Fundamentals of Neuroscience; 4th Edition,

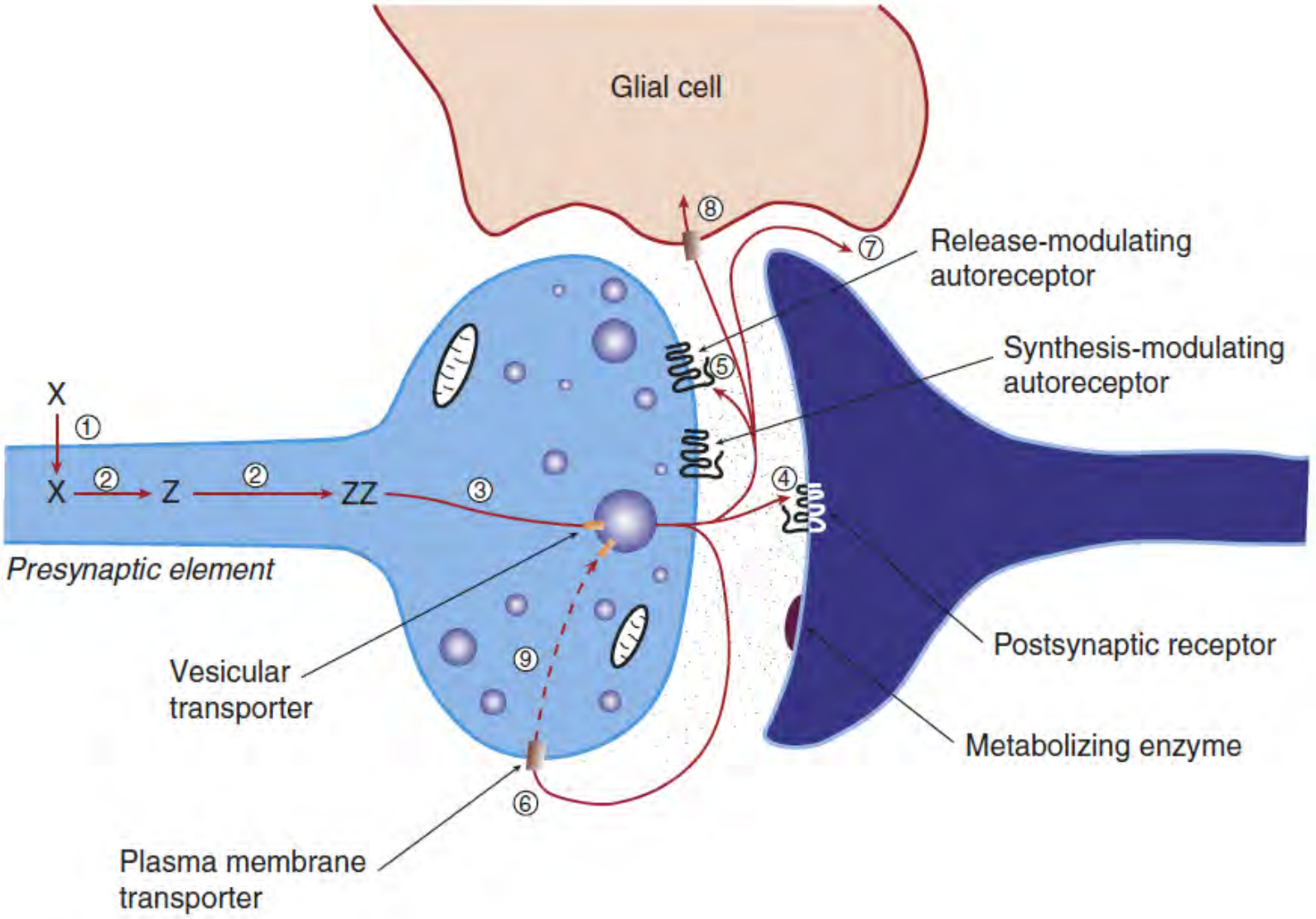
9/6/2019	**8-9 am**	**Fundamentals of Neuroscience (Chapters 3&4) - Cellular & Subcellular Components of Nervous Tissue**	**751 Neuro Conf rm 3717**
9/13/2019	8-9 am	Fundamentals of Neuroscience (Chapters 5&6) – Membrane Potential, AP, Neurotransmitters	751 Neuro Conf rm 3717
9/20/2019	**8-9 am**	**Fundamentals of Neuroscience (Chapters 7&8) - Neurotransmitter Release & Neurotransmitter Receptors**	**751 Neuro Conf rm 3717**
9/27/2019	8-9 am	Fundamentals of Neuroscience (Chapters 7&8) – Intracellular Signaling, Postsynaptic Potentials & Synaptic Integration	751 Neuro Conf rm 3717

Spinal cord & injuries
Neuroendocrinology
Neuropsychiatric disorders
Neuroimmune disorders

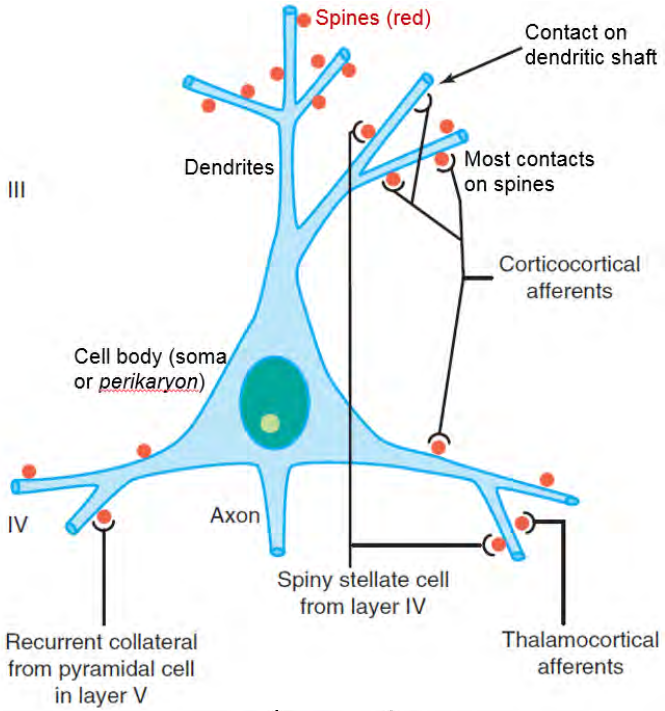
Release of Neurotransmitters

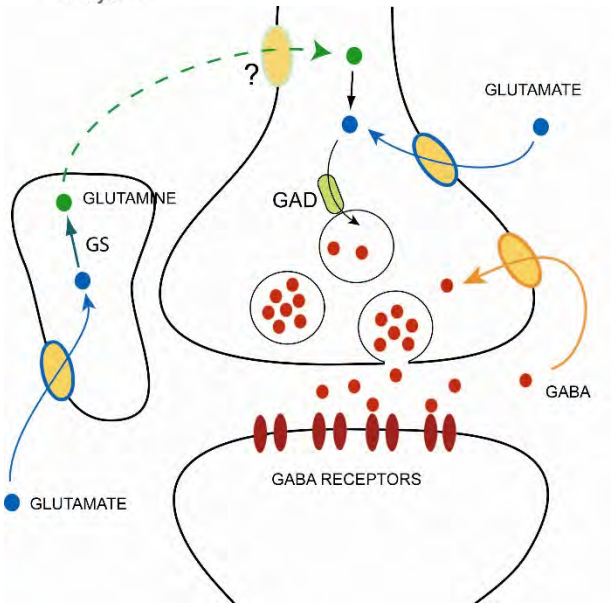
- Ernesto Solis, Jr.
- September 20, 2019

Neurons communicate at synapses with chemical neurotransmission

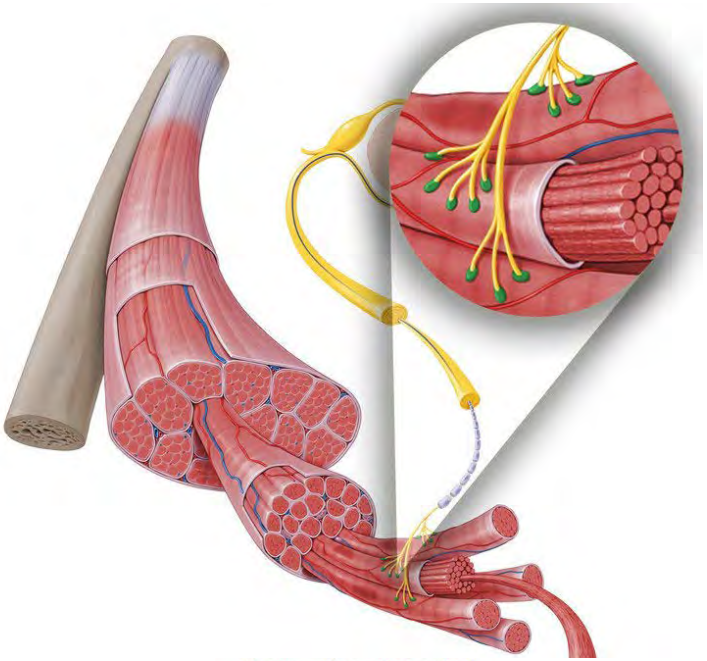


Brain Synapse

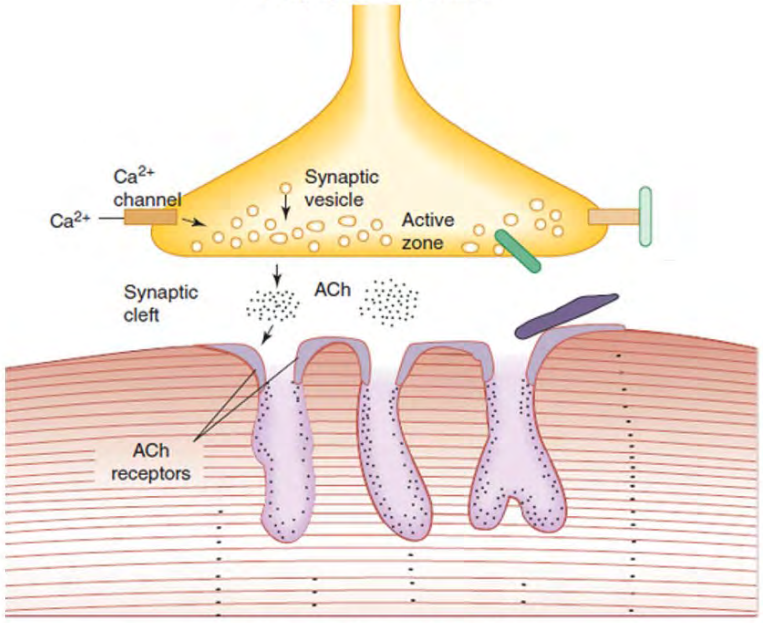




Neuromuscular Junction Synapse



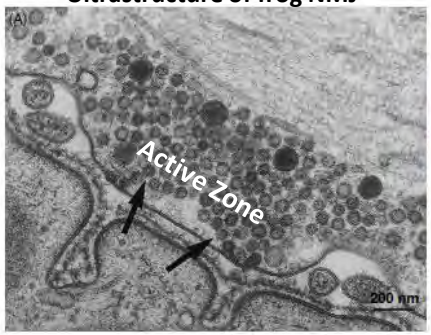
Presynaptic nerve terminal



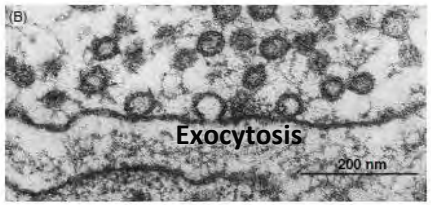
Postsynaptic muscle membrane

Ultrastructure of frog NMJ

Before stim'n (few SVs docked)

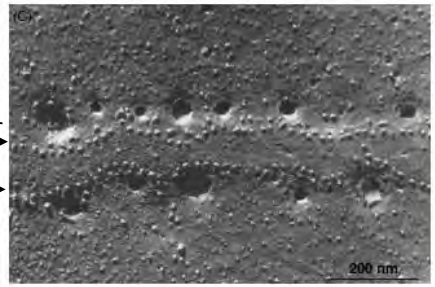


5 s after stim'n



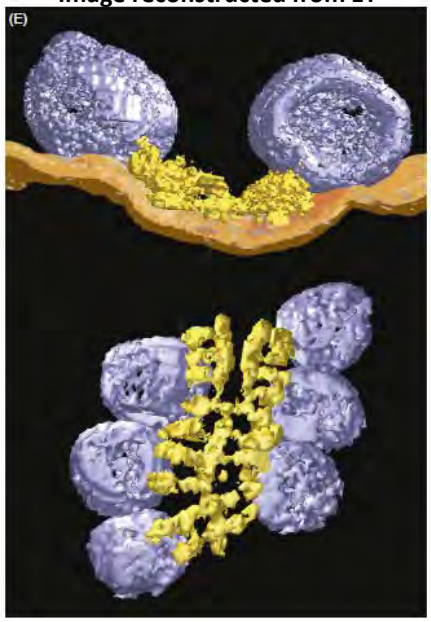
Presyn. face

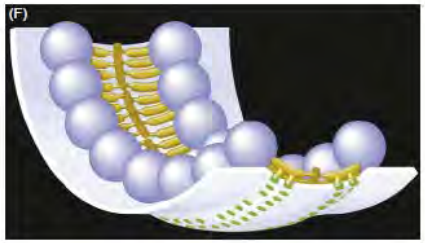
SVs near channel rows (50 nm)



Electron tomography

Image reconstructed from ET





- The active zone (AZ) of the NMJ is a highly structured specialization of the membrane and cytoskeleton.
- To secrete thousands of neurotransmitter (NT) molecules rapidly, terminals package NT in enclosed organelles (i.e. synaptic vesicles, SV).
- SV = 50 nm in diameter
- SV differ in appearance depending on NT (GLU and Ach stored in small, clear SVs, peptide NTs stored in large dense-core vesicles).
- At NMJ, AP triggers release of ~300 SVs, and at central synapse, AP triggers release of 5-10 SVs.

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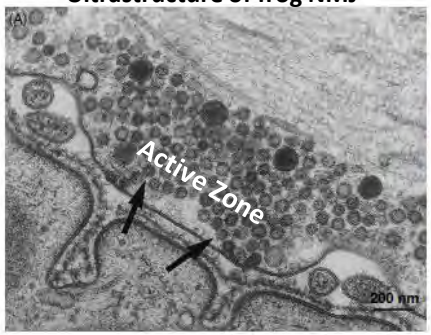

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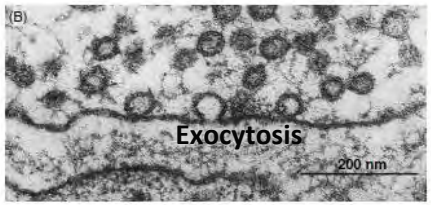

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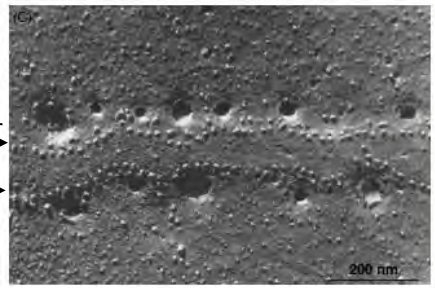

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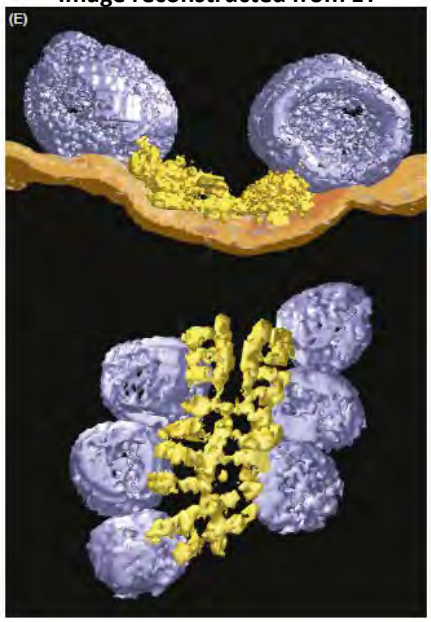

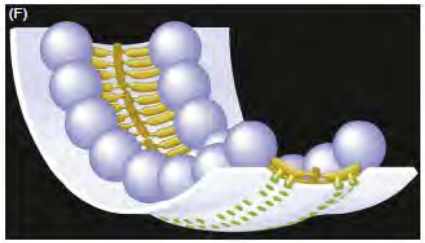

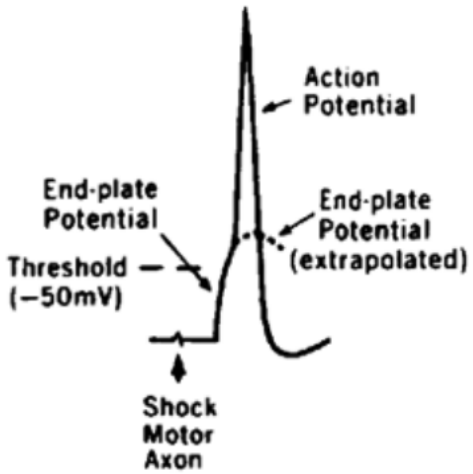

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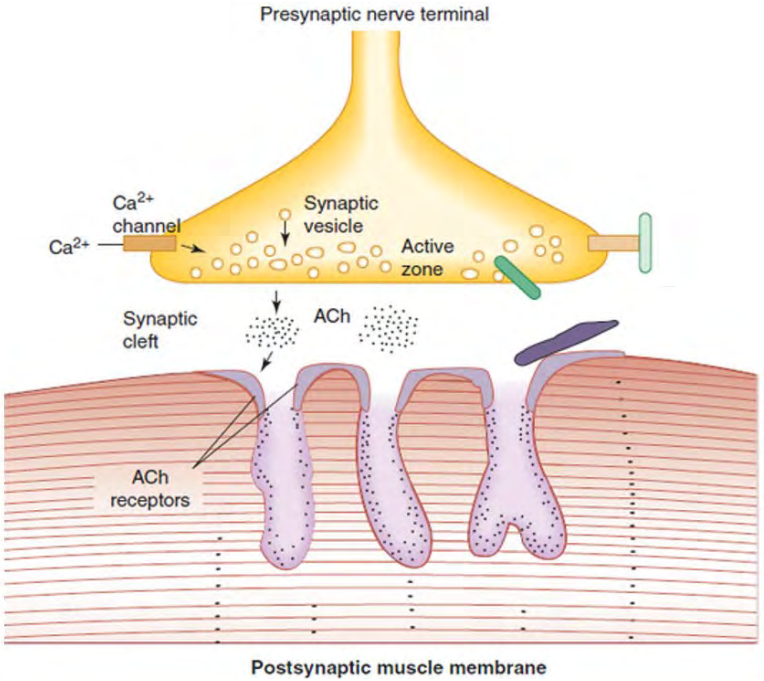
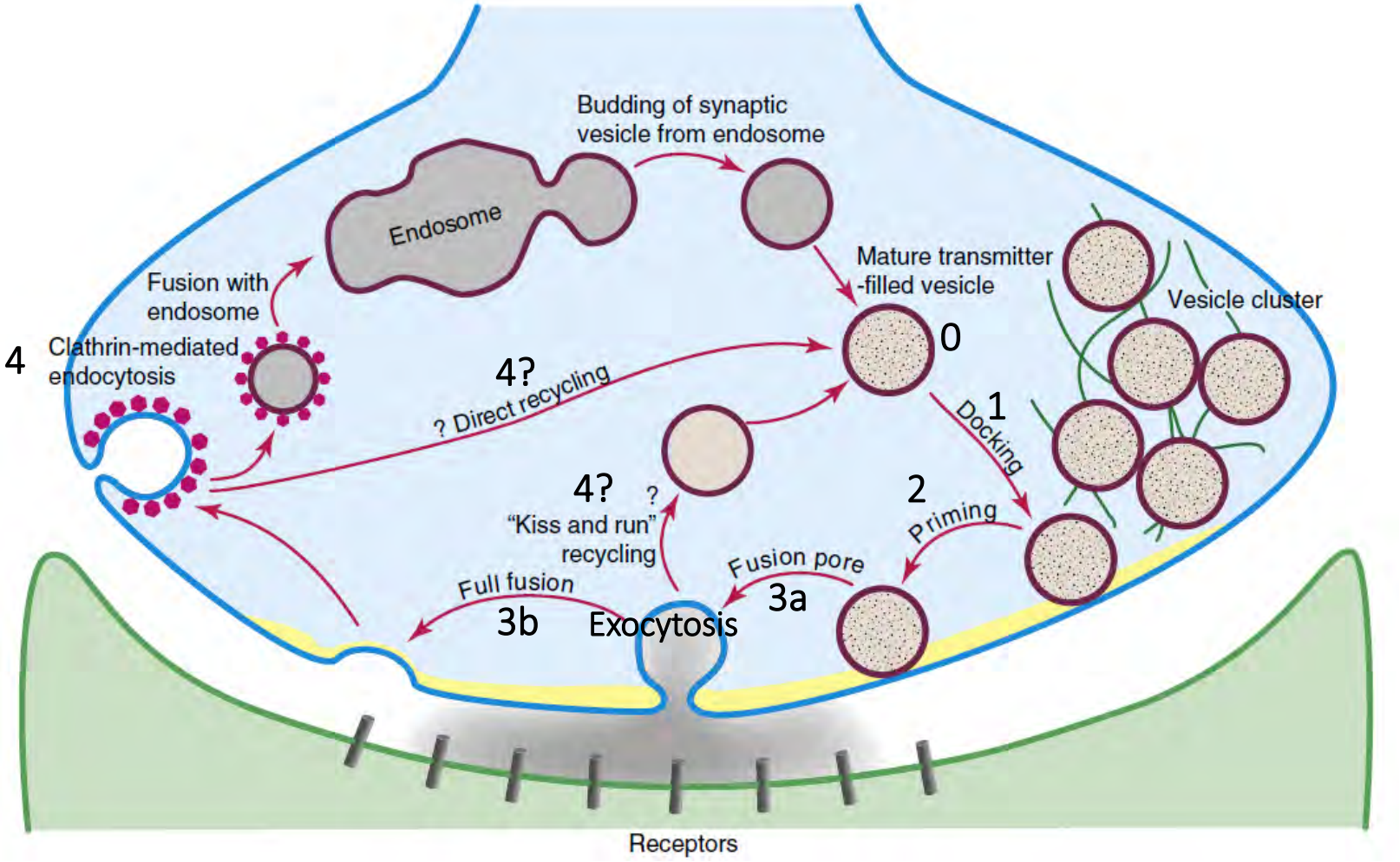
- At NMJ, AP triggers release of ~300 SVs, and at central synapse, AP triggers release of 5-10 SVs. .

- Synaptic cleft = 100 nm wide from pre- to postsynaptic membrane
- At neuromuscular junctions, <u>one</u> SV diffuses across synaptic cleft in 2 ms reaching [1 mM] at postsynaptic receptors (up to 2,000 will bind Ach).
- <u>One</u> nerve ending will have ~1,000 active zones, <u>one</u> AP causes a SV to fuse in ~1/3 of the AZs (300 quanta in 1.5 ms).
- After 0.5 ms delay, the postsynaptic muscle fiber is depolarized, reaching a peak of tens of millivolts typically sufficient to generate an AP (causing muscle contraction).



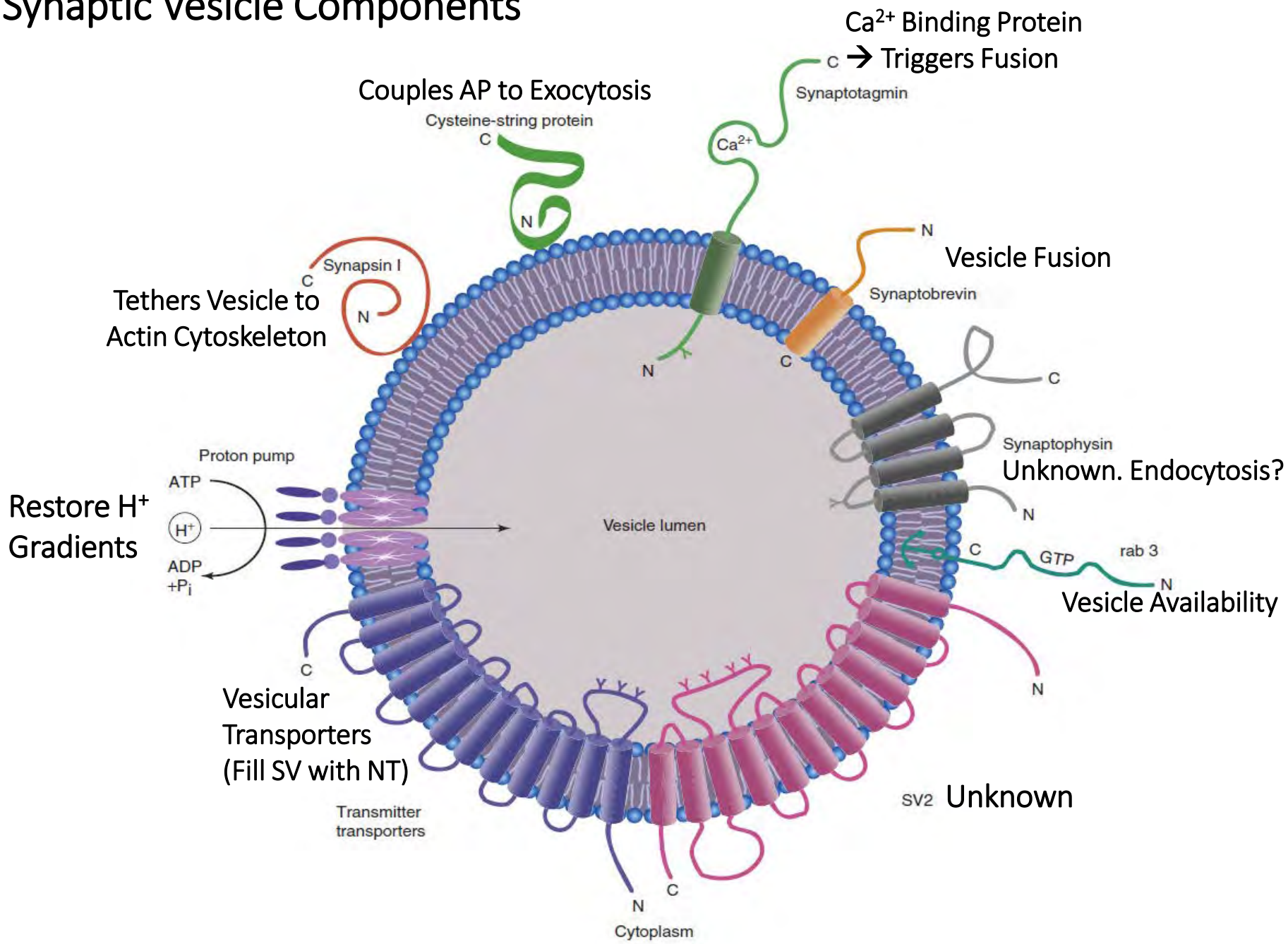
The life cycle of a synaptic vesicle



Transmitter-filled SVs can be observed in clusters in the vicinity of the active zone.

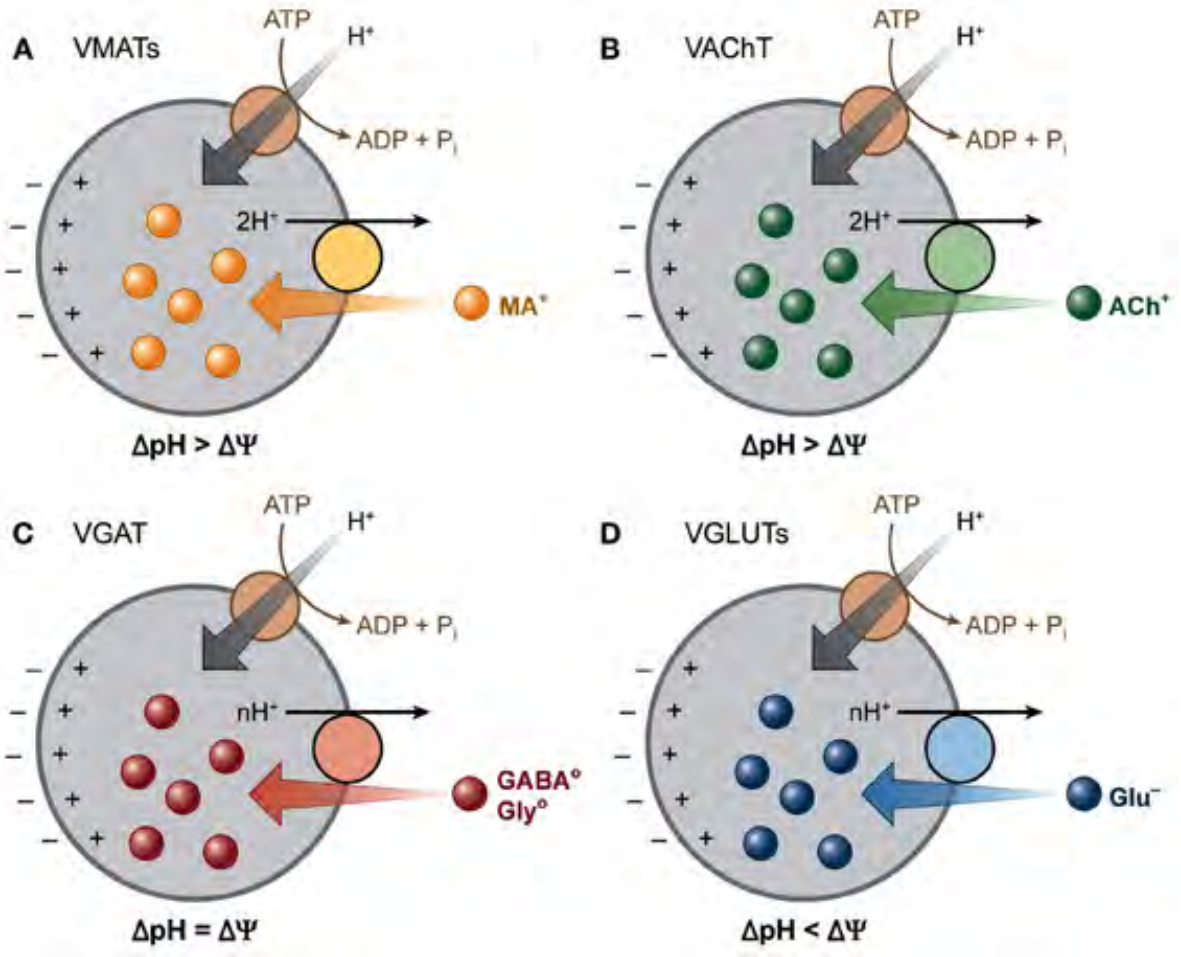
1. **Docking.** Some SVs are recruited to sites within the active zone in a process called docking.
2. **Priming.** These vesicles subsequently are primed for release.
3. **Exocytosis.** The rise in cytosolic Ca2+ that occurs during an action potential triggers the opening of a fusion pore between some of the primed, docked vesicles and the plasma membrane. Transmitter exits through this fusion pore.
4. **SV Recovery.** Three pathways are proposed by which the now empty vesicle can be recovered and returned to the releasable pool:
   - 4-1) direct reclosing of the fusion pore and reformation of the SV, often called "kiss and run";
   - 4-2) complete fusion (i.e., the flattening of SV onto the PM) followed by clathrin-mediated endocytosis, clathrin coat removal, and return of the SV to the releasable pool;
   - 4-3) complete fusion and recycling as in the second pathway, but the endocytosed vesicle fuses first with an endosome and mature vesicles are subsequently formed by budding from the endosome. After or during this recycling process, the vesicle must be refilled with transmitter.

Synaptic Vesicle Components



Vesicular Storage Via Vesicular Transporters

- Protection against enzymatic degradation (inactivation)
- Readily available for rapid release



V-ATPases Create:
- Proton Gradient (Ψ)
- pH Gradient (pH ~5.5)

<u>(A & B): Monamines (DA, NE, 5HT) & Ach (+)</u>
- Relies on pH gradient

<u>(C): GABA/glycine (neutral)</u>
- Relies on both

<u>(D): Glutamate (–)</u>
- Relies on the proton gradient

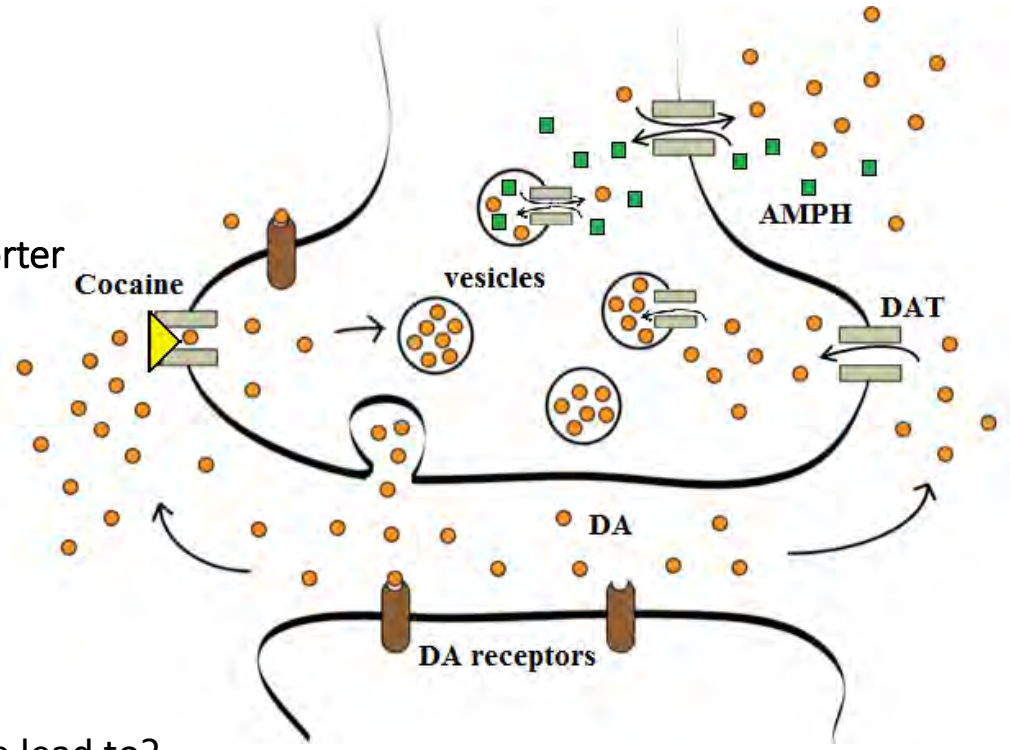
(Van Liefferinge et al., Front Cellular Neuro, 2013)

*Recent research: Simultaneous DA/GLU NT release (same SV)

Amphetamine Actions on the Dopamine Transporter

AMPH > COC to evoke DA release

- ✧ Uptake inhibition
- ✧ AMPH-induced DAT internalization
- ✧ AMPH-induced DA efflux (non-exocytotic)
- ✧ Displace DA from SVs



What medical condition can AMPH/METH use lead to?

Amphetamine/methamphetamine users have higher risk for developing Parkinson's Disease

Drug and Alcohol Dependence 120 (2012) 35–40

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

2012

Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs

Russell C. Callaghan[a,b,*], James K. Cunningham[c], Jenna Sykes[a], Stephen J. Kish[a,d]

Conclusion: These data provide evidence that <u>METH/AMPH users have above-normal risk for developing PD (76% > controls)</u>.

Drug and Alcohol Dependence 146 (2015) 30–38

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

2015

Methamphetamine/amphetamine abuse and risk of Parkinson's disease in Utah: A population-based assessment

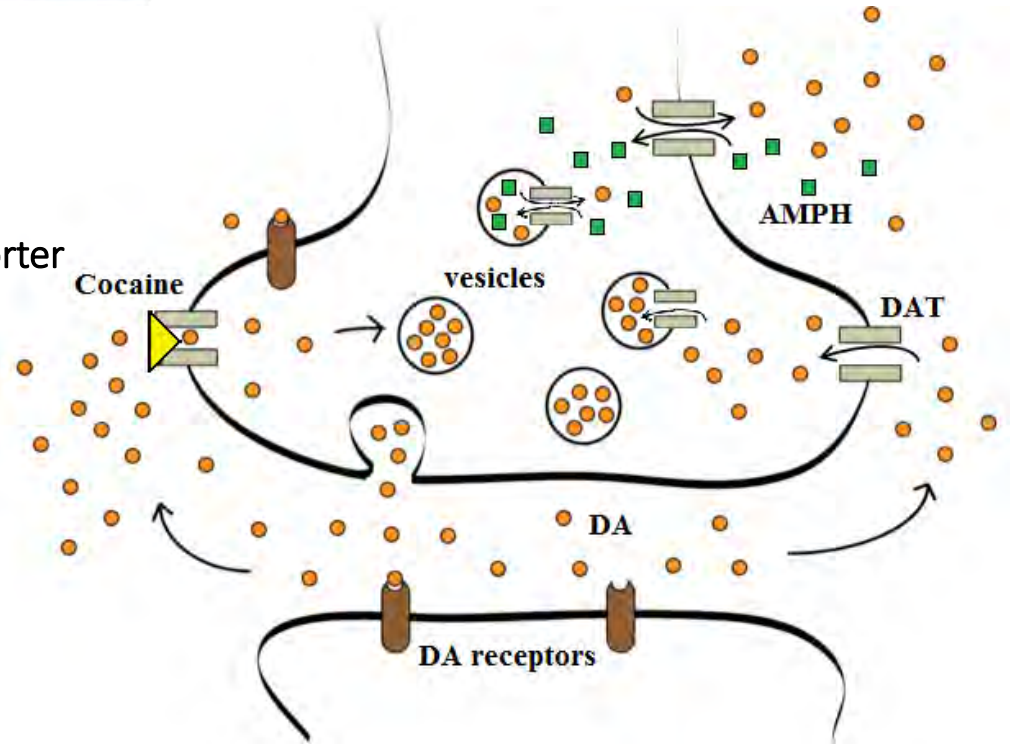
Karen Curtin[a,b,*], Annette E. Fleckenstein[c,g], Reid J. Robison[d,e], Michael J. Crookston[d], Ken R. Smith[b,f], Glen R. Hanson[c,g]

Conclusion: Observed a <u>near 3-fold increased risk of PD in METH/AMPH users vs. controls</u> which; supports that PD risk in users may be higher than previous estimates.

Amphetamine Actions on the Dopamine Transporter

AMPH > COC to evoke DA release

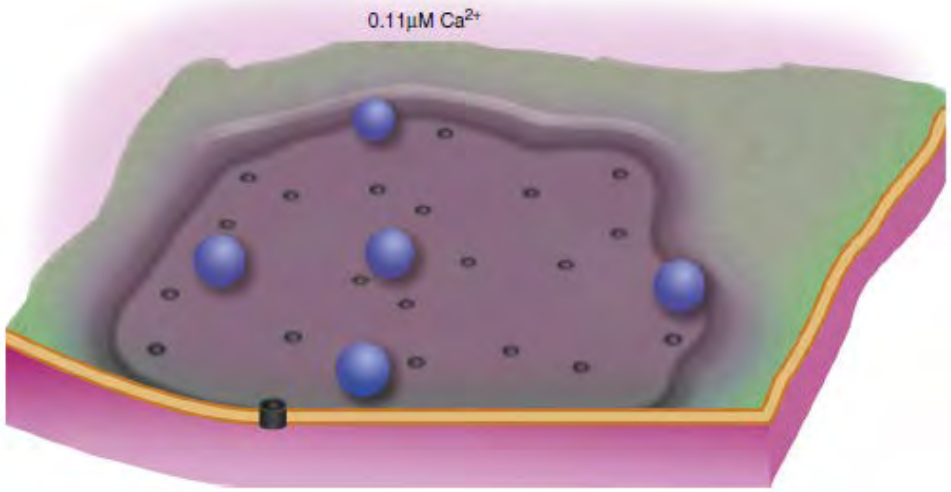
- ✧ Uptake inhibition
- ✧ AMPH-induced DAT internalization
- ✧ AMPH-induced DA efflux (non-exocytotic)
- ✧ Displace DA from SVs
- ✧ Disrupts e^- transport chain in complex I of mitochondria → E depletion, ROS formation → DAergic neuron death



Calcium microdomains regulate synaptic vesicle exocytosis

- Extracellular [Ca^{2+}] = 1.5-2 mM; intracellular [Ca^{2+}] = 0.1 µM (buffered by mitochondria and ER); e/i = 15,000 to 40,000:1
- Following an AP, Ca^{2+} influx raises [Ca^{2+}] in terminal from 0.1 to 0.11 µM (as measured with [Ca^{2+}] indicator dyes).

Q: Why does this small [Ca^{2+}] increase lead to exocytosis?



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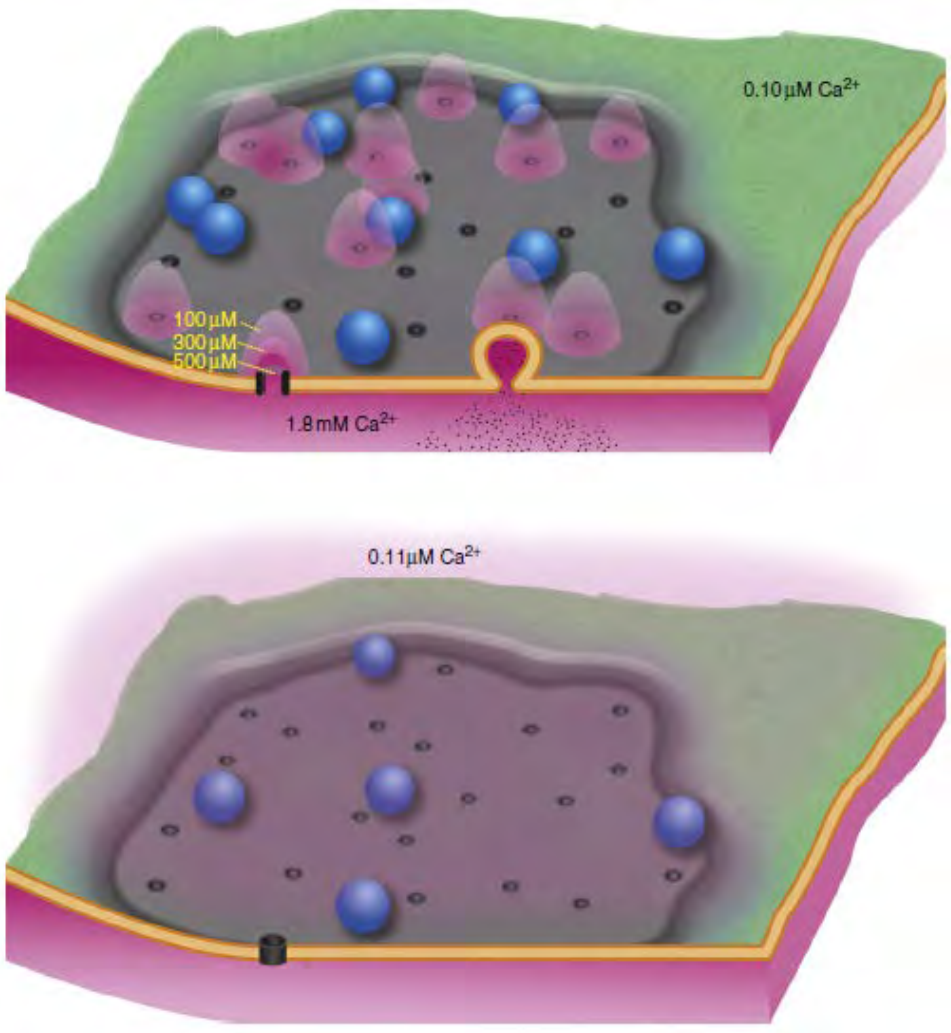

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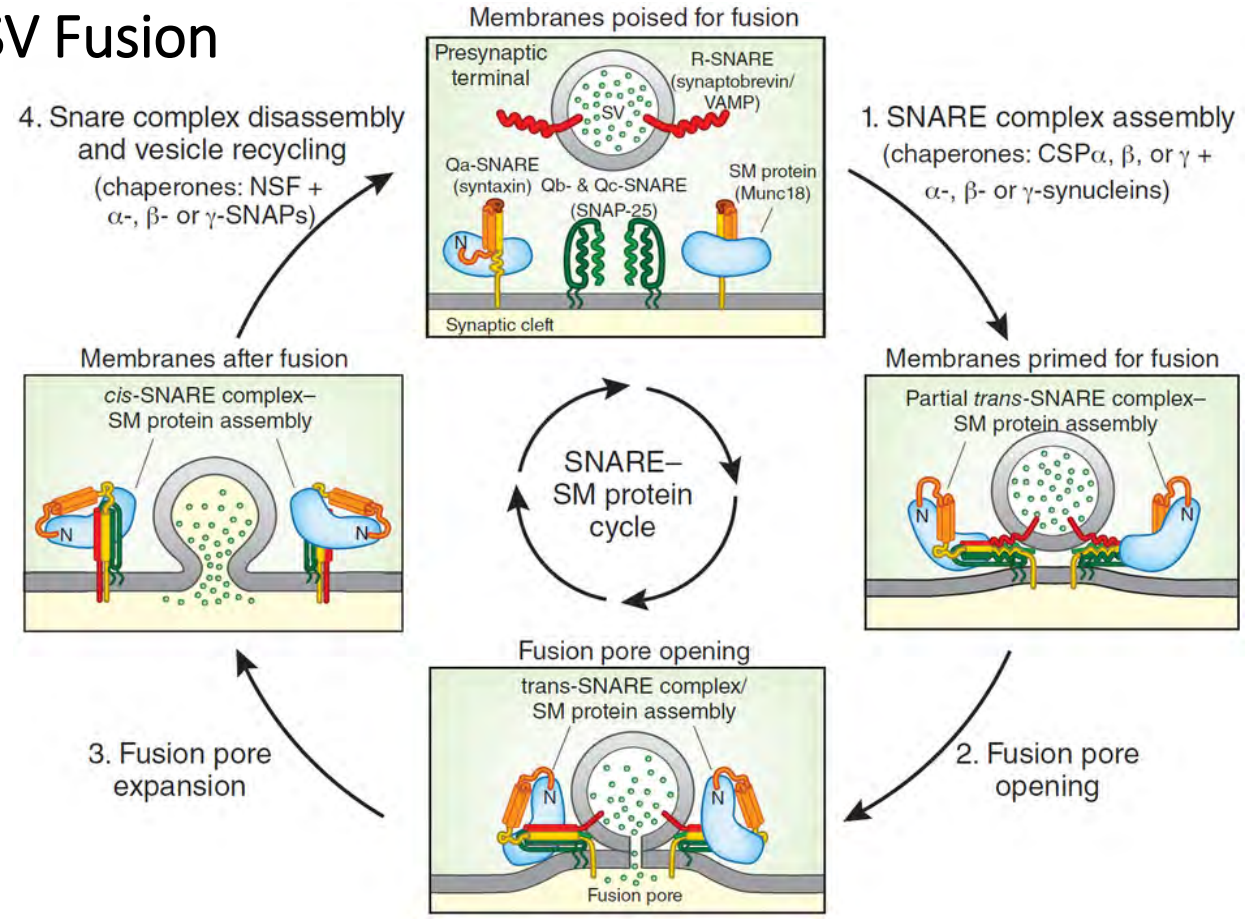
Q: Why does this small [Ca^{2+}] increase lead to exocytosis?

