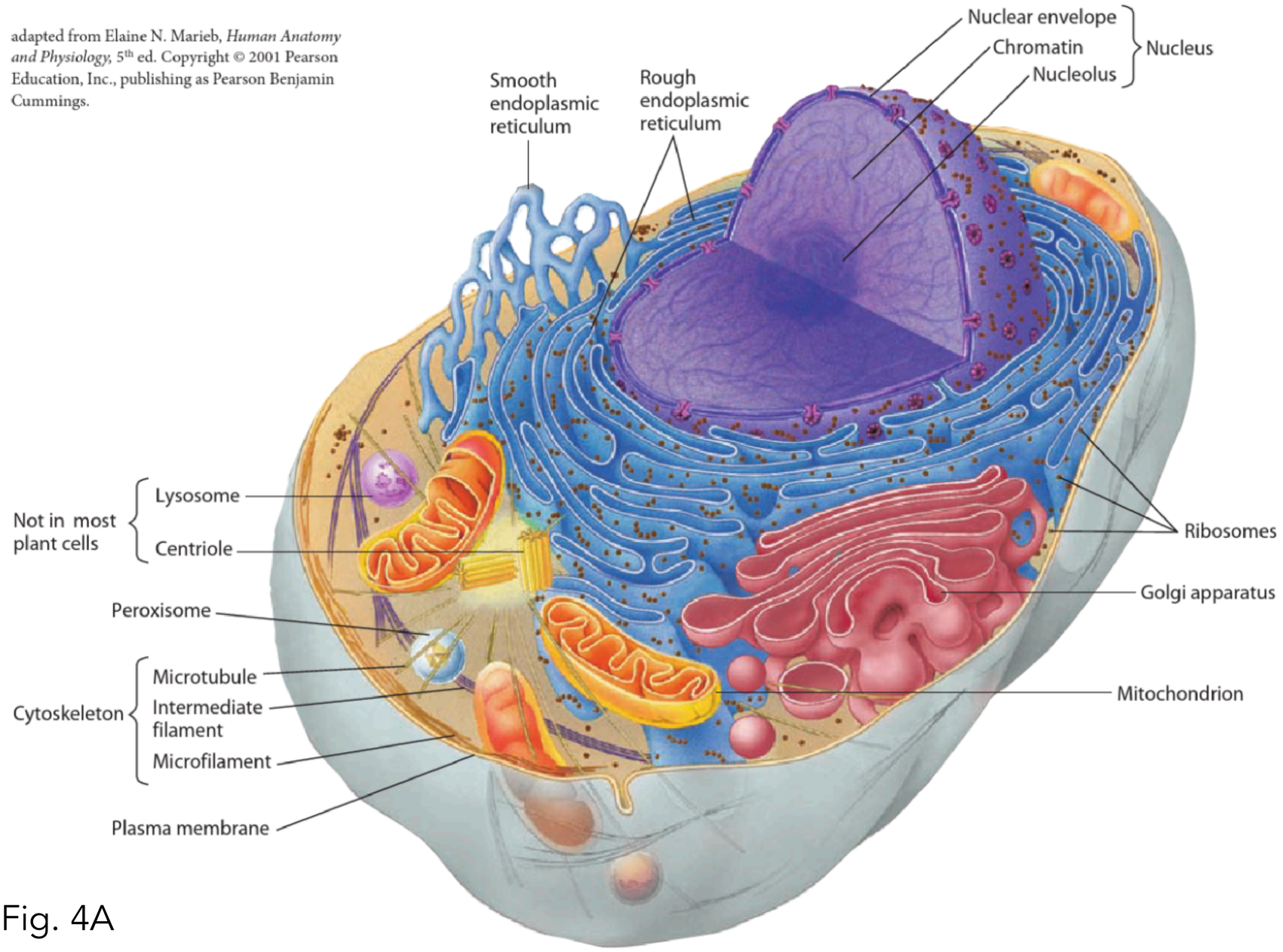
Revision

Fides Zenk 27.05.25

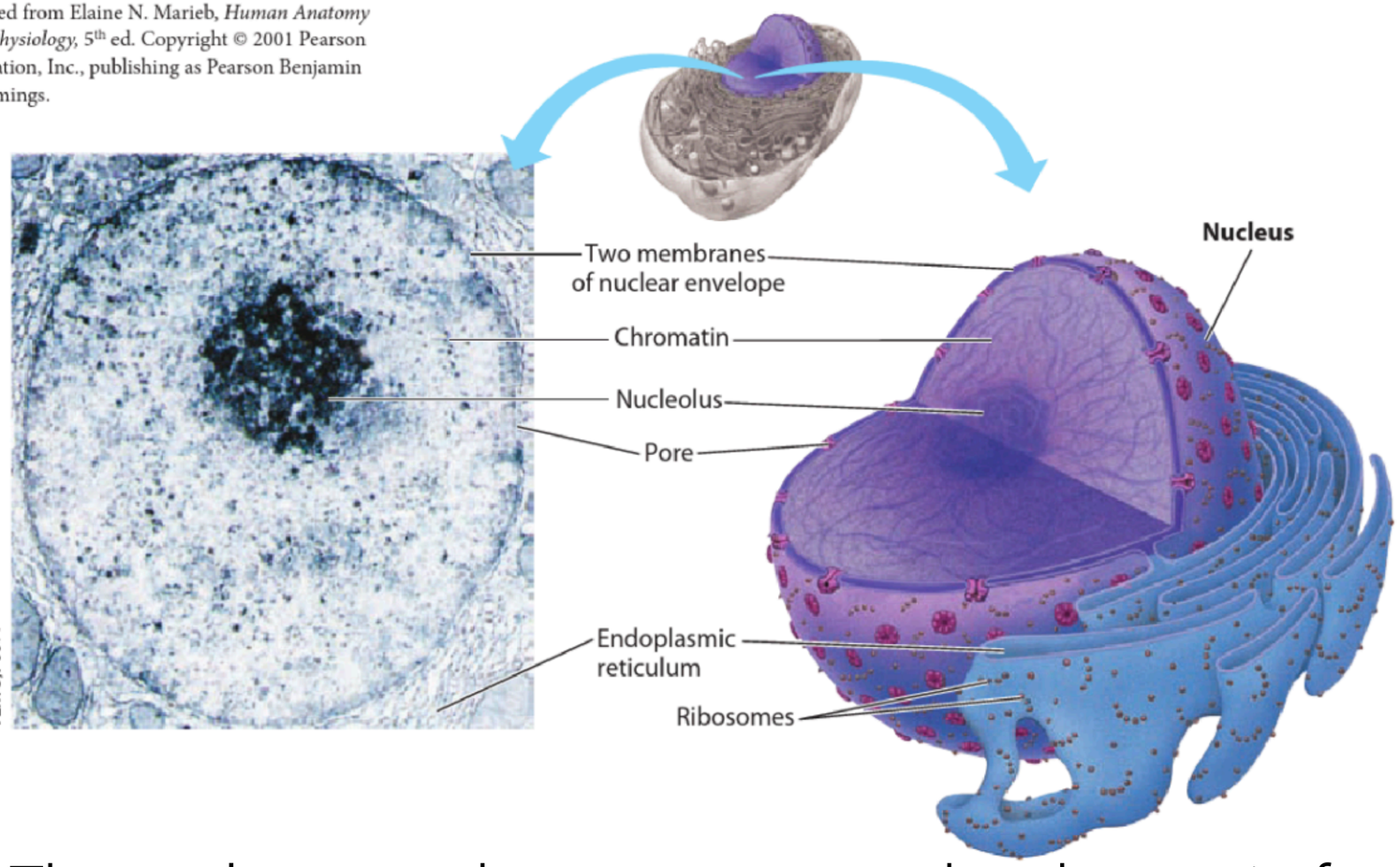
What are the building blocks of a cell?



Campbell, Biology Fig. 4A

The nucleus

adapted from Elaine N. Marieb, *Human Anatomy and Physiology*, 5th ed. Copyright © 2001 Pearson Education, Inc., publishing as Pearson Benjamin Cummings.



Courtesy of Richard Rodewald/Biological Photo Service

TEM 3,700×

The nucleus stores the genetic material (chromatin = DNA+structural components) of the cell.

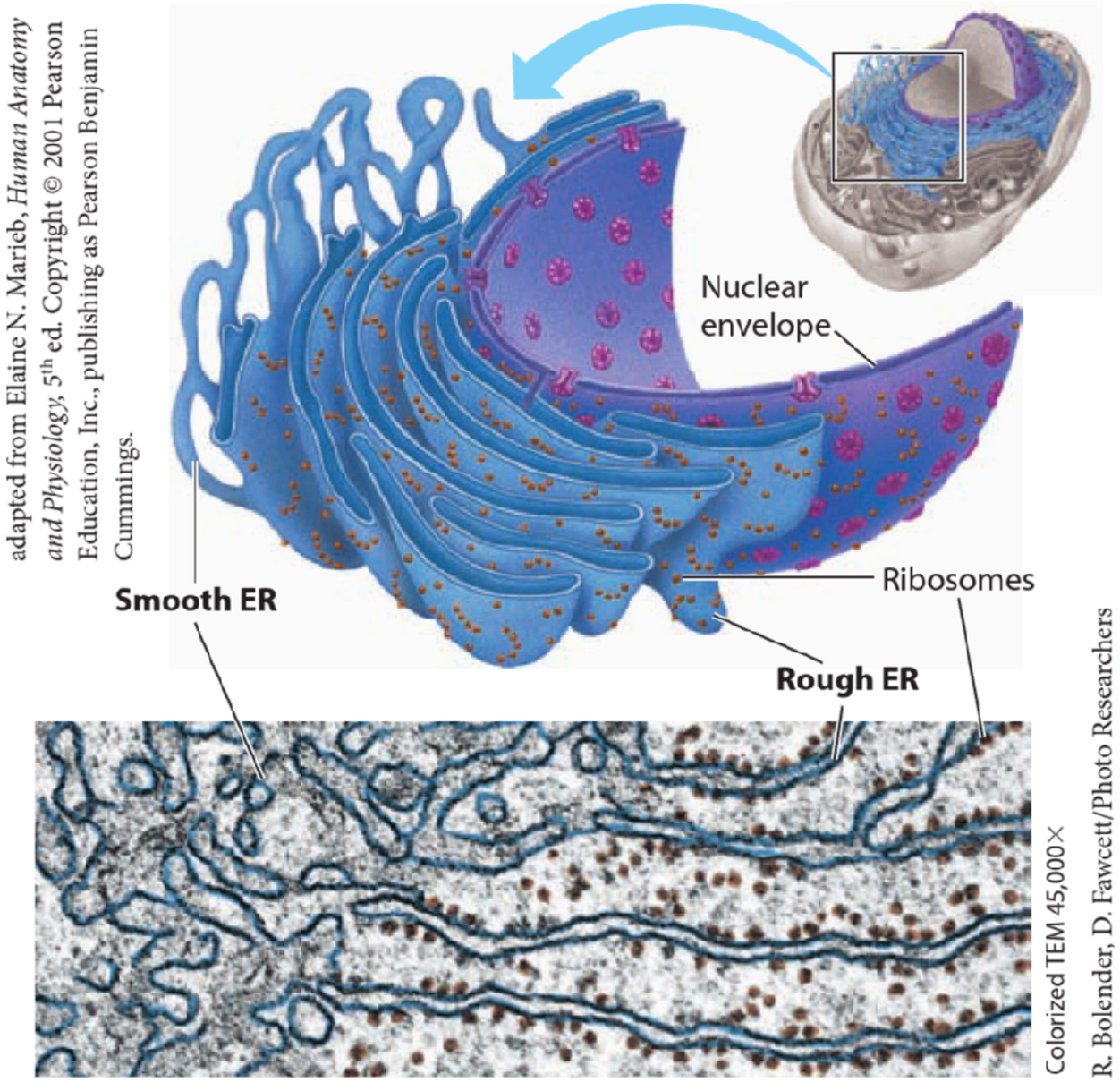
The DNA contains all the instructions on how to make the proteins of the cell.

The nuclear membrane separates the chromatin from the rest of the cell.

Nuclear pores allow communication and the export and import of mRNA and proteins.

Campbell, Biology Fig. 5

The endosplamic reticulum



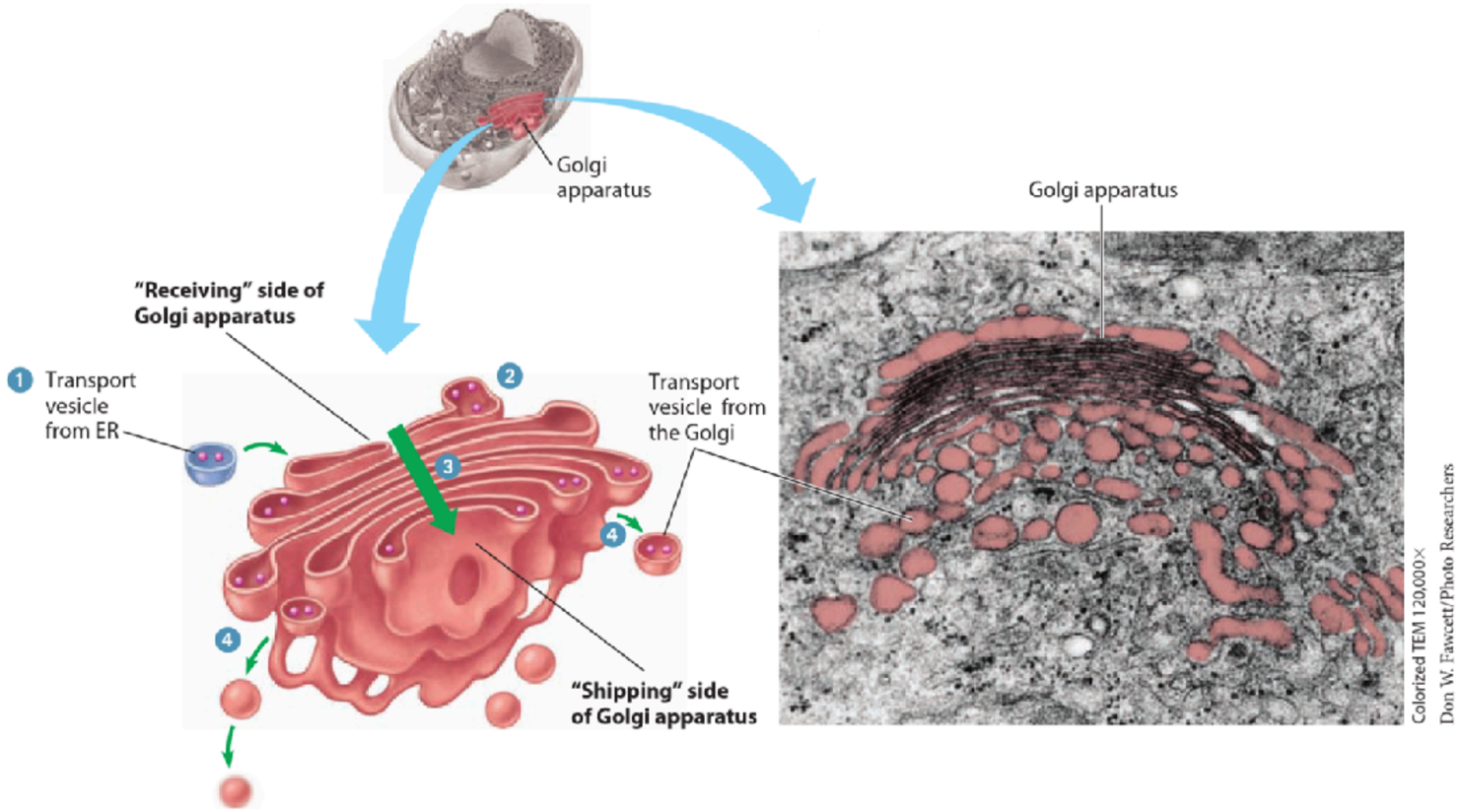
The endoplasmic reticulum is directly attached to the nuclear membrane.

mRNA exported from the nucleus is translated into protein by the ribosomes that are bound or freely floating around the endoplasmic reticulum.

These proteins will have a variety of functions in the cell. Some will go back inside the nucleus.

Campbell, Biology Fig. 8A

The Golgi



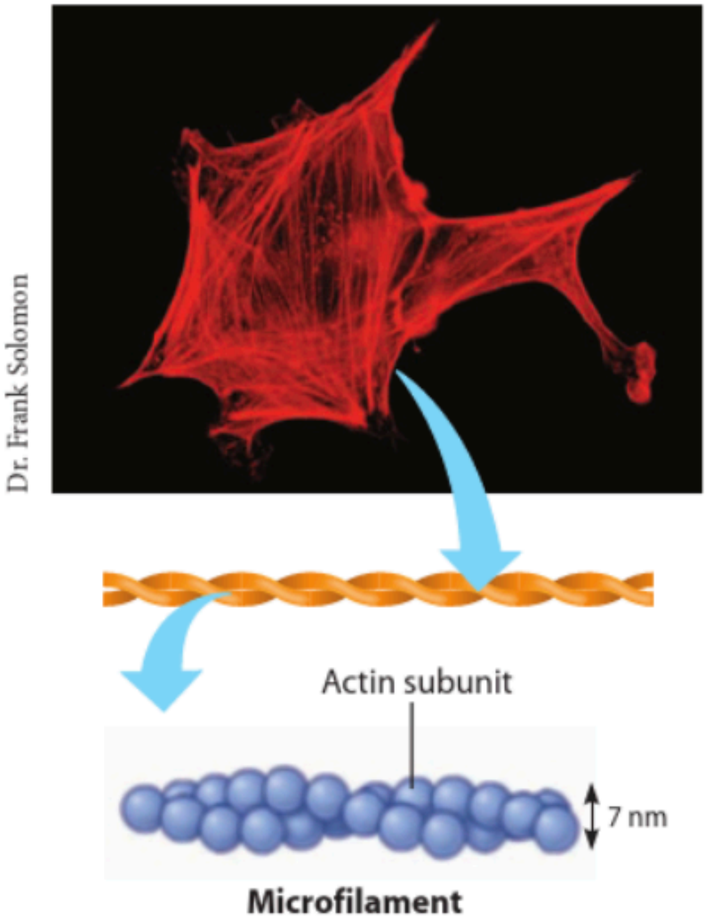
The Golgi is the packaging center of the cell. It receives proteins from the ER and adds chemical modifications (sugar, phsophate, sulfates).

Takes care of sorting and sending to a destination (membrane, lysosome, secretion)

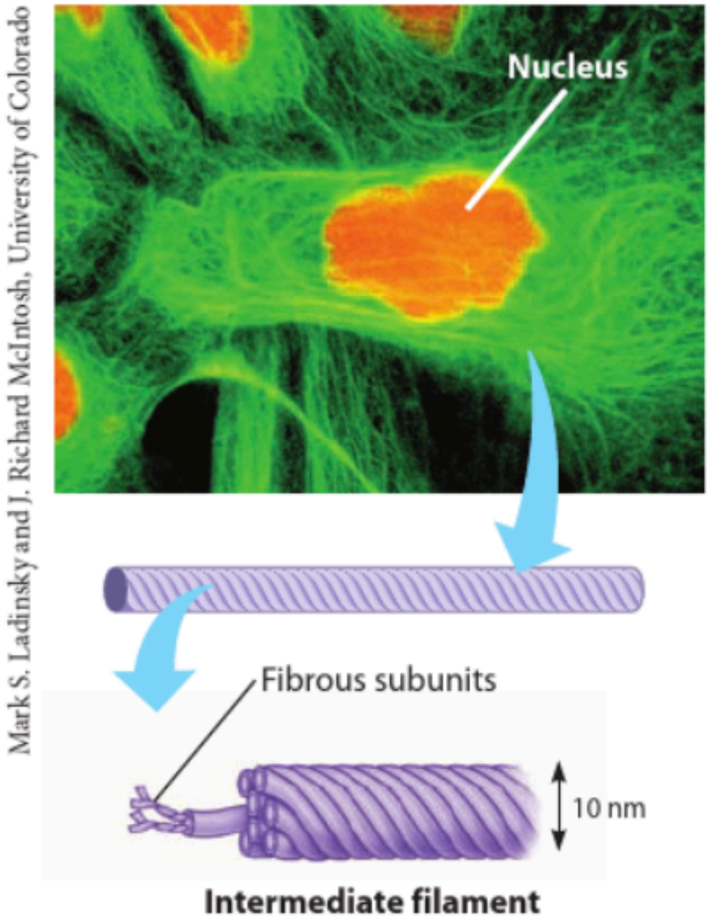
This process will be very important in later lectures! Why?

Campbell, Biology Fig. 9

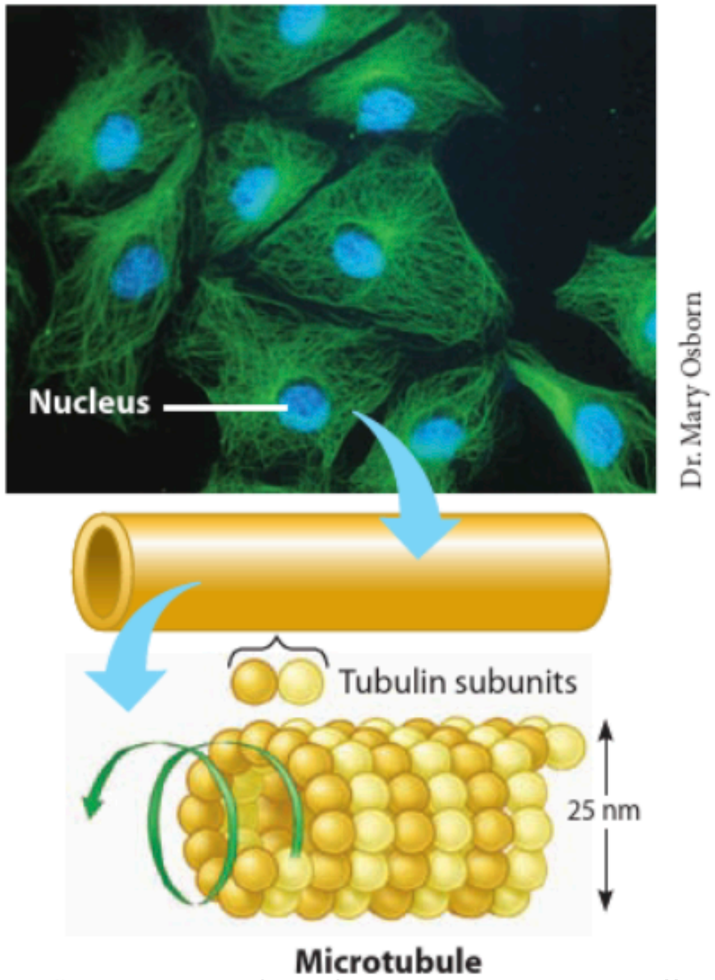
Cytoskeleton



Mechanical support of the cell shape in movement, cell division, endocytosis and muscle contraction



Mechanical support of the cell shape, Lamins support the nuclear membrane



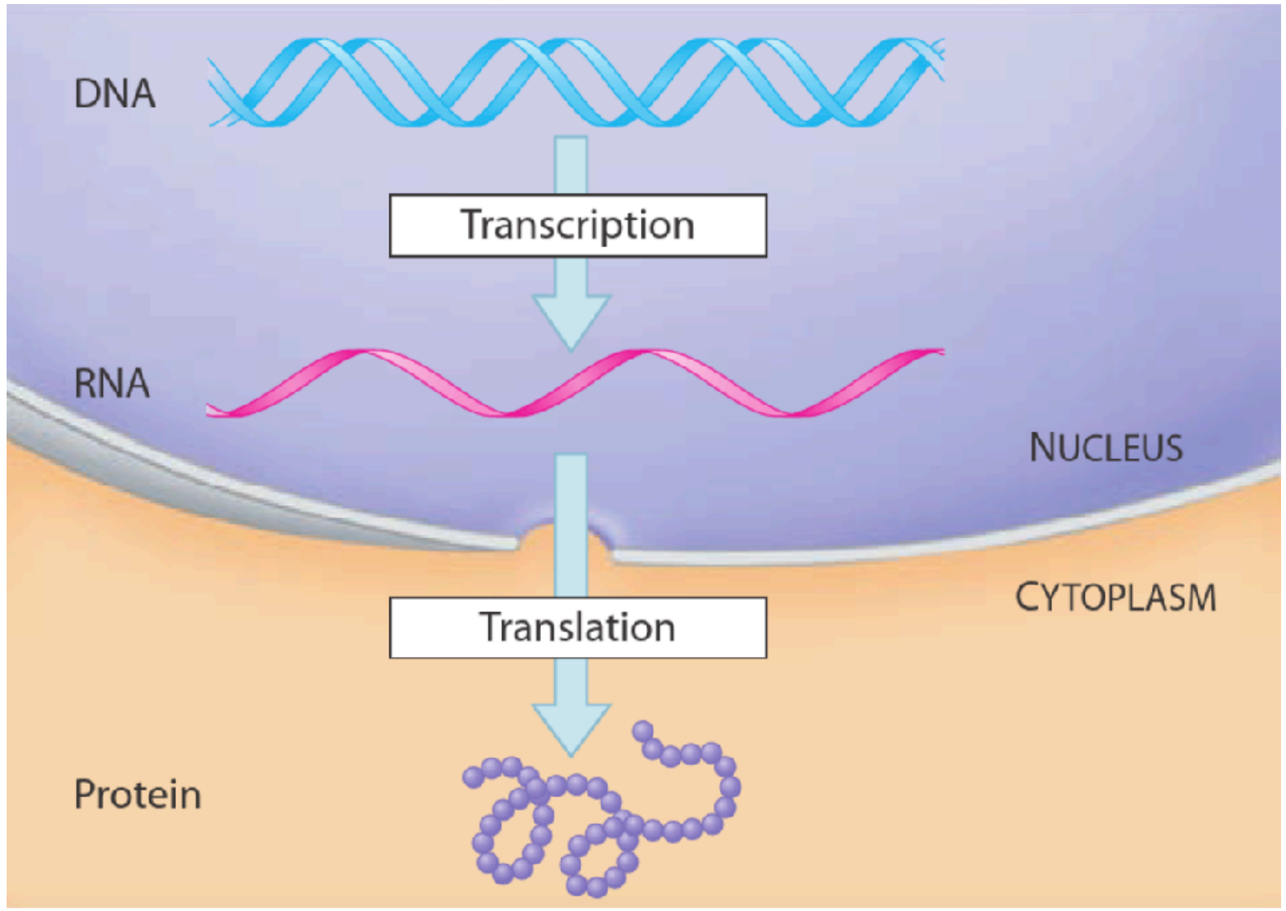
Segregate the chromosomes in cell division, intracellular transport, cell motility (cilia, flagella)

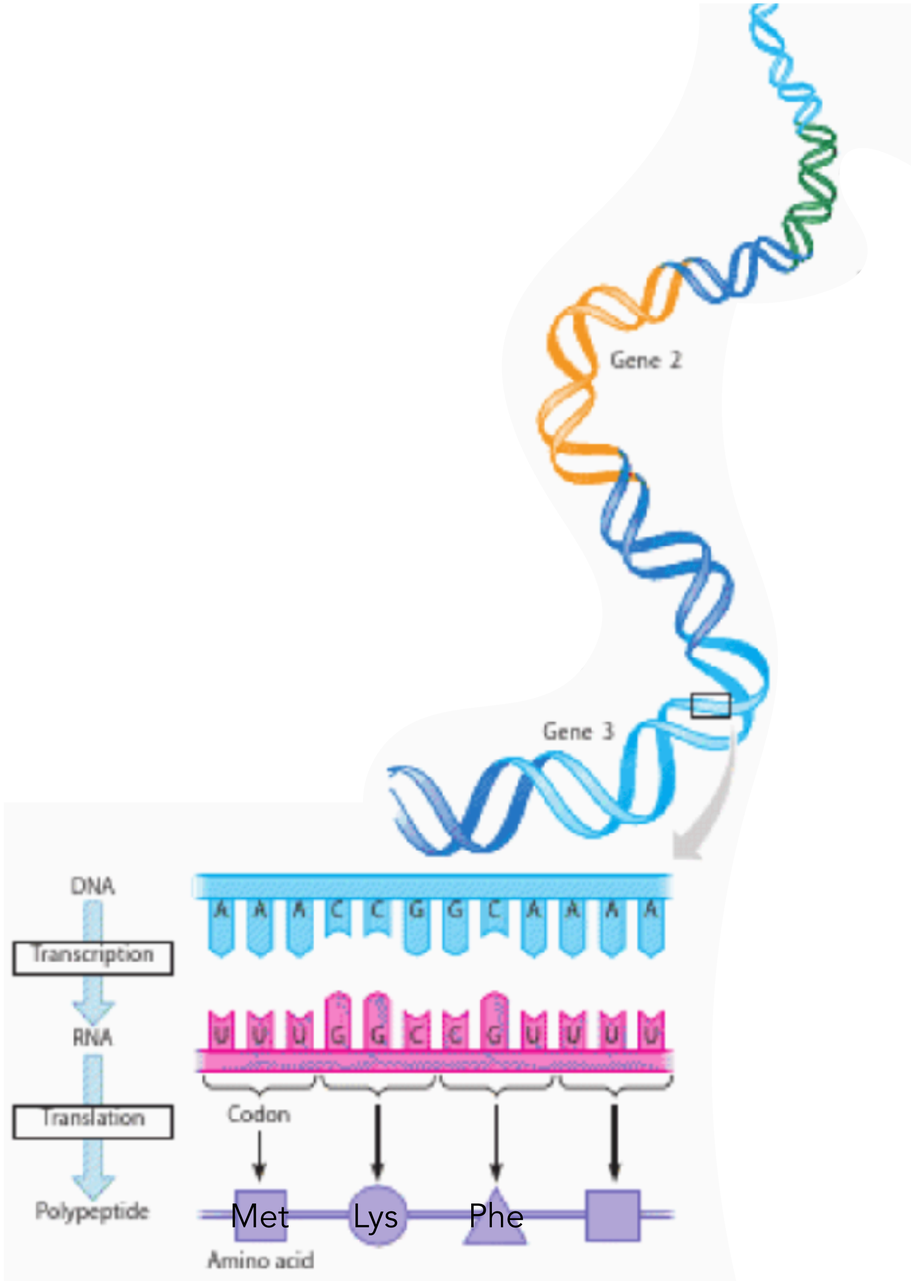
This will be very important in later lectures! Why?

