Tampere University

Cellular Biophysics – Cell Signaling

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Contents of the lecture

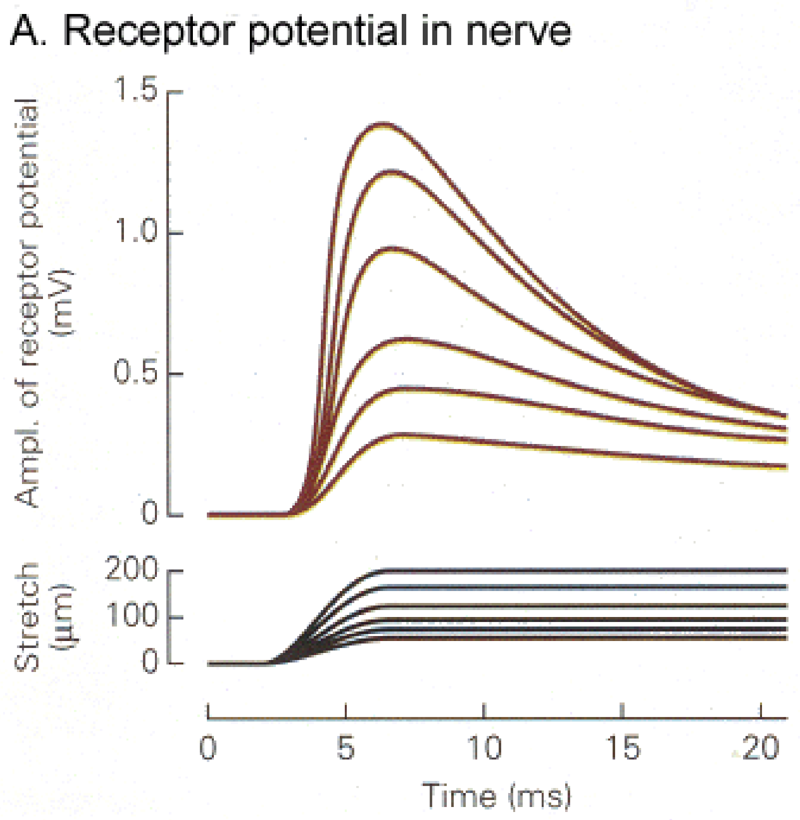
- Action potential
- Voltage-gated ion channels
- Gap junctions
- Ionotropic and metabotropic receptors
- Neuronal communication
- Cardiac action potential

Graded potentials - recap

- Electrical signals in cells: responses to stimuli which change the resting membrane potential

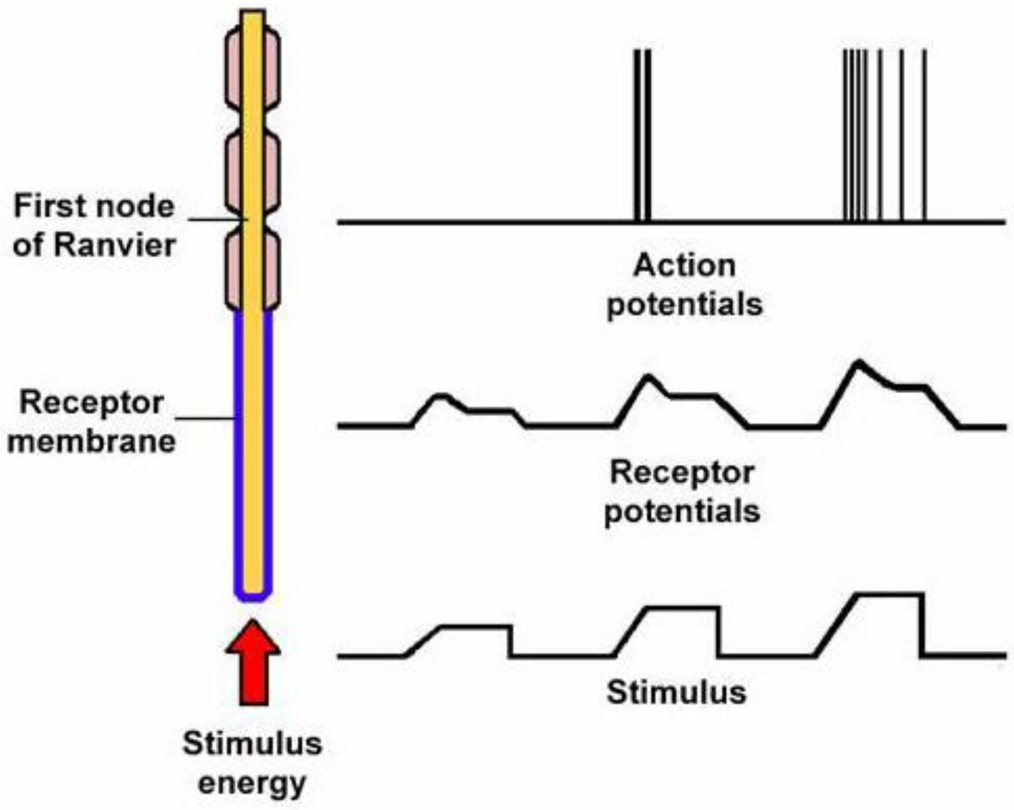
- **Receptor potentials** are due to activation of sensory neurons by external stimuli (light, sound, heat, stretch...)

    - **Graded potentials**: magnitude of the sensory stimulus coded to the amplitude of the change in the membrane potential



Action potentials (AP) - recap

- Constant duration
- Amplitude independent of the magnitude of the stimulus
- Stimulus strength / magnitude coded to the frequency of action potentials
- Key players: voltage-gated ion channels



Voltage-gated ion channels

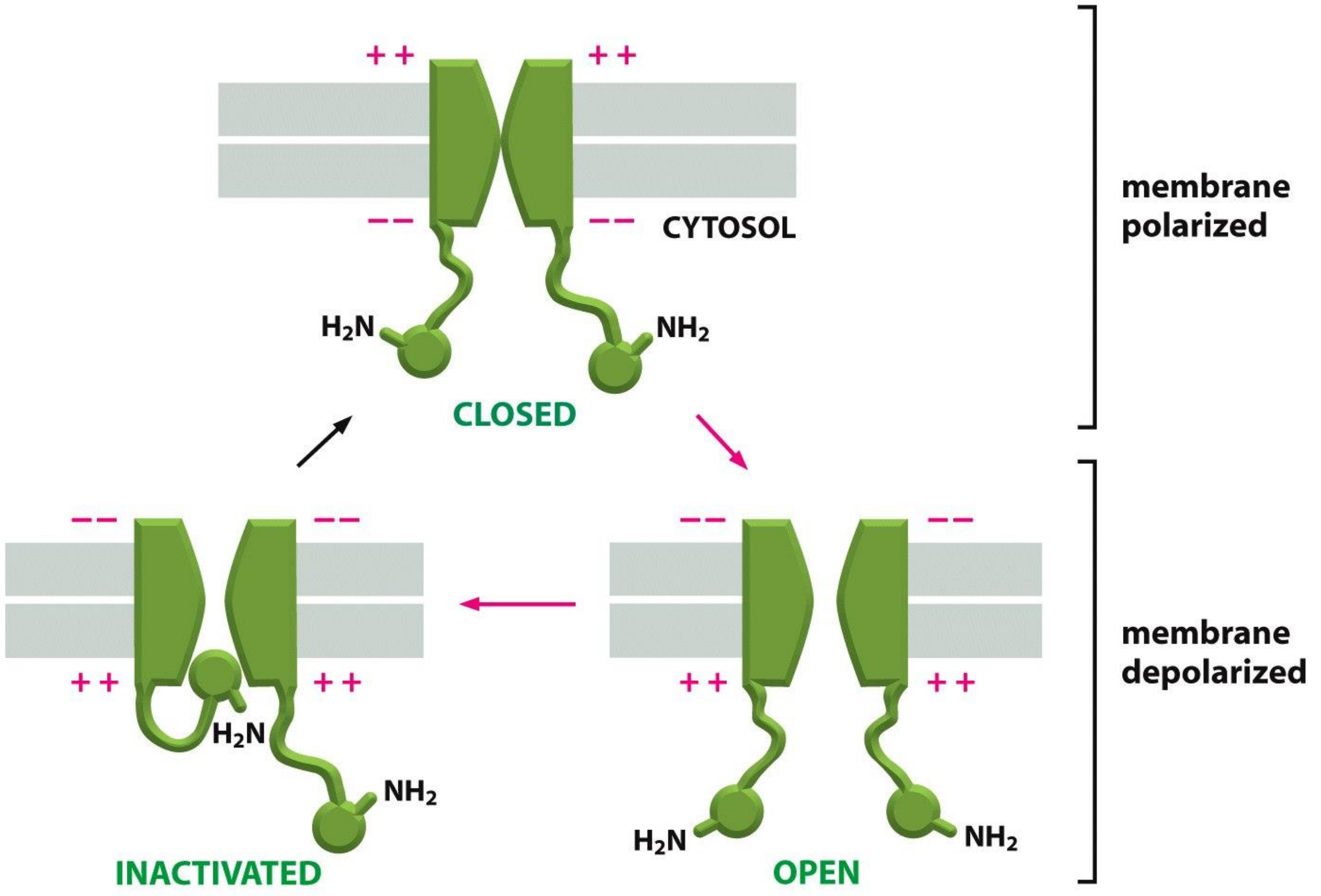
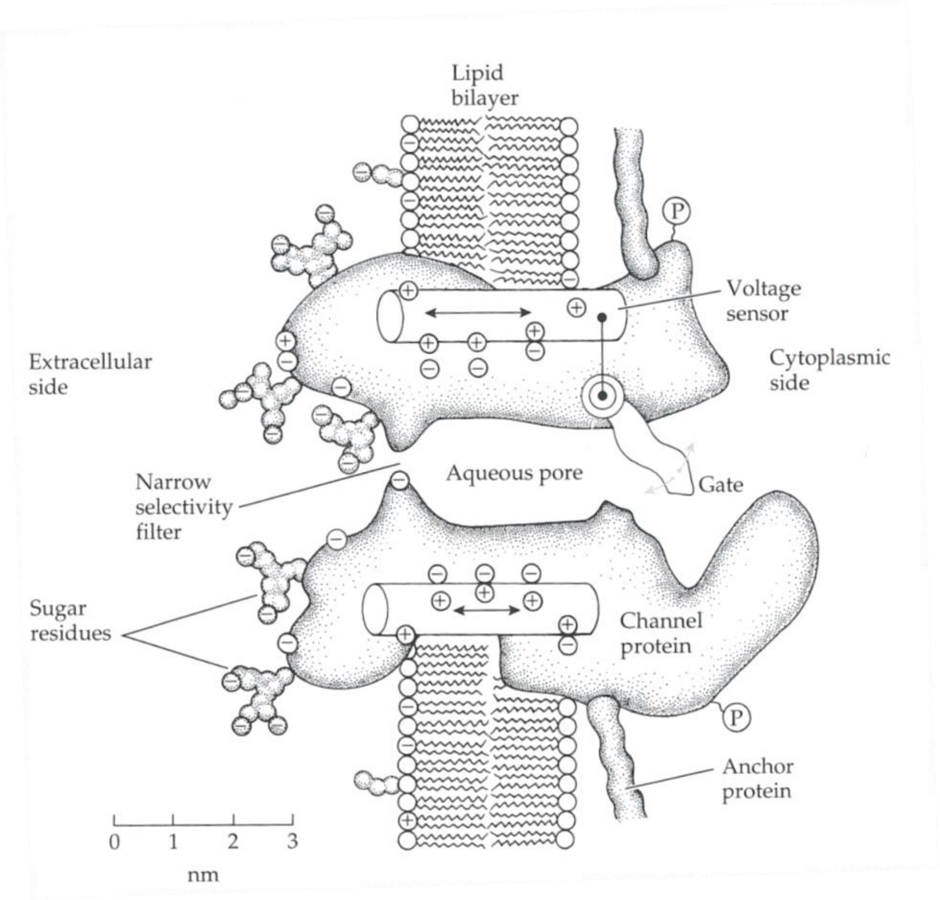


Figure 11-31 *Molecular Biology of the Cell* (© Garland Science 2008)

