Synaptic transmission: nerve-muscle synapse

Based on Ch 9 Principle of Neural Science Kandel

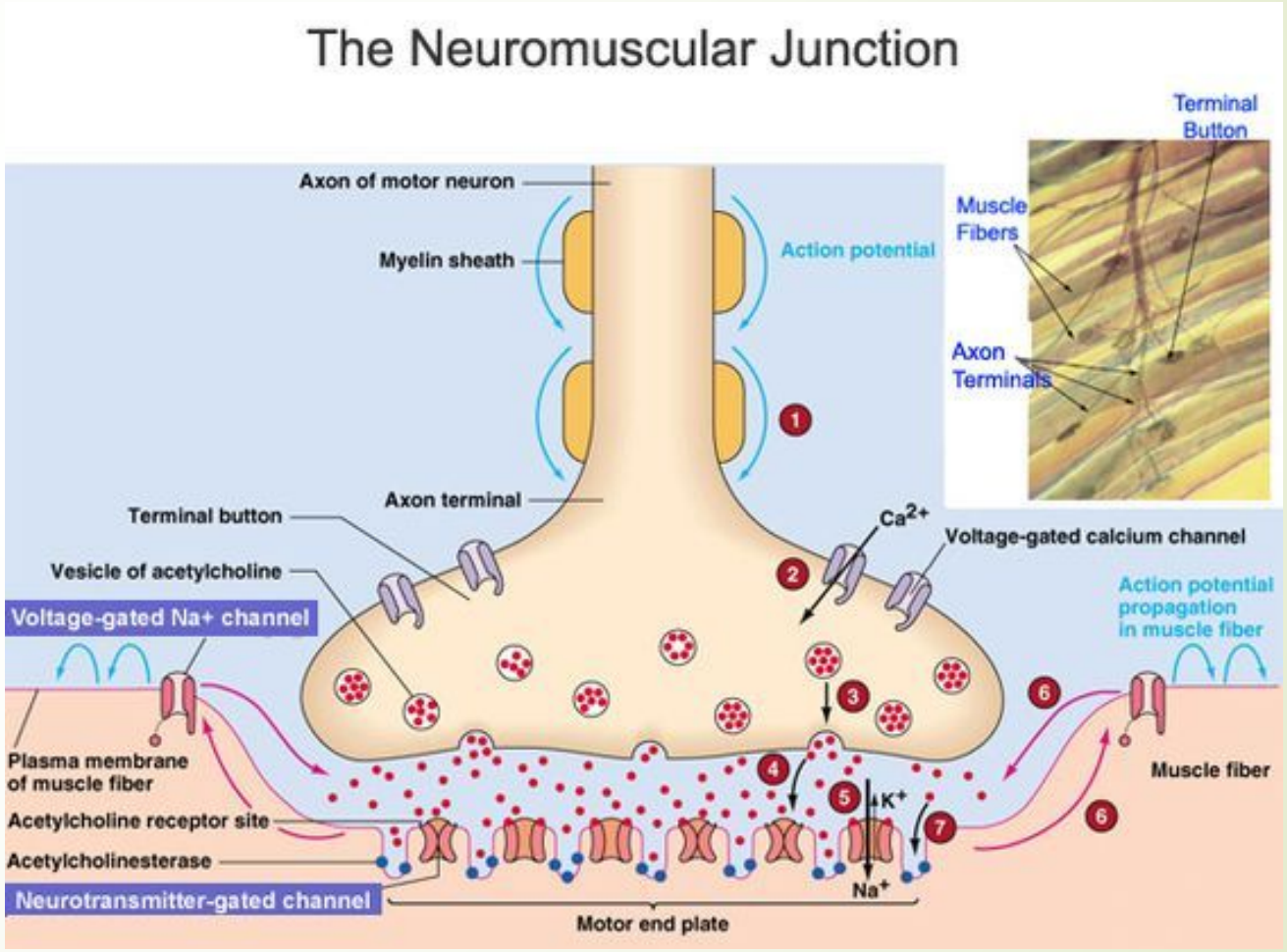
- Communication between neurons in the brain relies mainly on chemical synapses (see also chapter 8 Kandel).
- Much of the present understanding on the function of these synapses comes from studies of synaptic transmission at the nerve-muscle synapses = junction between a motor neuron and a skeletal muscle fiber
- Nerve-muscle synapse is an ideal site for studying chemical signaling because it is relatively simple and accessible to experimentation.
- Muscle cell is large enough to accommodate the two or more electrodes needed to make electrical measurements.
- Muscle cell normally receive signals from just one presynaptic axon = easy model

Chemical signaling at the nerve-muscle synapse = relatively simple mechanism

- Release of neurotransmitter from the presynaptic nerve directly opens a single type of ion channel in the post synaptic membrane.

Neuromuscular junction: example of directly gated synaptic transmission

Motor neuron innervates the muscle at a specialized region of the muscle membrane called the **end plate**, where the motor axon loses its myelin sheath and splits into several branches. The ends of the fine branches form multiple expansions, **synaptic buttons** from which the motor neuron releases its transmitter.

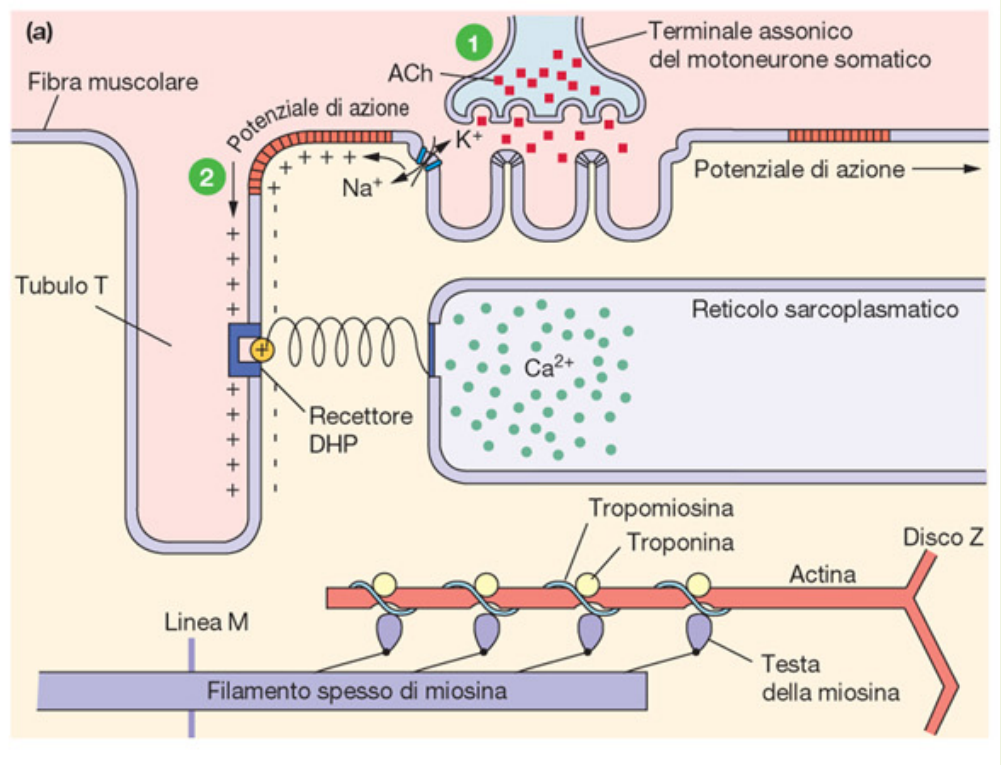


Neuromuscular junction: example of directly gated synaptic transmission

The trasnmitter released by the motor axon terminal is **Acetylcholine (ACh)** and the receptor on the muscle membrane is the nicotinic type of Ach receptor (**nACh**).

Presynaptic and postsynaptic membranes are separated by a synaptic cleft of about 100nm wide. Within the cleft is a basal lamina composed of collagen and other extracellular matrix proteins.

Acethilcholinesterase is anchored to the collagen fibrils on the basal laminae.



In the muscle cell, the region below the crest of the junctional fold and extending into the fold, the membrane **is reach in voltage-gated Na+ channels**

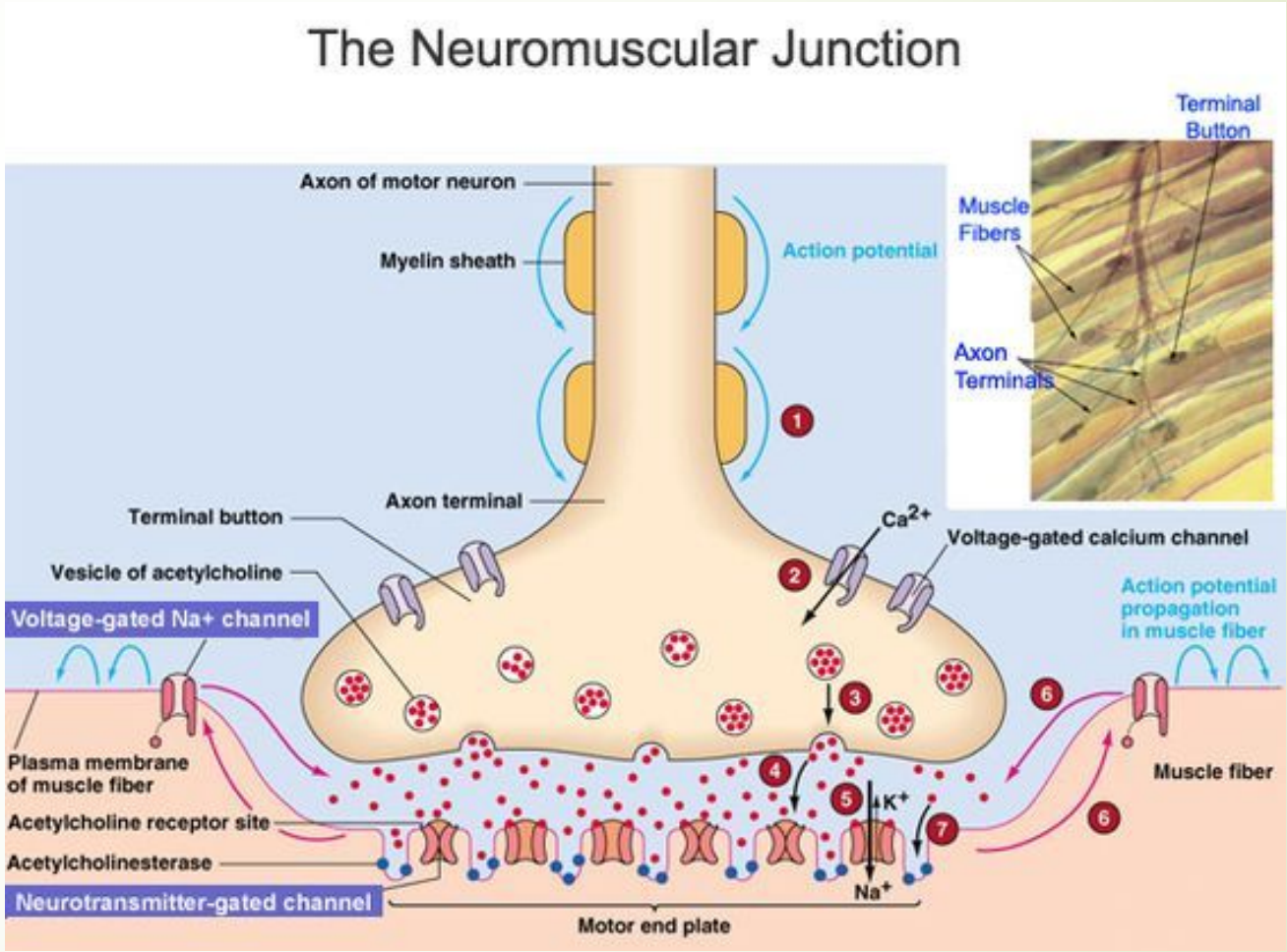
Neuromuscular junction: example of directly gated synaptic transmission

Each **synaptic button** contains all the machinery required to release neurotransmitter:

Synaptic vesicles containing ACh;

Active zones, specialized for neurotransmitter release, where synaptic vesicles are clustered;

Voltage-gated Ca2+ channels that allow Ca2+ influx that in turn triggers the fusion of the synaptic vesicles with the plasma membrane at the active zone, releasing the content of the vesicles into the synaptic cleft by exocytosis



- The **release** of the transmitter from the motor nerve terminal opens nAchR at the end late

= rapid **membrane depolarization** with a resulting excitatory postsynaptic potential **(EPSP)**, the **end-plate potential (EPP)** at the nerve-muscle synapse.

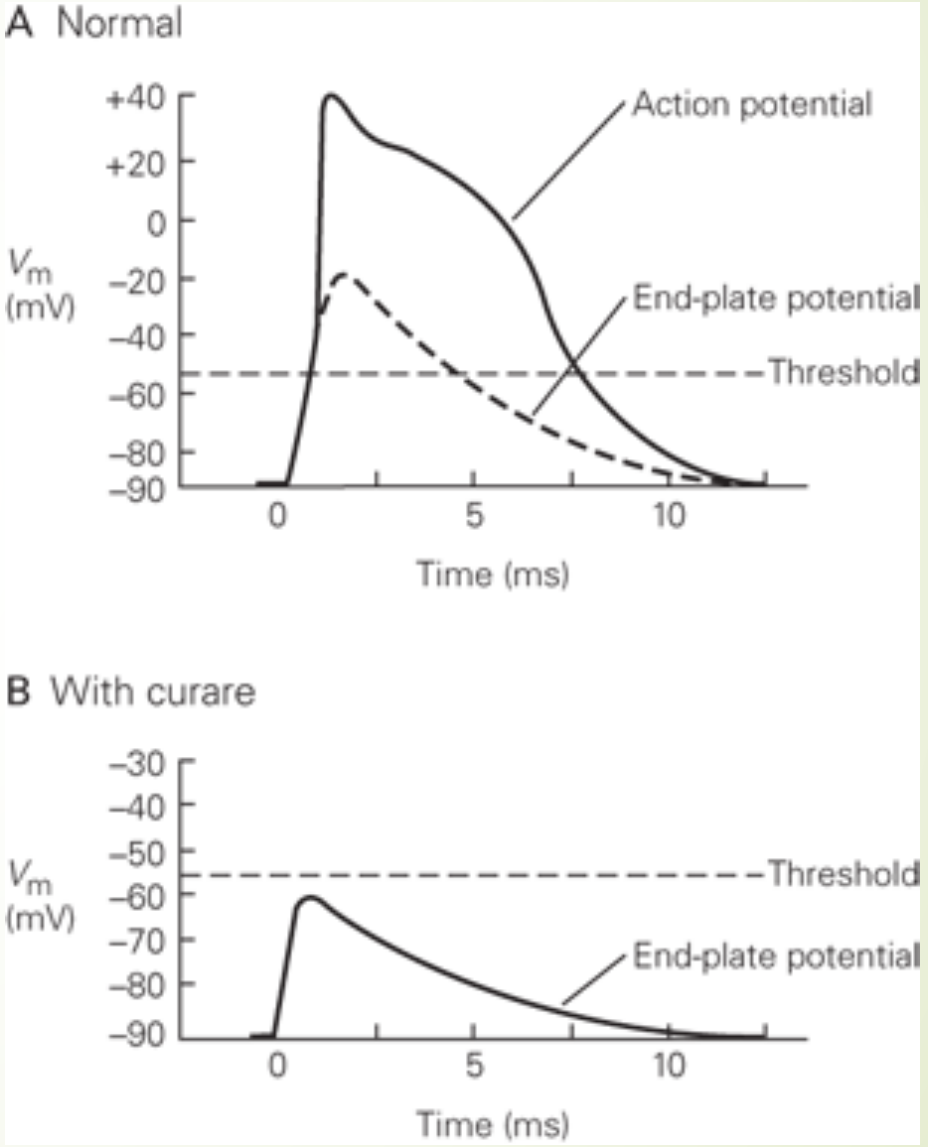
Stimulation of a single motor neuron cell produces a synaptic potential of approximately **70 mV**

- this charge in membrane potential usually is large enough to rapidly **activate Nav** in the junctional folds
- **Conversion of end-plate potential to Action potential** that propagates along the muscle fiber.
- In CNS most presynaptic neurons produce a postsynaptic potential less than 1 mV in amplitude = input from many presynaptic neurons is needed to generate Action potential

End-plate potential EPP

End-plate potential was first studies in 1950s by Fatt and Katz using intracellular voltage recordings.

