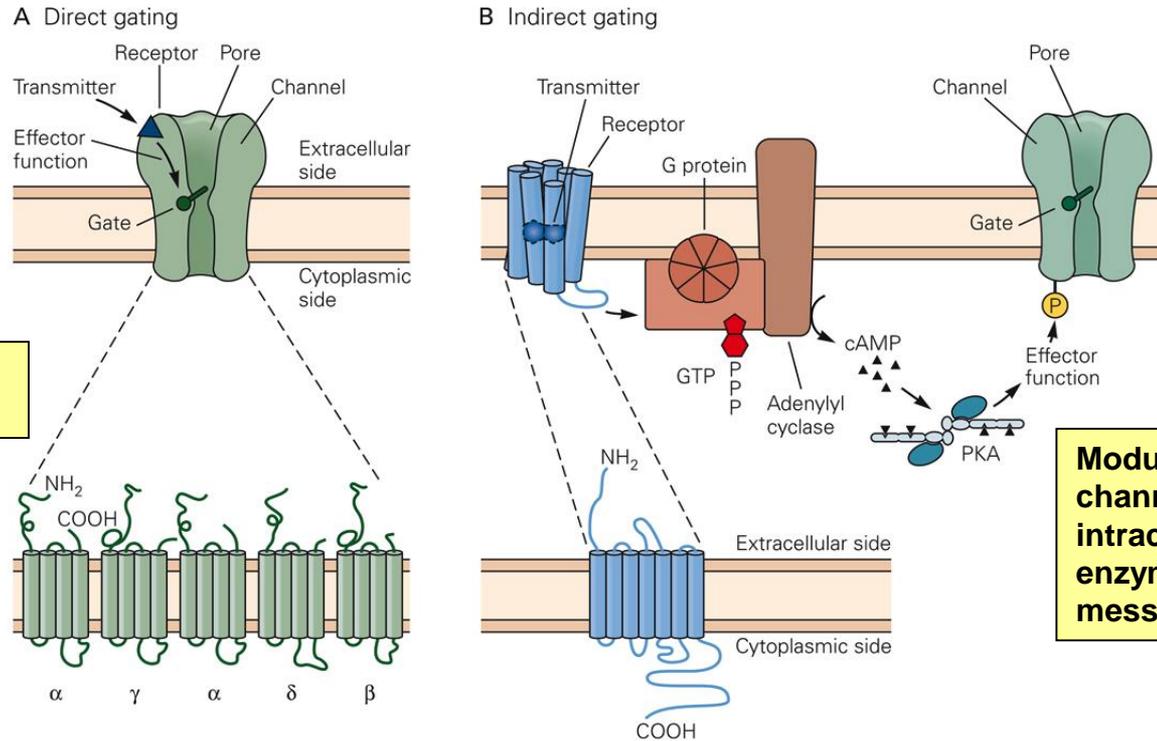


# Activation and signal transduction of neurotransmitter receptors



**Direct activation of an ion channel**

**Modulation of an ion channel through intracellular effector enzymes and second messengers**

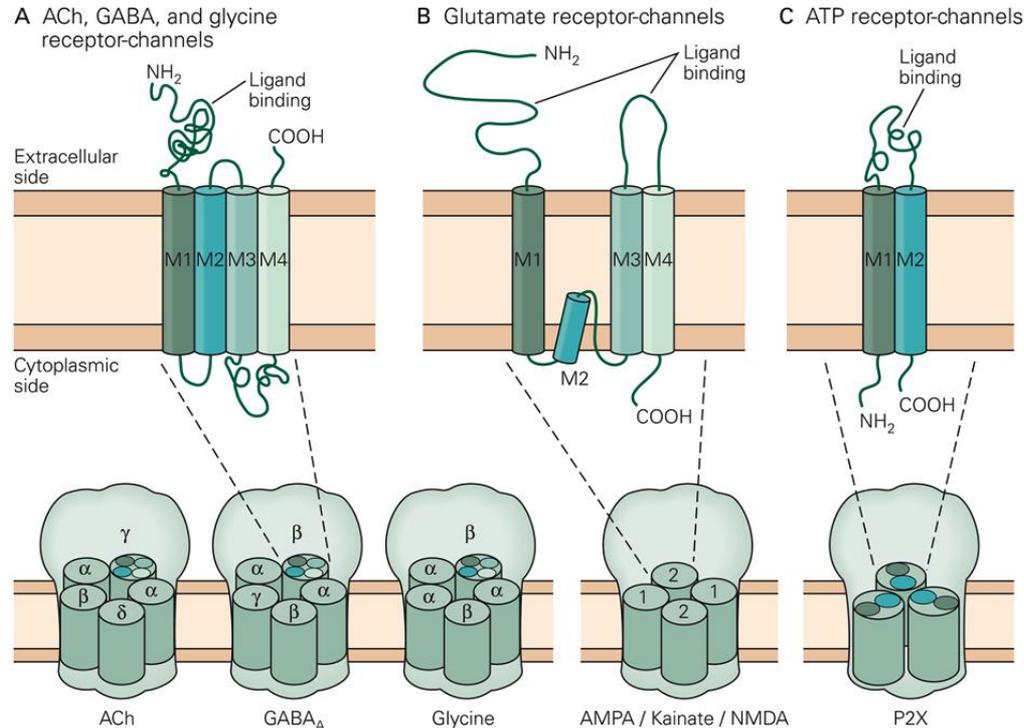
**Figure 8-9** Neurotransmitters open postsynaptic ion channels either directly or indirectly.

**A.** A receptor that directly opens ion channels is an integral part of the macromolecule that also forms the channel. Many such ligand-gated channels are composed of five subunits, each of which is thought to contain four membrane-spanning  $\alpha$ -helical regions.

**B.** A receptor that indirectly opens an ion channel is a distinct macromolecule separate from the channel it regulates. In one large family of such receptors, the receptors are composed of a

single subunit with seven membrane-spanning  $\alpha$ -helical regions that bind the ligand within the plane of the membrane. These receptors activate a guanosine triphosphate (GTP)-binding protein (G protein), which in turn activates a second-messenger cascade that modulates channel activity. In the cascade illustrated here the G protein stimulates adenylyl cyclase, which converts adenosine triphosphate (ATP) to cAMP. The cAMP activates the cAMP-dependent protein kinase (PKA), which phosphorylates the channel (P), leading to a change in function.

# The general architecture of ligand-gated ion channels



subunit

Oligomeric subunit assembly

**Figure 10-7** The three families of ligand-gated channels.

A. The nicotinic ACh, GABA<sub>A</sub>, and glycine receptor-channels are all pentamers composed of several types of related subunits. As shown here, the ligand-binding domain is formed by the extracellular amino-terminal region of the protein. Each subunit has a membrane domain with four membrane-spanning  $\alpha$ -helices (M1–M4) and a short extracellular carboxyl terminus. The M2 helix lines the channel pore.

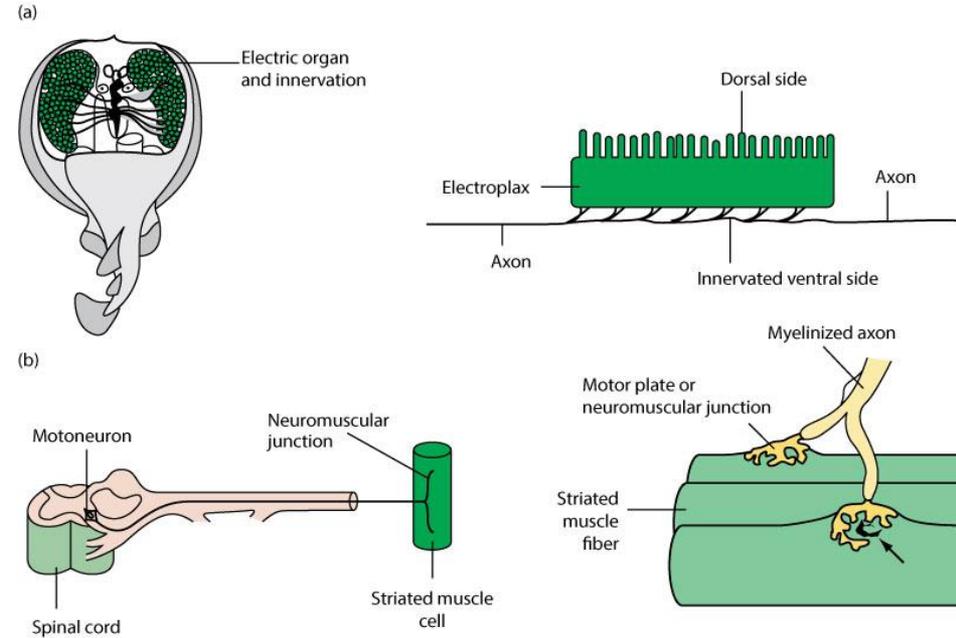
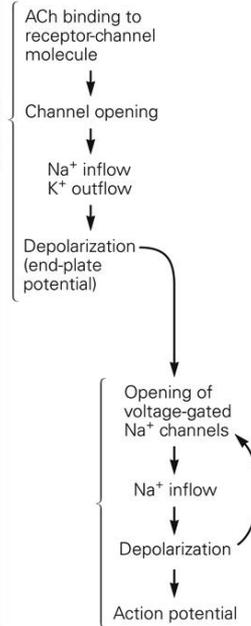
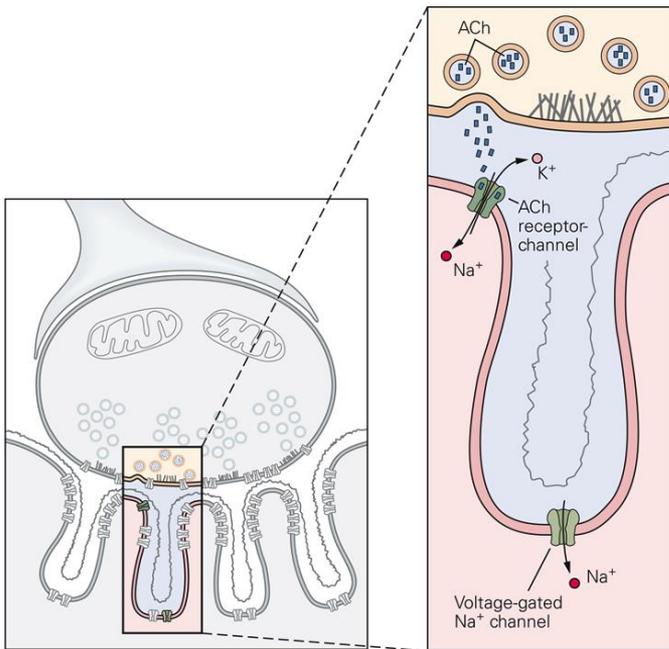
B. The glutamate receptor-channels are tetramers, often composed of two different types of closely related subunits (here denoted 1 and 2). The subunits have a large extracellular amino terminus, a membrane domain with three membrane-

spanning  $\alpha$ -helices (M1, M3, and M4), a large extracellular loop connecting the M3 and M4 helices, and an intracellular carboxyl terminus. The M2 segment forms a loop that dips into and out of the cytoplasmic side of the membrane, contributing to the selectivity filter of the channel. The glutamate binding site is formed by residues in the extracellular amino terminus and in the M3-M4 extracellular loop.

