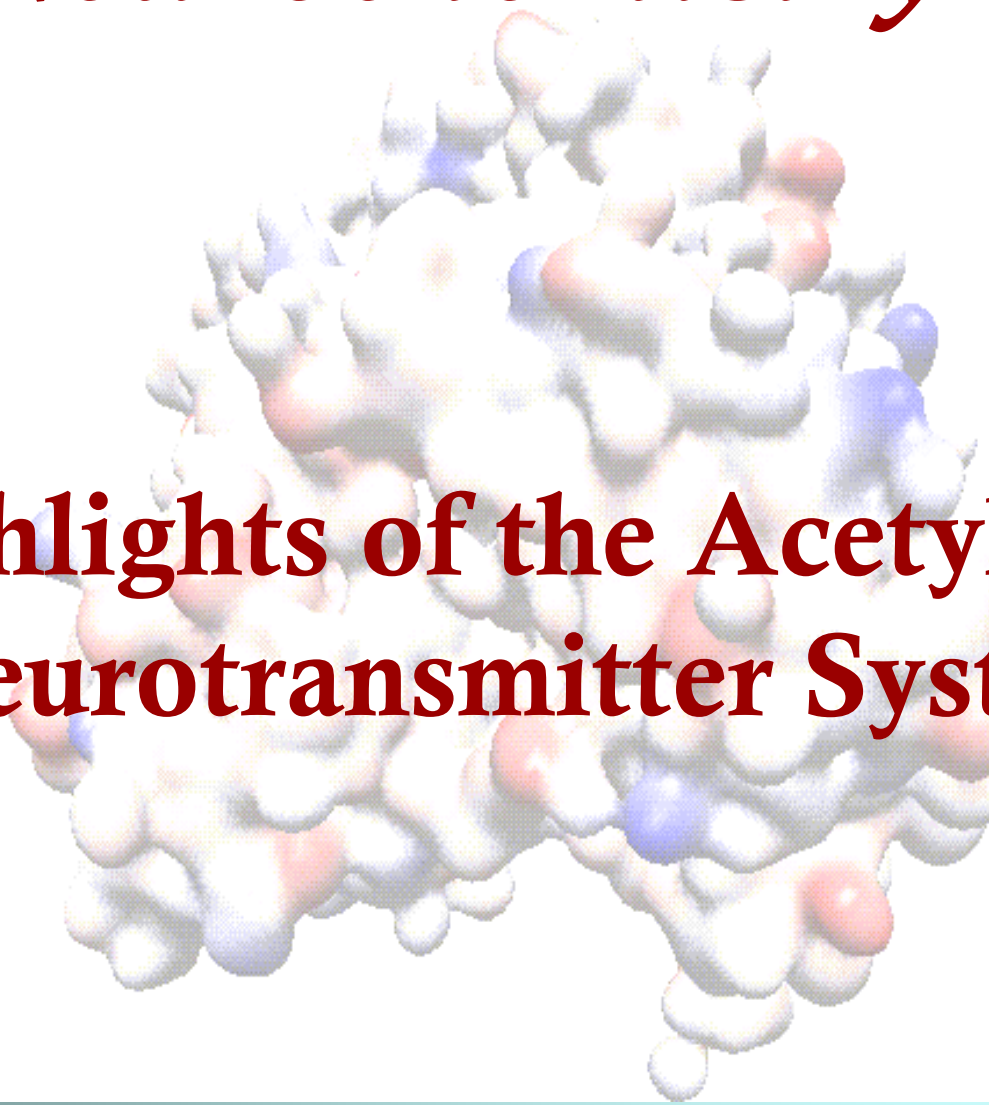
Neurochemistry 417



"Highlights of the Acetylcholine Neurotransmitter System"

The Acetylcholine System

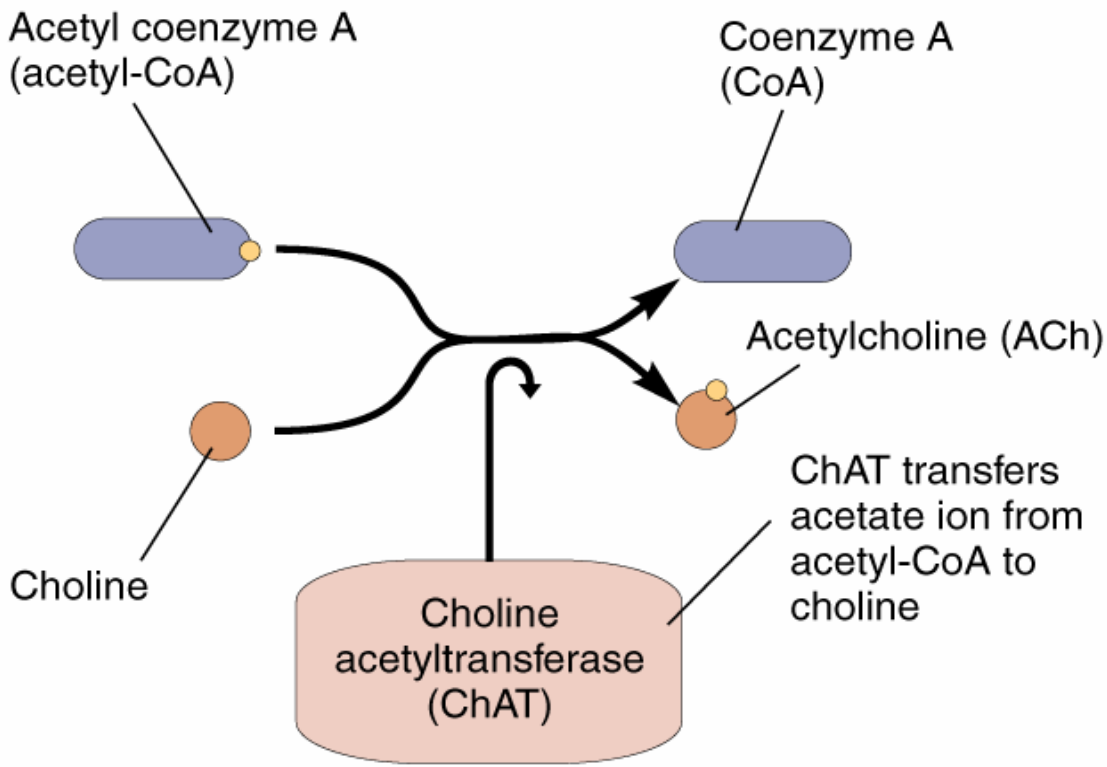
Outline

I. Acetylcholine (ACh) as a neurotransmitter
II. Synthesizing and metabolizing enzymes
    - choline Acetyltransferase (ChAT)
    - acetylcholinesterase (AChE)
III. Receptors and mechanism of ACh action
    - nicotinic receptor
    - muscarinic receptor

