Neurotransmitter Actions

Katya Likhtik

- What is a neurotransmitter?
- Under what conditions and how are they released?
- What happens to the presynaptic and the postsynaptic neuron once they are released?

The 18th and 19th C debate about the nature of communication in the nervous system: Electrical or Chemical??

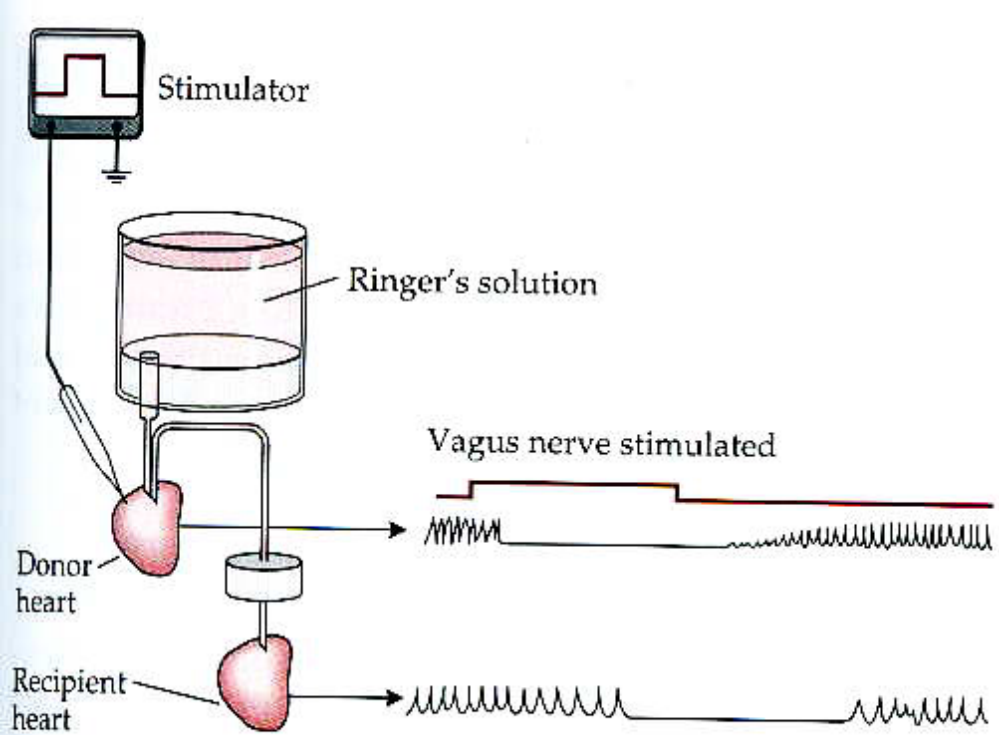
After a presynaptic neuron is stimulated the delay is about 0.3 ms for the postsynaptic neuron to respond. This is too long for electric transmission.

If you stimulate the postsynaptic neuron , no response in the presynaptic one. Polarization of communication between neurons.

Stimulation of presynaptic neuron may result in postsynaptic inhibition. Difficult to explain in terms of direct passage of electrical event.

No relationship between the magnitude of the pre and postsynaptic electrical event.

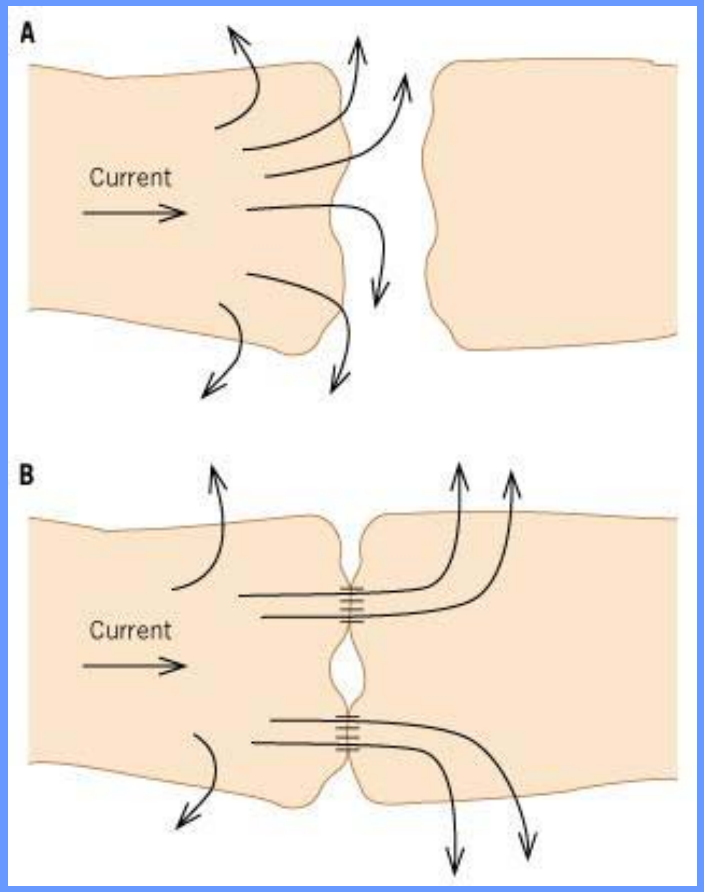
Otto Lowei (1921)



6.15 LOEWI'S EXPERIMENT. Stimulating the cardiac branch of the vagus nerve of a donor frog heart arrested the heartbeat. Ringer's solution perfusing the donor heart was transferred to the recipient heart. After 15 seconds, the beat in the second heart was also arrested.

"Vagusstoff" (actually Acetylcholine)

Not to say that there aren't electrical synapses!!



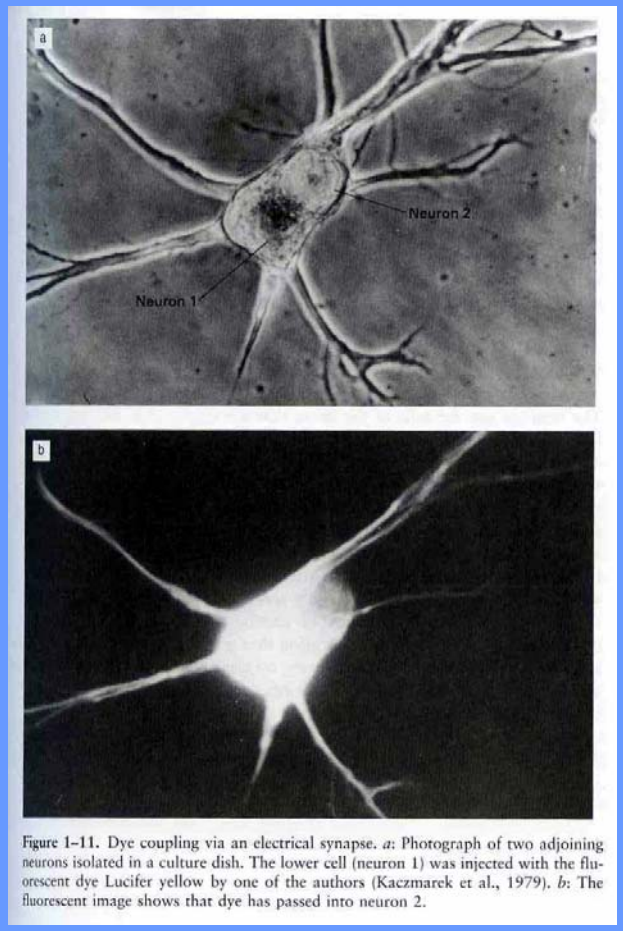


Figure 1–11. Dye coupling via an electrical synapse. *a*: Photograph of two adjoining neurons isolated in a culture dish. The lower cell (neuron 1) was injected with the fluorescent dye Lucifer yellow by one of the authors (Kaczmarek et al., 1979). *b*: The fluorescent image shows that dye has passed into neuron 2.

Gap junctions – cell to cell pores that allow ions and very small molecules to pass from the cytoplasm of one cell to the next.

Before a substance can be called a neurotransmitter:

1. Presynaptic terminal should contain a store of the substance (preferably in a sequestered form)

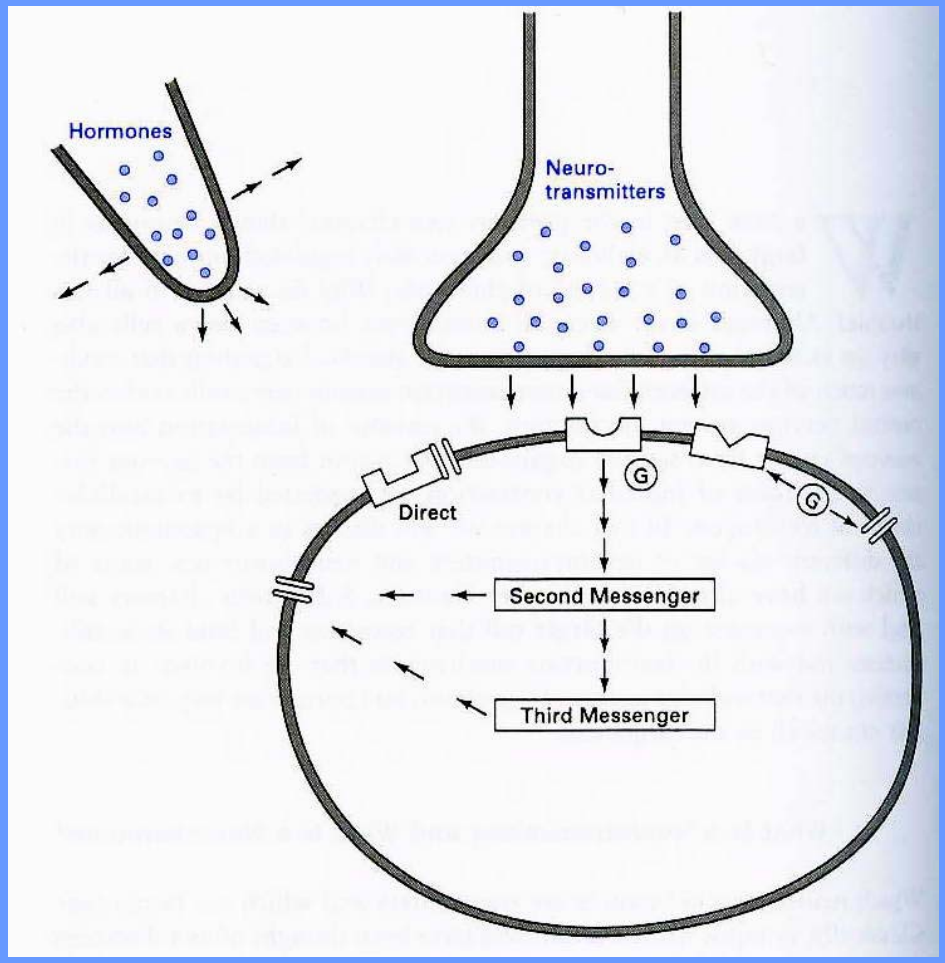
2. Applying the substance to a postsynaptic cell should mimic the effects caused by stimulating the presynaptic terminal

3. If a drug is known to block a neurotransmitter, it should have the same effect on this transmitter if it's applied exogenously

4. A mechanism for the synthesis of this trasmitter must exist (including the appropriate precursors/enzymes in the terminal)

5. A mechanism for inactivation of the transmitter must exist (catabolic enzymes for its degradation/ reuptake system, etc)

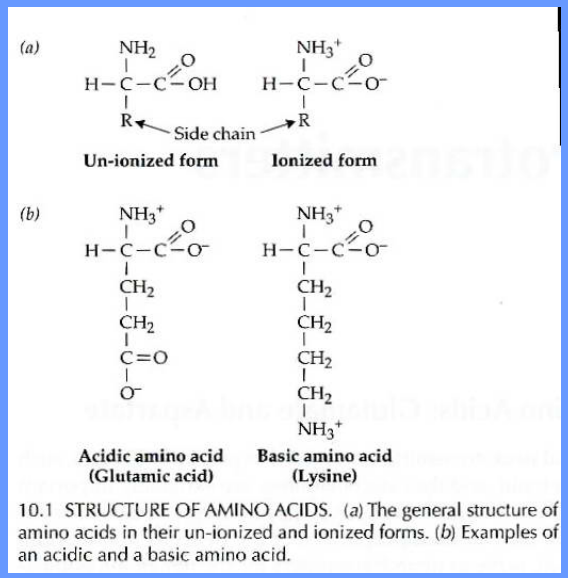
The type of communication between neurons discussed in this class



What kinds of neurotransmitters are there?