C. The ATP receptor-channels (or purinergic P2X receptors) are trimers. Each subunit possesses two membrane-spanning  $\alpha$ -helices (M1 and M2) and a large extracellular loop that binds ATP. The M2 helix lines the pore.

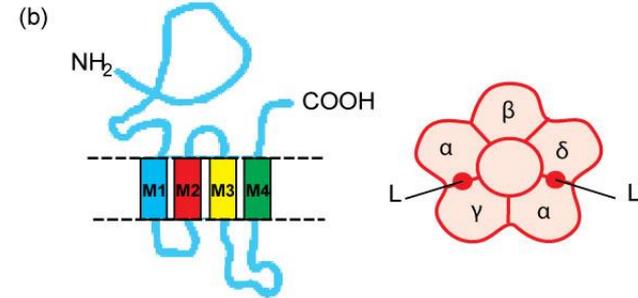
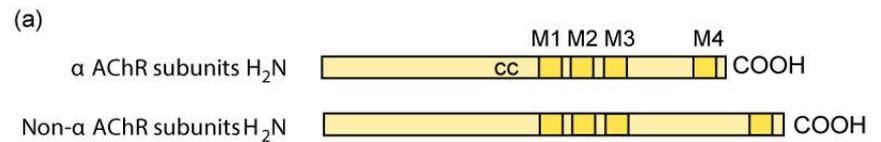
# The **nicotinic Acetylcholine receptor (nAChR)** is the best-studied of ionotropic receptors

**nAChR** is a cationic channel permeable to  $\text{Na}^+$  e  $\text{K}^+$



The **electric organ of the electric ray** is an example of preparation in which nicotinic receptors have been extensively studied. On a dissected *Torpedo* (left) we can see the electric organs and their innervation. These organs constitute electroplax membranes (right) which are modified muscle cells that do not contract. Nicotinic receptors are present on the ventral side of the postsynaptic membrane of the electroplax. The electroplax are simultaneously activated and the summation of their electric discharges can be of the order of 500 V.

# The nAChR of the neuromuscular junction is a cationic channel made of 5 subunits: (2) $\alpha$ , $\beta$ , $\gamma$ , $\delta$

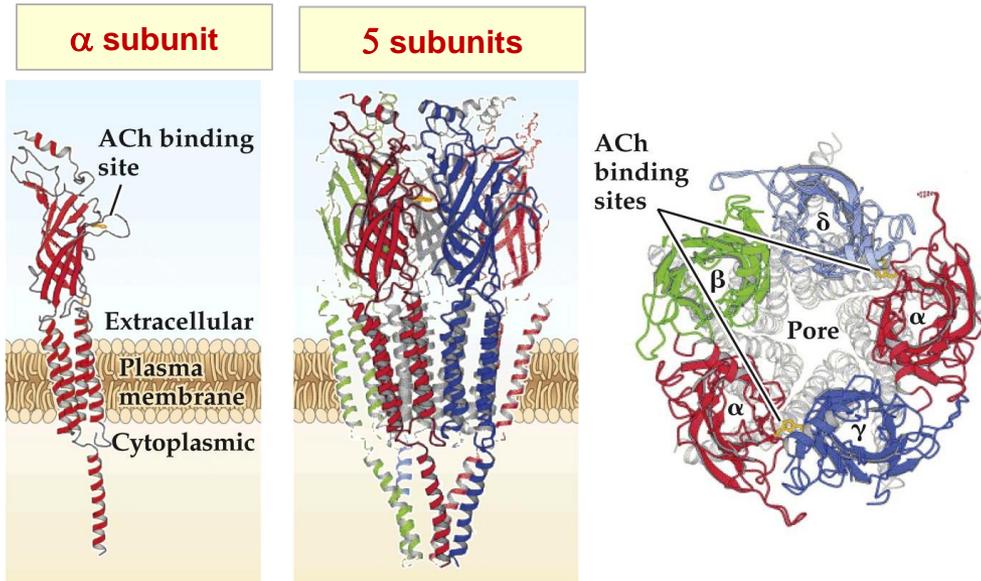


(a) Schematic representation of the primary sequence of the  $\alpha$ - ( $\alpha_1$ - $\alpha_9$ ) and non- $\alpha$ - ( $\beta_1$ - $\beta_4$ ,  $\gamma$ ,  $\epsilon$ , and  $\delta$ ) subunits of the nAChR. M1-M4, transmembrane domains; CC, Cys-Cys pair found in the  $\alpha$ -subunits from both muscle- and neuronal-type nAChRs.

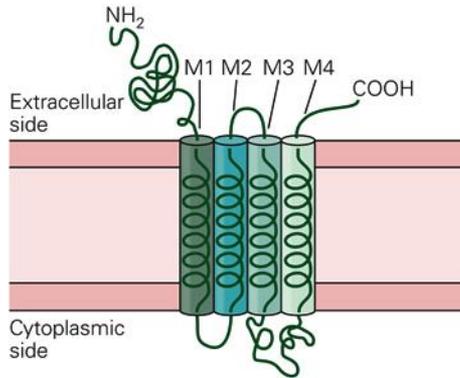
(b) Diagram of the tertiary organization of nAChRs. (Left) Schematic representation of the transmembrane organization of a single subunit. (Right) Schematic representation of the oligomeric organization of muscle-type nAChR. The pentameric nAChR is formed by two  $\alpha$ -subunits and three non- $\alpha$ -subunits. The two ligand binding sites (L) are located at the interfaces of one  $\alpha$ -subunit and one non- $\alpha$ -subunit.

(c) Architecture of the extracellular and transmembrane domains of the nAChR from electron micrographs at 4 Å (0.4 nm) resolution. Individual subunits are in different colours ( $\alpha$ , red;  $\beta$ , green;  $\gamma$ , blue;  $\delta$ , light blue). The extracellular domains are  $\beta$ -sandwiches formed from two anti-parallel  $\beta$ -sheets perpendicular to the membrane topped by an  $\alpha$ -helix. The transmembrane domains of each subunit contain four  $\alpha$ -helices. (Right) Plan view from the synaptic cleft. The five subunits form a ring. A water filled vestibule runs through the extracellular domains down to the channel and through the membrane. The four M2 domains (in bright colors) of the four subunits line the channel.

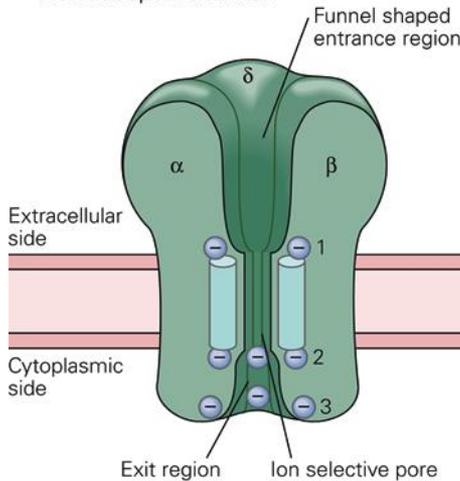
(c)



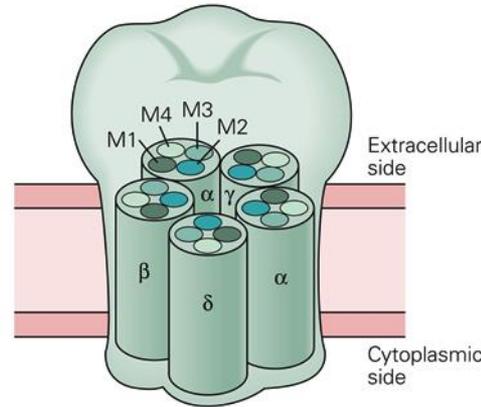
**A** A single subunit in the ACh receptor-channel



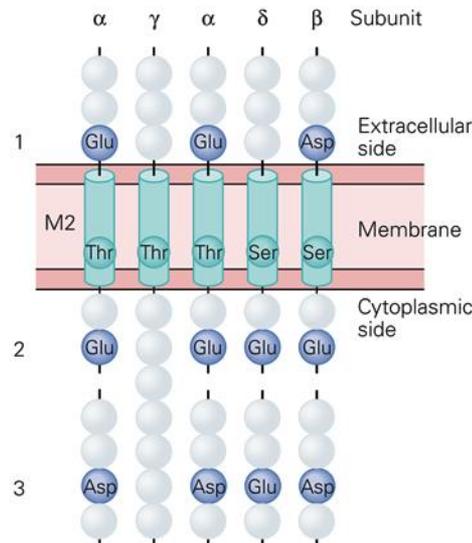
**C** Functional model of ACh receptor-channel



**B** Arrangement of subunits surrounding the channel pore



**D** Amino acid sequence of channel subunits



# Molecular bases of the selective ion permeability of nAChR

**Figure 9-14** The ACh receptor subunits are homologous membrane-spanning proteins.

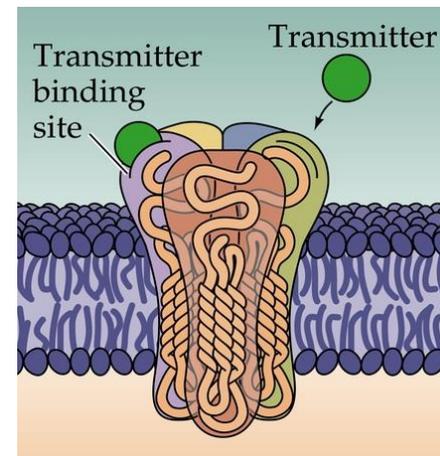
**A.** Each subunit contains a large extracellular N-terminus, four membrane-spanning  $\alpha$ -helices (M1–M4), and a short extracellular C-terminus. The N-terminus contains the ACh-binding site, and the membrane helices form the pore.

**B.** The five subunits are arranged such that they form a central aqueous channel, with the M2 segment of each subunit forming the lining of the pore. Note that the  $\gamma$ -subunit lies between the two  $\alpha$ -subunits. (Dimensions are not to scale.)

**C.** According to one model, negatively charged amino acids on each subunit form three rings of negative charge around the pore. As an ion traverses the channel it encounters these rings of charge. The rings at the external (1) and internal (3) surfaces of the cell membrane may serve as prefilters that help repel anions and form divalent cation blocking sites. The central ring near the cytoplasmic side of the membrane bilayer (2) may contribute more importantly to establishing the specific cation selectivity of the selectivity filter, which is the narrowest region of the pore.

