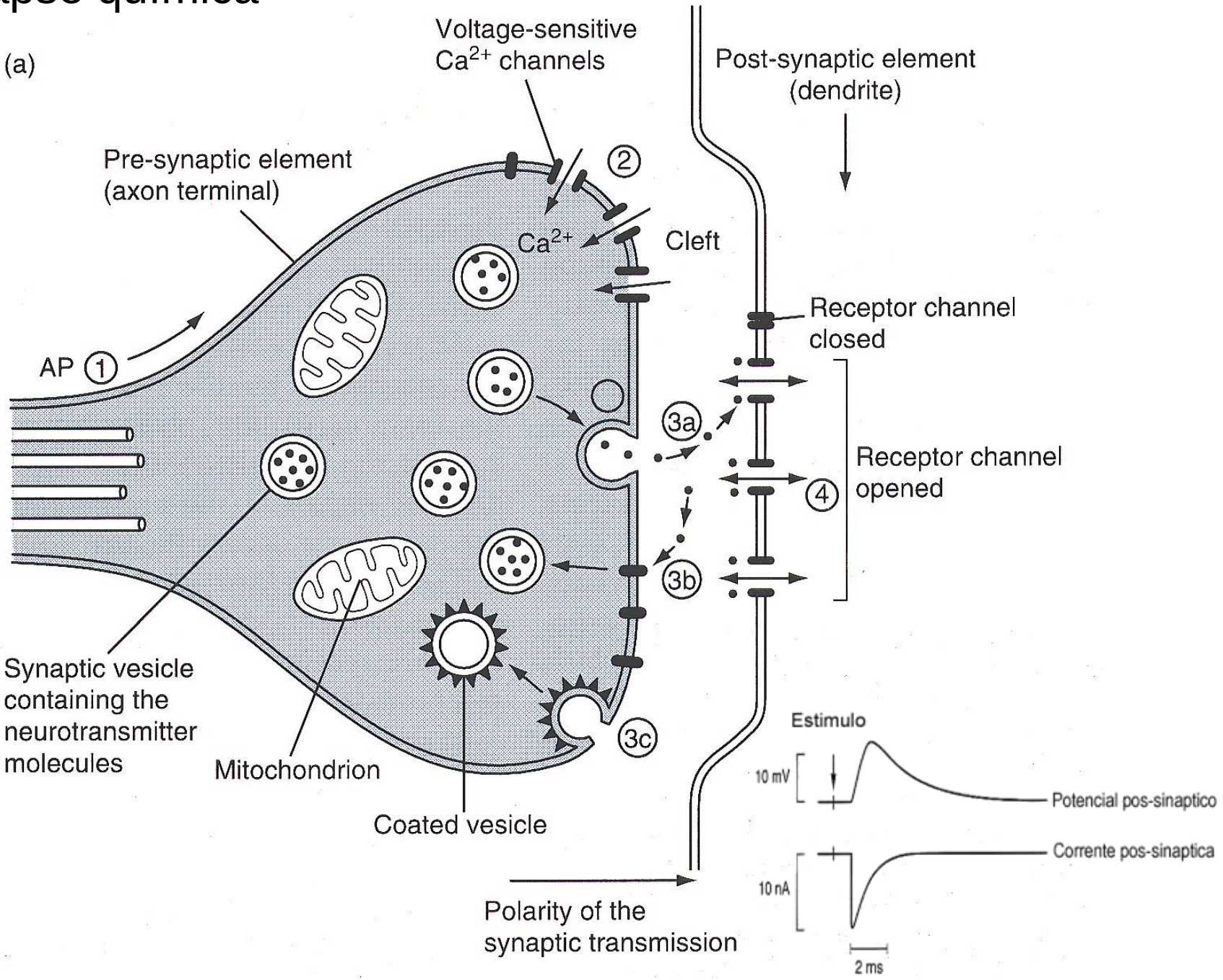
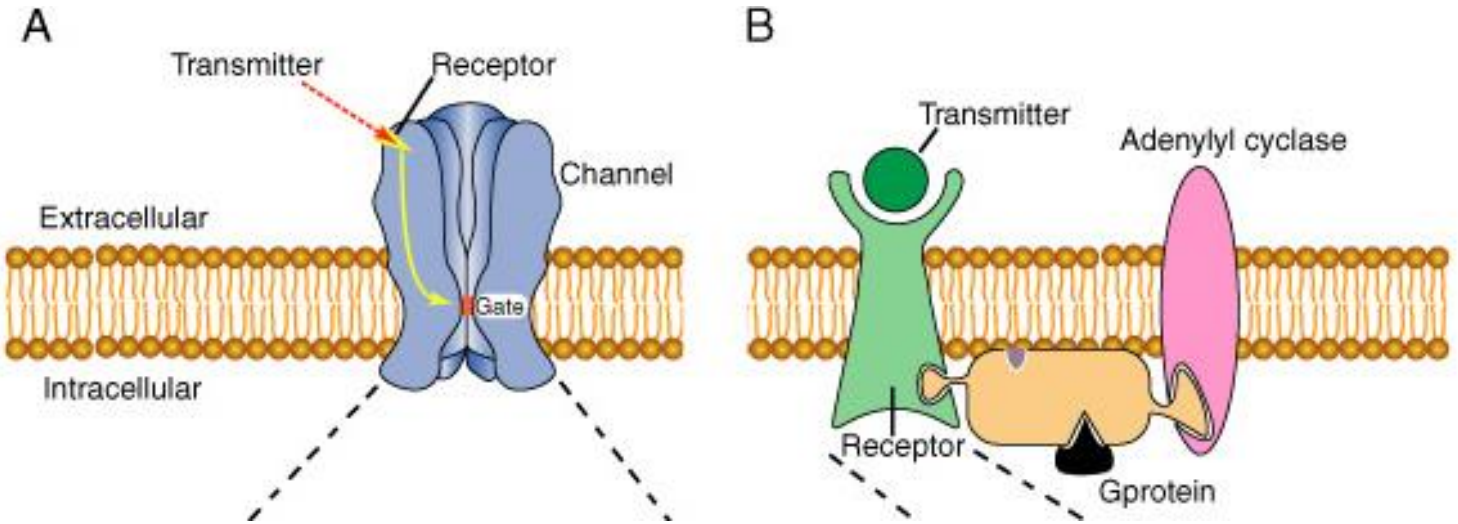
O Lado pós-sináptico

-Receptores ionotrópicos.
-Receptores metabotrópicos.
-Cleareance de transmissores.

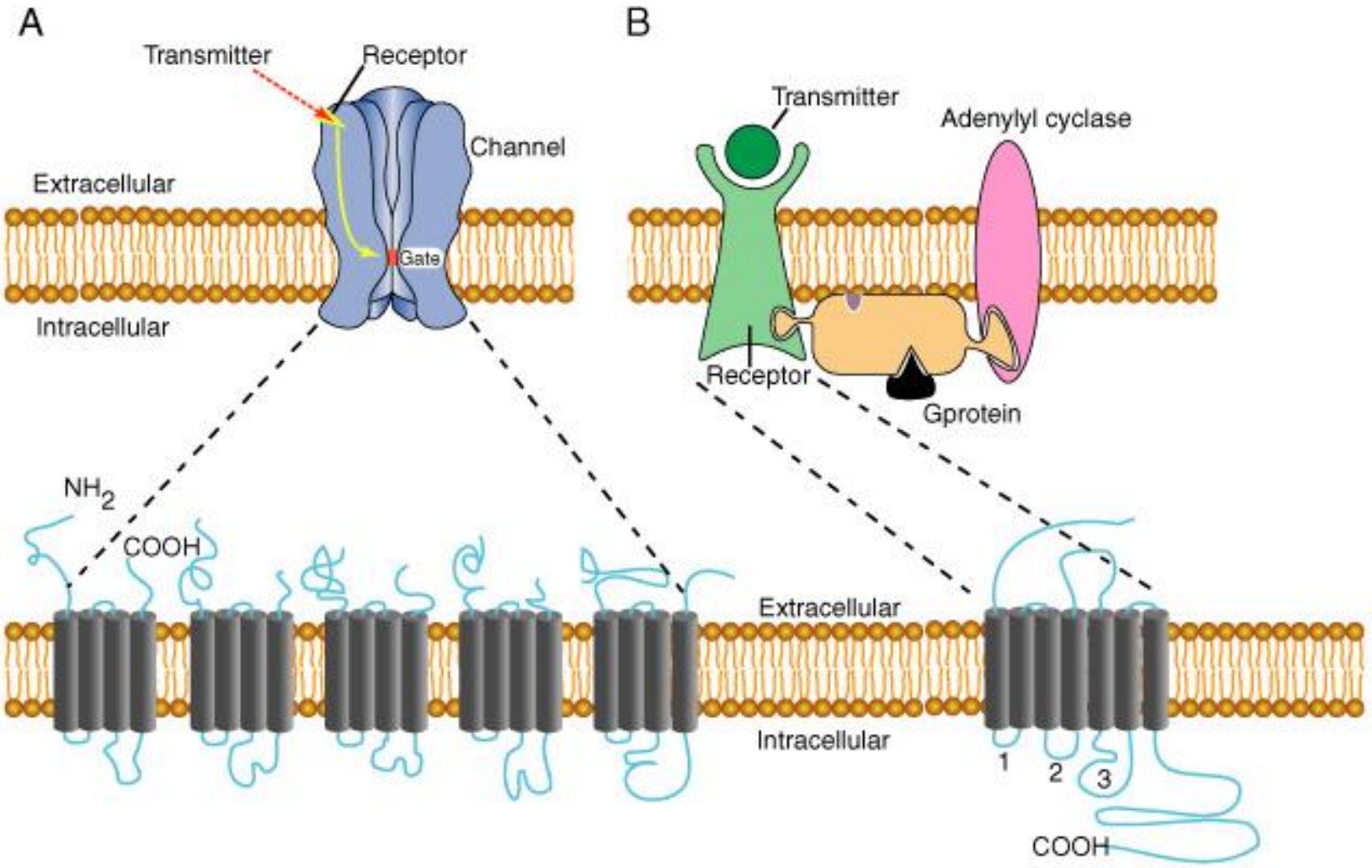
Sinapse química



Os receptores de neurotransmissores são classificados como ionotrópicos (A) e metabotrópicos (B)

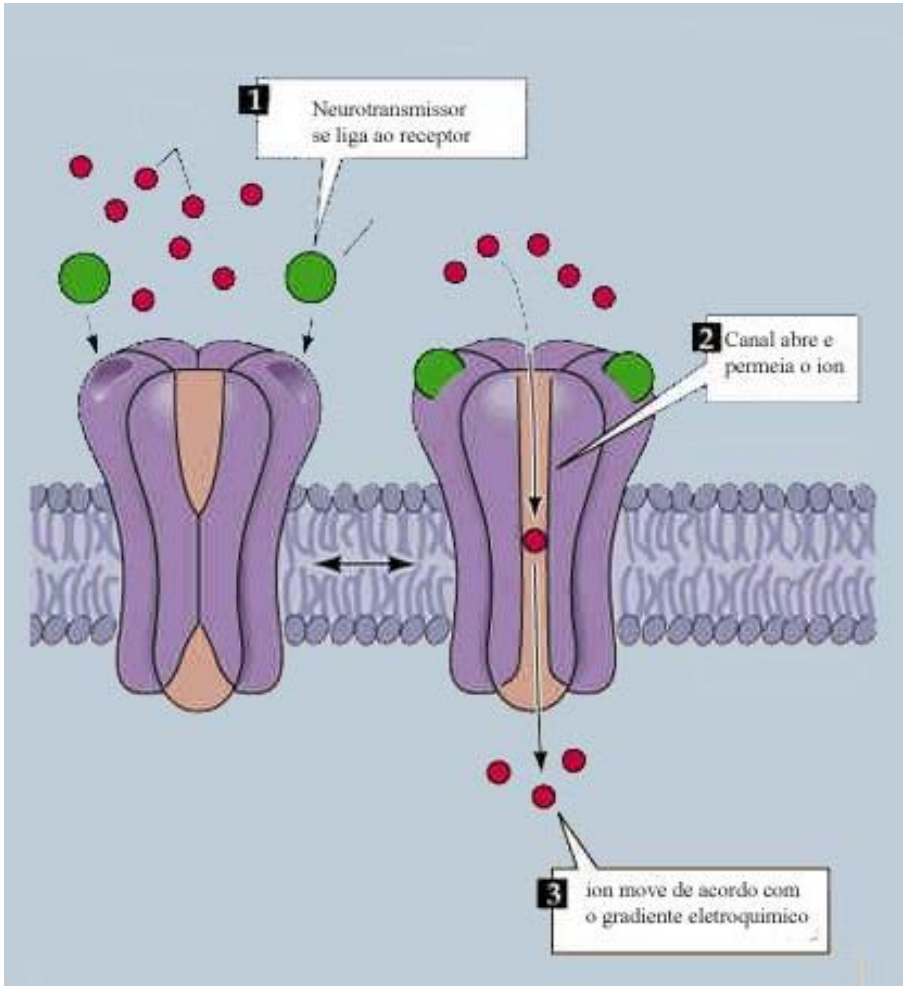


Os receptores de neurotransmissores são classificados como ionotrópicos (A) e metabotrópicos (B)



Os neurotransmissores se ligam aos receptores situados na membrana pós-sinaptica.
receptores ionotrópicos = responsaveis pela resposta rápida. Abrem canais iônicos na membrana pós-sinaptica

O transmissor na JNM dos vertebrados é a acetilcolina (ACh)
Ela se liga aos receptores nicotinicos (ionotrópicos), abrindo canais catiônicos



transmissores excitatórios (permeiam cátions)

Ach-nicotinico

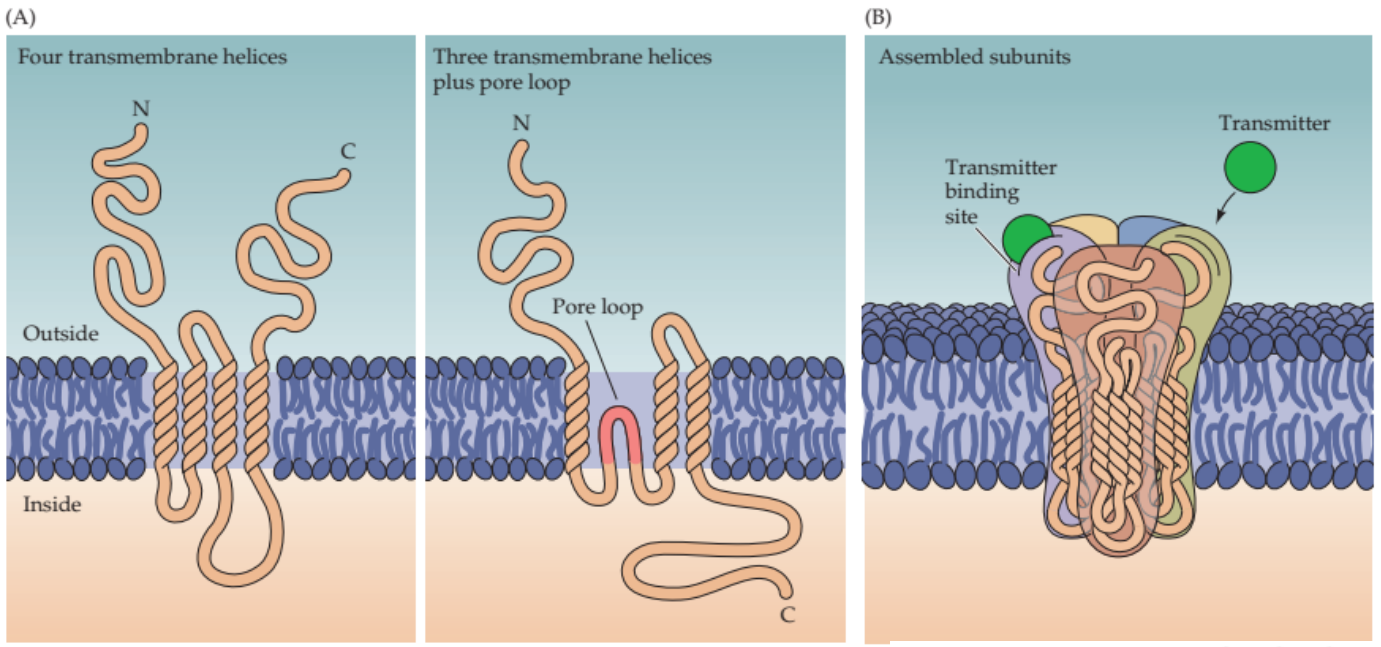
Glutamato-AMPA, Kainato, NMDA

transmissores inibitórios (permeiam ânions)

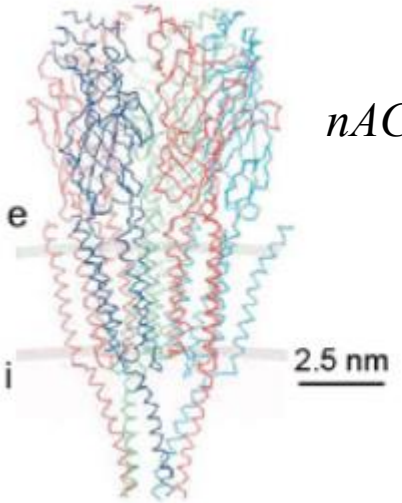
GABA-$GABA_A$

Glicina

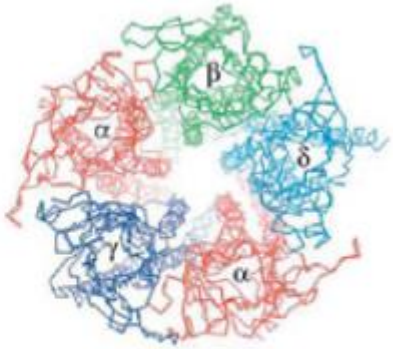
Estrutura geral dos receptores ionotrópicos



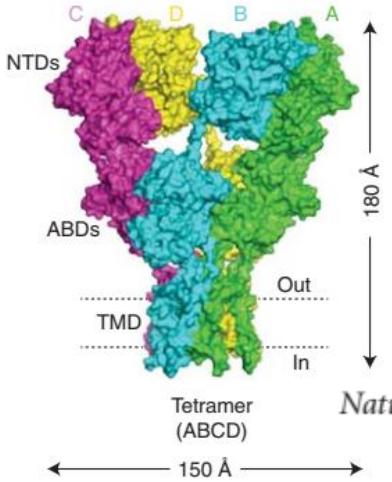
NEUROSCIENCE: Third Edition



nAChR



J. Mol. Biol. **346**, 967–989



GluA2

Nature **462:** 745–756.

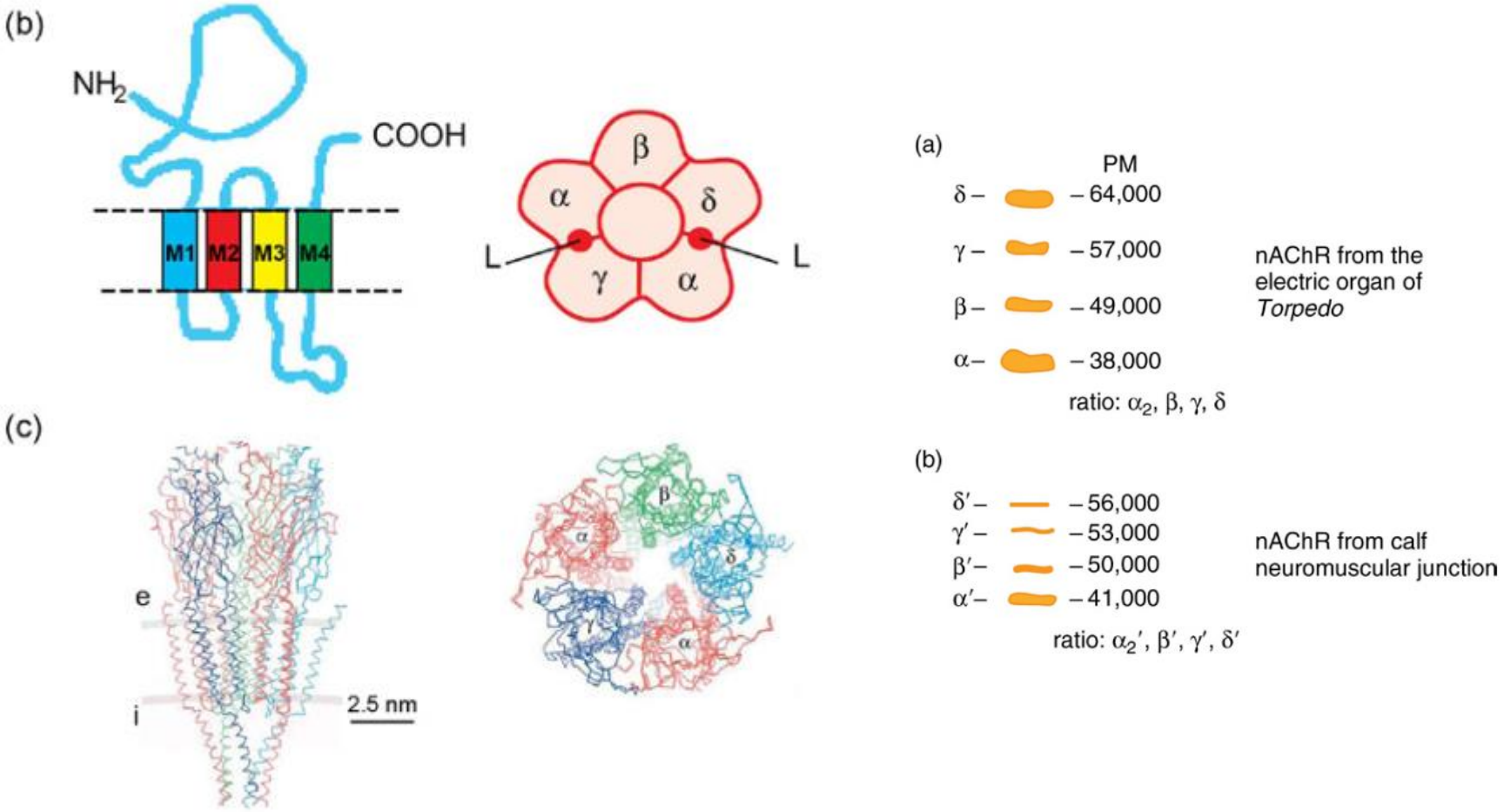
Várias subunidades compõem os receptores ionotrópicos

glutamato

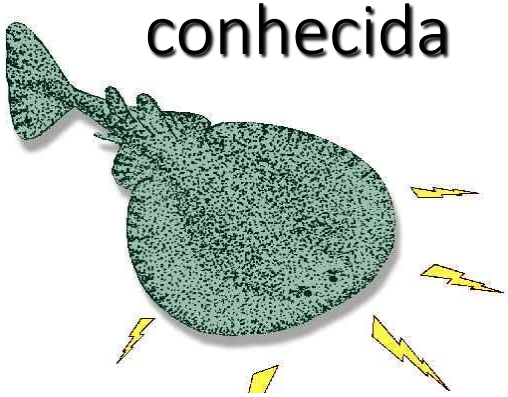
Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nACh	Serotonin	Purines
Subunits (combination of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5	α_{1-7}	α1	α_{2-9}	5-HT_3	P_{2X1}
	Glu R2	NR2A	Glu R6	β_{1-4}	α2	β_{1-4}		P_{2X2}
	Glu R3	NR2B	Glu R7	γ_{1-4}	α3	γ		P_{2X3}
	Glu R4	NR2C	KA1	δ	α4	δ		P_{2X4}
		NR2D	KA2	ε	β			P_{2X5}
				ρ_{1-3}				P_{2X6}
								P_{2X7}

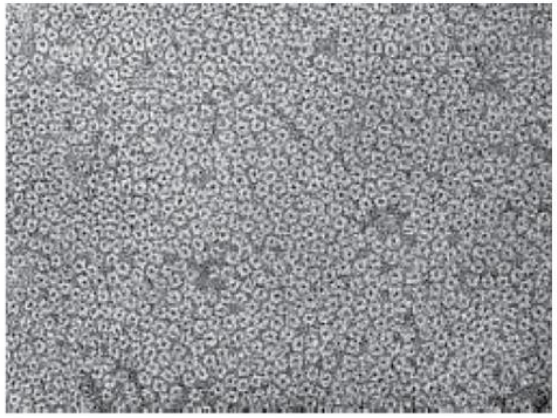
NEUROSCIENCE: Third Edition

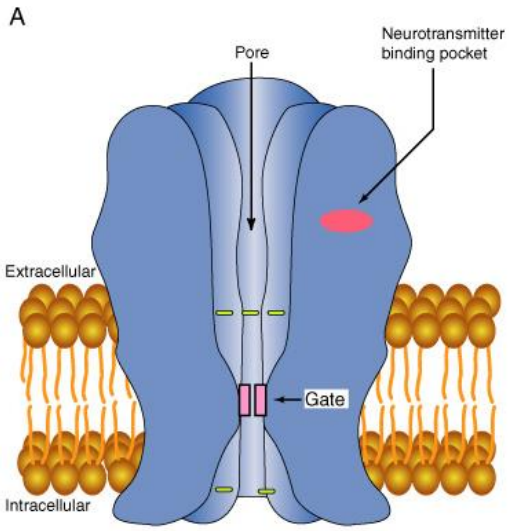
O Receptor nicotínico é um pentâmero

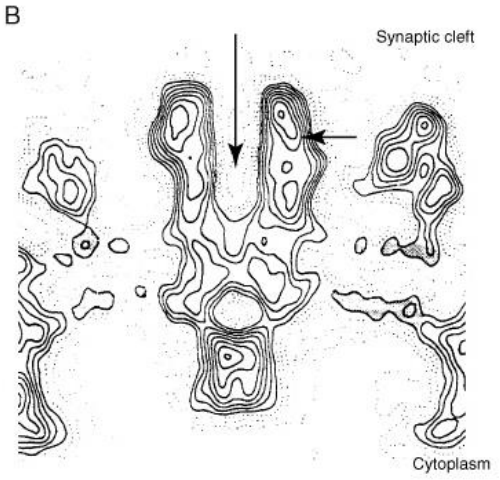


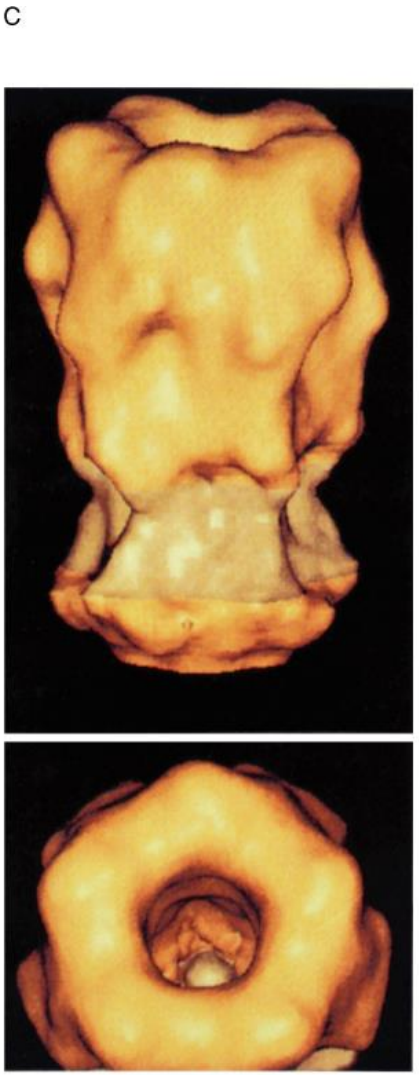
A estrutura do receptor nicotínico é a melhor conhecida