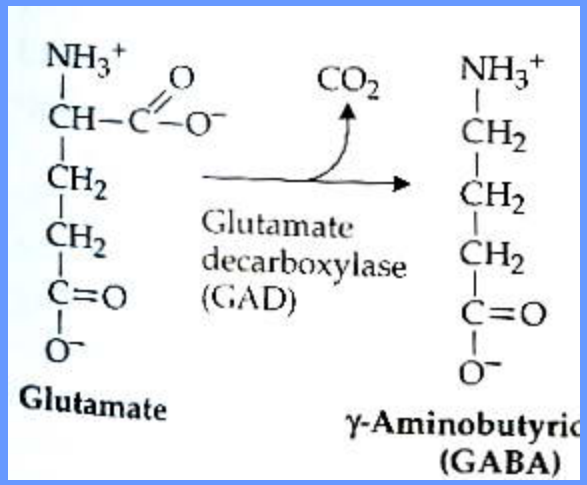
AMINO ACID transmitters

Glutamate



10.1 STRUCTURE OF AMINO ACIDS. (*a*) The general structure of amino acids in their un-ionized and ionized forms. (*b*) Examples of an acidic and a basic amino acid.

GABA (γ-aminobutyric acid)

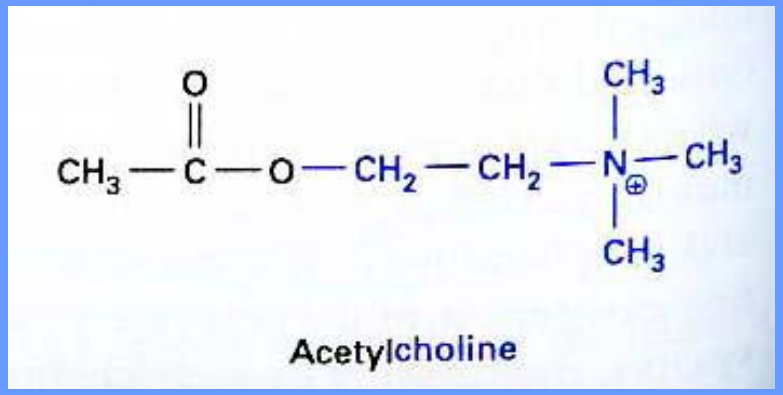


Most transmitters are small, water-soluble molecules containing amine and (in the case of amino acid transmitters) carboxyl groups. These chemical groups cause the transmitters to be ionized at physiological pH and thus reduces the probability of passing the blood-brain-barrier (BBB!).

Acetylcholine (Ach)

Present both in the central and peripheral nervous systems (CNS &PNS)

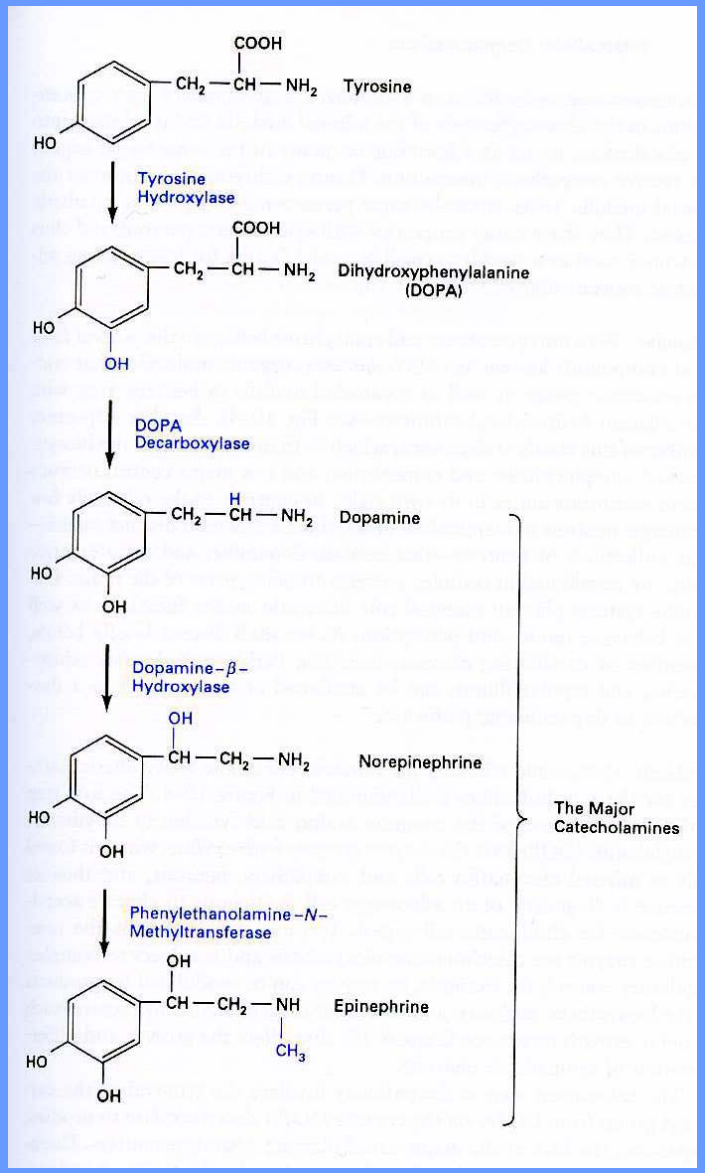
Synthesized by the combination of AcetylCoA, which is a product of the Krebbs cycle in the mitochondria, and choline, which is obtained from food (egg yolk, legumes).

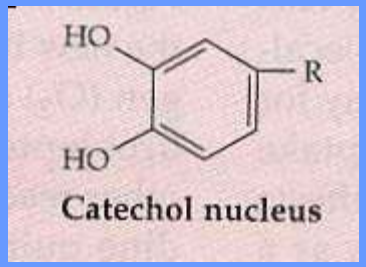


Acetylcholine

In the PNS, it is the transmitter of the neuromuscular junction- between neurons and all types of muscle (cardiac/smooth/skeletal) and thus is responsible for muscle contraction.

The monoamine neurotransmitters





Dopamine (DA) – concentrated in neurons of the Ventral Tegmental Area (VTA) and in the substantia nigra of the basal ganglia. Important for motion, mood, reward, schizophrenia, etc

NE – first discovered in the sympathetic branch of the autonomic nervous system. Cell groups containing NE found in the locus coerulus (LC), which projects all over the brain and partakes in The sleep-wake cycle, attention, vigilance.

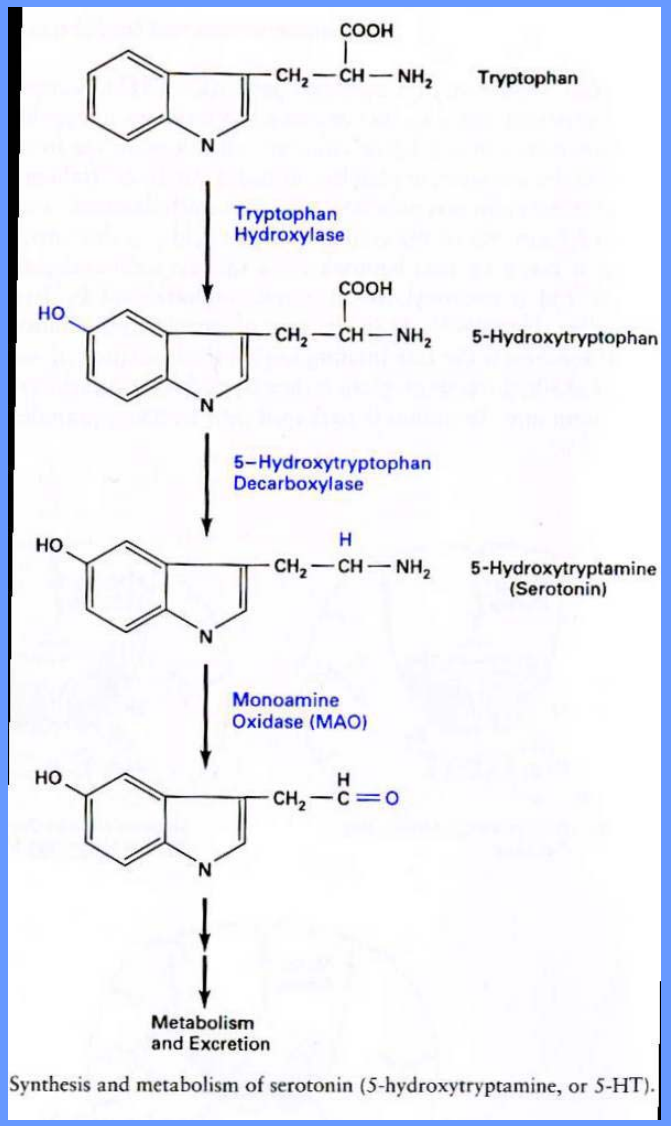
EPI – sympathoexcitatory, found in the adrenal medulla and in cell groups of the medulla (oblongata)

Serotonin (5HT)

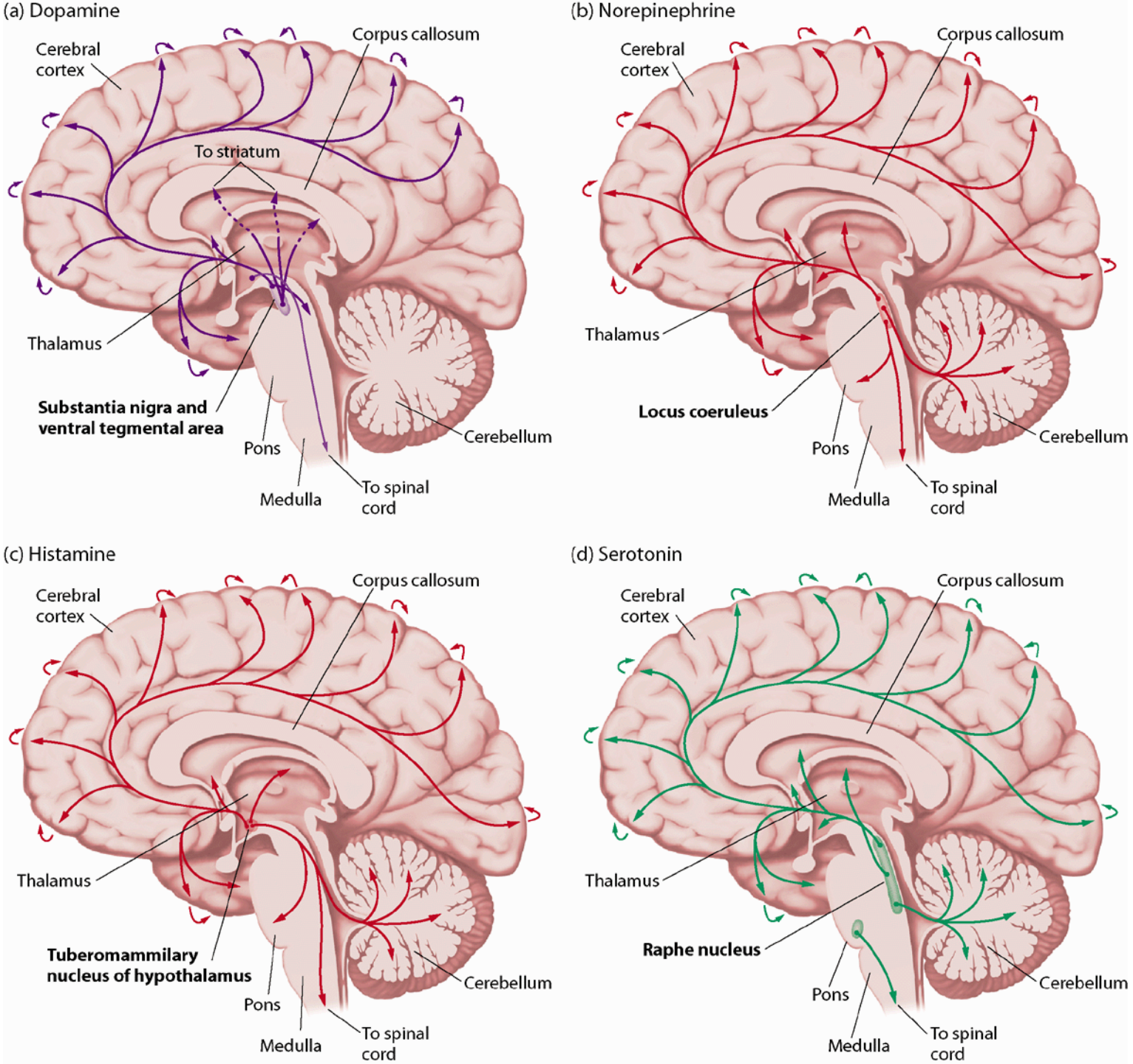
First identified as an element found in the blood that aided its clotting and produced vasoconstriction (originating from “serum” + having an effect on muscle “tone” resulted in the name serotonin)

5HT neurons found mostly in the raphe nuclei that are located in the brainstem and that innervate all major brain areas

5HT has been found to be important for food intake, aggression, mood. It is manipulated by antipsychotic drugs. Variations of serotonin (slight changes in its chemical structure) result in hallucinogens such as LSD, ecstasy, mescaline.



