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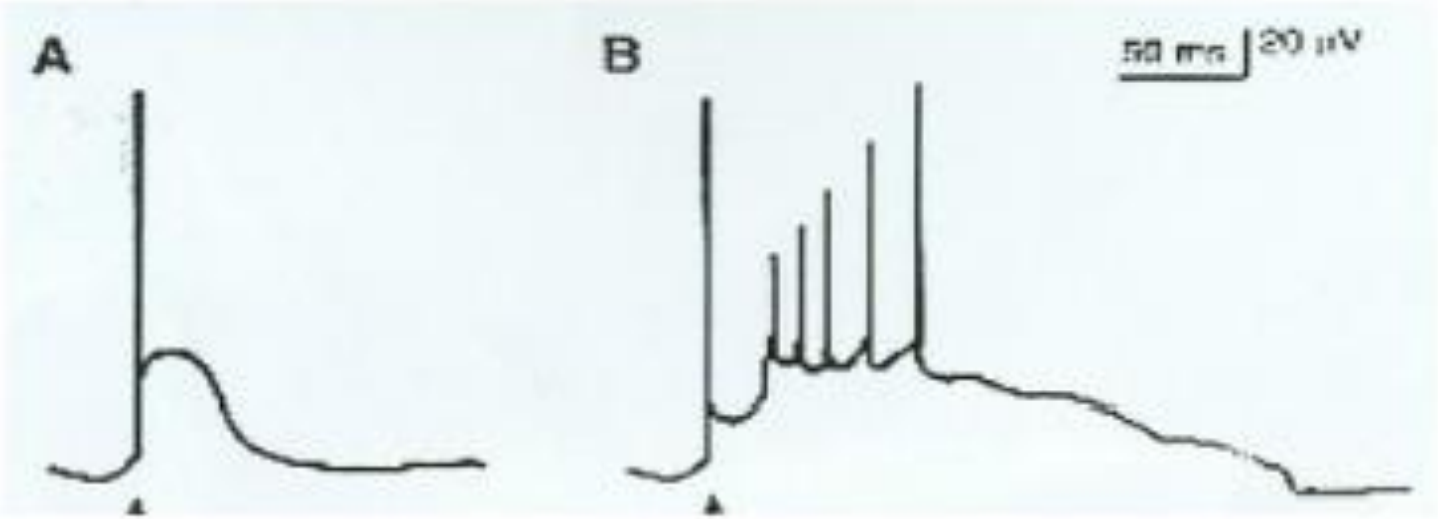
Le canalopatie nelle epilessie

Bodo Wentz (1983): tonic clonic seizures

Genova febbraio 2015

L'epilessia è un disturbo neurologico che si manifesta con crisi ricorrenti sostenute dalla scarica parossistica di un gruppo di neuroni della corteccia cerebrale.

la scarica di un neurone



scarica di un neurone normale **scarica di un neurone epilettico**

FOREP ONLUS
Fondazione per la Ricerca sull'Epilessia e Sindromi Correlate

Classificazione

Si distinguono in

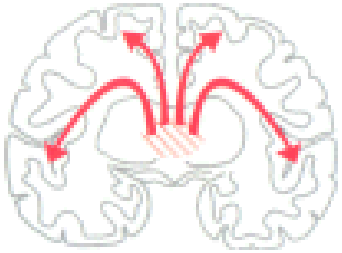
- IDIOPATICHE (indipendenti da lesioni cerebrali)
- SINTOMATICHE (derivanti da lesioni cerebrali o malformazioni del cervello)
- CRIPTOGENETICHE (la causa scatenante non è conosciuta)

Nella prima la predisposizione alla crisi è presumibilmente costituzionale, nella seconda si collega a lesioni di varia origine (traumi cranici, encefaliti pregresse, tumori) dell'encefalo.
Dal punto di vista clinico si distinguono:

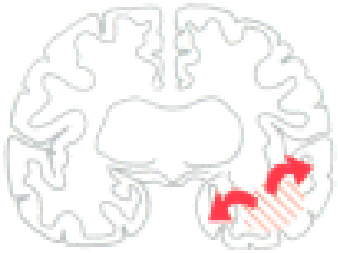
- un'epilessia generalizzata, in cui la sofferenza interessa tutte le zone corticali,
- una parziale, in cui la sofferenza è localizzata in un numero ristretto di aree.

Tra le epilessie parziali merita un posto a parte l'epilessia psicomotrice, legata a una sofferenza del lobo temporale. L'epilessia generalizzata si distingue in grande male e piccolo male.

LICE
Lega Italiana contro l'Epilessia



a) Generalised seizure:
both halves of the brain are affected simultaneously

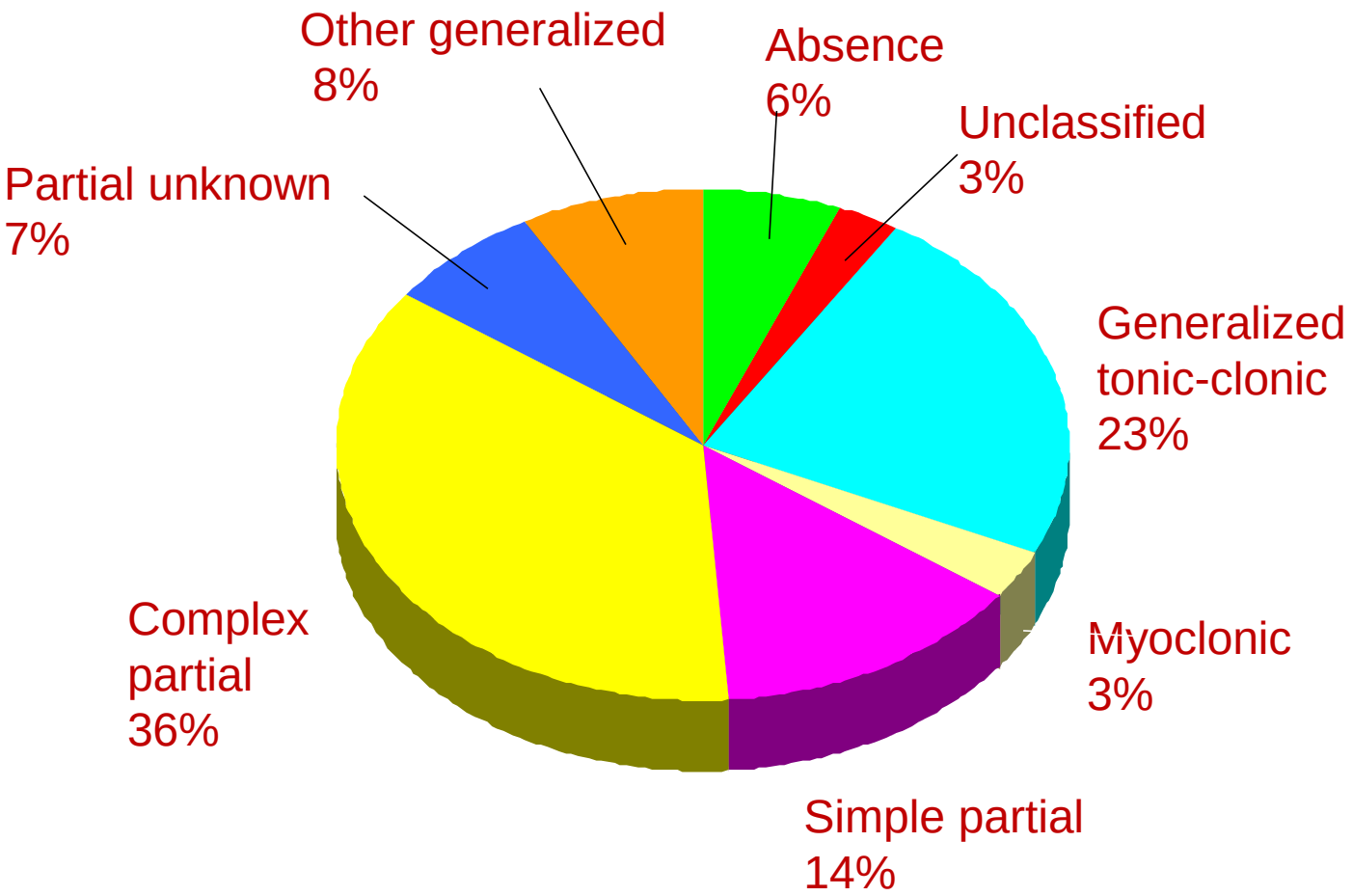


b) Focal seizure:
the seizure activity begins in one half of the brain

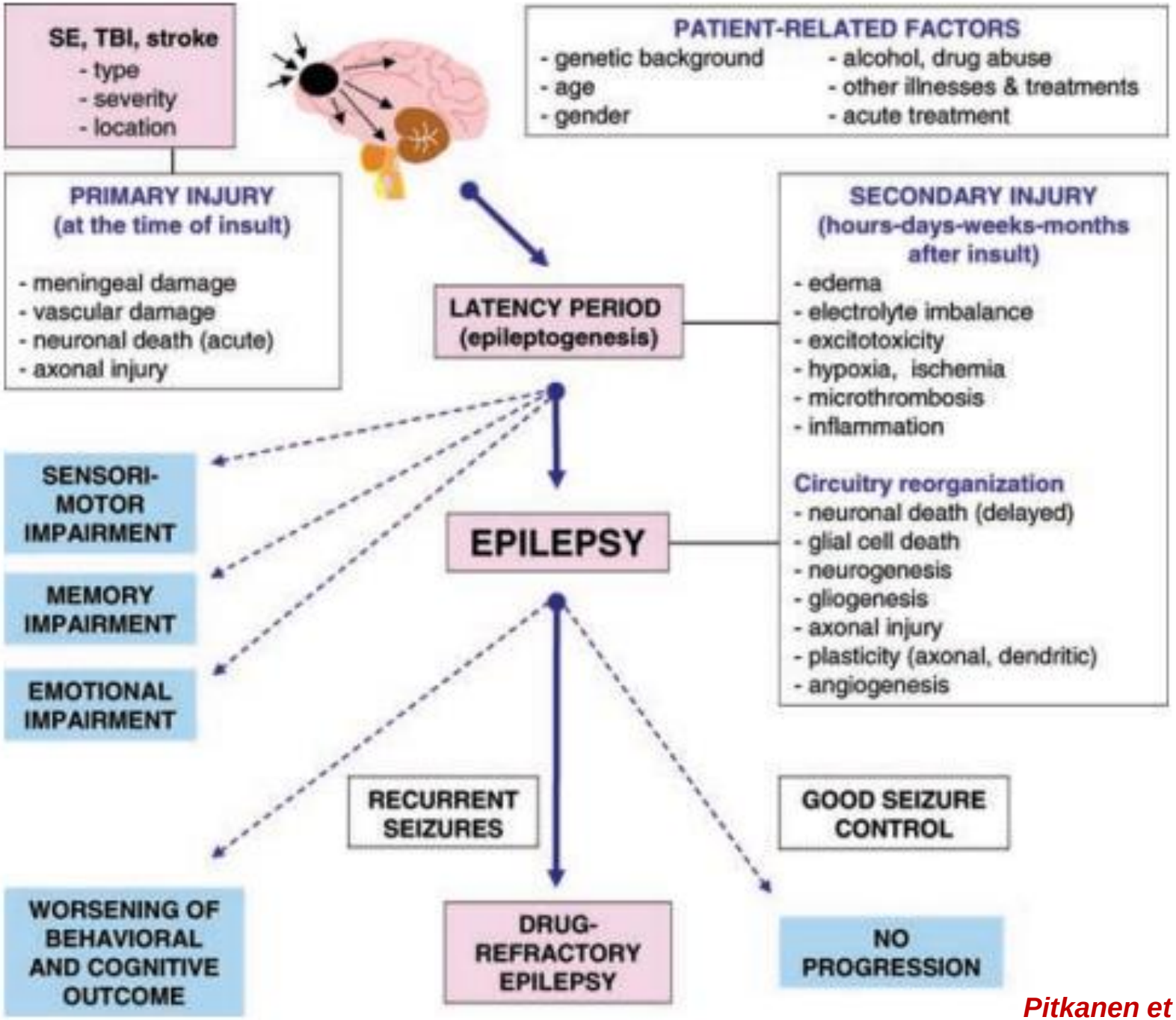
Epileptic seizures can be divided into the following types:

Generalised seizures	Focal seizures	Non-specific seizures
e.g. grand mal, absence	e.g. partial complex seizures, one-sided myoclonic seizures	e.g. seizures in new-borns

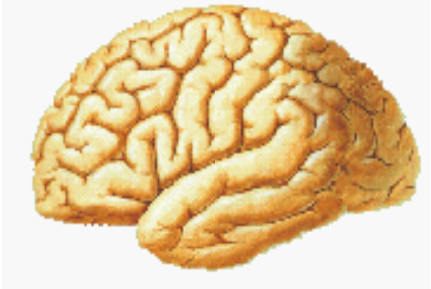
Incidence of Epilepsy Seizure Types

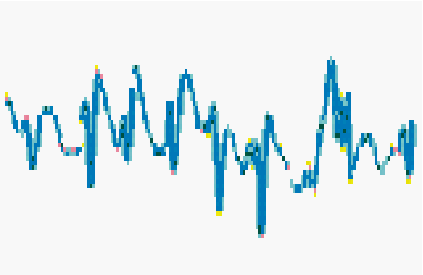


Hauser WA. *Epilepsia.* 1992;33(suppl 4):S6-S14.

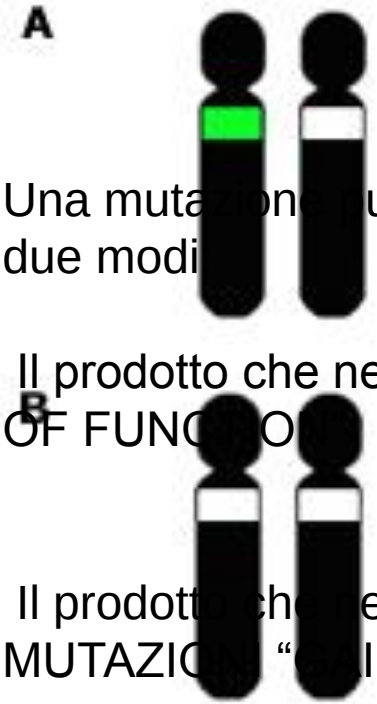


Pitkanen et al., 2007





autosomal dominant nocturnal frontal lobe epilepsy	**subunità mutanti nicotiniche neuronali α4 e β2**
generalized epilepsy with febrile seizures plus	**subunità α1 e β1 del canale voltaggio-dipendente del sodio e la subunità γ2 del $GABA_A$**
childhood absence epilepsy	**mutazioni sulla γ2 del $GABA_A$**
juvenile myoclonic epilepsy	**mutazioni sulla α1 del $GABA_A$**



Una mutazione può determinare un cambiamento fenotipico essenzialmente in due modi

Il prodotto che ne deriva ha una funzione ridotta o assente MUTAZIONI “LOSS OF FUNCTION”

Il prodotto che ne deriva ha acquisito una nuova funzione anomala MUTAZIONI “GAIN OF FUNCTION”

Loss of function. A. Mutazione in un gene recessivo. Il prodotto mutato non comporta alcuna perdita di funzione perché l’allele sano compensa per la perdita. **B.** La mutazione in entrambe gli alleli comporta una situazione di loss of function.

Una mutazione può determinare un cambiamento fenotipico essenzialmente in due modi:

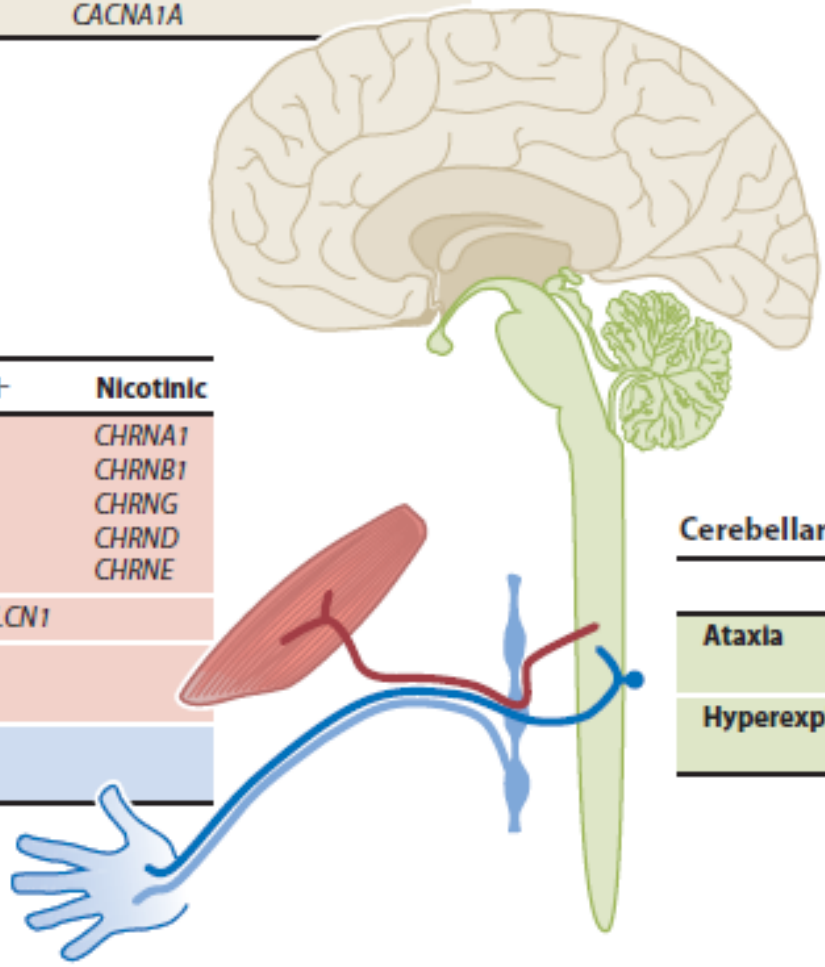
Il prodotto che ne deriva ha una funzione ridotta o assente MUTAZIONI “LOSS OF FUNCTION”

Il prodotto che ne deriva ha acquisito una nuova funzione anomala MUTAZIONI “GAIN OF FUNCTION”

Situazione normale e gain of function. A. I due alleli presenti sui due geni sono sani. **B.** Mutazione in uno dei due alleli dominanti. La mutazione comporta una situazione di gain of function.

