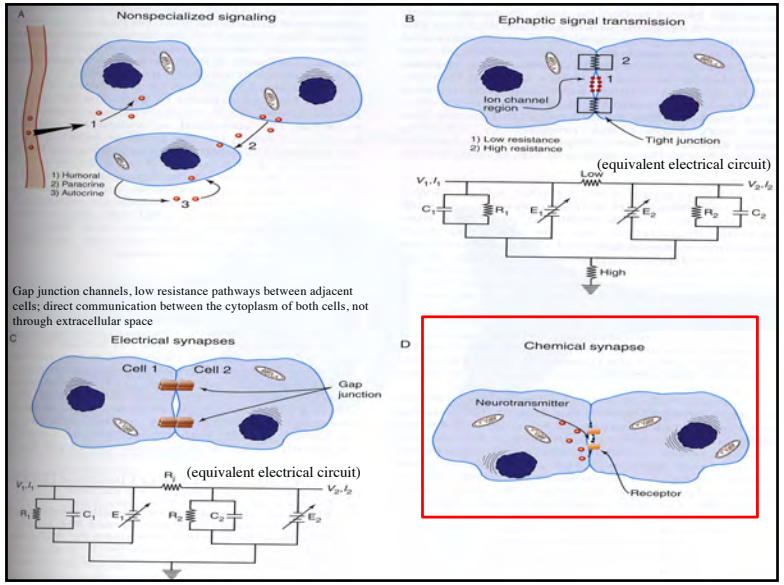
Molecular and Cellular Biology

08.3. Cell Signalling III:

Neurotransmitters & Receptors

Prof. Dr. Klaus Heese



Criteria for a Neurotransmitter

Neurotransmitter are endogenous substances that are released from neurons, act on receptor sites that are typically present on membranes of postsynaptic cells, and produce a functional change in the properties of the target cell:

1) A neurotransmitter must be synthesized by and released from neurons. This means that the presynaptic neuron should contain a transmitter and the appropriate enzymes need to synthesize the neurotransmitter. Synthesis in the axon terminal is not an absolute requirement. For example, peptide transmitters are synthesized in the cell body and transported to distant sites, where they are released.
2) The substance should be released from nerve terminals in a chemically or pharmacologically identifiable form. Thus, one should be able to isolate the transmitter and characterize its structure using biochemical or other techniques.
3) A neurotransmitter should reproduce at the postsynaptic cell the specific events (such as changes in membrane properties) that are seen after stimulation of the presynaptic neuron.
4) The effect of a putative neurotransmitter should be blocked by competitive antagonists of the transmitter in a dose-dependent manner. In addition, treatments that inhibit synthesis of the transmitter candidate should block the effects of presynaptic stimulation.
5) There should be active mechanisms to terminate the action of the putative neurotransmitter (enzymatic or reuptake by neuron / glia).

The Process of Chemical Neurotransmission can be Divided into Five Steps

1) Synthesis of the neurotransmitter in the presynaptic neuron
2) Storage of the neurotransmitter and/or its precursor in the presynaptic nerve terminal
3) Release of the neurotransmitter into the synaptic cleft
4) Binding and recognition of the neurotransmitter by target receptors
5) Termination of the action of the released transmitter

Classical Neurotransmitters

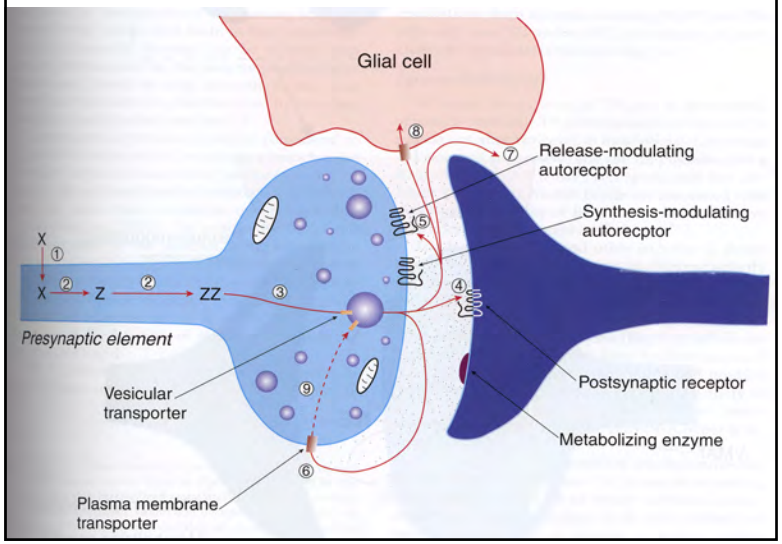
1) Acetylcholine, biogenic amines, amino acids
2) Others

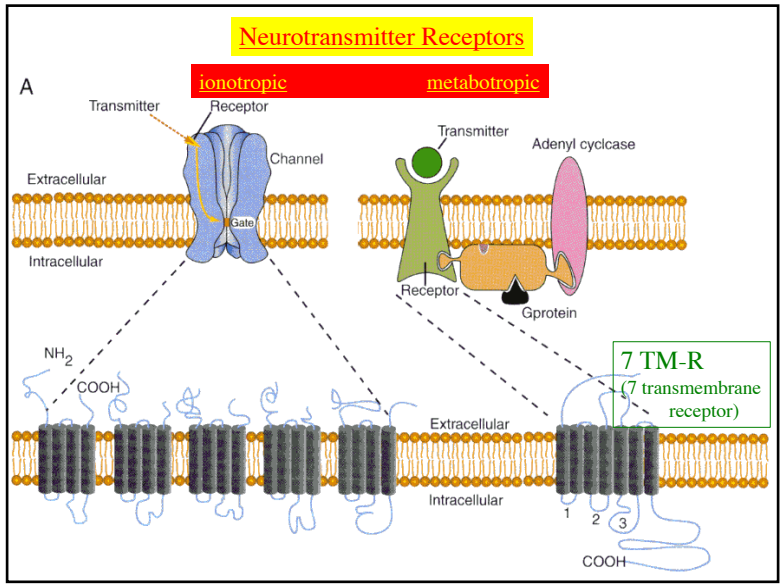
Storage vesicles for classical transmitters are smaller,

classical transmitters are subject to active reuptake by presynaptic cell and thus can be viewed as homeostatically conserved; in contrast, there is no energy-dependent, high-affinity reuptake process for non-classical transmitters.

