NEUROCHEMISTRY – Brief Review

UNIVERSITY OF PNG
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DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
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Membrane potential

- **Membrane potential**: due to electrical potential in cells, resulting from separation of electrical charges across cell membrane;
- Electrically negative inside cell compared to outside;
- **K^+ ions** are higher inside cell compared to outside;
- **Na^+ ions** are higher outside cell compared to inside;
- **Neuron is an excitable cell, because it is capable of generating and conducting electrical impulse by temporarily reversing its membrane potential**;
- Membrane potential is maintained by **Na^+, K^+-ion pump** with **ATPase** activity;
- Pumps Na^+ out of cell and K^+ into the cell;

What are the two modes of impulse transmission?

- **Electrical mode of transmission**:
- Impulse transmission along axon – Membrane transmission:
  - Involves propagation of Action Potential along axonal membrane;
- **Chemical modes of transmission**:
- Impulse transmission across synapse:– Synaptic transmission;
- Impulse transmission from neuron to muscle:– Neuromuscular transmission;
  - Involves Neurotransmitters;

What factors induce ions to cross cell membrane?

- Three major factors that can induce ions to cross cell membrane are:
  - Difference in concentration of ions on both sides of the membrane;
  - Difference in electrical potential on both two sides of the membrane;
  - Action of an ion pump;

What are ion channels?

- **Ion channels:** special openings in cell membranes for passive movement of ions;
- Ion movements based on concentration and electrical gradients are passive, but require the presence of **ion channels;**
- Each channel is highly selective for a particular ion,
  - Determined by size of central pore and electrical charges of amino acids residues in the polypeptide structure of the pore;

What are the different types of ion channels?

- **Leakage channels:**
  - They are open to the flow of ions all the time;
- **Gated channels:**
  - Can open or close through conformational changes;
  - It may involve a change in diameter of central pore, or movement of protein component in the pore;

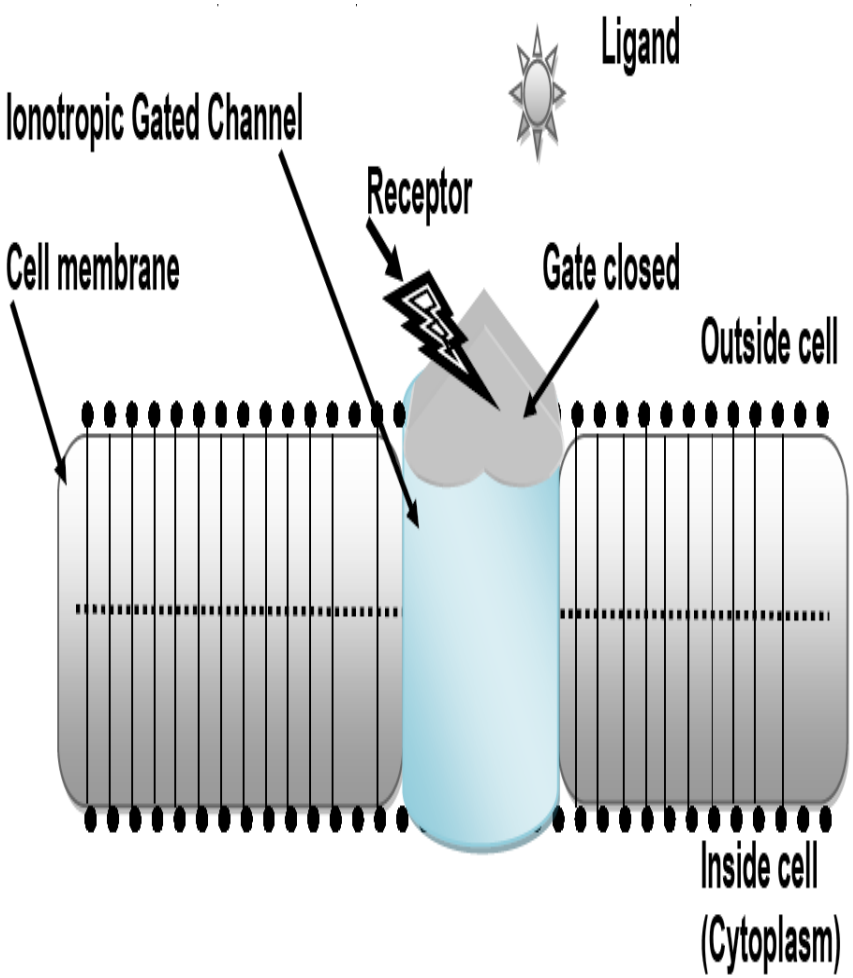
Major types of Ion-Gated Channels are:

- Stretch Sensitive Channels;
- Ligand Gated Ion Channels;
- Voltage Gated Ion Channel;

