PHYSIOLOGY OF NEUROTRANSMITTERS

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Neurotransmitter and Receptors

Synaptic transmission: The release of neurotransmitter by a presynaptic cell and the detection and response of the neurotransmitter by the postsynaptic cell

Objective of these lectures: to learn the specific mechanisms of the principal neurotransmitters, to introduce basic neuropharmacology. Touch on physiological role now, explore in later lectures in more depth.

Classification of neurotransmission

Fast neurotransmission

Neurotransmitter directly activates ligand-gated ion channel receptor

Neuromodulation

Neurotransmitter binds to G-protein coupled receptor to activate a chemical signaling cascade

Outline

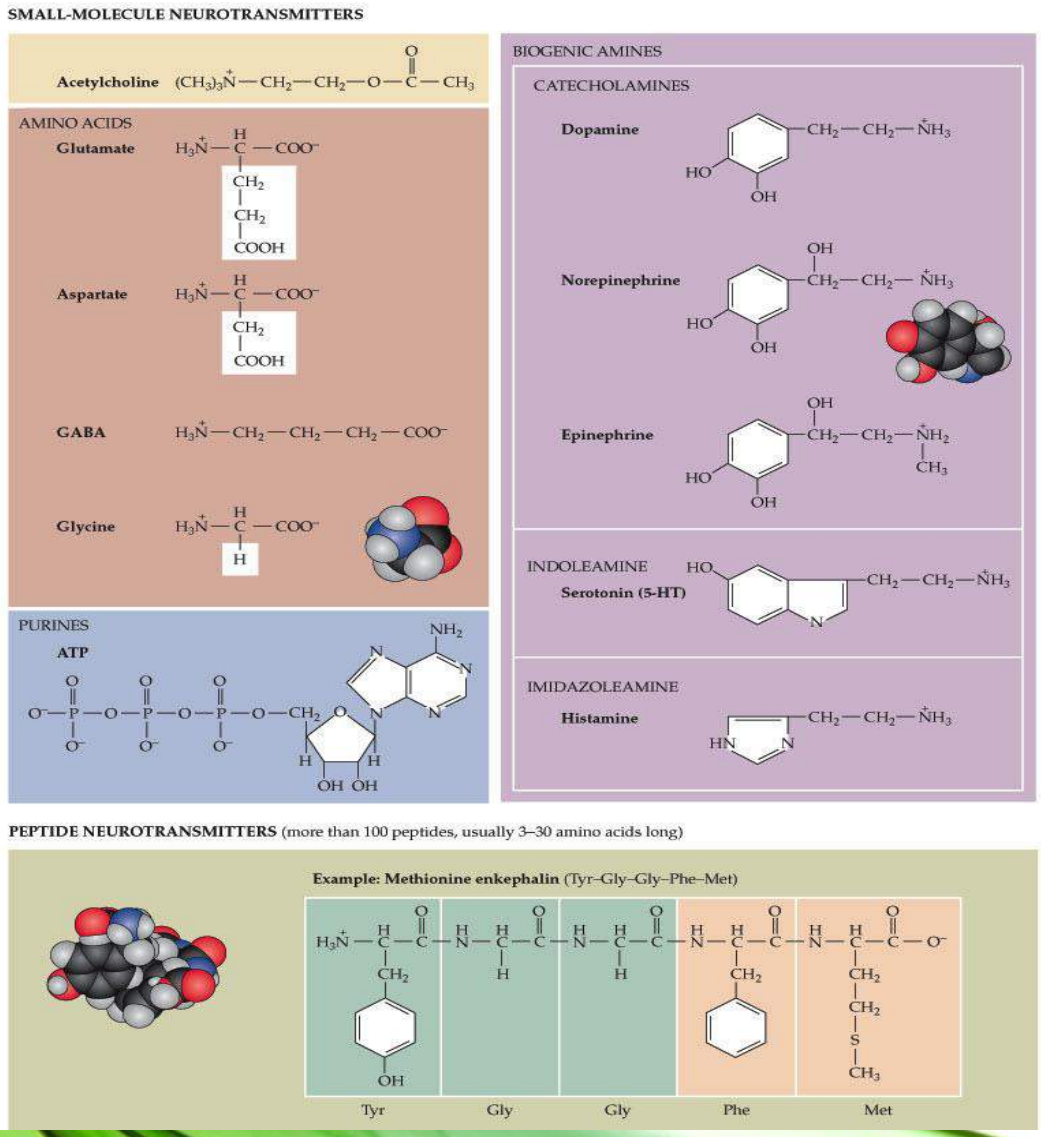
Survey of neurotransmitter structures

Fast neurotransmission: glutamate, GABA, glycine, acetylcholine
- Metabolism and vesicular transport
- Reuptake and degradation
- Receptor systems
- Pharmacology: agonists and antagonists
- Synaptic integration

Neuromodulation: catecholamines, serotonin, histamine, neuropeptides
- Overview of G-protein signaling
- Metabolism and vesicular transport
- Reuptake and degradation
- Receptor systems, coupling, downstream targets
- Pharmacology: agonists and antagonists

Unconventional neurotransmitters
- endocannabinoids, NO

Survey of the major neurotransmitters



A word about classifying neurotransmitters

Some neurotransmitters have fast and neuromodulatory modes of function, some exclusively one type or the other

Fast mode: ion channel receptors (ionotropic receptors)

Modulatory mode: G-protein coupled receptors (metabotropic receptors)

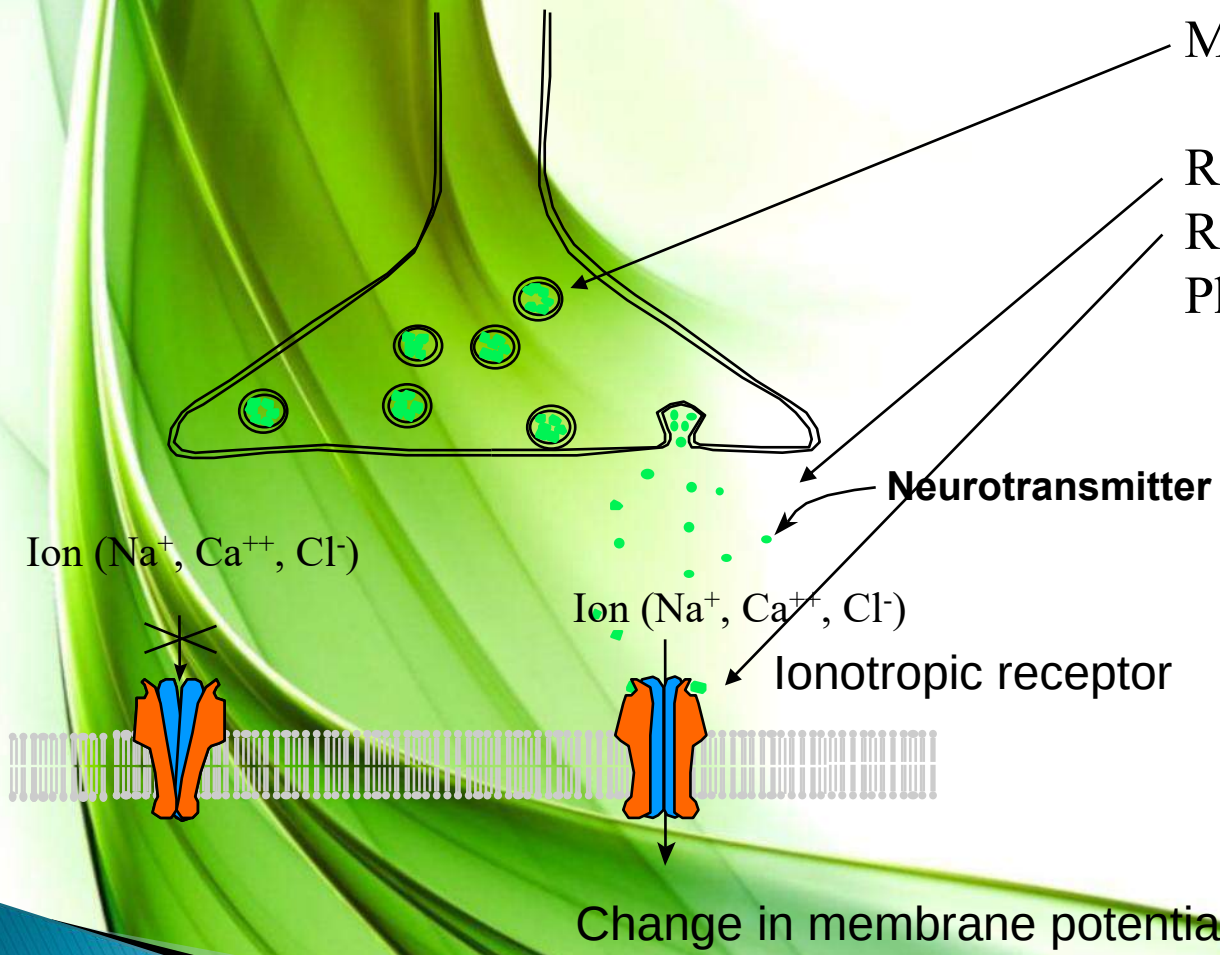
(C)

Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nAChR	Serotonin	Purines
Subunits (combination of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5	α_{1-7}	α1	α_{2-9}	5-HT_3	P_{2X1}
	Glu R2	NR2A	Glu R6	β_{1-4}	α2	β_{1-4}		P_{2X2}
	Glu R3	NR2B	Glu R7	γ_{1-4}	α3	γ		P_{2X3}
	Glu R4	NR2C	KA1	δ	α4	δ		P_{2X4}
		NR2D	KA2	ε	β			P_{2X5}
				ρ_{1-3}				P_{2X6}
								P_{2X7}

(B)

Receptor class	Glutamate	$GABA_B$	Dopamine	NE, Epi	Histamine	Serotonin	Purines	Muscarinic
Receptor subtype	Class I	$GABA_B$ R1	$D1_A$	α1	H1	5-HT 1	A type	M1
	mGlu R1	$GABA_B$ R2	$D1_B$	α2	H2	5-HT 2	A1	M2
	mGlu R5		D2	β1	H3	5-HT 3	A2a	M3
	Class II		D3	β2		5-HT 4	A2b	M4
	mGlu R2		D4	β3		5-HT 5	A3	M5
	mGlu R3					5-HT 6	P type	
	Class III					5-HT 7	P2x	
	mGlu R4						P2y	
	mGlu R6						P2z	
	mGlu R7						P2t	
	mGlu R8						P2u	