Epilepsy and migraine

	Na^+	K^+	Ca^{2+}	$GABA_A$	Nicotinic
Epilepsy	*SCN1A* *SCN1B* *SCN2A*	*KCNQ2* *KCNQ3* *KCNMA1*	*CACNA1H*	*GABRA1* *GABRB3* *GABRG2*	*CHRNA2* *CHNRA4* *CHRNB2*
Migraine	*SCN1A*		*CACNA1A*		



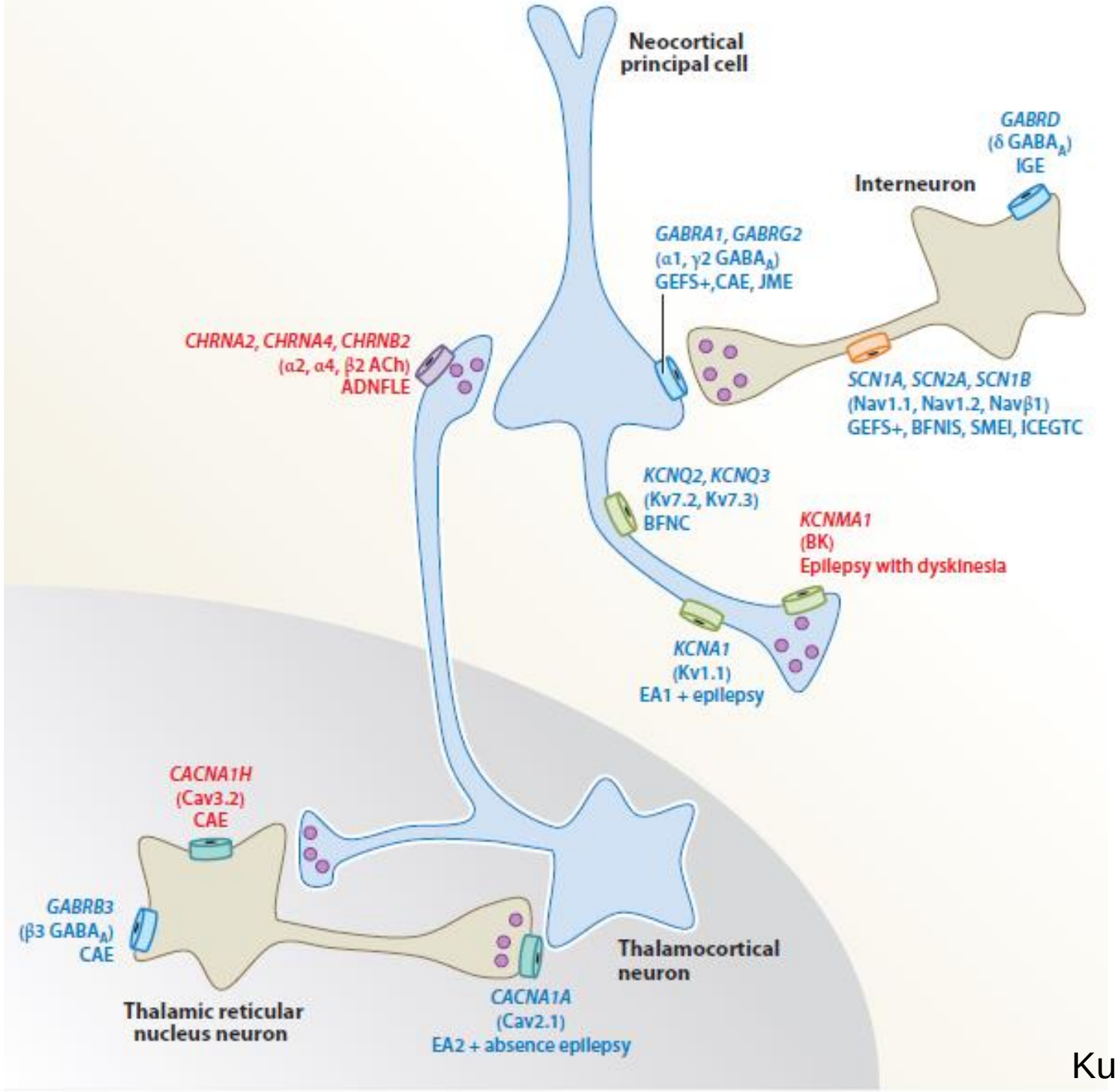
Neuromuscular disorders

	Na^+	K^+	Ca^{2+}	Cl^-	Nicotinic
Myasthenia **Fetal akinesia**					*CHRNA1* *CHRNB1* *CHRNG* *CHRND* *CHRNE*
Myotonia	*SCN4A*			*CLCN1*	
Periodic paralysis	*SCN4A*	*KCNJ2*	*CACNA1S*		
Pain **Erythema**	*SCN9A*				

Cerebellar ataxia and excessive startle

	K^+	Ca^{2+}	Glycine
Ataxia	*KCNA1* *KCNC3*	*CACNA1A*	
Hyperexplexia			*GLRA1* *GLRB*

Kullmann 2010



Kullmann 2010

What do the monogenic epilepsies tell us about epilepsy mechanisms?

Gene	*Protein*		*Phenotype*
SCN1A	Na^+ channels	α subunit of $Na_V1.1$	GEFS+, SMEI, FHM
SCN2A		α subunit of $Na_V1.2$	BFIS
SCN1B		β_1 subunit	GEFS+
KCNMA1	K^+ channels	BK channel	Epilepsy + paroxysmal dyskinesia
KCNQ2		M current	BFNC
KCNQ3			
KCNA1		Kv1.1	EA1 ± epilepsy
CACNA1A	Ca^{2+} channels	P/Q-type Ca^{2+} channel	EA2 ± epilepsy, FHM, SCA6
CLCN2	Cl^- channel	ClC-2	IGE
CHRNA4	Nicotinic ACh receptors	α_4 subunit	ADNFLE
CHRNB2		β_2 subunit	
GABRG2	$GABA_A$ receptors	γ_2 subunit	GEFS+
GABRA1		α_1 subunit	JME, absence epilepsy
LGI1	Not channels	Epitempin	ADPEAF / LTLE
EFHC1		Myoclonin1	JME

Epilepsy: the simple view

Prediction

Epilepsy should result from:

Loss of function mutations causing decreased inhibition

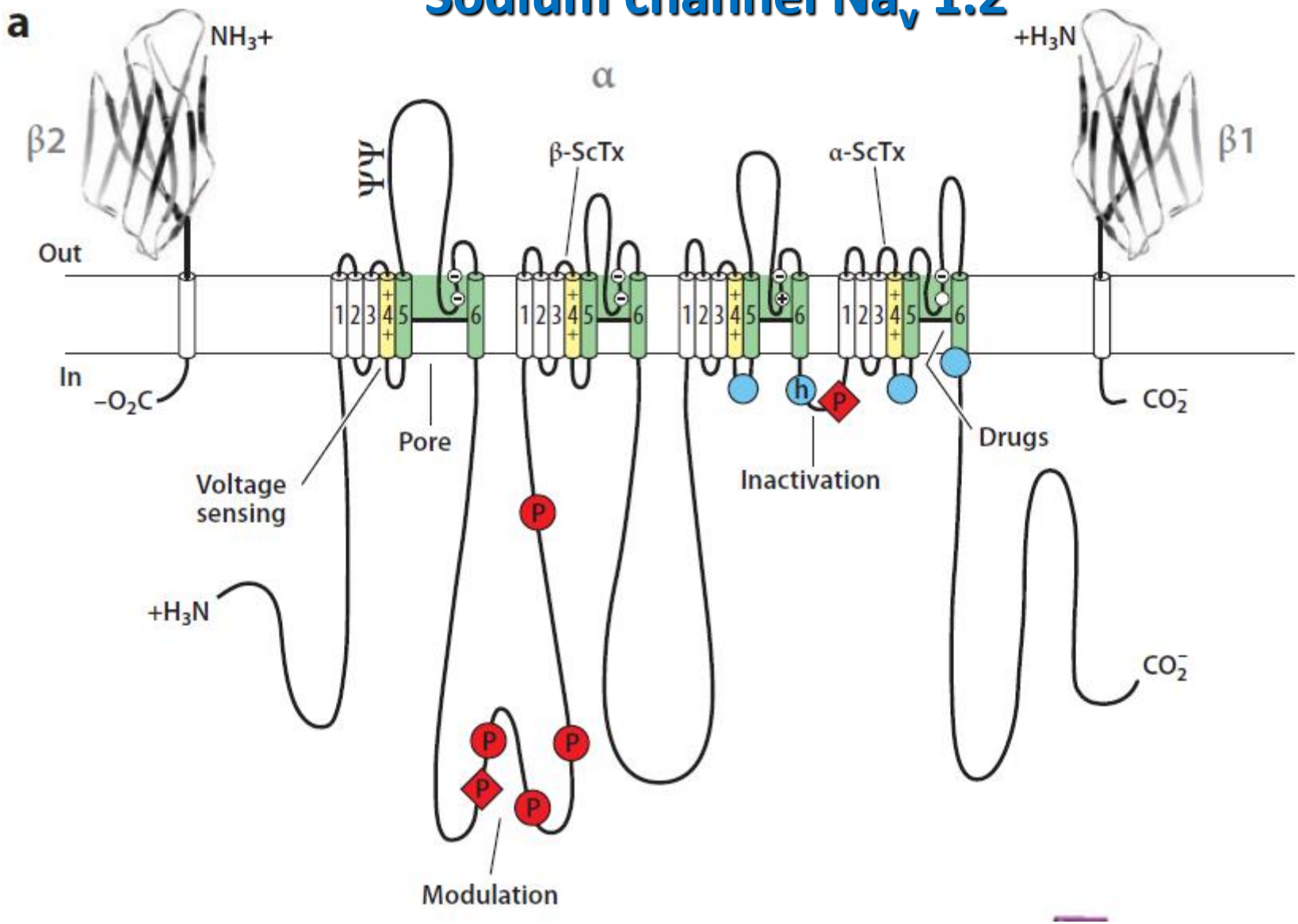
- $GABA_A$ receptors
- K^+ channels ✔

Gain of function mutations causing increased excitation

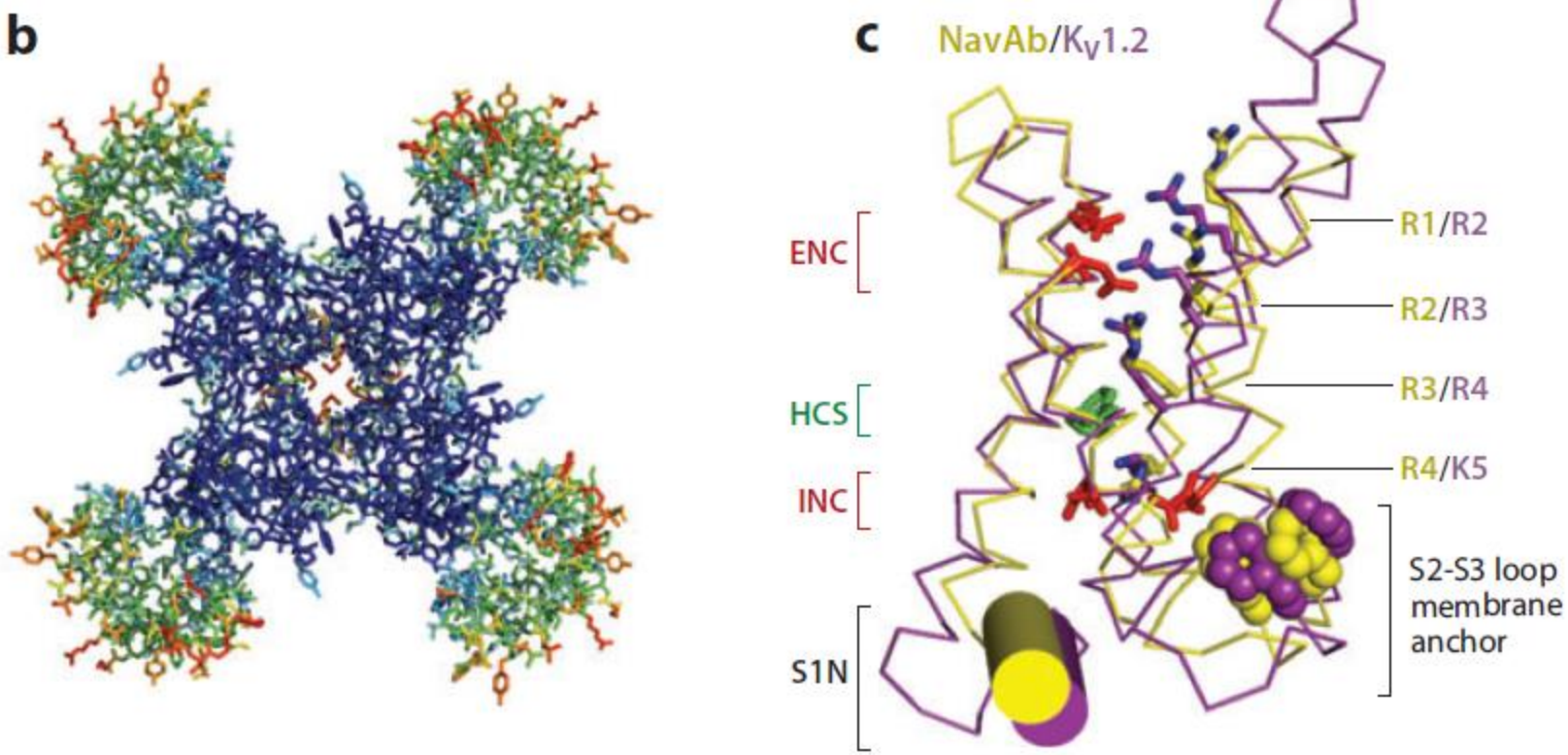
- glutamate receptors ✔
- Na^+ channels ✕
- Ca^{2+} channels ?

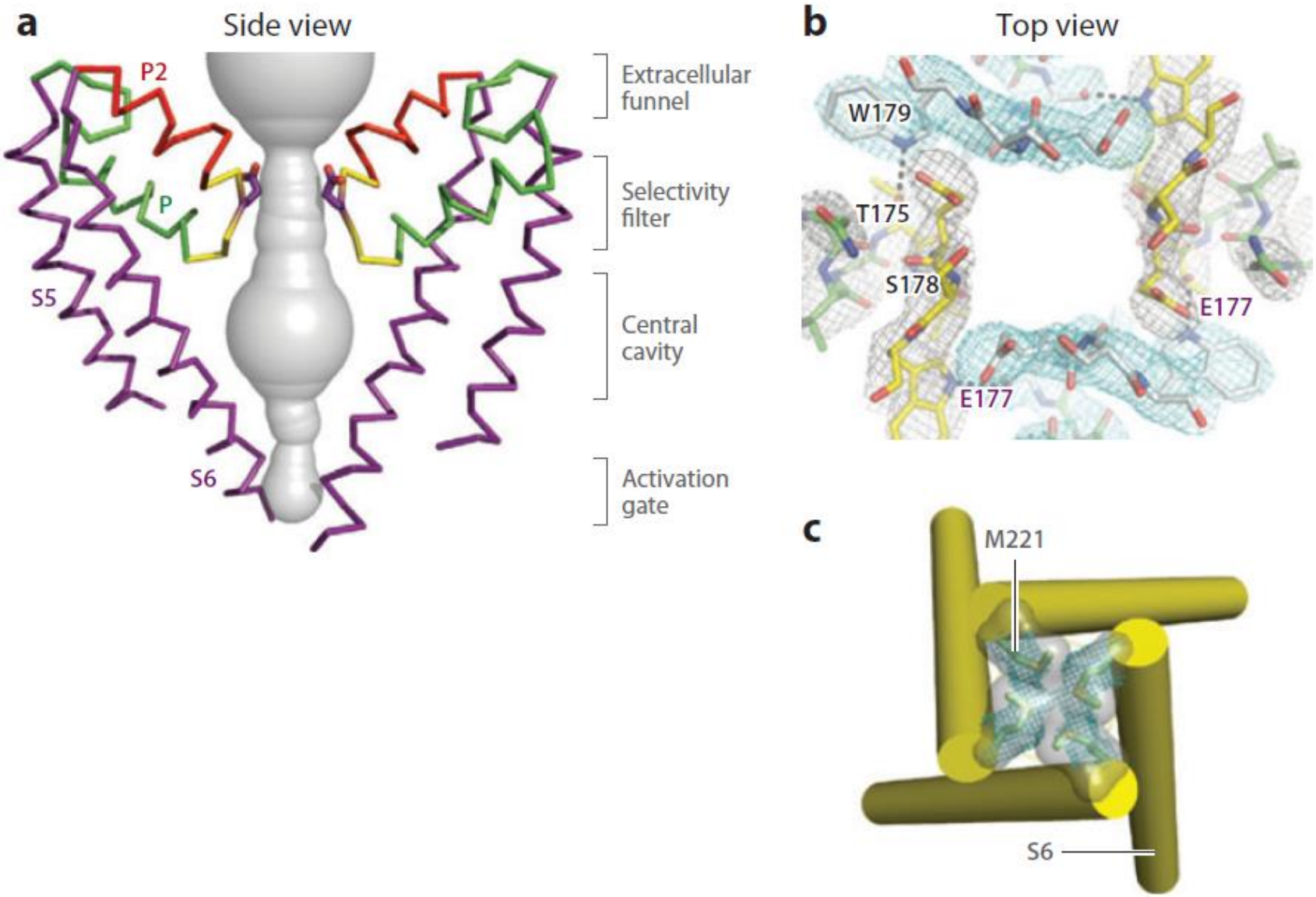
?