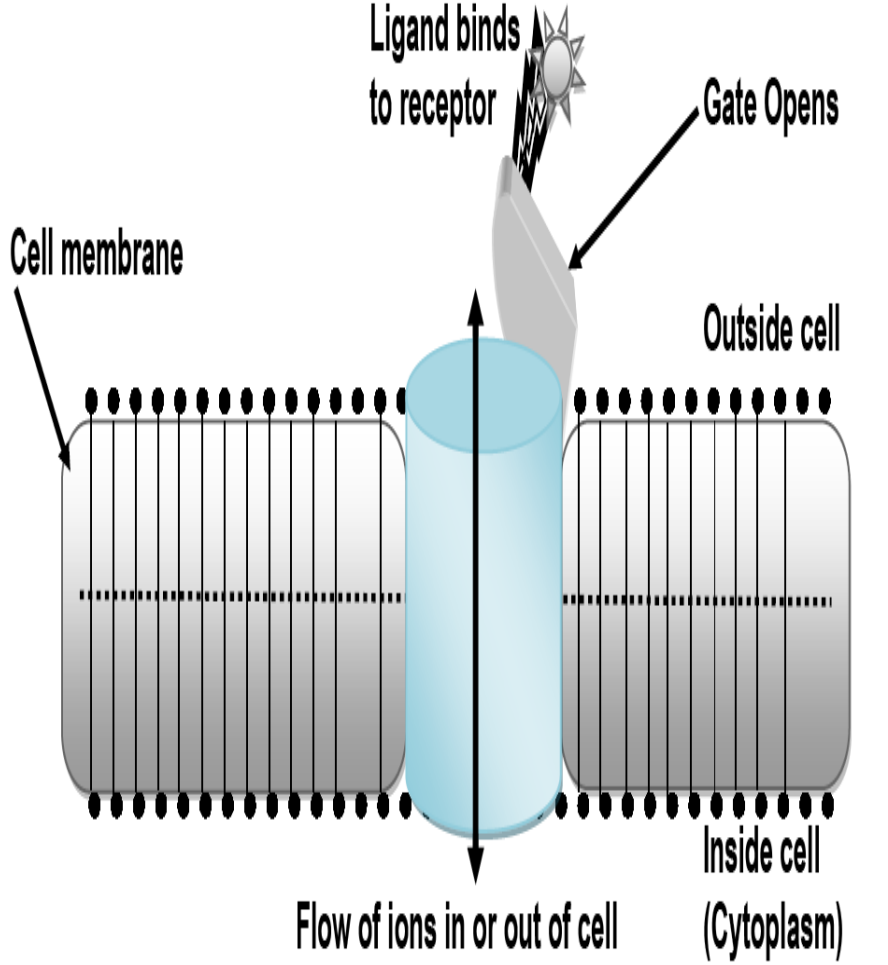
What are the different types of Ion-Gated channels?

- **Ligand Gated Ion Channels** or Ligand Sensitive Channels (**Figs. 1 to 4**)
  - Open or close in the presence of a signaling molecule,
  - Involve in signal transduction across synapses and neuromuscular junctions;
  - Ligand Gated Channels are usually highly distributed at Postsynaptic sites and have specific type of receptors,

Figs 1 & 2: Ligand Gated Ionotropic Channel

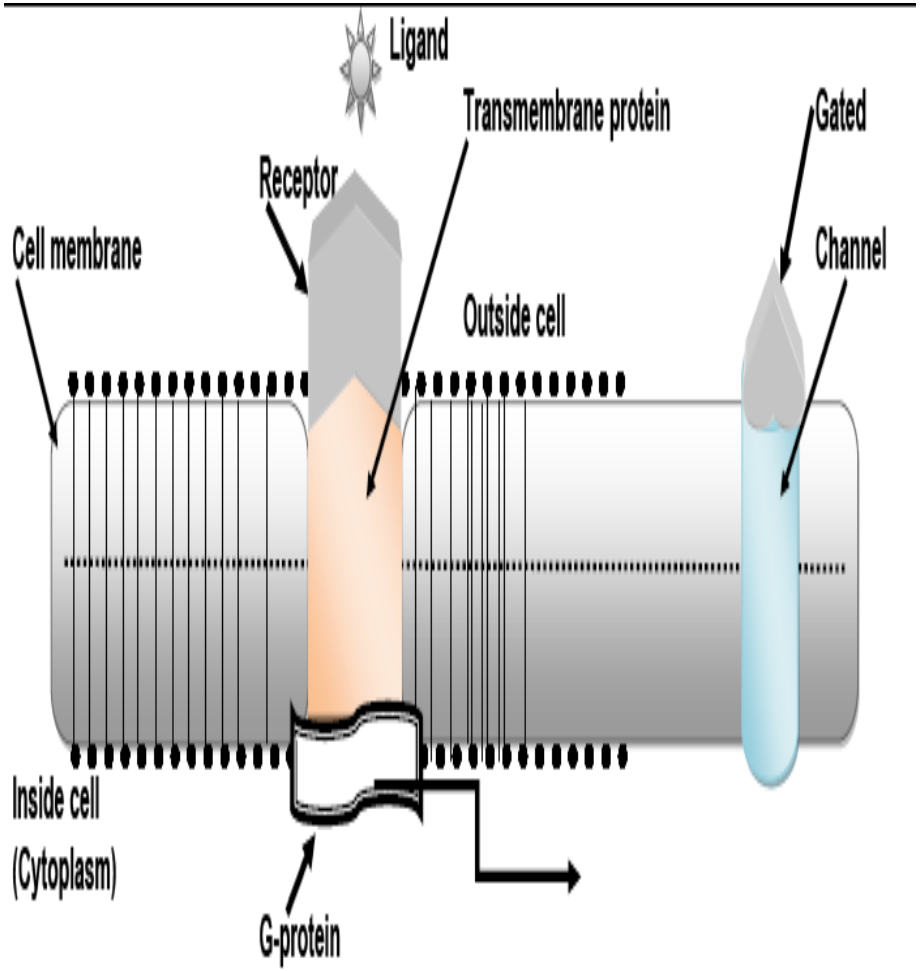


Schematic diagram showing Ionotropic Receptor on Ionotropic Ion-Gated Channel when gate is closed; Ligand is not in contact with Receptor