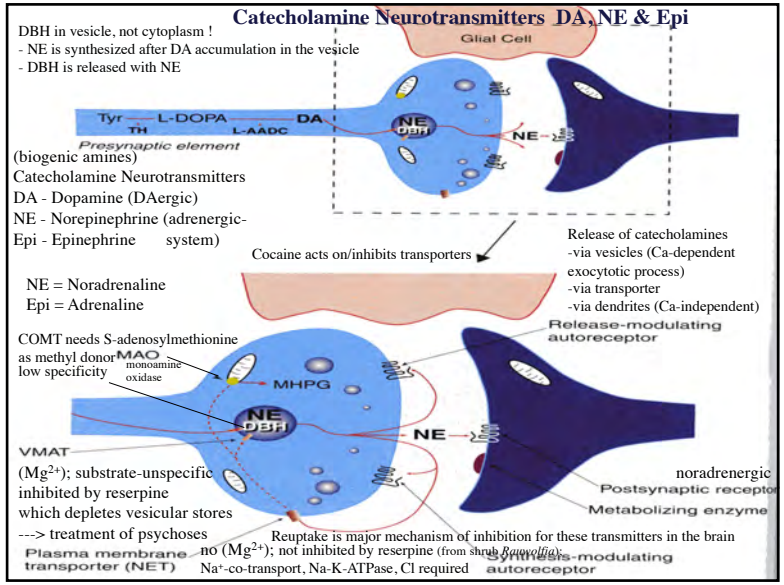
Most classical transmitters are synthesized in the nerve terminal by enzymatic action;
peptides, however, are synthesized in the soma from a precursor protein and are then transported to the nerve terminal.

Life cycle of a Classical Neurotransmitter





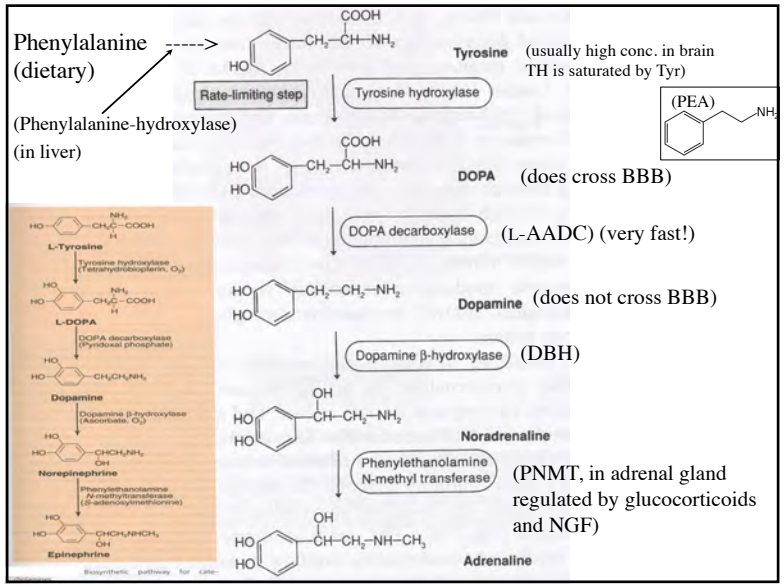
Catecholamine Neurotransmitters DA, NE & Epi

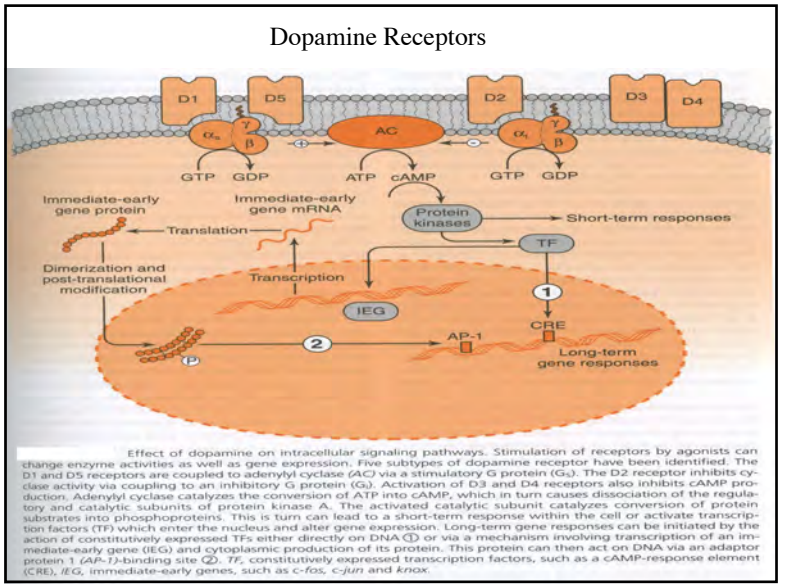


Dopamine

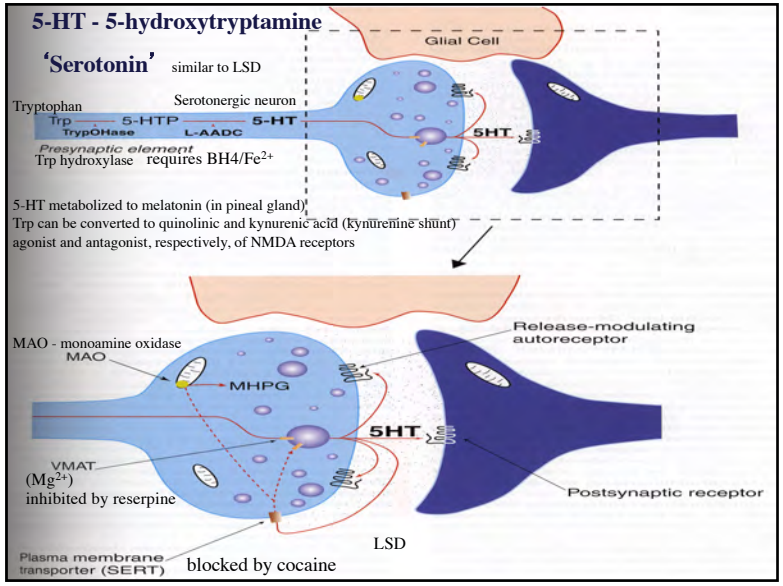
&

NA-system





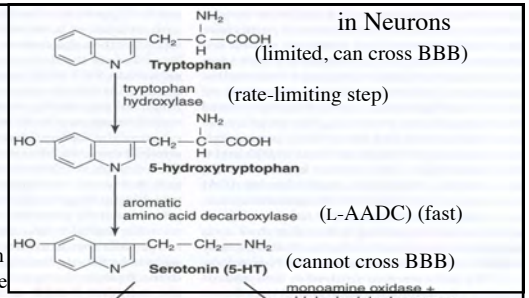
5-HT-system



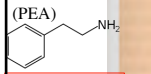
5-HT:

1% in brain;
In the blood (in platelets) and induces contractions of smooth muscle organs; high concentration in intestinal mucosa where it causes contraction of intestinal smooth muscle

in Neurons



(PEA)

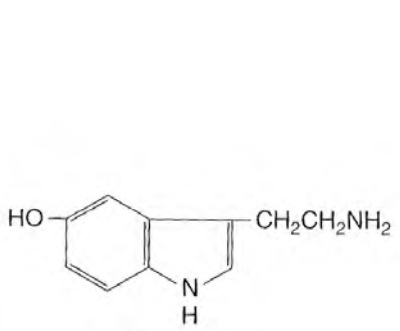


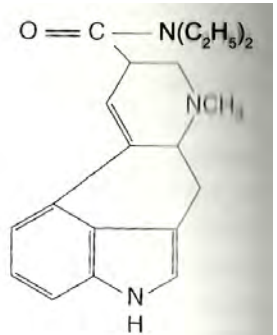
(compare with Dopamine)

Phenylethylamine (PEA) is found in abundance in cacao. Because PEA is heat sensitive, much of the PEA in conventional cooked and processed chocolate is missing. PEA is the chemical that we produce in our bodies when we fall in love. This is likely one of the main reasons why love and chocolate have such a deep connection. PEA also plays a role in increasing focus and alertness.