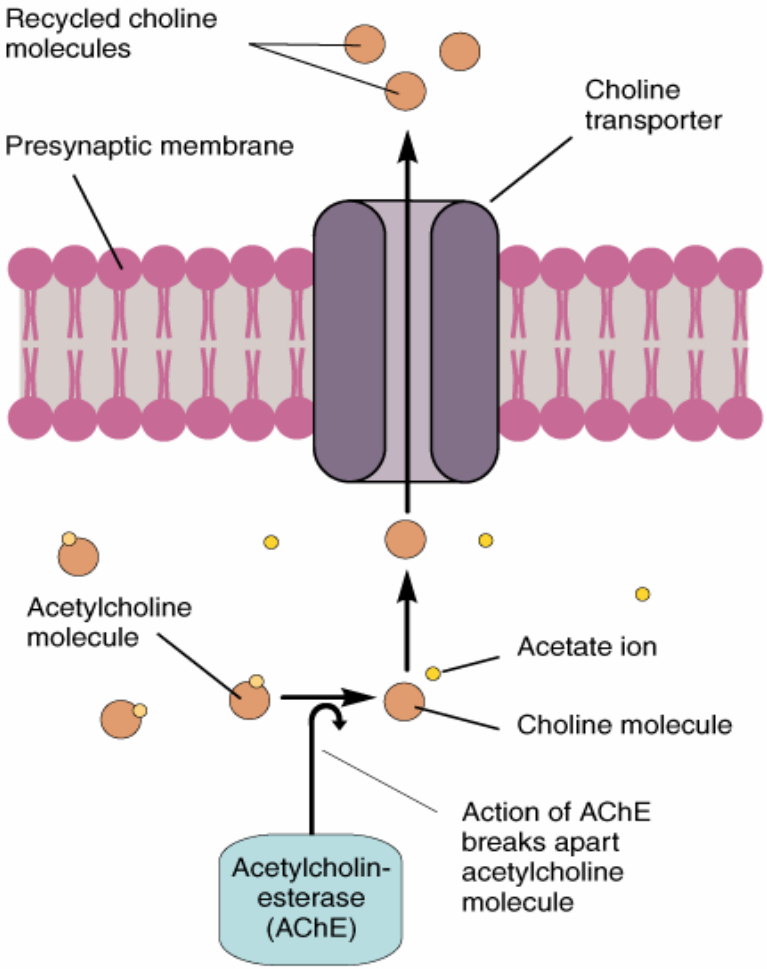
Acetylcholine:

- It is the first neurotransmitter to be discovered
- Acetyl Choline (Ach) is secreted by many nerves in the autonomic nervous system
- It is also the main neurotransmitter at the neuromuscular junction.
- It is synthesised from two common chemicals, Acetate and Choline.
- After release Ach is broken down by the enzyme Acetylcholinesterase.