- Answer: The SV release mechanisms respond to localized, high [Ca^{2+}] in microdomains.
- In brief period in which Ca^{2+} channels open, the cytosol near channels is flooded with Ca^{2+} (100-500 µM reached in 200 µs).
- Diffusion and buffering return Ca^{2+} to basal levels within milliseconds (the [Ca^{2+}] gradient around mouth of the channel completely dissipates and only the small Ca^{2+} net rise remains (i.e. residual [Ca^{2+}] as detected by fluorescent indicator dyes).
- An AZ may have more than 100 Ca^{2+} channels in its membrane, and a single SV may be within 50 nm of as many as 10 Ca^{2+} channels.
- Since multiple Ca^{2+} channels can open, Ca^{2+} entering through nearby channels can summate in overlapping microdomains.
- Different subtypes of Ca^{2+} channels with different electrophysiological kinetics can influence release (i.e. high- vs. low-voltage activated Ca^{2+} channels).

Proteins involved in SV Fusion



(Thomas Südhof, Nature Medicine, 2013)

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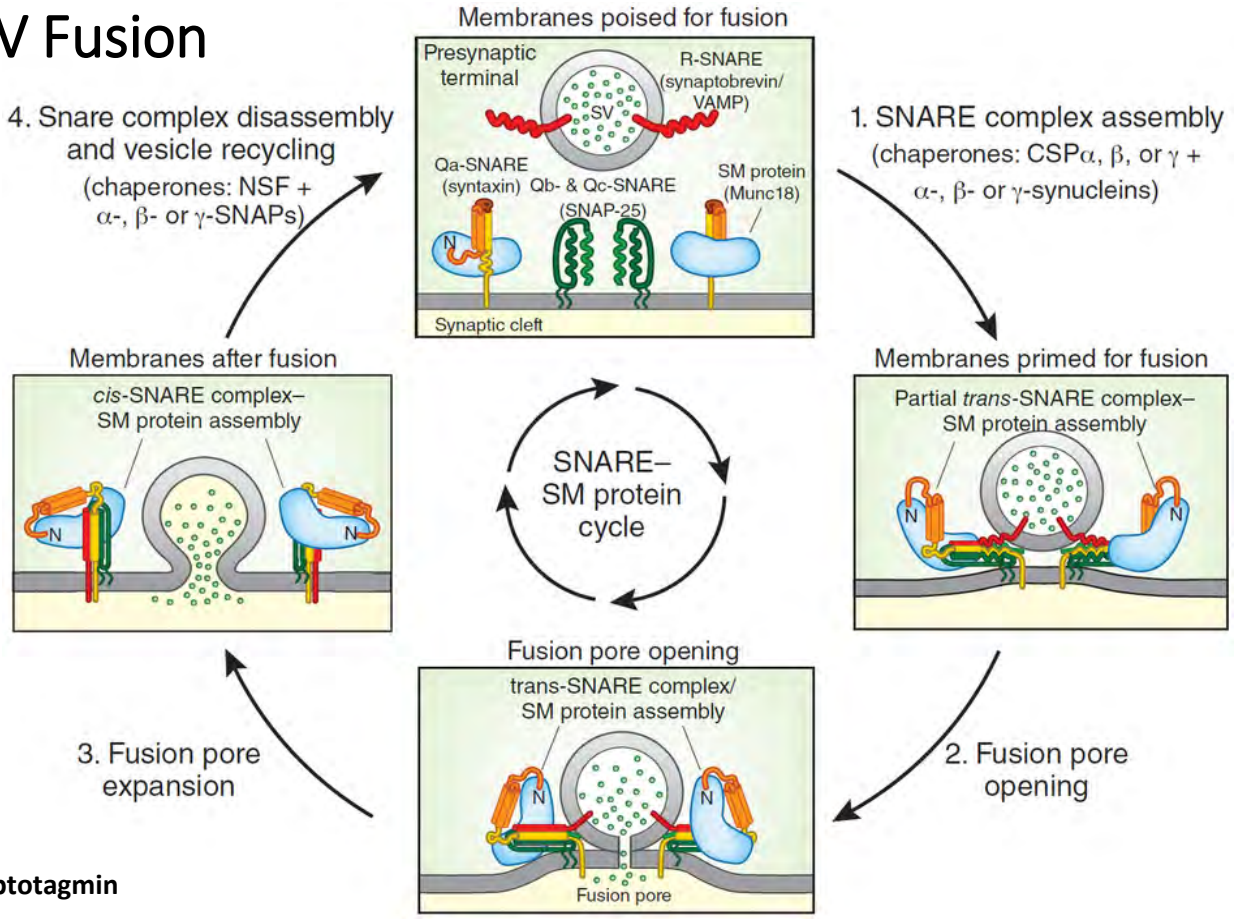

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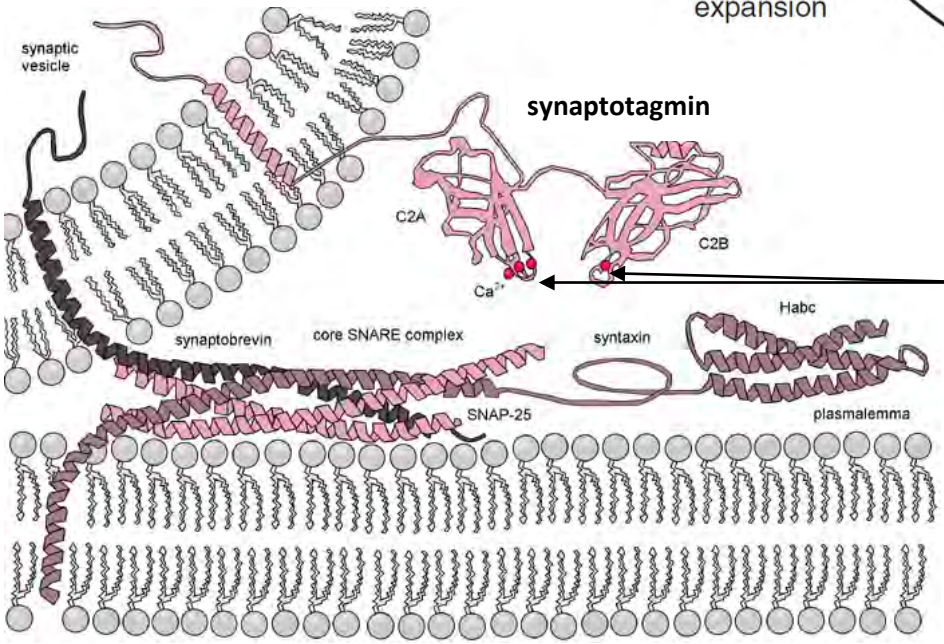
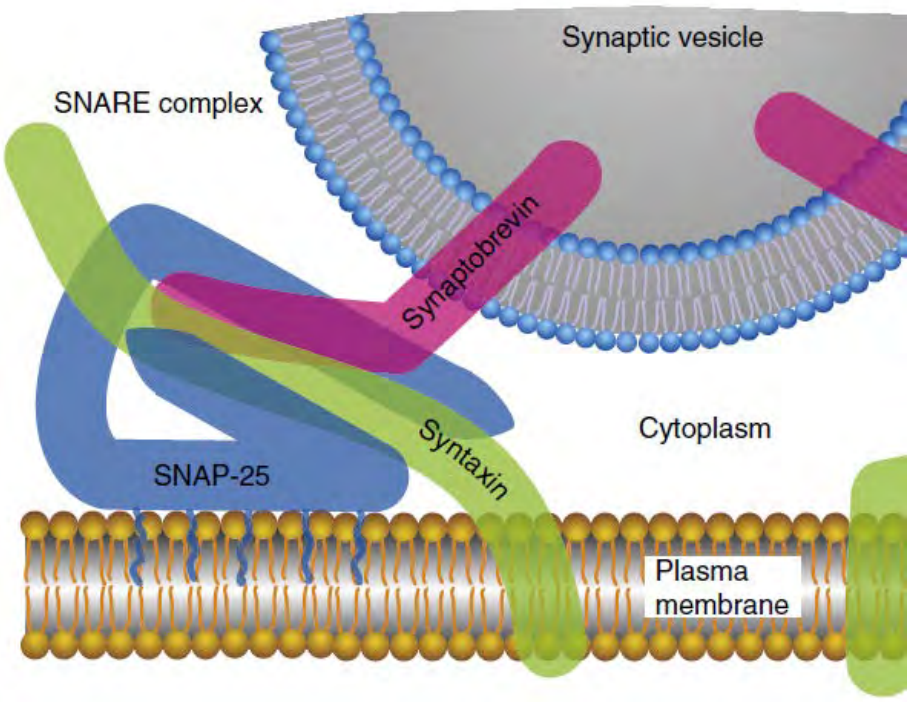


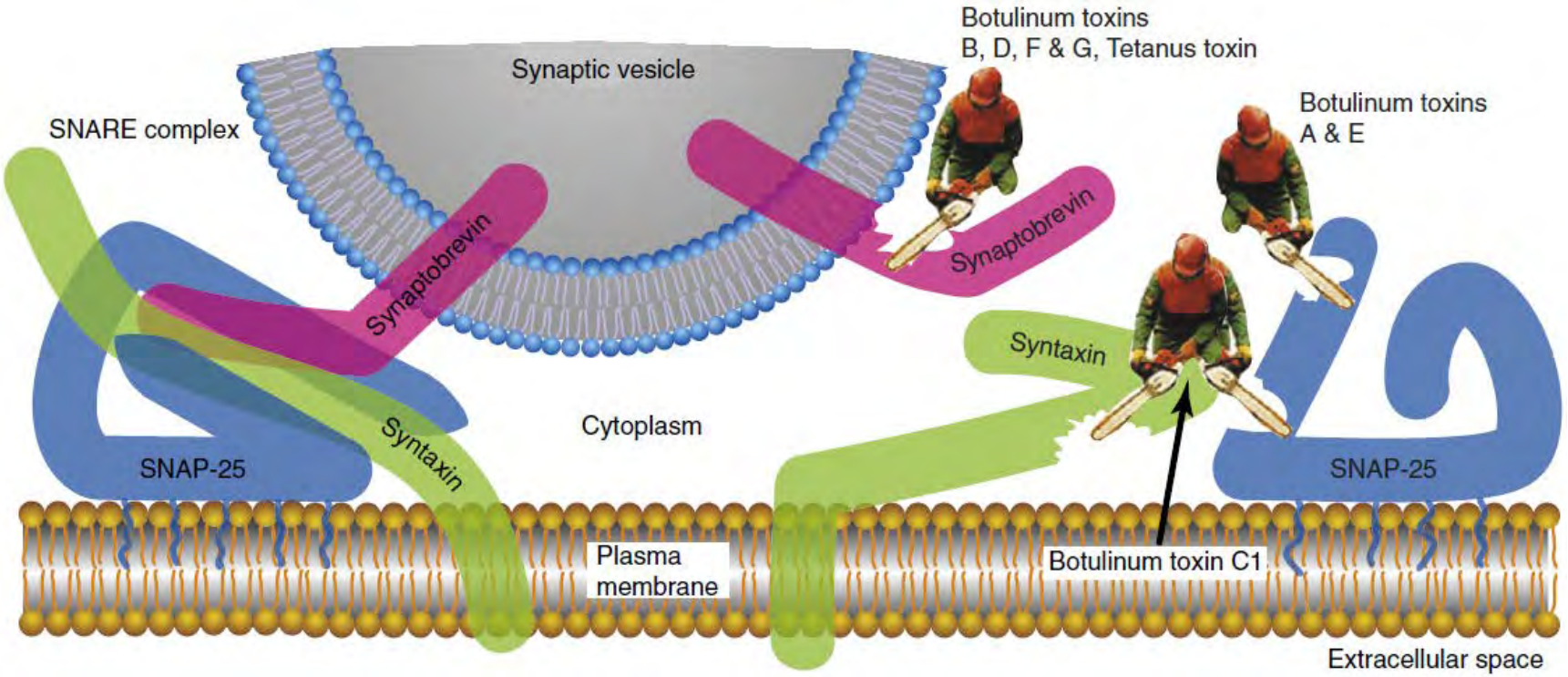
Ca^{2+} binds to Ca^{2+}-binding domain in synaptotagmin inducing conformational change to begin SV fusion.

SNARE complex proteins tether SV to presynaptic plasma membrane



- The SNARE complex brings the SV and PM into close proximity and represents one of the last steps in vesicle fusion.
- Vesicular VAMP, also called synaptobrevin, binds with syntaxin and SNAP-25 that are anchored to the PM.

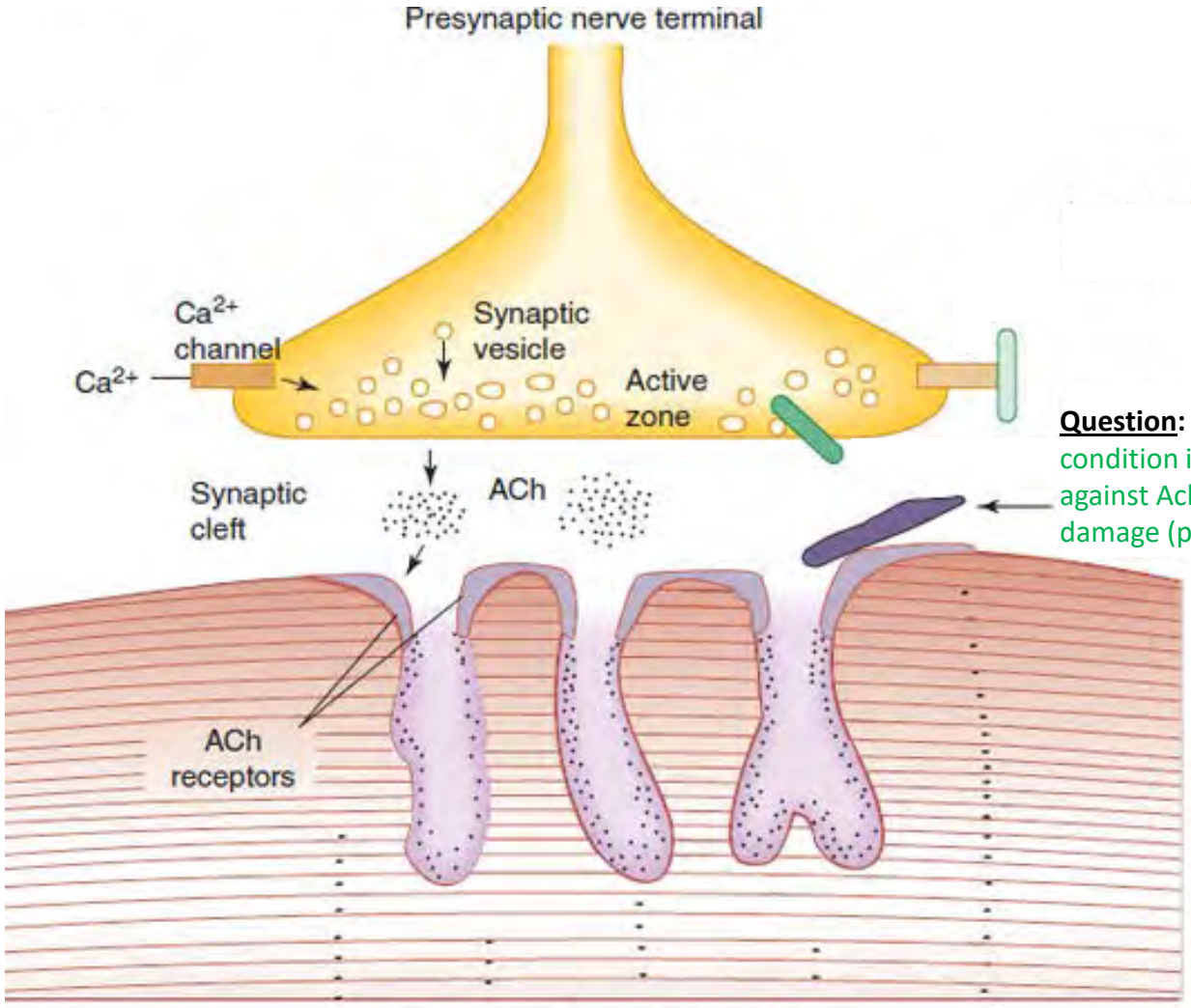
SNARE complex proteins are cleaved by bacterial neurotoxins



Question: What would happen to a person exposed to these neurotoxins?

- The SNARE complex brings the SV and PM into close proximity and represents one of the last steps in vesicle fusion.
- Vesicular VAMP, also called synaptobrevin, binds with syntaxin and SNAP-25 that are anchored to the PM.
- **Tetanus toxin** and the **botulinum toxins**, proteases that cleave specific SNARE proteins as shown, can block transmitter release.

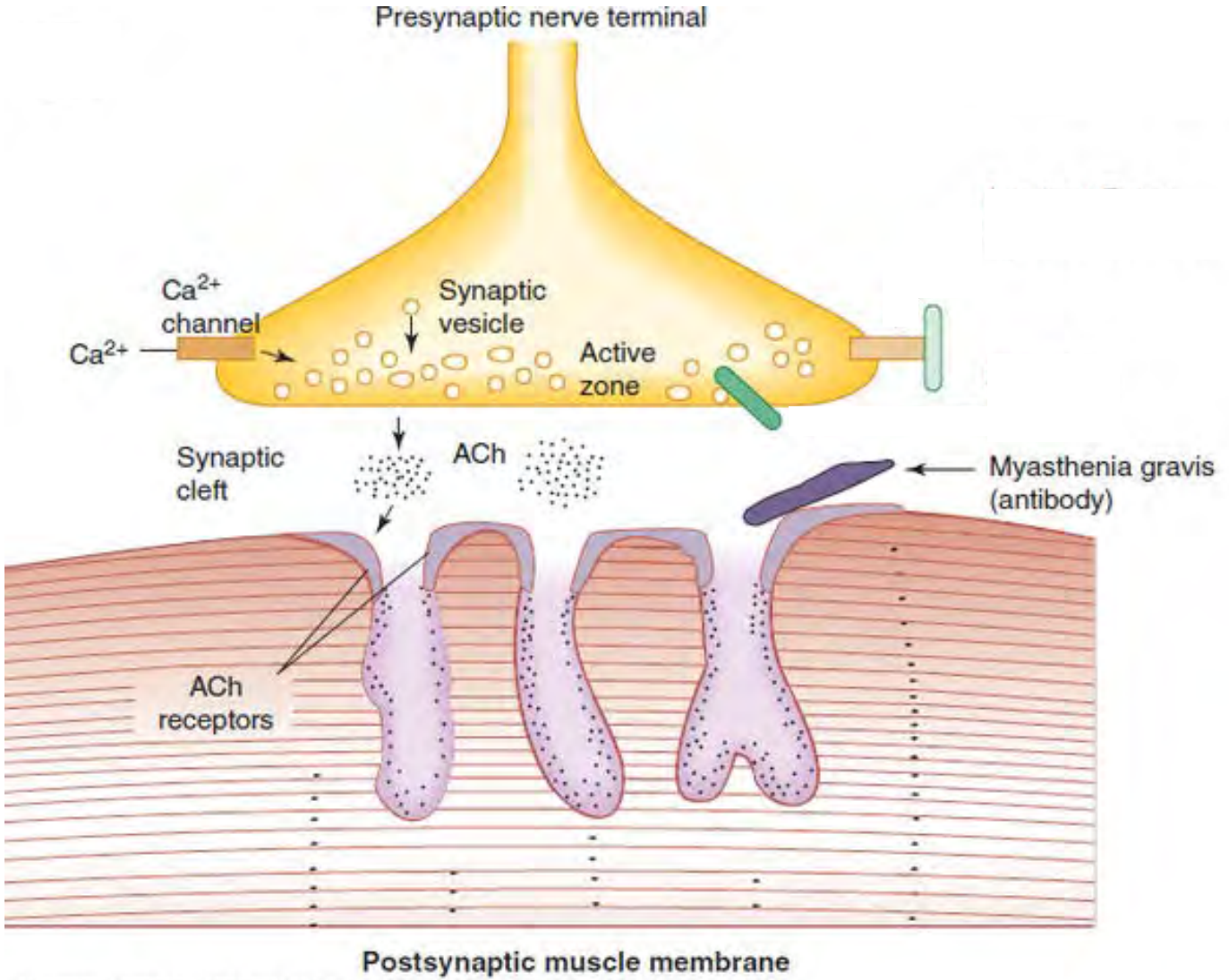
Disorders affecting the NMJ



Source: Aaron L. Berkowitz: Clinical Neurology and Neuroanatomy: A Localization-Based Approach

Question: What is the medical condition in which autoantibodies against AchRs leads to receptor damage (preventing binding of Ach)?

Disorders affecting the NMJ



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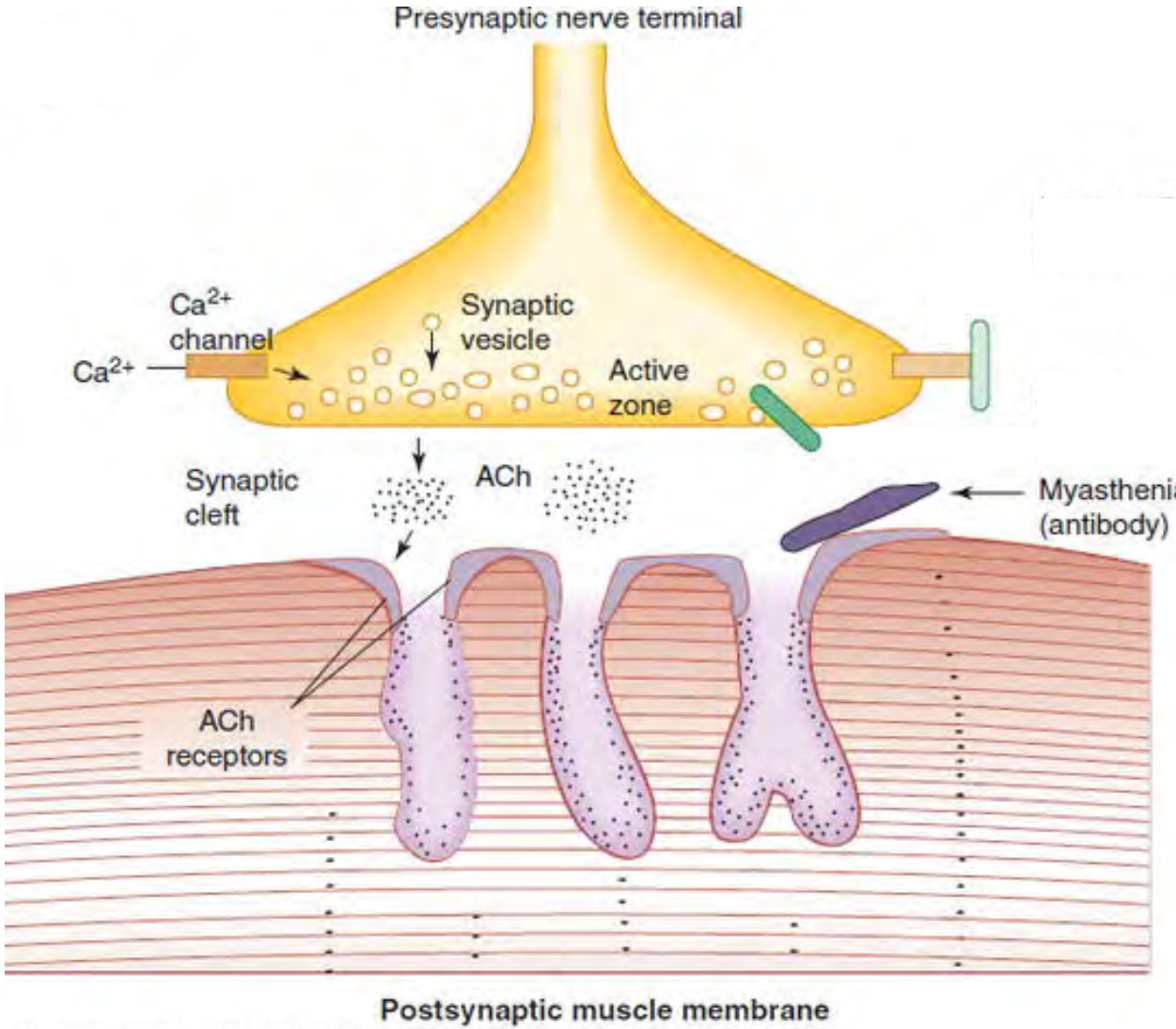
Question: How is Myasthenia gravis treated?

Disorders affecting the NMJ



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Pyridostigmine /Mestinon (AchE inhibitor)

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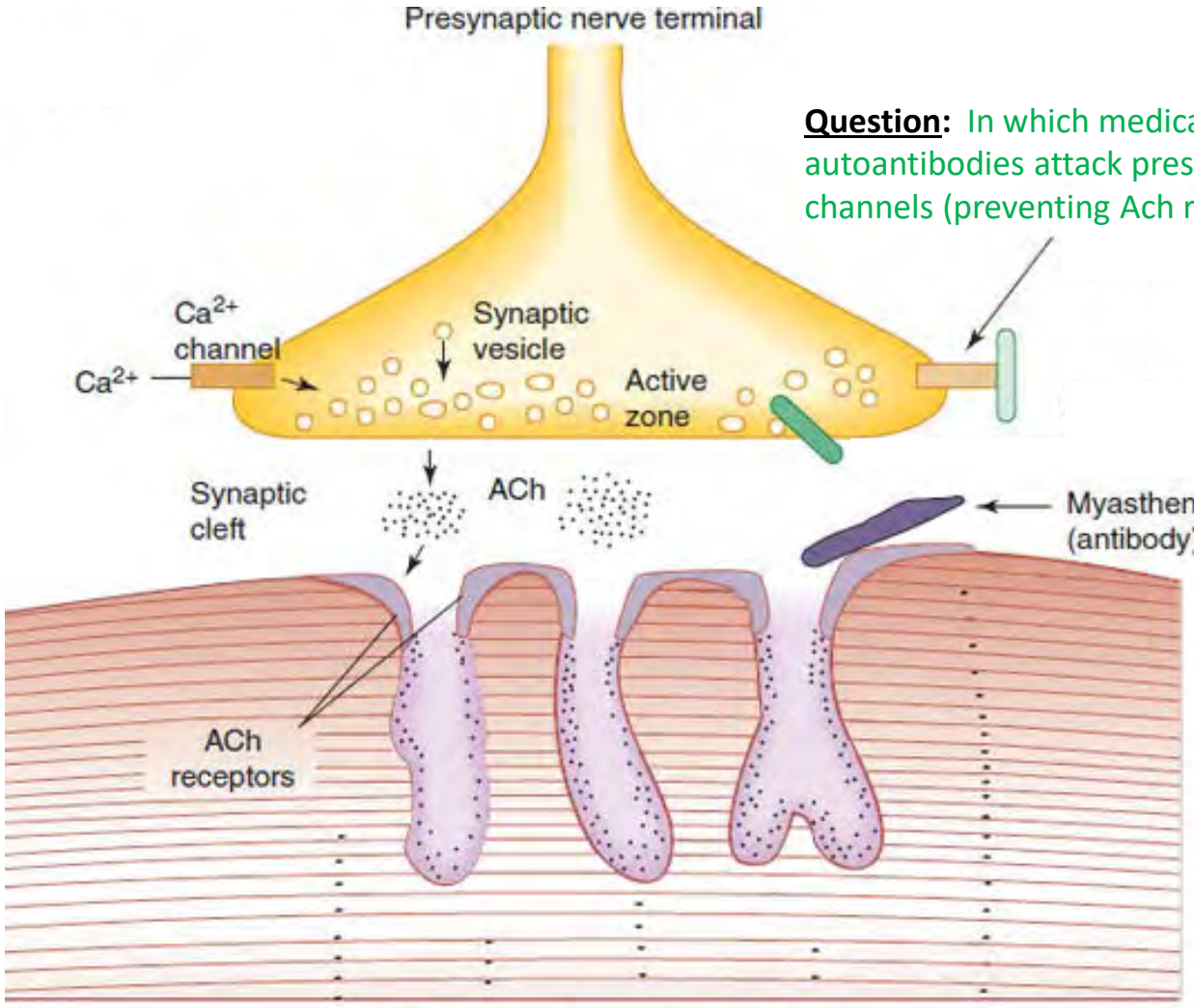
Disorders affecting the NMJ

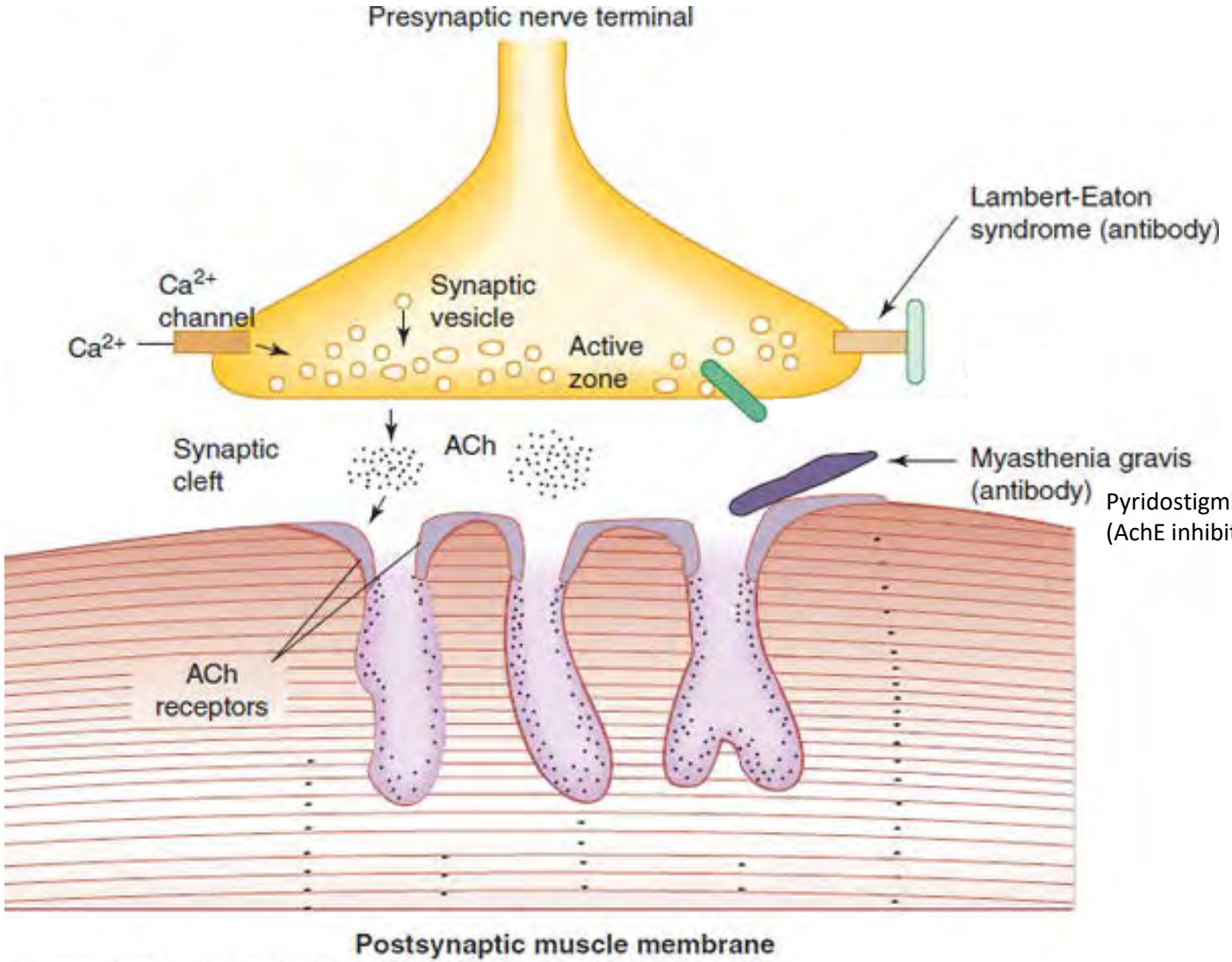


Source: Aaron L. Berkowitz: Clinical Neurology and Neuroanatomy: A Localization-Based Approach

Question: In which medical condition do autoantibodies attack presynaptic Ca^{2+} channels (preventing Ach release)?

Pyridostigmine /Mestinon (AchE inhibitor)

Disorders affecting the NMJ

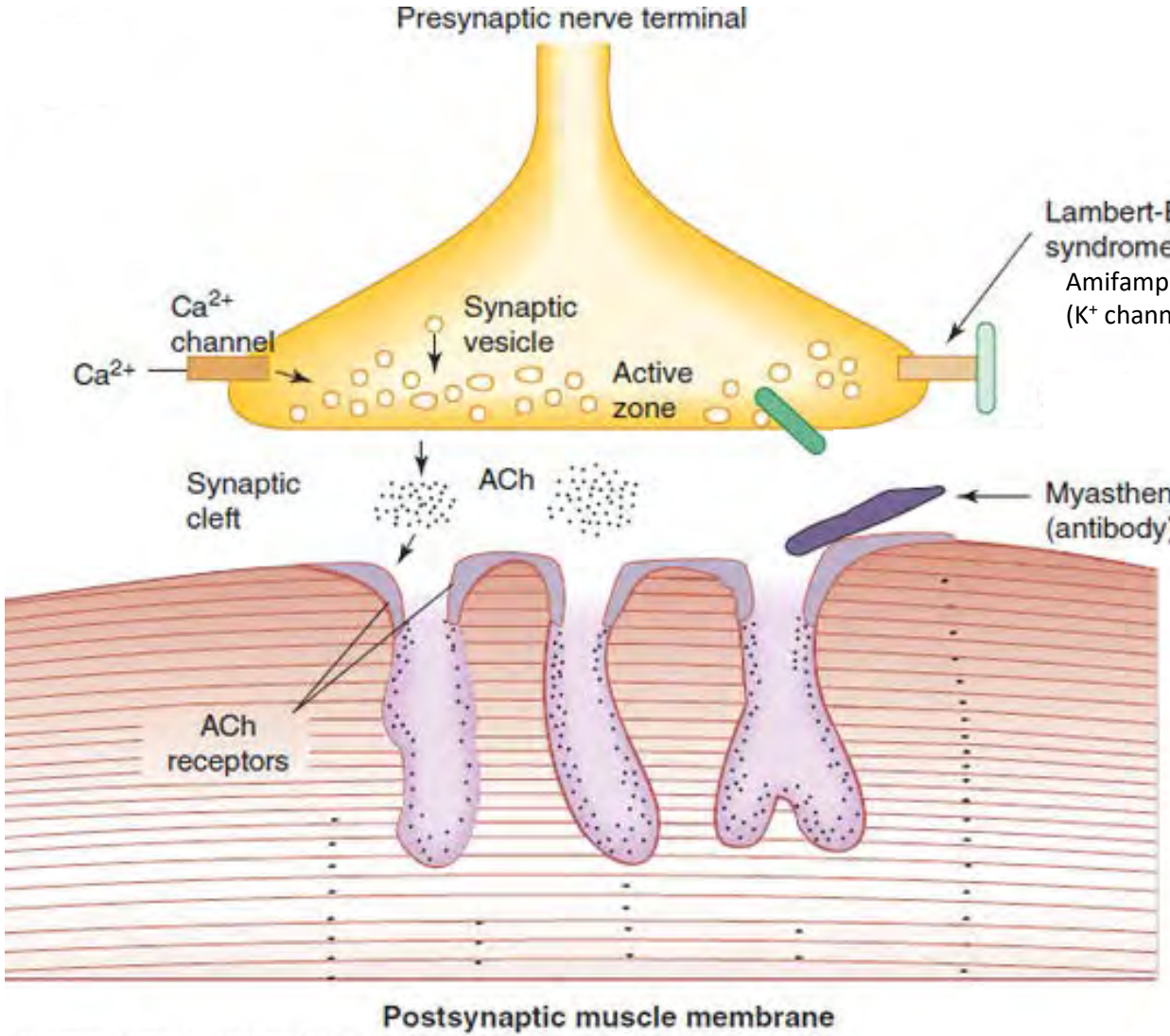


Source: Aaron L. Berkowitz: Clinical Neurology and Neuroanatomy: A Localization-Based Approach

Pyridostigmine /Mestinon (AchE inhibitor)

Question: How is LEMS treated?

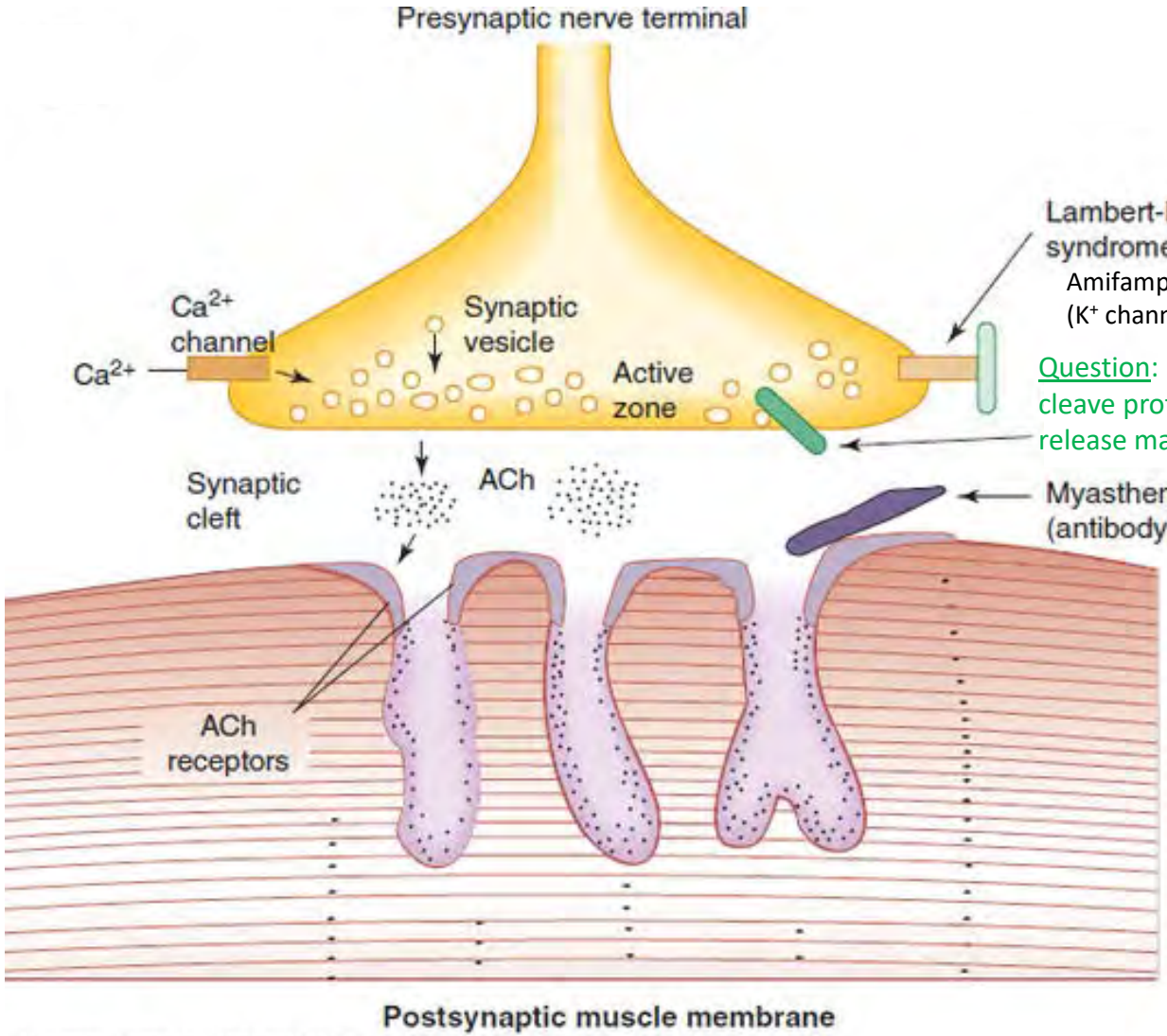
Disorders affecting the NMJ



Source: Aaron L. Berkowitz: Clinical Neurology and Neuroanatomy: A Localization-Based Approach

Question: How is LEMS treated?