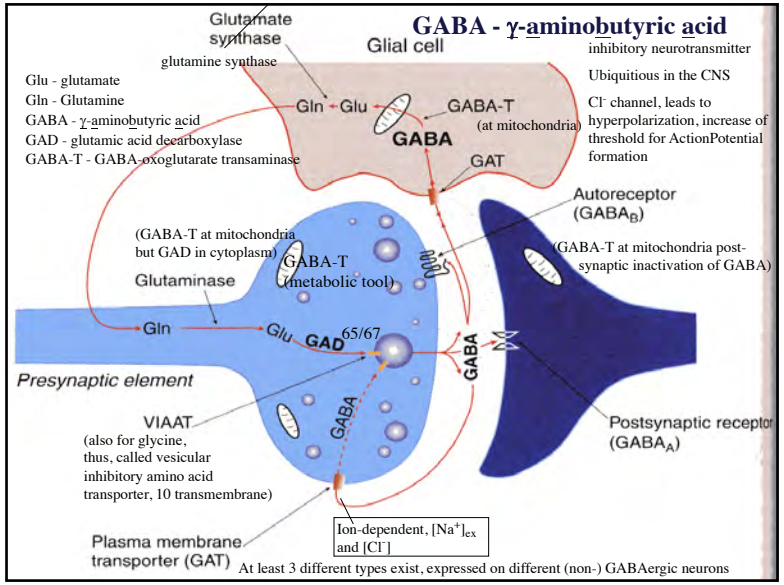
The biosynthesis and catabolism of serotonin. Note that in the pineal gland, serotonin is converted enzymatically to melatonin.

LSD, a hallucinogetic drug

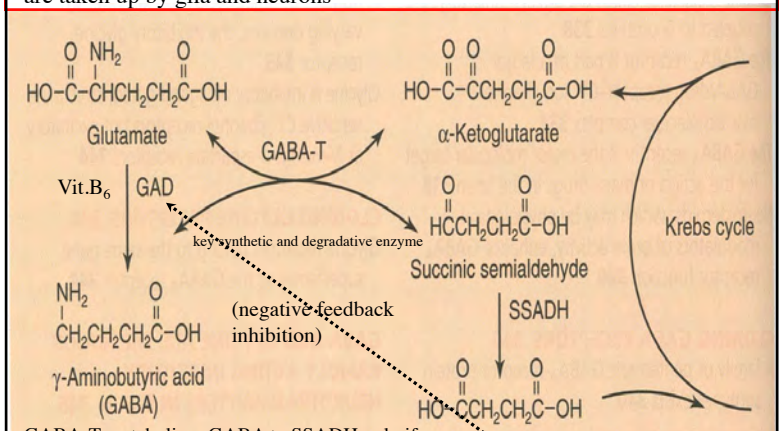




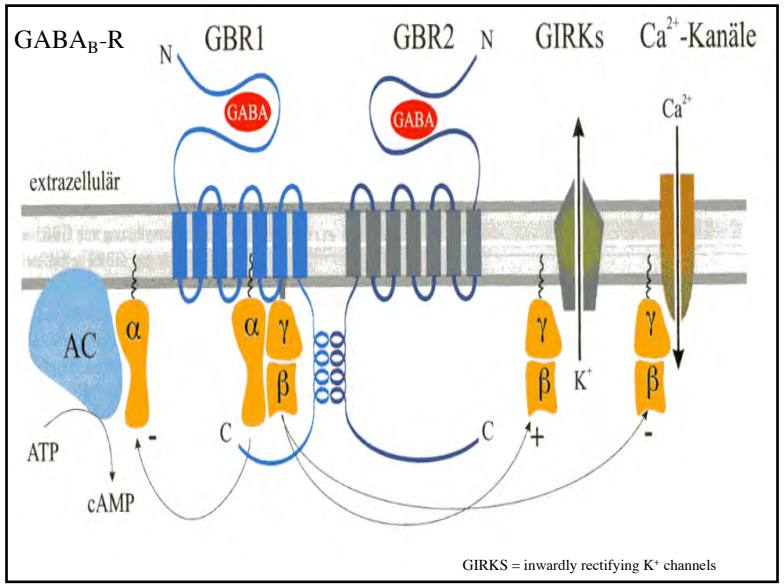
GABA-system



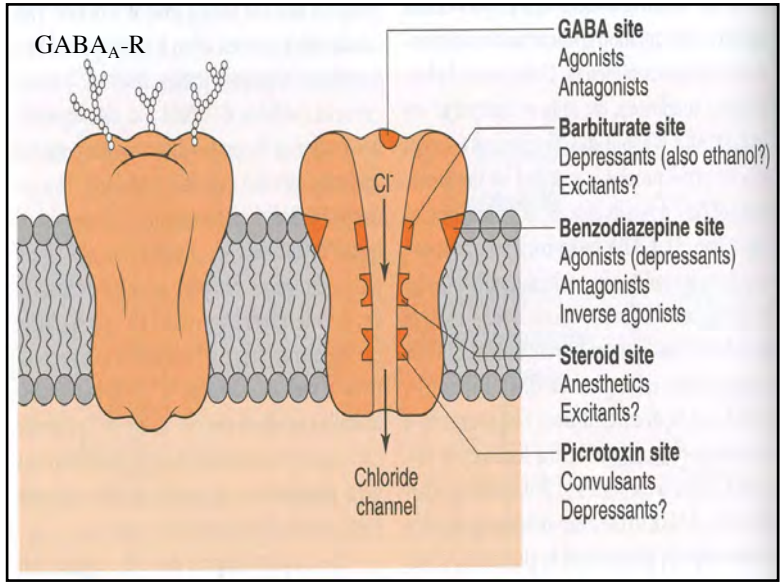
Major difference between catecholamines and amino acid neurotransmitters
the latter are derived from glucose metabolism and
are taken up by glia and neurons



