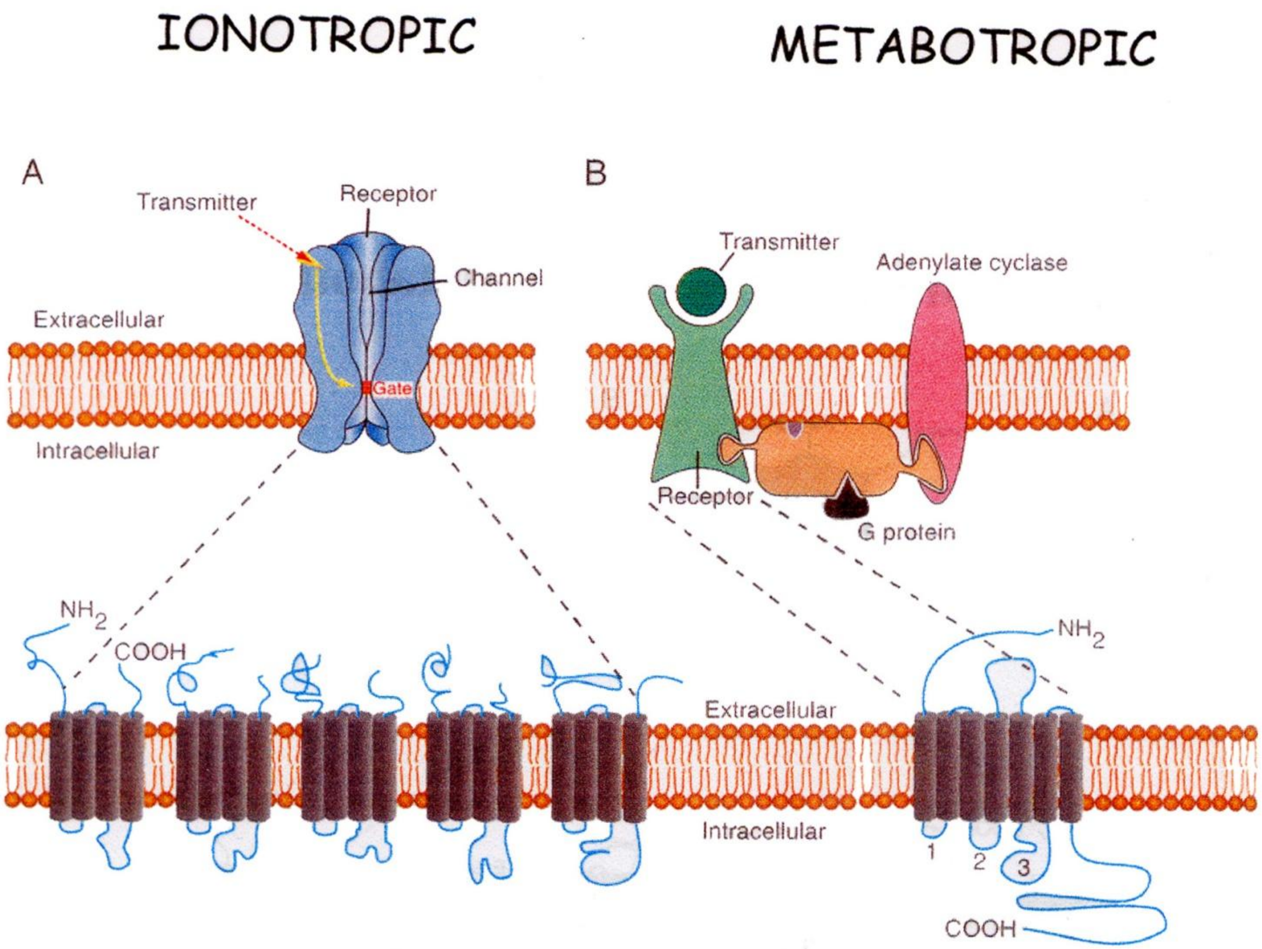
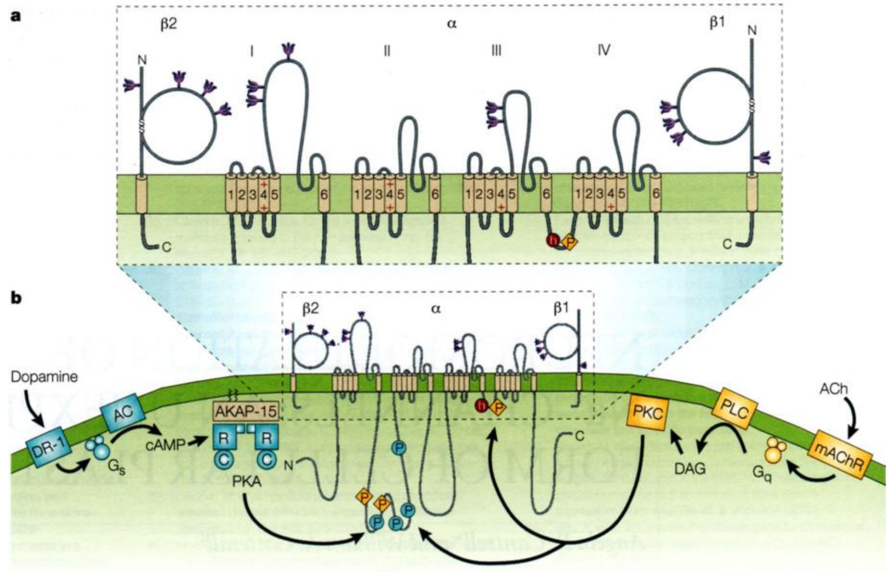
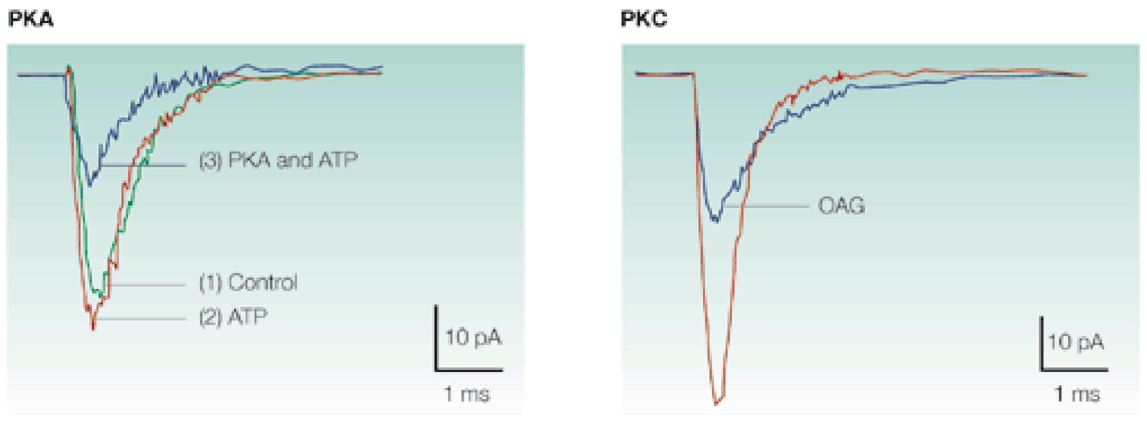
Neurotransmitters act on specific receptors



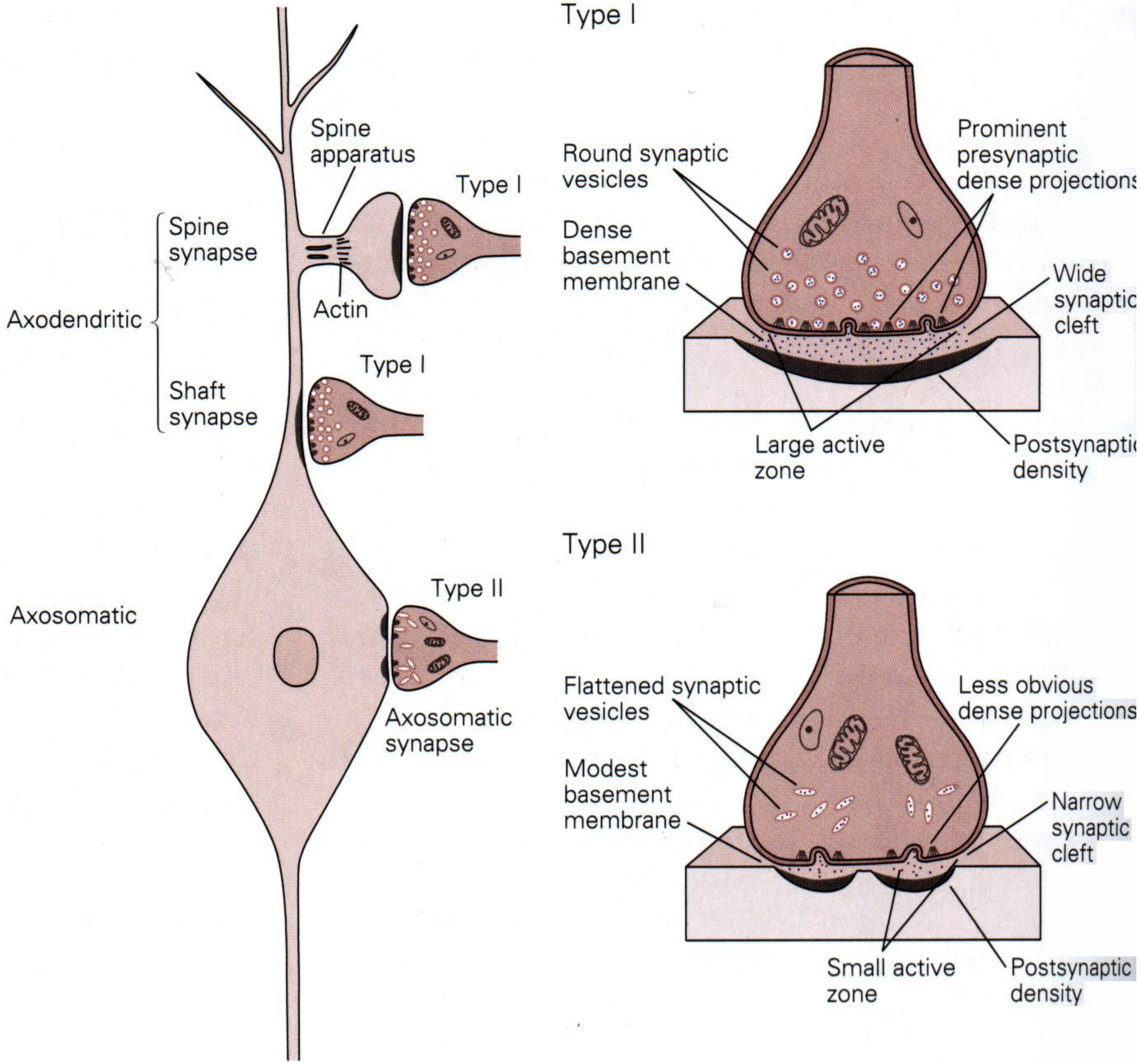


Modulation of Na^{+} channels by neurotransmitters acting through PK has important effects on neuronal activity influencing action potential generation and synaptic transmission



Cantrel & Catteral, Nature Neurosci, 2001

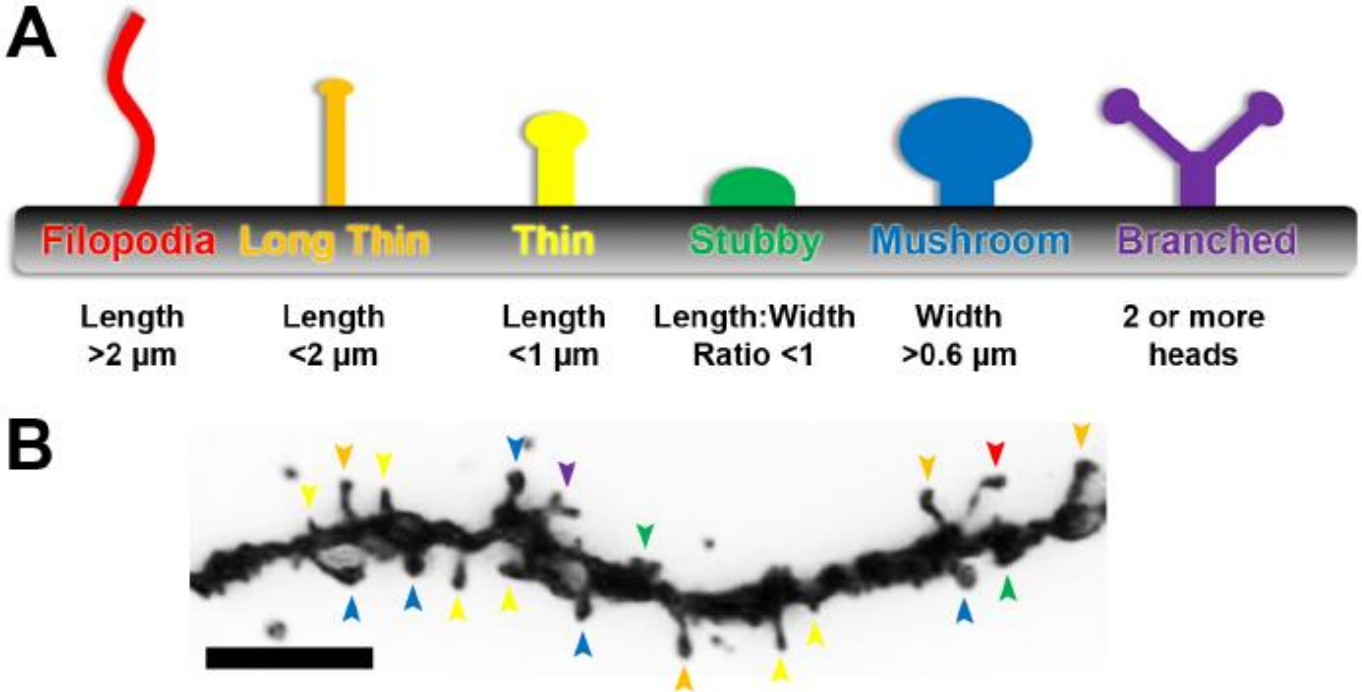
Neurotransmitter	Ionotropic receptors	Metabotropic receptors	
Amino acids			
GABA	Yes (inhibitory)	Yes	
Glutamate	Yes (excitatory)	Yes	
Glycine	Yes (inhibitory)	Yes	
Biogenic amines			
Dopamine		Yes	Catecholamines deriving from tyrosine
Noradrenaline		Yes	
Adrenaline		Yes	
Serotonin	Yes (excitatory)	Yes	from tryptophan
Histamine		Yes	from histidine
Purinergic			
Adenosine		Yes	
ATP	Yes (excitatory)	Yes	
Acetylcholine	Yes (excitatory)	Yes	
Neuropeptides (many)		Yes	



Excitatory

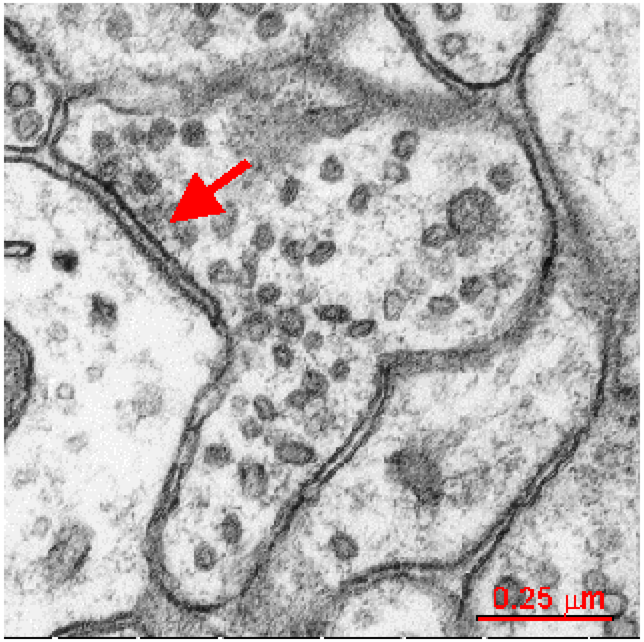
asymmetric

Inhibitory


A
Filopodia
Long Thin
Thin
Stubby
Mushroom
Branched
Length >2 µm
Length <2 µm
Length <1 µm
Length:Width Ratio <1
Width >0.6 µm
2 or more heads
B