Disorders affecting the NMJ



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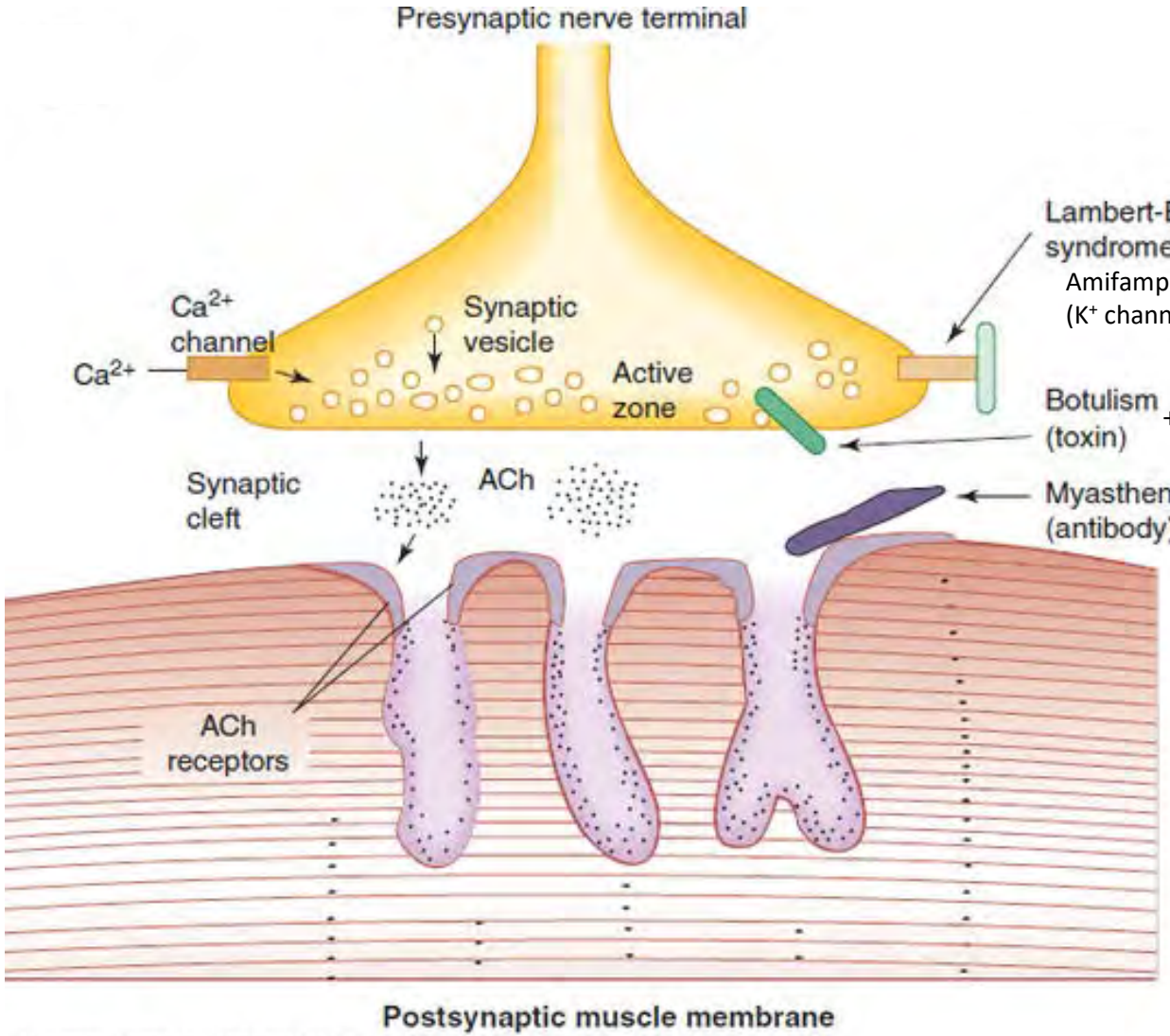

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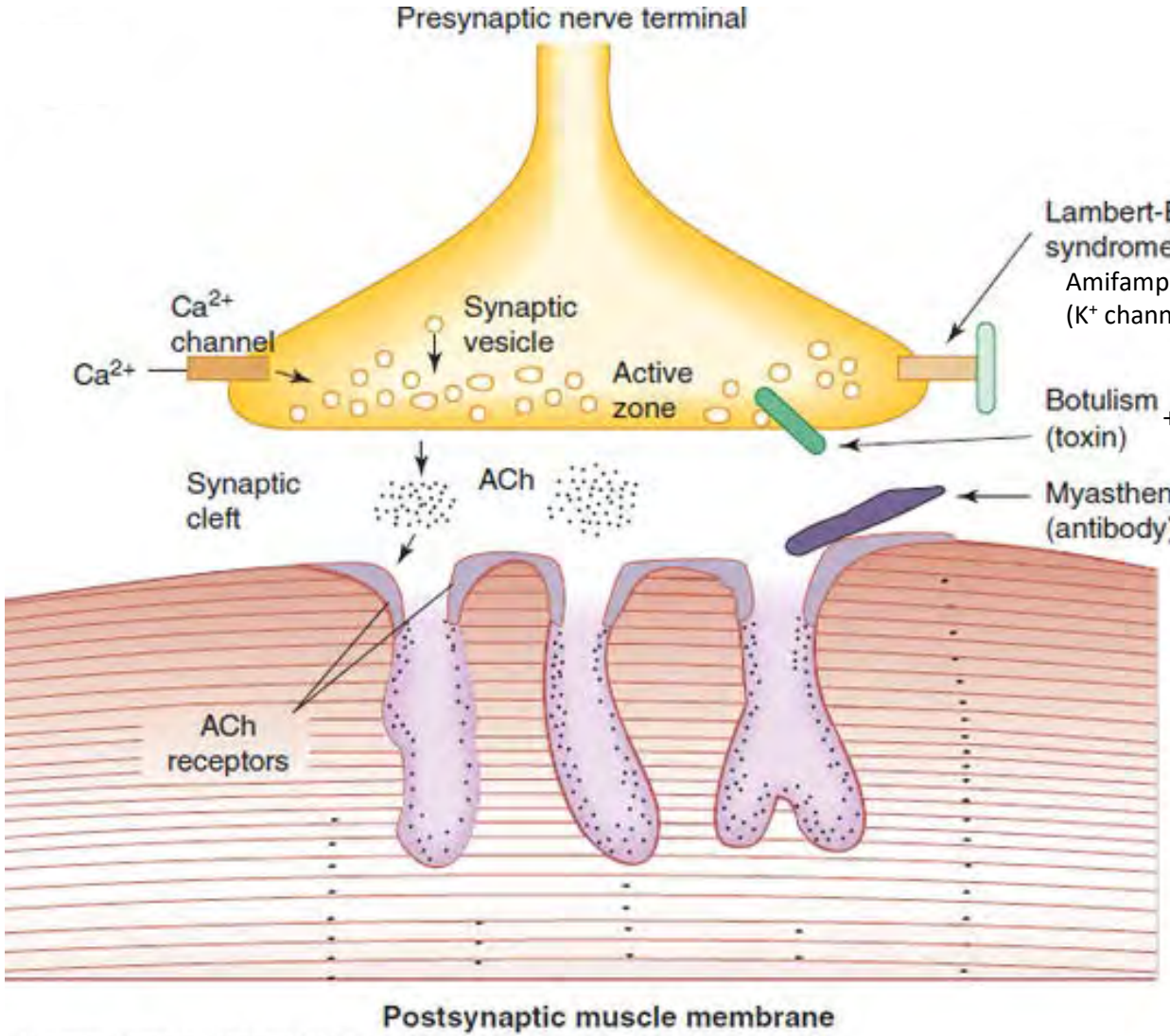
Question: How are botulism and tetanus treated?

Disorders affecting the NMJ



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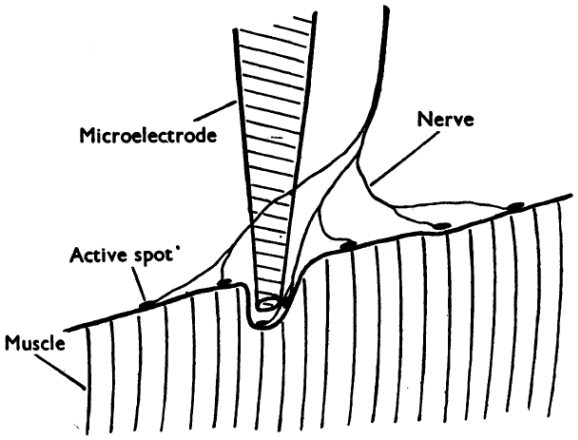
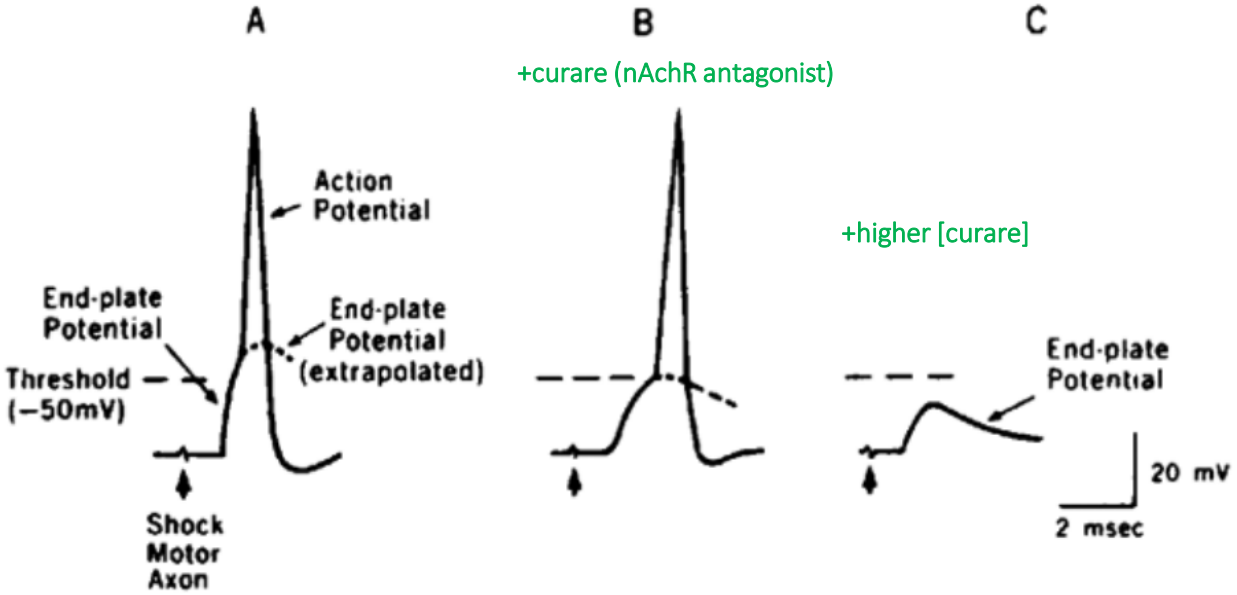
Question: How are botulism and tetanus treated?

Botulism (antitoxin, breathing/eating assistance)

Tetanus (antibiotics)

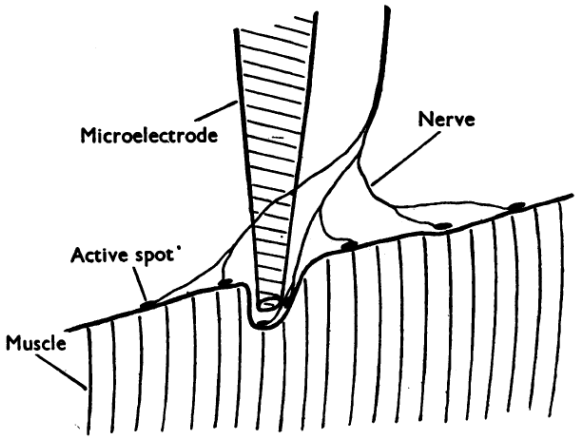
Curare can reduce End Plate Potential (EPP) at NMJ without affecting postsynaptic AP



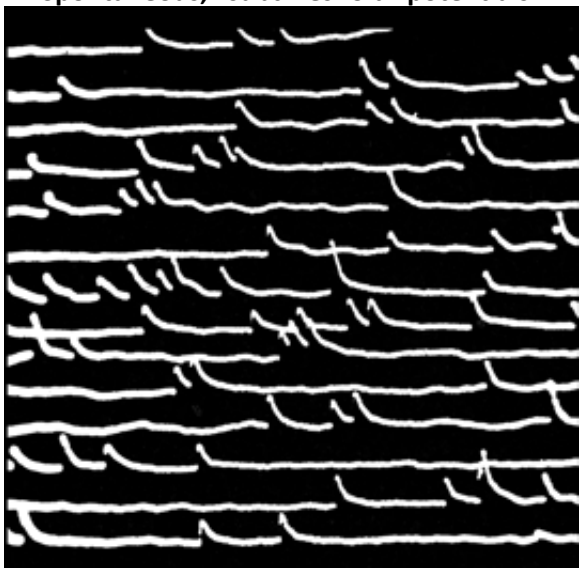


Neurotransmitter Release is Quantal – NMJ recordings

Sir Bernard Katz



Spontaneous, "subthreshold" potentials



(Fatt & Katz, 1952)

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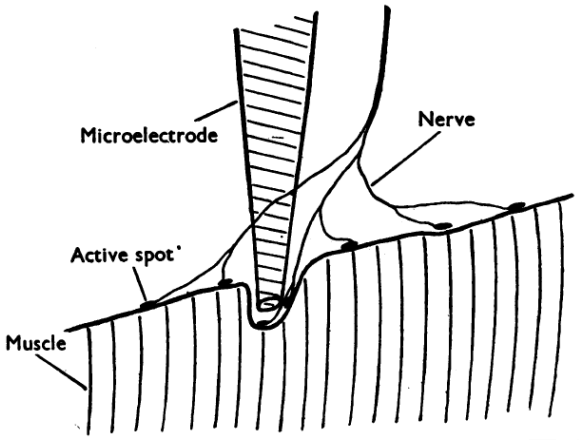

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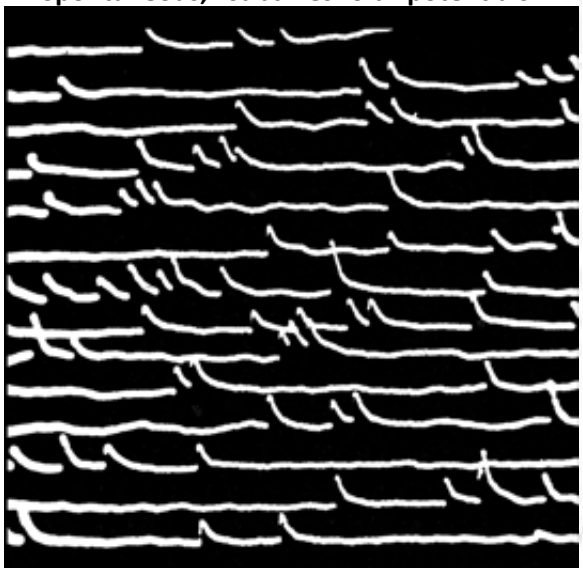
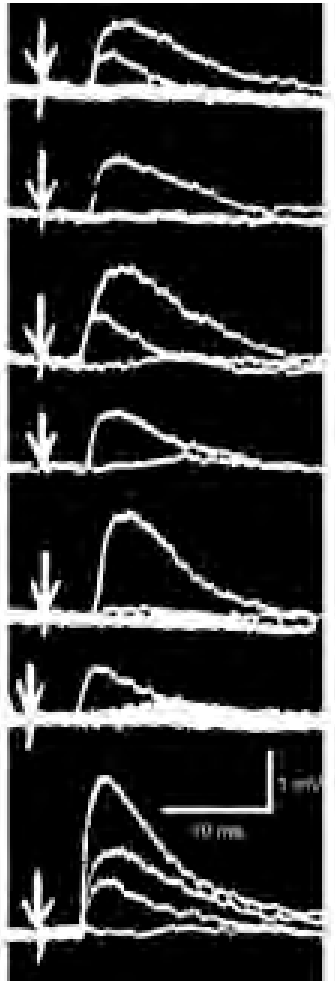


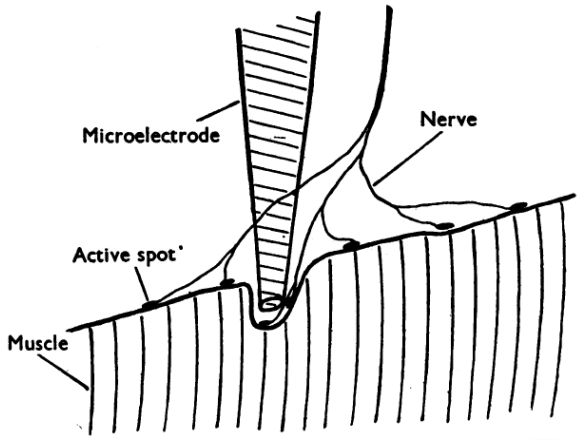
(Fatt & Katz, 1952)

Potentials following nerve stimulation in low [$Ca2^+$]



(Castillo & Katz, 1954)

Neurotransmitter Release is Quantal – NMJ recordings



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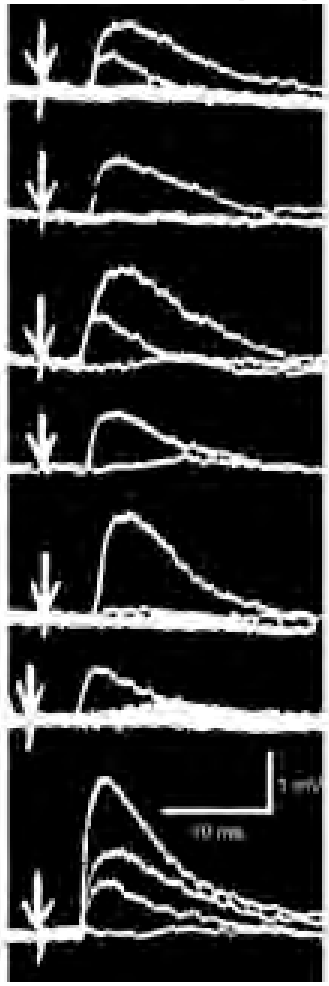

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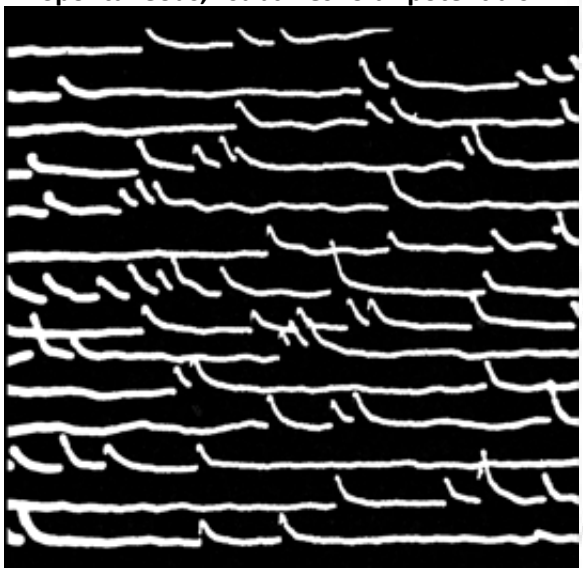
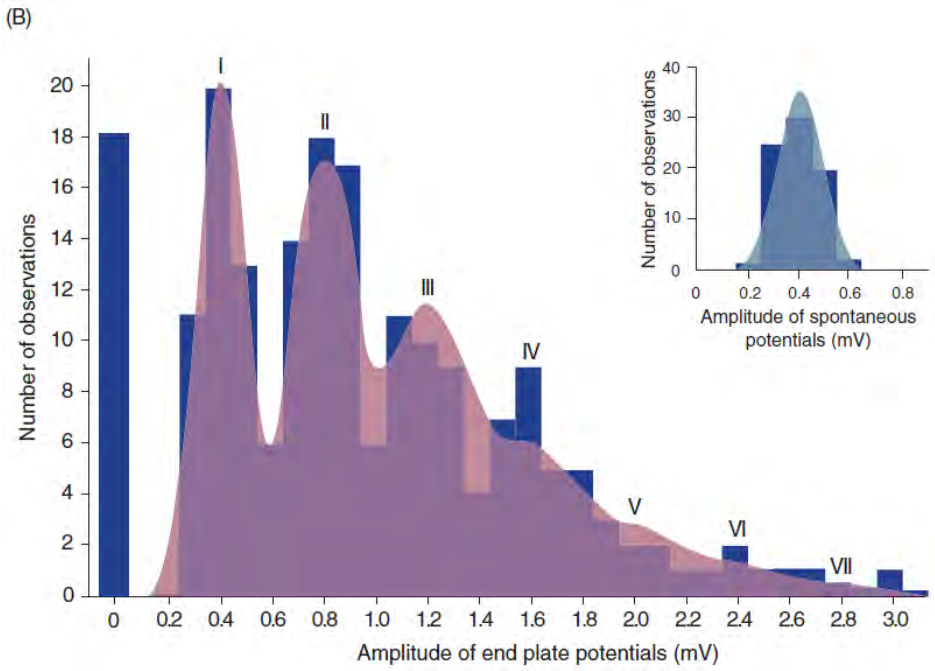


(Fatt & Katz, 1952)

(Castillo & Katz, 1954)



QUANTUM: the amount of neurotransmitter contained within 1 SV

- Neurotransmitters are released in discrete quanta
- Small, spontaneous release of NTs without stimulation
- End plate potential (EPP) consists of summation of quanta (minis)

Katz Model of Quantal Release (as studied at NMJ)

1. Action potential raises probability of vesicle fusion.

2. Several quanta are available for release and each provides the same electrical signal to the postsynaptic cell.

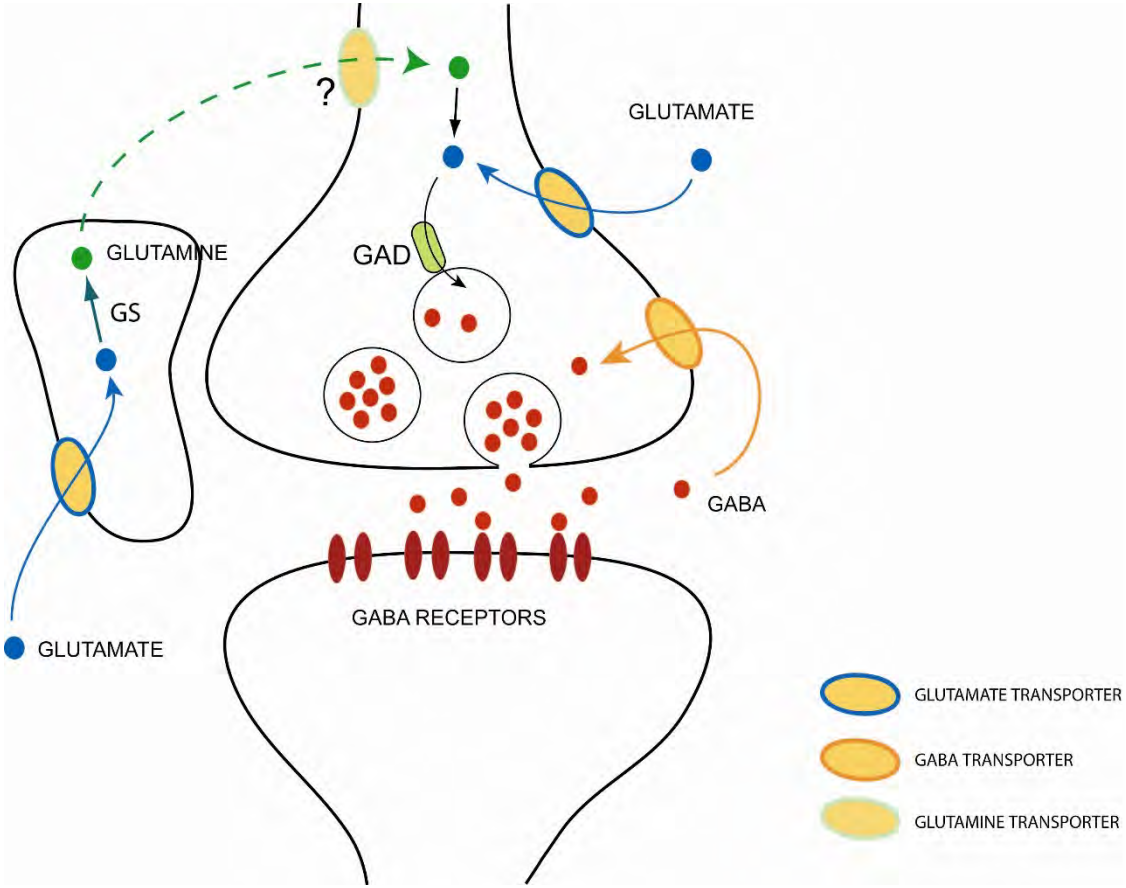
3. Average release probability: quanta released (m) = # of available quanta (n) * average release probability (p) **[m=n*p]**

4. Probability of quanta release follows a Poisson distribution

Katz Model of Quantal Release (as studied at NMJ)

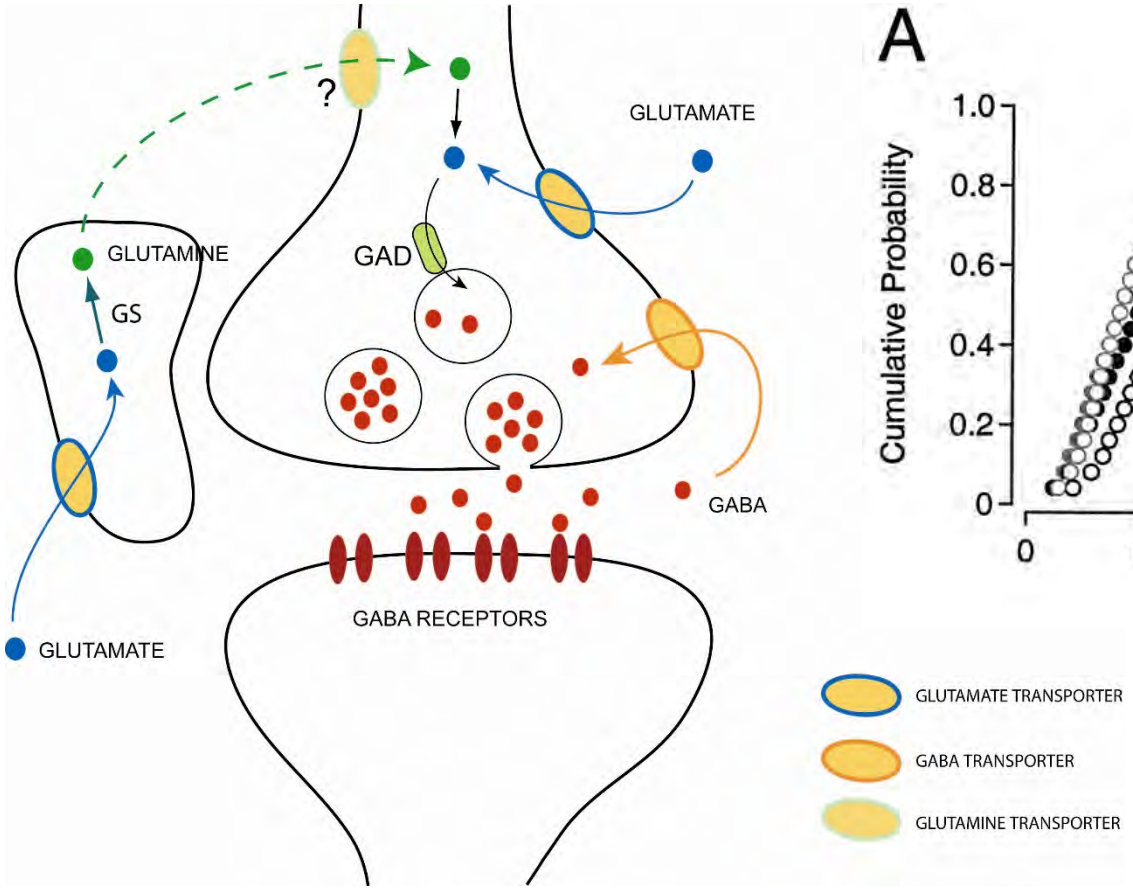
Describes the neuromuscular junction **BUT NOT** a CNS synapse

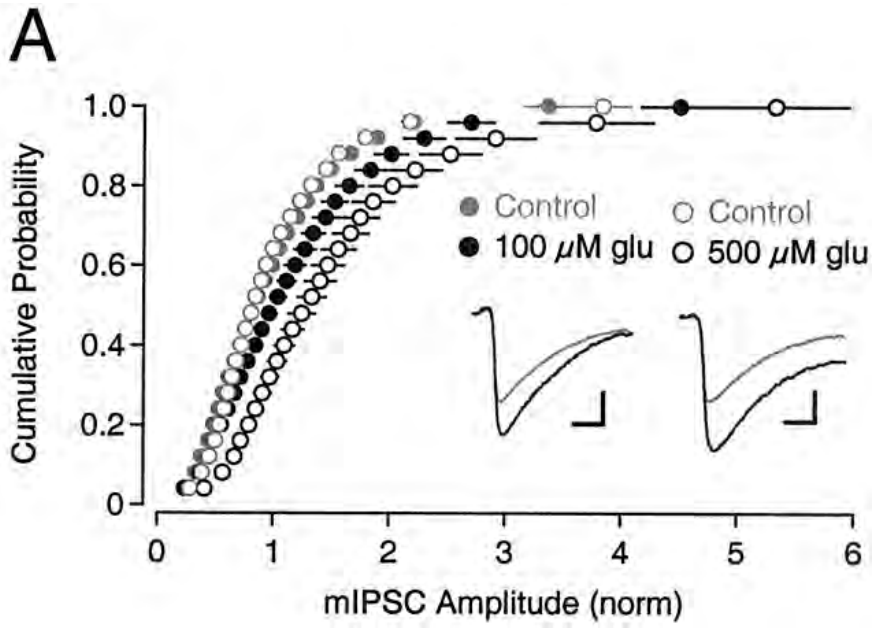
- Quantum varies in size (strength/NT content)
- Different Ca^{2+} channel subtypes changes release probabilities
- Voltage properties of postsynaptic cells prevents quanta sumation
- Receptor saturation: easy for a single quantum to saturate
- Silent Synapses: No receptors are present



Neuronal Glutamate Uptake Contributes to GABA Synthesis and Inhibitory Synaptic Strength

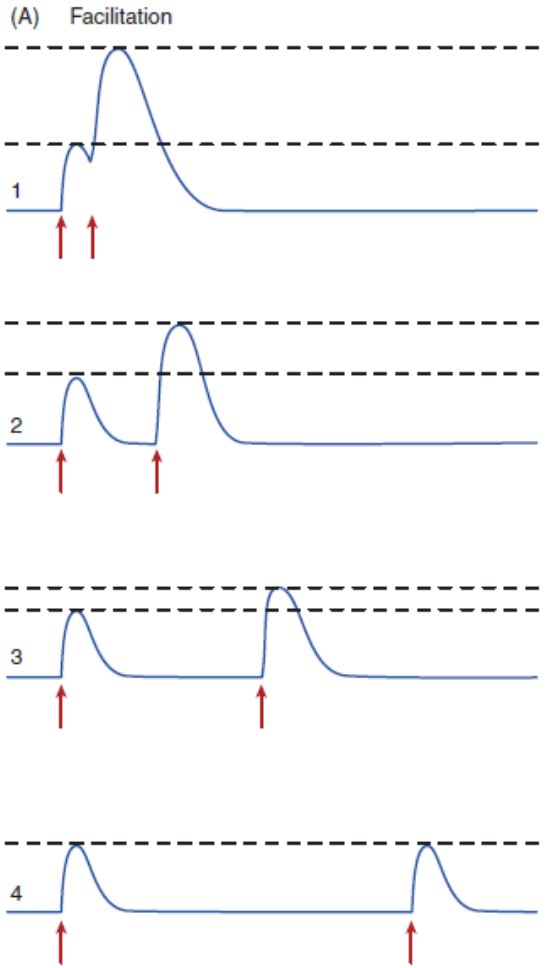
Gregory C. Mathews[1,2] and Jeffrey S. Diamond[1]
[1]Synaptic Physiology Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-4066, and
[2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287





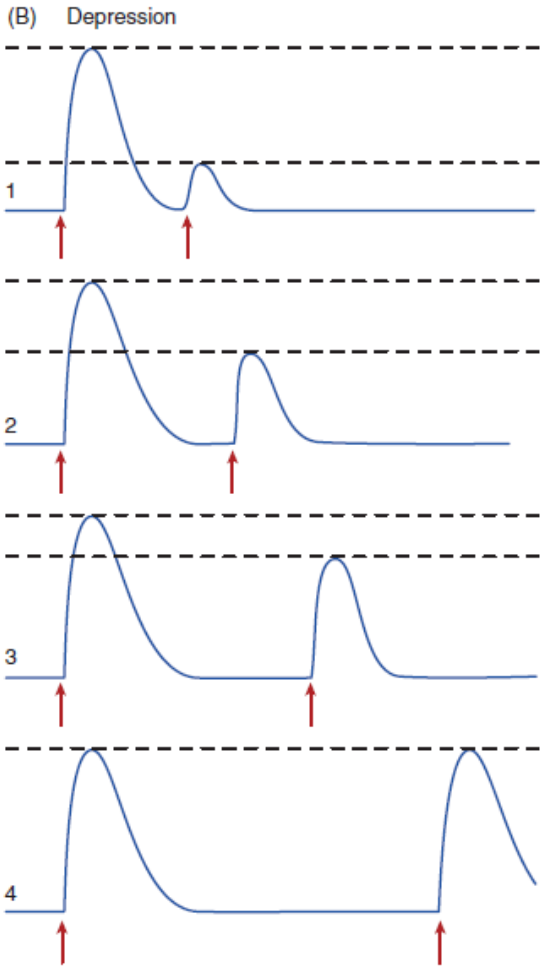
Exogenously applied glutamate increases mIPSC amplitudes.
(A) Addition of 100 or 500 μM glutamate (*glu*) increased mIPSC amplitude compared with control.

Short Term Synaptic Plasticity



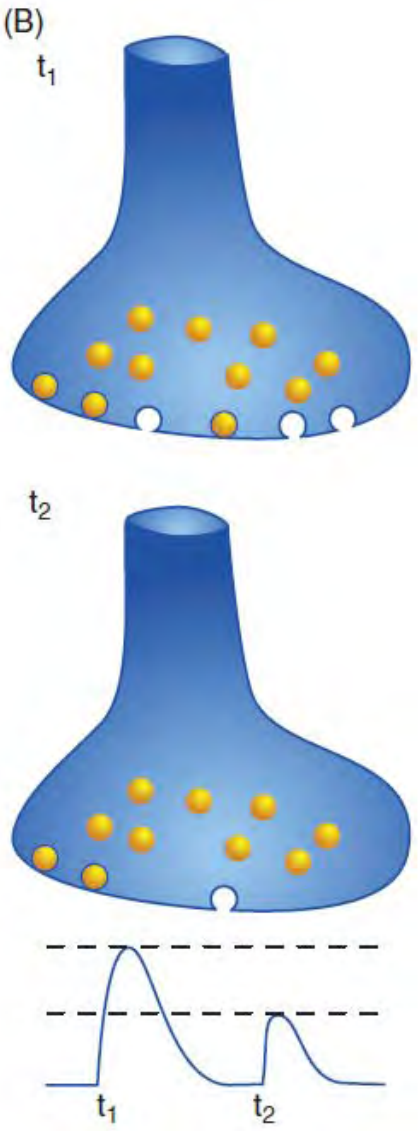
Facilitation

- Residual Ca^{2+}



Depression

- Depletion readily releasable SVs
- Autoinhibition (PreS receptors)
- ↓ Receptor Sensitivity



With multiple presynaptic neurons synapsing on a single postsynaptic neuron, transmission strength can depend upon the previous history of the synapse.

Some cells in the retina use ribbon synapses to encode visual information

- Ribbon synapses are present in vertebrate sensory systems such as in auditory hair cells, in vestibular hair cells, photoreceptors, and retinal bipolar cells.
- They are also found in lower vertebrate pinealocytes in the pineal gland, fish lateral lines, and electroreceptors, as well as in frog saccular or turtle hair cells.
- Some ribbon-type synapses maintain the highest rates of exocytosis documented so far, releasing up to hundreds of SVs per second at an individual synapse for an extended period of time.

Direct pathway

Photoreceptors (Rods and Cones)

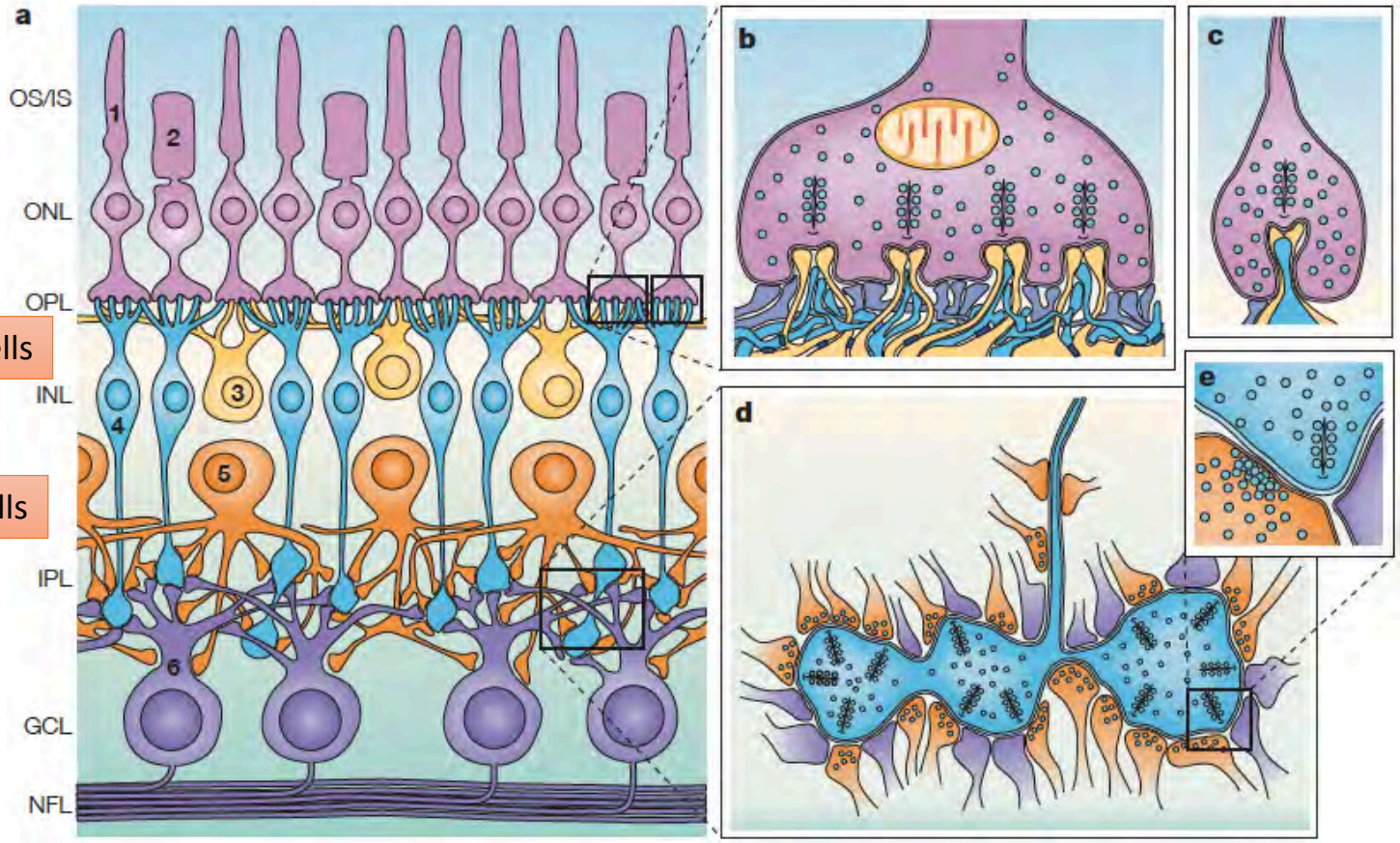
Horizontal Cells

(Rod/Cone) Bipolar Cells

Amacrine Cells

Retinal Ganglion Cells

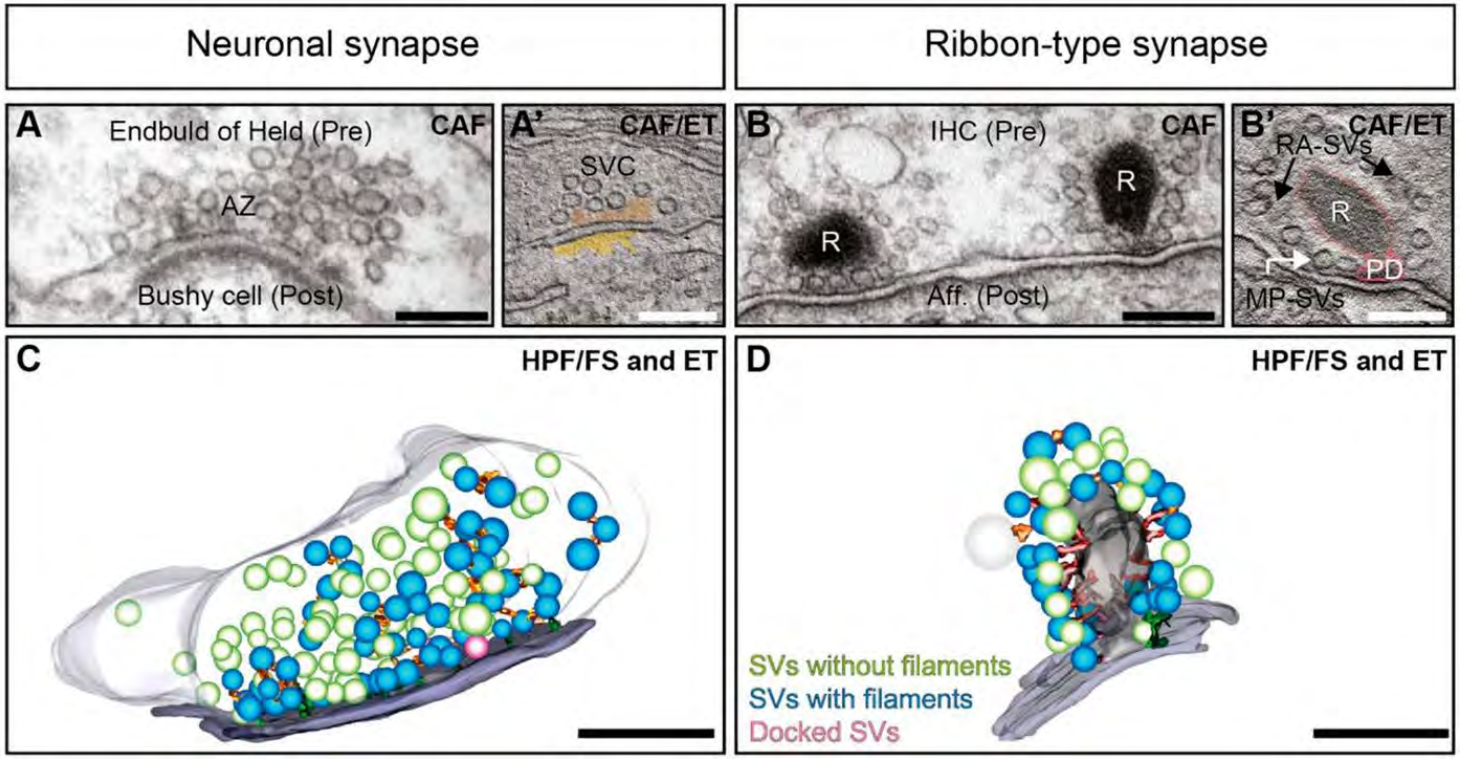
To LGN (via optic nerve)



Ribbon synapses in retina communicate to many cells simultaneously

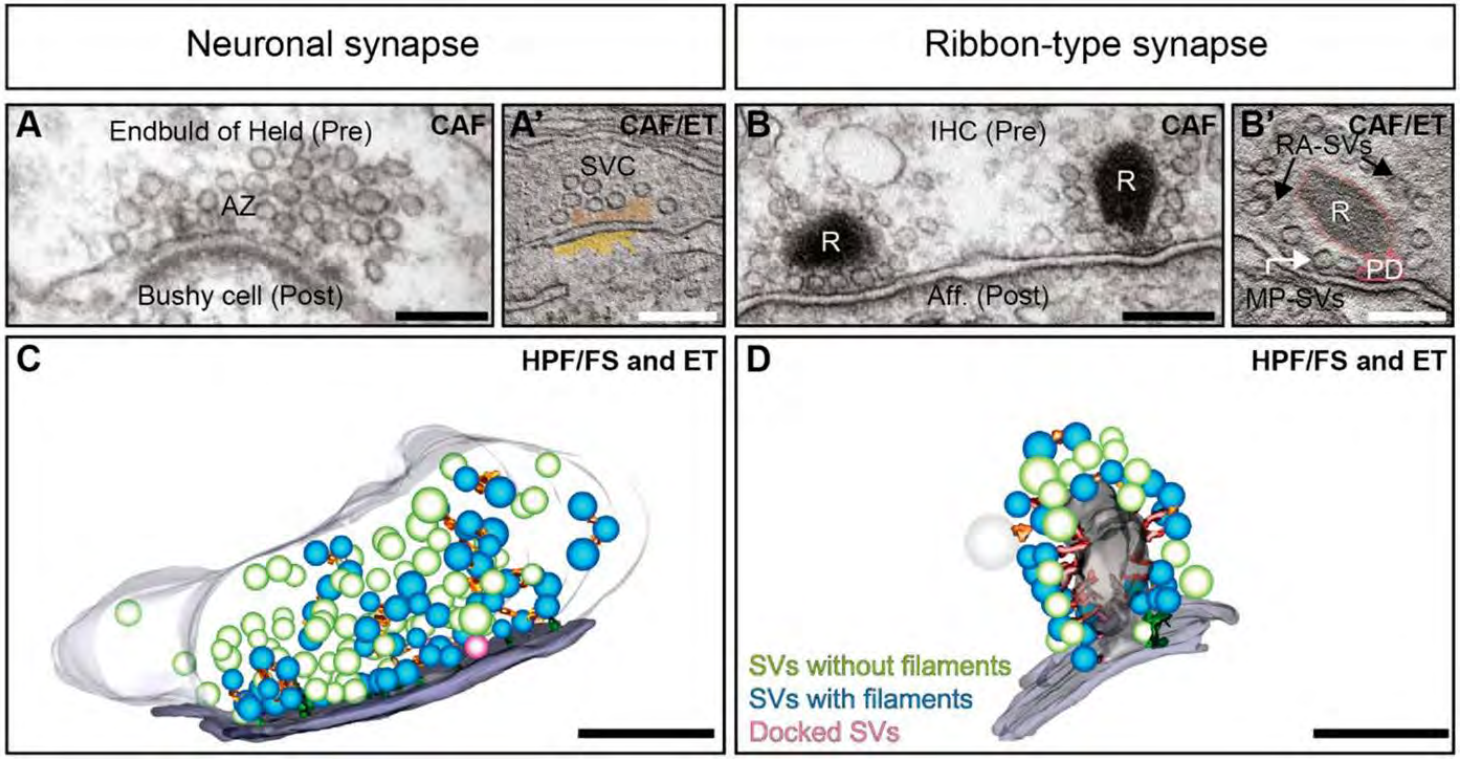
- Photoreceptors (rods/cones) to Bipolar and Horizontal Cells
- Bipolar Cells to Amacrine and Retinal Ganglion Cells

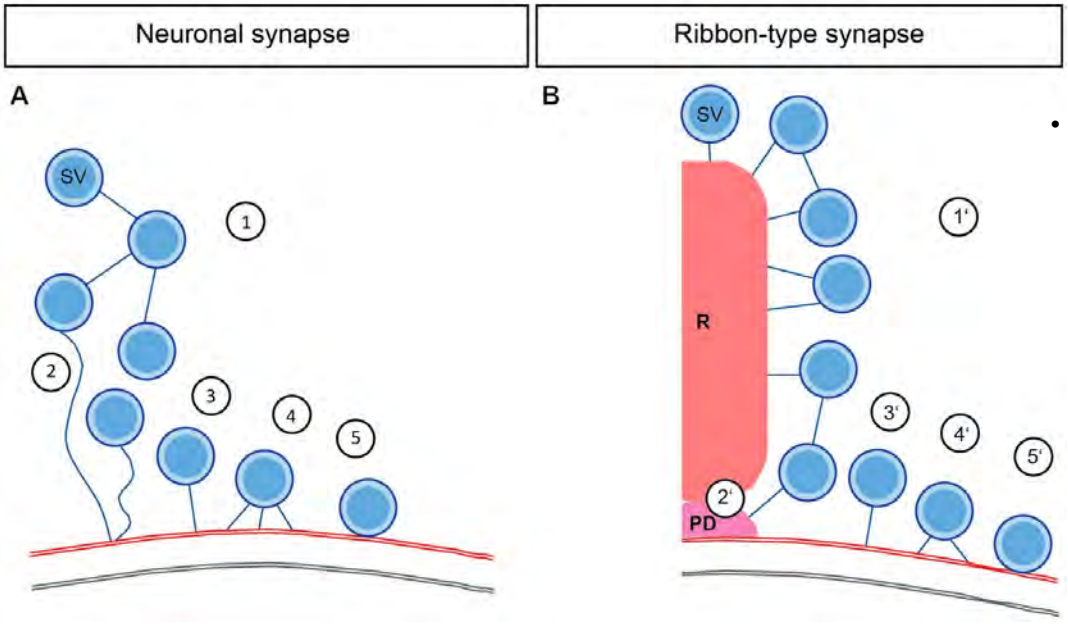
(Wazzle, Nature Reviews 2004)



- Ribbon synapses share a structural specialization appearing as a large electron-dense projection, the synaptic ribbon, which can reach in the photoreceptor a size of several hundreds of nanometers, and this way is capable to cluster a large number of SVs.

