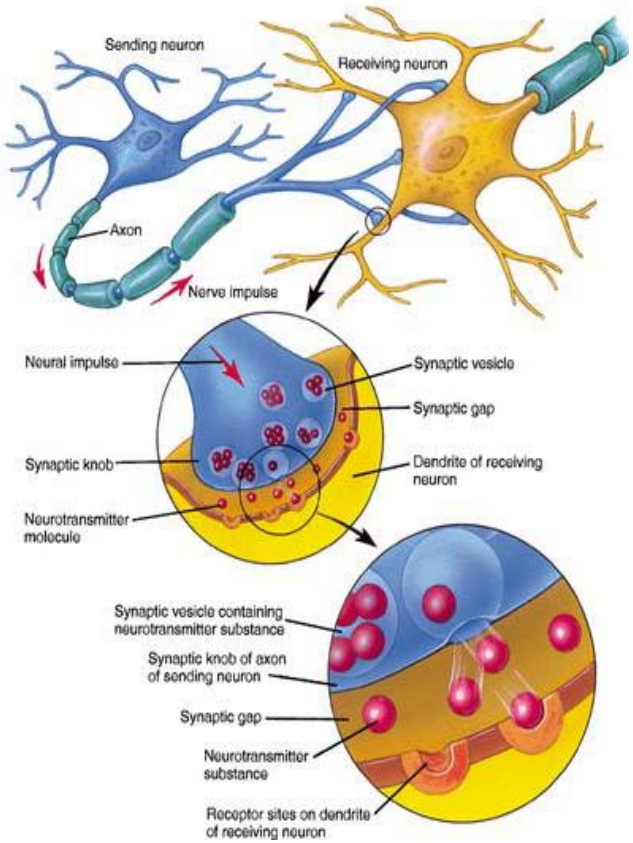
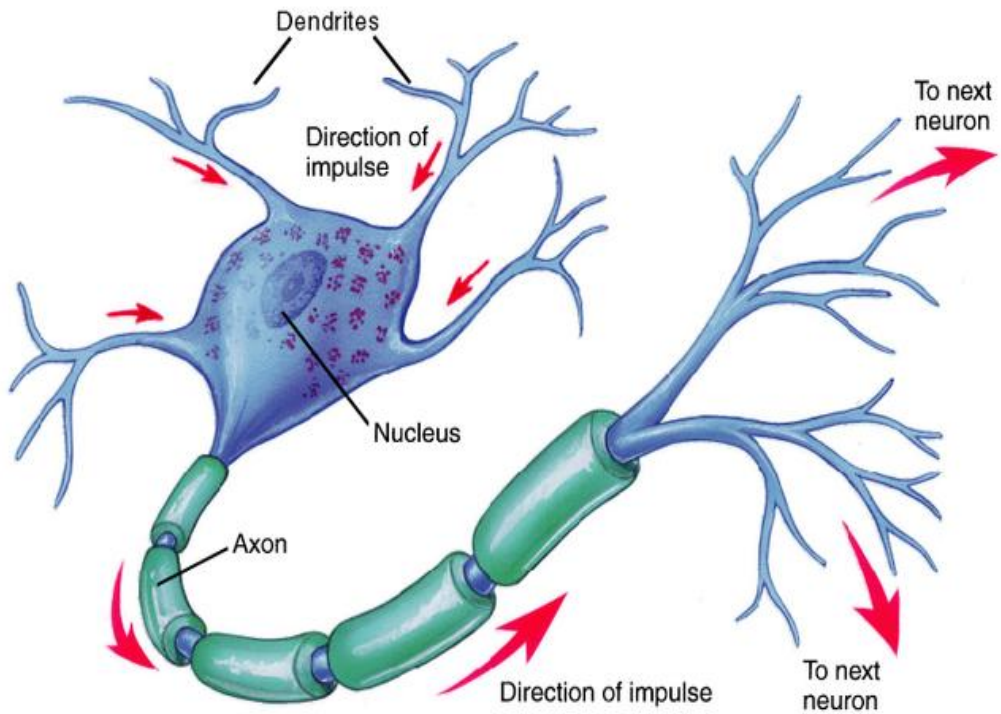
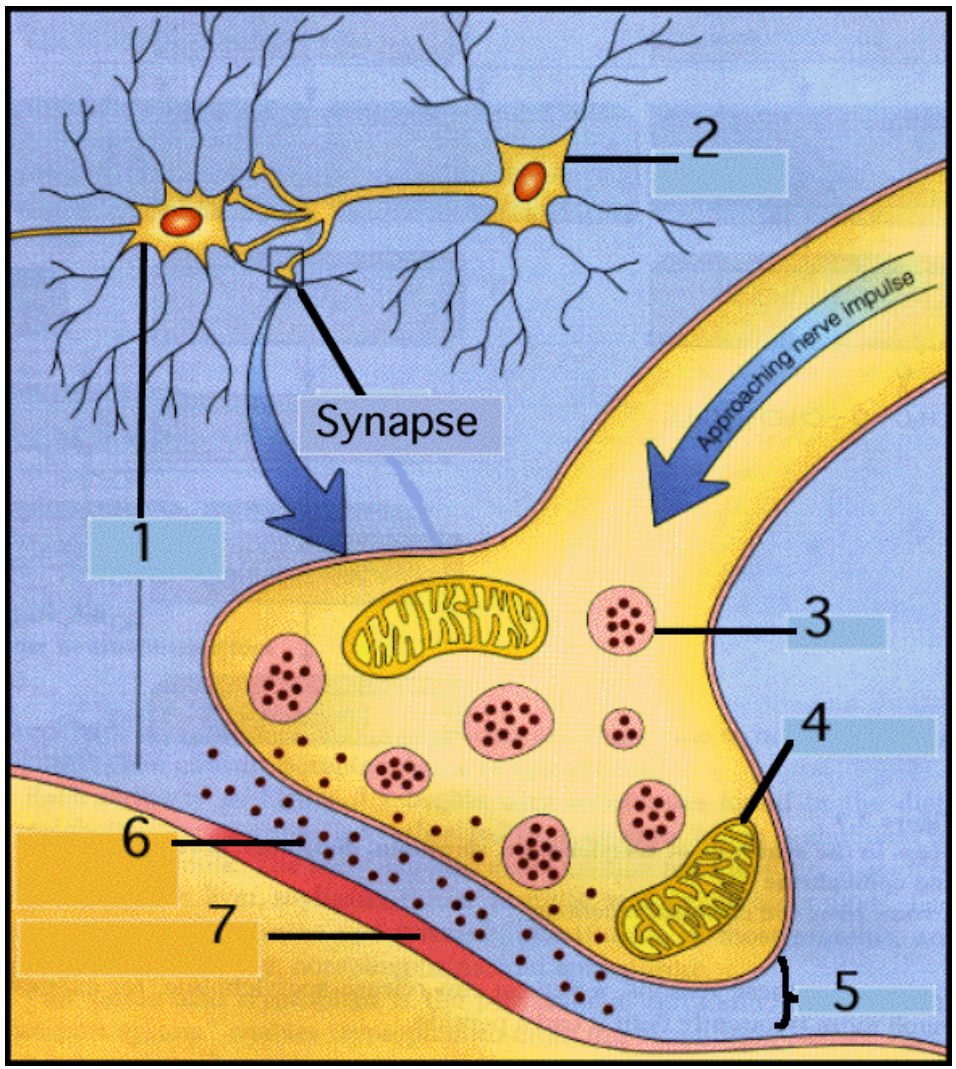


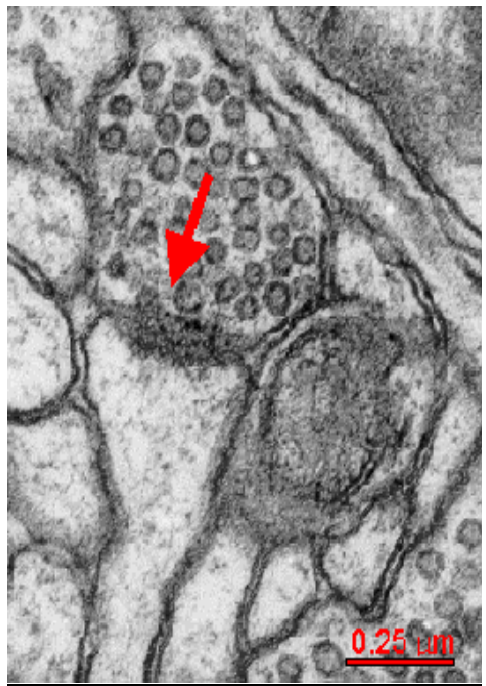


Mehmet Emin Uslu
Burcu Kasapoğlu
Can Küçük

Bio 417 Neurochemistry Course

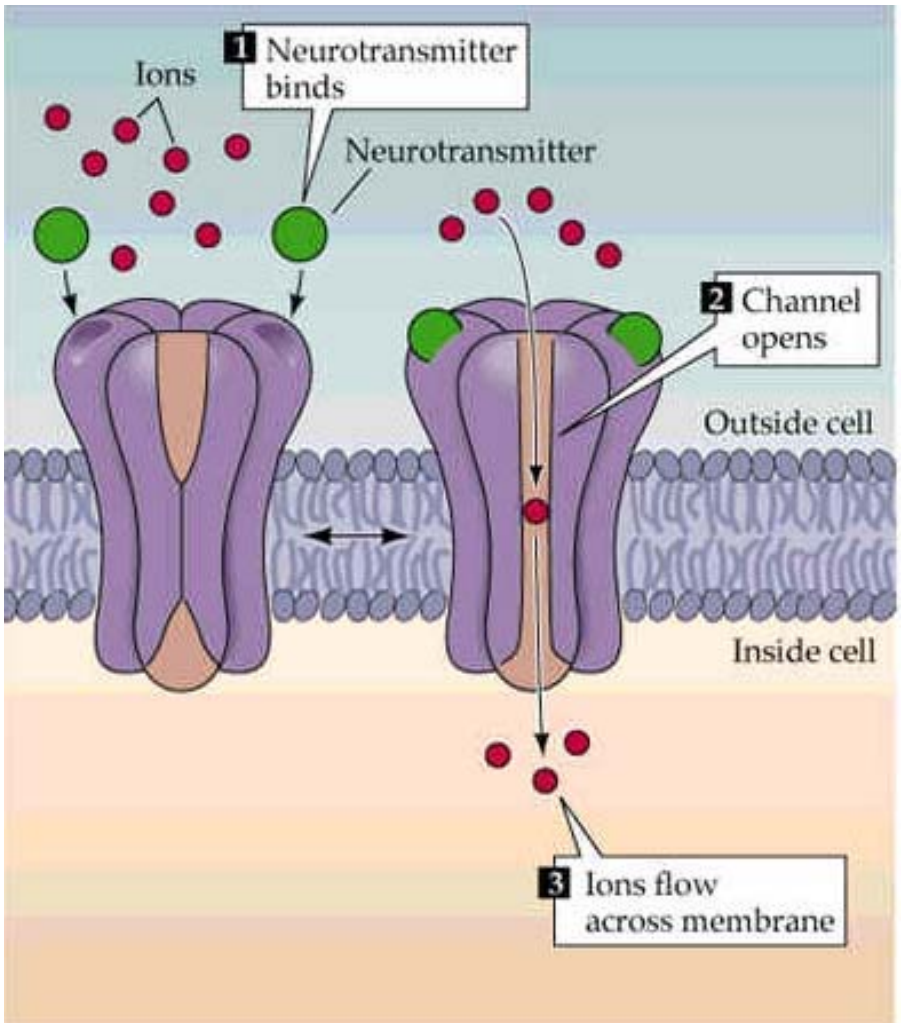
Neuron-neuron communication – the synapse





