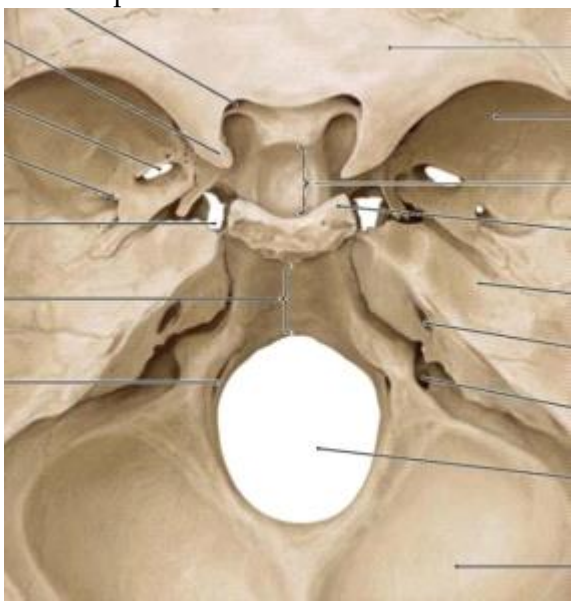
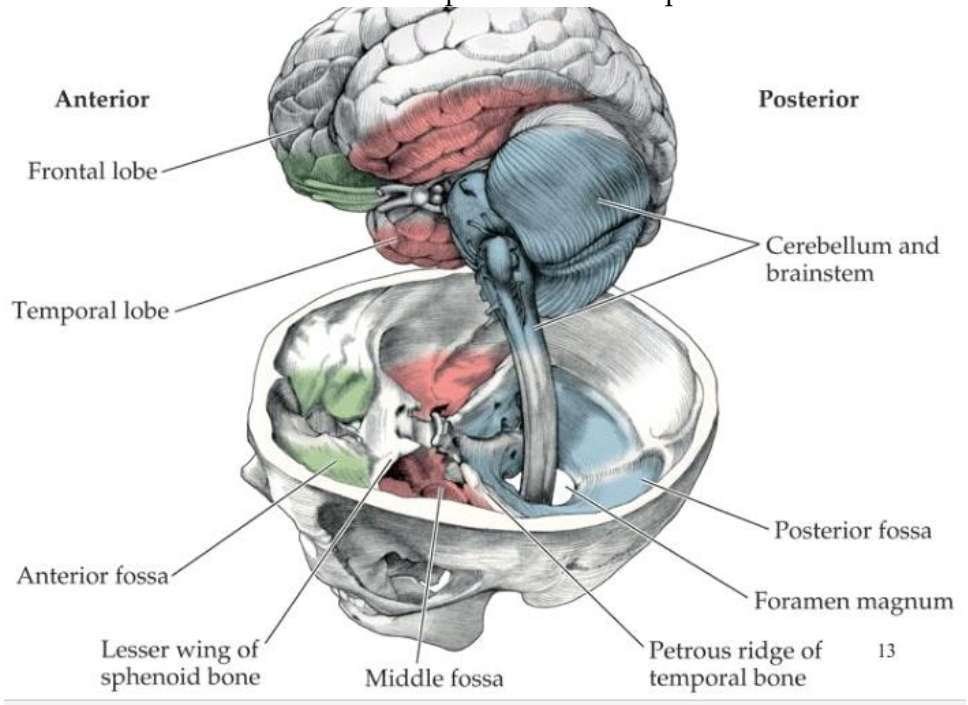
0. Introduction to Neuroanatomy

	We do not need to memorize every label in the Atlas Anatomy Understanding is more important than tiny anatomical details
Why the soft brain is not damaged by its pointy skull?	**Foramen magnum** - Entrance to Spinal cord, nerves, meninges, blood vessels Brain has a very soft texture - It would get damaged by the pointy skull if it was not protected 
What is the part of the skull that touches: **a) Frontal lobe** **b) Temporal lobe** **c) Cerebelum**	The brain and skull have a 1 to 1 spatial relationship 

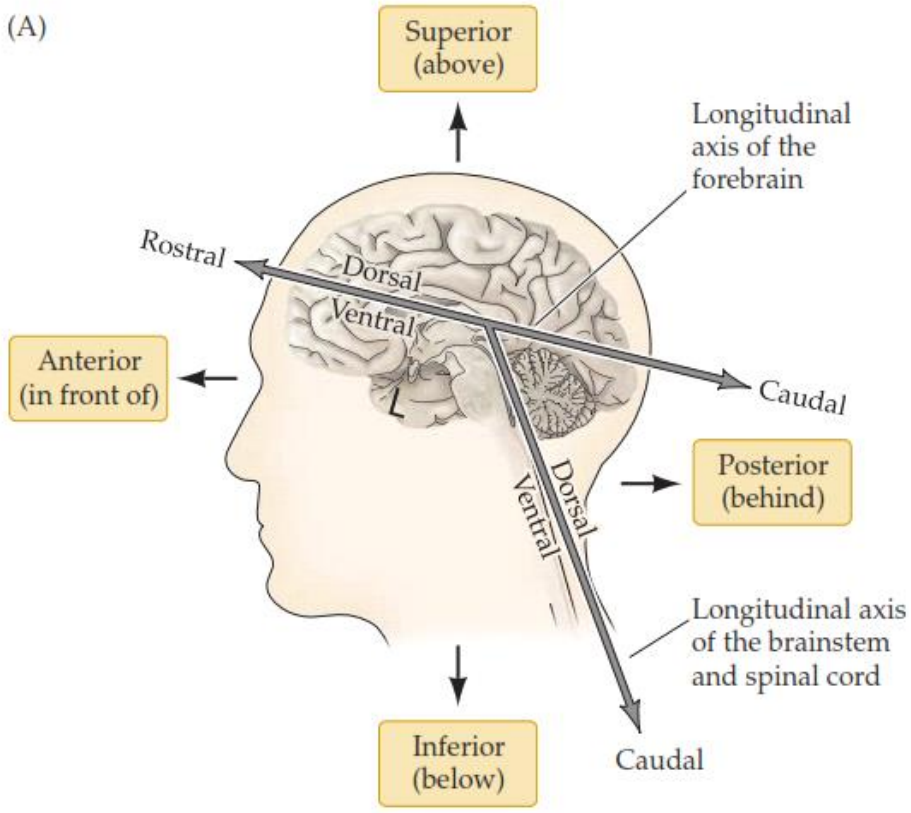
What is the difference between dorsal/ventral and superior/inferior?

Planes of the head

Dorsal/ventral - Top and lower side (in humans, brain and spinal cord are in different plance because we are bipedal)

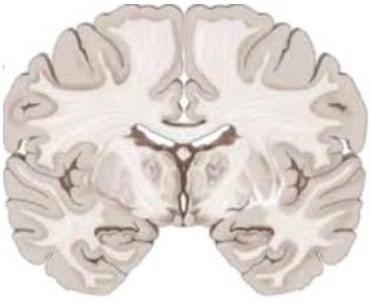
Superior and Inferior - Up and Down (absolute position)

Rostral and caudal - Nose and tail side
Anterior and posterior - Front and Back (absolute position)

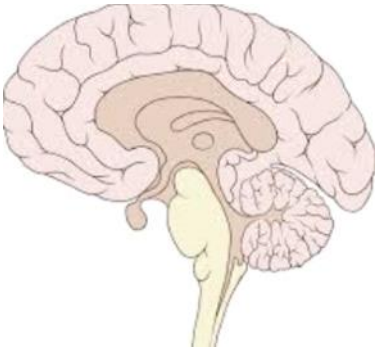


Medial and lateral - From the middle to the side

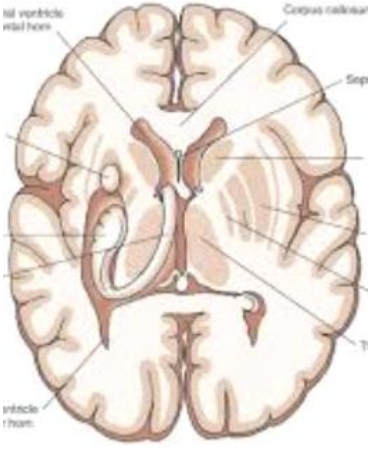
Coronal

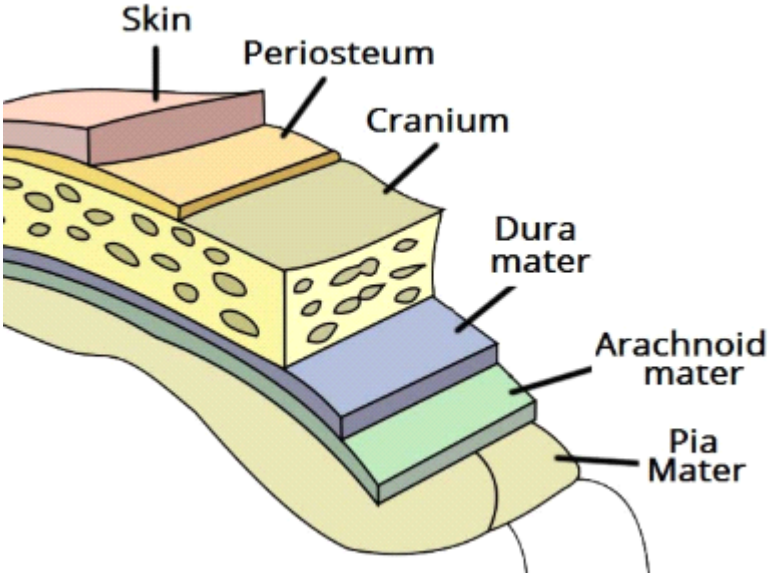


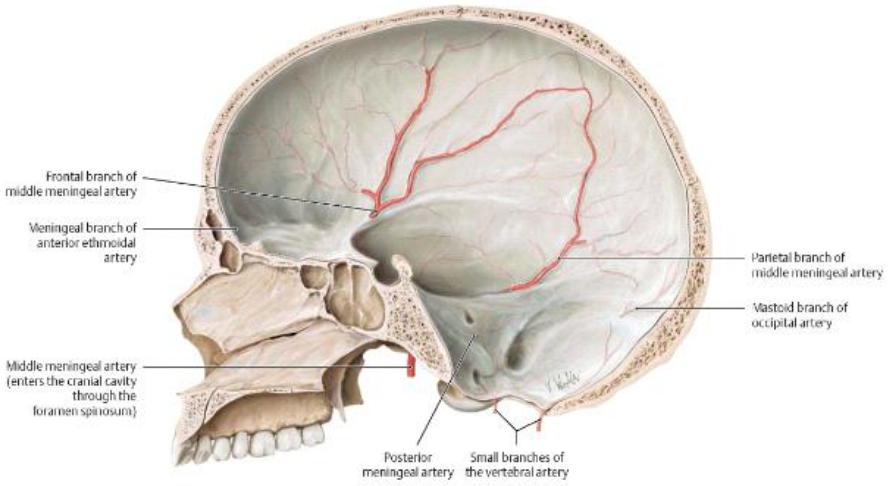
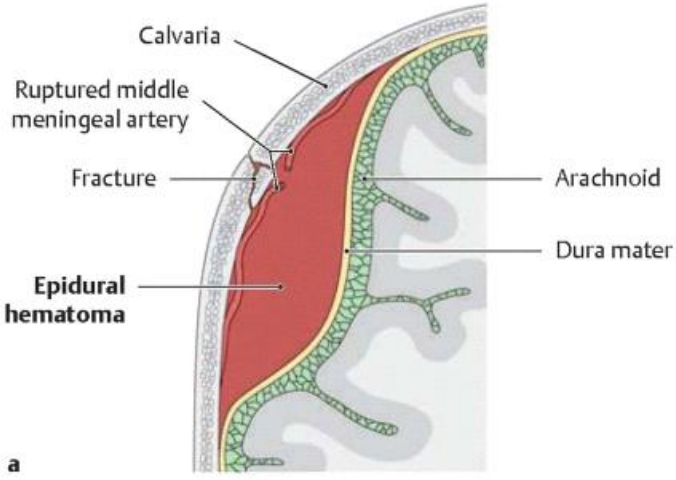
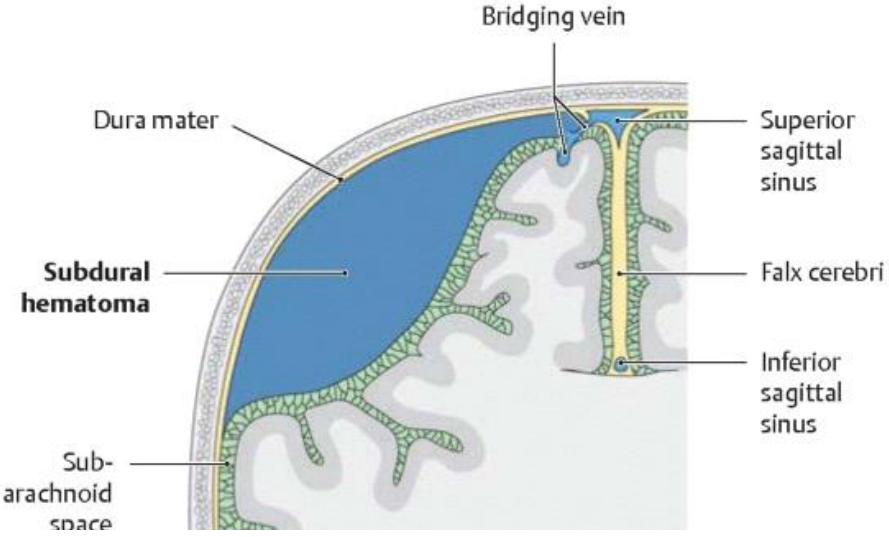
Sagital - Mid or parasagital



