

The role of Glutamate and GABA in the Epileptic process

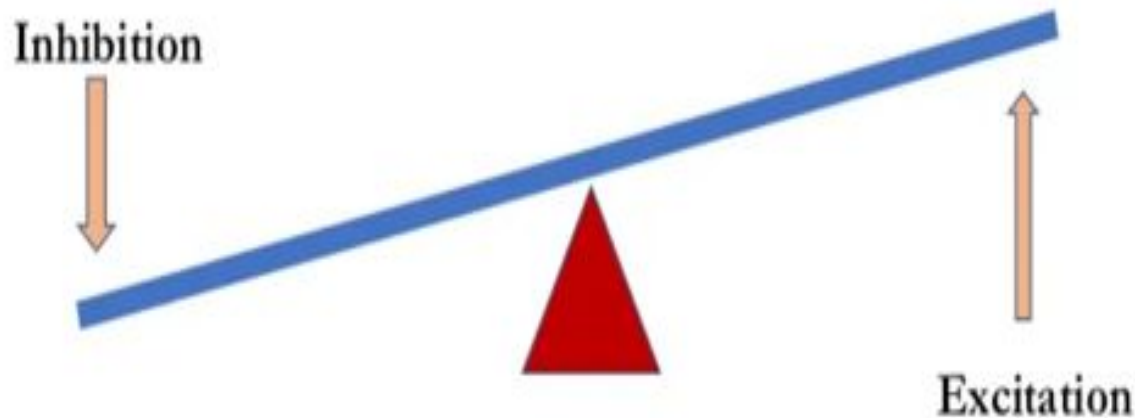
By

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Definitions

- A seizure is the clinical manifestation of an abnormal, excessive, hyper synchronous discharge of a population of **cortical neurons**.
- Epilepsy is a disorder of the CNS characterized by **recurrent seizures** unprovoked by an acute systemic or neurologic insult.
- Epileptogenesis is the sequence of events that turns a normal neuronal network into a hyper excitable network.

Mechanism of acute symptomatic/ provoked seizures



Mechanism of epilepsy

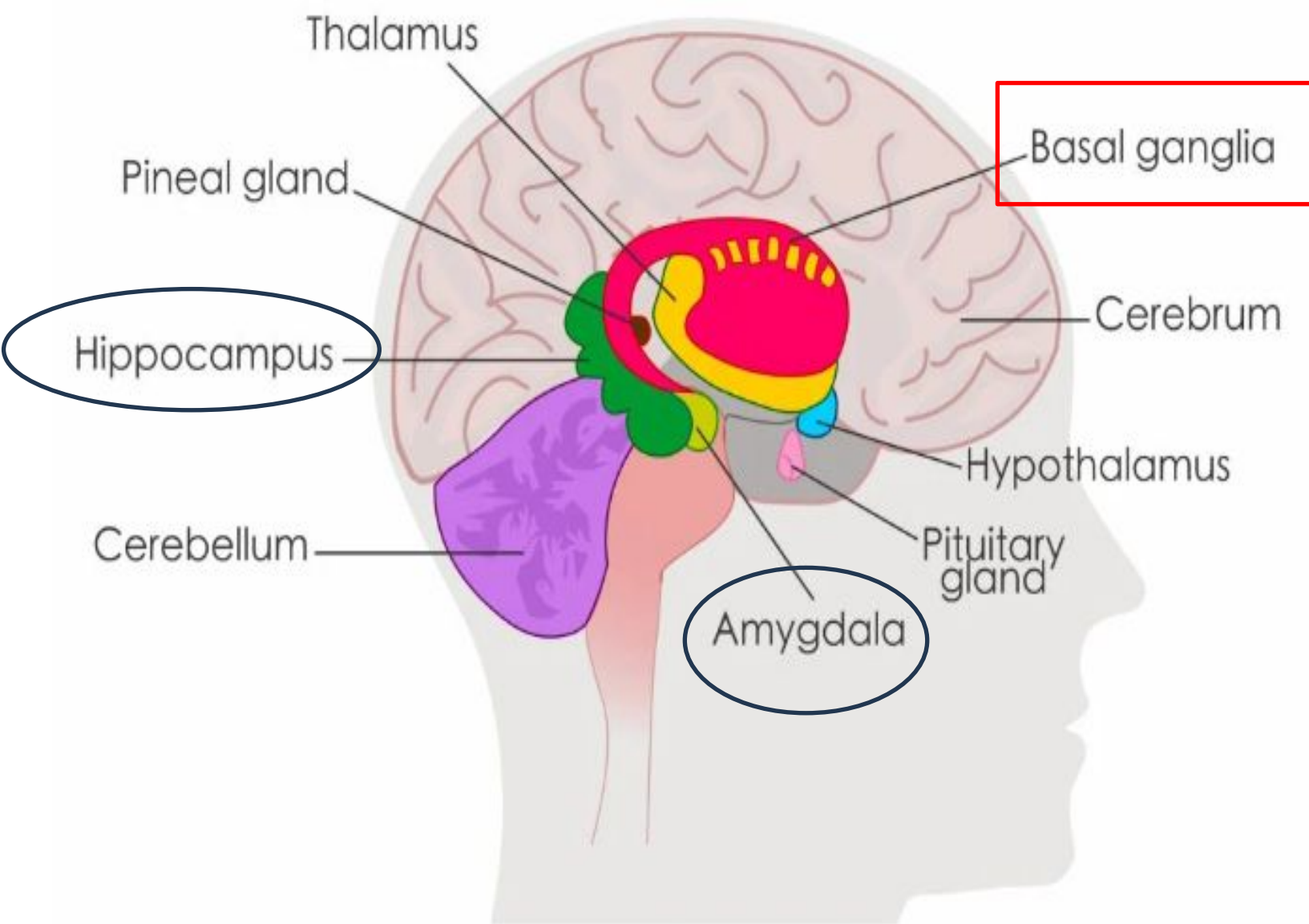
- In epilepsy there is predisposition to recurrent seizures.
- Seizures are unpredictable and relatively rare.
- Many known mutations and models do not affect excitation or inhibition.
- Mechanism of epilepsy is not just about excitation- inhibition imbalance.
- It should also explain the episodic nature of seizures and the varied etiology.

Epileptogenesis

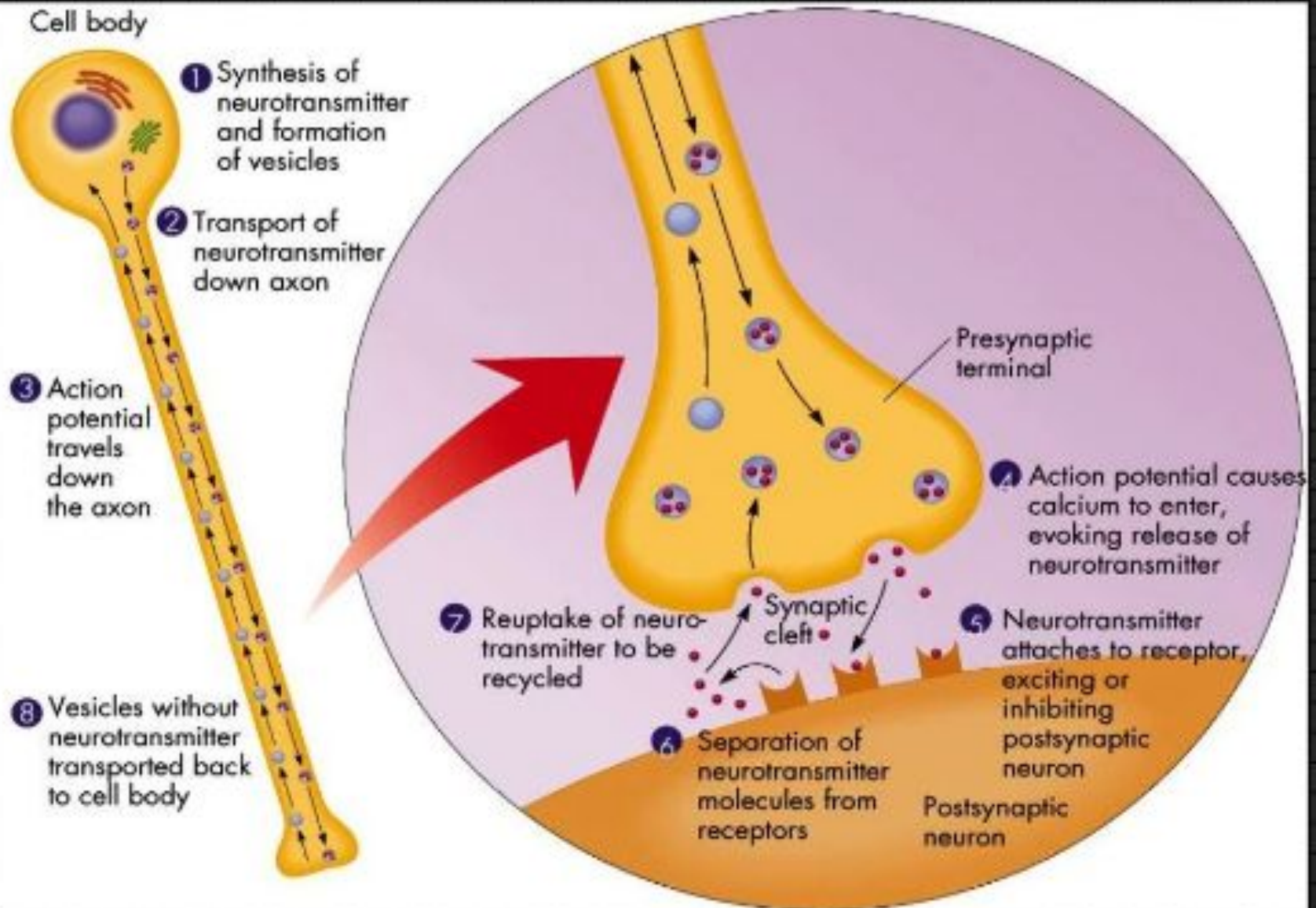
- Approximately 50% of patients who suffer a **severe head injury** will develop a seizure disorder.
- In a significant number of these patients, the seizures will not become clinically evident for months or years.
- This "silent period" after the initial injury indicates that in some cases the epileptogenic process involves a **gradual transformation of the neural network over time**.
- Changes occurring during this period could include **delayed necrosis** of inhibitory interneurons (or the excitatory interneurons driving

Epileptogenesis

- An important experimental model of Epileptogenesis is *kindling*
- Daily, **subconvulsive stimulation** (electrical or chemical) of certain brain regions such as the hippocampus or amygdala result in electrical afterdischarges, eventually leading to ***stimulation-induced*** clinical seizures, and in some instances, ***spontaneous*** seizures.
- This change in excitability is ***permanent*** and presumably involves long-lasting biochemical and/or structural changes in the CNS.
- Alterations in **glutamate** channel properties, selective **loss of neurons**, and **axonal reorganization**.

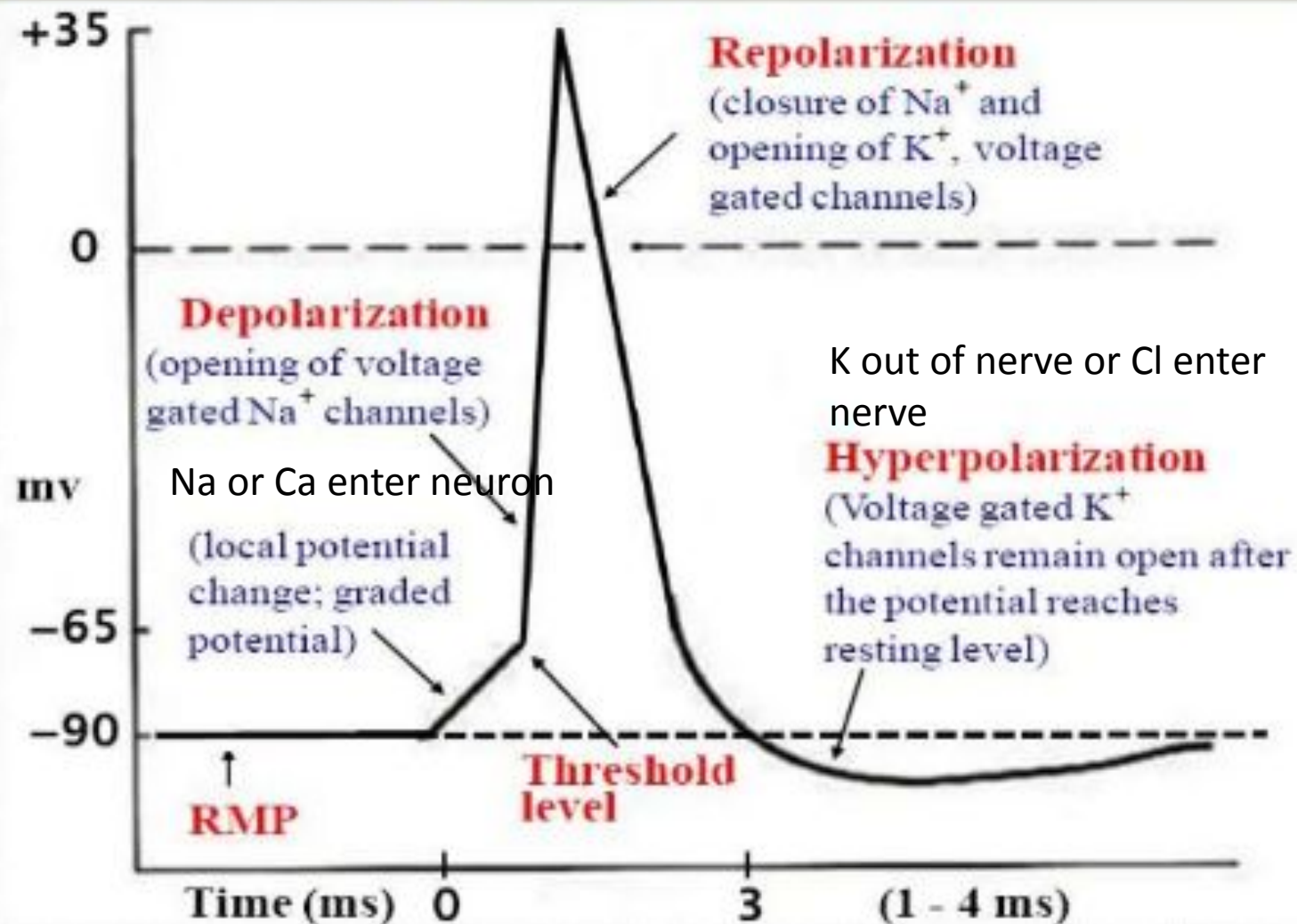


Synapse



0 The basic mechanism of neuronal excitability is the *action potential*.

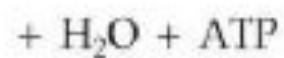
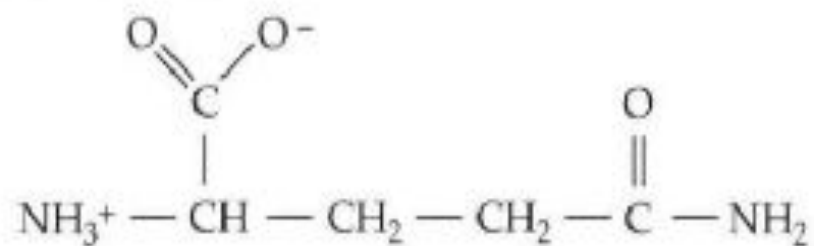
Action potential