Synthesis and metabolism of serotonin (5-hydroxytryptamine, or 5-HT).



Adapted from Purves D., Augustine, G., and Fitzpatrick, D. (2001). *Neuroscie□nce*, 2nd edition. Sunderland, MA: Sinaur Associates

Vesicle transport to the terminal is via microtubules

The synthesis of vesicles occurs in the cell soma, where they pinch off the Golgi apparatus and are transported to the terminal along microtubules in the axon.

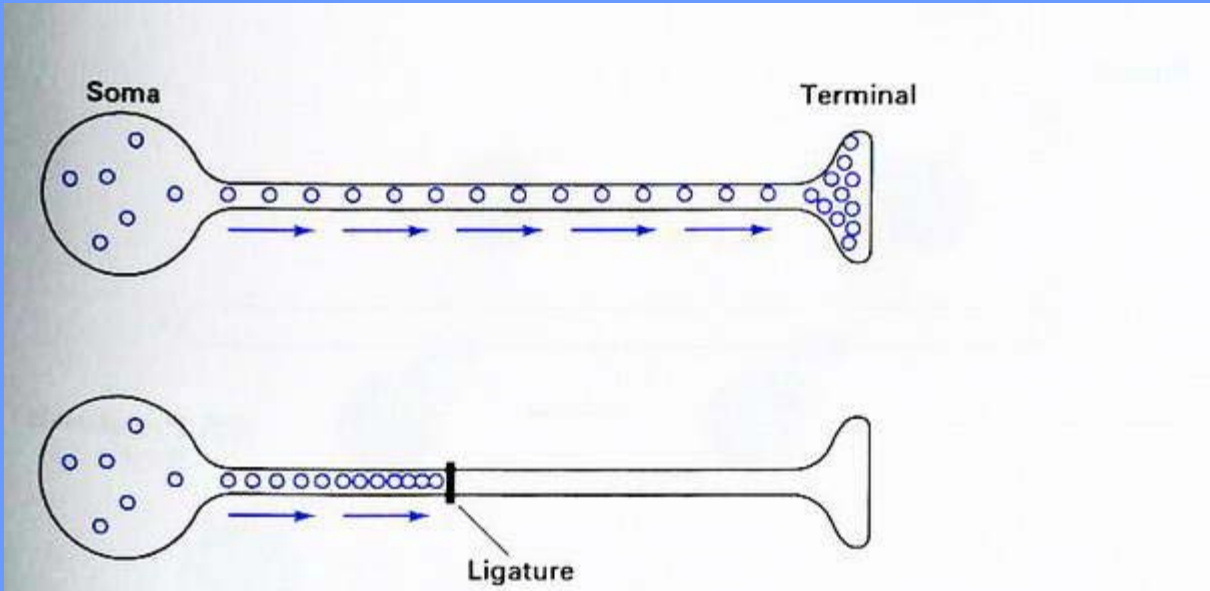
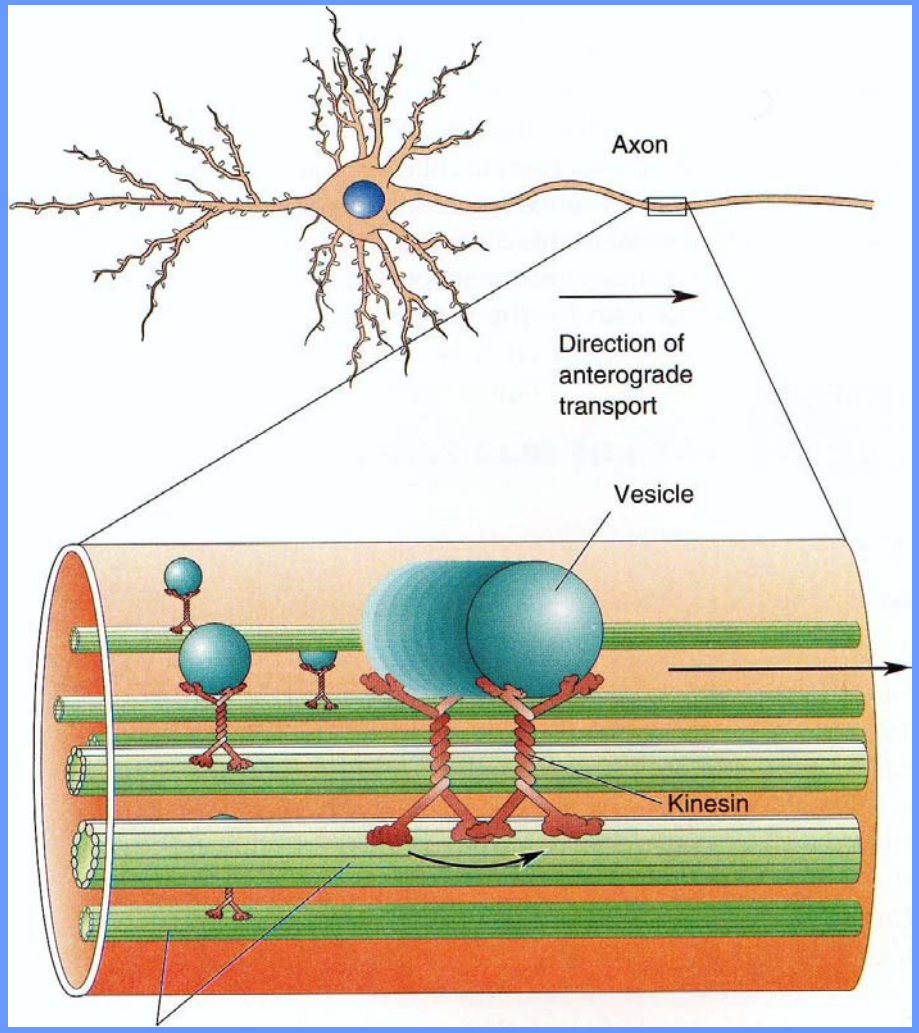


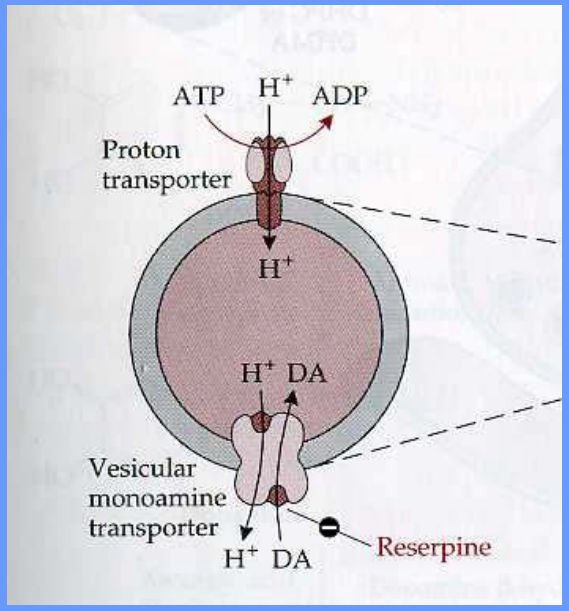
Figure 2–9. Vesicles are transported from soma to axon terminal. Vesicles and organelles are synthesized in the cell body and transported (arrows) by an active process down the axon toward its terminal. When the axon is tied off (ligated), vesicles are seen to accumulate in the axon on the side of the ligature proximal to the cell body. Experiments of this type were first done by Paul Weiss and colleagues in the 1930s.

Vesicles are transported to the terminal via an active process



Vesicle transport requires ATP hydrolysis by a small molecular “motor” called kynesin.

Loading neurotransmitters into vesicles at the terminal



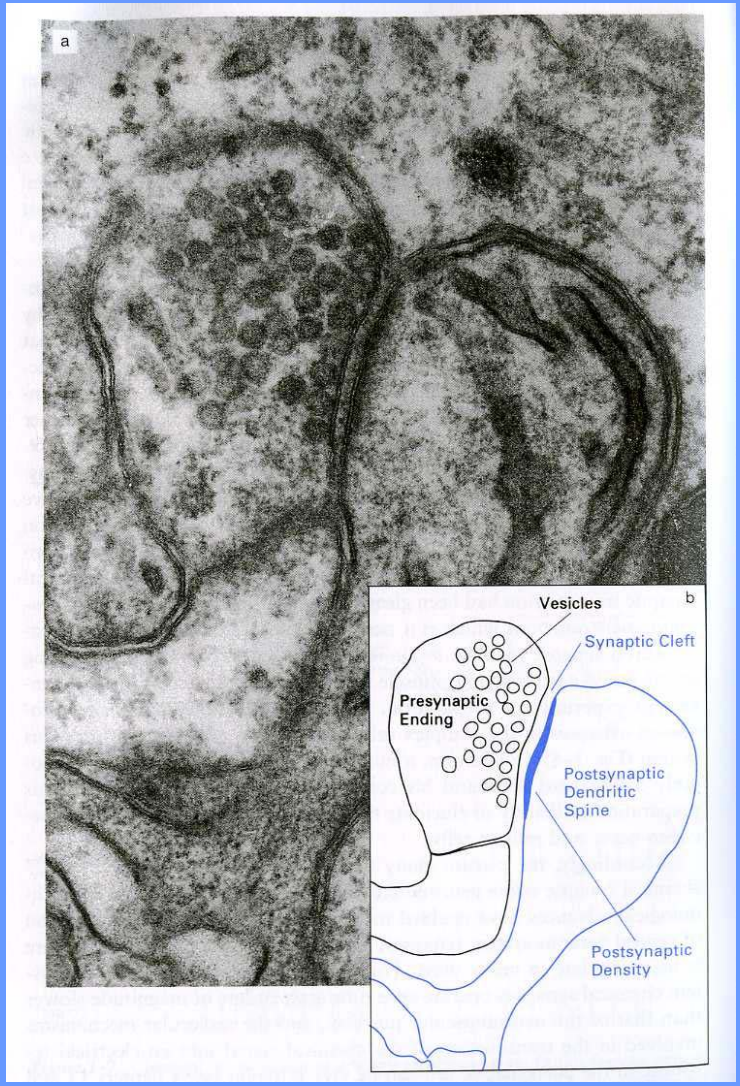
The proton pump hydrolizes ATP and produces a proton gradient and membrane potential across the granule membrane. The amine transporter binds an uncharged amine and couples its transport INTO the vesicle with the H^+ exit OUT of it. The amine then becomes protonated at the acidic pH of the vesicle and is stuck inside the vesicle.

