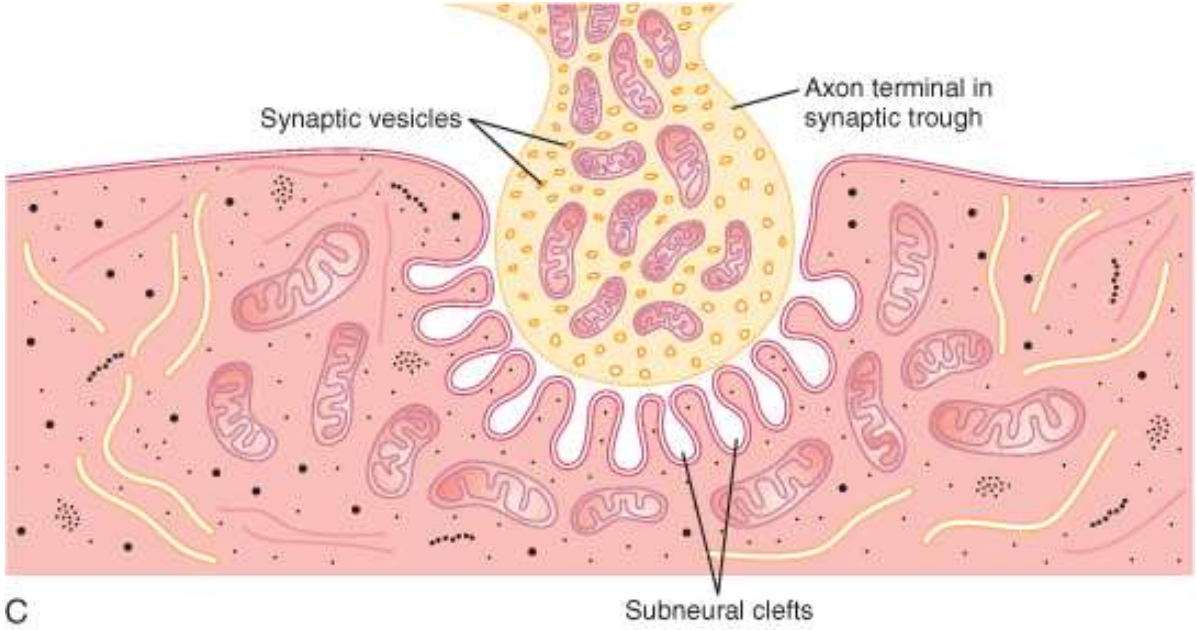
Neuro-muscular junction



Dr Gauri Apte

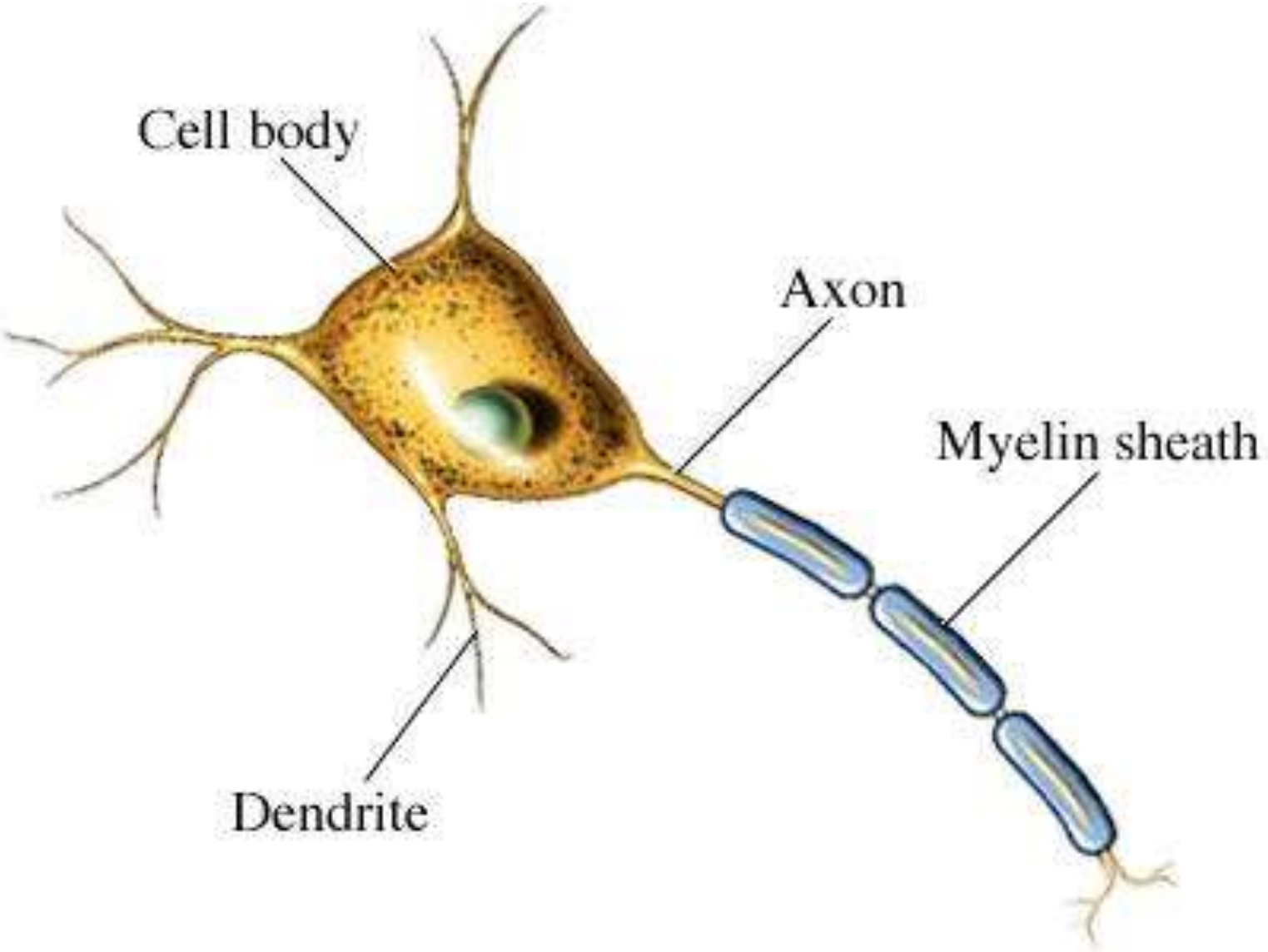
Competencies covered

PY3.4: Describe the structure of neuromuscular junction and transmission of impulses.

