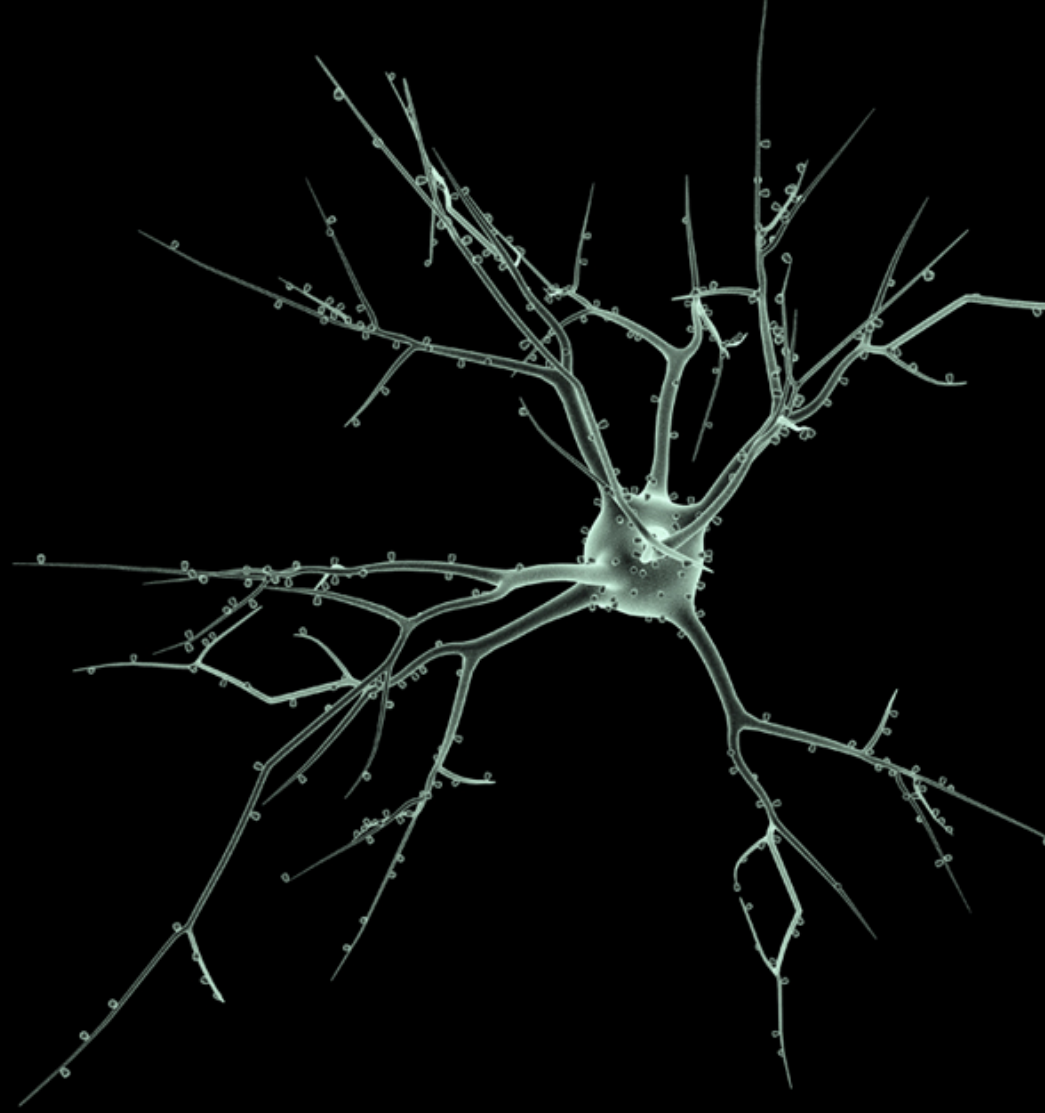
The Ionotropic $GABA_A$ Receptor



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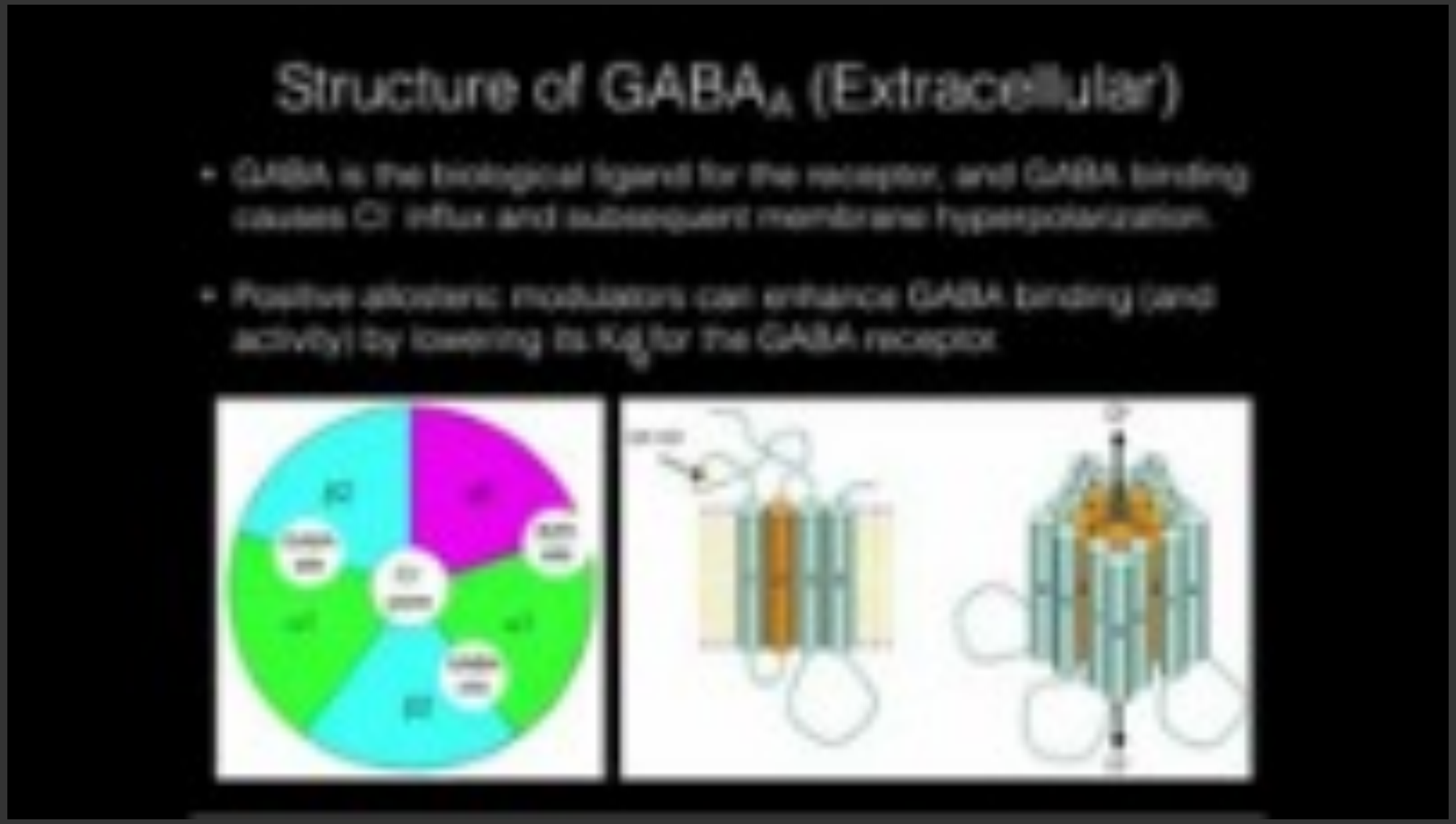
https://www.elsevier.com/books/cellular-and-molecular-neurophysiology/hammond/978-0-12-374127-1

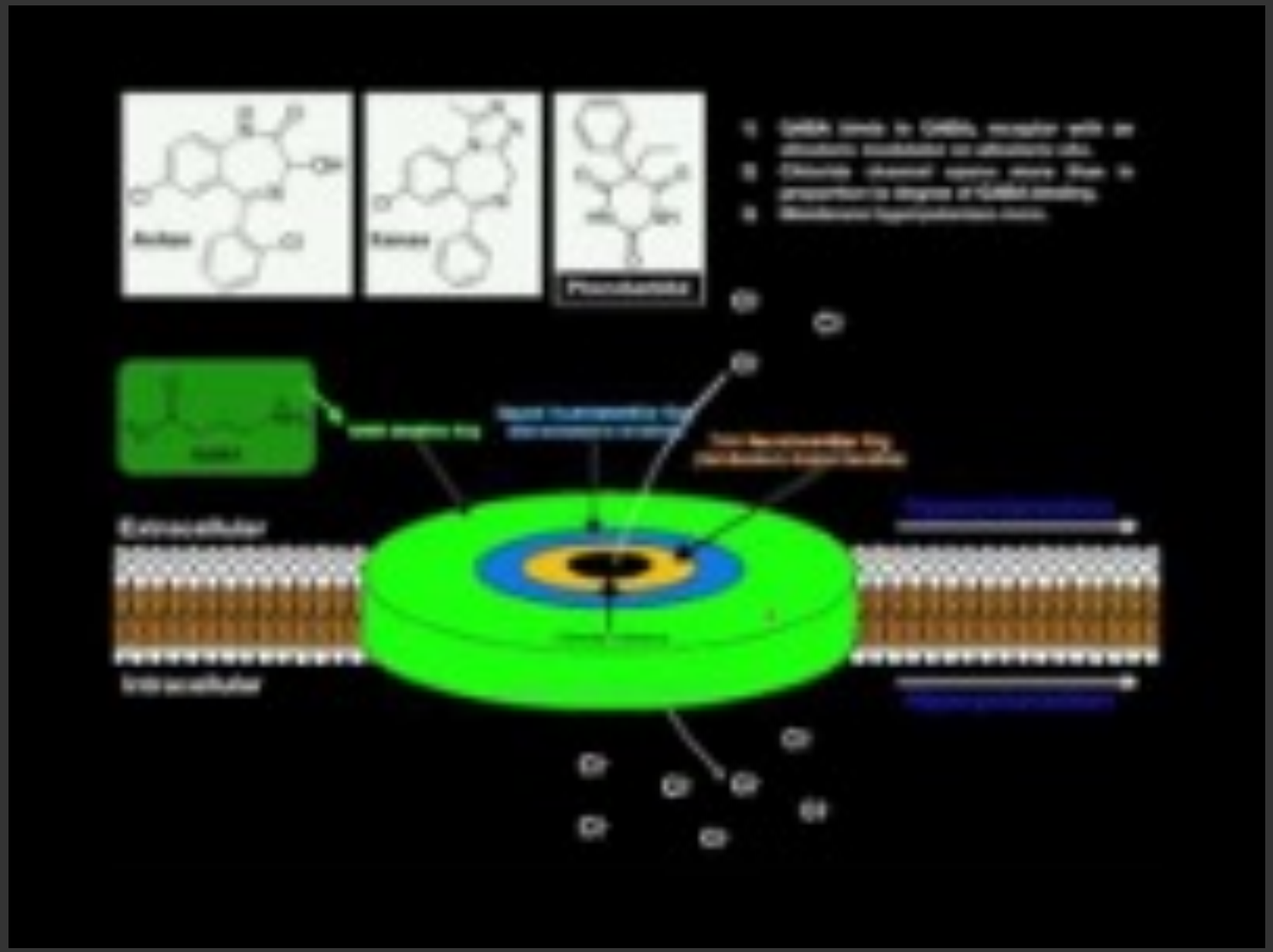
The $GABA_A$ Receptor & Positive Allosteric Modulation

https://www.youtube.com/watch?v=pVJoU7c-7S4



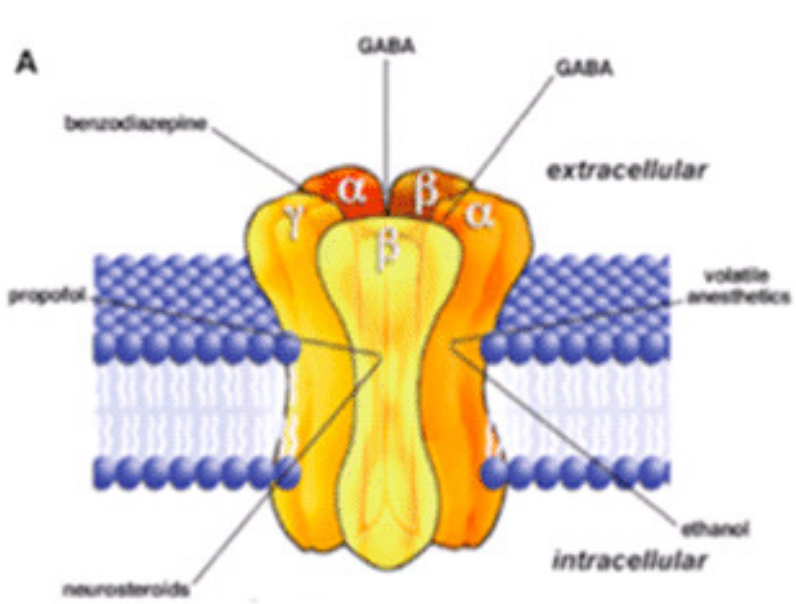
The Mechanism of Benzodiazepines | The GABA Receptor and Allosteric Modulation