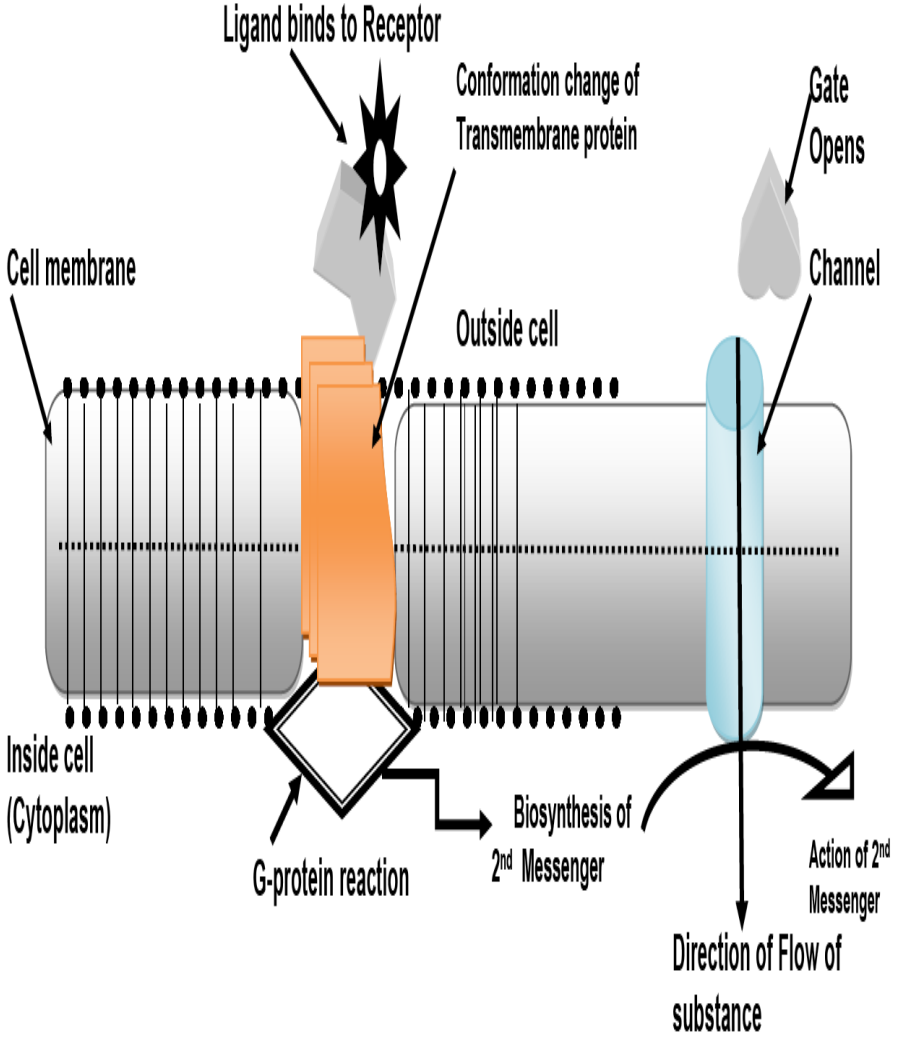


Schematic diagram showing Ionotropic Receptor on Ionotropic Ion-Gated Channel when Ligand binds to receptor and gate opens for flow of ions either in or out of cell

Figs 3 & 4: Ligand Gated Metabotropic Channel



Schematic diagram of Metabotropic Receptor on Metabotropic Ion-Gated Channel when gate is closed; Ligand is not in contact with Receptor