✦ Glutamate:

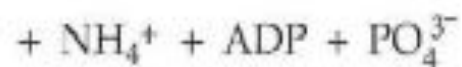
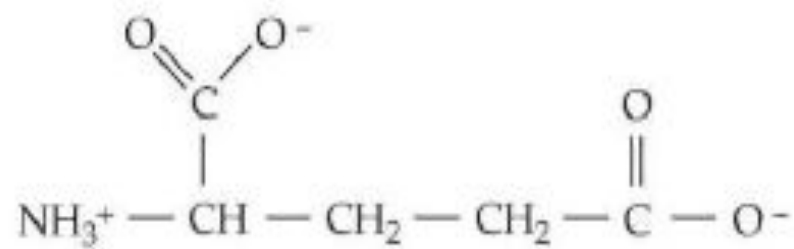
- is a powerful excitatory **neurotransmitter** that is released by nerve cells in the **brain**.
- It is responsible for sending signals between nerve cells, and under normal conditions it plays an important role in learning and memory.

Glutamine

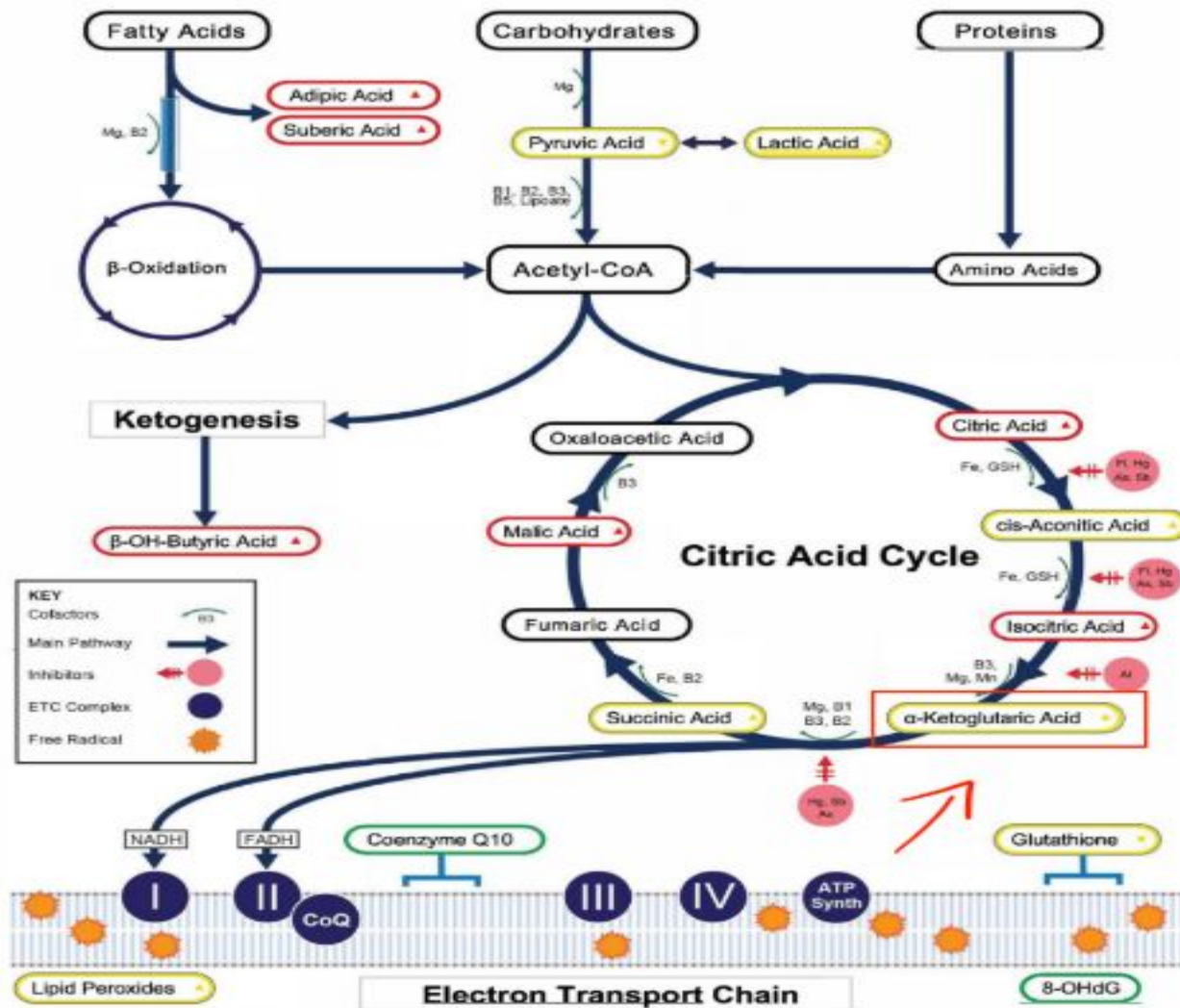


Glutaminase

Glutamate



- Glutamate synthesis:
- Glutamate is formally classified as a non-essential amino acid, because it can be synthesized (in sufficient quantities for health) from alpha-Ketoglutaric acid, which is produced as part of the citric acid cycle.



- **Glutamate transport:**
- Glutamate cannot cross the blood-brain barrier unassisted, but it is actively transported out of the nervous system by a high affinity transport system, which maintains its concentration in brain fluids at a fairly constant level.
- glutamate EAAT(excitatory amino acid transporter) and VGLUT (vesicular glutamate transporter). are found **in neuron**

GLUTAMATE SYNTHESIS

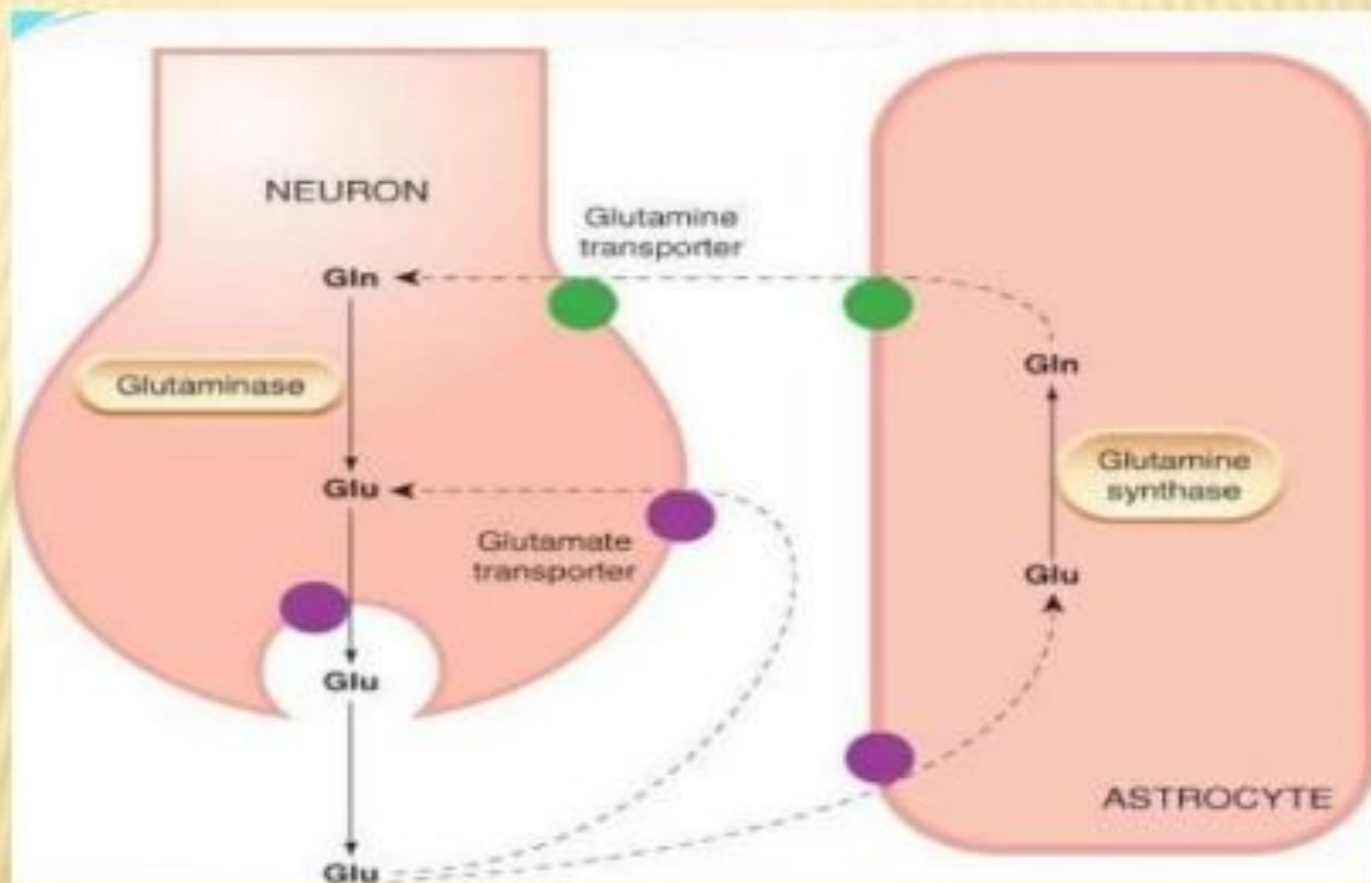
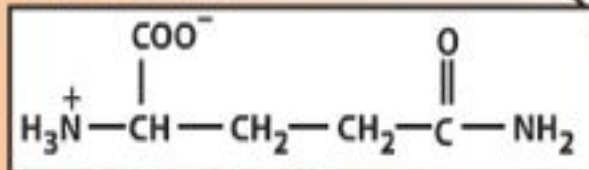


FIG. SOURCE- Elsevier Ltd. Rang et.al. Pharmacology.