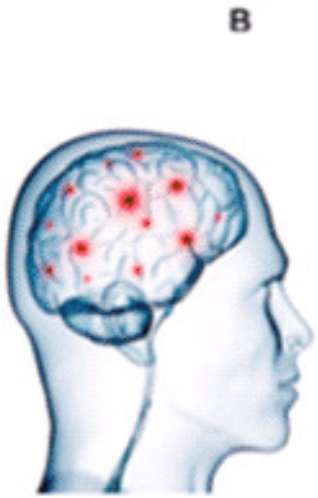
https://www.youtube.com/watch?v=BPWgGbBLebI

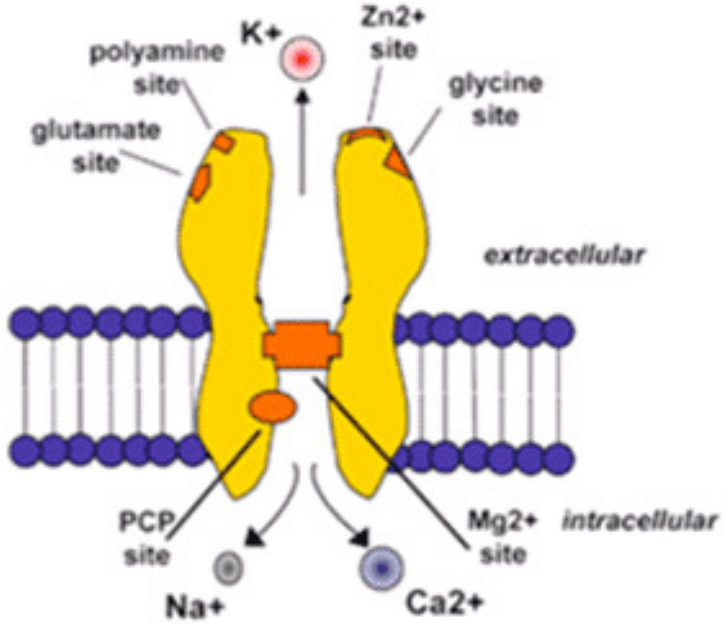


$GABA_A$ Receptors
(inhibiting brain cell firing)

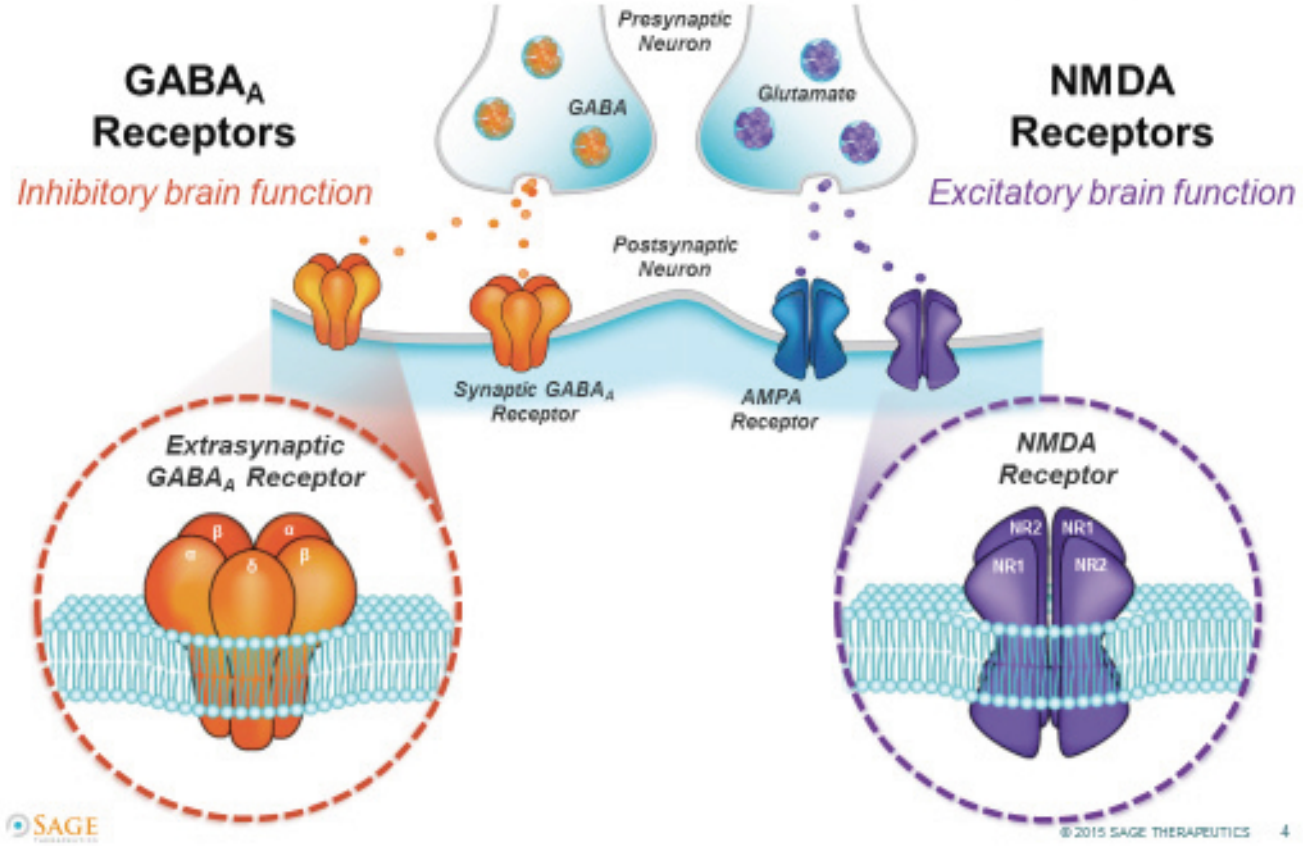
NMDA Receptors
(causing brain cell firing)