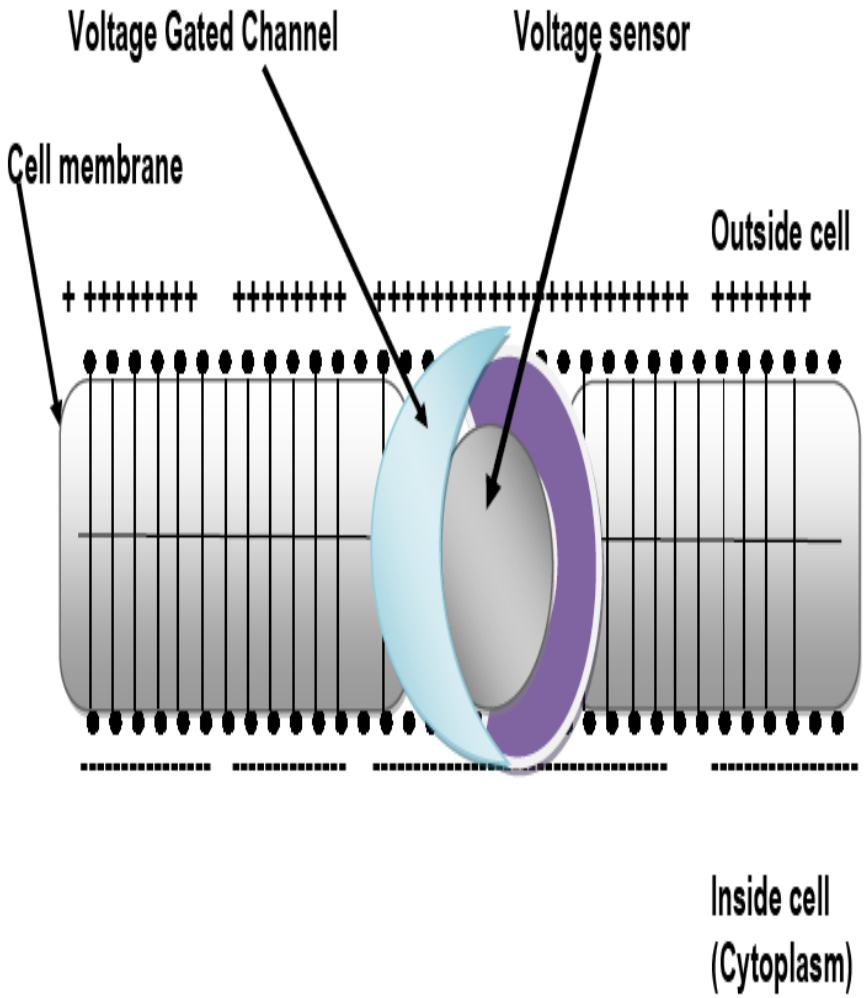


Schematic diagram of Metabotropic Receptor on Metabotropic Ion-Gated Channel: Ligand binds to Receptor; Conformational change of transmembrane protein; Activation of G-protein leading to biochemical reactions to produce 2nd messenger, whose action signals opening of channel gate.

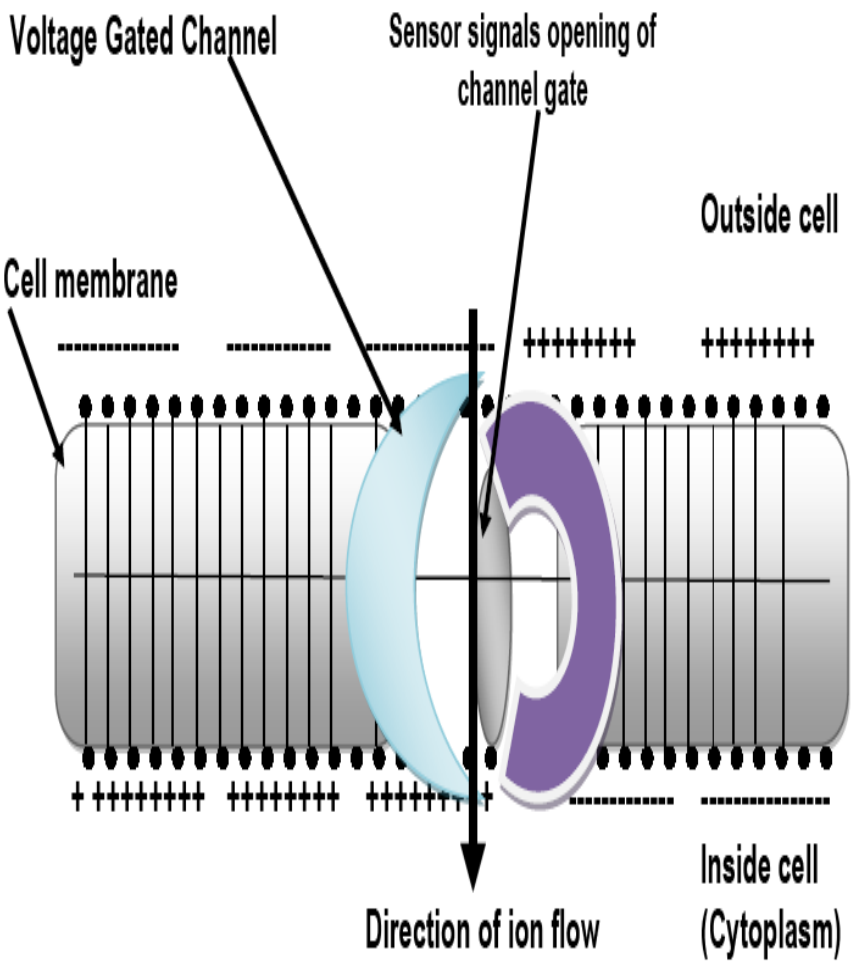
- **Voltage Gated ion channels** or voltage sensitive channels (**Figs. 5 & 6**):
  - Open or close in response to electrical potential differences across the cell membrane,
  - Forms the basis for conduction of nerve signals along axons,

- **Stretch sensitive channels**
  - Opens or closes when mechanical force is applied,
  - Mediates mechanical sensitivity

Figs 5 & 6: Voltage Gated Ion channels



Schematic diagram showing Voltage Gated Channel with sensor when gate is closed; Status of Resting Membrane potential



Schematic diagram of Voltage-Gated Channel; Wave of depolarization changes membrane potential, Voltage sensor detects the change and signals opening of channel for movement of ions;

What are receptors?