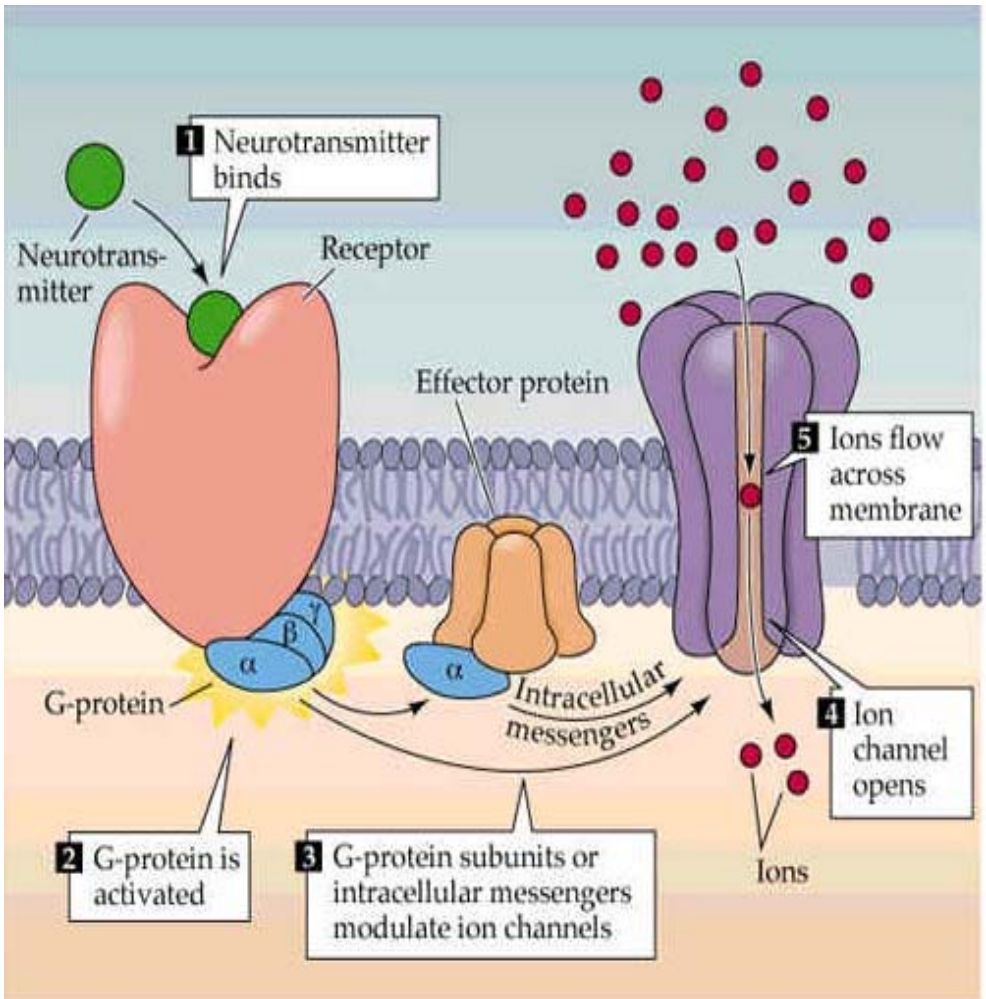
- Neurons use neurotransmitters to communicate

Ionotropic Neurotransmitter Receptors



- Ligand gated ion channels
- Fast neurotransmission
  - Inhibitory
    Neurotransmitter causes chloride influx and hyperpolarization
  - Excitatory
    Neurotransmitter causes sodium influx and depolarization

Metabotropic Neurotransmitter Receptors



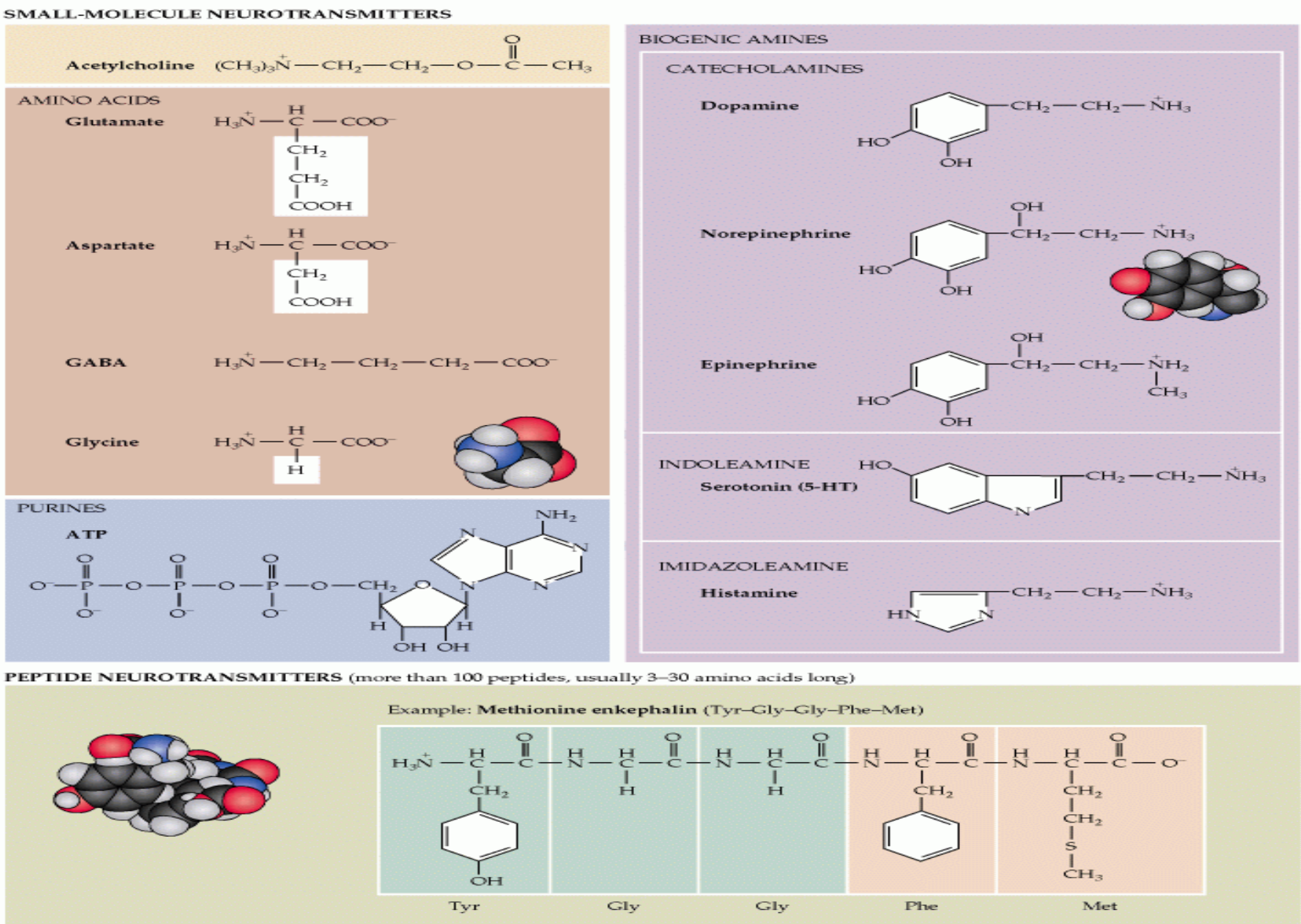
- Induction of second messenger systems
  - Receptor coupled to G-protein
  - Activates intracellular enzyme systems to produce an intracellular signal, the second messenger
- Slow neurotransmission – neuromodulation

Summary - Neurotransmission

A comparison of direct and indirect neurotransmitter actions.

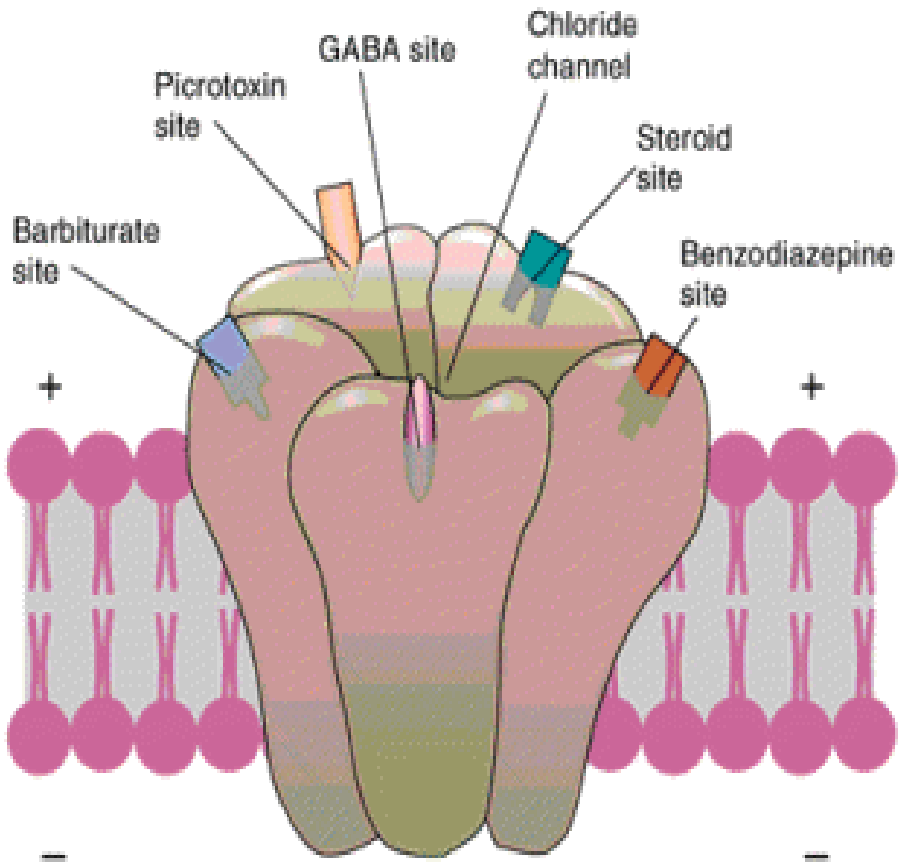
- Direct neurotransmitter action
- Inhibitory synaptic transmission between two neurons.
- Acetylcholine indirectly opens potassium channels in cardiac-muscle cells.
- Norepinephrine promotes the activation of voltage-dependent calcium channels in cardiac-muscle cells

