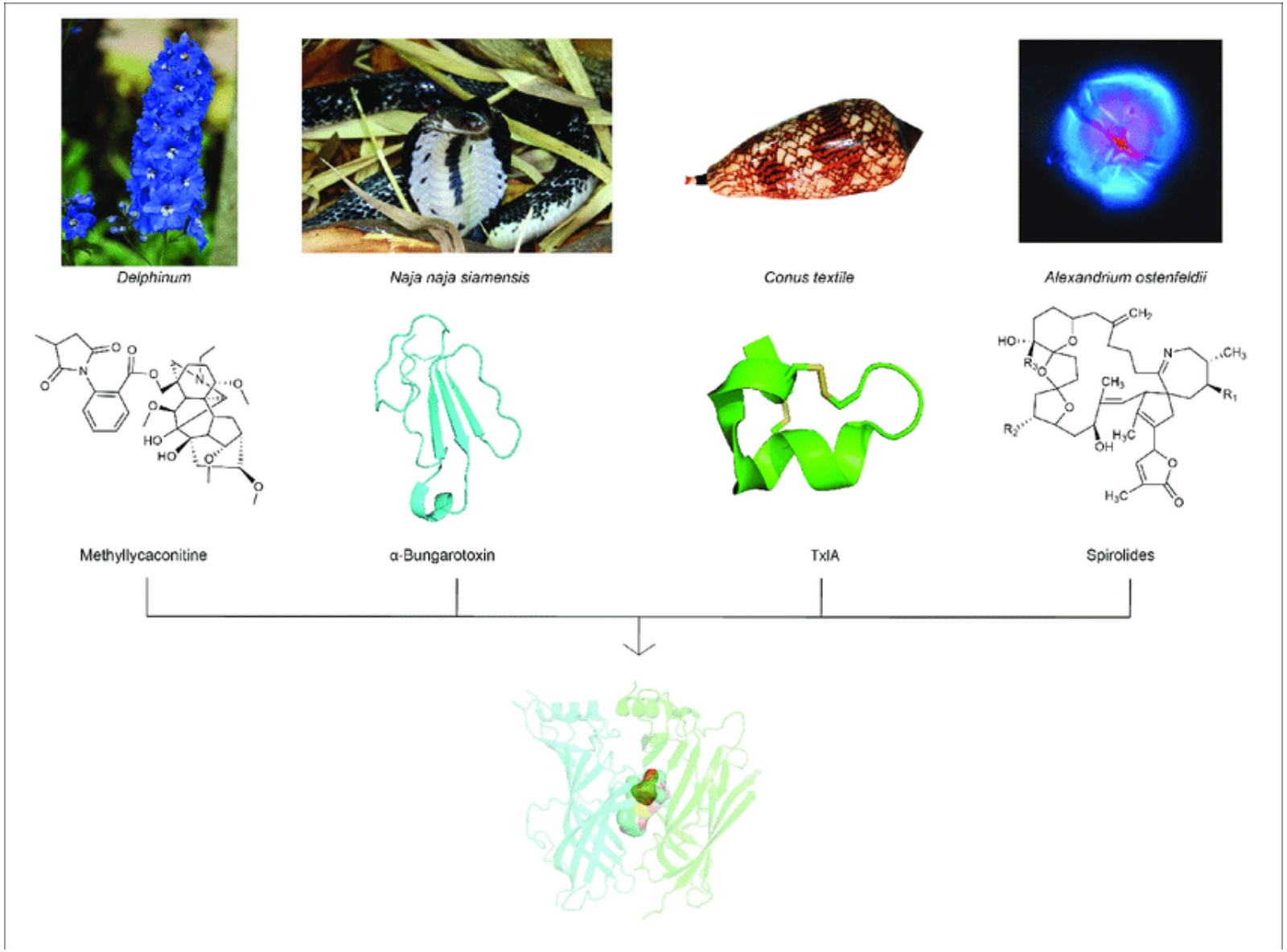


Subunit topology





Toxins blocking Nicotinic Receptor

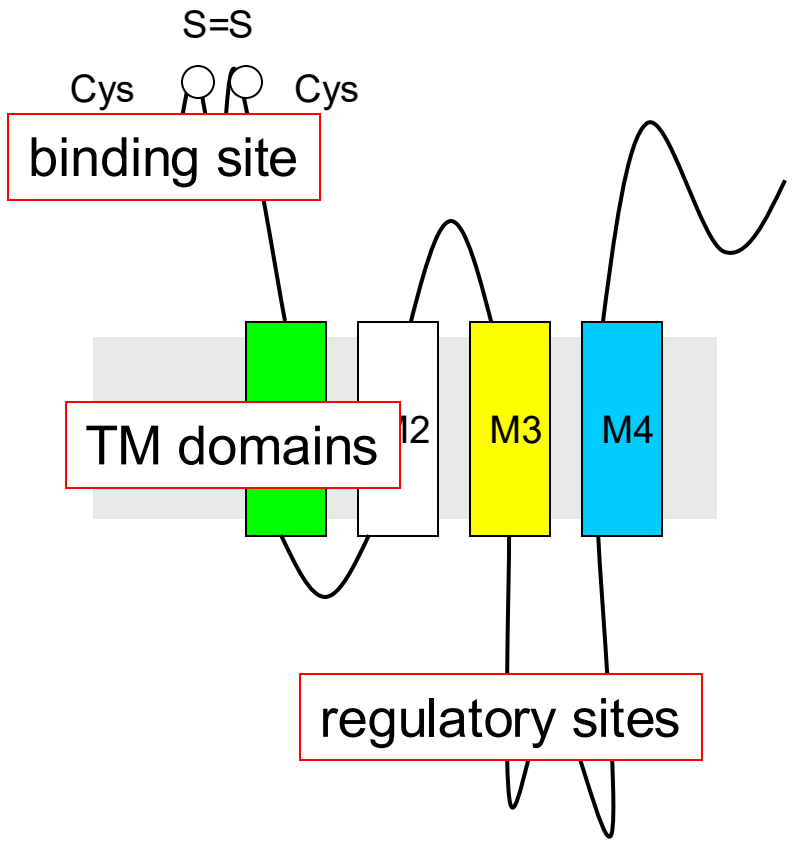


Nicotinic Receptor Mutations & Diseases

Congenital Myasthenic Syndrome
(α1, β1, δ, ε)

Autosomal Dominant Nocturnal
Frontal Lobe Epilepsy (α4, β2)

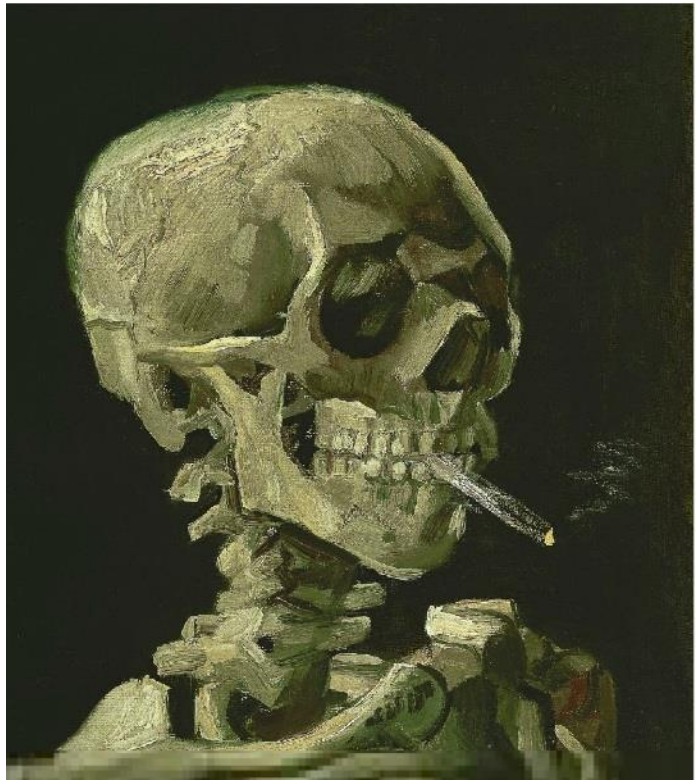
Addiction to nicotine
(α3, α5, β2, α6, β3)



Identification in patients
Functional studies on human muscle
Functional studies on recombinant nAChRs
Genetically modified animal models

Nicotine addiction

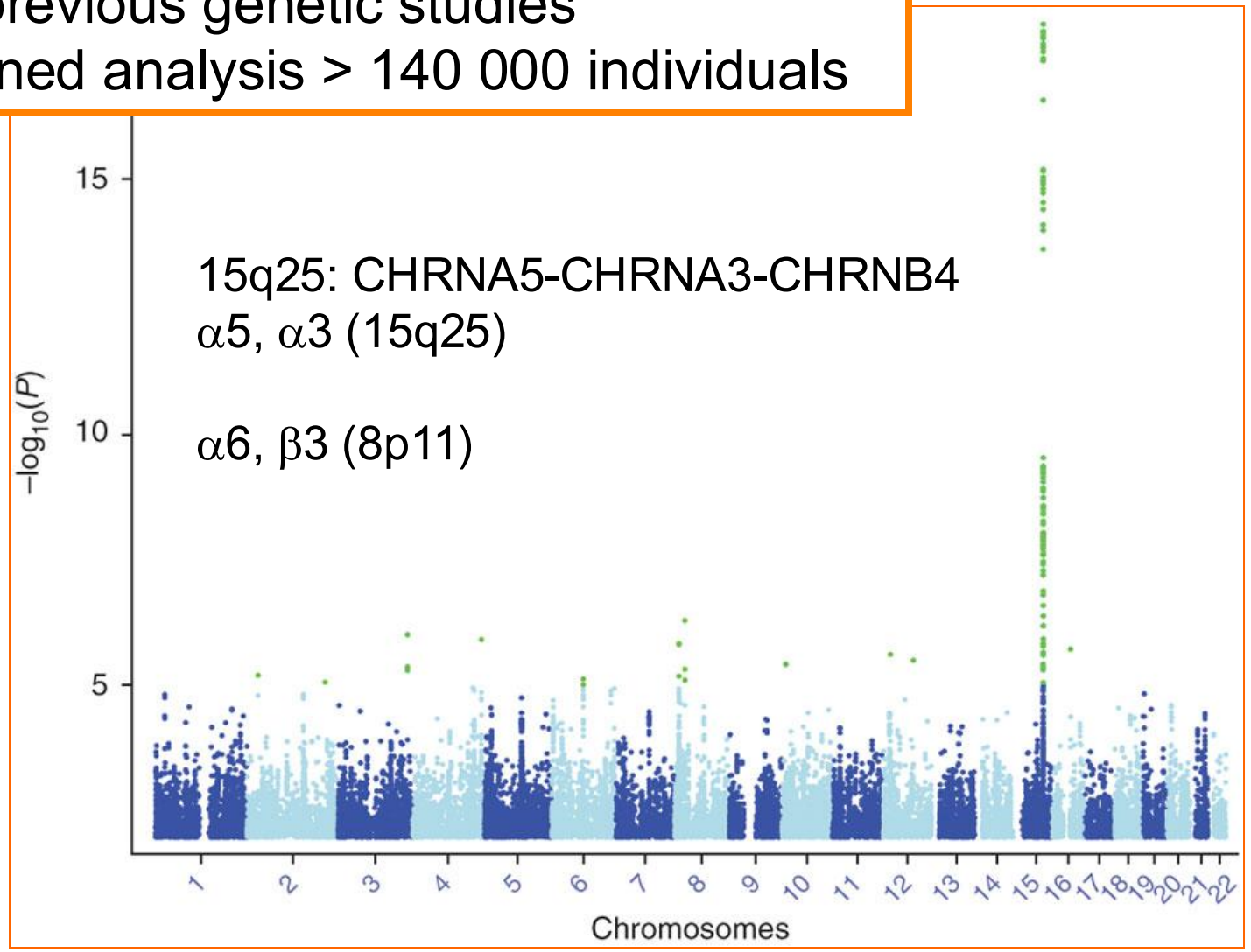
Tobacco use is the leading cause of preventable death in developed countries, causing about 5 million deaths/year wordwide. Its use is increasing in developing countries, further raising death toll.



Vincent van Gogh (1853-1890)
Van Gogh Museum, Amsterdam

nAChR & Nicotine addiction

3 genome-wide association studies (2010)
other previous genetic studies
Combined analysis > 140 000 individuals



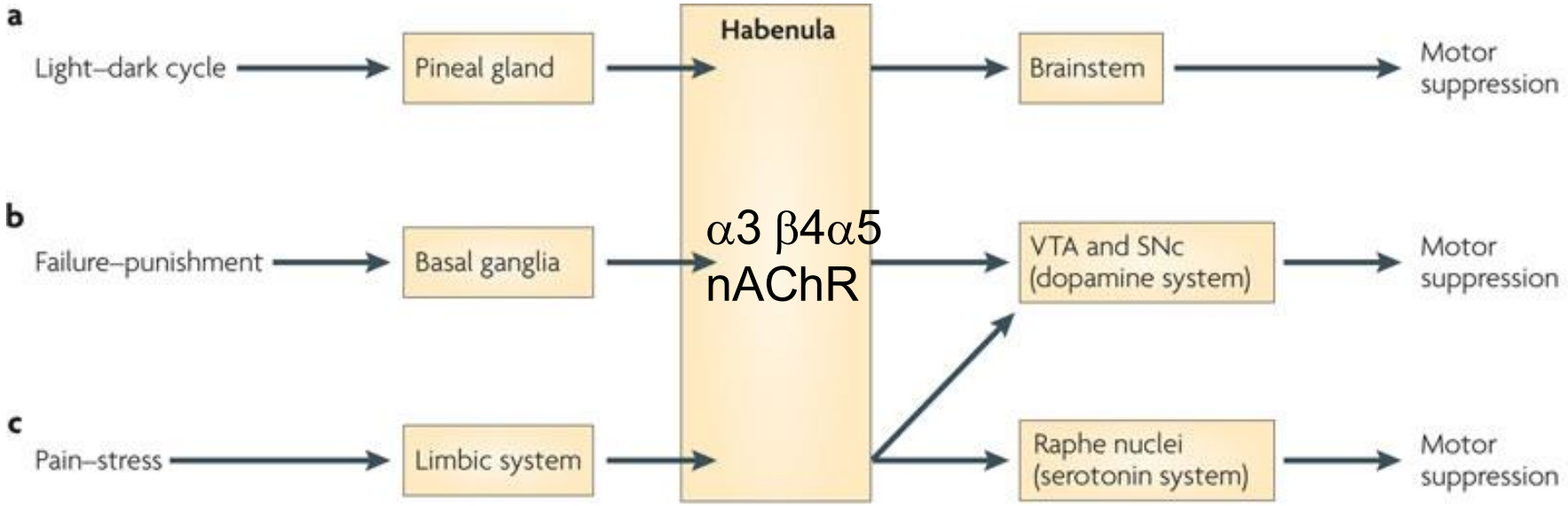
Nature Genetics, May 2010

D398N α5 & Nicotine addiction

Bierut et al., Am J Psych 2008

D398N α5 & Nicotine addiction

α3α5β4 in
- PNS (ganglion neurones)
- medial habenula (epithalamus)

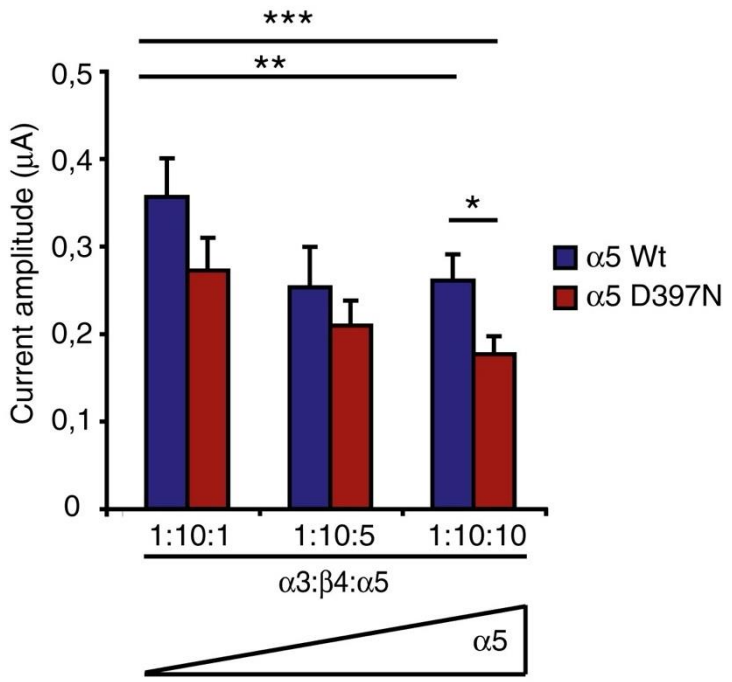


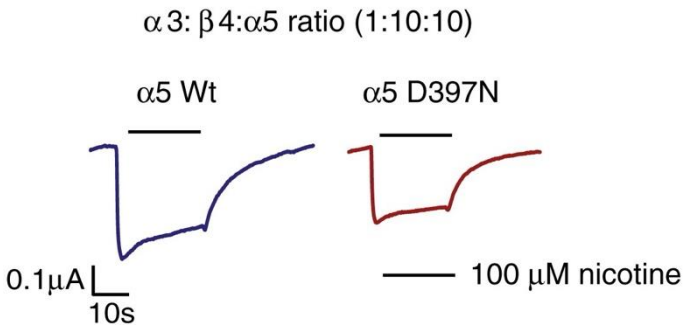
Nature Reviews | Neuroscience
2010

D398N α5 & Nicotine addiction

$\alpha5_{D398N}$ induces LOSS-of-function in $\alpha3\beta4\alpha5$ nAChR

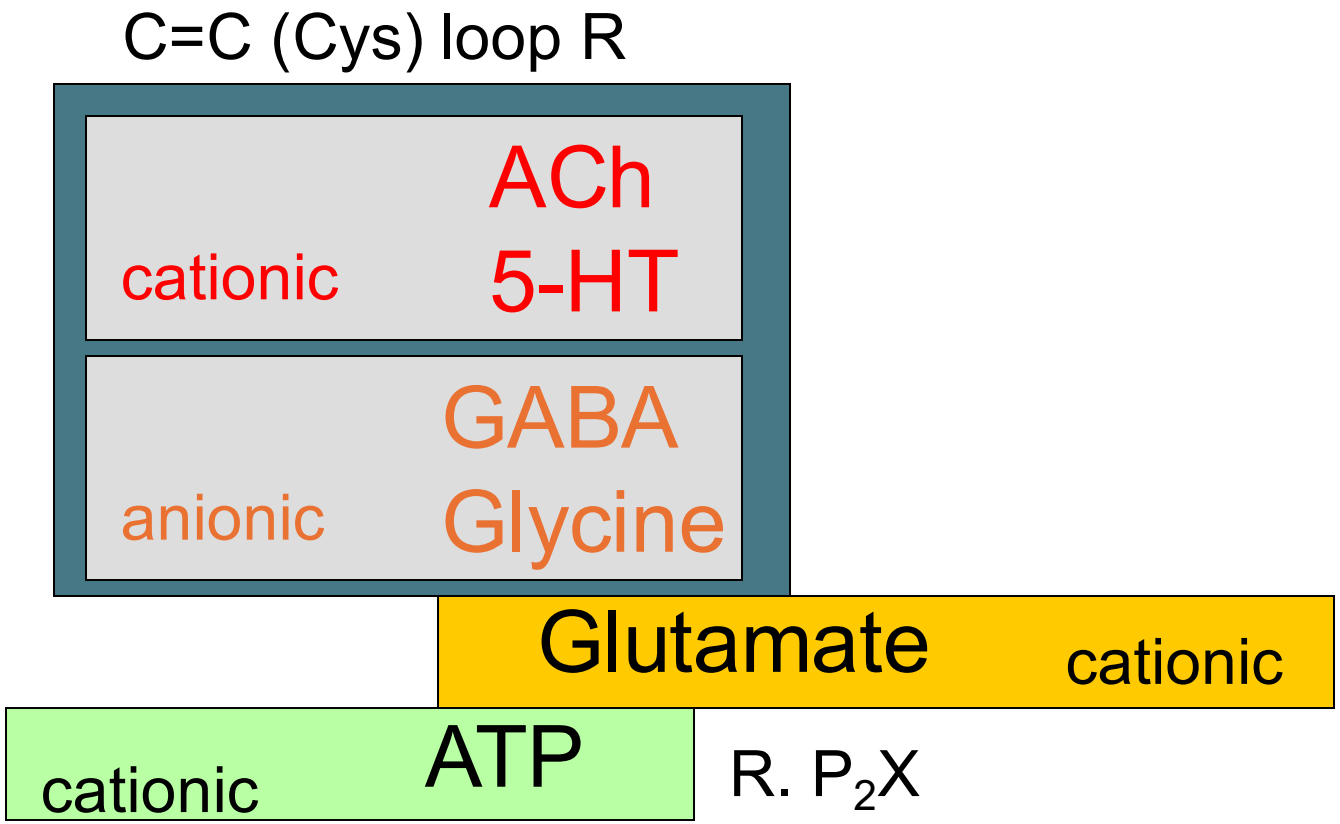
$\alpha5_{D398N}$ reduces aversion to nicotine allowing enhanced consumption



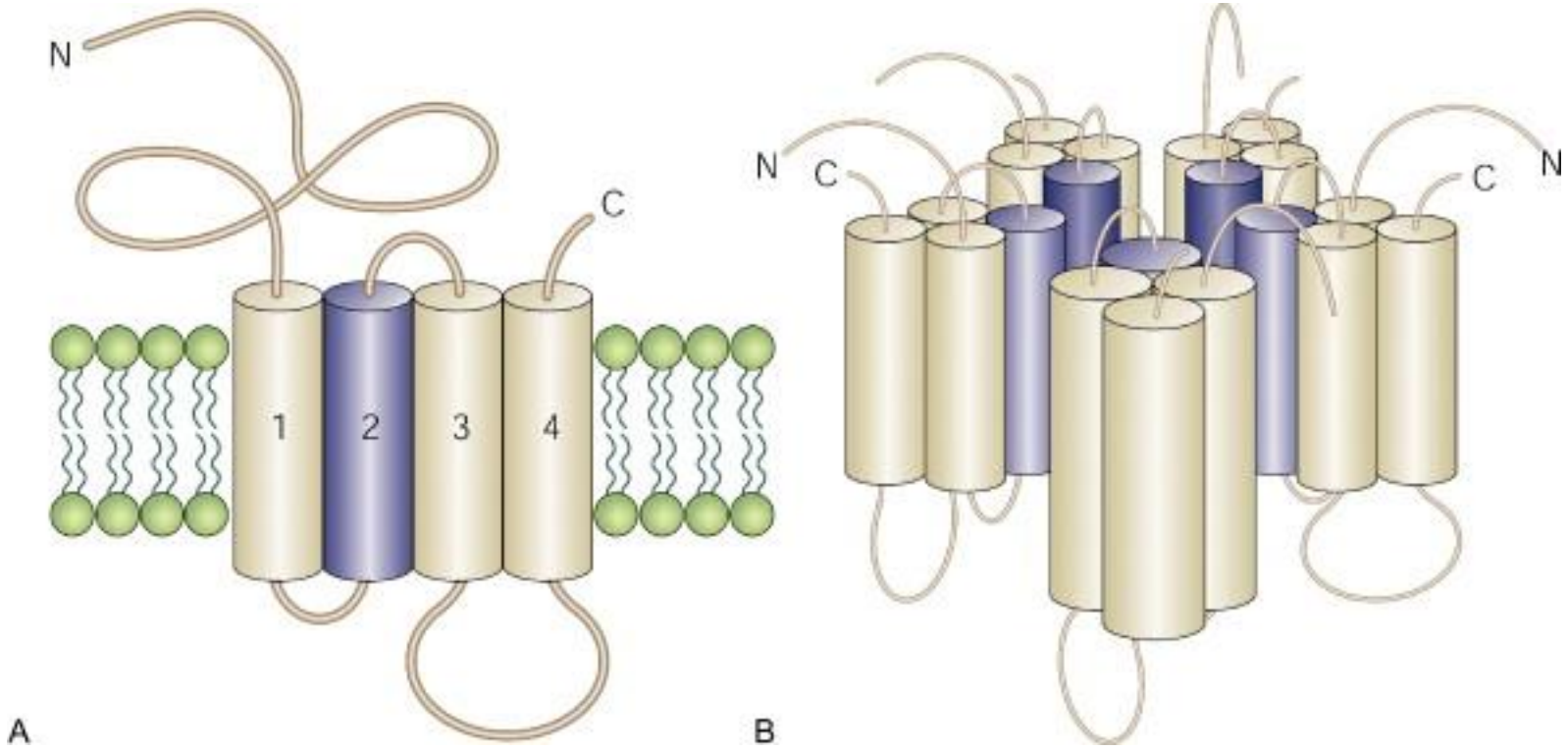


Frahm et al. (2011) Neuron 70: 522-535

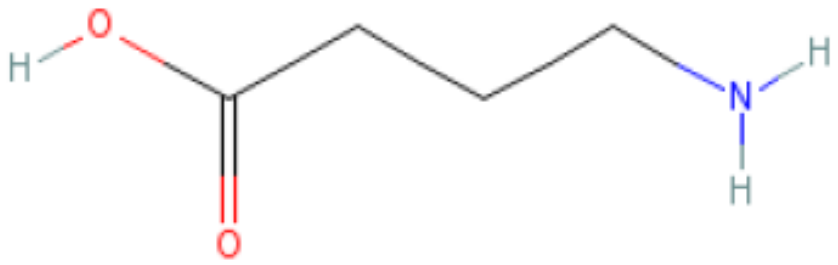
Ionotropic receptor families



Cys-loop receptors: Ionotropic GABA Receptors



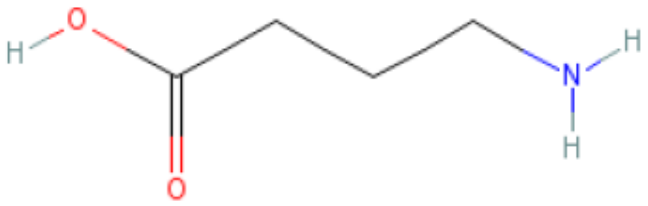
Fast GABAergic transmission



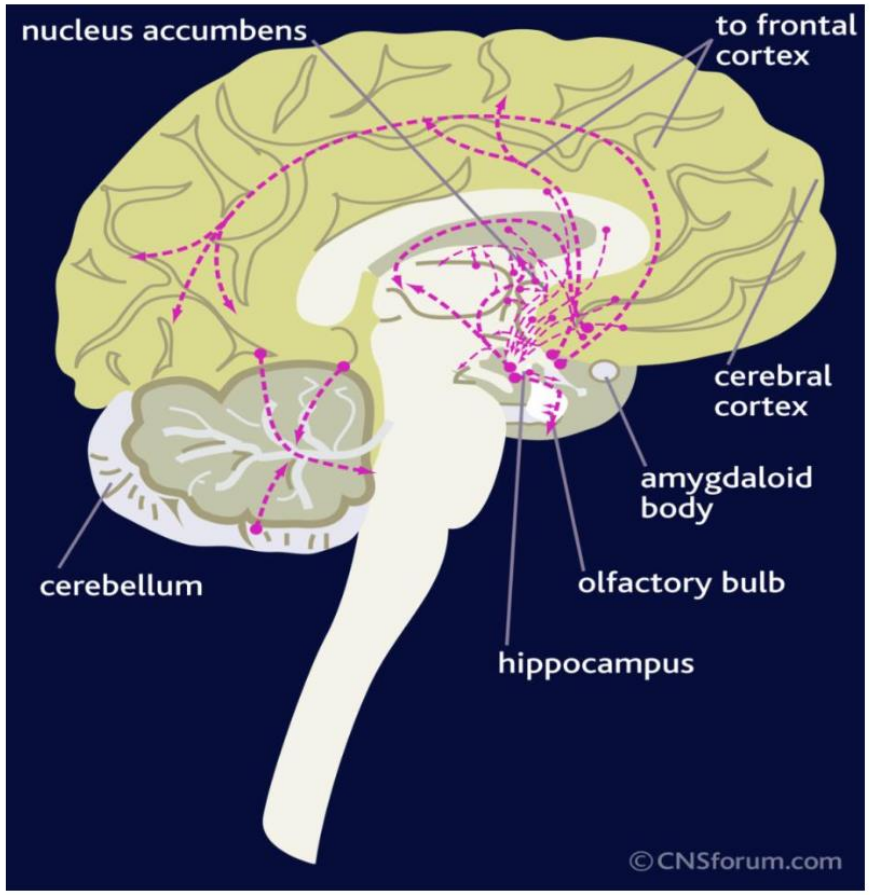
γ-amino butyric acid (GABA)

Gamma Amino Butyric Acid (GABA)

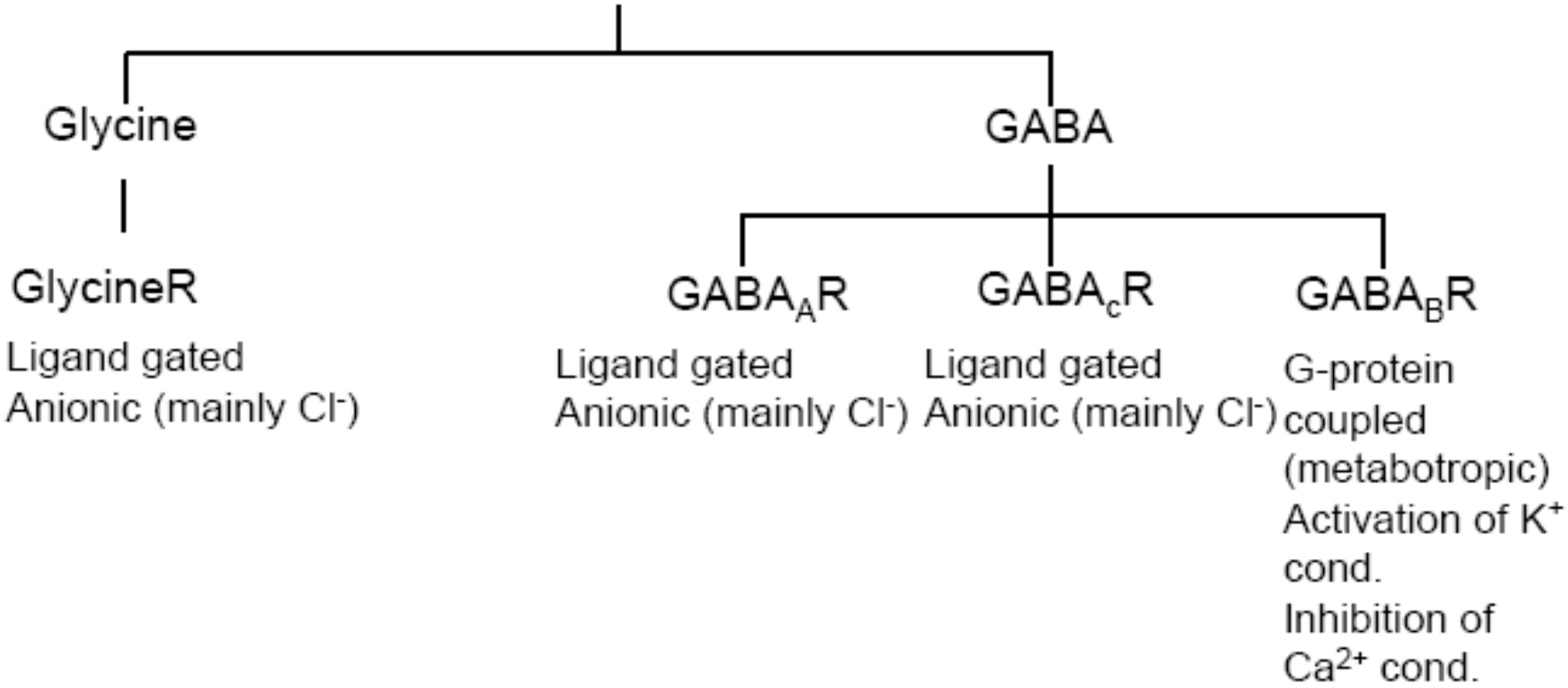
GABA is the chief inhibitory neurotransmitter in the mammalian central nervous system. It plays the principal role in reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone.



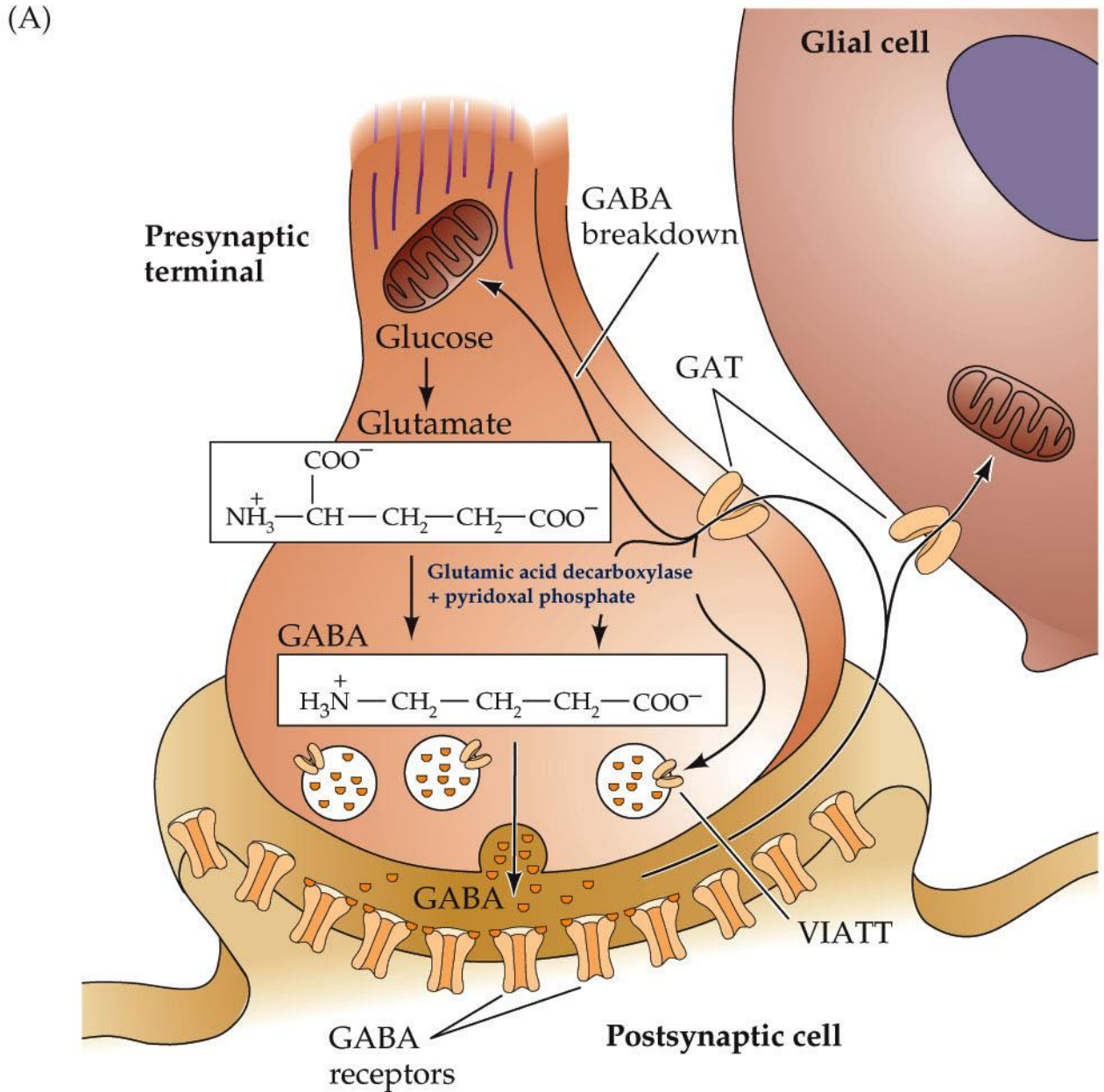
γ-amino butyric acid (GABA)



Inhibitory transmission

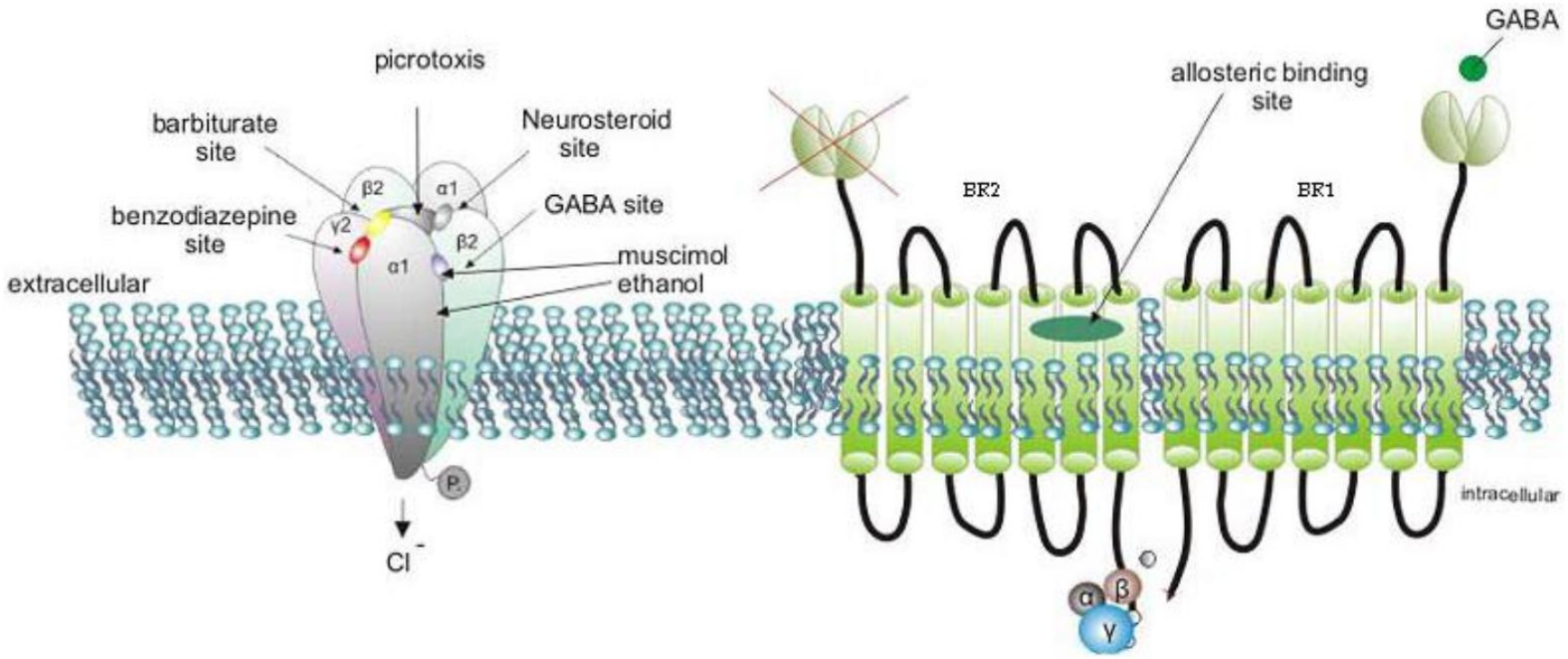


GABA metabolism



GABA RECEPTORS

GABA binds to both $GABA_A$ and $GABA_B$ receptors.