- Receptor is a membrane bound protein with high affinity for specific molecule;
- It responds to the presence of signaling molecule by initiating a chain of events that brings about a response by the neuron;
- Each receptor is specific for a particular signaling molecule and for close chemical analogs of the signaling molecule

What type of receptors are in Ion-Gated channels?

- **Ionotropic Receptors**: (**Fig. 1 & 2**)
  - Cause opening or closing of ion channels and quick, short lasting electrical response;
  - Receptor protein may act as ion channel that opens or closes in presence of signaling molecule (Ligand);
  - Receptor may be coupled to adjacent channel;
- **Metabotropic Receptors: (Fig. 3 & 4)**
  - Exerts effect via biochemical cascade that takes longer to develop than Ionotropic receptor, but lasts longer,
  - Exerts its effect via G protein (GTP binding proteins),
  - Usually act via 2nd Messenger system;

What are the types of Voltage Gated Ion channels?

- **Two types** of voltage gated ion channels are present in the membrane:
- **Sodium voltage** gated channel: Permeable to Na^+
- **Potassium voltage** gated channel: Permeable to K^+
- Both are sensitive to changes in Membrane Potential;
- Conformational changes occurs as Potential alters;
- Na^+ voltage gated channel has two gates:
  - Activation Gate,
  - Inactivation Gate
- K^+ channel has only one gate;

What are Ion Pumps, give example?

- Ion pumps need energy (ATP) to pump ions against the concentration gradient,
- Movement through Ion pump is a multi-step process, and may be up to 100 times slower than ion channel,
- Examples: Na^+/K^+ ion pump, Ca^{2+} ion pump, Cl^- ion pump

Na+ / K+ ion exchange pump:

- In a cycle **3 Na^+ ions** are moved out of cell in exchange for **2 K^+ ions** moved into the cell,
- Energy is obtained from ATP;
- Enable neurons to generate differences in concentration of ions across membrane that is essential to function;
- Used to restore membrane potential after wave of depolarization;

MEMBRANE TRANSMISSION

What is the mechanism for the resting potential in neuron?

- Exact chemical mechanisms not fully understood,
- Generally agreed that electrical polarity of neuron and of excitable cells in general, is due to unequal distribution of Na^+ and K^+ ions on both sides of neuronal membrane;
- Neuron has:
  - Low internal concentrations of Na^+ and Cl^- ions,
  - High internal concentration of K^+ ions,
  - Abundance of small electrically charged organic molecules;

- **At Rest**, neurons are electrically negative in the inside compared to the outside,
- One explanation is that at rest the permeability of the neuronal membrane for K^+ ions is much higher than that for Na^+ ions;
- Thus, K^+ ions freely diffuse out creating a potential to balance concentration gradient of K^+ ions inside the cell;
- This process on completion gives the so-called "**Resting Potential**" of the neuron;

How are voltage-gated ion channels related to Action Potential?

- **Action Potential:**
- Action potential is a temporary change in membrane potential that is transmitted along the axon
- It is usually initiated in the cell body, travels in one direction normally;
- It can be separated into Phases:

Resting phase:

- Sodium activation gates are closed,
- Sodium inactivation gates are open, and Potassium gate is closed;
- Resting membrane potential is – 70mv inside the neuron;

Depolarization phase:

- Action potential begins with activation gates of Na^+ channels opening,
- Na^+ ions enter neuron causing depolarization, leading to the Spike of the Action Potential;
- Excess Na^+ ions enters causing **Reversal of Potential,** becoming briefly more Positive on the inside of the neuron

Re-polarization phase:

- Sodium inactivation gates close and Potassium gate open;
- Causes Na^+ ions to stop entering neuron and K^+ ions to exit neuron, leading to Re-polarization;
- Until membrane is Re-polarized it cannot be stimulated, called the **Absolute Refractory Period;**

Hyper-polarization:

- Excess Potassium exits the neuron causing a brief **hyper-polarization,**
- Sodium activation gates close and Potassium gates begin closing,
- Na^+, K^+ - ion pump begins to re-establish resting membrane potential (pumping Na^+ ions out and K^+ ions in);
- **An energy process requiring constant supply of ATP;**
- **Inhibition of energy supply to nervous system can lead to inhibition of impulse transmission along the axon;**
- During hyper-polarization the membrane can be stimulated but only with a greater than normal depolarization, the **Relative Refractory Period;**

