Basic mechanisms of cell communication:

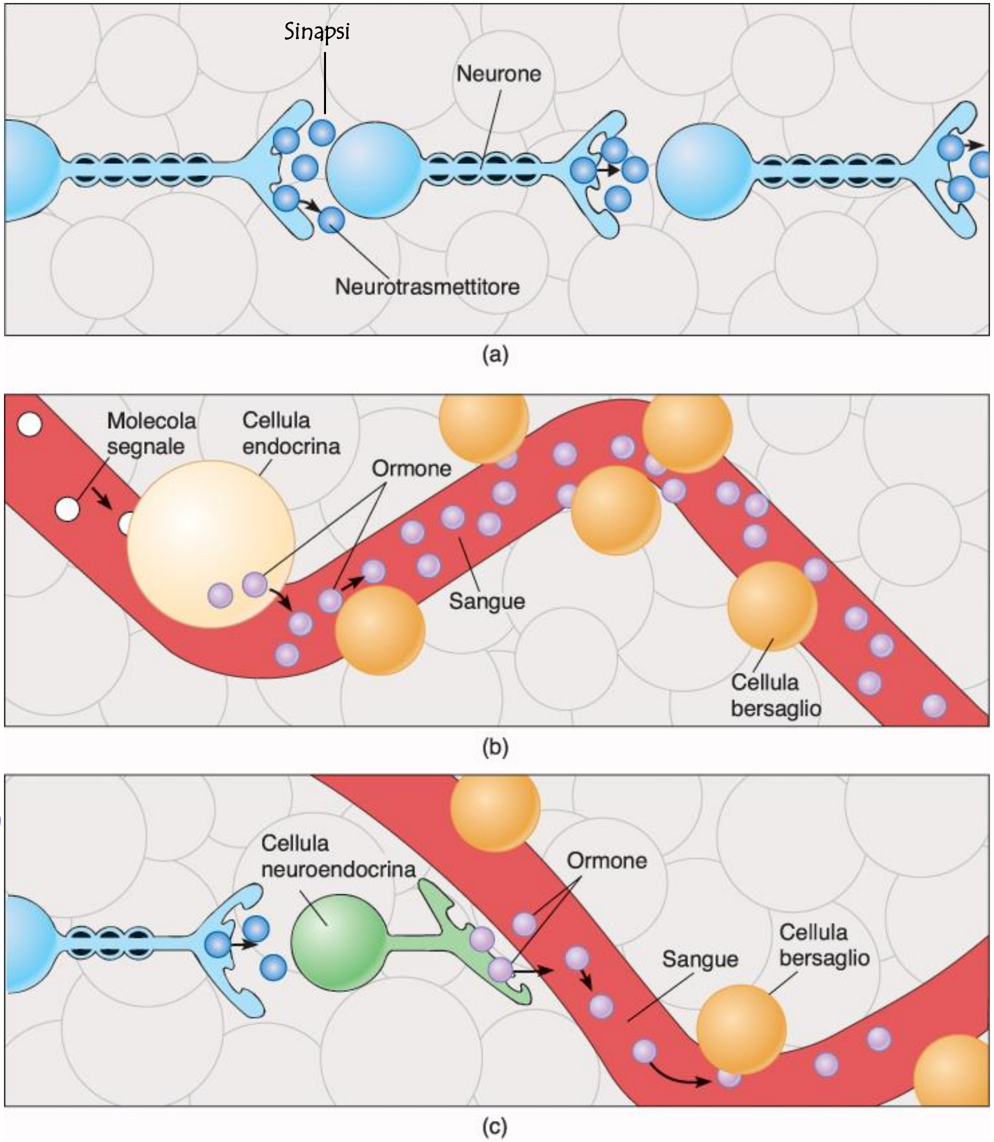
Neuronal communication

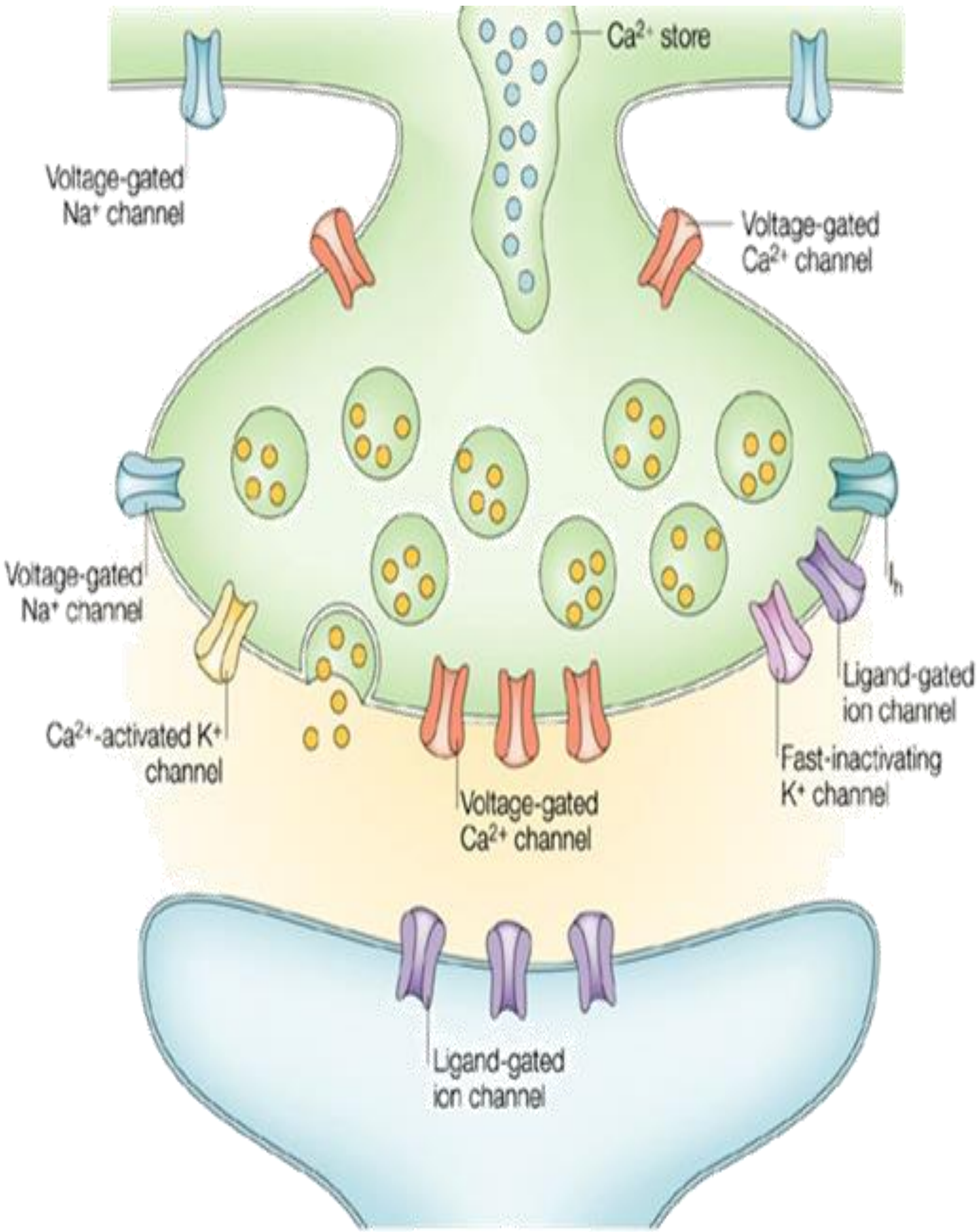
Quick answer
fast
muscular and secretory cells

Endocrine communication:

Slow response
durable
Cell functions, metabolism

Neuroendocrine communication



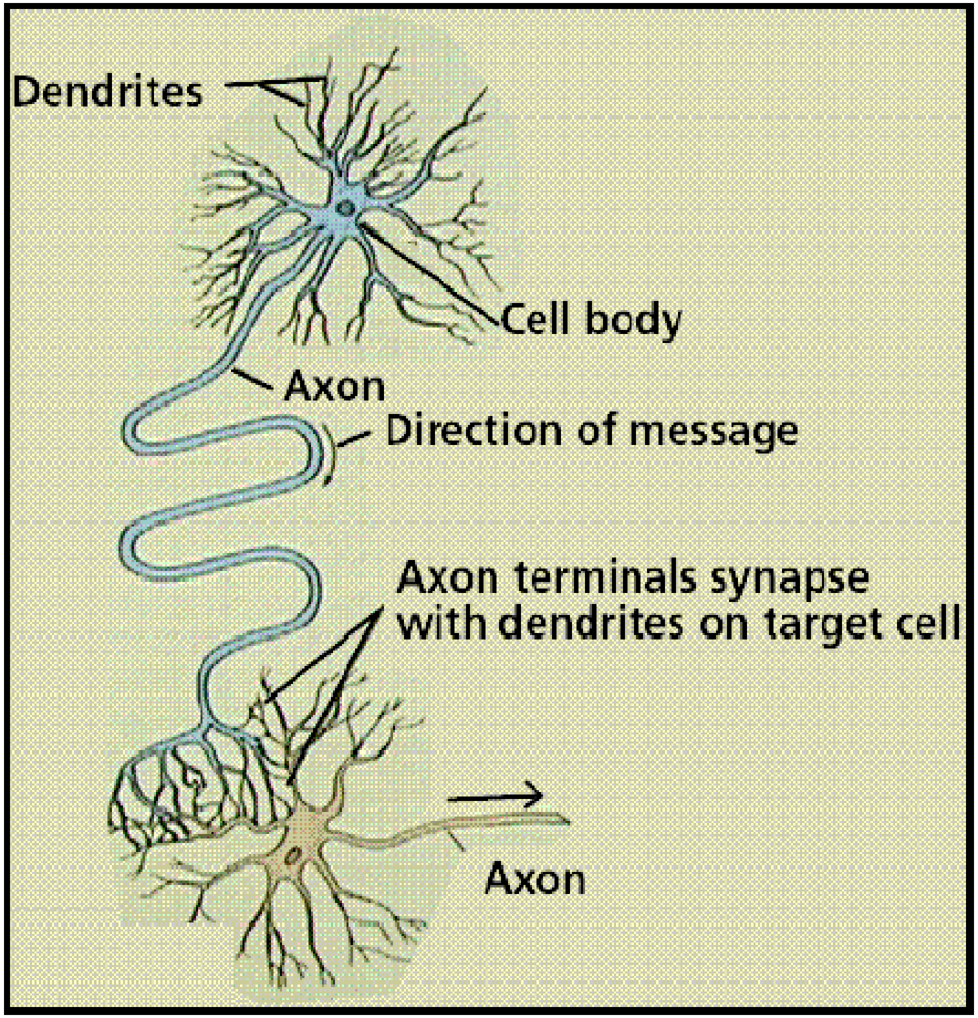


Synaptic Transmission

Synapses: what are they?

Specialized contact sites that allow the transmission of the nerve signal from one neuron to another

Connecting elements between nerve cells



A synapse is a site of close contact between a neuron and a target cell, such that an electrical signal in the neuron leads to a change in the probability that the target cell will produce an action potential.

-if the probability increases, the synapse is excitatory

-if the probability decreases, the synapse is inhibitory

Synaptic transmission is the closest example of intercellular communication

- Synaptic transmission

the target cell is distant <1 µm

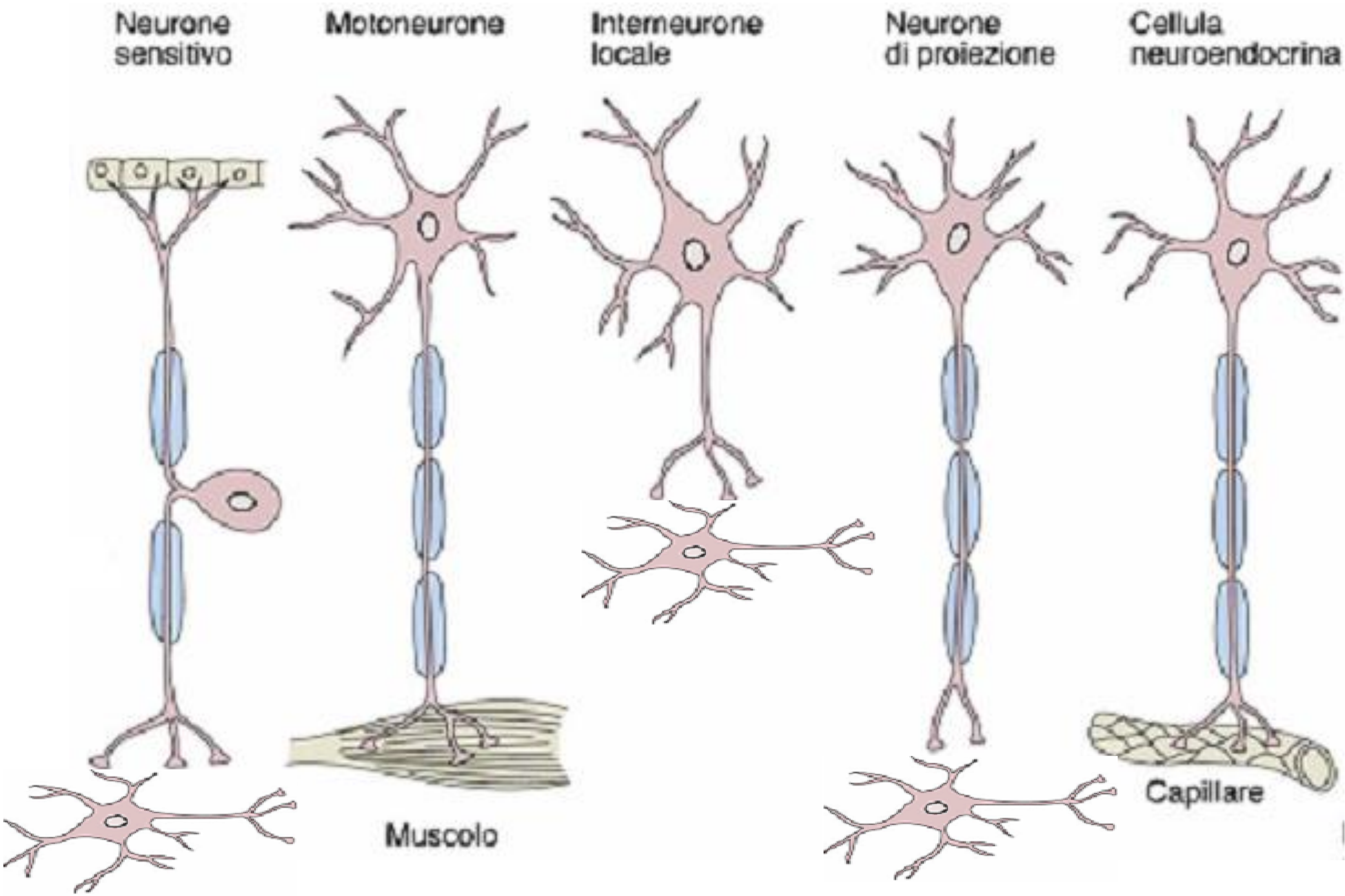
- Paracrine transmission

the target cell is usually a few µm away

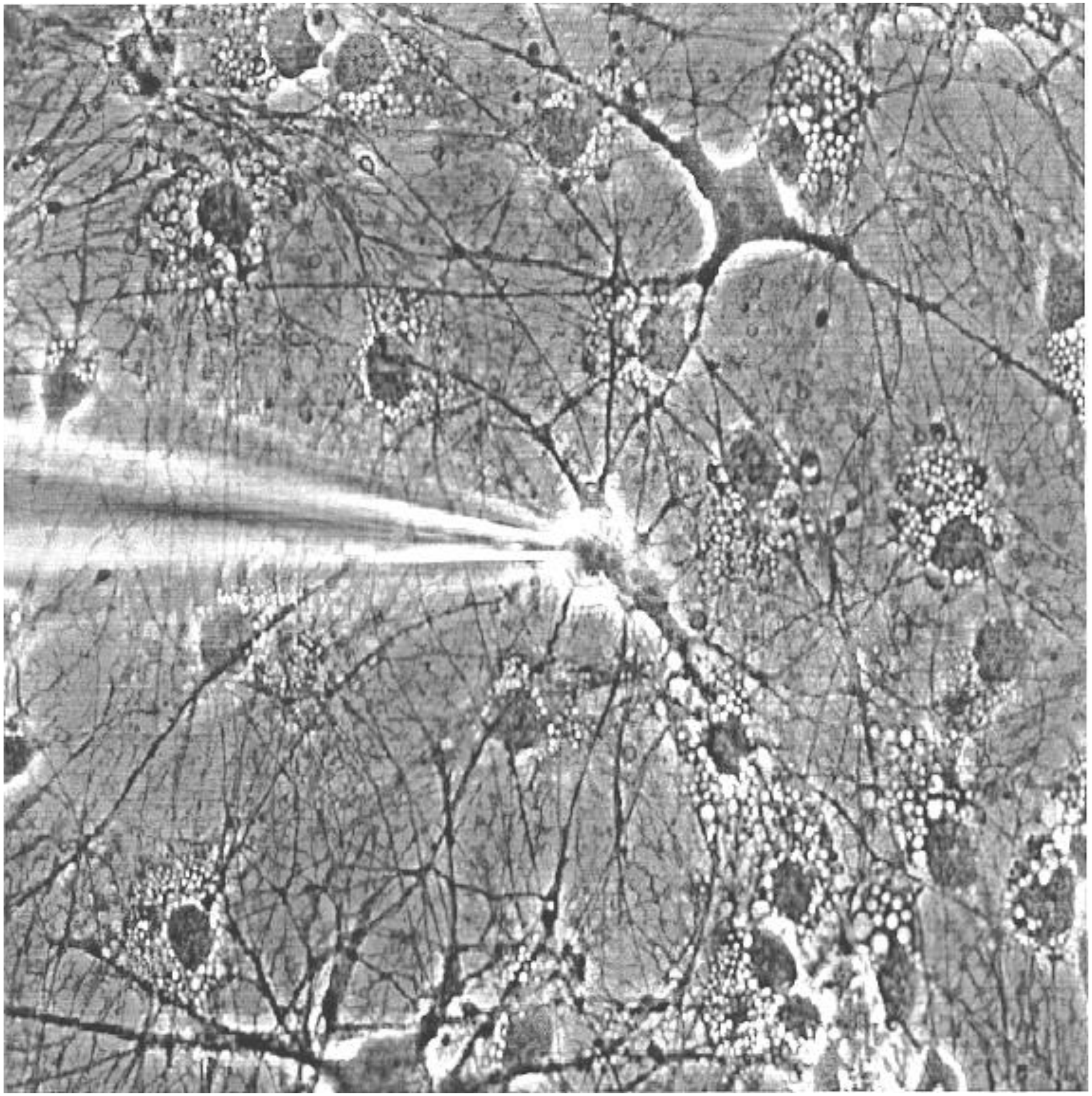
- Humoral transmission

the target cell can be anywhere in the body

Neurons and targets



Neural networks



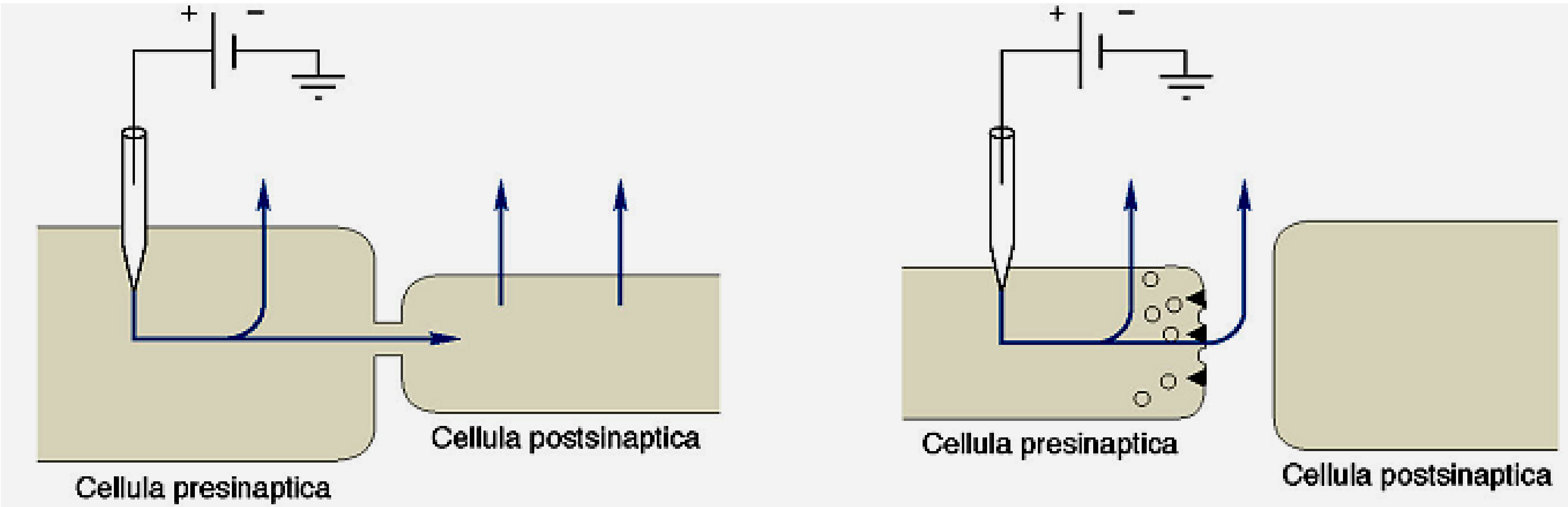
General structure

A synapse is made up of
specialized structures
both in the presynaptic termination
and in the postsynaptic cell

Electrical synapses vs. chemical synapses

Electrical

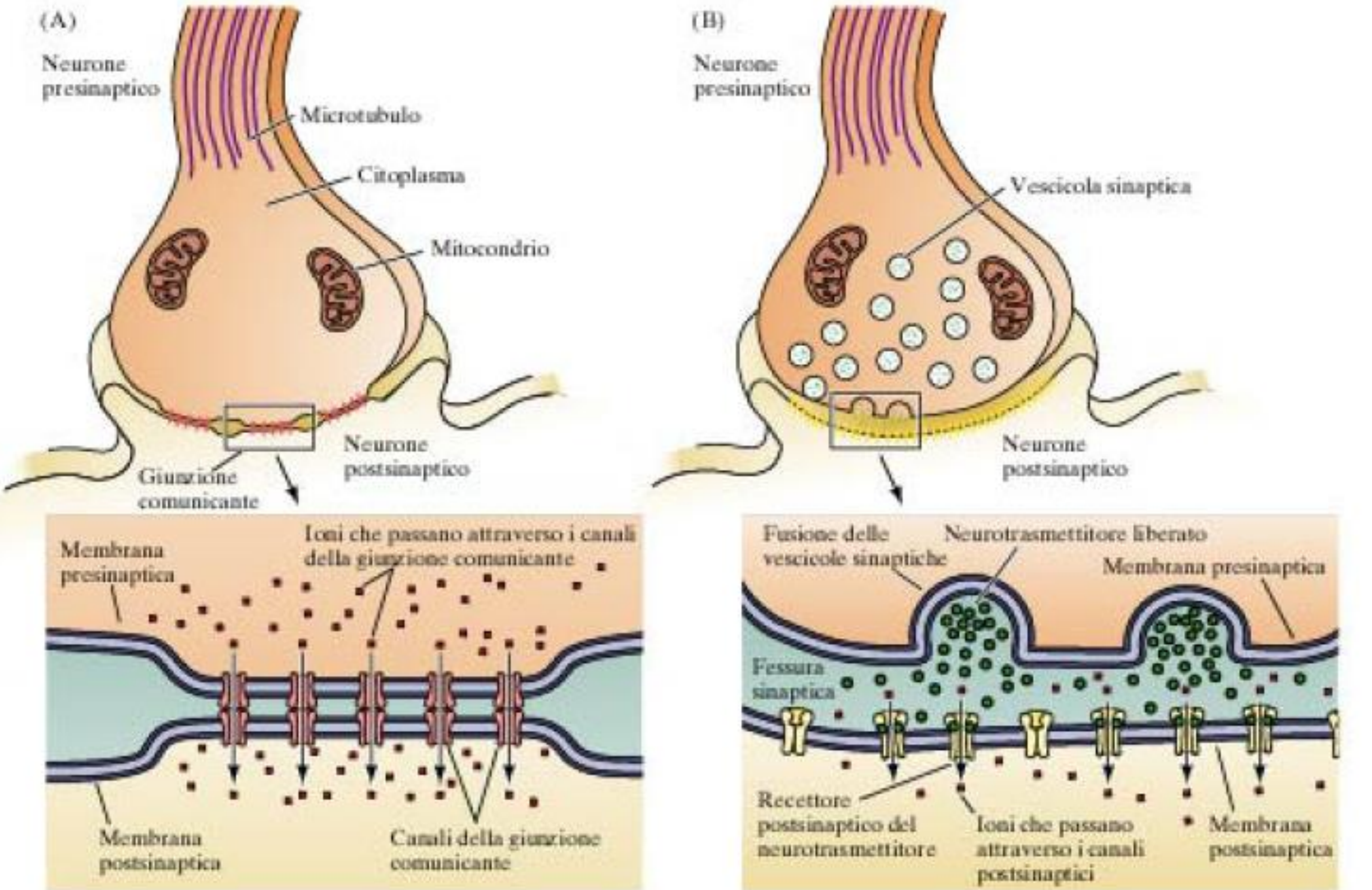
Chemical

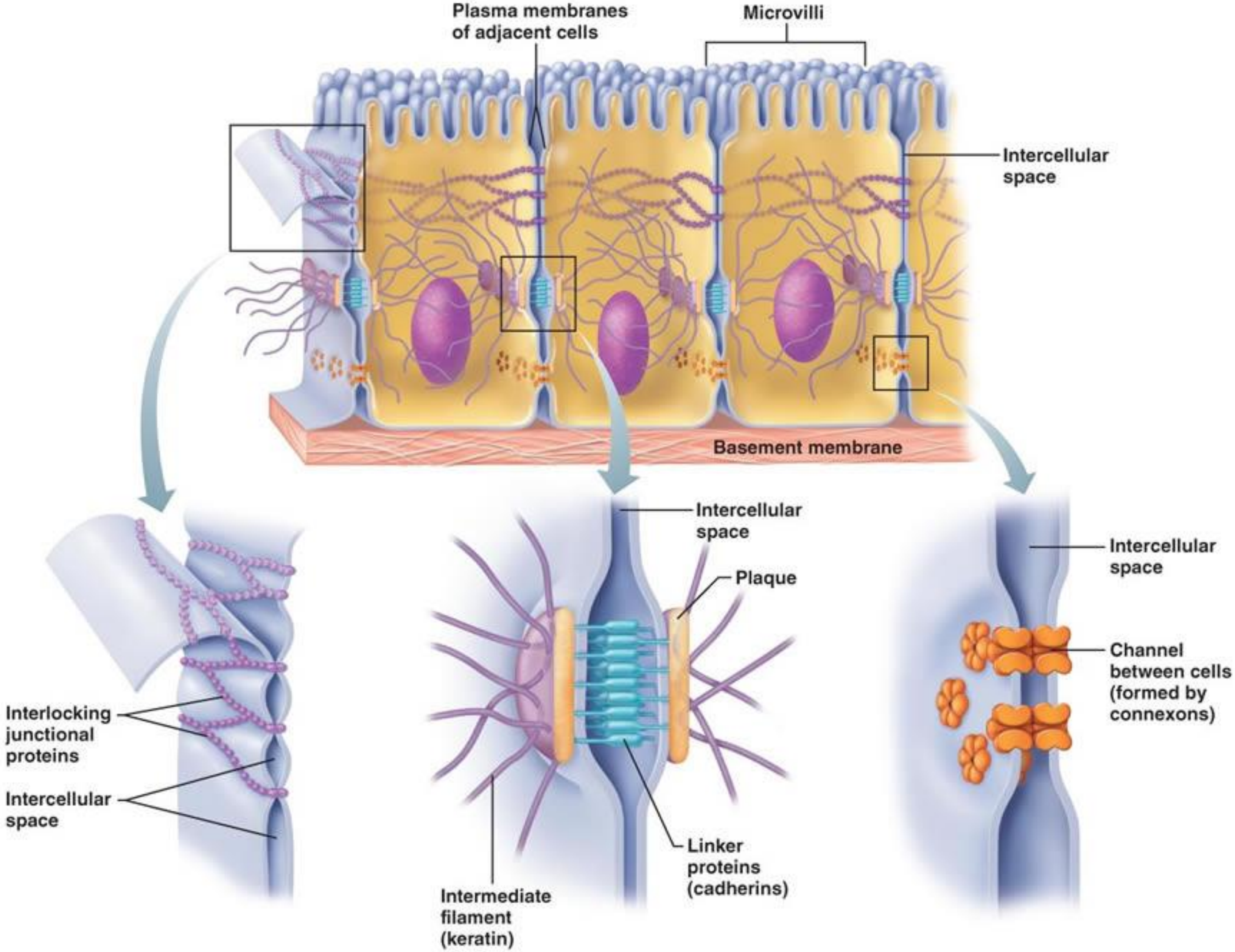


The variation of the membrane potential of a cell is transmitted to another adjacent for direct current flow.
They are located in the CNS and peripheral of vertebrates and invertebrates where it is necessary a high speed of connection and synchronization in the activity of neighboring neurons

The variation of the membrane potential of a cell is transmitted to an adjacent cell by releasing a chemical transmitter triggered by the action potential of the presynaptic neuron.
The chemical transmitter acts on specialized proteins of the post-synaptic membrane, changing its permeability to ions

Electrical synapses vs. chemical synapses





(a) Tight junctions: Impermeable junctions prevent molecules from passing through the intercellular space.

(b) Desmosomes: Anchoring junctions bind adjacent cells together like a molecular "Velcro" and help form an internal tension-reducing network of fibers.

(c) Gap junctions: Communicating junctions allow ions and small molecules to pass for intercellular communication.

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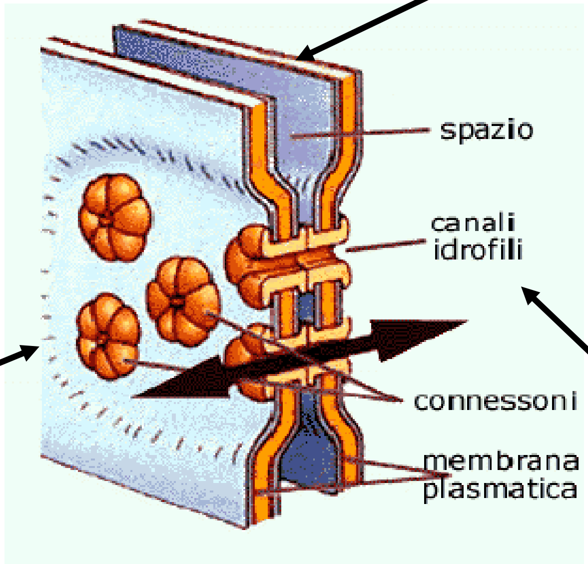
Electrical synapses morphology

the wave of depolarization of the action potential passes from one cell to another through a specialized structure,
the gap junction

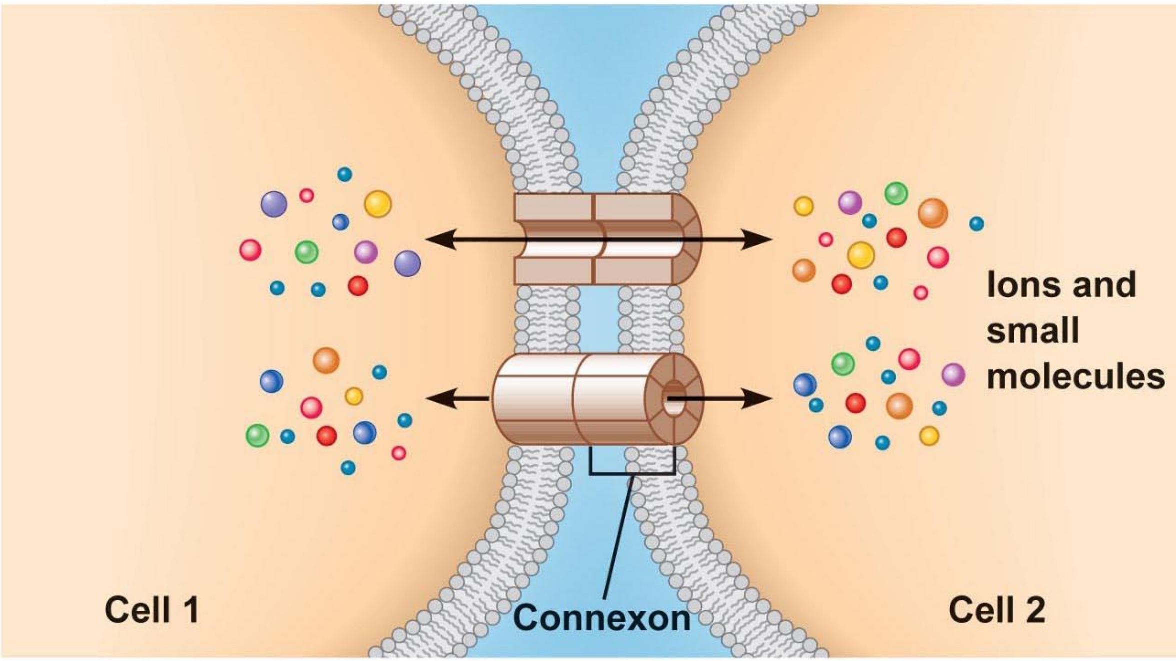
The electric synapses are joined by a plate-shaped gap, between which a space of less than 3 nm runs.



Connexon:
it is made up of 6 protein subunit arranged in hexagon that surround a channel permeable to water.

They are among the largest pores that are known. Their diameter is such as to allow the passage of the largest cell ions and organic micromolecules

The electric synapses allow direct flow of ionic current from one cell to another



(a) Direct communication through gap junctions

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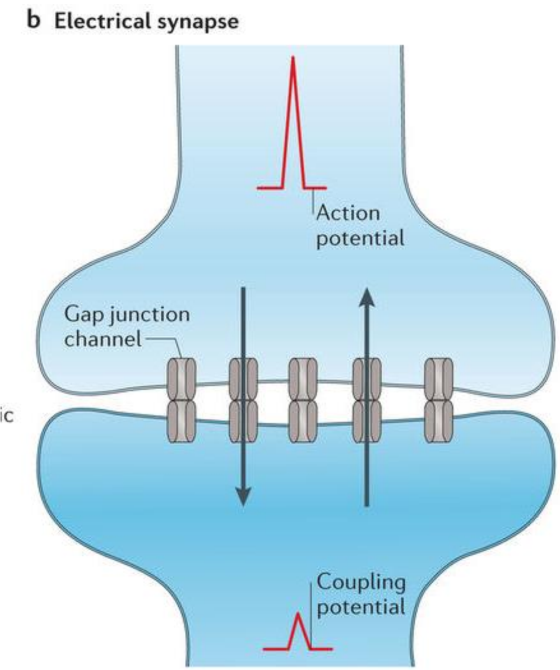

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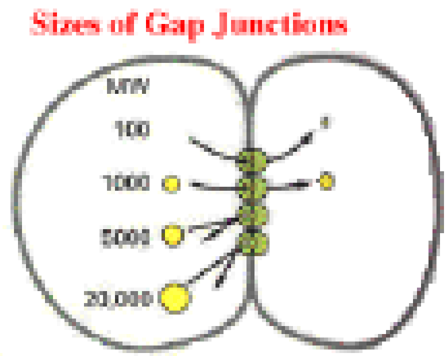
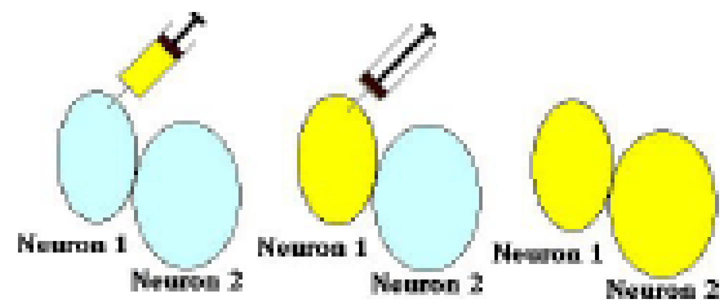
Small molecules can easily pass between cells connected through gap-junction (connexons)

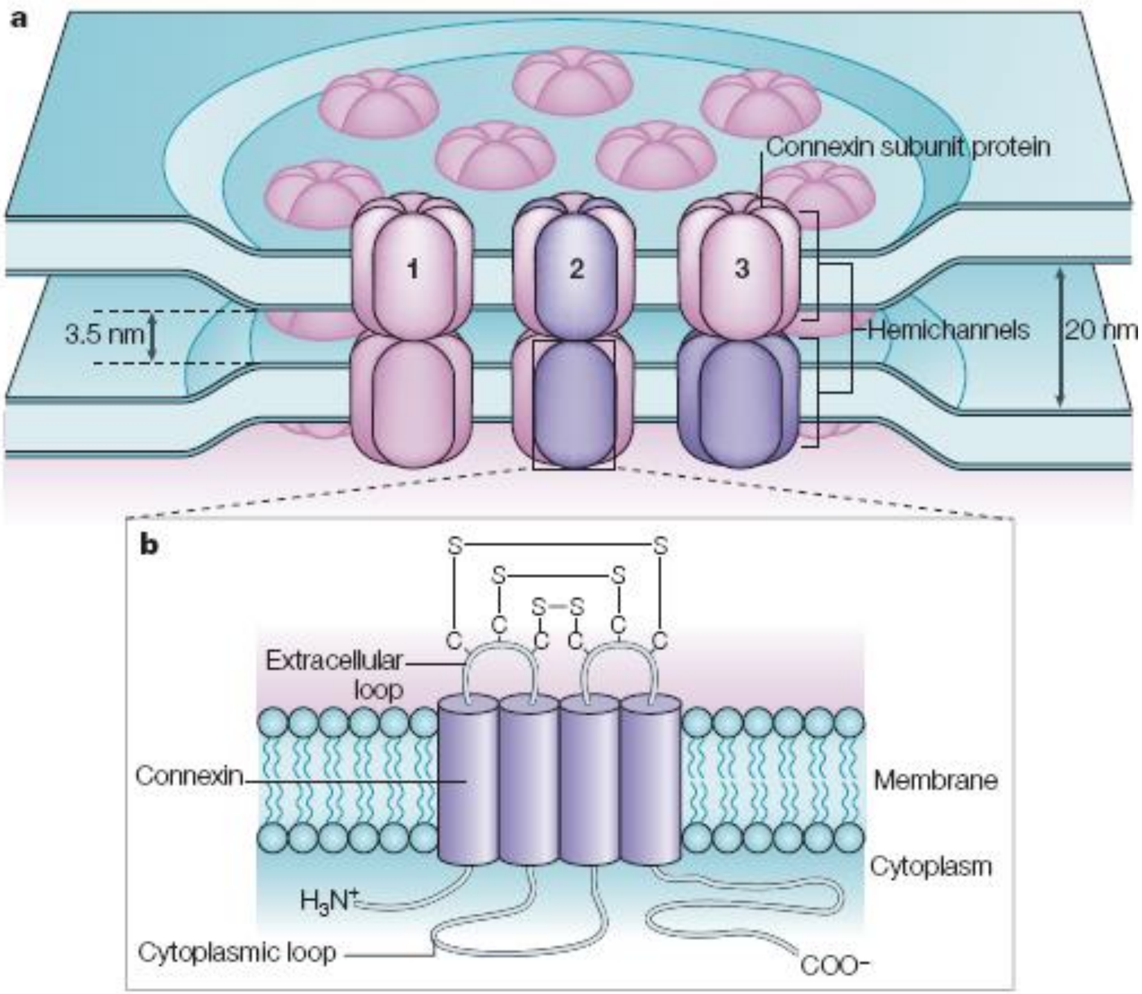
- Weight molecules up to ~ 2 kDa can pass from one cell to another electrically connected to it

- Fluorescent molecules such as yellow lucifer pass through gap-junctions - the connected cells are called "dye-coupled."