Fast neurotransmission, simplified



Metabolism and vesicular transport

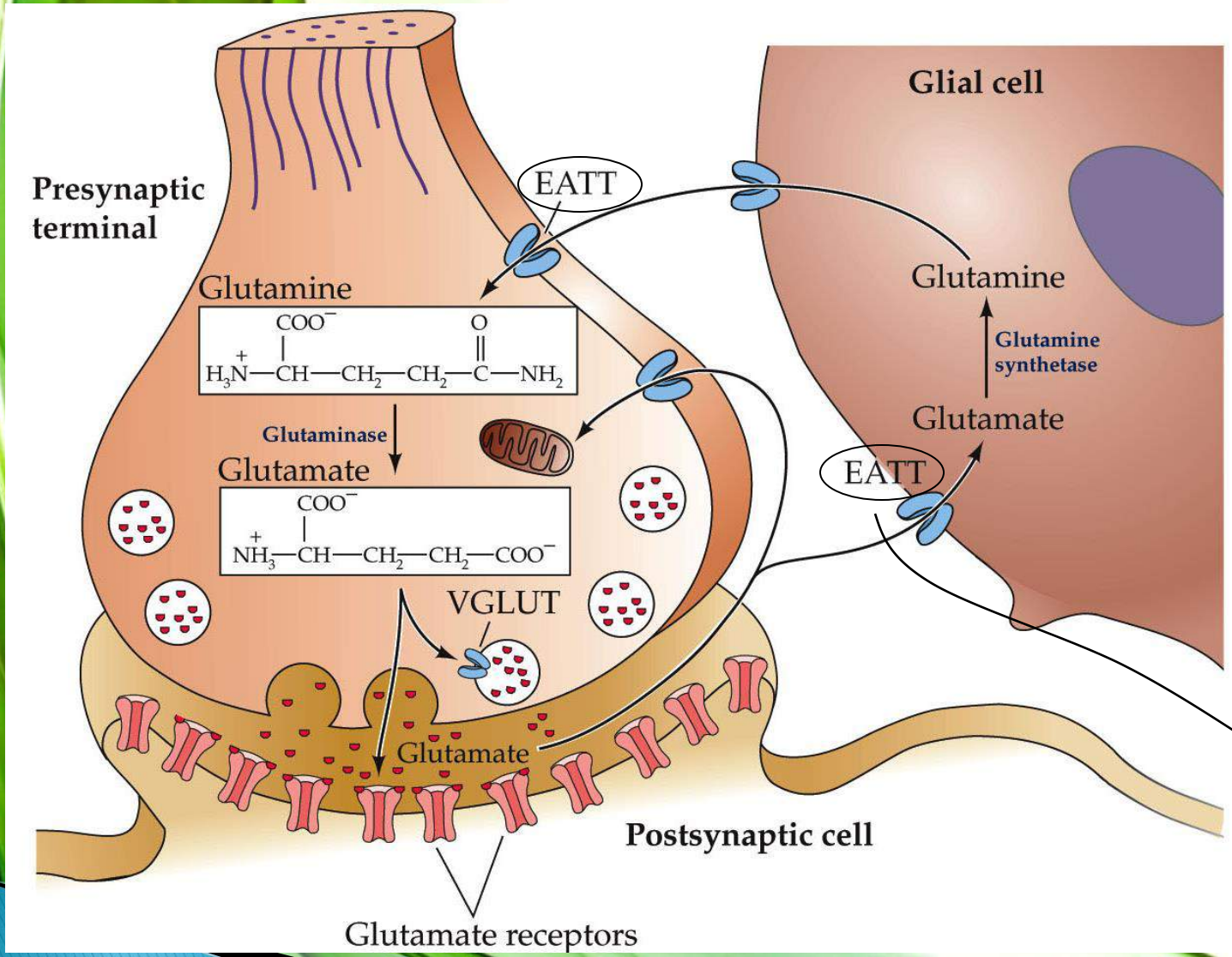
Reuptake and degradation

Receptor systems

Pharmacology: agonists and antagonists for study and therapy

Glutamate fast neurotransmission

Synthesis, packaging, reuptake, degradation



(error - should be EAAT)

(C)

Receptor	AMPA	NMDA	Kainate
Subunits (combi-nation of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5
	Glu R2	NR2A	Glu R6
	Glu R3	NR2B	Glu R7
	Glu R4	NR2C	KA1
		NR2D	KA2

Molecular diversity of glutamate receptors:

3 types, based on sensitivity to pharmacological agents: AMPA, kainate, N-methyl d-aspartate (NMDA)

AMPA: homotetramers or heterotetramers assembled from Glu R1-4 subunits

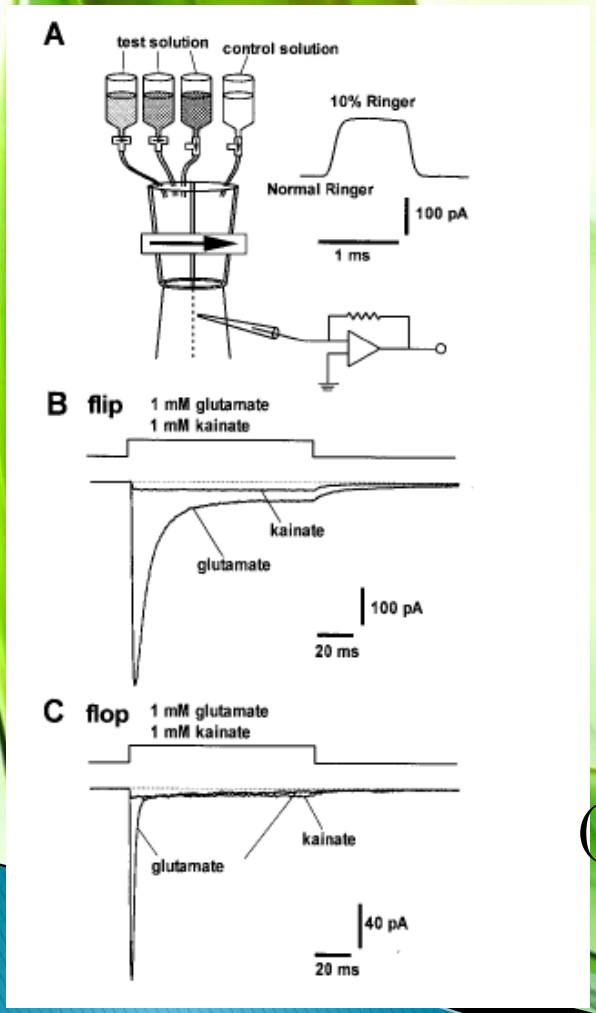
NMDA: heterotetramers that contain an NR1 subunit, and a subunit from the NR2 family

Kainate: heterotetramers containing subunits from the KA1,2 family, and from the GluR5-7 family

AMPA receptor functional diversity

Mixing and matching of subunits (see GABA receptors for examples)

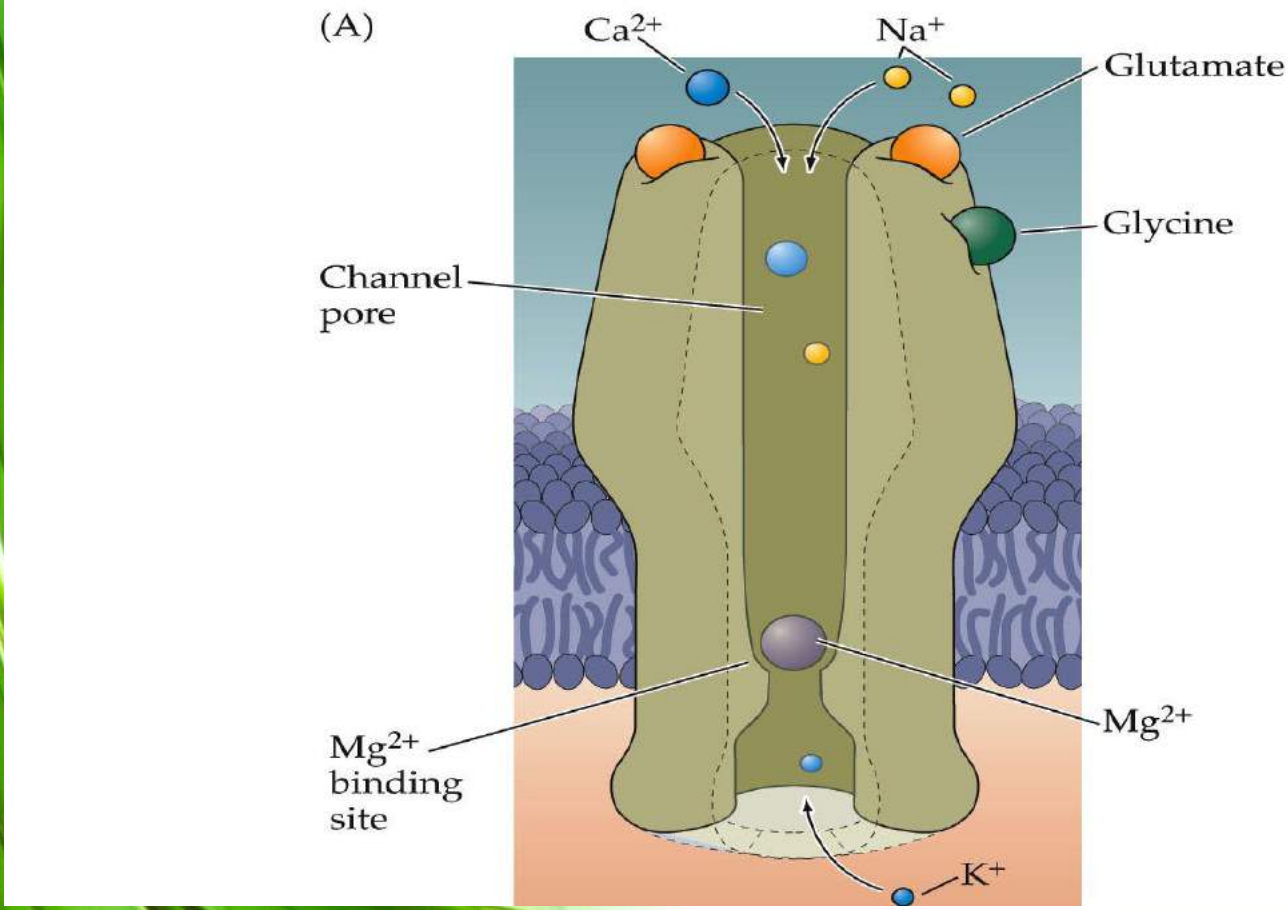
Further diversity generated by alternative splicing, editing



Flip and flop splice forms desensitize at different rates, both have rapid onset kinetics