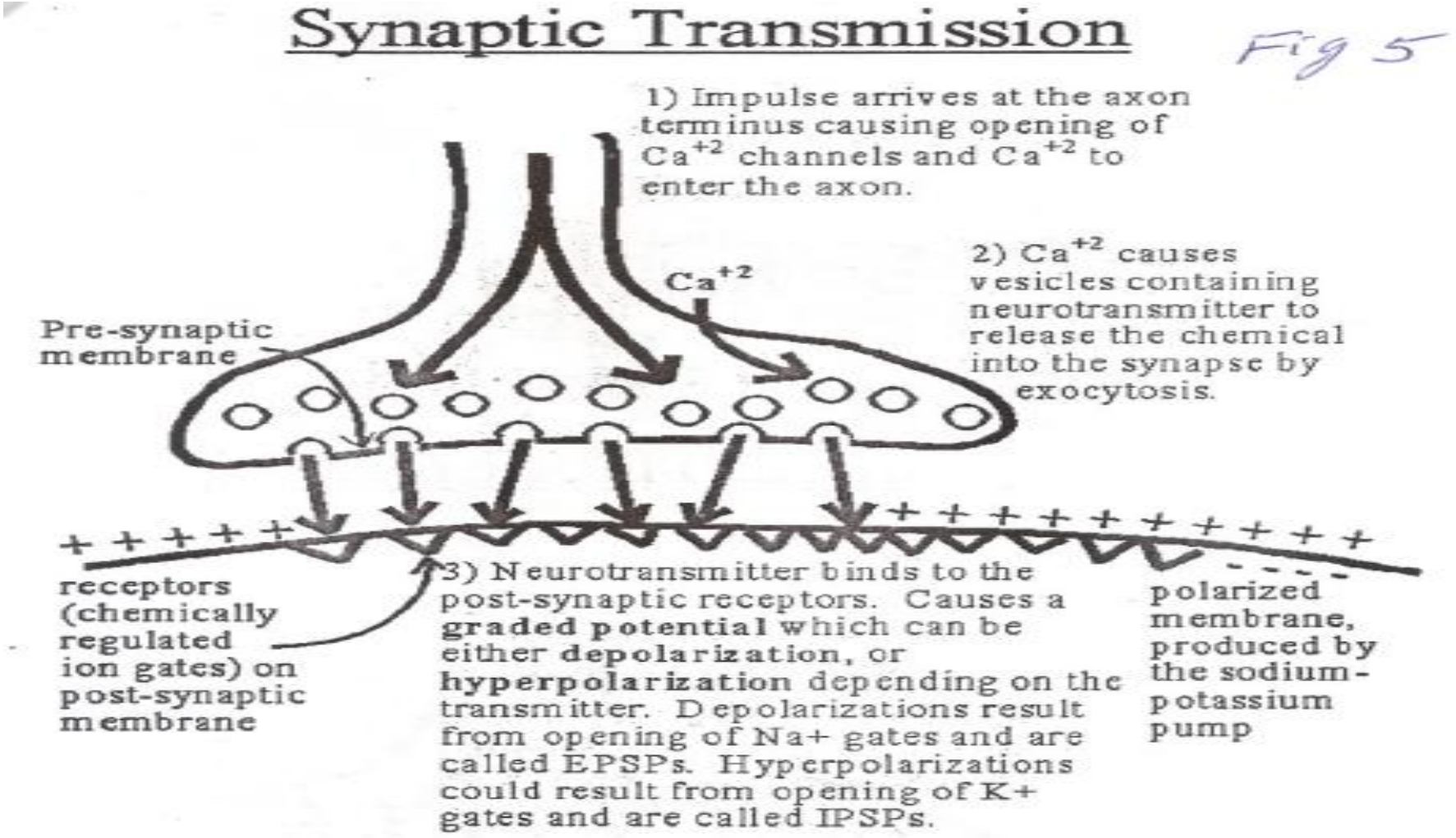
IMPORTANT TO NOTE!!

- **Action potential are self-propagated and once started it progresses along the axon membrane;**
- **It is all-or-none, that is, there are no different degrees of action potentials: You either have one or you do not;**
- **Action potential** is due to **transient changes in permeability of neuronal membrane to Na^+ ions and K^+ ions;**
- **Tetrodotoxin** a highly **potent Neurotoxin from Puffer fish**, blocks conduction of impulses along **Axons,**
  - It leads to respiratory paralysis by binding very tightly to Na^+ ion channels, thus blocking membrane transmission along axons;
- Signals that make the **cytoplasm more Positive** are said to **Depolarize the membrane**, those that make it **more Negative** are said to **Hyperpolarize the membrane**;

SYNAPTIC TRANSMISSION:
Impulse transmission across synapse (Fig.7)

- Synaptic transmission: propagation of impulses across synapse
  - Axon can make contact along a second neuron: on Dendrites (Axo–Dendritic Synapse), (Axo– Somatic Synapse) or Axons (Axo– Axonal Synapse);
- Neurotransmitters transmit Impulses across synapse,
- Neurotransmitter is released, diffuses across synaptic cleft to Post-synaptic cell, then binds specific receptors;
- Neurotransmitter binds to receptor and opens channels (ligand-gated channels) allowing Na^+ and K^+ ions to flow in and out of the Postsynaptic cell, respectively;

Fig. 7: Synaptic Transmission



- **Excitatory Postsynaptic Potential (EPSP) is produced If the ions depolarize the Postsynaptic cell;**
  - Neurotransmitters that produce EPSPs: Acetylcholine, Epinephrine, and Norepinephrine;
- **Inhibitory Postsynaptic Potential (IPSP) is produced if ions Hyper-polarizes (cytoplasm more Negative) the membrane**
  - Neurotransmitters that produce IPSPs: Glycine and GABA (Gamma Amino-Butyric Acid);
- Depolarization of postsynaptic membrane initiates a new action potential in the postsynaptic neuron;
- Neurotransmitter action on receptors on the postsynaptic membrane may be terminated either by:
  - Specific enzymes,
  - Reuptake, or
  - Diffusion into Glea cells;

