Neurotransmitter Release & Receptors

Ben Richardson, PhD

Department of Pharmacology

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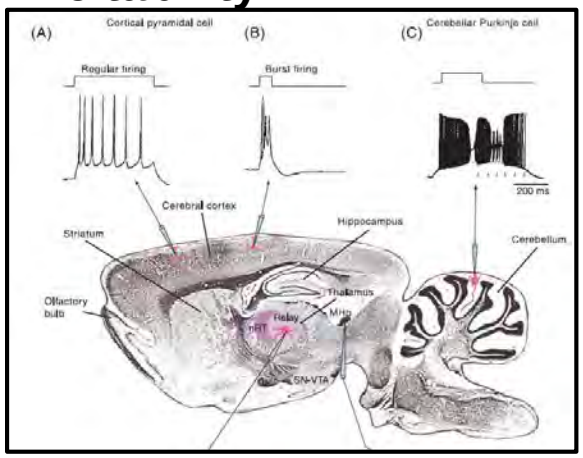
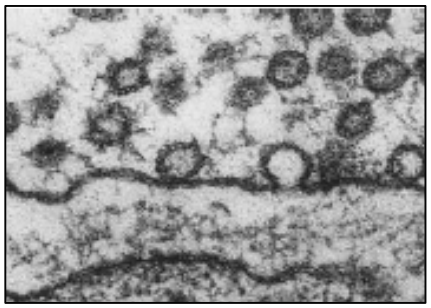
FOUNDATIONAL PRINCIPLES OF THE BRAIN'S DYNAMIC INTERACTIONS

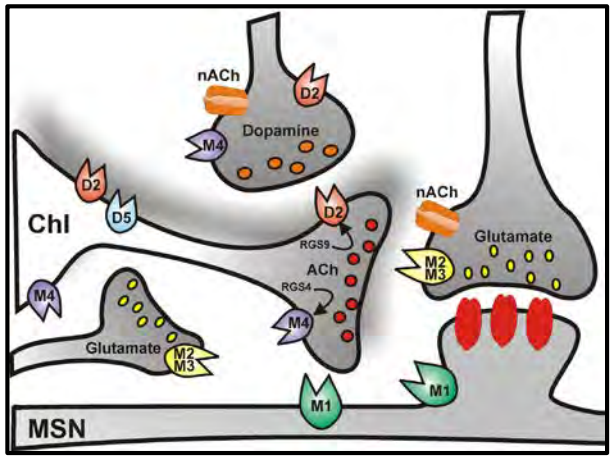
Membrane Excitability



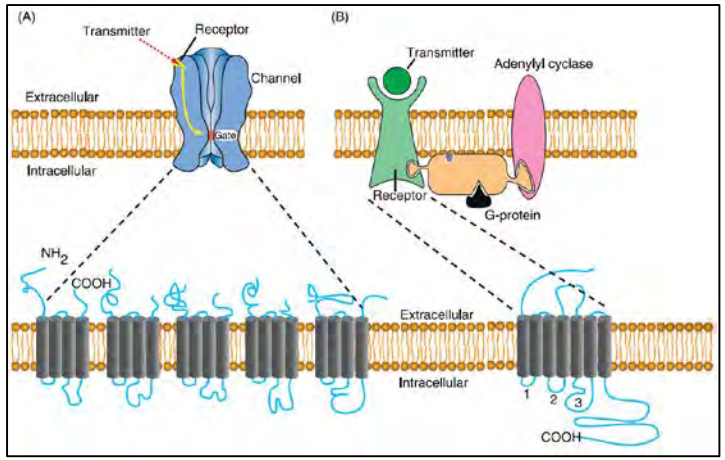
NT Release



Chemical Neurotransmission



Receptor Activation

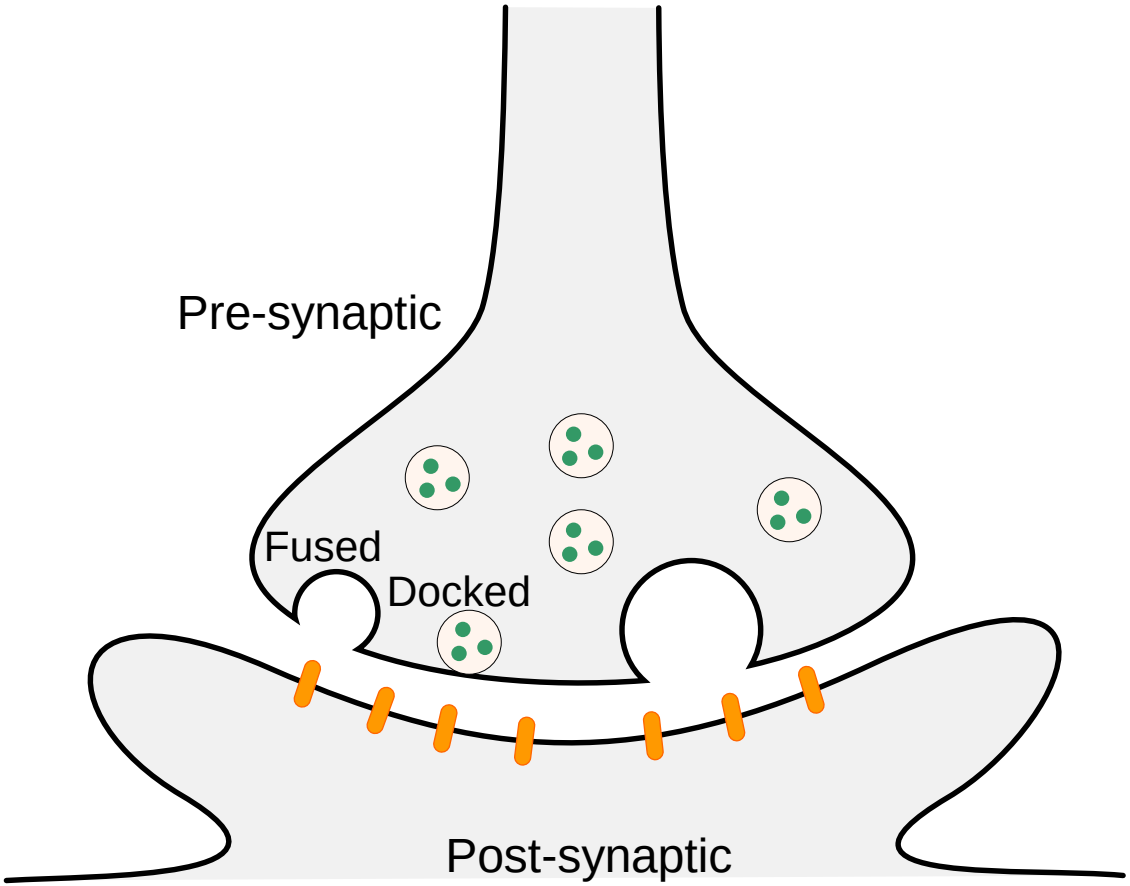


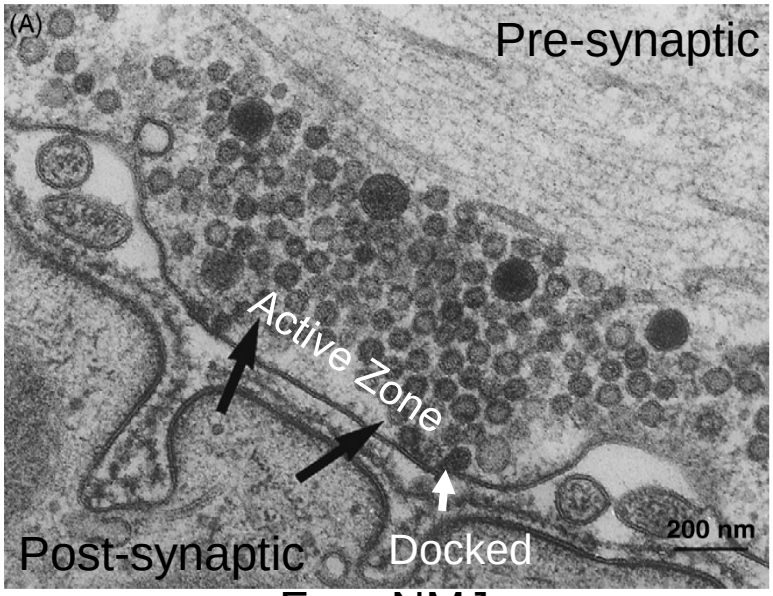
Bonsi et al., 2011. Front. Neuroanat.
Fund. Neuroscience, 4th Ed.

Part 1: Neurotransmitter Release

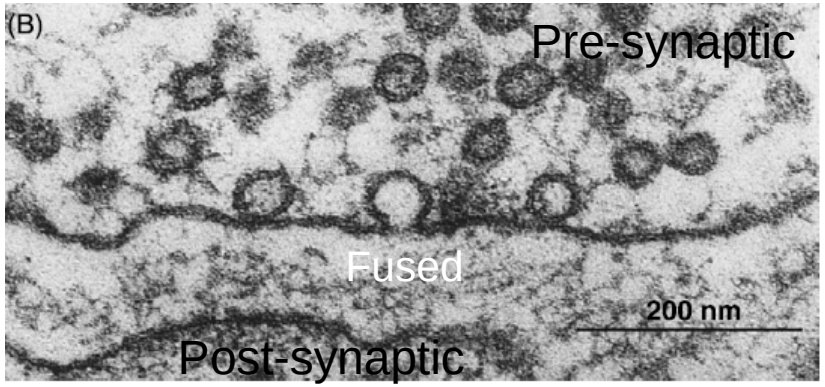
- NTs in Vesicles
- Mechanisms of Vesicle Fusion
- Vesicle Cycling
- Quantal Release
- Short-term plasticity

NEUROTRANSMITTERS IN VESICLES





Frog NMJ



1-4 Active zones (fusion sites)/varicosity or bouton

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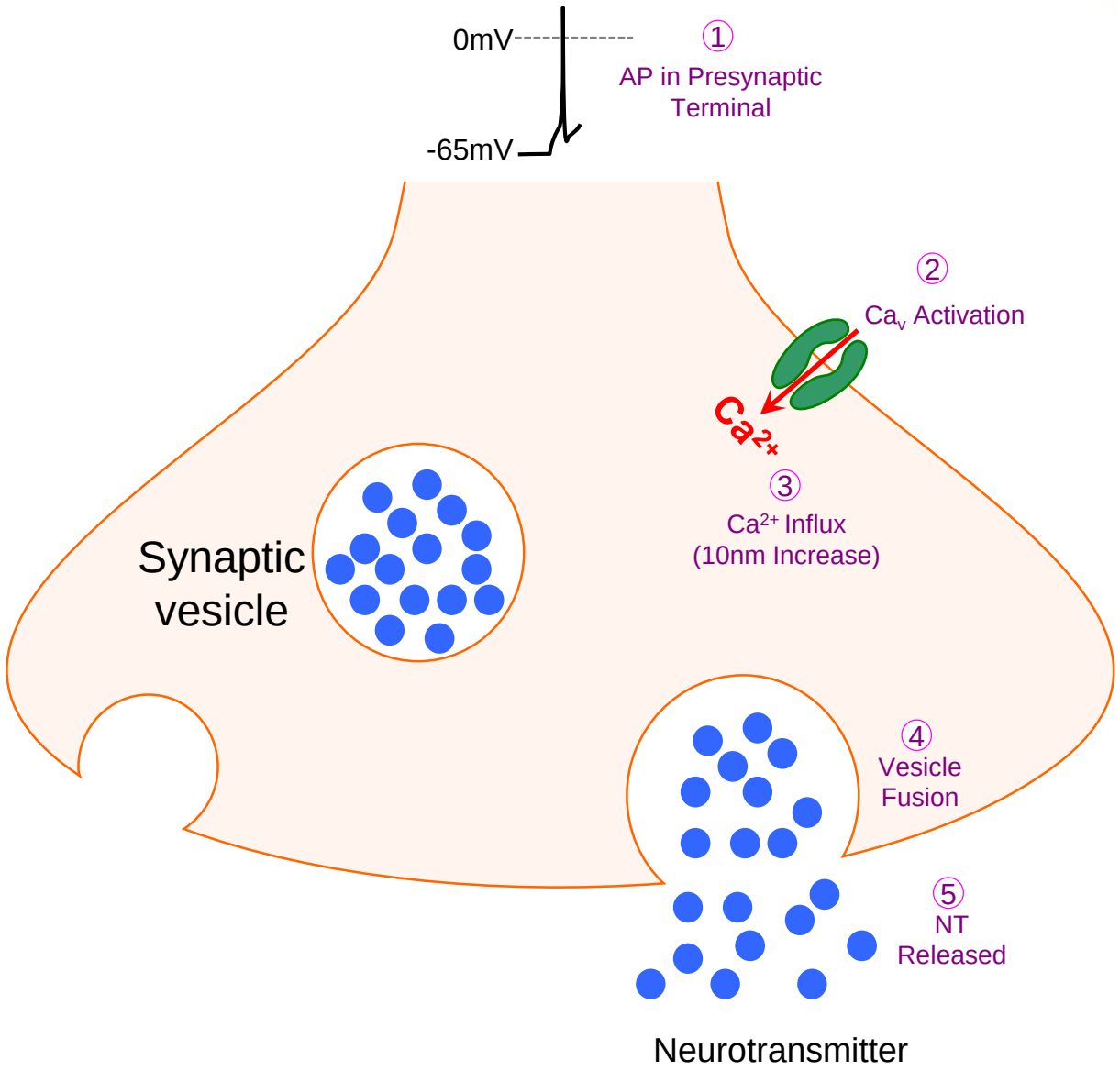
DEPOLARIZATION CAUSES VESICLE FUSION