(Chakrabarti and Wichmann, Int J Mol Sci., 2019)





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CHAPTER 8

Neurotransmitter Receptors

Binds the NT and transmits the signal to postsynaptic neuron.

*Response type is dependent upon:

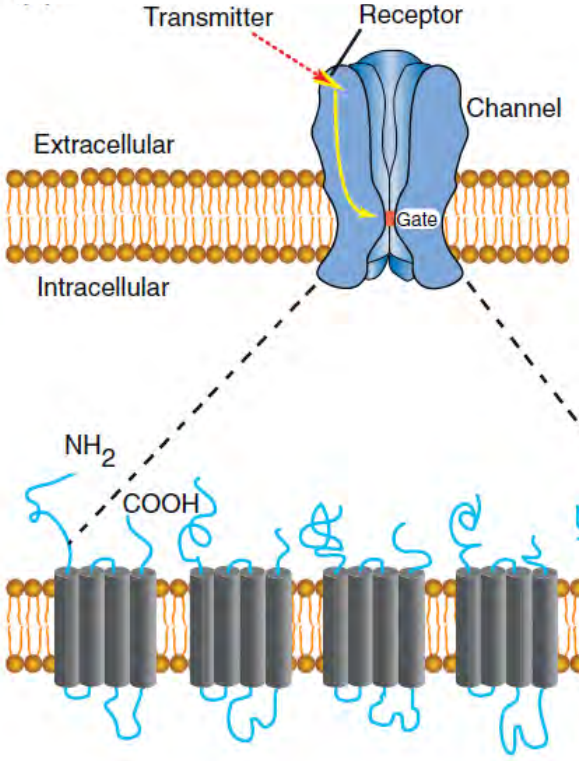
- The type of receptor (excitatory / inhibitory)

*Response magnitude is dependent upon:

- Number of receptors present in the synapse
- "State" of the receptors
- Amount of transmitter release (Quanta)

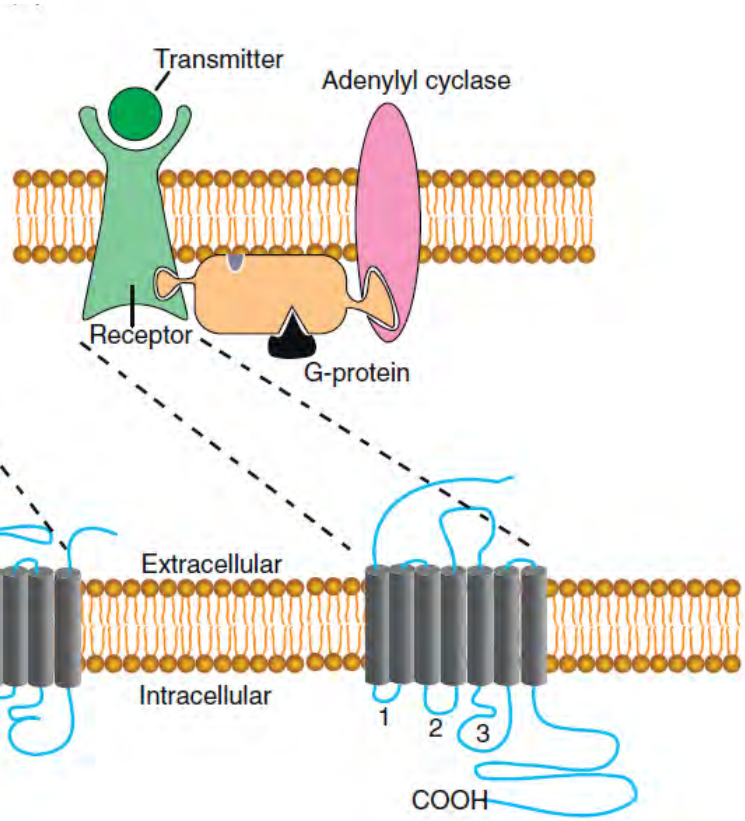
Two Types:

Ionotropic



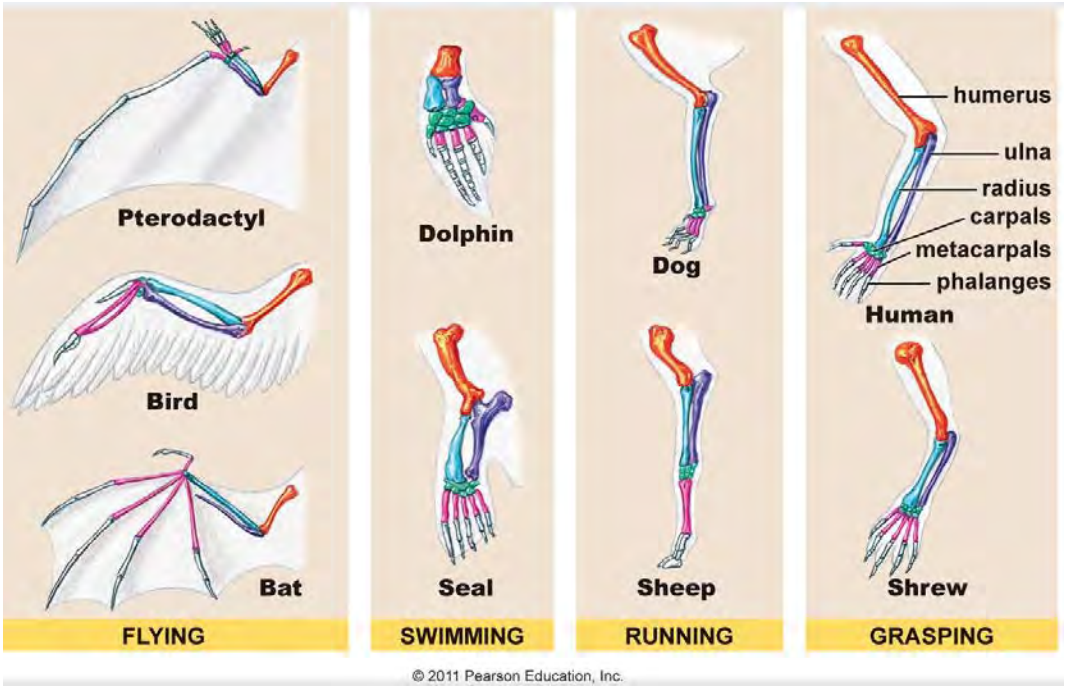
- Multiple proteins/subunits (4-5) combine to form ion channel
  - *Closed → impermeable to ions
  - *Open → ions flow down [ ] gradients
- Ligand-gated ion channels
- **Fast** (milliseconds time scale)
- Induce fast excitatory/inhibitory neurotransmission

Metabotropic (G-protein coupled receptor)



- Single polypeptide (7 TM domains), or can composed of dimers
- Activates G-protein receptors (GDP→GTP)
- Activated G-proteins couple to downstream effectors to alter their activity
- Often open or close neighboring ion channels
- **Slow** (signal transduction lasts tenths of seconds to hours)
- Effects span a broad range of time domains providing CNS with a rich source for temporal information processing that is subject to constant modification

Structure helps to determine function



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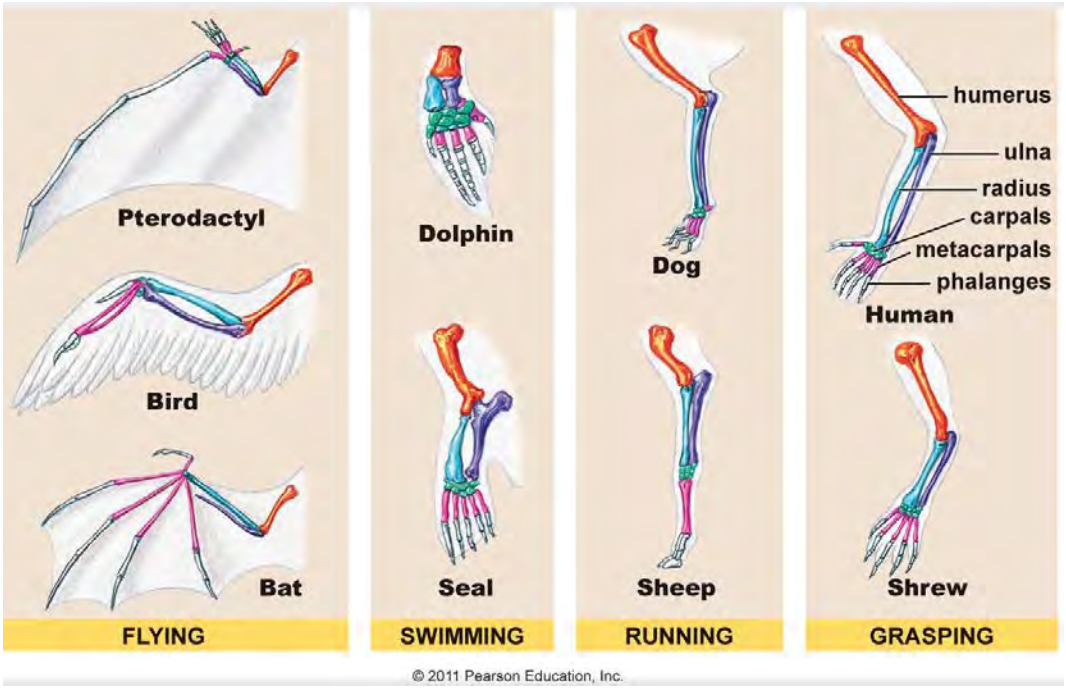
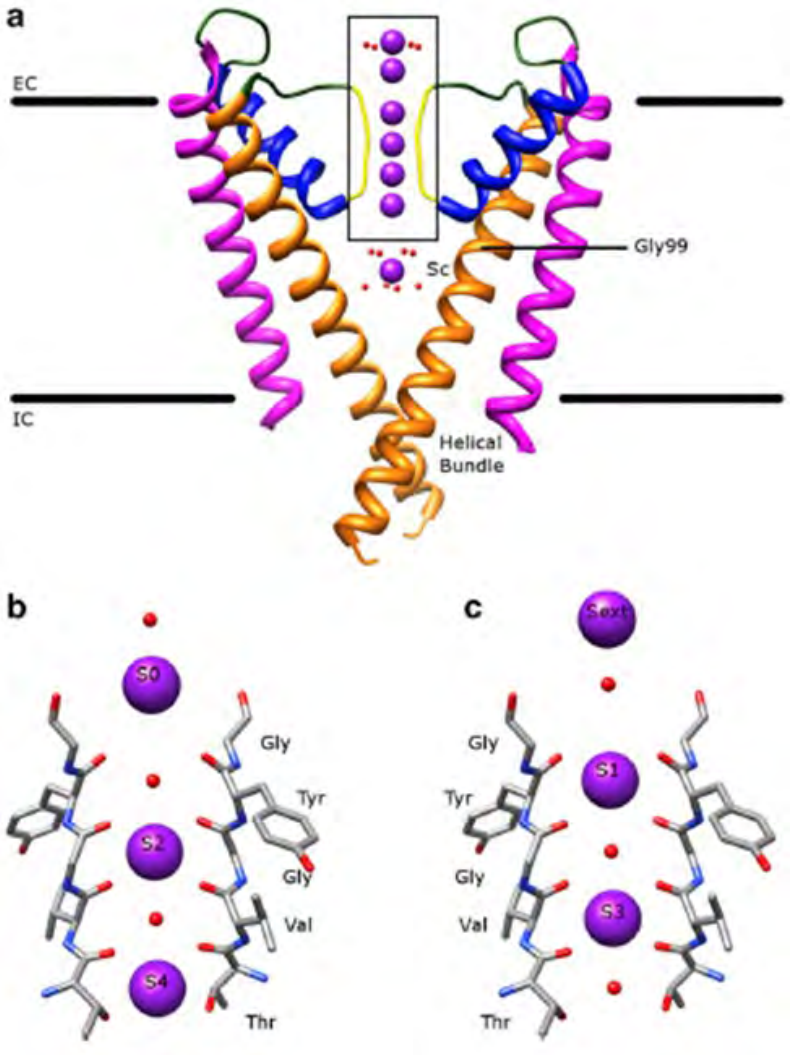
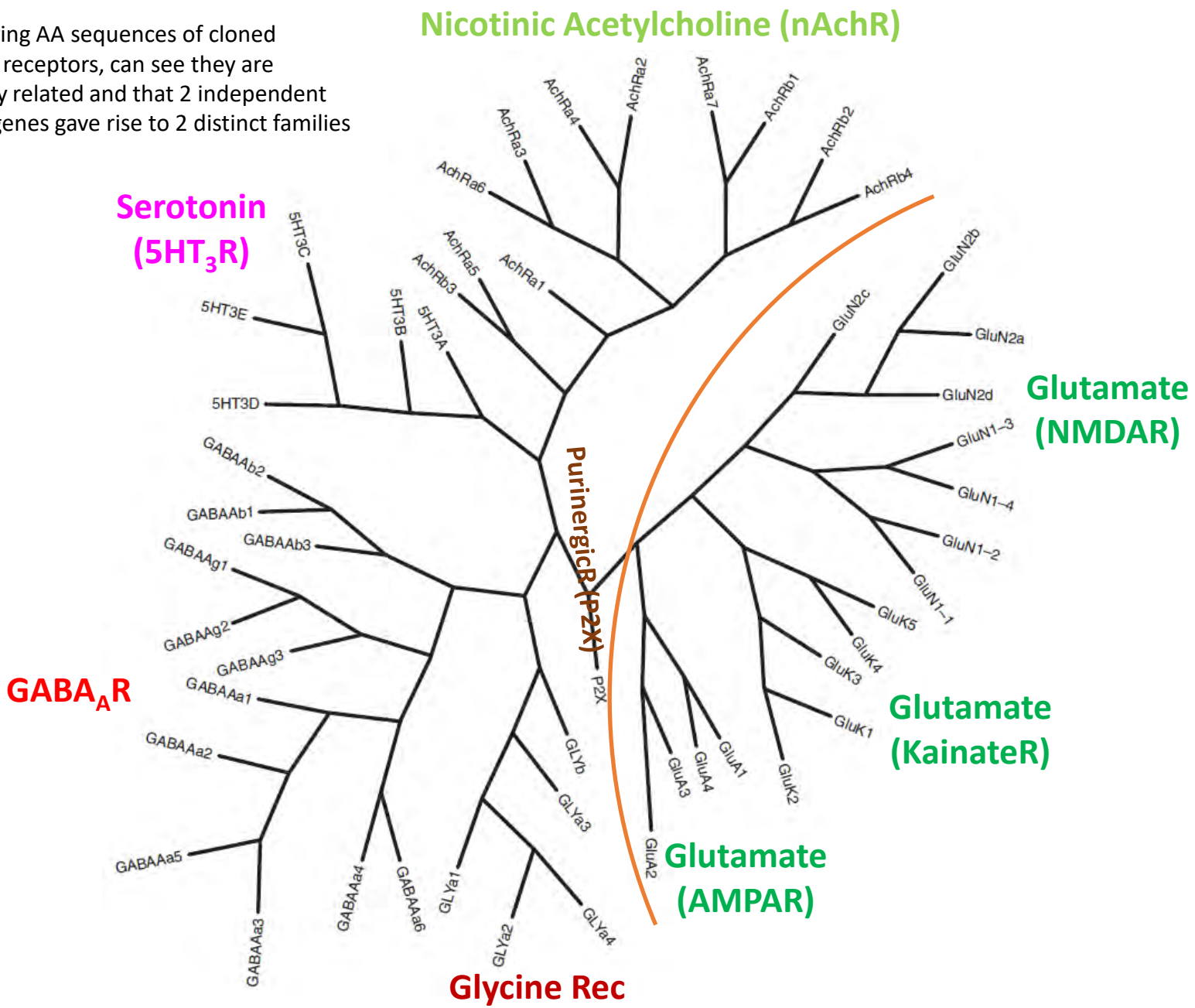




Voltage-gated K^+ channel has selectivity filter
(backbone carbonyls coordinate K^+ ions that are largely stripped of their hydration shells)

Evolutionary relationships of the ionotropic receptor family

By comparing AA sequences of cloned ionotropic receptors, can see they are structurally related and that 2 independent ancestral genes gave rise to 2 distinct families



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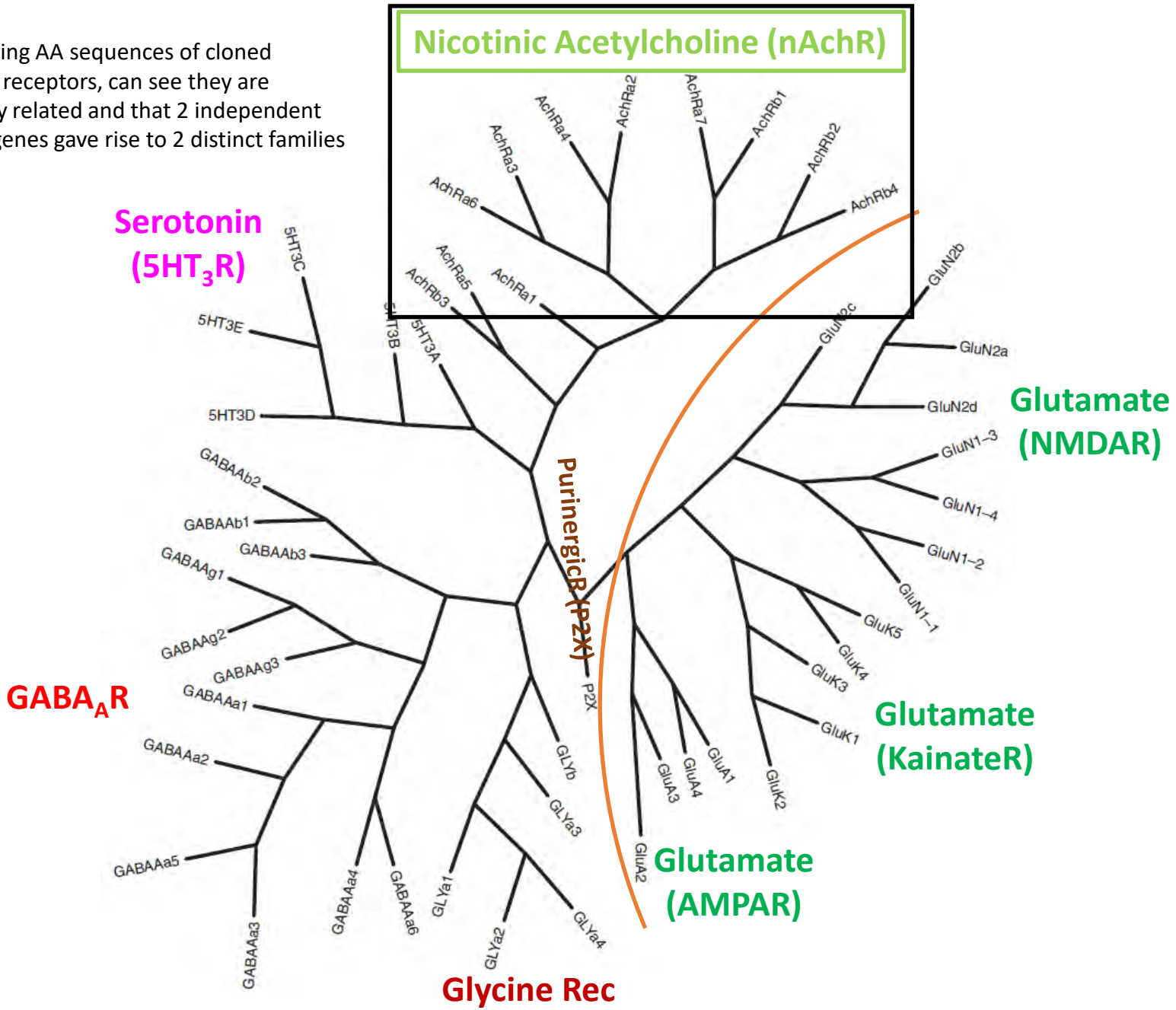
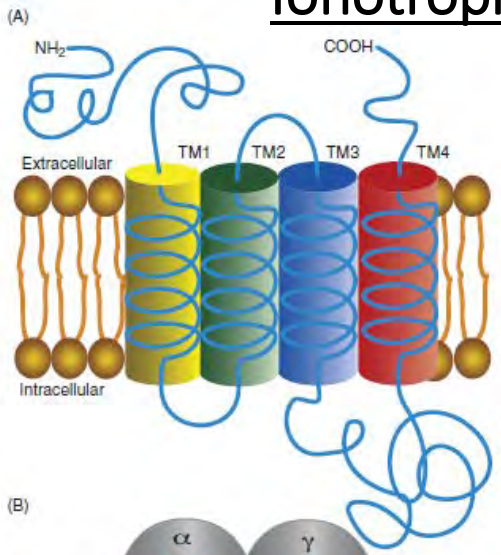
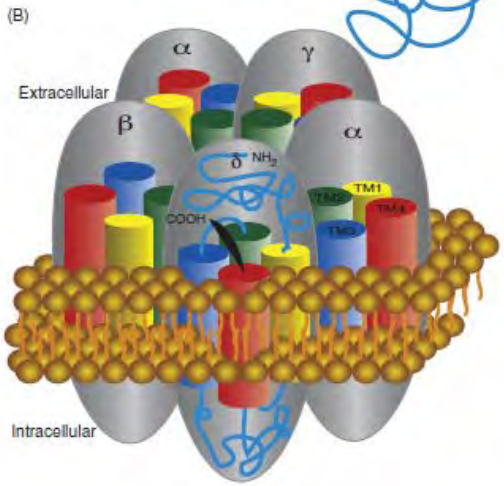
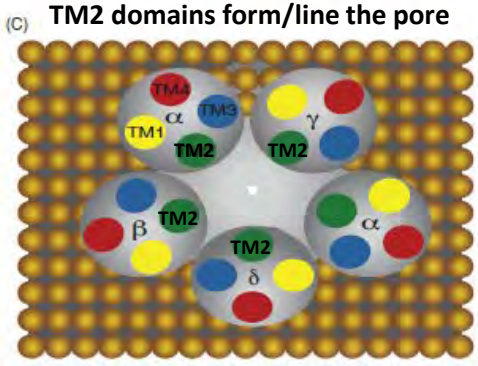


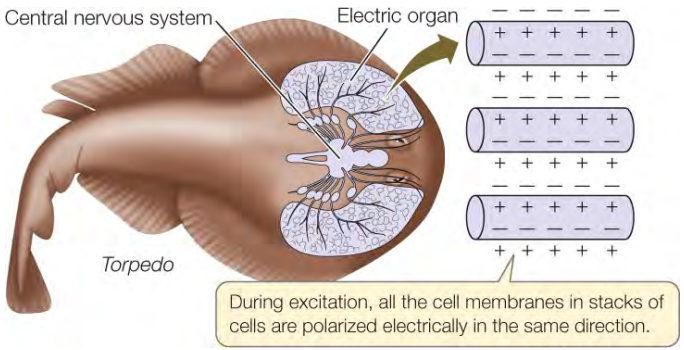
Ionotropic: Nicotinic Acetylcholine Receptor (nAChR)



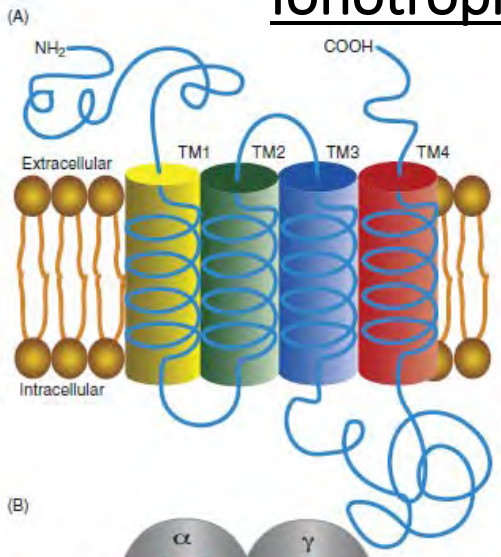




- Isolated from *Torpedo californica* in 1982
- Named for agonist: nicotine
- 4 different subunits: α,β,γ,δ
- Each subunit has 4 transmembrane segments
- Two alpha subunits per receptor
- TM2 domains form ion channel
  - Negative charged AA's line the pore
  - Selectivity filter for Na^+, K^+, Ca^{2+}
- When open ions flow across their concentrations gradients
  - Na^+ & Ca^{2+} → inward
  - K^+ → outward



Ionotropic: Nicotinic Acetylcholine Receptor (nAChR)



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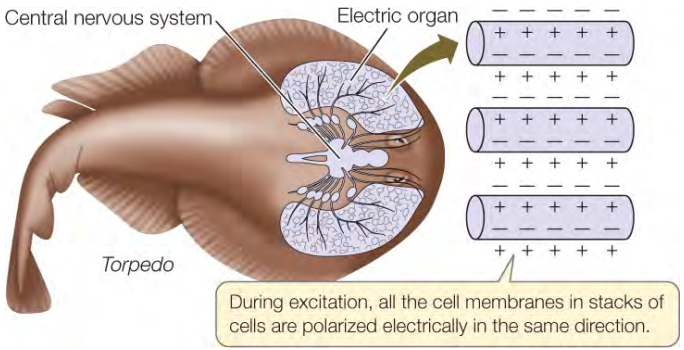
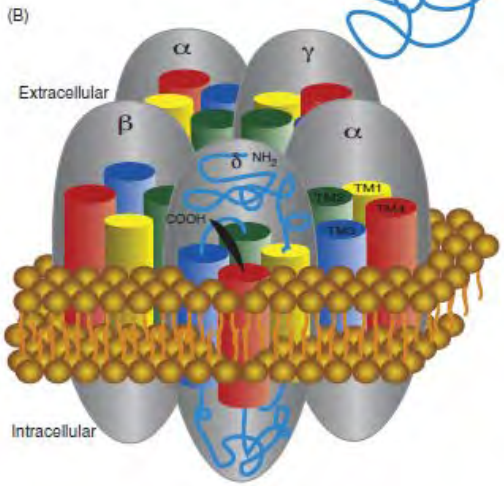
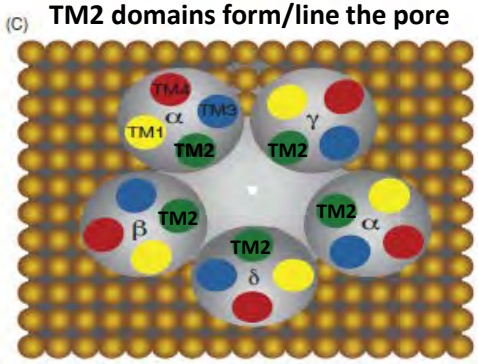
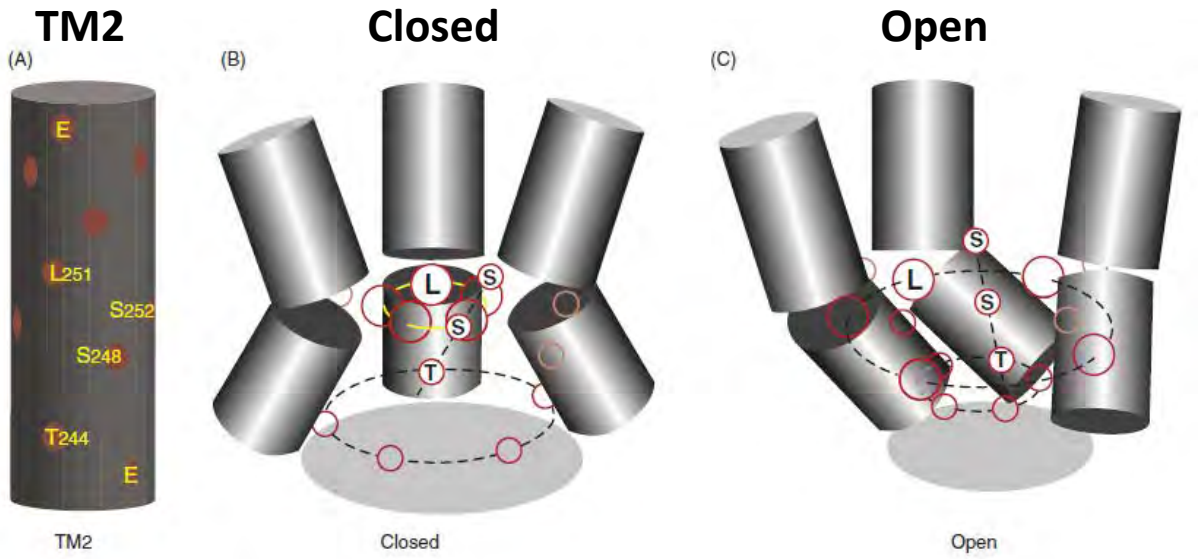




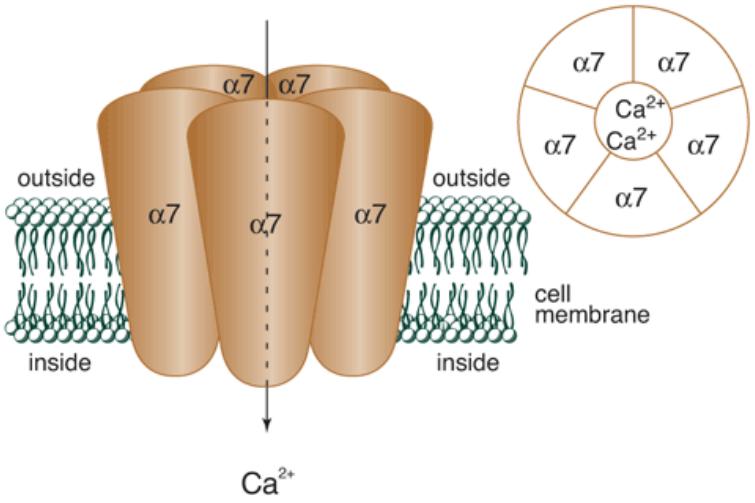




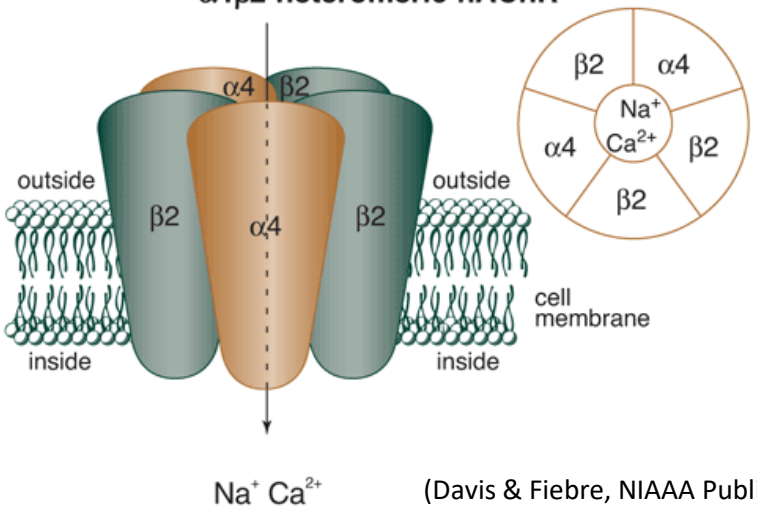
- Each AChR has at least 2 α subunits
  - Location of Ach binding site (2 bound ACh for pore opening)
- Binding for 1st ACh promotes binding of 2nd (cooperativity)
- 2nd ACh binding results in rotation of TM2 segments
  - Pore opens instantaneously (20 μs)

nAChR Assembly

Five α7 subunits form an α7 homo-oligomeric nAChR



Two α4 and three β2 subunits form an α4β2 heteromeric nAChR

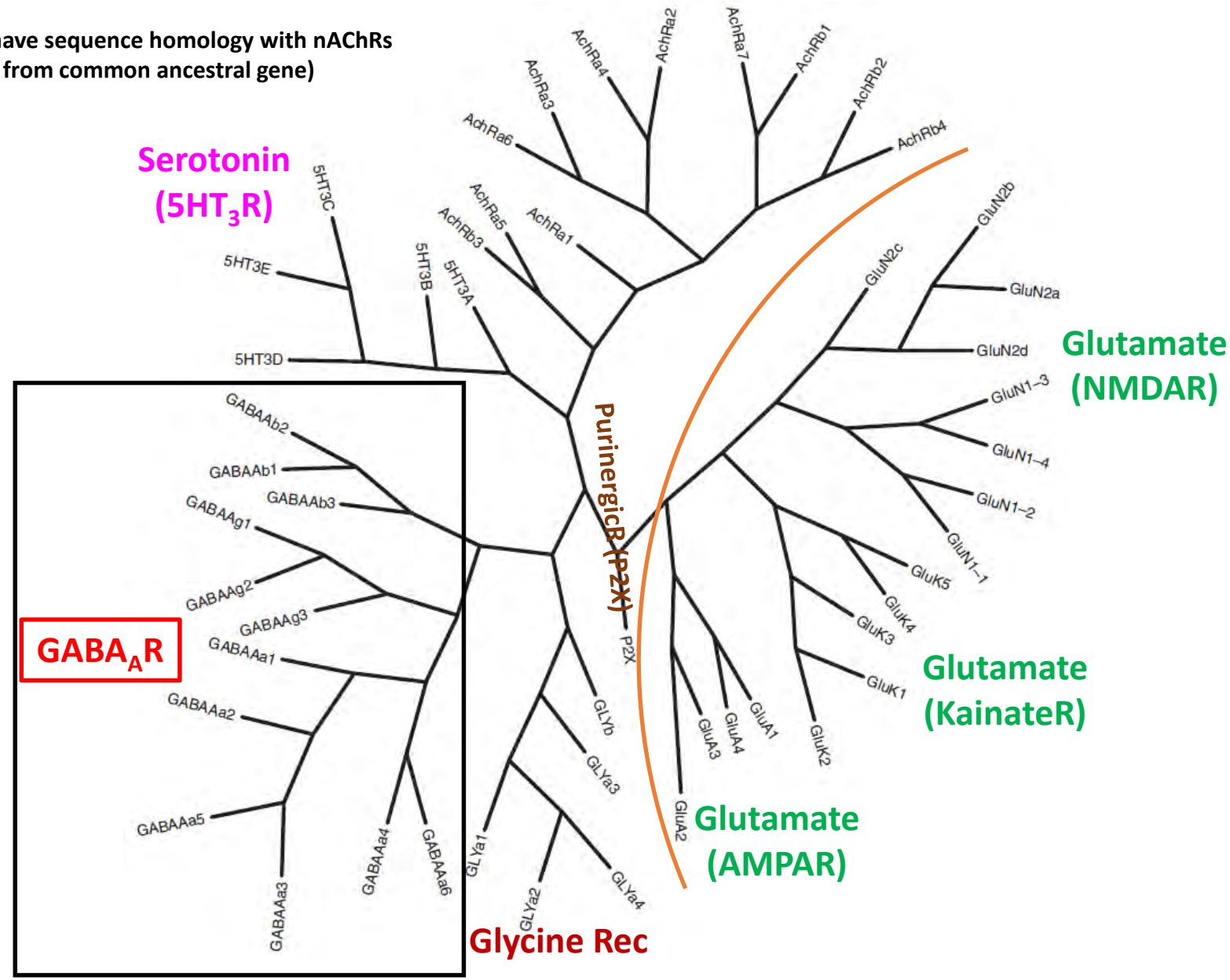


(Davis & Fiebre, NIAAA Publications)

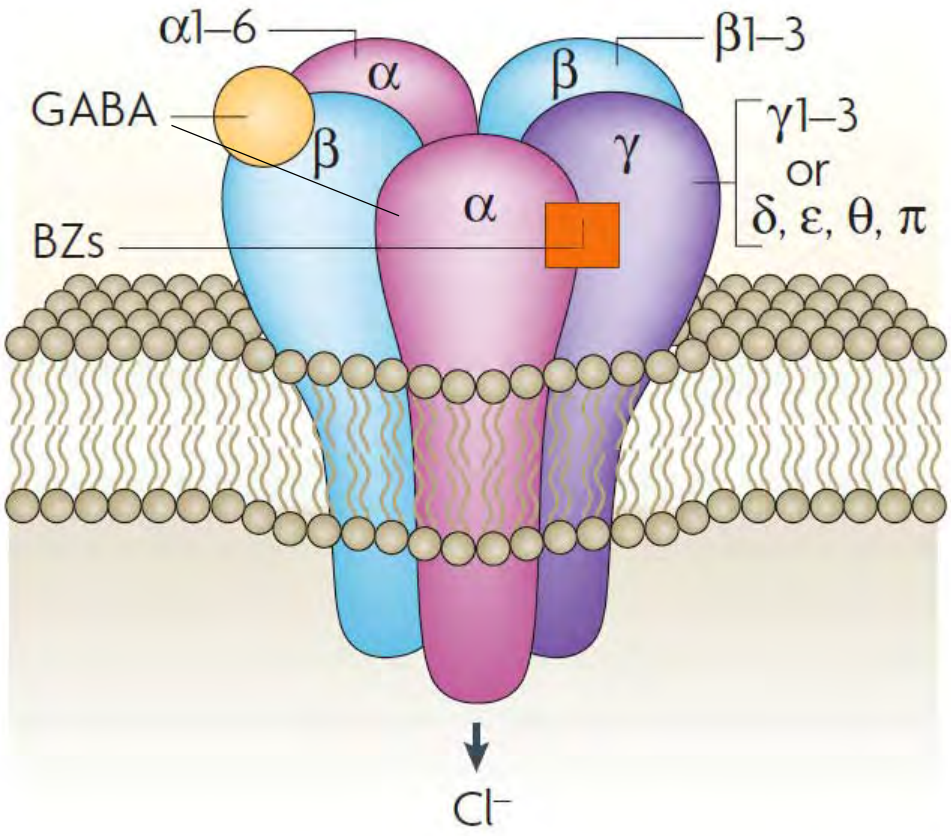
- 208 possible confirmations
- Muscle: $(\alpha 2)_2\beta 1\delta\varepsilon$
- Neuronal: α7
  - Homopentamer
  - α-Bungarotoxin (irreversible)
  - Ca^{2+} influx
- Aβ2
  - Heteropentamer
  - Nicotine binding
  - Ca^{2+}/Na^{+} influx
- Subunits Δ Ca^{2+}/Na^{+} Permeability
- Desensitization Varies (0.1-20s)
- Excitatory Transmission

Evolutionary relationships of the ionotropic receptor family

$GABA_AR$ have sequence homology with nAChRs (diverged from common ancestral gene)



Ionotropic: $GABA_A$ Receptor



Jacob et al., Nature Reviews Neuroscience, 2008

- Like nAChRs, **$GABA_AR$** is composed of 5 subunits forming a heteropentamer of 275 kDa
- Seven types of **$GABA_AR$** subunits (+ subtypes) are found in the brain (+ 1 more in the retina)
- A mix of **$GABA_AR$** subunits associate to form heterogeneous receptors with distinct pharmacological and electrophysiological properties
- Predominant **$GABA_AR$** in the brain and spinal cord has stoichiometry of two α1s, two β2s, and one γ2
- Selective for Cl^- (inhibitory/hyperpolarizing current)
- Selectivity conferred by AA residues at TM2 near end of pore
- **Agonists** (↑ Cl^- influx → hyperpolarization/inhibition)
  - Barbituates (prolongs open state of the channel)
  - Benzodiazepines (↑ channel opening frequency)
- **Antagonists** (↓ Cl^- influx → disinhibition)
  - Picrotoxin (binds to the channel preventing Cl^- influx)
  - Bicuculline (decreases GABA binding)
  - Steroid metabolites (progesterone, corticosterone and testosterone) have potentiating effects
  - Penicillin (binds within channel pore)
  - All of these at high [ ] can produce seizures

THE JOURNAL OF COMPARATIVE NEUROLOGY 359:154–194 (1995)

$GABA_A$-Receptor Heterogeneity in the Adult Rat Brain: Differential Regional and Cellular Distribution of Seven Major Subunits

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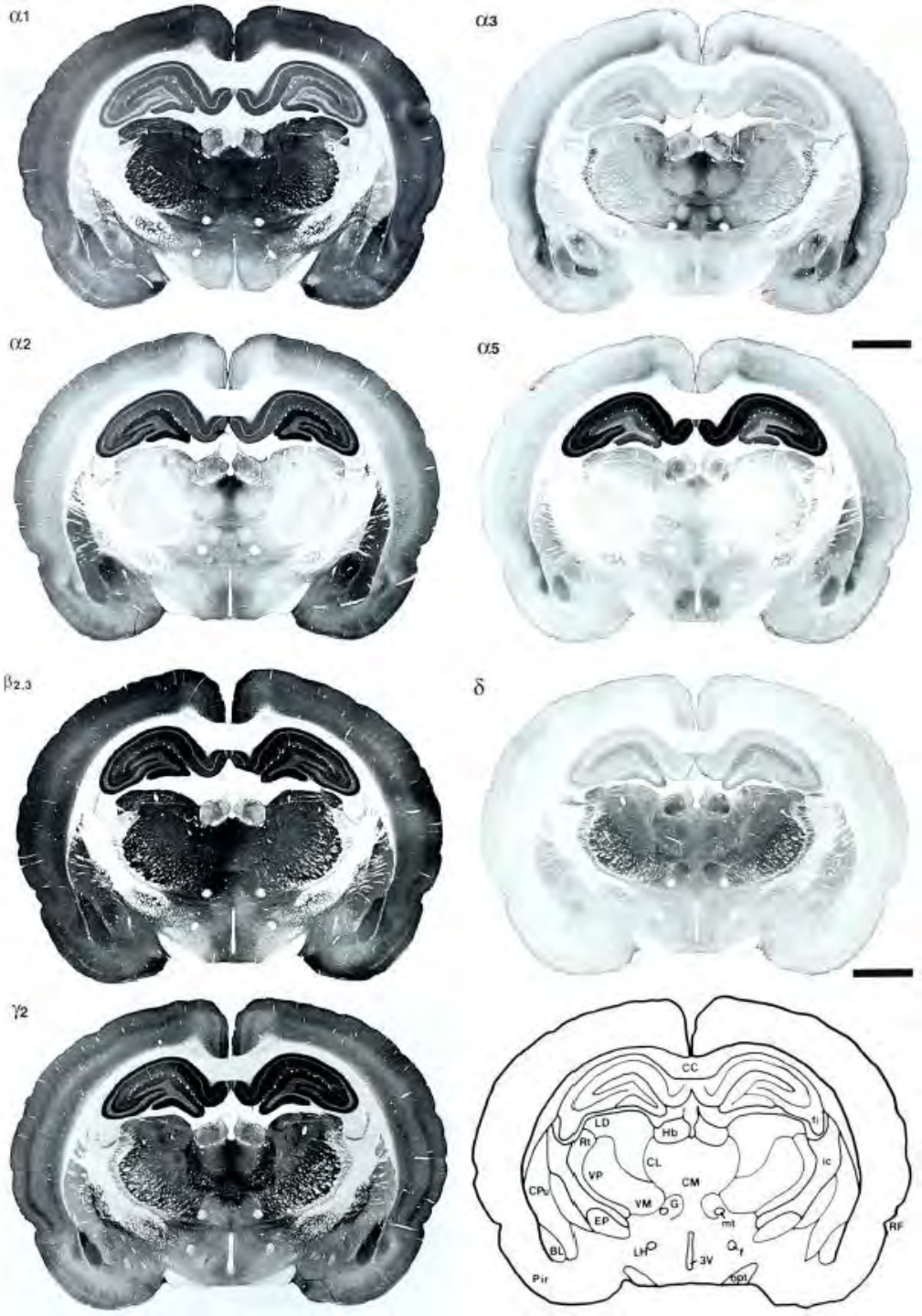
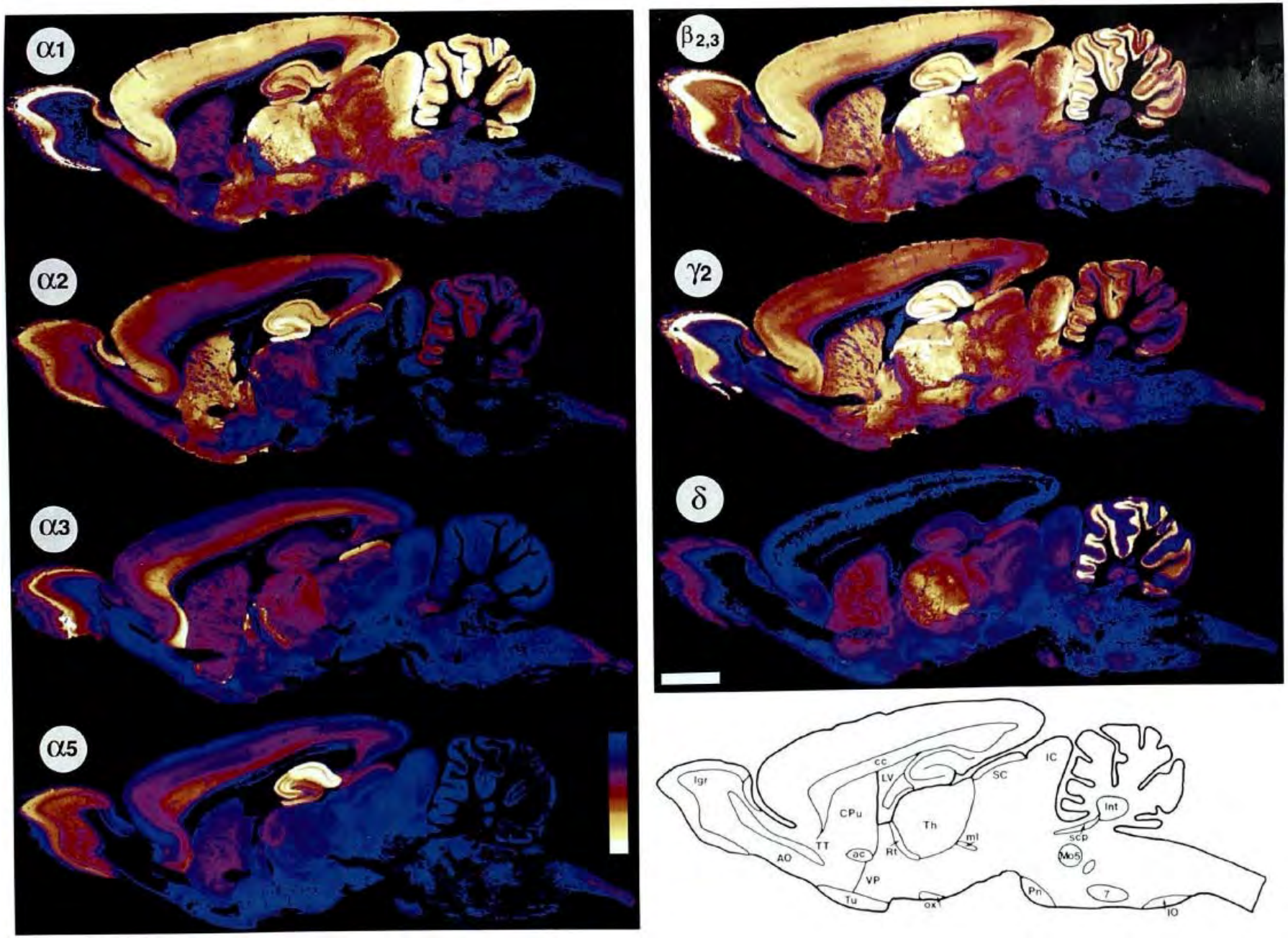
Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland

$GABA_A$-receptors display an extensive structural heterogeneity based on the differential assembly of a family of at least 15 subunits (α1–6, β1–3, γ1–3, θ, ρl–2) into distinct heteromeric receptor complexes. The subunit composition of receptor subtypes is expected to determine their physiological properties and pharmacological profiles, thereby contributing to flexibility in signal transduction and allosteric modulation. In heterologous expression systems, functional receptors require a combination of α-, β-, and γ-subunit variants, the γ2-subunit being essential to convey a classical benzodiazepine site to the receptor. The subunit composition and stoichiometry of native $GABA_A$-receptor subtypes remain unknown. The aim of this study was to identify immunohistochemically the main subunit combinations expressed in the adult rat brain and to allocate them to identified neurons. The regional and cellular distribution of seven major subunits (α1, α2, α3, α5, β2,3, γ2, δ) was visualized by immunoperoxidase staining with subunit-specific antibodies (the β2- and β3-subunits were covisualized with the monoclonal antibody bd-17). Putative receptor subtypes were identified on the basis of colocalization of subunits within individual neurons, as analyzed by confocal laser microscopy in double- and triple-immunofluorescence staining experiments. The results reveal an extraordinary heterogeneity in the distribution of $GABA_A$-receptor subunits, as evidenced by abrupt changes in immunoreactivity along well-defined cytoarchitectonic boundaries and by pronounced differences in the cellular distribution of subunits among various types of neurons. Thus, functionally and morphologically diverse neurons were characterized by a distinct $GABA_A$-receptor subunit repertoire. The multiple staining experiments identified 12 subunit combinations in defined neurons. The most prevalent combination was the triplet α1/β2,3/γ2, detected in numerous cell types throughout the brain. An additional subunit (α2, α3, or δ) sometimes was associated with this triplet, pointing to the existence of receptors containing four subunits. The triplets α2/β2,3/γ2, α3/β2,3/γ2, and α5/β2,3/γ2 were also identified in discrete cell populations. The prevalence of these seven combinations suggest that they represent major GABAA-receptor subtypes. Five combinations also apparently lacked the β2,3-subunits, including one devoid of γ2-subunit (α1/α2/γ2, α2/γ2, α3/γ2, α2/α3/γ2, α2/α5/δ). These combinations were selectively associated with small neuron populations, thereby representing minor $GABA_A$ receptor subtypes. These results provide the basis for a functional analysis of $GABA_A$-receptor subtypes of known subunit composition and may open the way for unproved therapeutic approaches based on the development of subtype-selective drugs.