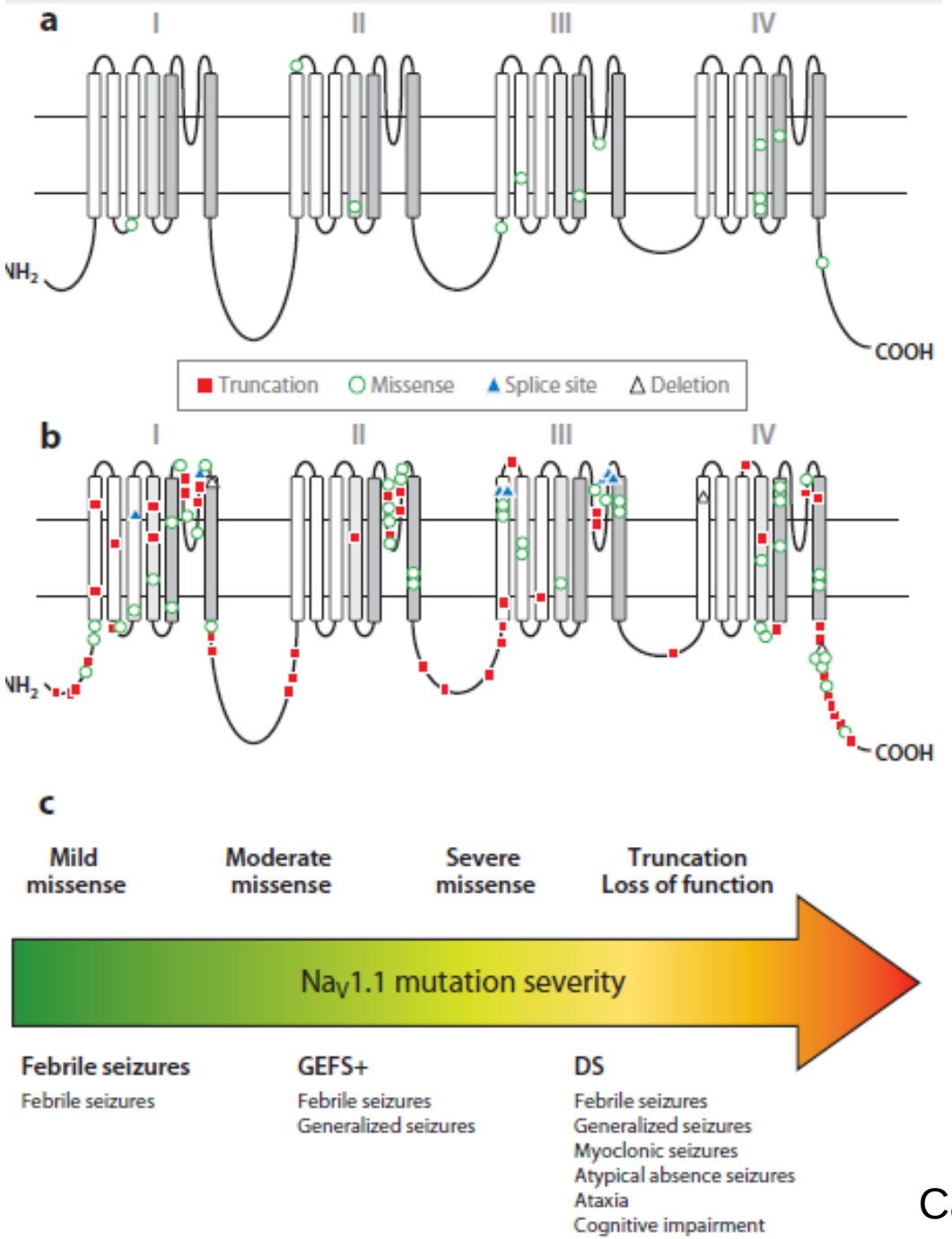
Sodium channel Na_v 1.2



Catterall 2014

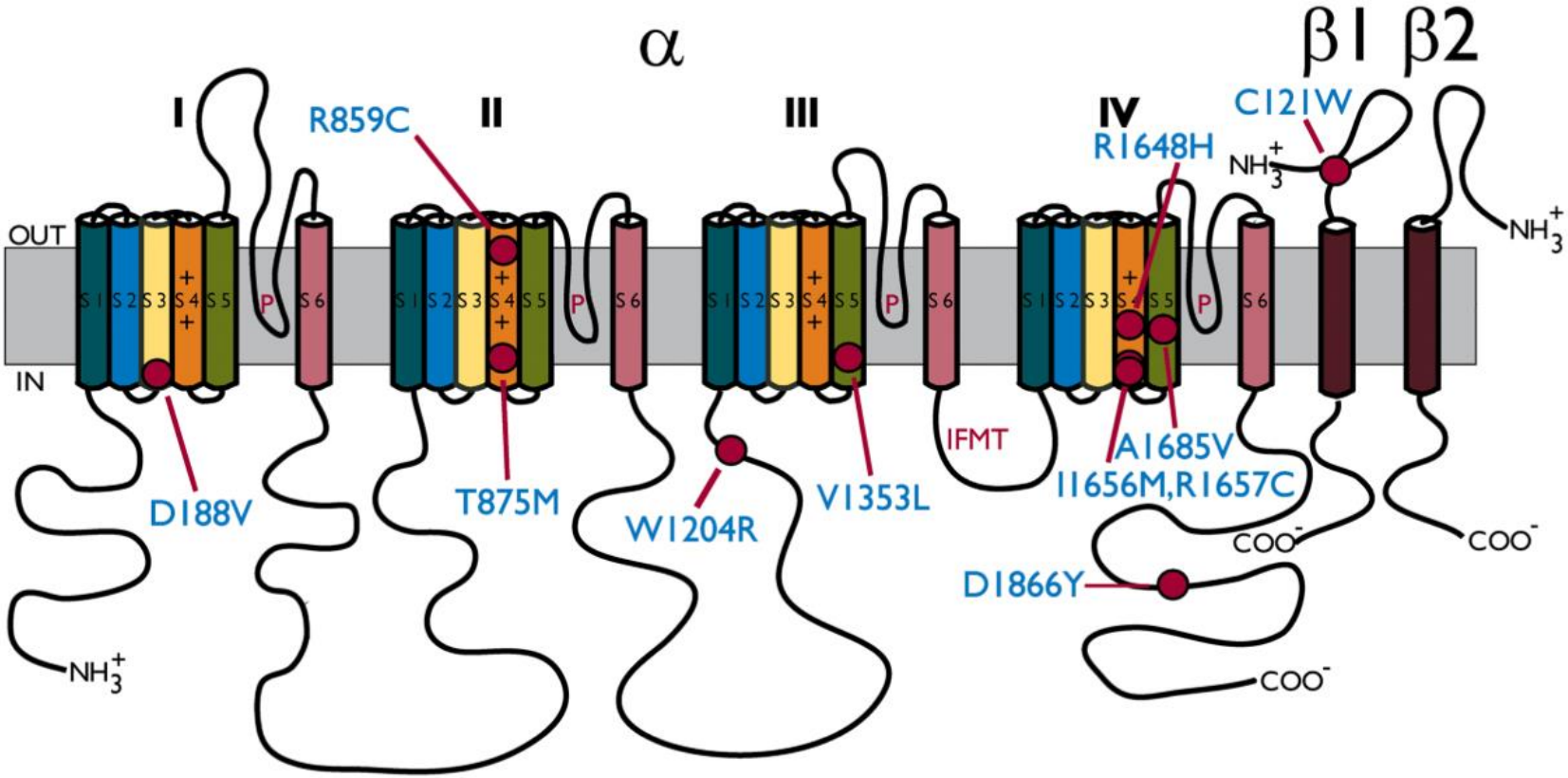

b
c
NavAb/Kv1.2
ENC
HCS
INC
S1N
R1/R2
R2/R3
R3/R4
R4/K5
S2-S3 loop
membrane
anchor


a
Side view
P2
P
S5
S6
Extracellular funnel
Selectivity filter
Central cavity
Activation gate
b
Top view
W179
T175
S178
E177
E177
c
M221
S6



Catterall 2014

Epilepsy-Causing Mutations in SCN1A



Escayg and Goldin 2010

Table 1

Functional Effects of the *SCN1A* Sodium Channel Mutations that Cause GEFS+

Increased Sodium Channel Activity			
Mutation	**Channel**	**Cell Type**	**Effects**
D188V	$rNa_v1.2$	HEK	↓ Use-dependence, Faster recovery from slow inactivation
T875M	$rNa_v1.1$	*Xenopus* oocytes	↑ Persistence
W1204R	$rNa_v1.1$	*Xenopus* oocytes	Negative voltage-dependence
W1204R	$hNa_v1.1$	tsA201	↑ Persistence
R1648H	$rNa_v1.1$	*Xenopus* oocytes	↓ Use-dependence, Faster recovery
R1648H	$hNa_v1.1$	tsA201	↑ Persistence
R1648H	$hNa_v1.4$	tsA201	Faster recovery
R1648C	$rNa_v1.1$	tsA201	↑ Persistence
D1866Y	$rNa_v1.1$	*Xenopus* oocytes	↑ Persistence
Decreased Sodium Channel Activity			
Mutation	**Channel**	**Cell Type**	**Effects**
R859C	$rNa_v1.1$	*Xenopus* oocytes	Positive Voltage-dependence, Slower recovery from slow inactivation, ↓ Current
T875M	$rNa_v1.1$	*Xenopus* oocytes	↑ Slow inactivation
T875M	$hNa_v1.4$	tsA201	↑ Fast and slow inactivation
V1353L	$hNa_v1.1$	tsA201	No sodium current
I1656M	$hNa_v1.1$	tsA201	Positive voltage-dependence
R1657C	$hNa_v1.1$	tsA201	Positive voltage-dependence, ↓ Current
A1685V	$hNa_v1.1$	tsA201	No sodium current

Escayg and Goldin 2010

Ligand-gated channelopathies

GABA ↓

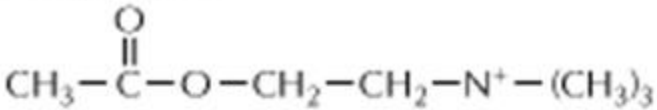
GLUTAMATE ↑

Pre and postsynaptic modifications

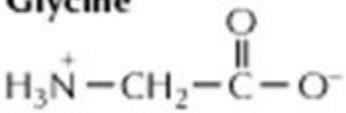
Physiological or structural properties of receptors