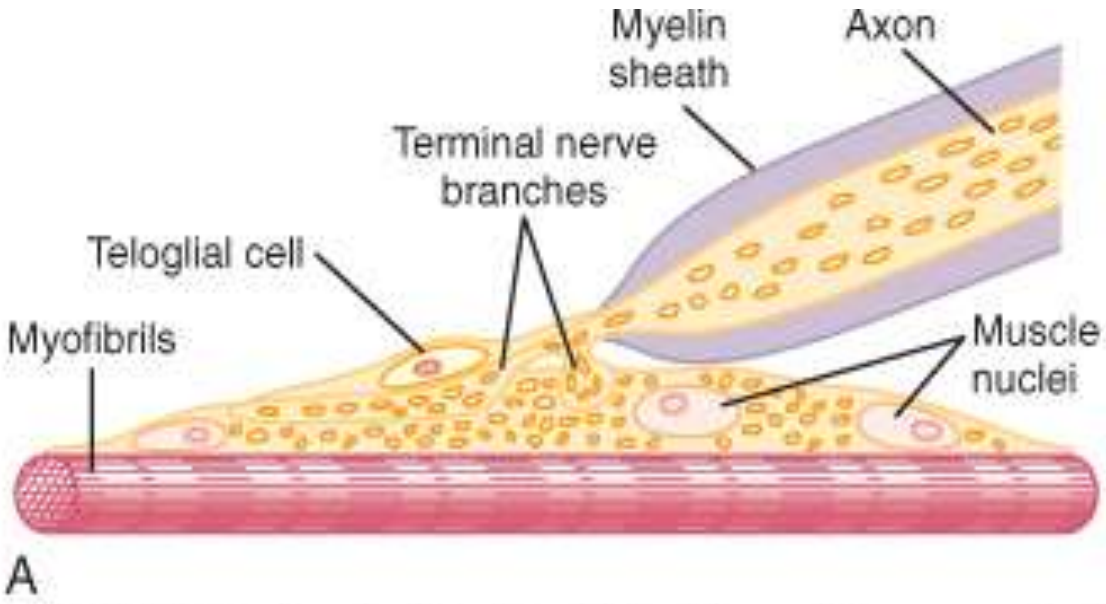
PY3.5: Discuss the action of neuromuscular blocking agents.
PY3.6: Describe the pathophysiology of myasthenia gravis.

Neuro-muscular junction

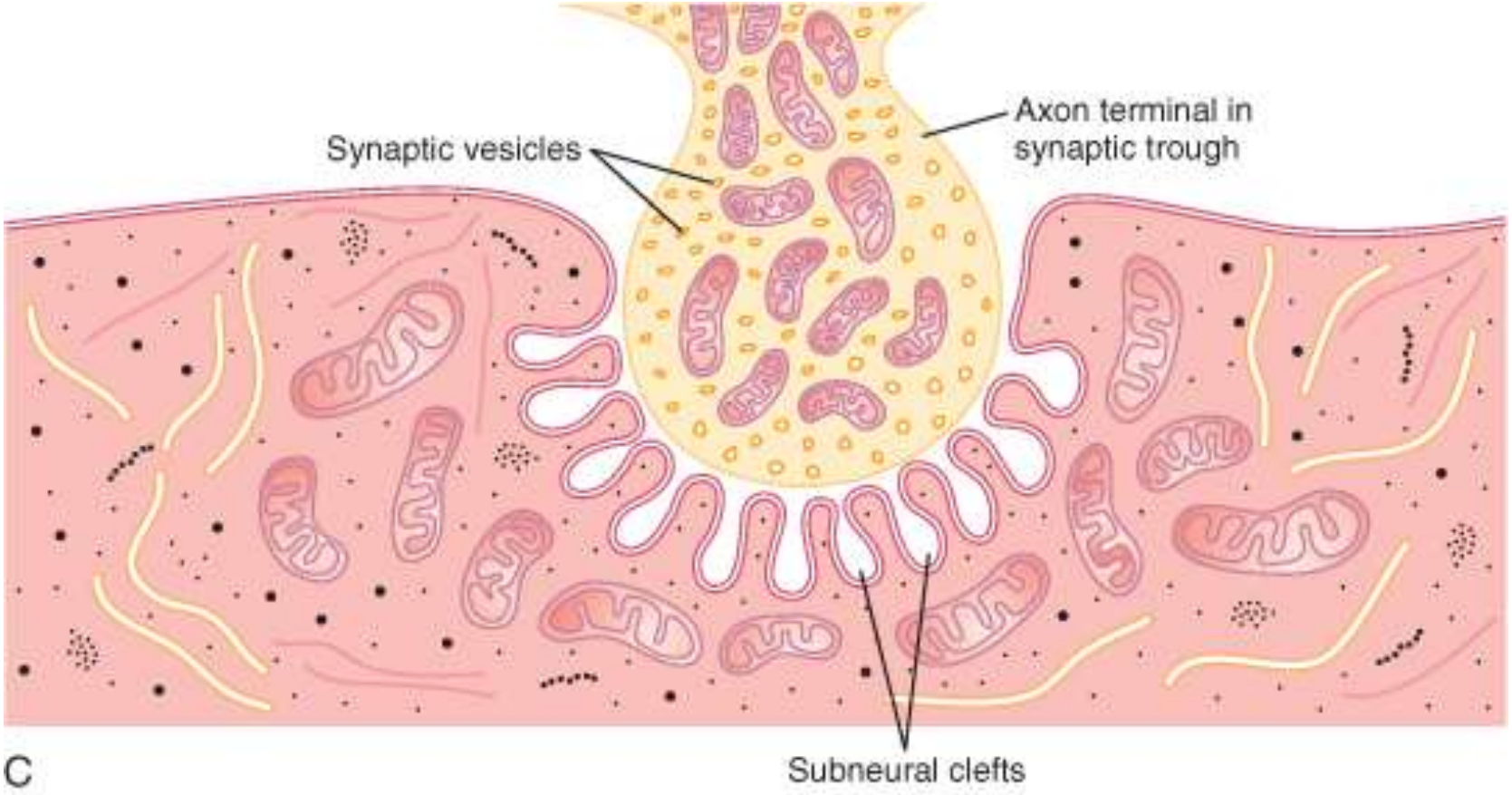
- The skeletal muscle fibers are innervated by large, myelinated nerve fibers that originate from the **anterior horn cells** of the spinal cord or **cranial nerve nucleus** in brainstem.
- Each nerve fiber branches and stimulates from three to several hundred skeletal muscle fibers.
- A nerve fiber with all the muscle fibers supplied by it, together form a **Motor unit**.
- Each nerve ending makes a junction, called the neuromuscular junction/ motor end plate, with the muscle fiber near its midpoint.


