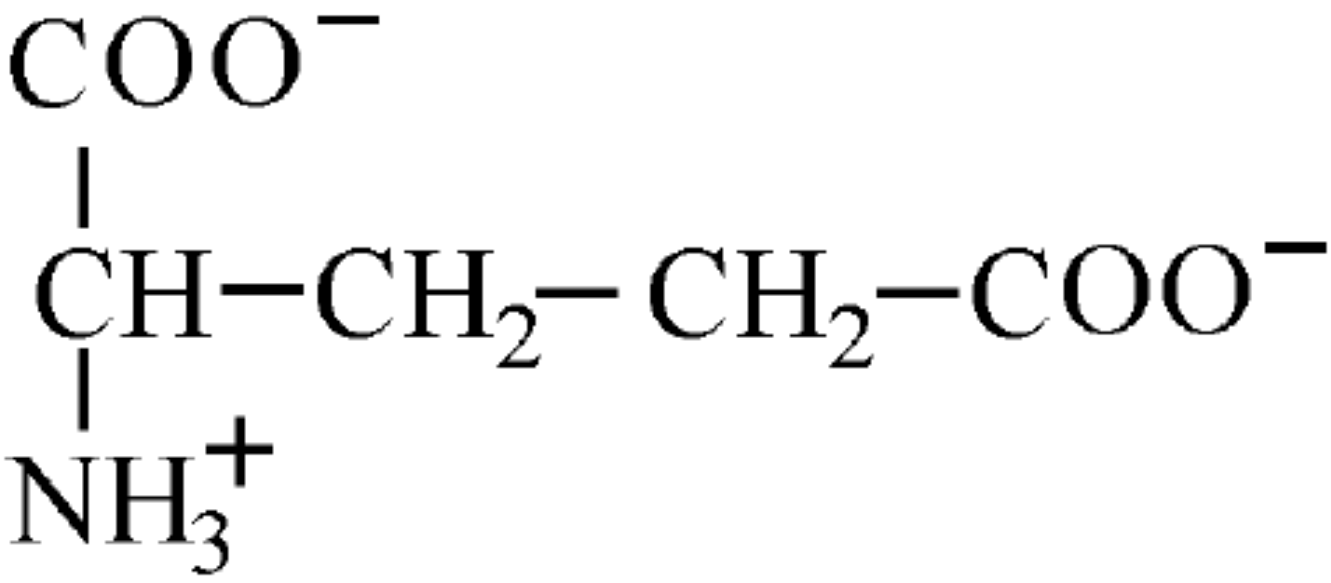
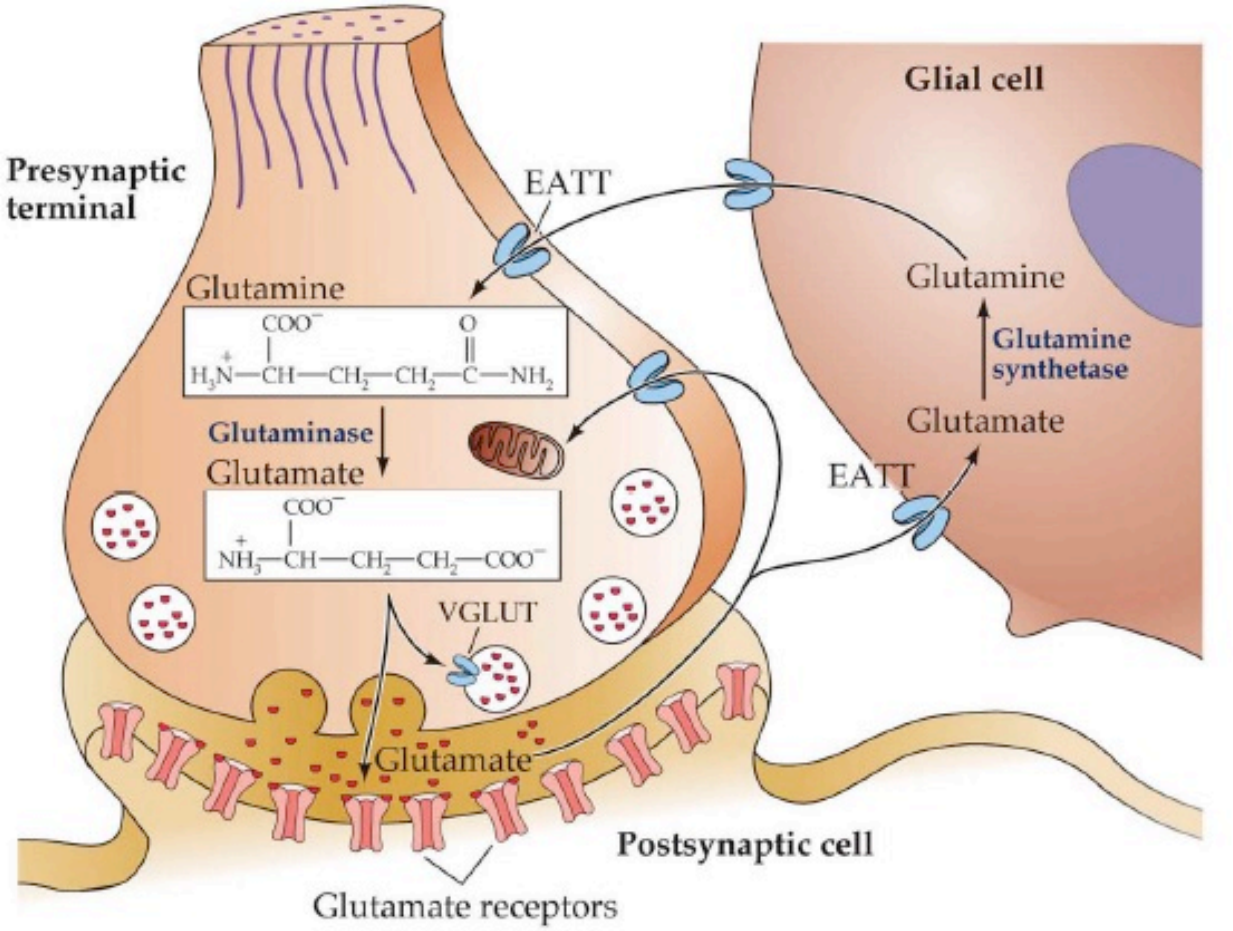
Glutamate Receptors

Glutamate



Glutamate cycle

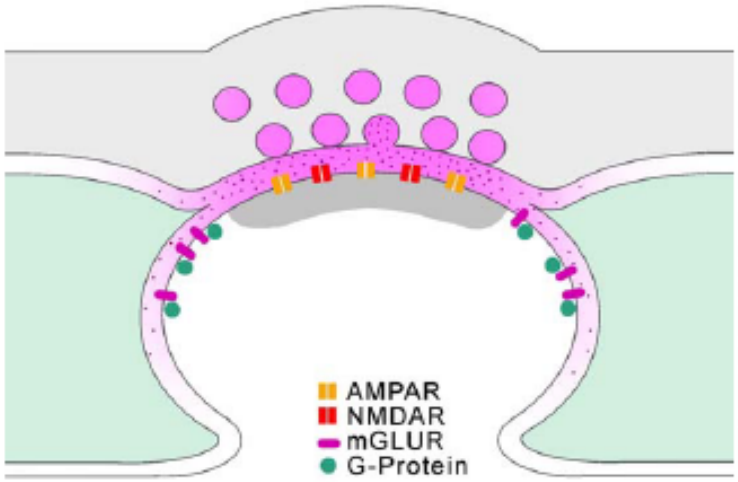


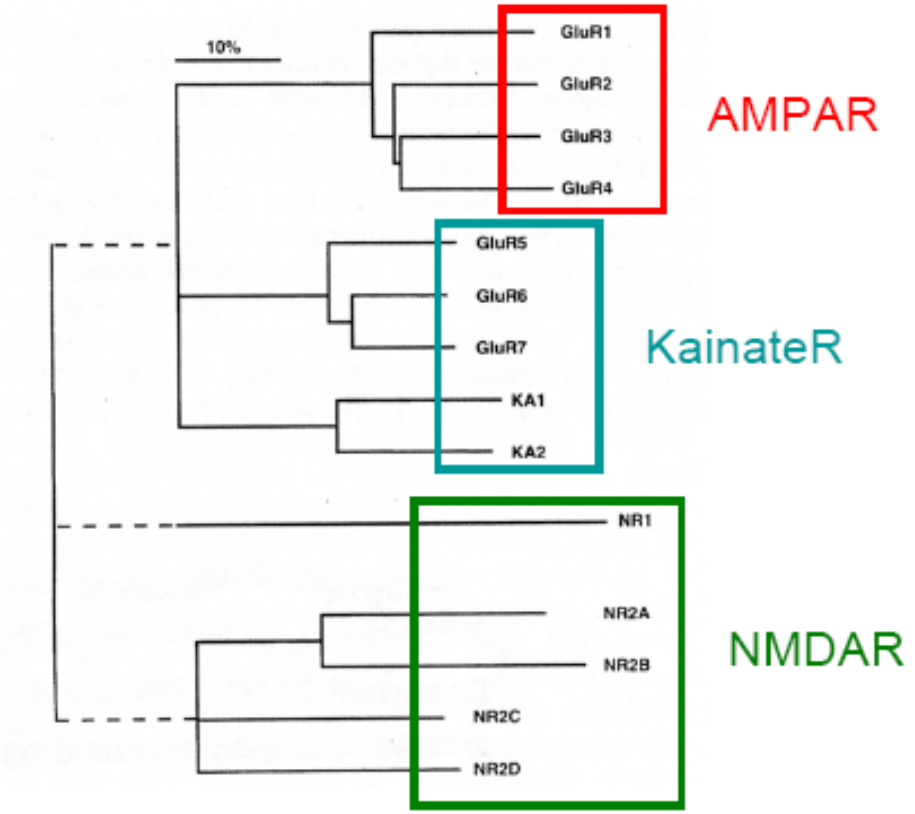
EATT: Excitatory amino acid transporter
VGLUT: Vesicular glutamate transporter

Excitatory Glutamatergic Synaptic Transmission

- Metabotropic
  - mGLURs
    - G-protein coupled
- Ionotropic
  - AMPAR
  - NMDAR
  - KainateR

Ligand gated
Na+/K+





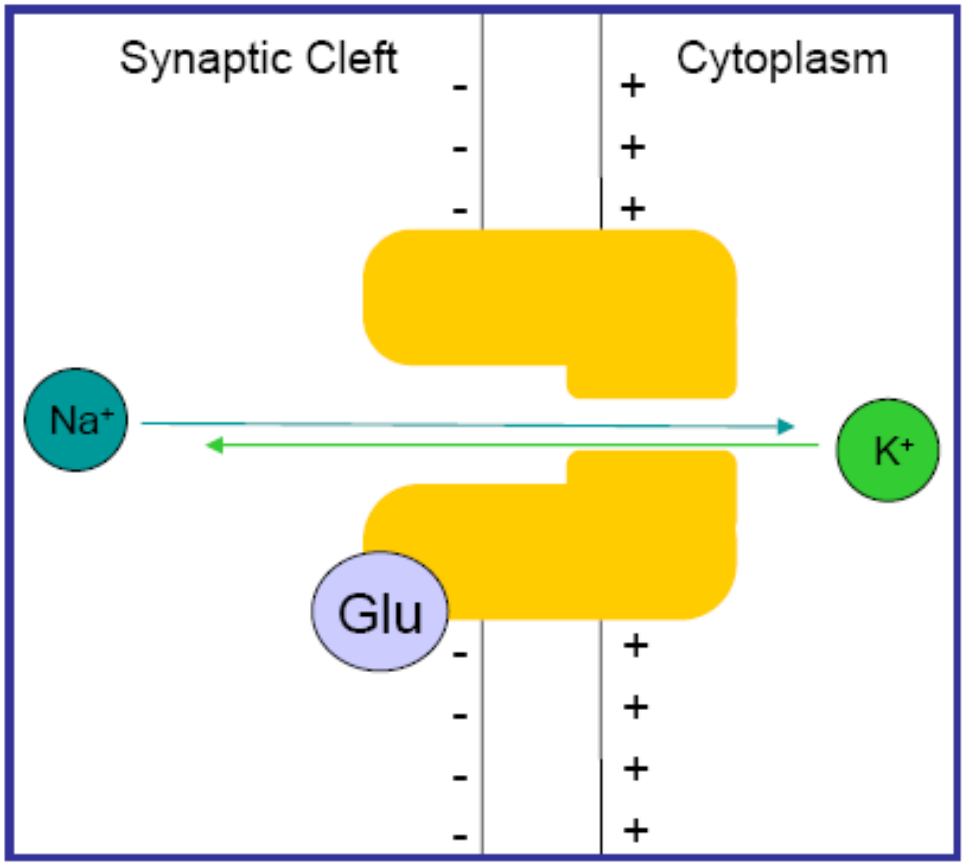
Ionotropic Glutamate receptors

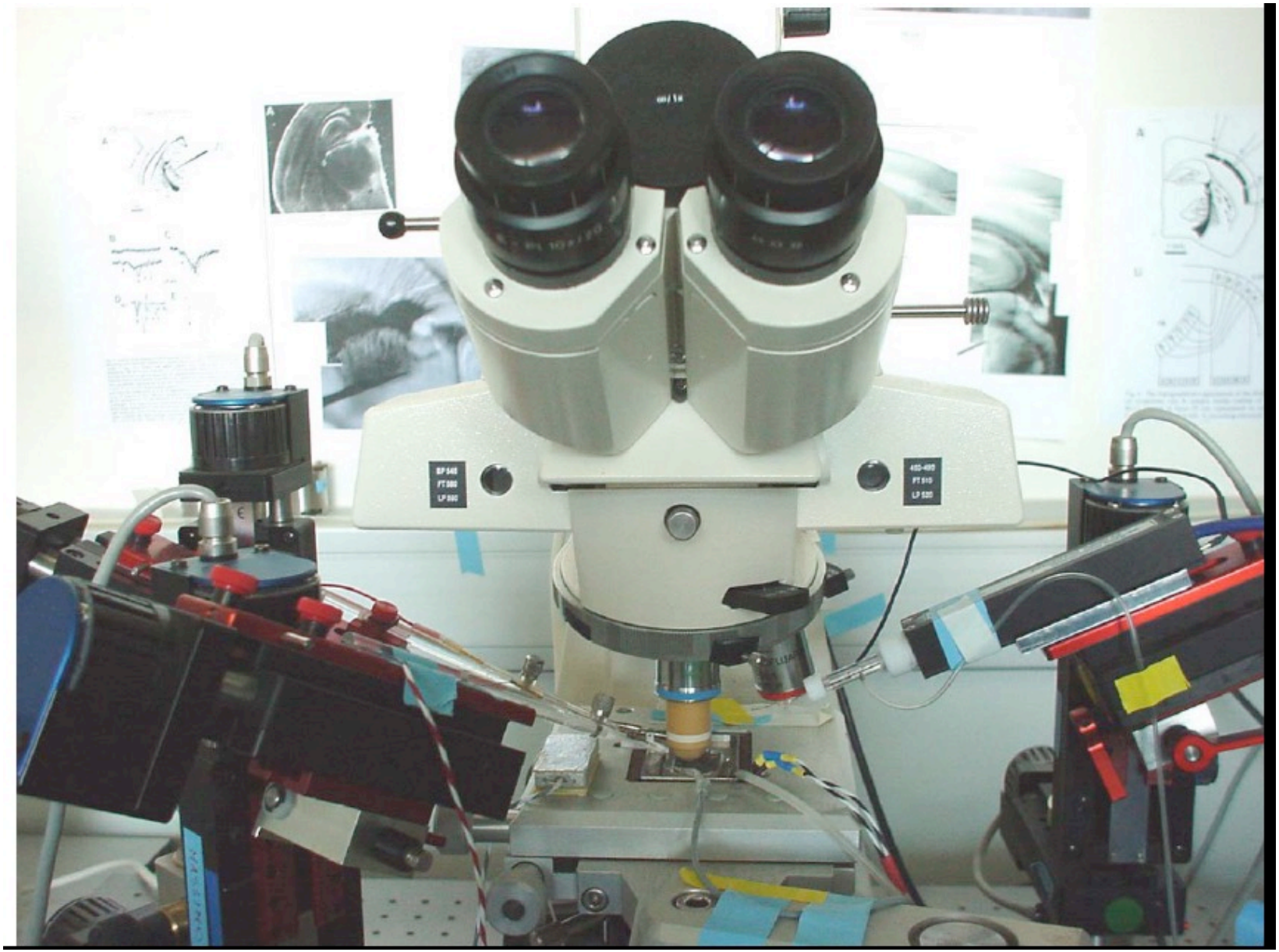
IGluR are widespread in the central nervous system, where more than 80% of the excitatory synapses are glutamatergic.
IGluR are also present in the sense organs.