► Biosynthesis of Acetylcholine

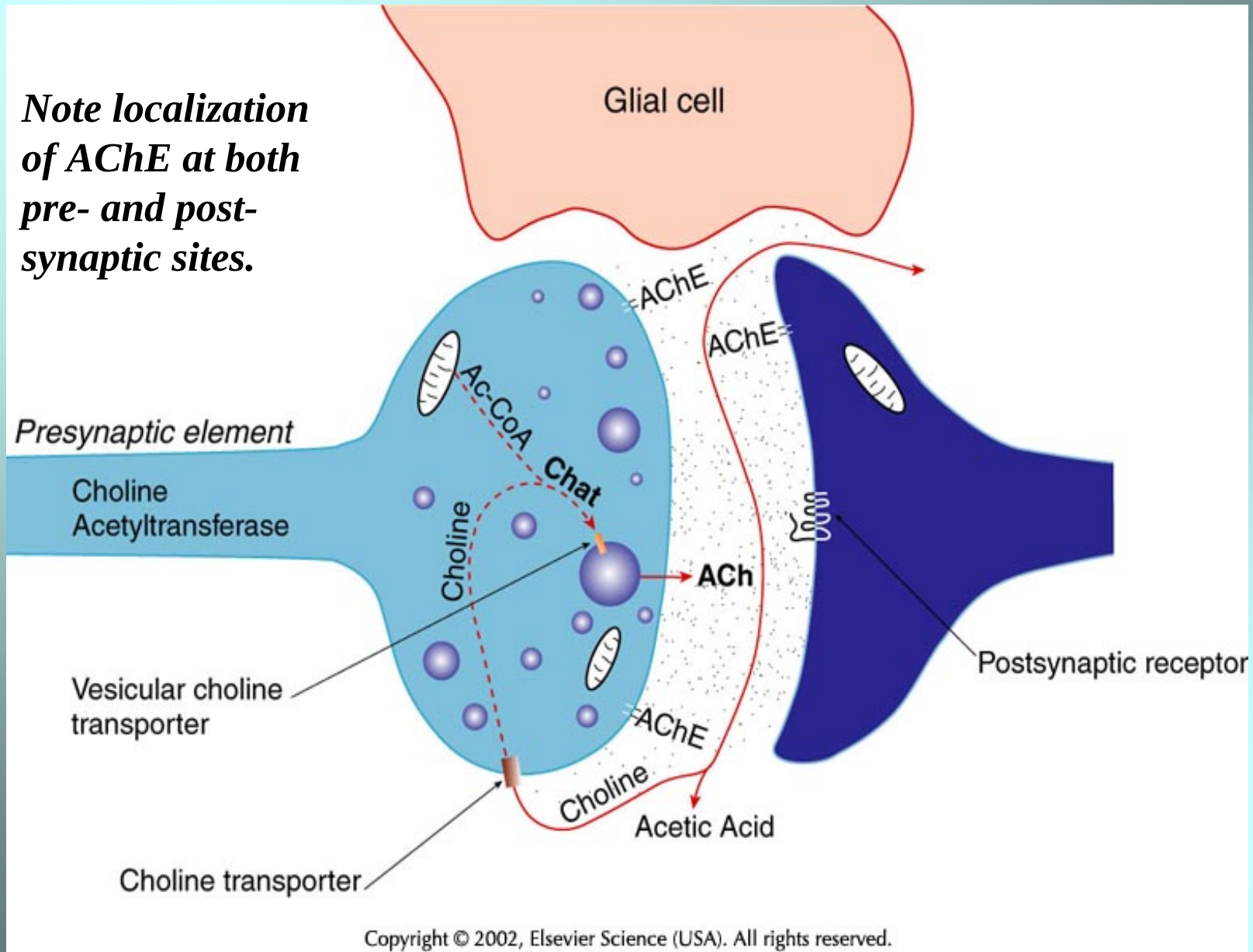


► The Destruction of Acetylcholine by Acetylcholinesterase and the Reuptake of Choline



Overview of the ACh Neurotransmitter System: Release, Action, Uptake, Synthesis and Degradation of ACh

Note localization of AChE at both pre- and post-synaptic sites.

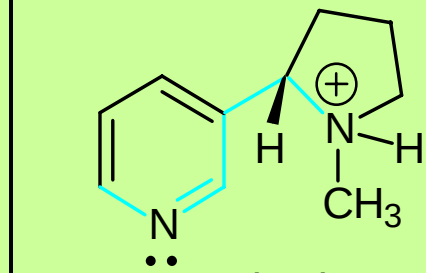


Receptors:

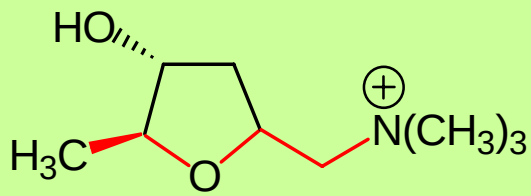
- There are two main types of cholinergic receptor.
    - Nicotinic
    - Muscarinic
- Nicotinic and muscarinic ACh receptors are named after two substances which bind to those receptor subtypes.
- Nicotine binds to nicotinic, but not muscarinic ACh receptors.
- Muscarinic receptors are named after Muscarine, found in some species of mushroom.

Structure-Function Correlations for ACh

How can ACh bind two different receptor types?

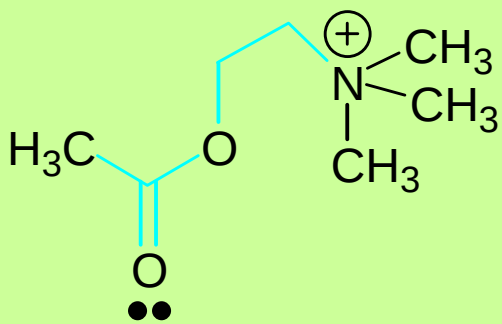


nicotine

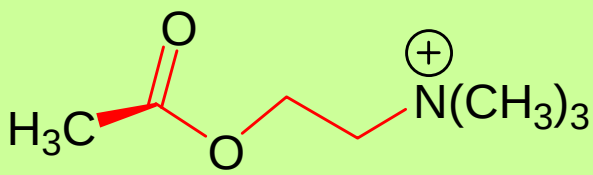


muscarine

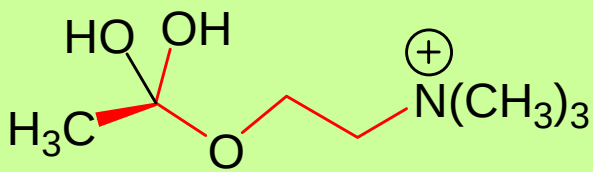
Certain flexible structures can adopt conformations or be "isosteres" (functional groups that mimic other functional groups). The acetylcholine molecule has been drawn in two forms (colors) to emphasize the mimicry.



acetylcholine



acetylcholine



acetylcholine hydrate (TS analog)

Nicotinic Receptors

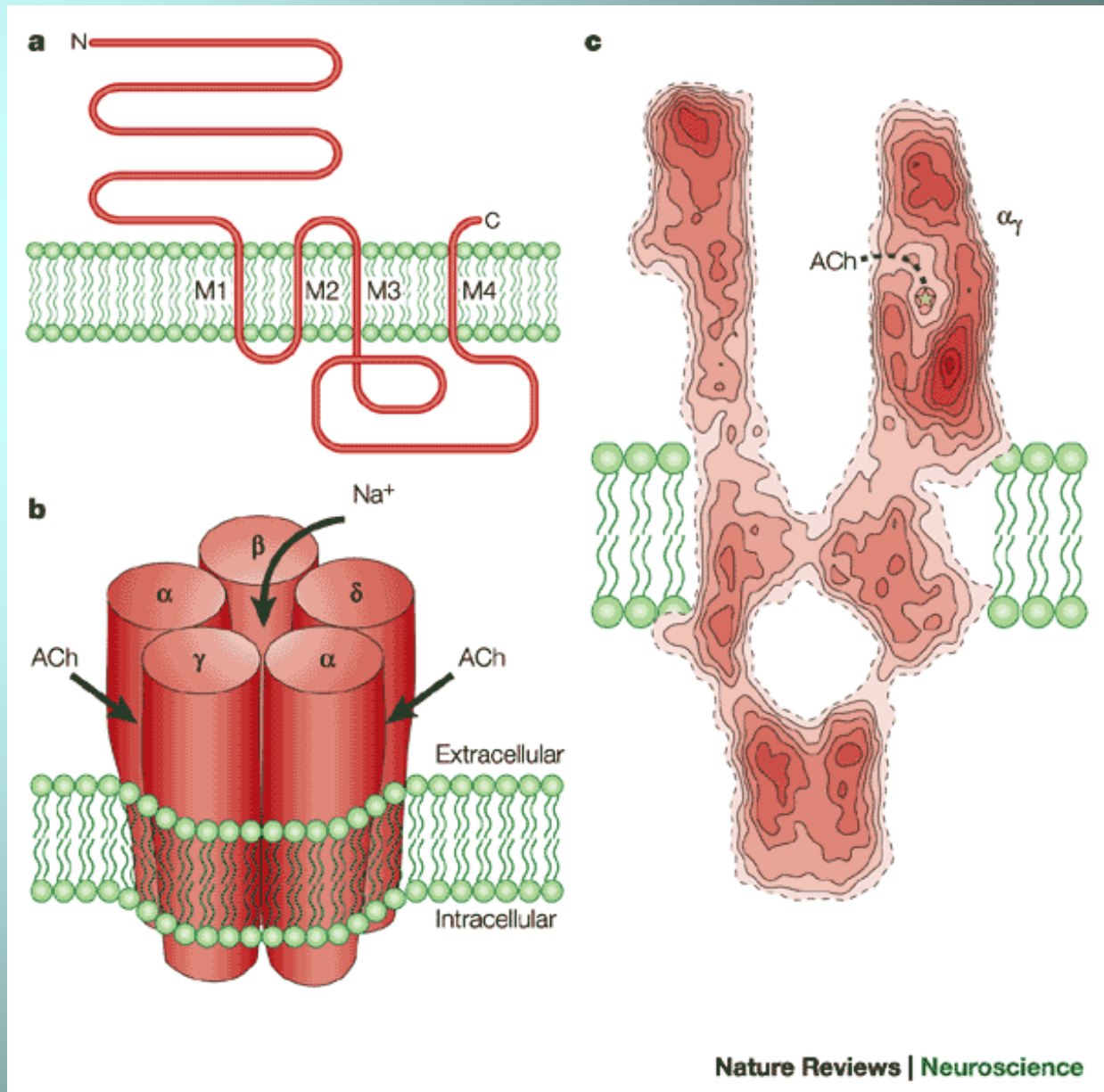
(ionotropic – allows passage of ions):