Differential Regional and Cellular Distribution of Seven Major $GABA_A$ Receptor Subunits



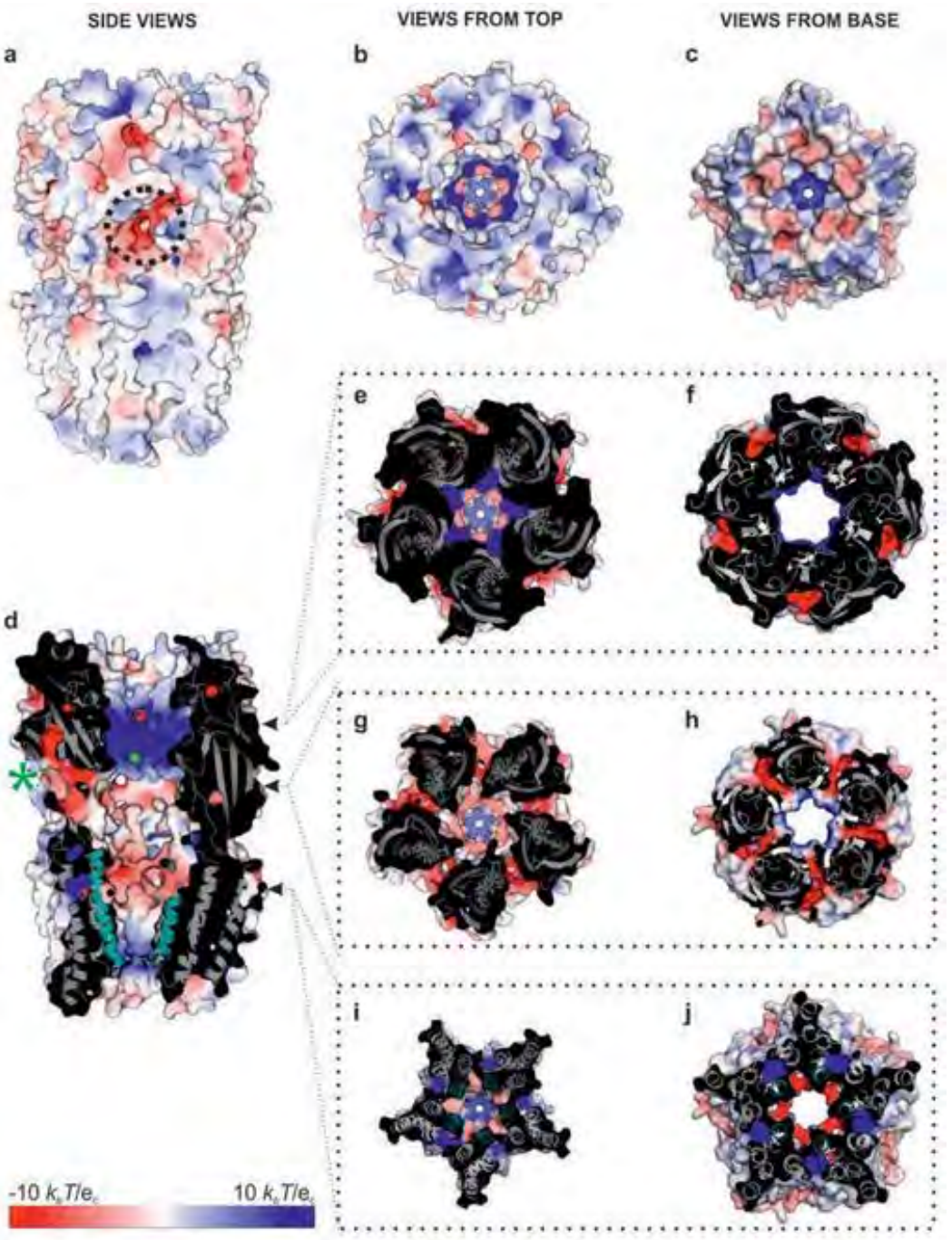
(Fritschy and Mohler, J Comp Neurol., 1995)

Ionotropic: $GABA_A$ Receptor

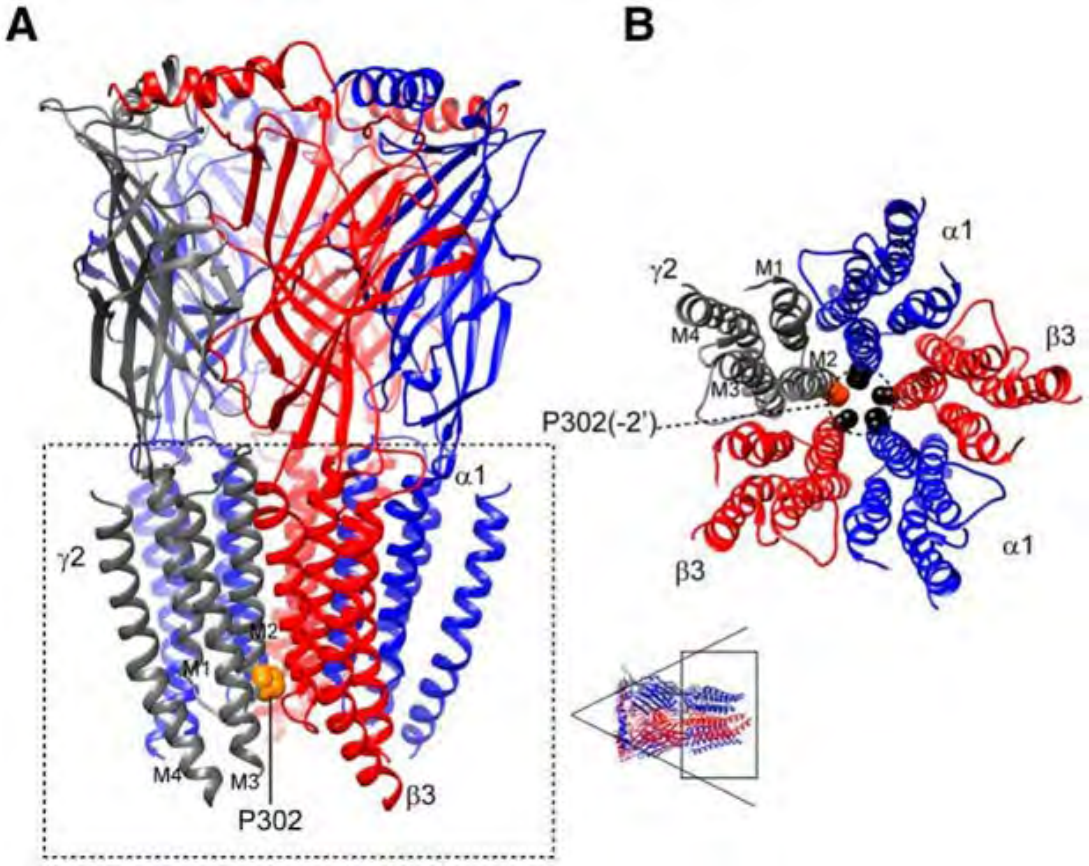
Crystal structure of a human $GABA_A$ receptor (Nature, 2014)

Paul S. Miller[1] & A. Radu Aricescu[1]

*Paul Miller and Radu Aricescu report the first X-ray crystal structure of the human $GABA_A$ receptor.

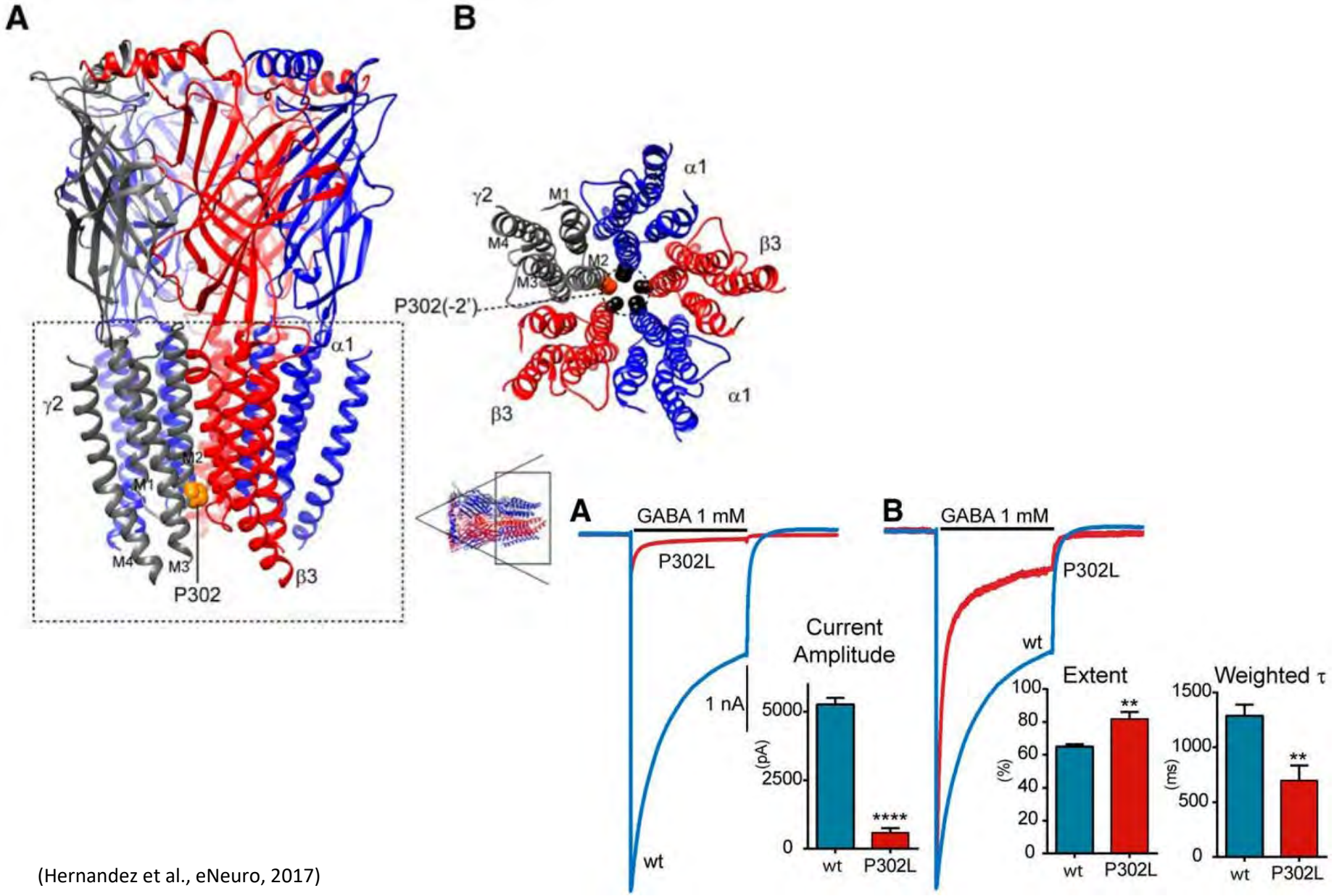


The *de novo* γ2(P302L) subunit was an evolutionary conserved residue in the pore region of the $GABA_A$ receptor



(Hernandez et al., eNeuro, 2017)

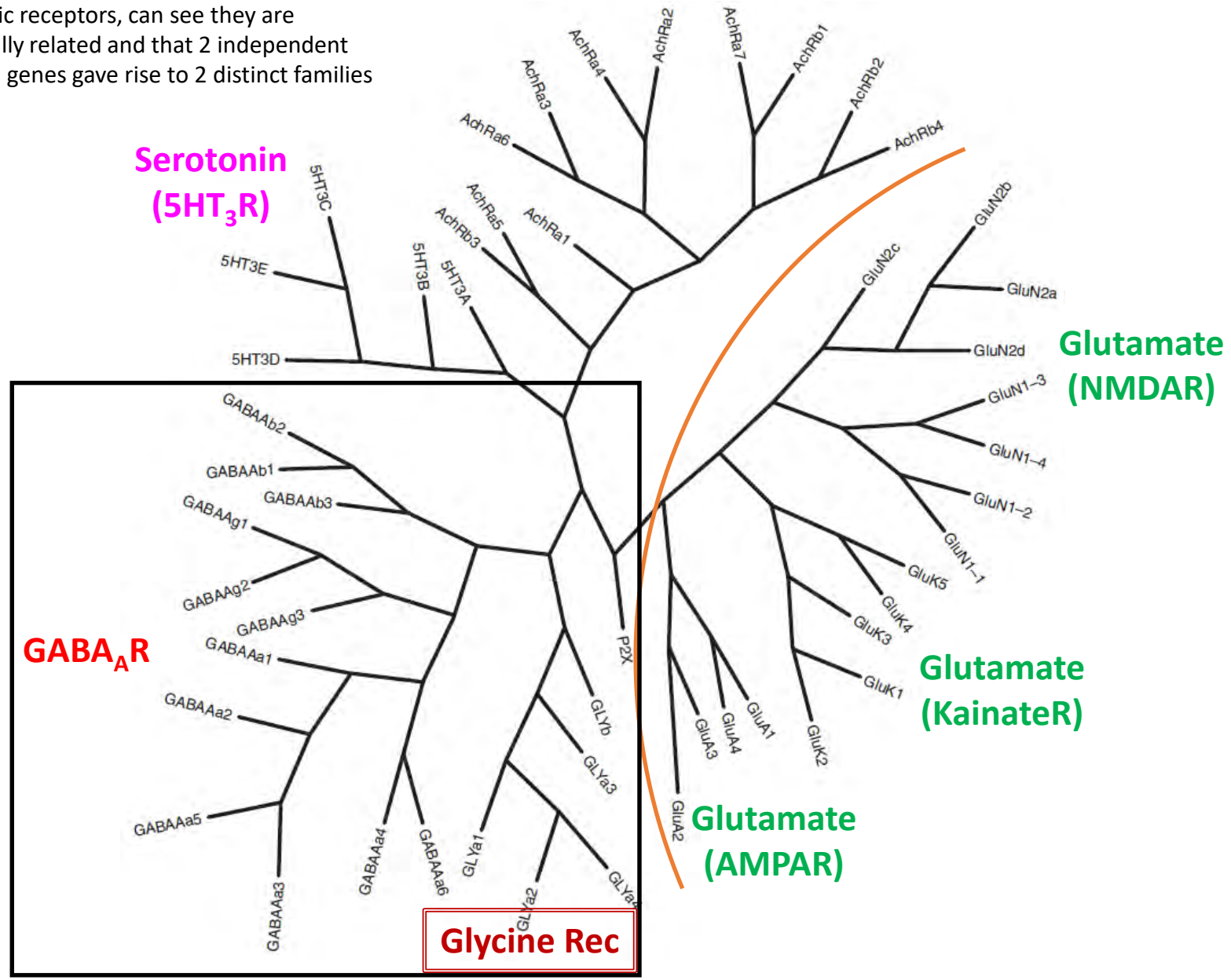
The *de novo* γ2(P302L) subunit mutation reduces GABA-activated currents and enhances desensitization



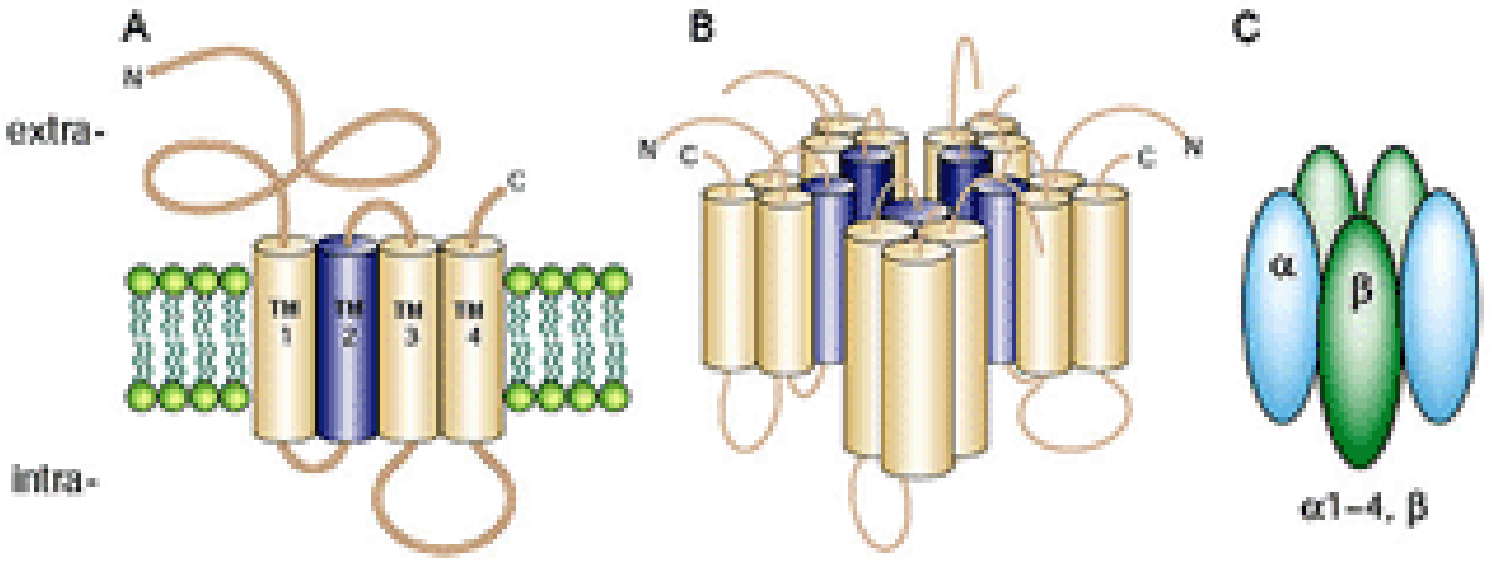
(Hernandez et al., eNeuro, 2017)

Evolutionary relationships of the ionotropic receptor family

By comparing AA sequences of cloned ionotropic receptors, can see they are structurally related and that 2 independent ancestral genes gave rise to 2 distinct families



Ionotropic: Glycine Receptor



(Moss & Smart, 2001)

- Closely related to $GABA_A$ Receptor (not as diverse)
  - Major inhibitory receptors of brain stem and spinal cord
  - Ion channels permeable to the anion Cl^- (similar conductance to $GABA_AR$)
  - Strychnine (rat poison) is a potent antagonist
- Heteropentamer composed of 2 main subunits (α and β)
  - Most likely (3α2β)
  - α subunits are pore-forming unit (single expression of α subunits in oocytes result in functional glycine receptors)
  - β subunits are modulatory (e.g. affect sensitivity to picrotoxin)

**Changing 3 AA/residues in TM2 segment can change selectivity from (–) to (+)

Evolutionary relationships of the ionotropic receptor family

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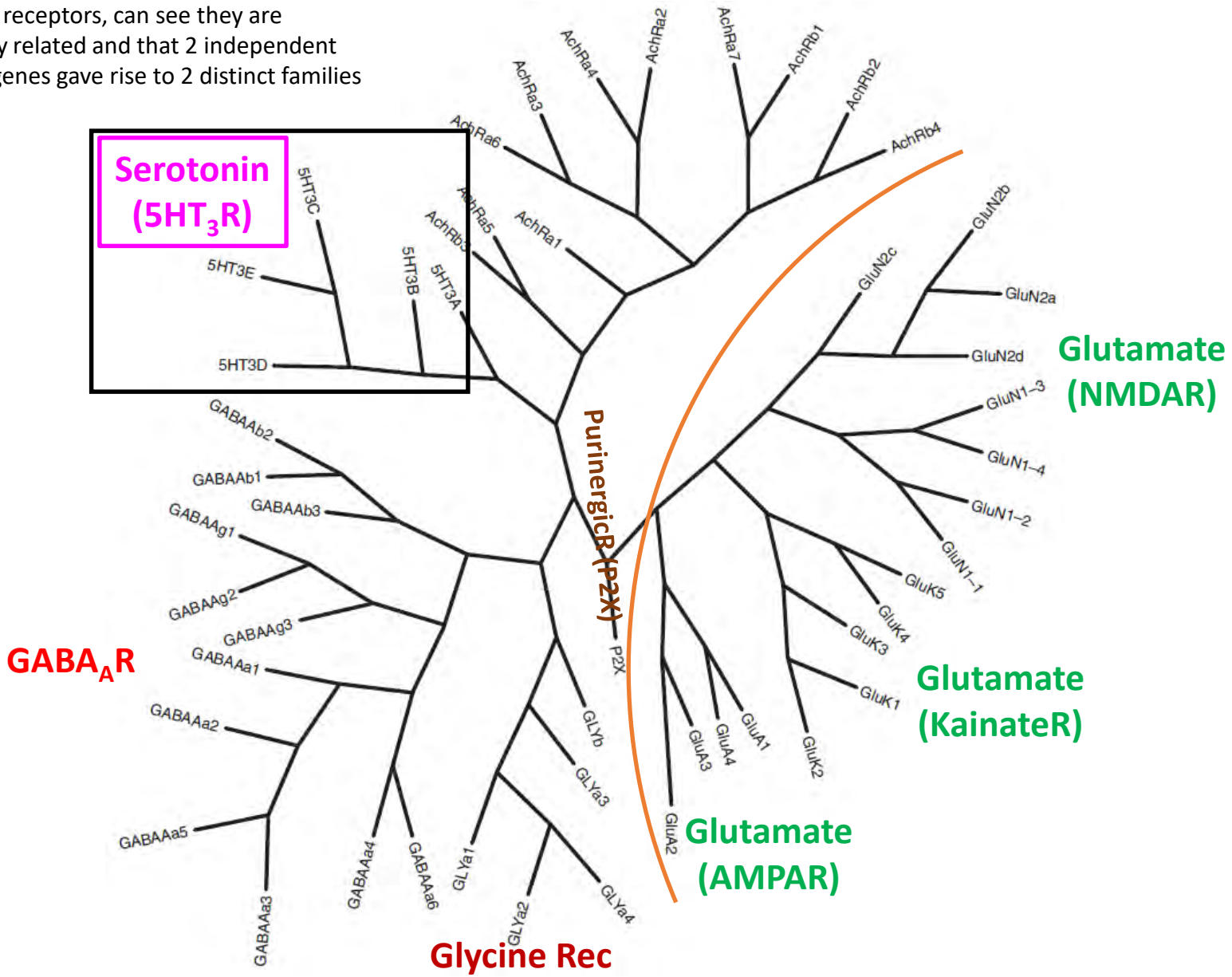
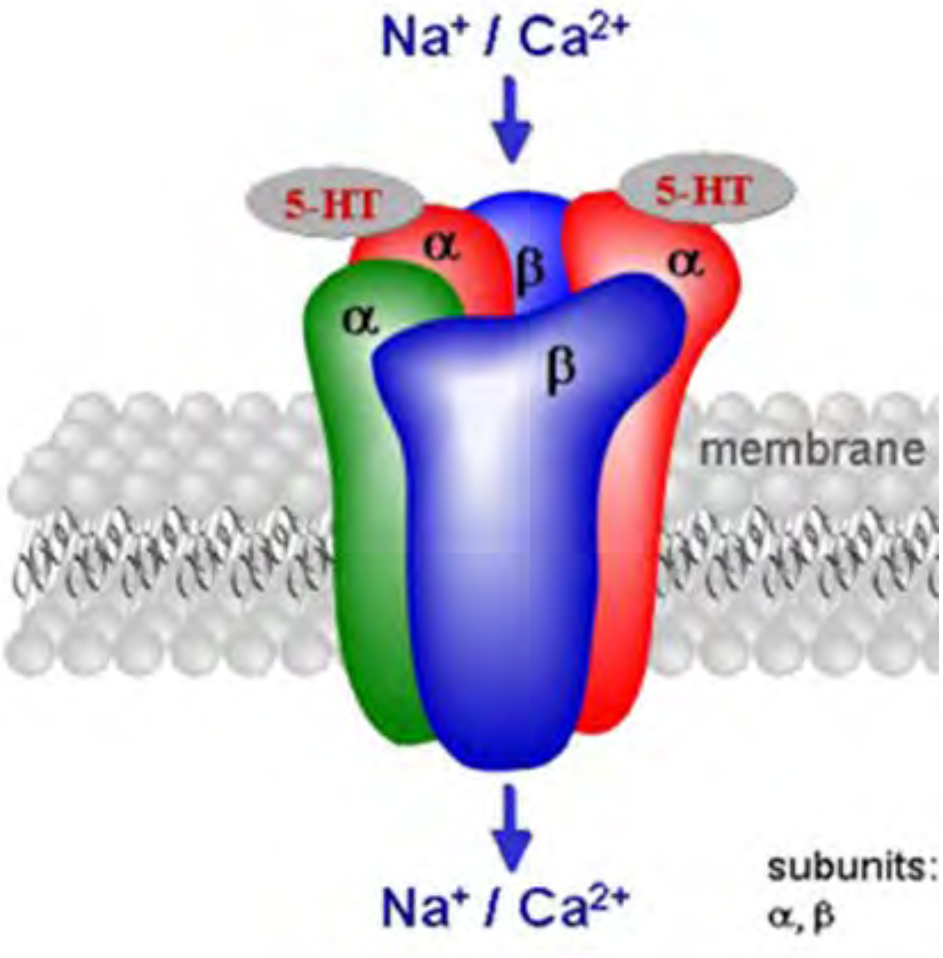


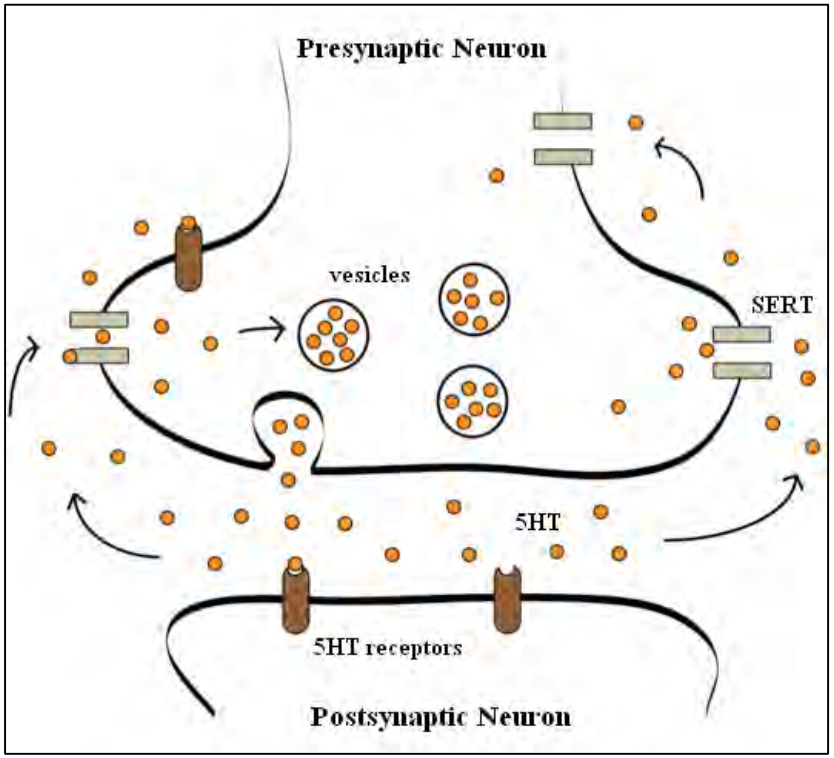
Ionotropic: Serotonin Receptor (5-HT_3R)



- Subunits: 5-HT_{3A-E}
  - Homo or heteropentamer
  - Similar to nAChRs
- Permeability:
  - $Na^+/K^+/Ca^{2+}$
- Slow Channel Opening
  - 10x slower
- Slow Desensitization
  - 1-5 seconds
- Antagonists
  - Antiemetics
  - Anxiolytics
  - Antipsychotics

(Rammes et al., Molecular Psychiatry, 2004)

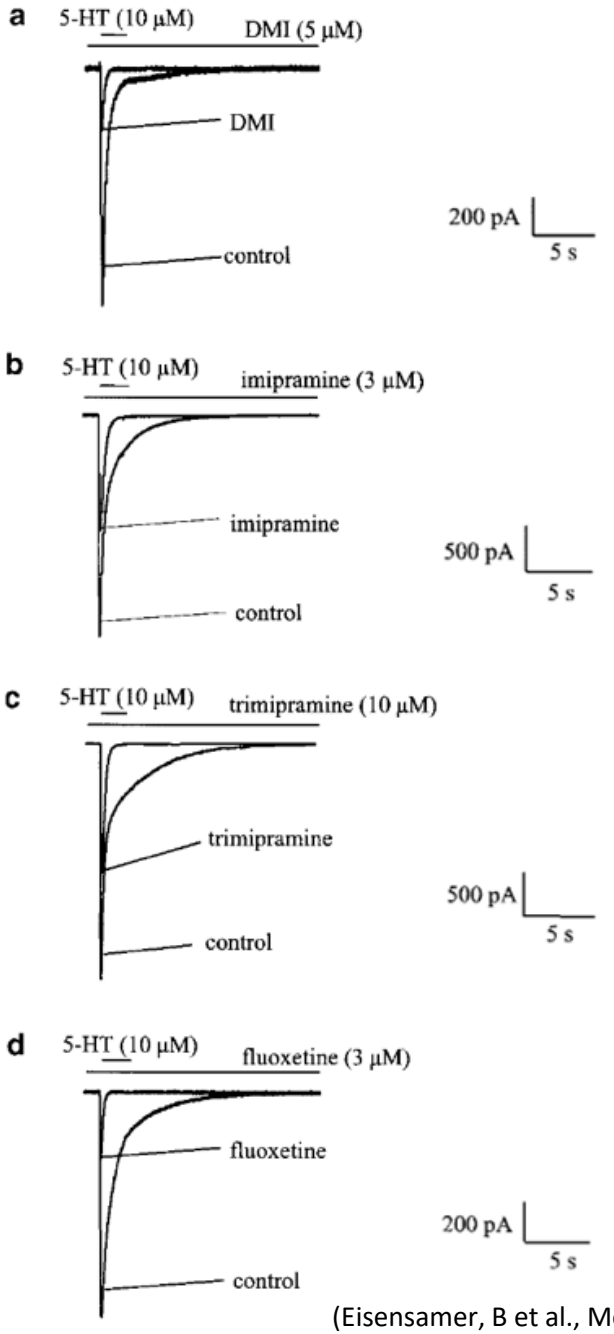
$5HT_3$ receptors are implicated in treatment of depression



Antidepressants

SERT inhibitor

5HT receptor antagonist

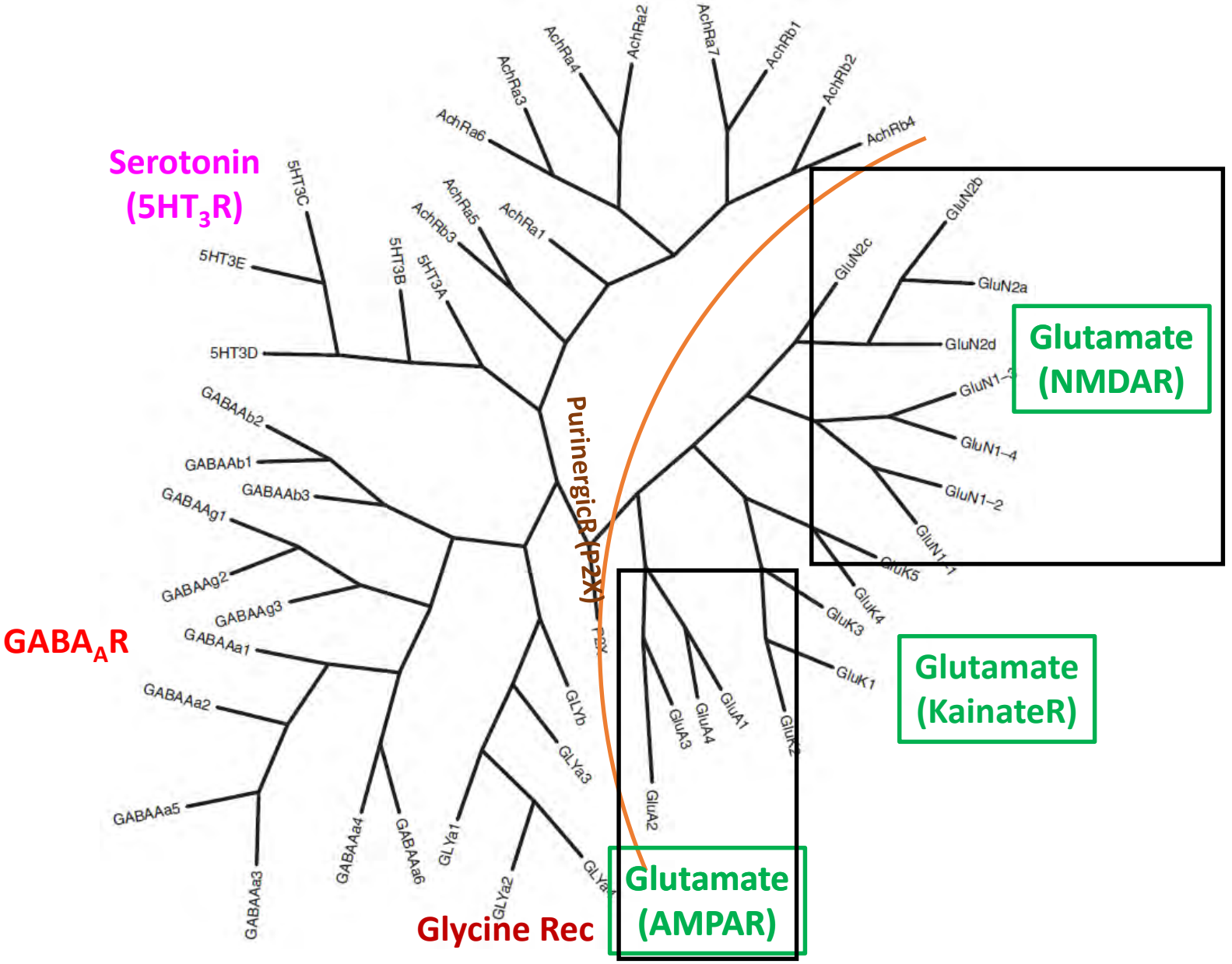


(Eisensamer, B et al., Mol. Psych., 2003)

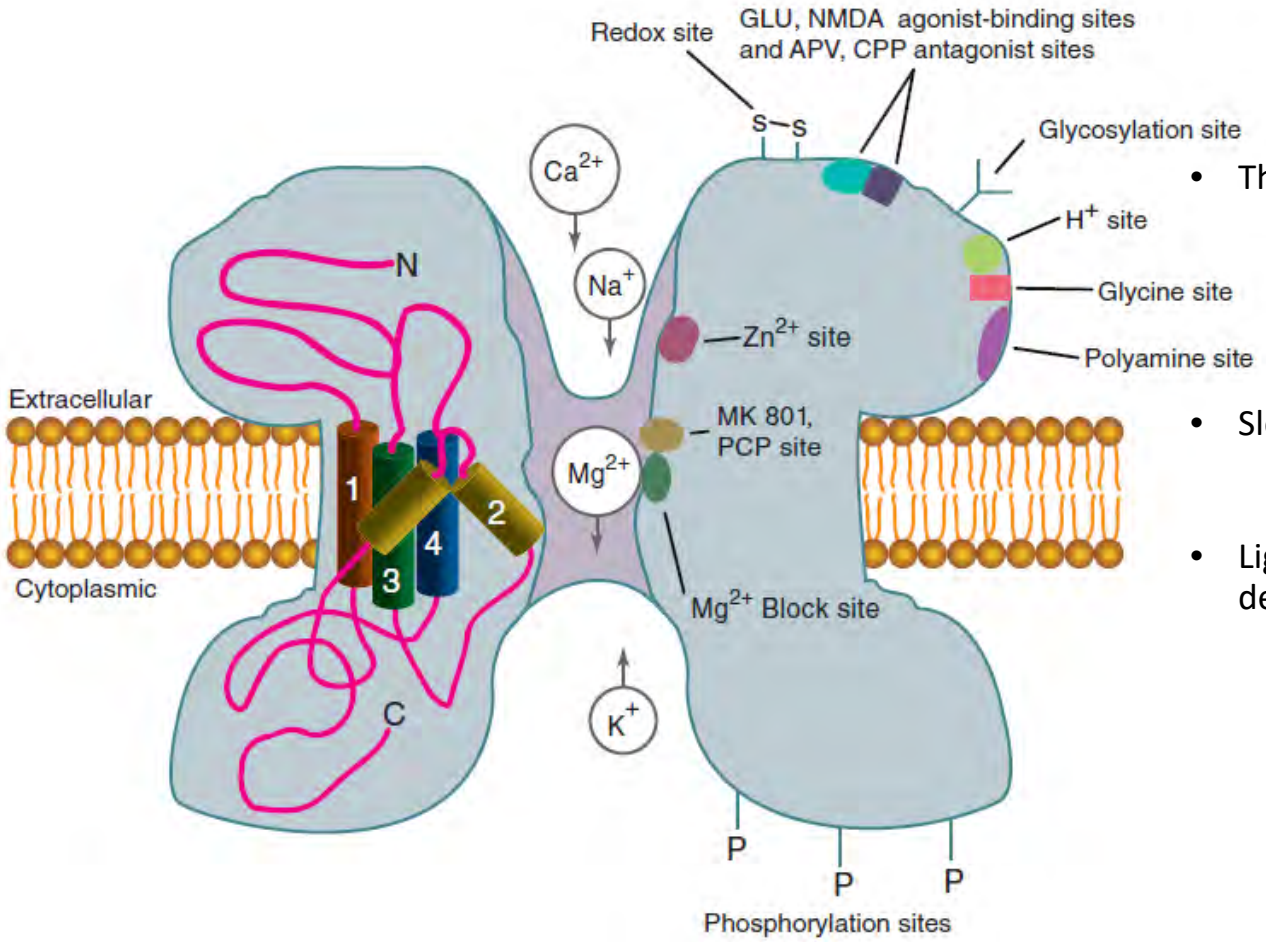
Ionotropic: Glutamate Receptors

- Non-NMDA
  - AMPA Receptors: Amino-3-hydroxy-5-methylisoxazoleproprionic acid (AMPA) → GluA1-GluA4
  - Kainate (KA) Receptors: → GluK1-GluK5
    - Subunit assembly determines their properties
- NMDA Receptors
  - N-methyl-D-aspartate (NMDA) → GluN1, GluN2A, GluN2D, GluN3A-GluN3B
  - Ca^{2+} Permeable - Excitatory

Ionotropic: Glutamate Receptors



Ionotropic: NMDA Receptors (NMDARs)



- Three Main Characteristics
  - Mg^{2+}-dependent, voltage sensitive channel blocker.
  - Glycine is a co-agonist
  - Large Ca^{2+} Permeability
- Slowest activating ionotropic GluRs
- Ligand-gated ion channel that is voltage dependent.
  - Receptor binding opens pore, but pore becomes blocked by Mg^{2+} or Zn^{2+}
  - Membrane must be depolarized to remove this block allowing influx of Ca^{2+} / Na^+

NMDA Receptor Antagonists

Competitive: blocks agonist binding site without activating the receptor

- Selfotel- anxiolytic, but with Phencyclidine (PCP)-like effects

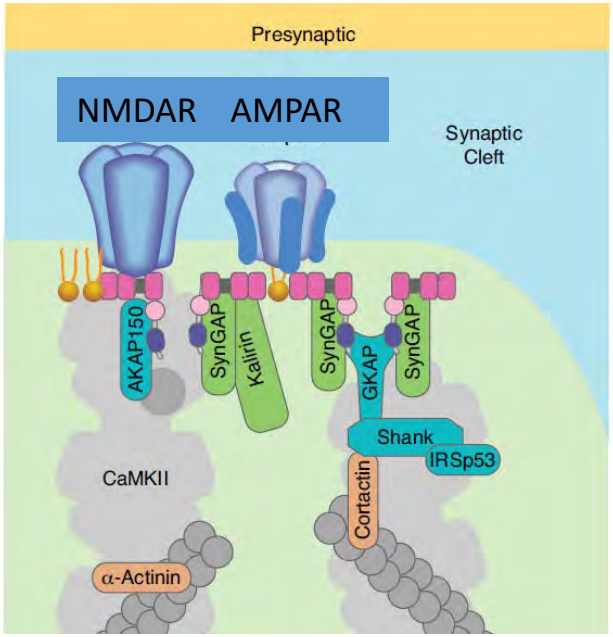
Channel Blockers: blocks channel pore (needs to be open for binding)