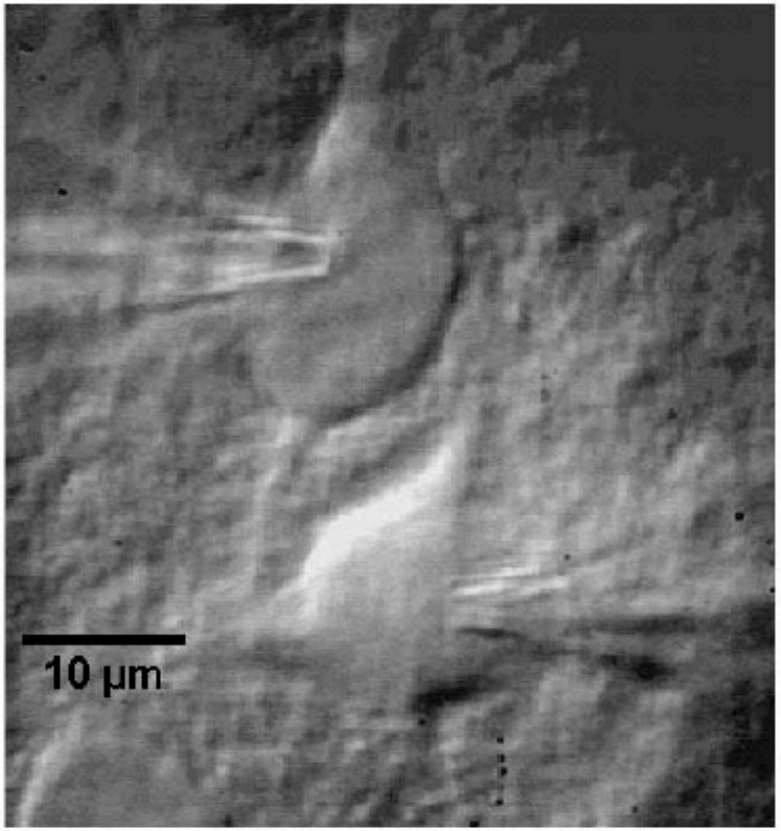
Given the great functional diversification of the formations in which they operate, it should not be surprising that they are differentiated into many types (with different conductivity, ionic selectivity and pharmacological sensitivity) but have the same character of cationic channels (always excitatory).

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

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

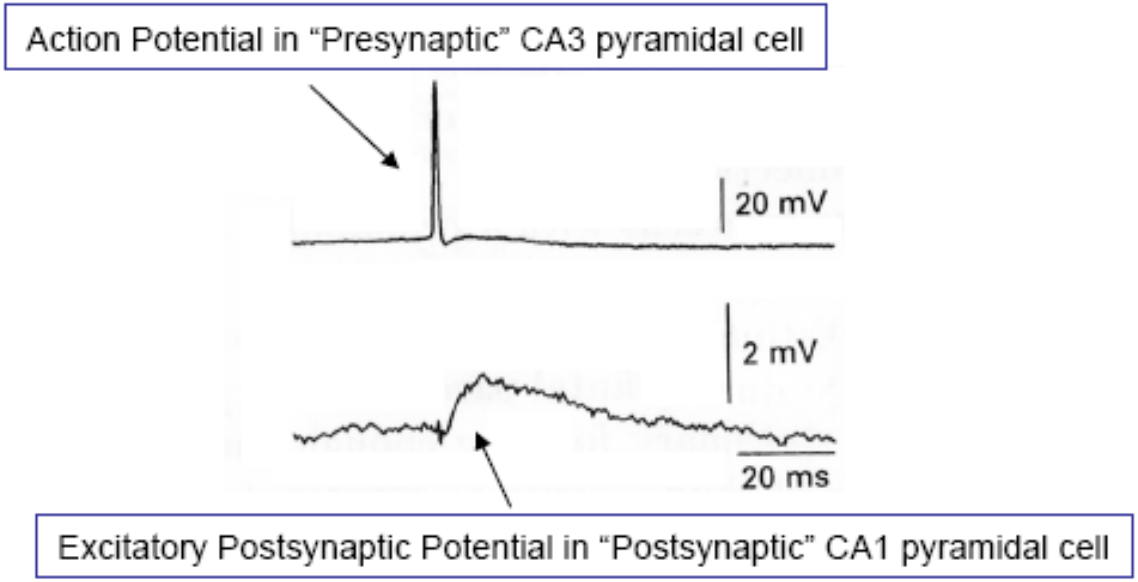





10 µm

Excitatory Post-synaptic Potential

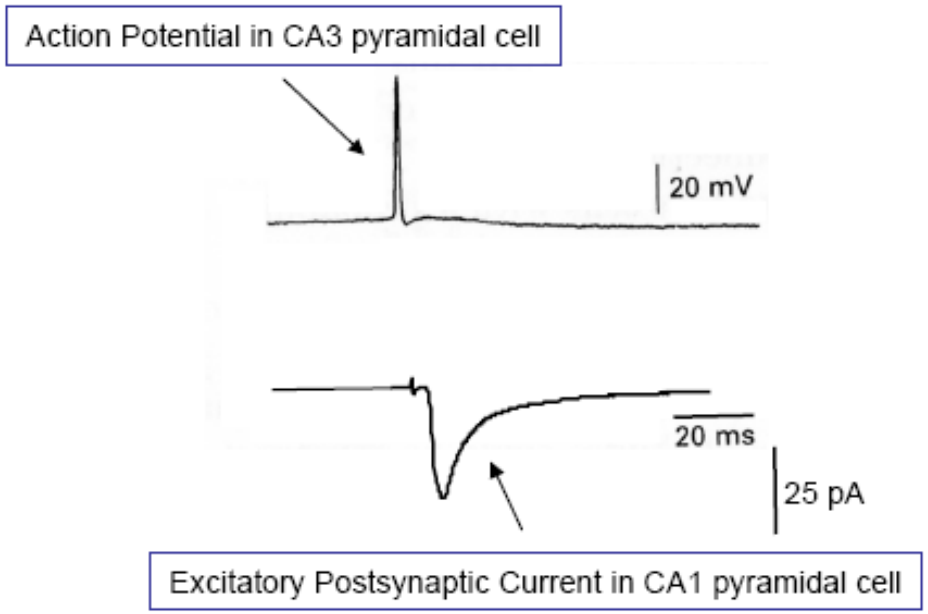
The EPSP



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

Excitatory Post-synaptic Current

The EPSC

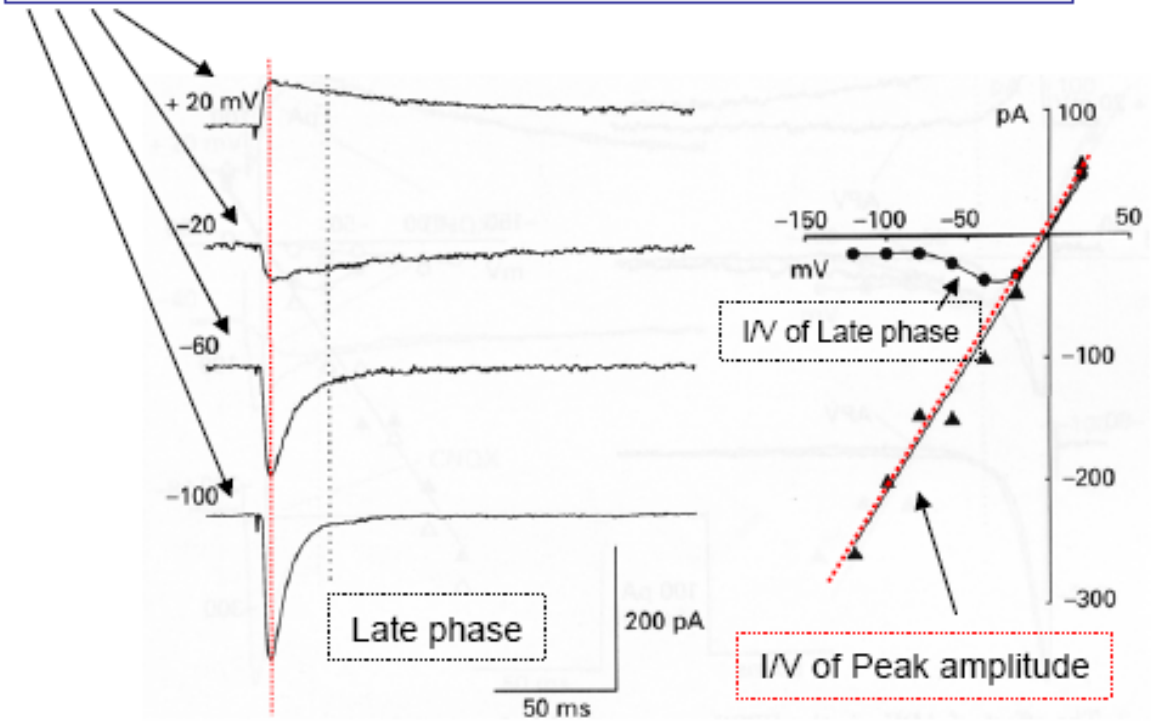


Presynaptic neuron is recorded in the Current Clamp configuration: measure the membrane potential (V_m)
Postsynaptic neuron is recorded in the Voltage Clamp configuration: measure the membrane current (I_m)

I-V Curve

The EPSC

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

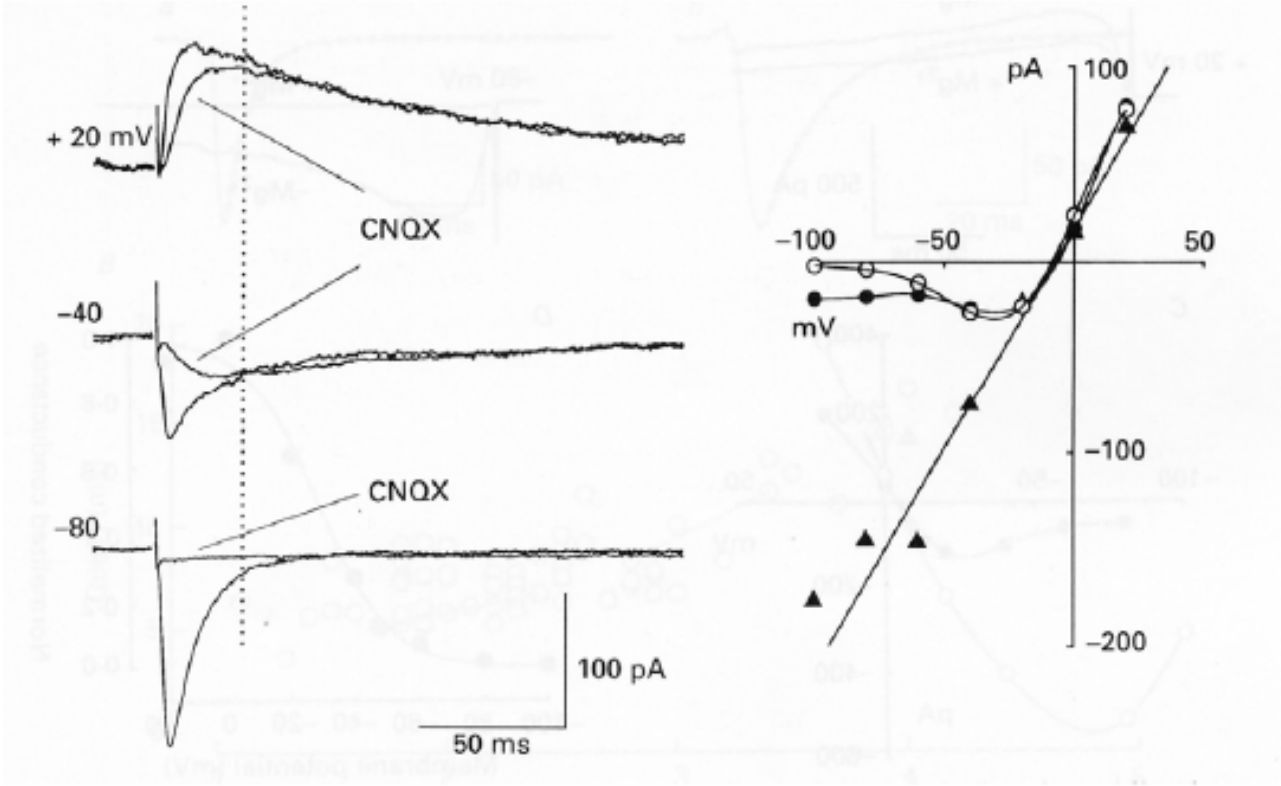


Peak amplitude

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

There are two pharmacologically distinct EPSC components … ..AMPA receptors mediate the fast component

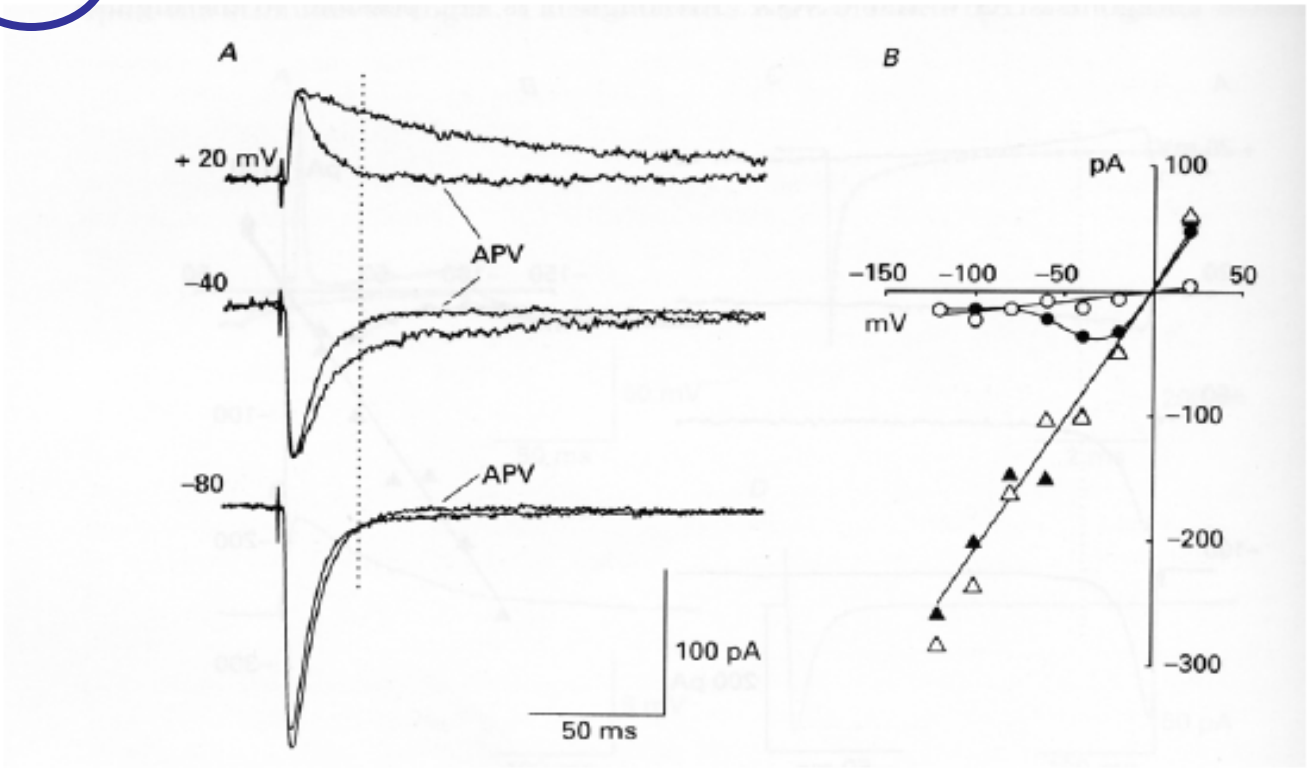
CNQX is an AMPA receptor antagonist and blocks the fast component



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

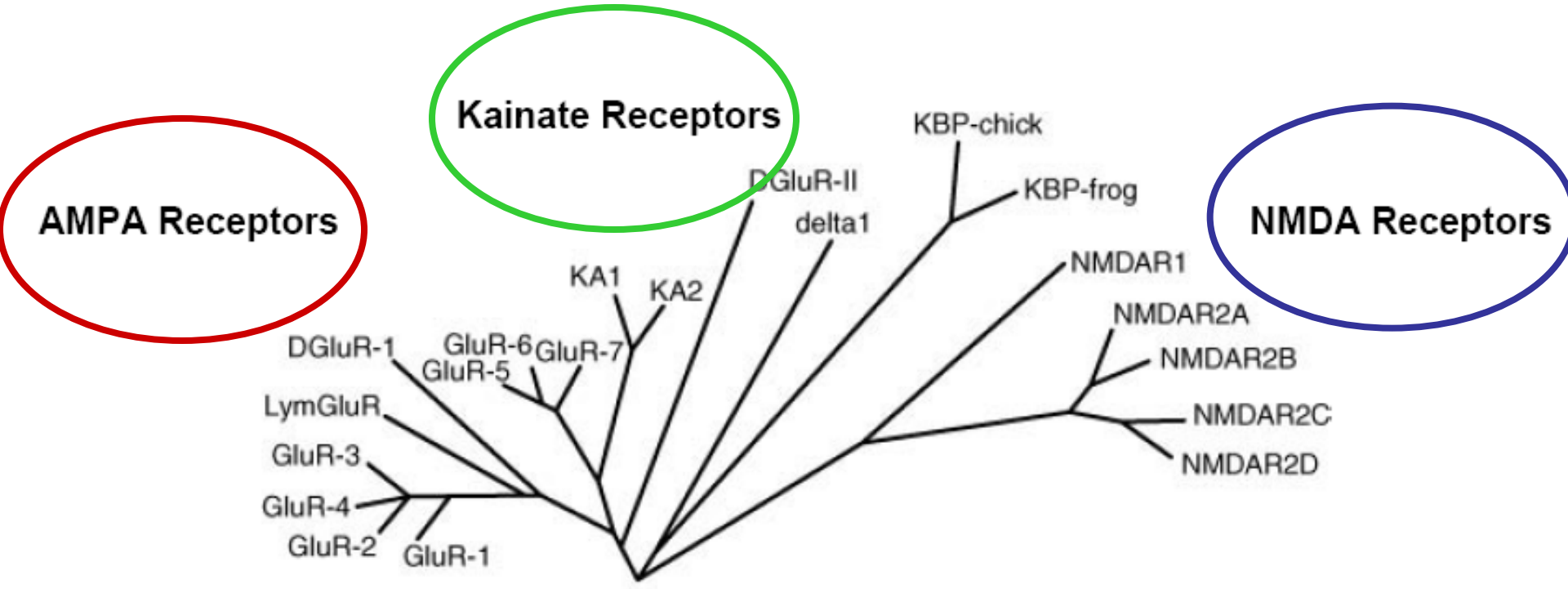
There are two pharmacologically distinct EPSC components … ..NMDA receptors mediate the slow component