0-2 vesicles released/AP/active zone

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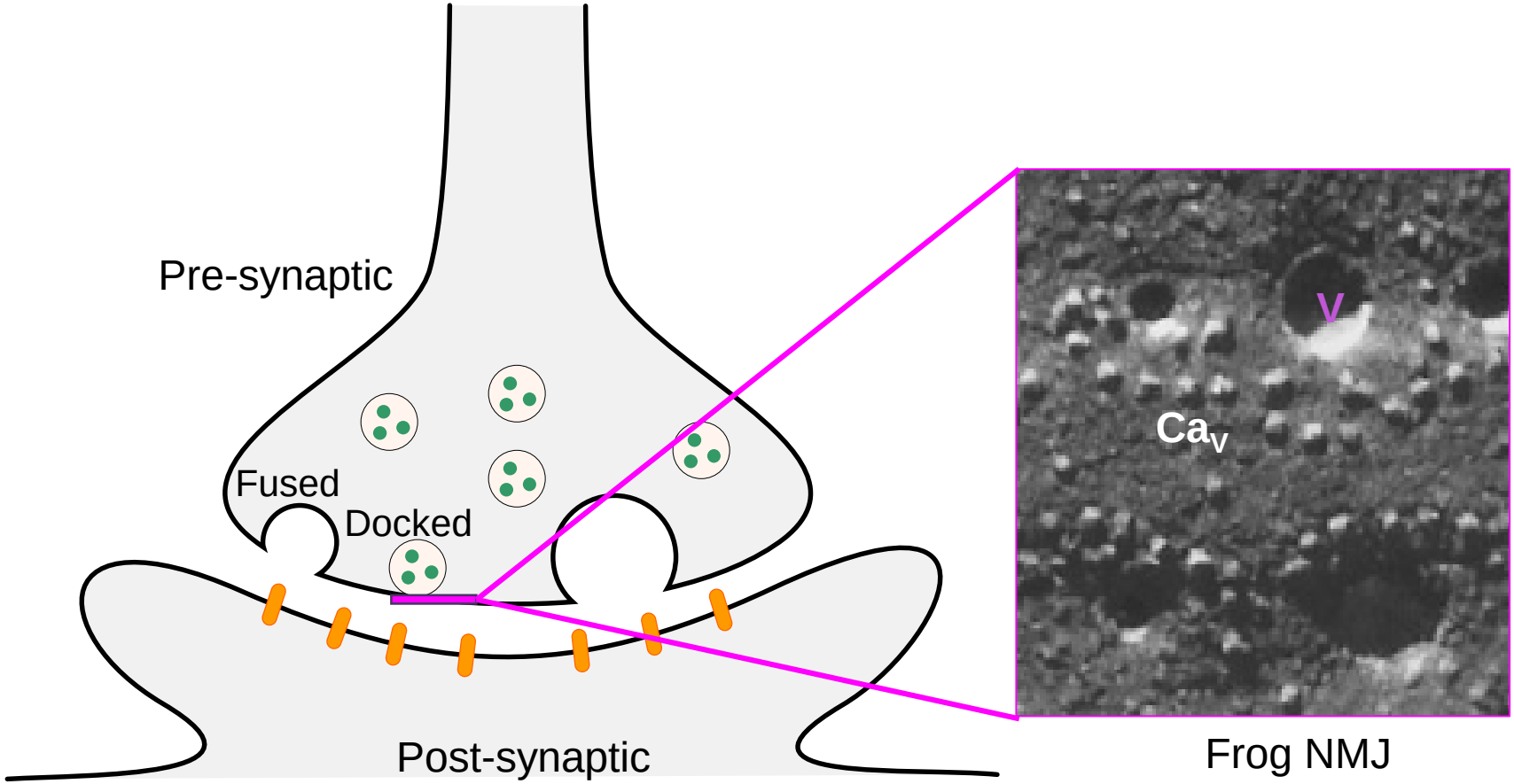
VESICLES FUSE NEAR Ca^{2+} CHANNELS



Why is fusion limited to areas near Ca^{2+} channels?

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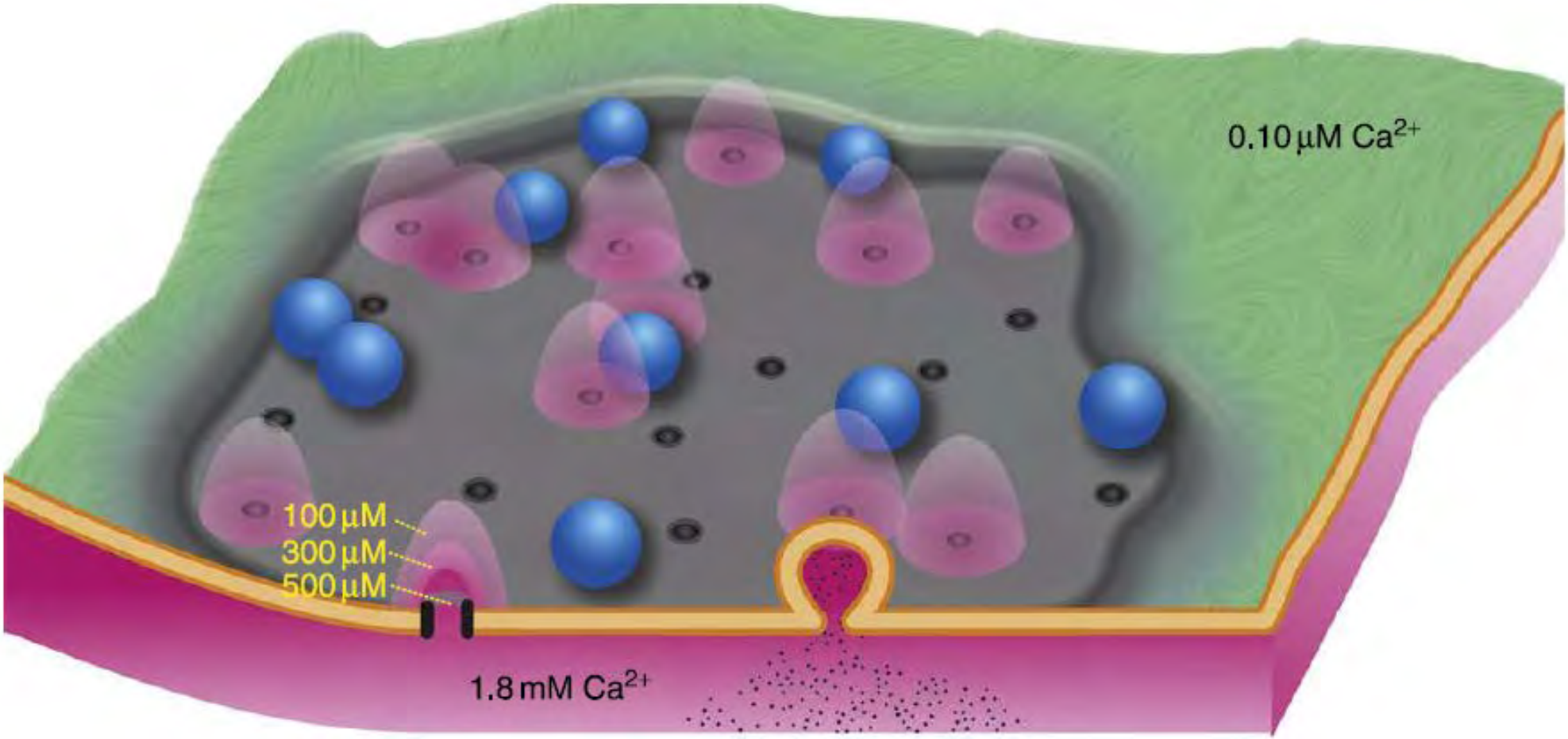
VESICLES FUSE NEAR Ca^{2+} CHANNEL-DENSE MICRODOMAINS

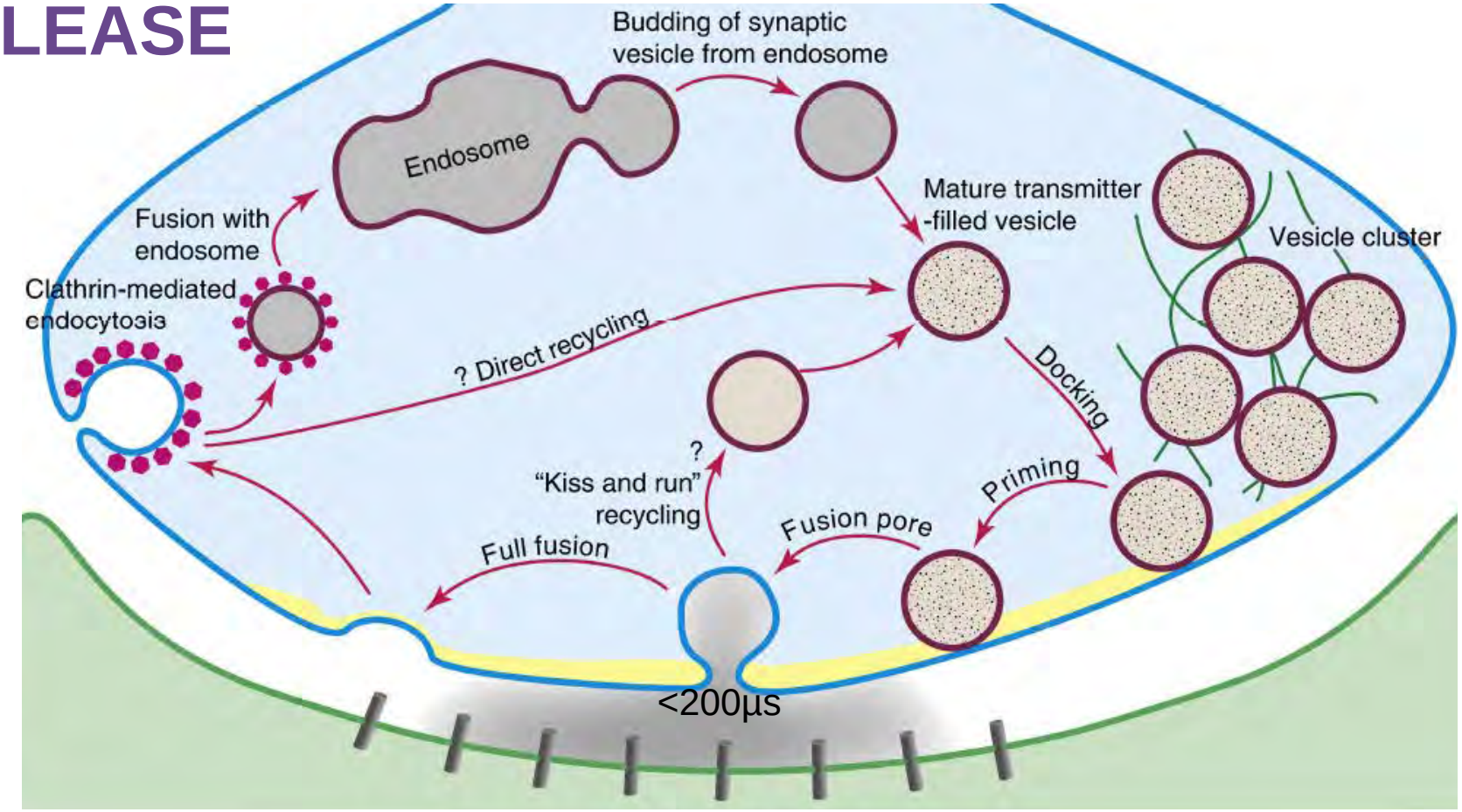


- Total $[Ca^{2+}]_i$ changes are small
- "Local" $[Ca^{2+}]$ changes may be >1000X

- Low variability & high reliability

Fund. Neuroscience, 4th Ed.

AN EFFICIENT ENDO-EXOCYTOSIS CYCLE IS NECESSARY TO MAINTAIN RELEASE



Docked: tethered to release site
Primed: ready for fusion

-Local synthesis of new vesicles and associated protein *de novo* is inefficient.
e.g. 5Hz firing rate with 200 vesicles/terminal = <1m max duration of response

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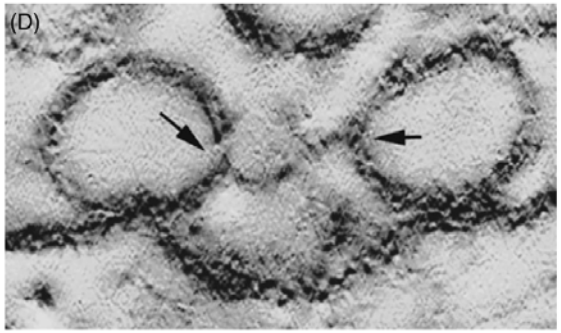
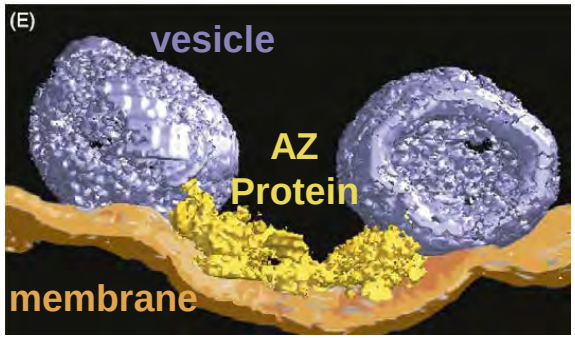
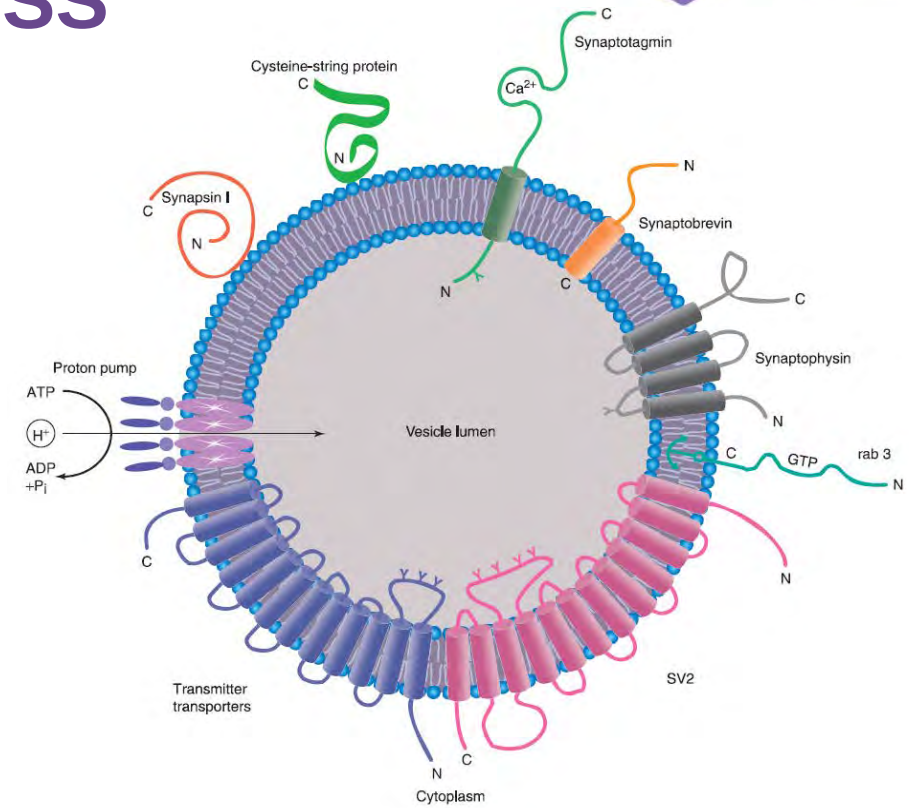
VESICLE FUSION IS A COORDINATED Ca^{2+}-DEPENDENT PROCESS