Molecular organization and gap-junction topology



Goran Söhl et al Nature reveiw neurosci 2005

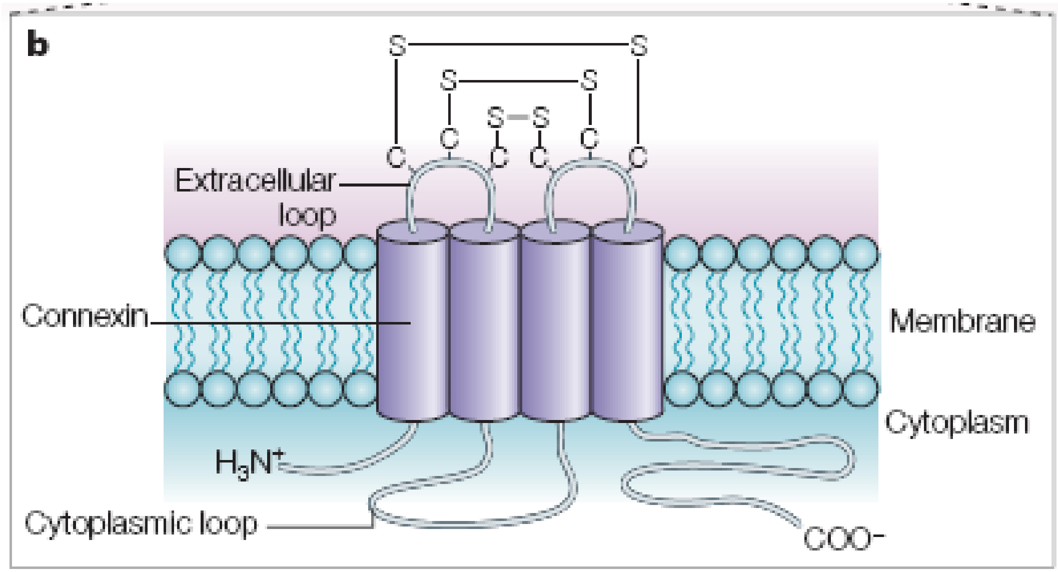
Three types of gap-junction
homomeric/homotypic
heteromeric
heterotypic

Homotypic or heterotypic gap junctions comprise two identical or two different types of hemichannel, respectively.

Homomeric or heteromeric hemichannels
are composed of one or more connexin (or possibly pannexin) isoforms, respectively.

Each hemichannel represents an assembly of six connexin protein subunits.

Connessine

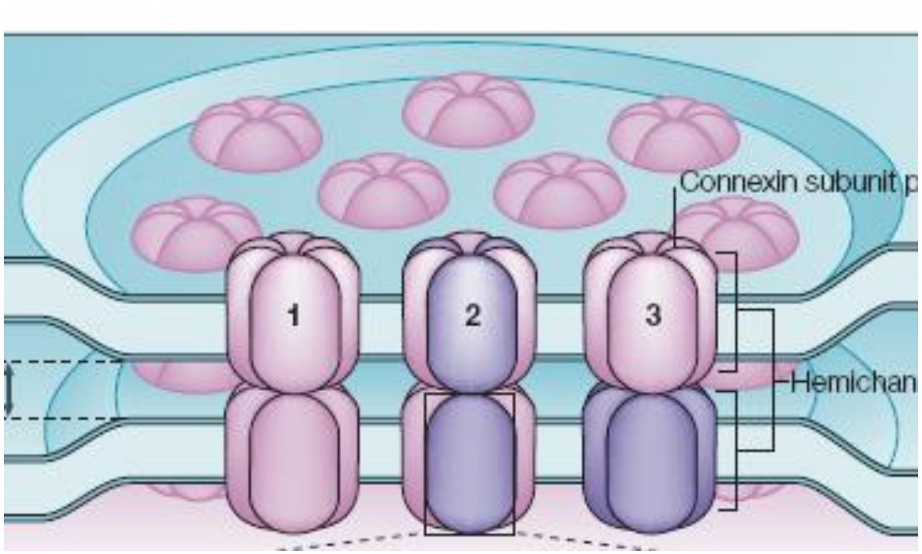


Connexin protein subunits are tetra-spanning membrane proteins that share three conserved extracellular cysteine residues, which are crucial for docking.

The subunits vary mainly in their cytoplasmic loop and carboxy-terminal region.

S–S represents conserved disulphide bonds in the extracellular domains of connexins.

A cosa servono le gap junction quando coesistono con le sinapsi chimiche?



Improve network activity by synchronizing a large number of neuronal sets and their oscillatory activity (perception, memory and learning)

Due to the size of the pores (16-20 Å diameter) ions passe(above all K +) but also second messengers such as IP3 and cAMP

Where gap junctions are expressed?

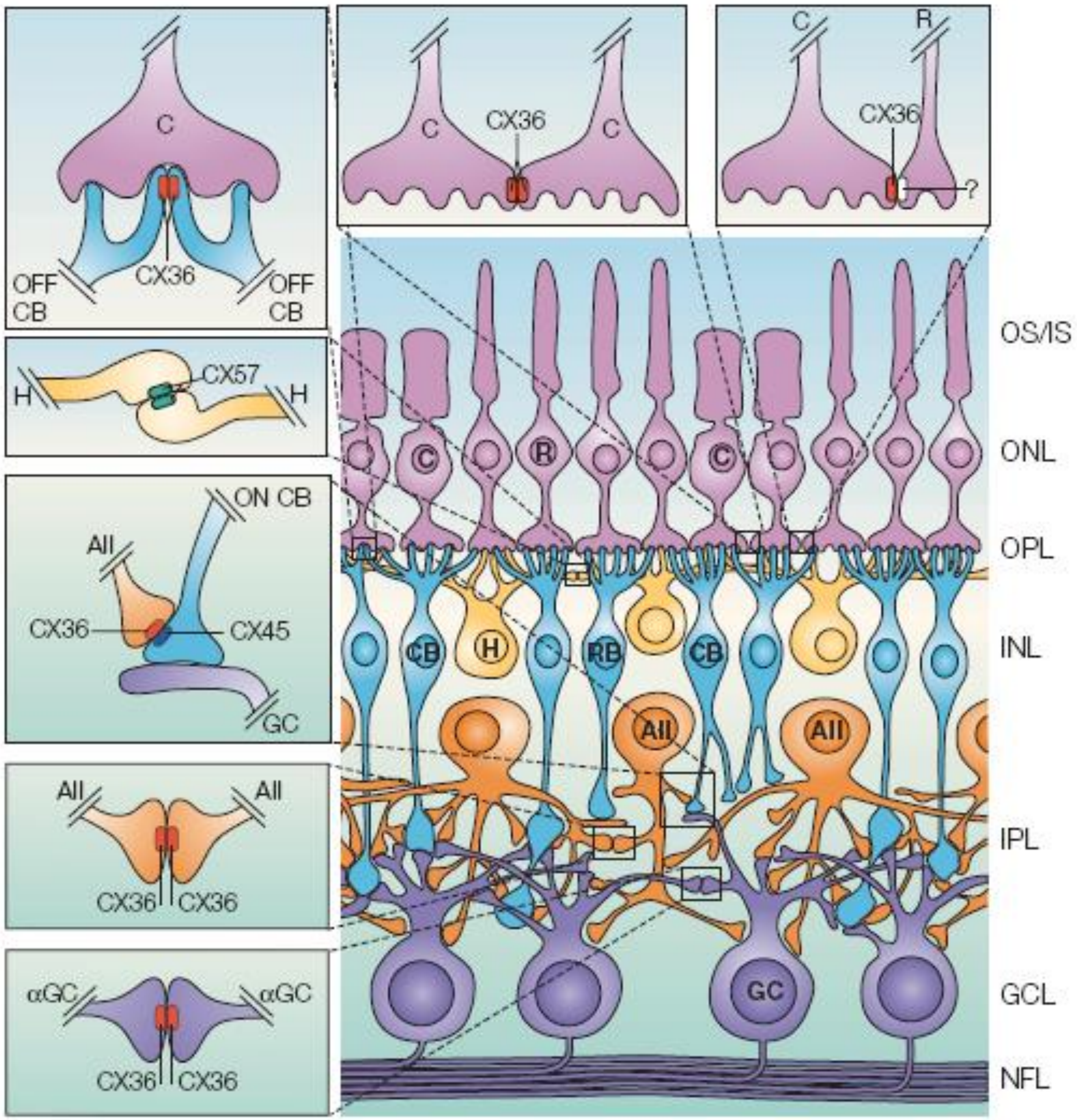
Table 1 | **Properties of protein subunits in electrical synapses**

Gap junction protein	Amino acid residues	Unitary conductance	Half-inactivation voltage (V_0)	Ability to form heteromeric channels	Site of main expression
CX36	321	10–15 pS[107]	±75 mV[107]	n.d.	Interneurons[33], olivary nucleus, cone photoreceptor cells[81]
CX45	396	32±8 pS[108]	±13.4 mV[108]	Yes (CX43/CX45)[109]	Distinct neuronal subtypes[27], vascular smooth muscle cells[106], conductive myocardiocytes[106]
CX57	492	n.d.*	n.d.	n.d.	Horizontal cells[29]
PX1	426	550 pS[54] (‡PX1)	n.d.	Yes[5] (§PX1/§PX2)	‖Principal neurons[30]
PX2	607	n.d.	n.d.	Yes (§PX1/§PX2)[5]	‖Principal neurons[30]

*The mouse CX57 protein with the correct carboxy-terminal region[29] has not yet been analysed. ‡Human protein. §Mouse protein. ‖Expression based on *in situ* hybridization. CX, connexin; n.d., not determined; PX, pannexin.

In the Hippocampus the gamma oscillations depend on gap junctions

Where gap junctions are expressed?



Electrical coupling between retinal cells

Where gap junctions are expressed?

- **During development**

All cells in the initial stage of the embryo are coupled with gap junctions. This coupling, and its subsequent loss, can be related to the fate of the cells.

- **In the epithelia**

Many epithelia are made by sheets of paired cells. This coupling is regulated: damaged cells are decoupled from their neighbors.

- **In the heart**

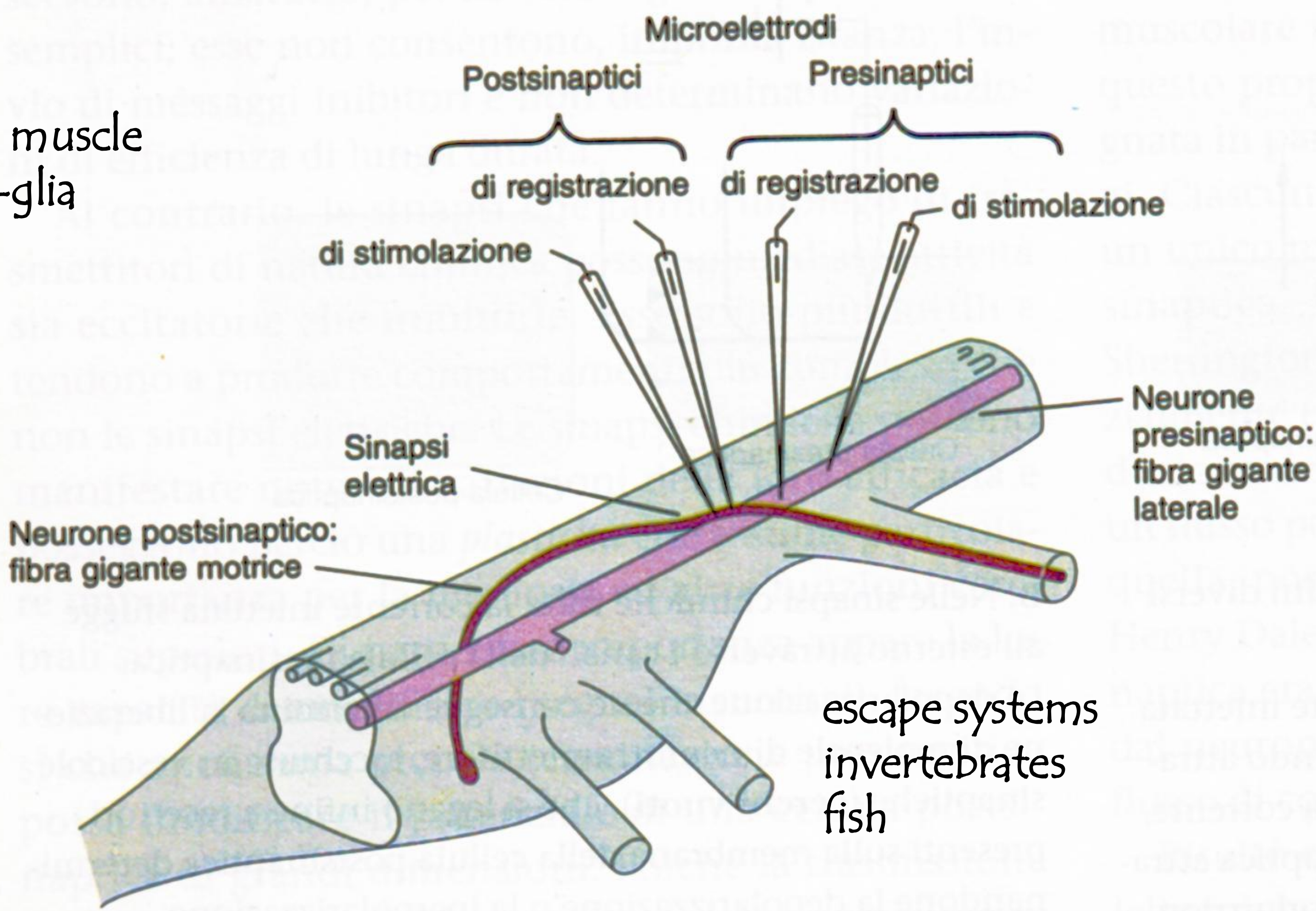
the heart muscle cells are coupled in a syncithium, which allows conduction of the cardiac action potential in a coordinated manner.

- **In the central nervous system**

the coupling in the CNS seems to be important for the generation of coordinated behaviors of cell populations, and, in invertebrates for the rapid escape reaction.

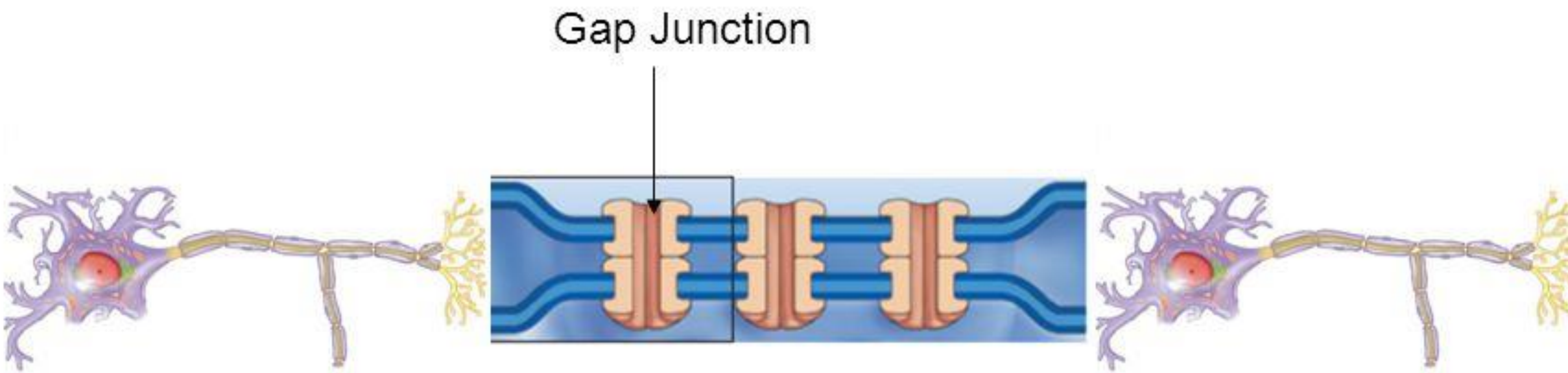
Where gap junctions are expressed?

Heart
smooth muscle
neuron-glia



escape systems
invertebrates
fish

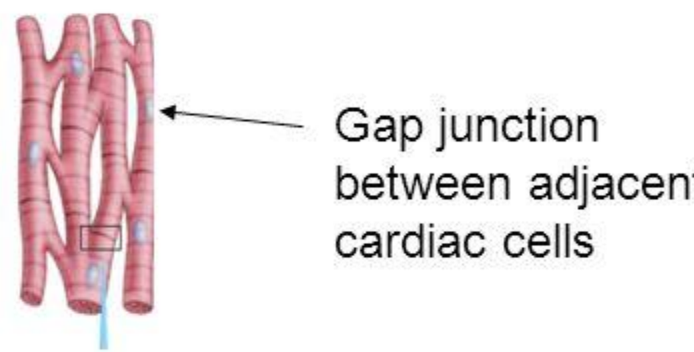
Electrical SYNAPSE



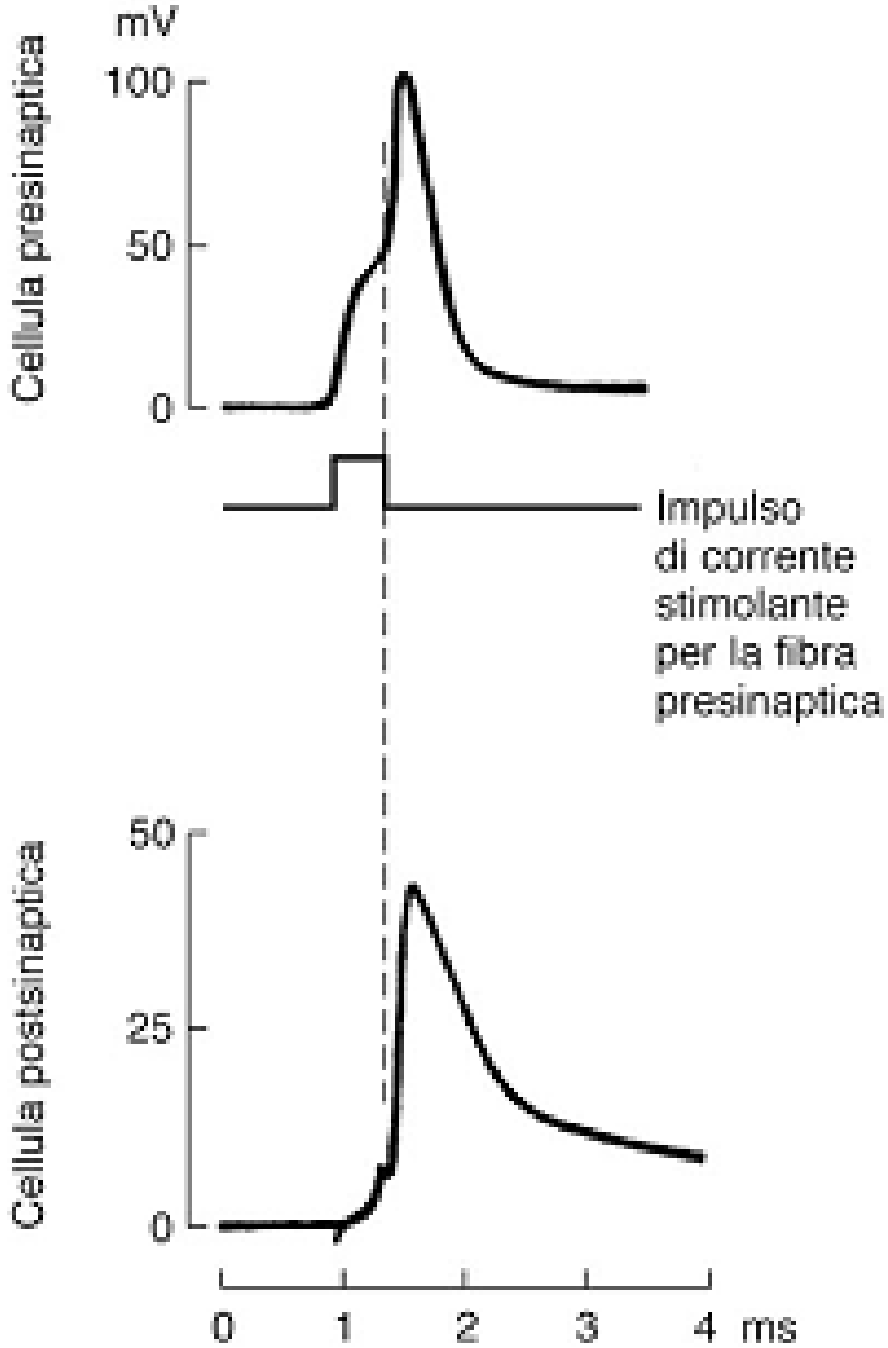
Action potential moves DIRECTLY between neurons

EXAMPLES:

Smooth Muscle
Cardiac Muscle



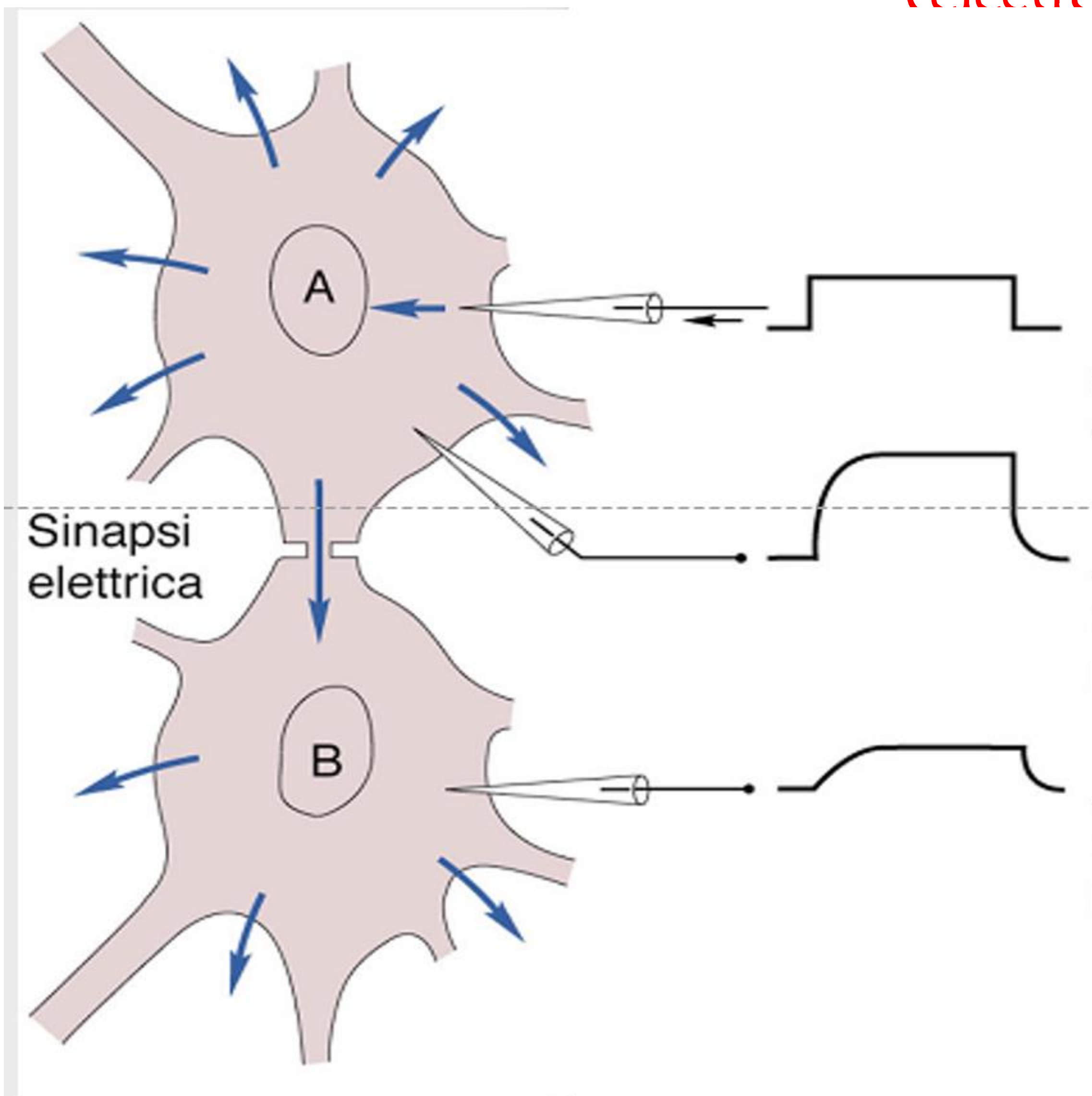
Speed



mV
100
50
0
Cellula presinaptica
Impulso
di corrente
stimolante
per la fibra
presinaptica
50
25
0
Cellula postsinaptica
0
1
2
3
4 ms

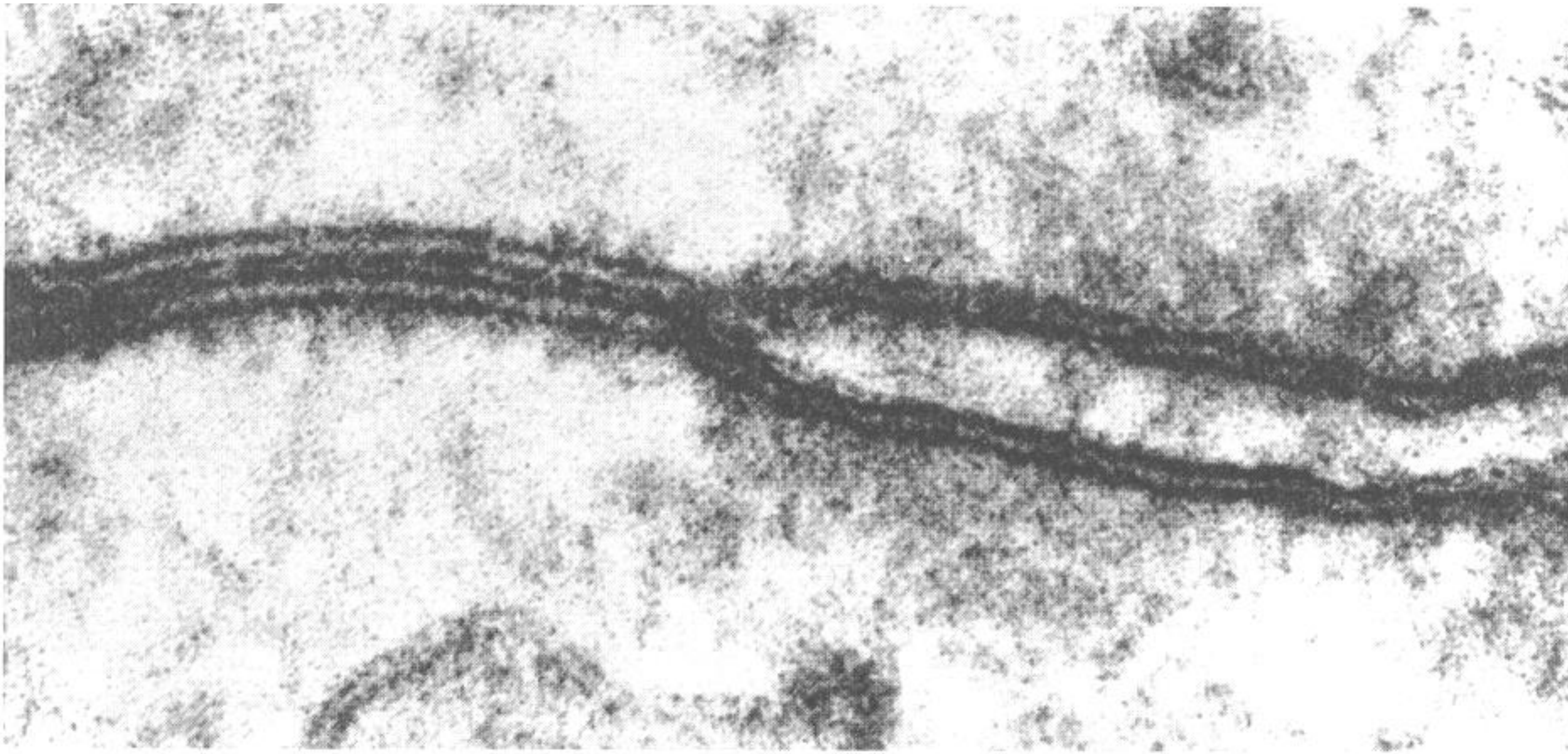
Activation also subthreshold

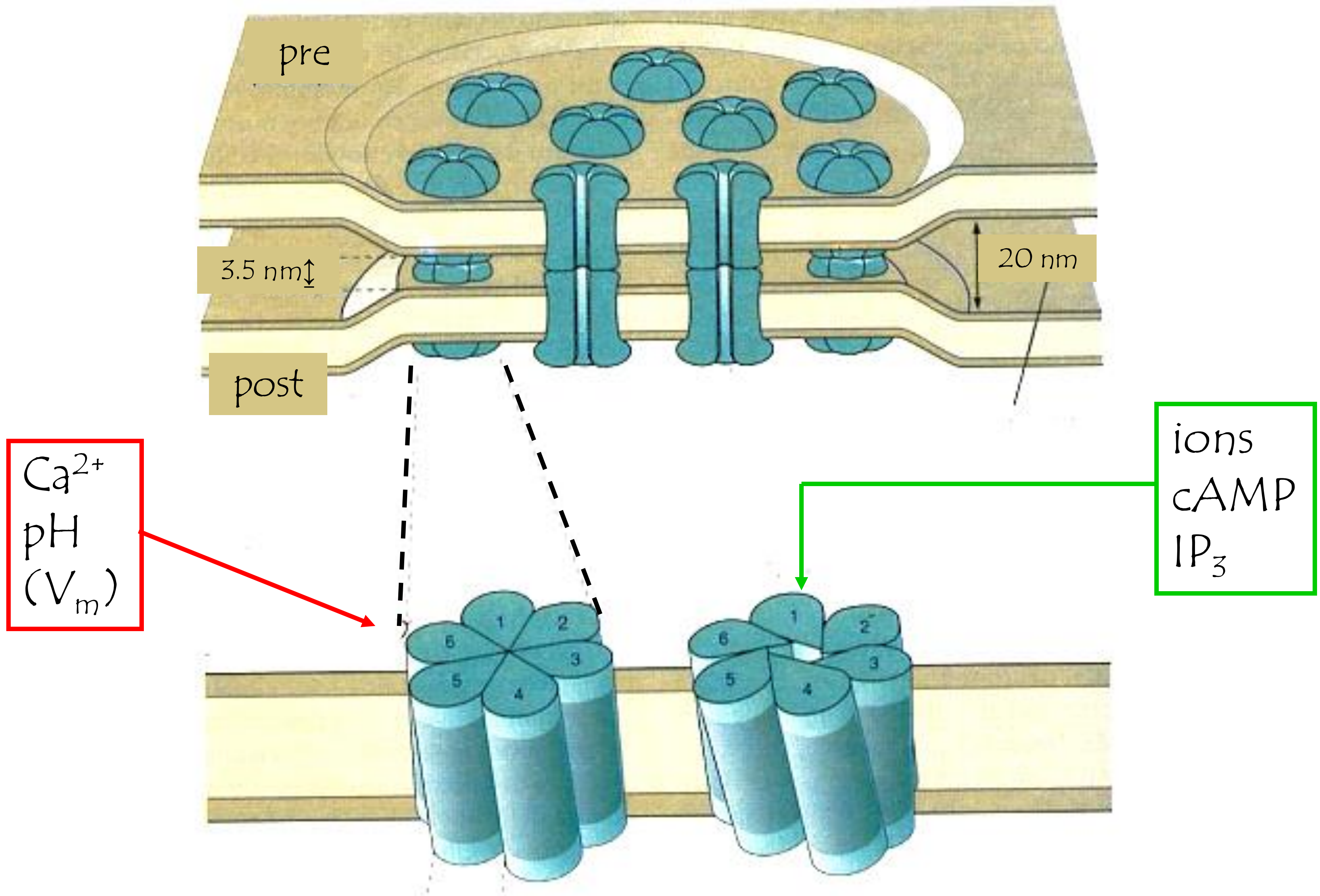
(electrotonic propagation)



Stimulating current pulse

The channels pass through 2 membranes





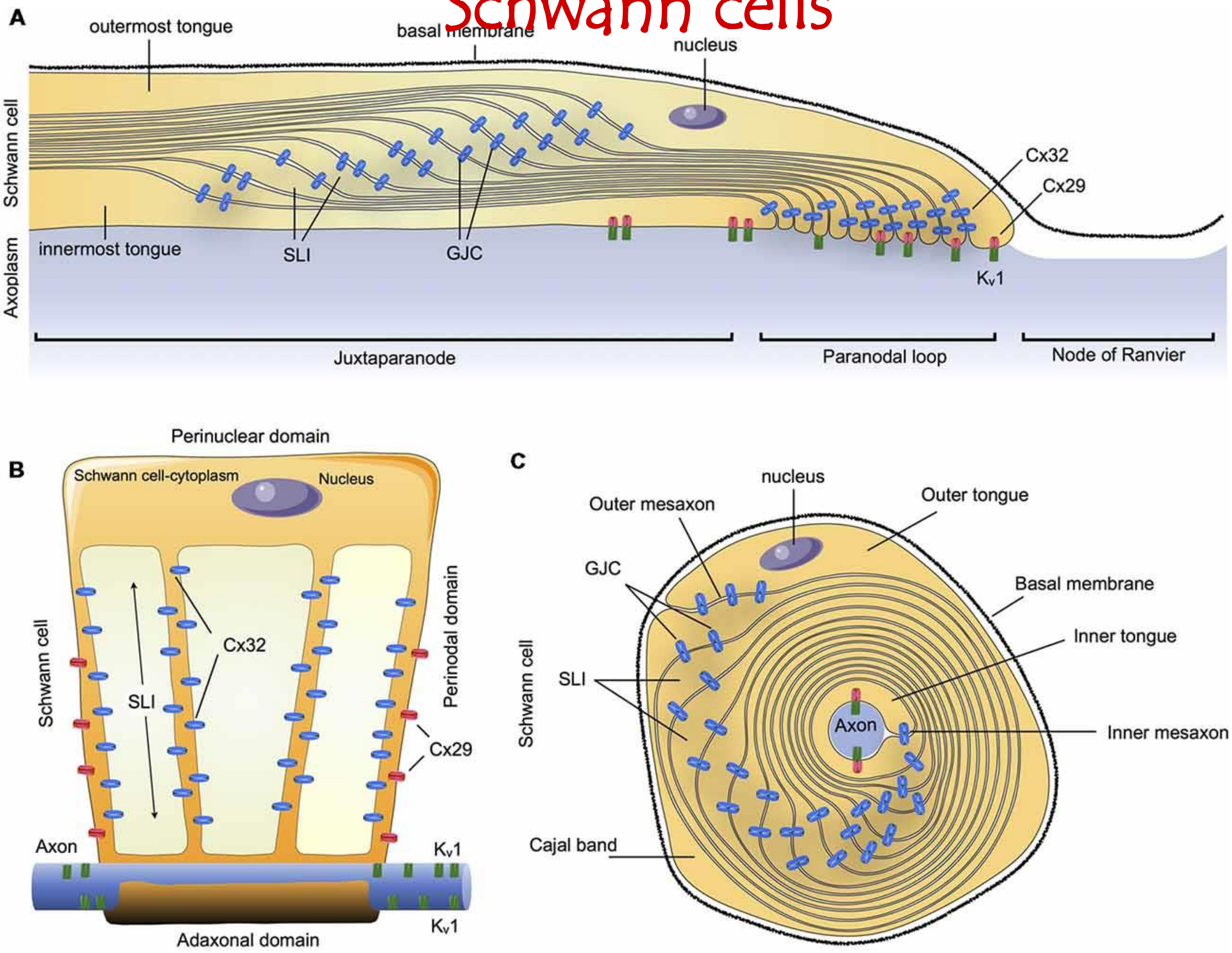
pre
3.5 nm
20 nm
post
Ca^{2+}
pH
(V_m)
ions
cAMP
IP_3
1
2
3
4
5
6
1
2
3
4
5
6

Metabolic coupling due to gap junction: Schwann cells

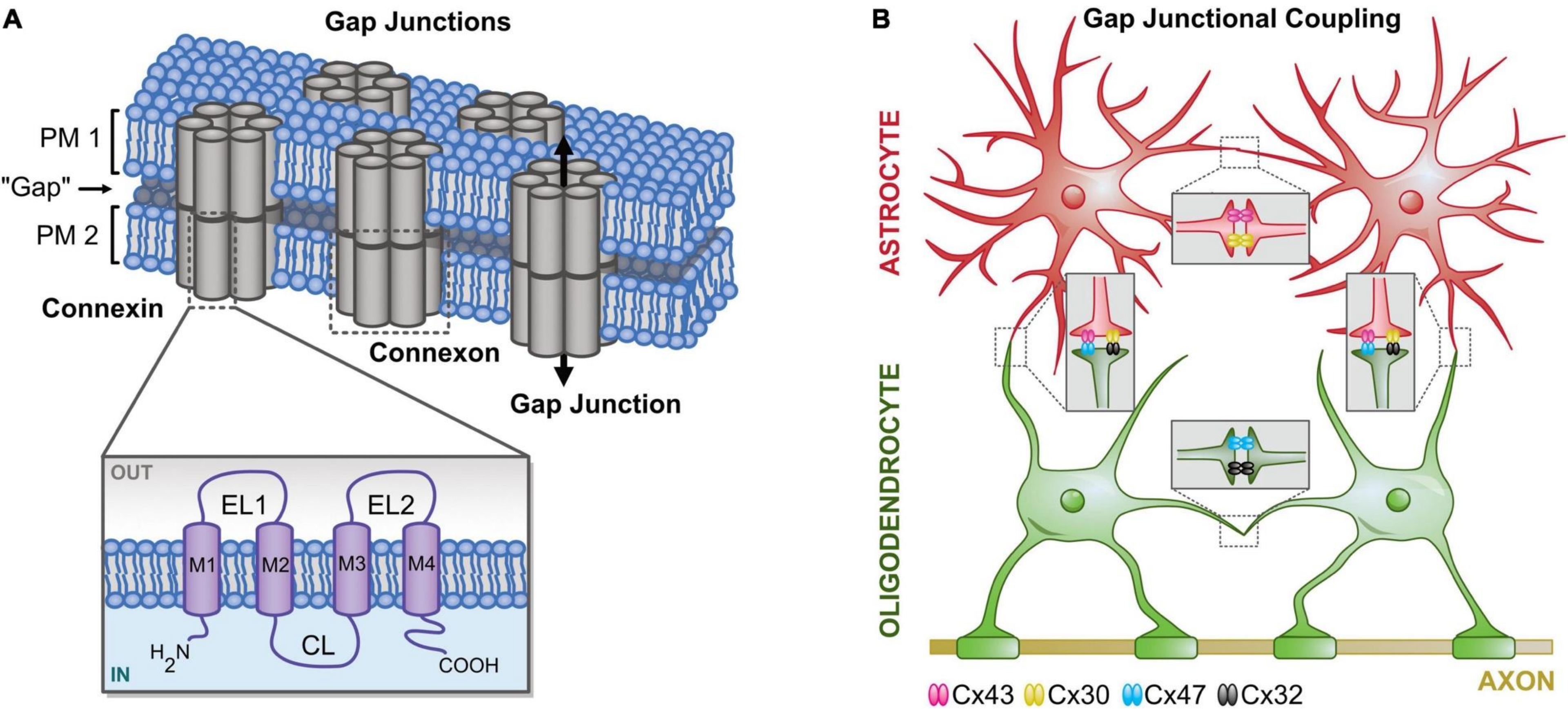
Charcot-Marie-Tooth Syndrome

- Demyelinating disease of peripheral nerves → sensory-motor neuropathy.
- There is a form linked to the X chromosome.
- The X-linked form is associated with point mutations in a connexin gene expressed by Schwann cells (Cx32).
- → alteration of metabolite flows from the outer (perinuclear) region of the Schwann cell to the internal myelin layers.