Neurotransmitter Families



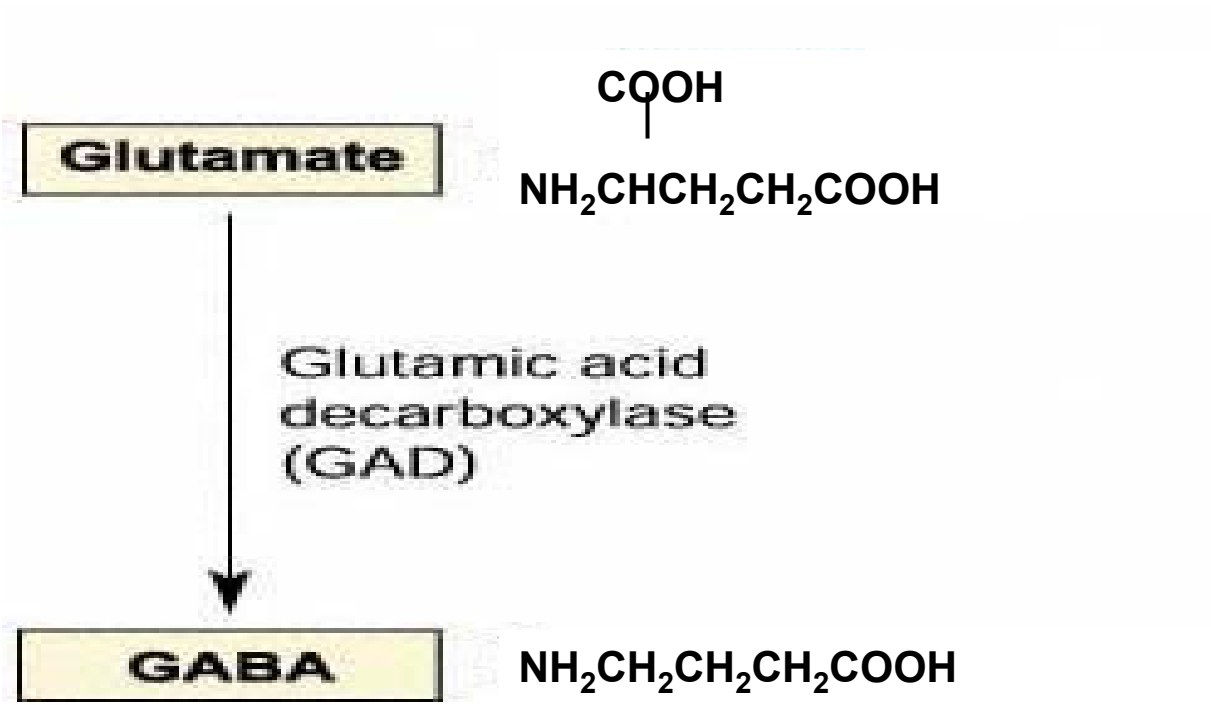
GABA

- inhibitory NT, discovered by Eugene Roberts in 1950
- predominately in interneurons
- synthesized from glutamate
- GABA receptors:
  - $GABA_A$: Ionotropic control of a Cl^- channel (IPSPs)
  - $GABA_B$: Metabotropic control of a K^+ channel (IPSPs)
  - $GABA_C$: Metabotropic control of a Cl^- channel (IPSPs)



- Although GABA is usually termed as an inhibitory neurotransmitter, because the Cl- equilibrium (20 vs 110 mM) is more negative than the resting cells, in embryonic neurons the intracellular Cl- is increased substantially (80-120 vs 110 mM)
- Thus in embryonic neurons, GABA can cause strong depolarization
- The switch to a “mature” neuron phenotype is mediated by the KCC2 K+ Cl- cotransporter which lowers internal Cl-

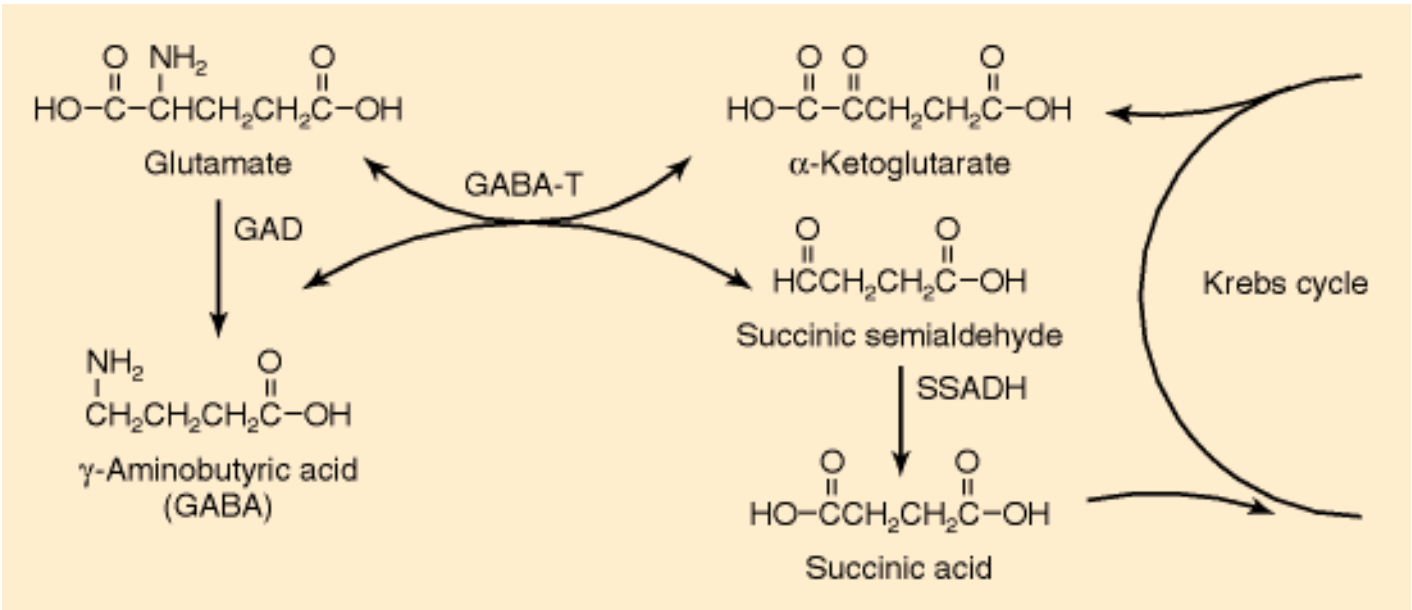
Synthesis of GABA



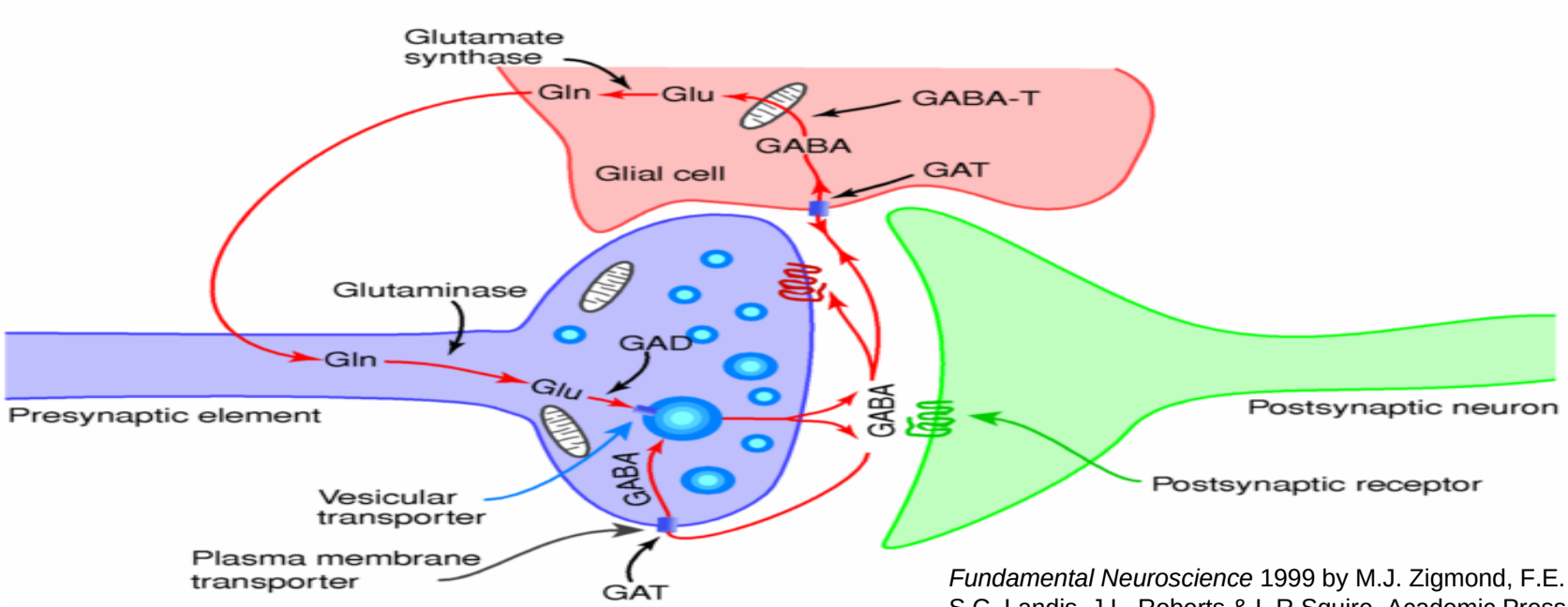
Neuroscience – Exploring the Brain 2nd Edition 2001 by M.F. Bear, B.W. Connors & M.A. Paradiso. Lippincott, Williams & Wilkins, Baltimore MD, USA. ISBN: 0683-30596-4

Enzymes of GABA metabolism

- Glutamic Acid Decarboxylase
- GABA-Transaminase
- Succinic Semialdehyde Dehydrogenase
- GABA synthesis represents a shunt of the TCA cycle
  - 8-10% of glucose carbon is funneled through the GABA shunt



Cartoon of a GABAergic synapse



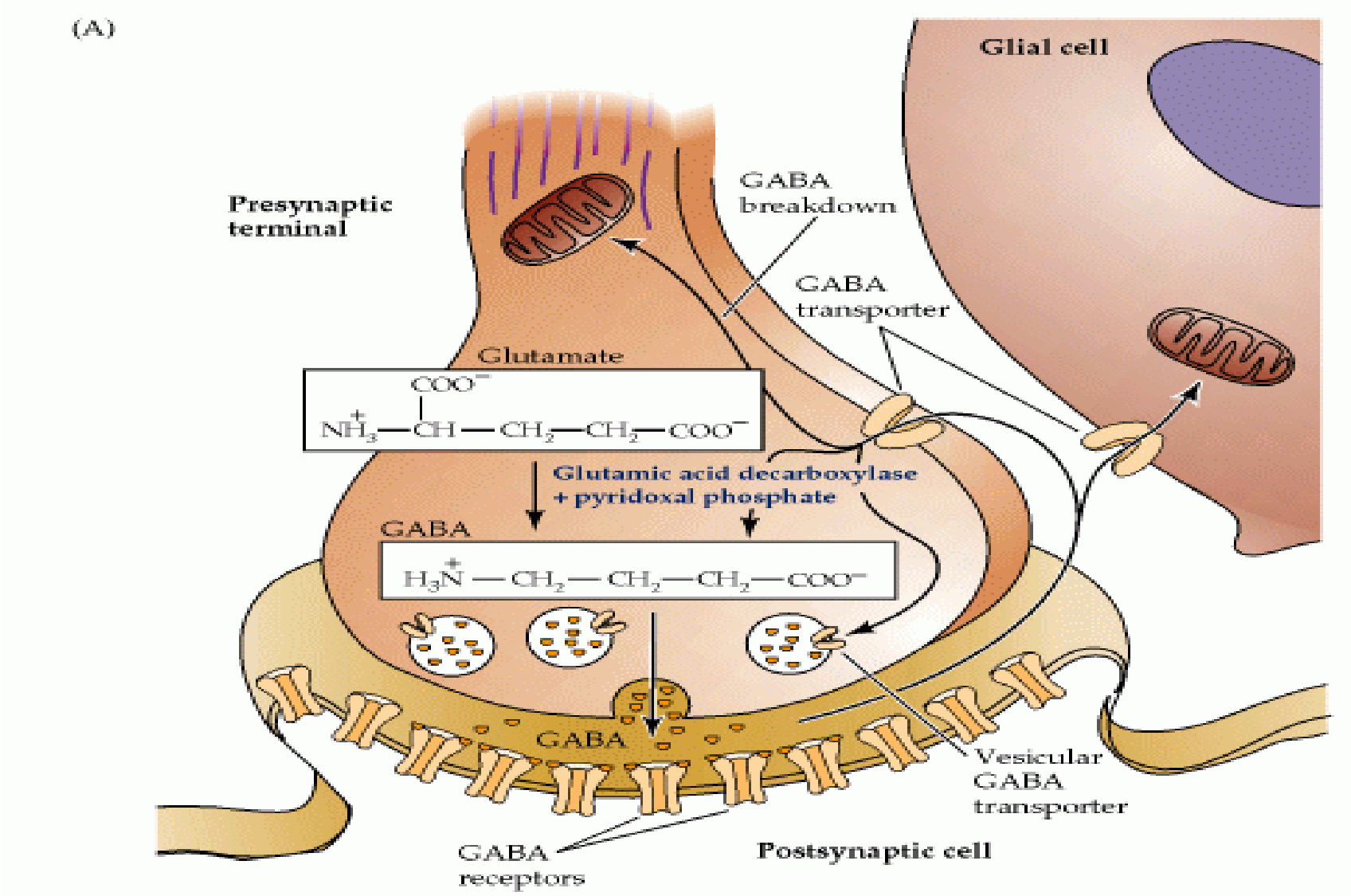
Fundamental Neuroscience 1999 by M.J. Zigmond, F.E. Bloom, S.C. Landis, J.L. Roberts & L.R.Squire. Academic Press, San Diego CA, USA. ISBN: 0-12-780870-1