They isolated end-plate potential by applying **CURARE** to reduce the amplitude of EPSP below the threshold for action potential.

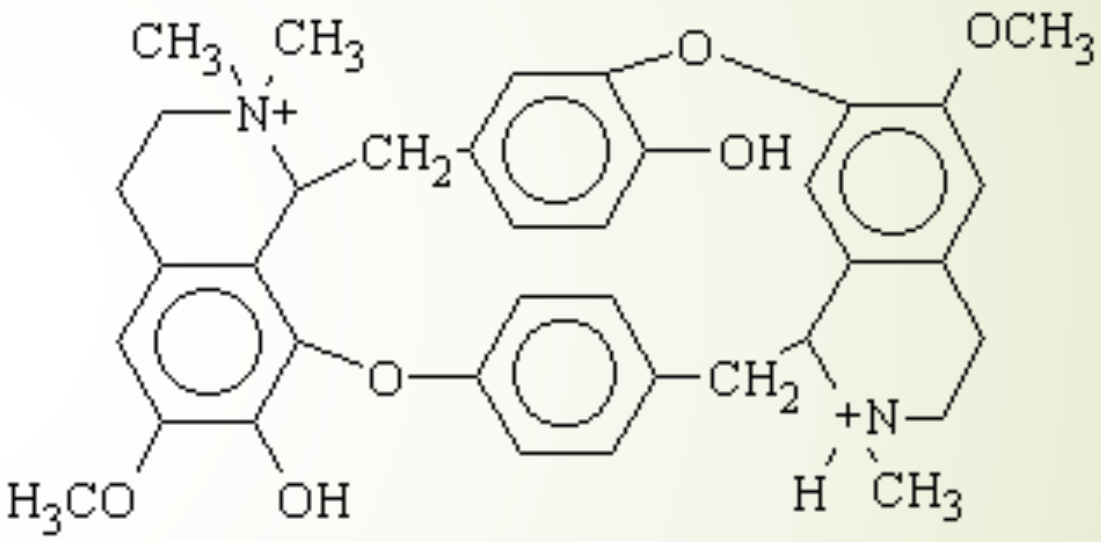


End-plate potential

Curare is a mixture of plant and toxins used by South American Indians to paralyze their quarry.

Tubocurarine is the purified active agent

It blocks neuromuscular transmission by binding to nAChR preventing activation by ACh

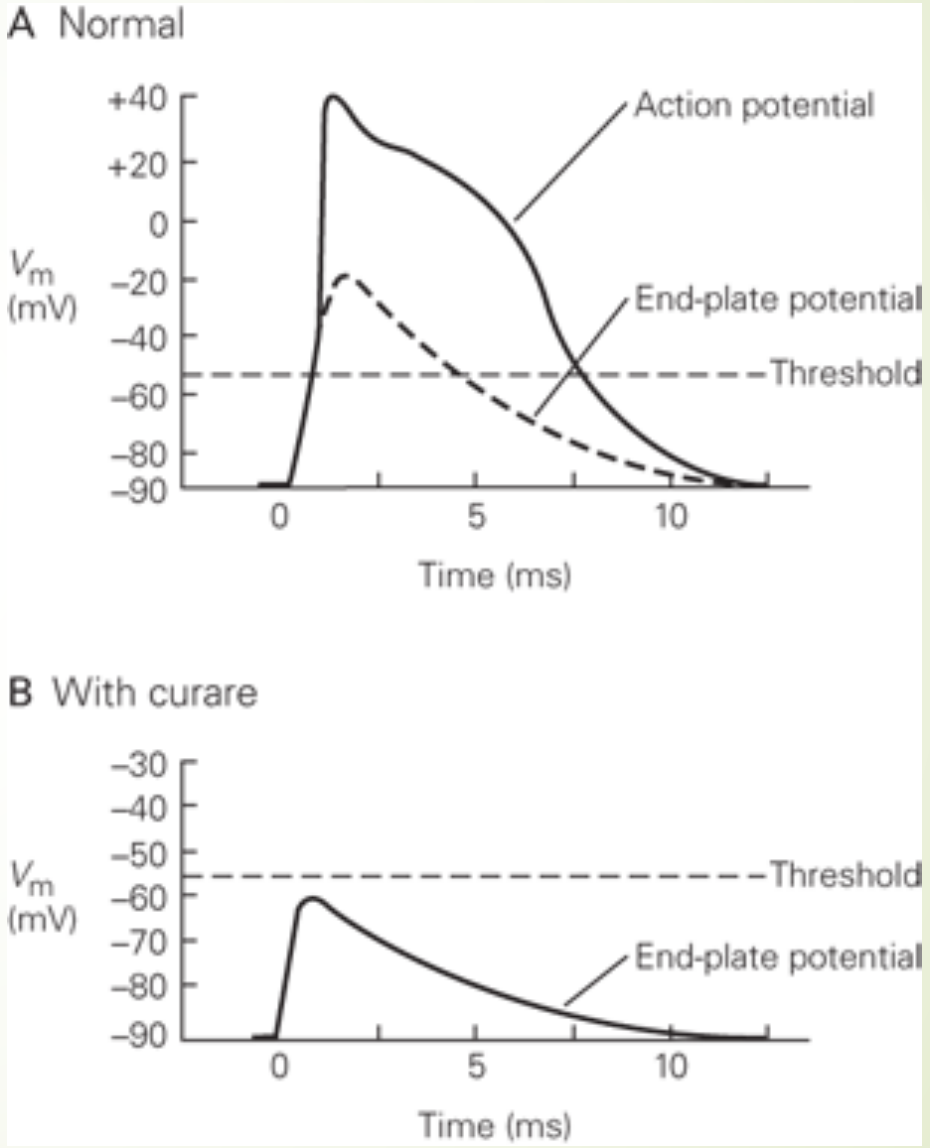


d-Tubocurarine

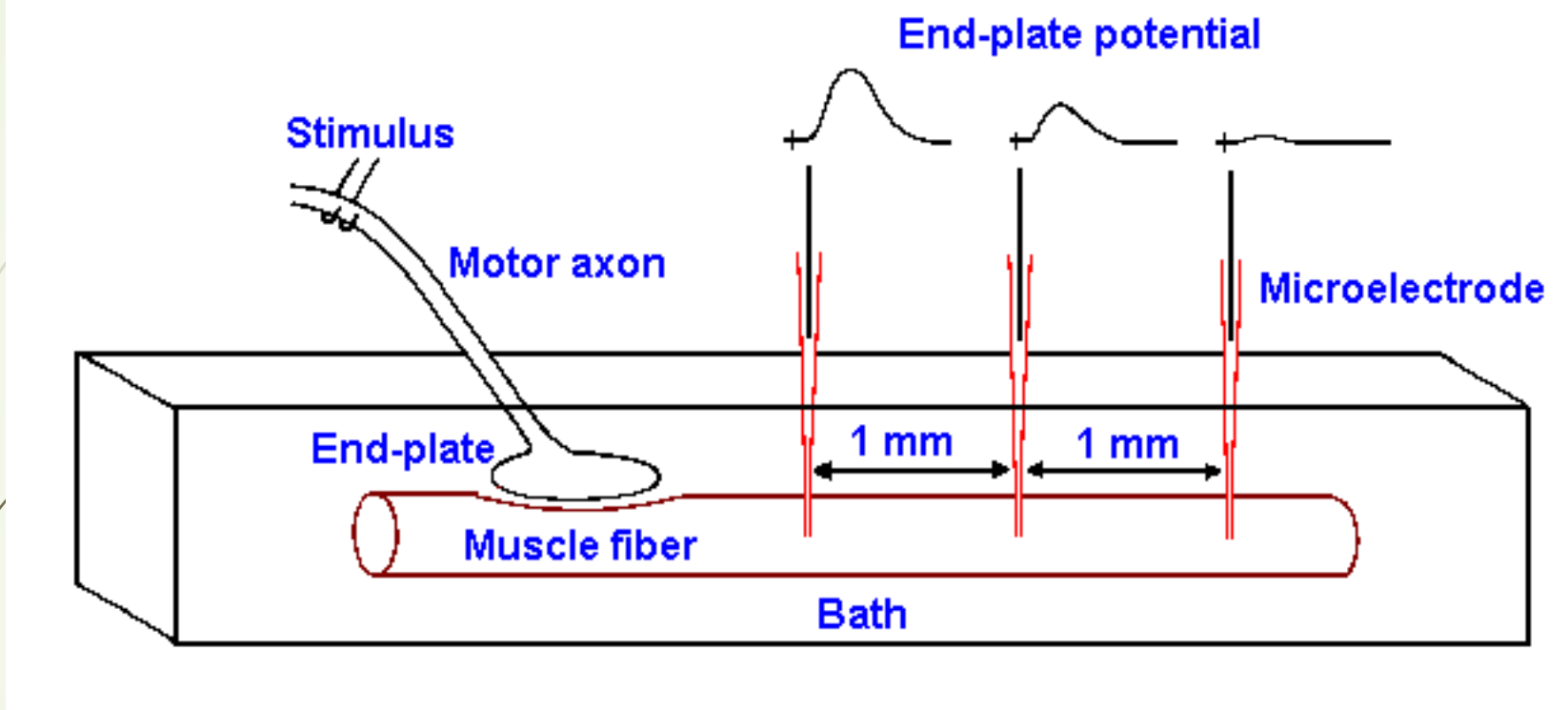
End-plate potential

The End-plate potential in **B** was recorded by low doses of curare which block only a fraction of the nACh receptors.

Values of Vm (resting V) (-90mV) and action potential are typical of vertebrate skeletal muscles



End-plate potential

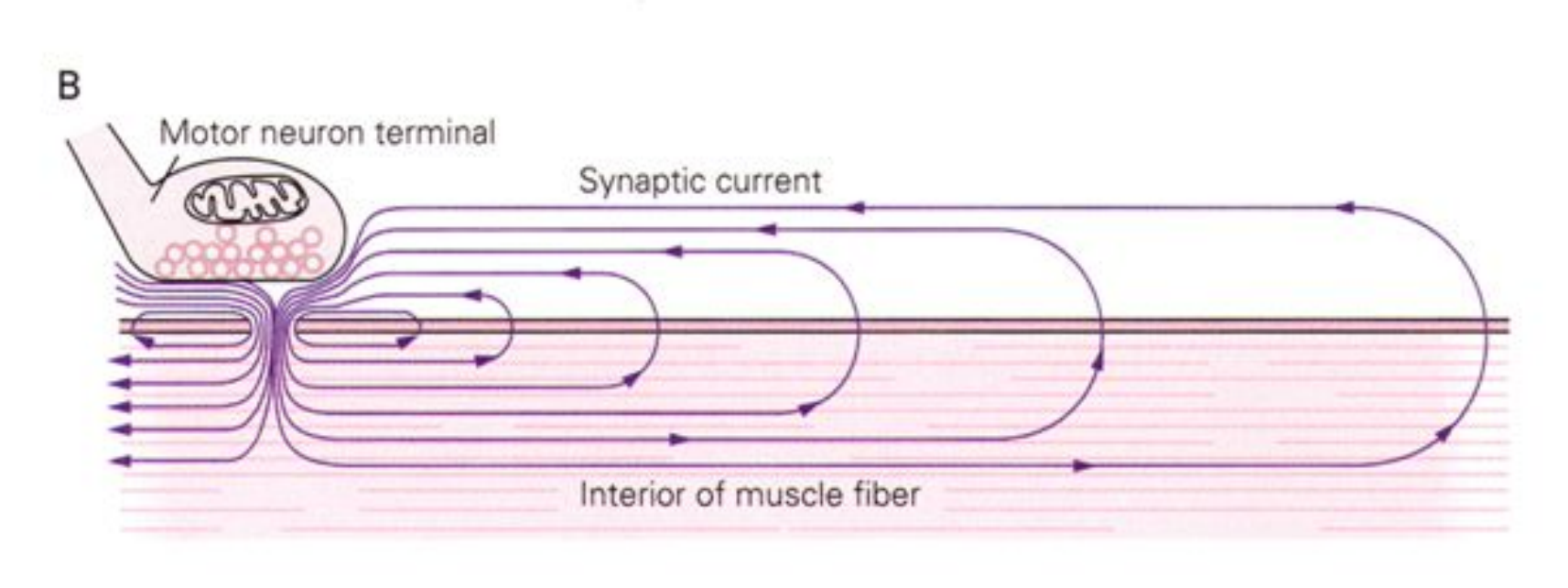


The End-plate potential decrease progressively with distance.

Fatt and Katz concluded that end-plate potential is generated by an inward current that is confined to the end-plate and then spreads passively away.

This is because nAChR are concentrated there opposite to presynaptic terminal from which Ach is released.

End-plate potential



The decay results from the leakiness of the muscle fiber membrane. Because charge must flow in a complete circuit, the inward synaptic current at the end plate gives rise to a return outward current through the resting channels and across the membrane (capacitor).

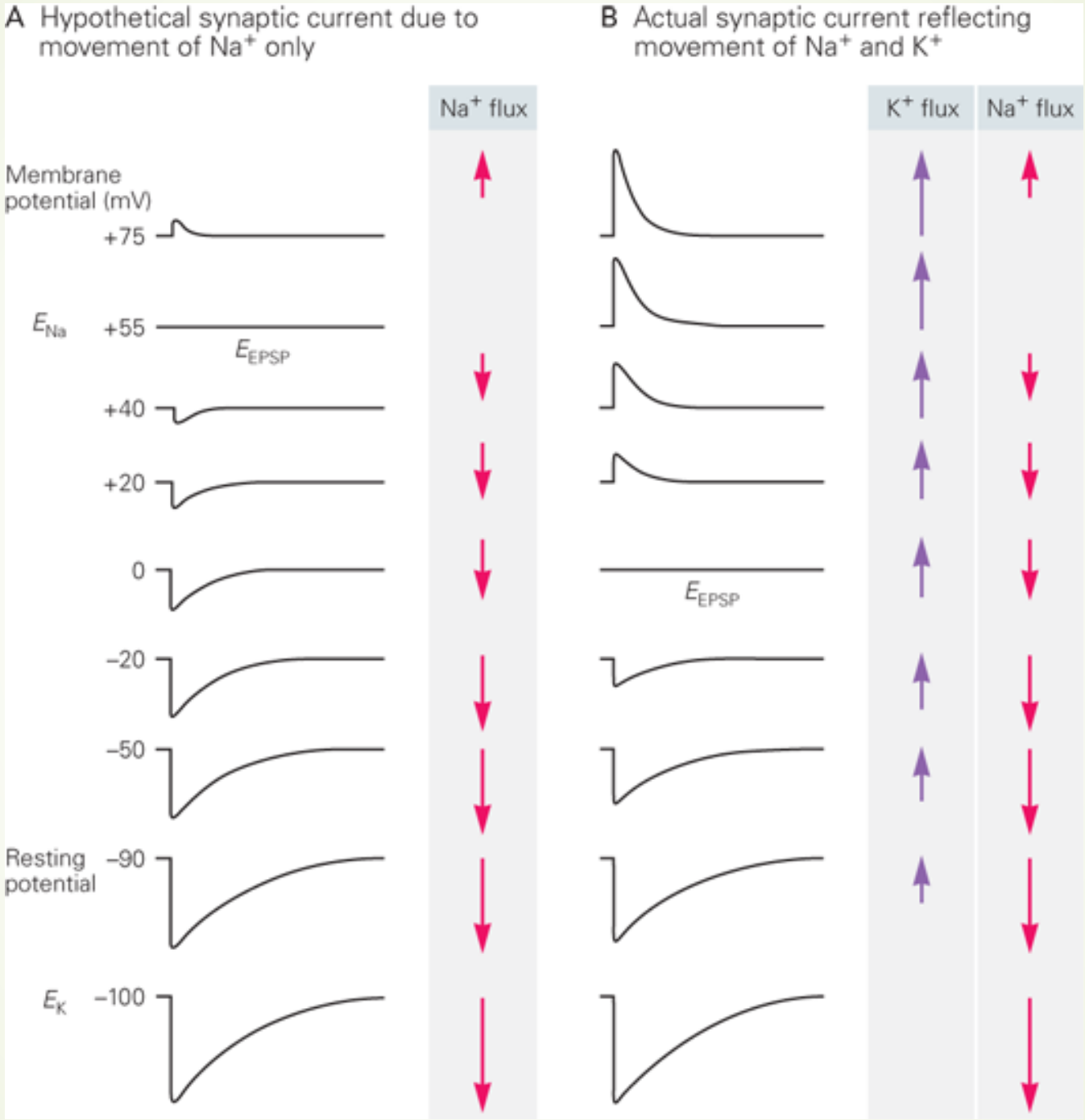
End-plate potential

- Why the opening of Ach receptor channels produce an inward current and depolarizing EPP?
- Which ions move through ACh-gated channels?