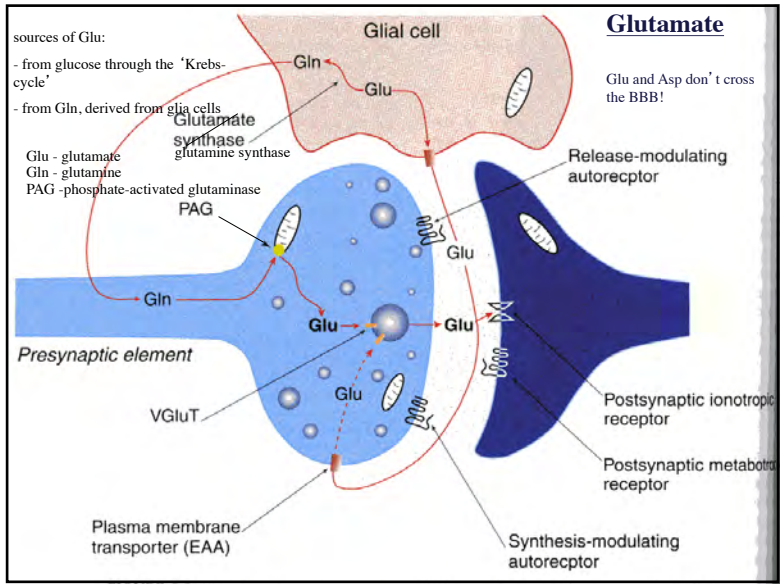
GABA-T metabolizes GABA to SSADH only if α-Ketoglutarate is present to receive the amino group from GABA (to generate then Glu)

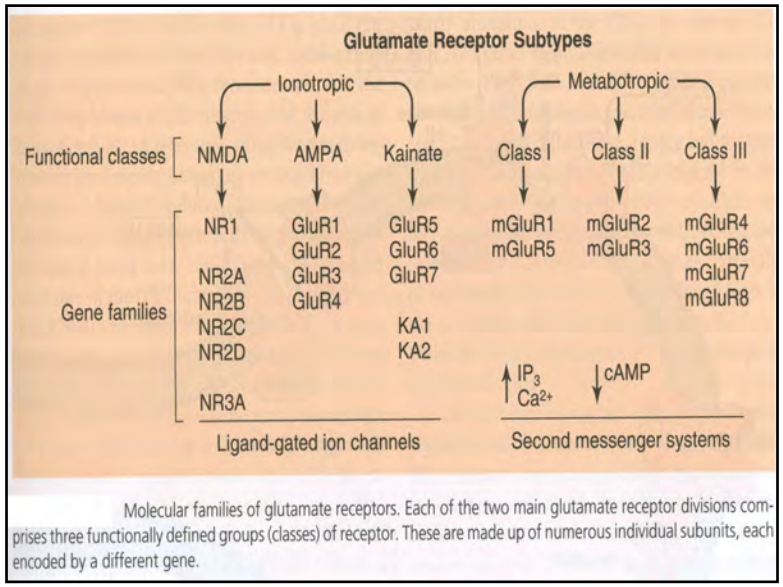


GIRKS = inwardly rectifying K^+ channels



Glutamate-system

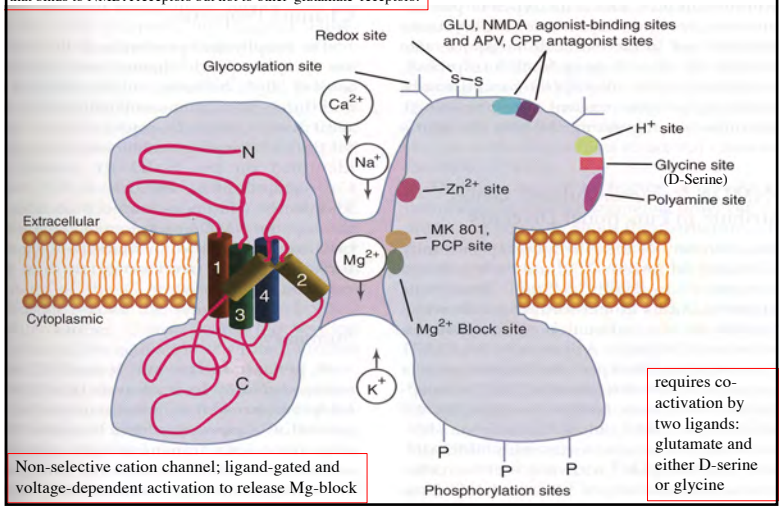




Molecular families of glutamate receptors. Each of the two main glutamate receptor divisions comprises three functionally defined groups (classes) of receptor. These are made up of numerous individual subunits, each encoded by a different gene.

NMDA-receptor, binds NMDA, ionotropic

NMDA (N-methyl-D-aspartate) is the name of a selective agonist that binds to NMDA receptors but not to other 'glutamate' receptors.

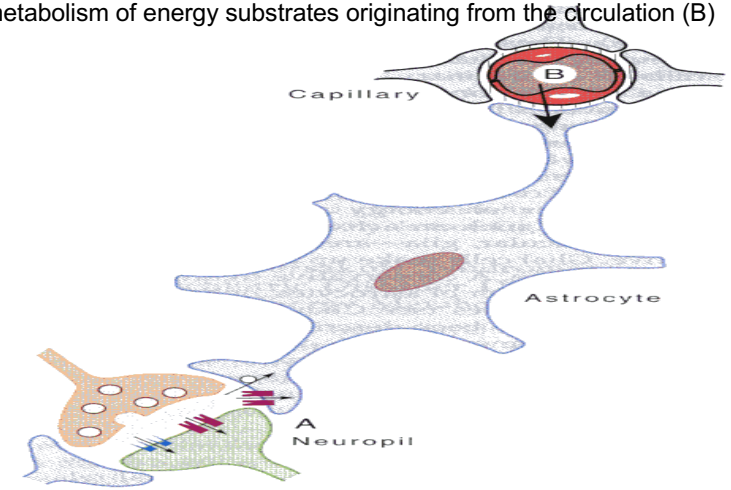


Non-selective cation channel; ligand-gated and voltage-dependent activation to release Mg-block

requires co-activation by two ligands: glutamate and either D-serine or glycine

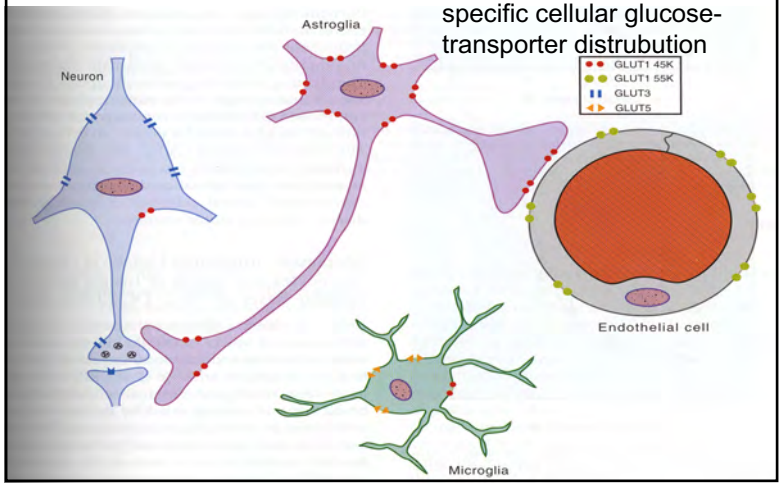
Astrocytes as mediators between capillaries and neuropil