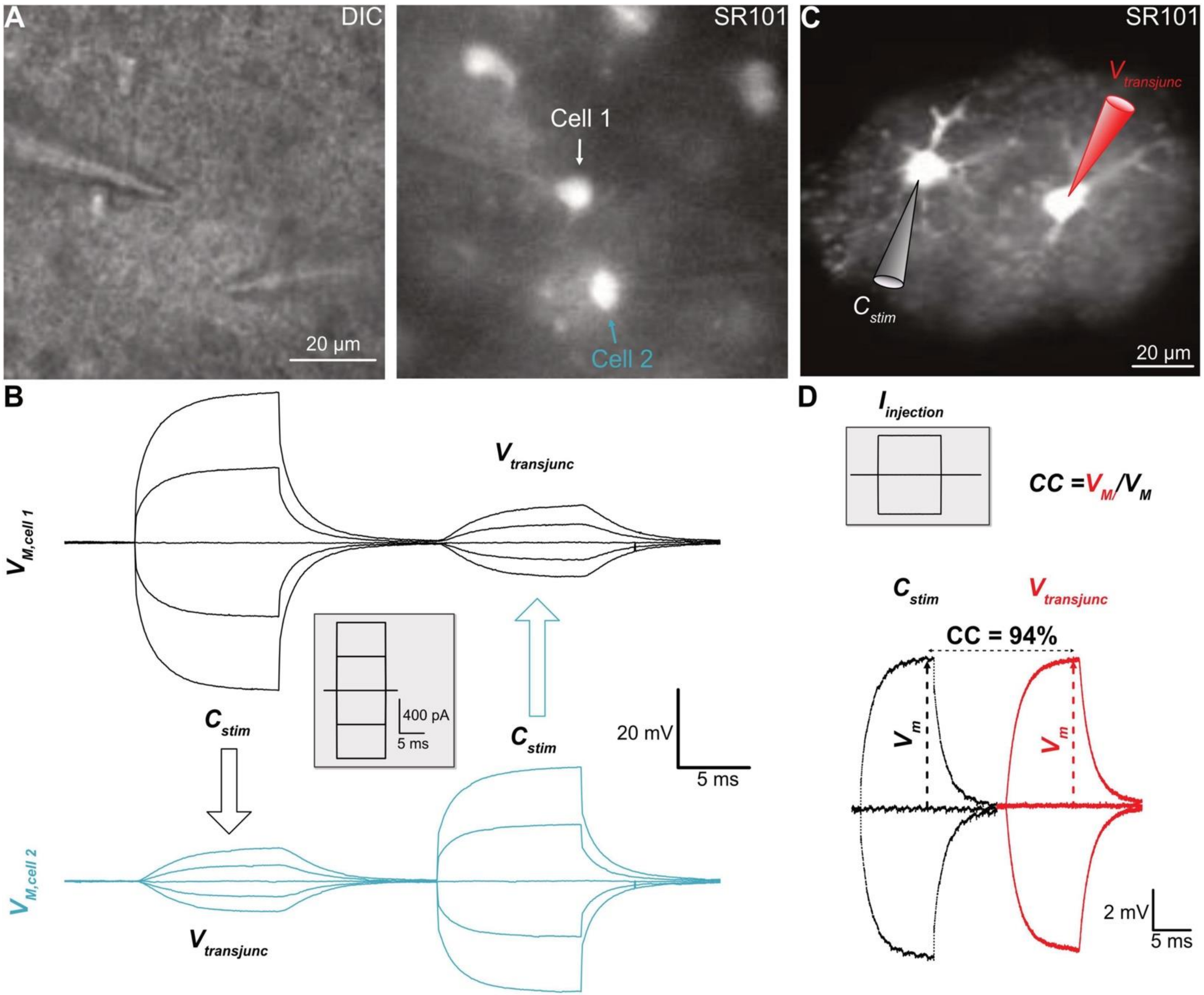
Metabolic coupling due to gap junction: Schwann cells



Electrical coupling due to gap junction:



Electrical coupling due to gap junction:



Metabolic coupling due to gap-junction: astrocytes

Ca2 + waves induced by neuronal activity

Glutamate release

↓

activation of metabotropic astrocytic glutamate receptors

↓

release of Ca2 + IP3-mediated

↓

Ca^{2+} signal amplification (CICR)

↓↑

propagation of Ca2 + signal via gap junction

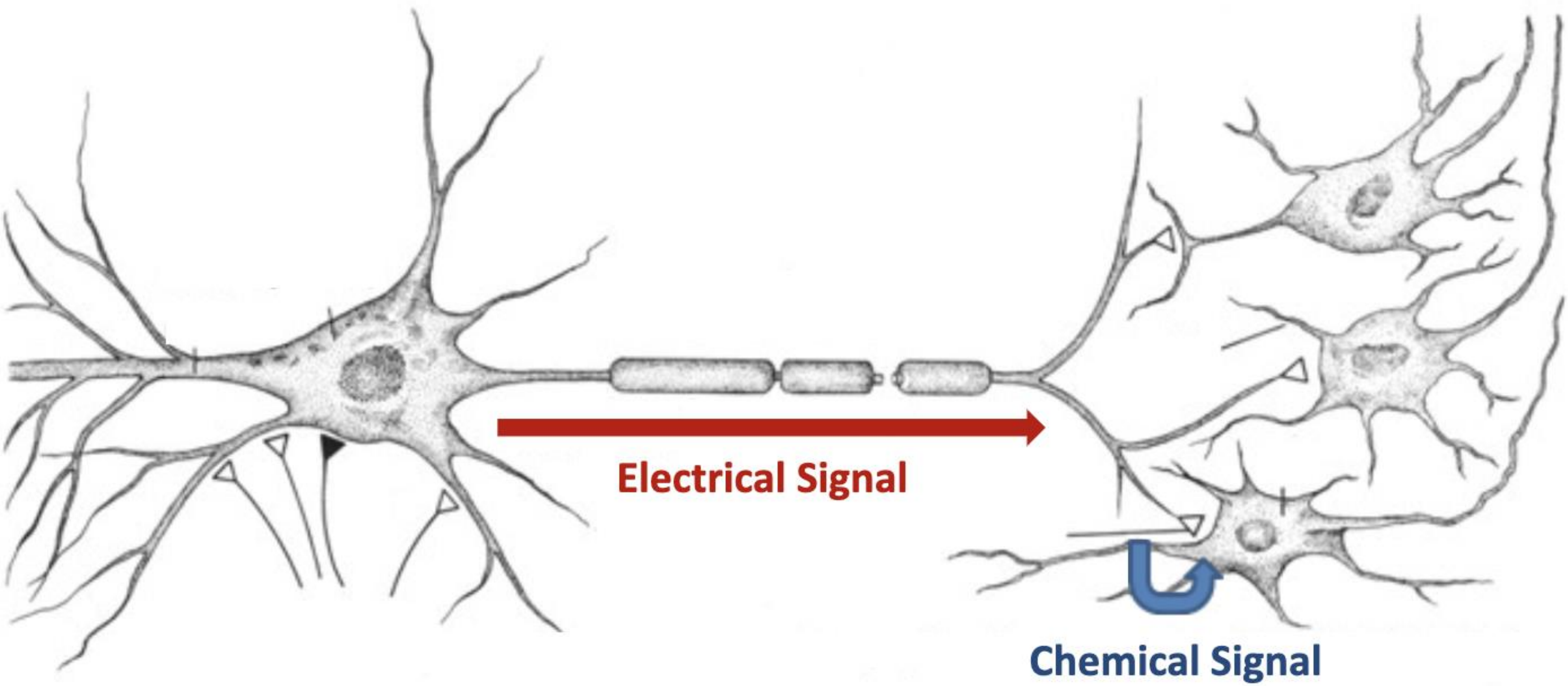
↓

NOS activation → NO production

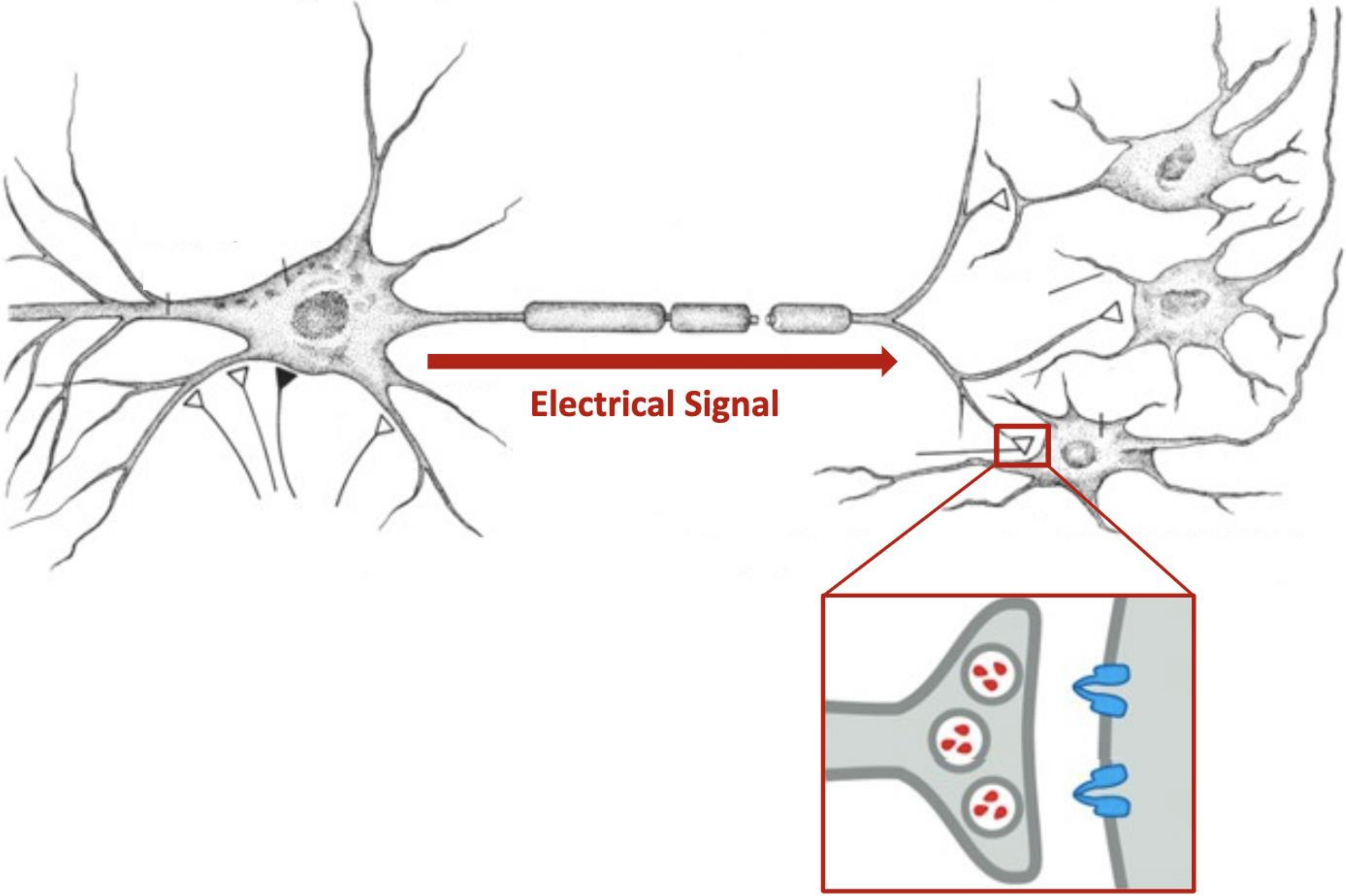
↓

vasodilation

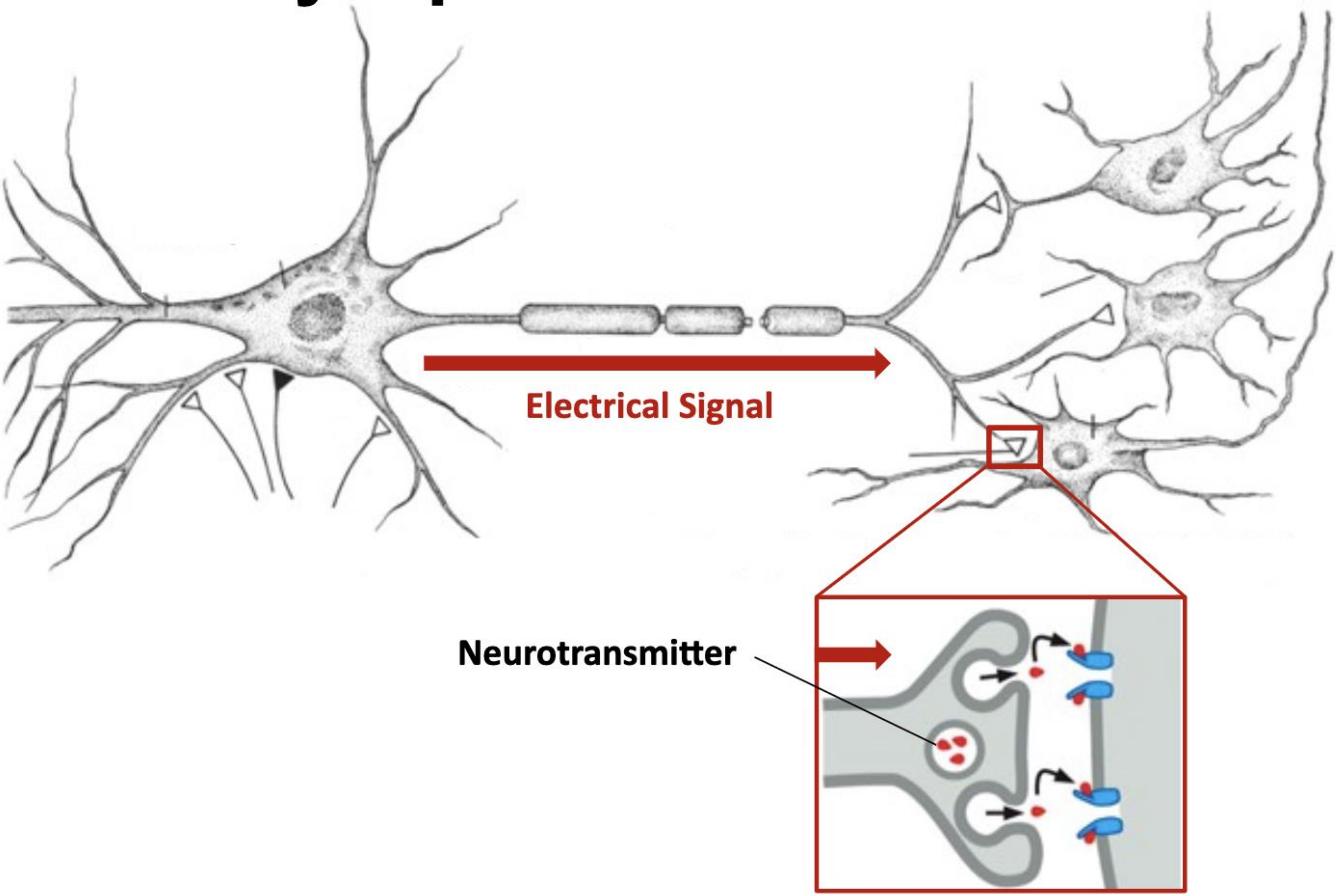
Converting an Electrical Signal to Chemical Signal



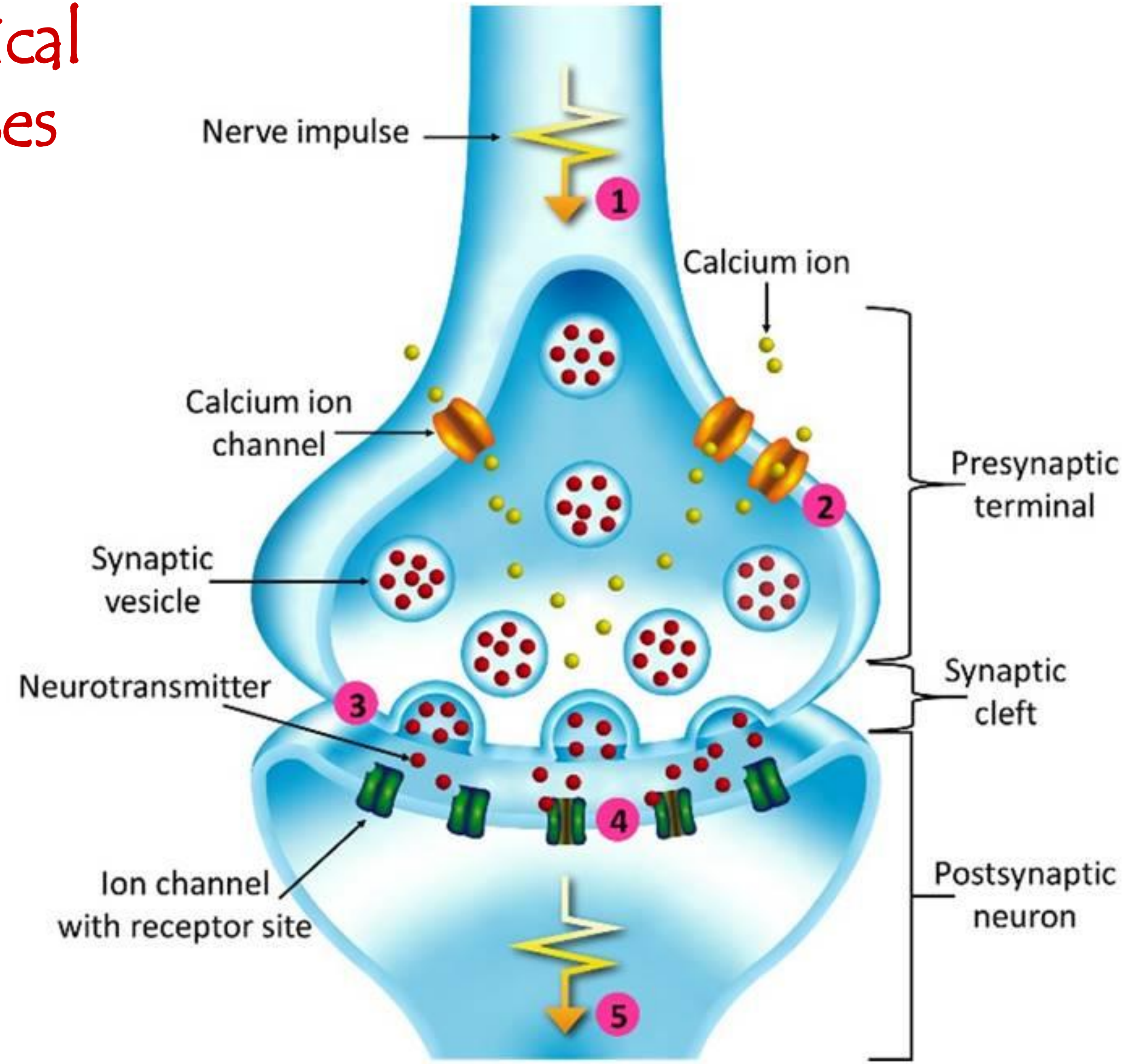
Synaptic Transmission



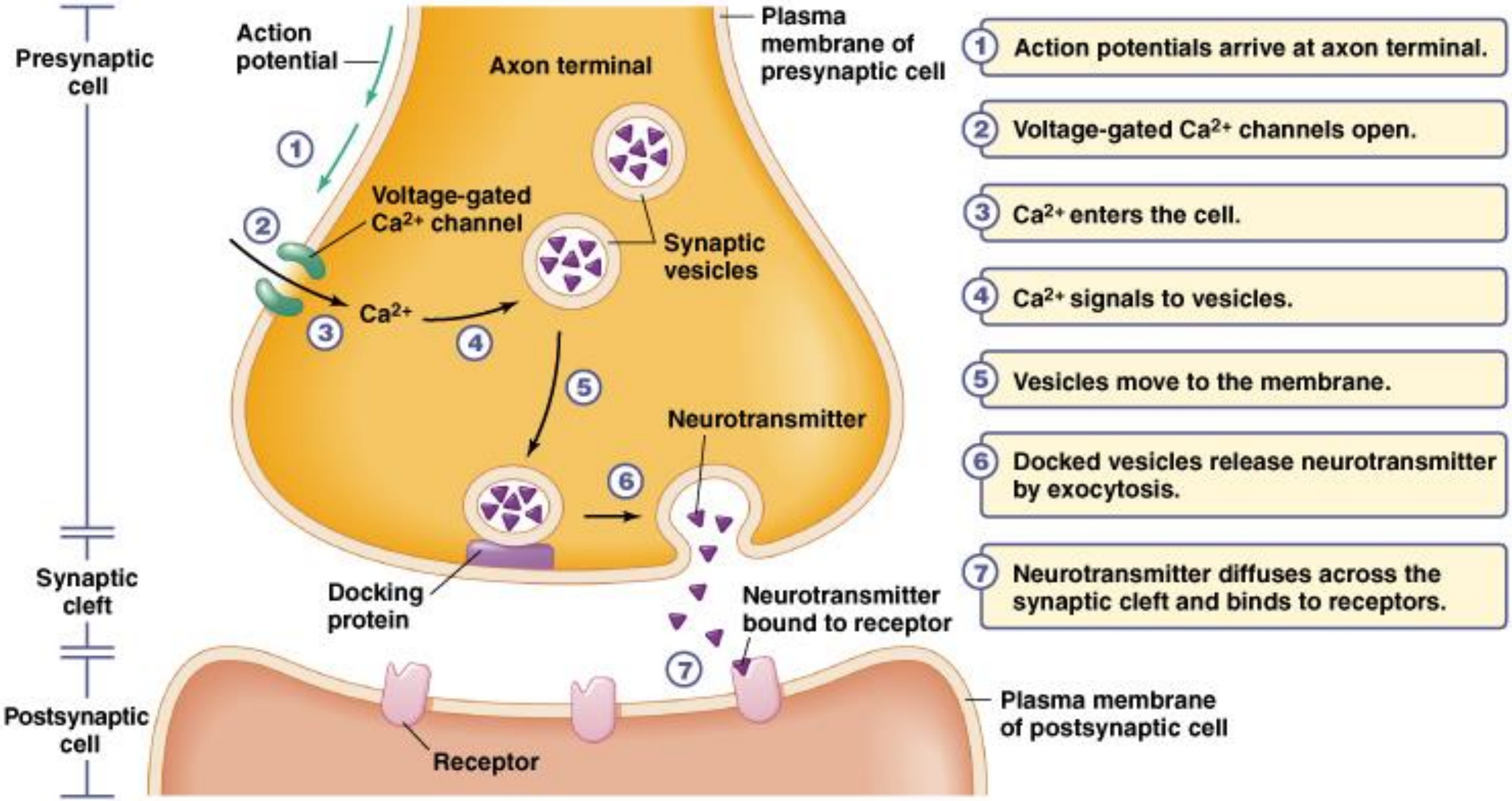
Synaptic Transmission



Chemical synapses



Chemical synapses



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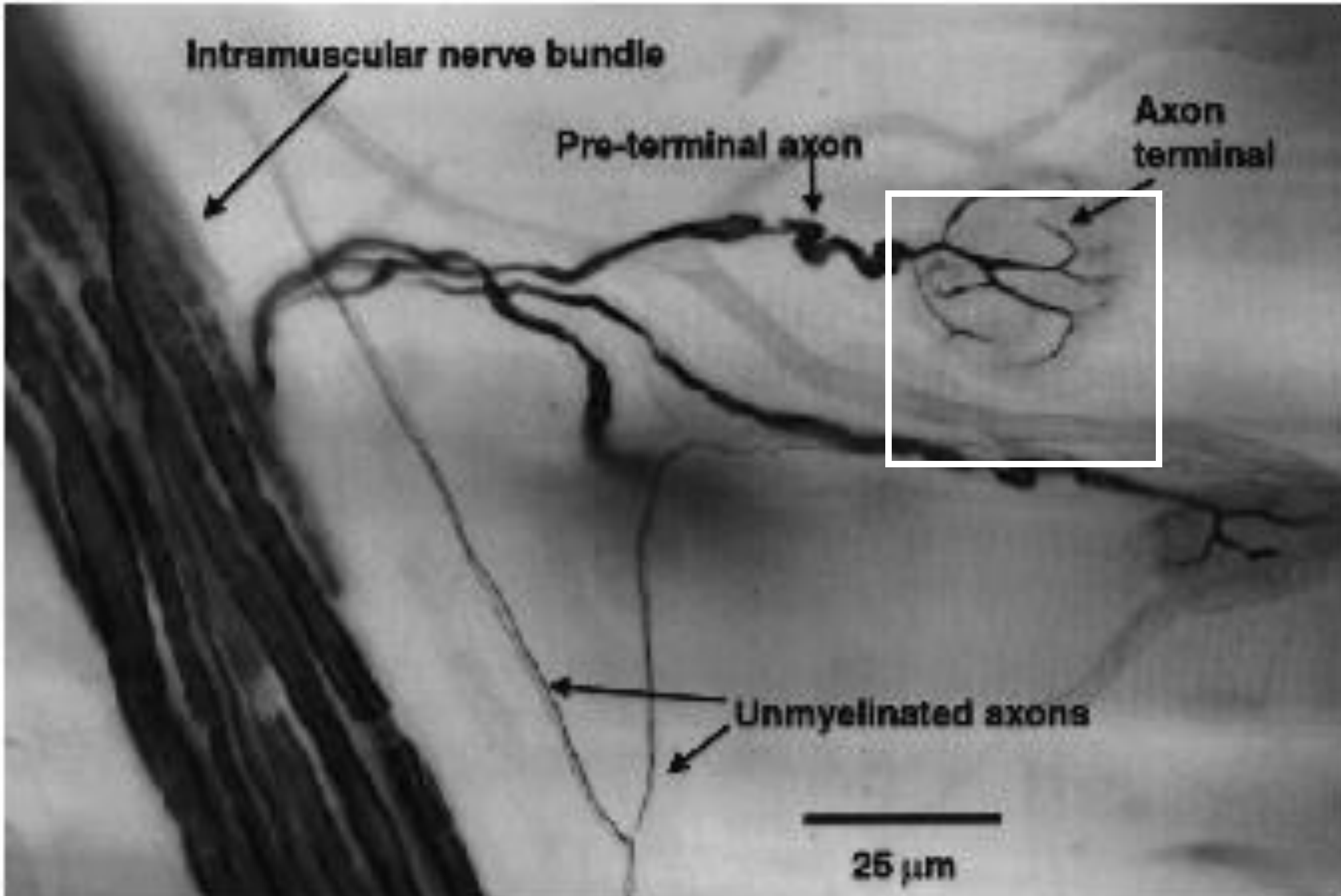
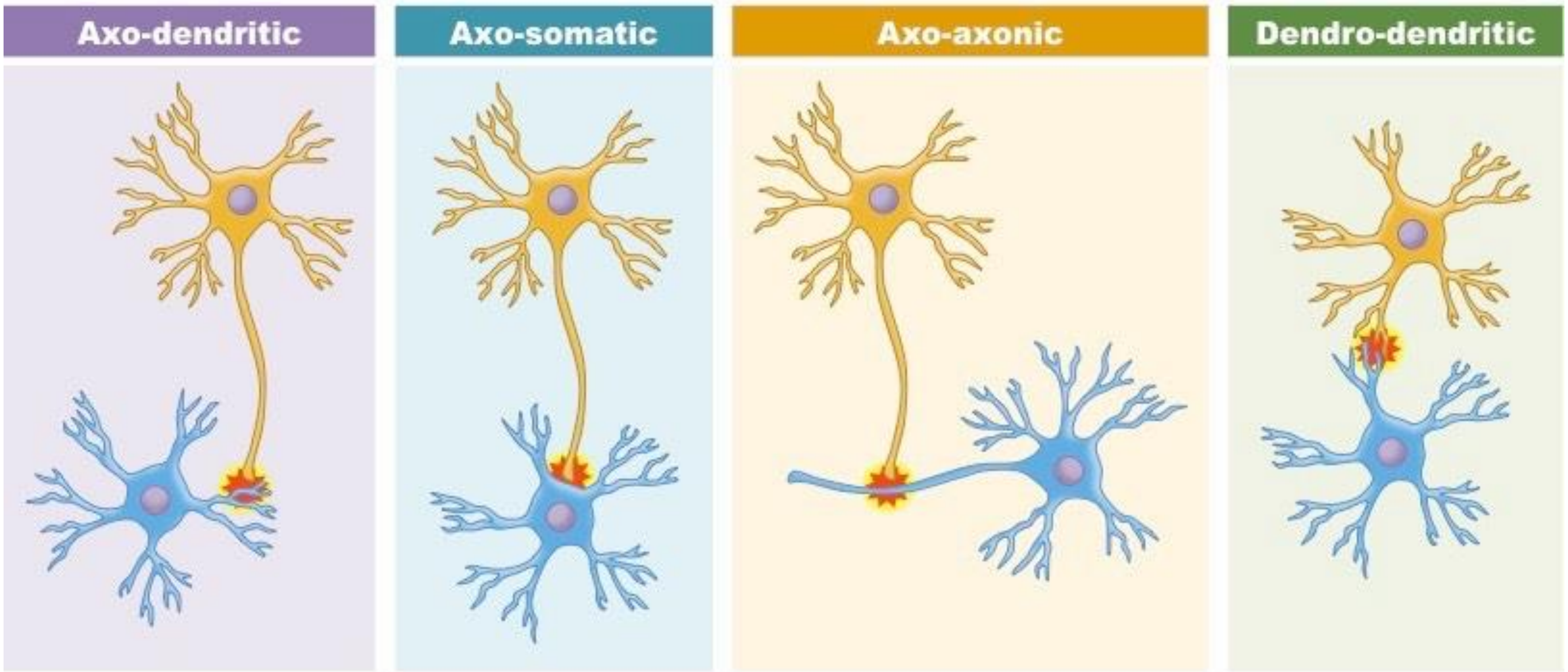
Chemical synapses



The neuromuscular junction

CNS synapses

Depending on which the postsynaptic part is invvolved

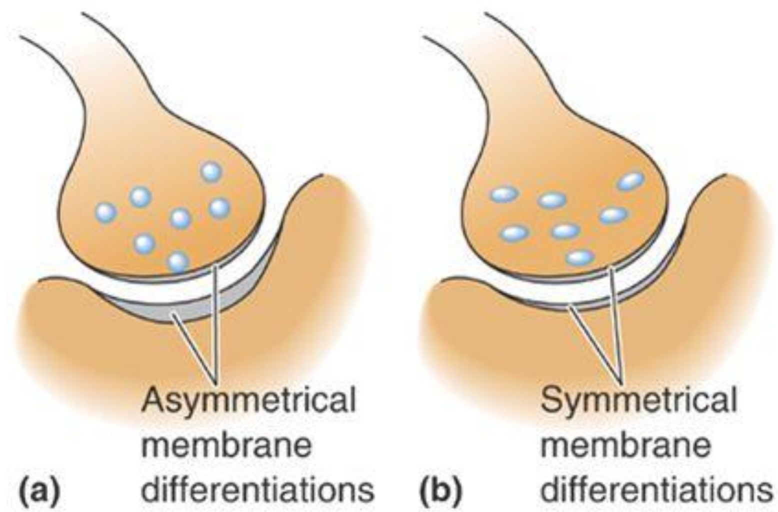


CNS synapses – "Gray types"

Depending on the relationship between the pre-and post-synaptic termination thickness, the following is distinguished:

The Geometry of Excitatory and Inhibitory Synapses

- Excitatory synapses
  - Gray's type I morphology
  - Spines: Excitatory synapses
- Inhibitory synapses
  - Gray's type II morphology
  - Clustered on soma and near axon hillock



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Why have chemical synapses?