Campbell, Biology Fig. 16

The information of how to produce proteins is stored in the nucleus

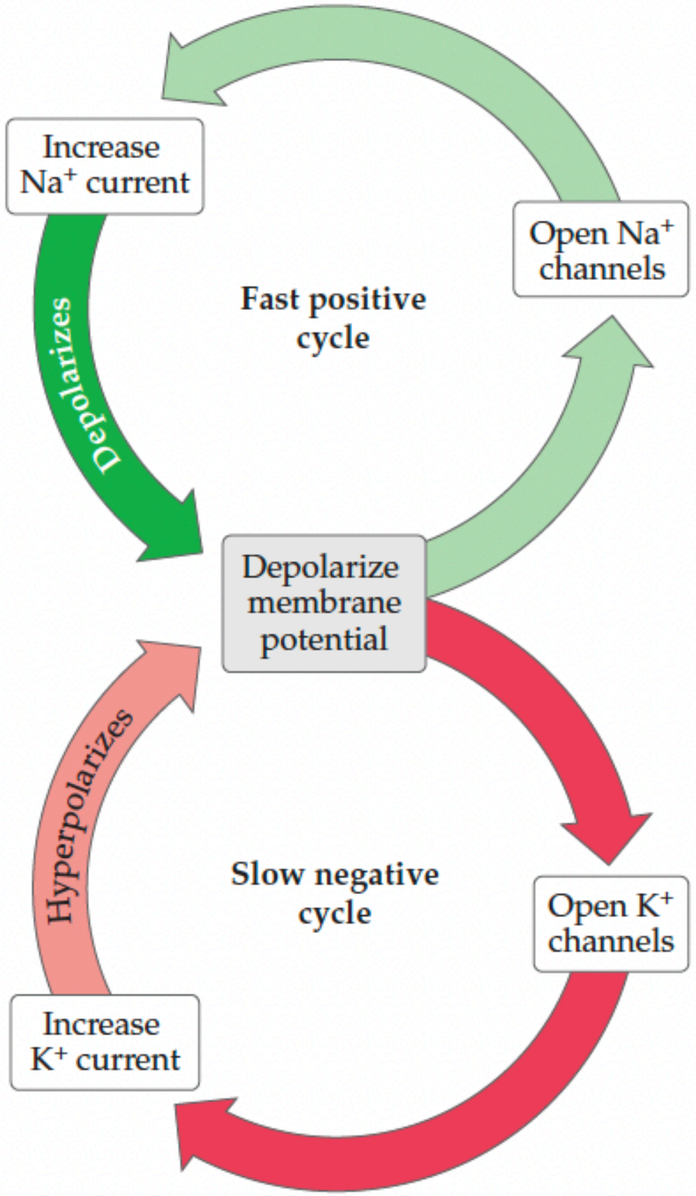
The central dogma of molecular biology

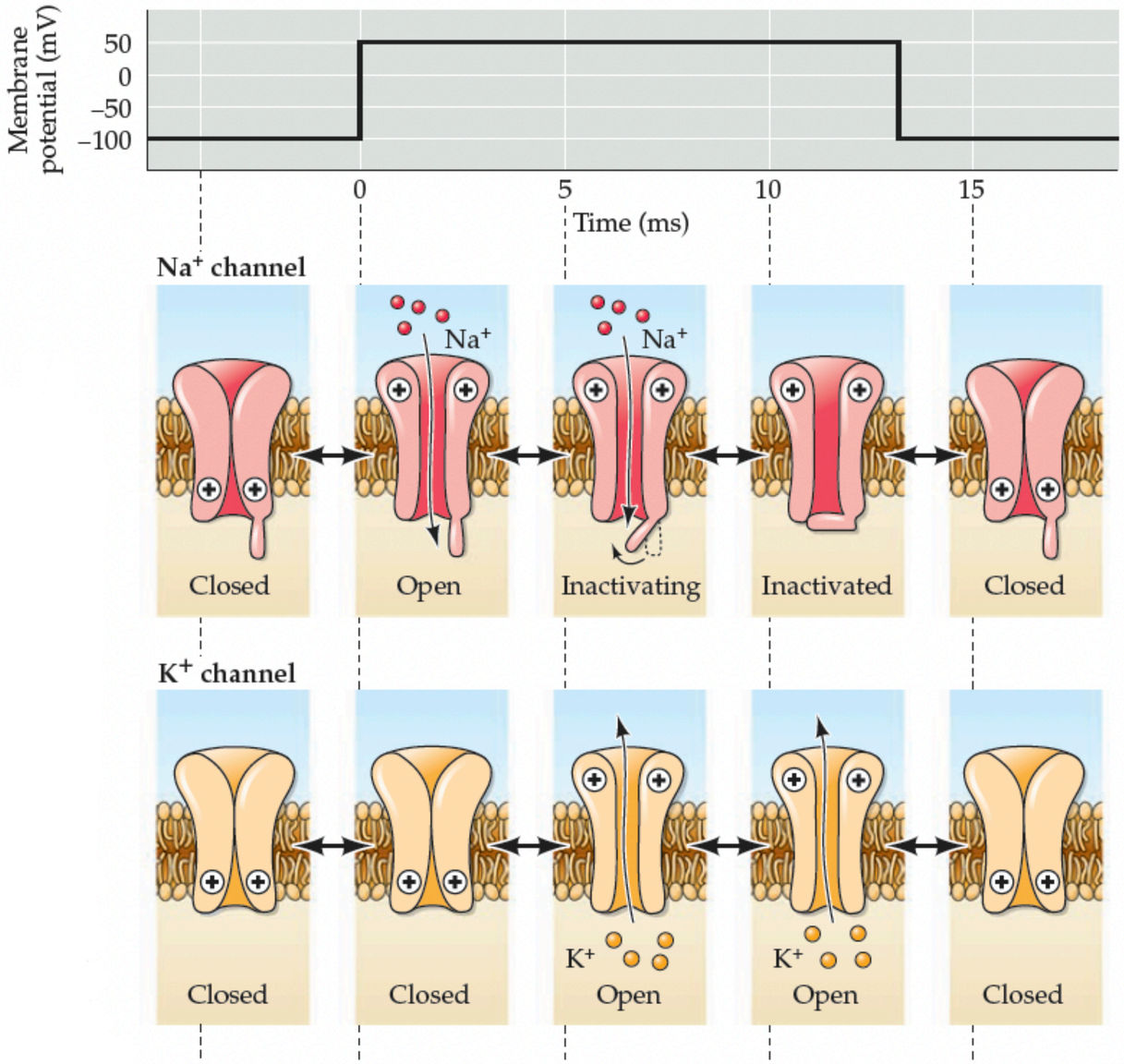




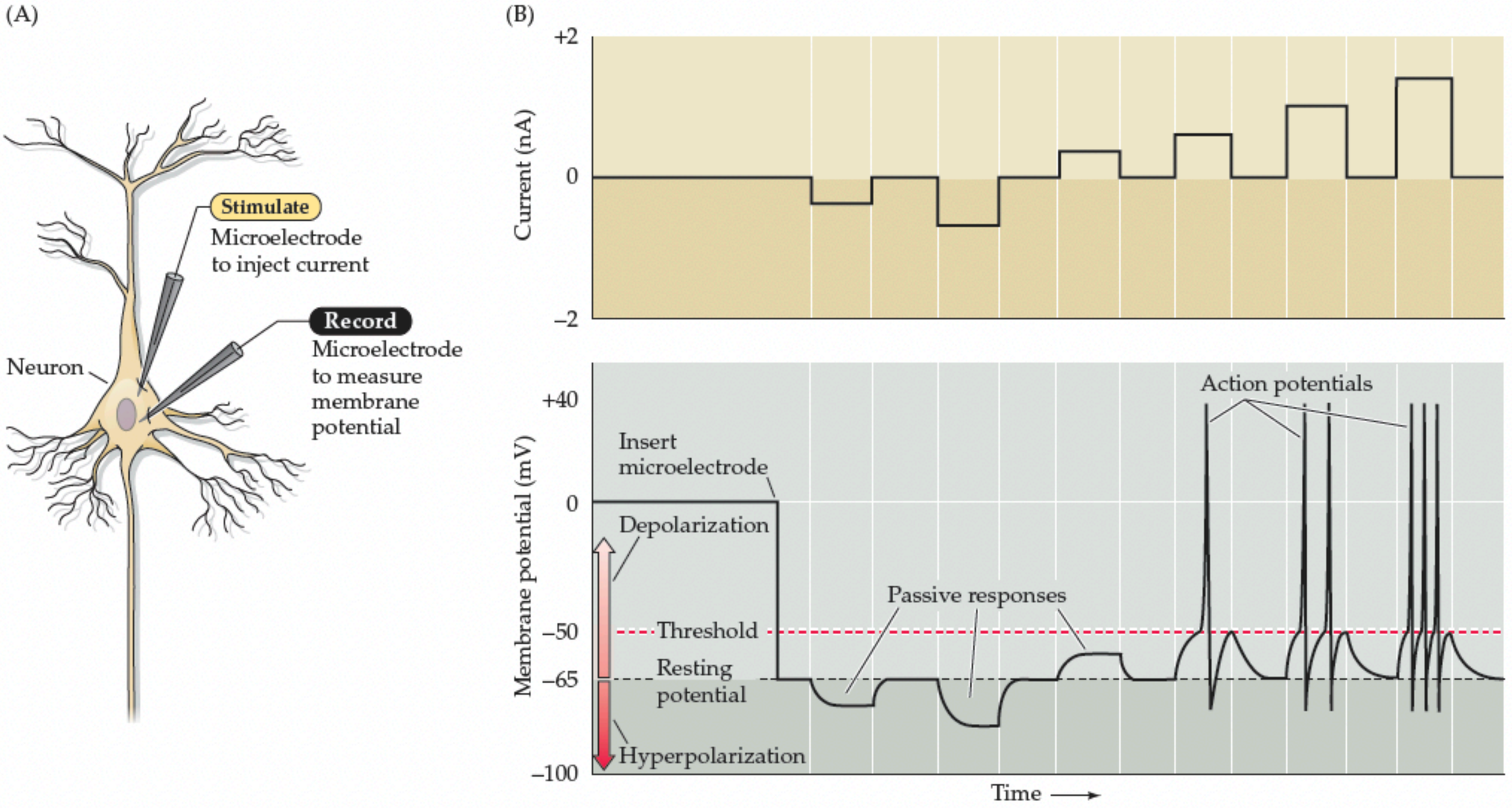
Campbell, Biology Fig. 6A

Voltage gated channels are responsible for these dynamics





Electrical signals above a threshold depolarize the membrane

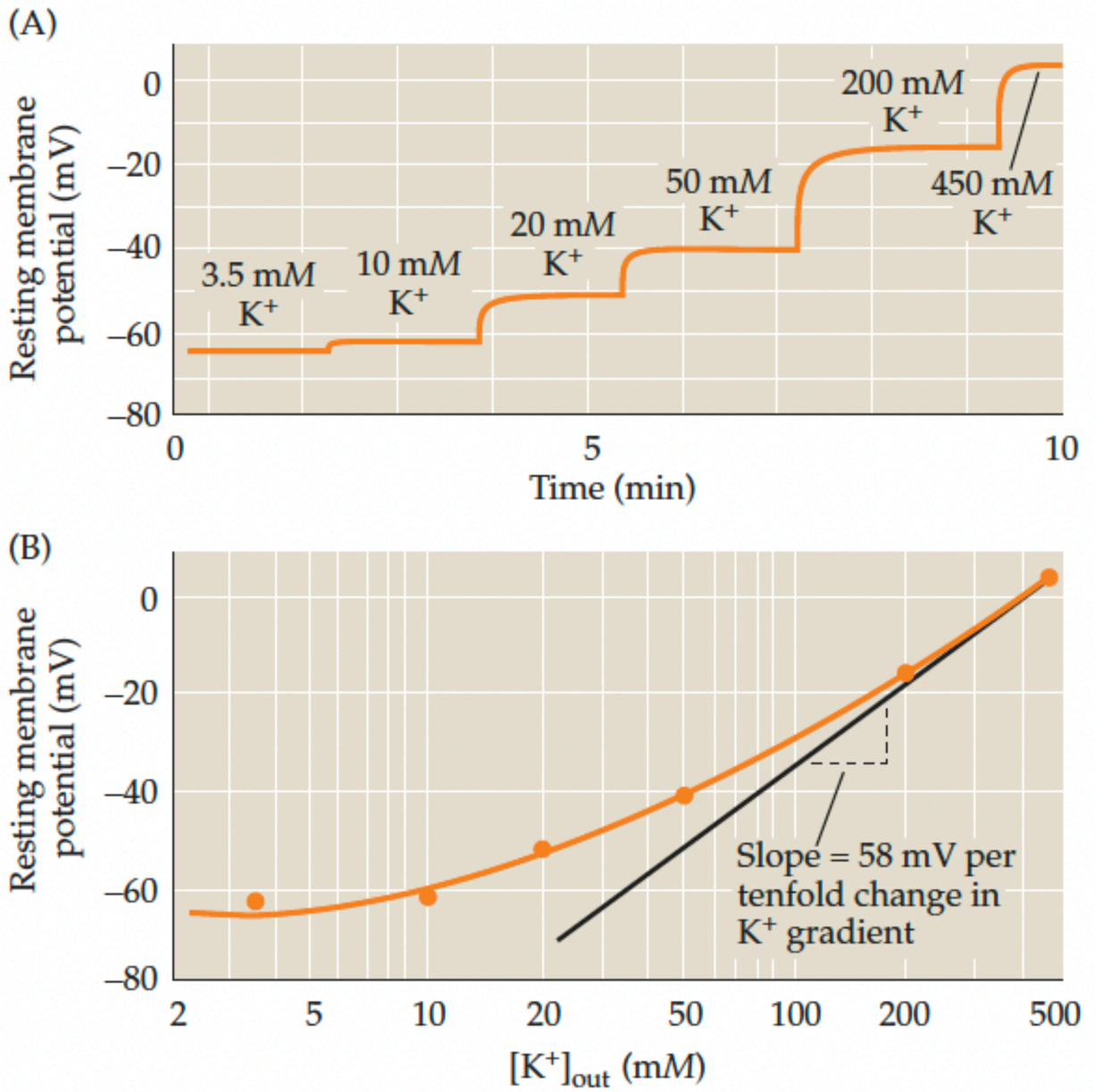


The resting potential of a membrane is determined by the K+ gradient

$$V_m = 58log_{10}\frac{P_K[K]_{out} + P_{Na}[Na]_{out} + P_{Cl}[Cl]_{in}}{P_K[K]_{in} + P_{Na}[Na]_{in} + P_{Cl}[Cl]_{out}}$$

According to Goldman's equation the Membrane potential is close to K^+ potential (-58 mV)

Increasing extracellular K^+ should depolarize the membrane by 58 mV per 10-fold increase in K^+

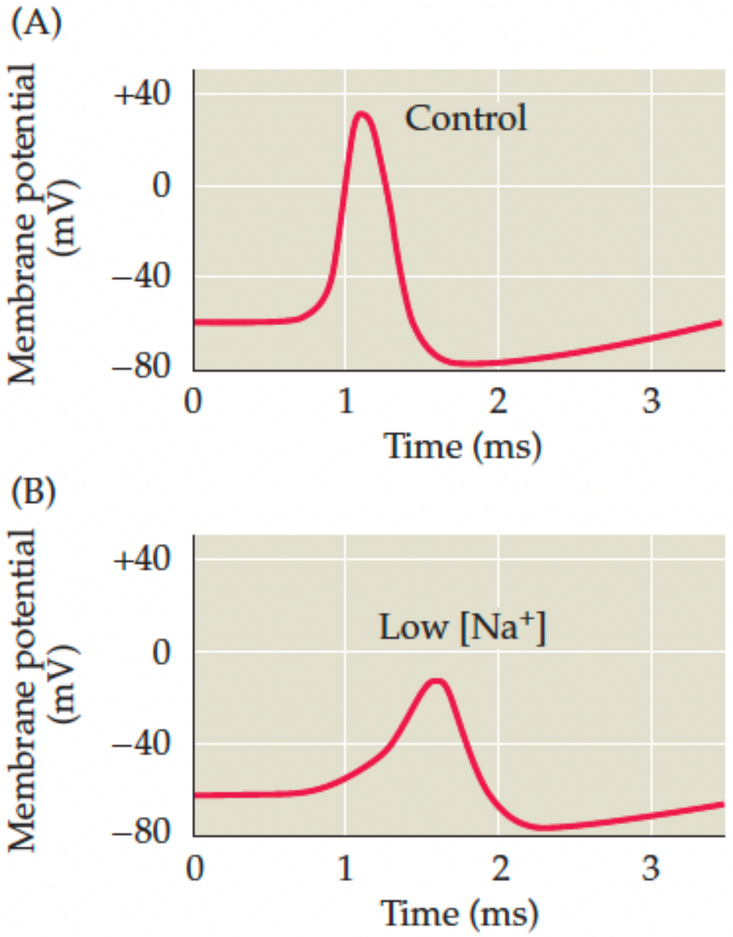


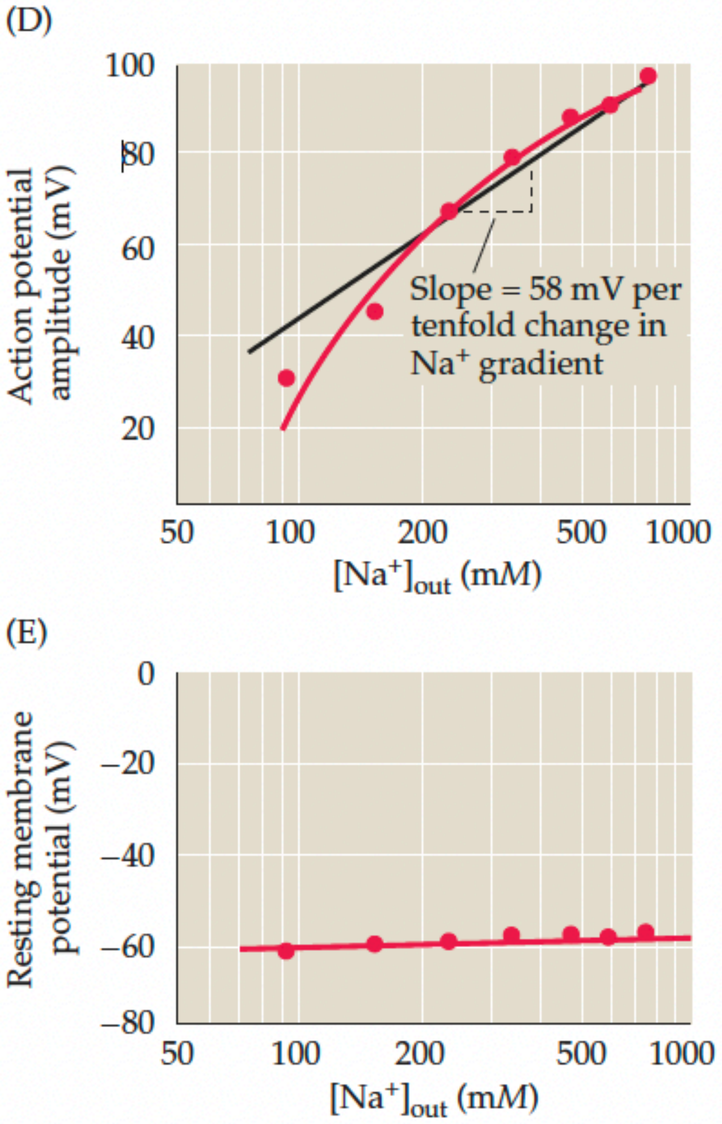
The action potential of a membrane is determined by Na^+ flow

$$V_m = 58log_{10}\frac{P_K[K]_{out} + P_{Na}[Na]_{out} + P_{Cl}[Cl]_{in}}{P_K[K]_{in} + P_{Na}[Na]_{in} + P_{Cl}[Cl]_{out}}$$

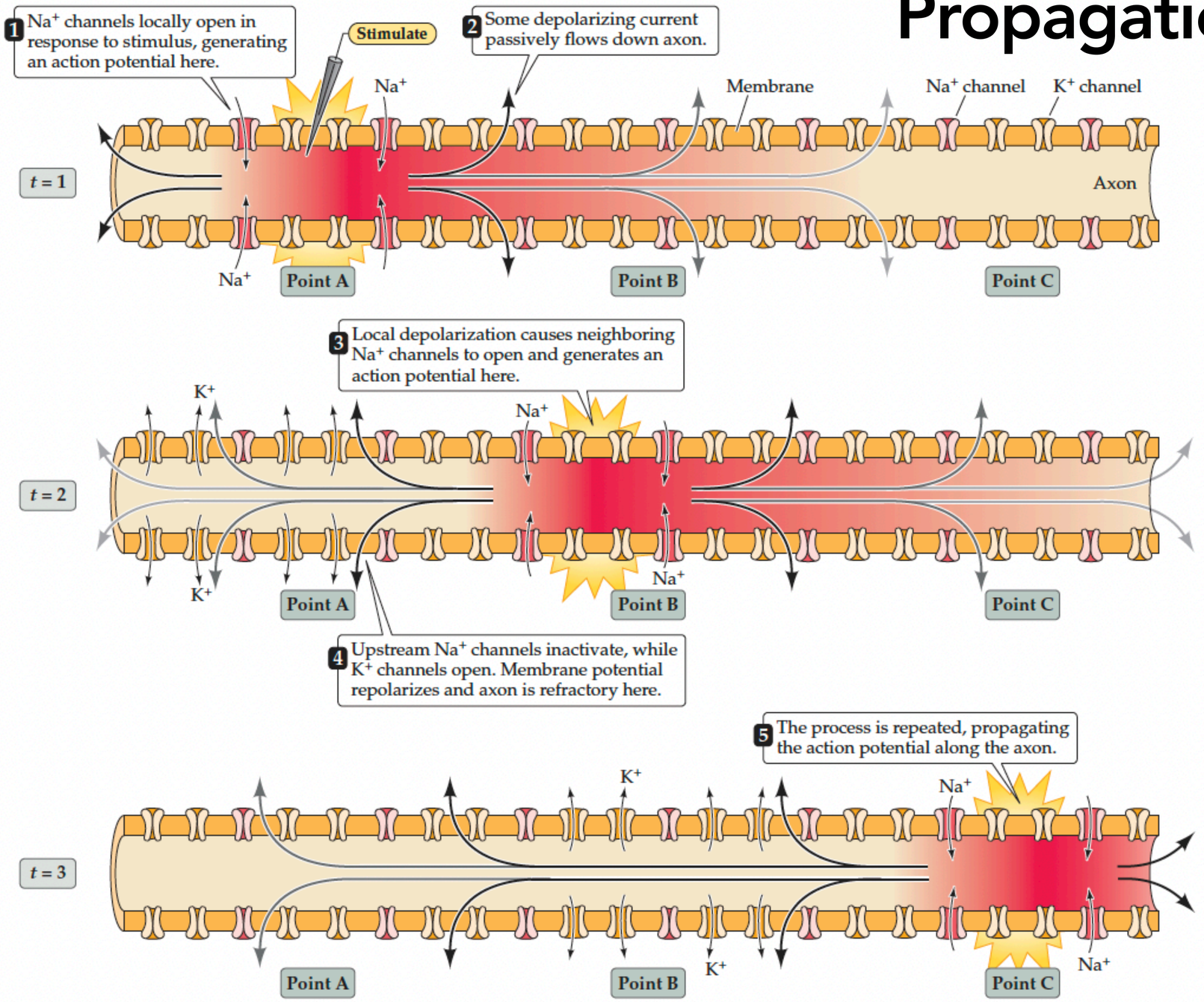
According to Goldman's equation the Membrane potential is close to Na^+ potential (+58 mV)

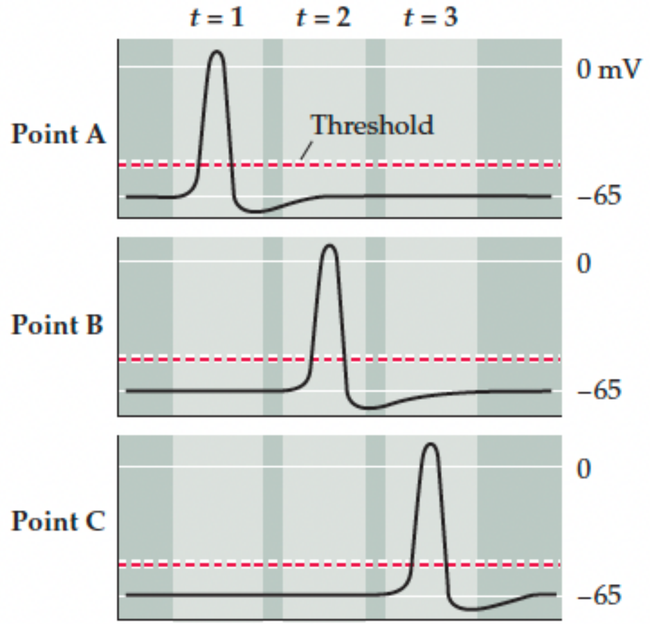
Decreasing extracellular Na^+ should reduce the membrane potential during an action potential and have no effect on the resting potential





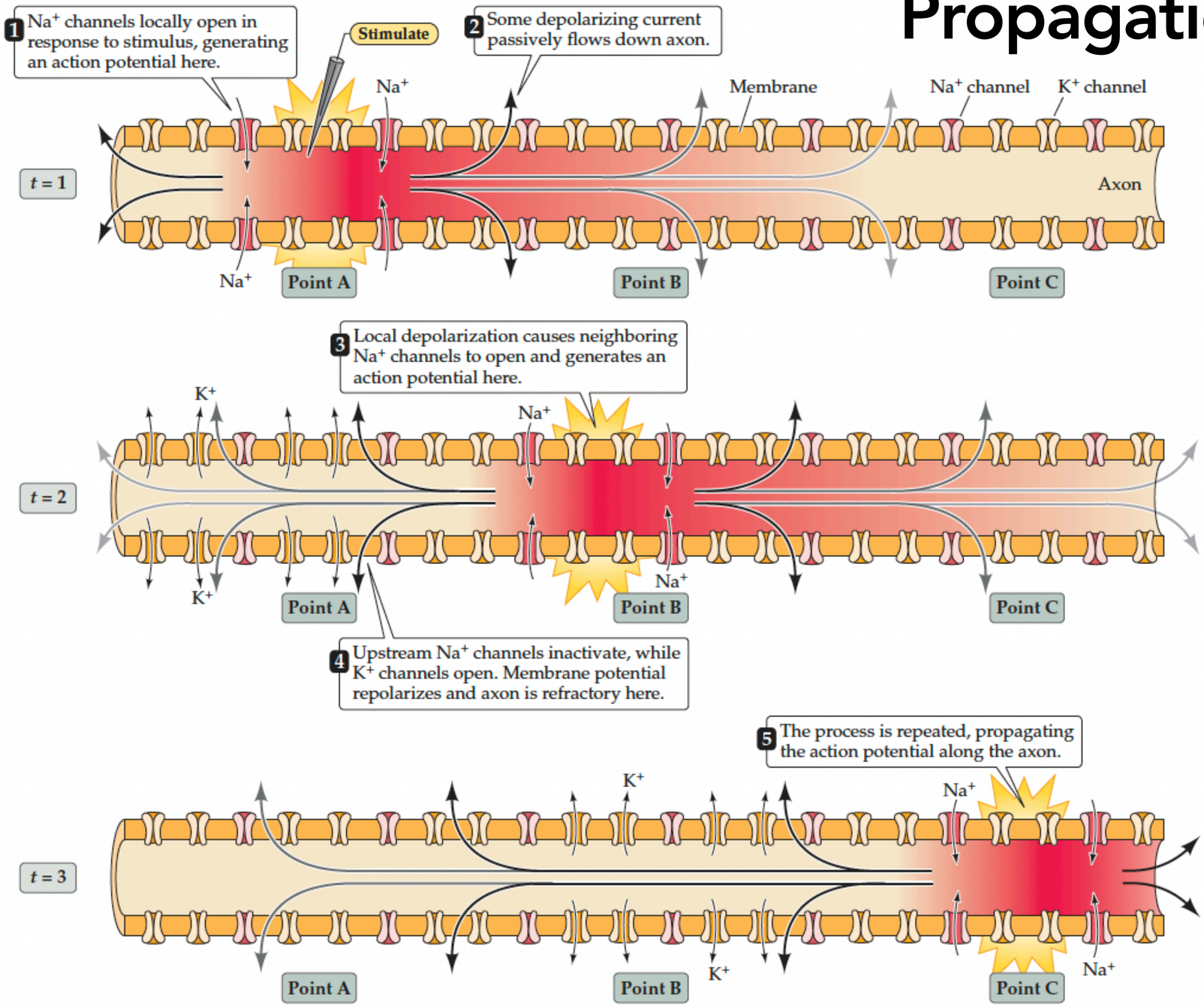
Propagation of action potentials





Action potentials spread with a directionality because the ion channels remain refractory

Propagation of action potentials



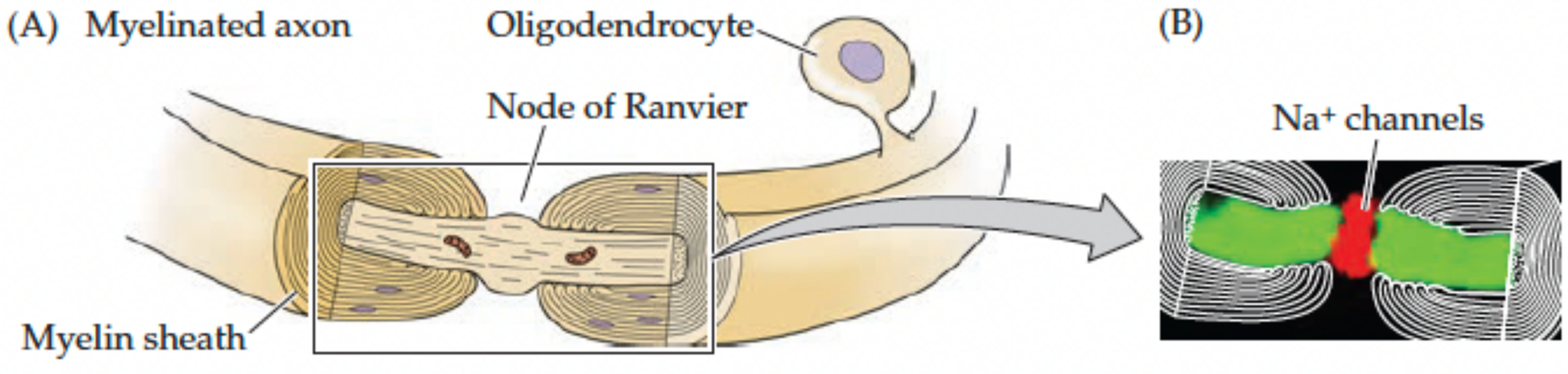
In the Squid neurons have a diameter of 500 um and action potentials spread at 25 m/s

That's good but how can it be improved?

Propagation of action potentials

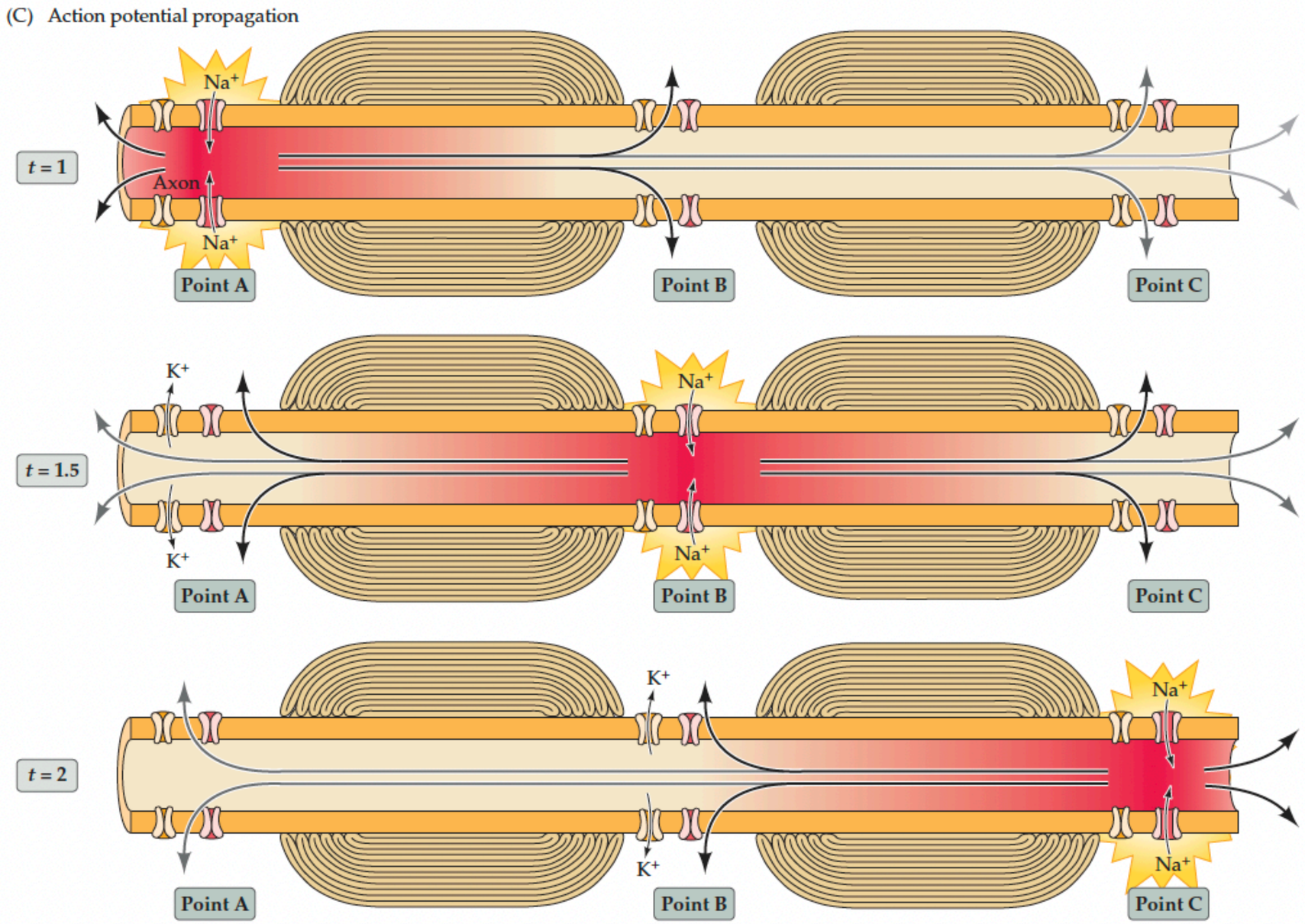
Mammalian neurons are only a few um in size and action potentials spread at velocities up to 120 m/s

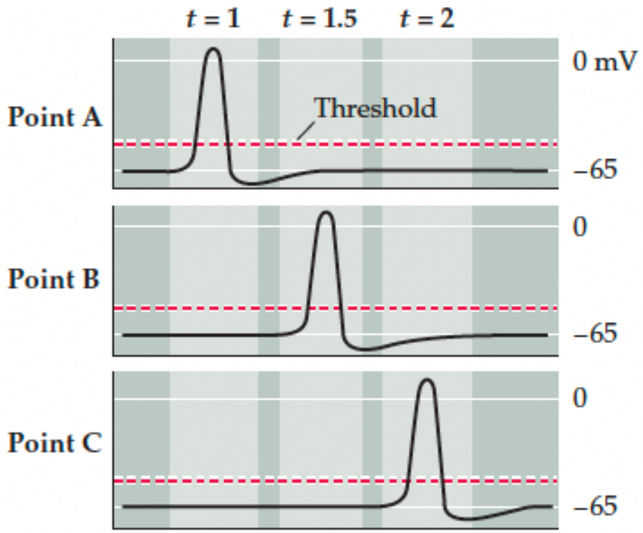
How does that work?



Myelin generated by oligodendrocytes isolates the neuron and leaves only few regions free (Nodes of Ranvier).

Propagation of action potentials

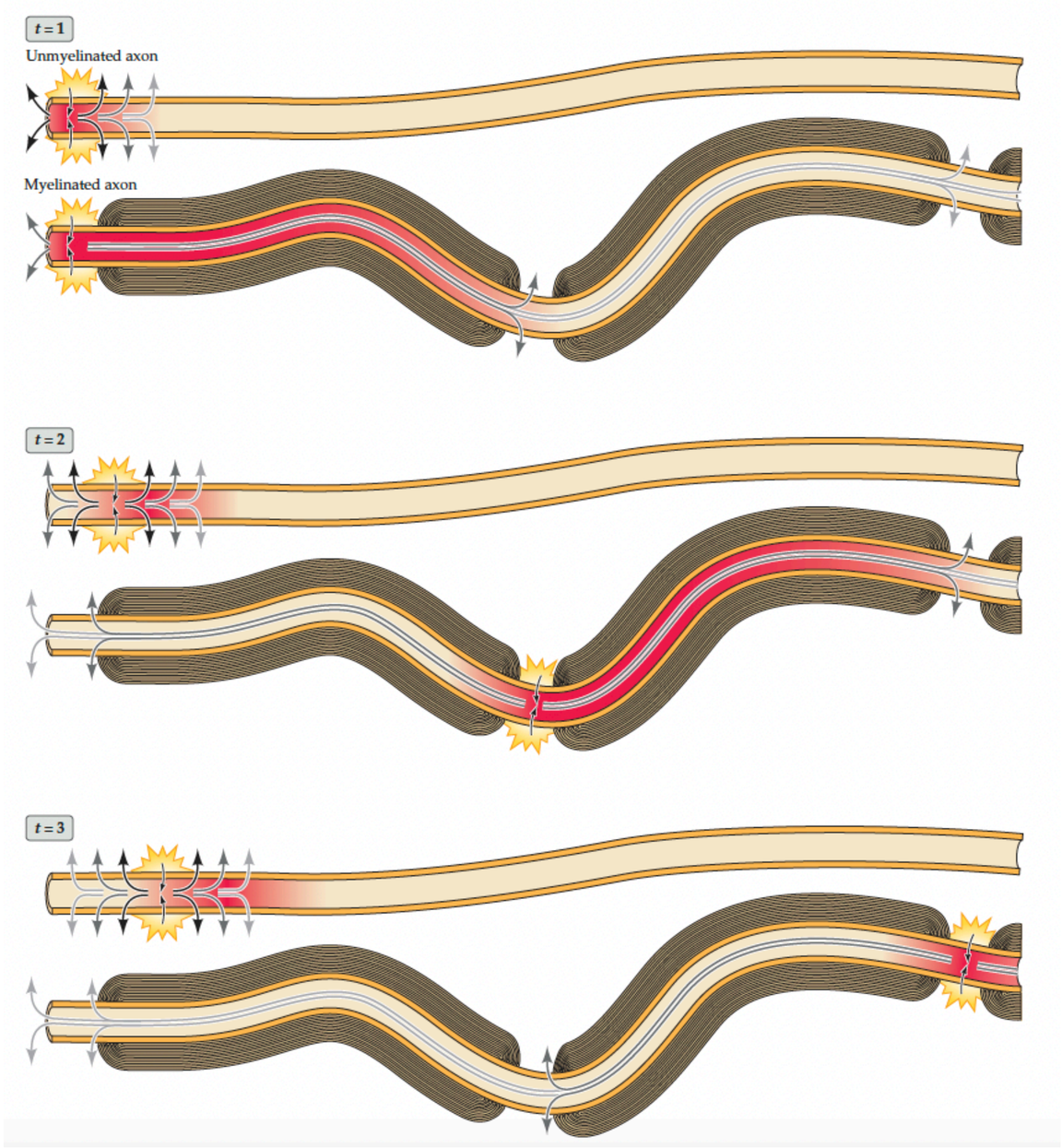




Action potentials only initiate at the Nodes of

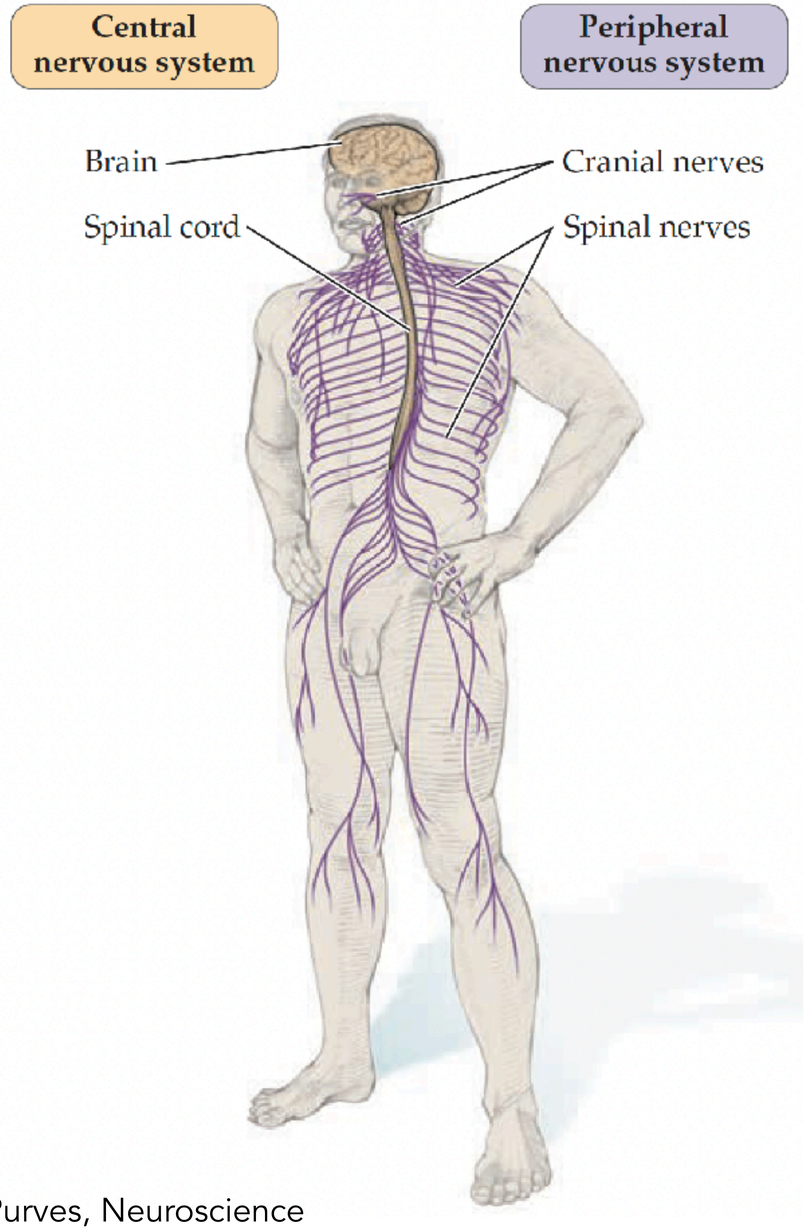
Saltatory Propagation

Saltatory propagation of action potentials is much faster



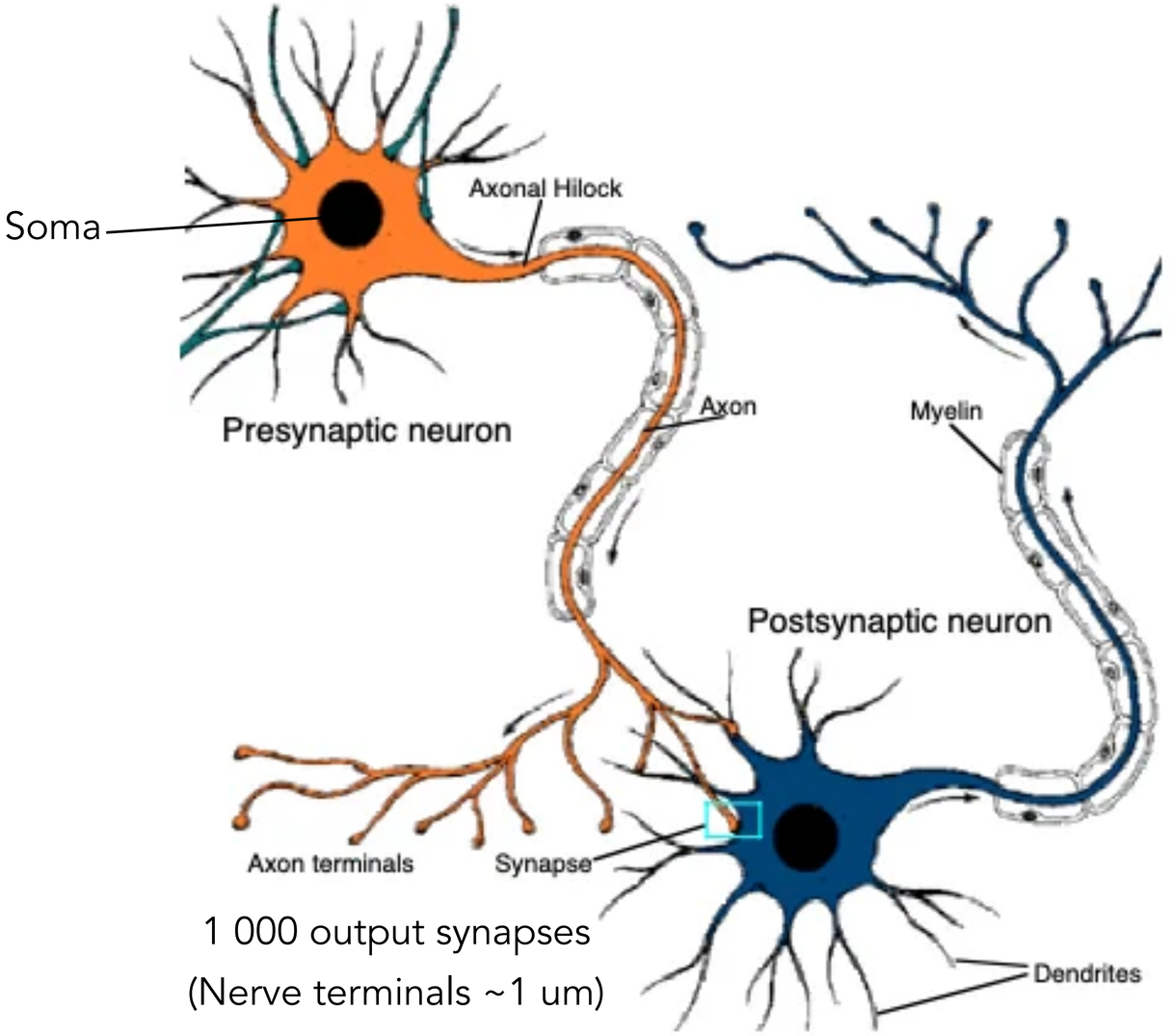
We will further look into the propagation of action potentials over distances next week.