Astrocytes sense synaptic activity (A) and couple it with uptake and metabolism of energy substrates originating from the circulation (B)

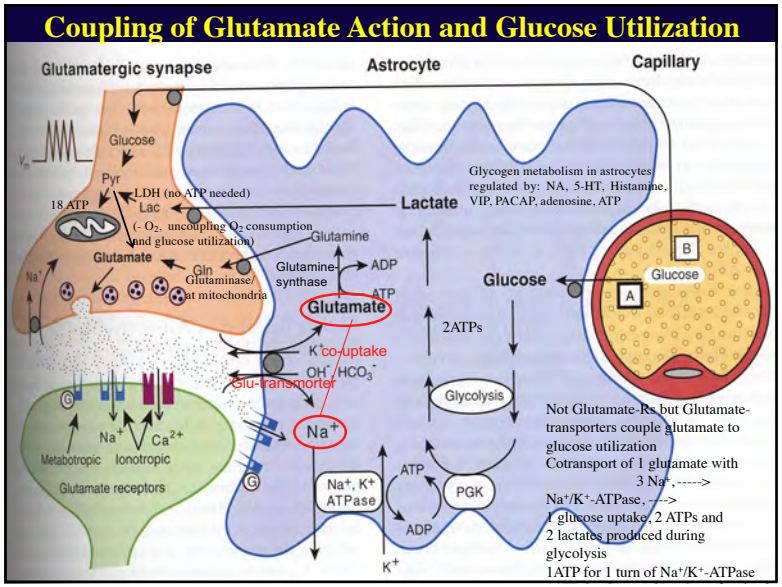


in brain: ~ 0.5-2.0 mM glucose in extracellular space; basal rate of glucose utilization is higher in astrocytes than in neurons, with values of about 20 and 6 nmol per milligram of protein per minute, respectively.

specific cellular glucose-transporter distrubution



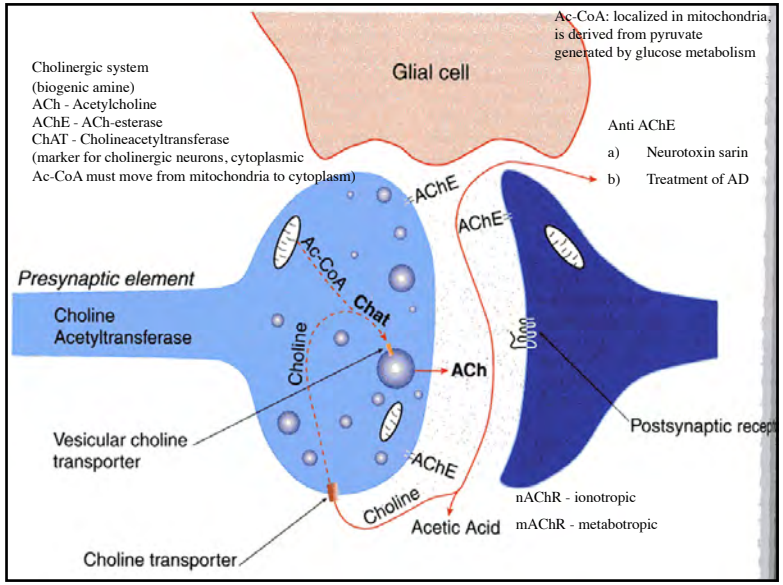
Coupling of Glutamate Action and Glucose Utilization

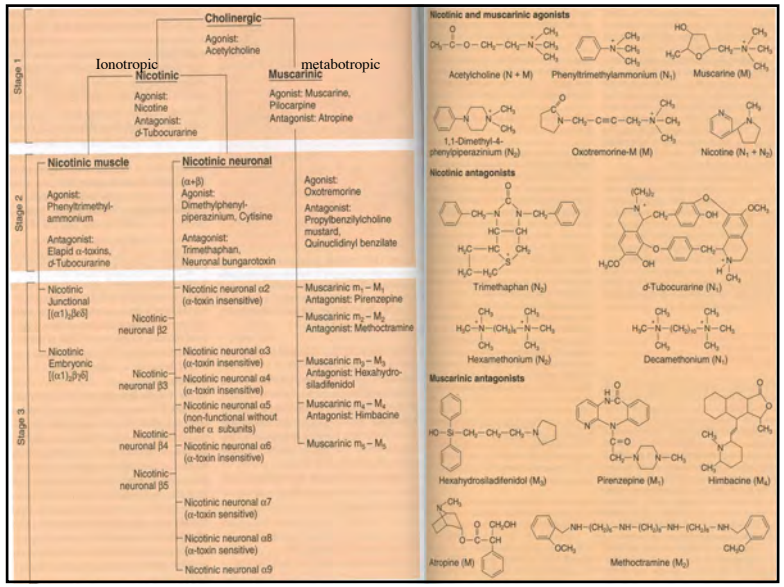


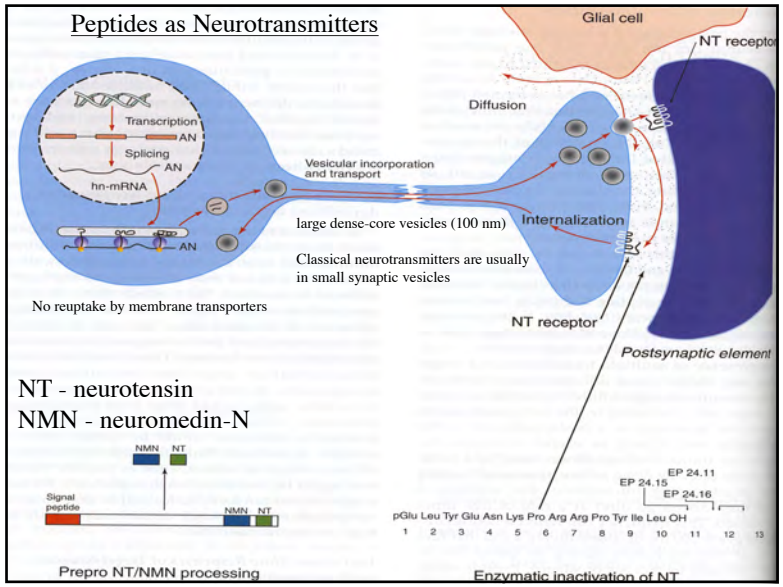
Acetylcholine (ACh)

Important Neurotransmitter in CNS and of e.g.: motorneurons, preganglionic sympathetic neurons, and neurons innervating

sweat glands







Major differences between 'classical' (Glu, GABA and ACh)- and peptide (for instance neurotensin (NT)) neurotransmitters:

In most cases, genes encoding peptide transmitters give rise to a prohormone which is incorporated into secretory granules after transcription, it is then acted on by peptidases to form the peptide transmitter, thus, peptide transmitters differ from classical transmitters by being synthesized in the soma rather than axon terminal. The active transmitter thus must be transported in vesicles to the nerve terminal.