Regulation of inhibition is important:

Too much - **loss of consciousness**

Too little - **seizures may occur**

The GABA-ergic Synapse

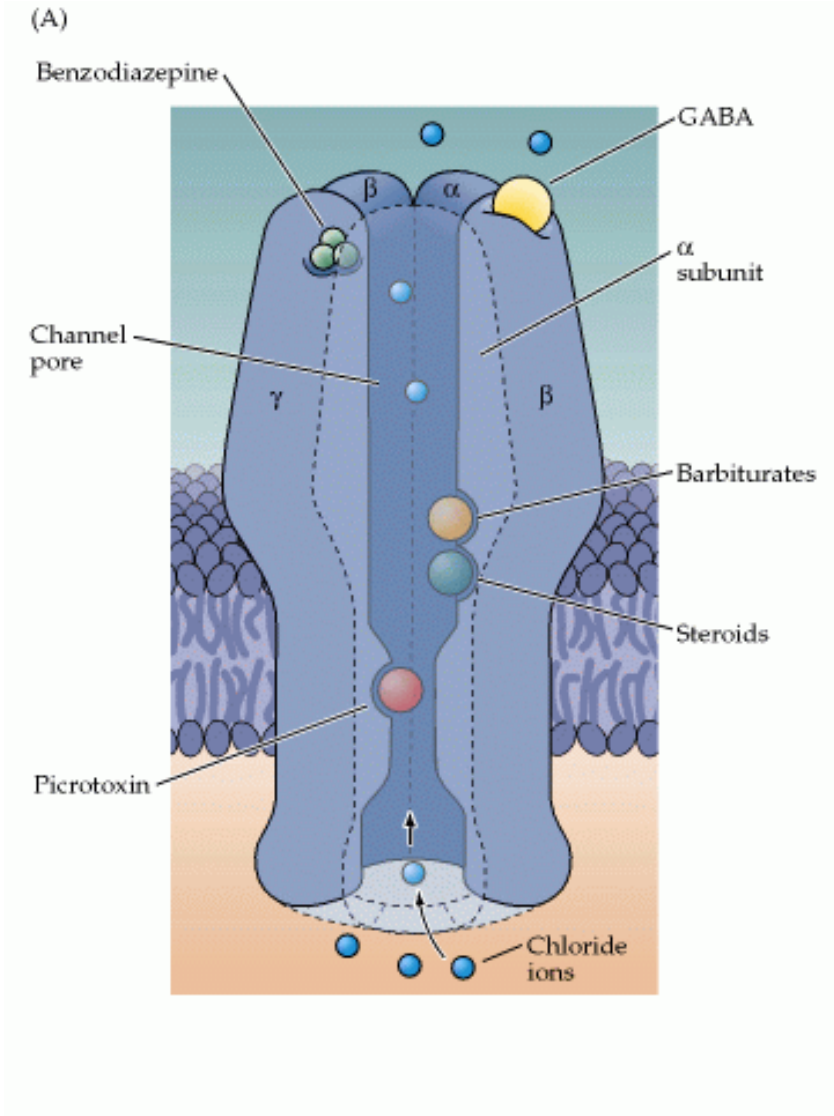


GABA receptor heterogeneity

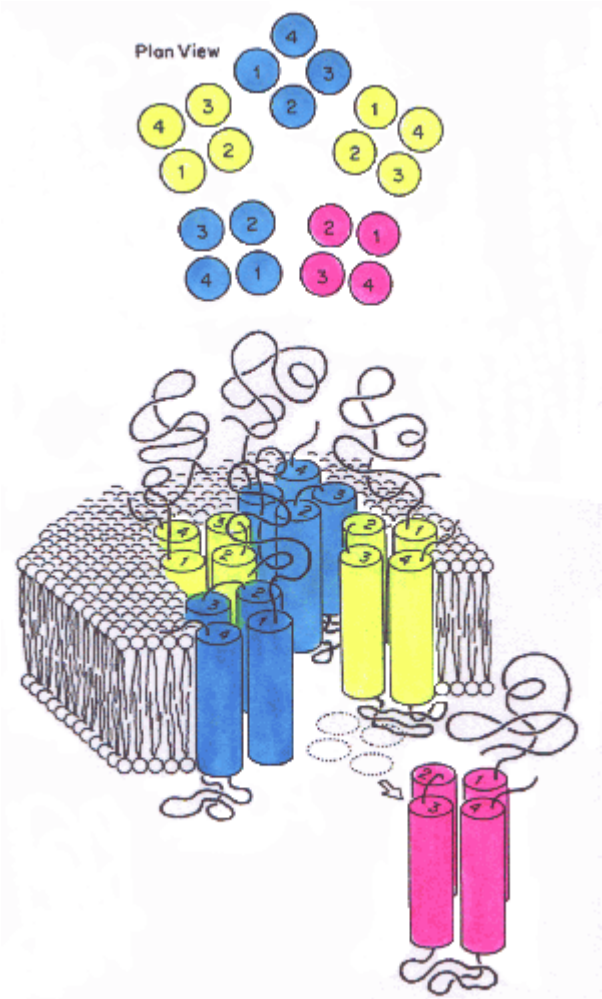
Isoform	Abundance	Location	Pharmacology/property
$\alpha1\beta2\gamma2$	40%	Most brain areas; hippocampal, cortical interneurons, cerebellar Purkinje cells	Common coassembly; BZ-typel; Zn-insensitive
$\alpha2\beta3\gamma2$	15%	Spinal cord motoneurons, hippocampal pyramidal cells	BZ-type II; moderately Zn-sensitive
$\alpha3\beta\gamma2/3$	10%	Cholinergic, monaminergic neurons	BZ-type II, abecarnil-sensitive
$\alpha2\beta\gamma1$	10%	Bergmann glia, thalamus, hypothalamus	BZ inverse agonist-enhanced
$\alpha5\beta3\gamma2/3$	3%	Hippocampal pyramidal cells	BZ-type II, zolpidem-insensitive, moderate Zn-sensitivity
$\alpha6\beta\gamma2$	2%	Cerebellar granule cells	BZ agonist-insensitive, moderate Zn-sensitivity
$\alpha6\beta\delta$	3%	Cerebellar granule cells	Insensitive to all BZ, GABA high affinity high Zn-sensitivity steroid-insensitive
$\alpha4\beta\gamma$	2%	Cortical, hippocampal pyramidal cells; striatum	BZ agonist-insensitive, low steroid sensitivity
$\alpha4\beta2\delta$	4%	Thalamus, dentate granule cells	Insensitive to all BZ, GABA high affinity high Zn sensitivity, steroid-insensitive
All other	11%	Throughout CNS	

$GABA_A$ Receptors

- a postsynaptic ionotropic receptor increasing Cl- conductance across the postsynaptic membrane.
- similar structure to that of the pentameric nAChR receptor

