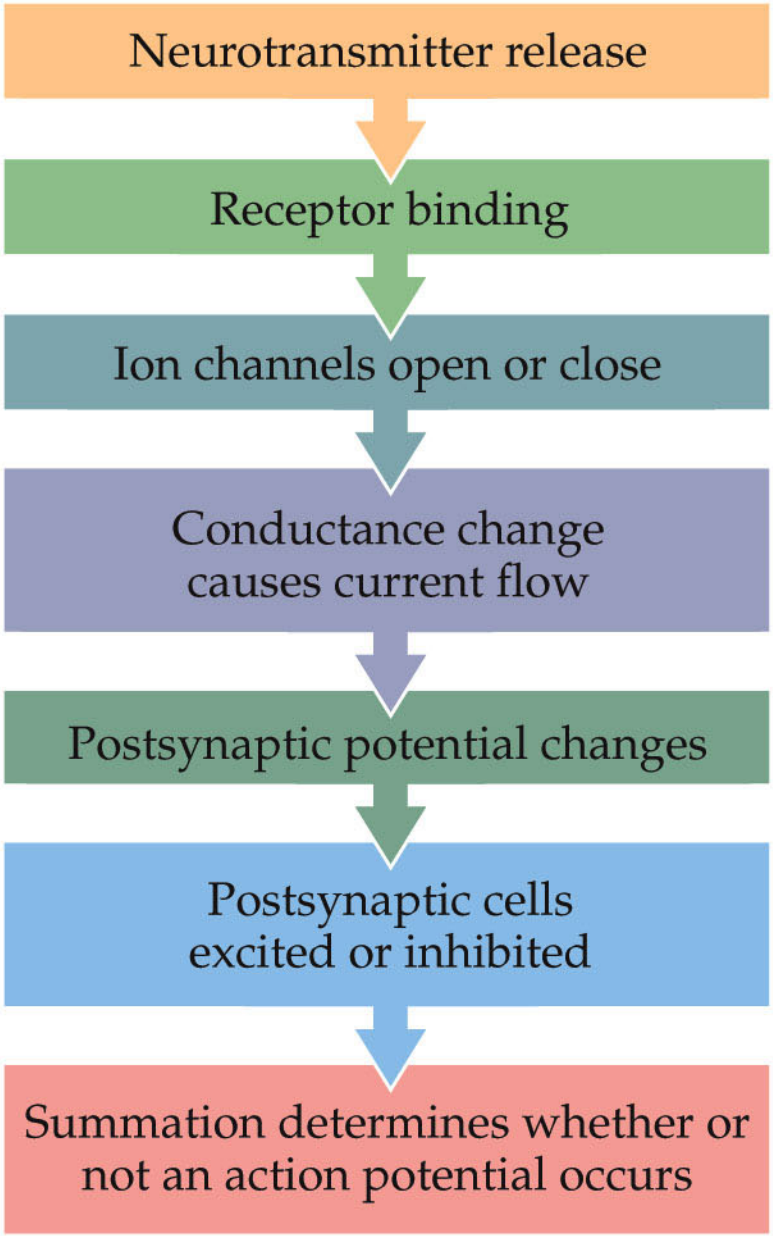
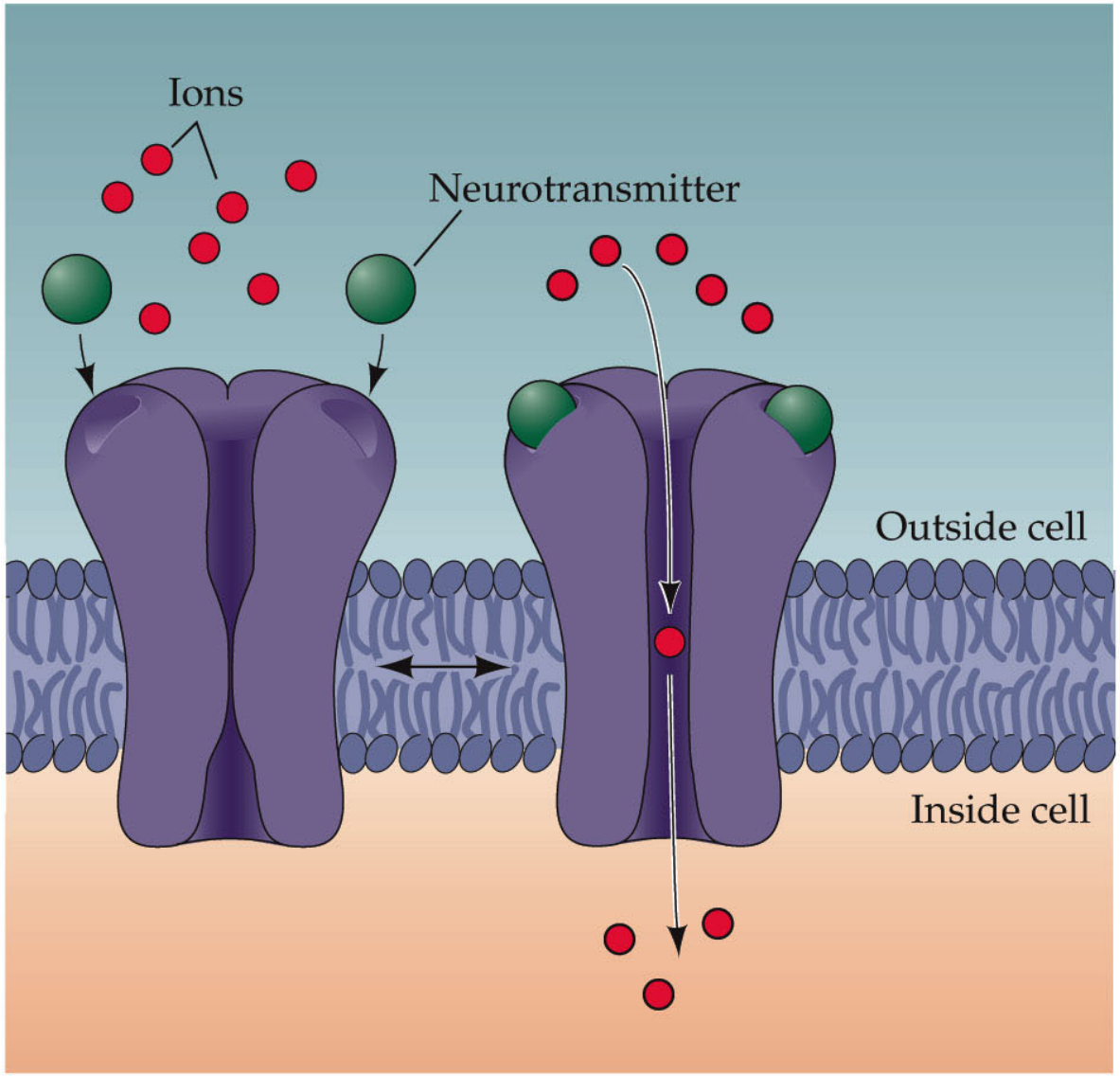
Ligand gated ion channels

Cys-loop pentameric receptors
nAChR
GABAR

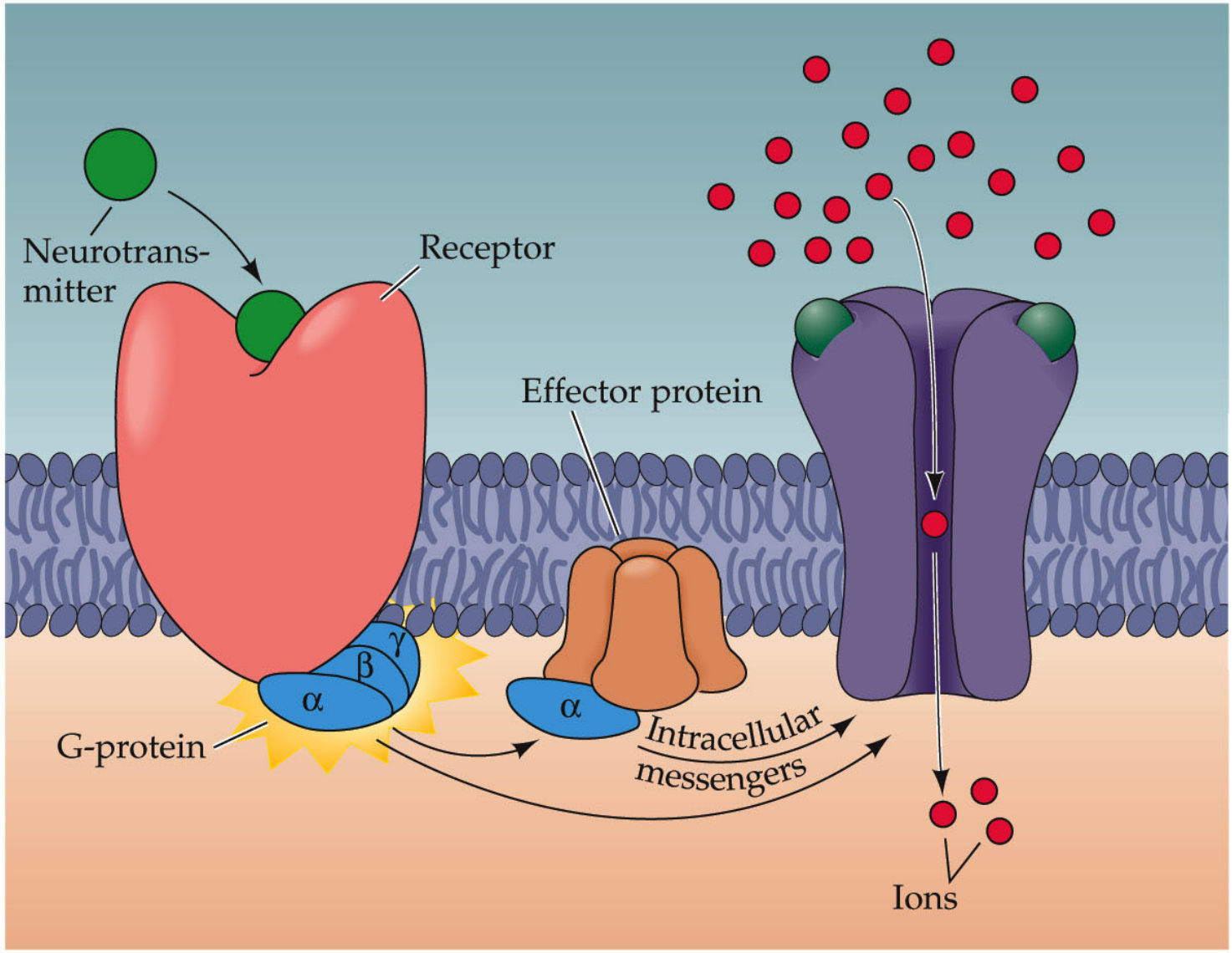

Neurotransmitter release
Receptor binding
Ion channels open or close
Conductance change causes current flow
Postsynaptic potential changes
Postsynaptic cells excited or inhibited
Summation determines whether or not an action potential occurs

(A) LIGAND-GATED ION CHANNELS



***NEUROSCIENCE, Fourth Edition,* Figure 5.23 (Part 1)**

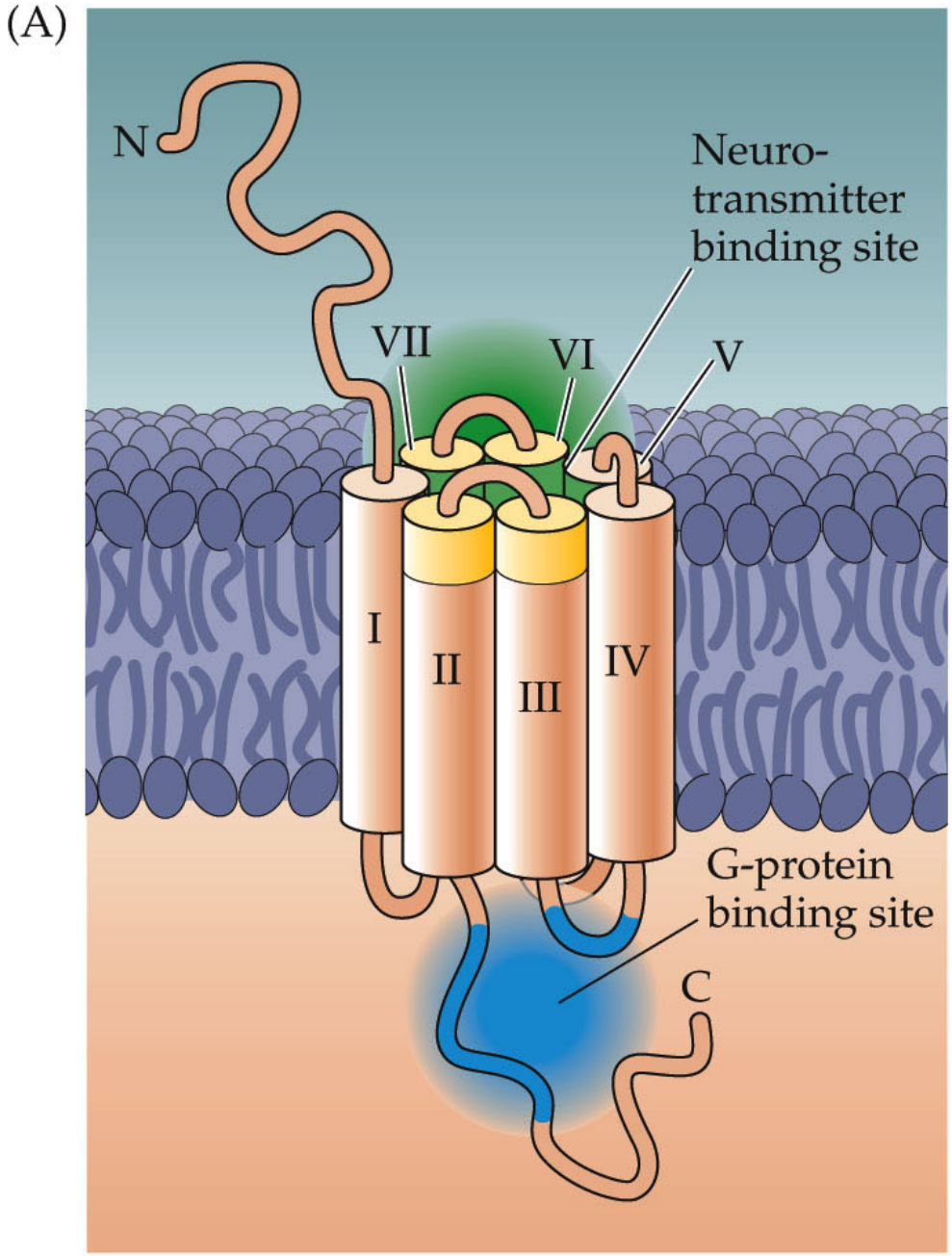
(B) G-PROTEIN–COUPLED RECEPTORS



***NEUROSCIENCE, Fourth Edition,* Figure 5.23 (Part 2)**

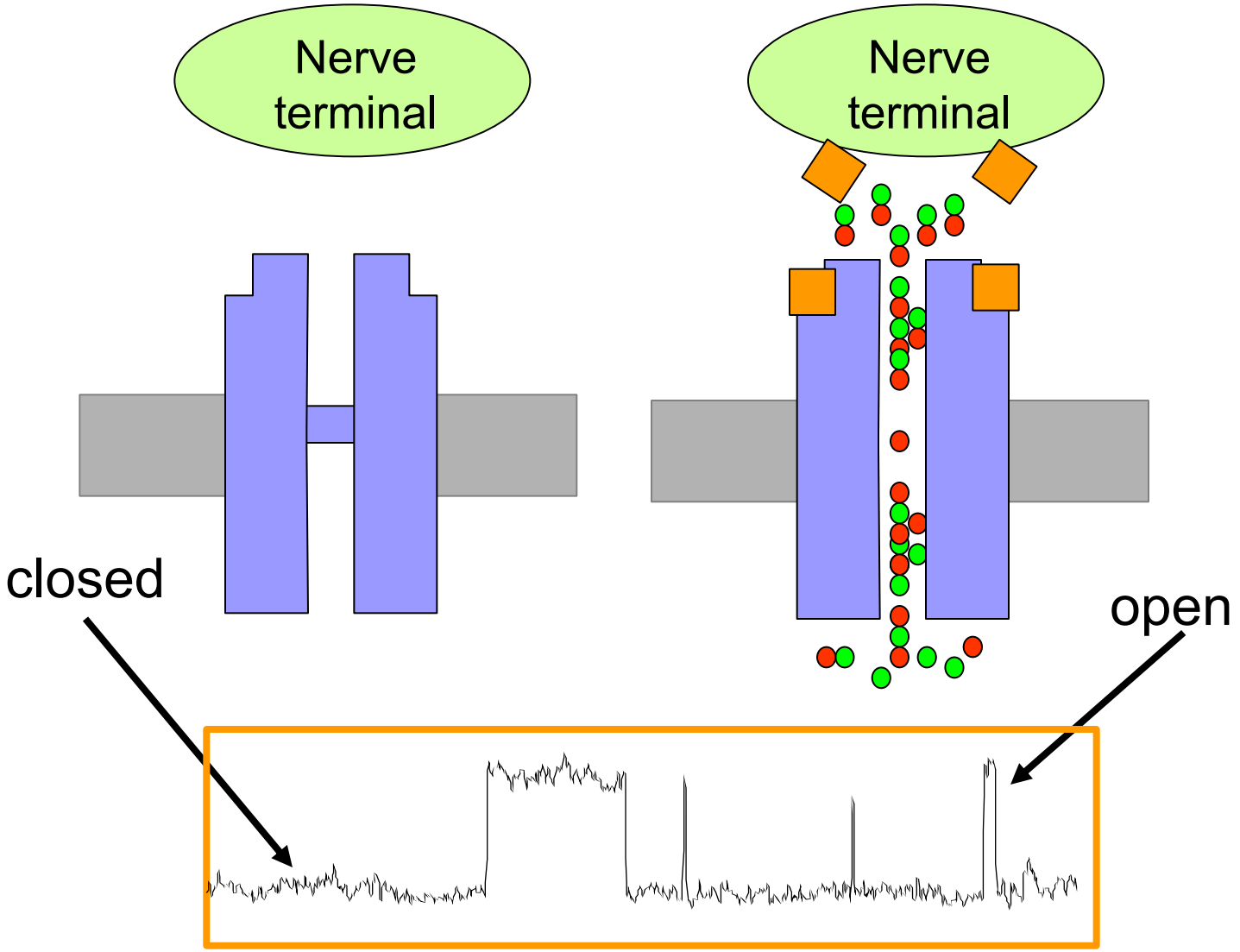
NT recptors:

Agonist	Ionotropic ligand-gated channels (fast 0.1 - 100 ms)	Metabotropic G-protein-coupled receptors (slow 0.05 – 100 s)
ACh	Nicotinic (Cationic)	Muscarinic
Glutamate	AMPA, NMDA, Kainate (Cationic)	mGluR
GABA	$GABA_A$ (Anionic)	$GABA_B$
Glycine	Glycine (Anionic)	—
Serotonin	5-HT_3 (Cationic)	5-$HT_{1,2,4-7}$
ATP (a purine)	P2Y (Cationic)	P2X
Histamine	—	H_1, H_2, H_3
Catecholamines	—	α_1, α_2, β, D_1, D_2
Anandamide	—	Cannabinoid R
Odorants	—	>500 odorant receptors[b]
Tastants	Some	Some[b]



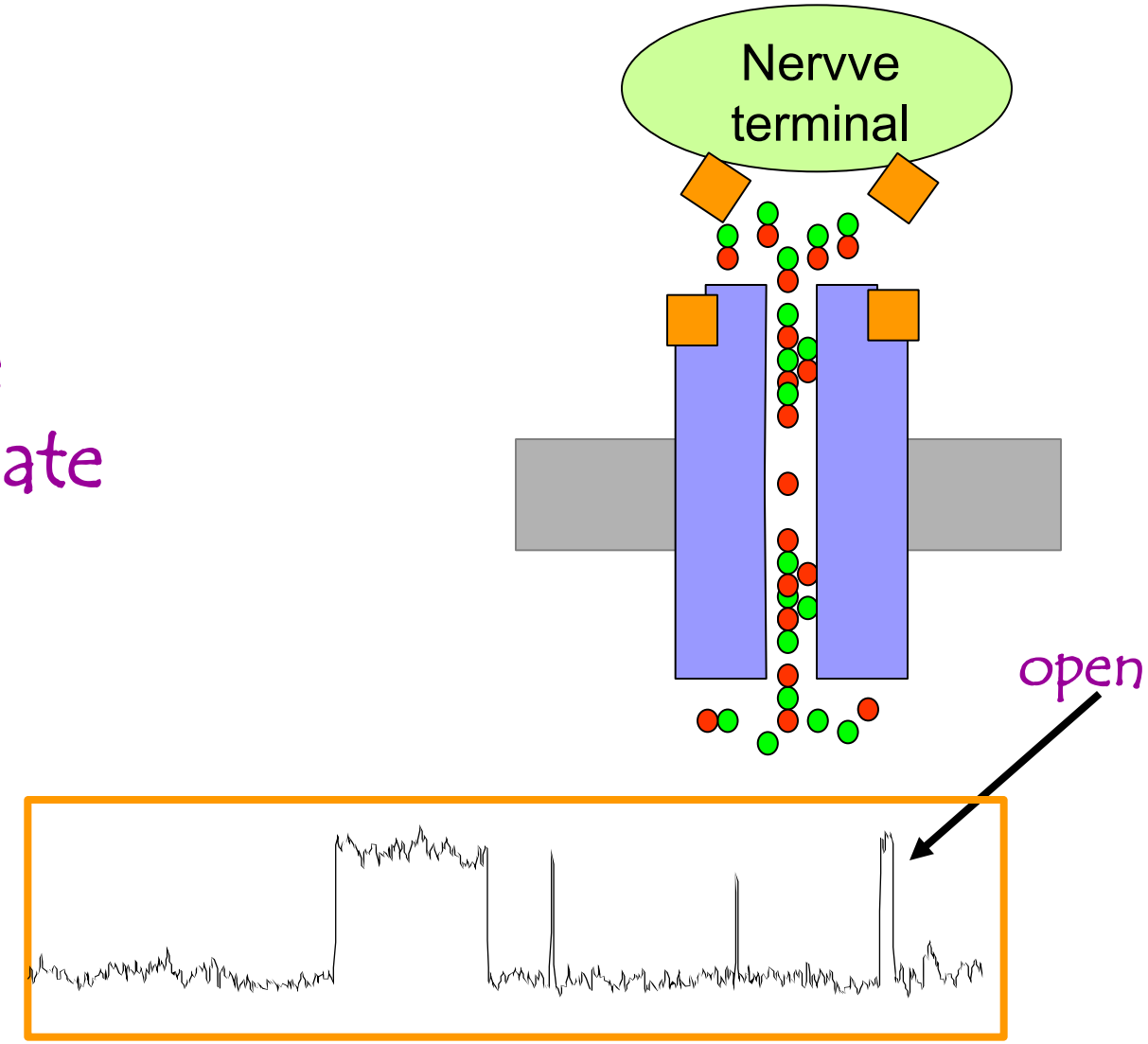
***NEUROSCIENCE, Fourth Edition,* Figure 6.5 (Part 1)**

