Cours: Pharmacologie des récepteurs GABA

Récepteurs GABA → Nous traitons surtout les récepteurs $GABA_A$

I. Bases

1. Vue d'ensemble, introduction:
    1. où est-ce qu'ils se trouvent dans le système nerveux?
    2. Autres récepteurs/canaux inhibiteurs
    3. La synapse inhibitrice

2. Fonction moléculaire/cellulaire des récepteurs GABAA
    1. Composition, Fonction
    2. Pharmacologie des récepteurs GABAA → les benzodiazépines
3. Effet anxiolytique:
    1. Définition
    2. Comment évaluer
4. Autres modulateurs au récepteur GABAA: anesthésiques, éthanol (?)

II. Développements

1. Une vue moléculaire du site des benzodiazépines sur le récepteur GABAA
    1. Localisation du site benzo
    2. Vers des benzodiazépines spécifiques
        1. Les bases (expériences sur les souris) → le rôle des sous-unités α
        2. Effets des substances sous-unité-spécifiques

2. Un rôle pour les récepteurs GABAA extrasynaptiques

3. Cibles pharmacologiques autres que les récepteurs GABAA dans le système GABAergique

2 sortes de récepteurs GABA existent:

-récepteur-canal à Cl^- → $GABA_A$

-récepteur GPCR → $GABA_B$

Les deux sont largement distribué dans le système nerveux, les récepteurs GABAA sont post-synaptique, tandis que les récepteurs GABAB sont localisés pré- ou post-synaptique

Comparison GABAA and B and glycine receptors, (table RD p472)

Table 32.2 Properties of inhibitory amino acid receptors

	$GABA_A$			$GABA_B$	Glycine
	Receptor site	**Modulatory site (benzodiazepine)**	**Modulatory site (others)**		
Endogenous agonists	GABA	?DBI	Various neurosteroids (e.g. progesterone metabolites)	GABA	Glycine, β-alanine, taurine
Other agonists	Muscimol	Anxiolytic benzodiazepines (e.g. diazepam)	Barbiturates, steroid anaesthetics (e.g. alphaxolone)	Baclofen	–
Antagonists	Bicuculline	Flumazenil	–	Phaclofen, CGP 35348 & others	Strychnine
Channel blockers	Picrotoxin	Picrotoxin	Picrotoxin	Not applicable	–
Effector mechanisms	Ligand-gated chloride channel	Ligand-gated chloride channel	Ligand-gated chloride channel	G-protein-coupled receptor; inhibition of adenylate cyclase	Ligand-gated chloride channel
Location	Widespread; mainly GABA-ergic interneurons	Widespread; mainly GABA-ergic interneurons	Widespread; mainly GABA-ergic interneurons	Pre- and postsynaptic; widespread	Postsynaptic; mainly in brainstem and spinal cord
Function	Postsynaptic inhibition (fast ipsp)	Postsynaptic inhibition (fast ipsp)	Postsynaptic inhibition (fast ipsp)	Presynaptic inhibition (decreases Ca^{2+} entry Postsynaptic inhibition (increases K^+ permeability)	Postsynaptic inhibition (fast ipsp)

GABA, gamma-aminobutyric acid; DBI, diazepam-binding inhibitor; ipsp, inhibitory postsynaptic potential.

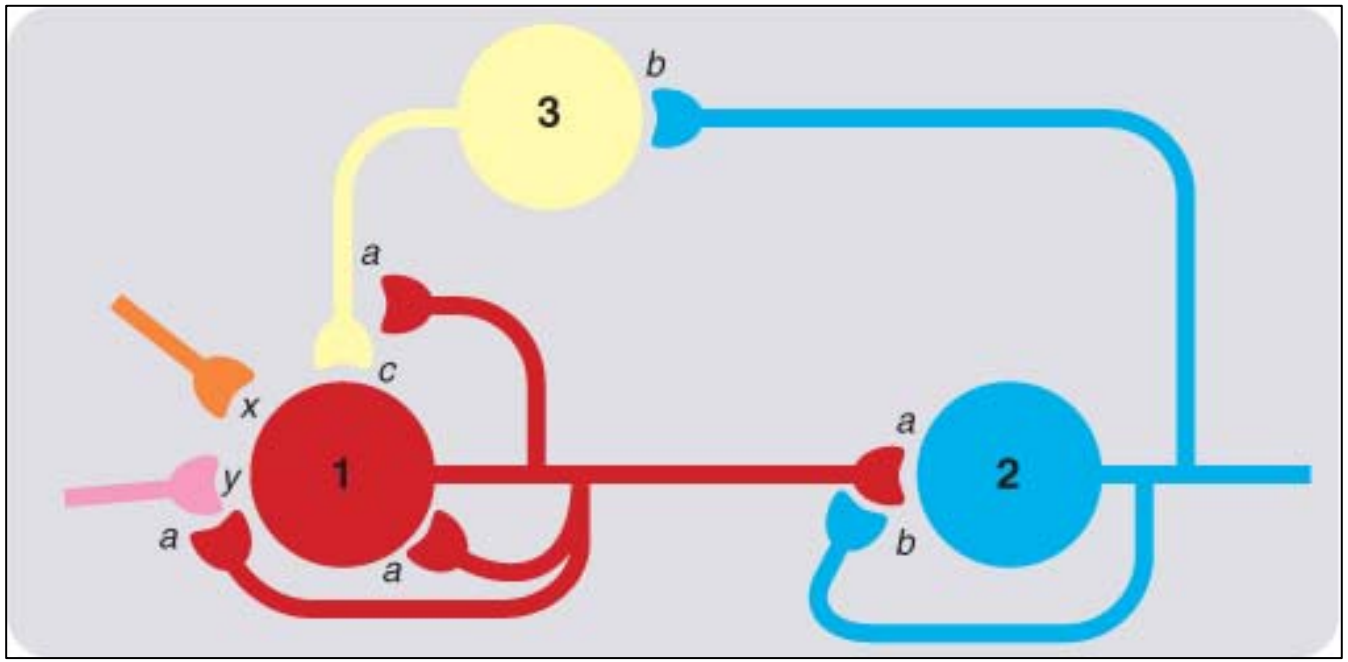


Figure 31.2 **Simplified scheme of neuronal interconnections in the CNS.** Neurons 1, 2 and 3 are shown releasing transmitters *a*, *b* and *c*, respectively, which may be excitatory or inhibitory. Boutons of neuron 1 terminate on neuron 2, but also on neuron 1 itself, and on presynaptic terminals of other neurons that make synaptic connections with neuron 1. Neuron 2 also feeds back on neuron 1 via interneuron 3. Transmitters (*x* and *y*), released by other neurons are also shown impinging on neuron 1. Even with a such a simple network, the effects of drug-induced interference with specific transmitter systems can be difficult to predict.

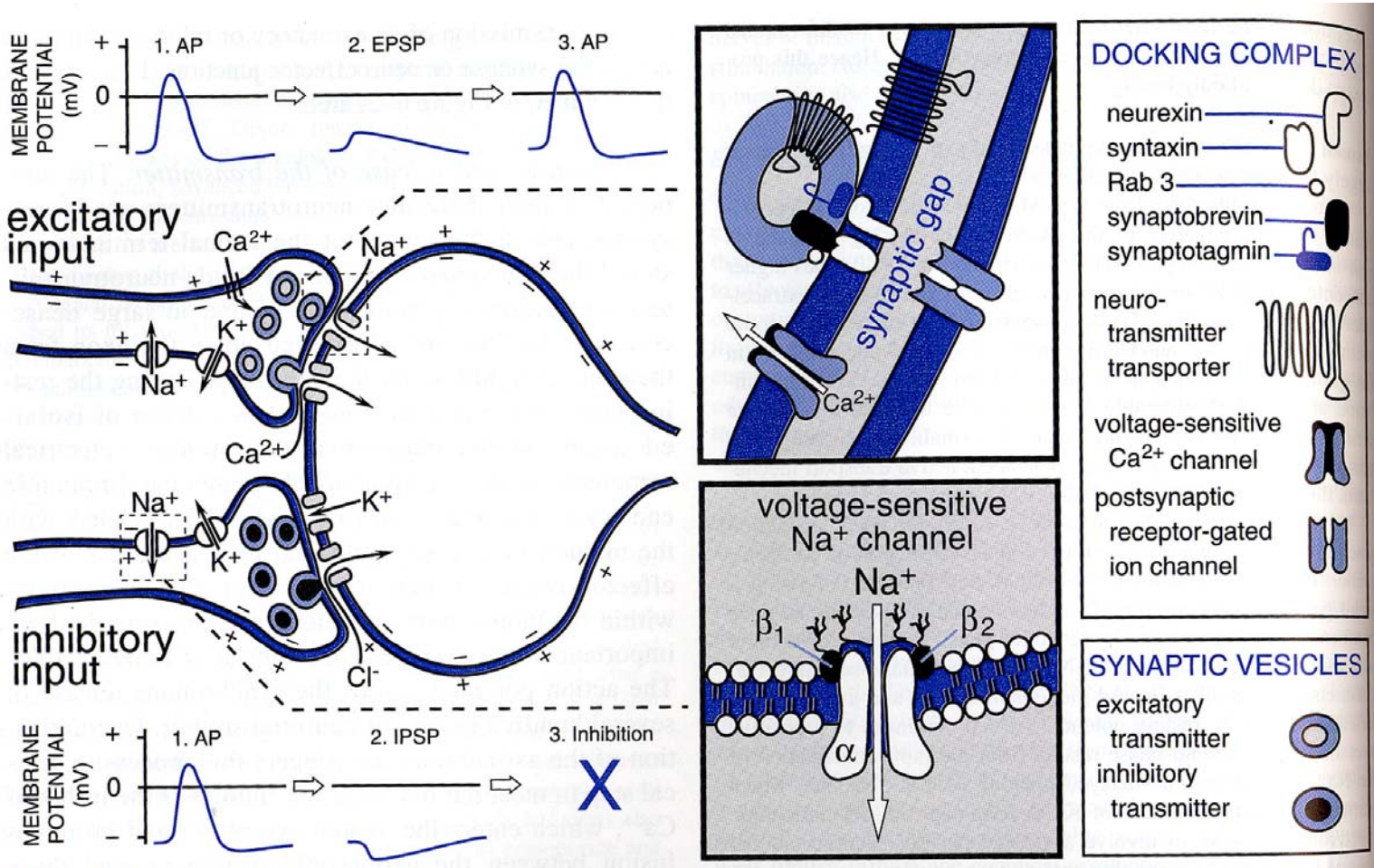
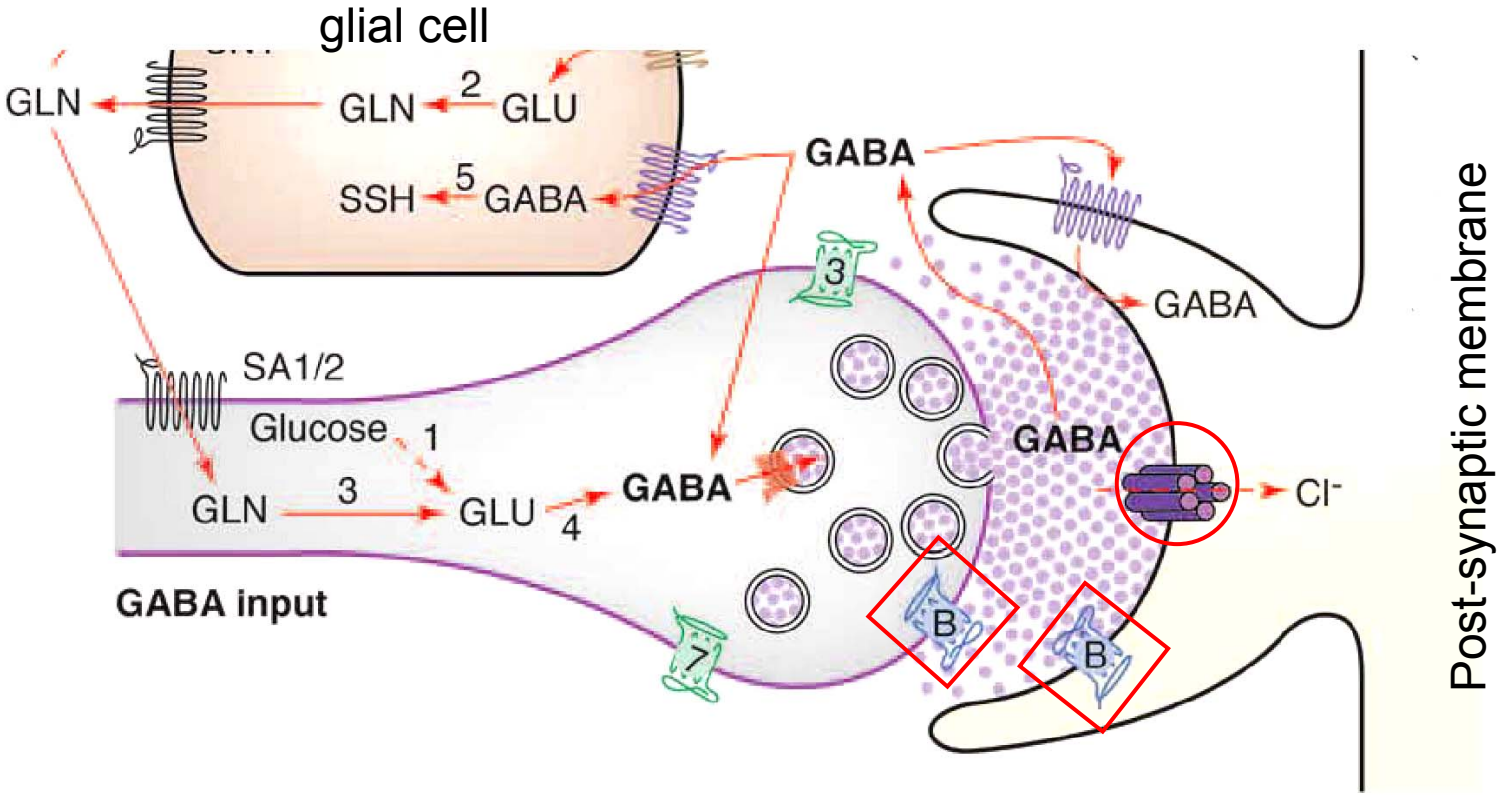


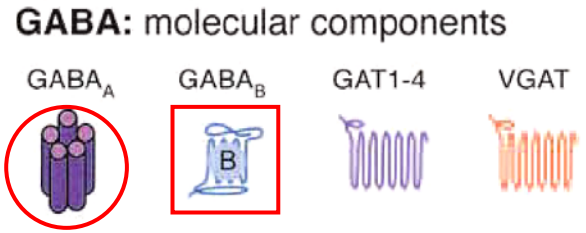
Figure 6–2. ***Steps involved in excitatory and inhibitory neurotransmission.*** *1.* The nerve action potential (AP) consists of a transient self-propagated reversal of charge on the axonal membrane. (The internal potential E_i goes from a negative value, through zero potential, to a slightly positive value primarily through increases in Na^+ permeability and then returns to resting values by an increase in K^+ permeability.) When the AP arrives at the presynaptic terminal, it initiates release of the excitatory or inhibitory transmitter. Depolarization at the nerve ending and entry of Ca^{2+} initiate docking and then fusion of the synaptic vesicle with the membrane of the nerve ending. Docked and fused vesicles are shown. *2.* Combination of the excitatory transmitter with postsynaptic receptors produces a localized depolarization, the excitatory postsynaptic potential (EPSP), through an increase in permeability to cations, most notably

hyperpolarization, the
an be prevented, how-
ction, by reuptake into
; Catterall, 1992; Jahn