Vesicles aggregate at the presynaptic terminal at the synapse

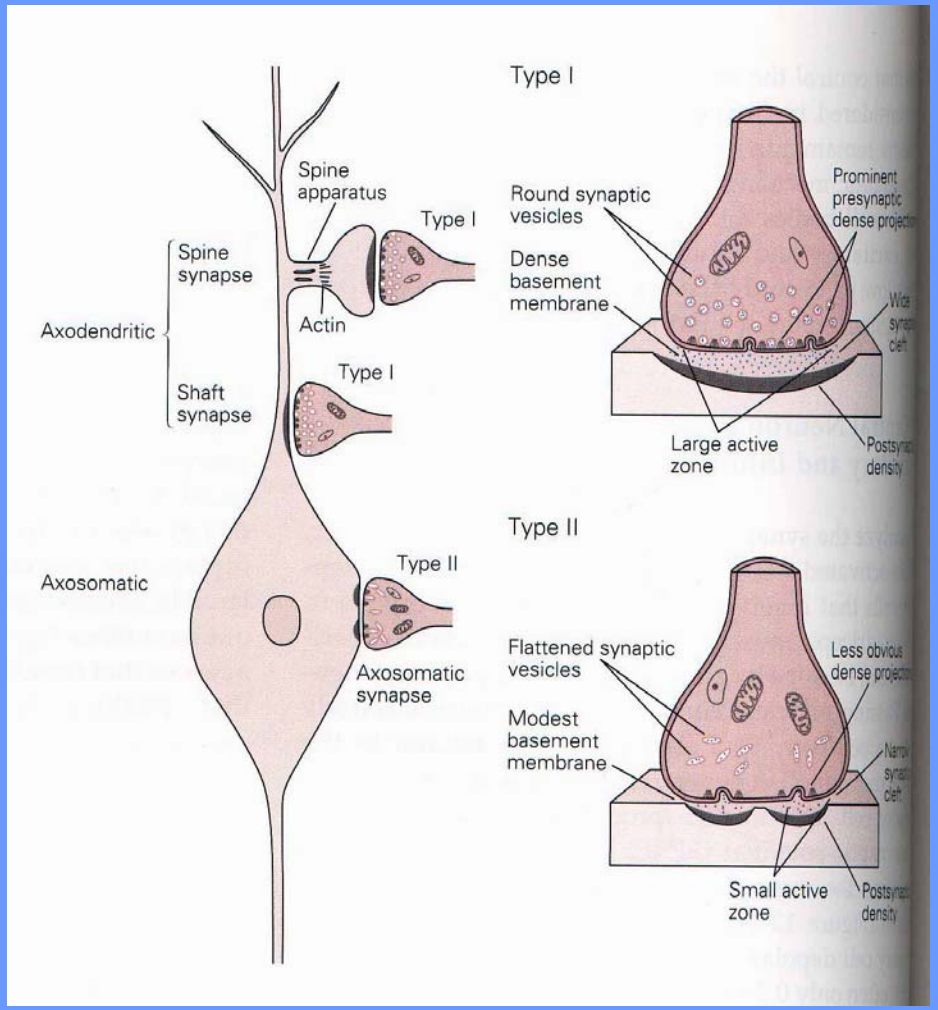
A synaptic cleft in the CNS separates the pre and postsynaptic terminals by about 20-30 nm

Synapses can be axodendritic, axosomatic and axoaxonic

Active zones- areas of the presynaptic membrane that are sites of vesicle attachment and neurotransmitter release



Gray's Type I and Type II synapse structure

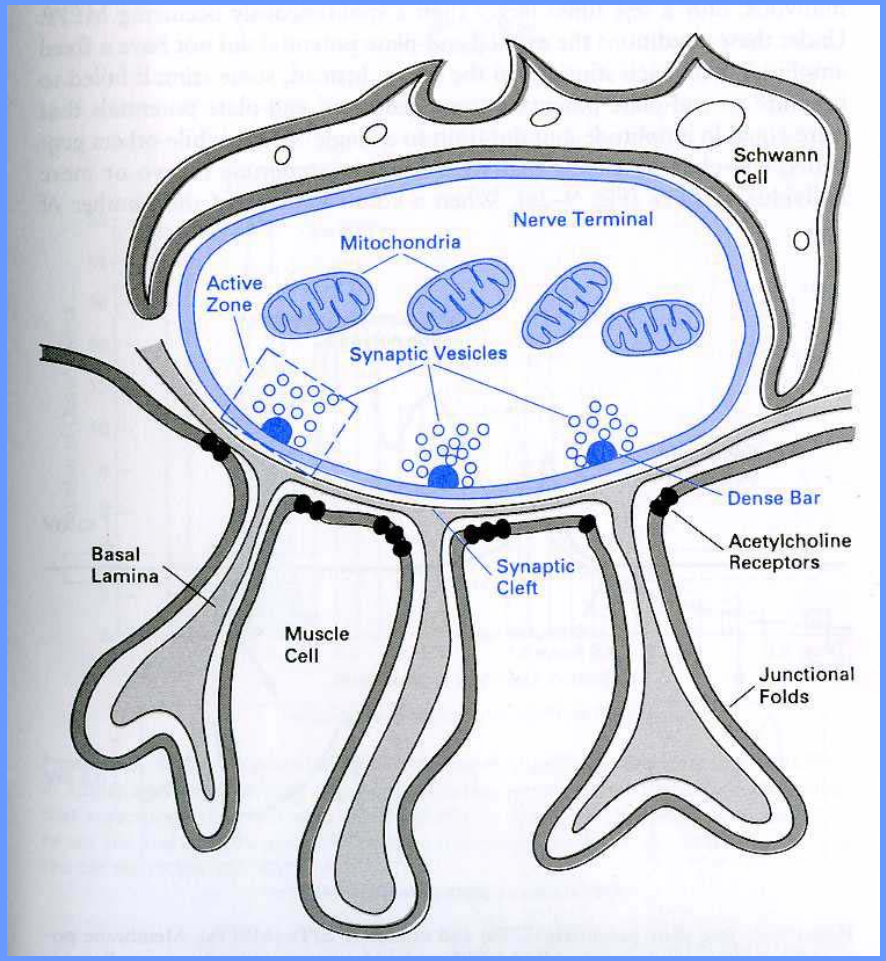


Note the differences in localization of synapses, vesicle shape, the shape of the synaptic cleft.

Type I - usually glutamatergic, excitatory

Type II - usually GABAergic, inhibitory

The structure of the neuromuscular junction (NMJ) in the PNS



The junctional folds found on the postsynaptic side of the NMJ increase the number of Ach receptors that are exposed to release of neurotransmitter, resulting in very efficient transmission

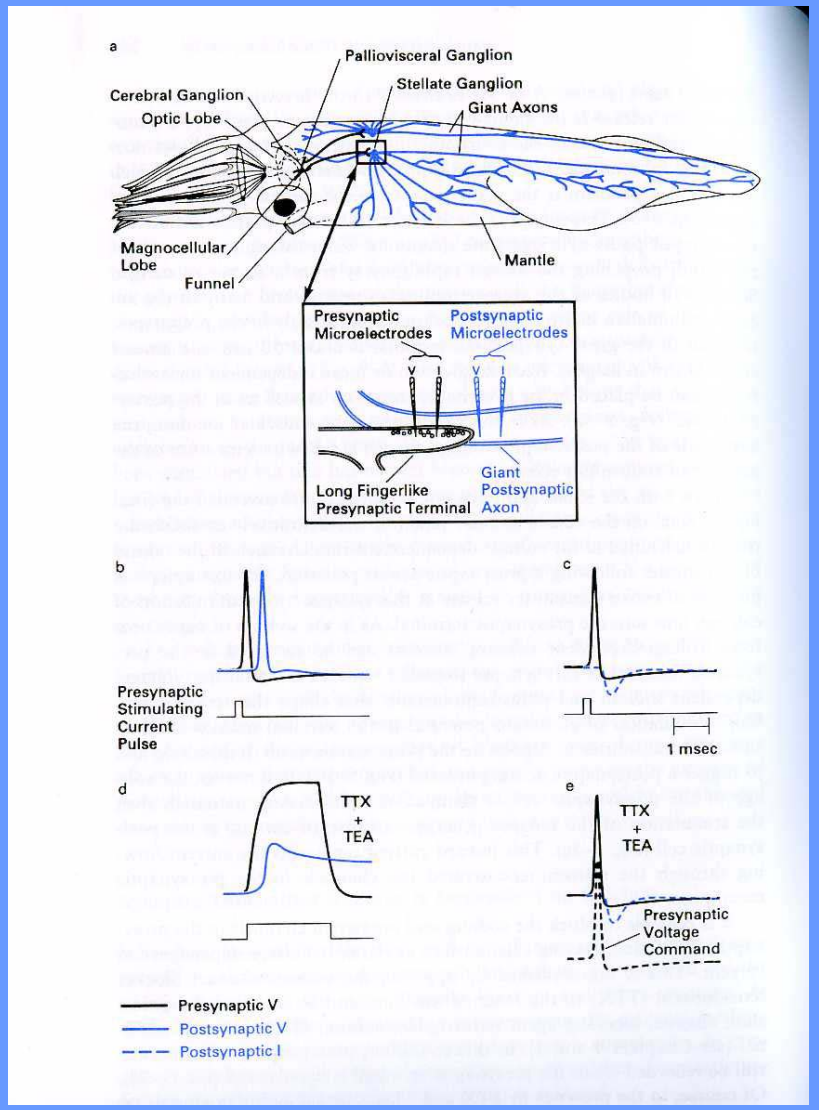
Neurotransmitter Release is Ca++ dependent

Release of neurotransmitters is dependent upon the entry of Ca^{++} into the presynaptic terminal through voltage sensitive Ca^{++} channels, which are clustered near release sites.

Voltage increases sufficient to open Ca^{++} channels usually occurs with the arrival of an action potential to the terminal. The terminal can also be stimulated with an electrode to increase voltage.

TTX blocks Na^{+} channels

TEA blocks K^{+} channels



Vesicle Exocytosis

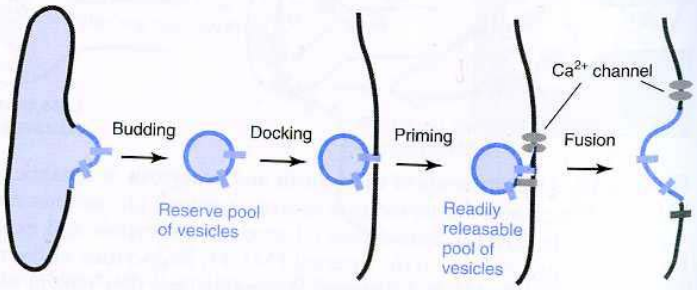
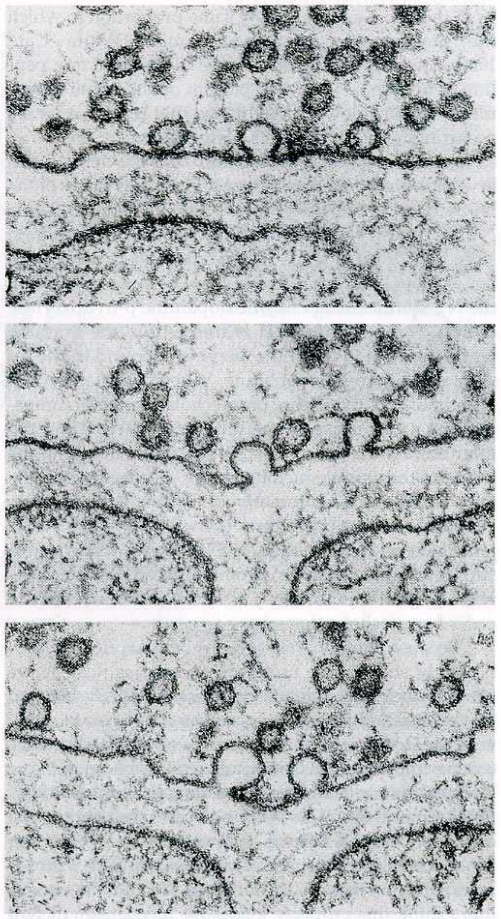


Figure 8–11. Steps in the movement of synaptic vesicles from internal membranes to fusion with the plasma membrane.

The time between docking and exocytosis is less than 200 ms. In order for vesicles to dock and to be primed for release, ATP is necessary. Thus mitochondria are present at the terminal.



6.17 EXOCYTOSIS at the frog neuromuscular junction. Three electron micrographs of frog neuromuscular junctions are shown at high magnification (×145,000). A single electrical stimulus was applied to the motor nerve, after which the tissue was frozen extremely rapidly. The drug 4-aminopyridine was used to prolong action potential duration, thereby greatly increasing transmitter release from the terminals. In each micrograph, several vesicles are seen fused with the presynaptic membrane, presumably in the act of exocytosis. Such fusion events occurred only at the active zones opposite the junctional folds of the motor end plate. (From Heuser, 1977.)