**D.** The amino acid sequences of the M2 and flanking regions of each of the five subunits. The horizontal series of amino acids numbered 1, 2, and 3 constitute the three rings of negative charge (part C). The aligned serine and threonine residues within M2 help form the selectivity filter.

# Each ionotropic receptor subtype is made of a combination of different subunits

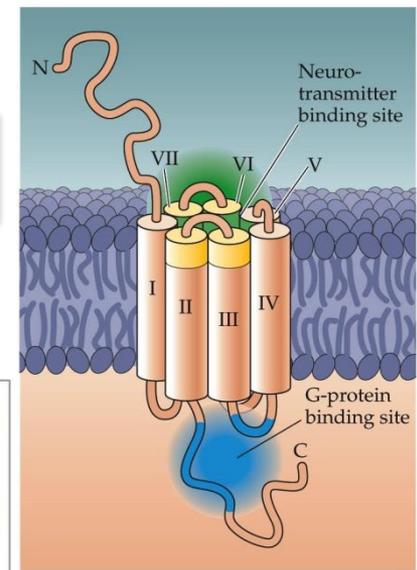


Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nACh	Serotonin	Purines
Subunits (combination of 4 or 5 required for each receptor type)	GluA1	GluN1	GluK1	$\alpha_{1-6}$	$\alpha_{1-6}$	$\alpha_{1-10}$	5-HT <sub>3A</sub>	P <sub>2X1</sub>
	GluA2	GluN2A	GluK2	$\beta_{1-3}$	$\beta$	$\beta_{1-4}$	5-HT <sub>3B</sub>	P <sub>2X2</sub>
	GluA3	GluN2B	GluK3	$\gamma_{1-3}$		$\gamma$	5-HT <sub>3C</sub>	P <sub>2X3</sub>
	GluA4	GluN2C	GluK4	$\delta$		$\delta$	5-HT <sub>3D</sub>	P <sub>2X4</sub>
		GluN2D	GluK5	$\epsilon$		$\epsilon$	5-HT <sub>3E</sub>	P <sub>2X5</sub>
		GluN3A		$\rho_{1-3}$				P <sub>2X6</sub>
		GluN3B		$\theta$				P <sub>2X7</sub>
				$\eta$				

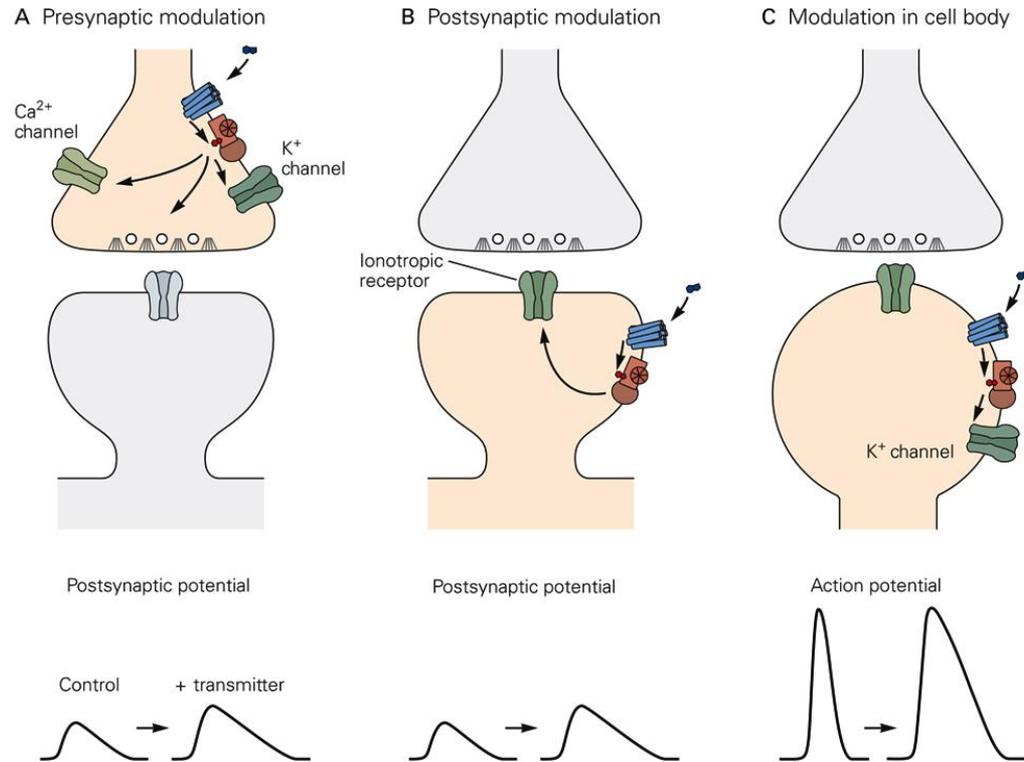
# Multiplicity of metabotropic receptors

(C)

Receptor class	Muscarinic	Glutamate	GABA <sub>B</sub>	Dopamine	Adrenergic	Histamine	Serotonin	Purines
Receptor subtype	M <sub>1</sub>	Class I	GABA <sub>B1</sub>	D <sub>1</sub>	Alpha	H <sub>1</sub>	5-HT <sub>1A</sub>	Adenosine
	M <sub>2</sub>	mGlu <sub>1</sub>	GABA <sub>B2</sub>	D <sub>2</sub>	α <sub>1A</sub>	H <sub>2</sub>	5-HT <sub>1B</sub>	A <sub>1</sub>
	M <sub>3</sub>	mGlu <sub>5</sub>		D <sub>3</sub>	α <sub>1B</sub>	H <sub>3</sub>	5-HT <sub>1D</sub>	A <sub>2A</sub>
	M <sub>4</sub>	Class II		D <sub>4</sub>	α <sub>1D</sub>	H <sub>4</sub>	5-HT <sub>1E</sub>	A <sub>2B</sub>
	M <sub>5</sub>	mGlu <sub>2</sub>		D <sub>5</sub>	α <sub>2A</sub>		5-HT <sub>1F</sub>	A <sub>3</sub>
		mGlu <sub>3</sub>			α <sub>2B</sub>		5-HT <sub>2A</sub>	P2Y
		Class III			α <sub>2C</sub>		5-HT <sub>2B</sub>	P2Y <sub>1</sub>
		mGlu <sub>4</sub>			Beta		5-HT <sub>2C</sub>	P2Y <sub>2</sub>
		mGlu <sub>6</sub>			β <sub>1</sub>		5-HT <sub>4</sub>	P2Y <sub>4</sub>
		mGlu <sub>7</sub>			β <sub>2</sub>		5-HT <sub>5A</sub>	P2Y <sub>6</sub>
		mGlu <sub>8</sub>			β <sub>3</sub>		5-HT <sub>6</sub>	P2Y <sub>11</sub>
							5-HT <sub>7</sub>	P2Y <sub>12</sub>
								P2Y <sub>13</sub>
								P2Y <sub>14</sub>



# At synapses metabotropic receptors have mostly a modulatory role



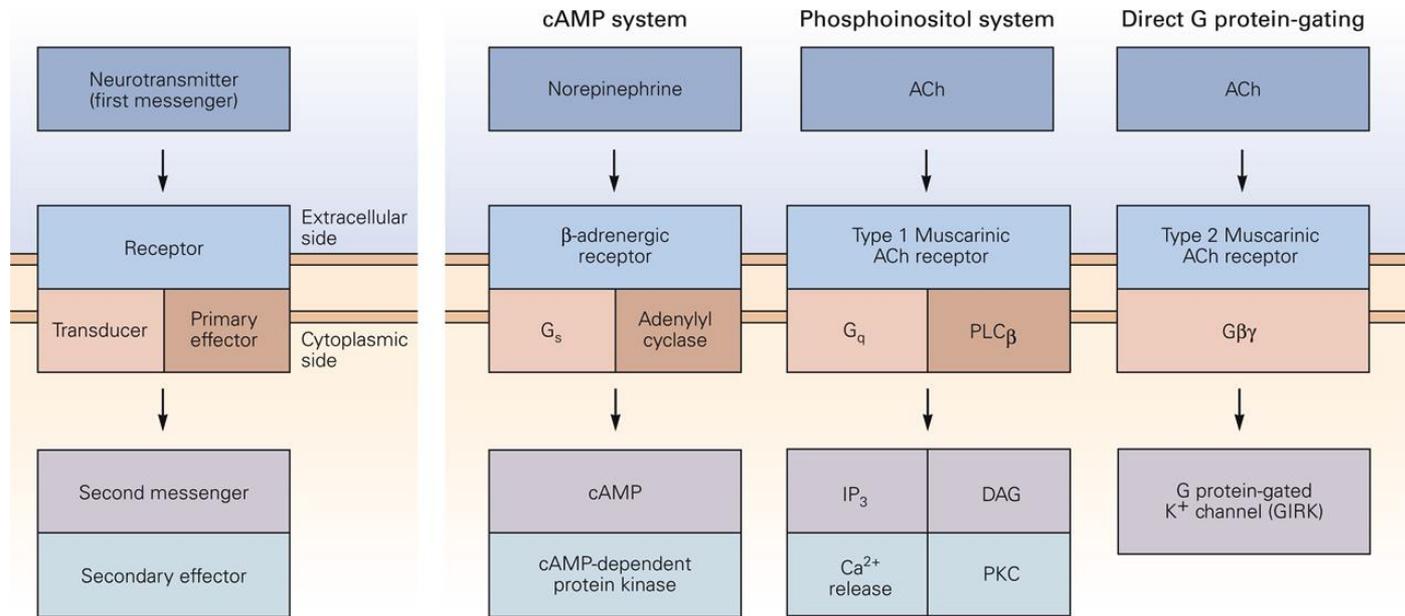
**Figure 11–10** The modulatory actions of second messengers can occur at three cellular sites.

A. In the presynaptic neuron second messengers can modulate the activity of  $K^+$  and  $Ca^{2+}$  channels, as well as the transmitter release machinery, to regulate the efficacy of transmitter release and thus the size of the fast postsynaptic potential mediated by ionotropic receptors.

B. In the postsynaptic neuron second messengers can alter directly the amplitude of postsynaptic potentials by modulating ionotropic receptors.

C. Second messengers can also affect the function of resting and voltage-gated channels in the soma and dendrites, thus altering a variety of electrical properties of the cell, including resting potential, input resistance, length and time constants, threshold, and action potential duration (as illustrated here).