Cell body
Axon
Myelin sheath
Dendrite

Neuro-muscular junction

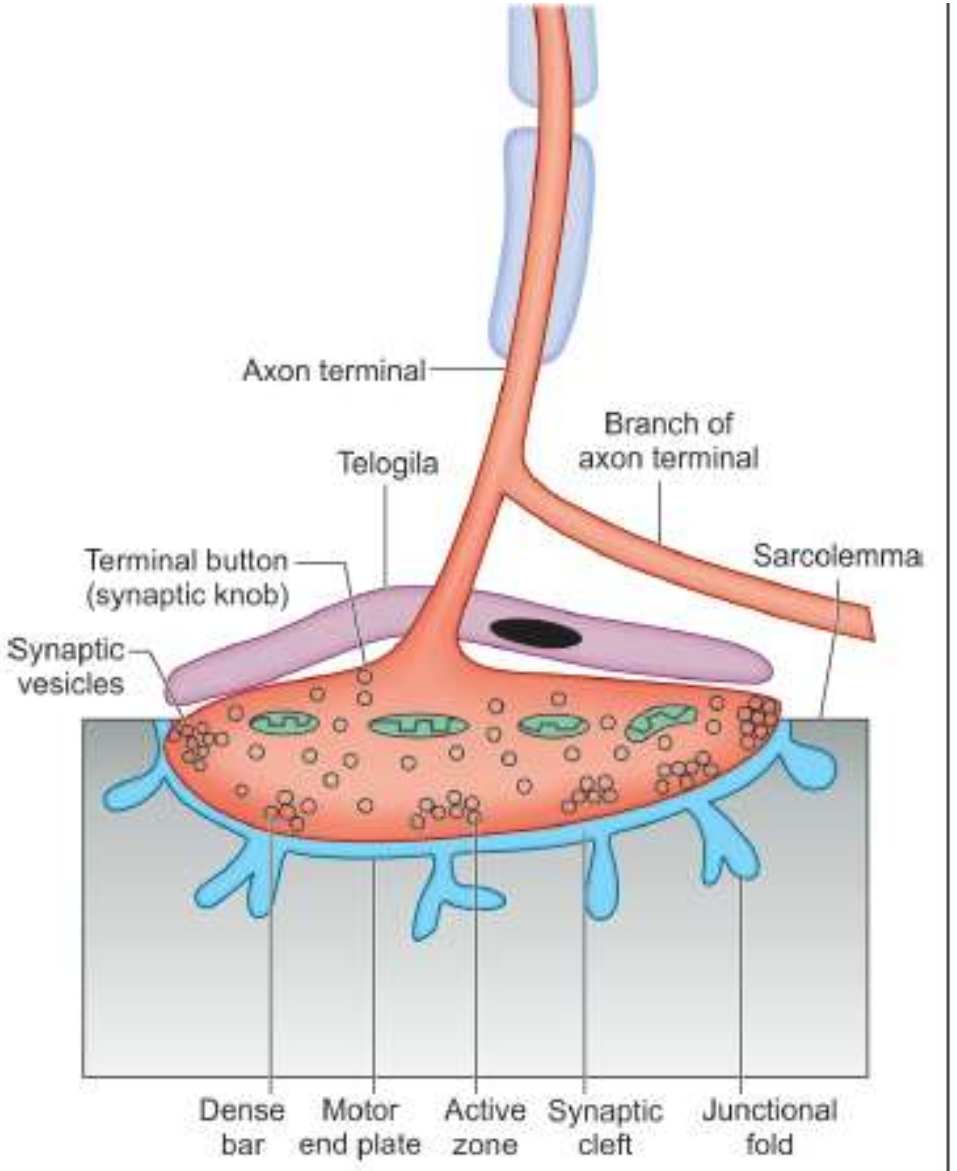


Neuro-muscular junction



Structure of NMJ

- Broadly divided into three parts: Presynaptic, synaptic, and postsynaptic portions.



Presynaptic Portion : Axon terminal/ axon button

- Dialated end of the nerve.
- The terminals are covered by Schwann cells, known as **teloglia** (glial cells at terminals).
- Contains mitochondria, **acetyl choline vesicles**, calcium channels.
- The terminal button lies in the groove (**synaptic trough**) on the surface of the muscle fiber.
- The vesicles are clustered around specific points called active zones.
- The membrane at the active zones is modified to form dense bar that contains numerous **voltage-gated Ca2+ channels**.