GABA

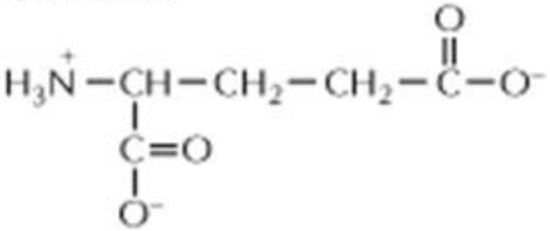
Acetylcholine



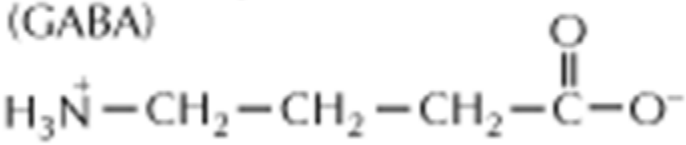
Glycine

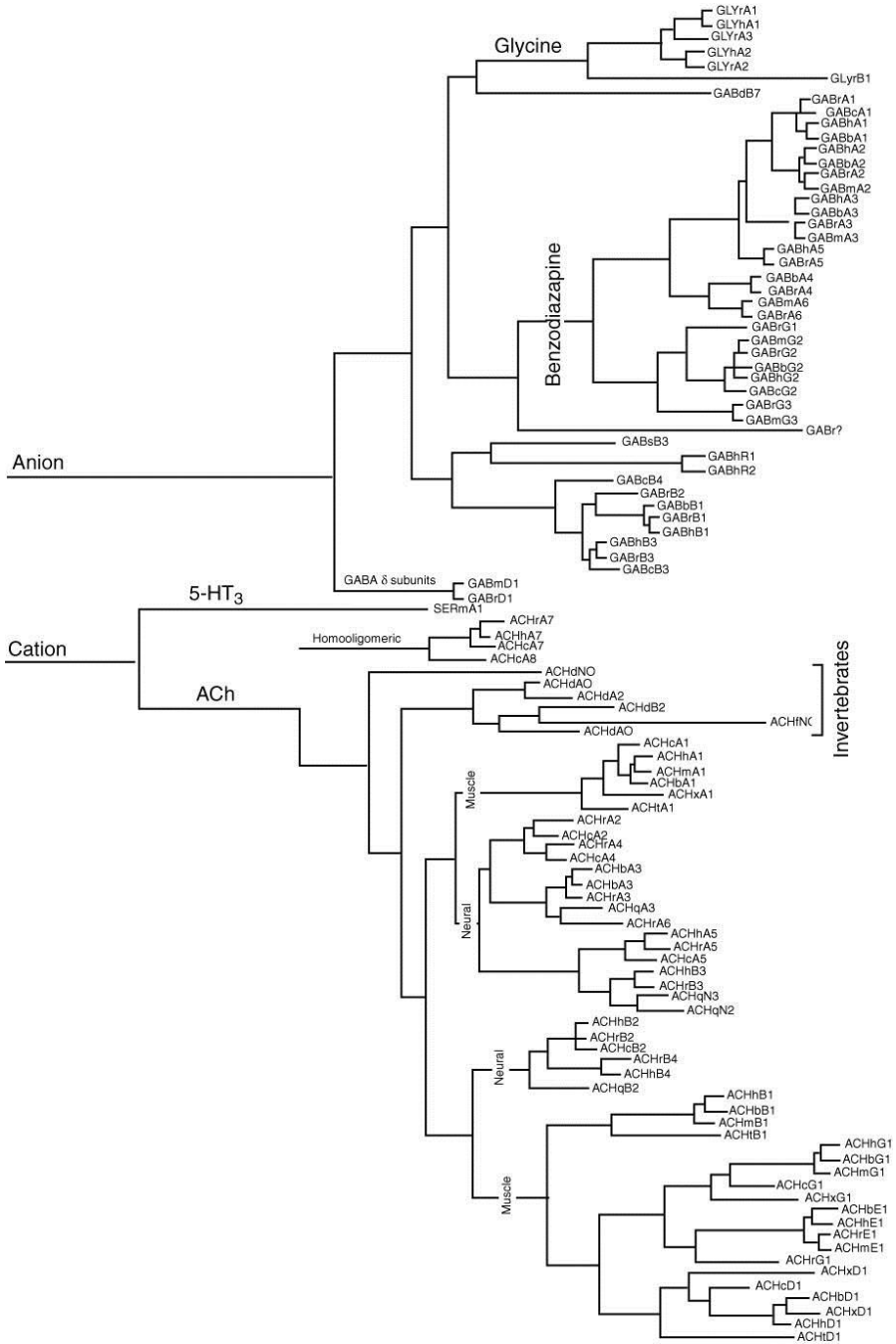


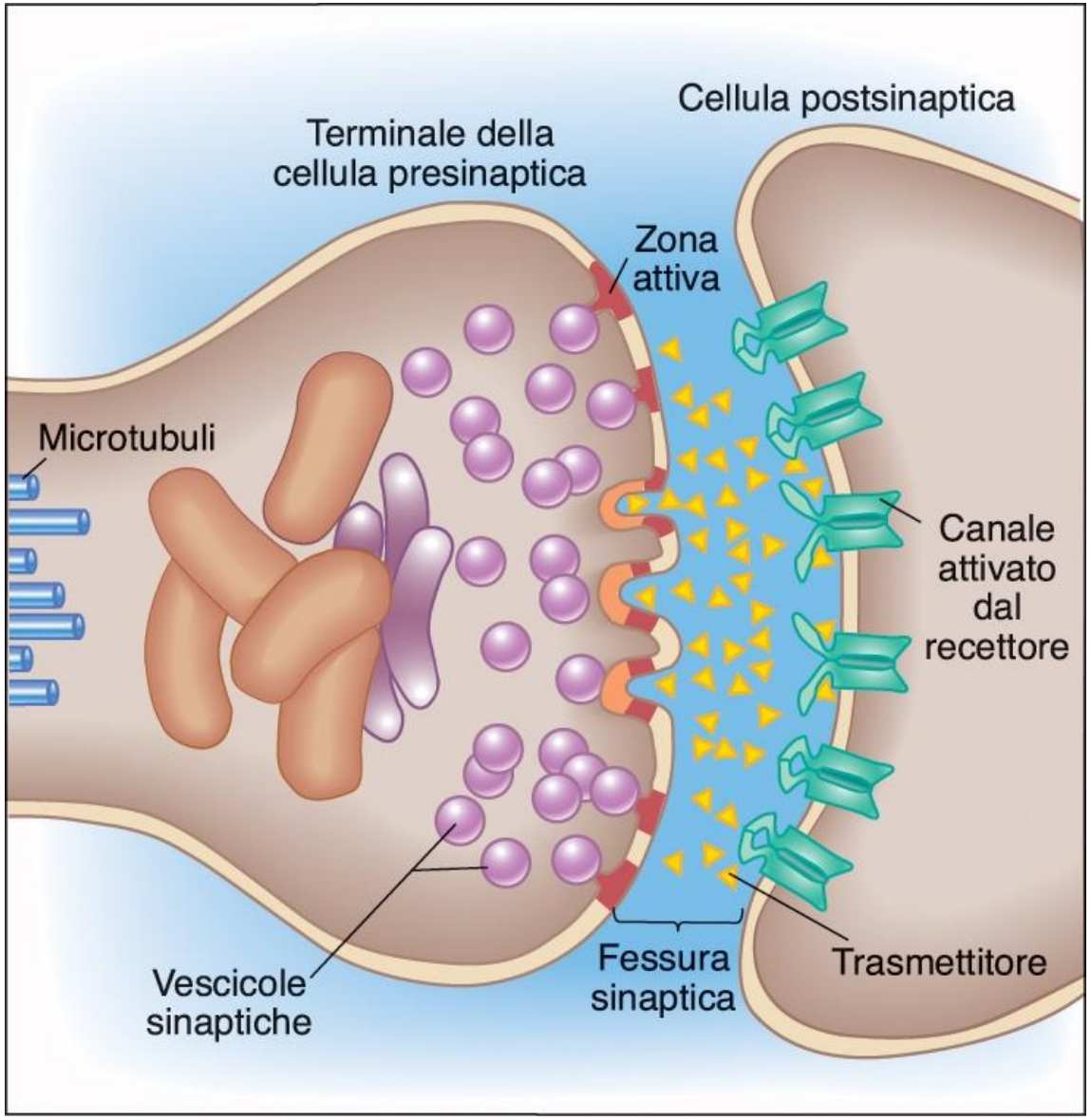
Glutamate



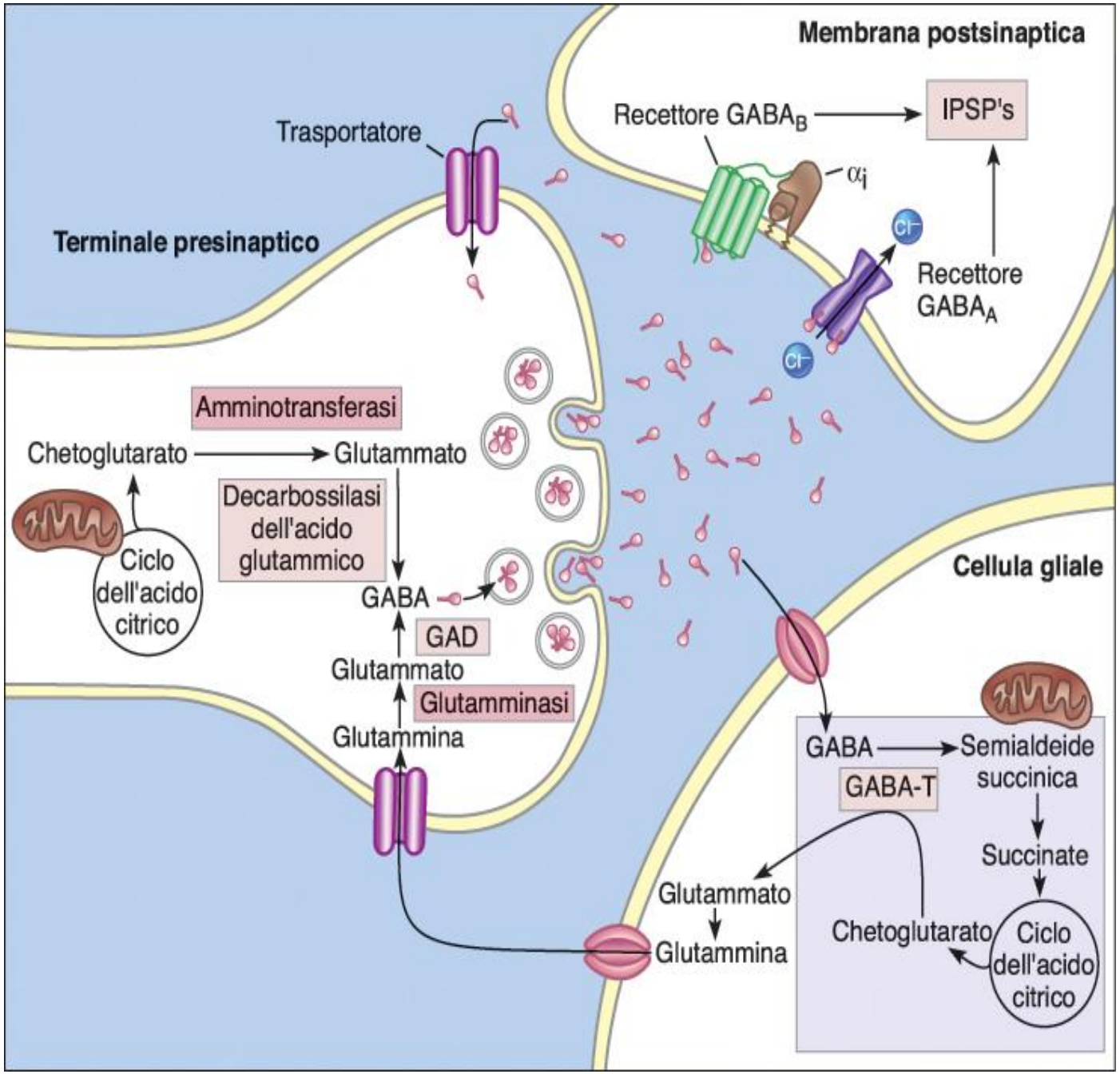
γ-Aminobutyric acid
(GABA)

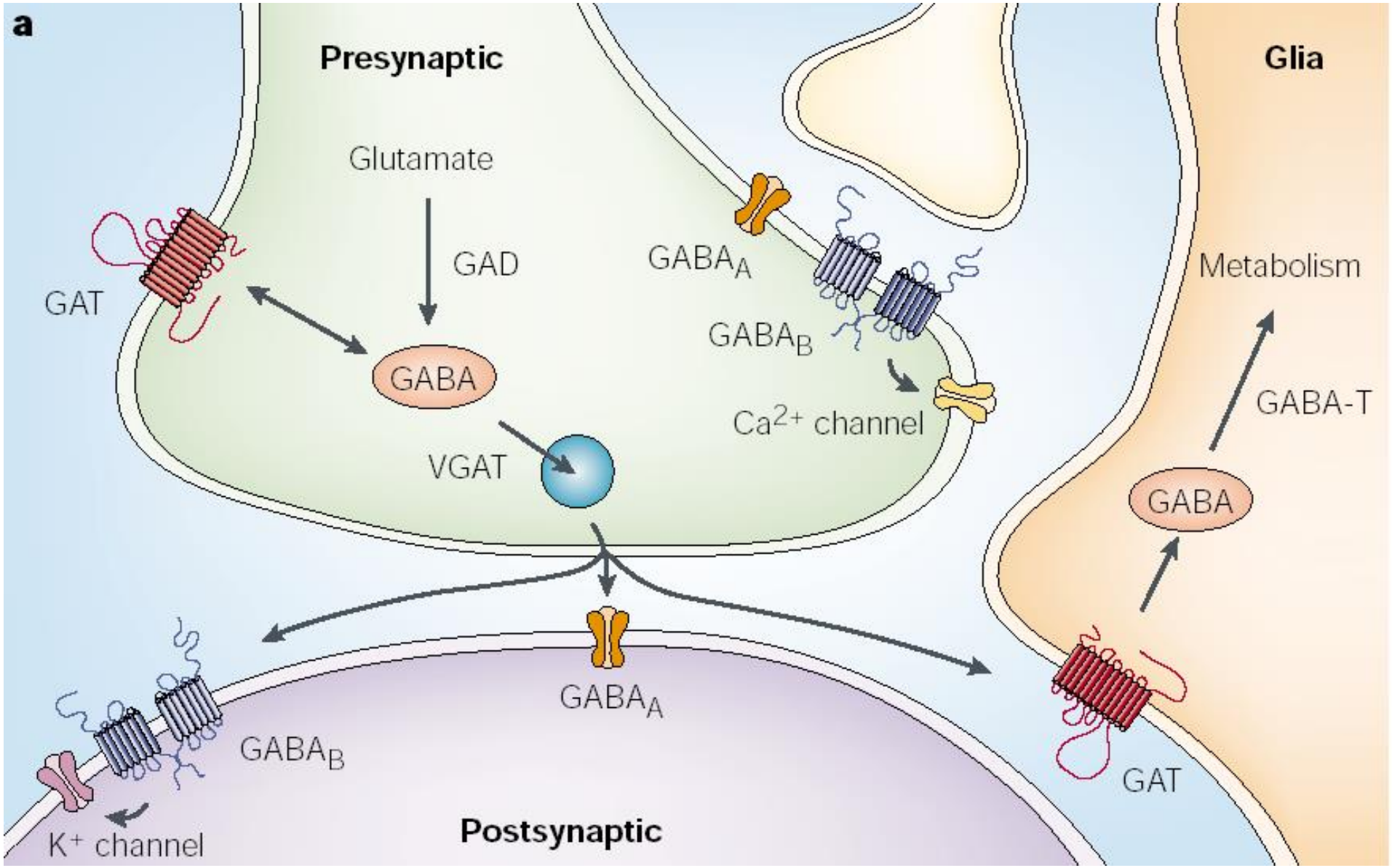


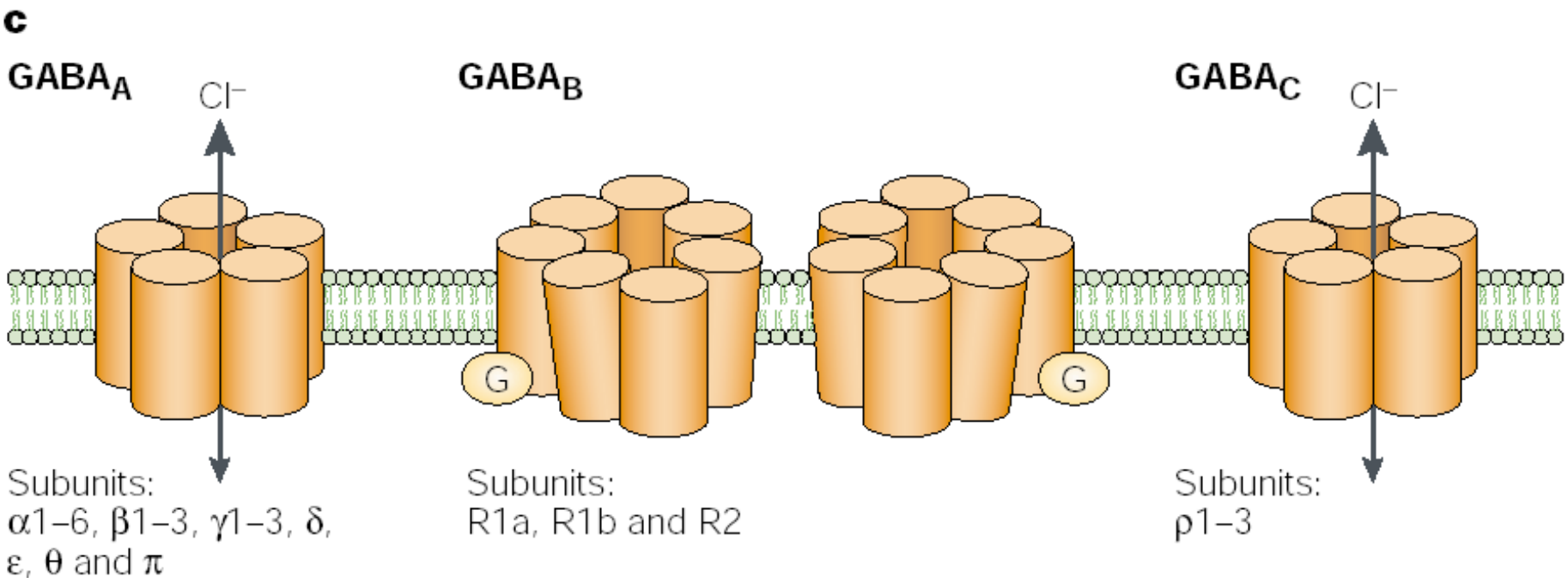



Cellula postsinaptica
Terminale della
cellula presinaptica
Zona
attiva
Microtubuli
Canale
attivato
dal
recettore
Vescicole
sinaptiche
Fessura
sinaptica
Trasmettitore

(a)


Membrana postsinaptica
Recettore GABA$_B$
IPSP's
Trasportatore
α_i
Terminale presinaptico
Cl^-
Recettore GABA$_A$
Cl^-
Amminotransferasi
Chetoglutarato
Glutammato
Decarbossilasi dell'acido glutammico
Ciclo dell'acido citrico
GABA
GAD
Glutammato
Glutamminasi
Glutammina
Cellula gliale
GABA
Semialdeide succinica
GABA-T
Succinate
Glutammato
Chetoglutarato
Ciclo dell'acido citrico
Glutammina


a
Presynaptic
Glia
Glutamate
GAD
GABA
VGAT
GAT
GABA$_A$
GABA$_B$
Ca^{2+} channel
Metabolism
GABA-T
GABA
GABA$_A$
GABA$_B$
K$^+$ channel
GAT
Postsynaptic



Recettori per il GABA (acido γ-amminobutirrico)

$GABA_A$-Rs

- Recettori $GABA_A$ (ionotropo)

  - Recettori ionotropi ligando-dipendenti

  - Funzionano essi stessi da canali ionici permettendo il passaggio di Cl^- se aperti dal neurotrasmettitore

- Sono attivati da: GABA
  Muscimolo
  Isoguvacina

- Sono inibiti da: Bicucullina
  Gabazina

$GABA_B$-Rs

- Recettori $GABA_B$ (metabotropo)

  - Sono associati a canali Ca^{2+} e K^+ attraverso l'attivazione di proteine G

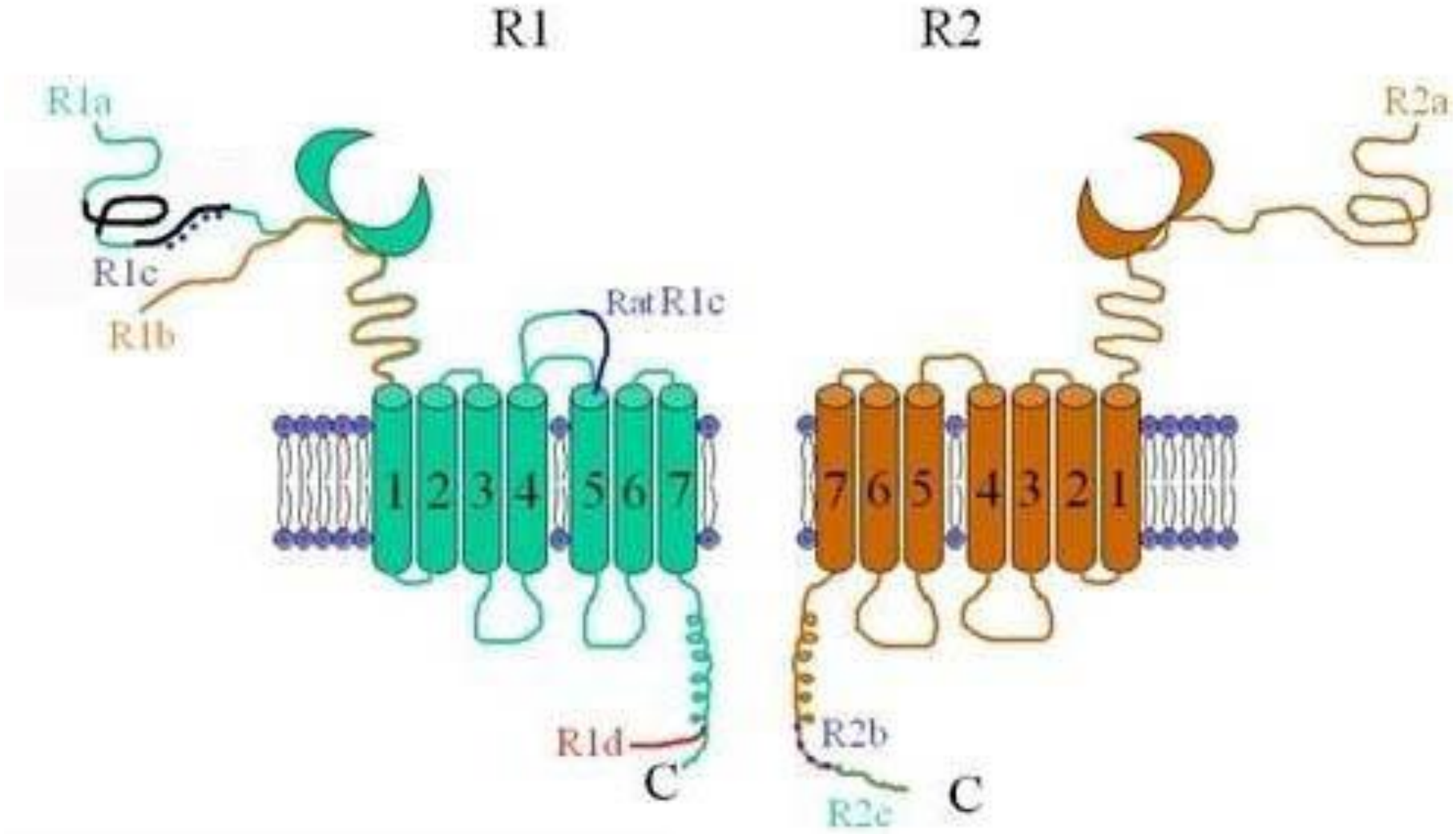
  - Sono attivati da: GABA
    Baclofene
  - Sono inibiti da: faclofene
    saclofene

$GABA_C$-Rs

- Recettori $GABA_C$

- Sono considerati un sottotipo di recettori $GABA_A$. Derivano da varie isoforme della subunità ρ e sono associati a canali per il Cl^-. È tessuto specifico per la retina.

- Sono attivati da: GABA
  CACA ac. Amminocrotonico- 4- cis
  TACA ac. Amminocrotonico-4- trans
- Sono inibiti da: TPMPA ac. metilfofidico
- Sono resistenti a: Bicucullina
  Barbiturici
  Benzodiazepine
  Baclofene



$GABA_B$-Rs

Caratteristiche	$GABA_A$	$GABA_B$	$GABA_C$
Meccanismo	**Ionotropo**	**Metabotropo**	Ionotropo
Subunità	**αβγδεπθ**	**----------**	ρ
Tempo di apertura	**2-20 ms**	**----------**	150 ms
Farmacologia			
GABA	**10 μM (EC_{50})**	**10 μM (EC_{50})**	1 μM (EC_{50})
Muscimolo	**Agonista**	**Inattivo**	Agonista parziale
Baclofen	**Inattivo**	**Agonista**	Inattivo
Bicucullina	**Antagonista competitivo**	**Inattivo**	Inattivo
Picrotoxin	Antagonista non competitivo	Inattivo	Antagonista non competitivo

GABA site
Agonists
Antagonists

BARBITURATE site
Depressants (also ethanol?)
Excitants?

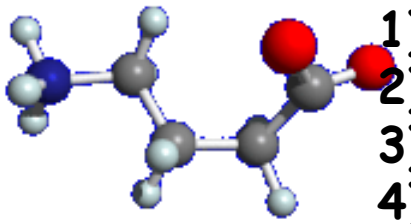
BENZODIAZEPINE site
Agonists (depressants)
Antagonists
Inverse agonists

STEROID site
Anesthetics
Excitants?

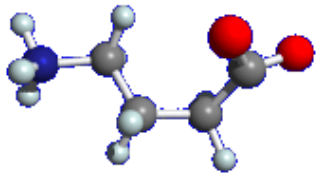
PICROTOXININ site
Convulsants
Depressants?