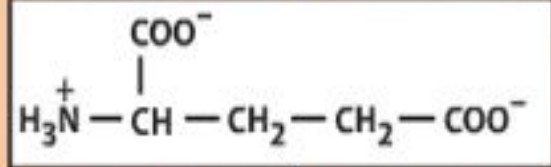
Presynaptic terminal

Glutamine



Glutaminase

Glutamate



VGLUT

Glutamate

EAAT

Glutamine synthetase

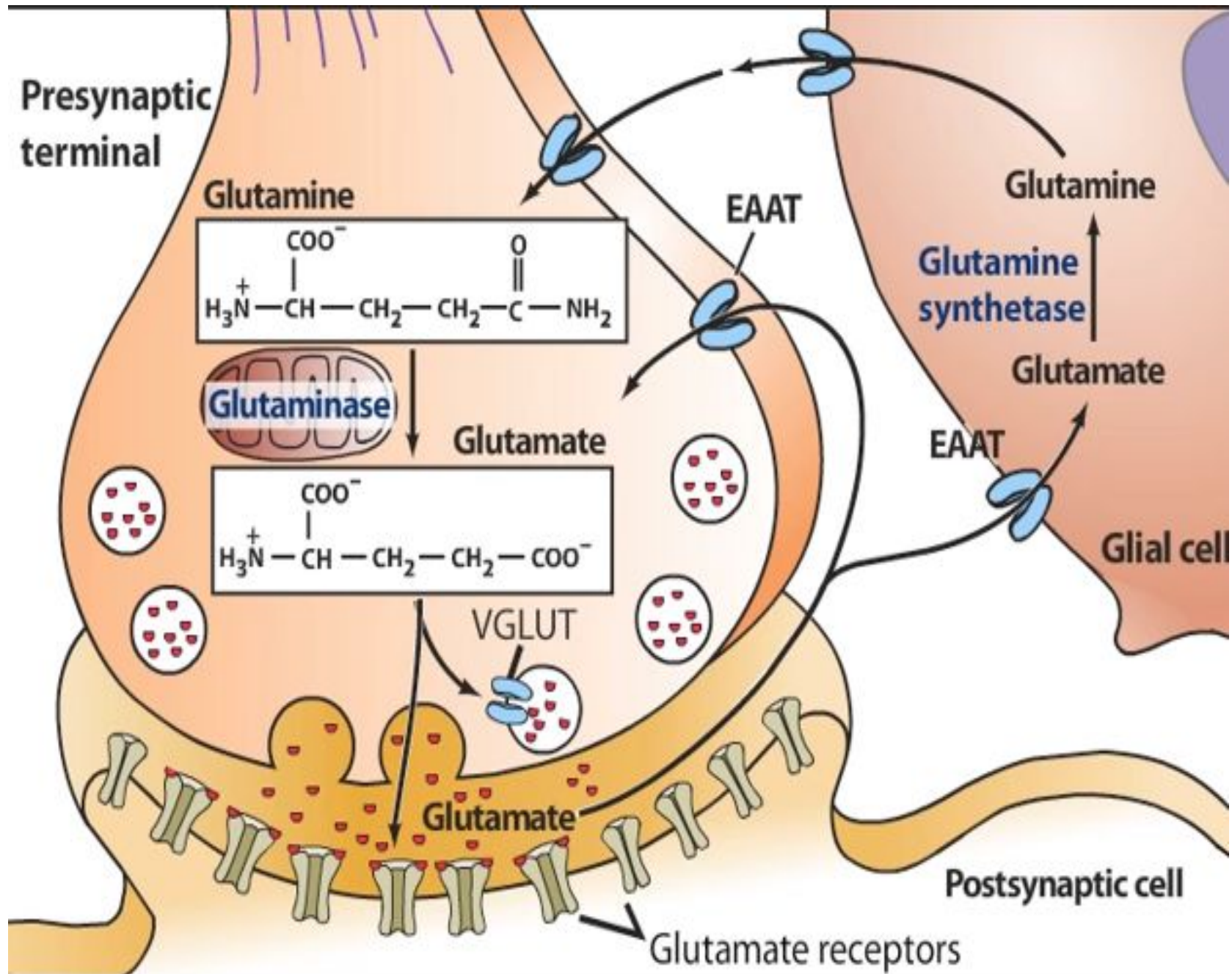
Glutamate

EAAT

Glial cell

Postsynaptic cell

Glutamate receptors





- Glutamate is widely distributed excitatory neurotransmitter in the CNS, where its concentration is much higher than in other tissues.

- Synthesized within the brain from:

- ✓ Glucose (via Kreb's cycle/ α -ketoglutarate)
- ✓ Glutamine (from glial cells)

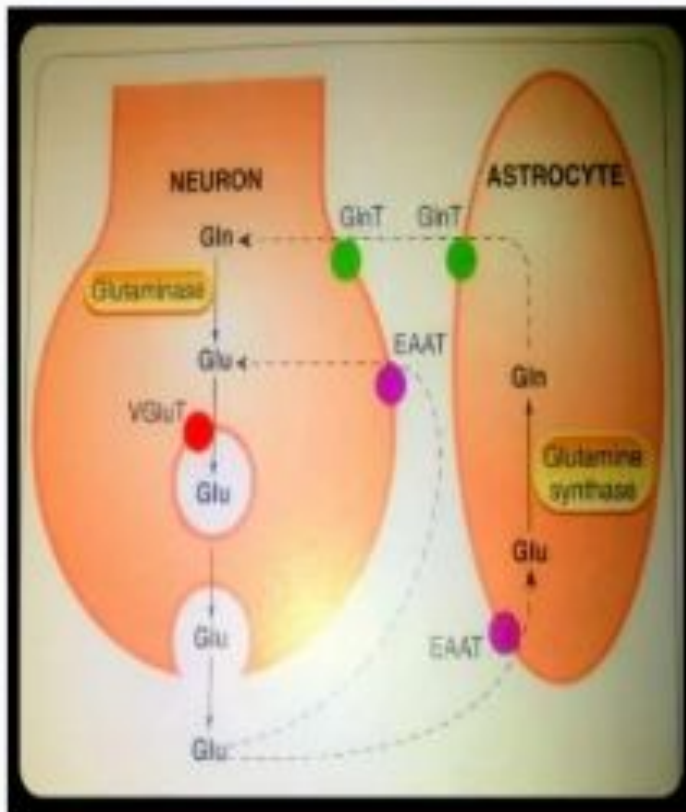
- Glutamate is a precursor of GABA which is inhibitory neurotransmitter in the CNS.

- They are produced in the mitochondria, transported into the cytoplasm, and packaged into synaptic vesicles.

GLUTAMATE RECEPTORS

- ✗ Glutamate receptors are synaptic receptors located primarily on the membrane of neuronal cells.
- ✗ Glutamate (glutamic acid) is abundant in the human body, but particularly in the nervous system & prominent in the human brain & body's most prominent neurotransmitter, the brain's main excitatory neurotransmitter.
- ✗ It is also precursor for GABA, the brain's main inhibitory neurotransmitter.

- It acts through both ligand gated ion channel (ionotropic receptor) and G-protein coupled receptor (Metabotropic receptor)



Released Glutamate is captured partly by neurons and partly by astrocytes, which convert most of it into the glutamine.

- **EAAT** : Excitatory amino acid transporter

- **GlnT** : Glutamine transporter

- **VGLUT**: Vesicular glutamate transporter
uploading glutamate into synaptic vesicles

Transport of Glutamate (Glu) and Glutamine (Gln) by neurons and astrocytes

GLUTAMATE RECEPTORS

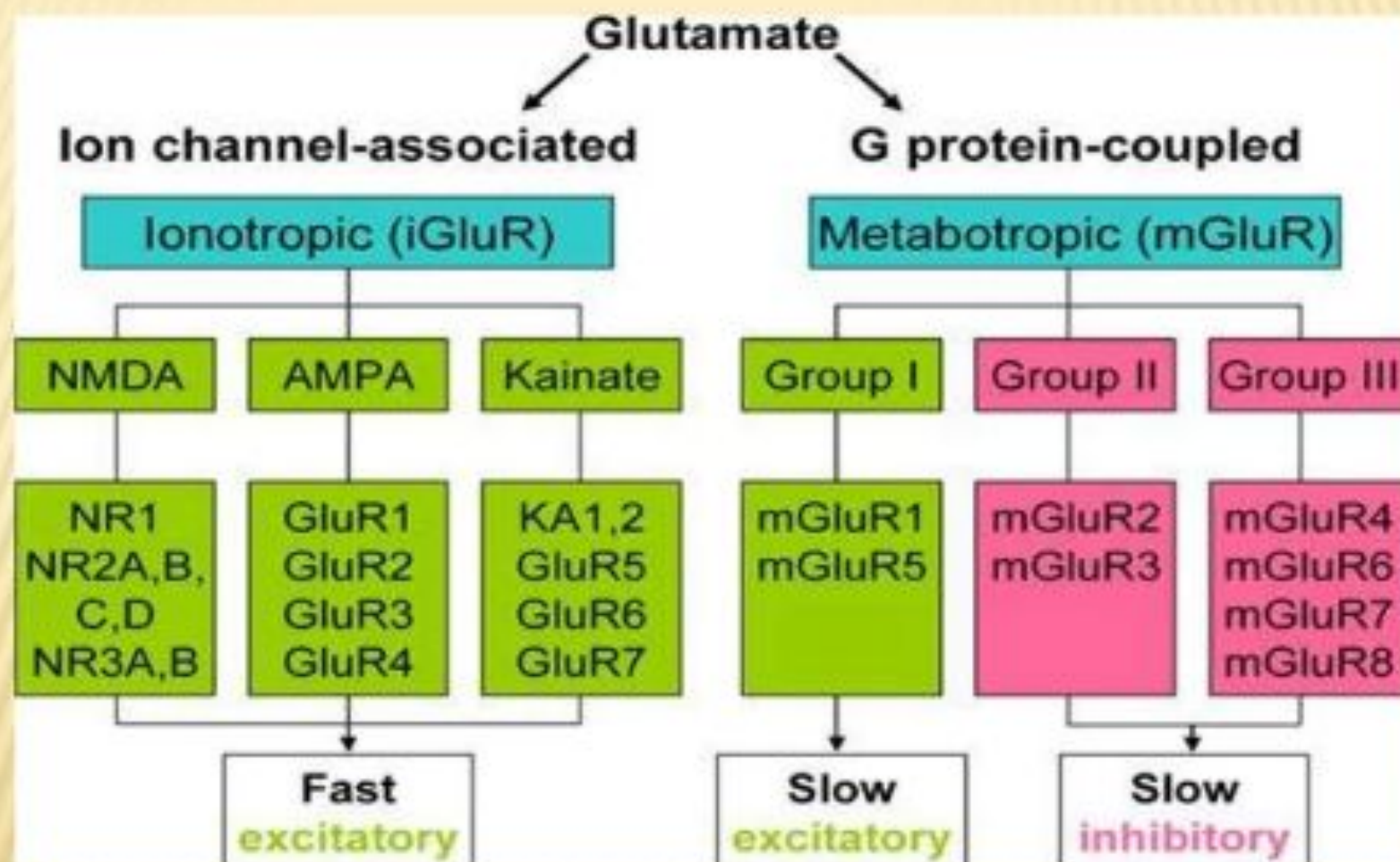


FIG SOURCE:- www.frontiersin.org *Classification of glutamate receptors.*



Three main subtypes of Ionotropic Glutamate Receptor:

1. **NMDA Receptor** (N-methyl-D-aspartate)
2. **AMPA Receptor** (amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)
3. **Kainate Receptor**

(named according to their specific agonists.)

Binding study shows that ionotropic glutamate receptors are most abundant in **cortex**, **basal ganglia** and **sensory pathways**. NMDA and AMPA receptors generally co-localised, but kainate receptors have a much more restricted distribution.

Ionotropic Glutamate Receptor

Ionotropic Glutamate receptors are **ligand gated** type of ion channels and get activated when ligand gets bind to the receptor.

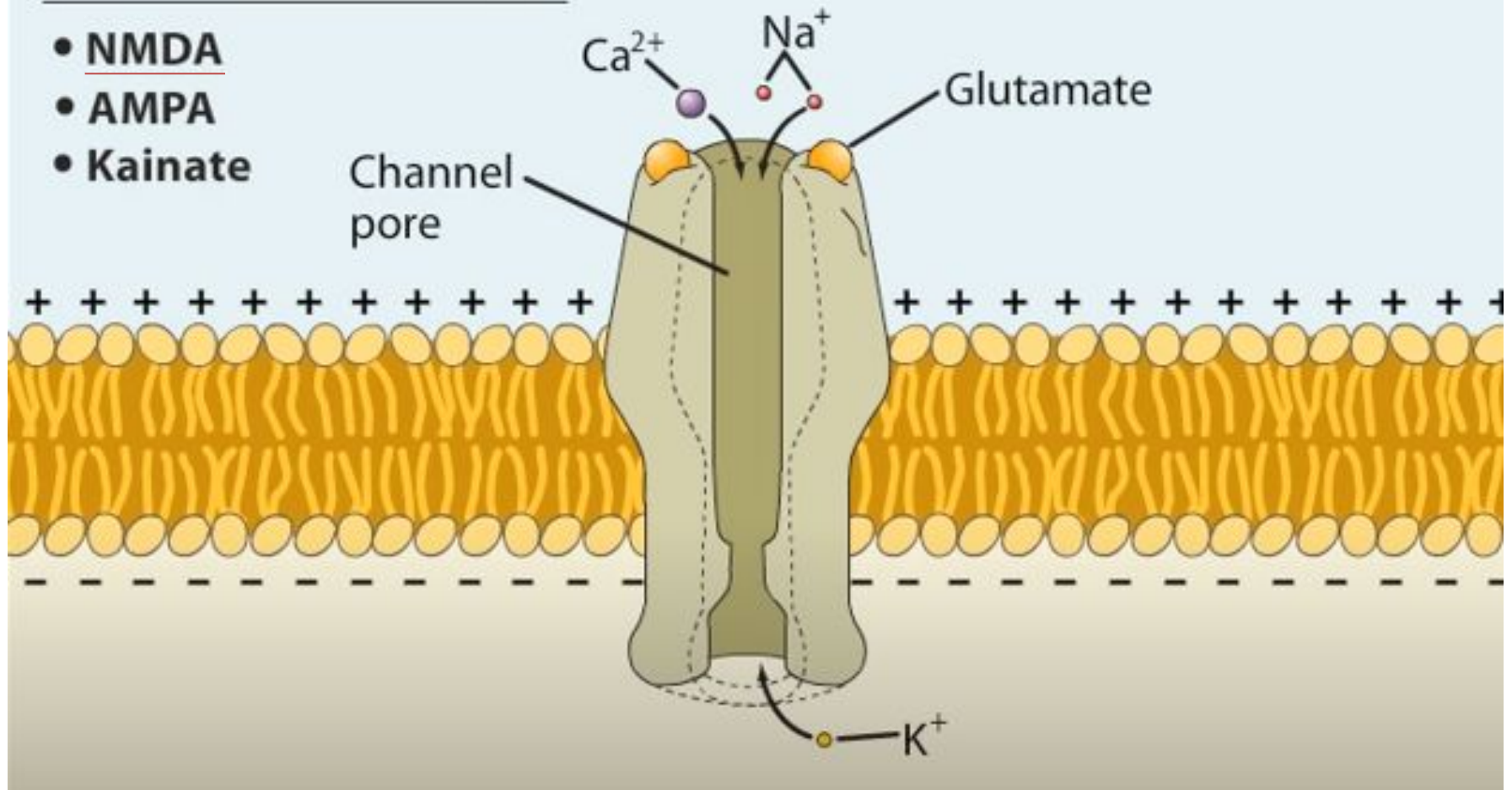
All of the ionotropic glutamate receptors are nonselective cation channels, allowing the passage of Na^+ and K^+ , and in some cases small amounts of Ca^{2+}

Upon binding, the agonist will stimulate direct action of the central pore of the receptor, an ion channel, allowing ion flow and causing excitatory postsynaptic current (EPSC). This current is depolarizing and, if enough glutamate receptors are activated, may trigger an action potential in the postsynaptic neuron.