How does information travel within the nervous system?



Purves, Neuroscience

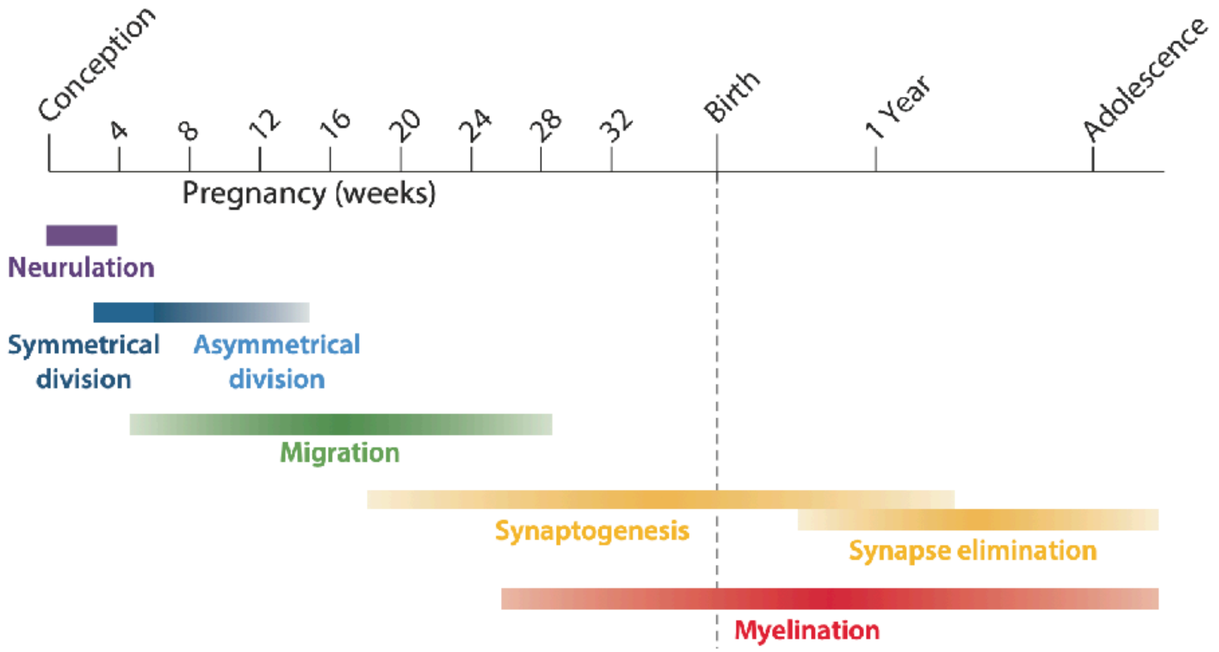
10 000 inputs to dendrites and soma (receive)



1 000 output synapses
(Nerve terminals ~1 um)

https://insidelearningmachines.com/neural_networks_explained/

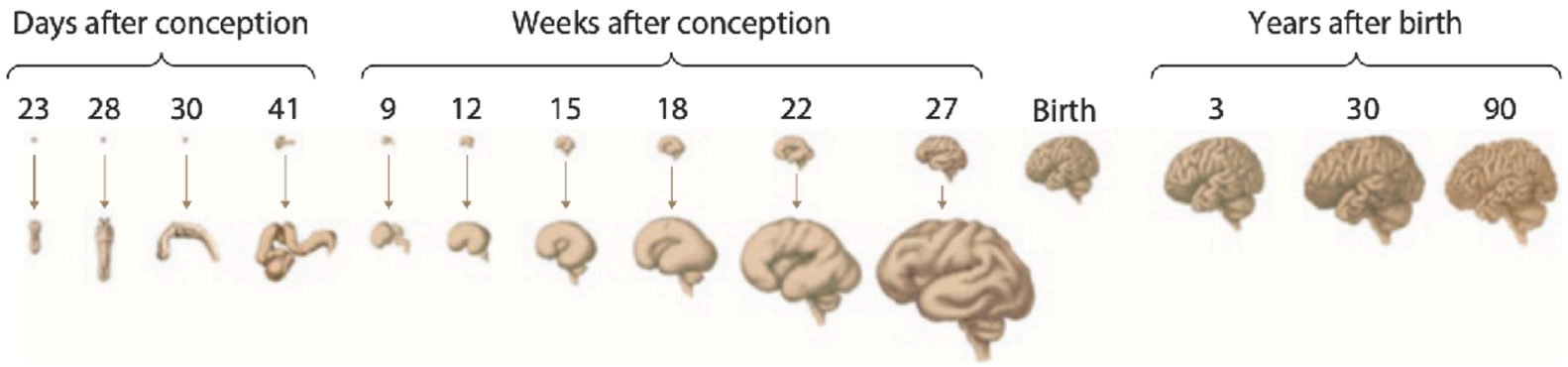
How are neurons connected? The chemical synapse



Most synapses are made in the third trimester of development to connect active neurons.

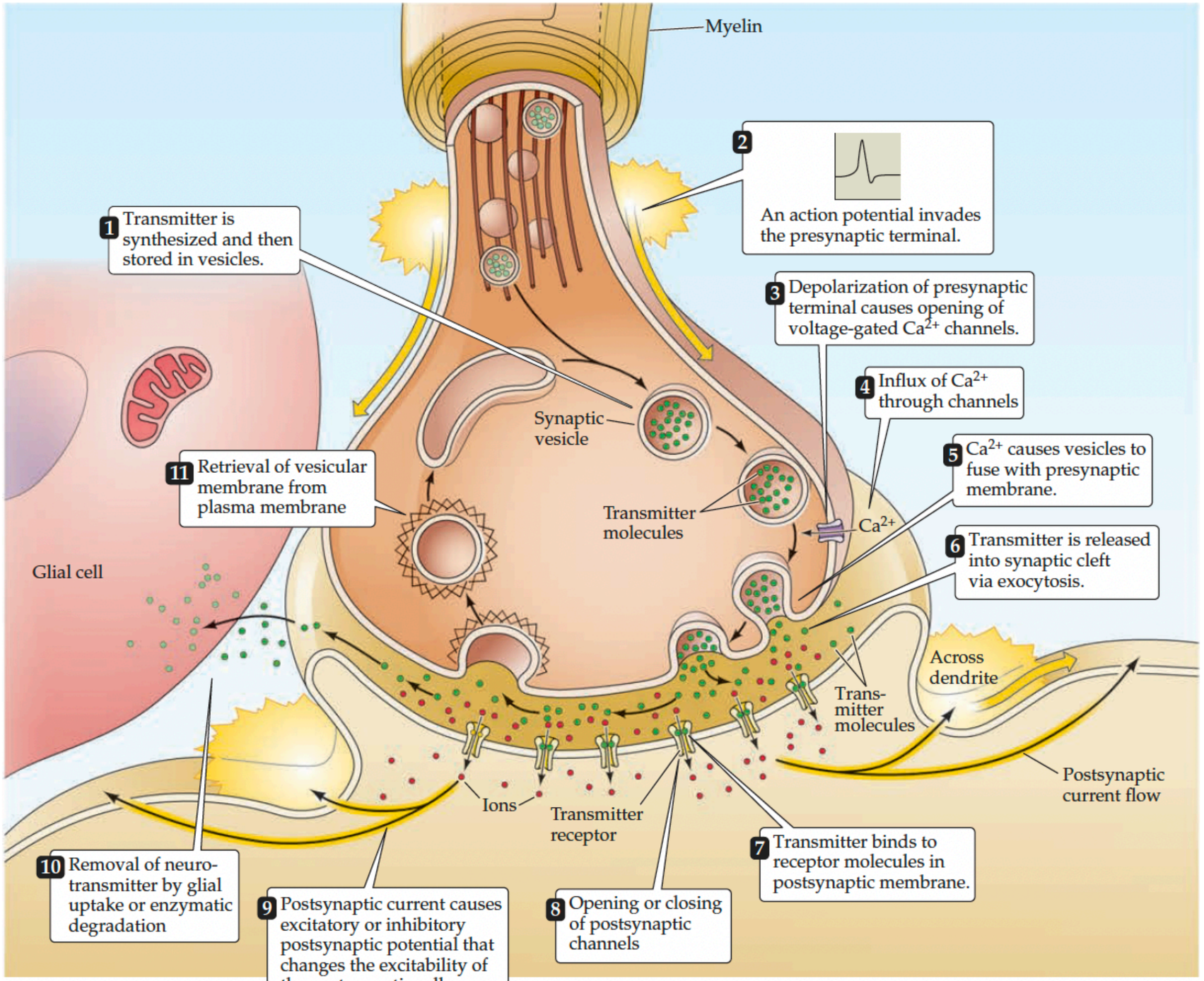
Synapses are then pruned after birth.

Most of the myelination happens after birth.



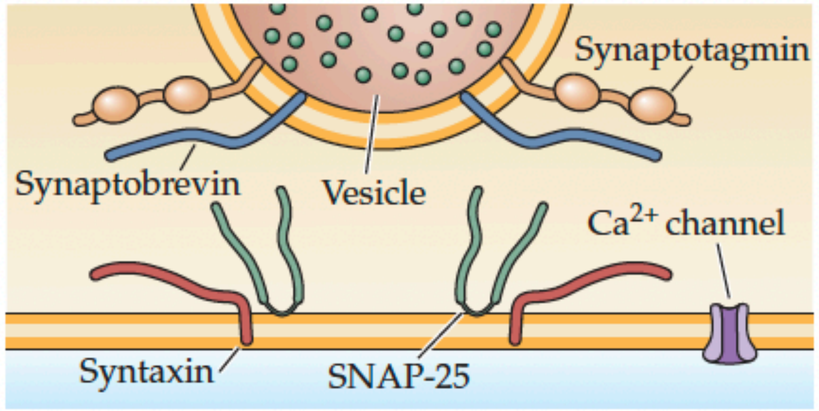
Faust, Gunner, Schafer (2021) Nature Reviews Neuroscience

Processes at the chemical synapse

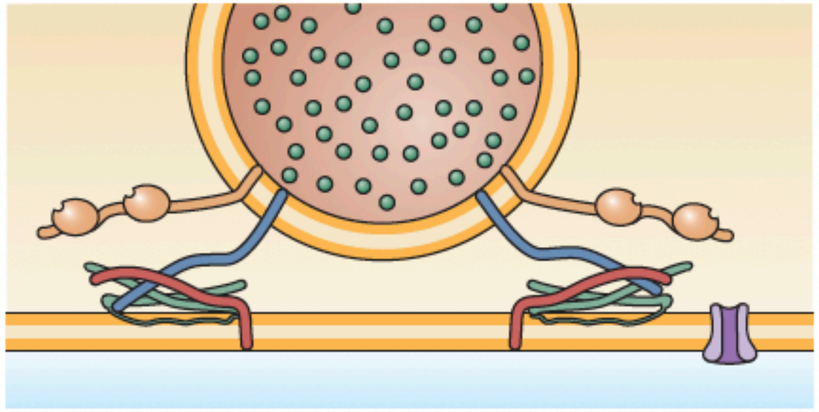


Exocytosis of neurotransmitter vesicles

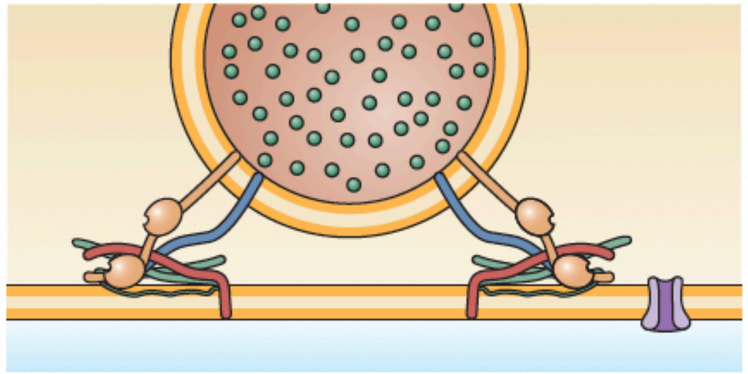
(1) Free SNARES on vesicle and plasma membranes



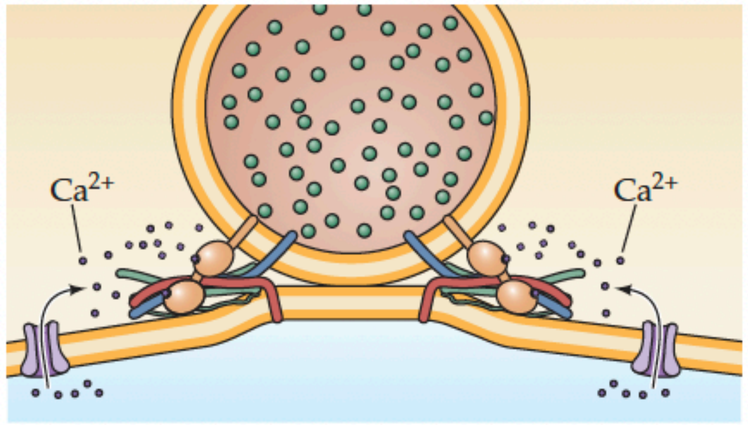
(2) SNARE complexes form as vesicle docks



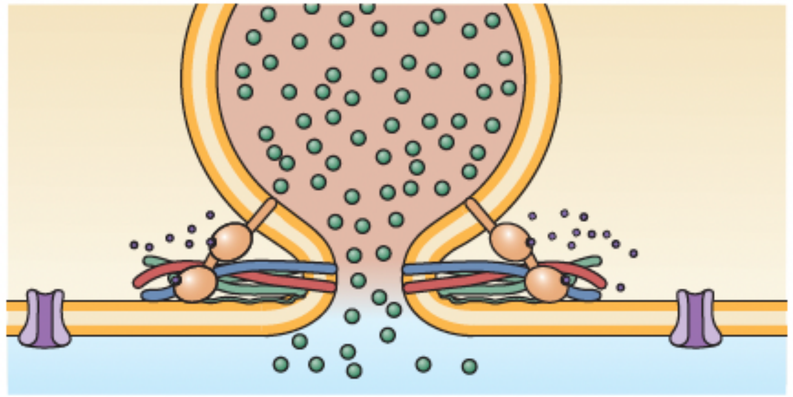
(3) Synaptotagmin binds to SNARE complex



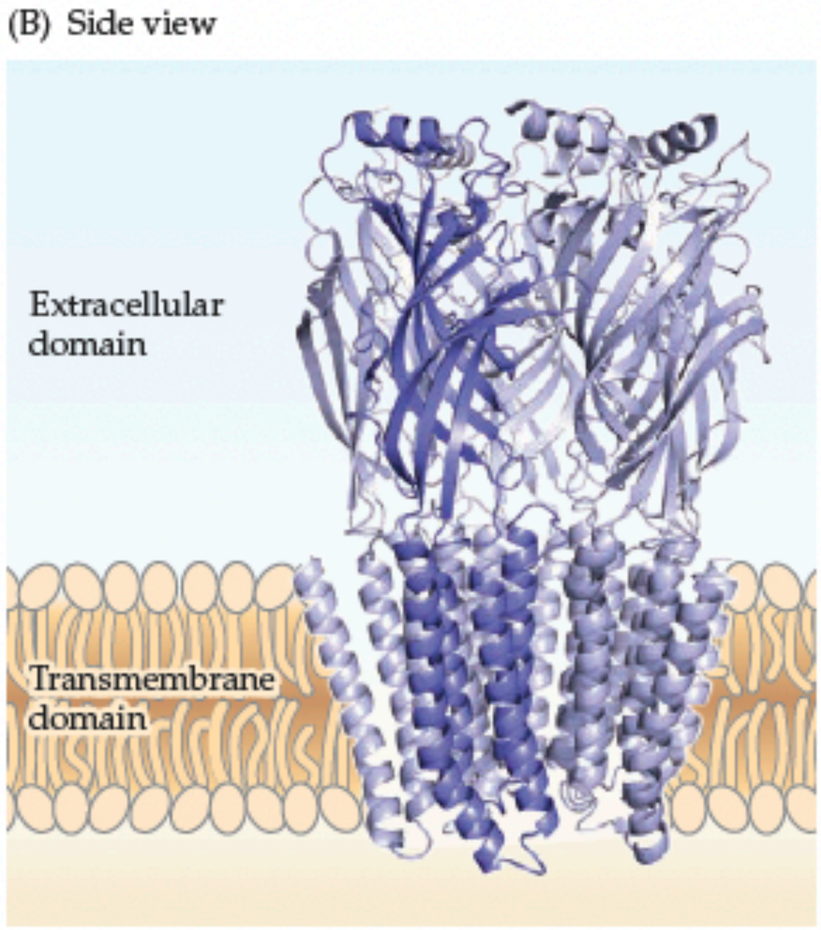
(4) Entering Ca^{2+} binds to synaptotagmin, leading to curvature of plasma membrane, which brings membranes together

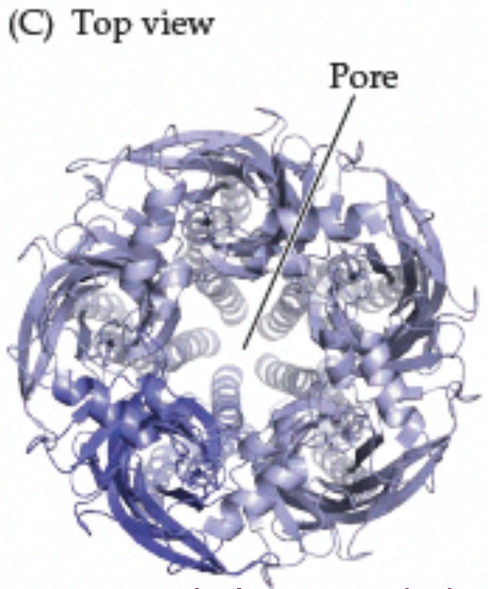


(5) Fusion of membranes leads to exocytotic release of neurotransmitter



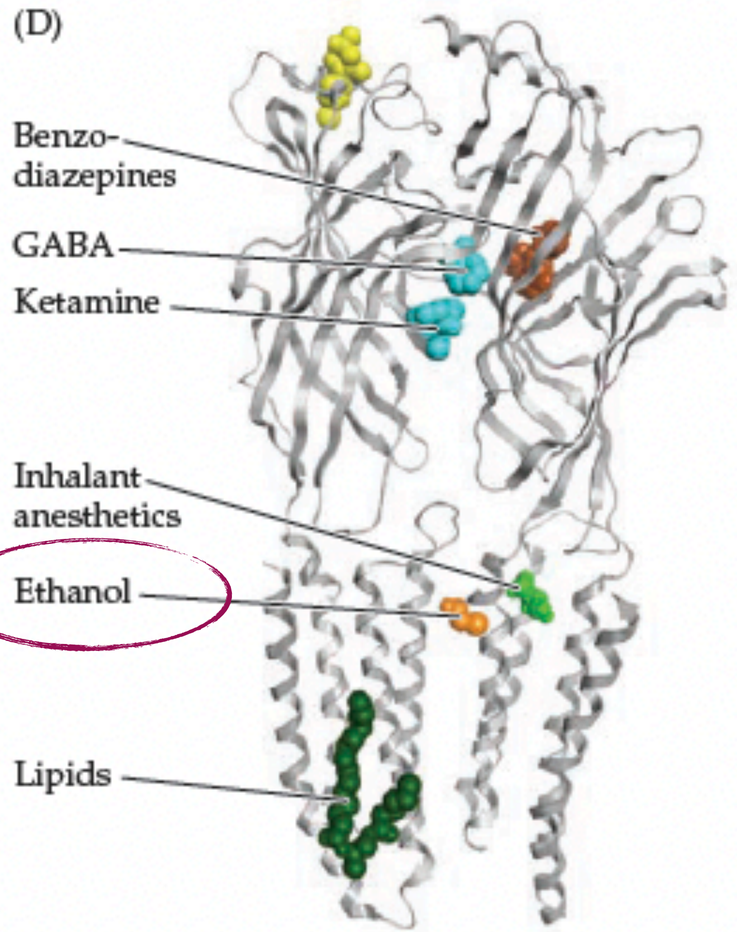
Ionotropic GABA receptors have many binding sites for modulators





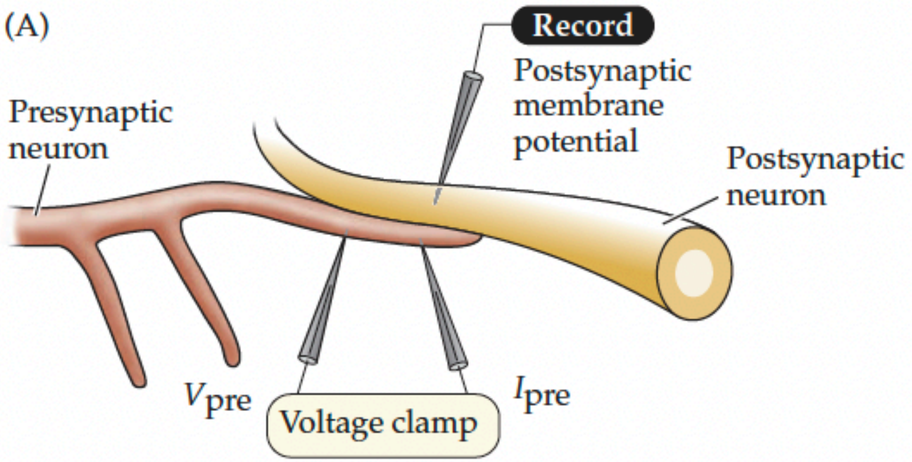
Modulates inhibitory signalling

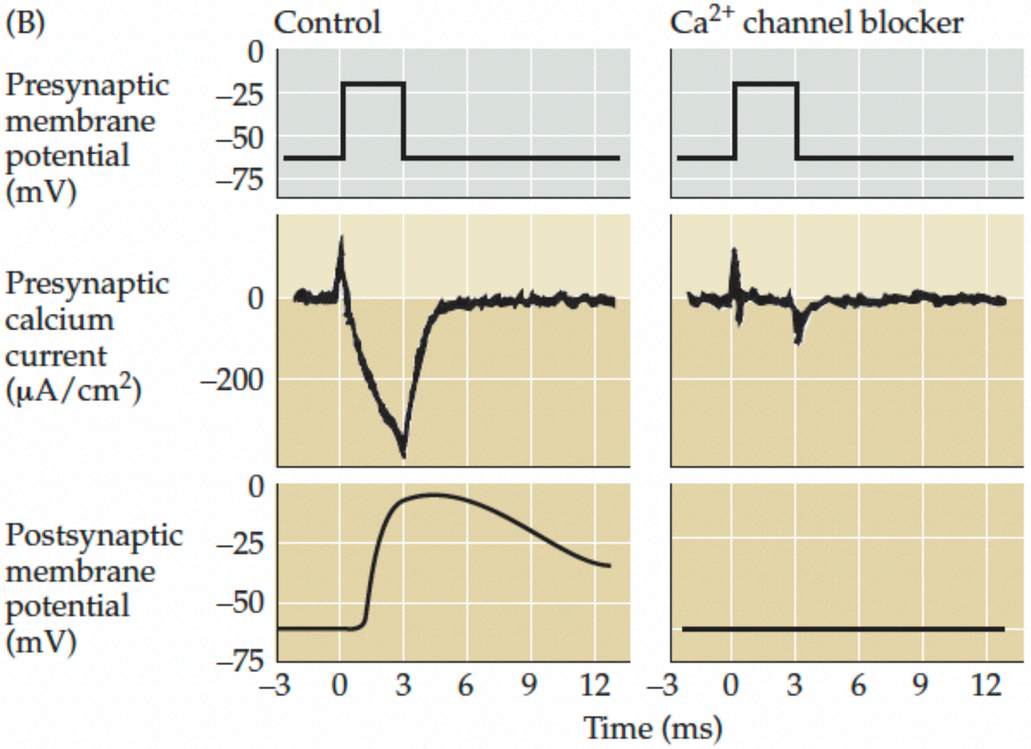
GABA opens Cl- channels which leads to inhibition of transmission



We will talk more about this in the last lecture about Neuropharmacology

Calcium is needed for transmission at the synapse

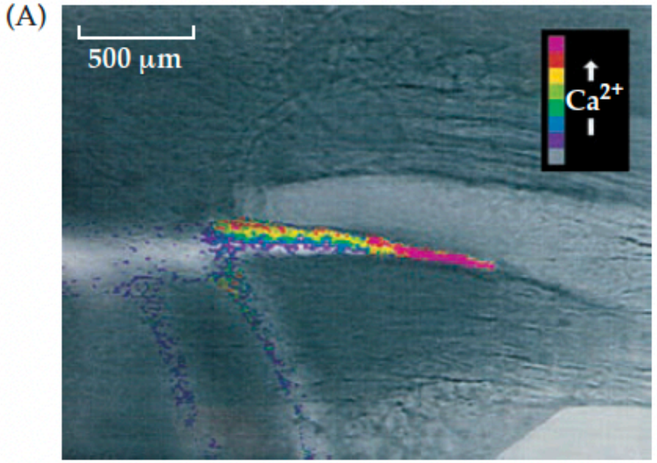




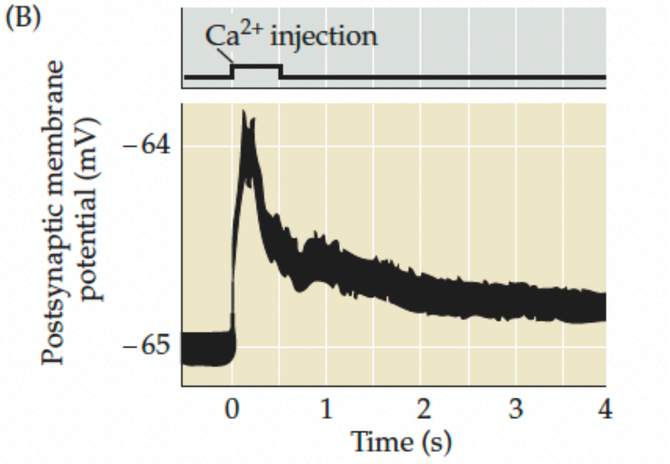
Calcium is needed to allow the fusion of neurotransmitter-filled vesicles at the presynaptic membrane

Calcium is needed for transmission at the synapse

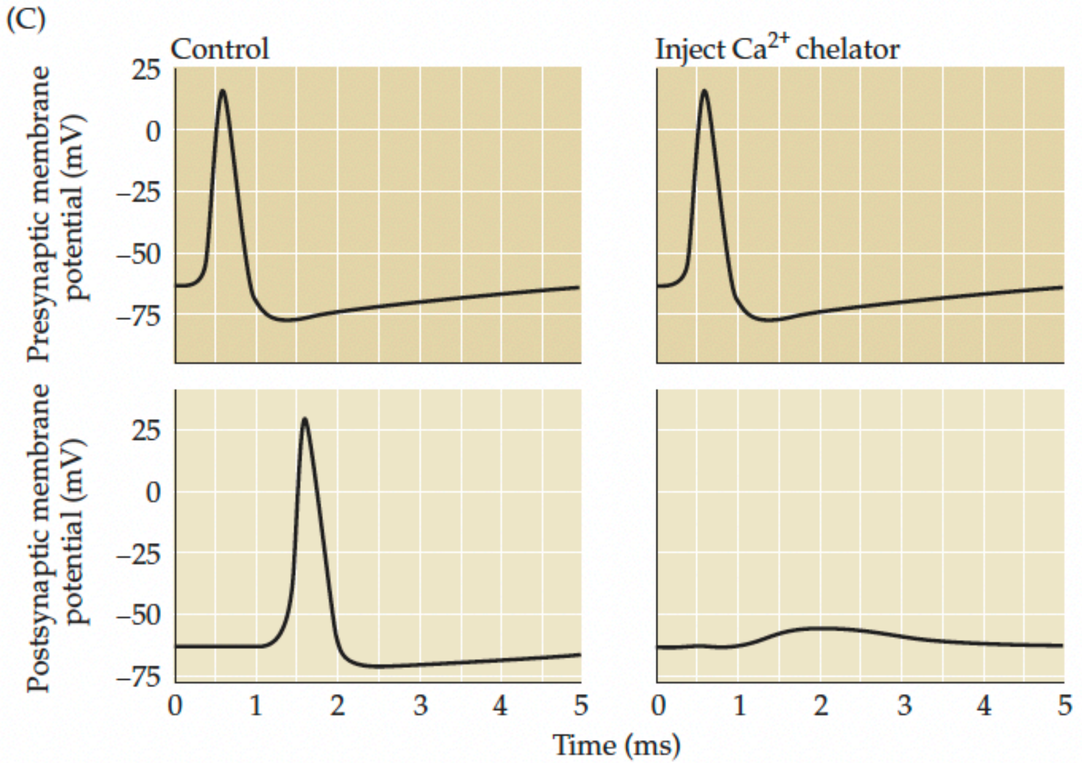
Different experimental evidences



A calcium wave arrives at the synapse before the release of neurotransmitters

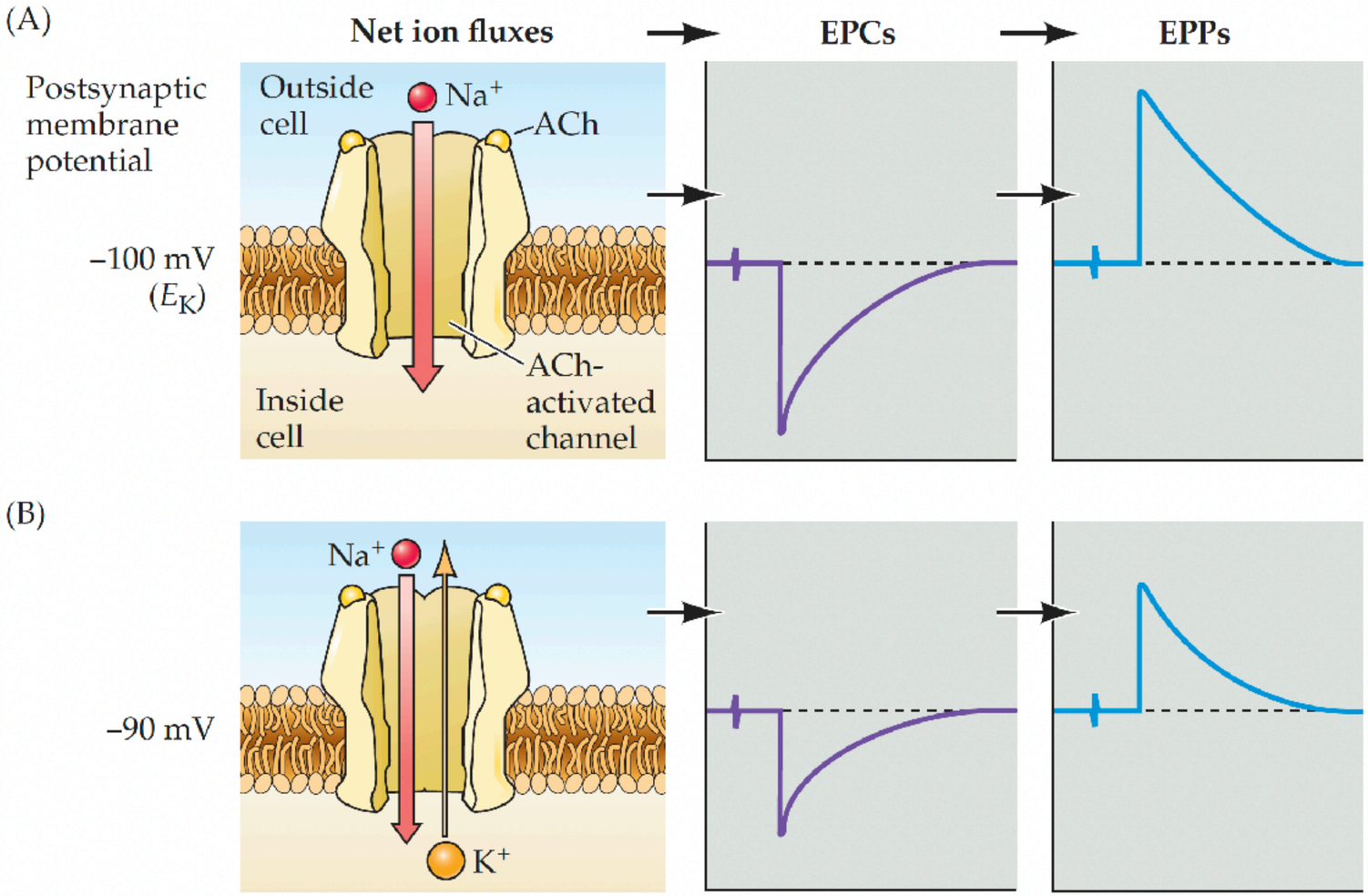


Calcium injection causes depolarization at the postsynaptic membrane



Calcium binding agents (chelator) abolishes signal transmission to the postsynaptic membrane

Testing the role of individual ions in generation the post-synaptic potential through patch clamp



Similar setup to voltage clamp experiments from last week!