Cl^-

Chloride channel

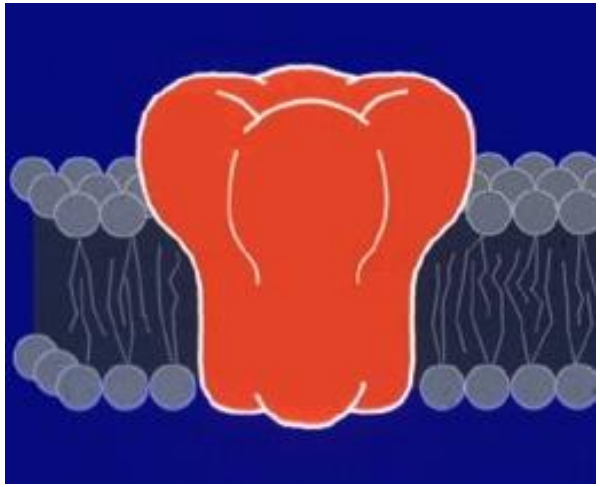


1) Media la neurotrasmissione inibitoria
2) Ionotropo, selettivo per gli ioni Cl^-
3) Pentamero (2α, 2β, γ)
4) Siti di legame per farmaci quali barbiturici e benzodiazepine



two classes of $GABA_A$ receptors

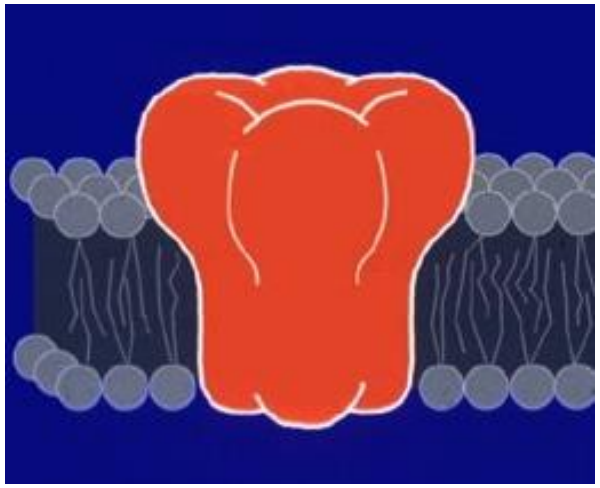
γ2 receptors



Low affinity Zn^{2+}-insensitive

Phasic

α5/δ/αx/βx receptors



High affinity Zn^{2+}-sensitive

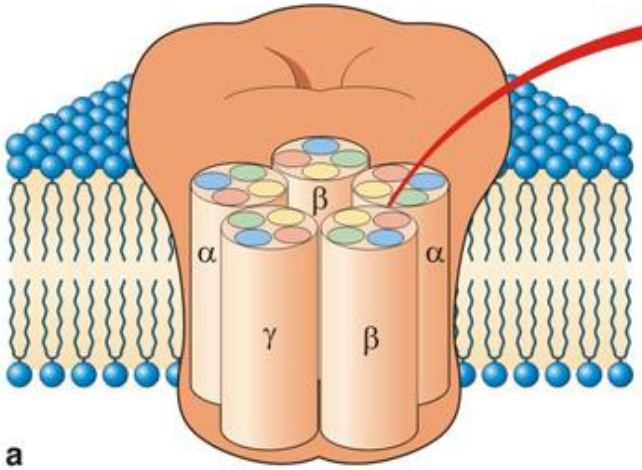
Tonic

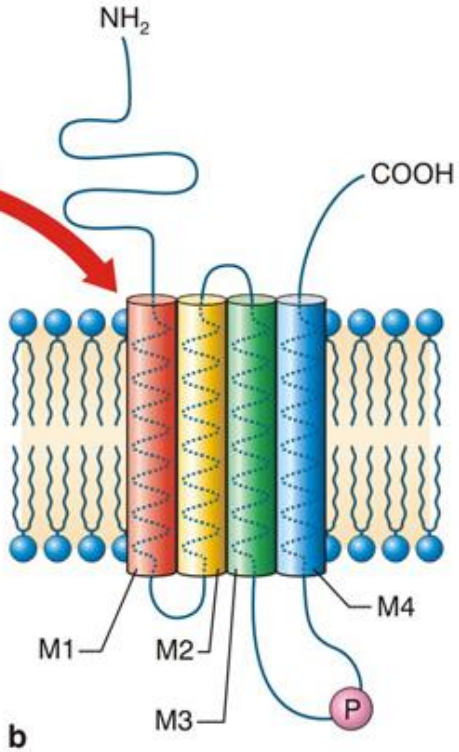
Table 16-1. Distributions and Novel Pharmacological and Physiological Properties of the Major $GABA_A$ Receptor Subtypes in the Rat Brain

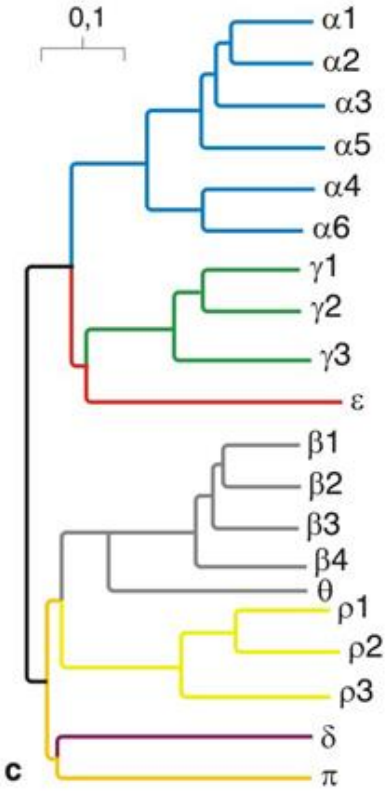
Isoform	Relative abundance	Location	Pharmacology/property
$\alpha1\beta2\gamma2$	40%	Most brain areas; hippocampal, cortical interneurons; cerebellar Purkinje cells	Common coassembly BZ-type I Zn-insensitive
$\alpha2\beta3\gamma2$	15%	Spinal cord motoneurons, hippocampal pyramidal cells	BZ-type II Moderately Zn-sensitive
$\alpha3\beta\gamma2/3$	10%	Cholinergic, monaminergic neurons	BZ-type II, abecarnil-sensitive
$\alpha2\beta\gamma1$	10%	Bergmann glia, thalamus, hypothalamus	BZ inverse agonist-enhanced
$\alpha5\beta3\gamma2/3$	3%	Hippocampal pyramidal cells	BZ-type II, zolpidem-insensitive, moderate Zn-sensitivity
$\alpha6\beta\gamma2$	2%	Cerebellar granule cells	BZ agonist-insensitive, moderate Zn-sensitivity
$\alpha6\beta\delta$	3%	Cerebellar granule cells	Insensitive to all BZ, GABA high affinity high Zn-sensitivity steroid-insensitive
$\alpha4\beta\gamma$	2%	Cortical, hippocampal pyramidal cells; striatum	BZ agonist-insensitive, low steroid sensitivity
$\alpha4\beta2\delta$	4%	Thalamus, dentate granule cells	Insensitive to all BZ, GABA high affinity high Zn sensitivity, steroid-insensitive
All other	11%	Throughout CNS	

BZ, benzodiazepine.Modified from McKernan and Whiting [20] with permission.

$GABA_A$-Rs

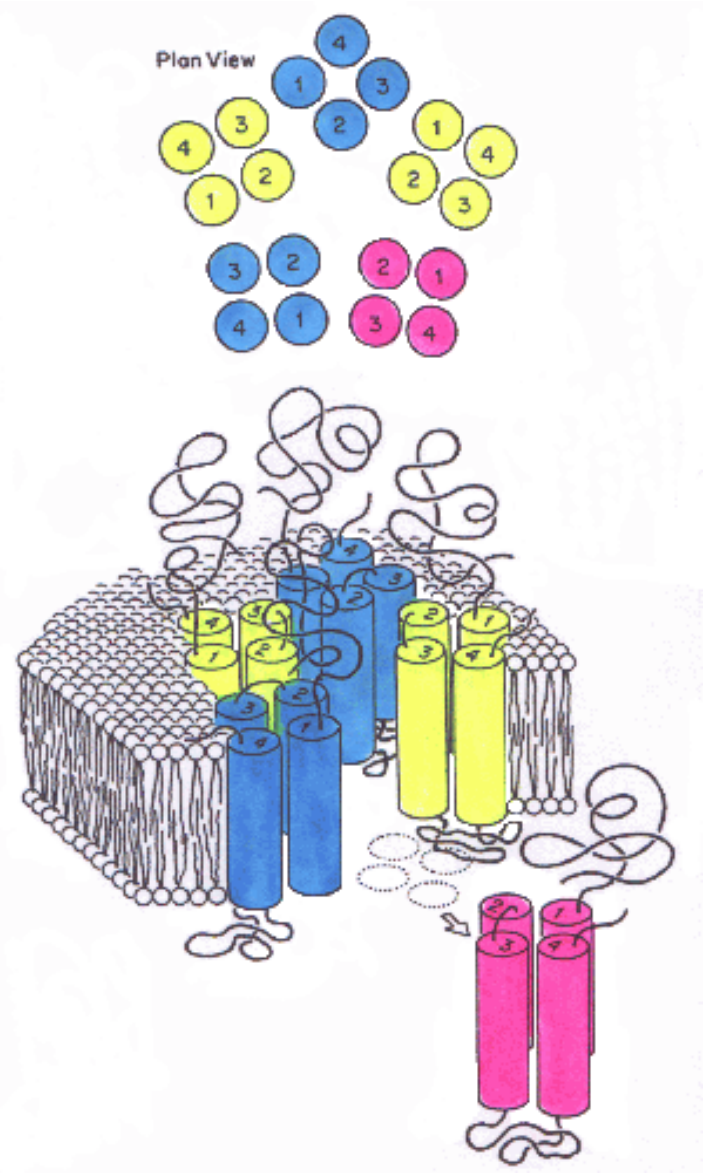


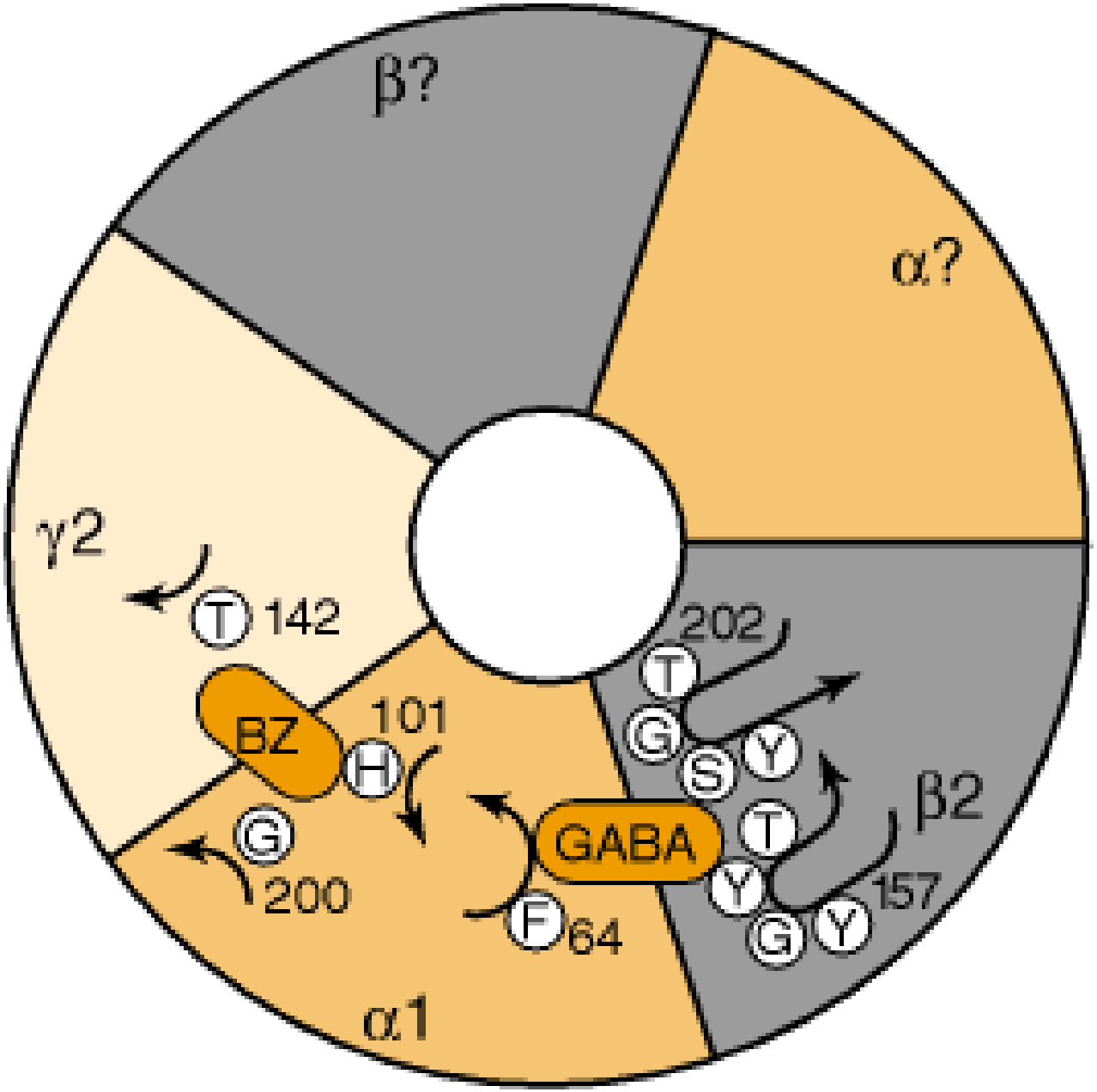




$GABA_A$-Rs

- Pentamerica
- 4 domini transmembranari
- Loop intracellulare fra i domini 3 e 4
- Sono stati identificati 16 geni responsabili per le subunità del recettore
- Le subunità sono state divise in 7 classi α1-6, β1-3, γ1-3, δ, ε, π e ρ1-3
- la maggior parte dei sottotipi di recettori $GABA_A$ siano formati da subunità α, β e γ




β?
α?
γ2
T
142
BZ
101
H
G
200
GABA
F
64
α1
202
T
G
S
Y
T
Y
G
Y
157
β2

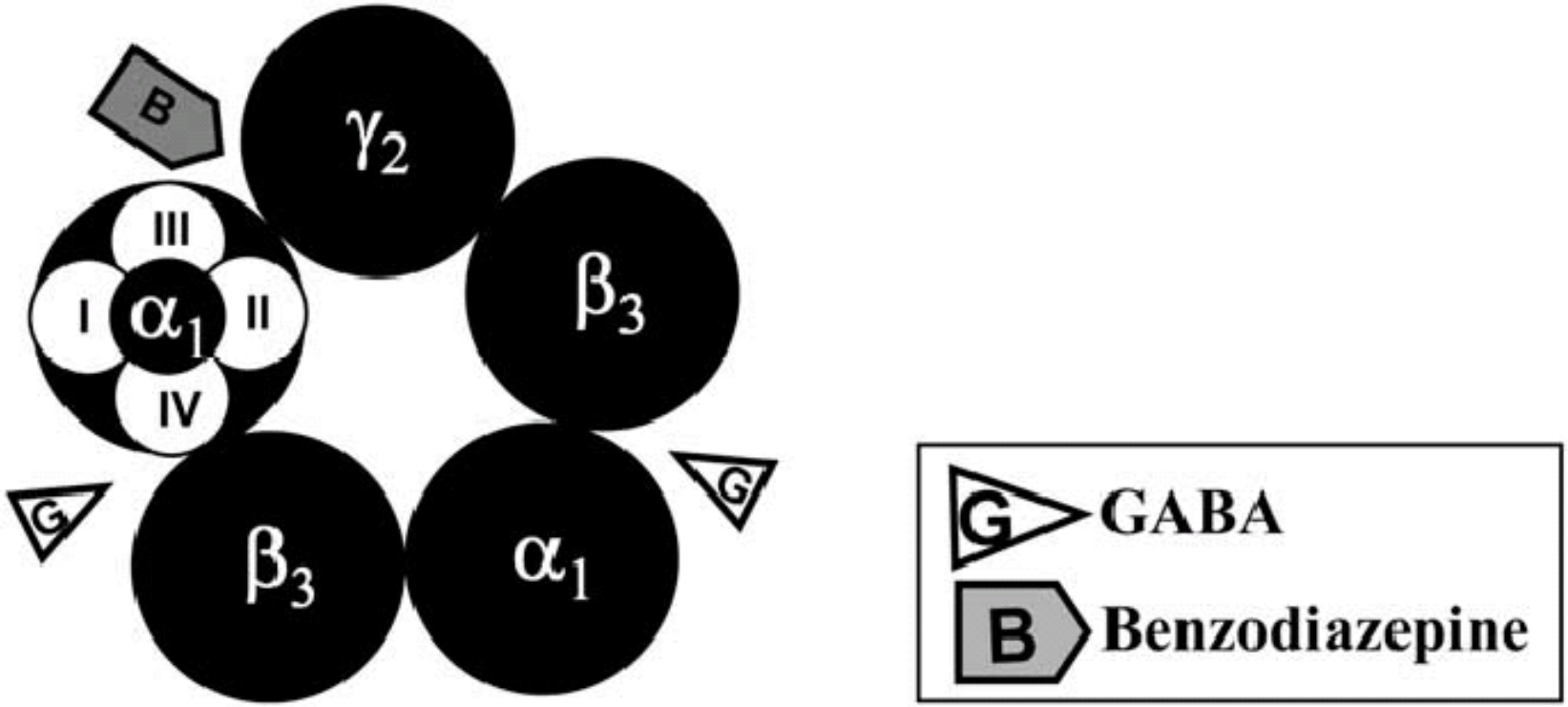
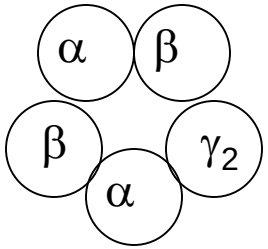


Fig. 1. Model of $GABA_A$ receptors with location of two binding sites for GABA and one for benzodiazepine allosteric modulators.