GABA A Receptor

GABA B Receptor

$GABA_A$ receptors are ligand-gated channel receptors

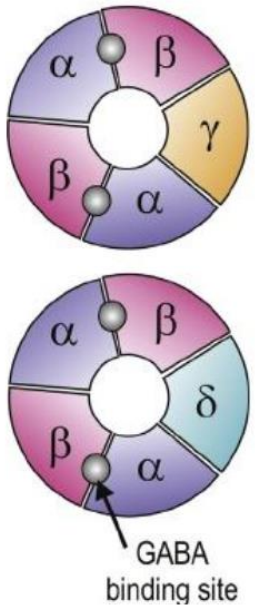
$GABA_B$ receptors are members of the 7-TM G protein-coupled receptors

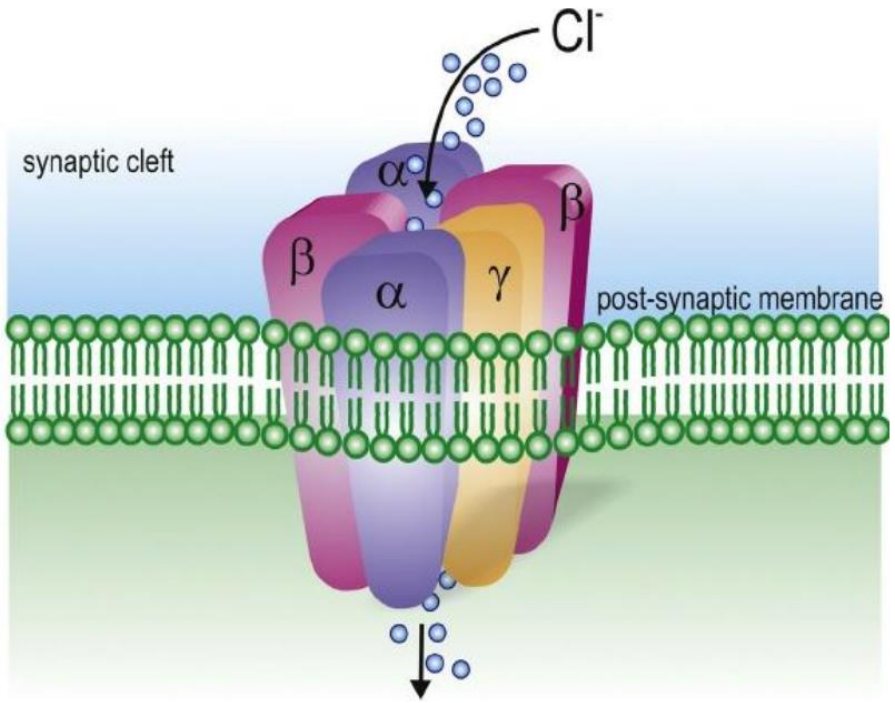
Fast GABAergic transmission : GABAA Receptors

- The γ-aminobutyric acid, type A ($GABA_A$) receptor is a chloride-conducting receptor composed of **α, β,** and **γ subunits** assembled in a pentameric structure forming a central pore.
- The majority of $GABA_A$ Rs are believed to be expressed as heteromeric complexes of **2α, 2β,** and **1 γ** subunit

- The **2β** subunits represent the **BINDING SITE** for the ligand (GABA).

- Both subunits must be occupied by GABA to allow receptor activation. The first binding facilitates the second (**cooperation**)



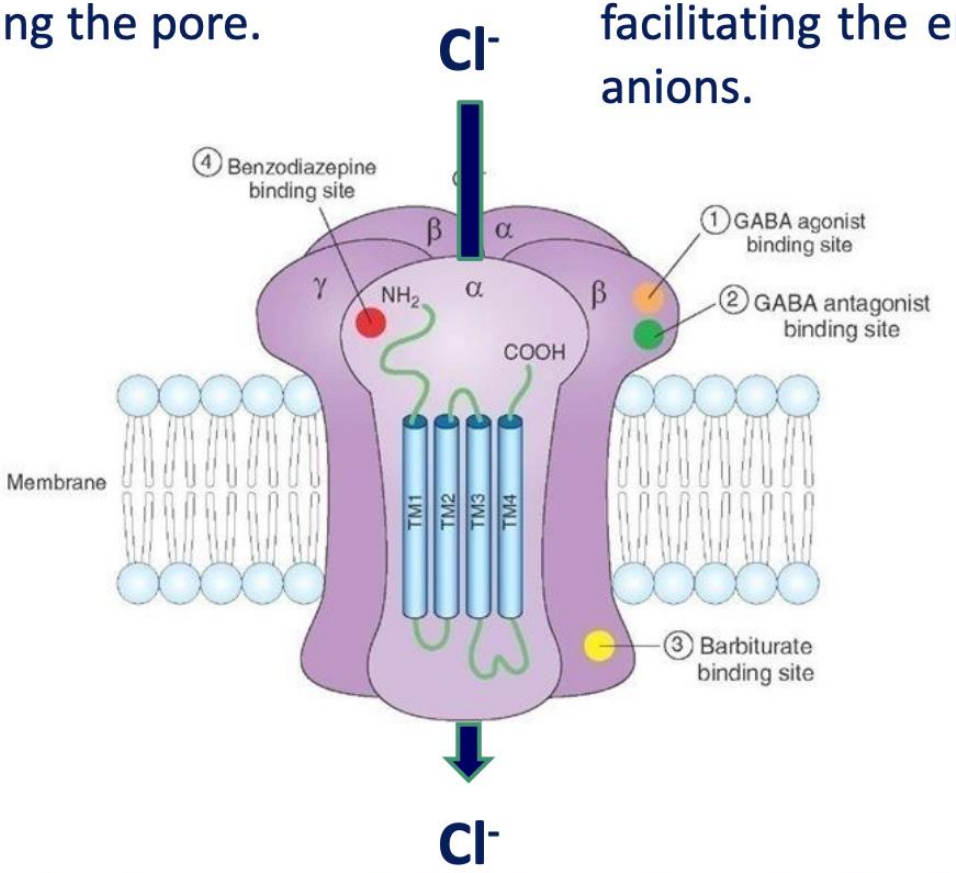


GABAA Receptors

- Each subunit has a large extracellular agonist binding domain and four transmembrane domains (M1–M4), with the second transmembrane (M2) domain lining the pore.

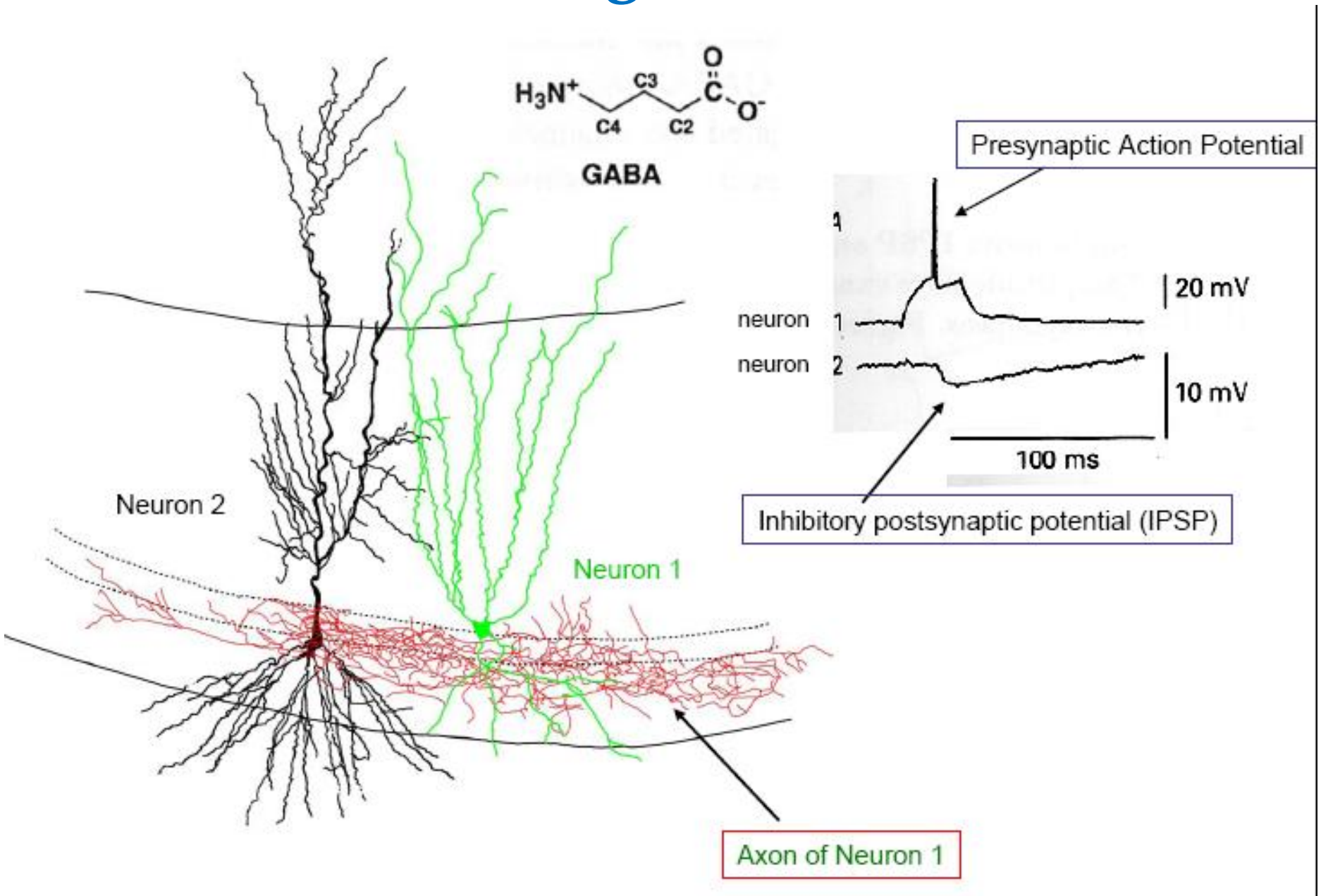
- The M2 sequence of each TSM forms the **SELECTIVITY FILTER** facilitating the entry of anions.

- The **α** subunits represent the **ALLOSTERIC SITE,** a modulatory region where binding of ligands different from GABA may facilitate/obstacolate the GABA/RECEPTOR interaction.

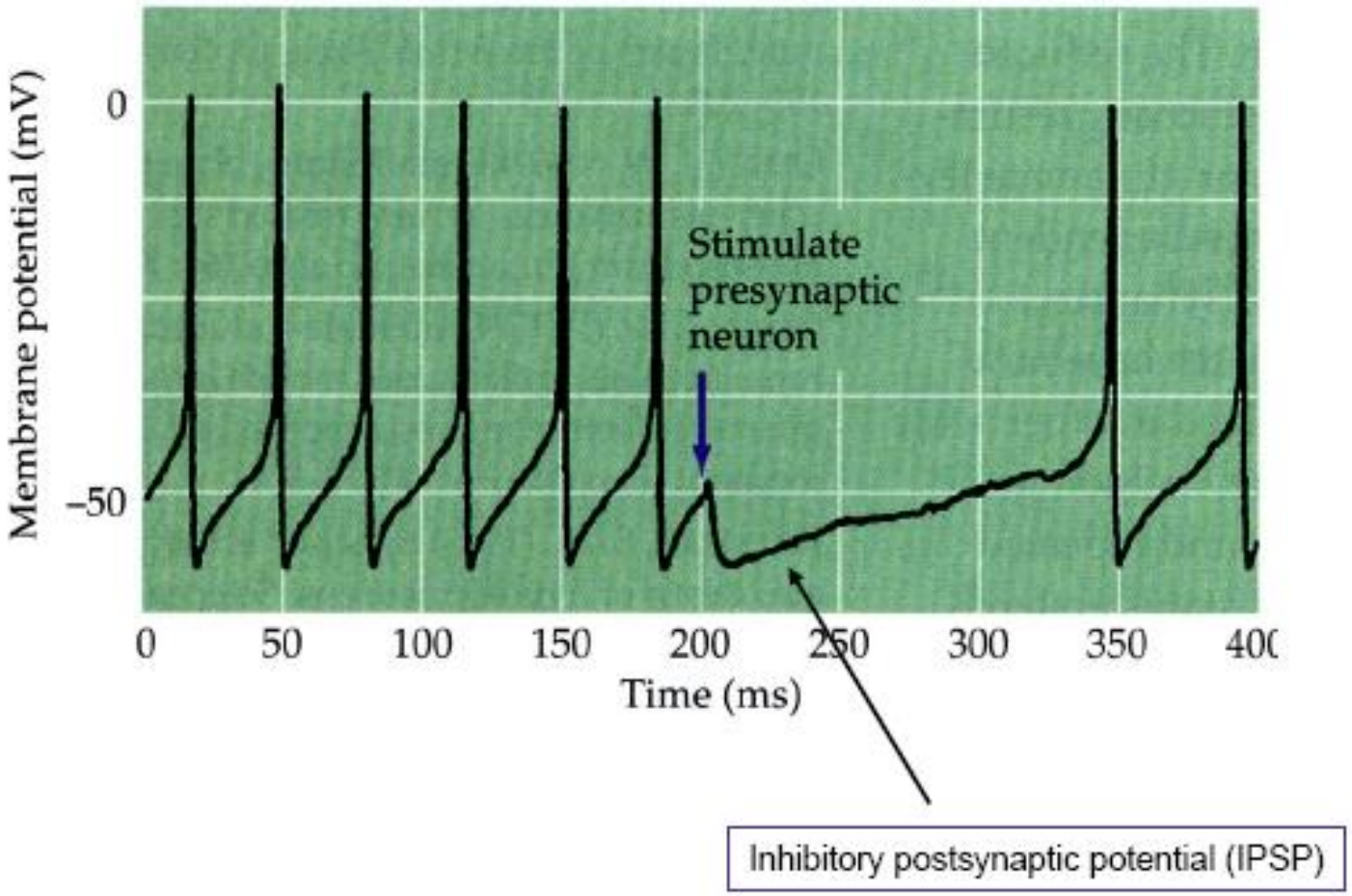


- Another ligand site is present on the deep part of the channel, on the **β subunit.** Binding on this site allows a different modulation of channel opening that may excede the GABA-mediated effects

Fast GABAergic transmission



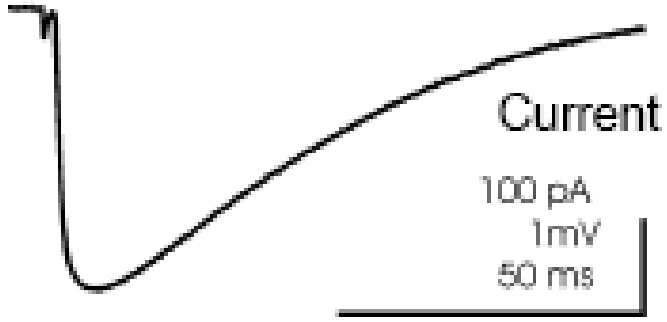
GABA action on neuronal firing



IPSPs are mediated by a Cl^- conductance : GABAA Receptor

At macroscopic level

Voltage clamp: Inhibitory postsynaptic current (IPSC); outward current



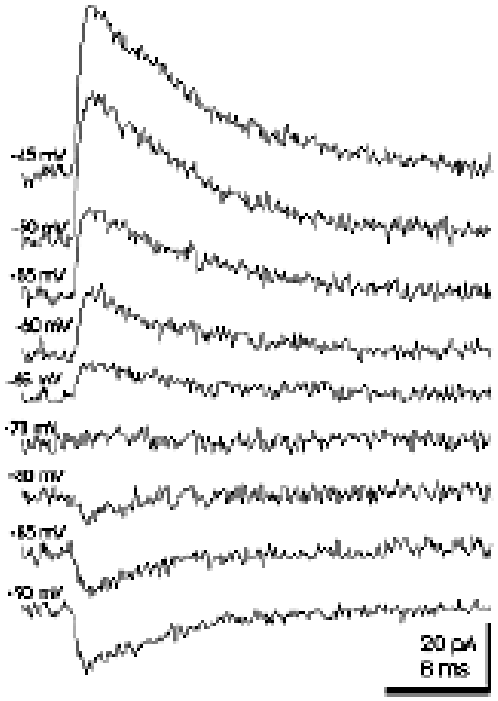
Current clamp: Inhibitory postsynaptic potential (IPSP); hyperpolarization

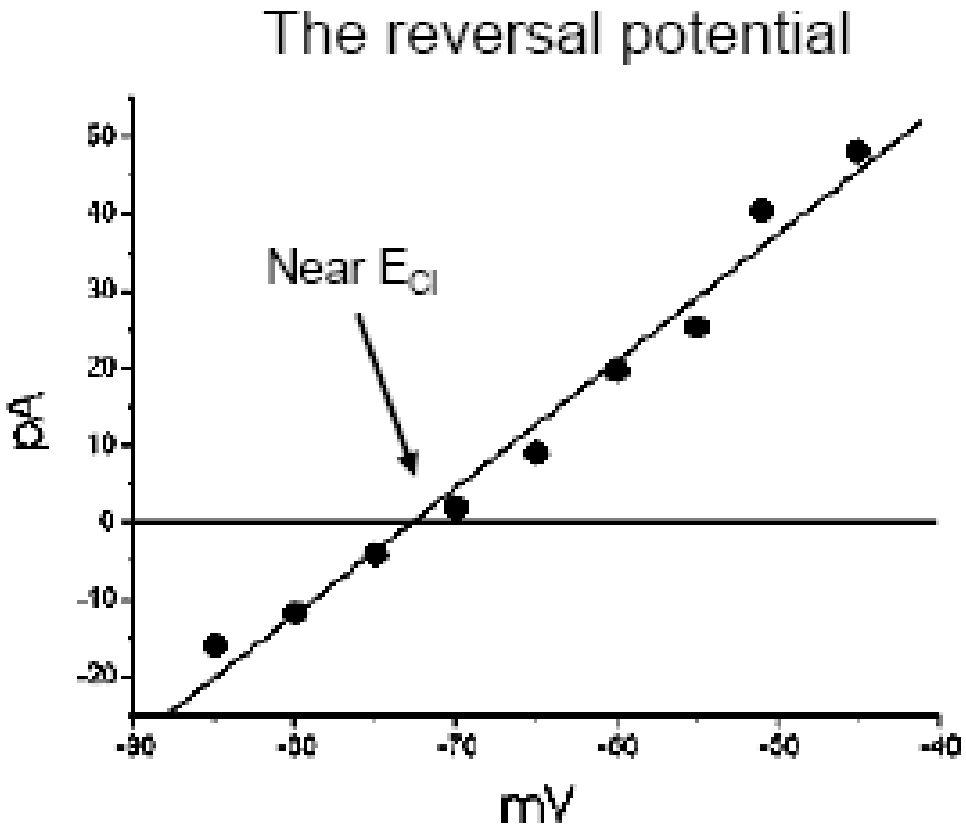
Influence of the postsynaptic membrane potential on GABAergic currents .... How does Cl^- move?

I-V curve

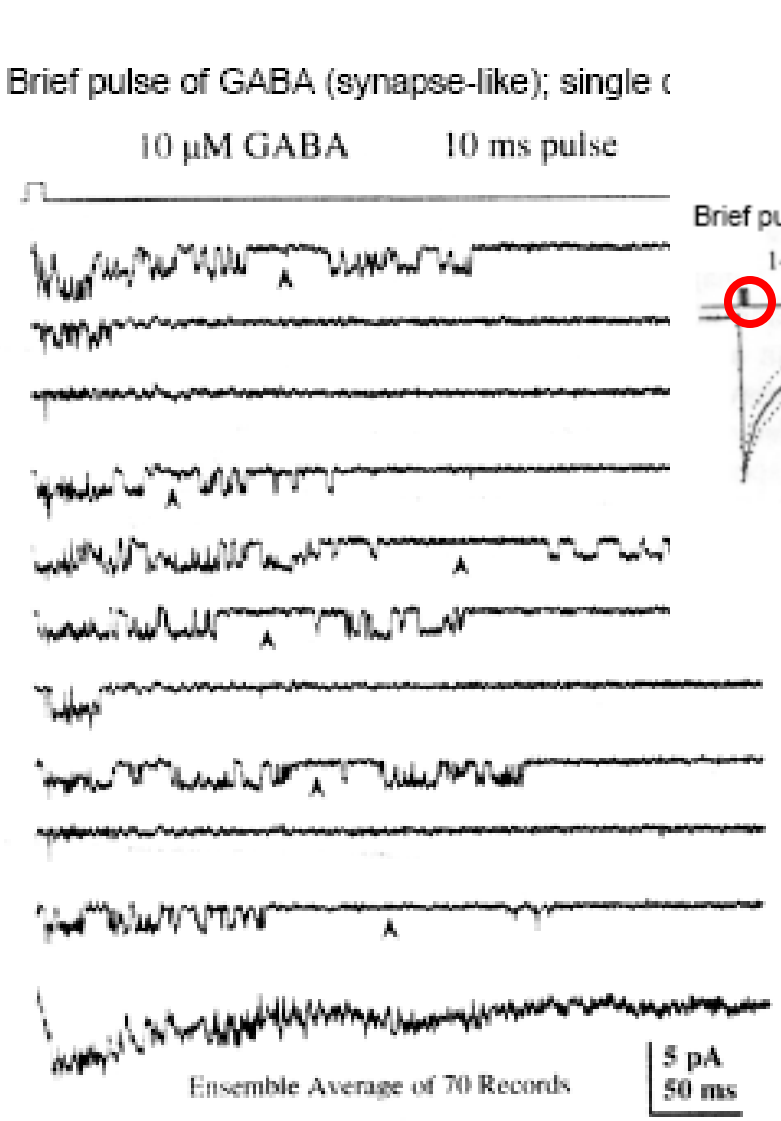
IPSCs

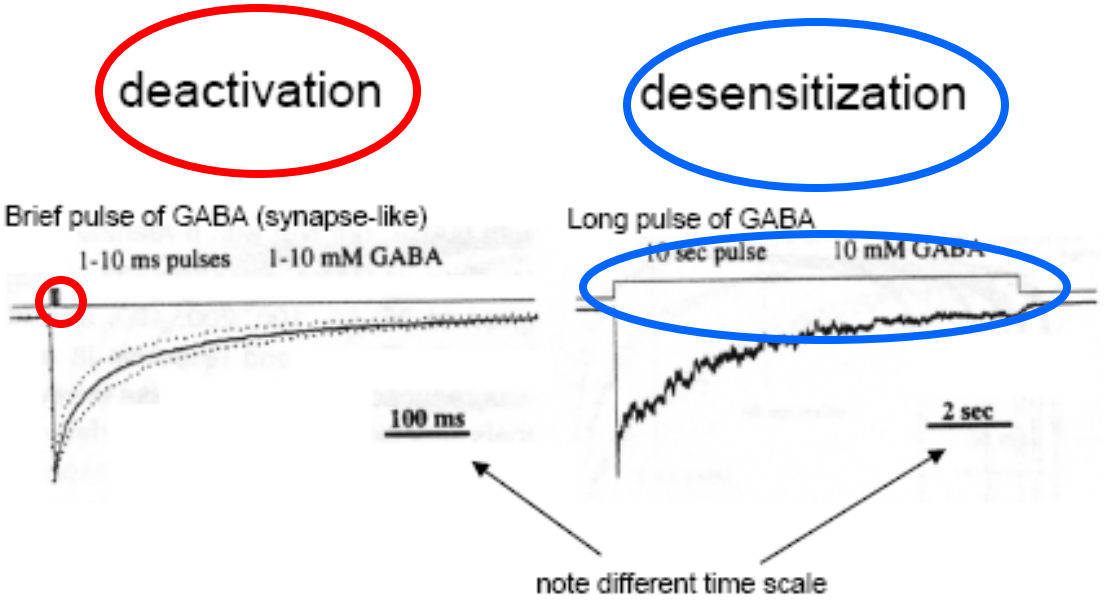
The postsynaptic neuron is Voltage-Clamped at different potentials





Kinetics of GABAA receptors



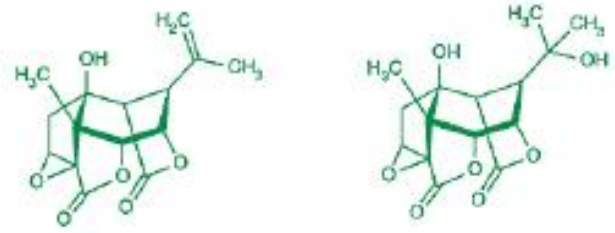


Antagonists of GABAA receptors lead to epileptic seizures

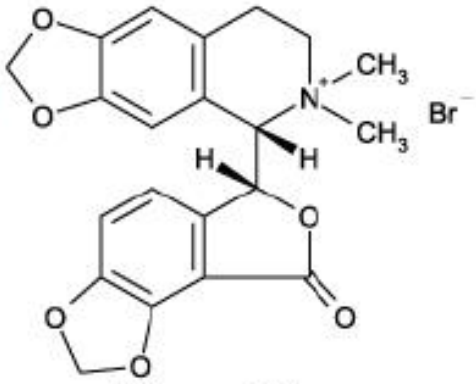
Anamirta cocculus

Dicentra cucullaria

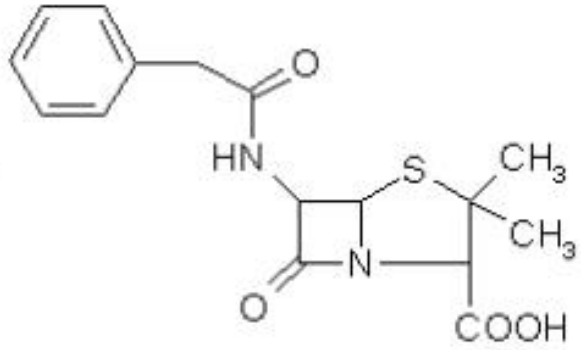
Penicillium notatum



Picrotoxin

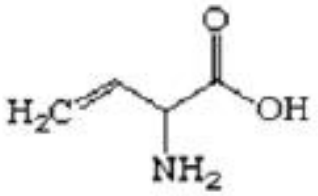


Bicuculline



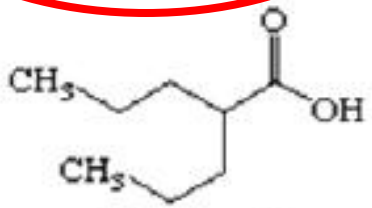
Penicillin

GAD (glutamic acid decarboxylase) catalyzes the formation of GABA from glutamic acid



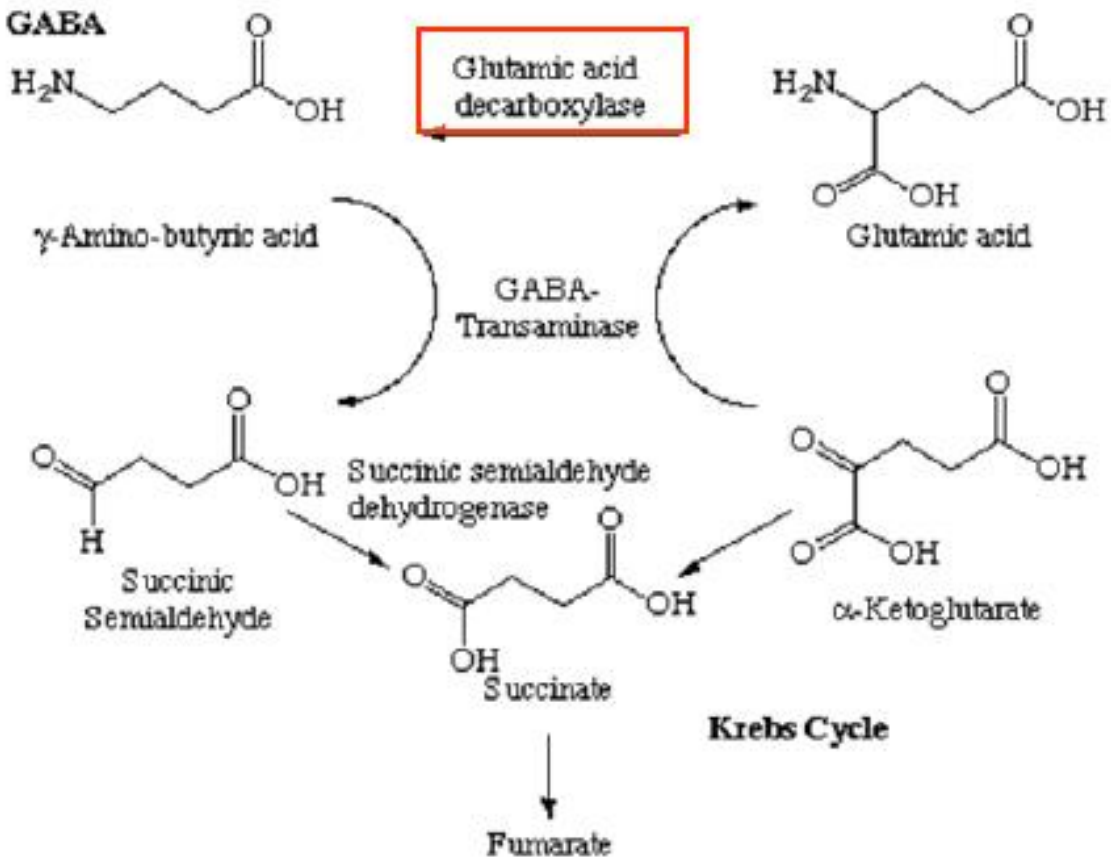
Allylglycine

The GAD inhibitor allylglycine, inhibit GABA formation and cause convulsions.

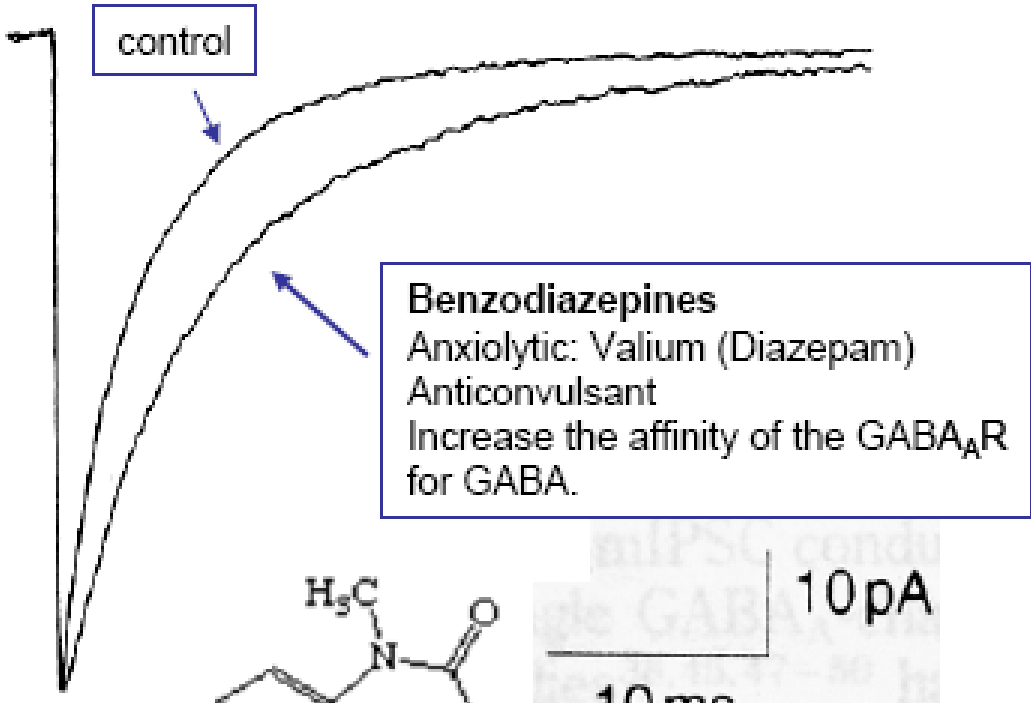


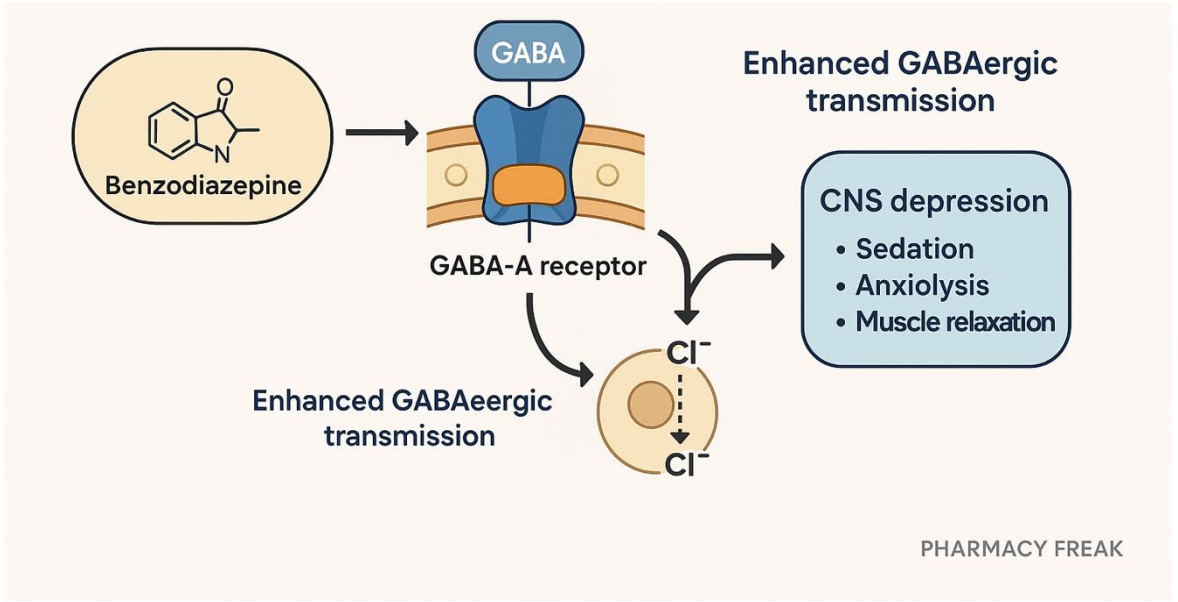
Valproic acid

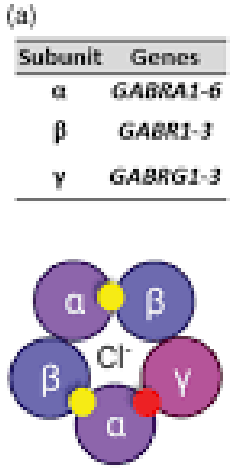
Sodium valproate (or valproic acid) blocks GABA transaminase activity, thereby elevating GABA levels. Treatment of Epilepsy.