Depolarized: more positive potential

Hyperpolarized: more negative potential

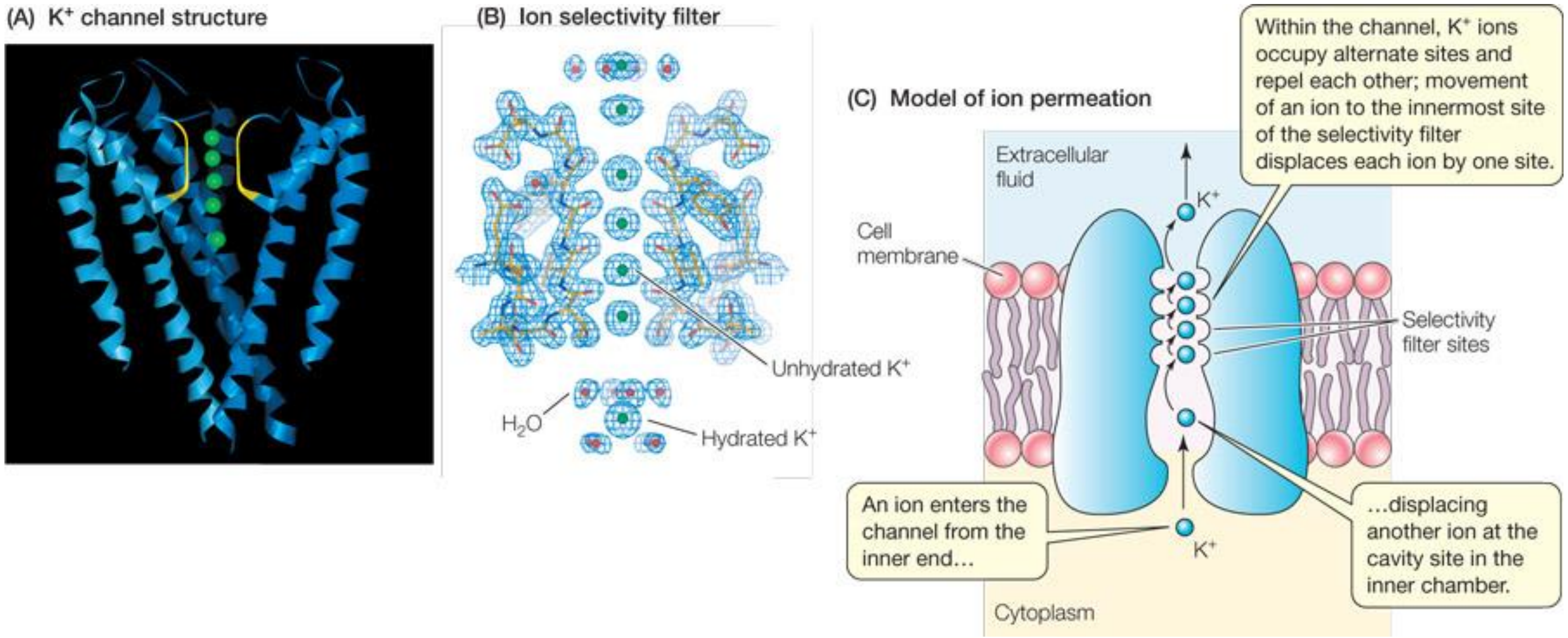
Voltage-gated ion channels



B. Hille: Ion Channels of Excitable Membranes, 3rd Ed., Sinauer

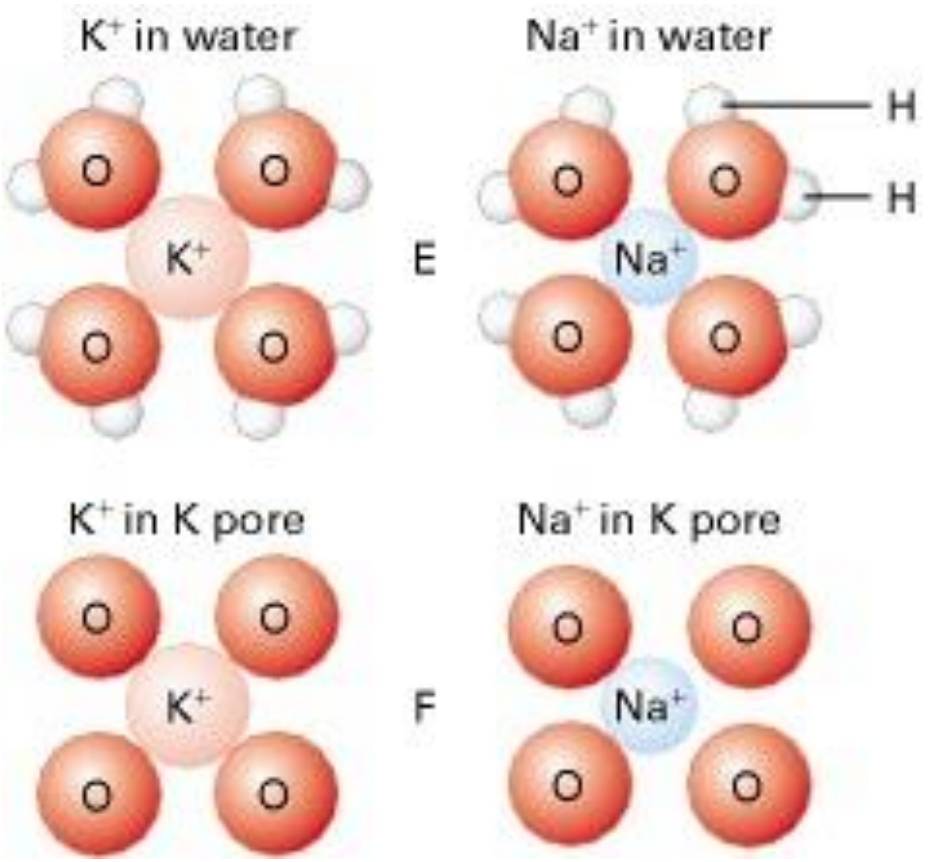
- Large transmembrane proteins
- Narrow selectivity filter in the pore pathway makes the channel ion-specific
- Voltage sensing portion of the ion channel highly positively charged
  - Responsible for detecting changes in transmembrane potential => trigger channel the opening or closing

Ion selectivity in voltage-gated K$^+$ channel



Animal Physiology, 4th ed

Ion channel selectivity



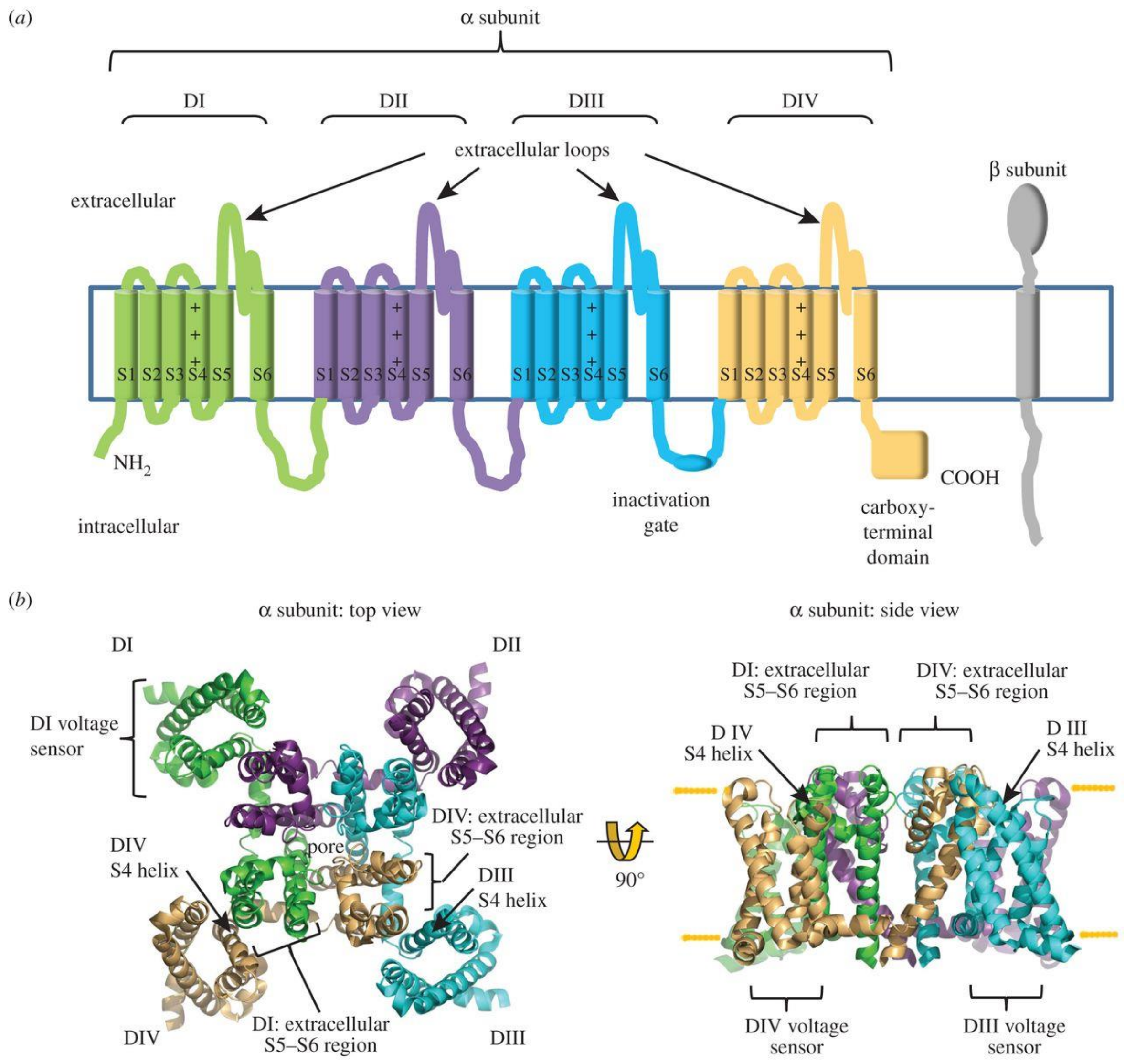
“K^+ ions (hydrated in solution) lose their bound water molecules as they pass through the selectivity filter and become coordinated to four backbone carbonyl oxygens in the channel-lining loop of each P segment.“

“Na^+ ions, being smaller, cannot perfectly coordinate with these oxygens. They pass through the channel only rarely.“

Molecular Cell Biology. 4th ed.

Voltage-gated ion channel structure: Na^+ channel as an example

- Four transmembrane domains (DI-DIV) create the pore
  => α-subunit
- Each domain has 6 transmembrane amino acid helices (S1-S6); S1-S4 serve as voltage sensing region; S5&S6 form the ion selective pore
- Intracellular loop makes the inactivation gate
- Modulatory β-subunit modulates channel functioning

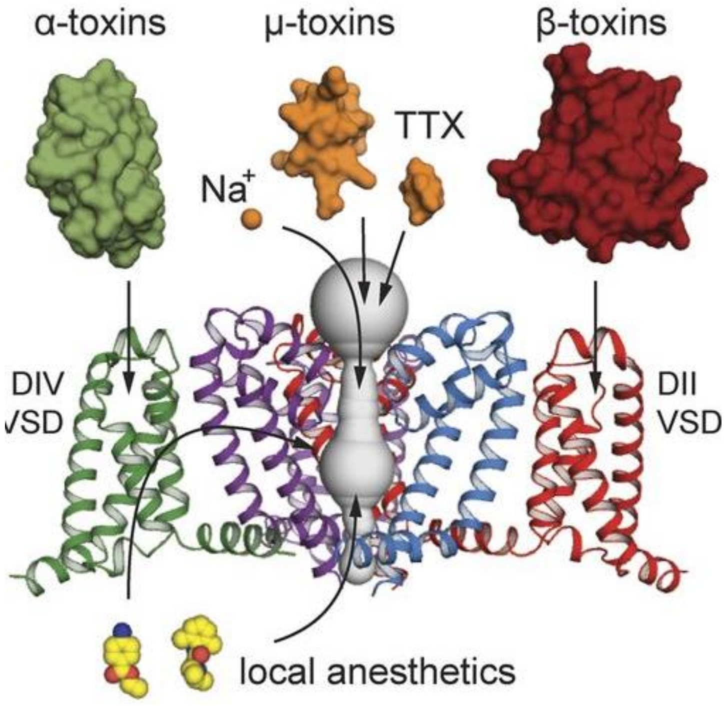


Namadurai et al. 2014, Open Biology

Opening and closing of voltage-gated Na^+ channels

Bagneris et al. JGP, DOI: 10.1085/jgp.201411242

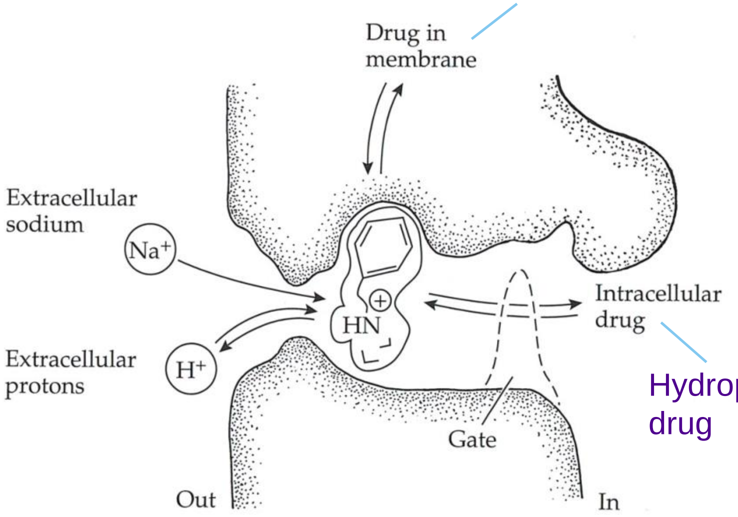
Blocking channels with drugs: Na^+ channel as an example



Ahern et al. 2016, J. Gen. Phys.