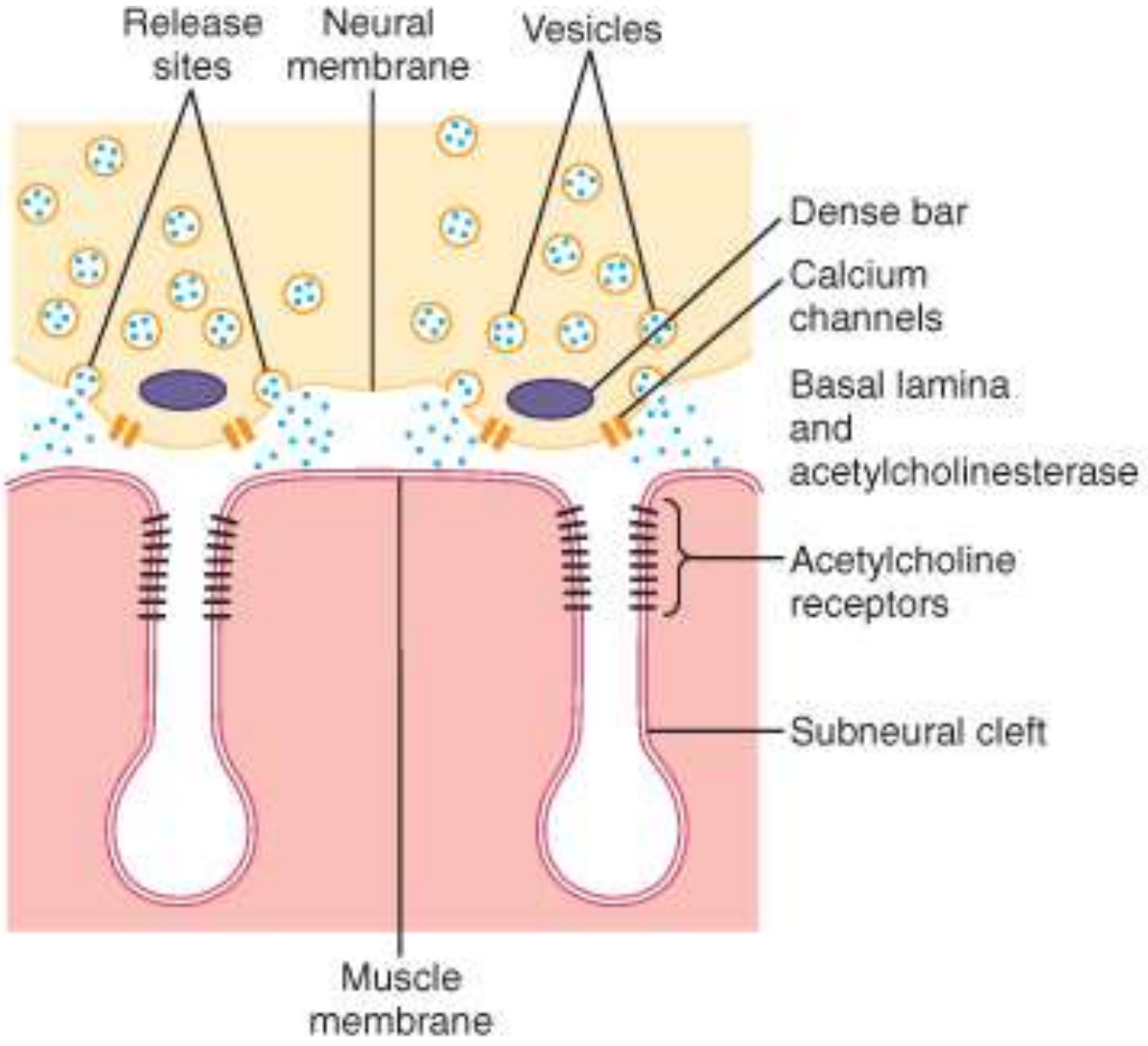
Subneural cleft

- This is the gap between the terminal button and the muscle fiber, which is about **40–100 nm wide**.
- Contains cholinesterase enzyme
- Acetylcholinesterase rapidly hydrolyzes ACh into acetate and choline.

The postsynaptic portion

- Also known as the end-plate membrane (motor endplate).
- The area of the end-plate membrane increases many times as it is thrown into several folds called **junctional folds**.
- The end-plate membrane contains numerous **Ach receptors (AChRs),** which are concentrated at the crests of the junctional folds.
- It also contains voltage-gated Na+ channels.

Neuro-muscular junction

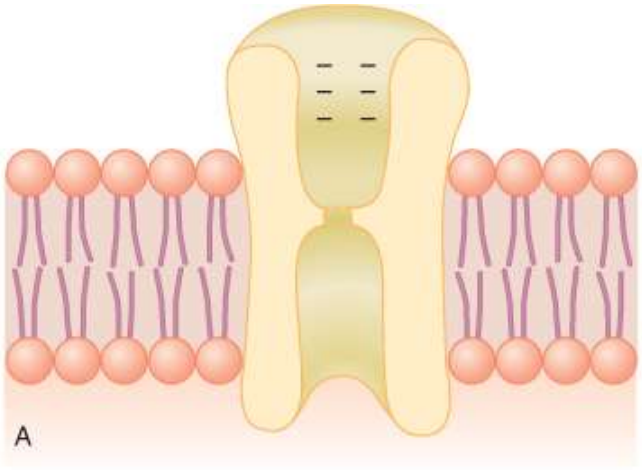


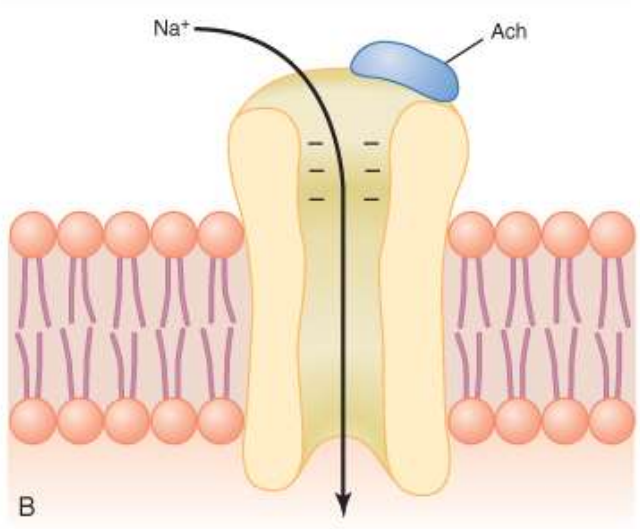
Acetylcholine Receptors at Neuromuscular Junction