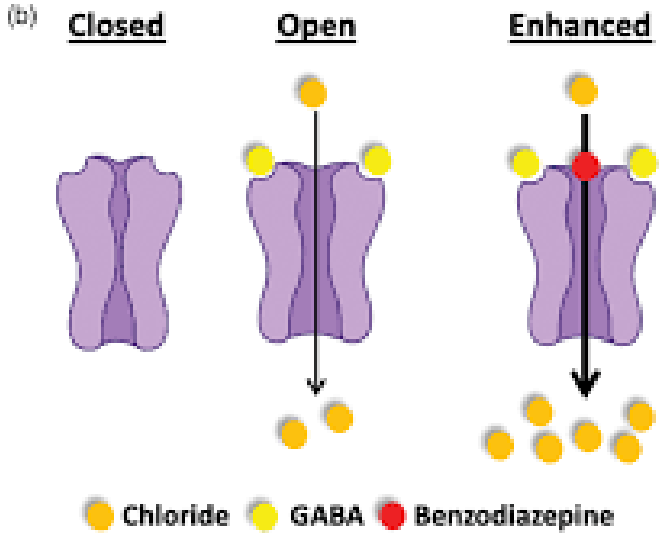


Allosteric modulators of GABAA receptors

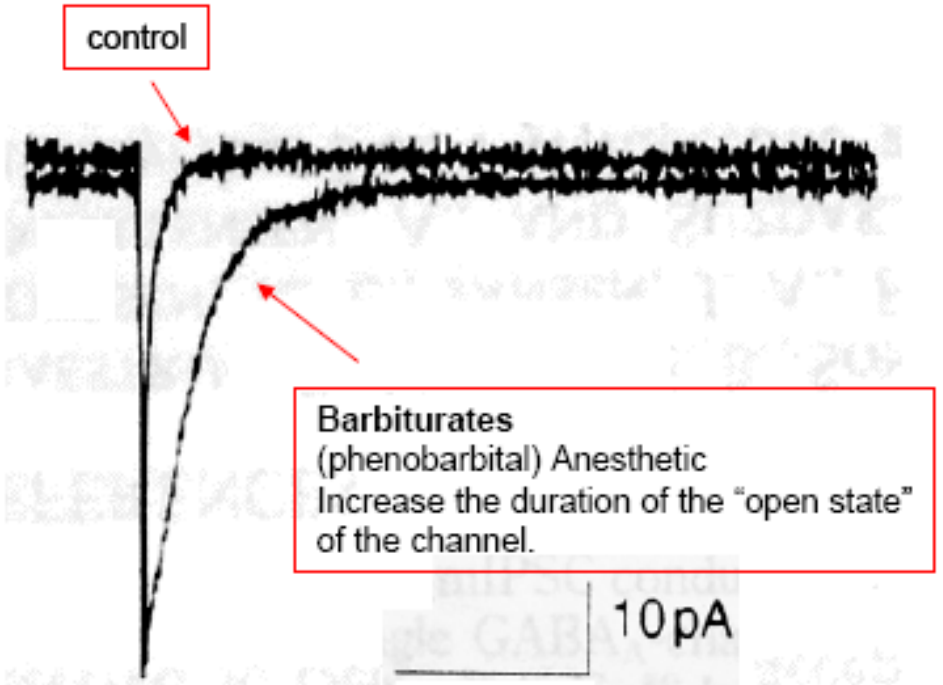




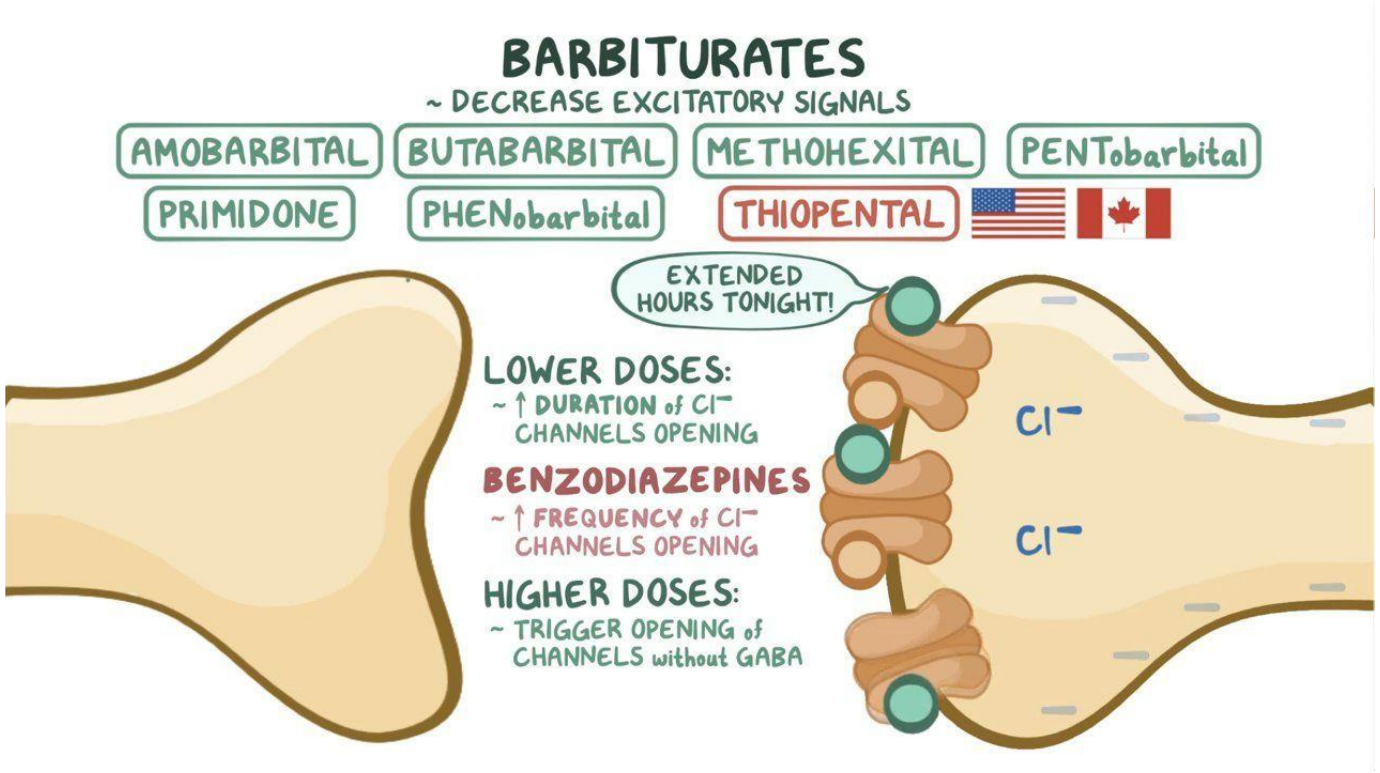




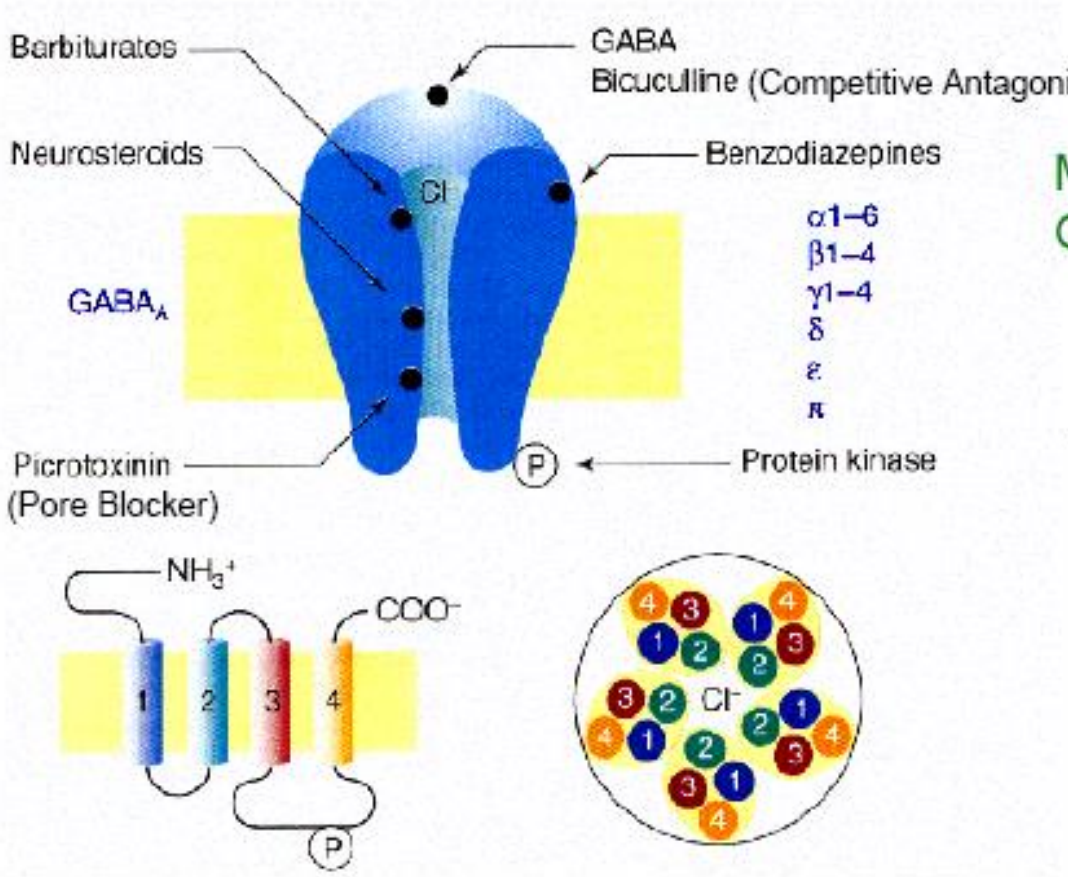
Allosteric modulators of GABAA receptors



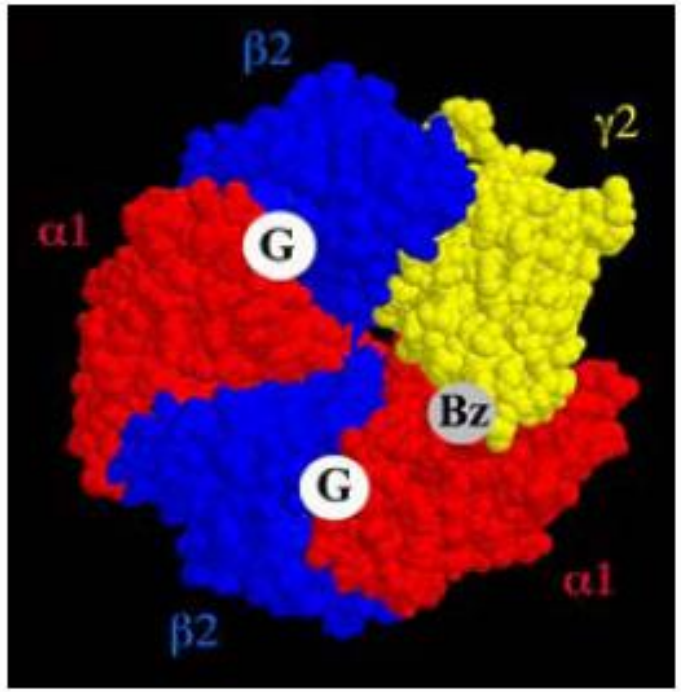
Phenobarbital



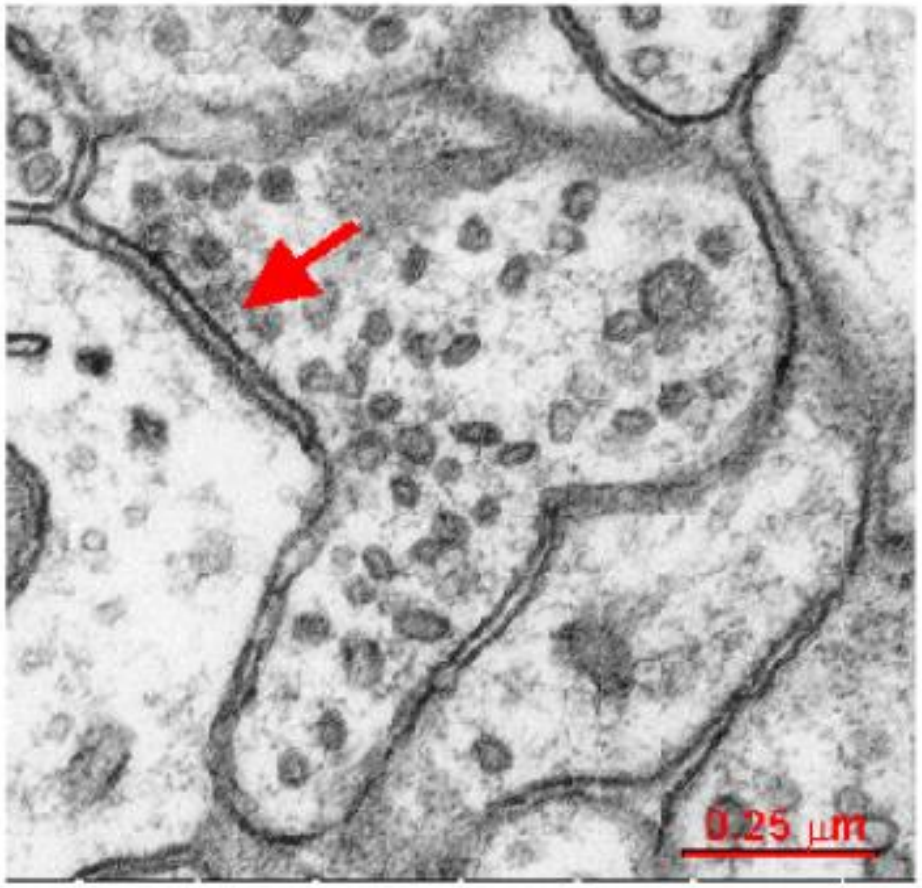
Structure of GABAA receptor



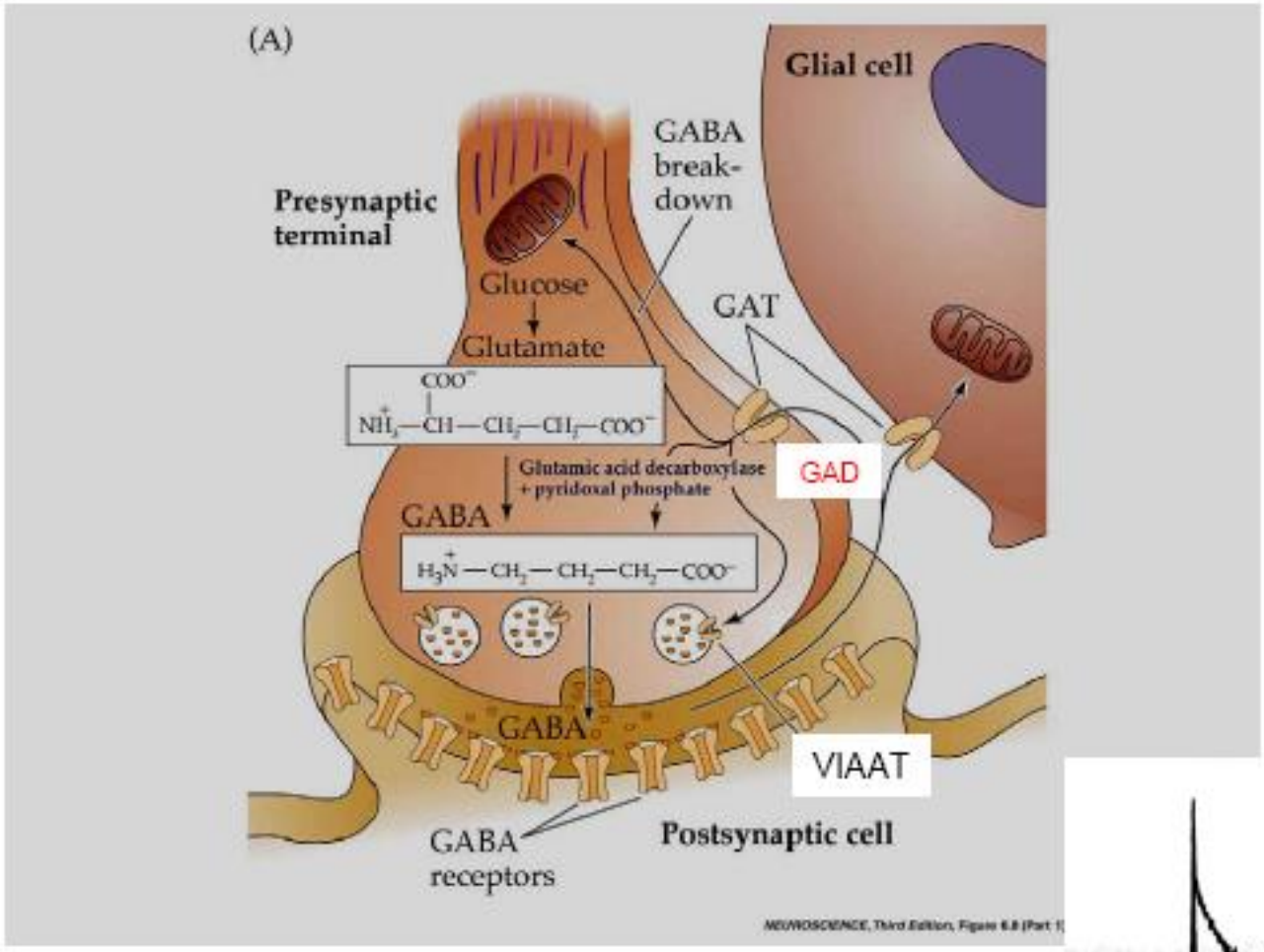
More than 20 genes known for GABAA R

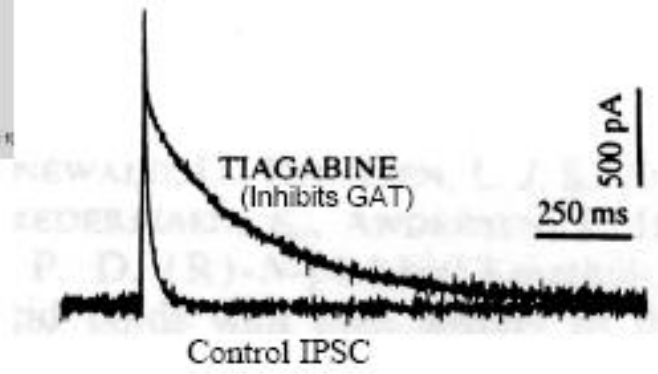


At the synaptic level, GABAA receptors are found in symmetric, inhibitory synapses (no postsynaptic density)



Synthesis and uptake of GABA in nerve endings

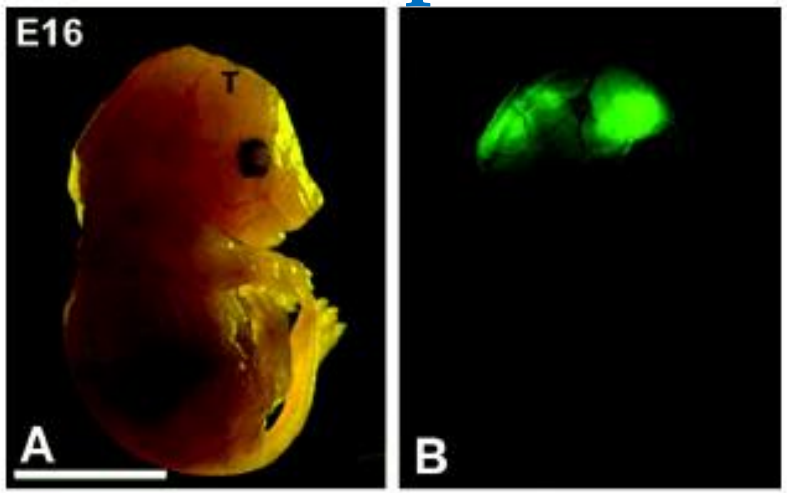


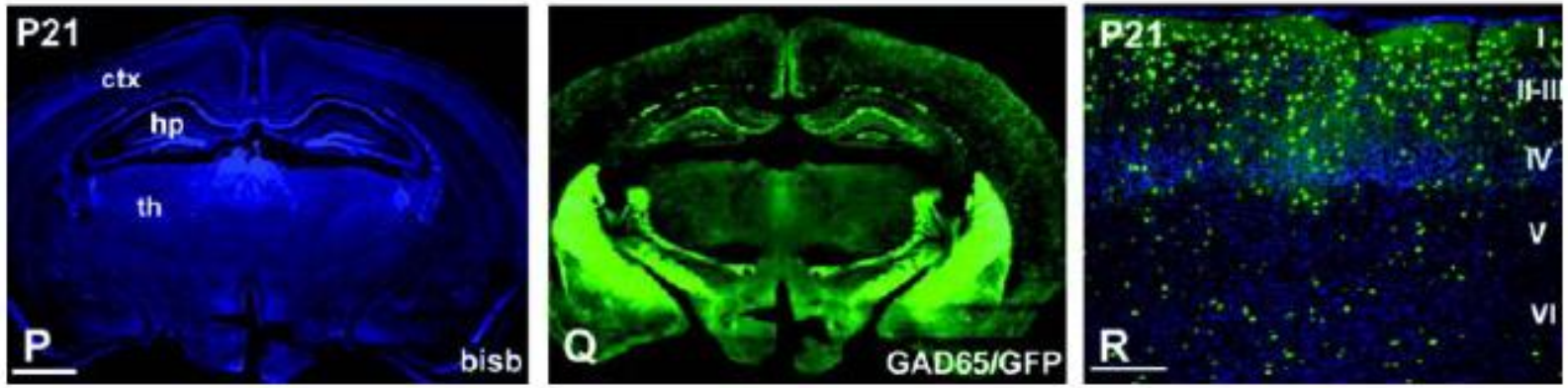


GAT: GABA Transporter
VIAAT (or VGAT): Vesicular Inhibitory Amino Acid Transporter
GAD: Glutamic Acid Decarboxylase

GABAA receptor distribution

A transgenic mouse that expresses the green fluorescent protein (GFP) in GAD positive neurons.

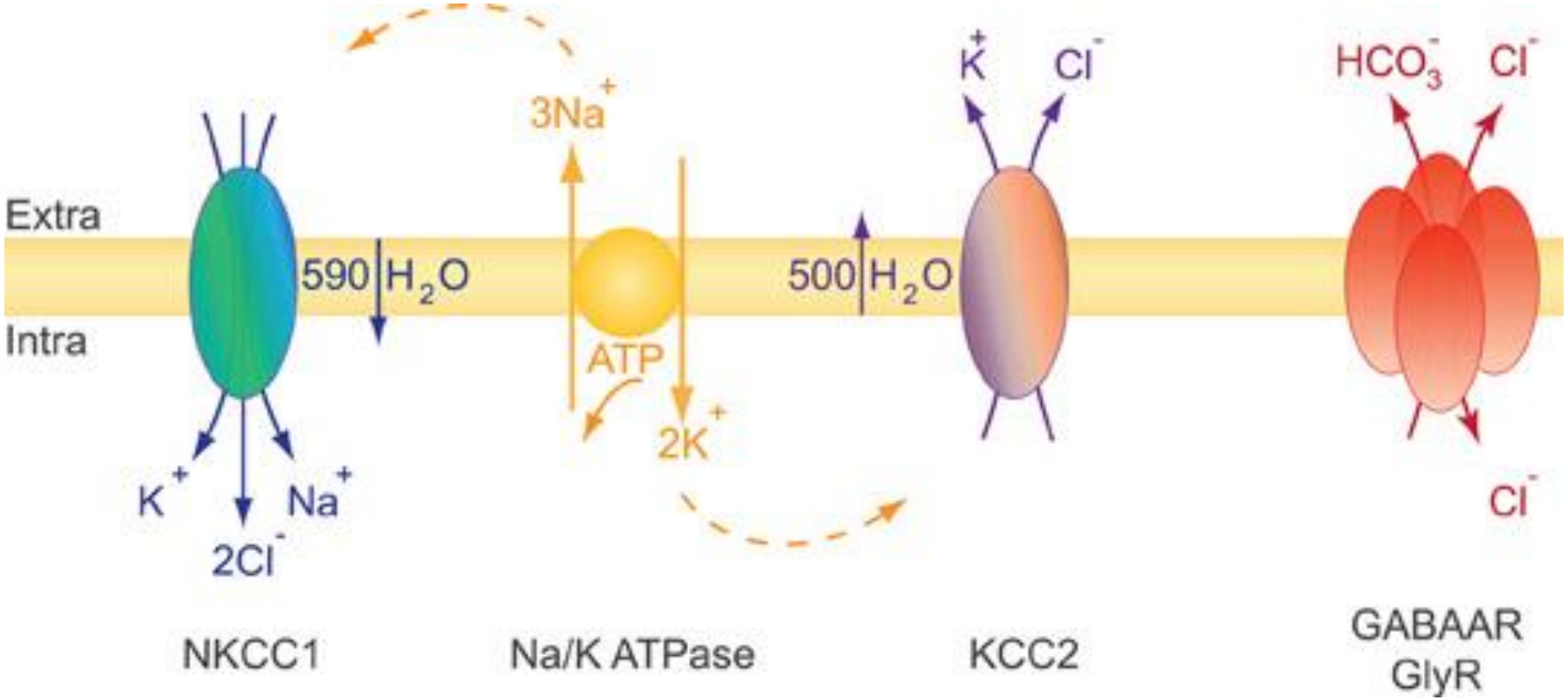




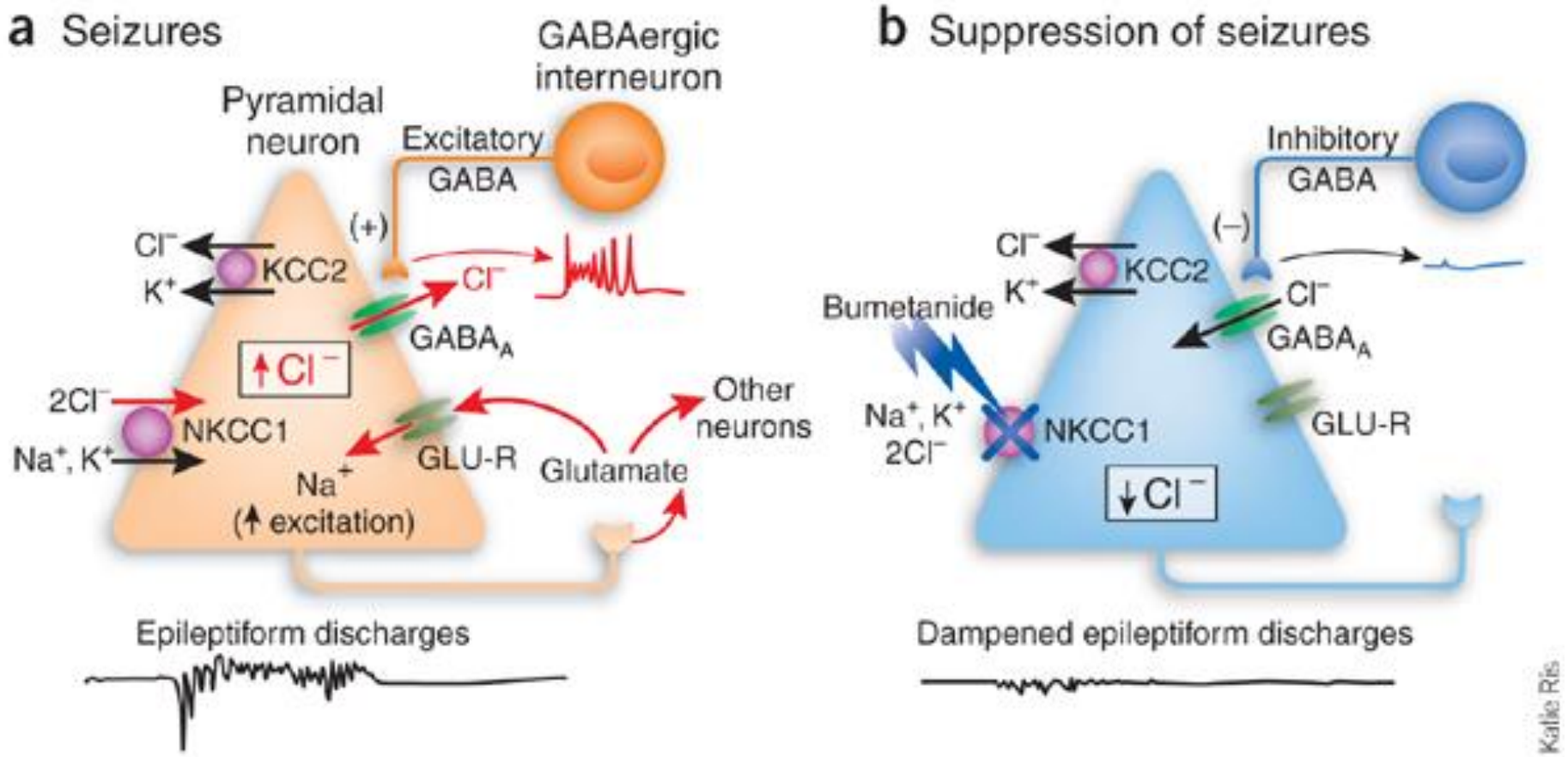
Paulsen 2004

How does Cl^- move?