Local anesthetics:

Hydrophobic drug

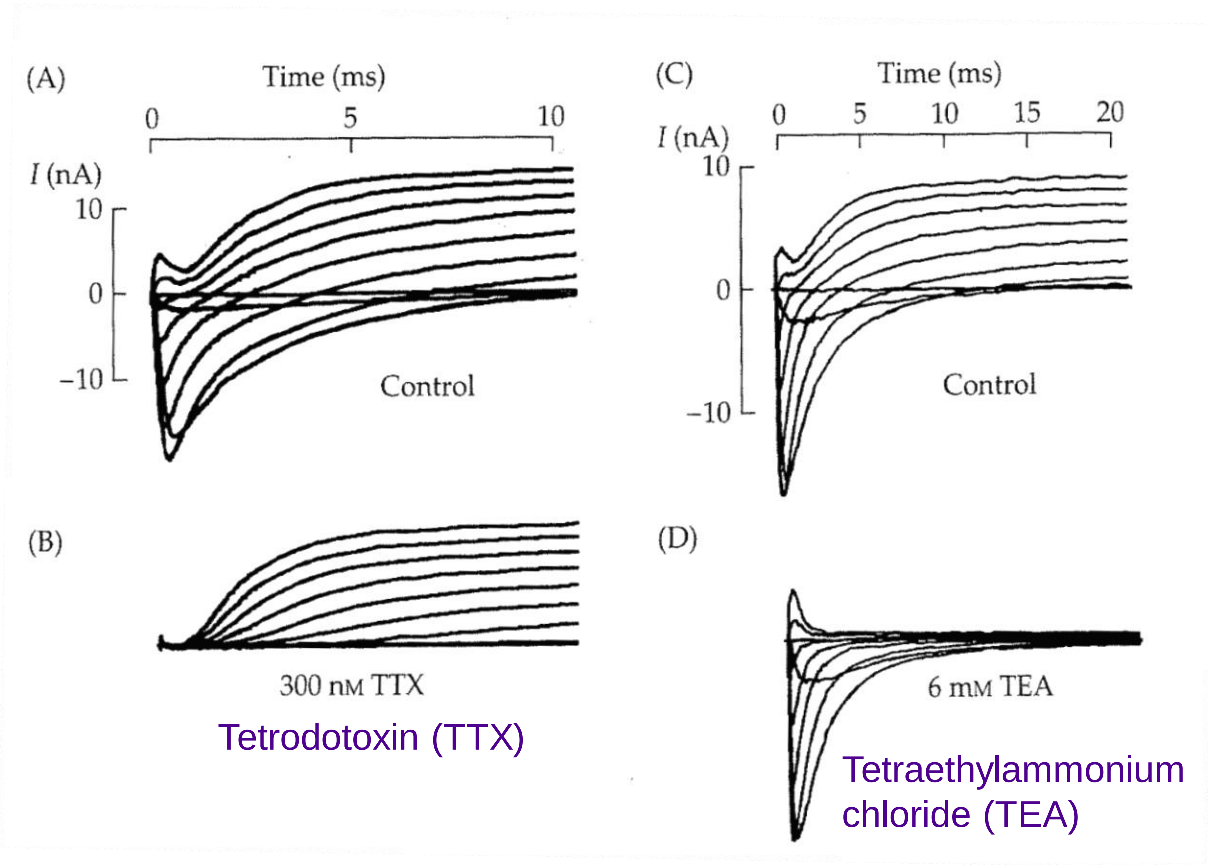


Hydrophilic drug

B. Hille: Ion Channels of Excitable Membranes, 3rd Ed., Sinauer

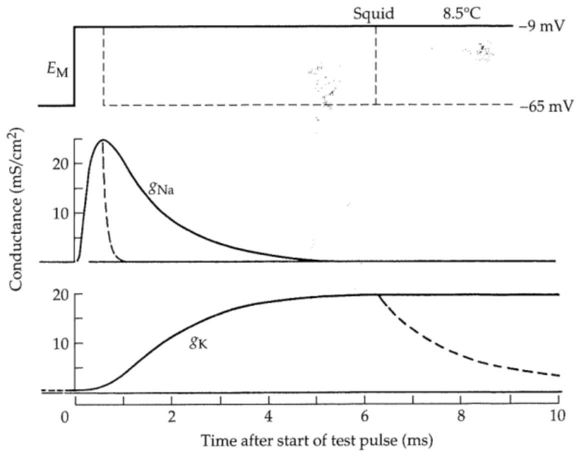
Important e.g. in anesthesia, treatment of epilepsy, bipolar disorder, chronic pain, and cardiac arrhythmia.

Blocking Na⁺ and K⁺ channels in patch clamp recordings



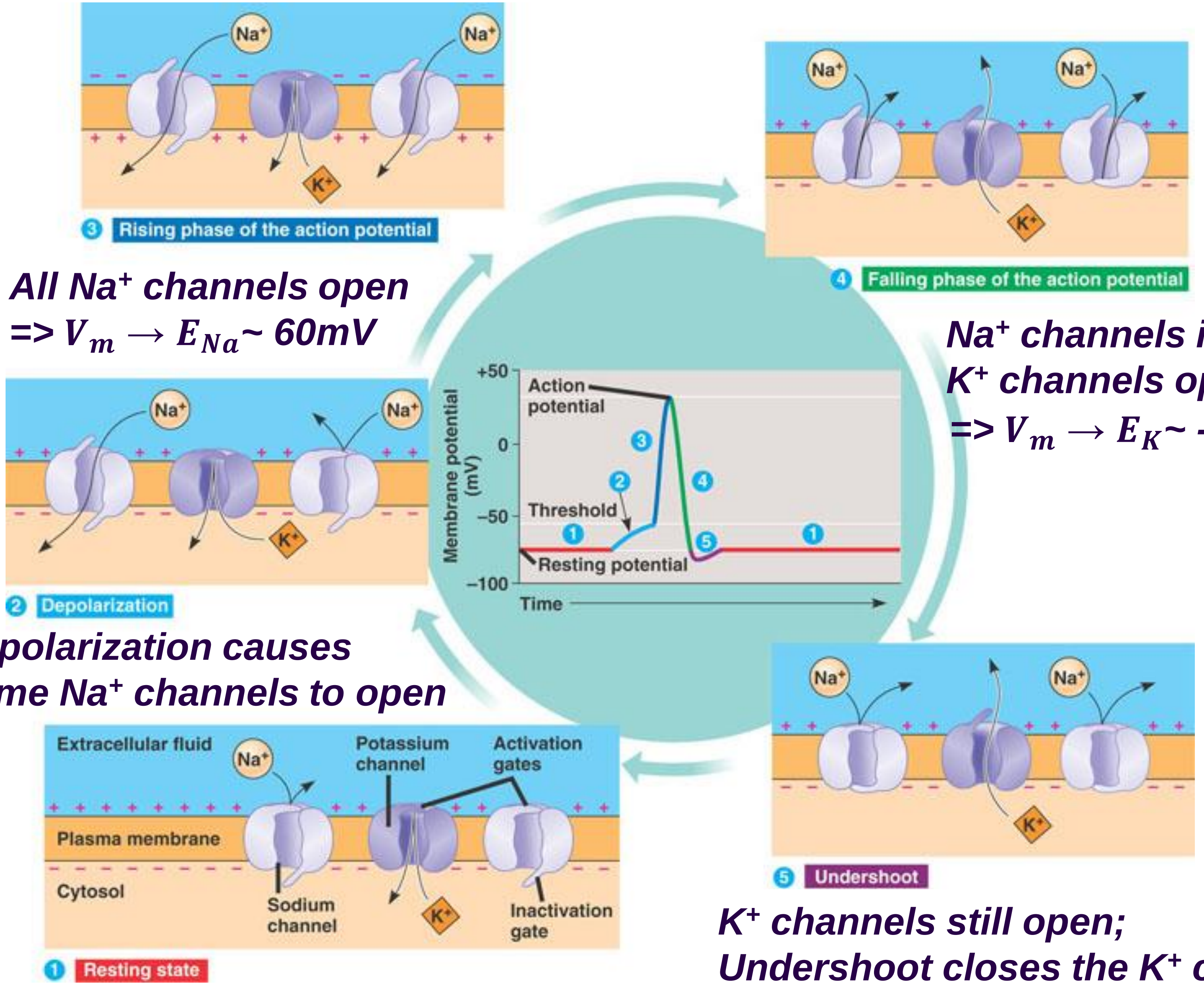
Tetrodotoxin (TTX)

Tetraethylammonium chloride (TEA)



Note:
No inactivation gate in voltage-gated K^+ channels

Voltage-gated channels in action potential generation



All Na$^+$ channels open
=> $V_m \rightarrow E_{Na}$~ 60mV

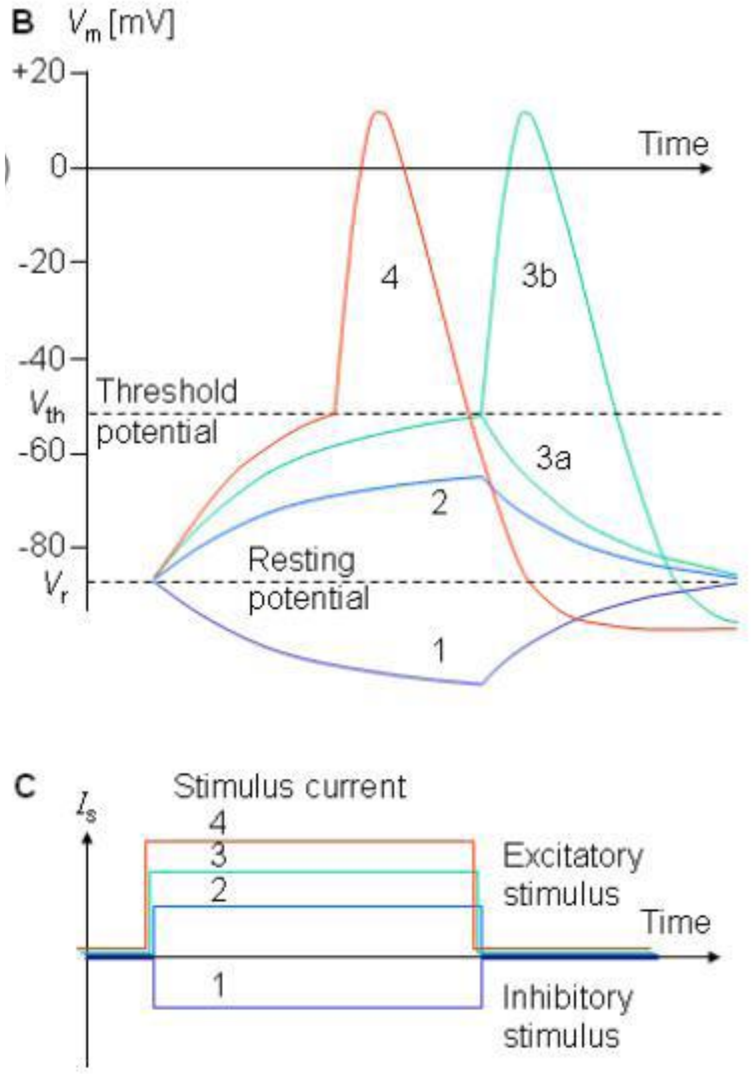
Na$^+$ channels inactivate;
K$^+$ channels open
=> $V_m \rightarrow E_K$~ -80mV

Depolarization causes
some Na$^+$ channels to open

K$^+$ channels still open;
Undershoot closes the K$^+$ channels

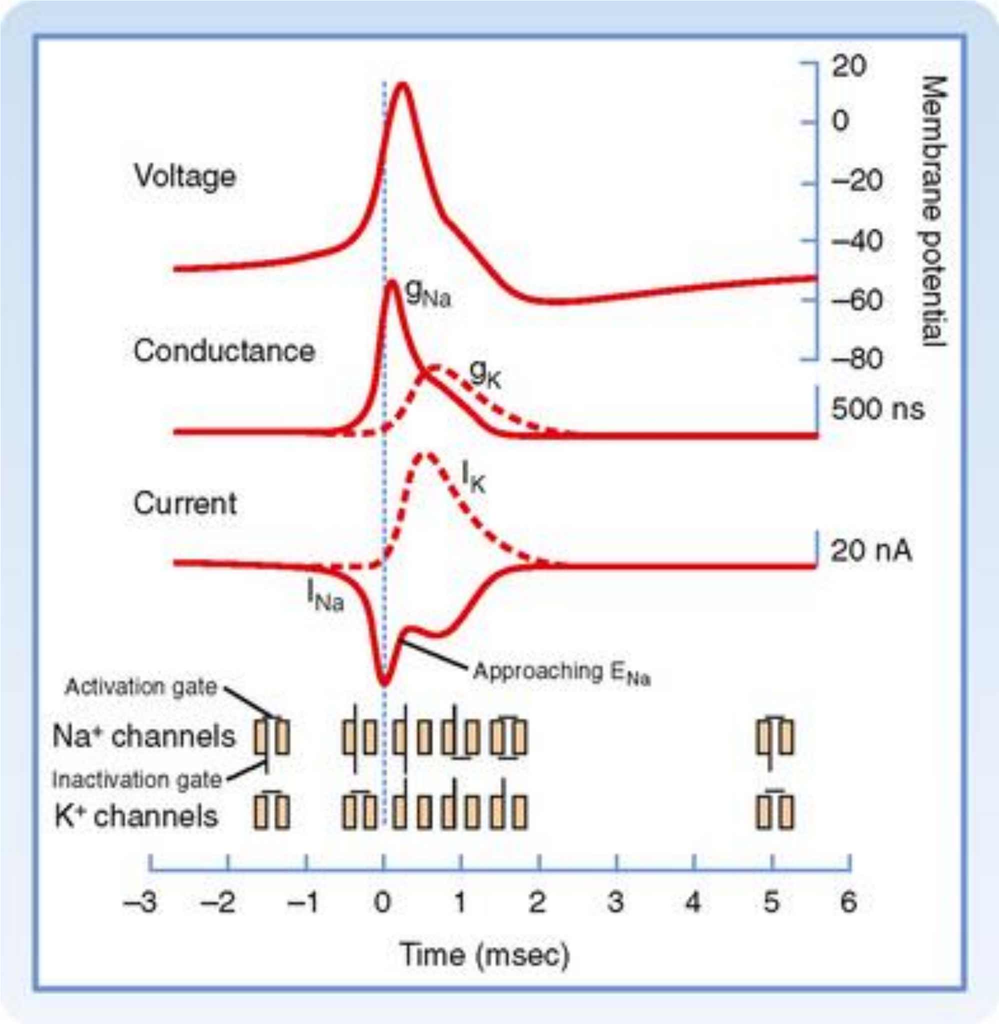
Threshold for stimulus

- AP initiates at a certain membrane potential called **treshold**
- At rest, there is outflow of K^+
- Membrane depolarization increases inflow of Na^+
- This inflow further depolarizes the cell
- If stimulus brings inflow of Na^+ to the same levels with K^+ outflow, the threshold is reached
- AP is fired if there is net gain of Na^+ ions inside the cell triggering the positive feedback loop

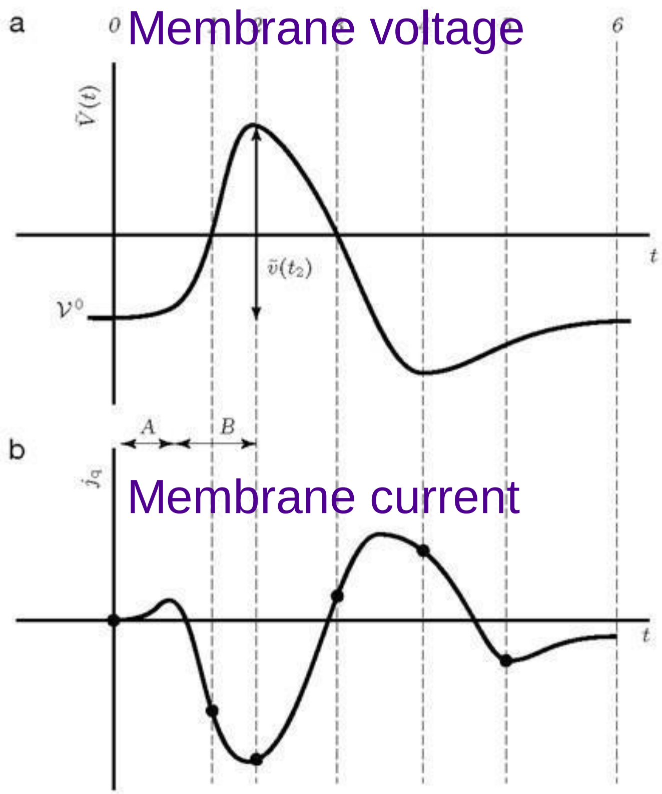


http://www.bem.fi/book/ Fig 2.8

Action potential: Changes in membrane voltage and current



Membrane voltage (a) vs.
total membrane current (b)



Squires et al: Fundamental Neuroscience, 2nd ed. San Diego, CA, Academic Press, 2002.