- quaternary structure indicates five subunits (two alpha, beta, delta, and gamma) that preferentially bind nicotine and are found in all autonomic ganglia and at the neuromuscular endplate of striated muscle.
- at least eight different sequences of *alpha* subunits and three different sequences of *beta* subunits of the nicotinic receptor have been identified.
- the nicotinic receptors were isolated with the help of the electric eel/ray, which have a copious concentration of these receptors far more than the human brain.

species:	ACh-R conc.:
ray	1000 nmol/kg tissue
eel	50-100 nmol/kg tissue
human brain	0.1-1.0 nmol/kg tissue

Nicotinic ACh Receptors (AChR)

(**a)** threading pattern of receptor subunits through the membrane. (**b)** A schematic representation of the quaternary structure showing the arrangement and location of the two ACh-binding sites (between an α- and a γ-subunit, and an α- and δ-subunit), (**c)** cross-section through the 4.6-Å structure of the receptor determined by electron microscopy (*Torpedo* membrane in ice). Dashed line indicates proposed path to binding site.

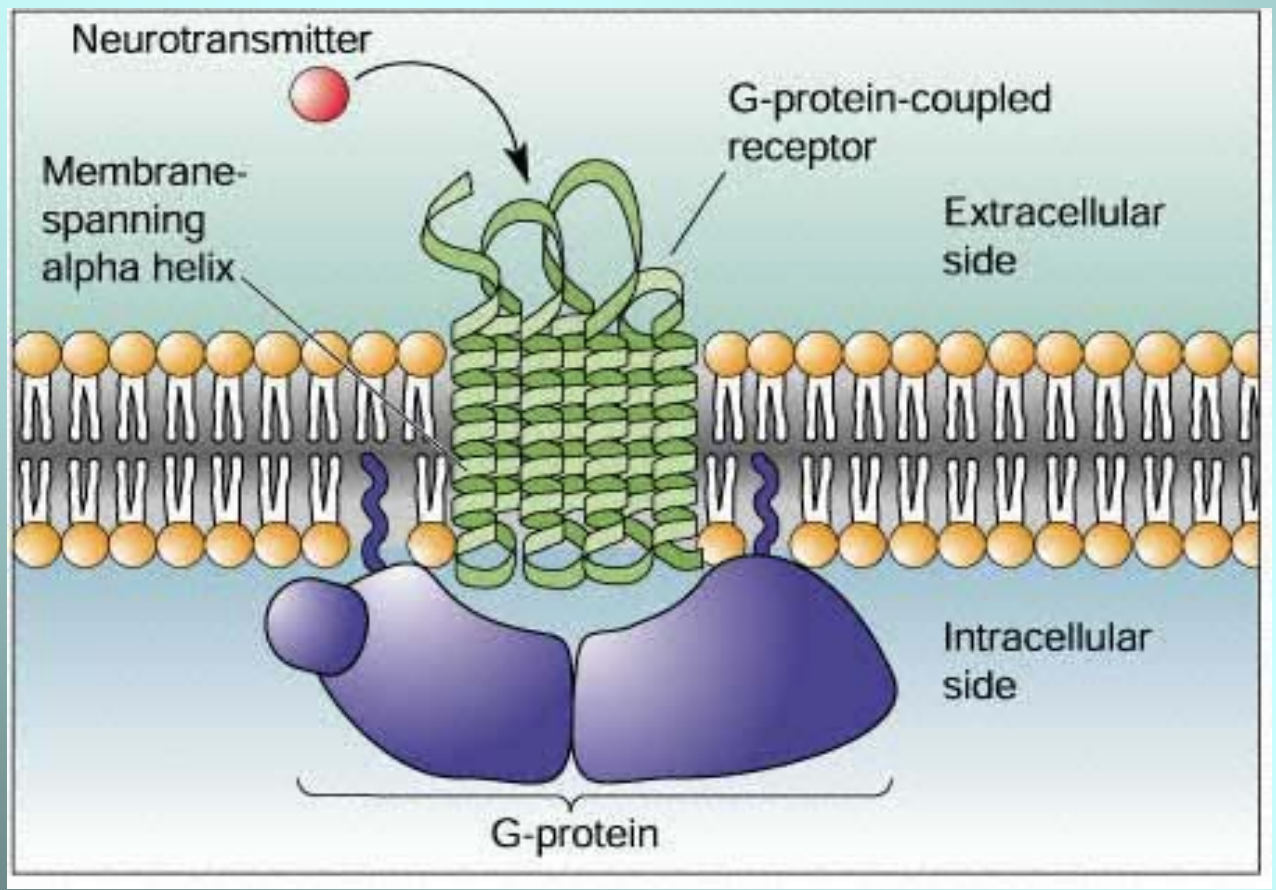


Nicotinic receptors:

- When ACh interacts with a nicotinic ACh receptor, it opens a Na+ channel and Na+ ions flow into the membrane.
- This causes a depolarization, and results in an EPSP.
- Thus, ACh is excitatory on skeletal muscle.
- The electrical response is **fast, and short-lived**

Muscarinic Receptors

- Receptor is linked to a G-protein
  - The G-protein activates channels or enzymes indirectly
  - Responses are **diverse, slower, and longer-lived**



Muscarinic Acetylcholine Receptors					
	M_1	**M_2**	**M_3**	**M_4**	**M_5**
Distribution	Cortex, hippocampus	Heart	Exocrine glands, GI tract	Neostriatum	Substantia nigra
Antagonists	Pirenzepine	AF-DX 116	pF-HHSiD		
Agonists	Xanomeline, CDD-0097				
G protein	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$
Intracellular response	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ

Muscarinic Receptors

(G-protein coupled receptor GPCR; old term- metabotropic): bind muscarine and occur at post-ganglionic parasympathetic terminals -> control peristalsis, glandular secretion, pupil constriction, vasodilation and heart rate reduction.

At least five distinct muscarinic receptor genes have been cloned and sequenced. The subtypes differ in their ability to couple to different G proteins and, hence, to elicit [different] cellular signaling events.

When a GPCR is activated it couples to a G-protein initiating the exchange of GDP for GTP, activating the G-protein. “Activated” G-proteins then couple to many downstream effectors and alter the activity of enzymes (phosphorylation).

Muscarinic Receptors (cont.)

- Far more stereospecific and structure specific than the nicotinic receptor.

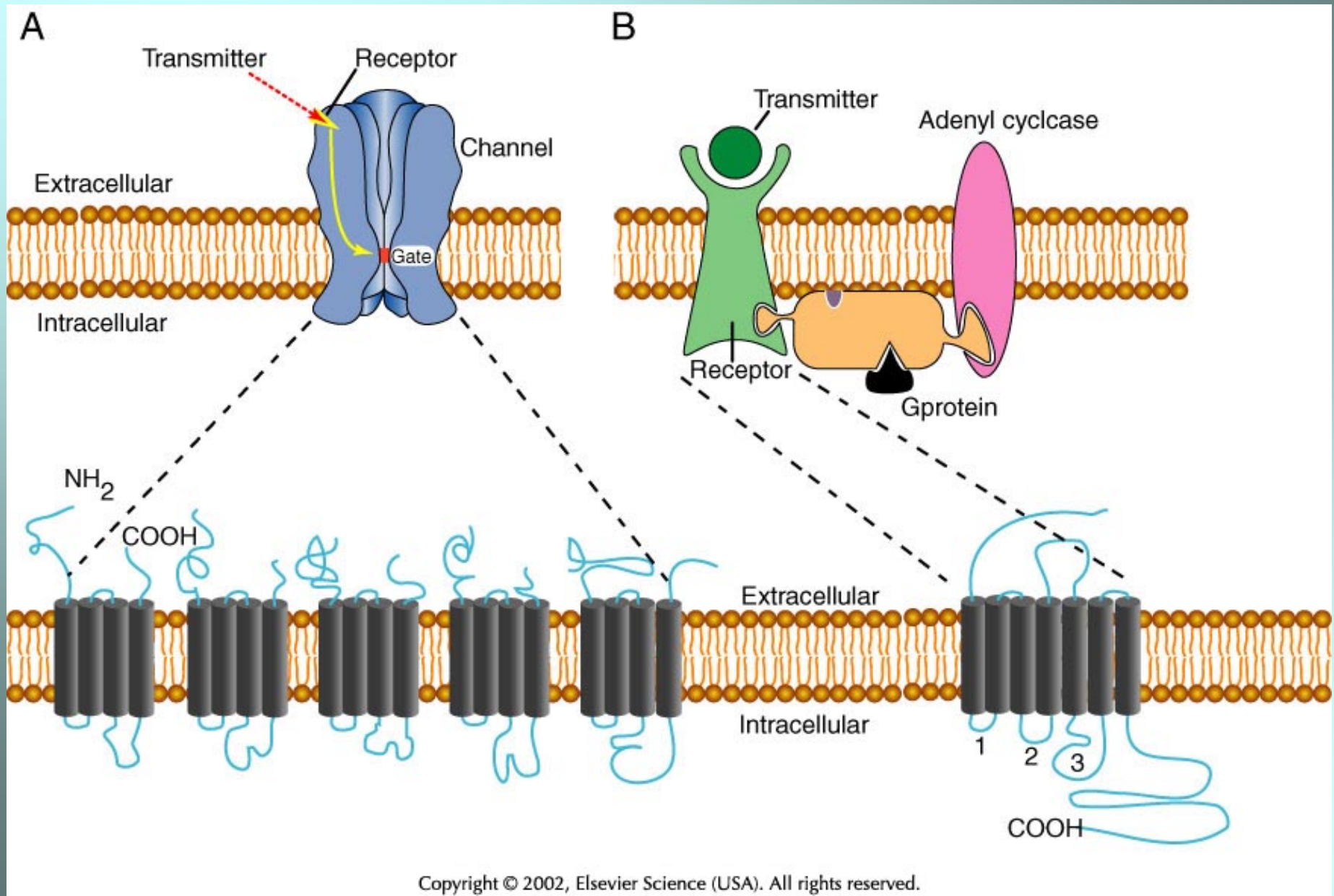
- Typically made up of seven transmembrane domain regions and the neurotransmitter typically binds 10-15 angstroms deep into the receptor.