→Signalisation le long des axons: Nav, Kv → potentiel d'action
→Exocytose, relâchement des neurotransmetteurs: Nav, Cav
→Signalisation à la synapse, glutamate, acétylcholine, glycine, GABA

Localisation des récepteurs GABA



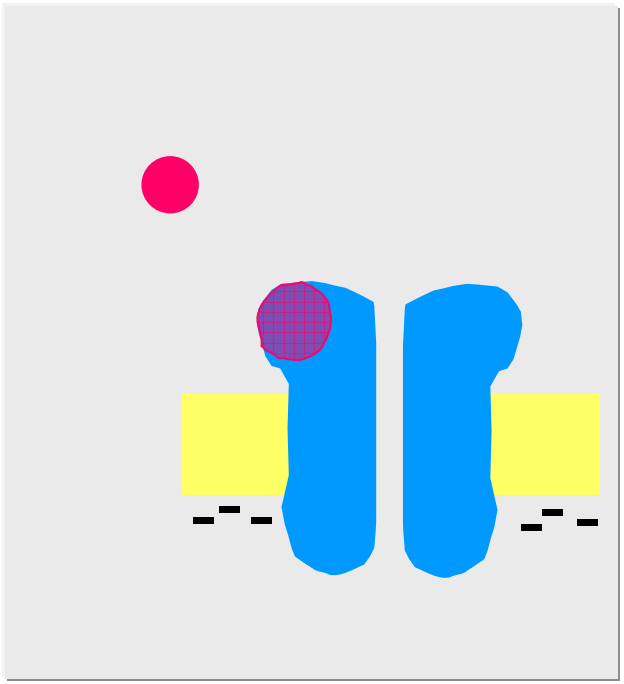


2. Fonction moléculaire/cellulaire des récepteurs GABAA

1. Fonction/Structure

- canal à Cl^- *(membre de la famille des récepteurs-canaux)*
- activation par liaison du neurotransmetteur GABA
  → hyperpolarise (le plus souvent) la cellule
  → excitabilité ↓
  (stabilisation du potentiel membranaire)

- voie principale inhibitrice dans le SNC



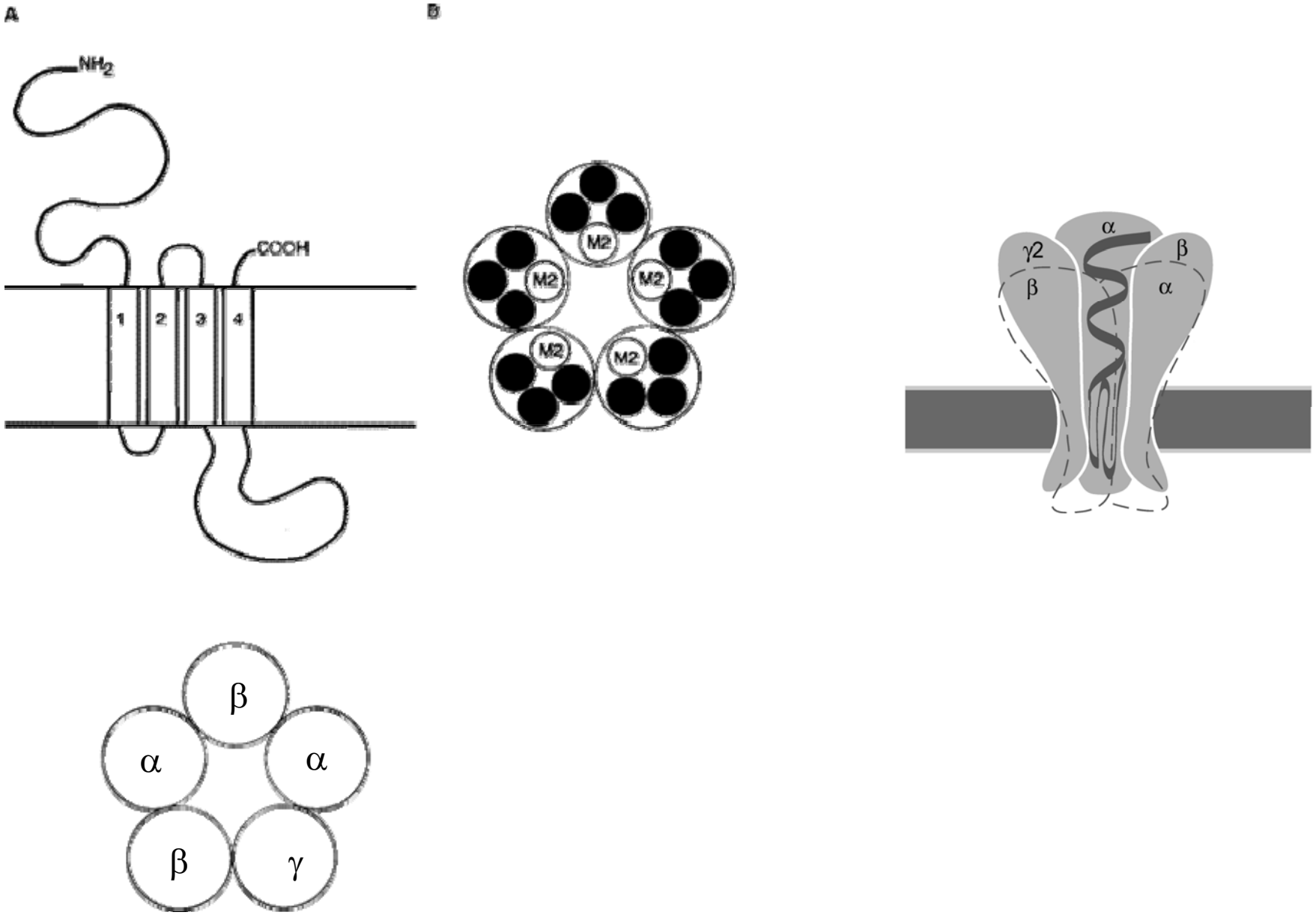
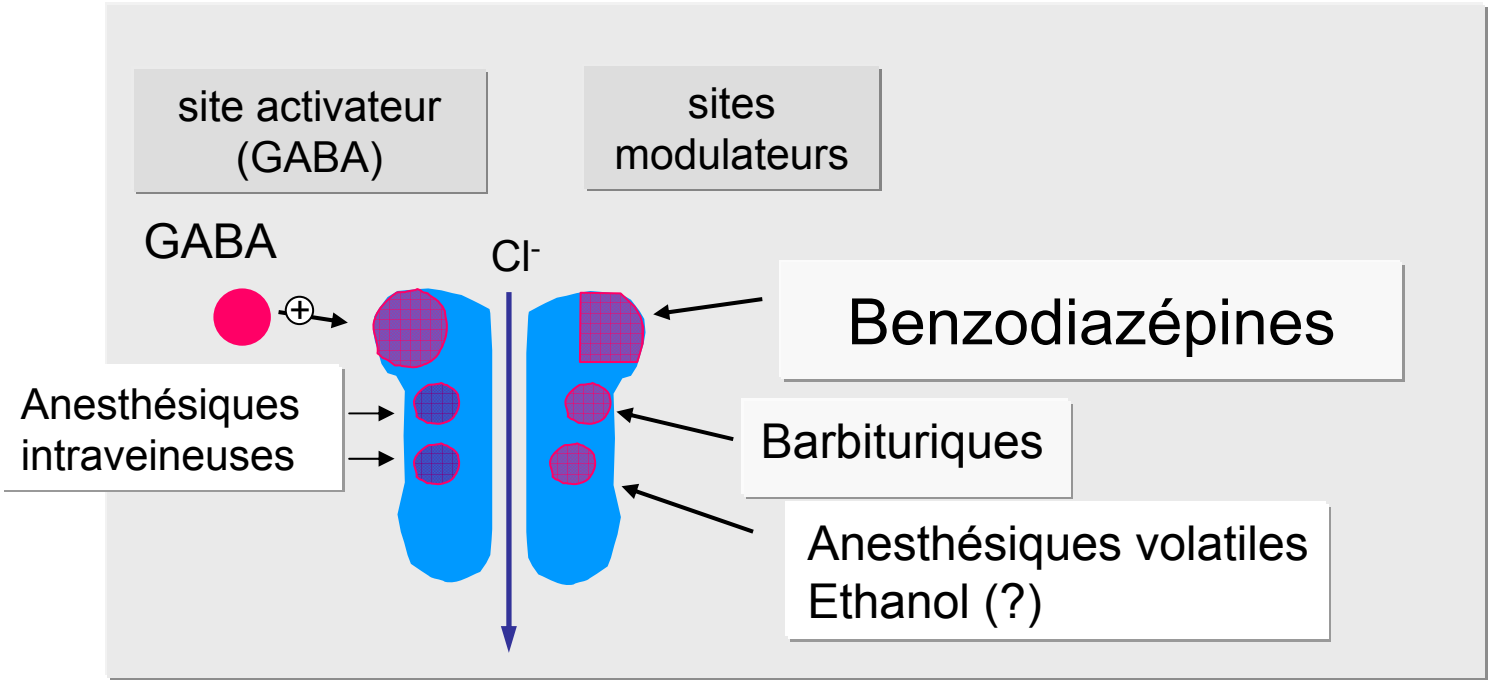


Figure 1 Representations of GABA-activated ligand gated ion channels: A, general structure of one of the five protein subunits showing four membrane spanning regions; B, pentameric arrangement of the protein subunits showing the second membrane spanning region lining the pore of the ion channel; C, heteromeric makeup of a GABAA receptor with two α1, two β2, and one γ2 protein subunits; and D, homomeric makeup of a GABAC receptor with five ρ1 protein subunits. Chebib and Johnston, J. Med. Chem. 43, 1427

2.2. Pharmacologie des récepteurs GABAA



Activateur

molulateur

I.2.2.1. Les benzodiazépines

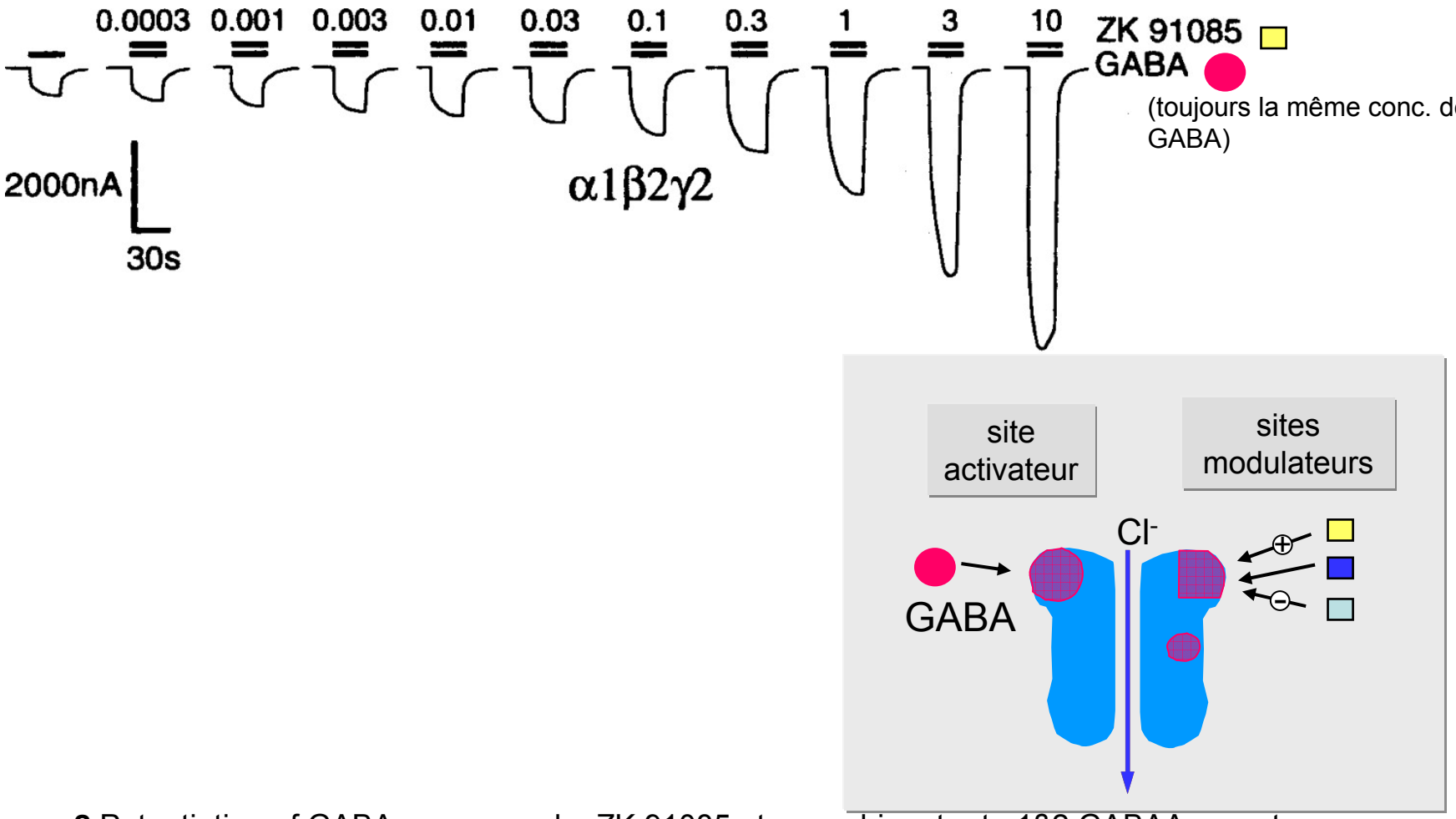
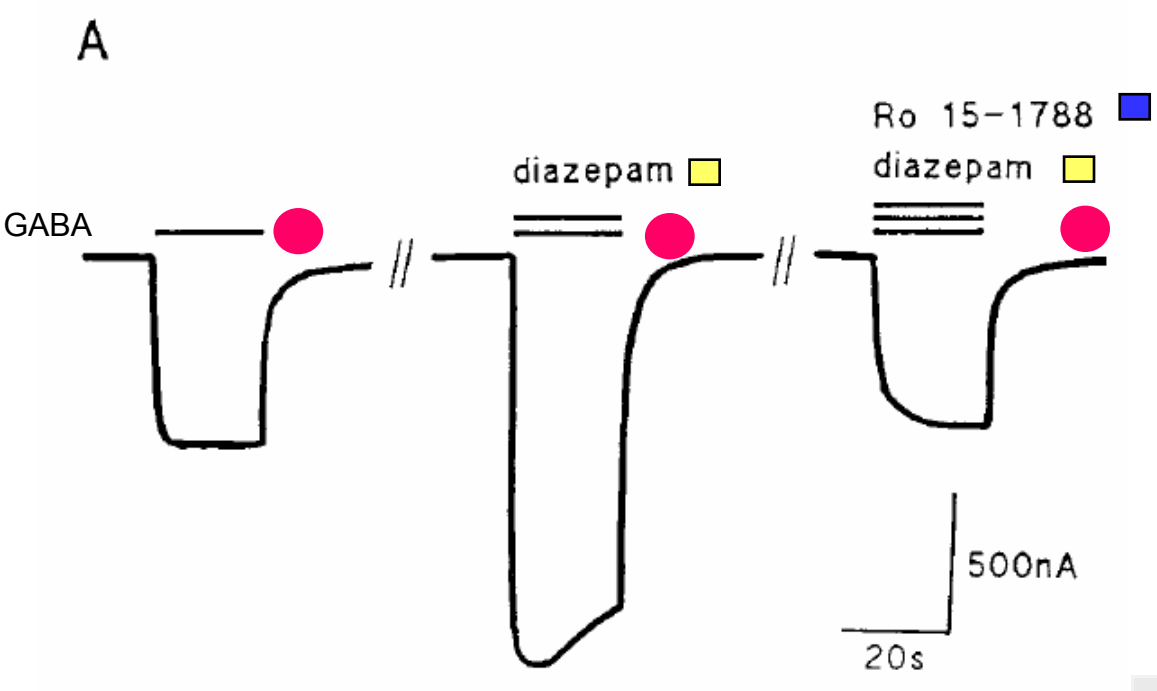
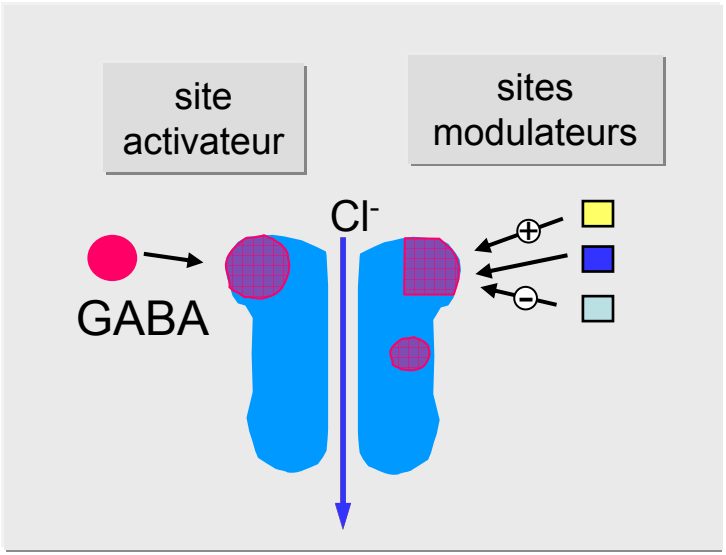