Chemical synapses can modify the input signal:

1. Amplification

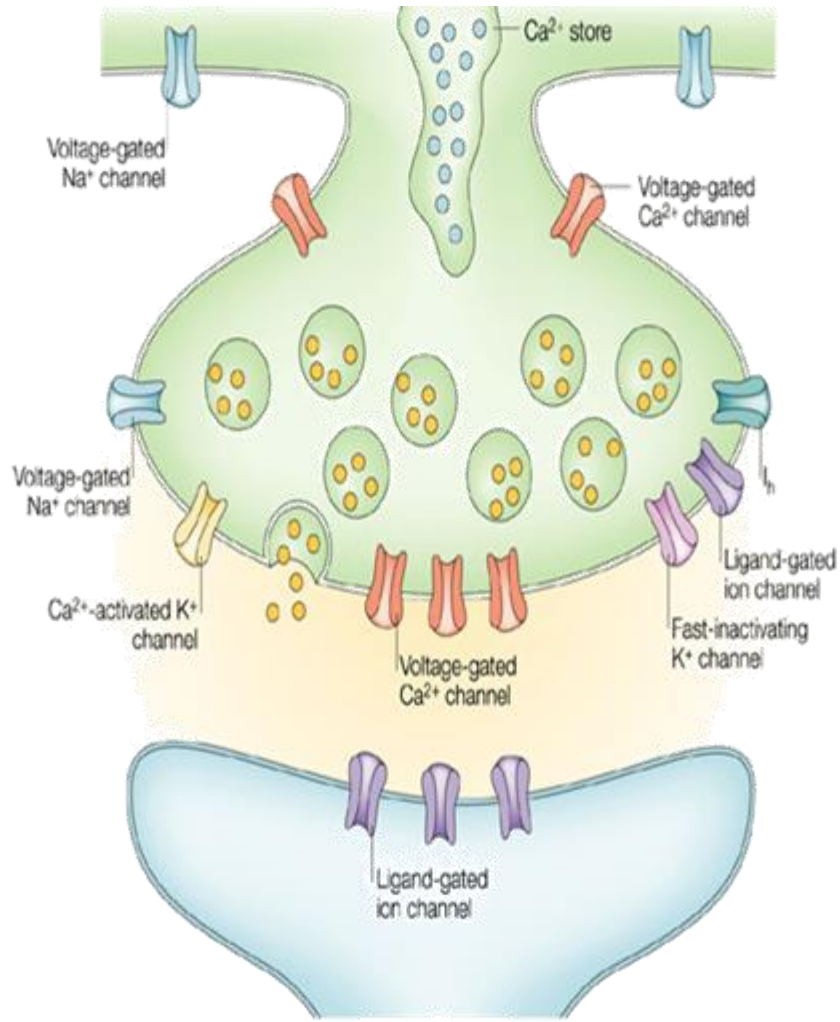
2. Reversal

3. Time extension

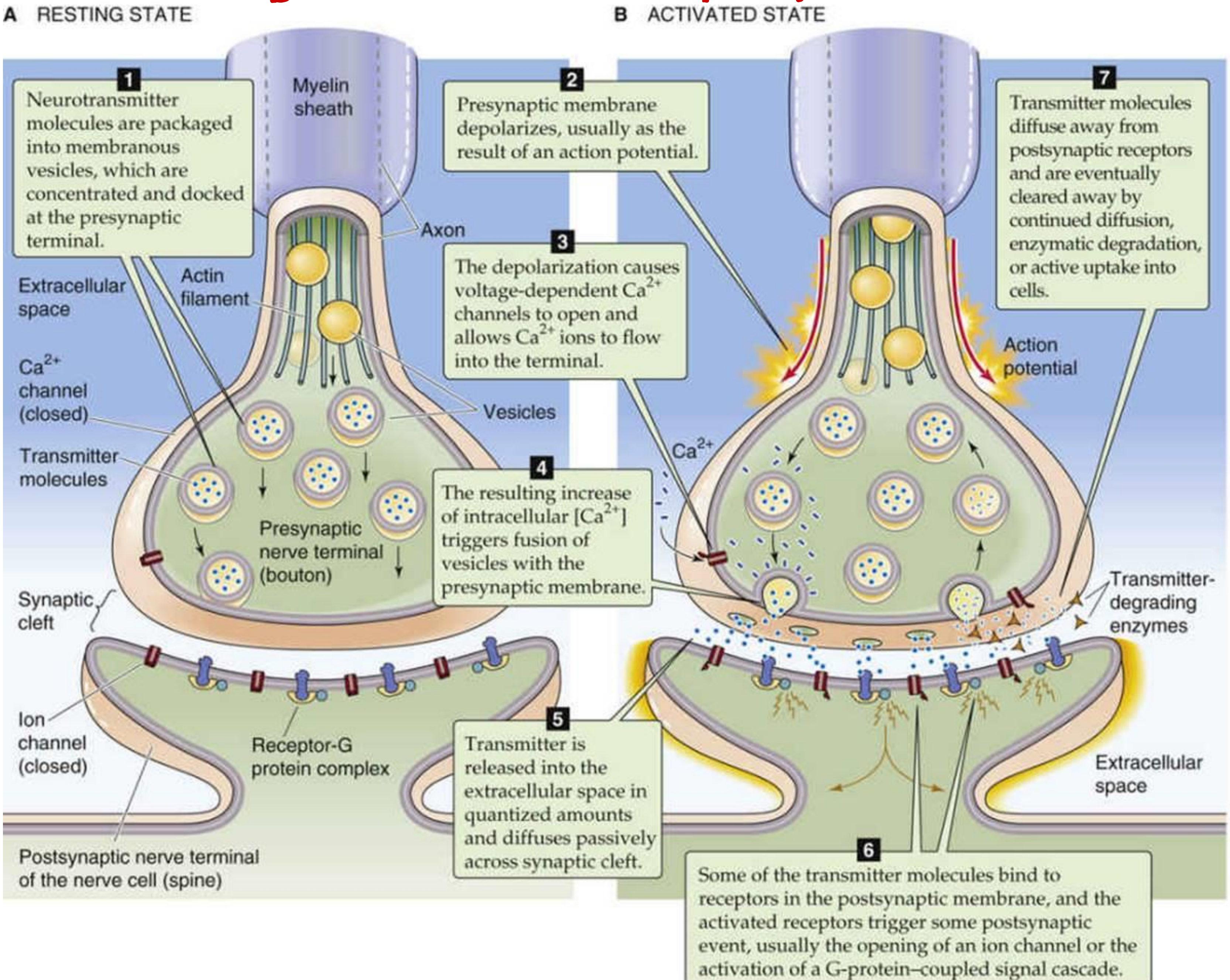
4. Plasticity phenomena

Sequence of events (chemical synapses)

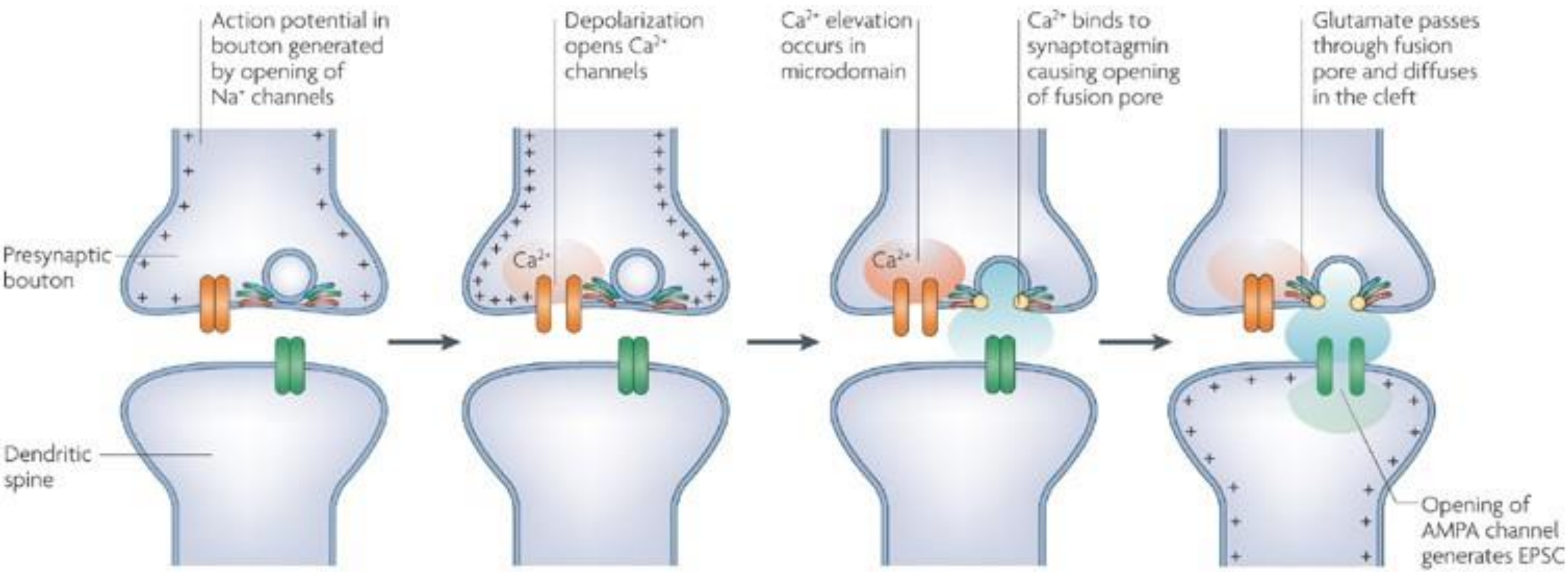
1. Arrival of the action potential in the axon terminal
2. Activation of activated voltage calcium channels
3. Ca2 + input into the terminal that stimulates vesicle fusion with the plasma membrane
4. Release of the neurotransmitter in the synaptic space
5. Linking the neurotransmitter to postsynaptic membrane receptors
6. Postsynaptic changes due to different membrane permeability



Resting and activated synapses



Sequence of events (chemical synapses)



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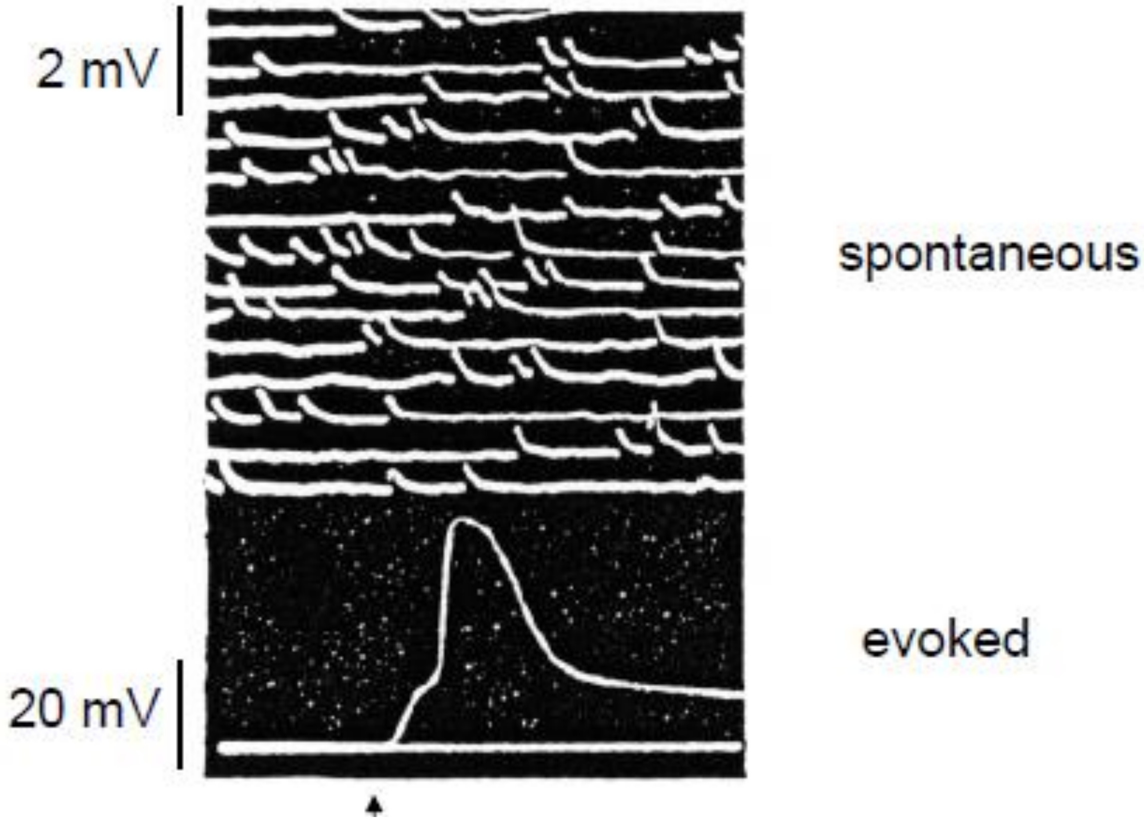
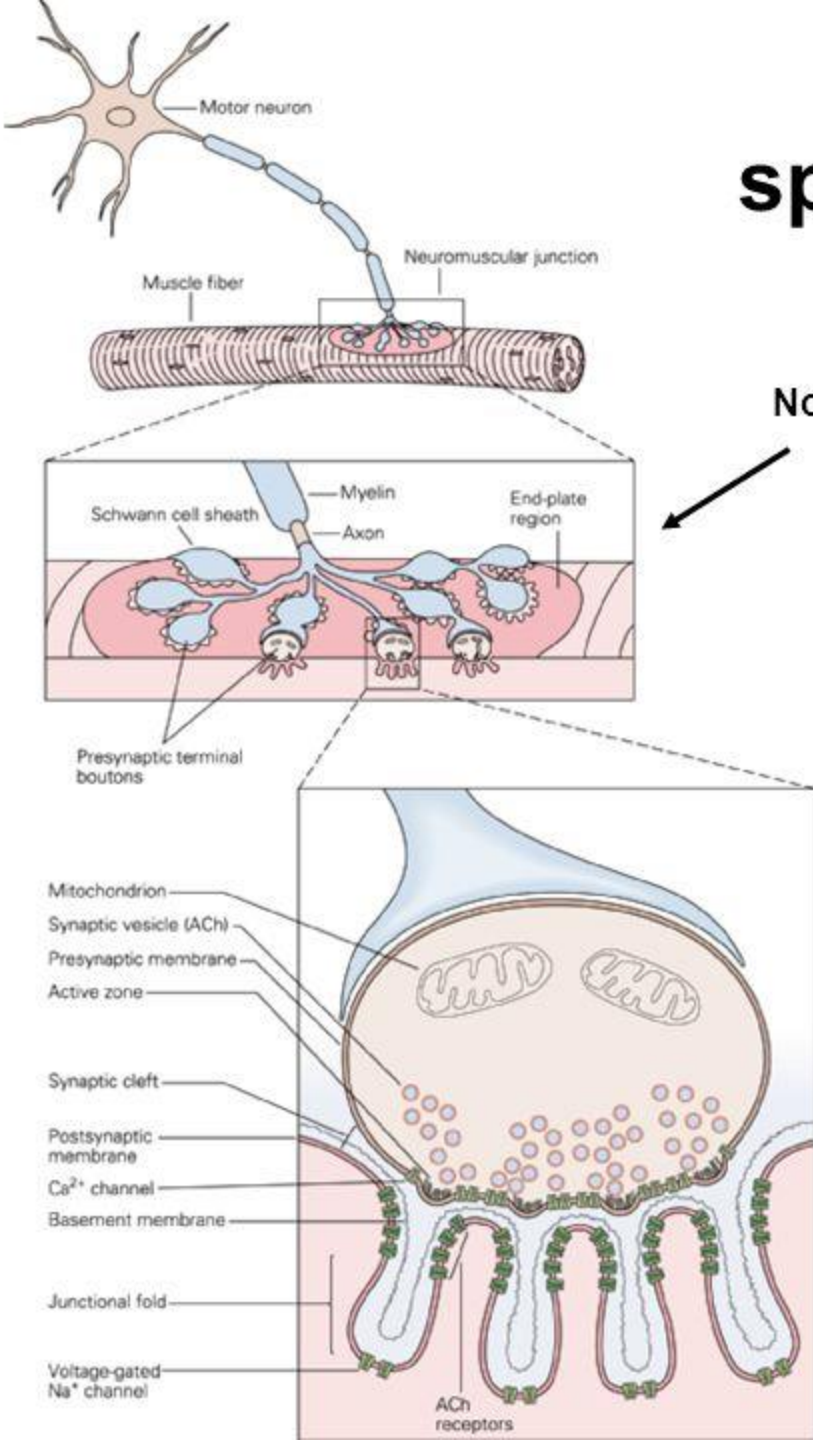
Resting and activated synapses: minis

The discovery of miniature endplate potentials; "minis"

Sir Bernard Katz



Nerve-evoked and spontaneous synaptic transmission

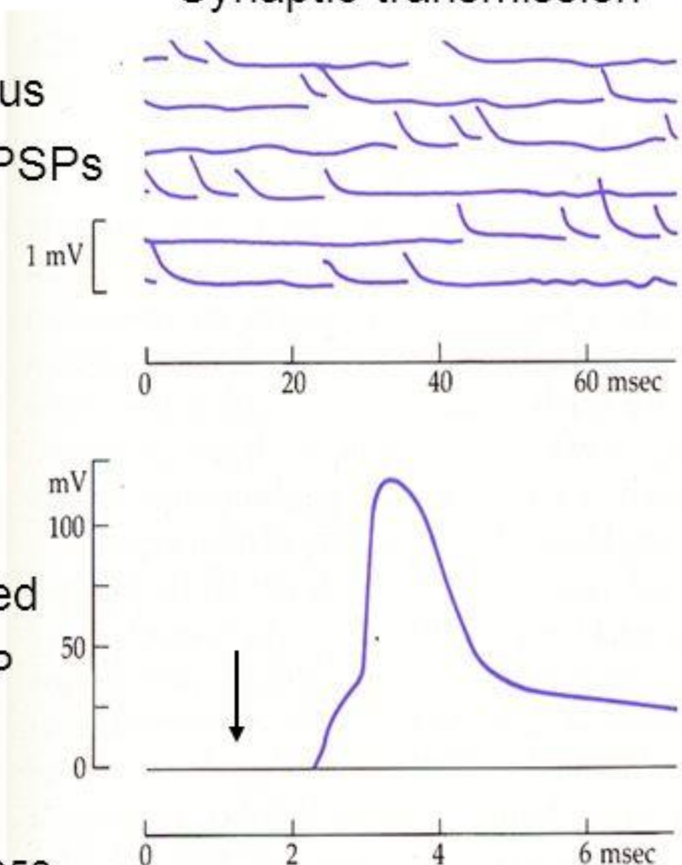


Note multiple release sites

Synaptic transmission

Spontaneous miniature EPSPs

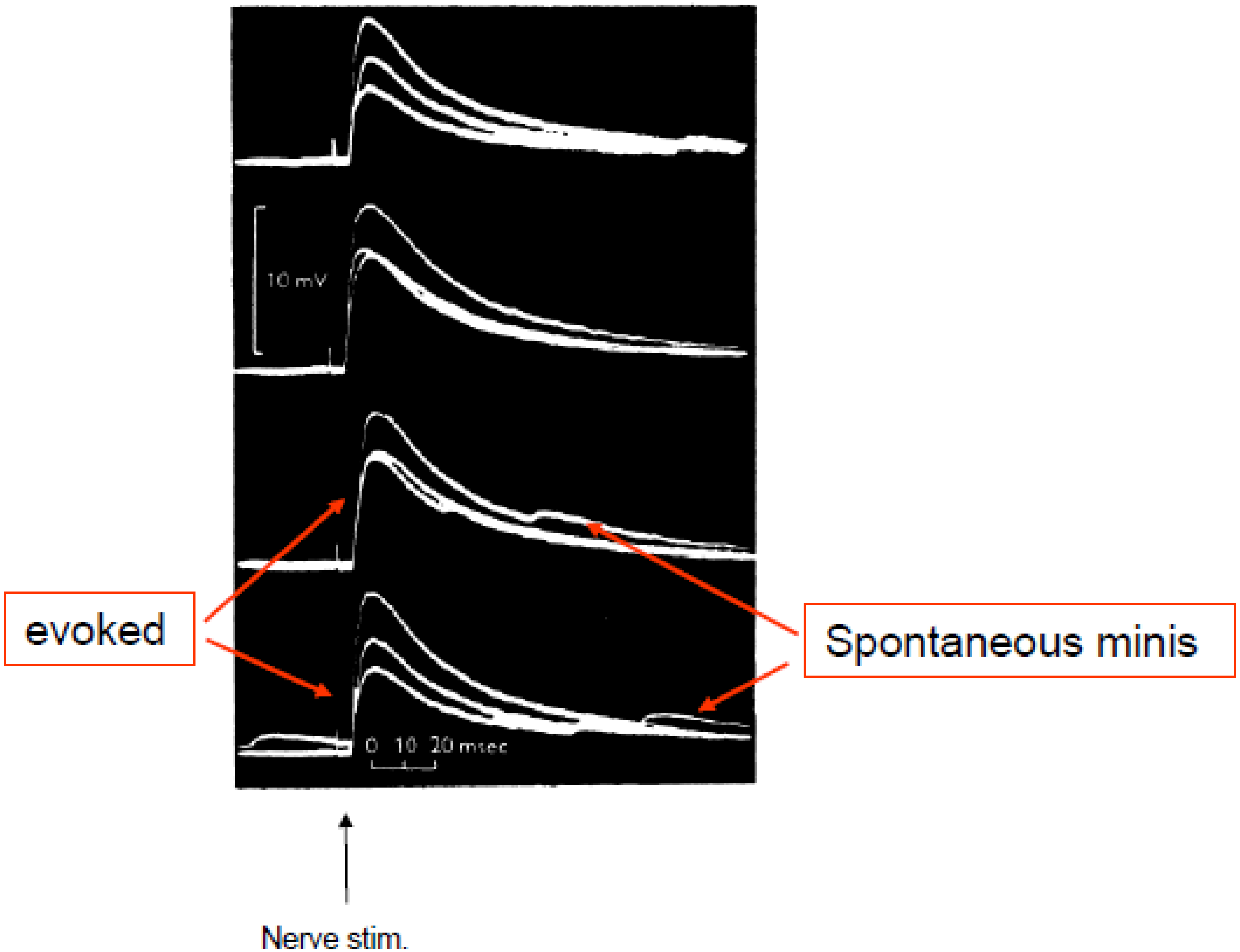
Nerve-evoked EPSP + AP



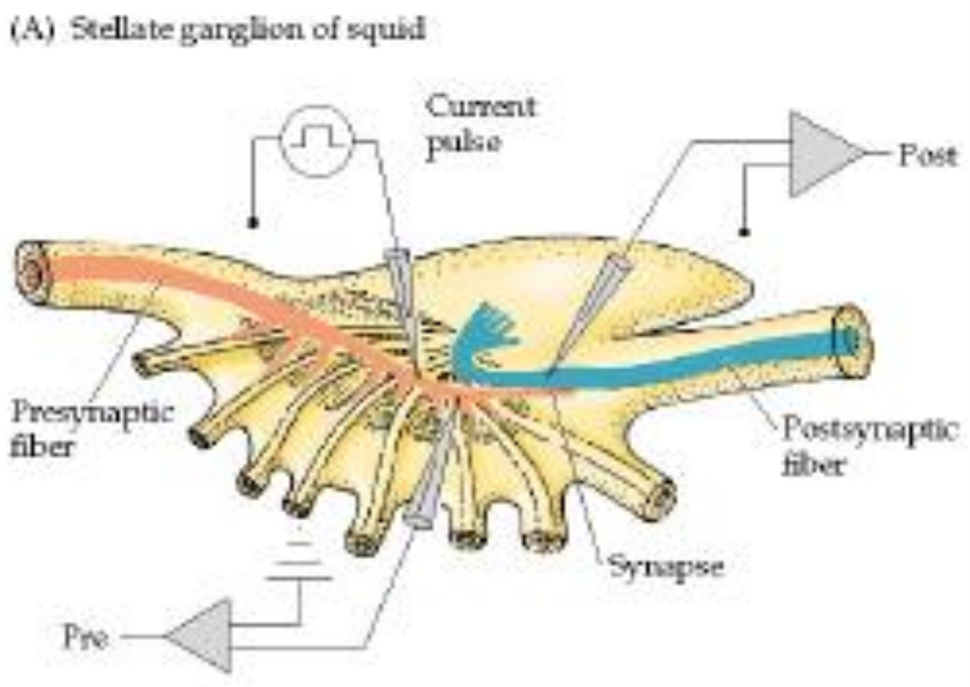
Fatt & Katz, 1952

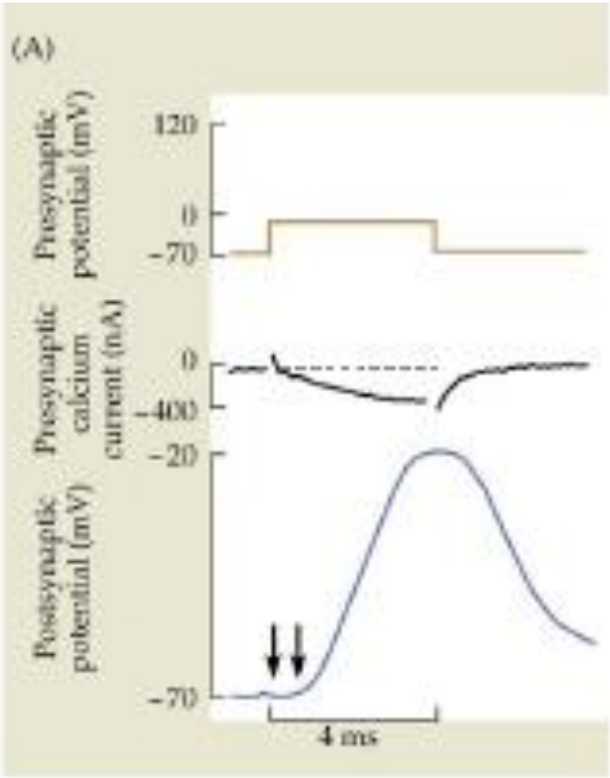
Ca^{++} is necessary for transmitter release

Low Ca^{++} in the perfusion medium



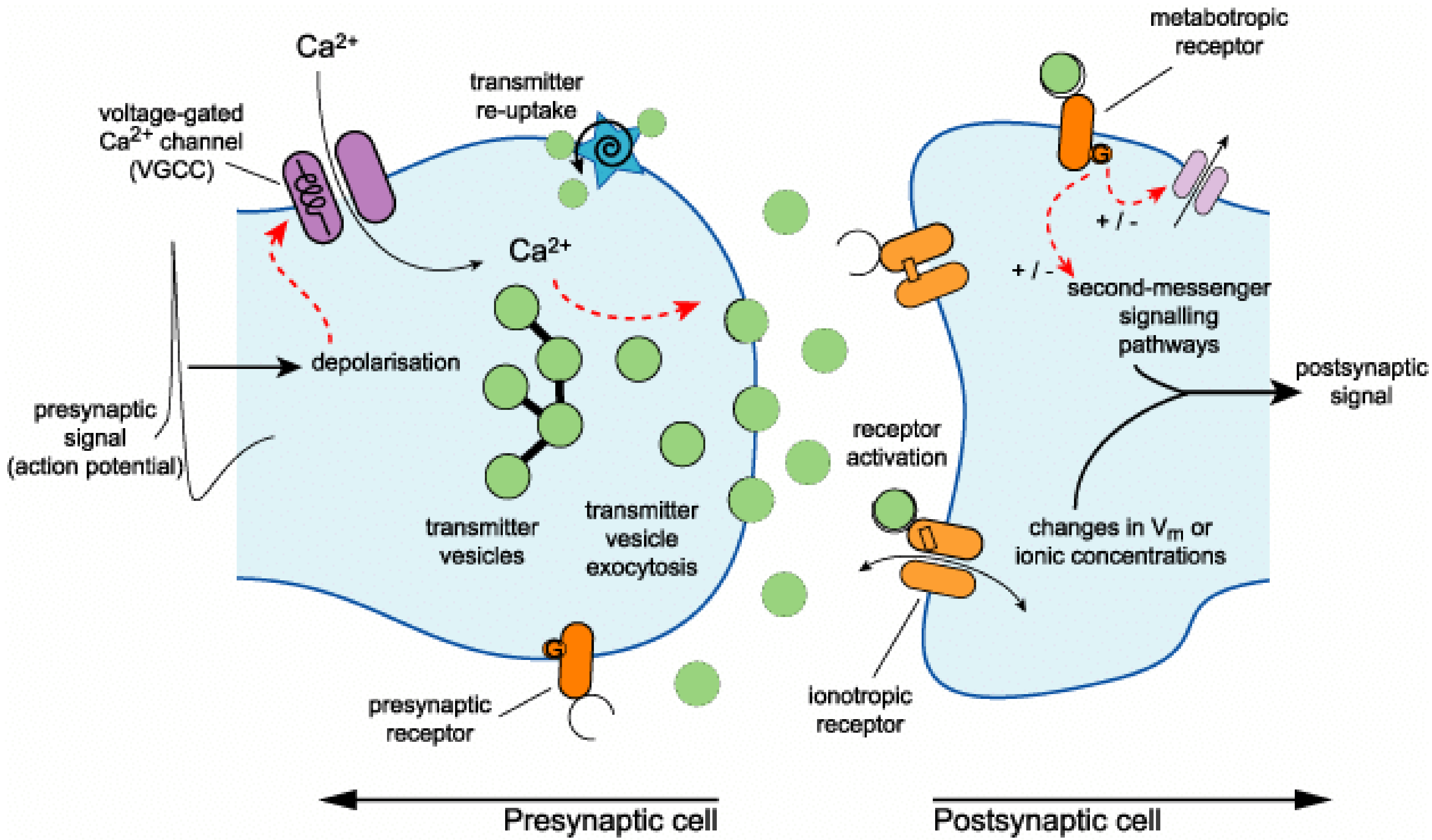
Ca^{2+} channels open in response to depolarization



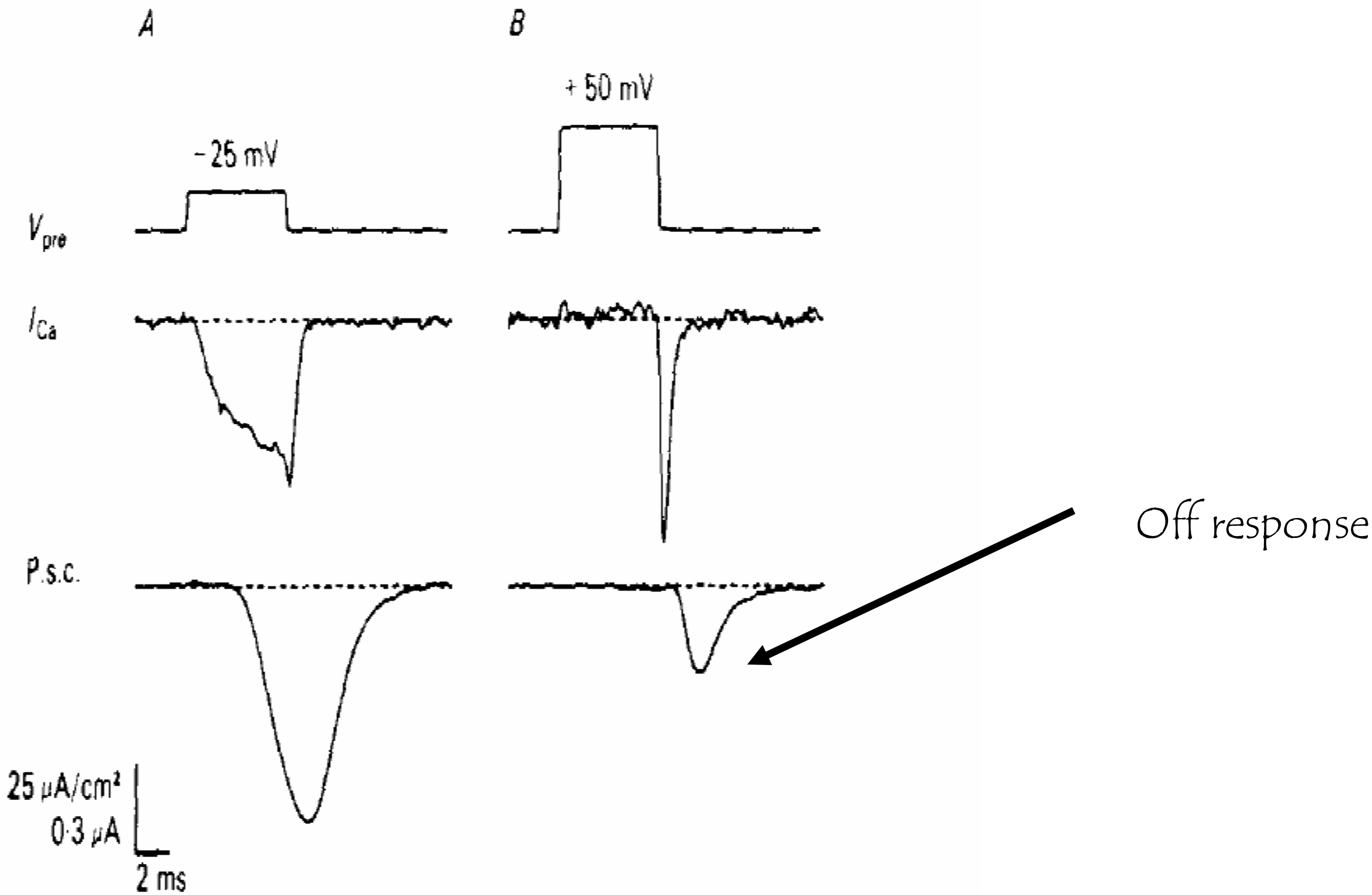


Sinapsi gigante del calamaro per isolare le correnti al calcio

Ca^{2+} channels open in response to depolarization

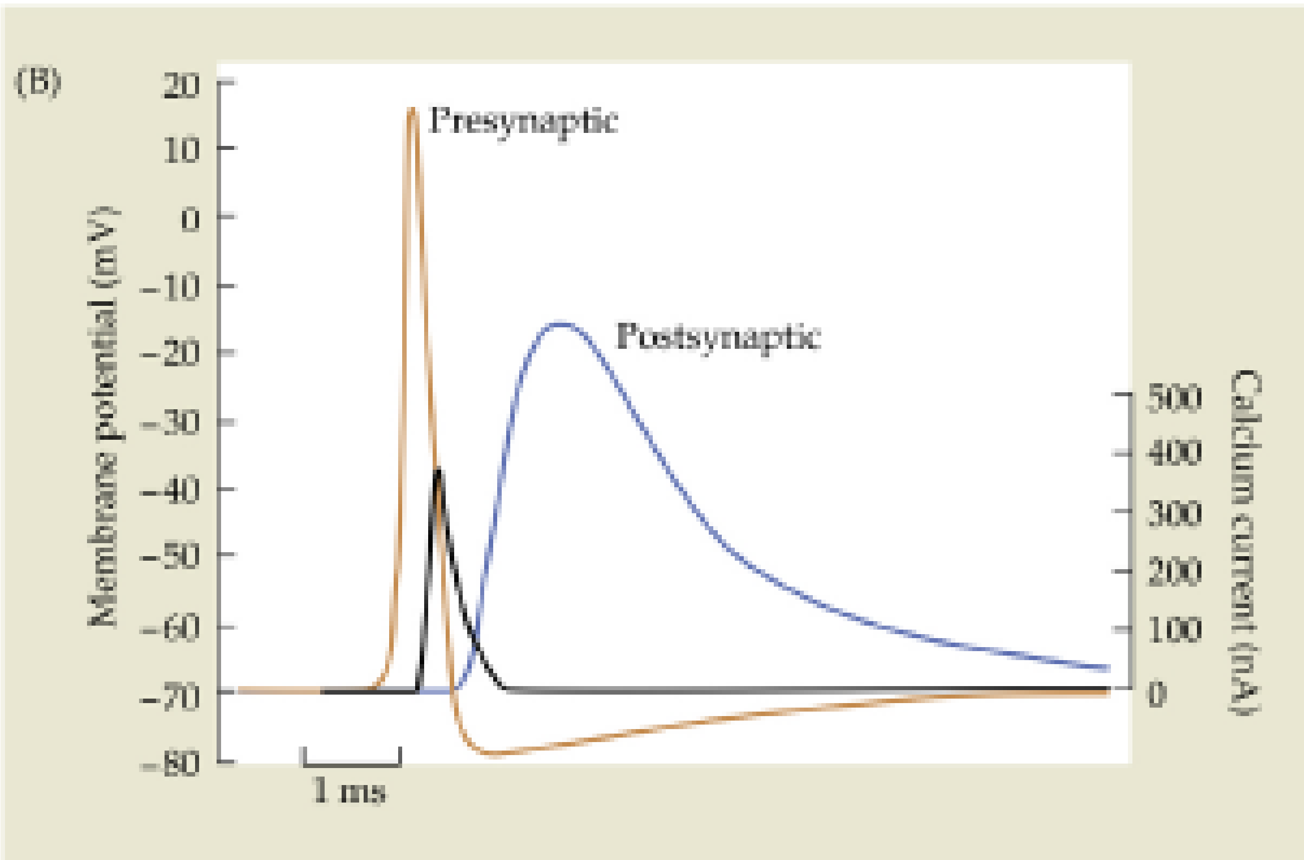


A strong depolarization suppresses the calcium currents until the end of the depolarizing impulse



Augustine G, Charlton M, Smith S (1985) Calcium entry into voltage-calmped presynaptic terminals of squid. J Physiol 367:143-162.