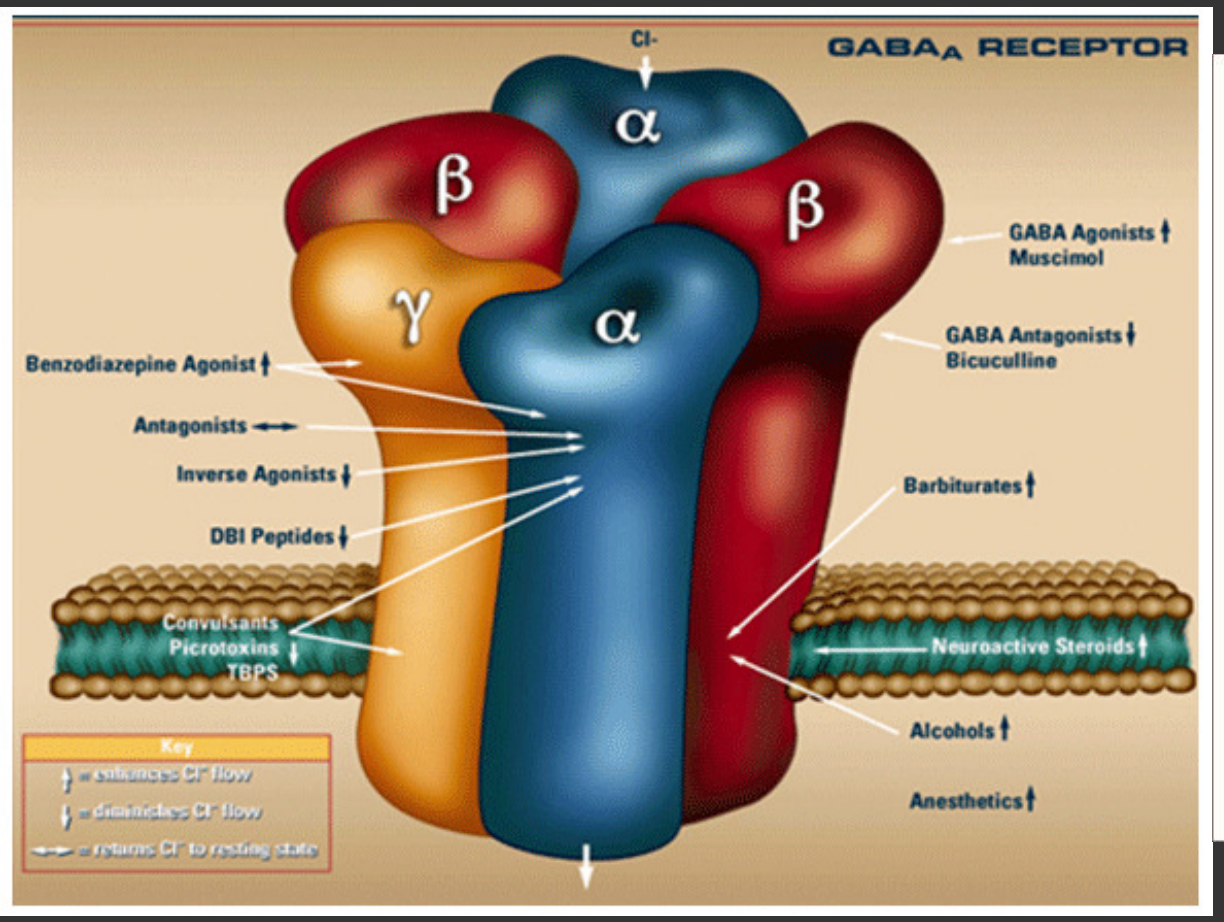


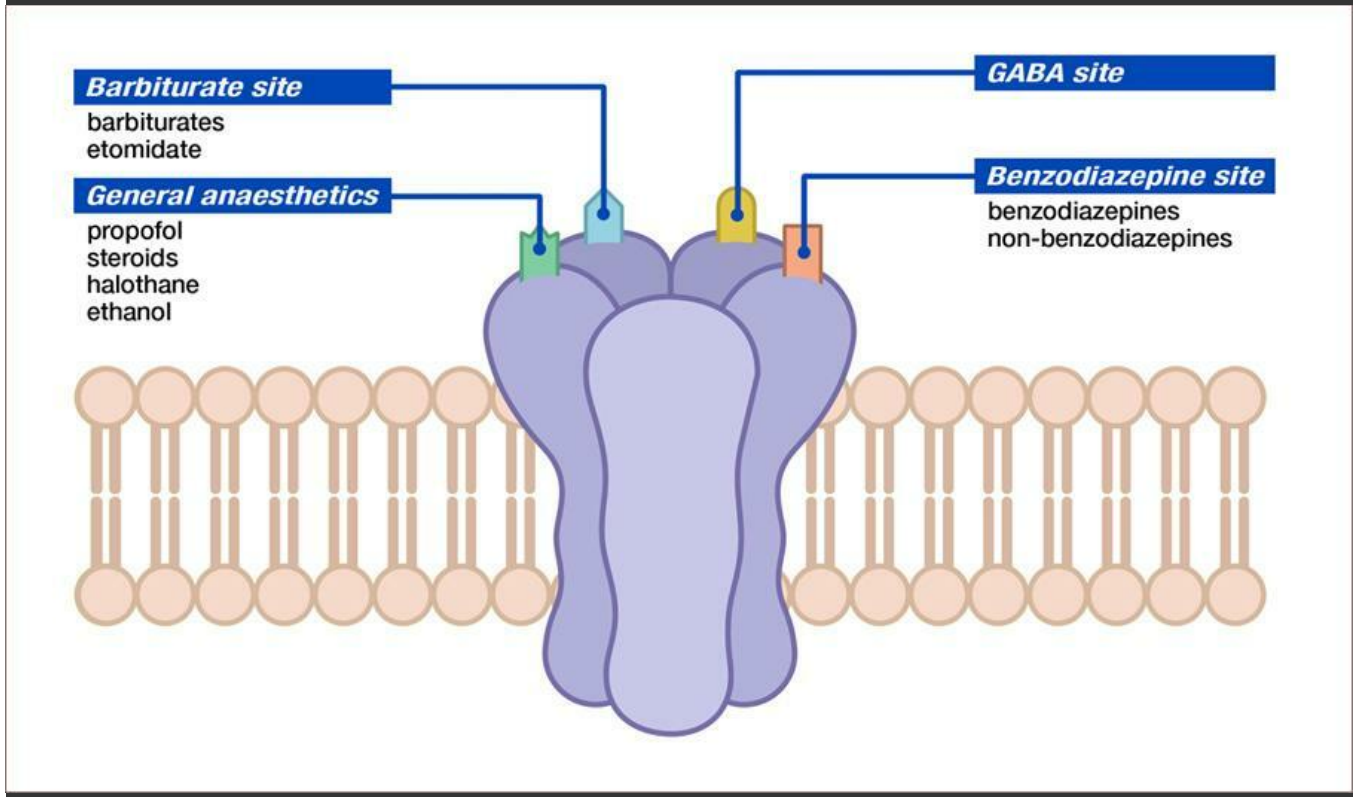


Targeting Key Receptor Systems that Regulate Essential Brain and CNS Signaling



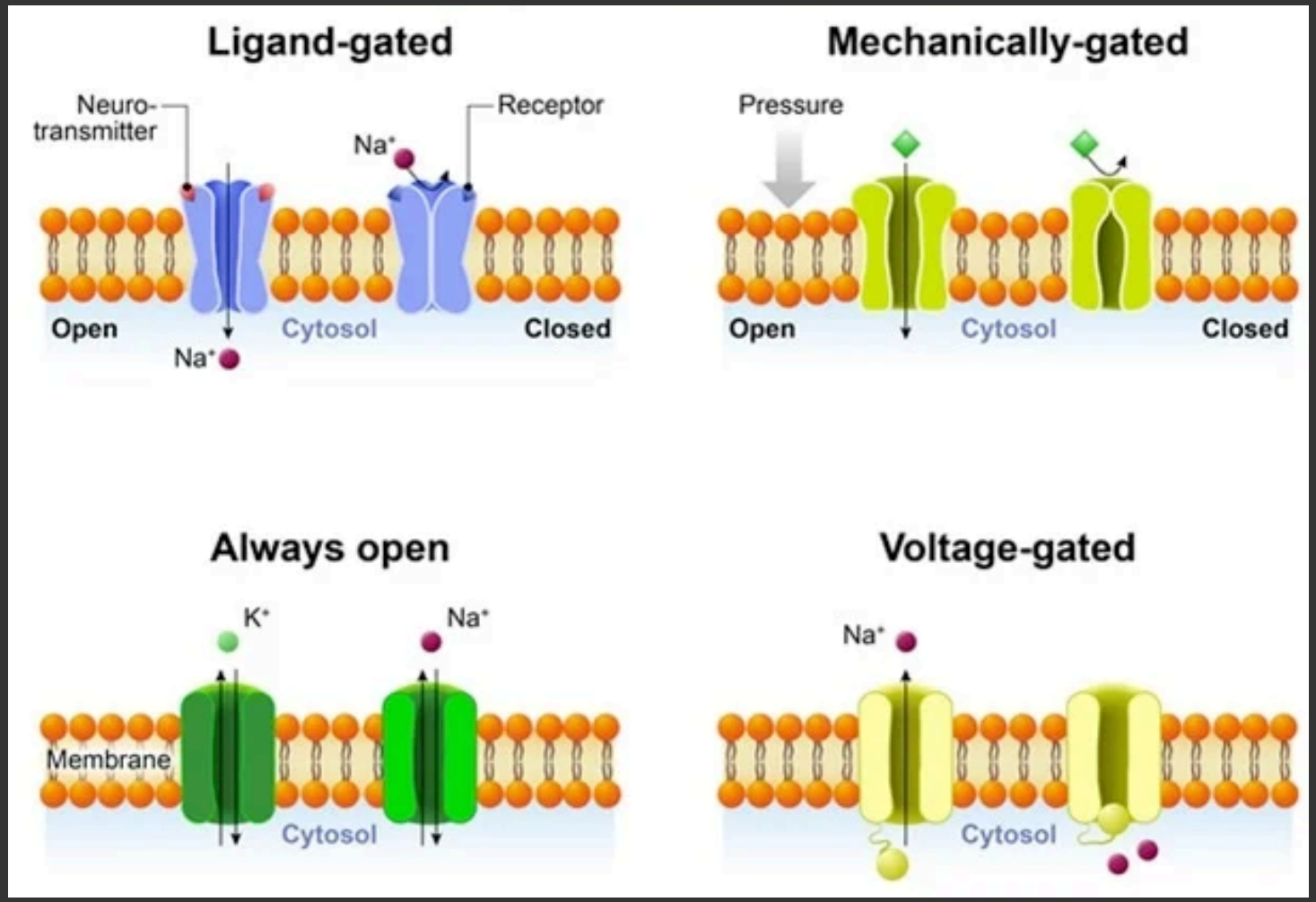
SAGE

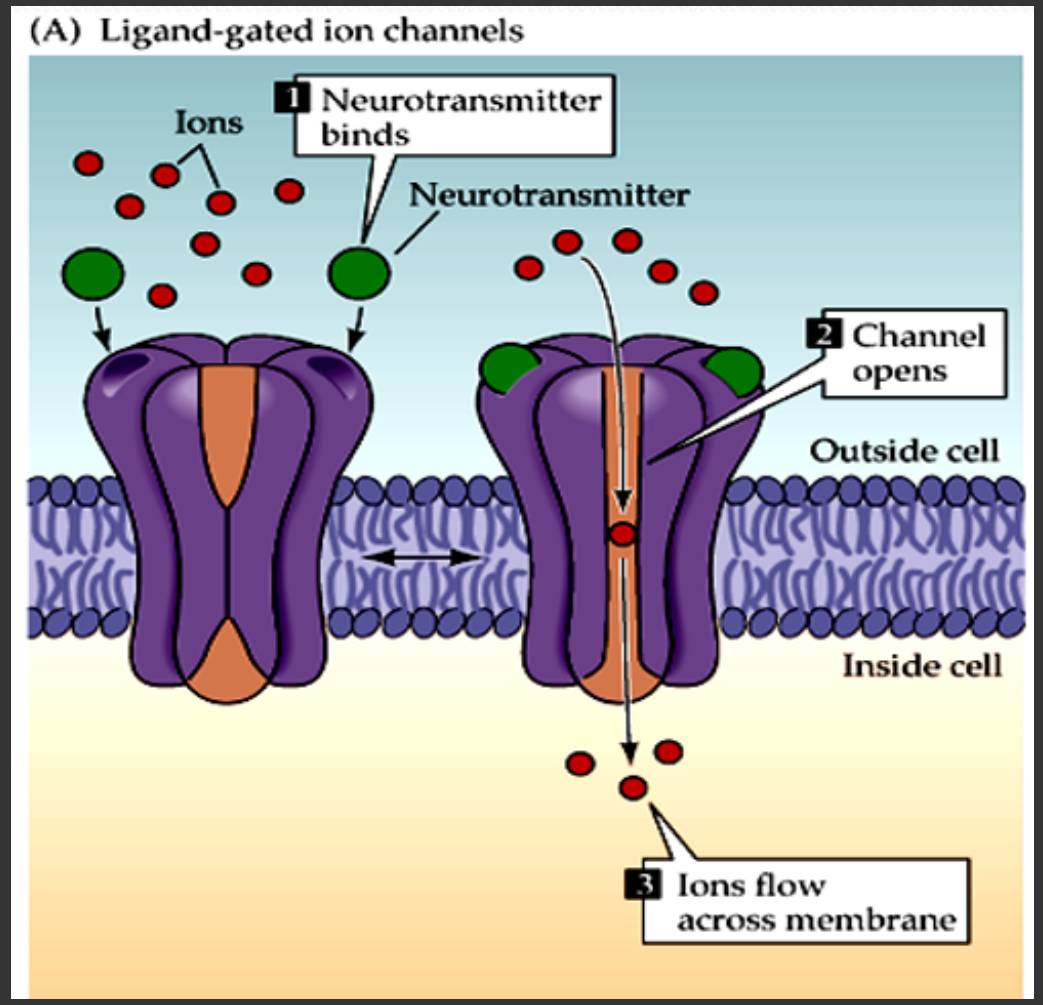

GABA$_A$ RECEPTOR
Cl-
α
β
β
γ
α
GABA Agonists
Muscimol
GABA Antagonists
Bicuculline
Benzodiazepine Agonist
Antagonists
Inverse Agonists
DBI Peptides
Barbiturates
Convulsants
Picrotoxins
TBPS
Neuroactive Steroids
Alcohols
Anesthetics
Key


Barbiturate site
barbiturates
etomidate
General anaesthetics
propofol
steroids
halothane
ethanol
GABA site
Benzodiazepine site
benzodiazepines
non-benzodiazepines

Double-Liganded Ion Channel

Just has two places for receptors to bind ?





Drug Interactions

Synergestists

- inhaled general anesthetic (to competitive)
- depolarizing blockers (to competitive)
- Ca++ blockers
- some antibiotics (aminglycosides, tetracyclins due to Ca++ chelation, polymixin B, colistin)