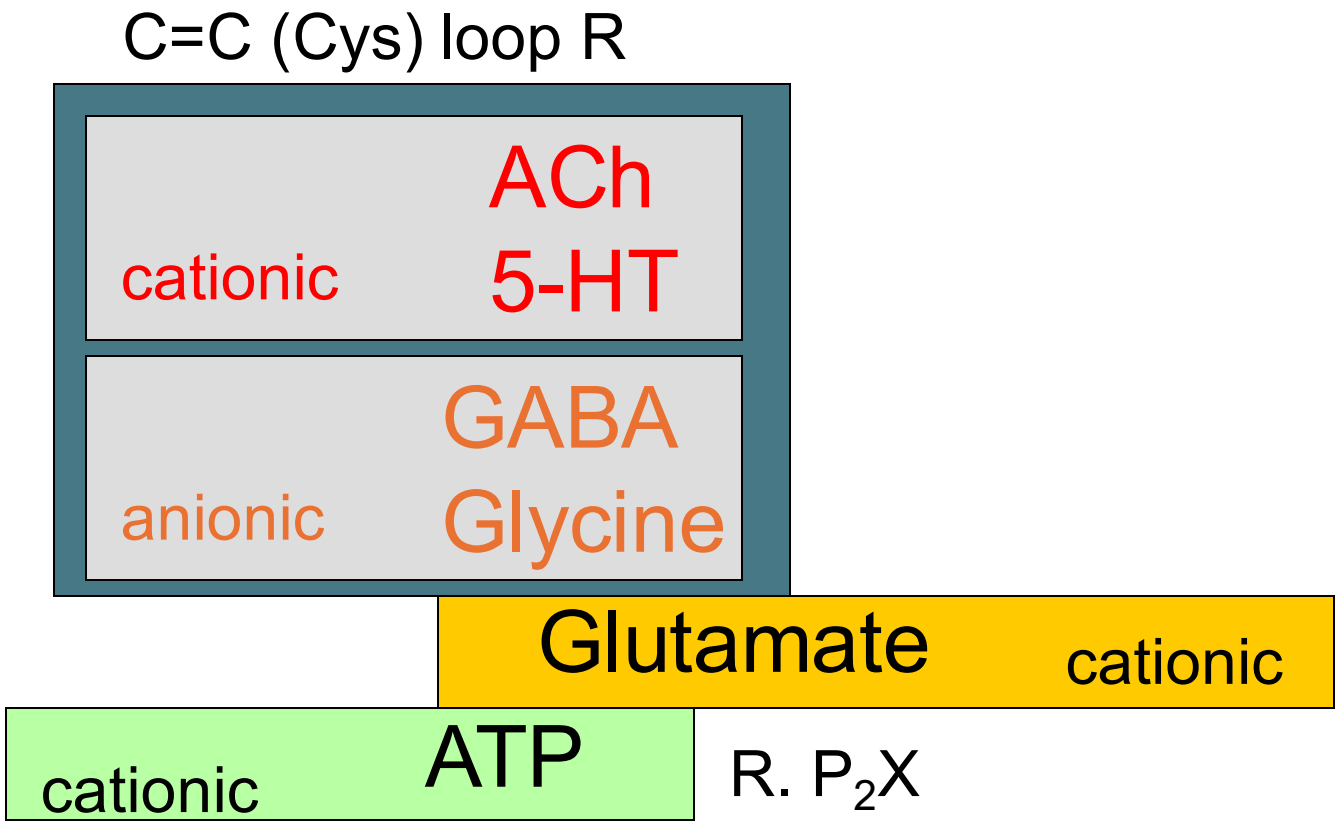
Cl^- equilibrium



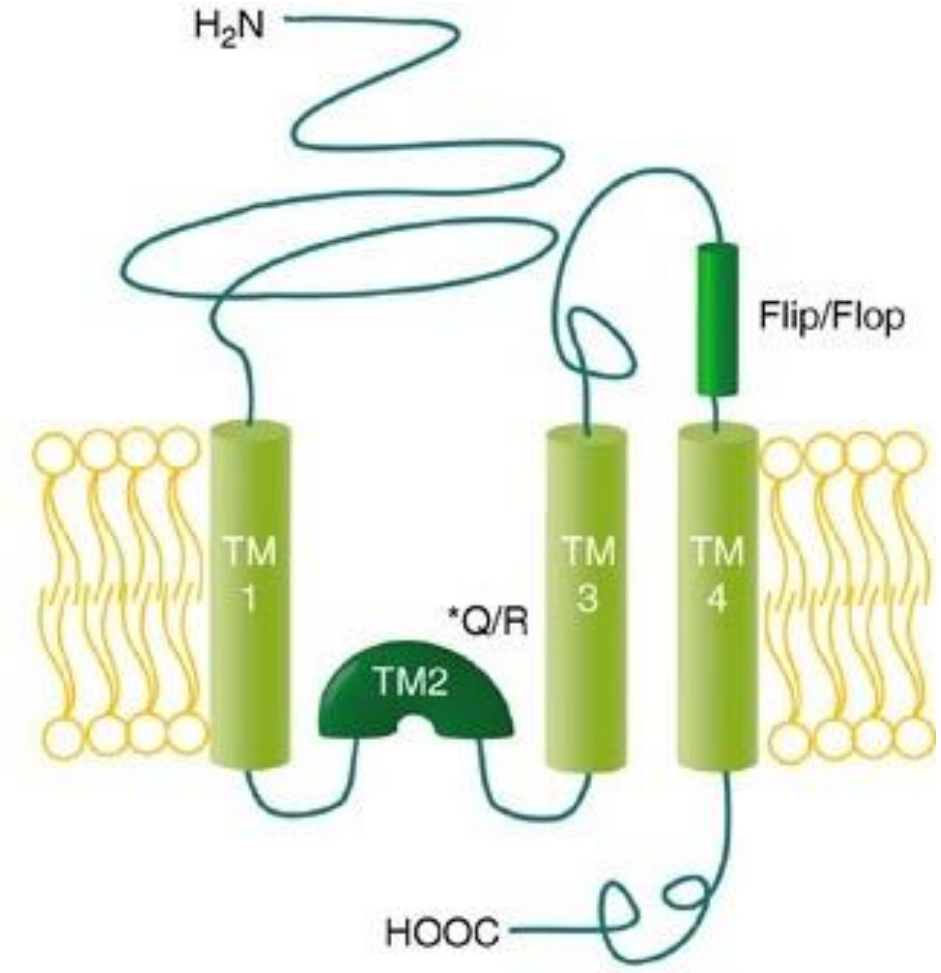
Cl^- equilibrium



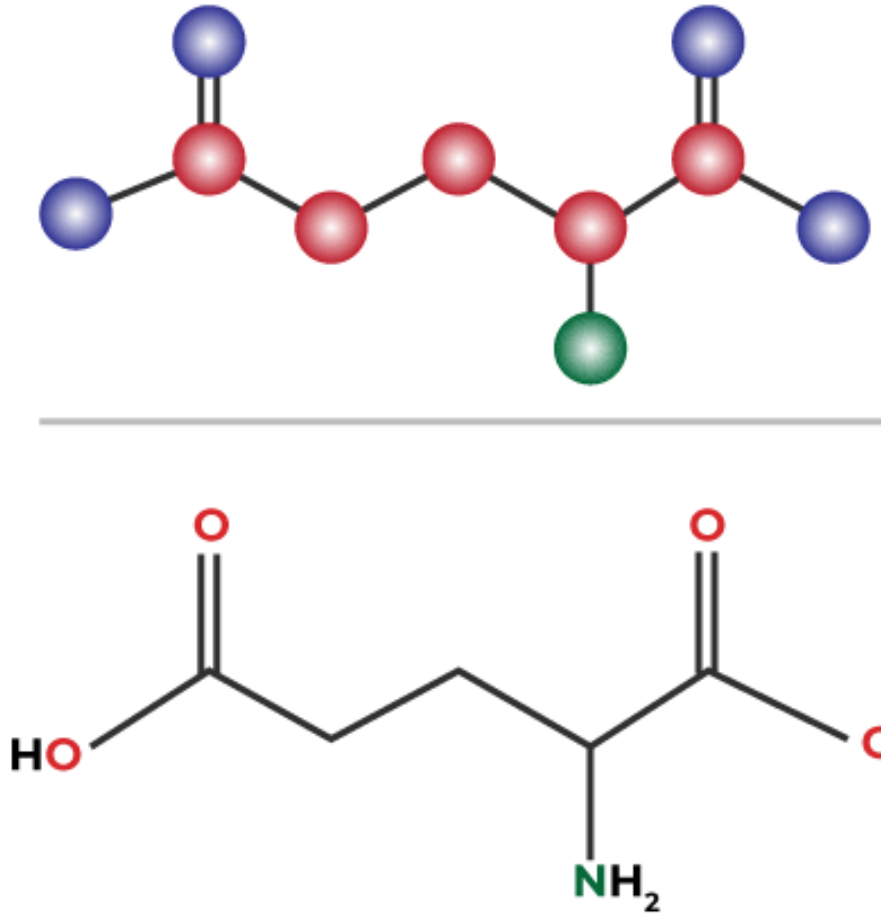
Ionotropic receptor families



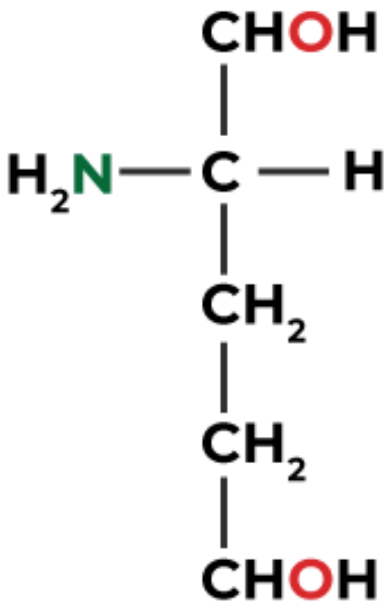
Ionotropic Glutamate Receptors



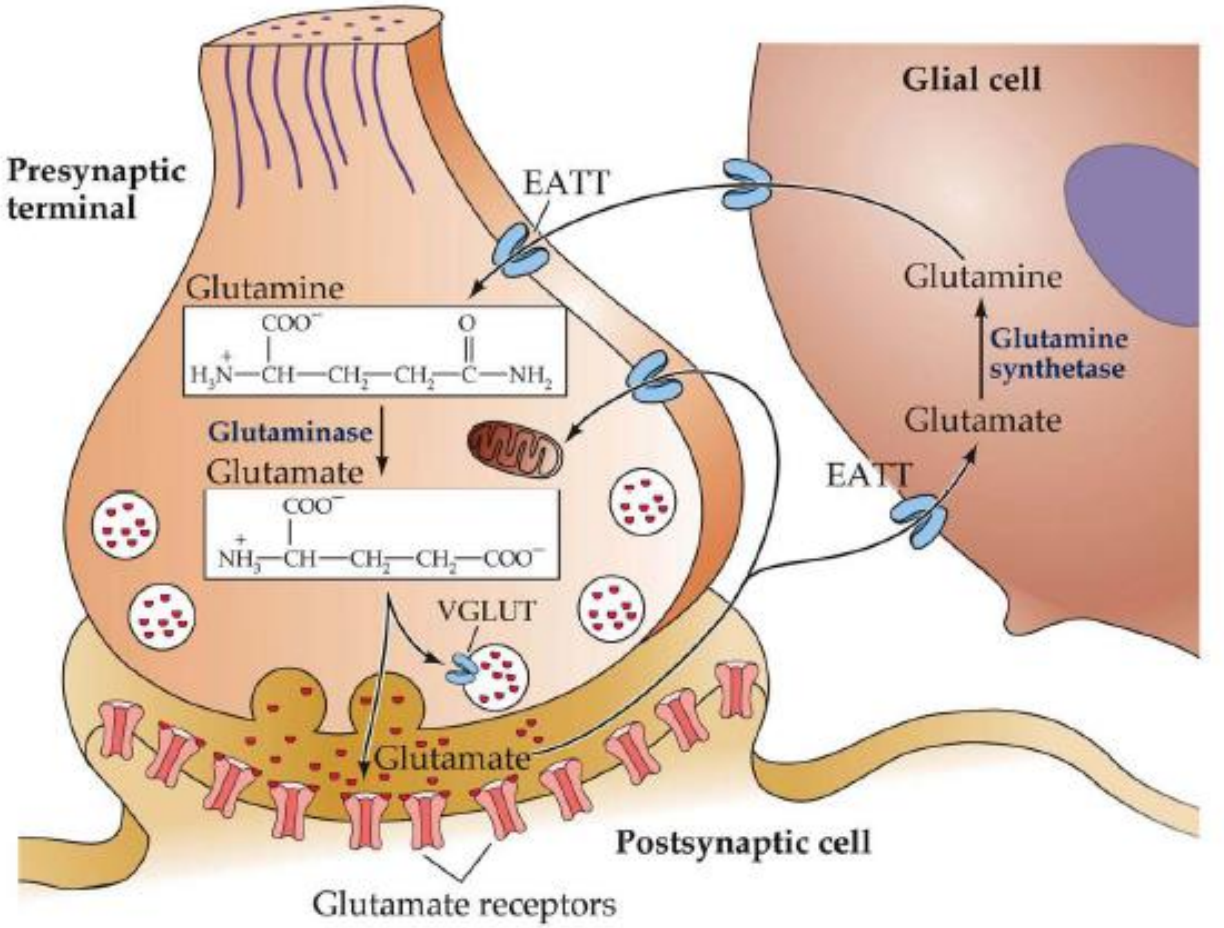
Glutamate



Glutamic Acid
Structure

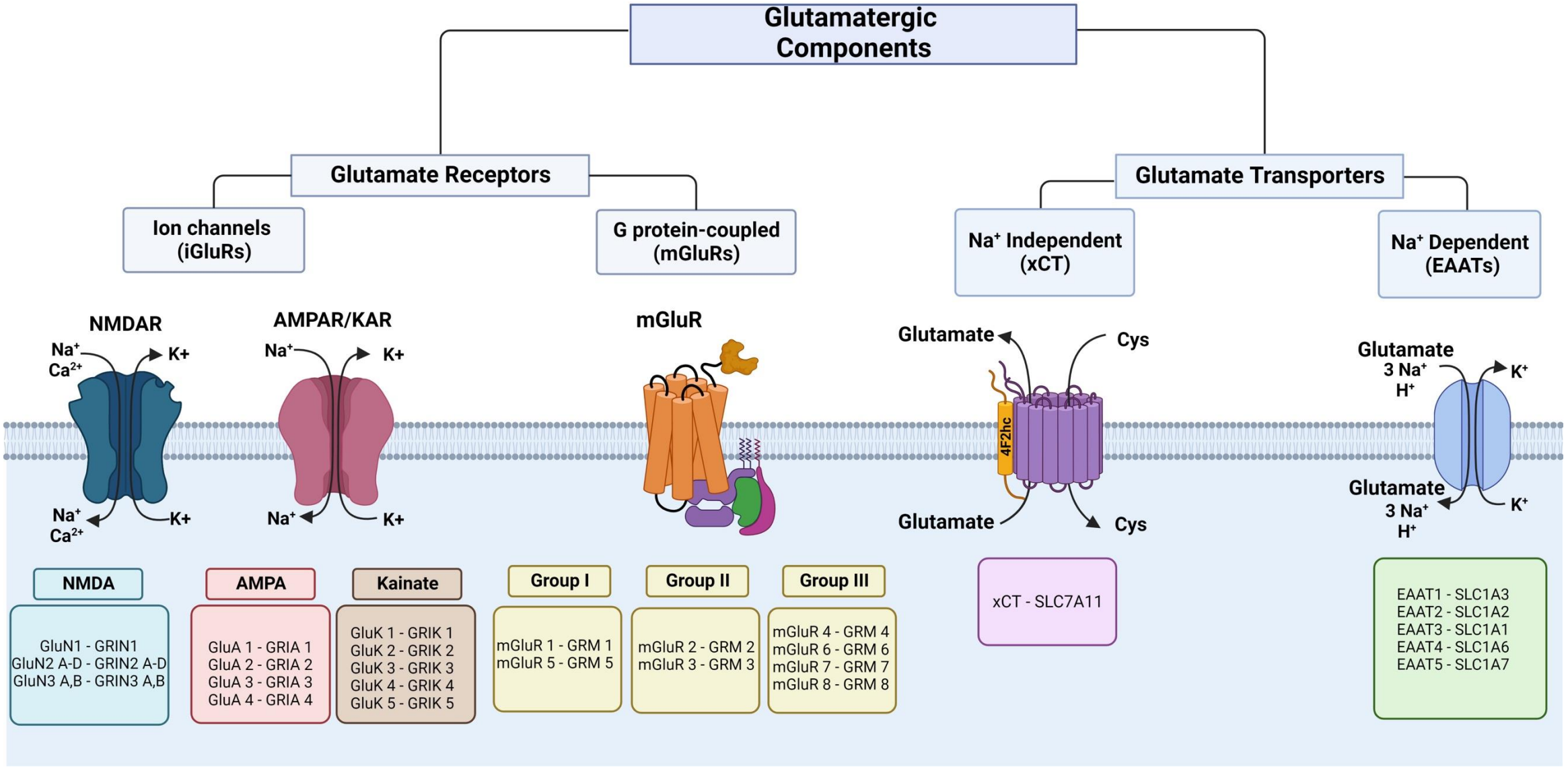


Glutamate cycle



EATT: Excitatory amino acid transporter
VGLUT: Vesicular glutamate transporter

Ionotropic Glutamate Receptors



Ionotropic Glutamate receptors

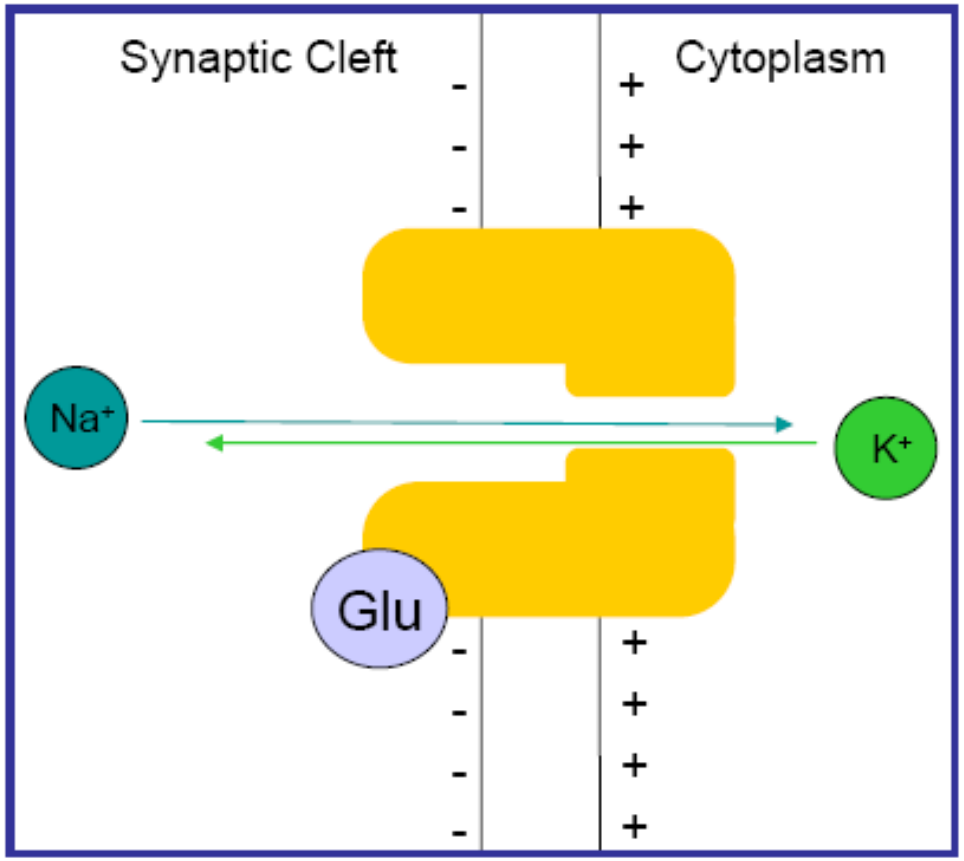
IGluR are widespread in the central nervous system, where more than 80% of the excitatory synapses are glutamatergic.
IGluR are also present in the sense organs.

Given the great functional diversification of the formations in which they operate, it should not be surprising that they are differentiated into many types (with different conductivity, ionic selectivity and pharmacological sensitivity) but have the same character of cationic channels (always excitatory).

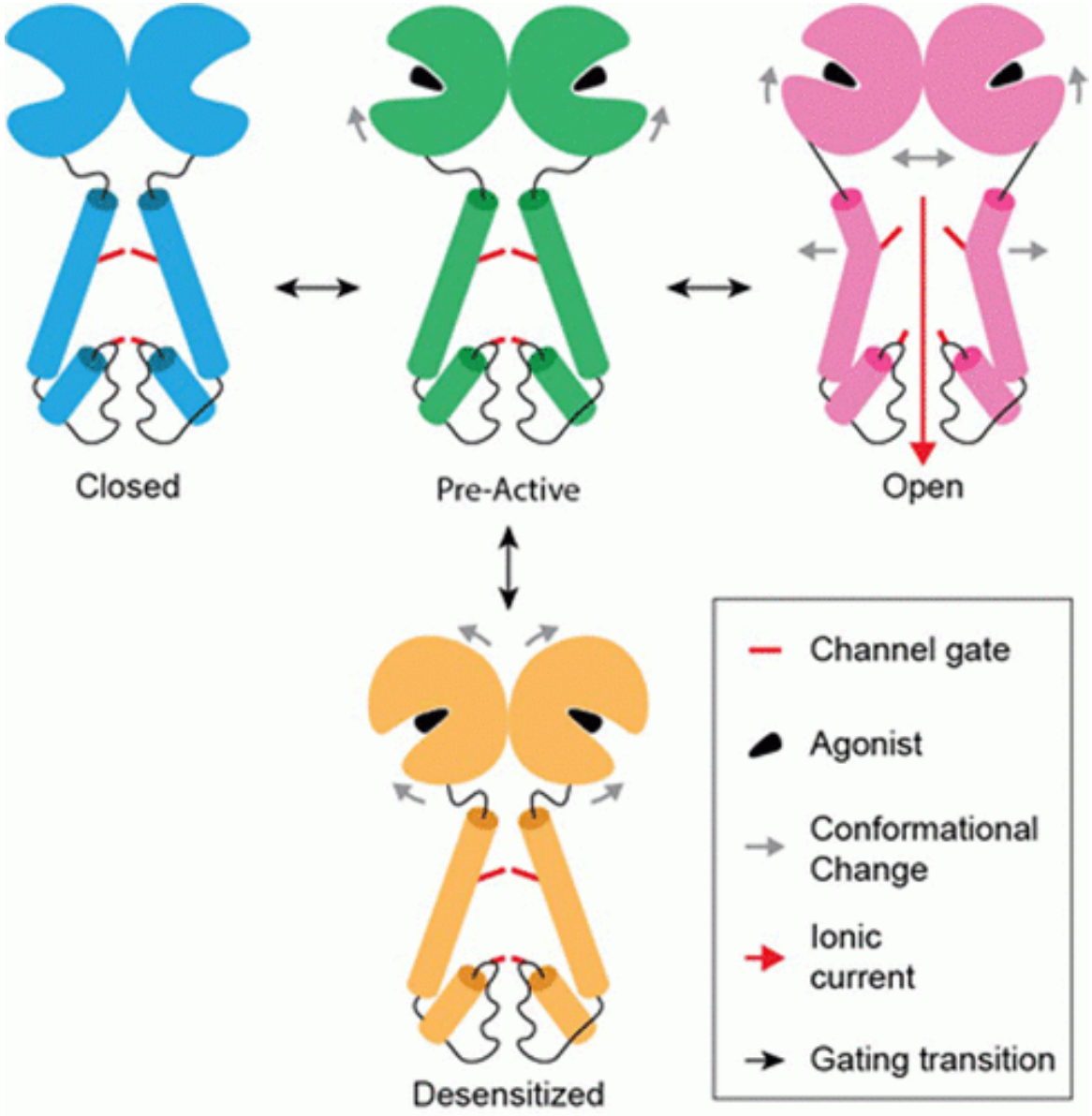
The molecular structure of iGluR differs significantly from the "model" of nAchR, and in some ways resembles that of voltage-gated ion channels ....

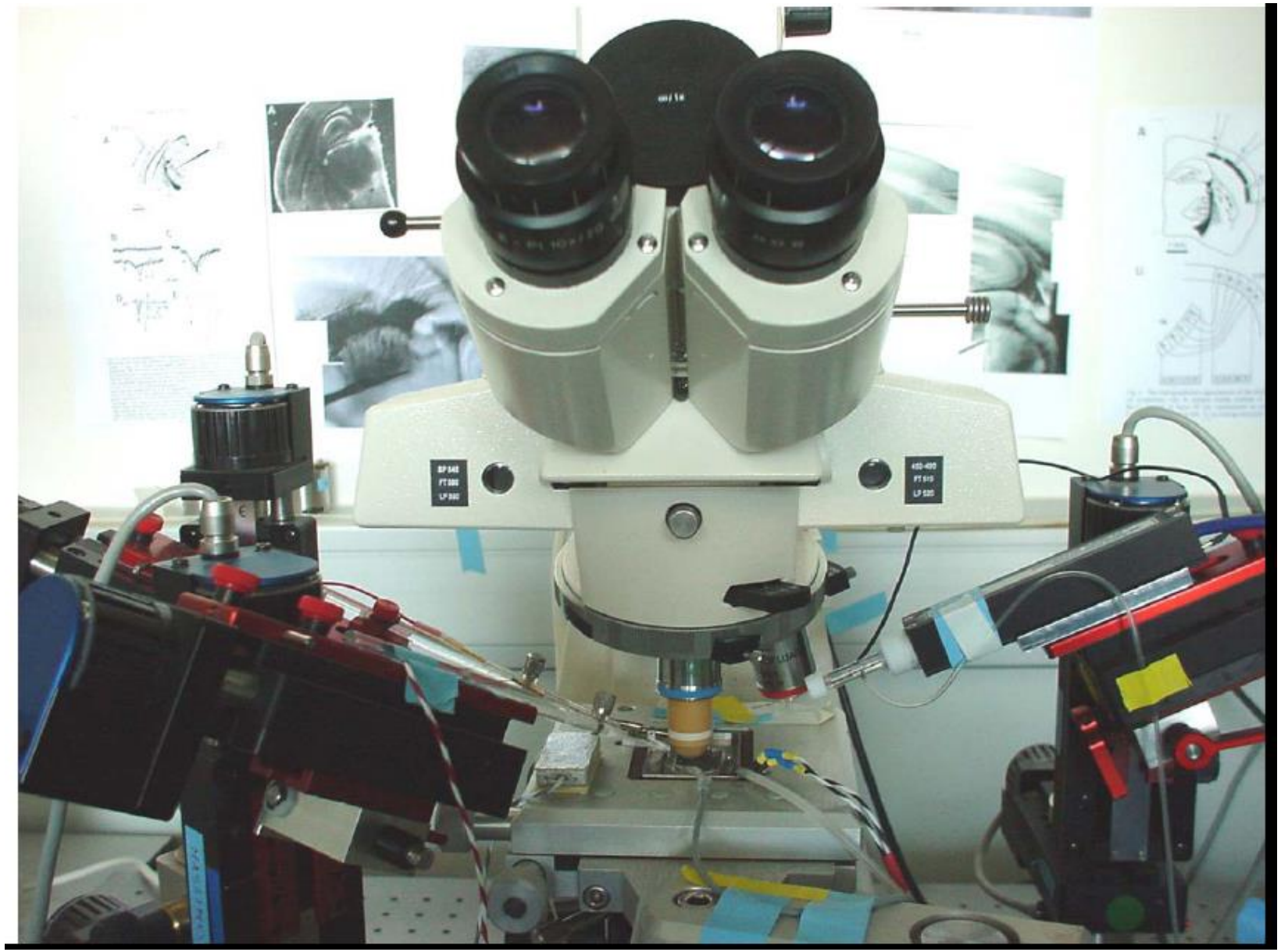
Ionotropic Glutamate receptors

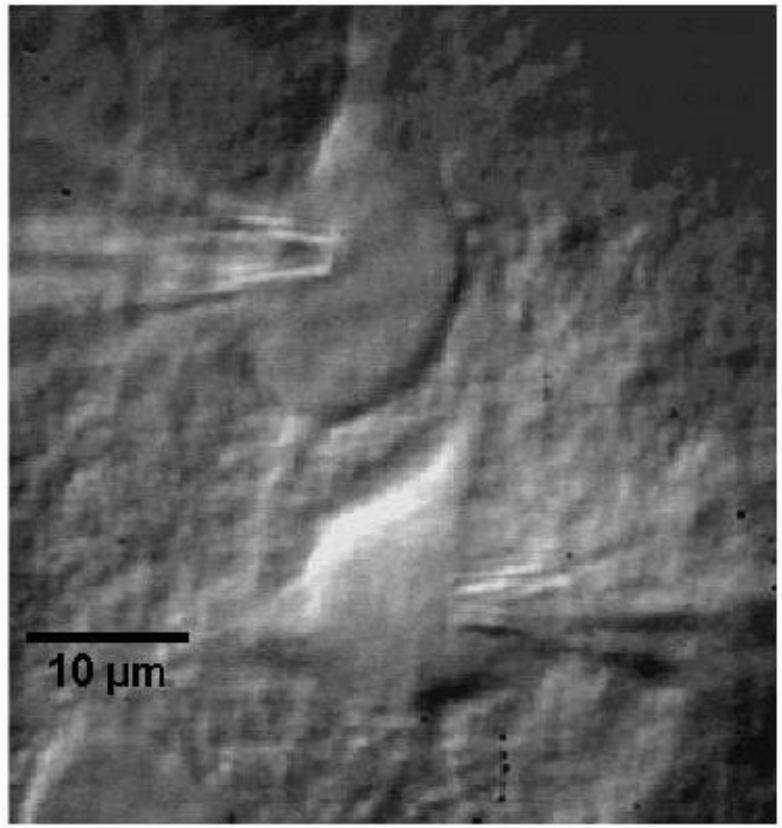
AMPA, NMDA and Kainate receptors opens a non-selective cationic conductance



Ionotropic Glutamate receptors - Gating

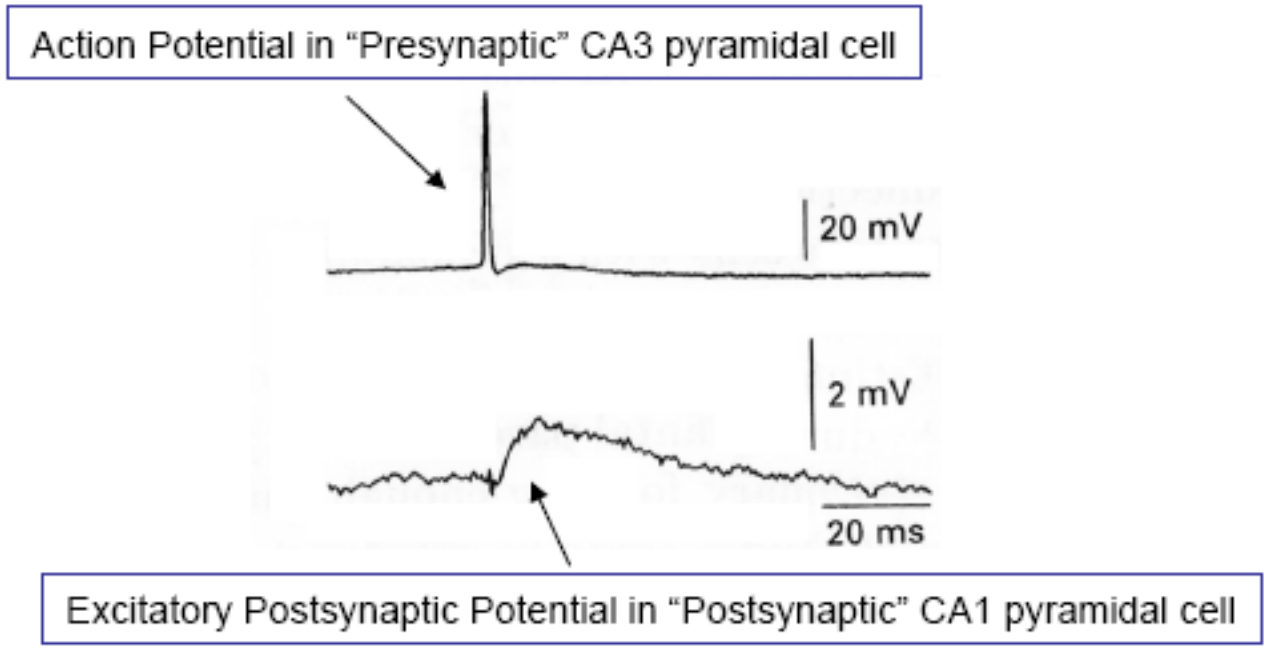





10 µm

Excitatory Post-synaptic Potential

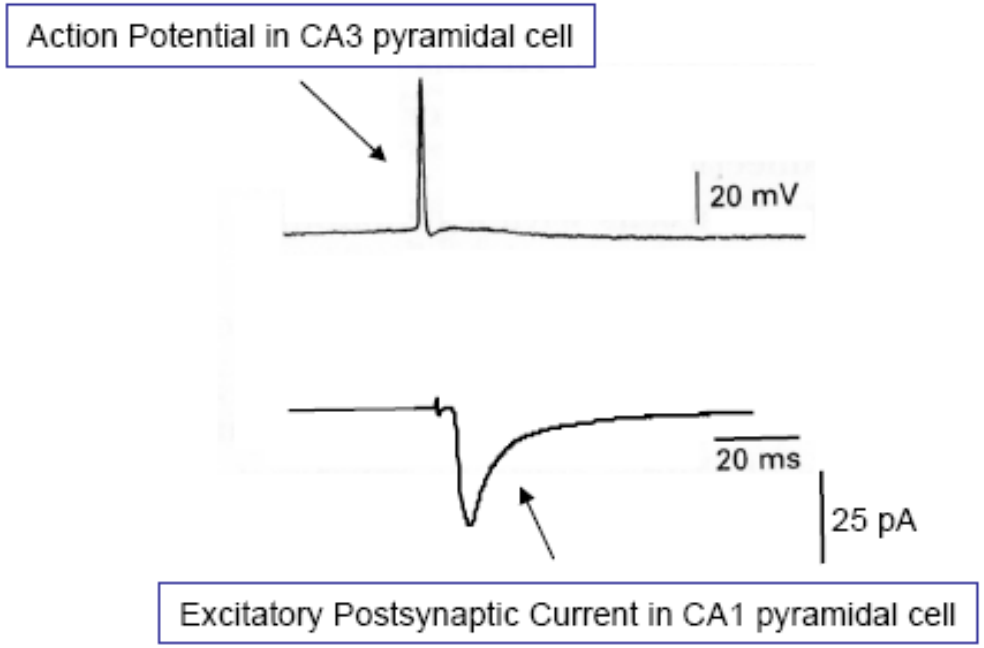
The EPSP



Both pre- and postsynaptic neurons are recorded in the Current Clamp configuration

Excitatory Post-synaptic Current

The EPSC

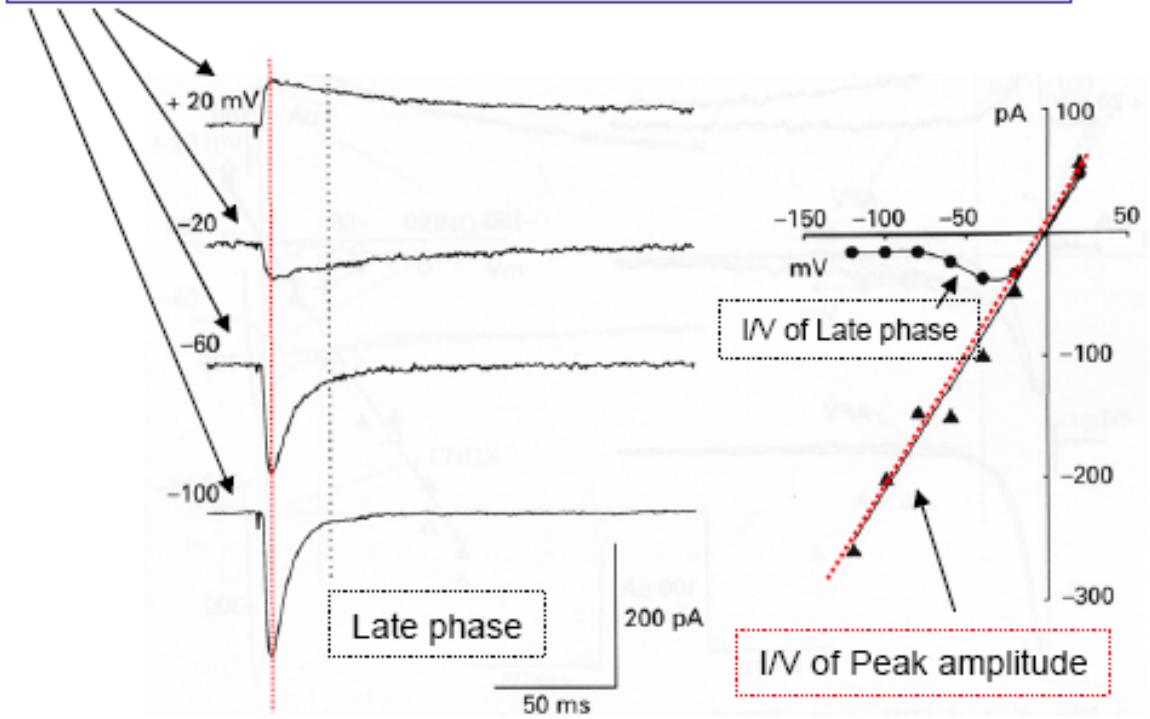


Presynaptic neuron is recorded in the Current Clamp configuration: measure the membrane potential (V_m)
Postsynaptic neuron is recorded in the Voltage Clamp configuration: measure the membrane current (I_m)

I-V Curve

The EPSC

Excitatory Postsynaptic Currents (EPSCs)
The postsynaptic neuron is Voltage-Clamped at different potentials

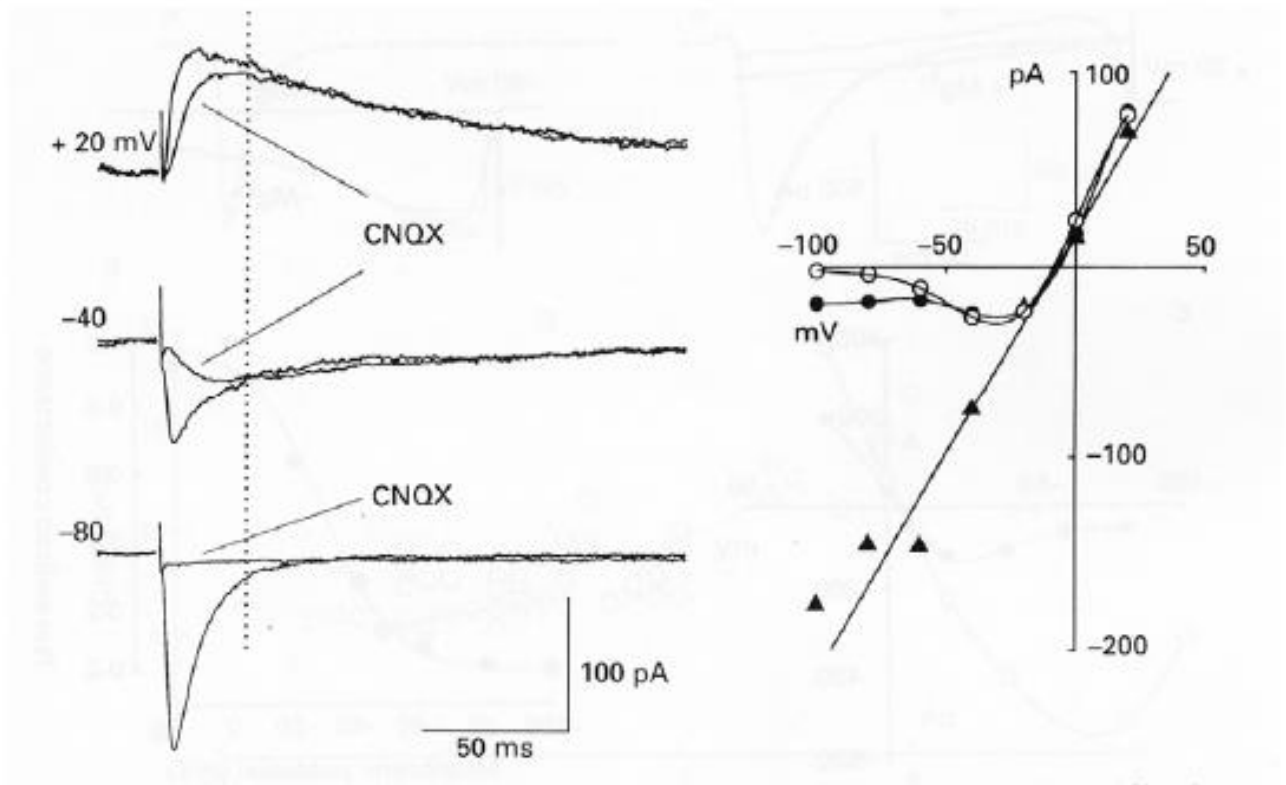


Peak amplitude

Note: Time course of EPSC slower at depolarized potentials
E_{rev} ~0

Two pharmacologically distinct EPSC components: AMPA receptors mediate the fast component

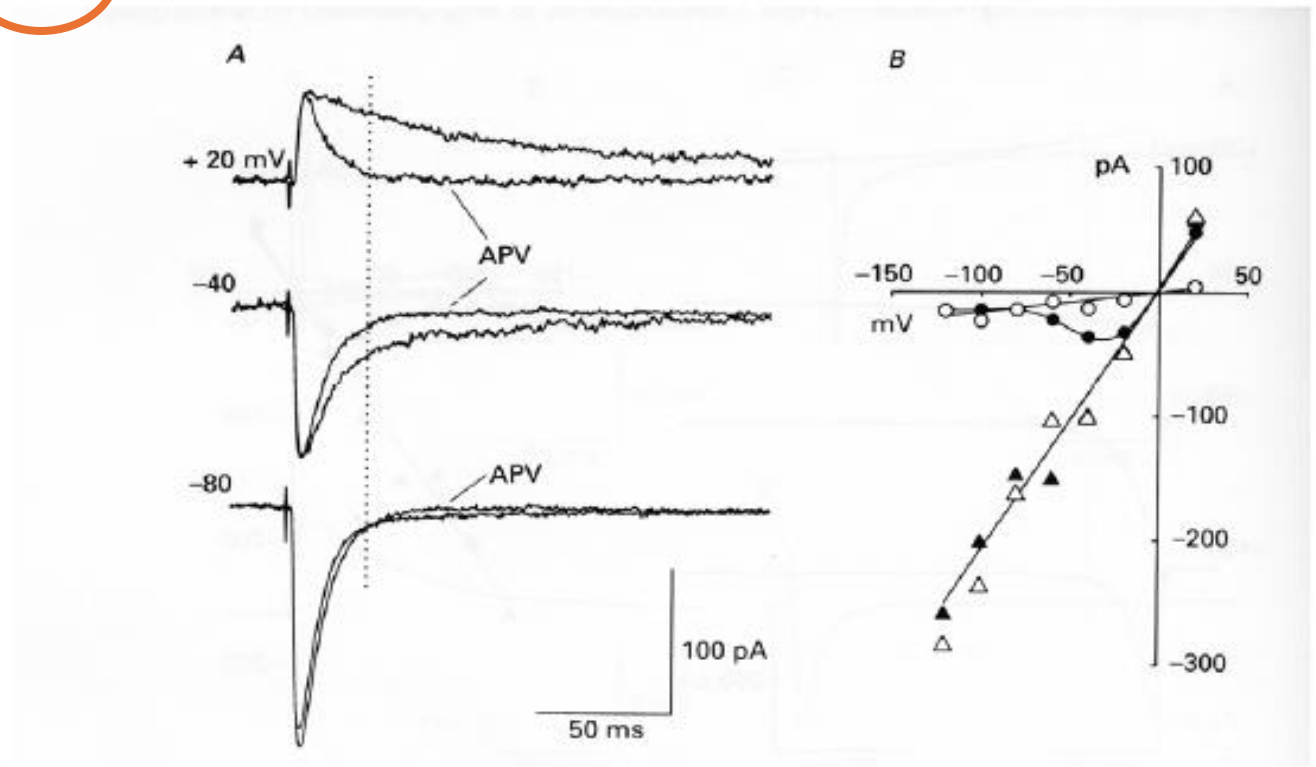
CNQX is an AMPA receptor antagonist and blocks the fast component



AMPA receptors I/V relationship is linear (with some exceptions)