Action potentials (continued)

- Depolarization and repolarization occur via diffusion, they do not require active transport
- Once AP completed, Na^+/ K^+-ATPase pump extrudes Na^+ and recovers K^+
- All or none:
  - When threshold reached, maximum potential change occurs
  - Duration is the same, channels open only for a fixed period of time (Na^+ channels transient type, K^+ channels sustained type but closed by hyperpolarization of the membrane)
- Coding for Stimulus Intensity:
  - Increased frequency of AP indicates greater stimulus strength

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Action potentials (continued)

- Action potentials occur in several types of animal cells, called excitable cells
  - Neurons, muscle cells, endocrine cells

- In neurons, they play a central role in cell-to-cell communication.
  - APs in neurons are known as nerve impulses or spikes
  - The temporal sequence of action potentials generated by a neuron is called a spike train
  - A neuron that emits an action potential is said to fire.

- In muscle cells, an action potential is the first step in the chain of events leading to contraction.

- In beta cells of the pancreas, APs are suggested to provoke the release of insulin.

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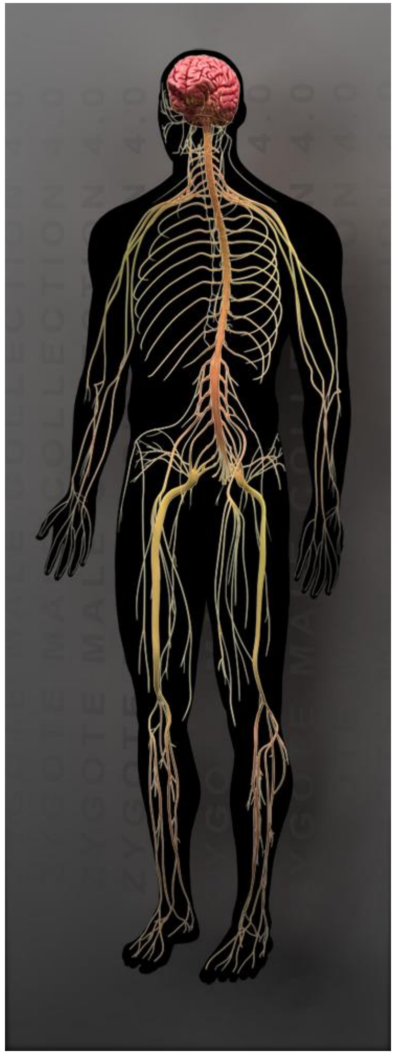
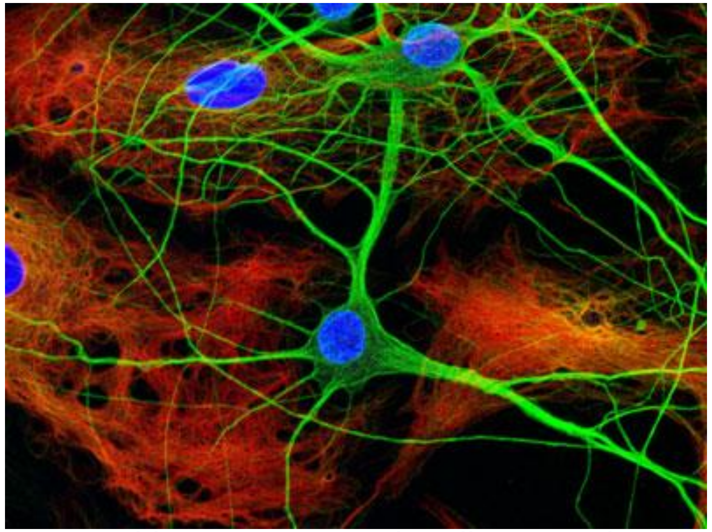
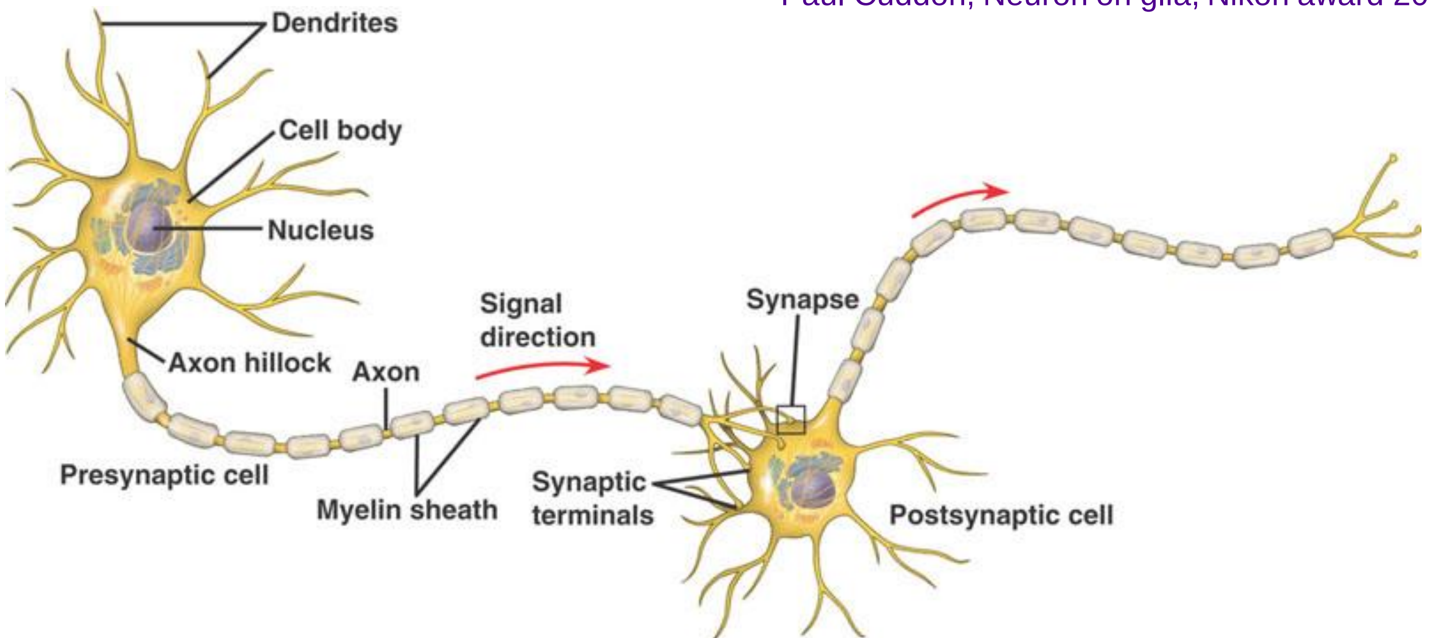
Neuronal communication



- Our nervous system is based on neuronal communication
- In neuronal cells, APs propagate down the axons
- Axons can be long, in spinal motor neurons even > 1 m



Paul Cuddon, Neuron on glia, Nikon award 2005

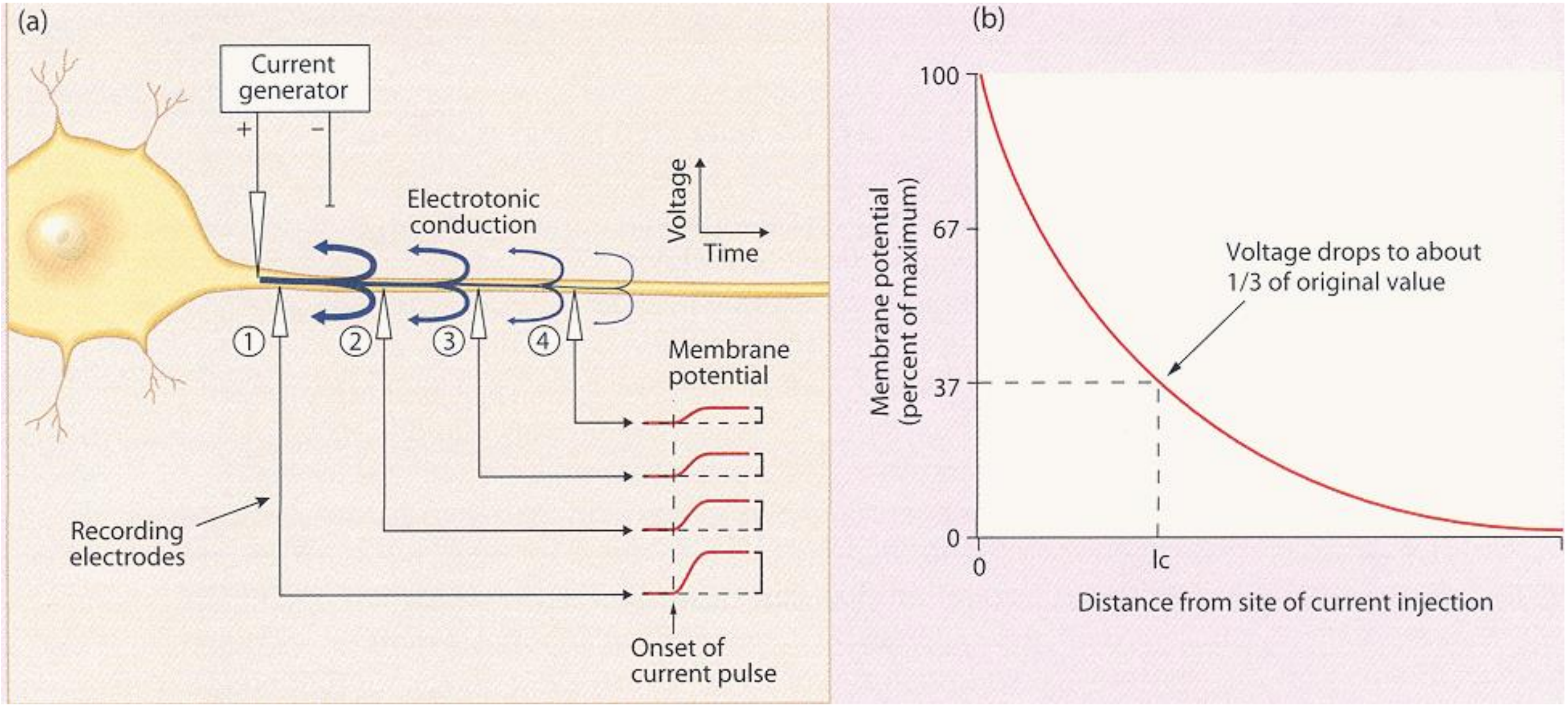


Conduction velocity is critical!

- Rate of AP conduction limits the flow of information within the nervous system and impairments in it cause sever diseases

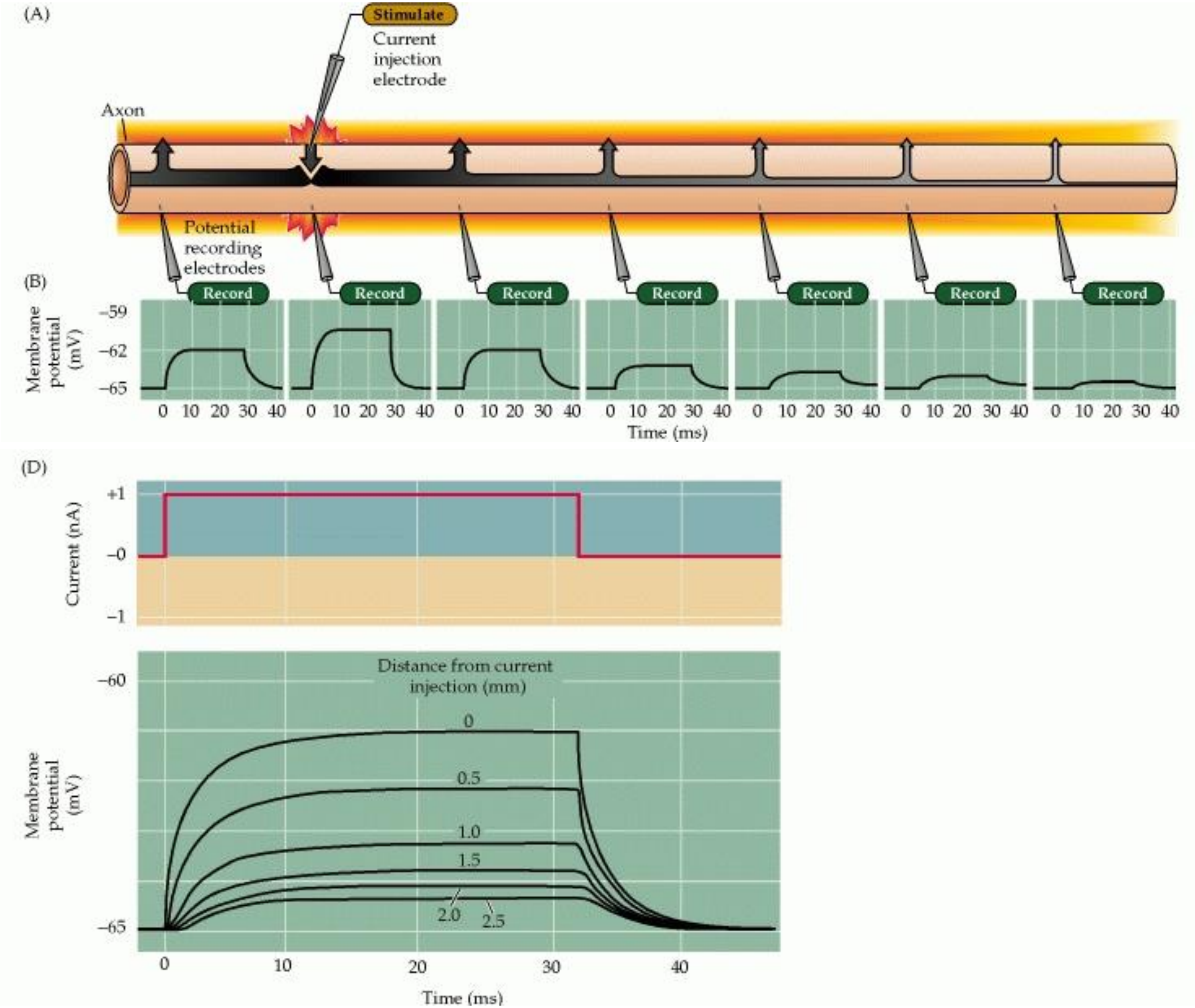
- Depends on passive and active flow of current