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



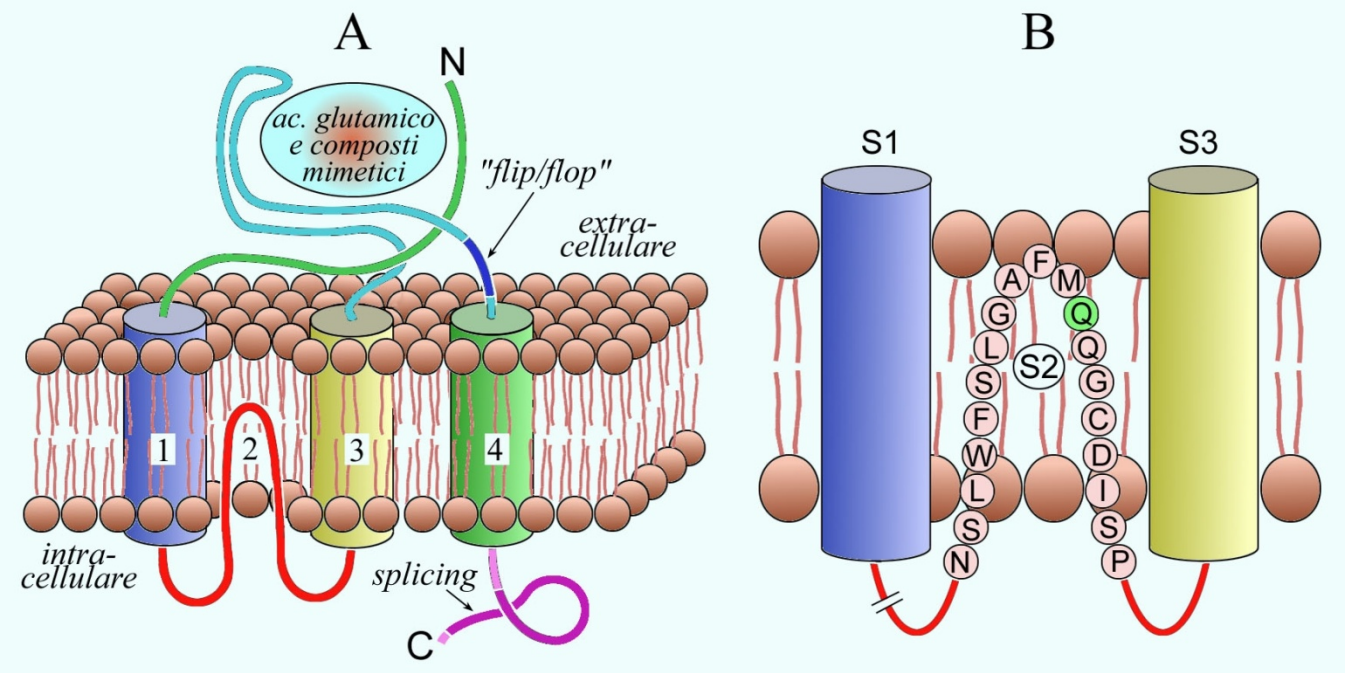
NMDA receptors I/V relationship is not linear

Ionotropic Glutamate Receptor Family



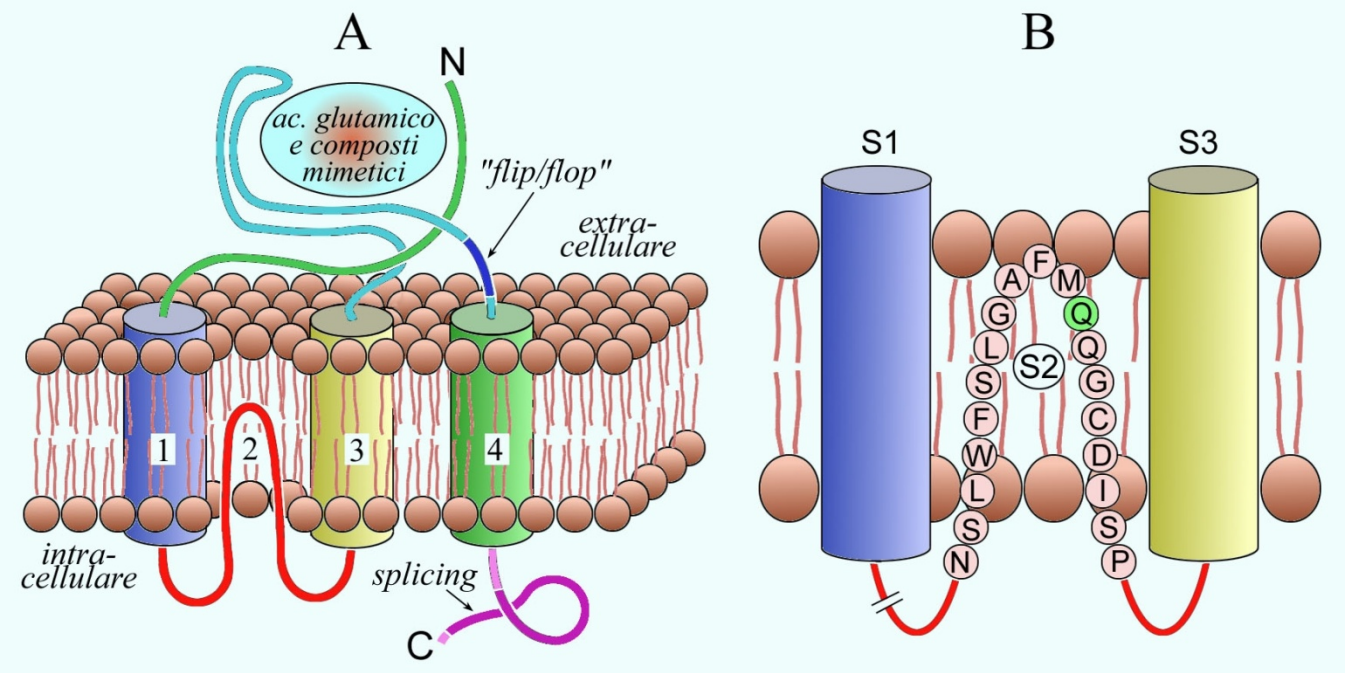
GluR structure

Assembly of *4 subunits*



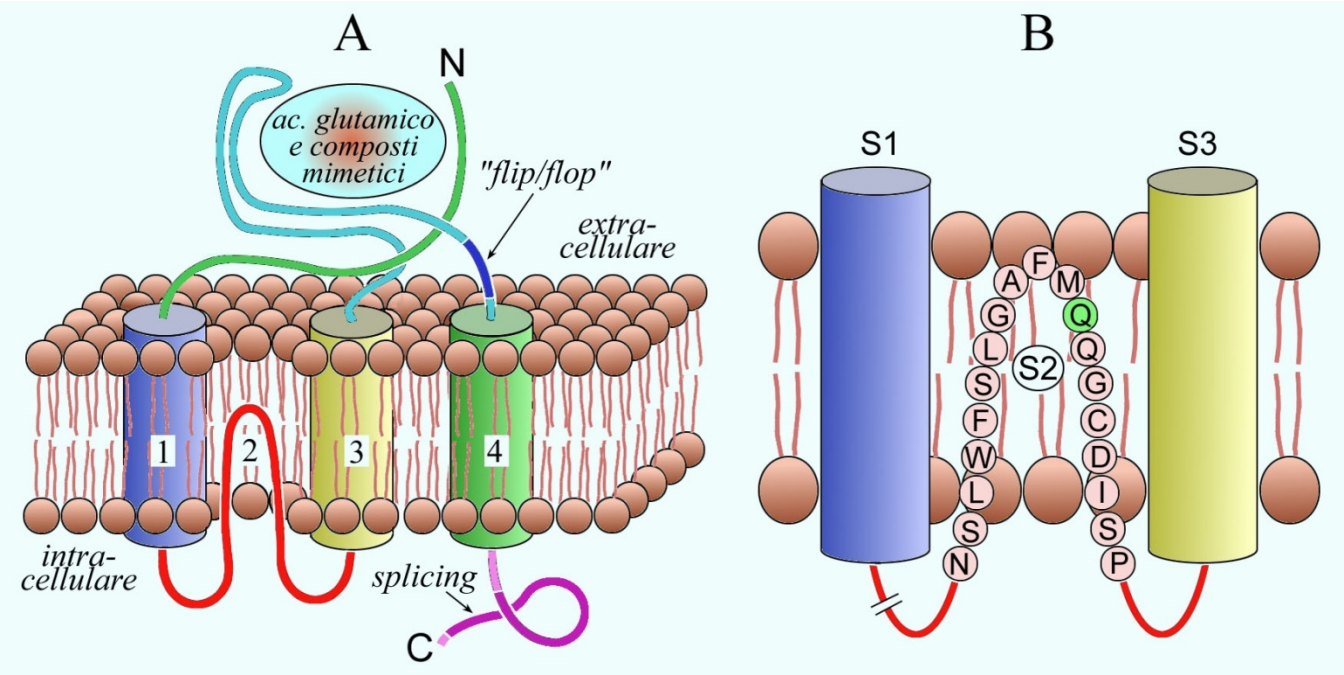
in each subunit the second hydrophobic domain (improperly referred to as "S2"), after entering the membrane from the cytoplasmic side, is reflected forming a loop (a sort of "P region") and returns to the cytoplasm without crossing it.

GluR structure



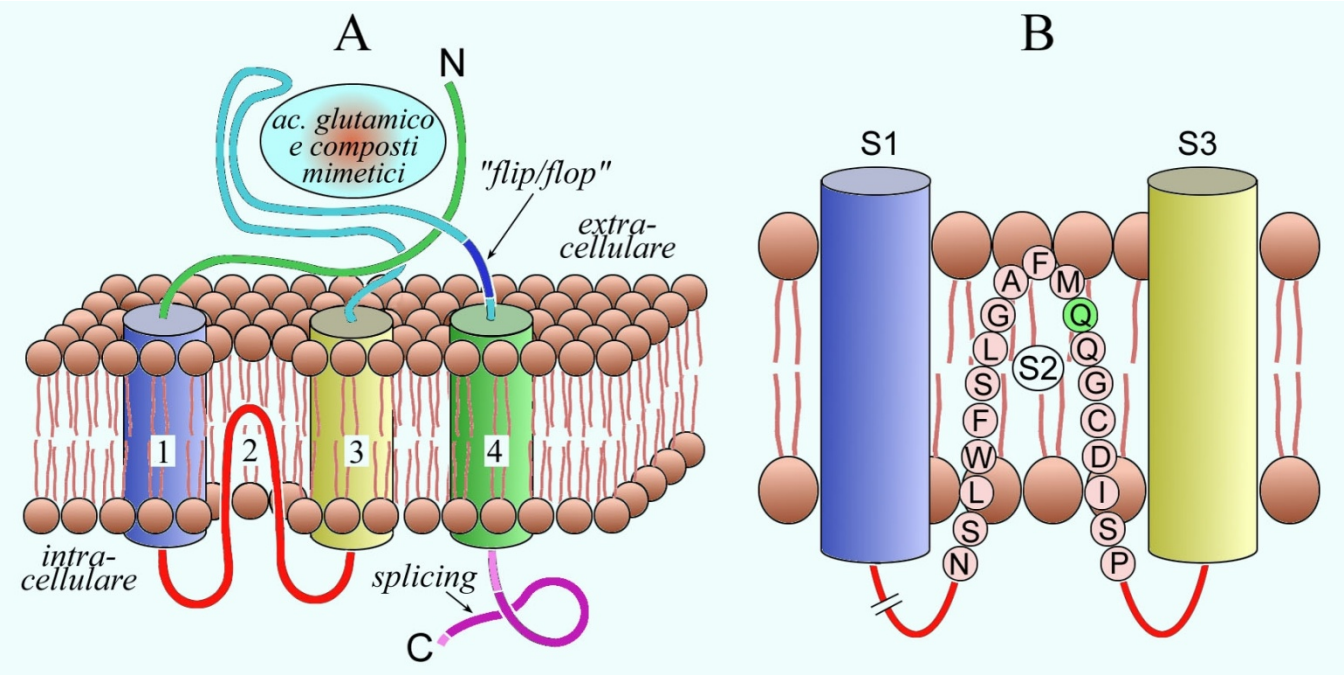
this missed crossing causes the iGluR subunits to have only three transmembrane segments (and not four) and that the C-terminal end of the whole amino acid chain is directed towards the intracellular medium (and not towards the extracellular medium, as it is as a rule for other ionotropic receptors).

GluR structure



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.

GluR structure



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 ahuge "ball", inside which is the binding site for the neurotransmitter.

GluR Pharmachology

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".