All produce excitatory postsynaptic current, but the speed and duration of the current is different for each type.

GLUTAMATE RECEPTORS

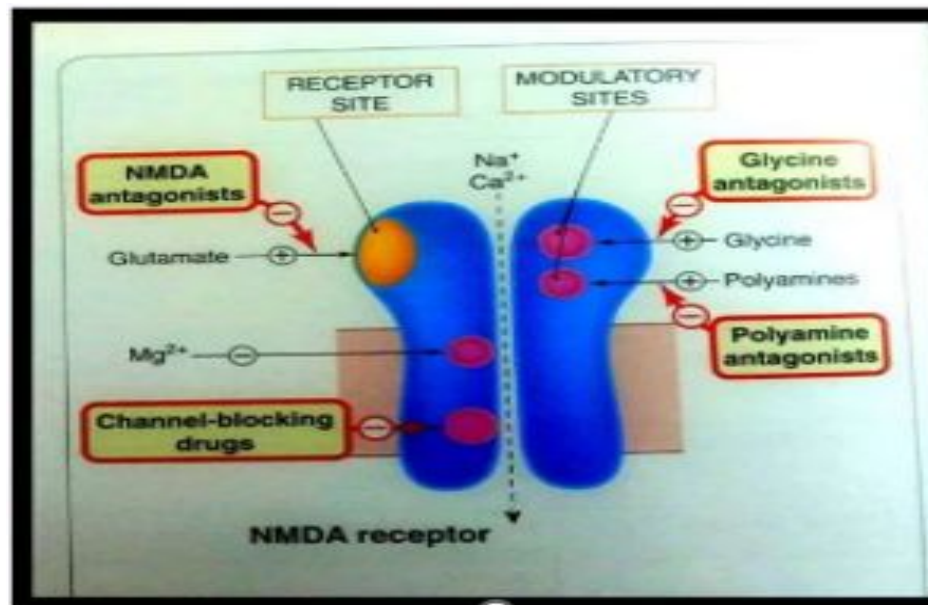
- NMDA
- AMPA
- Kainate



NMDA Receptor

It is both voltage gated and ligand gated, (it requires co-activation by 2 ligands glutamate and glycine).

- The activation of NMDA receptor requires binding of a ligand (glutamate) to the GluN2 subunits, membrane depolarization to remove the Mg^{2+} block of the channel and prevent excessive activation
- and Binding of an essential co-agonist, glycine to the GluN1 subunits.

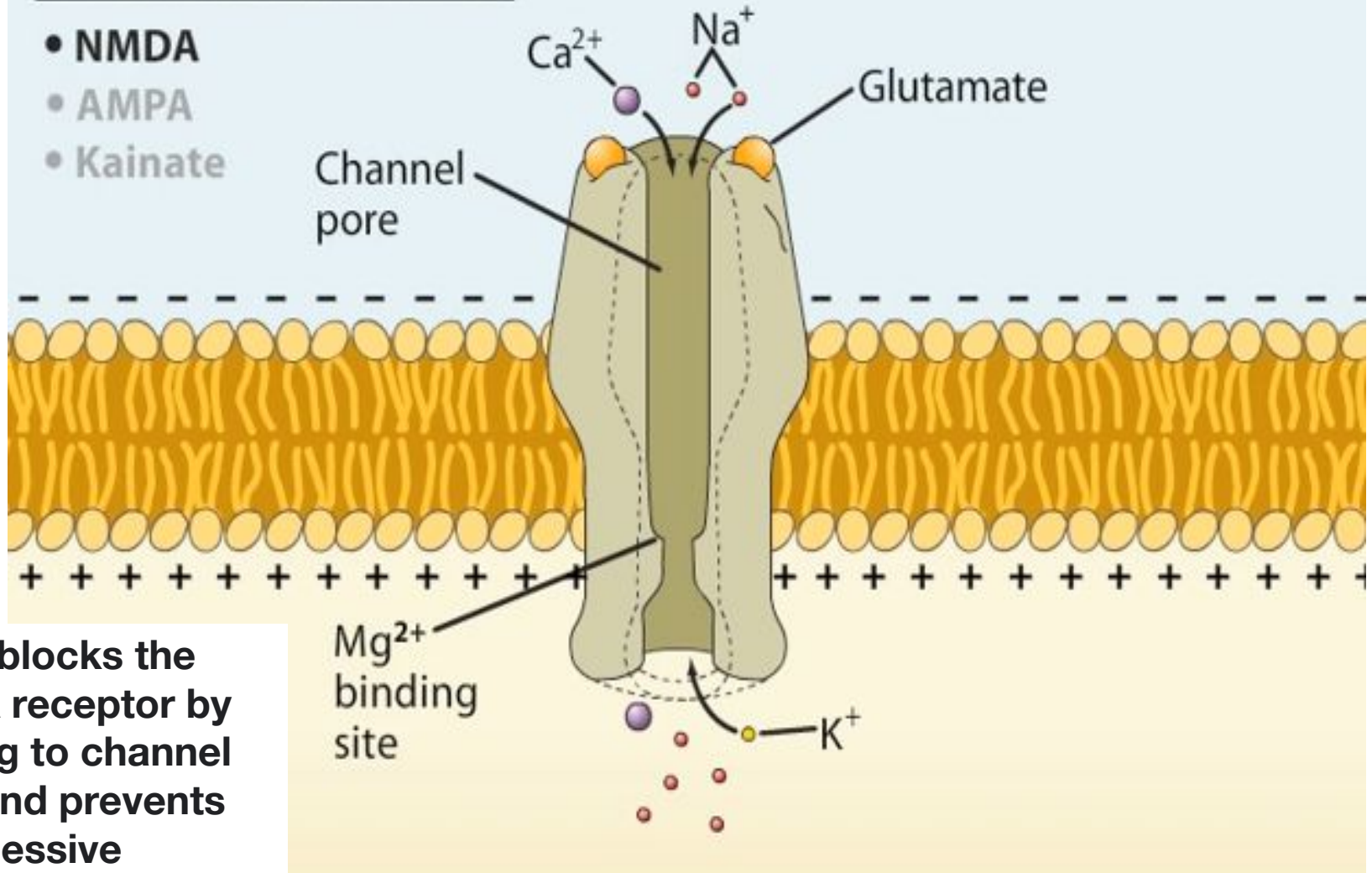


- Activation of NMDA receptor is particularly effective in Ca^{2+} entry.
- They are readily blocked by Mg^{2+} (voltage dependence)
- Glycine and Glutamate both require for NMDA receptor activation, binding site of both are different and both have to be occupied for the channel to open.
- Subunits: GluN₁, GluN₂A-D, GluN₃A-B
- **Drugs act through NMDA receptors:**

Agonists	Antagonists
Cycloserine Aspartate antagonist: ligand that binds to receptor, prevents receptor function	Ketamine Phencyclidine methadone Dexomethorphan Pethidine Nitrous oxide Acamprosate Amantadine Memantine

GLUTAMATE RECEPTORS

- NMDA
- AMPA
- Kainate



Mg²⁺ blocks the NMDA receptor by binding to channel pore and prevents its excessive activation.



AMPA Receptor

- 4 types of subunits: GluA1-4
- AMPA receptors have 4 types to which agonist can bind one for each subunit.
- The channel gets open when ligand gets bind to the extracellular transmembrane domains which then moves towards each other. To open the channel their should be two sites occupied over the receptor.
- Fast excitatory synaptic transmission
- AMPA permeable to calcium and other cations such as sodium potassium.

Drugs act through AMPA receptors:

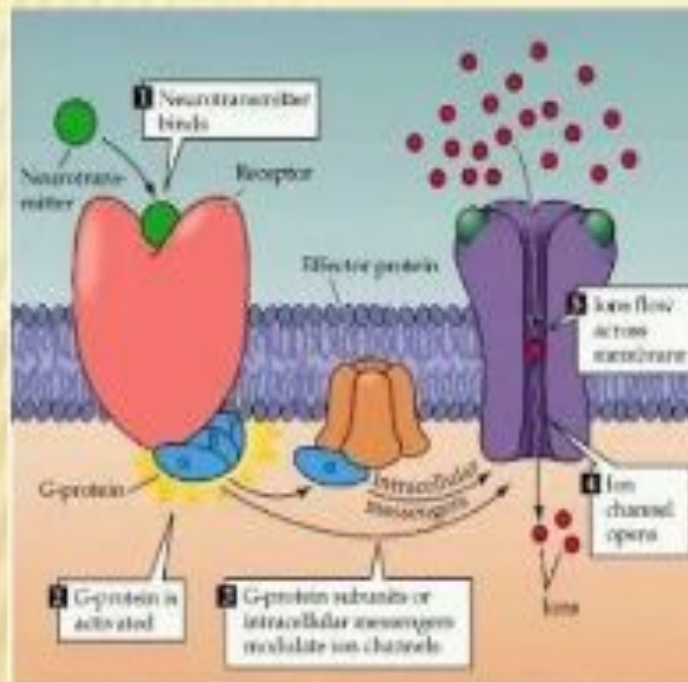
Agonists	Antagonists
Glutamate AMPA Domoic acid	Ethanol Tezampanel Anticonvulsants

Kainate Receptor

- 5 Subunits: GluK1-5
- Permeable to Na^{2+} , K^{+} but less permeable to Ca^{2+}
- Postsynaptically : Excitatory neurotransmission
- Presynaptically : Inhibitory neurotransmission (through GABA)

Agonists	Antagonists
Glutamate Kainate Domoate	ethanol Kynurenic acid anticonvulsant

METABOTROPIC GLUTAMATE RECEPTORS



- ✗ Metabotropic glutamate receptor (mGlu) are G-protein coupled receptors activated by glutamate the major excitatory neurotransmitter of the CNS.

- **METABOTROPIC GLUTAMATE RECEPTORS**

- 8 different metabotropic glutamate receptors (mGlu1–8).
- Divided into three groups.
- Group 1 mGlu receptors located **post-synaptically** and are largely excitatory.
- Group 2 and 3 mGlu receptors **presynaptic** receptors ↓ synaptic transmission and neuronal excitability.