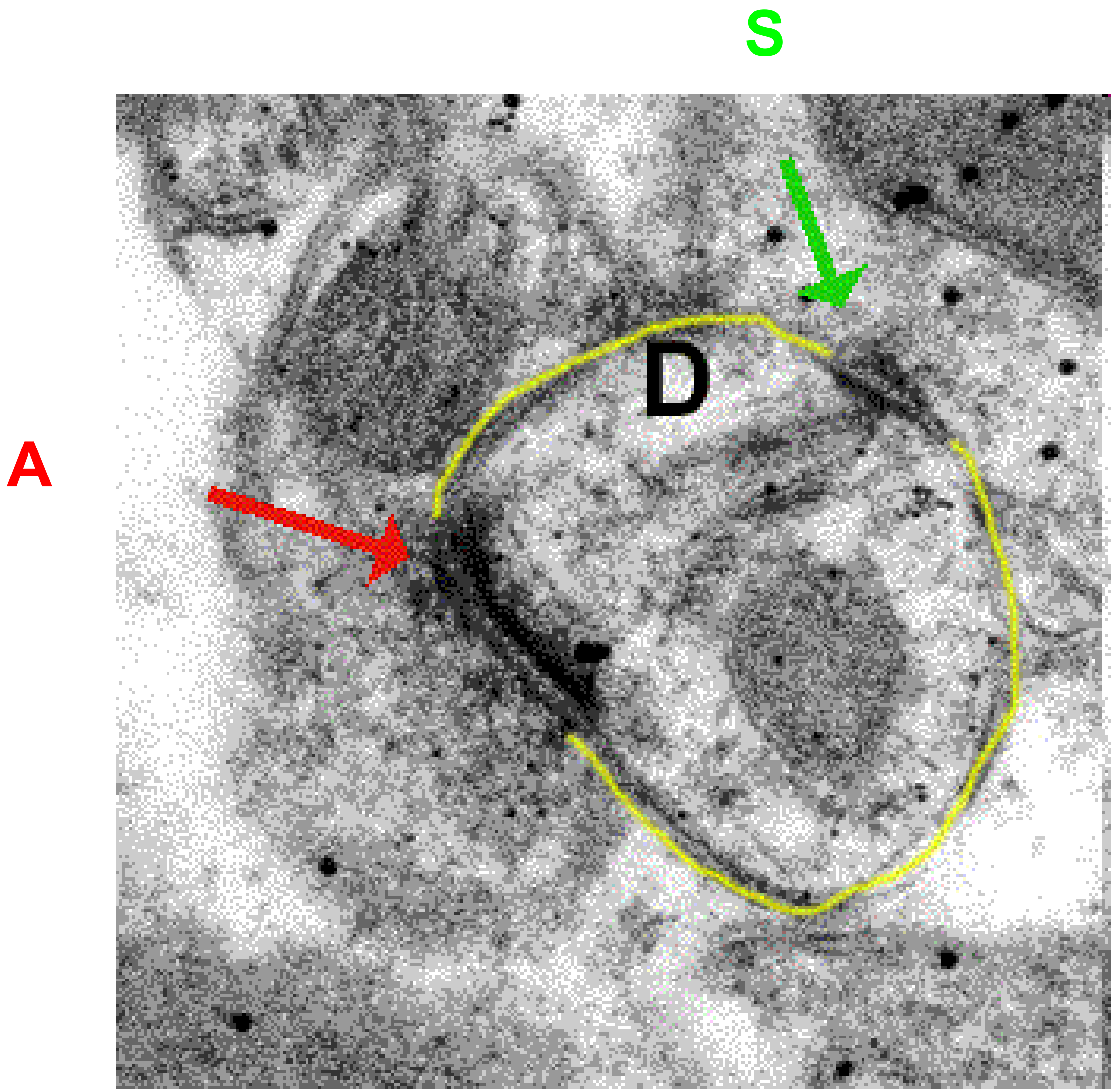
Asymmetric Glu synapse

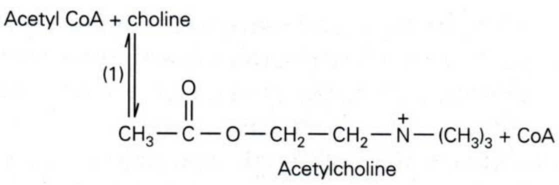
Symmetric GABAergic synapse



not prominent PSD

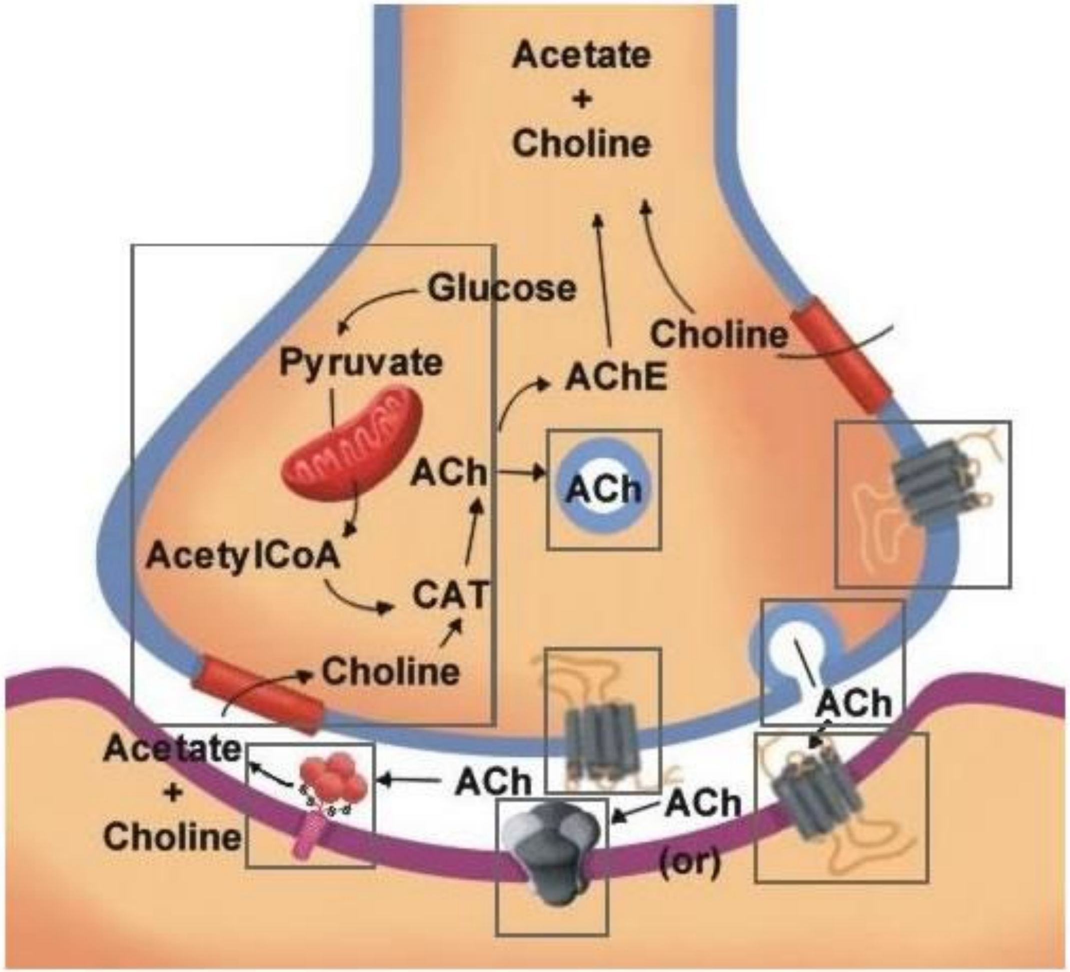
Symmetrical and asymmetrical synapses on single dendrites





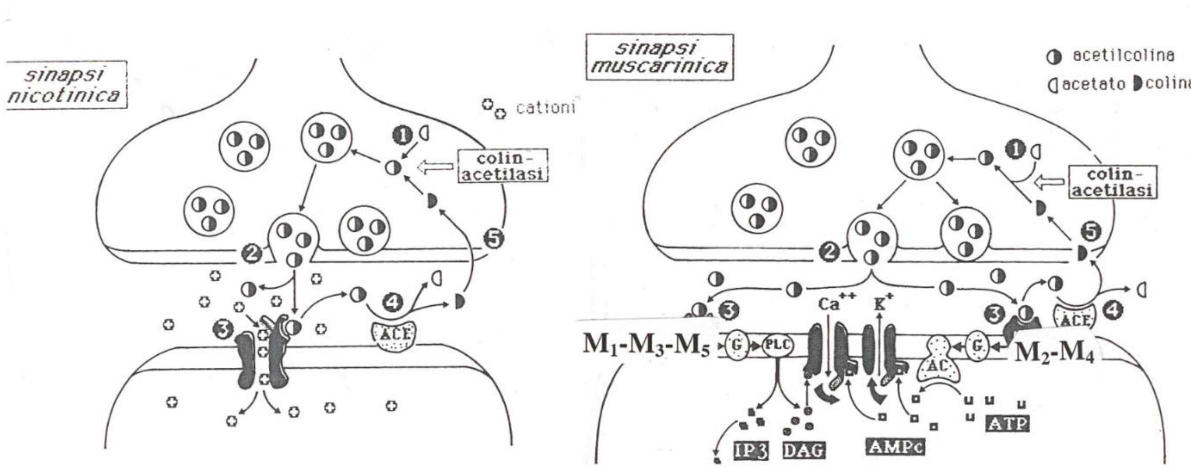
Acetylcholine does not directly come from an AA.

Choline acetyltransferase ChAT: acetil-CoA + colina ⇄ CoA + ACETYLCHOLINE



AChE breaks down ACh stopping its action at the synapse

Nicotinic and muscarinic receptors



- Farmacologia delle sinapsi colinergiche

	Azione	**Nicotiniche**	**Muscariniche**
a livello postsinaptico		Acetilcolina	
a livello postsinaptico	attivazione dei recettori *(agonisti)*.	nicotina tetra-metil-ammonio	muscarina pilocarpina oxotremorina
a livello postsinaptico	blocco dei recettori *(antagonisti)*	d-tubocurarina gallamina esametonio pentolinio α-bungarotossina	atropina scopolamina (ioscina) pirenzepina

Amanita muscaria

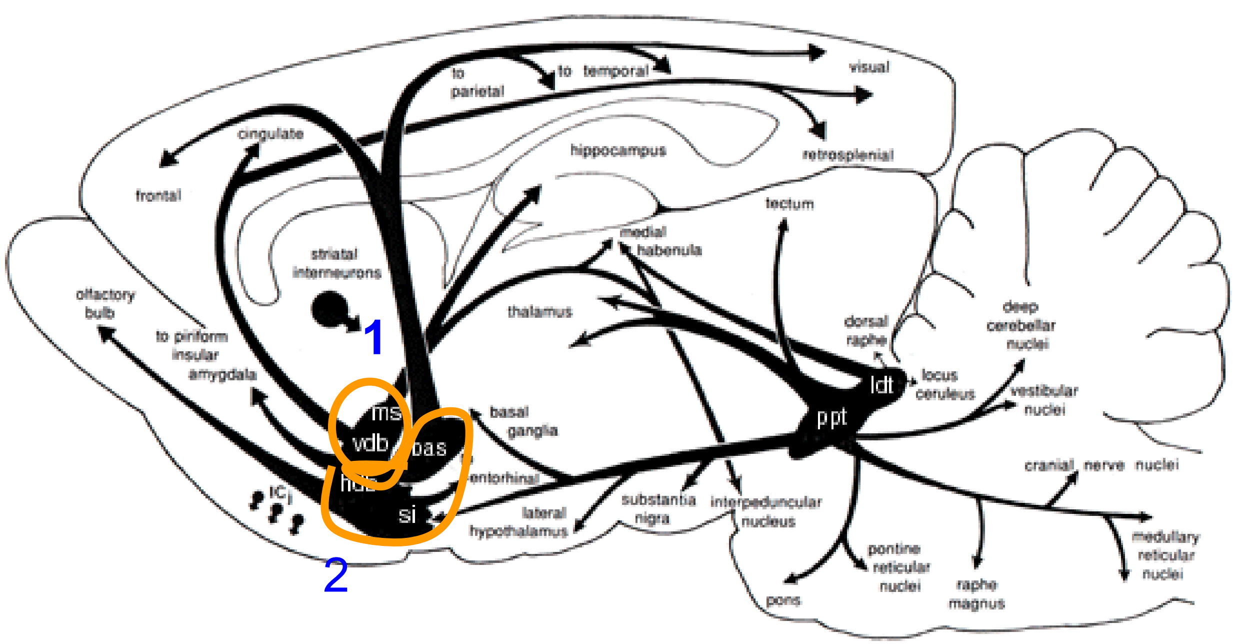
Taglietti e Casella, Elementi di Fisiologia e Biofisica della cellula, La Goliardica Pavese

Two groups of cholinergic neurons in **BASAL FOREBRAIN**

(1) the medial septal group (medial septal nucleus and vertical diagonal band: ms and vdb) that project cholinergic axons to the hippocampus and parahippocampal gyrus

(2) the nucleus basalis group (nucleus basalis, substantia innominata and horizontal diagonal band: bas, si, hdb) that project cholinergic axons to all parts of the neocortex, parts of limbic cortex and to the amygdala.

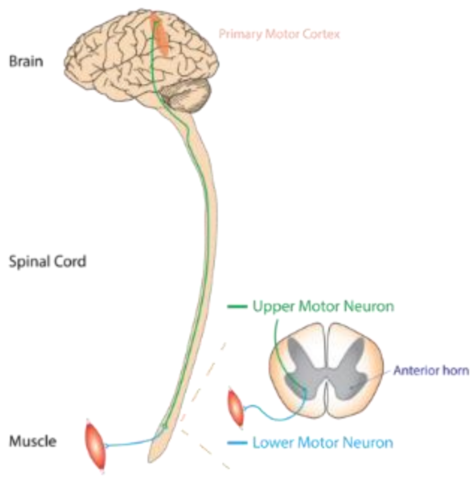
The cholinergic **PONTOMESENCEPHALON** neurons (laterodorsal tegmental and pedunculopontine tegmental nuclei: ldt and ppt) project onto hindbrain, thalamus, hypothalamus and basal forebrain.

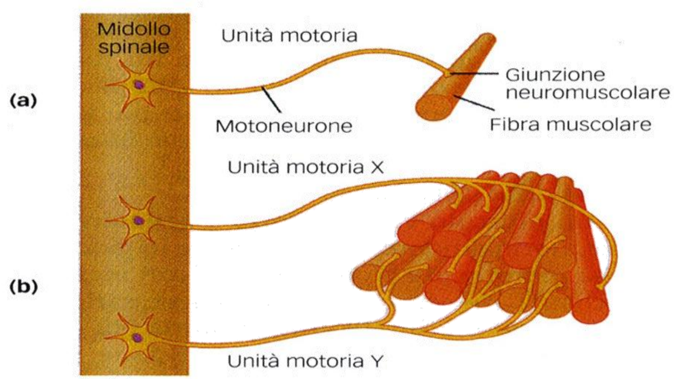


After Woolf, 1991

Central cholinergic pathways are ideally suited to regulate global functions that depend on the cerebral cortex; such functions include attention, arousal, motivation, memory and consciousness (Woolf, 1991; 1996).

Ach is also used by all the spinal MNs.

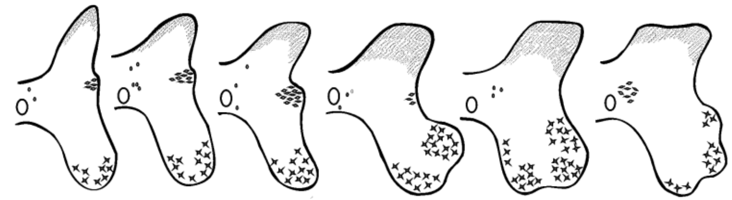




Distribution of positively-labelled cholinergic nerve cells in selected spinal segments

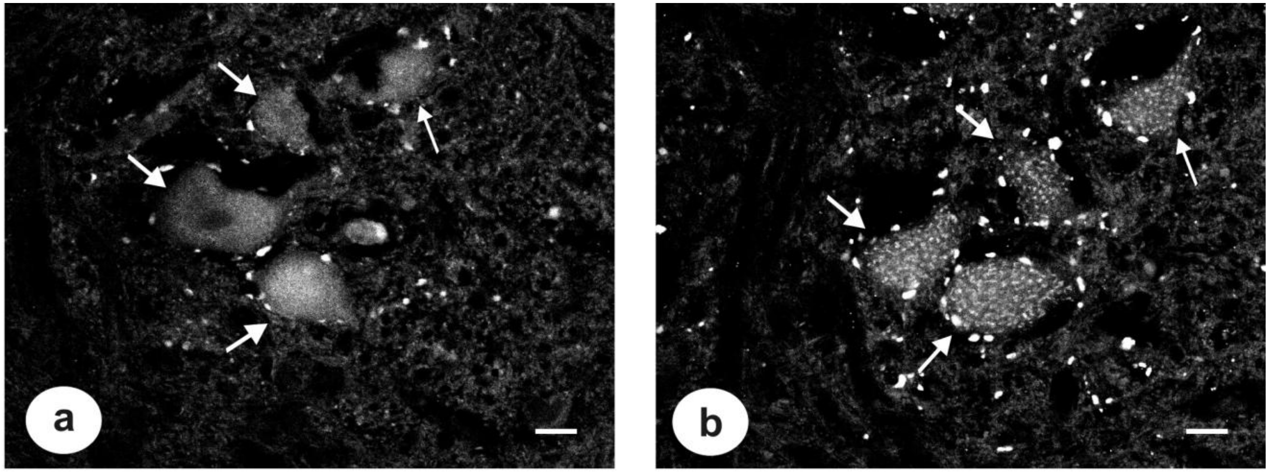
detected by immunostaining

Choline acetyltransferase (ChAT)
catalyzing the formation of ACh



Calka et al., Veterinarni Medicina, 53, 2008 (8): 434–444

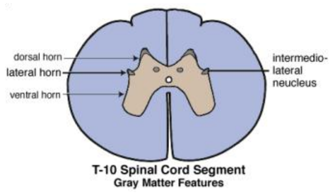
Vesicular acetylcholine transporter (VAChT)
able to load ACh into synaptic vesicles

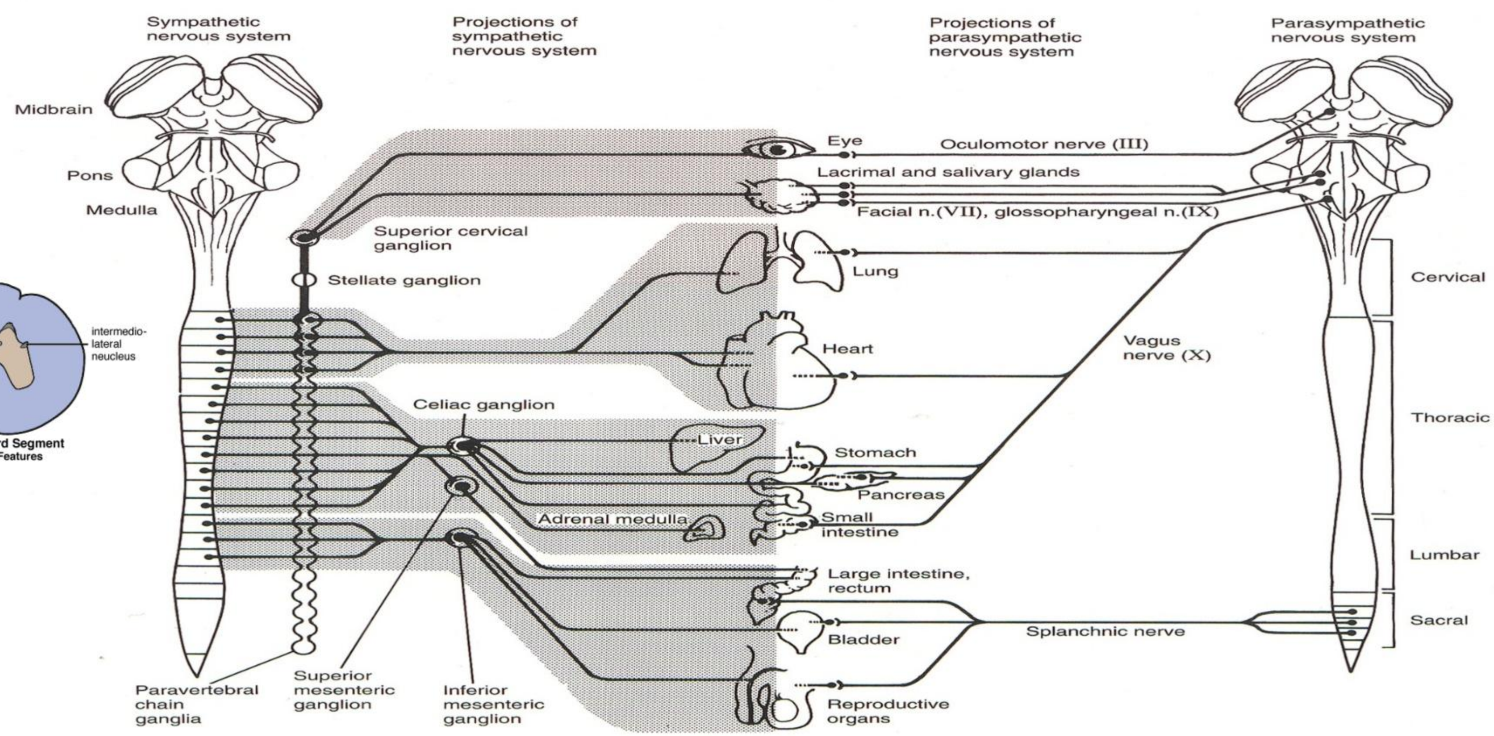


A pair of photomicrographs demonstrating different nature of the ChAT and VAChT stainings of the same ventral horn cells on the consecutive sections; (**a**) “smooth” ChAT-positive motor neurons (arrows) and (**b**) “clumpy” VAChT-immunoreactive cells (arrows).

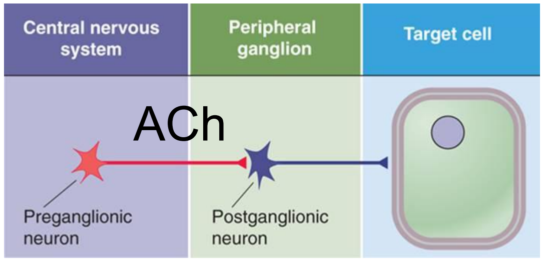
SYMPATHETIC PARASYMPATHETIC

Autonomic NS





ACh is the neurotransmitter of all the preganglionic neurons and in parasympathetic post-ganglionic neurons.