Horizontal/axial

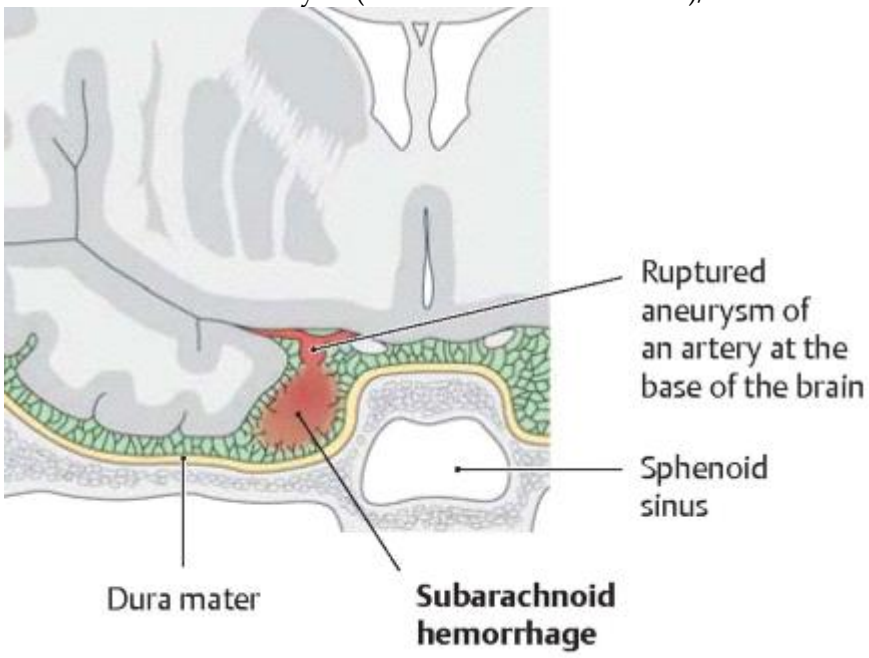


What are the three protective layers of the brain?	Protective layers of the brain **Dura Matter** Meningeal layer Periosteal layer **Arachnoid** - Attached to the skull **Bridging vein** - Cross the arachnoid and Dura Matter Subdural hemorrhage - People get older, their brains shrink, bridging veins are more likely to rupture **Pia Mater** - Fibrous layer impermeable to fluid Maintains CSF inside the brain 

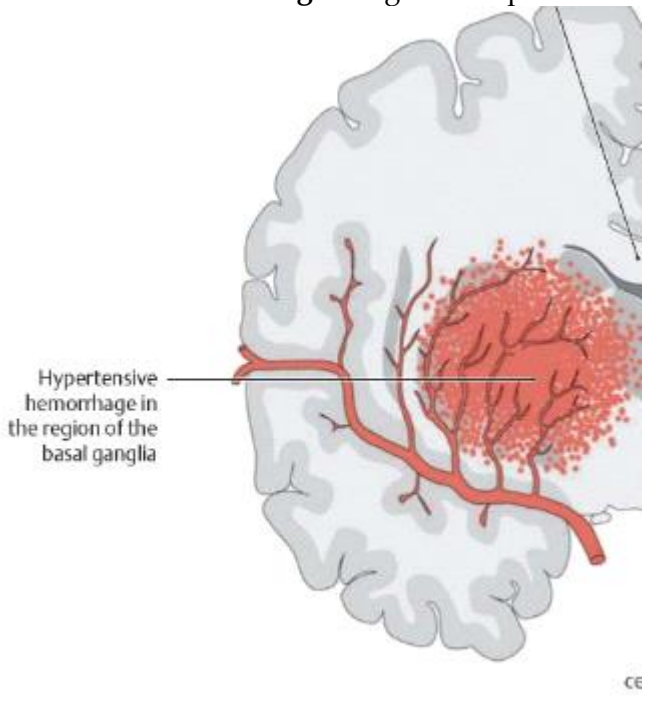
	These three also cover the spinal cord
What is the 'biker's artery' and why does it have this name?	Skull is very thin at the temporal bone - Prone to hematoma Frontal branch of the **middle meningeal artery** - Bikers artery (prone to injury) 
What are the possible causes to a subdural hemorrhage? **What are the possible causes to a subarachnoid hemorrhage?** **What is the most likely cause to an intracerebral hemorrhage?**	**Epidural hematoma** - High pressure  **Subdural hemorraghe** - Low pressure Rupture bridging vein (shaking a baby/getting older) 

b

Subarachnoid - Aneurysm(dilation of a blood vessel)/trauma



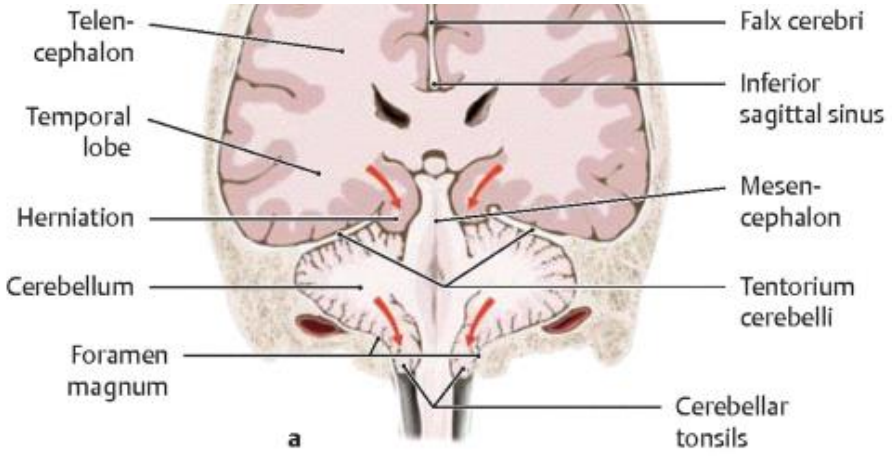
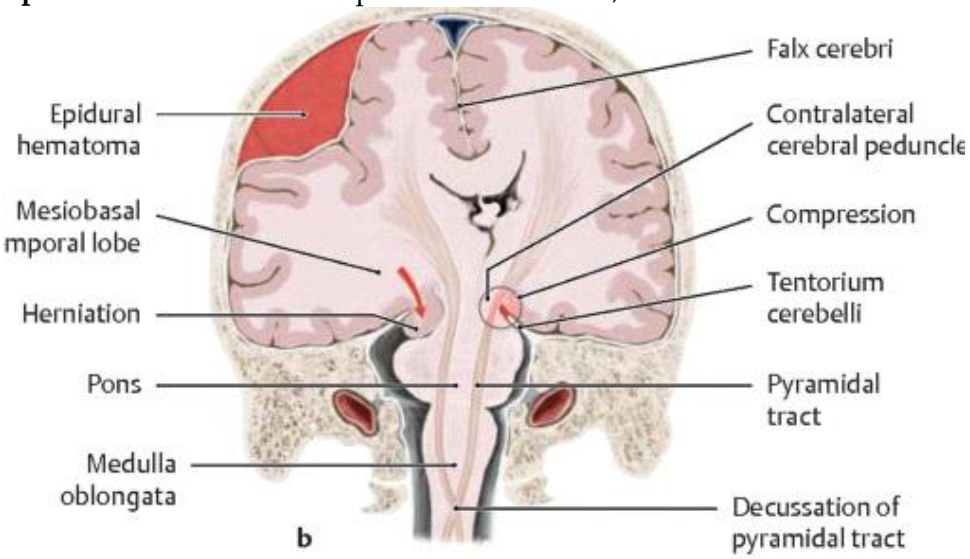
Intracerebral hemorrhage - High blood pressure

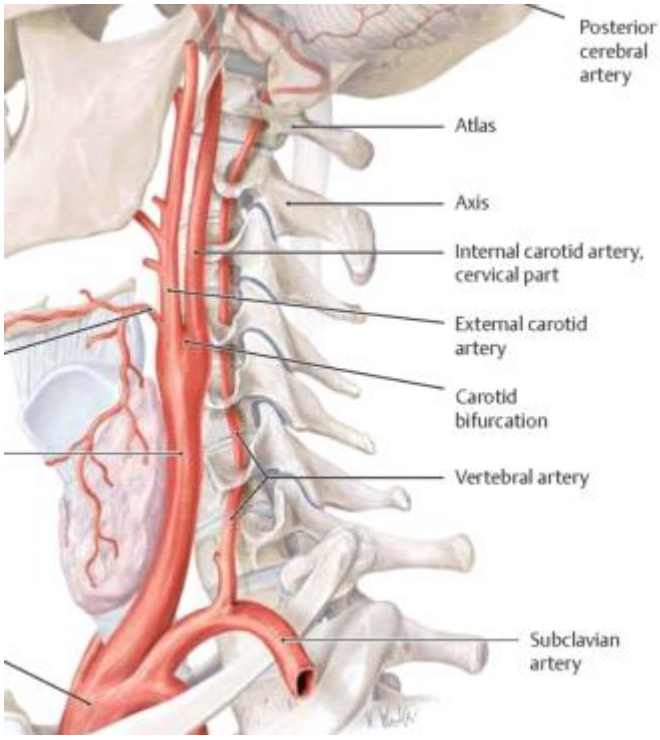


Define Herniation

What happens to a brain with the increased pressure of an epidural

Herniation - Brain can be pushed downward into its caveties
Worst symptoms come from pressure at the midbrain

hematoma?	 **Epidural hematoma** - Compression in one side, herniation at the other 
Where does the vertebral artery enter the brain?	**Vertebral artery** - Enters via foramen magnum with the spinal cord **Common carotid** - Internal carotid, external carotid

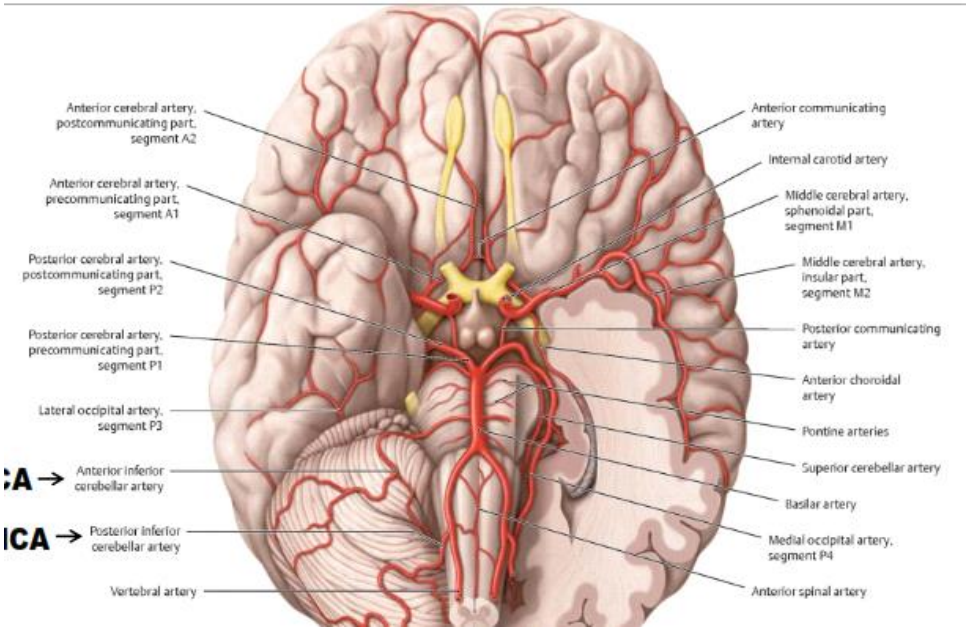


What are the three main cerebral arteries?

What are the two main cerebellar arteries?