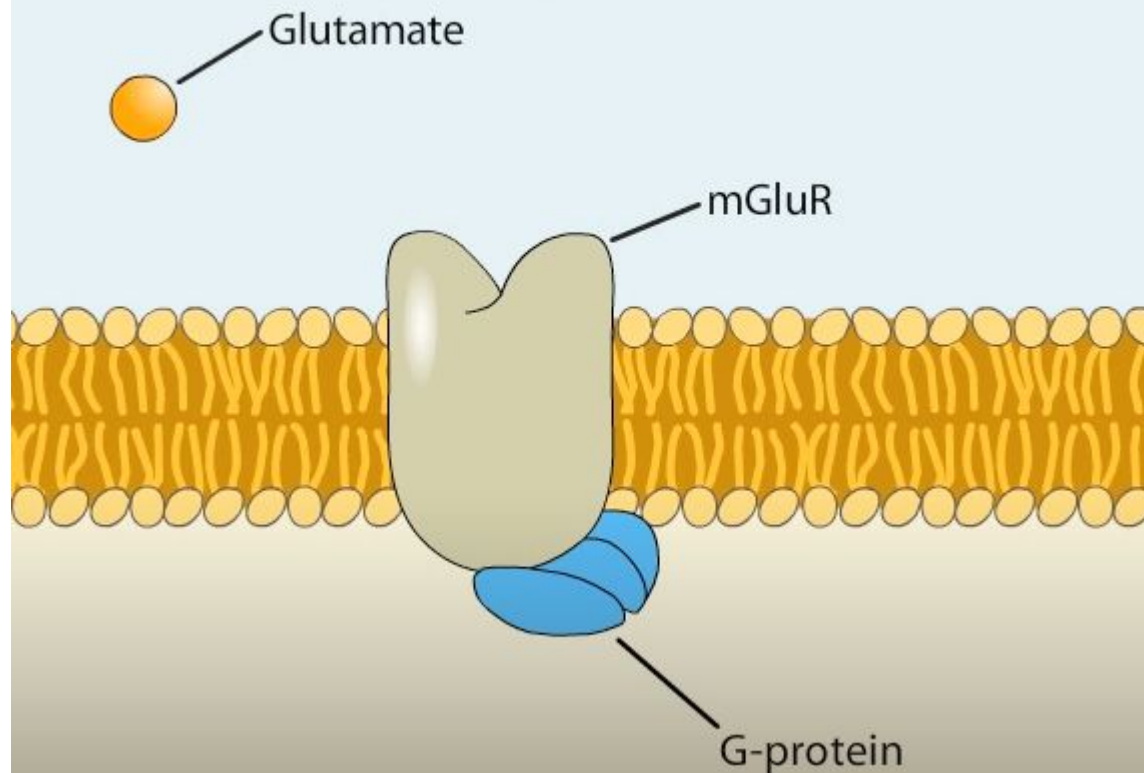
Metabotropic Glutamate Receptor

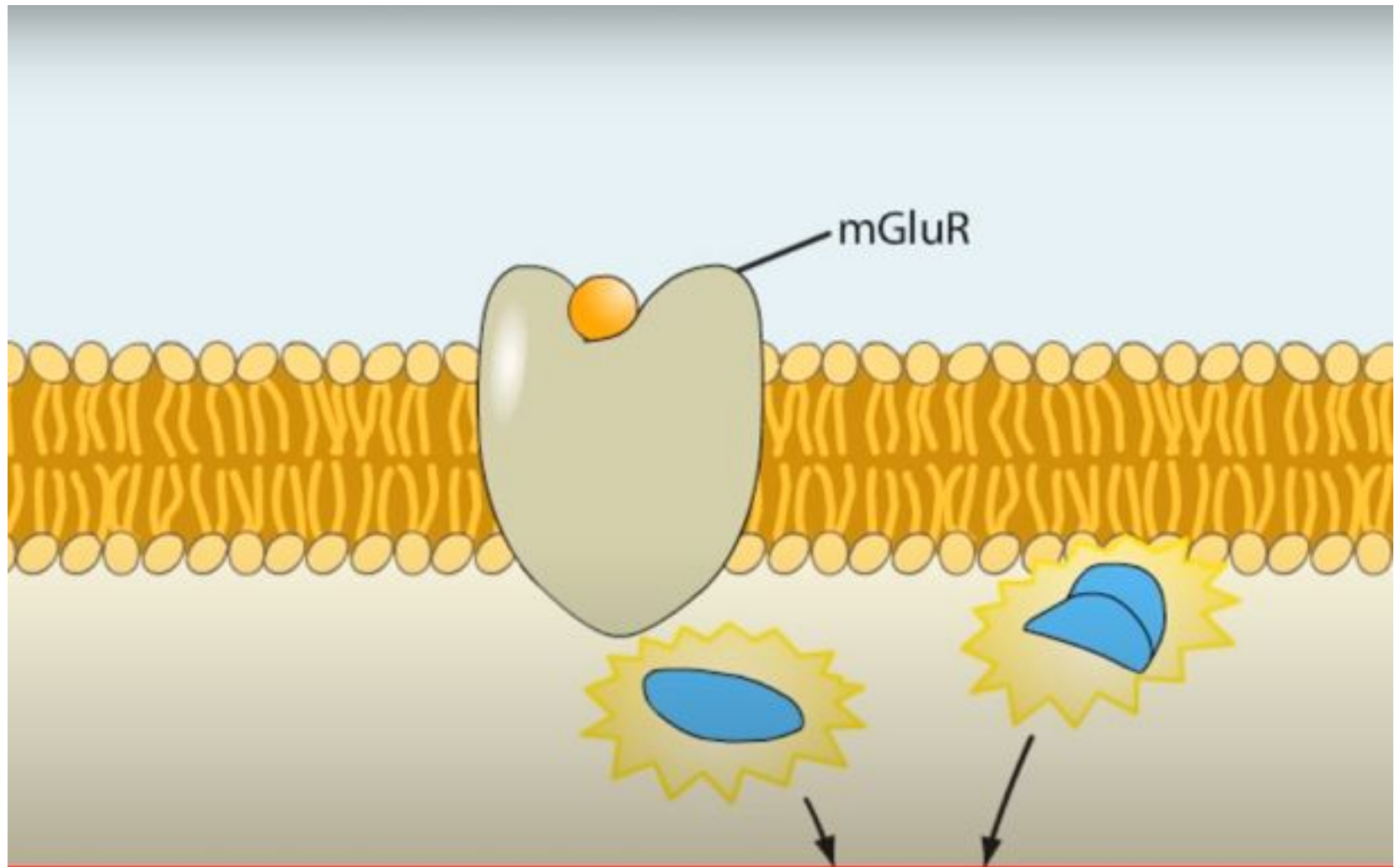
- The metabotropic glutamate receptors, or mGluRs, are a type of glutamate receptor that are active through an indirect metabotropic process.

Classification

Group 1	Group 2	
mGlu 1, mGlu 5	mGlu 2, mGlu 3	
G_q	$G_{i/o}$	
$\uparrow IP_3/DAG$, $\uparrow se Ca^{2+}$	$\downarrow se cAMP$	
Somatodendric	Somatodendritic and nerve terminals	

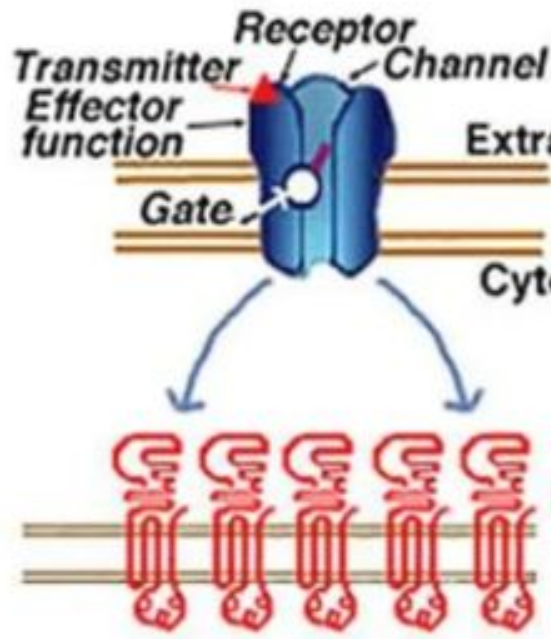
METABOTROPIC GLUTAMATE RECEPTORS





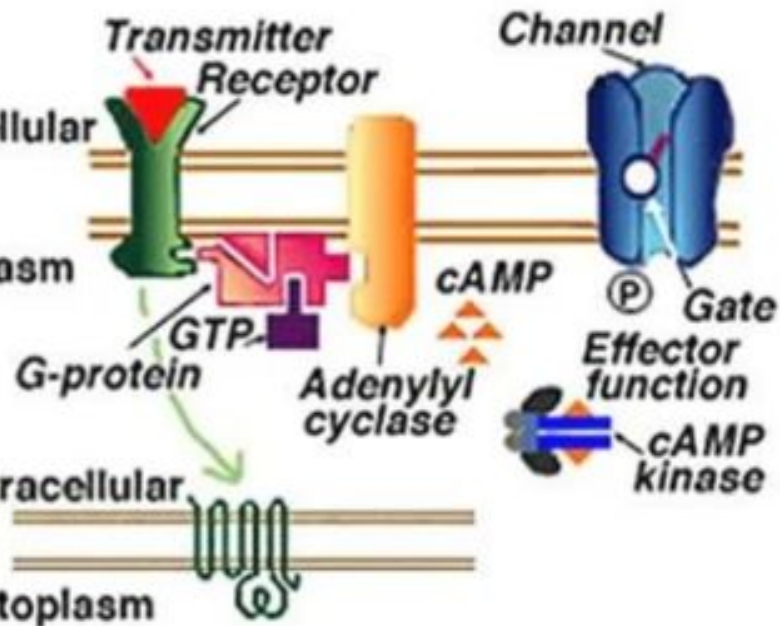
Signaling cascade

Ionotropic receptor

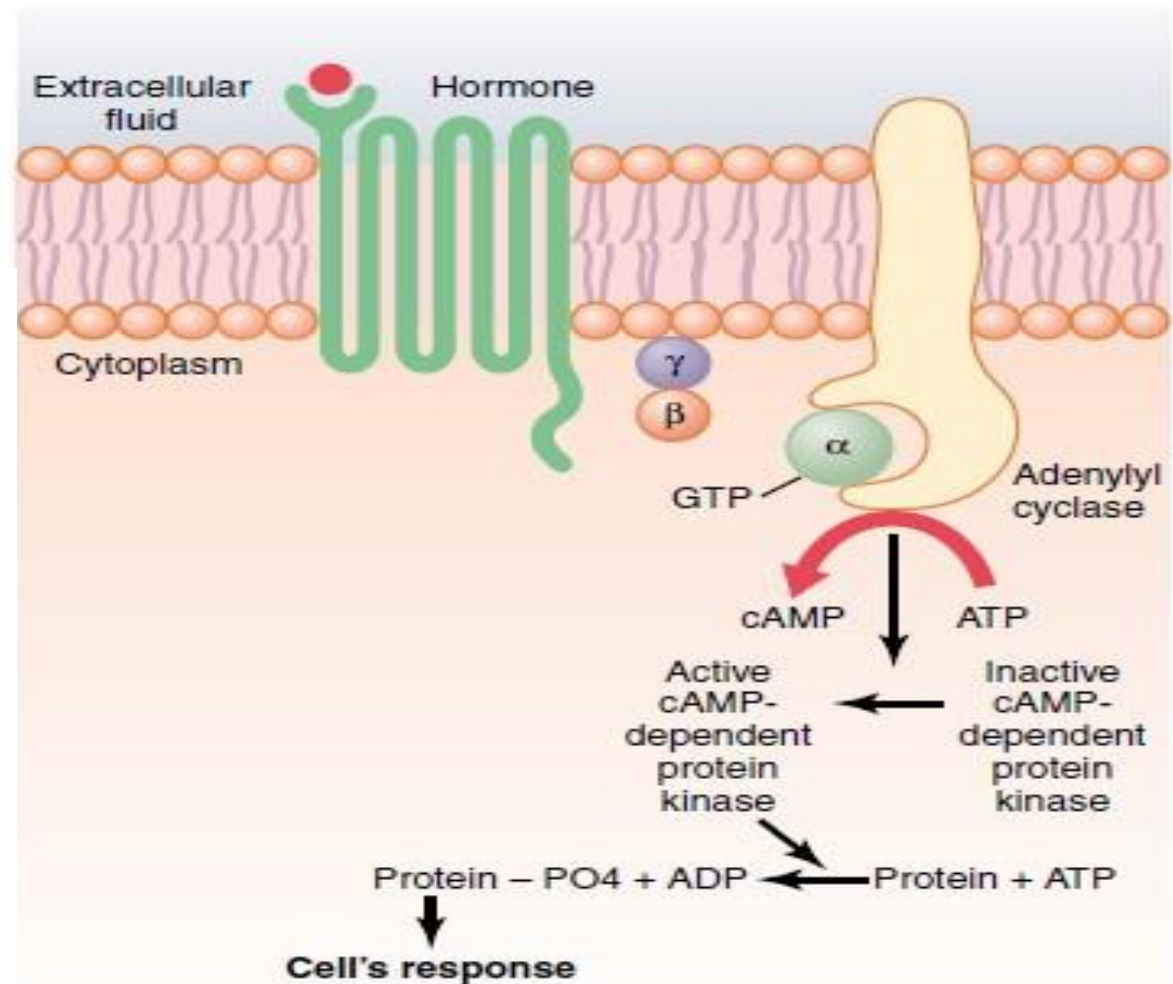
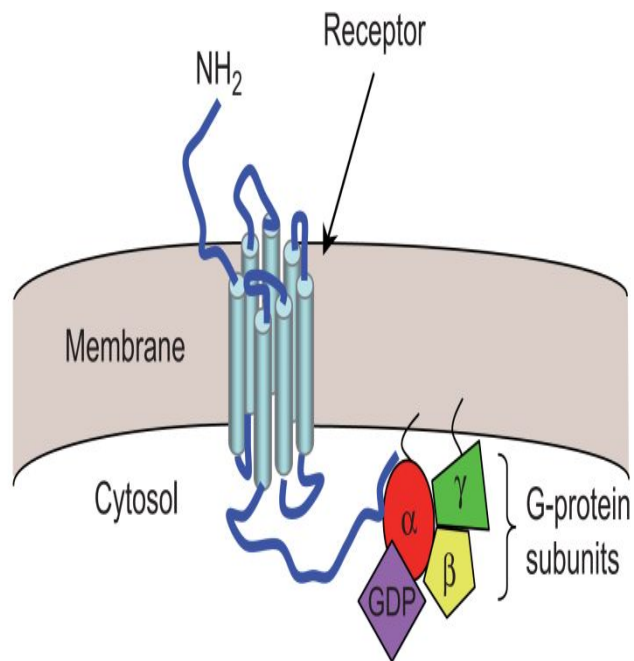


Ionotropic receptors and their associated ion channels form one complex (top). Each iGluR is formed from the co-assembly of multiple (4-5) subunits.

Metabotropic receptor



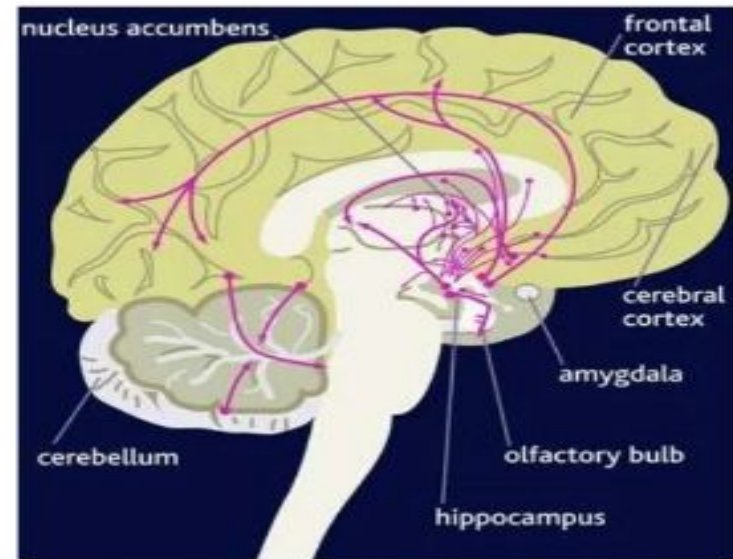
Metabotropic receptors are coupled to their associated ion channels by a second messenger cascade (top). Each mGluR is composed of one polypeptide, which is coupled to a G- protein.



Cyclic adenosine monophosphate (cAMP) mechanism by which many hormones exert their control of cell function. ADP, adenosine diphosphate; ATP, adenosine triphosphate.