EM Reconstruction







Core (SNARE) Complex: Essential for Fusion

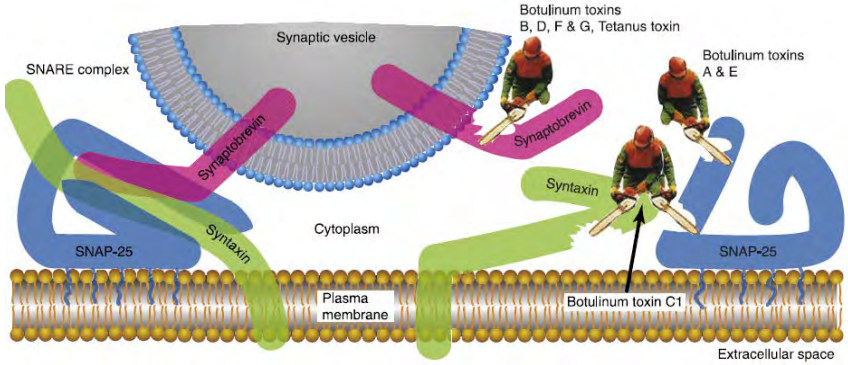


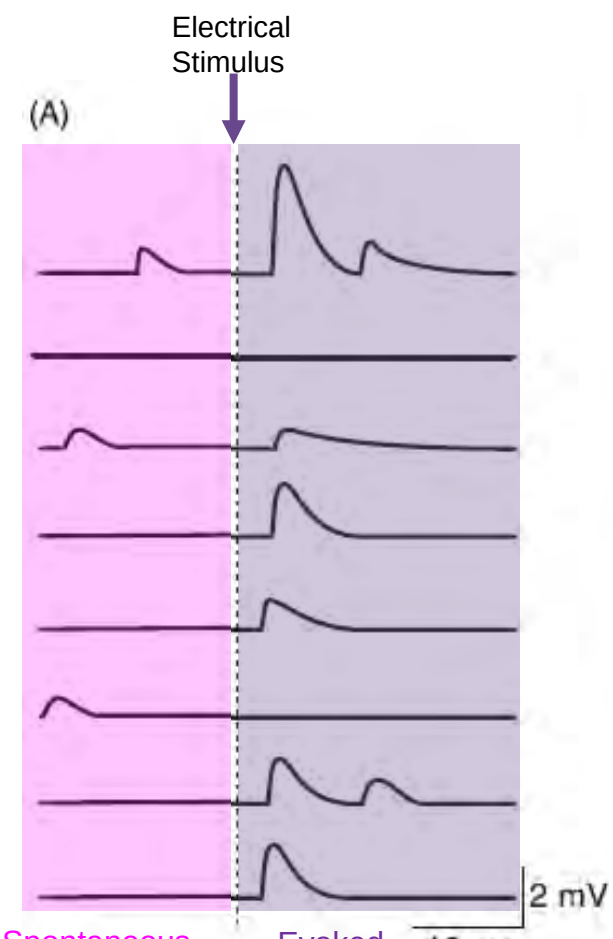
TABLE 7.1 Function of Synaptic Vesicle Proteins

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 Levetiracetam Binding
Synaptophysin	Unknown, endocytosis?

Fund. Neuroscience, 4th Ed.

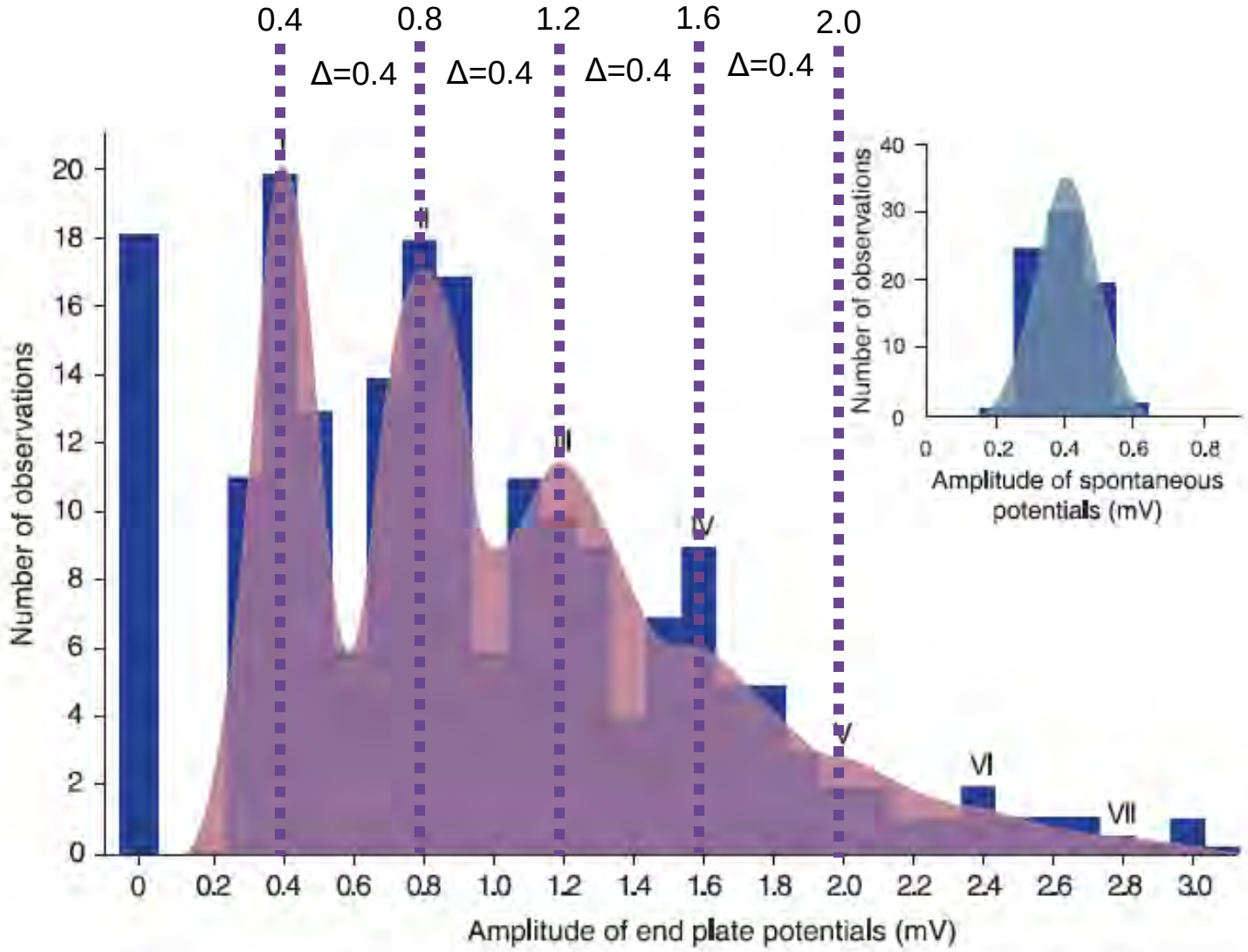
QUANTAL RELEASE SHAPES SYNAPTIC RESPONSES

Minimum amplitude responses are due to release of a single vesicle.
**Large amplitude responses are built from responses to multiple single vesicles*



<u>Spontaneous</u>
-AP Independent
-Single Vesicle

<u>Evoked</u>
-AP Dependent
-0+ Vesicles



AP Depolarization raises release probability to promote synchronous fusion of additional vesicles

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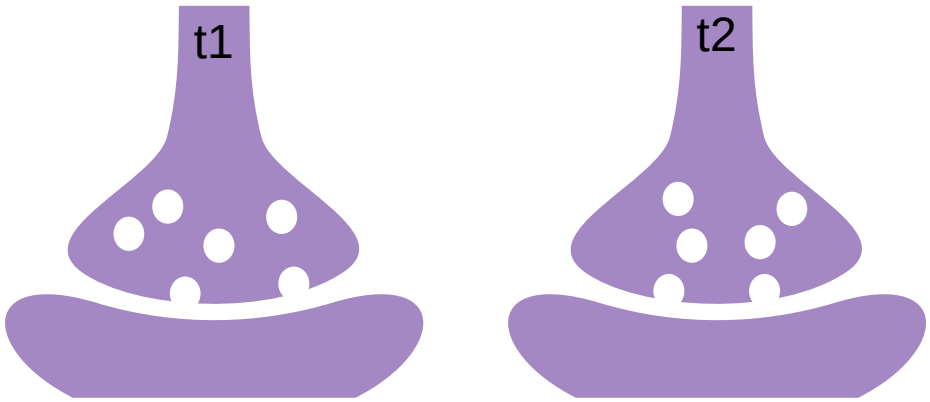
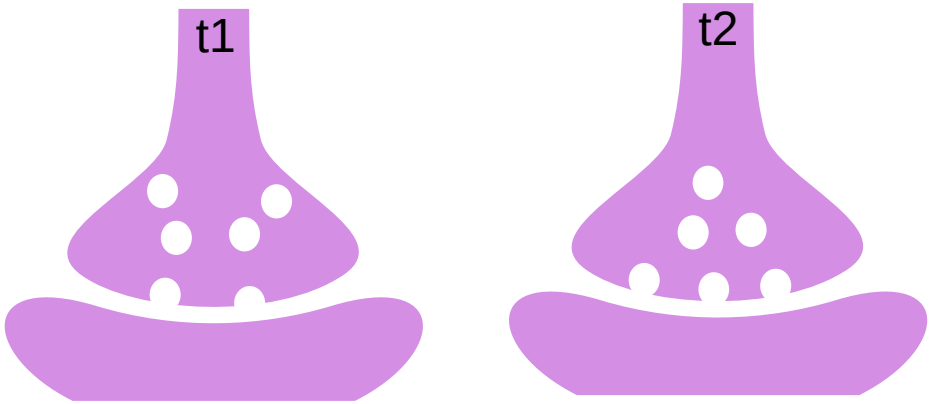
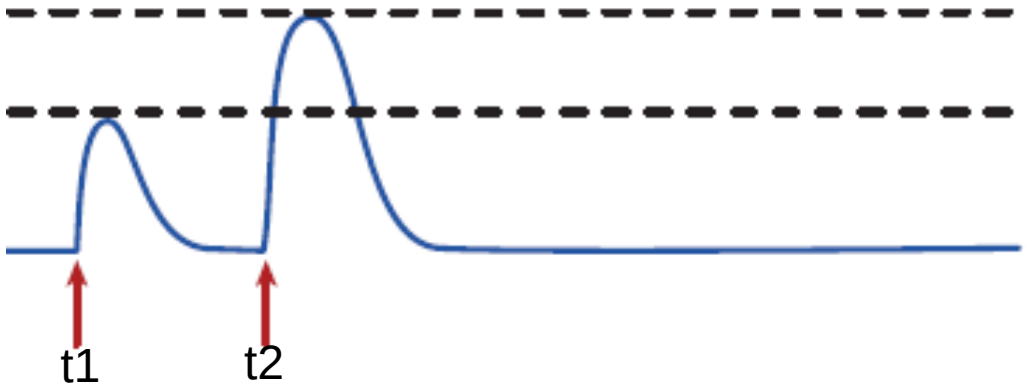
CAVEATS TO QUANTAL RELEASE

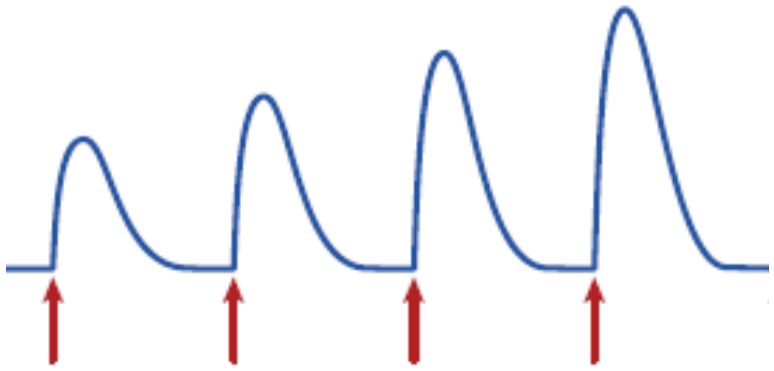
- Nonuniformity of Quanta (vesicle content, receptors)
- Nonuniformity of release sites (Ca^{2+} channel variation)
- Variation in membrane excitability
- Receptor saturation
- Silent synapses (release w/out receptors)