Important answer will come from determining E_{EPSP}

$$I_{EPSP} = g_{EPSP} \times (V_m - E_{EPSP})$$

E_{EPSP} can be calculated by altering Vm in a voltage clamp experiments and measuring I_{EPSP}


A Hypothetical synaptic current due to movement of Na+ only
B Actual synaptic current reflecting movement of Na+ and K+
Na+ flux
K+ flux
Na+ flux
Membrane potential (mV)
+75
ENa
+55
EEPSP
+40
+20
0
–20
–50
Resting potential
–90
EK
–100
EEPSP

End-plate potential

- E_{EPSP} is not due to a single ion species but to a combination of ions:
- The nAChR are equally permeable to both Na+ and K+
- The E_{EPSP} = 0mV because is the weighted average of the Vrev for Na+ and K+

End-plate potential

At the E_{EPSP} the total net current is 0

$$I_{Na+} + I_{K+} = 0$$

$$I_{Na+} = g_{Na+} \times (V_m - V_{Na+})$$

$$I_{K+} = g_{K+} \times (V_m - V_{K+})$$

We can replace V_m with V_{EPSP} because at the reversal potential $V_m = E_{EPSP}$

$$g_{Na+} \times (E_{EPSP} - V_{Na+}) + g_{K+} \times (E_{EPSP} - V_{K+}) = 0$$

$$E_{EPSP} = \frac{(g_{Na} \times E_{Na}) + (g_K \times E_K)}{g_{Na} + g_K}$$

End-plate potential

If we devide top and bottom for g_K

$$E_{EPSP} = \frac{E_{Na}(g_{Na} \ / \ g_K) + E_K}{(g_{Na} \ / \ g_K) + 1}$$

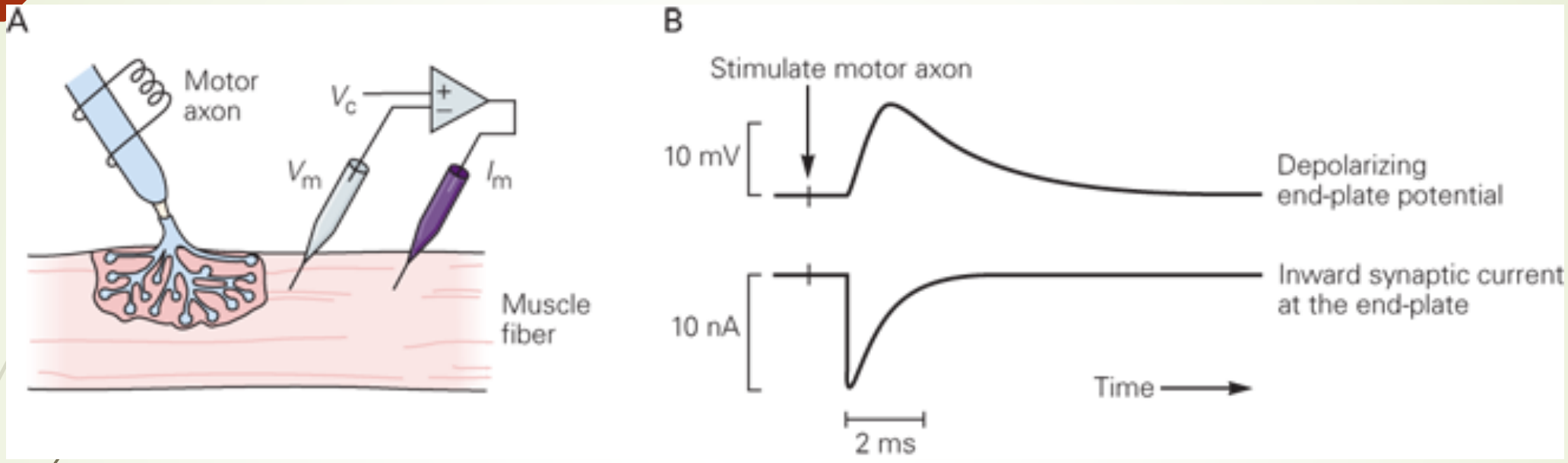
This equation can also be used to solve for the ratio $g_{Na} \ / \ g_K$ if one knows $E_{EPSP}\, E_{Na}$ and E_K

$$\frac{g_{Na}}{g_K} = \frac{E_{EPSP} - E_K}{E_{Na} - E_{EPSP}}$$

For the neuromuscular junction $E_{EPSP} = 0mV$, $E_K = -100mV$, $E_{Na} = +55mV$

$\frac{g_{Na}}{g_K}$ = 1.8 indicating that the cunductance of the nAChR for Na+ is slighly higher than for K+

End-plate potential

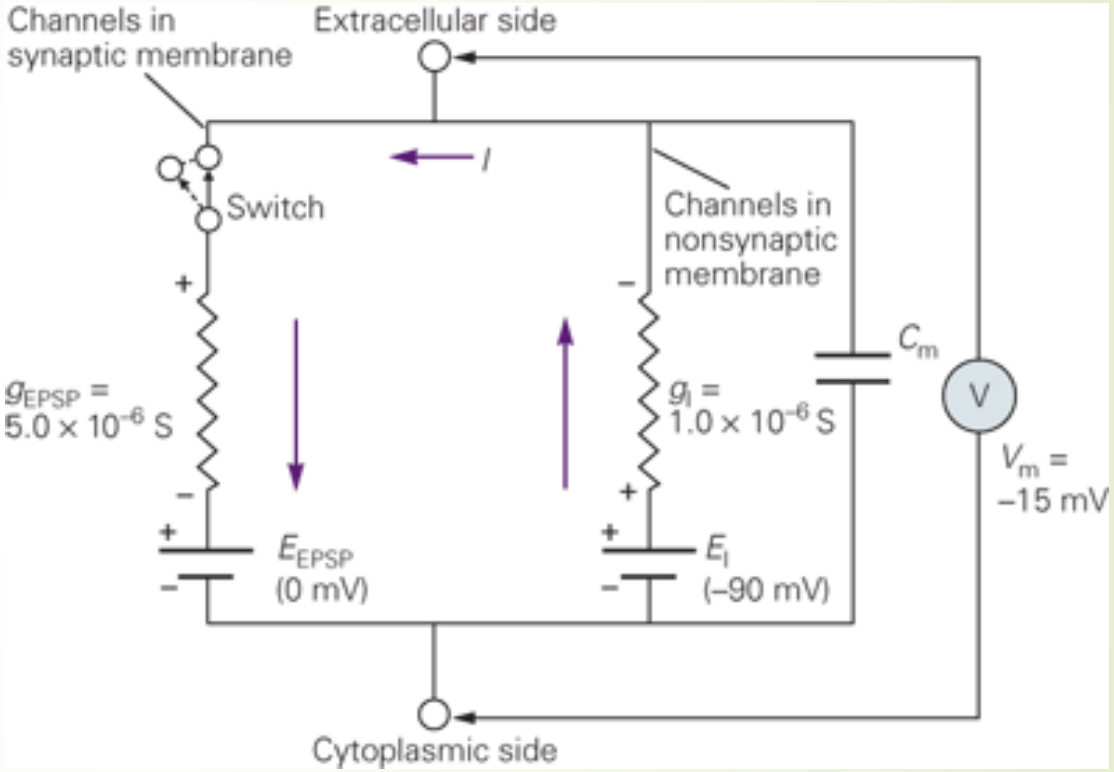


The End-plate current can be recorded in V clamp:

End-plate I rises and decay more rapidly than the resultant EPP

Equivalent circuit for End-Plate Current

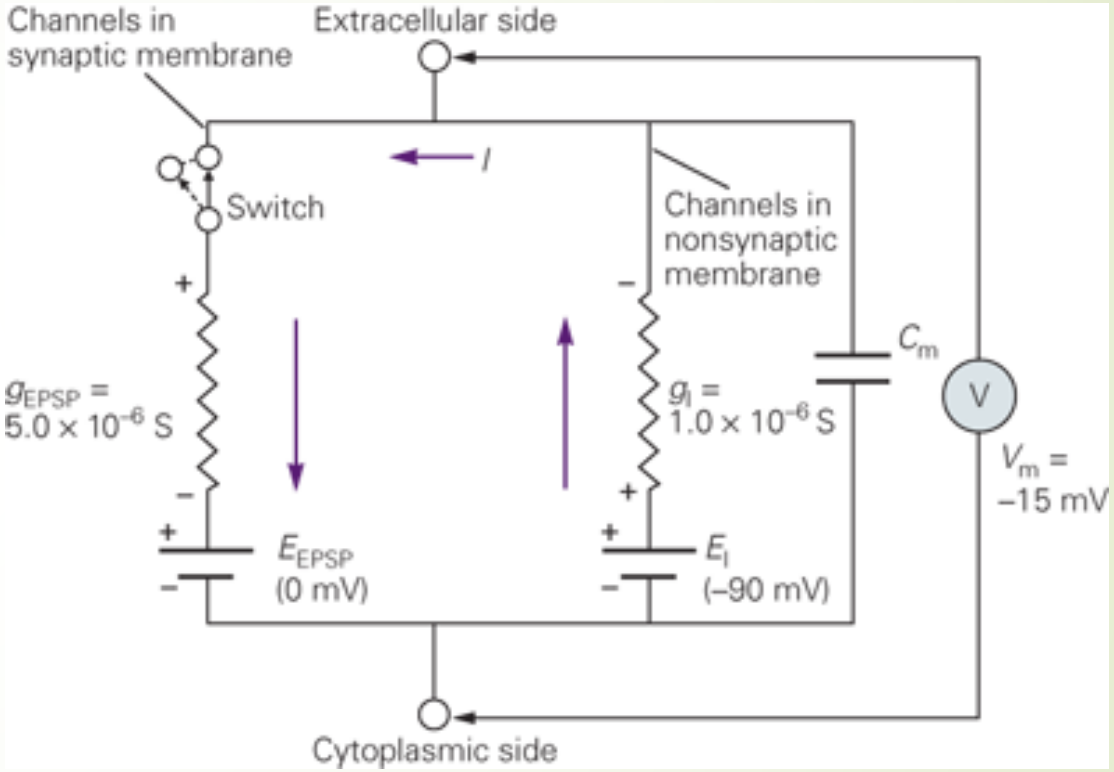
The current through AChR can be described by Ohm's law. However, to fully describe how the electrical current generates the EPP, all the resting channels in the surrounding membrane must be considered. Moreover we must also take in account the capacitive properties of the membrane and the "batteries" determined by the distribution of Na+ and K+ inside and outside of the cell.



Equivalent circuit for End-Plate Current

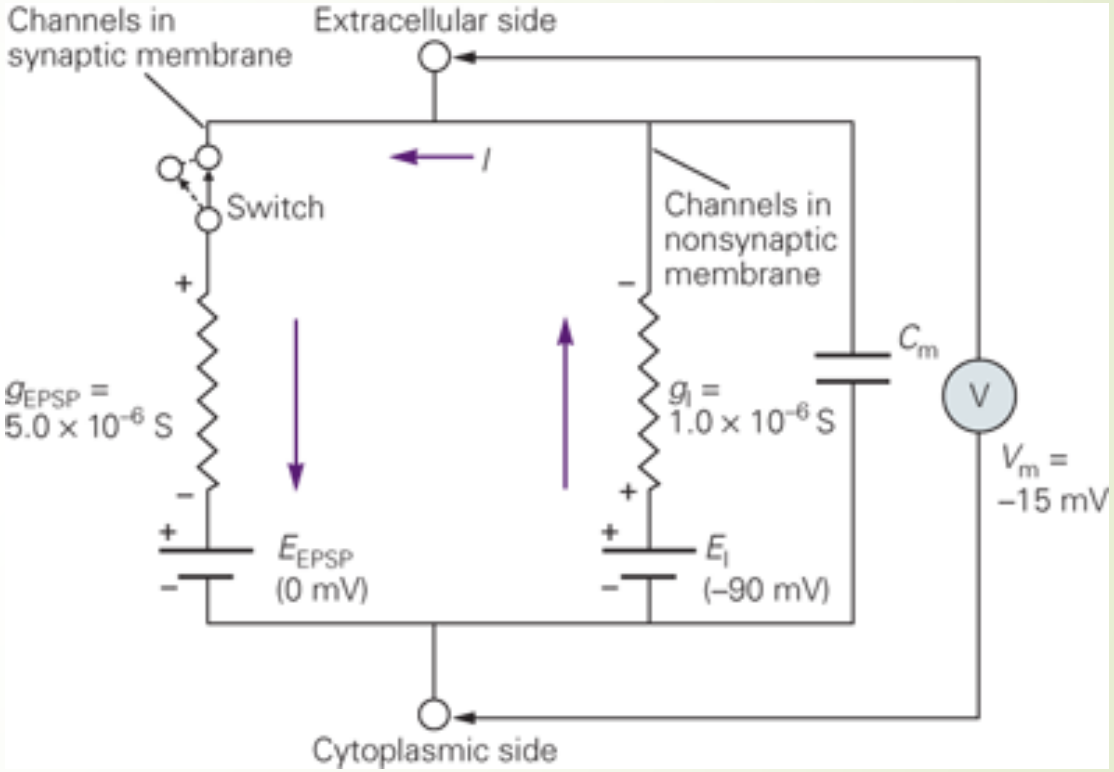
The end-plate region can be represented with an equivalent circuit composed by 3 branches:

1. Synaptic current through nAChR
2. Return current through the resting channels (non synaptic membrane)
3. Current across the lipid bilayer, acting as a capacitor



Equivalent circuit for End-Plate Current

End-plate current is carried by both Na+ and k+, we could represent the synaptic branch as 2 parallel branches, each representing the flow of each individual ion species. However Na+ and K+ flow in the same channel. It is therefore more correct to combine the Na+ and K+ current pathways into a single conductance g_{EPSP} represneting the nAChR.



Equivalent circuit for End-Plate Current

$g_{EPSP} = n \times \gamma$

n = number of open channels

γ = single channel conductance

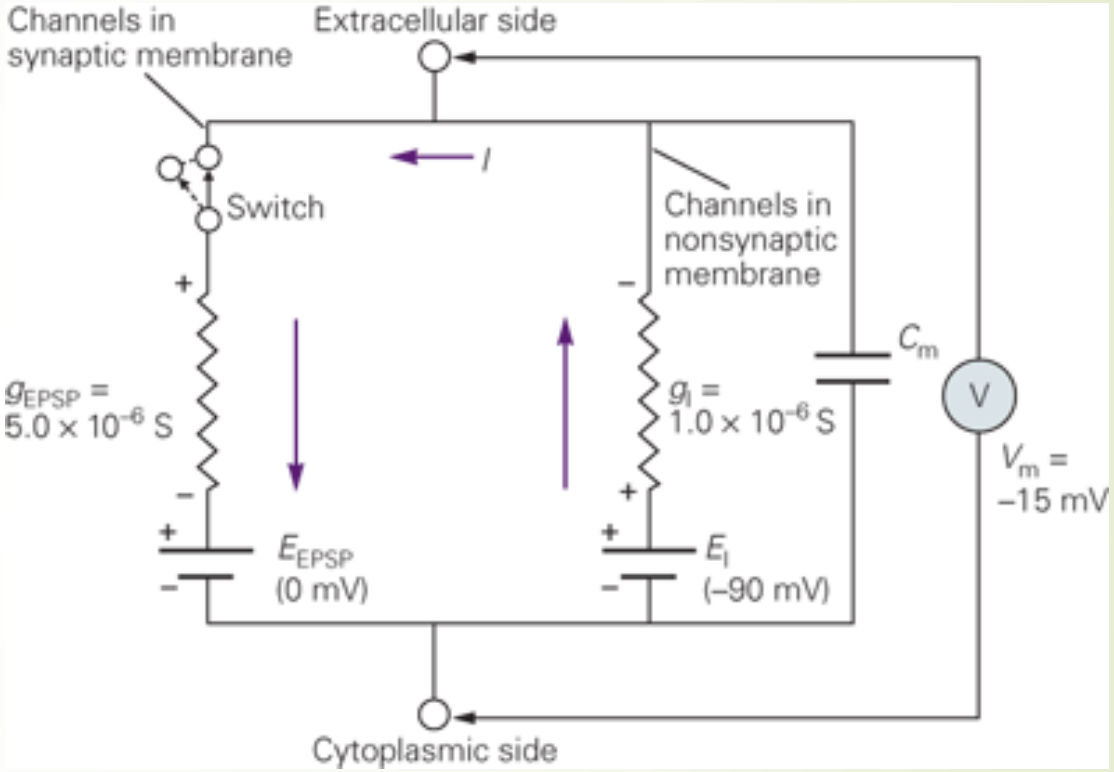
$n = N \times p_{open}$

N = total number of channels

p_{open} depends on ACh concentration (ligand-gated channels)

When a presynaptic Action potential causes the relaease of Ach, the g_{EPSP} increases to a value approximately 5μS.