Major arteries:

Anterior cerebral artery
Middle cerebral artery
Posterior cerebral artery

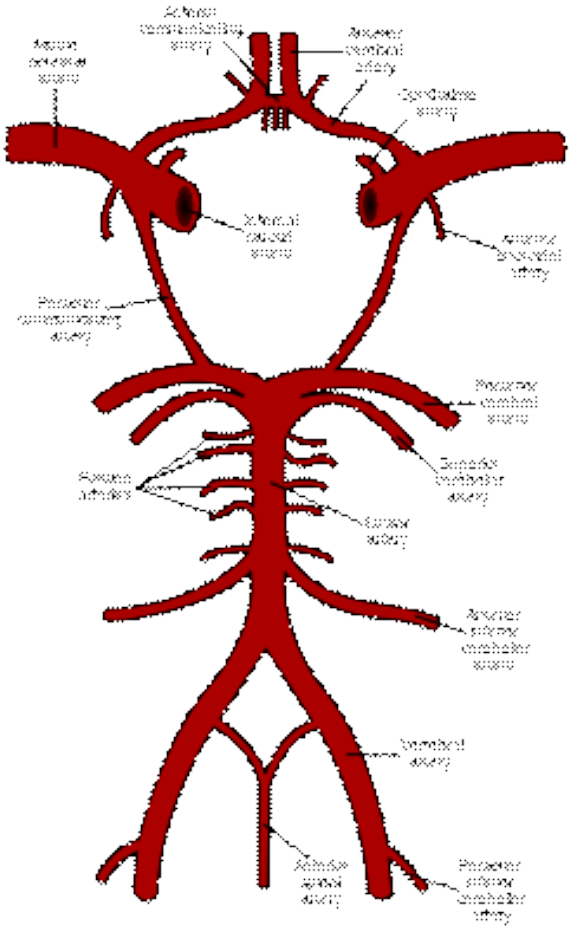
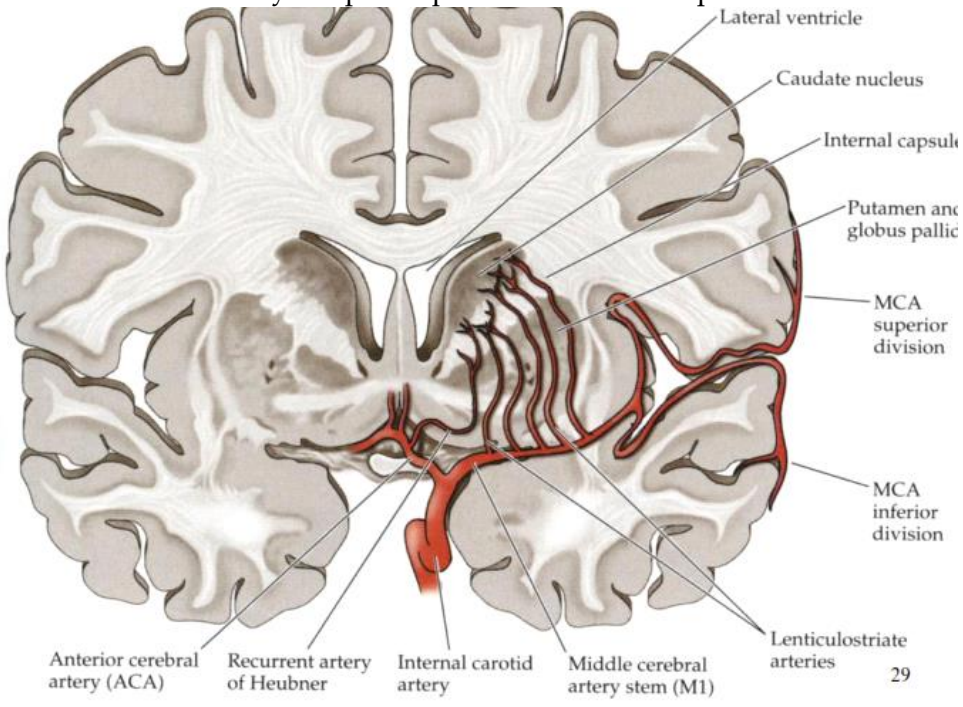


To the cerebellum:
AICA - **Anterior inferior cerebellar artery**
PICA - **Posterior inferior cerebellar artery**

Is the Circle of Willis present in every human brain? Is there an advantage to its

Posterior communicating artery + Anterior communicating artery = **Circle of Willis**

The brain has a complete circle - If one part is damaged, other parts can provide blood flow

presence?	**Anastomoses** = Connections between separate veins 
	Middle cerebral artery - Superior portion and inferior portion 
Which brain part has two different cerebral arteries	Areas covered by cerebral artery Damage to a particular artery relates to damage to a particular brain

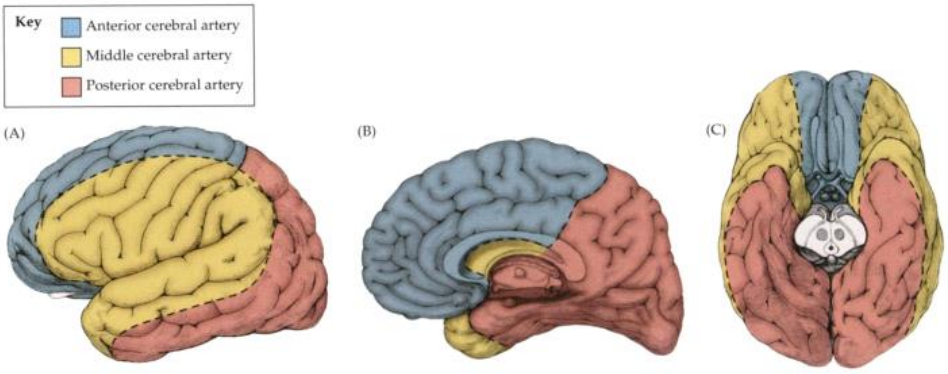
as input?

region

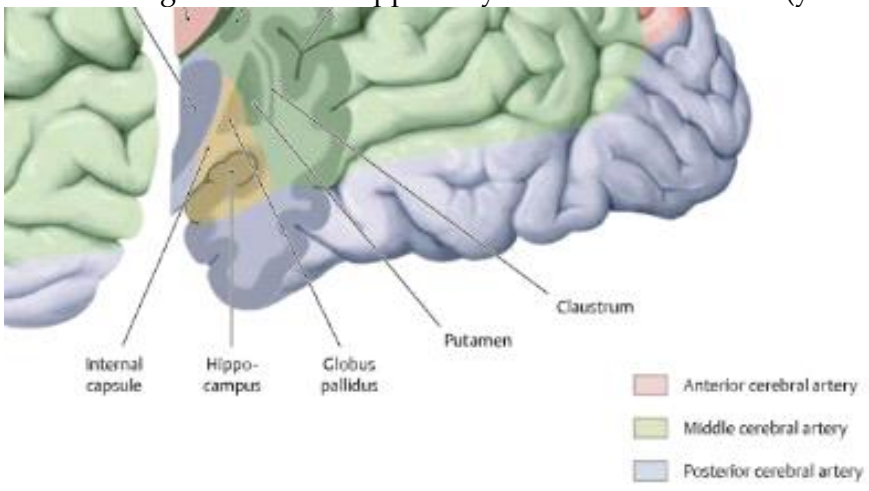
Which area is covered by the anterior cerebral artery?

Which area is covered by the middle cerebral artery?

Which area is covered by the posterior cerebral artery?

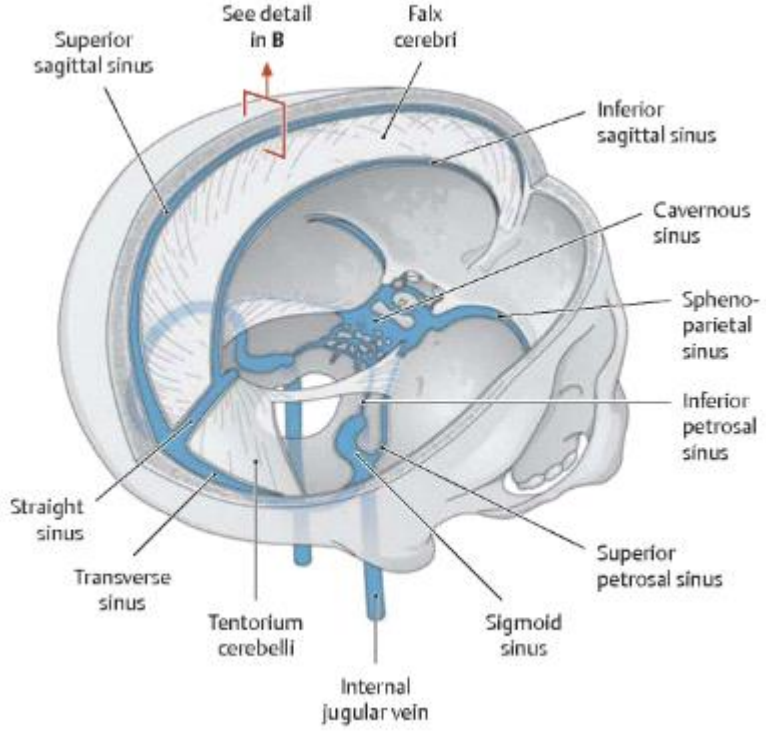


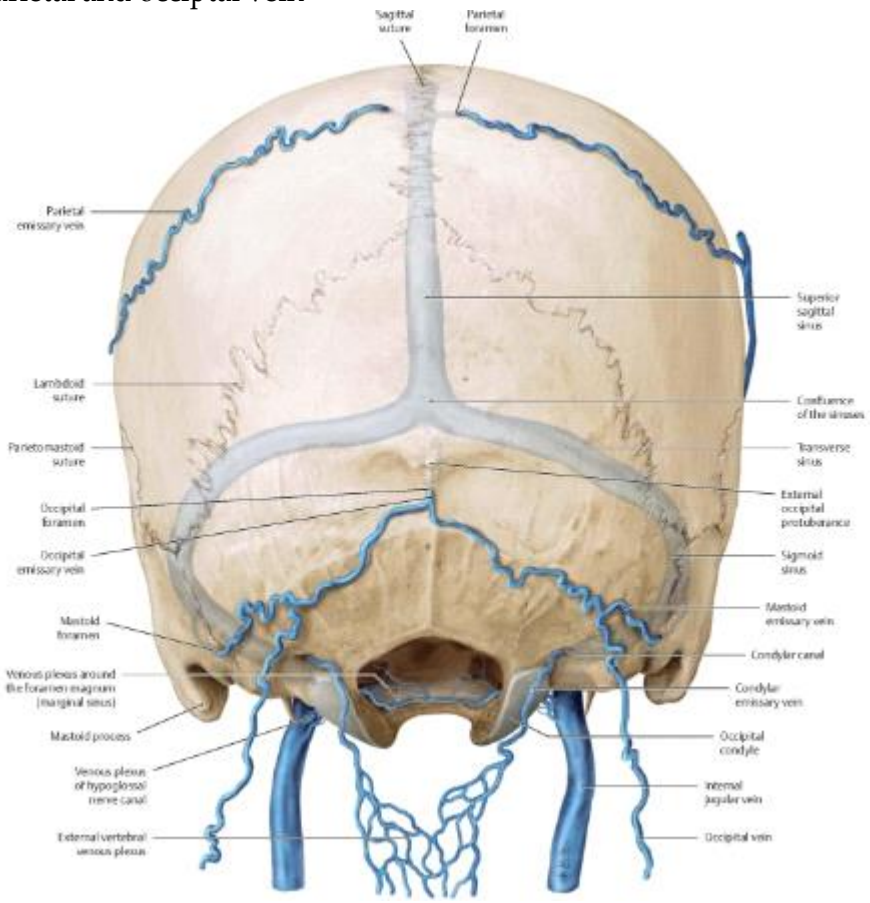
There are regions that are supplied by two different arteries (yellow)

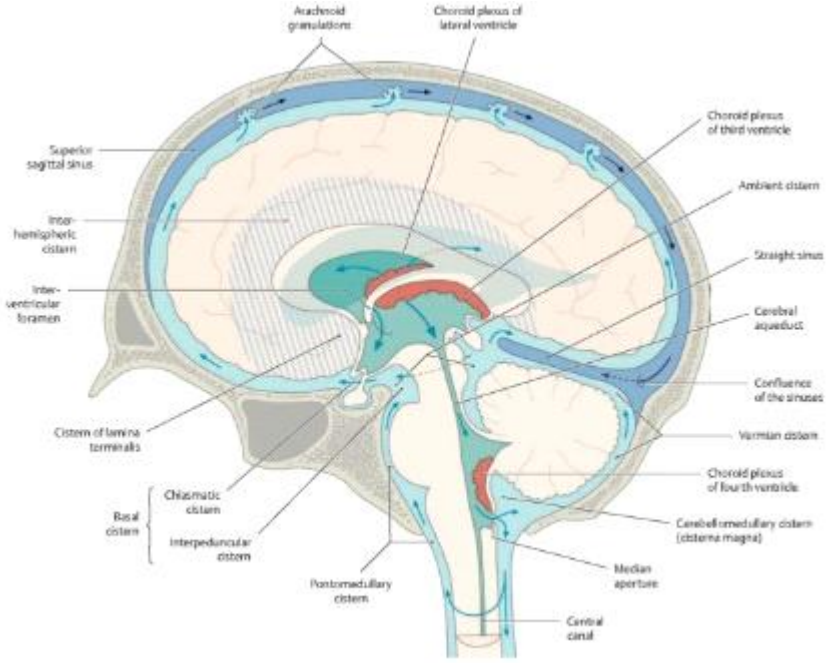


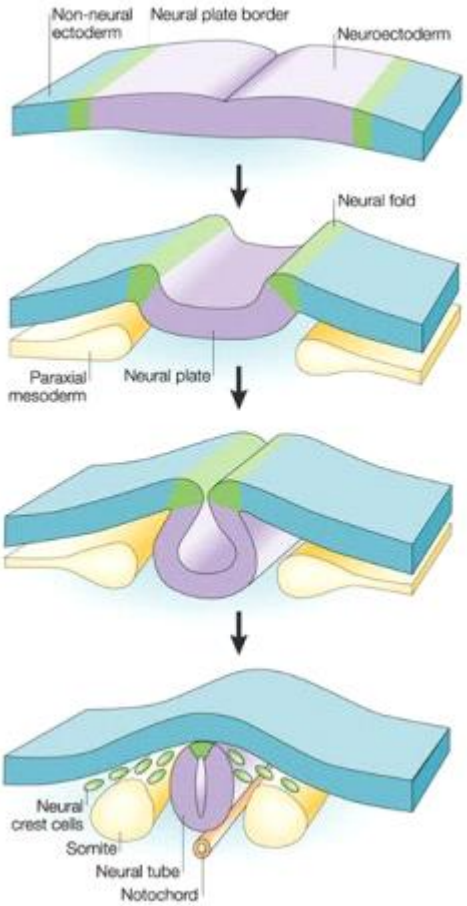
What is the function of a sinus?