- PCP, ketamine, dizocilpine (MK-801) – potent; produce dissociative hallucinogenic psychosis
- memantine – low affinity / faster dissociation: FDA approved for treatment of AD

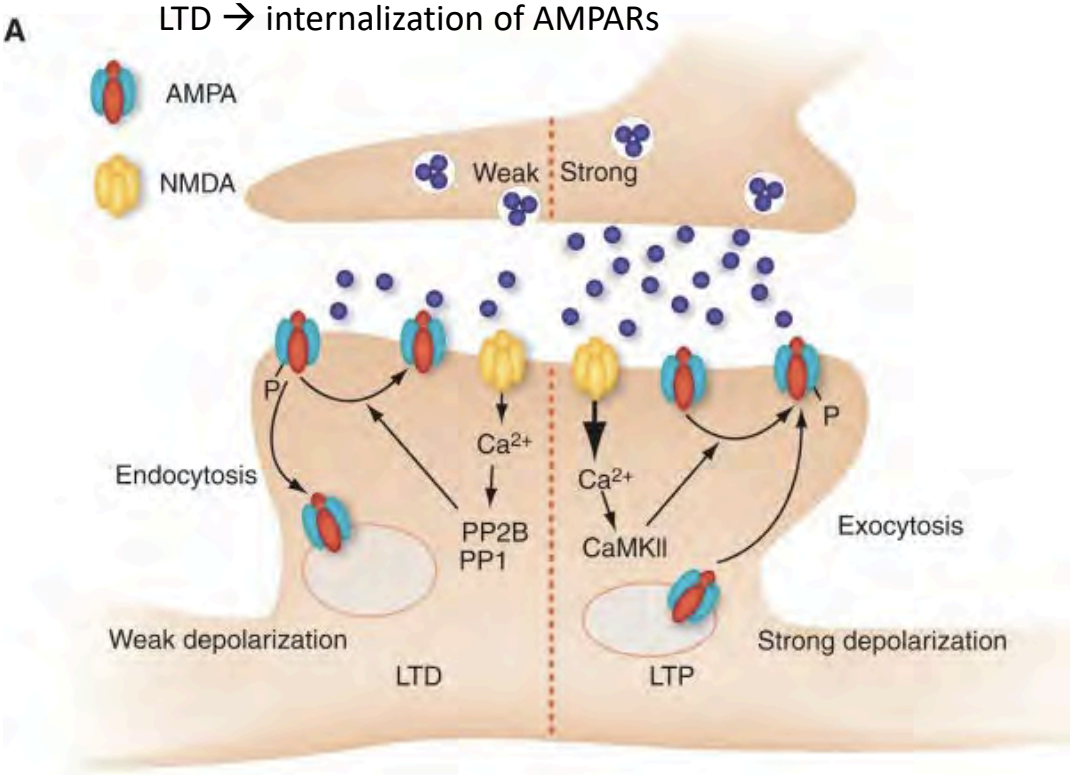
Involved in learning, memory, synaptic plasticity, LTP, LTD, excitotoxicity, neurodegeneration

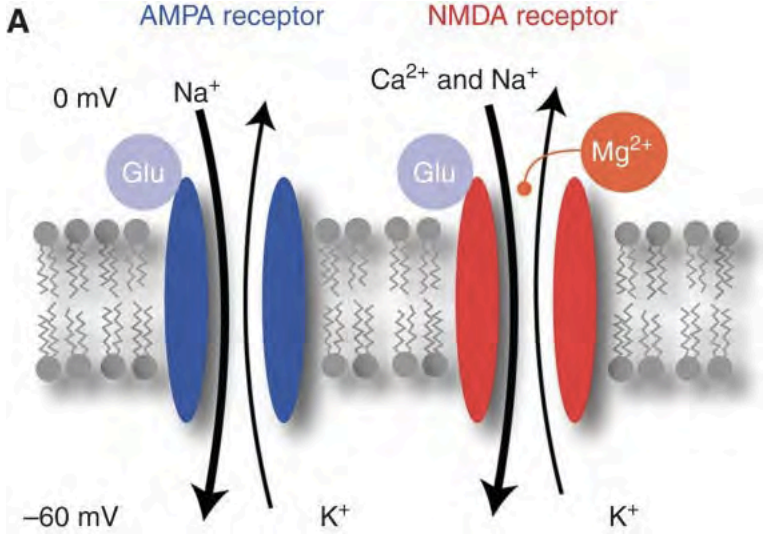
Glutamate Receptor (NMDAR-AMPAR) Cluster

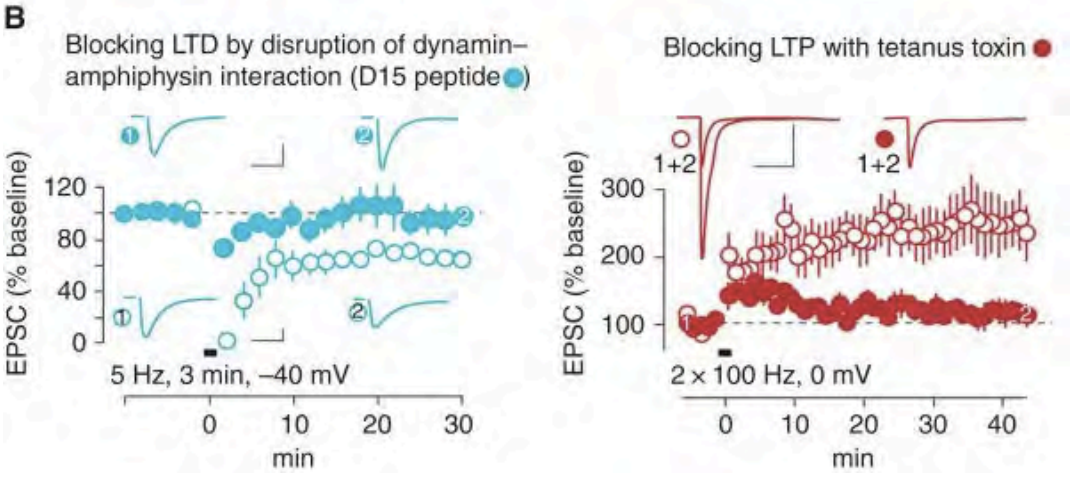
- Concentrated at synapses by intracellular scaffolding proteins
- Important for NMDA activation
- Linked to Ca^{2+}/Calmodulin Protein Kinase II (CaMKII)



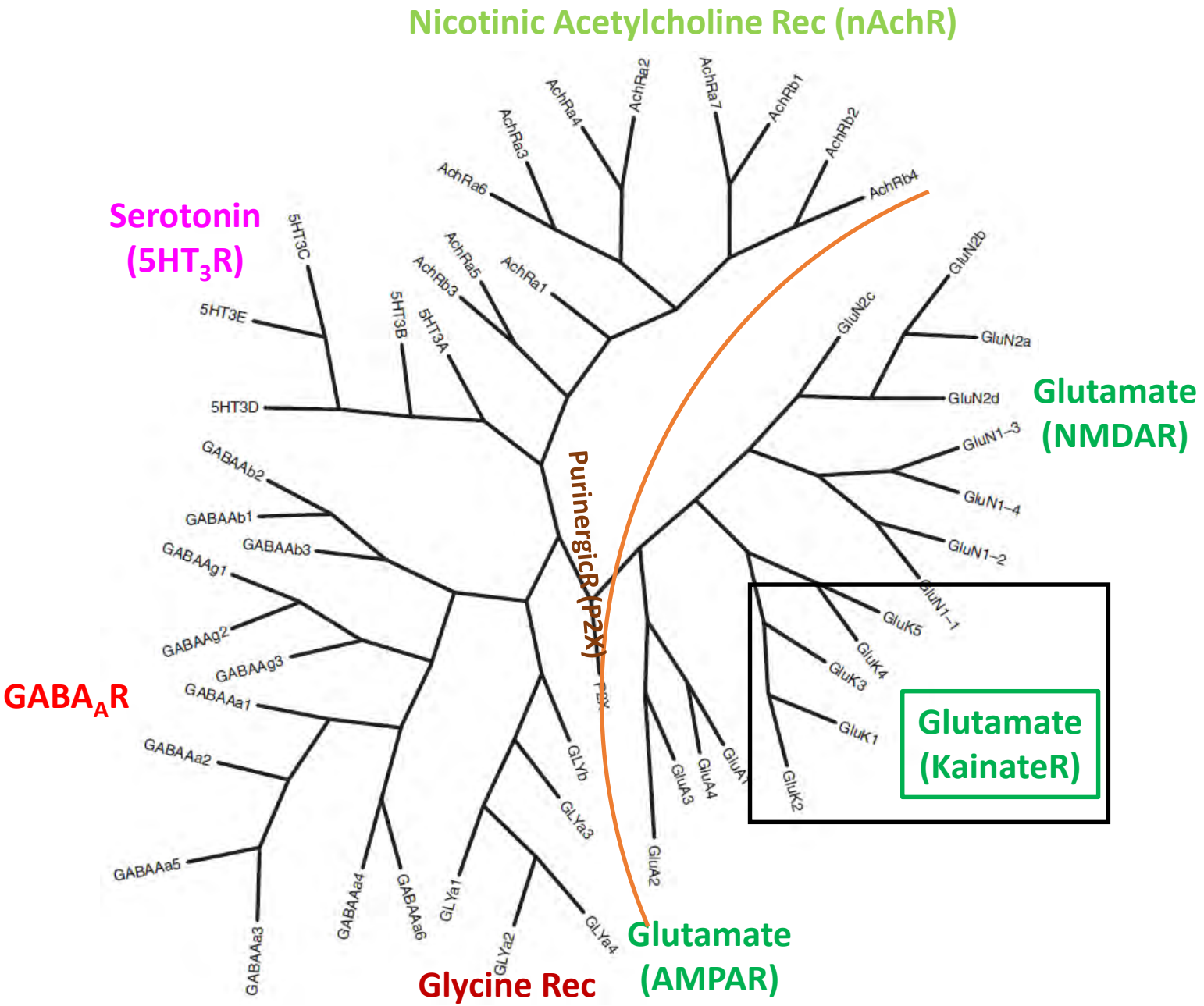
Links to intracellular signaling:
LTP → insertion of AMPARs
LTD → internalization of AMPARs



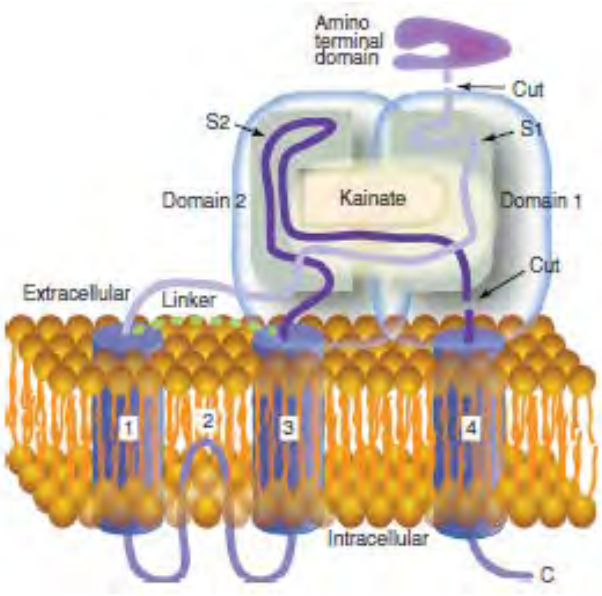




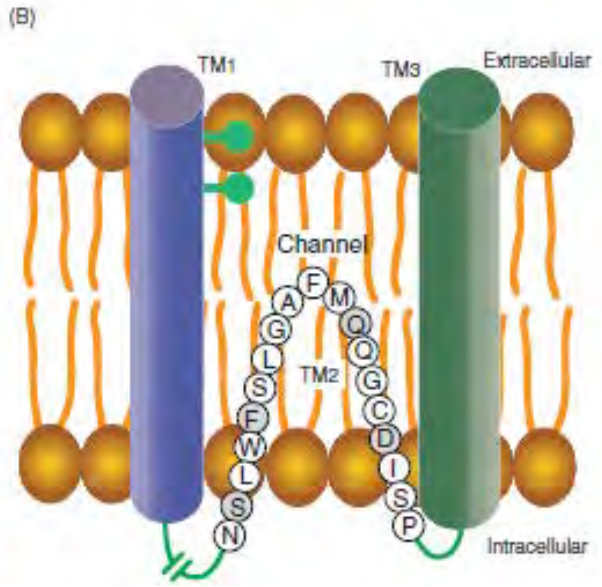
Ionotropic: Glutamate Receptors



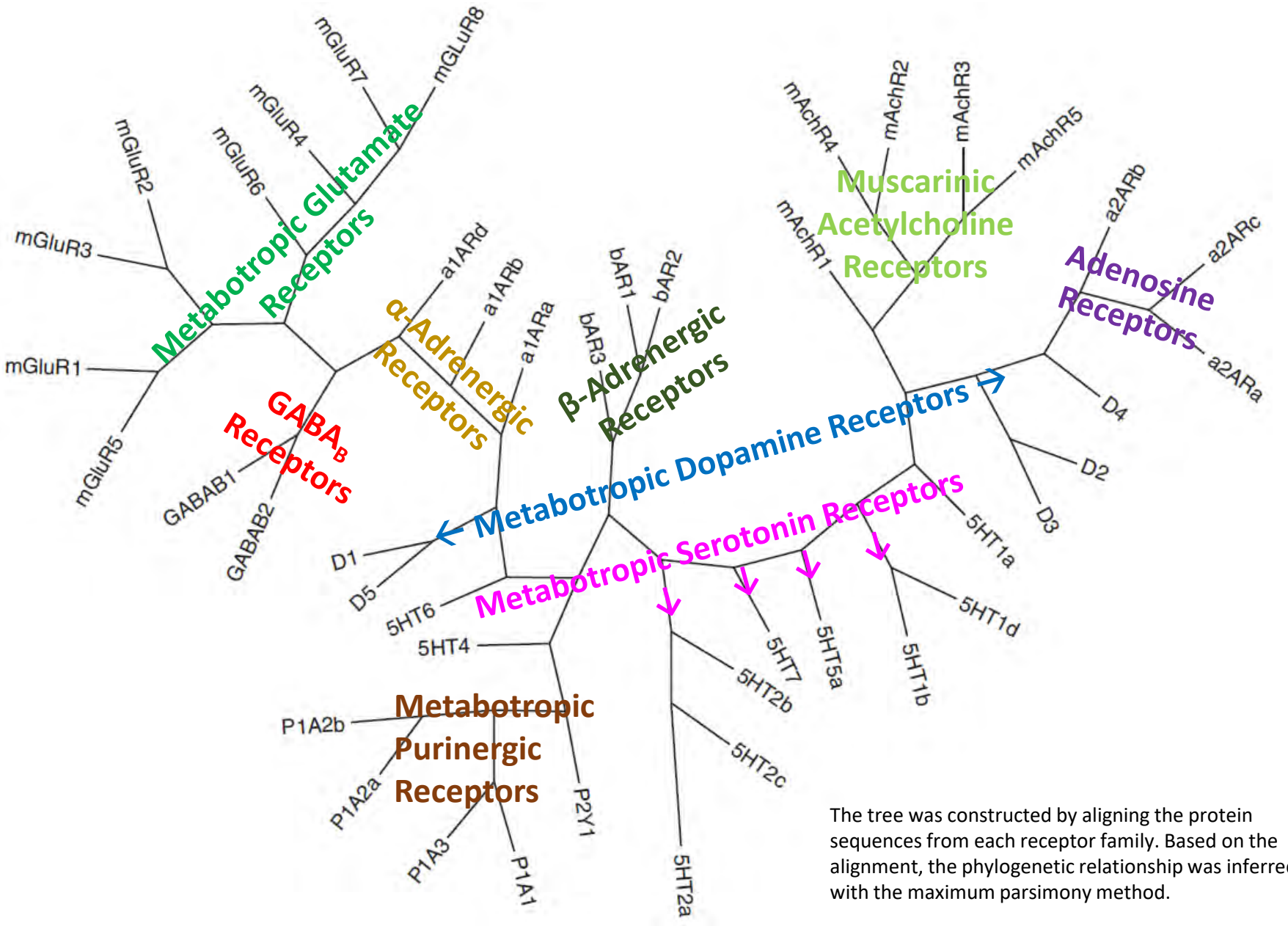
Ionotropic: Glutamate (Kainate) Receptors Structure



- 4 Transmembrane Domains
  - TM2 does not pass through membrane
- Tetrameric
- Twice the size of AChR's
  - Large extracellular amino terminus for receptor assembly & trafficking
  - Ligand binding domain
  - Permeable to Na+ and K+



Evolutionary relationship of the metabotropic/GPCR family

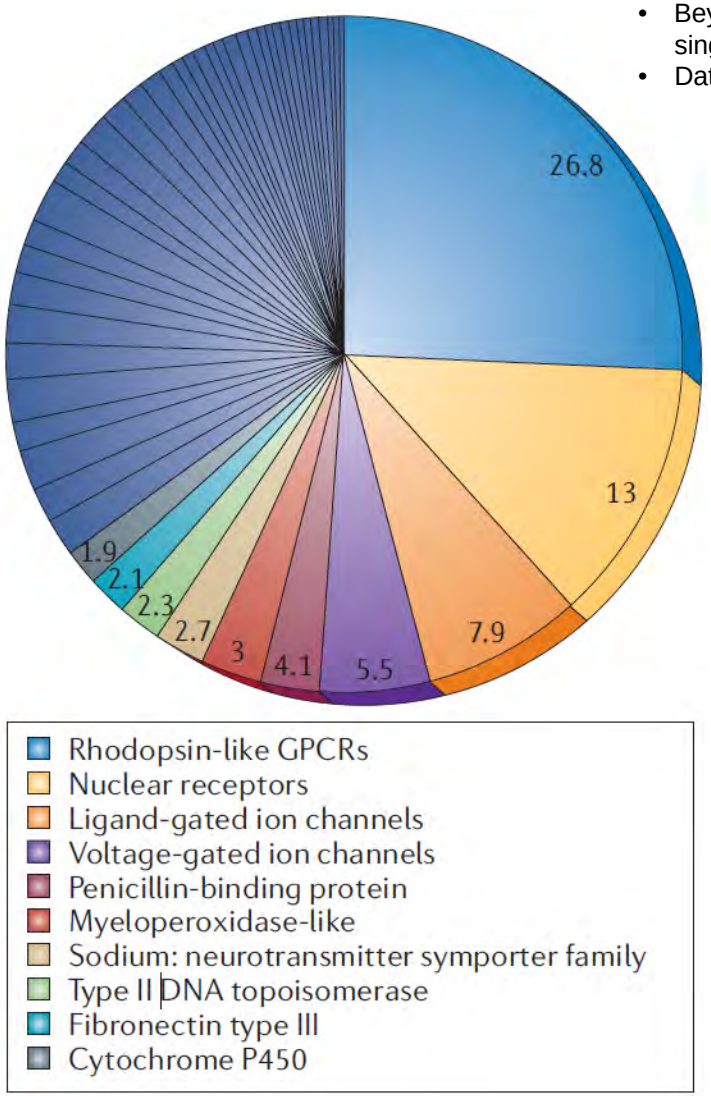


The tree was constructed by aligning the protein sequences from each receptor family. Based on the alignment, the phylogenetic relationship was inferred with the maximum parsimony method.

G-Protein Coupled Receptors (GPCRs) → Metabotropic

Gene-family distribution of current drugs per drug substance.

- The family share as a % of all FDA-approved drugs is displayed for the top ten families.
- Beyond the 10 most commonly drugged families, there are a further 120 domain families or singletons for which only a few drugs have been successfully launched.
- Data based on 1,357 dosed components from >20,000 approved products, FDA, Dec. 2005.



(Overington et al., Nature Rev, 2005)

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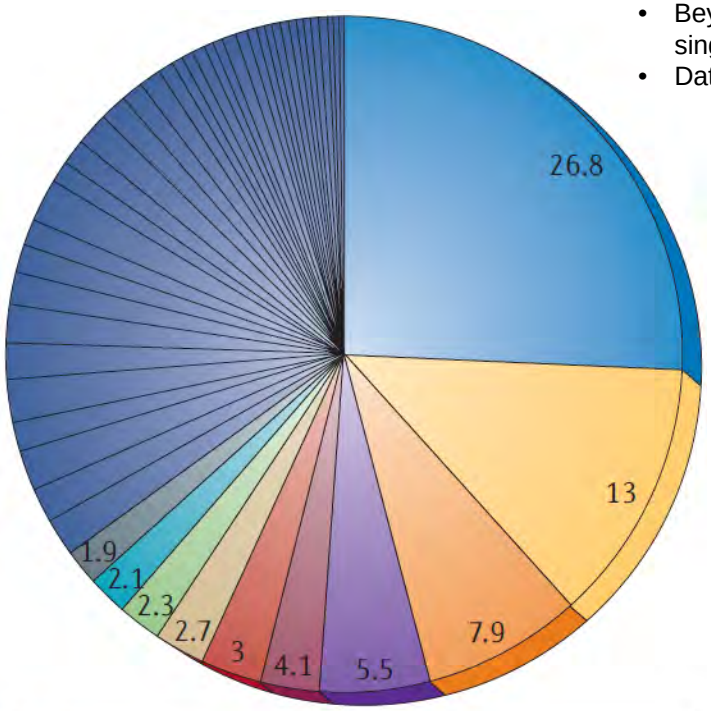
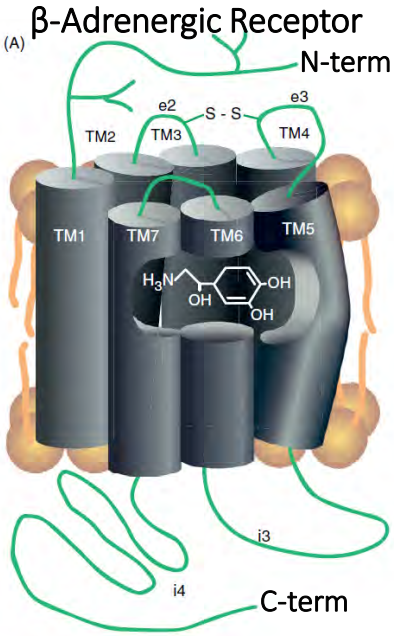
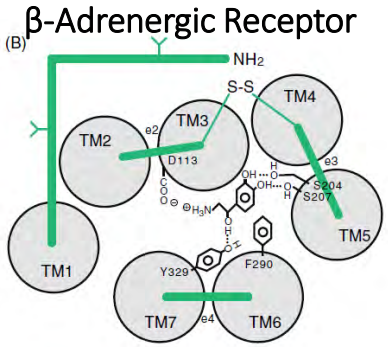
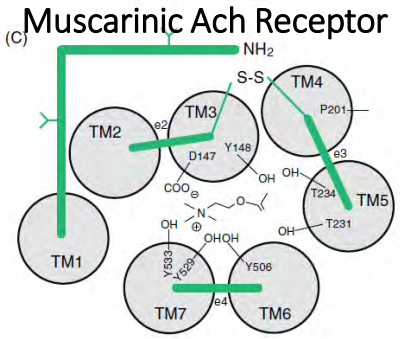


(Overington et al., Nature Rev, 2005)

Structure of GPCRs (7 TM segments)

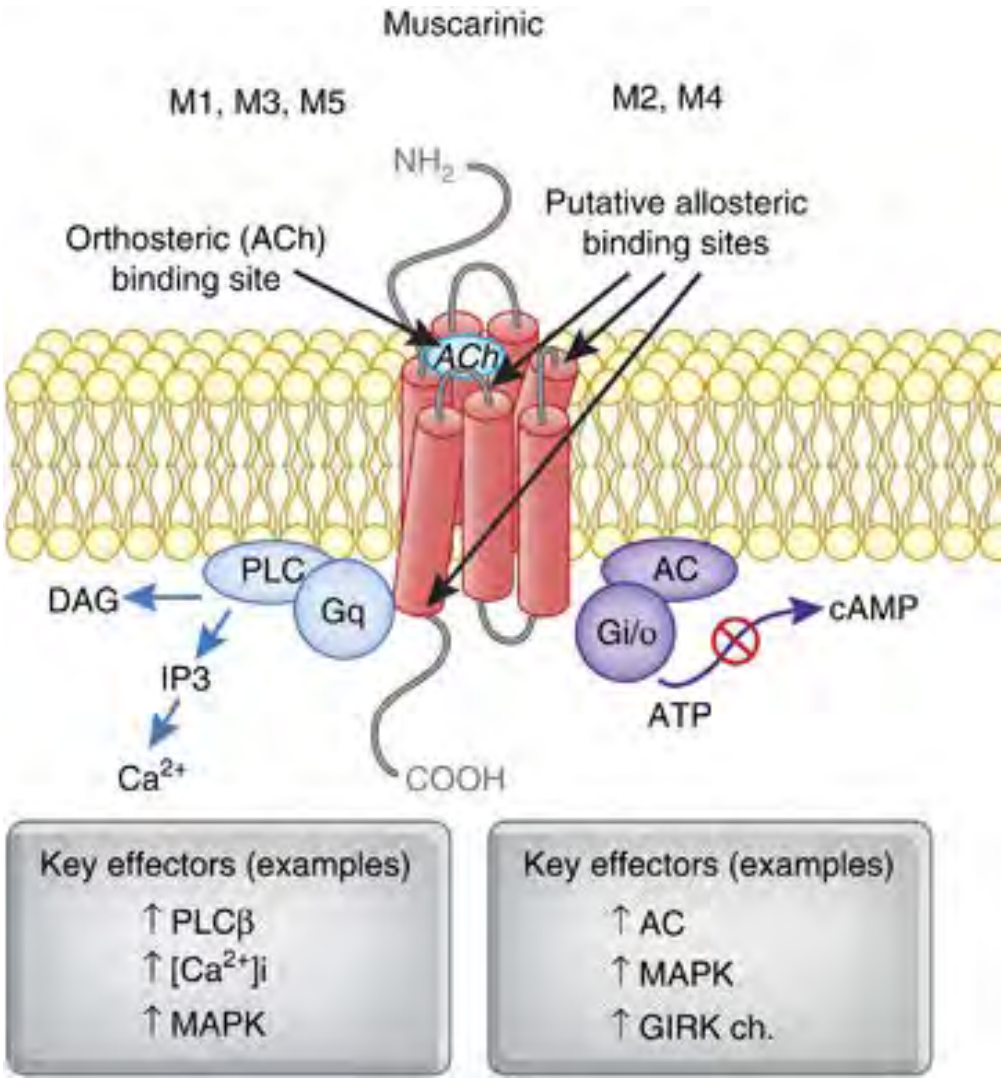






- GPCRs constitute a large protein family of receptors (over 800 identified from sequencing human genome) that detect molecules outside the cell and activate internal signal transduction pathways and, ultimately, cellular responses.
- Coupling with G proteins, they are called seven-transmembrane receptors because they pass through the cell membrane 7 times.
- N-terminus = extracellular / C-terminus = intracellular
- Transmitter binding site is buried in the center of the 7-TM ring
  - Stabilizes activated state of the receptor

Metabotropic: Muscarinic ACh Receptors (mAchRs)



- 5 members (M1-M5)
- Pre- & postsynaptically
- Feedback loops regulate ACh release
- Ion channel alterations

- Antagonists
  - Atropine (parasympathetic)
  - N-methylscopolamine (motion sickness)

(Jones et al., Neuropsychopharm., 2012)

Table of Serotonin Receptor Subtypes	
Receptor Name	**Location, Effects, Agonists and Antagonists**
5-HT_{1A}	Location: CNS Effects: Regulates sleep, feeding, and anxiety Antagonists: Yohimbine
5-HT_{1B}	Location: CNS Effects: neuronal inhibition, behavioral changes Antagonists: Yohimbine.
5-HT_{m}	Location: CNS, vascular Effects: Locomotor, vasoconstriction Antagonists: Yohimbine. Agonists: Sumatriptan
5-HT_{3A}	Location: CNS, smooth muscle, platelets Effects: cellular excitiation, behavior, muscle contraction, vasoconstriction Antagonists: LSD, Chlorpromazine
5-HT_{3B}	Location: Stomach Antagonists: Yohimbine, Chlorpromazine
5-HT_{2C}	Location: CNS Effects: Anxiety
~~5-HT_{3}~~	Location: Sensory nerves Effects: Vomiting
5-HT_{4}	Location: CNS, ENS Effects: Gut Motility
5-HT_{5A}	Location: CNS Effects: Unknown
5-HT_{6}	Location: CNS Effects: Unknown
5-HT_{7}	Location: CNS, ENS, blood vessels Effects: Unknown

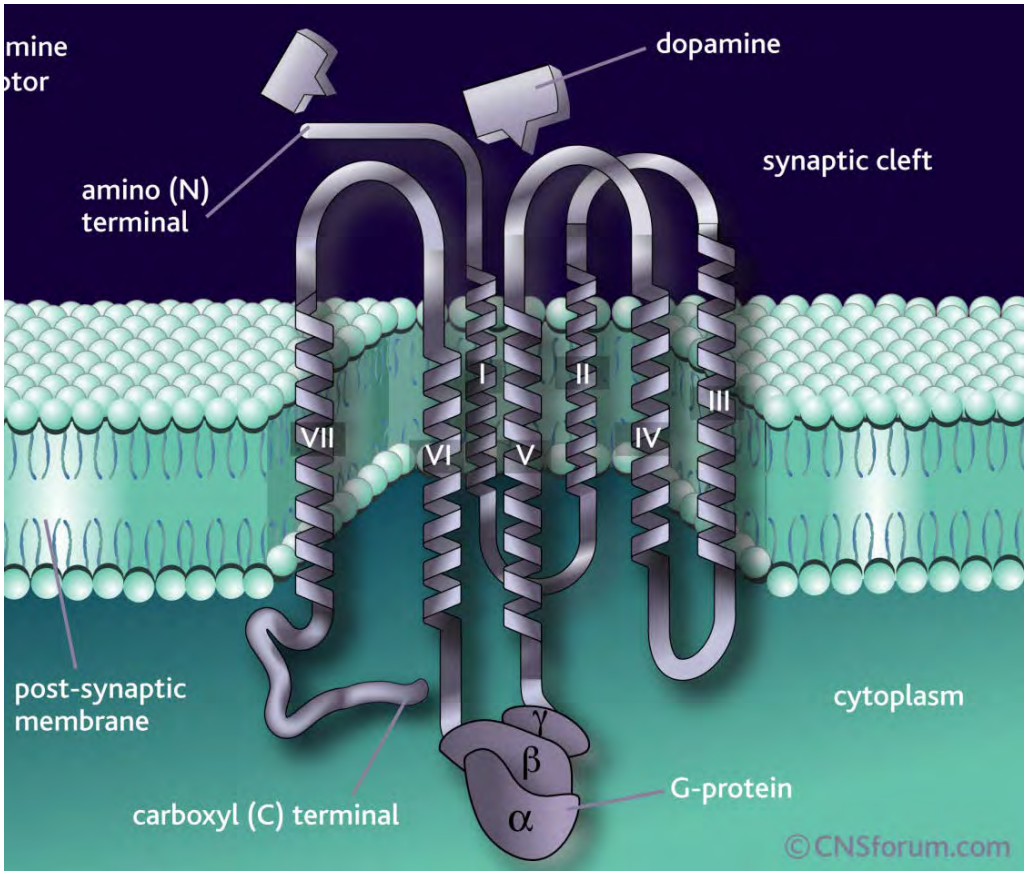
Adapted from Mohammad-Zadeh et al, 2008

Metabotropic

5-HT Receptors

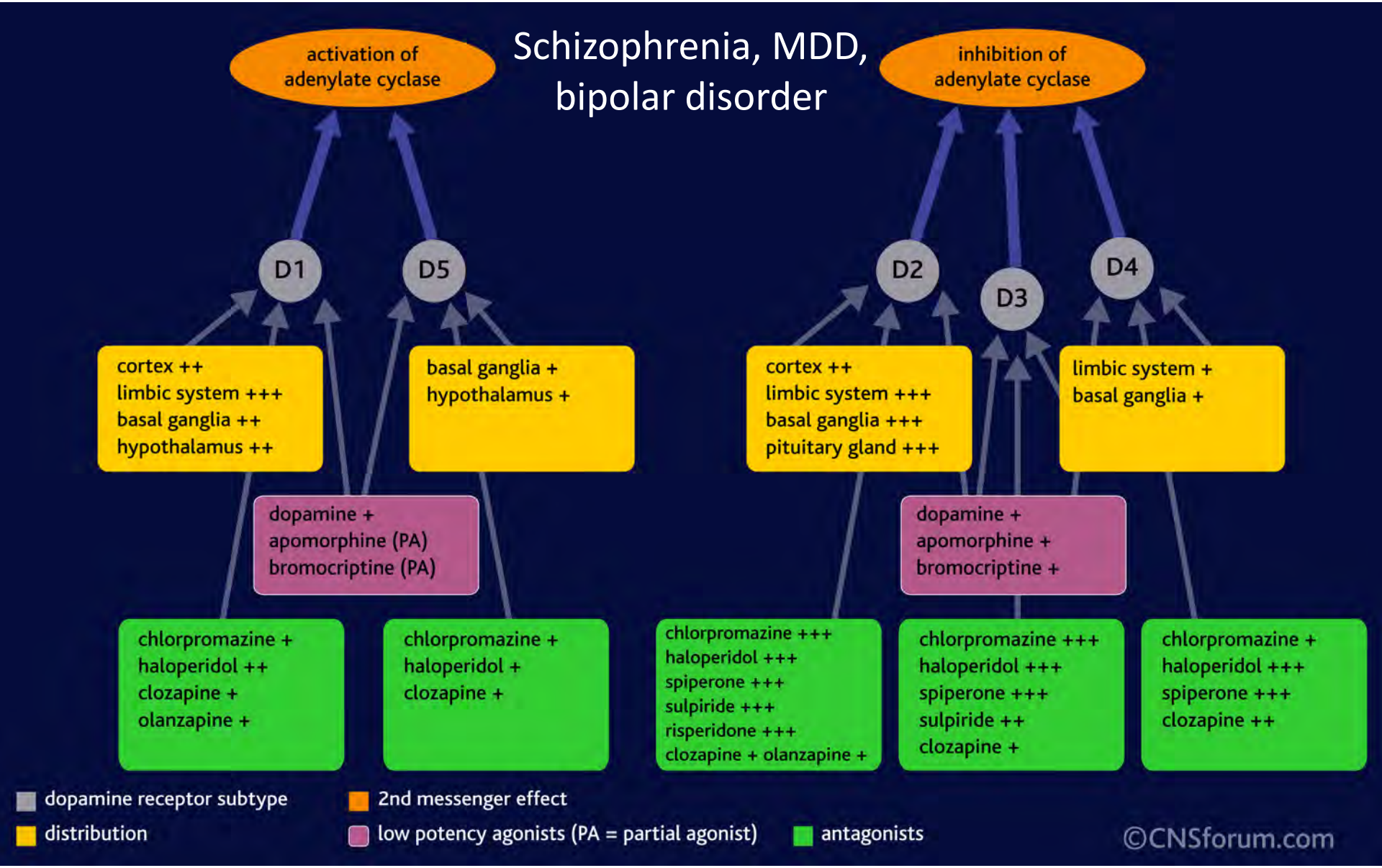
- Raphe Nucleus
- Regulates
  - Sleep
  - Mood
  - Hunger
  - Circadian Rythyms
- 7 Subtypes
  - 5-HT1 – 5-HT7

Metabotropic: Dopamine Receptors

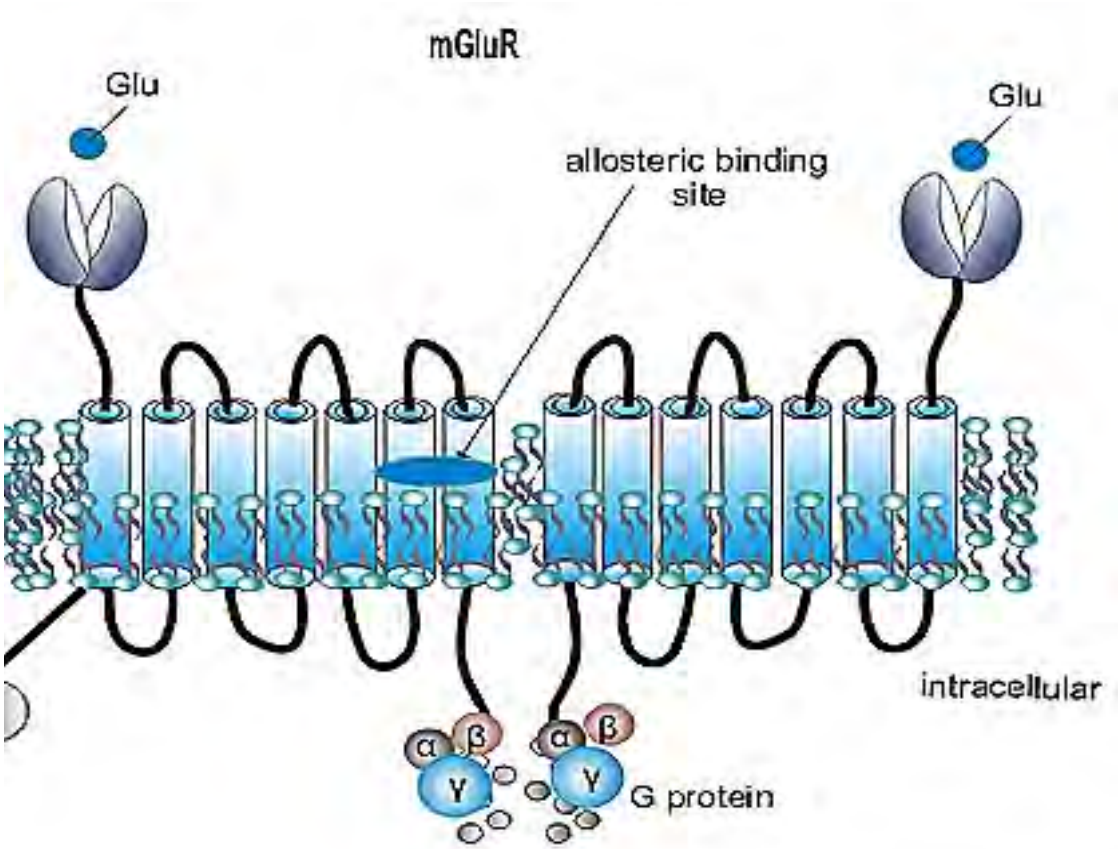


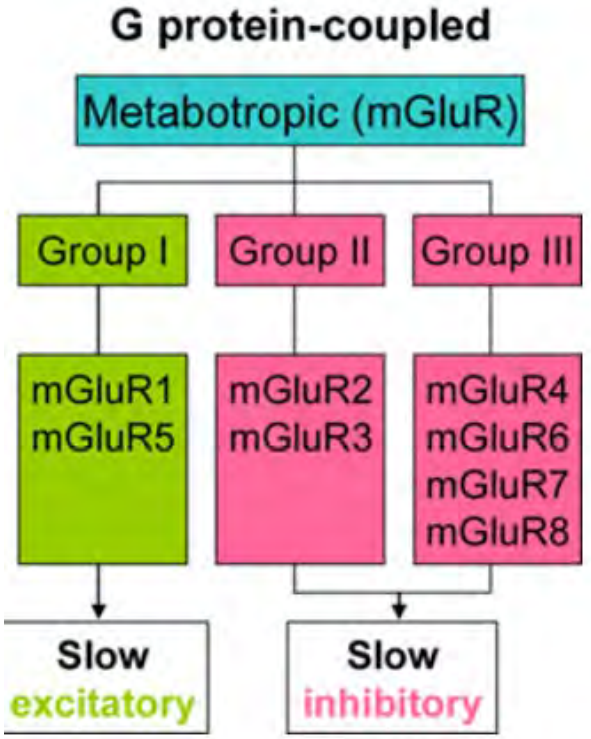
- Localization:
  - Corpus Striatum
  - Cortex, Basal Ganglia, Hypothalamus
- 5 Subtypes / 2 Families
- D1-like (D1 & D5)
  - Activate Adenylyl Cyclase
- D-2-like (D2, D3, D4)
  - Inhibit Adenylyl Cyclase
- Pre & Postsynaptic
  - autoreceptor

Metabotropic: Dopamine Receptor Pharmacology



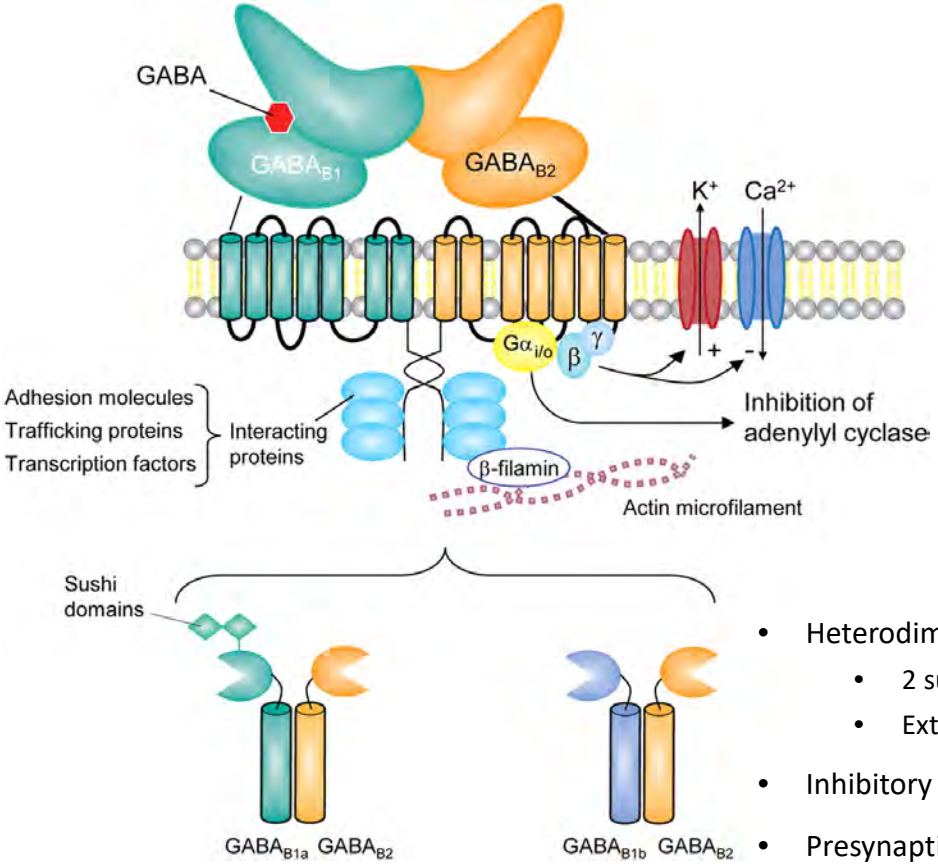
Metabotropic Glutamate Receptors (mGluRs)



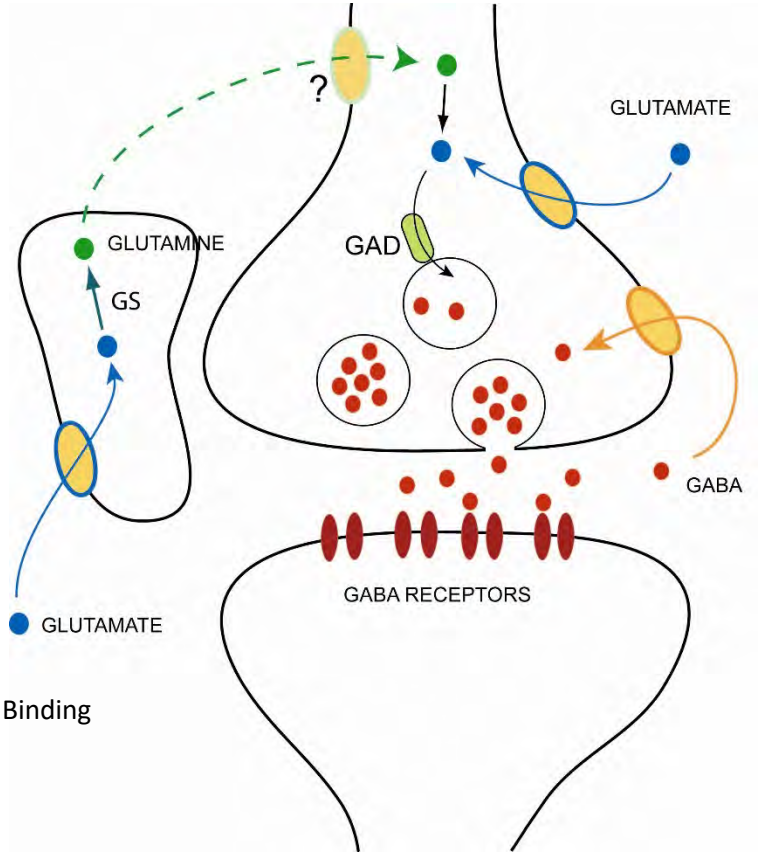


- Dimerize – Glutamate binds to both for activation?
- Glutamate Binding – extracellular N-terminus
- Group I – Postsynaptic: activate adenylyl cyclase or PLC
- Group II/III – Pre & postsynaptic: inhibit adenylyl cyclase / $\downarrow Ca^{2+}$ $\uparrow K^{+}$

Metabotropic: $GABA_B$ Receptors



(Bennaroch, Neurology, 2012)

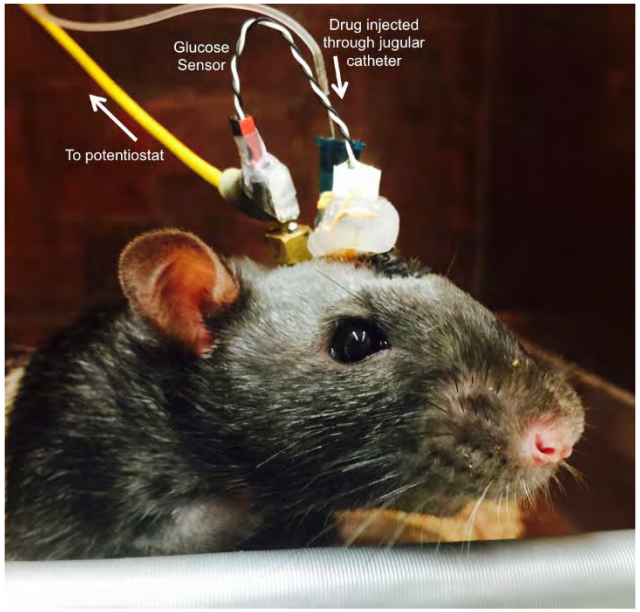


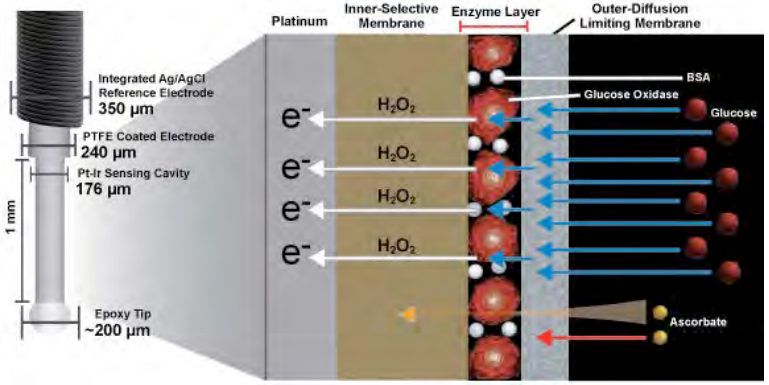
- Heterodimer
  - 2 subunits
  - Extracellular Binding
- Inhibitory
- Presynaptic
  - No GPCR
  - Opens K^+
  - Closes Ca^{2+}
- Postsynaptic
  - GPCR
  - Inhibits AC to open K^+
- Agonist: Baclofen
  - MS, Cerebral Palsy
  - Alcoholism?