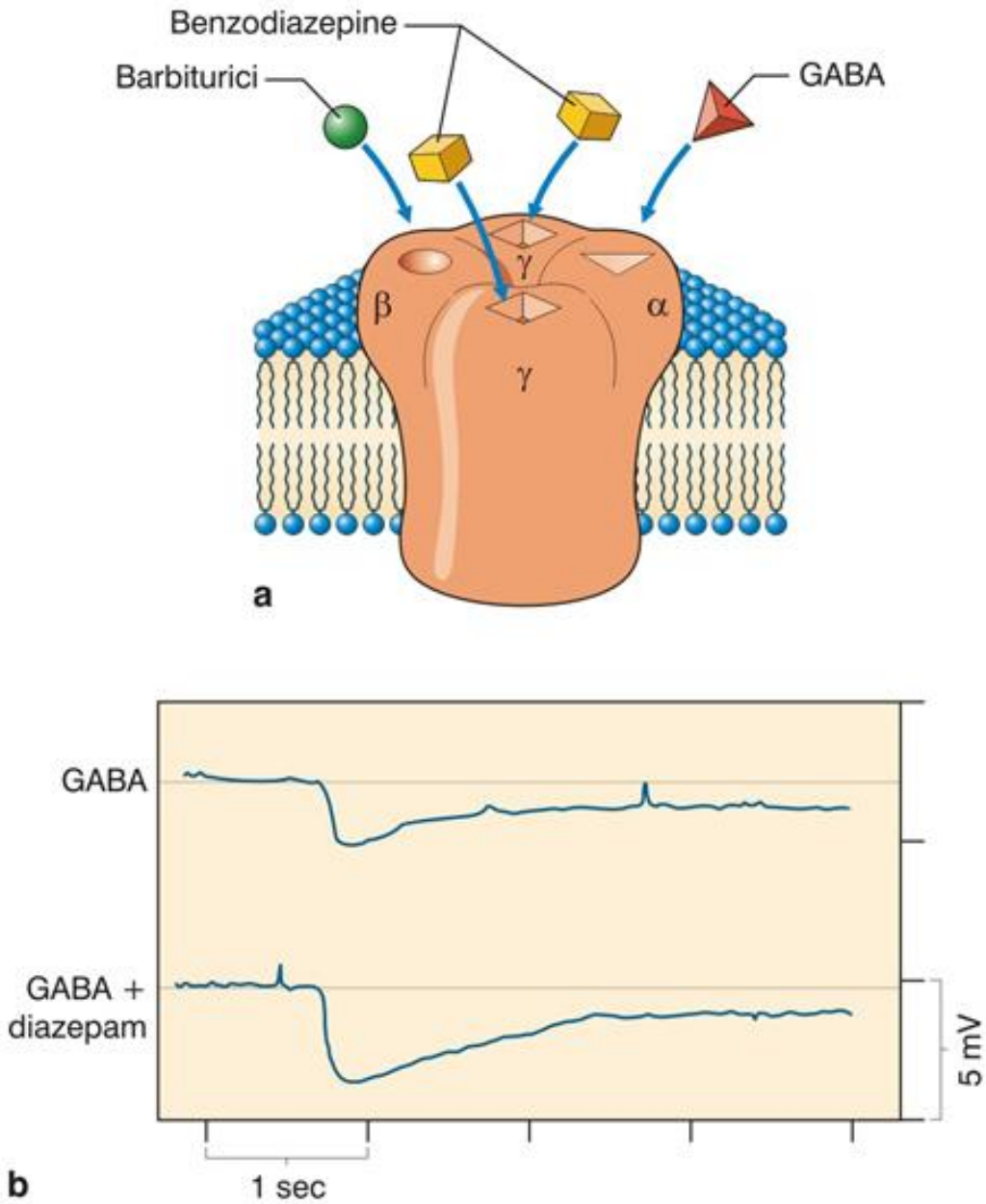
$GABA_A$-Rs

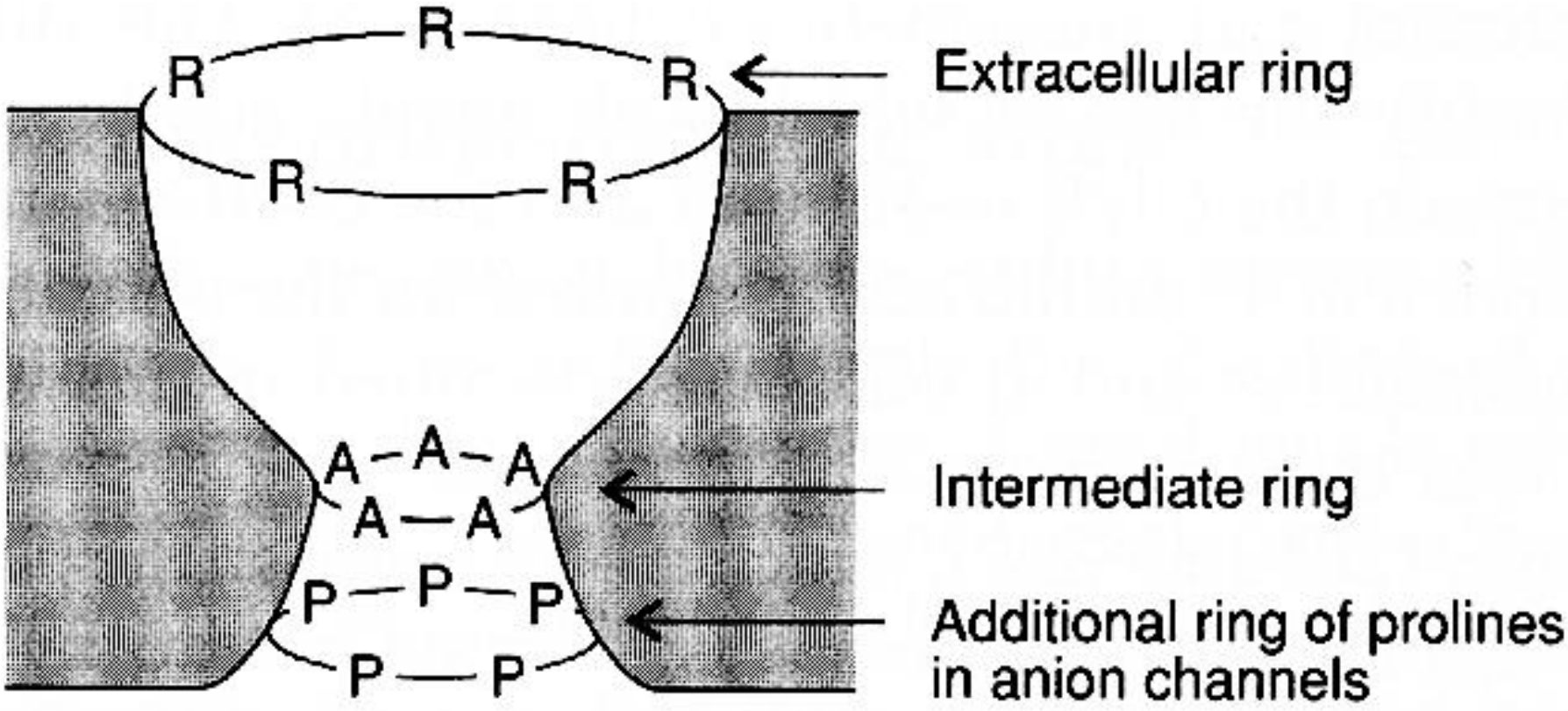


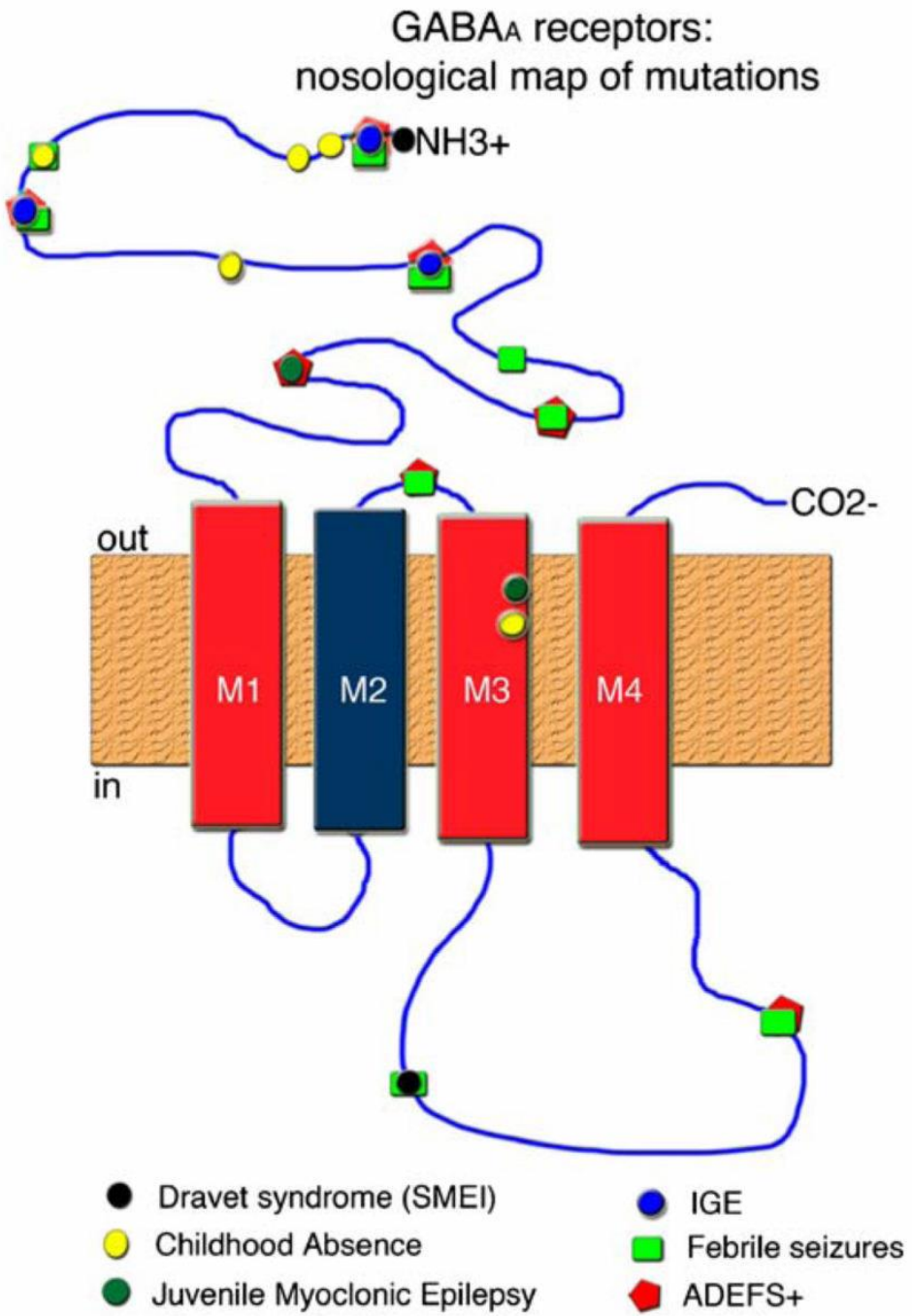
Probabilita' di apertura e e durata delle aperture condizionata da:

- benzodiazepine (+γ_2)
- barbiturici
- anestetici (-ε)
- alcol
- ormoni steroidei (δ)

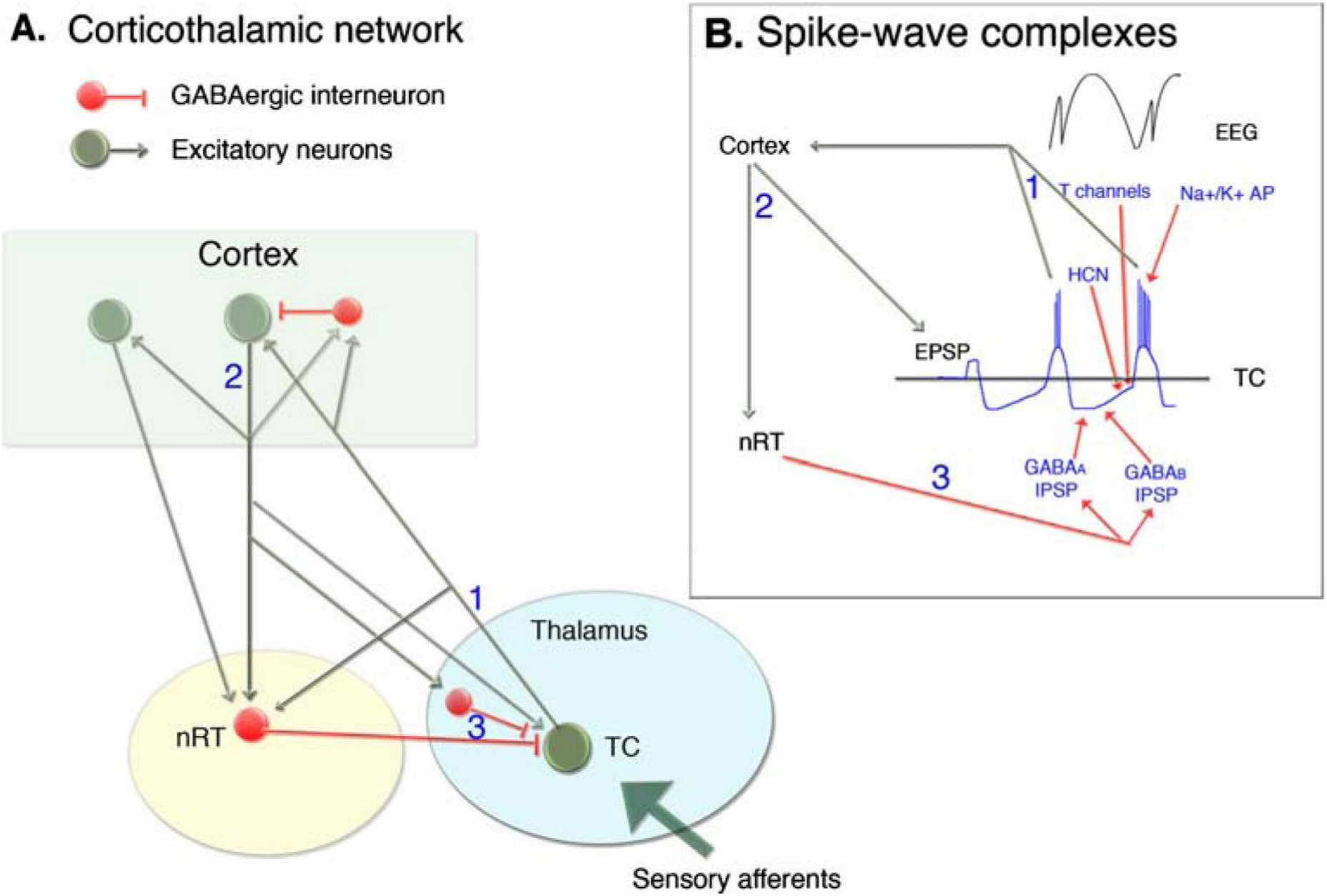
Gene	Protein	Chromosome location (human)
GABRA1	α1	5q31.1–33.2
GABRA2	α2	4p12–p13
GABRA3	α3	Xq28
GABRA4	α4	4p14–q12
GABRA5	α5	15q11–q13
GABRA6	α6	5q31.1–33.2
GABRB1	β1	4p12–p13
GABRB2	β2	5q31.1–33.2
GABRB3	β3	15q11–q13
GABRG1	γ1	4p14–q21.1
GABRG2	γ2	5q31.1–33.2
GABRG3	γ3	15q11–q13
GABRD1	δ1	1p
GABRE1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




R
R
R
R
R
Extracellular ring
A
A
A
A
A
Intermediate ring
P
P
P
P
P
Additional ring of prolines
in anion channels