Ligand gated ion channels

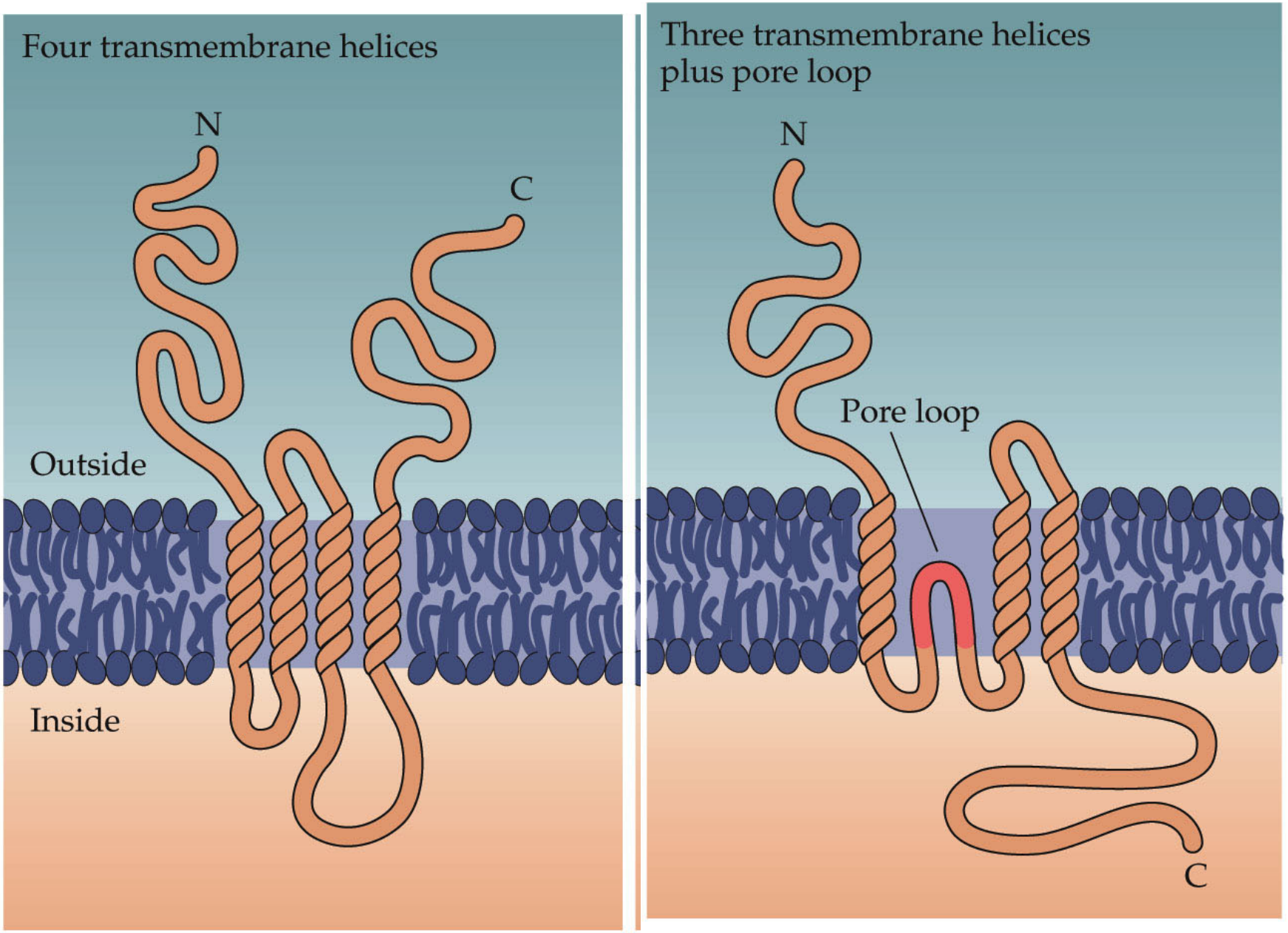


Neurotransmitters

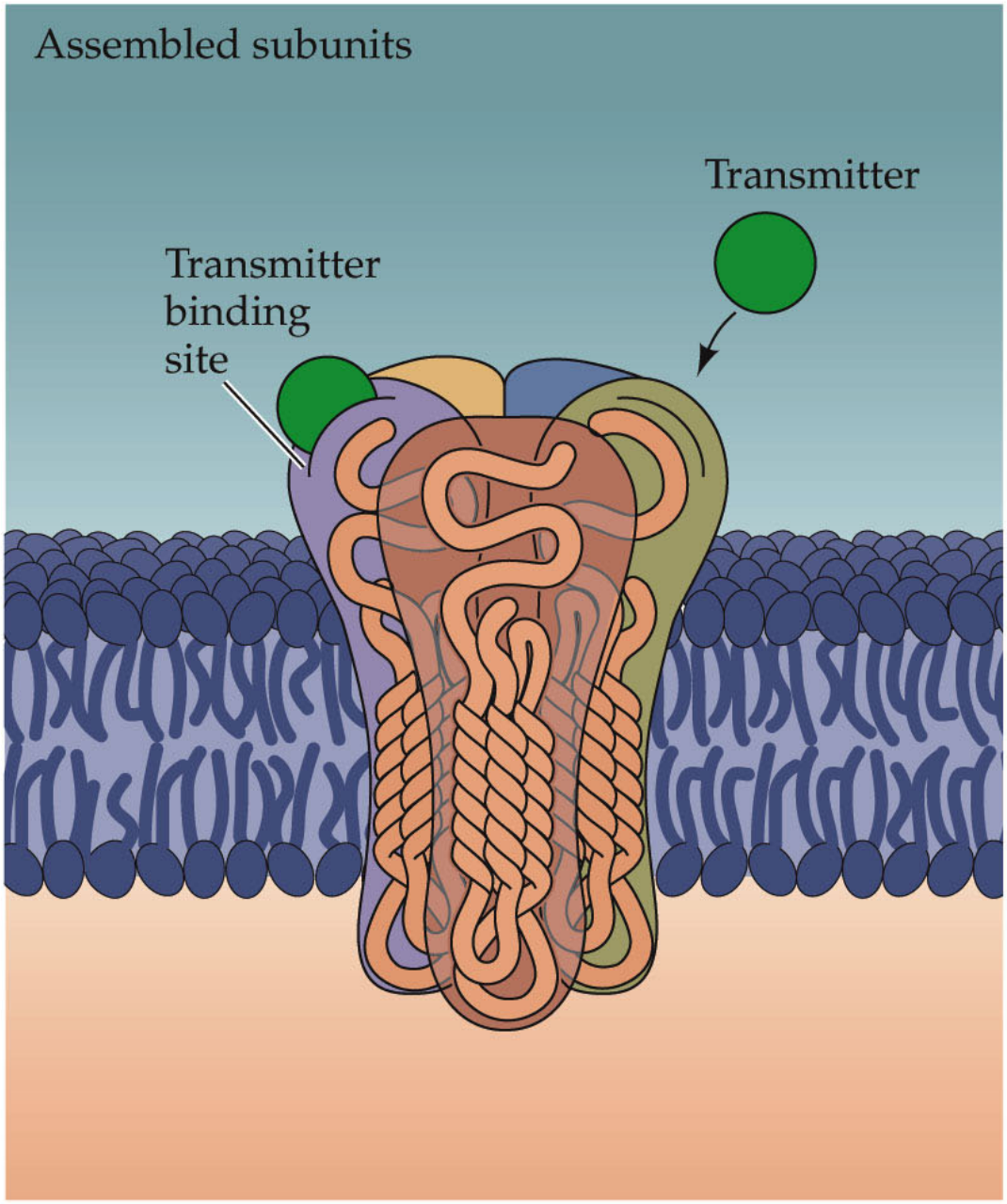
ACh
5-HT
GABA
Glycine
Glutamate
ATP



General structure



General structure



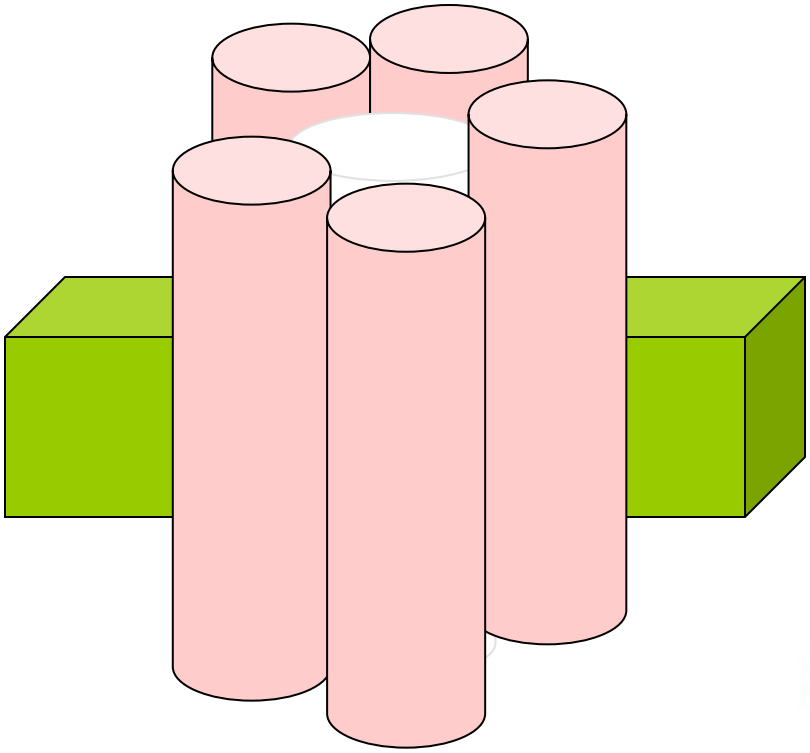
Except for rare exceptions, ionotropic receptors are heteropolymers, consisting of four or five subunits arranged in a circle, which can be present in many variations.

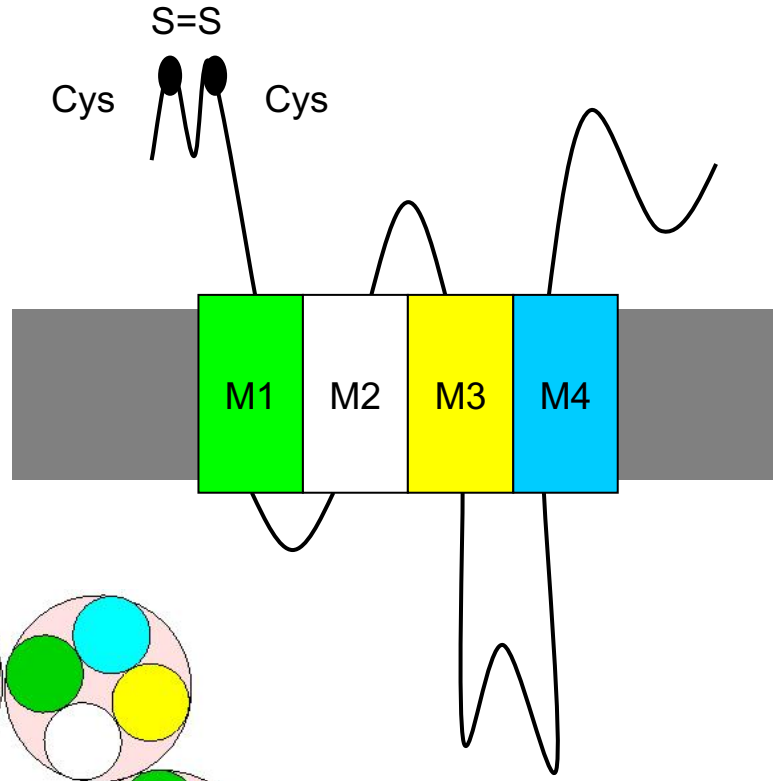
The structural analysis of the ionotropic receptors molecules suggests to classify them in two large families, derived from two distinct ancestral genes:

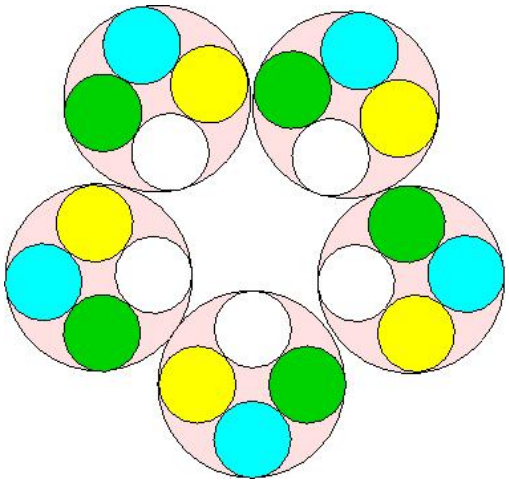
a)the family that has as its protoype the nicotinic acetylcholine receptor (nAchR) and its like; - nAchR represents the reference model for the whole class of ionotropic receptors;

b)the family which has the ionotropic receptor for ac. glutamic (iGluR).

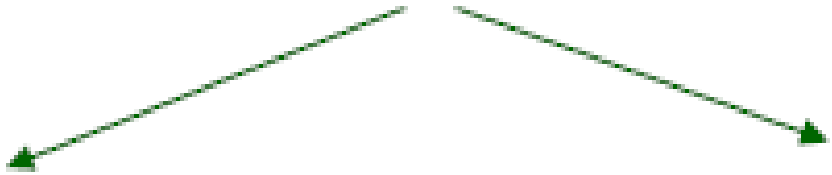
Cys Loop receptors







Ligand Gated Ion Channels in Vertebrates



Mixed Anionic

Cl^-
HCO_3^-

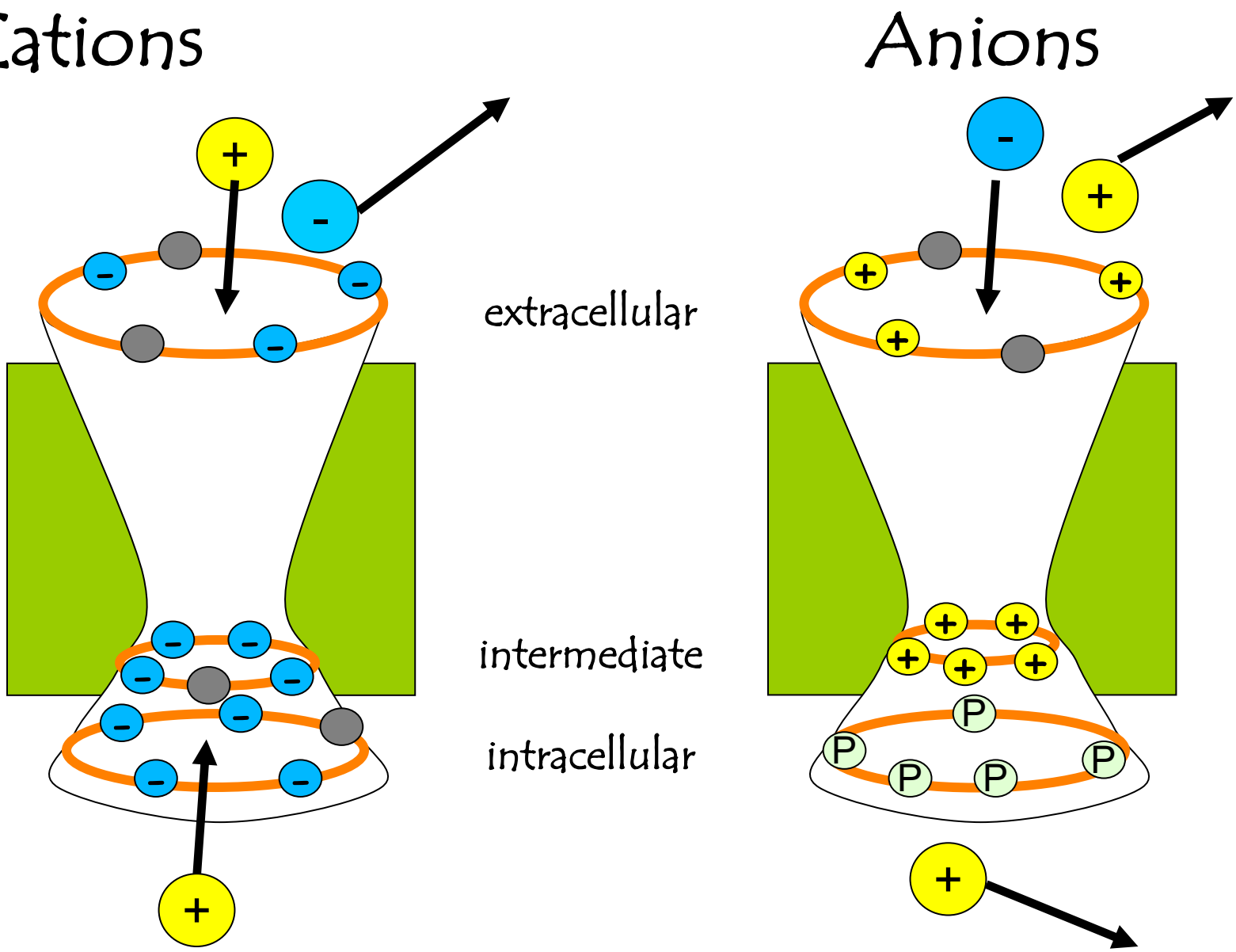
$GABA_AR$
GlycineR

Mixed Cationic

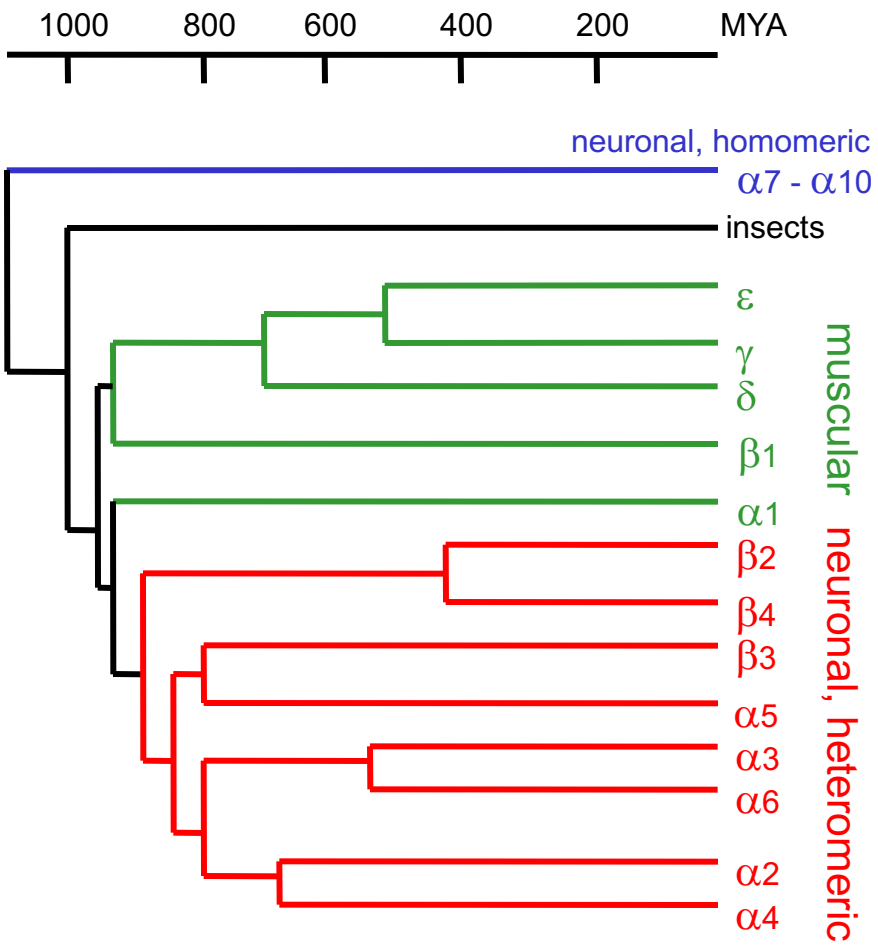
Na^+
K^+
(Some also Ca^{2+})

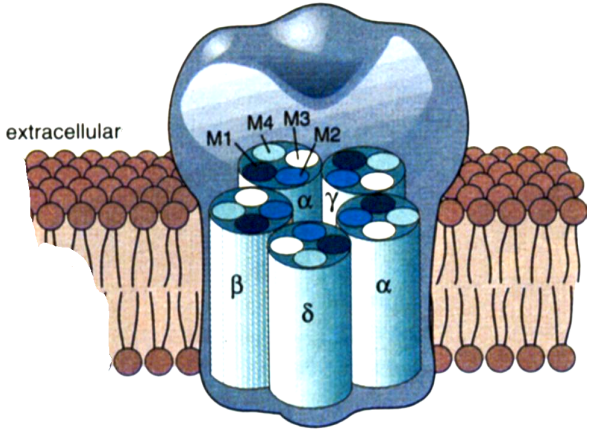
nAchR
GluR
$5\text{-}HT_3R$
PurineR

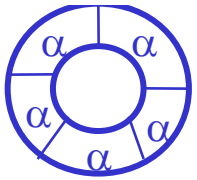
Selectivity

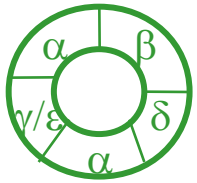


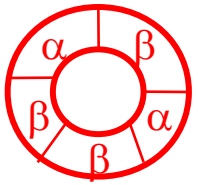
Nicotinic receptos





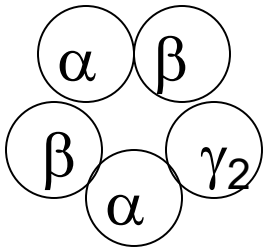






nAChRs are hetero- o omo- pentamers

$GABA_A$ Receptors

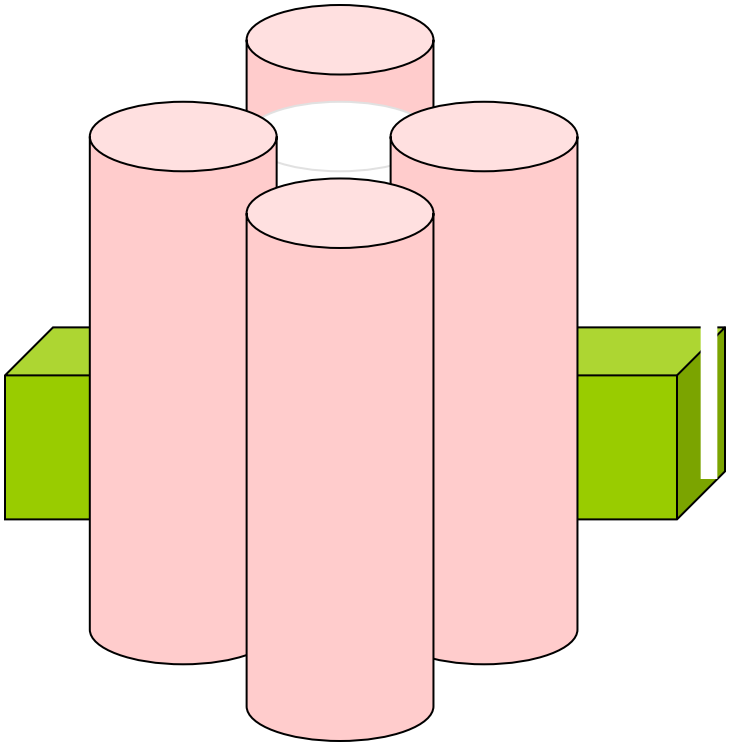


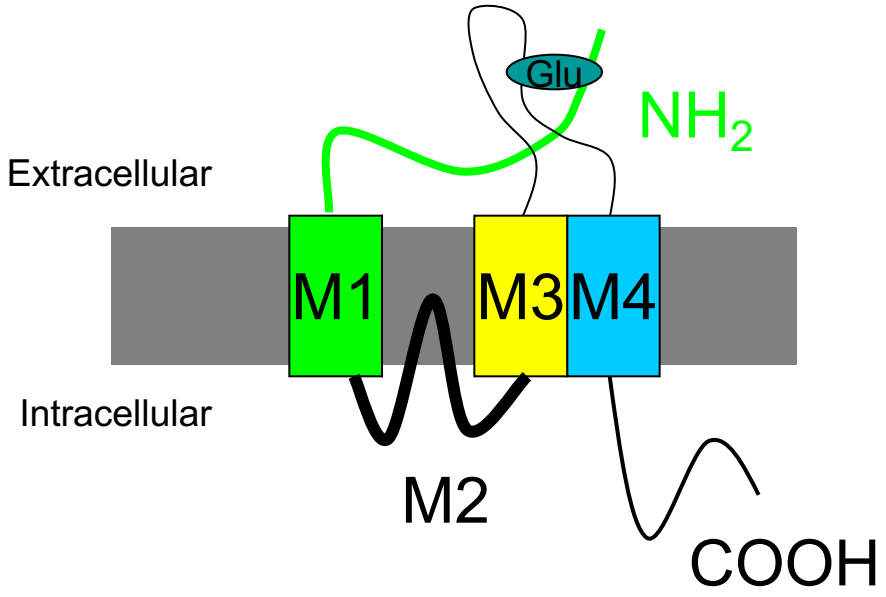
Opening Probability and duration of the openings are conditioned by:

benzodiazepines
barbiturates
anesthetics
Alcohol
steroid hormones

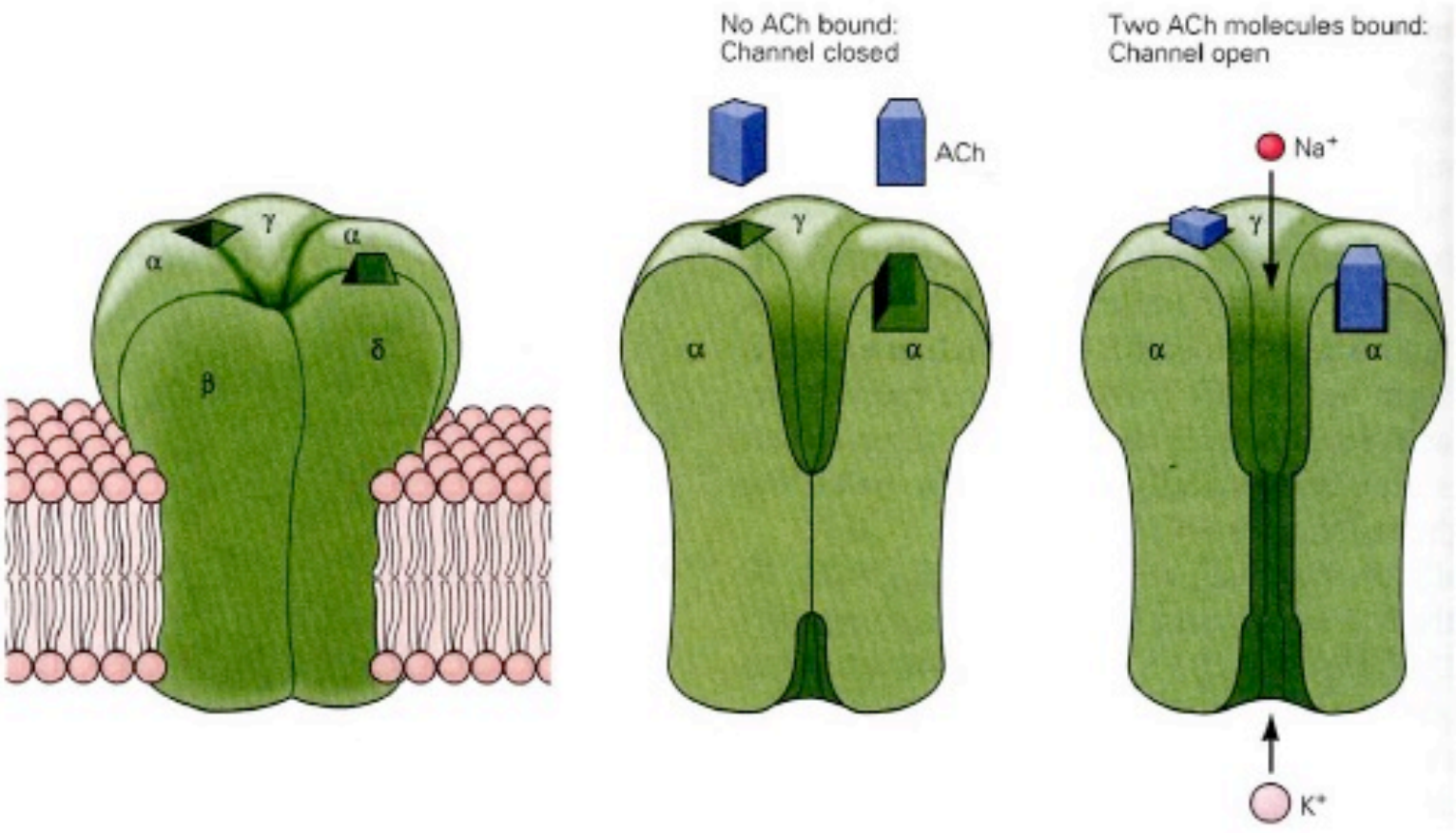
Gene	Protein	Chromosome location (human)
GABRA1	α1	5q31.1–33.2
GABRA2	α2	4p12–p13
GABRA3	α3	Xq28
GABRA4	α4	4p14–q12
GABRA5	α5	15q11–q13
GABRA6	α6	5q31.1–33.2
GABRB1	β1	4p12–p13
GABRB2	β2	5q31.1–33.2
GABRB3	β3	15q11–q13
GABRG1	γ1	4p14–q21.1
GABRG2	γ2	5q31.1–33.2
GABRG3	γ3	15q11–q13
GABRD1	δ1	1p
GABRE1	ε	Xq28
	ρ1	6q14–q21
	ρ2	6q14–q21

Glutamatergic Receptors





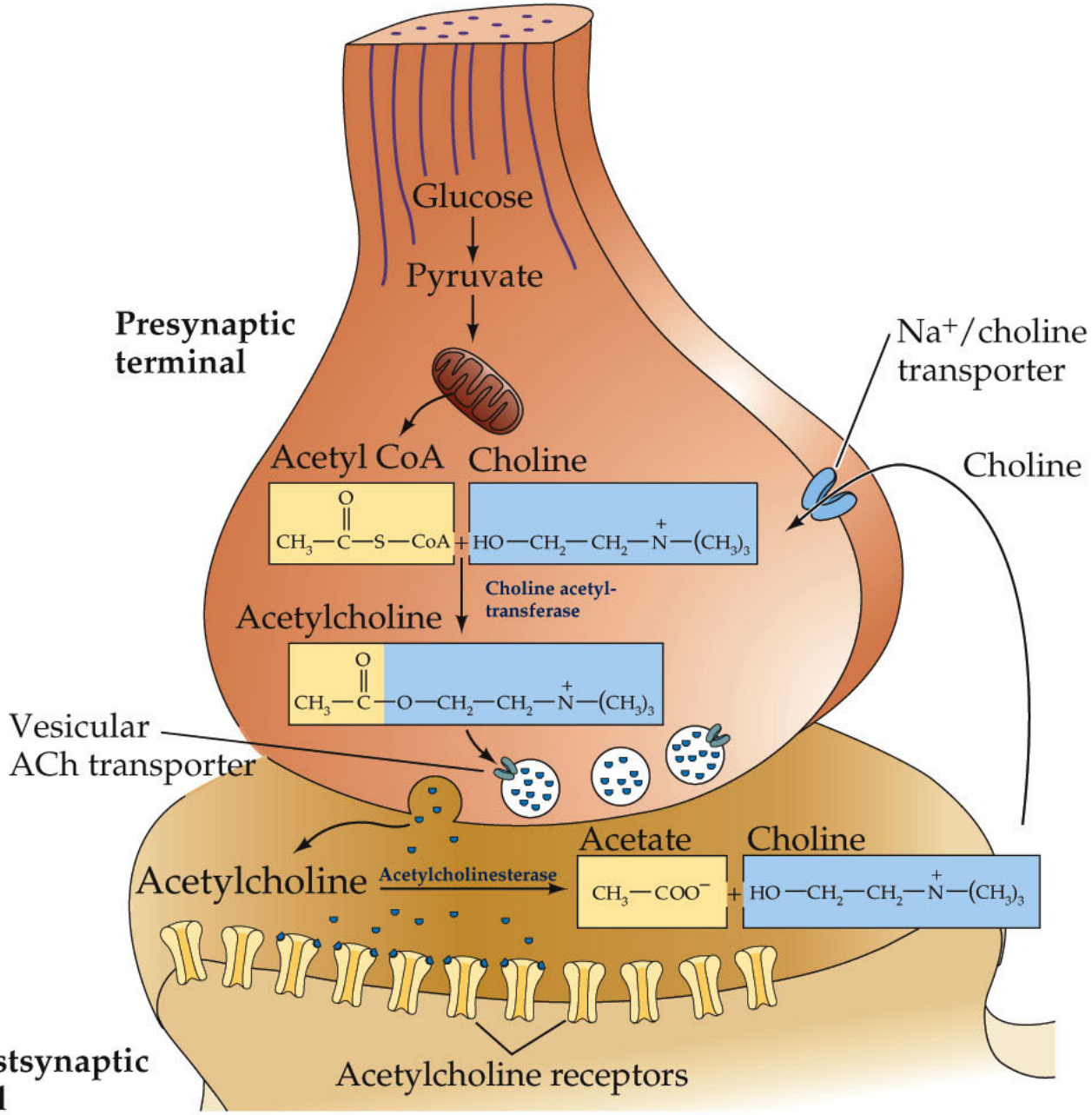
Nicotinic Acethylcholine Receptors nAChR



$$R \underset{k_{-1}}{\overset{A,\ k_1}{\rightleftharpoons}} AR \underset{k_{-2}}{\overset{A,\ k_2}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

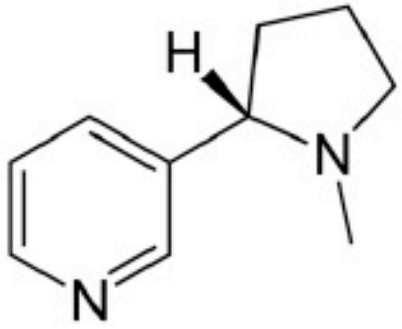
(closed) (closed) (closed) (open)

Ach metabolism

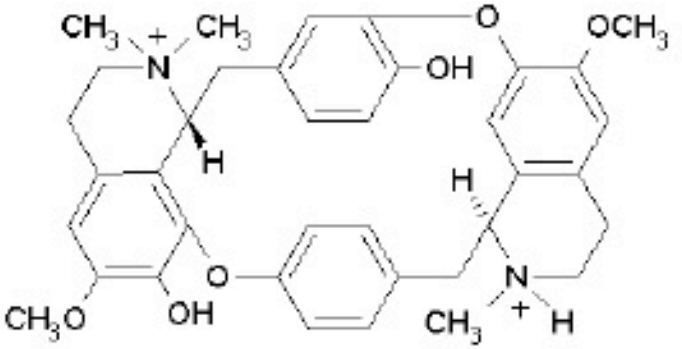


Substances that interact with nicotinic transmission

Nicotine



Curare



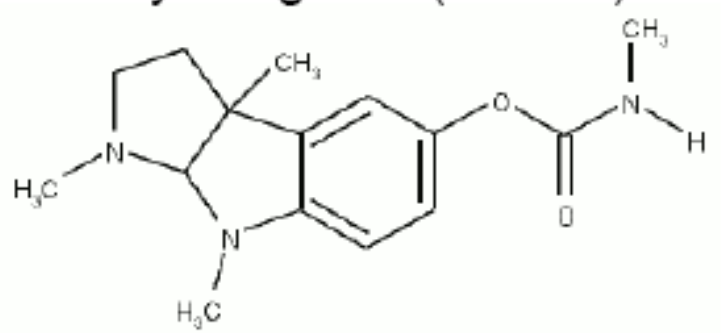
· 5H2O 2Cl⁻

Nicotina tabacum

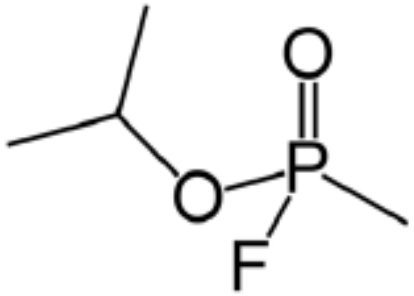
Strychnos Toxifera

Substances that interact with nicotinic transmission

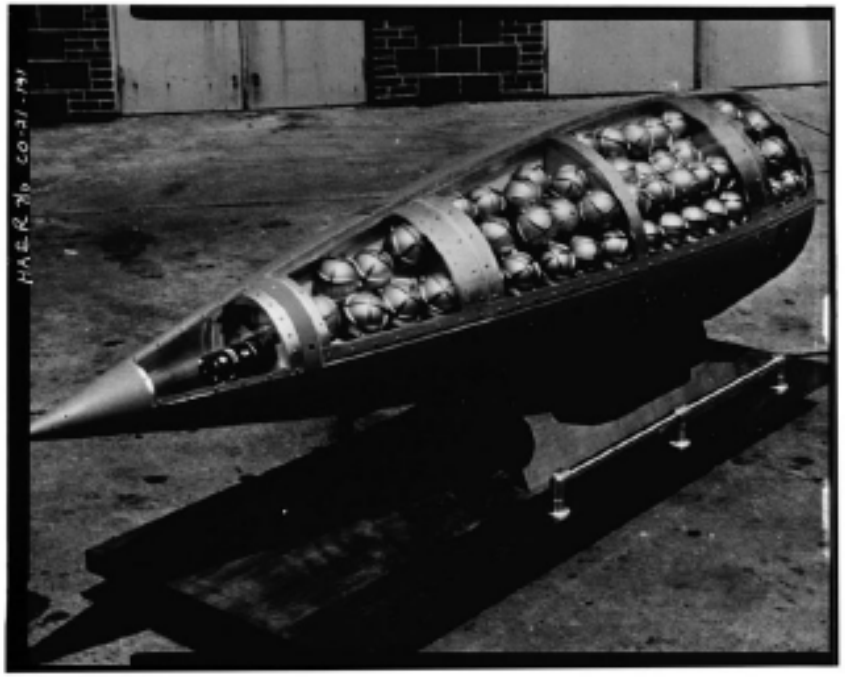
Physostigmine (eserine)



Nerve Gas (Sarin)

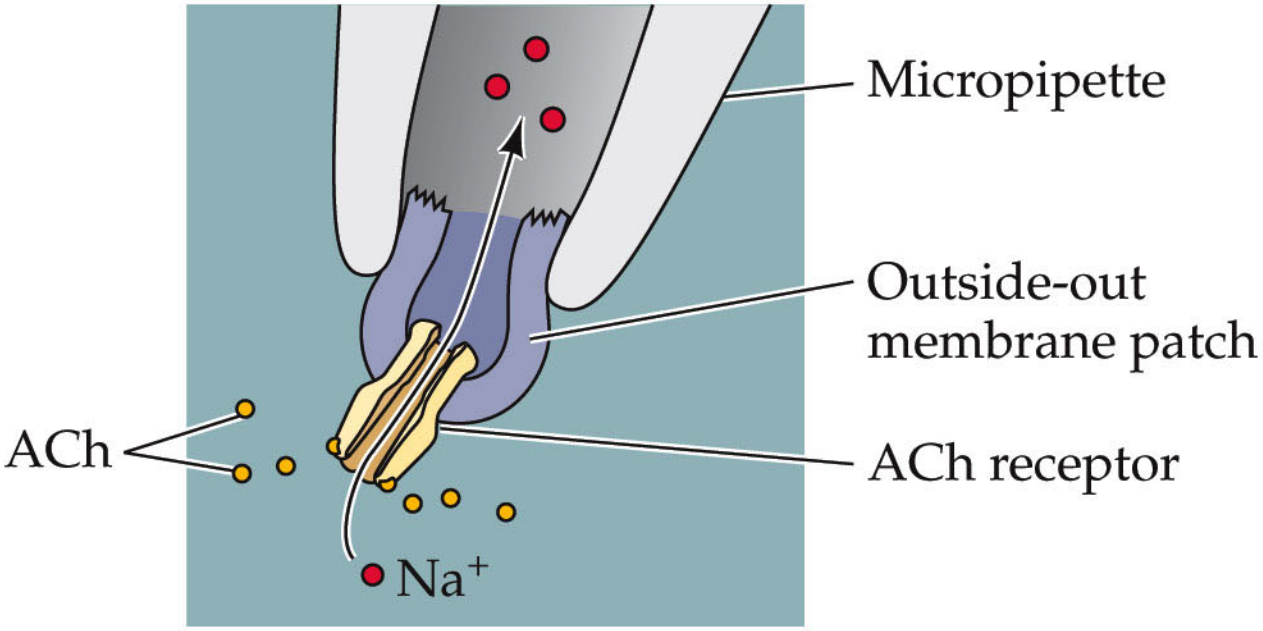


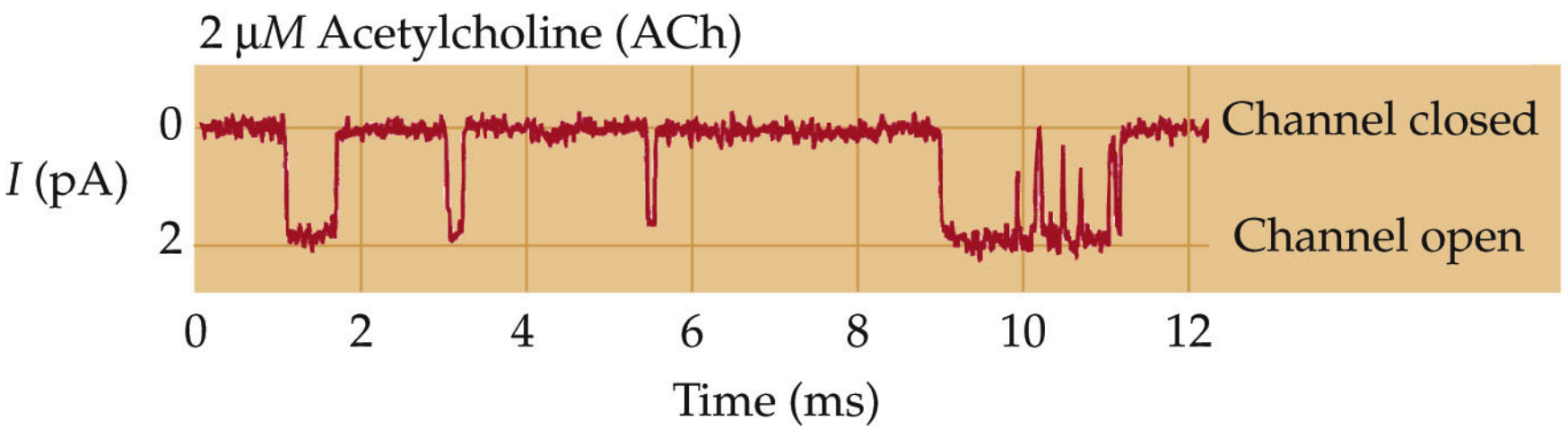
Physiostigma Venenosum
(Calabar bean)