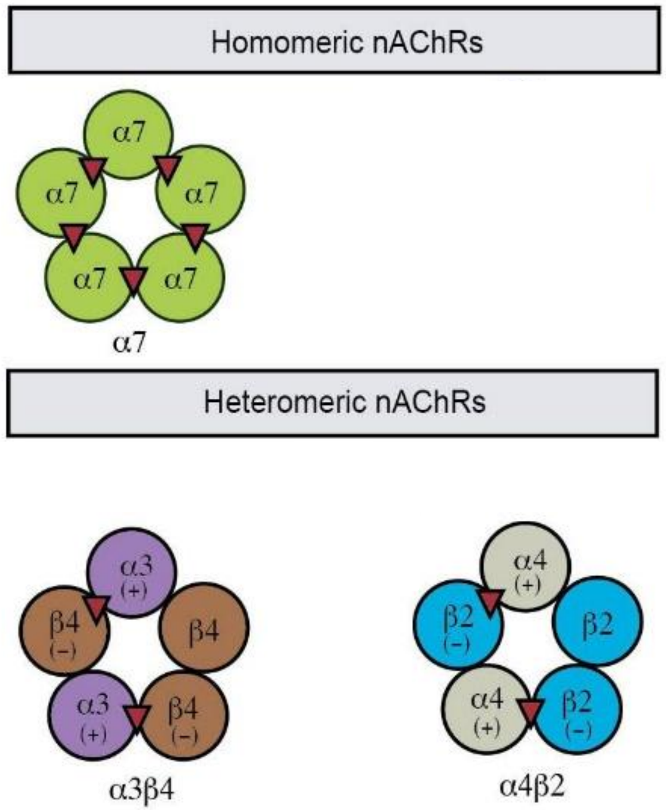
Kandel et al., Principles of Neural Science, Elsevier

Decline, disruption or alterations of nicotinic cholinergic mechanisms contribute to dysfunctions such as:

epilepsy, schizophrenia, Parkinson’s disease, autism, dementia, Alzheimer’s disease and addiction.

Subunit composition of neuronal ionotropic AChRs

Permeable to Na^+, K^+, Ca^{2+}



αBgt
pCa/Na ~10

αBgt
pCa/Na ~0.2

9 different α subunits (α2-α10) and three β subunits (β2- β4) have been found expressed in vertebrate neuronal tissue

mAChRs

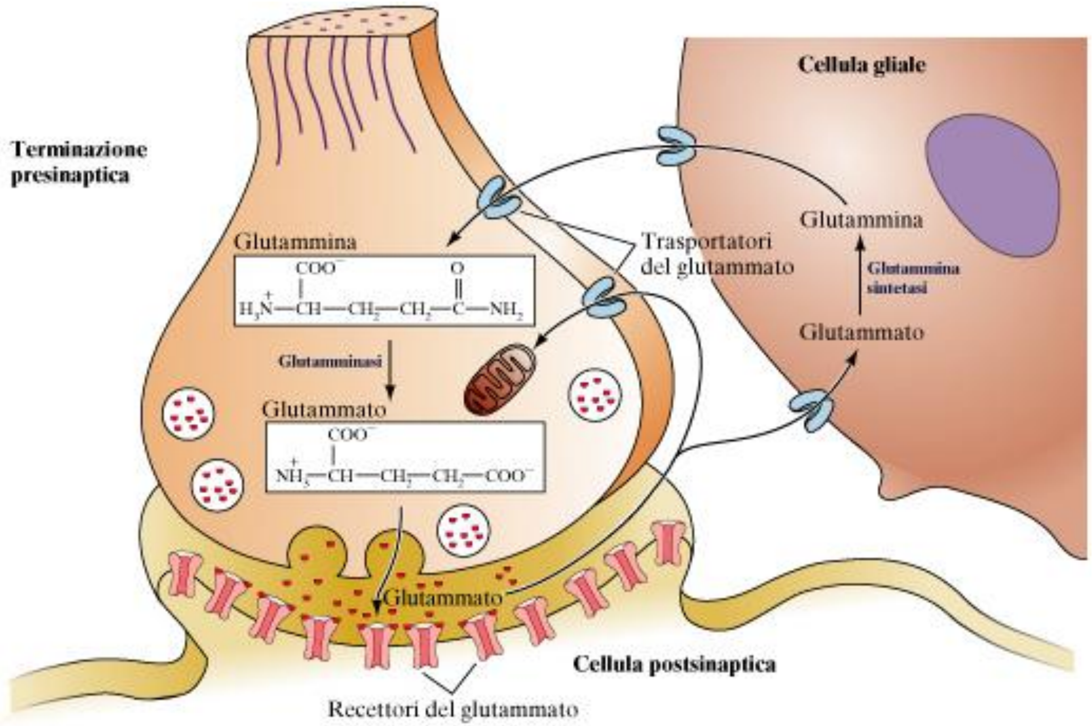
The predominant mAChR in CNS is M1 located **post-synaptically**.
Generally together with M3 and M5 activation leads to an increase in neuronal activity
M5 are highly localized in *substantia nigra*

M2 and M4 located predominantly **presynaptically** in brainstem and thalamus, in general negatively regulate the neurotransmitter release.
M4 mostly prominent in striatum

Glutamatergic synapses

Glutamate is present in high concentration in ALL the cells, because it is synthesized by normal metabolism in all the neurons (even non-glutamatergic), but it is stored in vesicles only in part of them (glutamatergic neurons)

Glutamatergic transmission



EAA: Na^+-dependent excitatory AA secondary active transporters that take Glutamate up into the cell against its concentration gradient.
To date, five different glutamate transporters subtypes have been cloned (EAAT 1-5)

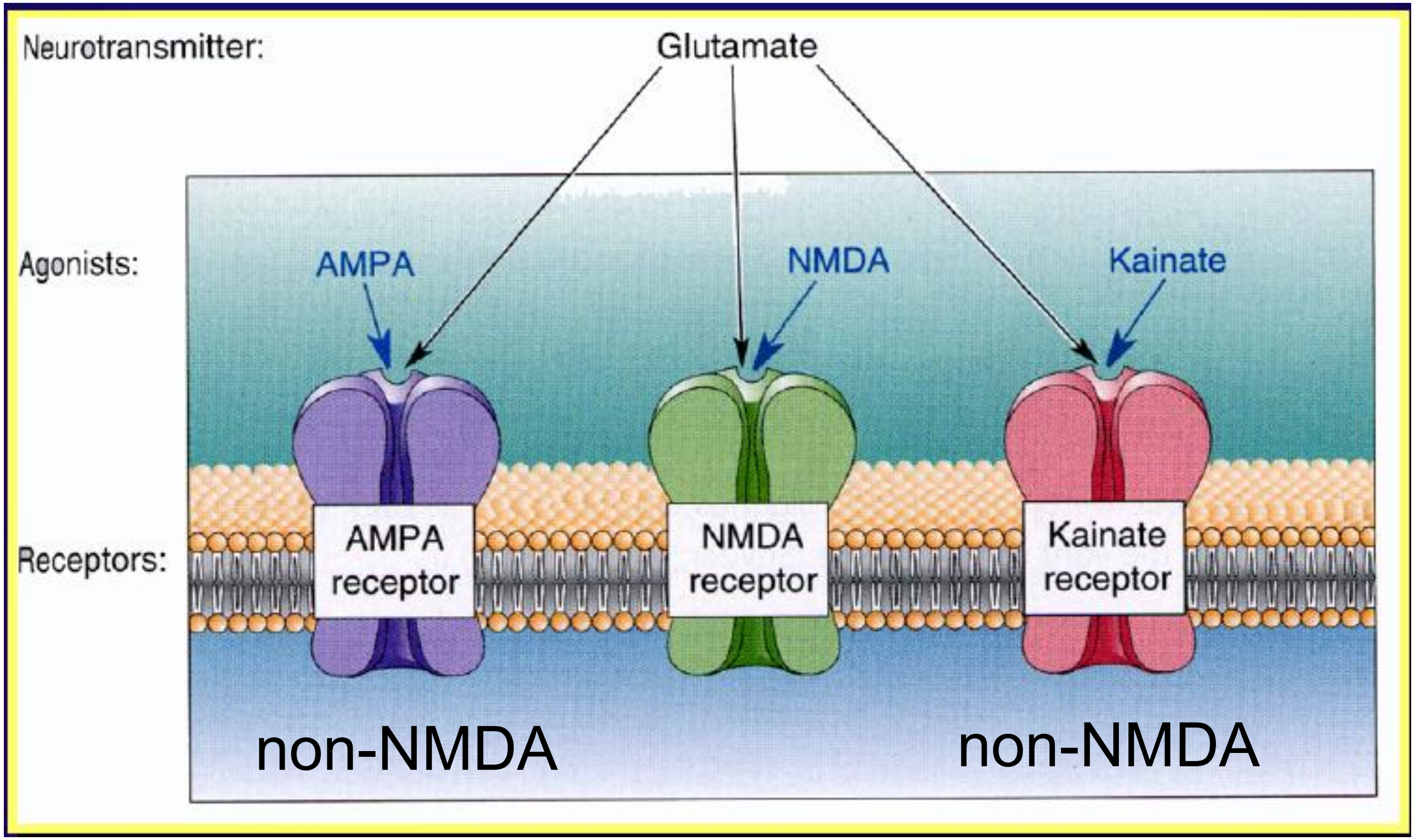
To date, five different glutamate transporters subtypes have been cloned (EAAT 1-5)

Glutamate Transporters Subtype	Rodent Homologue	Cell Type	DISTRIBUTION
EAAT1	GLAST	Astrocytes, oligodendrocytes	Cerebellum, cortex, spinal cord
EAAT2	**GLT-1**	**Astrocytes**	**Through the brain and spinal cord**
EAAT3	EAAC1	Mostly neurons. Also found in cells of glial origin (i.e., oligodendrocytes, glioma cells)	Hippocampus, striatum, cerebellum
EAAT4	EAAT4	Purkinje cells	Cerebellum
EAAT5	EAAT5	Photoreceptor and bipolar cells	Retina

Magi et al., Int J Mol Sci. 2019 20(22): 5674.

Na^+-dependent excitatory amino acid transporters (EAATs): glutamate–aspartate transporter (GLAST), glutamate transporter-1 (GLT-1), excitatory amino acid carrier1 (EAAC1).

Ionotropic glutamate receptors

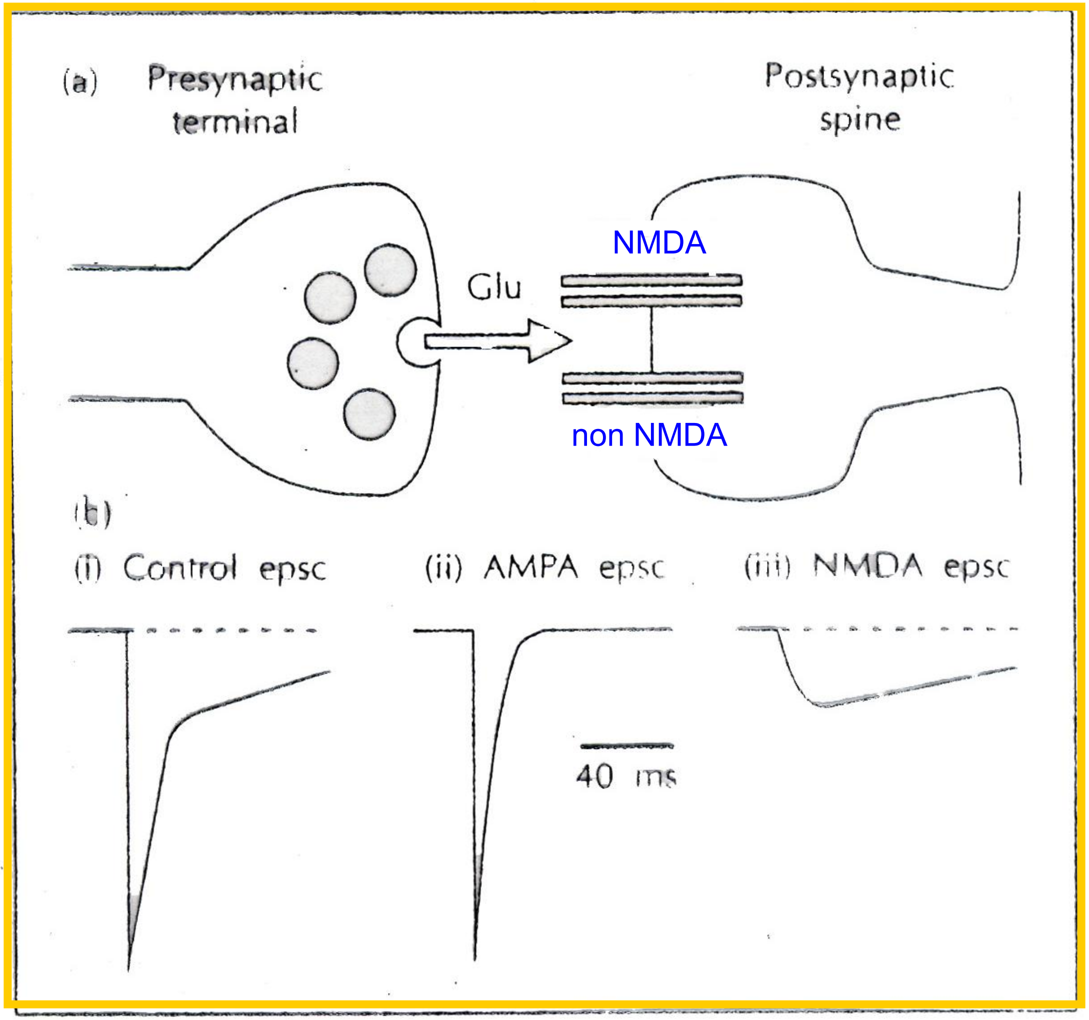


On the basis of their binding affinity for certain substances:

The names derive from the selective synthetic agonists that can be used to distinguish them

Excitatory post-synaptic currents

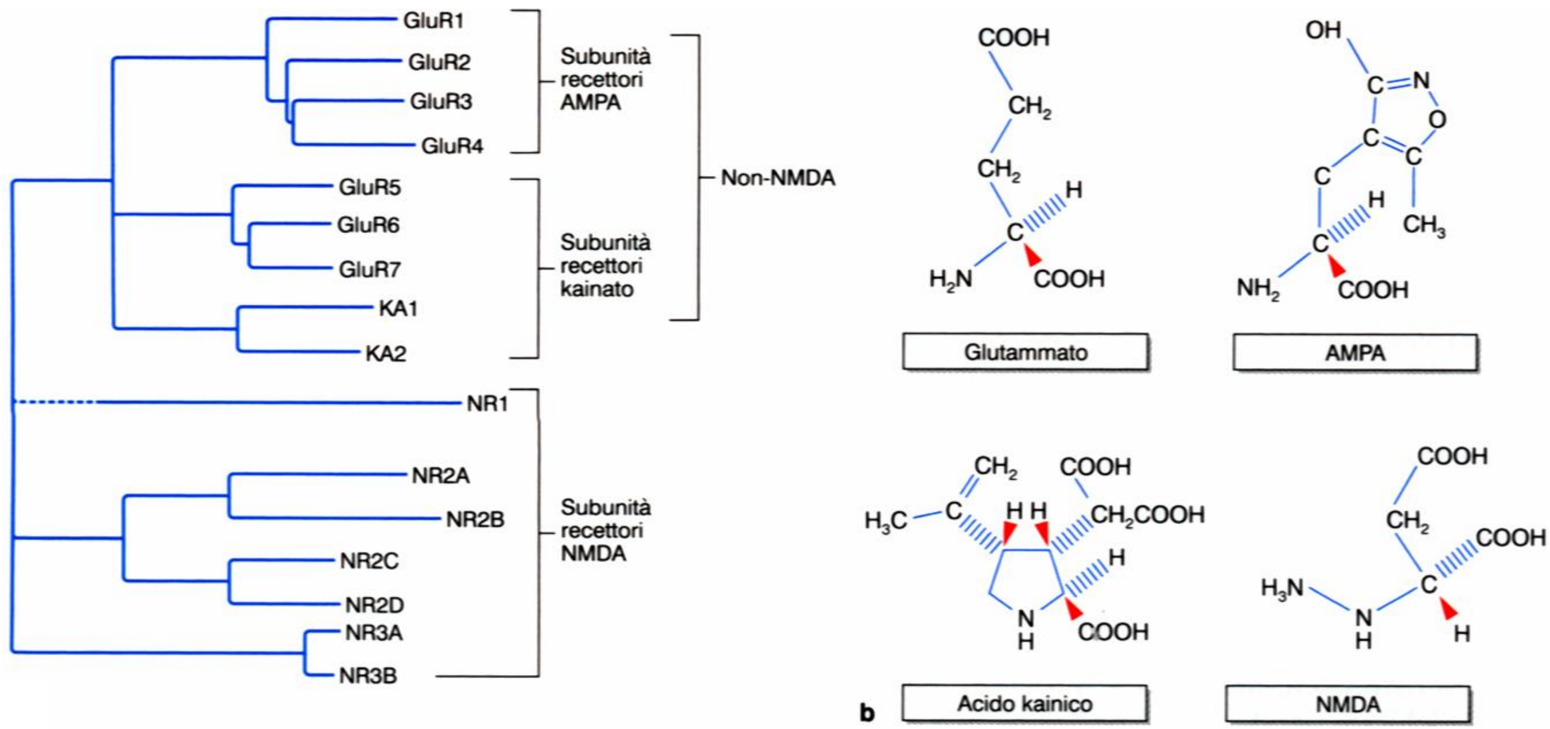
GLUTAMATE diffuses in the synaptic cleft



Glutamate receptor subunits

4 large subunits form a central ion channel pore.

Sequence similarity among all known glutamate receptor subunits, suggests they share a similar architecture.