Galanopoulou 2010

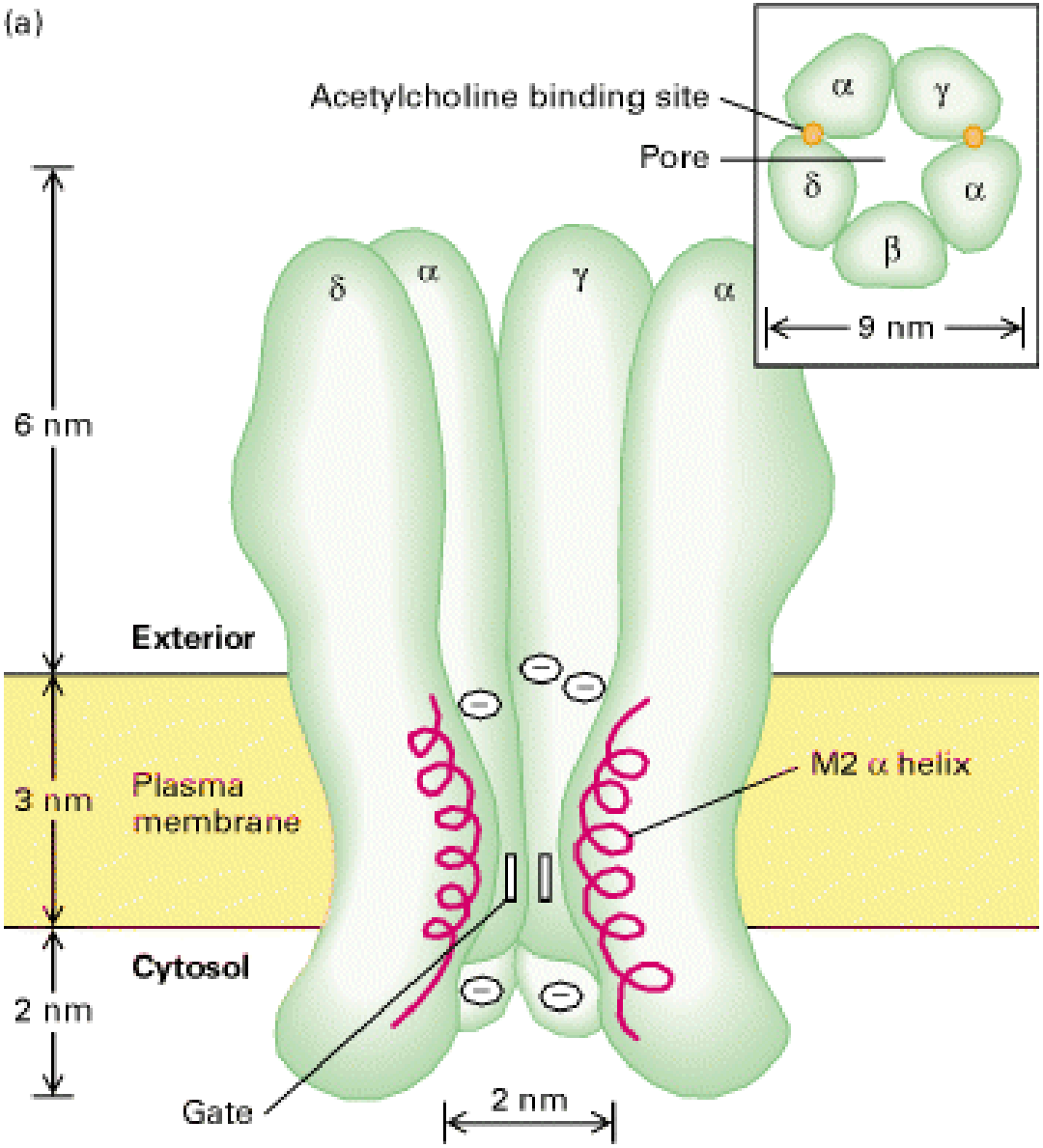


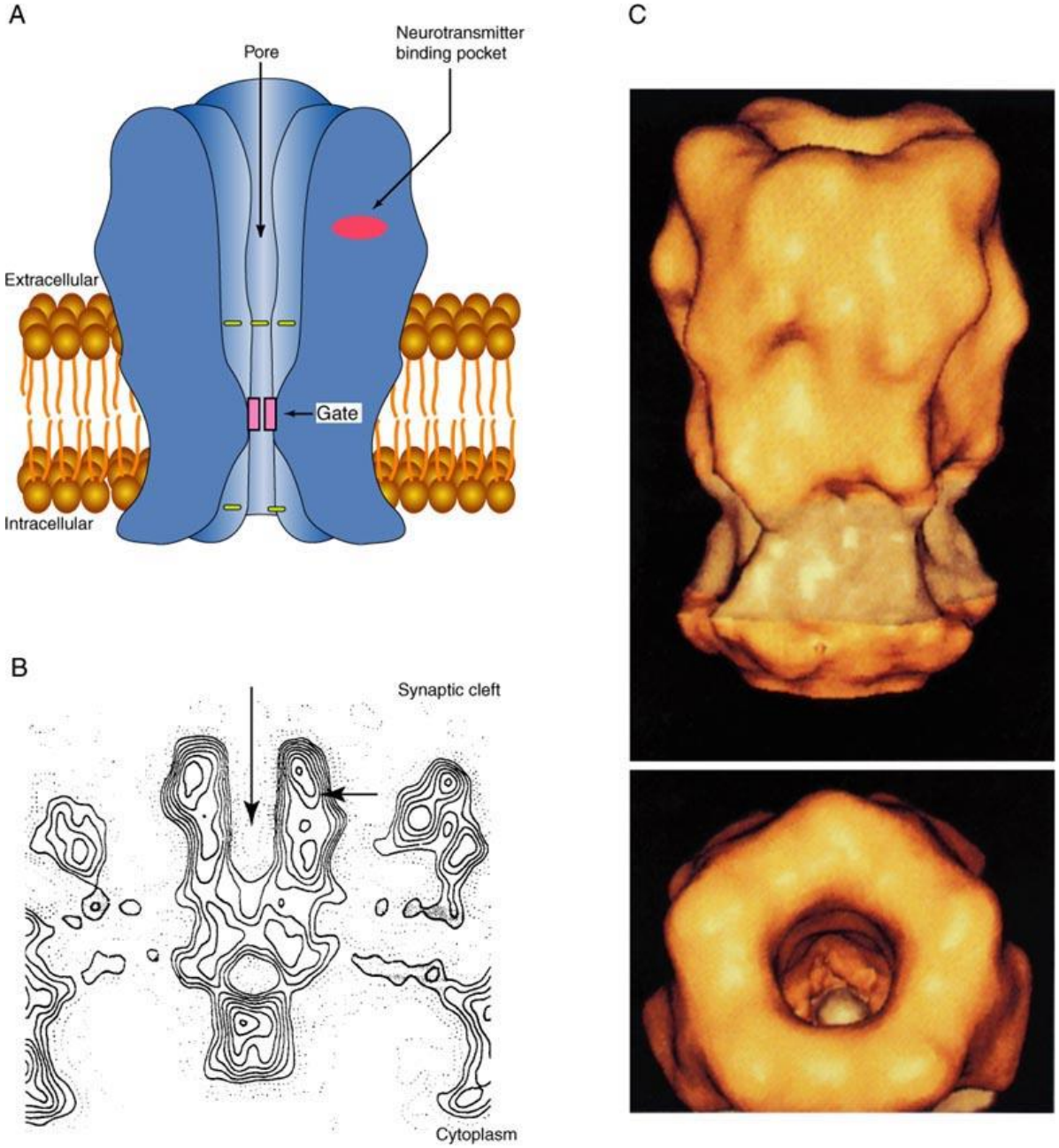
Galanopoulou 2010

GABAA receptor mutations in epilepsy and seizures

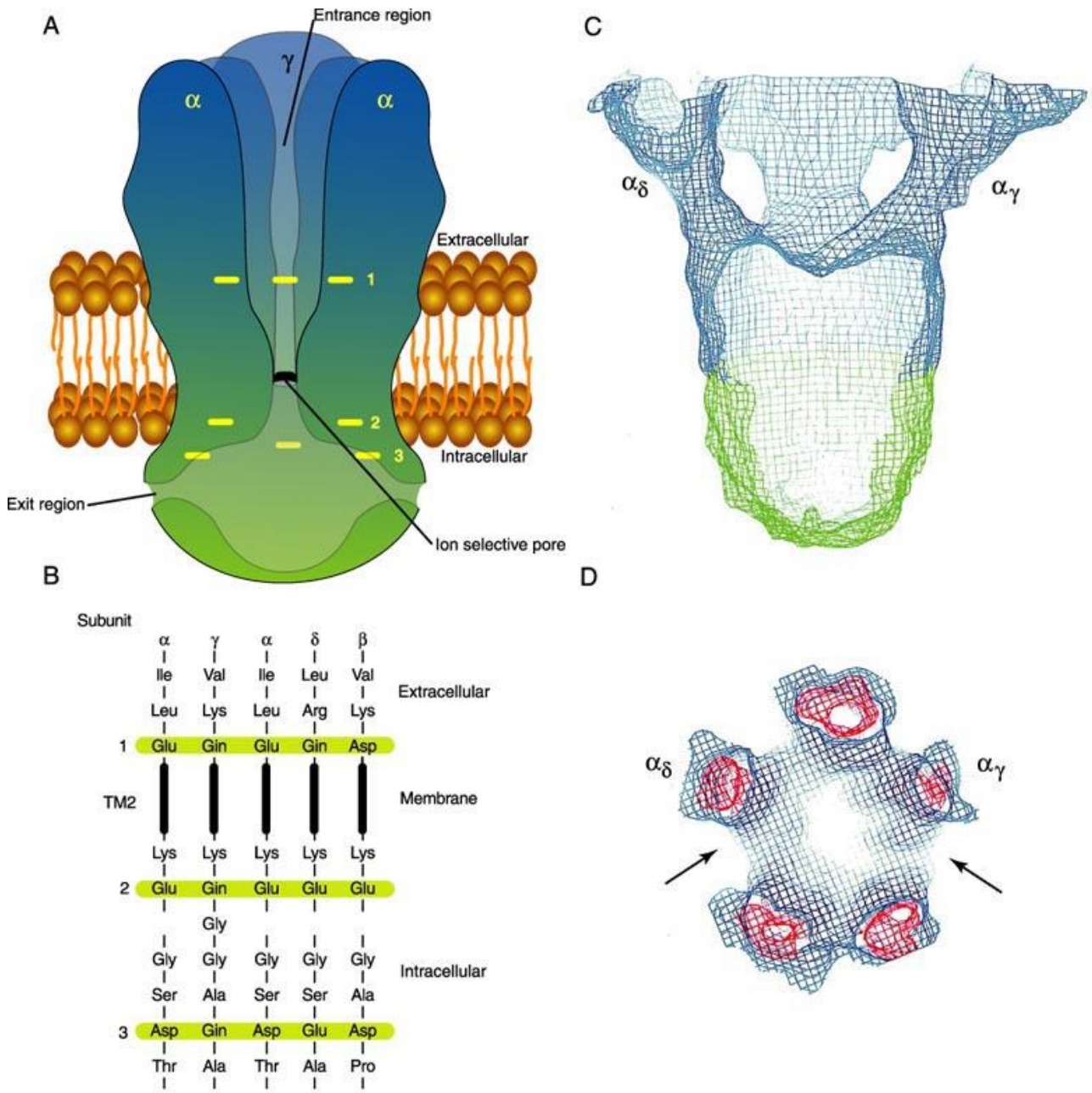
Seizure type / epilepsy	Mutation	Type of mutation	Site of mutation	Functional effect of mutation	Reference
Autosomal dominant JME	GABRA1 (A322D)	Missense	M3 domain	Low amplitude GABA currents reduced surface expression; increased GABA EC50	[56,57]
	GABRD (R220H	Missense	Extracellular domain	Low amplitude GABA currents; impaired trafficking?	[46]
CAE	GABRA1 (975delC; S326fs328X)	Frameshift mutation Premature termination	M3 domain	Nonsense-mediated mRNA decay; degradation in ER	[16,17]
	GABRA6 (R46W)	Missense	Extracellular domain	No change in GABAA current	[18]
	GABRB3 (–897T/C polymorphism)		Promoter region	Decreased transcription (impaired N-Oct-3 binding)	[19]
	GABRB3 (P11S; S15F, G32R)	Missense	Extracellular domain	Hyperglycosylation?	[20]
	GABRB3	Association analysis			[15]
CAE + febrile seizures	GABRG2 (R43Q)	Missense	Extracellular domain	Decreased surface expression; ER retention; Altered benzodiazepine / GABA binding?	[24,57,126] [26,27,32,34]
	(IVS6 + 2 T<G)	Exon 6 skipping Premature termination	M1–M2 linker	Exon skipping, premature termination ER retention and degradation vs nonfunctional protein	[37]
ADEFS+	GABRG2 (K289M)	Missense	M2–M3 linker (extracellular)	Decreased GABAA current amplitude; Intact DZ potentiation	[38]
	GABRG2 (W390X)	Premature termination	M3–M4 linker (intracellular)	?	[127]
	GABRD (E177A) (R220C)	Missense	Extracellular domain	Low amplitude GABA currents impaired trafficking?;	[46]
	GABRA4 (T320A)	Missense		? Also seen in controls	[18]
IGE, ADEFS+ febrile seizures	GABRP (V10M)	Missense	Extracellular domain	?	[18]
	GABRE (Y38S, E52K)	Missense	Extracellular domain	? Also present in controls	[18]
SMEI ADEFS+	GABRG2 (Q351X)	Premature	M3-M4 linker	ER retention	[42,121]
SMEI	GABRG2 (Q40X)	Premature termination	Extracellular domain	ER retention	[49]
Febrile seizures	GABRG2(R139G)	Missense	Extracellular domain	Increased fast phase desensitization, reduced sensitivity to diazepam	[44]
	GABRE (G66S)	Missense	Extracellular domain	?	[18]
Angelman syndrome	15q11-13 deletion	Deletion, maternally inherited	15q11-13	Decreased expression of involved genes (may include GABRB3, GABRA5, GABRG3)	[60]