Passive flow of current

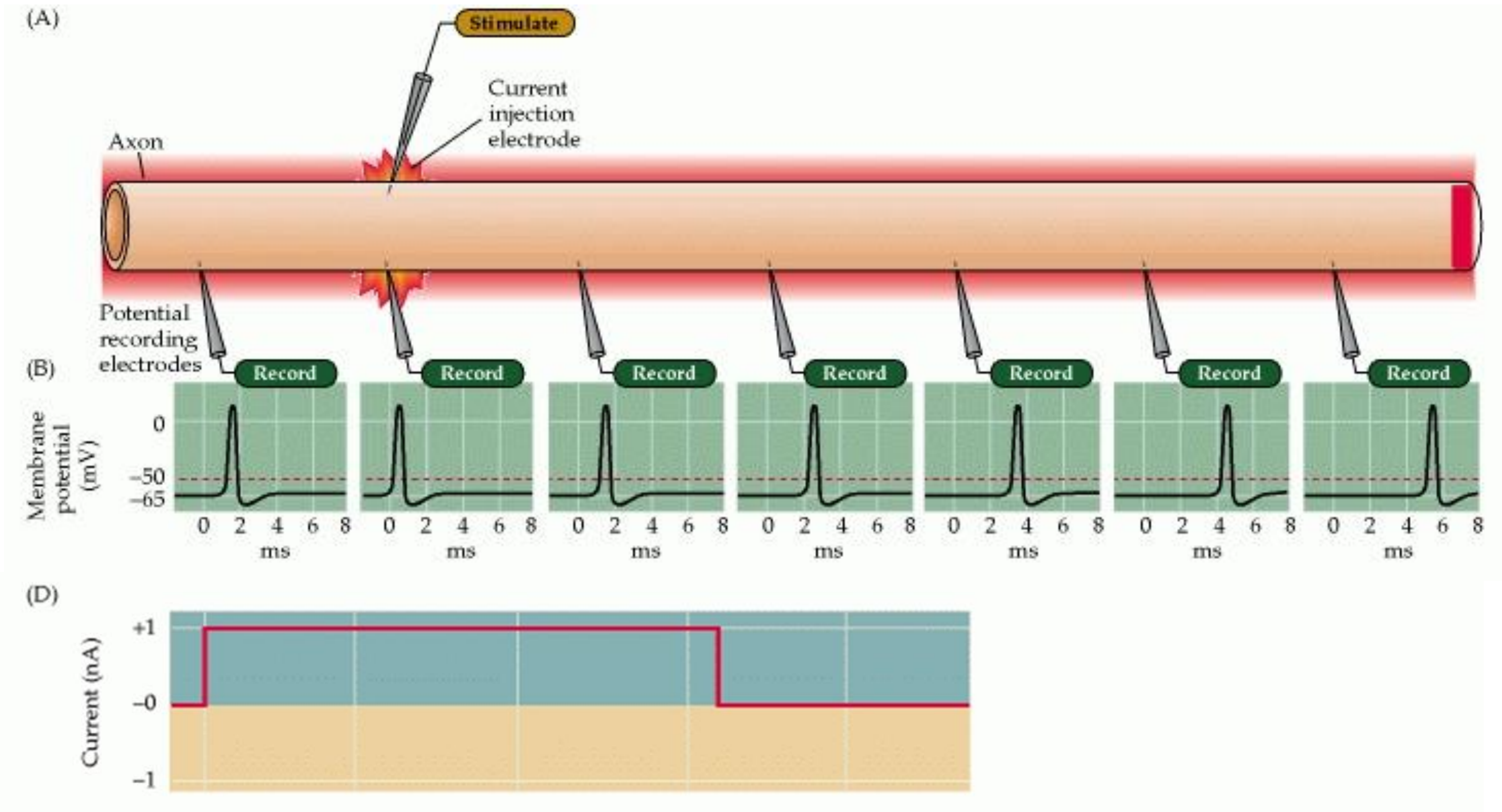


- Axon is a poor electrical conductor
- With increasing distance from the stimulus, the amplitude of the potential change decays exponentially

Passive flow of current



Active flow of current (=AP propagation)



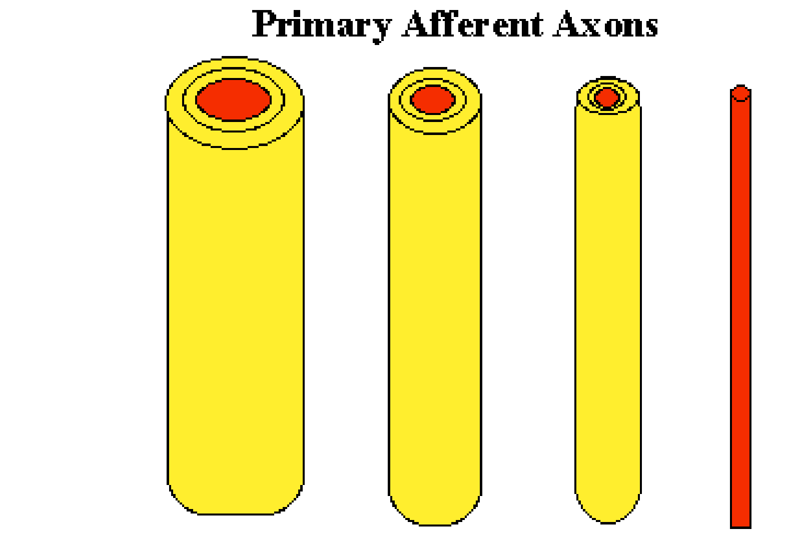
- Amplitude constant
- Signal can travel long distances unchanged
- How is this possible?

Systems to increase conduction velocity

- Increase the axon diameter => increases the internal resistance and improves passive current flow
  - Only a limited possibility due to practical issues with large axon diameters
- Insulate the axonal membrane => reduces the ability of current to leak out of the axon and improves passive current flow
- Insulation: wrapping axons in myelin (layers of glial membranes)

Myelinated axon has high conduction velocity

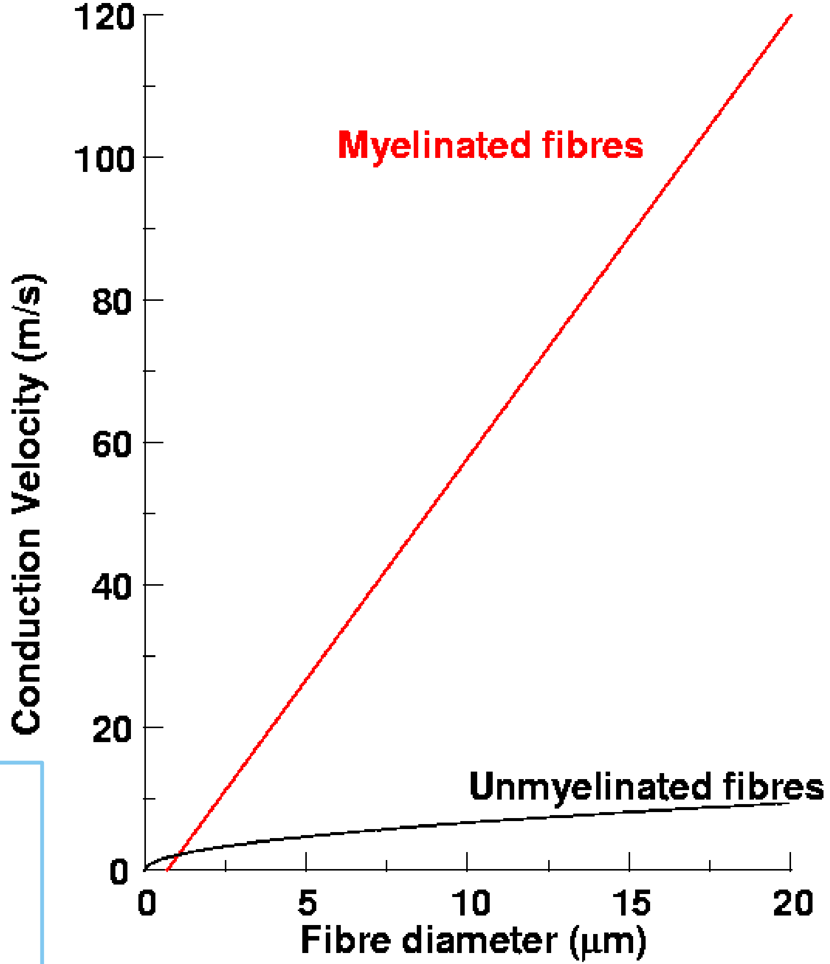
Effect of axon diameter



Axon Type	Aα	Aβ	Aδ	C
Diameter (µm)	13-20	6-12	1-5	.2-1.5
Speed (m/s)	80-120	35-75	5-35	.5-2.0

- *Impairments in myelination lead to sever diseases such as multiple sclerosis (MS disease)*

Effect of myelination



Systems to increase conduction velocity

- Increase the axon diameter => increases the internal resistance and improves passive current flow
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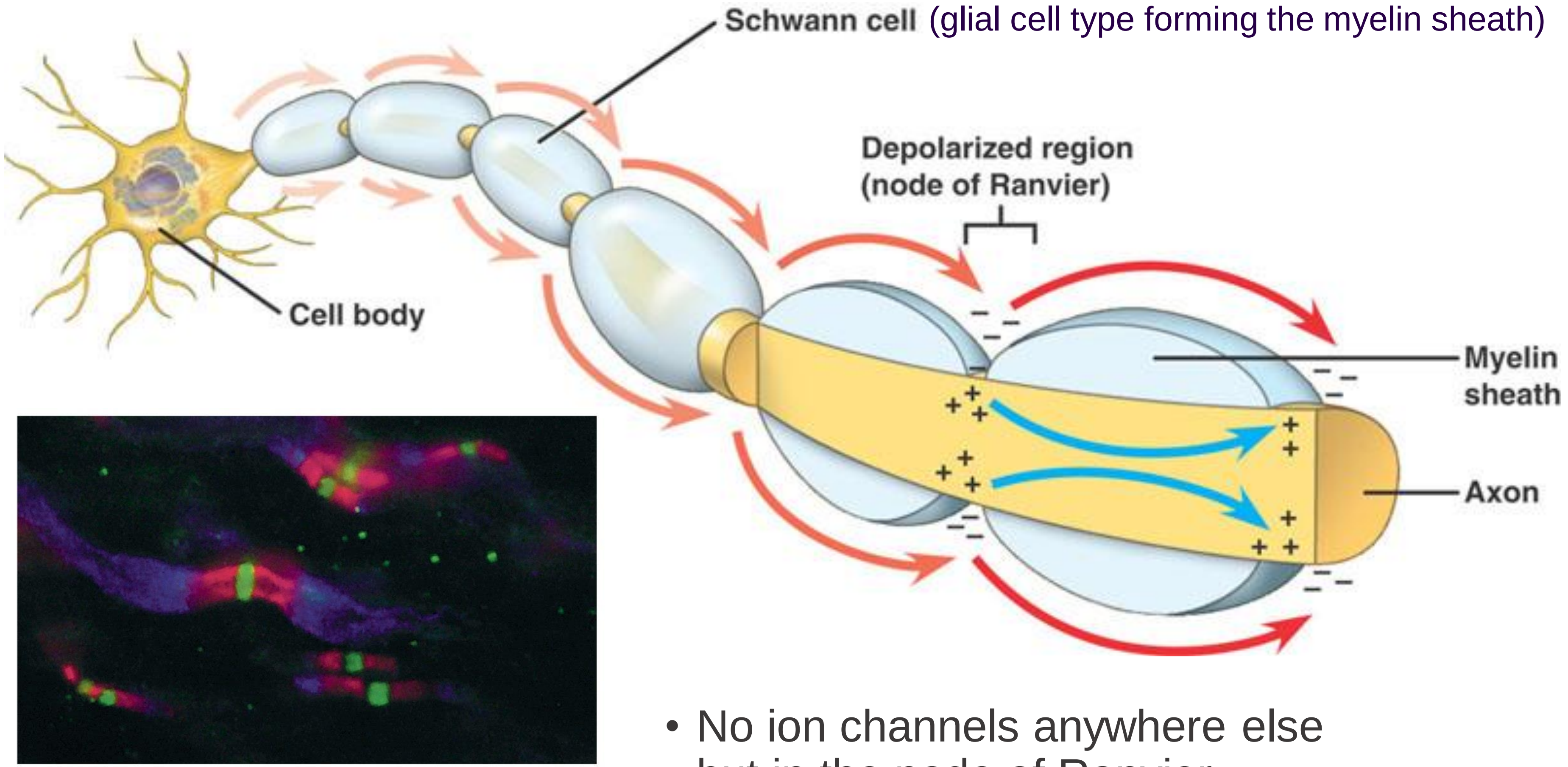
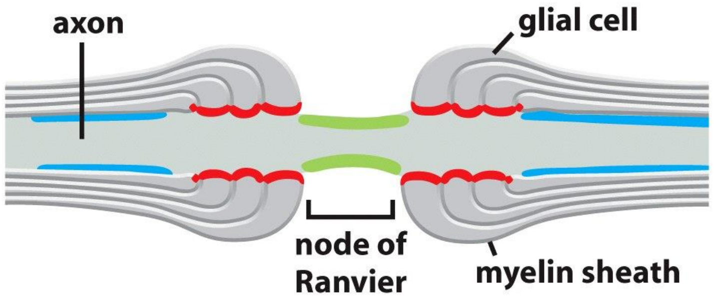
- Insulate the axonal membrane => reduces the ability of current to leak out of the axon and improves passive current flow
- Insulation: wrapping axons in myelin (layers of glial membranes)
- Final trick: disconnect insulation at specific points => time-consuming process of generating APs occur only at these gaps in insulation and active current flow improves (APs “jump” from node to node)

Myelinated axon





- No ion channels anywhere else but in the node of Ranvier
- These nodes boost the signal and prevent it from decaying

Figure 11-32c *Molecular Biology of the Cell* (© Garland Science 2008)

- How does the action potential as an electrical signal travel from one neuron to another?