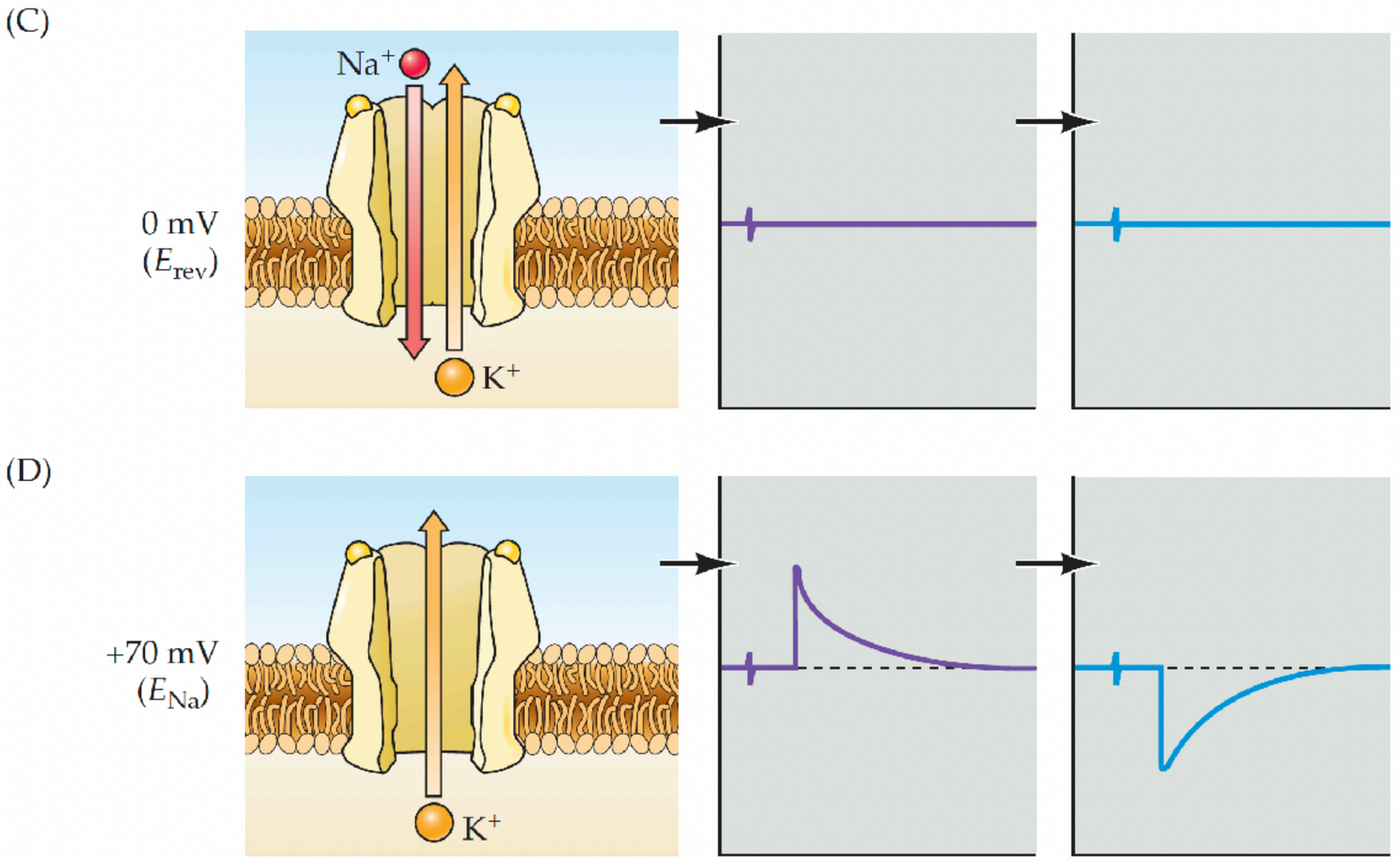
At -100 mV there is a strong driving force on Na+ to enter the cell.

Inward current - depolarisation at the postsynaptic membrane.

At -90 mV there is a strong driving force on Na+ to enter the cell and a small driving force on K+ to exit the cell.

Inward current - depolarisation at the postsynaptic membrane.

Testing the role of individual ions in generation the post-synaptic potential through patch clamp



Similar setup to voltage clamp experiments from last week!

At 0 mV the membrane is at the **reversal potential**.

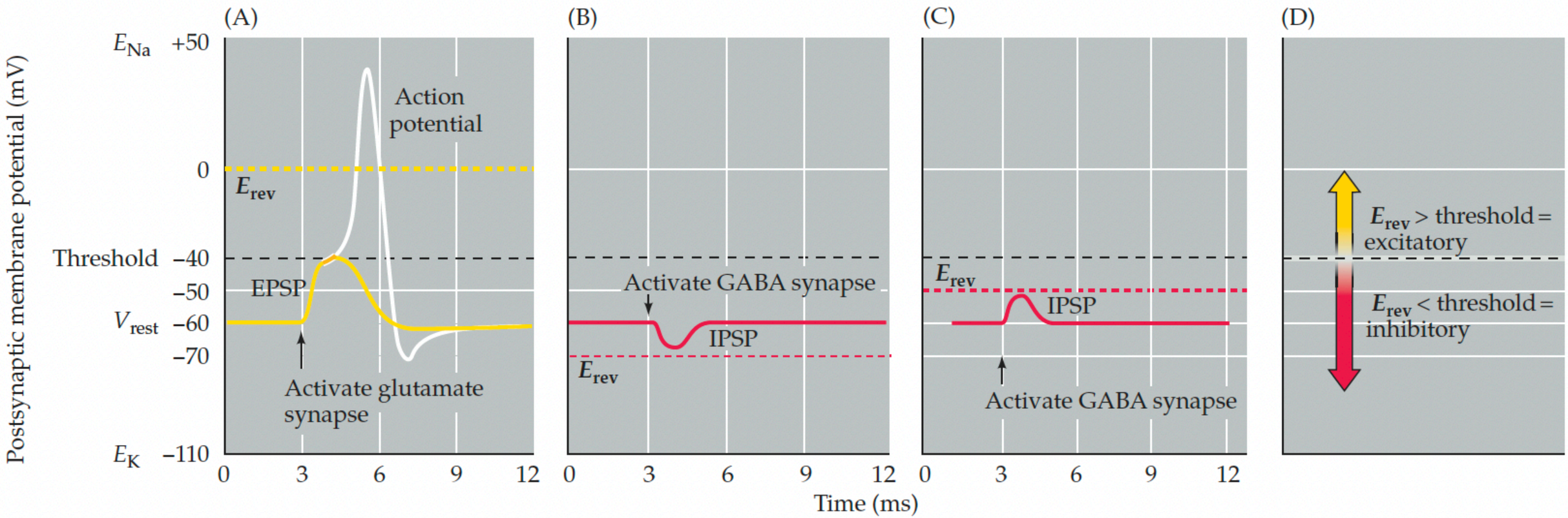
All ions are in electrochemical equilibrium - no movement!

At +70 mV there is a strong driving force on K+ to exit the cell.

Outward current - hyperpolarisation at the postsynaptic membrane.

NO ACTION POTENTIAL = INHIBITION

Generation of excitatory and inhibitory postsynaptic potentials

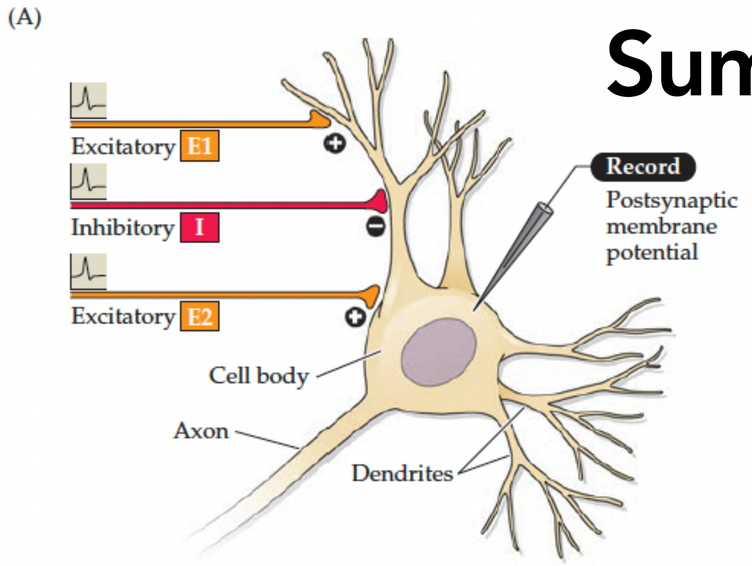


The general rule of postsynaptic action is:

If the reversal potential is more positive than threshold, excitation results; inhibition occurs if the reversal potential is more negative than threshold.

Reversal potential depends on the synapse and the channel composition

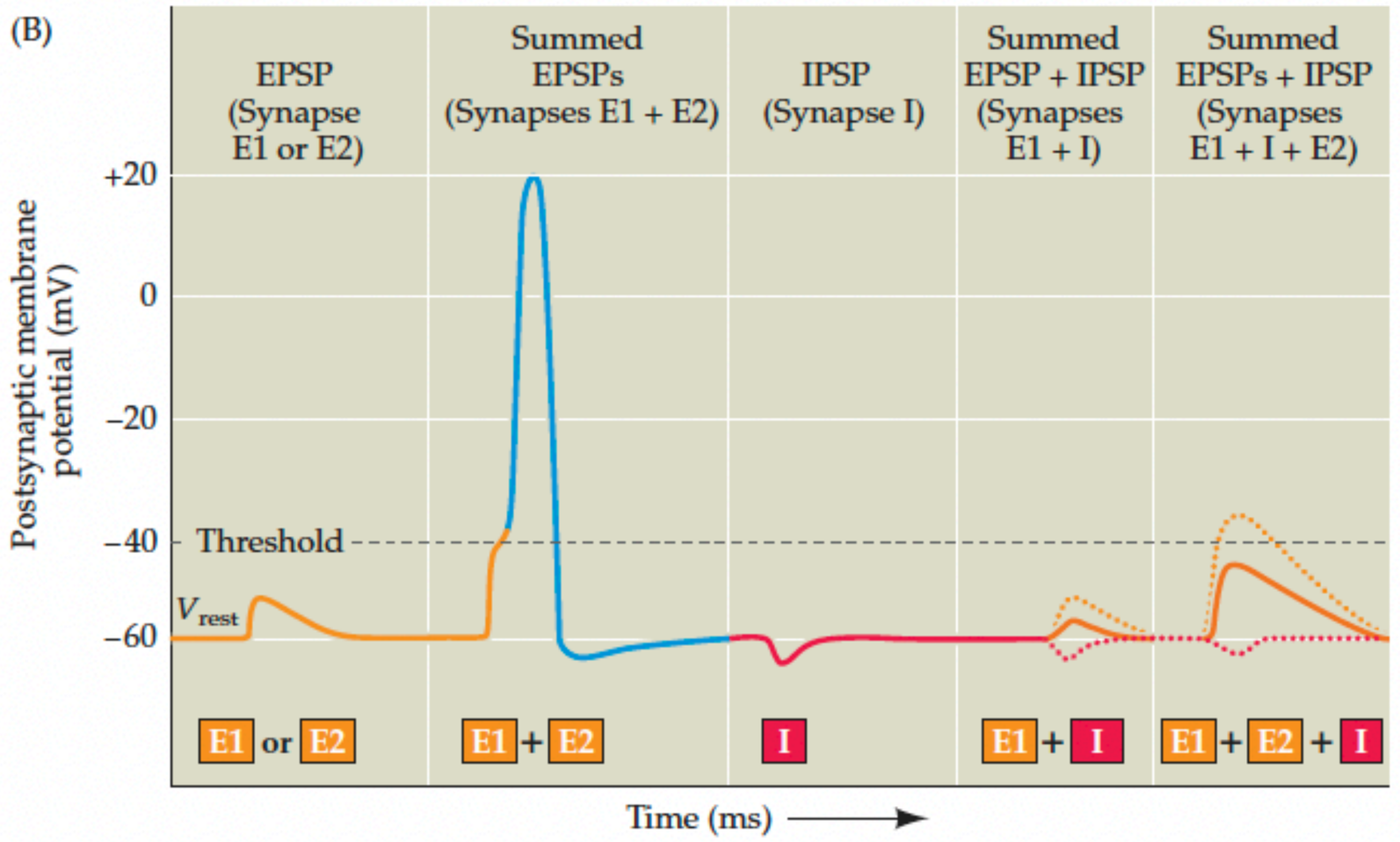
Summation of excitatory and inhibitory signals



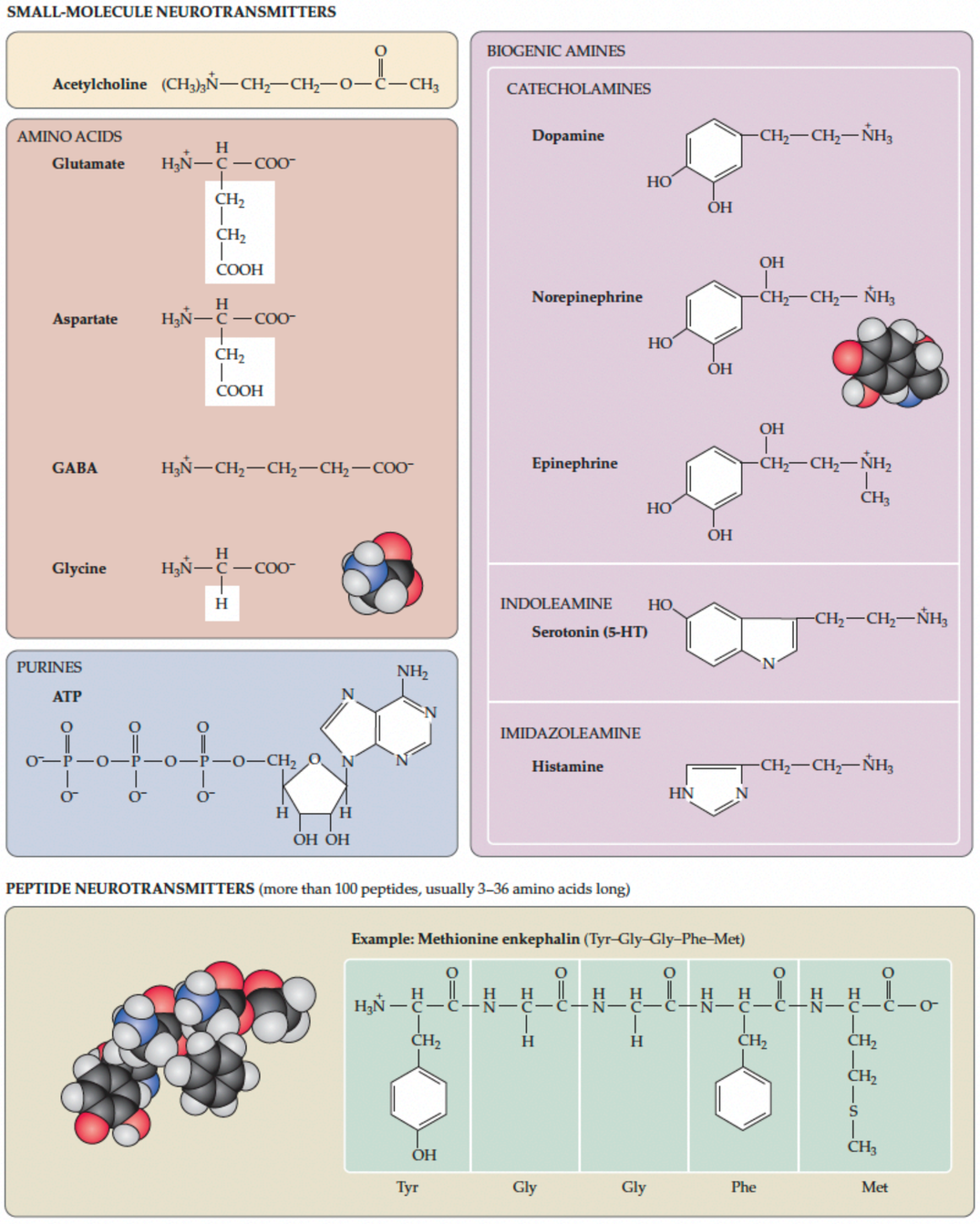
An excitatory signal at one of the many synapses of a neuron is often not enough to trigger an action potential and signal transmission!

Summation of excitatory (and inhibitory signals) is needed.

Dashed line individual signals.



Overview of common neurotransmitters and their action



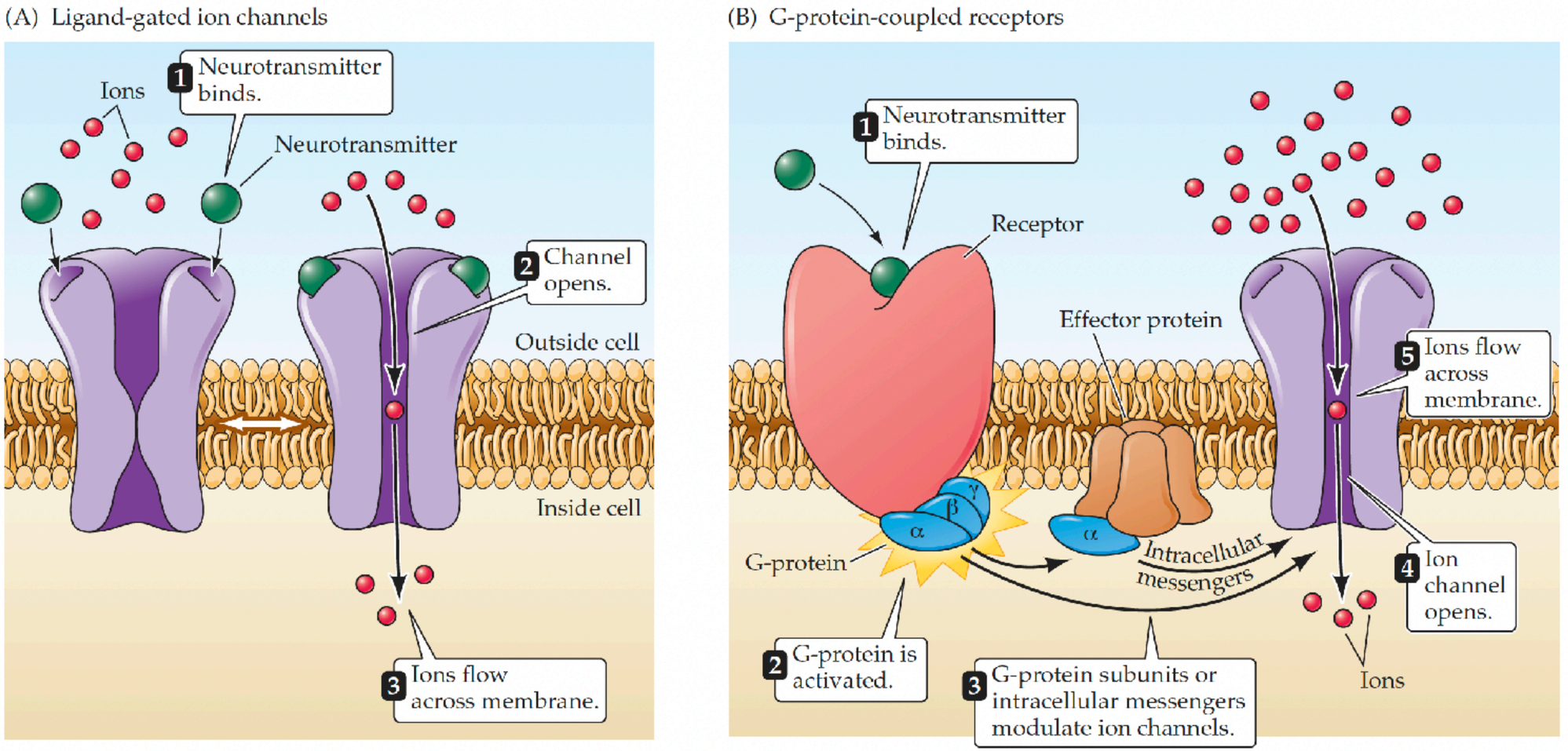
Neurotransmitter	Postsynaptic effect[a]
ACh	Excitatory
Glutamate	Excitatory
GABA	Inhibitory
Glycine	Inhibitory
Catecholamines (epinephrine, norepinephrine, dopamine)	Excitatory
Serotonin (5-HT)	Excitatory
Histamine	Excitatory
ATP	Excitatory
Neuropeptides	Excitatory and inhibitory
Endocannabinoids	Inhibits inhibition
Nitric oxide	Excitatory and inhibitory

Remember!

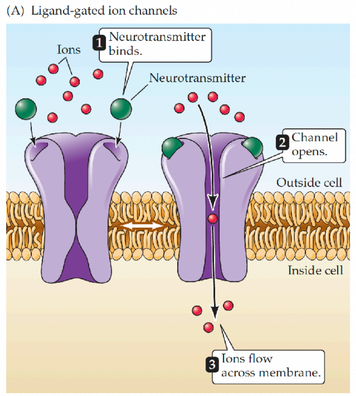
Two classes of transmitter receptors at the postsynaptic membrane

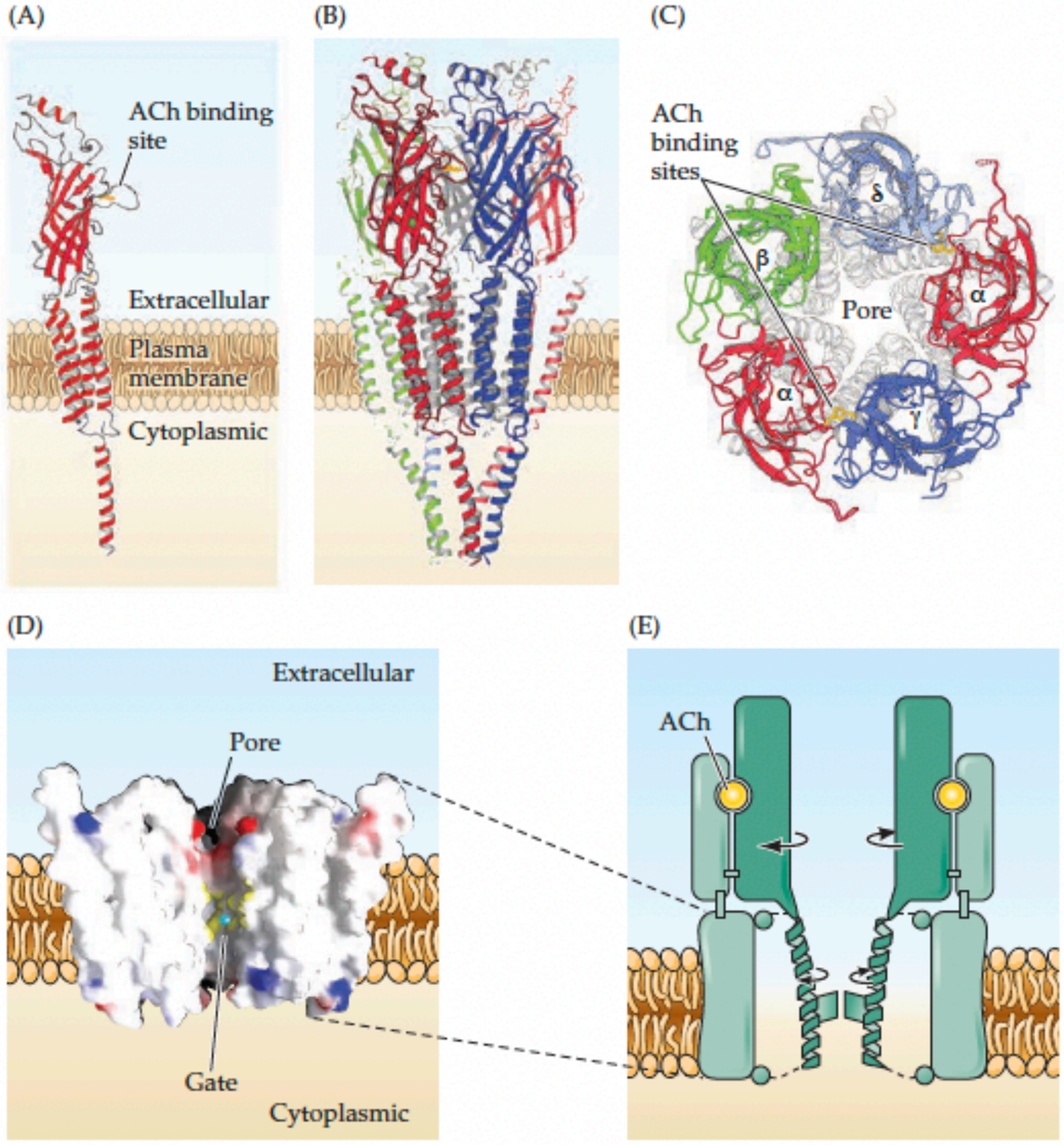
Ionotropic receptors directly bind to ion channel and cause opening

Metabotropic rely on an intermediate signalling step to open an ion channel



Ionotropic transmitter receptors



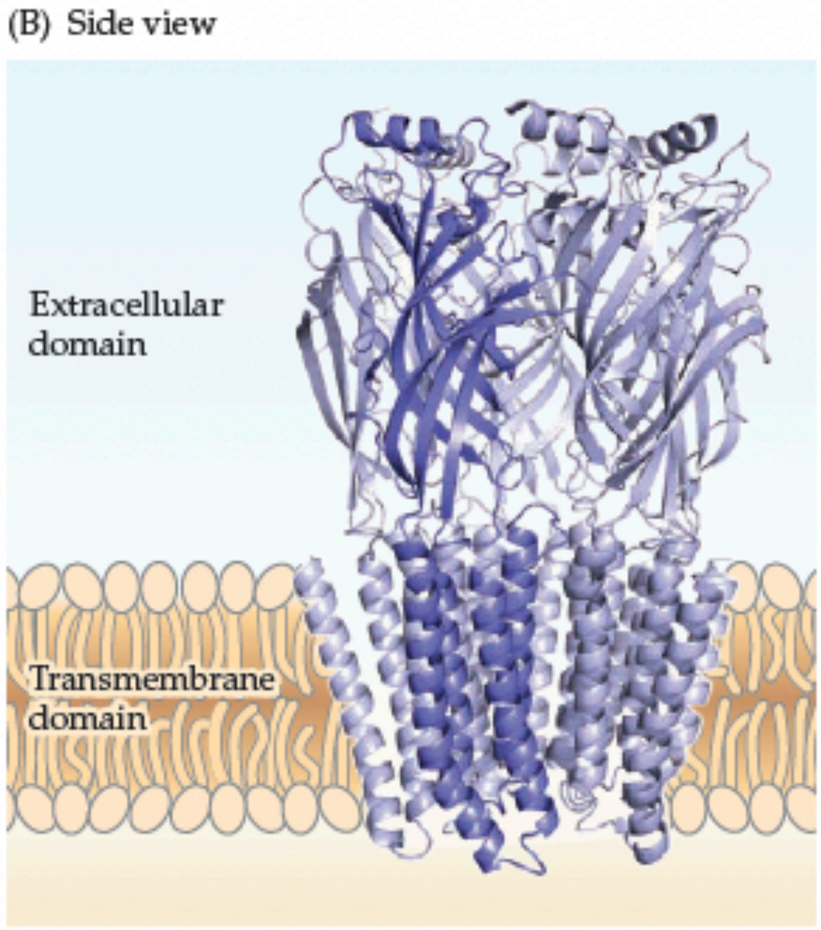


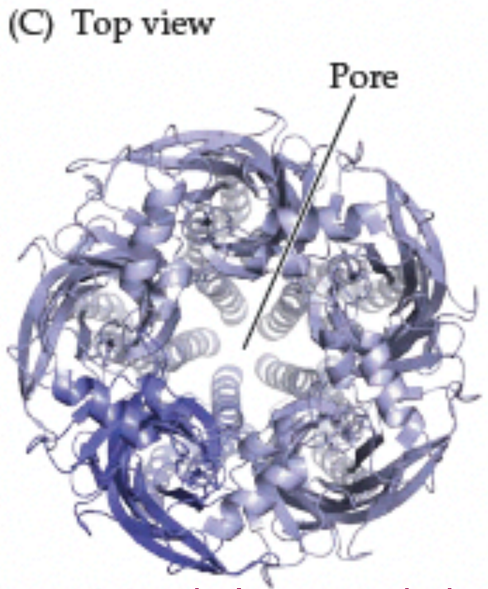
Nicotine is an agonist of the receptor

The nicotinic Acetylcholine receptor is a ionotropic receptor.

This receptor becomes permeable to Na+ and K+ upon ACh binding.

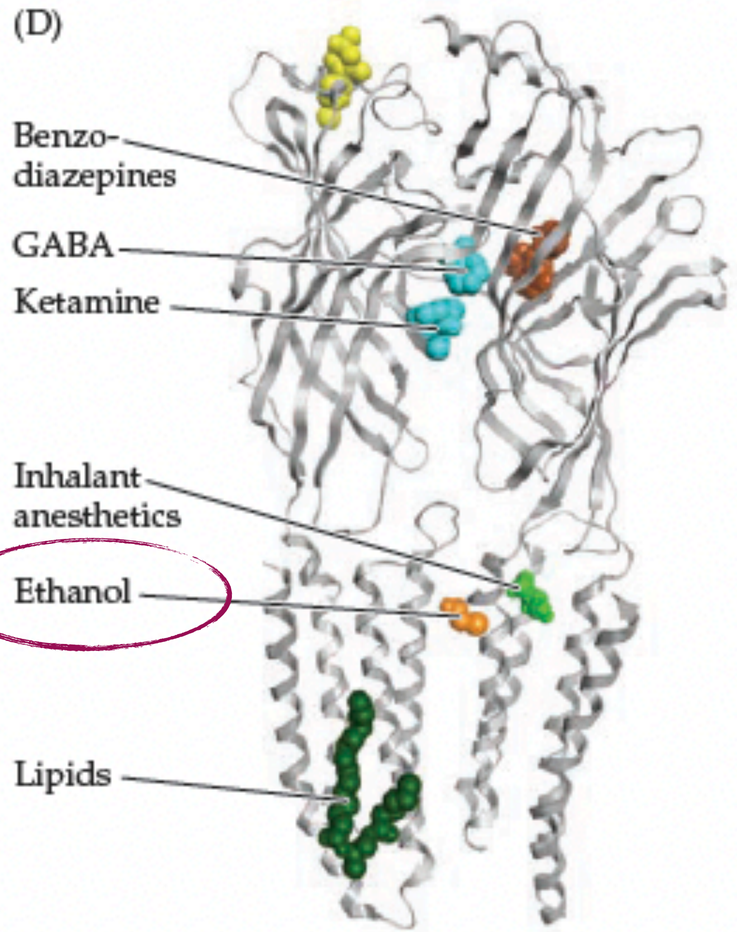
Ionotropic GABA receptors have many binding sites for modulators





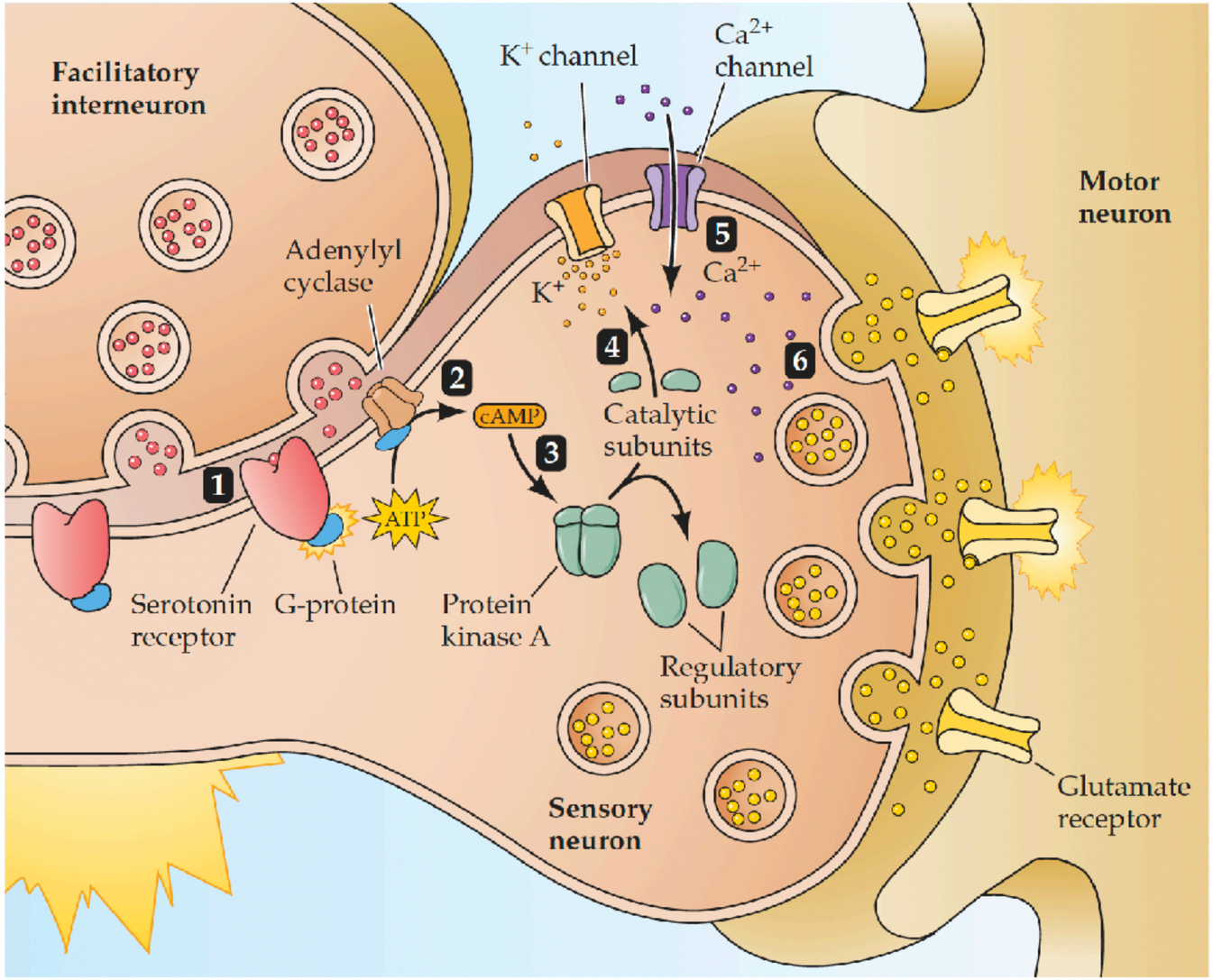
Modulates inhibitory signalling

GABA opens Cl- channels which leads to inhibition of transmission



We will talk more about this in the last lecture about Neuropharmacology

Short-term sensitization and synaptic enhancement



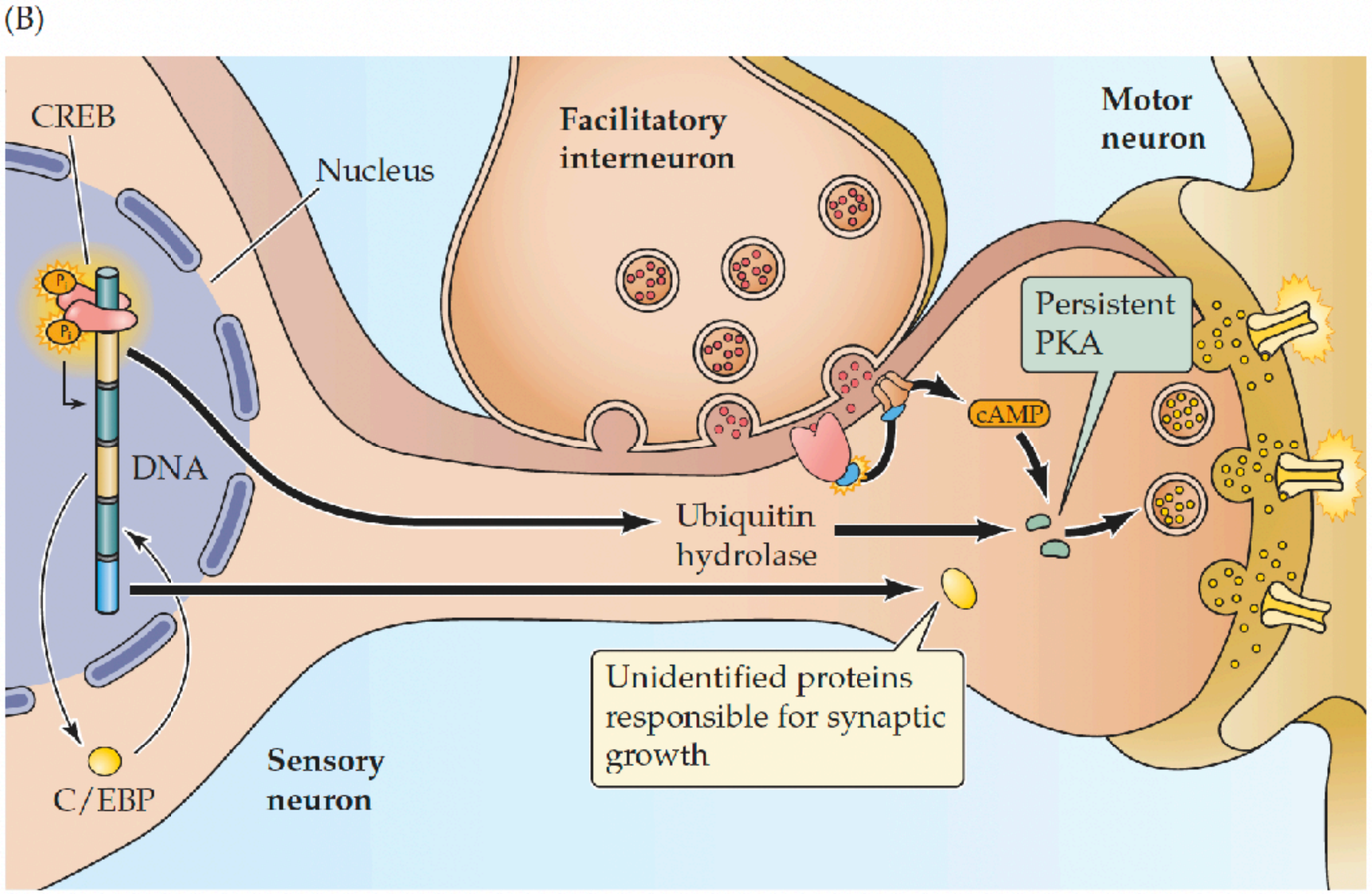
The motorneuron is activated by the summed synaptic potentials of the inter and sensory neurons.