# Metabotropic receptors transduction mechanisms



**Figure 11-5** Synaptic second-messenger systems involving G protein coupling follow a common sequence. The signal transduction pathways illustrated here involve similar steps (left). Chemical transmitters arriving at receptor molecules in the plasma membrane activate a closely related family of G proteins (the transducers) that activate different enzymes or channels (the primary effectors). The activated enzymes produce a second messenger that activates a secondary effector or acts directly on a target (or regulatory) protein.

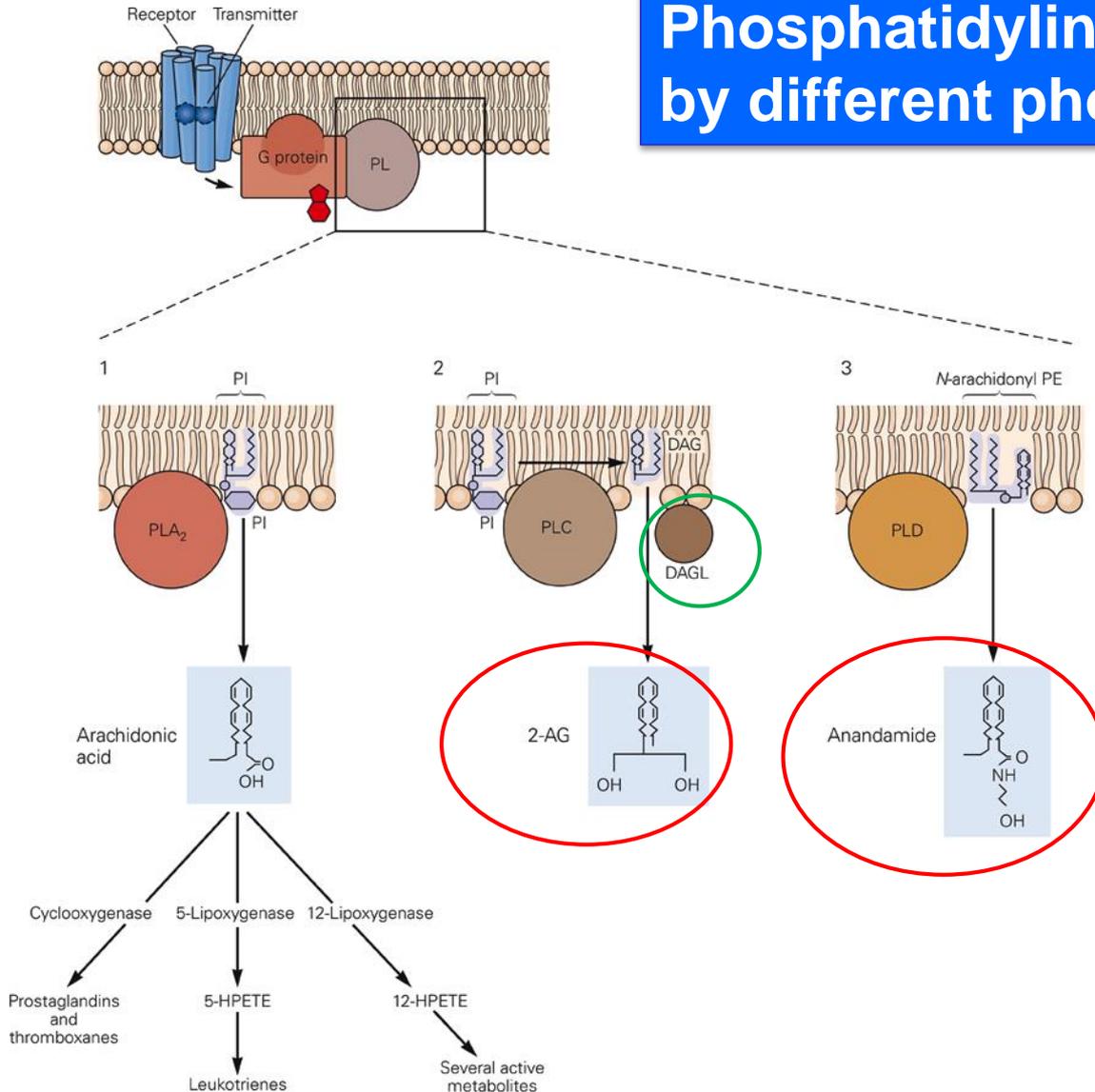
**cAMP system.** This pathway can be activated by a transmitter-bound  $\beta$ -adrenergic receptor, which acts through the  $G_s$  protein  $\alpha_s$ -subunit to activate adenylyl cyclase. Adenylyl cyclase produces the second messenger cAMP, which activates PKA. The G protein here is termed  $G_s$  because it stimulates the cyclase. Some receptors activate a  $G_i$  protein that inhibits the cyclase.

**Phosphoinositol system.** This pathway, activated by a type 1 muscarinic acetylcholine (ACh) receptor, uses the  $G_q$  or  $G_{11}$  type

of G protein (with  $\alpha_q$ - or  $\alpha_{11}$ -subunits, respectively) to activate a primary effector, phospholipase C $\beta$  ( $PLC\beta$ ). This enzyme hydrolyzes the phospholipid, phosphatidylinositol 4,5-bisphosphate ( $PIP_2$ ), yielding a pair of second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ( $IP_3$ ). In turn,  $IP_3$  releases  $Ca^{2+}$  from internal stores, whereas DAG activates protein kinase C (PKC). The drop in membrane  $PIP_2$  levels can directly alter the activity of some ion channels.

**Direct G protein-gating.** This pathway represents the simplest synaptic mechanism for G protein-coupled receptor action. Acetylcholine (ACh) acting on type 2 muscarinic receptors activates the  $G_i$  protein, leading to functional dissociation of the  $\alpha_i$ -subunit and  $\beta\gamma$  complex. The  $\beta\gamma$  complex interacts directly with a G protein-gated inward-rectifying  $K^+$  channel (GIRK), leading to channel opening and membrane hyperpolarization.

# Phosphatidylinositide hydrolysis by different phospholipases



**Figure 11-7** Three phospholipases generate distinct second messengers by hydrolysis of phospholipids containing arachidonic acid.

**Pathway 1.** Stimulation of G protein-coupled receptors leads to activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) by the free βγ subunit complex. Phospholipase A<sub>2</sub> hydrolyzes phosphatidylinositol (PI) in the plasma membrane, leading to the release of arachidonic acid, a 20-carbon fatty acid that is a component of many phospholipids. Once released, arachidonic acid is metabolized through several pathways, three of which are shown. The 12- and 5-lipoxygenase pathways both produce several active metabolites; the cyclooxygenase pathway produces prostaglandins and thromboxanes. Cyclooxygenase is inhibited by indomethacin, aspirin, and other nonsteroidal anti-inflammatory drugs. Arachidonic acid and many of its metabolites modulate the activity of certain ion channels.

**Pathway 2.** Other G proteins activate phospholipase C (PLC), which hydrolyzes PI in the membrane to generate DAG (see Figure 11-6). Hydrolysis of DAG by a second enzyme, diacylglycerol lipase (DAGL), leads to production of 2-arachidonylglycerol (2-AG), an endocannabinoid that is released from neuronal membranes and then activates G protein-coupled endocannabinoid receptors in the plasma membrane of other neighboring neurons.

**Pathway 3.** Elevation of intracellular Ca<sup>2+</sup> activates phospholipase D (PLD), which hydrolyzes phospholipids that have an unusual polar head group containing arachidonic acid (*N*-arachidonylphosphatidylethanolamine [*N*-arachidonyl PE]). This action generates a second endocannabinoid termed anandamide (arachidonylethanolamide). (HPETE, hydroperoxyeicosatetraenoic acid.)

# The GABA<sub>A</sub> receptor

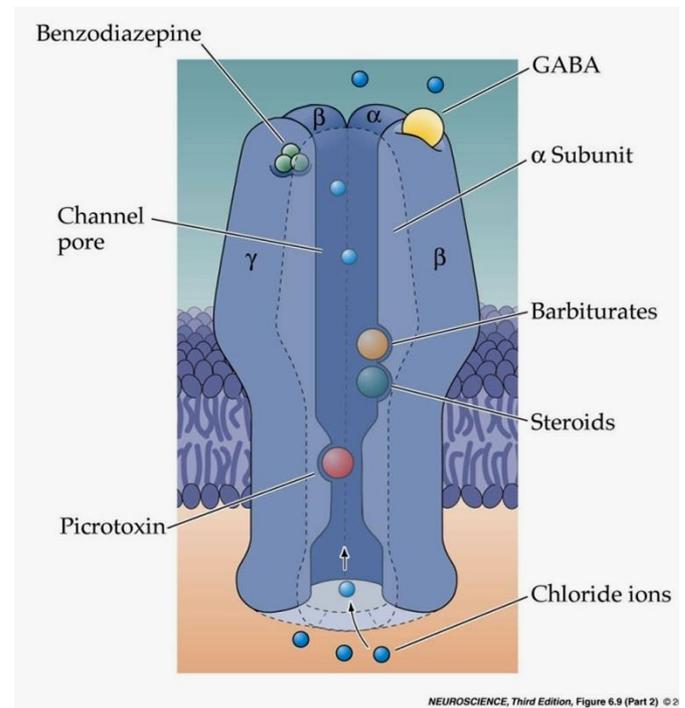
Receptor activity is modulated by a series of molecules (drugs such as sedatives, anxiolitics, anesthetics, ...)

Benzodiazepines positively modulates GABA<sub>A</sub> receptors

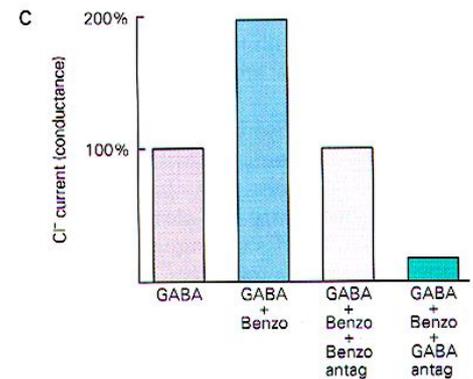
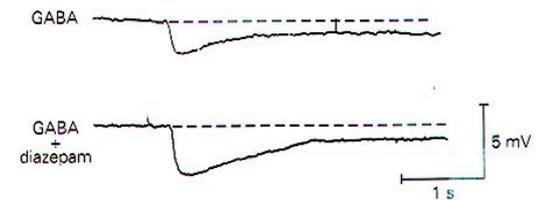
A. Structural model of the GABA<sub>A</sub> chloride channel. The channel protein contains at least five different subunit types, of which only three are illustrated here ( $\alpha$ ,  $\beta$ ,  $\gamma$ ). Benzodiazepines bind to the  $\gamma$  subunits, GABA to the  $\alpha$  subunit, and barbiturates to the  $\beta$  subunit. All the subunits contribute to forming the Cl<sup>-</sup> channel. When GABA binds to GABA<sub>A</sub> receptors the Cl<sup>-</sup> channels open and the influx of Cl<sup>-</sup> hyperpolarizes the cell.