The peak of the calcium currents is in the descending phase of the action potential



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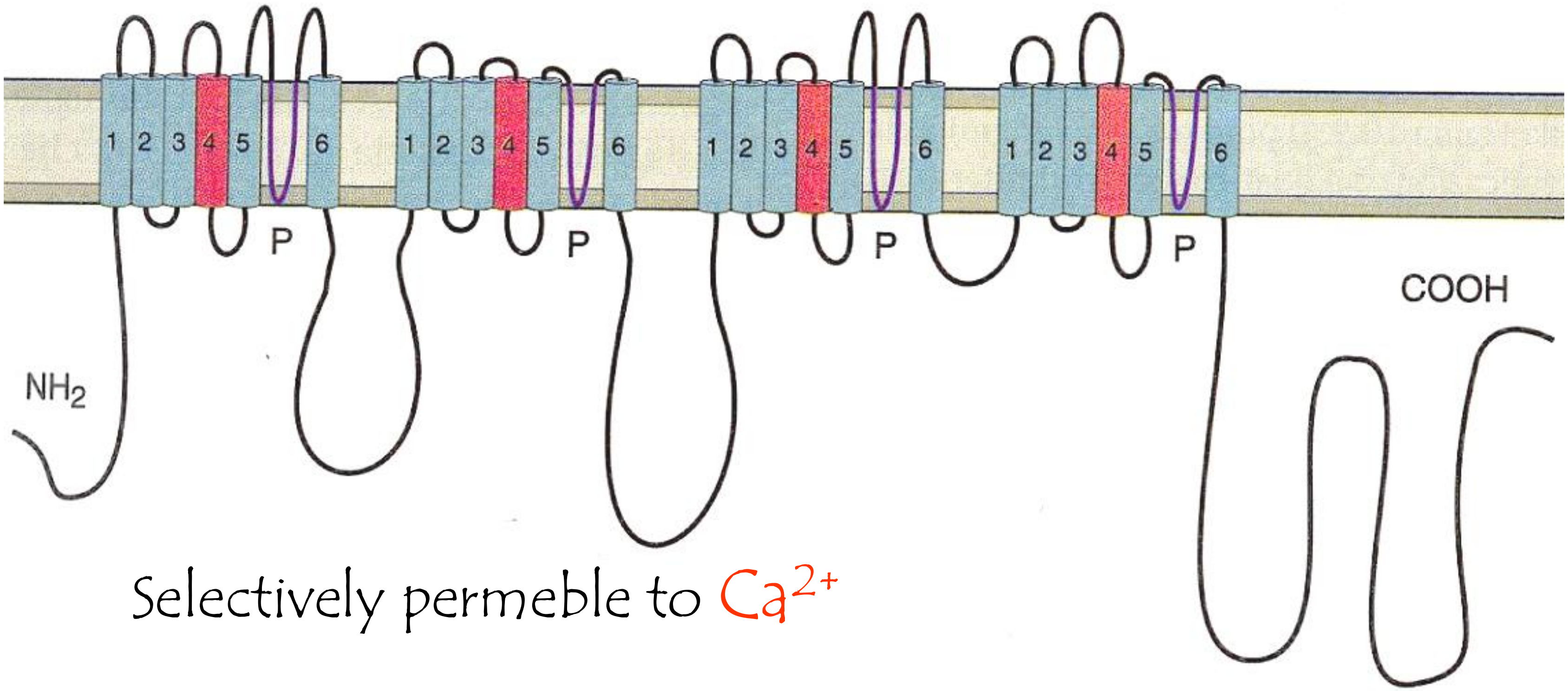
Lavoro basato sulla sinapsi gigante del calmato a varie T

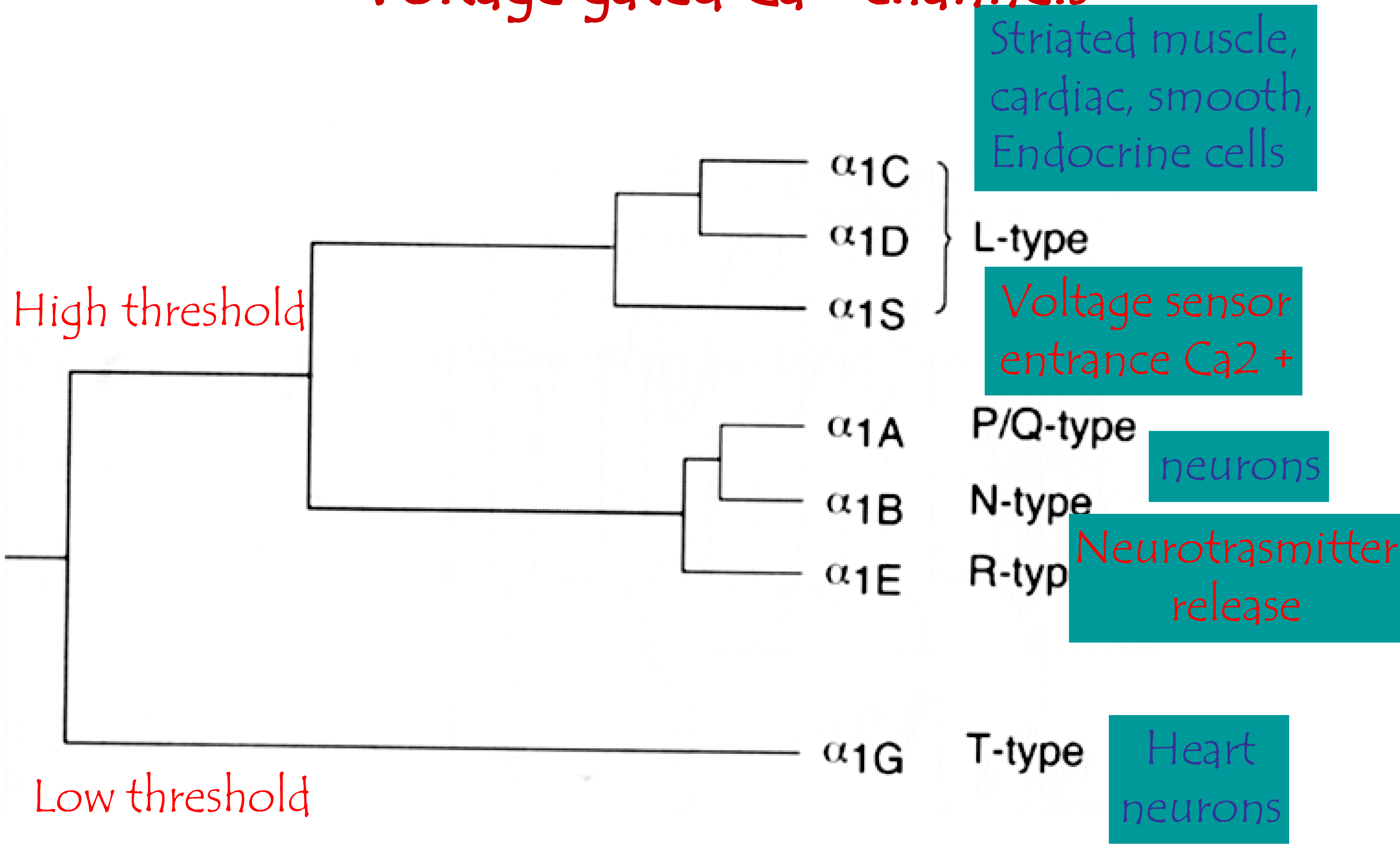
Voltage gated Ca^{2+} channels



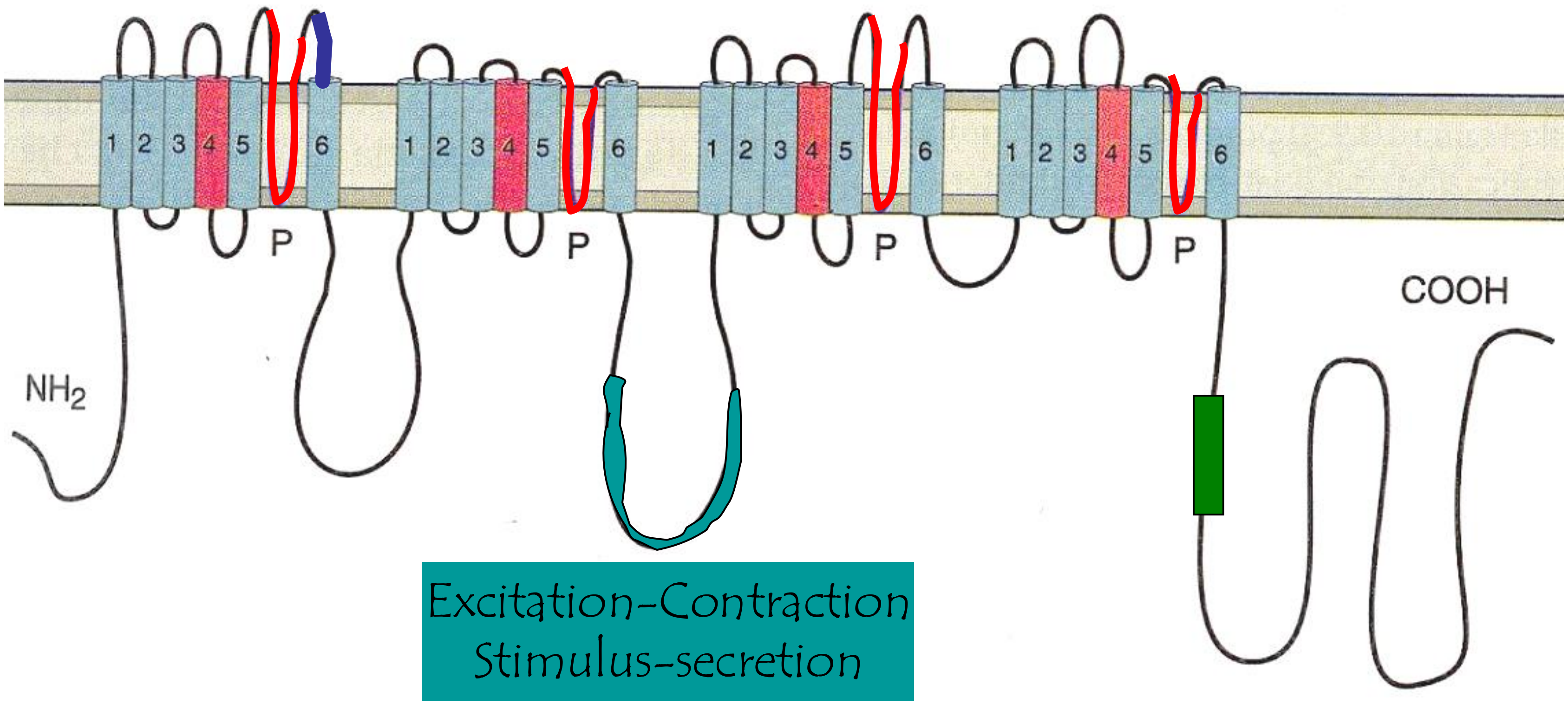
Selectively permeble to Ca^{2+}

They transduce electrical signals into metabolic signals

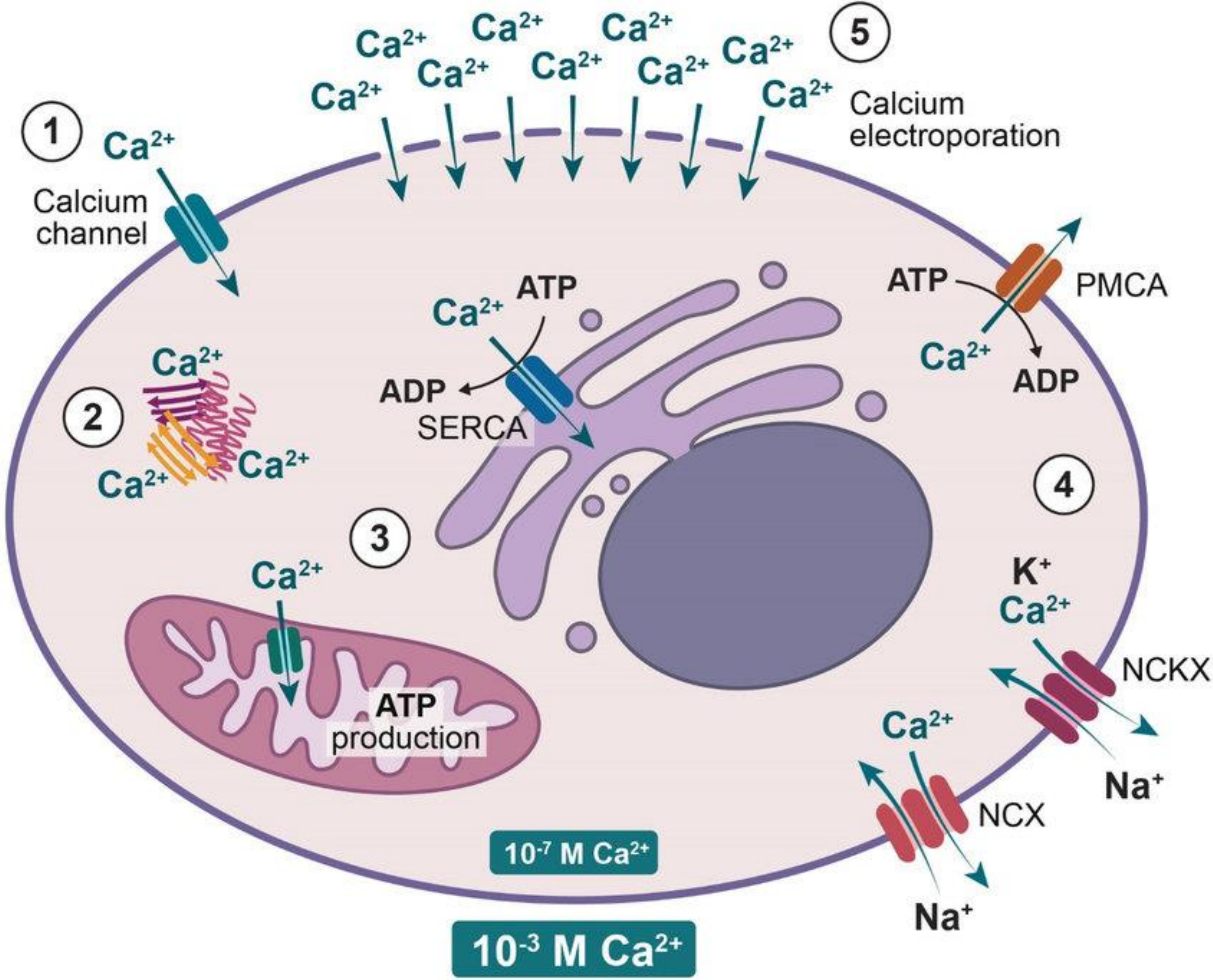
Voltage gated Ca^{2+} channels



Voltage gated Ca^{2+} channels



- Pore sodium permeability in the absence of calcium
- Voltage-dependent inactivation of conformational change at the entrance of the canal
- Inactivation Ca^{2+}-dependent



5
Calcium electroporation
1
Calcium channel
2
3
4
ATP
ADP
SERCA
PMCA
ATP production
K+
NCKX
Na+
NCX
10^{-7} M Ca^{2+}
10^{-3} M Ca^{2+}
Ca^{2+}

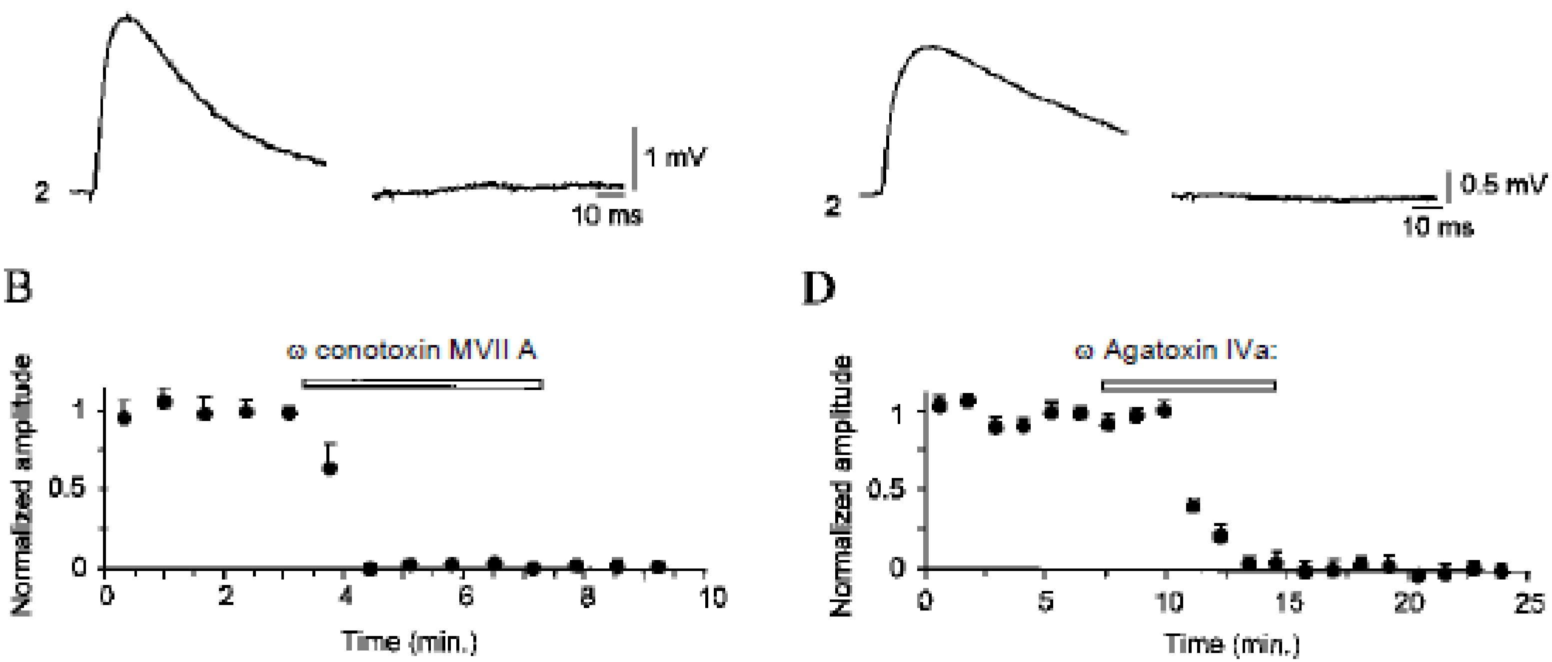
Which Ca^{2+} channel is responsible for Transmitter release?

Conus Granulatus

conotoxins

Agelenopsis Aperta

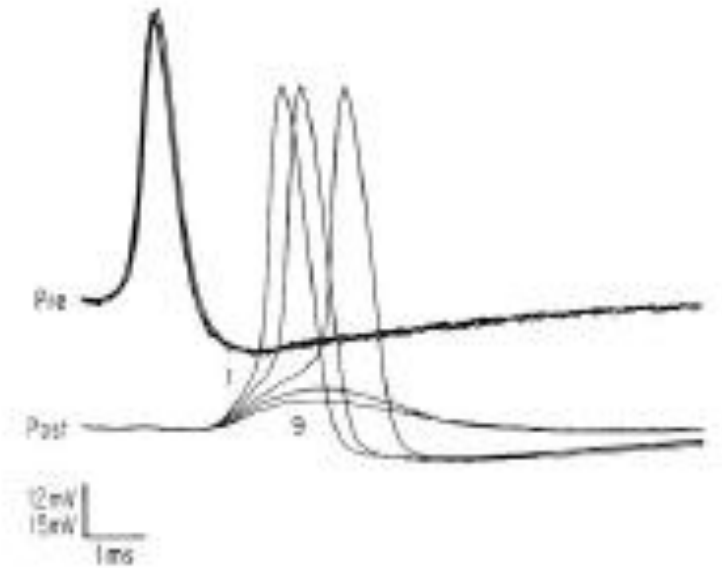
Which Ca^{2+} channel is responsible for Transmitter release?

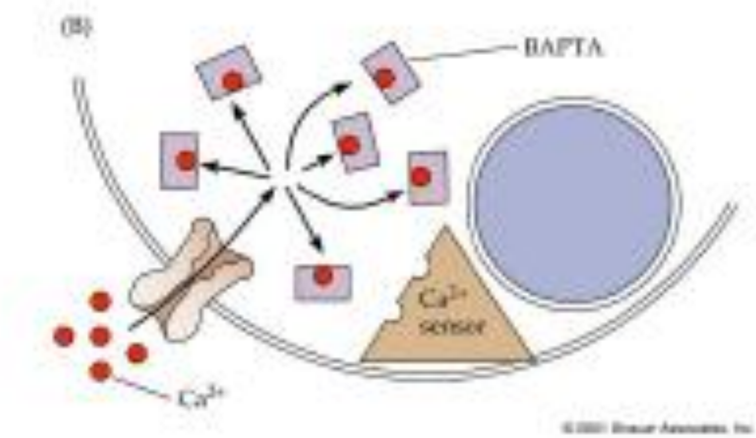


ωconotoxin MVII A :Blocks N-type Ca2+ channels

ω Agatoxin IVa: Blocks P-type Ca2+ channels

Ca2 + is necessary for the release of NT

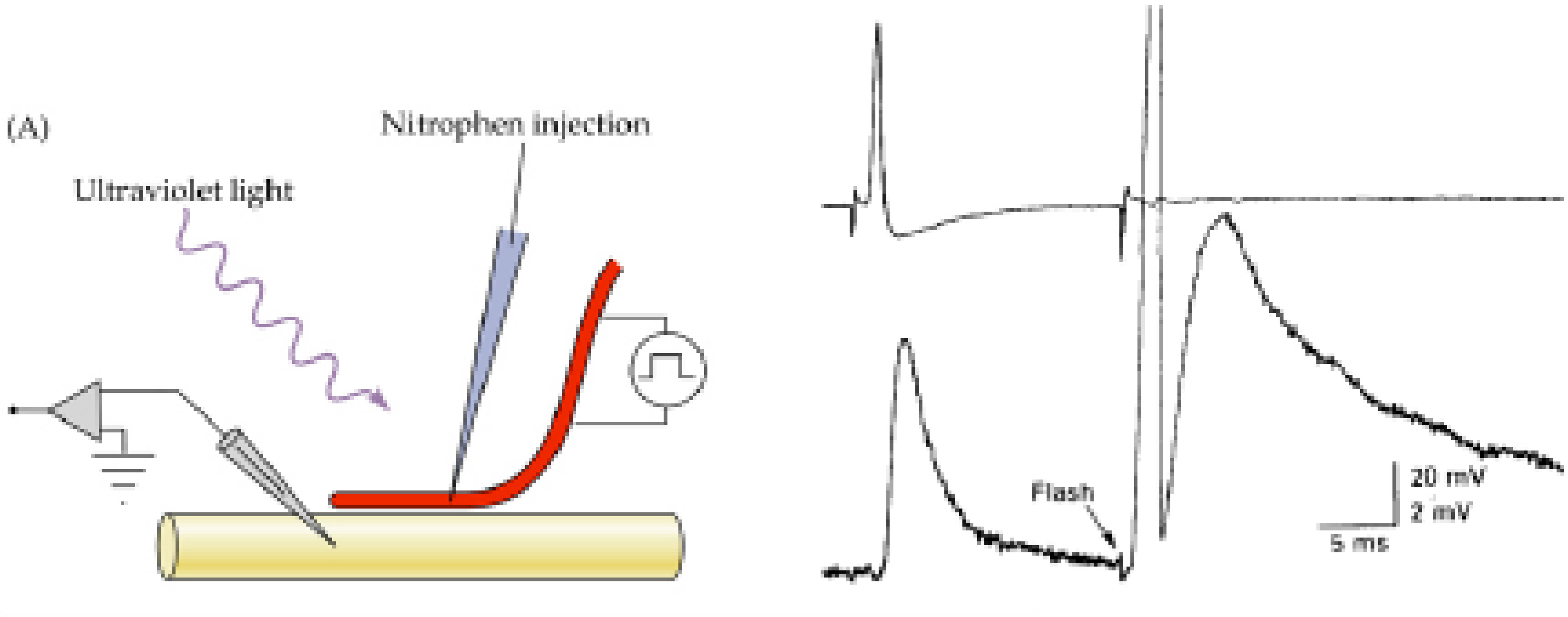




- Consequences of BAPTA injection into the presynaptic terminal: slowing of release

Adler EM, Augustine GJ, Duffy SN, Charlton MP (1991) Alien intracellular calcium chelators attenuate neurotransmitter release at the squid giant synapse. J Neurosci 11:1496-1507.

Ca2 + is sufficient for the liberation of NT



Consequences of the arificial increase of [Ca2 +] i in the terminal: increase in the probability of release.

Delaney K, ZuckerRS (1990) Calcium released by photolysis of DM-nitrophenstimulates transmitter release at squid giant synapse. J Physiol 426:473-498.

What happens if we interfere with NT removal?

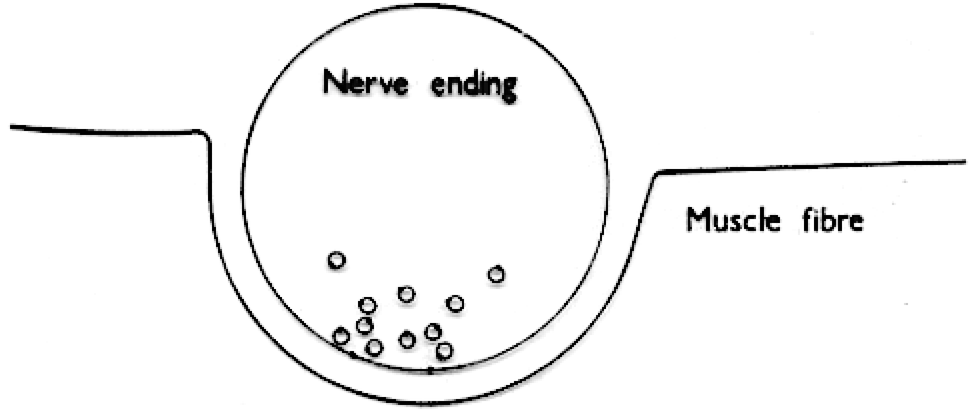
AChE blockers at the neuromuscular junction
-sarin, GB
-Malathion®

Blockers of the re-uptake of the amine transmitters

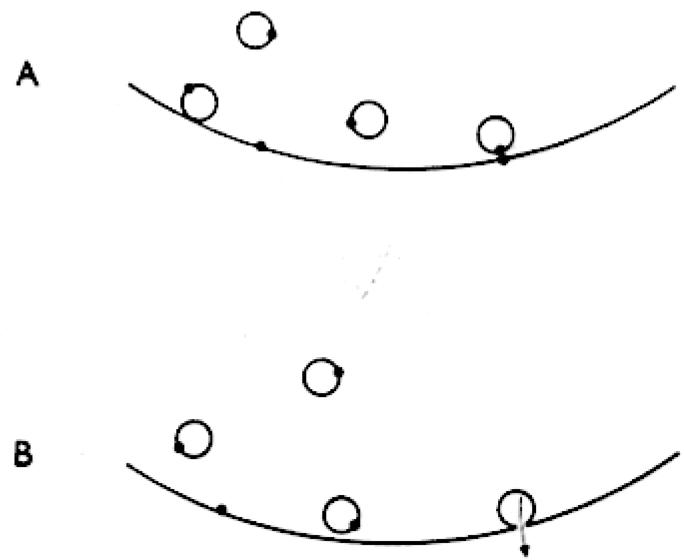
-dopamine -cocaine
-serotonin -fluoxetine(Prozac®)

$$A + R \leftrightarrow AR + A \leftrightarrow A2R \leftrightarrow A2R^* \leftrightarrow A2R$$

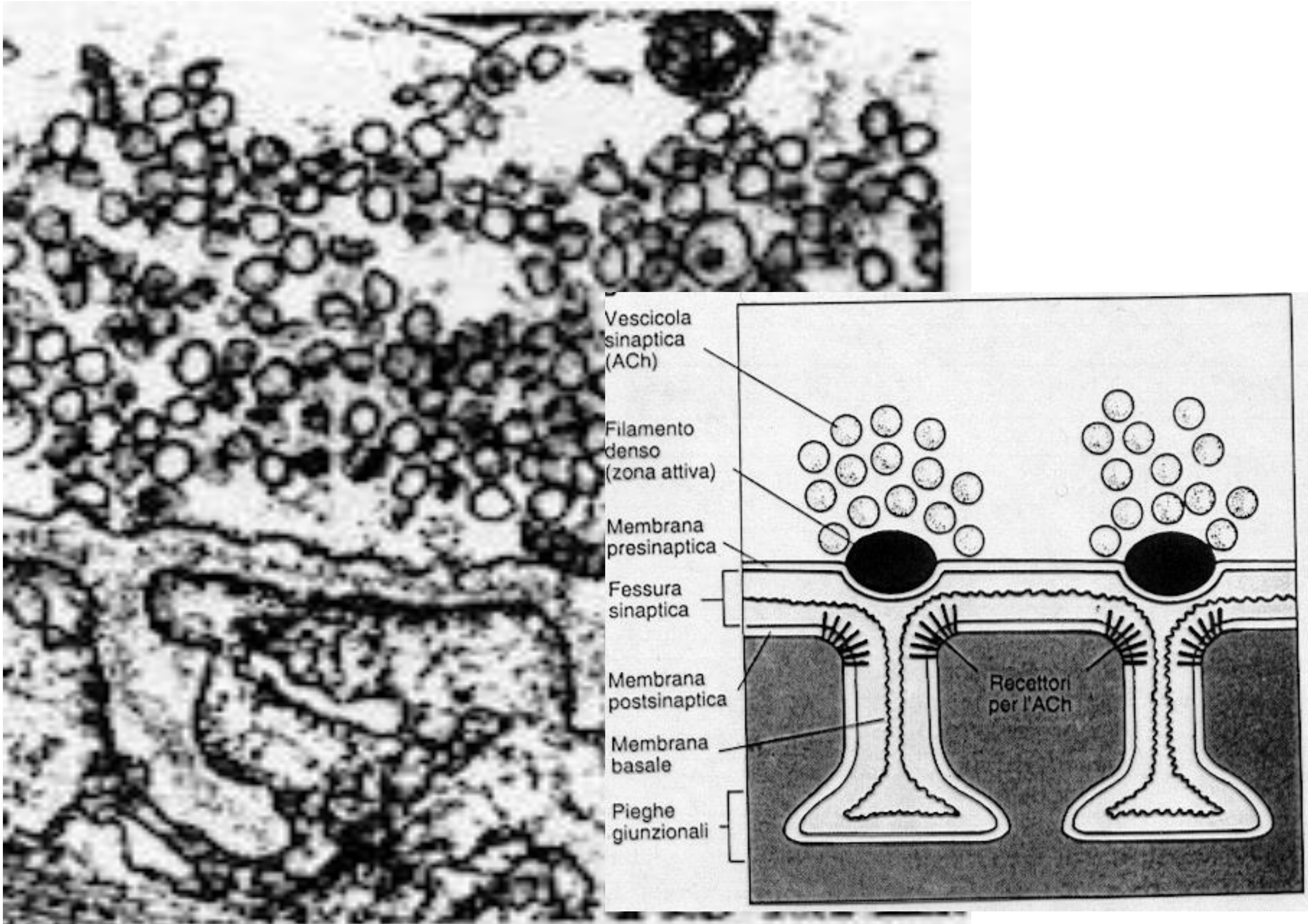
Quantal release : (a theory that explains the facts)



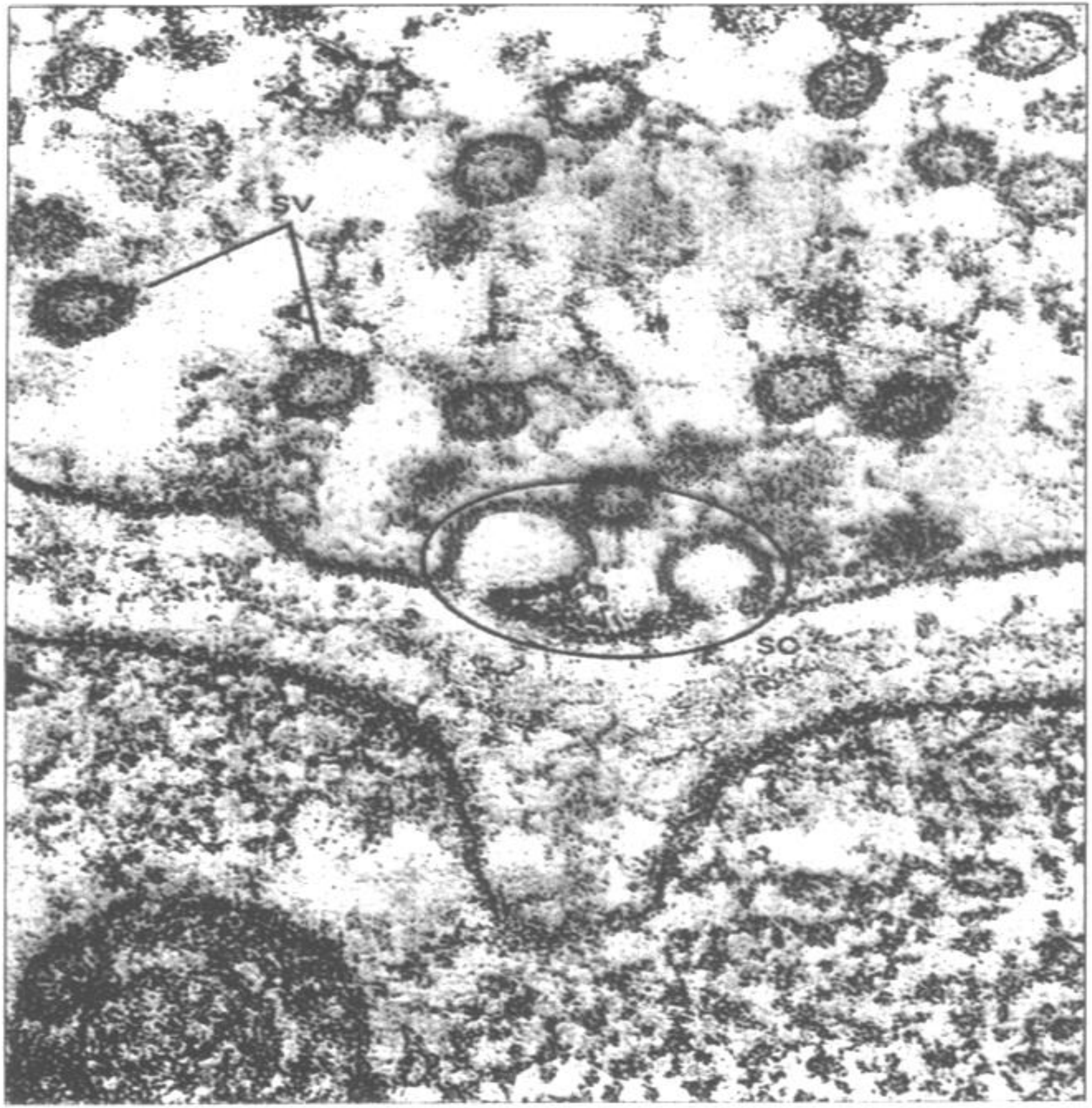
The presynaptic terminal releases quanta of neurotransmitter which act on the postsynaptic membrane, depolarizing it



Del Castillo & Katz, 1957

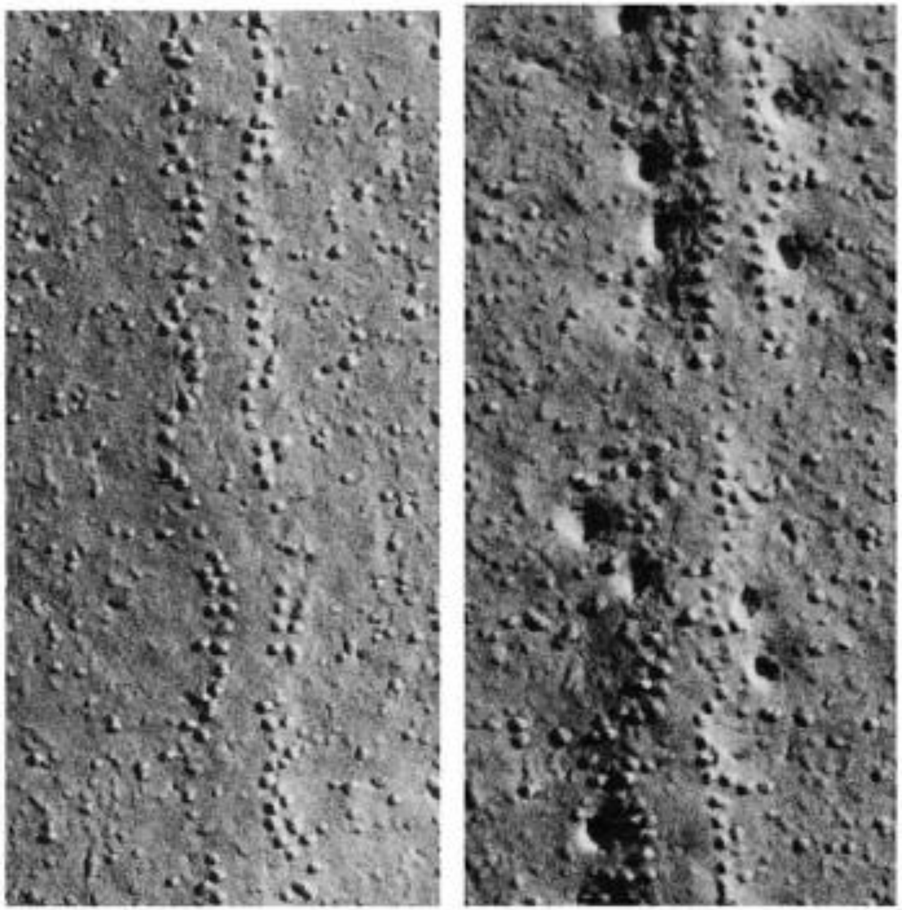


Vescicola sinaptica (ACh)
Filamento denso (zona attiva)
Membrana presinaptica
Fessura sinaptica
Membrana postsinaptica
Membrana basale
Pieghe giunzionali
Recettori per l'ACh

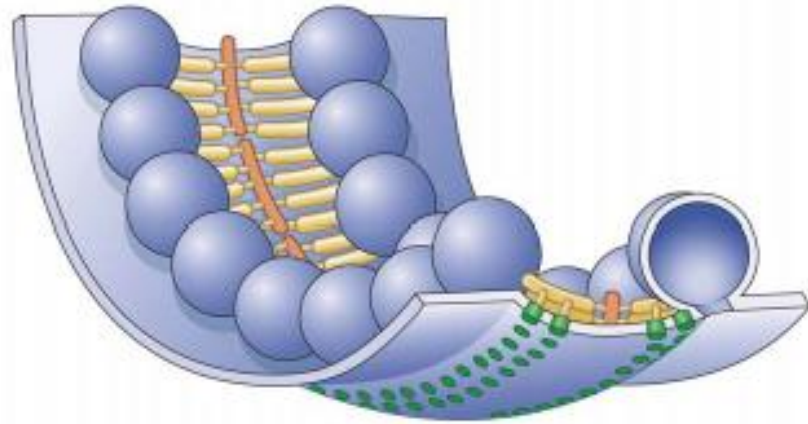

SV
SO

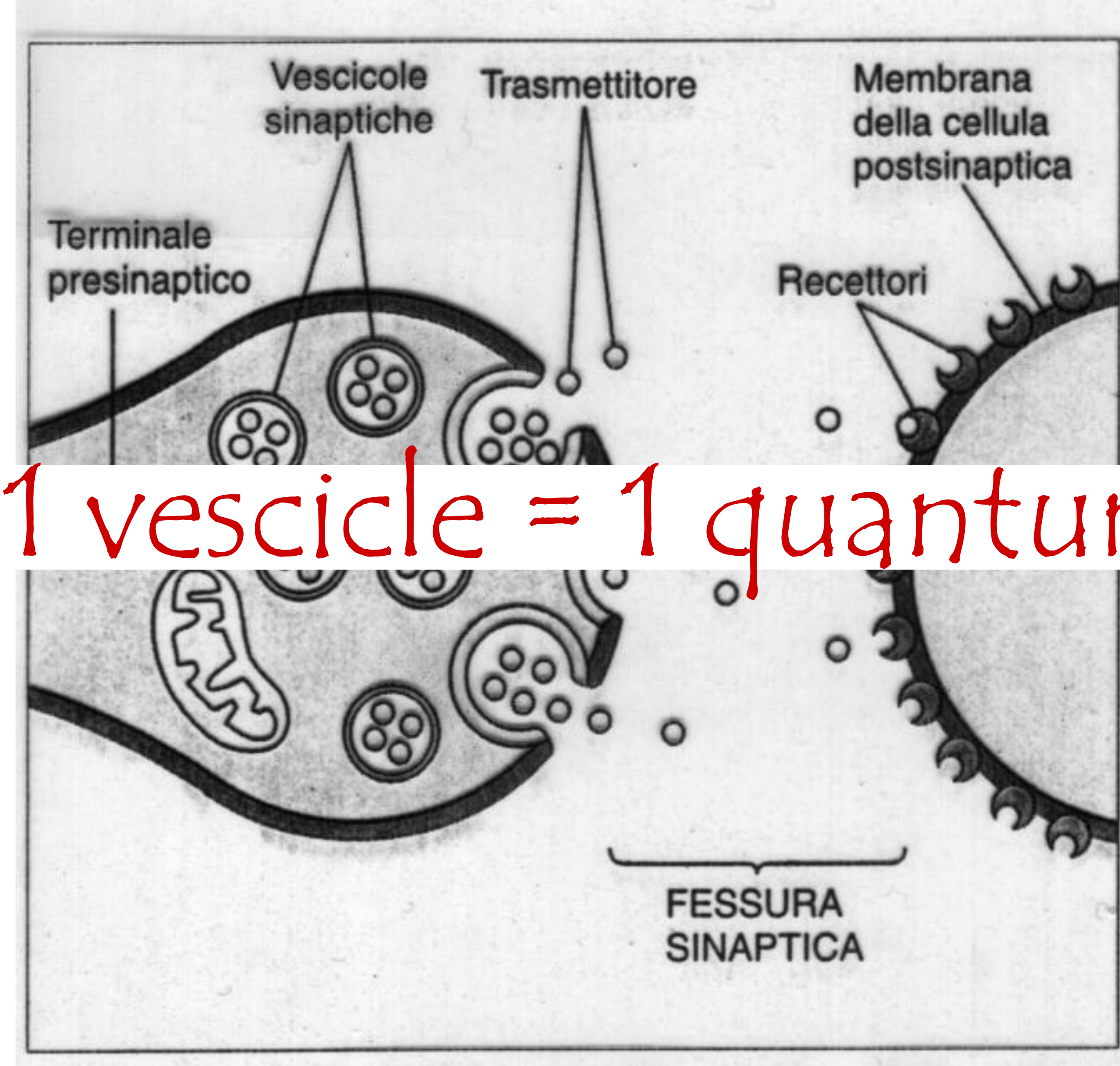
Release is vesicular

Synaptic Vesicle exocytosis captured by quick freezing; (Hauser & Reese)