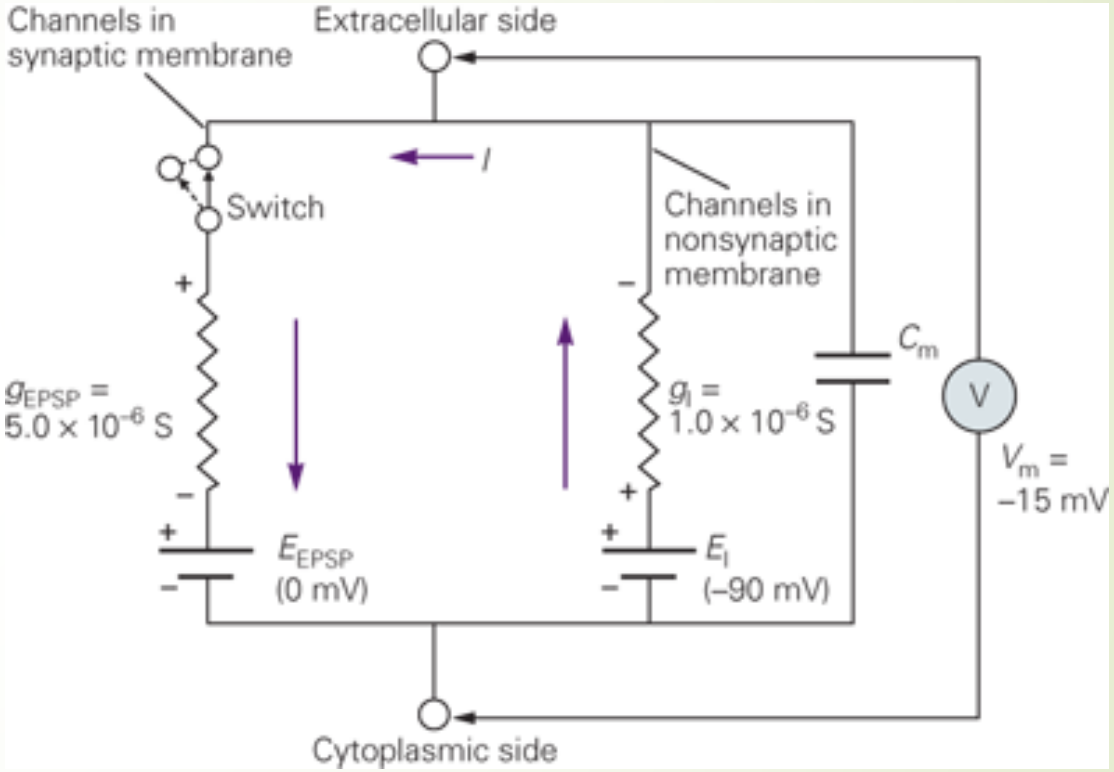
This is **about five time** the conductance of the parallel branch representing the resting leakage channels(g_l)



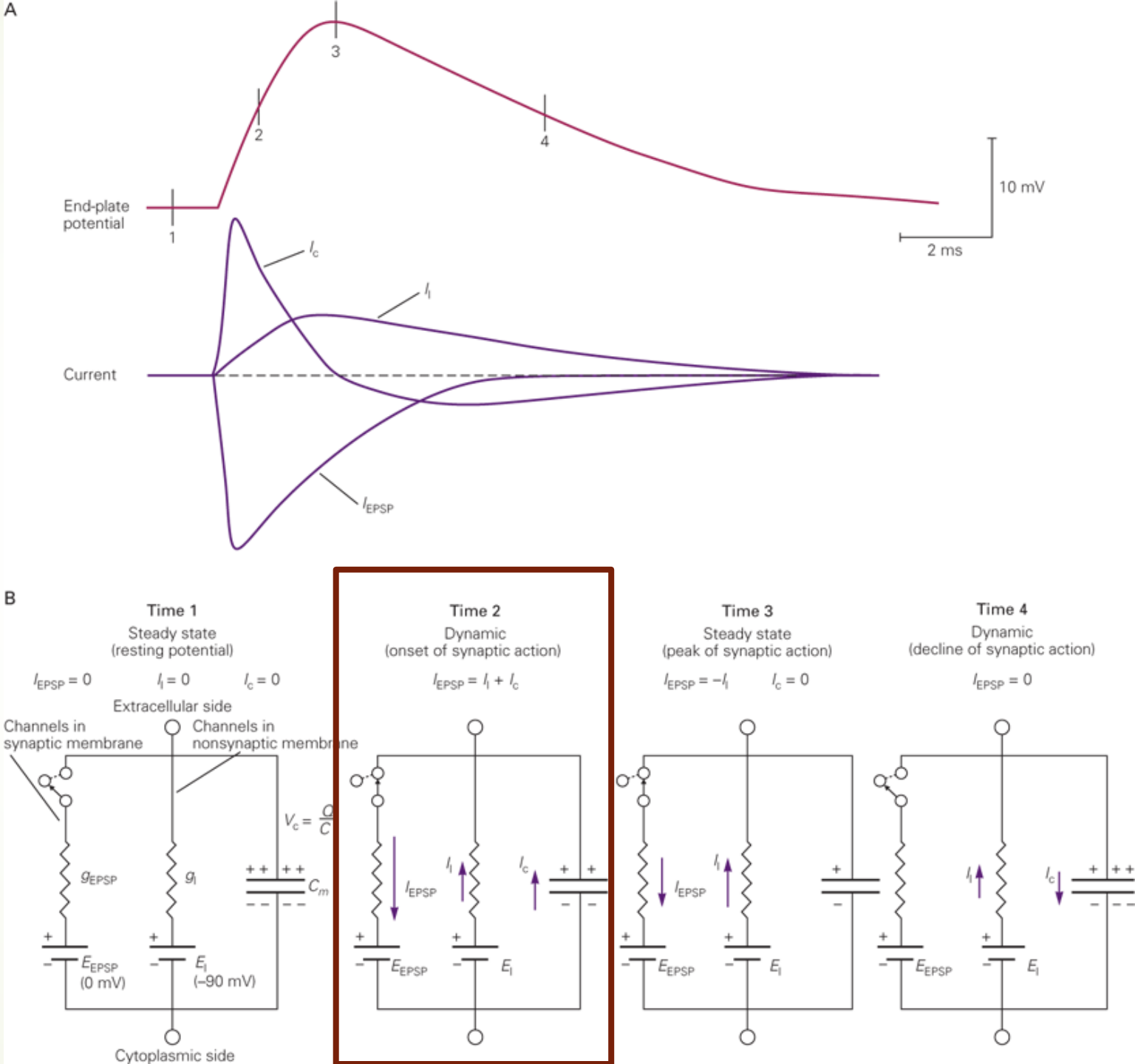
Equivalent circuit for End-Plate Current

g_{EPSP} is in series with a battery (E_{EPSP}) with a value given by the reversal potential for the I_{EPSP} = 0mV that is the weight algebric sum of the Na+ and K+ equilibrium.

$I_{EPSP} = g_{EPSP} \times (V_m - E_{EPSP})$

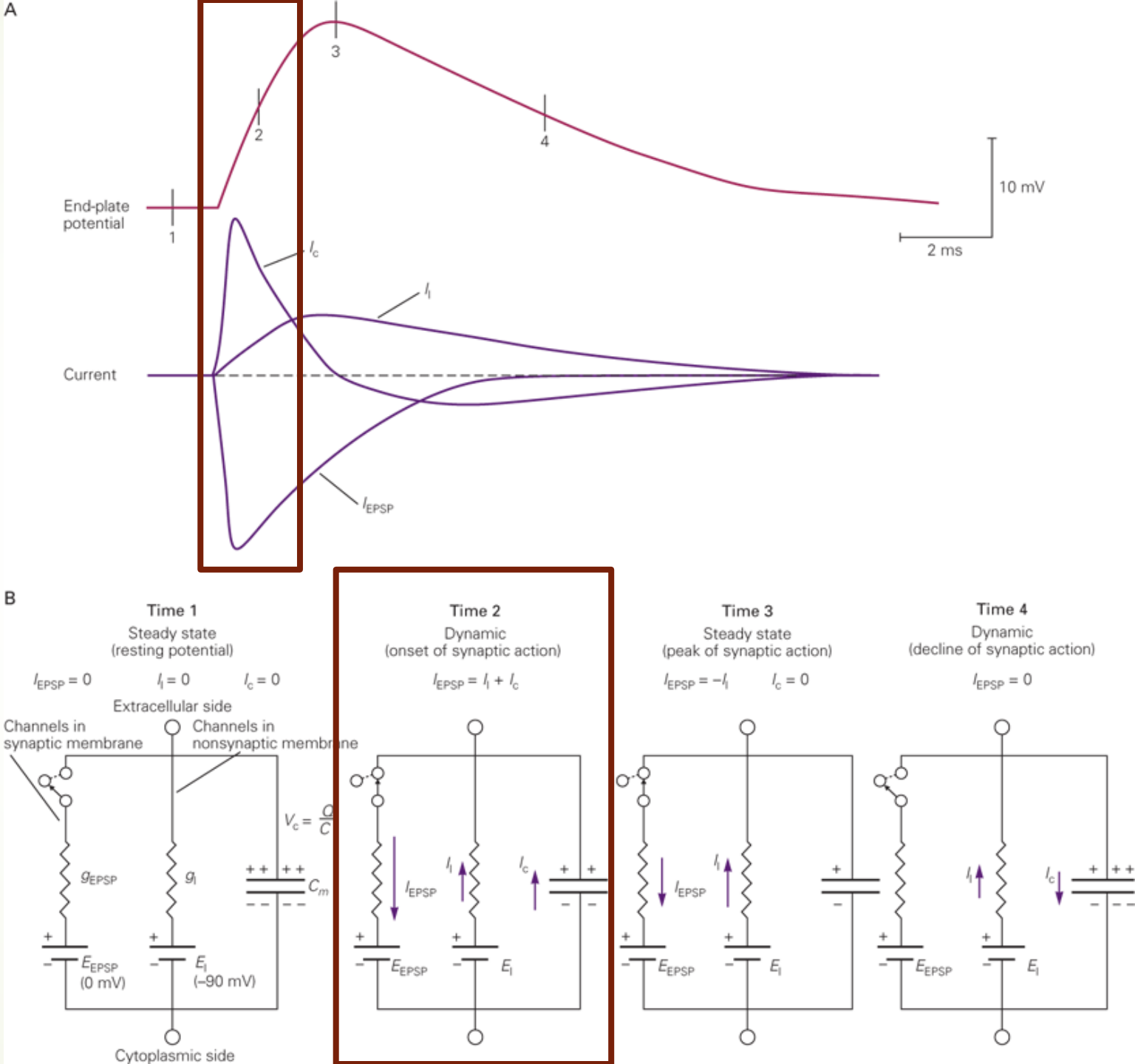


At the onset of the excitatory synaptic action (dynamic phase) inward current (I_{EPSP}) is generated by the nAChR because of the increase conductance to Na+ and K+ and the large inward driving force for Na+ at the resting potential of -90mV



At the beginning of EPP, V_m is still close to its resting value, E_I.

- The outward driving force on current through the resting channels (V_m – E_I) is low
- Most of the current leaves the cell as capacitive current and the membrane depolarize rapidly



As the cell depolarizes, the outward driving force on current through the resting channels increases, while the inward driving force through the nAChR decreases. Concomitantly, as the [ACh] decreases, nAChR begin to close, and the inward current is exactly balanced by outward current trhough I_I ($I_{EPSP} = I_I$)

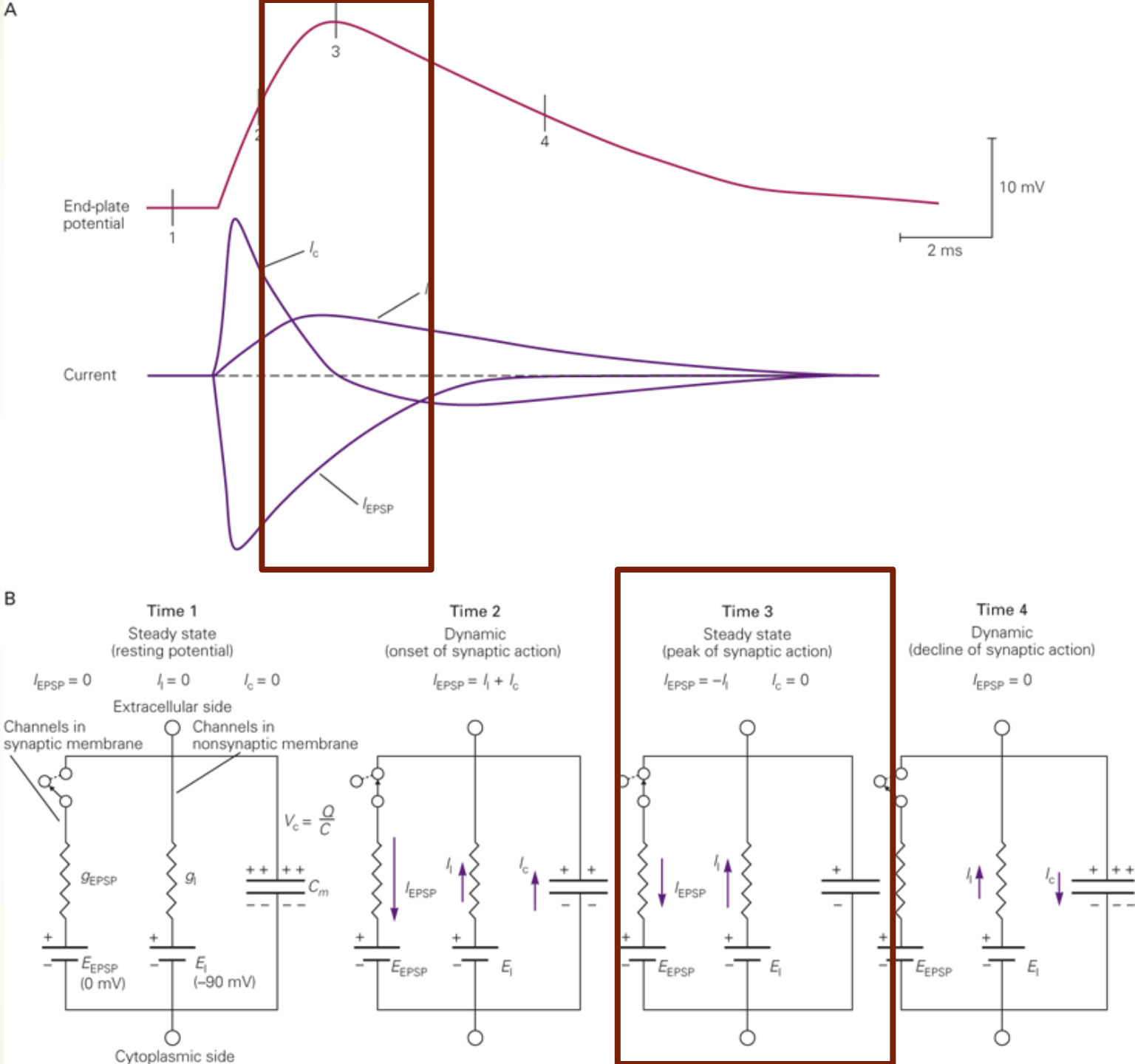
At this point there is no current into the capacitor ($I_C = 0$)