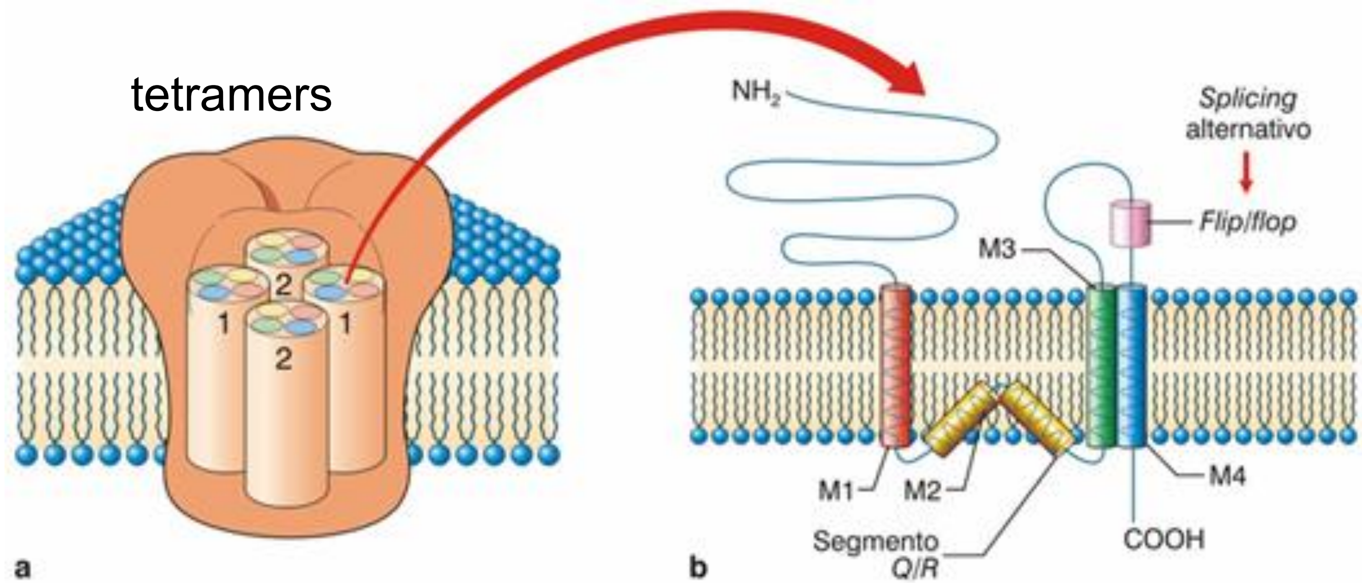
Non-NMDA R can be homo- and heteromers whereas NMDAR are only heteromers

AMPA Receptors

Permeable to Na^+ and K^+

homo- and heteromers

tetramers

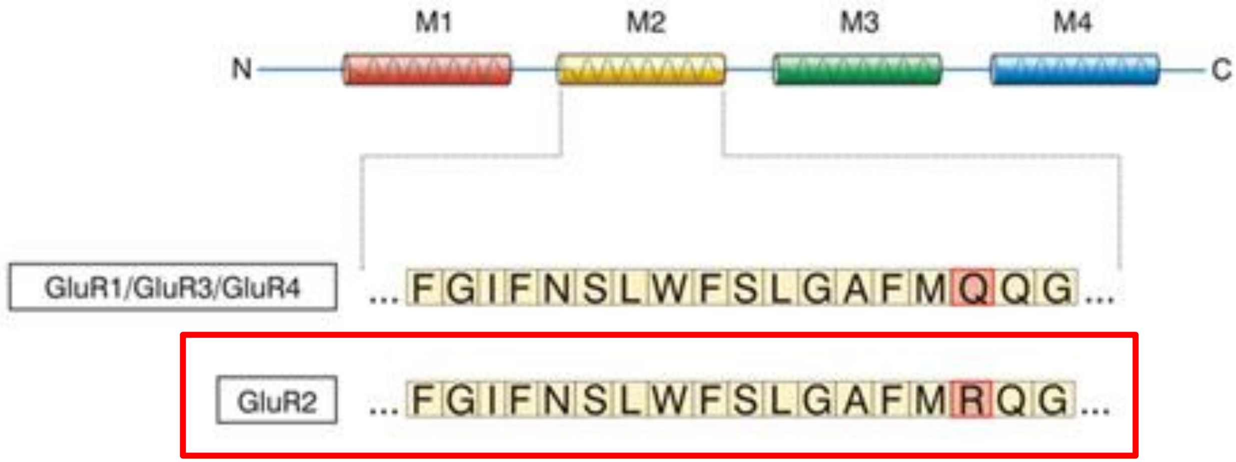


GluR1
GluR2
GluR3
GluR4

agonists	*antagonists*
AMPA	*CNQX*
KA	*NBQX*
domoic	*GYKI53655*

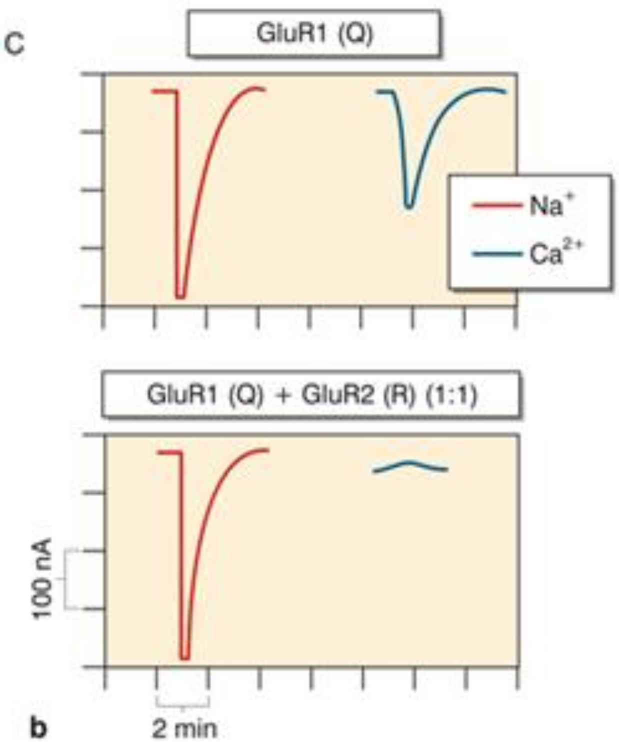
The resulting **diversity in receptor composition** contributes to differences in receptor biogenesis, trafficking, posttranslational modifications, cellular distribution, and biophysical properties

If GluR2 subunit is absent AMPARs become Ca^{2+} permeable



the substitution of a glutamine (Q, neutral) with arginine (R,+) in GluR2 blocks the Ca^{2+} permeability

Na^+ K^+ and Ca^{2+} permeable

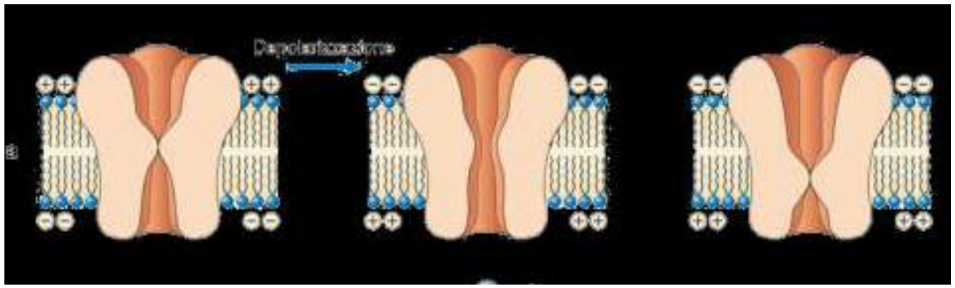


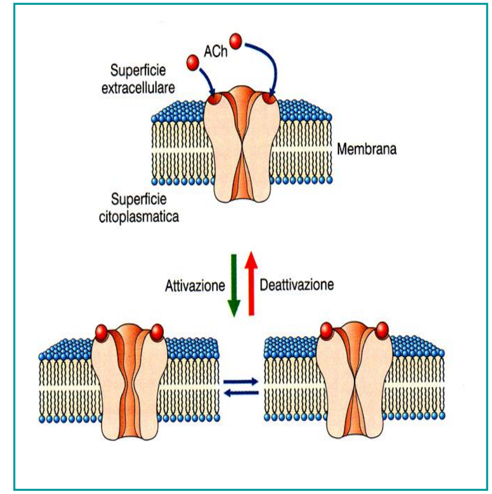
no Ca^{2+} permeable

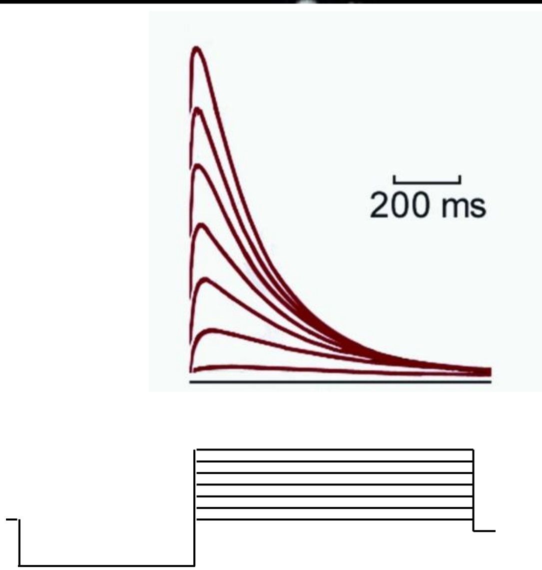
Inactivation/Desensitization

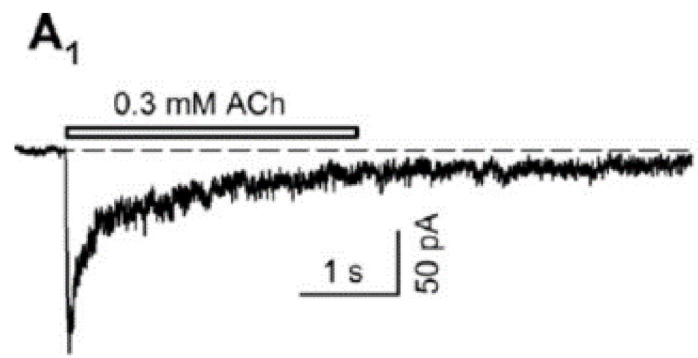
Voltage-gated channels

Ligand-gated channels









INACTIVATION

DESENSITIZATION

Desensitization can also lead to the reduction of postsynaptic currents upon repetitive synaptic neurotransmitter release to prevent the over-activation of receptors.

The desensitization of ionotropic receptors is a relatively transient process
There is a conformational change of the receptor. Phosphorylation is an important post-translational covalent modification. The detailed molecular mechanism is still not clear.

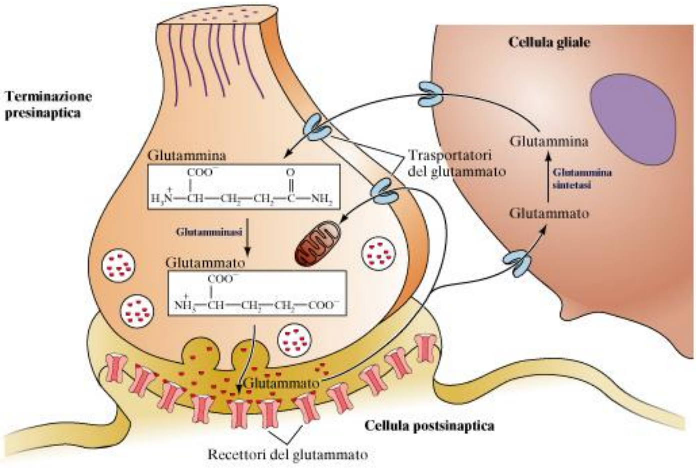
Desensitization of metabotropic receptors can involve a block in the activity of signaling, post-translational modification of the channel or subsequent receptor internalisation.

Glutamate vesicle transporters VGLUT (1-3)

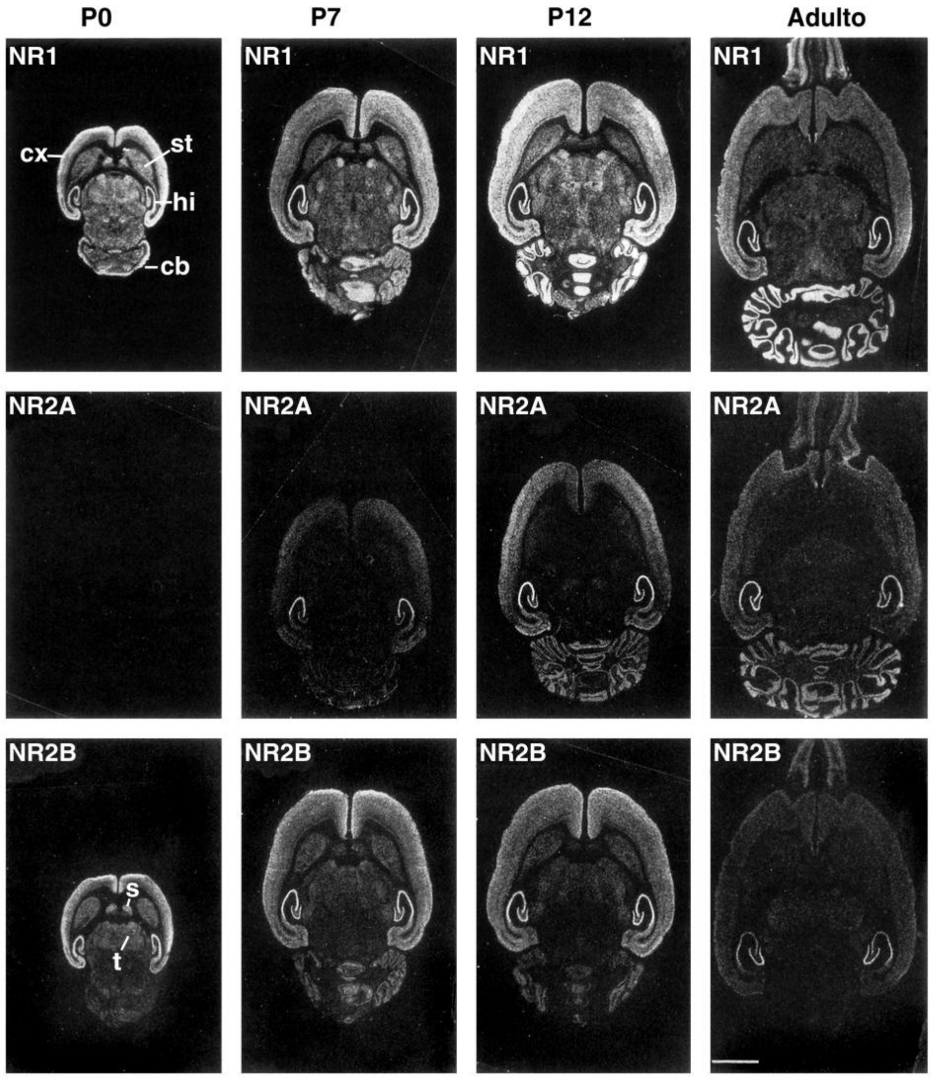
and glutamate reuptake system (EAAT)

can be used to identify glutamate receptors

but pay attention,
reuptake system can be glial !!!



Developmental switch of NMDAR subunits



Dominant subunits

NR1 + NR2B

syn and extrasyn R

↓

NR1 +NR2A

synaptic

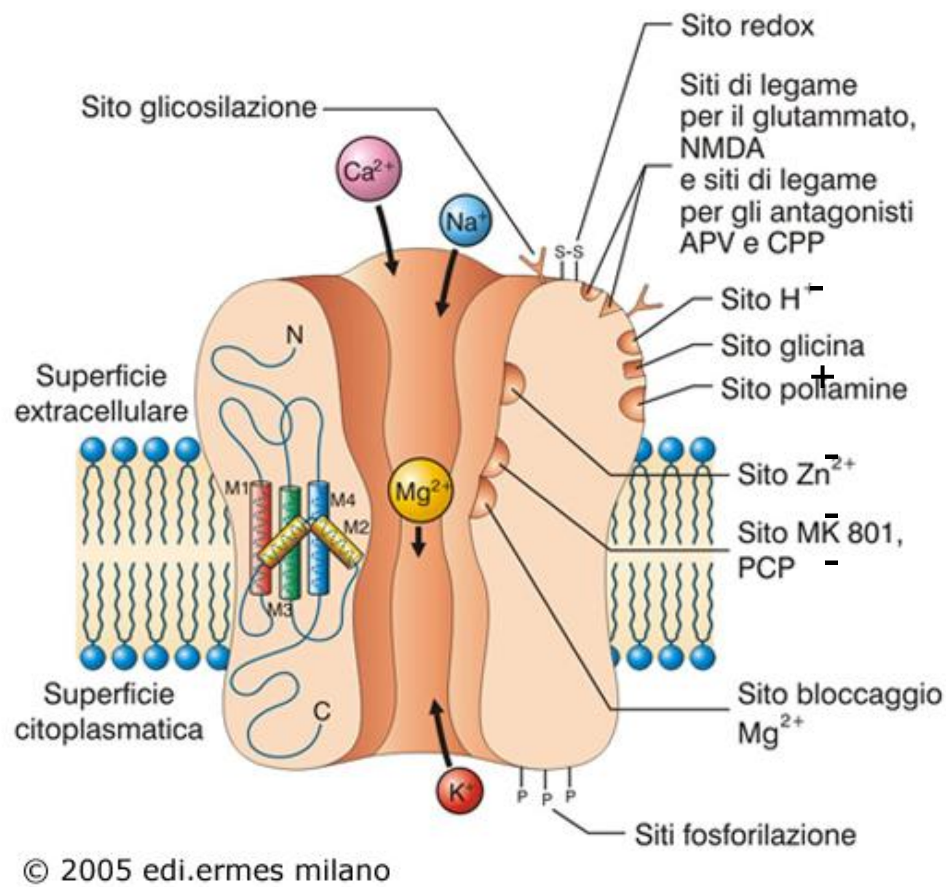
NMDA receptors, heterotetramers

play a key role in brain development, synaptic plasticity, and memory formation

2NR1 + NR2 (A-D)
Co-activated by binding of glycine and glutamate.

Two binding site for glutamate, one on each NR2 subunit.

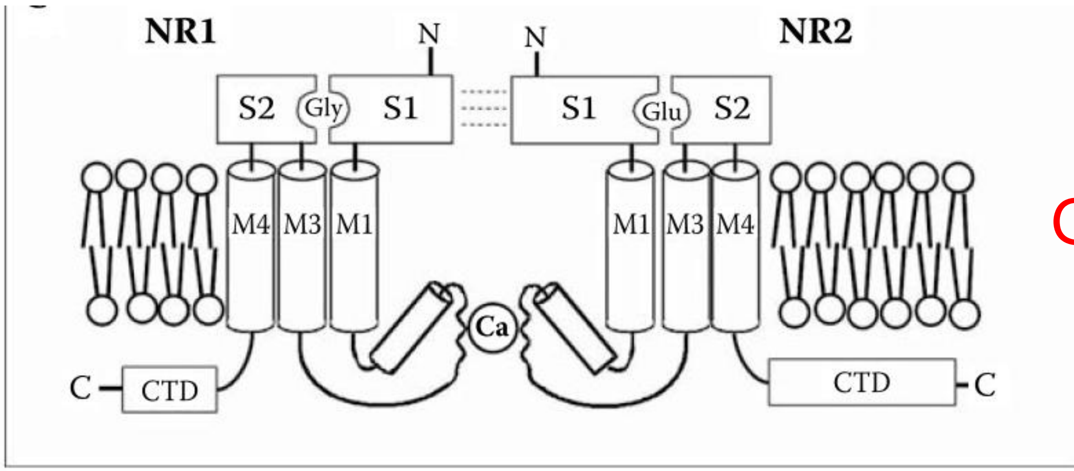
glycine binds NR1



NMDARs have high affinity for glutamate, are highly permeable to Ca^{2+} and are specifically blocked by ketamine, kinurenic acid, MK-801, memantine

The 2 NMDA subunits able to bind Glycine and Glutamate, coagonists of NMDARs

Gly



Glu

Biology of NMDA receptors, NIH

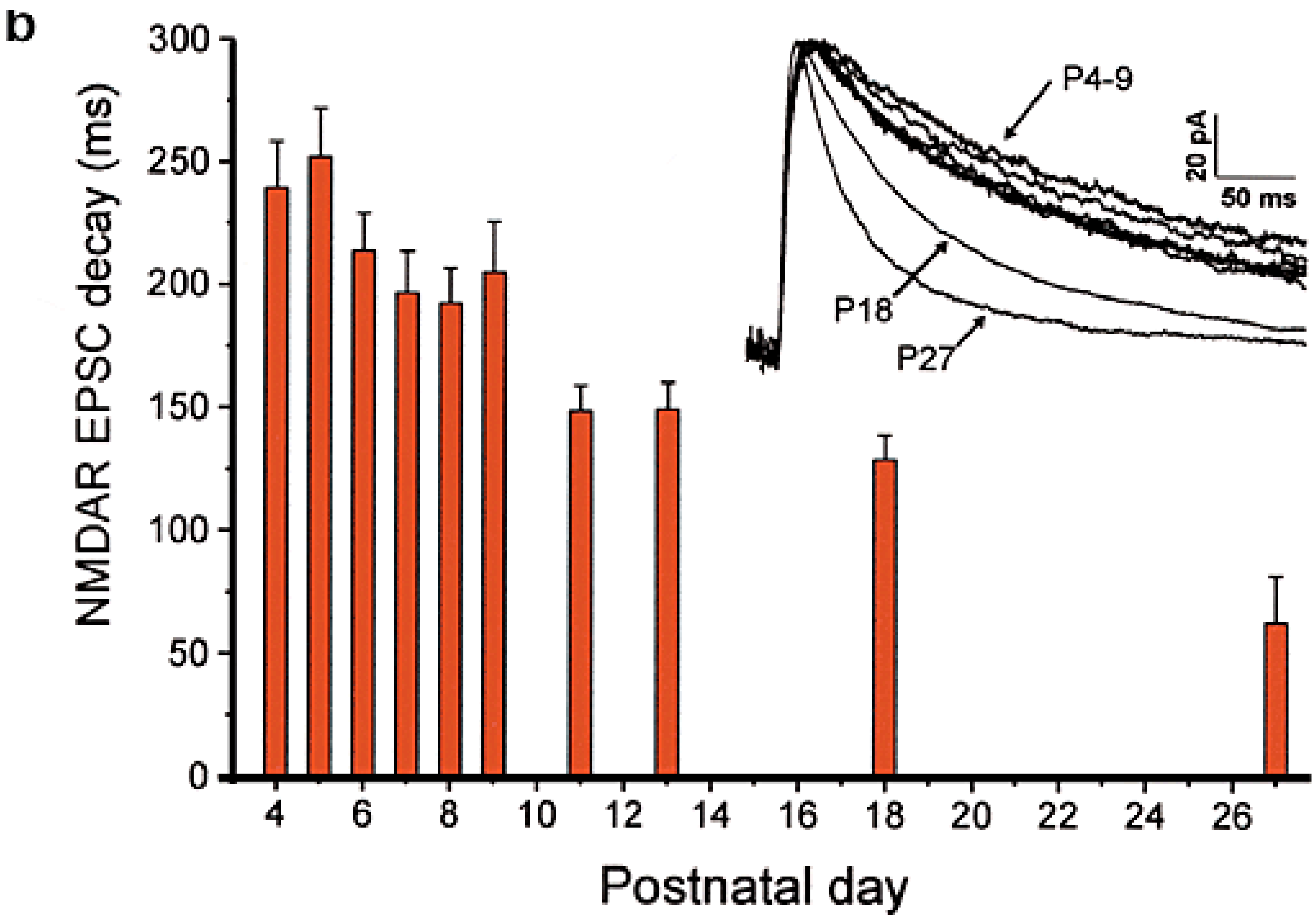
EPSP

Gating properties of single channels influence the amplitude, shape and duration of post-synaptic potentials.