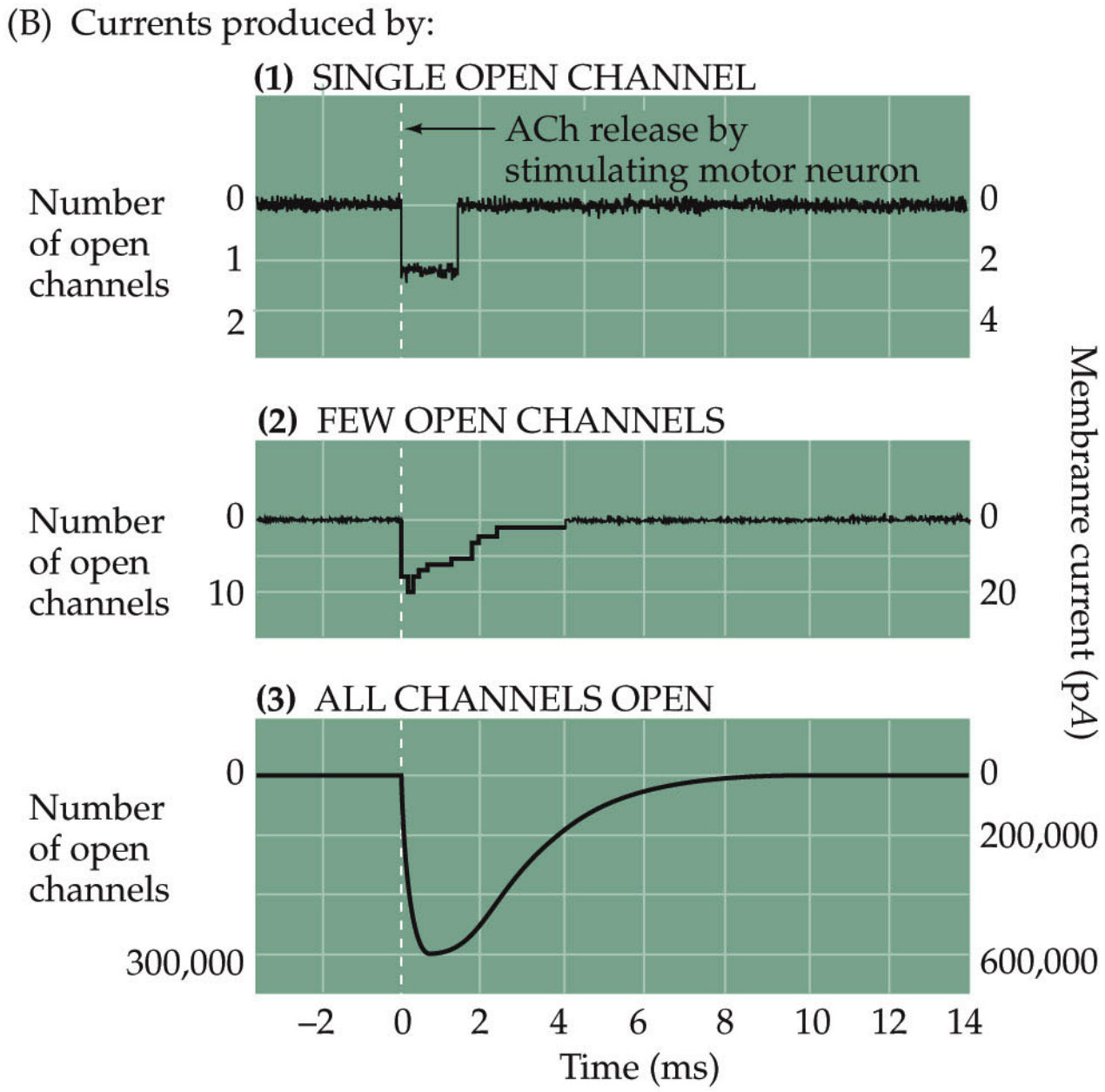
nAChR at NMJ

(A) Patch clamp measurement of single ACh receptor current

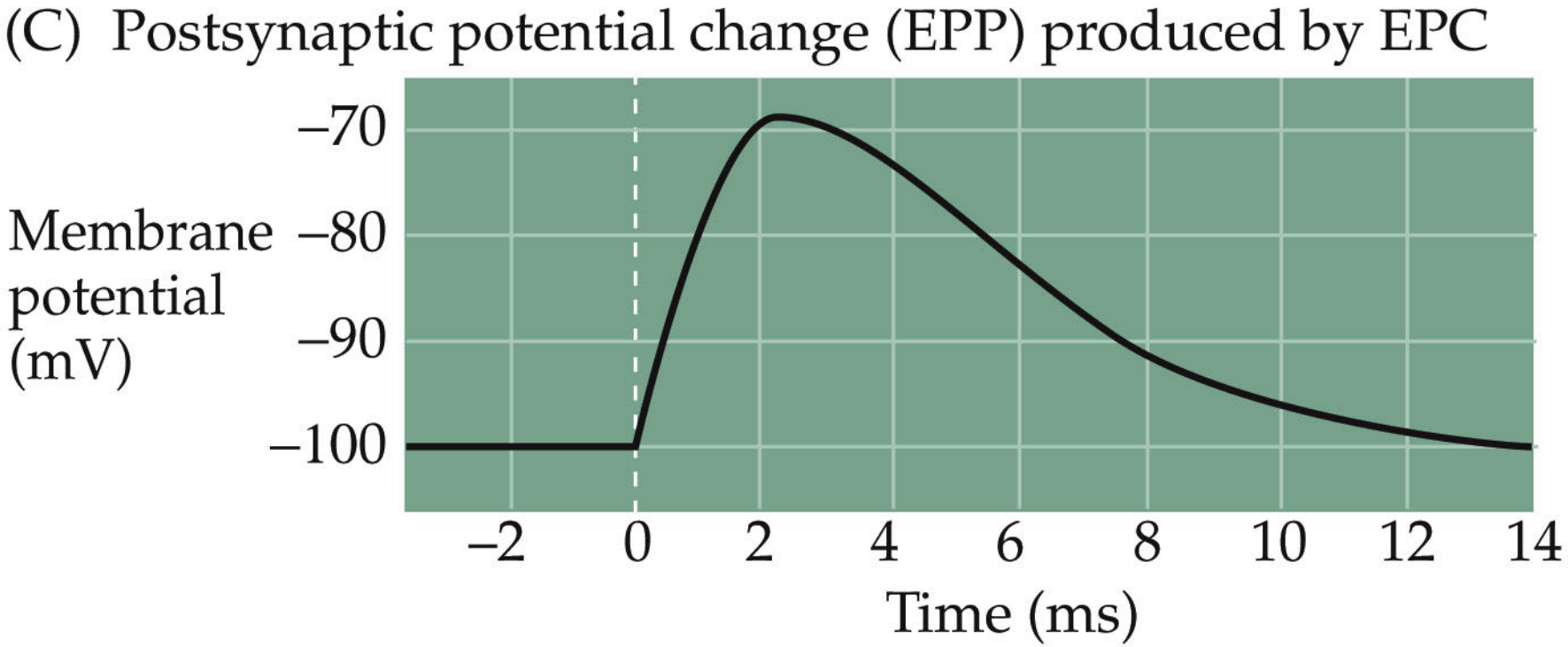




nAChR at NMJ

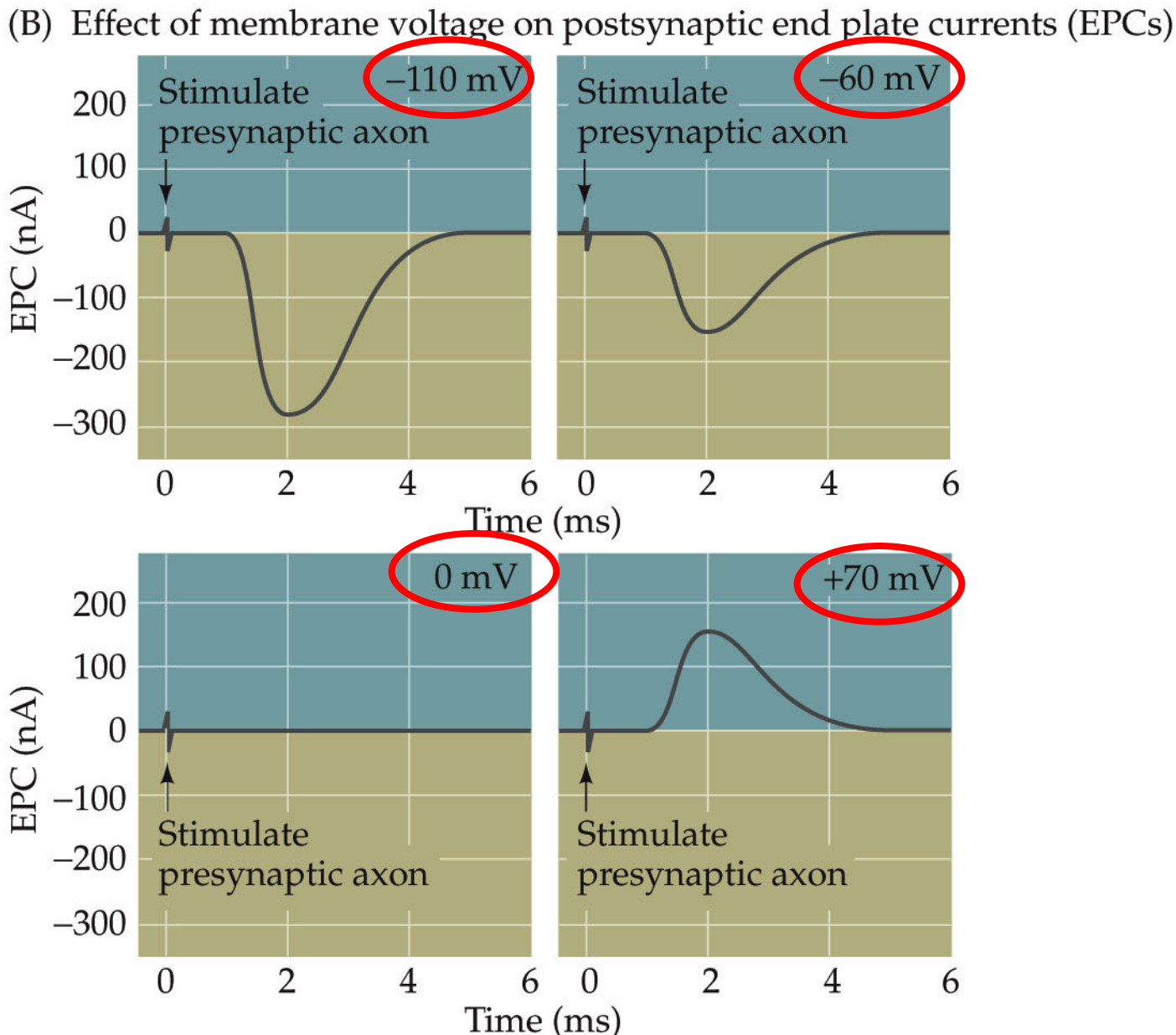

(B) Currents produced by:
(1) SINGLE OPEN CHANNEL
ACh release by stimulating motor neuron
Number of open channels
0
1
2
0
2
4
(2) FEW OPEN CHANNELS
Number of open channels
0
10
0
20
(3) ALL CHANNELS OPEN
Number of open channels
0
300,000
0
200,000
600,000
Membranre current (pA)
–2
0
2
4
6
8
10
12
14
Time (ms)

nAChR at NMJ

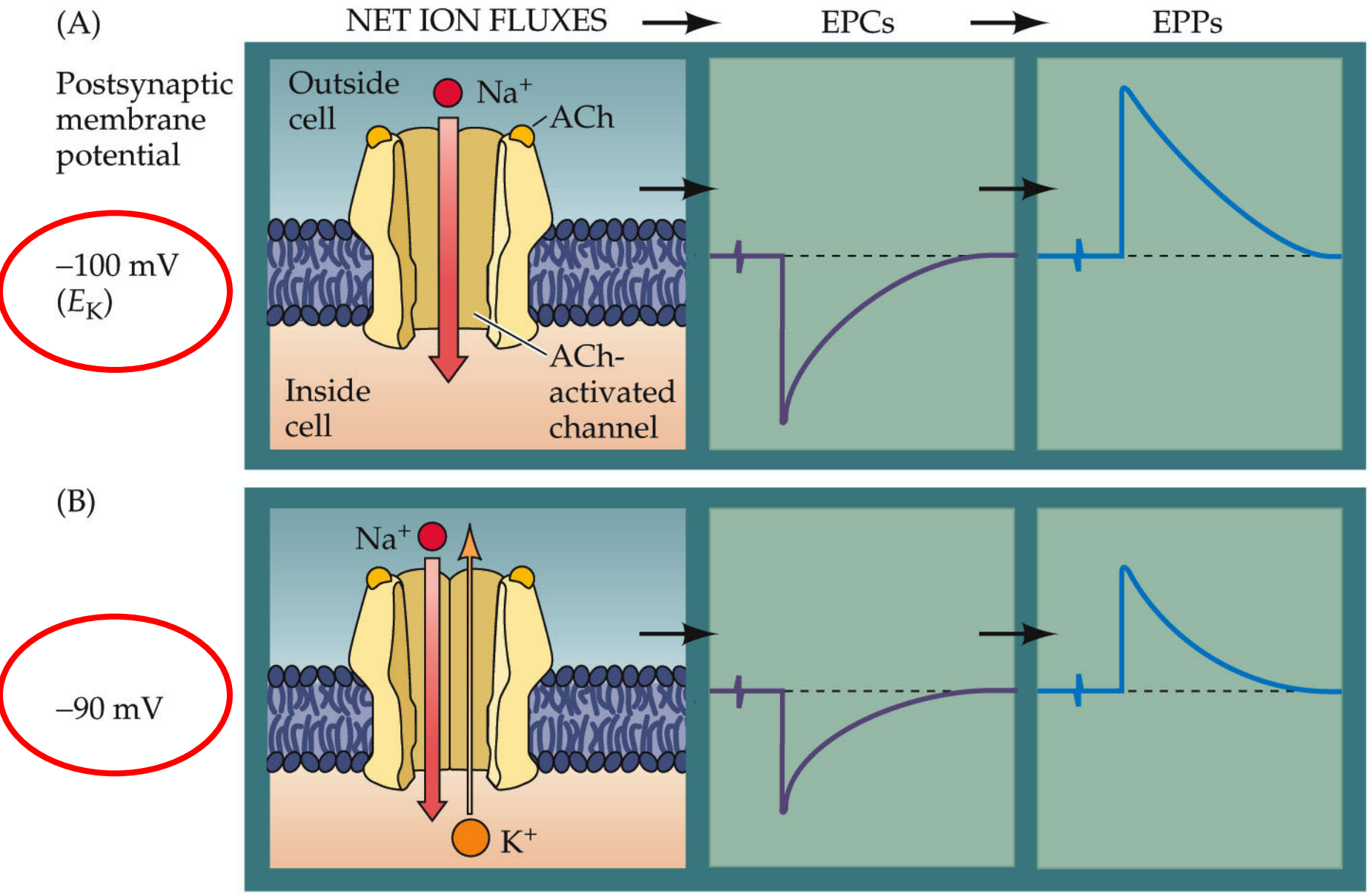


***NEUROSCIENCE, Fourth Edition,* Figure 5.16 (Part 3)**

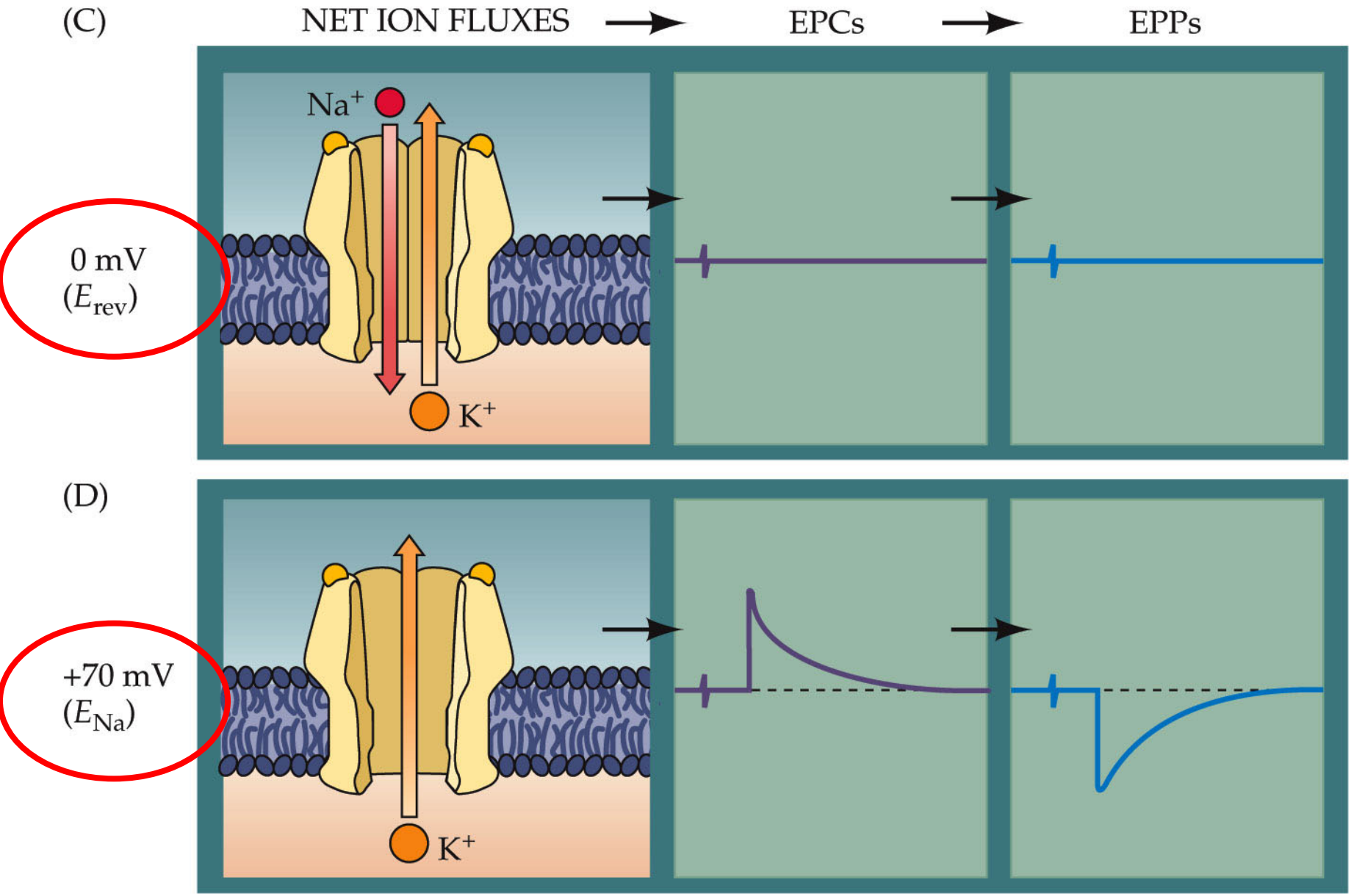
Influence of the postsynaptic membrane potential on the nicotinic (muscular) currents



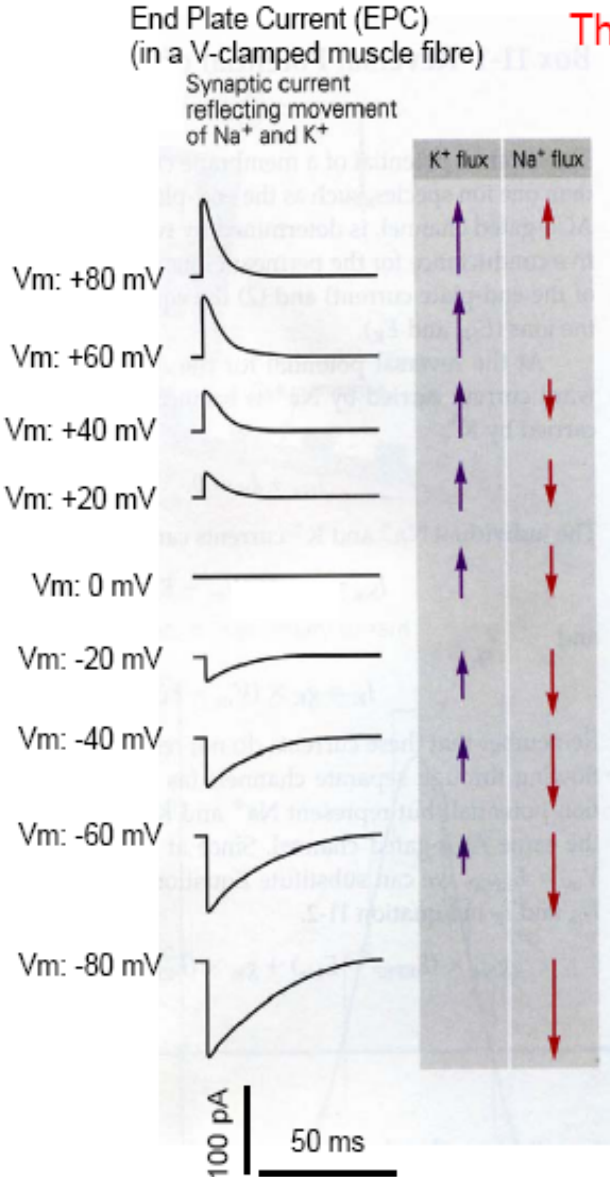
Na^+ & K^+ contribute to the current generated by the activation of nACh .... How do they move?



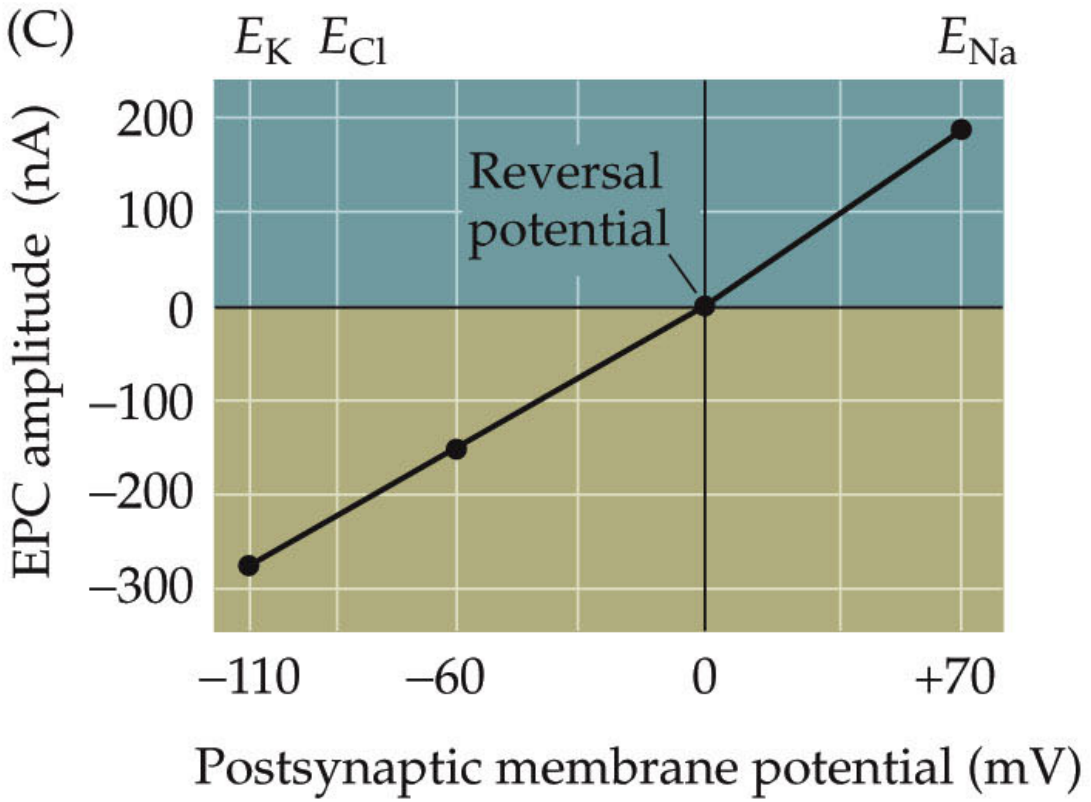
Na^+ & K^+ contribute to the current generated by the activation of nACh .... How do they move?



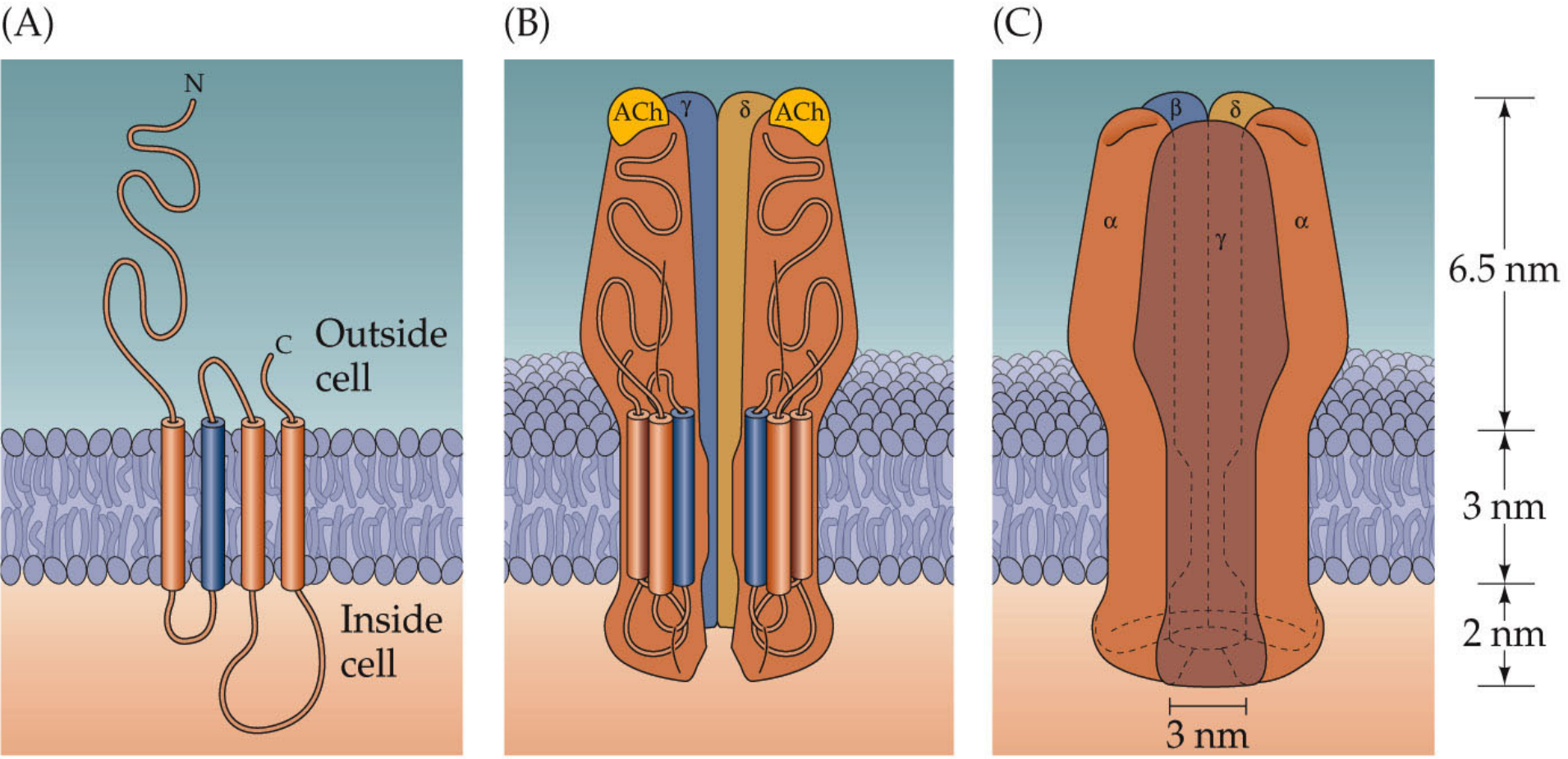
Influence of the postsynaptic membrane potential on the nicotinic (muscular) currents



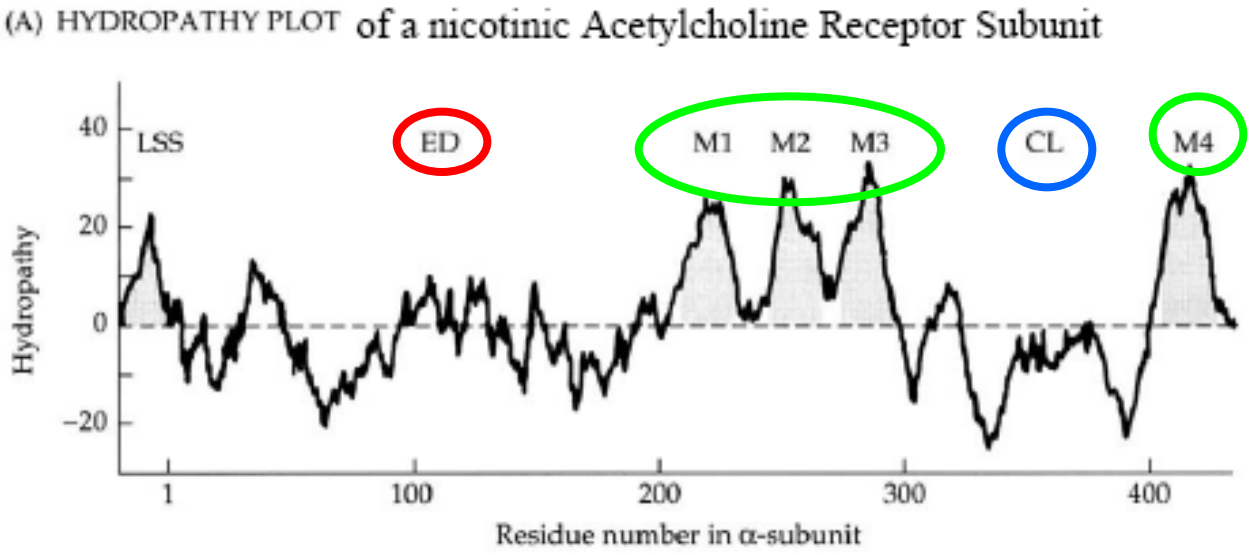
I-V curve



nAChR structure



nAChR structure



LSS: Leading signal sequence (cleved in mature AchR)

ED: Extracellular domain

M1-4 Membrane spanning segment

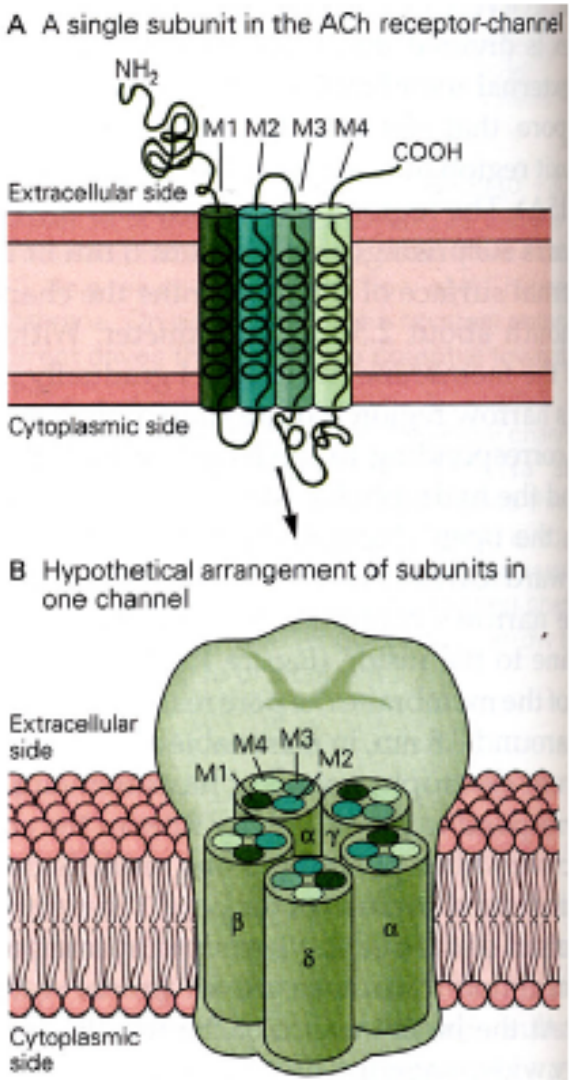
CL: Cytoplasmatic Loop

Distribution of polar and nonpolar AA provides info on how the AA sequence spans the membrane