- Acetylcholine receptors (AChRs) at NMJ are **nicotinic type.**

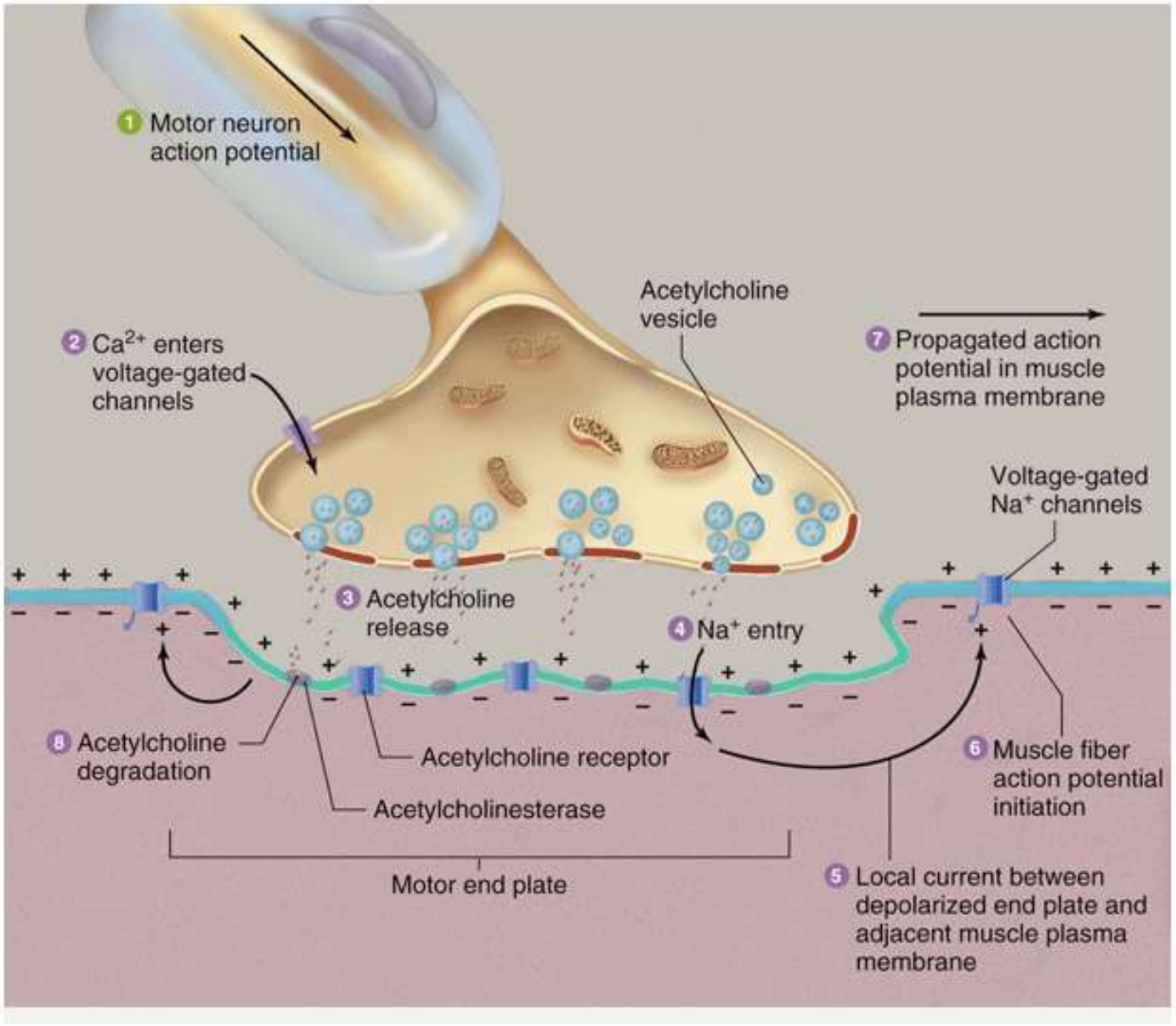
- The AChR protein is a **chemically gated** ion channel that opens when two molecules of ACh bind to it.

Receptor for Ach





Events in neuromuscular transmission



MECHANISMS OF NEUROMUSCULAR TRANSMISSION

Presynaptic Events

1. Arrival of an action potential at the axon terminal **depolarizes the membrane** of the terminal buttons.
2. This causes activation and **opening of the voltage-gated calcium channels**, which leads to **calcium influx**.
3. Increased cytoplasmic calcium concentration leads to **migration of neurotransmitter vesicles** to the presynaptic membrane.
4. The vesicles **fuse with the membrane** and discharge their contents into the synaptic cleft by **exocytosis**.

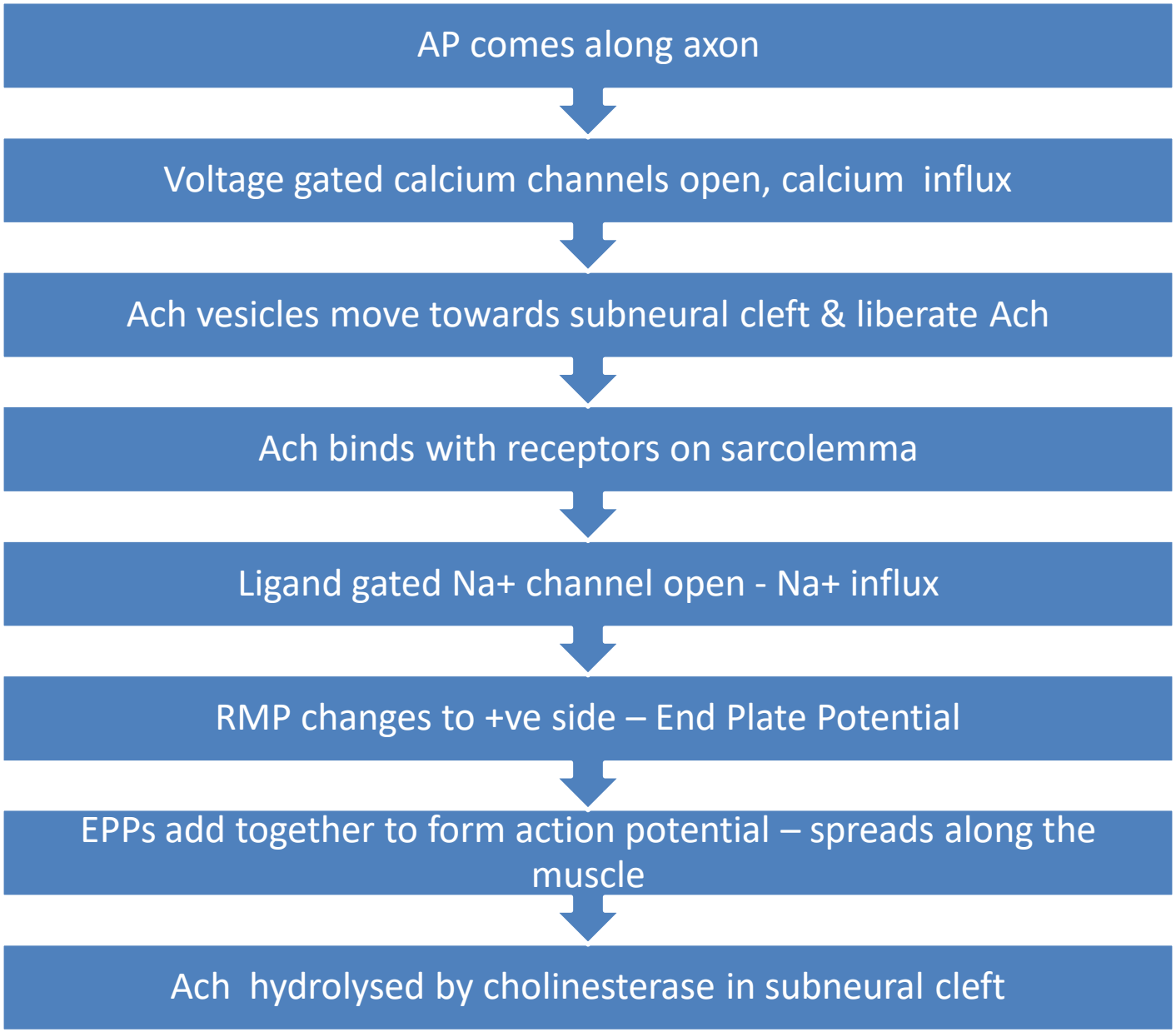
EVENTS AT THE SYNAPTIC CLEFT

The released ACh molecules diffuse across the synaptic cleft and **bind to the receptors present at the postsynaptic membrane**.