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 -

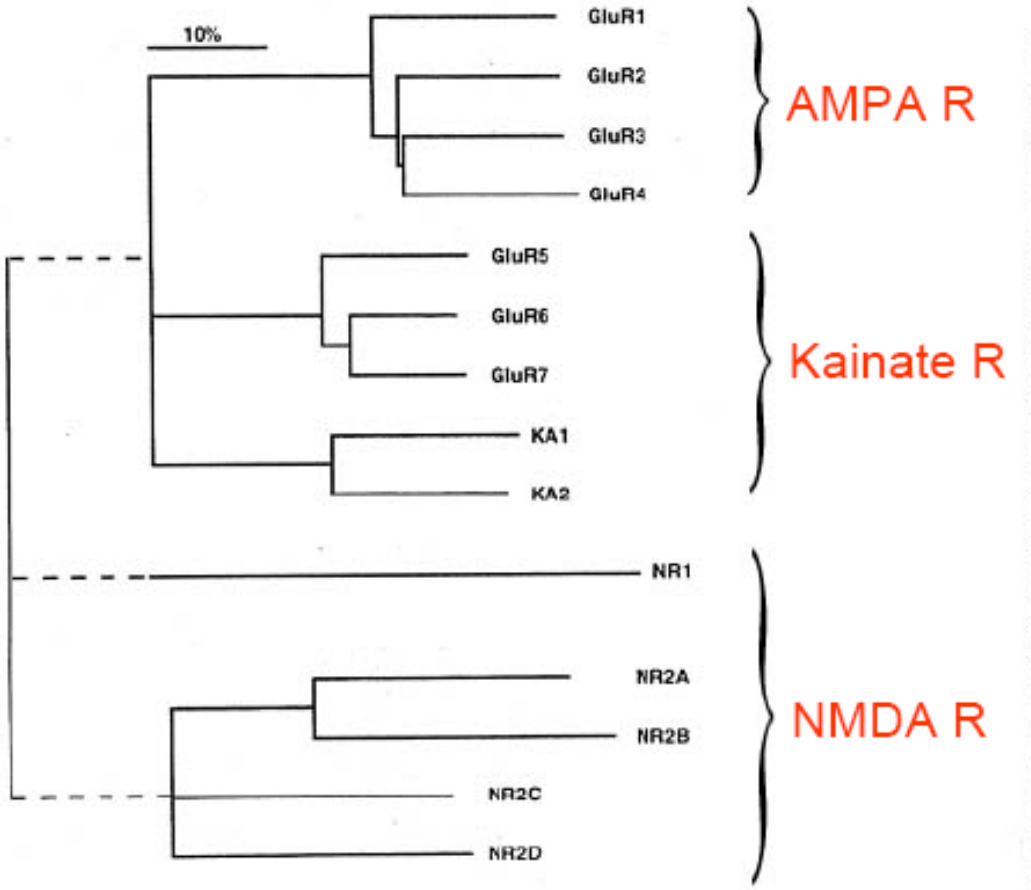
Assembling of GluR

However, generally

NMDA receptors are very permeable to Ca2 +

Non-NMDA receptors are little or not permeable to Ca2 +

The Ionotropic Glutamate Receptor Family



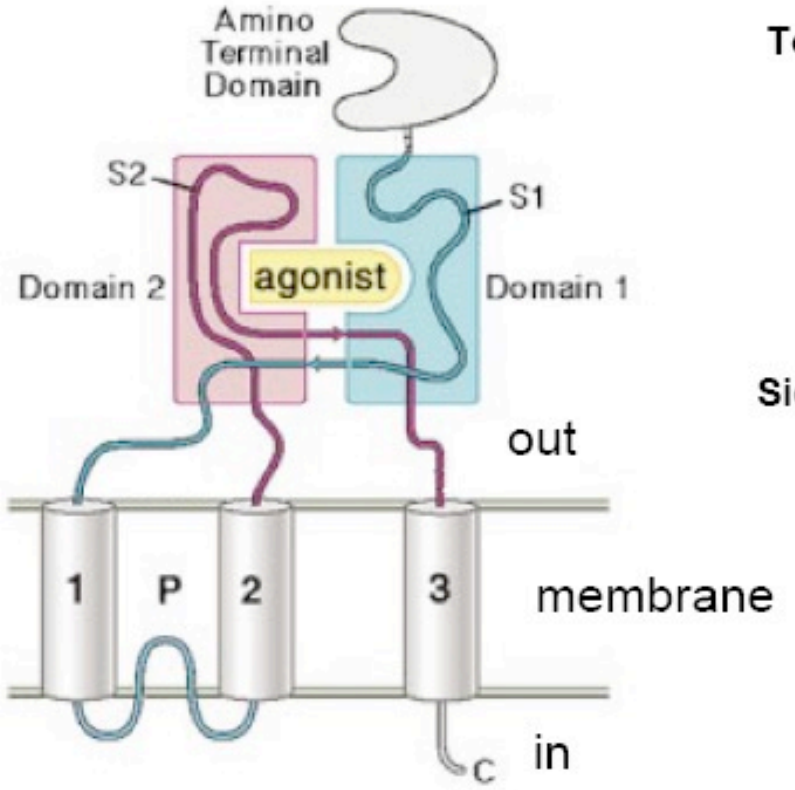
AMPA Receptors

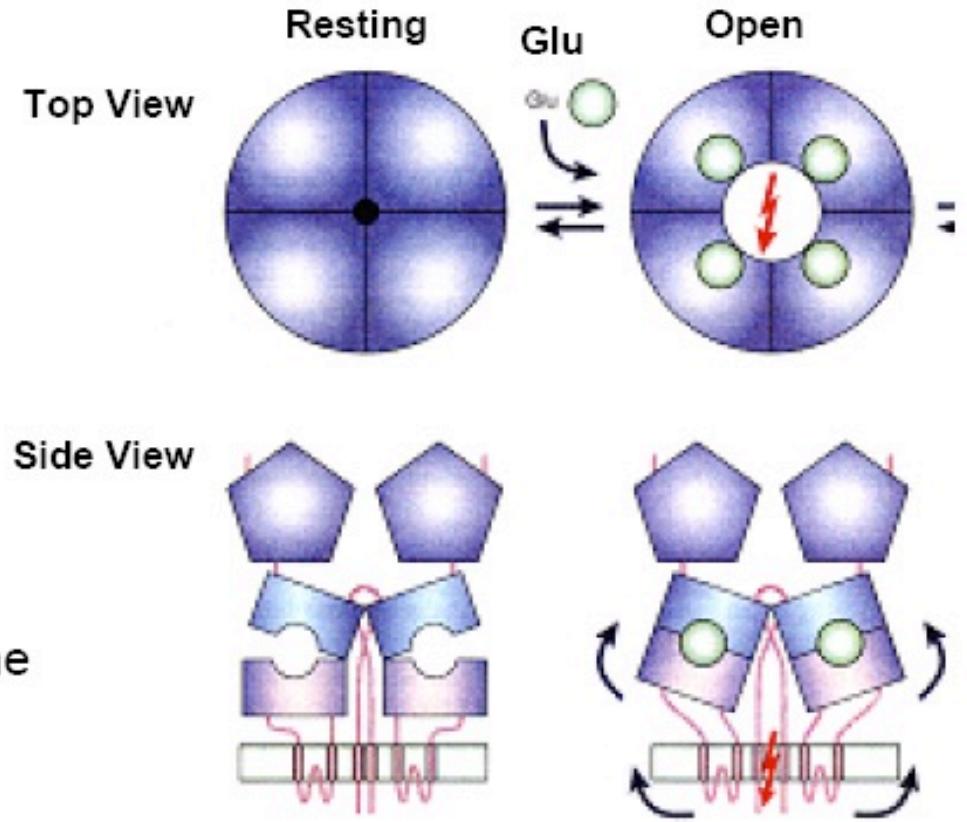
Receptors for AMPA have kinetics ofactivation / inactivation and desensitizationvery fast.

- They are permeable to Na + and little to Ca2 +
- They are located in the postsynaptic membrane
- They are responsible for the excitatory response (depolarizing) rapid typical of glutamatergic synapses.

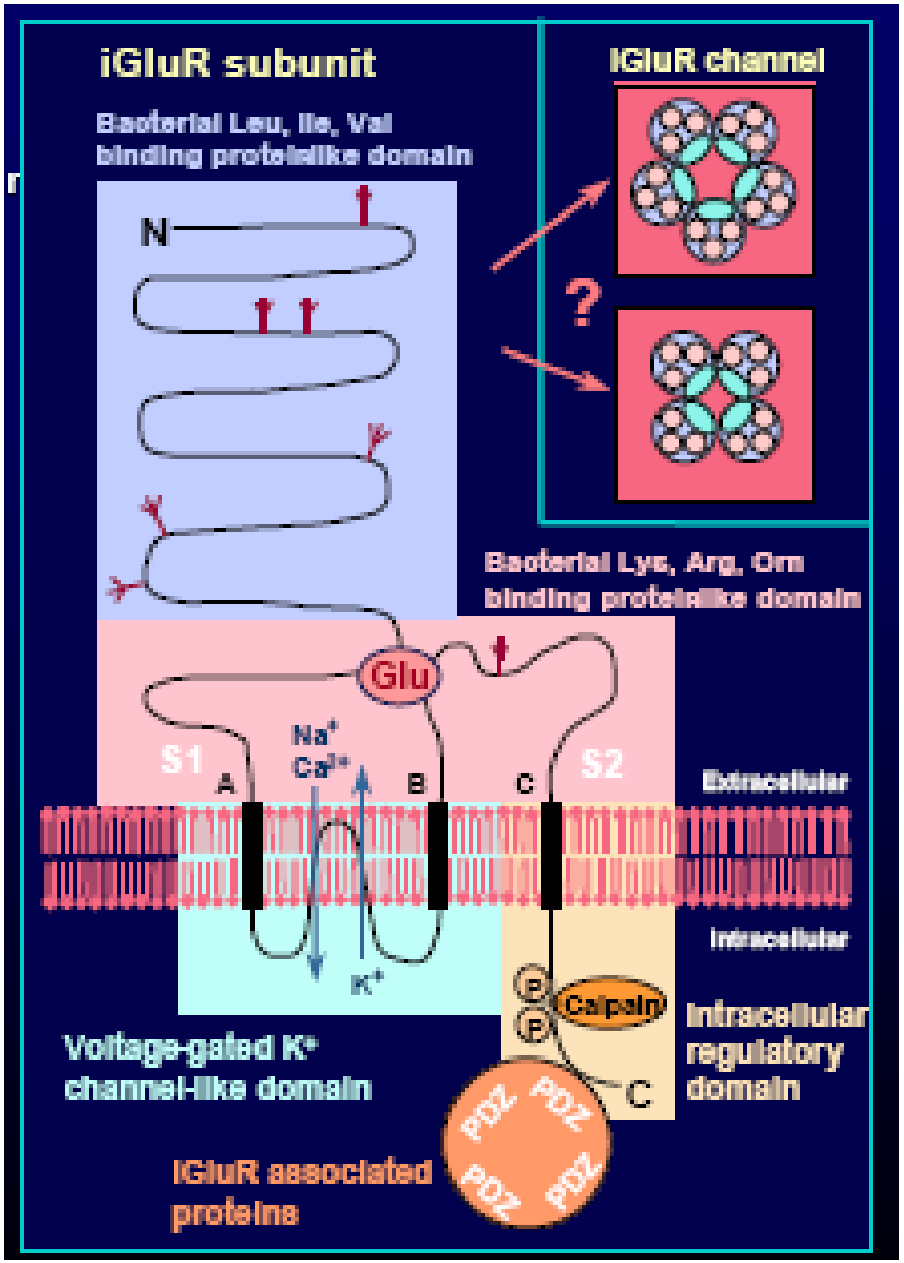
Four Genes Code For AMPARs (GluR1-4).
A functional AMPAR is made of four subunits (tetramer).

Domains of the AMPAR subunit

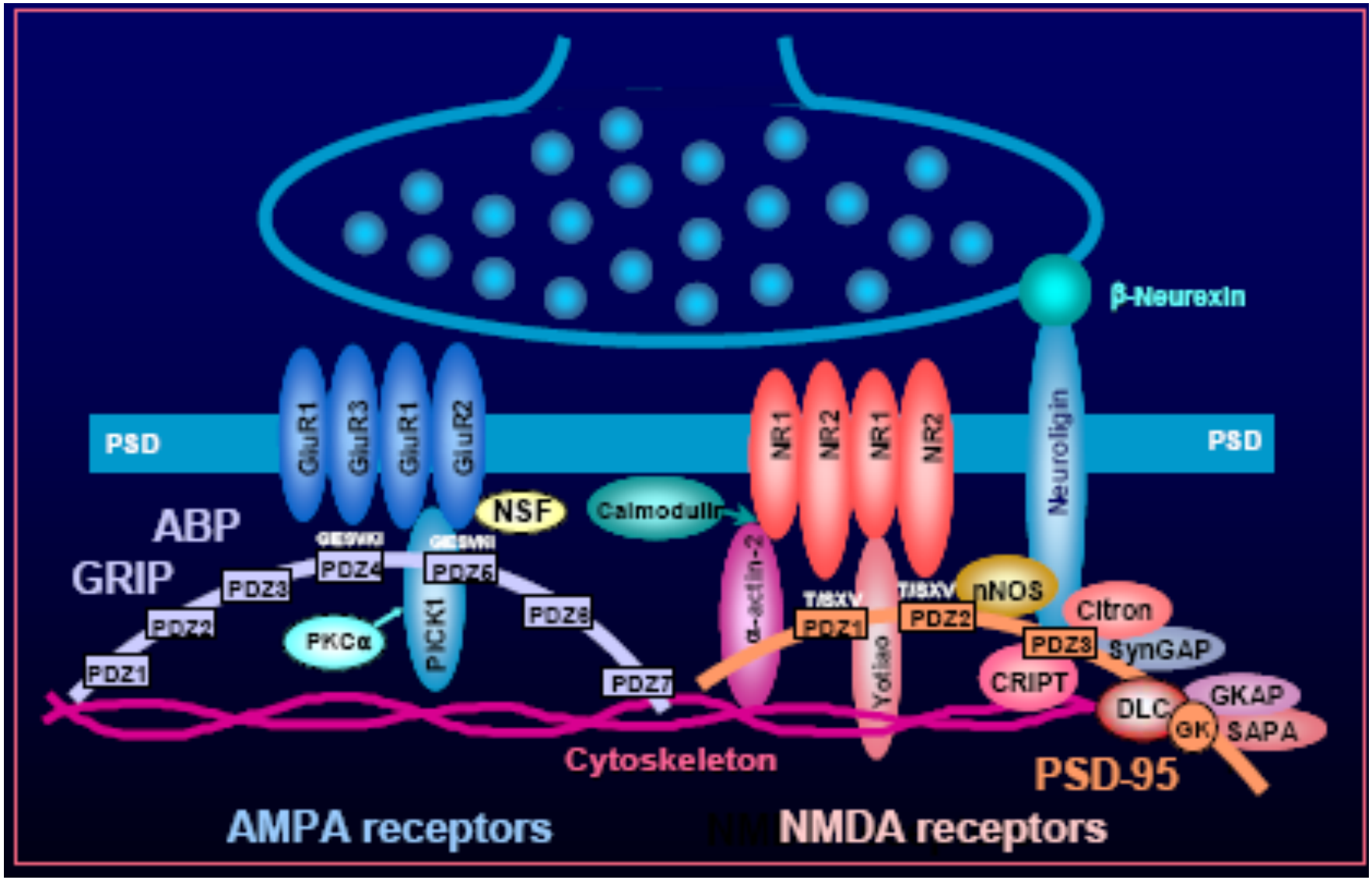




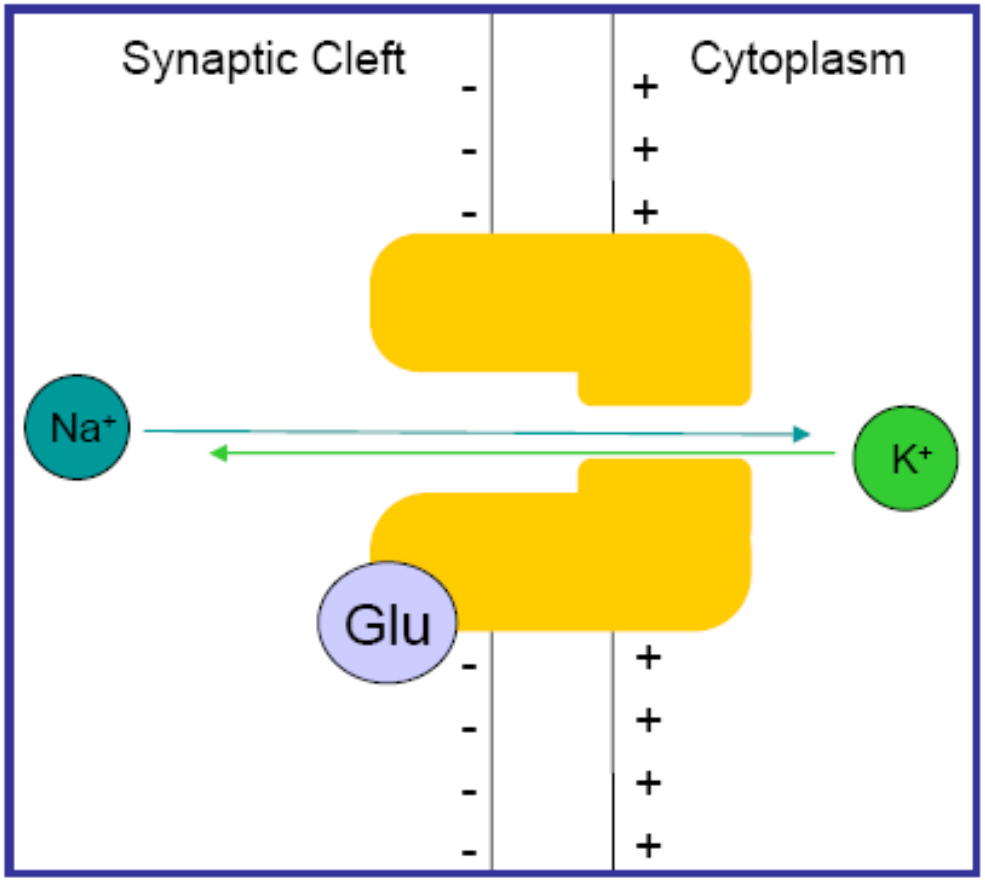
Schematic representation of the transmembrane topology of ionotropic glutamate receptors



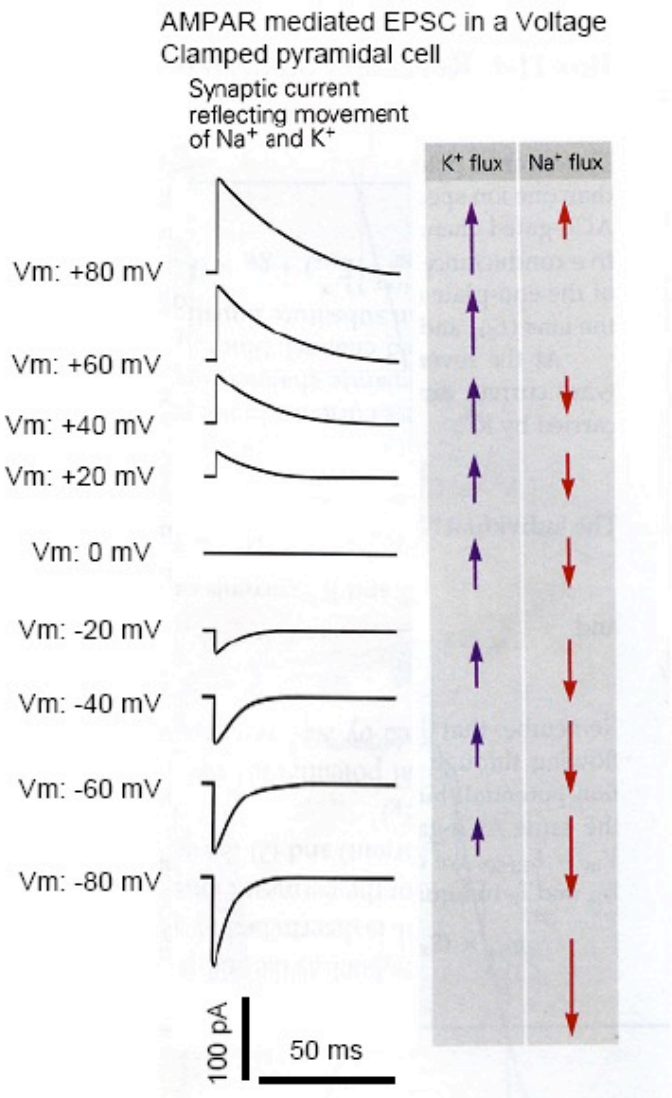
The synaptic protein network associated with AMPA and NMDA receptors



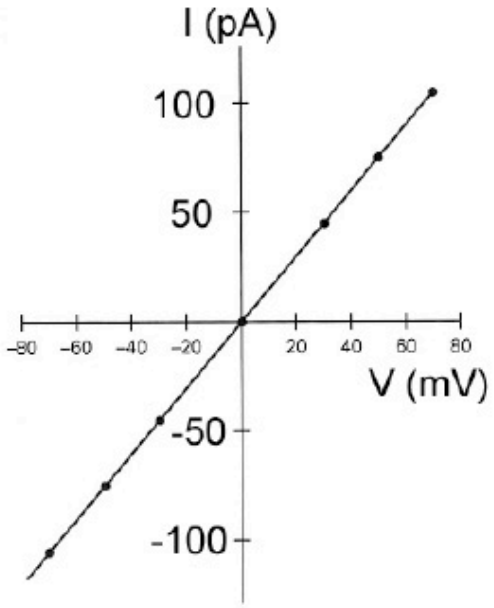
The AMPAR opens a non-selective cationic conductance