Since

$Ic/Cm = \Delta V/\Delta t$

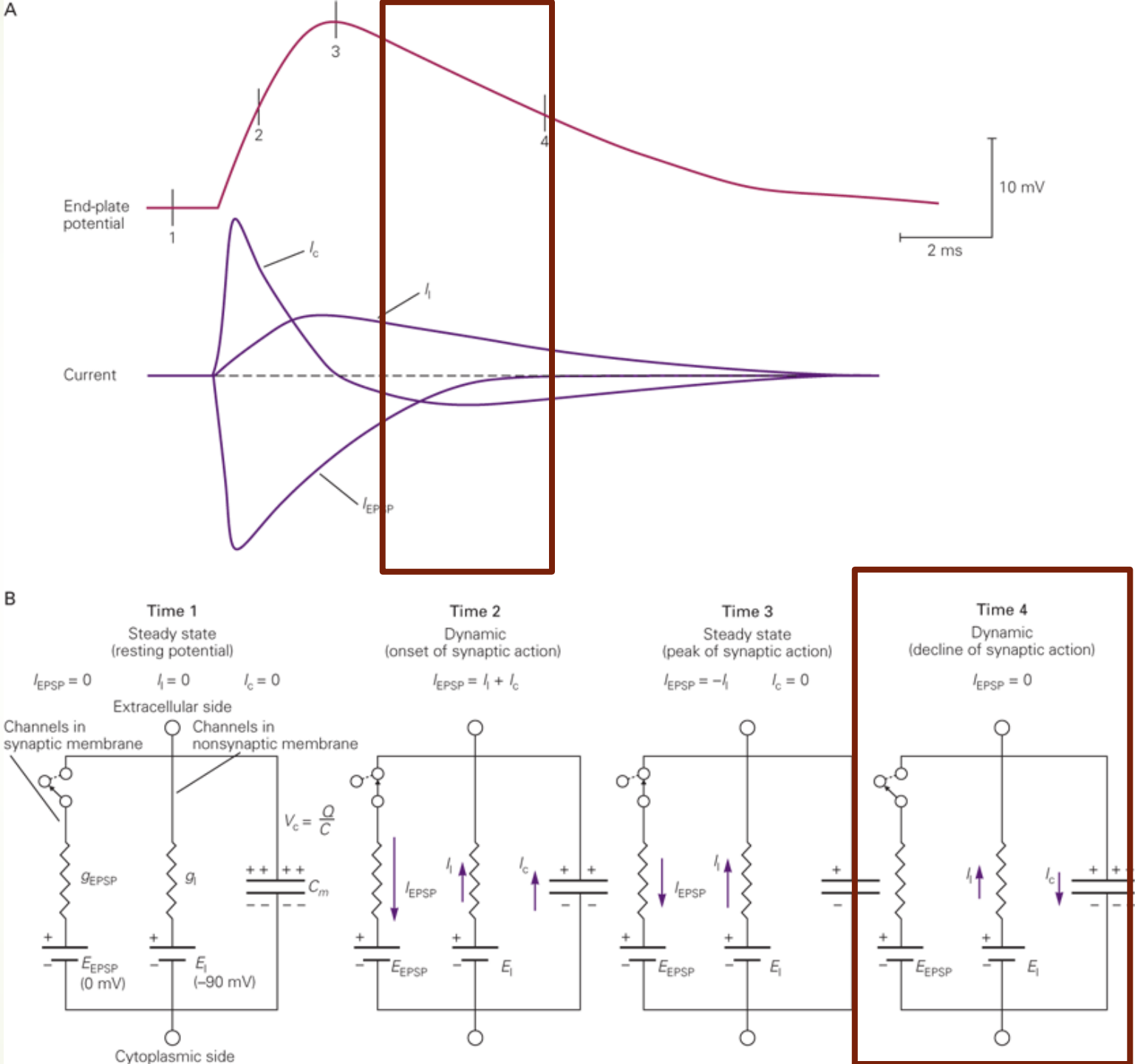
$\Delta V/\Delta t = 0$

Steady state value

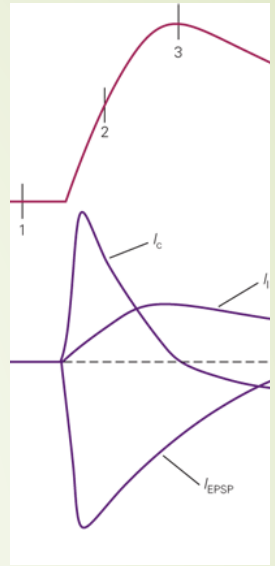


As the nAChR close, I_{EPSP} decreases further. Now I_{EPSP} and I_I are no longer in balance and the E_{EPSP} repolarize, because the outward current through the leakage channels (I_I) becomes larger than the inward current (I_{EPSP}). During most of the decay of the E_{EPSP} there is no current through the nAChR because all of these channels are closed.

Current instead passes through the membrane only as outward current carried by g_I and balanced by current across the membrane capacitor (g_c)



Vm at the steady state value at EPP peak can be calculated:



$I_{EPSP} + I_l = 0$

$I_{EPSP} = g_{EPSP} \times (V_m - E_{EPSP})$

$I_l = g_l \times (V_m - E_l)$

$g_{EPSP} \times (V_m - E_{EPSP}) + g_l \times (V_m - E_l) = 0$

We can solve for V_m by expand the two products and rearrange them

$(g_{EPSP} \times V_m) + (g_l \times V_m) = (g_{EPSP} \times E_{EPSP}) + (g_l \times E_l)$

By factoring out V_m on the left side

$$Vm = \frac{(g_{EPSP} \times E_l) + (g_l \times E_l)}{g_{EPSP} + g_l}$$

In the specific case of the stady state:

g_{EPSP} = 5μS; gl = 1μS; E_{EPSP} = 0mV; El = -90mV

Vm = -15mV

Why the opening of Ach receptor channels at the end-plate is not as selective for one ion species as the Voltage gated channels?

- This is mainly due to the diameter of the pore.

Electrophysiological measurements suggest that it may be up to 0.8nm in diameter, an estimate based on the size of the largest organic cation that can permeate the channel.

For example, tetramethylammonium (TMA) is approximately 0.6nm in diameter. In contrast, Nav are only permeant to organic cation that are smaller than 0.5 x 0.3nm in cross section and Voltage-gated K+ channels will only conduct ions less than 0.3nm in diameter.

Why the opening of Ach receptor channels at the end-plate not as selective for one ion species as the Voltage gated channels?

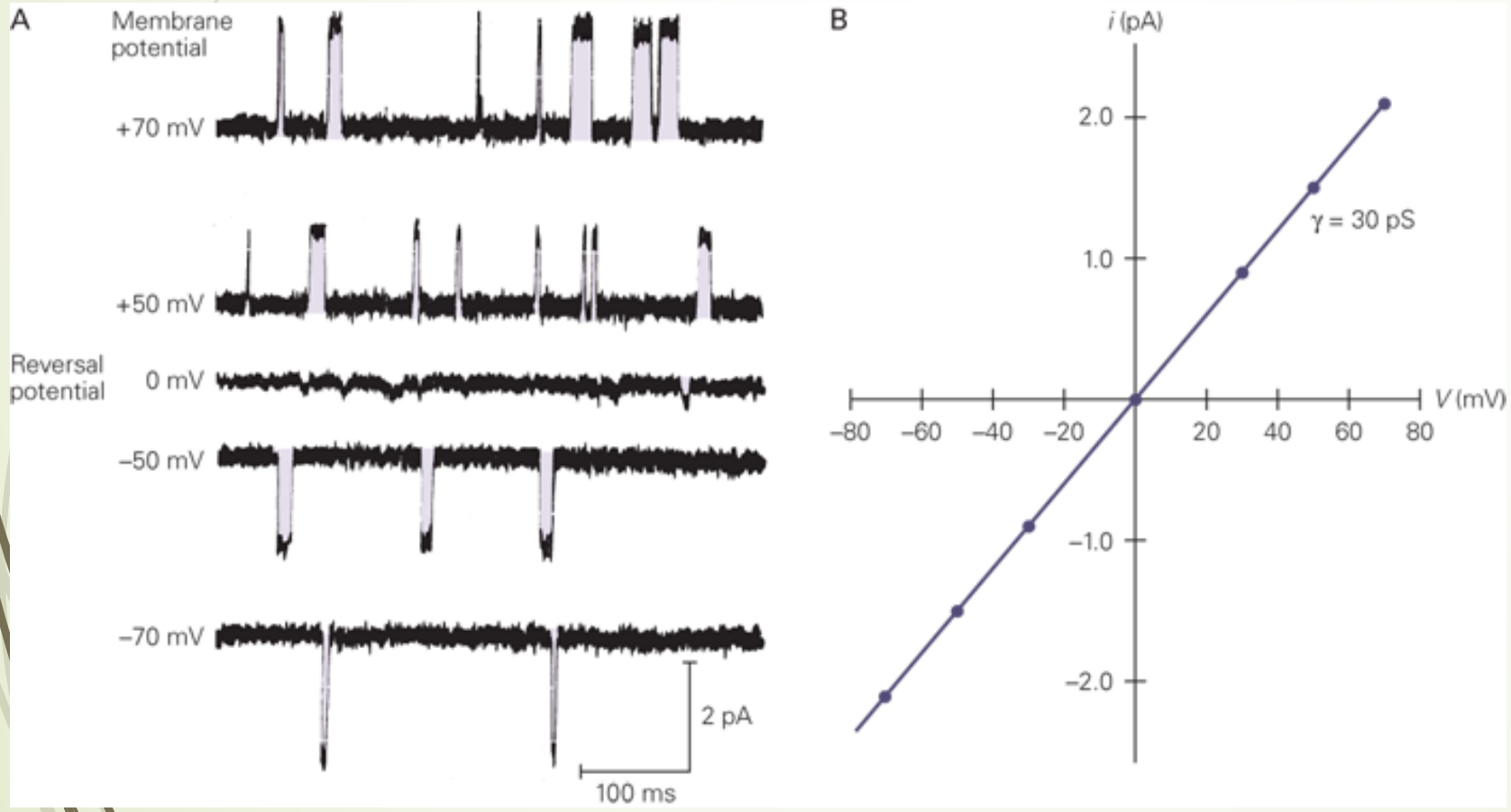
- This is mainly due to the diameter of the pore.

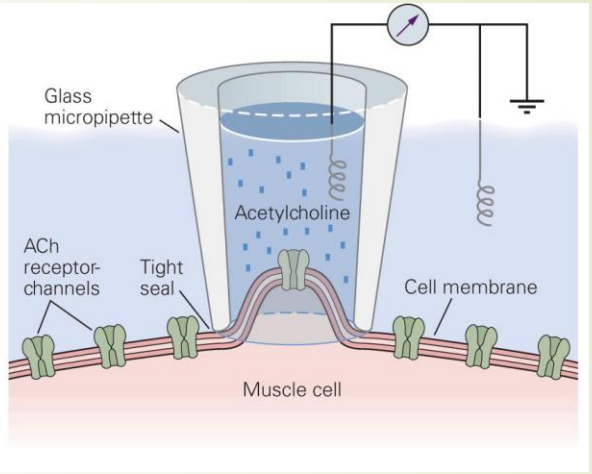
The relatively large diameter of the nAChR pore is though to provide a water-filled environment that allows cation to diffuse through the channel unempeded.

This explain why the pore does not discriminate between Na+ and K+. This also explain why divalent cation such as Ca2+, can permeate the channel. Anion are excluded, however by the presence of fixed negative charges in the channel

Single channel recordings of nAChR

- The first successful recording of single AChR channels currents from skeletal muscle cells was done by Neher and Sakmann in 1976



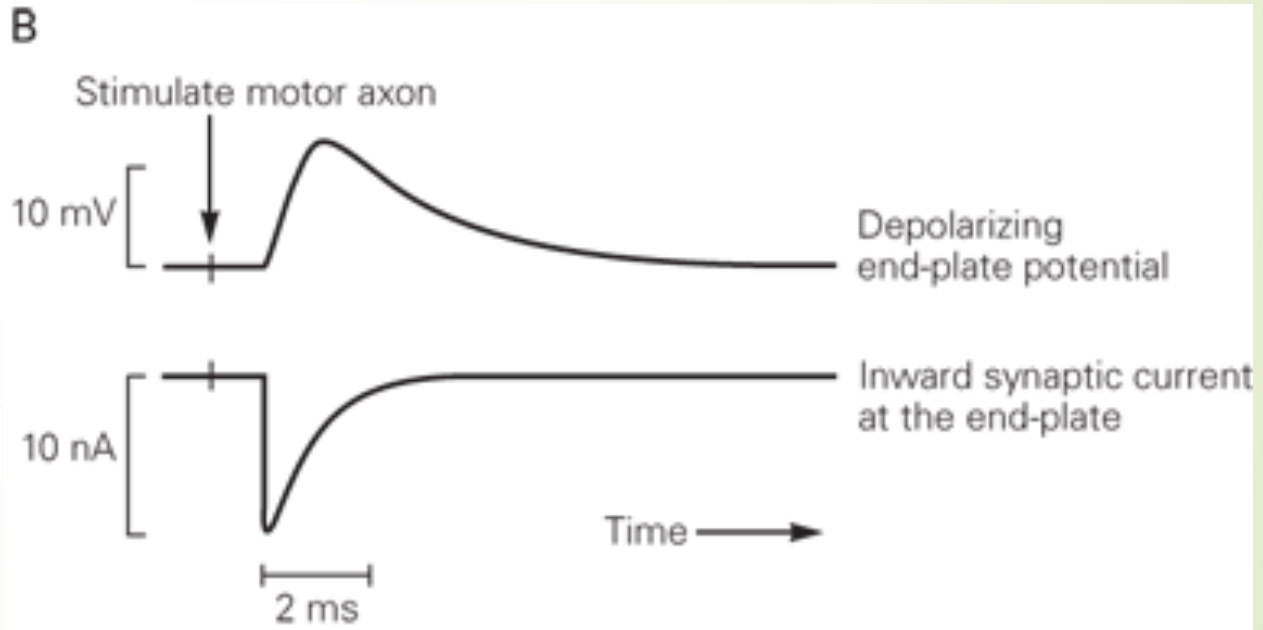


I-V is lienear, indicating that the single channel conductance is constant and does not depend on the voltage.