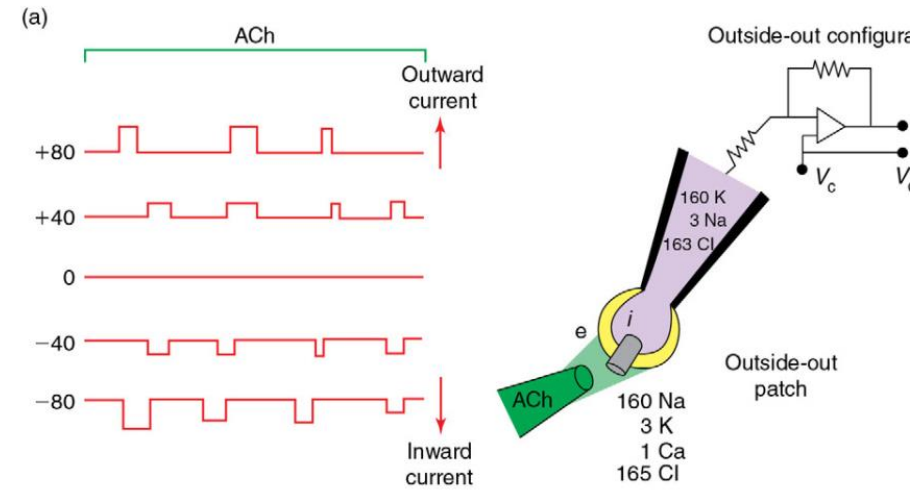


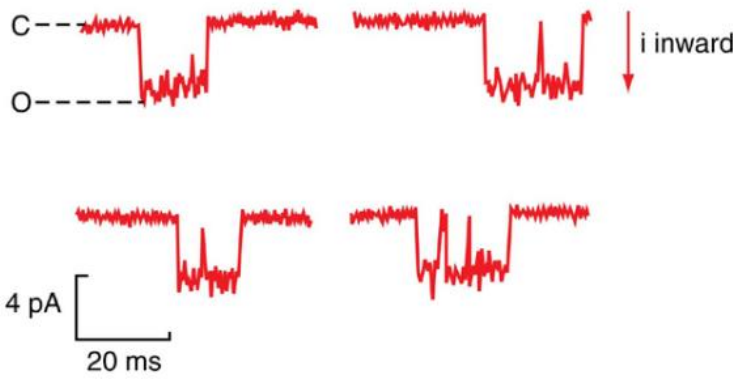


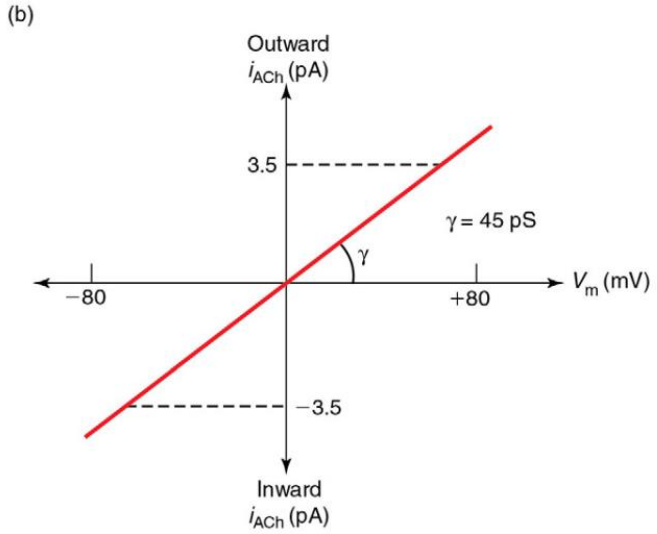


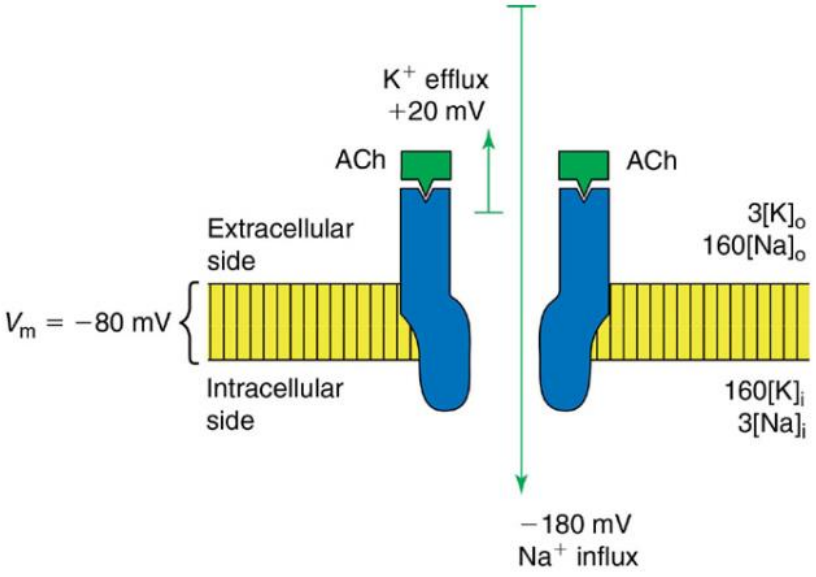


Correntes unitárias através do receptor nicotínico

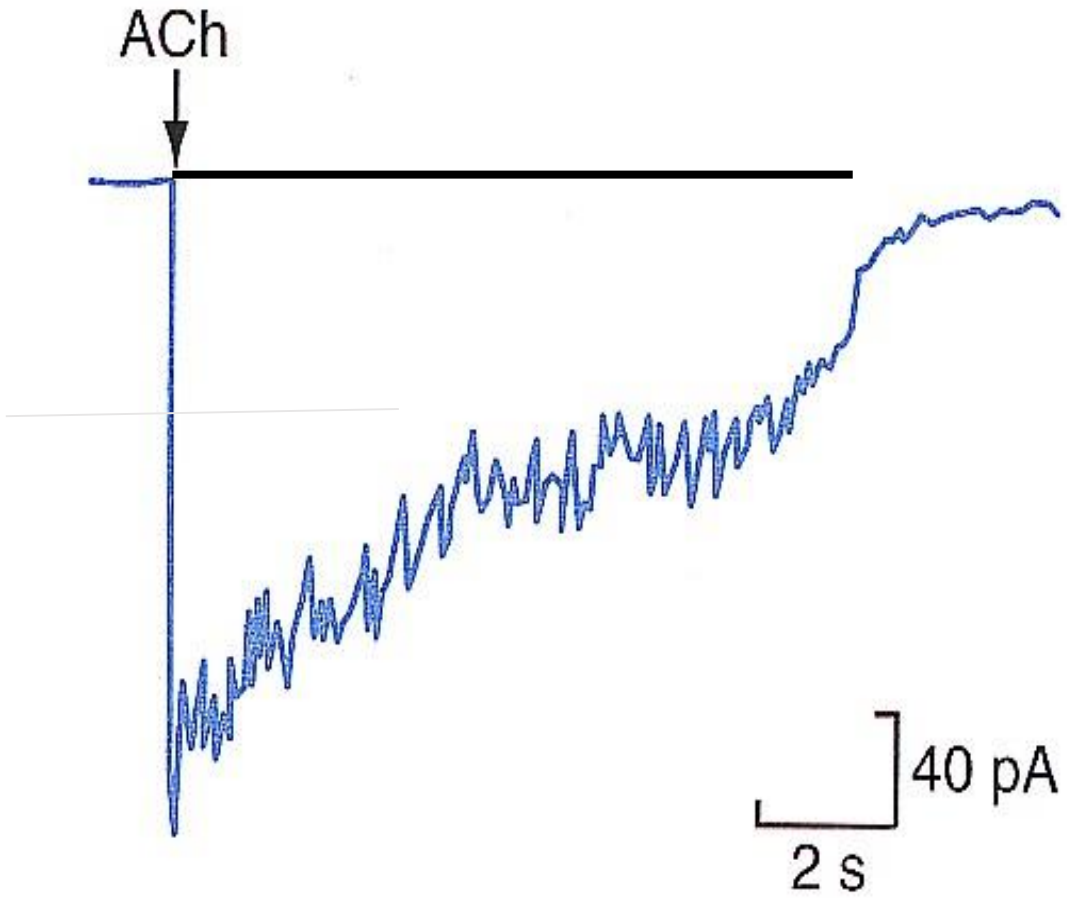








Correntes TOTAIS da membrana através do receptor nicotínico (*whole cell patch clamp*)



Correntes sinápticas através do receptor nicotínico

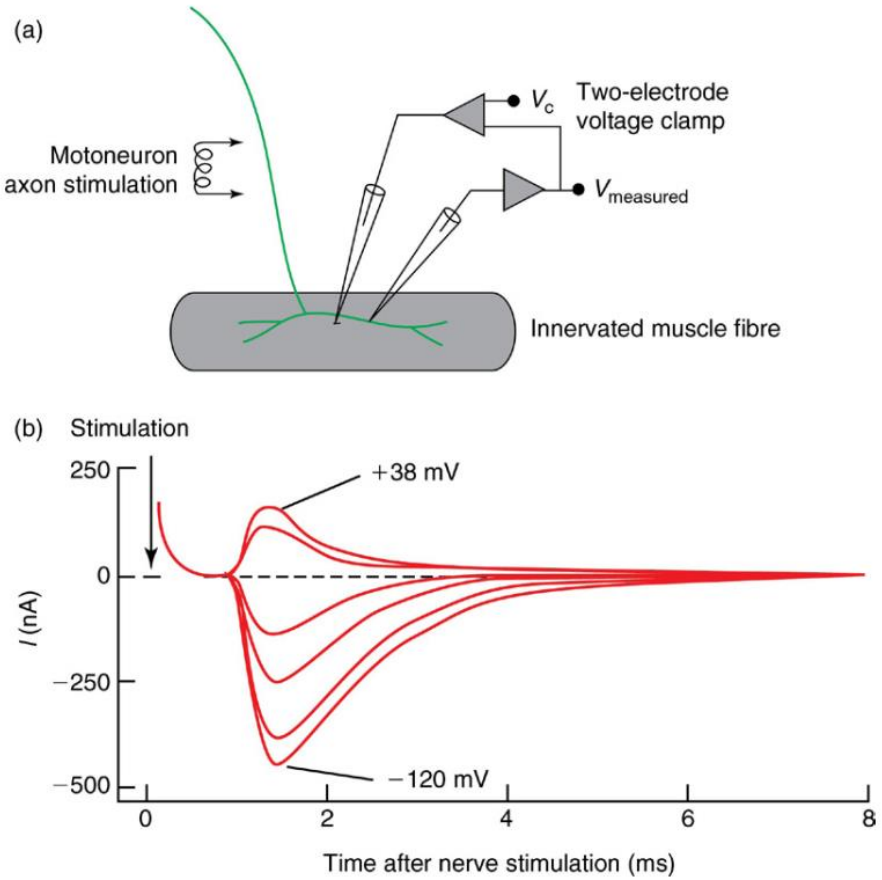


FIGURE 8.21 Motor endplate current. (**a**) Current recorded in response to a stimulation of the nerve fiber under two-electrode voltage clamp. (**b**) This current is inward for negative membrane potentials and outward for positive voltages. As in the case of the unitary current, the motor endplate current reverses around 0 mV (frog's neuromuscular junction). Muscular action potentials are blocked by voltage clamping the corresponding muscle region. The muscle contraction induced by the inward current can be blocked by the destruction of T tubules (with a hyperosmotic shock). Adapted from Magleby KL, Stevens CF (1972) A quantitative description of end plate currents. *J. Physiol. (Lond.)* **223**, 173–197, with permission.

Receptores nicotínicos podem ser homoméricos ou heteroméricos

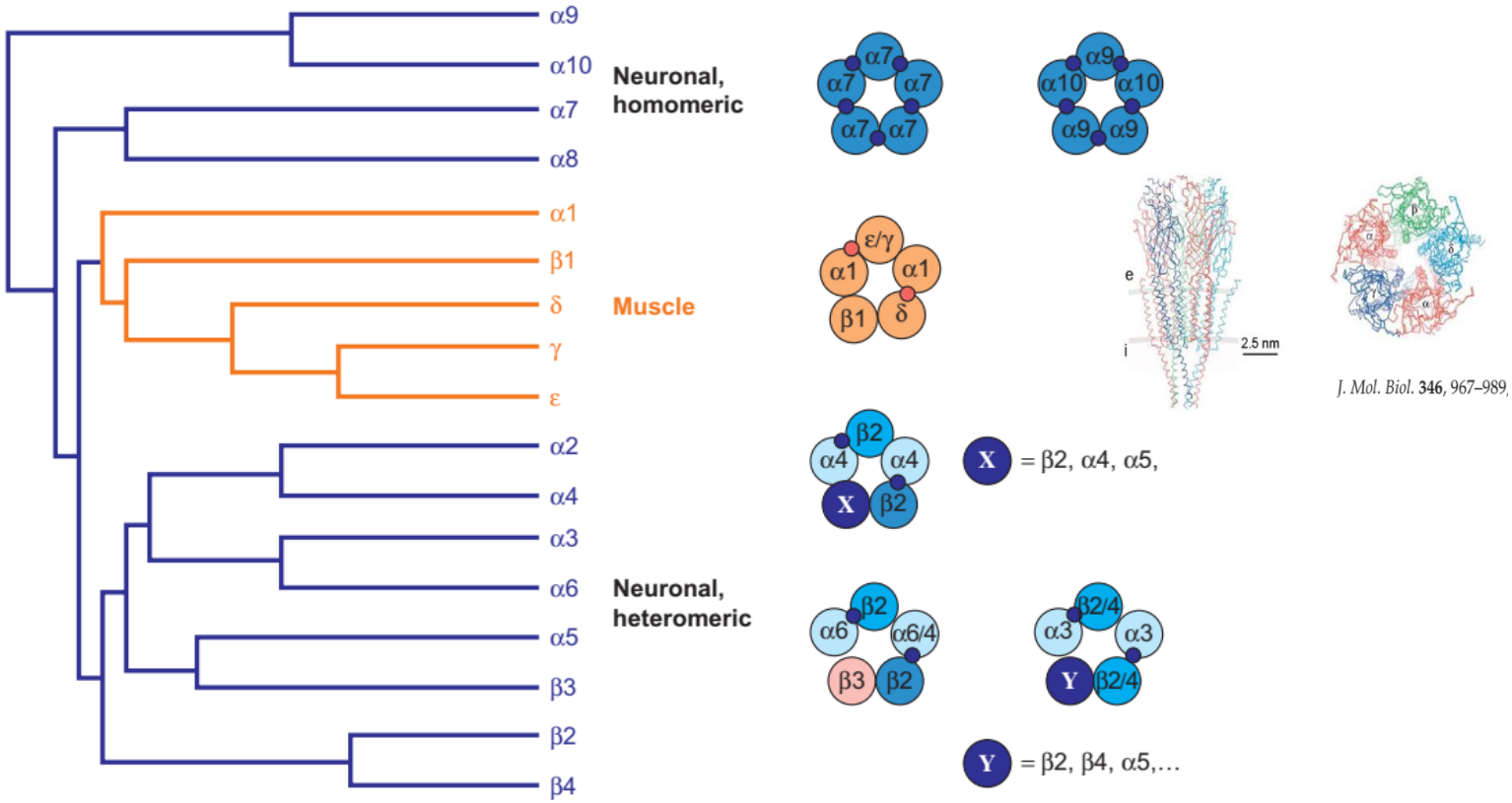
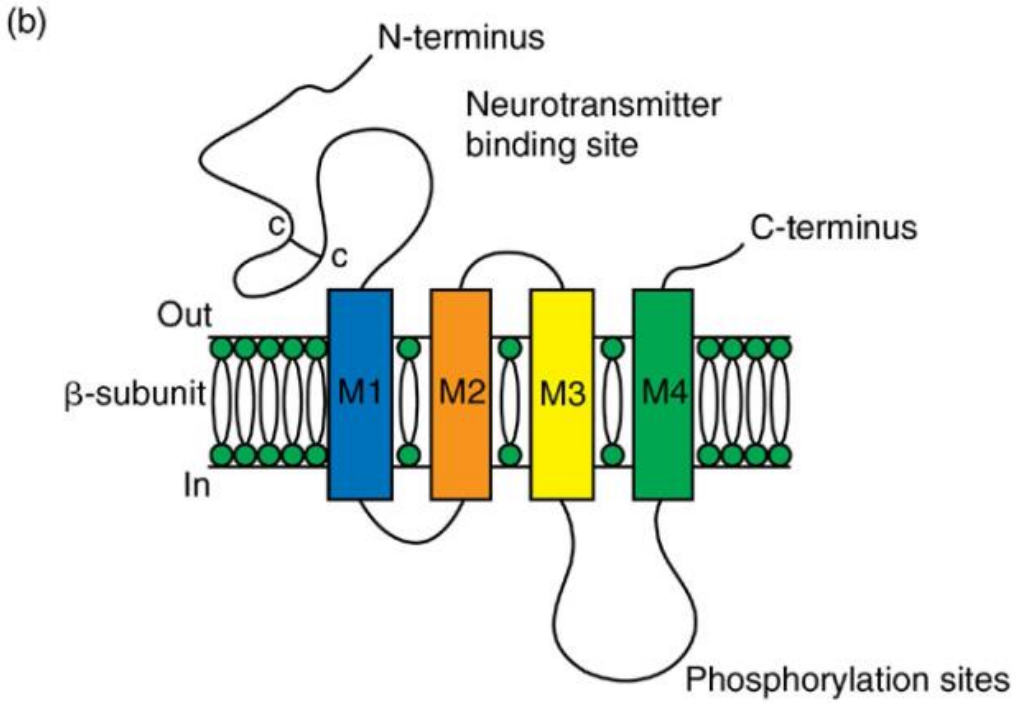
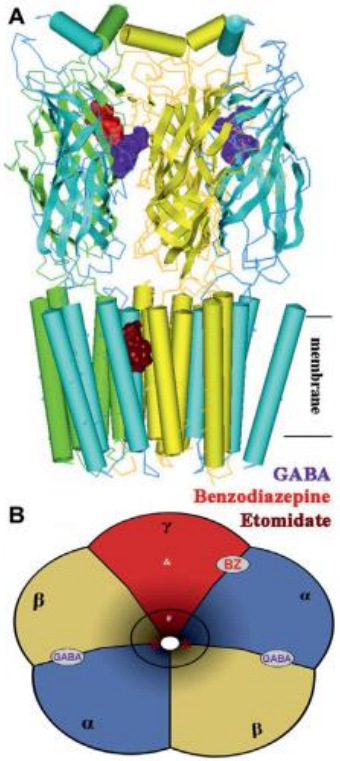
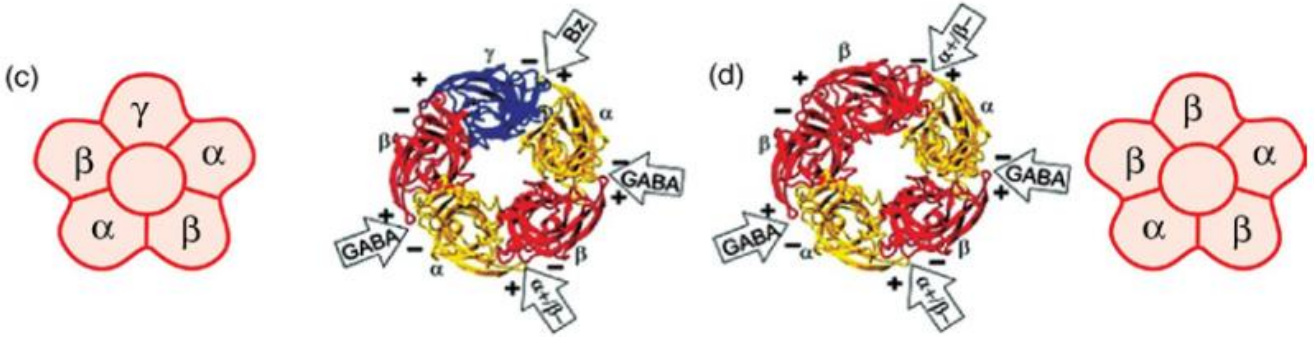


FIGURE 13-11 **Heterogeneity of nAChR family of subunits.** Phylogenetic relationship of vertebrate nAChR subunits, adapted from Le Novère & Changeux (1995). The subunit composition of native nAChRs is illustrated on the right. Putative agonist-binding sites are indicated by dark circles between principal and complimentary adjacent subunits.

O Receptor $GABA_A$ é um pentâmero também

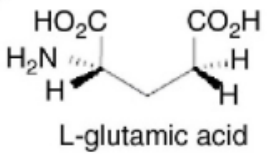




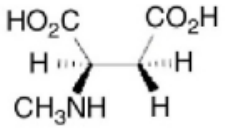


Os receptores glutamatérgicos são divididos em 3 tipos baseados em sua farmacologia

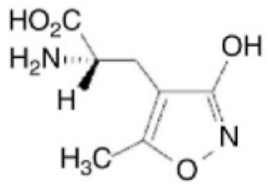
(b)



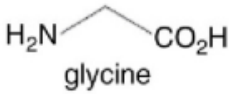
L-glutamic acid



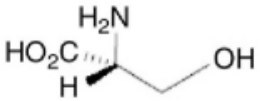
NMDA



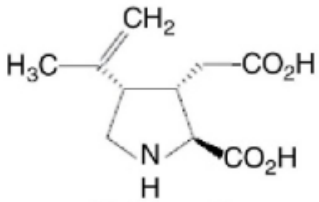
S-AMPA



glycine

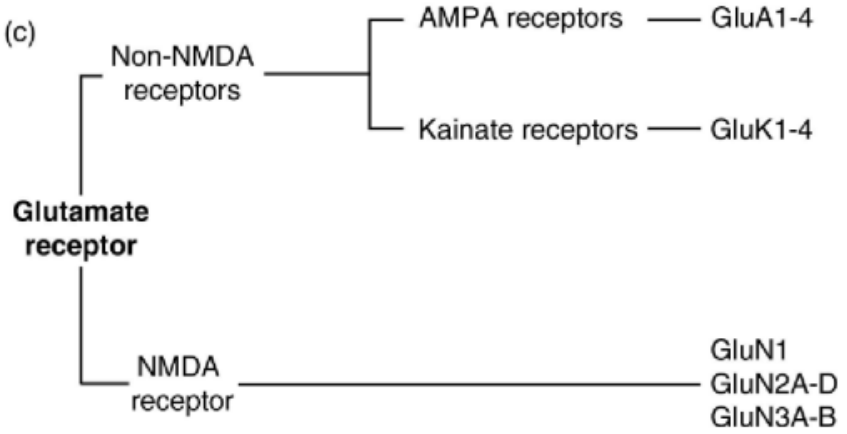


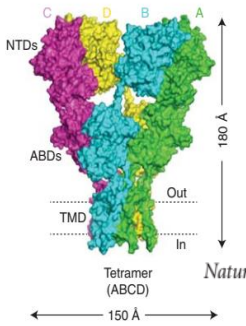
D-serine



Kainic acid

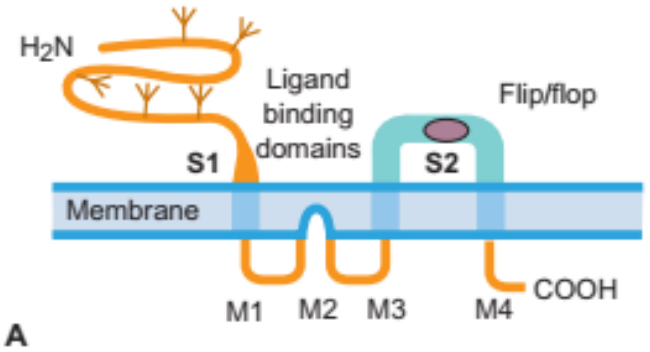
(c)





GluA2

Nature **462:** 745–756.



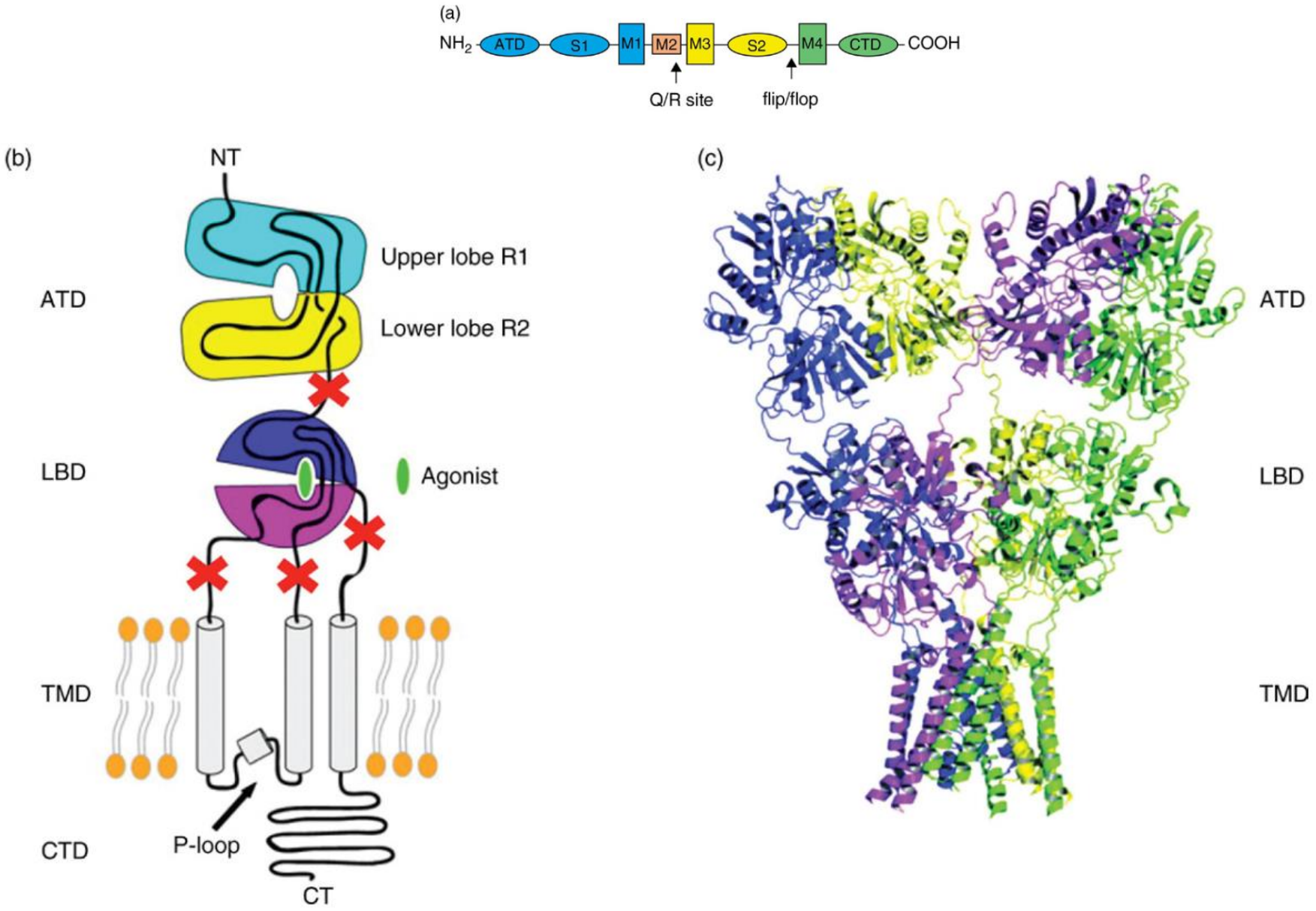
A

Os receptores glutamatérgicos são divididos em 3 tipos

(a)