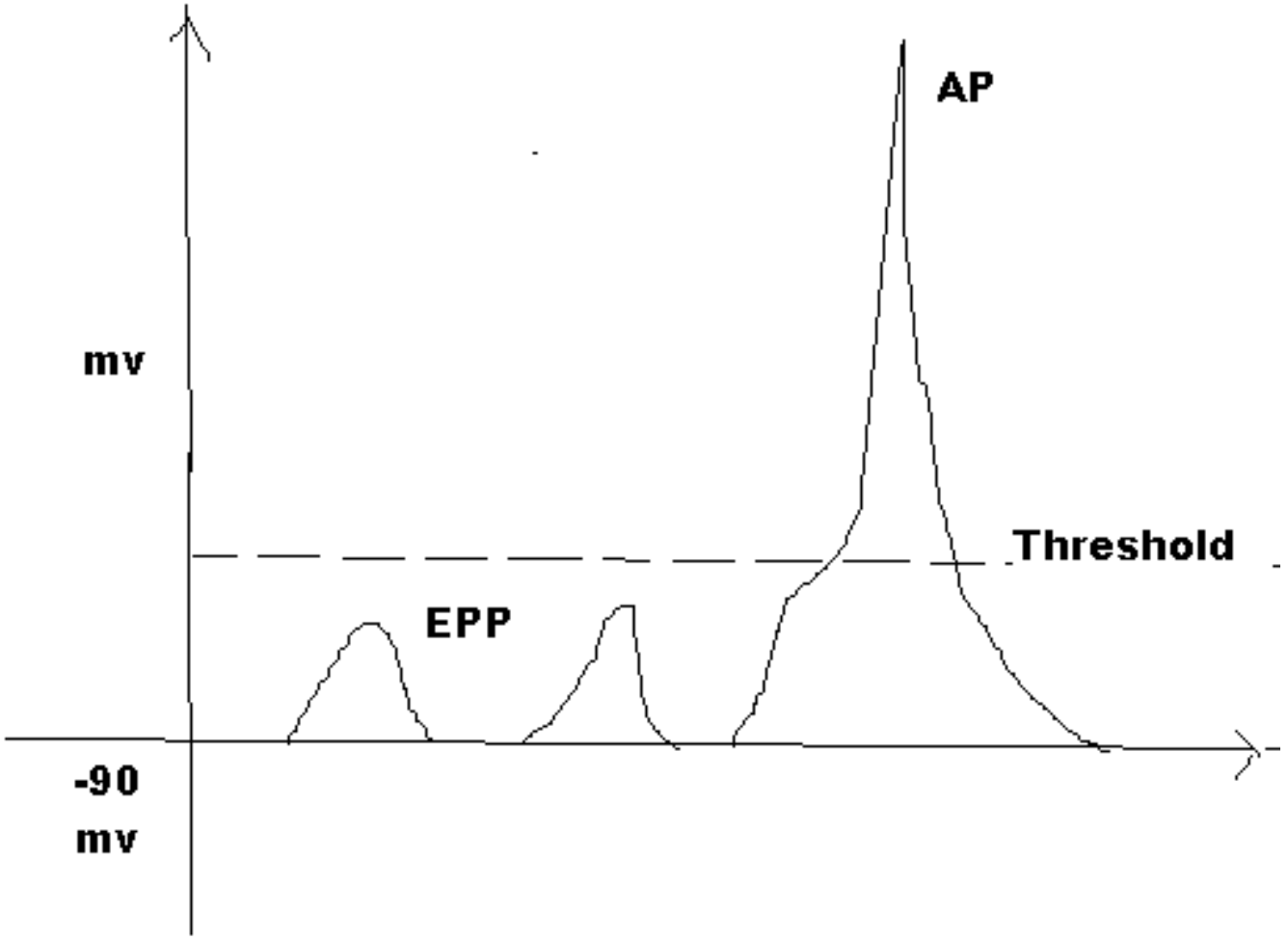
1. Some Ach molecules are **hydrolyzed by AChE**, and some ***diffuse out of the synaptic cleft***.
2. Remaining Ach is **sufficient enough** to produce an action potential.

EVENTS AT THE END-PLATE

1. The binding of ACh to AChR in the end-plate membrane allows Na+ influx, producing a graded potential known as **end-plate potential (EPP)**.
2. End-plate potential causes the firing level and opens the voltage-gated Na+ channels resulting in an **action potential.**
3. The action potential propagates along the sarcolemma.
4. The binding of ACh to AChR is **loose and reversible**.
5. This leads to ***closure of ion channels***, ***cessation of endplate potential,*** and consequently action potential.
6. The acetate diffuses away into the extracellular fluid and choline is taken up by the presynaptic terminal to be reused for the synthesis of new ACh.


AP comes along axon
Voltage gated calcium channels open, calcium influx
Ach vesicles move towards subneural cleft & liberate Ach
Ach binds with receptors on sarcolemma
Ligand gated Na+ channel open - Na+ influx
RMP changes to +ve side – End Plate Potential
EPPs add together to form action potential – spreads along the muscle
Ach hydrolysed by cholinesterase in subneural cleft

End plate potential & action potential in muscle membrane



End-plate potential

- The end-plate potential (EPP) is like a graded potential, the amplitude of which is proportionate to the amount of neurotransmitters released.
- The EPP is always a depolarizing potential.
- Stimulation of a single motor neuron produces an EPP of about 70 mV. EPP of this magnitude is much bigger than that required (15 mV)

Miniature End-plate Potential

- Spontaneously occurring potentials of minute amplitude (0.1–4 mV) in the end-plate membrane at rest.
- They occur **several times a second.**
- Cause: *release of the contents of a single ACh vesicle* by exocytosis (**a quantal event**) that opens around **2,000 ACh channels** in a very small area of the end-plate membrane.