Termination of peptide transmitter action differs from that of classical transmitters, being achieved mainly by enzymatic means and diffusion.

Peptides: lack of a specific high-affinity active reuptake process and there is much less specificity in the enzymatic inactivation of peptide transmitters. [For example, a metalloendopeptidase that inactivates enkephalins, small pentapeptide opioid-like transmitters, is frequently called enkephalinase but is also critically involved in he inactivation of several other neuropeptides.]

Major differences between 'classical' (Glu, GABA and ACh)- and peptide (for instance neurotensin (NT)) neurotransmitters:

Termination (inactivation) of classical transmitters (small molecules that are derived from either amino acids (Glu, GABA) or intermediary metabolism; usually synthesized by the sequential action of key enzymes, in the general vicinity of where they are to be released) takes place by a specific high-affinity active reuptake mechanism (Glu, GABA) to remove the transmitter from the extracellular space, and by enzymatic means (ACh), or both mechanism.

One final difference in the inactivation of peptide and classical transmitter is the product. Once classical transmitters are catabolized, the resultant metabolites are inactive at the transmitter receptor.

However, certain peptide fragments derived from the enzymatic 'inactivation' of peptide transmitters are biologically active. An example is angiotensinI ---> II (more active than I).

It is therefore sometimes difficult to distinguish between synthetic processing and inactivation.

Major differences between 'classical' (Glu, GABA and ACh)- and peptide (for instance neurotensin (NT)) neurotransmitters:

The peptide that is stored in vesicles and then released is therefore considered the transmitter, although the actions of certain peptidases may lead to other biologically active fragments later on/upon release.

five major steps involved in neurotransmission at CNS synapses

(1) Invasion of action potential into presynaptic terminal.
(2) Ca2+ influx into the nerve terminal through activation (opening) of voltage-dependent (gated) Ca2+ channels (VGCC).
(3) Docking (fusion) of synaptic vesicles with the terminal membrane (Exocytosis) and discharge of vesicular contents (neurotransmitters)
(4) Diffusion of neurotransmitters into the synaptic cleft and activation of (binding to) postsynaptic receptors.
(5) Diffusion and/or uptake (enzymatic inactivation) of neurotransmitters to terminate their actions.

the major ions that contribute to shape the action potential and the basic properties of their channels.

(1) Na+ and K+ ions are responsible for shaping the action potential. The Na+ current underlies the rising phase of action potential, whereas the K+ current is responsible for the decaying phase (re-polarization) of action potential.
(2) The properties of Na+ channels:
a. The Na+ channel displays threshold where activation starts to occur.
b. The Na+ channel shows the regenerative activity (self-reinforcing) that underlies an overshoot of action potentials. Because of this property, the action potential can conduct along the axon and muscle fibers without attenuating its amplitude.
c. The Na+ channel exhibits an inactivation process, which determines the refractory period of action potential regeneration.
d. Tetrodotoxin (TTX) and cocaine selectively block the Na+ channel activation.
(3) The properties of K+ channels:
a. The activation of K+ channels proceeds depending on the membrane depolarization.
b. The K+ channels does not exhibit an inactivation with maintained membrane depolarization, which is in a sharp contrast to the Na+ channel activation.
c. TEA (tetraethylammonium) selectively blocks the K+ channel activation.

the three major types of synaptic connections and two other forms of synaptic interactions

(1) Axo-somatic synapses.
(2) Axo-dendritic synapses.
(3) Axo-axonic synapses.

(4) Dendro-dendritic interactions (Dendritic release of neurotransmitters).
(5) Retrograde interactions (signaling/transmission).

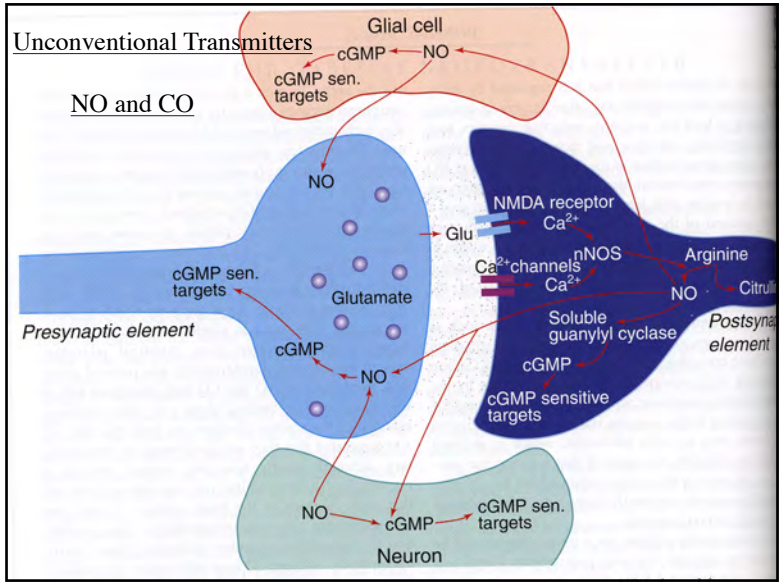
(6) Heterosynaptic interactions (Spillover transmission).
(7) Receptor cross-talks.

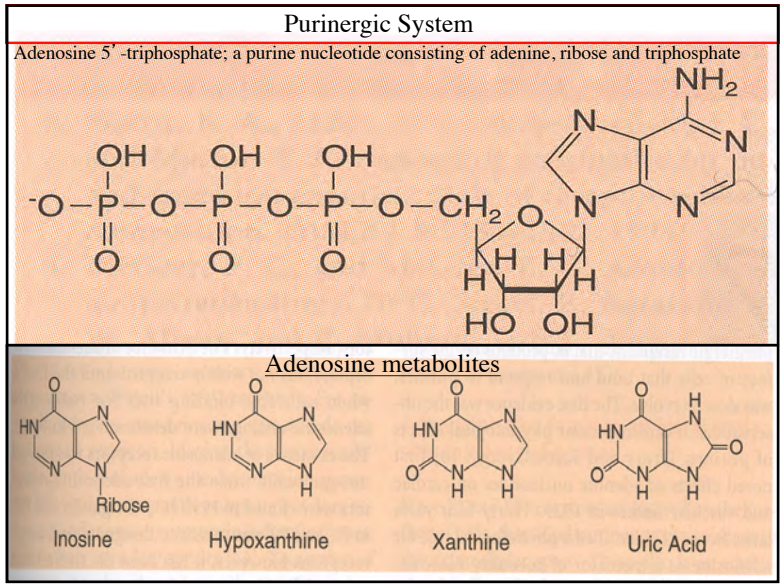
the three possible mechanisms that underlie modulation (i.e., gain changes) of neurotransmission at CNS synapses