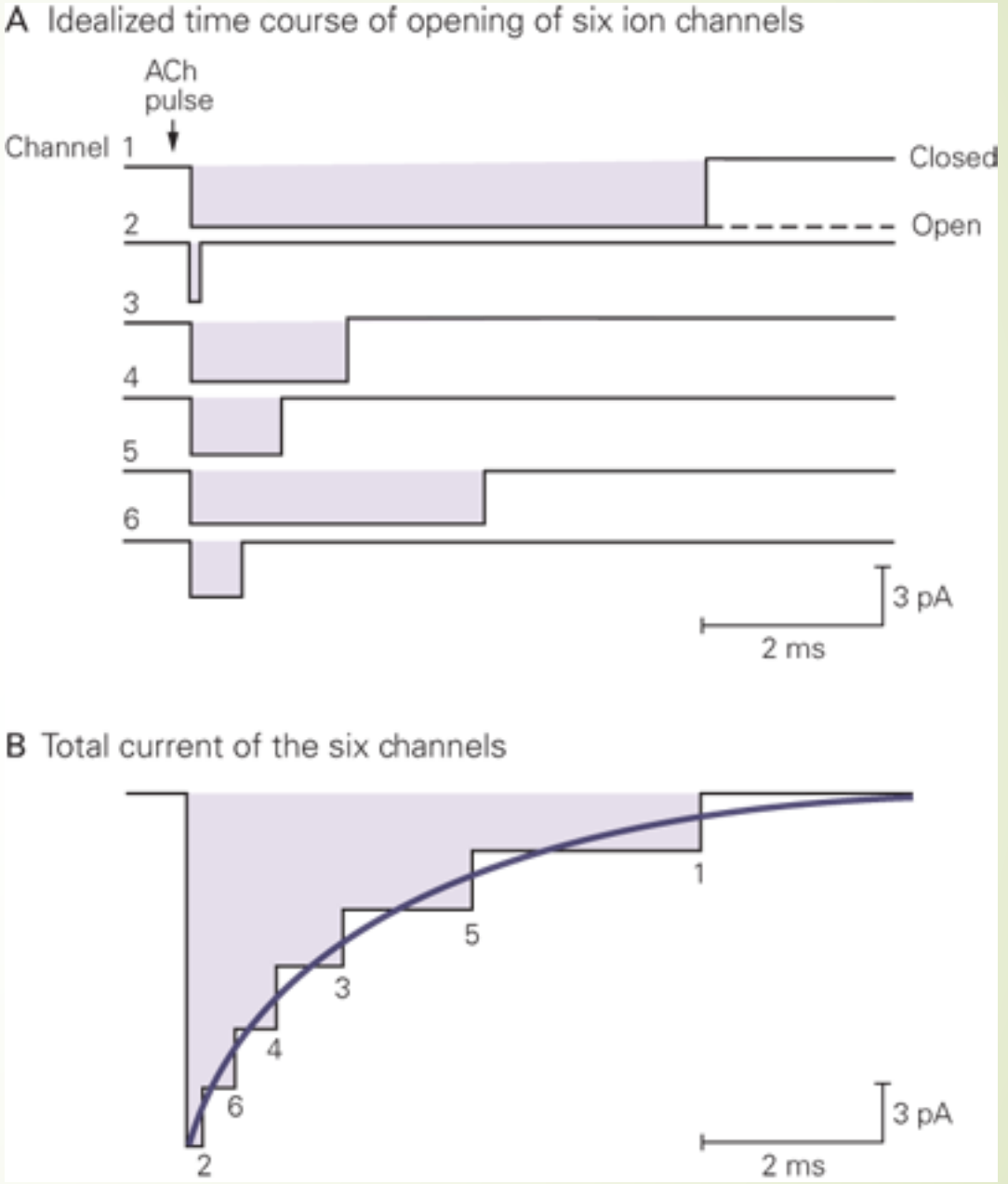
Erev = 0

- Stimulation of a motor nerve releases a large quantity of Ach into the synaptic cleft. ACh rapidly diffuses across the cleft and binds AChR, causing more than 200000 receptor-channels to open:
- Total I_{EP} = -500nA
- I single channel = -2,5pA

- How do small step-like changes in current through 200000 individual nAChR produce a smooth waveform of the end-plate current?



- Rapid opening of nAChR produce a large increase in total conductance of end-plate membrane, g_{EPSP}, and produce the rapid increase in I_{EPSP}.
- ACh rapidly decrease in the cleft (less than 1ms) because of the enzymatic hydrolysis and diffusion
- Channels close in a random manner
- The random close of a large number of small unitary currents causes the total I_{EPSP} to appear to decay smoothly



The summed conductance of all open channels in a large population of AChR is the total synaptic conductance

$g_{EPSP} = n \times \gamma$ n = number of open channels

γ = single channel conductance

$n = N \times p_{open}$ N = total number of channels

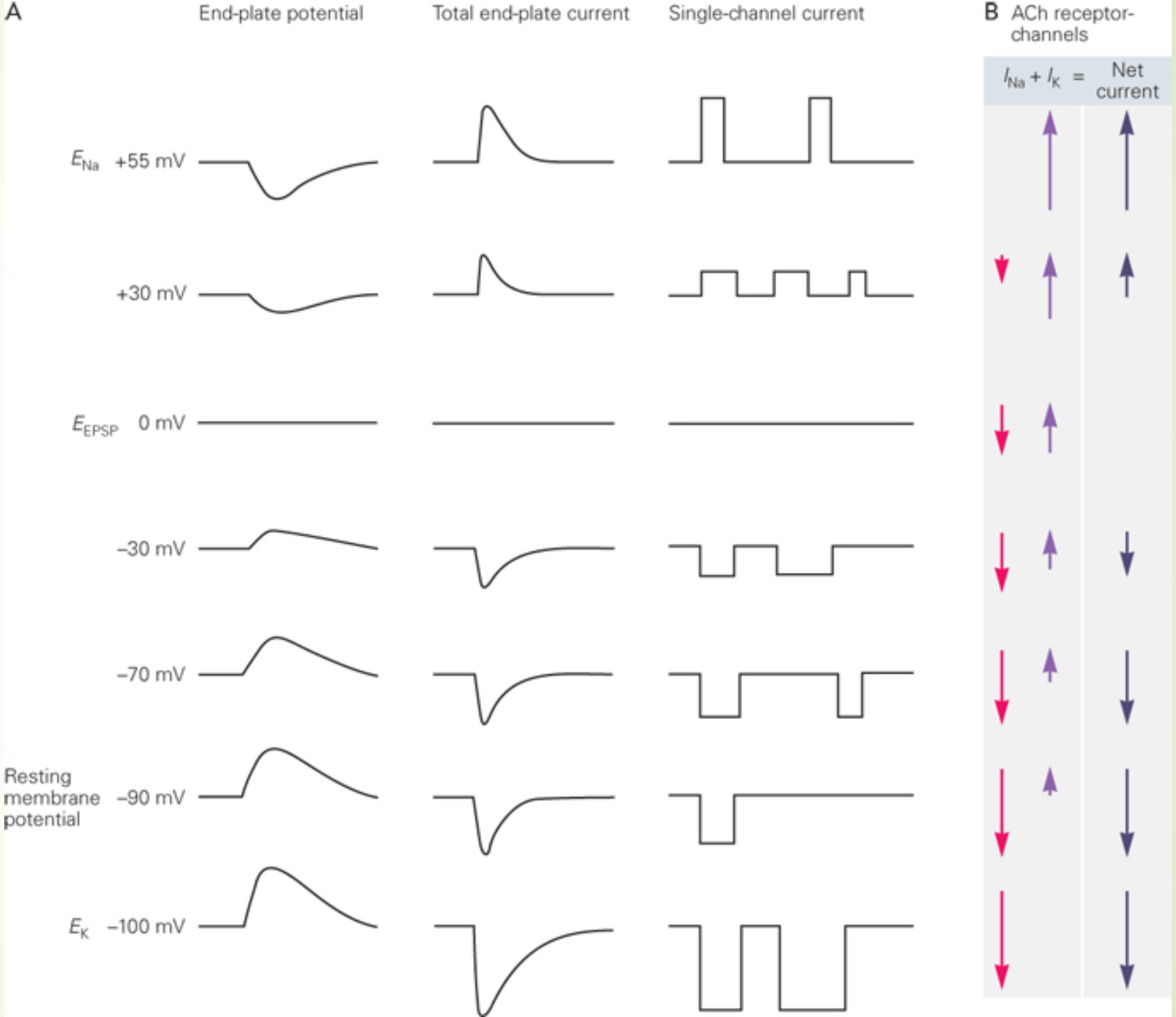
p_{open} depends on ACh concentration (ligand-gated channels)

$$I_{EPSP} = N \times p_{open} \times \gamma \times (V_m - V_{EPSP})$$

$$I_{EPSP} = n \times \gamma \times (Vm - V_{EPSP})$$

The total I_{EPSP} depends on

1) N;
2) p_{open} ;
3) conductance of the channels;
4) driving force of the ions $V_m - V_{EPSP}$



Action Potential

- Two different Voltage-gated channels activated sequentially:

Nav selective for Na+ and Kv selective for K+

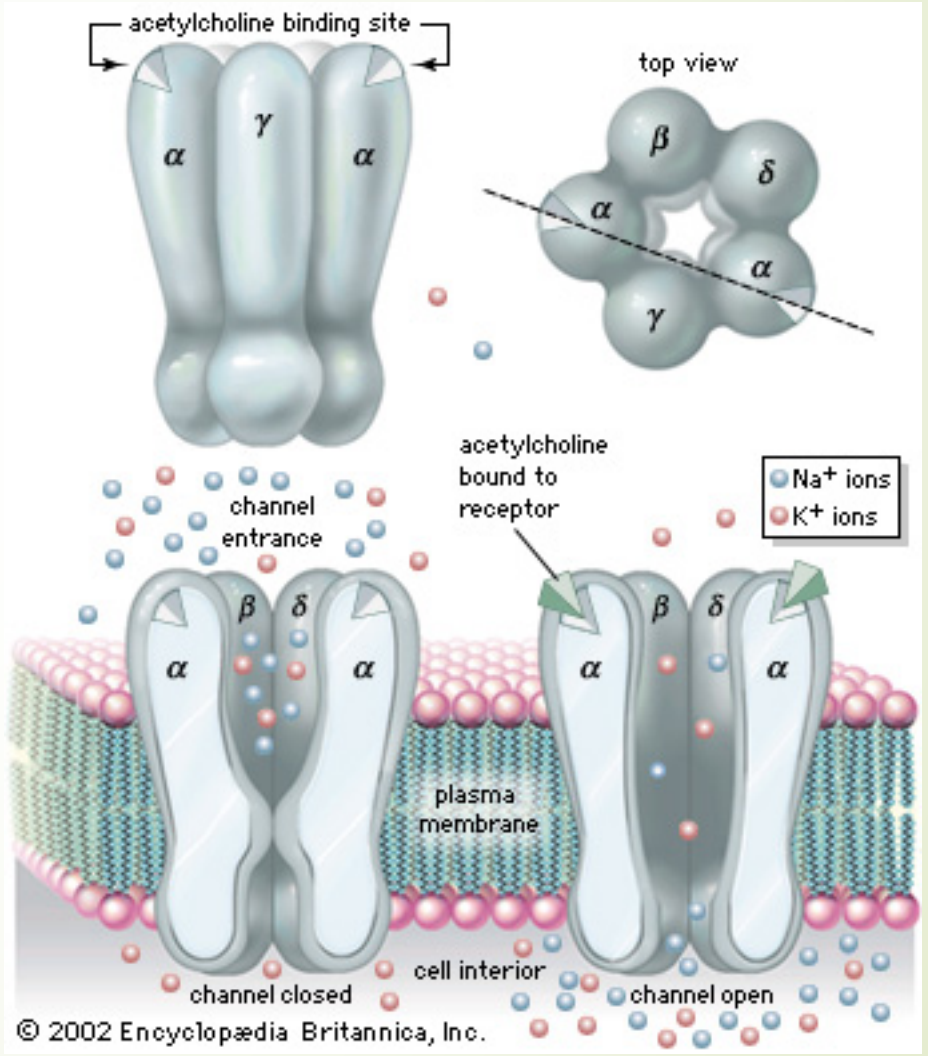
- Na+ flux through Nav is regenerative: by increasing the depolarization of the cell, the Na+ influx opens more Nav channels. This properties is responsible for the ALL or NONE nature of action potential

EPP

- One channel Na+ and K+ permeable allowing both ions to pass with nearly equal permeability
- The number of nAChR opened during the synaptic potential is fixed by the amount of ACh available. The depolarization produced by Na+ entry does not lead to the opening of more nAChR
- **To trigger and Action Potential EPP must recruit neighboring Nav**

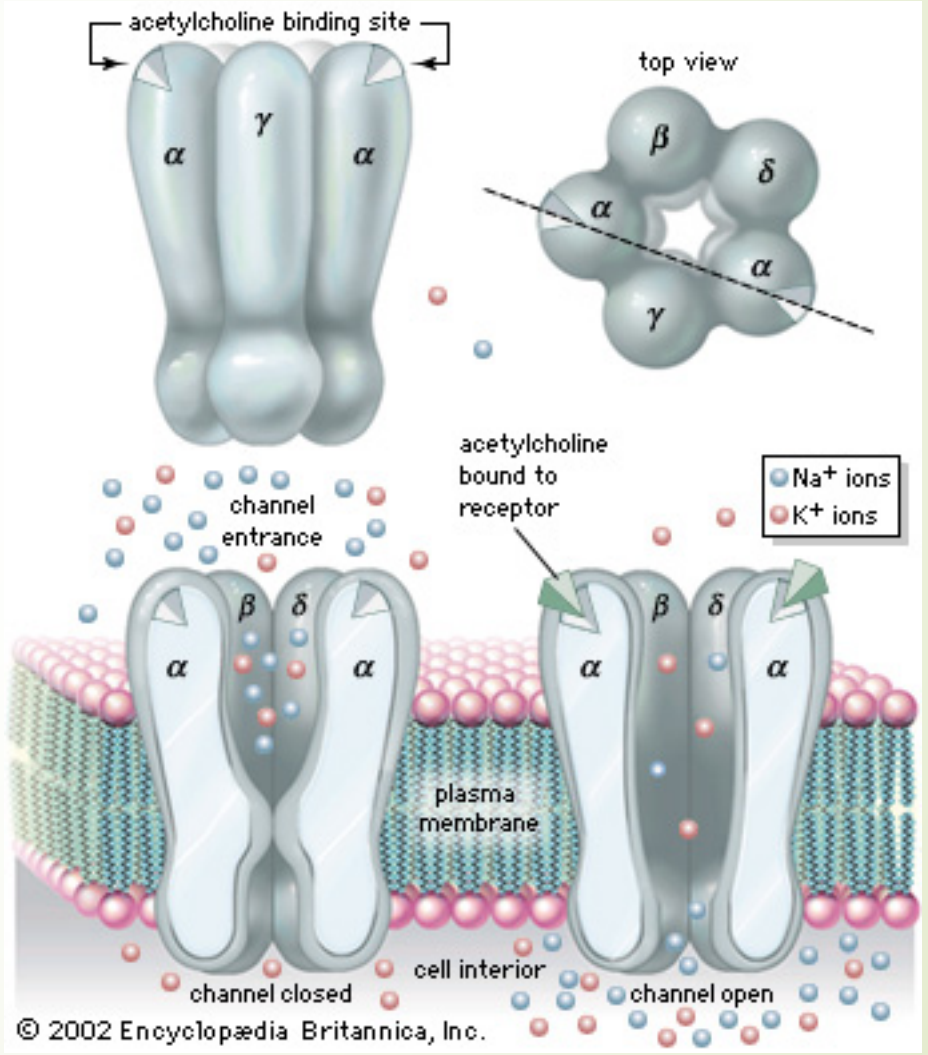
Muscle nACHR

Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of the Cys-loop ligand-gated ion channels (LGICs), which also includes the GABA, glycine, and 5-HT3 receptors.
The characteristic feature of this superfamily is a conserved sequence of 13 residues flanked by linked cysteines in the Nterminal domain of each subunit.
The first nAChR subtype was purified from the **electric organs of the fishes Torpedo and Electrophorus**, and four types of subunits, namely, α, β, χ, and δ, were identified.
ACh binding site is situated in beteween each α subunit and the neighboring γ or δ subunits.