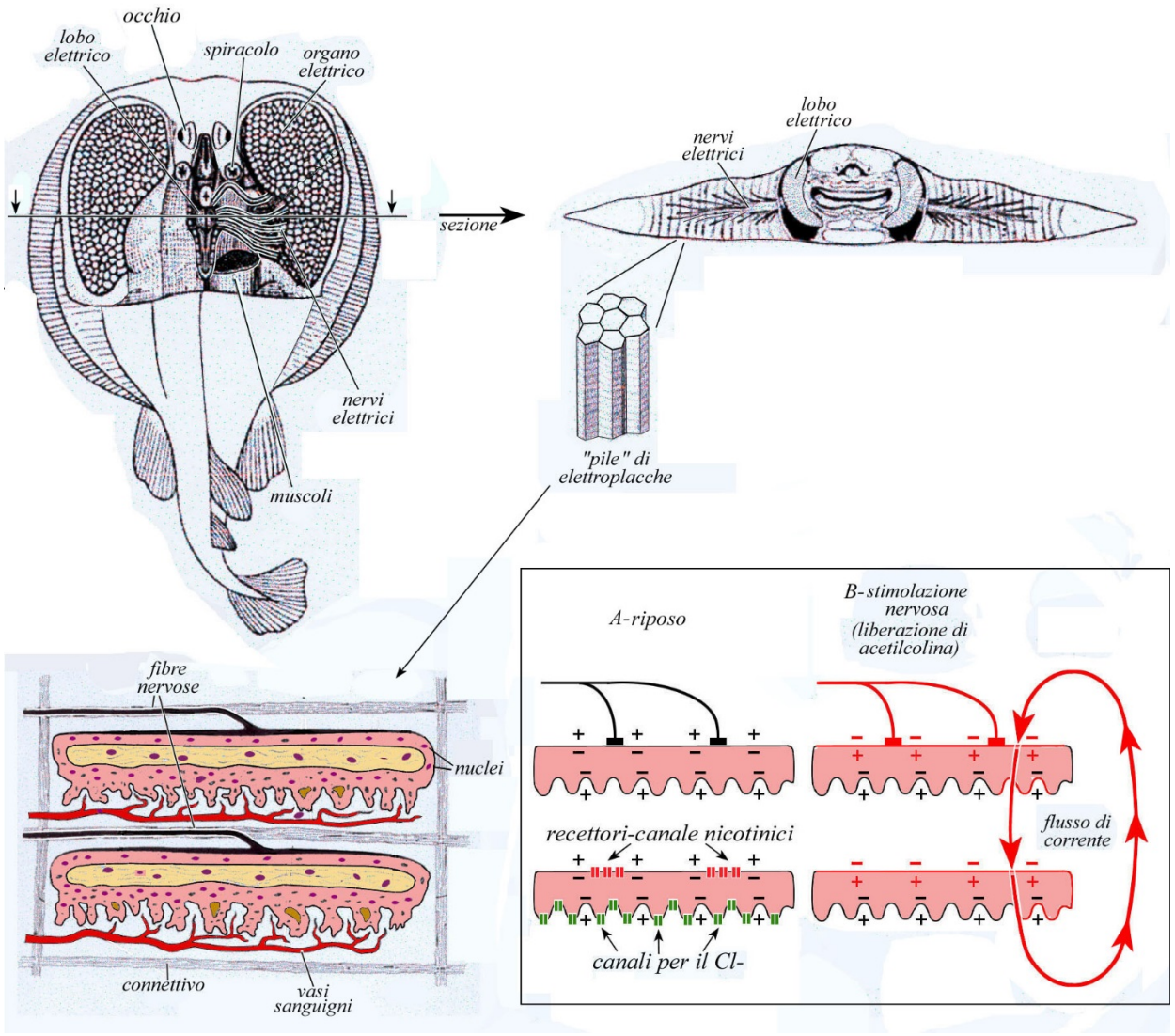
5 subunits each with 4TM domains (hydrophobic regions)

External end Wide mouth 2.5 nm

Narrowest diameter ~0.8 nm



the molecules of the best known specimen: (nAchR) T, are present in the membranes of the electric organ of the Torpedo (Torpedo) in such a large quantity as to constitute "almost crystalline" structures.

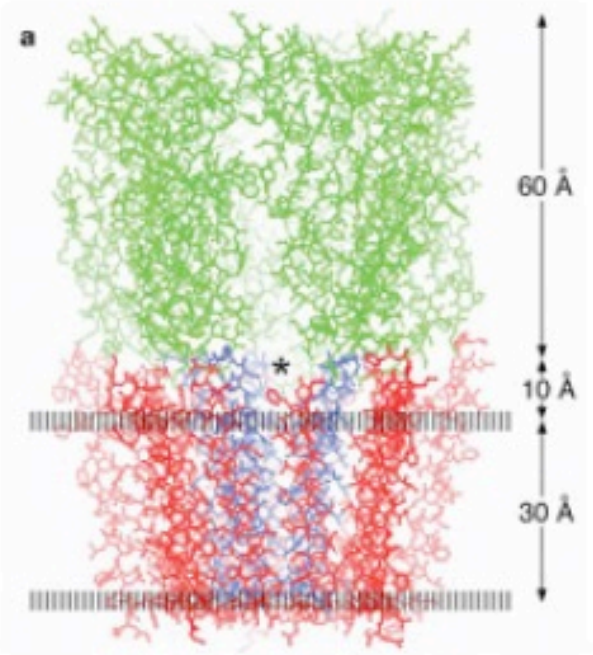


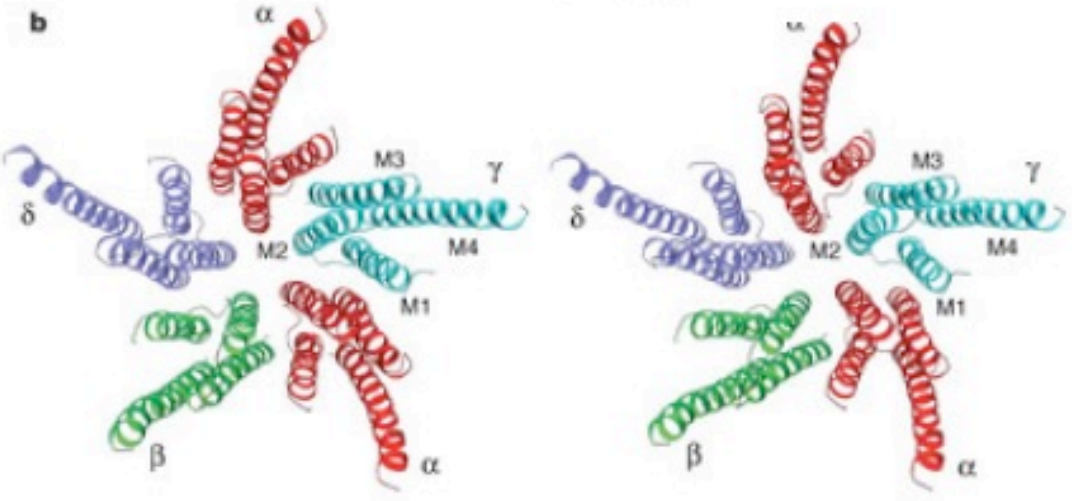
α-bungarotoxin (Cobra) binds to nAchRs with very high affinity, facilitating protein extraction and purification

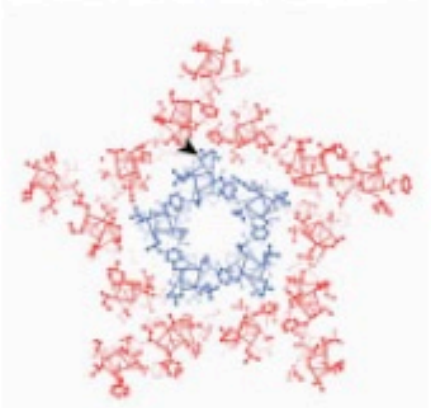
the structure of the (nAchR) T molecule is resolved to the atomic level (with X-ray diffraction).

nAChR structure

Structure of the AchR (from EM)

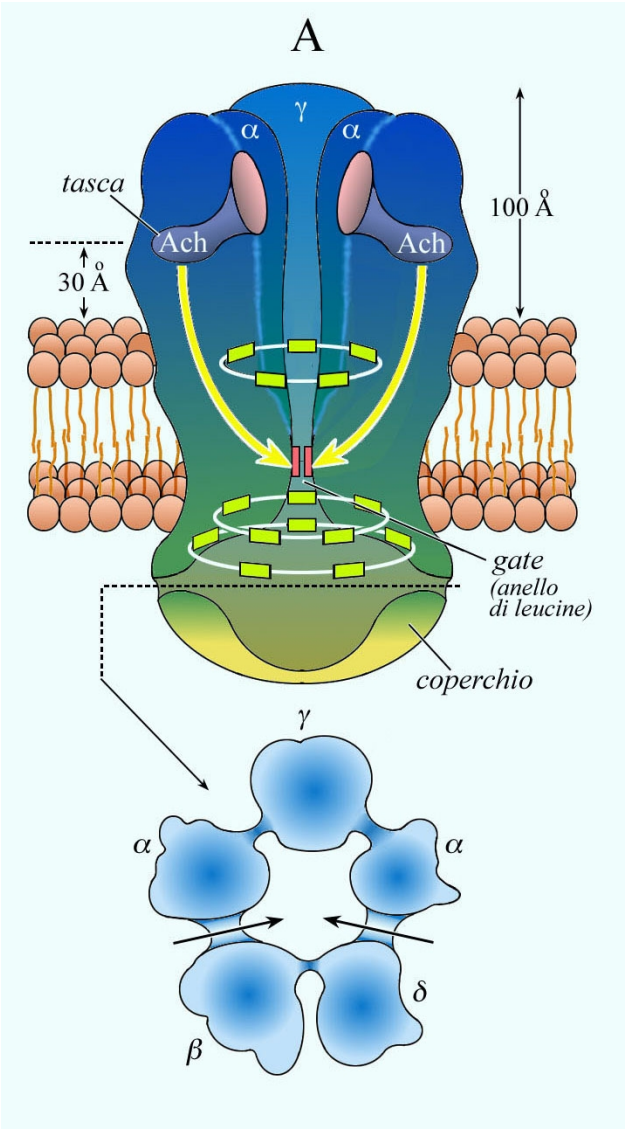






From Torpedo Electric Organ; Unwin 2003

nAChR structure

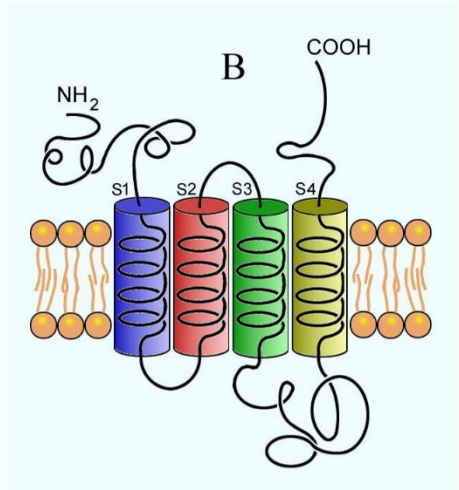


large transmembrane molecule.

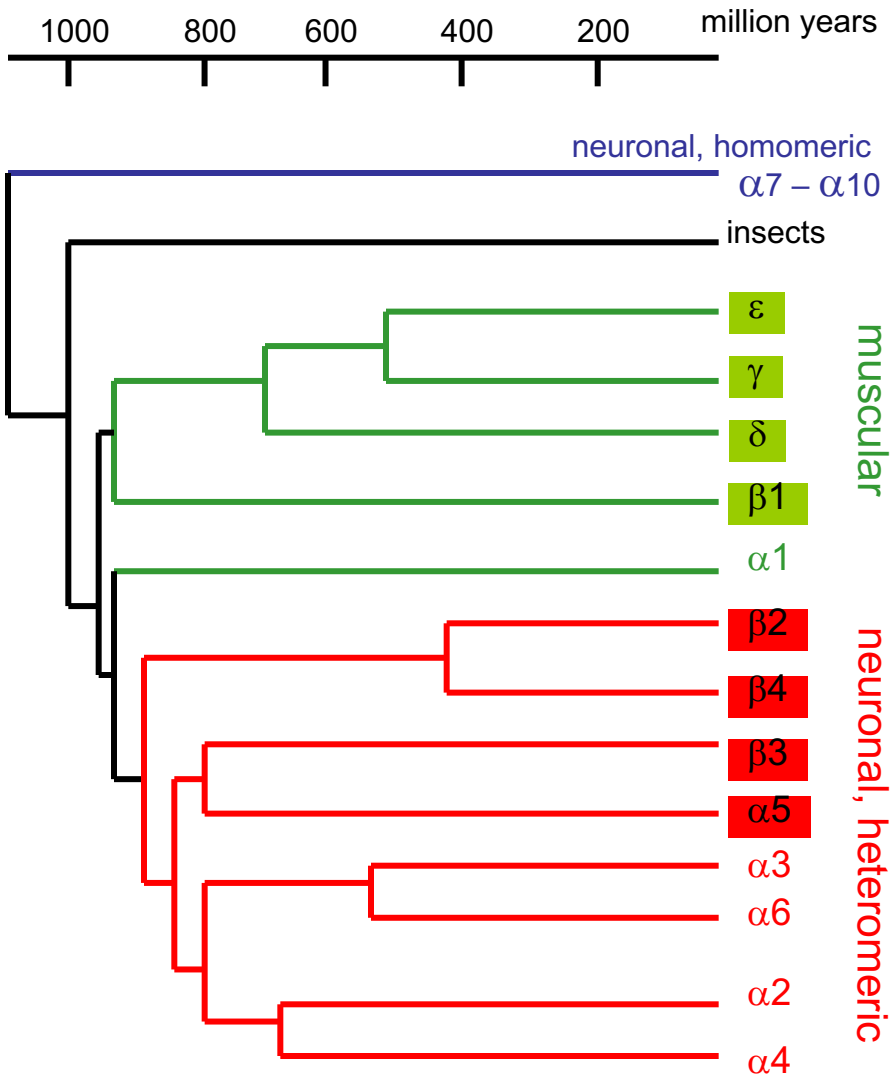
The 5 subunits (αβγδ) cross the double phospholipid layer with 4 STM, are arranged at the top of a pentagon, in which a γ subunit is interposed between two α subunits.

Extracellular domains 100 Å

M2 domains form the *pore.*



Nicotinic Receptors



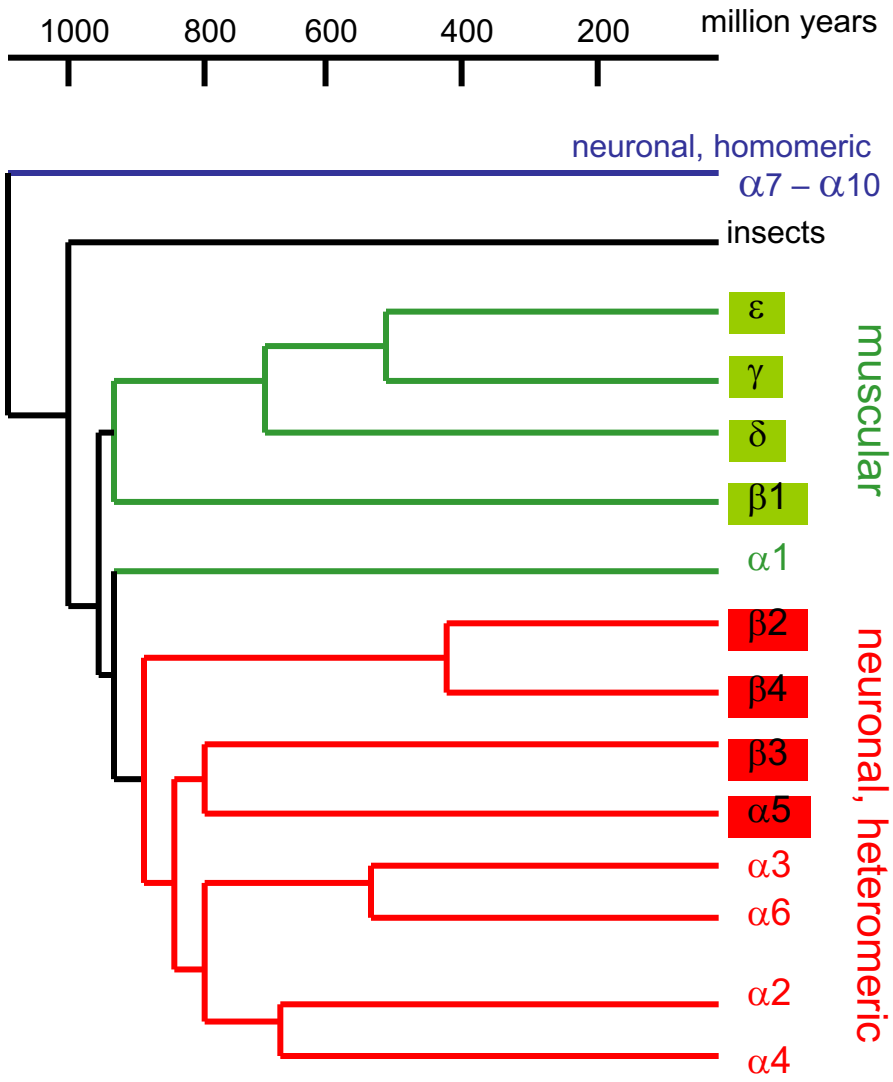
17 genes in vertebrates

Subunit expression:
regional & developmental regulation

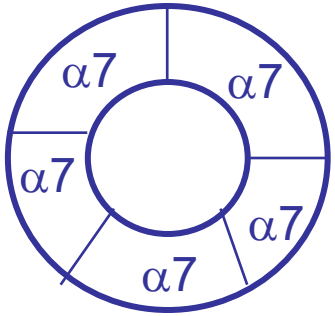
Subunit composition:
nAChR-channel functional properties

Nicotinic Receptors

nAChRs are homo- or hetero-pentamers

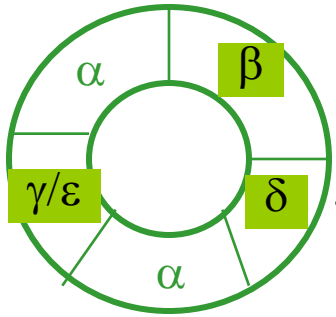


17 genes in vertebrates



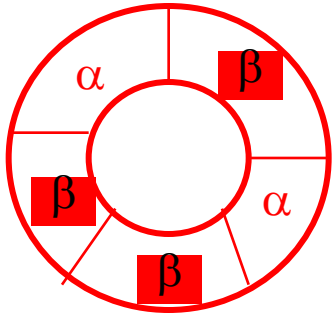
Subunit expression: regional & developmental regulation

Subunit composition: nAChR-channel functional properties



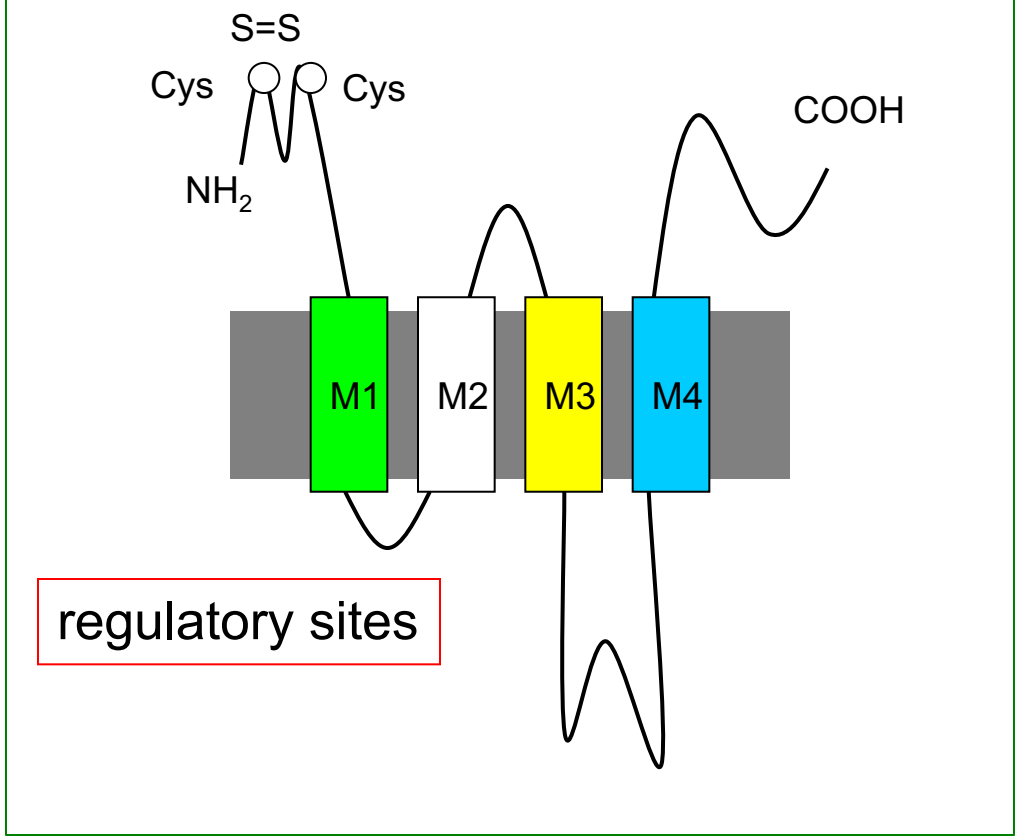
γ-nAChR (30 pS; 5 ms)

ε-nAChR (50 pS; 1 ms)
regulated by innervation

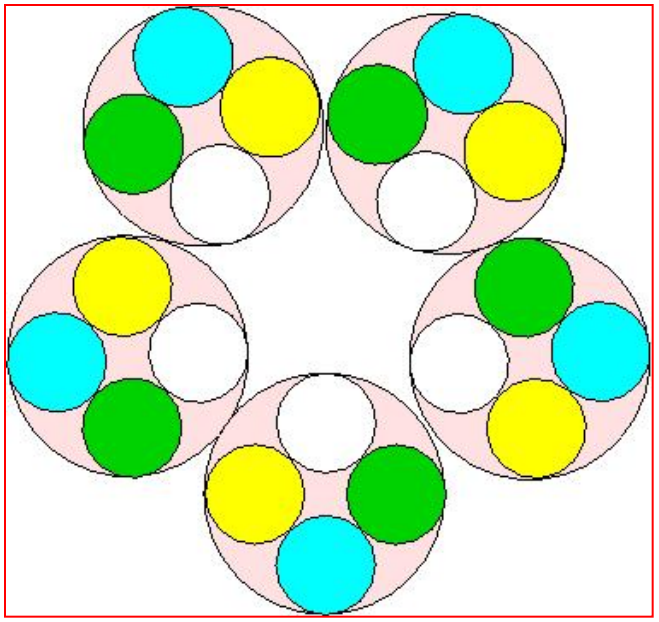


Subunit topology

binding site

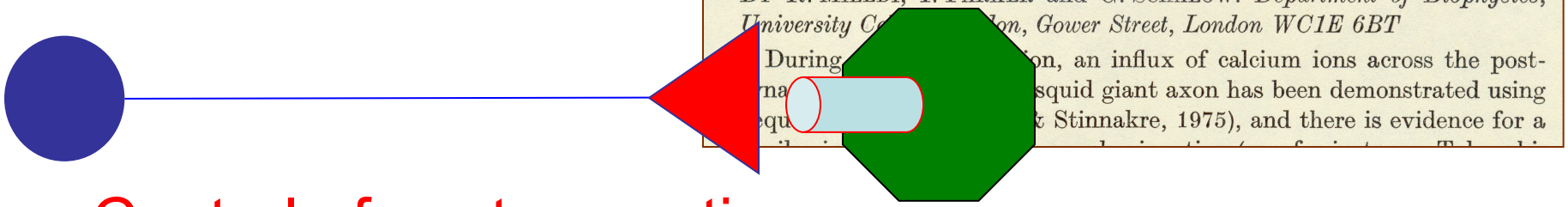


regulatory sites



nAChR-channels

select for cations
are permeable to Ca^{2+}



[*From the Proceedings of the Physiological Society*, 18–19 *February* 1977
Journal of Physiology, **268**, 32–33 *P*]