The Reversal Potential



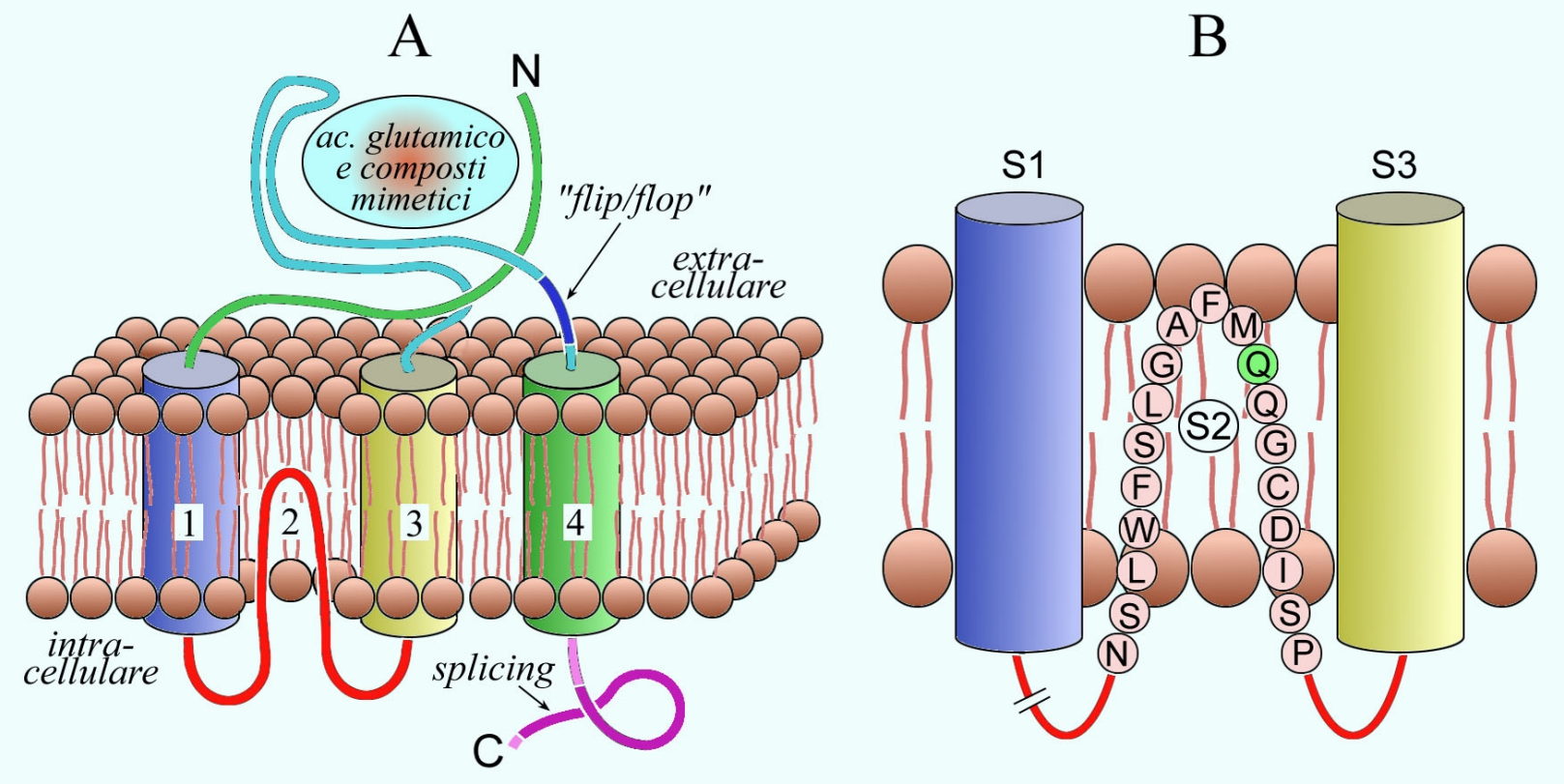
The I/V plot



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

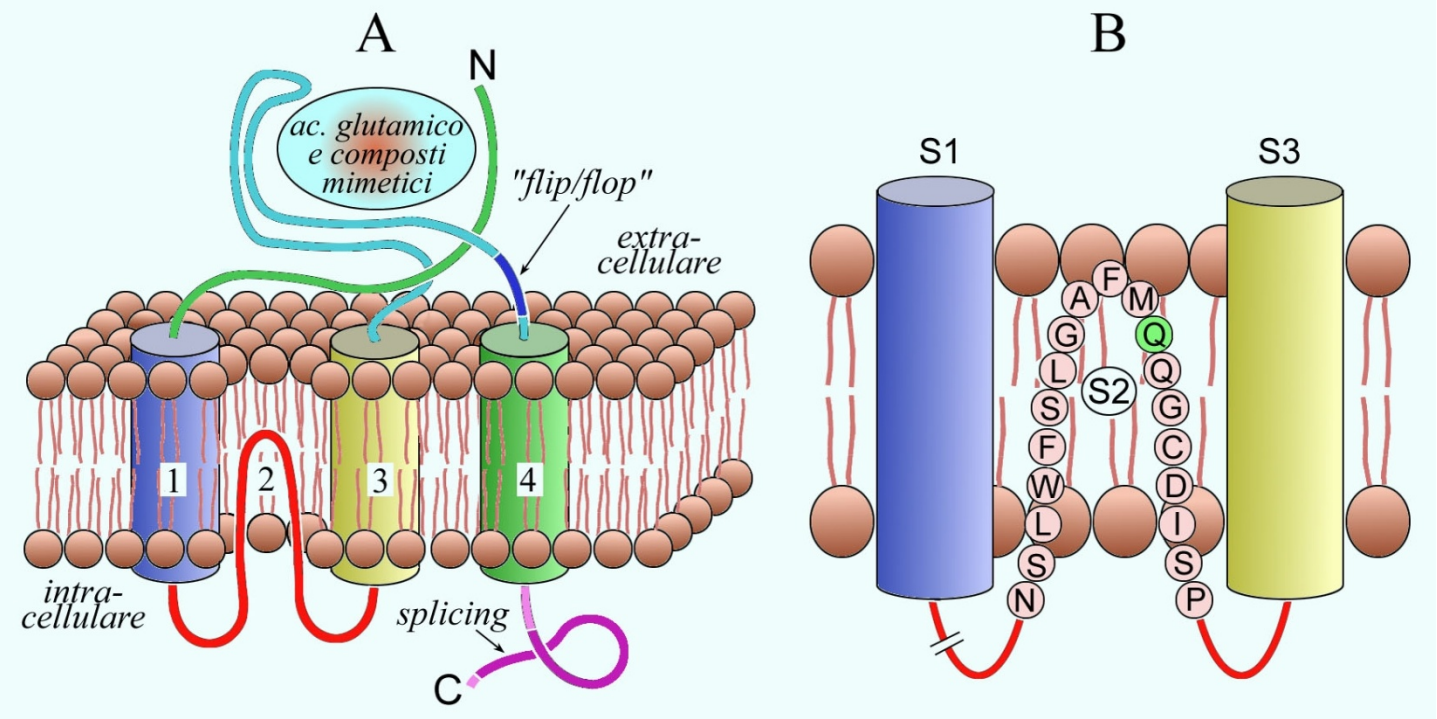
E_{rev}= 0 mV: @ 0 mV: I_{Na}= -I_K

AMPA receptor Splicing



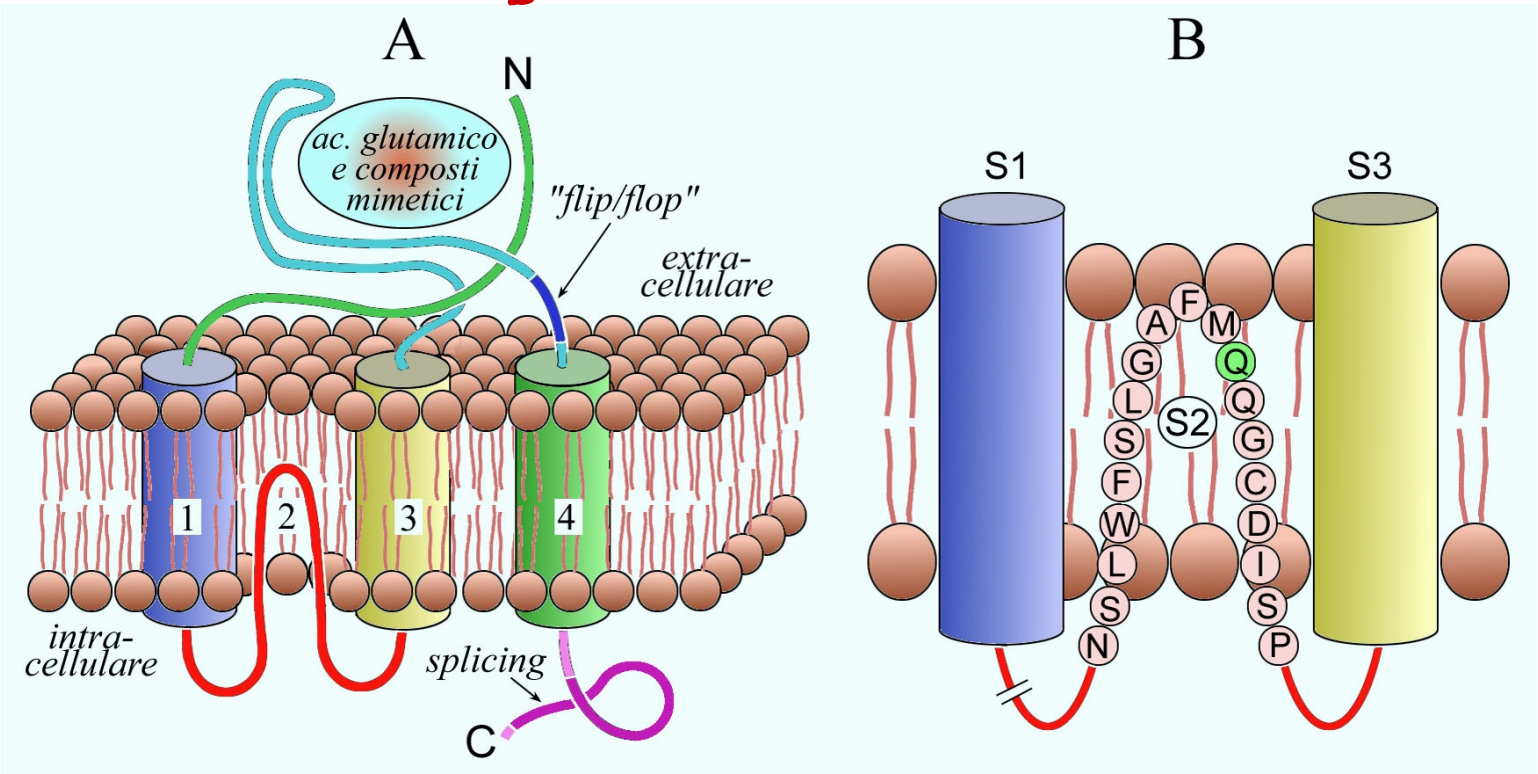
Splicing concerns mainly the C-terminal tract (purple) of polypeptide chains, that interact with cytoskeletal proteins; it is thought that different C-terminal sequences constitute as many "addresses" differentiated for the different types of iGluR.

AMPA receptor Splicing



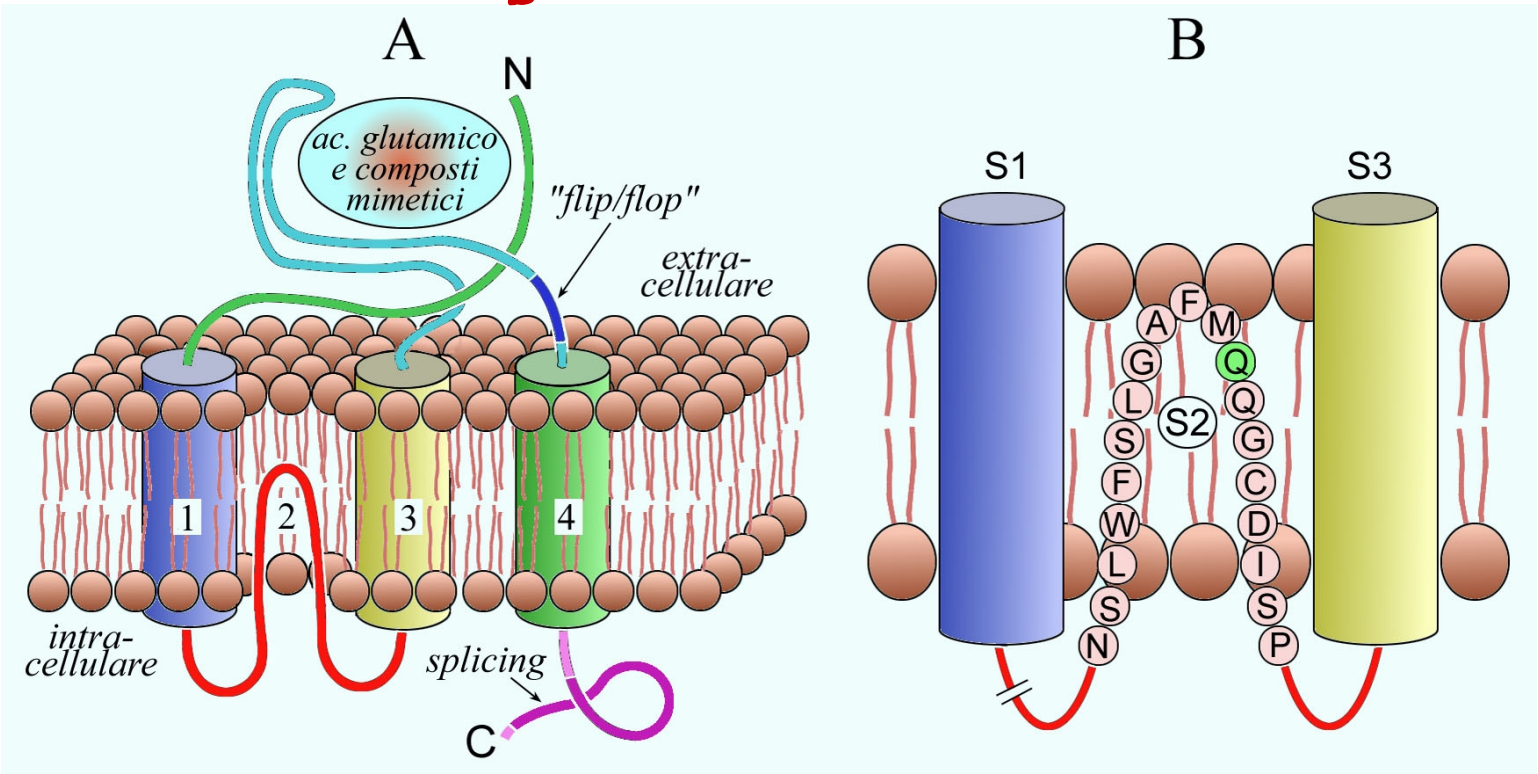
Another segment of the "spliced" (blue) chains is interposed between the STM S4 and the extracellular "ball". this segment (at least in the GluR1-GluR4 subunits of AMPA receptors) can occur in two variants, called "flip" and "flop", which give the receptor a very different kinetics of desensitization: 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.

AMPAR editing



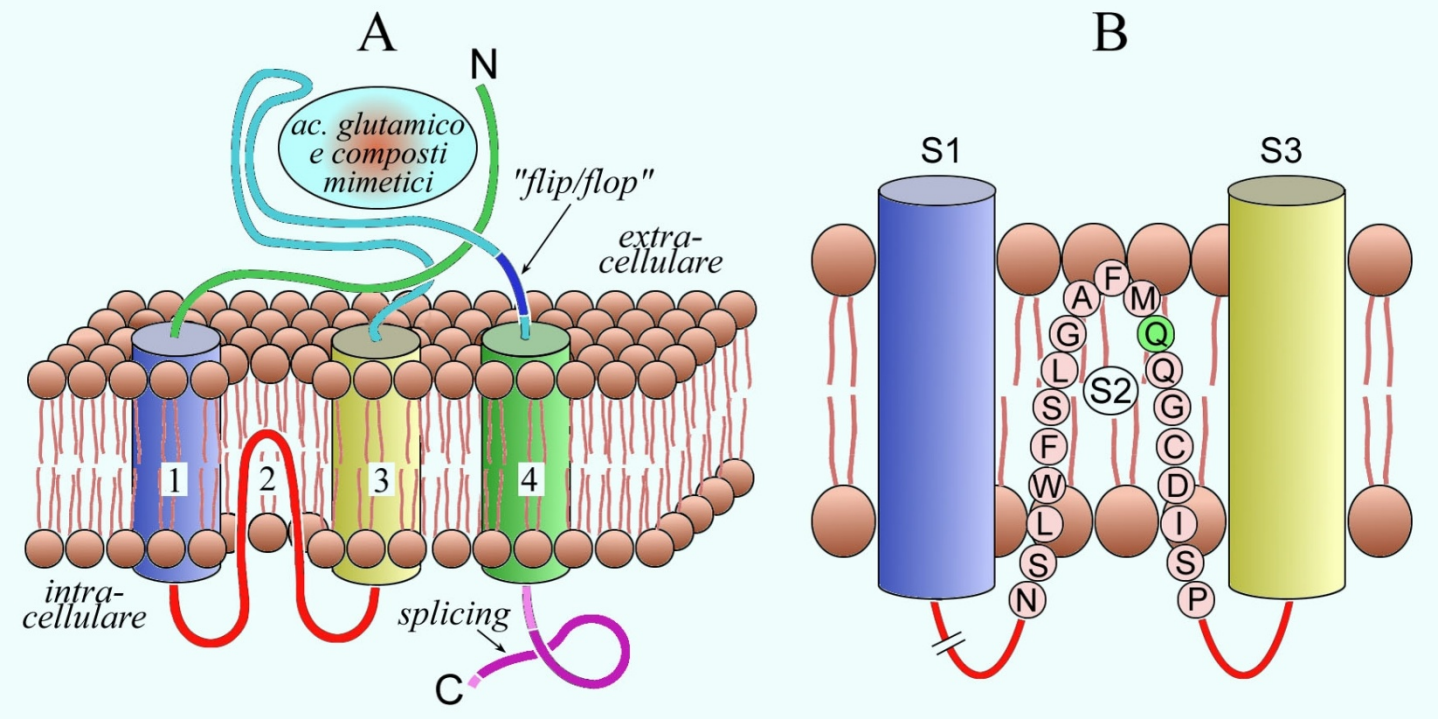
A further possibility of diversification in iGluR isoforms is the reorganization of mRNAs known as "editing", through which an element of the pre-mRNA nucleotide sequence can be modified enzymatically, thus changing the amino acid that will be encoded by the mature mRNA.