AMPA-mediated EPSP 1-5 ms
NMDA-mediated EPSP 50-200 ms
GABA-mediated IPSP 10-30 ms

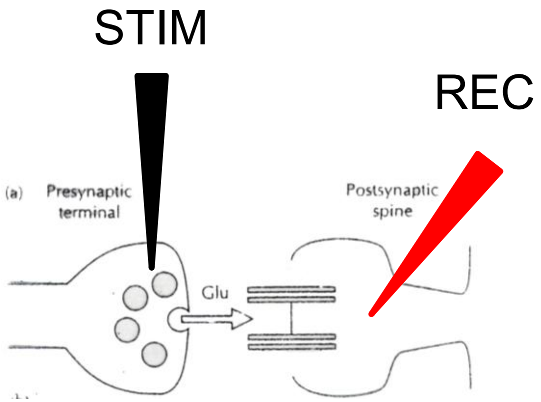
Faster kinetics in mature EPSC

thalamocortical synapses

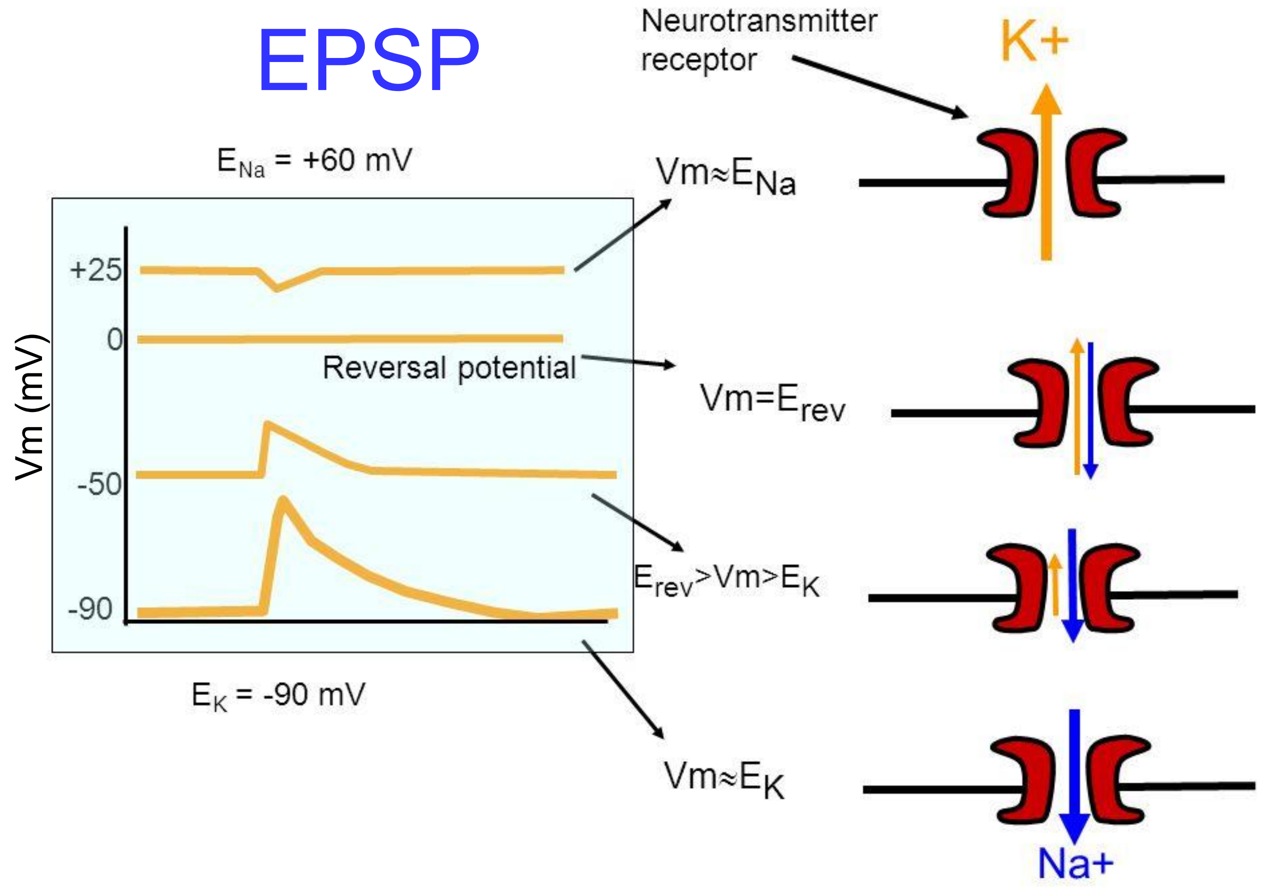


Barth and Malenka, 2001

Ligand gated cation channels



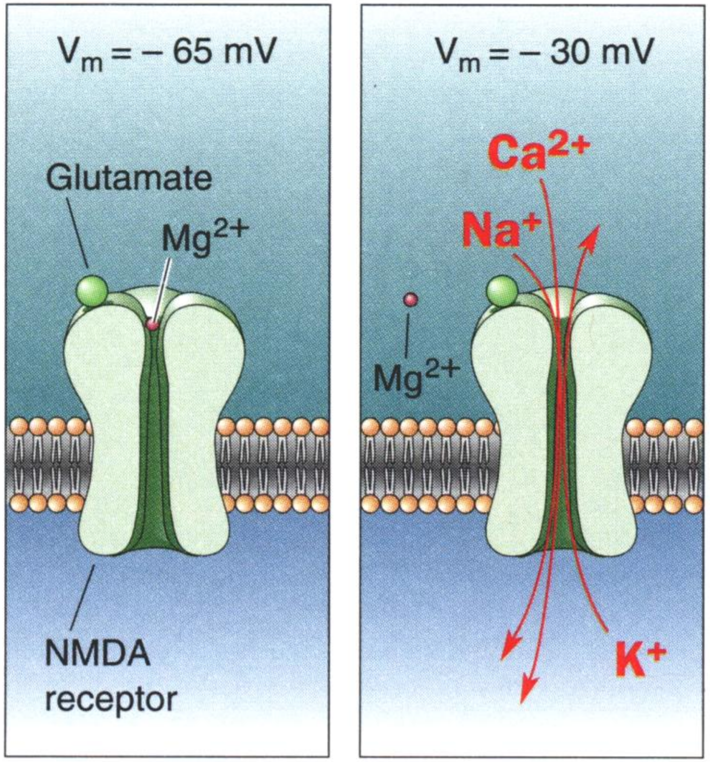
EPSP



$V_m = E_{rev}$ when the net current = 0

Inward current = outward current

At a membrane potential close to the resting, NMDARs are blocked by Mg^{2+}

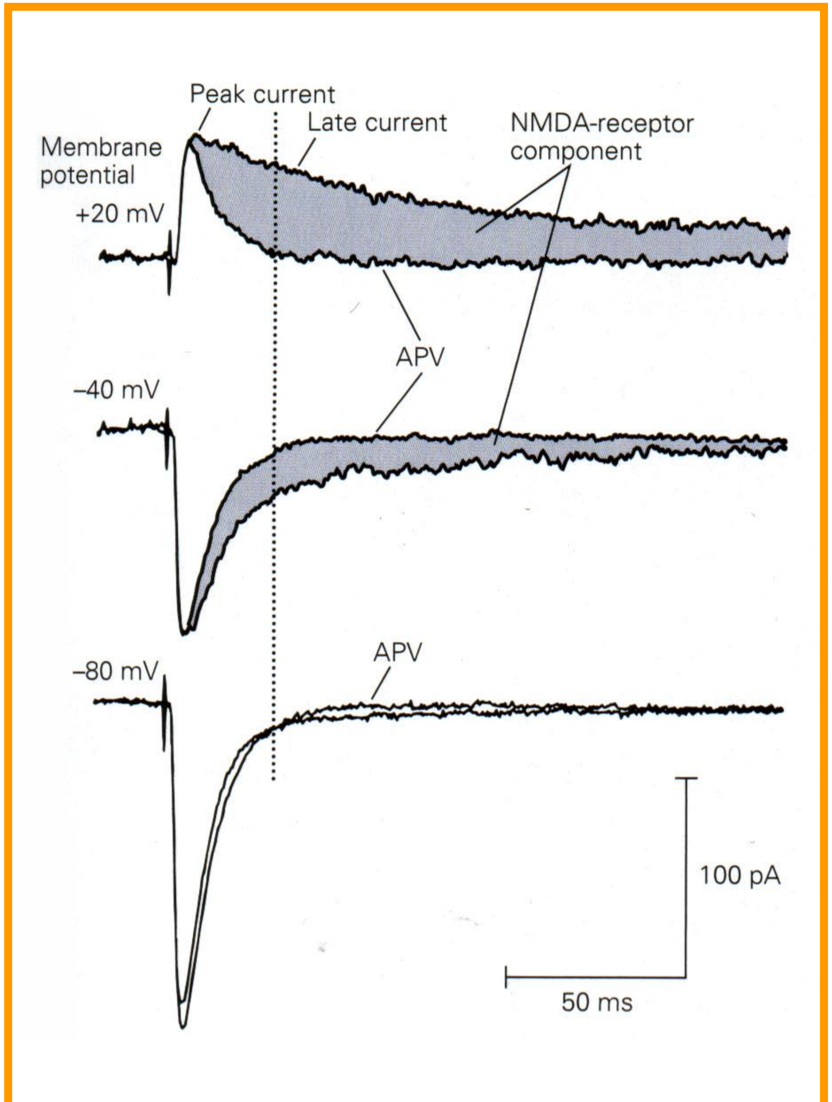


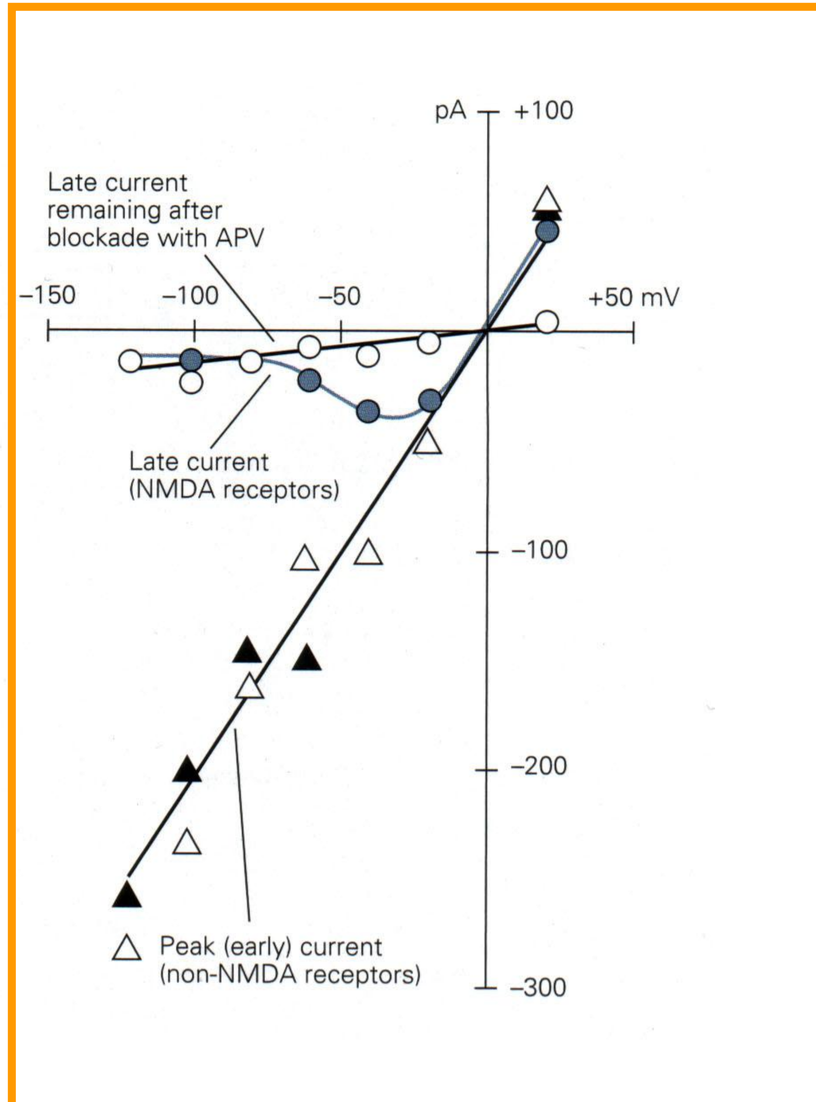
Glutamate

Glutamate and depolarization

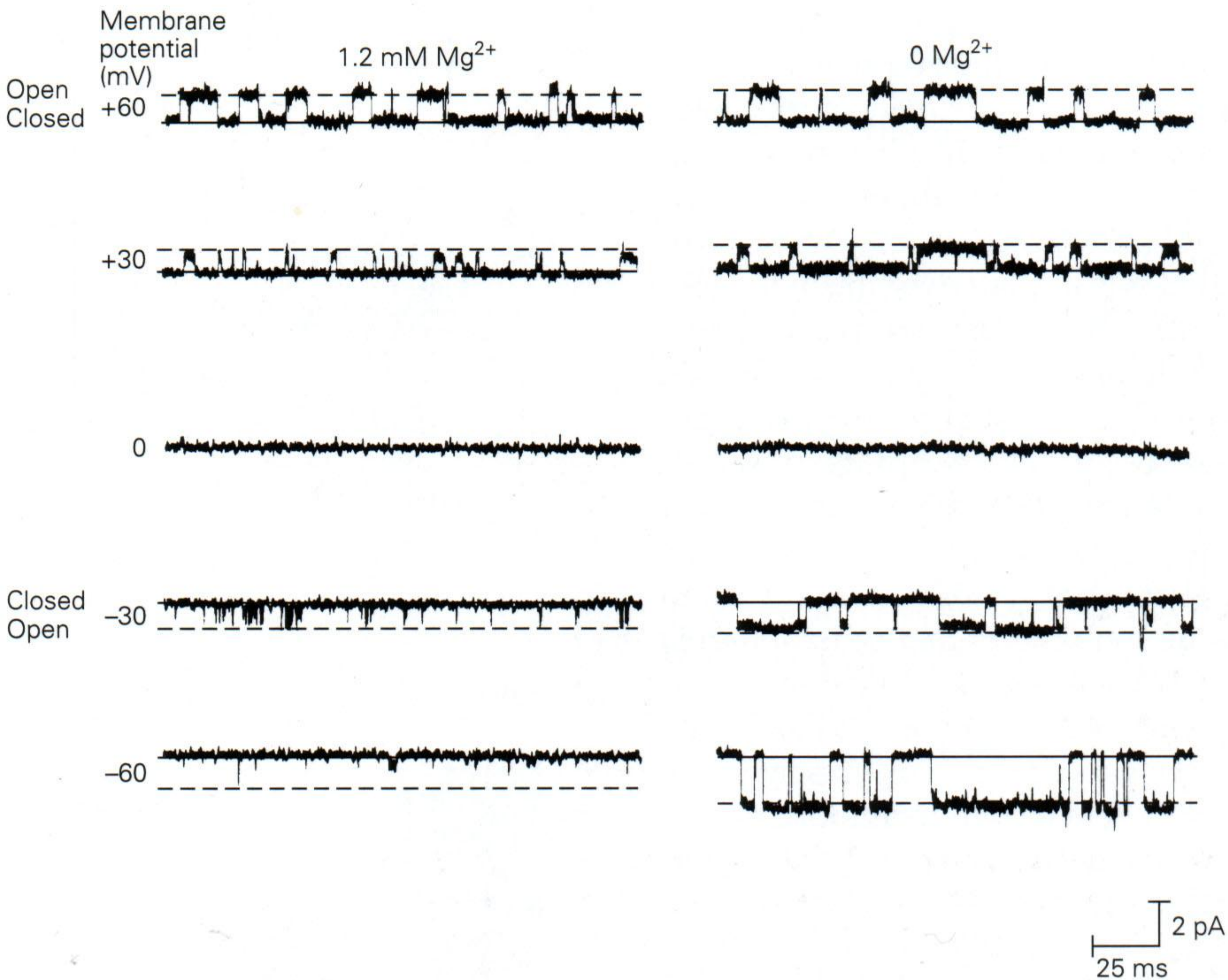
Early and late components of glutamatergic synaptic currents

I-V relationship of the synaptic current



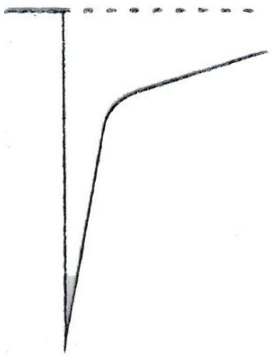


NMDAR- voltage-dependent Mg^{2+} block



In conclusion…

AMPARs and KARs are extremely fast receptors; at high glutamate concentrations, they open within less than 1 ms to produce fast excitatory currents and deactivate or desensitize within a few milliseconds when glutamate concentrations rapidly drop or remain elevated, respectively.



In contrast, NMDARs respond more slowly, with activation times in the 10 ms range and deactivation on the order of hundreds of milliseconds, depending on the GluN subtype. Moreover, NMDARs show no fast ligand-induced desensitization and have a higher Ca^{2+} permeability which allows them to potently induce downstream signaling cascades.

NMDARs can represent the trigger of multiple neuronal death cascades (glutamate excitoxicity) leading to apoptosis and necrosis, mainly as a consequence of the massime intracellular Ca^{2+} influx.

No known extracellular enzymes that can degrade glutamate, the maintenance of low extracellular concentrations relies on the balance of the opposite functions of uptake and release.