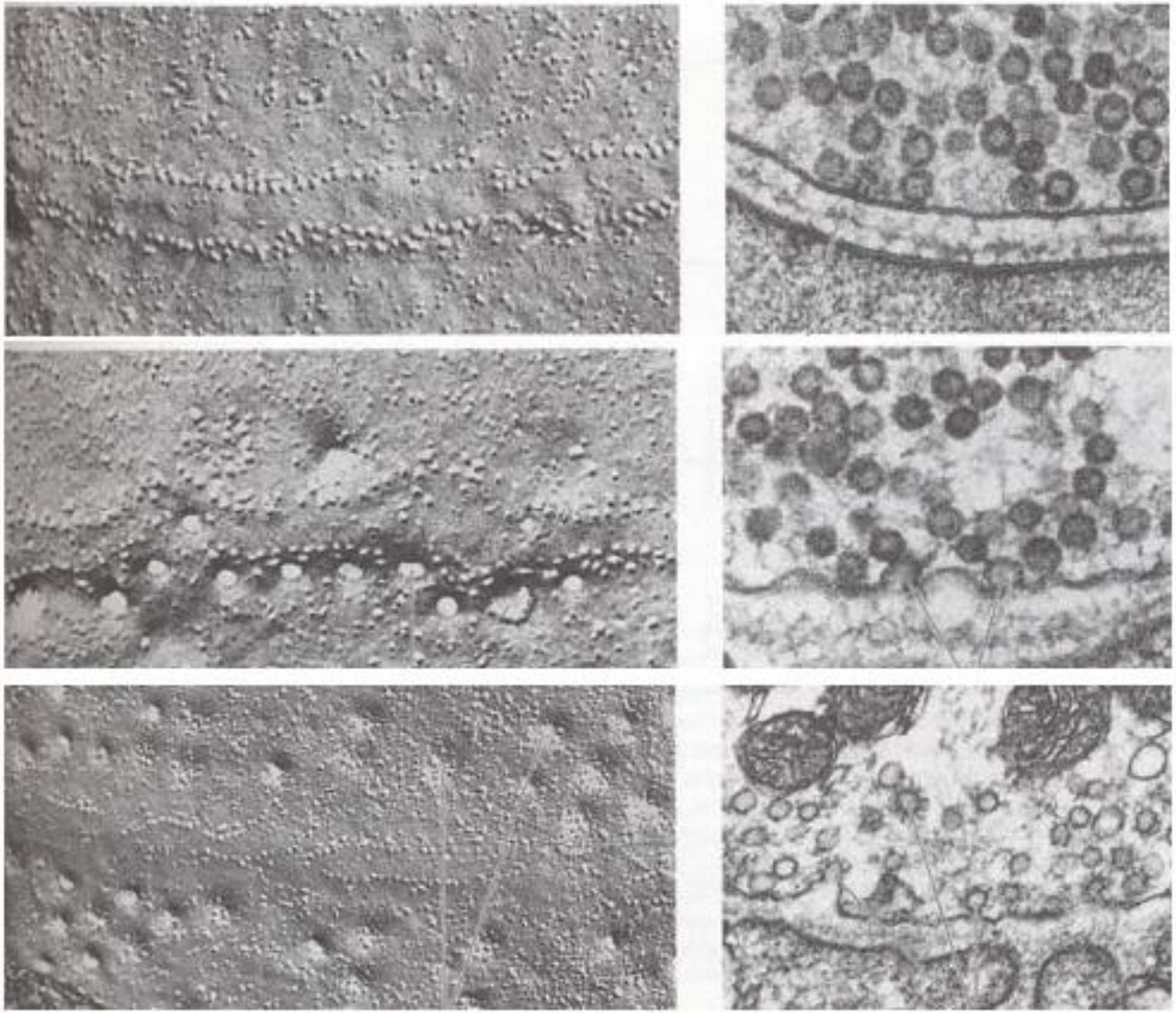
(C)



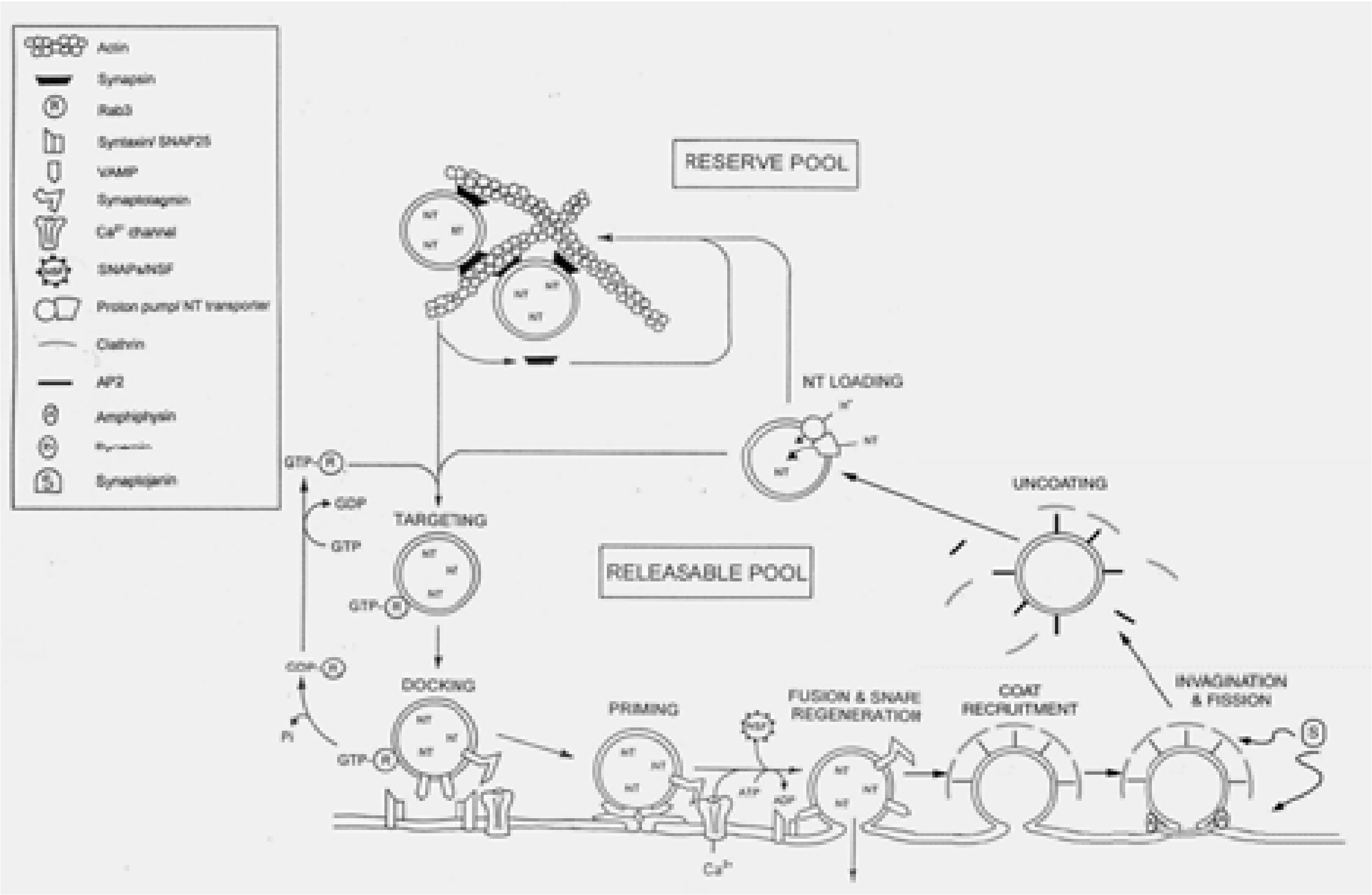


1 vescicle = 1 quantum

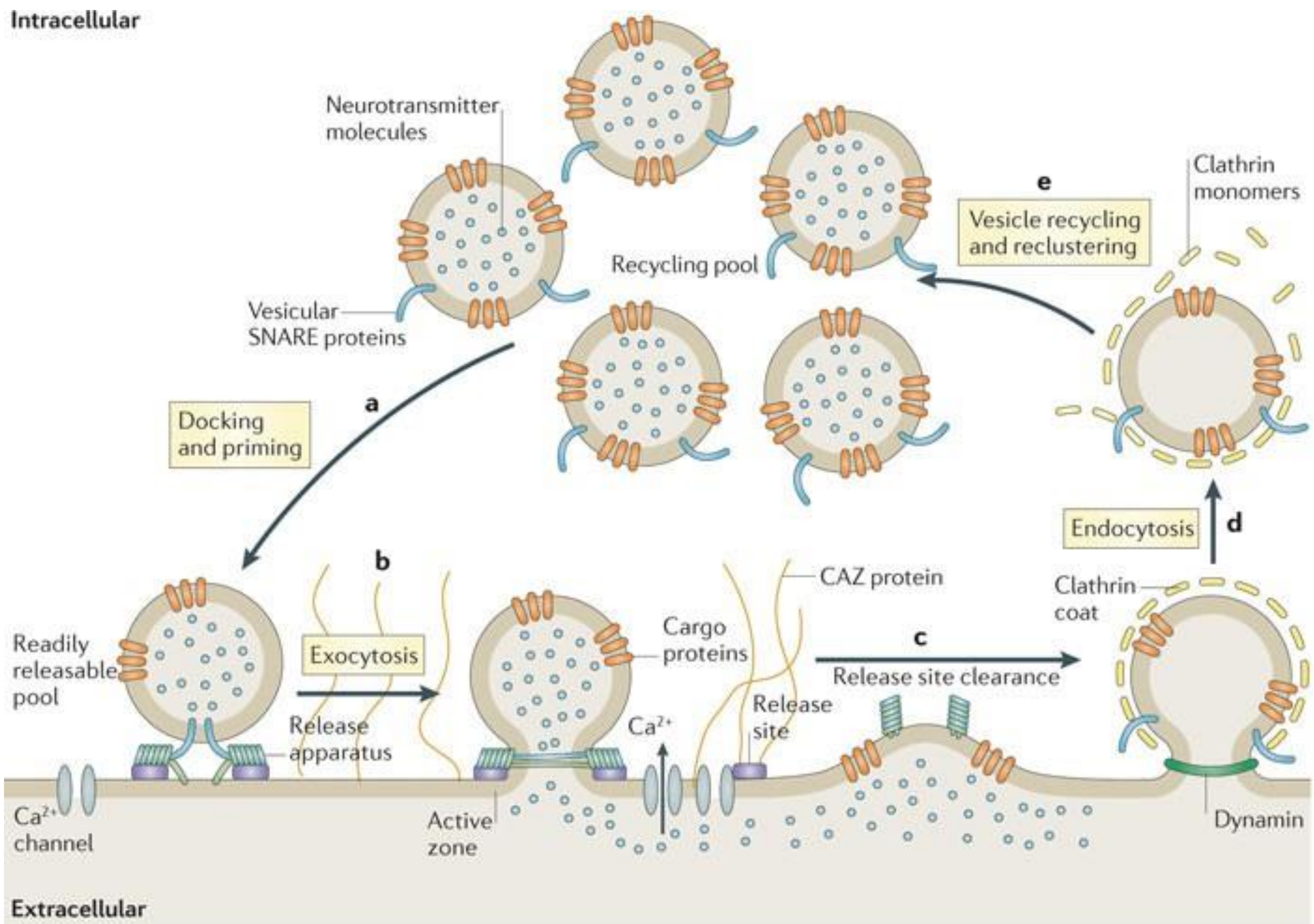
The exo-endocytotic cycle of synaptic vesicles



The exo-endocytotic cycle of synaptic vesicles



The exo-endocytotic cycle of synaptic vesicles



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Synaptic vesicle pools

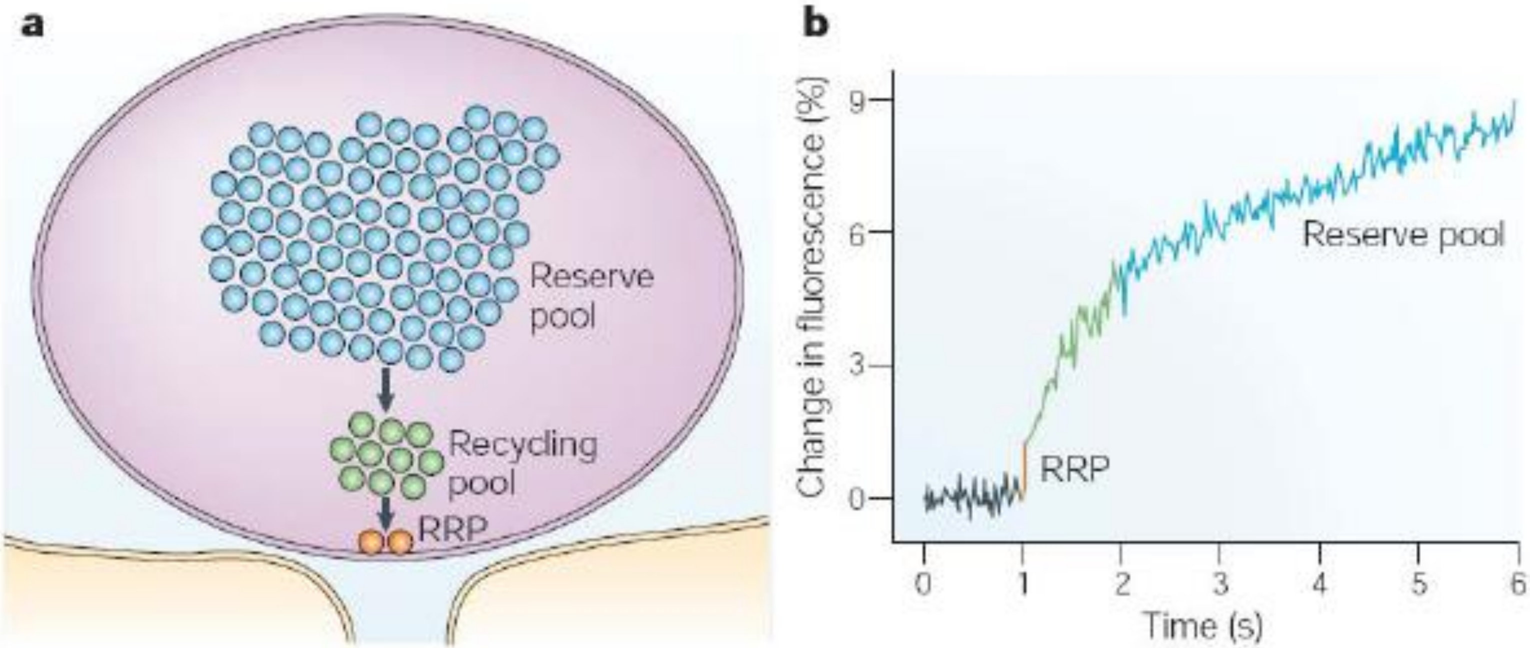
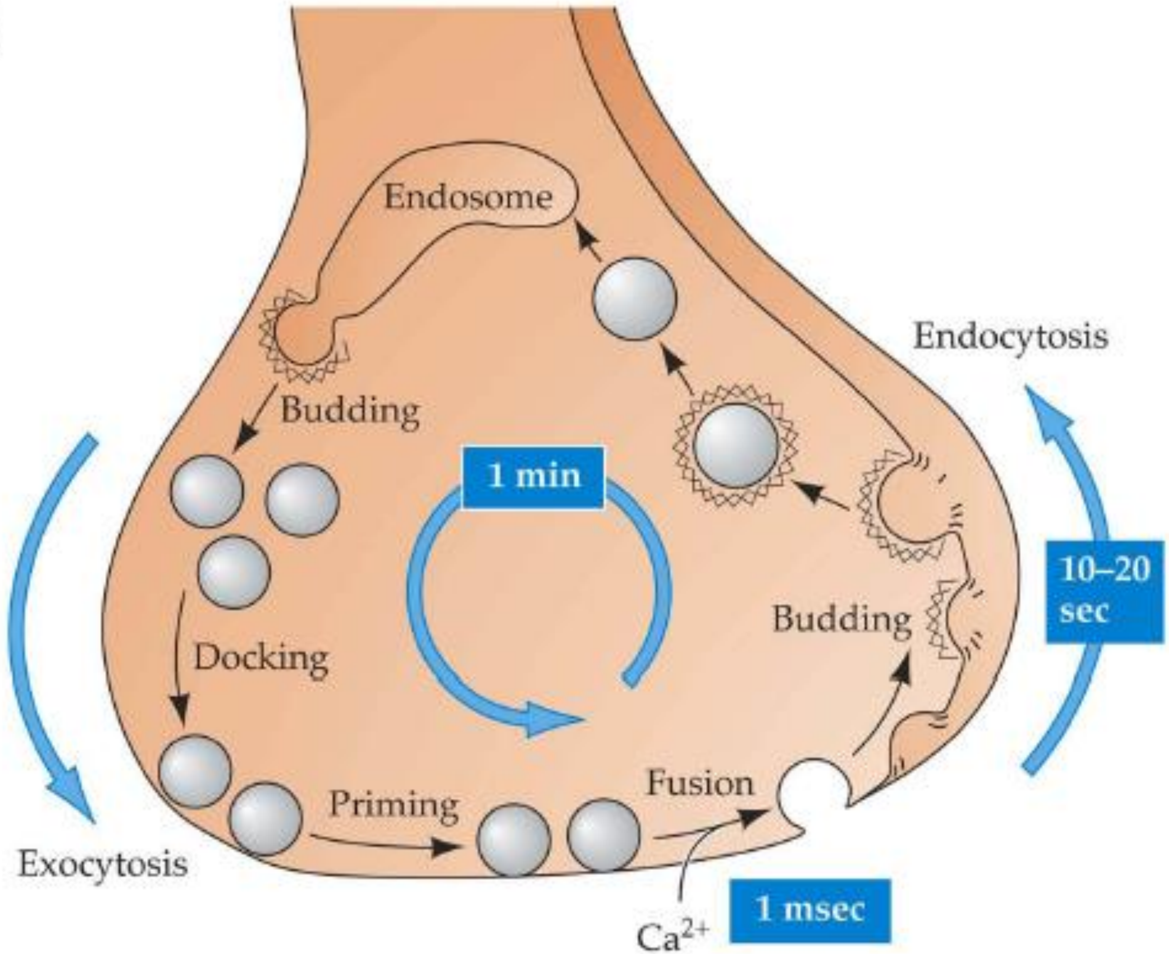


Figure 1 | **Three vesicle pools. a** | The classic three-pool model. The reserve pool makes up ~80–90% of the total pool, and the recycling pool is significantly smaller (~10–15%). The readily releasable pool (RRP) consists of a few vesicles (~1%) that seem to be docked and primed for release. **b** | Three kinetic components of release (indicating release of three vesicle pools) on depolarization of goldfish bipolar cells. The cell was stimulated in the presence of the styryl dye FM 1-43, and the increase in fluorescence gives a direct measure of exocytosis. Panel **b** modified, with permission, from REF. 12 © (1999) Blackwell Scientific Publishing.

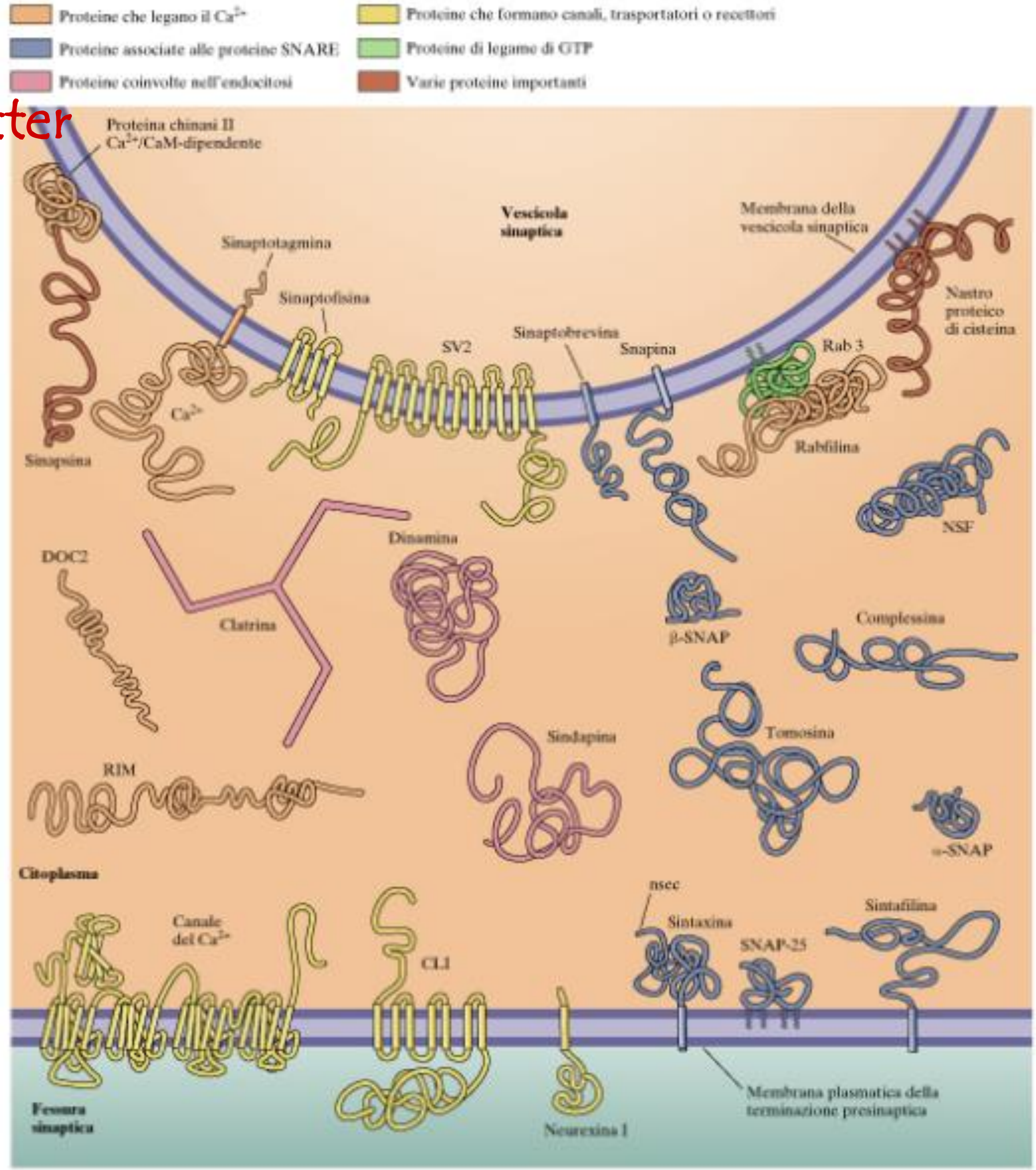
Vesicular Cycling Kinetics

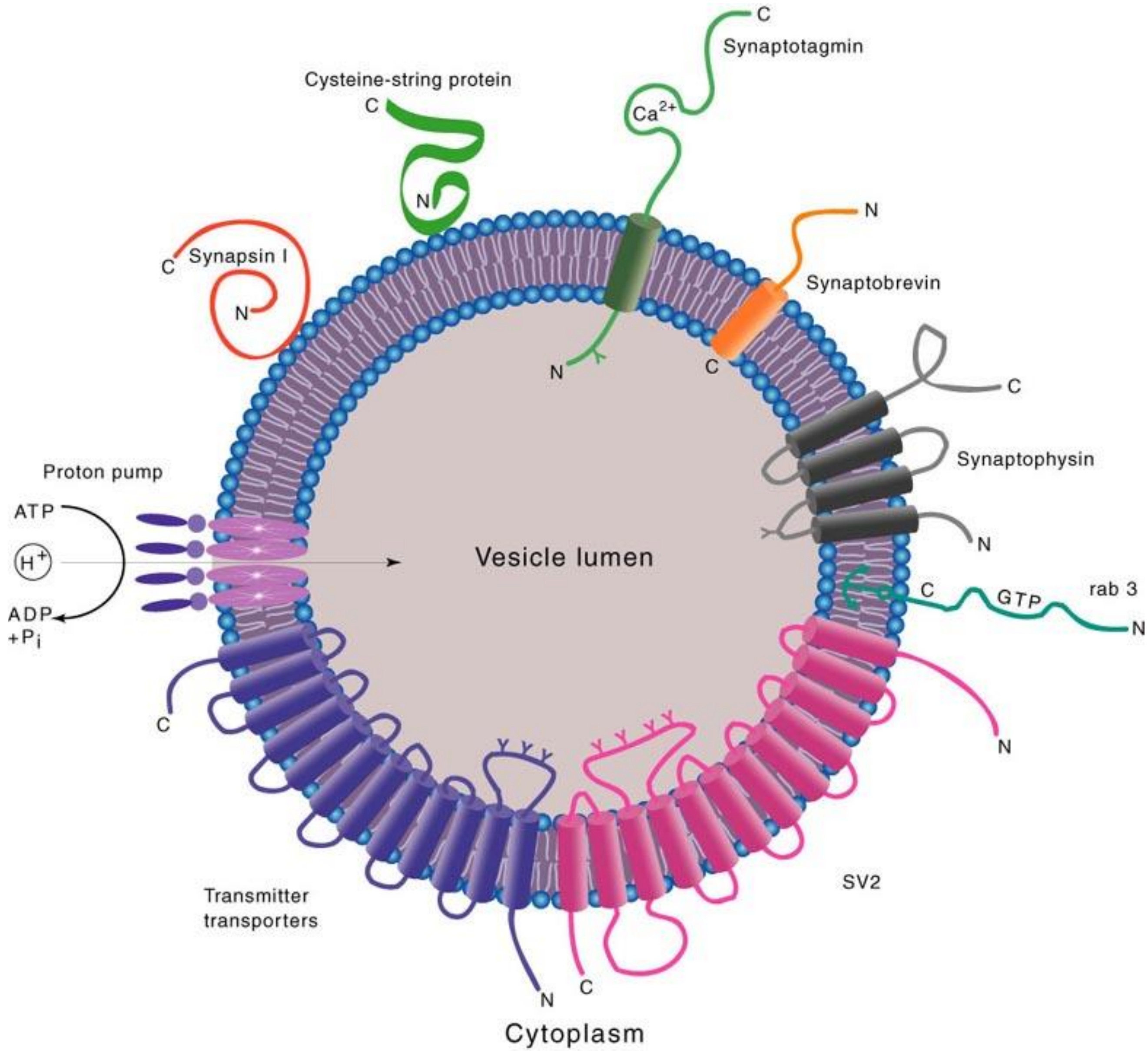
(E)



NEUROSCIENCE, *Third Edition*, Figure 5.9 (Part 2) © 2004 Sinauer Associates, Inc.

Presynaptic proteins involved in neurotransmitter release





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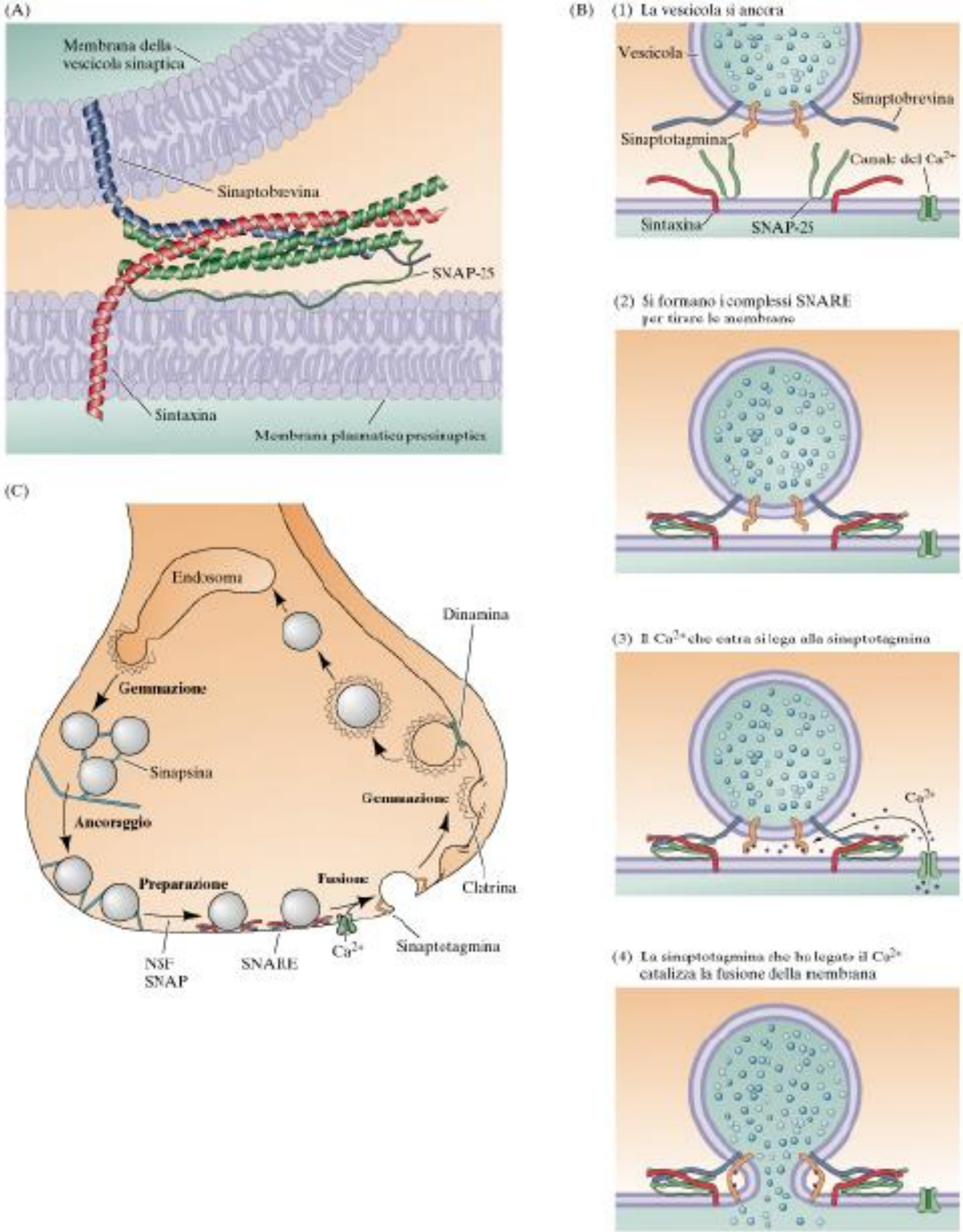
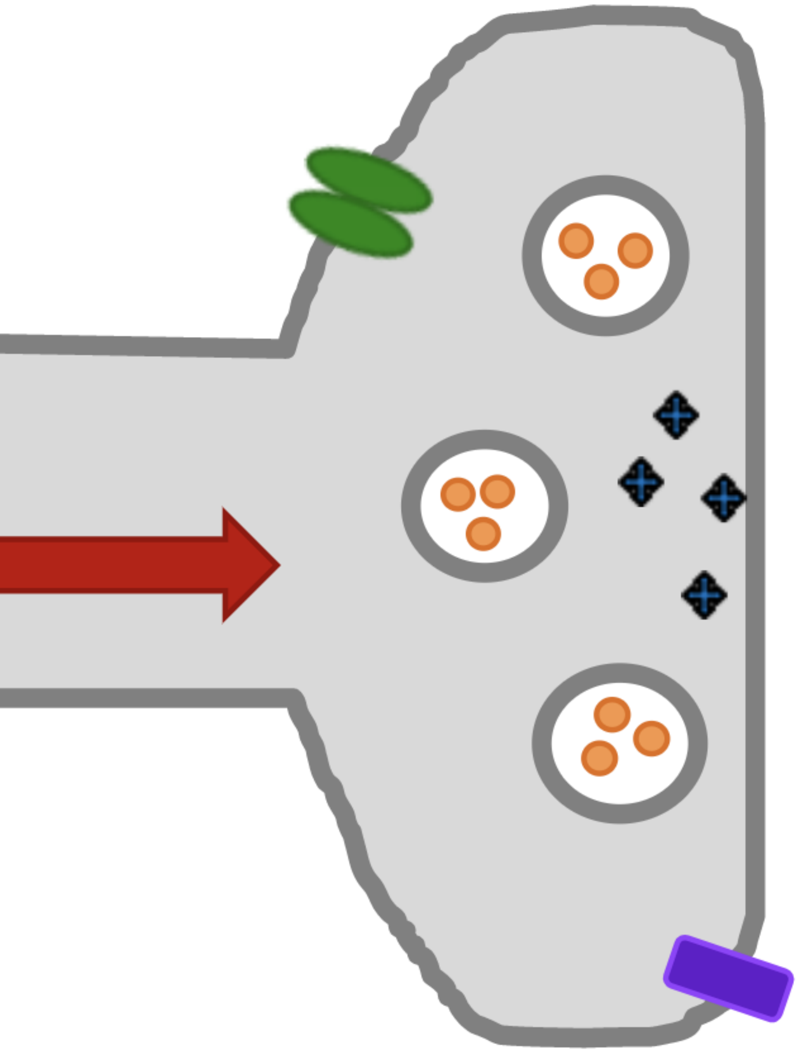
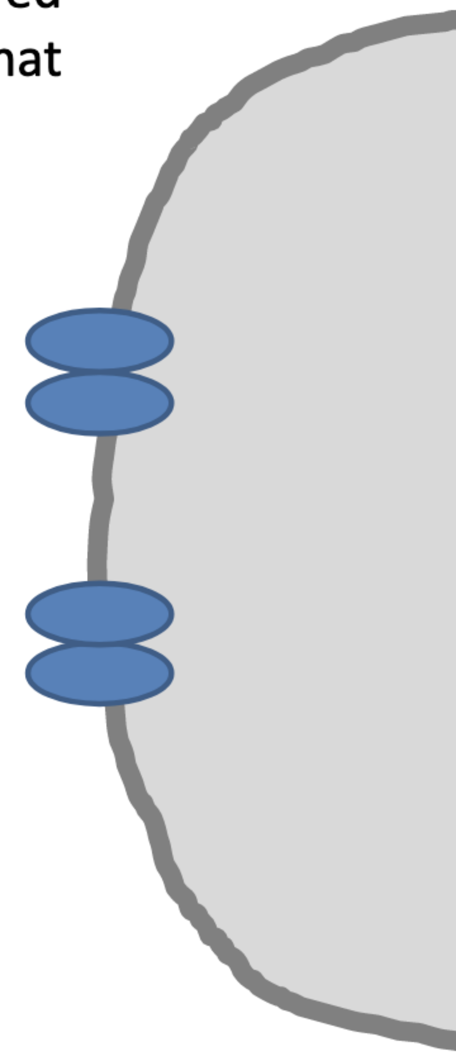
Molecular mechanisms of NT liberation



What would happen if…

You took a drug that destroyed the Ca^{2+} sensitive proteins that fuse synaptic vesicles to the membrane???



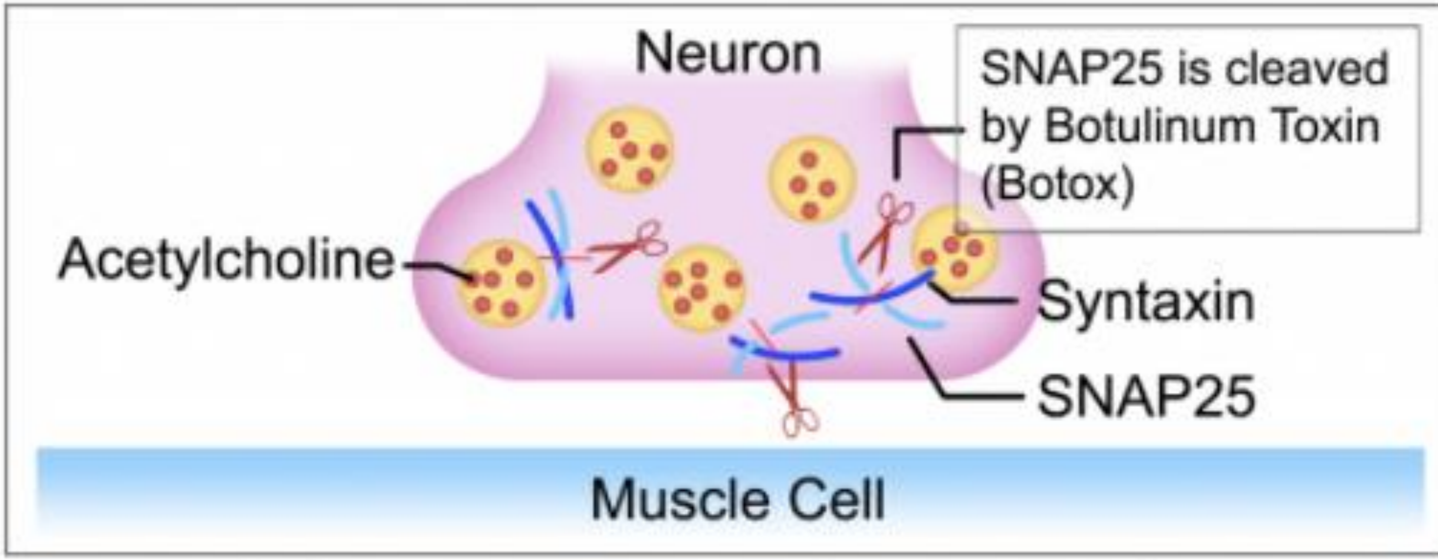


That's How Botox Works!

Botox destroys the proteins that fuse synaptic vesicles with the membrane.

By stopping vesicle release, Botox prevents muscle contraction which prevents wrinkles!