NEUROMUSCULAR TRANSMISSION

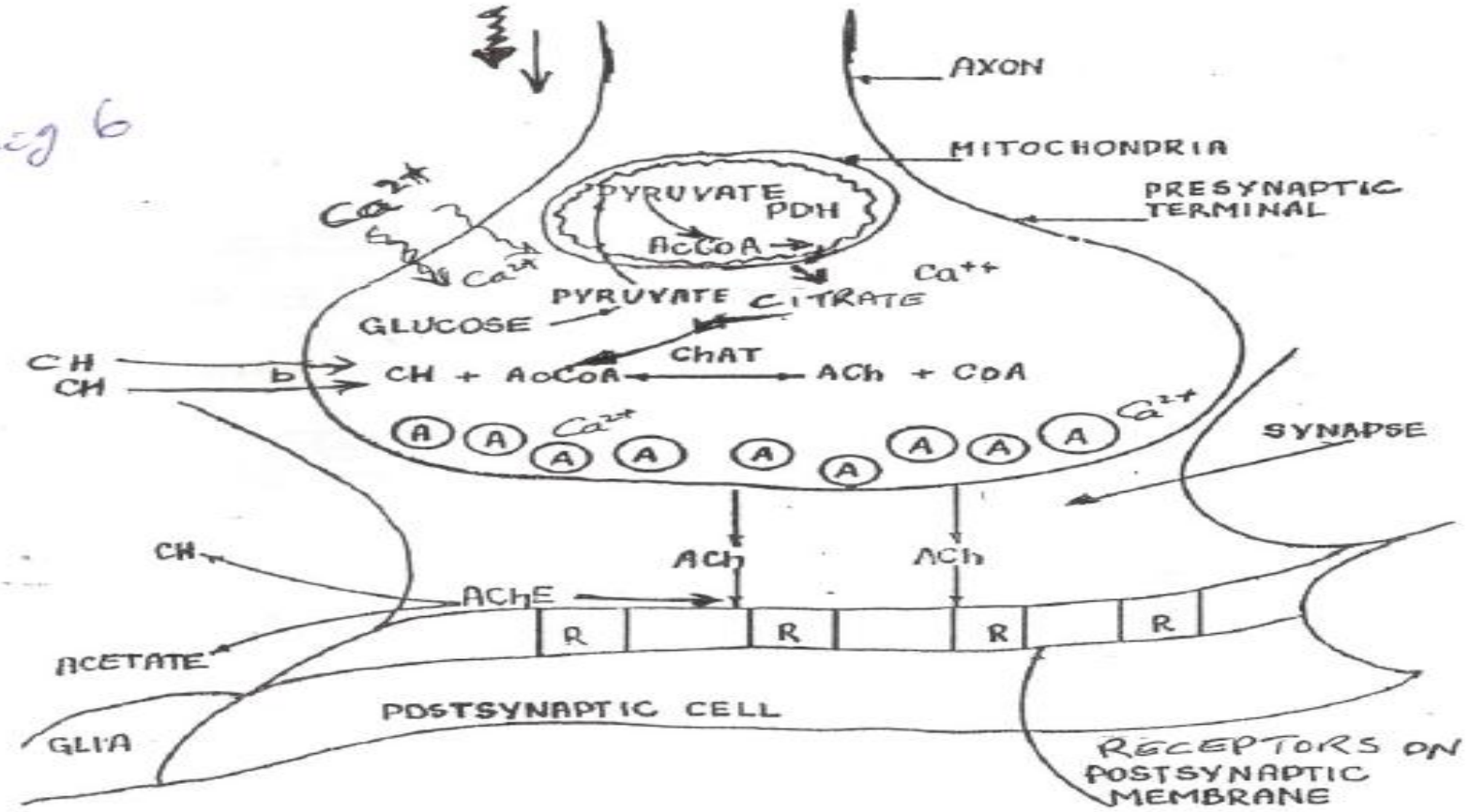
- Impulse transmission from neuron to skeletal muscle fiber, (neuromuscular junction);
- Action potential occurring at the site is called Neuromuscular Transmission;
- At a neuromuscular junction, the axon subdivides into numerous terminal buttons that reside within depressions formed in the motor end-plate;

Outline the steps involved in Neuromuscular Transmission (Fig. 8)

- Action potential (inhibited by Tetrodotoxin) arrives at the axonal terminal (Presynaptic Knob),
- Ca channels open, Ca^{2+} ions rushes into axonal terminal, because Ca^{2+} outside is much higher than Ca^{2+} inside;
- Axonal terminal is loaded with vesicles containing the neurotransmitter, Acetylcholine (ACh);
- Ca^{2+} causes some of the vesicles to fuse with the membrane and release their ACh (inhibited by Botulinum toxin);
- ACh diffuses across neuromuscular junction and binds ACh receptors (inhibited by Curare) on postsynaptic membrane;
- Binding causes ion channels to open;
- Flow of ions depolarize membrane, producing EPSP;