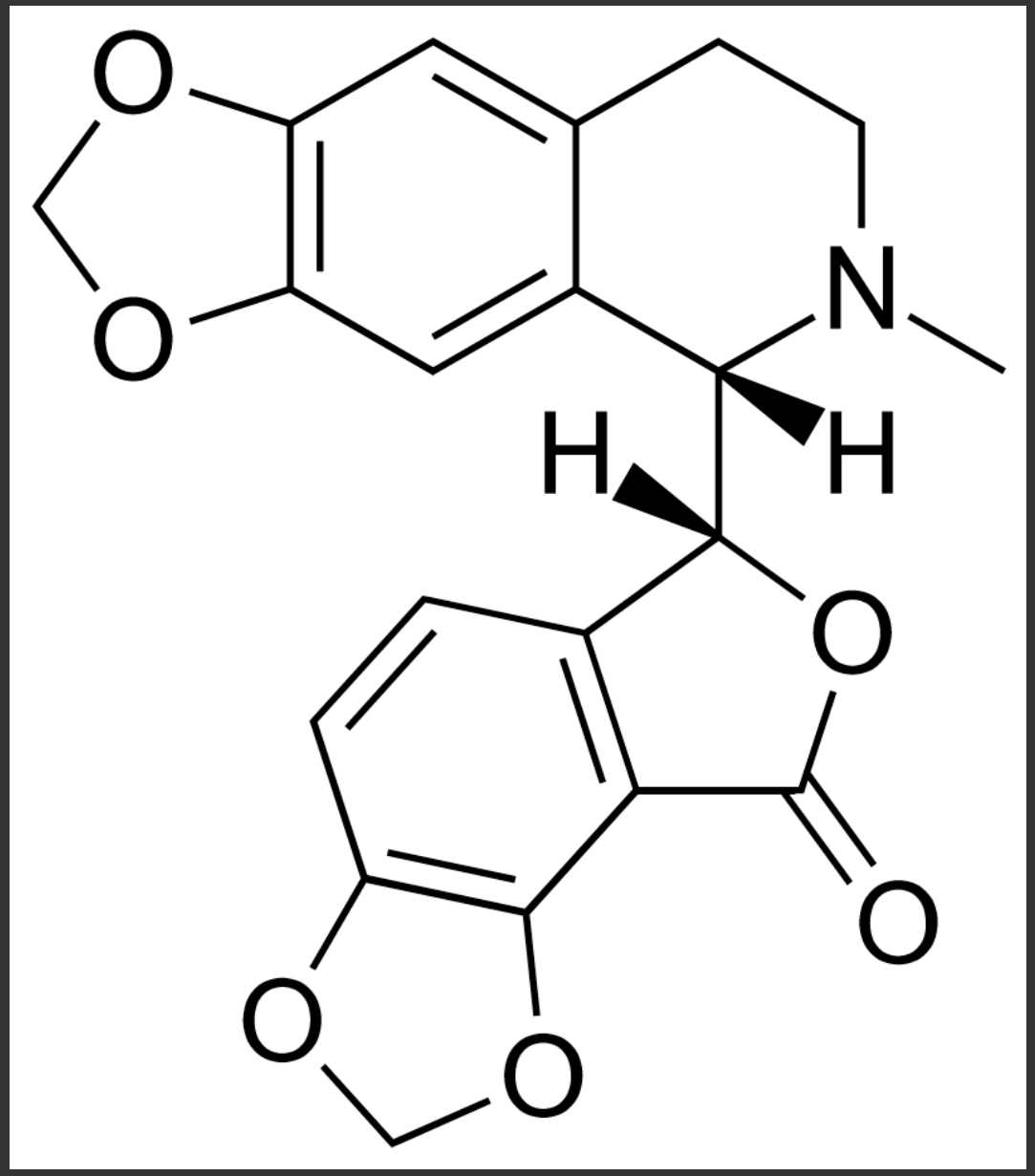
Antagonists

- neostigmine, pyridoine (these are AntiChE-ase)
- potassium in muscles

Bicuculline

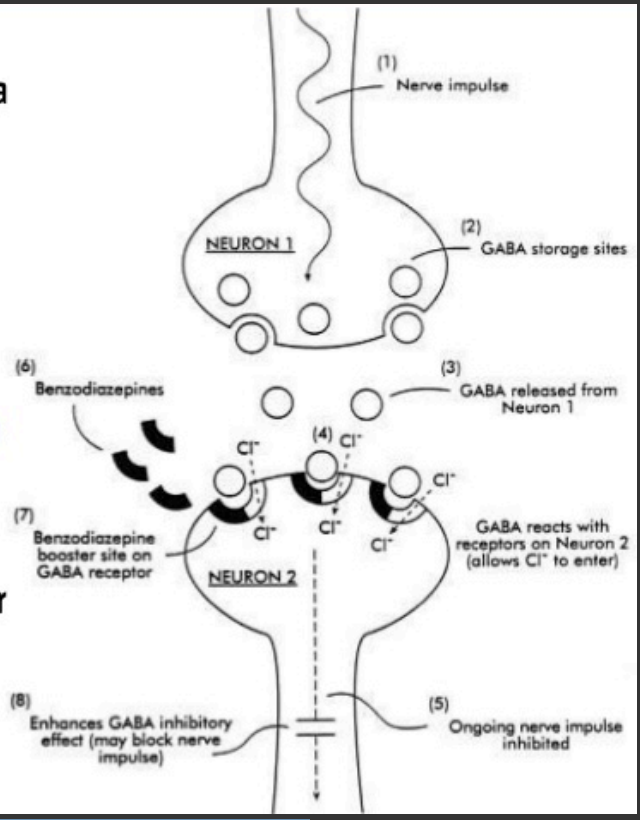
- light-sensitive competitive

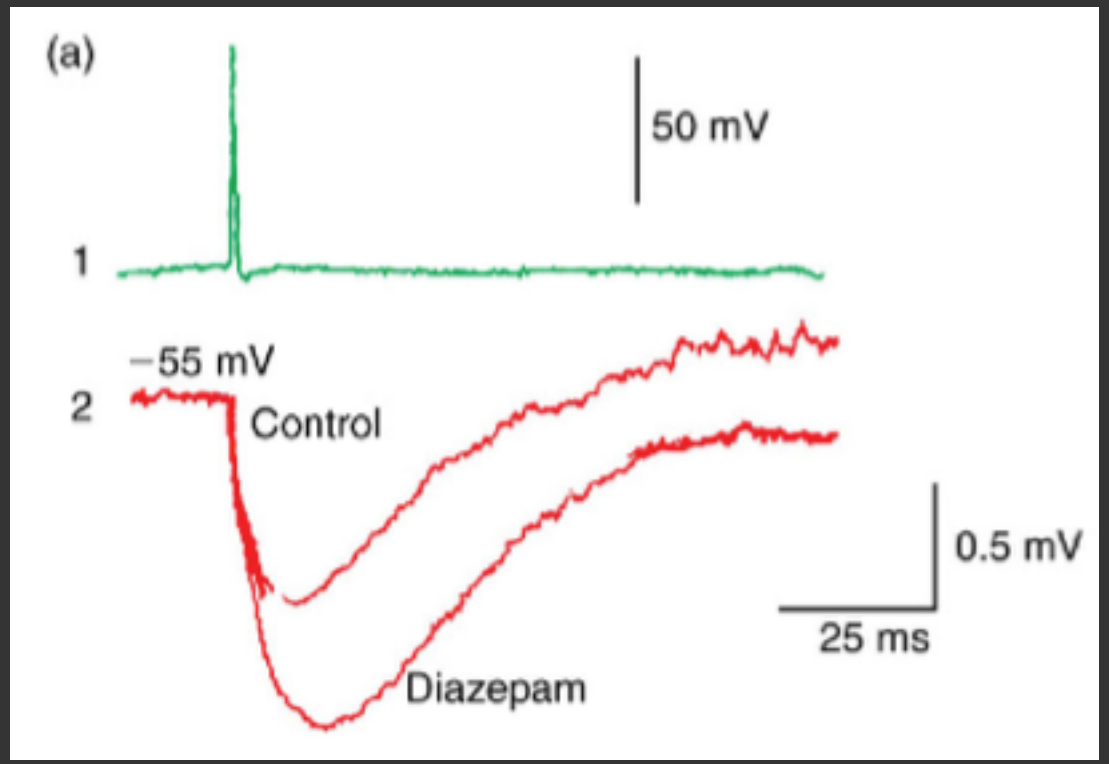
  antagonist of $GABA_A$ receptors

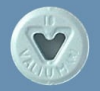


Benzodiazepine

- **Benzodiazepene** is a sedative that can be taken to provide a calming effect
- **Thus having an agonistic effect by imitating the action of GABA thus inhibiting an overly active physiological response caused by exposure to phobic stimulus.**
- **By allowing more negatively charged chloride ions to enter the postsynaptic neuron**
- **Similar affect to alcohol**

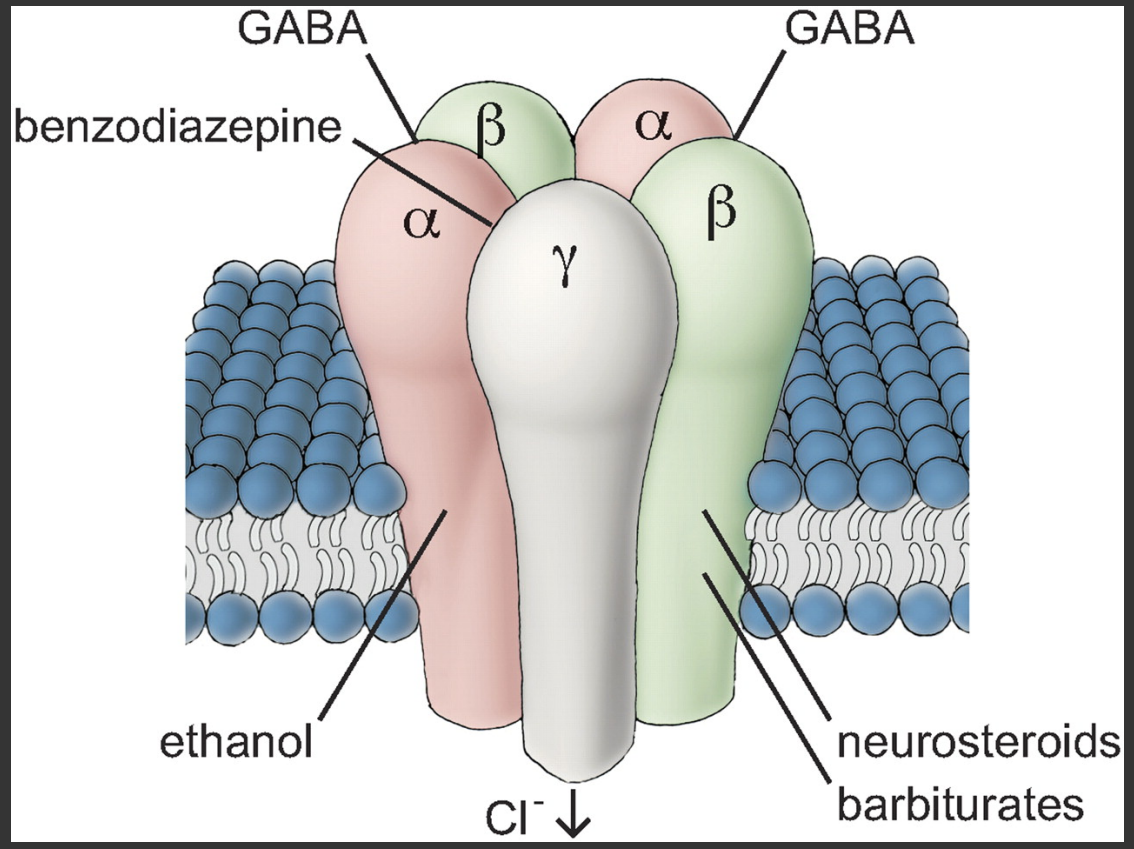


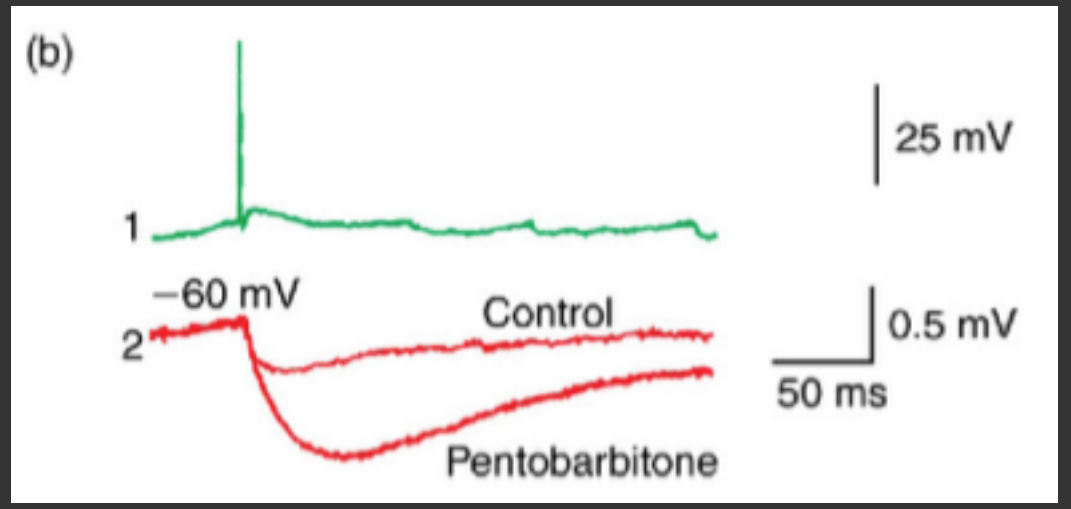


Many of the most commonly prescribed benzos are those which are most readily abused, including:	ALPRAZOLAM (Xanax) 	CLONAZEPAM (Klonopin) 	CHLORDIAZEPOXIDE (Librium) 
DIAZEPAM (Valium) 	LORAZEPAM (Ativan) 	TEMAZEPAM (Restoril) 	TRIAZOLAM (Halcion) 

- A single spike is triggered in the presynaptic GABAergic interneuron in response to a current pulse.
- It evokes an IPSP in the postsynaptic pyramidal neuron.
- This IPSP is increased in amplitude and duration in the presence of a benzodiazepine

Barbiturates





- Similar experiment in a different cell pair with pentobarbitone sodium (250 m M), a barbiturate.
- Modulation of bistratified cell IPSPs and basket cell IPSP by pentobarbitone sodium, diazepam and Zn2+: dual recordings in slices of adult hippocampus

- Slow down brain activity by increasing the activity of GABA.
- The result is drowsy or calming feeling.