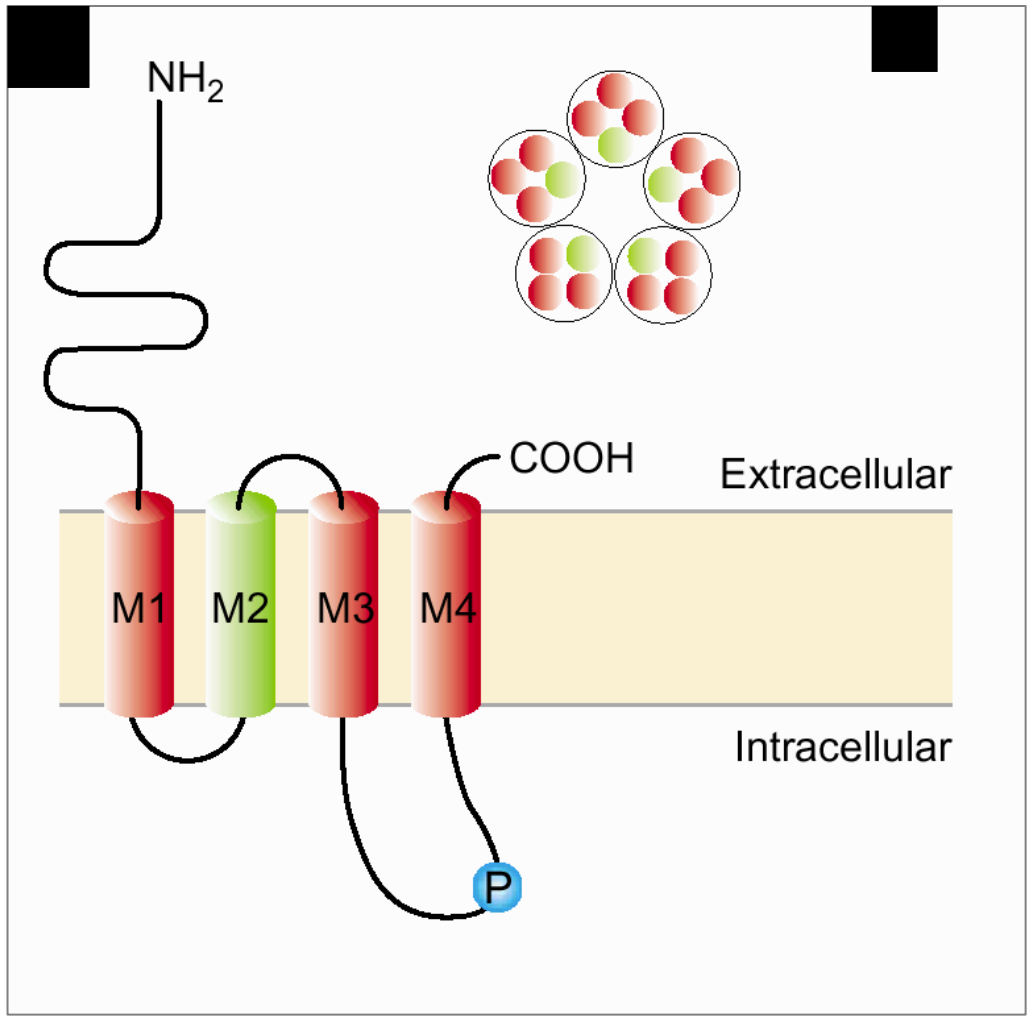


$GABA_A$ Receptor Structure



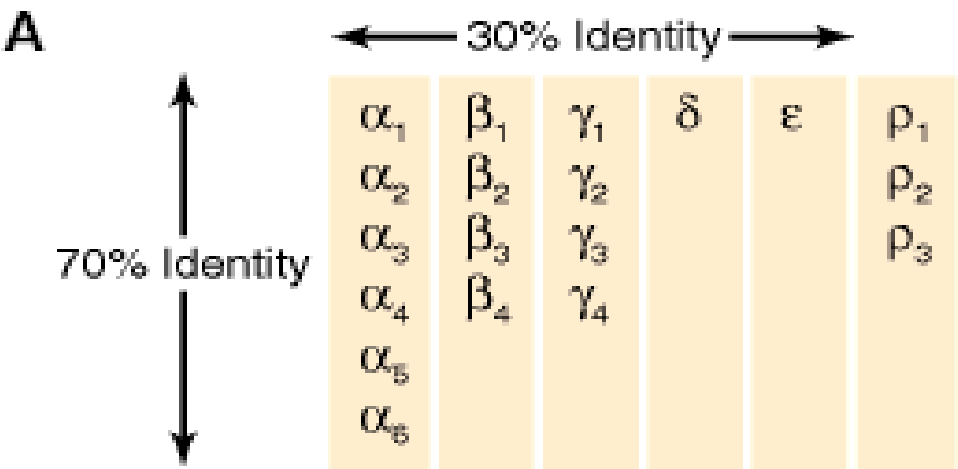
- Heteropentameric glycoprotein of closely related polypeptides (50-60kDa)
- Each $GABA_A$ receptor subunit contains 4 TMDs with the M2 domain forming the walls of the channel pore
- The amino terminus of each subunit is extracellular and the intracellular loop between TM3 and TM4 contains sites of protein-protein interaction as well as phosphorylation

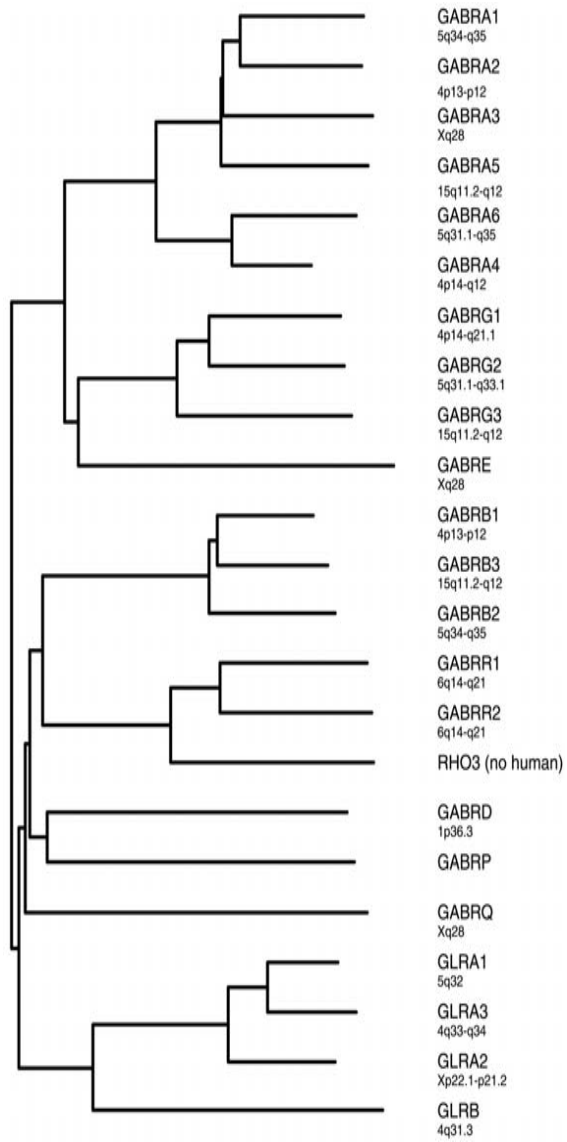
$GABA_A$ receptors



$GABA_A$ R subunits

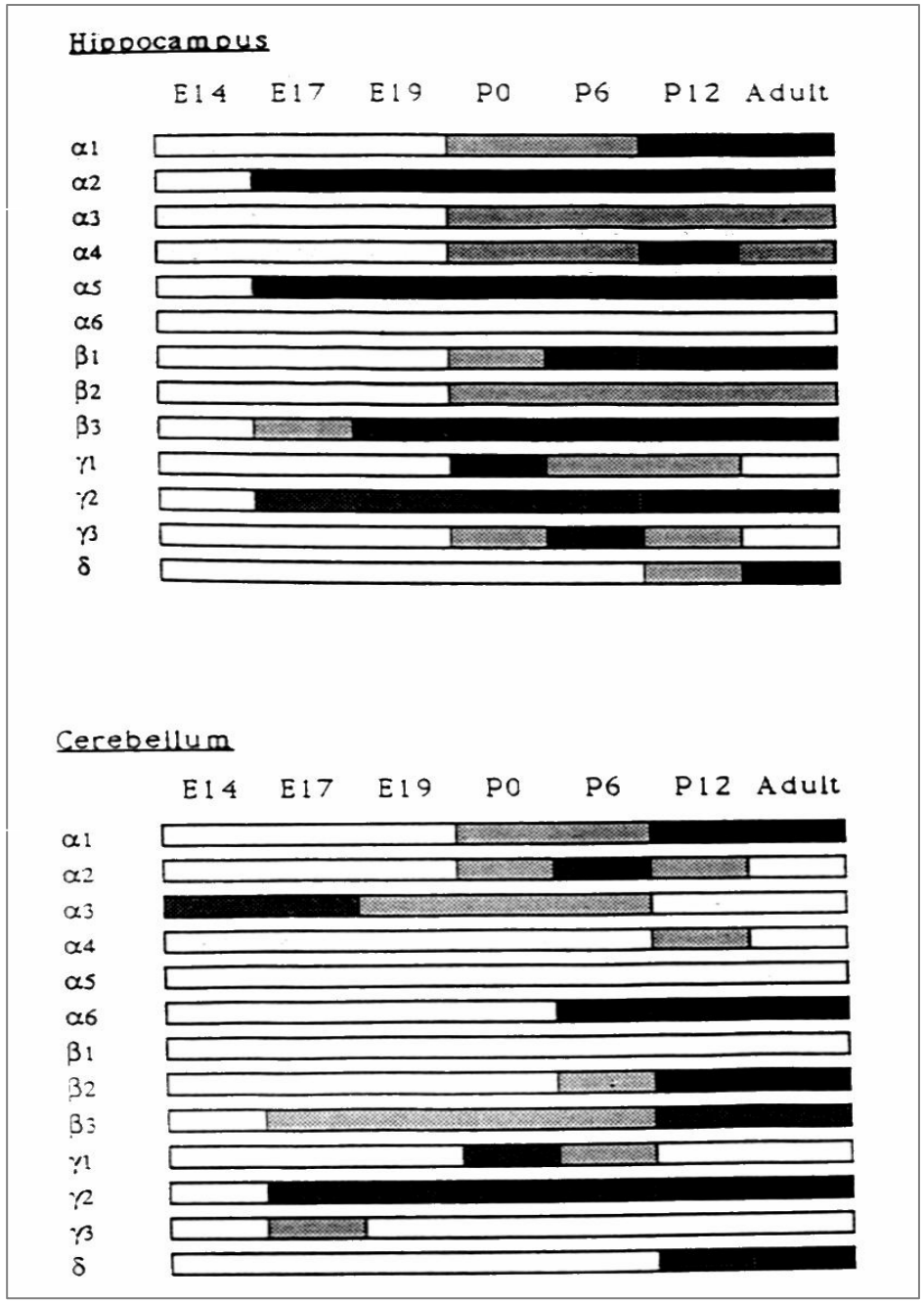
- over 20 different $GABA_A$ R subunits cloned
- expression of the subunit isoforms developmentally and regionally controlled.
- properties of GABA receptors depending on the individual subunit isoforms
- differences in gating, regulation and ligand binding to the receptors they form



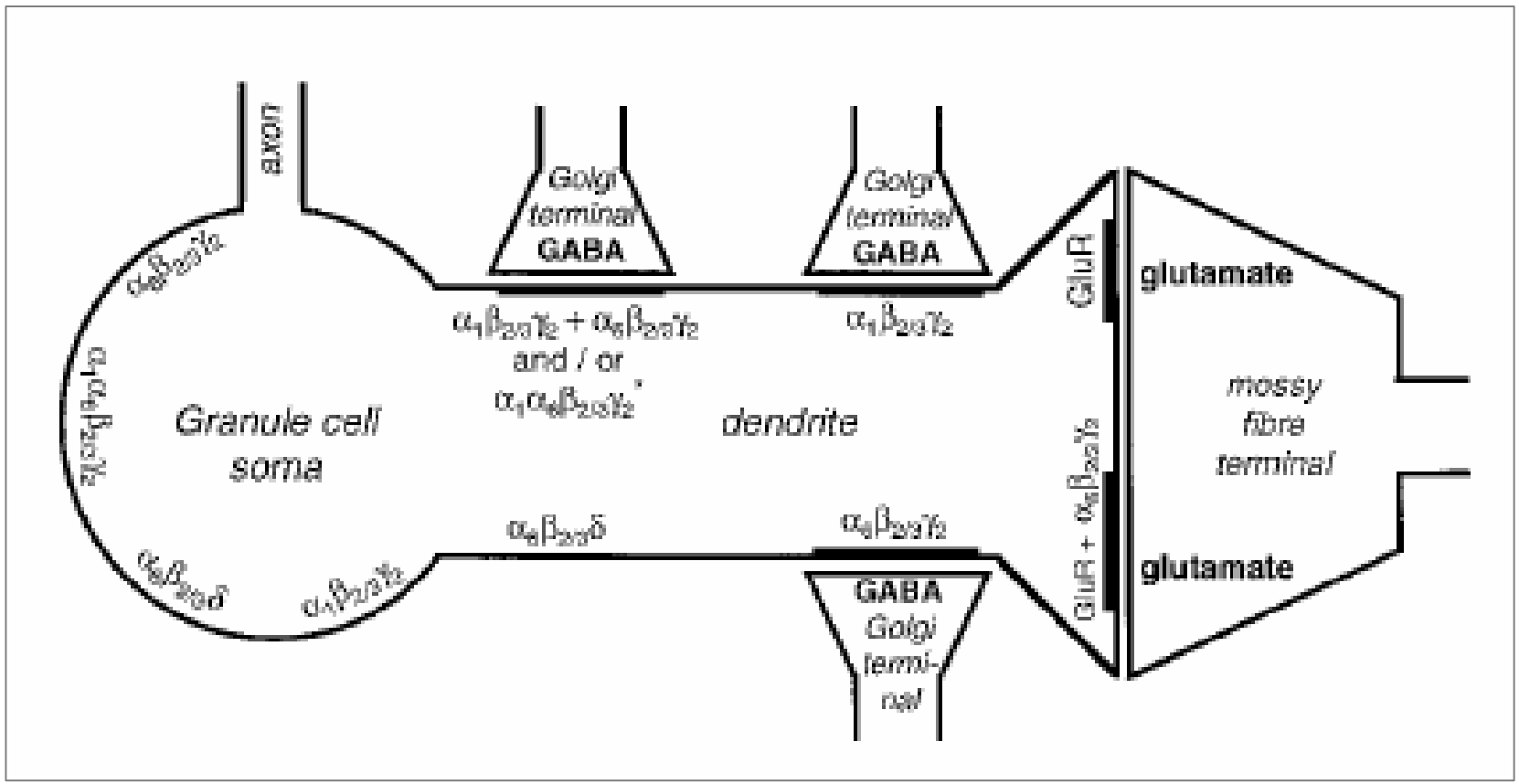


Expression of $GABA_A$ receptor subunit mRNAs in selected regions of the embryonic and postnatal rat brain