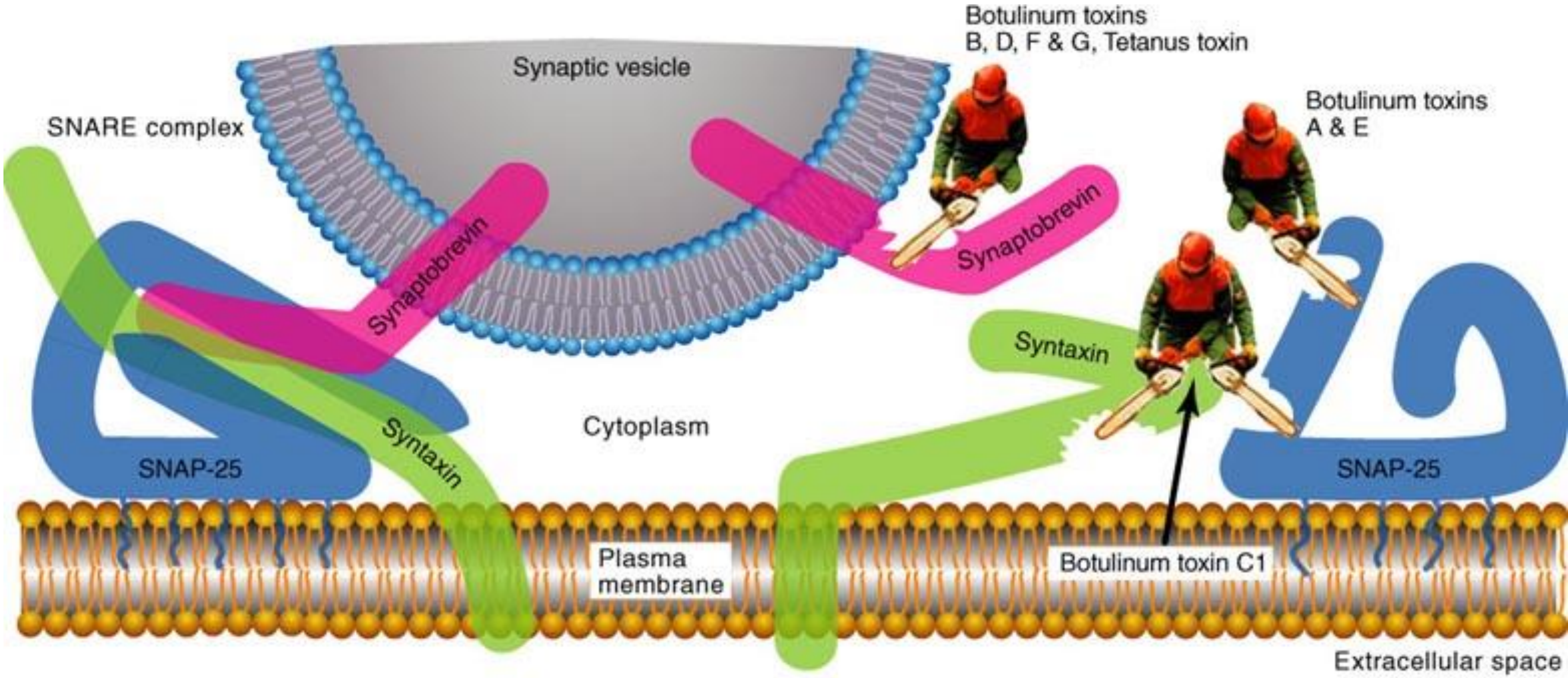
BOTOX IN THE NEUROMUSCULAR JUNCTION



Acetylcholine stays in Vesicles

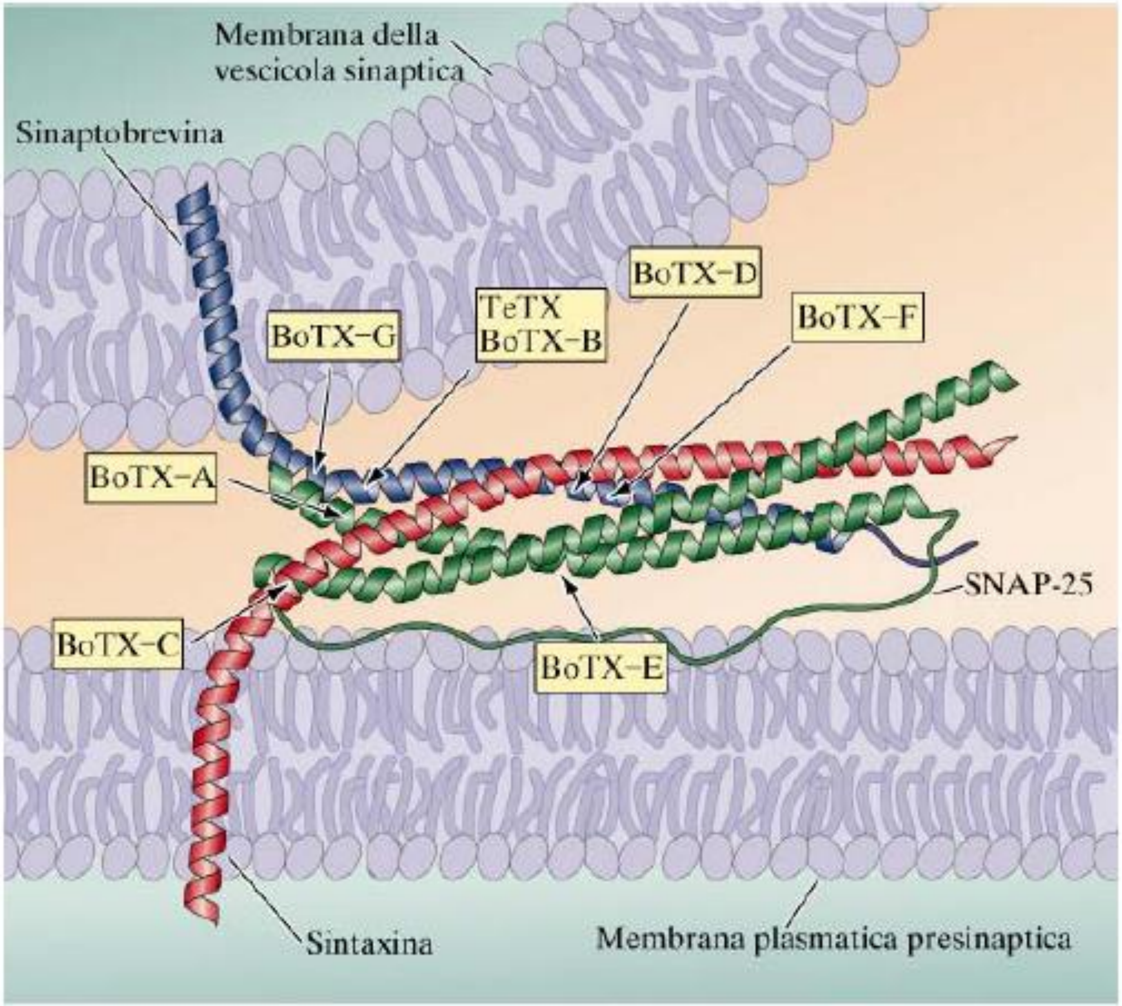
⬇ ⬇

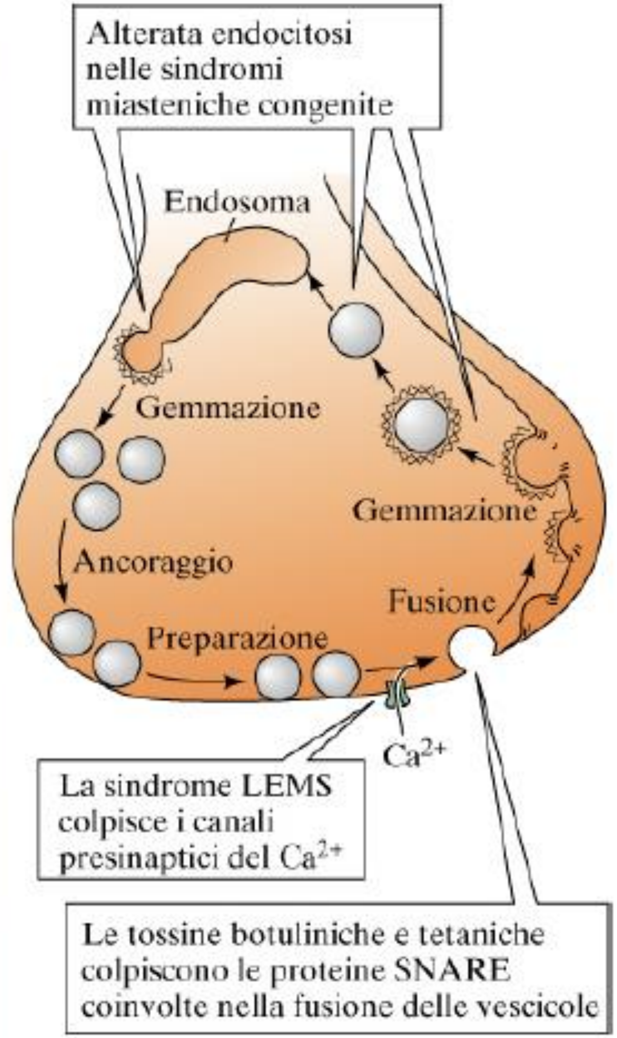
Muscle becomes paralyzed



Botulinum toxins
B, D, F & G, Tetanus toxin
Synaptic vesicle
Botulinum toxins
A & E
SNARE complex
Synaptobrevin
Synaptobrevin
Syntaxin
Cytoplasm
Syntaxin
SNAP-25
SNAP-25
Plasma
membrane
Botulinum toxin C1
Extracellular space

Toxins that interfere with the liberation of NT

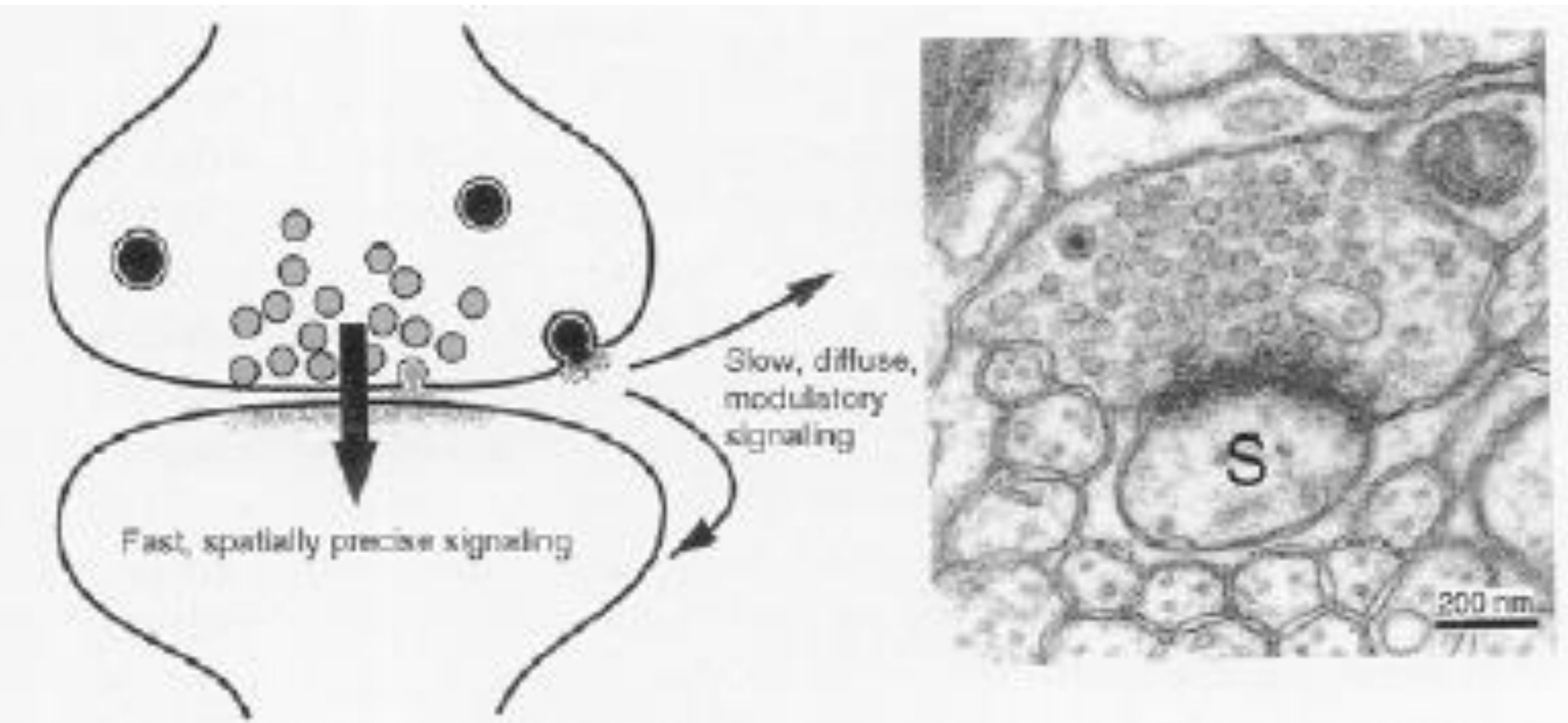




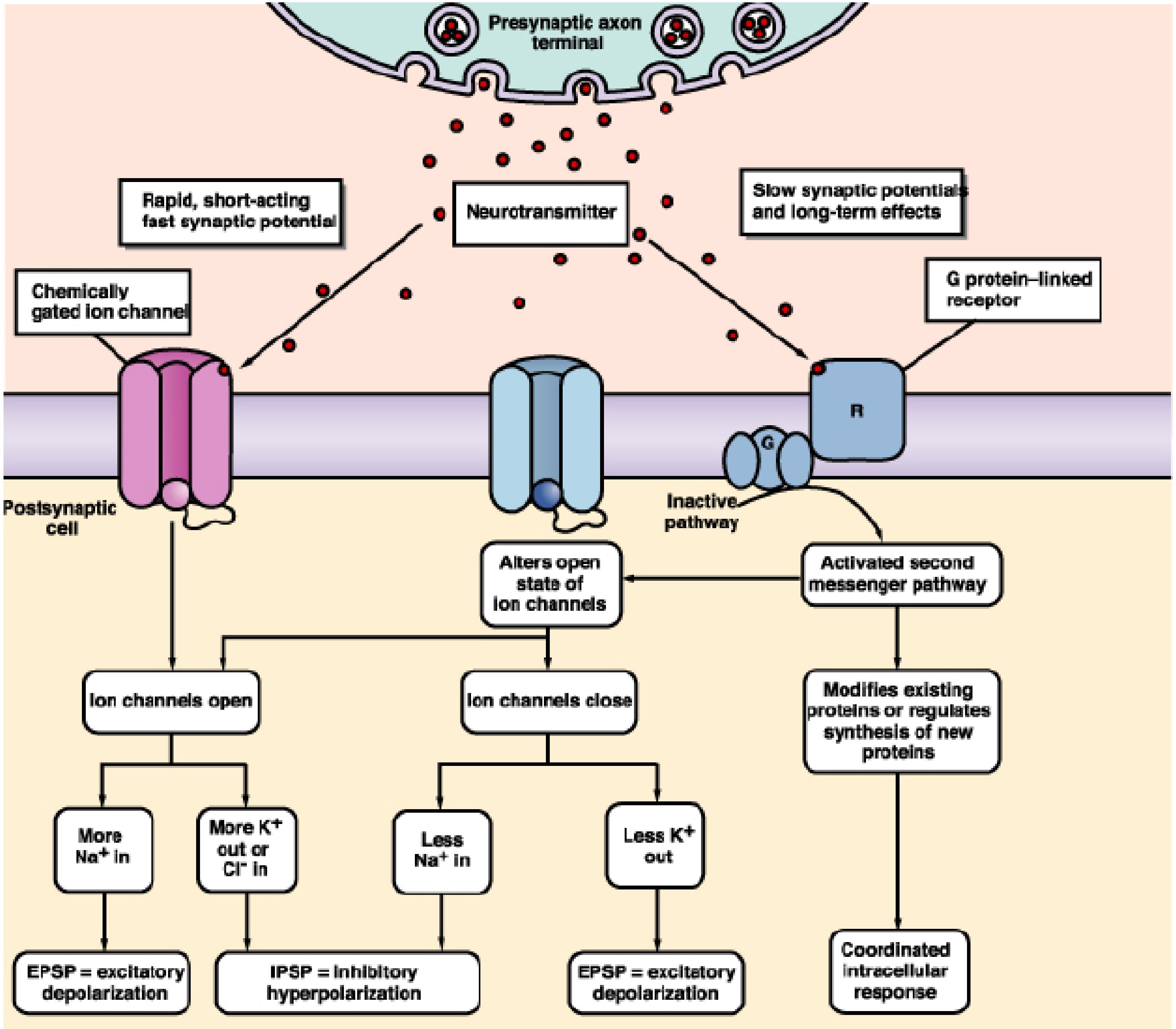
Classical and peptide neurotransmitters have different functions

CLASSIC NEUROTRASMECTORS:
- rapid and precise synaptic action
- self-sufficient terminal
- specific to SN

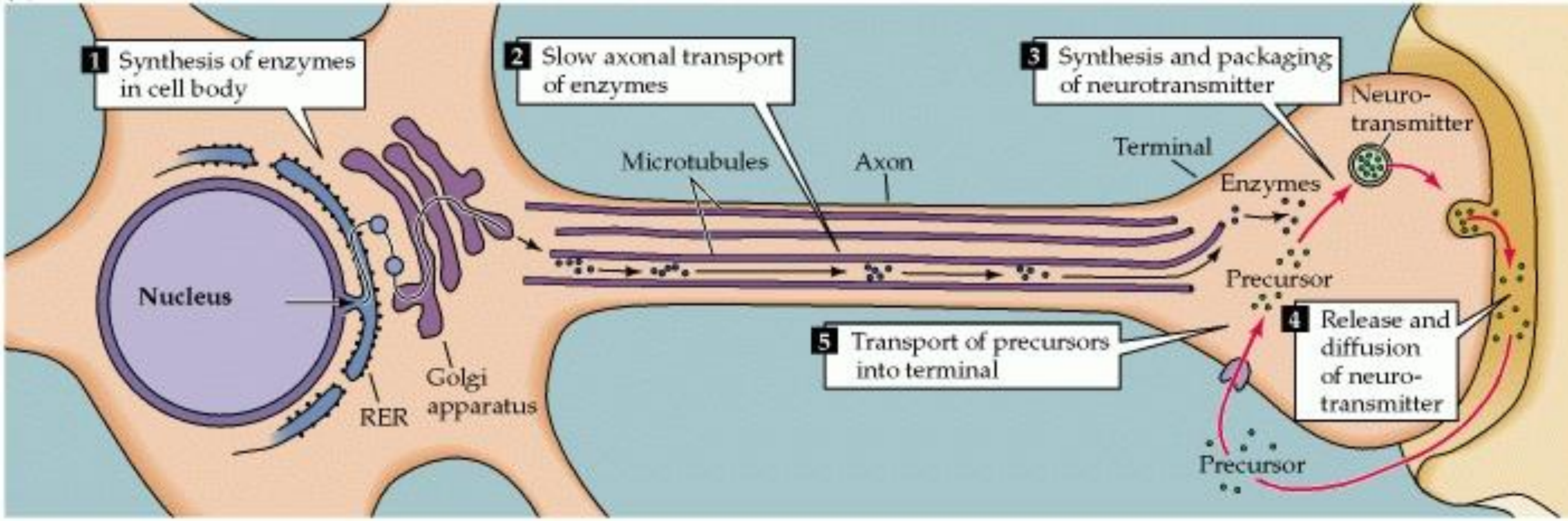
PEPTIDIC NEUROTRASMETTERS:
- slower and more modulatory paracrine action
- release requires intense electrical activity
- non self-sufficient terminal
- common to the endocrine system



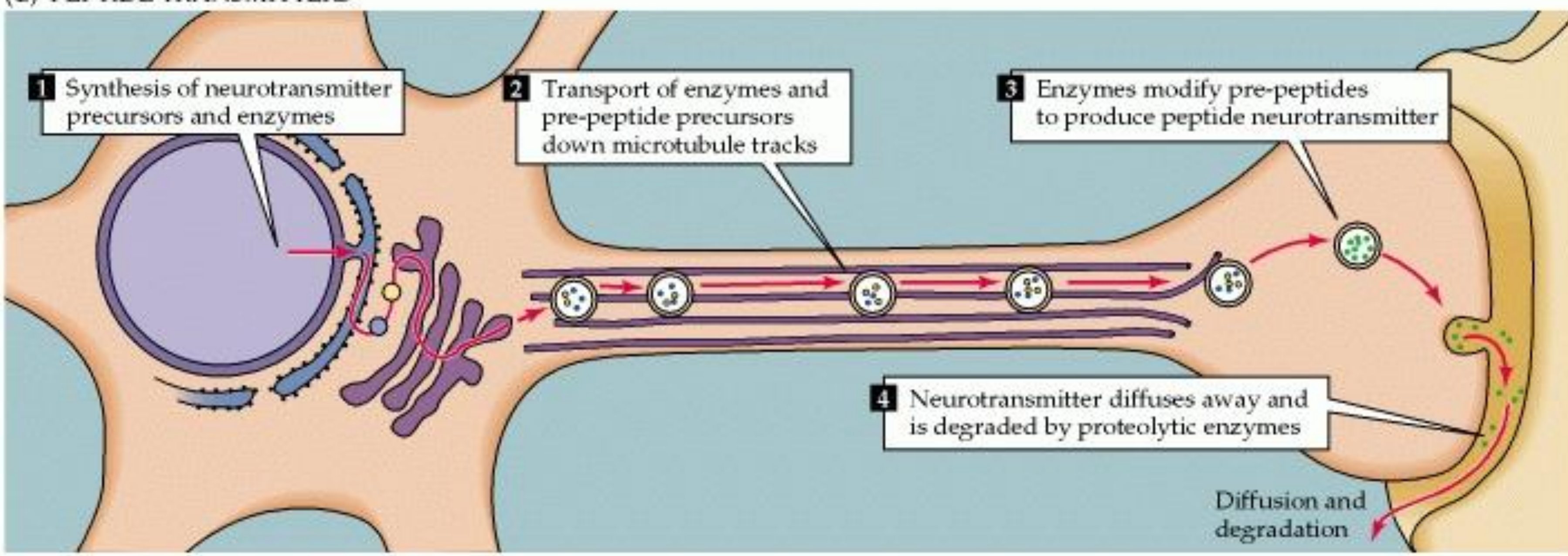
Neurotransmitter action

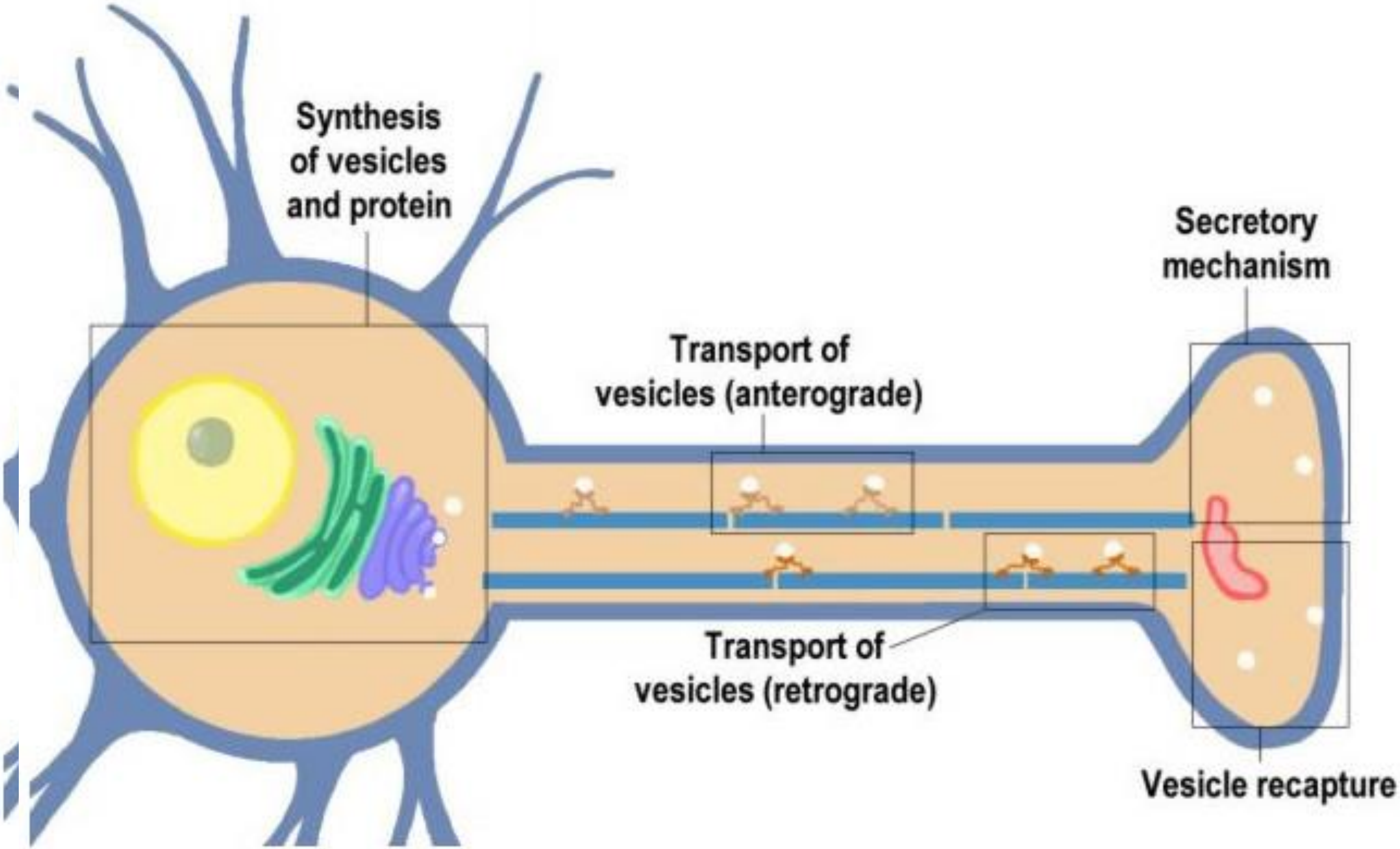


(B) SMALL-MOLECULE TRANSMITTERS

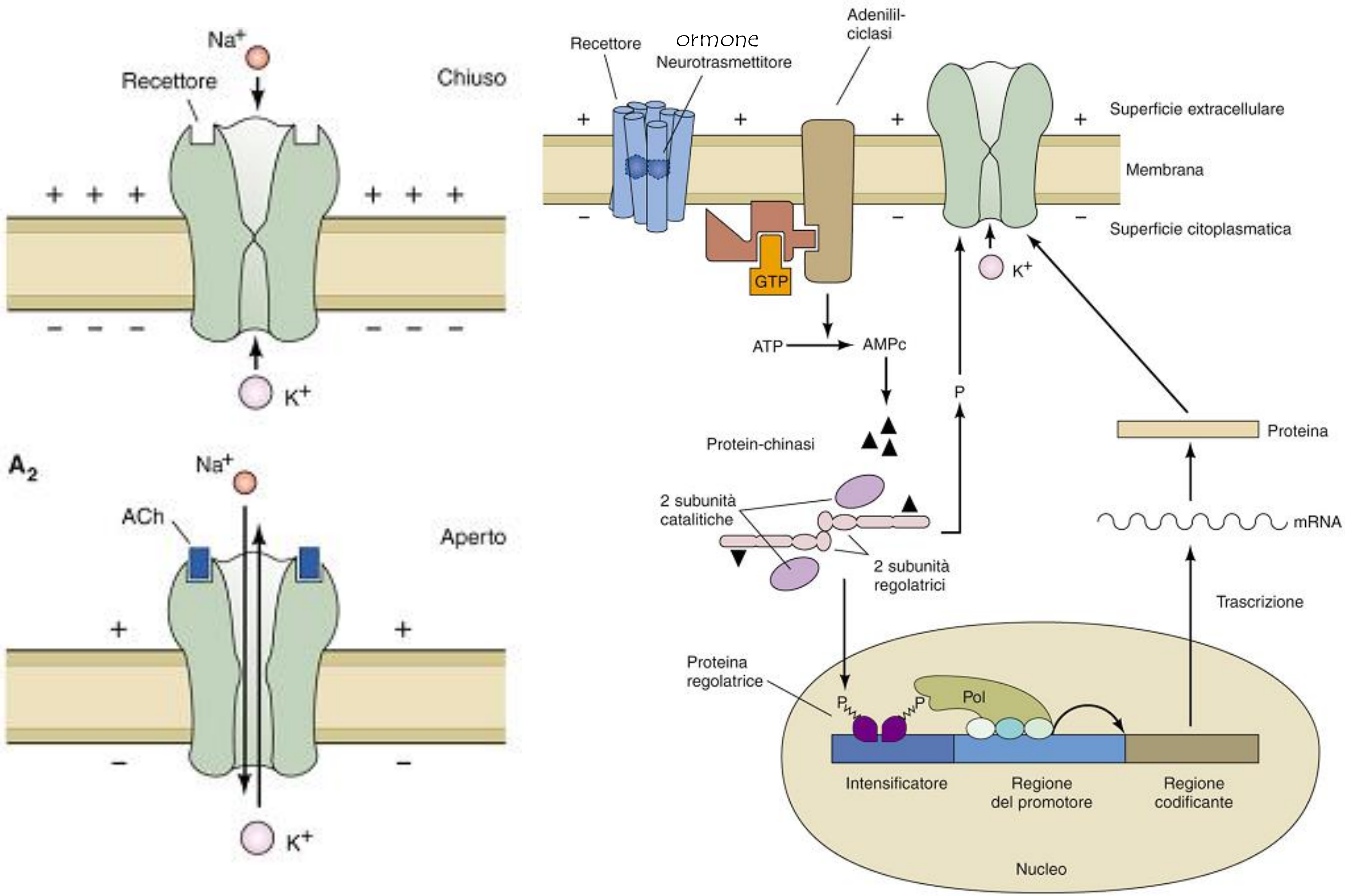


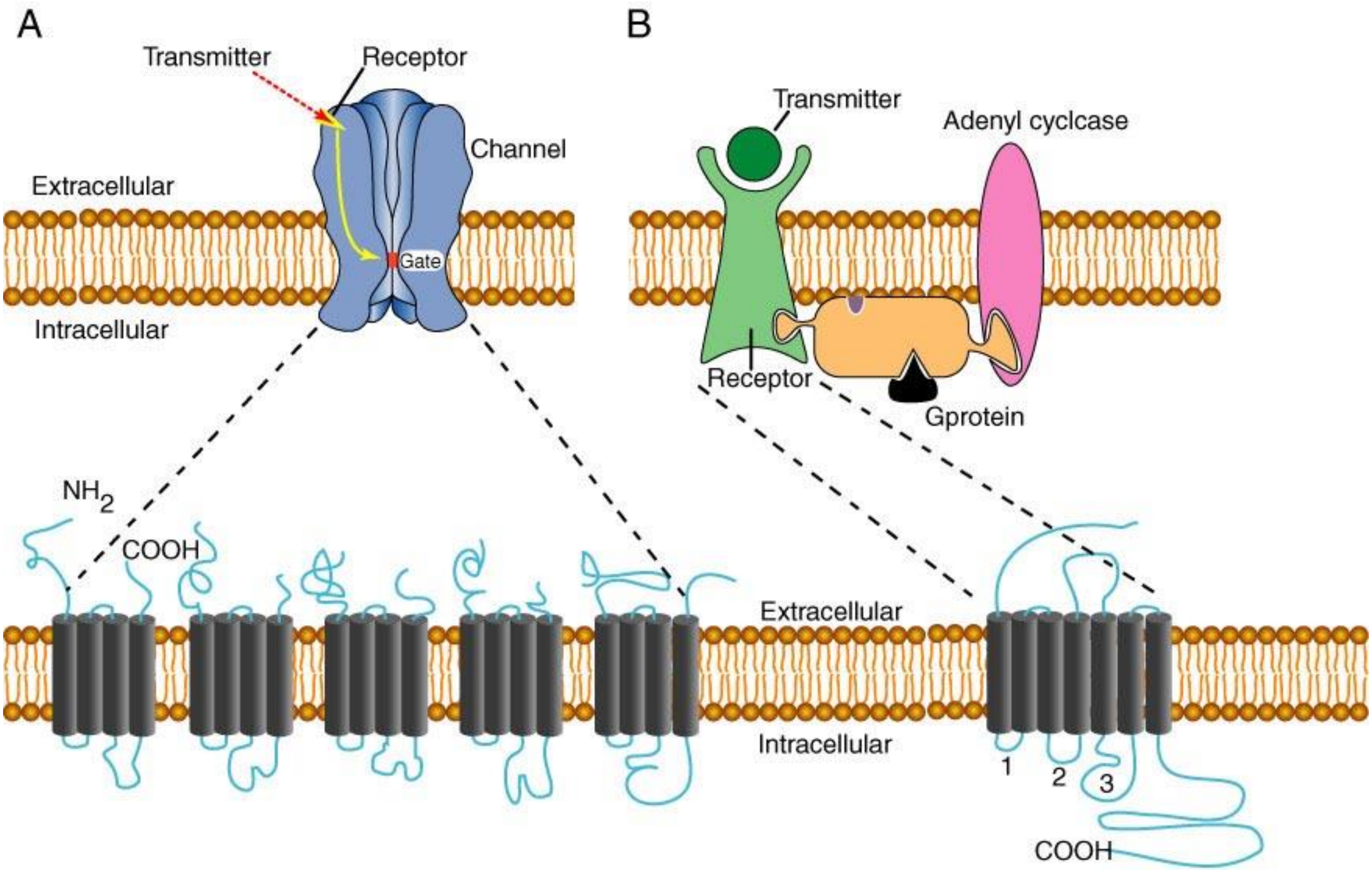
(C) PEPTIDE TRANSMITTERS




Synthesis
of vesicles
and protein
Secretory
mechanism
Transport of
vesicles (anterograde)
Transport of
vesicles (retrograde)
Vesicle recapture

Neurotrasmitter receptors





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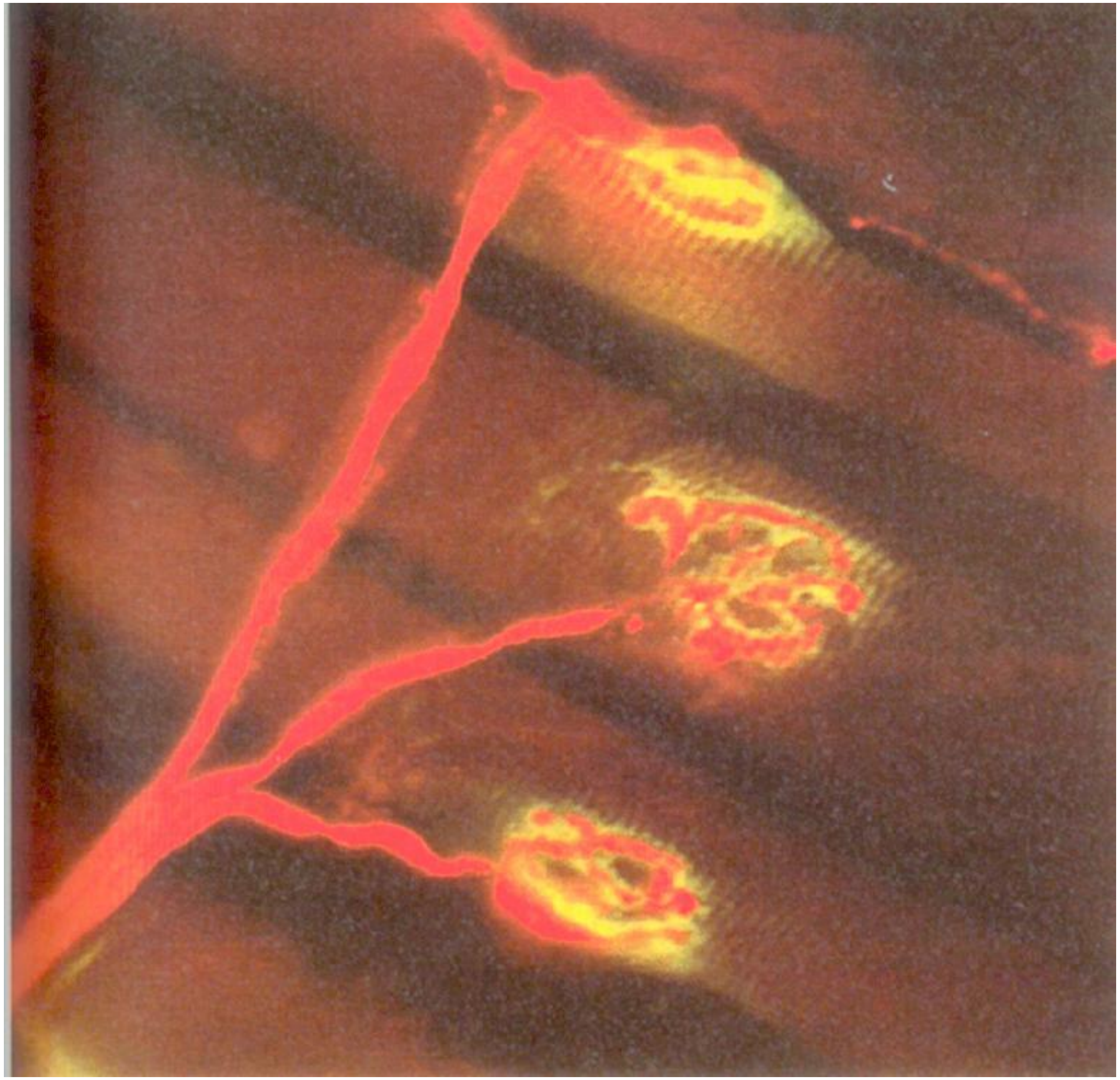
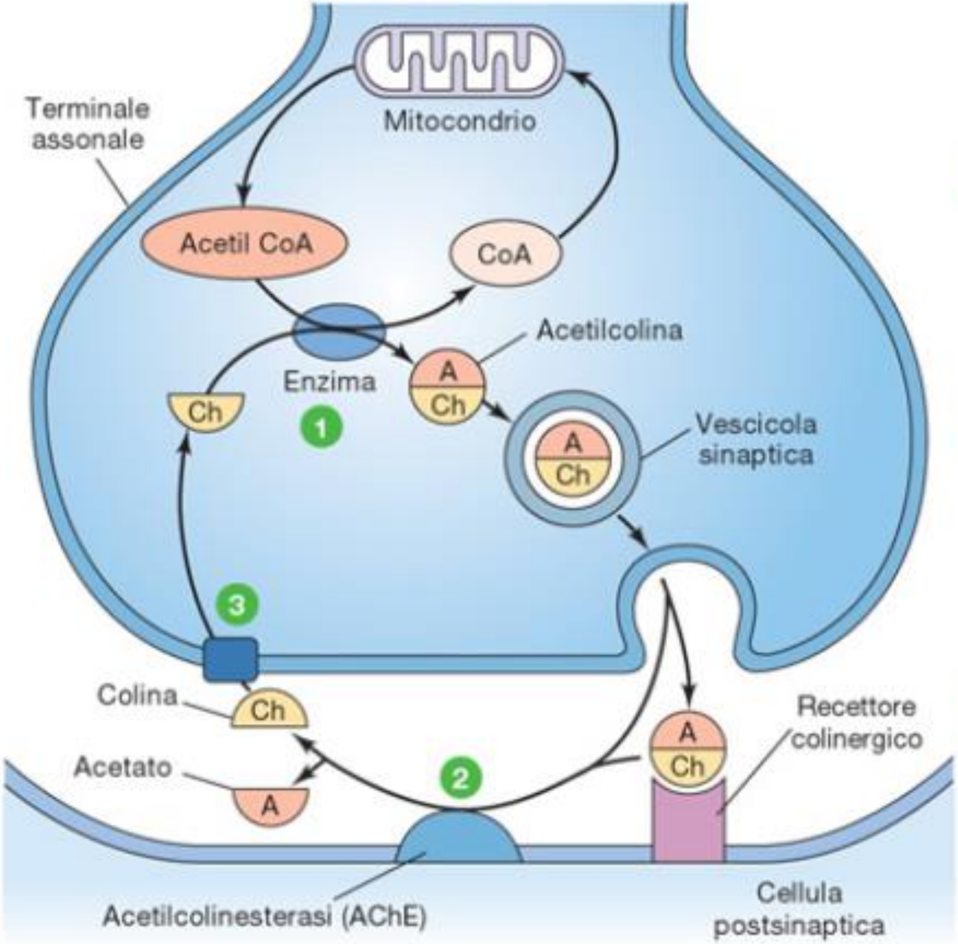
NMJ