SYNAPTIC FACILITATION



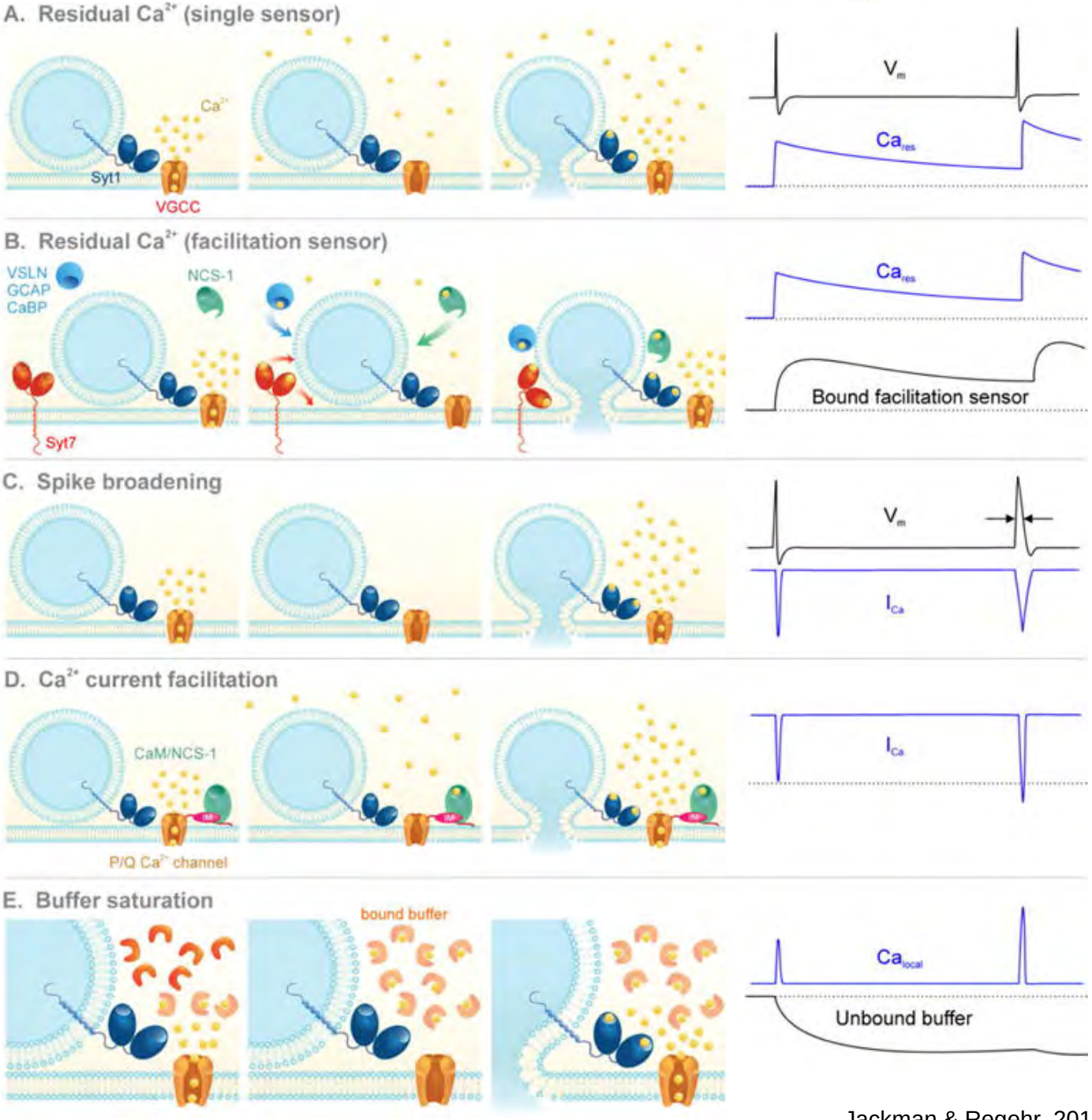






Fund. Neuroscience, 4th Ed.

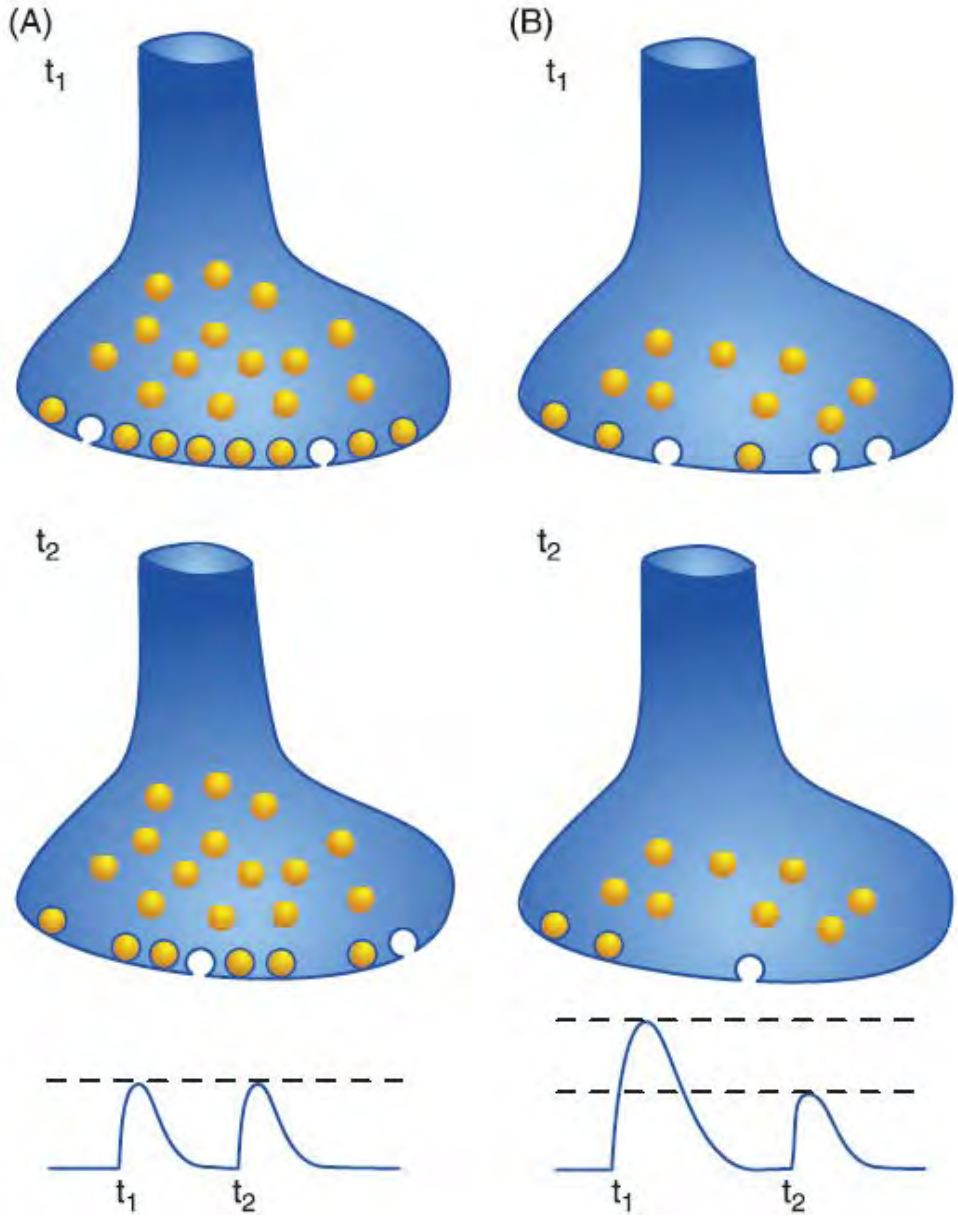
MECHANISMS OF SYNAPTIC FACILITATION



Jackman & Regehr, 2017

SYNAPTIC DEPRESSION

- Depletion of Readily Releasable Vesicles
  - high release probability
  - small readily releasable pool
- Autoinhibition (e.g presynaptic autoreceptors)
- Receptor Desensitization

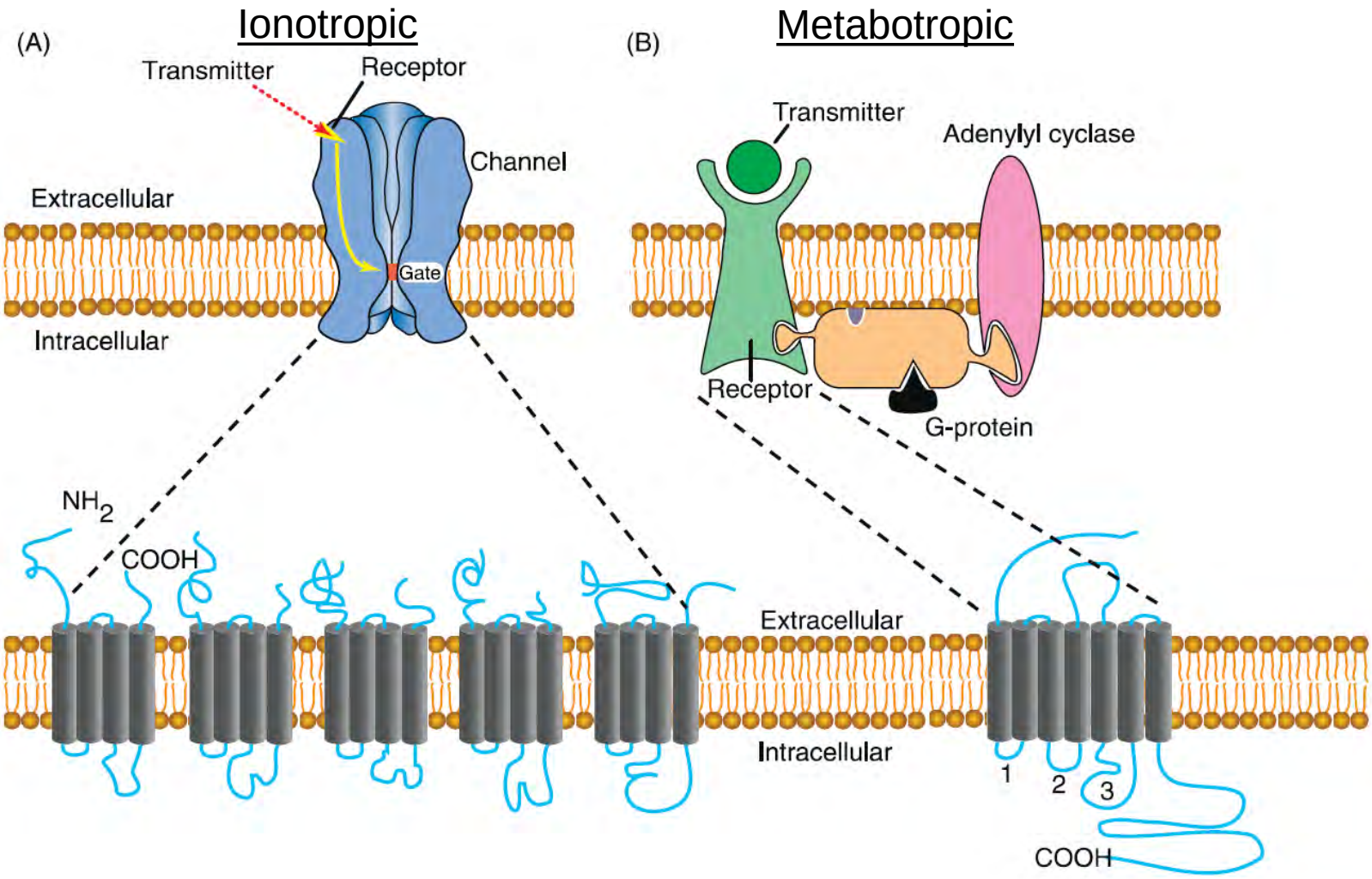


Fund. Neuroscience, 4th Ed.

Part 2: Neurotransmitter Receptors

- Ionotropic & Metabotropic Receptors
- Glutamate Receptors (AMPAR, NMDAR, Kainate, mGluR)
- GABA Receptors ($GABA_AR$, $GABA_BR$)
- Glycine Receptors
- Acetylcholine (nAChR, mAChR)
- Dopamine Receptors
- Adrenergic Receptors
- Serotonin Receptors
- Receptor modification & Plasticity