Sinus - Deoxygenated blood is transported out of the skull
Collects in the jugular vein



What is an emissary vein? In which skull bones are they present?	Some of those vein exit the skull = **Emissary vein** Parietal and occiptal vein  If there is trauma and the blood flow is reversed - Problem because it does not have the blood brain barrier
What is the apparent weight of a brain due to the CSF buoyancy? **What is the structure that produces CSF? And which structure absorbs it?**	**Cerebrospinal Fluid** Produced by choroid plexus Absorbed by Arachnoid granulations (cauliflower) Increase brain buyancy Bouyancy generated by the CSF makes the brain float - Seemingly weigh 50 g instead of 1500 g
How many mililiters of CSF are present in a	Red zone - Produces Blue - Absorbs

human brain?	 This generates flow 125 ml in a human brain = renewed constantly
	Blood Brain Barrier 20% of the oxygen 600 km in capillaries
	Development of the brain - Very soon after conception Start as a plate Becomes a tube



What are the three divisions of the neural tube? What are the five structures they generate?

Forebrain - Telecephalon and diencephalon
Midbrain - Mesencephalon
Hindbrain - Metencephalon and myelencephalon

What the tube will become?

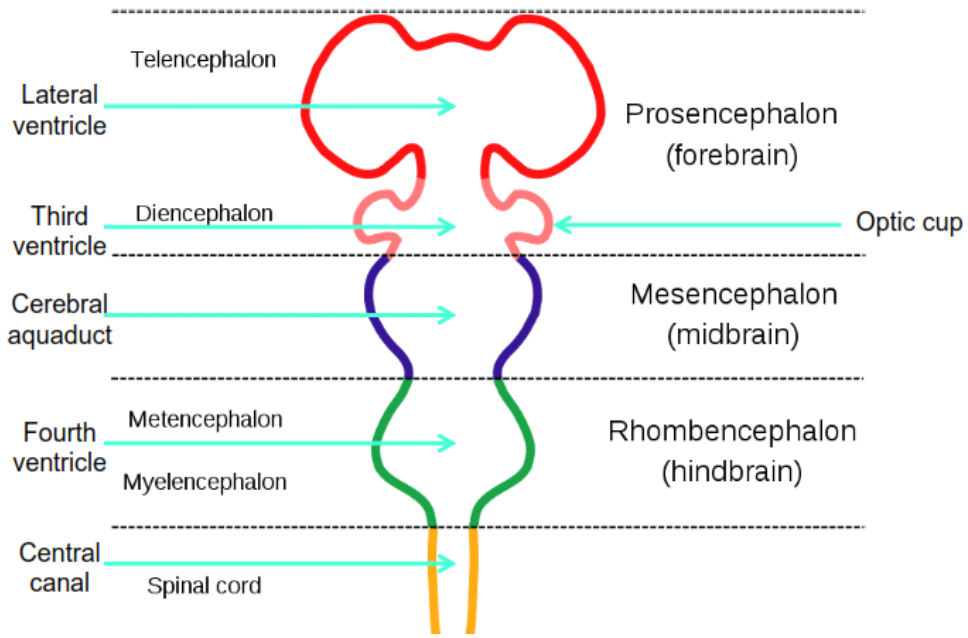
What is the ventricle associate with the telencephalon?

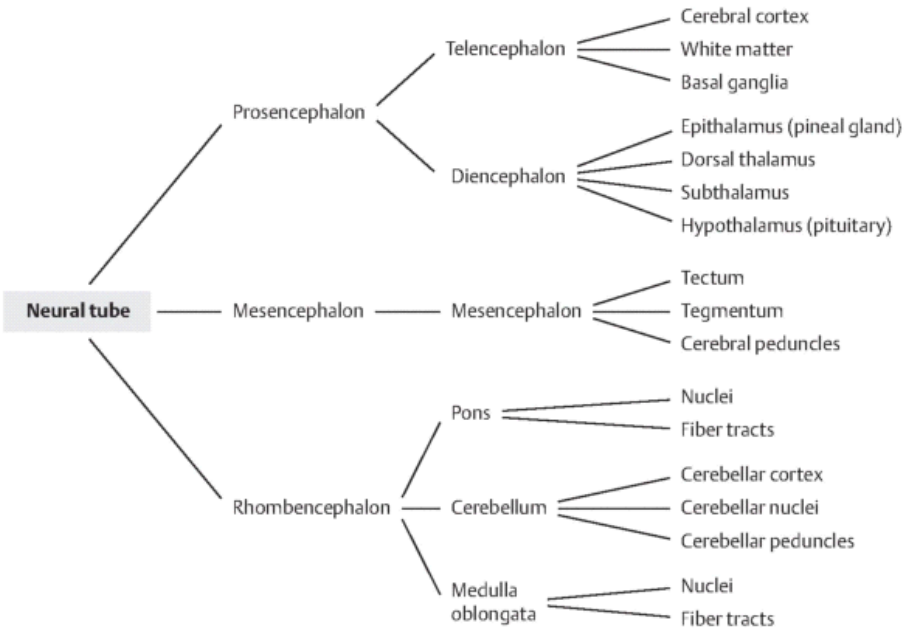
What is the ventricle associate with the diencephalon?

What is the ventricle associate with the mesencephalon?

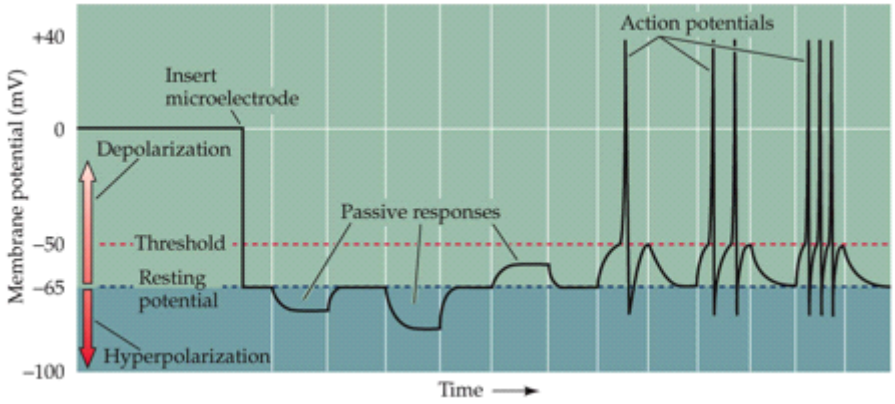
What is the ventricle associate with the rhombencephalon?

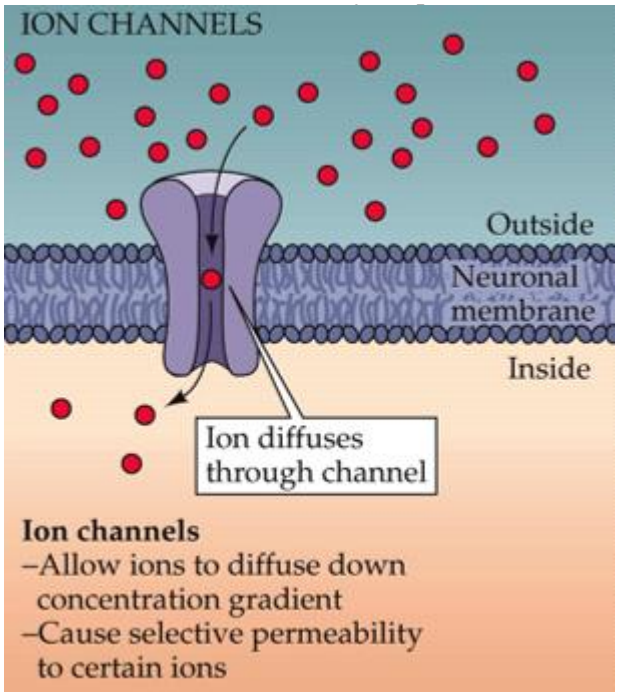
What is the ventricle associate with the spinal



cord?	
Why doesn't the ventricular system look like a normal tube?	Ventricular system - Does not look like a tube Different flexures 1) Cephalic (grows quicker than the other parts) 2) Cervical 3) Pontine

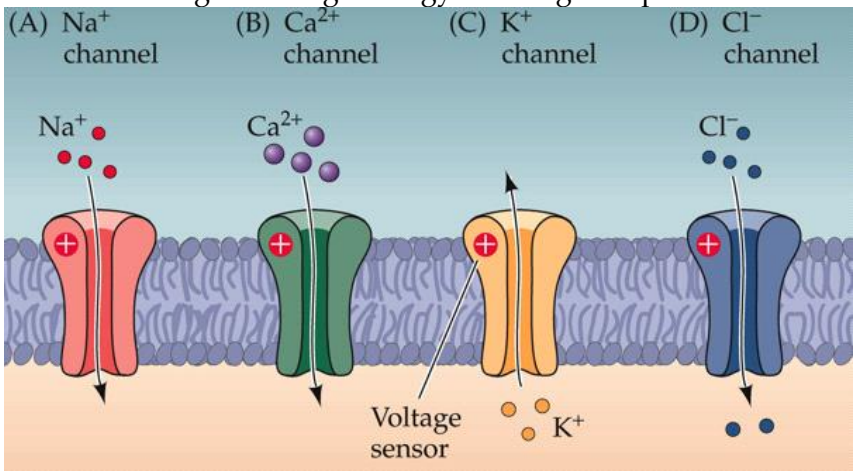
1. Neurophysiology - Ions channels & Resting membrane potential

What do neurons have in common? **What is the functional advantage to use electricity instead of chemical communication?**	Brain has the most diverse cell types in the body What neurons have in common: - Resting membrane potential and use electricity to communicate **What is the advantage of electrical signalling?** Electricity is used because it is faster than chemical communication
What is a neuronal threshold?	Resting membrane potential = **-65 mv** Threshold = **-50 mv**  Below threshold - Passive responses Above threshold - Active responses
Define the cellular function of ion channels and their two main different types.	**Ion channels** - Proteins that allow ions to move towards their concentration gradient Ions cannot pass the membrane because they are hydrophylic and the cell membrane is hydrophobic



Ligand-gated - Need a chemical ligand to change the protein chains

Voltage-gated - No chemical ligand, only changes in the electrical field give enough energy to change the protein chains



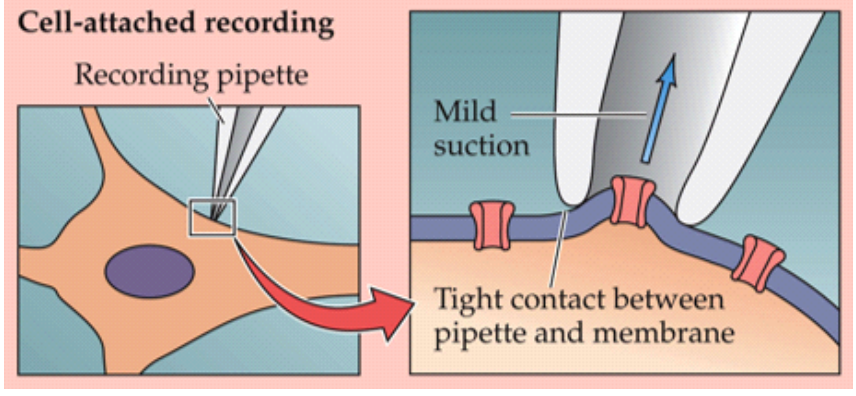
Na, Ca, K, Cl channels

What is the technique of patch clamp? How does it work?