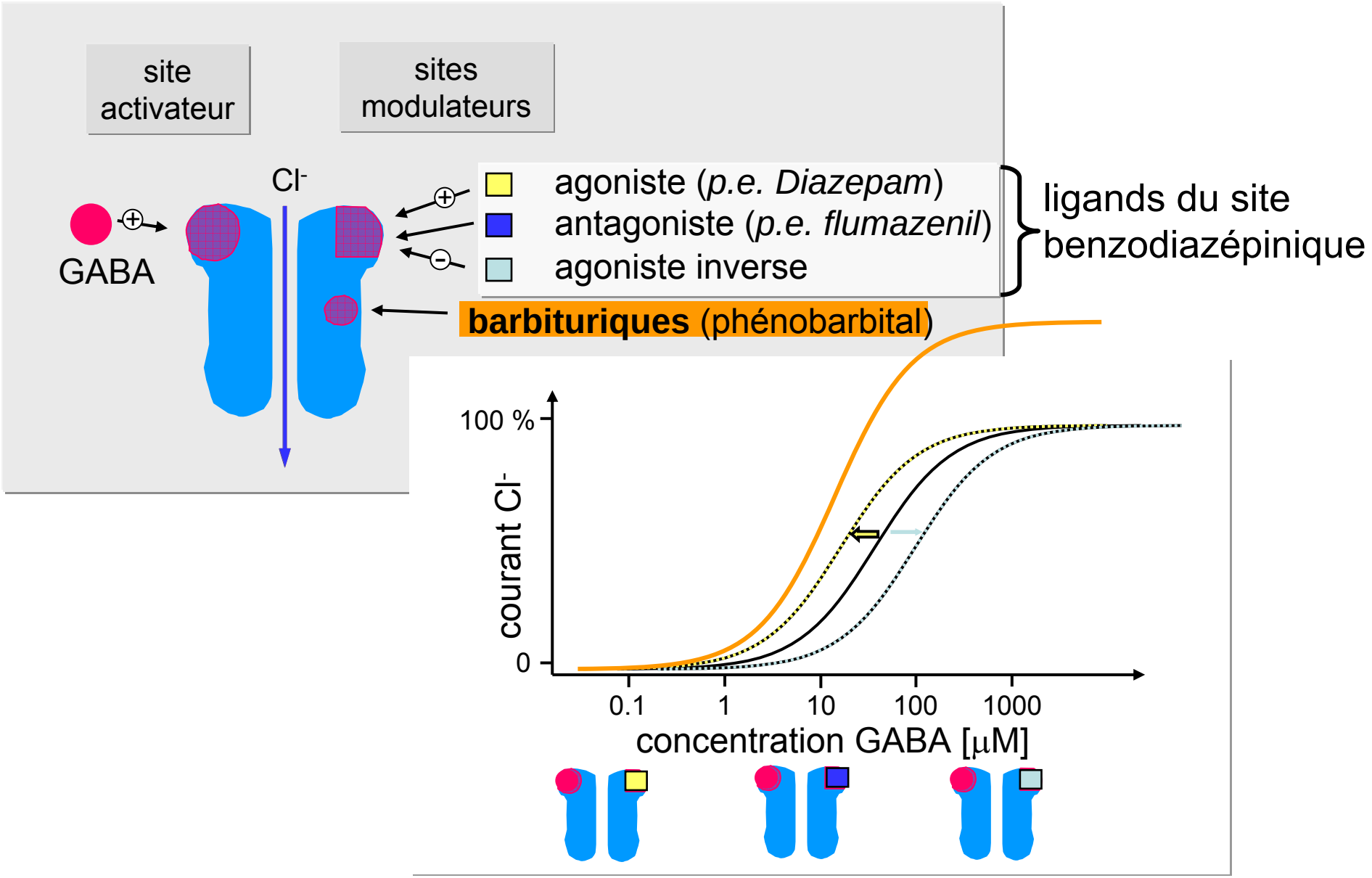
Figure 2 Potentiation of GABA responses by ZK 91085 at recombinant rat a1ß2 GABAA receptors expressed in *Xenopus laevis* oocytes. Application of GABA alone resulted in approximately 5% of the maximal current amplitude. Increasing concentrations of ZK 91085 were coapplied with GABA. Periods of drug application are indicated by horizontal bars above the current records. Concentrations of ZK 91085 are shown in µM.





From Buhr et al., JBC 272, 11799–11804, 1997, concentrations GABA, Ro: 1 μM

Mécanisme d ’action sur le récepteur



Usages cliniques

Points communs des benzodiazépines:

→ les effets pharmacologiques:

- anxiolytique
- sédatif
  - hypnotique
  - amnésiant
- myorelaxant
- anticonvulsivant

→ pharmacodynamie:

efficacité; différences dans la *puissance* égalisés par le dosage

Différences

→ pharmacocinétique

- demi-vie (de < 6h à >48 h, due aux différences dans le *métabolisme et l'excrétion*)

Quelques exemples d ’applications:

Substance	Marque	Demi-vie(h)	Utilisations principales
midazolam	Dormicum©	2.5	hypnotique, anesthésique
oxazépam	Seresta ©	10	anxiolytique
clonazépam	Rivotril ©	40	anticonvulsivant, antiépileptique

Efféts indésirables

- sédation

- dépression respiratoire (grande marge thérapeutique)

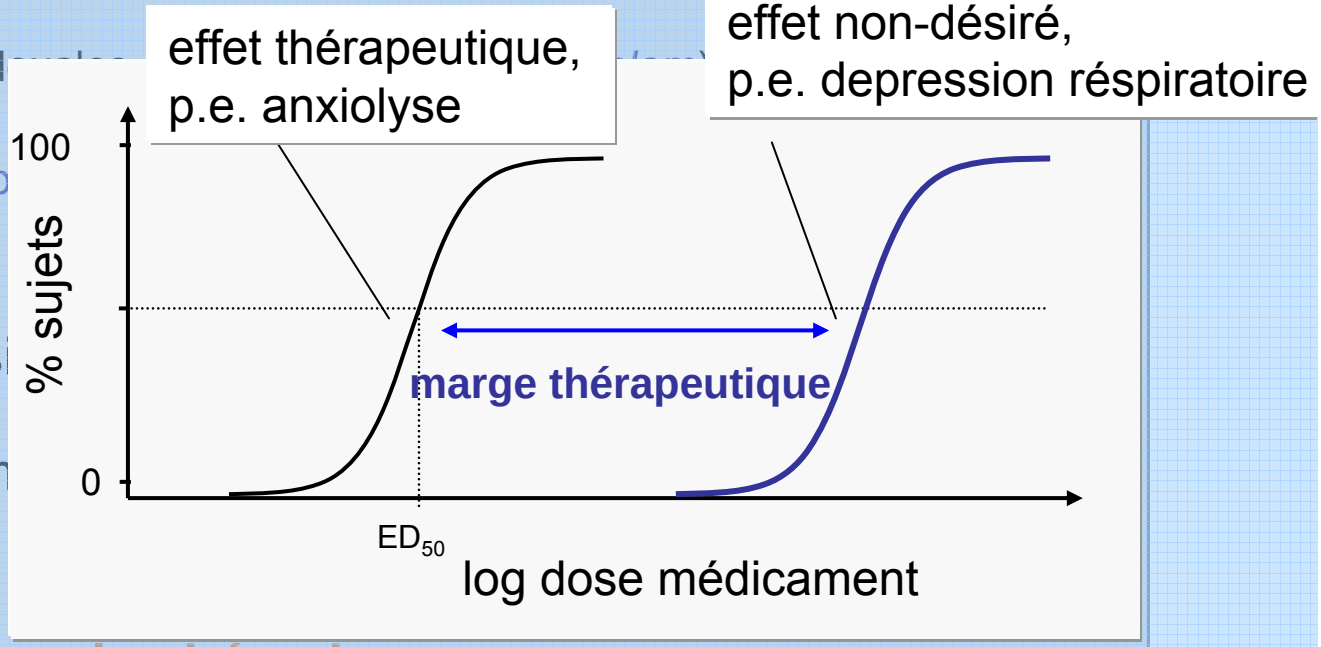
fortement augmenté en cas d 'association à un autre dépresseur du SNC (alcohol, opioïde)

- amnésie antérograde

- rares réactions parad

- tolérance (surtout p
  épileptique)

-dépendance physi
convulsions)
→ problèn
(rebond)



→ **conclusions pour la thérapie:**

→ pour thérapies d 'une durée limitée

→ diminution graduelle de la dose à la fin de la thérapie

Effets indésirables

- sédation
- dépression respiratoire (grande marge thérapeutique)

fortement augmenté en cas d 'association à un autre dépresseur du SNC (alcohol, opioïde)

- amnésie antérograde

- rares réactions paradoxales (agitations, anxiété; *triazolam*)

- tolérance (surtout pour les effets sédatifs, plus lentement pour l'effet anti-épileptique)

-dépendance physique → syndrome de sevrage (anxiété, insomnie, convulsions)

→ problèmes avec benzodiazépines à action courte (rebond)

→ conclusions pour la thérapie:

→ pour thérapies d 'une durée limitée

→ diminution graduelle de la dose à la fin de la thérapie

3. L'effet anxiolytique

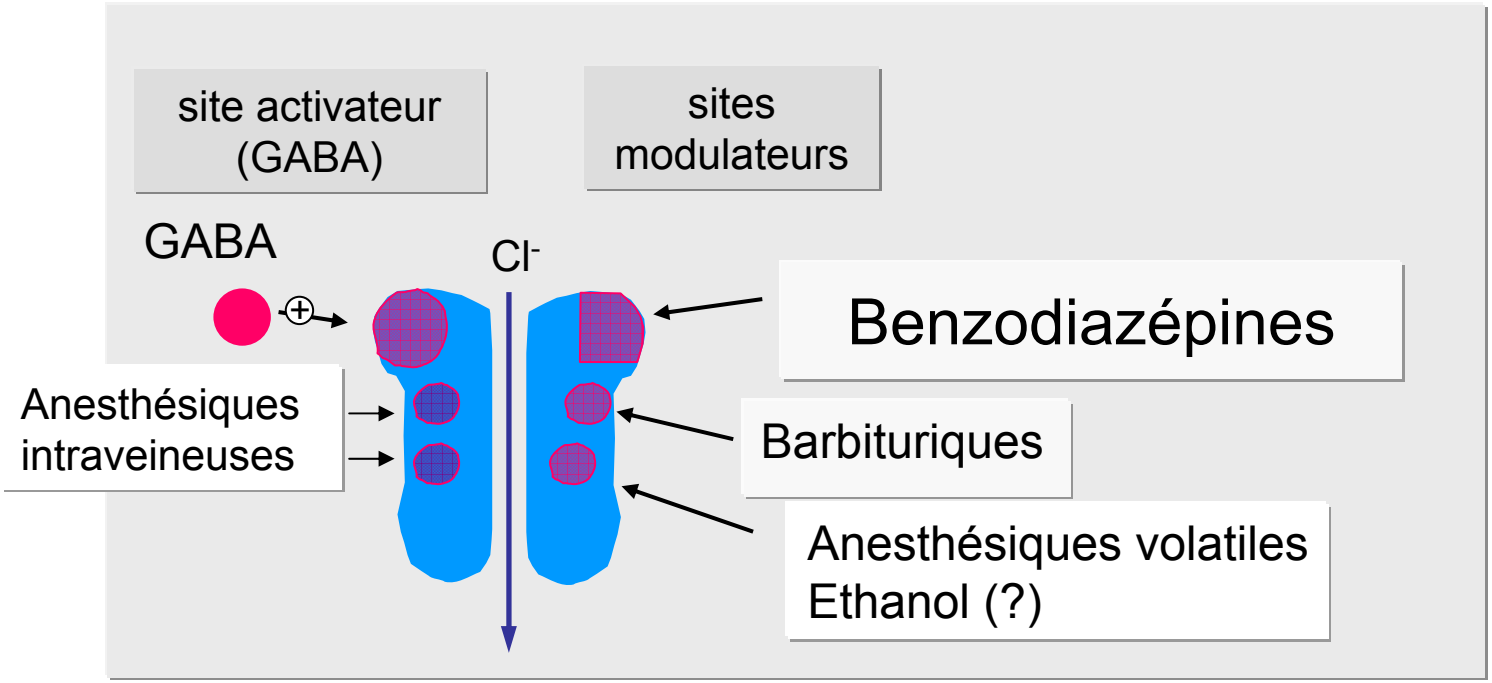
Anxiety disorders as recognised clinically include:
- *generalised anxiety disorder* (an ongoing state of excessive anxiety lacking any clear reason or focus)
- *panic disorder* (attacks of overwhelming fear occurring in association with marked somatic symptoms, such as sweating, tachycardia, chest pains, trembling, choking, etc): such attacks can be induced even in normal individuals by infusion of sodium lactate , and the condition appears to have a genetic component
- *phobias* (strong fears of specific things or situations, e.g. snakes, open spaces, flying, social interactions)
- *Post-traumatic stress disorder* (anxiety triggered by insistent recall of past stressful experiences).

Comment tester?

The elevated plus maze shown on the right is also used to test for anxiety-related behavior in mice. The test is based on the same conflict between *tendency to explore* a novel environment and aversive properties of a brightly lit open arm. However, this test also includes height above the floor and openness of the arms. Two arms are completely open while the other two alternating arms are enclosed with clear Plexiglas. Anxiolytic drugs increase the number of entries and time spent in the open arms. This test is also sensitive to both anxiogenic and anxiolytic effects.