IONOTROPIC AND METABOTROPIC RECEPTORS

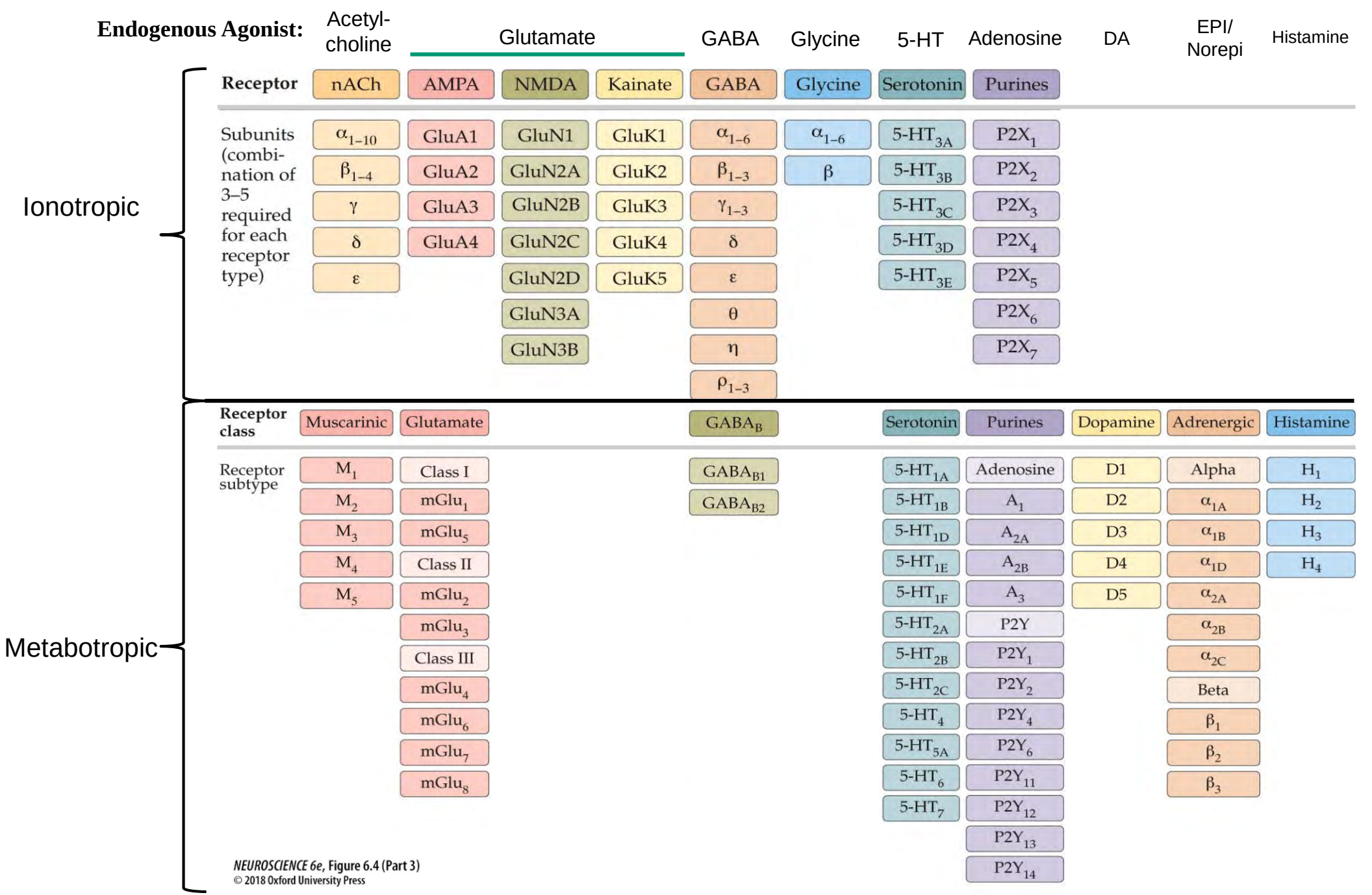


-Composed of 4-5 Individual Subunits
-Fast On/Off
-Selective Ion Flux

-A single protein couples to effectors
-Slow On/Off
-$G_s/G_i/G_q$

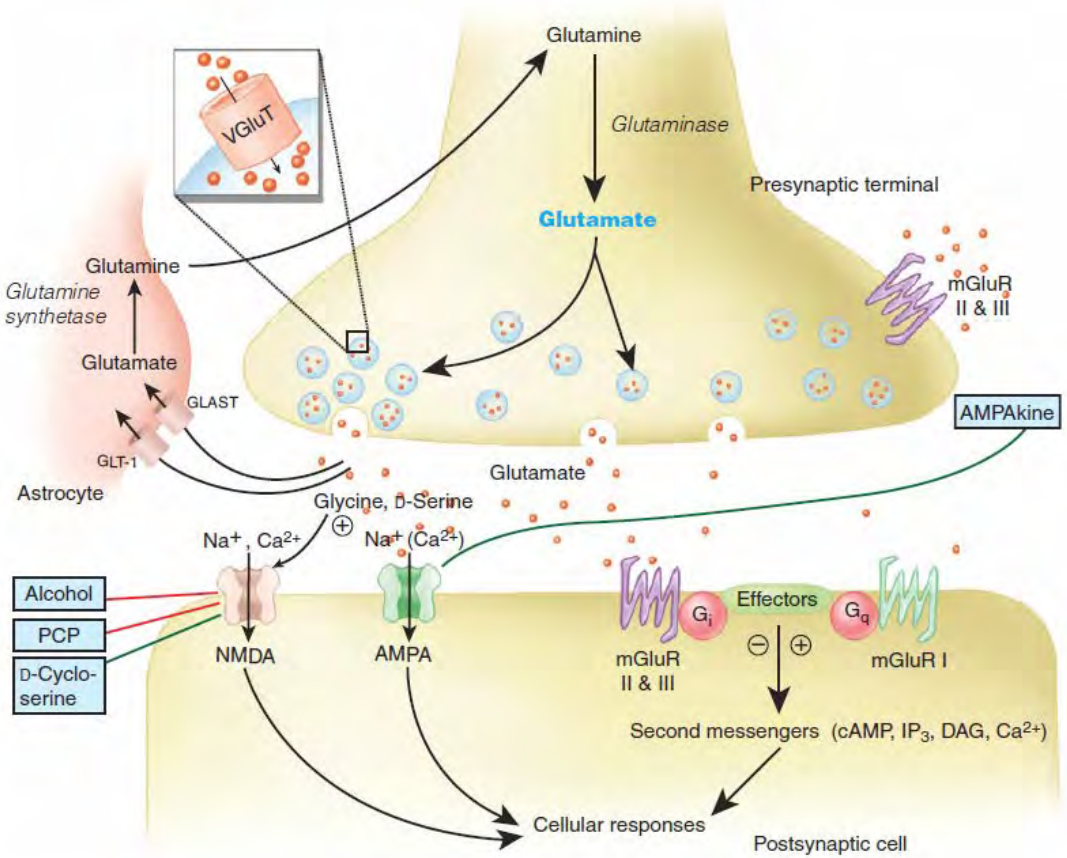
Fund. Neuroscience, 4th Ed.

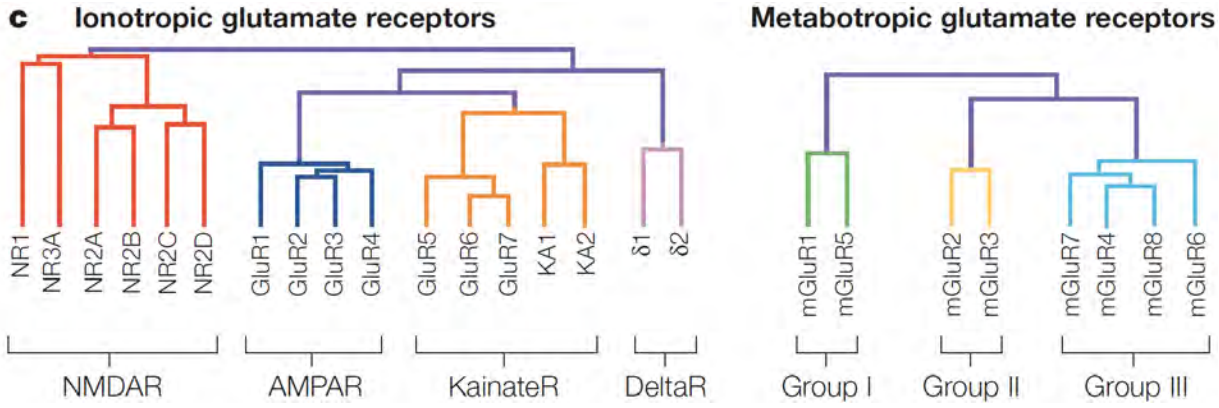
Neurotransmitters and Their Receptors



NEUROSCIENCE 6e, Figure 6.4 (Part 3)

GLUTAMATE RECEPTORS

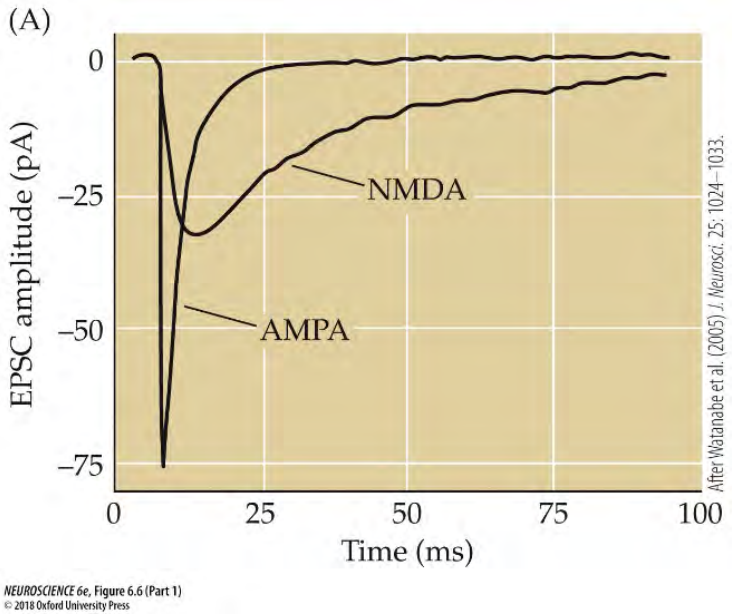


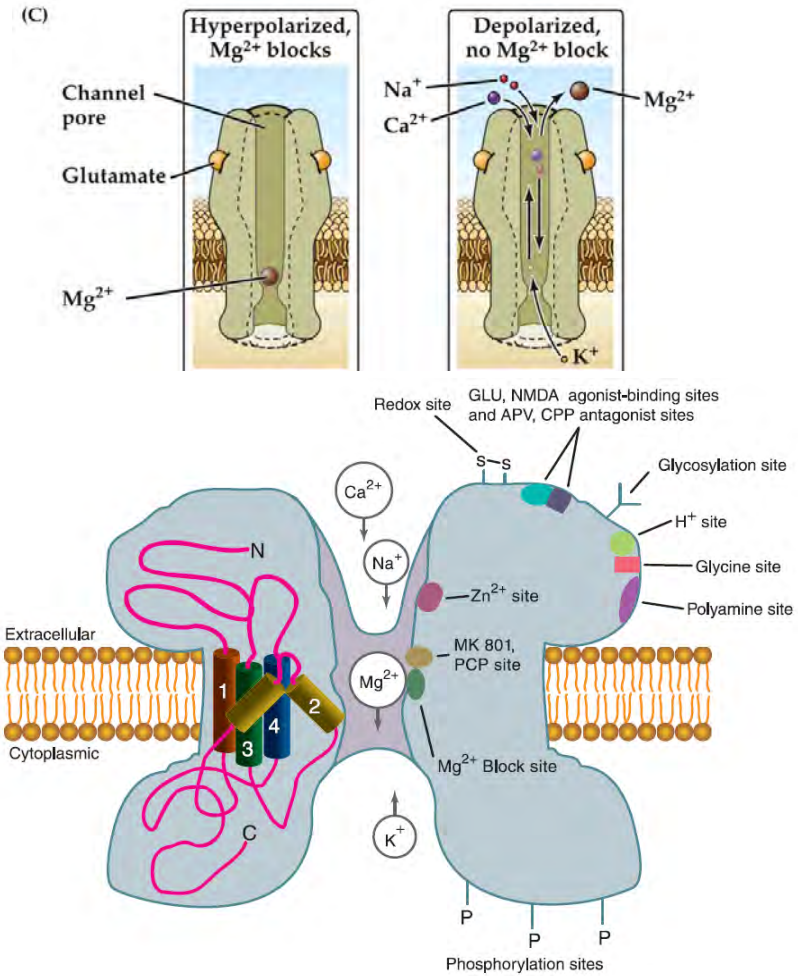


-Tetrameric ion channels
-Fast on/off

Molecular Neuropharmacology; Nestler, et al. 3rd Ed.

IONOTROPIC GLUTAMATE RECEPTORS





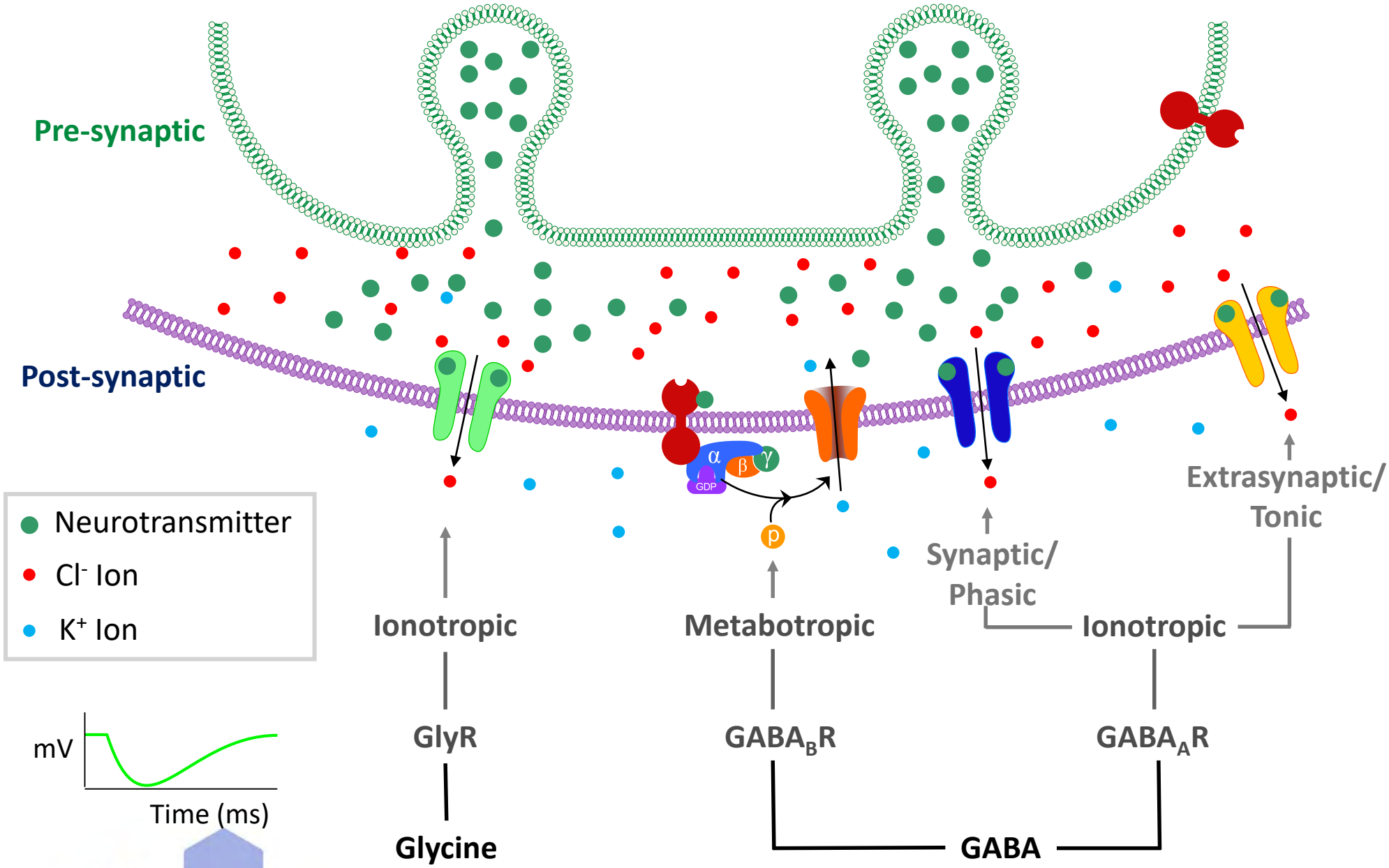
AMPA receptors:

- Fast on/off response
- Na^+ and Ca^{2+} permeable
- Underlie long-term potentiation (**LTP**)

NMDA receptors:

- Blocked by Mg^{2+} at resting membrane potentials – require depolarization to be activated
- Highly Ca2+ permeable
- Driving force of synaptic plasticity underlying learning and memory

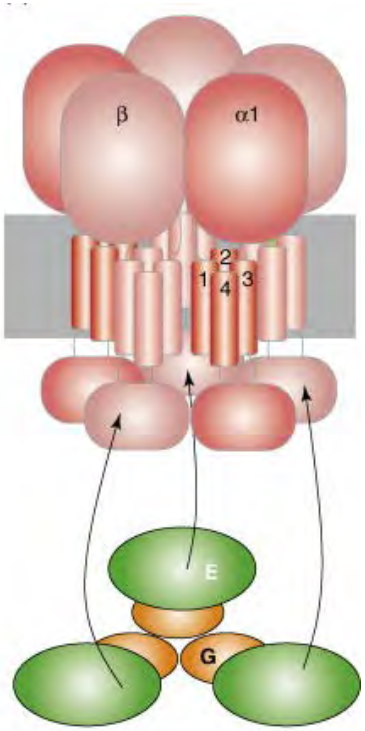
LIGAND-GATED INHIBITORY RECEPTORS



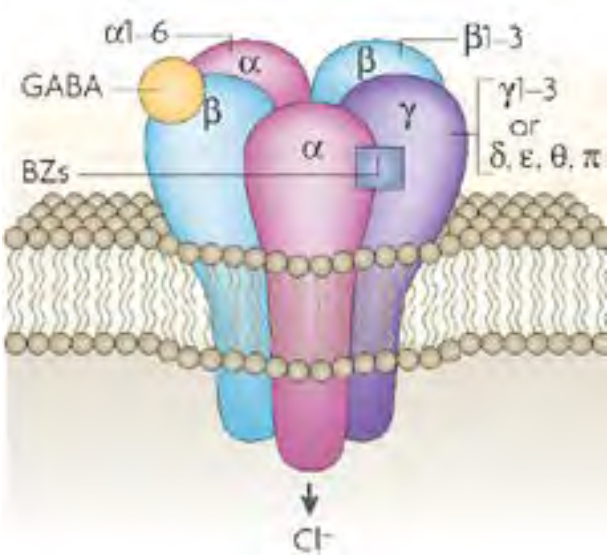
$GABA_A$ AND GLYCINE RECEPTORS

- Ligand-Gated
- Selectively Fluxes Cl^- ions
- Pentameric Protein Complexes
- GlyR: 5 subunits
  - α_{1-4} or β_1
- $GABA_AR$: 19 subunits
  - α_{1-6}, β_{1-3}, γ_{1-3}, δ, ρ, ε_{1-3}, θ, π