- Found on smooth muscle, like heart

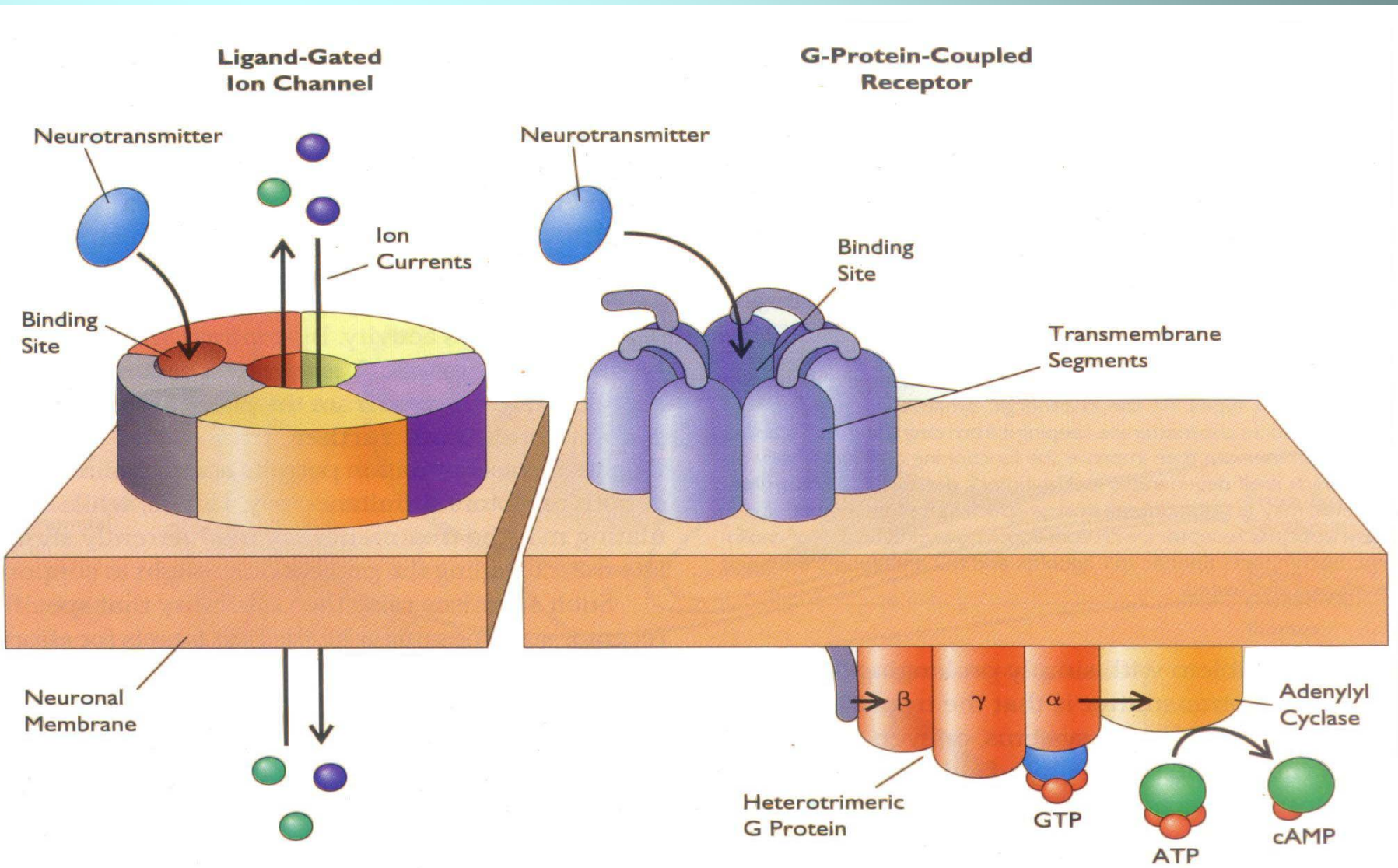
- When ACh binds to a muscarinic receptor, it causes K+ ions to leave the muscle, hyperpolarizing the membrane and causing a IPSP