Cell-to-cell signaling in general

- Paracrine signaling:
  - Cells within an organ secrete regulatory molecules that diffuse through the extracellular matrix to nearby target cells
- Endocrine signaling:
  - Cells of endocrine glands secrete hormones into extracellular fluid
- Synaptic signaling:
  - Means by which neurons regulate their target cells
- Gap junctions:
  - Signal can directly travel from one cell to the next through fused membrane channels
- *For a target cell to respond to a hormone, neurotransmitter, or paracrine regulator, it must have specific receptor proteins for these molecules*

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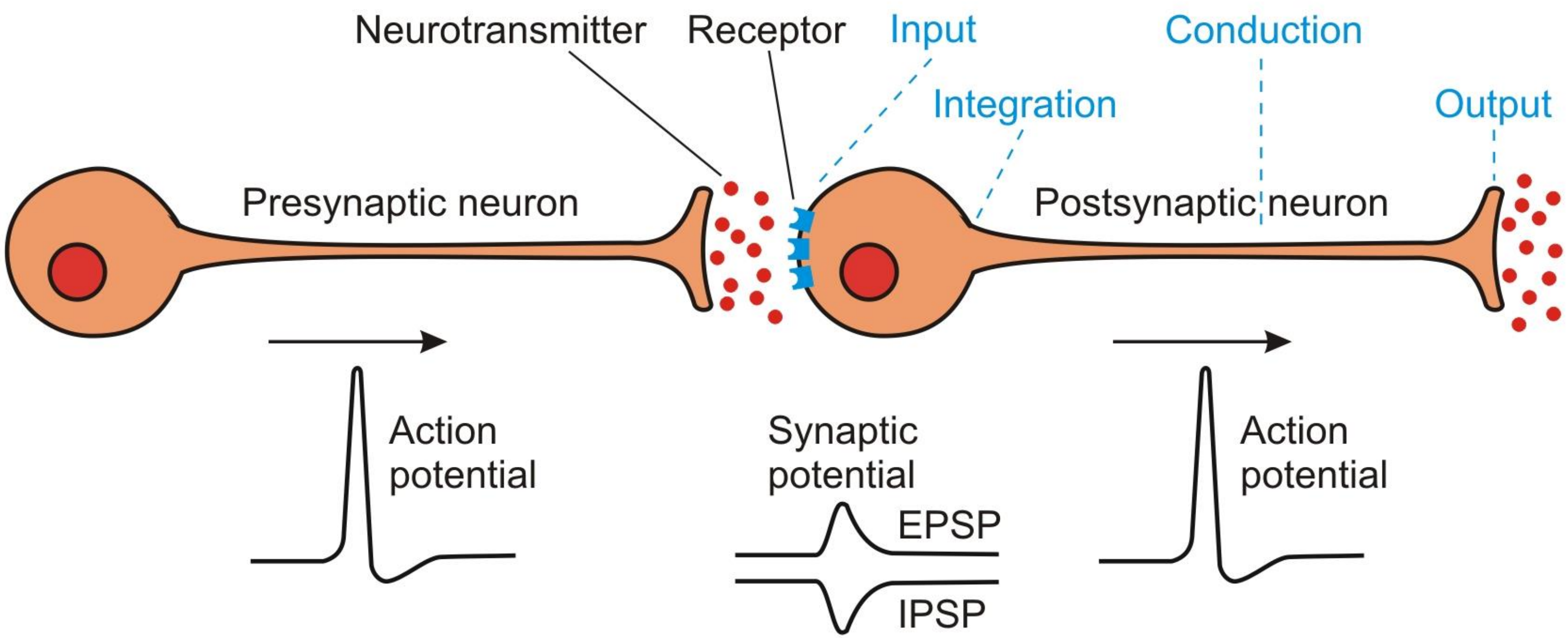
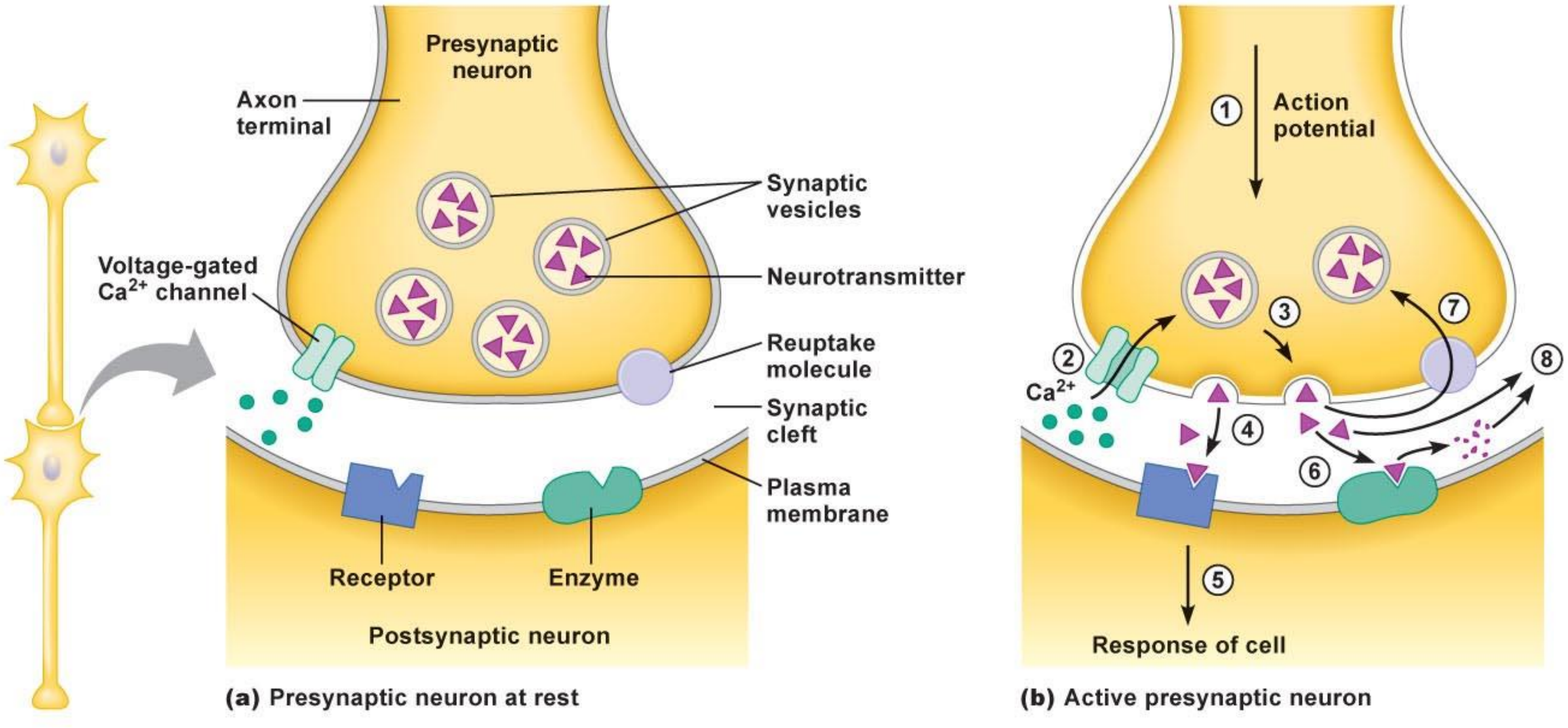
Neuronal communication



- Action potential signalling inside the cell, neurotransmitter signalling between the cells
- Connections can be:
  - Excitatory (inducing Excitatory Postsynaptic Potential (EPSP))
  - Inhibitory (inducing Inhibitory Postsynaptic Potential (IPSP))

Neurotransmitter release

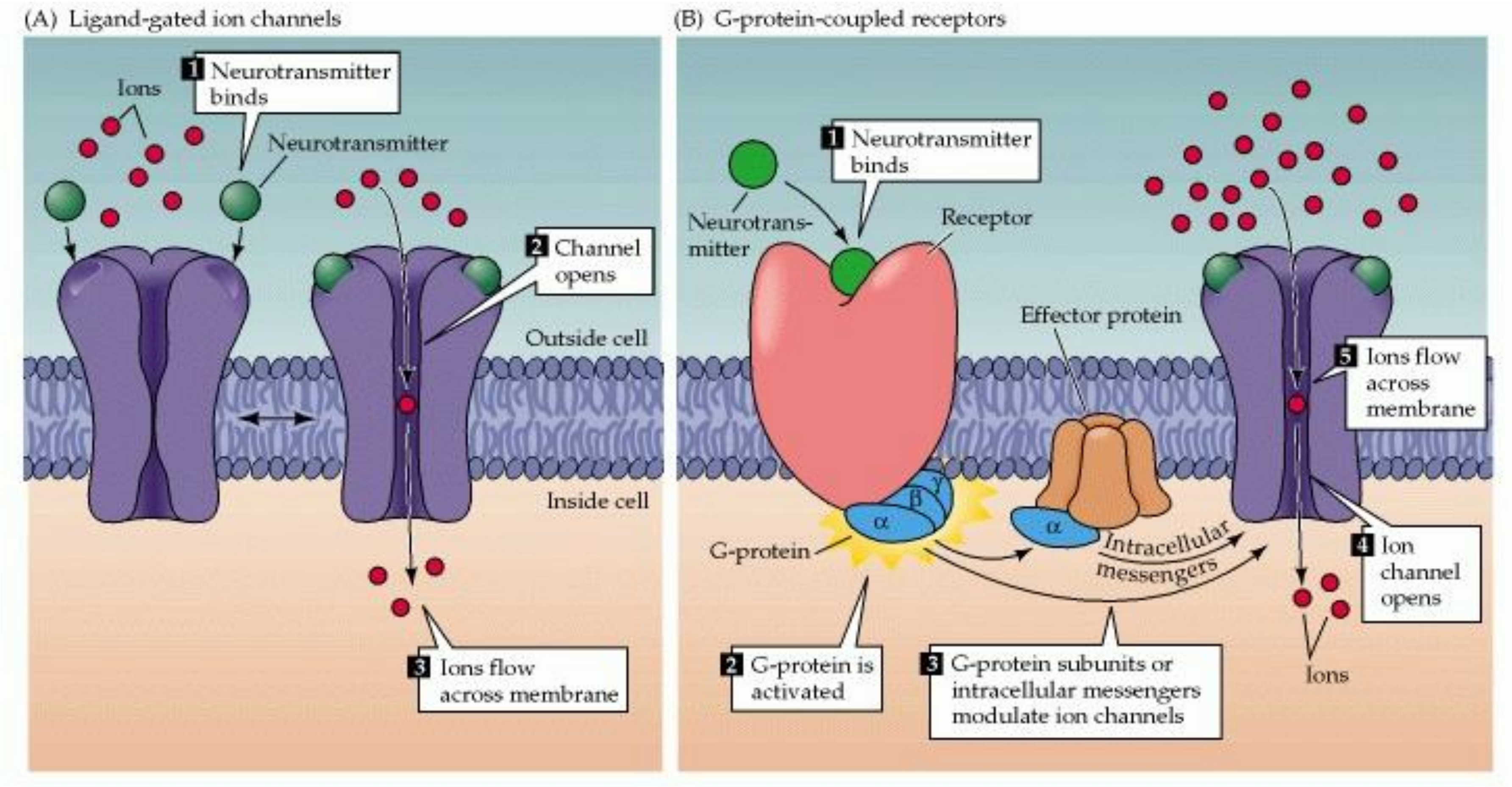


- Change in membrane potential causes voltage-gated Ca^{2+} channels to open => neurotransmitter release from synaptic vesicles
- Neurotransmitter molecules bind to receptors in the target cell membrane