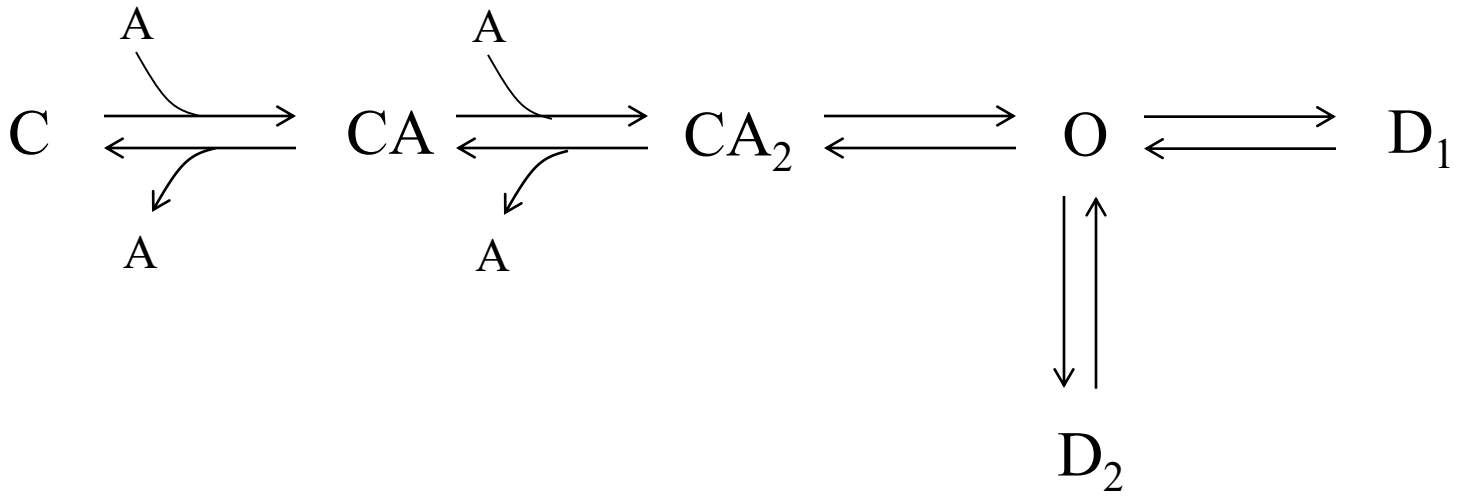
iGluRs	Non-NMDA receptors		NMDA receptors	
	AMPA receptors	KA receptors	Glu site	Gly site
Selective agonists	AMPA	Kainate (< 1 μM) ATPA (GluK1)	NMDA	Glycine
Non-selective agonists	Glutamate		Glutamate	–
Selective antagonists	GYKI 53655 GYKI 52466 NBQX (1 μM)	SYM 2081 UBP 310 (GLUK2/K5)	D-AP5	5,7-dichloro-kynurenate
Non-selective antagonists	CNQX DNQX NBQX (>1 μM)		–	CNQX
Channel blockers			MK 801 ketamine	

O Receptor glutamatérgico ionotrópico



Modelos cinéticos

Receptor nicotínico



C = fechado

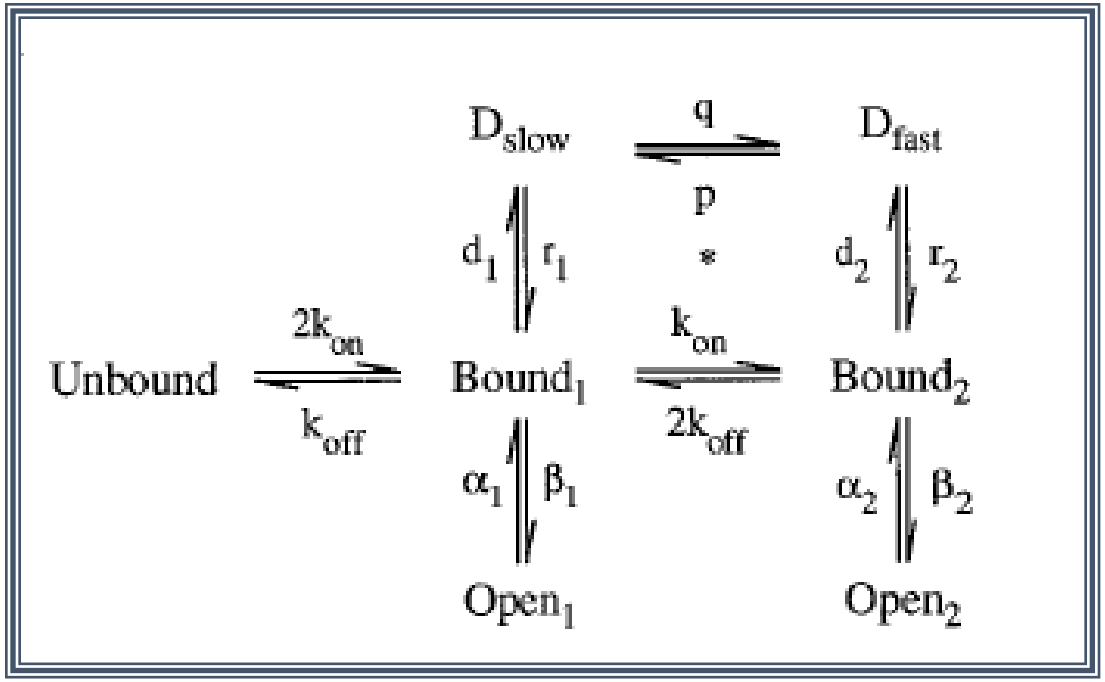
O= aberto

D = dessensibilizado

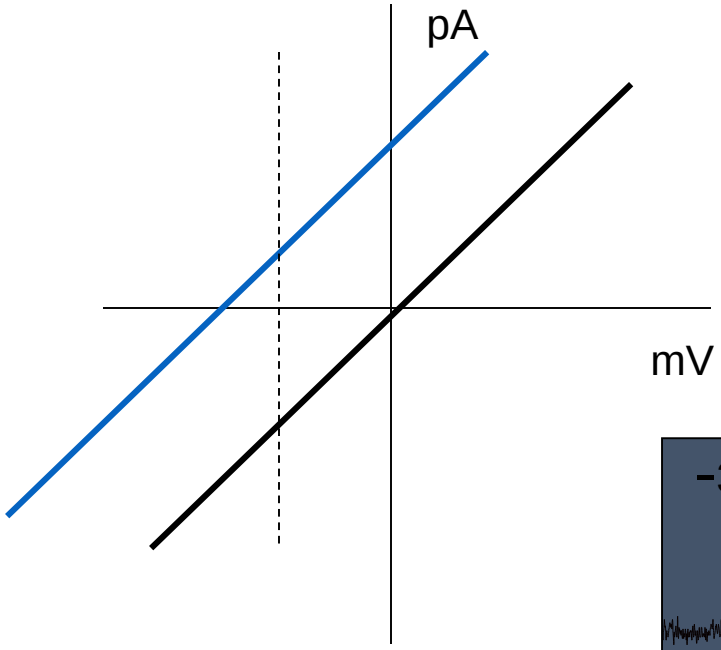
A = Acetilcolina

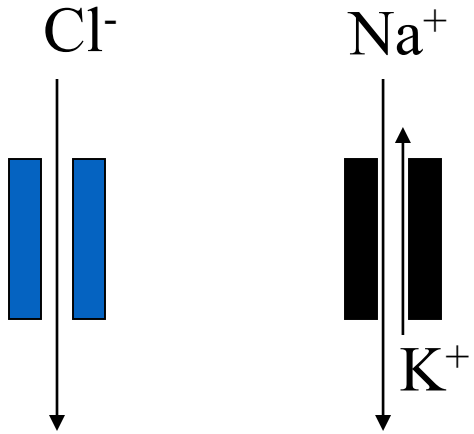
Modelos cinéticos

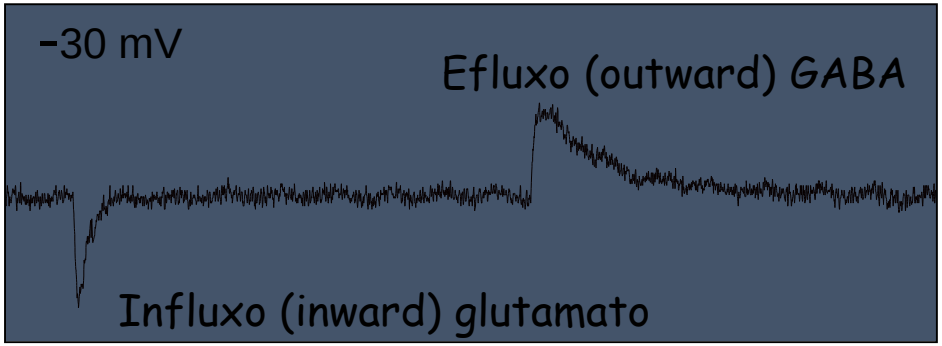
Receptor $GABA_A$



Polaridade/reversão



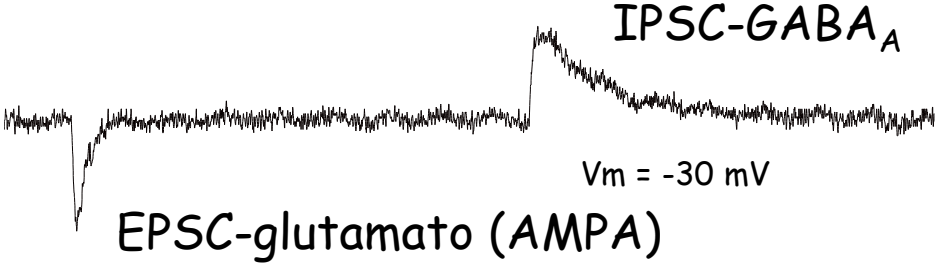




glutamato

GABA

Sinapses inibitórias e excitatórias



$$V_m = \left(\frac{g_s}{g_s + g_m}\right) E_{psp} + \left(\frac{g_m}{g_m + g_s}\right) E_m$$

$E_{rev(GABAA)} = E_{revCl} \sim -80$ mV

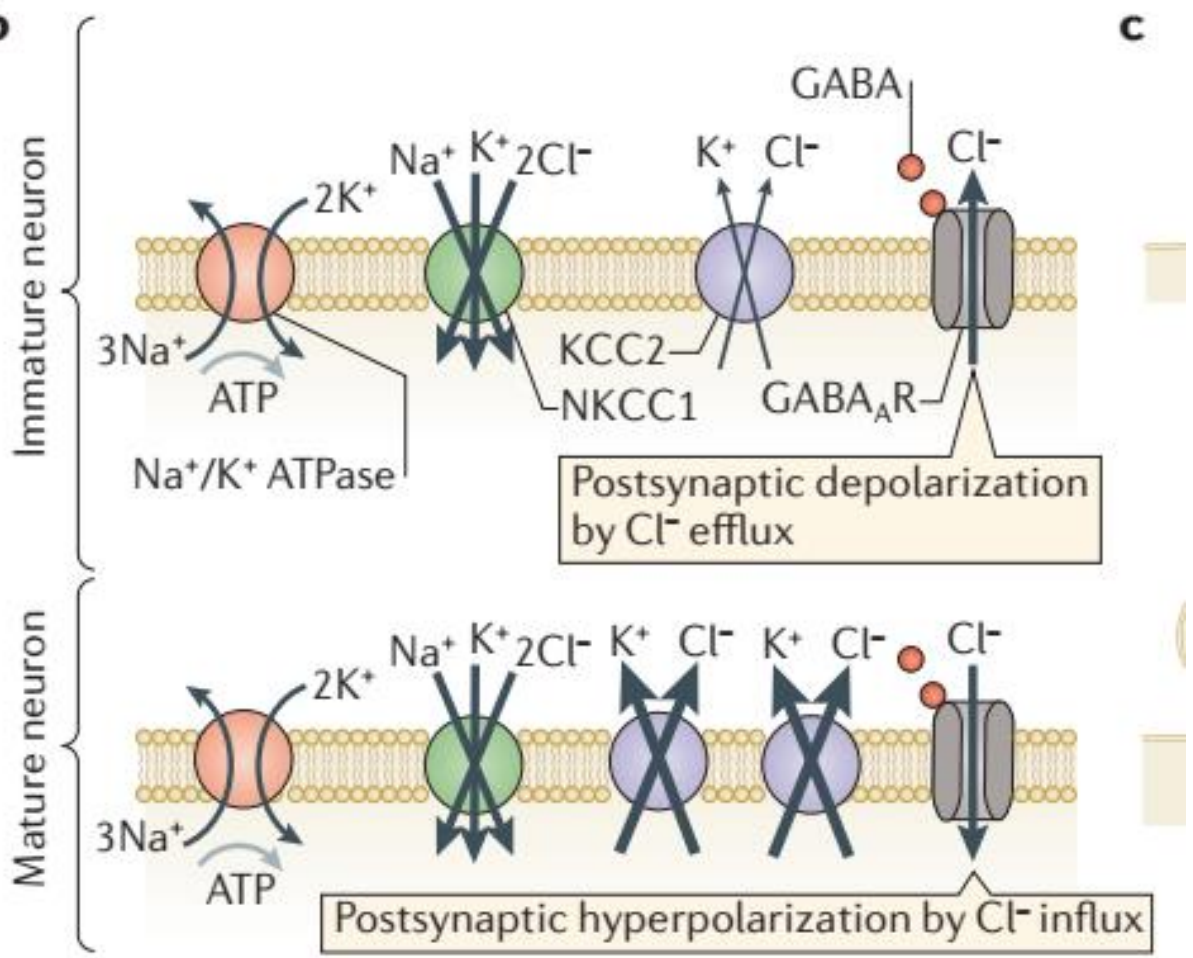
$E_{rev(AMPA)} \sim 0$ mV

- Sinapses excitatórias levam o E_m próximo de 0 mV (despolarizam)
- Sinapses inibitórias estabilizam o E_m próximo de repouso

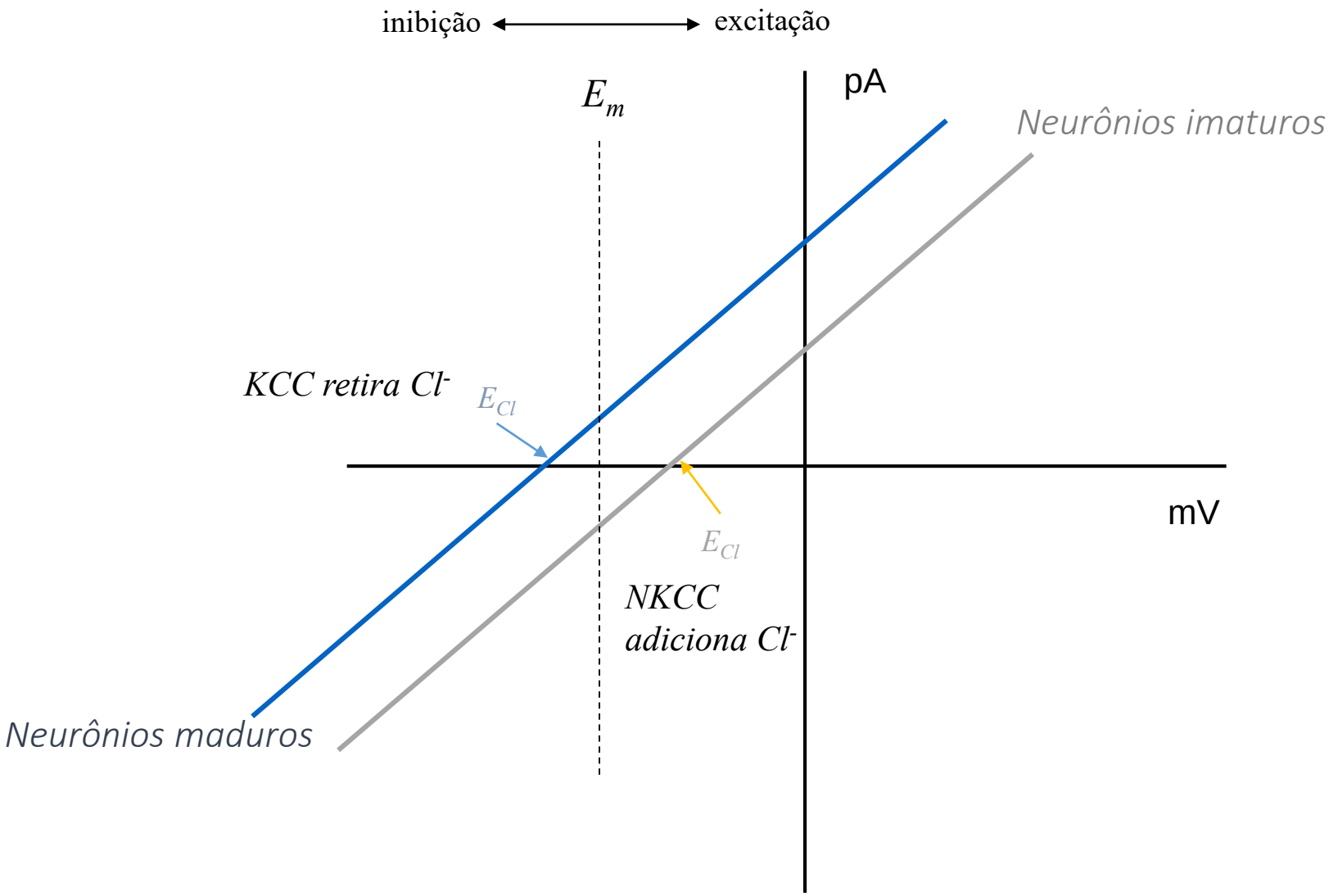
O gradiente de cloreto nos neurônios é influenciado por transporte ativo secundário

KCC = co-transportador potássio/cloreto

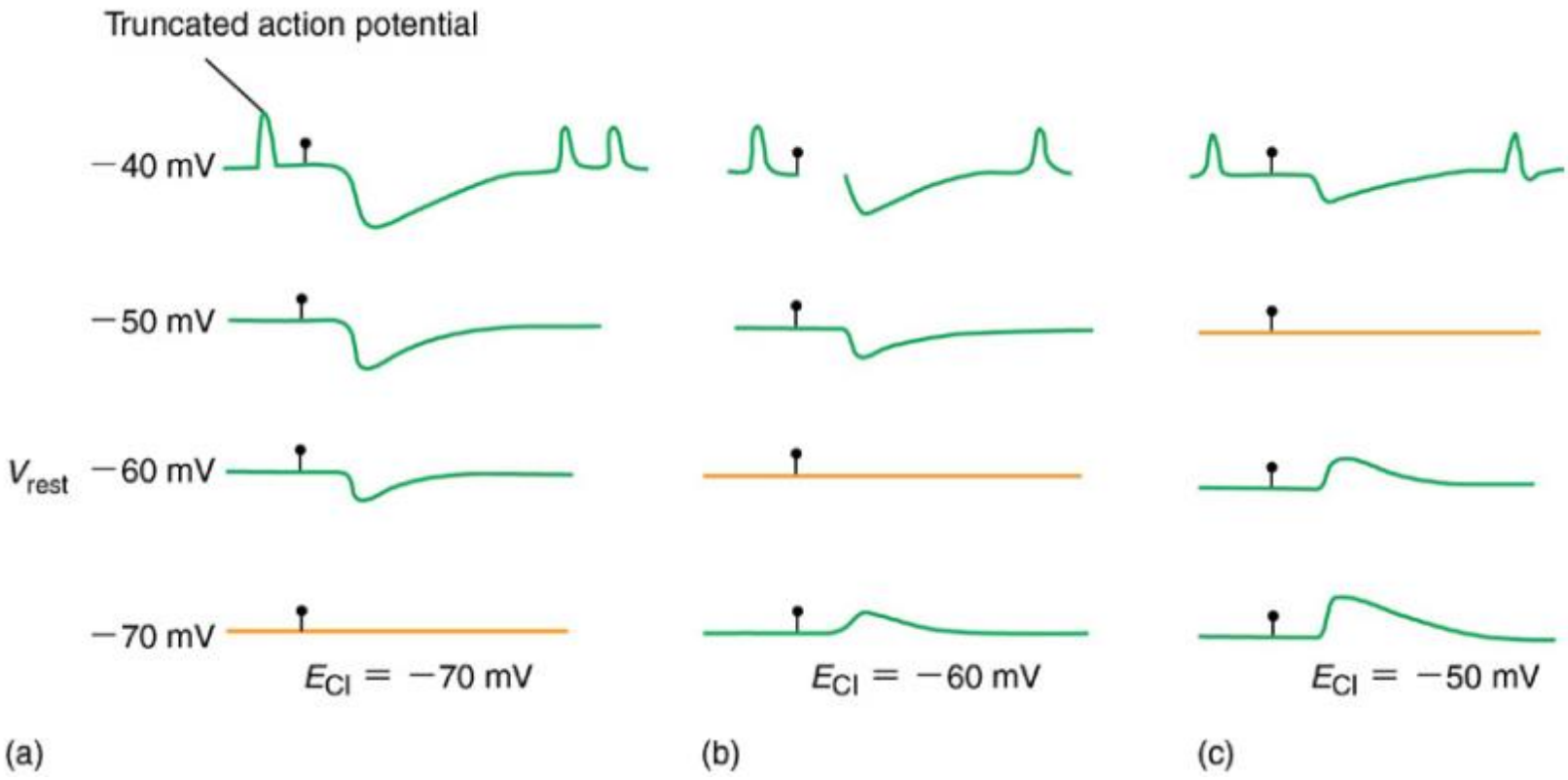
NKCC = co-transportador sódio/potássio/cloreto



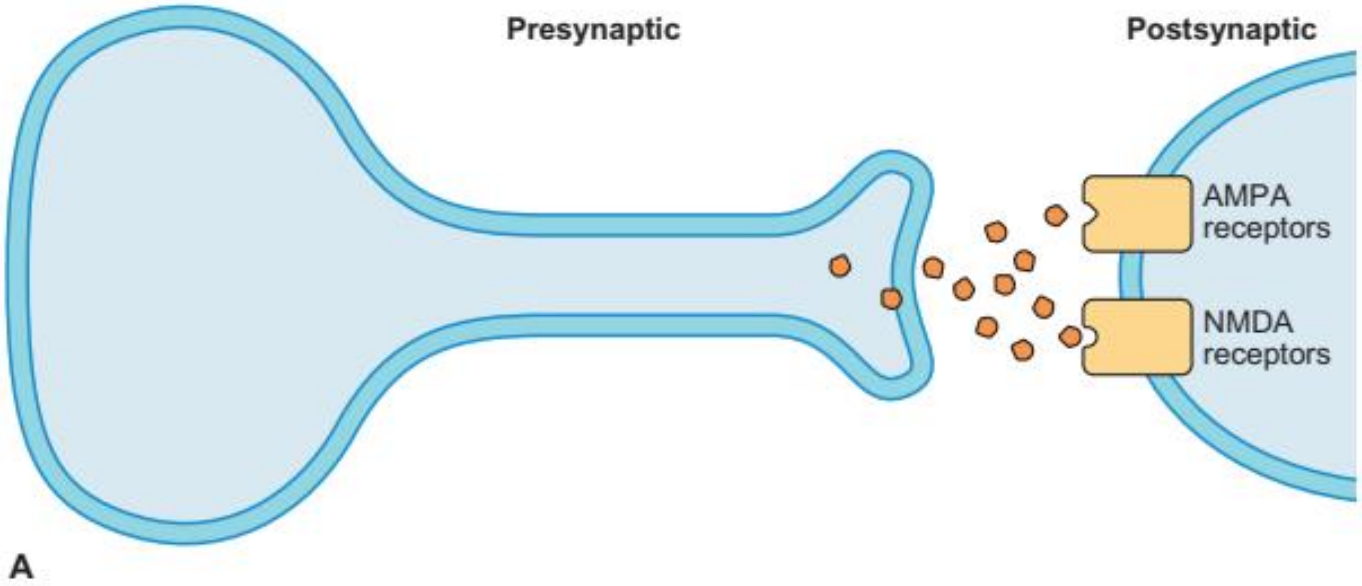
GABA pode ser excitatório dependendo da E_{Cl}



GABA pode ser excitatório dependendo da E_{Cl}

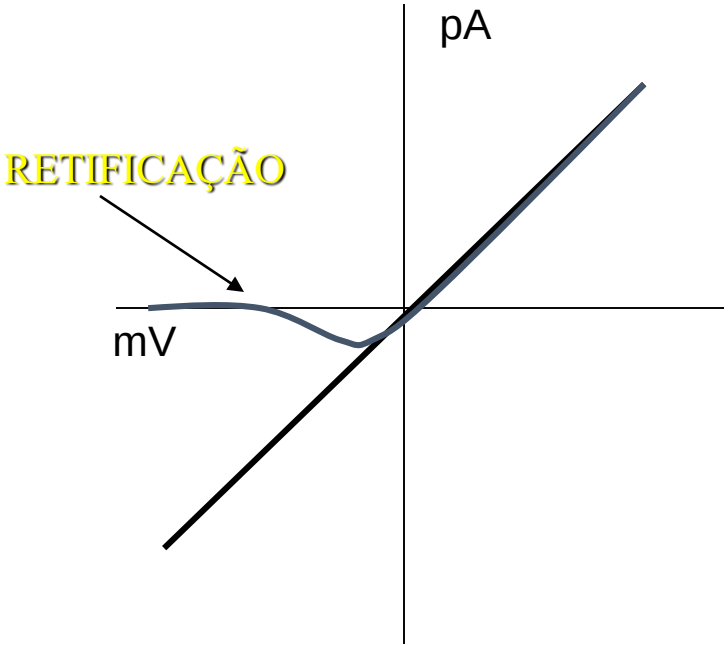


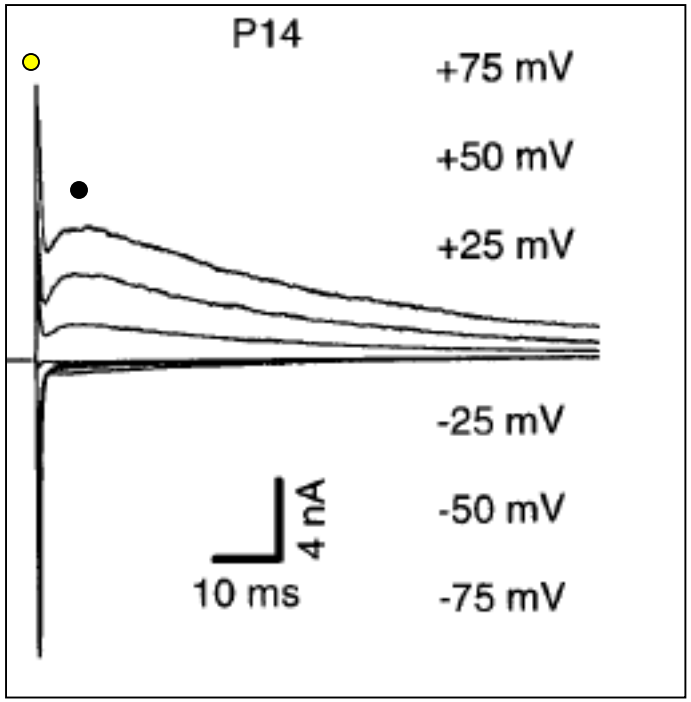
O glutamato ativa dois tipos de receptores com características biofísicas distintas: AMPA/kainato e NMDA