B. Diazepam, a benzodiazepine, is an effective drug in treating generalized anxiety disorders. The traces compare the responses of a mouse spinal cord neuron to GABA, the major inhibitory neurotransmitter in the brain, and to GABA in the presence of diazepam. Diazepam increases the affinity of the receptor for GABA and thus increases the Cl<sup>-</sup> conductance and the hyperpolarizing current.

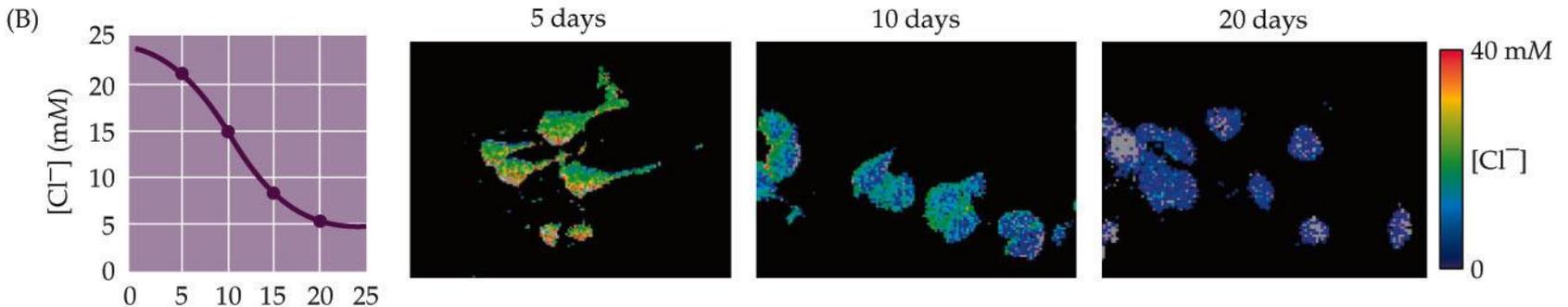
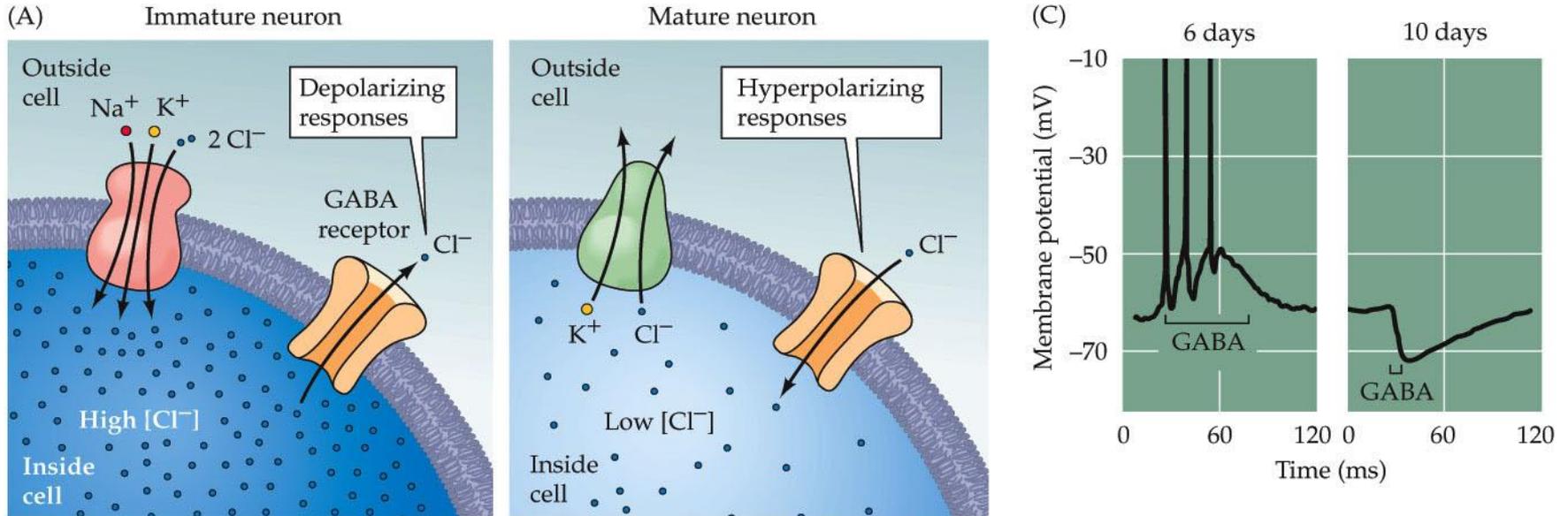
C. Benzodiazepine (Benzo) modulates Cl<sup>-</sup> flux through the channel by enhancing the effect of GABA, which itself enhances the influx of Cl<sup>-</sup> into the nerve cell. As a result, basal levels of GABA become more effective in gating the channel. Benzodiazepine antagonists prevent enhancement of GABA effects but do not reduce the basal conductance of Cl<sup>-</sup>. GABA antagonists prevent gating of Cl<sup>-</sup> channels in spite of the presence of benzodiazepines.



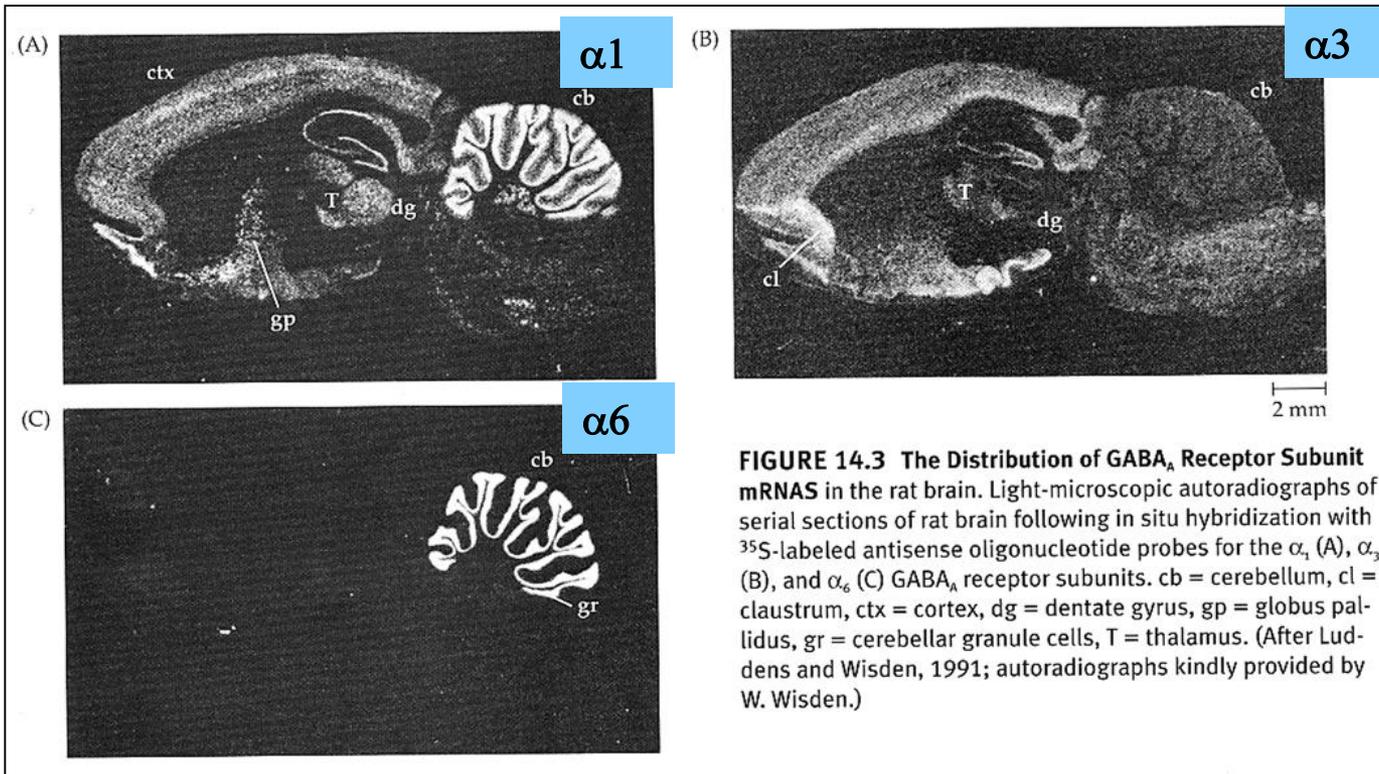
NEUROSCIENCE, Third Edition, Figure 6.9 (Part 2) © 2