Alternatives:
Tests de conflit
Comportement agressif

I. 4. Autres modulateurs au récepteur GABAA



Activateur

molulateur

Les anesthésiques généraux

-Plusieurs anesthésiques généraux volatiles et d'injection augmentent la sensibilité des canaux GABAA pour le GABA (anesthésiques volatiles: *agents halogénés, comme halotane, isoflurane*; agents d'injection: *propofol, barbituriques, etomidate, neurostéroides*)
-Les sites de liaison se trouvent sur les parties M2 et M3 des sous-unités α et β
-Certains de ces substances agissent aussi sur des autres cibles comme les récepteurs glycine (comme activateurs) et des canaux acétylcholines nicotiniques neuronales (comme inhibiteurs)
-Autres cibles de certains anesthésiques d'inhalation sont les canaux glutamate du type NMDA et le canaux K à « two-pore domains »

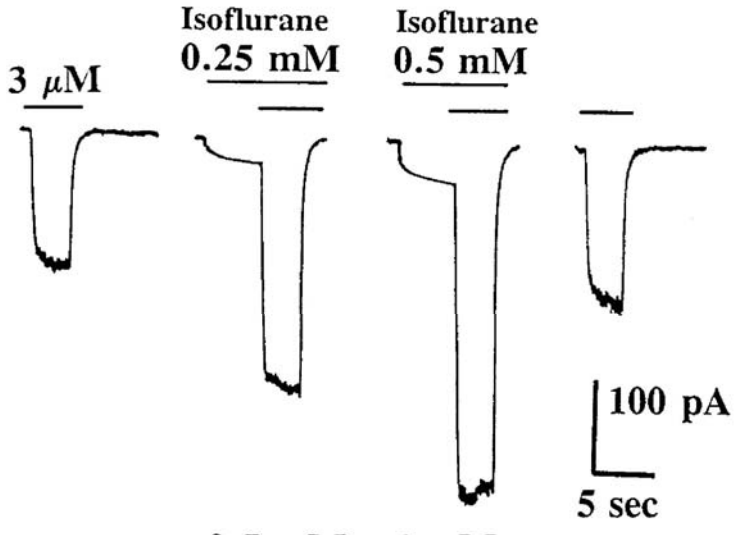


Figure: effet de l'anesthésique isoflurane sur le courant GABA

CMLS, Cell. Mol. Life Sci. Vol. 55, 1999

Ethanol

- Un verre de vin contient ~14 g éthanol

- Une concentration de 0.05 % correspond à 10.5 mM

- Il existe des évidences indirectes pour un effet de l'éthanol sur les récepteurs GABAA

-Par contre, sur des récepteurs clonés exprimés dans des cellules, les effets de l'éthanol sont très faibles ou nécessitent des concentrations énormes

-p.e., dans une étude qui identifiait un site de liaison de l'éthanol sur les récepteurs GABAA, la concentration d'éthanol utilisée était de 200 mM! (*Mihic, S.J. et al., Nature 385, p389, 1997*)

-Une étude récente a proposé un effet à des concentrations de 1-3 mM sur des récepteurs GABAA contenant la sous-unité δ (*Wallner et al., PNAS 100, 15218, 2003*). Ces résultats n'ont pas pu être confirmés par des autres groupes de recherche (*Borghese et al., J. Pharm Exp Ther 316, 1360 (2006))*

II. Développements

1. Une vue moléculaire du site benzodiazépine

1.1. Localisation du site benzodiazépine

Benzodiazepines bind to the interface between α and γ subunits

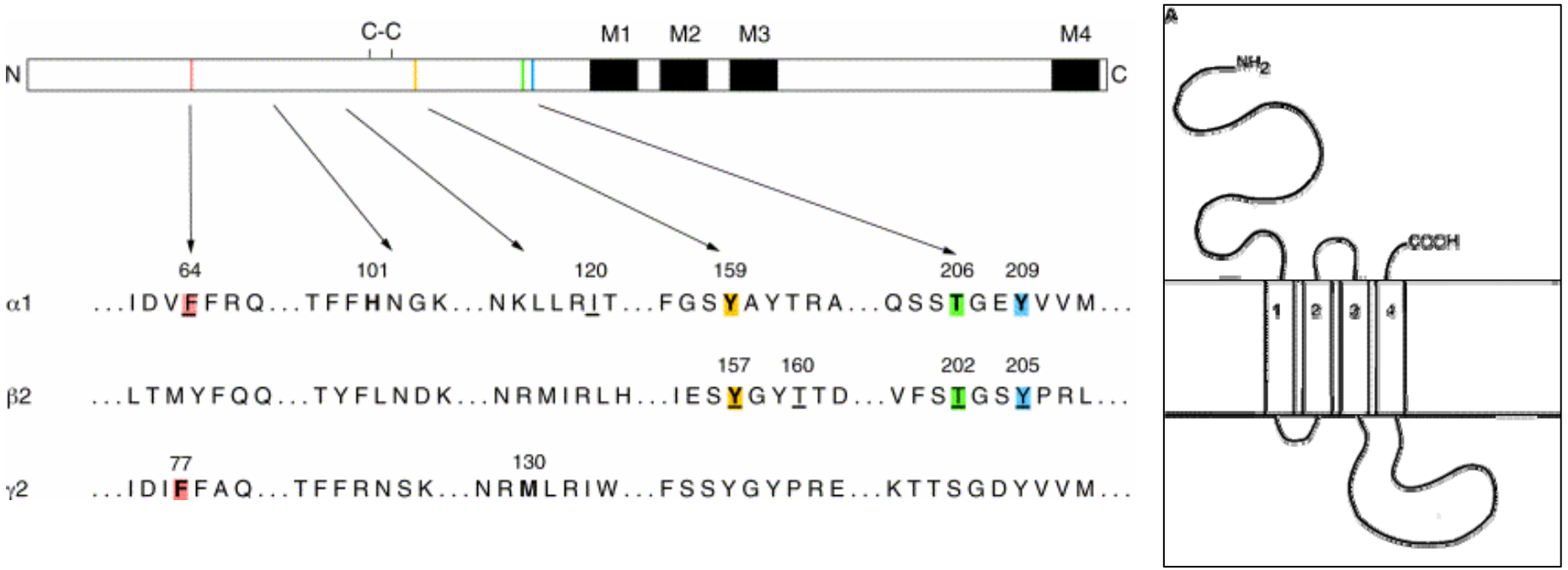
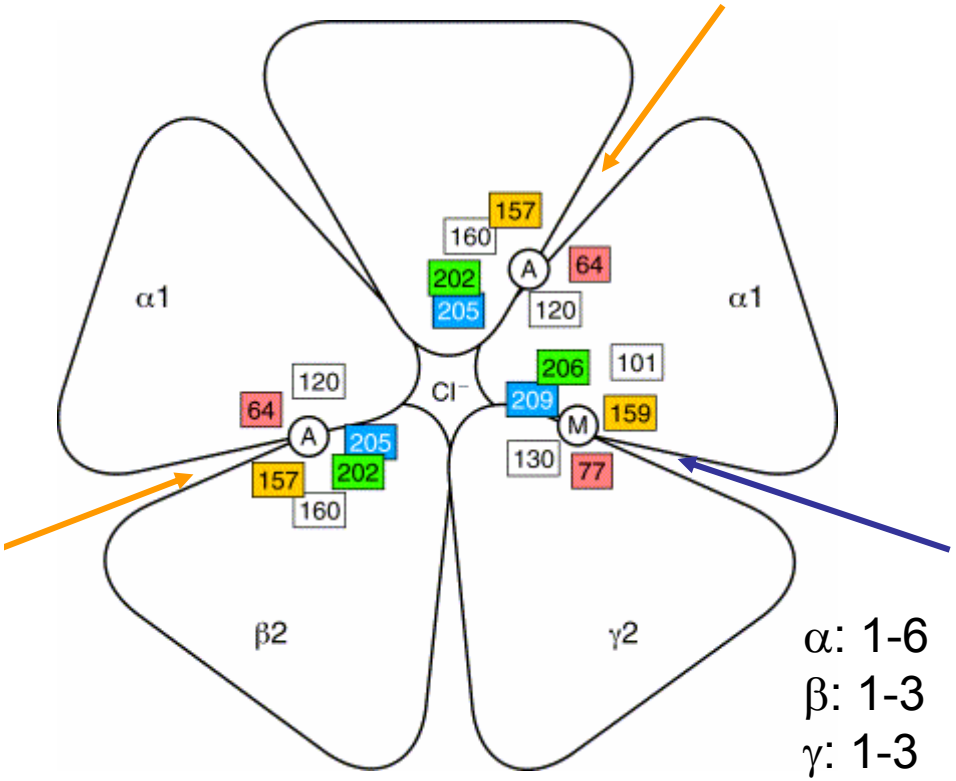
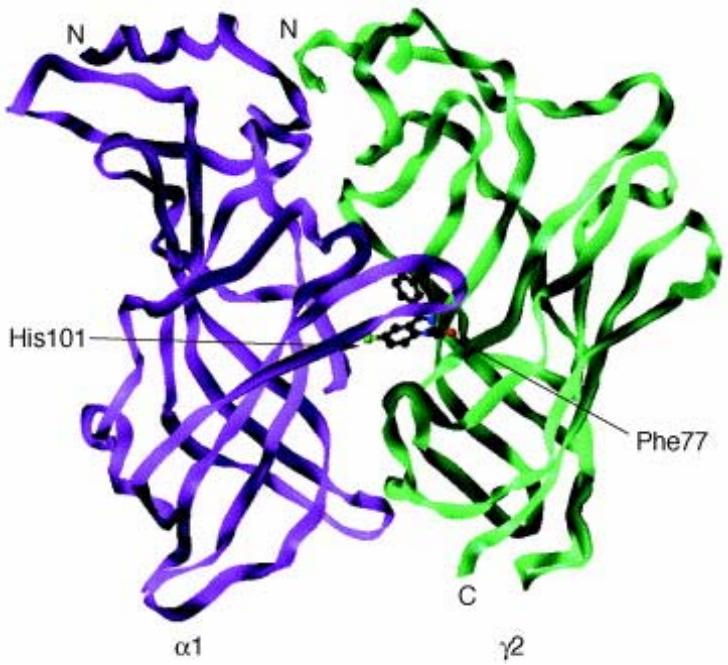


Fig. 1. Homology of amino acid residues involved in agonist and modulatory drug interactions. An alignment of the rat subunit isoforms α1, ß2 and γ2 is shown. All subunits have a similar structure, with the N-terminal half carrying a putative S-S bridge and glycosylation sites exposed to the extracellular space. Four transmembrane helices, called M1–M4 are located in the C-terminal half. The numbers indicate amino acid residues of the corresponding mature subunit isoform. Amino acid residues putatively involved in agonist (GABA) binding are shown underlined, **amino acid residues putatively involved in binding of ligands of the modulatory site for benzodiazepine-type drugs are shown in bold face**. For the latter case, only those residues are emphasized that affect binding by a factor of at least tenfold. Amino acid residues of agonist and modulatory sites identical or directly homologous to each other are shown in the same colour. Both binding sites are located in the N-terminal, extracellular part of the subunits. (Sigel and Buhr, TiPs, 1998)

Benzodiazepines bind to the interface between α and γ subunits



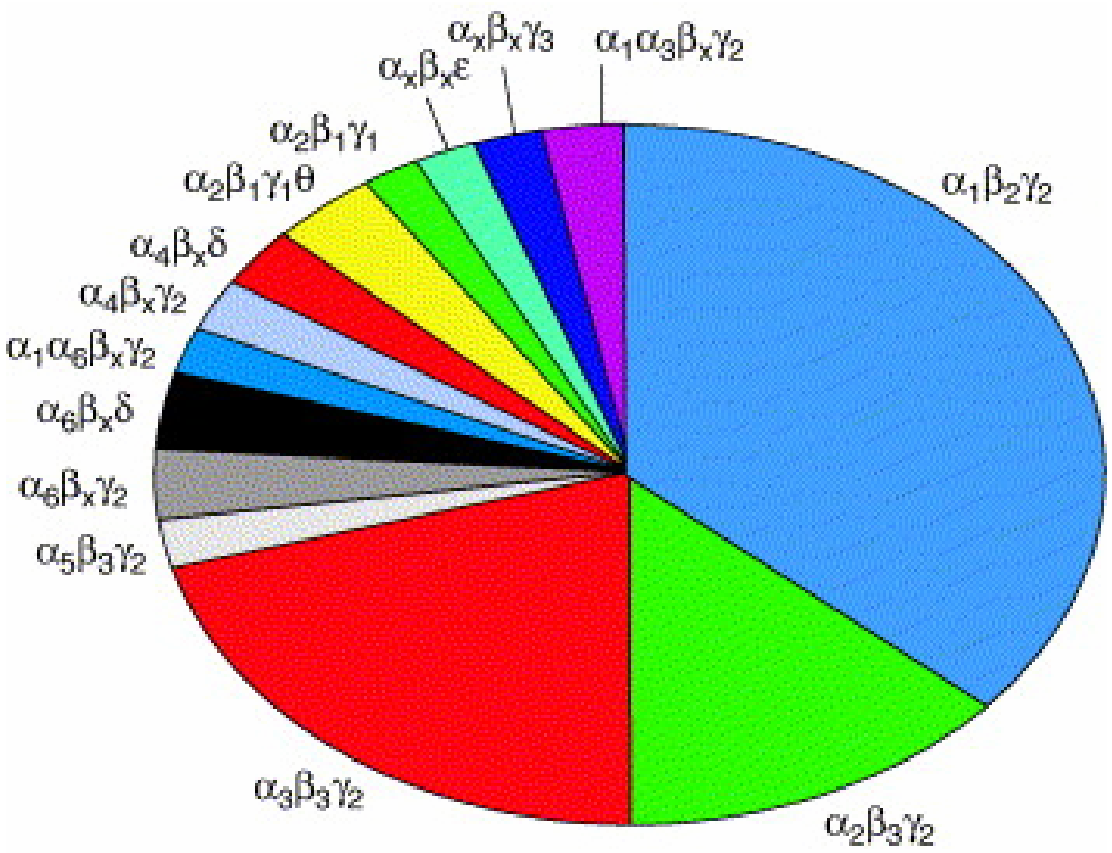
α: 1-6
β: 1-3
γ: 1-3
(δ, ε, θ, π, ρ 1-3)



3D model of the benzodiazepine site

Fig. 2. (left figure) Hypothetical model of the GABAA receptor with the agonist and modulatory sites at subunit interfaces. Five subunits are arranged around a central Cl− ion-selective pore, that opens as a consequence of agonist (GABA) binding. The numbers indicate amino acid residues of the corresponding mature rat subunit isoform. Amino acid residues of agonist and modulatory sites identical or directly homologous to each other are shown in the same colour. A, agonist (GABA) binding site; M, modulatory site for benzodiazepine-type ligands. As two subunits contribute to each, the agonist and to the modulatory binding site, they must be located at subunit interfaces. If the modulatory site is occupied, this fact is allosterically communicated to the agonist binding site whose function is altered as a consequence. Sigel and Buhr, TiPS, 1998

Certains récepteurs GABA ne sont pas modulé par les benzodiazépines



α: 1-6
β: 1-3
γ: 1-3
(δ, ε, θ, π, ρ 1-3)

Figure 1. Pie chart illustrating the approximate abundance of different GABAA receptor subtypes in the mammalian brain. Subscript x is indicated where the particular subunit is not known. Whithing PJ, Drug Discov Today 2003, 8, 445.