Questions

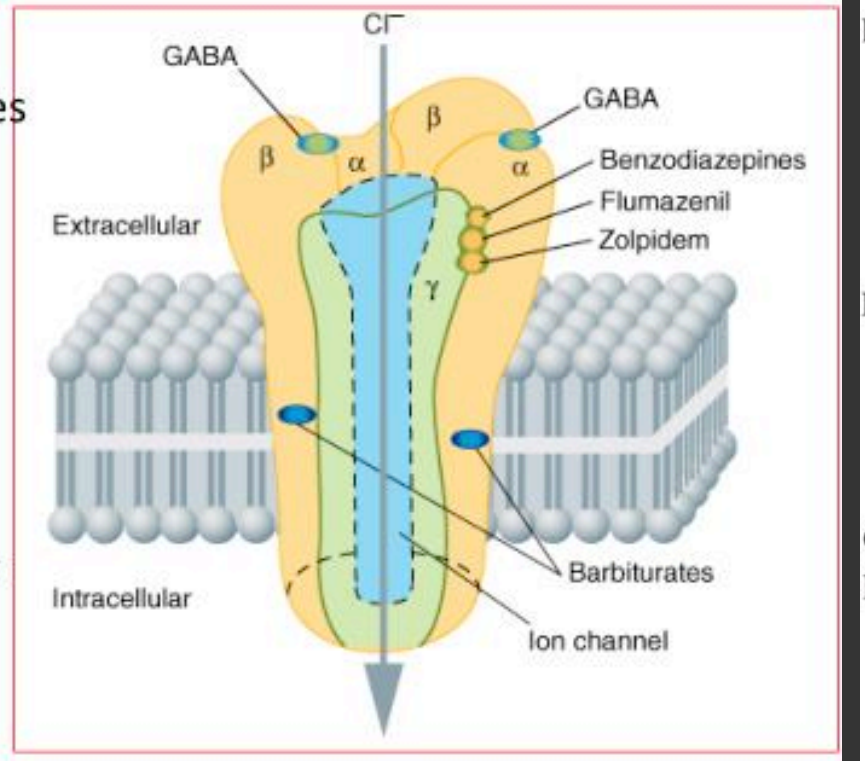
- How does a $GABA_A$ receptor mediate the binding of GABA into a transient hyperpolarization of the membrane?
  - How is the agonist binding signal transmitted down to the channel (coupling) and transduced in opening of the ion channel (gating).
  - What are the permeant ions?
  - Does GABA induce the entry of negatively charged ions?
    - Or does it induce the exit of positively charged ions?

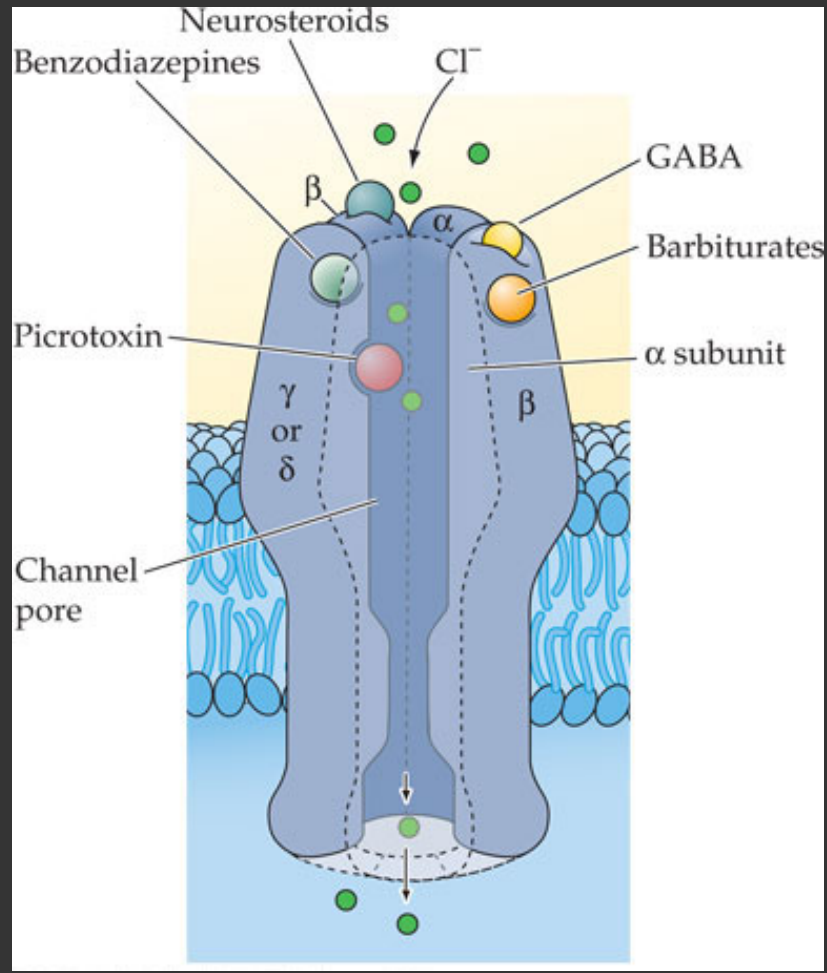
Questions

- Do benzodiazepines and barbiturates act directly on $GABA_A$ receptors?
- Are there selective and distinct binding sites on the receptor for each of these drugs?
- How do they potentiate the hyperpolarizing effect of GABA?
- Are there other modulators of $GABA_A$ receptors?

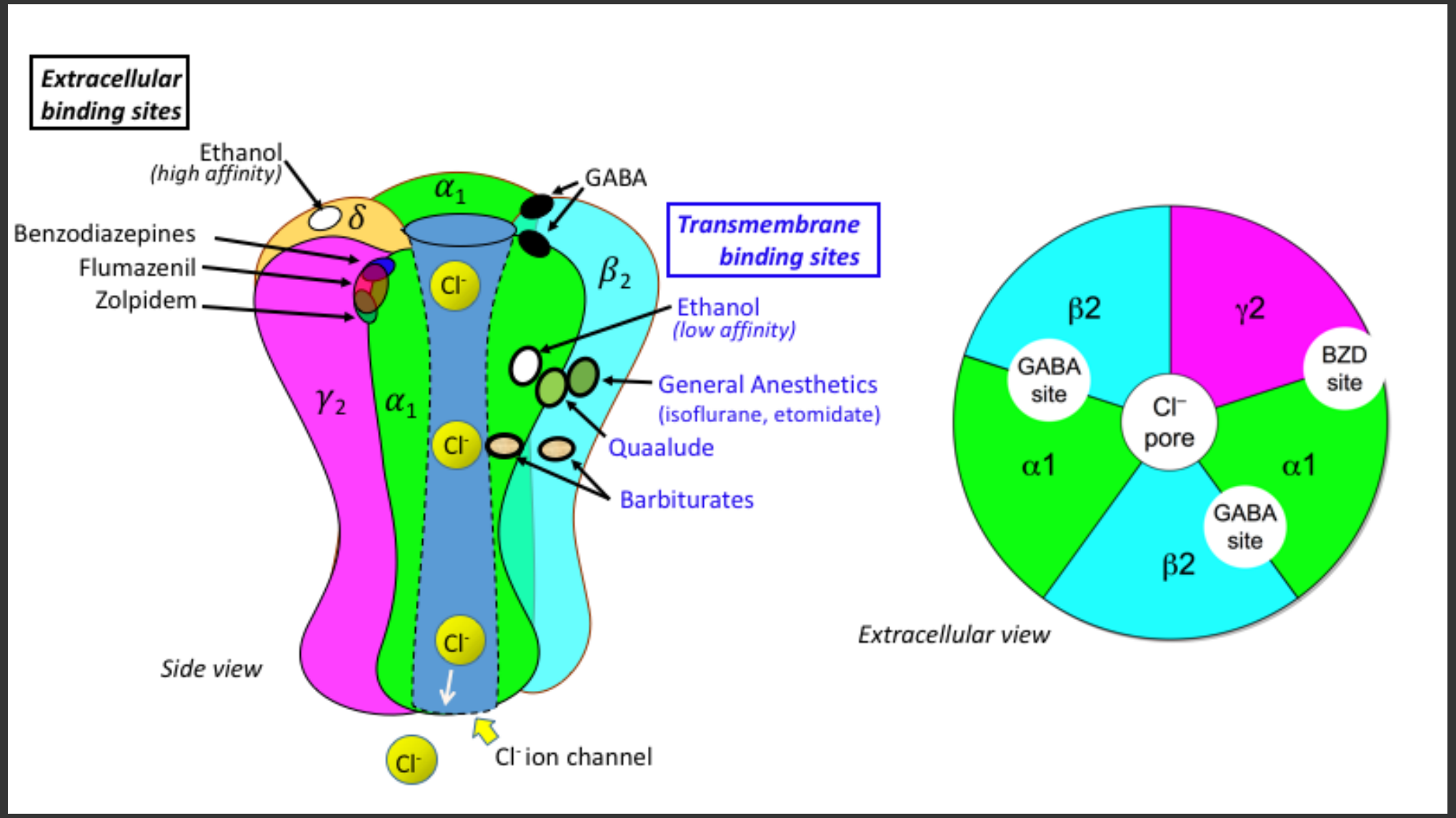
$GABA_A$ Receptor Subunits

- The GABA-A receptor has a pentameric structure assembled from subunits selected from multiple polypeptide classes (alpha, beta, gamma etc.)
- Multiple subunits of these classes have been characterized (i.e alpha 1,2,3,4,5,6, beta 1,2,3,4, and gamma 1,2,3).
- Receptors with an alpha-1 subunit are widely distributed through most brain areas and are the most abundant type. Their activation seems to induce sedative and amnestic effects
- Receptors with an alpha-2 subunit are localized mainly in the limbic system, cerebral cortex and striatum. Their activation seems to induce anxiolytic effects





$GABA_A$ Receptor Subunits



$GABA_A$ Receptor Subunits

- Subunit Names = α , β , γ , δ , ε , π , ρ

- Different Isoforms of Subunits α_{1-6} , β_{1-3} , γ_{1-3} , ρ_{1-3}
- Each Subunits contains 450-550 amino acids.
  - Subunit peptide structure is highly conserved between species

- The common elements of the subunit structure include
  - A large N-terminal hydrophilic domain exposed to the synaptic cleft (extracellular)
  - Three highly conserved hydrophobic transmembrane domains (M1, M2, M3),
    - a large, poorly conserved, hydrophilic domain of variable size and amino acid sequence, with putative phosphorylation sites, separating the M3 and M4 segments and located in the cytoplasm and a fourth transmembrane domain

$GABA_A$ Receptor Subunits

- The segment M2 of each of the subunits composing the $GABA_A$ receptor is thought to line the channel (as for the nAChR) and to contribute to ion selectivity and transport.

- A small number of amino acids within the M2 sequence is responsible for anionic versus cationic permeability.

- The M3–M4 linker is the intracellular domain that binds the cytoskeleton.
- $GABA_A$ receptors (with nAChRs) are part of the cys-loop pentameric ligand-gated ion channel superfamily.
  - their α-subunits have a characteristic cysteine-cysteine pair in the N-terminal extracellular domain and the disulfide bond between the two cysteines forms a characteristic loop.