Diferenças cinéticas dos receptores

Receptores glutamatéricos AMPA x NMDA



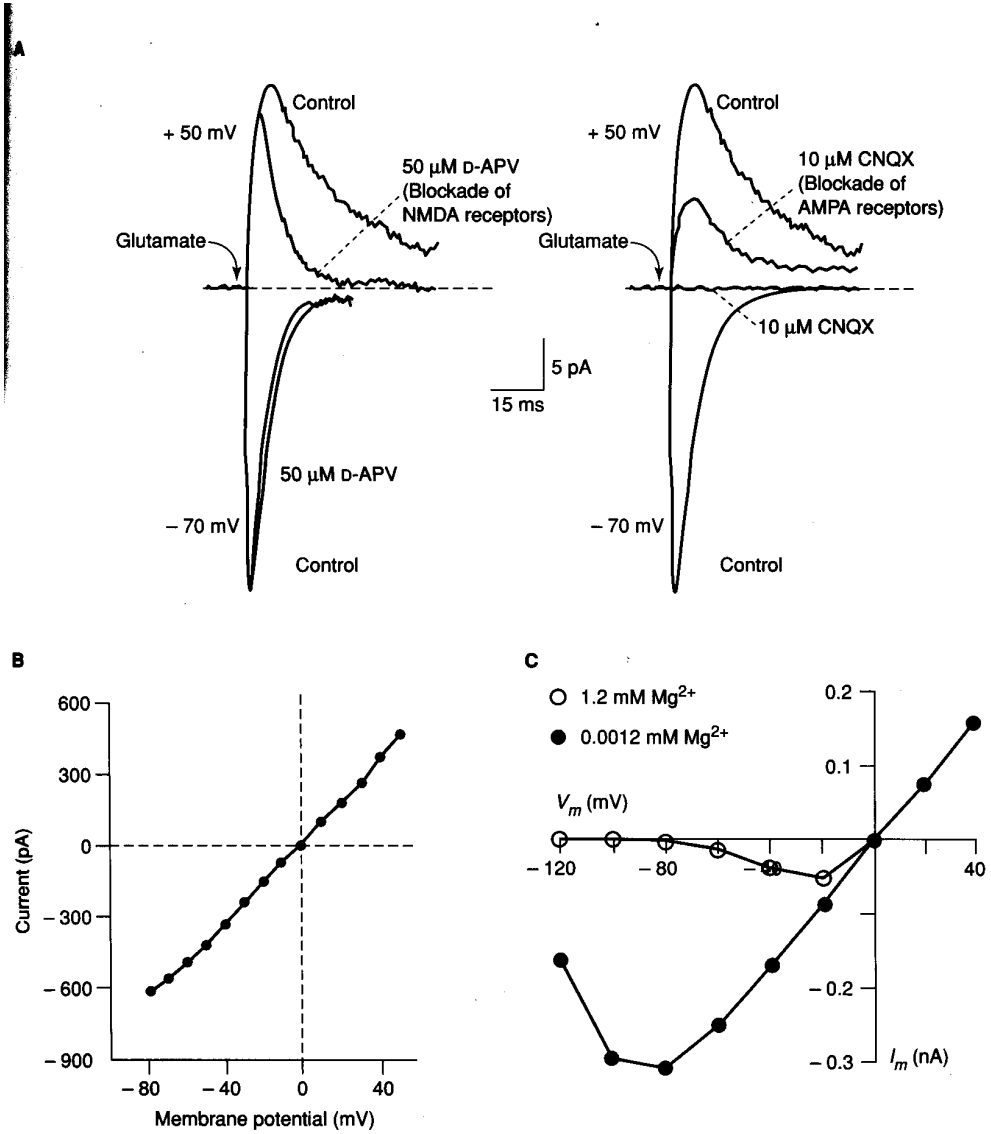


AMPA

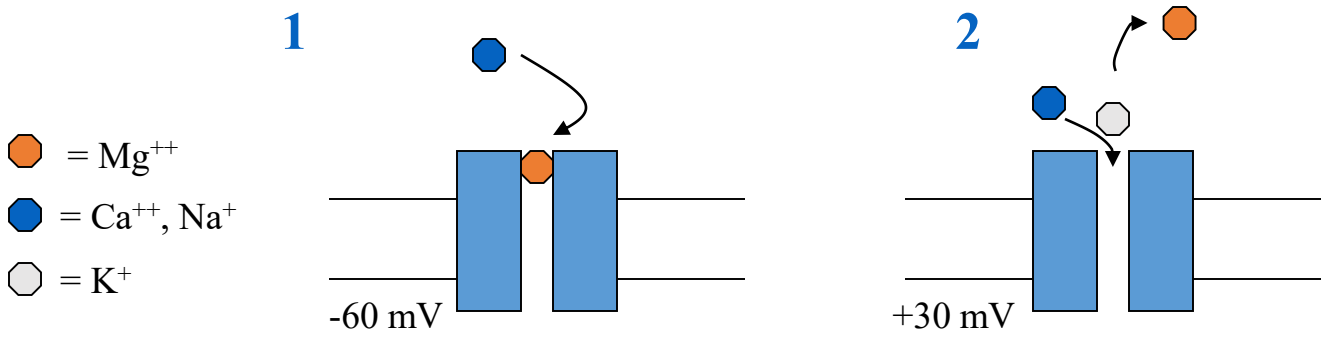
NMDA

- NMDA
- AMPA

O Receptor NMDA é bloqueado pelo Mg^{++} em potenciais hiperpolarizados



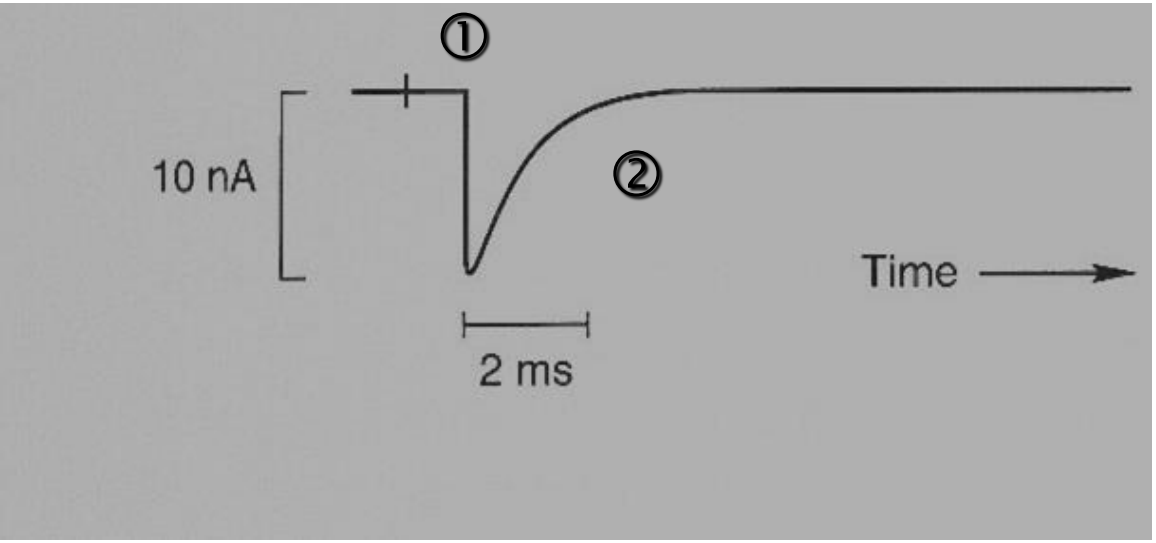
O Receptor NMDA é um detector de coincidência.



1. Bloqueio da entrada de cátios pelo magnésio externo no repouso
2. Em potenciais positivos a saída dos cátions expulsa o magnésio

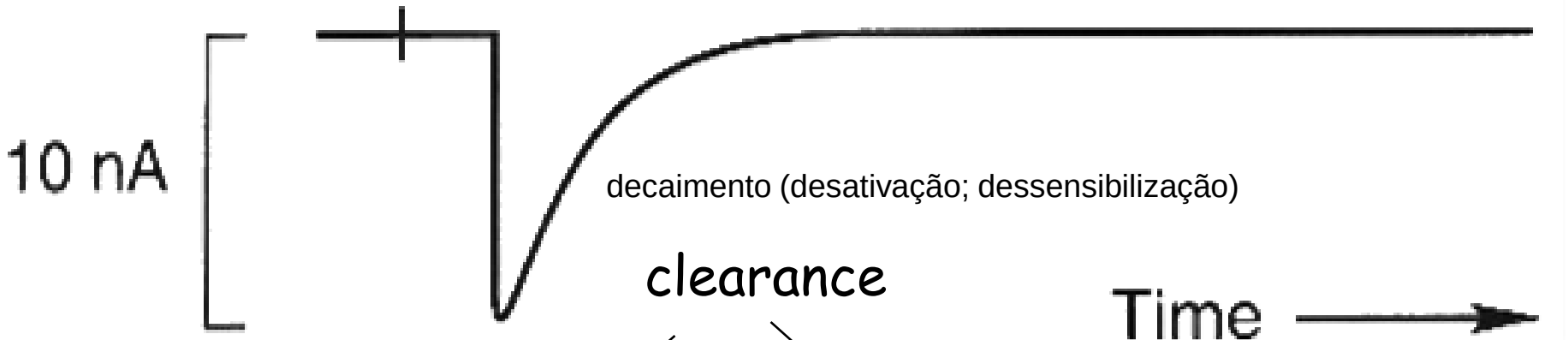
Apenas quando a despolarização da célula pós-sináptica for grande o suficiente (>0 mV) é que o receptor NMDA se abre e permite o influxo de cálcio

Morfologia funcional do evento sináptico
Análise de correntes



1. Ativação (rise-time)
2. Decaimento
    1. Desativação
    2. Dessensibilização

Decaimento

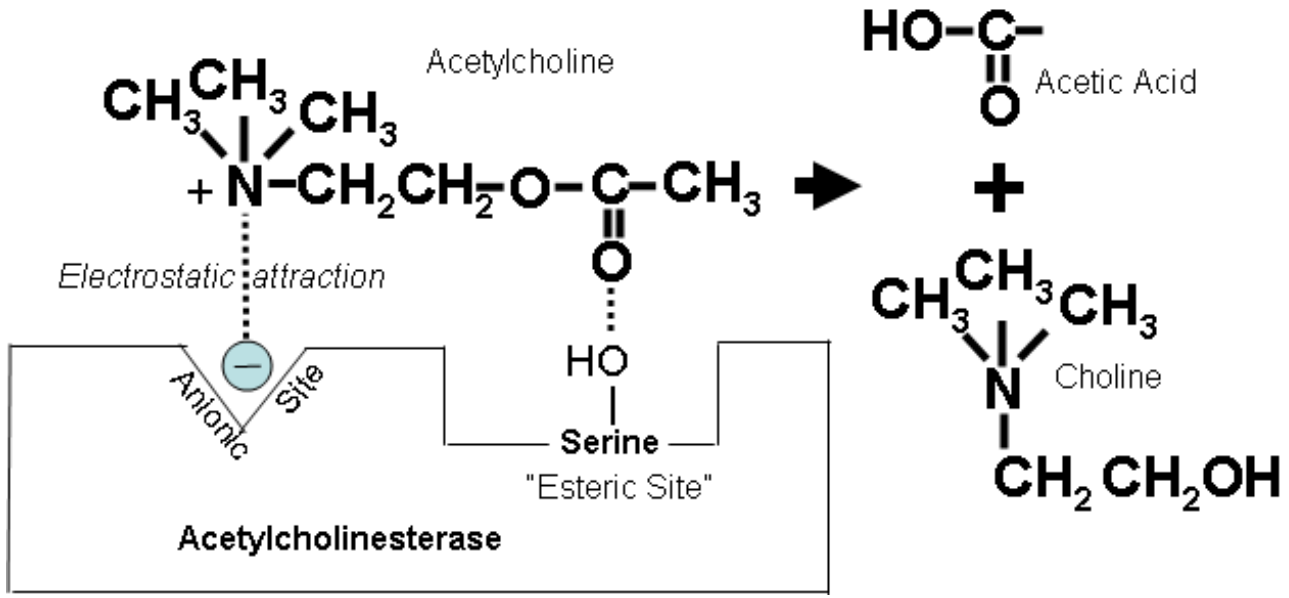


difusão do transmissor para fora da fenda sináptica

degradação enzimática (acetilcolina)

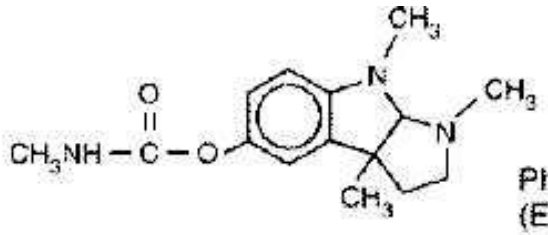
remoção por transportadores gliais e/ou neuronais

Acetilcolinaesterase cliva a acetilcolina em colina e acetato

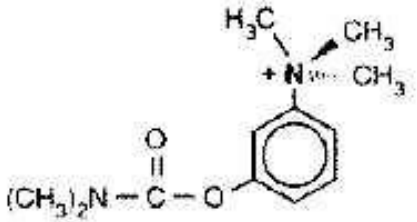


Inibidores da acetilcolinaesterase tem funções terapêuticas, de inseticidas e são usadas como armas químicas

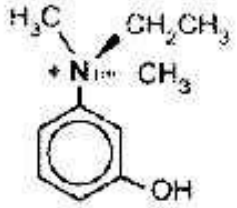
reversíveis



Physostigmine
(Eserine)

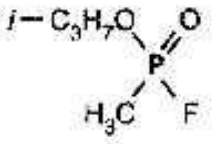


Neostigmine
(Prostigmin)

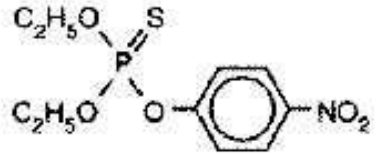


Edrophonium
(Tensilon)

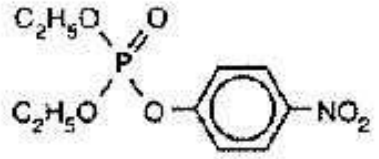
irreversíveis - organofosforados



Sarin
("nerve gas")

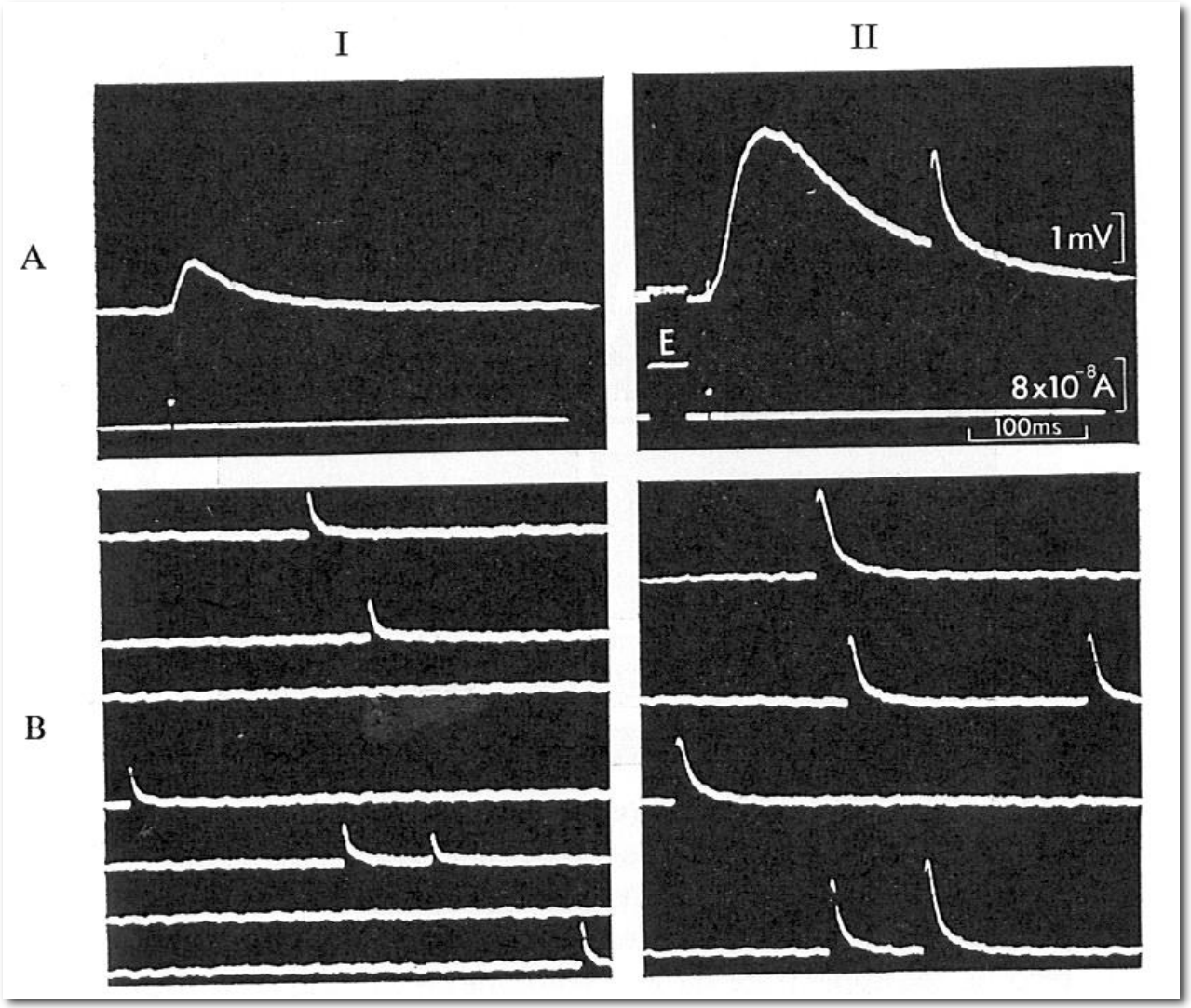


Parathion
(insecticide
precursor)

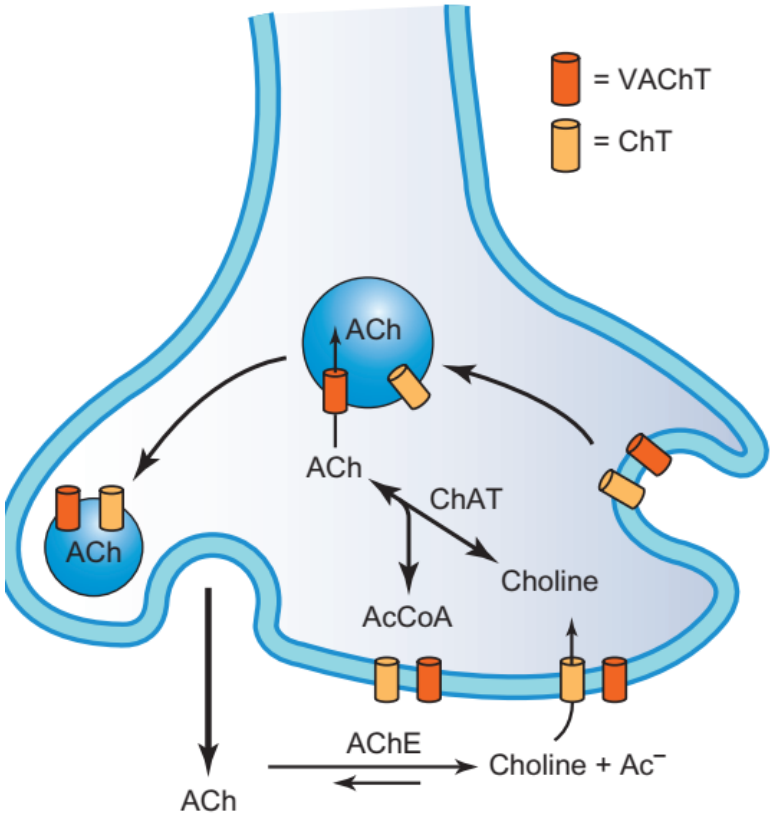


Paraoxon
(active product
of parathion)

Inibição da acetilcolinaesterase por eserina (II) aumentou a amplitude e duração de tanto os mEPSPs (A) quanto dos EPSPs (B)



A colina é recaptada pré-sinapticamente para resintese de acetil-colina



O glutamato é capitado por transportadores astrocíticos e é reciclado como glutamina para os terminais axonais.

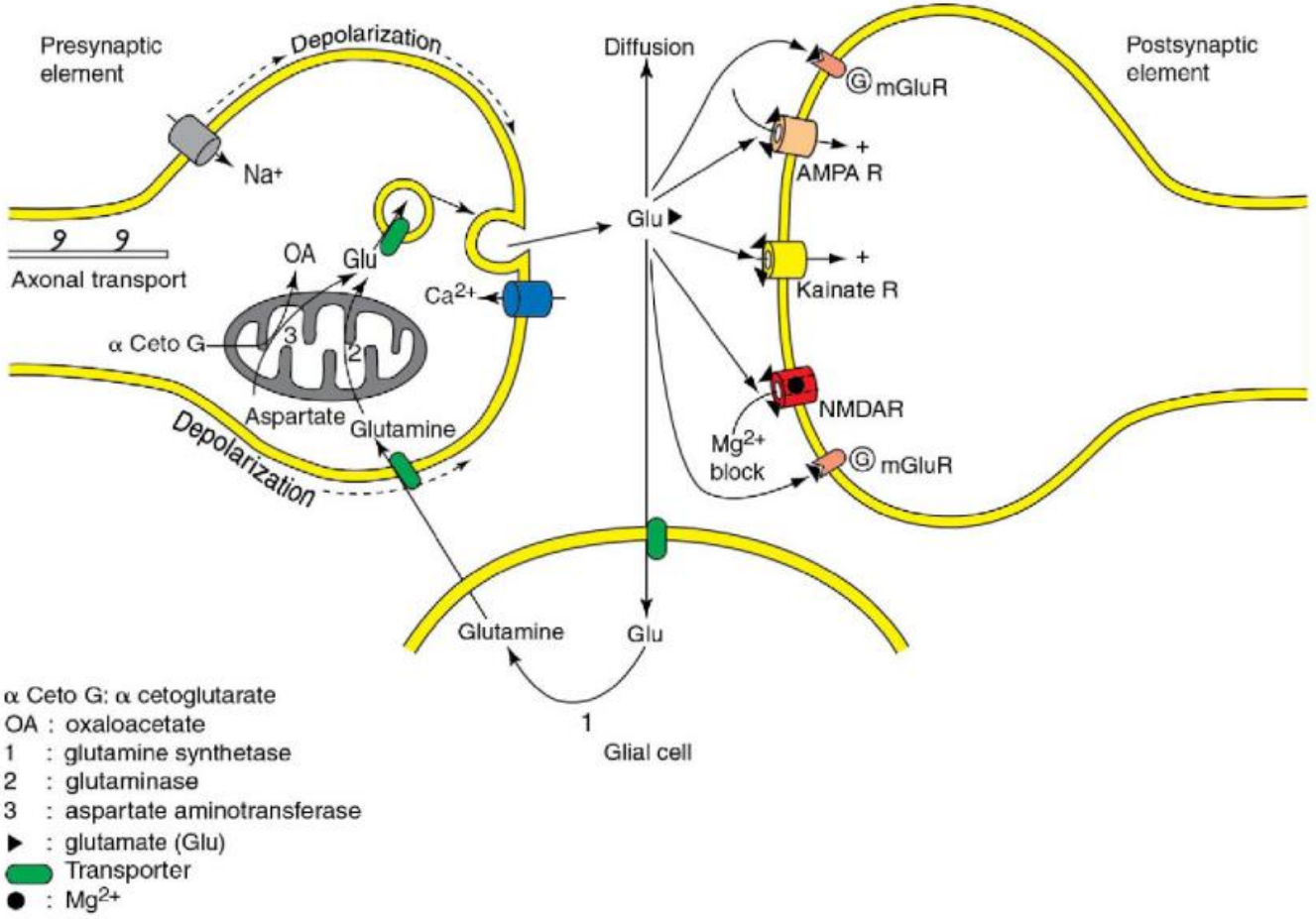
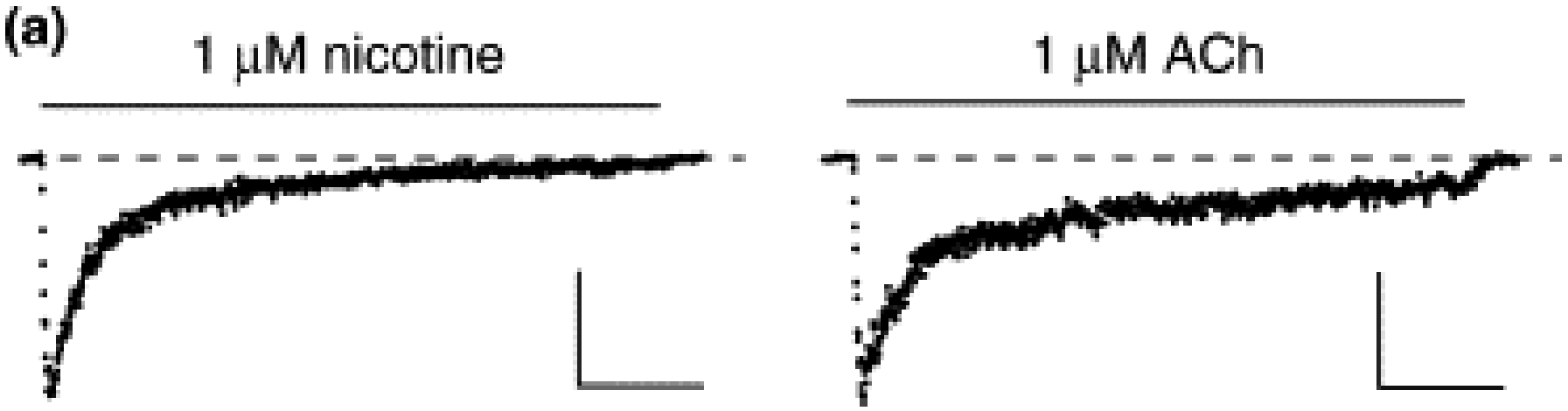
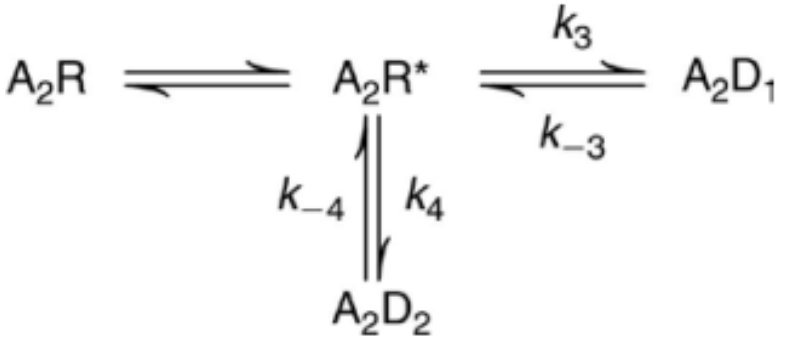


FIGURE 10.13 **The glutamatergic synapse.** Functional scheme of a glutamatergic synapse where ionotropic and metabotropic glutamate receptors are co-localized. Presynaptic receptors are omitted. The enzymes (1 to 3) and mitochondria are carried to axon terminals via anterograde axonal transports. Glutamate synthesized in mitochondria of the presynaptic element is transported actively into synaptic vesicles by a vesicular carrier. A percentage of the glutamate released in the synaptic cleft is uptaken into presynaptic terminals and glial cells by transporters. Inset shows iGluRs antagonists. Inset from Mody I (1998) Interneurons and the ghost of the sea. *Nat. Neurosci.* **1**, 434–436, with permission.