-Le pentamer contient 2 sous-unités α, 2 sous-unités β et une autre sous-unité
-Les canaux contenant une sous-unité γ2 possèdent un site de liaison pour les benzodiazépines
-Ceux qui contiennent une sous-unité α4 ou α6 ne sont pas sensible à la majorité des benzodiazépines utilisés

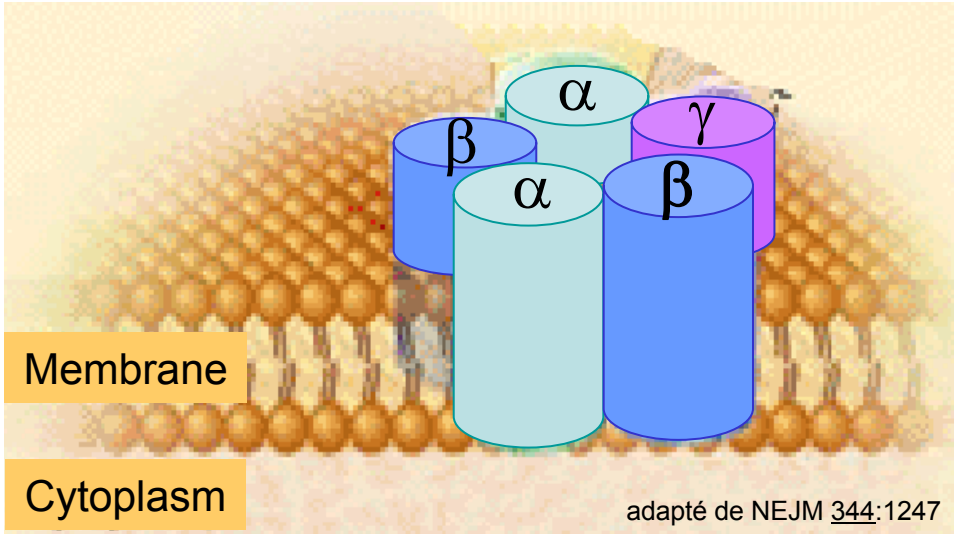
1.2. vers des benzodiazépines plus sélectives

Problème:
Les benzodiazépines ont un très large spectre d ’effets

→ Il serait souhaitable d ’avoir des benzodiazépines (ou analogues) plus sélectifs, par exemple des substances qui...

- sont anxiolytique sans être sédatif
- n ’induisent pas de tolérance et dépendance

→ → le large spectre d'effets des benzodiazépines est potentiellement du à la diversité des récepteurs GABAA



→ Corrélation entre sous-type de récepteur et rôle physiologique?

Pour tester si les sous-populations de récepteurs $GABA_A$ ont des différents rôles, deux approches sont possibles en principe:

-1. tester les effets de substances qui agissent spécifiquement sur le site benzodiazépine d'un sous-type de canal GABAA

-2. tester les effets des benzodiazépines non-spécifiques sur des animaux qui portent une mutation qui détruit le site aux benzodiazépines dans une population de canaux spécifiques (p.e. ceux contenant la sous-unité $\alpha 1$)

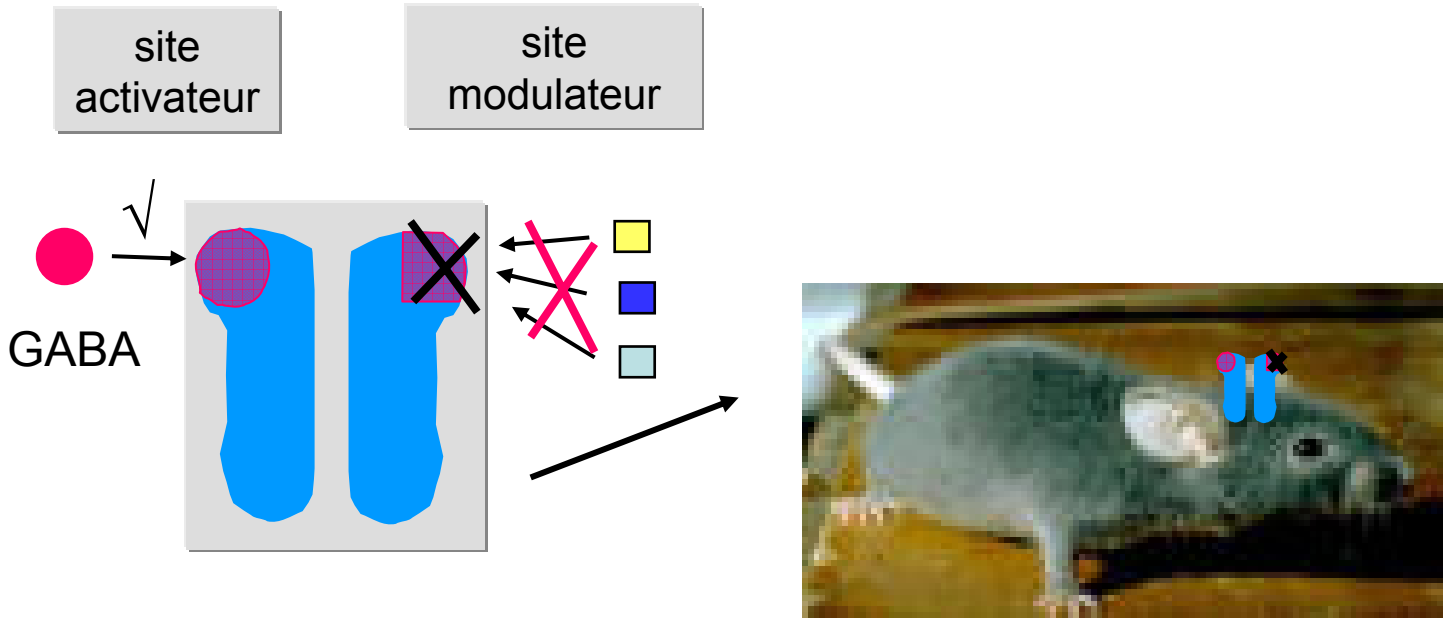


Figure: principe de l'approche 2

Benzodiazepine actions mediated by specific γ-aminobutyric acid$_A$ receptor subtypes

Uwe Rudolph*, Florence Crestani*, Dietmar Benke*, Ina Brünig*, Jack A. Benson*, Jean-Marc Fritschy*, James R. Martin, Horst Bluethmann & Hanns Möhler*

* Institute of Pharmacology, University of Zürich, and Swiss Institute of Techn
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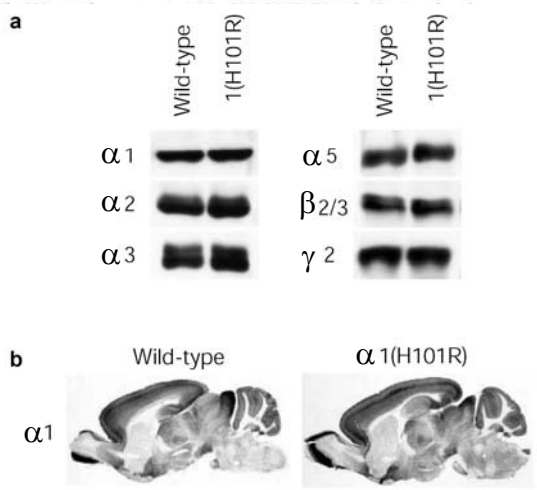
L'approche 2 a été appliquée et a produit des informations intéressantes. L'équipe de Mohler avait remplace la sous-unité α1 par une α1 dont le site benzodiazépine était muté (αH101R)



c

Displacement of [^{3}H]Ro15-4513, K_i (nM)		
	Wild-type	α1(H101R)
Clonazepam	3 ± 2	> 10,000
Diazepam	28 ± 15	> 10,000
Zolpidem	13 ± 5	> 10,000

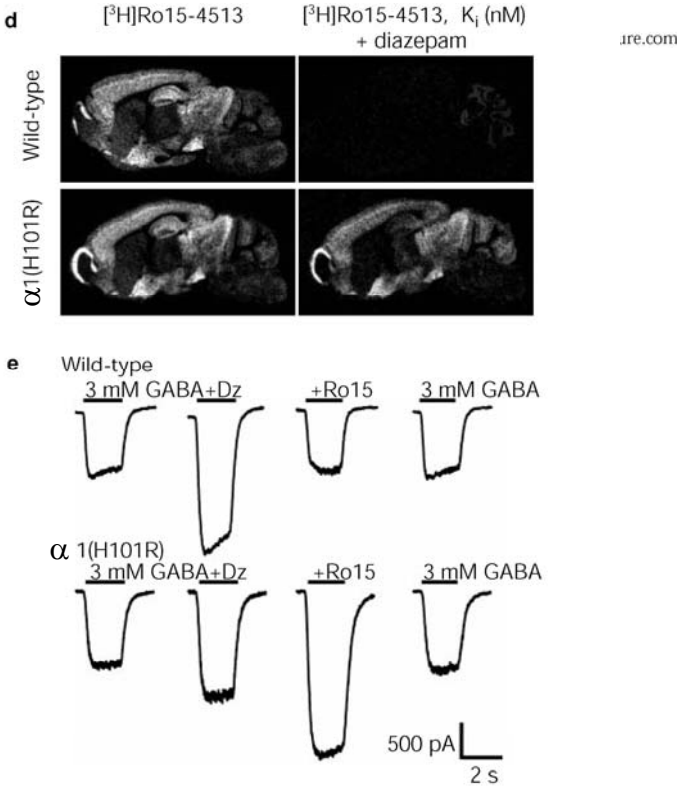
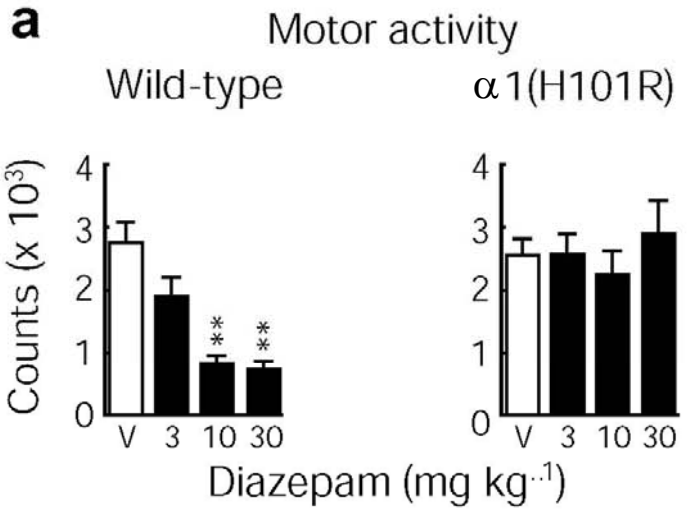
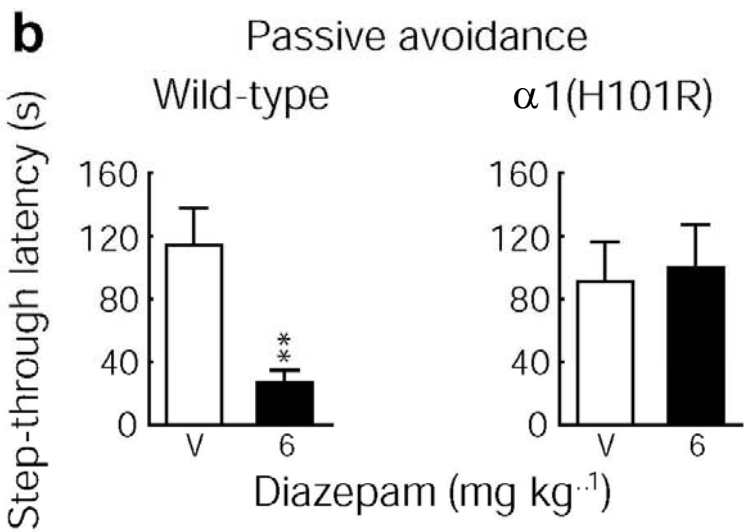


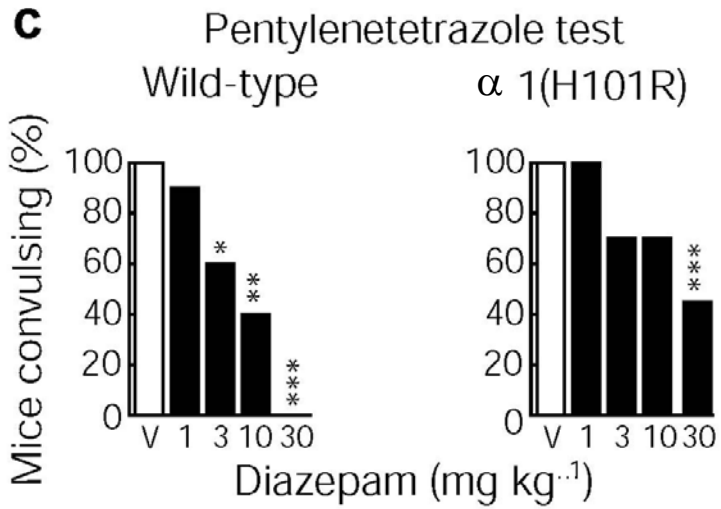
Figure 2 Biochemical, morphological and electrophysiological characteristics of GABA$_A$ receptors in α1(H101R) mice. **a**, Western blots of GABA$_A$ receptor subunit proteins in wild-type and α1(H101R) mice with antisera recognizing GABA$_A$ receptor subunits α1, α2, α3, α5, β2/3 and γ2. **b**, Immunohistochemical regional distribution of the α1 subunit in parasagittal brain sections from wild-type (left) and α1(H101R) mice (right). **c**, Displacement potencies of several benzodiazepine-site ligands at wild-type and α1(H101R) receptors, immunoprecipitated with an α1-subunit-specific antiserum. **d**, Autoradiographic distribution of total [^{3}H]Ro15-4513-binding sites (left) and diazepam-insensitive [^{3}H]Ro15-4513-binding sites (right) in wild-type mice (top) and α1(H101R) mice (bottom). Parasagittal brain sections were incubated with 20 nM [^{3}H]Ro15-4513 in the absence (left) or presence of 100 μM diazepam (right). Low levels of diazepam-insensitive binding in wild-type mice represent the receptors containing the α4 or α6 subunit. **e**, Patch-clamp analysis of GABA responses in dissociated cerebellar Purkinje cells. The potentiation by diazepam (Dz) was 134 ±19% (mean ±s.e.m.; n = 12) in cells of wild-type mice and was reduced to 71 ±14% (n = 10) in cells of α1(H101R) mice ($P < 0.05$, t-test). The inverse agonist Ro15-4513 (Ro15) displayed an agonistic response in the mutant cells.