AMPAR editing



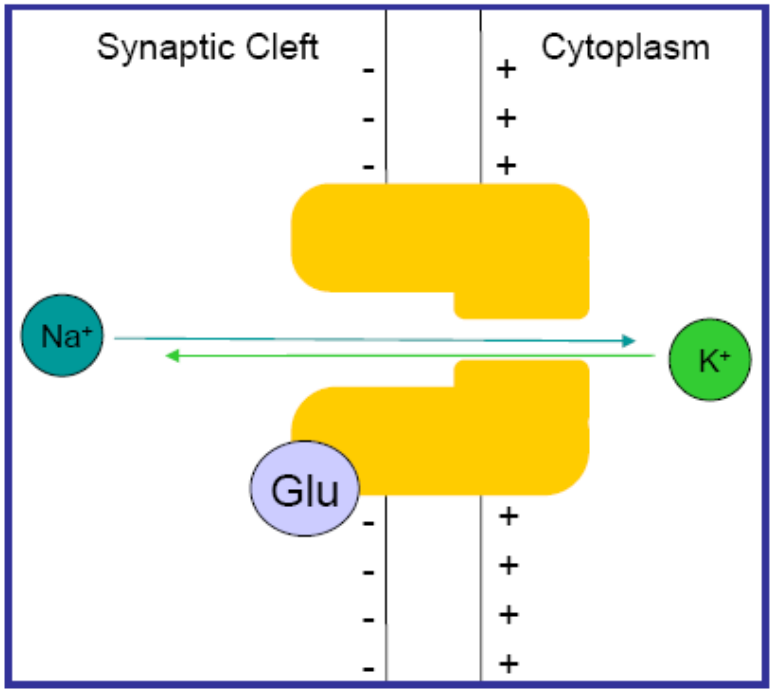
An important "editing site" has been identified in the S2 section of many subunits (shown in green in B). In this "site" may be a glutamine (Q, as specified by the "code" contained in the DNA), or an arginine (R), when the codon of mRNA for glutamine (CAG) is modified enzymatically in that Arginine (CGG).

AMPAR editing

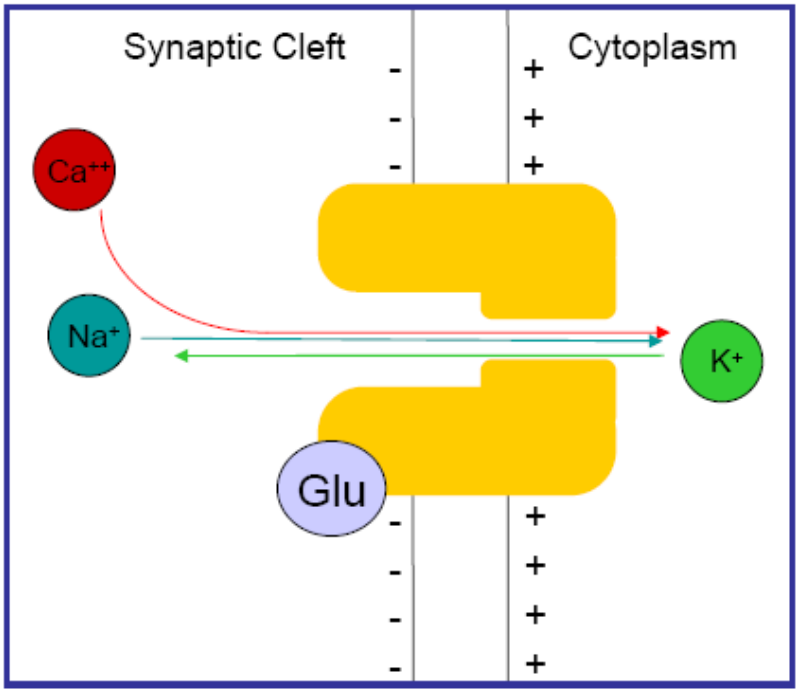


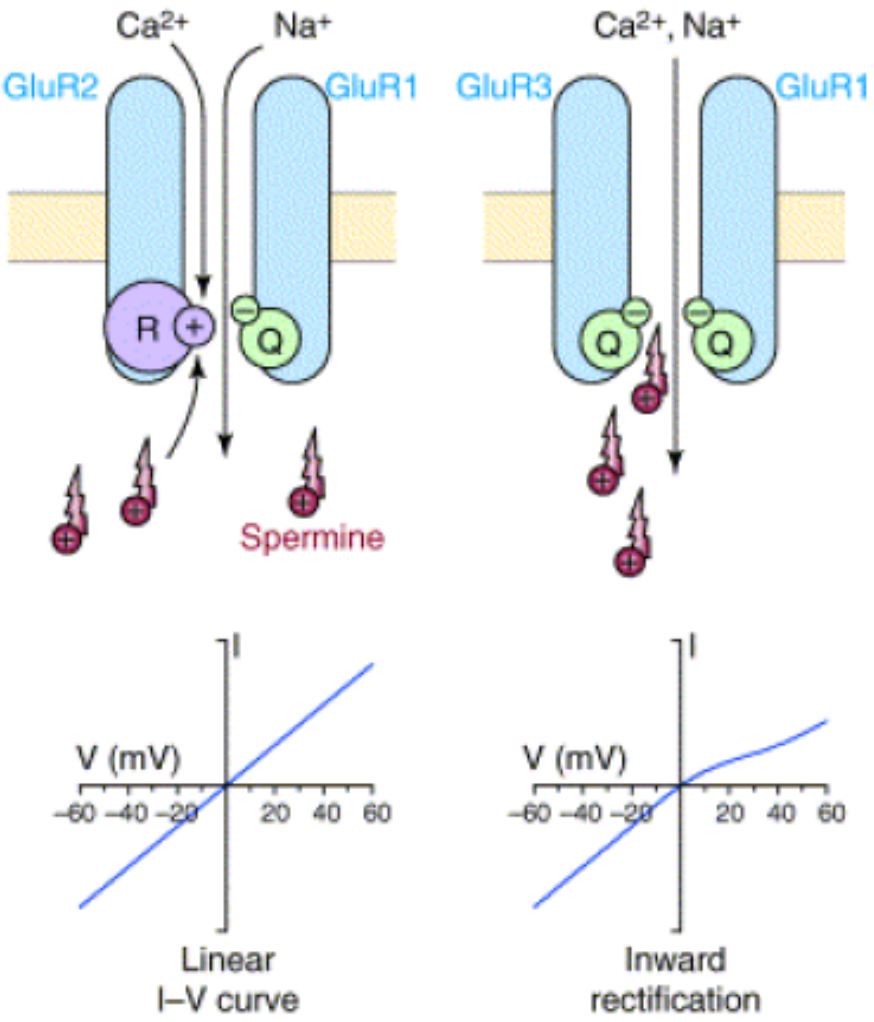
The functional interest for this "editing" comes from the fact that it influences the receptor-channel Ca2 + permeability: it has been observed that the same subunit contributes positively to the Ca2 + permeability of the whole molecular complex when the "site" is in the version "Q" (occupied by a glutamine),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

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



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

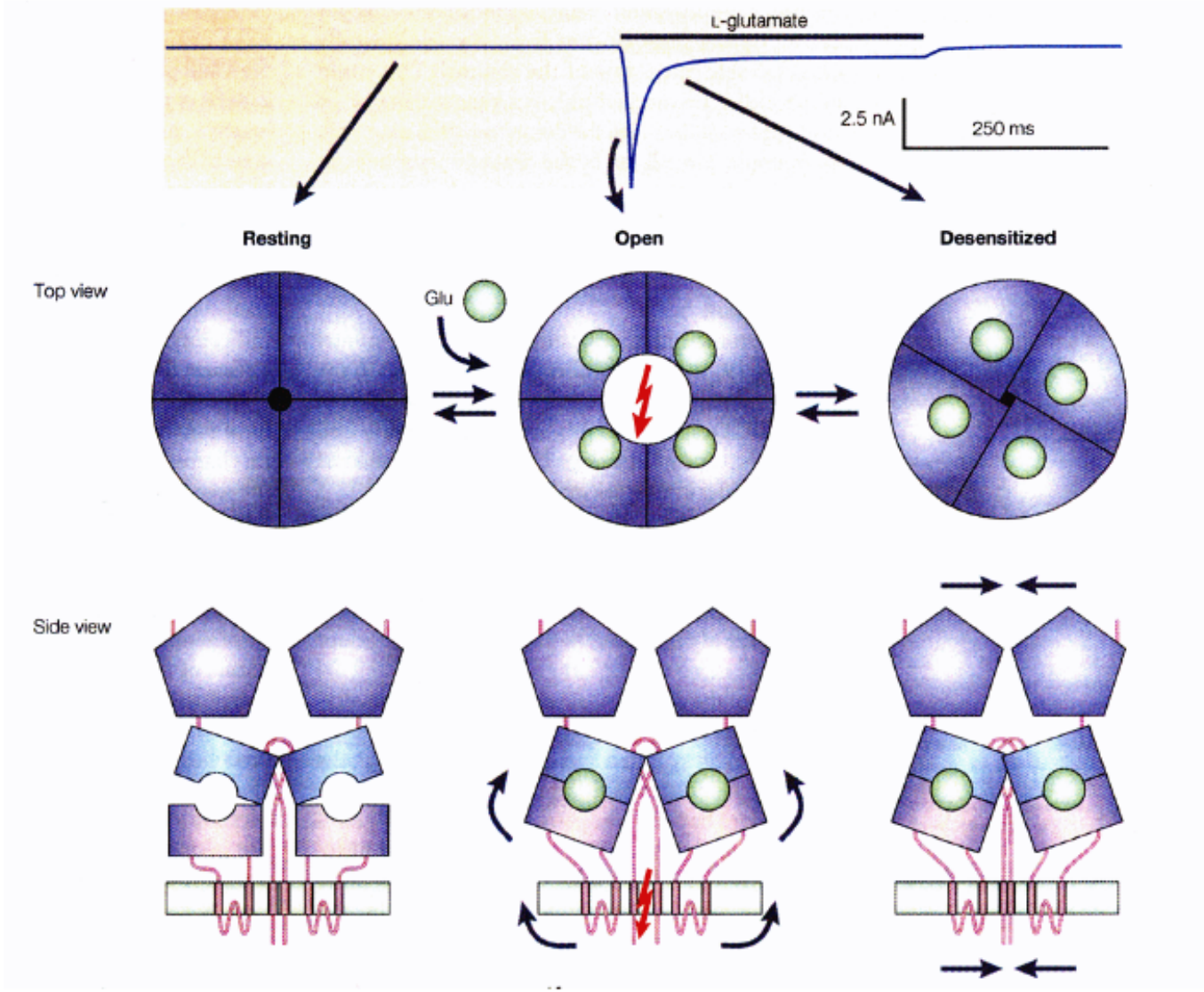




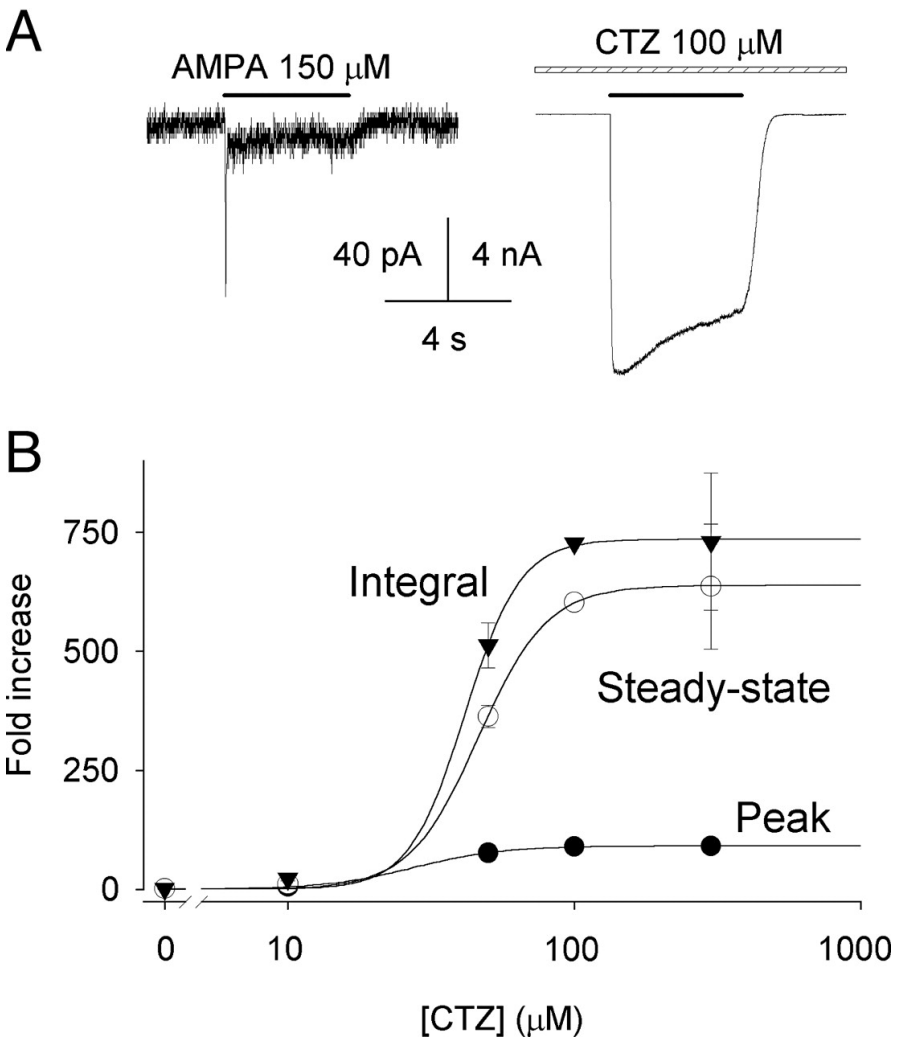
AMPAR containing the GLUR2 subunit have a linear I/V plot