In Vivo Electrochemistry: detection of **glucose** and **oxygen** with biosensors

- Fixed-potential amperometry

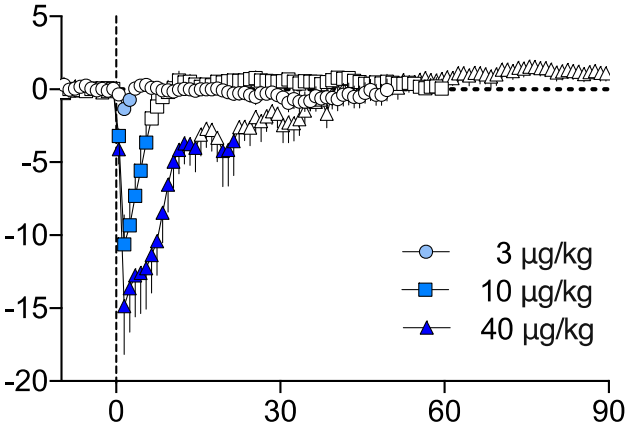
- Adult male Long-Evans rats (460±40 g)
- Stereotaxic surgery
  - * Cannula implantation into NAc
  - * Catheter in jugular vein (daily heparin flush)





- Glucose biosensors coated with glucose oxidase; glucose detected by oxidation at a Pl-Ir electrode (V_h = +0.6 V) and currents are recorded with 1-s time points

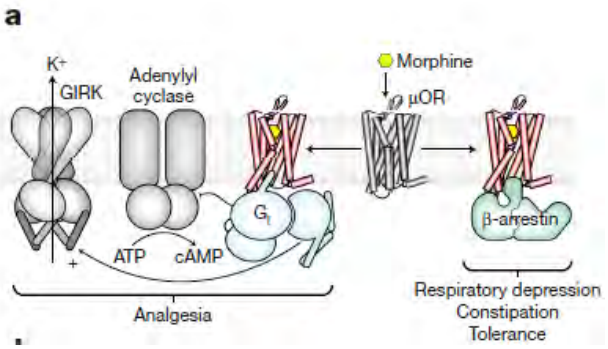
Effect of iv **fentanyl** (**MOR agonist**) on:



- O2 decrease → CO2 increase → central vasodilation → increase of glucose entry into the brain

(Solis, Jr. et al., Neuropsychopharm., 2018)

Structure-based discovery of opioid analgesics with reduced side effects



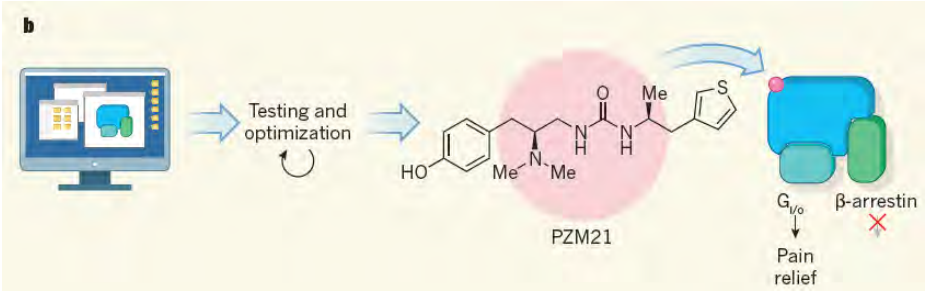
(Manglik et al., Nature, 2016)

DRUG DISCOVERY

Designing the ideal opioid

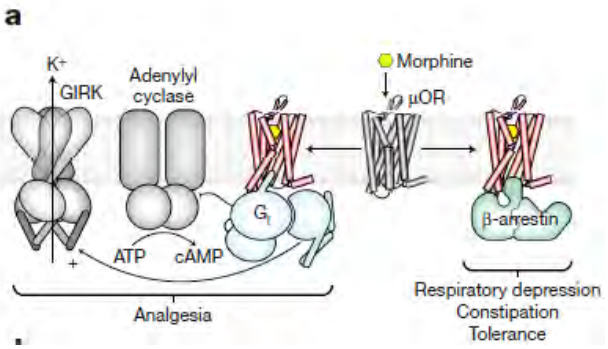
The development of a drug that mimics the pain-relieving activity of opioid compounds, but has fewer side effects, points to an effective strategy for the discovery of many types of drug. SEE ARTICLE P.185

BRIGITTE L. KIEFFER



- 3 million commercially available compounds tested (computationally docked to MOR binding pocket)
- 1 million+ configurations for each compound
- 2,500 best-fitting molecules selected, identified chemotypes unrelated to known opioids

Structure-based discovery of opioid analgesics with reduced side effects



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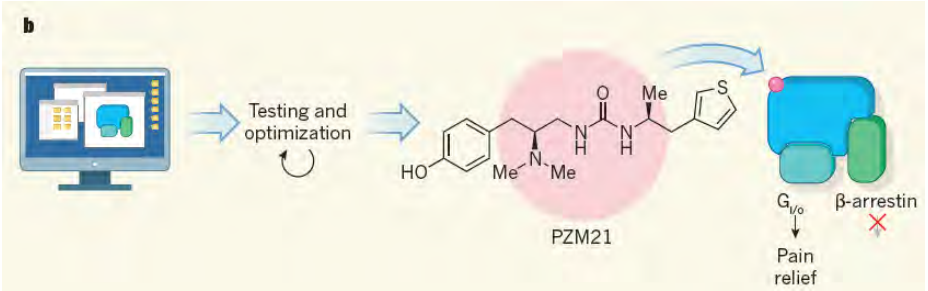

- 3 million commercially available compounds tested (computationally docked to MOR binding pocket)
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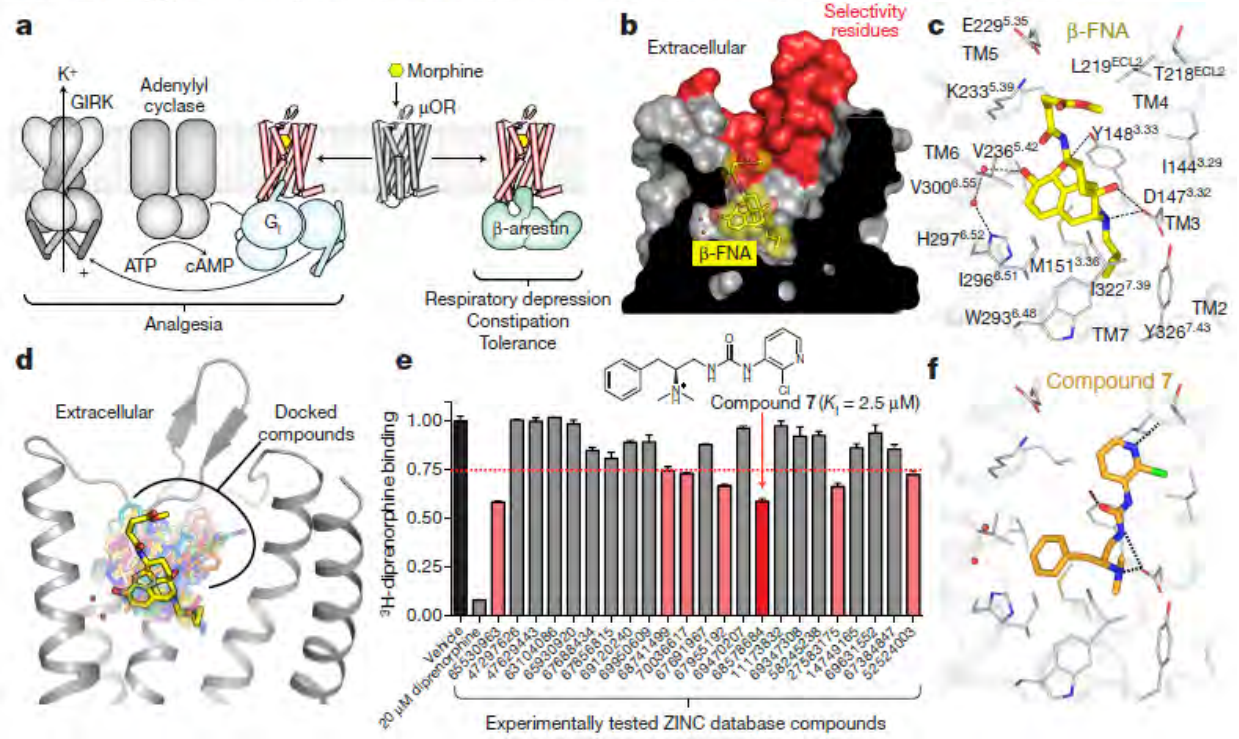
Structure-based discovery of opioid analgesics with reduced side effects



(Manglik et al., Nature, 2016)

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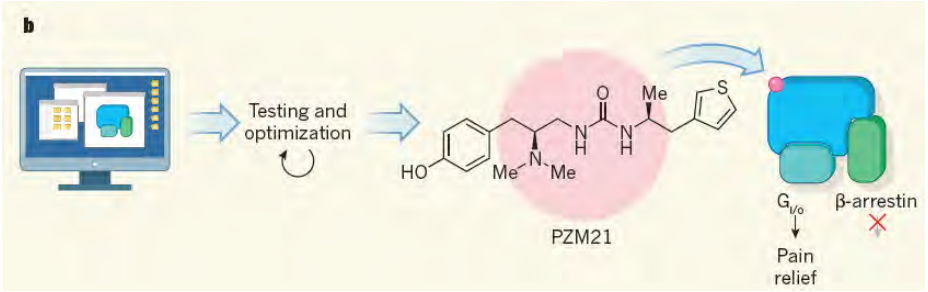
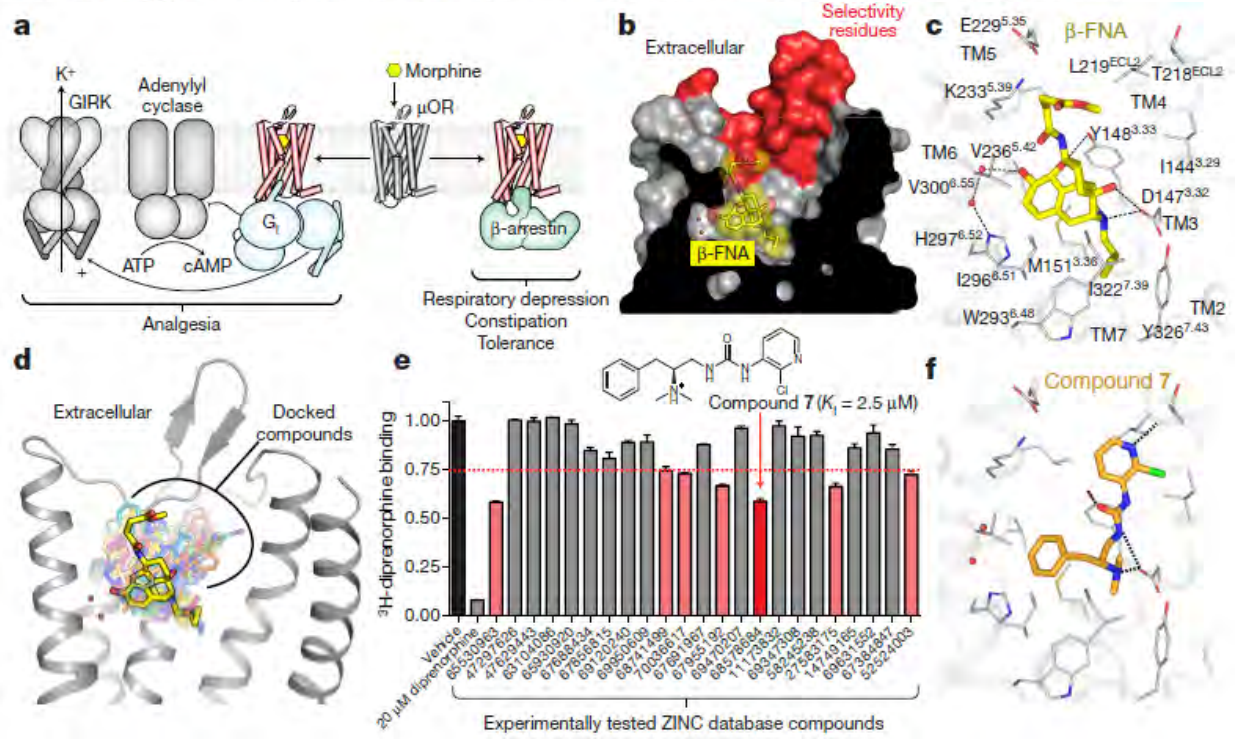
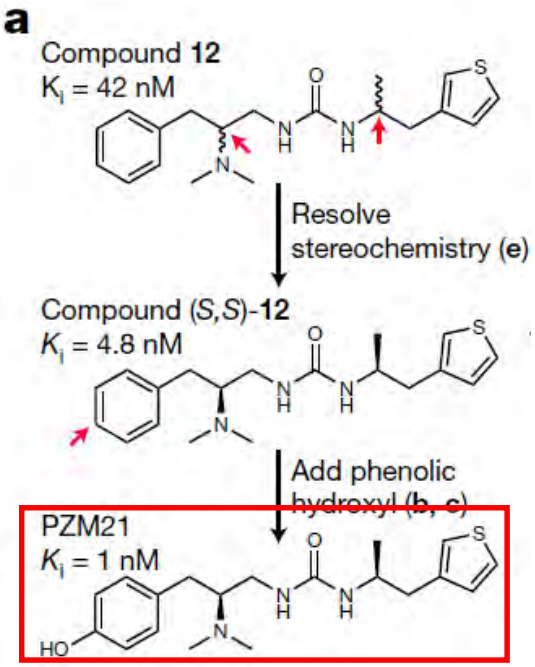


- 3 million commercially available compounds tested (computationally docked to MOR binding pocket)
- 1 million+ configurations for each compound
- 2,500 best-fitting molecules selected, identified chemotypes unrelated to known opioids
- 23 tested experimentally
- Structure-guided optimization led to PZM21, which has a better side-effect profile in animals

Structure-based discovery of opioid analgesics with reduced side effects





(Manglik et al., Nature, 2016)

Questions?

List of neurological conditions and disorders (according to Wikipedia) = 389 total

- # 22q13 deletion syndrome
- A Abulia
- Achromatopsia
- Agraphia
- AIDS – neurological manife
- Akinetopsia
- Alcoholism
- Alien hand syndrome
- Allan–Herndon–Dudley sy
- Alternating hemiplegia of
- Alzheimer's disease
- Amaurosis fugax
- Amnesia
- Amyotrophic lateral scler
- Aneurysm
- Angelman syndrome
- Anosognosia
- Aphasia
- Apraxia
- Arachnoiditis
- Arnold–Chiari malformati
- Asomatognosia
- Asperger syndrome
- Ataxia
- Attention deficit hyperact
- ATR-16 syndrome
- Auditory processing disor
- Autism spectrum disorder
- B Behçet's disease
- Bipolar disorder
- Bell's palsy
- Blindsight
- Brachial plexus injury
- Brain injury
- Brain tumor
- Brody myopathy
- C Canavan disease
- Capgras delusion
- Carpal tunnel syndrome
- Causalgia
- Central pain syndrome
- Central pontine myelinoly
- Centronuclear myopathy
- Cephalic disorder
- Cerebral aneurysm
- Cerebral arteriosclerosis
- Cerebral atrophy
- Cerebral autosomal domi
- Cerebral dysgenesis–neur
- Cerebral gigantism
- Cerebral palsy
- Cerebral vasculitis
- Cerebrospinal fluid leak
- Cervical spinal stenosis
- Charcot–Marie–Tooth dise
- Chiari malformation
- Chorea
- Chronic fatigue syndrome
- Chronic inflammatory demy
- Chronic pain
- Cluster Headache
- Cockayne syndrome
- Coffin–Lowry syndrome
- Coma
- Complex regional pain syndr
- Compression neuropathy
- Congenital distal spinal mus
- Congenital facial diplegia
- Corticobasal degeneration
- Cranial arteritis
- Craniosynostosis
- Creutzfeldt–Jakob disease
- Cumulative trauma disorder
- Cushing's syndrome
- Cyclothymic disorder
- Cyclic vomiting syndrome
- Cytomegalic inclusion body
- Cytomegalovirus Infection
- D Dandy–Walker syndrome
- Dawson disease
- De Morsier's syndrome
- Dejerine–Klumpke palsy
- Dejerine–Sottas disease
- Delayed sleep phase disord
- Dementia
- Dermatomyositis
- Developmental coordinatio
- Diabetic neuropathy
- Diffuse sclerosis
- Diplopia
- Disorders of consciousness
- Distal hereditary motor neu
- Distal spinal muscular atrop
- Distal spinal muscular atrop
- Down syndrome
- Dravet syndrome
- Duchenne muscular dystrop
- Dysarthria
- Dysautonomia
- Dyscalculia
- Dysgraphia
- Dyskinesia
- Dyslexia
- Dystonia
- E Empty sella syndrome
- Encephalitis
- Encephalocele
- Encephalopathy
- Encephalotrigeminal angion
- Encopresis
- Enuresis
- Epilepsy
- Epilepsy-intellectual disability
- Erb's palsy
- Erythromelalgia
- Essential tremor
- Exploding head syndrome
- F Fabry's disease
- Fahr's syndrome
- Fainting
- Familial spastic paralysis
- Febrile seizures
- Fisher syndrome
- Friedreich's ataxia
- Fibromyalgia
- Foville's syndrome
- Fetal alcohol syndrome
- Fragile X syndrome
- Fragile X-associated tremor/at
- Frontotemporal dementia
- Functional Neurological Disord
- G Gaucher's disease
- Generalized epilepsy with febri
- Gerstmann's syndrome
- Giant cell arteritis
- Giant cell inclusion disease
- Globoid cell leukodystrophy
- Gray matter heterotopia
- Guillain–Barré syndrome
- Generalized anxiety disorder
- H HTLV-1 associated myelopathy
- Head injury
- Headache
- Hemicrania Continua
- Hemifacial spasm
- Hemispatial neglect
- Hereditary motor neuropathies
- Hereditary motor neuropathies
- Hereditary spastic paraplegia
- Heredopathia atactica polyneu
- Herpes zoster oticus
- Herpes zoster
- Hirayama syndrome
- Hirschsprung's disease
- Holmes–Adie syndrome
- Holoprosencephaly
- Huntington's disease
- Hydranencephaly
- Hydrocephalus
- Hypercortisolism
- Hypoxia
- I Immune-mediated encephalon
- Inclusion body myositis
- Incontinentia pigmenti
- Refsum disease
- Infantile spasms
- Inflammatory myopathy
- Intracranial cyst
- Intracranial hypertension
- Isodicentric 15
- J Joubert syndrome
- K Karak syndrome
- Kearns–Sayre syndrome
- Kinsbourne syndrome
- Kleine–Levin syndrome
- Klippel Feil syndrome
- Krabbe disease
- Kufor–Rakeb syndrome
- Kugelberg–Welander disease – se
- L Lafora disease
- Lambert–Eaton myasthenic syndr
- Landau–Kleffner syndrome
- Lateral medullary (Wallenberg) sy
- Learning disabilities
- Leigh's disease
- Lennox–Gastaut syndrome
- Lesch–Nyhan syndrome
- Leukodystrophy
- Leukoencephalopathy with vanish
- Lewy body dementia
- Lissencephaly
- Locked-in syndrome
- Lou Gehrig's disease – see Amyotr
- Lumbar disc disease
- Lumbar spinal stenosis
- Lupus erythematosus – neurologic
- Lyme disease
- M Machado–Joseph disease
- Macrencephaly
- Macropsia
- Mal de debarquement
- Megalencephalic leukoencephalo
- Megalencephaly
- Melkersson–Rosenthal syndrome
- Menieres disease
- Meningitis
- Menkes disease
- Metachromatic leukodystrophy
- Microcephaly
- Micropsia
- Migraine
- Miller Fisher syndrome
- Mini-stroke (transient ischemic at
- Misophonia
- Mitochondrial myopathy
- Mobius syndrome
- Monomelic amyotrophy
- Morvan syndrome
- Motor neurone disease – see Amy
- Motor skills disorder
- Moyamoya disease
- Mucopolysaccharidoses
- Multi-infarct dementia
- Multifocal motor neuropathy
- Multiple sclerosis
- Multiple system atrophy
- Muscular dystrophy
- Myalgic encephalomyelitis
- Myasthenia gravis
- Myelinoclastic diffuse scleros
- Myoclonic Encephalopathy of
- Myoclonus
- Myopathy
- Myotubular myopathy
- Myotonia congenita
- N Narcolepsy
- Neuro-Behçet's disease
- Neurofibromatosis
- Neuroleptic malignant syndro
- Neuromyotonia
- Neuronal ceroid lipofuscinosi
- Neuronal migration disorders
- Neuropathy
- Neurosis
- Niemann–Pick disease
- Non-24-hour sleep–wake disc
- Nonverbal learning disorder
- O O'Sullivan–McLeod syndrome
- Occipital Neuralgia
- Occult spinal dysraphism seq
- Ohtahara syndrome
- Olivopontocerebellar atrophy
- Opsoclonus myoclonus syndr
- Optic neuritis
- Orthostatic hypotension
- Otosclerosis
- Overuse syndrome
- P Palinopsia
- Pantothenate kinase-associa
- Paresthesia
- Parkinson's disease
- Paramyotonia congenita
- Paraneoplastic diseases
- Paroxysmal attacks
- Parry–Romberg syndrome
- PANDAS
- Pelizaeus–Merzbacher diseas
- Periodic paralyses
- Peripheral neuropathy
- Pervasive developmental dis
- Phantom limb / Phantom pair
- Photic sneeze reflex
- Phytanic acid storage disease
- Pick's disease
- Pinched nerve
- Pituitary tumors
- PMG
- Polyneuropathy
- Polio
- Polymicrogyria
- Polymyositis
- Porencephaly
- Post-polio syndrome
- Posttraumatic stress disorder
- Postherpetic neuralgia
- Postural hypotension
- Postural Orthostatic Tachycardi
- Prader–Willi syndrome
- Primary lateral sclerosis
- Prion diseases
- Progressive hemifacial atrophy
- Progressive multifocal leukoenc
- Progressive supranuclear palsy
- Prosopagnosia
- Pseudotumor cerebri
- Q Quadrantanopia
- Quadriplegia
- R Rabies
- Radiculopathy
- Ramsay Hunt syndrome type I
- Ramsay Hunt syndrome type II
- Ramsay Hunt syndrome type III
- Rasmussen encephalitis
- Reflex neurovascular dystrophy
- Refsum disease
- REM sleep behavior disorder
- Repetitive stress injury
- Restless legs syndrome
- Retrovirus-associated myelopat
- Rett syndrome
- Reye's syndrome
- Rhythmic movement disorder
- Romberg syndrome
- S Saint Vitus dance
- Sandhoff disease
- Sanfilippo syndrome
- Schilder's disease (two distinct
- Schizencephaly
- Sensory processing disorder
- Septo-optic dysplasia
- Shaken baby syndrome
- Shingles
- Shy–Drager syndrome
- Sjögren's syndrome
- Sleep apnea
- Sleeping sickness
- Snatiation
- Sotos syndrome
- Spasticity
- Spina bifida
- Spinal and bulbar muscular atro
- Spinal cord injury
- Spinal cord tumors
- Spinal muscular atrophy
- Spinal muscular atrophy with re
- Spinocerebellar ataxia
- Split-brain
- Steele–Richardson–Olszewski syndrome – see Progressive supranu
- Stiff-person syndrome
- Stroke
- Sturge–Weber syndrome
- Stuttering
- Subacute sclerosing panencephalitis
- Subcortical arteriosclerotic encephalopathy
- Superficial siderosis
- Sydenham's chorea
- Syncope
- Synesthesia
- Syringomyelia
- T Tarsal tunnel syndrome
- Tardive dyskinesia
- Tarlov cyst
- Tay–Sachs disease
- Temporal arteritis
- Temporal lobe epilepsy
- Tetanus
- Tethered spinal cord syndrome
- Thalamocortical dysrhythmia
- Thomsen disease
- Thoracic outlet syndrome
- Tic Douloureux
- Todd's paralysis
- Tourette syndrome
- Toxic encephalopathy
- Transient ischemic attack
- Transmissible spongiform encephalopathies
- Transverse myelitis
- Traumatic brain injury
- Tremor
- Trichotillomania
- Trigeminal neuralgia
- Tropical spastic paraparesis
- Trypanosomiasis
- Tuberous sclerosis
- Tinnitus
- U Unverricht–Lundborg disease
- V Vestibular schwannoma
- Von Hippel–Lindau disease
- Viliuisk encephalomyelitis
- Visual Snow
- W Wallenberg's syndrome
- Werdnig–Hoffmann disease – see Spinal muscular atrophy
- Wernicke's encephalopathy
- West syndrome
- Whiplash
- Williams syndrome
- Wilson's disease
- Y Y-Linked hearing impairment
- Z Zellweger syndrome

389 Total # of neurological diseases listed in Wikipedia

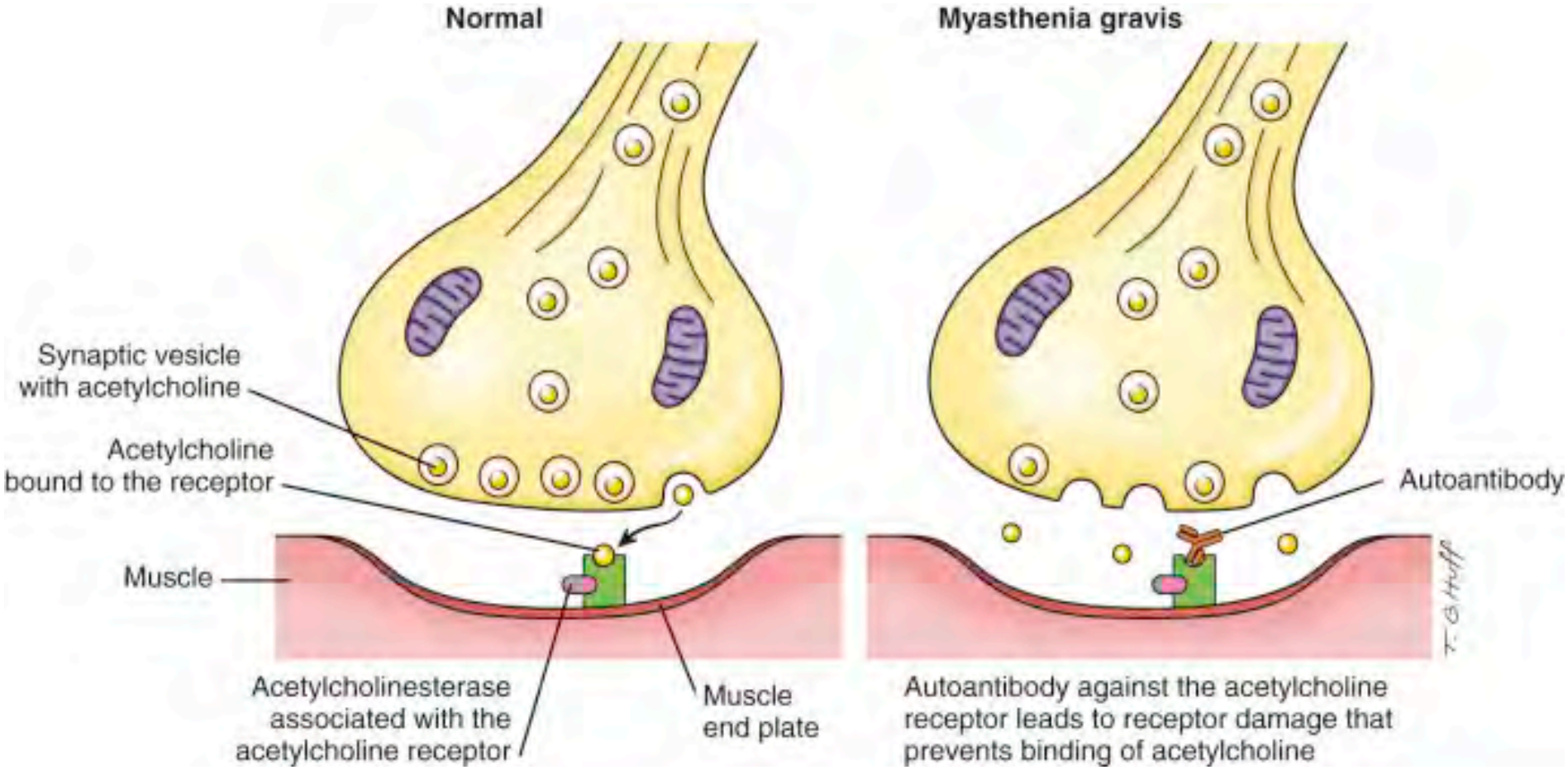

Normal
Myasthenia gravis
Synaptic vesicle with acetylcholine
Acetylcholine bound to the receptor
Muscle
Acetylcholinesterase associated with the acetylcholine receptor
Muscle end plate
Autoantibody
Autoantibody against the acetylcholine receptor leads to receptor damage that prevents binding of acetylcholine

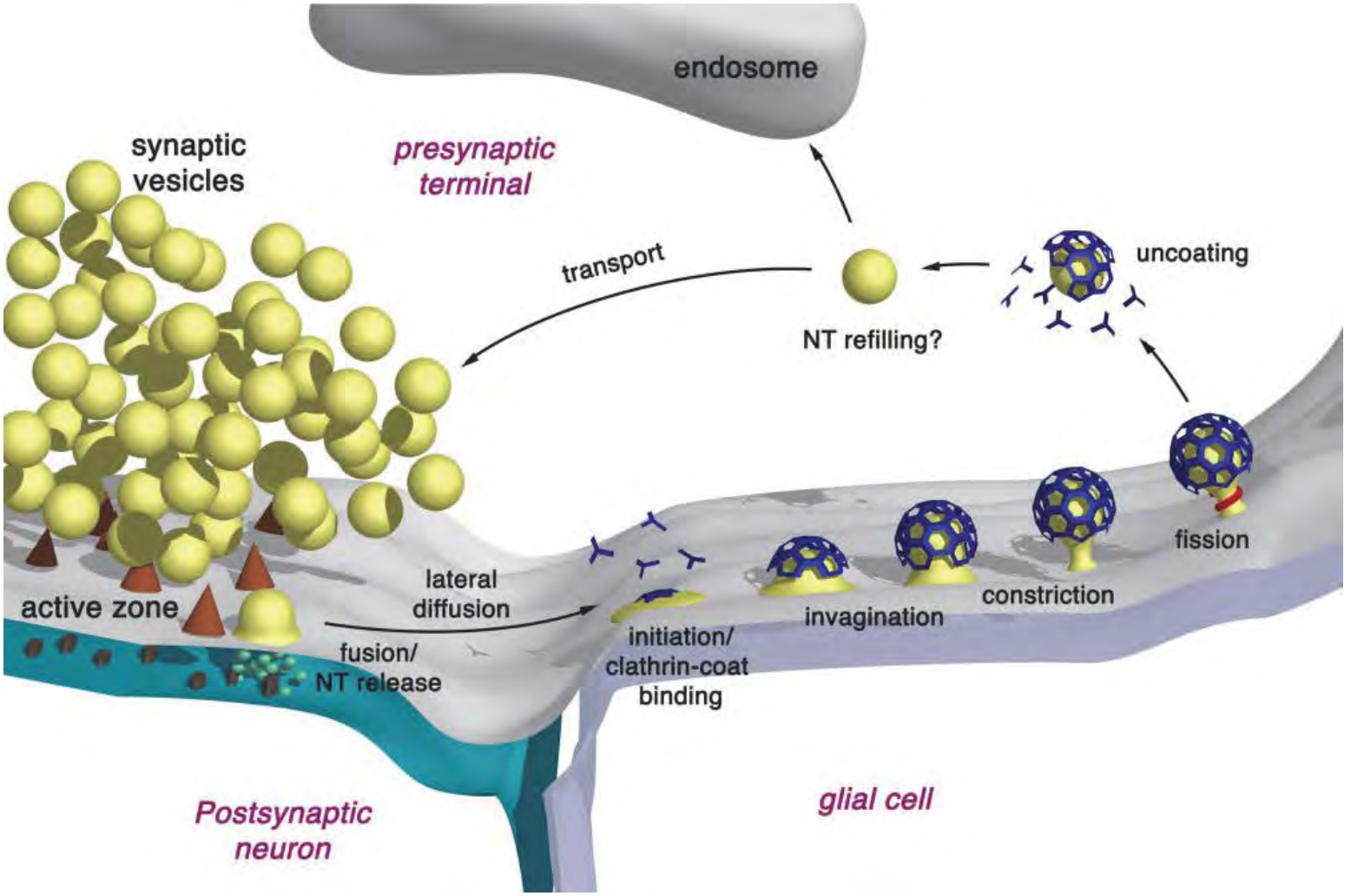
TABLE 7.1 Function of Synaptic Vesicle Proteins

Protein	Function
Proton pump	Generation of electrochemical gradient of protons
Vesicular transmitter transporter	Transmitter uptake into vesicle
VAMP/synaptobrevin	Component of SNARE complex; acts in a late, essential step in vesicle fusion
Synaptotagmin	Ca^{2+}-binding trigger for fusion and component of vesicle docking at release sites via interactions with SNARE complex and lipid; promotes clathrin-mediated endocytosis by binding AP-2 complex
Rab3	Possible role in regulating vesicle targeting and availability
Synapsin	Likely to tether vesicle to actin cytoskeleton
Cysteine string protein	Promotes reliable coupling of action potential to exocytosis
SV2	Unknown
Synaptophysin	Unknown, endocytosis?

TABLE 7.2 Additional Proteins Implicated in Transmitter Release

Protein	Function
Syntaxin	SNARE protein present on plasma membrane (and on synaptic vesicles to a lesser extent); forms core complex with SNAP-25 and VAMP/synaptobrevin; essential for late step in fusion
SNAP-25	SNARE protein present on plasma membrane (and on synaptic vesicles to a lesser extent); forms core complex with syntaxin and VAMP/synaptobrevin; essential for late step in fusion
Nsec-l/munc-18	Syntaxin-binding protein required for all membrane traffic to the cell surface
Complexin	Syntaxin-binding protein; may stabilize an intermediate in core complex formation
Snapin	Binds SNAP-25; associated with synaptic vesicles; unknown function
NSF	ATPase that disassembles SNARE complex; likely to disrupt complexes after exocytosis
a-SNAP	Cofactor for NSF in SNARE complex disassembly
unc-13/munc-13	Active zone protein; vesicle priming for release; modulation of transmission by diacyl glycerol and Protein Kinase C
Rabphilin	C2 domain protein; Ca^{2+}-binding protein; binds rab3 and associates with synaptic vesicle; modulation of transmission (?)
DOC2	Ca^{2+}-binding C2 domain protein; binds munc-18 and SNAREs; regulates spontaneous fusions and asynchronous release
RIM1 and related proteins	Active zone proteins; bind rab3; modulation of transmission
Piccolo	Likely scaffolding protein at active zones
Bassoon	Likely scaffolding protein at active zone
Exocyst (sec6/8 complex)	Marks plasma membrane sites of vesicle fusion; not needed for synaptic vesicle fusion

Endocytosis – Recovery of Synaptic Vesicles



1) After exocytosis, vesicles diffuse laterally away from the active zone.
2) Clathrin binds to the vesicle leading to invagination.
3) Dynamin (GTPase) forms a ring around the constricting vesicle and its hydrolysis leads to separation from the PM.
4) Vesicles are refilled with neurotransmitter and returned to the active zone.

(Shupliakov and Brodin, Exp Cell Res., 2010)

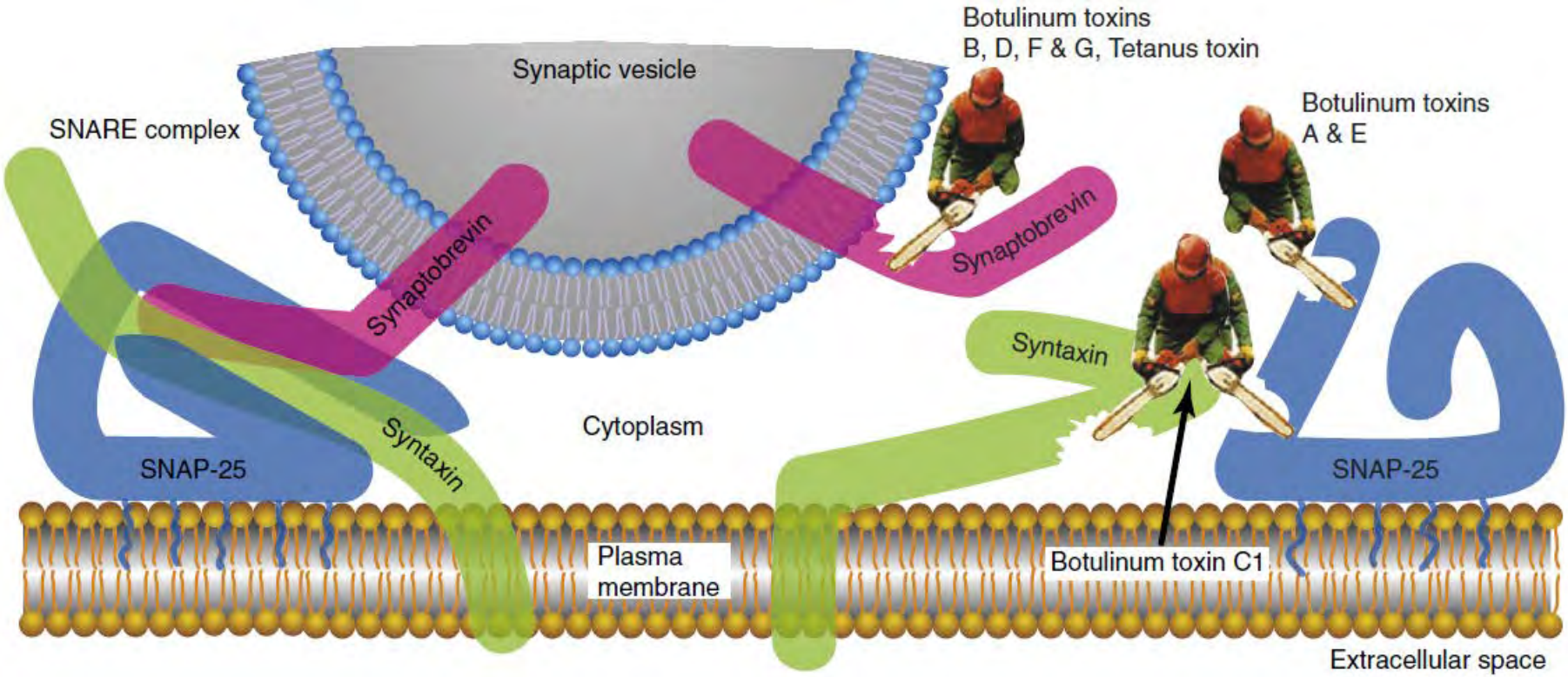


FIGURE 7.5 SNARE proteins and the action of clostridial neurotoxins. The SNARE complex shown at the left brings the vesicle and plasma membranes into close proximity and likely represents one of the last steps in vesicle fusion. Vesicular VAMP, also called synaptobrevin, binds with syntaxin and SNAP-25 that are anchored to the plasma membrane. Tetanus toxin and the botulinum toxins, proteases that cleave specific SNARE proteins as shown, can block transmitter release.

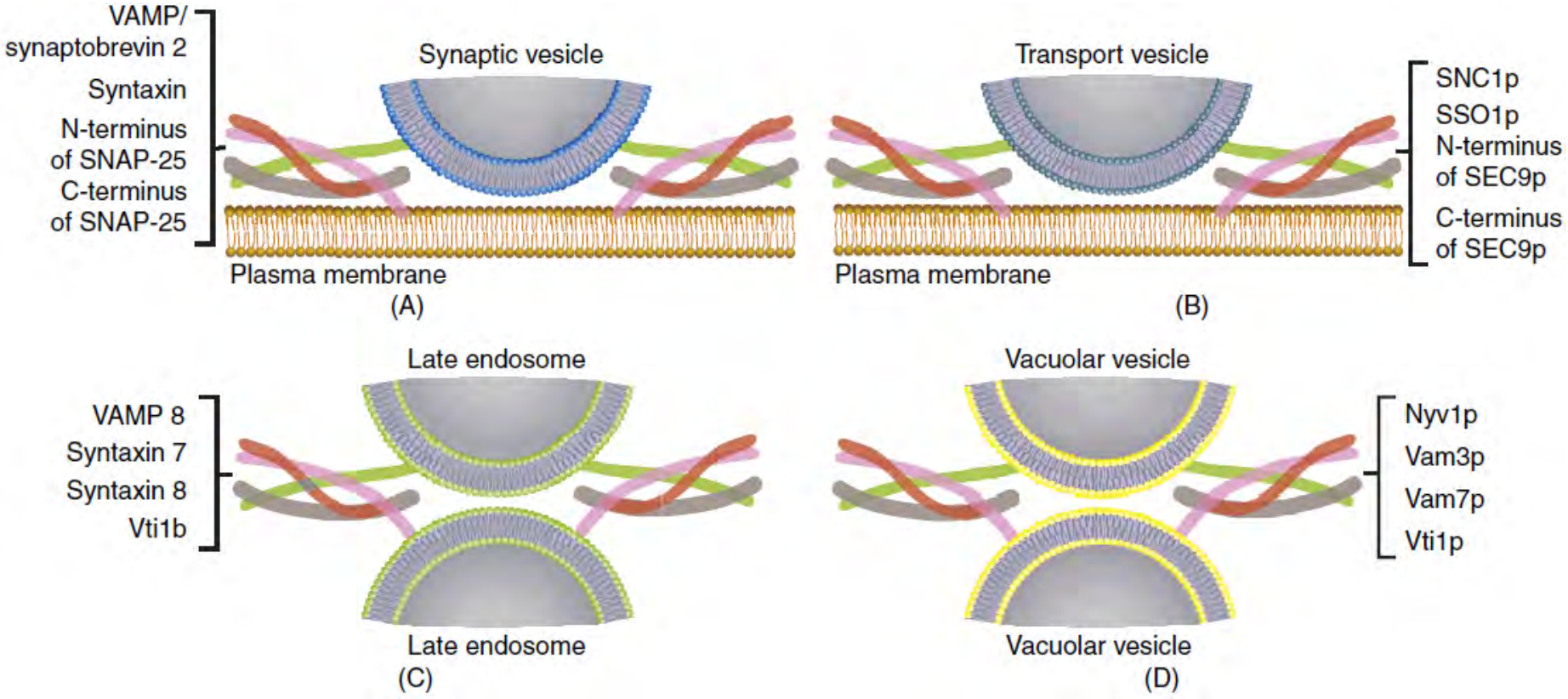


FIGURE 7.6 Neurotransmitter release shares a core mechanism with many membrane fusion events within eukaryotic cells. The fusion of synaptic vesicles (A) is driven by a particular complex of four coiled-coil domains contributed by three different proteins. Exocytosis in yeast (B), the fusion of late endosomes in mammalian cells (C), and the fusion of vacuolar vesicles in yeast (D) exemplify the closely related four-stranded coiled-coil complexes required to drive fusion in other membrane-trafficking steps.