A paradox

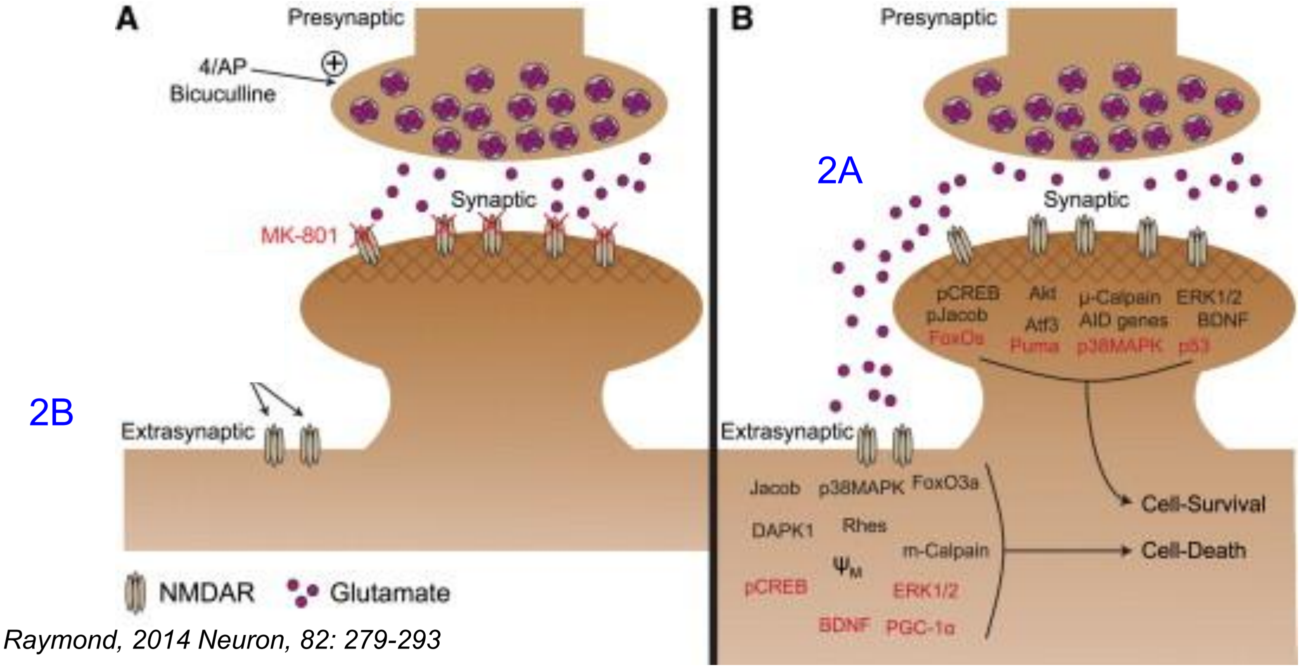
1. Activation of NMDAR can promote cell survival and plasticity

2. NMDAR-induced excitotoxicity is thought to contribute to the cell death associated with certain neurodegenerative diseases, stroke, epilepsy, and traumatic brain injury.
More recently, NMDAR has been found to be expressed on various types of cancer cells and NMDAR signaling has been implicated in tumor growth.

Synaptic and extrasynaptic NMDARs

a long-standing paradox, different subcellular localization and subunit composition

Synaptic NMDARs mediate physiological plasticity, gene transcription and survival
Extrasynaptic NMDARs trigger cell death pathways



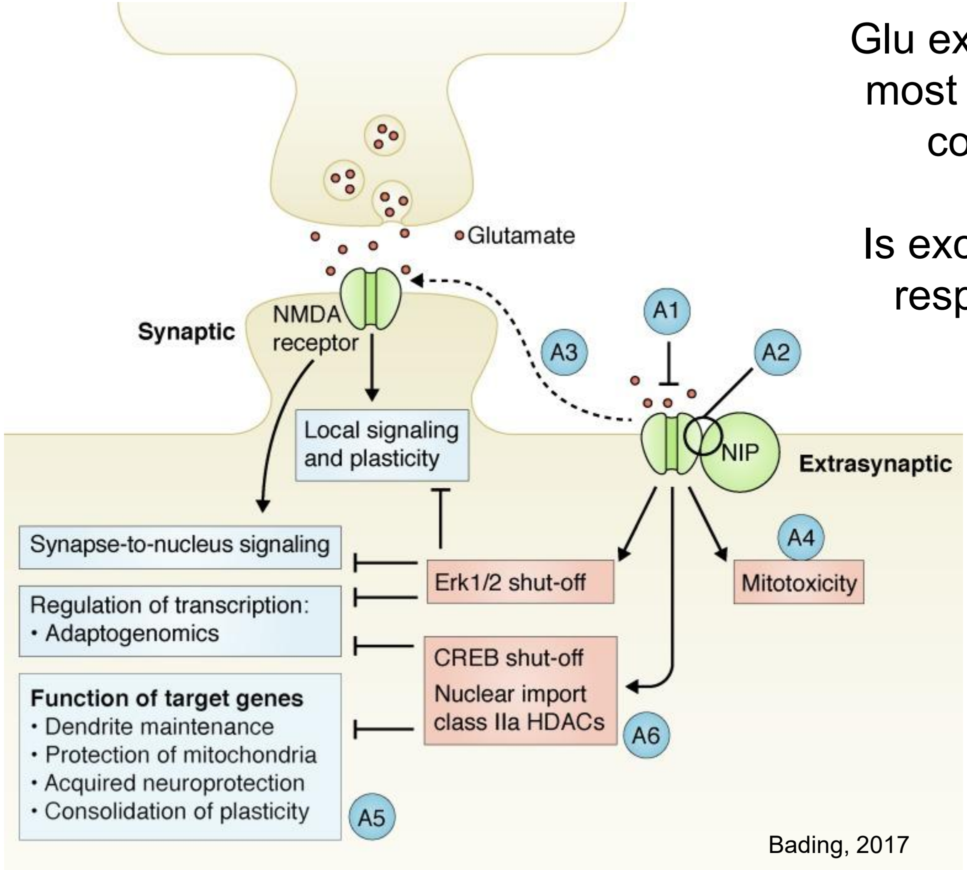
Parsons and Raymond, 2014 Neuron, 82: 279-293

In adult hippocampal neurons one quarter of extrasynaptic NMDARs are perisynaptic

MK-801 <u>use-dependent</u> noncompetitive open channel blocker used to block synaptic receptors (the channel must be open to block it)

Memantine, a noncompetitive, moderate affinity NMDAR antagonist preferentially blocks tonically-activated extrasynaptic NMDARs

Differential signals by synaptic and extrasynaptic NMDARs



Bading, 2017

Glu excitotoxicity is implicated in all most common neurodegenerative condition AD, PD, ALS, MS

Is excessive activation of NMDAR responsible for killed neurons?

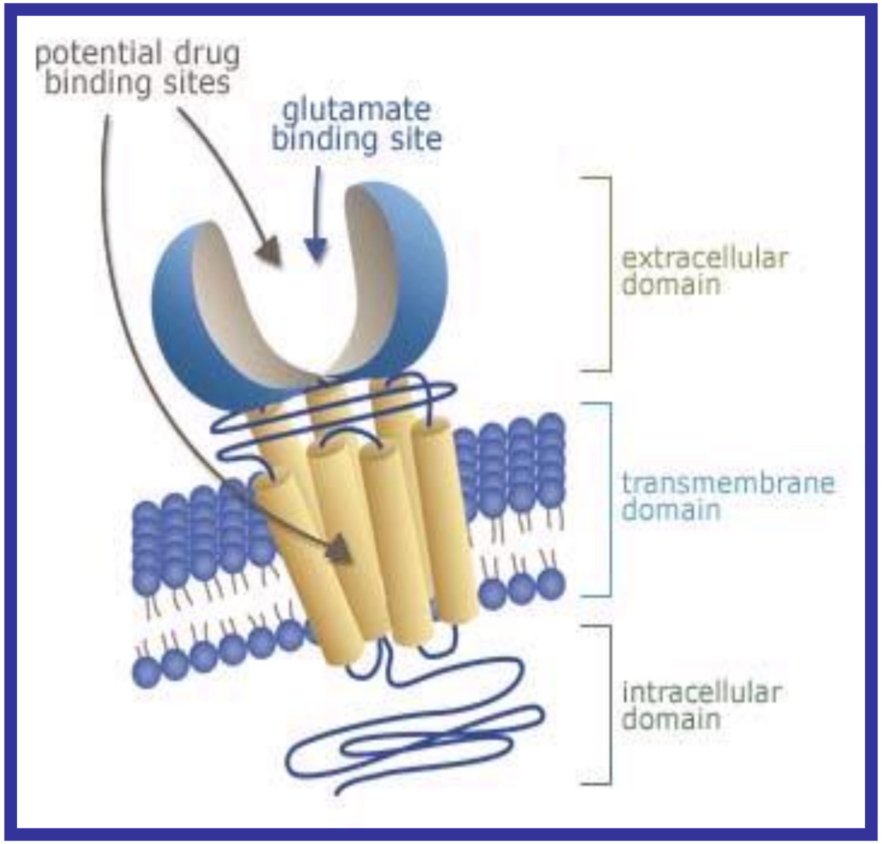
Synaptic NMDAR stimulation increases extracellular signal-regulated kinase 1/2 (ERK1/2) activation, cAMP response element-binding protein (CREB) phosphorylation and brain-derived neurotrophic factor (BDNF) expression, enhances antioxidant defense, and provides neuroprotection, whereas extrasynaptic NMDAR stimulation does the opposite

A shift in the balance from synaptic towards extrasynaptic NMDAR signalling may be an important factor in the aetiology of neurodegenerative diseases

- In Huntington's disease, mutant huntingtin causes a specific increase in extrasynaptic NMDAR currents.

- In acute ischaemic trauma, cell death may be caused in part by an upregulation and activation of extrasynaptic NMDARs

8 types of mGLU receptors have been cloned classified in 3 subfamilies based on sequence homologies and their coupling to specific enzyme systems.

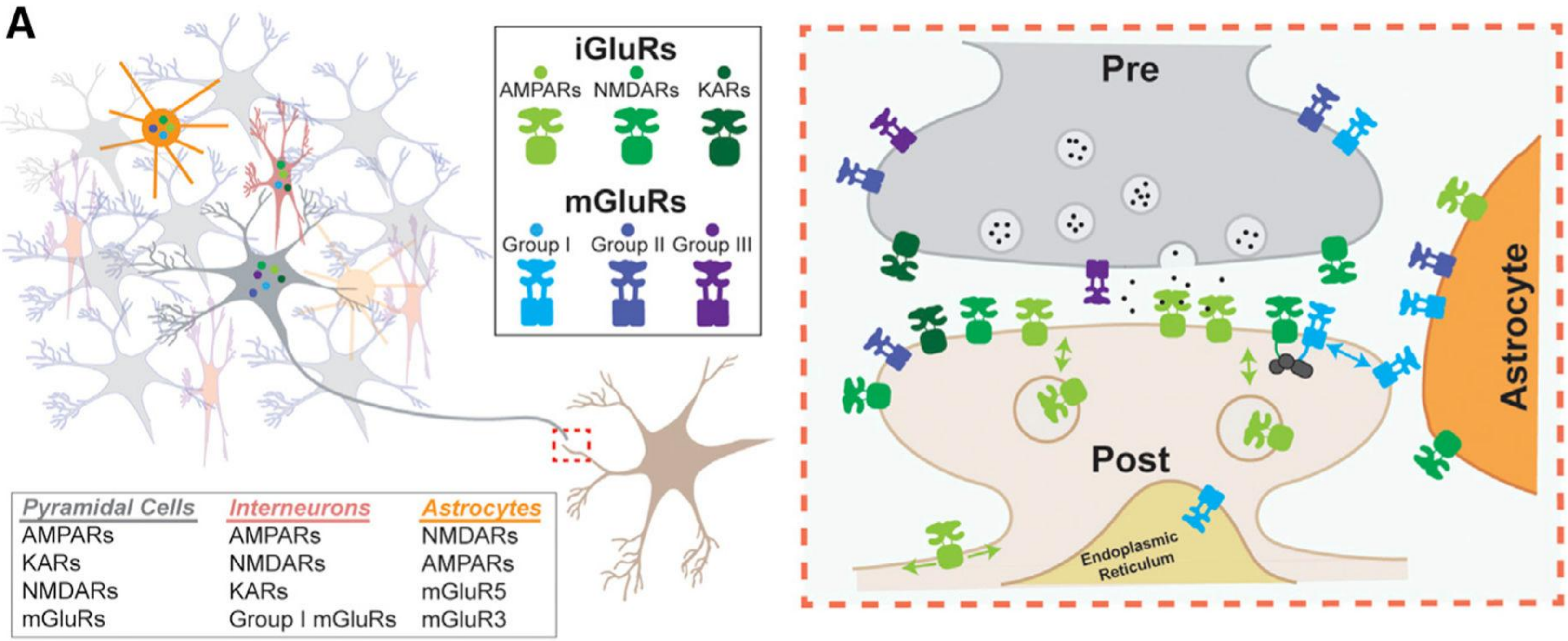


Group I: phospholipase C activation, (mGluR1, mGluR5)

Group II: negatively coupled to adenylate cyclase (mGluR2, mGluR3)

Group III: negatively coupled to adenylate cyclase (mGluR4,mGluR7,mGluR8, mGluR6)

A major challenge is understanding how the complex structural and biophysical properties of GluRs manifest in the context of synaptic signaling dynamics



Reiner and Levitz, *Neuron*, 2018

Holistic approach, everything must be studied as a whole

The activation of different GluRs is determined by the complex changes in glutamate location, concentration, affinity and gating properties of each receptor subtype

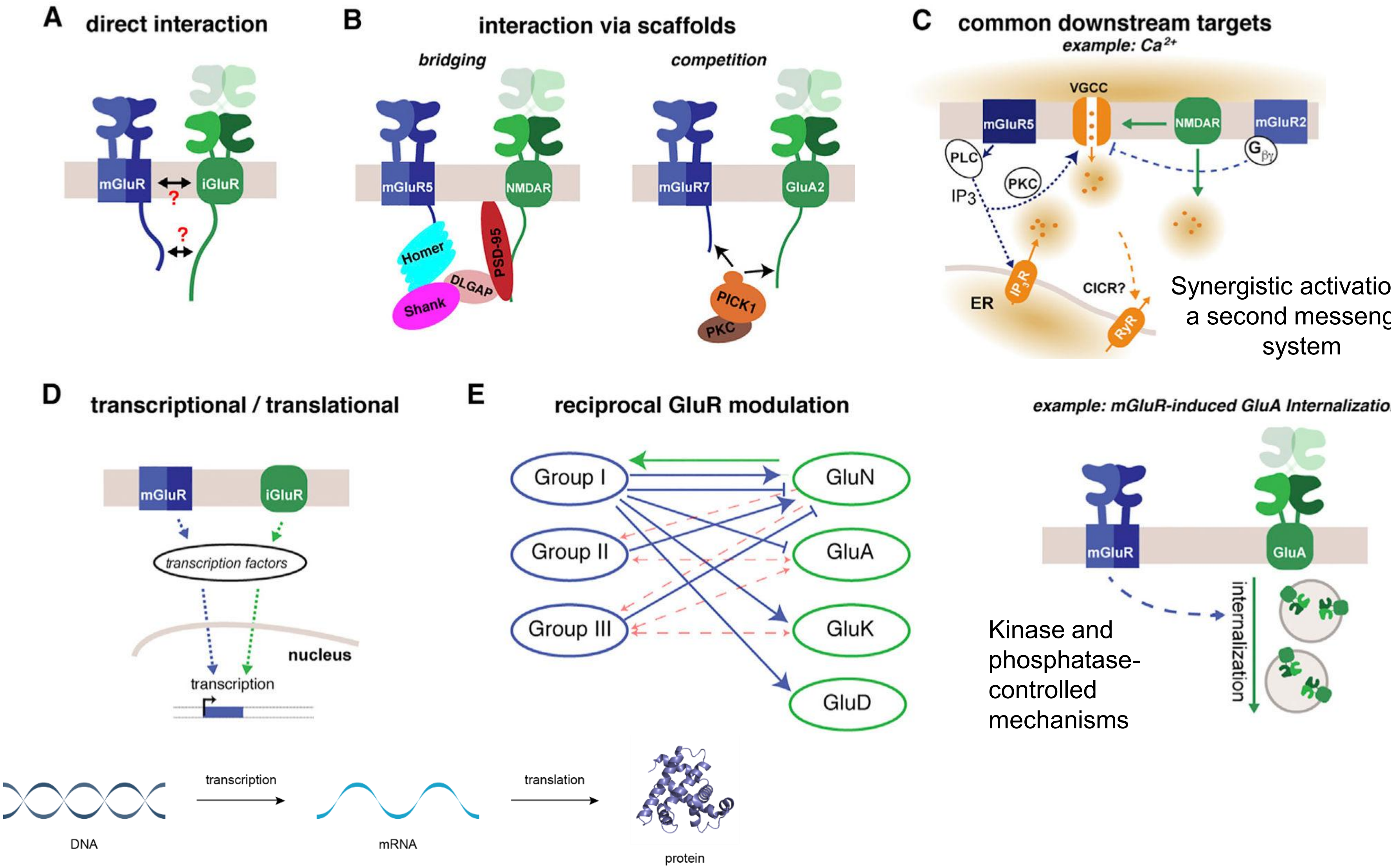
Different affinity for Glu

NMDAR has high glutamate sensitivity EC_{50} 0.4 - 4 μM

Most **mGluRs** respond to Glu in the μM to tens of μM range

AMPAR and **KAR** hundreds of μM to mM range

Ionotropic and metabotropic receptors signal in concert
Do they talk to each other?



Synergistic activation of a second messenger system

Kinase and phosphatase-controlled mechanisms

Reiner & Levitz, Neuron 2018

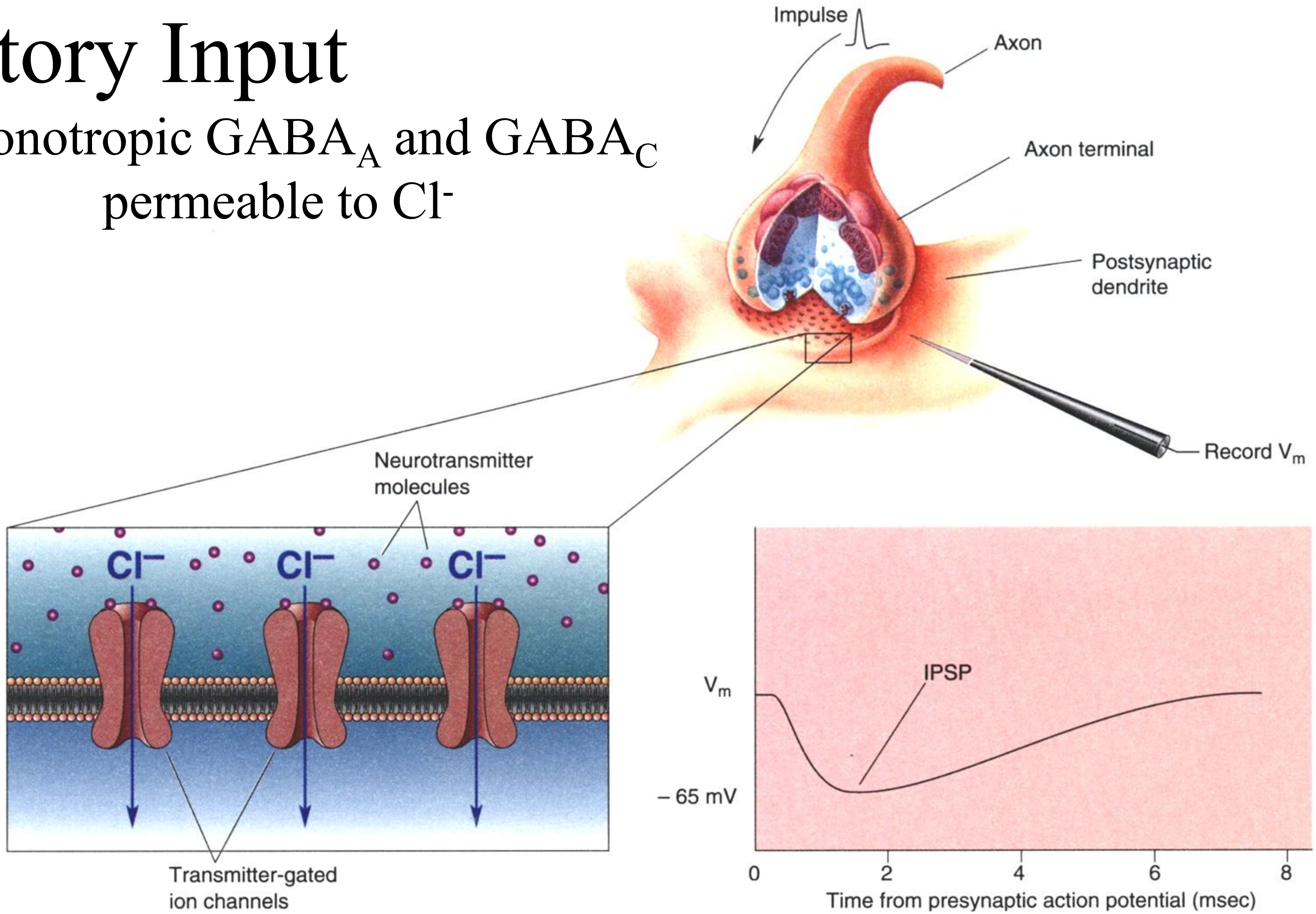
Anomalies in glutamatergic neurotransmission will give origin to a wide series of neurologic and psychiatric diseases.