What it means to say that channels are stochastic?

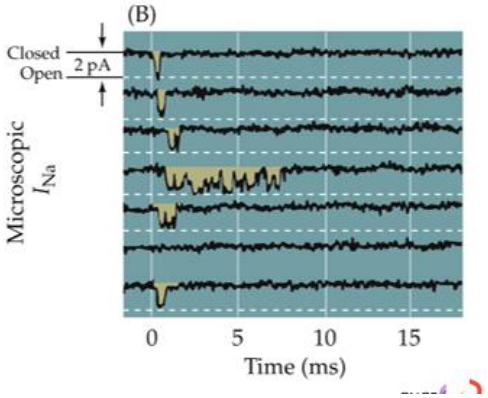
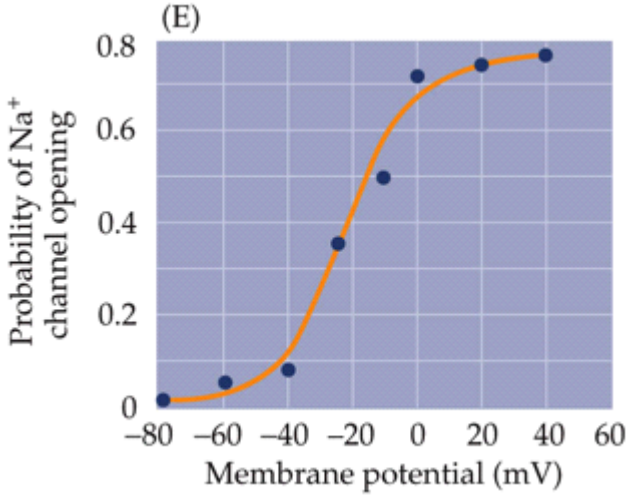
Patch clamp - Nobel prize in 1991

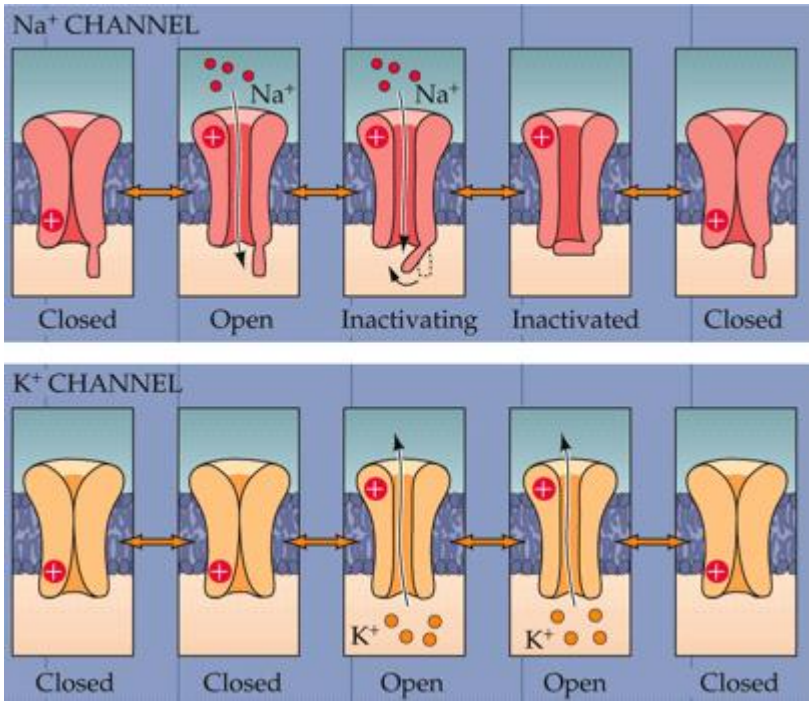
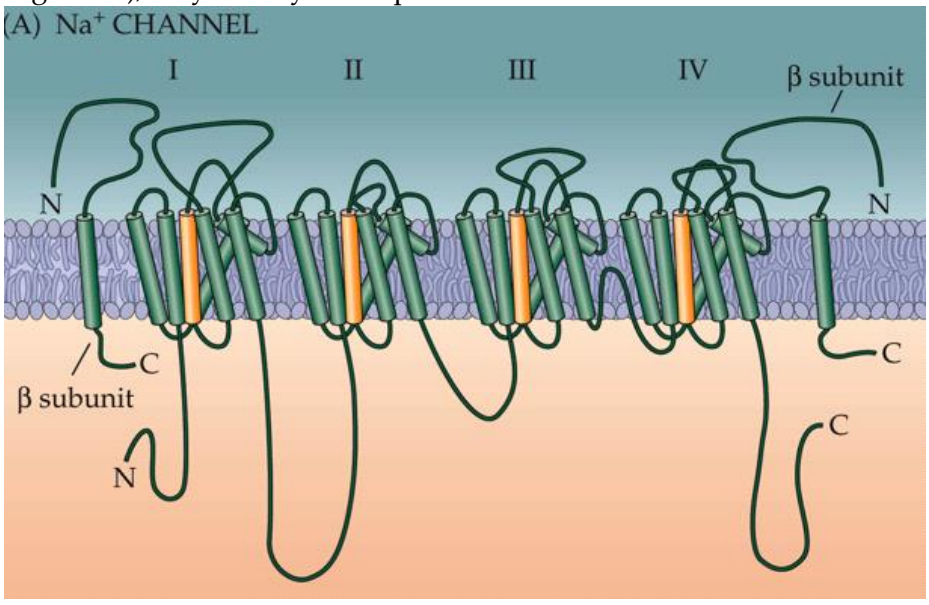
Cell-attached recording

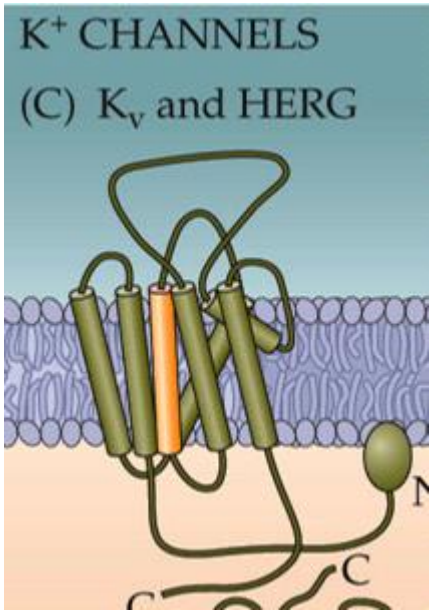


Closed channel - No signal

Open channel - Baseline signal (total current equals the sum of

	millions of tiny channels)  **Na channel** - Closes very quickly **K channel** - Open for a longer period of time Channels are stochastic - There is a chance of being either open or closed at all times  Probability of a channel opening is voltage dependent
Define 'inactivation site' present in sodium	Na channel - Has a **inactivation site** (in a different place than the part that regulates open/closed state)

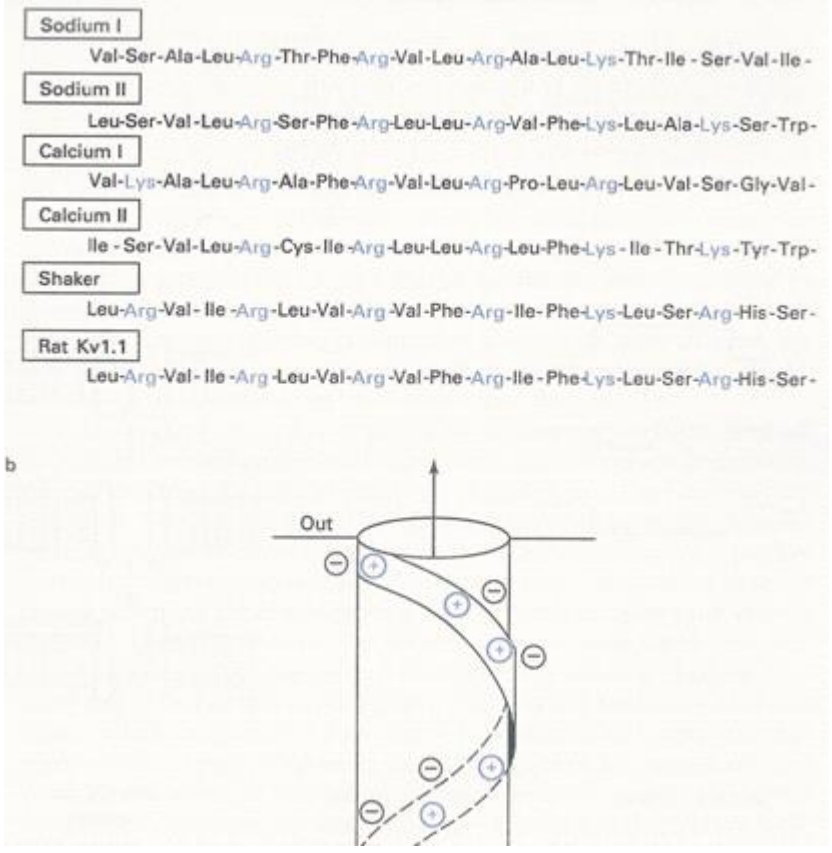
channels.	 *Sodium channels may be open but inactive - Sodium ions cannot flow* Na channels open fast K channels open slower
What is the structural difference between sodium and potassium channels?	Na channel - Alpha subunit (**four** domains with **six** transmembranes segments), may or may not express beta subunits  K channels - **Four separate** subunits (**six** transmembrane segments) come together to form a channel



Define the important of the S4 domain from the potassium channel and its molecular underpinings.

Voltage dependence - Voltage sensor causes change of conformation of the channel

Gating of **S4 domain** - Every third aminoacid has a positive charge



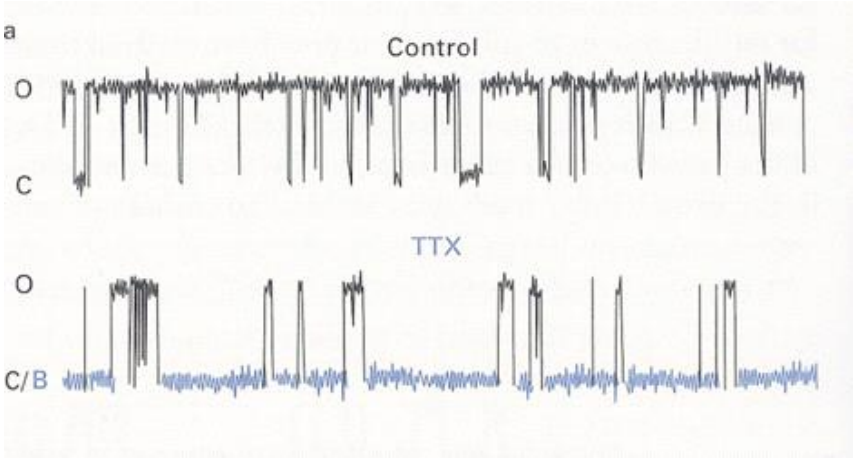
Change in this domain cause the voltage-gated channels to move

How can toxins be used to study ion channels?

Ion selectivity - Certain aminoacids interact only with certain ions (physical barrier against larger ions)

Toxins - Evolutionary target ion channels very especifically

Tetradotoxin (from pufferfish) blocks sodium channels



Conotoxin (from snail) blocks potassium channels

How can a single aminoacid mutation cause a disease like cystic fibrosis?

What is a probable cause for migraines?

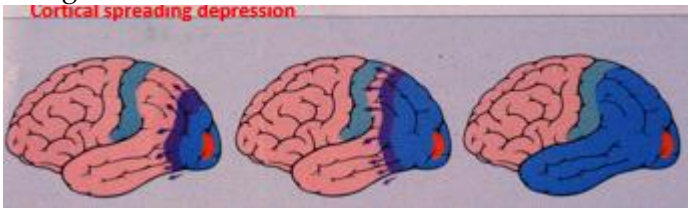
Ion channels and disease

Cystic fibrosis - Chloride channel

Epilepsy - Potassium channel

Migraine - Calcium channel

Cortical spreading depression - Wave of depolarization that goes across the cortex



Activate pain regions around the brain (the brain itself does not have nociceptors)

Obesity - TRP (calcium) channel

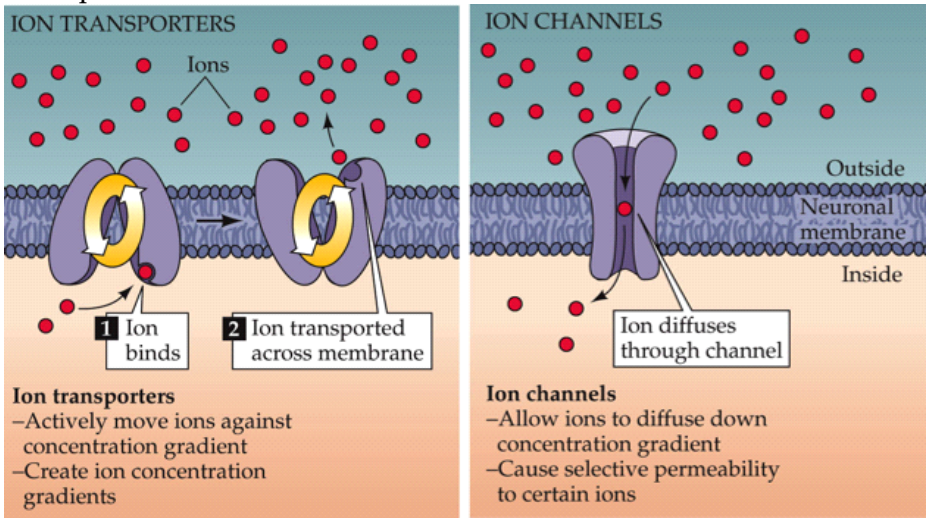
How can positive ion cause a resting negative membrane potential?