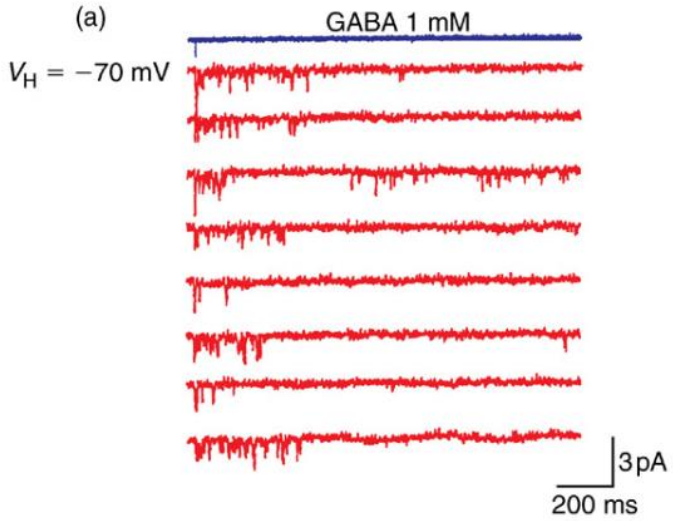
Decaimento das correntes sinápticas

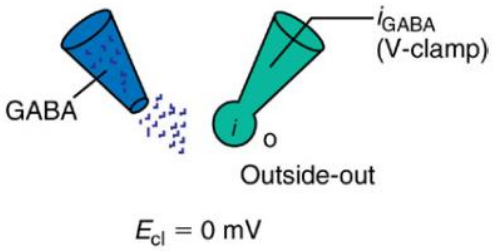


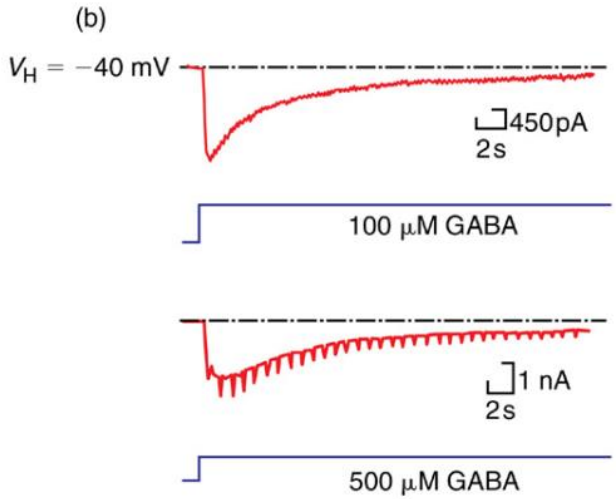
Dessensibilização = fechamento do canal na presença do agonista

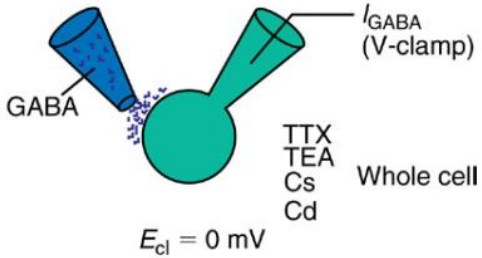


A dessenssibilização dos receptores contribui para o decaimento da corrente sináptica

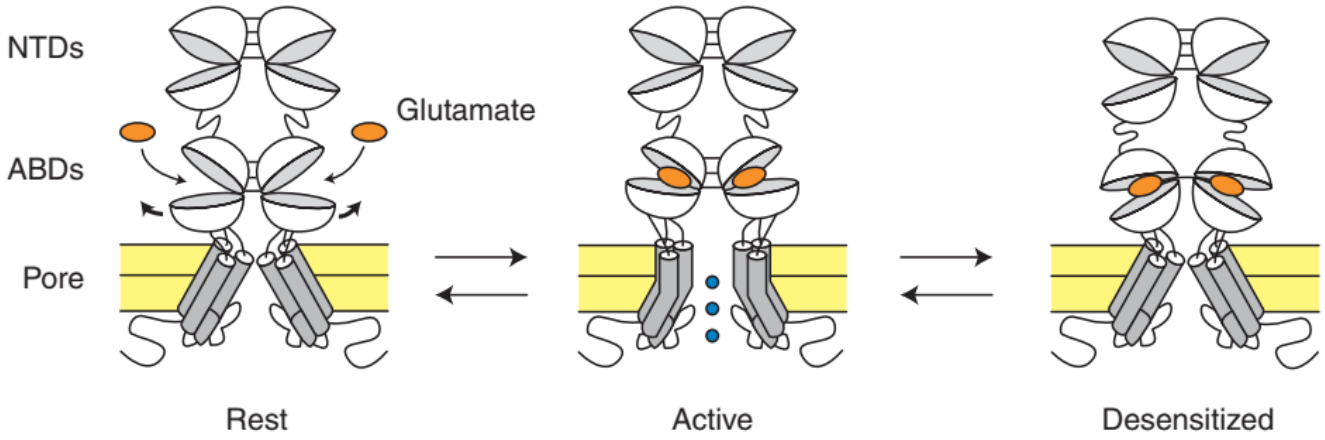


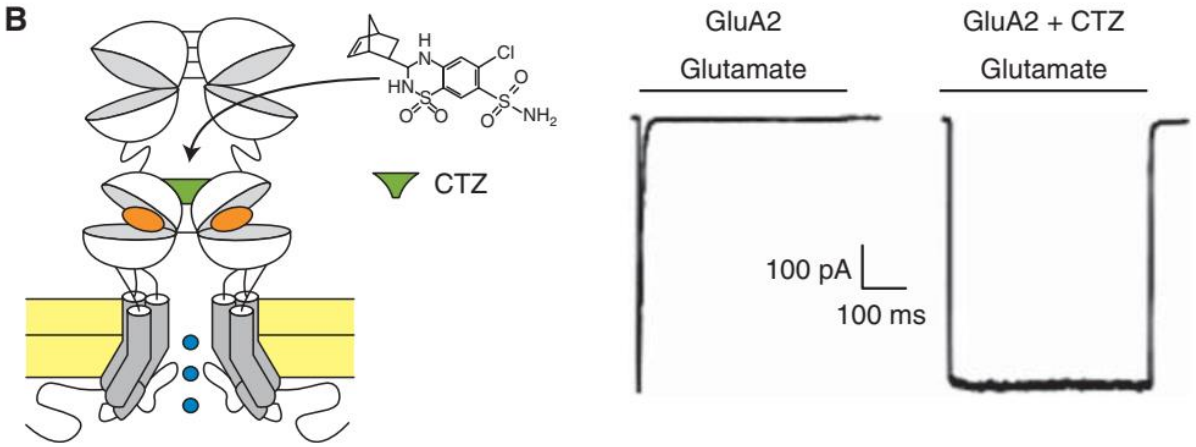






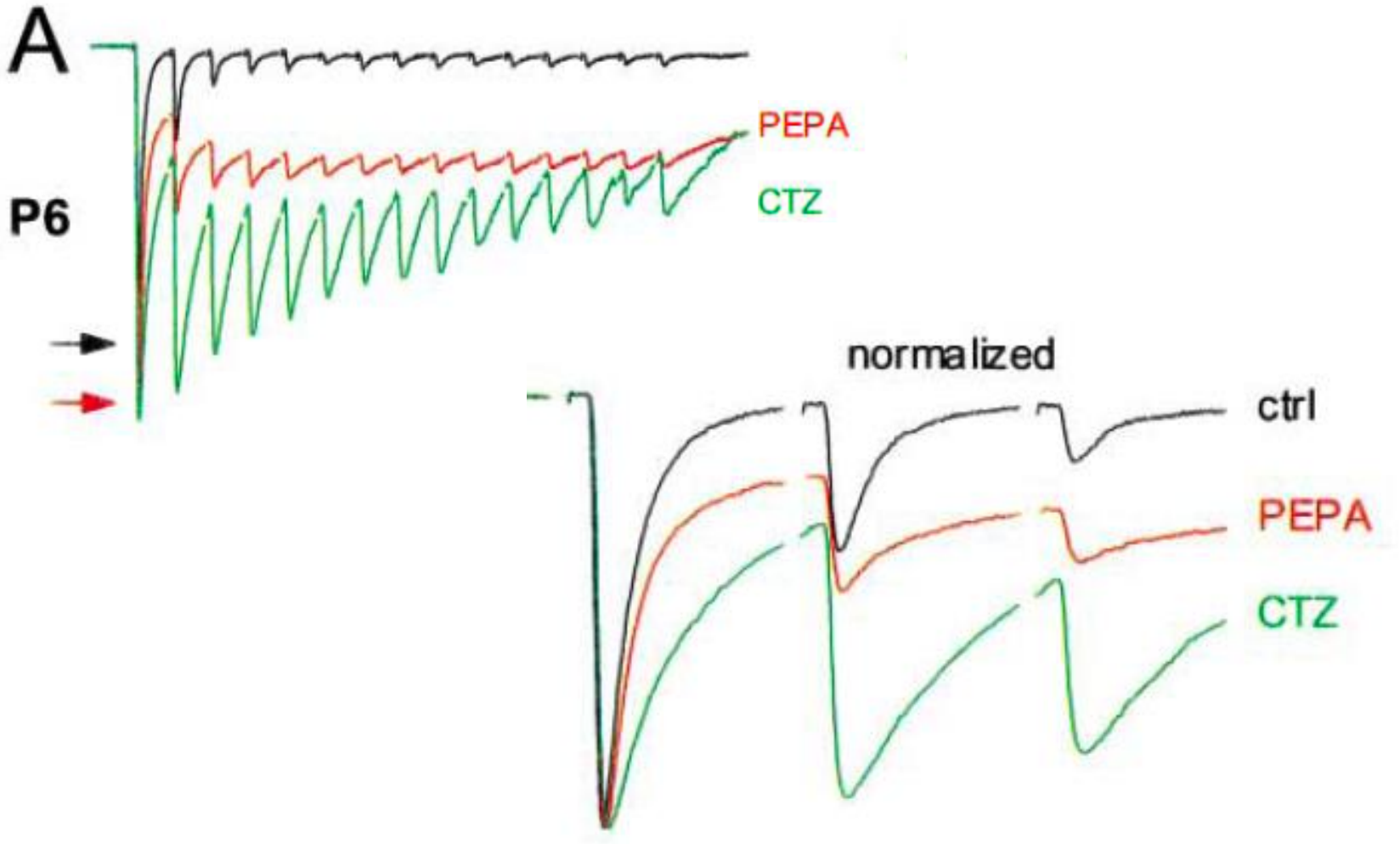
Mecanismo de dessensibilização do receptor AMPA



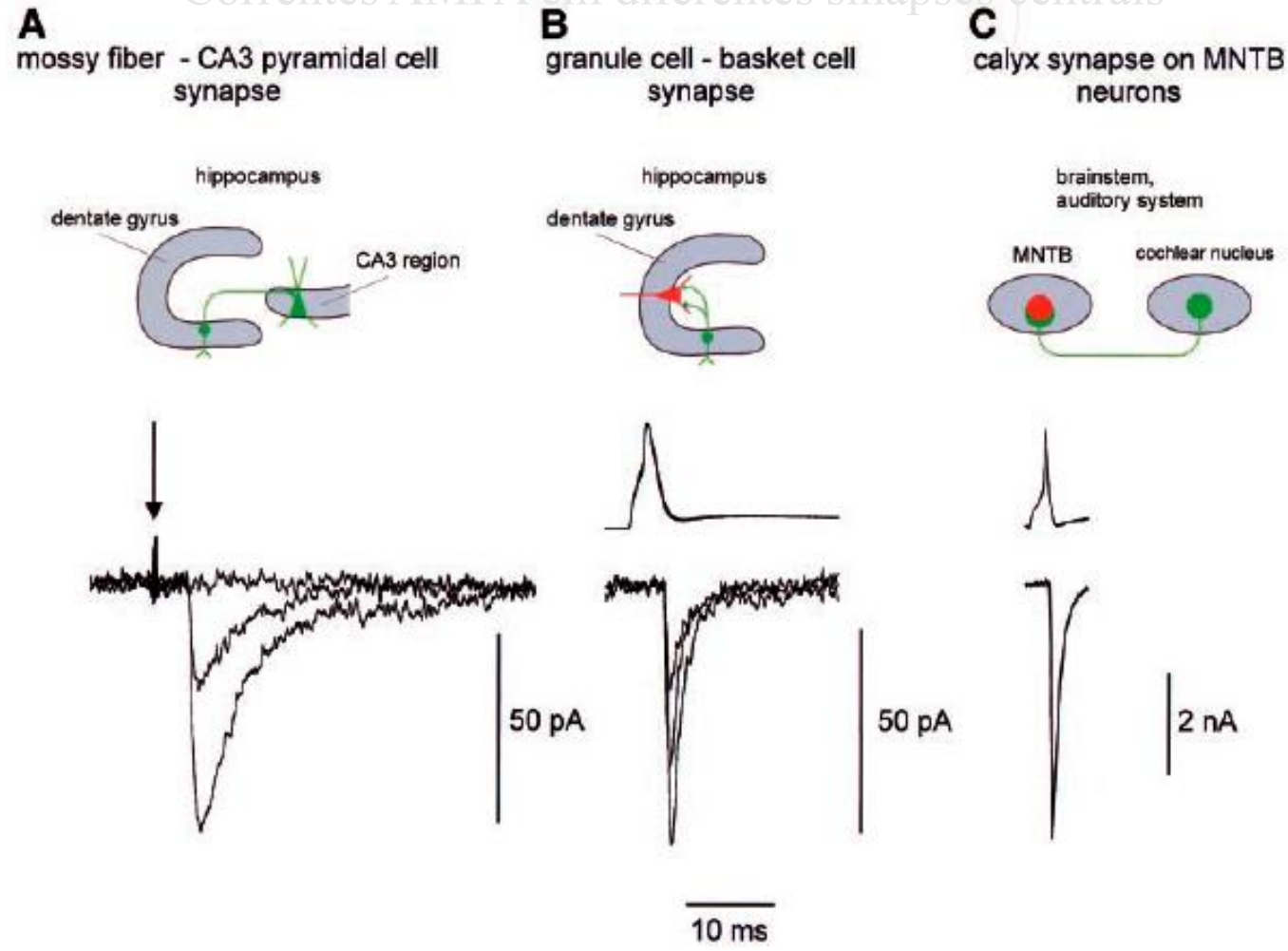


Cold Spring Harb Perspect Biol 2012;4:a009662

Algumas drogas como a ciclotiazida previnem a dessesssibilização do recepor AMPA revelando uma depressão devido a dessenssibilização desses receptores

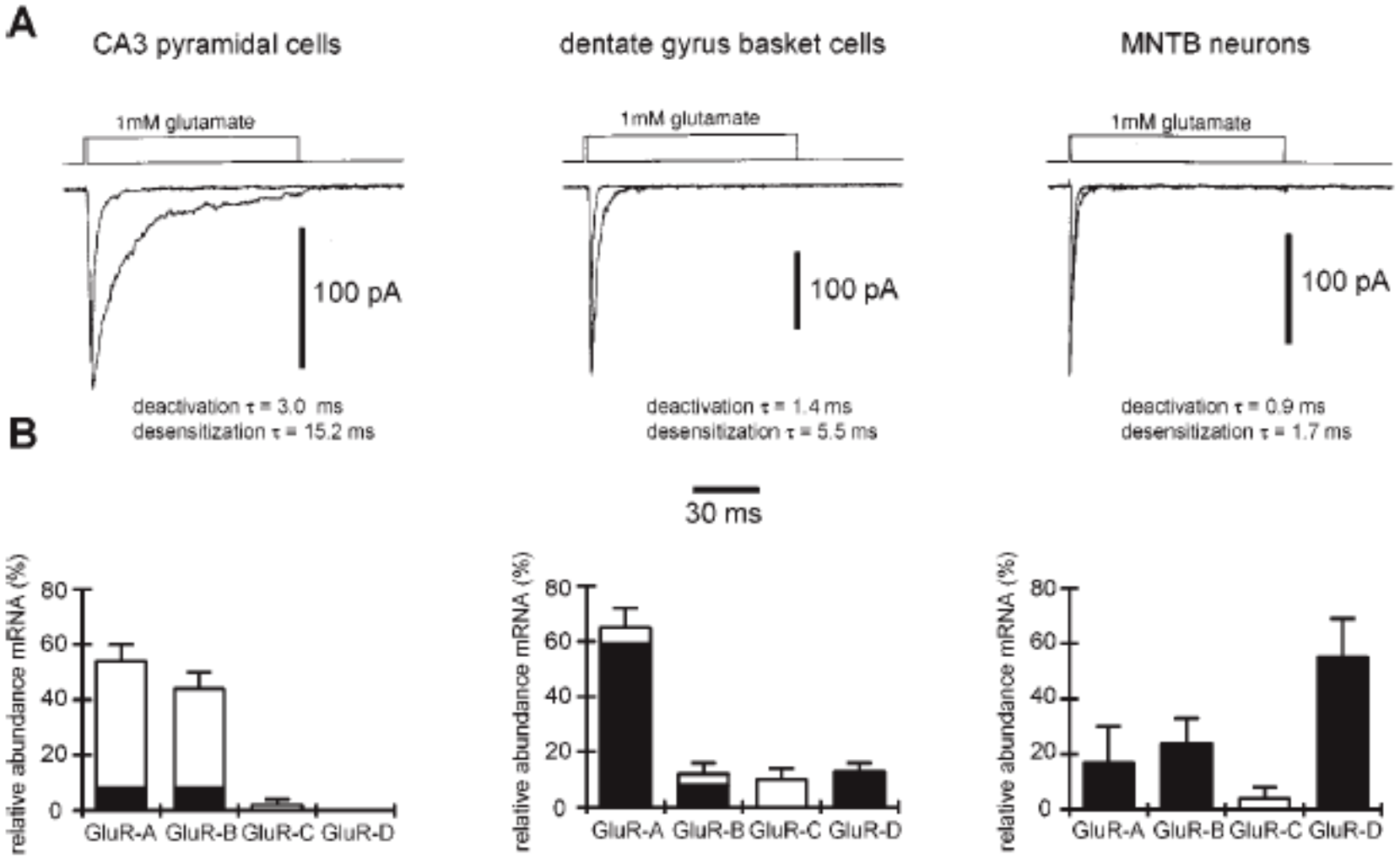


Diferentes sinapses possuem receptores com características diferentes



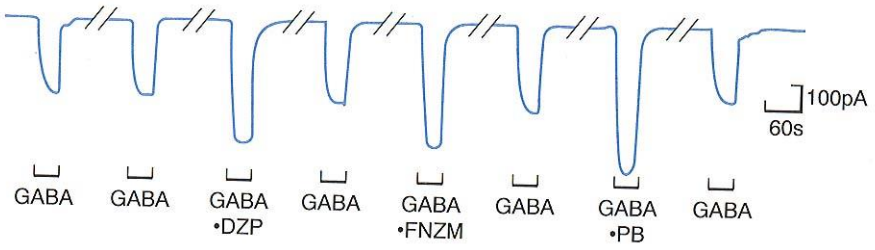
Diferentes sinapses possuem receptores com características diferentes devido as diferenças nas composições de subunidades

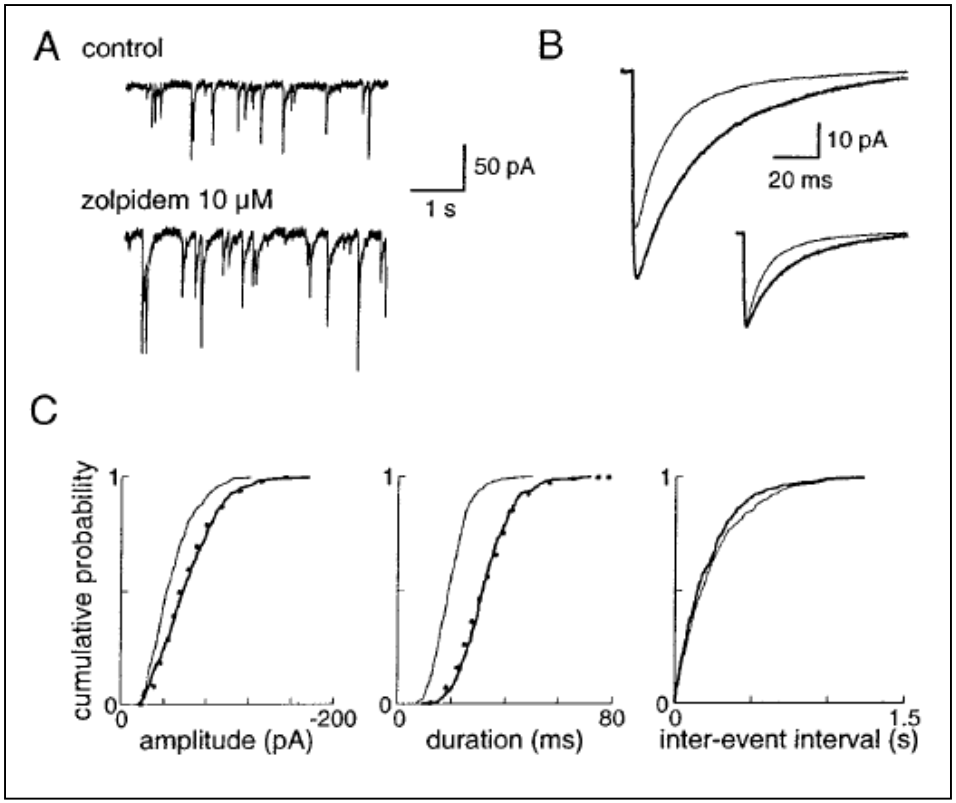
Correntes AMPA em diferentes sinapses centrais e distribuição dos diferentes tipos de subunidades



Certos agonistas modificam a cinética dos receptores

benzodiazepínicos nos receptores $GABA_A$



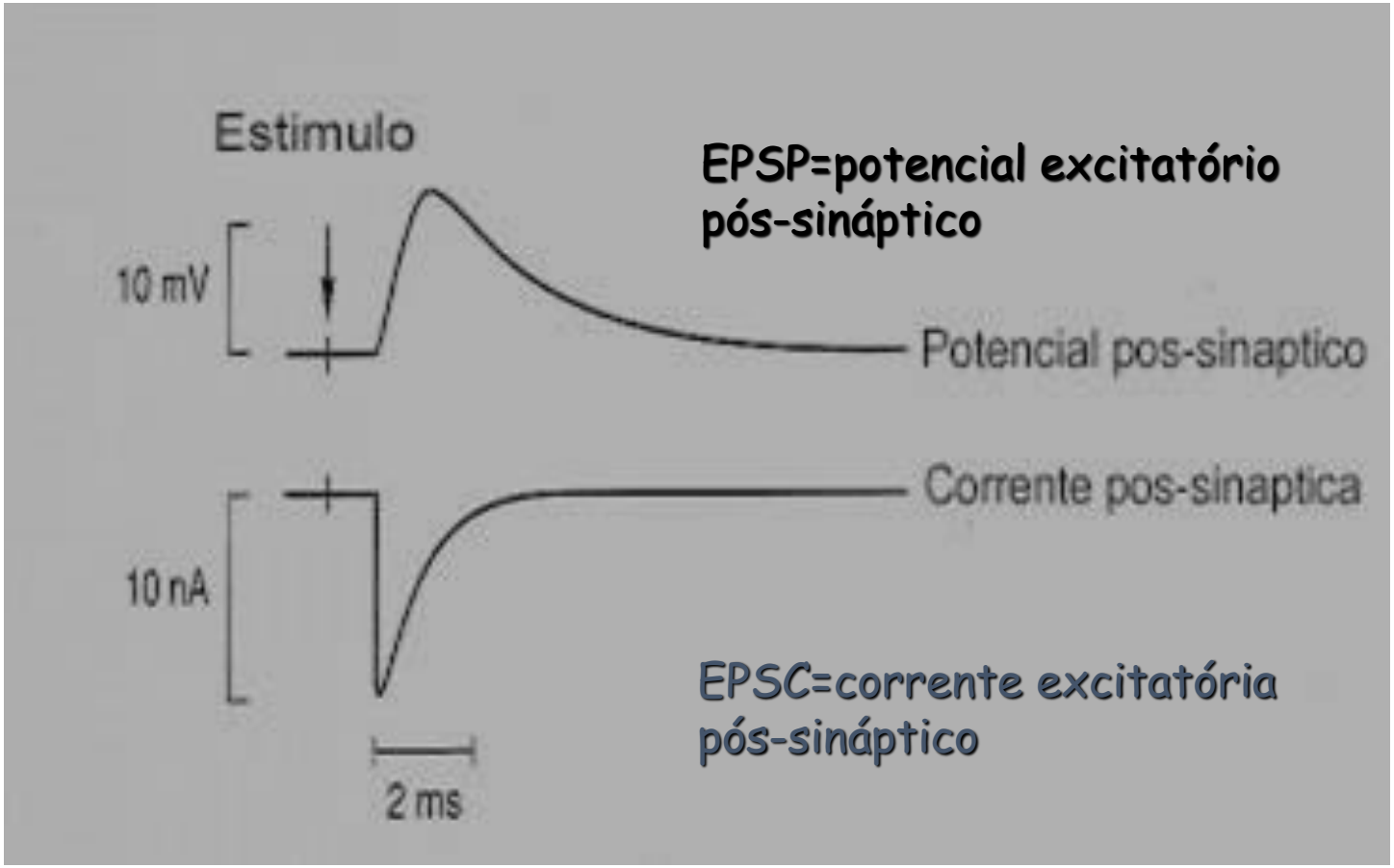


-Aumento na afinidade.
-Diminuição da velocidde de desativação.
-Ausência de efeito pré-sináptico.

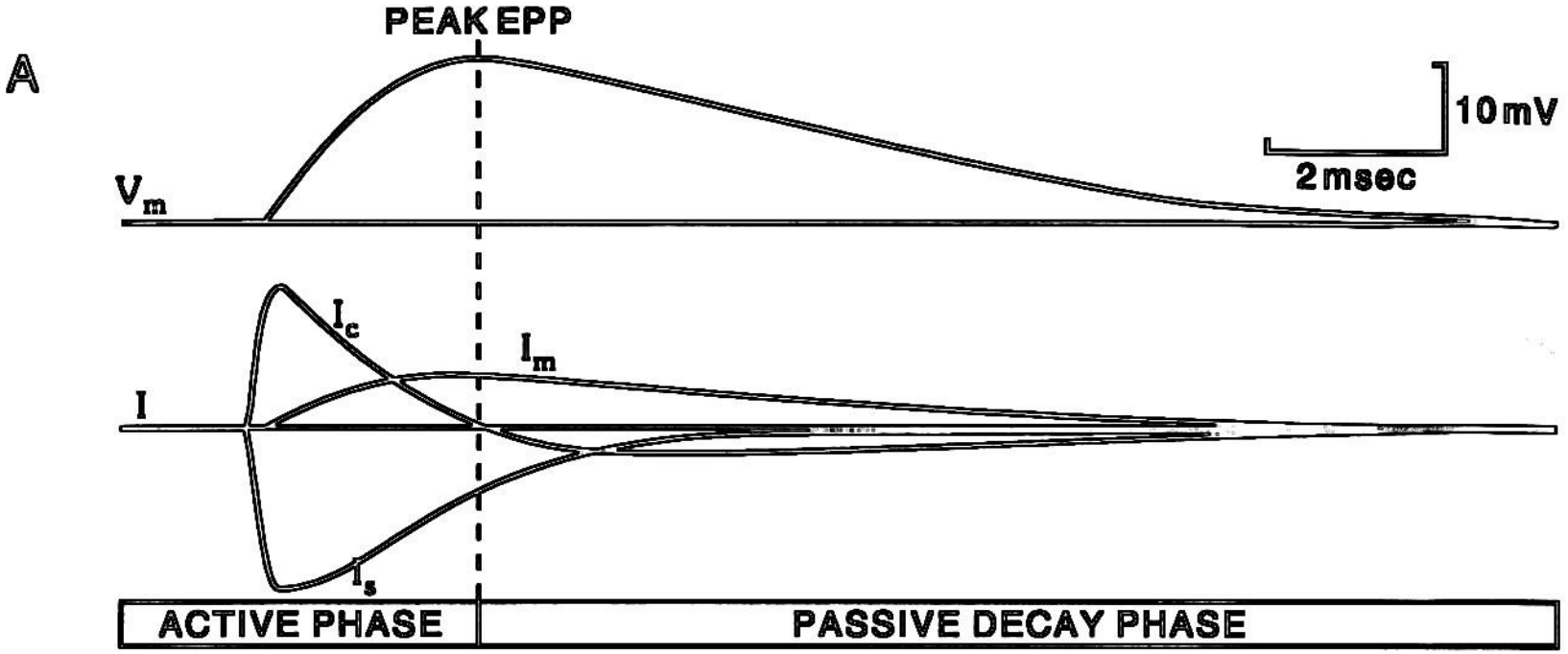
-Barbiturattos, álcool aumentam a duração das aberturas do canal

Obs. Zolpidem apesar de não ser uma BZD atua no sítio da BZD

O fluxo iônico pelos receptores ionotrópicos (corrente) gera uma mudança de potencial da membrana

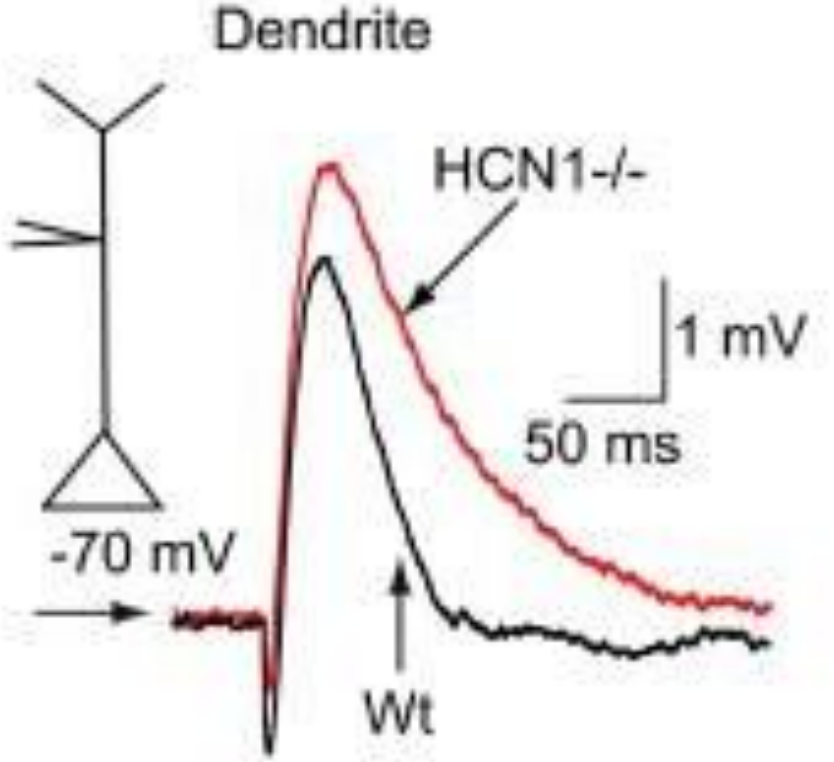


A corrente sináptica (Is) em conjunto com as correntes intrínsicas (Im) e capacitivas (Ic)“moldam” o potencial sináptico resultante

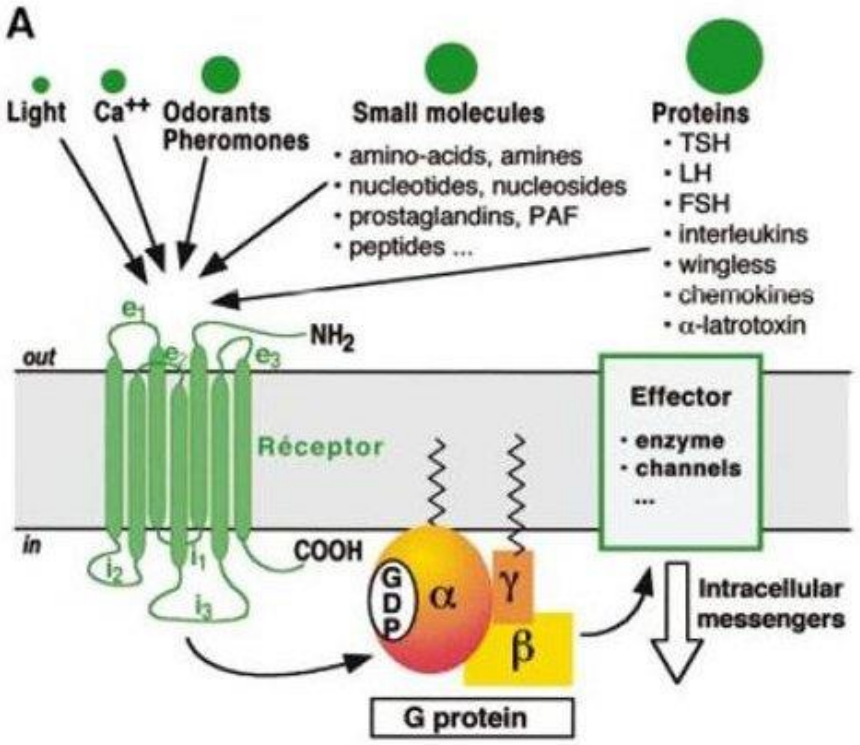


Correntes iônicas no soma e dendrito modulam os potenciais pós-sinápticos.