Synthesis and recycling of Acetylcholine



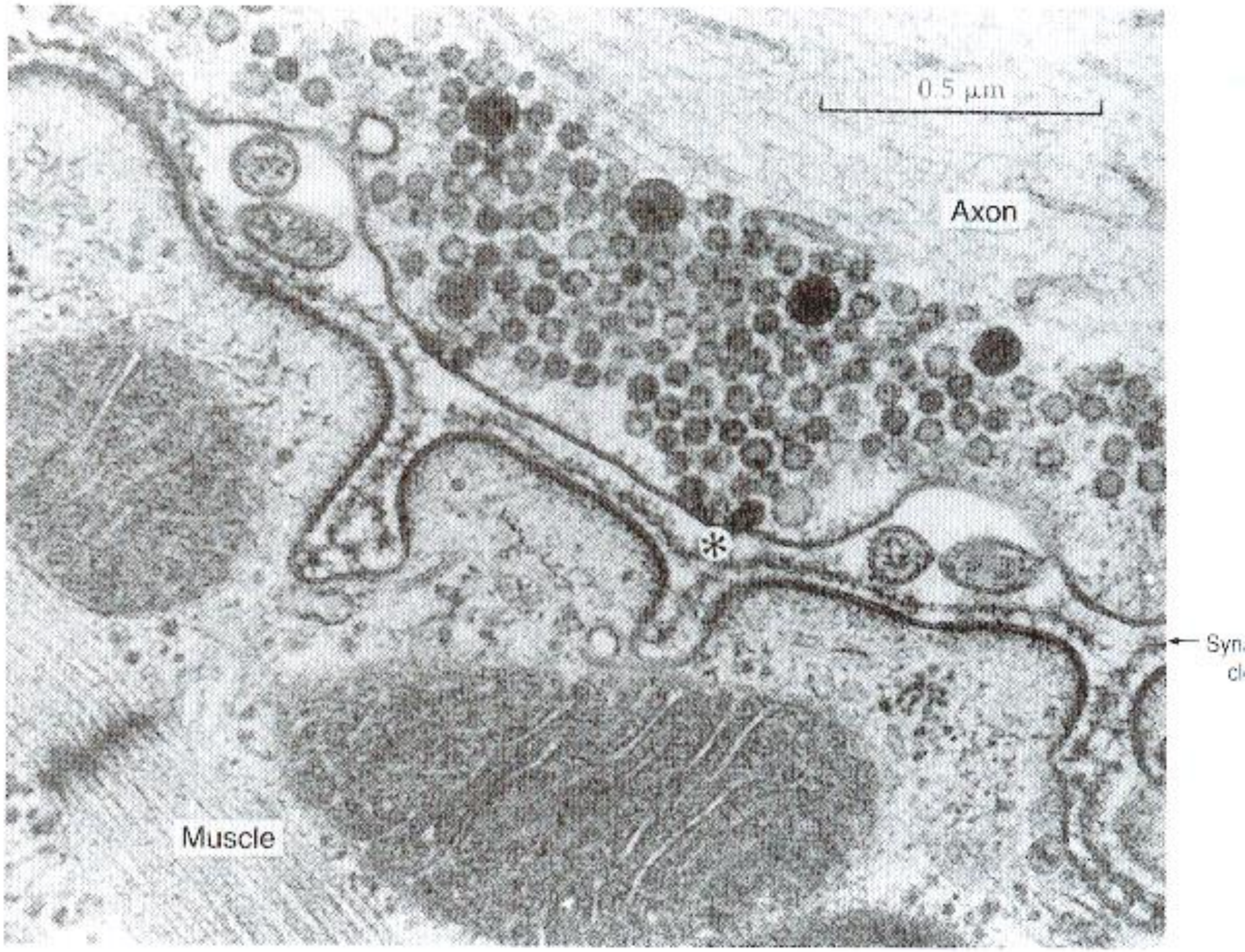
1 L'**acetilcolina** (ACh) è formata a partire da colina e acetil CoA.

2 Nella fessura sinaptica l'ACh viene rapidamente degradata dall'enzima **acetilcolinesterasi**.

3 La colina viene ritrasportata nel terminale assonico e viene utilizzata per sintetizzare altra ACh.

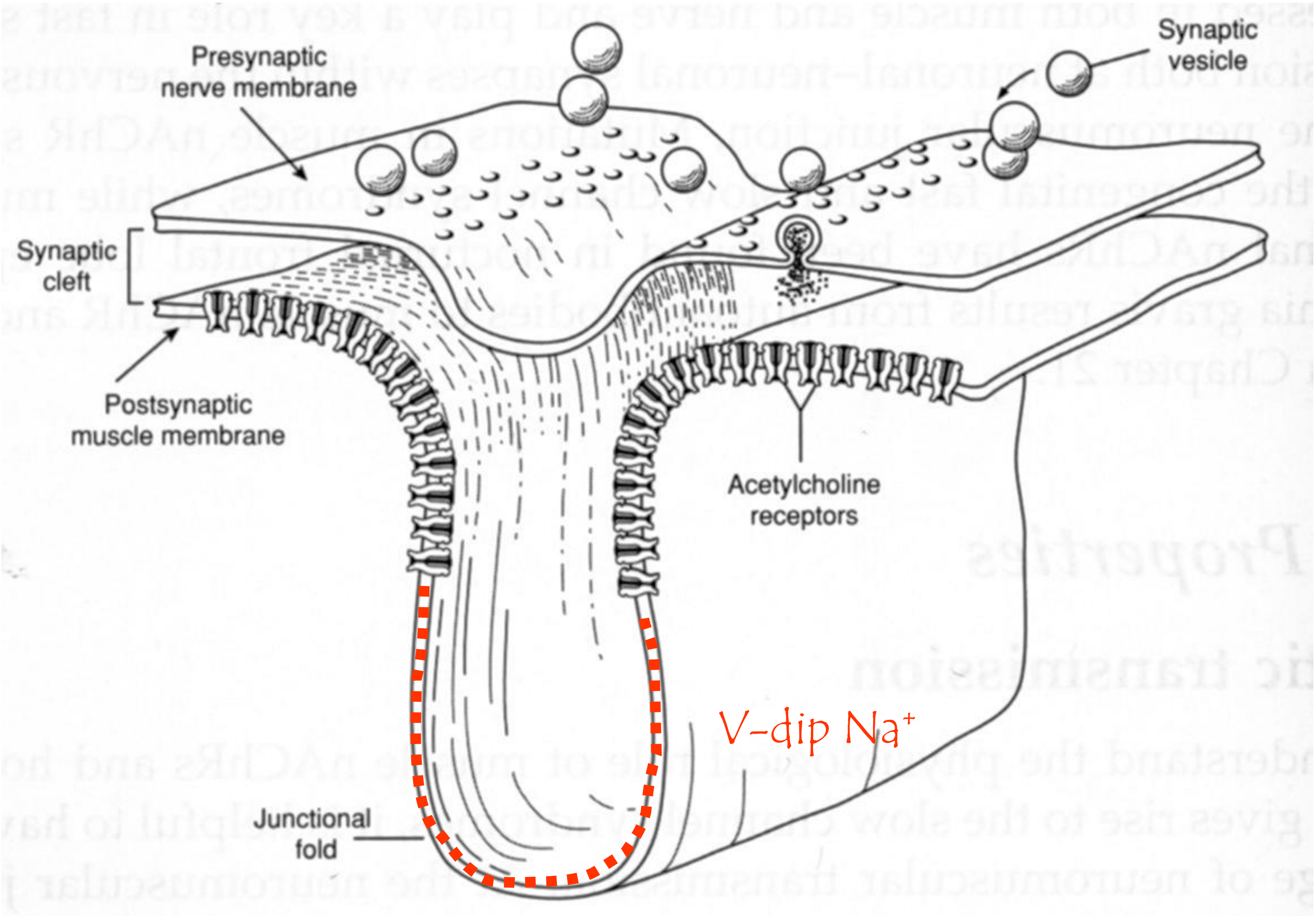
NMJ

A



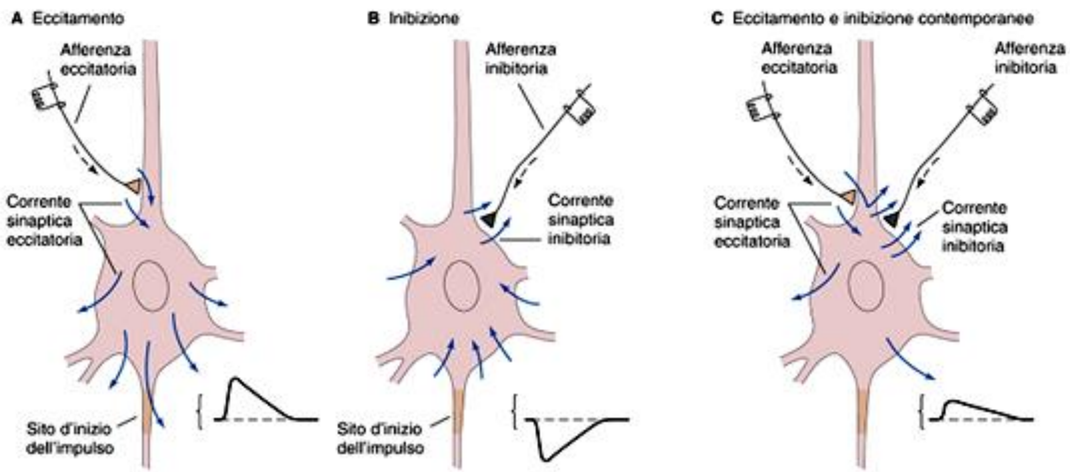
B

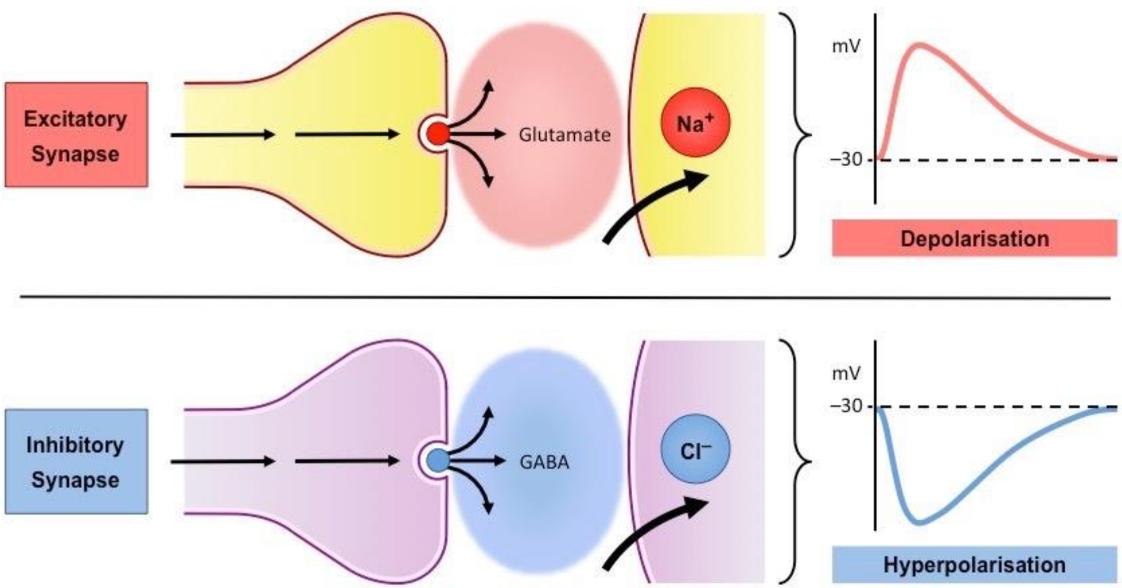
Scheme



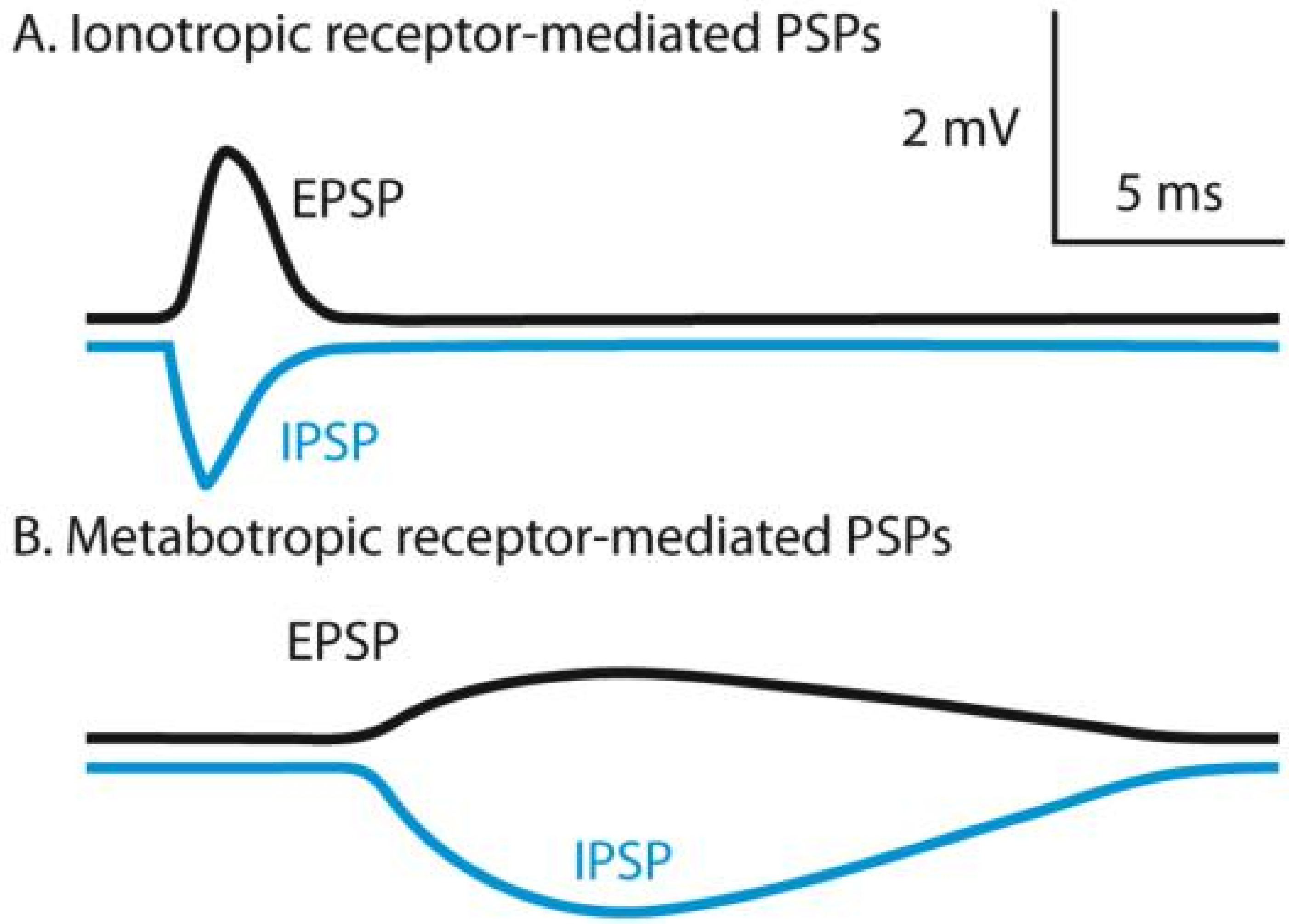
Presynaptic nerve membrane
Synaptic vesicle
Synaptic cleft
Postsynaptic muscle membrane
Acetylcholine receptors
Junctional fold
V-dip Na+

Excitatory and inhibitory synapses

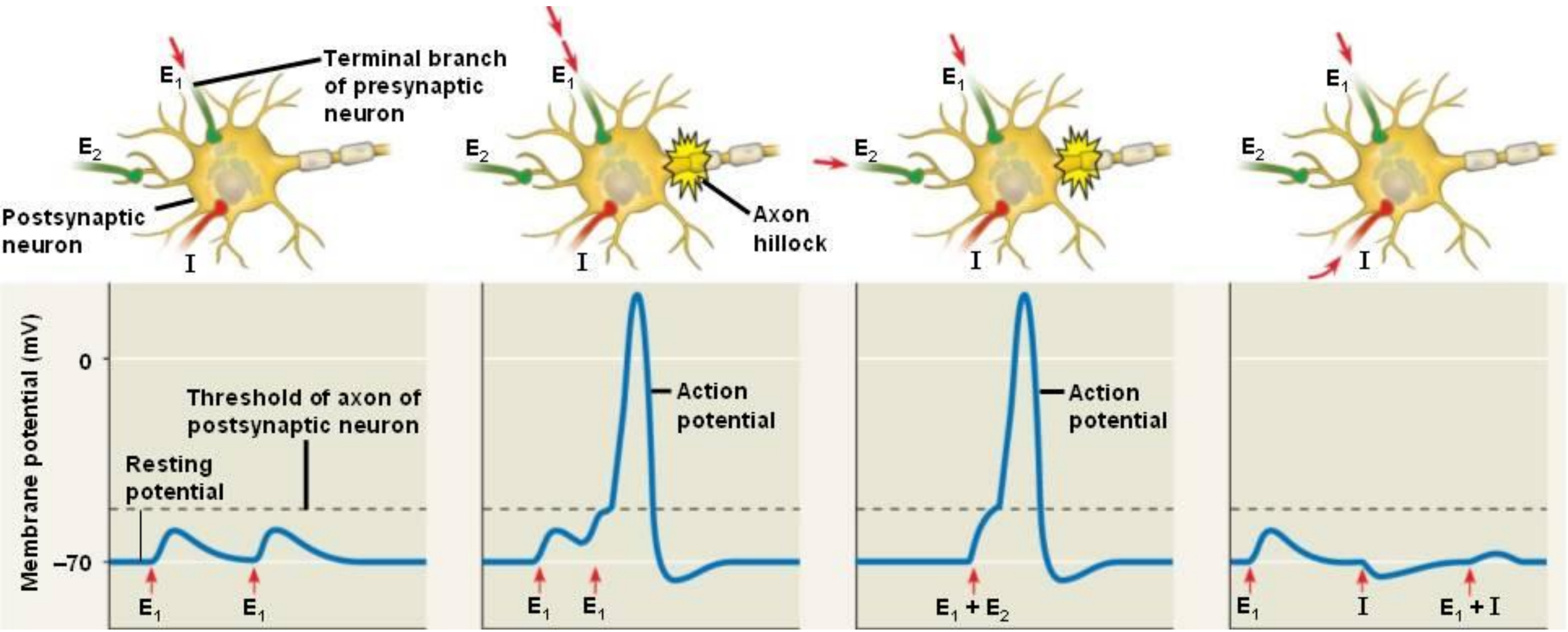




Ionotropic vs. metabotropic PSPs



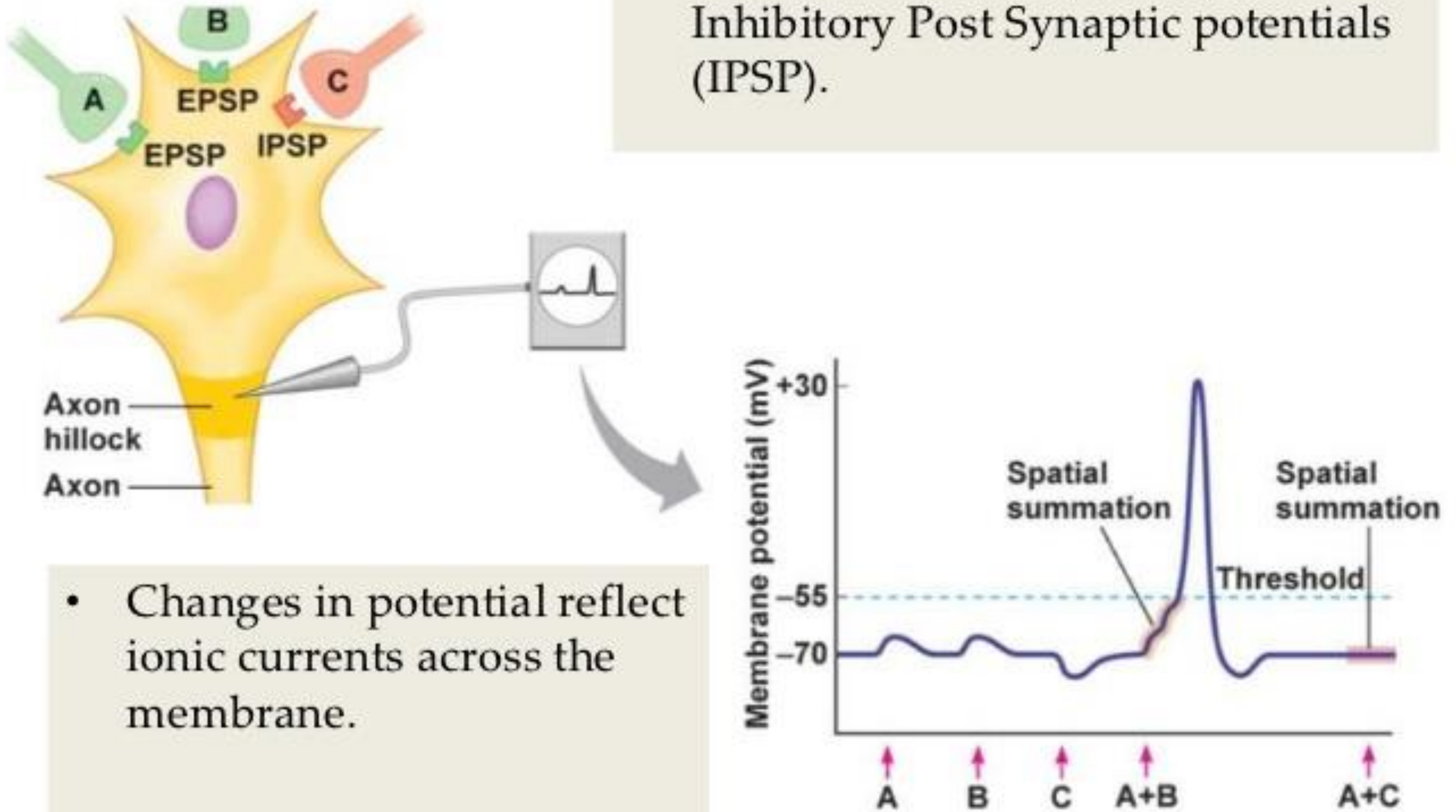
EPSP



Terminal branch of presynaptic neuron
E_1
E_2
Postsynaptic neuron
I
Axon hillock
Membrane potential (mV)
0
–70
Threshold of axon of postsynaptic neuron
Resting potential
Action potential
Action potential
E_1
E_1
E_1
E_1
$E_1 + E_2$
E_1
I
$E_1 + I$

Excitatory and Inhibitory Synapses

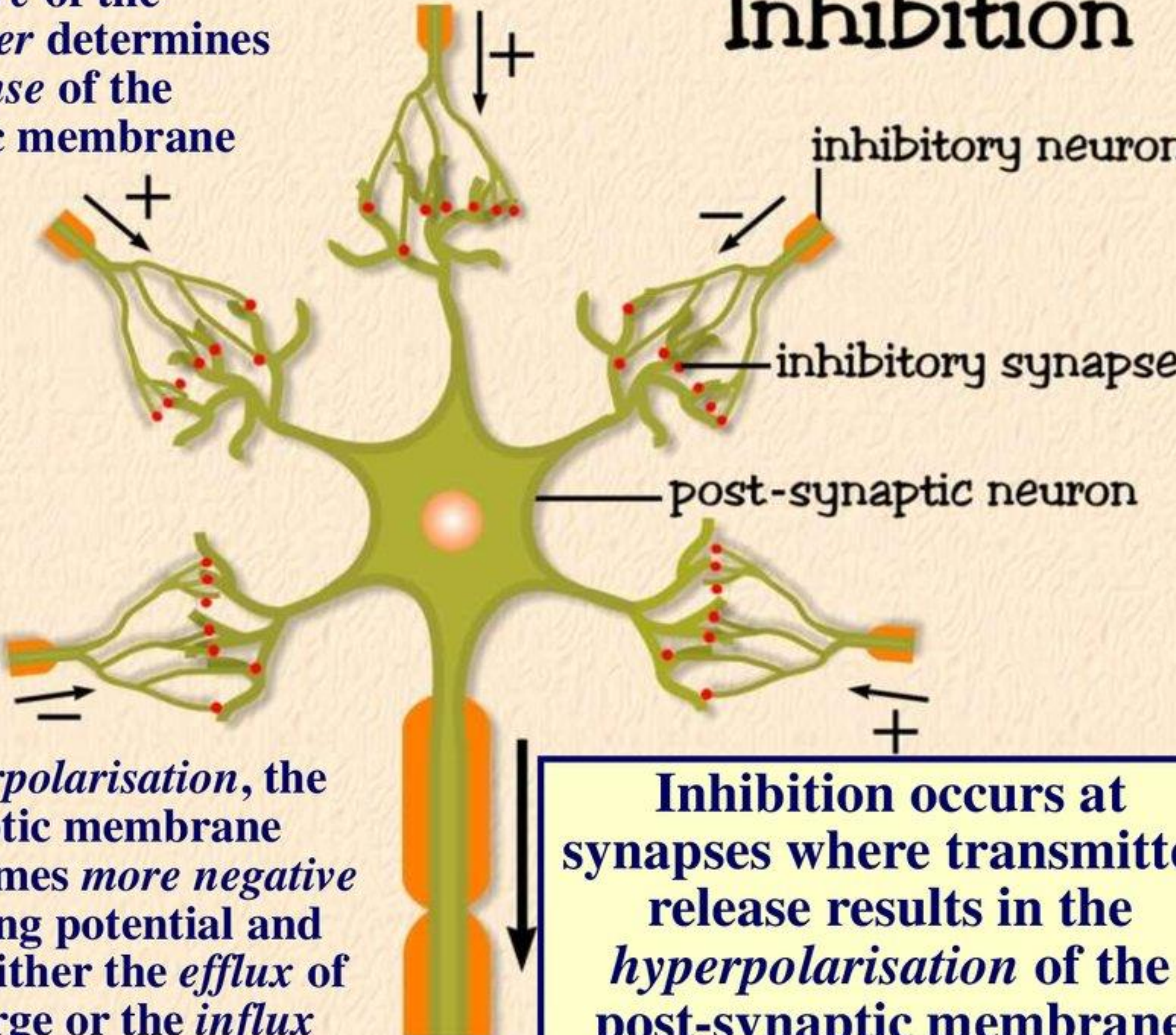
- Presynaptic action potentials result in either Excitatory (EPSP) or Inhibitory Post Synaptic potentials (IPSP).



- Changes in potential reflect ionic currents across the membrane.

Inhibition

The *nature* of the *neurotransmitter* determines the *response* of the post-synaptic membrane

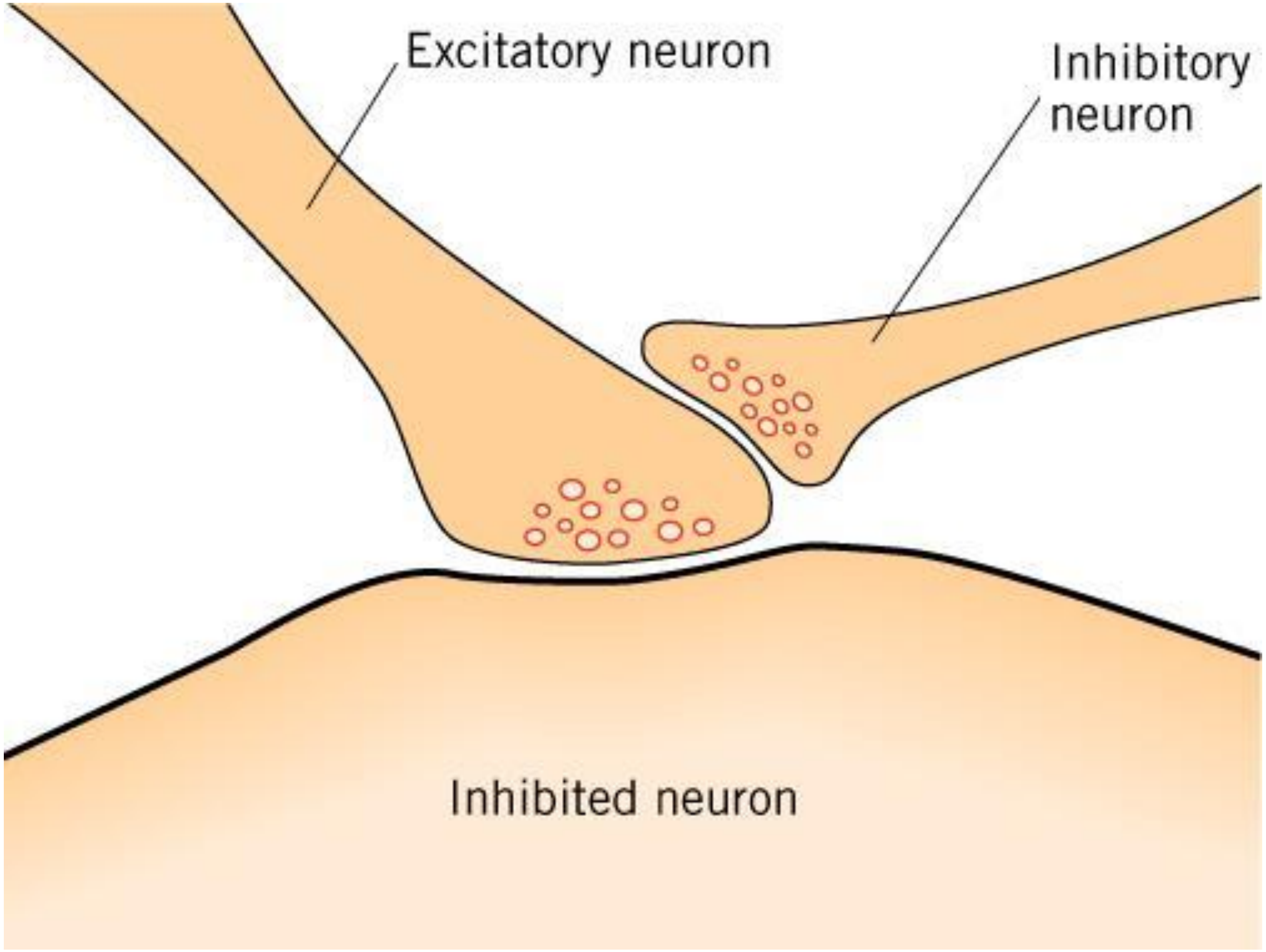


During *hyperpolarisation*, the post-synaptic membrane potential becomes *more negative* than its resting potential and results from either the *efflux* of positive charge or the *influx* of negative charge

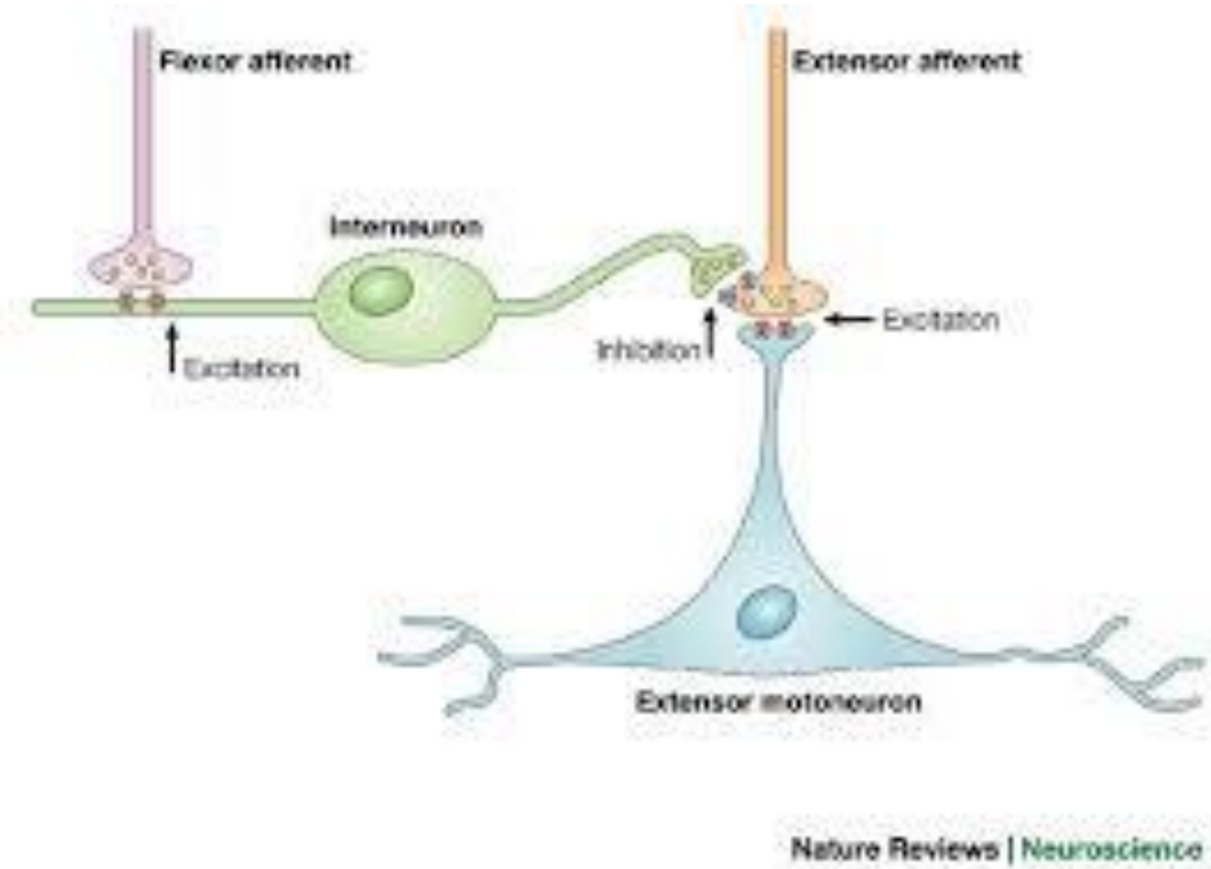
Inhibition occurs at synapses where transmitter release results in the *hyperpolarisation* of the post-synaptic membrane

NEXT

Excitatory and inhibitory synapses coexist and modulate with each other

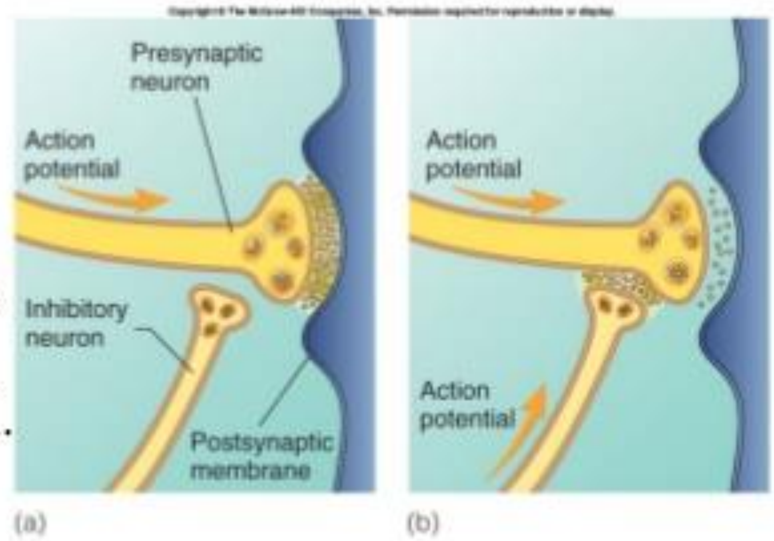


Excitatory and inhibitory synapses coexist and modulate with each other



Presynaptic Inhibition and Facilitation

- **Axoaxonic synapses**: axon of one neuron synapses with the presynaptic terminal (axon) of another. Many of the synapses of CNS
- **Presynaptic inhibition**: reduction in amount of neurotransmitter released from presynaptic terminal. Endorphins can inhibit pain sensation
- **Presynaptic facilitation**: amount of neurotransmitter released from presynaptic terminal increases. Glutamate facilitating nitric oxide production



Presynaptic inhibition

- **<u>Mechanism:</u>**

- **GABA** opens the **Cl^- channels** in the excitatory presynatpic terminal → increase **Cl^- influx** → leading to decrease in the amplitude of the action potentials arriving at the knob → **decrease Ca^{2+} influx** → **decrease the amount** of neurotransmitter released → decrease in post-synaptic response **(↓ EPSP)**