The sensory neuron releases serotonin which activates a G-protein coupled receptor leading to increase in cAMP.

cAMP activates Protein kinase A (PKA), which inhibits K+ channels and increases the influx of Calcium.

Sustained calcium increases the amount of neurotransmitters being released which leads to a stronger postsynaptic potential.

Long-term sensitization and synaptic enhancement



PKA activates CREB - a transcriptional regulator that binds to specific target motifs in the DNA CREs (cAMP responsive elements)

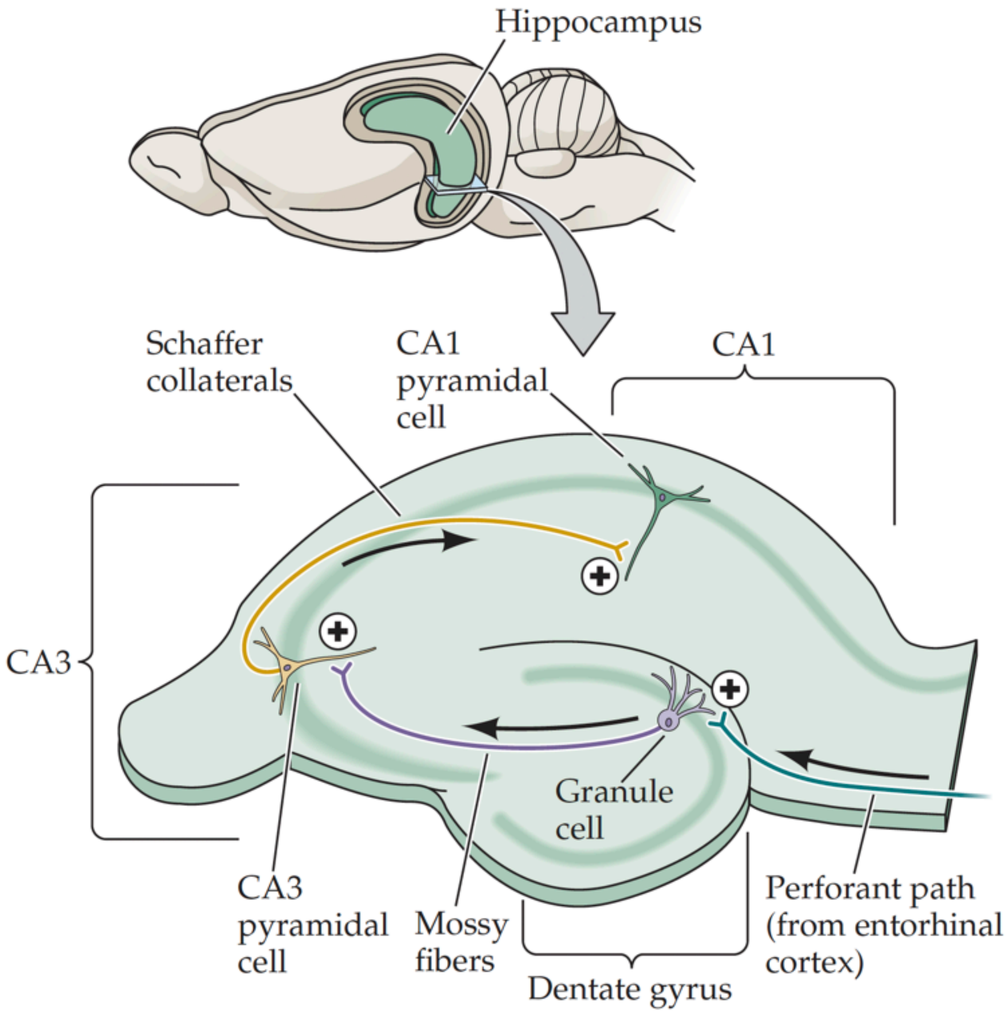
Two identified genes are now transcribed: Ubiquitin hydrolase and C/EBP.

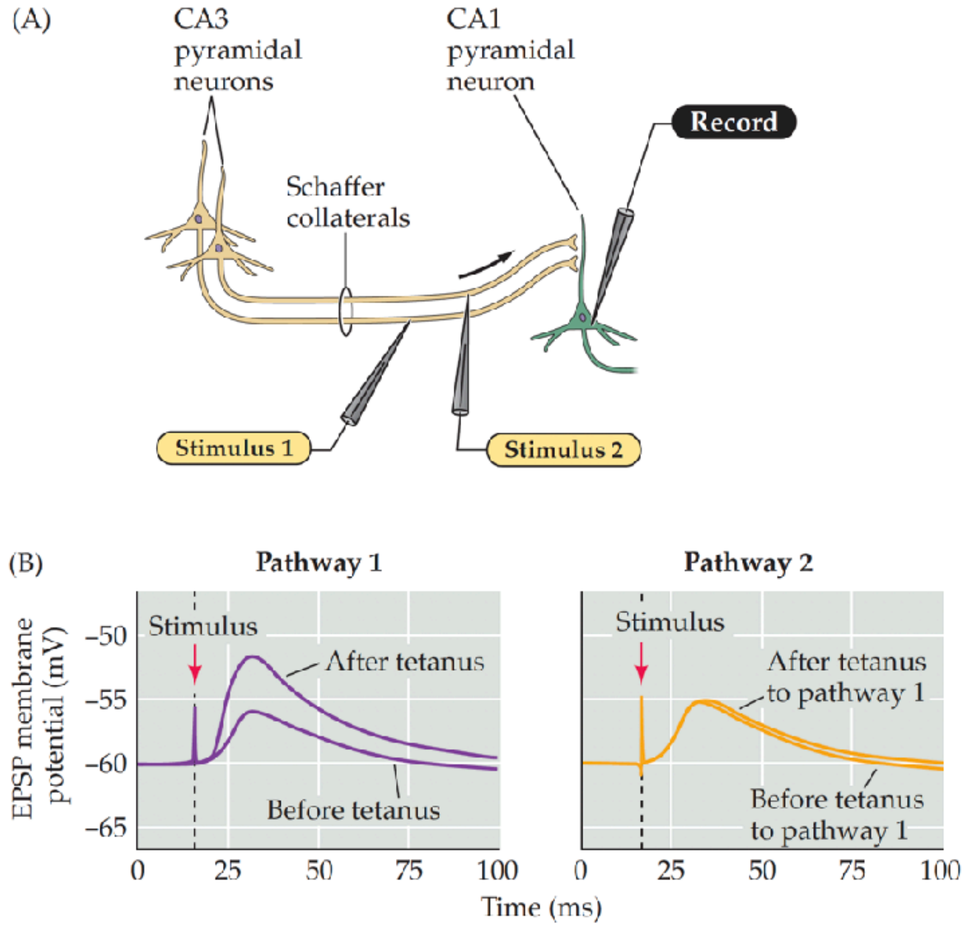
C/EBP activates the transcription of various genes involved in synaptic growth.

Ubiquitin hydrolase removes the regulatory subunit of PKA, making PKA active of an extended time, facilitating Calcium signalling.

Blocking protein synthesis block long-term sensitization.

Long-term potentiation (LTP) and depression (LTD) in the hippocampus

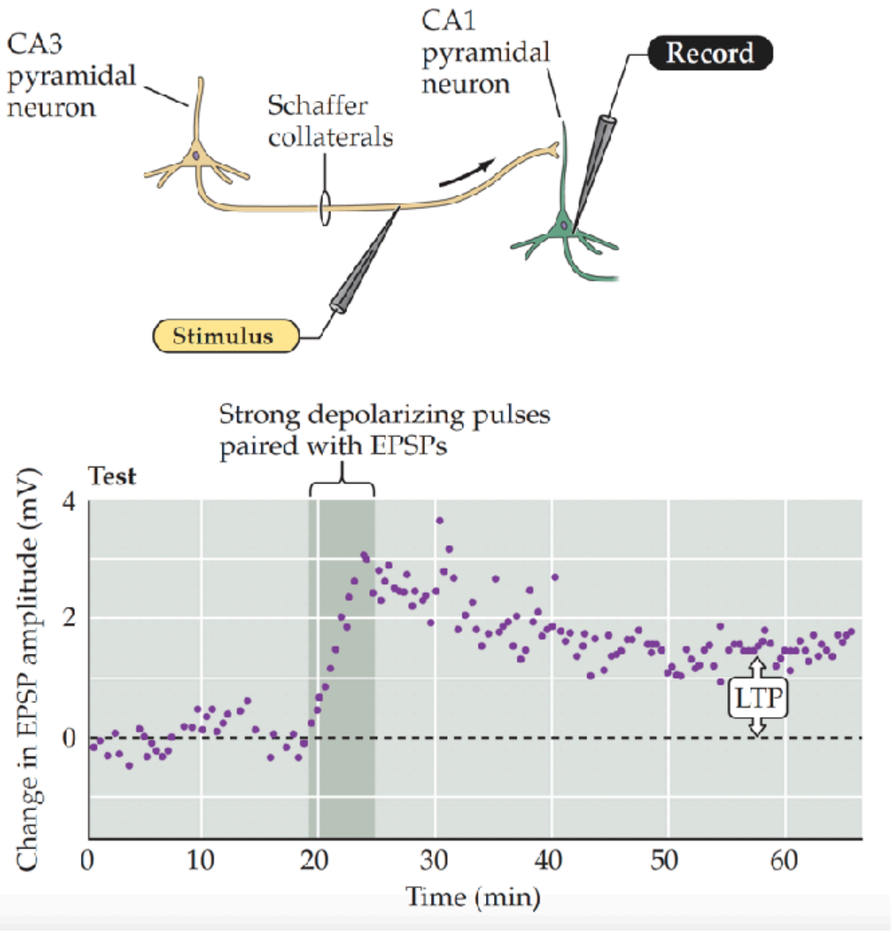


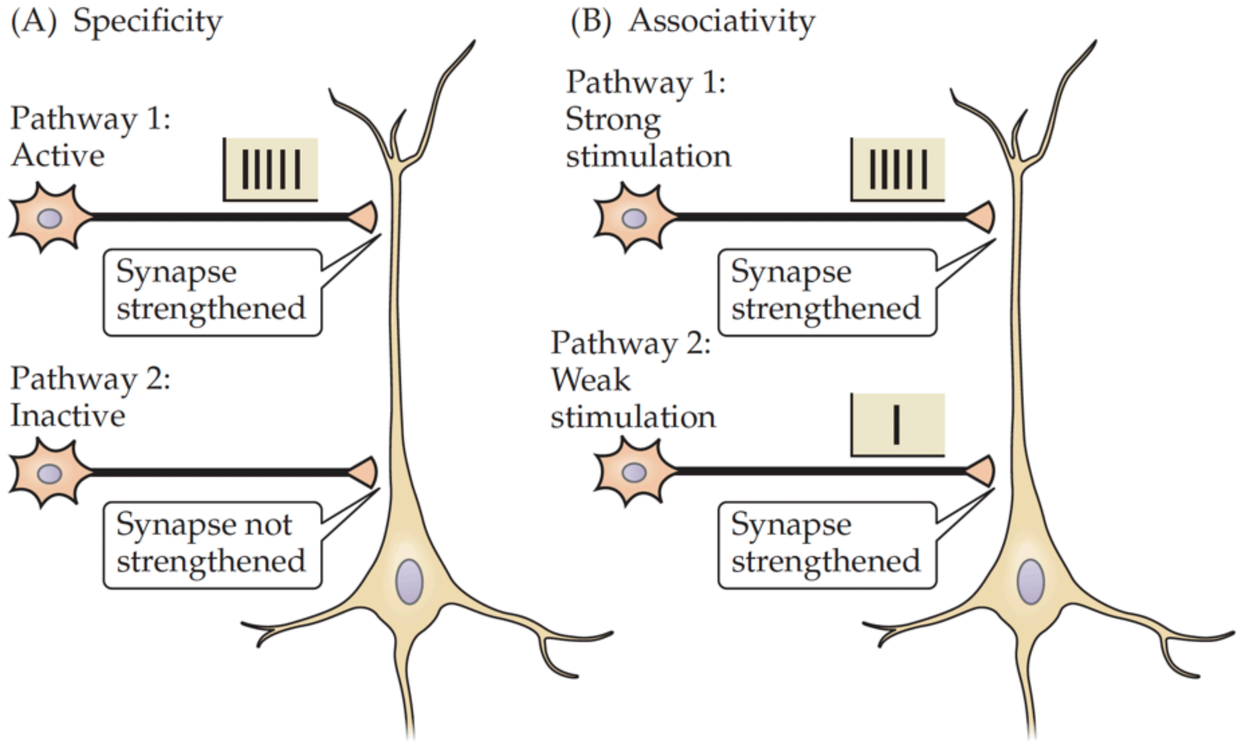


Brief, high frequency train of stimuli causes a long-lasting increase in post-synaptic potential

Long-term potentiation (LTP) and depression (LTD) in the hippocampus

Pairing a stimulus with a depolarisation also causes long-term-potentiation

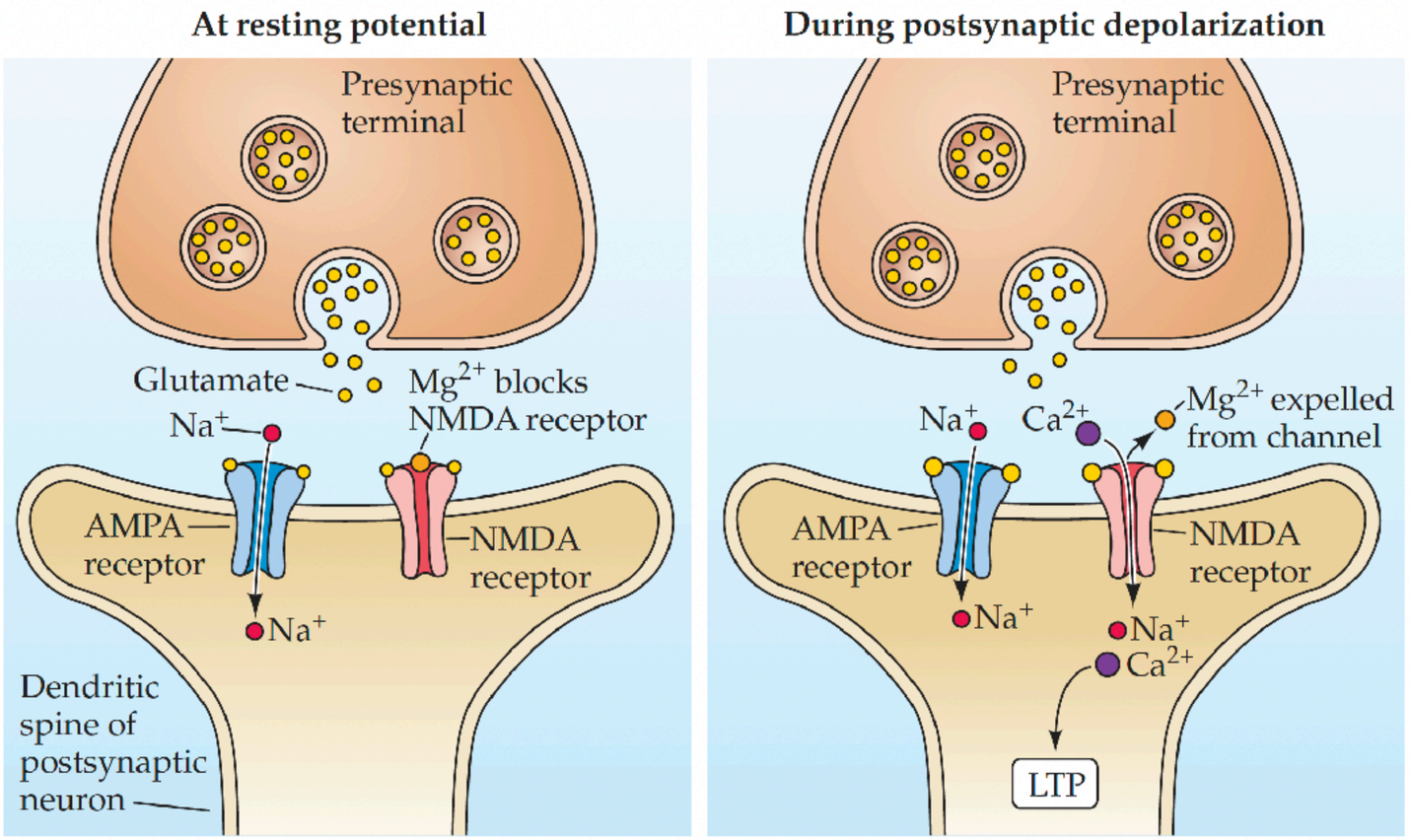




This phenomenon also explains associativity. If one pathway is weakly activated while another is active, they will undergo LTP.

Carla Shatz:
Fire together, wire together
Fire out of sync lose your link

NMDA receptors are needed for LTPs



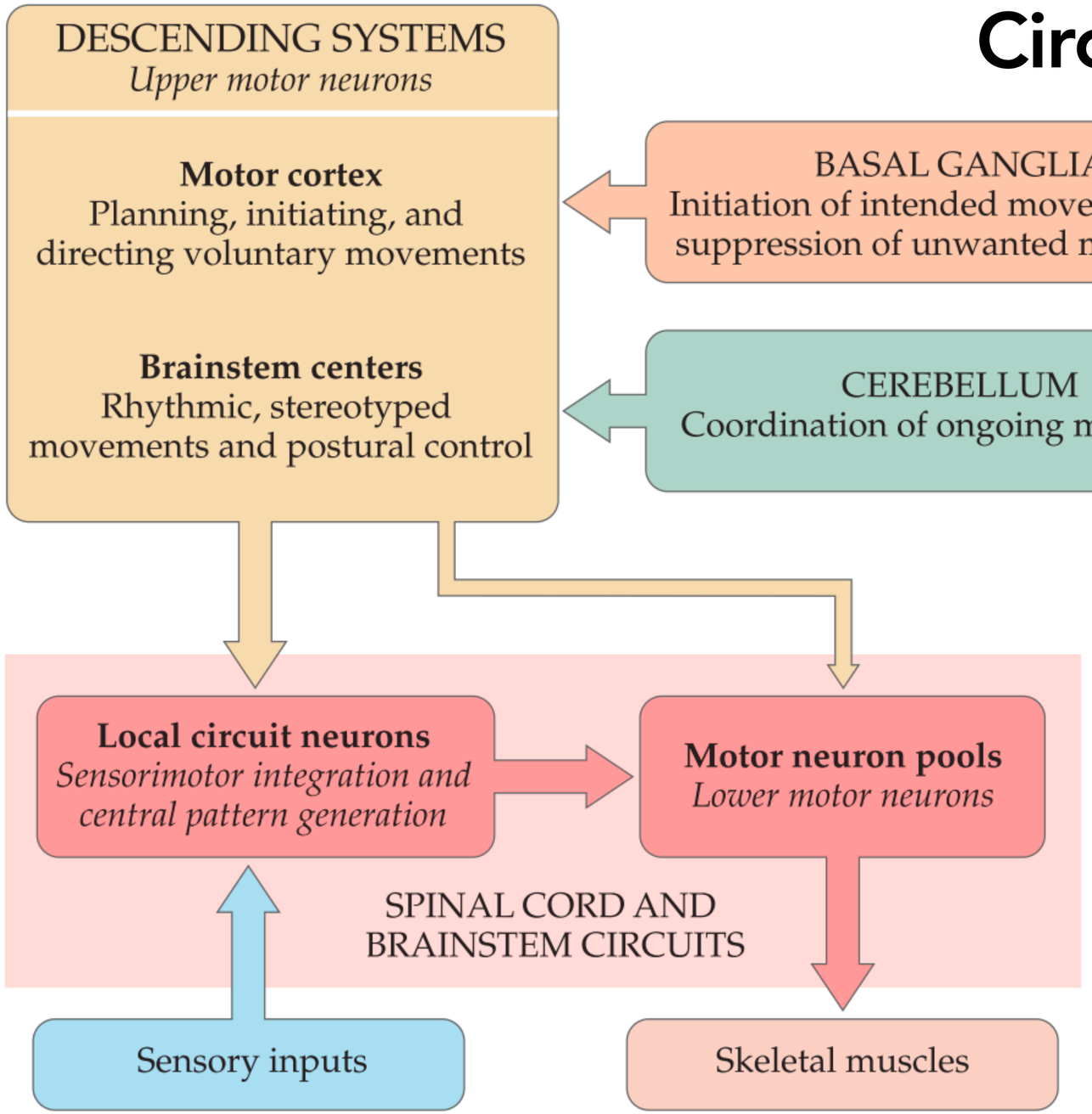
NMDA receptors (glutamate) only open upon prolonged depolarization in high-frequency stimulation. Only then can calcium enter through the channel.

Blocking NMDA receptor prevents LTP.

The sustained Calcium increase causes more AMPA receptor to be incorporated at the postsynapse supporting LTP. (The number of NMDA receptor does not change)

Previously silent synapses do not have AMPA.

Circuits that control movements



- Reflexive movements – Involuntary, automatic responses to stimuli (e.g., knee-jerk reflex).
- Rhythmic movements – Repetitive and semi-automatic motions (e.g., walking, chewing).
- Voluntary movements – Goal-directed actions controlled by the motor cortex (e.g., reaching for an object).

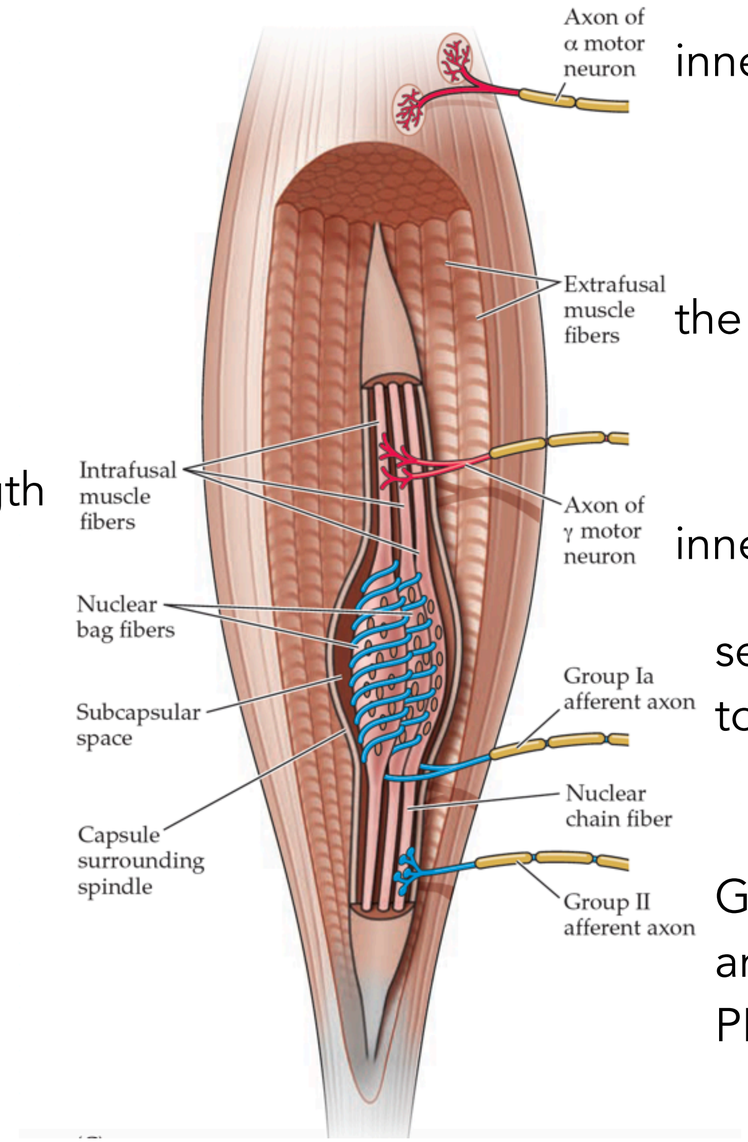
4 Systems controlling movement:

- Spinal Cord (Brain stem circuits)
- Cerebellum
- Basal Ganglia
- Descending Systems in the cerebral cortex and brainstem

Purves 16.1

Stretch reflex circuitry

"measure" muscle strength



innervate "working muscle fibers"

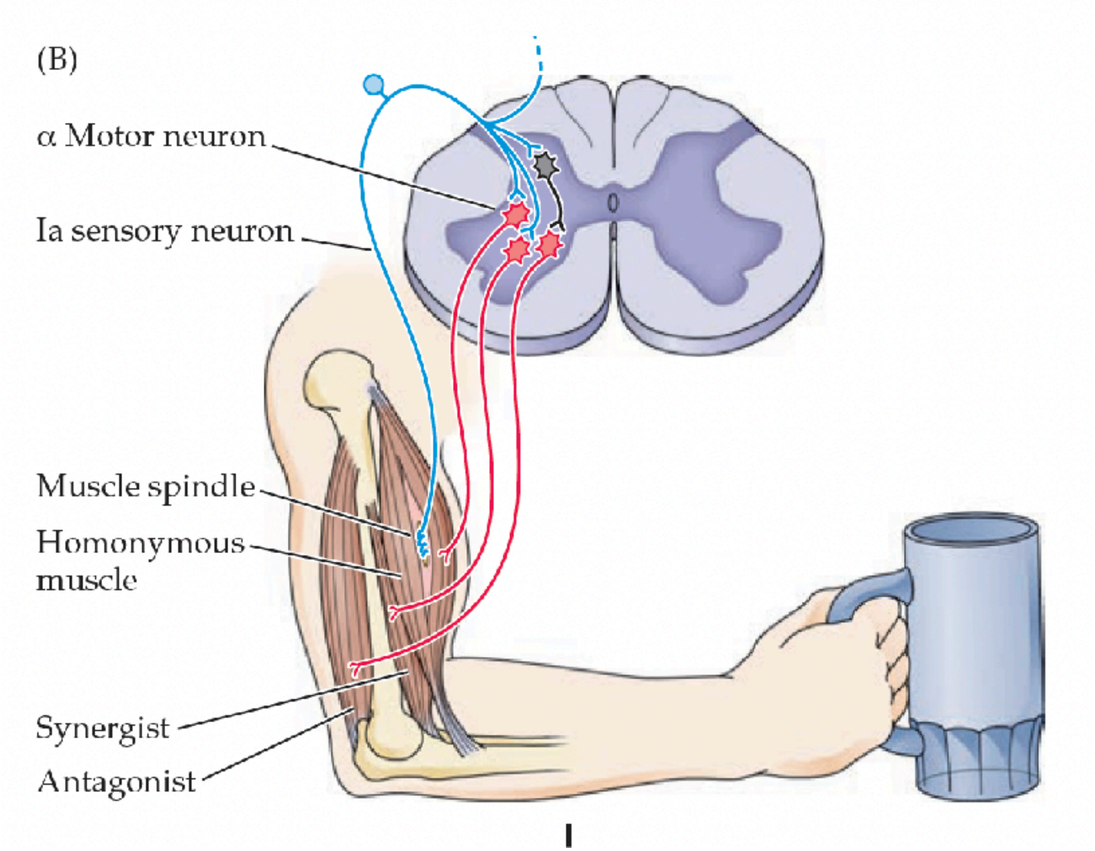
the "working" muscle fibers

innervate "working muscle fibers"

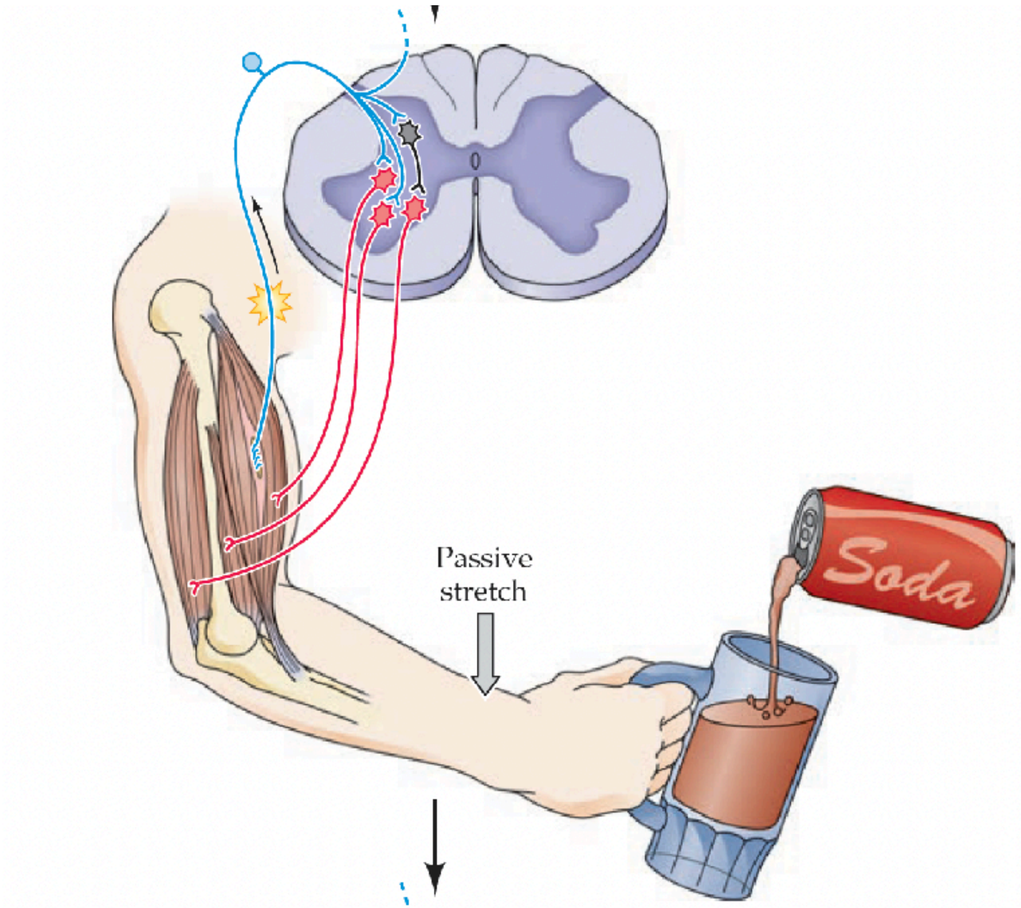
sensory axons conducting the signal towards the CNS

Group I a and II neurons are the largest and fastest conducting neurons in the PNS

Stretch reflex circuitry

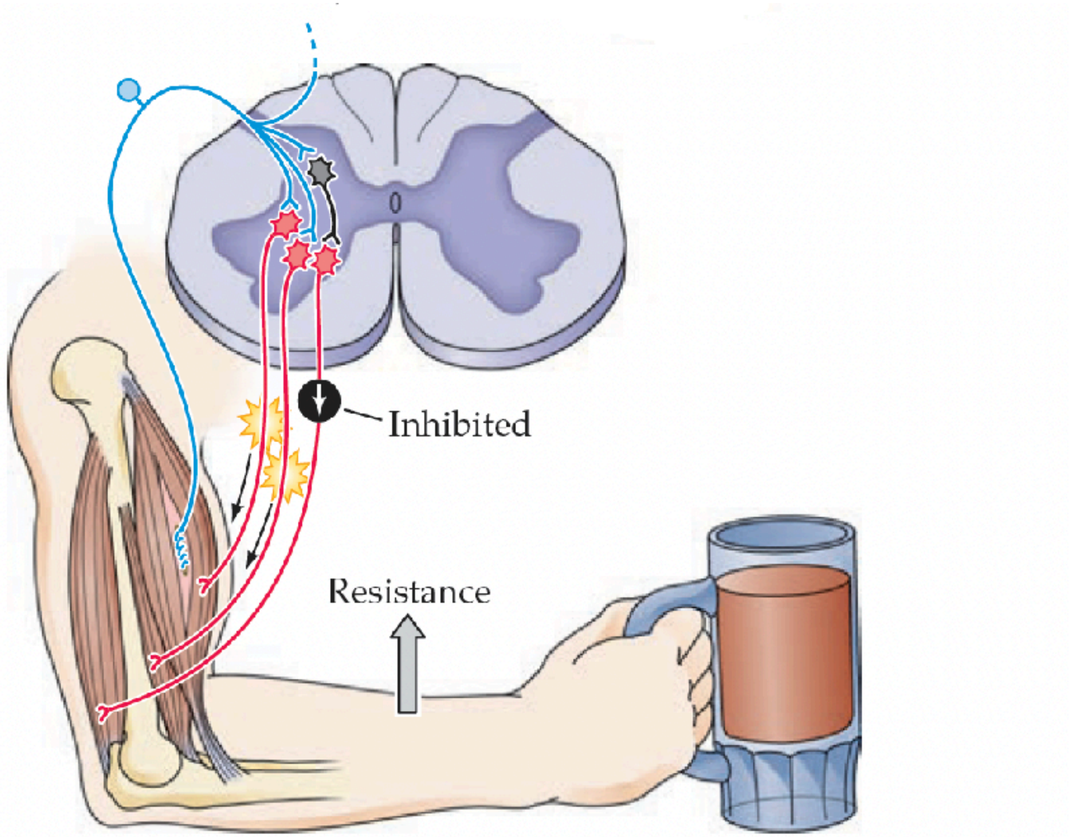


Arm at rest



Group I a sensory detects stretch and directly signals to α motor neuron

Stretch reflex circuitry

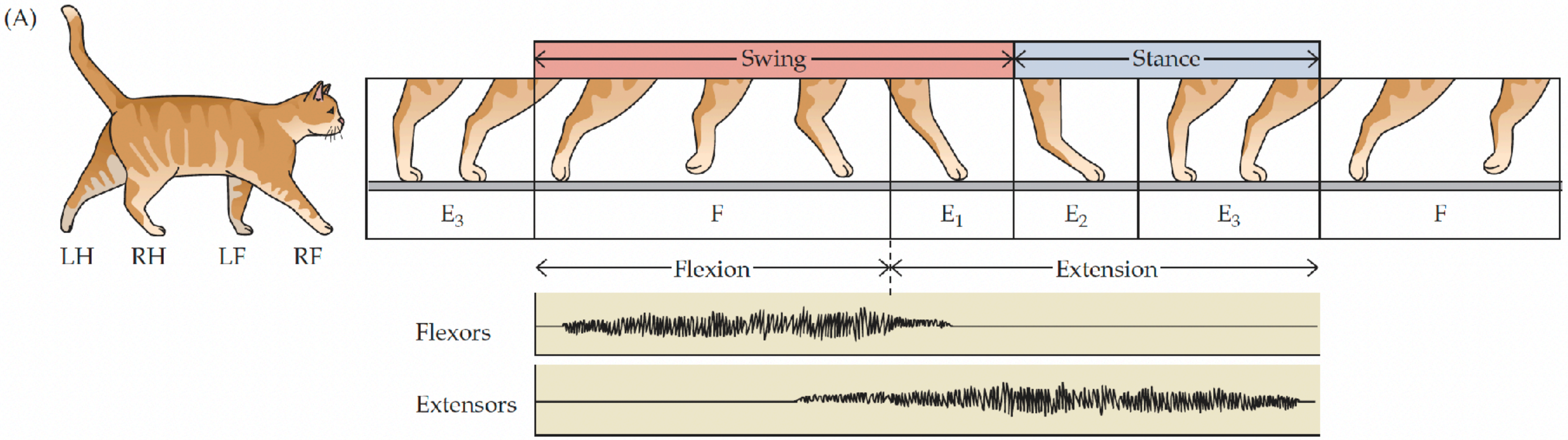


α motor neuron fires and synergist contracts

Group I a sensory gets relayed and sends an inhibitory signal to relax the antagonist

This only happens through local circuits, these signals do not enter the brain!