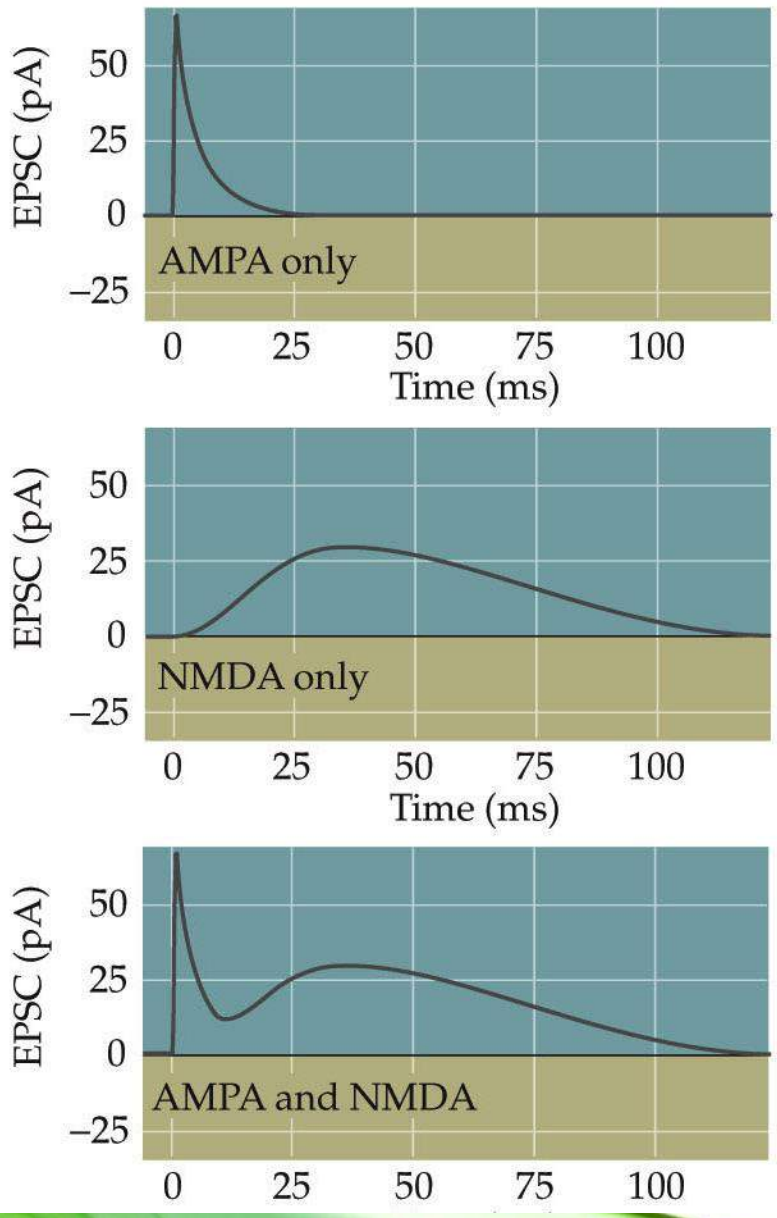
(gluR2 homomers shown)

NMDA receptors



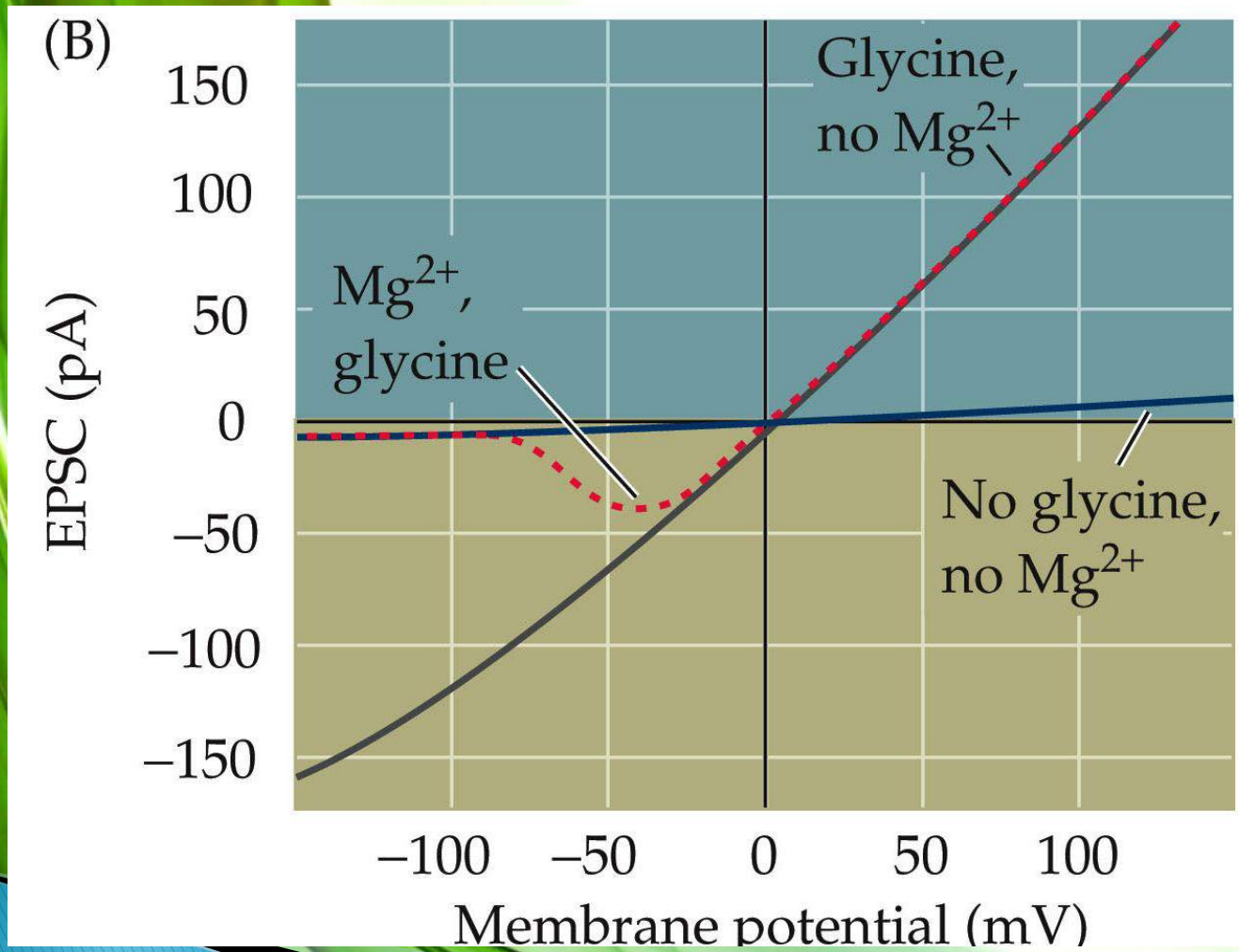
NMDA receptors show slow onset and decay kinetics

Some synapses have both glutamate receptor types, and produce a two-component synaptic current

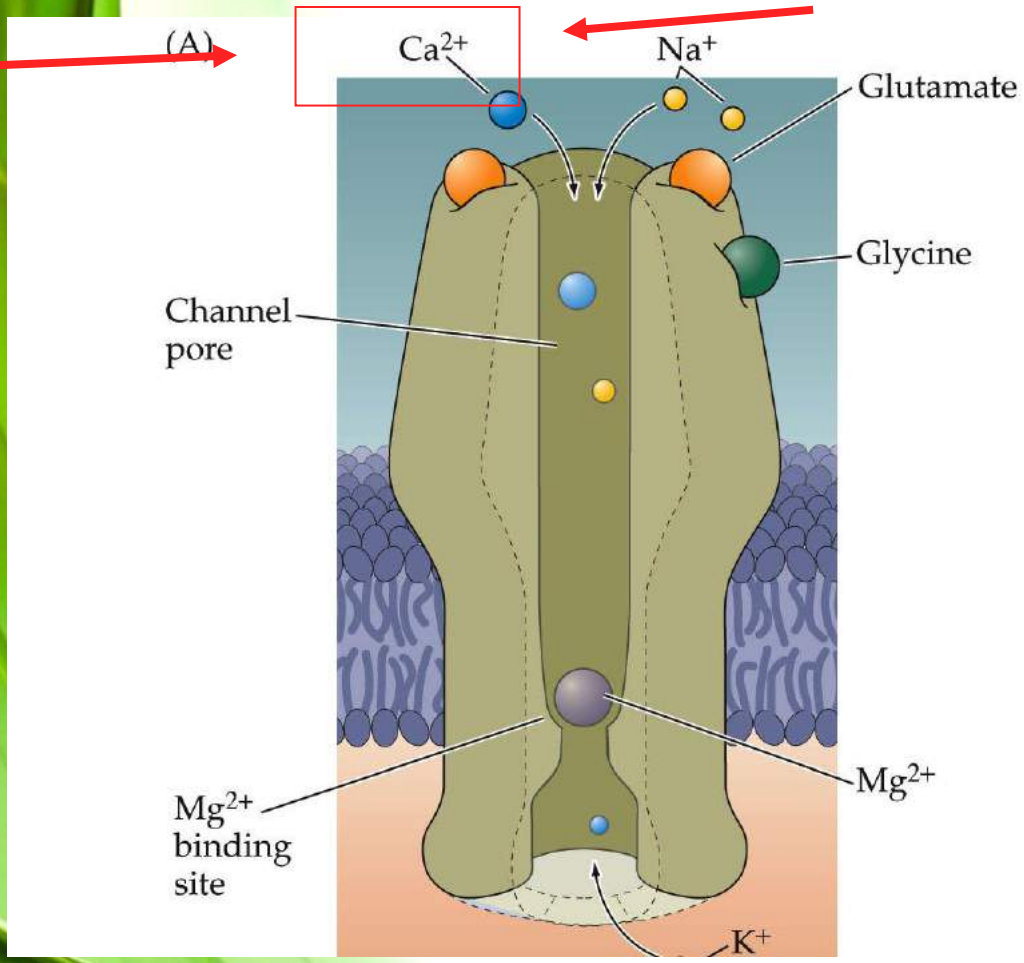


NMDA receptors are strongly rectifying because of Mg^{++} block

Coincidence detector in learning and memory



NMDA receptors are calcium permeable



This property is particularly significant because calcium is a second messenger that plays many important regulatory roles