- Thus , ACh causes heart muscle to slow down

Review of ionotropic and GPC receptor differences

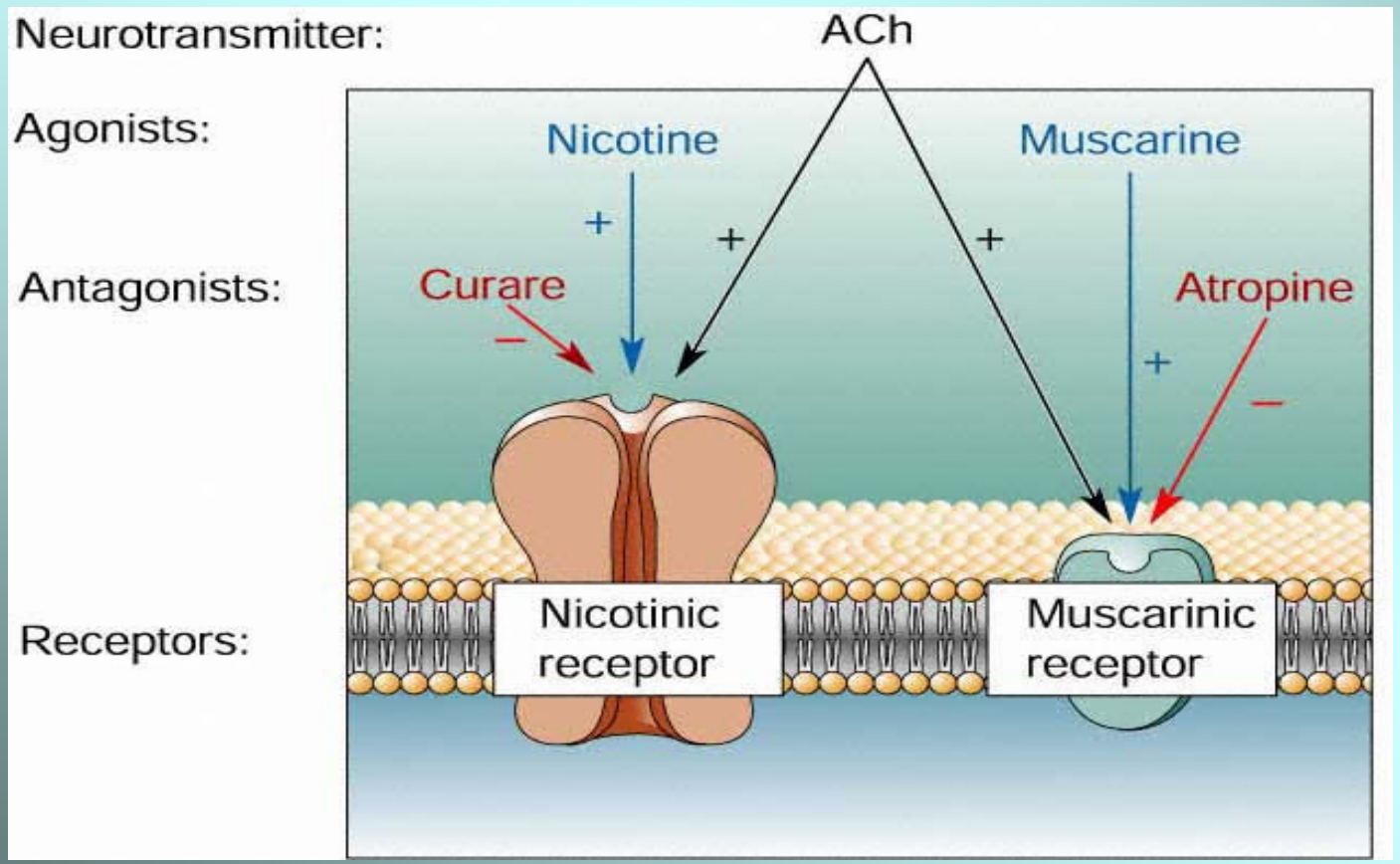


Review of Basic Receptors Types: Ion channel and G-protein coupled.



Acetylcholine Receptors

- *Nicotinic* receptors are **ionotropic**
  - Found in NMJ and in the brain (blocked by curare)
- *Muscarinic* receptors are **metabotropic** (and there are several sub-types)
  - Found in smooth muscles (heart, gut) and the brain. Atropine blocks muscarinic receptors (belladonna)



Summary – ACh Neurotransmitter System

- Acetylcholine is synthesized in the neuron by acetylation of choline with acetyl CoA.
- The choline is brought into the cell by a transporter.
- Synthesized ACh is stored in vesicles.
- Vesicles release ACh during neurotransmission – ACh binds with nicotinic and muscarinic receptors.
- Nicotinic receptors are ionotropic and muscarinic receptors are G-protein coupled receptors (GPCRs).
- The titer of ACh is controlled by acetylcholinesterase, which hydrolyzes ACh into choline and acetic acid (recycle).
- Various toxins and poisons have paved the way to understanding this neurotransmitter system.

Select Resources for the Acetylcholine System

Squire, Bloom, McConnell, Roberts, Spitzer, and Zigmond (2003) "Fundamental Neuroscience," 2nd Edition; Academic Press.

http://www.neuroguide.com/cajal_gallery.html (some nice animations of neurotransmitters; need high speed line)

http://synapses.bu.edu (neuroanatomy to neurochemistry – great pics and great info)

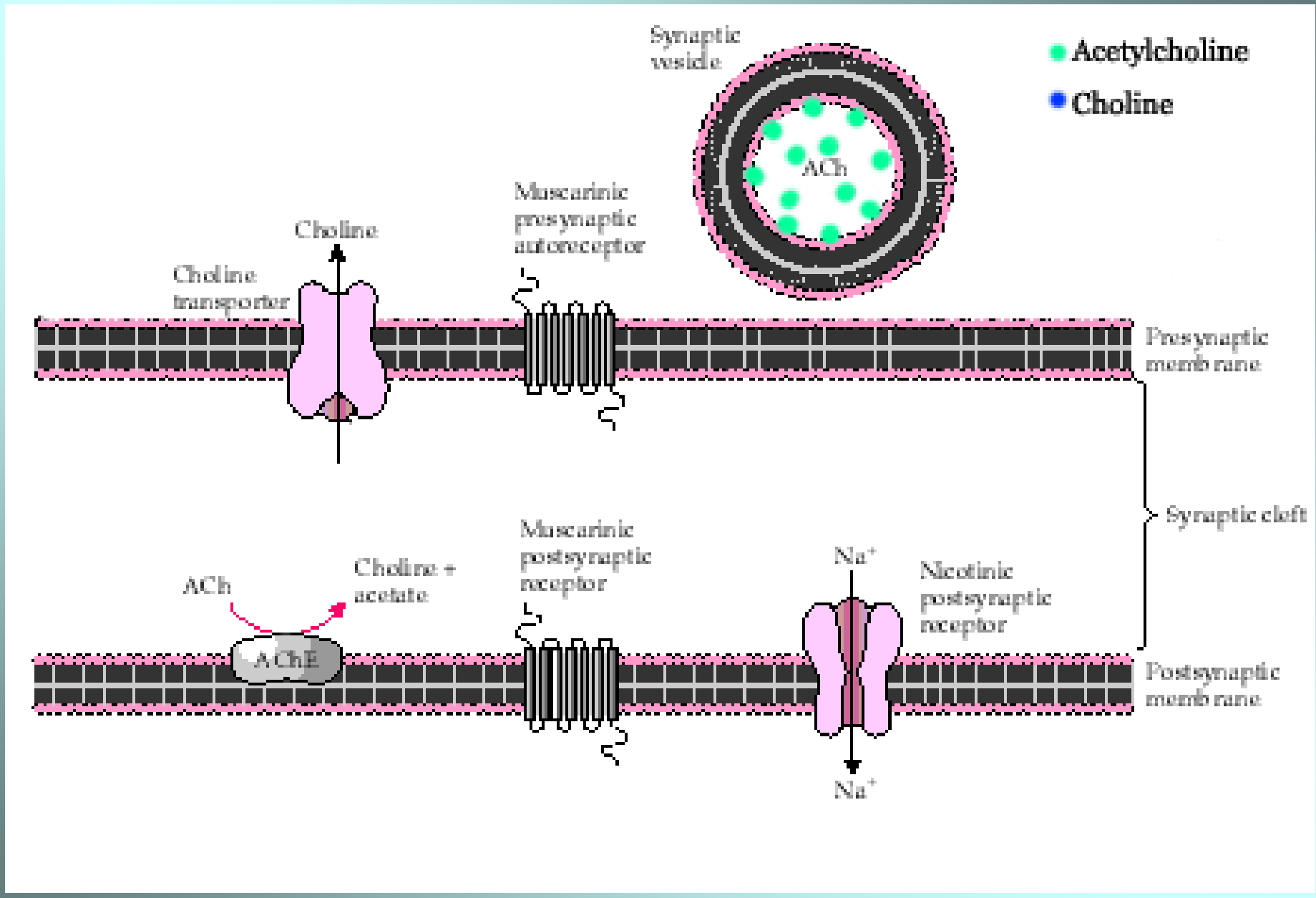
http://sunny.mpimf-heidelberg.mpg.de/people/vkitzing/AChR.html (modeling the Ach receptor; good overall coverage – basic graphics)