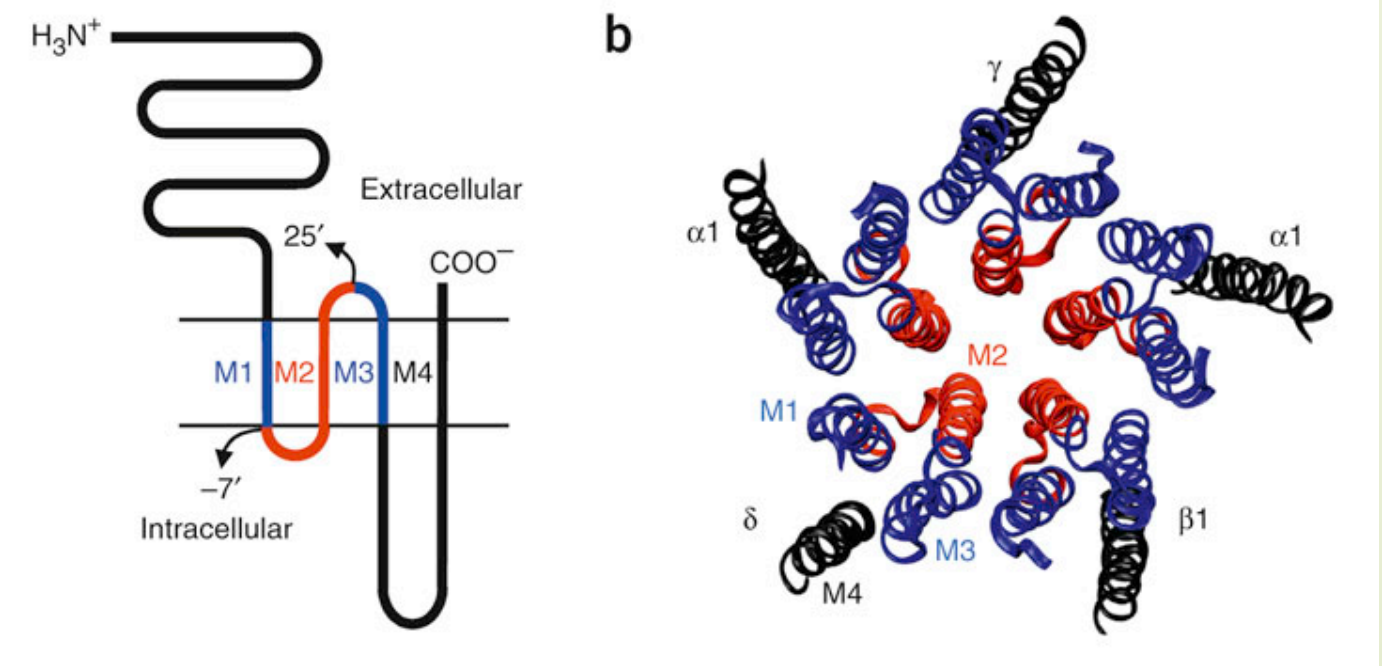
Muscle nACHR

In vertebrates, the 17 known homologous nAChR subunits (α1–α10, β1–β4, γ, δ, and ε) assemble into a variety of pharmacologically distinct receptor subtypes. The **muscle nAChR** is a heteropentamer, with a subunit stoichiometry of $\alpha 1_2\beta 1\gamma\delta$ in the embryo, similar to that in Torpedo ($\alpha 2\beta\gamma\delta$), whereas in adults, the γ subunit is replaced by the ε subunit ($\alpha 1_2\beta 1\varepsilon\delta$).
Muscle and Torpedo nAChRs are often named **muscle-type nAChRs**.
2 ACh must bind two the channels to open efficiently. The inhibitory snake venom α-bungarotoxin also bind to the ACh binding sites in the α subunit



Muscle nACHR

Each subunit present a huge extracellular N-term and 4 a helics (M1-M4). M2 is the helics that delimites the pore.



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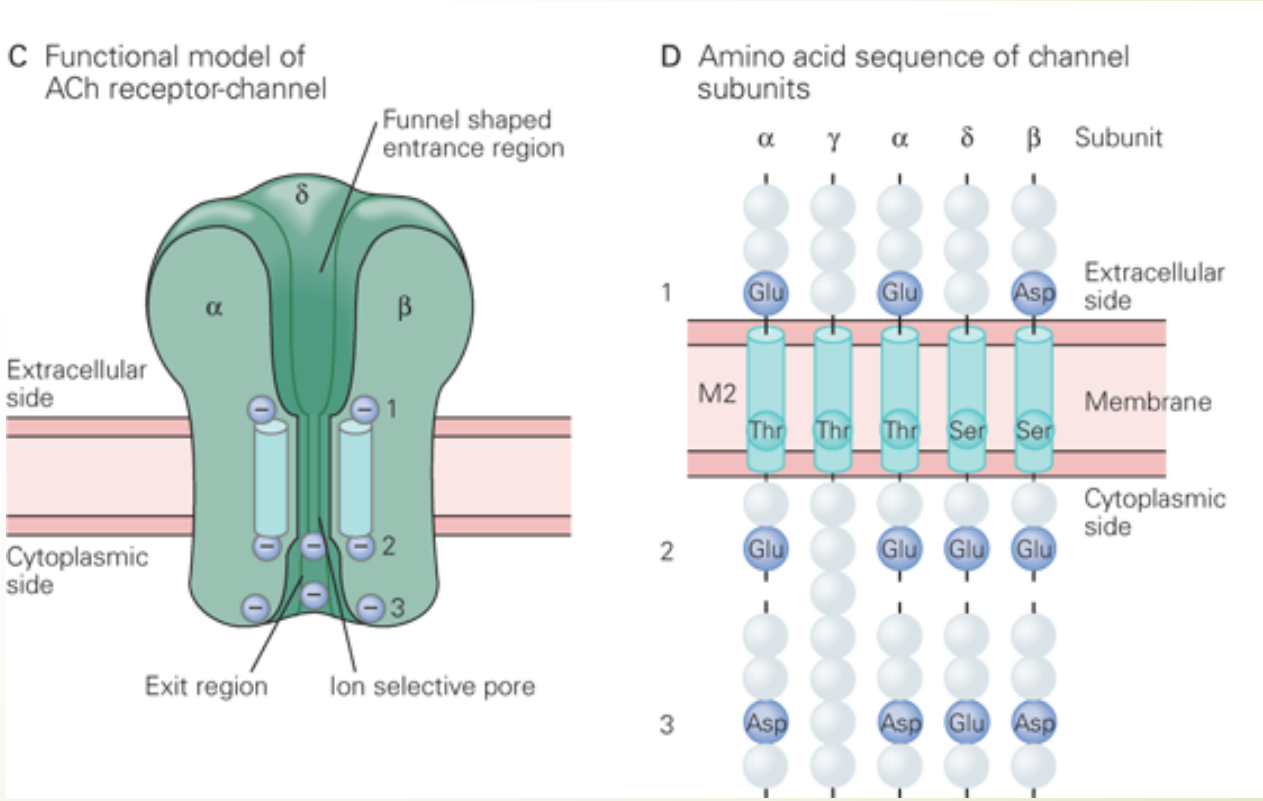
M2 in α and β subunits presents negative charges repeated 3 times forming therefore 3 rings that play an important role in the channel's selectivity for cations. The aligned Ser and Thr residues within the M2 help forming the selectivity filter

Certain local anesthetic drugs block the channels by interacting with one ring of polar Ser residues and two rings of hydrophobic residues in the central region of the M2 helix, midway through the membrane



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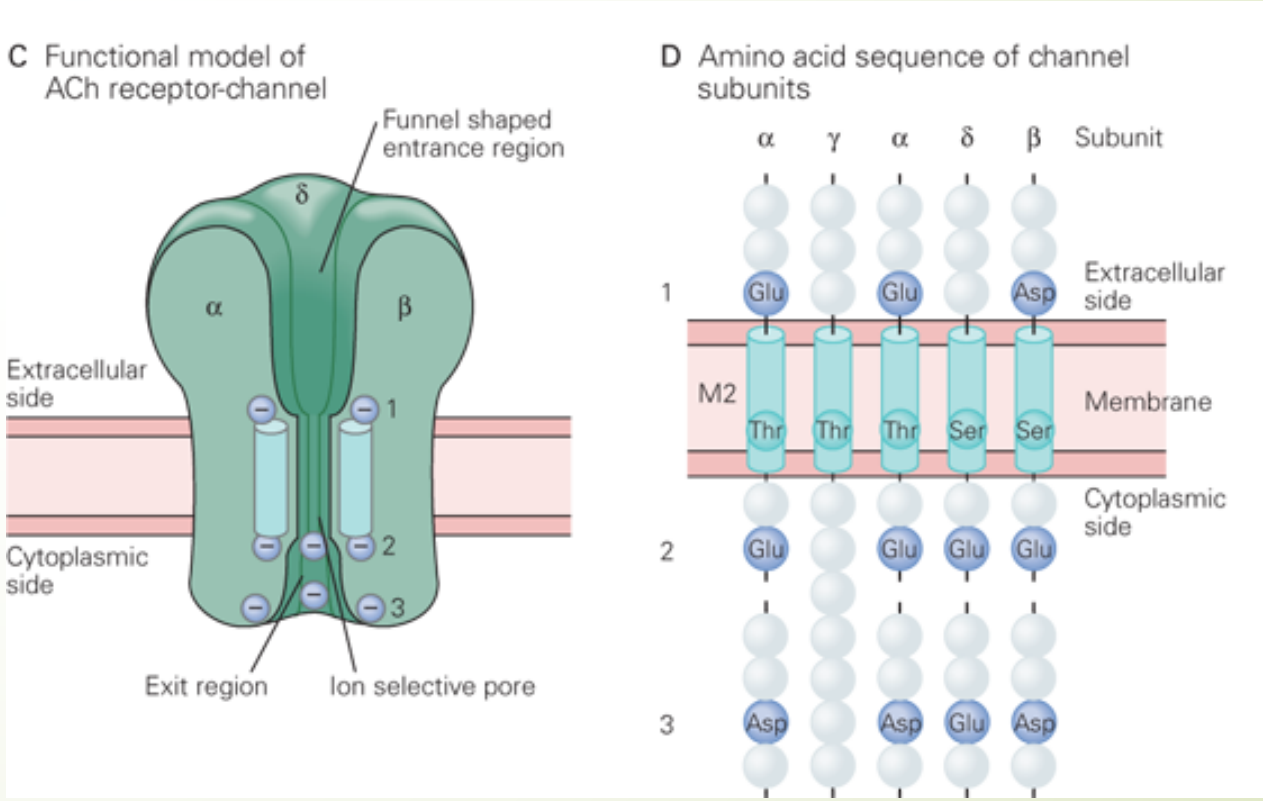
The pore complex can be divided in three regions:
Large **extracellular portion** that contains Ach binding site
Narrow **transmembrane pore** selective for cations
Large **exit region** at the internal membrane surface

extracellular portion is surprisingly large, about 6nm in length. The extracellular end of the pore has a wide mouth approximately 2.5nm in diameter.
Within the bilayer the pore gradually narrows.



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The first milestone in understanding the structure of nAChRs in atomic detail was the elucidation of the X-ray crystal structure of the molluscan Lymnaea stagnalis AChBP (L-AChBP) at 2.7 A ° resolution (Brejc, K., et al, Nature 2001)which is homologous to the Nterm of muscle nAChR:

Like the nAChRs, AChBP assembles into a homopentamer with ligand-binding characteristics that are typical for a nicotinic receptor; unlike the nAChRs, however, **it lacks the domains to form a transmembrane ion channel**.

The molluscan AChR is a soluble protein secreted by glial cells into the extracellular space. At cholinerginc synapses in snails it acts to reduce thesize of the excitatory postsynaptic potential.

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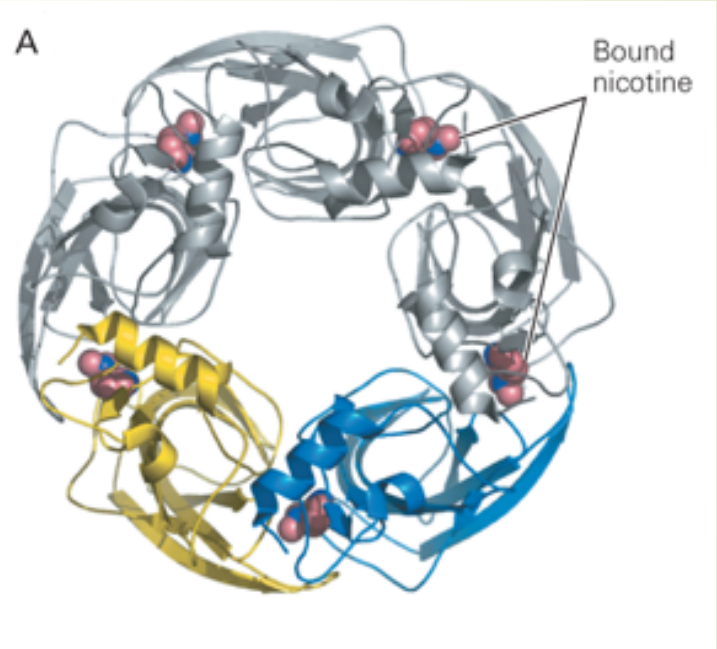
The resolved dimensions of the AChBP (a 62 A ° high cylinder, with a diameter of 80 A ° and a central 18 A ° diameter hole) are in good agreement with those estimated for the Torpedo nAChR-ECD by EM studies. When viewed along the fivefold axis, the AChBP resembles a toy windmill, with blade-like monomers.

The walls of the protein are seen to surround a large vestibule, which presumebly funnels ions toward the narrow transmembrane domain of the receptor.

Each subunit bind one molecule of nicotine at the Ach binding site, located at the interface of two subunits = TOT 5 ACh. This is similar to some neuronal nAChR



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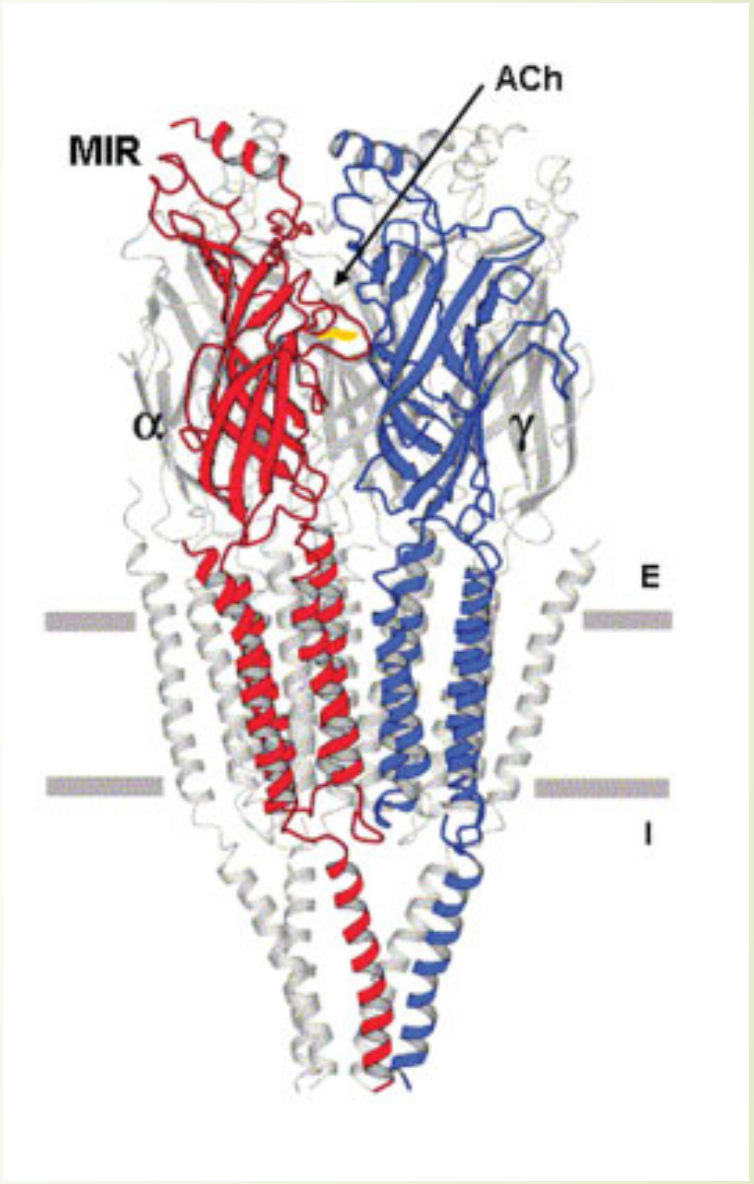
The structure of the AChBP and a subsequent 4 A ° resolution EM study of the Torpedo nAChR (Unwin et al 1995, 2003) were used to create the refined 4 A ° model of the whole receptor in its closed state.