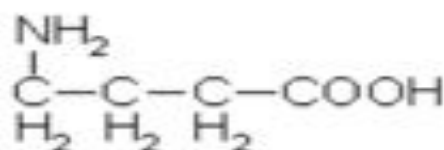
γ -AMINOBUTYRIC ACID

GABA



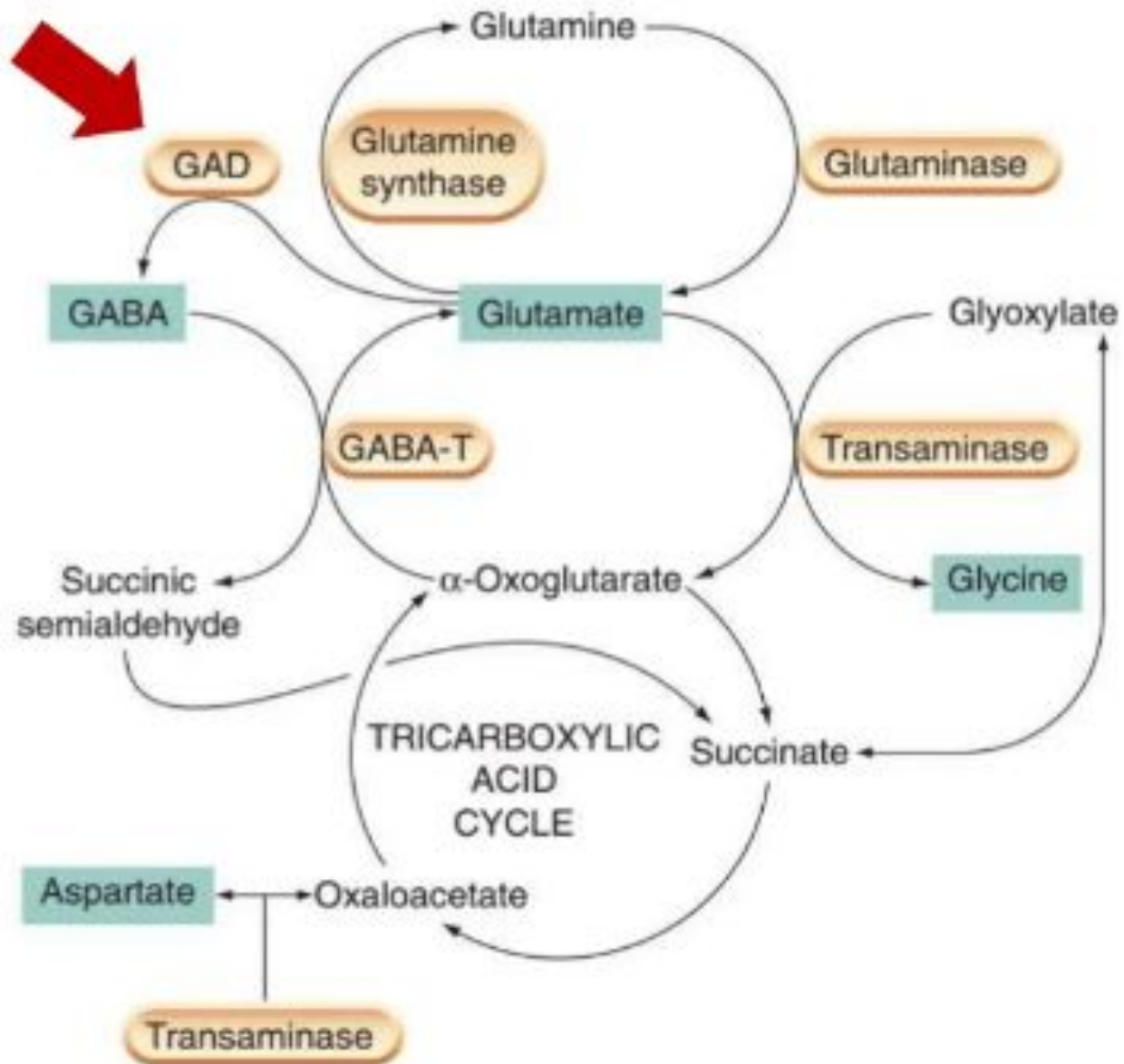
γ -AMINO BUTYRIC ACID (GABA)

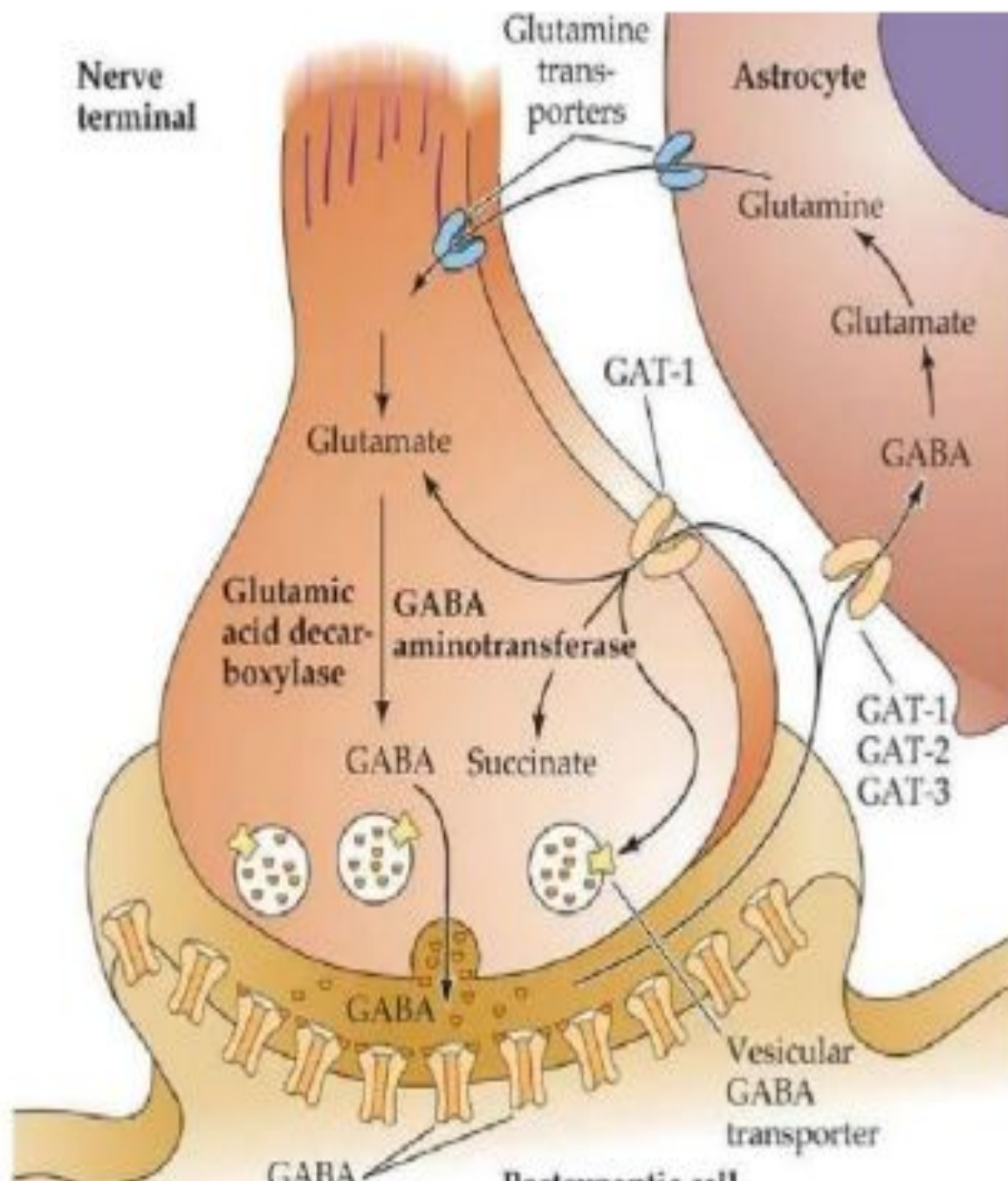
- Synthesized directly from **glutamate**.
- GABA is the most important **inhibitory** neurotransmitter
- Present in high concentrations in the CNS, preventing the brain from becoming overexcited.
- If GABA is lacking in certain parts of the brain, **epilepsy** results.

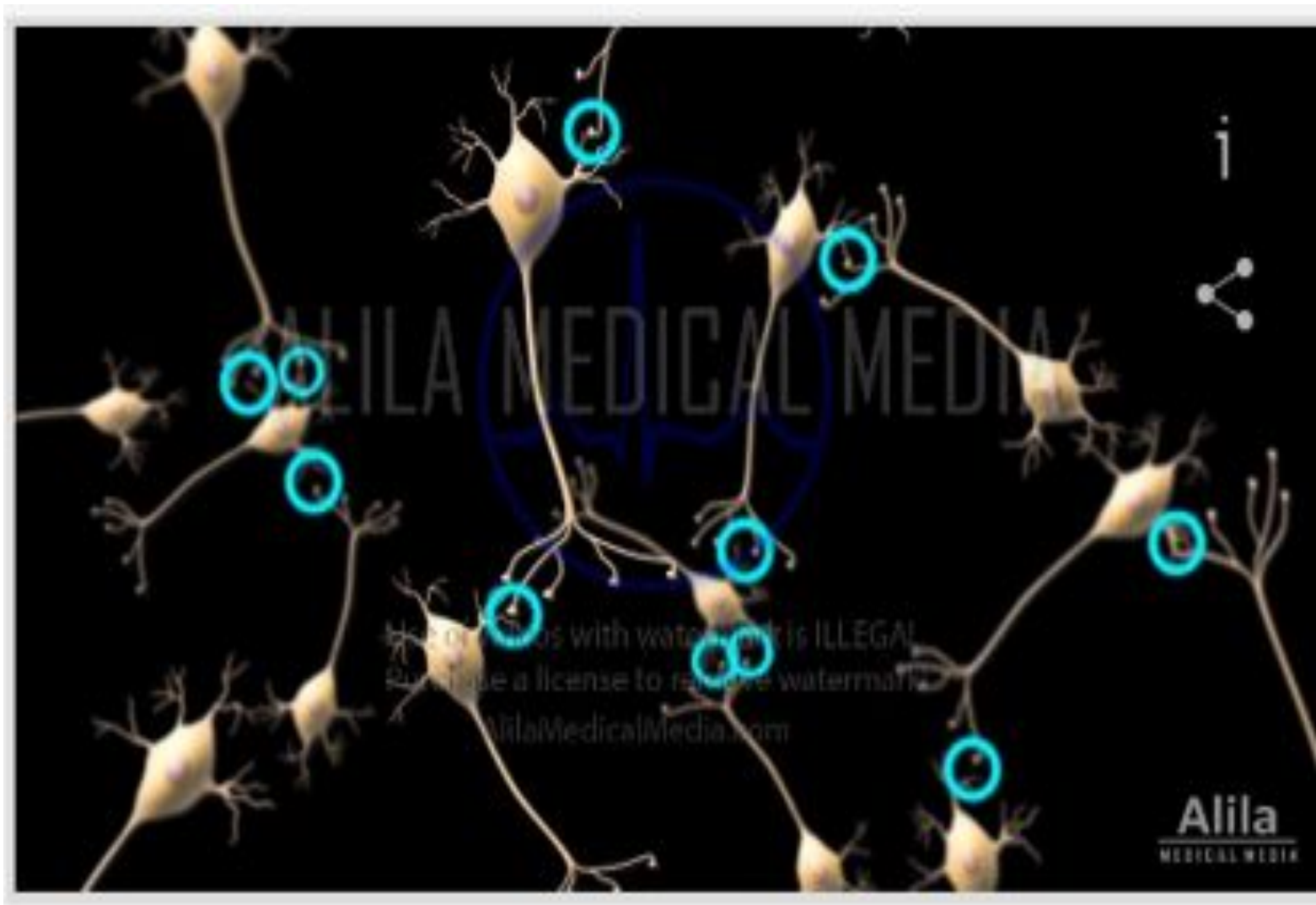


GABA

Glutamic acid decarboxylase (GAD) is **the rate-limiting enzyme that catalyses the conversion of glutamate to GABA, the main inhibitory neurotransmitter of the CNS**







It about half of synapses in the brain across through GABA receptors therefore become responded