Pharmacology

AMPA

agonists: AMPA, glutamate

antagonists: CNQX, NBQX

Kainate

agonists: kainic acid, glutamate

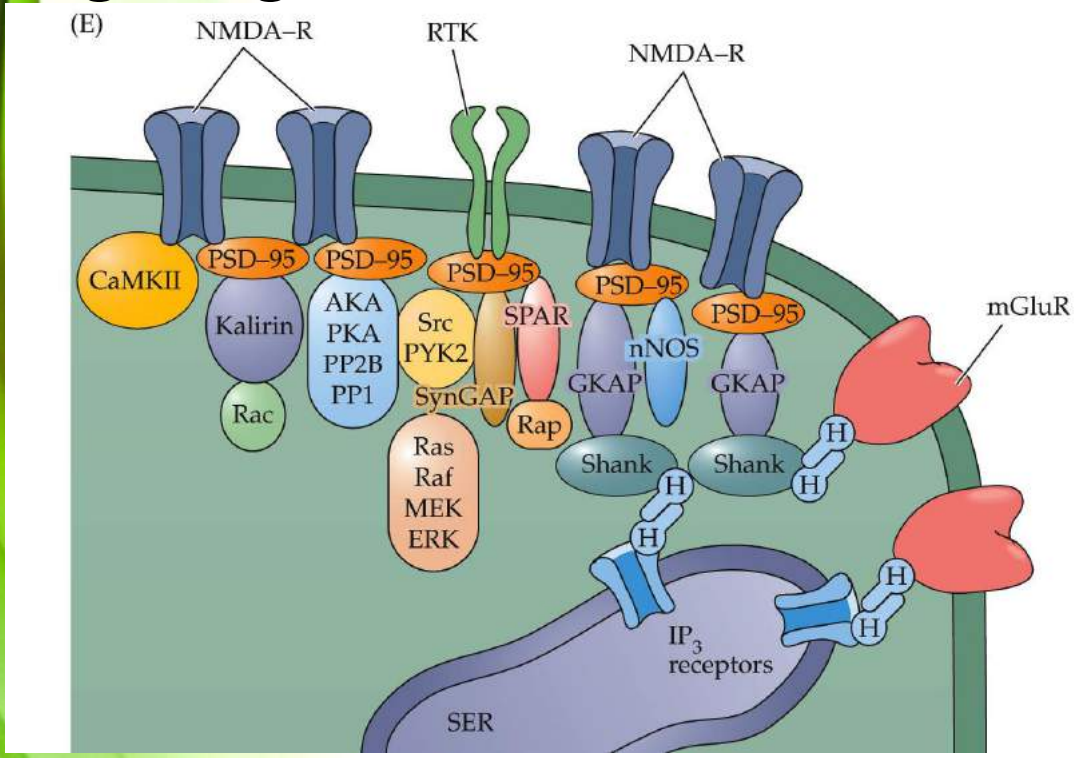
antagonist: CNQX

NMDA

agonists: glutamate, aspartate, NMDA

antagonists: D-APV, D-AP5, MK-801, Ketamine, Phencyclidine, (Mg^{++})

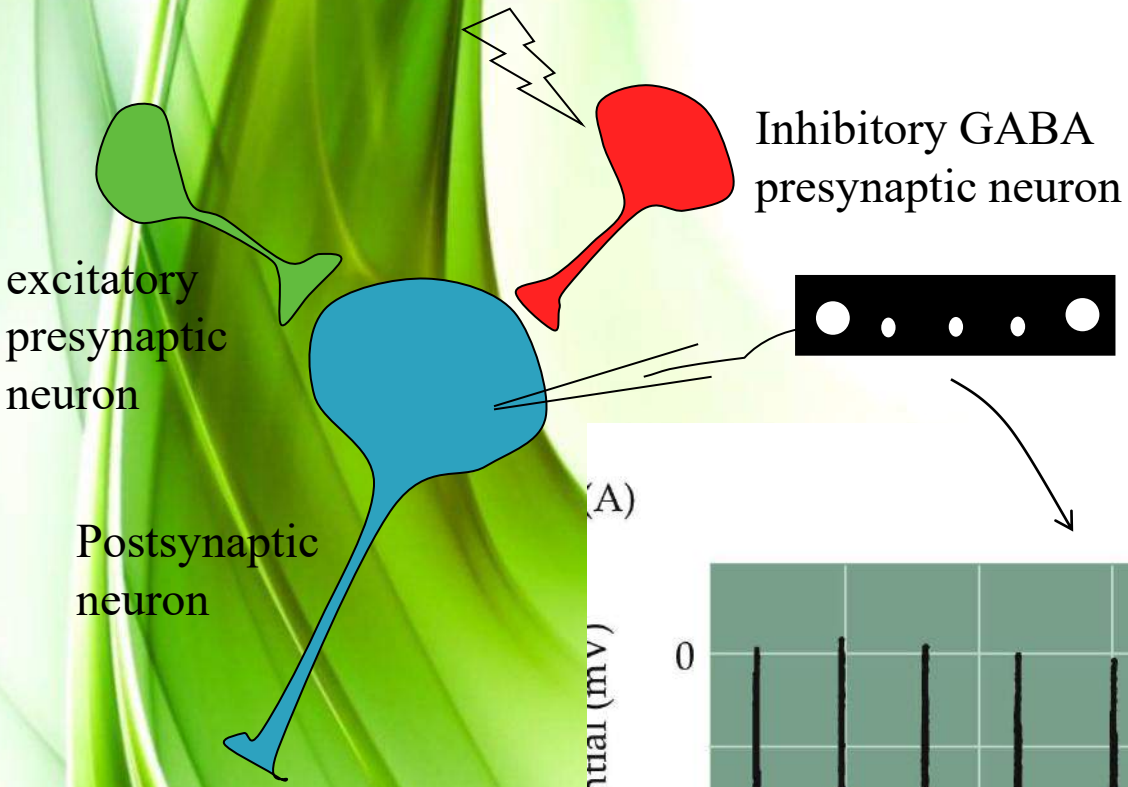
Glutamate receptors are physically tethered at synapses and associated with signaling molecules

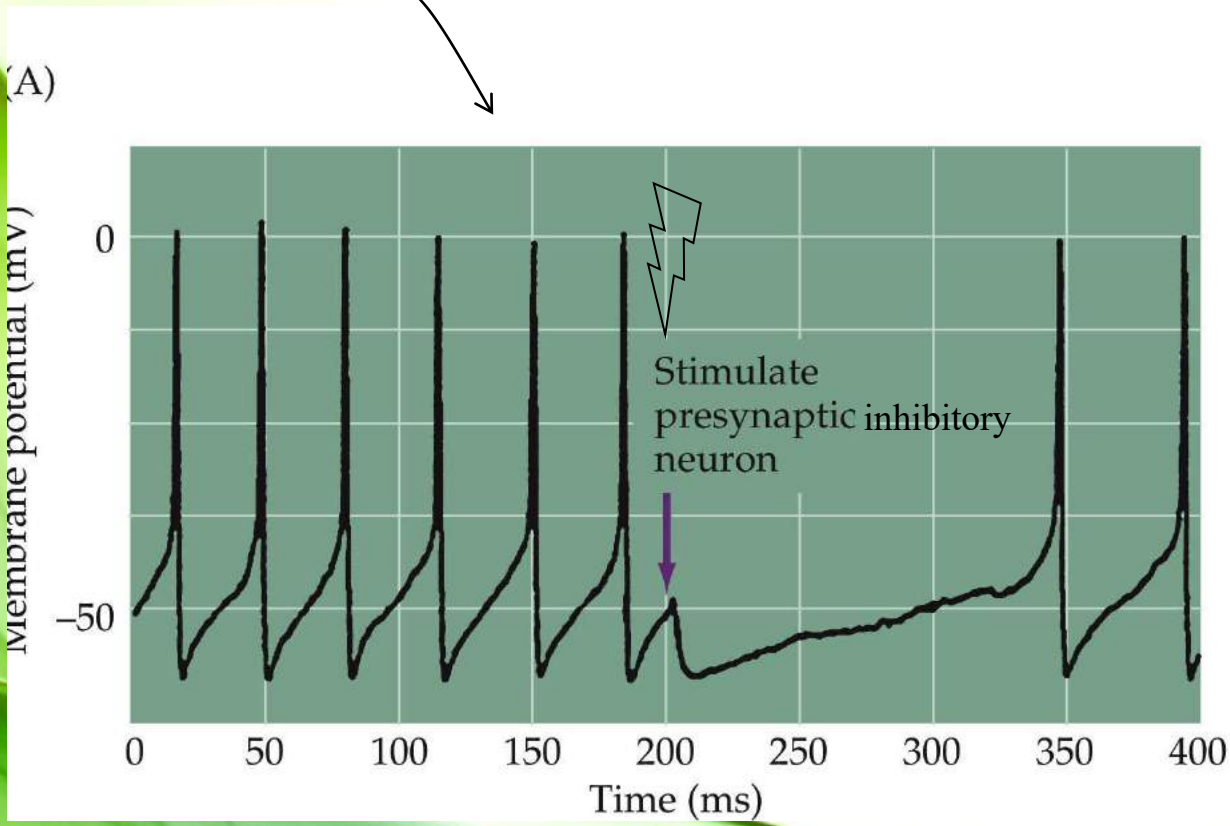


AMPA receptors interact with GRIP, SAP-97 and others

Synaptic strength and Ca^{++} permeability of glutamate postsynaptic complexes is a major determinant of synaptic plasticity

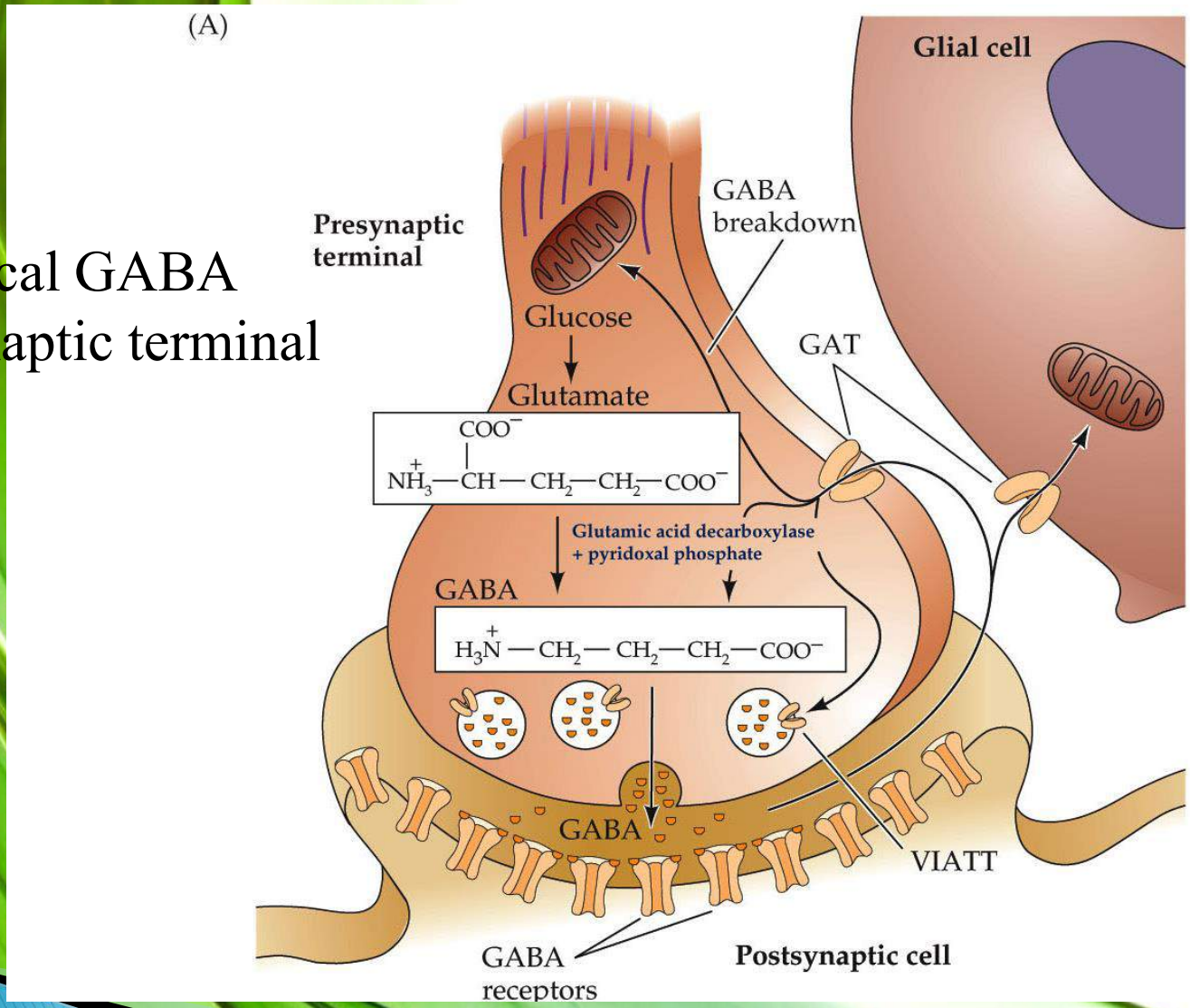
Inhibitory neurotrans-mission prevents excitation of the post-synaptic neuron