Testing drug-induced sedation (a), amnesia (b) and anticonvulsant activity (c)





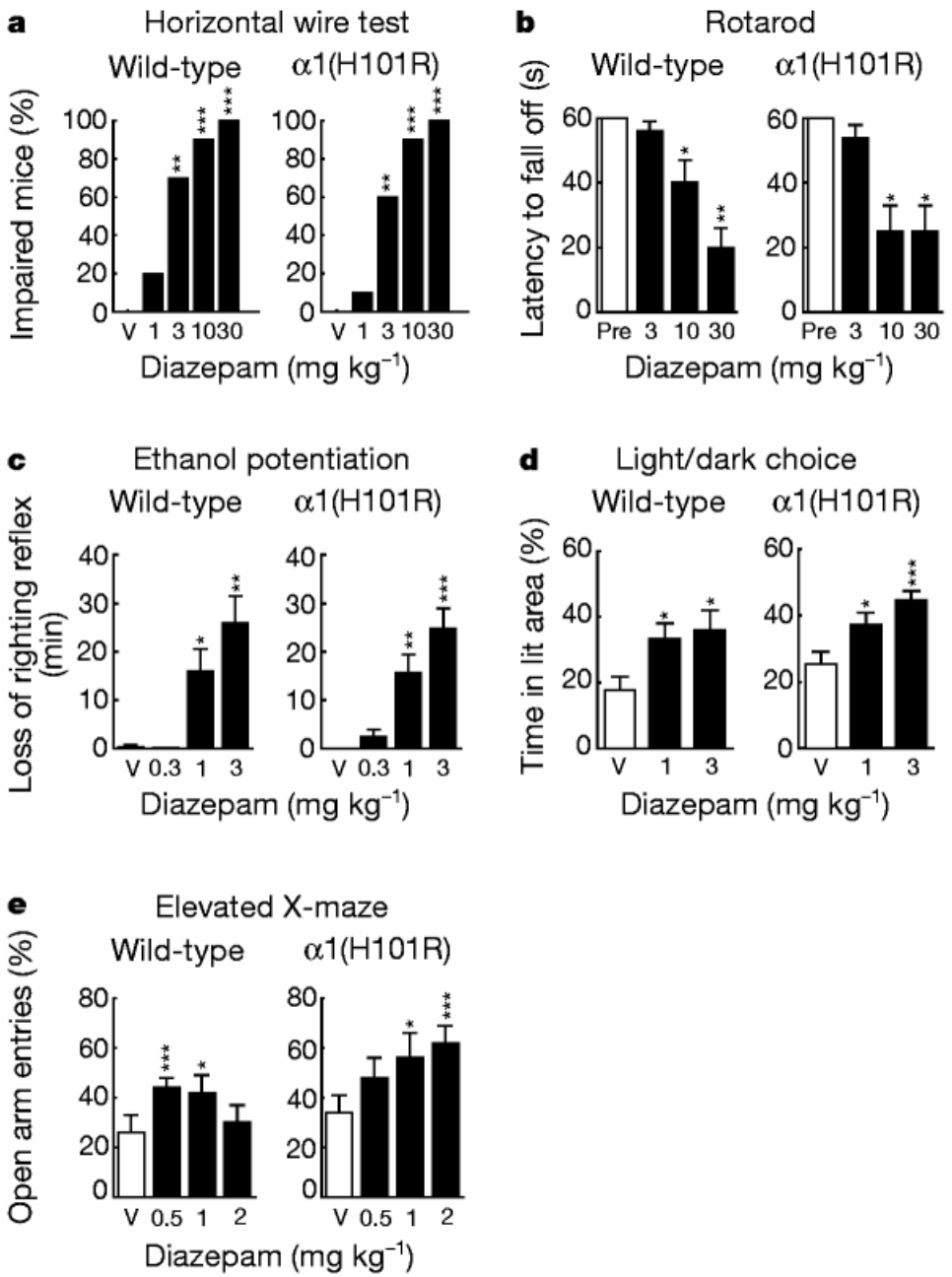
b) 2 chambers, one dark, one well lit. Once the mouse enters the dark chamber, it receives an electrical shock. One day later this procedure is repeated and the latency to entering the dark chamber is measured.



(pentylenetetrazole induit des convulsions)

Figure 3 Behavioural assessment of drug-induced sedation, amnesia and anticonvulsant activity in wild-type and α1(H101R) mice. a, Diazepam induced a dose-dependent decrease in motor activity in wild-type but not in α1(H101R) mice (n = 16 per group). b, In a passive avoidance paradigm, diazepam shortened the latency to re-enter the dark compartment 24 h after training in wild-type mice but not in α1(H101R) mice (n = 10 per group). c, Partial anticonvulsant activity of diazepam against pentylenetetrazole (120 mg kg^{-1} i.p.) in α1(H101R) compared with wild-type mice (n = 10–11 per group). All results are given as means ±s.e.m. Asterisk, $P < 0.05$; double asterisks, $P < 0.01$; triple asterisks, $P < 0.001$. Diazepam was administered p.o.; V, vehicle.

Behavioral assessment of myorelaxant, motor-impairing, ethanol-potentiating and axiolytic-like actions



Horizontal Wire Test: Mice were lifted by their tails and allowed to grasp a horizontally strung wire (20 cm high, 1mm diameter, 15 cm long) with their forepaws and then released. The trials were executed two times at 30 min after injection. The number of animals from a total of ten per treatment group that did not grasp the wire with the forepaws or actively grasp the wire with at least one hind paw within 3 s was determined. In the vehicle treatment group, this number was consistently zero.

For the **righting reflex test**, mice were placed on their back, and the time (in min) to right themselves (all four paws under their body) was recorded. Mice received an intraperitoneal injection of 3 g/kg 15% ethanol solution, 30 min after having been treated with either vehicle or diazepam

Figure 4 Behavioural assessment of myorelaxant, motor-impairing, ethanol-potentiating and anxiolytic-like actions of diazepam in wild-type and α1(H101R) mice. **a**, Dose-dependent impairment of the grasping reflex in wild-type and α1(H101R) mice (n = 10–11 per group). **b**, Dose-dependent decrease of the latency to fall off the rotating rod in wild-type and α1(H101R) mice (n = 10 per group). **c**, Dose-dependent potentiation of the sedative effect of a 15% ethanol solution in wild-type and α1(H101R) mice (n = 5–15 per group). **d**, Dose-dependent increase of the percentage of time spent in the lit area in wild-type and α1(H101R) mice (n = 10 per group) tested in a light/dark choice paradigm. **e**, Dose-dependent increase of the number of open arm entries (in percentage of total number of arm entries) in both wild-type and α1(H101R) mice (n = 8–11 per group) exposed to an elevated X-maze. Results are given as means ± s.e.m. Asterisks, $P < 0.05$; double asterisks, $P < 0.01$; triple asterisks, $P < 0.001$. Diazepam was administered p.o.; V, vehicle.

TABLE 1 Overview of selected phenotypes of $GABA_A$ receptor subunit knockout (KO) and knockin (KI) mice

α	1	KI: Mediation of sedative action of diazepam KI: Mediation of anterograde amnesic action of diazepam KI: Mediation of anticonvulsant action of diazepam (partial) KO: Reduction of body weight (30%) KO: No spontaneous seizures, but seizure susceptibility increased KO: Tremor KO: Normal locomotor activity and motor performance	(29, 30, 47, 79)
	2	KI: Mediation of anxiolytic action of diazepam KI: Mediation of myorelaxant action of diazepam (partial)	(80, 87)
	3	KI: Mediation of myorelaxant action of diazepam (partial)	(80, 87)
	5	KO: Improved performance in a hippocampus-dependent task (spatial learning) KI: Facilitation of trace fear conditioning KI: Mediation of myorelaxant action of diazepam (partial)	(32, 49)
	6	KO: Posttranslational loss of subunit in cerebellum KO: increased expression of TASK-1 K^+ channels	(37, 53)
β	2	KO: Increased locomotor activity in novel environment KO: No spontaneous seizures	(30)
	3	KO: Cleft palate KO: Neonatally lethal (ca. 90%) KO: Hyperactive KO: Spontaneous seizures KO: Hyperresponsive KO: Motor impairment KI: Immobilizing action of etomidate and propofol absent KO, KI: Immobilizing action of halothane and enßurane: diminished potency	(38, 57, 59, 99)
γ	2	KO-Homozygotes: Neonatally lethal Defects in postsynaptic clustering of $GABA_A$ receptors KO-Heterozygotes: Reduction of synaptic clustering, e.g., in hippocampus Chronic anxiety Heightened responsiveness in trace fear conditioning and ambiguous cue discrimination	(39, 41, 65)
δ		KO: Attenuation of responses to neuroactive steroids KO: Reduced ethanol consumption KO: Attenuated withdrawal from chronic ethanol exposure KO: Reduced anticonvulsant effect of ethanol	(70, 102)
ρ	1	KO: Alteration of the excitation/inhibition balance between second and third retinal neurons	(76)

α1 est responsable pour: sédation, amnésie antérograde

α2 est responsable pour: anxiolyse, myorelaxation

La présence de α5 rend les souris un peu moins performantes dans des tests de mémoire et d'apprentissage (la sous-unité α5 est présent dans le hippocampe)

Par contre, le développement de tolérance et dépendance n'a pas pu être attribué à un sous-type spécifique

Annu. Rev. Pharmacol. Toxicol. 2004.44:475-498.

Les observations obtenues des souris « knock-in » ont pu être confirmé avec des ligands spécifiques

Table 1

$GABA_A$ receptor subtype ligands.

Drug	Main activity	Interaction with recombinant $GABA_A$ receptors[a]	References
Benzodiazepine site ligands			
Zolpidem	Hypnotic	Preferential af" nity for a1	[49]
Zaleplone	Hypnotic	Preferential af" nity for a1	[49]
Indiplon	Hypnotic	Preferential af" nity for a1	[50]
L-838 417	Anxiolytic	Comparable af" nity at a1, a2, a3 and a5 subtypes. Partial agonist at a2, a3 and a5 (but not a1) subtypes	[3]
Ocinaplon	Anxiolytic	Comparable af" nity at a1 a2, a3 and a5 subtpyes. Partial agonist at a2, a3 and a5 subtypes. Nearly full agonist at a1	[51]
SL 651 498	Anxiolytic	Agonist at a2 and a3. Partial agonist at a1 and a5 subtypes	[52]
TPA 023	Anxiolytic	Partial agonist at a2 and a3 subtypes. Antagonist at a1 and a5 subtypes	[7]
TP003	Anxiolytic (at high receptor occupancy)	Comparable af" nity at a1, a2, a3 and a5 subtypes. Selective agonist ef" cacy at a3 subtype	[22]
ELB139	Anxiolytic	Selective receptor pro" le uncertain	[53]
L-655 708	Memory enhancer, anxiogenic	Partial inverse agonist, with preference for a5 subtype	[8 ,9,30,51]
a3 IA	Anxiogenic	Weak inverse agonist at a3	[21]
Ligands at modulatory sites other than the benzodiazepine site			
Ethanol	Anxiolytic, sedative	High sensitivity (3 mM) at a4(a6)b3d[b]; medium sensitivity (30 mM) at a4(a6)b2d[b]; low sensitivity (100 mM) at a4(a6)b3g2	[54]
Neurosteroids (e.g. 3a,5a-THDOC)	Anxiolytic, sedative Anaesthetic	High sensitivity at d-containing subtypes[b] and at a1 and a3 receptors in combination with b1	[55]
Intravenous anaesthetics (etomidate propofol)	Sedative, anaesthetic	Act on receptor subtypes containing b3 (i.e. mainly a2 and a3 subtypes)	[38]
Dihydroquinoline (compound 4)	Anxiolytic	Agonist ef" cacy at a2 but not a1 subtype	[20]
GABA site			
Gaboxadol	Hypnotic	Partial agonist at a1 and a3 subtypes. Full agonist at a5 subtype. Agonist at a4b3d receptors	[56]

[a]Data should be treated with caution, as recombinant receptors that are expressed in foreign host cells might not give an accurate re" ection of their neuronal counterparts. [b]GABA is a weak partial agonist on d-containing receptors, which largely explains the strong modulatory response of ligands acting on d-containing receptors [57]. THDOC, 5a-pregnane-3a,21-diol-20-one.

Enhanced Learning and Memory and Altered GABAergic Synaptic Transmission in Mice Lacking the α5 Subunit of the GABAA Receptor

Neil Collinson et al., J. Neurosci 2002

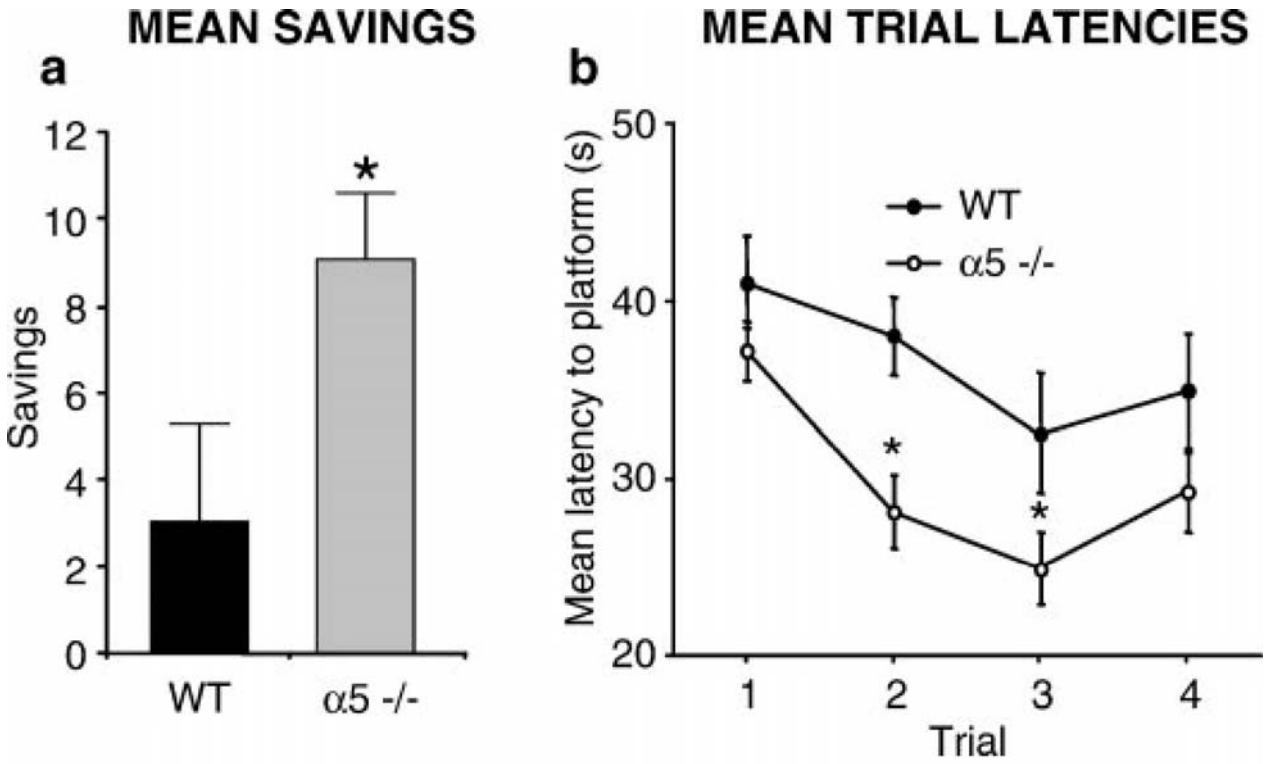


Figure 2. Enhanced performance of α5 -/- mice in the matching-to-place version of the water maze test. Eighteen WT and 20 α5 -/- mice were used in this test. a, The difference in time taken between trial 1 and trial 2 (savings) to find the hidden platform over the 10 d testing period is shown. α5 -/- mice made significantly higher savings compared with WT mice. b, α5 -/- mice were significantly quicker (*) at finding the hidden platform for both trial 2 and trial 3.