Neurons spend a lot of energy to maintain a negative membrane potential

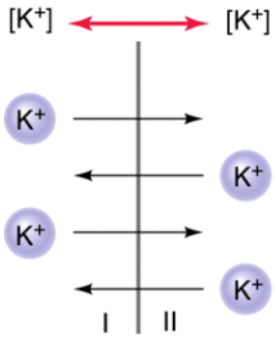
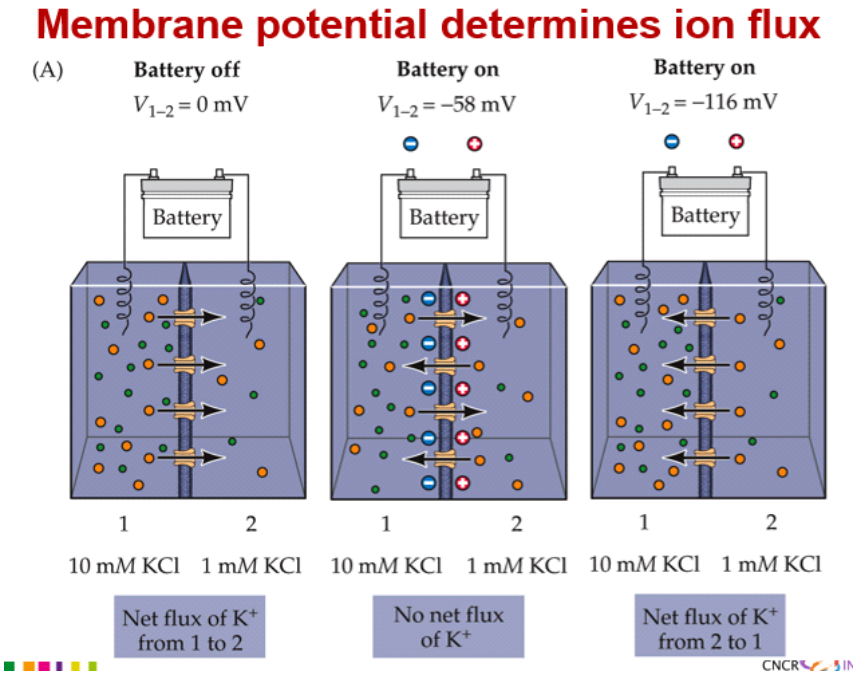
Positive ions can cause depolarization or hyperpolarization - It depends on **the direction in which they are flowing**

What is the functional difference between transporters and channels?

Transporters and channels move ions across neuronal membranes



Transporters - Actively move ions against their concentration

	gradient **Channels** - Passively more ions towards their concentration gradient Need the concentration difference generated by the transporters
What does it mean that a cell is at 'equilibrium' between electrical potential and concentration gradient?	**Equilibrium** - The amount of movement among compartments is equal (there is movement!)  **Concentration gradient** - Chemical driving force Electrical potential & concentration gradient - Electrical and chemical driving force
	Membrane potential determines ion flux Battery off - Chemical driving force wins Battery on - Electrical force balances out chemical driving force If the electrical potential is big enough, ions may move against their concentration gradient 
Describe the difference between Nerst and Goldman's equation.	**Nerst equation** - Describe how ions move $Ex = \frac{RT}{} \ln \frac{[X^+]_o}{}$

Why is calcium concentration so low in the intracellular space?

What is the equilibrium potential for the four main ions?

$$\frac{RT}{zF}\ln\frac{[X^+]_o}{[X^+]_i}$$

In mammal temperatures and using 10-base log: **58**

$$58\log_{10}\frac{[X^+]_o}{[X^+]_i}$$

Goldman equation - Includes the permeability of the membrane for different ions

$$Vm = \frac{RT}{zF}\ln\frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o}$$

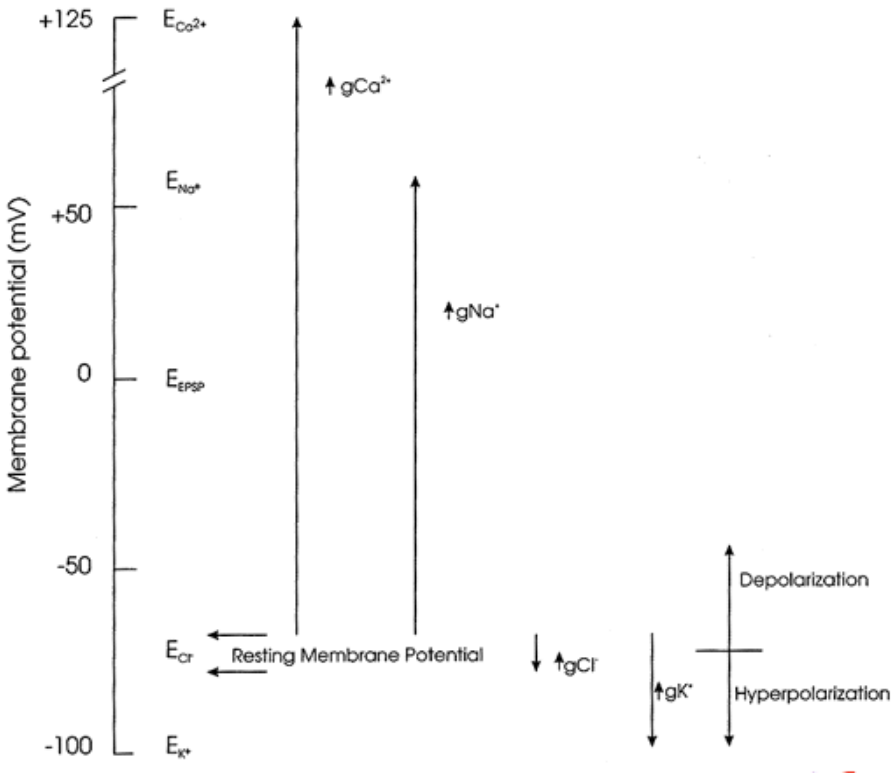
Table - **Extracellular and intracellular neuron concentration**

TABLE 2.1 Extracellular and Intracellular Ion Concentrations

Ion	Concentration (mM) Intracellular	Extracellular
Squid neuron		
Potassium (K^+)	400	20
Sodium (Na^+)	50	440
Chloride (Cl^-)	40–150	560
Calcium (Ca^{2+})	0.0001	10
Mammalian neuron		
Potassium (K^+)	140	5
Sodium (Na^+)	5–15	145
Chloride (Cl^-)	4–30	110
Calcium (Ca^{2+})	0.0001	1–2

Calcium needs to be low because it is a **finely regulated** second messenger

Equilibrium potential of **sodium and calcium are very positive**
EP of **cloride and potassium are very negative**



Epsp - Excitatory post-synaptic neuron (both sodium and potassium are going through - net = 0)

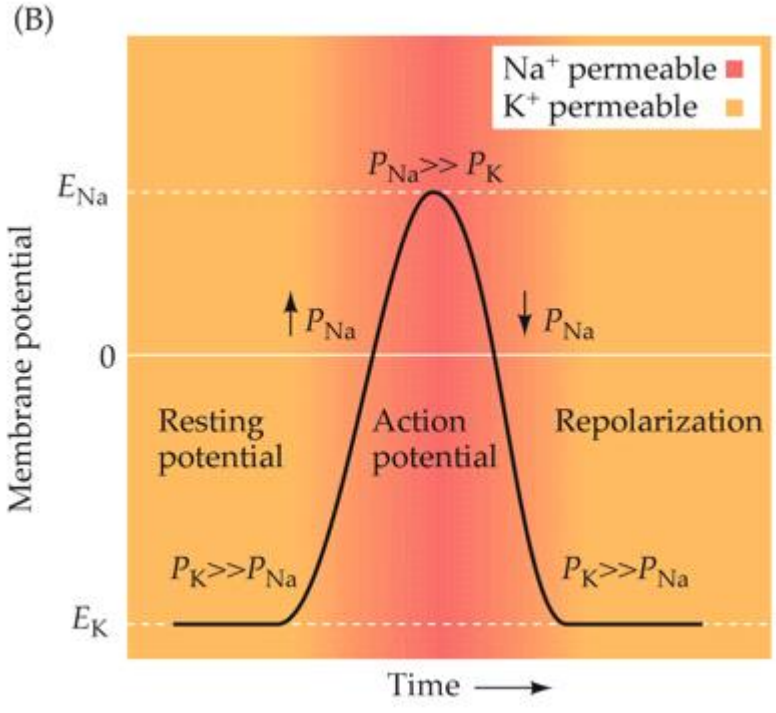
Describe the change in permeability of sodium and potassium during an action potential.

Why is sodium not so important to determine the resting membrane potential?

Why is GABA an excitatory neurotransmiter before birth? How is that explained at a molecular level?

Permeability of the membrane **changes during action potentials**

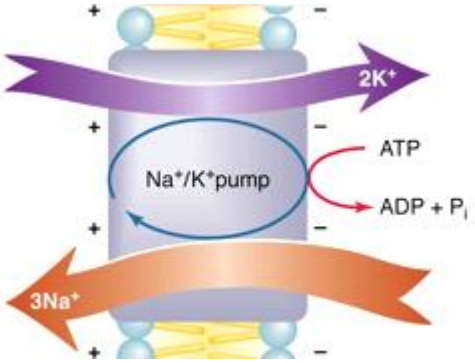
At the peak - Very high permeability for sodium, very low for potassium



Ion pumps maintain membrane potential at rest

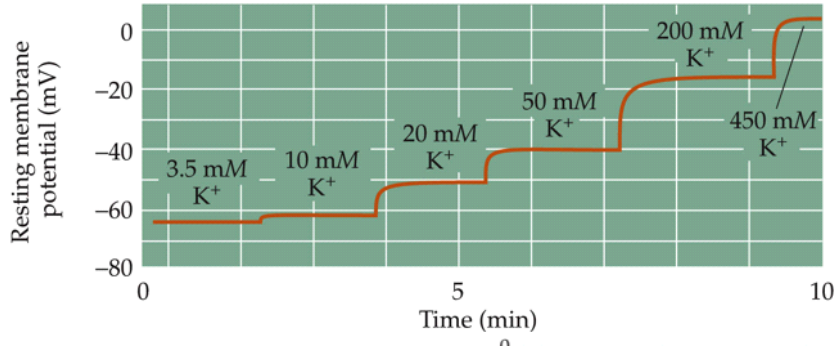
Na+/K+ pump - High potassium and low sodium inside the cell

3 sodium, 2 potassium



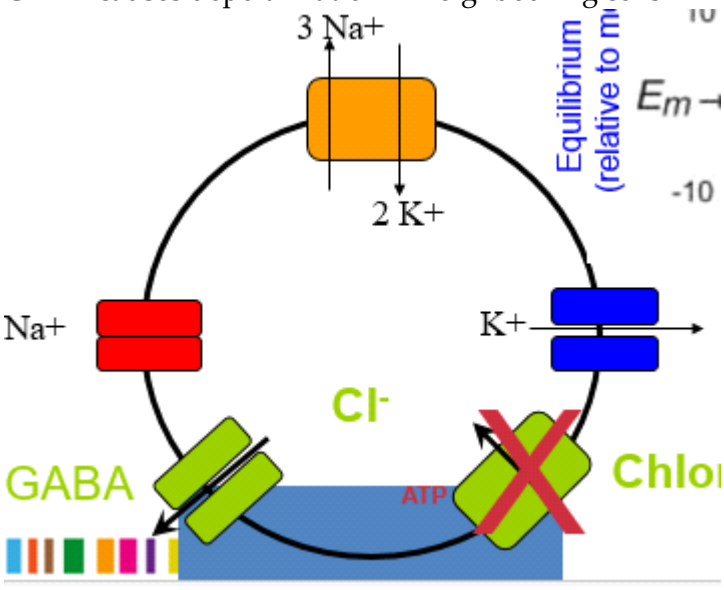
Potassium leaves the cell - Inside becomes negative!