Efeito da deleção dos canais catiônicos HCN.



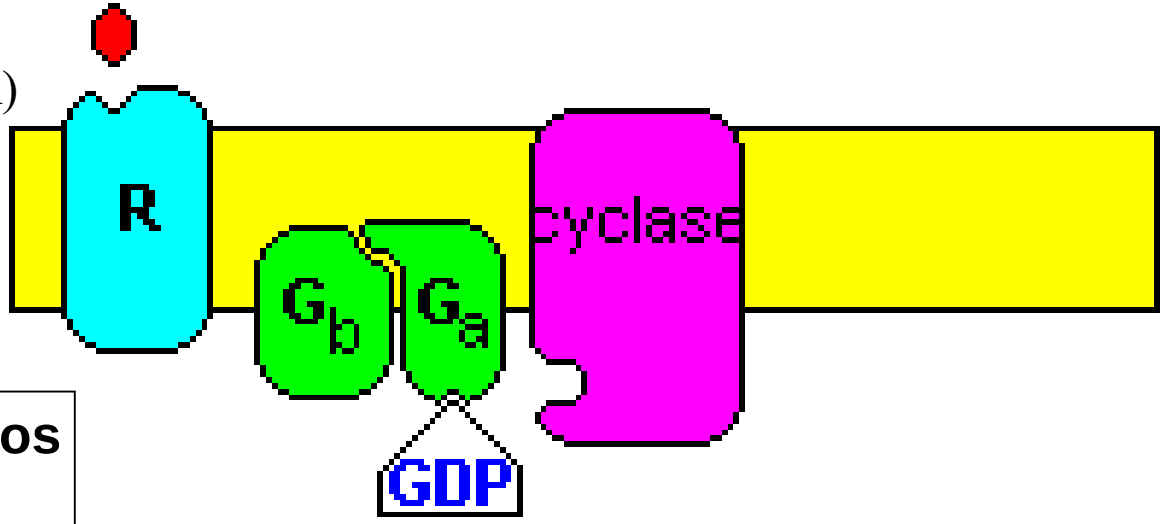
Recetpores ligados a proteínas G com 7 segmentos transmembrana (7M-GPCR)



Reproduced with permission from
"Molecular tinkering of G protein-coupled receptors: an evolutionary success"
The EMBO Journal Vol. 18 No. 7 pp.1723-1729, 1999

A maioria dos receptores metabotrópicos ativam proteínas G

Efeitos pré-sinápticos:
-inibição de canais de cálcio
-ativação de canais de potássio (GIRK)

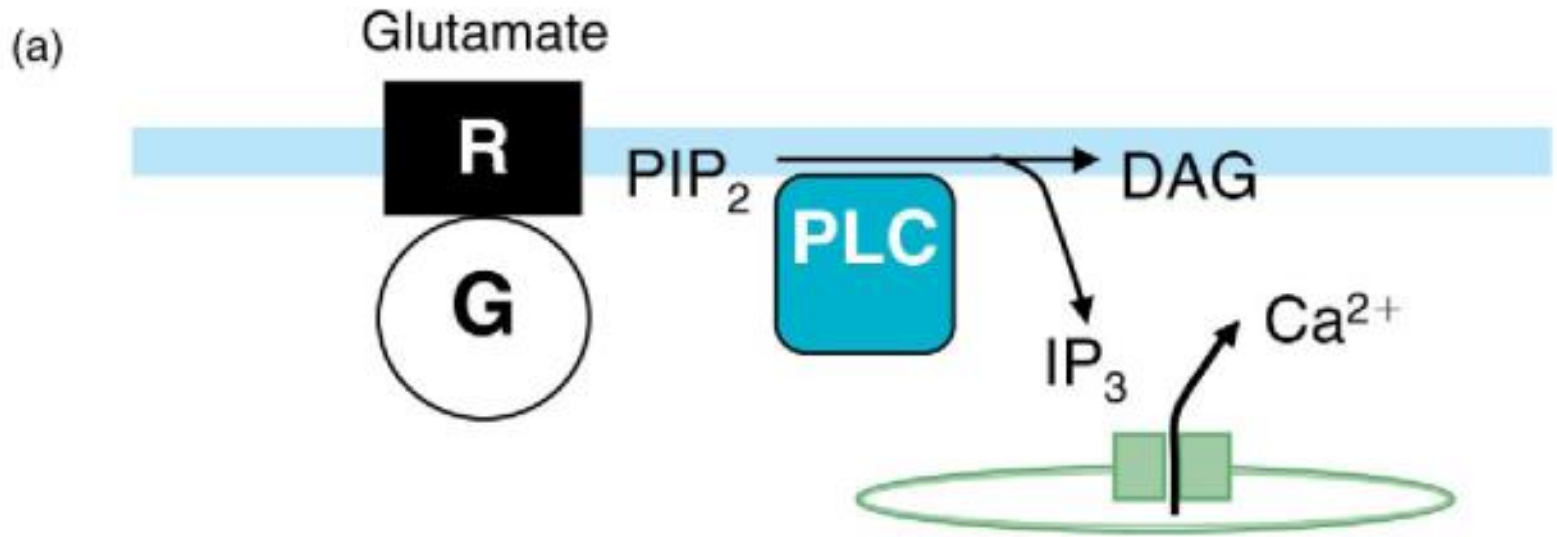


transmissores modulatórios (metabotrópicos)

glutamato
GABA-$GABA_B$
adrenalina
serotonina
dopamina
adenosina

- Modificação de canais para íons
- Modificação de função de proteínas
- Alteração da expressão gênica

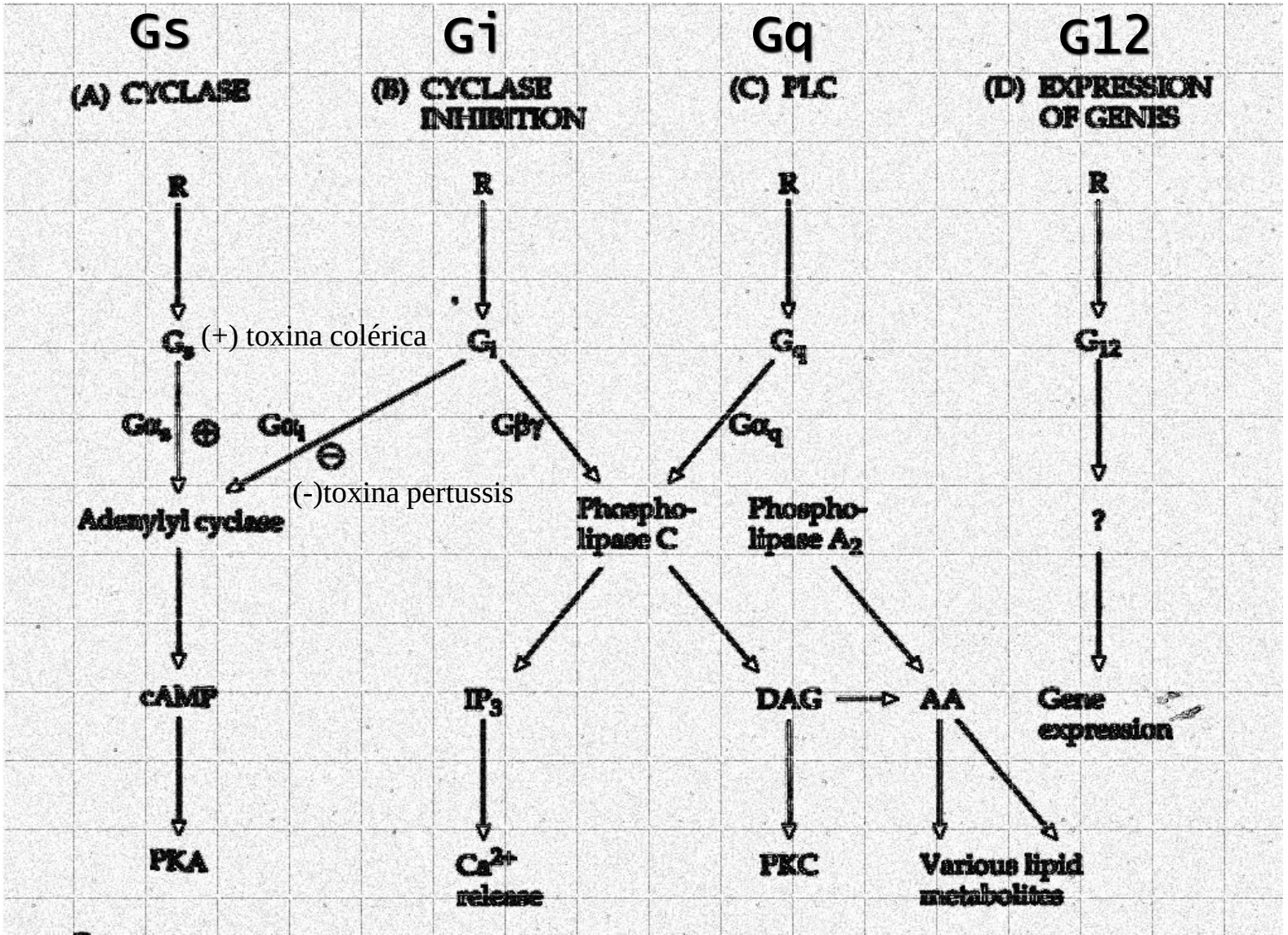
Uma via de sinalização de mGluRs



Variedade de Proteínas G

15 genes em mamíferos

~1500 receptores identificados

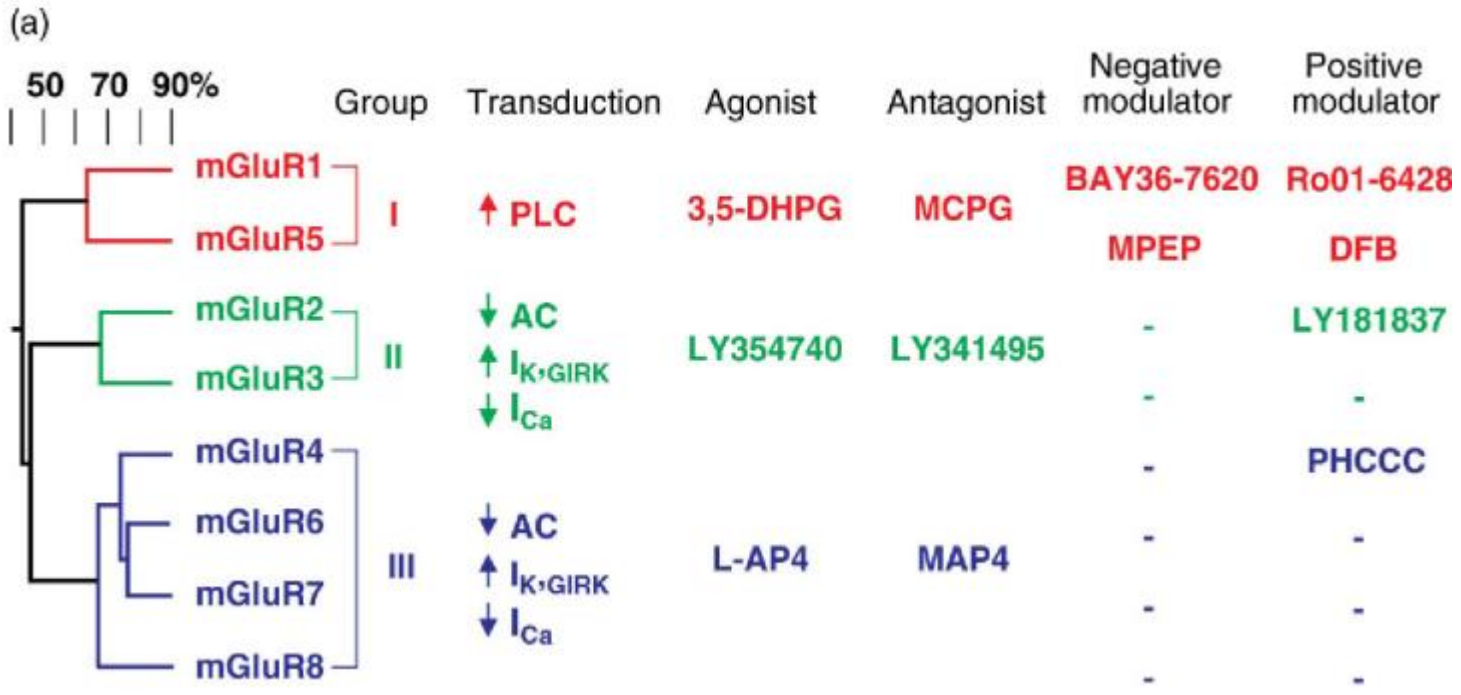


Diversidade dos receptores metabotrópicos

(B)

Receptor class	Glutamate	$GABA_B$	Dopamine	NE, Epi	Histamine	Serotonin	Purines	Muscarinic
Receptor subtype	Class I	$GABA_B$ R1	$D1_A$	α1	H1	5-HT 1	A type	M1
	mGlu R1	$GABA_B$ R2	$D1_B$	α2	H2	5-HT 2	A1	M2
	mGlu R5		D2	β1	H3	5-HT 3	A2a	M3
	Class II		D3	β2		5-HT 4	A2b	M4
	mGlu R2		D4	β3		5-HT 5	A3	M5
	mGlu R3					5-HT 6	P type	
	Class III					5-HT 7	P2x	
	mGlu R4						P2y	
	mGlu R6						P2z	
	mGlu R7						P2t	
	mGlu R8						P2u	

Diversidade dos mGlurs



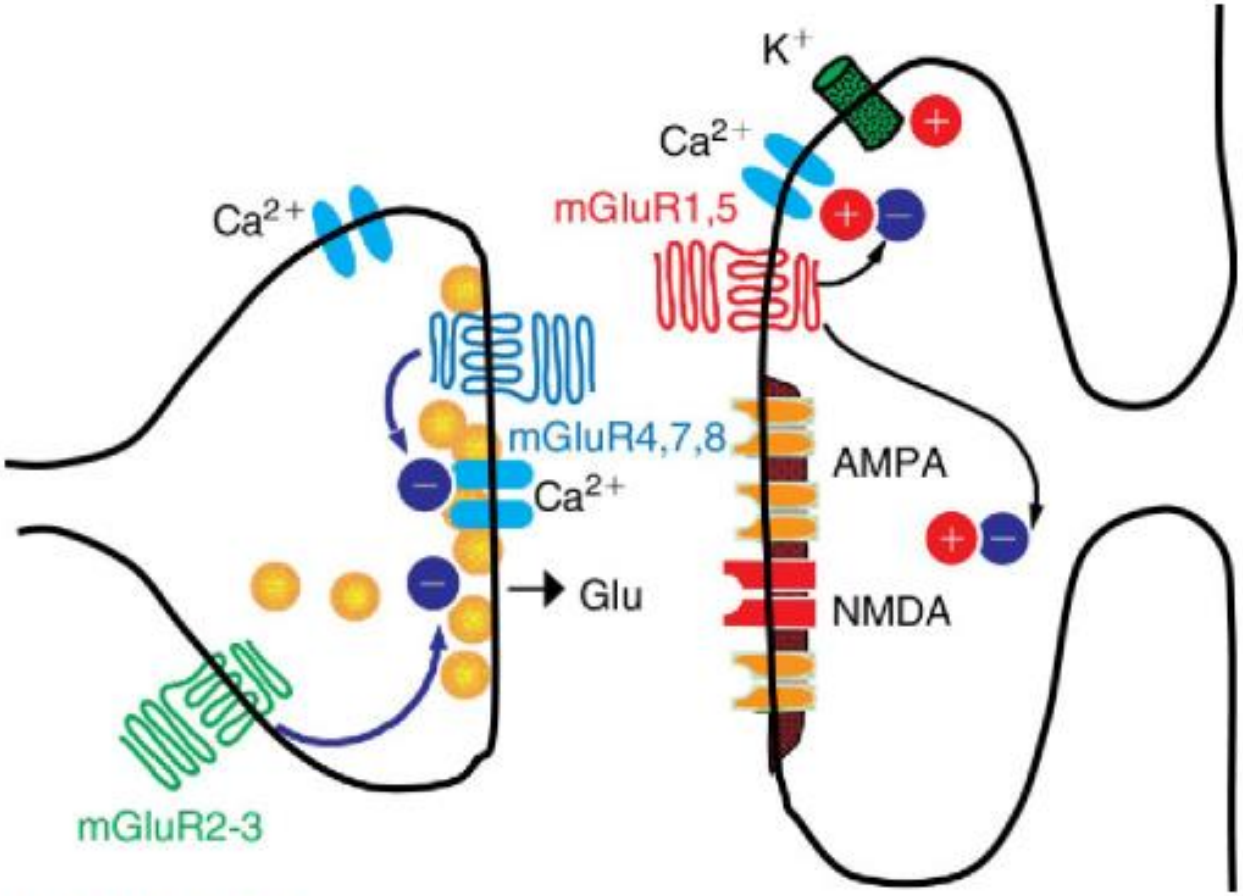
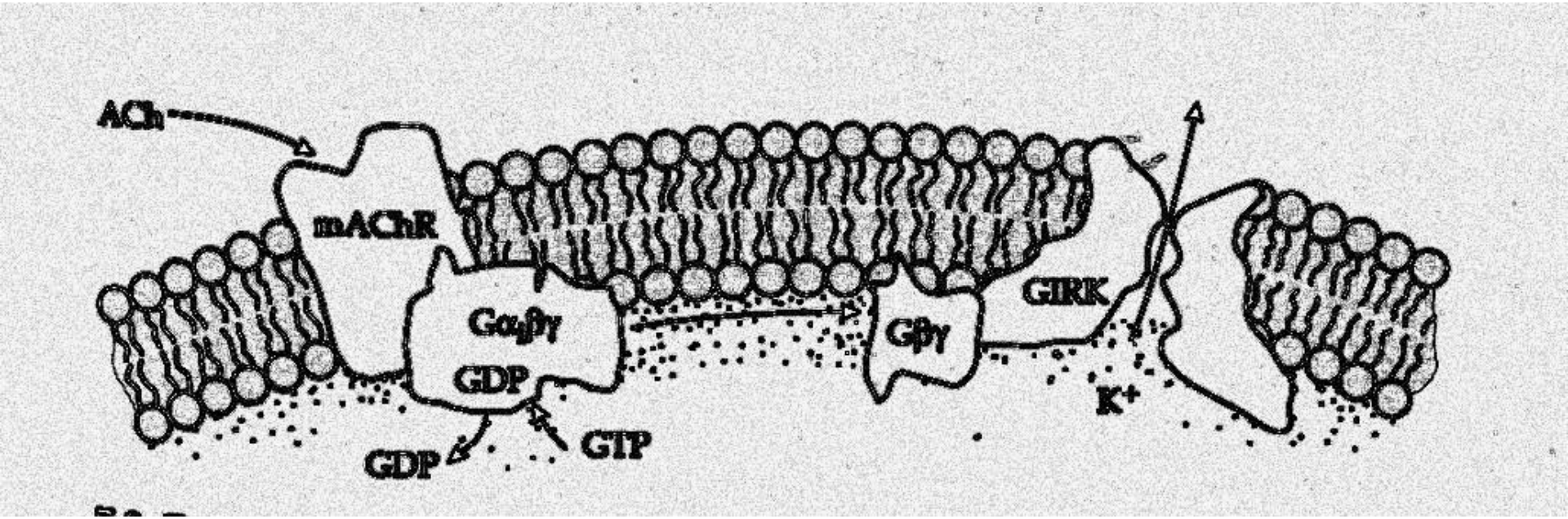


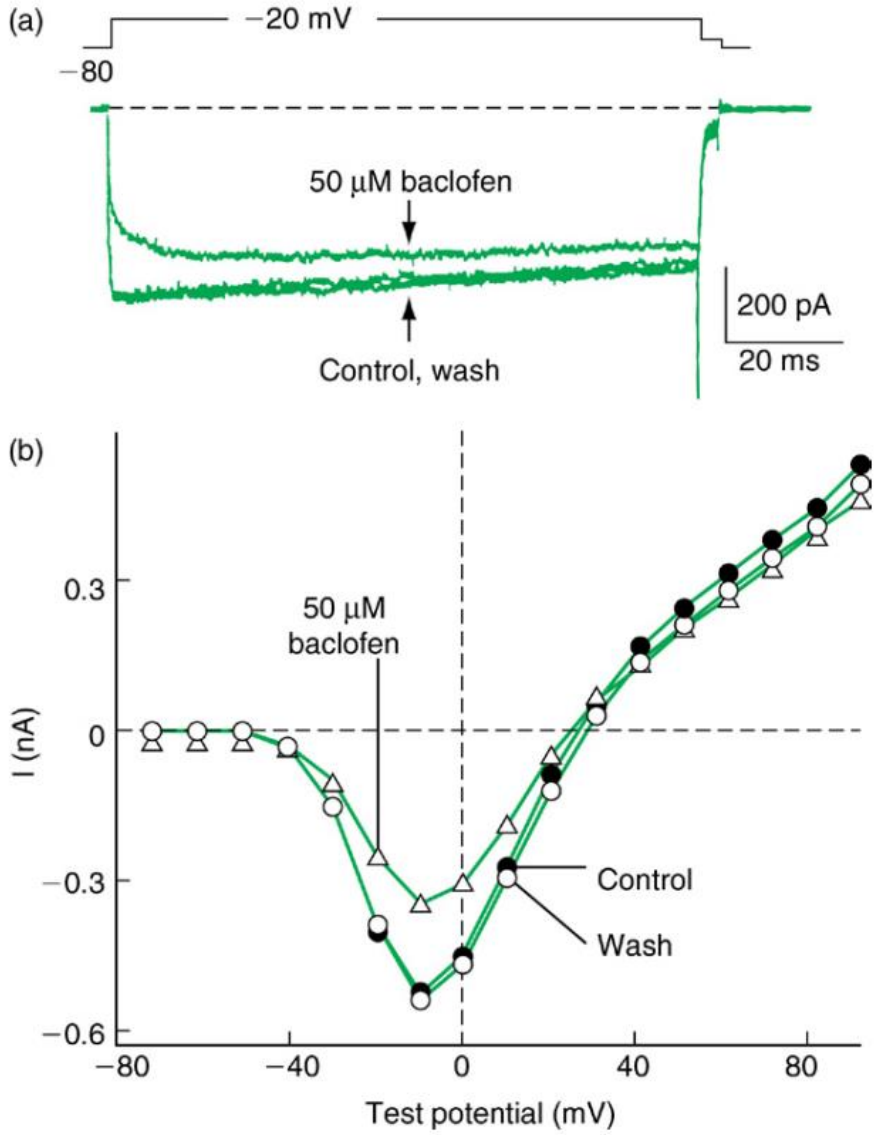
FIGURE 12.6 **Schematic representation of a glutamatergic synapse with the modulatory roles of mGluRs.** Note group-I mGluRs (1 and 5) are mostly located in the postsynaptic spines, on the side of the postsynaptic density, from where they regulate postsynaptic ionotropic glutamate receptors, as well as Ca^{2+} and K^{+} channels. Group-II (mGluR2) and group-III (mGluR4, 7 and 8) are mostly presynaptic, regulating neurotransmitter release by, at least, inhibiting Ca^{2+} channels.