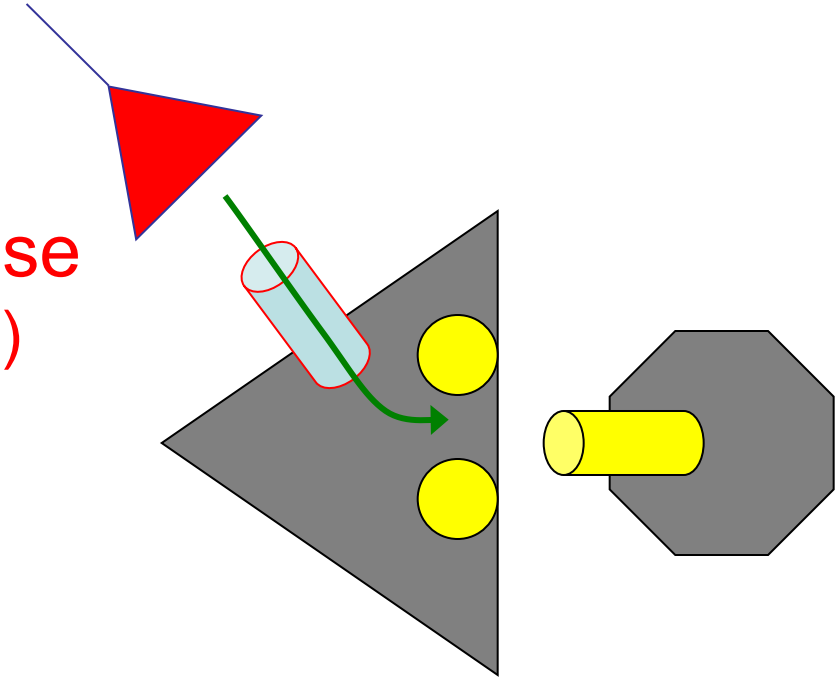
Calcium entry across the post-junctional membrane during transmitter action

By R. Miledi, I. Parker and G. Schalow. *Department of Biophysics, University Co[illegible]don, Gower Street, London WC1E 6BT*

During [illegible]on, an influx of calcium ions across the post-[illegible]squid giant axon has been demonstrated using [illegible]equ[illegible] Stinnakre, 1975), and there is evidence for a

Control of post-synaptic processes

Control of transmitter release
(dopamine in nicotine addiction)



Ca^{2+} permeability of recombinant nAChR

nAChR	P_f
h α7	11.4%
r α7	8%
h α1β1εδ	7.5 %
m/r α1β1εδ	4.2 %
m/r/h α1β1γδ	2.8 %
h α3β4	2.7 %
chick	4.5 %
h α4β2	2.6 %
chick	2.9 %
h α4β4	1.5 %
chick	2.1 %

Fucile (2004) Cell Calcium 35:1-8
Fucile et al. (2006) J Physiol 573: 35-43

P_f depends on subunit composition & SPECIES

Ca^{2+} permeability of recombinant nAChR

nAChR	P_f
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chick	2.1 %

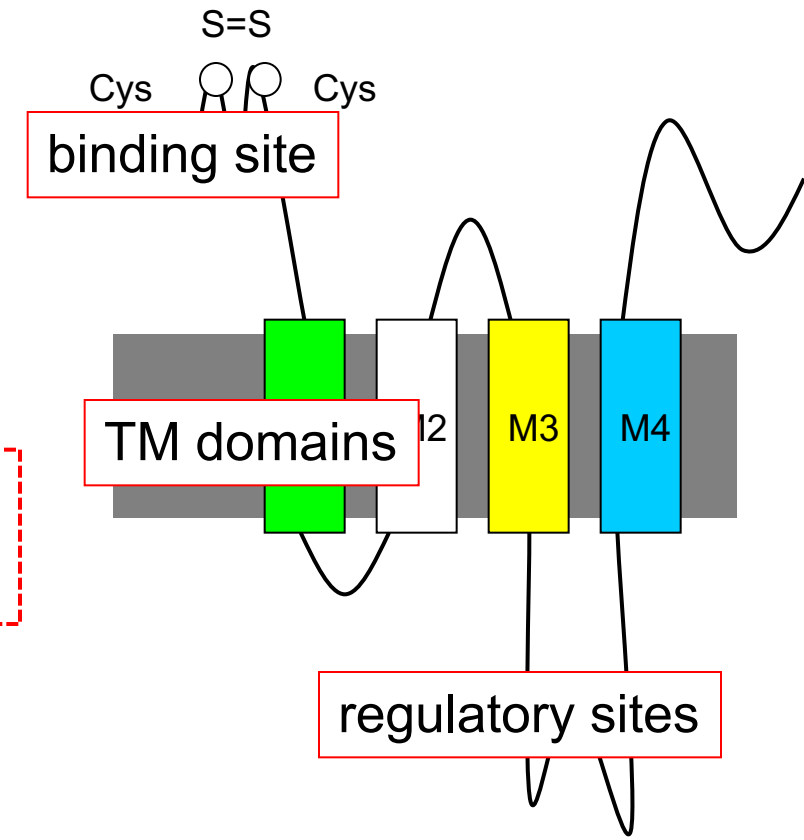
Fucile (2004) Cell Calcium 35:1-8
Fucile et al. (2006) J Physiol 573: 35-43

Mutations & Disease

Congenital Myasthenic Syndrome
(α1, β1, δ, ε)

Autosomal Dominant Nocturnal
Frontal Lobe Epilepsy (α4, β2)

Addiction to nicotine
(α3, α5, β2, α6, β3)



Identification in patients
Functional studies on human muscle
Functional studies on recombinant nAChRs
Genetically modified animal models

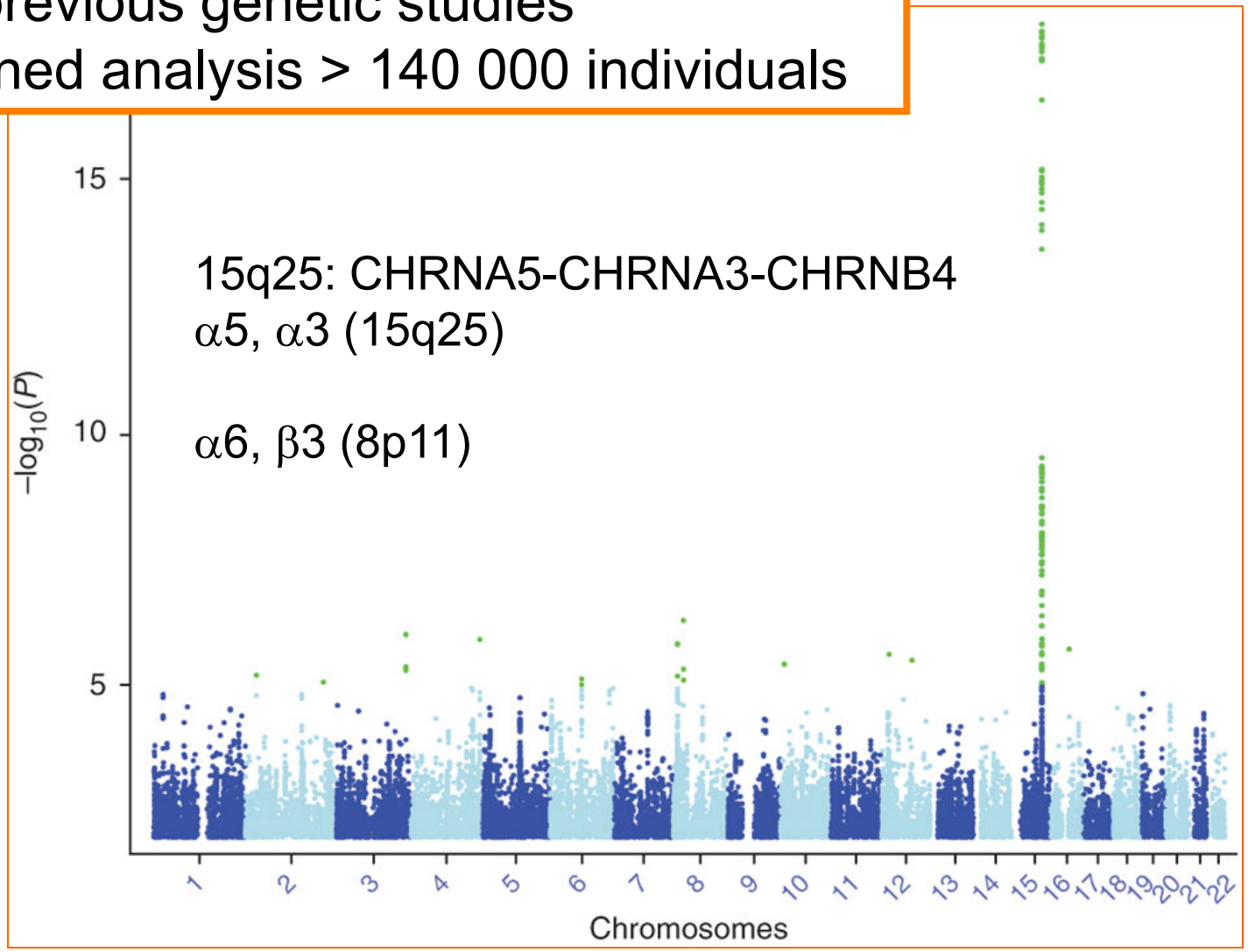
Nicotine addiction

Tobacco use is the leading cause of preventable death in developed countries, causing about 5 million deaths/year wordwide. Its use is increasing in developing countries, further raising death toll.

Vincent van Gogh (1853-1890)
Van Gogh Museum, Amsterdam

nAChR & Nicotine addiction

3 genome-wide association studies (2010)
other previous genetic studies
Combined analysis > 140 000 individuals



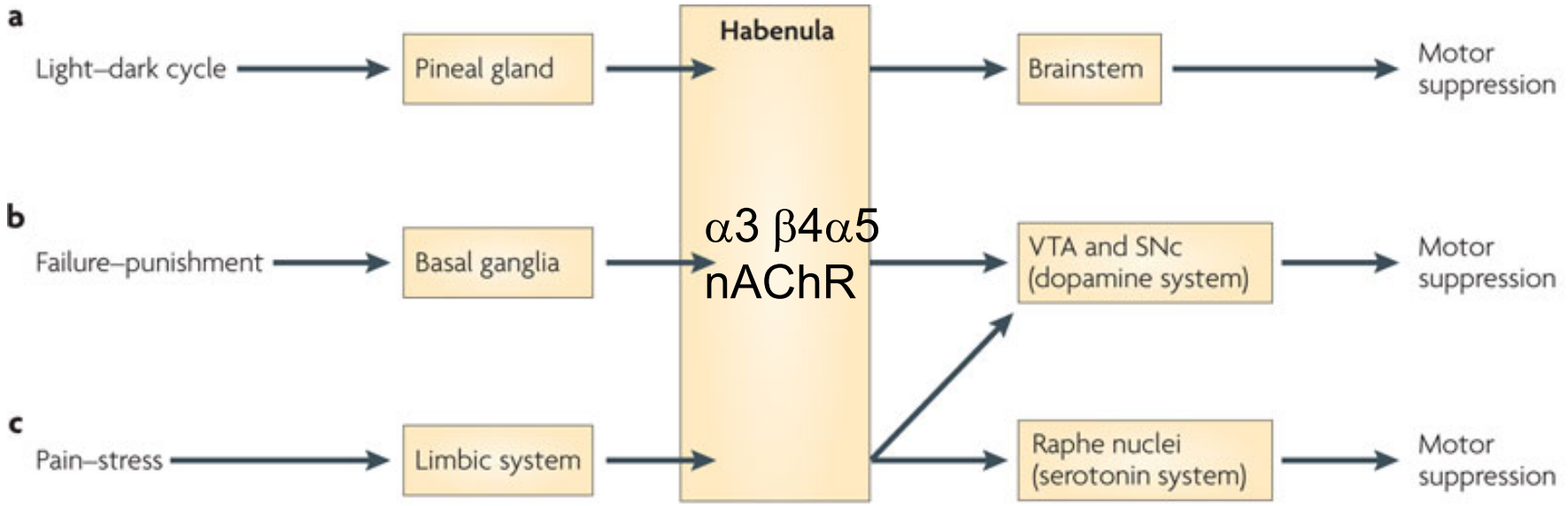
Nature Genetics, May 2010

D398N in α5 & Nicotine addiction

Bierut et al., Am J Psych 2008

D398N in α5 & Nicotine addiction

α3α5β4 in
- PNS (ganglion neurones)
- medial habenula (epithalamus)

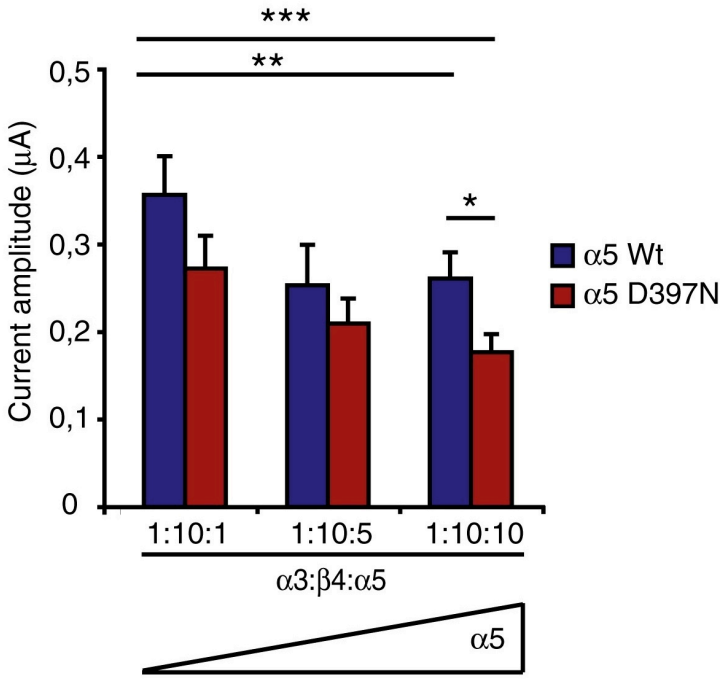


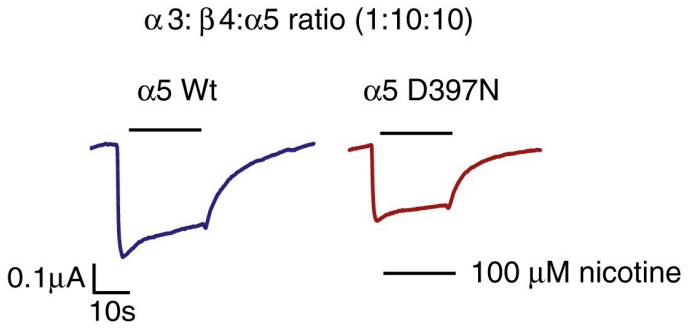
Nature Reviews | Neuroscience
2010

D398N in α5 & Nicotine addiction

$\alpha5_{D398N}$ induces LOSS-of-function in α3β4α5 nAChR

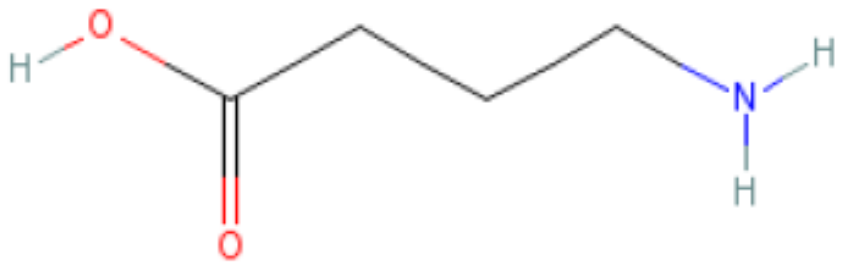
$\alpha5_{D398N}$ reduces aversion to nicotine allowing enhanced consumption





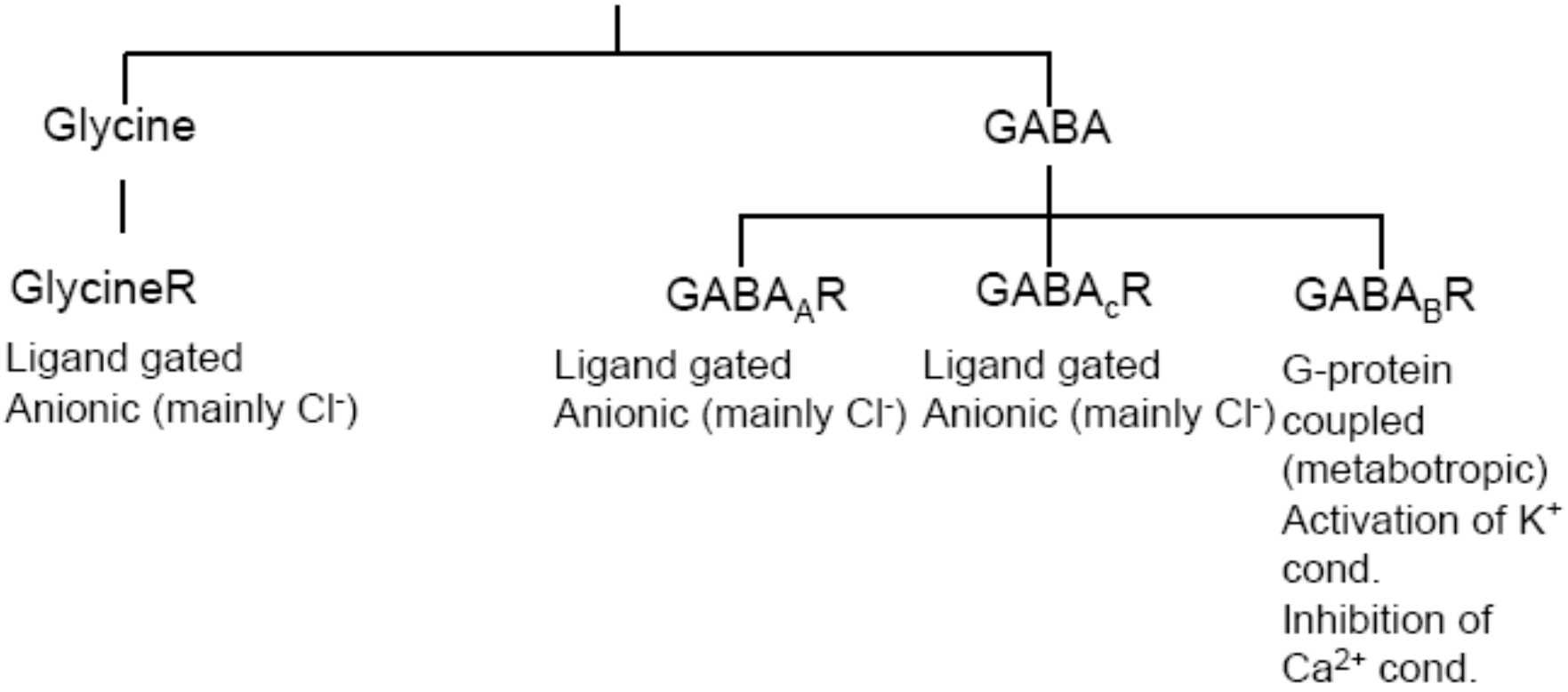
Frahm et al. (2011) Neuron 70: 522-535

Fast GABAergic transmission

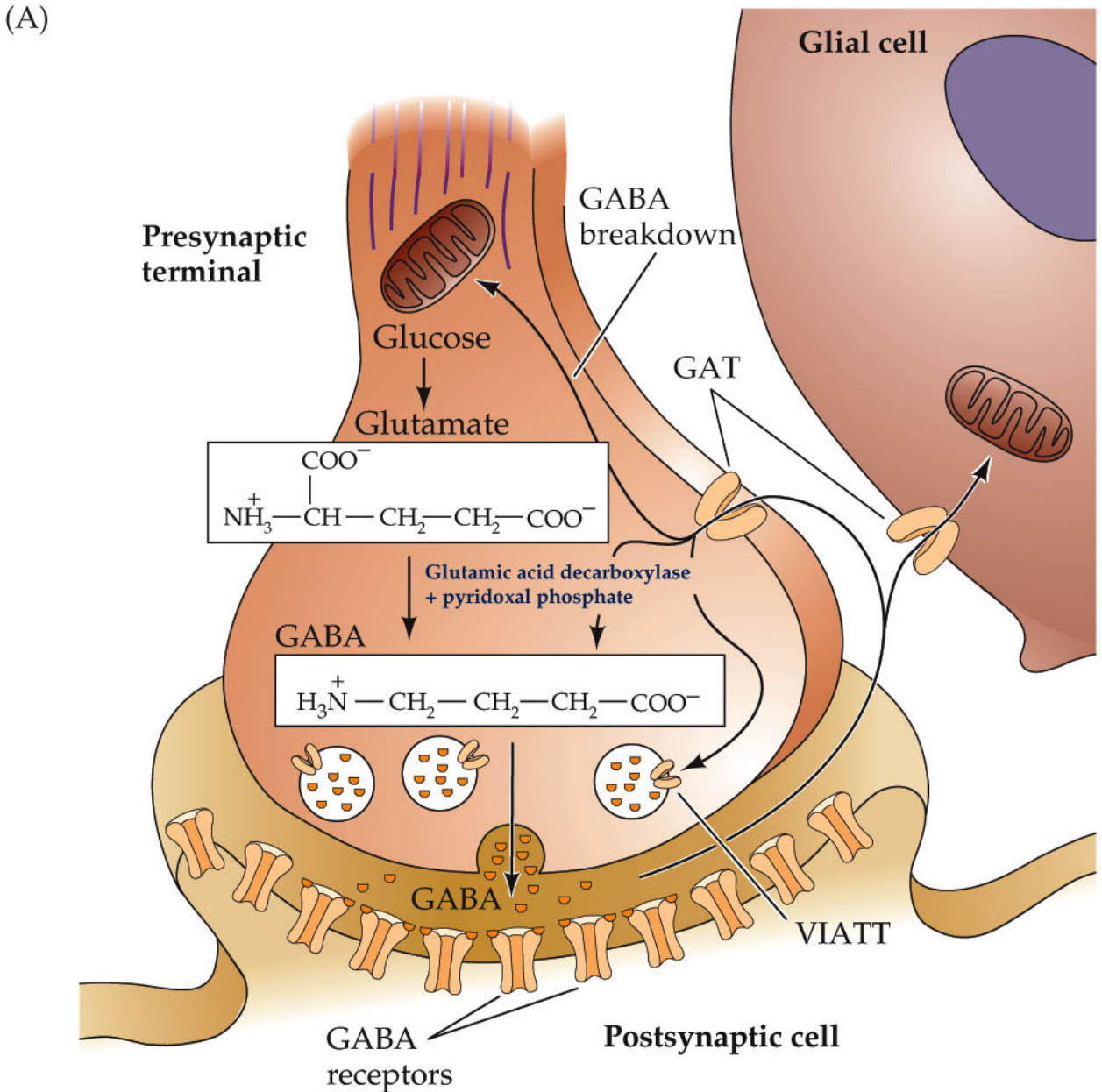


γ-amino butyric acid (GABA)