Ionotropic and metabotropic receptors

Direct neurotransmitter action:
Ionotropic receptor

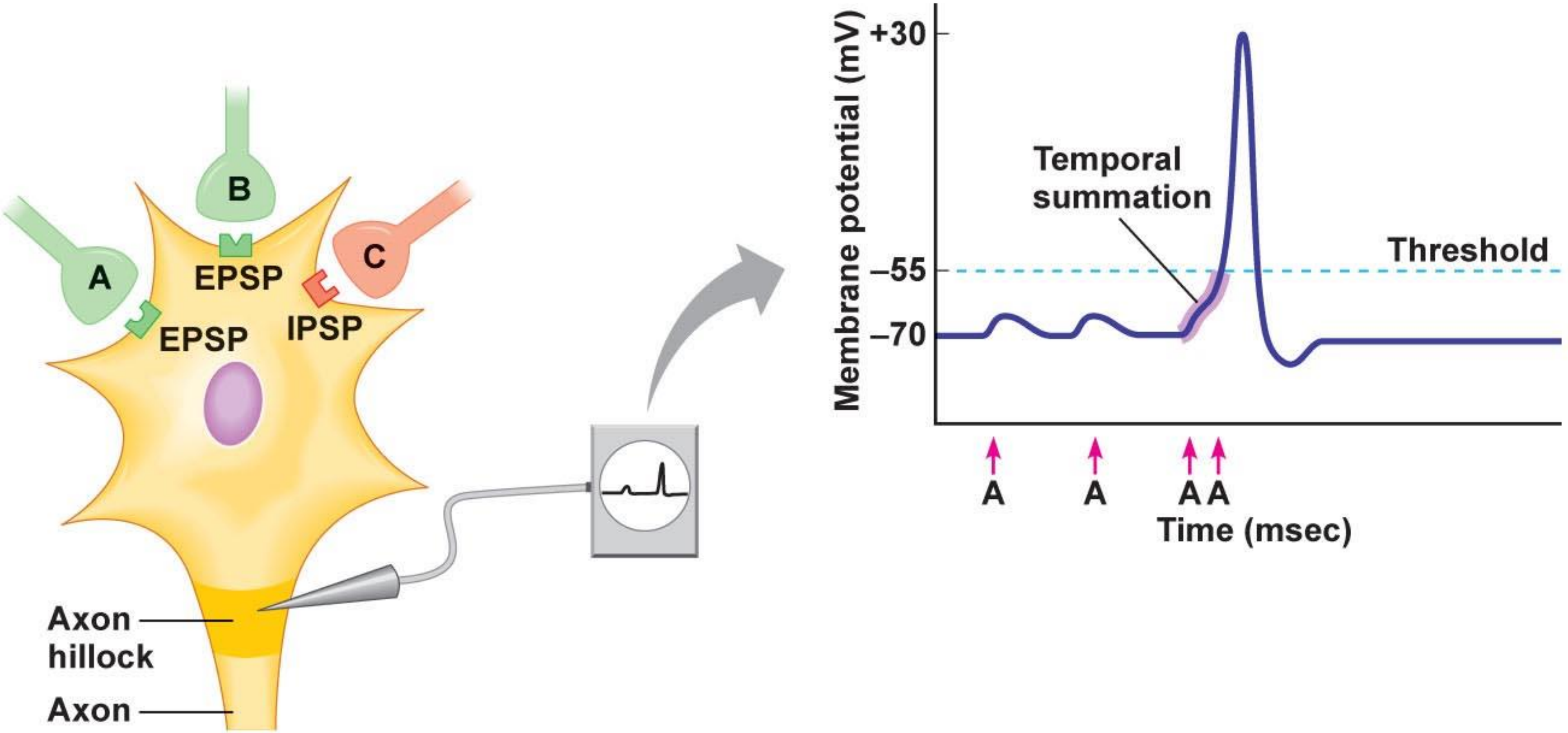
Indirect neurotransmitter action:
Metabotropic receptor



Purves (Ed.), Neuroscience

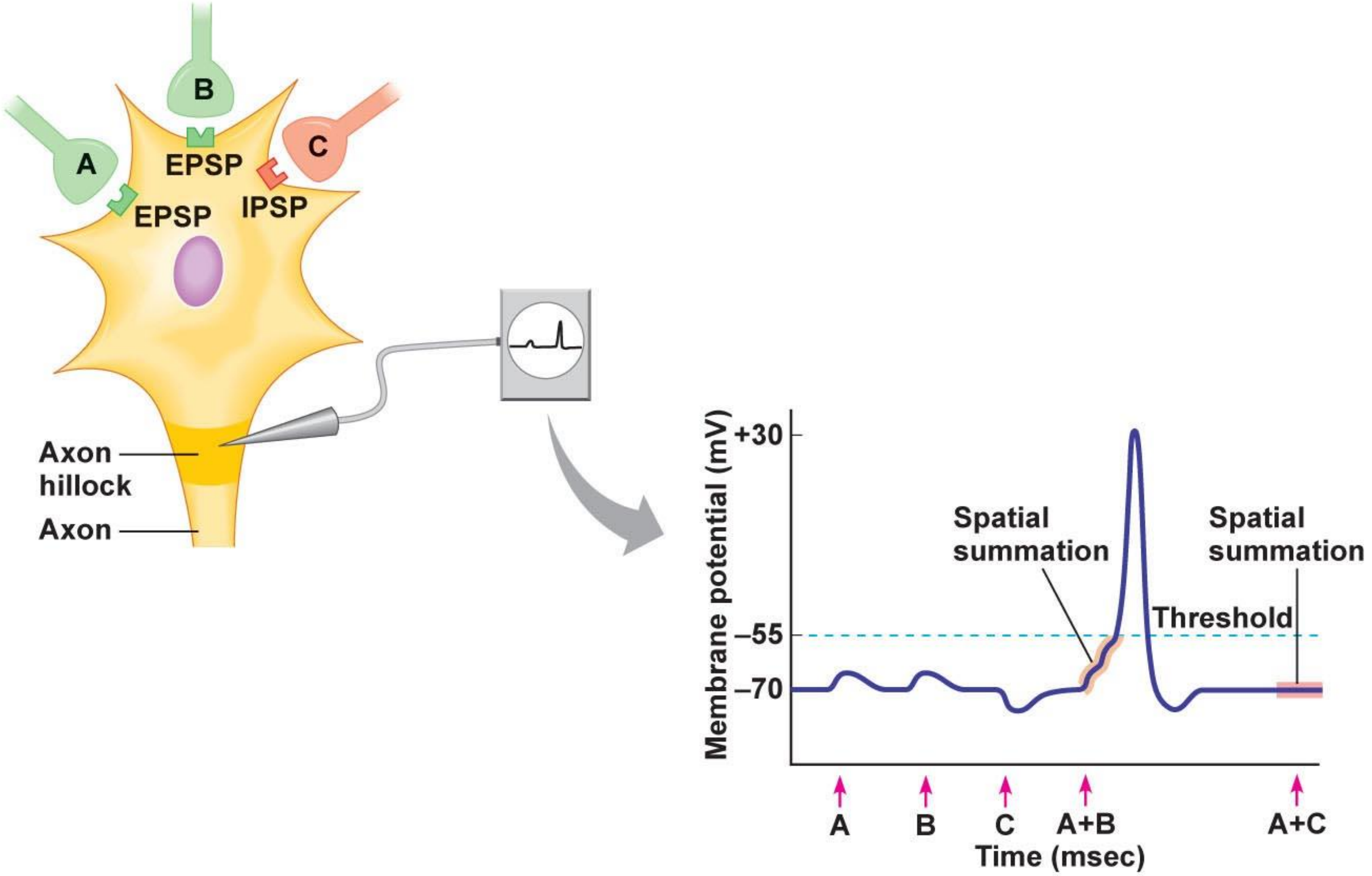
Neuronal communication - temporal summation

- Presynaptic cell signals are summed *temporally* in postsynaptic cell

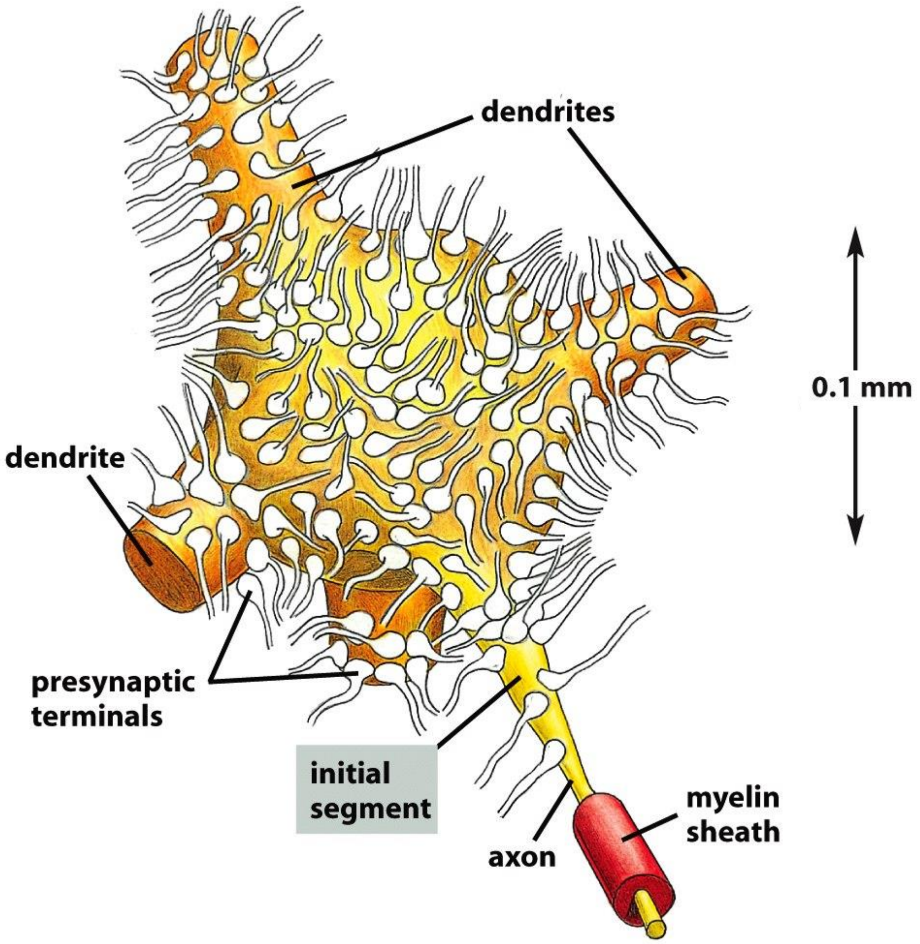


Neuronal communication - spatial summation

- Presynaptic cell signals are summed *spatially* in postsynaptic cell



Synaptic connections to *one cell*



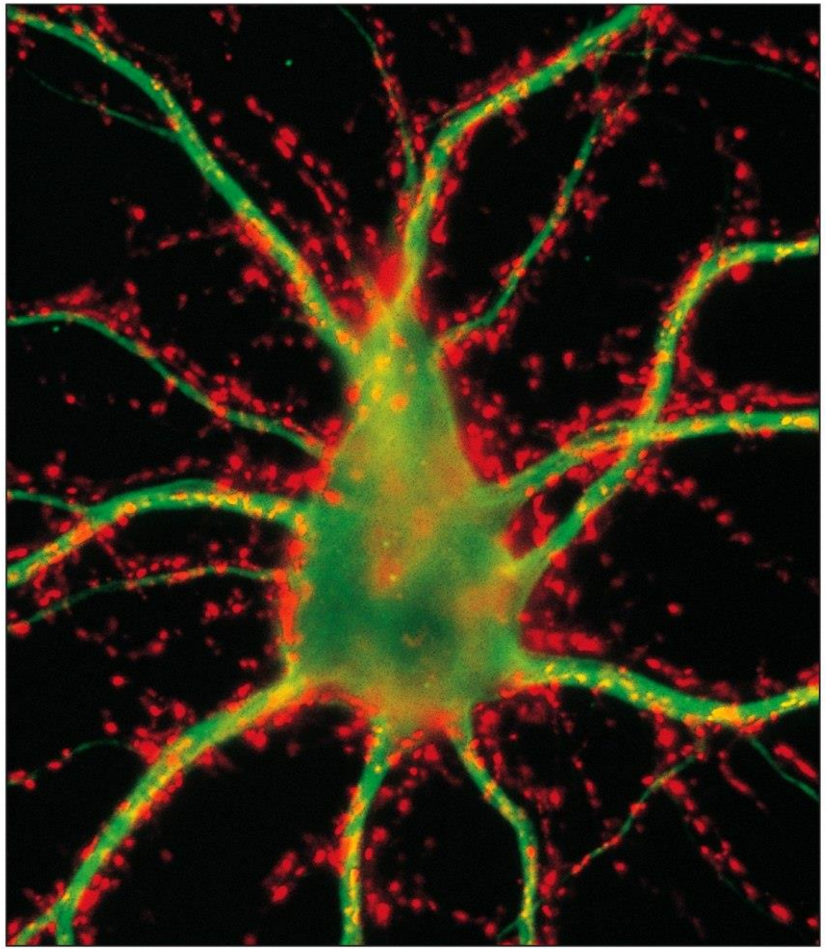
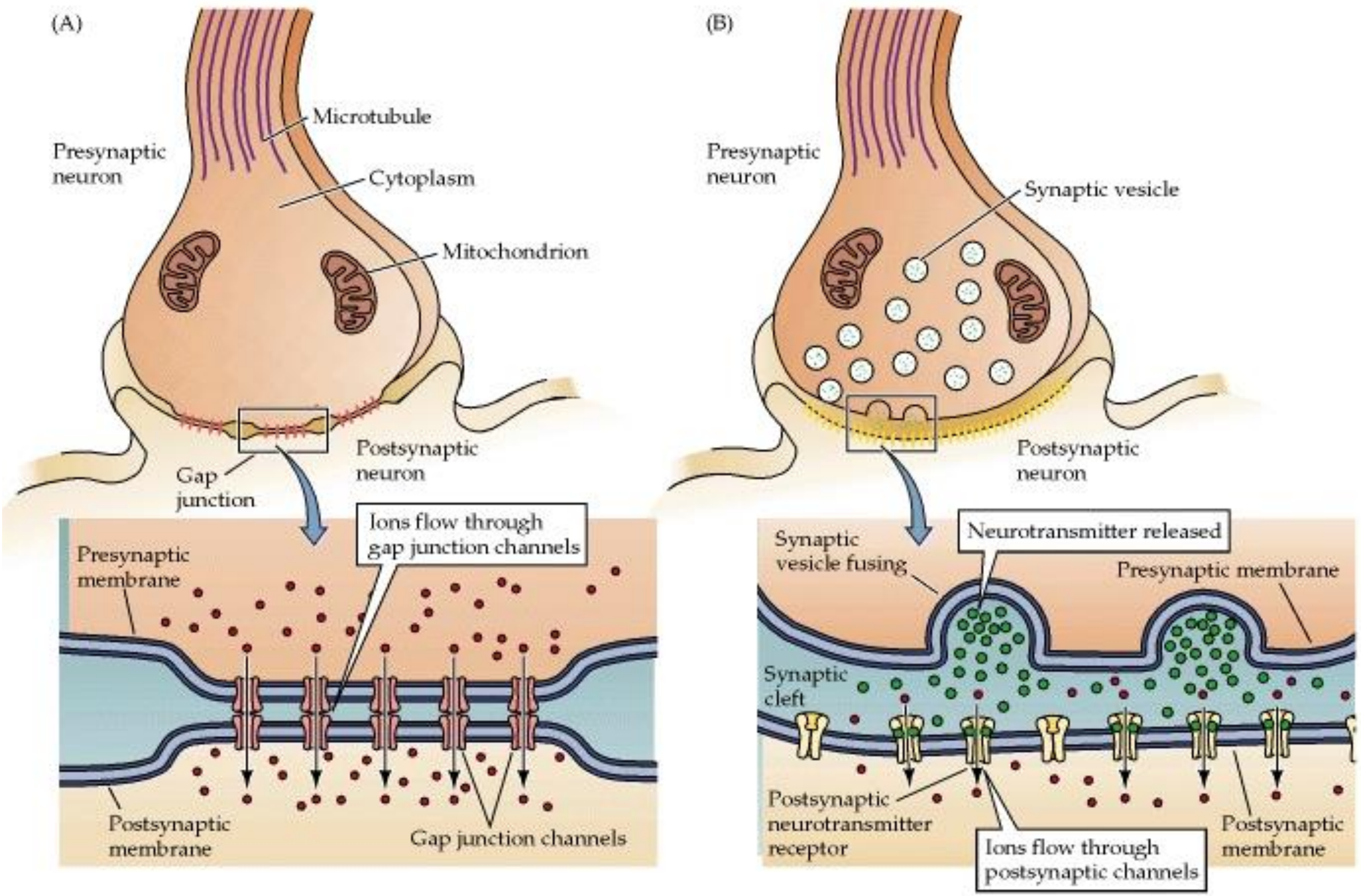