As subunidades βγ também tem atividade

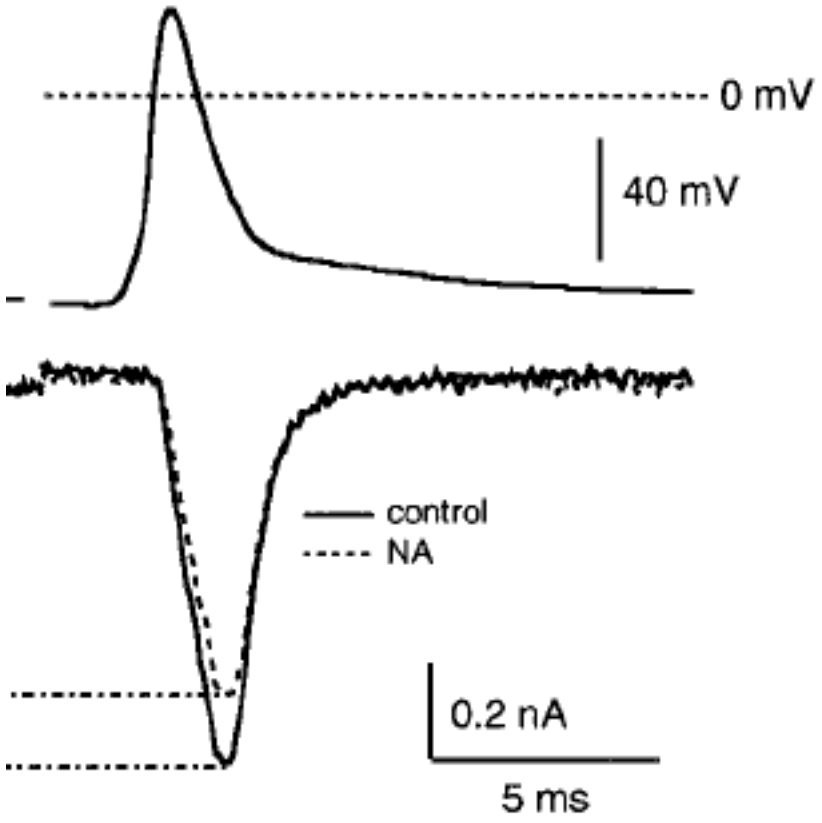
Ativação direta de canais de K (GIRK)
Inibição direta de canais de Ca

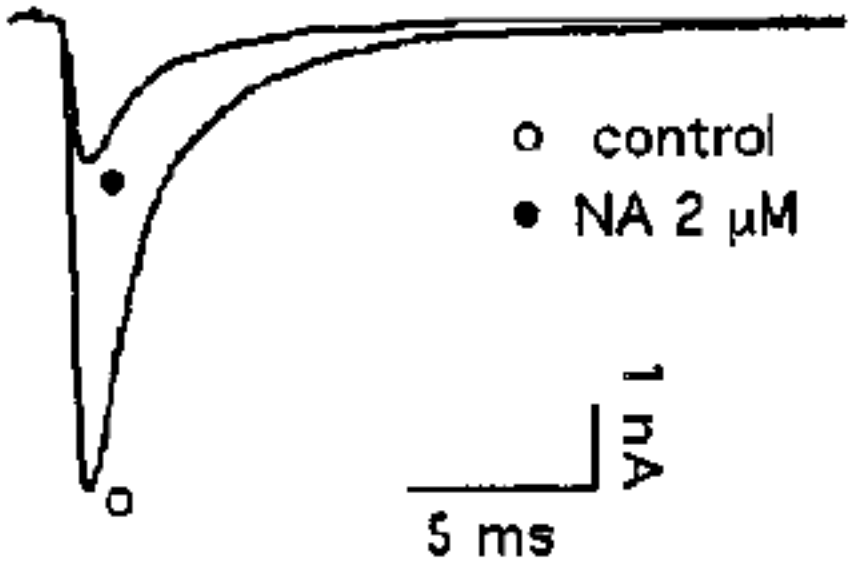


Inibição de correntes de cálcio pela ativação dos receptores GABAB

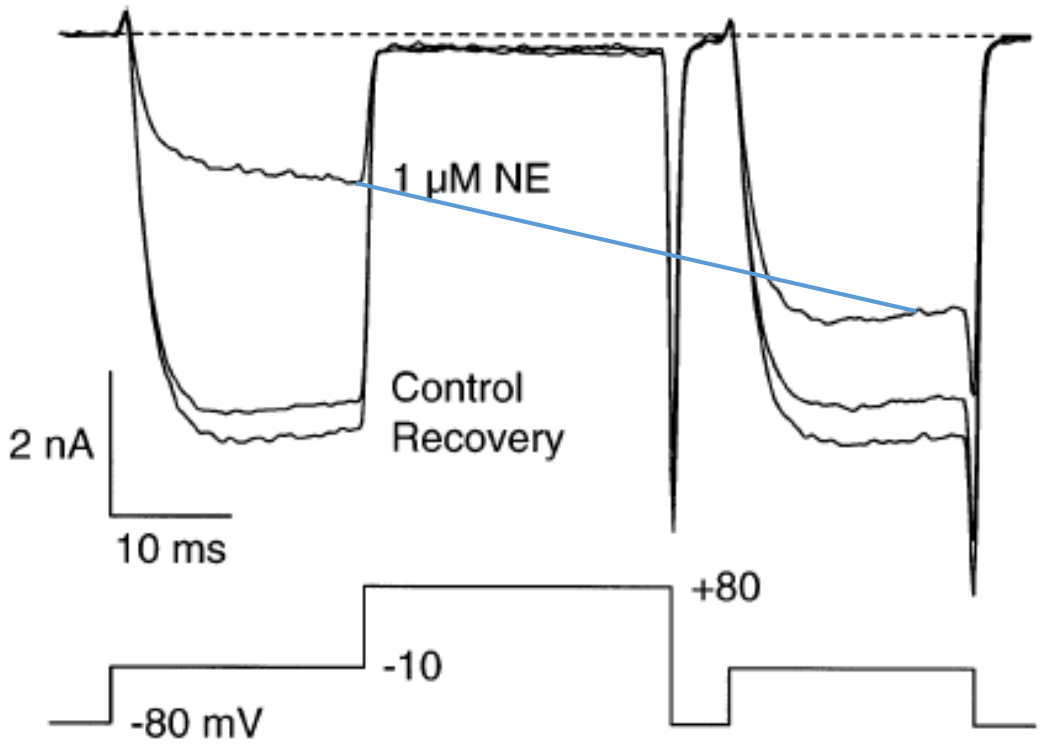


A inibição de correntes de cálcio pré-sinápticas por ativação de receptores pré-sinápticos inibe a liberação de transmissores



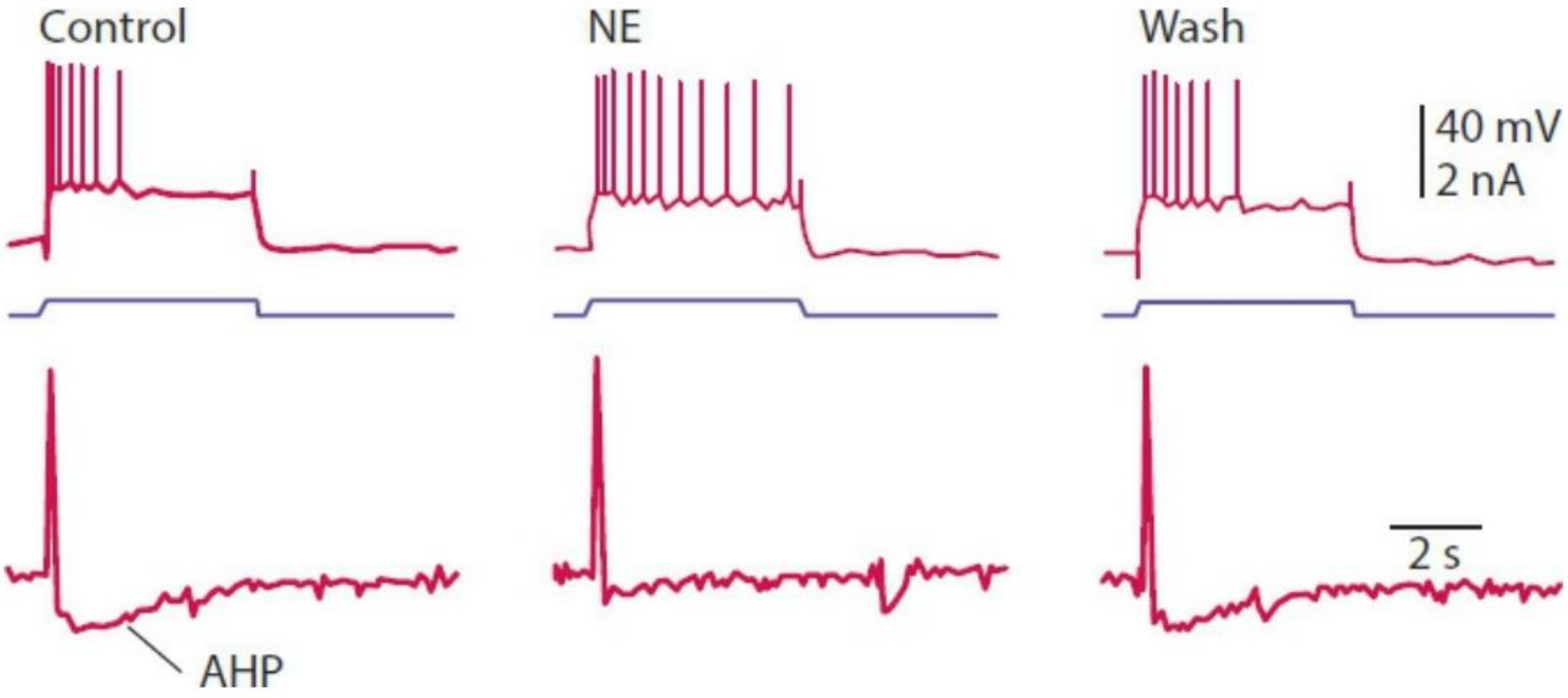


A inibição das correntes de cálcio por subunidades βγ é revertida por despolarização

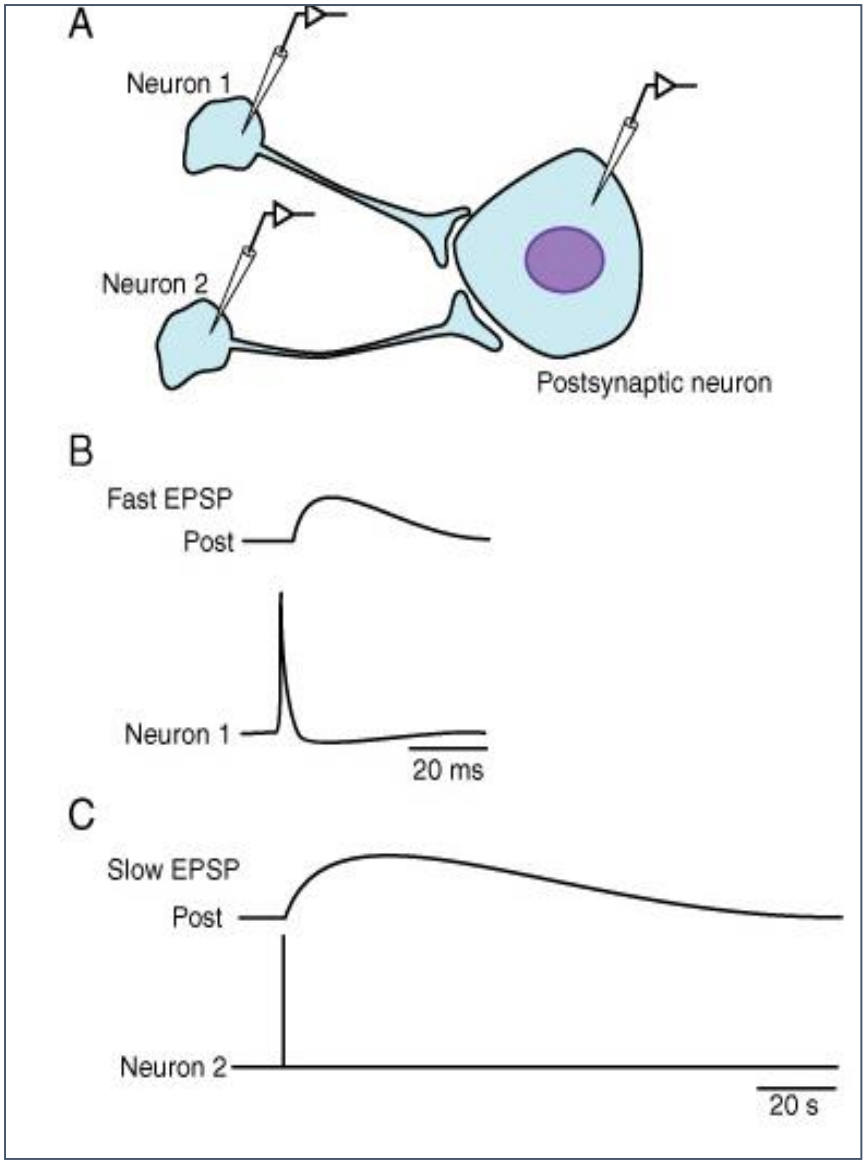


Neurotransmissores podem alterar a excitabilidade da membrana pós-sináptica via receptores metabotrópicos

Ação da noradrenalina (NE) no disparo de PAs em neurônios hipocampais

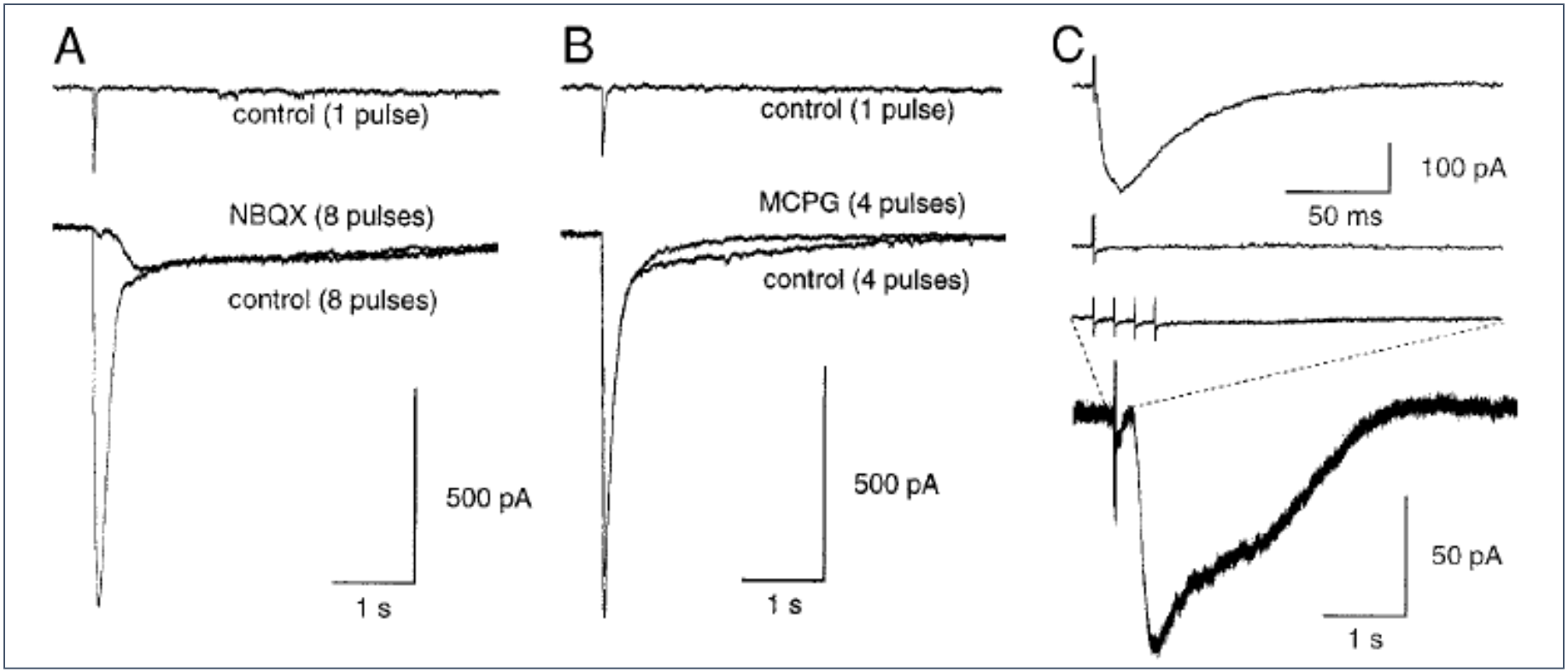


Receptores metabotrópicos podem gerar PSPs lentos

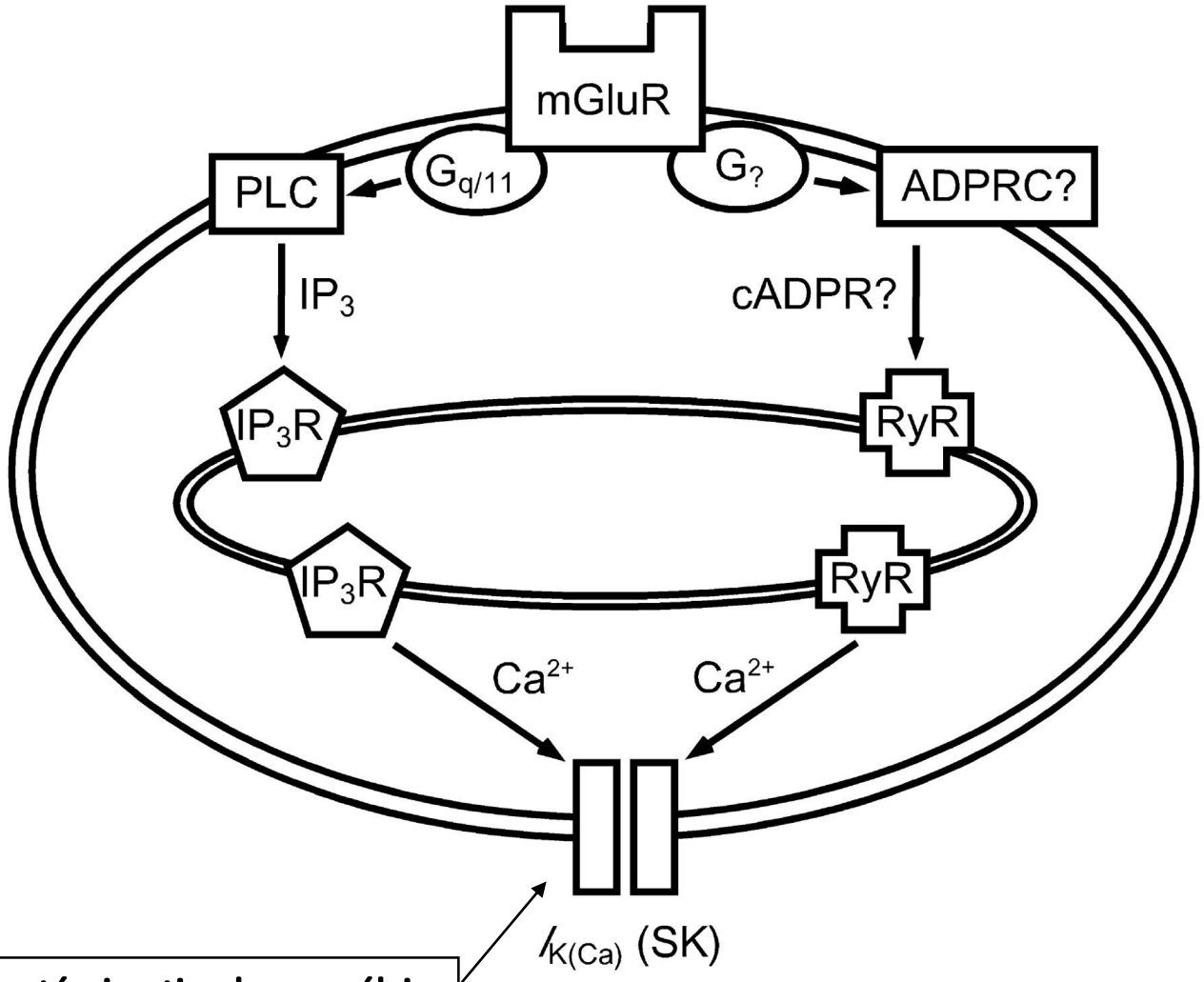


Postsynaptic Current Mediated by Metabotropic Glutamate Receptors in Cerebellar Purkinje Cells

FILIPPO TEMPIA, MARIA CONCETTA MINIACI, DAVIDE ANCHISI, AND PIERGIORGIO STRATA
Department of Neuroscience, University of Turin, 10125 Turin, Italy

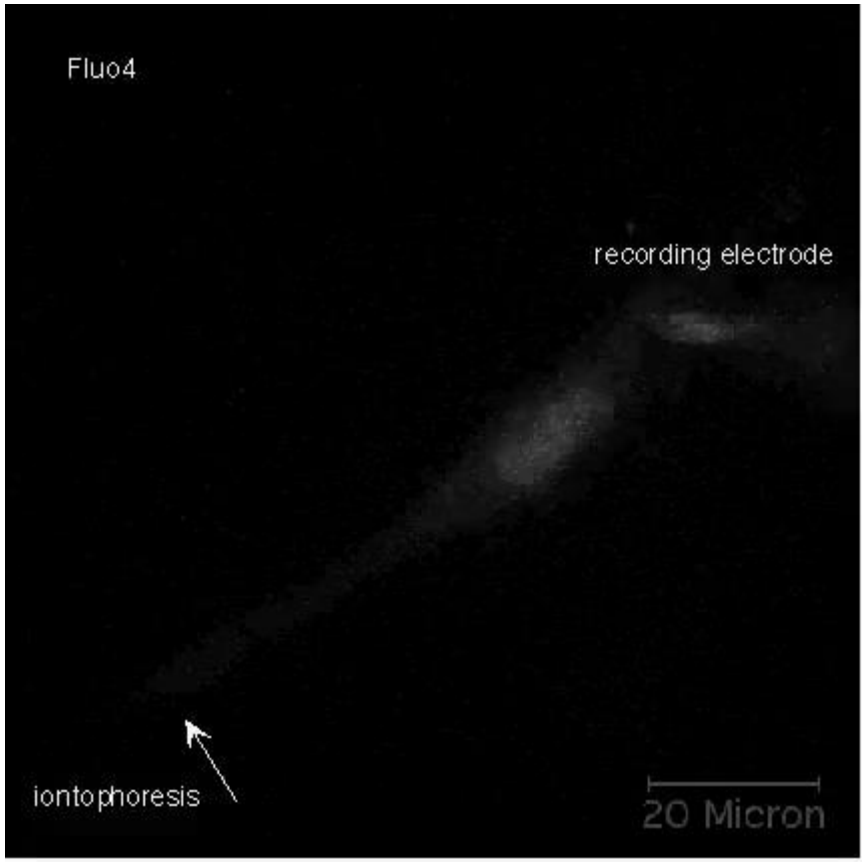


Ativação de receptores metabotrópicos pode mobilizar cálcio de estoques intracelulares

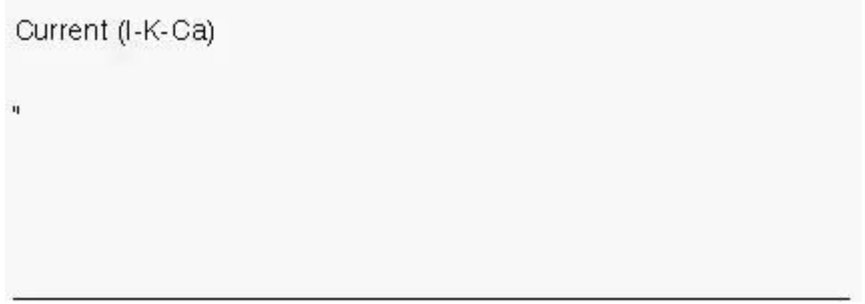


Canal de potássio ativado por cálcio

Aplicação iontoforética de glutamato

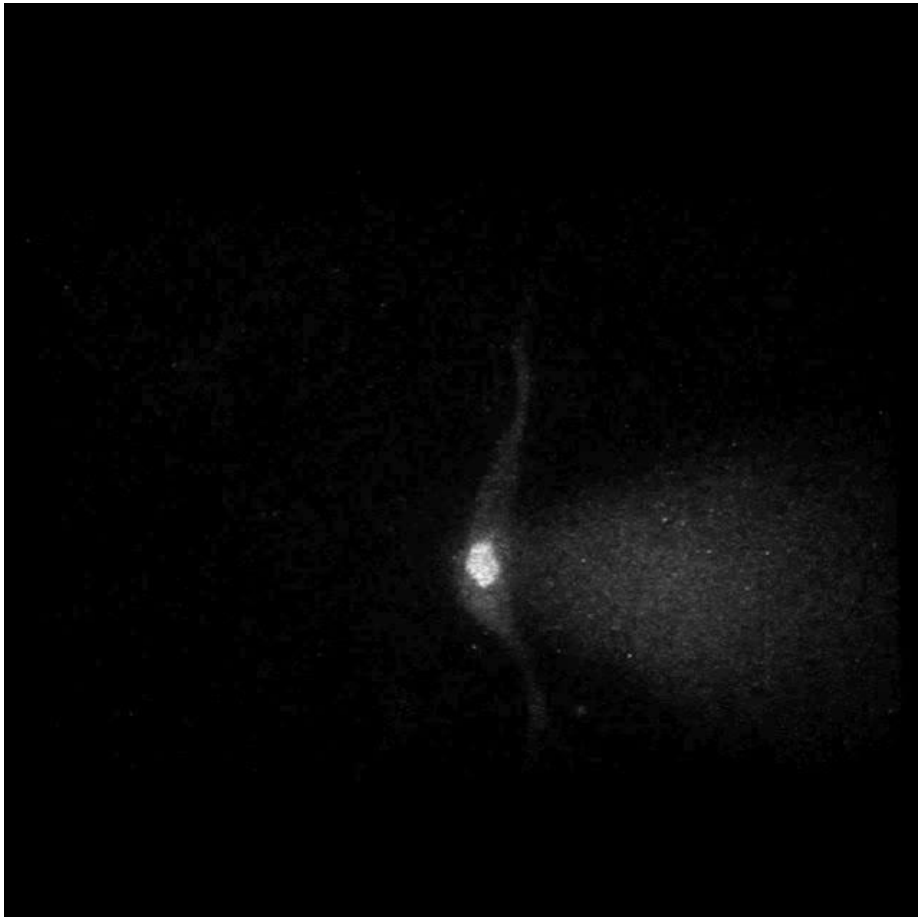


Cálcio



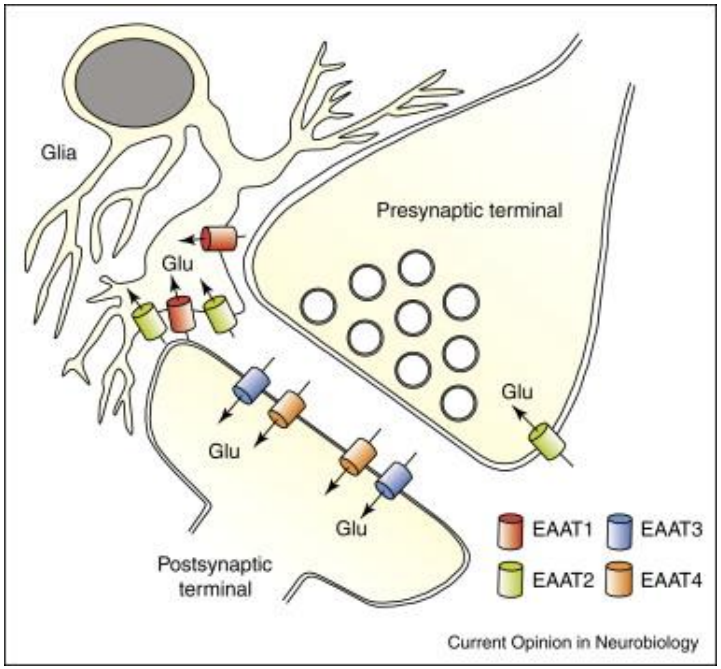
IK_{Ca}

Onda de cálcio gerada por mGLUR em resposta a ativação sináptica



Neurotransmissores são removidos do meio extracelular por meio de co-transporte com o sódio