In this model, which constitutes the second milestone in our understanding of nAChR structure in atomic detail, the receptor was shown to have a total length of about 160 A ° normal to the membrane plane and to be divided in three domains:



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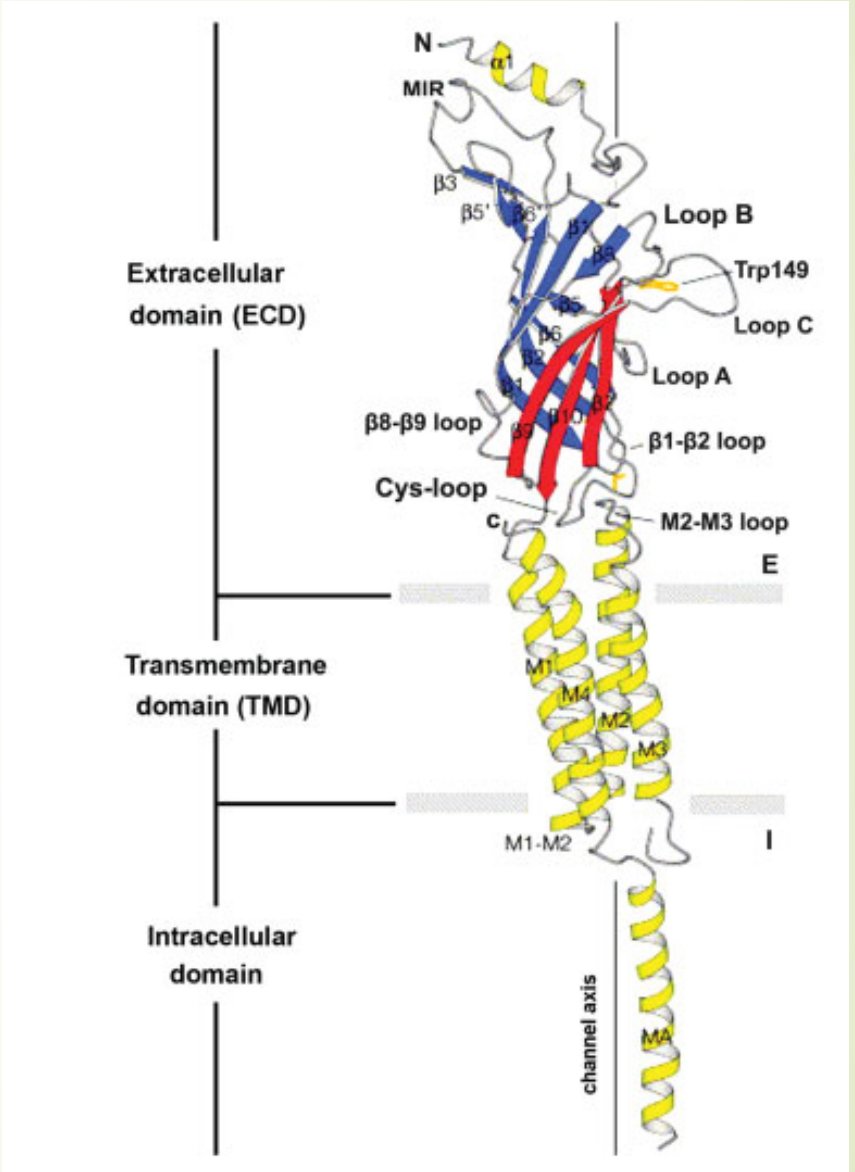
(a) an Nterminal ECD, or ligand-binding domain, which shapes a about 60 A°, a long central vestibule with a diameter of About 20 A ° , and has two binding sites for ACh,
(b) a transmembrane domain (TMD), components of which form about 40 A ° long water-filled narrow pore, containing the gate of the channel,
(c) an intracellular domain, which shapes a smaller vestibule than the extracellular one and which has narrow lateral openings for the ions, and a short C-terminal extracellular tail.

The subunits of the Torpedo nAChR all have a similar size (maximum dimensions 30 A ° x 40 A ° x 160 A °) and the same three-dimension fold.



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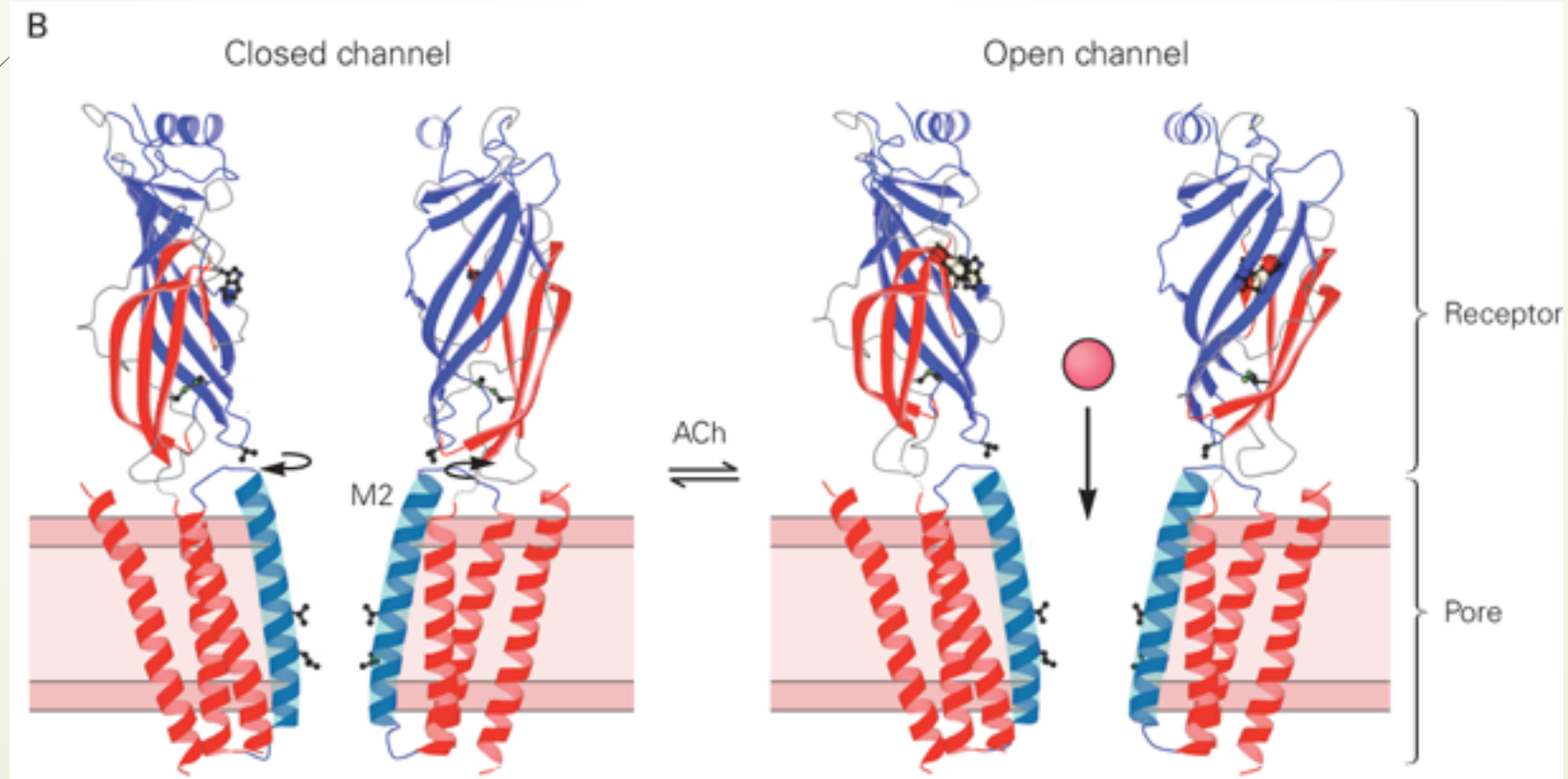
The pore limiting M2 segments are inclined toward the central axis of the channel, so that the pore narrows continuously from the outside of the membrane to inside.



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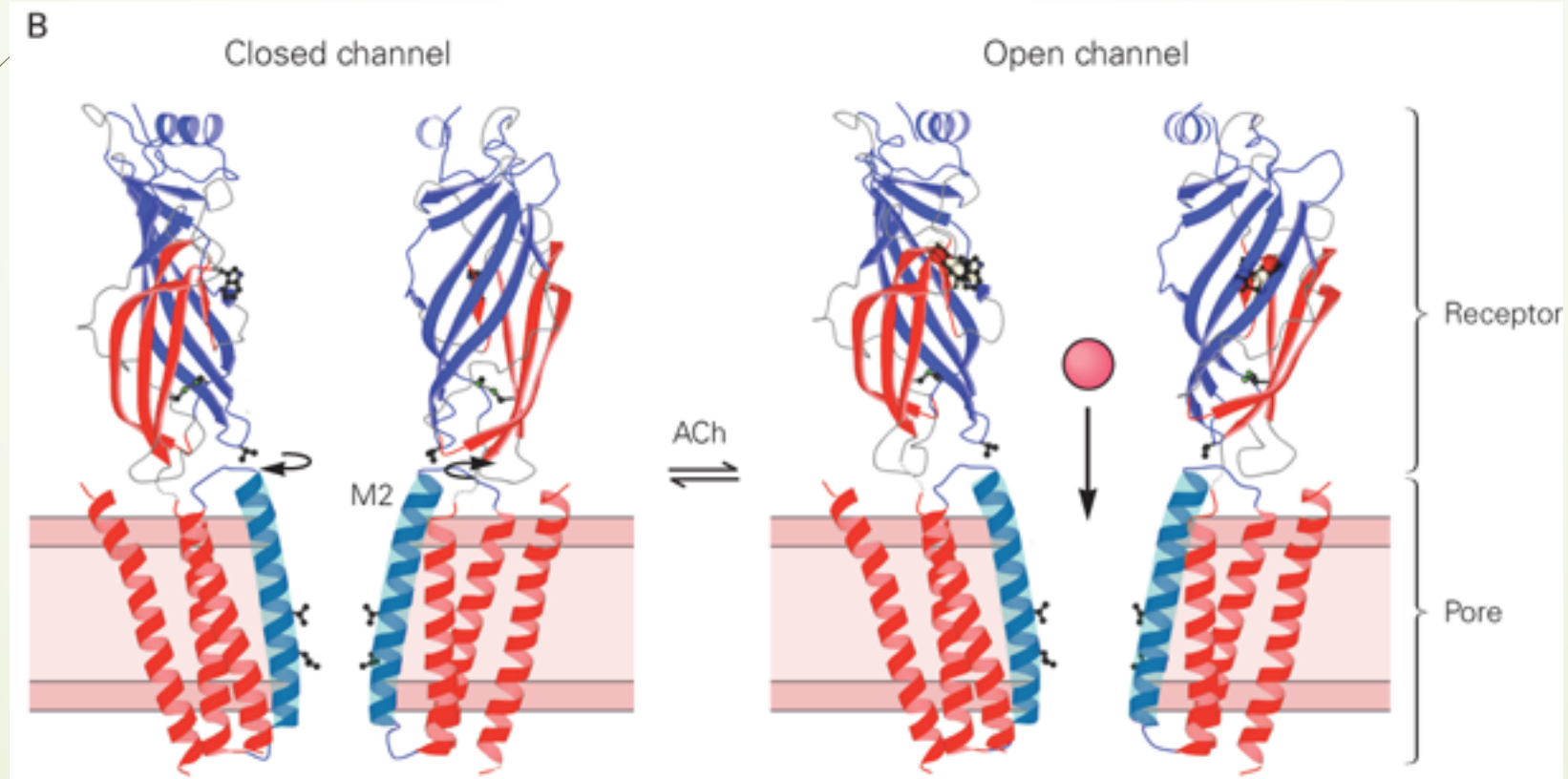
In the closed state a ring of hydrophobic residues is thought to constrict the pore in the middle of the M2 helix to a diameter of less than 0.6 nm. This hydrophobic rings may act as the channel's gate, providing a steric and energetic barrier that prevents ion conduction



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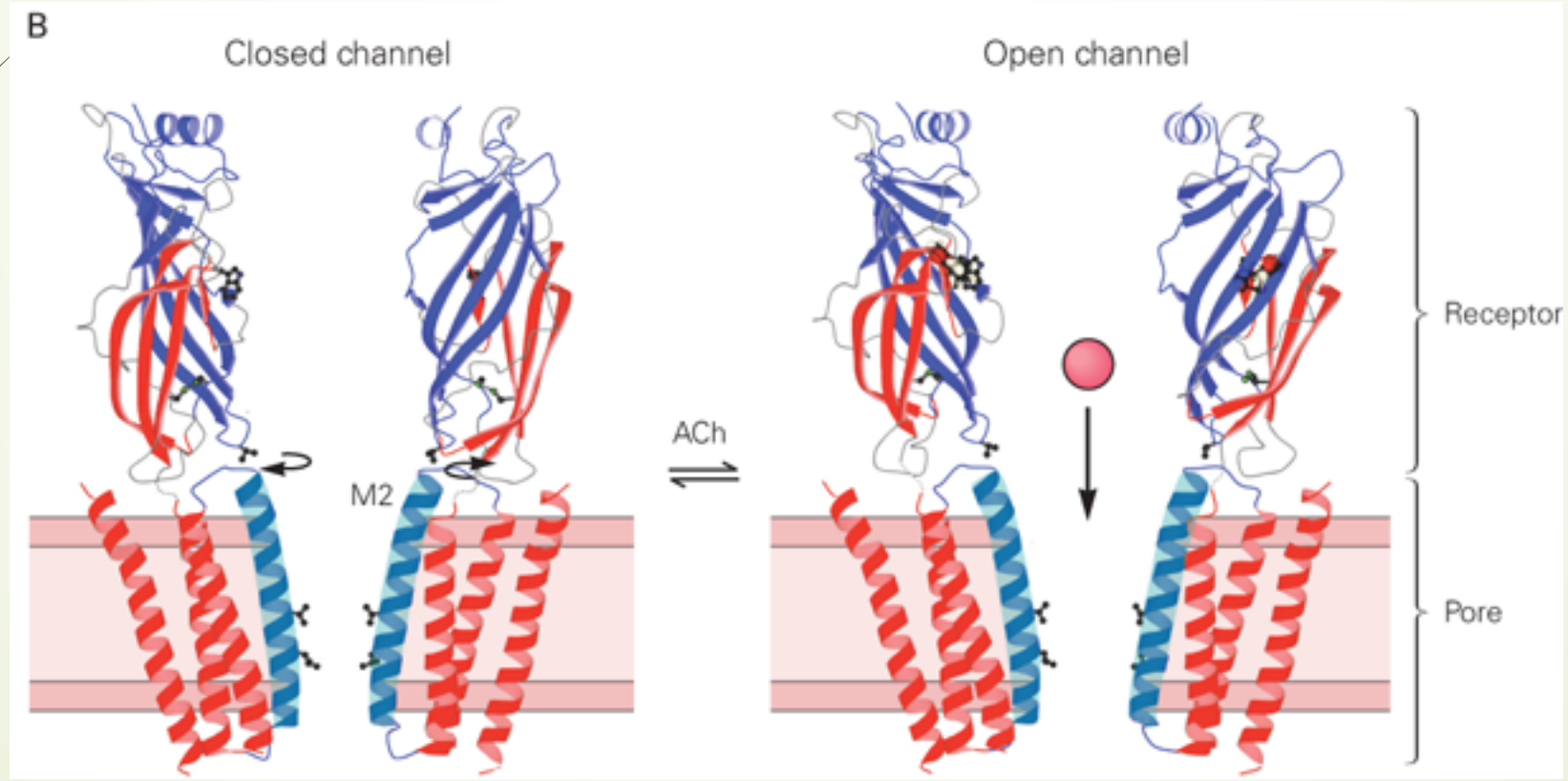
The binding of Ach is thought to trigger a rotation of the extracellular binding domain that is somehow coupled to an opposite rotation in the M2 helixes, widening the constriction in the middle of the M2 to around 0.8 to 0.9nm, enabling ion permeation



Muscle nACHR

Other important informations come from:

- the X-ray crystal structure of the mouse nAChR a1-ECD complexed with a-Btx:

Dellisanti, C. D., Yao, Y., Stroud, J. C., Wang, Z. Z., and Chen, L. (2007) Crystal structure of the extracellular domain of nAChR a1 bound to a-bungarotoxin at 1.94A ° resolution. **Nat. Neurosci.** 10, 953–962.

- the X-ray crystal structure of ELIC and more recently of GLIC, two prokaryotic pentameric LGICs, which are cation-selective channels and considered to be the ancestors of nAChRs. ELIC-ECD and GLIC-ECD have both similar structure with the AChBP and the Torpedo nAChR-ECDs, but lack the N-terminal a-helix and the Cys-loop:

Hilf, R. J. and Dutzler, R. (2008) X-ray structure of a prokaryotic pentameric ligand-gated ion channel. **Nature** 452, 375–379.

Hilf, R. J. and Dutzler, R. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. **Nature**, 2009.

Bocquet, N., Nury, H., Baaden, M., Le Poupon, C., Changeux, J. P., Delarue, M., and Corringer, P. J. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. **Nature**, 2009