# Excitatory actions of GABA during development



# GABA<sub>A</sub> receptor subunit distribution is spatially regulated in the brain



GABA

$\alpha_{1-7}$

$\beta_{1-4}$

$\gamma_{1-4}$

$\delta$

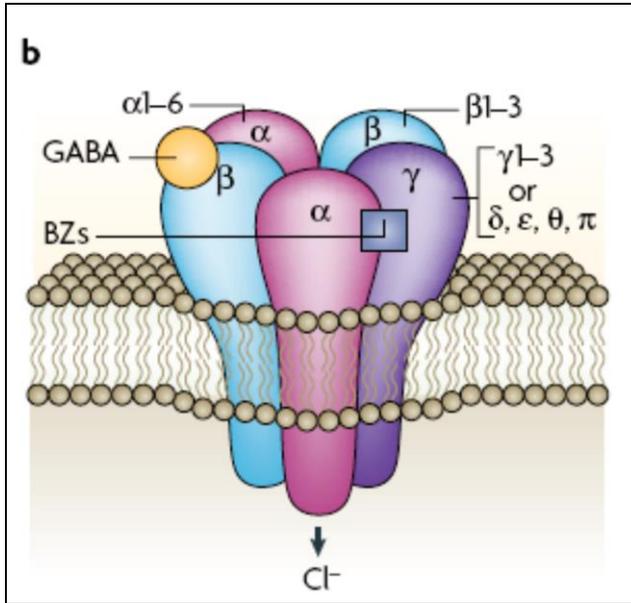
$\epsilon$

$\rho_{1-3}$

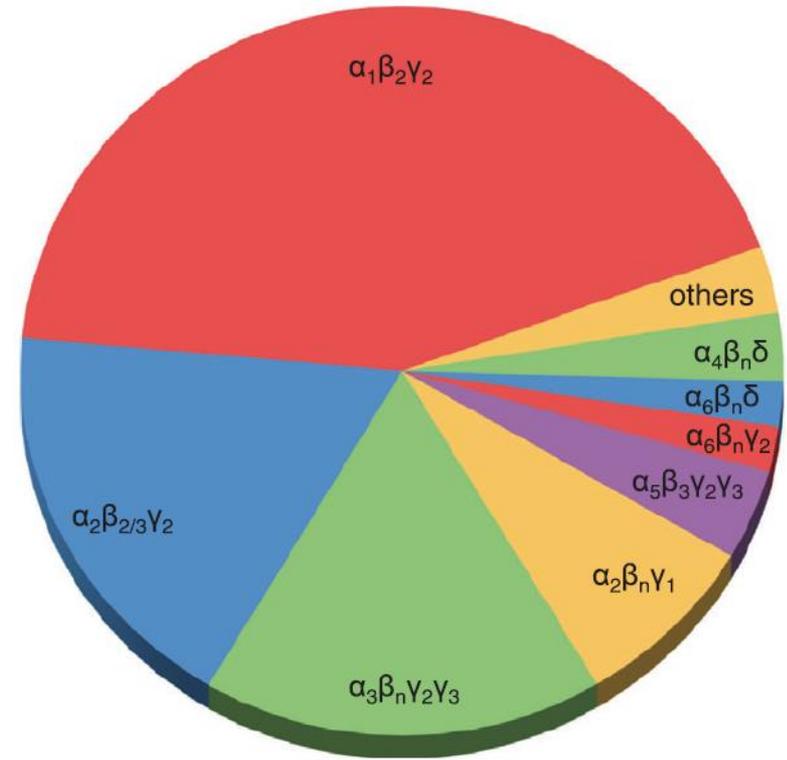
$\eta$

# GABA<sub>A</sub>Rs are made of variable combinations of at least 18 different subunits (each receptor is a heteropentameric Cl<sup>-</sup> channel)

- GABA
- α<sub>1-7</sub>
- β<sub>1-4</sub>
- γ<sub>1-4</sub>
- δ
- ε
- ρ<sub>1-3</sub>
- η



Jacob et al., 2008  
Nature Reviews Neurosci  
doi:10.1038/nrn2370

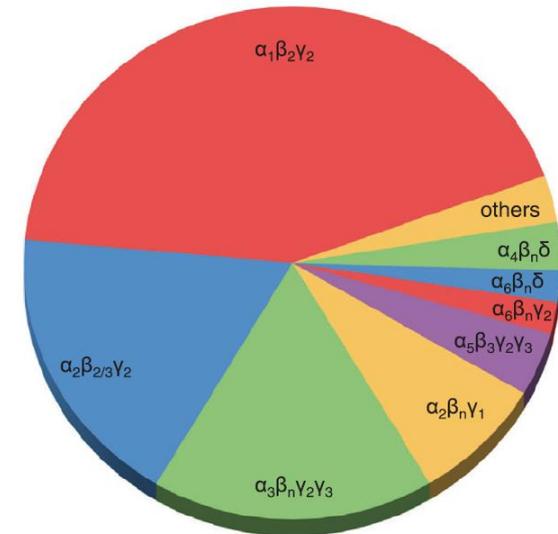
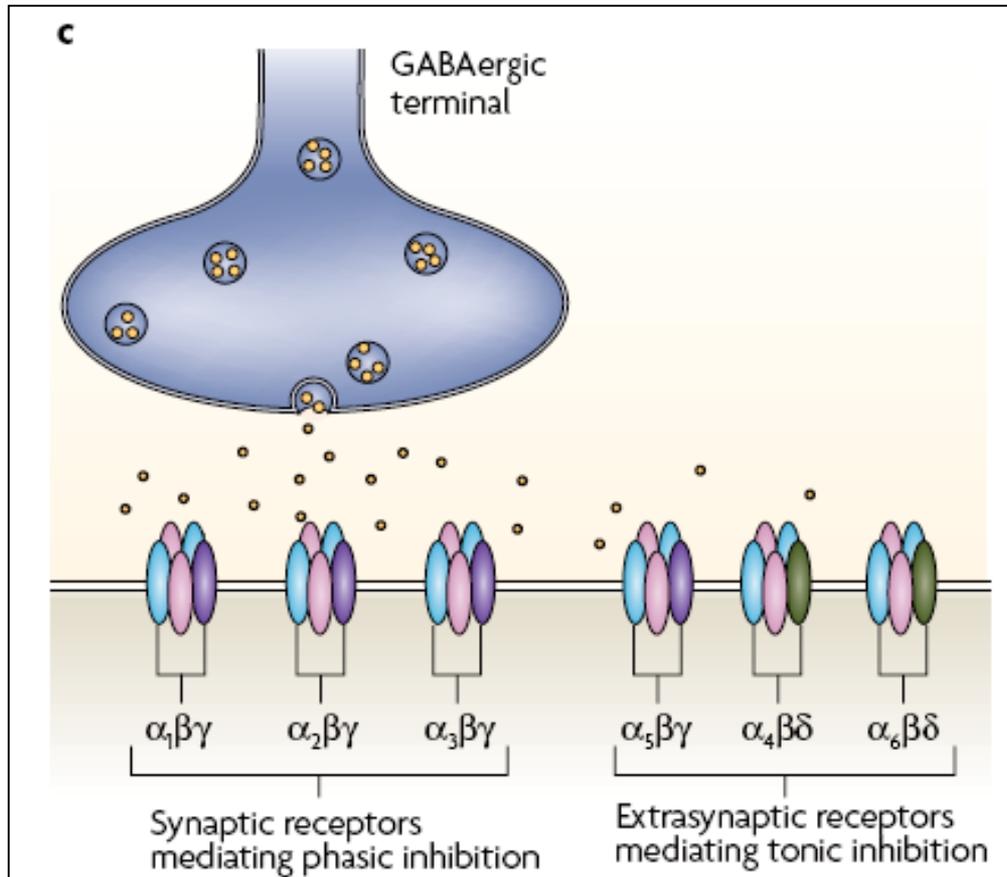


The relative expression of GABA<sub>A</sub> receptor subunit combinations in the rodent brain.

Stephens et al., 2016  
Genes Brain Behav.  
doi: 10.1111/gbb.123212016

Many different subunit combinations are theoretically possible; however, only a limited number of these combinations can actually exit the ER and access the neuronal cell surface. The majority of studies agree that most GABA<sub>A</sub>Rs expressed on the surface of neurons are composed of two α subunits, two β subunits and one γ subunit

# GABA<sub>A</sub>R subunit compositions at synaptic sites differ from those located at extra-synaptic sites

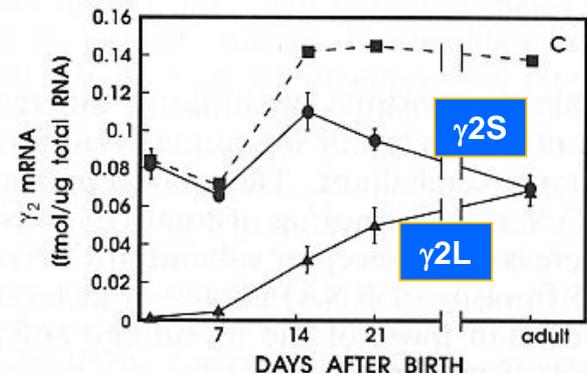
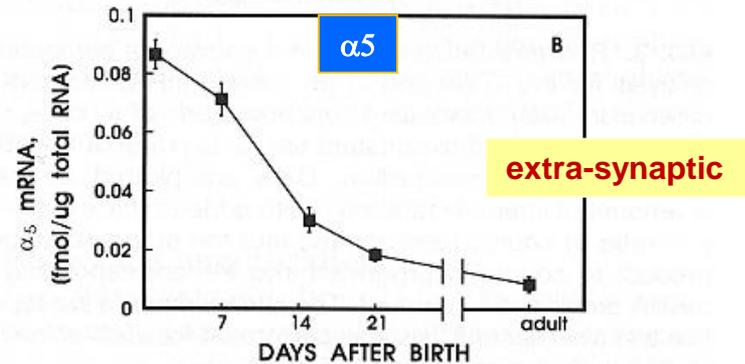
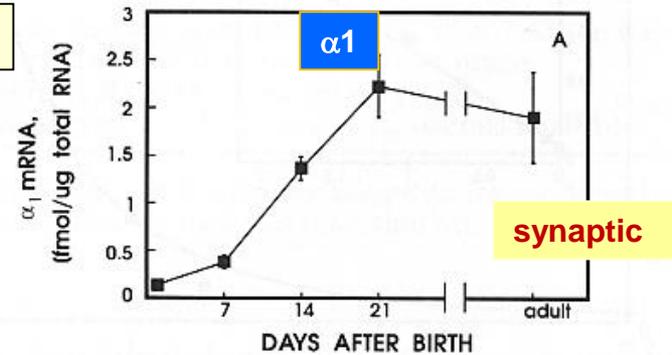


GABA<sub>A</sub>Rs composed of α(1–3) subunits together with β and γ subunits are thought to be primarily **synaptically localized**, whereas α<sub>5</sub>βγ receptors are located largely at **extrasynaptic** sites. Both these types of GABA<sub>A</sub>R are BZ sensitive. By contrast, receptors composed of α(4 or 6)βδ are BZ insensitive and localized at extrasynaptic sites.

Expression levels of GABA<sub>A</sub> receptor subunits are strictly regulated during development

$\gamma_2$  subunit regulation involves both the transcription rate and the type of splicing occurring at different developmental stages