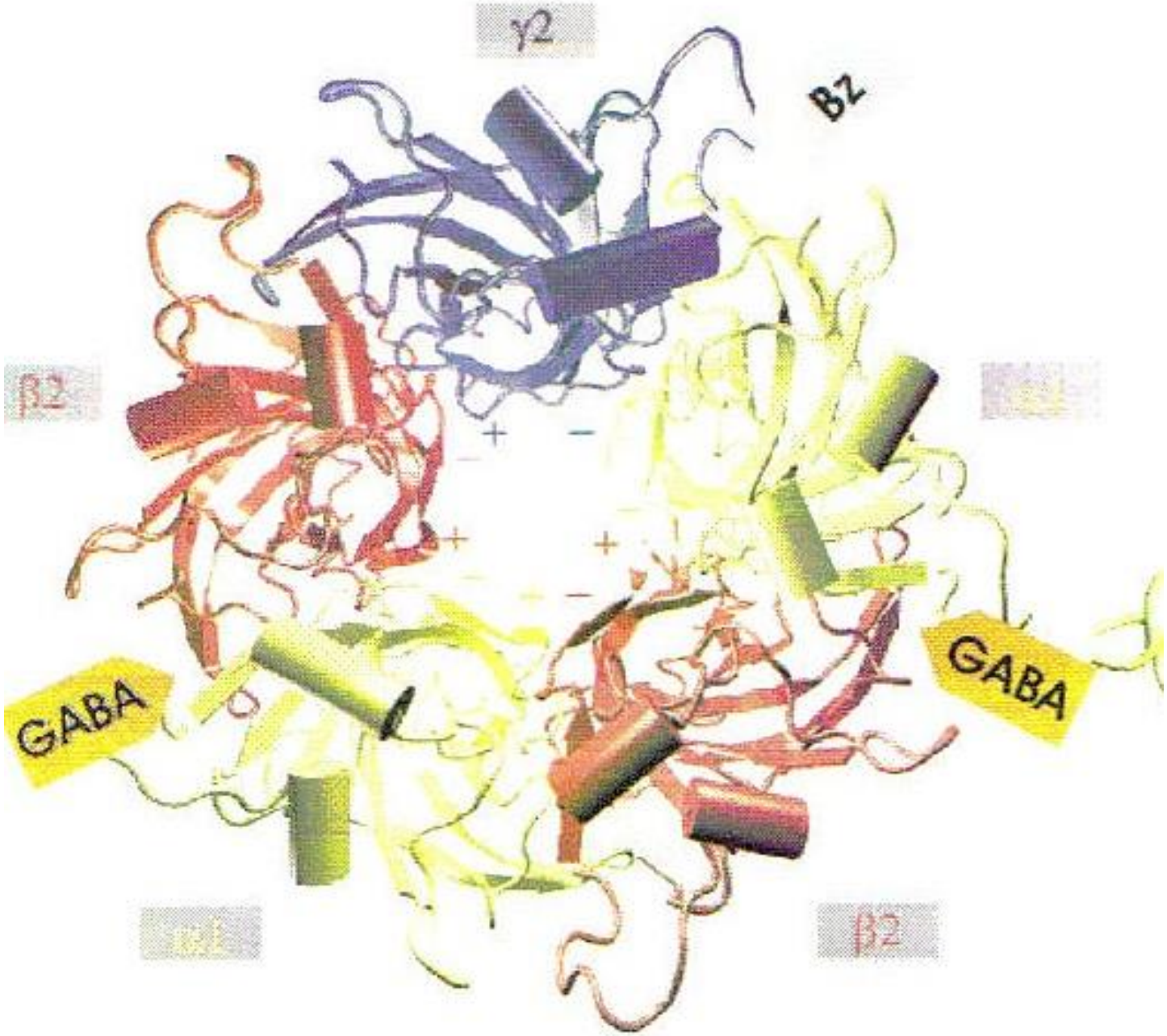
Acetylcholine-Rs

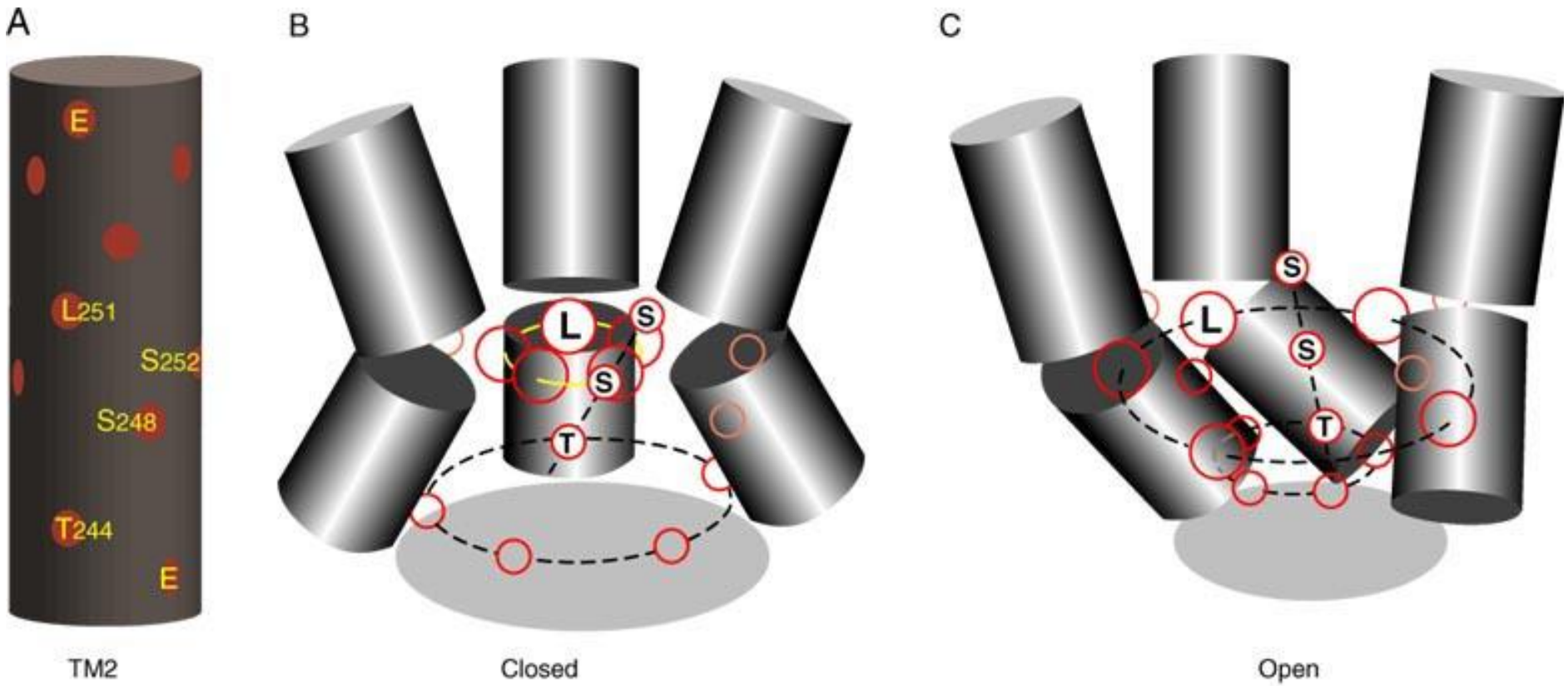


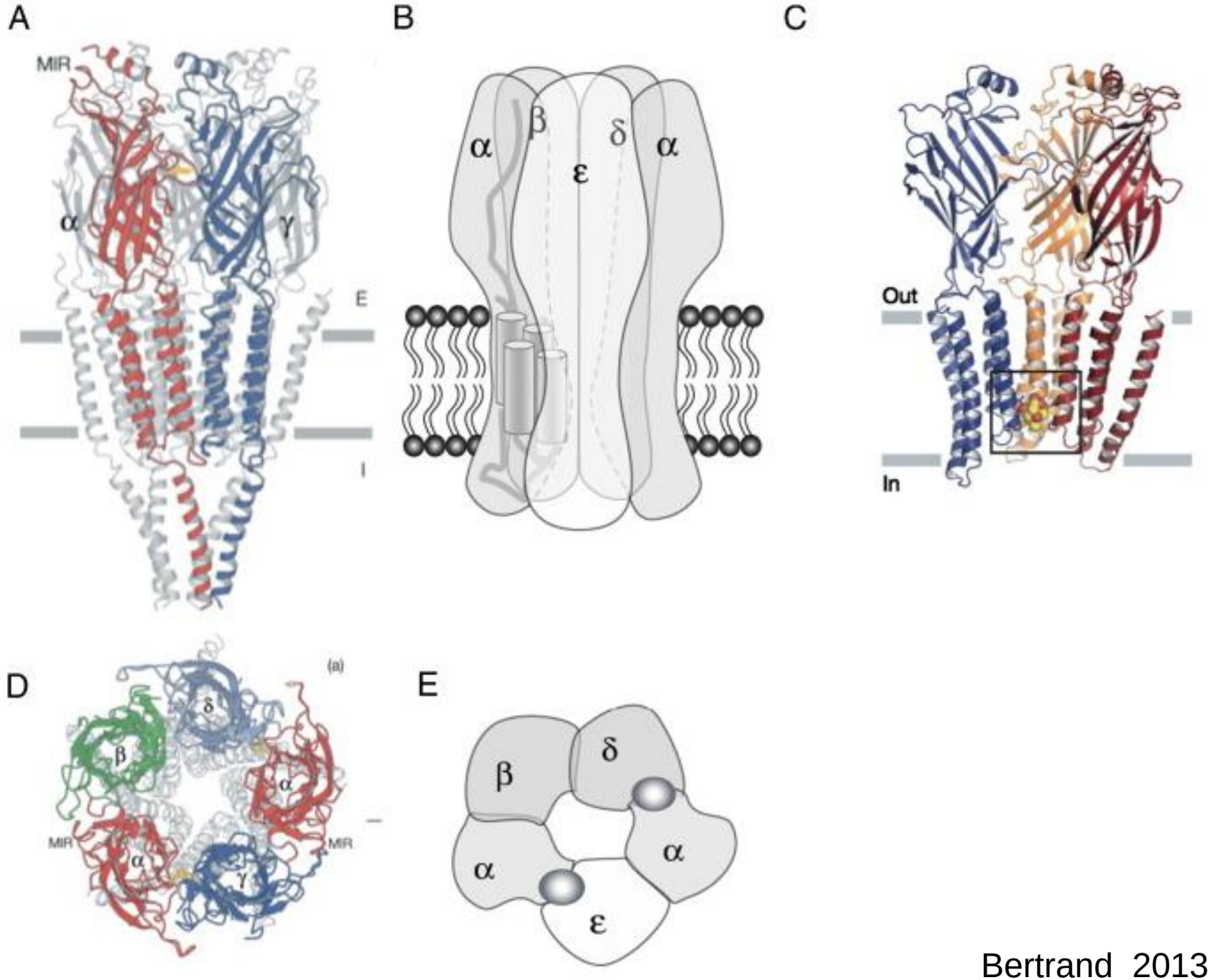


Unwin 1993




γ2
B2
β2
GABA
GABA
β2


A
E
L251
S252
S248
T244
E
TM2
B
L
S
S
T
Closed
C
S
L
S
T
Open



Bertrand 2013

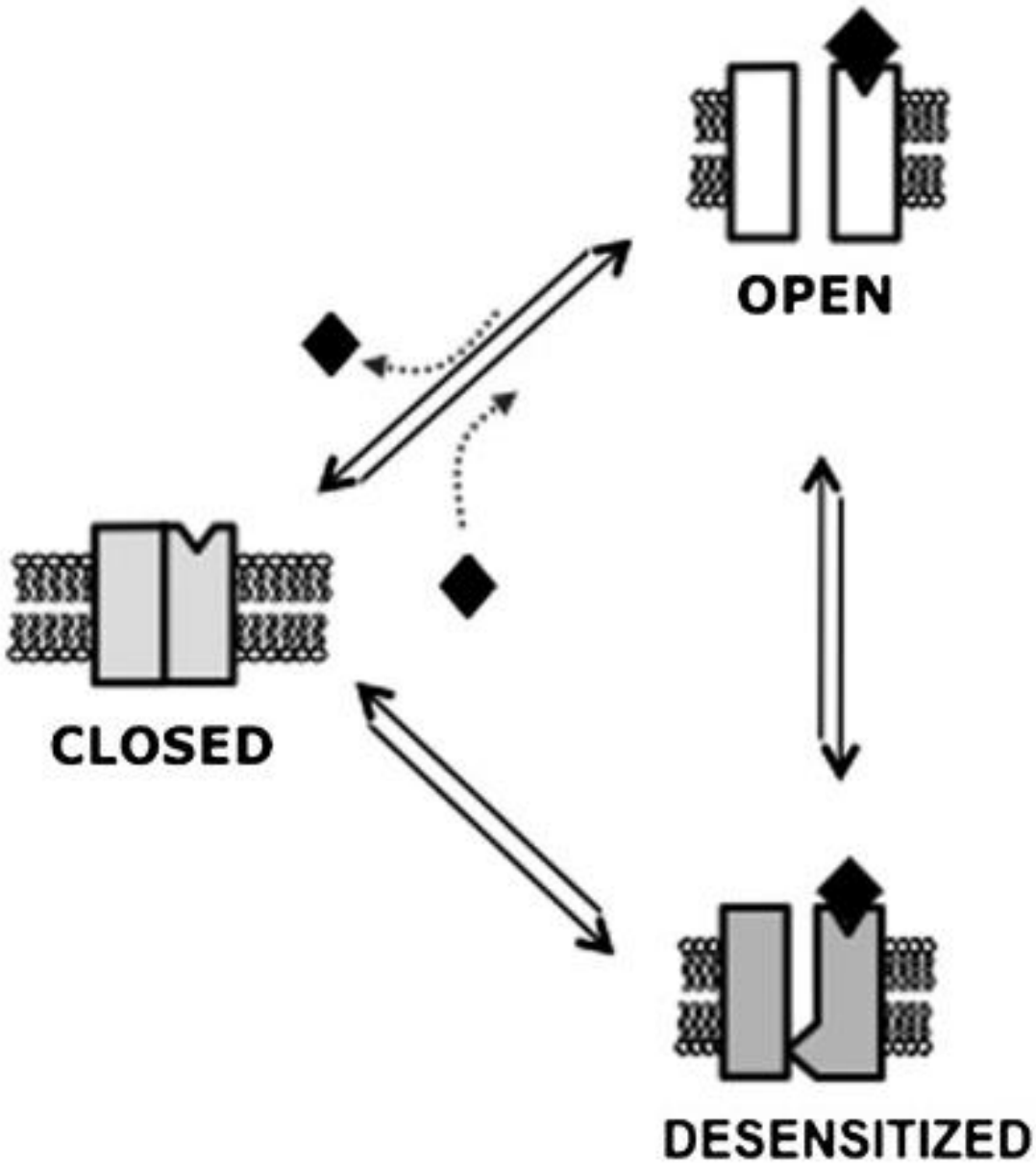

OPEN
CLOSED
DESENSITIZED

Table 1
Distribution of nAChR subtypes in the central nervous system (adapted from Gotti & Clementi, 2004).

	α3	α4	α5	α6	α7	β2	β3	β4
Cerebral cortex								
Frontal		●	●		●	●		
Parietal		●				●		
Occipital								
Temporal	●	●	●	●	●	●	●	●
Insular								
Enthorinal cortex	●	●			●	●		●
Subiculum	●	●			●	●		●
Hippocampus	●	●			●	●		●
Thalamus								
Basal ganglia								
Caudate	●	●			●	●		
Putamen	●	●	●	●	●	●	●	
Globus pallidus								
Brainstem								
Midbrain		●				●		
Pons								
Medulla oblongata								
Cerebellum	●	●		●	●	●		●

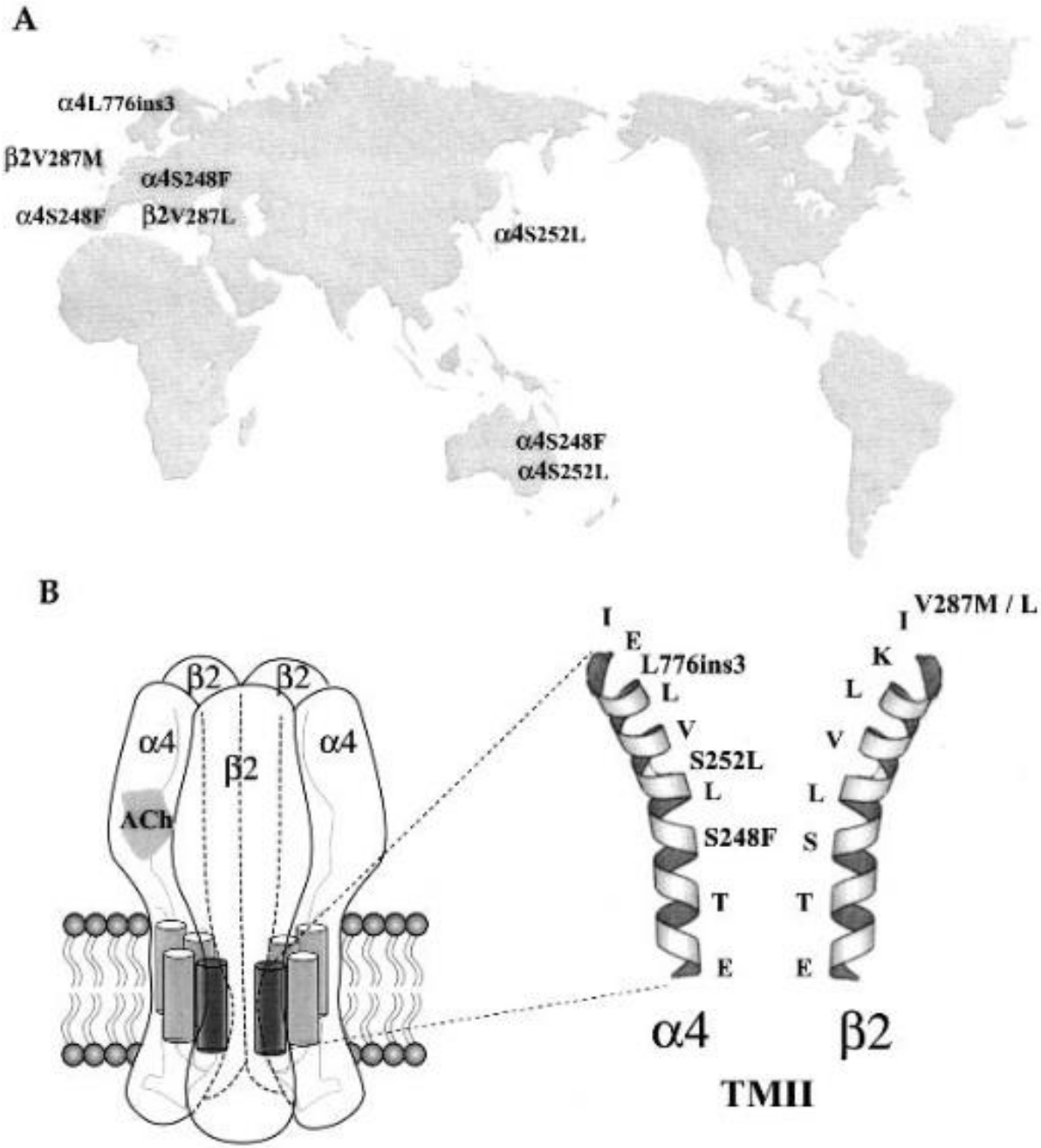
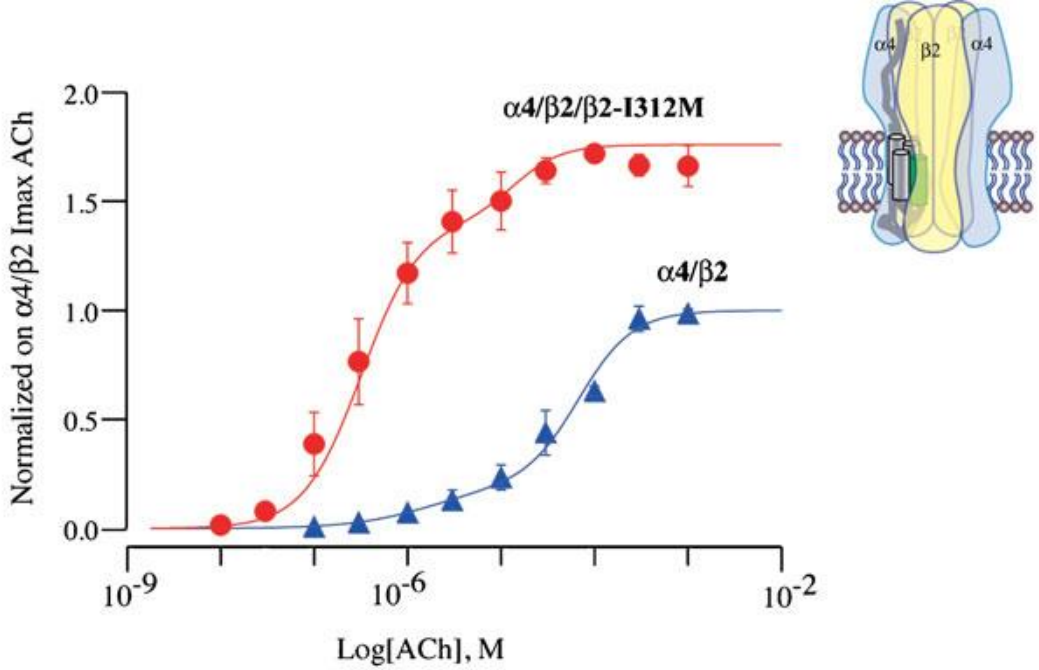
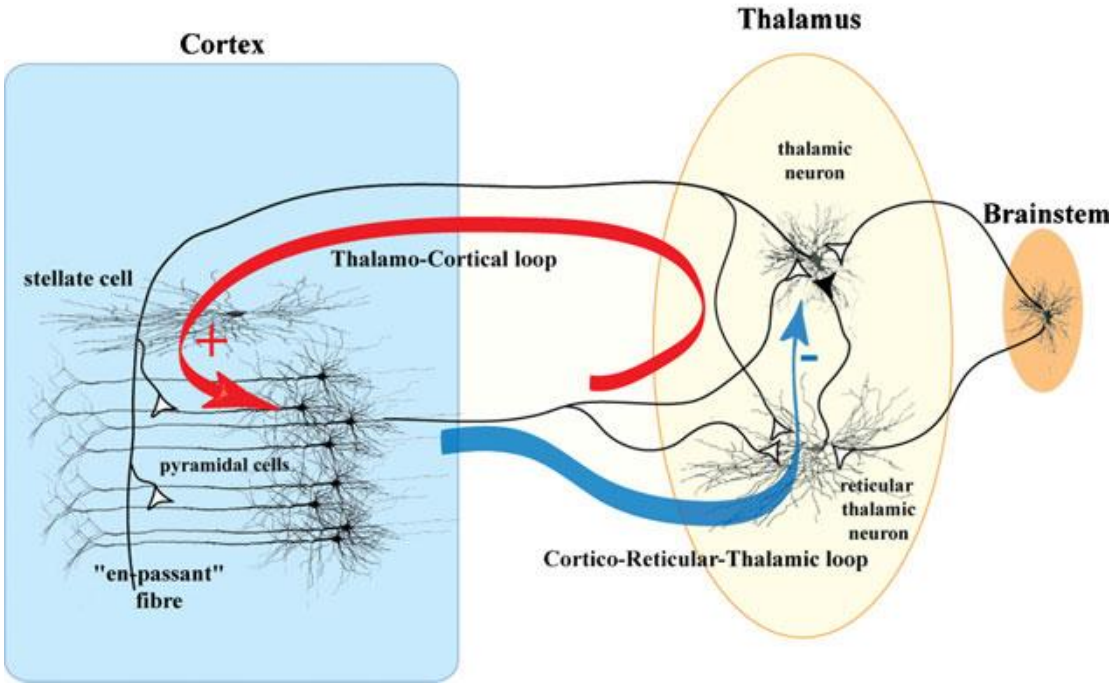


FIG. 1. Geographic and physical distribution of the different mutations associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). **A:** Worldwide map with the localization of the families in which the different ADNFLE mutations were identified. **B:** Schematic representation of a neuronal α4β2 nicotinic acetylcholine receptor (nAChR) with, in inset, a blowup of the second transmembrane (TMII) domain and the location of the different mutations identified in **A.**

Bertrand et al., 2002





Steinlein and Bertrand, 2010