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

AMPA Receptors Desensitize Over Prolonged Exposure to Glutamate

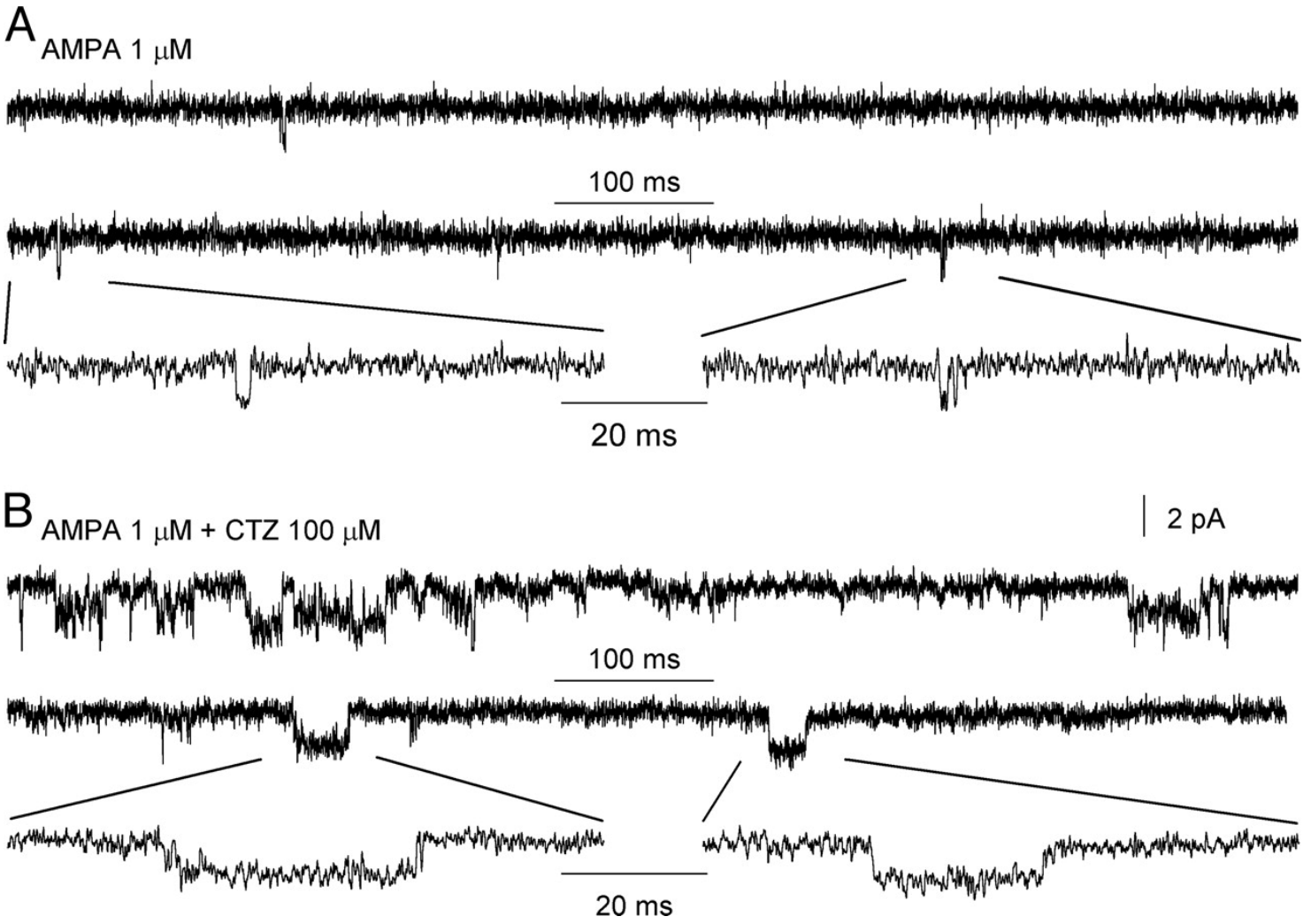


Effects of CTZ on whole-cell currents elicited by AMPA



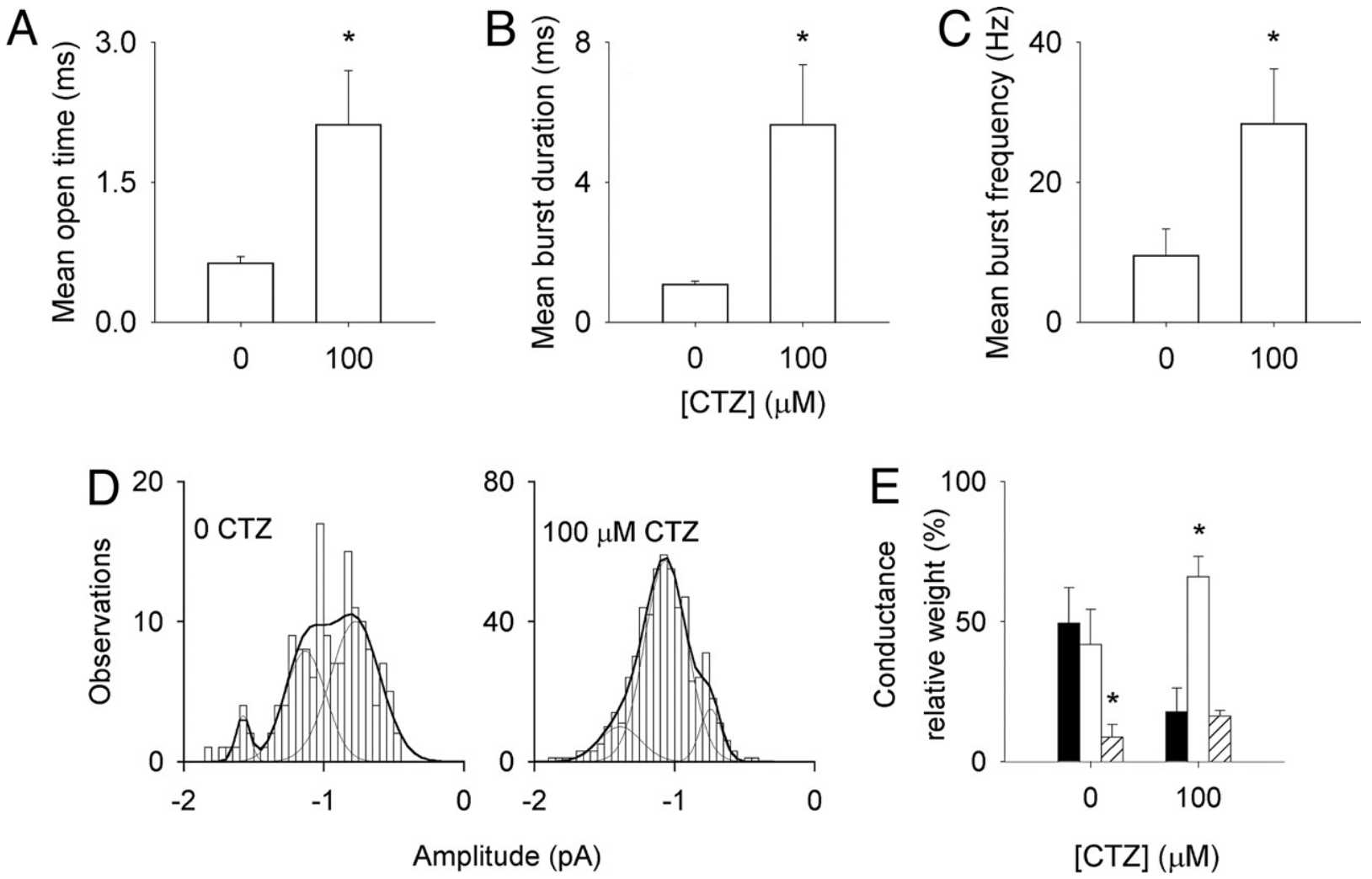
Fucile S. et.al. PNAS 2006;103:2943-2947

Effects of CTZ on single-channel currents



Fucile S. et.al. PNAS 2006;103:2943-2947

Effects of CTZ on single-channel properties



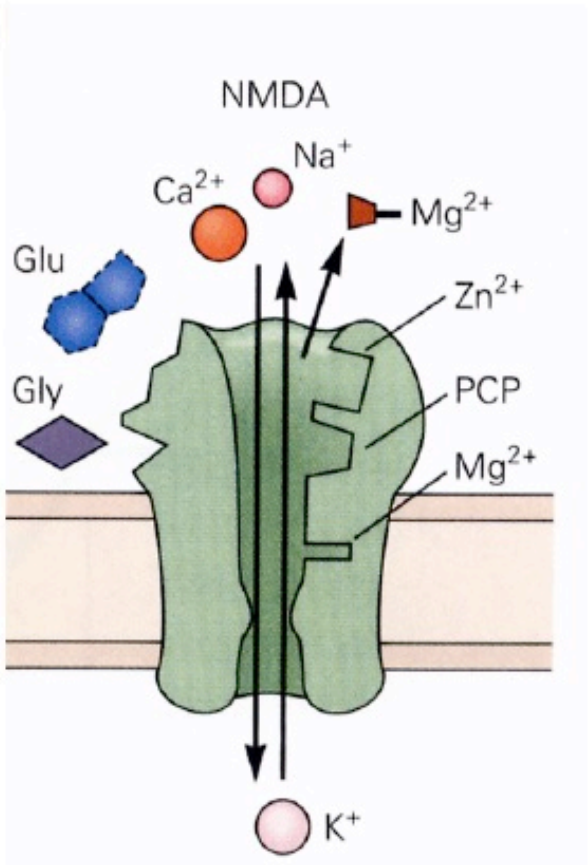
Fucile S. et.al. PNAS 2006;103:2943-2947

Kainate receptors

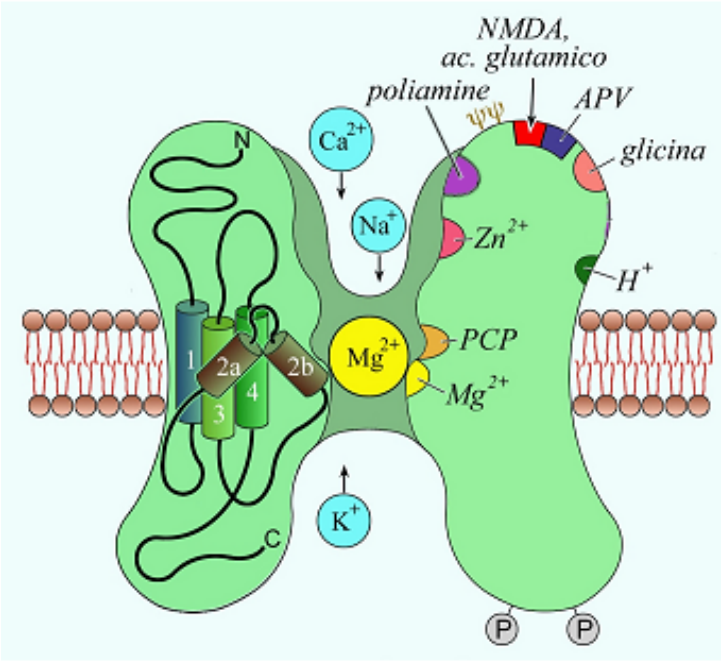
- KAR are widespread throughout the SNC (but less abundantreceptors for AMPA)
- KAR are permeable to Ca+
- Responsible for rapid synaptic transmission
- Possible presynaptic functions: control on the NT release

NMDA Receptors

5 Genes Code for NMDARs (NR1 and NR2A-D).
A functional NMDAR is made of four subunits (tetramer).
The NR1 subunit is obligatory. It forms heteromers with the NR2.
The NR2 subunits bind Glutamate
NMDARs are permeable to Ca^{2+}

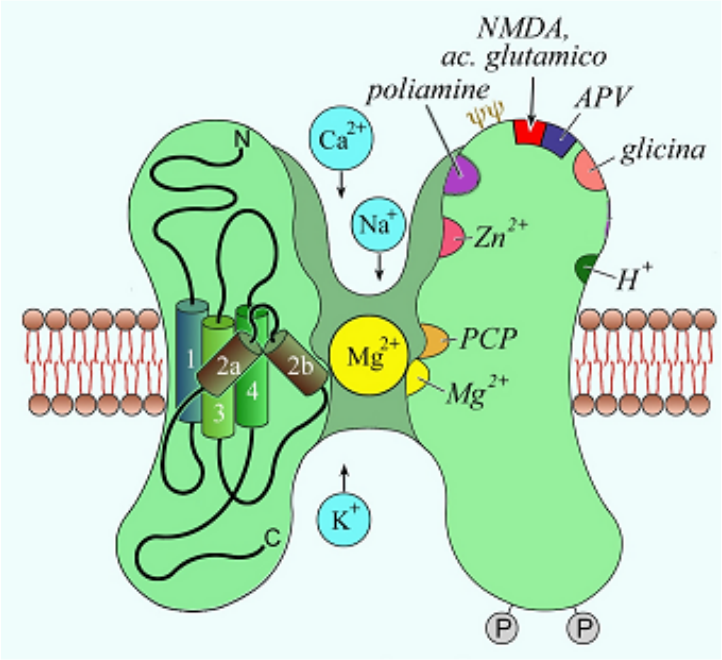


NMDA Receptors



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

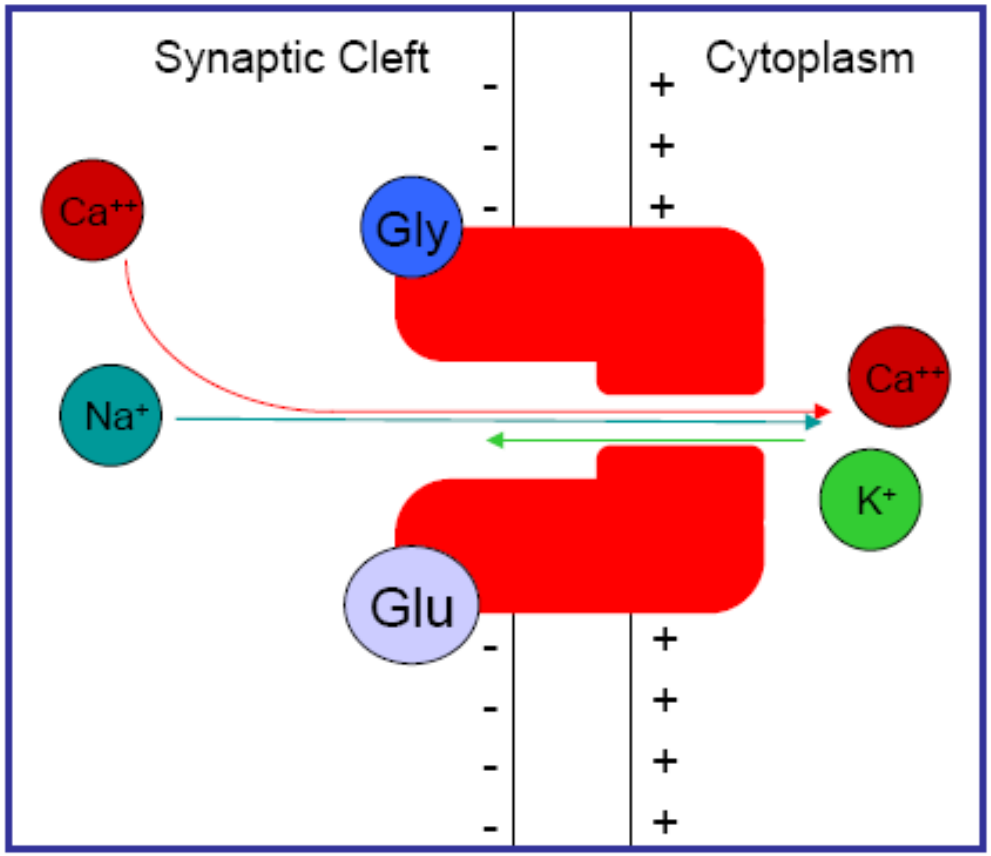


NMDAR have a considerable voltage-dependence: a unique property among all ionotropic receptors.

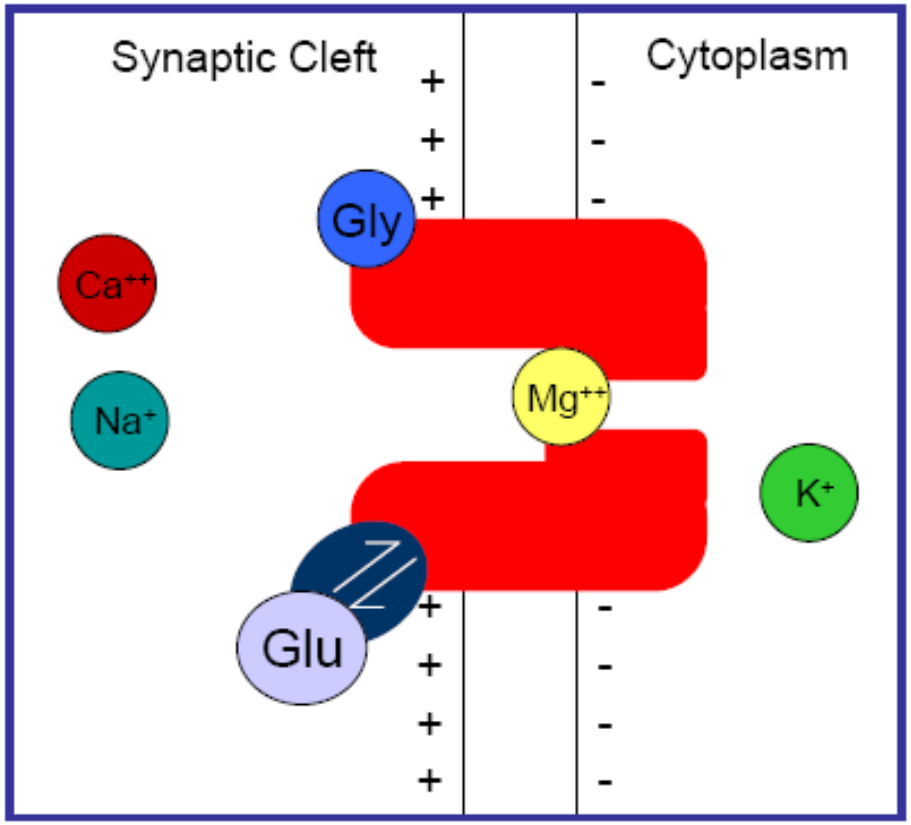
This voltage-dependence is interpreted as a voltage-dependent block of extracellular Mg2+, which recalls the voltage-dependent block of the intracellular Mg2+ of the inward rectifiers.