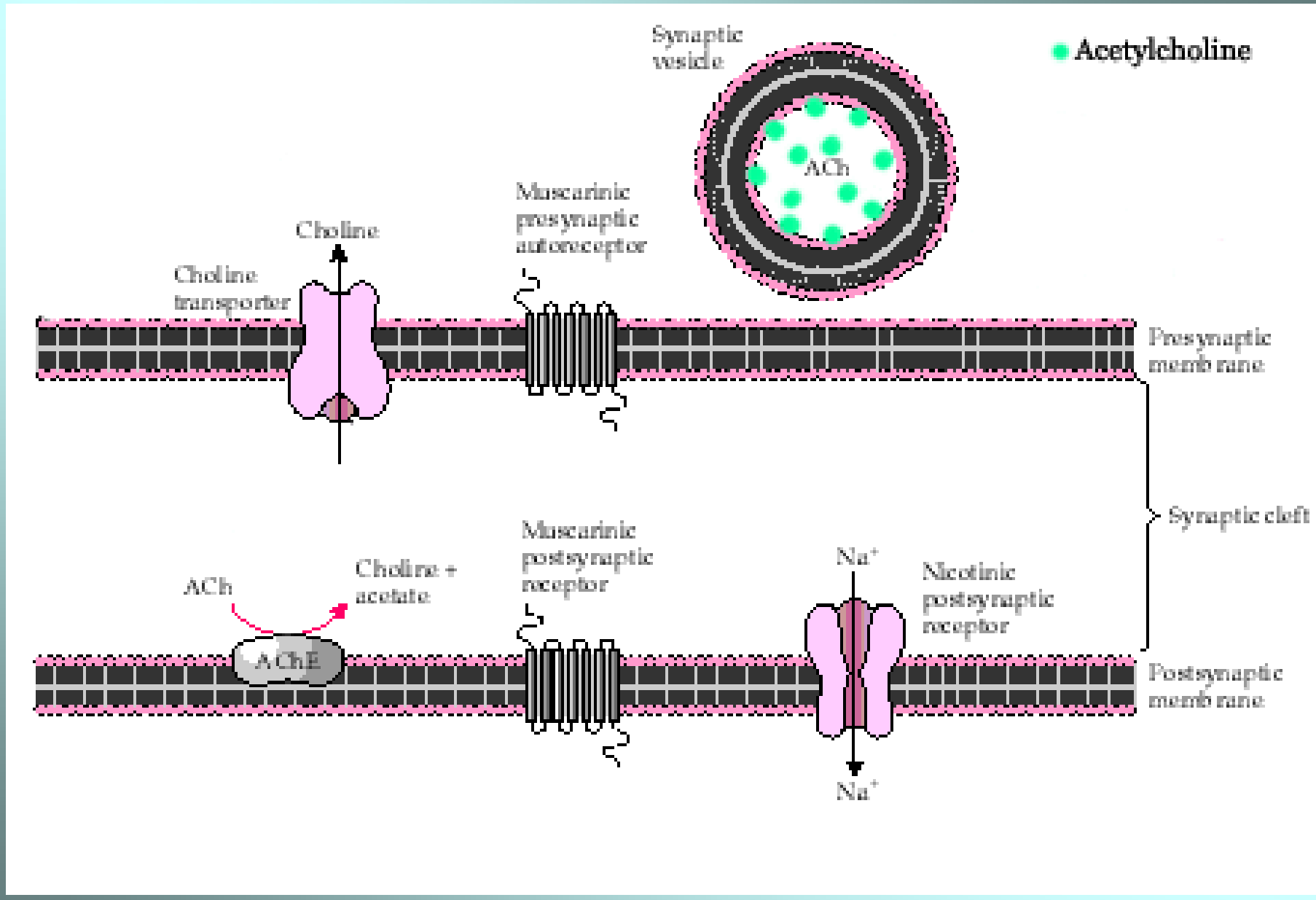
http://www.neuro.wustl.edu/neuromuscular/mother/acetylcholine.htm (everything you wanted to know about the muscarinic and nicotinic receptors in tabular forms with links; easy to navigate and thorough)

http://www.dartmouth.edu/~rpsmith/Cholinergic_Transmission.html (covers cholinergic transmission in detail)

http://www.rcsb.org/pdb/ (protein database – where you can download 3-D structural information on proteins)

http://molsim.vei.co.uk/weblab/ (WebLab Viewer – a freeware viewer for molecules in multiple formats; use in combination with the protein database *.pdb to visualize proteins)


Synaptic vesicle
Acetylcholine
Choline
ACh
Muscarinic presynaptic autoreceptor
Choline
Choline transporter
Presynaptic membrane
Synaptic cleft
Muscarinic postsynaptic receptor
Na+
Nicotinic postsynaptic receptor
ACh
Choline + acetate
AChE
Postsynaptic membrane
Na+


Synaptic vesicle
Acetylcholine
ACh
Muscarinic presynaptic autoreceptor
Choline
Choline transporter
Presynaptic membrane
Synaptic cleft
Muscarinic postsynaptic receptor
Na+
Nicotinic postsynaptic receptor
ACh
Choline + acetate
AChE
Postsynaptic membrane
Na+