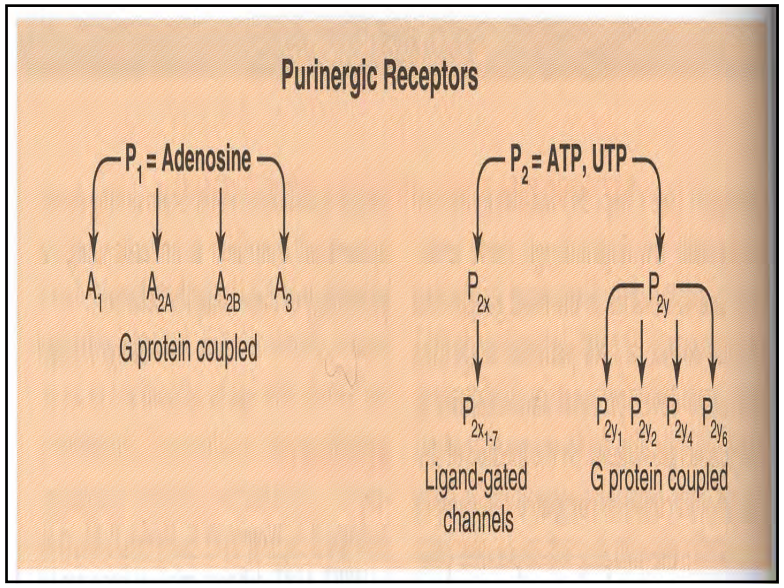
(1) Presynaptic mechanisms: either increase or decrease of neurotransmitter release. Monoamines, such as serotonin (5-HT), nor-adrenaline and dopamine, modulate the release of neurotransmitters.
(2) Postsynaptic mechanisms: (a) receptor efficacy can be changed by protein phosphorylation, and (b) the number of receptors at the synaptic site can be changed by enhanced or decreased trafficking (exocytosis or endocytosis) of receptor molecules through intracellular Ca2+-dependent signaling pathways, such as CaMKII-mediated protein phosphorylation.

the differences between ionotropic and metabotropic receptors.

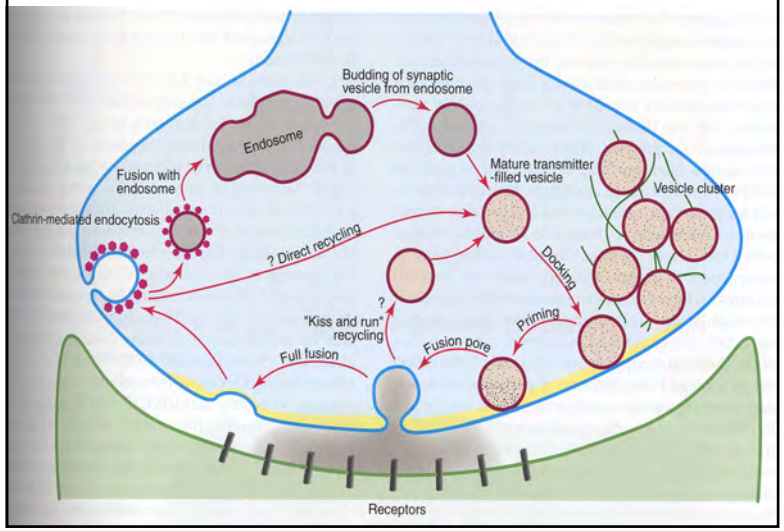
(1) The ionotropic receptor consists of a binding site for the neurotransmitter and an ionophore (ion pore) for allowing ion permeability (influx or efflux) within a single receptor molecule. Therefore, the ionotropic receptor is suitable for direct transmission which allows a fast point-to-point signaling at morphologically defined synapses.
(2) The metabotropic receptor has a binding site for the neurotransmitter and activates a GTP-binding protein, thereby coupling to modulation of the membrane ion channel activity (direct channel modulation) and/or intracellular signaling pathways (short-term action and long-term action via changes in transcription). Therefore, the metabotropic receptor is suitable for slow indirect transmission which allows integration (modulation) of synaptic gain with temporal and spatial domains.