$GABA_A$ Receptor Subunits – Binding Characteristics

- $GABA_A$ Receptor Synthesis :
  - Need only 5 subunits at a time from a pool of thousands of combinations
  - Two approaches to solve for subunit combinations:
    - a comparative study of the functional properties of receptors expressed in oocytes or in transfected mammalian cells from known combinations of cloned subunits (with the restriction that Xenopus oocyte does not automatically assemble a channel composed of all injected subunits)
    - a comparative study of the distribution of the various subunit mRNAs in the brain using the in situ hybridization technique

- "the transient expression in transfected cells of identical a- or b -subunits gives functional homomeric $GABA_A$ receptors"
  - i.e. receptors which induce a current in the presence of GABA.
  - This current is blocked by GABA A antagonists and potentiated by barbiturates
    - but is unaffected by benzodiazepines.
  - These properties can be attributed to the conserved structural features of all the subunits.

$GABA_A$ Receptor Subunits – Binding Characteristics

- The amino acids identified by site-directed mutagenesis to affect channel activation by GABA are in the b-subunit.
- For example, cysteine (C) substitution of b 2 Glu 155 alters both channel-gating properties and impairs agonist binding as it results in spontaneously opened $GABA_A$ channels.
- A model of the G AA R agonist-binding site predicts that b 2 Glu 155 interacts with the positively charged moiety of GABA.
- In the a1 -subunit, the mutation of phenylalanine (F) 64 to leucine (L) also impairs activation of the GABA channel indicating a role for this a-subunit residue in GABA binding.
- Therefore, identified domains of the b -subunit and of the neighboring a-subunit contribute to the GABA binding site.
- It is thought that most abg receptors are p tameric with a stoichiometry 2a, 2 b and 1 g , which is consistent with data indicating that GABA sites are located at the interface between a- and b -subunits and that there are two GABA sites per receptor
- GABA receptors have a relatively low affinity for GABA, of the order of 10–20 m M.

Evidence for the Binding of Two GABA Molecules

- Analysis of dose–response curves suggests the binding of two GABA molecules prior to opening of the channel

- The response studied, the peak amplitude of the total current I_{GABA} evoked by GABA in whole-cell patch clamp recording, is proportional to the square of the dose of GABA (but only at low doses of GABA)

$$I_{GABA} = f[GABA]^2$$

Evidence for the Binding of Two GABA Molecules

- At very low doses of GABA, when receptor desensitization is negligible, it seems that upon binding of two GABA molecules to the receptor, the conformational change of the receptor channel to an open state is favored. These observations can be accounted for by the following model :

$$2\,\mathrm{G} + \mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{G} + \mathrm{GR} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{G_2R} \underset{k_{\alpha}}{\overset{k_{\beta}}{\rightleftharpoons}} \mathrm{G_2R^*}$$

- where G is GABA; R is the $GABA_A$ receptor in the closed configuration; GR or G2 R is the mono- or doublyliganded $GABA_A$ receptor in the closed configuration; and G2 R* is the doubly-liganded $GABA_A$ receptor in the open configuration.

The Reversal Potential of the GABA Current Varies with the Chloride Equilibrium Potential (E_{Cl})

- The ionic selectivity of the channel is studied in outside-out patch clamp recordings from cultured spinal neurons.
- This patch clamp configuration allows control of the membrane potential as well as the composition of the intracellular fluid.
- When the intracellular and extracellular solutions contain the same Cl – concentration (145 mM), the unitary current evoked by GABA reverses at E rev= 0 mV.
- If part of the intracellular Cl – is replaced with nonpermeant anions such as isethionate (HO-CH2 -CH2 -SO3), for a 10-fold change in intracellular Cl – concentration a shift in the reversal potential of approximately 56 mV is observed
- This value approaches very closely that of 58 mV predicted by the Nernst equation for E Cl at 20°C
- Changes in extracellular Na +or K + concentration have very little effect on the reversal potential of the $GABA_A$ response.
- These results demonstrate that the $GABA_A$ channel is selectively permeable to Cl– .

In Physiological Extracellular and Intracellular Solutions, the GABAA Current Recorded in Isolated Spinal Neurons Reverses at -60 mV

- Using the technique of patch clamp recording one can record the unitary currents (iGABA) across the GABA A channel (spinal neurons in culture, cell-attached configuration).
- The GABA present in the solution inside the recording pipette (5 m M) evokes outward single channel currents at −30, 0 and +20 mV
- The magnitude of the single-channel current increases with depolarization suggesting that the reversal potential for the GABA A response is negative to −30 mV.
- The i/V curve, obtained by plotting the unitary current i GABA against the membrane potential V, shows in this experiment a reversal potential of the GABA-induced current around −60 mV
  - Thus, at a potential close to the resting membrane potential (−60 mV), the current evoked by GABA is not detectable.
- At potentials more depolarized than rest (e.g. −30 mV) (Figure 9.5b), an outward current is recorded whose magnitude increases with depolarization of the postsynaptic membrane.
- As E_{Cl} is close to the resting membrane potential (−60 mV) in physiological intracellular and extracellular solutions, the electrochemical gradient for the Cl− ions ($V - E_{Cl}$) for $V = E_{Cl} = -60$ mV is close to 0 mV.
- The net flux of Cl −ions at a potential close to rest is therefore null or very small:
  - no current is recorded even though the GABAA channels are open.
- On the other hand, as the membrane potential depolarizes, the net flux of Cl −ions becomes inward.
- An inward net flux of negative charges corresponds to an outward current.
- At potentials more depolarized than Vrest , an outward current is recorded
- At potentials more hyperpolarized than −60 mV, iGABA is inward (the net flux of Cl − ions is outward) but of very small amplitude.

Figure 9.3

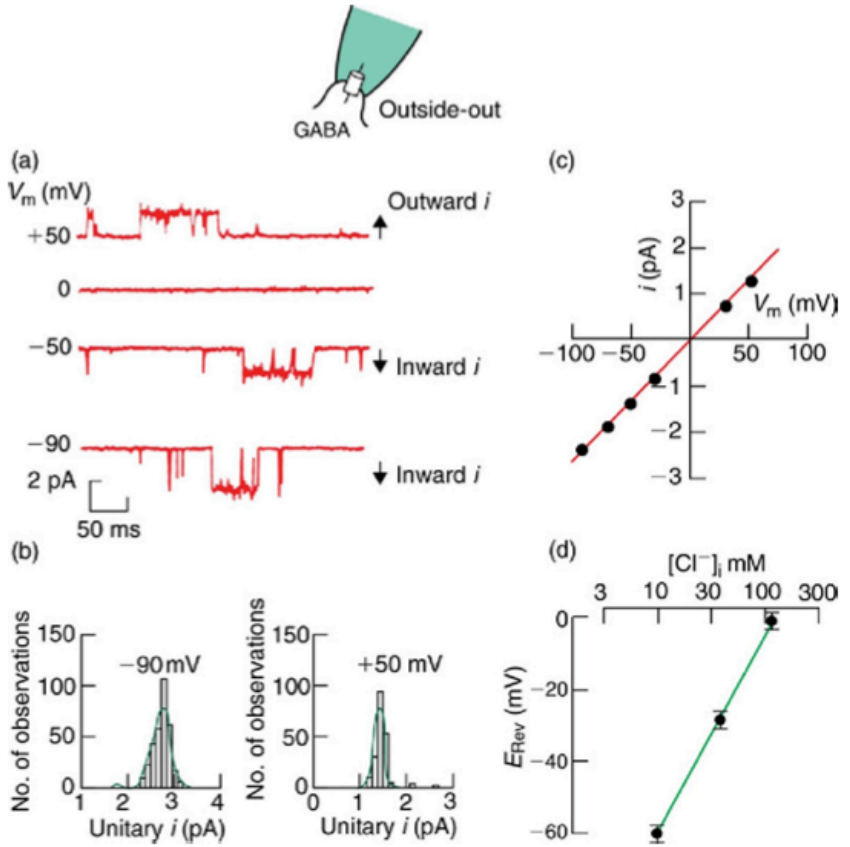


FIGURE 9.3 Variations of the reversal potential of the $GABA_A$ response as a function of the Cl^- equilibrium potential. The single-channel current *i* flowing across the $GABA_A$ channel is recorded in cultured mouse spinal neurons (outside-out patch clamp recording; equal concentrations of Cl^- on both sides of the patch: 145 mM). **(a)** In the presence of GABA (10 mM), the single-channel current *i* is outward at $V_m = +50$ mV (upward deflection), null at $V_m = 0$ mV and inward at $V_m = -50$ mV or -90 mV (downward deflections). **(b)** The distribution of single channel currents *i* in different patches of membrane held at $V_m = -90$ mV (left) and +50 mV (right) shows the existence of a single peak of current of -2.70 ± 0.17 pA and 1.48 ± 0.10 pA, respectively. These two values give a single channel conductance γ equal to 30 pS ($\gamma = i/V_m$ as $E_{rev} = 0$ mV). **(c)** i/V curve obtained by averaging the most frequently observed single-channel currents. It is a straight line according to the equation $i = \gamma(V_m - E_{rev})$. The relationship is linear between $V_m = -90$ mV and +50 mV and the slope is $\gamma = 30$ pS. **(d)** Reversal potential of the $GABA_A$ response (in mV) as a function of the intracellular Cl^- concentration $[Cl^-]_i$ (in mM). Each point represents the mean value of E_{rev} from four different cells. Note that, at the three $[Cl^-]_i$ tested, E_{rev} (experimental value) is very close to E_{Cl} (calculated by the Nernst equation):

$[Cl^-]_i$ (mM)	E_{Cl} (mV)	E_{rev} (mV)
14.5	–58	–56
45	–29	–28
145	0	0

Figure 9.4

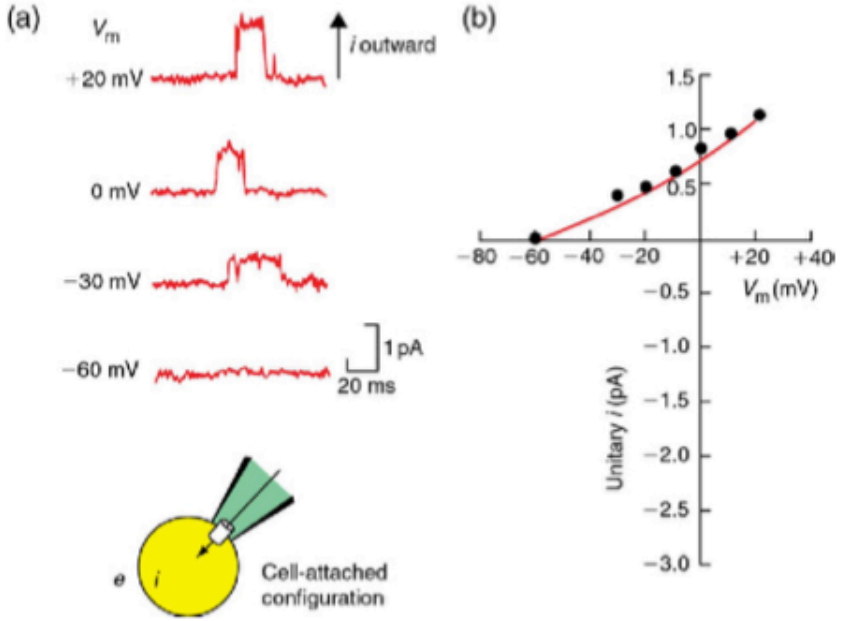


FIGURE 9.4 Activity of a single $GABA_A$ receptor channel in physiological solutions. The single-channel $GABA_A$ current recorded in cell-attached configuration in rat spinal neurons. **(a)** At $V_m = -30$, 0 and +20 mV respectively, the GABA present in the patch pipette at a concentration of 10 μM elicits an outward current (upward deflection). This current increases with depolarization of the patch. At $V_m = -60$ mV, no current is recorded. **(b)** i/V curve obtained by plotting the amplitude of the recorded unitary current i (pA) against the membrane potential V_m (mV). The intracellular medium is the physiological cytosol and the extracellular or intrapipette solution contains 144.6 mM Cl^-. The intracellular Cl^- concentration is estimated at 13 mM, which gives a value of −60 mV for the Cl^- reversal potential: $E_{Cl} = -58 \log(144.6/13) = -60$ mV. As all Na^+ ions are replaced by K^+ ions in the extracellular solution, the K^+ concentration is similar in both solutions, which gives a reversal potential for the K^+ current near 0 mV. The membrane potential values indicated in (**a**) and (**b**) are evaluated on the basis that the value 0 mV is the potential at which the K^+ currents across the K^+ channels are zero. From Sakmann B, Bormann J, Hamill OP (1983) Ion transport by single receptor channels, *Cold Spring Harbor Symposia in Quantitative Biology* **XLVIII**, 247–257, with permission.