Figure 11-40 *Molecular Biology of the Cell* (© Garland Science 2008)

Electrical vs. chemical synapse

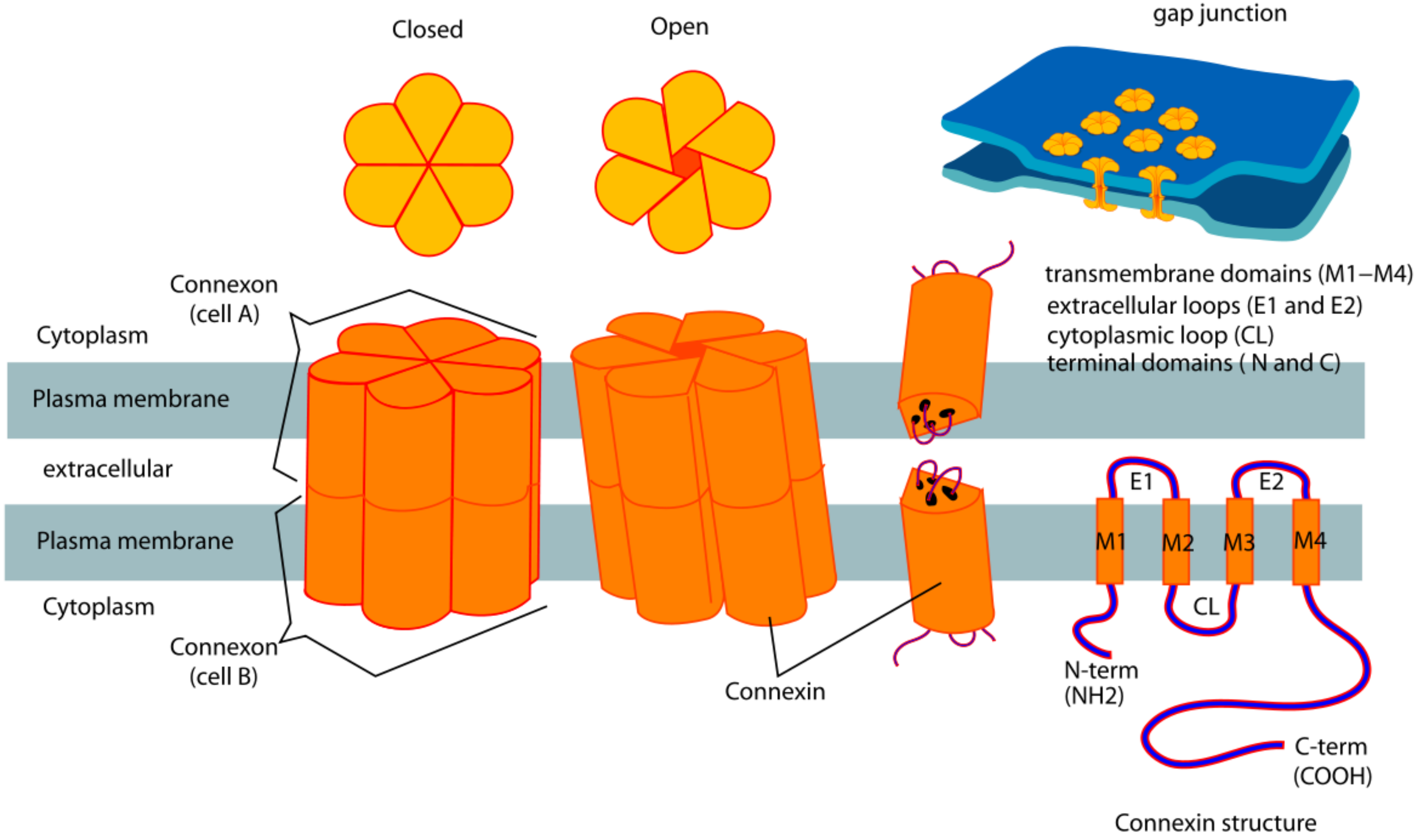
Electrical synapse

Chemical synapse



Gap junctions

- Direct coupling between cells – also known as *electrical coupling*
- Patches of channels, ~1.5 nm diameter
- Allow passage of ions and small molecules (amino acids, nucleotides, no proteins!)



Gap junctions and their “gating”

- Gap junctions are “gated”:
  - Closing at high extracellular Ca^{2+}-concentration
  - Decrease in intracellular pH closes gap junctions
  - Trans-membrane voltage modulates the opening of gap junctions

- Gap junctions permit changes in membrane potential to pass from cell to cell
  - Is the key factor for rhythmic contraction of the heart
  - Allows transmission of an action potential from cell to cell without the delay needed for release of neurotransmitter

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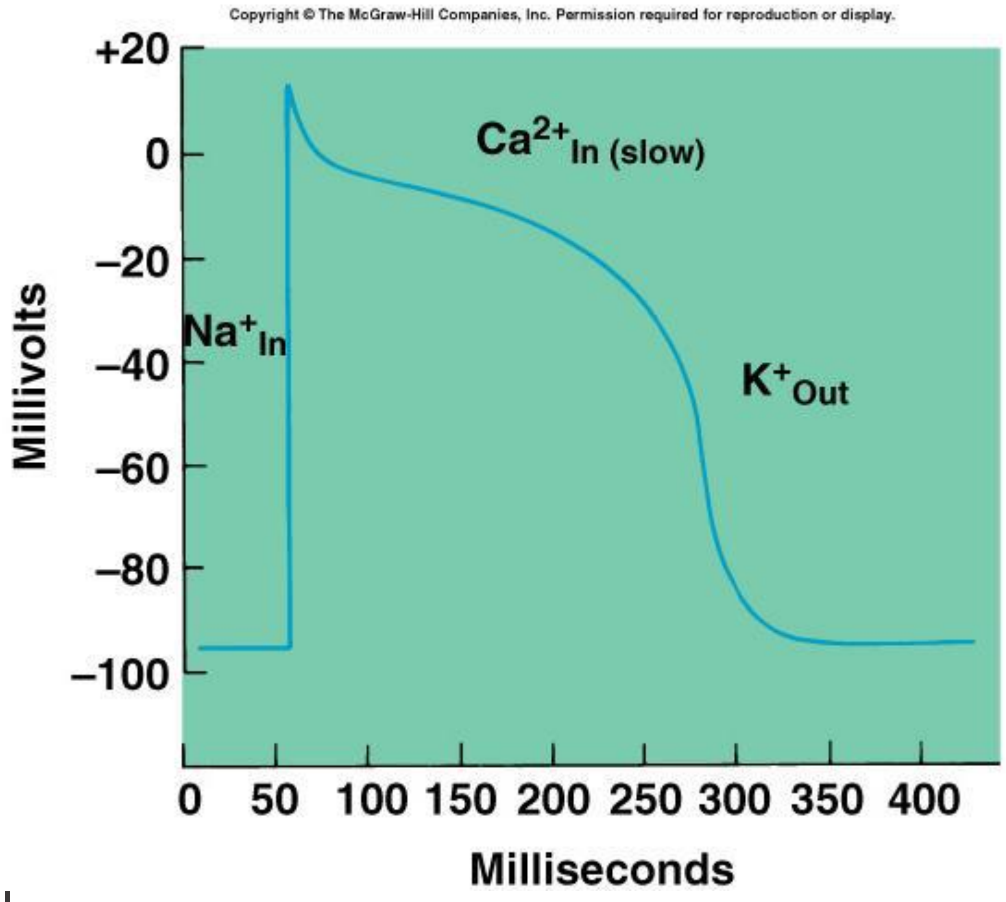
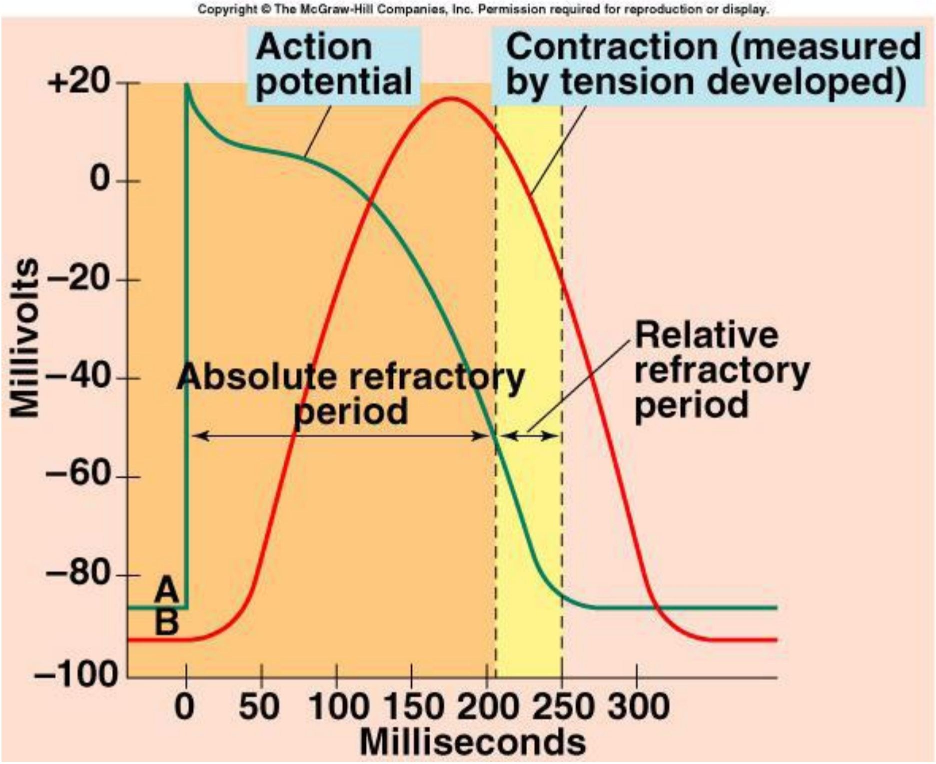
Cardiac action potential

- Gap junctions allow direct transmission of the depolarizing current from cell to cell => in cardiac muscle contraction, cells contract in unison
- Cardiac muscle requires extracellular Ca^{2+} ions for contraction
- The initiation and upshoot of the action potential: entry of Na^{+} ions into the cell
- An inward flux of Ca^{2+} ions through voltage-gated Ca^{2+} channels sustains the depolarization => longer duration for the action potential



Cardiac action potential - refractory period

- Heart contracts as syncytium
- Contraction lasts typically about 300 ms
- Refractory periods last almost as long as contraction
- Cardiac cell cannot be stimulated to initiate an action potential during the refractory period
  - Summation cannot occur
  - Cardiac muscle cannot be stimulated to contract again before it has relaxed



Take home message

- Voltage-gated ion channels (Na^+, K^+, Ca^{2+}), specific receptors and gap junctions are key players in the generation and propagation of action potentials in neurons and cardiac tissue
- Neuronal communication is based on action potential generation and propagation, and action potential conduction velocity limits the neuronal information flow
- Temporal and spatial summation are important concepts in neuronal communication
- Cardiac action potential has specific features due to the involvement of voltage-gated Ca^{2+} channels

- More in
  - http://www.bem.fi/book/
  - B. Hille: Ion Channels of Excitable Membranes, 3rd Ed., Sinauer
  - Purves (Ed.), Neuroscience, 2nd edition
    http://www.ncbi.nlm.nih.gov/books/NBK10799/

- Next lecture about sensory systems and their biophysical phenomena