The SNARE complex model of vesicular fusion

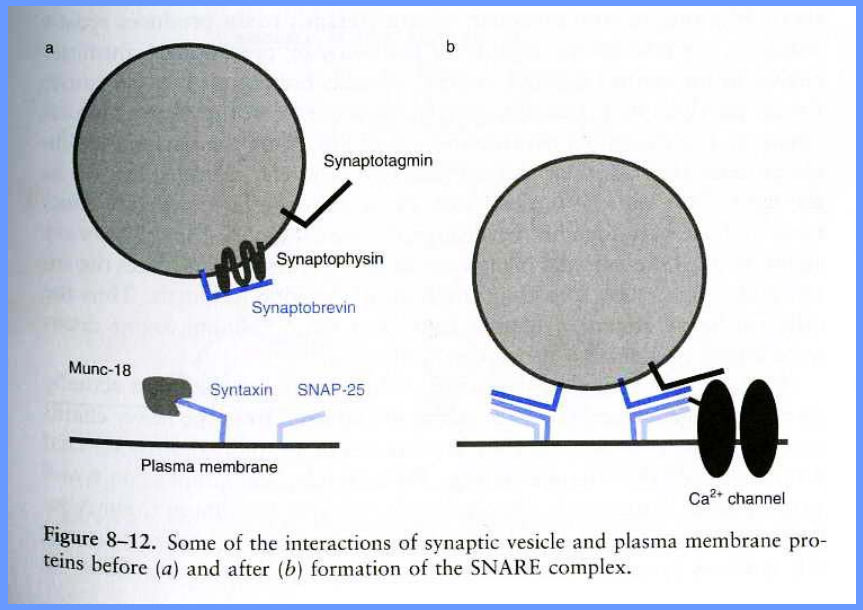


Figure 8–12. Some of the interactions of synaptic vesicle and plasma membrane proteins before (*a*) and after (*b*) formation of the SNARE complex.

As vesicles fuse with the membrane the SNARE complex forms as a result of the close association of proteins found on the membranes of the vesicle and plasma membrane.

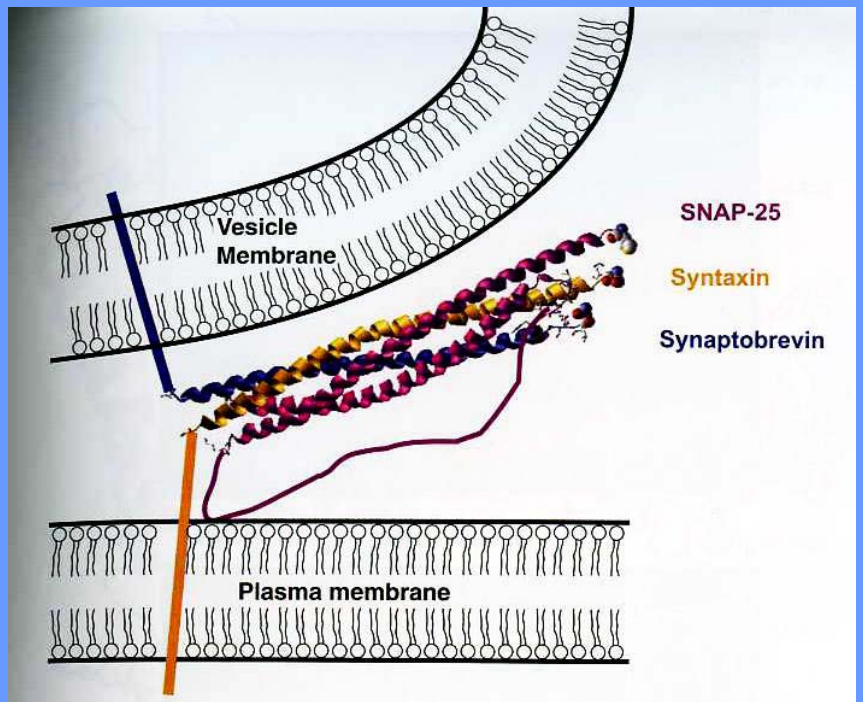


PLATE 6. Structure of the SNARE complex. The interactions of synaptobrevin, syntaxin, and SNAP-25 have been determined by X-ray crystallography. Locations of the vesicle and plasma membranes and the transmembrane regions of the proteins are drawn in for clarity (Sutton et al., 1998).

Toxins, such as botulinum toxin and tetanus toxin, selectively cleave different proteins of the SNARE complex, preventing vesicle fusion and neurotransmitter release, thus causing paralysis. (Botox is also used in plastic surgeries to eliminate wrinkles of the forehead.)

Vesicle endocytosis and recycling

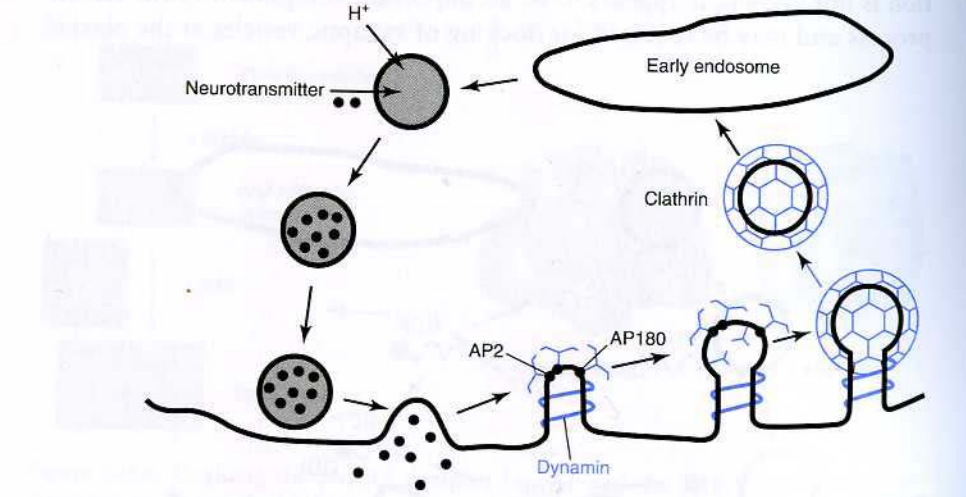


Figure 8–16. Recycling of synaptic vesicle membranes. After exocytosis, membranes are retrieved by the motor protein dynamin and are coated with clathrin. The membranes are then transported to the early endosome and reformed into new vesicles, which are refilled with neurotransmitter.

After neurotransmitter release, the vesicular membrane is coated with the protein clathring, thus identifying it for recycling. The clathrin coated pits are transferred to the endosome, where the membrane is reused for new vesicles and refilled with neurotranmitter.

Neurotransmitters are released in “quanta”

Spontaneous miniature end plate potentials (MEPPs) are usually about 0.5mV. Stimulation of the presynaptic terminal causes post-synaptic end plate potential (EPP) amplitudes to be at multiples of the MEPP. With iontophoretic application of Ach, it was estimated that about 5000 molecules (or a quantum) of Ach are released synchronously into the synaptic cleft to generate a single MEPP.

1950s, Katz et al., suggested that the packets of quanta of Ach correspond to neurotransmitter content of a single vesicle

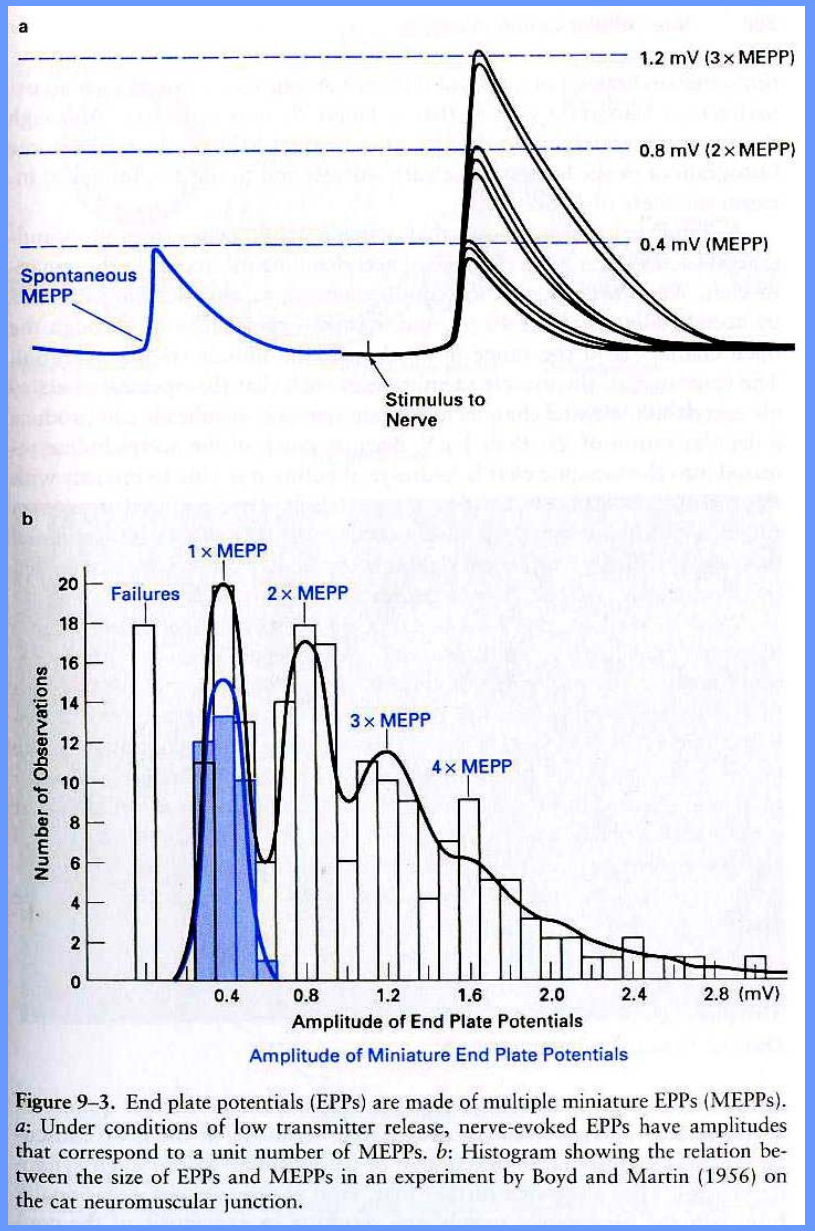


Figure 9–3. End plate potentials (EPPs) are made of multiple miniature EPPs (MEPPs). *a*: Under conditions of low transmitter release, nerve-evoked EPPs have amplitudes that correspond to a unit number of MEPPs. *b*: Histogram showing the relation between the size of EPPs and MEPPs in an experiment by Boyd and Martin (1956) on the cat neuromuscular junction.

Removal of the neurotransmitter from the synaptic cleft

Timely removal prevents desensitization of receptors and interference with new incoming signals.

Mechanisms

Enzymatic degradation:
Acetylcholinesterase

A nearly 100% efficient enzyme. Ach molecules are hydrolized as rapidly as they can diffuse into the active site. Forms acetate and choline.

Breaks down 14,000 Ach mols/sec (1 moleucle in 70 µsec)

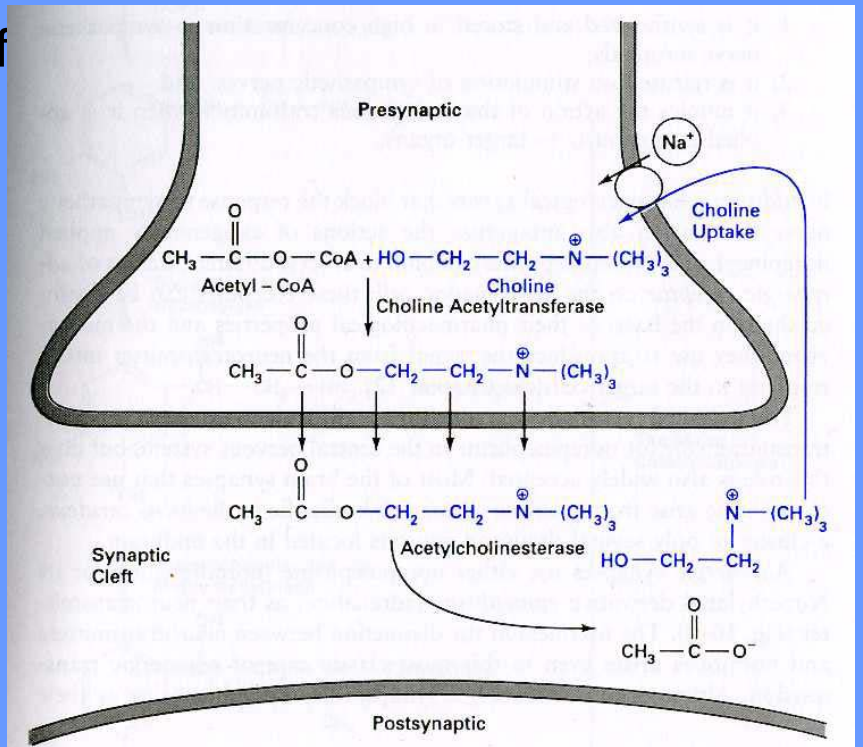


Figure 10–3. Synthesis, release, and degradation of acetylcholine. The transmitter is synthesized from choline and acetyl-CoA by the enzyme choline acetyltransferase. Following its release it is broken down rapidly by the enzyme acetylcholinesterase to choline and acetate. The choline can be taken back up into the terminal via a sodium-coupled choline transport system.