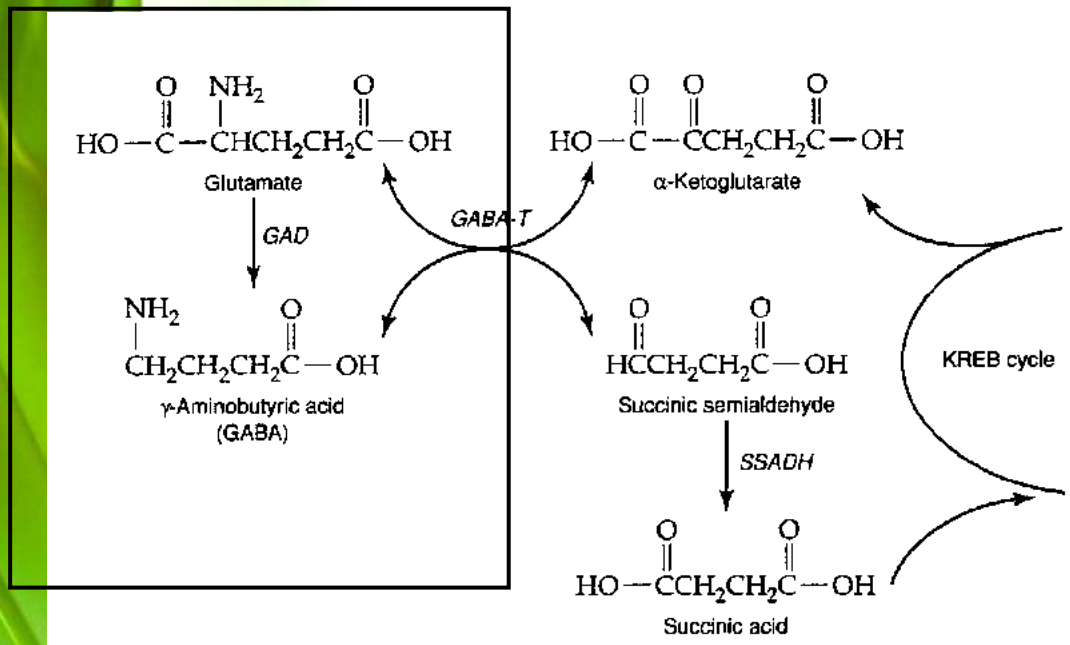


Whereas glutamate is the principal excitatory neurotransmitter, GABA is the principal inhibitory neurotransmitter in the brain

A typical GABA presynaptic terminal



GABA synthesis



Biosynthetic enzyme: GAD_{65}, GAD_{67}

GAD_{65} more highly enriched in nerve terminals, therefore might be more important for neurotransmission

GAD requires pyridoxal phosphate as cofactor (might be regulated by GABA and ATP)

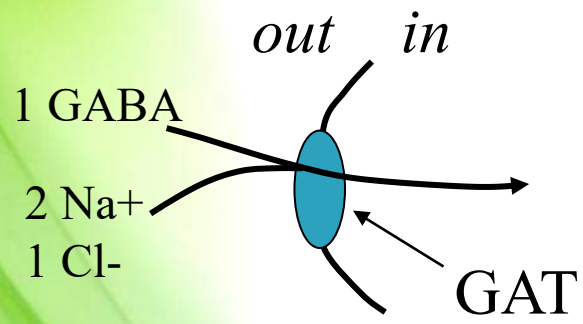
GABA release, reuptake

Vesicular release is the major mechanism

Uptake is mediated by plasma membrane transporters

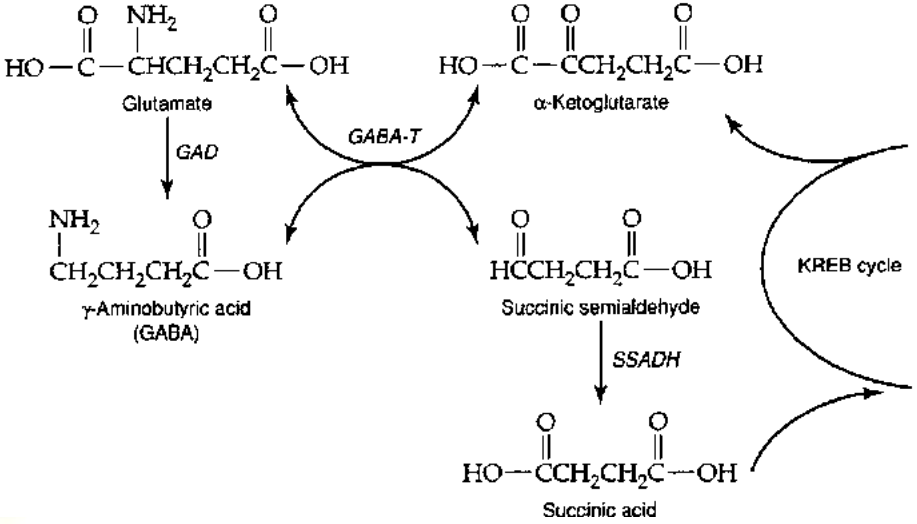
GAT-1, GAT-2, GAT-3, BGT-1

GAT1-3 in brain, BGT-1 in kidney but may also be in brain

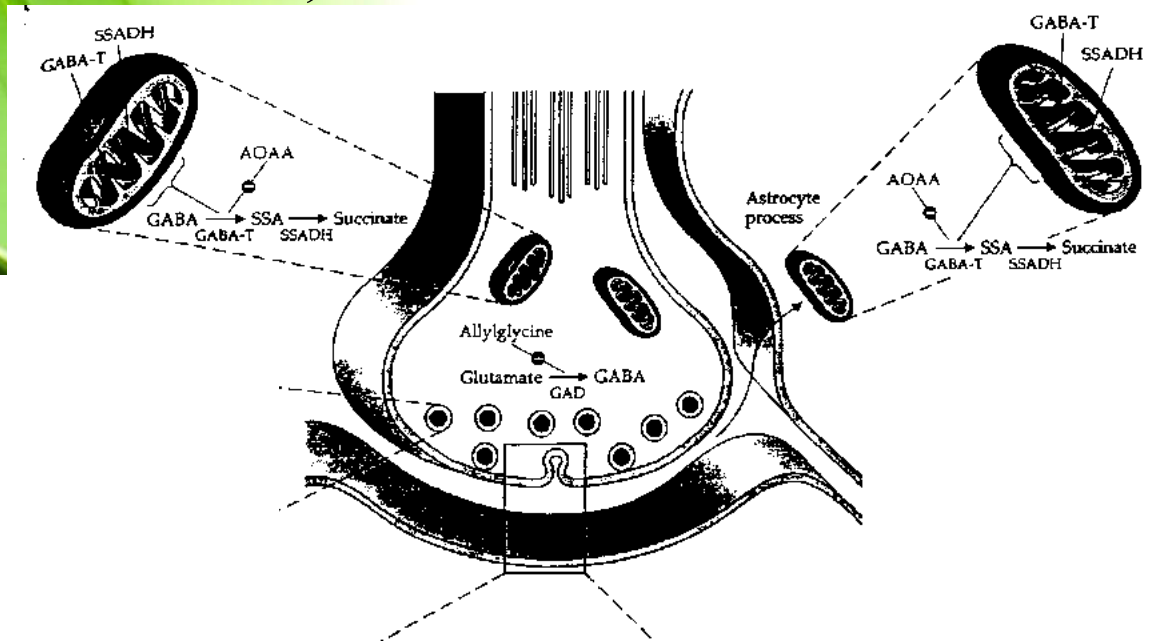


Degradation

GABA aminotransferase (aka GABA transaminase or GABA T)



Astrocytes and neurons, mitochondrial



Summary of GABA synthesis, release, reuptake, degradation

1. GABA is formed by removal of carboxyl group of glutamate, by the enzyme GAD
2. GABA is packaged into synaptic vesicles by VIAAT and released by depolarization
3. GABA may be taken up by nerve terminal by GAT proteins for repackaging into synaptic vesicles
4. GABA may be taken up by glial cells, where it undergoes reconversion to glutamate (amine group is transferred to α-ketoglutarate, generating glutamate and succinic semialdehyde)
5. Glutamate is transported back into nerve terminal, where it serves as precursor for new GABA synthesis