(*Laurie et al. 1992*)

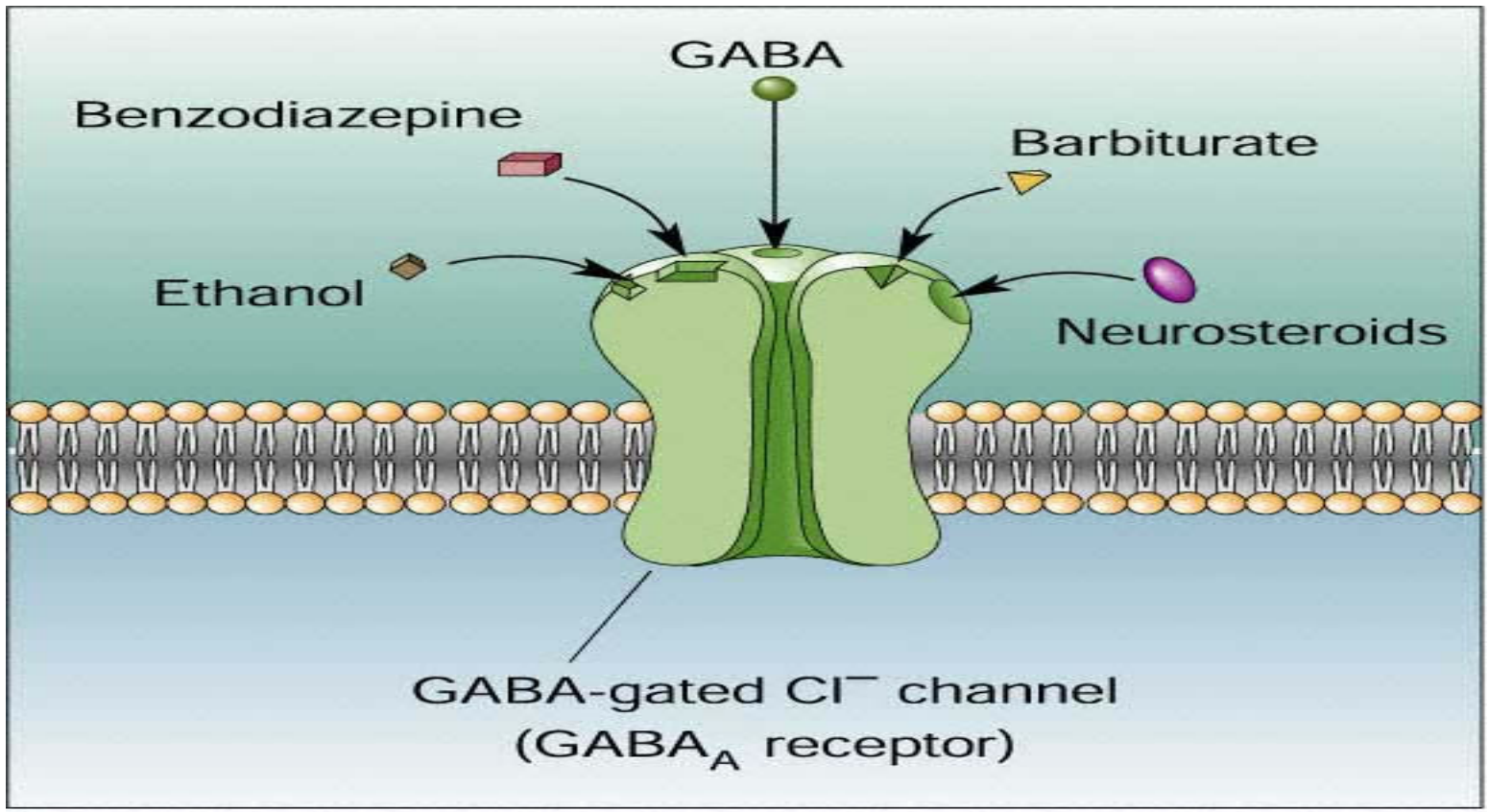


Distribution of $GABA_A$ Rs on cerebellar granule cells



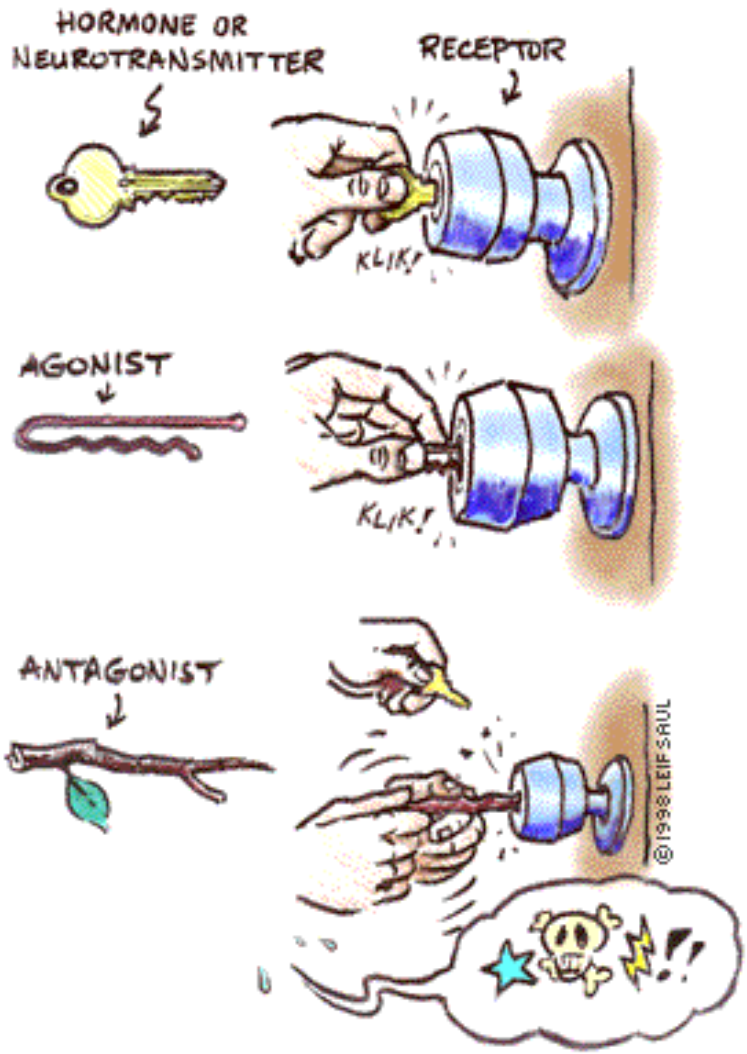
Nusser et al. J. Neurosci. 18, 1693-1703, 1998

The $GABA_A$ receptor - a target for drug action



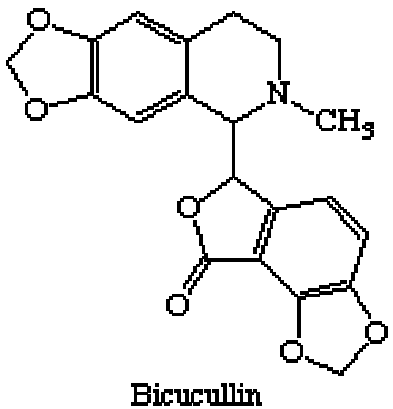
Neuroscience – Exploring the Brain 2nd Edition 2001 by M.F. Bear, B.W. Connors & M.A. Paradiso. Lippincott, Williams & Wilkins, Baltimore MD, USA. ISBN: 0683-30596-4

Agonists and Antagonists



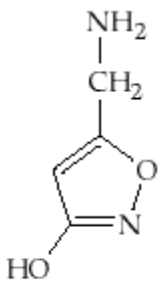
- Agonists
  - Structurally similar to neurotransmitter to activate receptor
- Antagonists
  - blocking receptor
  - Diverse structures often unrelated to neurotransmitter

1. Agonist/Antagonist Site

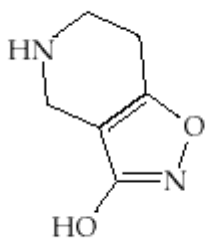


Bicucullin

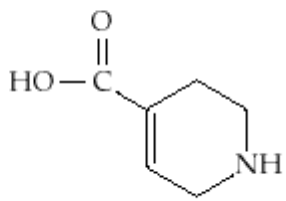
- Muscimol: a naturally occurring GABA analog isolated from the psychoactive mushroom Amanita muscaria and a selective $GABA_A$ agonist
- Bicuculline: a competitive antagonist and a convulsant.



Muscimol



THIP



Isoguvacine

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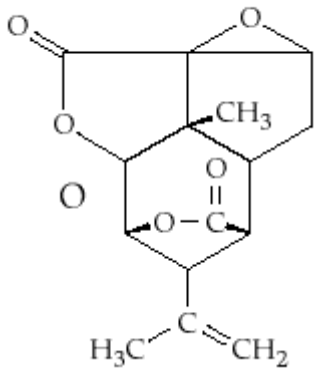
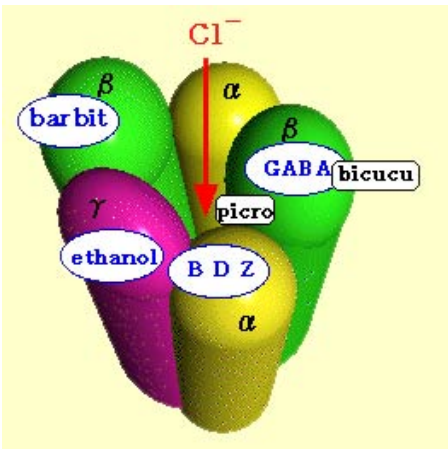
Fig. CS10.33-35

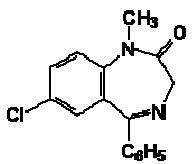
2. Picrotoxin site



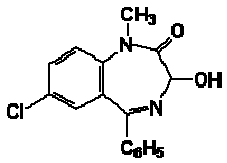


- Picrotoxin: a non-competitive GABA antagonist
- a channel blocker, causing a decrease in the mean open time of the channel
- also inhibit some forms of the GlyR