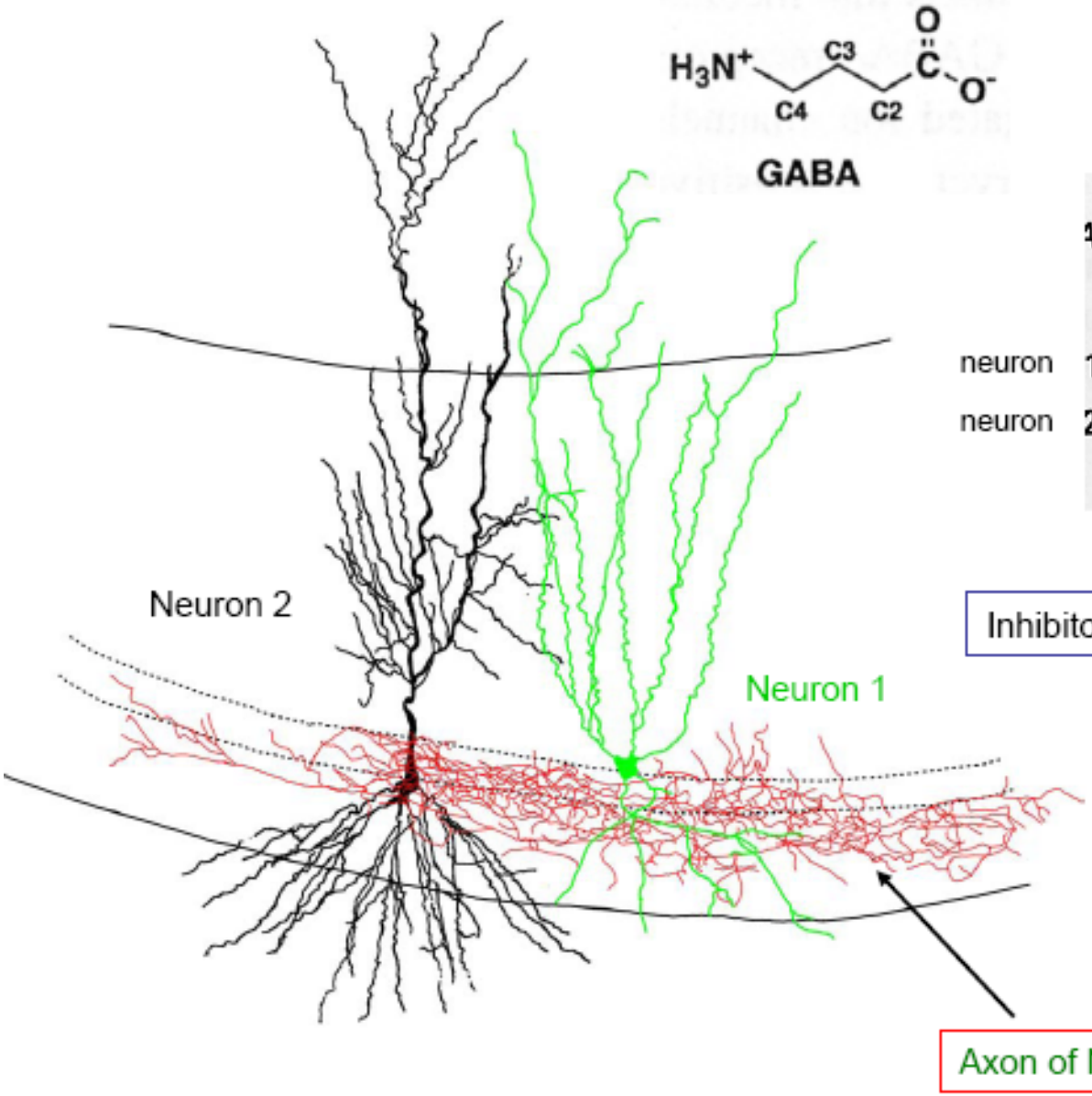
Inhibitory transmission

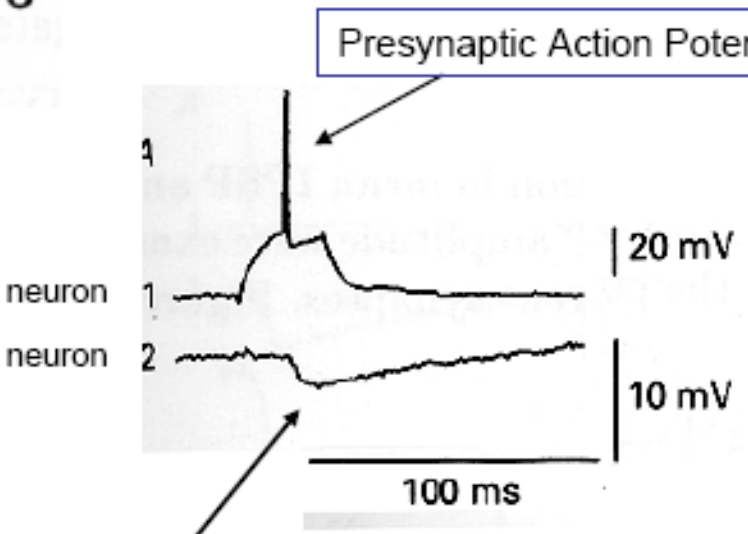


GABA metabolism

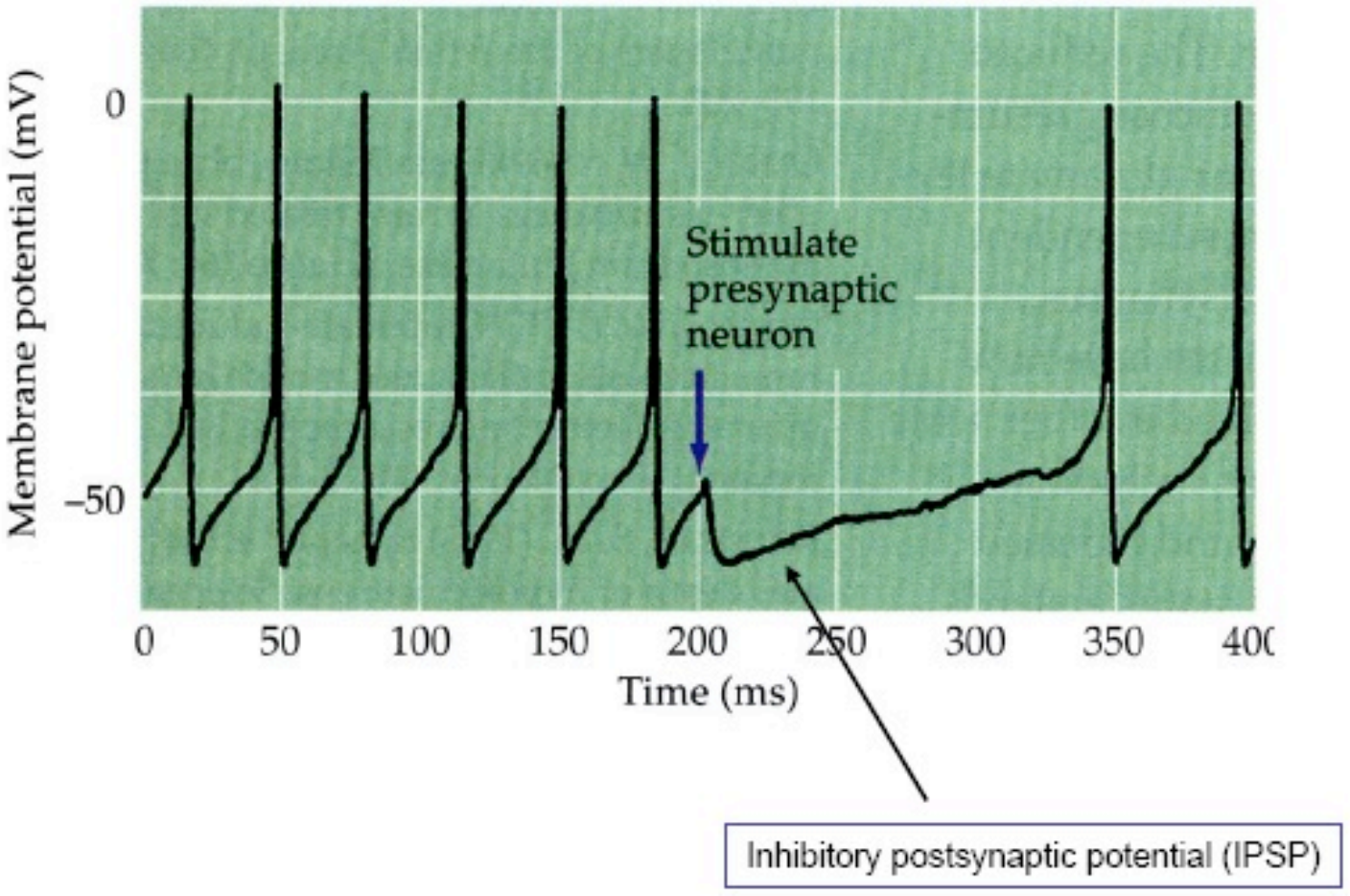


Fast GABAergic transmission



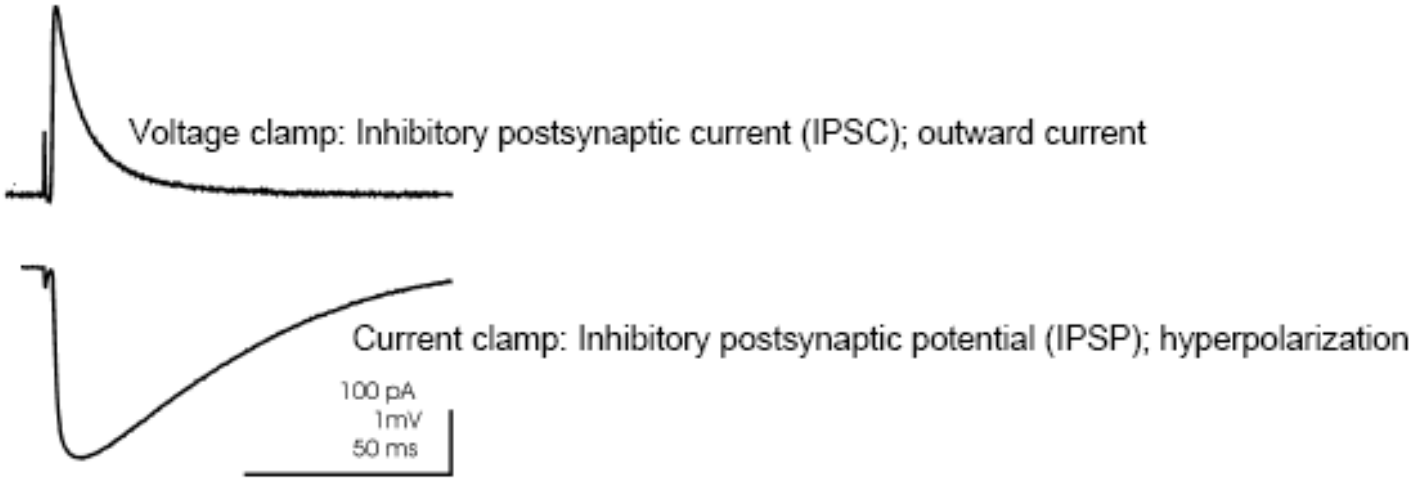


GABA action on neuronal firing



IPSPs are mediated by a Cl^- conductance : $GABA_A$ Receptor

At macroscopic level

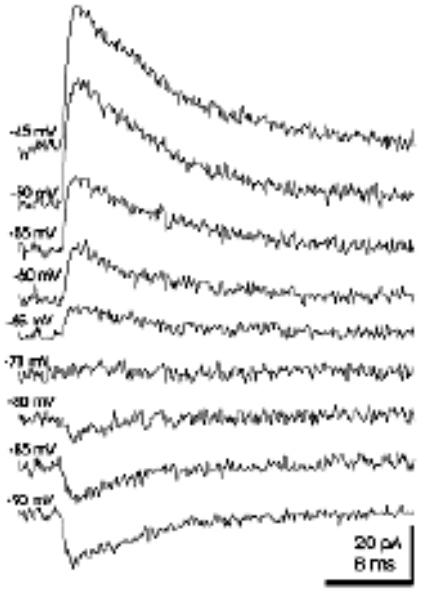


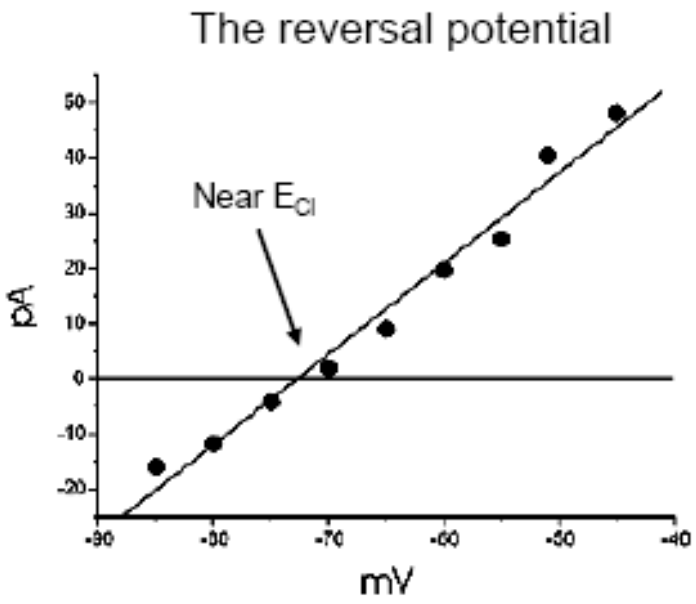
Influence of the postsynaptic membrane potential on GABAergic currents .... How does CL- move?

I-V curve

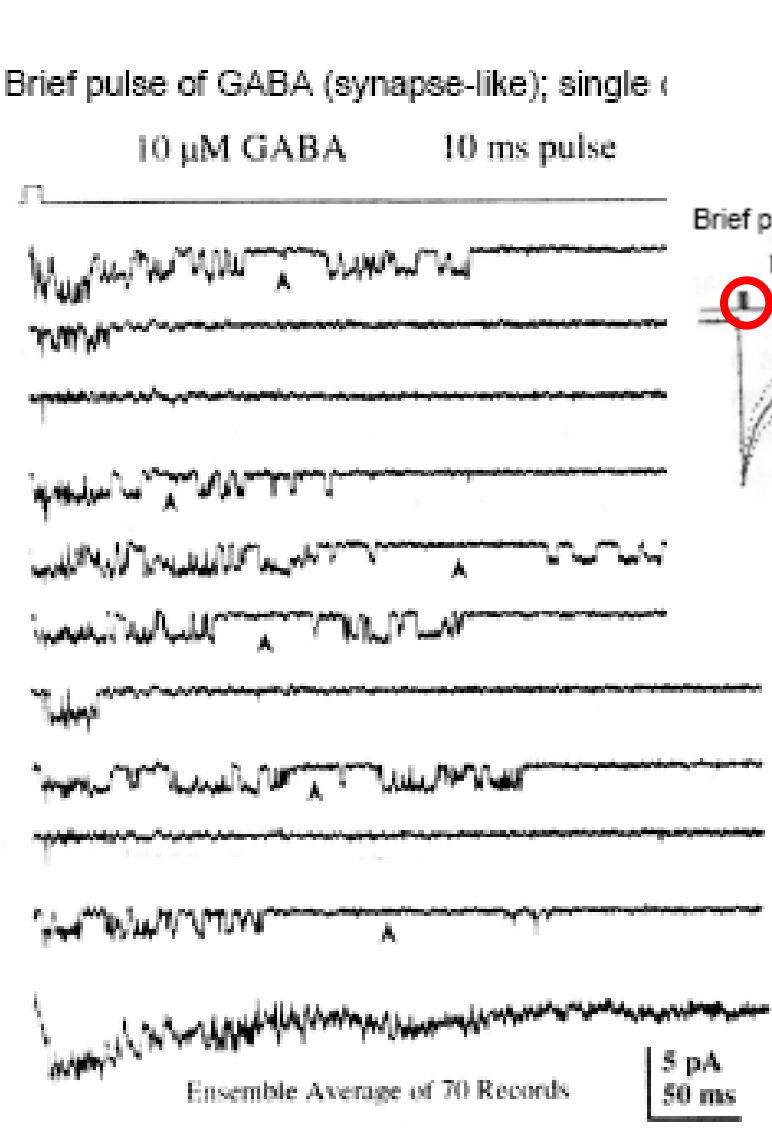
IPSCs

The postsynaptic neuron is Voltage-Clamped at different potentials



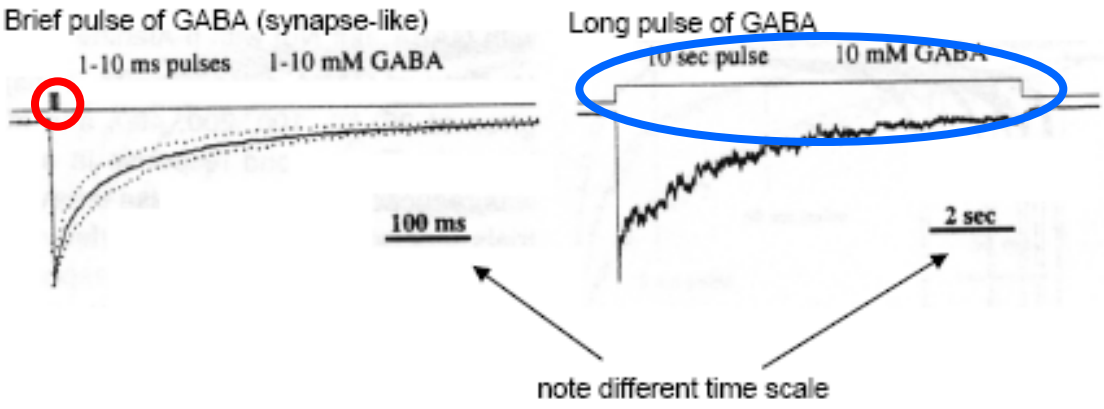


Kinetics of $GABA_A$ receptors



deactivation

desensitization

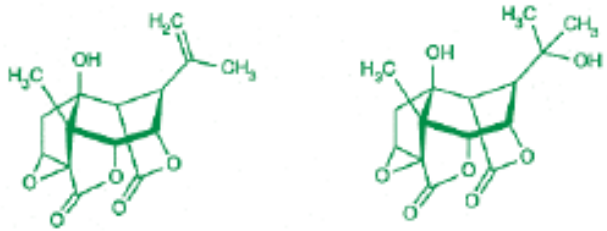


Antagonists of GABAA receptors lead to epileptic seizures

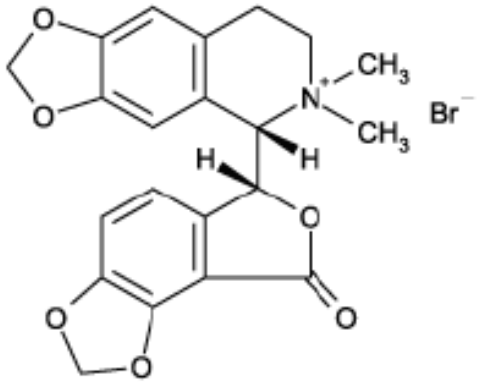
Anamirta cocculus

Dicentra cucullaria

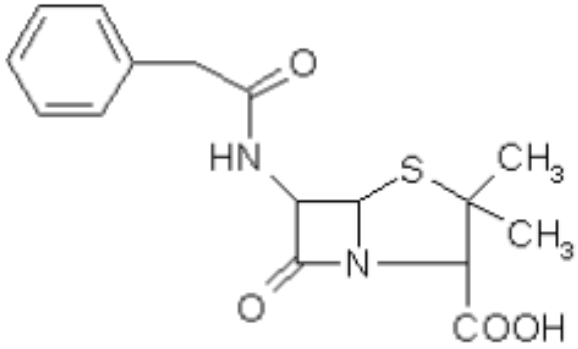
Penicillium notatum



Picrotoxin

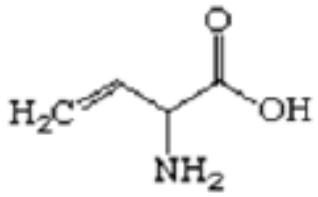


Bicuculline



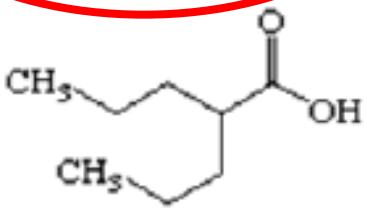
Penicillin

GAD (glutamic acid decarboxylase) catalyzes the formation of GABA from glutamic acid



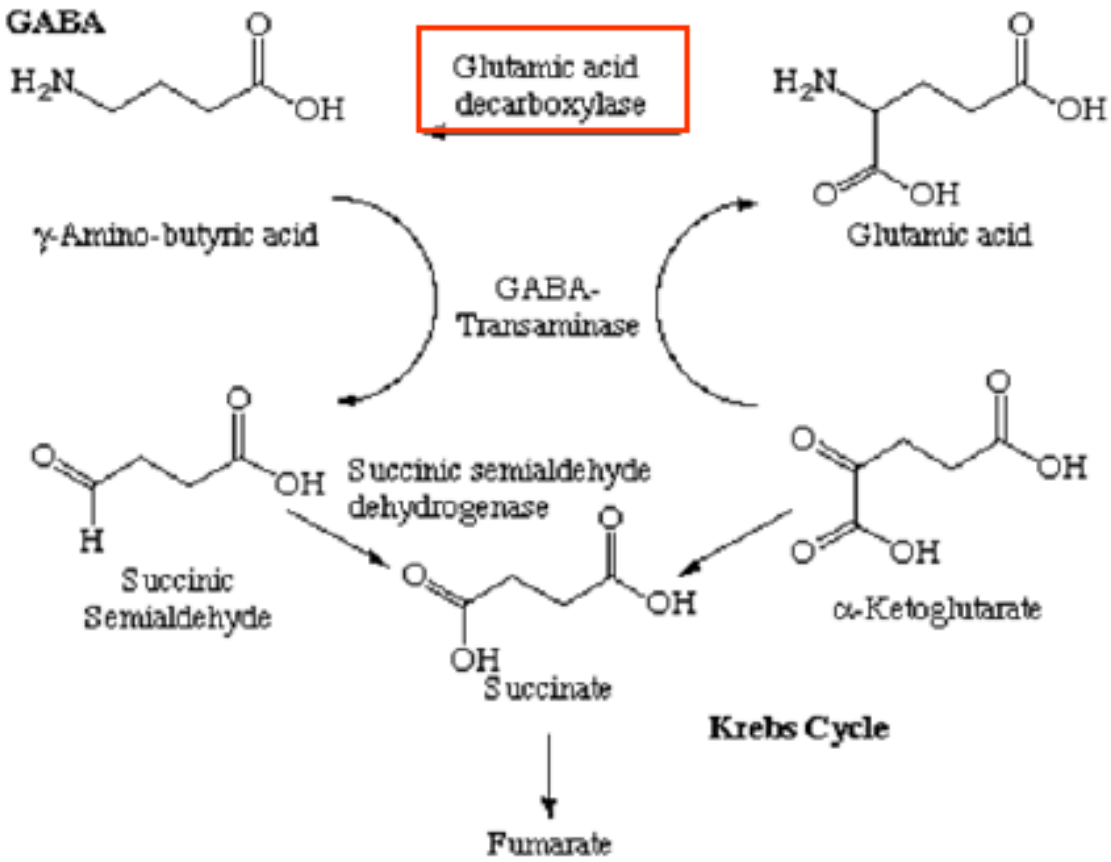
Allylglycine

The GAD inhibitor allylglycine, inhibit GABA formation and cause convulsions.