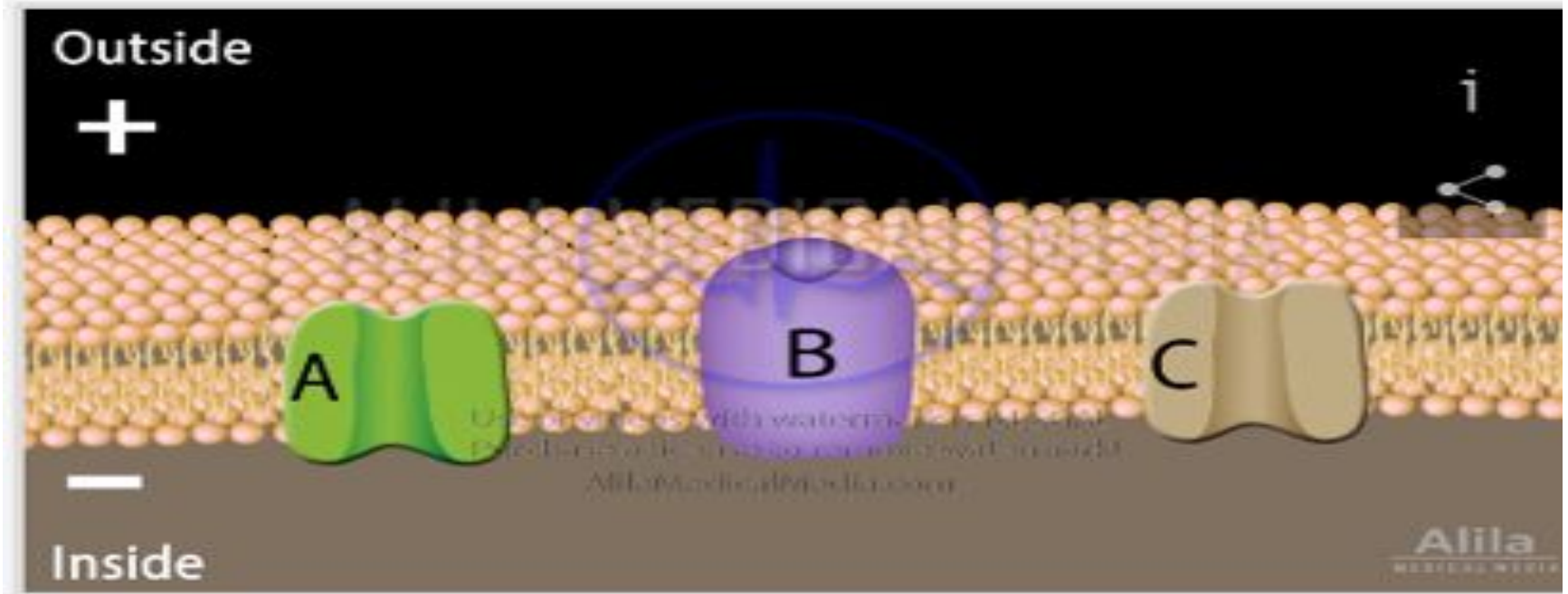
GABA receptors

THREE MAJOR TYPES

I. GABA_A

II. GABA_B

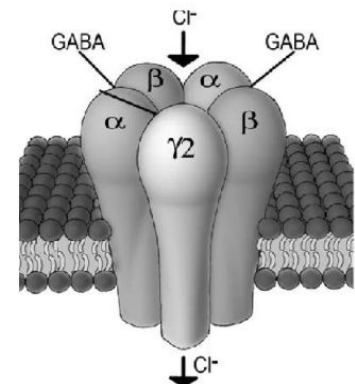
III. GABA_C



There are three types of GABA receptors :
GABA A, GABA B, GABA C

GABA_A RECEPTORS

- Ligand gated ion channel
- Distributed throughout the brain.
- It is a heteropentamer, made of five subunits .
- Ligand binds at the interface between α and β domain (2 α , 2 β , 1 γ)



- Different types of GABA A receptors present- depending on the type of subunit .
- Subunits – alpha(1-6), beta(1-3), gamma(1-3), delta, epsilon, pi, theta and rho.
- Different types of GABA A receptors - present in different regions of the brain , at different levels of development.

GABA_A receptor when activated, an increase in the conductance.



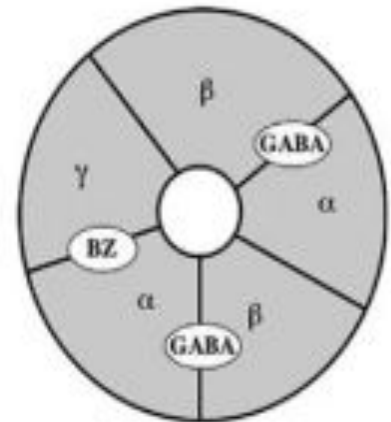
Increase in the influx of Cl⁻ ions causing membrane hyperpolarization.

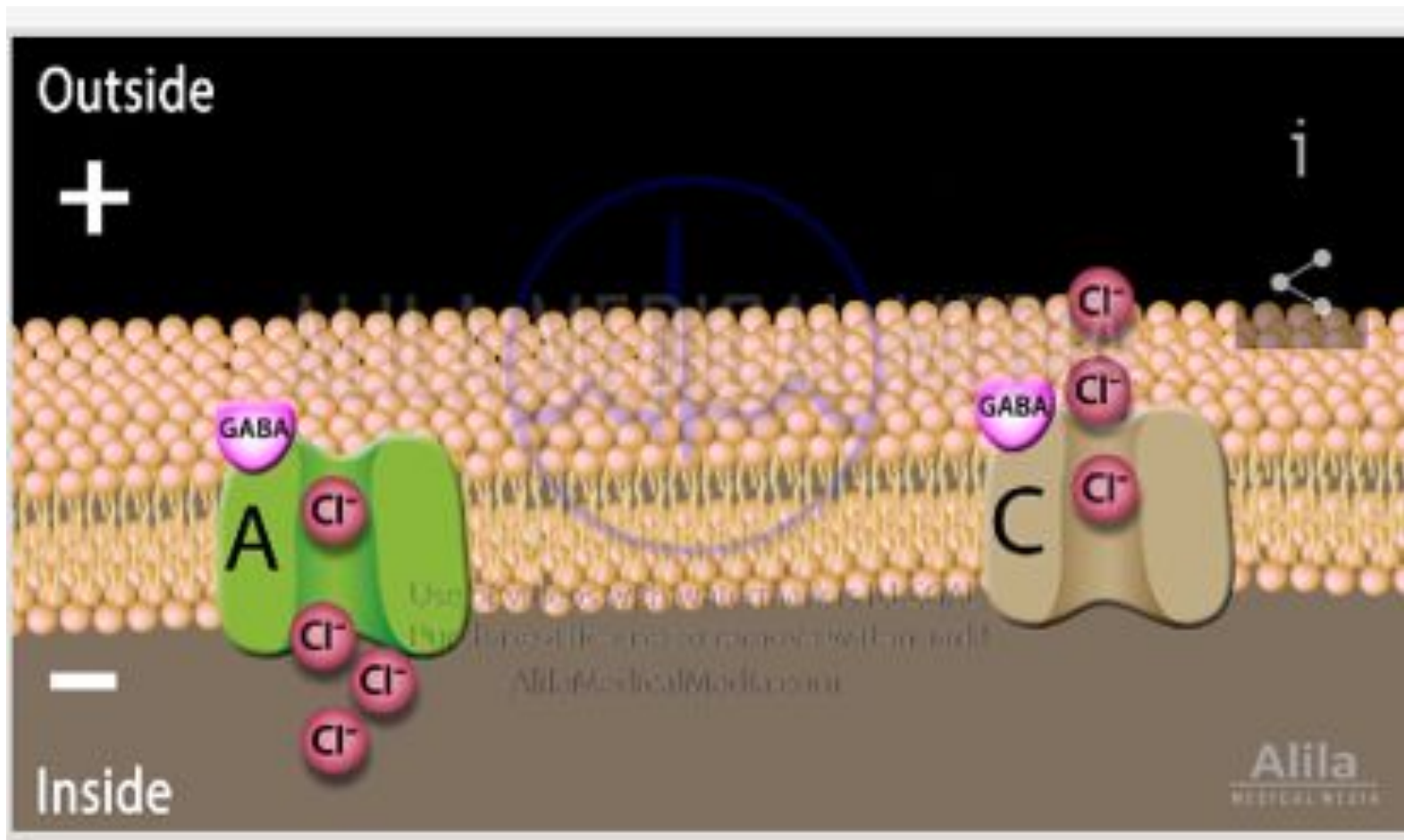


Increase in the threshold for generating action potential.



Inhibitory action



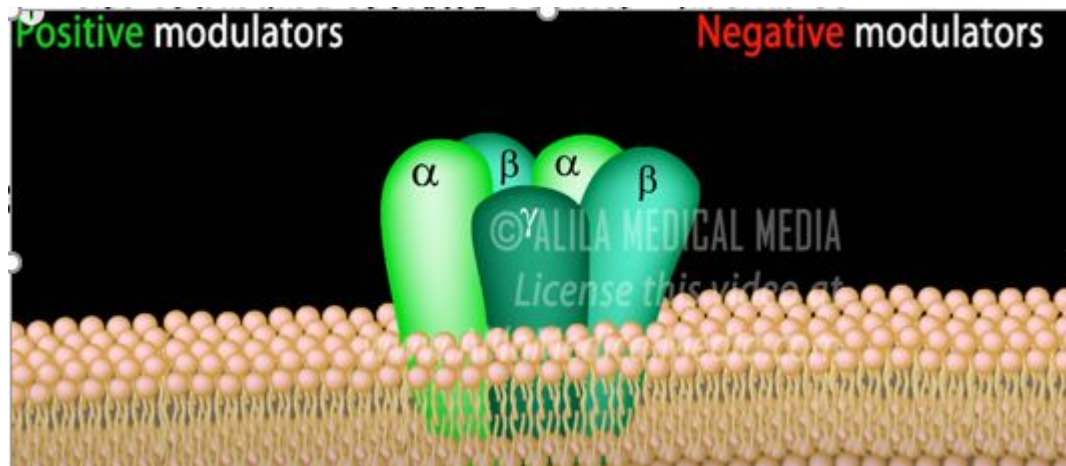


- When GABA bind receptor channel open to permit of Cl to release in side neuron cell
- This make high negativity (hyperpolarization) inhibitory effect

- **Allosteric modulation**

The site where modulators bind is different from the site of binding of GABA agonist – known as “allosteric” site . Modulator - “allosteric modulator”. The modulator has no activity of its own.

- ❖ **change in the shape and activity of a protein receptor that results from : combination with another substance at a point other than the chemically active site**



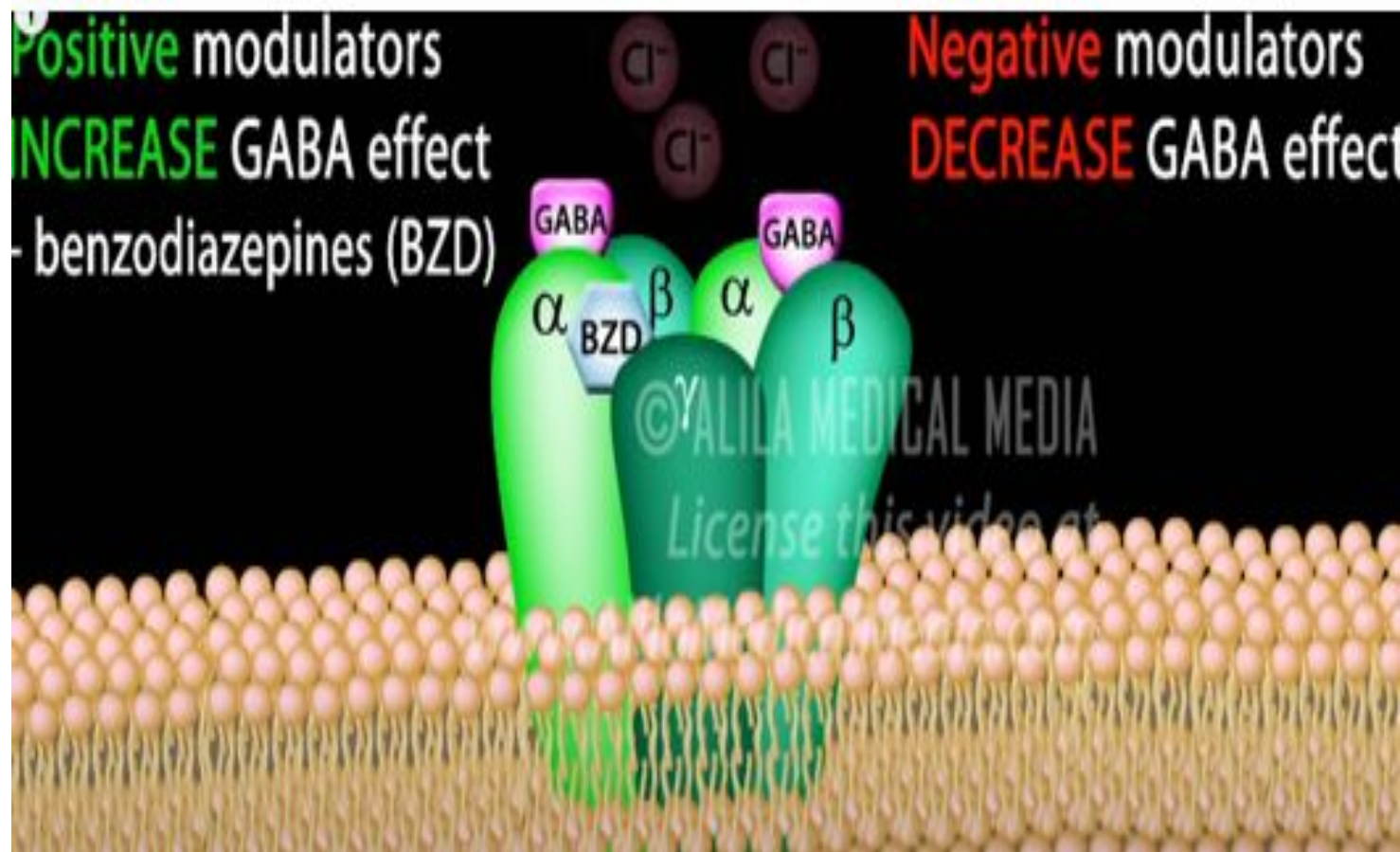
- **Positive Allosteric Modulation:** ligand binds to allosteric site and enhances the action of neurotransmitter. E.g. **BZD**
- **Negative Allosteric Modulation:** ligand binds to the allosteric site while an agonist is also bound and the channel opens less frequently. E.g. Bicuculline.

it blocks the inhibitory action of GABA receptors, the action of bicuculline mimics epilepsy; it also causes convulsions