- In muscle impulse causes depolarization to reach threshold;
- Action potential is generated in muscle membrane causes release of Ca^{2+} from Sarcoplasmic Reticulum,
- This triggers muscle contraction;
- At receptor site in neuromuscular junction the ACh is broken down to Acetate and Choline by the enzyme **Acetylcholinesterase;**
  - Enzyme is inhibited by Physostigmine, Nerve gases, Organophosphate insecticides;
- Choline is recycled: transported into the nerve terminal and used to produce ACh;

Fig. 8: Neuromuscular Transmission



PDH = PYRUVATE DEHYDROGENASE COMPLEX
R = CHOLINERGIC RECEPTORS
CH = CHOLINE
ChAT = CHOLINE ACETYL TRANSFERASE
AChE = ACETYLCHOLINESTERASE
b = SITES OF HIGH AFFINITY CHOLINE TRANSPORT SYSTEM
A = VESICLES CONTAINING ACETYLCHOLINE
ACh = ACETYLCHOLINE

CHOLINERGIC NERVE TERMINAL

(ADAPTED & MODIFIED FROM BRAIN RESEARCH REVIEW, VOL. 1, No. 3, 1979)

Stages in chemical neurotransmission and role of Ca^{2+} ions in the release of neurotransmitters

- Wave of depolarization reaches presynaptic terminal,
- Voltage-Gated Ca^{2+} ion channels open up;
- Ca^{2+} ions enter cell, increasing the concentration;
- Ca^{2+} ions activate **Calcium-Calmodulin-dependent Protein Kinase**;
  - Enzyme that Phosphorylates specific protein (**SYNAPSIN-1)** attached to surface of presynaptic membrane;
- **De-phosphorylated Synapsin-1** prevents synaptic vesicles from making contact with the Presynaptic membrane;

- **Phosphorylated Synapsin-1** dissociates from the membrane, allowing synaptic vesicles to attach to the presynaptic membrane;
- Synaptic vesicles release neurotransmitter into synaptic cleft via process of exocytosis;
- After the release Synapsin-1 is de-phosphorylated and displaces the empty synaptic vesicles;
- Displaced vesicles then take up neurotransmitter and start the cycle over again;
- Neurotransmitter that is released diffuses across the synaptic cleft, binds specific receptors on membrane of post-synaptic cell;

- Binding of neurotransmitter to receptors cause channels to open, for ions to flow in and out of the cell;
- Depolarization of post-synaptic membrane gives new action potential;
- Once signal has been delivered neurotransmitter must be removed from receptors;
- Some chemical or drugs may inhibit enzymes that break down neurotransmitters (examples Nerve gases, Physostigmine);
- Other drugs act by inhibiting recycling of neurotransmitters (examples Prozac, Cocaine);

What are the effects of chemical neurotransmitters on membrane receptors?

- Some neurotransmitters such as **Acetylcholine, Glycine, Glutamate, GABA** have an "inherent" biological activity such that the neurotransmitter act directly (**Ionotropic Receptors**) to cause increase in conductance of ions by binding to "Ligand-activated" ion channels at the post-synaptic membrane.;

- Other neurotransmitters, such as, **Norepinephrine, Dopamine** and **Serotonin**, have no direct activity but act indirectly via **2nd Messenger** systems to bring about the post-synaptic response;
- The 2nd Messenger systems involve compound such as, cAMP, cGMP, ITP, PGs, Epoxides and Ca^{2+} ions;
- The 2nd Messengers act in the Cytosol to activate target proteins, including Protein Kinases, which in turn act on substances such as, Ion channels, to produce the neurotransmission effect;

- If it is an Excitatory Neurotransmitter, then it causes depolarization of the post-synaptic cell;

- If it is an Inhibitory Neurotransmitter, then it causes Hyper-polarization by increasing Chloride ion conductance of the post-synaptic membrane, thus making it more difficult for the cell to depolarized;

Toxins and diseases that affect neuromuscular junction & synaptic transmission

- ACh release in NMJ is inhibited by **Botulinum toxin**;
- Glycine release in CNS is inhibited by **Tetanus toxin**;
- Black Widow Spider toxin, **Alpha-Latrotoxin**, stimulates fusion and depletion of neurotransmitter vesicles;
- **Acetylcholinesterase** is inhibited by Physostigmine, Nerve gases and Organophosphorus pesticides;

- Muscle ACh receptor is blocked by South American arrow poison, **Curare**;
- **Atropine**, inhibits ACh receptors of the Autonomic Nervous system (but not the NMJ);
- **Strychnine** binds to Glycine receptor and inhibits IPSPs in the spinal cord;
- **Cocaine** blocks the recycling of Dopamine and Norepinephrine in the brain; This has an excitatory effect;

Diseases affecting Synapses and Neuromuscular junctions

- **Eaton-Lambert syndrome**: patient produces antibodies that attack his own Ca channels, which results in low Ca in the synapse inhibiting the release of neurotransmitters;
- **Myasthenia gravis**: autoimmune disease that damages receptors in Cholinergic neurons;
- **Parkinson's disease**: cells in Substantia Nigra in the brain are deficient in Dopamine;
- **Clinical depression**: associated with low levels of Serotonin in parts of the brain;

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