Features of NM transmission

- High Safety factor
  - When motor signal comes along the axon, Ach release is very high, 3 times as much EPP is produced as required for normal transmission.
  - AP develops in muscle without wasting the signal.
- Fatigue
  - When axon is stimulated repeatedly, all Ach gets depleted & transmission fails.
  - Reversible, relieved by rest as Ach is resynthesized.

APPLIED ASPECT

BLOCKADE OF NEUROMUSCULAR TRANSMISSION

- The neuromuscular transmission is disrupted at different steps by **drugs, chemicals, toxins, and trauma**.
- Failure to generate required EPP leads to paralysis of the skeletal muscles.
- The drugs causing muscle relaxation are used during surgery and in some hyperactive states.
- The blockade can occur at presynaptic or postsynaptic level.

PRESYNAPTIC BLOCKADE

- **Botulinum Toxins (Blocks calcium channels)**

The bacterium *Clostridium botulinum* releases a toxin that causes a paralytic disease called **botulism.**

1. One of the most potent natural toxins
2. The **lethal dose** for an adult human is around 2–3 µg.
3. Inhibits the release of acetylcholine from the axon terminals resulting in cessation of muscle contraction (flaccid paralysis)

Clinical use of botulinum toxin:

For therapeutic & cosmetic purpose: Injection of highly diluted toxin produces local relaxation.

Treatment of Achalasia cardia, Strabismus, Age related wrinkles etc.

Can be used in **Bio-terrorism**.

Postsynaptic Blockade

- A variety of agents can act on the postsynaptic membrane interfering with generation of EPP by different mechanisms.

- They may be classified into competitive blockers and depolarizing blockers.

Competitive Blockers

Competitive blockers are agents that compete with ACh to bind to AChR on end-plate membrane. For examples, curare and gallamine.

- **ACh binding sites in the receptors remain occupied permanently**.
- **Ach fails to bind to its receptor**

Curare

Curare is a plant product used as a South American arrowhead poison for hunting.

The animal hit by such arrow suffers from **paralysis in all skeletal muscles** including the respiratory muscles.

Consequently, the animal dies of asphyxiation.

Gallamine

Gallamine is given before surgery to **relax the skeletal muscles**.

1. It reduces the required dose of general anesthetic, as well as bleeding and other complications.

Depolarizing Blockers

Drugs, like **succinylcholine** and **carbamylcholine,** have the biological activity of ACh, but they are not hydrolyzed by AChE. Therefore, their action is long lasting.

- When they bind to AChR, the **ion channels remain open** in the end-plate.
- The maintained depolarization keeps the ***voltage-gated Na+ channels in an inactivated state*** and ***blocks subsequent action potentials***.
- There are other drugs that produce the blockade by inactivating AChE reversibly or irreversibly.

Reversible Acetylcholinesterase Inhibitors

These agents compete with ACh to bind to AChE, thereby, **prevent the hydrolysis of ACh** by AChE.

The accumulated ACh at the synaptic cleft binds to AChR, leading to a **depolarizing block**.

1.This is of use in diseases like myasthenia gravis

2. **Neostigmine** and **physostigmine** (eserine) are examples of reversible AChE inhibitors.

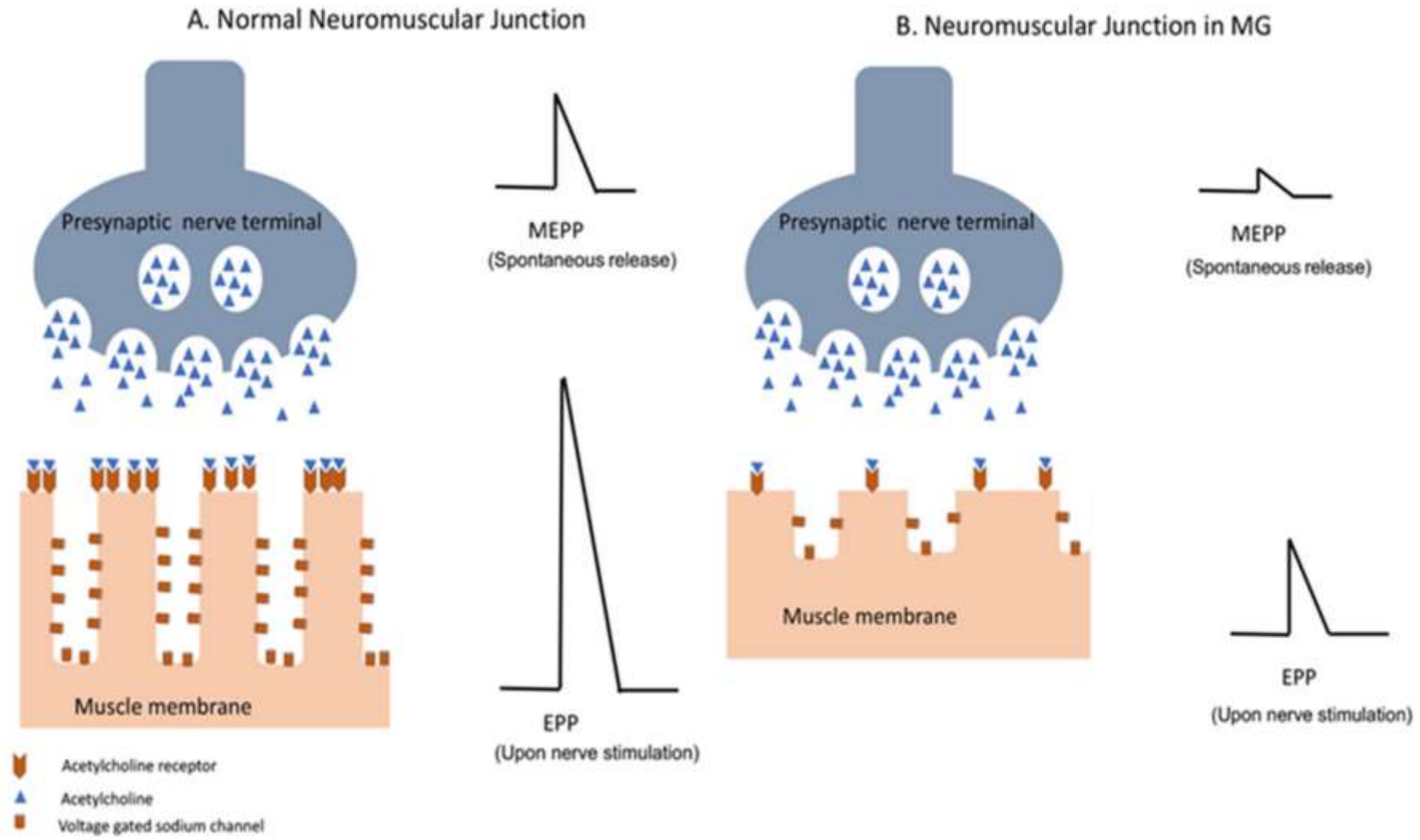
Irreversible Acetylcholinesterase Inhibitors

The **organophosphorus compounds** (pesticides like parathion, malathion, and baygon) and **nerve gases** used in chemical warfare, like diisopropyl fluorophosphate (DFP) and sarin, **bind to AChE very tightly**. This binding is irreversible.