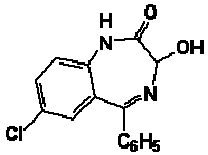
3. Benzodiazepine (BDZ) Site



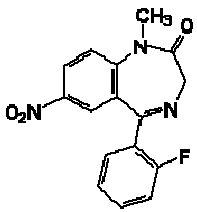
DIAZEPAM



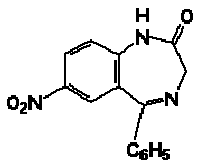
TEMAZEPAM



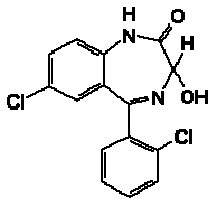
OXAZEPAM



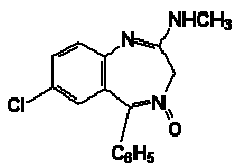
FLUNITRAZEPAM



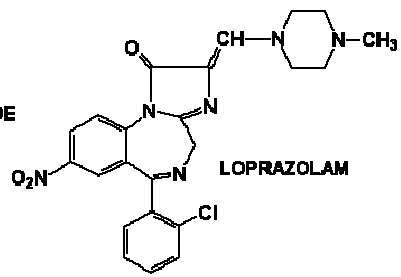
NITRAZEPAM



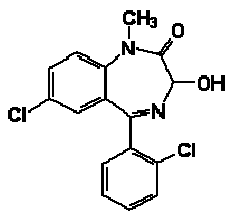
LORAZEPAM



CHLORDIAZEPOXIDE

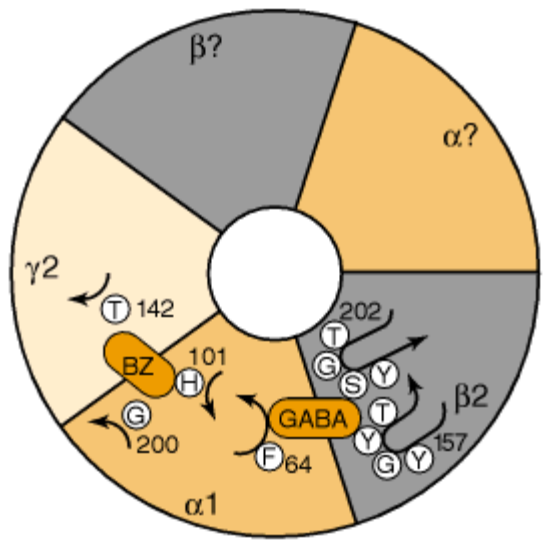


LOPRAZOLAM



LORMETAZEPAM

- GABA binds at the interface between α and β subunits
- BDZs bind at the interface between α and γ subunits
- Anxiolytic, sedative, hypnotic, antiseizure and muscle relaxants
- antagonists or inverse agonists



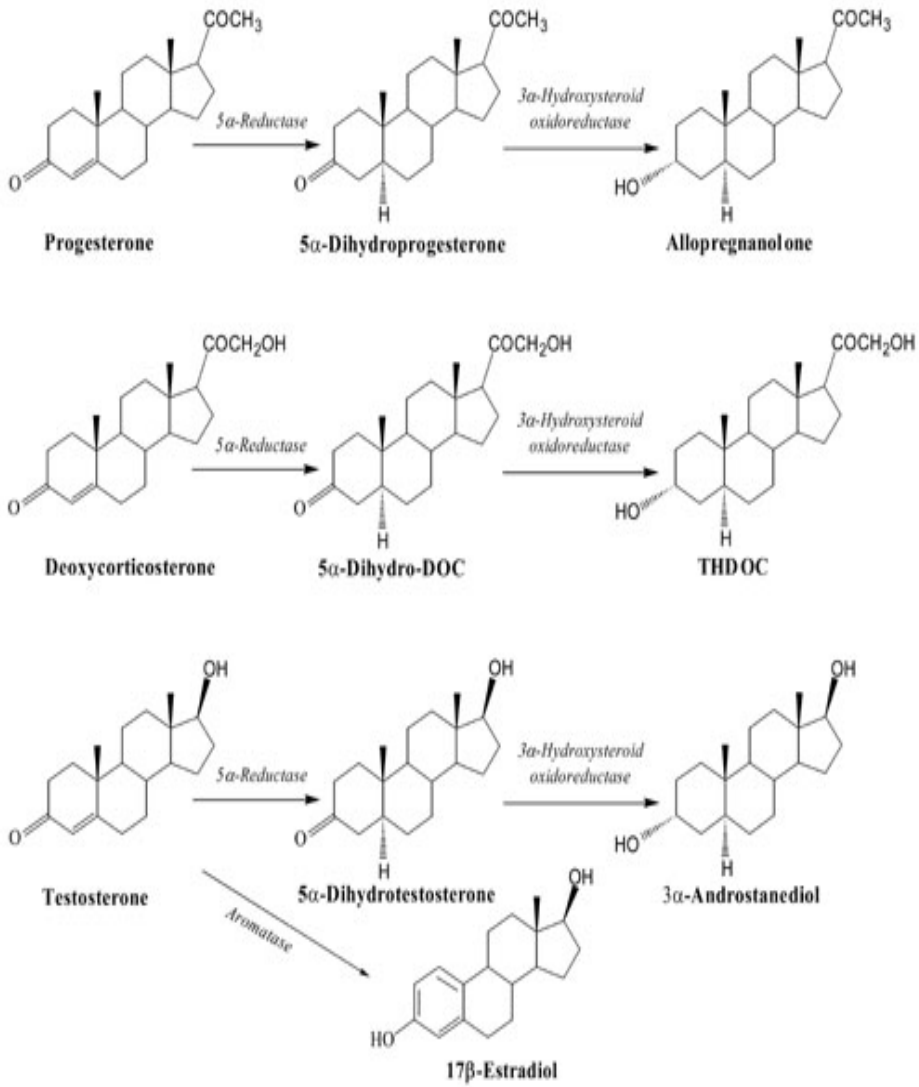
4. Barbiturate binding site

- examples: phenobarbital or pentobarbital
- sedative-hypnotic, anxiolytic and anti-convulsant effects
- enhanced postsynaptic responses to GABA by increasing the open time of the GABAA receptor channel
- occupancy of this site by barbiturates:

enhanced ligand binding to GABA and BDZ site and inhibited binding to the picrotoxin site.

5. Steroid hormone binding site

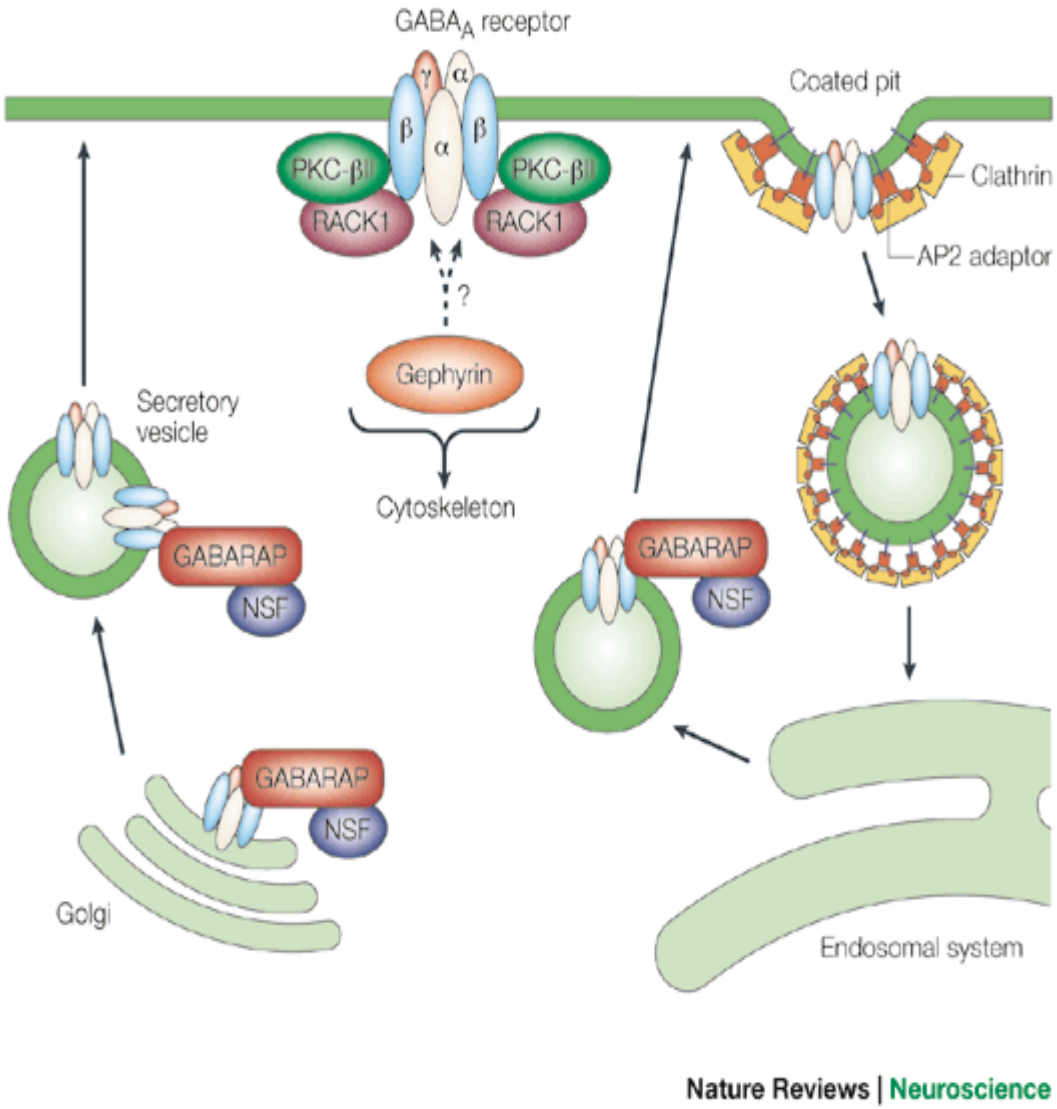
- Endogenously synthesized neurosteroids (mostly glial): progesterone (PREG), pregenolone sulfate (PREGS) and dehydroepiandrosterone sulfate (DHEAS)
- enhanced agonist binding to the $GABA_A$ receptor



Other binding sites on $GABA_A$ R

- Penicillin: inhibition of channel opening
- Ethanol: facilitation to channel opening by binding to specific hydrophobic regions of the $GABA_A$ receptor

$GABA_A$ Receptor Desensitization



- desensitization by internalization
- phosphorylation of GABA receptors by PKC, PKA and several other kinases to alter densensitization
- phosphorylation effective on gating of the receptor directly

The $GABA_B$ receptor

a ‘metabotropic’ receptor

a seven transmembrane domain receptor.

located both pre- and post-synaptically.