Therapeutic strategies may include a modification in:

- neurotransmitter release
- their link to the receptors
- the uptake

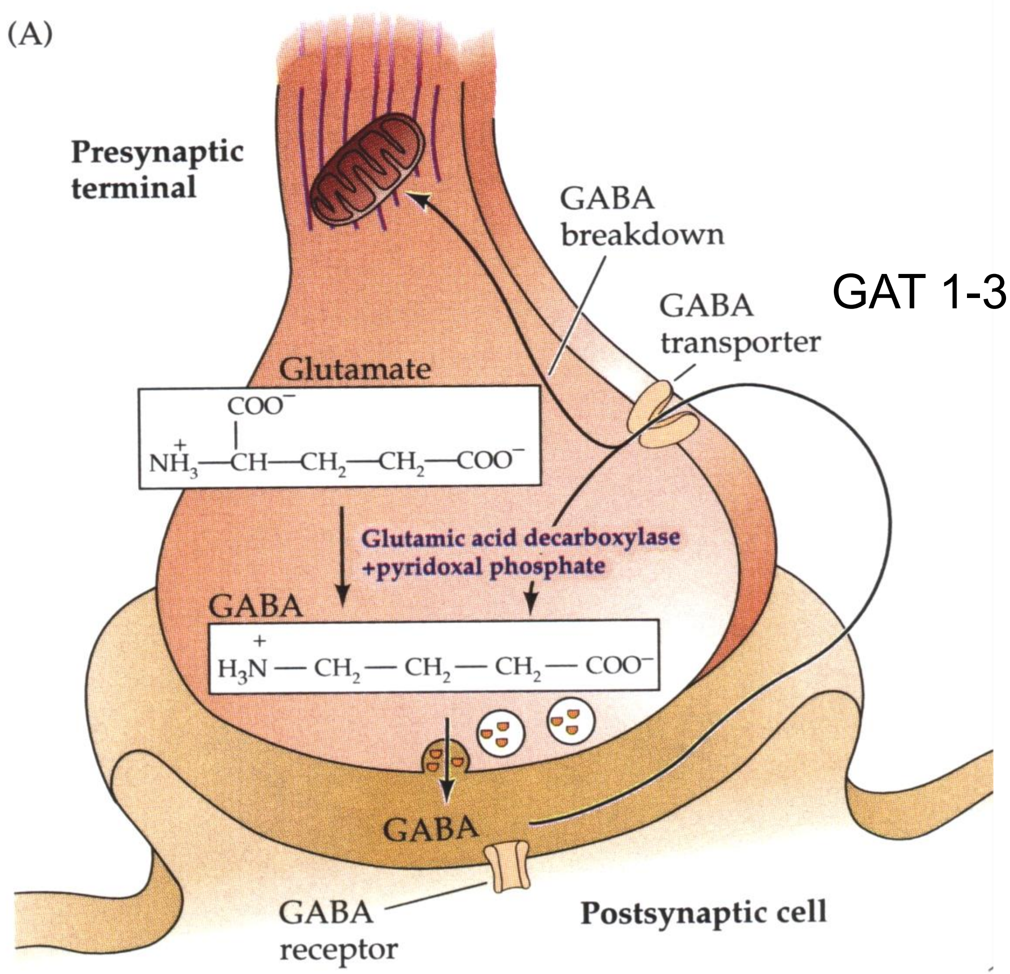
Inhibitory Input

- ionotropic $GABA_A$ and $GABA_C$ permeable to Cl^-



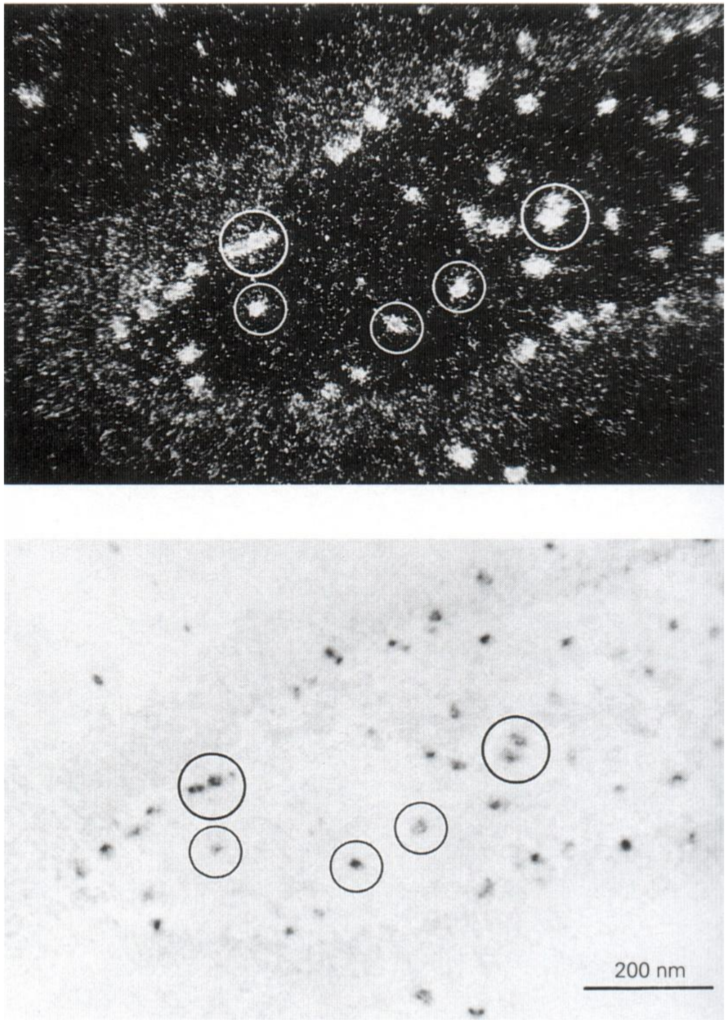
$GABA_A$Rs are blocked by bicuculline
$GABA_C$Rs are blocked by TPMPA

GABA is synthesized from glutamate by the enzyme, GAD which is found almost exclusively in GABAergic neurons. GAD require a cofactor derived from vitamin B_6.



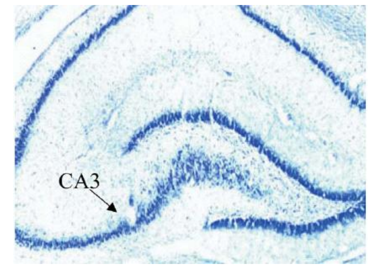
GAT 1-3 in neurons and glia

GABA synthesizing enzyme and transporters are co-localized in hippocampal neurons.

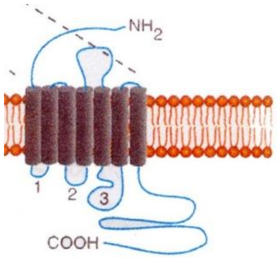


GAD

GAT-1



Metabotropic $GABA_B$ receptors

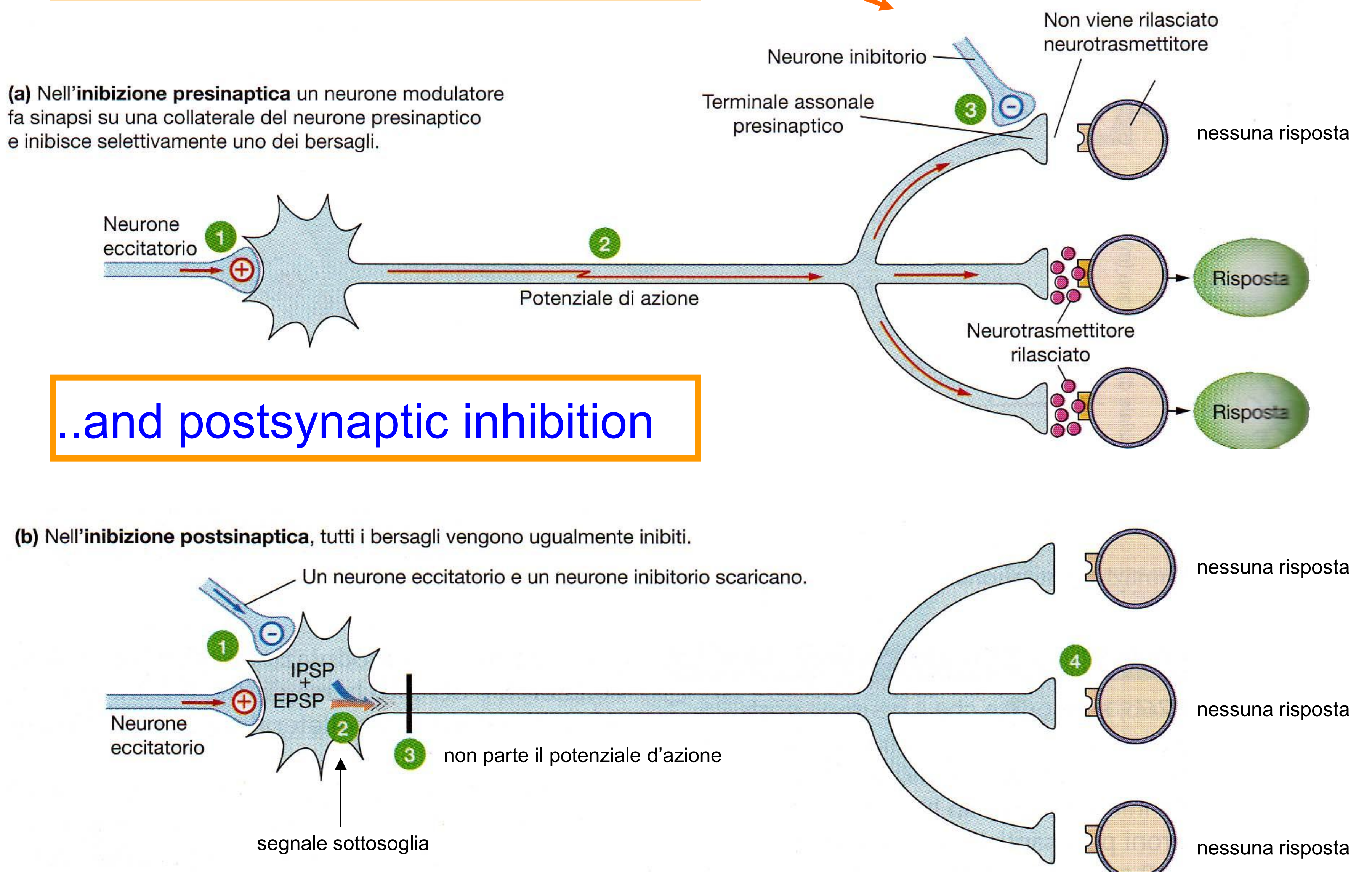


Presynaptic and postsynaptic inhibitory receptors

They are often linked via G-proteins to K^+ channels favoring their opening. $GABA_B$ receptor activation also reduces the activity of adenylyl cyclase and Ca^{2+} channels.

They are insensitive to bicuculline.
$GABA_B$ receptor blockers are CGP, Phaclofen, Saclofen.
Baclofen is a GABA analogue which acts as a selective agonist of $GABA_B$ receptors, used as a muscle relaxant.

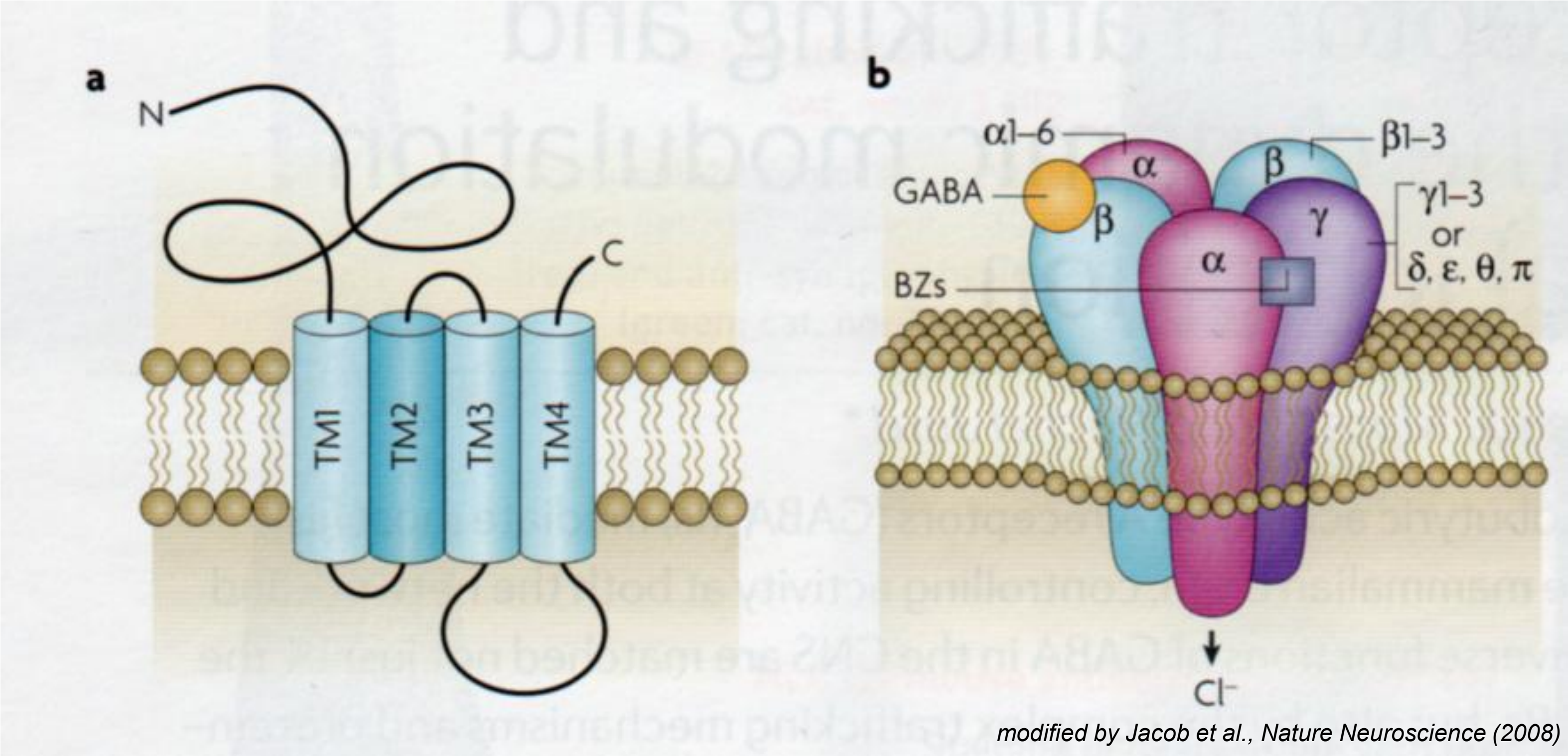
Presynaptic



..and postsynaptic inhibition

Fisiologia, Zanichelli

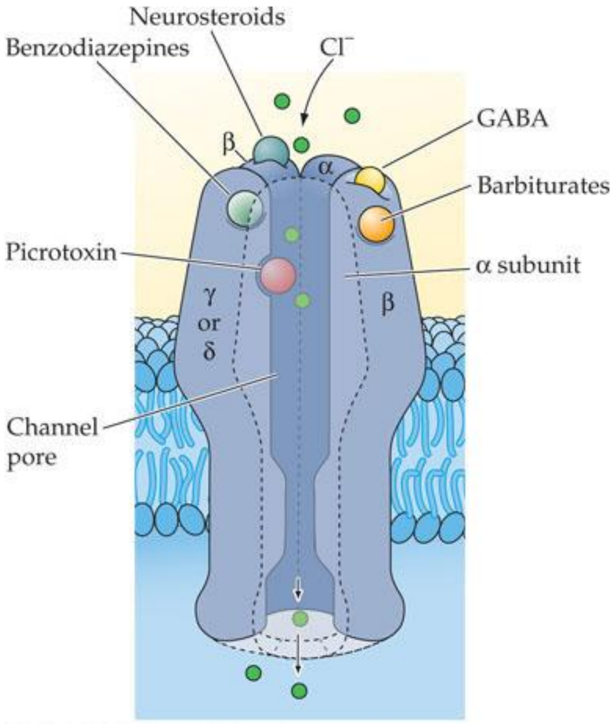
GABA$_A$R, major mature inhibitory receptors in the brain



Pentameric structure

α (1-6), β (1-3), γ (1-3), **δ**, **ε** (1-3), θ and π.

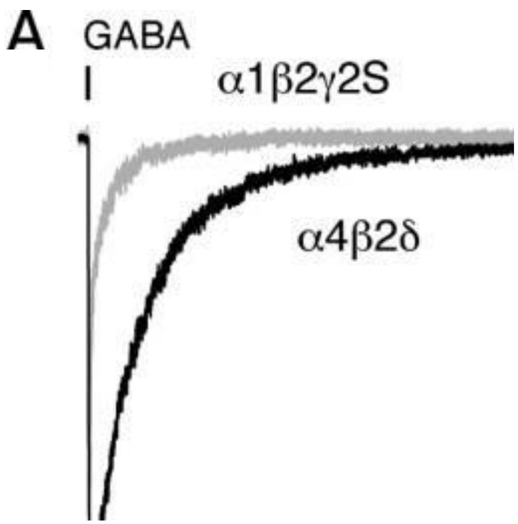
GABA$_A$ R are target of neurosteroids, barbiturates, benzodiazepine, all of them enhance the GABAR activity

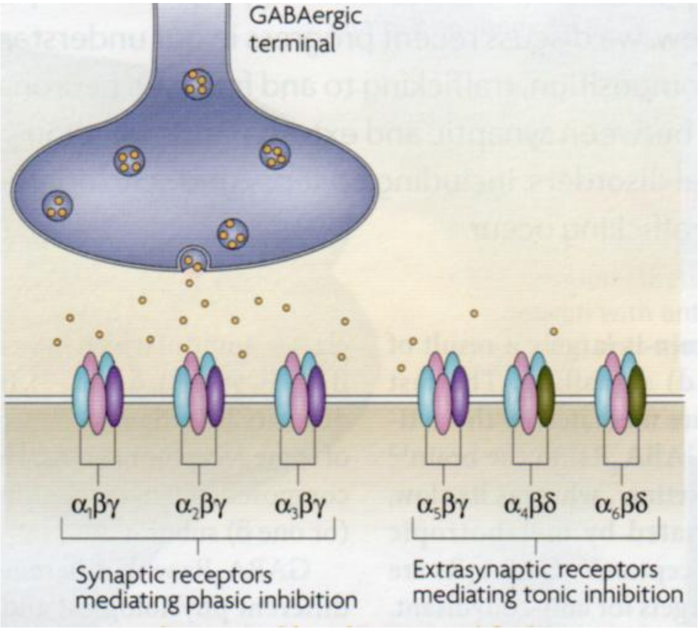


GABA$_A$ receptors are blocked by bicuculline and picrotoxin.