An Inverse Agonist Selective for α5 Subunit-Containing GABAA Receptors Enhances Cognition,

G. R. Dawson et al., J Pharm. Exp Therap. 2005

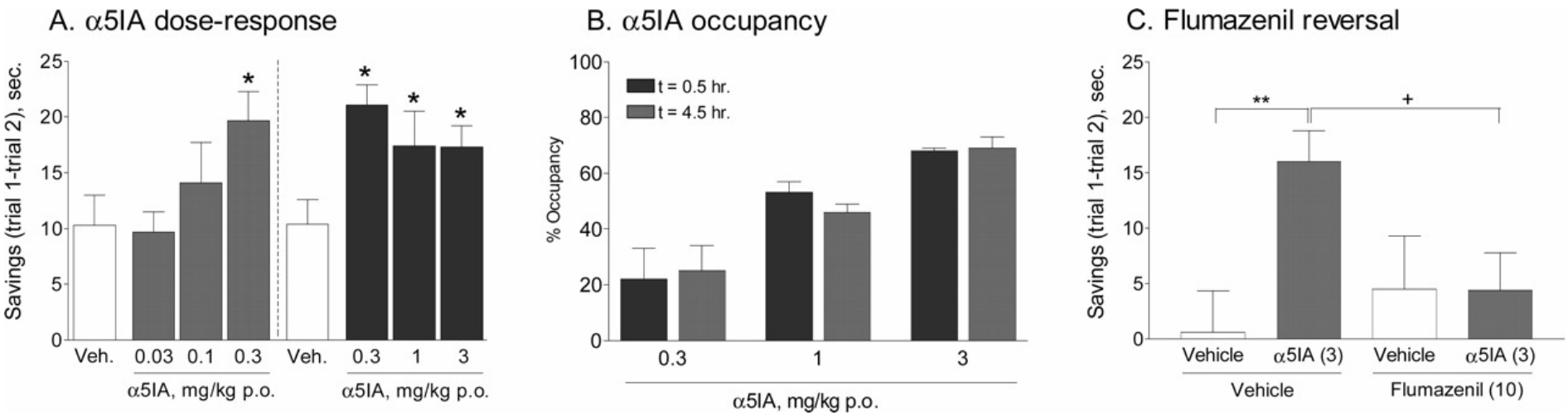


Fig. 8. 5IA enhances performance in the delayed-matching-to-position version of the Morris water maze. A, in two separate experiments, 5IA produced a significant enhancement in performance in the delayed-matching-to-position water maze as measured by the increase in the difference in time, averaged over 5–8 test days, between finding the hidden platform location in trial 2 compared with trial 1 (intertrial interval 4 h). In the low-dose experiment, 5IA had a minimal effective dose of 0.3 mg/kg, whereas in the high-dose experiment, the compound was effective at all three doses tested (0.3, 1, and 3 mg/kg). *, $p < 0.05$ versus vehicle-treated animals. B, occupancy of rat brain BZ sites by 5IA was measured 0.5 and 4.5 h postdosing (corresponding to the times of trials 1 and 2, respectively). Occupancy was dose-dependent but was not appreciably different at these time points. C, ability of 5IA (3 mg/kg p.o.) to increase the mean savings was prevented by the nonselective BZ antagonist flumazenil (10 mg/kg i.p.) given 15 min before trial 1, indicating that the effects of 5IA are mediated via the BZ site of GABAA receptors (presumably of the 5 subtype). Values shown are mean ± S.E.M. (n = 9–10 group). **, $p < 0.01$ compared with vehicle-vehicle group; +, $p < 0.05$ compared with 5IA-vehicle group.

Comme les sous-unités α jouent un rôle important pour l'effet des benzodiazépines, les sous-unités β sont importantes pour l'effet de quelques anesthésiques

Les propriétés anesthésiques d'étomidate sont majoritairement médié par la sous-unité β3, tandis que son effet sédatif est médié par la sous-unité β2. Le schéma illustre le rôle d'une acide aminée spécifique pour l'anesthésie

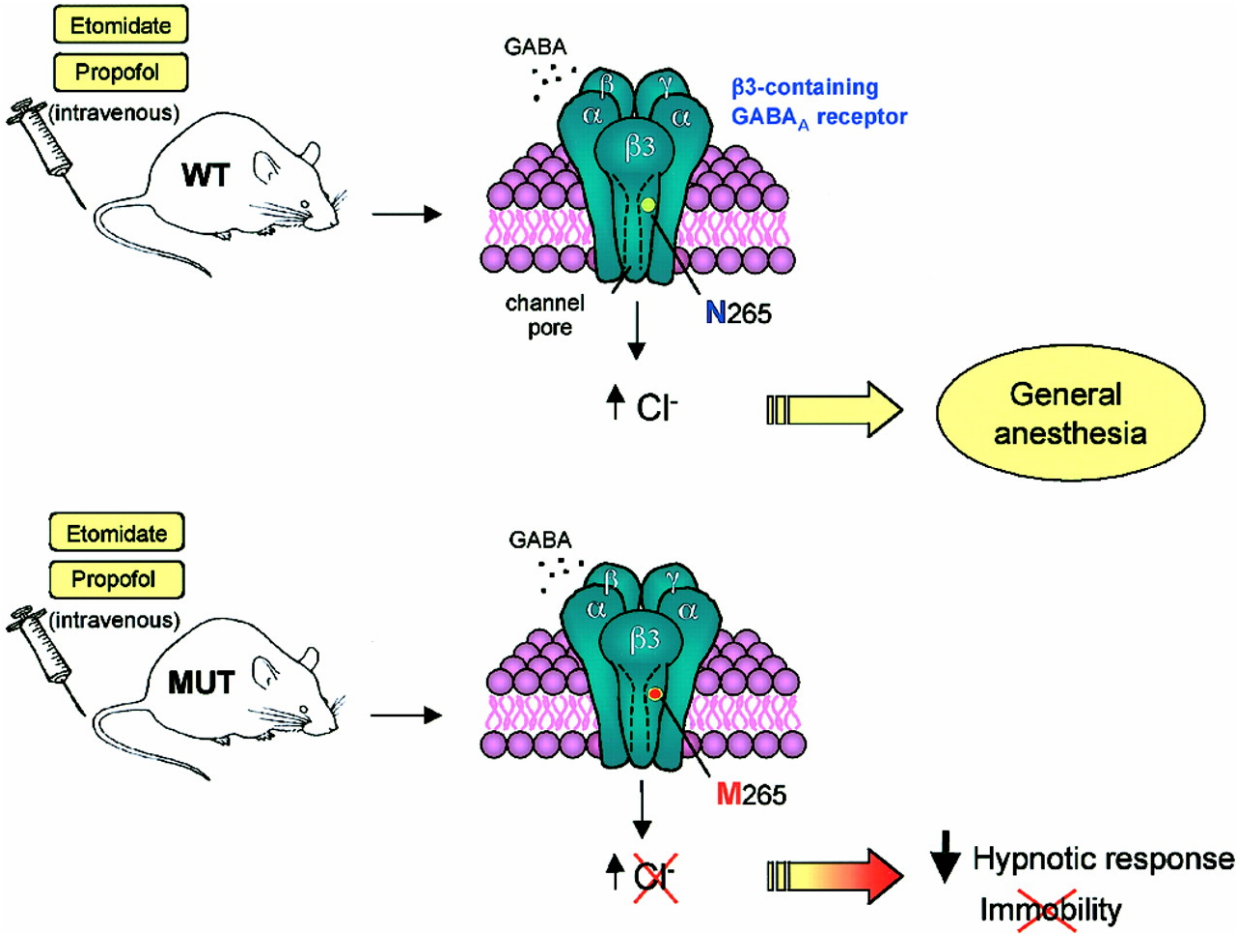
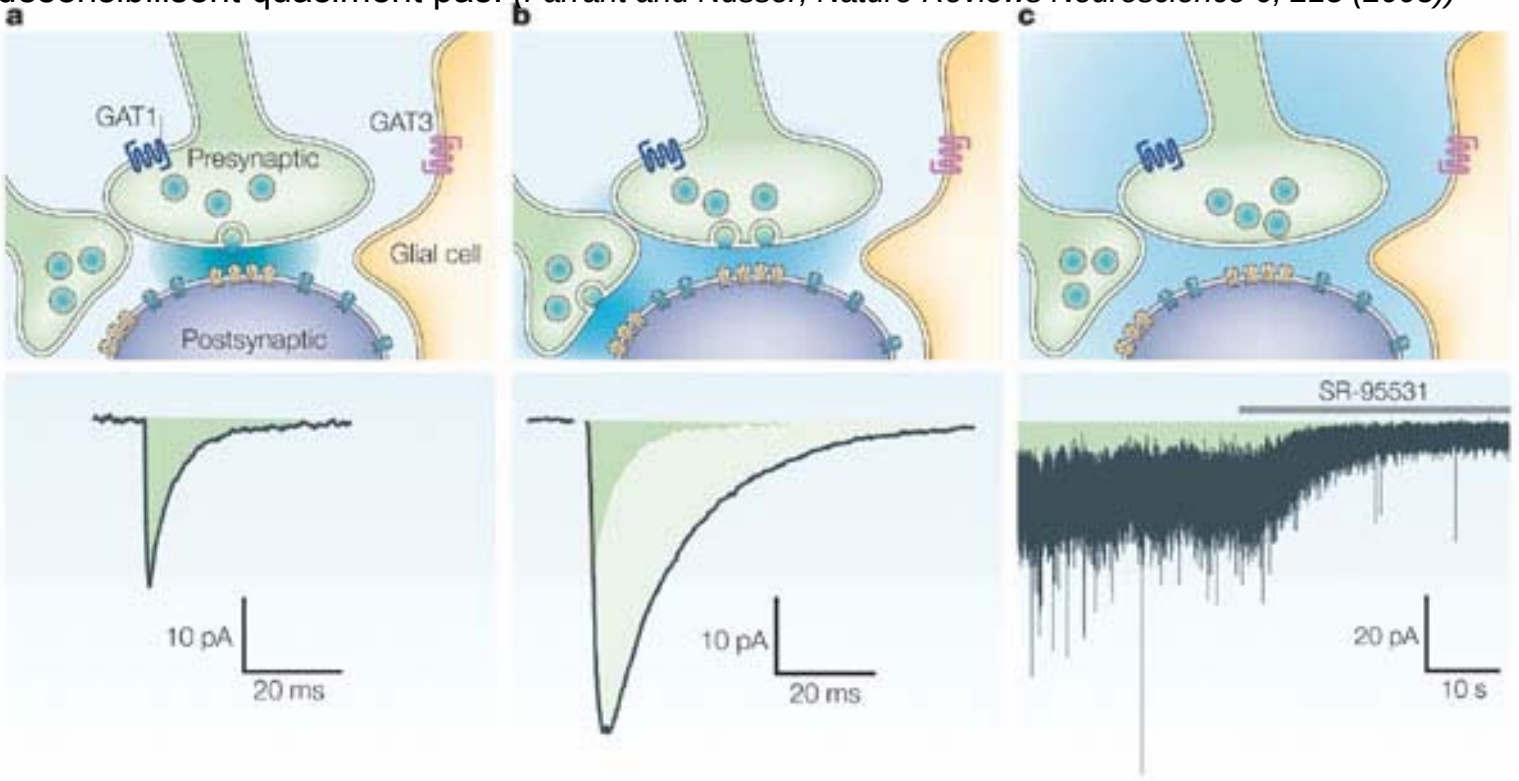


Figure 2. Schematic diagram illustrating that the general anesthetic actions of the i.v. drugs etomidate and propofol are largely dependent on an asparagine-265 residue located in the ß3 subunit of GABAA receptors. Mutation of this asparagine residue to a methionine (N265M) largely abolishes the anesthetic-induced increase in chloride ion flux through ß3-containing GABAA receptors, resulting in a decreased hypnotic response and a nearly complete absence of the immobilizing response in ß3(N265M) mutant mice (MUT). (Jurd R et al. Faseb J. 2003)

II.2. Les récepteurs GABAA extrasynaptiques ont une activité tonique qui peut co-déterminer une inhibition tonique

GABA peut arriver à des endroits externes à la fente synaptique. Les récepteurs GABAA extrasynaptiques contiennent pour la plupart la sous-unité δ. ils possèdent une sensibilité plus élevée au GABA et ne désensibilisent quasiment pas. *(Farrant and Nusser, Nature Reviews Neuroscience 6, 215 (2005))*

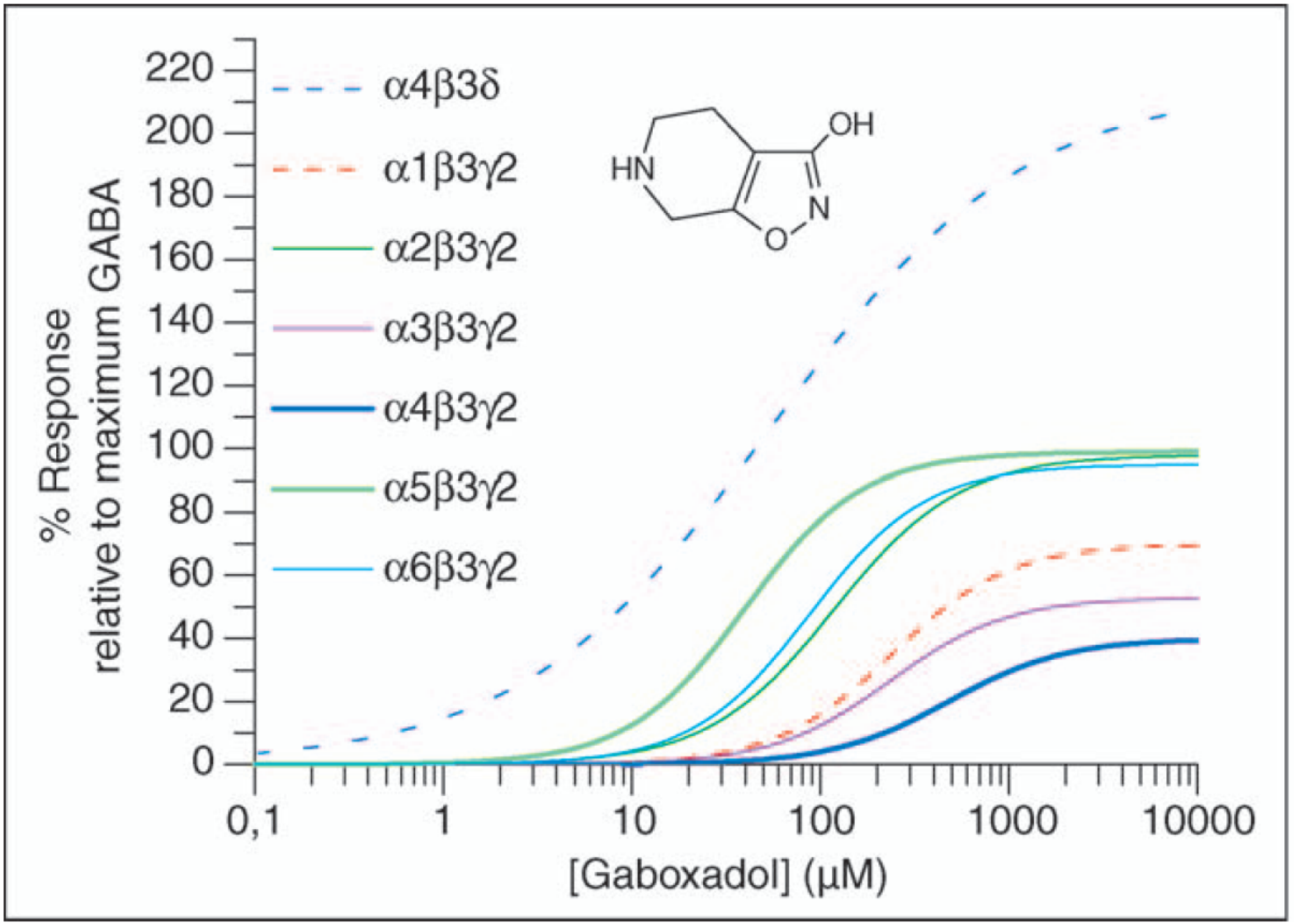


a) Un vésicule de GABA est relâché, b) plusieurs vésicules de GABA sont relâché, c) illustration qu'une parti du GABA arrive à des régions extrasynaptiques (malgré la présence des transporteurs GAT), où il active des récepteurs GABA