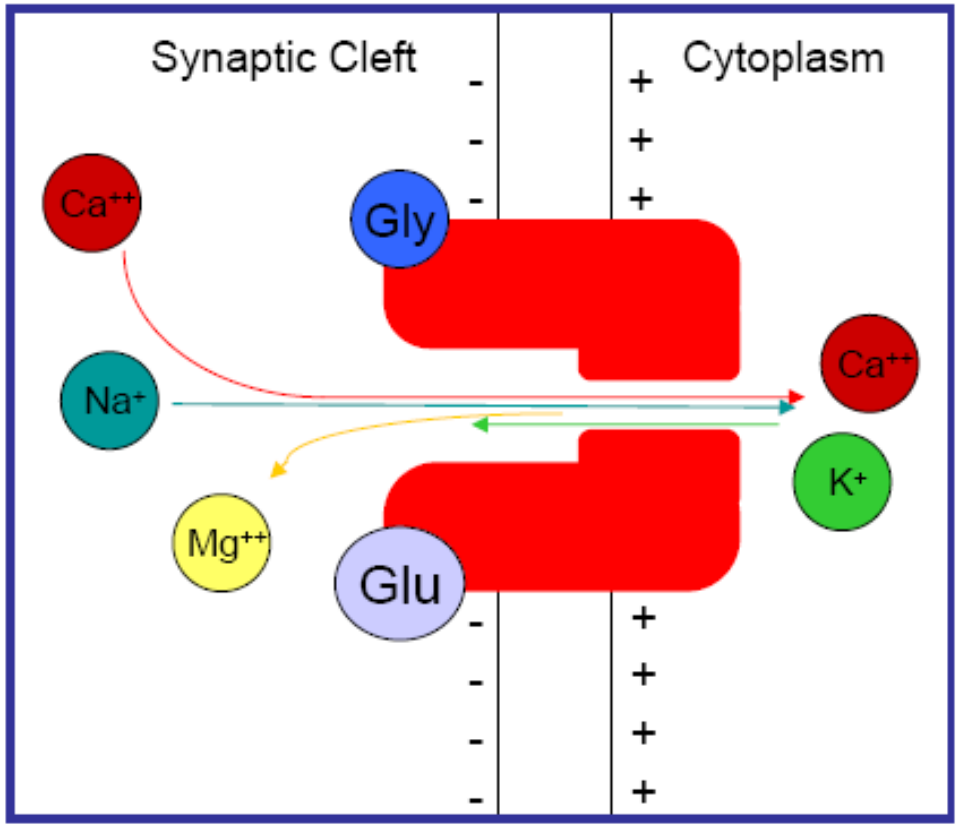
NMDARs are permeable to K+, Na+ and Ca++

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

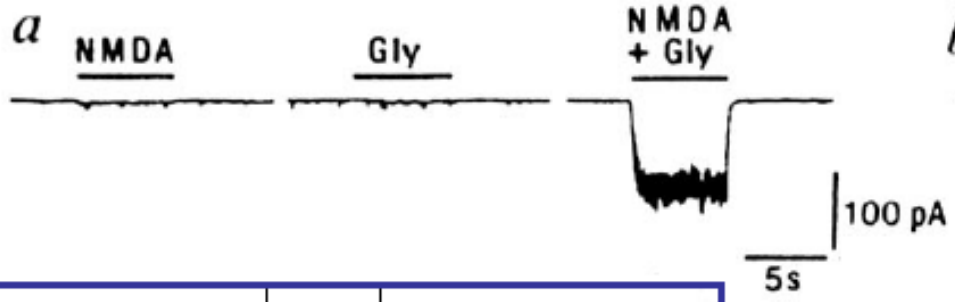


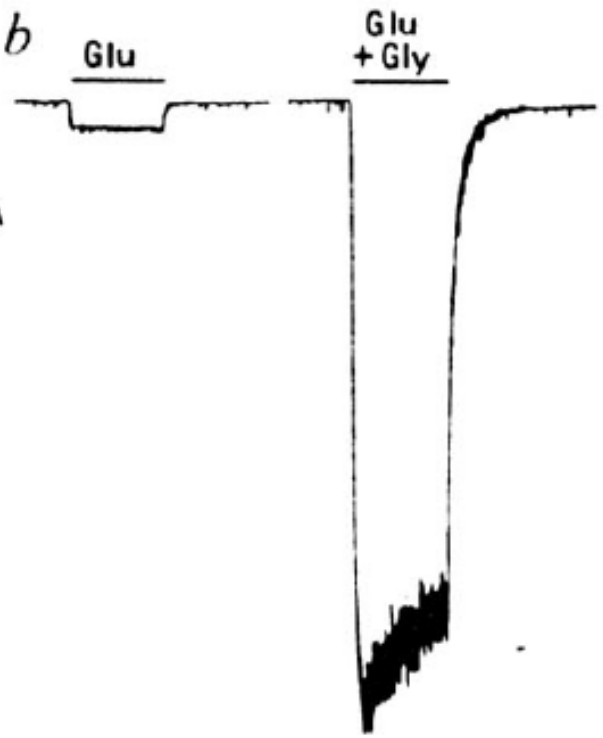
At negative potentials current through NMDARs is blocked by Mg^{++} (voltage dependent block)

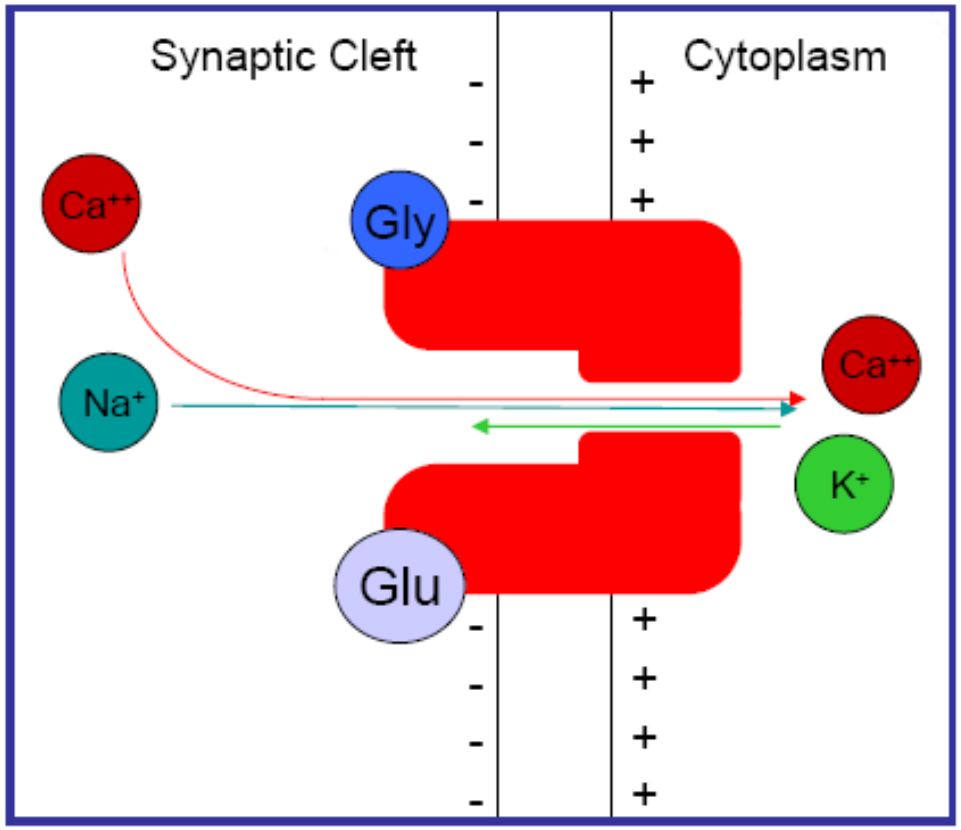




To Open, NMDA receptors necessitate a co-agonist: L-glycine (or D-serine)

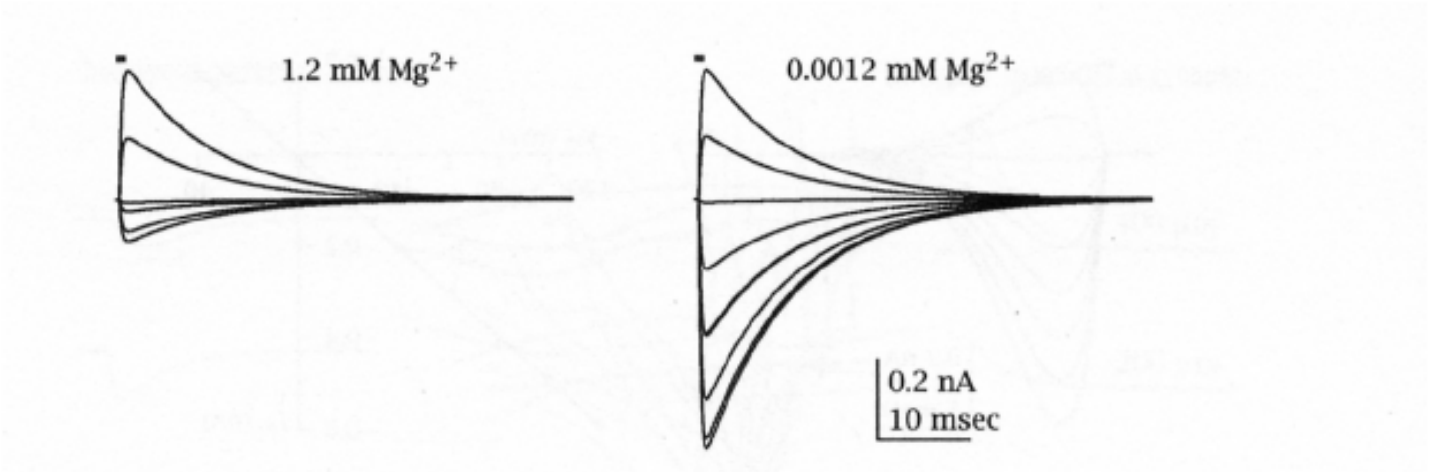


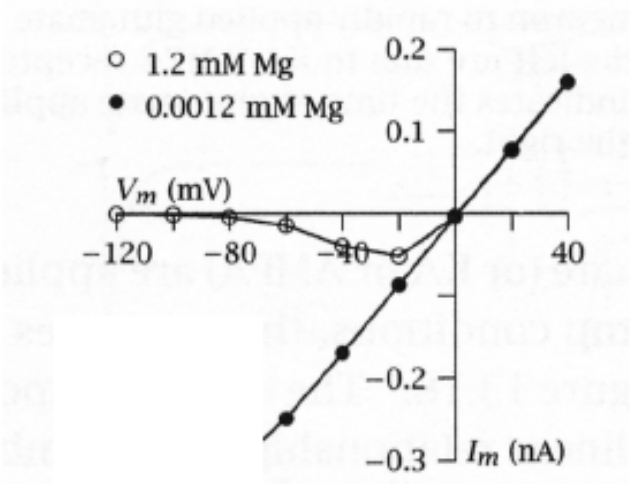




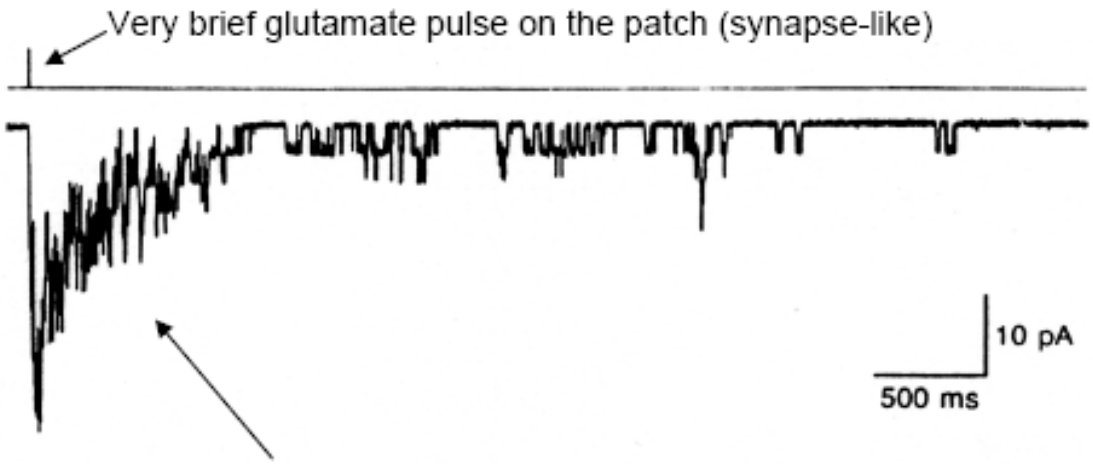
L-Glycine (and D-Serine) bind with very high affinity to the NMDA receptor (to the the NR1 subunit).
Normally, the "ambient" concentration of these molecules in the cerebrospinal fluid is sufficiently high for them to occupy their binding site on the NMDA receptor.

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



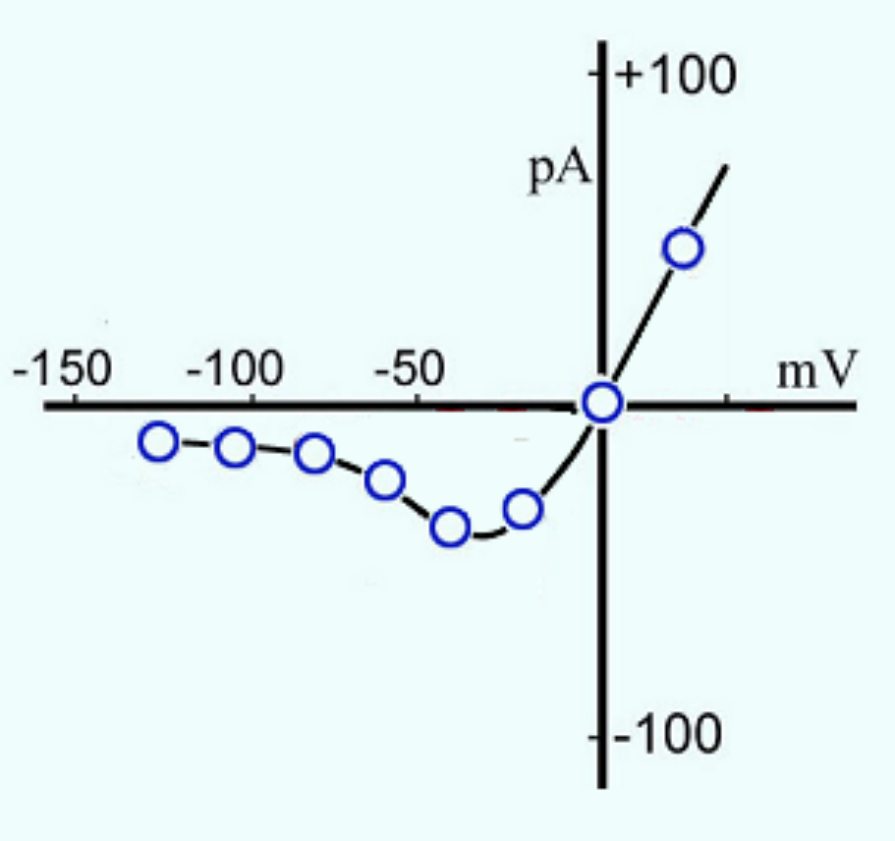


Gating of NMDA receptors is slow



Current through NMDA receptors in an outside-out patch of a neuronal membrane

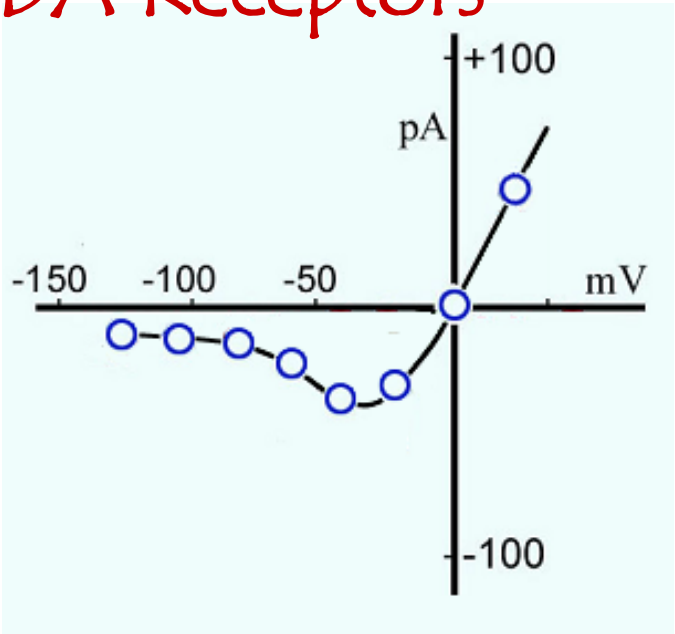
NMDA Receptors



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. glutamic and the channel goes into the "open" state, it does not conduct any current (due to the "plug").

NMDA Receptors

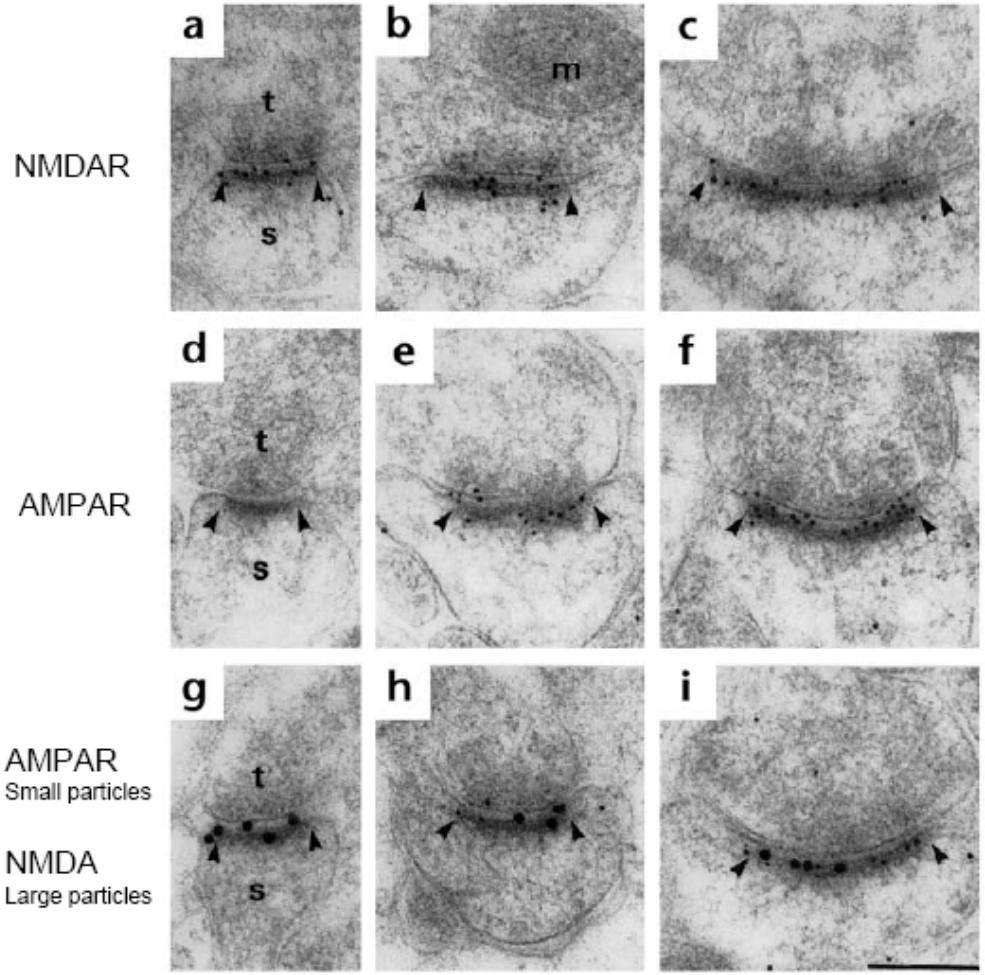


In order for the NMDA channel receptors to produce an EPSP in the postsynaptic membrane, the voltage-dependent plugs must be expelled by electrostatic repulsion, which requires the membrane to be depolarized.

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.

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.

NMDARs are coincidence detectors of pre- and postsynaptic activity