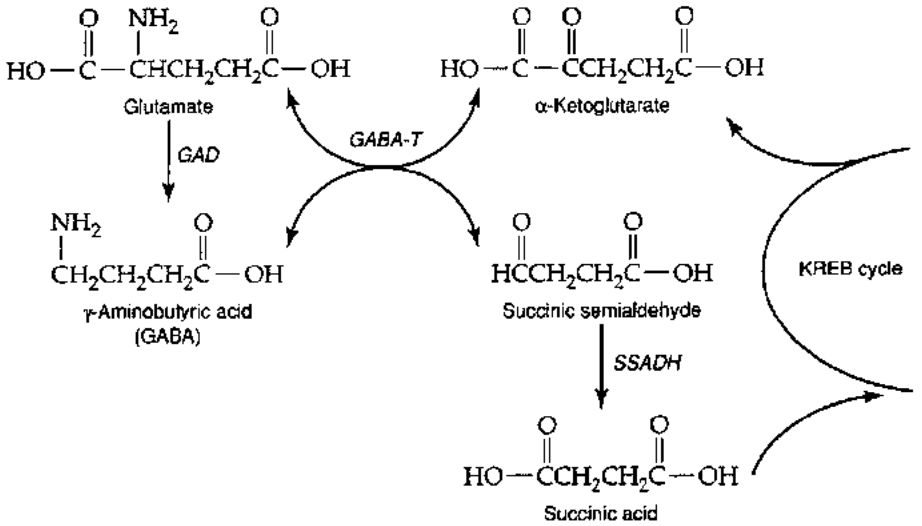
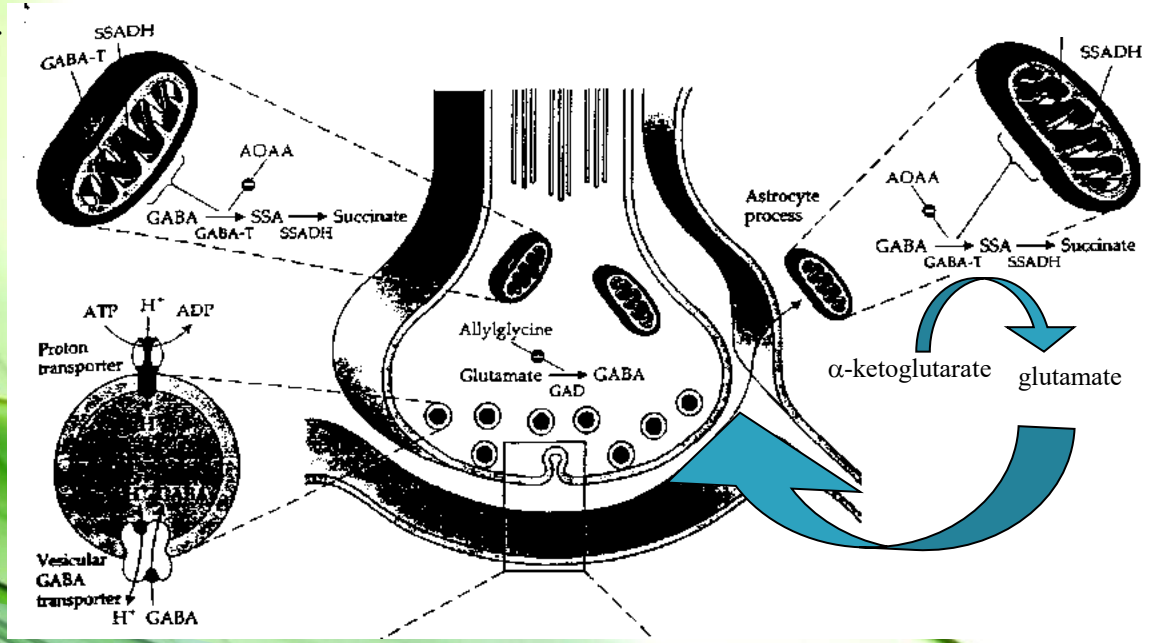


Figure 7–8. The GABA shunt. This metabolic pathway traces the synthesis and degradation of the neurotransmitter pool of GABA. GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase.

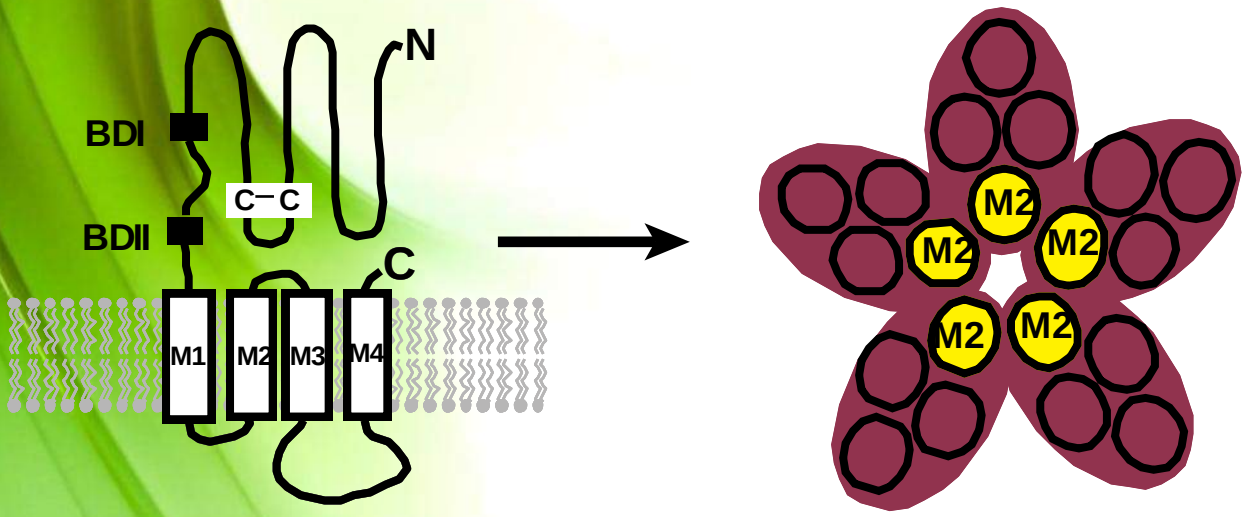


GABA receptors:

Fast GABA transmission mediated mainly by $GABA_A$ receptors, which are ligand-activated chloride channels. Some fast GABA transmission mediated by so-called $GABA_C$ receptors, which are a closely-related sub-family of $GABA_A$ receptors

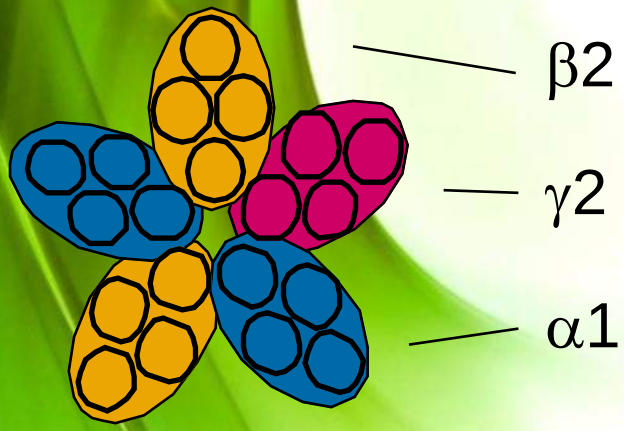
GABA also utilizes a metabotropic receptor called the $GABA_B$ receptor, described in Neuromodulation section.

Pentameric structure of $GABA_A$ receptors



$GABA_A$ receptors belong to the 'ligand-gated ion channel superfamily', which also includes nicotinic acetylcholine receptors, glycine receptors, and the 5-HT_3 serotonin receptor. Fine structure and function of this receptor class will be covered in more detail in the acetylcholine section, upcoming.

$GABA_A$ receptors are *hetero*multimers



subunits

-Alpha (1-6)
-Beta (1-4)
-Gamma (1-4)
-delta, epsilon, pi, theta
-Rho (1-3) - make up the $GABA_C$ receptor

Potentially thousands of different subunit combin-ations, or subtypes. Which really occur in the brain?

About 12 subtypes are prevalent

R.M. McKernan and P.J. Whiting – $GABA_A$-receptor structure

TABLE 1. Distribution of the major $GABA_A$-receptor subtypes in the rat brain

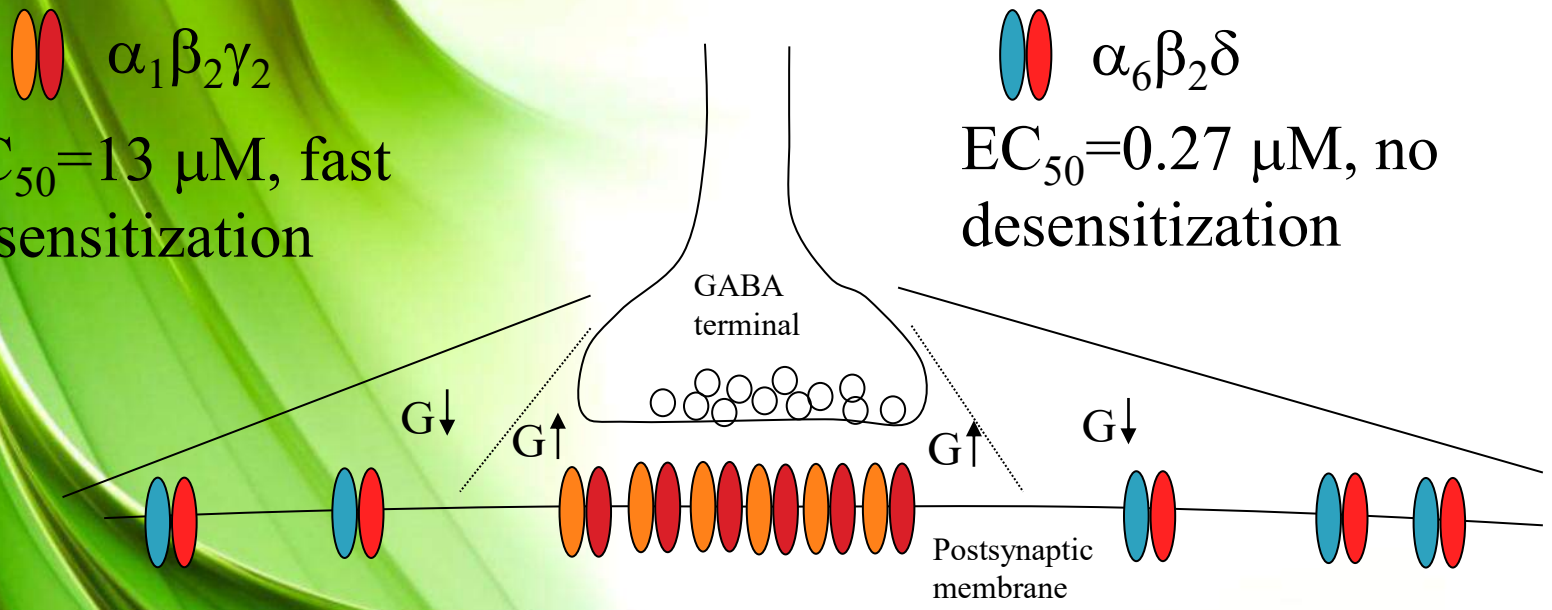
Subtype	Relative abundance in rat brain (%)	Location and putative function
α1β2γ2	43	Present in most brain areas. Localized to interneurones in hippocampus and cortex, and cerebral Purkinje cells
α2β2/3γ2	18	Present on spinal cord motoneurones and hippocampal pyramidal cells
α3βnγ2/γ3	17	Present on cholinergic and monoaminergic neurones where they regulate ACh and monoamine turnover
α2βnγ1	8	Present on Bergmann glia, nuclei of the limbic systems, and in pancreas
α5β3γ2/γ3	4	Predominantly present on hippocampal pyramidal cells
α6βγ2	2	Present on cerebellar granule cells
α6βδ	2	Present on cerebellar granule cells
α4βδ	3	Present in thalamus and hippocampal dentate gyrus
Other minor subtypes	3	Present throughout brain

Location and function are listed where these have been investigated, and are not comprehensive. Other minor subtypes include α1α6βγ2, α1α3βγ2, α2α3βγ2 and α5βγ2δ subtypes and are represented together as a small population.