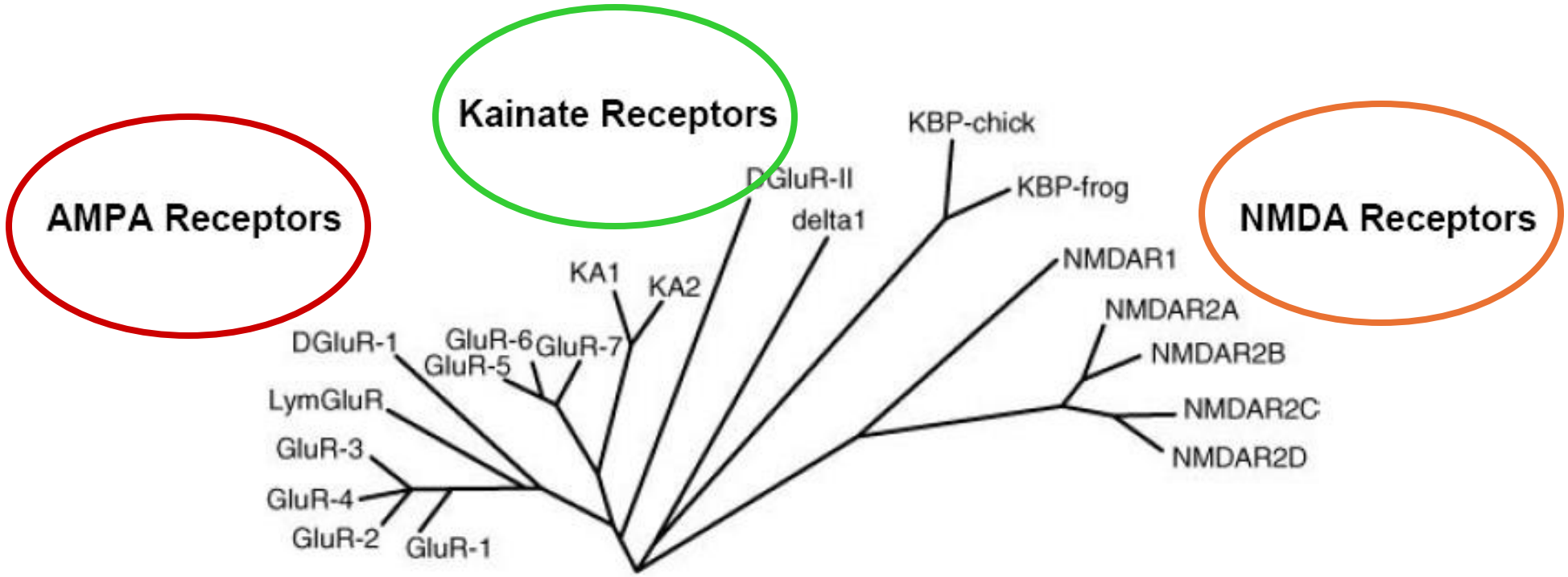
Two pharmacologically distinct EPSC components: NMDA receptors mediate the fast component

APV is an NMDA receptor antagonist and blocks the slow voltage dependent component



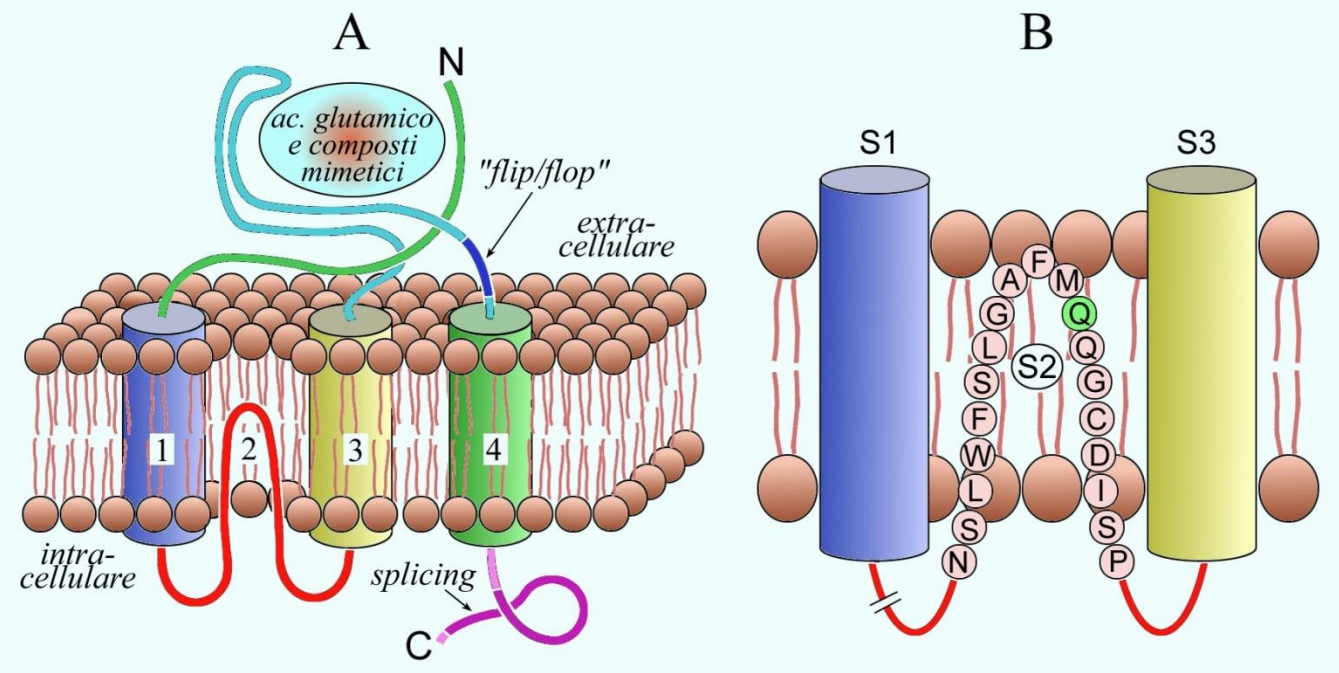
NMDA receptors I/V relationship is not linear

Ionotropic Glutamate Receptor Family



GluR structure

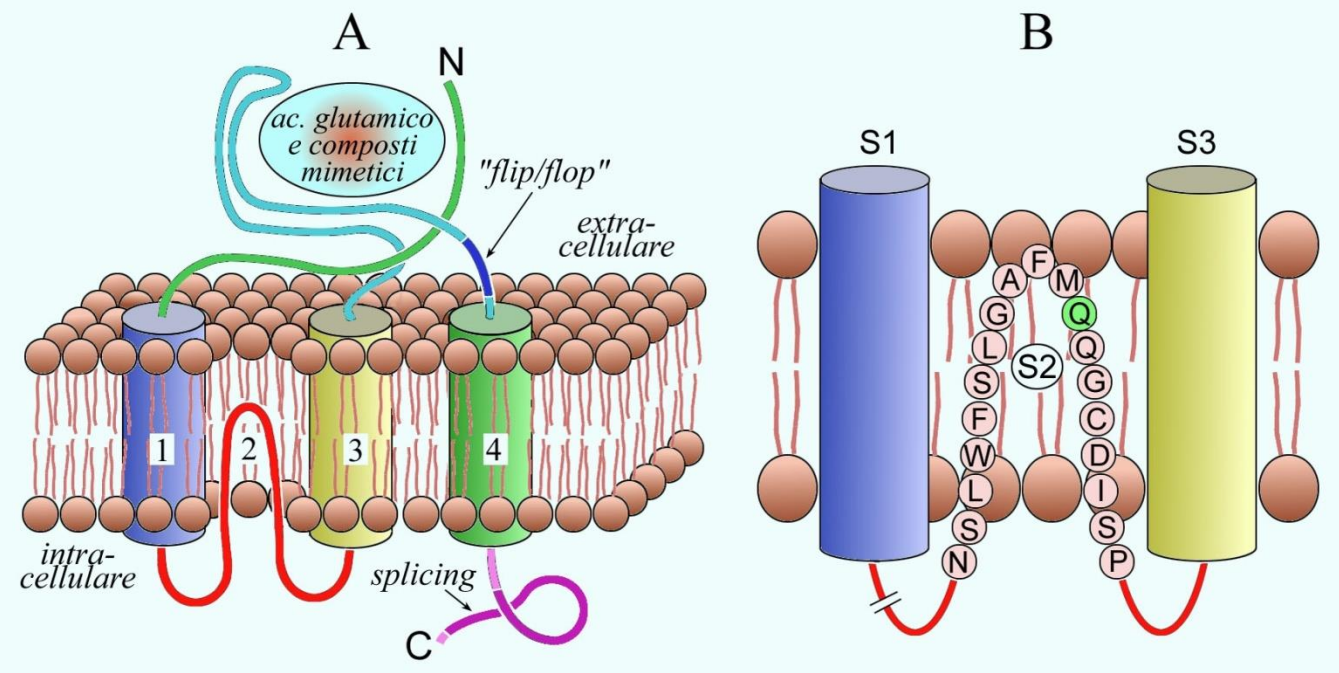
Assembly of *4 subunits*



In each subunit, the second hydrophobic domain (improperly referred to as "S2"), after entering the membrane from the cytoplasmic side, is reflected forming a loop (a sort of "P region") and returns to the cytoplasm without crossing it.

GluR structure

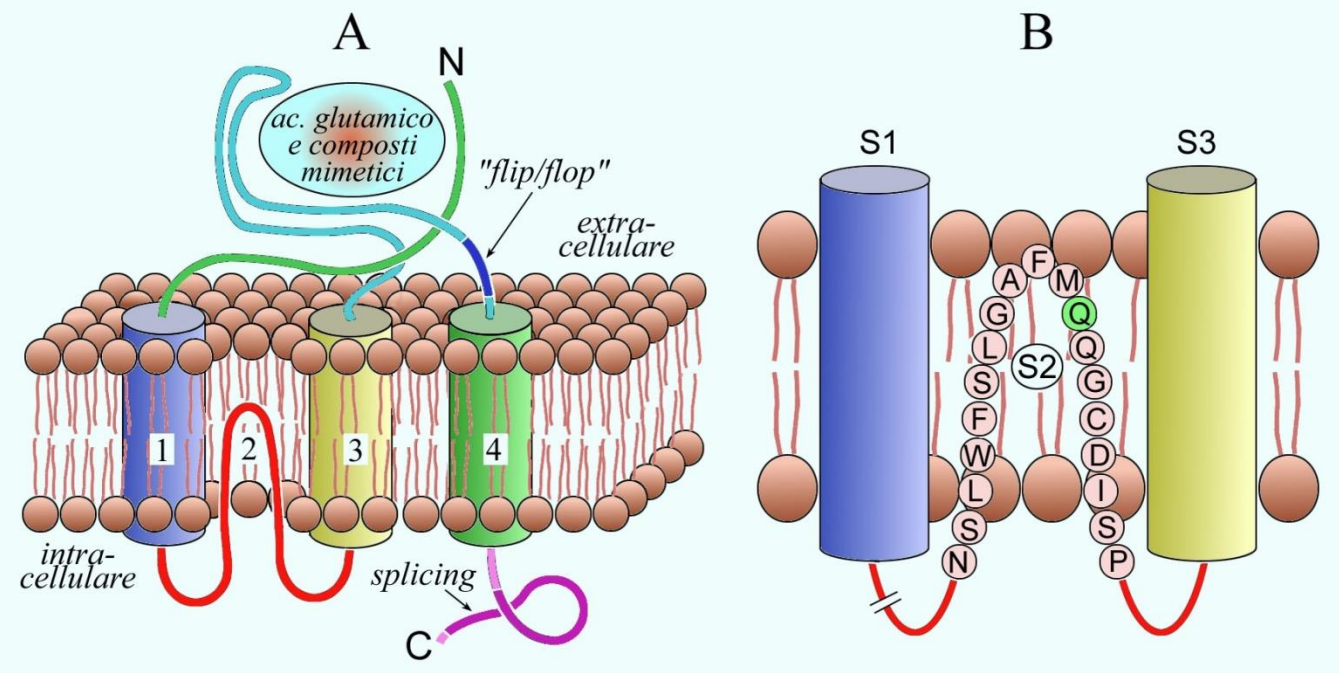
Assembly of *4 subunits*



This missed crossing causes the iGluR subunits to have only three transmembrane segments (and not four) and the C-terminal end of the whole amino acid chain is directed towards the intracellular medium (and not towards the extracellular medium, as is the rule for other ionotropic receptors).

GluR structure

Assembly of *4 subunits*



The length of the polypeptide chains is much greater (about twice as much). This explains why iGluR have a higher molecular weight, although they are tetrameric (and not pentameric) complexes. The long polypeptide chains of iGluR develop mainly in the extracellular medium, where the N-terminal ends and the S3-S4 connecting loops of the four subunits intertwine to form a huge "ball", inside which is the binding site for the neurotransmitter.

GluR Pharmacology

Among the different types of iGluR there are two subfamilies, based on their sensitivity to different mimetic compounds or antagonists of the natural neurotransmitter (glutamic acid):

a) **NMDA receptors, so called because activated by AC. N-Methyl-D-Aspartic.They are blocked by the AC. 2-Amino-5-Phosphono-Valerico (APV or AP5) and related compounds;**

b) **non-NMDA receptors, insensitive to NMDA.This subfamily is subdivided into two groups: that of the receptors activated by AMPA (a-Amino-3-hydroxy-5-Methyl-isossazol-Propionic Acid) and that of receptors activated by cainic acid (KA).All non-NMDA receptors are blocked by 6-Cyano-7-NitroQuinoXalin-2,3-dione (CNQX) and related compounds (NBQX, DNQX).**

In the same synapse (excitatory) both types of iGluR can be present at the same time; it is then said that they are "co-localized".

GluR Pharmacology

Agonists:

L-glutamate

AMPA

KA, ATPA

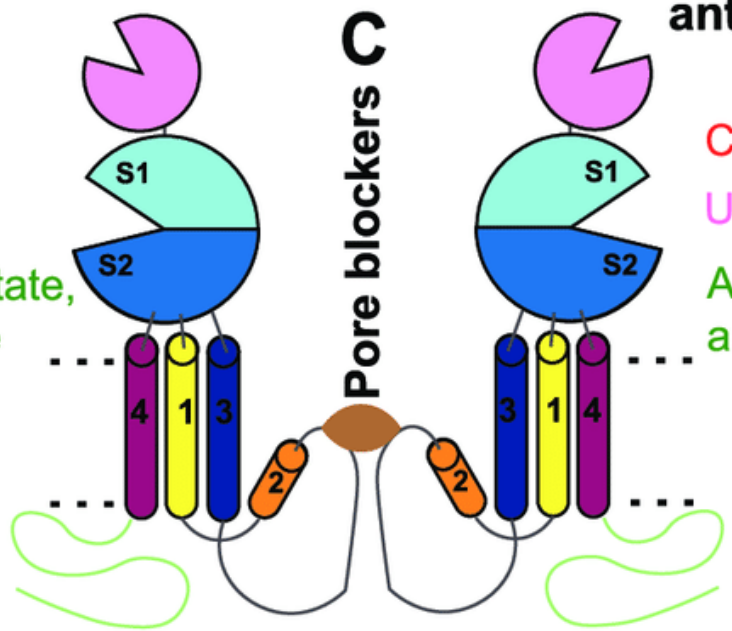
NMDA, aspartate,
homocysteate

Competitive antagonists:

CNQX, NBQX

UBP302

AP5/APV, CPP,
anti-GluN1-S2



JST

JST

Mg^{2+}, PCP,
MK-801, memantine

— for AMPAR

— for KAR

— for NMDAR

Assembling of GluR

It has been shown that ionotropic receptors for ac. glutamic can be formed by assembling many types of subunits, encoded by distinct genes.

- NMDA receptors are formed by subunits called NR ("NMDA Receptor").These can belong to 5 different types: NR1, NR2A, -2B, -2C and -2D.

-the NMDA receptor molecule always contains at least one specimen of the NR1 subunit, associated in a characteristic way, in the different parts of the brain, with a particular type of NR2 subunit.

Assembling of GluR

- **non-NMDA receptors are instead formed by subunits called GluR.These can belong to 7 different types: GluR1, .., GluR7).**
- **The assembly of GluR1, -2, -3 and -4 gives rise to the subfamily of AMPA receptors,**
- **The assembly of GluR5, -6 and -7 (probably together with two accessory subunits: KA1 and KA2) produces the subfamily of the kainate receptors.**

The considerable diversification in the biophysical and pharmacological properties of the various types of iGluR is due to the particular combination of subunits that make up their molecules, and is greatly enhanced by the fact that mRNAs of the various subunits can also be translated into various isoforms for "alternative splicing" .Furthermore, the "editing" process (enzyme modification of the mRNA) can intervene -

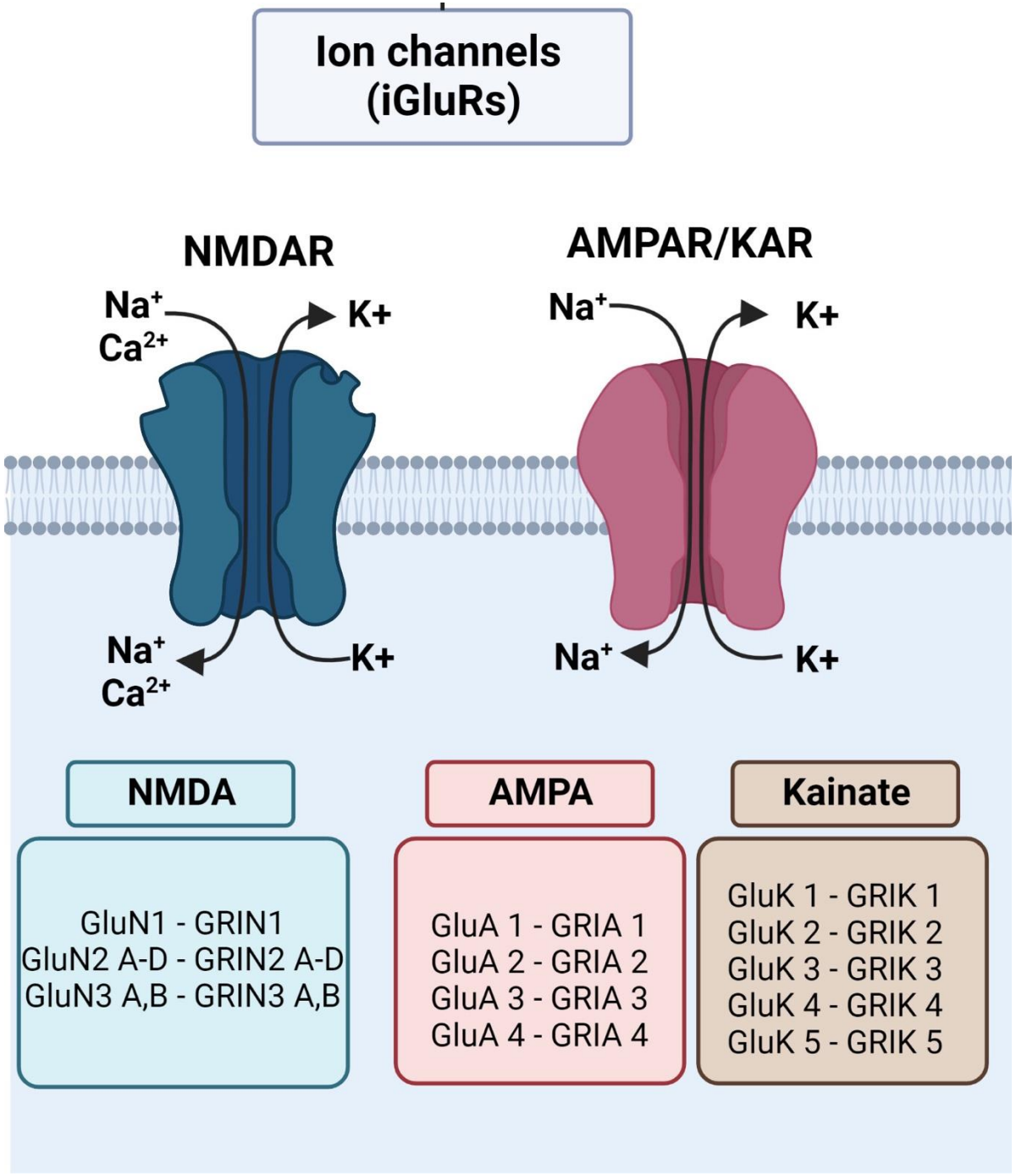
Assembling of GluR

However, generally

NMDA receptors are very permeable to Ca^{2+}

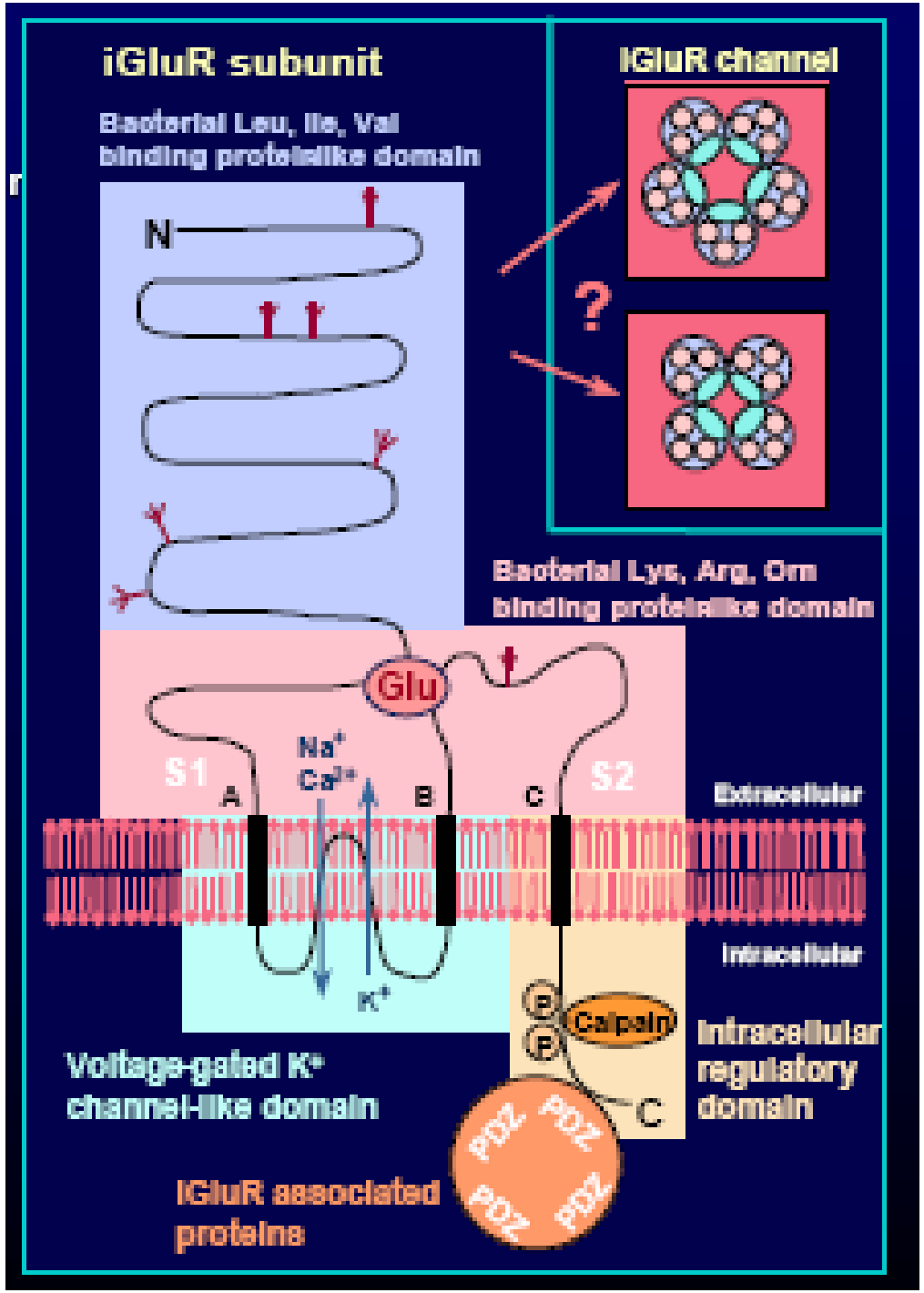
Non-NMDA receptors are little or not permeable to Ca^{2+}

Assembling of GluR

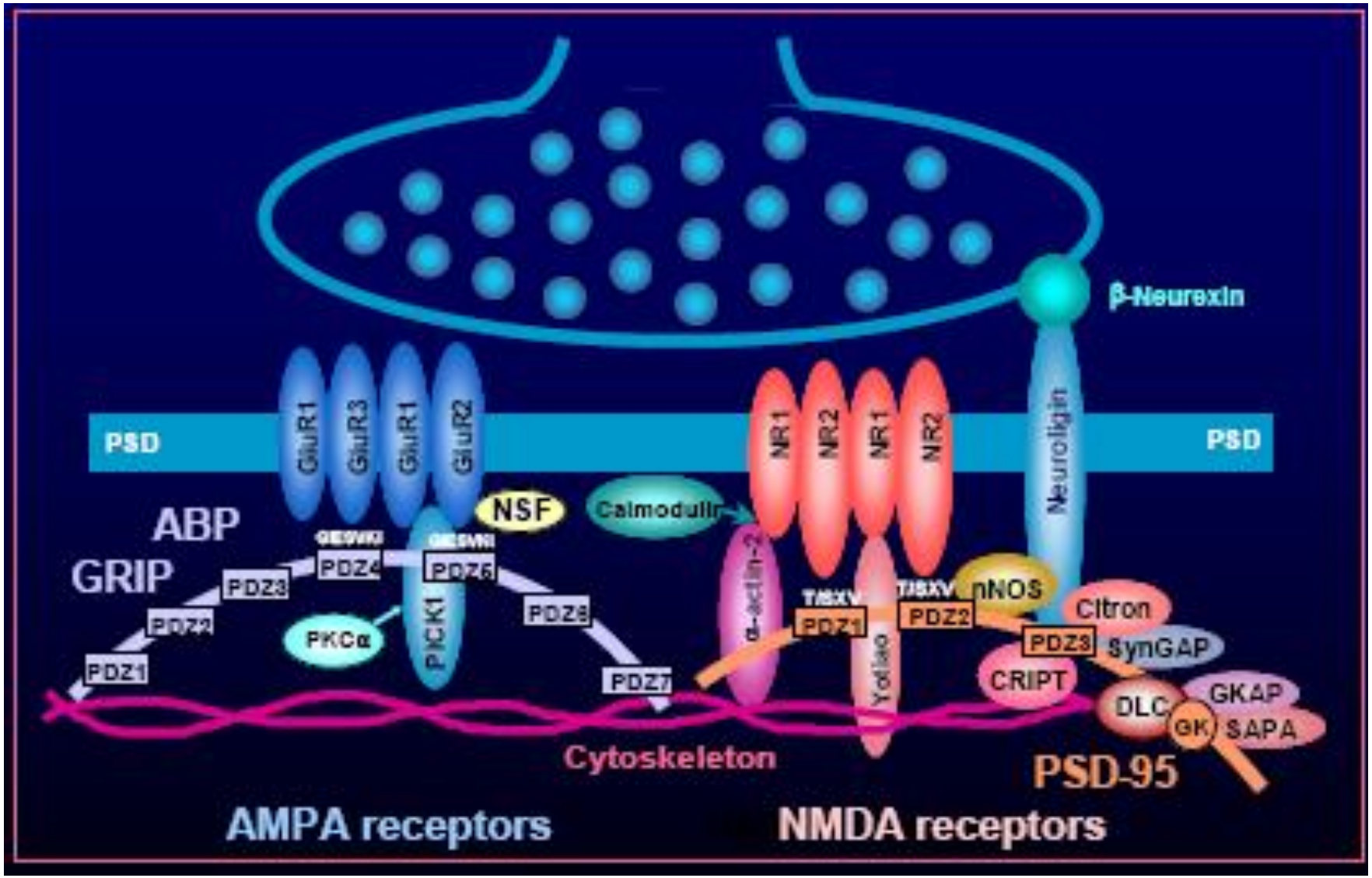


GluR Receptors at synapses

Schematic representation of the transmembrane topology of ionotropic glutamate receptors



GluR Receptors at synapses

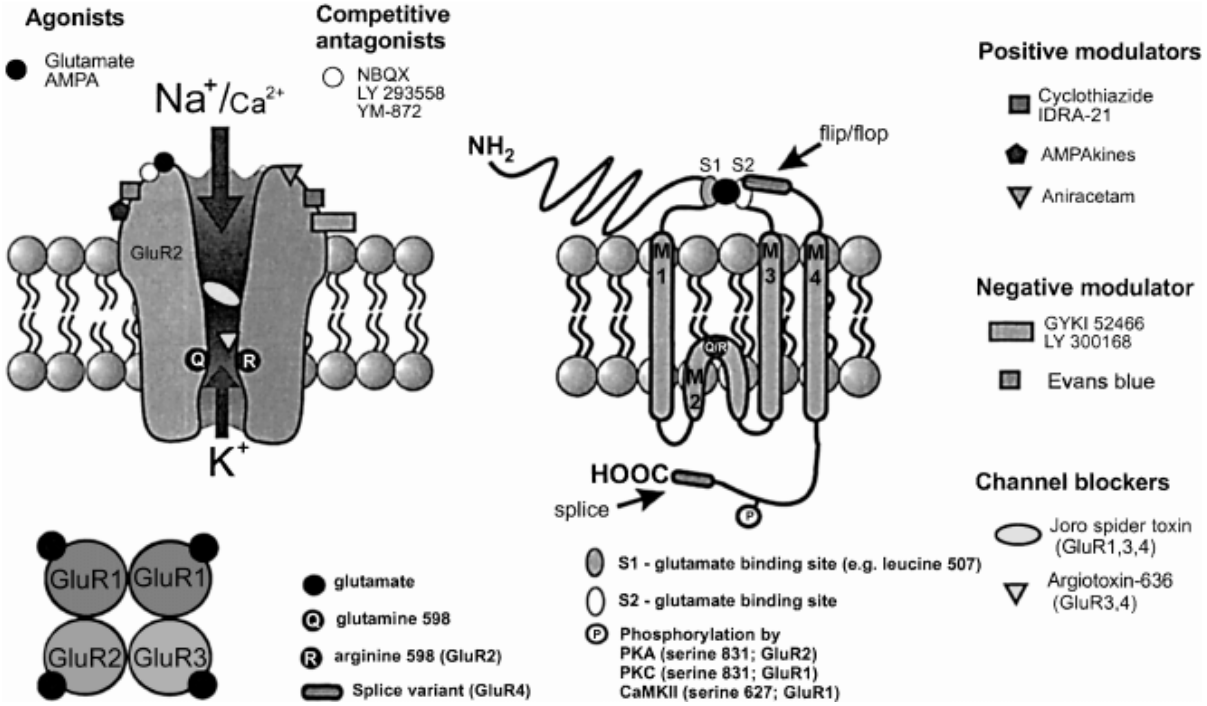


The synaptic protein network associated with AMPA and NMDA receptors

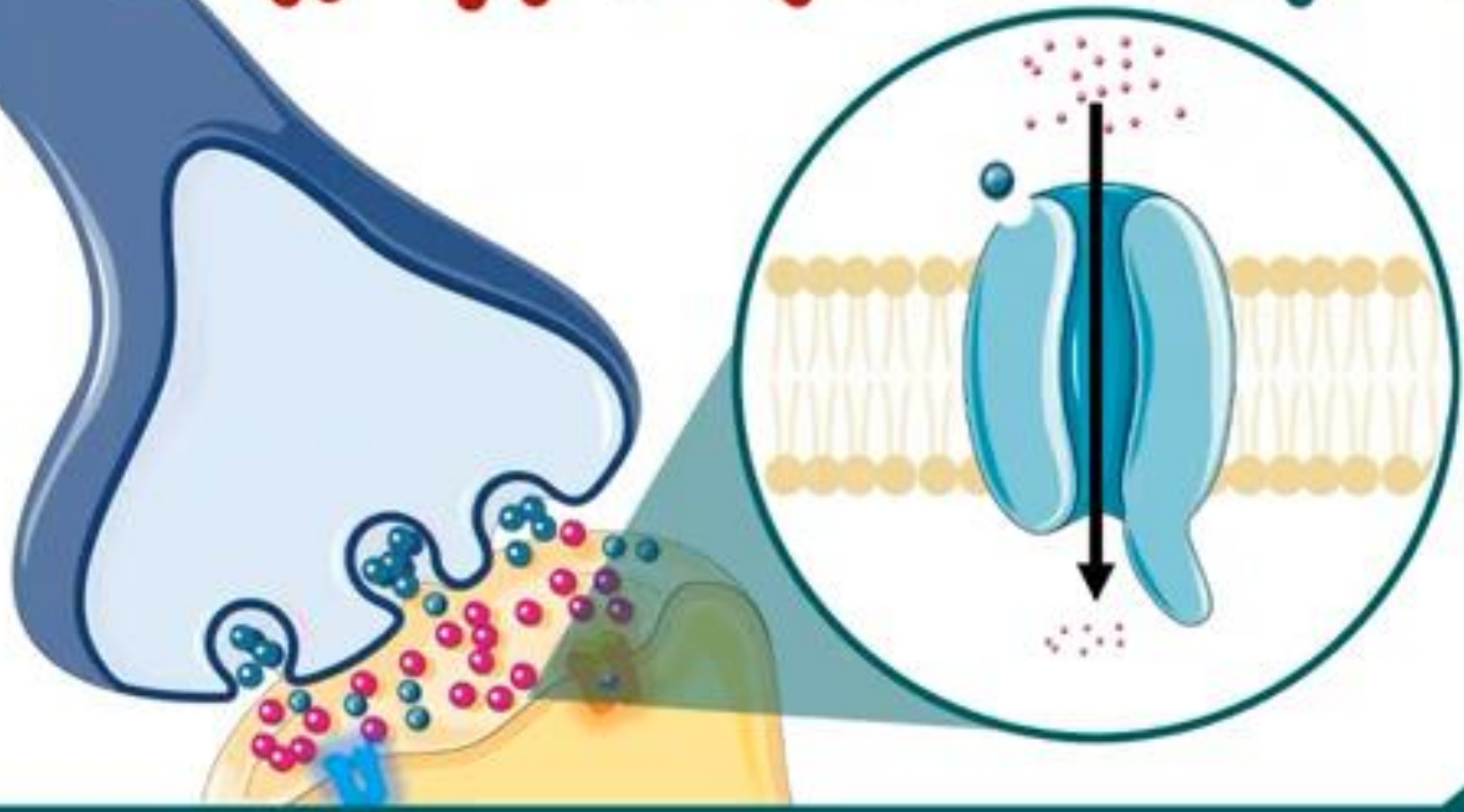
AMPA Receptors

Receptors for AMPA have kinetics of activation / inactivation and desensitization that are very fast.