- Na channels are closed - The membrane at rest is not very permeable to sodium
- Resting membrane potential is determined by K+ gradient



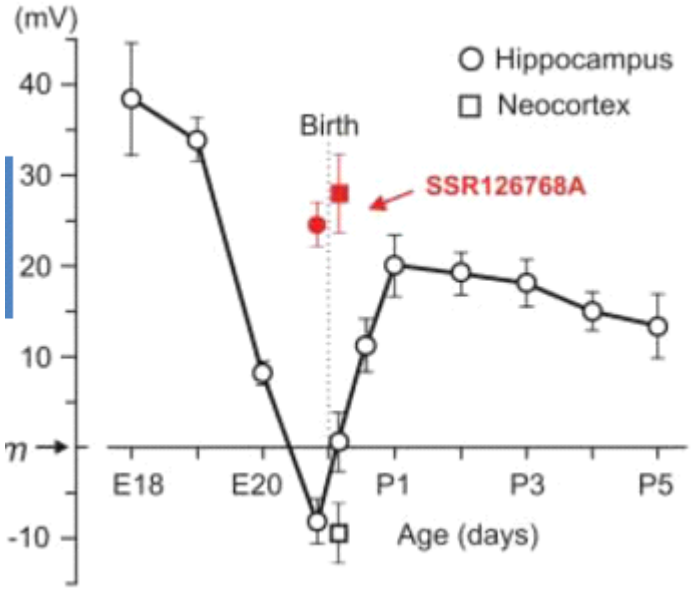
Chloride concentration is very different before and after birth

- GABA is an excitatory neurotransmiter because there is more chloride
- The fetus brain needs mechanism to prevent oxygen shortages
- GABA causes depolarization in neighbouring cells



After birth - Oxytocin close clhoride pump

SSR126768A - Oxytocin blocker



Why was the giant squid used as the model for studying action potentials?

Active properties of neurons

Squid giant axon - Nobel prize in 1963 (ionic mechanisms of action potentials)

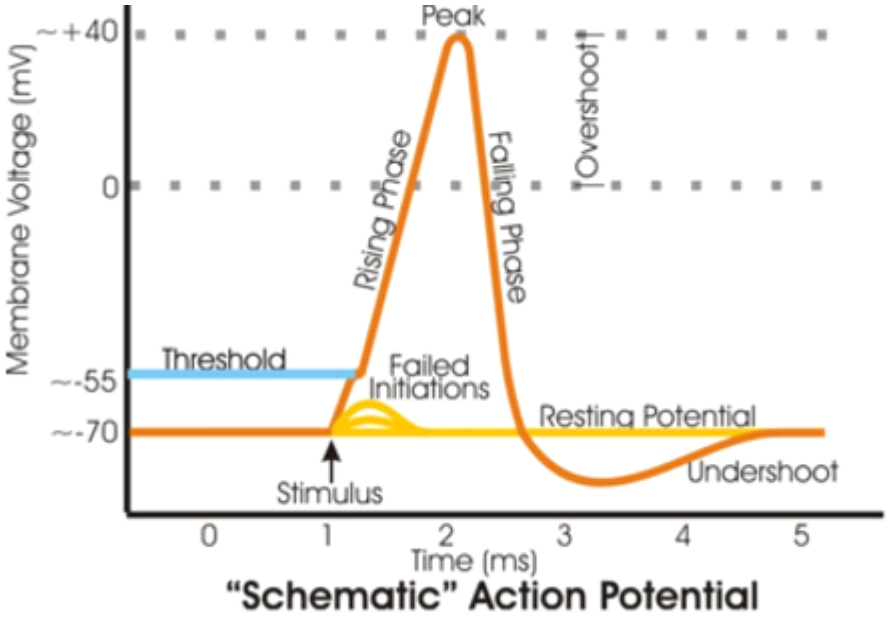
Squid evolved to be able to flee extremely quickly - Must have a large neuron to propagate the signal very quickly

Schematic action potential was discovered

Describe all the phases of an action potential in terms of the permeability of sodium and potassium.

Why is the sodium cycle a positive feedback and the potassium cycle a negative feedback loop?

Phases



Resting phase - Na channels closed, K channels open

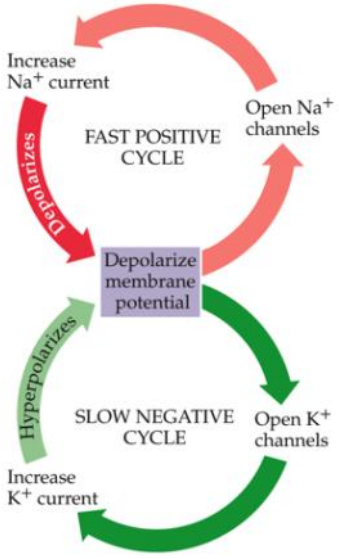
Rising phase - Occurs after a certain threshold has been passed, which cascades openings of sodium channels

Threshold changes overtime - You need a fast depolarization

Peak - No net flux (instant equilibrium)

Falling phase - Na channels closing, K channels opening
Undershoot phase - More potassium going out of the cell than during the resting phase

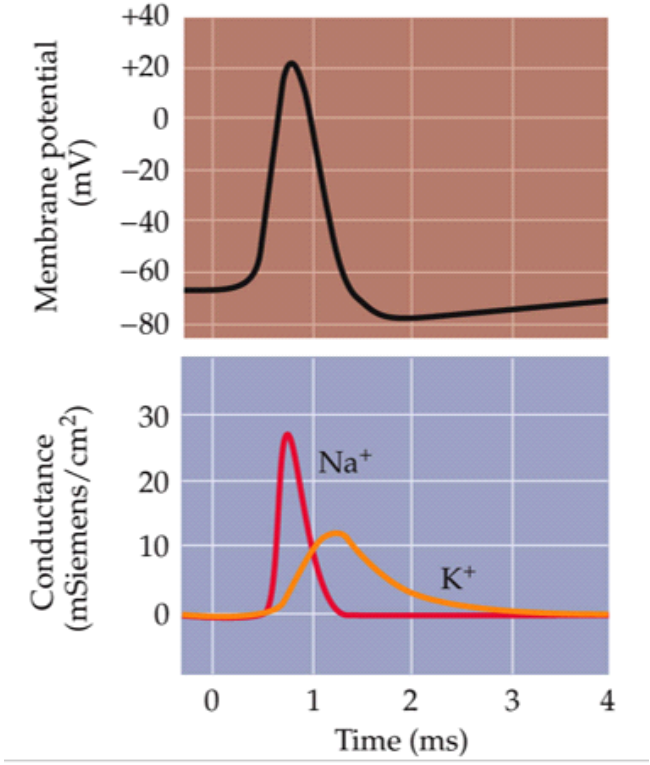
Feedback cycles



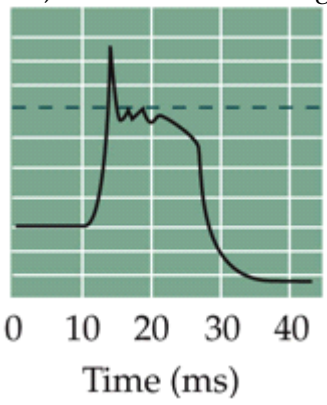
Sodium is positive feedback cycle and opens very quickly
Potassium is a negative feedback cycle and opens more slowly

What would happen if potassium channels had the same response time as sodium channels?

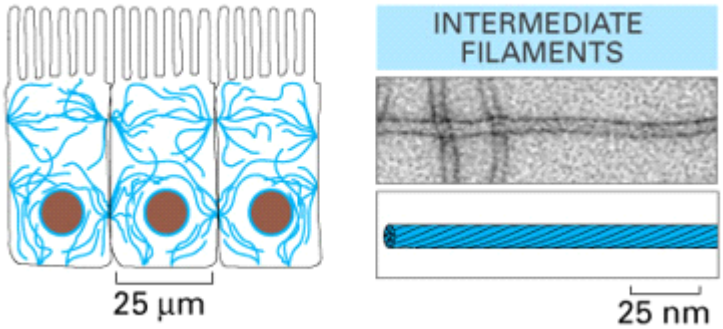
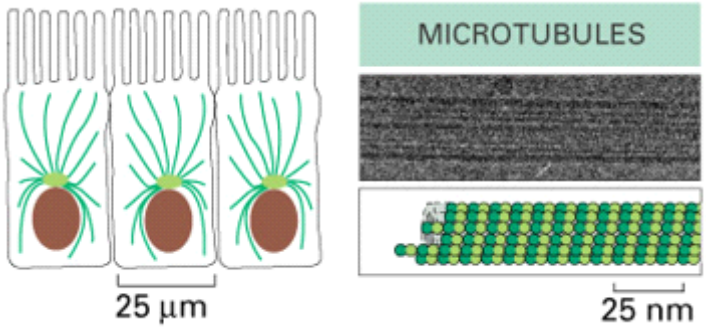
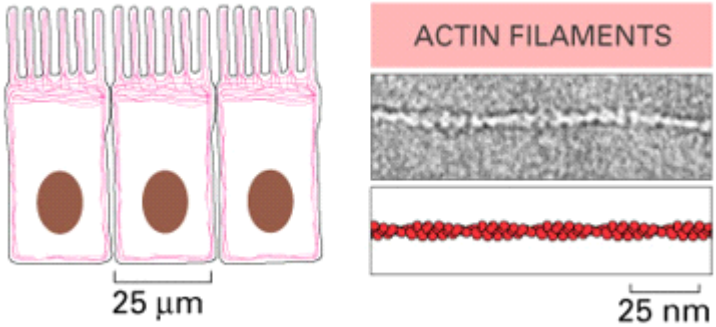
Membrane conductances during action potential
Sodium peaks first
Potassium peaks later



During resting conditions - Sodium permeability is extremely low
During action potentials - Sodium permeability increases many orders

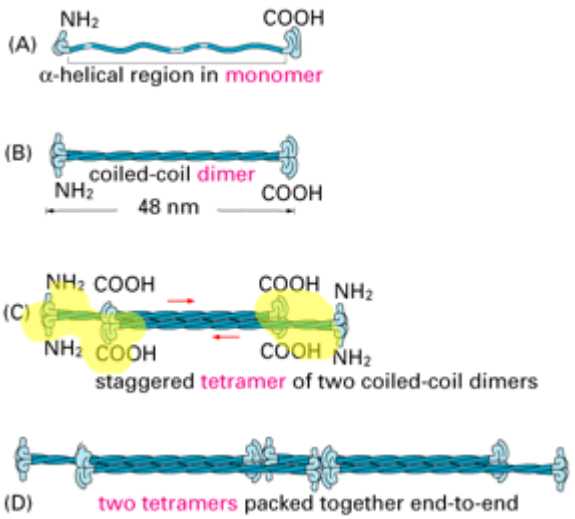
	of magnitude
Why there are such a variability in action potential shapes?	There are many shapes of action potential - It varies accordingly to the different ion channels expressed D) Calcium channels are expressed in pacemaker (in heart muscle cells) - Curve become bulgy 

2. Cytoskeleton (chap 17)

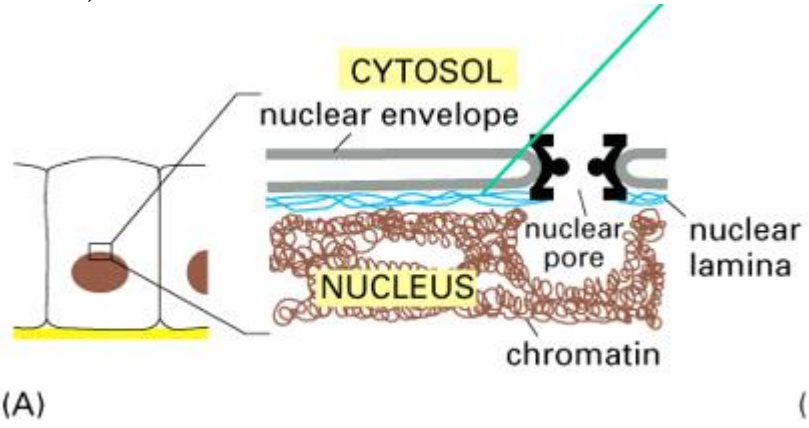
What are the three main components of the cytoskeleton? What length is their respective diameter?	Things are kept in place by the cytoskeleton **Components of the cytoskeleton** **Intermediary filaments** - Joins cells together (do not cross cells/not continuous); diameter of approximately 10 nm  **Microtubeles** - Have nucleation points (centrosomes), has a spider-like look; diameter of 25 nm  **Actin filaments** - Aligned along the cell membrane (cortex of the cell); diameter of 7 nm 
How are intermediary filaments formed? **What is the main**	**Intermediary filaments** Build from very simple structures - Monomers Superstructures - Coiled-coil dimers that keeps packing Generates a rope-like structure - The fact that they are not

function of intermediary filaments?