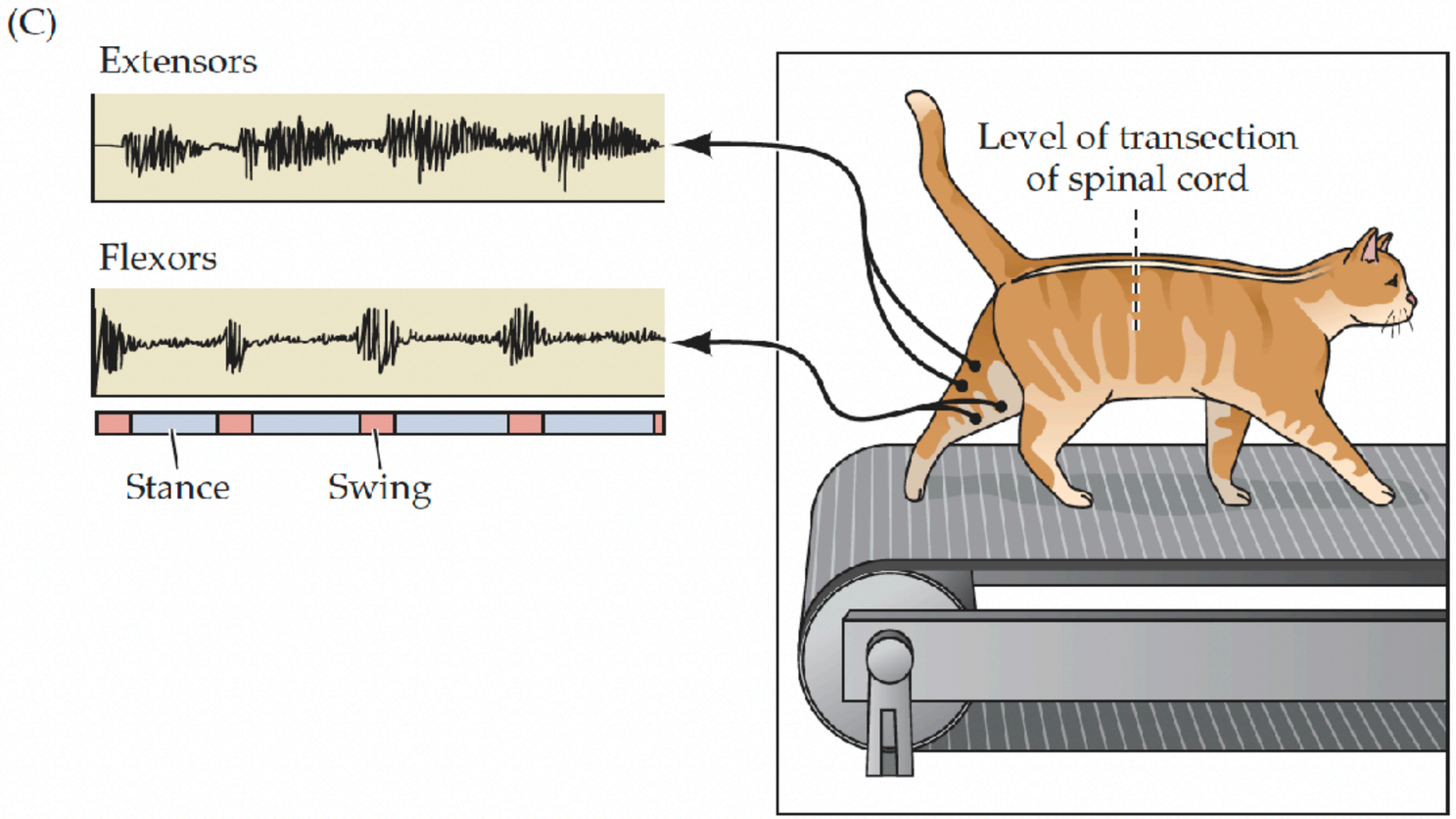
Locomotor control



The mammalian cycle of locomotion is organized by central pattern generators in the spinal cord.

In quadrupeds, changes in locomotor speed are accompanied by changes in the sequence of limb movements.

Locomotor control

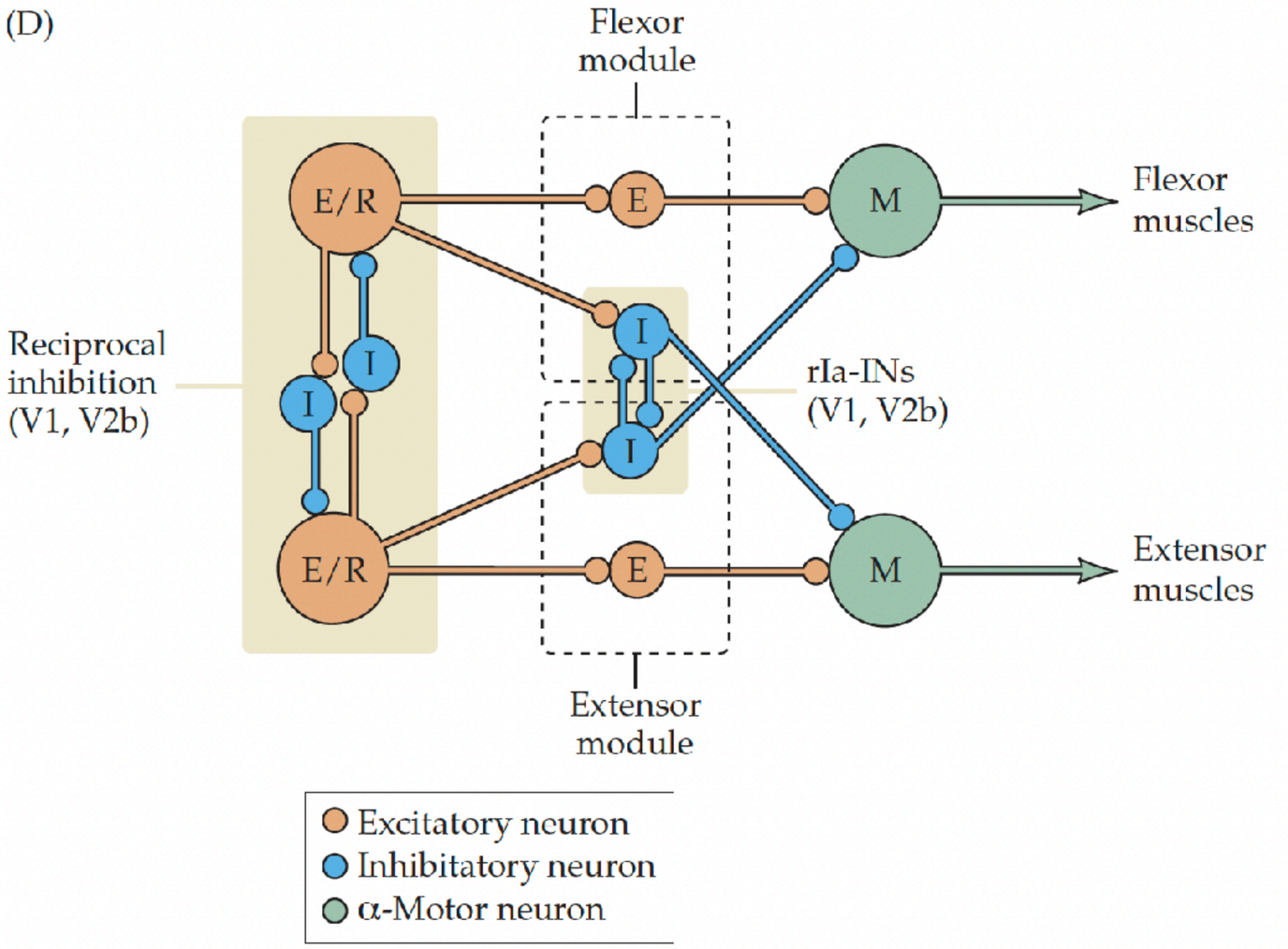


After transection of the spinal cord patterns are still generated (if the animal is supported)

Limb movement during locomotion is not dependent on sensory input, nor wholly on input from descending projections from higher centers.

Local circuitry provides for each limb a central pattern generator responsible for the alternating flexion and extension of the limb.

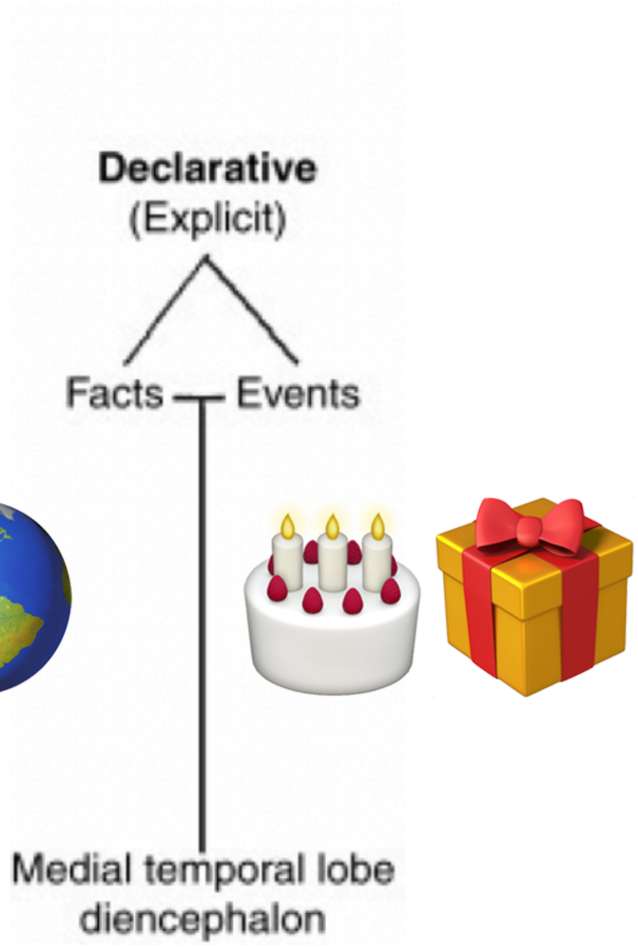
Central pattern generators in motor control

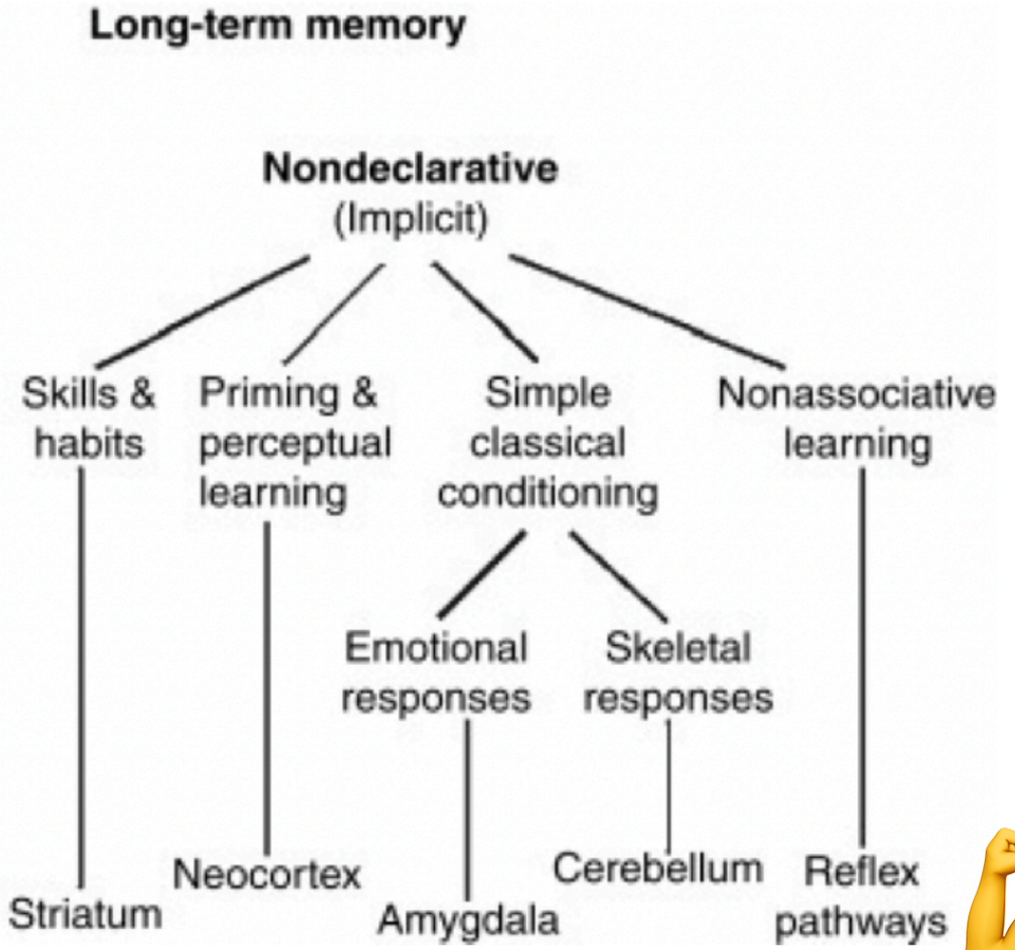


The central pattern generator comprises local circuit neurons that include excitatory glutamateric neurons coupled to one another and a variety of inhibitory GABAergic and glycinergic neurons

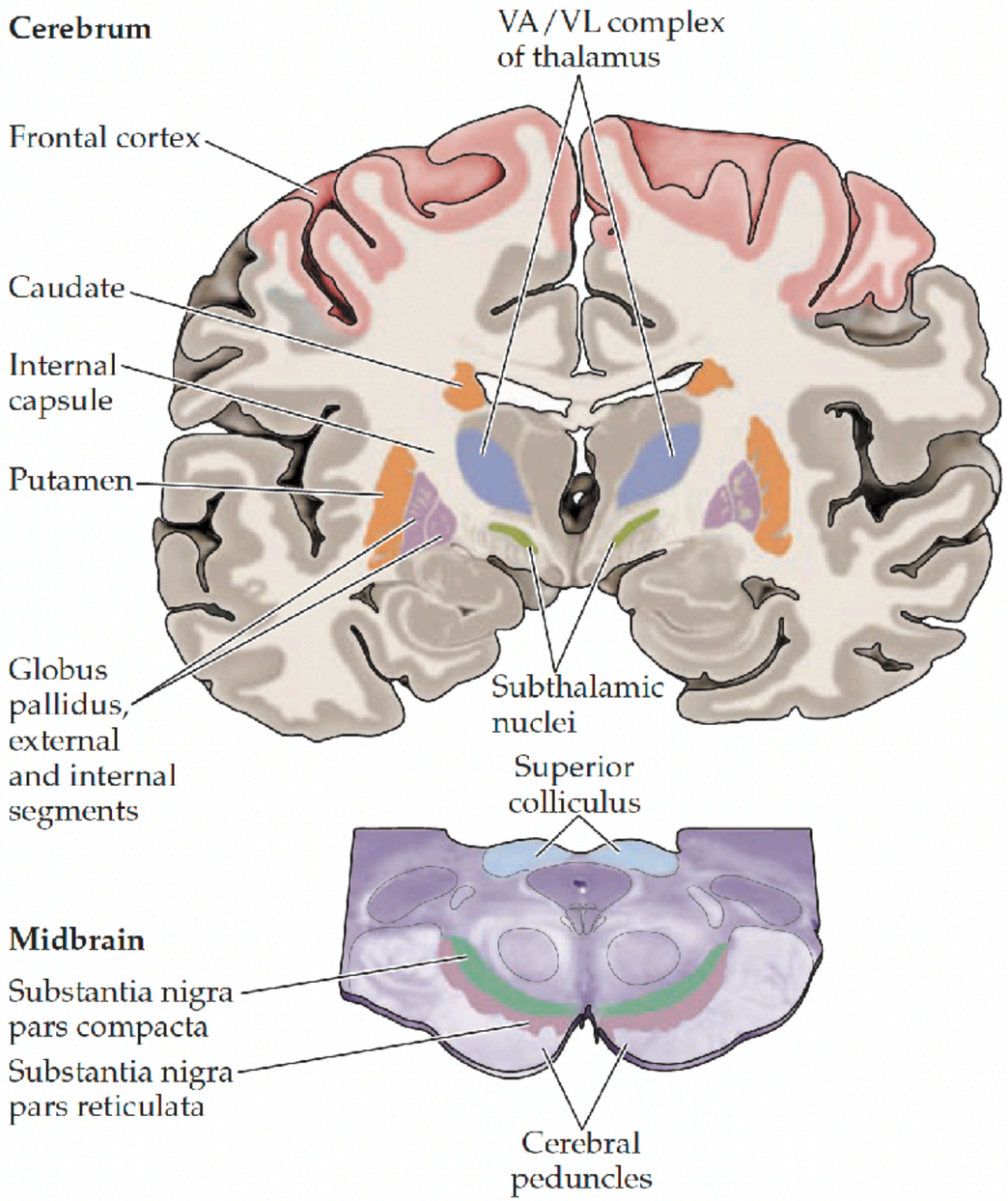
Reciprocal inhibition of antagonistic muscles at multiple sites (interneurons, Motor neurons)!

What type of movement are basal ganglia involved in?





Basal ganglia: anatomical structures



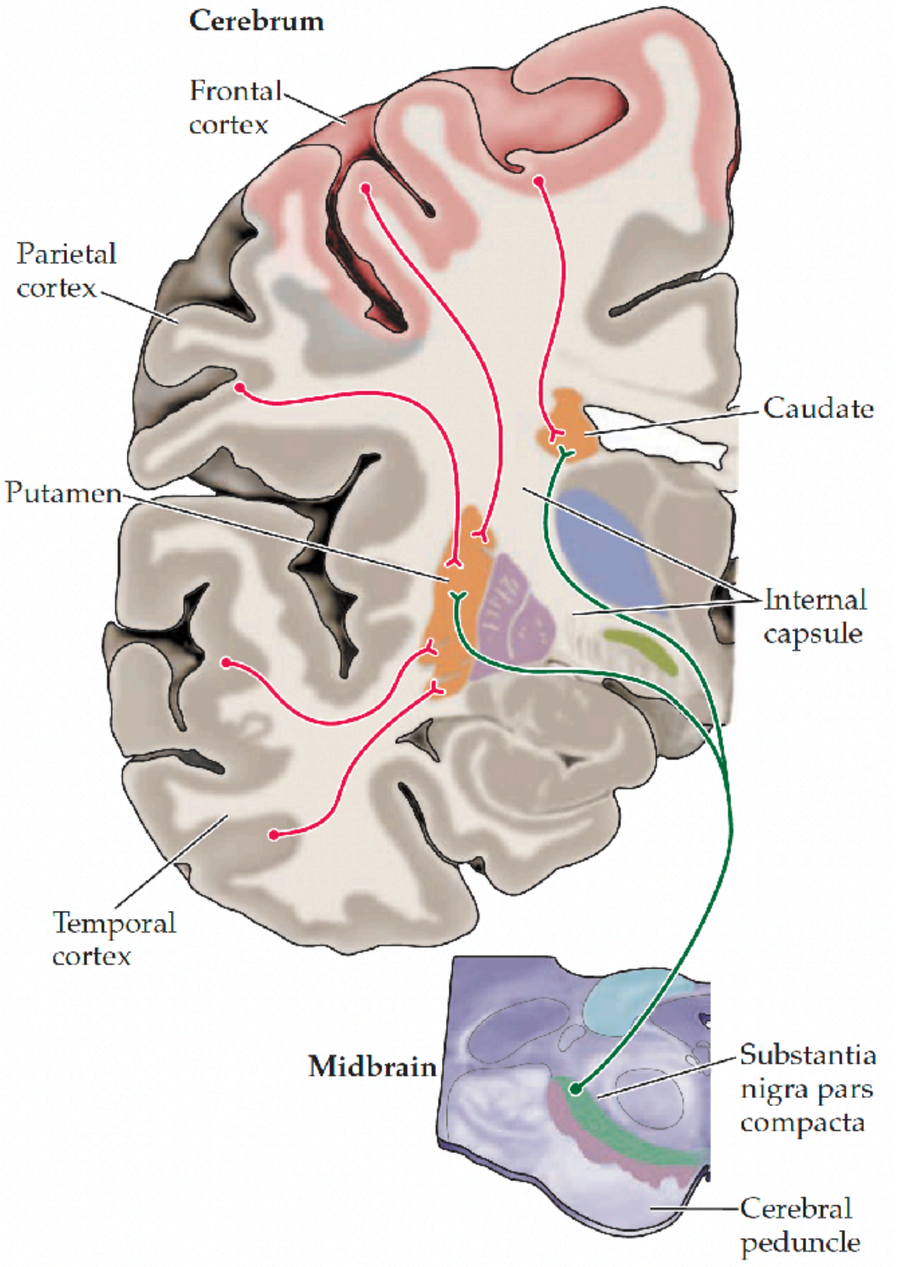
Caudate and Putamen for the Striatum

They receive input from the Cortex and Dopaminergic neurons of the Substantia nigra

They output to the Globus pallidus, Thalamus and Subthalamic Nuclei

Involved in iniating and stopping a movement

Anatomical organization of inputs to the basal ganglia



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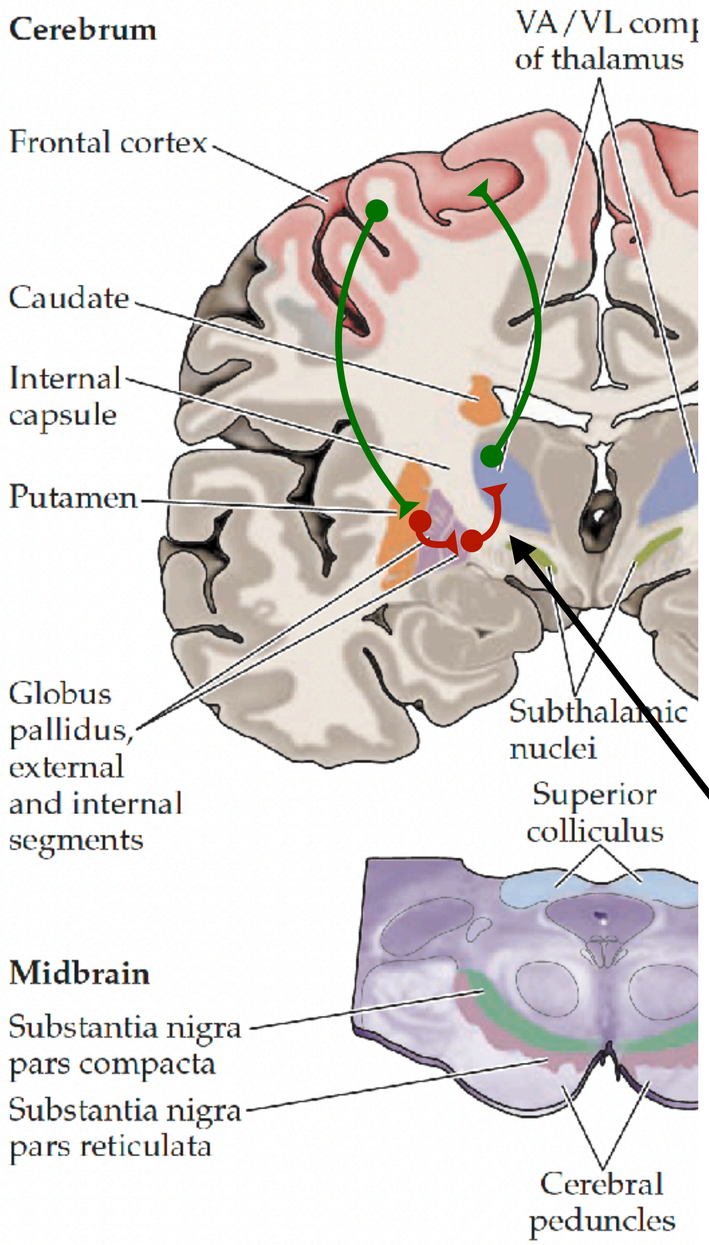
The direct pathway - Cortical - Basal Ganglia - Thalamo - Cortical loop



Glu - Excitatory

GABA - Inhibitory

Excitatory input from Cortex to medium spiny neurons in the Putamen

Inhibitory Medium spiny neurons connect to the Globus pallidus internal segments

Inhibitory from the Globus pallidus internal segments connect to the Thalamus. These neurons are tonically active and get inhibited now.

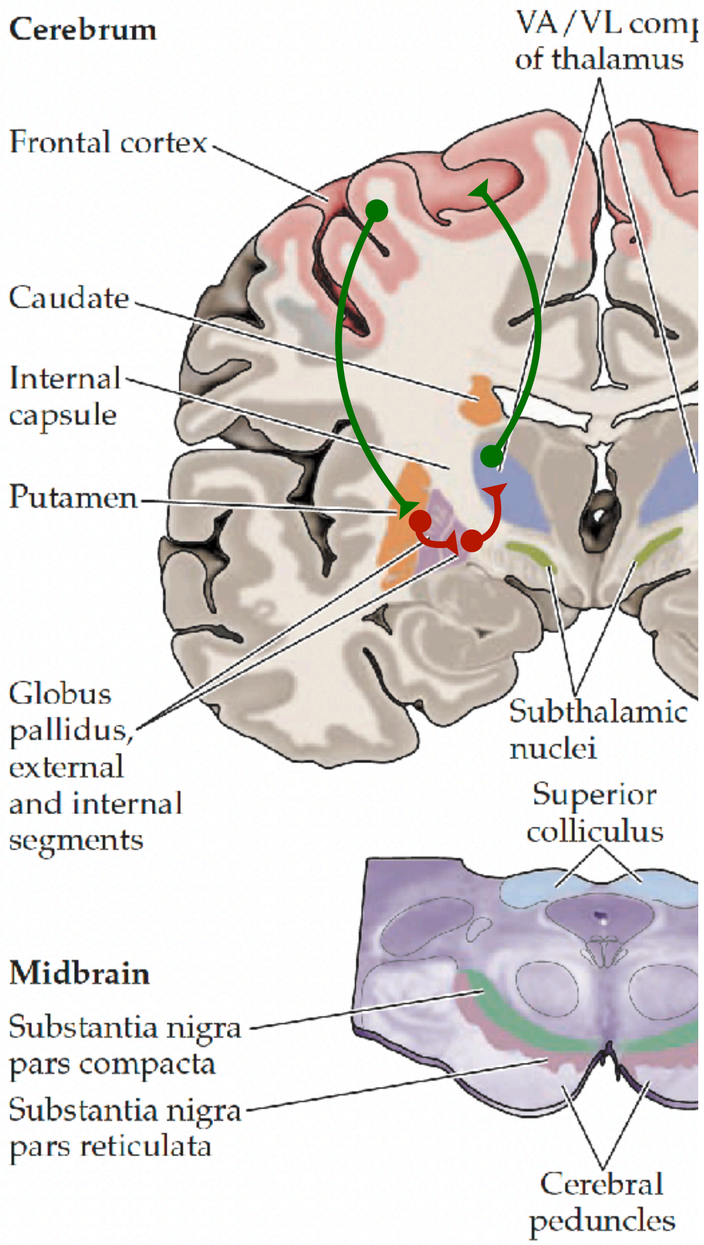
Upon inhibition of the tonically active inhibiting GPi neurons (pallidal neurons) the thalamus can send excitatory signals to the cortex. Otherwise the Thalamus is tonically inhibited.

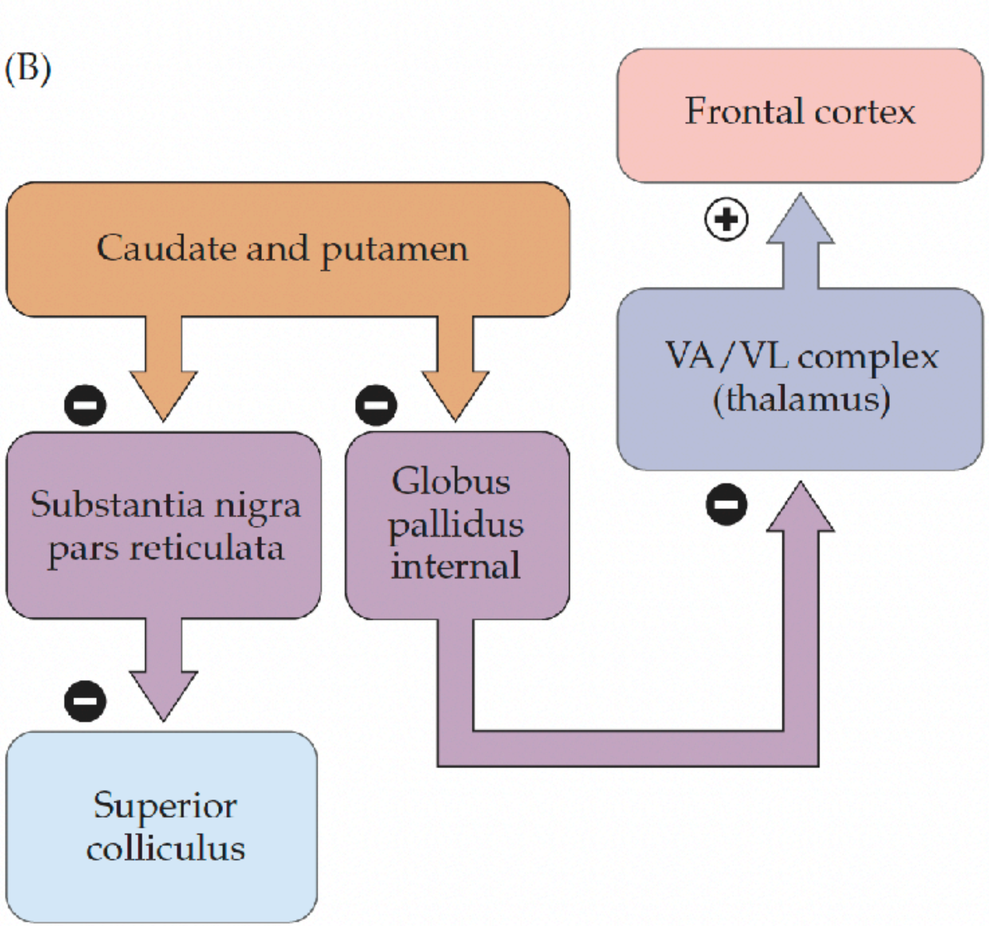
GPi neurons are tonically active when the circuit is at rest!