- **They are permeable to Na^+ and K^+ and little to Ca^{2+}**
- **They are located in the postsynaptic membrane**
- **They are responsible for the excitatory response (depolarizing) rapid typical of glutamatergic synapses.**



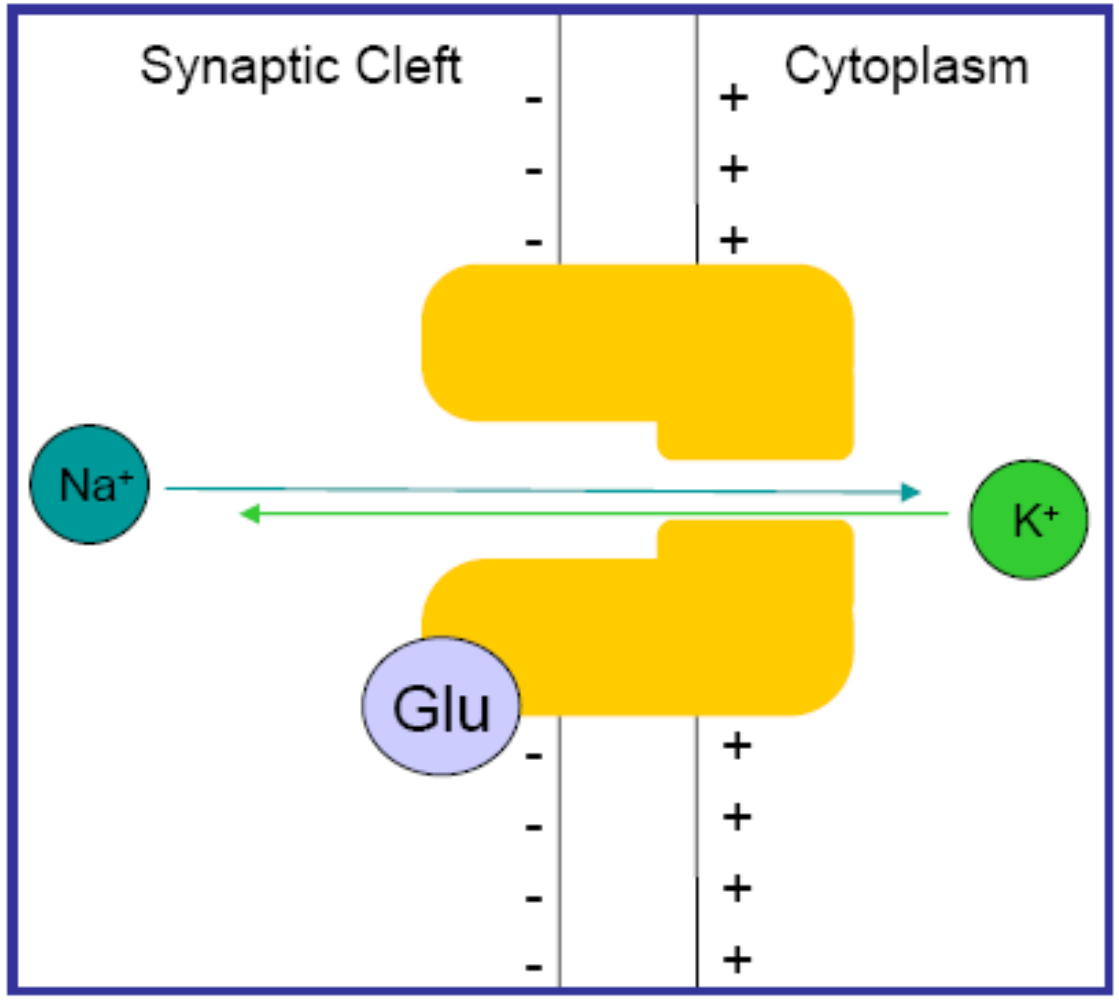
AMPA receptors



Neuro bio

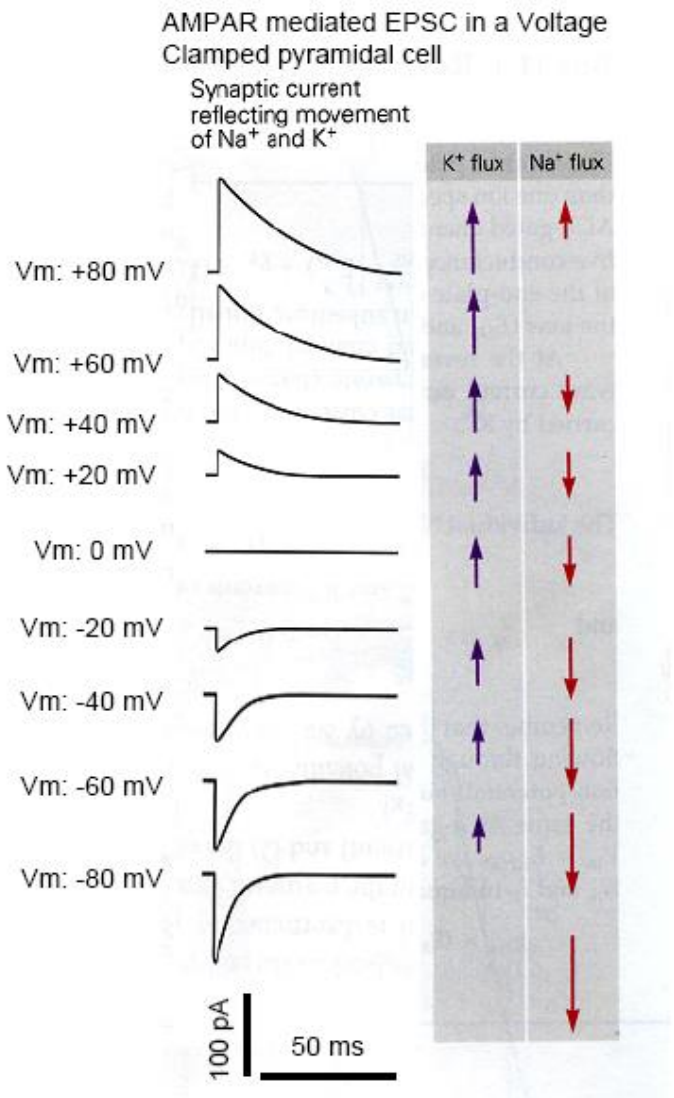
AMPA Receptors Conductance

The AMPAR opens a non-selective cationic conductance

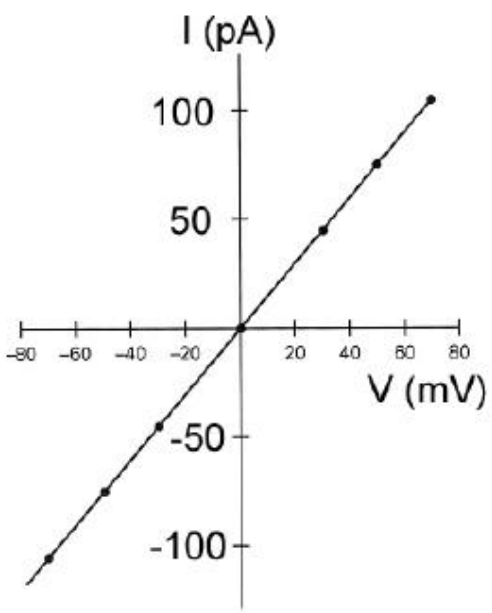


AMPA Receptors I-V curve

The Reversal Potential



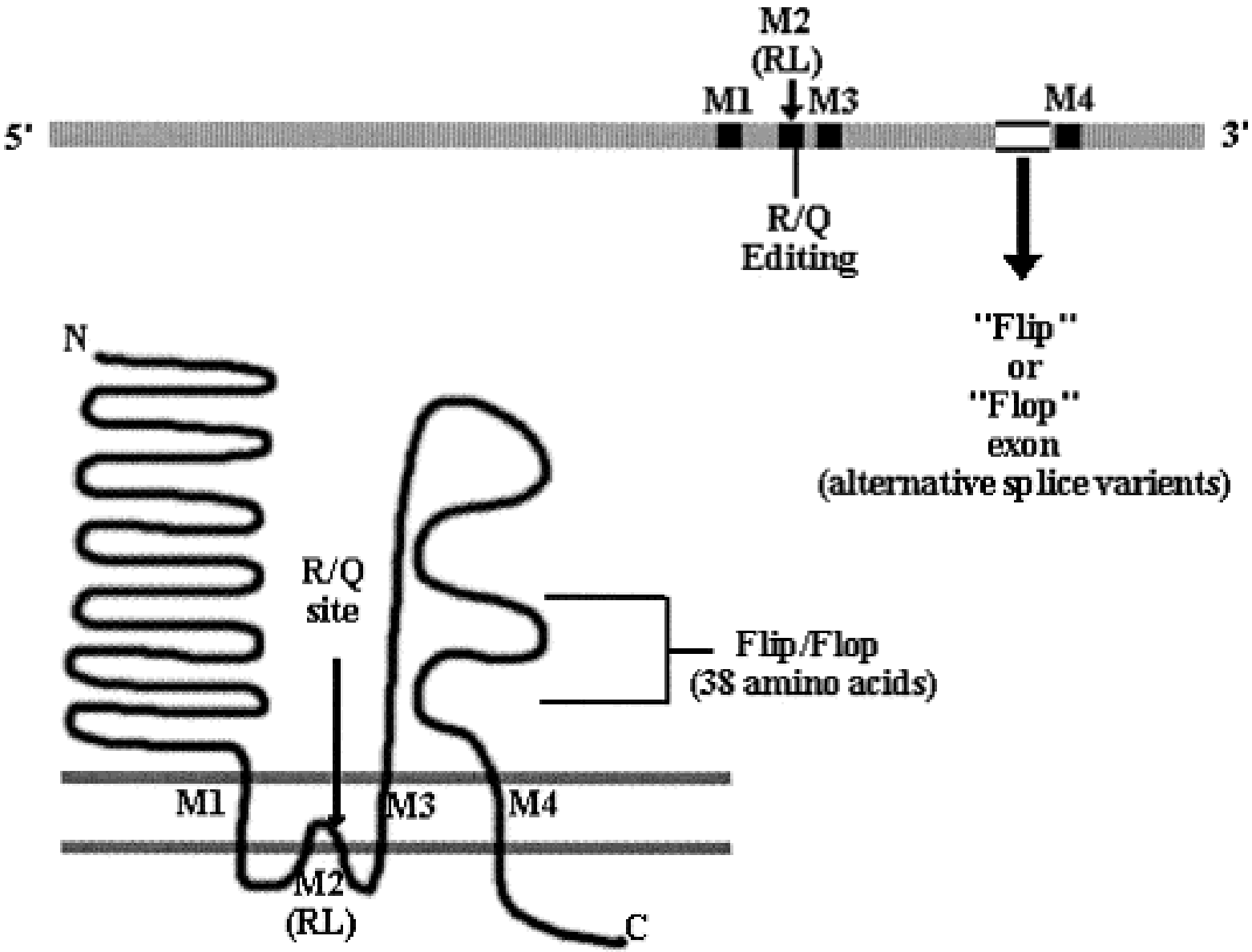
The I/V plot



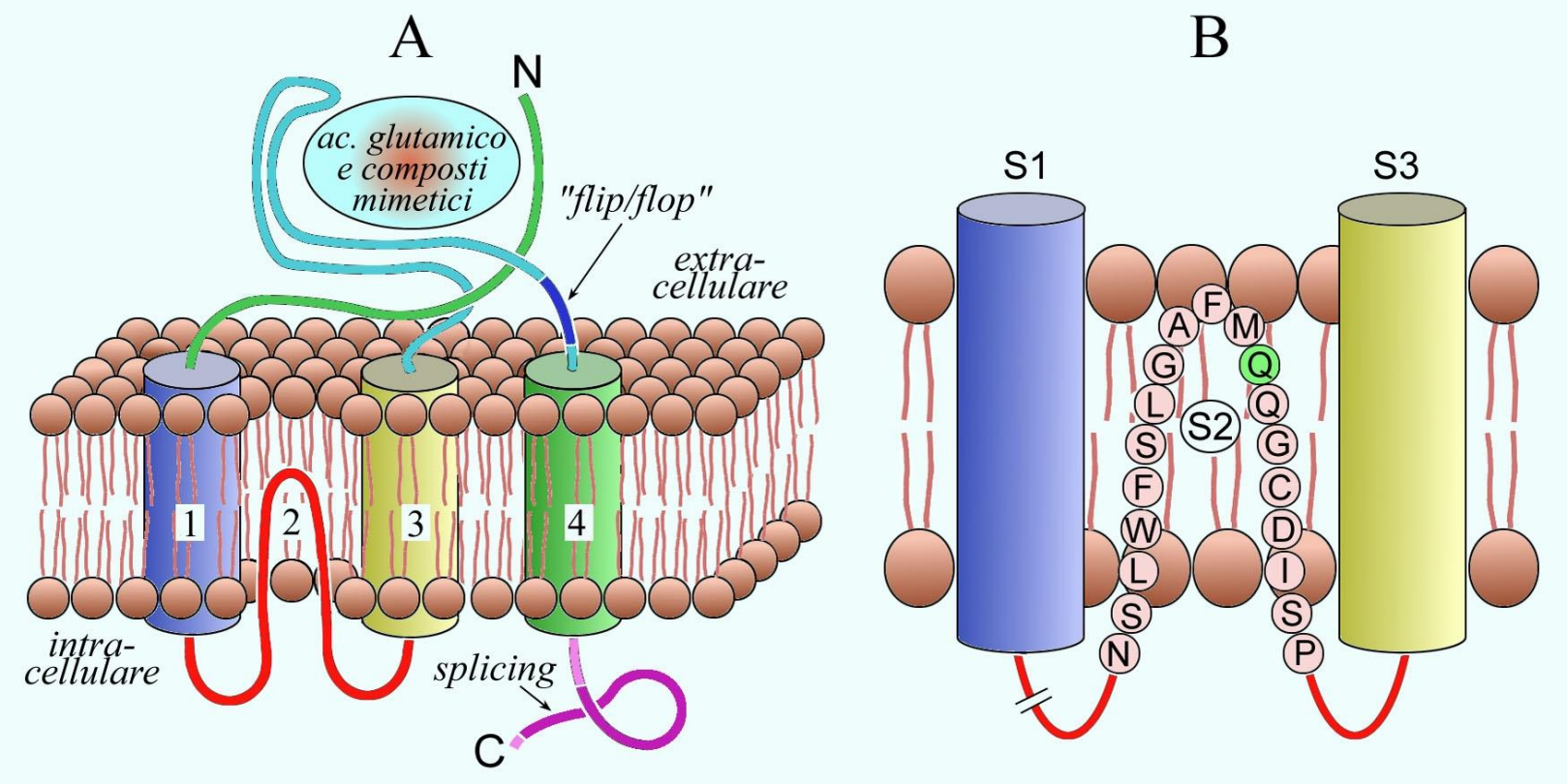
At negative potentials: I_{Na} inward > I_K outward

E_{rev}= 0 mV: @ 0 mV: I_{Na}= -I_K

AMPA receptor Splicing and editing

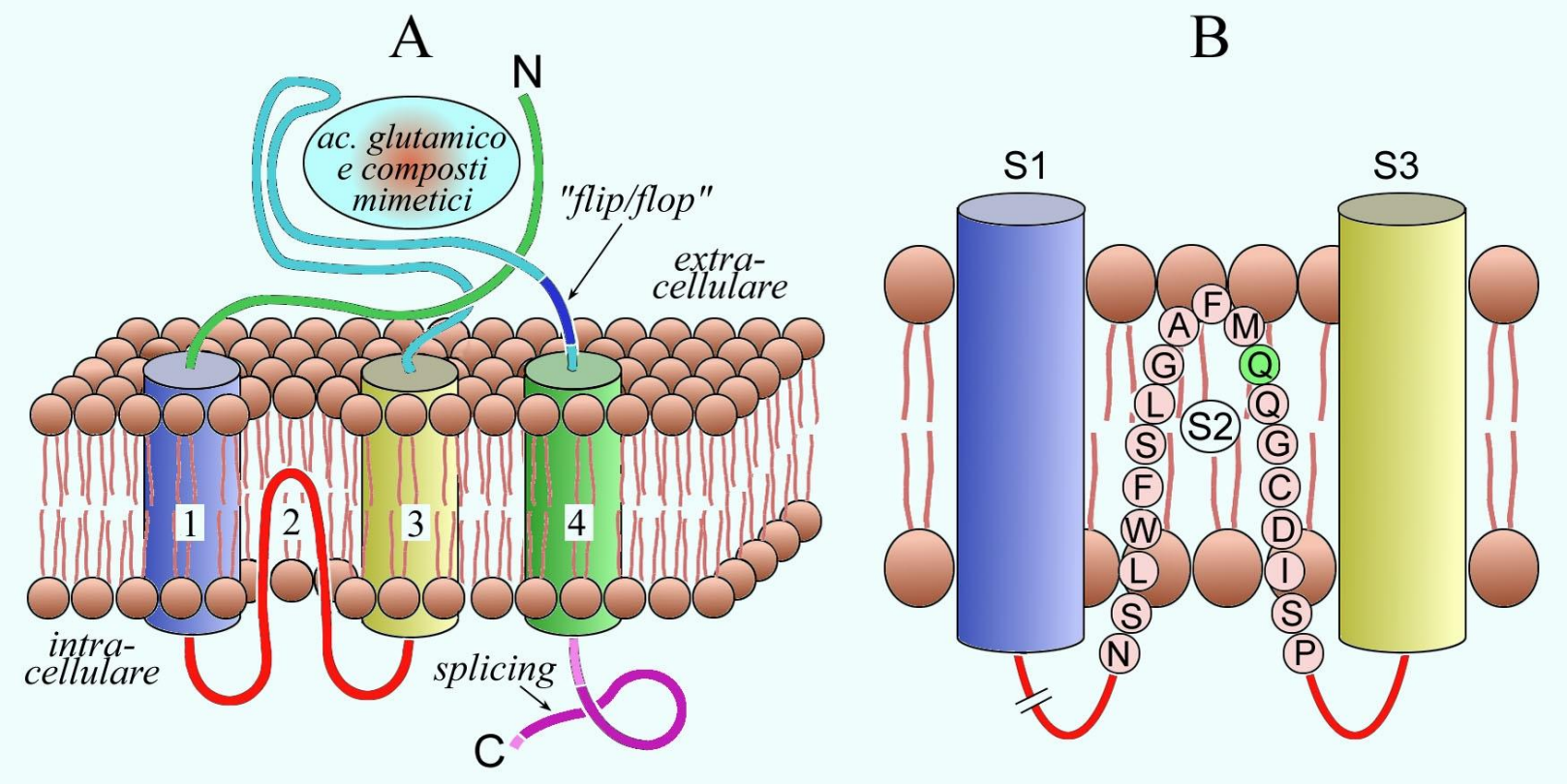


AMPA receptor Splicing



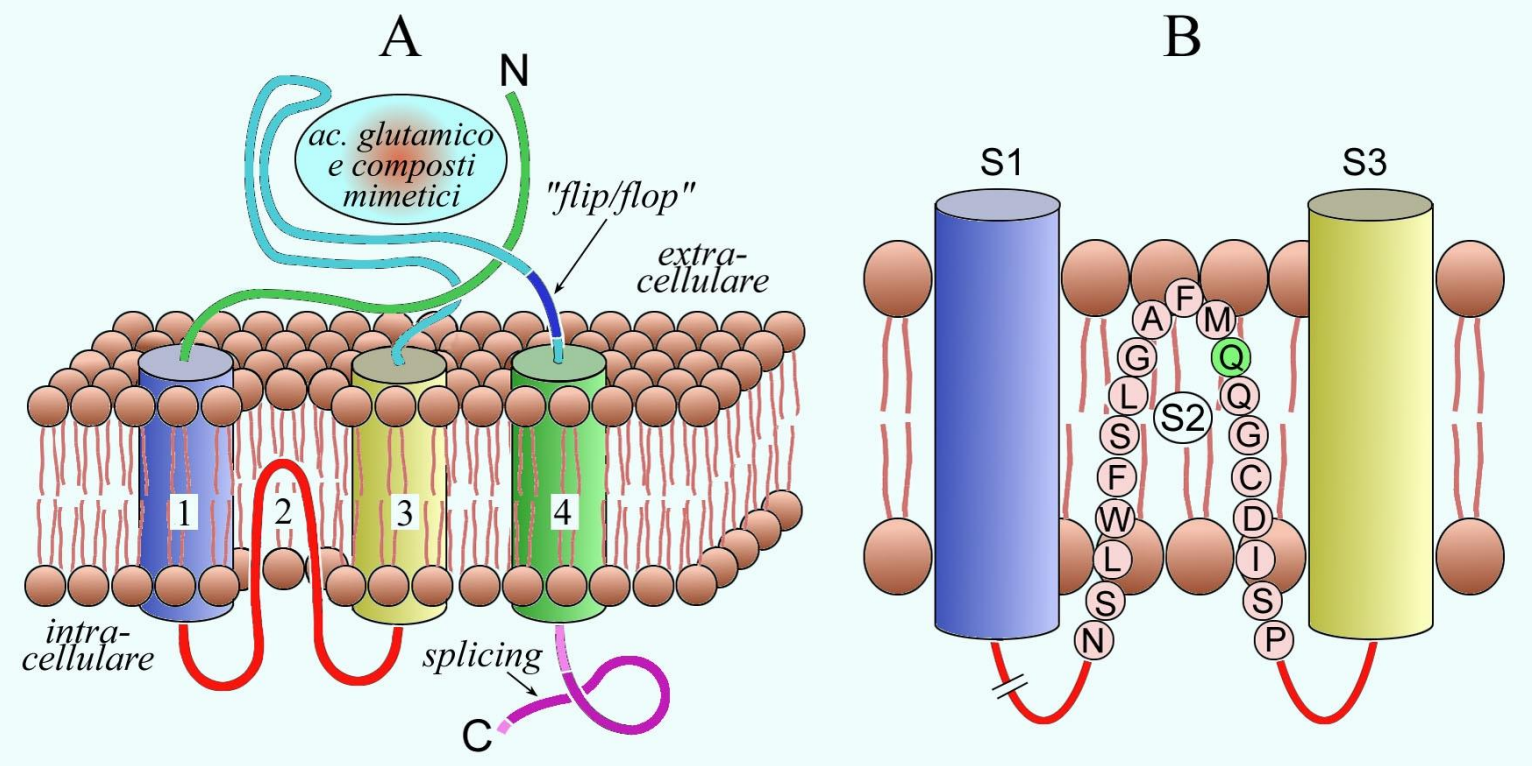
Splicing concerns mainly the C-terminal tract (purple) of polypeptide chains, which interact with cytoskeletal proteins; it is thought that different C-terminal sequences constitute as many "addresses" differentiated for the different types of iGluR.

AMPA receptor Splicing



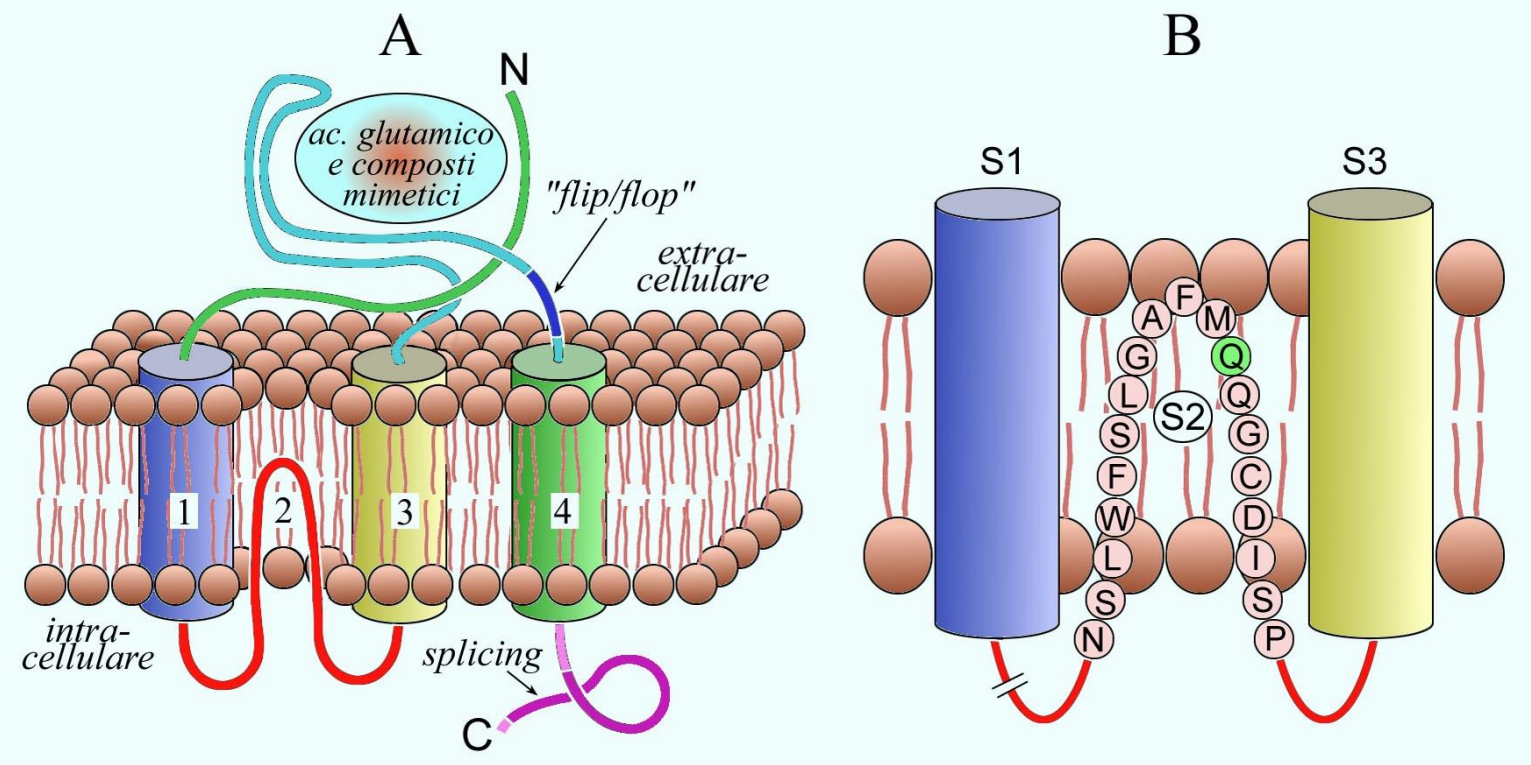
Another segment of the "spliced" (blue) chains is interposed between the STM S4 and the extracellular "ball". this segment (at least in the GluR1-GluR4 subunits of AMPA receptors) can occur in two variants, called "flip" and "flop", which give the receptor a very different kinetics of desensitization: very rapid (and the current is weaker) if the subunits are present in the "flop" version, slower (and the most intense currents) if they are present in the "flip" version.

AMPA Receptors editing



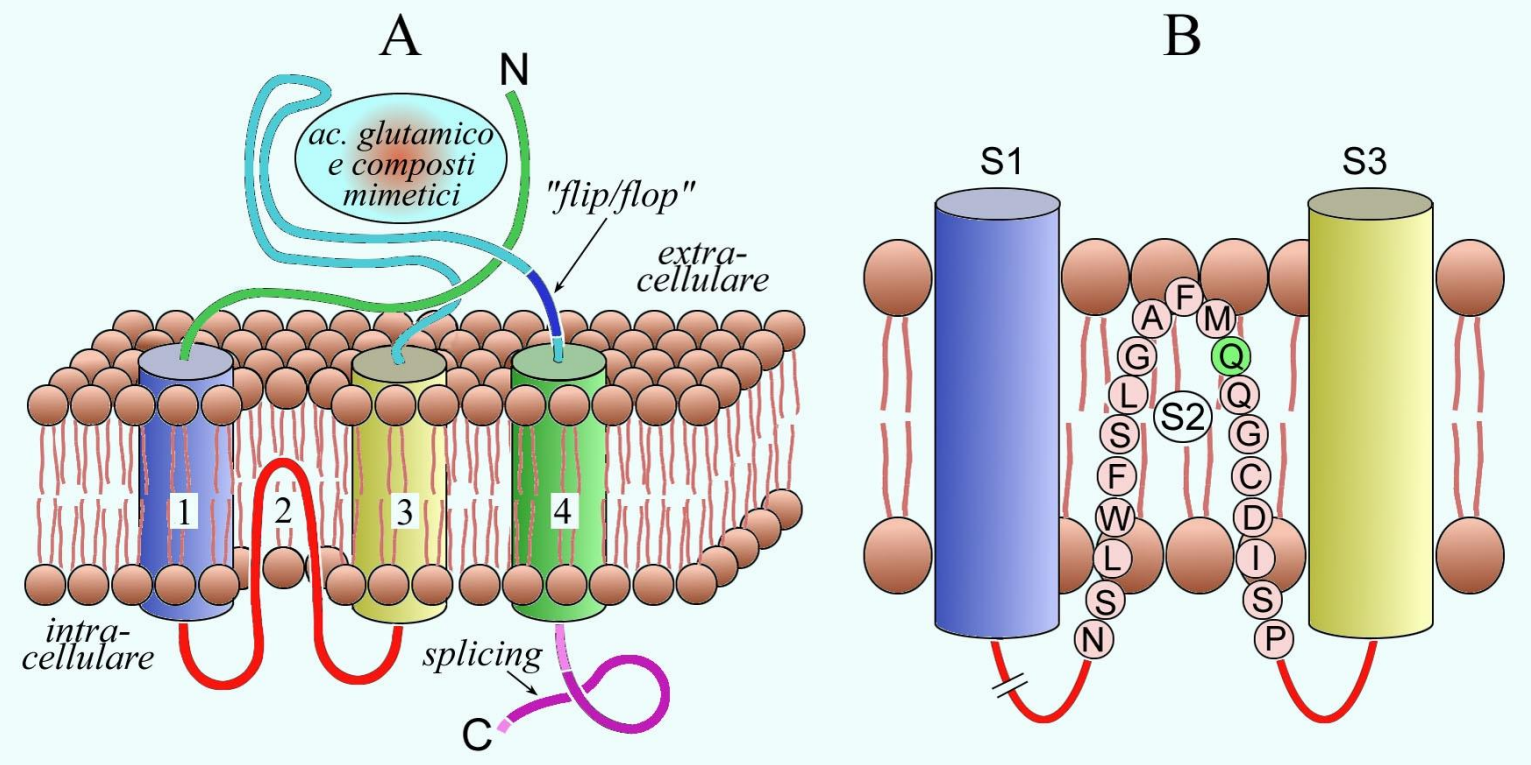
A further possibility of diversification in iGluR isoforms is the reorganization of mRNAs known as "editing", through which an element of the pre-mRNA nucleotide sequence can be modified enzymatically, thus changing the amino acid that will be encoded by the mature mRNA.

AMPA Receptors editing



An important "editing site" has been identified in the S2 section of many subunits (shown in green in B). In this, "site" may be a glutamine (Q, as specified by the "code" contained in the DNA), or an arginine (R), when the codon of mRNA for glutamine (CAG) is modified enzymatically to Arginine (CGG).

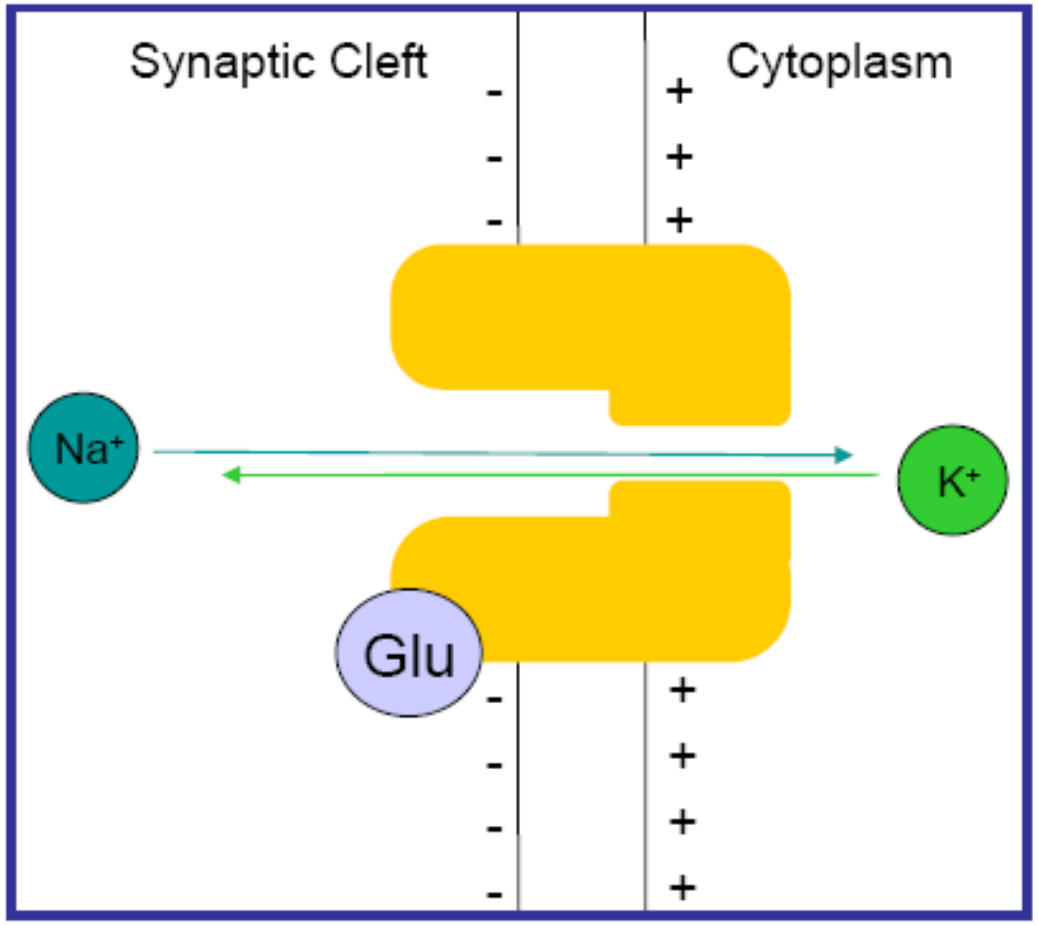
AMPA Receptors editing



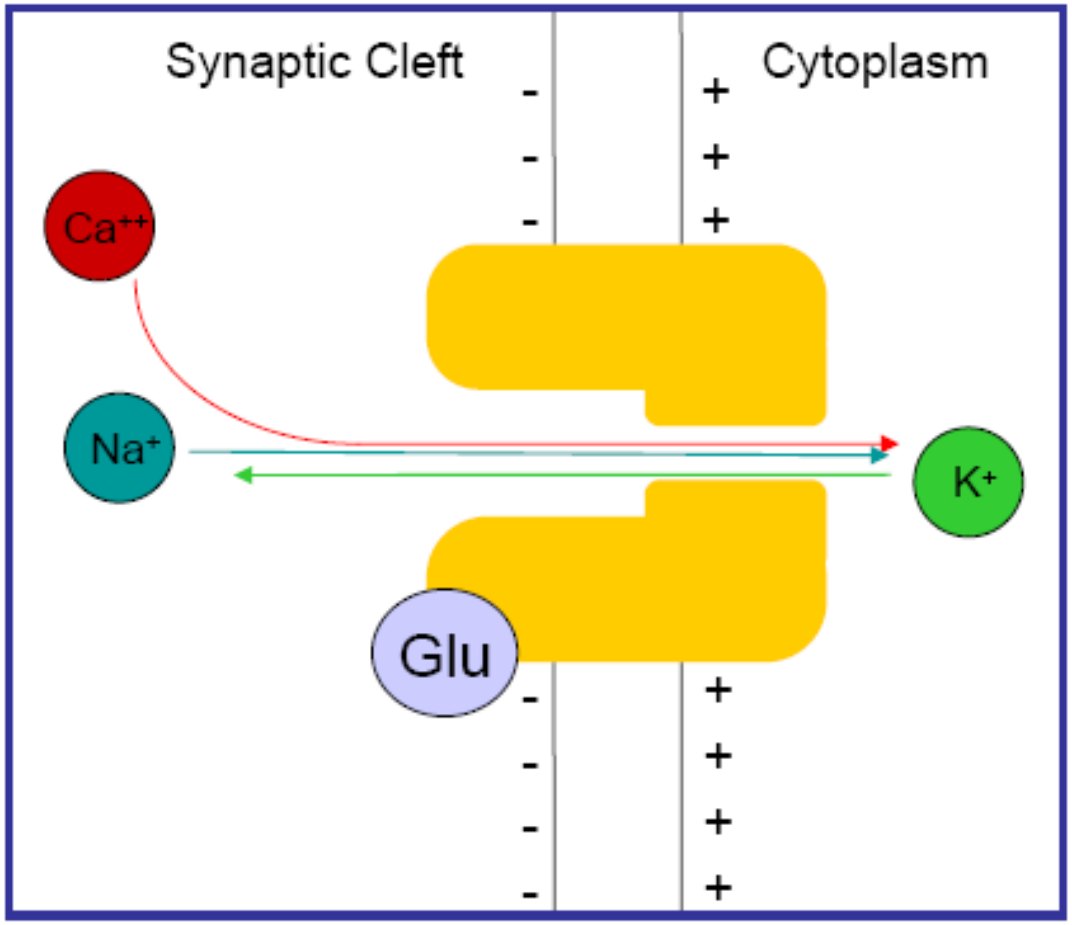
The functional interest for this "editing" comes from the fact that it influences the receptor-channel Ca2 + permeability: it has been observed that the same subunit contributes positively to the Ca2 + permeability of the whole molecular complex when the "site" is in the version "Q" (occupied by a glutamine), negatively when the "site" is in the "R" version (occupied by an arginine).