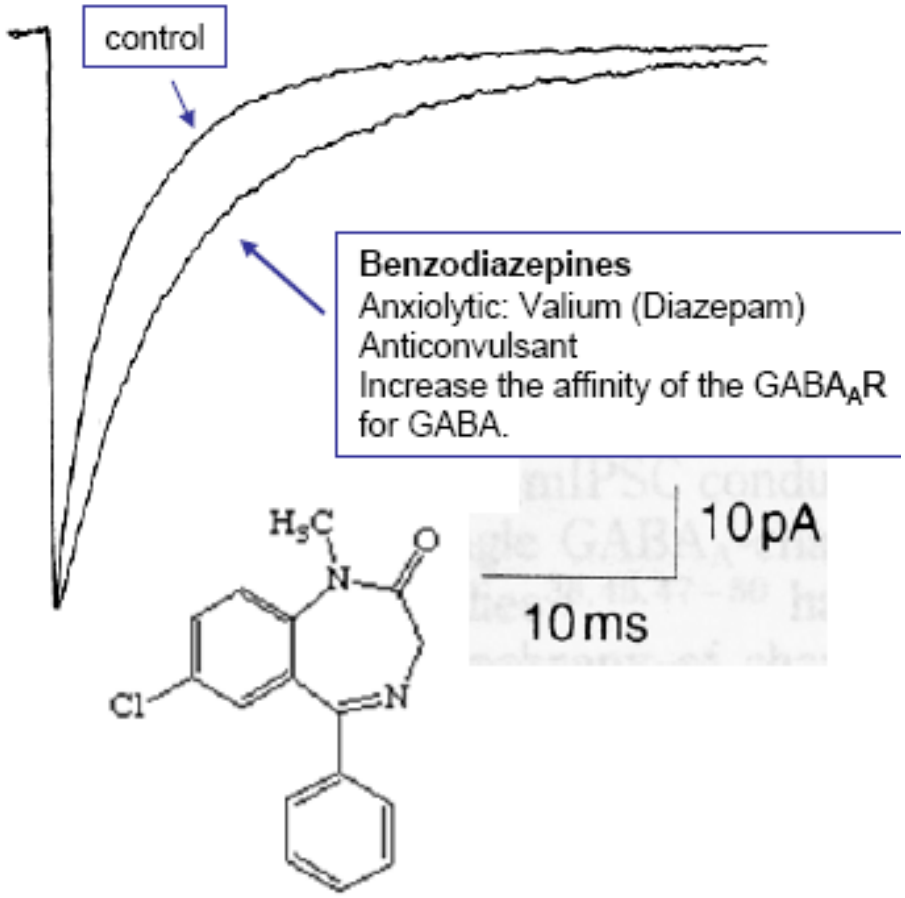


Valproic acid

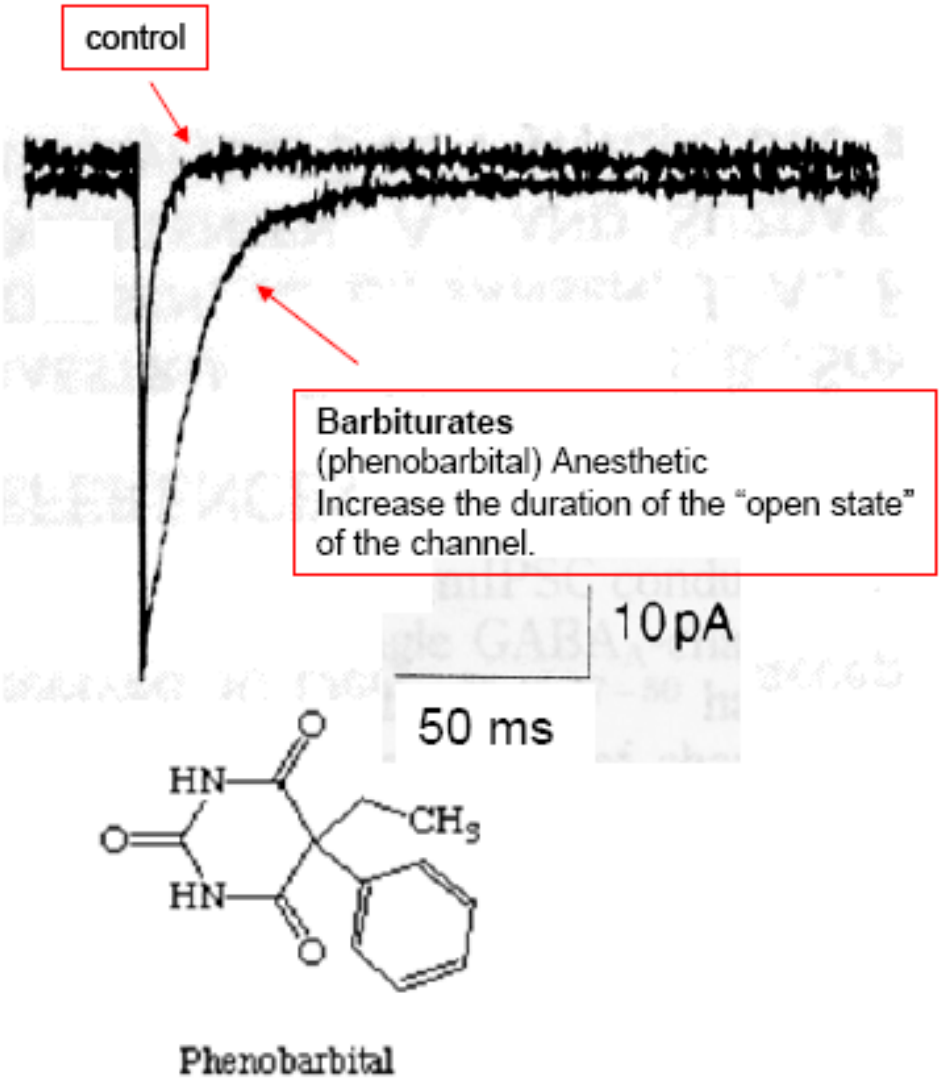
Sodium valproate (or valproic acid) blocks GABA transaminase activity, thereby elevating GABA levels. Treatment of Epilepsy.



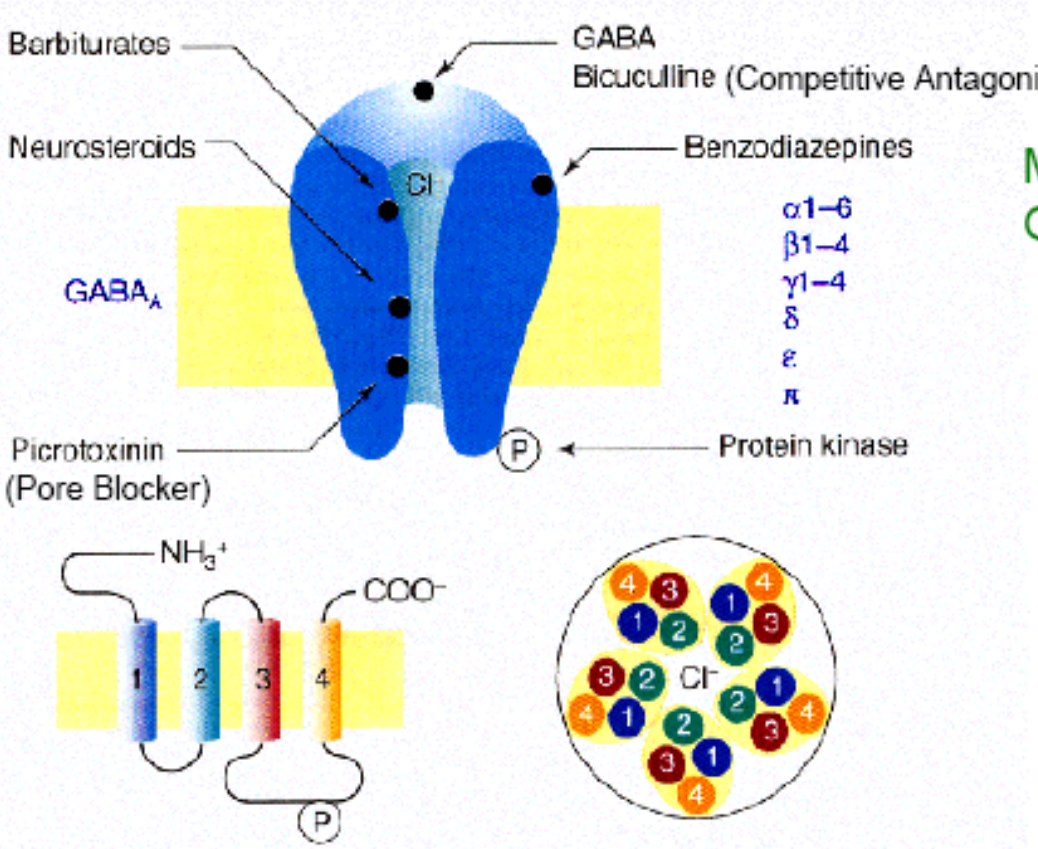
Allosteric modulators of GABAA receptors



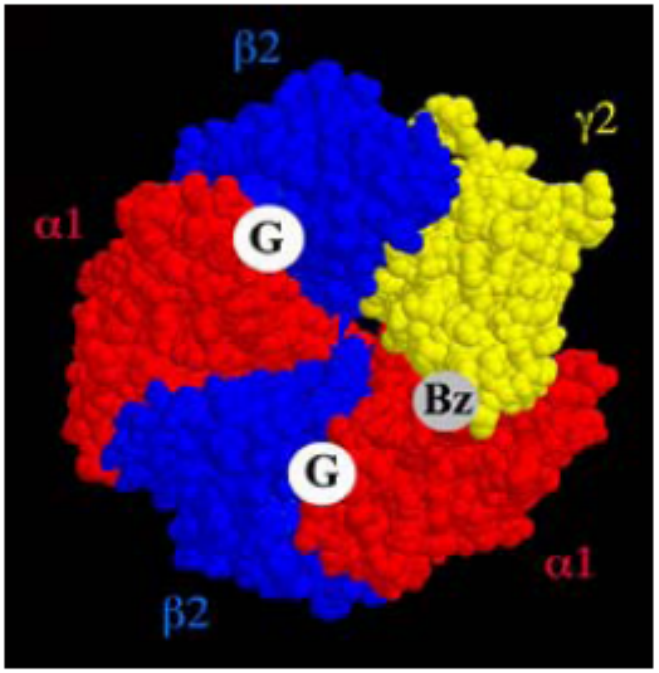
Allosteric modulators of GABAA receptors



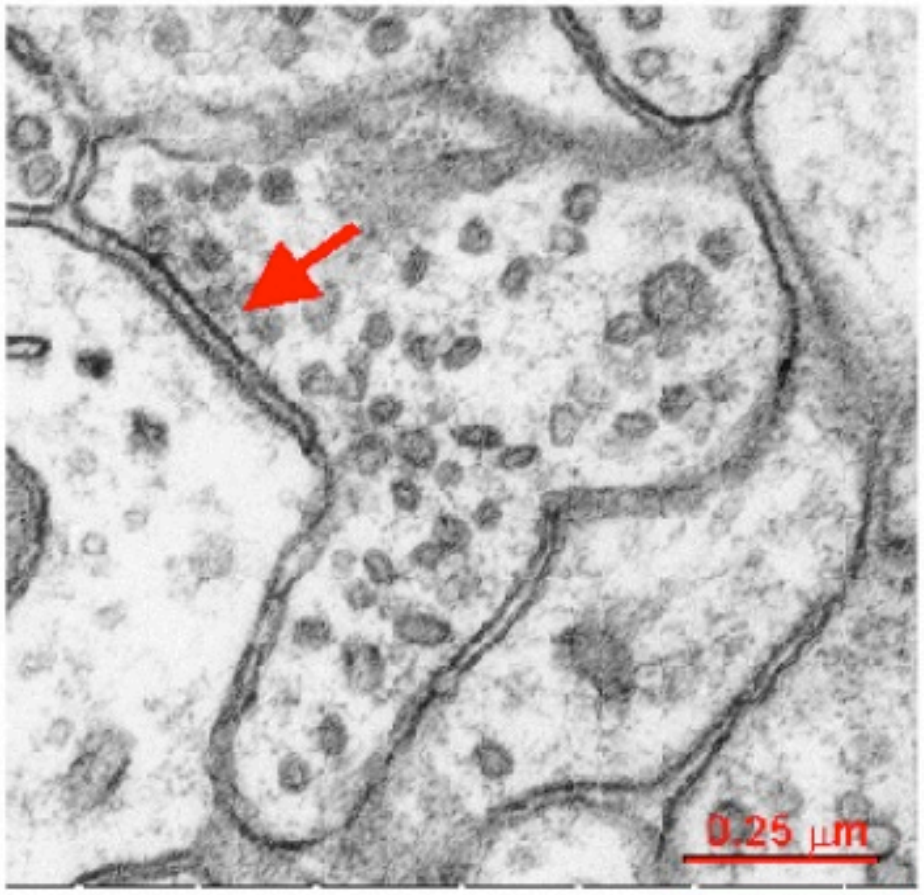
Structure of GABAA receptor



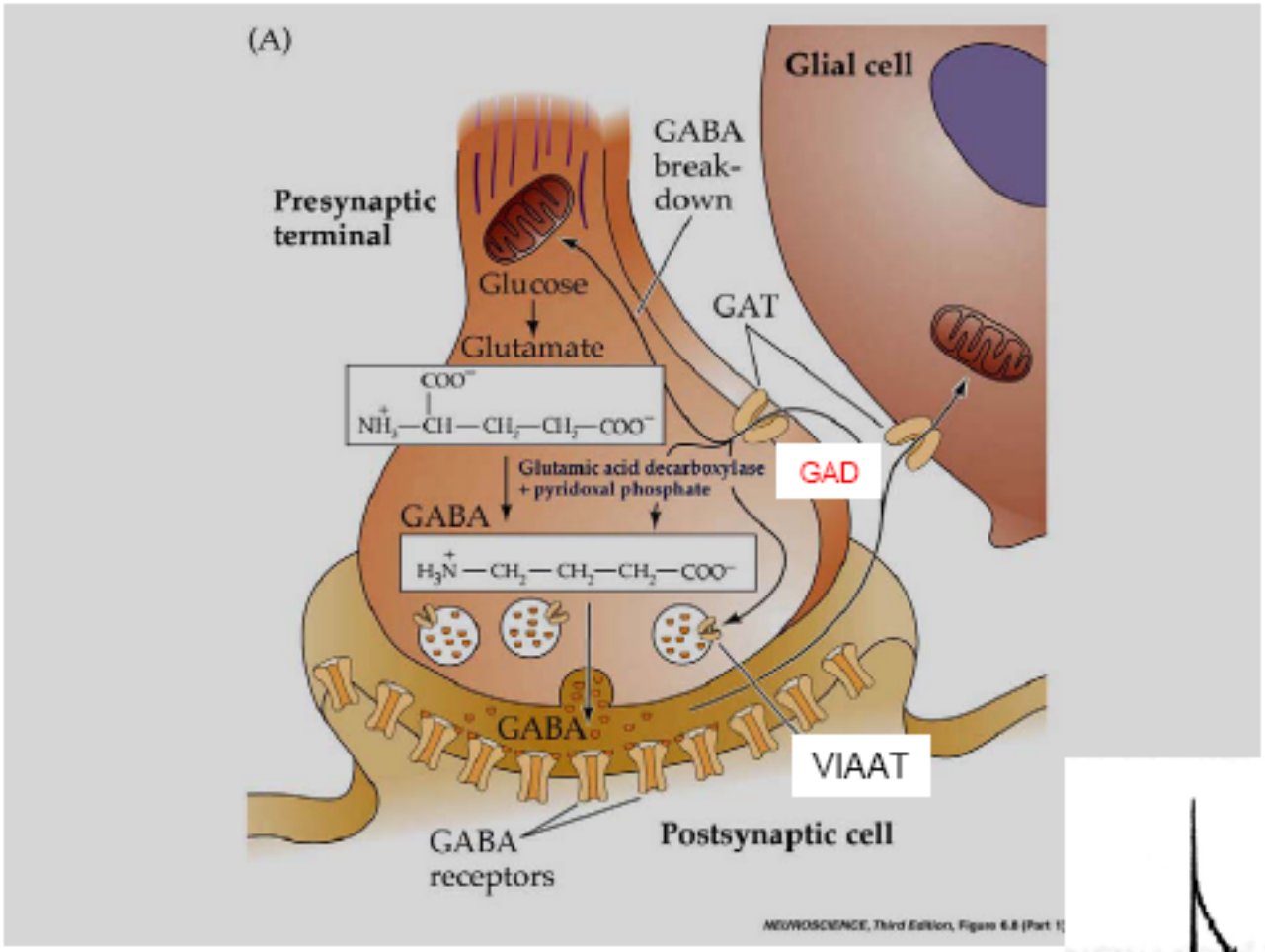
More than 20 genes known for GABAA R

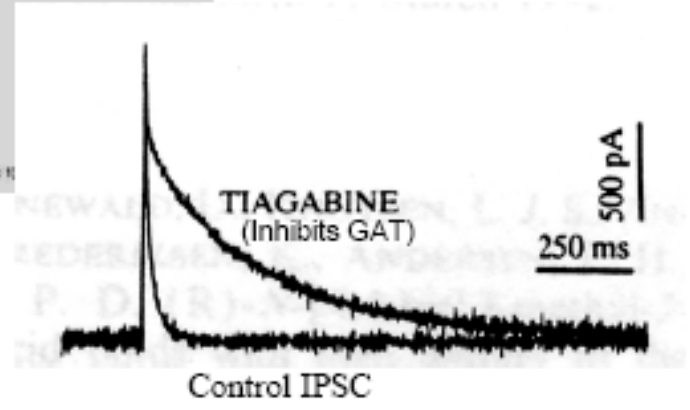


At the synaptic level, GABAA receptors are found in symmetric, inhibitory synapses (no postsynaptic density)



Synthesis and uptake of GABA in nerve endings

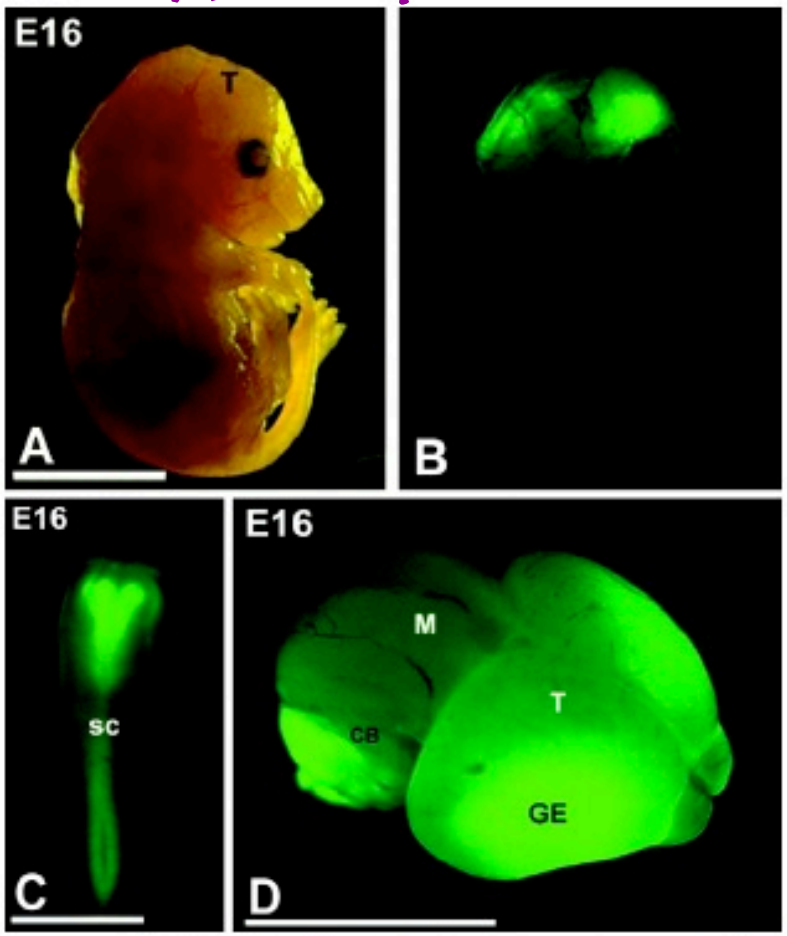


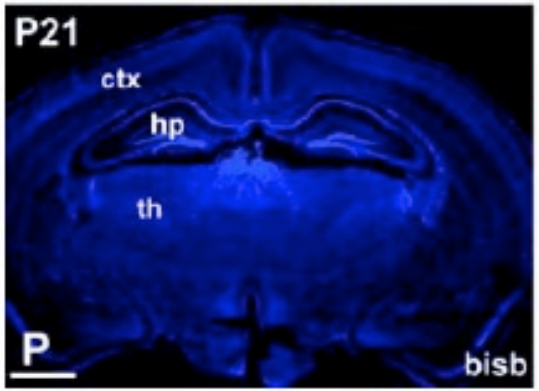


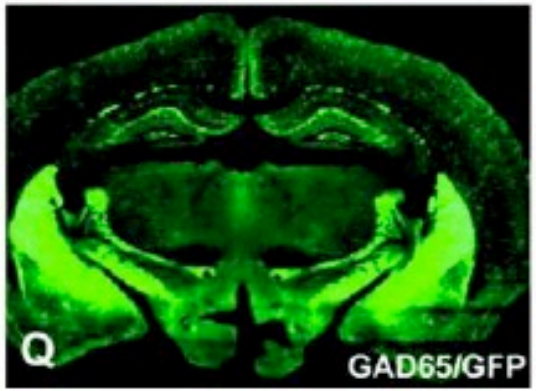
GAT: GABA Transporter
VIAAT (or VGAT): Vesicular Inhibitory Amino Acid Transporter
GAD: Glutamic Acid Decarboxylase

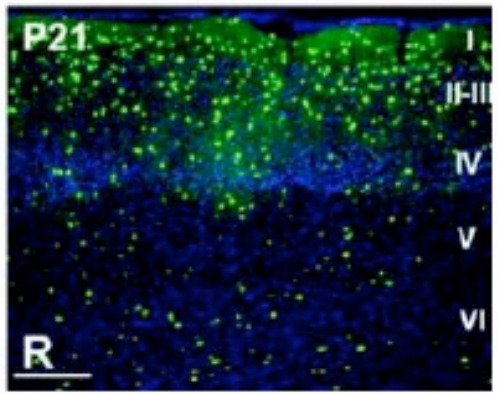
$GABA_A$ receptor distribution

A transgenic mouse that expresses the green fluorescent protein (GFP) in GAD positive neurons.





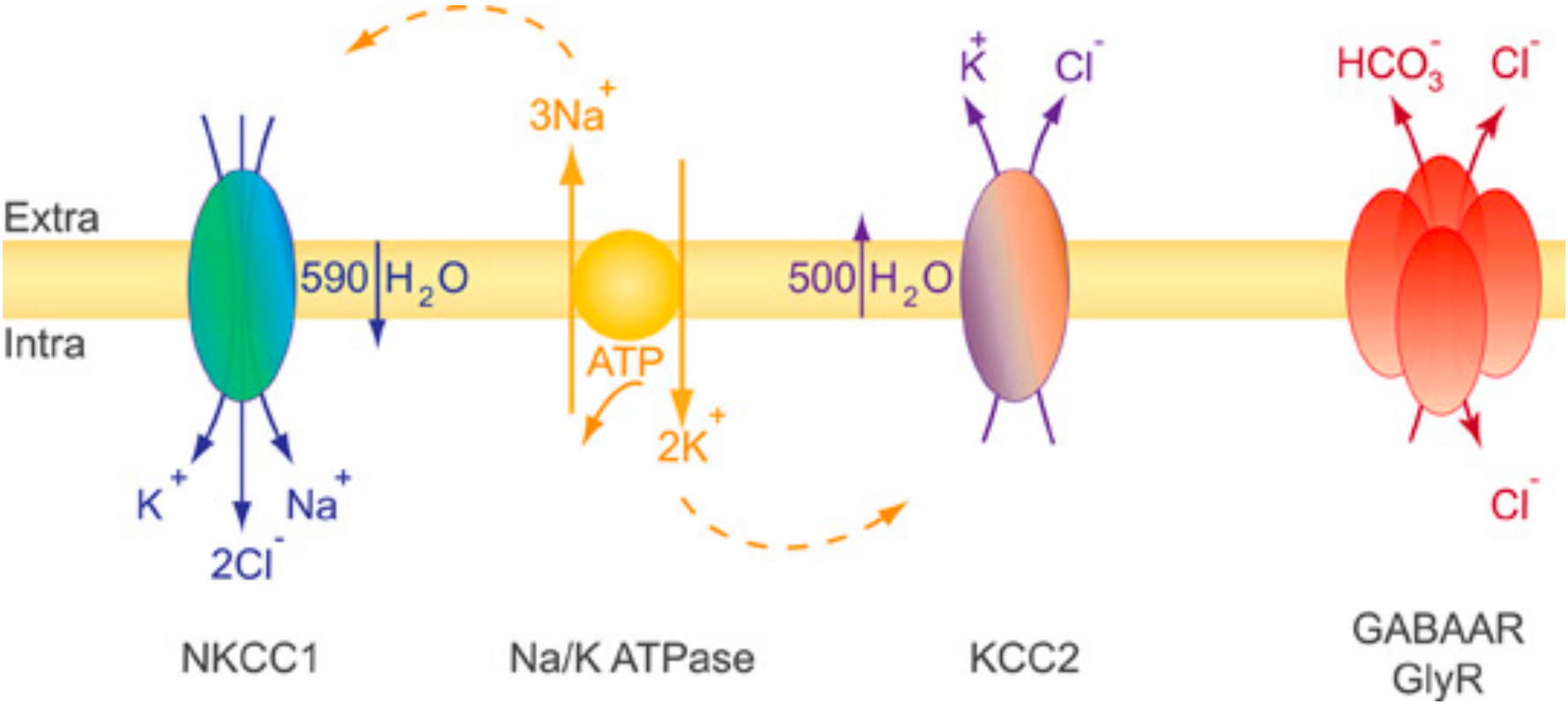




Paulsen 2004

How does Cl^- move?

Cl^- equilibrium



Cl^- equilibrium