The direct pathway - Cortical - Basal Ganglia - Thalamo - Cortical loop

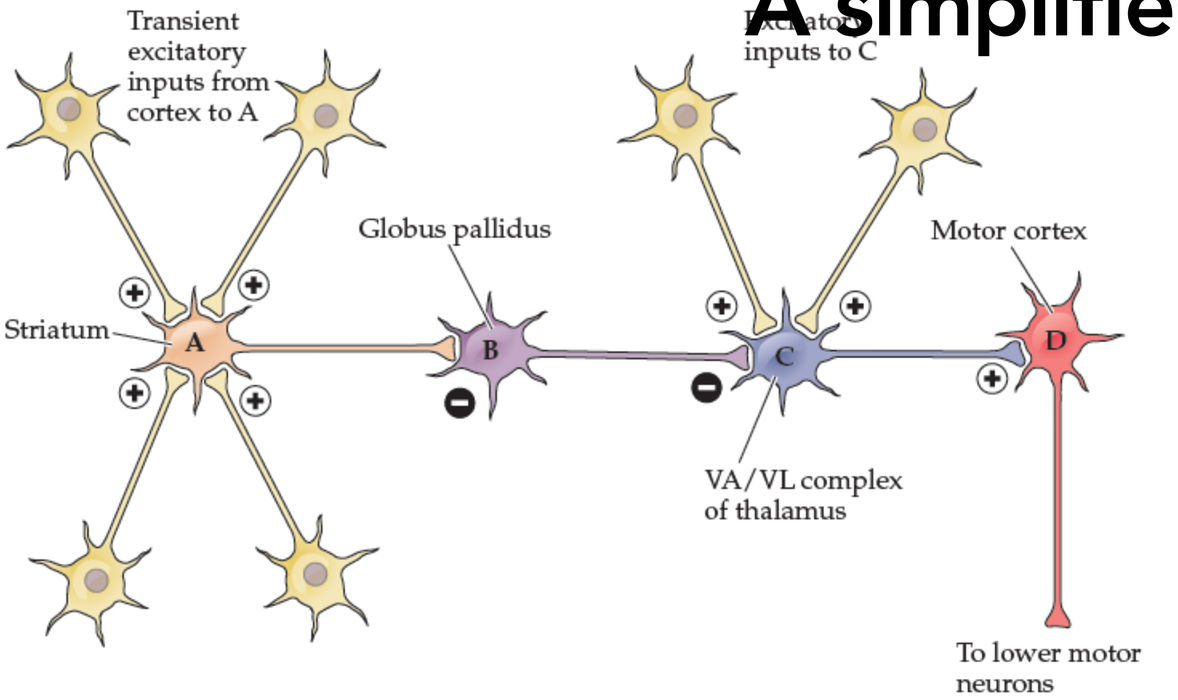
Glu - Excitatory

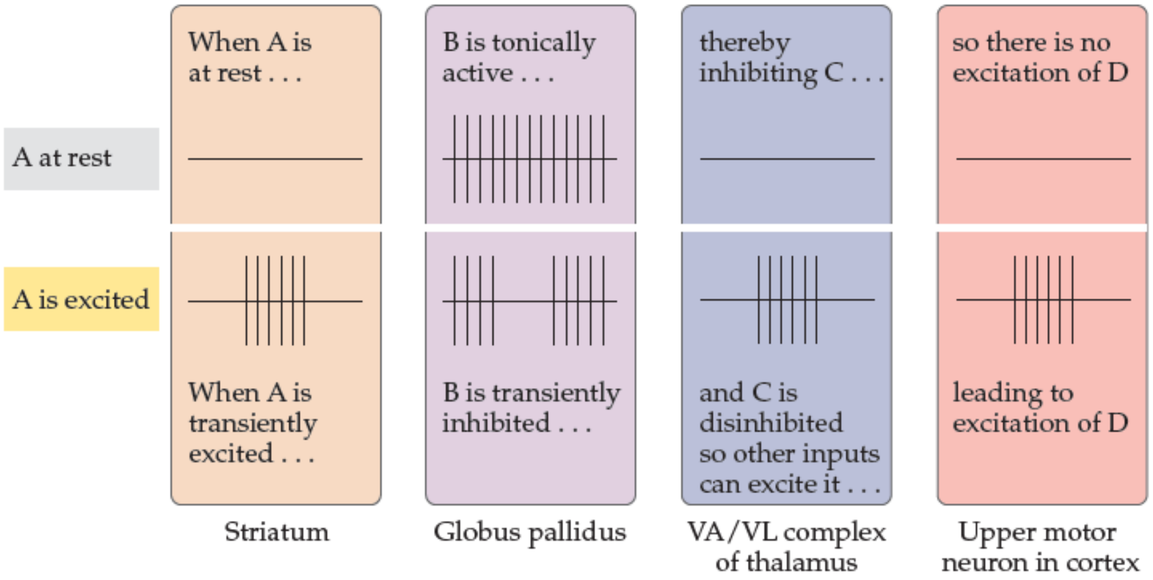
GABA - Inhibitory





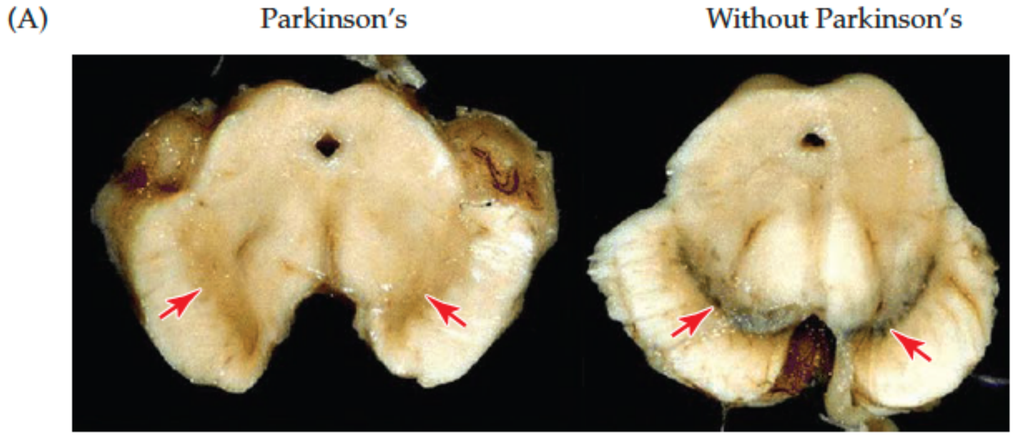
A simplified example of the direct pathway



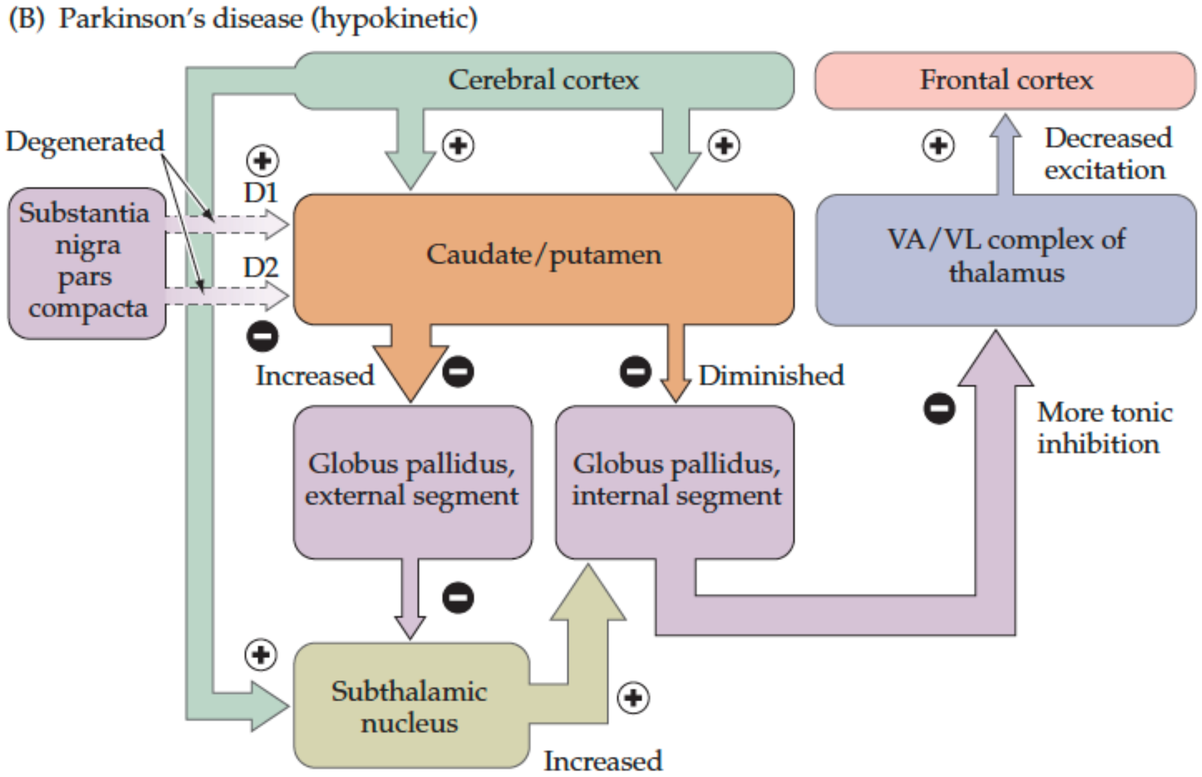


Excitatory input from the cortex inactivates the tonically active pallidal neurons which allows cortical neurons to fire and activate thalamic inputs to the cortex.

Dopamine neurons in Parkinsons



In Parkinsons patients dopaminergic neurons selective die in the substantia nigra

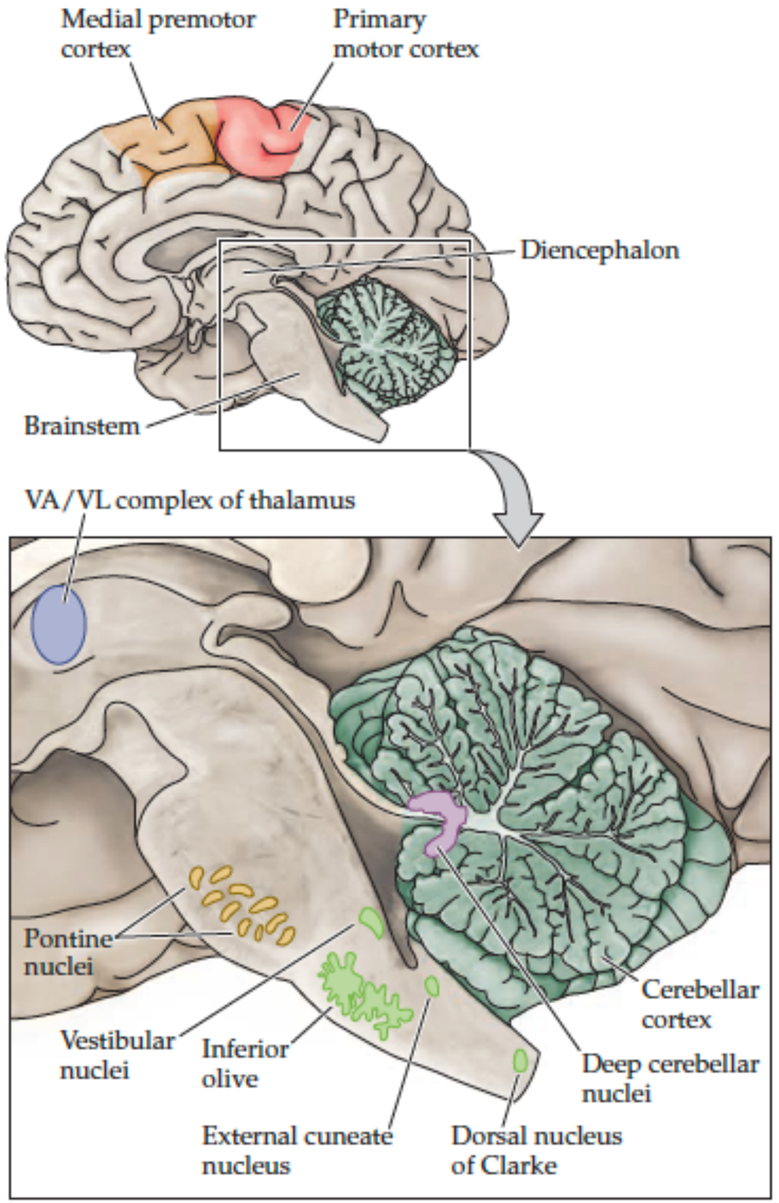


Regulatory inputs to the Caudate and Putamen are missing.

Leading to an increase in inhibition from the globus pallidus

And less excitatory inputs to the cortex

The cerebellum



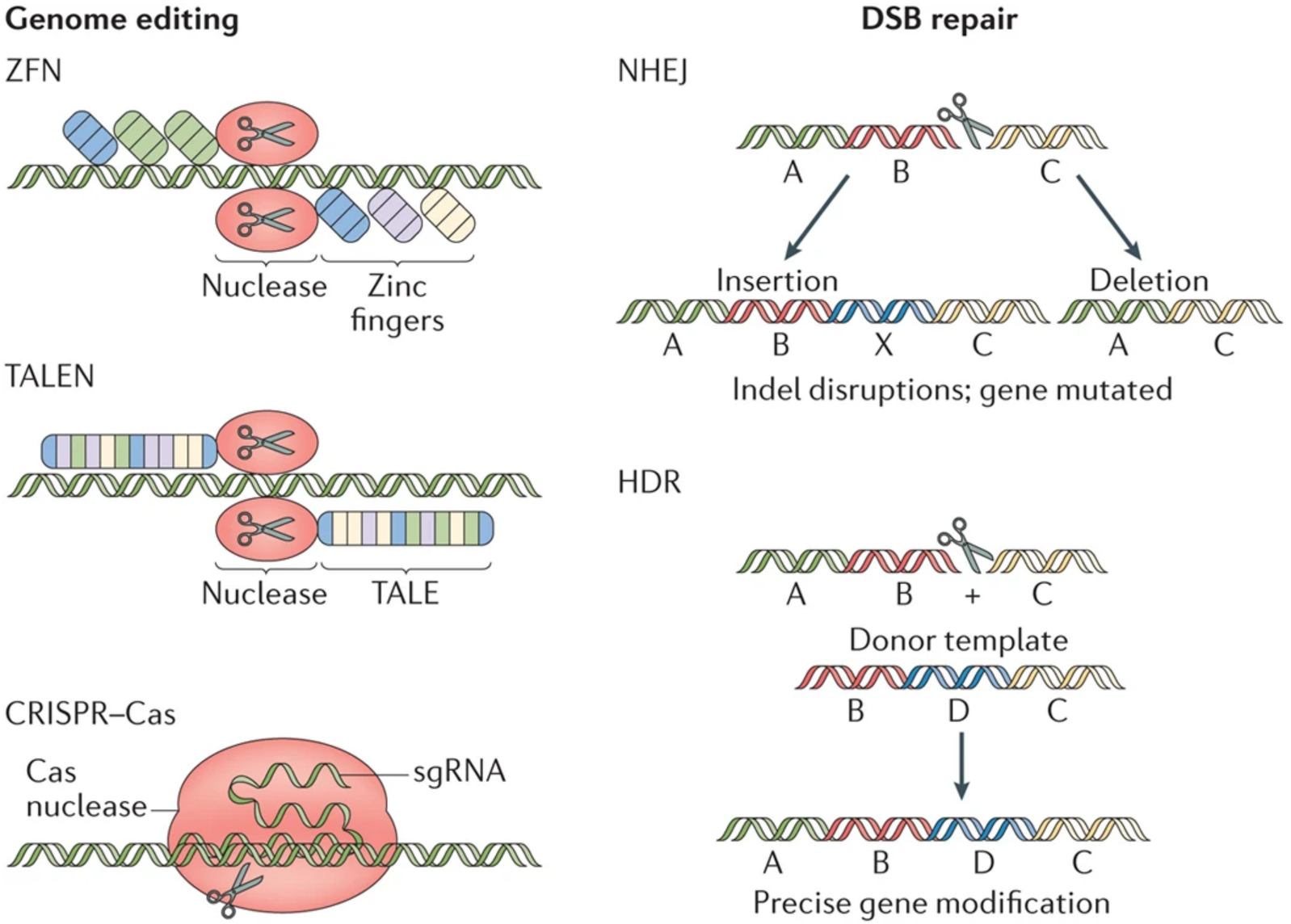
Cerebellum stands for little brain.

It comprises about 10% of the total volume of the brain but around 80% of its neurons.

Receives inputs from many brain regions (somatosensation, proprioception, visual, auditory, vestibular)

Damage to the cerebellum leads to problems with balance, spatial accuracy and temporal coordination. It also reduces motor learning.

How do you deplete a gene/protein?



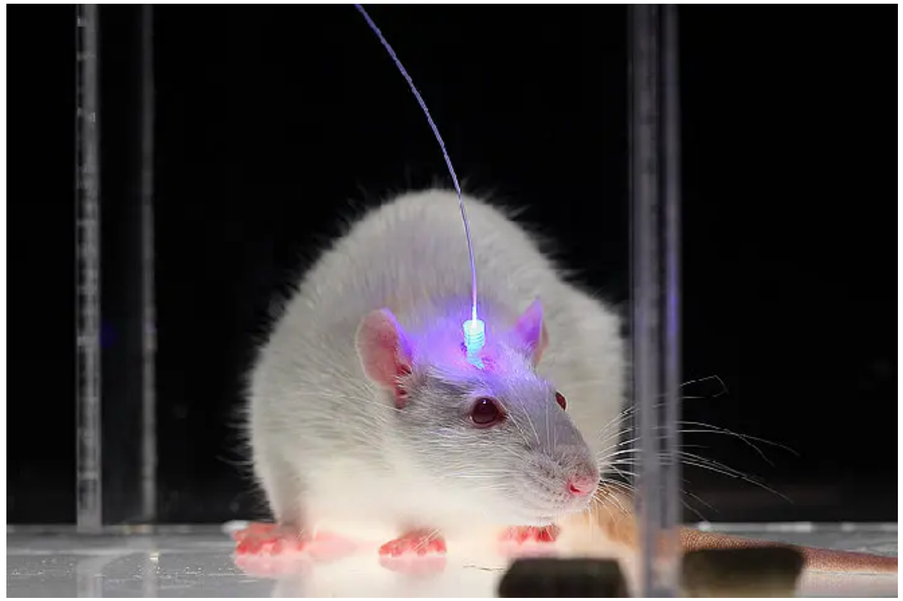
TALENs and ZFN can, to some extent, be designed to cut specific regions of the genome.

CRISPR Cas9/12a can be guided to a very specific location in the genome through an RNA and introduce a double strand break

Repair templates can be offered to integrate new pieces of DNA at the same locucs

WareJoncas, Ekker (2018) Nat. Rev. Nephr.

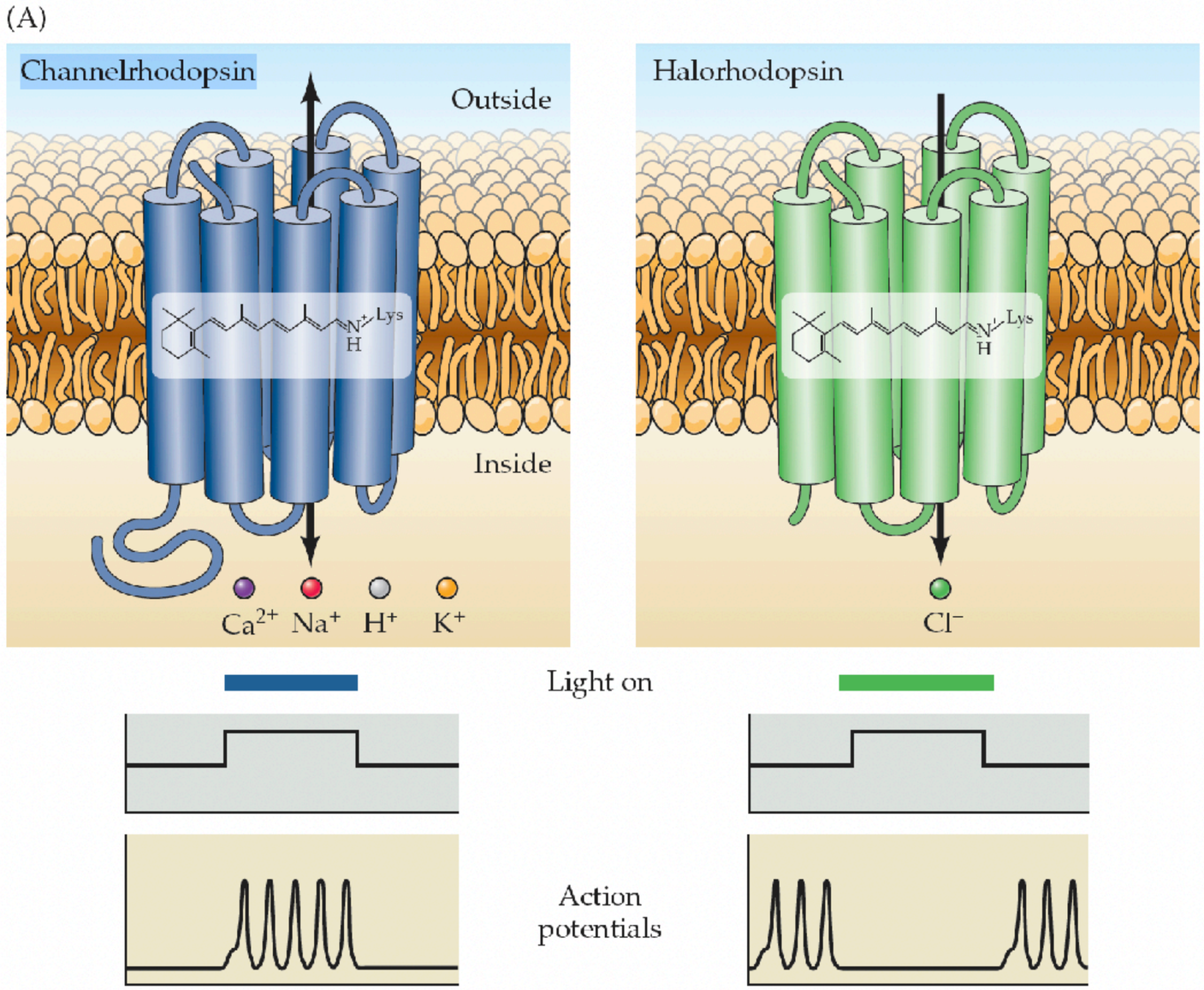
Using optogenetics to investigate circuits and individual neurons



https://www.nytimes.com/2011/05/17/science/

The behaviour of transgenic mice can be manipulated through light stimuli

Using optogenetics to investigate circuits and individual neurons



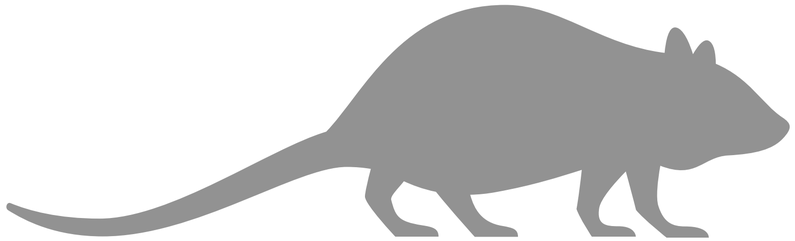
Channelrhodopsin and Halorhodopsin are bacterial proteins that can be expressed in neurons and controlled through light.

Upon light stimulation, cells get either depolarized or hyperpolarized (activated or inhibted).

Purves Neuroscience

Using optogenetics to investigate circuits and individual neurons

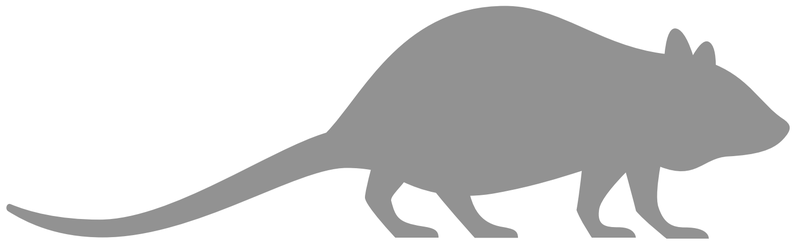
Expresses Cre in all neurons

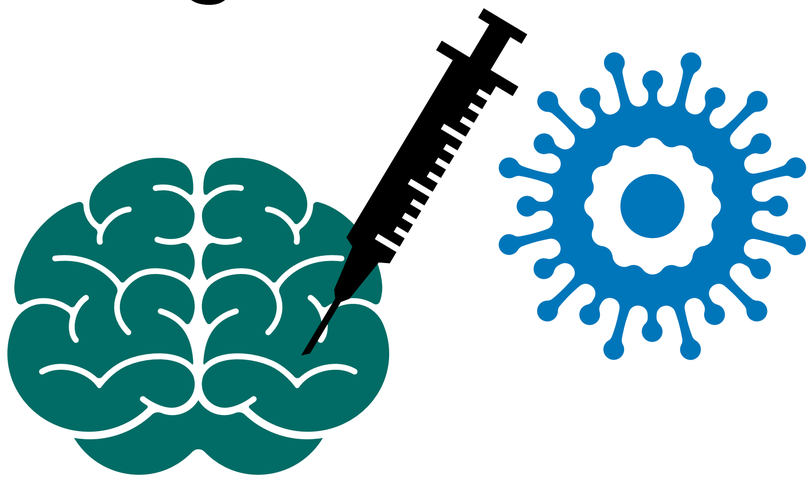


Promoter: hSyn >> Cre

Using optogenetics to investigate circuits and individual neurons

Expresses Cre in all neurons



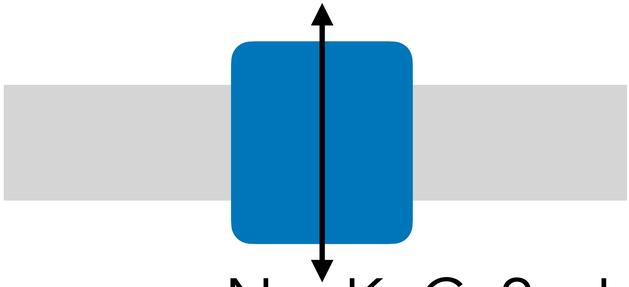


Promoter: hSyn >> Cre

Promoter: CAG STP ChR2

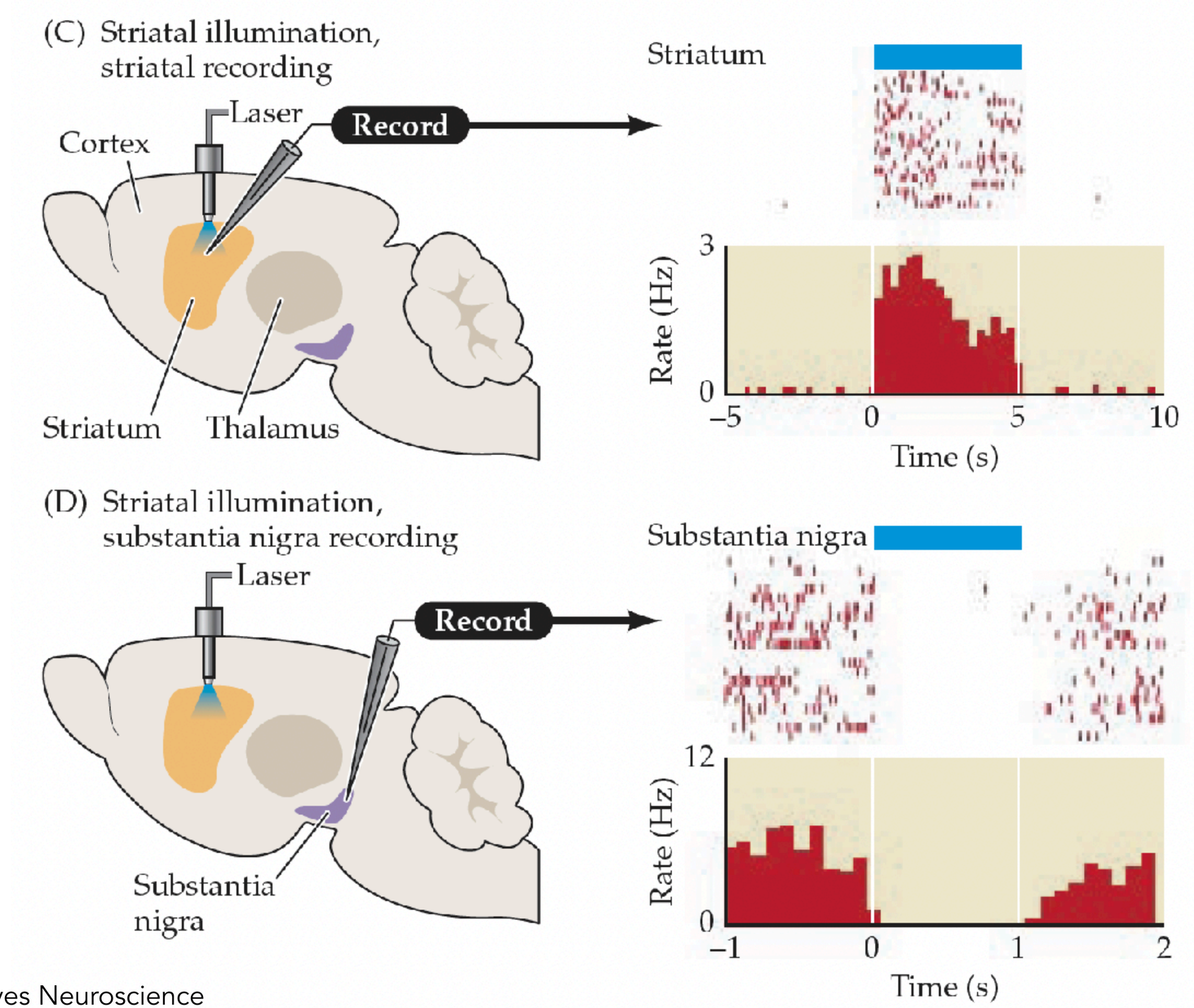
Cre

Promoter: CAG ChR2



Na, K, Ca2+,H

Using optogenetics to investigate circuits and individual neurons

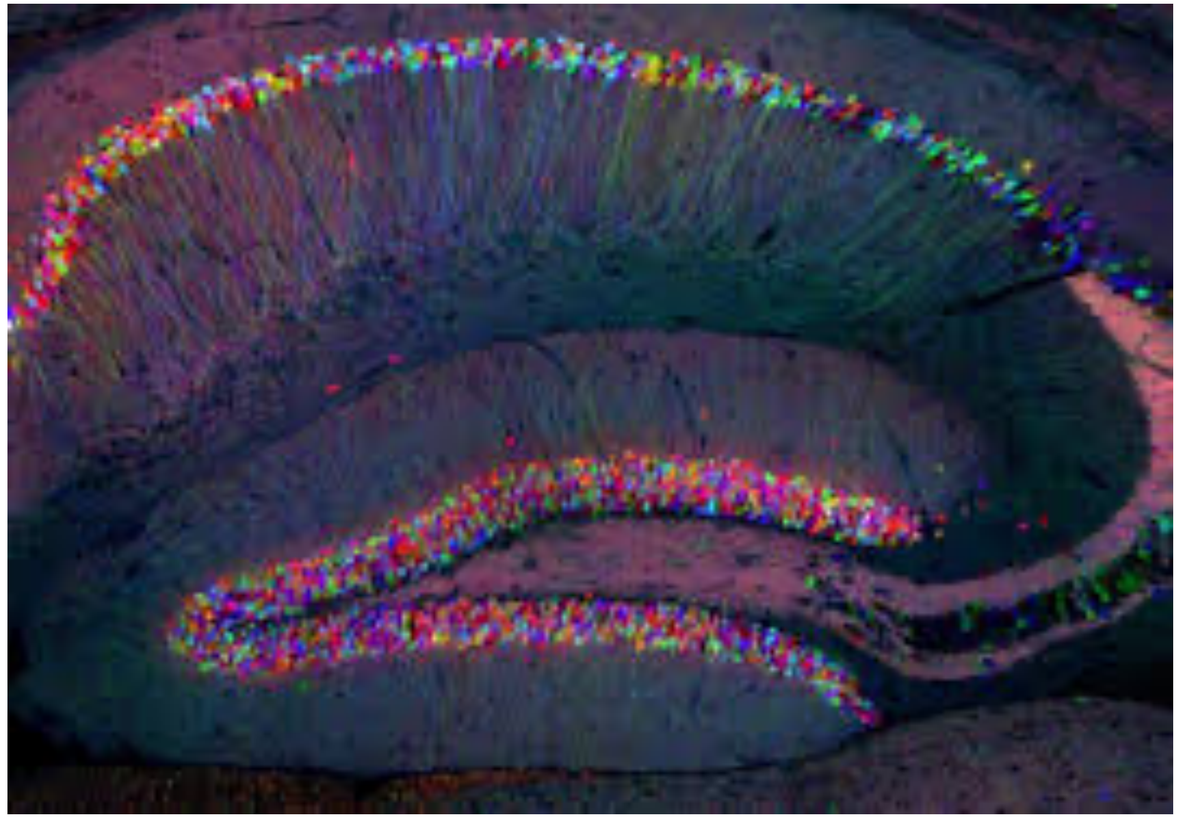


Neurons expressing channelrhodopsin in the striatum, where neurons have little or no spontaneous activity, fire robustly when illuminated.

Neurons in the substantia nigra, where neurons have a high frequency of spontaneous action potential activity can be "silenced" transiently by illumination in the striatum. The striatal axons release the inhibitory neurotransmitter GABA.

Purves Neuroscience

How do to label different celltypes with fluorescent proteins?



Brainbow makes use of labelling individual neurons with fluorescent proteins to discriminate them and trace their connectivity patterns

We need to combine different technologies to obtain this labelling.

We need a cell-type/neuron-specific Cre:

Promoter: hSyn >> Gene:Cre

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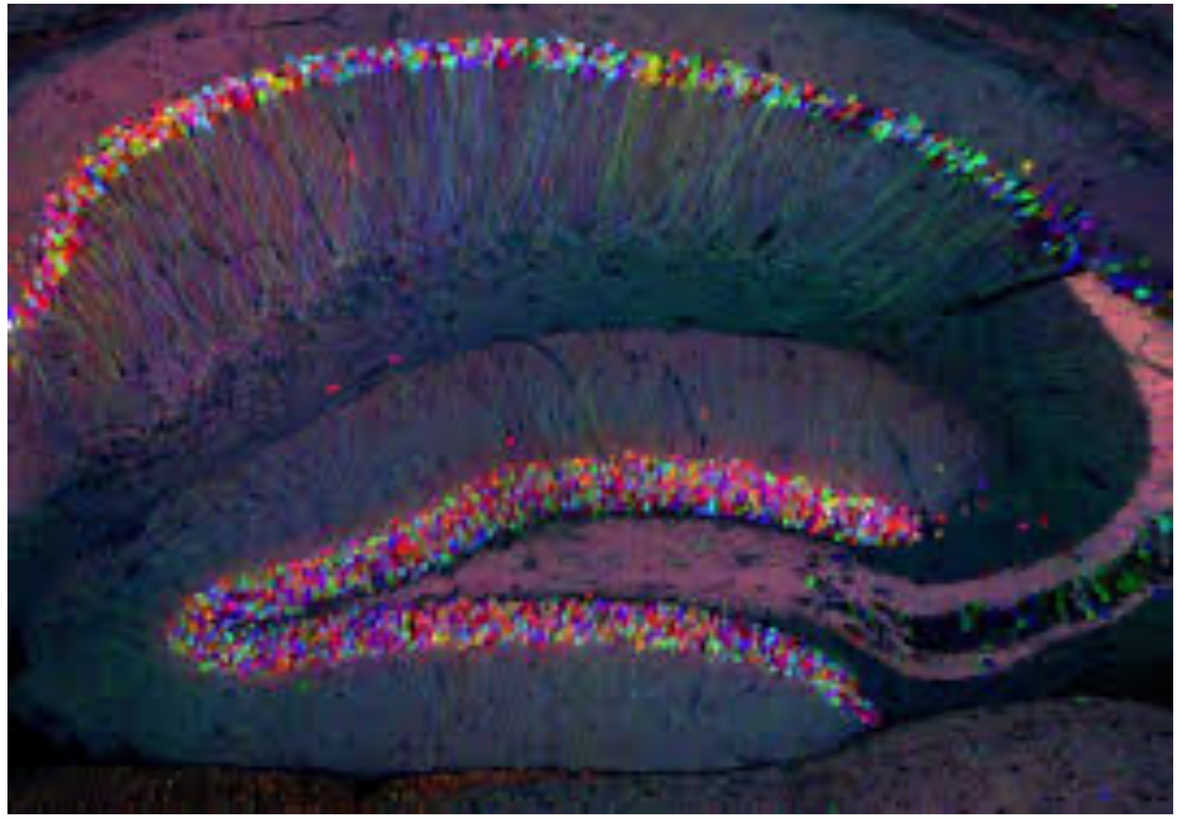

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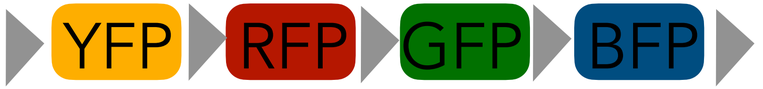

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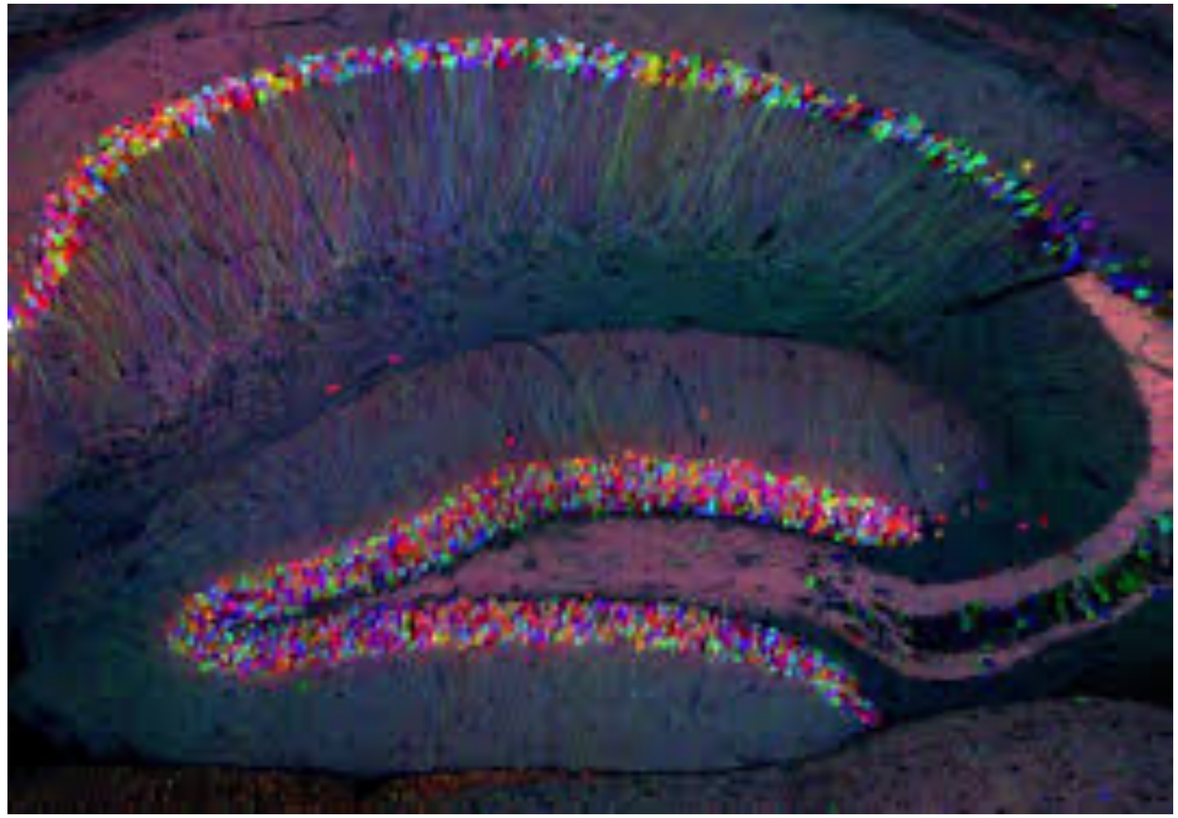

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YFP RFP GFP BFP

In each neuron, we can now find a random combination of these fluorescent proteins accounting to the different colours