Glycine Receptor

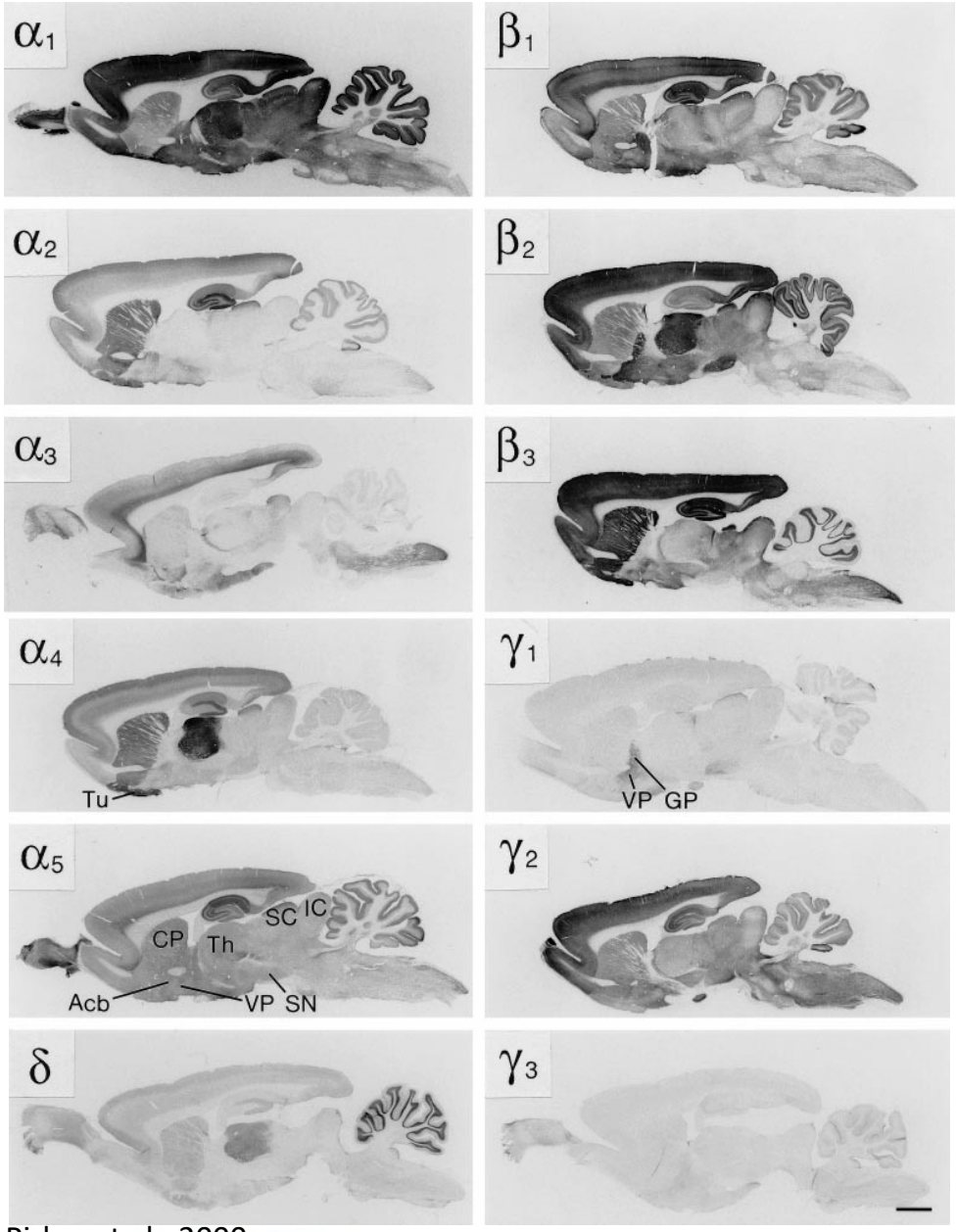


$GABA_A$ Receptor

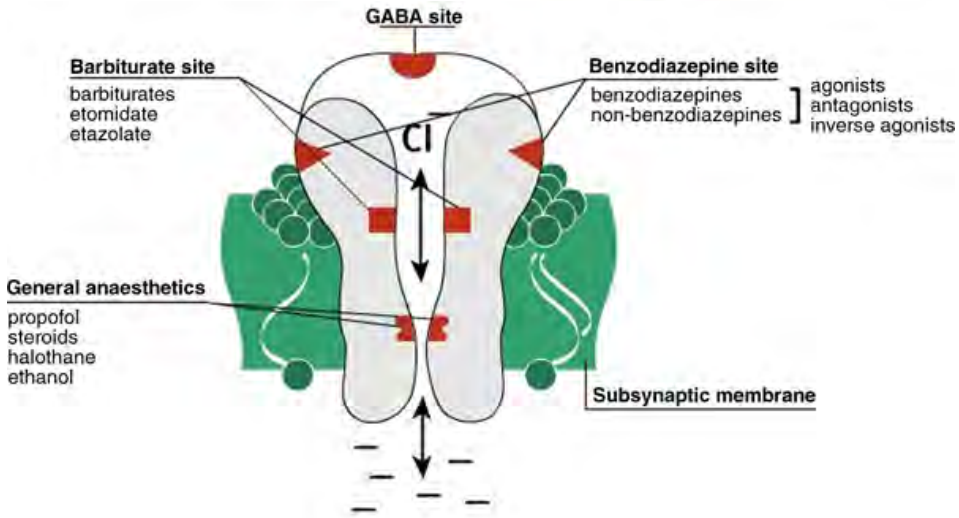


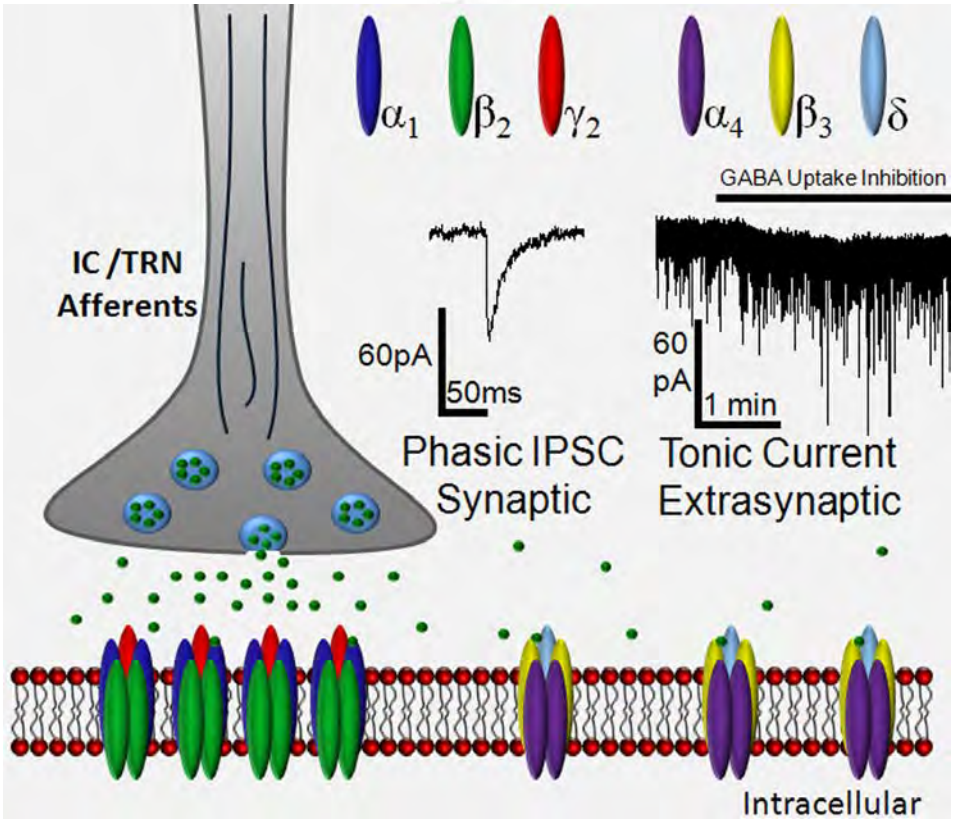
Fritschey et al., Trends in Neurosci, 2008
Jacob et al., Nature Reviews, 2008

$GABA_A$ RECEPTOR DIVERSITY



Pirker et al., 2000

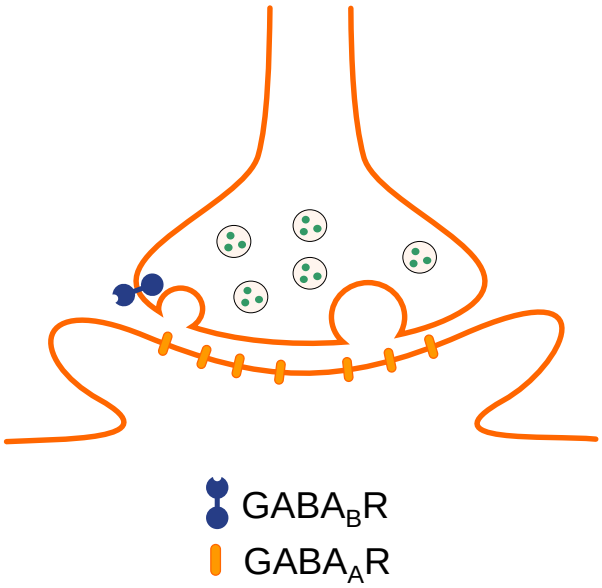


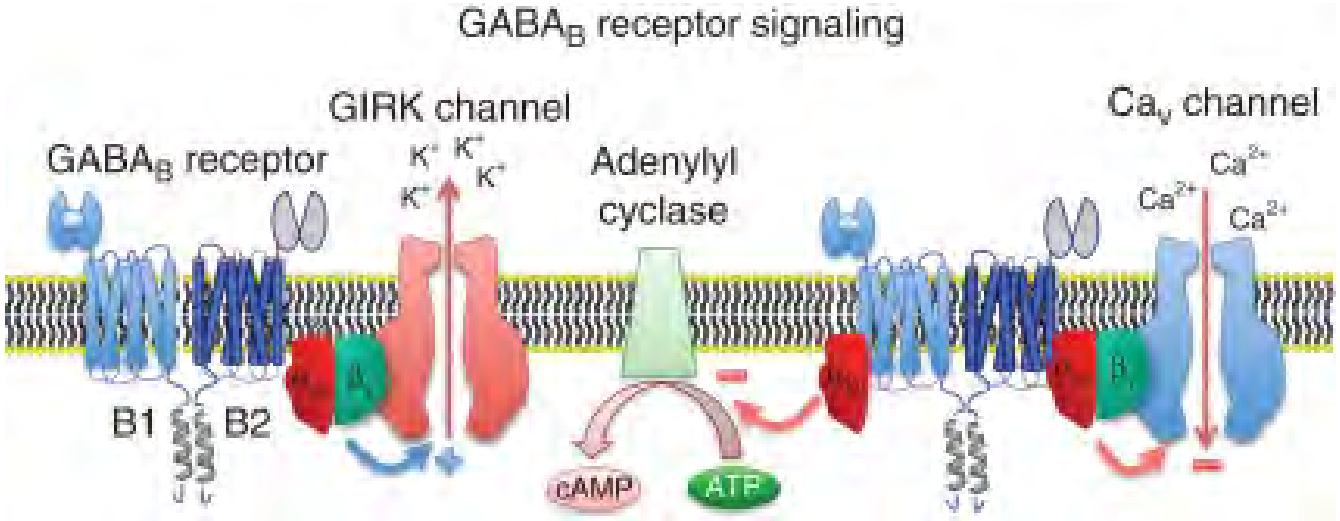


Adapted from Richardson et al., *PLoS ONE,* 2011

$GABA_B$ RECEPTOS

- Often a presynaptic autoreceptor
- Gi-coupled receptor
  - Activates inwardly-rectifying potassium channels (GIRKs)
  - inhibition of Cav channels
  - inhibition of adenylyl cyclase (cAMP production).
- Common agonists **<u>Baclofen</u>** (spasticity in Cerebral Palsy)

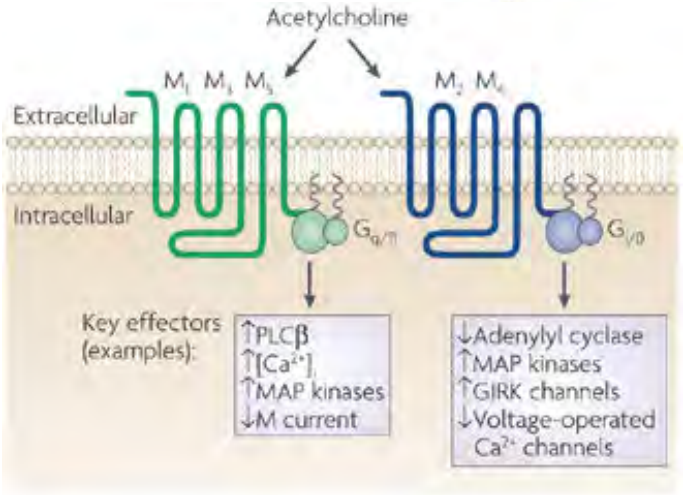




ACETYLCHOLINE RECEPTORS

Muscarinic ACh Receptors/mAChRs (M1-M5):