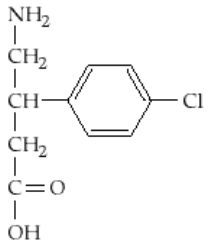
2 main effects of activating $GABA_B$ receptors: is **close Ca^{2+} channels** and **open K^+ channels**

Ca^{2+} channel closure **- inhibition of transmitter release**

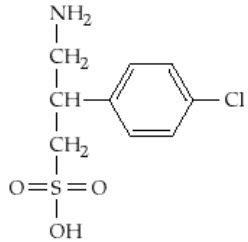
K^+ channel opening - **slow IPSP**

Baclofen: a selective $GABA_B$ receptor **agonist**

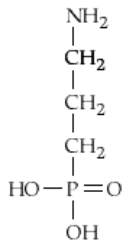
2-hydroxy-saclofen: a selective $GABA_B$ receptor **antagonist**



Baclofen



Saclofen



3-APPA

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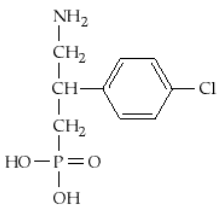
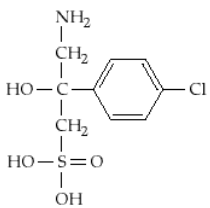
Feldman
Fundamentals of Neuropsychopharmacology
Fig. CS10.43-45



Phaclofen



2-Hydroxysaclofen

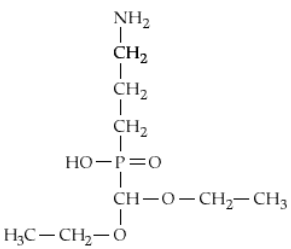


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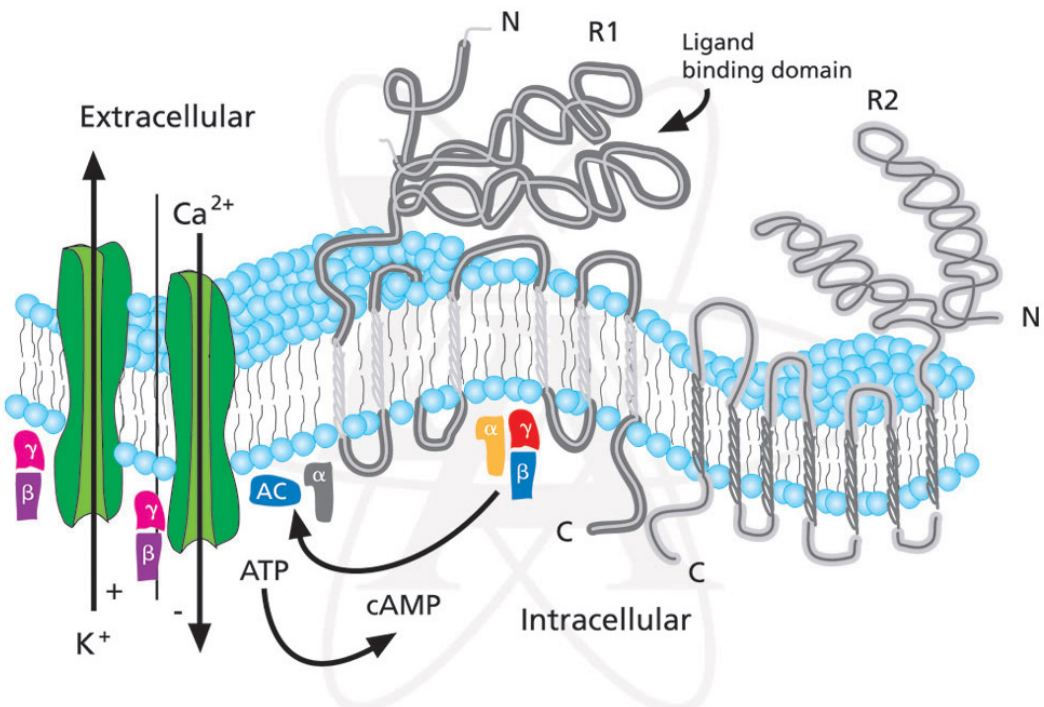
Fig. CS10.46-48

$GABA_B$ Receptors

- First discovered as mechanism for bicuculline-insensitive inhibition of release of other neurotransmitters.
- This effect of GABA could be mimicked by baclofen but not by muscimol

$GABA_B$ Receptor Structure



1) inhibition of adenylate cyclase

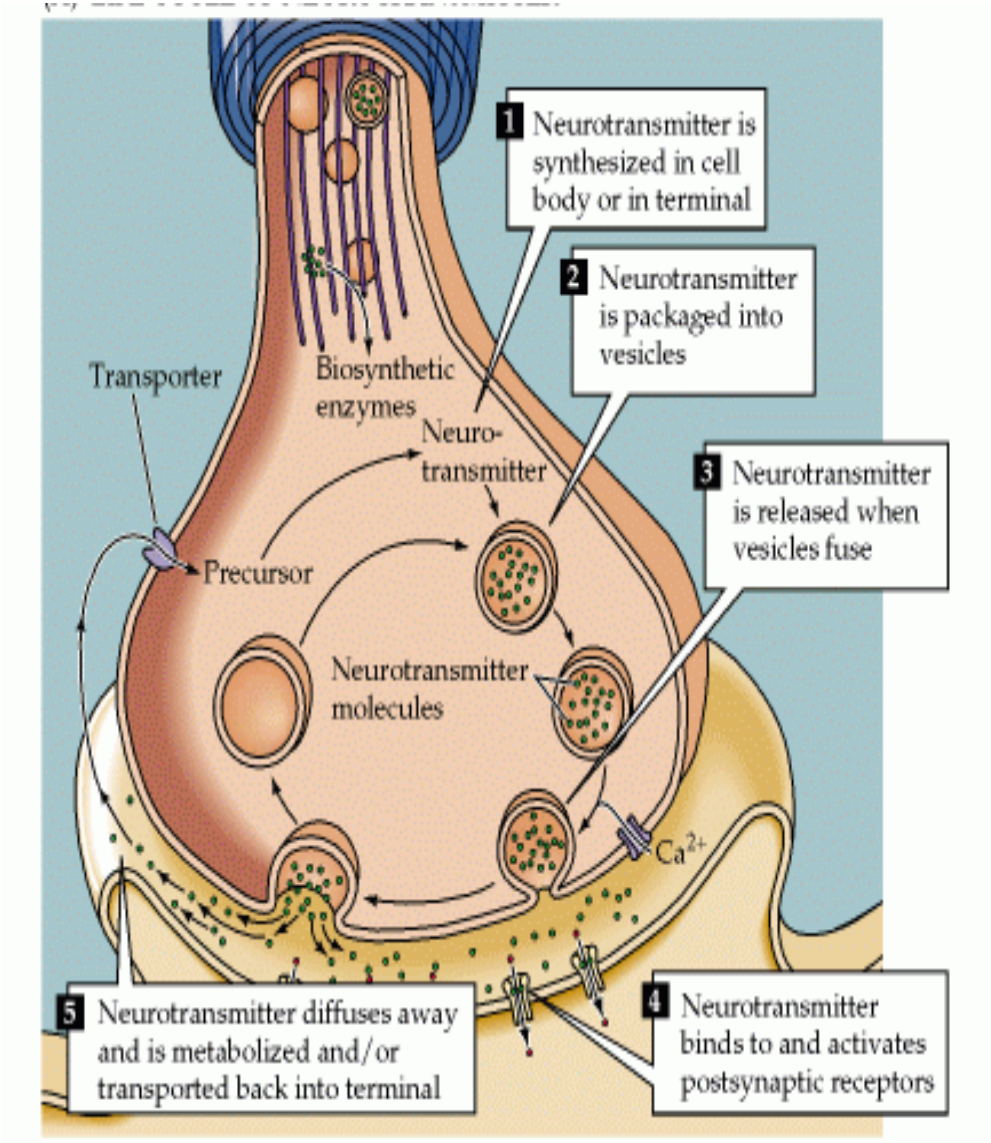
2) stimulation of phospholipase A2

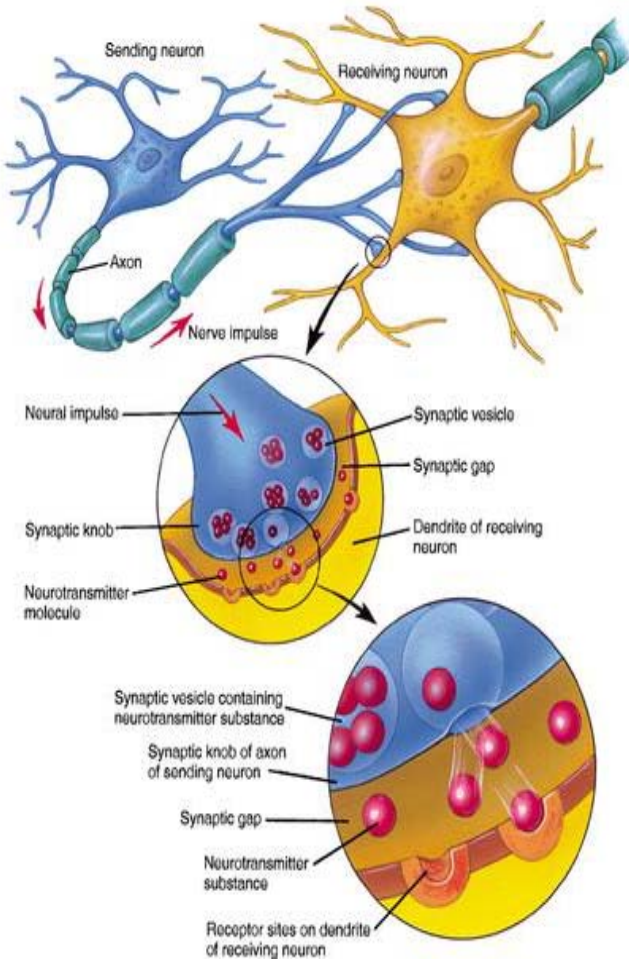
3) increase in K+ membrane conductance

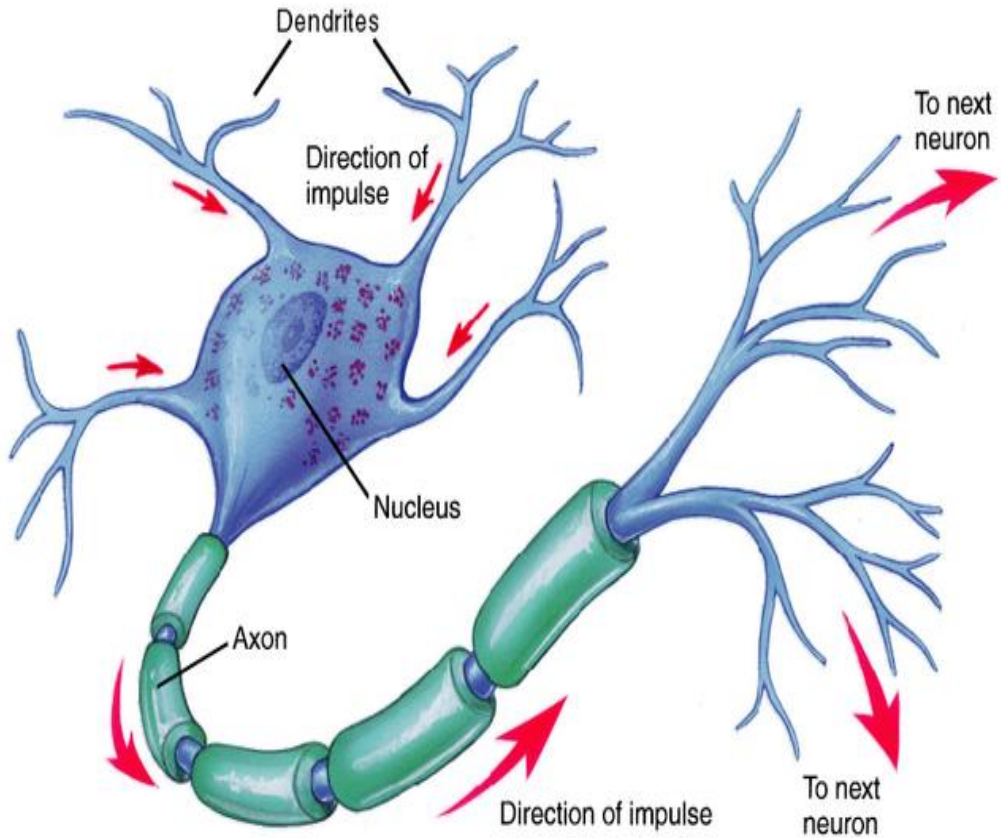
4) inhibition of voltage-dependent Ca^{+2} channels

GABA Transporters

- GABA removal from the synaptic cleft by a sodium dependent transporter with a high affinity for GABA (5-40 uM)
- The vesicular GABA transporter (GAT) dependent on electrical potential across the vesicular membrane







THANK YOU FOR YOUR ATTENTION!