Vesicle Fusion: Time Considerations

From action potential to neurotransmitter release it takes >200 µs

- Delay is due to the influx of Calcium and formation of the fusion pore.

Vesicular fusion must be a fast process

- Vesicles must already be present at the active site **or**
- in a fusion-ready complex that is triggered by Ca^{2+} influx

Other steps in the process can be slower

- Docking, priming, recycling, neurotransmitter filling

But not too slow, or else a neuron firing at 5 Hz can consume its vesicles in <1 minute.

- Once endocytosed, a vesicle can be filled and ready for release in 30 s.

CHAPTER

8

Neurotransmitter Receptors

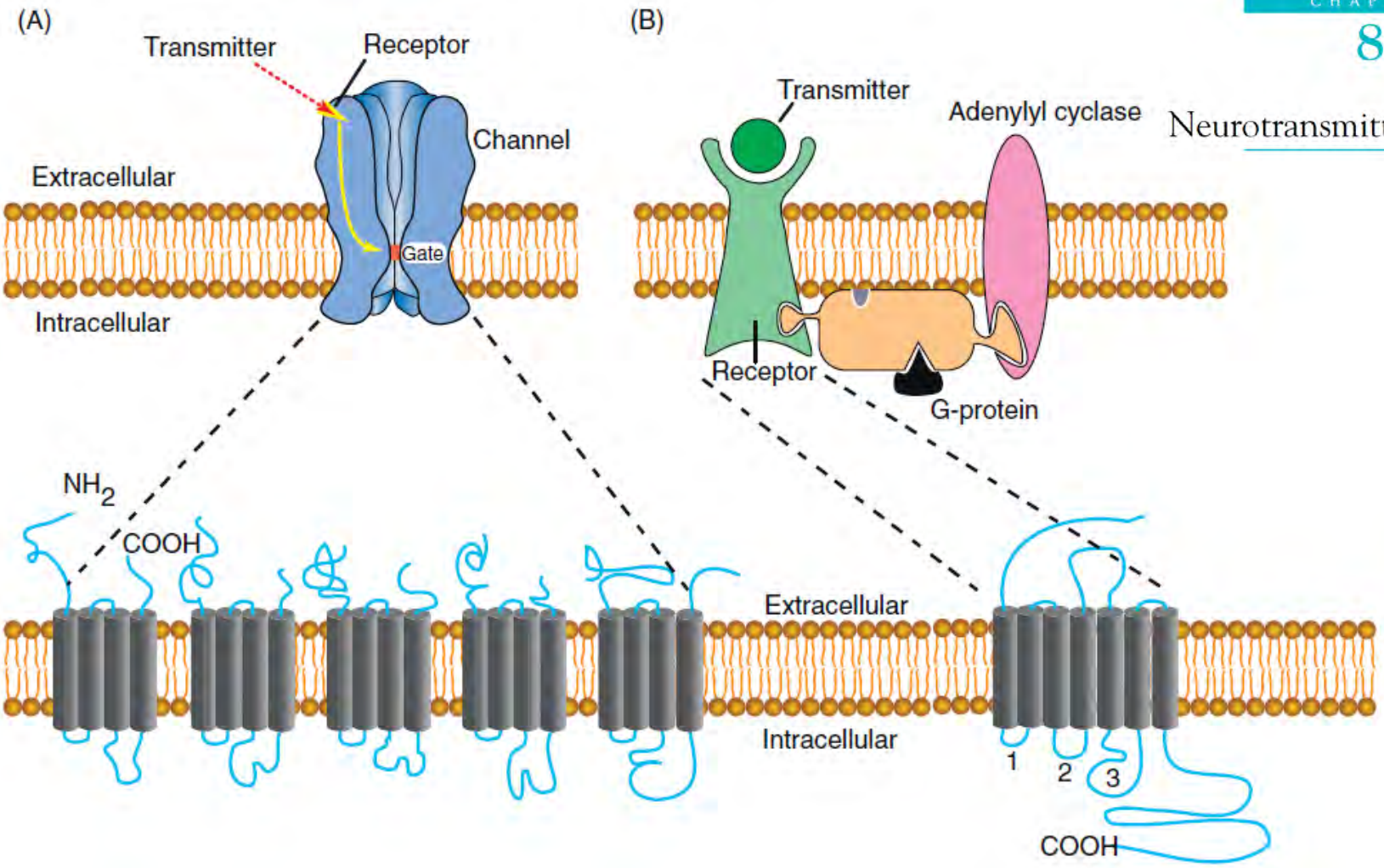


FIGURE 8.1 A comparison of the general structural features of ionotropic and G-protein couples receptors. (A) *Ionotropic receptors* bind transmitter, and this binding translates directly into the opening of the ion channel through a series of conformational changes. Ionotropic receptors are composed of multiple subunits. The five subunits that together form the functional nAChR are shown. Note that each of the nAChR subunits wraps back and forth through the membrane four times and that the mature receptor is composed of five subunits. (B) *G-protein coupled receptors* bind transmitter and, through a series of conformational changes, bind to G-proteins and activate them. G-proteins then activate enzymes such as adenylyl cyclase to produce cAMP. Through the activation of cAMP-dependent protein kinase, ion channels become phosphorylated, which affects their gating properties. GPCRs are single subunits or dimers. They contain seven transmembrane-spanning segments, with the cytoplasmic loops formed between the segments providing the points of interactions for coupling to G-proteins.

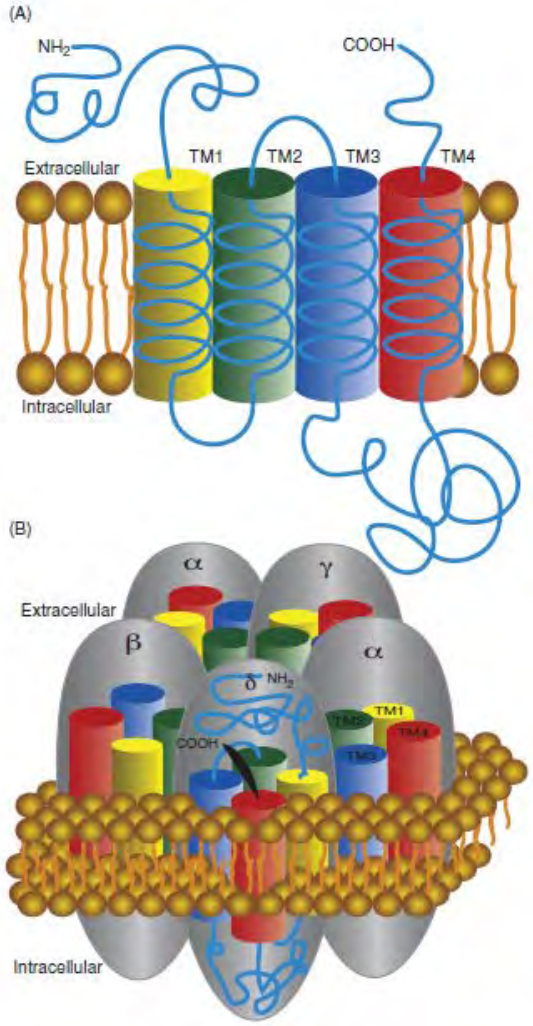


FIGURE 8.3 (A) Diagram highlighting the orientation of membrane-spanning segments of one subunit of the nAChR. The amino and carboxy termini extend in the extracellular space. The four membrane-spanning segments are designated TM1–TM4. Each forms an α helix as it traverses the membrane. (B) Side view of the five subunits in their approximate positions within the receptor complex. There are two α subunits present in each nAChR. (C) Top view of all five subunits highlighting the relative positions of their membrane-spanning segments, TM1–TM4, and the position of TM2 that lines the channel pore.

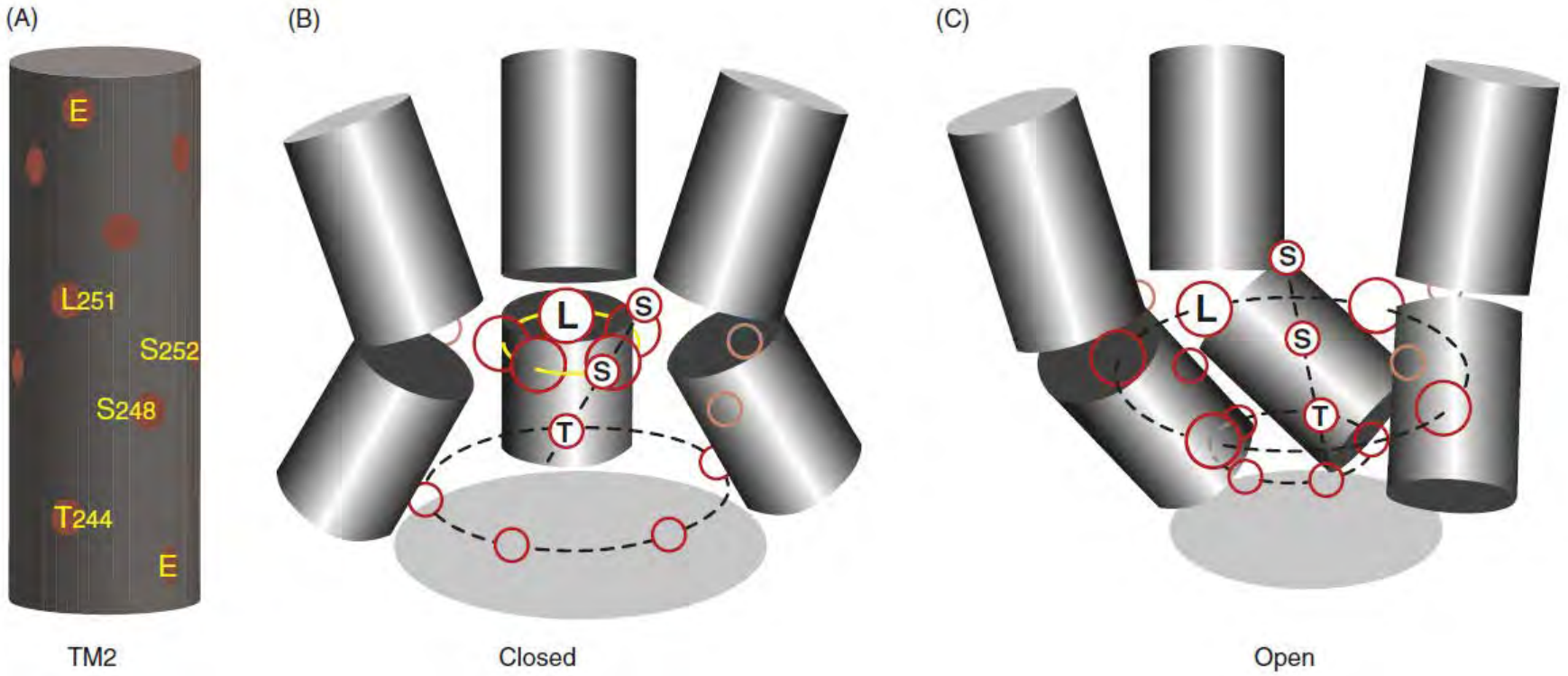


FIGURE 8.4 (A) Relative positions of amino acids in the TM2 segment of one of the nAChR *a* subunits modeled as an *a* helix. Glutamate residues (E) that form parts of the negatively charged rings for ion selectivity are shown at the top and bottom of the helix. (B) Arrangement of three of the five TM2 segments of the nAChR modeled with the receptor in the closed (ACh-free) configuration. In the closed configuration, leucine (L) residues form a right ring in the center of the pore that blocks ion permeation. (C) Arrangement of the three TM2 segments after ACh binds to the receptor. In the open configuration, construction formed by the ring of leucine (L) residues opens as the helices twist about their axes. Note that polar serine (S) and threonine (T) residues align when ACh binds, which apparently help the water-solvated ions travel though the pore. *Adapted from Unwin (1995).*

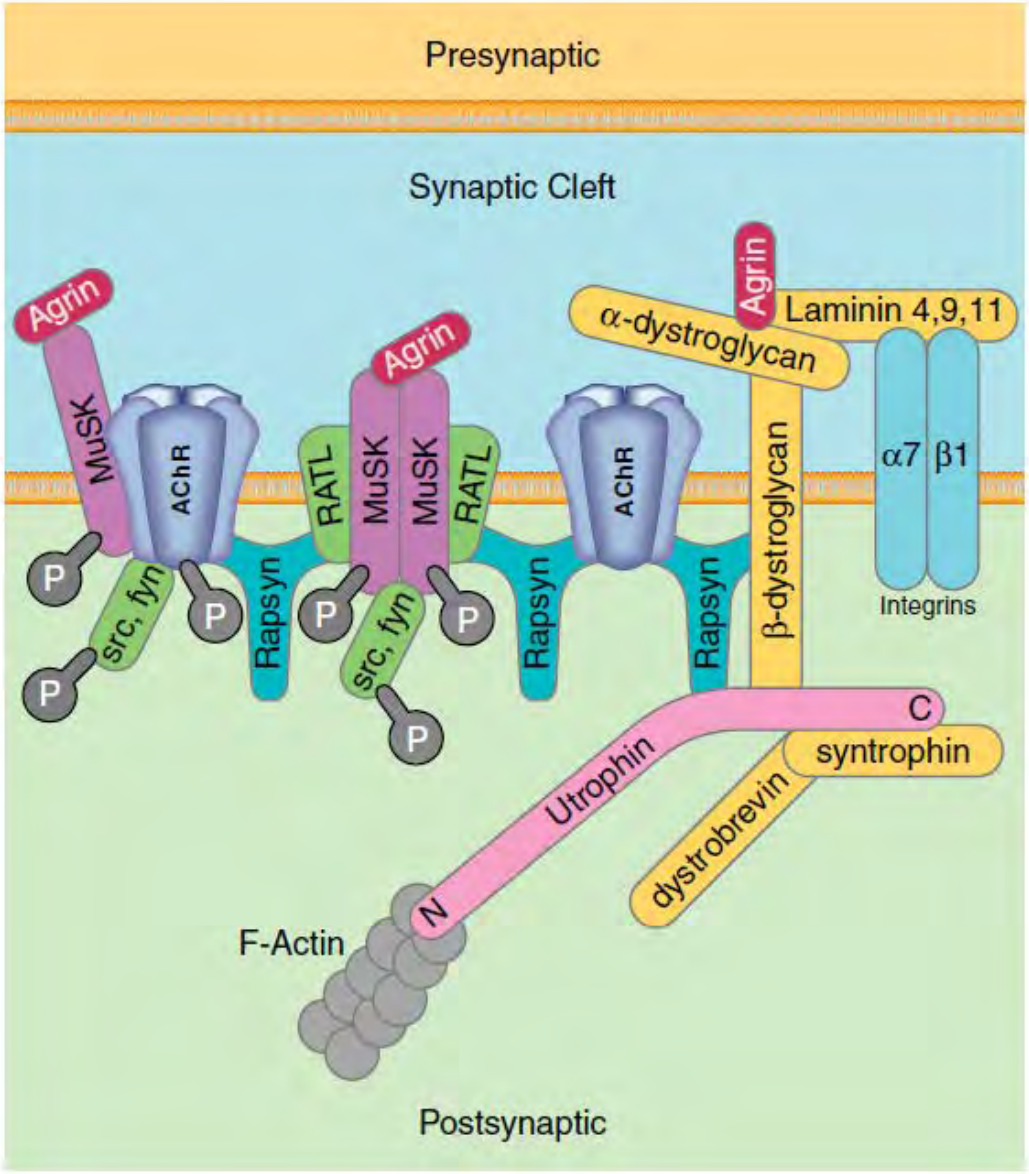


FIGURE 8.5 Diagram of nAChR clustering at the neuromuscular junction. Rapysn is a major anchoring protein at the neuromuscular junction that binds to itself and to the nAChR that concentrates and stabilizes nAChRs. The development and stabilization of the neuromuscular junction is mediated by a number of signaling cascades, only a few of which are shown. For example, agrin released from the presynaptic motor neuron binds to a number of proteins associated with the postsynaptic membrane including the tyrosine kinase MuSK (muscle specific kinase). MuSK activation by agrin recruits and activates the soluble tyrosine kinases src and fyn that further modify a number of proteins. RATL (rapsyn associated linker protein) is a membrane-bound protein that binds to both MuSK and to rapsyn to anchor MuSK at the neuromuscular junction. Agrin also interacts with the dystroglycans that make up the dystrophin complex important for the maintenance of the neuromuscular junction. Rapsyn also binds to the utrophin complex that anchors the overlying protein complex to the actin cytoskeleton. *Adapted from Willmann and Fuhrer (2002).*

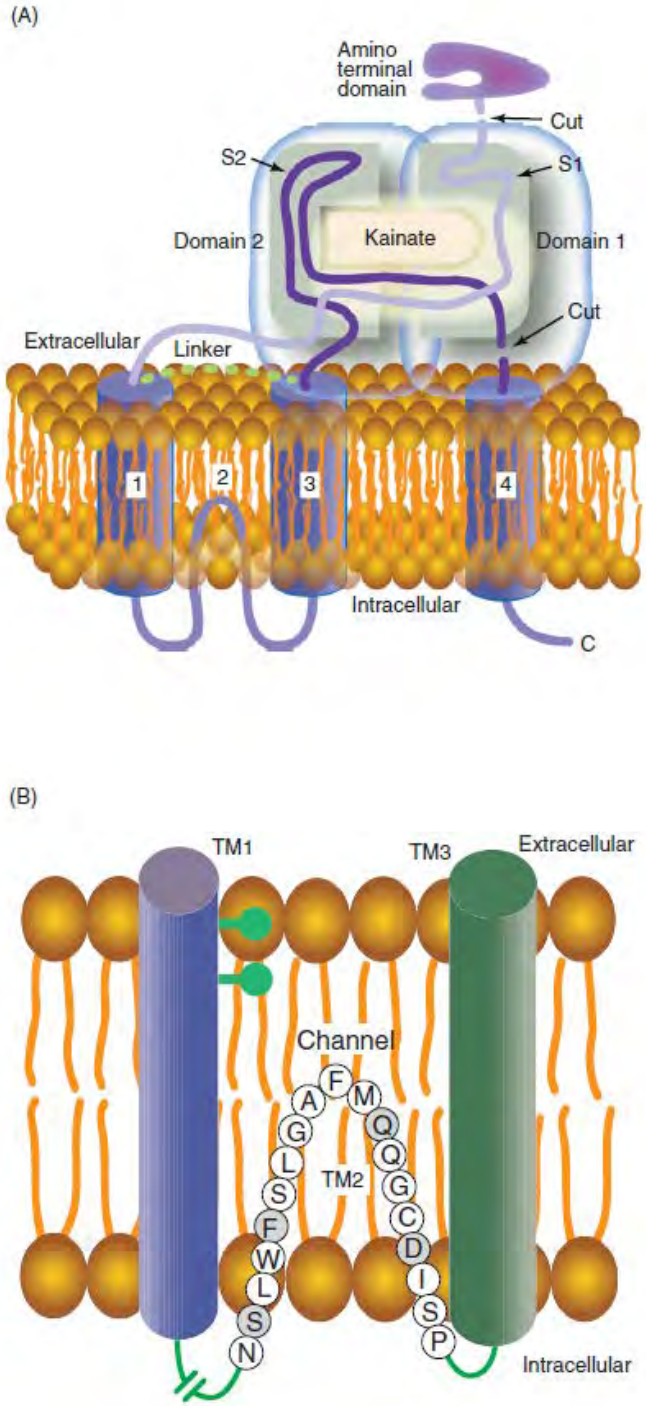


FIGURE 8.6 (A) Model of one of the subunits of the ionotropic glutamate receptor. Ionotropic glutamate receptors have four membrane-associated segments; however, unlike nAChR, only three of them completely traverse the lipid bilayer. TM2 forms a loop and exits back into the cytoplasm. This leaves the large N-terminal region extending into the extracellular space, whereas the C terminus extends into the cytoplasm. Two domains in the extracellular segments associate with each other to form the binding site for transmitter, in this example kainate, a naturally occurring agonist of glutamate. (B) Enlarged area of the predicted structure and amino acid sequence of the TM2 region of the glutamate receptor, GluR3. TM1 and TM3 are drawn as cylinders in the membrane flanking TM2. The residue that determines Ca^{2+} permeability of the non-NMDA receptor is the glutamine residue (Q) highlighted in gray. In NMDA receptors, an asparagine residue at this same position is the proposed site of interaction with Mg^{2+} ions that produce the voltage-dependent channel block. Serine (S) and phenylalanine (F), also shaded in gray, are highly conserved in the non-NMDA receptor family. The aspartate (D) residue is also conserved and is thought to form part of the internal cation-binding site. The break in the loop between TM1 and TM2 indicates a domain that varies in length among ionotropic glutamate receptors. *Adapted from Wo and Oswald (1995).*

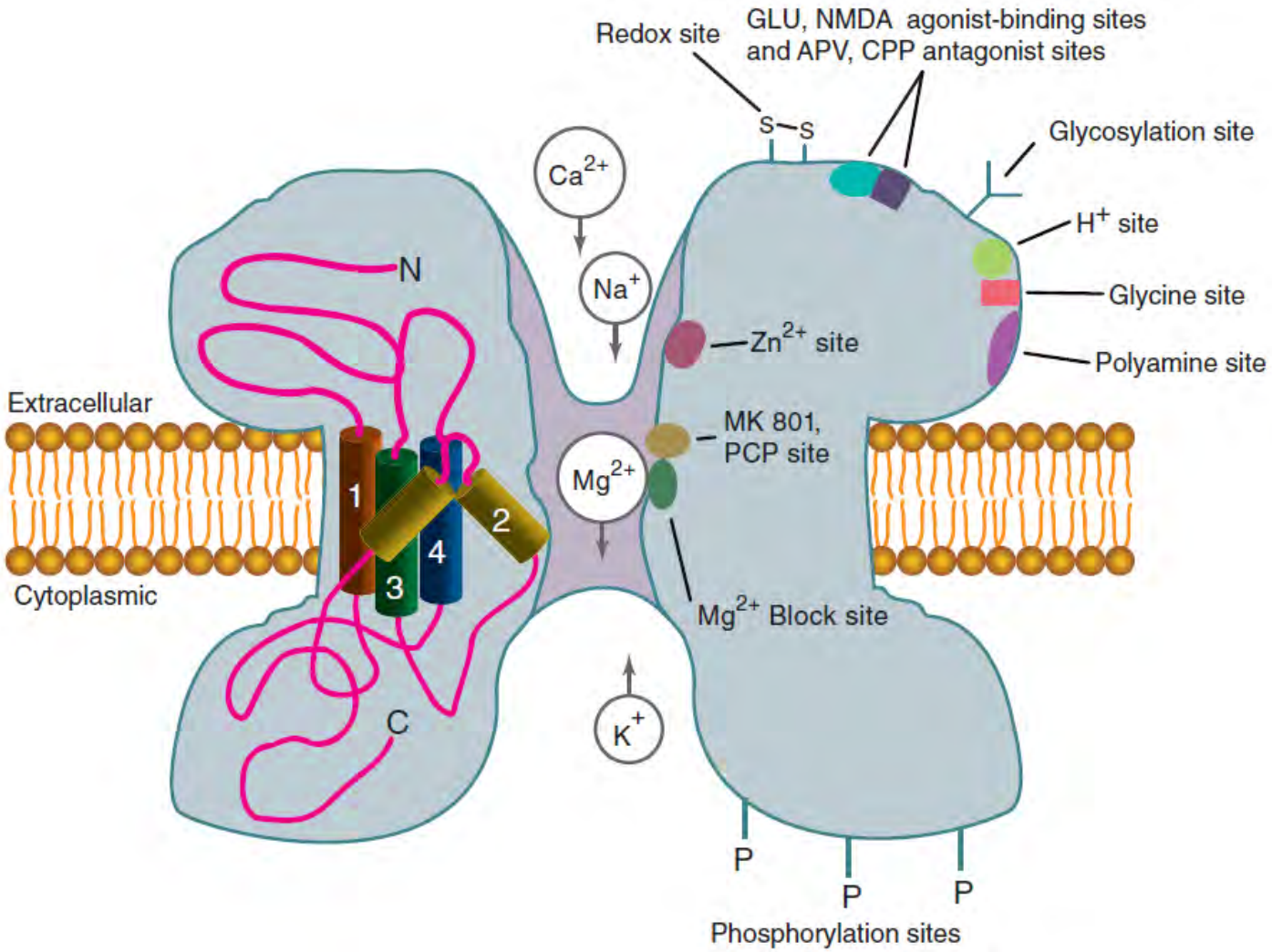


FIGURE 8.7 Diagram of an NMDA receptor highlighting binding sites for numerous agonists, antagonists, and other regulatory molecules. The location of these sites is a crude approximation for the purpose of discussion. *Adapted from Hollmann and Heinemann (1994).*

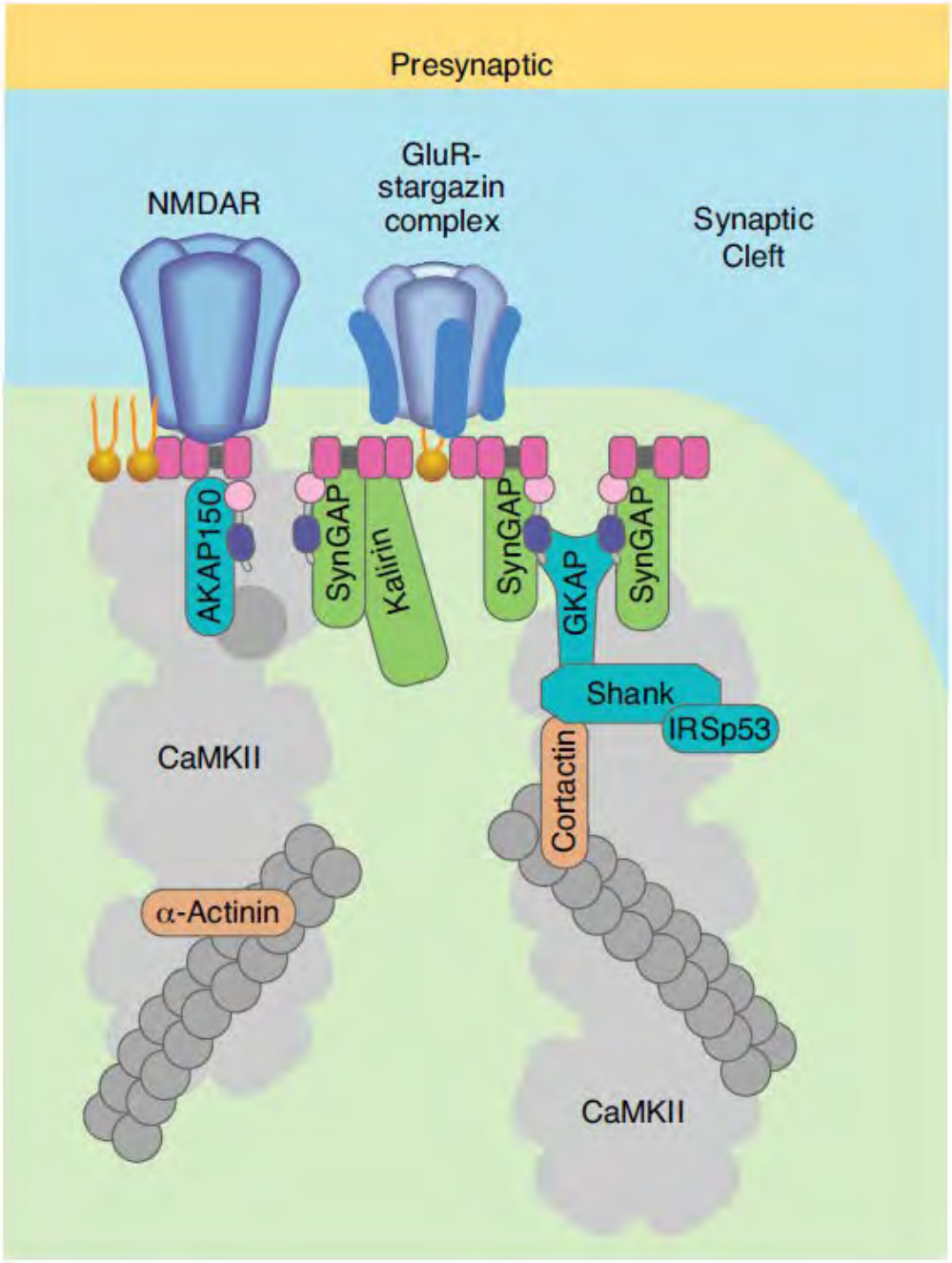


FIGURE 8.8 Diagram of glutamate receptor clustering at an excitatory synapse. The NMDA receptor interacts directly with PSD-95 through binding to one of PSD-95's three PDZ domains (the PDZ domains of PSD-95 are shown as pink squares). The AMPAR is associated with a protein called stargazin and stargazin interacts with one of the PDZ domains of PSD-95. Only a few of the many other signaling and scaffolding proteins at excitatory synapses are shown. AKAP150 is A-kinase anchoring protein of 150 kDa that binds to protein kinase A and other proteins, SynGAP is an abundant synaptic associated Ras GTP-ase activating protein that interacts with PSD-95, GKAP is a guanylate kinase associated protein that interacts with PSD-95, CaMKII is an abundant Ca^{2+}/calmodulin-activated protein kinase that interacts directly with the NMDAR. CaMKII also interacts with itself and with α-actinin, which is an actin-binding protein. This web of protein-protein interactions forms the electron dense structures called the postsynaptic densities visible in electon micrographs of excitatory synapses. *Adapted from Sheng and Hoogenraad (2007).*

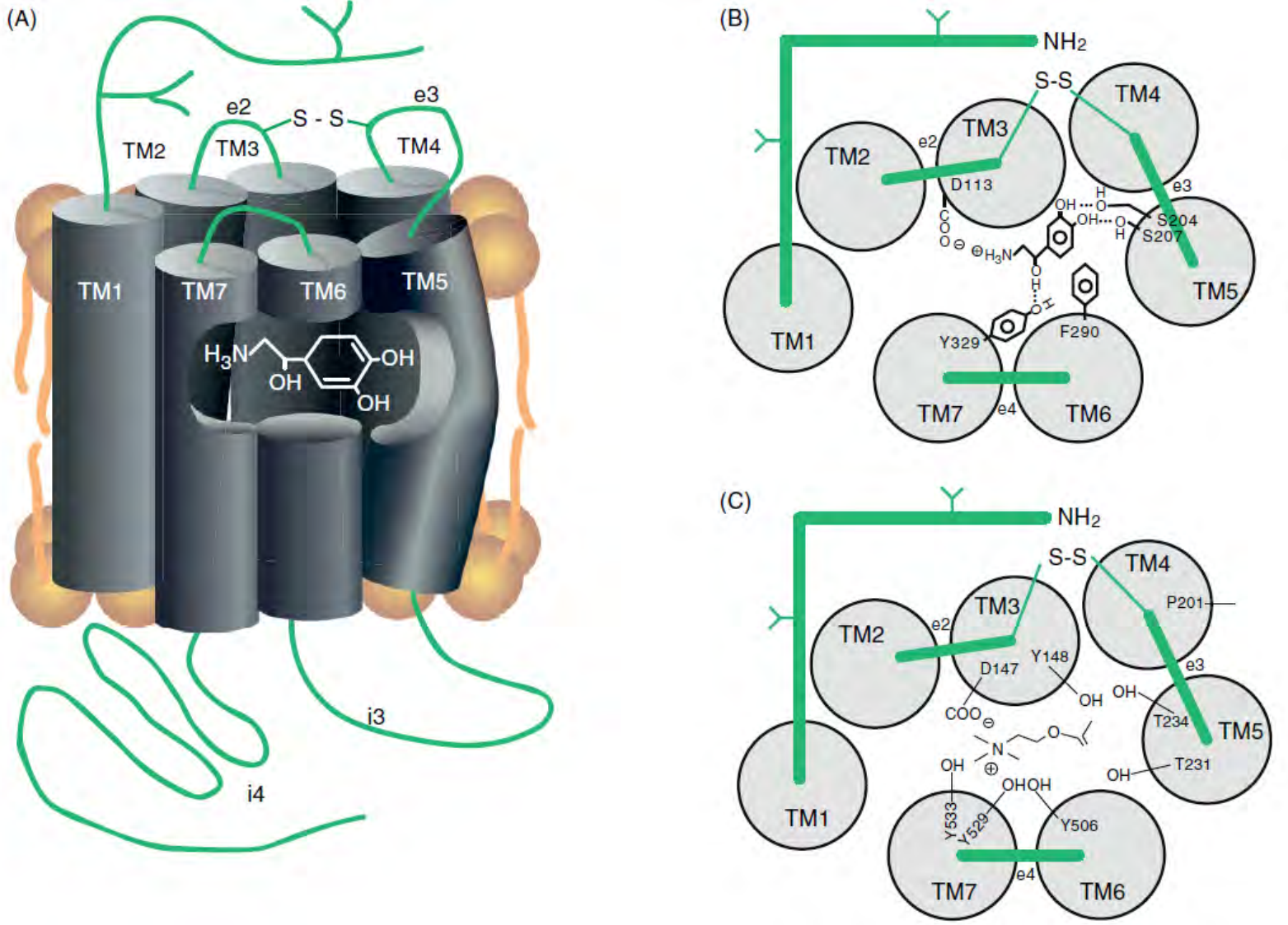


FIGURE 8.9 (A) Diagram showing the approximate position of the catecholamine-binding site in the βAR. The transmitter-binding site is formed by amino acids whose side chains extend into the center of the ring produced by the seven transmembrane domains (TM1–TM7). Note that the binding site exists at a position that places it within the plane of the lipid bilayer. (B) A view looking down on a model of the βAR identifying residues important for ligand binding. The seven transmembrane domains are represented as gray circles labeled TM1 though TM7. Amino acids composing the extracellular domains are represented as green bars labeled e1 through e4. The disulfide bond (–S–S–) that links e2 to e3 is also shown. Each of the specific residues indicated makes stabilizing contact with the transmitter. (C) A view looking down on a model of the mAChR identifying residues important for ligand binding. Stabilizing contacts, mainly through hydroxyl groups (-OH), are made with the transmitter on four of the seven transmembrane domains. The chemical nature of the transmitter (i.e., epinephrine versus Ach) determines the type of amino acids necessary to produce stable interactions in the receptor-binding site (compare B and C). *Adapted from Strosberg (1990).*

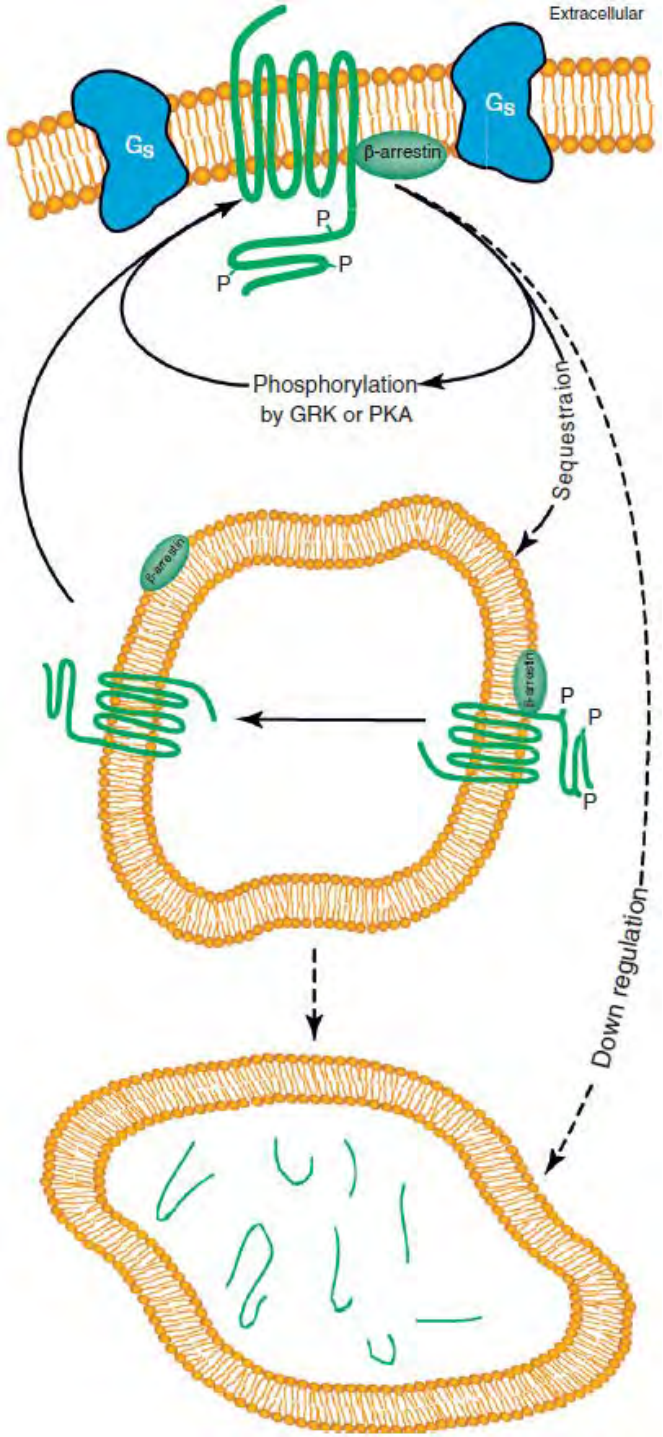
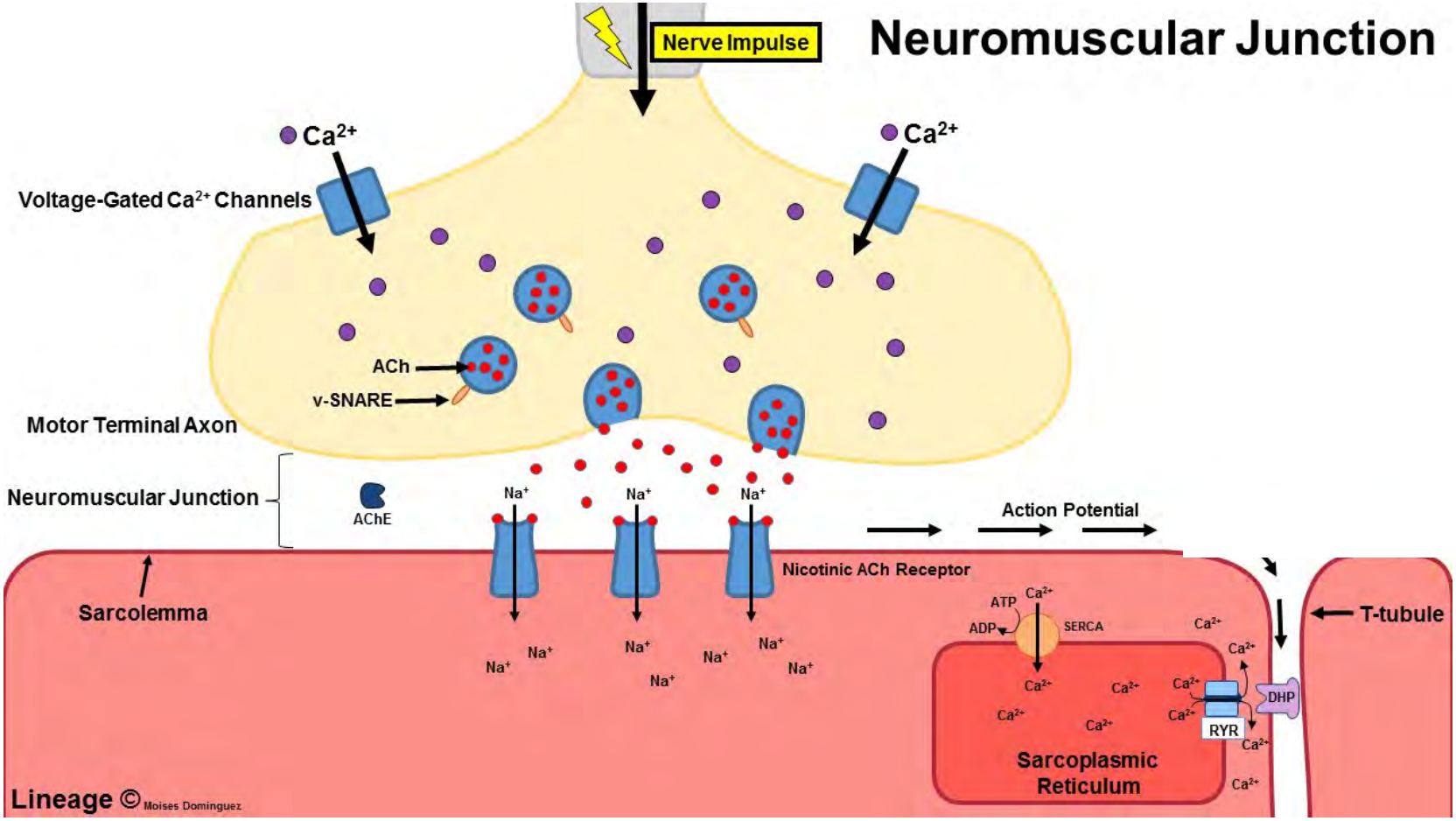


FIGURE 8.10 Intracellular pathways associated with desensitization of GPCRs. GPCRs are phosphorylated (noted with P) on their intracellular domains by PKA, GRK, and other protein kinases. The phosphorylated form of the receptor can be removed from the cell surface by a process called sequestration with the help of the adapter protein *β*-arrestin; thus fewer binding sites remain on the cell surface for transmitter interactions. In intracellular compartments, the receptor can be dephosphorylated and returned to the plasma membrane in its basal state. Alternatively, phosphorylated receptors can be degraded (down regulated) by targeting to a lysosomal organelle. Degradation requires replenishment of the receptor pool through new protein synthesis. *Adapted from Kobilka (1992).*

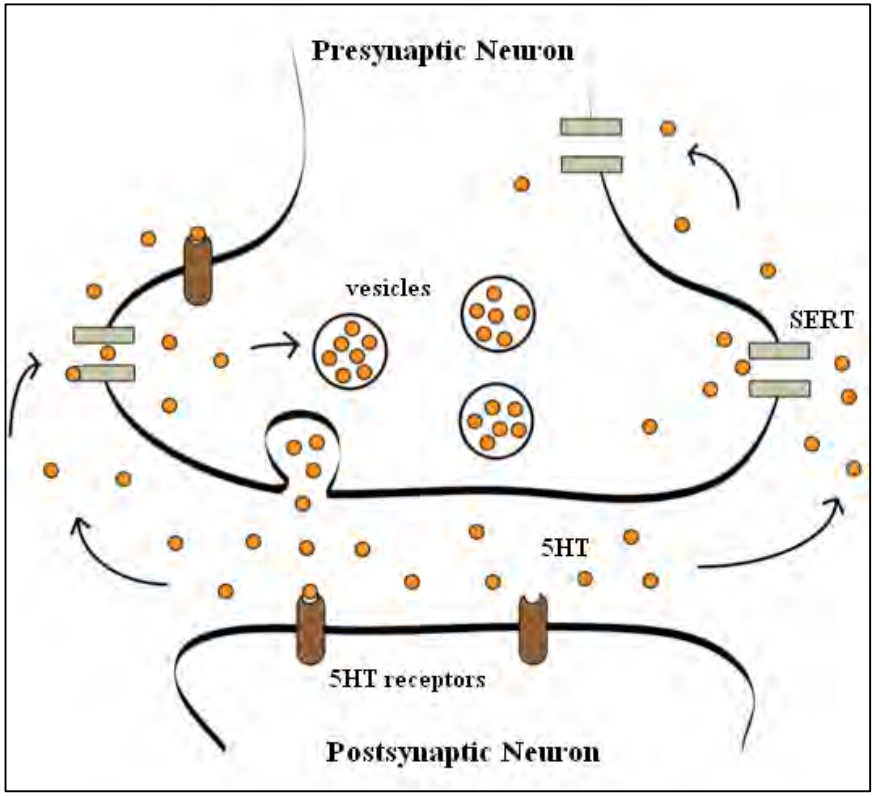
Disorders affecting the NMJ



Roles of Serotonin (5HT)

Serotonin and behavior

- Mood
- Aggression
- Appetite
- Sleep
- Libido
- Social



Serotonin and disease

- Low 5HT levels
  - Autism
  - Major depression
  - Bipolar disorder
  - Bulimia, anorexia
  - Social anxiety disorder
  - Seasonal affective disorder
  - Premenstrual syndrome
  - OCD
  - SIDS
  - Irritable Bowel Syndrome
  - Schizophrenia
  - Suicide
- Excessive 5HT levels
  - Chronic pulmonary hypertension
  - Serotonin syndrome

Disadvantages of antidepressants:

- long time for therapeutic benefits
- side-effects
- withdrawal

ELECTRIC ORGANS

- Electric organs are organs specialized for the production of an electric field outside the body.
- Built up from a large number of disc like cells, called electroplates.
- Electroplates embedded in a jelly like extracellular material and enclosed within a compartment of connective tissue.

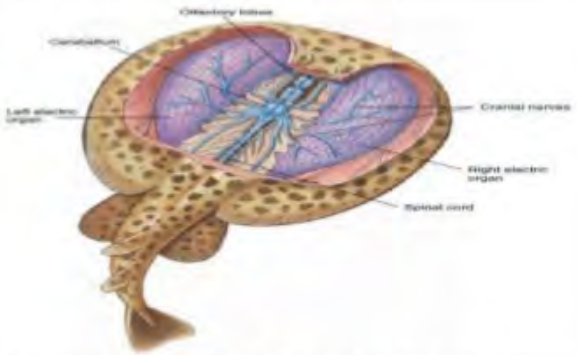


Diagram showing electric organs