Positive modulators
INCREASE GABA effect

Negative modulators
DECREASE GABA effect



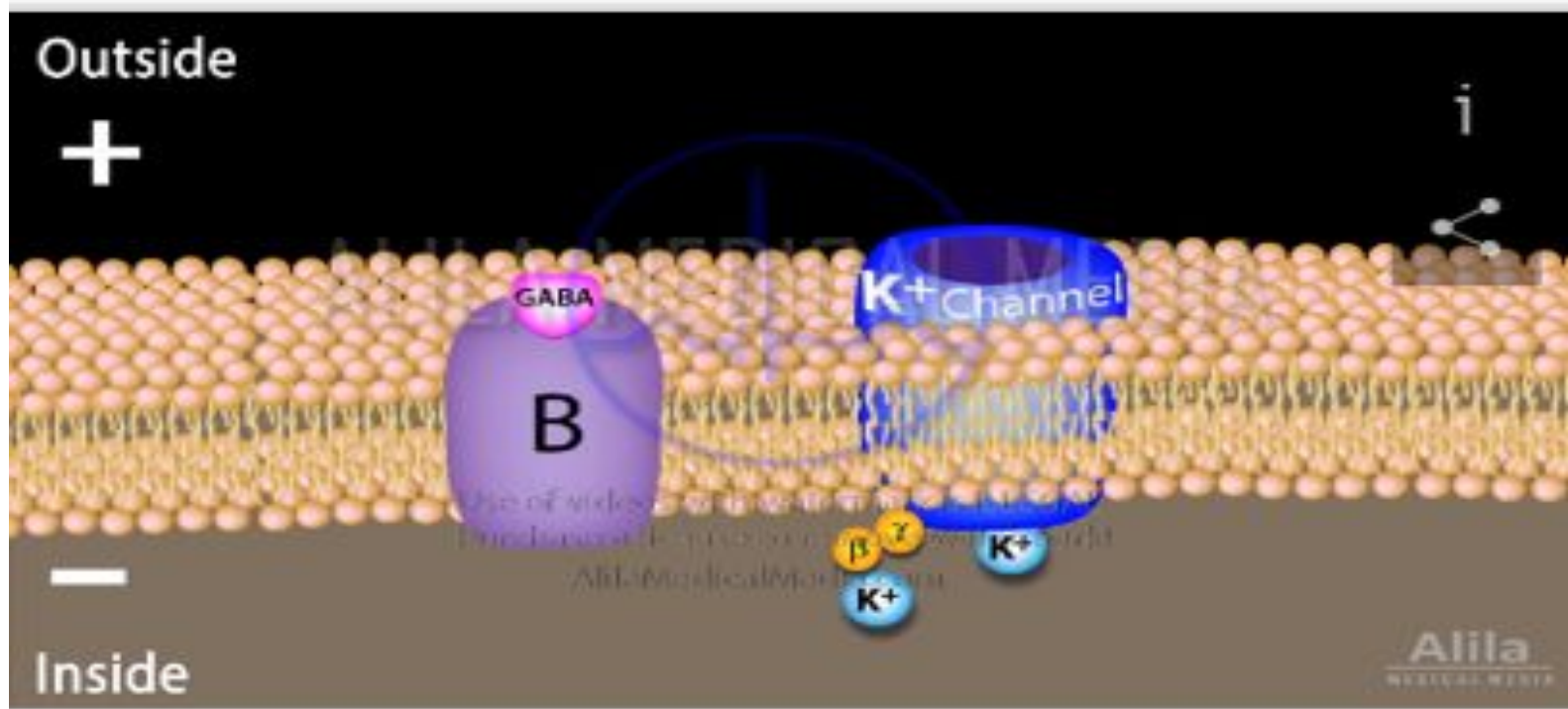


- Benzodiazepines increase inhibition effect of GABA
- Benzodiazepines produce relieve anxiety and muscle spasms, and reduce seizures

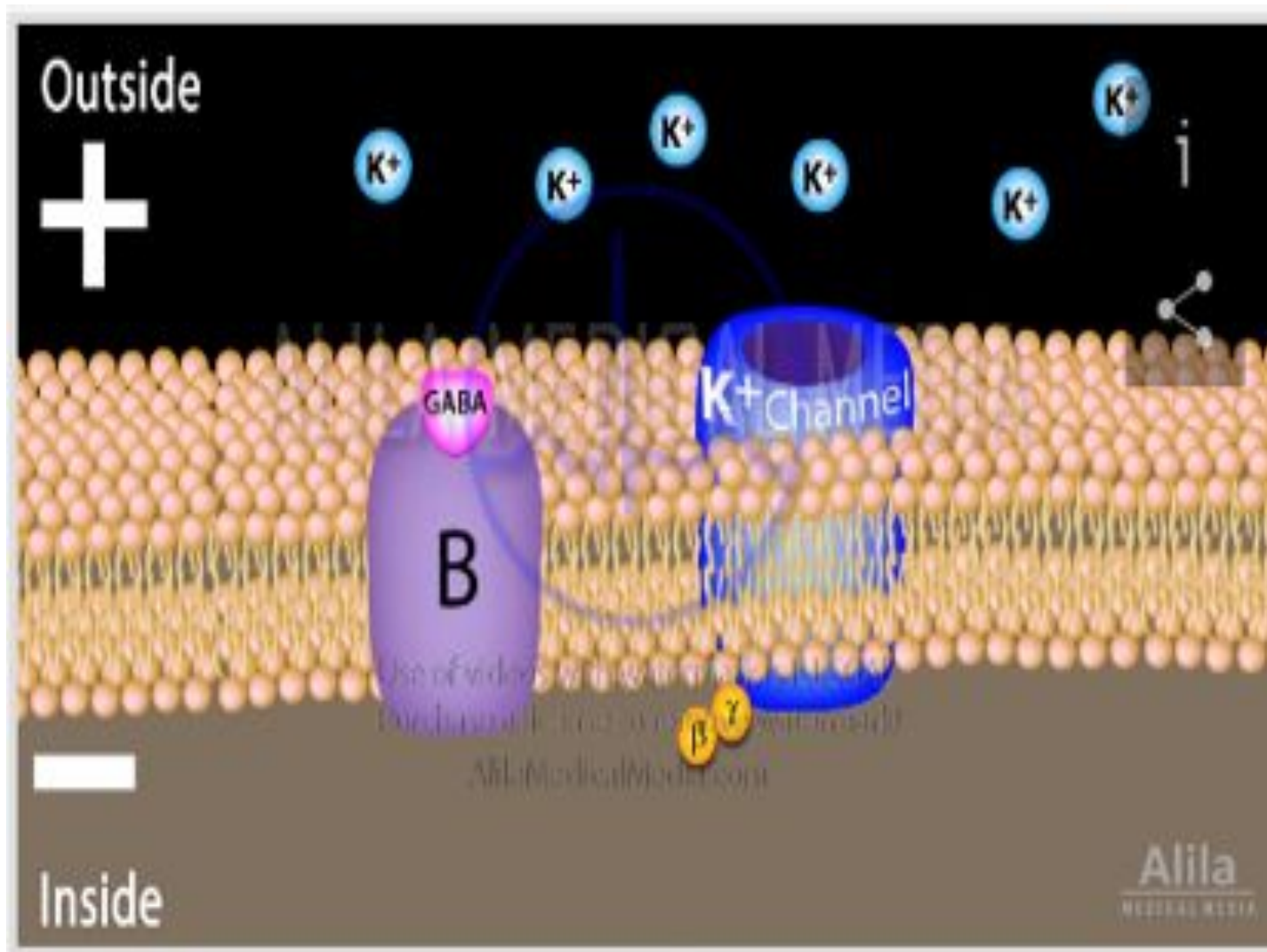
GABA_B RECEPTORS

- G-protein coupled receptor.
- Dimer of two, seven transmembrane spanning subunits held together by a coil – coil interaction btw their C-terminal tails.
- Activation of receptors occurs when GABA binds to the extracellular domain of the B1 subunit which produce an allosteric change in the B2 subunit which couples to the G-protein
- Widely distributed throughout the CNS.

- Located both pre- and post-synaptically.
- **Presynaptically**- auto-receptor. Inhibits voltage gated calcium channels, thus decrease neurotransmitter release.
- **Postsynaptically** – inhibitory – long lasting hyperpolarization by activating K^+ channel.



GABA B role through protein G activate K⁺ channel that permit to release K out the nerve(hyperpolarization)



K out of cell hyperpolarization

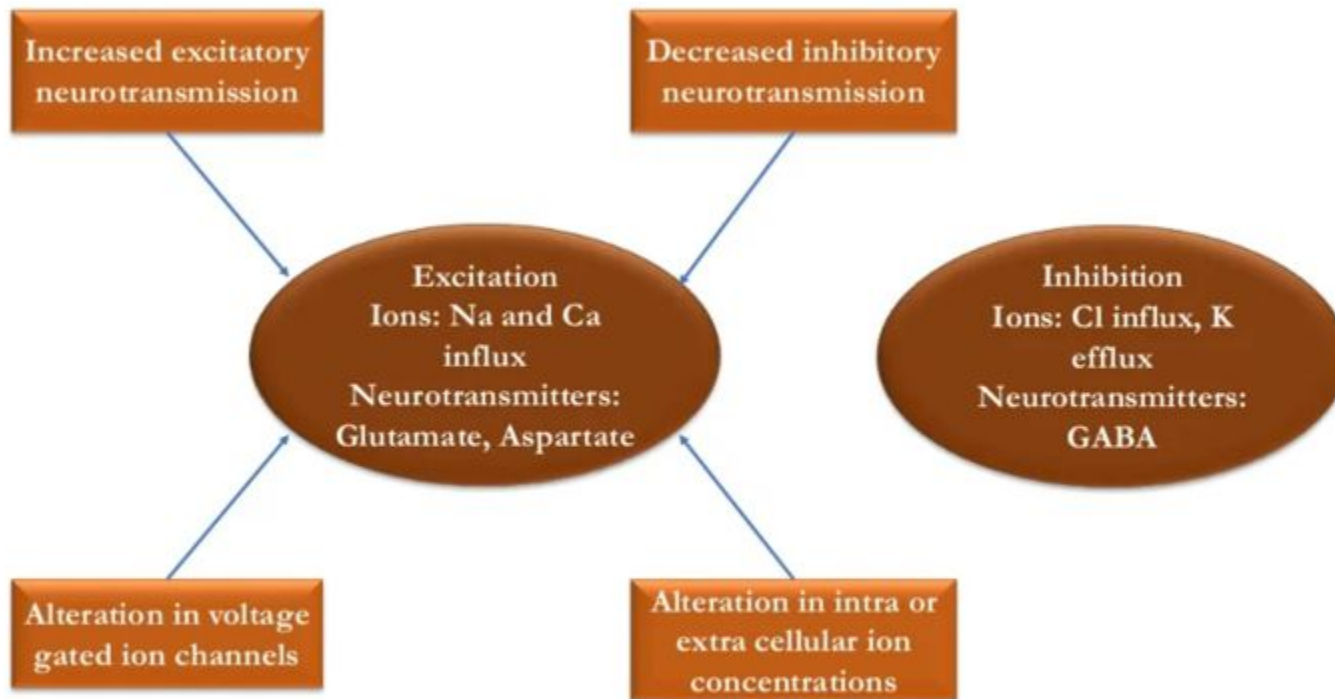
GABA_c RECEPTORS

- Transmitter gated chloride channel
- Retina,spinal cord,superior colliculus,pituitary
- Physiological role is not yet discovered.

GABA reduces:

- fear
- anxiety
- convulsions





A hyperexcitable state can also result when several synchronous subthreshold excitatory stimuli occur, allowing their temporal summation in the post synaptic neurons.

THERAPEUTIC APPLICATIONS

Drug/ Class	Mechanism	Use	Adverse Effects
BARBITURATES	↑ Duration GABA _A gated Chloride Channel	Anesthesia, Anticonvulsant, hyperbilirubinemia, Sedative-Hypnotics	Distortion of Sleep Architecture, Learning and Memory impairment, respiratory depression, abuse, laryngeal edema, hypersensitivity.
BENZODIAZEPINES	↑ Frequency GABA _A gated Chloride Channel	Anxiety, Insomnia, Induction, Sk muscle relaxation, Anticonvulsant, Alc Withdrawal	Drowsiness, fatigue, disorientation, lethargy, respiratory depression, abuse, impaired memory and learning, date rape
Z-DRUGS	Agonist at modulatory site at GABA _A R to which BZD bind	Insomnia	Rebound insomnia, hangover are less. Headache, daytime drowsiness, nightmares at high doses.

Role of neurotransmitters

GABA

Major inhibitory neurotransmitter.

GABA_A

Post synaptic.

Causes Cl influx and hyperpolarisation.

Agonists suppress seizures (BZD, barbiturates).

GABA_B

Presynaptic autoreceptors.

Can modulate synaptic release.

Associated with second messenger systems.

Causes K channel opening and hyperpolarizing current.

Glutamate

Major excitatory neurotransmitter.

Inotropic receptors

Fast synaptic transmission.

NMDA, AMPA, Kainate: Na influx.

NMDA: Ca influx. Can cause excitotoxicity and cell death due to excessive stimulation (eg in status).

Agonists trigger seizures.

Metabotropic receptors

Slow synaptic transmission.

Signal transduction involving membrane-associated G-proteins.

Stimulation results in variable effects.

Conclusion

- Epilepsy can arise due to various causes.
- Final common pathway is disturbance of excitation- inhibition balance (neuronal homeostasis) resulting in self reinforcing episodic shifts in neuronal and network excitability.
- This involves multiple neurotransmitters (primarily GABA and Glutamate), ion conductance (primarily Na, K, Ca, Cl) and genetic factors.
- Major role for interneurons and glial cells in epileptogenesis.
- Neuroinflammation plays a role in epileptogenesis.