Degradation of Catecholamines

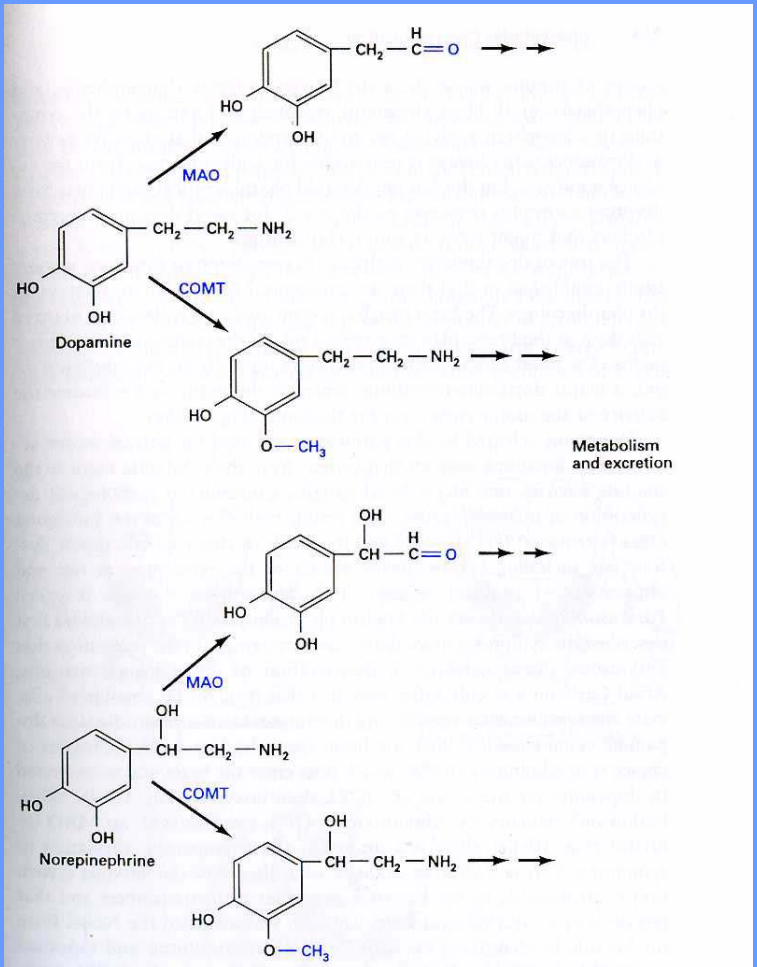


Figure 10–5. Metabolism of the catecholamines. Two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), catalyze the first steps in the degradation of the catecholamines.

Monoamine Oxidase (MAO) – an enzyme that catalyzes the neurotransmitter to its corresponding aldehyde. This form can be broken down further and excreted. MAO is located in the mitochondria of catecholaminergic terminals.

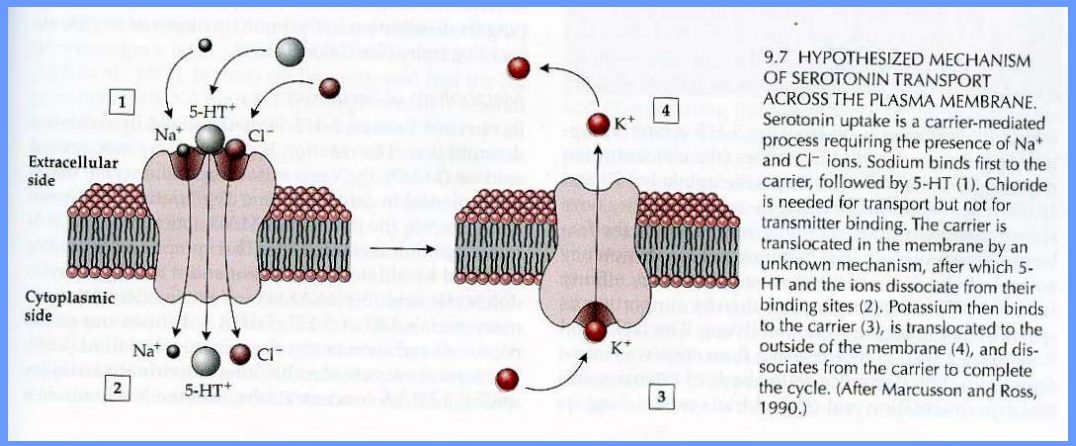
Catechol-O-Methyl-Transferase (COMT) – methylates a hydroxyl group of the catechol nucleus. The products can then also be excreted.

Antidepressant drugs act on these enzymes. E.g. Pargyline – a clinically effective antidepressant is an MAO inhibitor, increases DA levels in the CNS.

The most common mechanism: Reuptake by transporter molecules

Transporter molecules are in the presynaptic nerve terminals and glial cells

Transporter molecules have binding constants of <25 mM.



9.7 HYPOTHESIZED MECHANISM OF SEROTONIN TRANSPORT ACROSS THE PLASMA MEMBRANE. Serotonin uptake is a carrier-mediated process requiring the presence of Na^+ and Cl^- ions. Sodium binds first to the carrier, followed by 5-HT (1). Chloride is needed for transport but not for transmitter binding. The carrier is translocated in the membrane by an unknown mechanism, after which 5-HT and the ions dissociate from their binding sites (2). Potassium then binds to the carrier (3), is translocated to the outside of the membrane (4), and dissociates from the carrier to complete the cycle. (After Marcusson and Ross, 1990.)

serotonin

norepinephrine

Dopamine cocaine and amphetamine prevent reuptake of DA and NE

glutamate

GABA

glycine

choline (after acetylcholine is broken down, choline is taken into the presynaptic nerve terminal for reuse)

Postsynaptic actions of neurotransmitters are receptor dependent

Ligand-gated ion channels – activated by the biding of a neurotransmitter

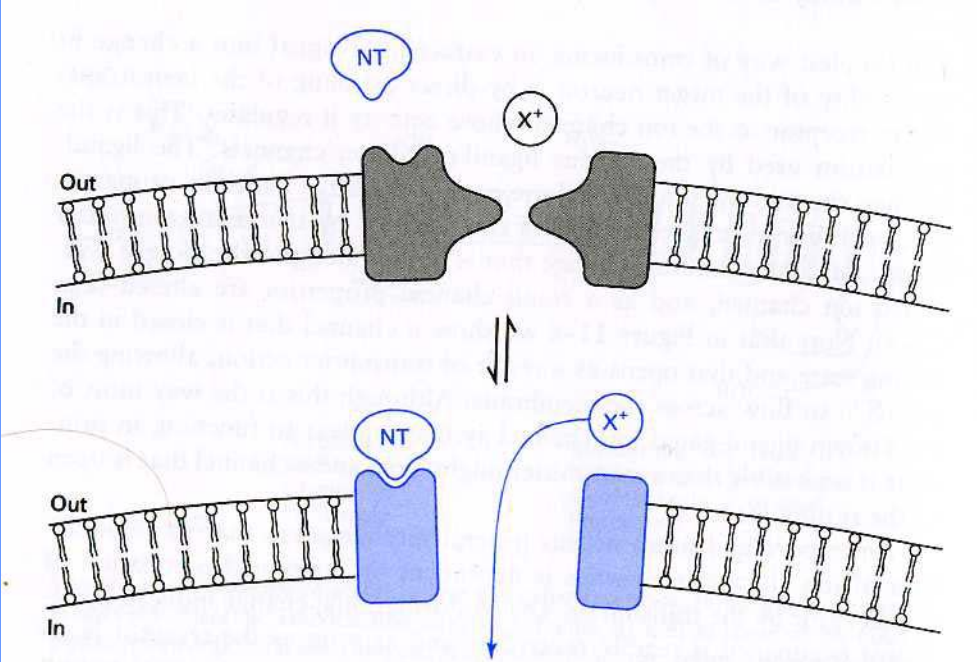


Figure 11–8. Direct receptor–channel coupling. In directly coupled receptor–channel systems the neurotransmitter (NT) binding site and the ion channel are intimately associated in a single macromolecular complex. Contrast with Figures 12–2 and 12–6.

Binding of the ligand stabilizes the active conformation of the receptor, thereby opening an ion channel, which is created by the arrangement of four to five receptor subunits.

e.g. Nicotinic receptor for Ach

An example of a ligand gated ion channel

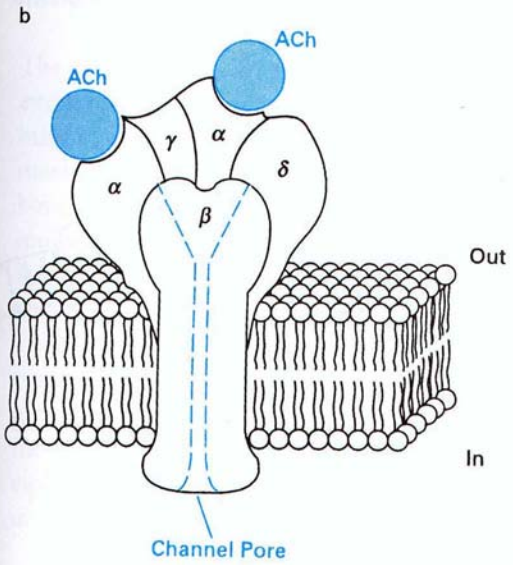


Figure 11–6. The nicotinic acetylcholine receptor from *Torpedo*. *a*: Polyacrylamide gel separation of the individual subunits of the purified nicotinic receptor. *b*: Cross-linking studies have demonstrated that the functional receptor molecule contains two α subunits, each with an acetylcholine (ACh) binding site, and one each of the β, γ, and δ subunits.

The nicotinic Ach receptor. It needs two molecules of Ach in order to open. It is permeable to cations. It is found in the NMJ, autonomic ganglia, hippocampus, thalamus, etc.

G-protein mediated receptors

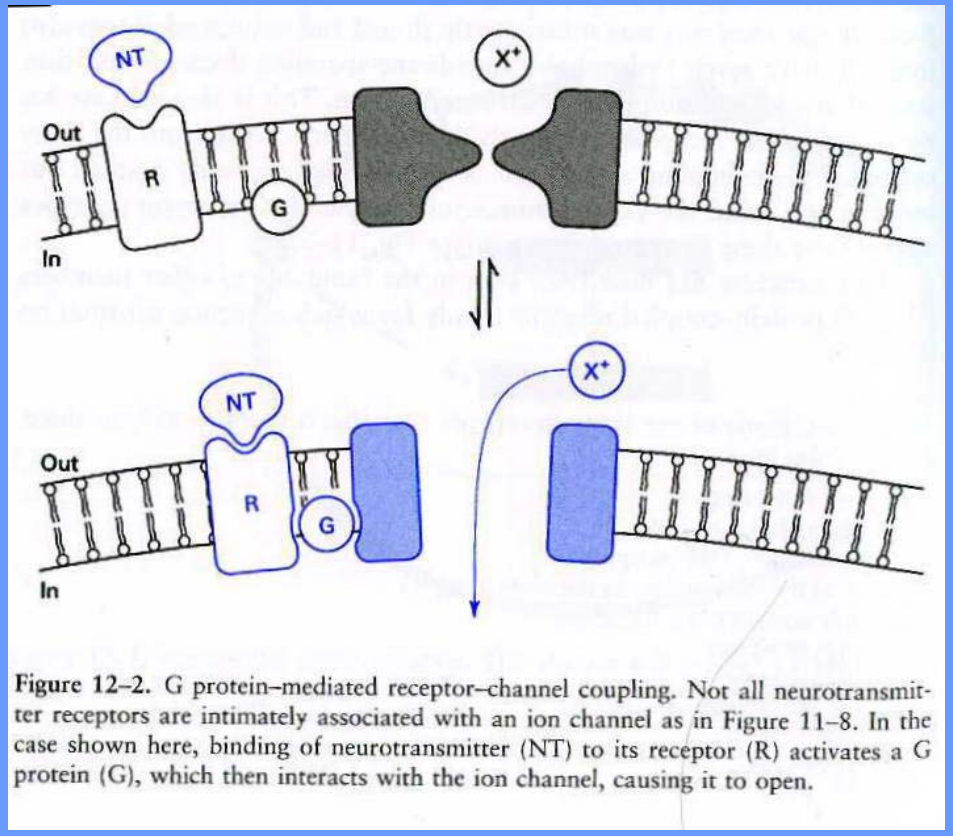


Figure 12–2. G protein–mediated receptor–channel coupling. Not all neurotransmitter receptors are intimately associated with an ion channel as in Figure 11–8. In the case shown here, binding of neurotransmitter (NT) to its receptor (R) activates a G protein (G), which then interacts with the ion channel, causing it to open.