AMPA Receptors Ca^{2+} permeability

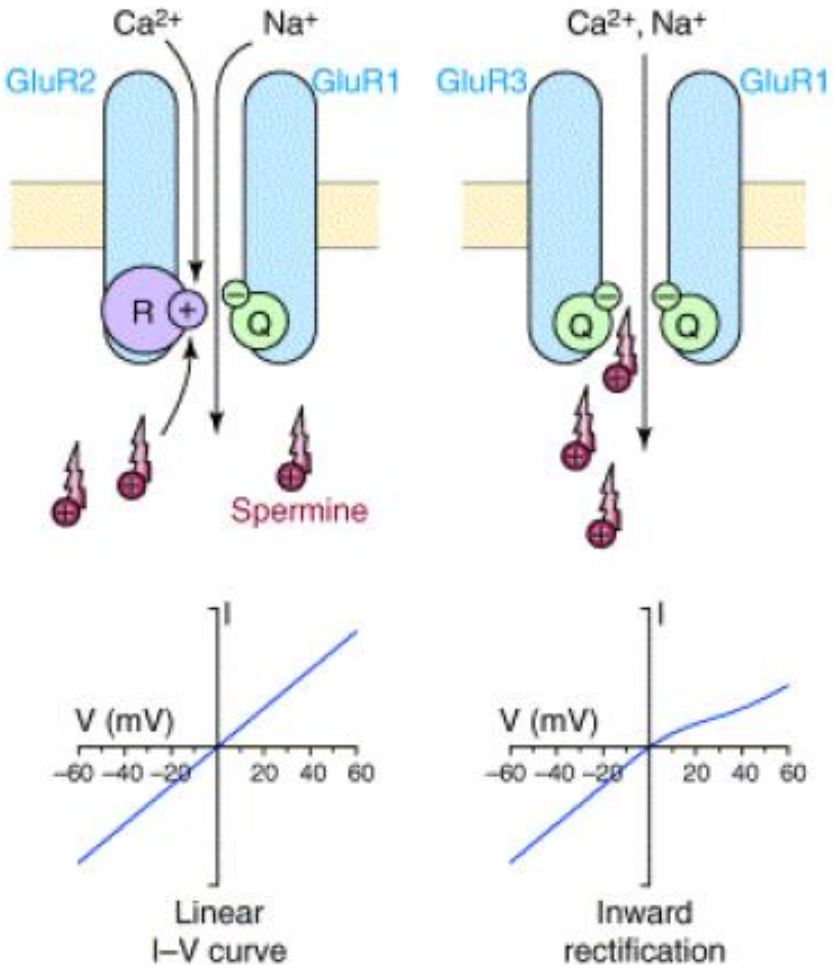
AMPAR containing the GLUR2
Subunit are permeable to K^+ and Na^+



AMPAR lacking the GLUR2
subunit are also permeable to Ca^{++}



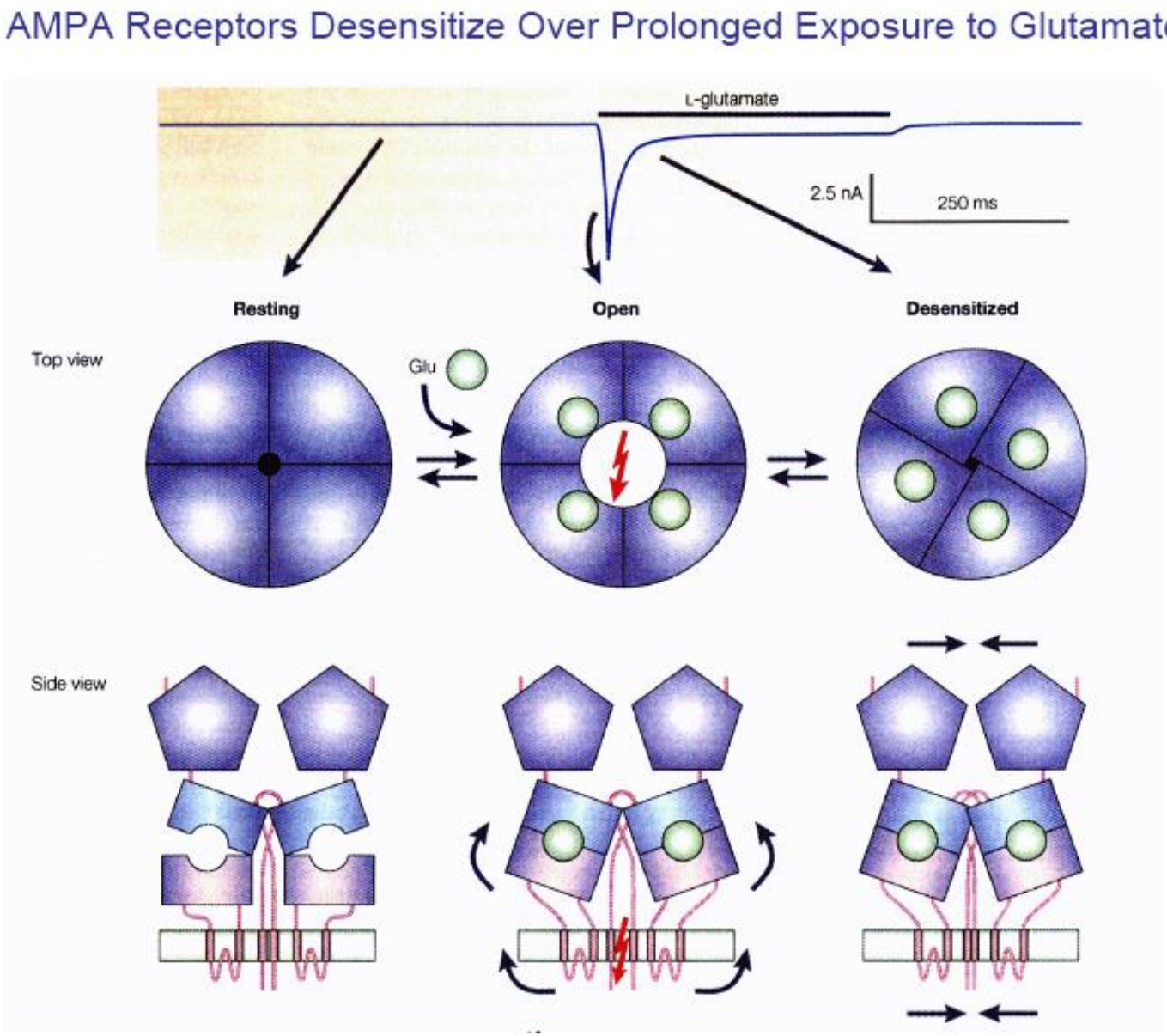
AMPA Receptors Ca^{2+} permeability



AMPAR containing the GLUR2 subunit have a linear I/V plot

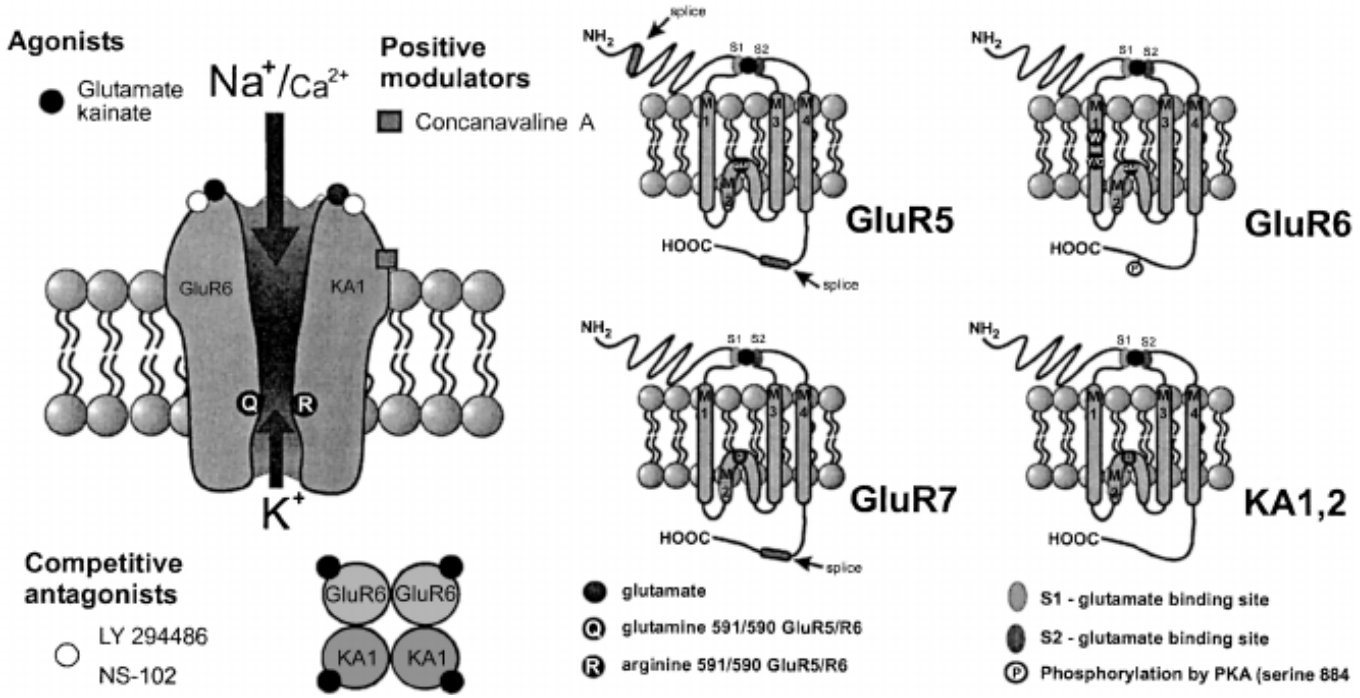
AMPAR lacking the GLUR2 subunit have a rectifying I/V plot

AMPA Receptors Ca^{2+} Desensitization



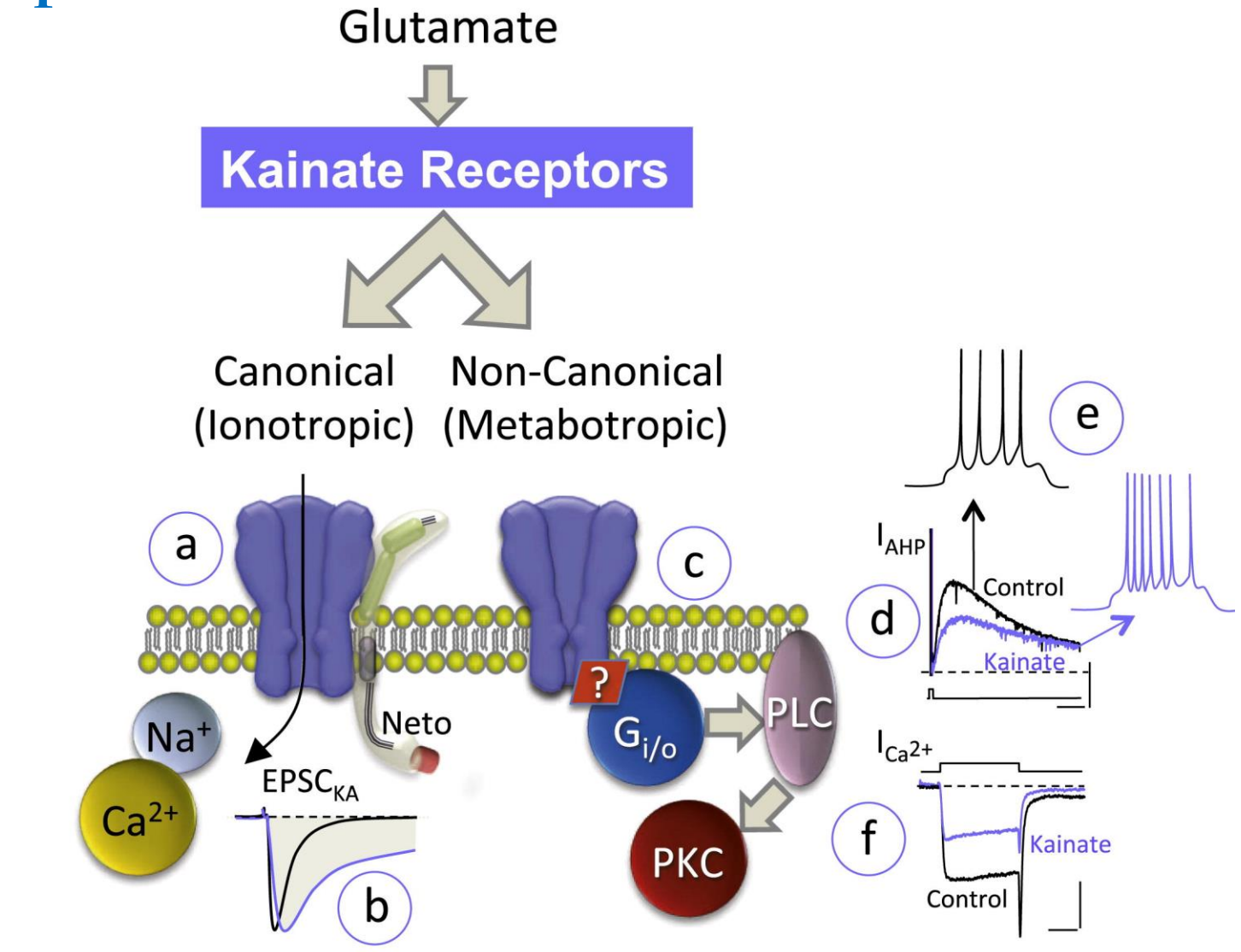
Kainate receptors

- KAR are widespread throughout the SNC (but less abundantreceptors for AMPA)
- KAR are permeable to Ca^{2+}
- Responsible for rapid synaptic transmission
- Possible presynaptic functions: control of the NT release



Non-Canonical Kainate Receptors (KARs)

Non-canonical KARs signal via G-protein-coupled (metabotropic) and intracellular pathways, not just ion flux.



Non-Canonical Kainate Receptors (KARs)

Key Non-Canonical Mechanisms

•Presynaptic Modulation: KARs regulate neurotransmitter release:

- **Facilitate or depress glutamate/GABA release depending on context and agonist concentration.**
- **Can act via G-protein, PKA, or PKC pathways—independently of ion channel activity.**

•Channel-Independent Signaling:

- **Increase intracellular Ca^{2+} via G-protein or distinct metabotropic-like mechanisms.**
- **Modulate release without opening ion channels.**

•PKC/PKA Pathways:

- **Engage second messenger cascades, influencing synaptic function and plasticity.**

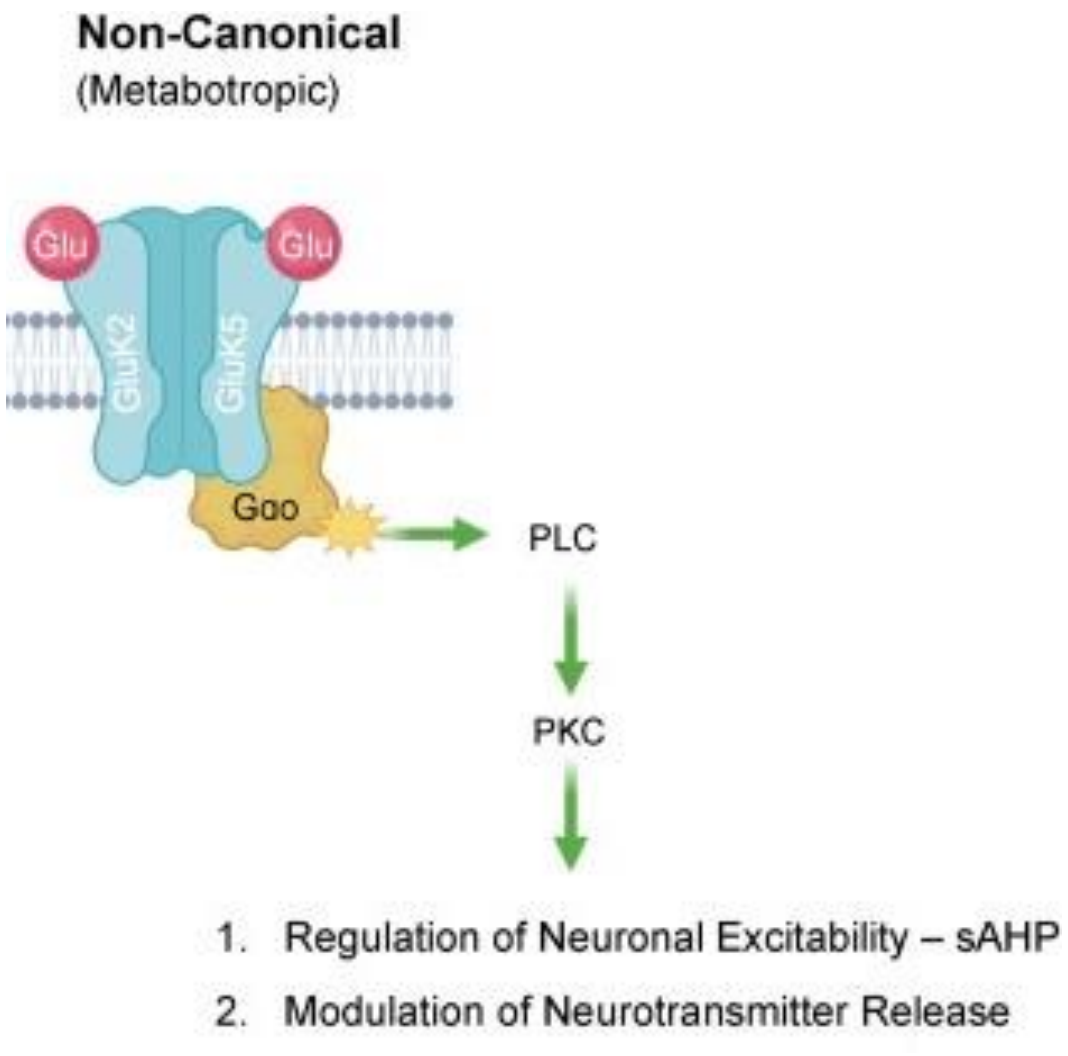
Relevance to Disease

•Epilepsy

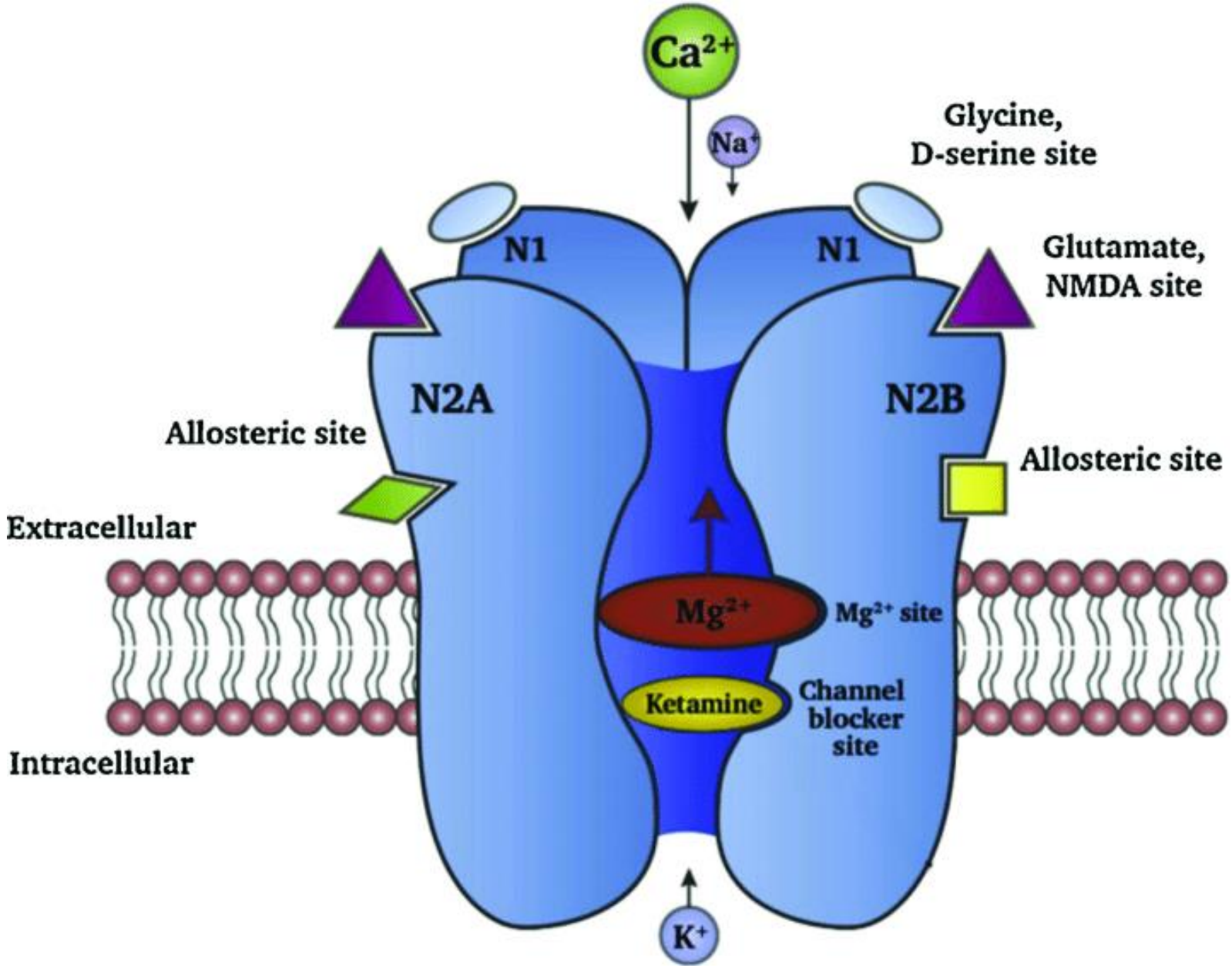
•Neurodegenerative diseases (e.g., ALS, Huntington's)

•Oligodendrocyte/myelin injury

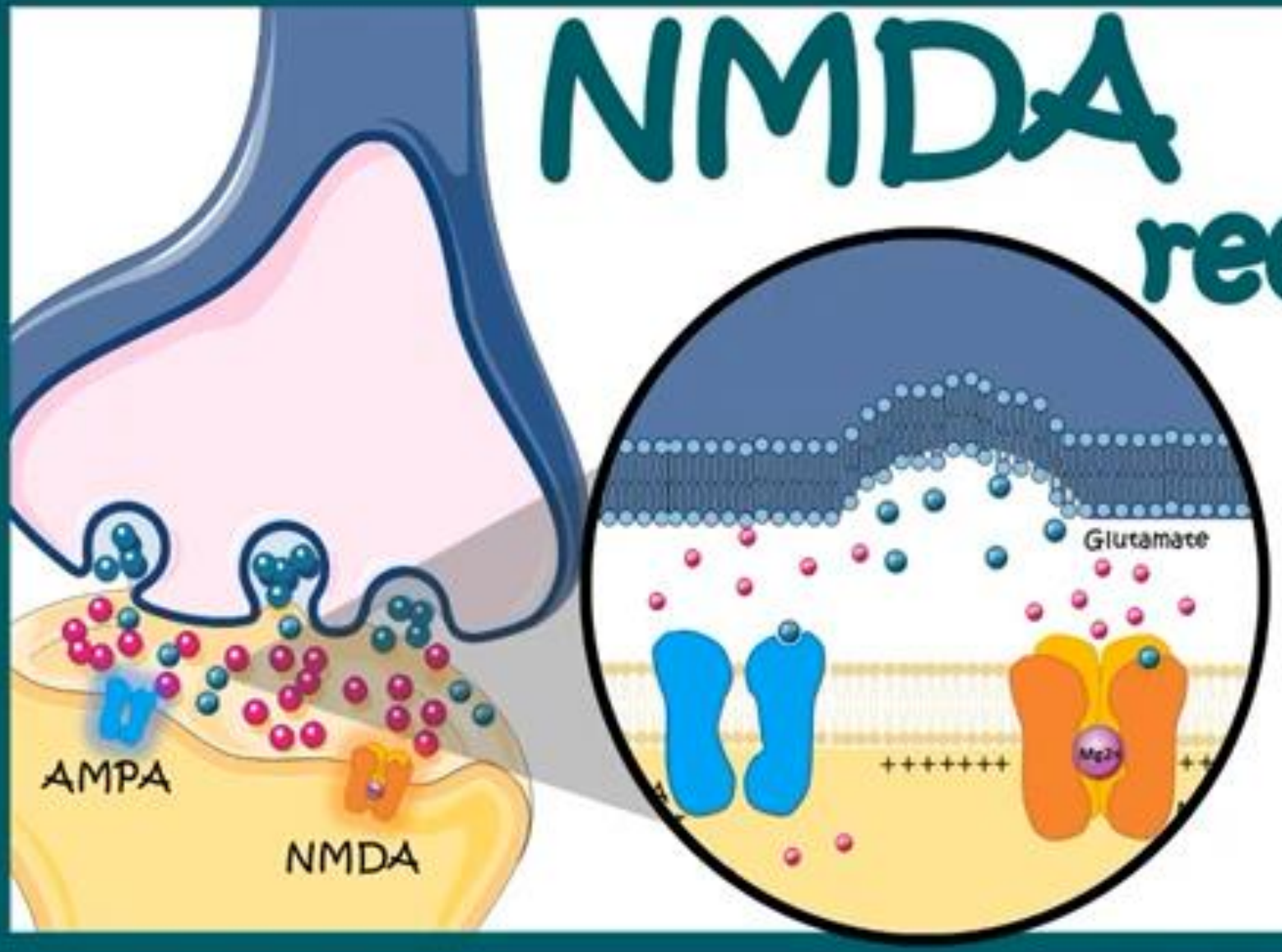
•Target for therapeutics distinct from AMPA/NMDA receptor modulators



NMDA Receptors

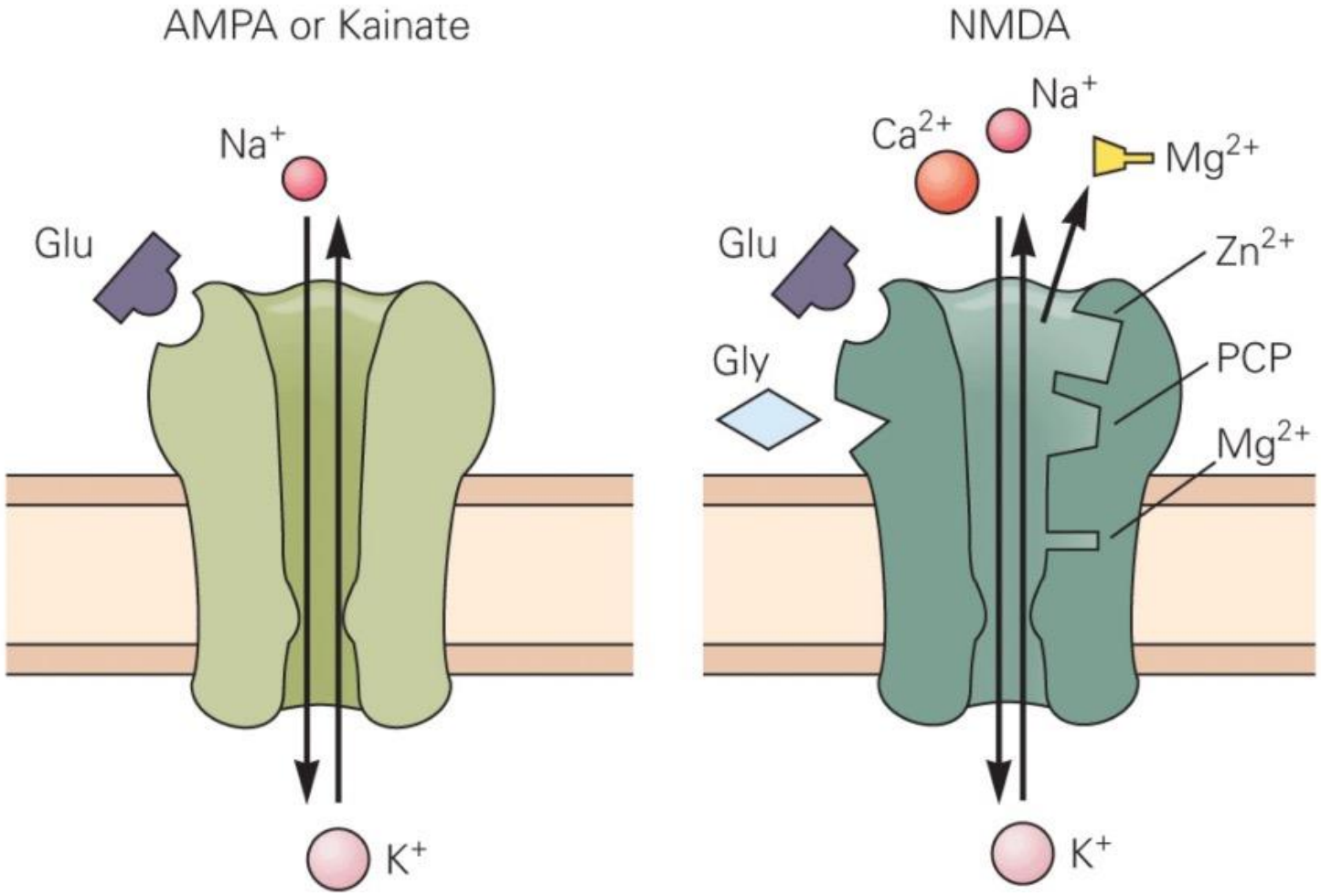


NMDA receptors



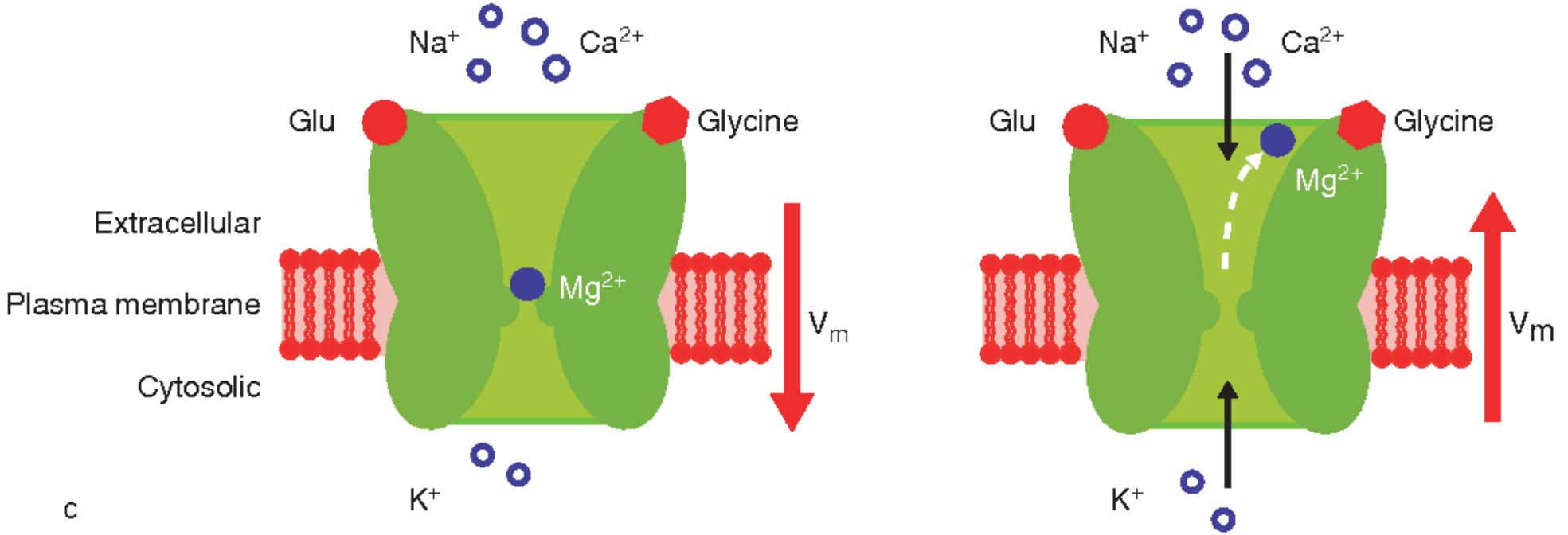
Neuro bio

NMDA Receptors



NMDAR display in their extracellular domains numerous binding sites for various agents that condition their permeability, such as Zn2+ ions and polyamines (spermine and spermidine). Curiously, the functioning of NMDA receptors requires the presence in the extracellular medium of glycine, which in other synapses acts as an inhibitory neurotransmitter.

NMDA Receptors are also Voltage Dependence



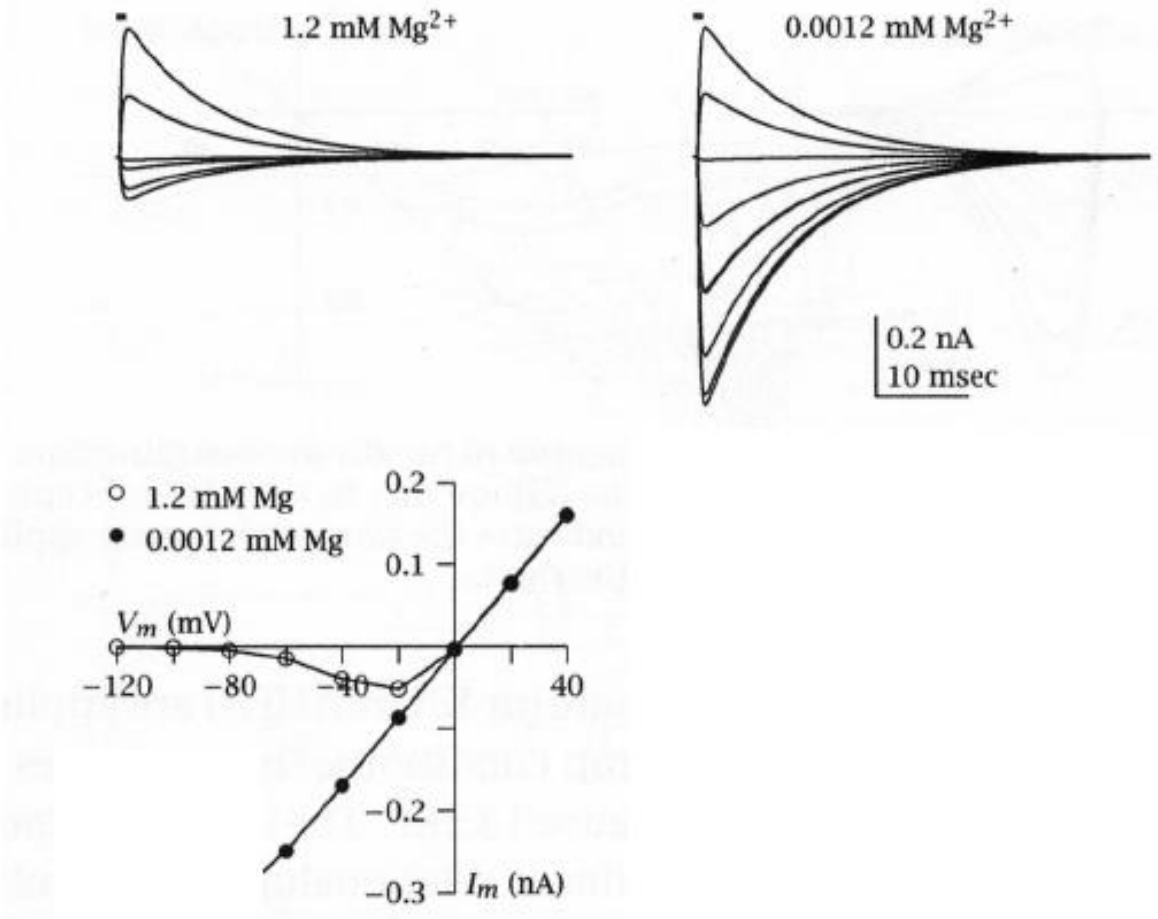
e 3 Magnesium block of NMDA receptors. (a) Voltage dependence of glutamate-induced currents in Mg^{2+}-free (circles

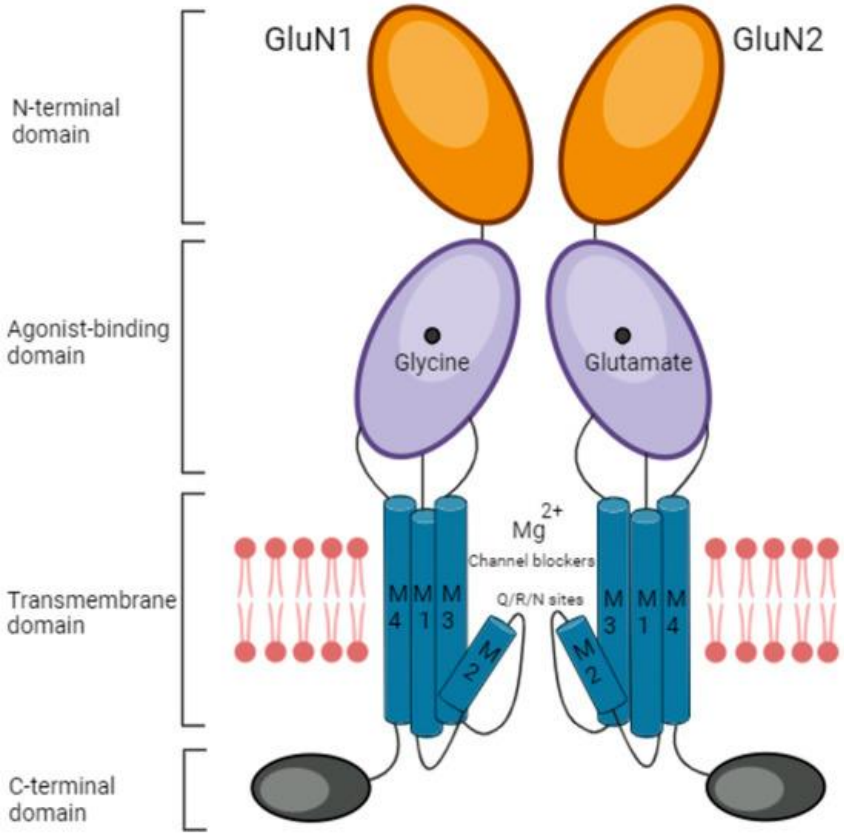
NMDAR have a considerable voltage-dependence: a unique property among all ionotropic receptors.

This voltage-dependence is interpreted as a voltage-dependent block of extracellular Mg^{2+}, which recalls the voltage-dependent block of the intracellular Mg^{2+} of the inward rectifiers.