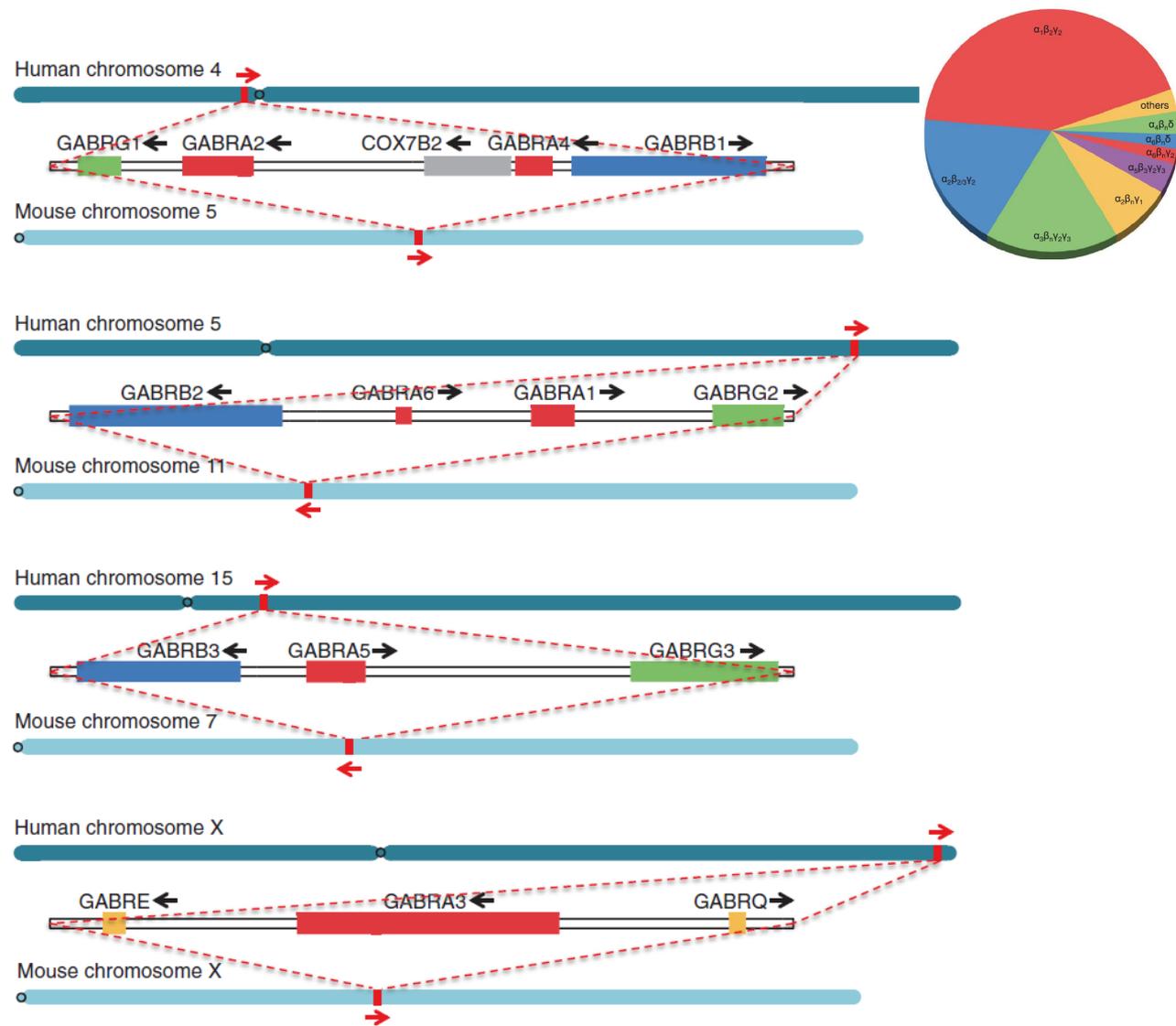
cerebellum



# Chromosomal locations of major GABA<sub>A</sub> receptor gene clusters

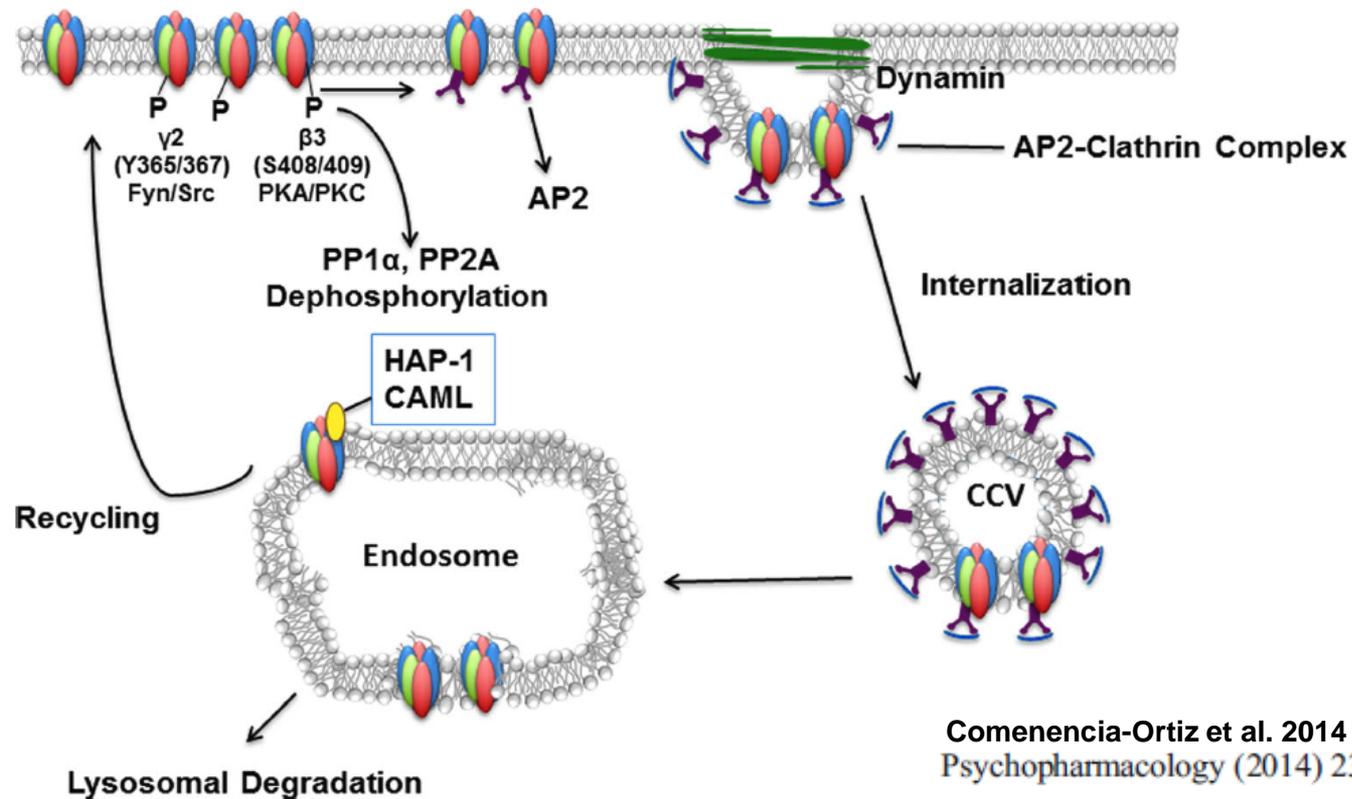
- Syntenic regions for the **human** clusters occur in the **mouse**, suggesting a highly conserved organization of these genes.
- Expression of those genes located on **human chr 4** predominate in rodent **embryo**, but these genes are generally down regulated in the **adult** rat, except in particular structures (cortical areas, hippocampus, etc..) where they continue to be highly expressed
- Three of the **chr 5 cluster** genes encode the most frequently expressed GABA<sub>A</sub>  **$\alpha 1\beta 2\gamma 2$  receptor**, while the **chr 4 genes** encode the GABA<sub>A</sub> receptor subunit group  **$\alpha 2\beta 1\gamma 1$**  that is found almost exclusively in the addiction-related mesolimbic pathways encompassing ventral tegmental area (VTA) and ventral striatal regions, consistent with **co-ordinated transcription within clusters**

Stephens et al., 2016  
Genes Brain Behav.  
doi: 10.1111/gbb.123212016



**Figure 2: Chromosomal locations of major GABA<sub>A</sub> receptor gene clusters.** The genes for individual subunits are present in clusters conserved across mammalian genomes. The figure shows the four main gene clusters and their relative locations on human and mouse chromosomes (shown on human and mouse chromosomes in red). The red arrow designates orientation along the chromosome. These clusters are expanded and the arrangement of the genes within them encoding the individual subunits displayed.  $\alpha$  subunit genes are shown in red,  $\beta$  subunit genes are blue,  $\gamma$  subunit genes are green. Other GABA<sub>A</sub> receptor subunit families are shown in yellow. The subunits are encoded by the following genes:  $\alpha 1$ , GABRA1;  $\alpha 2$ , GABRA2;  $\alpha 3$ , GABRA3;  $\alpha 4$ , GABRA4;  $\alpha 5$ , GABRA5;  $\beta 1$ , GABRB1;  $\beta 2$ , GABRB2;  $\beta 3$ , GABRB3;  $\gamma 1$ , GABRG1;  $\gamma 2$ , GABRG2;  $\gamma 3$ , GABRG3;  $\epsilon$ , GABRE;  $\theta$ , GABRQ. Black arrows indicate the direction of transcription for the individual genes.

# GABA<sub>A</sub> receptors endocytosis and recycling



**Fig. 3** GABA<sub>A</sub>R endocytosis and recycling. GABA<sub>A</sub>R endocytosis occurs primarily via mechanisms dependent on the formation of clathrin-coated vesicles. Clathrin- and dynamin-mediated endocytosis is regulated in a phospho-dependent manner via the interaction of specific motifs within the intracellular loop of the  $\beta$  and  $\gamma$  subunits with the  $\mu$  subunit of the AP2 protein. Phosphorylation of residues within the intracellular domains of  $\beta 3$  and  $\gamma 2$  subunits (by kinases PKC/PKA or

Fyn/Src, respectively) interferes with this interaction and therefore stabilizes GABA<sub>A</sub>Rs in the cell membrane. When endocytosed, GABA<sub>A</sub>Rs can be ubiquitinated and degraded via lysosomal degradation. Alternatively, the receptors can interact with regulatory lysosomal proteins such as HAP1, and CAML, which promotes the transport and recycling of receptors back to the cell surface (Arancibia-Cárcamo et al. 2009)

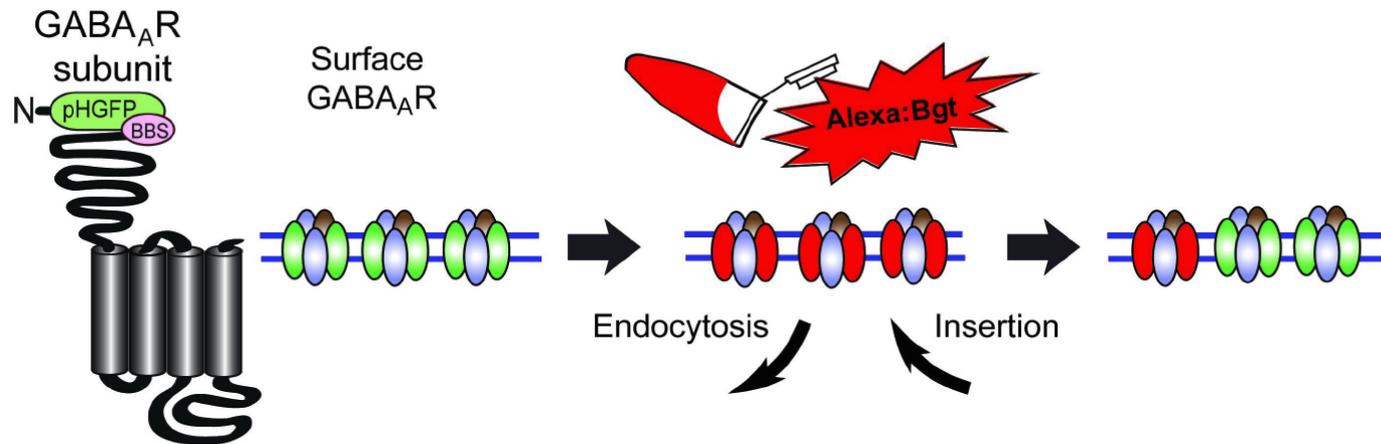
Video Article

# Using an $\alpha$ -Bungarotoxin Binding Site Tag to Study GABA A Receptor Membrane Localization and Trafficking

Megan L. Brady<sup>1</sup>, Charles E. Moon<sup>1</sup>, Tija C. Jacob<sup>1</sup>