1. **Sustained depolarization** of the end-plate resulting in no further contractions.
2. Poisoning with these agents lead to skeletal muscle paralysis and death from asphyxiation.

Myasthenia gravis

- Auto-immune disease – antibodies against Ach receptors – Neuromuscular transmission reduced



Pathophysiologic changes in NMJ

- Myasthenia gravis results from a decrease in the number of AChR
- Postsynaptic folds are flattened or simplified.
- Synaptic space is widened
- Due to these changes, though ACh is released normally, the magnitude of the end-plate potential falls below the threshold for initiating a muscle action potential.

Features of Myasthenia gravis

- 1. Fatigue: After a few motor nerve impulses, the successive contractile responses become feebler, causing fatigue (**decremental response**).
- 2. Women are affected more than men in a ratio of 3:2.
- 3. Progressive paralysis in muscles – limb muscles, facial muscles
- 4. Ptosis- drooping of upper eyelid, diplopia- double vision
- 5. Proximal limb muscles are commonly affected. In severe cases, respiratory muscle paralysis leads to death.

Ptosis in Myasthenia Gravis

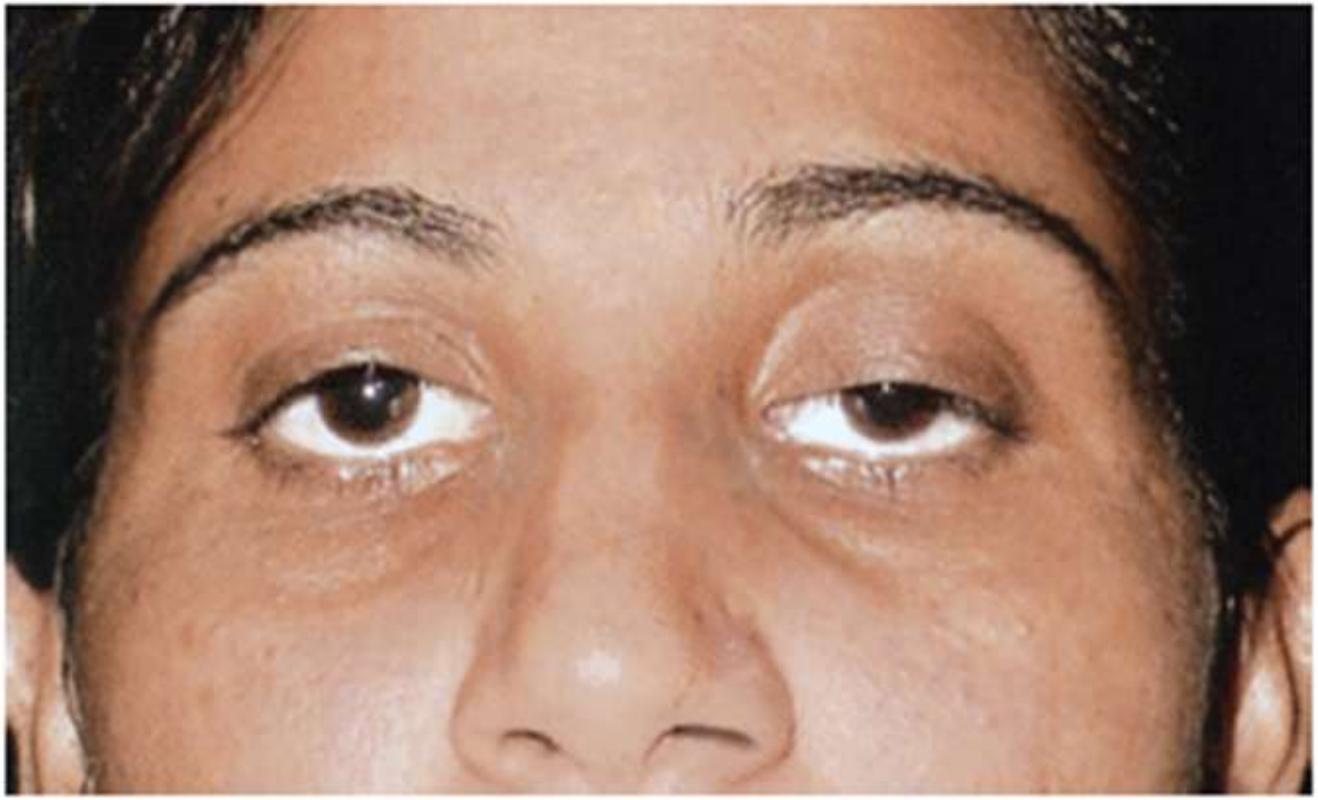


Fig. 29.3: Ptosis (as seen in myasthenia gravis). The left eye is more affected than the right eye of this female.

Courtesy: Figure 15.33 A. Khurana AK. Comprehensive Ophthalmology, 6th edition. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2015.

Physiological Basis of Treatment

- 1. Administration of AChE inhibitors:
  - increase the concentration of ACh at the neuromuscular junction.
  - Drugs, such as pyridostigmine and neostigmine
- 2. Thymectomy:
  - The immune responses mediated by T cells are part of the pathophysiologic mechanisms.
  - Thymectomy blunts down the immune response and improves the condition.
- 3. Immunosuppression:
  - Immunosuppressive drugs, glucocorticoids and azathioprine.
- 4. Plasmapheresis:
  - Removes AChR antibodies from plasma.

Lambert-Eaton Myasthenic Syndrome

- Production of autoantibodies against voltage-gated Ca2+ channels.
- The decreased Ca2+ influx in the presynaptic axon terminals results in impaired ACh release from the nerve endings.
- The muscular weakness is primarily seen in the limb muscles.
- Patients show **incremental response** to repetitive nerve stimulation as Ca+2 level raises with each action potential.
- Thus, with prolonged contractions, muscle strength increases.