GABA$_C$ receptors have an additional ρ (1-3) subunit, found predominantly in the retina (GABA$_C$). They are unaffected by BZ and barbiturates, not blocked by bicuculline.

$GABA_A$ mediated phasic and tonic inibition





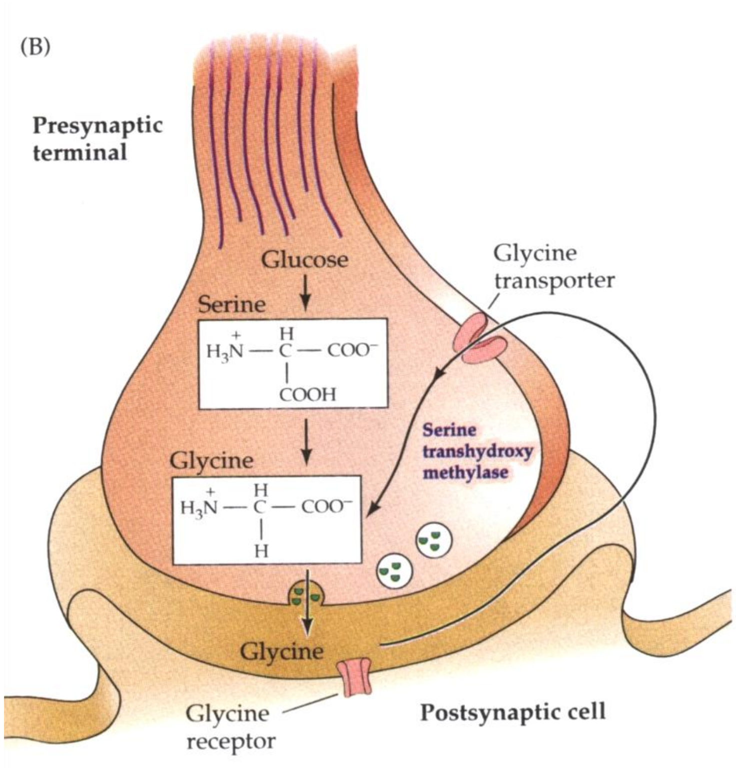
modified by Jacob et al., Nature Neuroscience (2008)

Phasic receptors with α1, α2, α3 or α5, β and γ are neurosteroid and BZ-sensitive, lower affinity for GABA, rapid desensitization.

Tonic receptors composed of α4, α5 or α6 subunits with β and δ, highly sensitive to neurosteroids and **insensitive to BZ**, **higher affinity for GABA**, **low desensitization.**

Glycine Receptors are the major mature inhibitory receptors in the spinal cord and brain stem

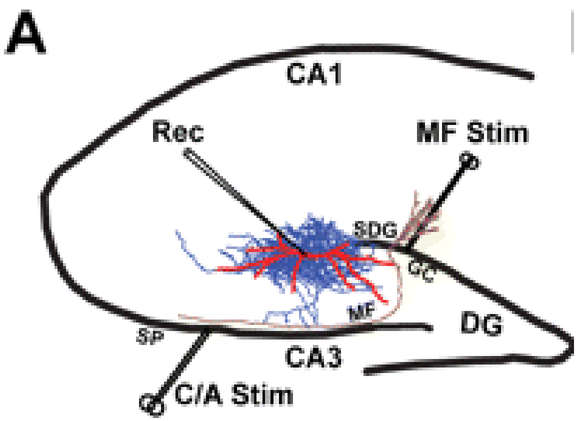
α (1-4) and 1β subunits



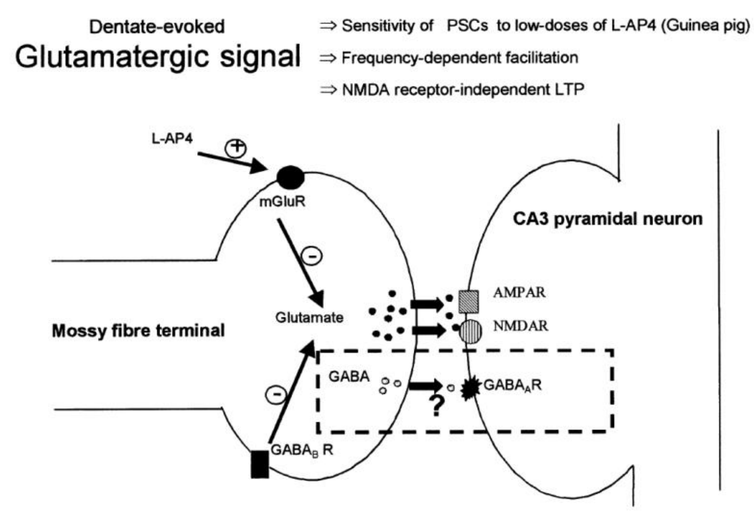
Strychnine is a potent antagonist of GlyRs, sensitive to picrotoxin.

one nerve/one transmitter principle might not be universally true

Co-transmitter hypothesis



GABA-like immunoreactivity present in MF !!

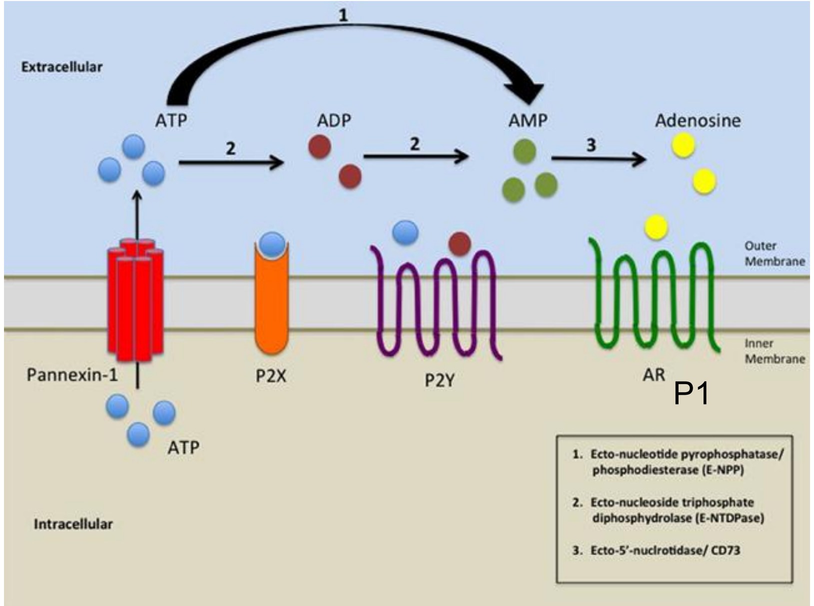


Epilepsia, Vol. 43, Suppl. 5, 2002

<u>In the immediate post-natal period</u> GABA is the only neurotransmitter released by MF. During development stimulation of MFs elicits in CA3 pyramidal neurons AMPA and $GABA_A$-mediated synaptic currents

PURINERGIC Receptors

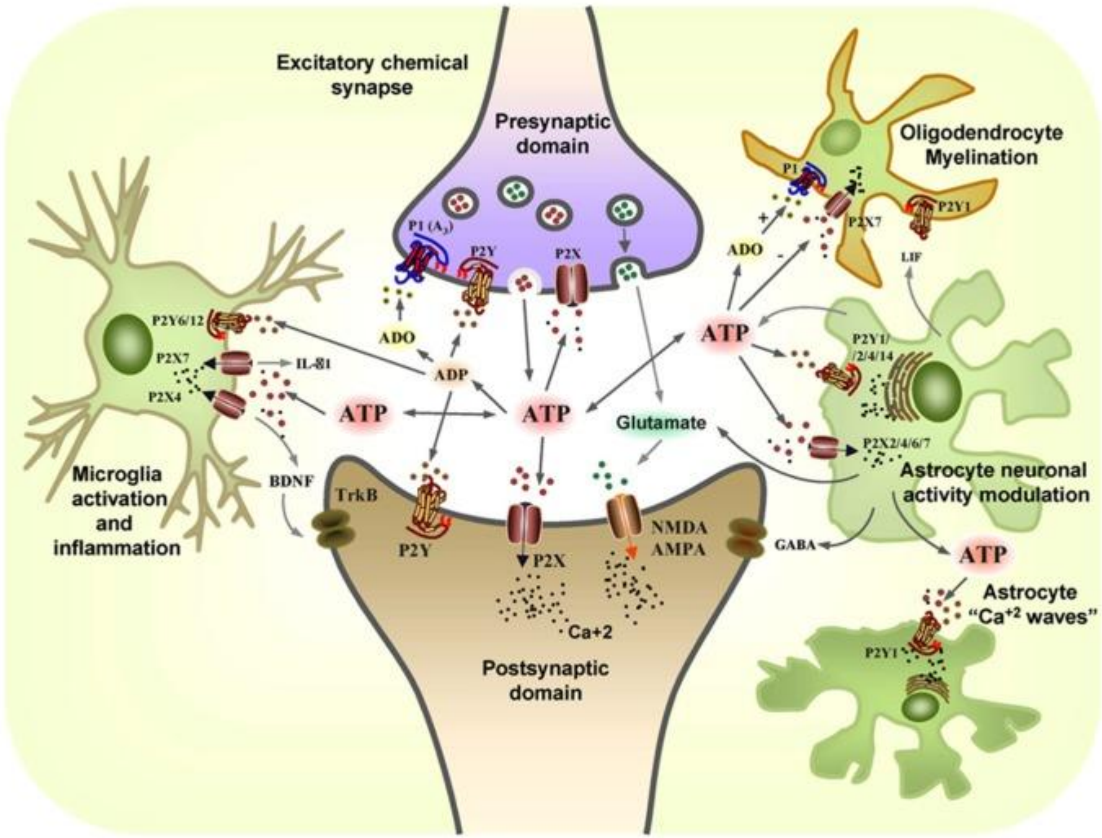
Adenosine 5′-triphosphate acts as an extracellular signalling molecule (purinergic signalling). Autocrine and paracrine activity



ATP and its metabolites activate P2Rs. P2X receptors are ionotropic receptors. P2Y receptors are G protein-coupled receptors.

Adenosine, a breakdown product of ATP, activates P1, G protein-coupled receptors (A1, A2A, A2B and A3)

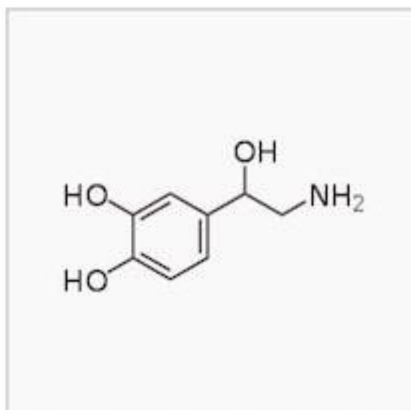
Coordinated action of glial and neuronal purinergic receptors in the CNS



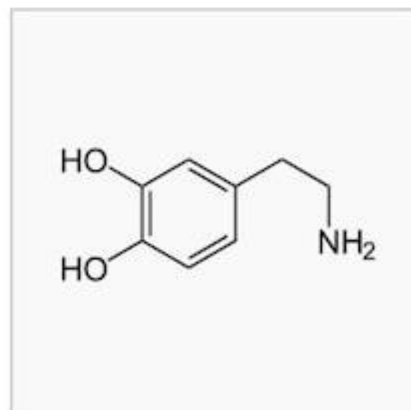
Ana del Puerto et al., 2013

Diffuse Modulatory Systems

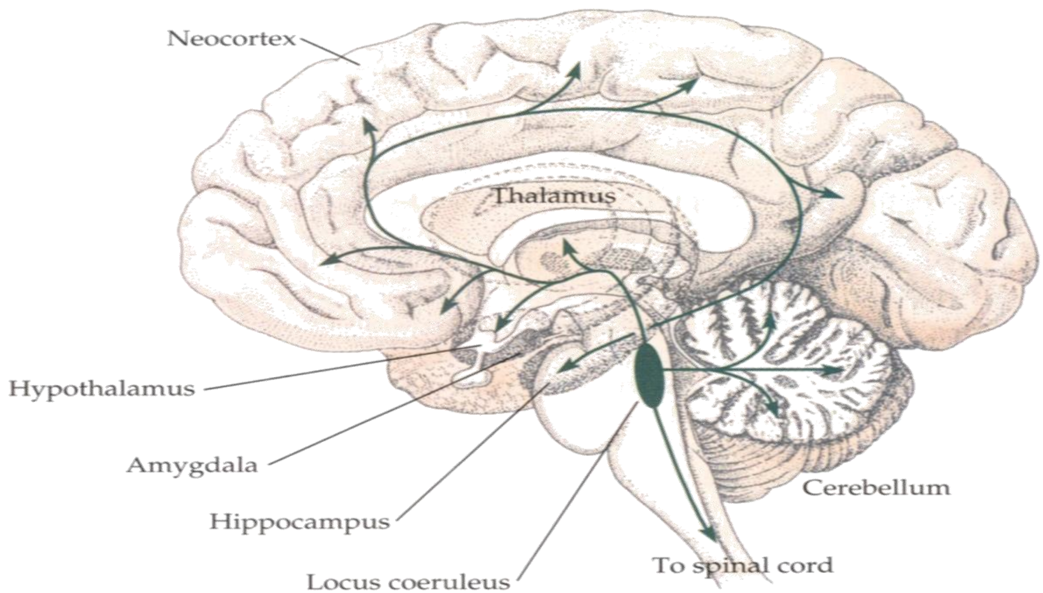
Noradrenaline and dopamine are the principal catecholamines formed by the AA tyrosine. All are formed by a catechol ring with a tail of amines.



Noradrenalina



Dopamina



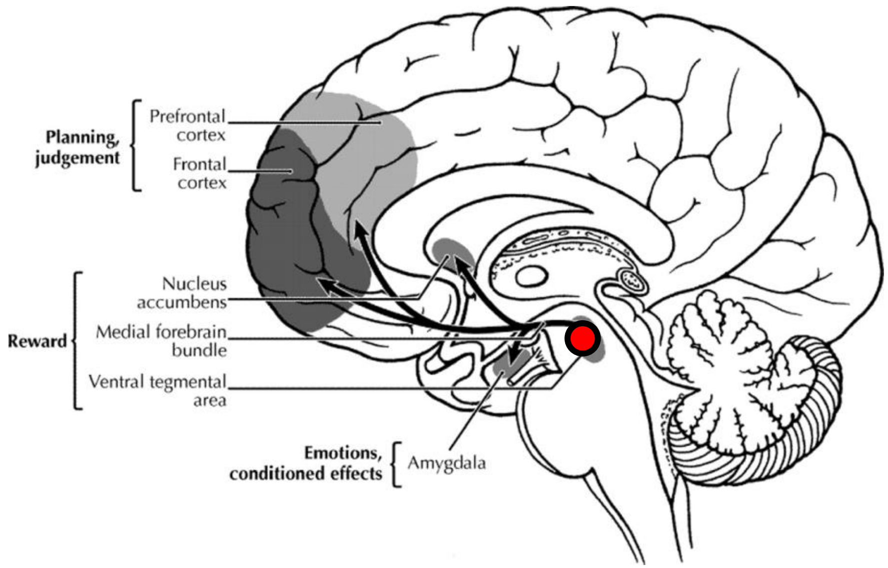
From NEURON to BRAIN, Sinauer Associates

In Autonomic NS, NA is the neurotransmitter of postganglionic symphatetic neurons. **There are α and β metabotropic adrenergic receptors.**

Noradrenergic system originates in the **locus coeruleus** (A6) and **lateral brainstem tegmentum** (A1, A2, A5, A7) in the PONT. From locus coeruleus noradrenergic fibers diffusely project to cortex, hippocampus, cerebellum, spinal cord. Neurons of lateral tegmentum project to thalamus and hypothalamus.

The locus coeruleus projections are part of the reticular system, they project to higher cerebral centers modulating arousal, attention and circadian rhythms.

D1-D5 metabotropic receptors



Dopamine neurons are found in either **substantia nigra** or the **Ventral Tegmental Area** (mesencepahlon). High levels of neuromelanin accounts for their dark colour. **Neurons from substantia nigra project to the striatum**. Neurons from VTA project to frontal cortex and part of the limbic system.

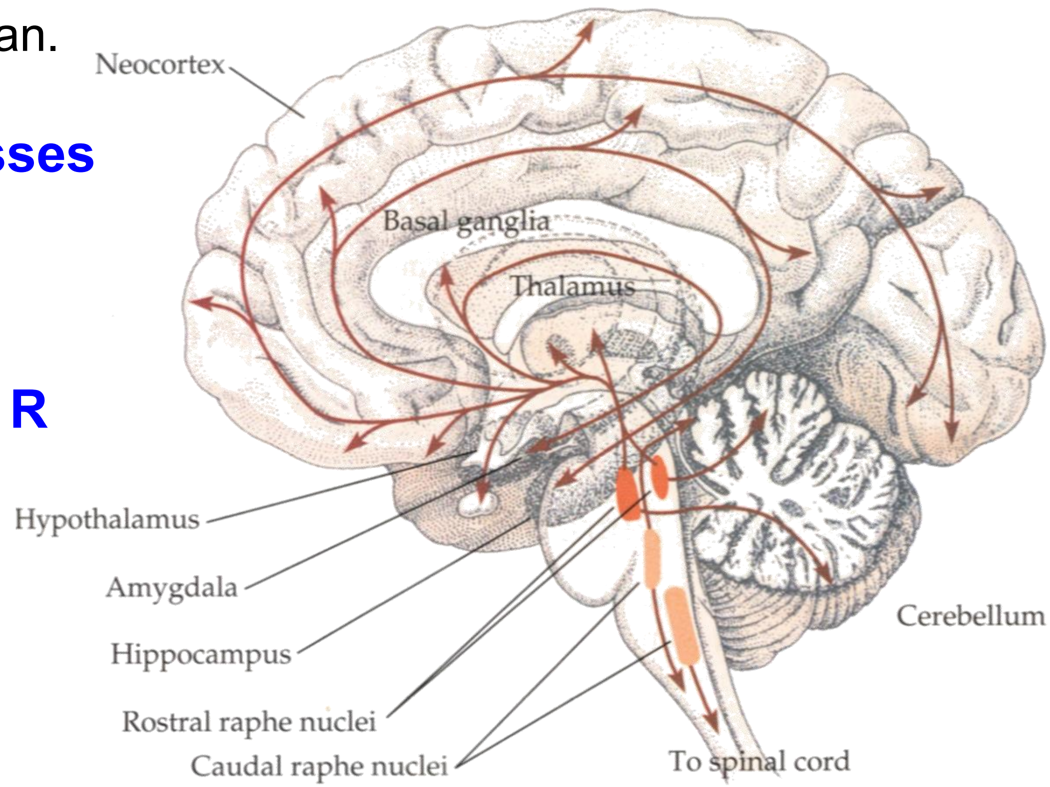
Dopaminergic neurons are involved in the regulation of movement, memory and pleasurable reward and motivation. High or low levels of dopamine are associated with several mental health and neurological diseases.

Serotonin modulates motor and sensory functions

5-HT derives from tryptophan.

Seven different subclasses
5 HT_1- $5HT_7$

5 HT_3
is the only ionotropic R



From NEURON to BRAIN, Sinauer Associates

Serotonergic cells are found in and around the **midline raphe nuclei** of the brainstem in pons and bulb. They are part of the reticular formation. The projections of these cells are widely distributed throughout the brain and spinal cord. They are involved in the control of the sleep-wake cycles and emotional behavior.