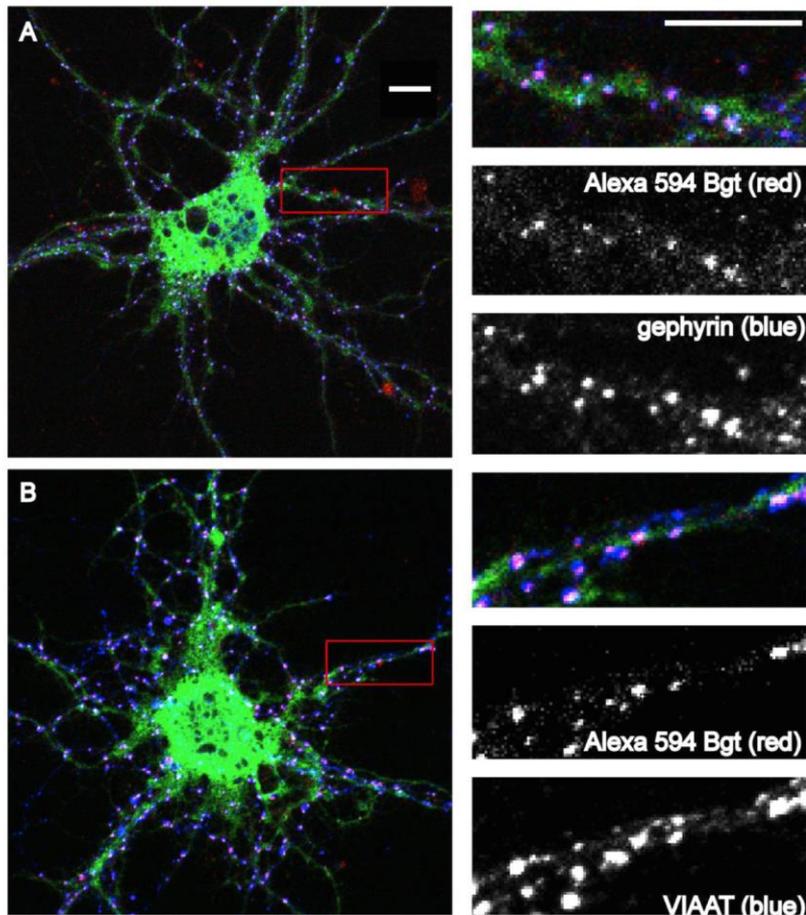
<https://www.jove.com/video/51365/using-an-bungarotoxin-binding-site-tag-to-study-gaba-receptor>

Date Published: 3/28/2014



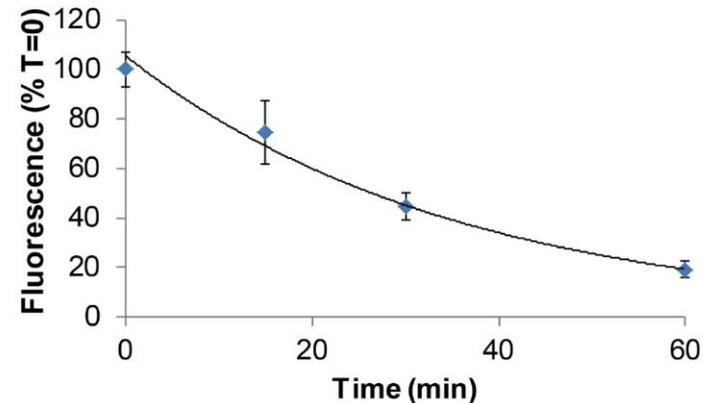
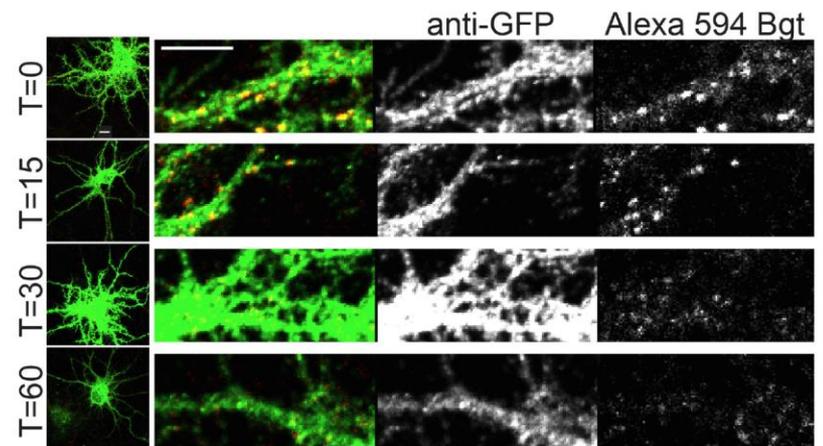
**Figure 1. Diagram of a BBS and pHGFP tagged GABAAR subunit and the endocytosis assay.** Endocytosis is measured by first applying a fluorescently labeled bungarotoxin to neurons or cells, followed by a brief wash to remove unbound toxin, then fluorescence loss is monitored as receptors are internalized.

$\alpha$ 2pHGFP+BBS, immunofluorescence



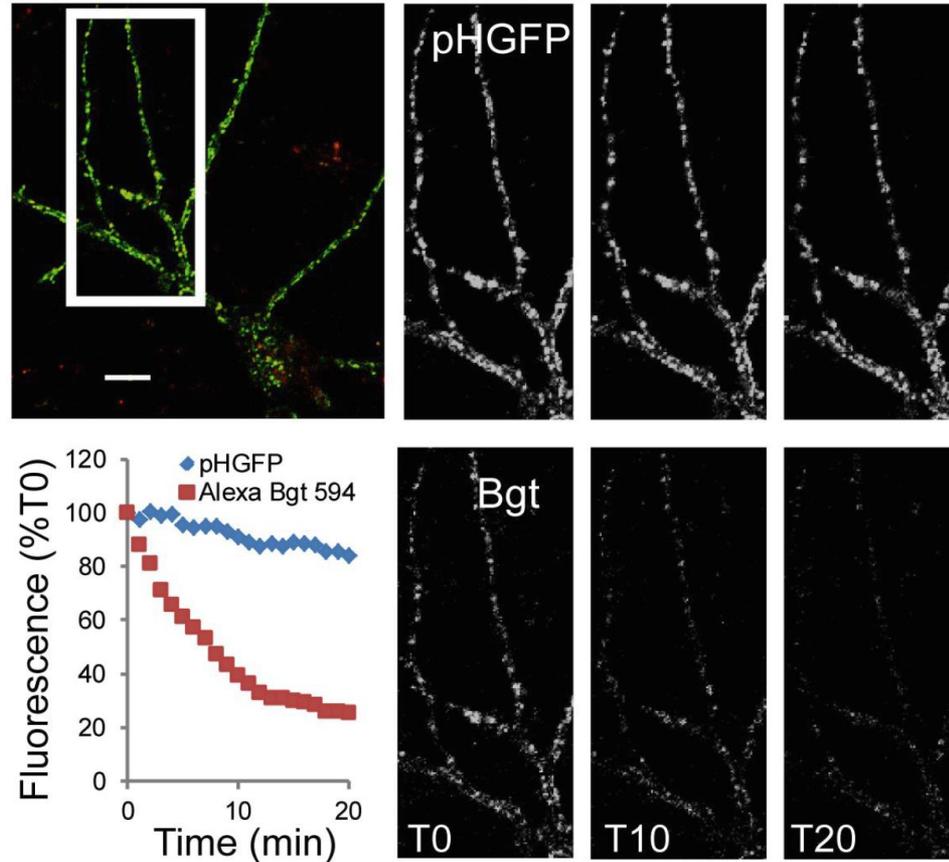
**Figure 2. Confocal imaging of BBS and pHGFP tagged GABAAR showing appropriate localization with inhibitory synapse components.**

Surface GABAAR in  $\alpha$ 2pHGFP+BBS expressing neurons were labeled with  $\alpha$ -bungarotoxin Alexa 594 (red), followed by immunostaining, enlargements of dendrites are shown to the right of each neuron. Merged panel ( $\alpha$ -bungarotoxin Alexa 594 in red, GFP in green, gephyrin or VIAAT in blue). Surface Bgt labeled GABAAR show colocalization with **A)** the postsynaptic inhibitory scaffold protein gephyrin and **B)** apposition to the presynaptic marker VIAAT. (Scale bars, 10  $\mu$ m.)



**Figure 3. Confocal imaging of GABAAR endocytosis in 15 DIV hippocampal neurons. A)** Surface GABAAR in  $\alpha$ 2pHGFP+BBS neurons were live-labeled with  $\alpha$ -bungarotoxin Alexa 594 (red), and then unbound  $\alpha$ -bungarotoxin Alexa 594 was removed by washing, followed by incubation at 37  $^{\circ}$ C. At T = 0, 15, 30, and 60 min, samples were removed and fixed. Total receptor number was assayed by permeabilization and staining with anti-GFP antibody (green). Panels are enlargements of dendrites (Scale bars, 10  $\mu$ m). **B)** Graph represents the surface GABAAR  $\alpha$ -bungarotoxin Alexa 594 fluorescence loss over time with endocytosis (normalized to T = 0) (10-12 neurons per time point, 4 processes analyzed per neuron; error bars represent MEAN  $\pm$  SEM).

$\beta$ 3pHGFP+BBS, live imaging in HBS, 37°C



**Figure 4. Live confocal imaging of GABAAR endocytosis in 15 DIV hippocampal neurons. A)** B3pHGFP+BBS containing surface GABAAR in neurons were live-labeled with  $\alpha$ -bungarotoxin Alexa 594 (red), followed by brief removal of unbound  $\alpha$ -bungarotoxin Alexa 594 by washing. Neurons were imaged by confocal microscopy at 37 °C in HBS for 20 min at a 1 min interval (Scale bars, 10  $\mu$ m). Bgt Labeled GABAARs were localized to the cell surface at T0, as seen by colocalization with the pHGFP signal at T0. Over the time course, Bgt labeled GABA AR signal decreases, as receptors endocytose, new pHGFP expressing receptors are continually being inserted, so the pHGFP signal remains nearly constant.