The single-channel conductance of $GABA_A$ channels is constant in symmetrical Cl – solutions, but varies as a function of potential in asymmetrical solutions

- In physiological conditions, Cl– concentration is approximately 10-fold lower in the intracellular than in the extracellular fluid, the unitary conductance γ_{GABA} varies with the membrane potential.

- The conductance in fact decreases progressively as the outward Cl– current decreases.
  - This phenomenon is called rectification.
    - This rectification (non-symmetrical inward and outward currents) results from the difference in Cl– concentration on either side of the membrane.

Mean Open Time of the $GABA_A$ Channel

- Brief openings (triangles) :
  - mean duration of around 2.5 ms
  - contribute little to the total current

- Burst of Openings (open circles) :
  - A burst is defined as a sequence of openings each one having a duration t_o , separated by brief closures of duration t_c
  - Brief durations are defined as less than 5 ms

Figure 9.6

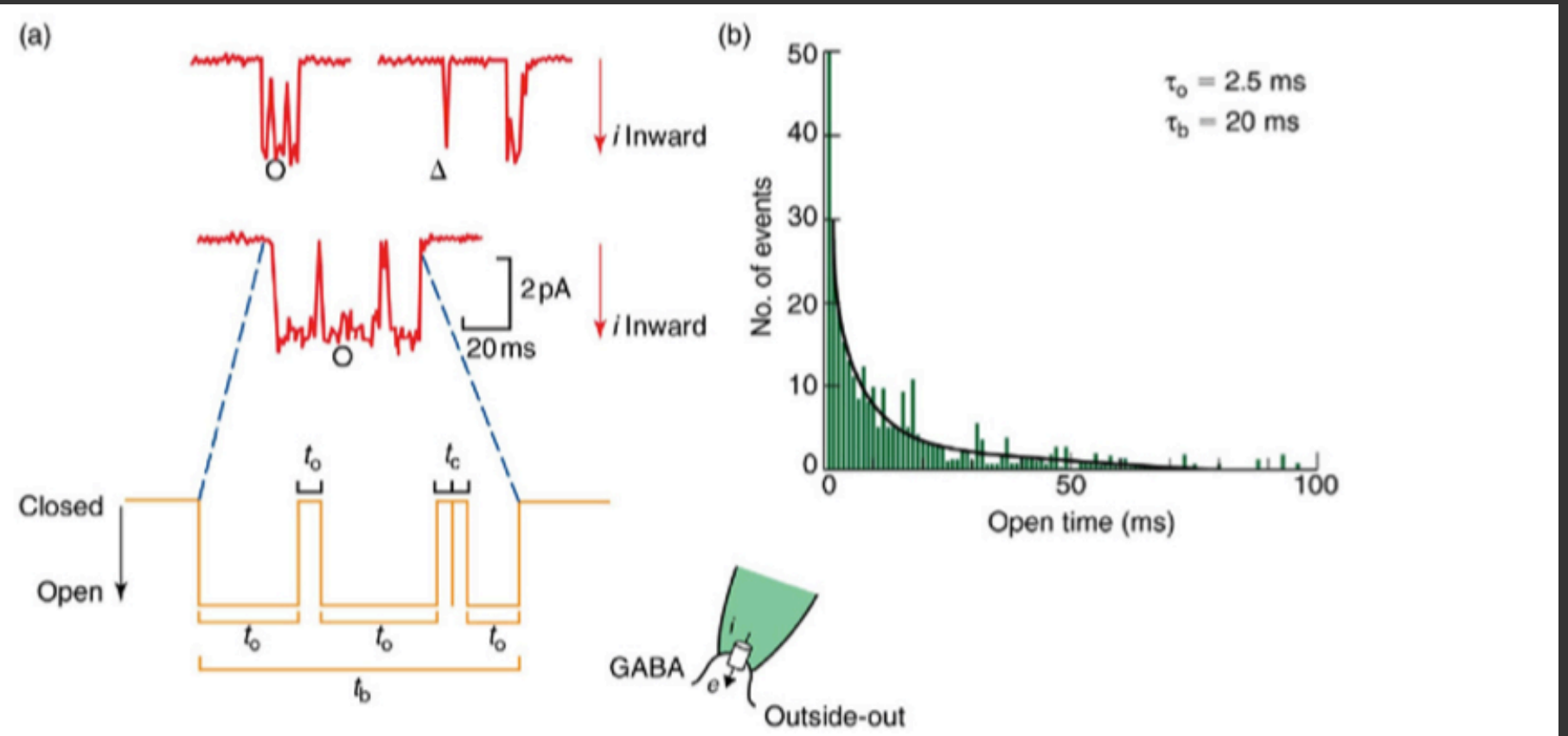
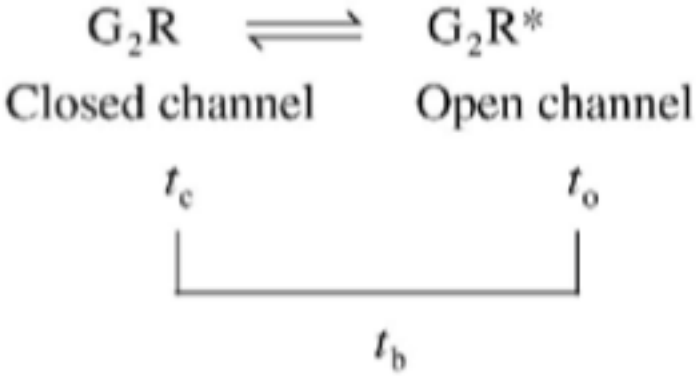


FIGURE 9.6 **Mean open time of $GABA_A$ channels.** Patch-clamp recording of the activity of the $GABA_A$ receptor channels from chromaffin cells of the adrenal medulla (outside-out configuration). The intracellular and extracellular Cl^- concentrations are similar and the membrane potential is maintained at −70 mV. **(a)** Inward unitary currents through a single $GABA_A$ channel evoked by GABA (10 μM). Brief openings (triangle) and bursts of openings (O, long duration openings interrupted by short closures defined in this experiment as less than 5 ms). **(b)** Histogram of open times measured in a homogeneous population of channels (mean value of i = −2.9 pA). The open times plotted on the graph represent the duration of short openings (t_o) and the duration of bursts of openings (t_b). The histogram is described by the sum of two exponentials with decay time constants of τ_o = 2.5 ms and τ_b = 20 ms. τ_o corresponds to the mean open time of short openings and τ_b to the mean open time of bursts of openings. From Borman J, Clapham DE (1985) γ-aminobutyric acid receptor channels in adrenal chromaffin cells: a patch clamp study, *Proc. Natl Acad. Sci. USA* **82**, 2168–2172, with permission.

Figure 9.6b

(**Figure 9.6b**). The openings and the brief closures observed within each burst in the presence of GABA are thought to correspond to fluctuations of the receptor between the double-liganded open state and the double-liganded closed state (before the two molecules of GABA leave the receptor site). Thus, upon a single activation by two molecules of GABA, the double-liganded receptor would open and close several times:



Silent Periods

- Silent periods separate single openings or bursts
- They are periods during which the channel is closed and the unitary current is zero
- In the presence of very low concentrations of GABA (when the receptor has a low probability to desensitize), they correspond to the G_2R, GR and R states of the $GABA_A$ receptor.
- Recordings in outside-out configuration show R and R* states of the $GABA_A$ receptor.
- Opening characteristics of the GABA receptor are very similar to those of the nicotinic receptor
  - However, the short openings observed within bursts are approximately twice as abundant in the case of the $GABA_A$ receptor.
    - This is explained by the fact that opening (b) and closing (a) rate constants have much closer values in the case of the $GABA_A$ receptor than in the case of the nicotinic receptor

The $GABA_A$ Receptor Desensitizes

- Recordings in outside-out configuration show a rundown of the frequency of opening of the $GABA_A$ channels upon prolonged application of GABA
  - Whereas neither the intensity of the unitary current nor the mean open time of the channels τ_0 appears to be affected