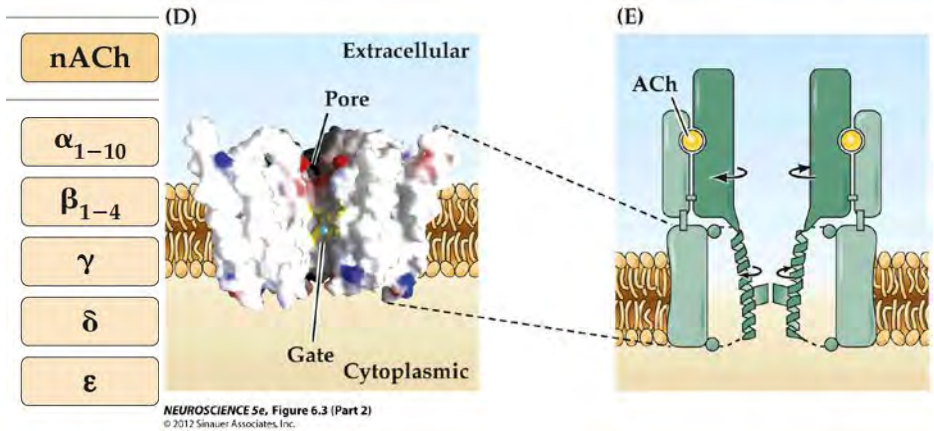
- M1 (odds) and M2 (evens) Classes
  - Gq-coupled (M_1, M_3, M_5): activate calcium release (IP3) and
  - Gi-coupled (M_2, M_4): inhibition of adenylyl cyclase (cAMP production) and K+ channel activation.
- Common off target site (anticholinergic side effects)

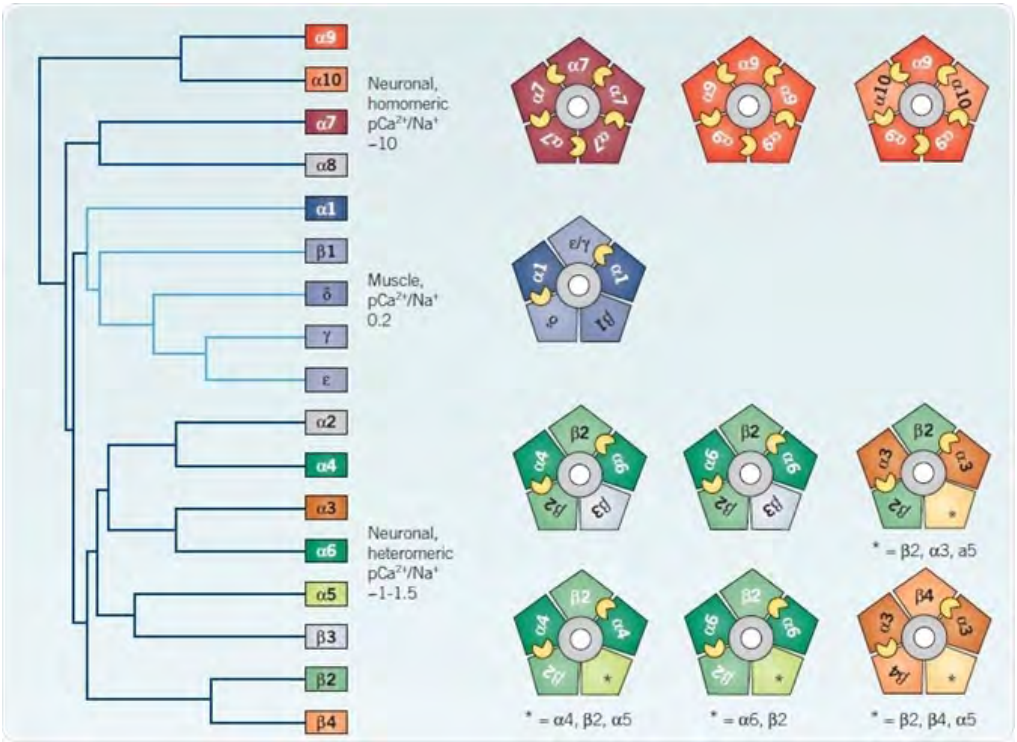


Nature Reviews | Drug Discovery

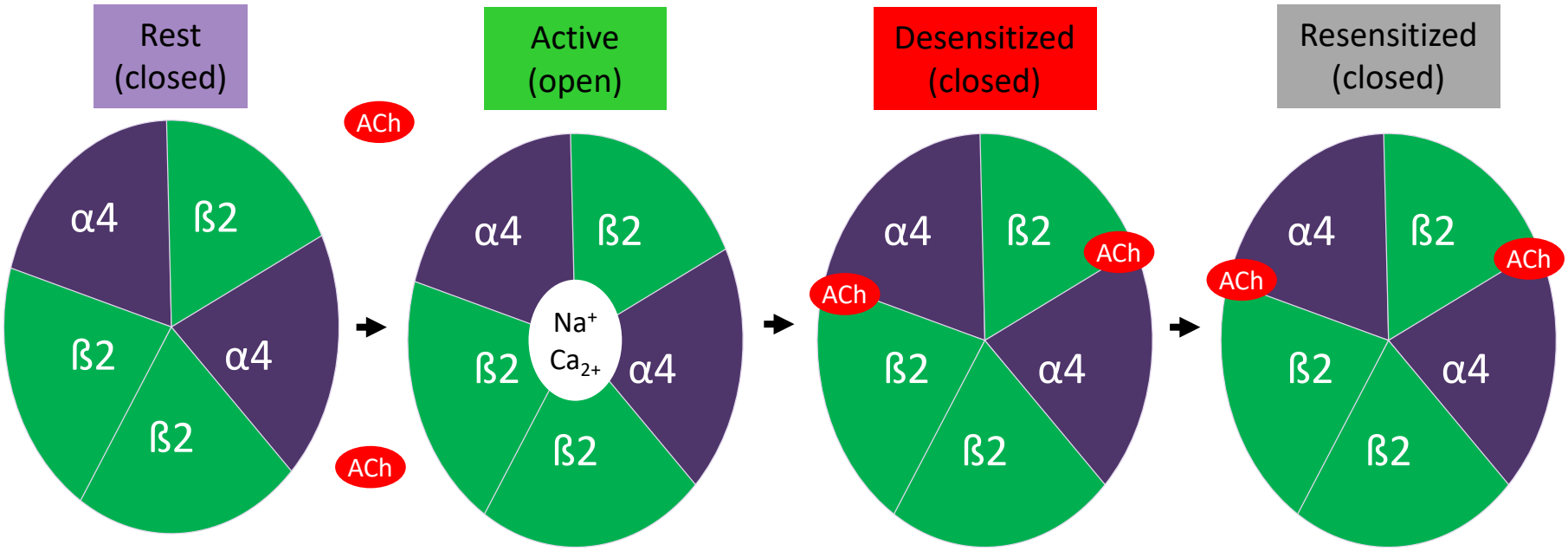
Nicotinic Ach Receptors/nAChRs:

- Heteropentamer (17 subunits)
- Tissue-specific subunit composition
- α subunits bind Ach
- Cation-permeable pore



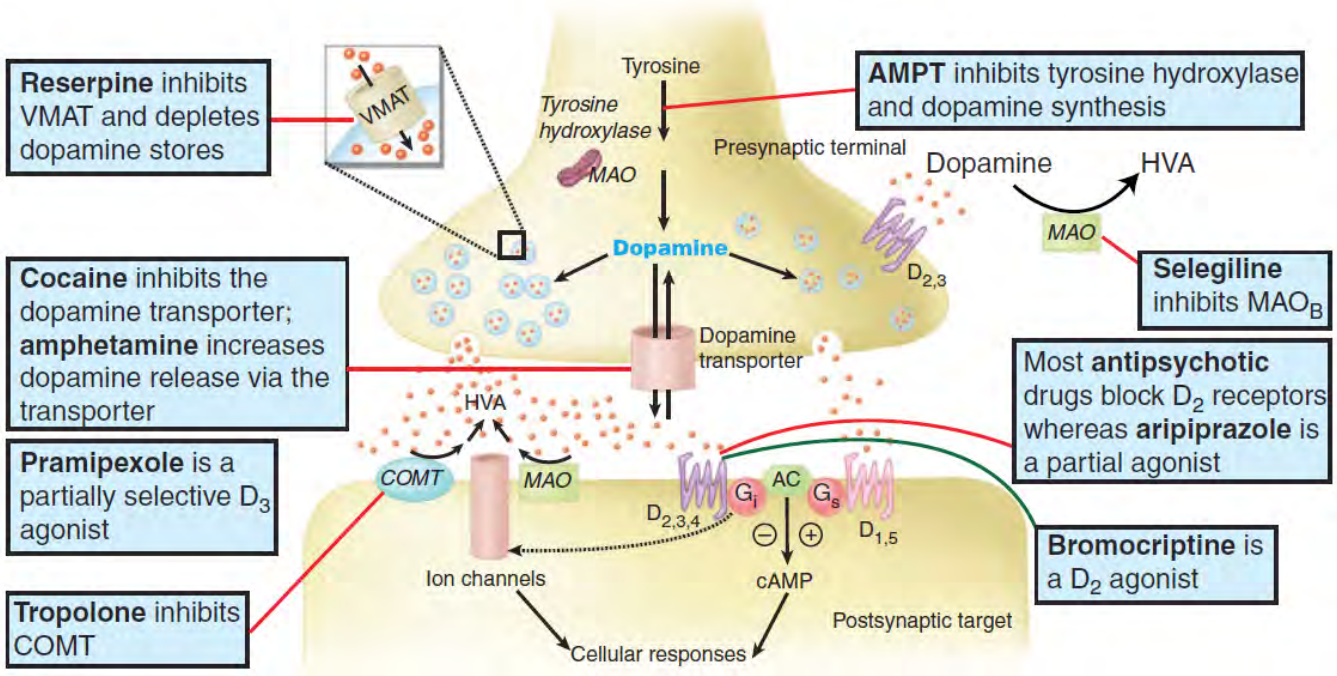


nACHR ACTIVATION

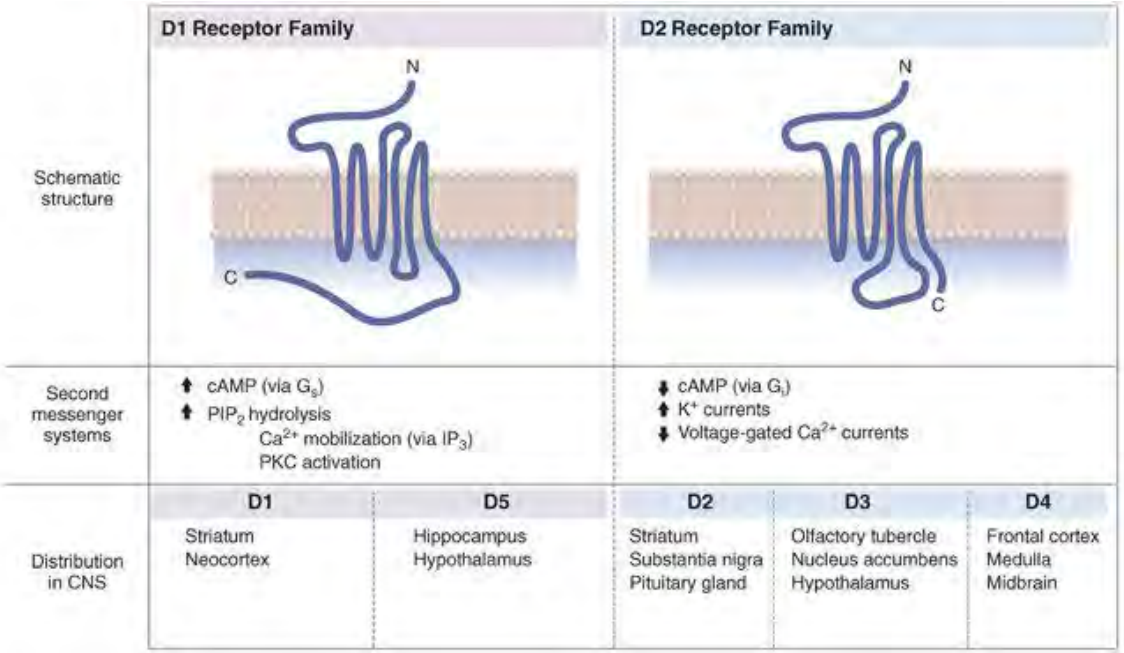


In the continued presence of ACh, nAChR undergoes an additional conformational change (desensitized) and no longer conducts current.

DOPAMINE RECEPTORS

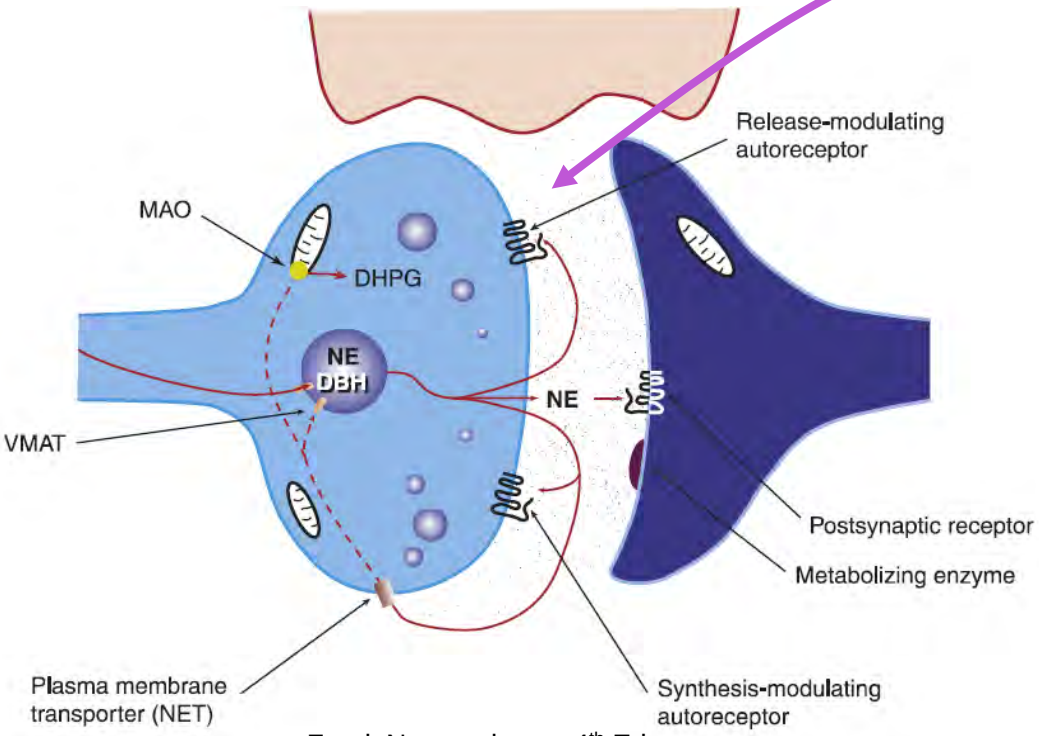


Molecular Neuropharmacology; Nestler, et al. 3rd Ed.