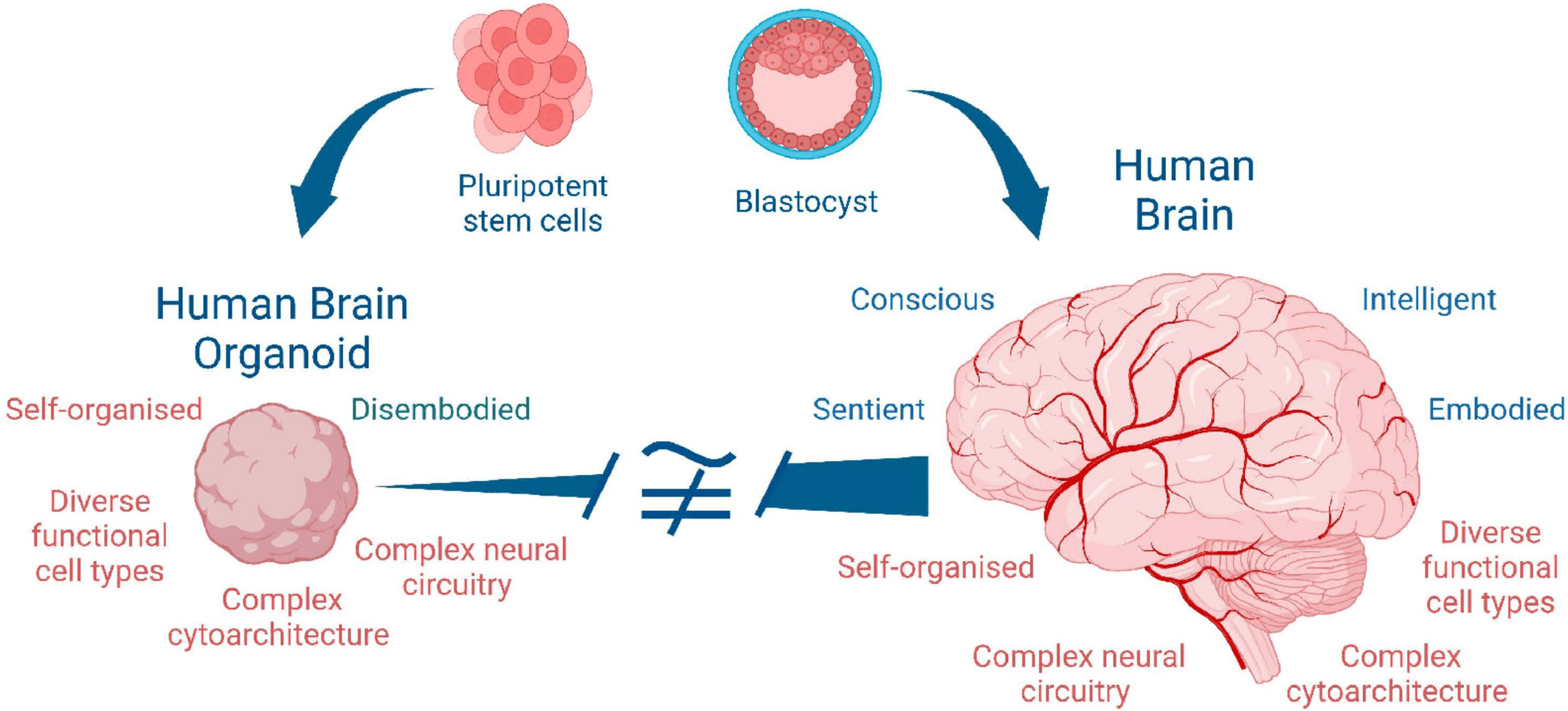
YFP BFP

YFP RFP BFP

YFP GFP BFP

RFP ….. and so on…

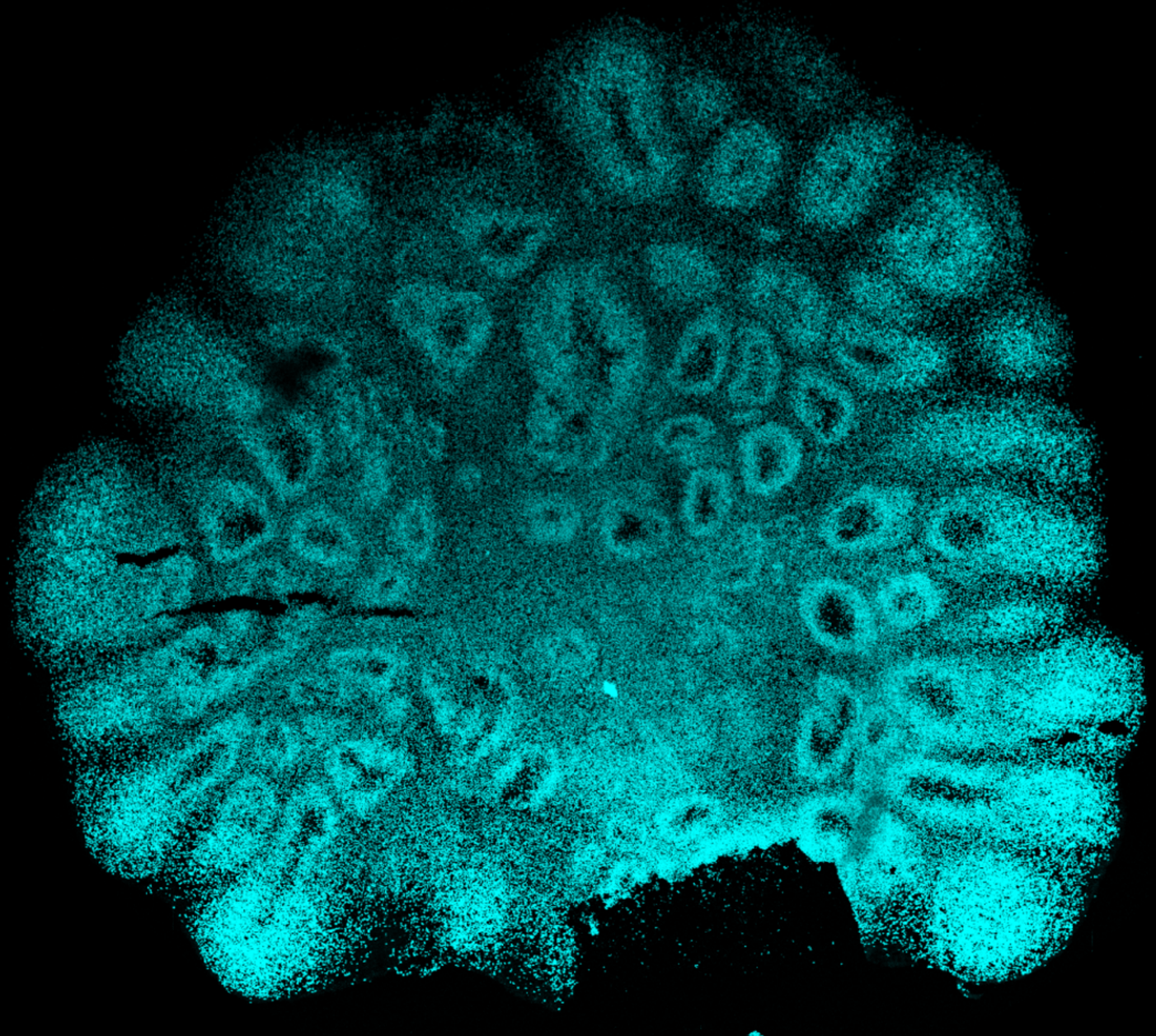
Building reliable in vitro models of the CNS



Molecular studies on human patient brains are almost impossible. Stem Cell models can help overcome this bottleneck.

Goddard et al. (2023) MDPI Organoids

Understanding Human Brain Development using Neural Organoids

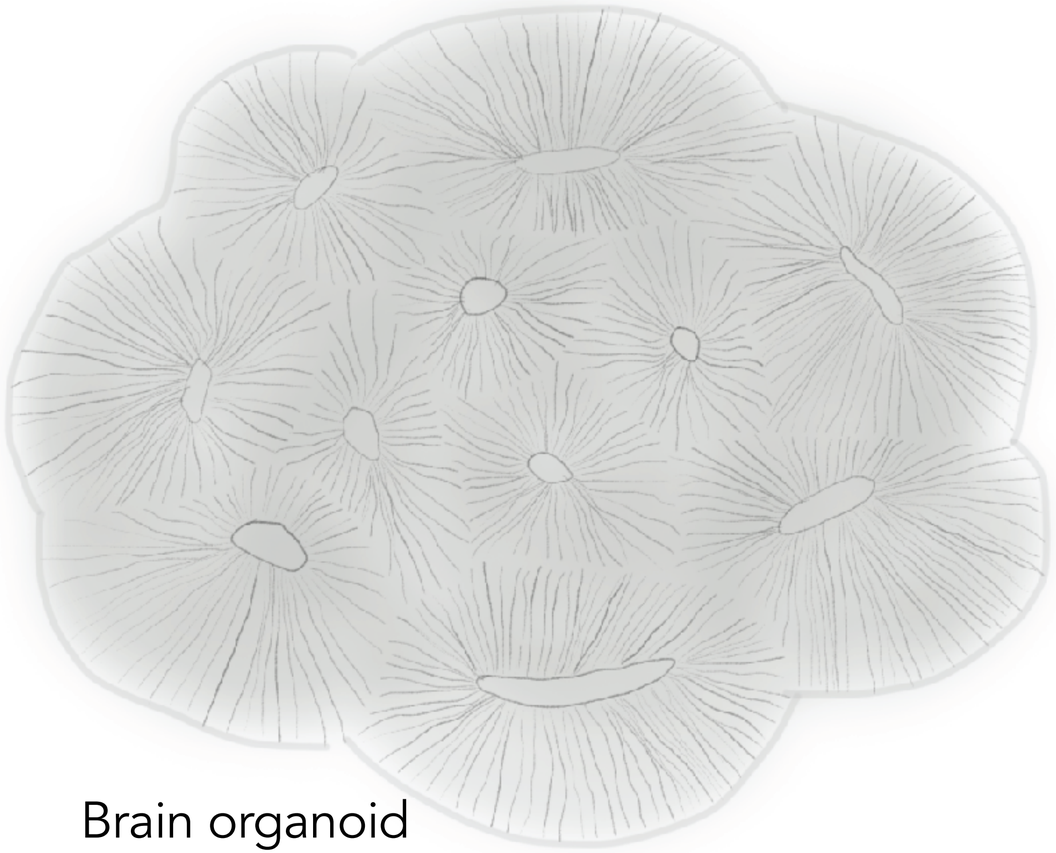


Human Neural Organoids recapitulate embryonic Development

iPCS

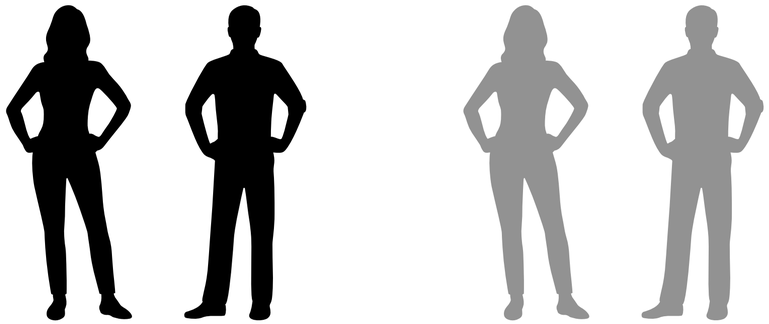
Embryoid Bodies

Neuroepithelium



Brain organoid

Human Neural Organoids are generated from iPSCs

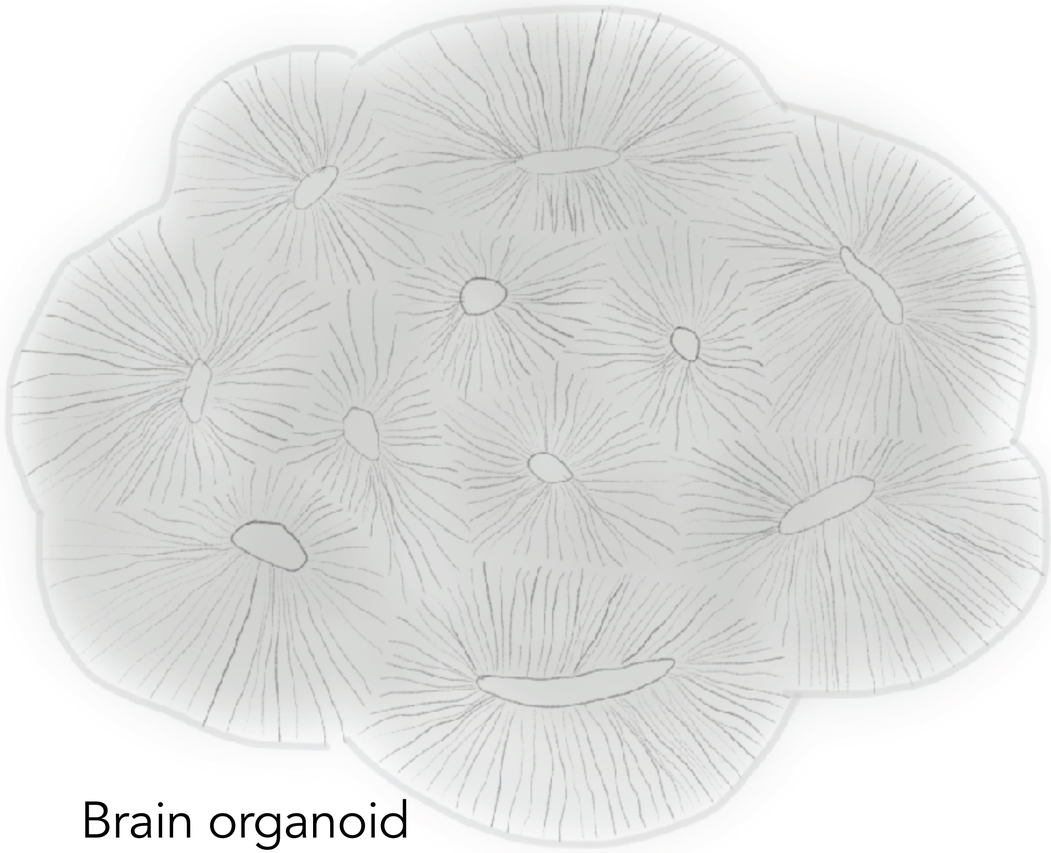


Healthy and Patient derived skin cells

iPCS

Embryoid Bodies

Neuroepithelium



Brain organoid

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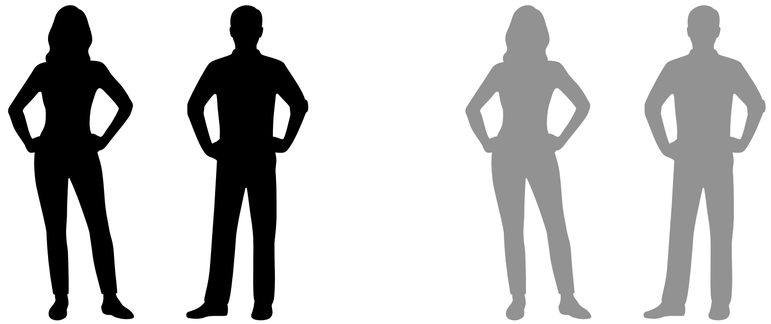


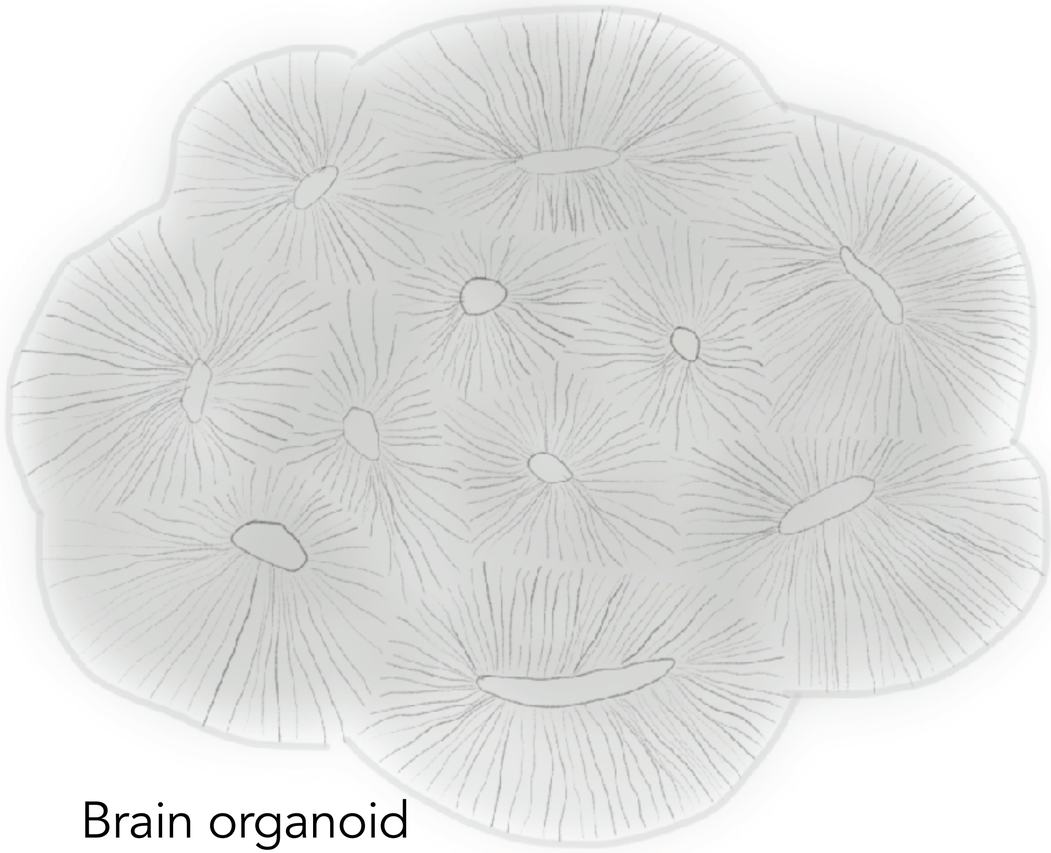
Healthy and Patient derived skin cells

Yamanaka Cocktail

iPCS

Embryoid Bodies

Neuroepithelium



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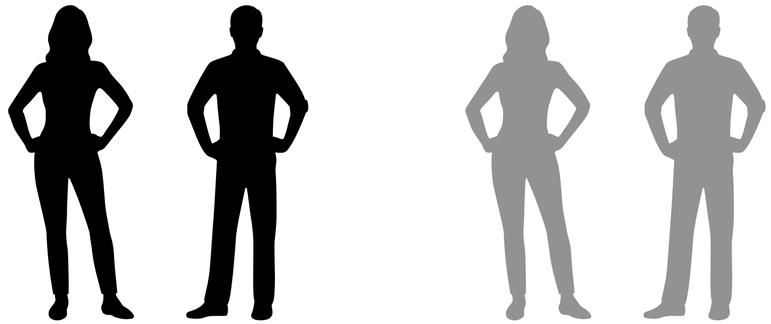


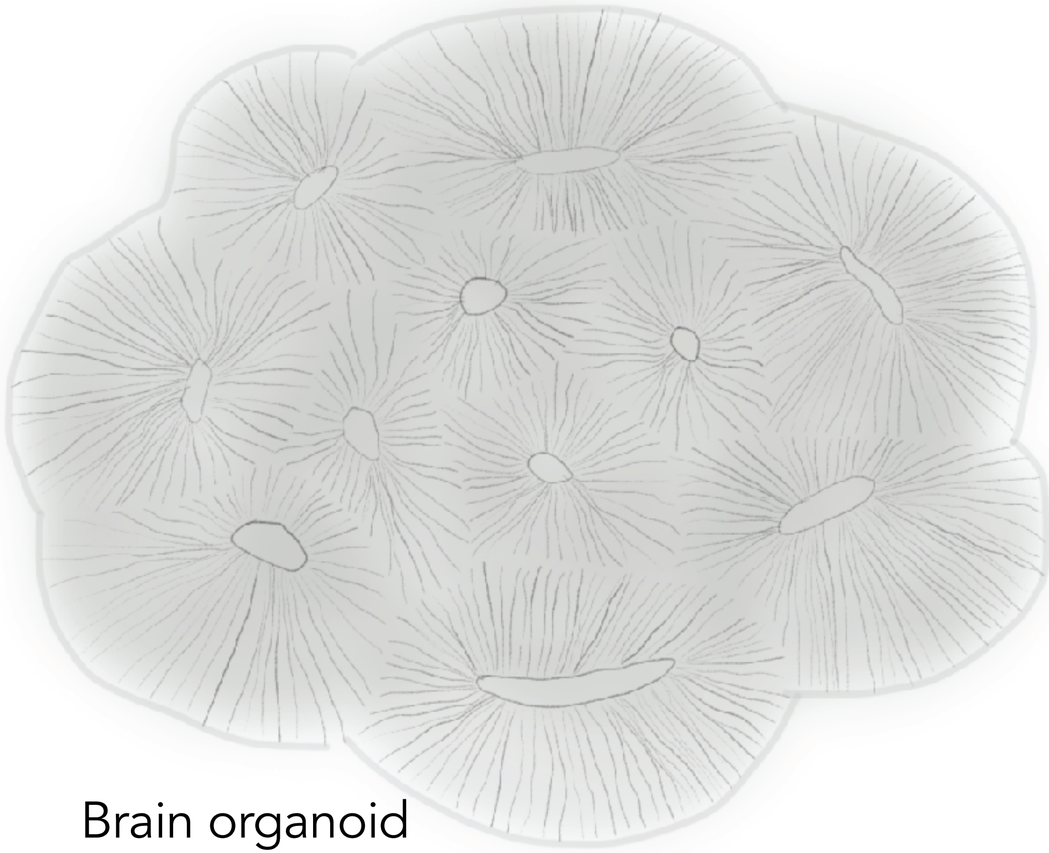
Healthy and Patient derived skin cells

Yamanaka Cocktail reprograms to pluripotency

iPCS

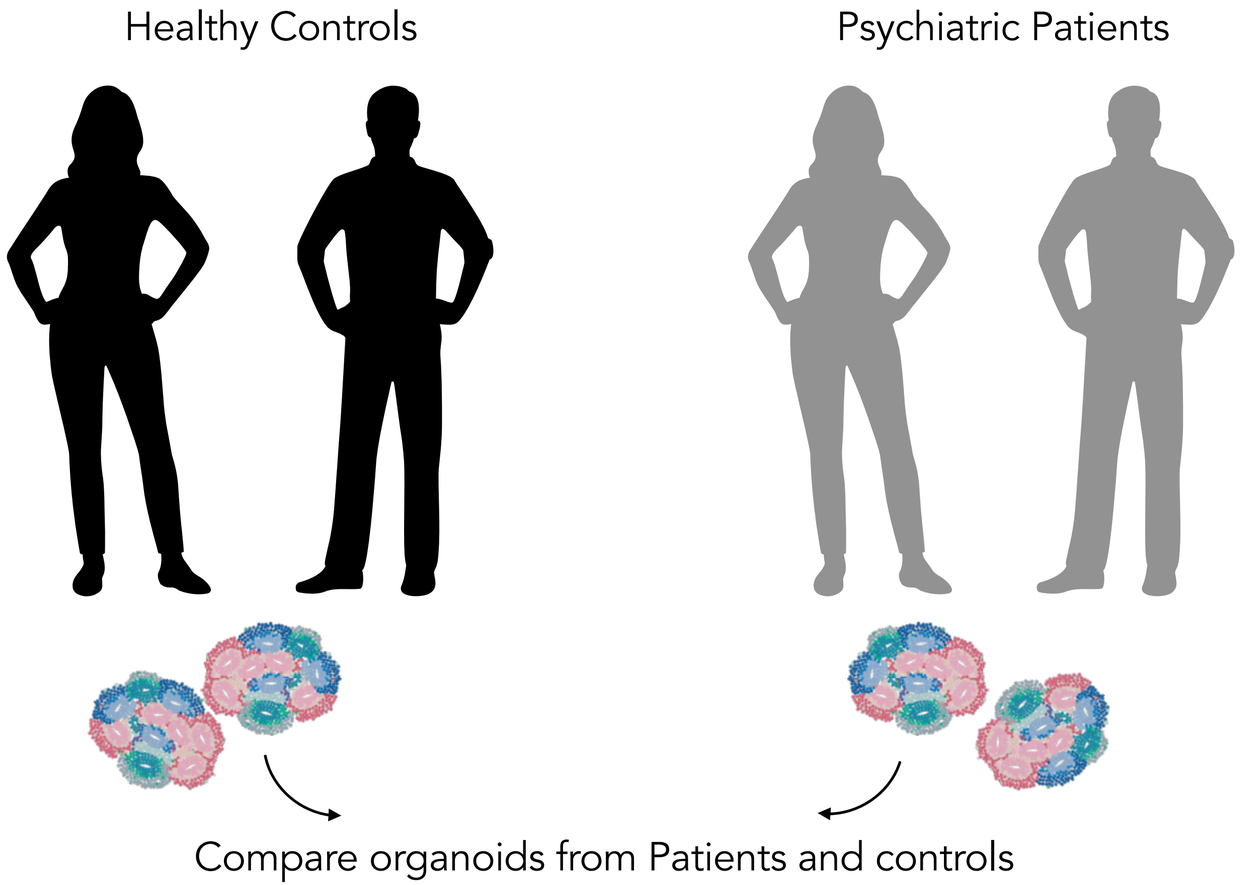
Embryoid Bodies

Neuroepithelium



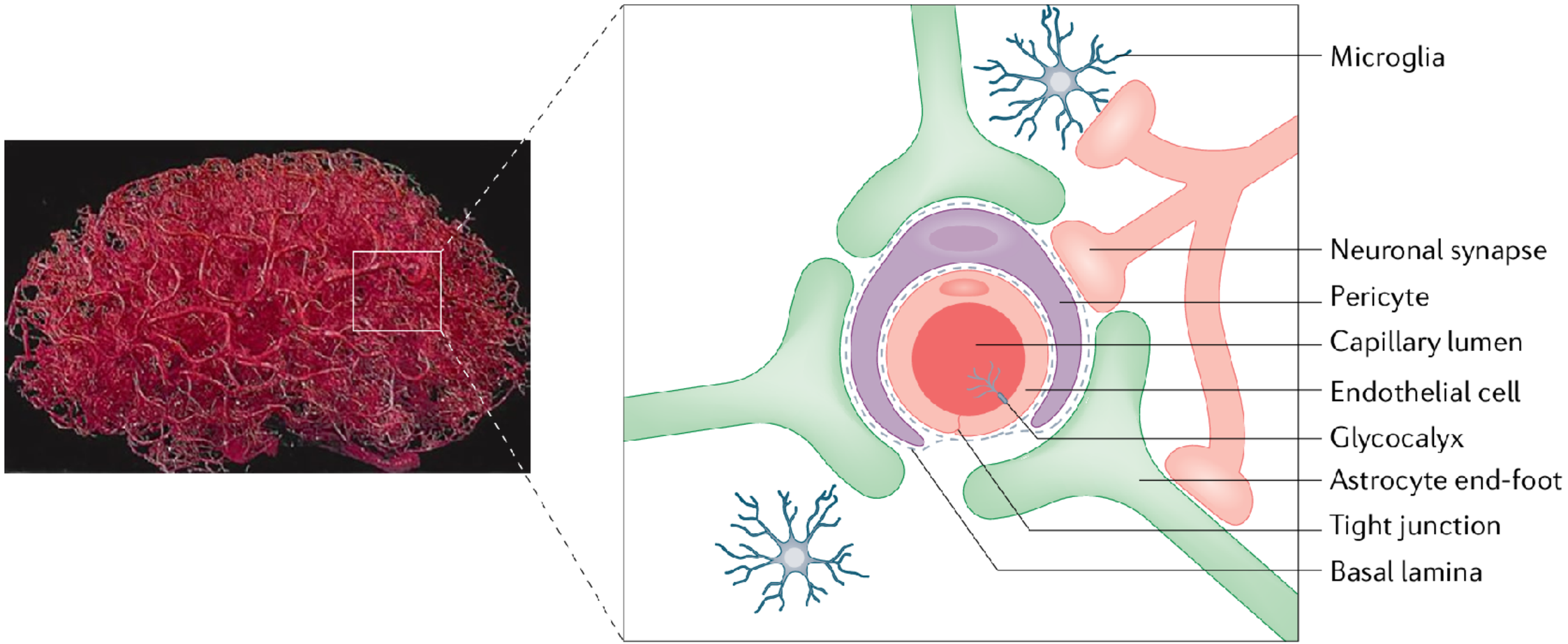
Brain organoid

Quantifying differences in Organoid Development



Organoids can serve as patient surrogates, aiding in the development and testing of personalised medicine.

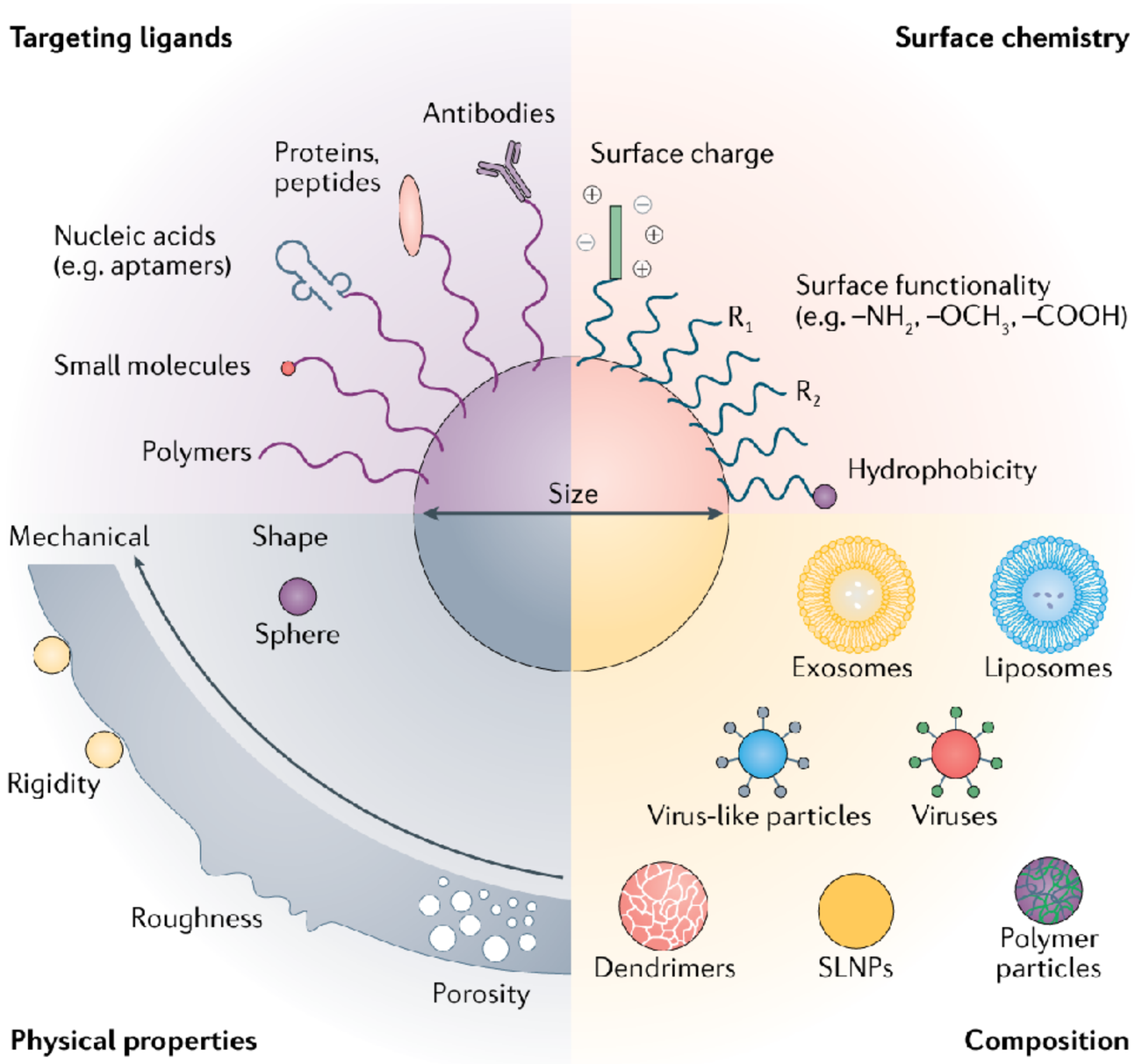
Blood Brain Barrier



The blood-brain barrier limits the diffusion of molecules from the blood system into the brain. It's one of the very few tight boundaries in the human boundaries in the human body.

Terstappen et al. (2021) Nature Reviews Drug Discovery

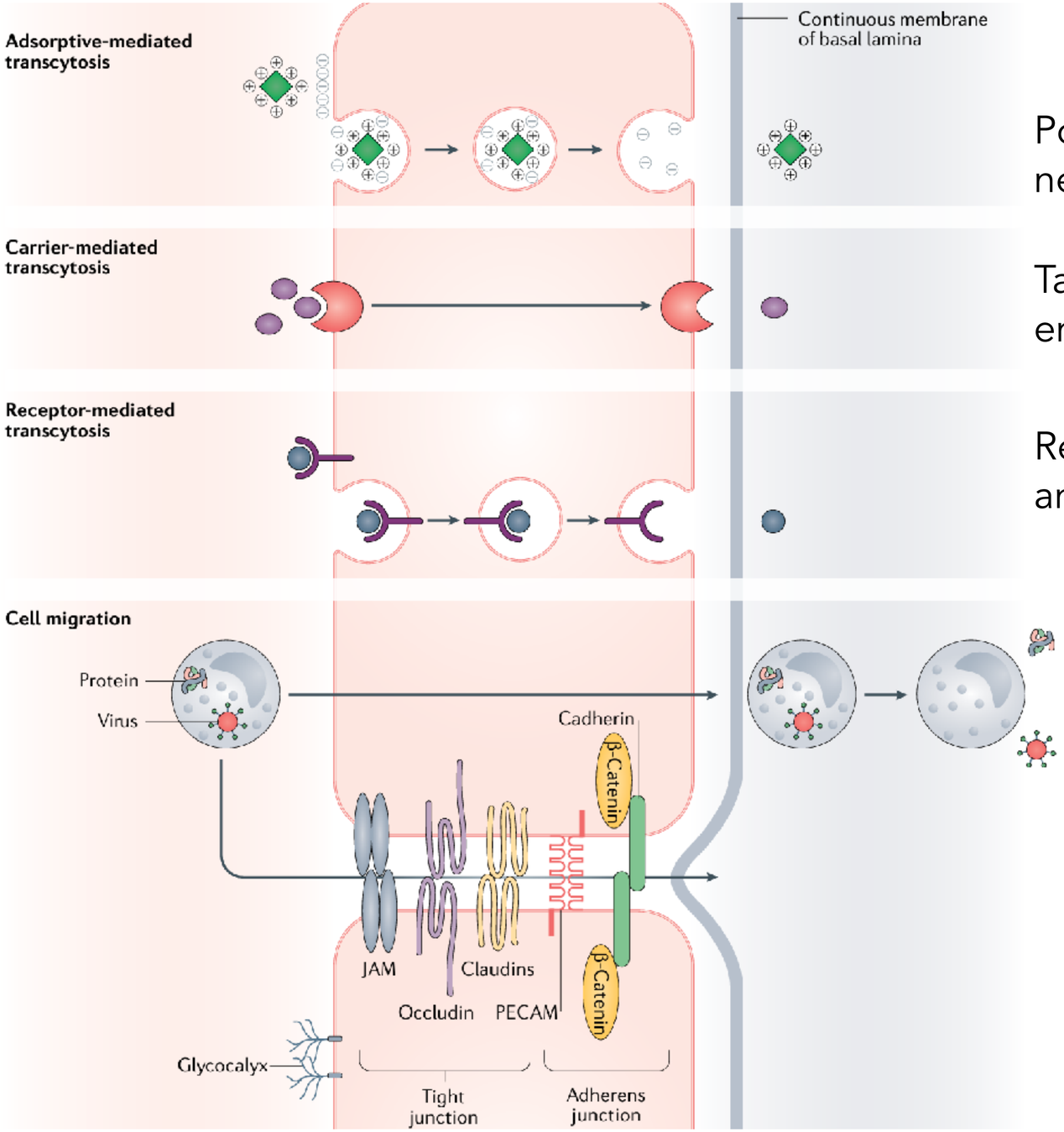
Blood Brain Barrier



Several factors influence the permeability of the BBB and novel tools try to effectively cross it.

Terstappen et al. (2021) Nature Reviews Drug Discovery

Blood Brain Barrier



Postitively charged molecules interact with the negatively charged glycocalyx

Target molecule binds to carrier and gets endocytosed

Receptor or antibody bind to target molecule and get endocytosed

Macrophages and monocytes (immune cells) can get endocytosed or travel through the pericellular space

Terstappen et al. (2021) Nature Reviews Drug Discovery