NMDA Receptors are also Voltage Dependence

NMDA receptors are blocked by external Mg^{2+} in a voltage dependent manner



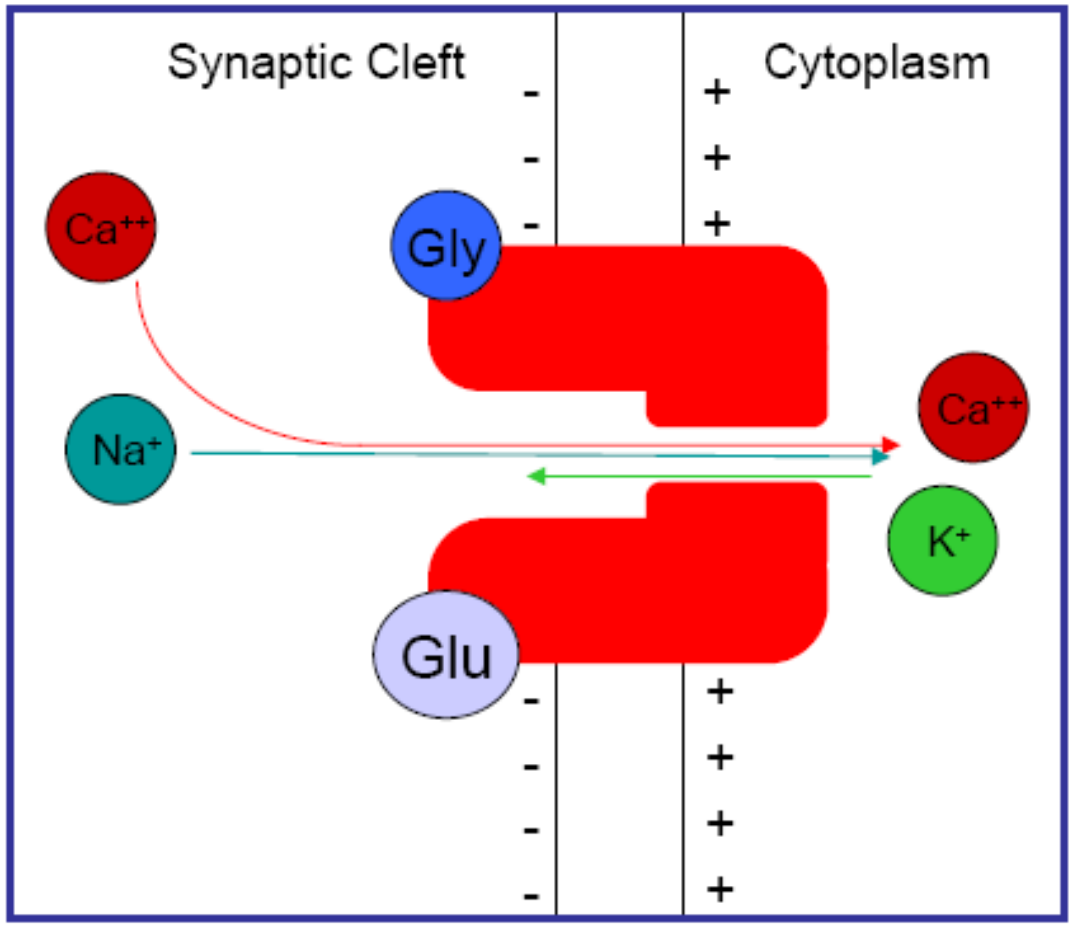


(b)

NMDA Receptors Permeability

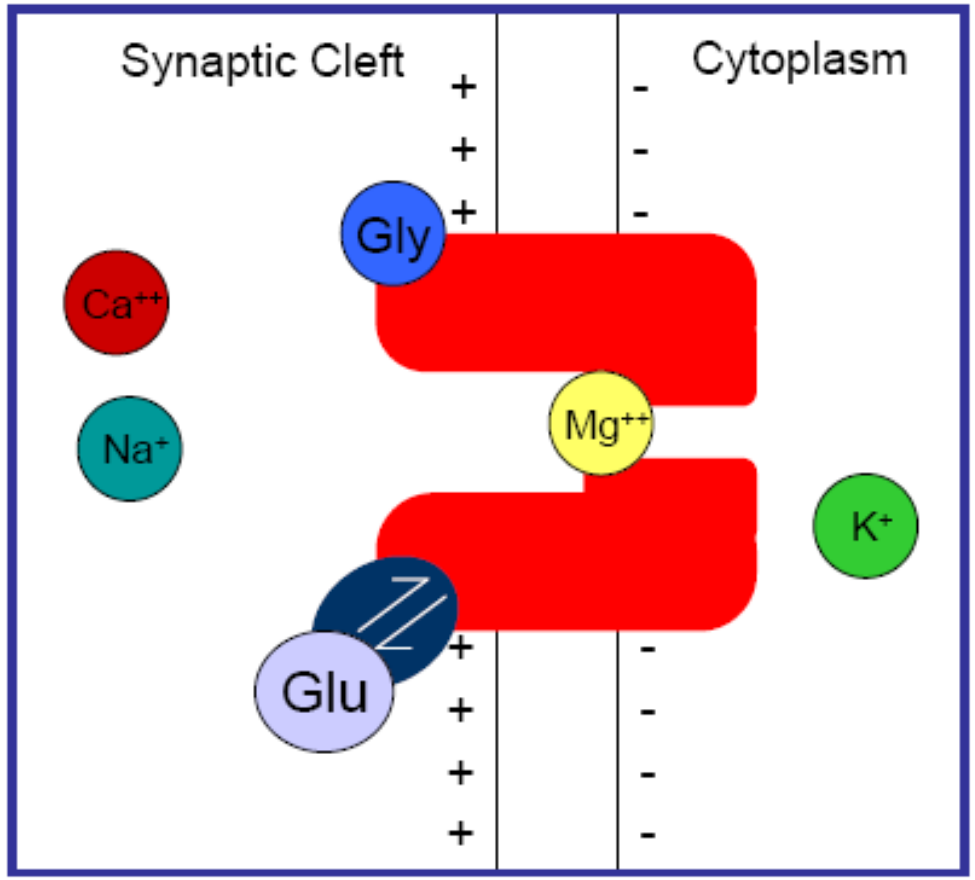
NMDARs are permeable to K+, Na+ and Ca++

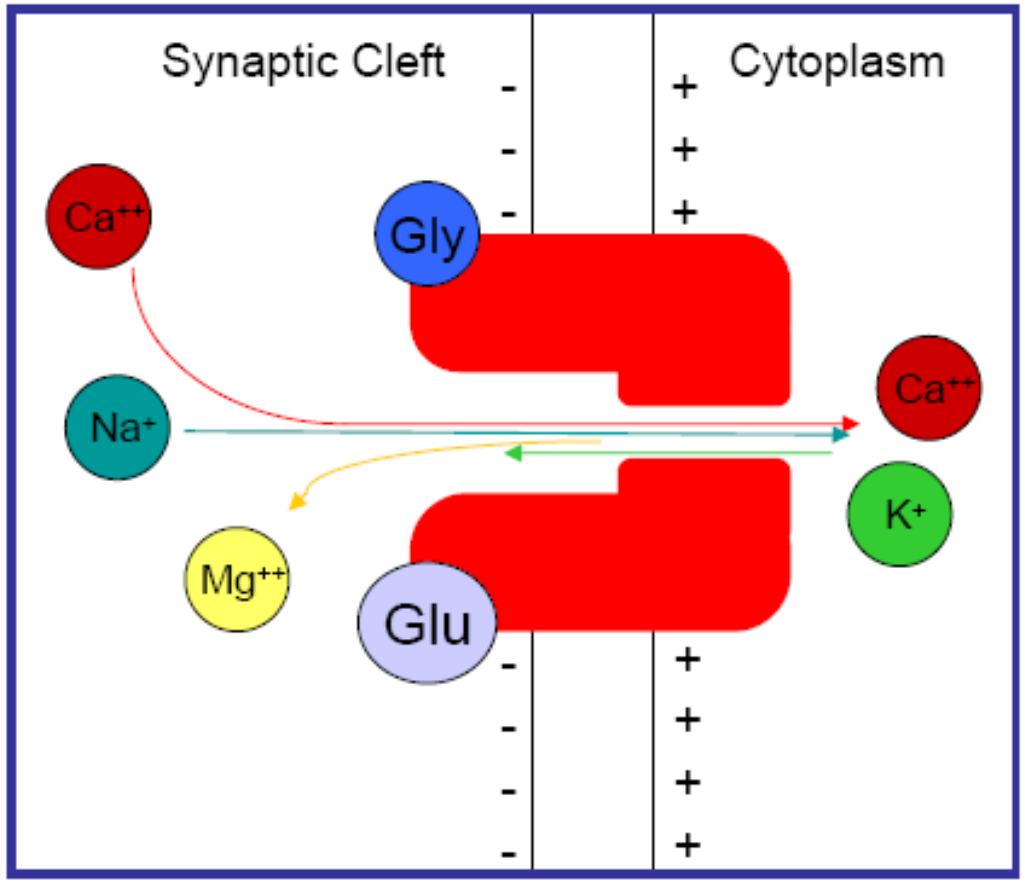
NMDARs has to bind to both glutamate and glycine in order to open



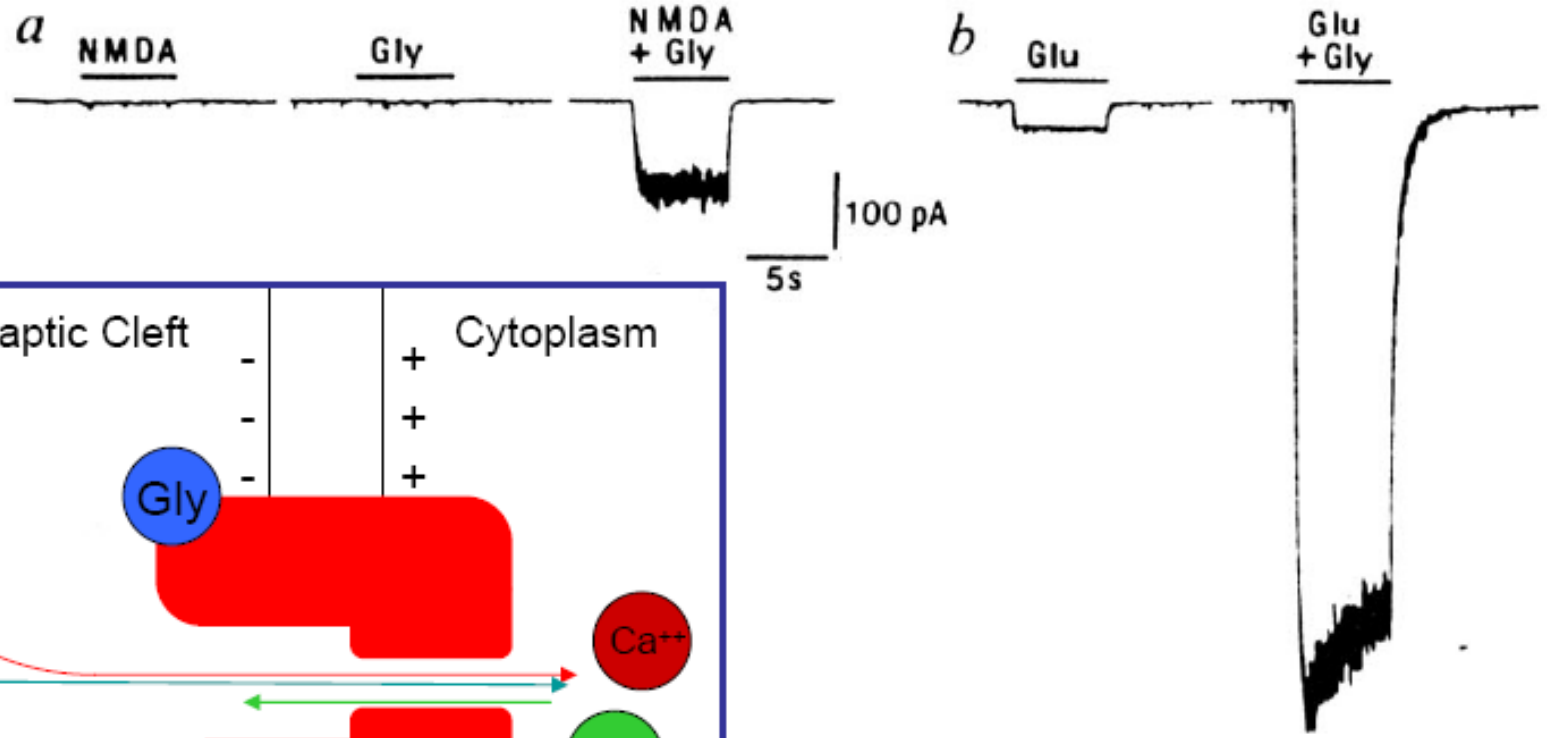
NMDA Receptors

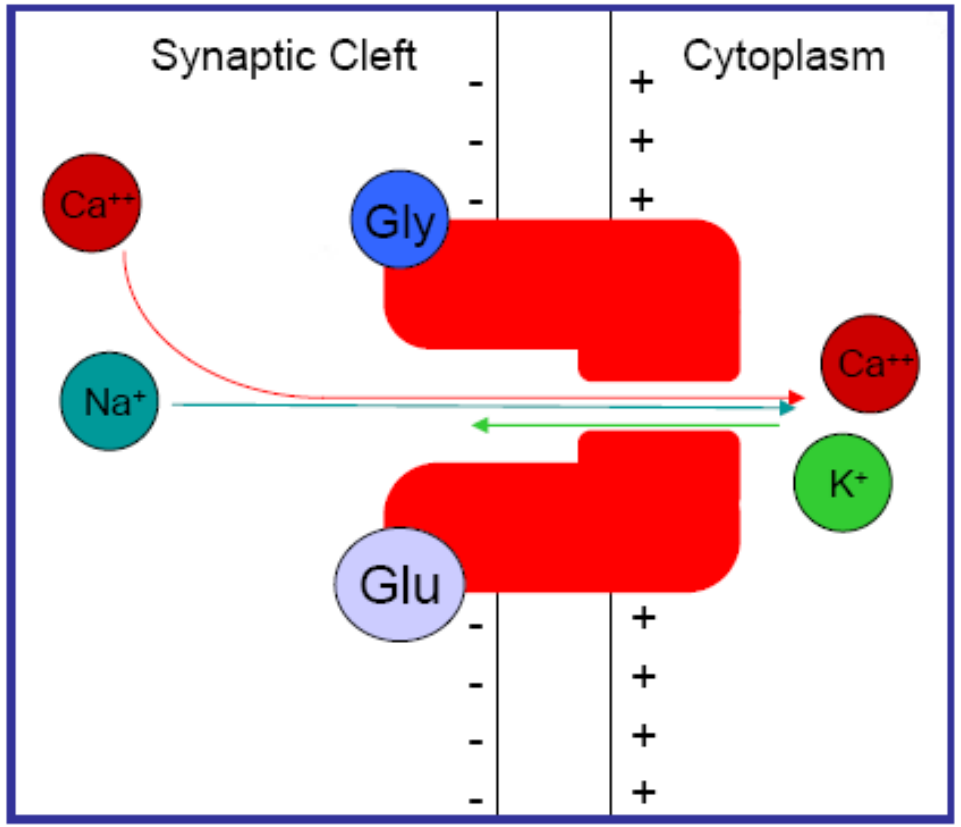
At negative potentials current through NMDARs is blocked by Mg^{++} (voltage dependent block)





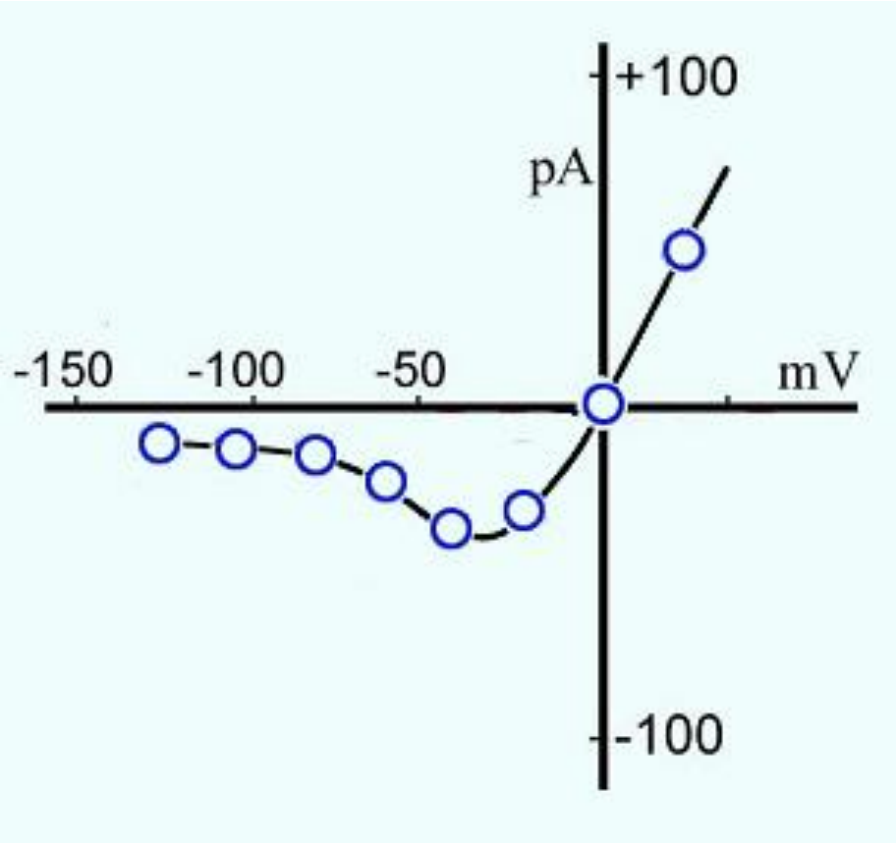
NMDA Receptors Activation Requires a Co-Agonist (L-Glycine or D-Serine)





L-Glycine (and D-Serine) bind with very high affinity to the NMDA receptor (to the the NR1 subunit).
Normally, the "ambient" concentration of these molecules in the cerebrospinal fluid is sufficiently high for them to occupy their binding site on the NMDA receptor.

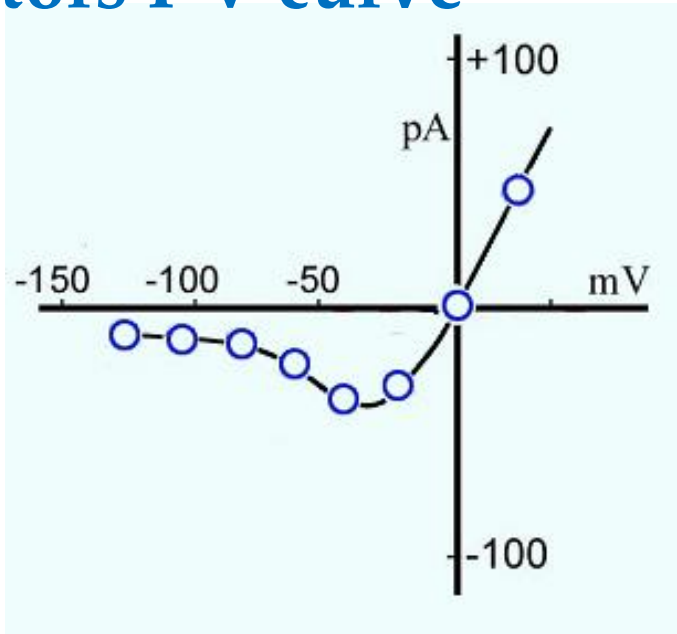
NMDA Receptors I-V curve



Considering also their molecular structure, they look like some "inward rectifiers" upside down !!

The voltage-dependence of the NMDA currents is interpreted as follows: at the resting potential, each channel is obstructed by an Mg2 + ion of extracellular origin so that, even if the receptor is activated by the AC. When glutamic acid binds to the channel, it goes into the "open" state, and it does not conduct any current (due to the "plug").

NMDA Receptors I-V curve

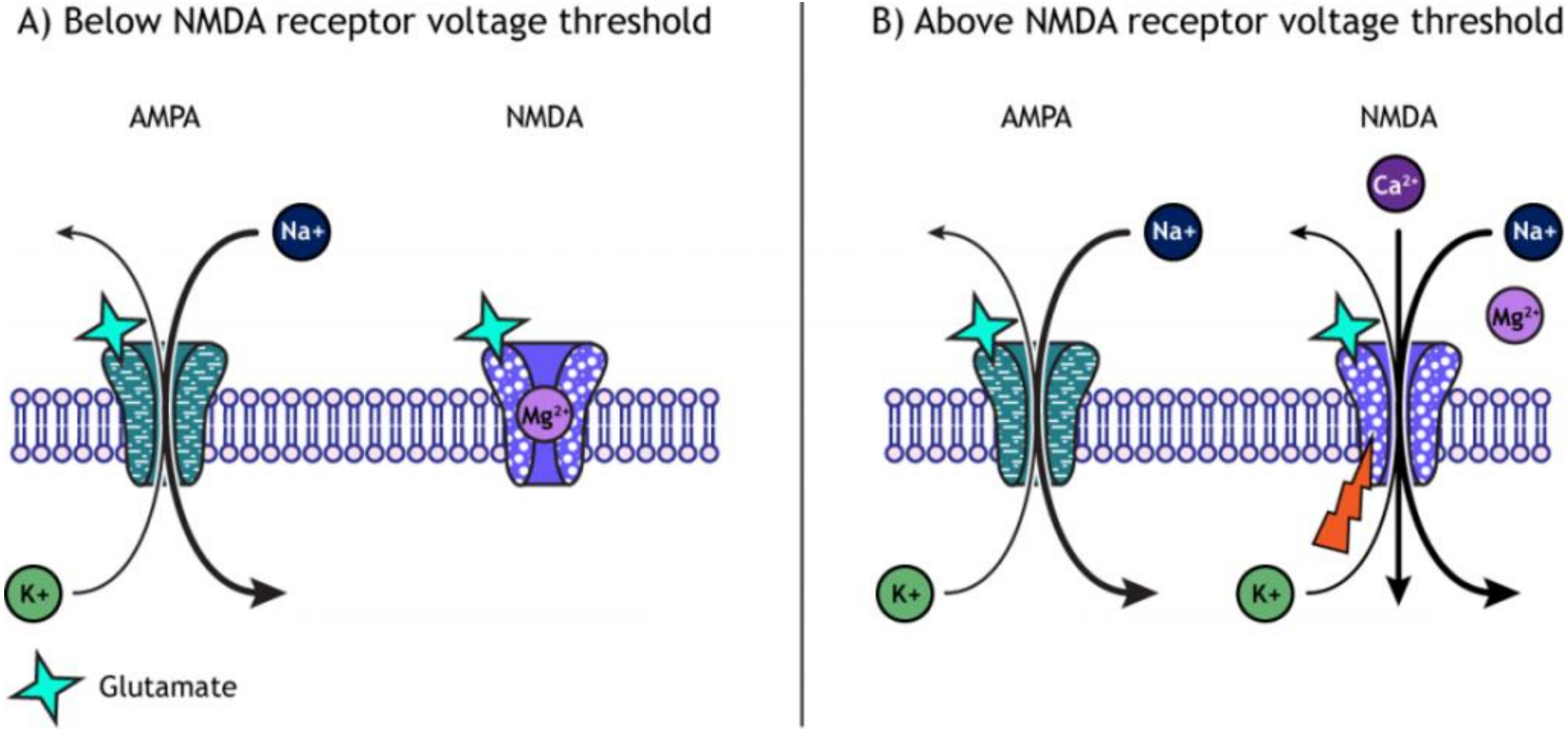


In order for the NMDA channel receptors to produce an EPSP in the postsynaptic membrane, the voltage-dependent plugs must be expelled by electrostatic repulsion, which requires the membrane to be depolarized.

When synapses express both types of iGluR, the depolarization necessary to remove the blockade of Mg2 + of the NMDA receptors is produced (when the Glu release occurs) from the primary activation of non-NMDA receptors..

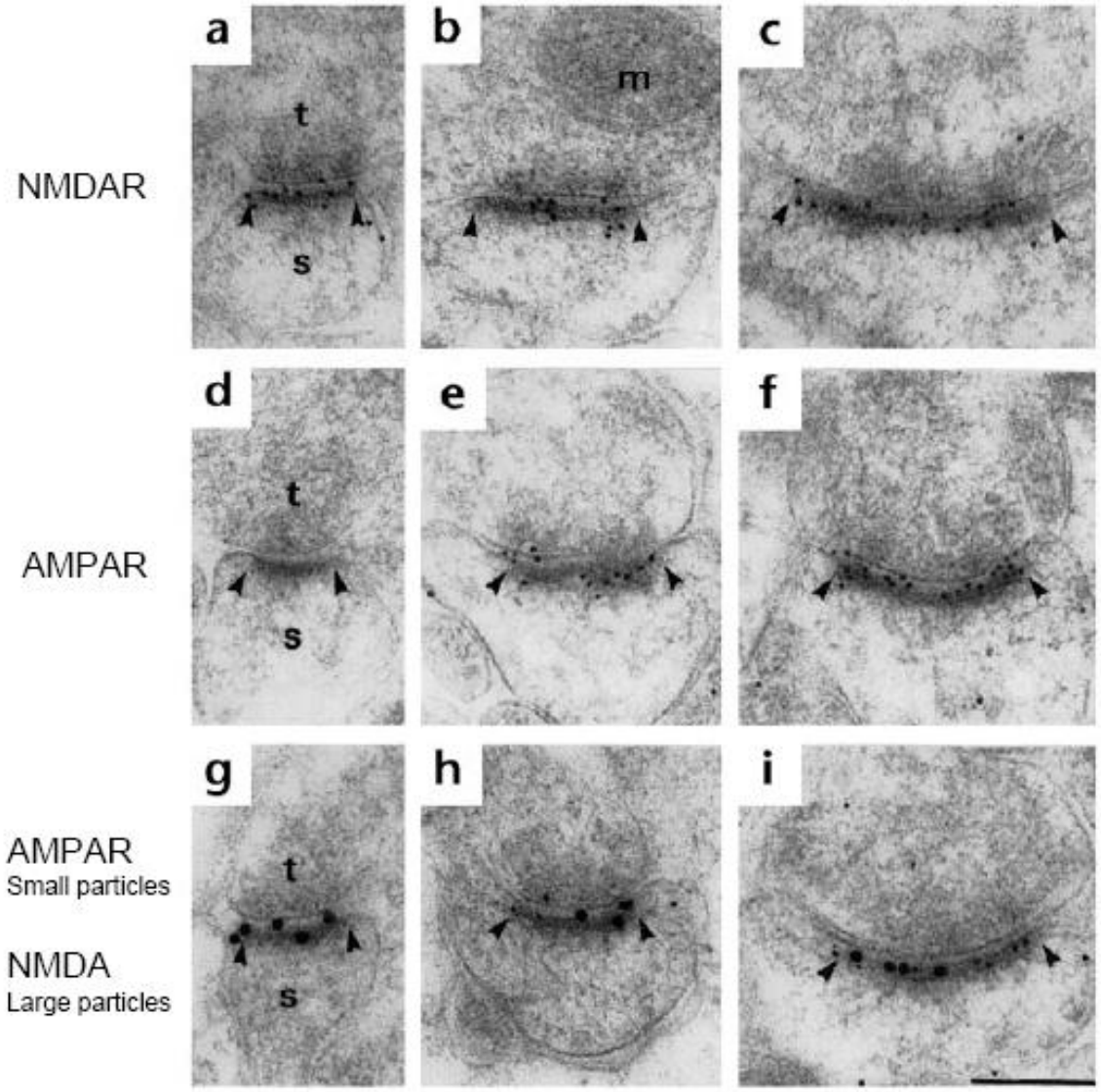
It is evident that, for small depolarizations of postsynaptic membrane, the contribution of NMDA receptors to the overall PPS will be null: it will become significant (and there will be a Ca2+ input) only if the depolarization (generally produced by the activation of non-NMDA receptors) will be sufficient to extrude the Mg2+ ions.

Can NMDA Receptors work in physiological conditions?



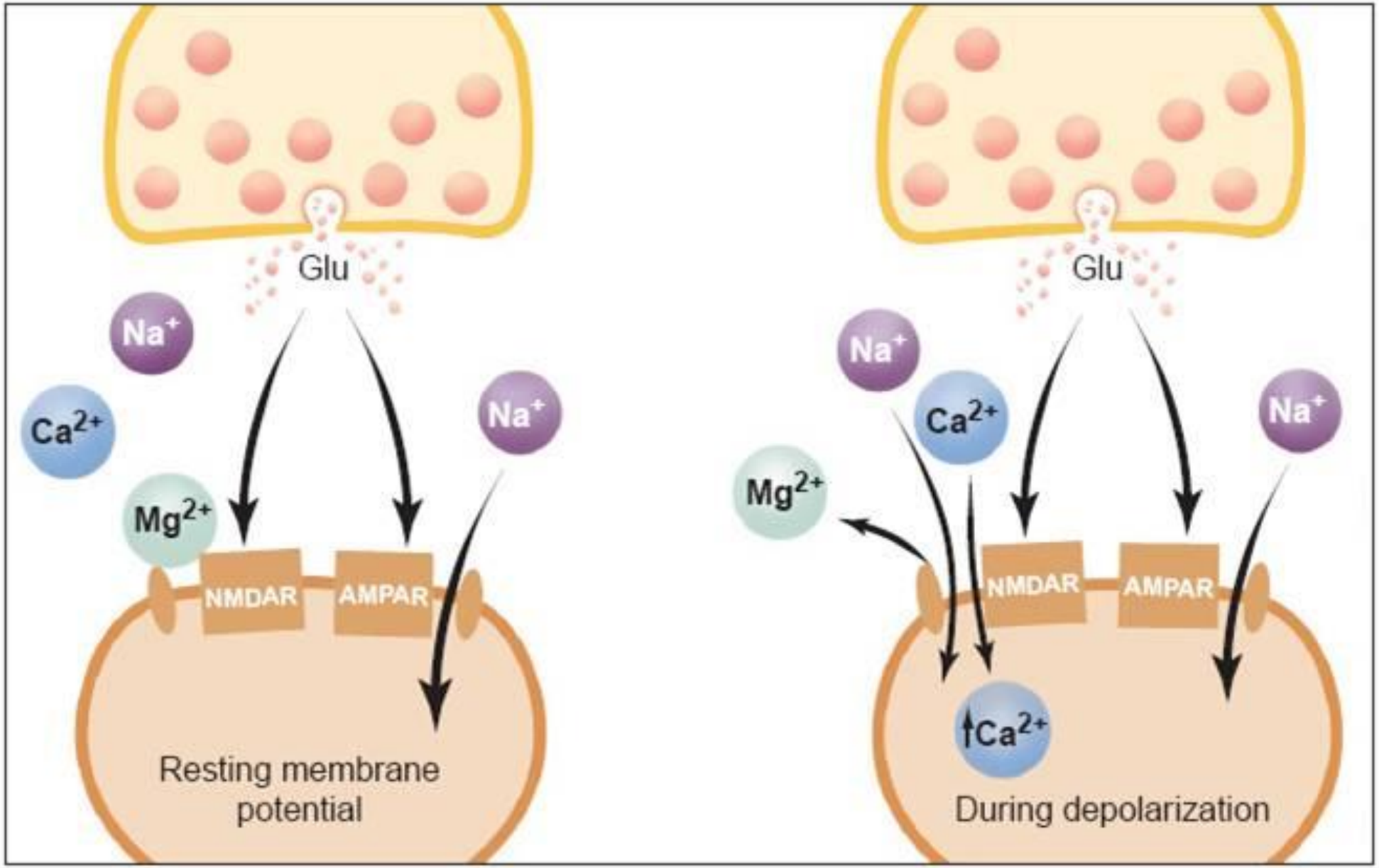
Can NMDA Receptors work in physiological conditions?

AMPA and NMDA receptors are localized at the same synapses

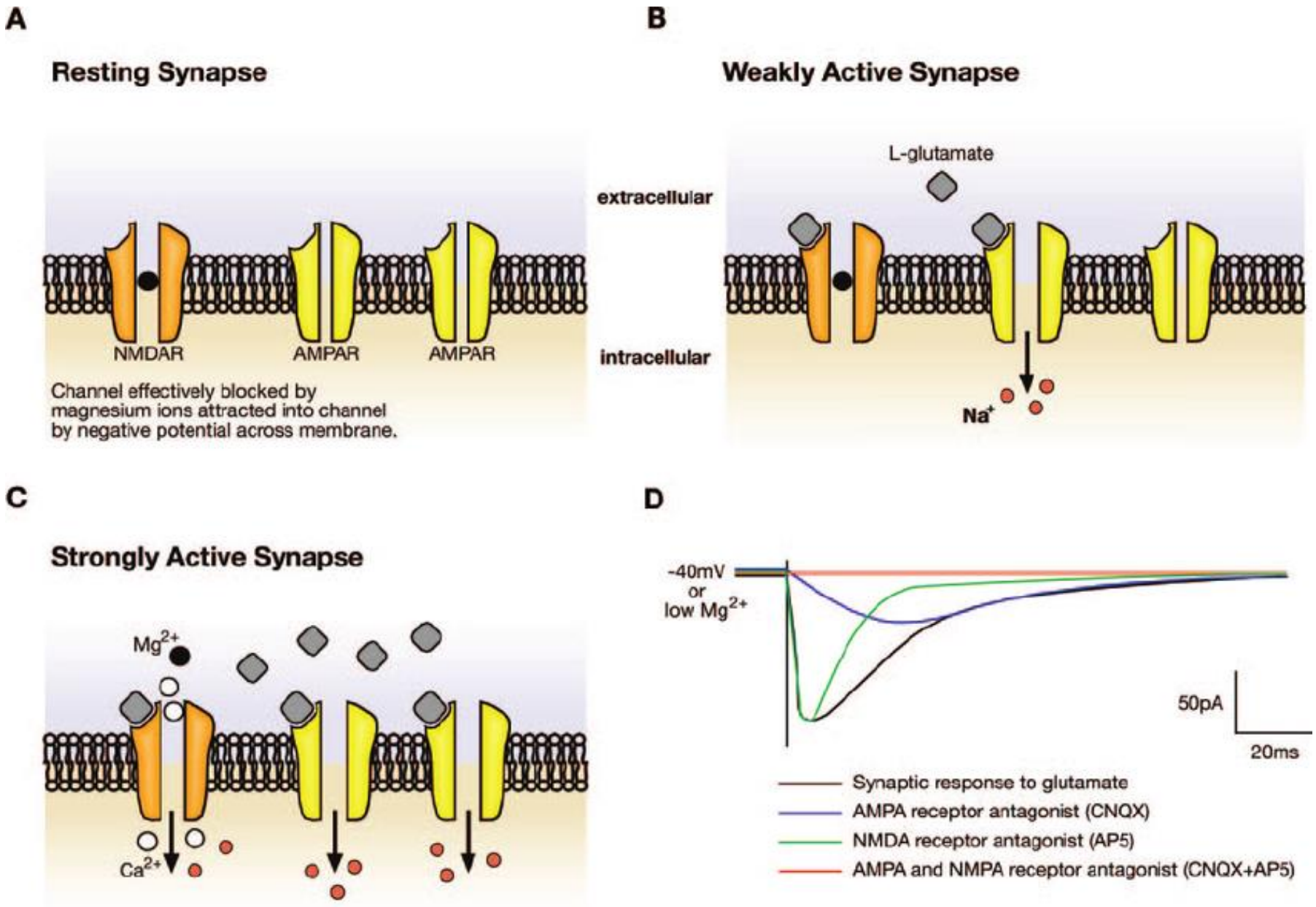


AMPA and NMDA receptor immunogold labeling at cortical synapses. Asymmetric synapses labeled with antibodies recognizing NMDA receptors **(a–c)** or AMPA receptors **(d–f)** or with antibodies to AMPA receptors (10-nm particles) followed by antibodies to NMDA receptors **(g–i**; 20-nm particles). Whereas large **(c, f, i)** and medium-sized **(b, e, h)** synapses contain both types of receptor, a subpopulation of the small synapses displays only NMDA receptors **(a, d, g)**. Arrowheads indicate extent of postsynaptic density. Each section corresponds to the PSD diameter, as identified in serial sections. Mitochondrion designated by 'm; terminal designated by 't'. Scale bar, 200 nm.

NMDA Receptors are coincidence detectors



NMDA Receptors are coincidence detectors



AMPA RECEPTORS
VERSUS
NMDA RECEPTORS

AMPA RECEPTORS	NMDA RECEPTORS
A type of glutamate receptor that participates in excitatory neurotransmission and also binds α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and acts as a cation channel	A type of glutamate receptor that participates in excitatory neurotransmission and also binds N-methyl-D-aspartate
Consist of four subunits, GluA1-4	Consist of a GluN1 subunit associated with one of the four GluN2 receptors, GluN2A-D
Only activated by glutamate	Activated by different agonists including glutamate
Agonist is α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	Agonist is N-methyl-d-aspartic acid
Activation results in the sodium and potassium influx	Activation results in sodium, potassium, and calcium influx
Do not contain a magnesium ion	Contain magnesium receptors
Responsible for the transmission of the bulk of the fast, excitatory synaptic signals	Responsible for the modulation of the synaptic response