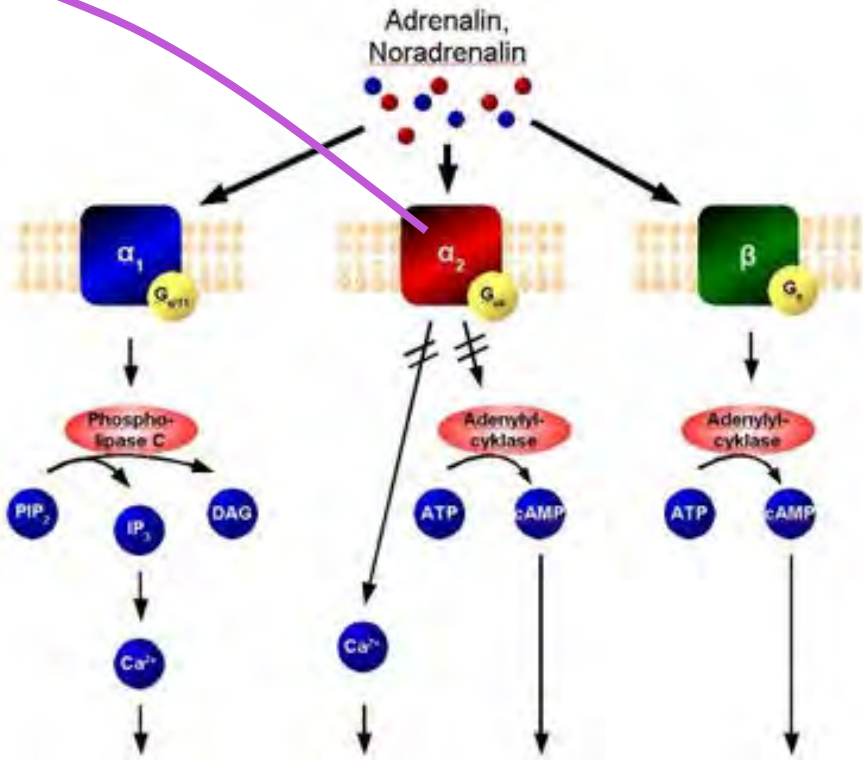


Principles of Pharmacology, Golan et al. 4th Ed.

ADRENERGIC RECEPTORS



Fund. Neuroscience, 4th Ed.

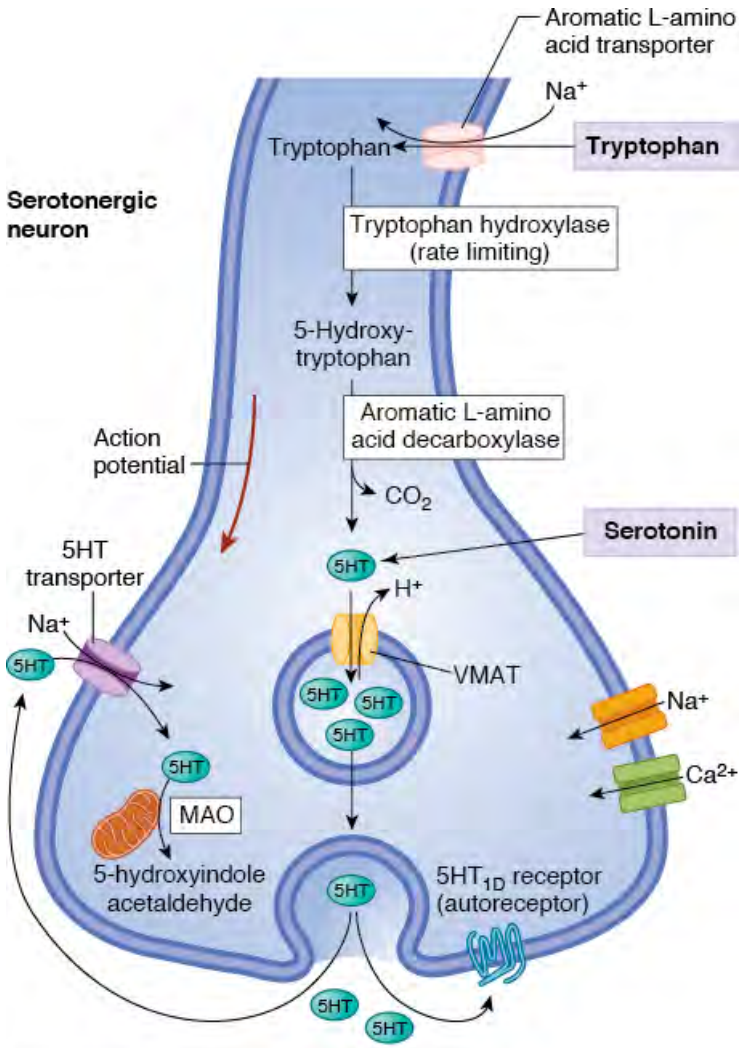


↑ $[Ca^{2+}]_{cyt}$

↑ K+ Efflux
(Hyperpol)
↓PKA
↓ $[Ca^{2+}]_{cyt}$

↑ cAMP
↑ PKA

SEROTONIN (5-HT) RECEPTORS



Source: Barrett KE, Barman SM, Boitano S, Brooks HL: *Ganong's Review of Medical Physiology:* www.accessmedicine.com

Metabotropic

- Serotonin
- 5-HT_{1A}
- 5-HT_{1B}
- 5-HT_{1D}
- 5-HT_{1E}
- 5-HT_{1F}
- 5-HT_{2A}
- 5-HT_{2B}
- 5-HT_{2C}
- 5-HT_{4}
- 5-HT_{5A}
- 5-HT_{6}
- 5-HT_{7}

Ionotropic

- Serotonin
- 5-HT_{3A}
- 5-HT_{3B}
- 5-HT_{3C}
- 5-HT_{3D}
- 5-HT_{3E}

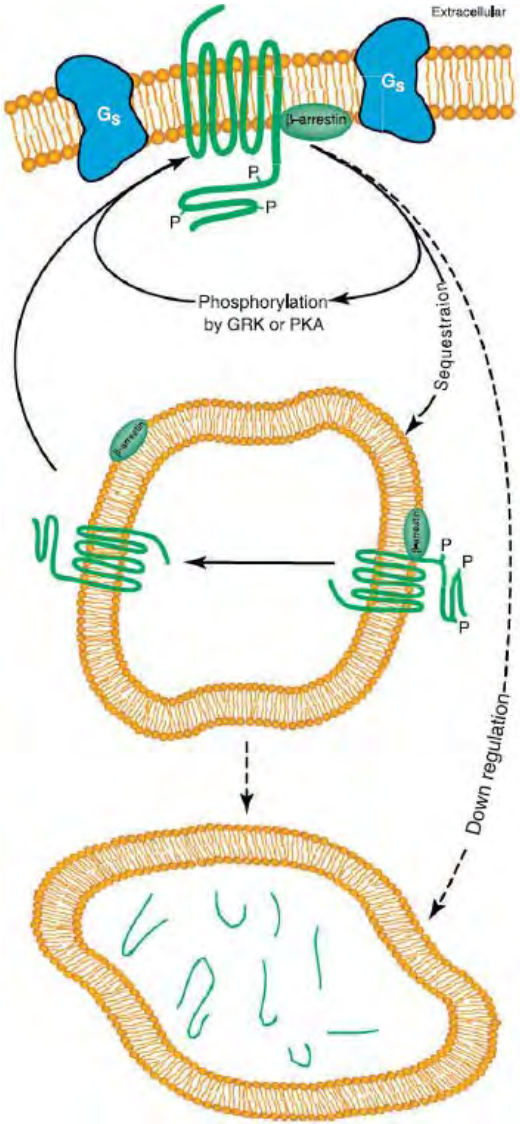
Physiological Roles of 5-HT Receptors Defined by Phenotypes in Knockout Mice

	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{3}	5-HT_{4}	5-HT_{5A}
Anxiety	↑[a]		↓[c]					
Aggression		↑[b]						
Heart defects				Lethal[d]				
Food intake					↑[e]			
Seizure susceptibility					↑		↑[g]	
Nociception						↓[f]		
Exploratory activity								↑[h]
Ethanol sensitivity								
Thermoregulation								

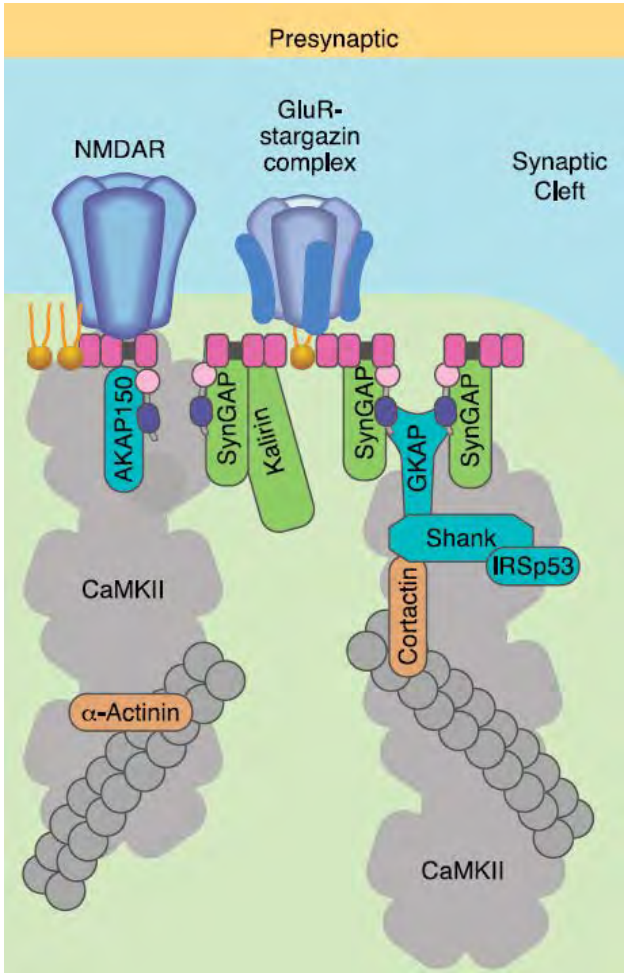
Arrow indicates direction of alteration of the trait.
[a]Parks et al., 1998; [b]Saudou et al., 1994; [c]Weisstaub et al., 2007; [d]Nebigil et al., 2000; [e]Tecott et al., 1995; [f]Zeitz et al., 2002; [h]Grailhe et al., 1999; [i]Bonasera et al., 2006; [j]Hedlund et al., 2003.

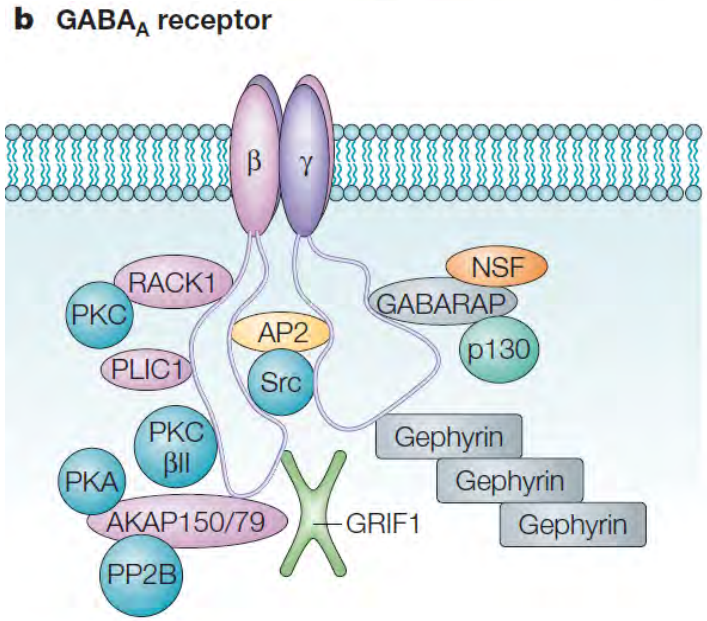
RECEPTOR MODIFICATION & PLASTICITY

B-Arrestin and GPCRs

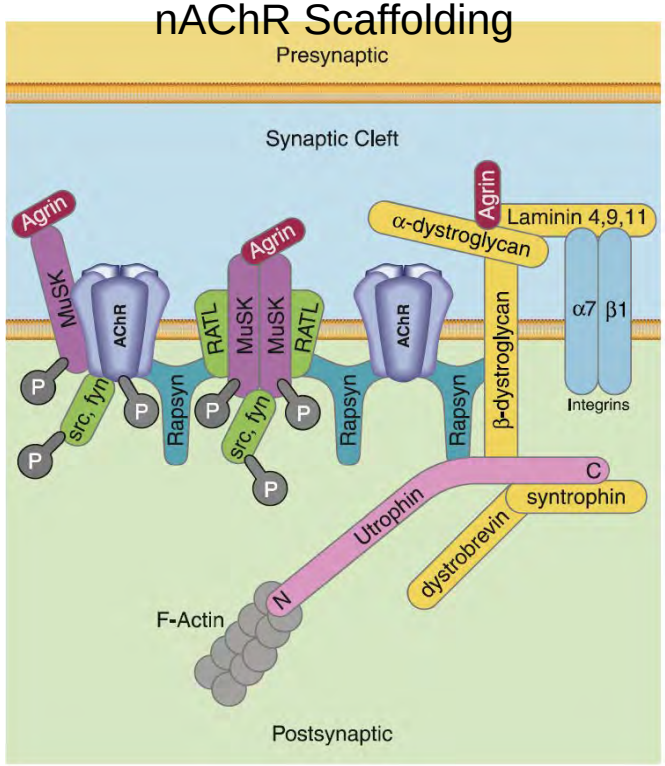


AMPAR & NMDAR Regulation





nAChR Scaffolding



Collingridge et al., Nat Rev Neuro, 2004
Fund. Neuroscience, 4th Ed.

Thank you!

Questions?

Ben Richardson

brichardson29@siumed.edu