What is the significance of this receptor diversity?

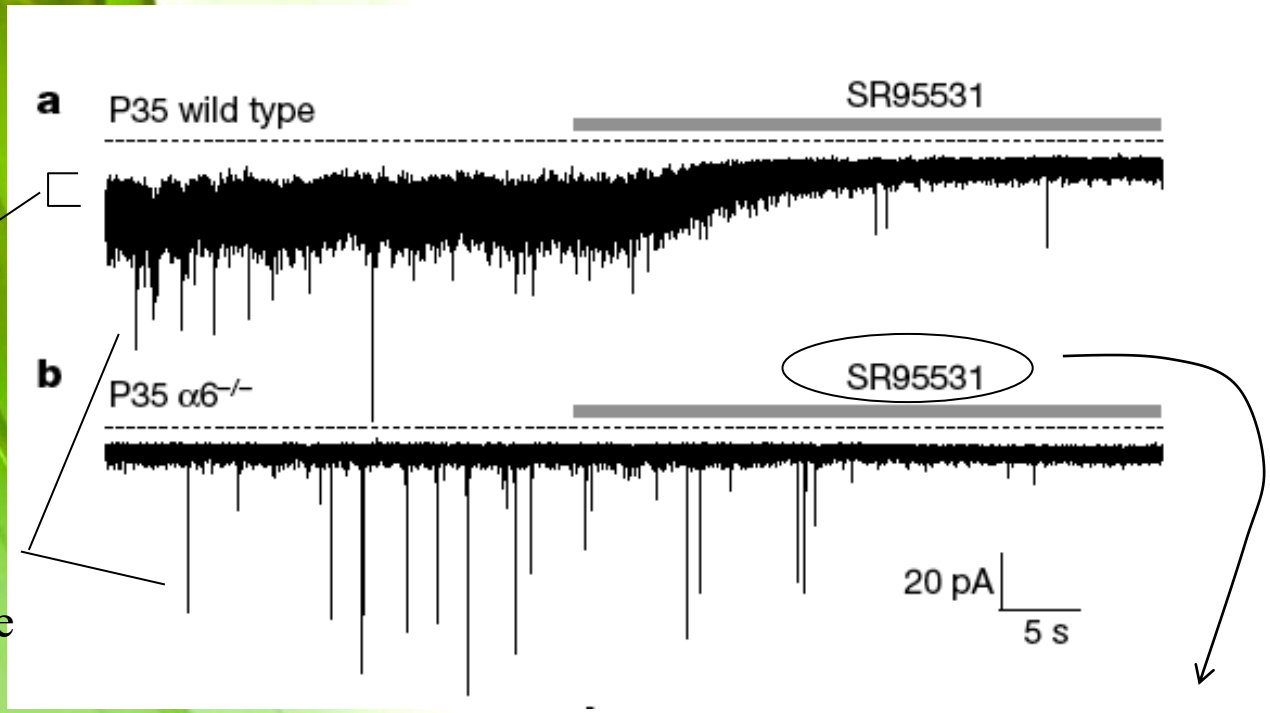
Different subunit combinations (receptor subtypes) confer different functional properties.

Those properties allow the receptors to do different jobs



Low GABA sensitivity of and fast desensitization $\alpha_1\beta_2\gamma_2$ are suited for phasic activity and high GABA concentrations found right at the synapse. High GABA sensitivity and lack of desensitization allows $\alpha_6\beta_2\delta$ to detect GABA that spills over from the active synaptic zone

Recording from cerebellar granule cells, showing both synaptic and extrasynaptic GABA responses



Extrasynaptic 'tonic' response

Phasic synaptic GABA response

Extrasynaptic tonic currents are dependent on the presence of an intact α_6 subunit gene

Inhibitor of all $GABA_A$ receptors, eliminates both phasic and tonic responses, showing that they are both GABA currents

$GABA_A$ receptor tethering at the synapse

Several proteins that are important for $GABA_A$ receptor tethering have been proposed, principally ‘gephyrin’, but the tethering mechanism is not well characterized.

$GABA_A$ receptor pharmacology

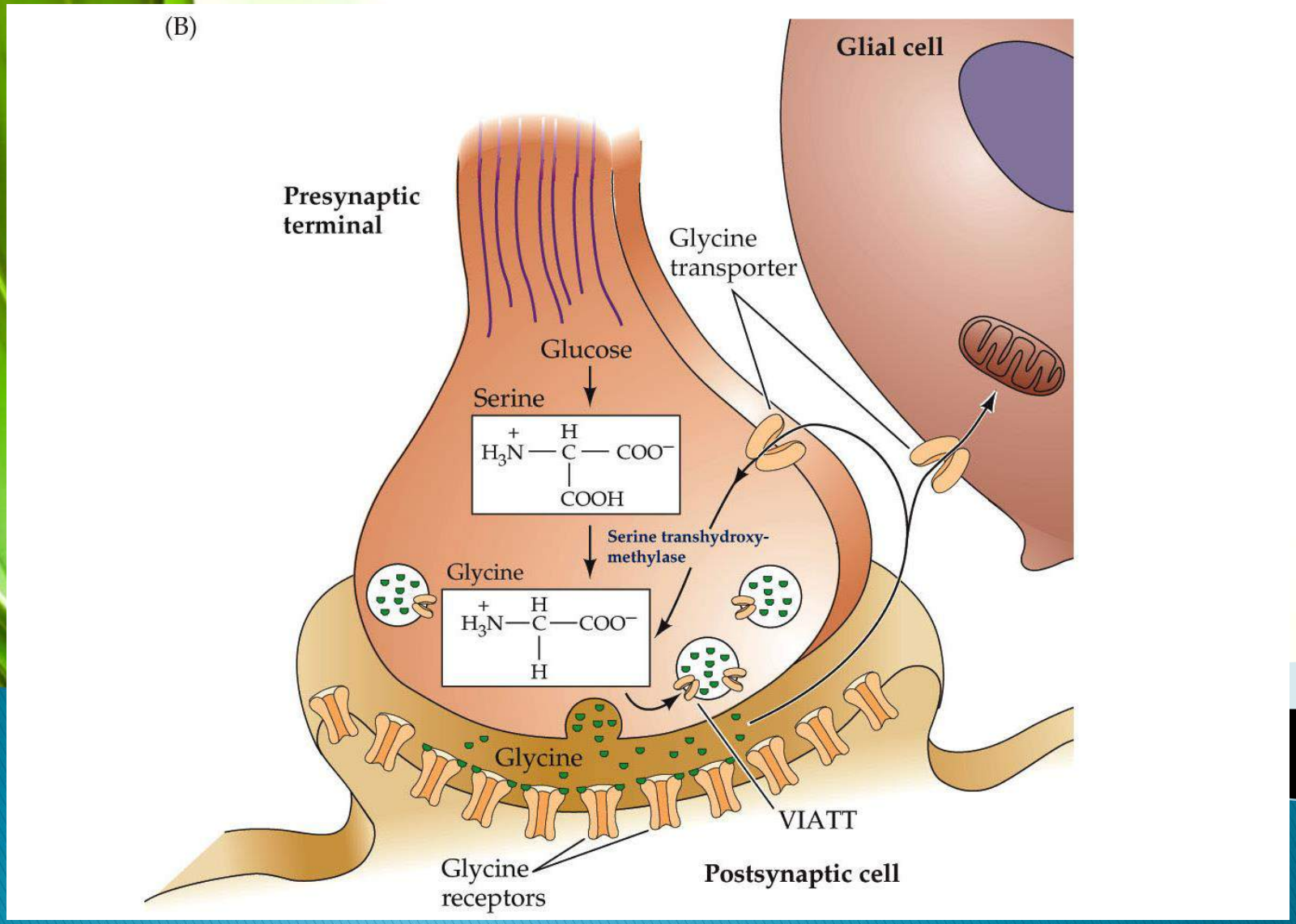
<u>Antagonists:</u>	
Bicucculine	competitive
SR95531 (gabazine)	competitive
Picrotoxin	mixed competitive, non-competitive
Penicillin G	open channel block
Pentelenetetrazole (PTZ)	open channel block
Pregnenolone sulfate	non-competitive

<u>Agonist:</u>
Muscimol
Barbiturates, neurosteroids (high concentrations)

<u>Enhancers:</u>
Benzodiazepines
Barbiturates, neurosteroids (low concentrations)

$GABA_A$ receptor antagonists are important research tools, but not clinically useful. $GABA_A$ receptor enhancement, but not direct agonism, is useful therapeutically in neurology.

Glycine neurotransmission



Summary of GABA synthesis, release, reuptake, degradation

1. Glycine is synthesized from serine by SHMT
2. Glycine is packaged into synaptic vesicles by VIAAT (same transporter as for GABA)
3. Glycine is removed from synapse by GLYT1 (glial, for clearance from synapse), and GLYT2 (neuronal, for re-uptake and packaging).
4. Glycine is cleaved by the glycine cleavage system