Rôle de l'activité tonique, indications de souris ko (*α5 est surtout extrasynaptique, avec une expression très restreinte, dans le hippocampe. La sous-unité δ est aussi préférentiellement exprimé au-dehors des synapses*)
-Les souris ko α5 apprennent mieux que les wild-type → rôle dans l'apprentissage
-Les souris ko δ ont des convulsions

a | The release of a single vesicle from a presynaptic terminal activates only those postsynaptic $GABA_A$ (γ-aminobutyric acid type A) receptors that are clustered in the membrane immediately beneath the release site (yellow). The diffuse blue shading indicates the spread of released GABA. The current record shows an averaged waveform of miniature inhibitory postsynaptic currents (mIPSCs) recorded in the presence of the sodium channel blocker tetrodotoxin. The area beneath the record is shaded to indicate the charge transfer. GAT, GABA transporter. b | Action potential-dependent release of multiple vesicles or evoked release from several terminals promotes GABA 'spillover', and activates both synaptic receptors and perisynaptic or extrasynaptic receptors (blue). The current record shows the larger and much slower averaged waveform of IPSCs evoked by electrical stimulation. The area of the mIPSC is superimposed for comparison. c | A low concentration of ambient GABA, which persists despite the activity of the neuronal and glial GABA transporters (GAT1 and GAT3), tonically activates high-affinity extrasynaptic receptors. The trace shows the 'noisy' tonic current that results from stochastic opening of these high-affinity $GABA_A$ receptors, with superimposed phasic currents (in this case, the synaptic events would be arising at sites not depicted in the schematic diagram). A high concentration (10 μM) of the $GABA_A$ antagonist gabazine (SR-95531) blocks the phasic IPSCs and tonic channel activity, causing a change in the 'holding' current and a reduction in current variance. The infrequent phasic events that remain in SR-95531 are glutamatergic excitatory postsynaptic currents. The shaded area beneath the current record before SR-95531 application represents the charge carried by tonically active $GABA_A$ receptors. The frequency of spontaneous IPSCs is relatively low and the tonic receptor activity generates a conductance several-fold larger than the averaged conductance that is carried by phasic IPSCs[42, 61]. The current records are from whole-cell patch-clamp recordings of granule cells in acute cerebellar slices from adult mice. The recordings were made with symmetrical chloride concentrations at a holding voltage of -70mV and a temperature of 25°C. pA, pico amp. Traces in panels a and b courtesy of S. G. Brickley and M. F. Trace in panel c modified, with permission, from Ref. 116

Gaboxadol – un agoniste sélective des récepteurs GABAA extra-synaptiques



Wafford and Ebert, Curr Opin Pharmacol (2006) **6**, 30

Gaboxadol

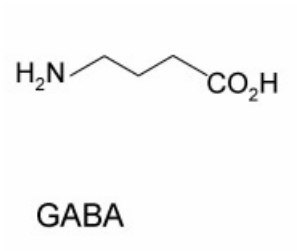
- Gaboxadol a comme cible les récepteurs GABAA contentant la sous-unité δ. La majorité des récepteurs GABAA contentant la sous-unité δ ont une localisation extrasynaptique (ainsi que les récepteurs $\alpha5\beta3\gamma2$)

- *in vivo*, la sous-unité δ s'assemble entre les sous-unités α exclusivement avec des sous-unités $\alpha4$ et $\alpha6$. La sous-unité $\alpha6$ est exprimé surtout dans le cervelet, et $\alpha4$ dans le thalamus, et dans le « dentate gyrus » de l'hippocampe.

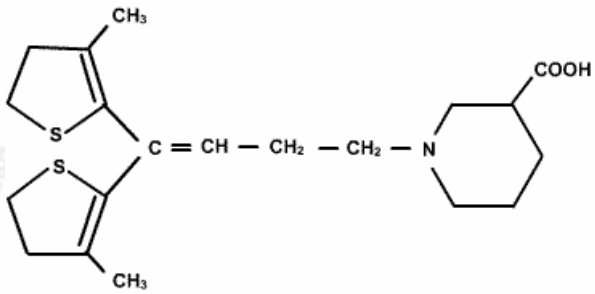
- les récepteurs GABAA extrasynaptiques sont exposés à des concentrations de GABA dans l'ordre de 1-5 μM (dans la synapse: mM). Les récepteurs GABAA extrasynaptiques ont une affinité pour le GABA plus élevé, comparé aux récepteurs GABAA synaptiques. Les récepteurs GABAA extrasynaptiques exercent une inhibition tonique (constante); par contre, les récepteurs GABAA synaptiques ont un effet inhibiteur seulement au moment d'activité synaptique.

- le thalamus joue un rôle important dans le contrôle du flux d'informations de la périphérie vers le cortex. Pendant le sommeil, l'entrée sensorielle est diminuée, mais l'activité thalamo-corticale continue (consolidation, traitement d'information dans le mémoire)

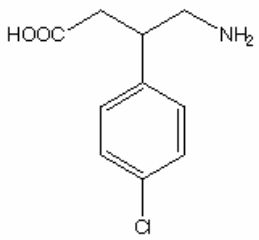
- Effets de Gaboxadol:
    - actuellement on pense que les effets de Gaboxadol sont surtout médiés par les récepteurs $\alpha4\beta2\delta$ dans le thalamus.
    - diminution du temps pour s'endormir, augmentation de la durée du sommeil
    - changement de « l'architecture » du sommeil: augmentation de l'activité SWS et SWA sans diminuer REM sleep (SWS= slow wave sleep, REM, rapid eye movement sleep, SWA, slow wave activity)



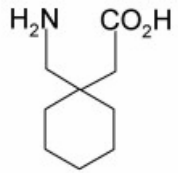
GABA



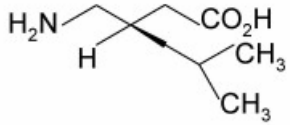
tiagabin



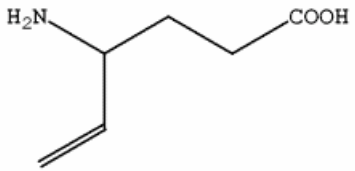
baclofen



Gabapentin

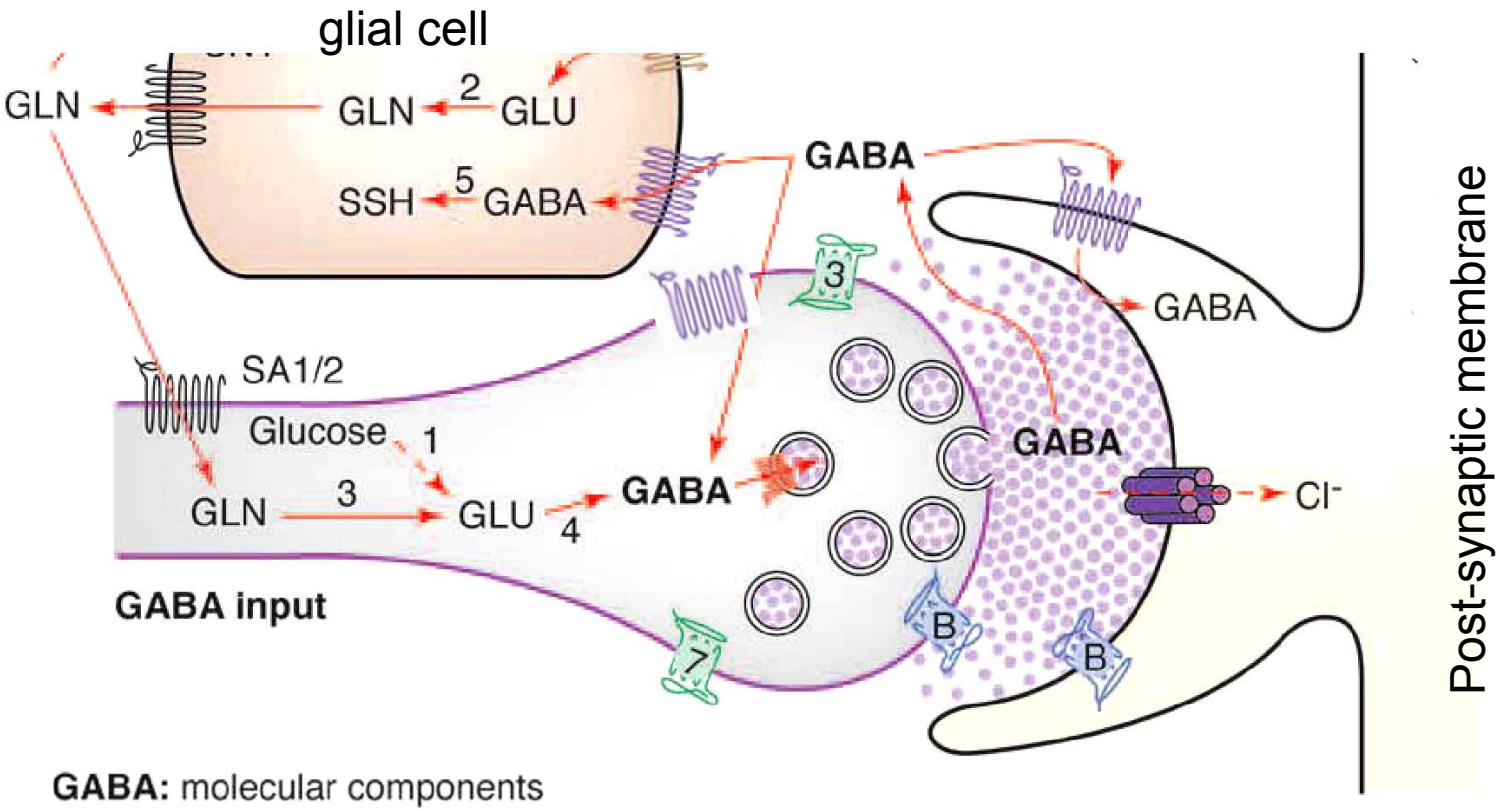


Pregabalin



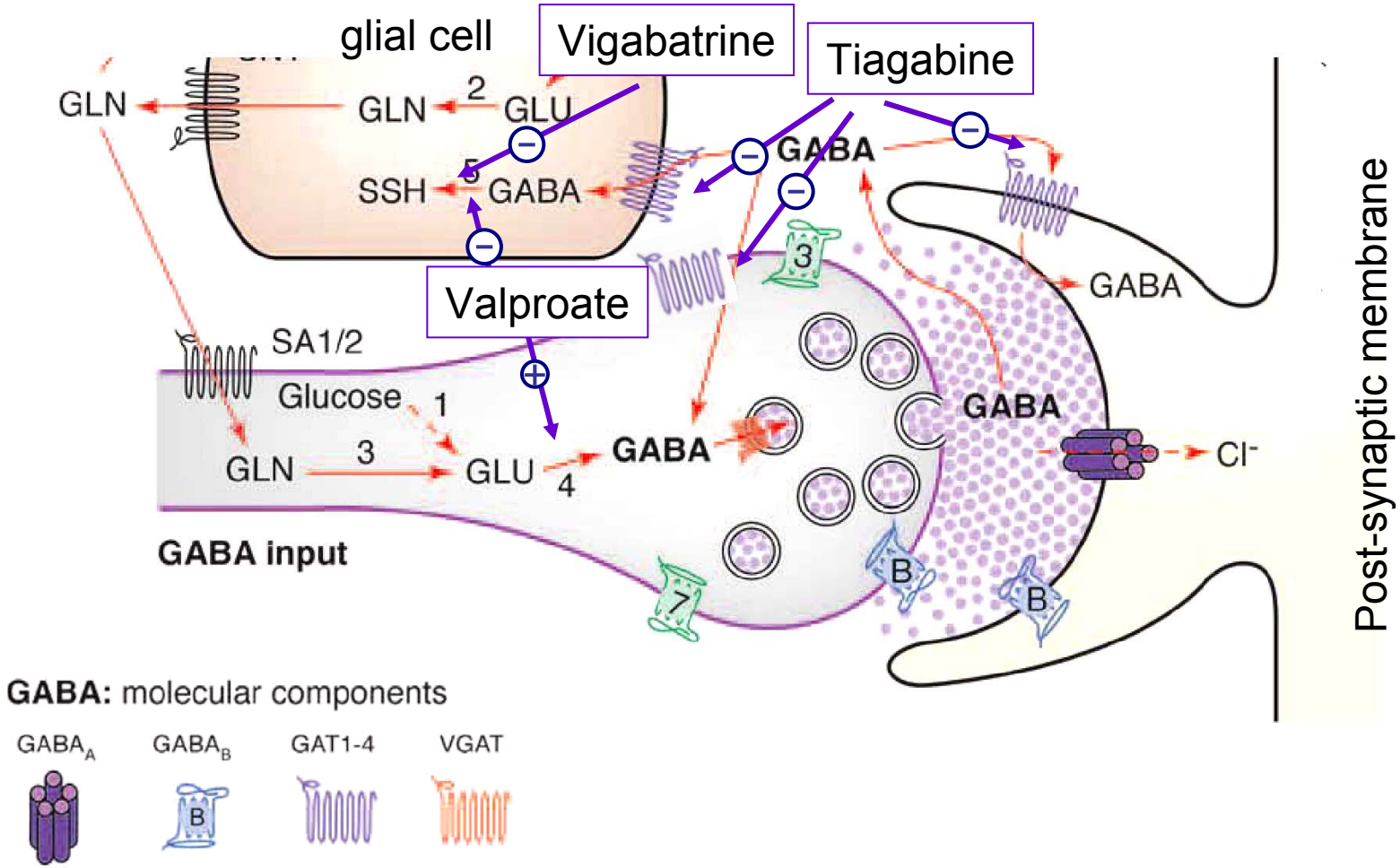
Vigabatrin

II.3. Approches pharmacologiques dans le système GABAergique, sans cibler le récepteur GABAA



<u>Transporteurs de GABA:</u>
GAT= GABA transporteur à la surface cellulaire
VGAT= transporteur GABA vésiculaire
<u>Formation de GABA:</u>
3), glutaminase, 4) glutamate décarboxylase
<u>Métabolisme de GABA</u>
GABA-T (=GABA-transaminase → acide succinique sémialdéhyde) et semi-succinique aldéhyde déhydrogenase (→ succinate, introduit en cycle Krebs)

Approches pharmacologiques dans le système GABAergique, sans cibler le recepteur GABAA



Valproate: dégradation ↘ synthèse ↗ de GABA

Tiagabine: re-uptake de GABA ↘ (inhibiteur de GAT1)

Vigabatrine: dégradation de GABA ↘ (inhibiteur de GABA-T)