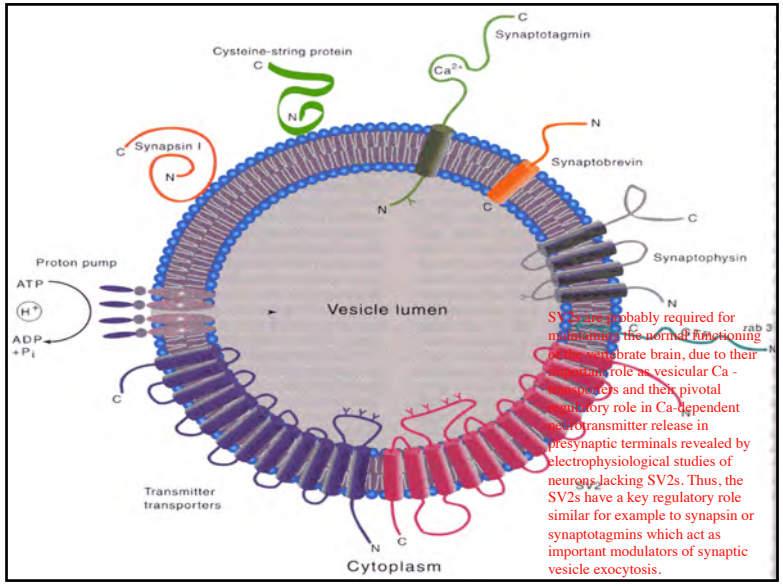




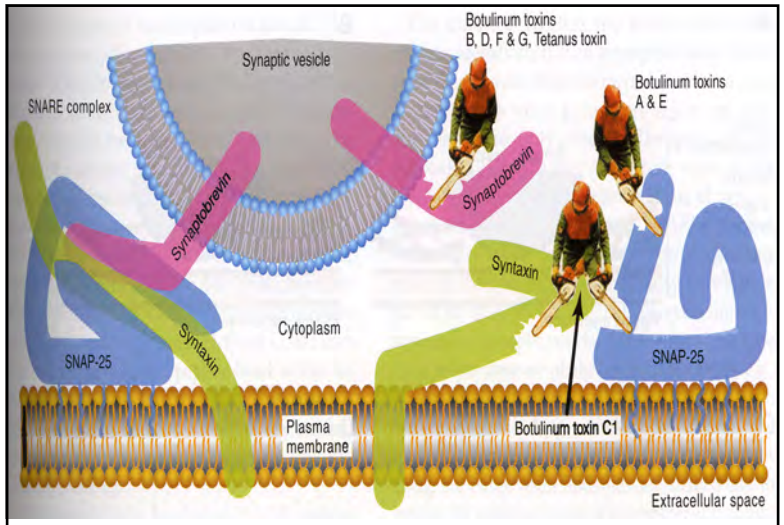


Life cycle of synaptic vesicles

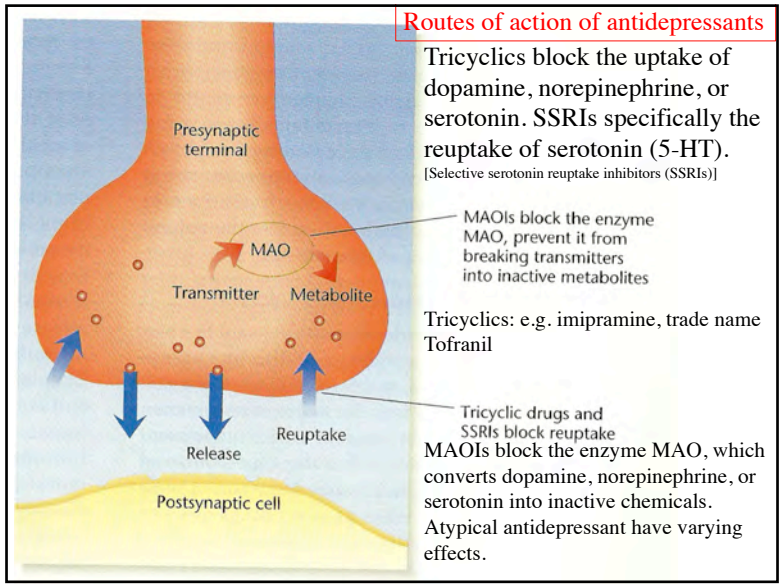




SV2s are probably required for maintaining the normal functioning of the vertebrate brain, due to their important role as vesicular Ca - transporters and their pivotal regulatory role in Ca-dependent neurotransmitter release in presynaptic terminals revealed by electrophysiological studies of neurons lacking SV2s. Thus, the SV2s have a key regulatory role similar for example to synapsin or synaptotagmins which act as important modulators of synaptic vesicle exocytosis.



Tetanus toxins and the botulinum toxins, proteases that cleave specific SNARE proteins as shown, can block transmitter release.



Routes of action of antidepressants

Tricyclics block the uptake of dopamine, norepinephrine, or serotonin. SSRIs specifically the reuptake of serotonin (5-HT).

[Selective serotonin reuptake inhibitors (SSRIs)]

MAOIs block the enzyme MAO, prevent it from breaking transmitters into inactive metabolites

Tricyclics: e.g. imipramine, trade name Tofranil

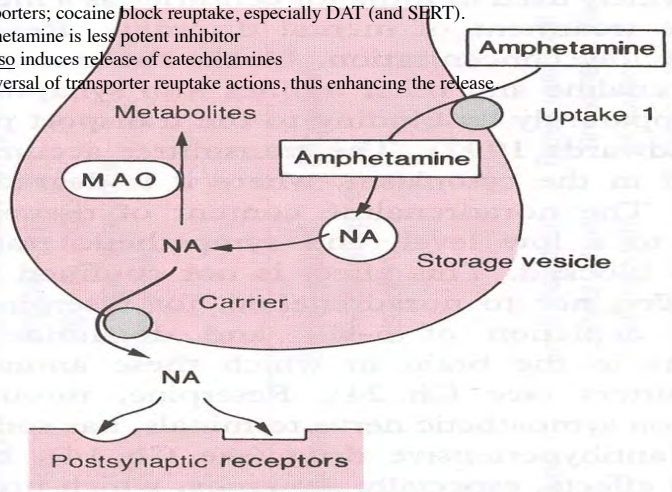
Tricyclic drugs and SSRIs block reuptake

MAOIs block the enzyme MAO, which converts dopamine, norepinephrine, or serotonin into inactive chemicals.

Atypical antidepressant have varying effects.

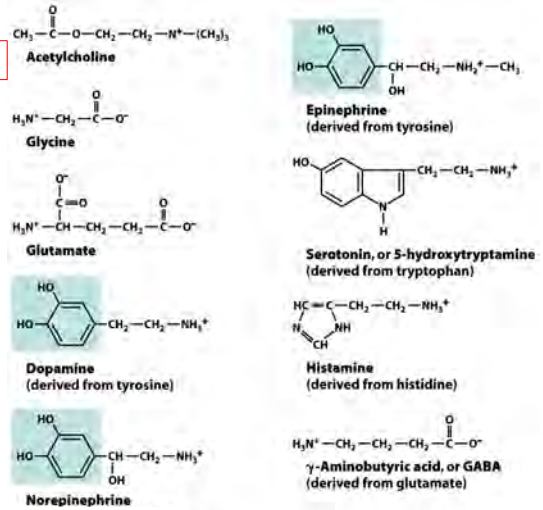
Amphetamine and cocaine block catecholamine and 5-HT transporters; cocaine block reuptake, especially DAT (and SERT). Amphetamine is less potent inhibitor but also induces release of catecholamines by reversal of transporter reuptake actions, thus enhancing the release

Amphetamine action



Drugs	Main Effects on Behavior	Main Effects on Synapses
Amphetamine	Excitement, alertness, elevated mood, decreased fatigue	Increases release of dopamine and several other neurotransmitters
Cocaine	Excitement, alertness, elevated mood, decreased fatigue	Blocks reuptake of dopamine and several other neurotransmitters
Methylphenidate	Increased concentration	Blocks reuptake of dopamine and others, but more gradually than cocaine does
Nicotine	Mostly stimulant effects	Stimulates nicotinic-type acetylcholine receptor, which (among other effects) increases dopamine release in nucleus accumbens
Opiates	Relaxation, withdrawal, decreased pain	Stimulates endorphin receptors
Cannabinoids (marijuana)	Intensified sensory experiences, distorted sense of time, decreased pain and nausea	Excites negative-feedback receptors on presynaptic cells; thereby puts the brakes on release of either glutamate or GABA
LSD	Distorted sensations	Stimulates serotonin type 2 receptors (5-HT_2)
Alcohol	Relaxation, decreased attention	Facilitates $GABA_A$ receptor

Neurotransmitters



Taking home message:

- Classical Neurotransmitters vs peptides
- Unconventional Transmitters
- Major difference between catecholamines and amino acid neurotransmitters
- Criteria for a neurotransmitter?
- Process (steps) of chemical neurotransmission?
- neuronal activity coupled to energy supply and controlled by by astrocytes