G-protein is a guanyl nucleotide-binding protein. In its inactive state it has a GDP bound to it, whereas in its active state it has a GTP bound to it. In its active state, the protein can interact with an effector system (such as an ion channel)

Second messenger systems

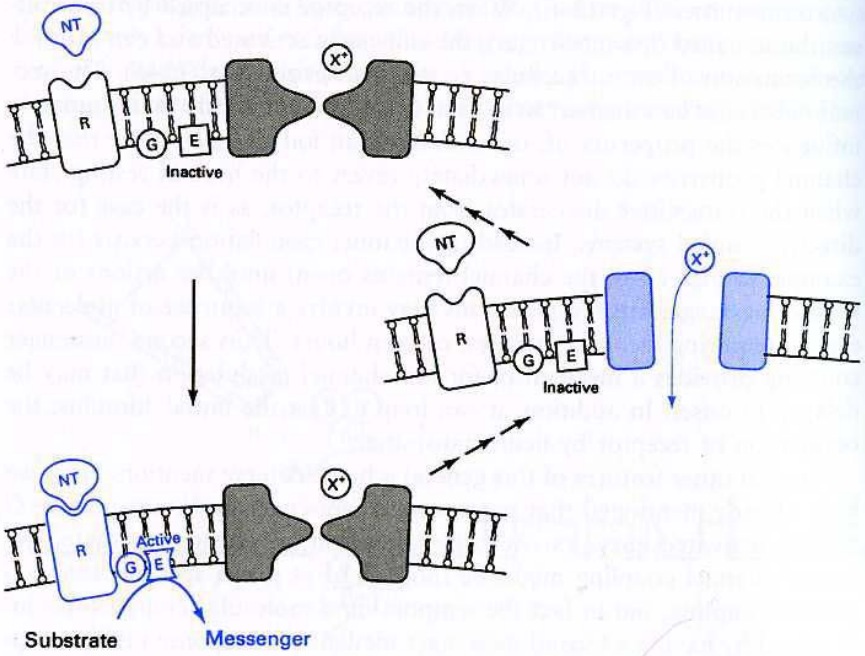
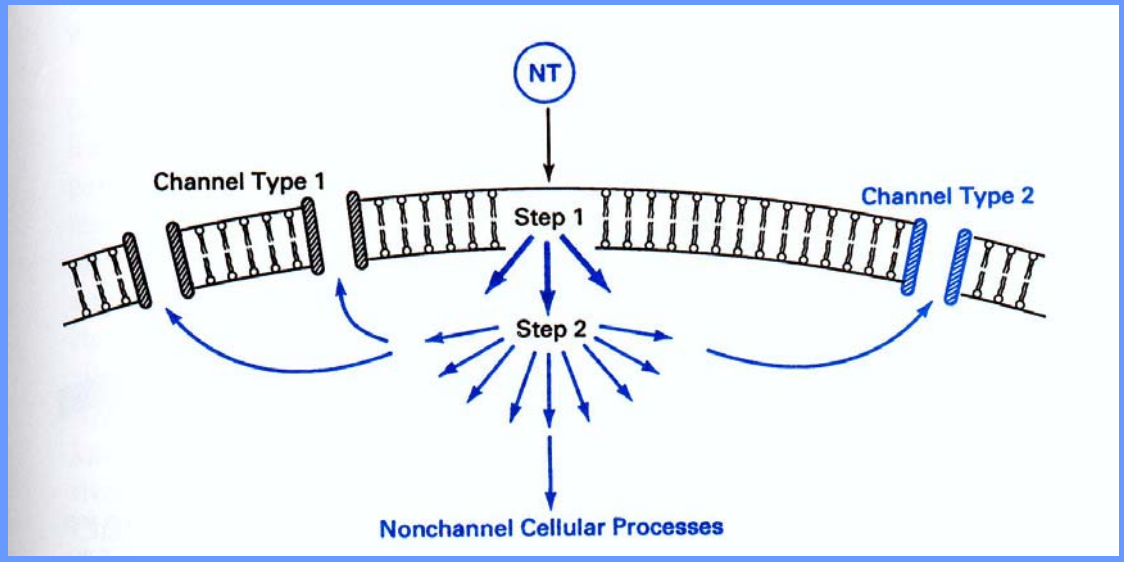


Figure 12–6. Second messenger–mediated receptor–channel coupling. In some cases neither the neurotransmitter receptor (R) nor the G protein (G) interacts directly with the ion channel. In these cases an intracellular second messenger influences ion channel activity. Contrast with Figures 11–8 and 12–2. E, enzyme regulated by G protein.

The G protein activates a second messenger which then activates any number of other mechanisms inside the cell, which can alter the intracellular calcium concentration, open/close channels, alter gene production

Amplification of second messenger message



The postsynaptic EPSP

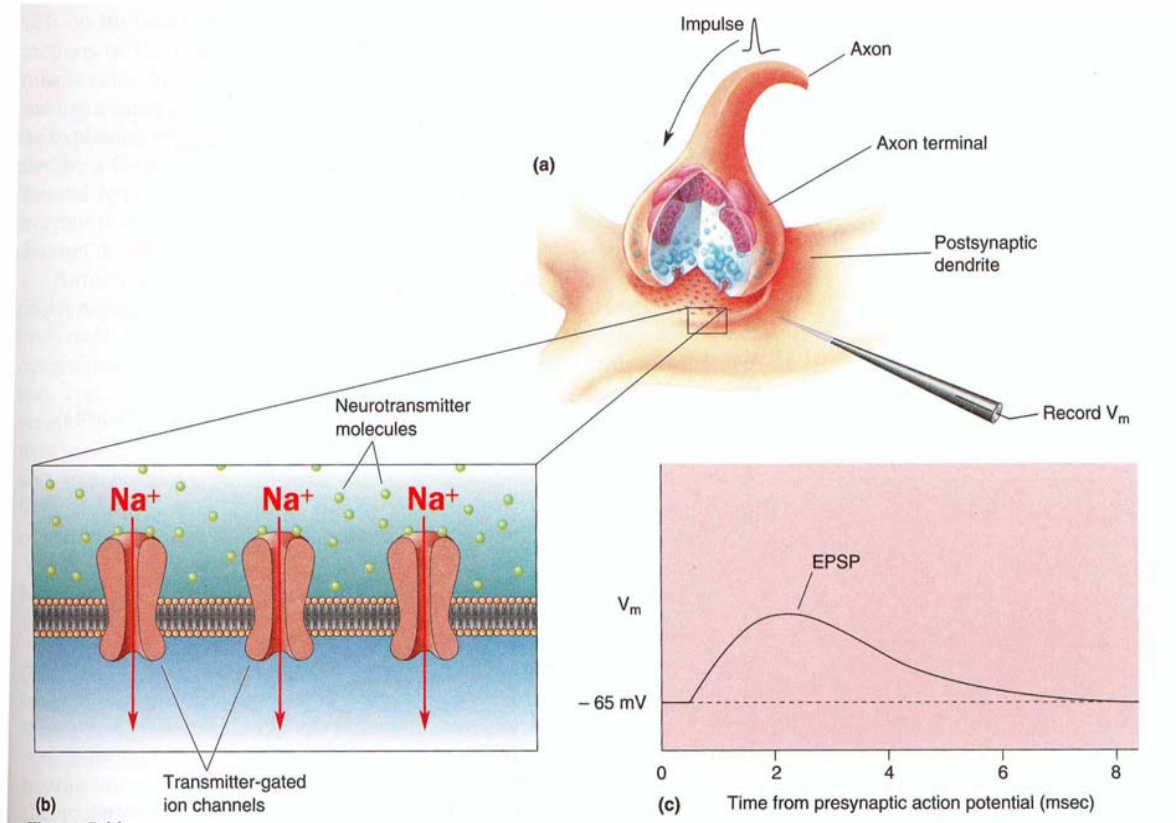


Figure 5.11
Generation of an EPSP. (a) An impulse arriving in the presynaptic terminal causes the release of neurotransmitter. **(b)** The molecules bind to transmitter-gated ion channels in the postsynaptic membrane. If Na^+ enters the postsynaptic cell through the open channels, the membrane will become depolarized. **(c)** The resulting change in membrane potential (V_m), as recorded by a microelectrode in the cell, is the EPSP.

When the channel opens, both Na+ and K+ can flow through the channel. Based on the resting membrane potential (near -70 mV) and the equilibrium potentials for each ion, we can see that there is a tremendous potential difference for Na+, yet a very small differential for K+. As a result, at rest, acetylcholine triggers a rapid influx of Na+. This influx of Na+ leads to an excitatory postsynaptic potential

The postsynaptic IPSP

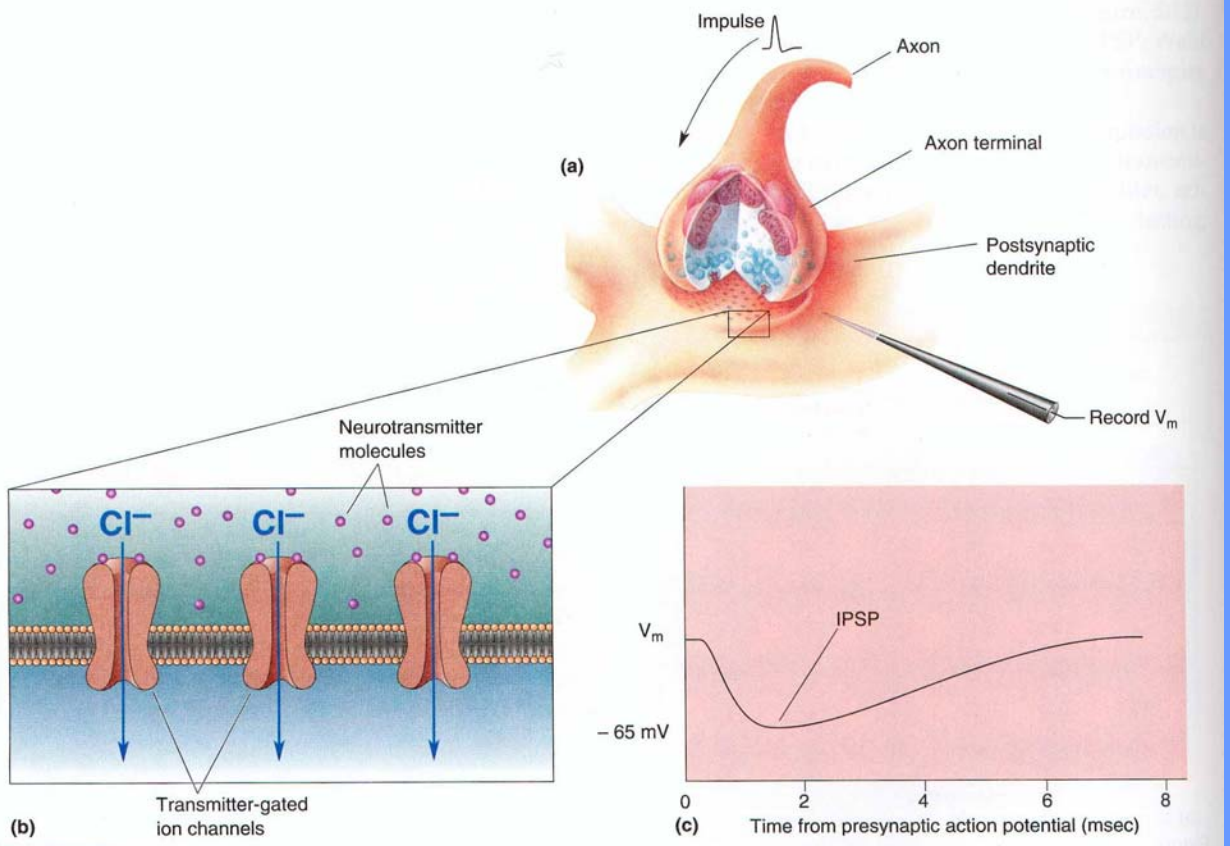


Figure 5.12
Generation of an IPSP. (a) An impulse arriving in the presynaptic terminal causes the release of neurotransmitter. **(b)** The molecules bind to transmitter-gated ion channels in the postsynaptic membrane. If Cl^- enters the postsynaptic cell through the open channels, the membrane will become hyperpolarized. **(c)** The resulting change in membrane potential (V_m), as recorded by a microelectrode in the cell, is the IPSP.