Transportadores extracelulares de glutamato



EAAT1 (GLAST1) - astrócitos

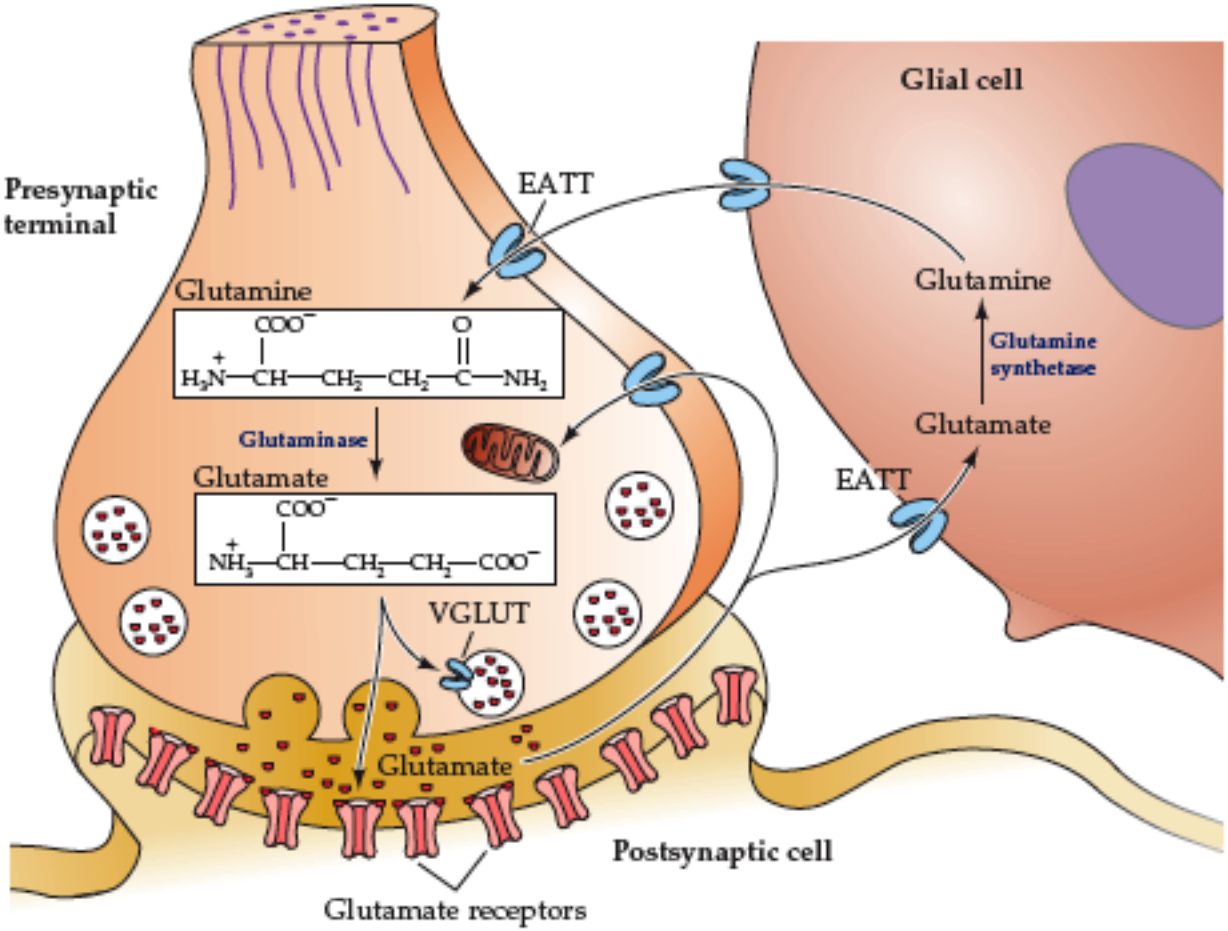
EAAT2 (GLT-1) - astrócitos, neurônios

EAAT3 (EAAC1) - neurônios

EAAT4 - neurônios de Purinje

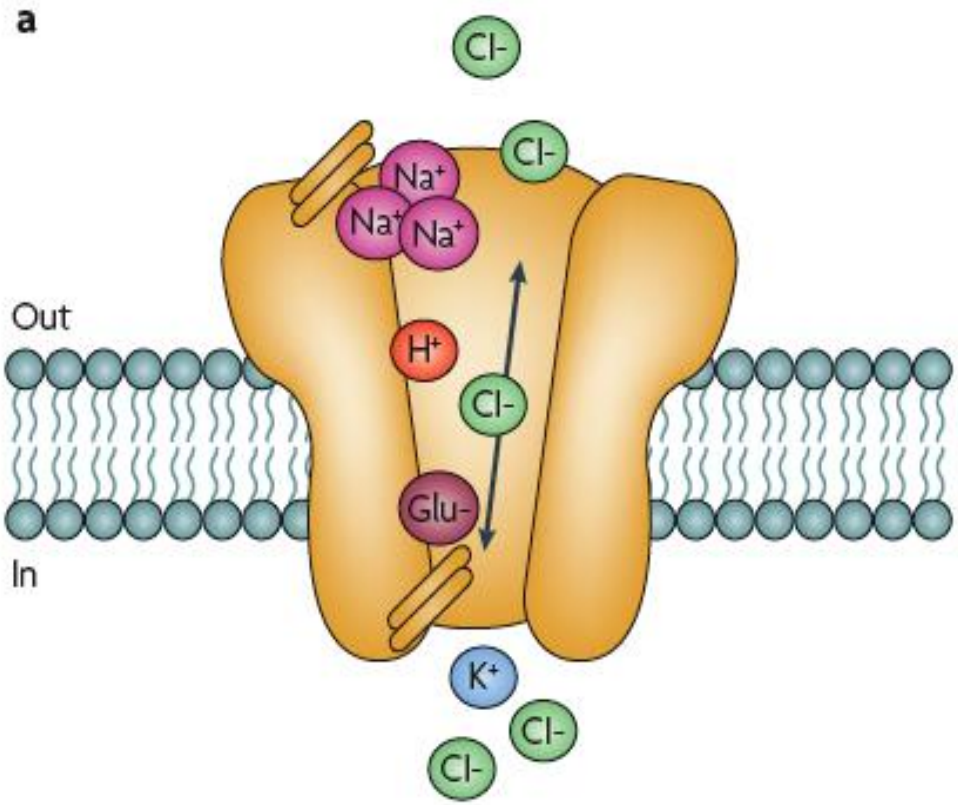
EAAT5 - retina

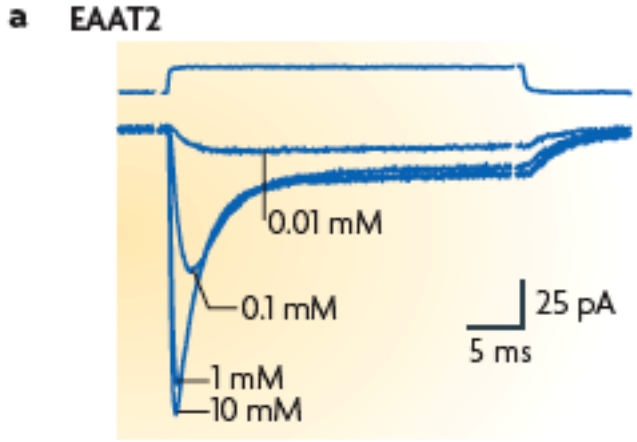
A glia “recicla” o glutamato captado como glutamina

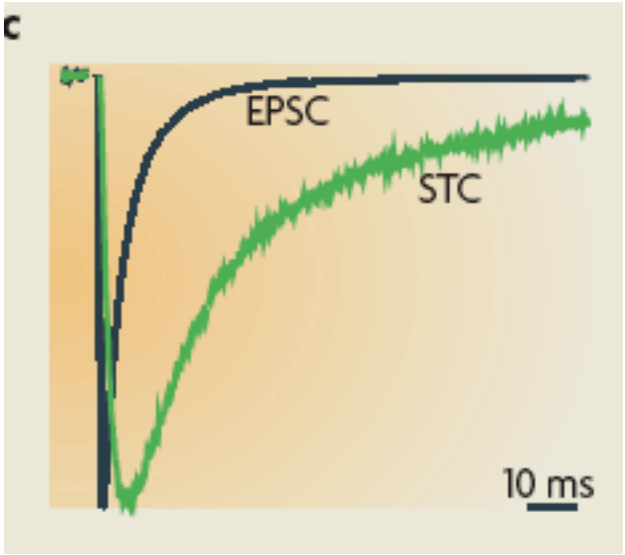


O transporte de glutamato é **eletrogênico** e envolve sódio, potássio, próton e cloreto

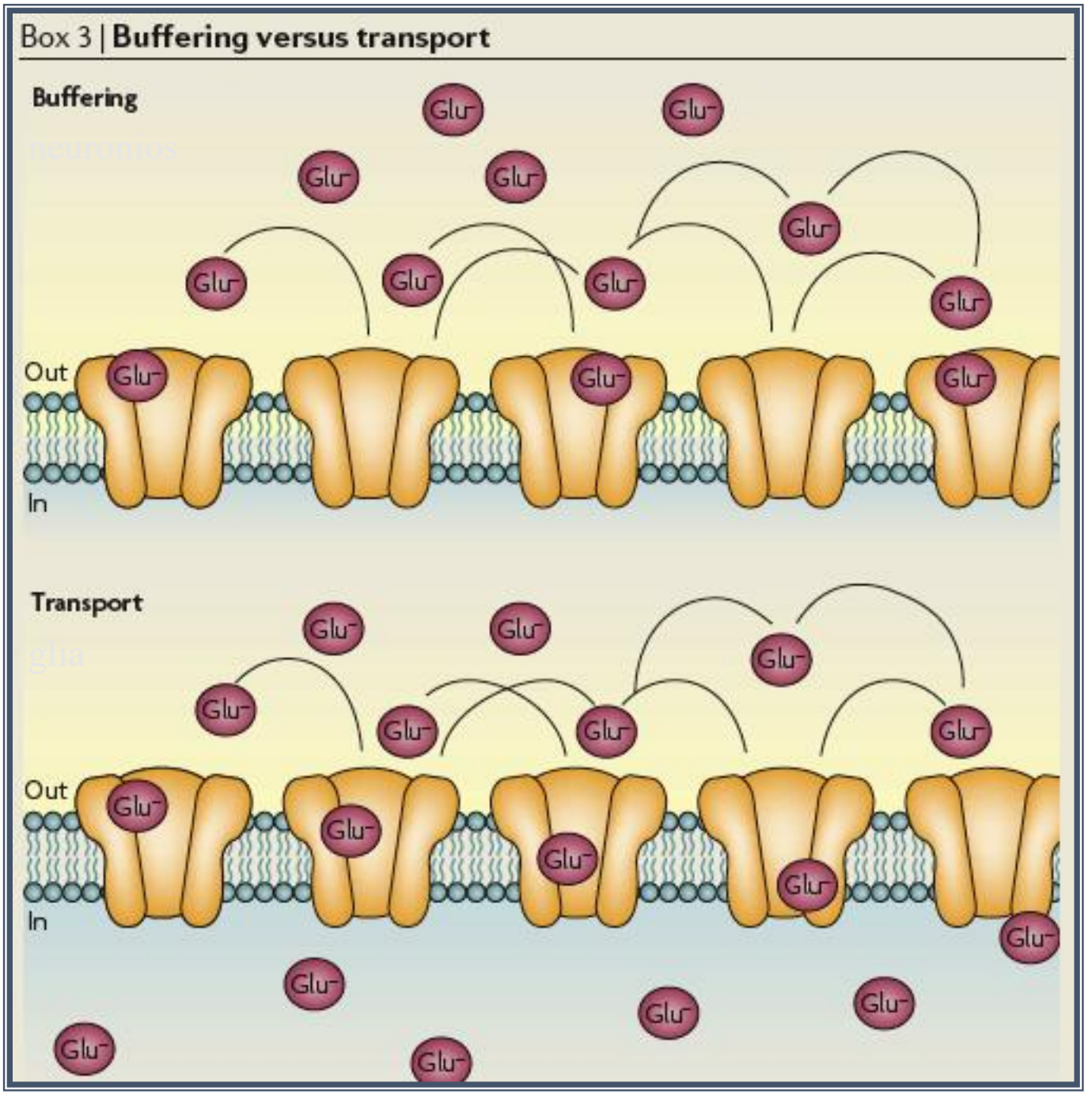
$(3Na^+ + 1Glu^- + 1H^+ - 1K^+ = \text{two positive charges})$,



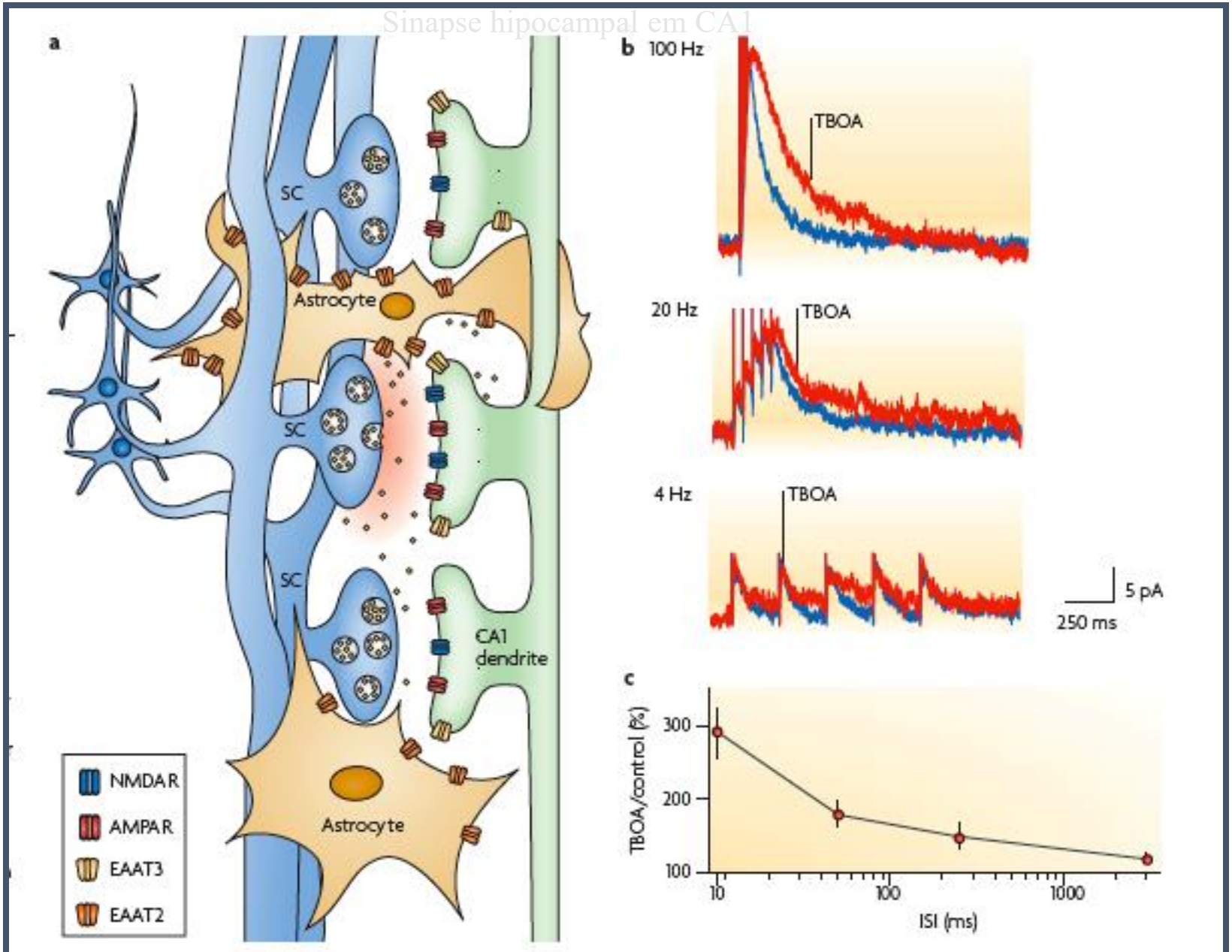


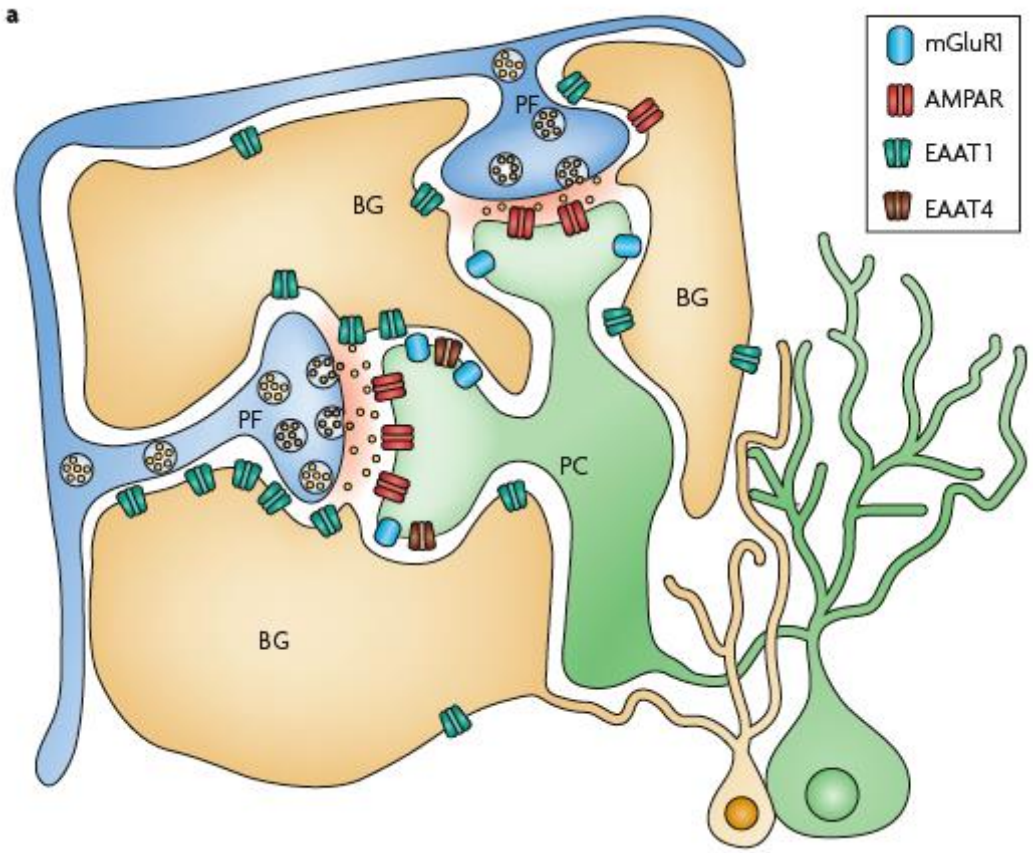


Alta afinidade e alta densidade nas sinapses tornam os transportadores de glutamato eficientes em tamponar rapidamente o excesso de glutamato extra-sináptico

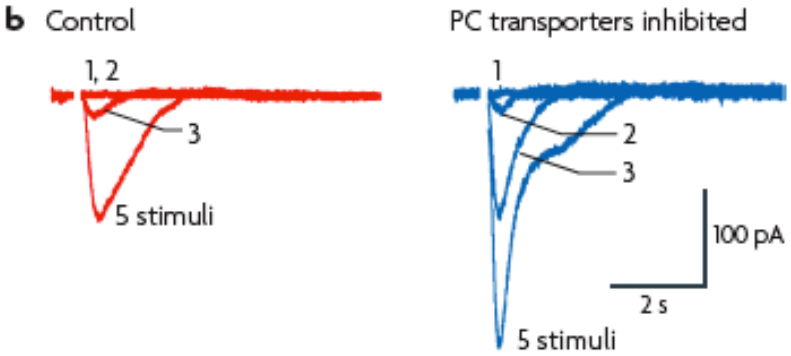


Funcionalidade da captação do glutamato sináptico

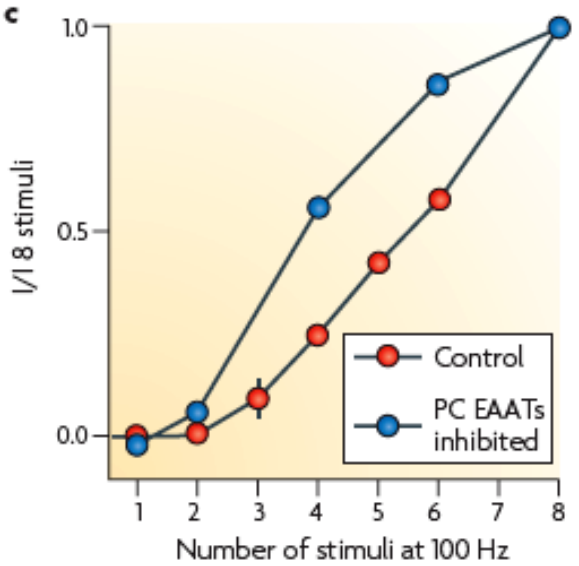




Correntes de mGluRs

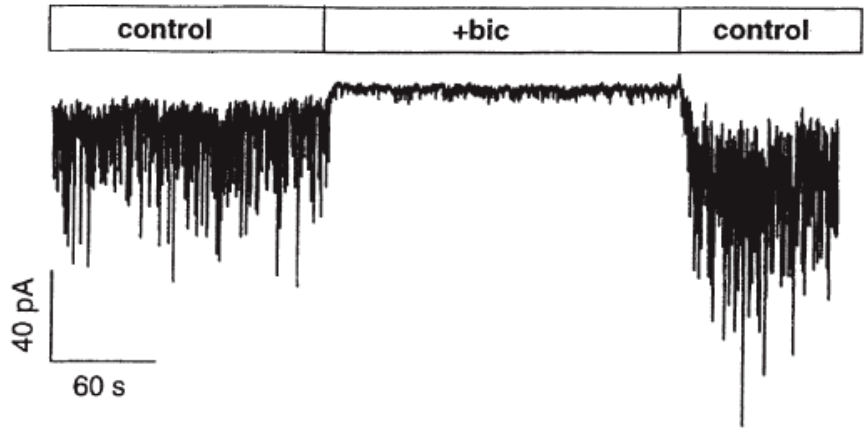


Os transportadores de glutamato limitam o *spill-over* de glutamato no cerebelo

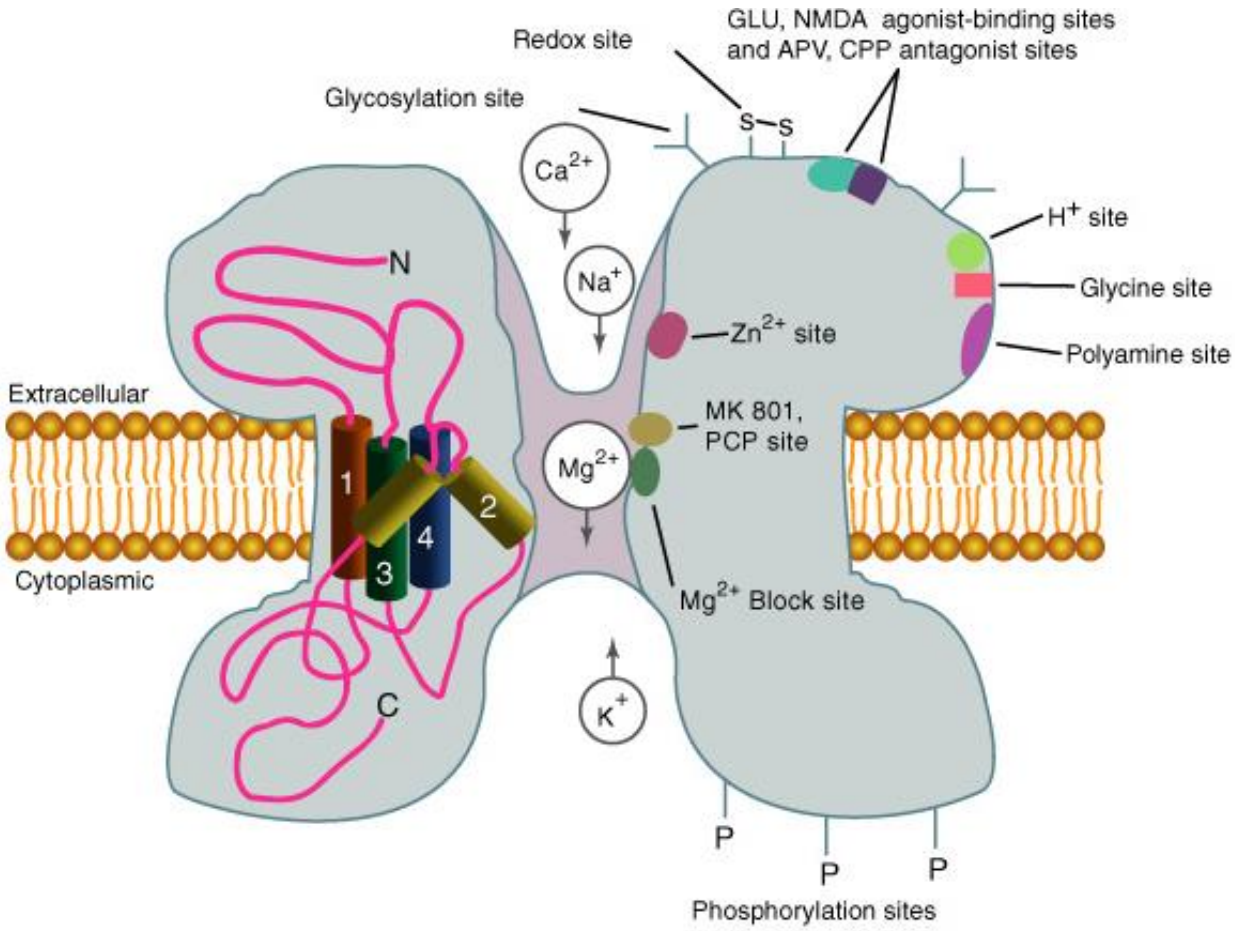


Receptores extra-sinápticos podem ser ativados tonicamente por transmissores que "escapam" da fenda sináptica *(spilled over)*

A

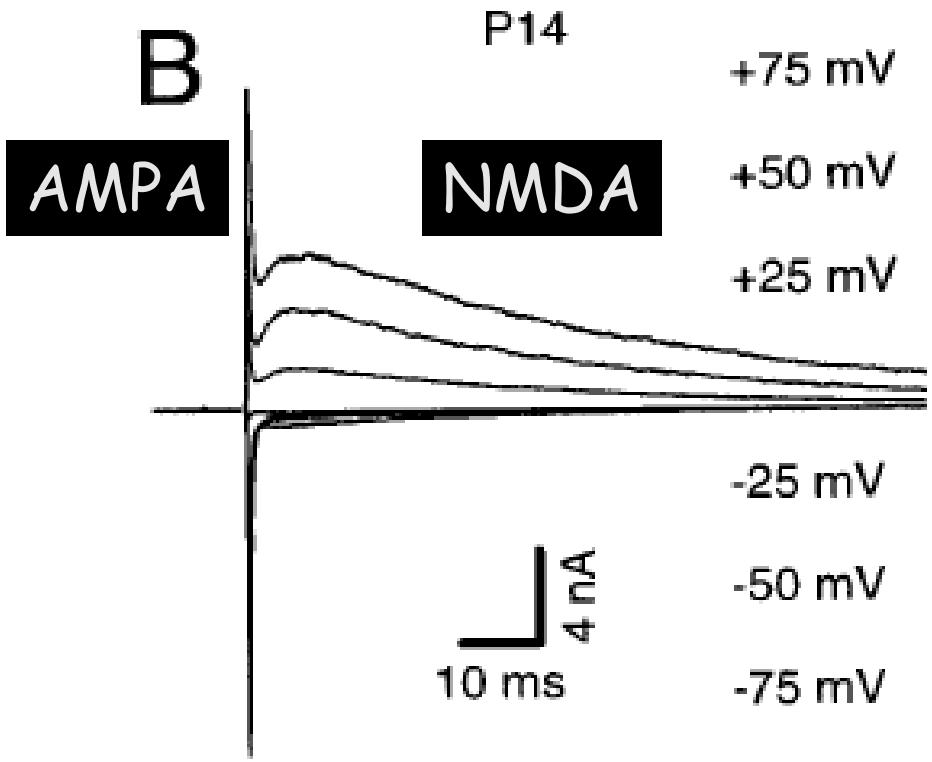


O Receptor glutamatérgico NMDA



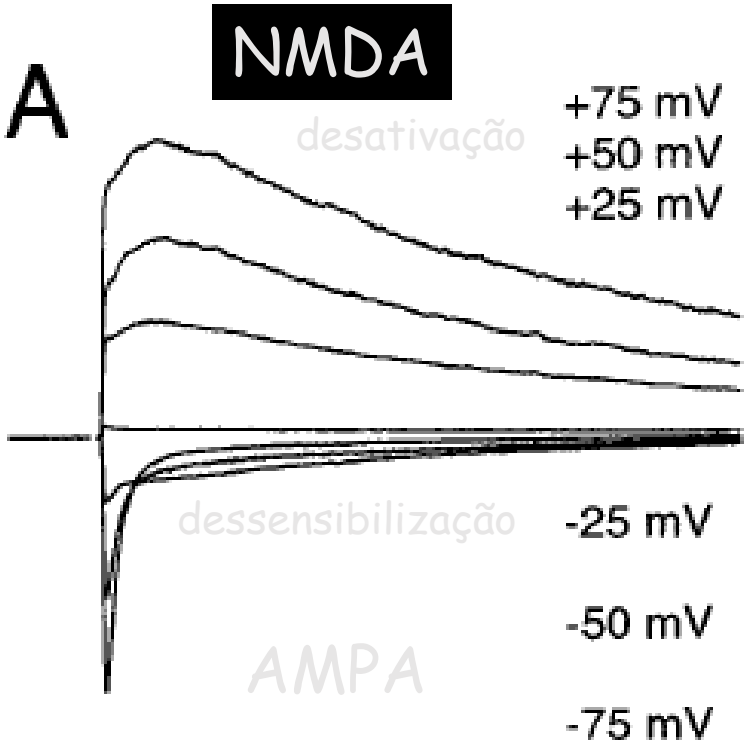
Receptores AMPA ativam mais rápido do que receptores NMDA

EPSCs glutamatérgicos evocados, registrados em diferentes potenciais de membrana



Receptores AMPA desensibilizam fortemente em comparação aos NMDA

EPSCs glutamatérgicos evocados, registrados em diferentes potenciais de membrana



O receptor GABAB é um **heterodímero**