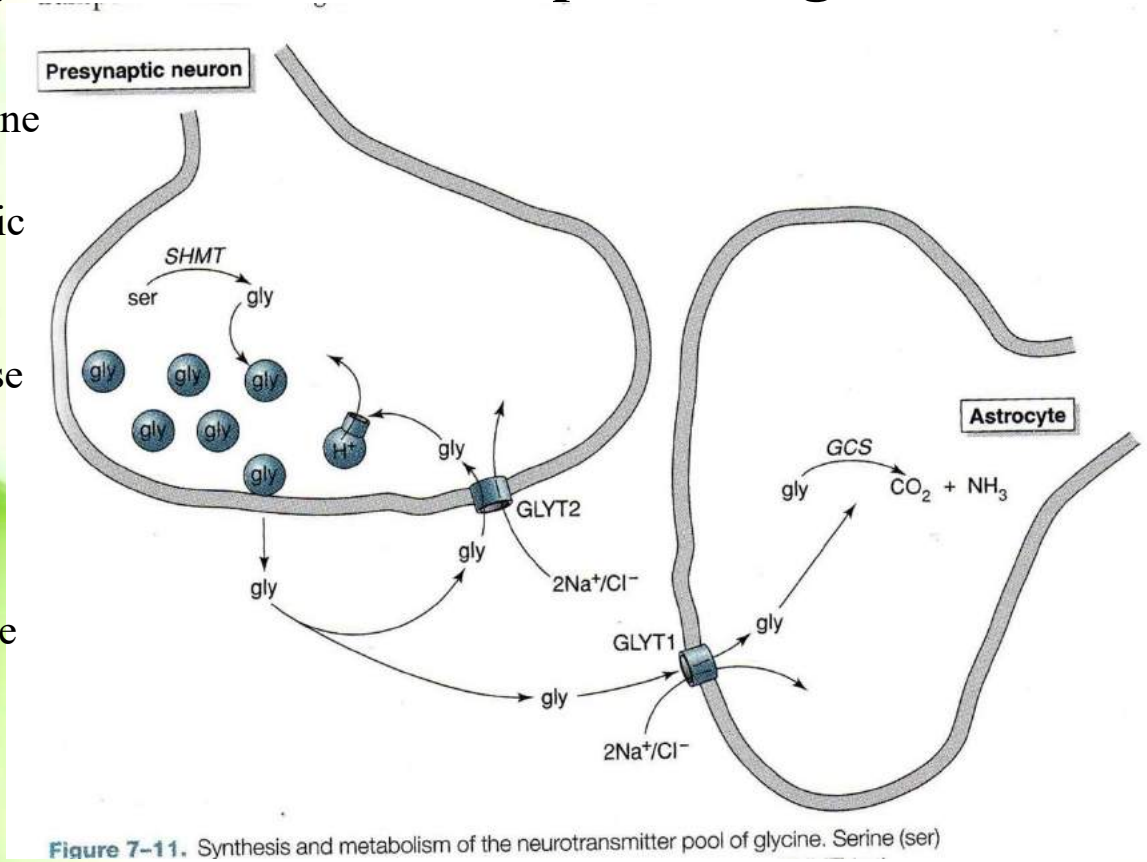


Figure 7–11. Synthesis and metabolism of the neurotransmitter pool of glycine. Serine (ser)

GCS: glycine cleavage system

Consists of 4 proteins

T protein

L protein

H protein

P protein

Glycine neurotransmission: receptors

Glycine is a neurotransmitter in its own right

Distinct from NMDA receptor co-agonist role

Ionotropic receptor, ligand-gated ion channel superfamily receptors, homologous to $GABA_A$ receptors

α1-4, βsubunits - α homomers in early development, αβ heteromers in adults

Major spinal cord inhibitory transmitter

Retinal, brainstem as well

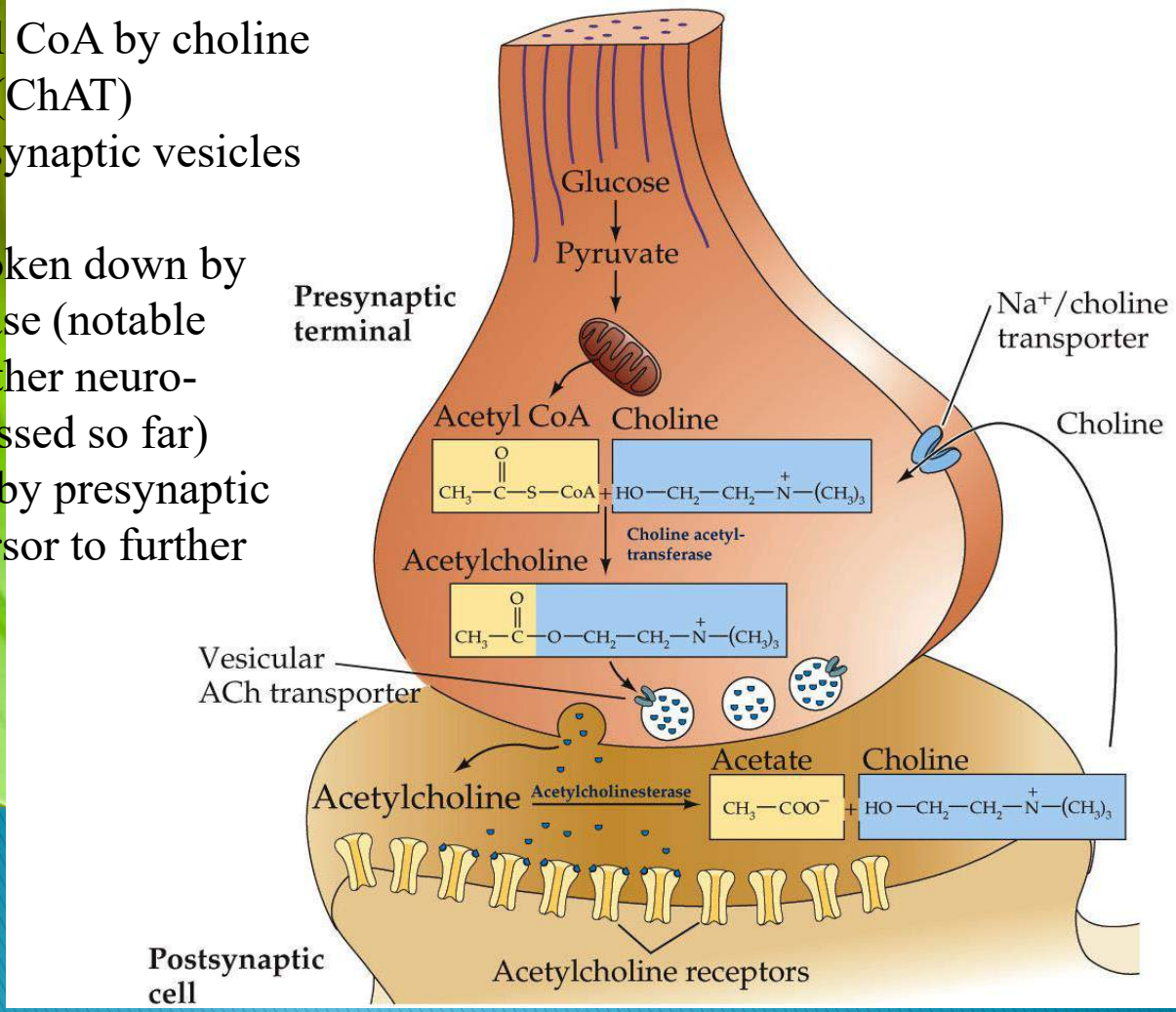
No allosteric regulators used as drugs

Strychnine is a competitive antagonist

Human mutations in glyR found in startle disease, hyperekplexia, 'Jumping Frenchman disease'

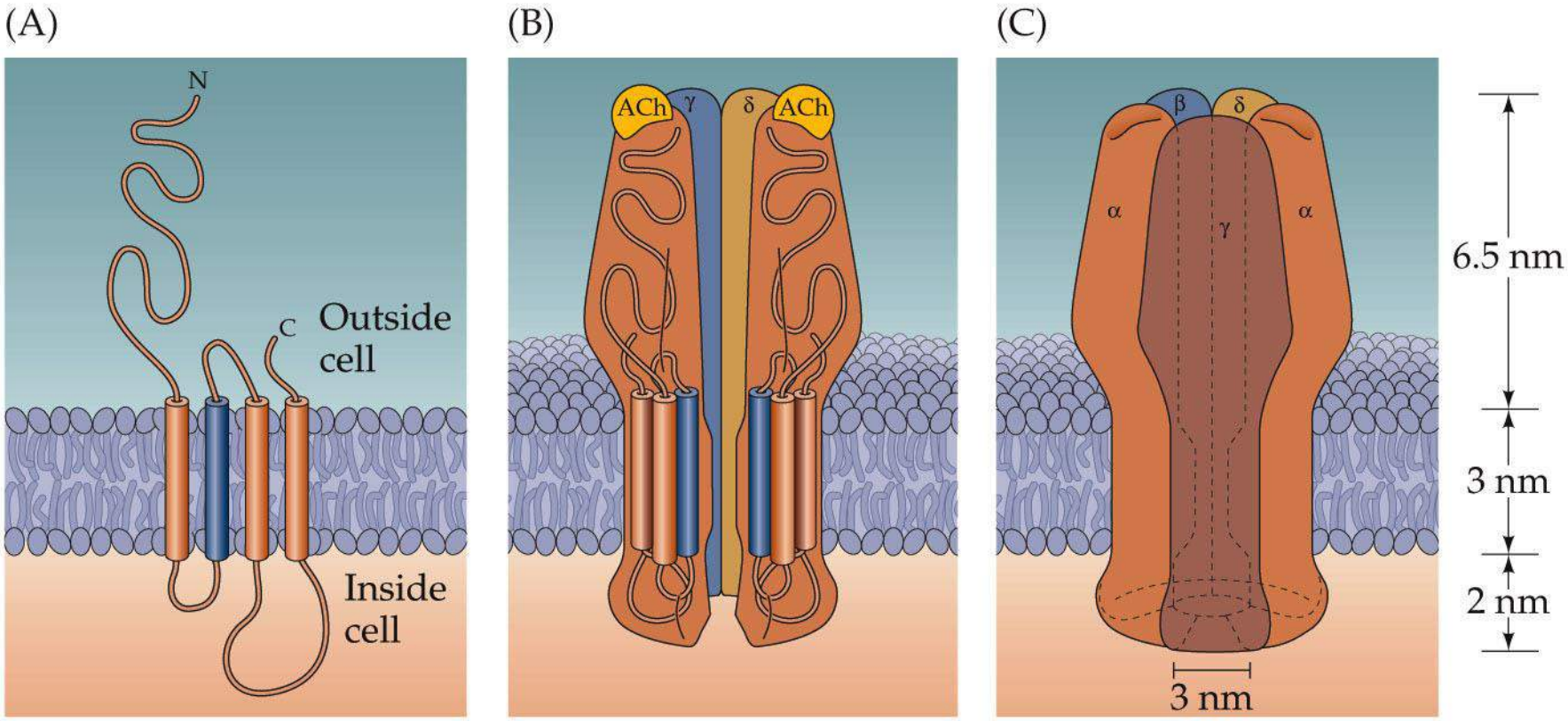
Acetylcholine neurotransmission

1. Acetylcholine synthesized from choline and acetyl CoA by choline acetyltransferase (ChAT)
2. ACh loaded into synaptic vesicles by VAchT
3. Released ACh broken down by acetylcholinesterase (notable difference from other neuro-transmitters discussed so far)
4. Choline taken up by presynaptic terminal as precursor to further ACh synthesis



Nicontinic acetylcholine receptors

Fast ACh neurotransmission utilizes ligand-gated ion channel superfamily receptors sensitive to nicotine, hence called nicotinic ACh receptors



Muscle nAChRs: 2α, β, ε, δ subunits in the ratio of 2α:β:ε:δ

Neuronal nAChRs: 3α:2β or α_7 homomers

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