An inhibitory postsynaptic potential is often mediated by the opening of chloride channels which cause an influx of Cl- into the cell

Modifying Mood and Easing Anxiety

Mood as predominant emotional state of an individual over time

- Antidepressant drug treatments

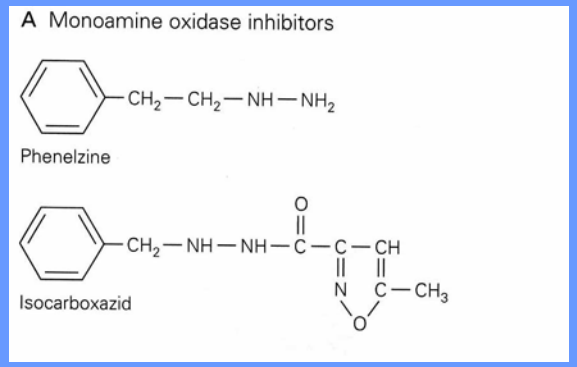
Emotion as transient response to environmental, interoceptive, or cognitive stimuli. Anxiety versus fear.

- Anxiolytic drug treatments

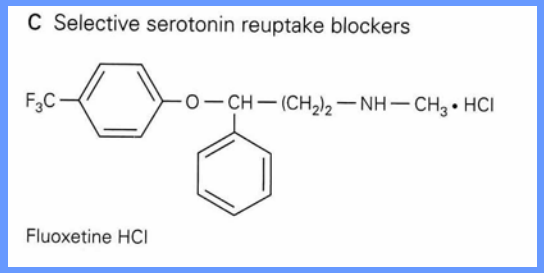
First Antideptressants - MAO Inhibitors

Monoamine depletion model of depression (1960s)

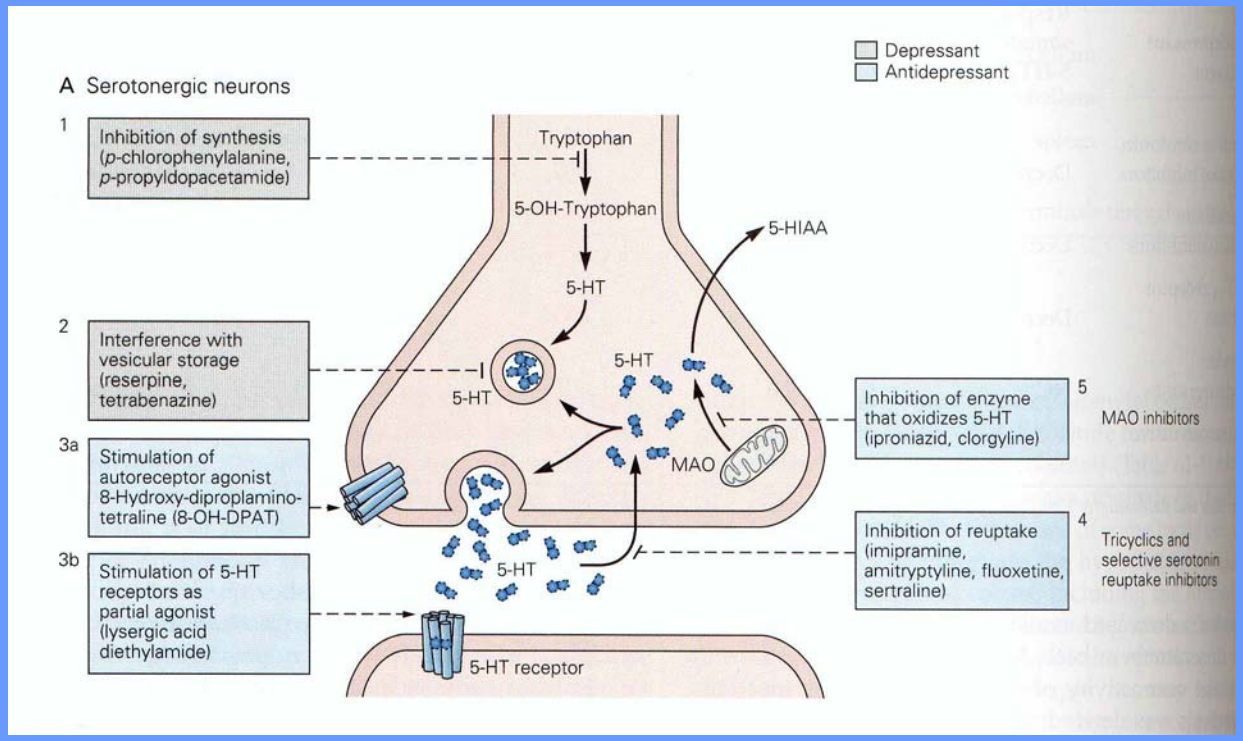
Mice given reserpine showed decreased locomotor activity, which was reversed by administration of MAO inhibitors (such as pargyline)



Monoamine Uptake inhibitors (imipramine) also proved useful for treatment of depression, suggesting a presynaptic mode of action



Action of antidepressants and other drugs at serotonergic synapses



SSRI- selective serotonin reuptake inhibitors

The Lag Time Enigma and Antidpressant Action

A substantial lag between the time an antidepressant is administered and relief of symptoms, suggested that changes occur post and not presynaptically.

Prompted the hypothesis that pathogenesis and treatment of depression involves a plasticity/adaptation in relevant neuronal pathways.

The current model suggests second messenger systems that upregulate postsynaptic signaling cascades and result in an increased production of BDNF (brain derived neurotrophic factor)

Psychotherapy and Medication can result in the same thing!!

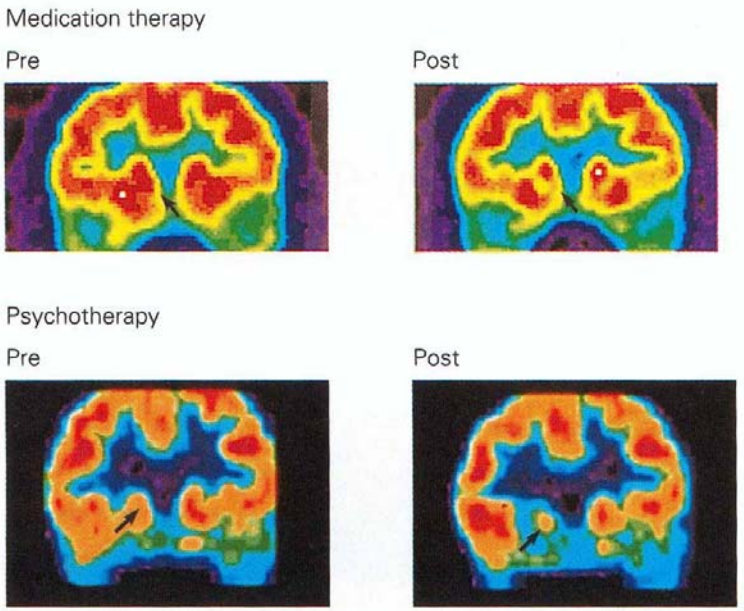


Figure 61-10 Patients with obsessive-compulsive disorder tend to show hyperactivity in the head of the caudate. This hyperactivity can be reduced in one of two ways: by selective serotonin reuptake inhibitors (**top**), medication therapy, or cognitive therapy (**bottom**), psychotherapy.

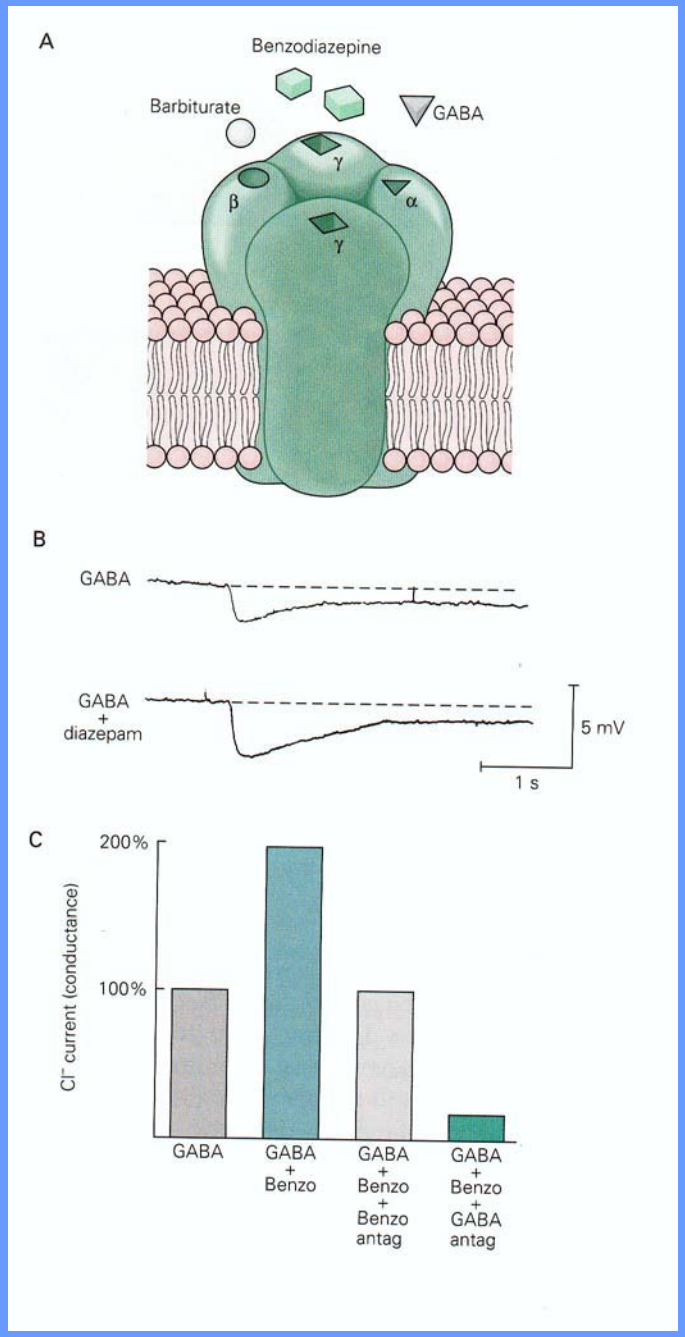
Pharmacotherapy of Anxiety

“Because I associated the attacks with driving, I reduced my driving to pure necessity. Eventually because I feared another attack and the consequent embarrassment, I avoided all but essential social contact. Ultimately I was frightened to leave my own home.”

An estimated 19 million Americans suffer from anxiety disorders (AADA - Anxiety Disorders Association of America)

Benzodiazepines: Acute anxiety, generalized anxiety disorder (GAD), panic

Antidepressants: Generalized anxiety disorder, panic, obsessive compulsive disorder



Pharmacotherapy of Anxiety

Diazepam - a benzodiazepine that is effective at treating GAD

Works via the GABAa channel (permeable to Cl-). It increases the affinity of GABA for the receptor, thus increasing the Cl- conductance and the hyperpolarizing current.

High concentrations of GABA receptors are found in the limbic system (“the emotional system”)