Figure 9.7

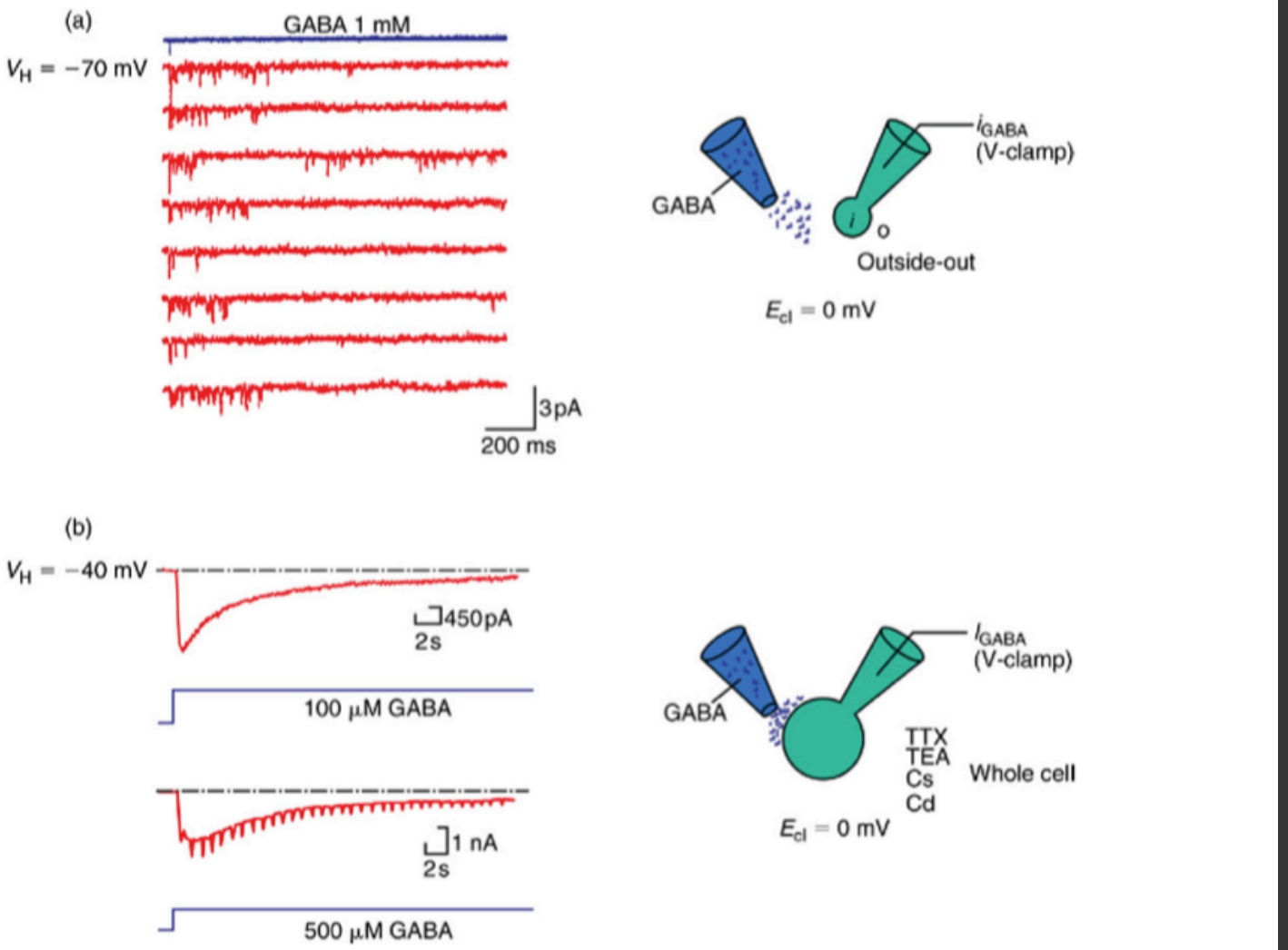


FIGURE 9.7 **Desensitization of the $GABA_A$ receptor. (a)** Outside-out patch excised from a cell transfected with $\alpha_1\beta_2\gamma_2$ cDNAs. A 2 ms pulse of GABA (1 mM) evokes single $GABA_A$ channel activity. **(b**, top**)** Patch clamp recording (whole-cell configuration, $V_H = -40$ mV) from a chick cerebral neuron. A prolonged application of GABA at high concentration (100 μM) evokes a total current I_{GABA} which decreases with time to almost zero. The total current I_{GABA} corresponds to the sum of the unitary currents i_{GABA}, passing through the open $GABA_A$ channels, while the other currents have been blocked with TTX and TEA as well as with Cs^+ and Cd^{2+} ions. **(b**, bottom**)** The same experiment in the presence of 500 μM of GABA and with hyperpolarizing voltage steps applied at a constant rate. The decrease in amplitude of the step current during the $GABA_A$ response shows that the decrease of I_{GABA} is associated with a decrease in G_m (as $i_{step} = Gm\ V_{step}$, V_{step} being constant), a decrease of i_{step} implies a decrease of G_m. There are symmetrical Cl^- concentrations in (**a**) and (**b**). Part (**a**) from Zhu WJ, Wang JF, Corsi L, Vicini S (1998) Lanthanum-mediated modification of $GABA_A$ receptor deactivation, desensitization and inhibitory synaptic currents in rat cerebellar neurons, *J. Physiol. (Lond.)* **511**, 647–661, with permission. Part (**b**) from Weiss DS, Barnes EM, Hablitz JJ (1988) Whole-cell and single-channel recordings of GABA-gated currents in cultured chick cerebral neurons, *J. Neurophysiol.* **59**, 495–513, with permission.

Pharmacology of the $GABA_A$ Receptor

- Benzodiazepines, barbiturates and neurosteroids enhance $GABA_A$ receptor current
- Whereas bicuculline, picrotoxin and β-carbolines reduce $GABA_A$ current, by binding to specific sites on the $GABA_A$ receptor channels.

Bicuculline and Picrotoxin

- Bicuculline and Picrotoxin reversibly decrease total $GABA_A$ current
  - They acer respectively competitive and non-competitive antagonists of the $GABA_A$ receptor

- Prior to GABA application, occasional brief spontaneous currents are recorded
- Following GABA application, bursting inward chloride currents are evoked
- These GABA-induced bursting currents are reversibly reduced in frequency by the concomitant application of bicuculline or picrotoxin.
- When the dose of GABA is increased and the dose of antagonist is kept constant, the inhibition by bicuculline is reduced whereas that by picrotoxin is unchanged
  - This shows that bicuculline is a competitive antagonist whereas picrotoxin is a non-competitive antagonist
- Bicuculline binds to the same receptor sites as GABA.
  - It is selective for the GABAA receptor and therefore serves as a good tool to identify GABAA -mediated responses.
- Picrotoxin in contrast binds to the ionic channel (it is a channel blocker).
  - Its binding site involves the M2 segment, the region thought to line the chloride ion channel.
  - GABA receptors abolishes antagonism by p toxin at concentrations up to 100 mM.
- The composition of residue 6 is highly conservative, implying that it is crucial for picrotoxin binding to ionophore, and most likely representing the epicenter of its binding pocket.
- Both bicuculline and picrotoxin are potent convulsants when administered intravenously or intraventricularly.

Allosteric Agonists

- Benzodiazepines, barbiturates and neurosteroids reversibly potentiate total GABAA current
  - They are allosteric agonists at the GABAA receptor
- Benzodiazepines and barbiturates are two classes of clinically active agents
- Barbiturates are hypnotic and anti-epileptic agents
- Benzodiazepines are anxiolytic agents, muscle relaxants and anticonvulsants.
- Various progesterone metabolites that are synthesized in the brain and thus called endogenous neurosteroids act directly on the GABAA receptor.

Receptor Sites

- Benzodiazepines, barbiturates and neurosteroids bind to the GABAA receptor at specific receptor sites
- First, it was shown that co-expression of a- or b -subunits with a g -subunit is required for the positive modulation of GABA-evoked Cl – currents by benzodiazepines and for photoaffinity labeling of the benzodiazepine receptor site
- It is now well established that benzodiazepines bind to the a+ g– extracellular interface

Figure 9.10

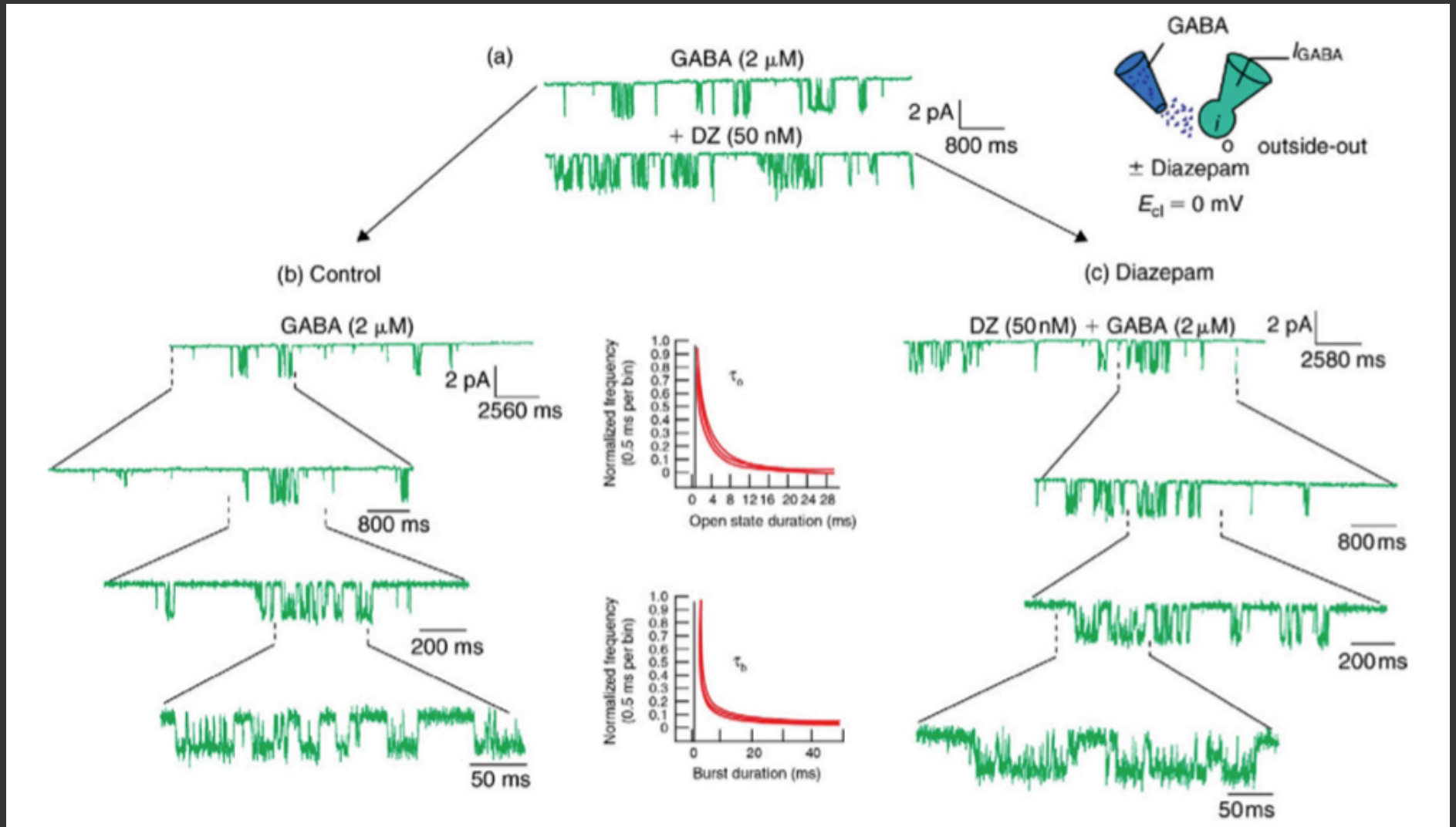


FIGURE 9.10 **$GABA_A$ single-channel current in the presence or absence of diazepam. (a)** Bursting inward currents in outside-out patches from spinal cord neurons evolved by GABA alone or GABA with diazepam (DZ). **(b,c)** The same experiment at increasing time resolution to demonstrate typical features of the unitary current. Open duration/frequency and bursts duration/frequency histograms for GABA are not significantly altered by addition of diazepam from 20 to 1000 nM (middle histograms). From Rogers CJ, Twyman RE, MacDonald RL (1994) Benzodiazepine and β-carboline regulation of single $GABA_A$ receptor channels of mouse spinal neurones in culture. *J. Physiol. (Lond.)* **475**, 69–82, with permission.

Benzodiazepines, Barbiturates and Neurosteroids Potentiate the $GABA_A$ Response

- In the presence of GABA, the benzodiazepine diazepam (DZ, 50 nM) increases the opening frequency of the channel but does not change iGABA amplitude nor the time spent by the channel in the open configuration at each opening.
- Consistent with this finding, diazepam decreases the mean closed time τ_c
  - i.e. the time spent by the channel in the closed configuration
- With decreasing or increasing doses, the effect of diazepam was less pronounced (U-shaped concentration dependency).
- Diazepam (50 nM) also increases the burst frequency without changing the mean burst duration (τ_b) nor the mean number of openings per burst.
- All the currents evoked by GABA alone or GABA with diazepam are blocked by bicuculline, thus showing that they are mediated by the GABAA receptor.
- Since the iGABA/V relationship shows that the unitary conductance is unchanged, it is hypothesized that diazepam alters the gating properties of the GABAA receptor channel
  - it increases the probability of the channel being in the open state, p_0

Benzodiazepines, Barbiturates and Neurosteroids Potentiate the $GABA_A$ Response

- The equation: $Np_0 * i_{GABA} = I_{GABA}$ tells us that when p_0 increases, I_{GABA} increases
  - Therefore, benzodiazepines should increase the amplitude of the total current I_{GABA} recorded in the whole-cell configuration
- The I/V curves show that the total currents I_{GABA} evoked in the presence or absence of benzodiazepines reverse at the same potential
- This indicates that the potentiation of I_{GABA} by these drugs is not the result of a change in the ion selectivity of the channel.
- Benzodiazepine agonists bind to a site distinct from that of GABA
  - It was first hypothesized that benzodiazepines do so by allosterically decreasing the GABA concentration needed to elicit half-maximal channel activity (EC50).
  - Another explanation is that benzodiazepines increase the adoption of a transitional receptor state, the preactivated state.
    - This state occurs after GABA binding, before p ceeding to channel activation.

Benzodiazepines, Barbiturates and Neurosteroids Potentiate the $GABA_A$ Response

- Barbiturates also increase the bicuculline-sensitive current I_{GABA} but via a different mechanism
  - they do not increase the frequency of GABAA channel openings, instead they increase the duration of single openings and bursts of native GABAA receptors or transfected $a_1\beta_1\gamma_2$ receptors.
  - An increase in the time spent in the open configuration at each opening results in an increase of the probability of the channel being in the open state and therefore in an increase of I_{GABA}
- Neurosteroids at physiological concentrations increase the total bicuculline-sensitive current I_{GABA}
  - Pregnenolone and allopregnenolone (3a-OH-DHP) in combination with GABA increases the GABAA channel activity
    - it increases the number of active channels in the patch and the channel open probability
  - On native GABAA receptors of spinal cord neurons, pregnenolone in combination with GABA also increases the duration of single and burst openings
  - Either one or both these effects on frequency of openings or opening duration result in an increase of p_0 and thus an increase of I_{GABA}
- In conclusion, benzodiazepines, barbiturates and neurosteroids can be considered as allosteric agonists of the GABAA receptor
  - They modulate the efficacy of activation of the receptor by GABA.
  - They act via distinct receptor sites on the GABAA receptor and via different mechanisms.