aligned means that they have more binding sites to other structures

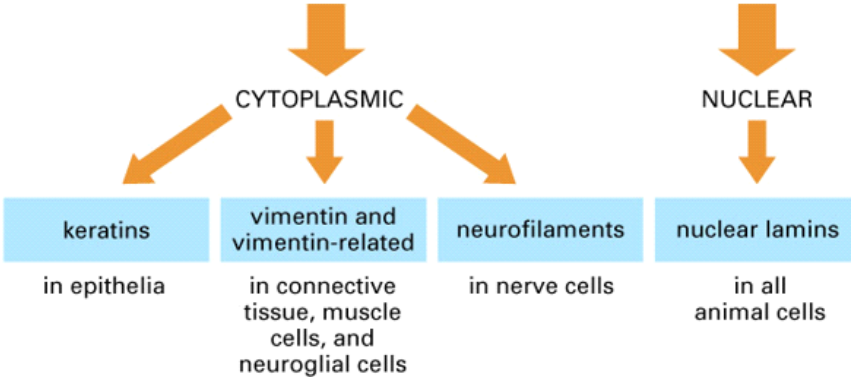


They **prevent cell rupture** - Give strength to the cell membrane and nuclear membrane (nuclear pores = no intermediary filaments)

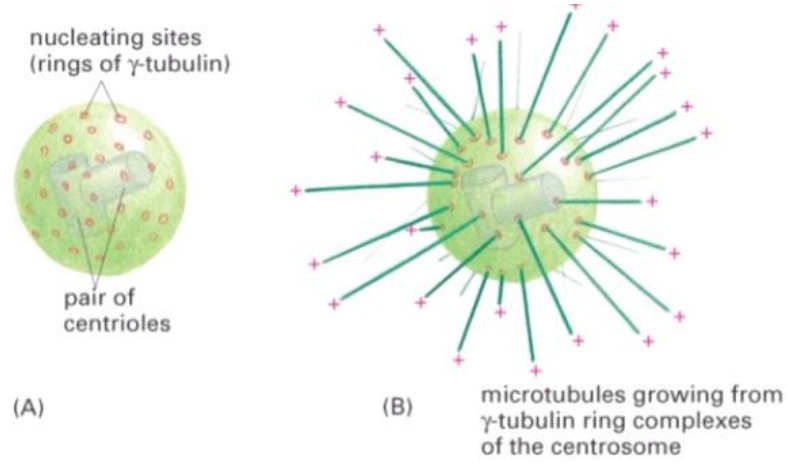
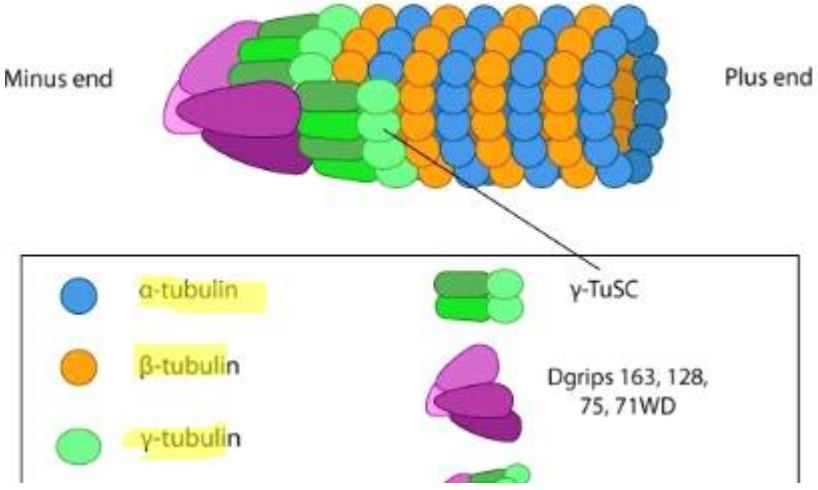
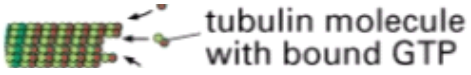


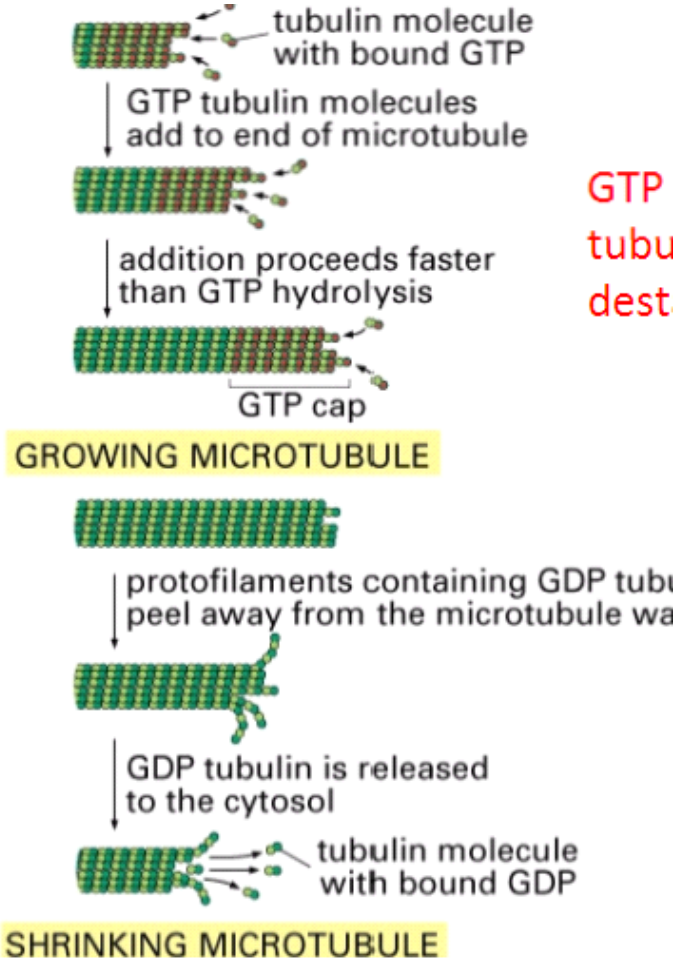
Nuclear intermediary filaments is stretched all the time - Pressure from the chromatin DNA

Found in cytoplasm (name varies depending on the location/encoded by different genes) and in the nucleus



Metaphor:
Membrane - Glass and curtains
Cytoskeleton - Concrete that makes the building

	There are parts of the DNA that are always bound to intermediary filaments - Needs to be organized in a particular way
Describe how microtubules grow are what are their main components.	**Microtubules** Grow from centrosomes  **Basal body** = Centrosomes in ciliated cells Organized from two proteins -alpha and beta Forms a heterodimers with a hollow tube superstructure Plus end - Part of the filament that grows Alpha-tubulin - Minus end;connects to the centrosome Beta-tubulin - Plus end; grows Gamma-tubulin - Center of nucleation  Gamma-tubulin - Starting points to cell division Microtubules grow independently
The process of microtubule growth is essentially stochastic. But how is directed in some way by the cell?	How do they grow? Tubulin binds with GTP GTP is present in the microtubule structure GTP is hydrolysed into GDP - Microtubules becomes less stable and prone to dissociation 



This process takes a lot of energy

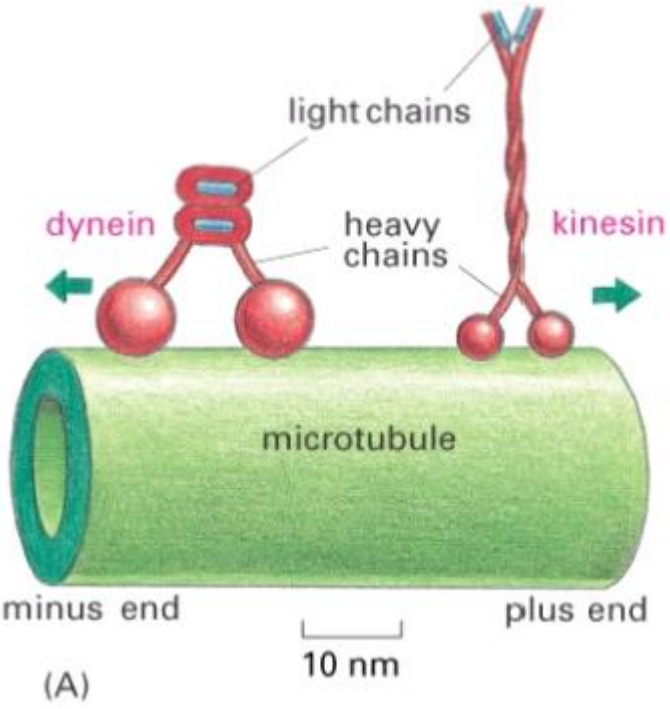
Selective stabilization of microtubules - Capping proteins protect the end of microtubules

Growth cannot be controlled (they are randomly growing), but can be directed

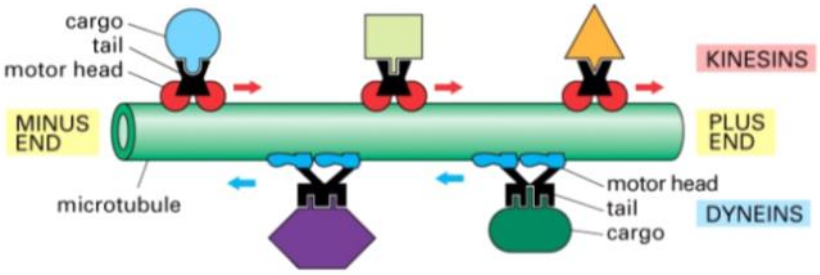
What is the function of kinesin and dynein?	**Motor proteins** - Drive transport in a neuron Movement occurs along the microtubules - Retrograde and anterograde transport

What is the function of the light chain and the heavy chain in motor proteins?

If kinesin always goes to the plus end of the microtubule and dynein always goes to the minus end, how come the cell doesn't accumulate these proteins at each extremity?



Motor proteins walk on microtubules - Two connection points

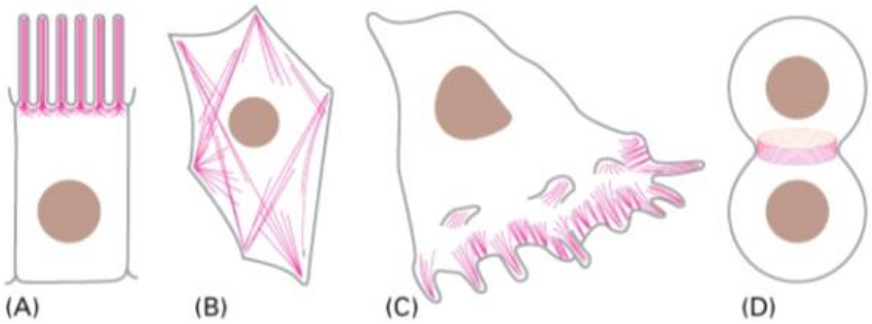


E.g. Kinesin (go to plus end) and dynein(go to minus end)
Built with two polypeptide chains

Light chain - Binds to cargo
Heavy chain - Motor head binds to microtubule
Spends ATP to dettach the motor head, not for the movement itself
All motor proteins need a adpative protein to carry cargo

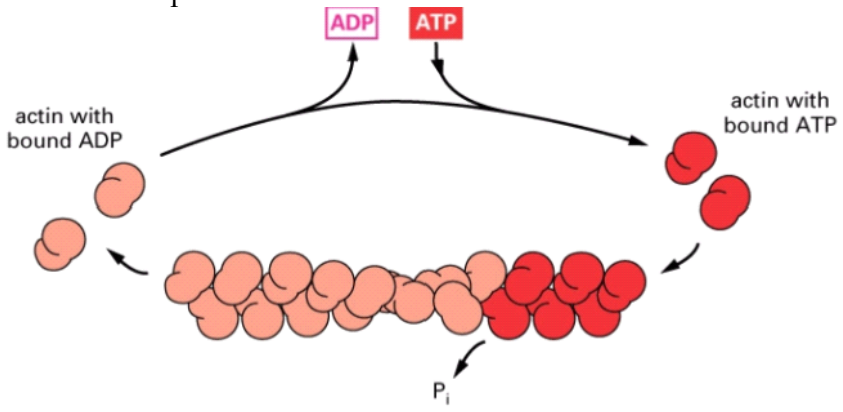
Why do kinesis not accumulate at the plus end and dyneins at the minus end?
Kinesins becomes cargo to dyneins to go back to the minus end
Dyneins becomes cargo to kinesis to go back to the plus end

Actin filaments - Dynamic regulated in the brain; important in celular motility and cytokinesis.



Often found close to the cell membrane
Makes cells pinch off during cell division

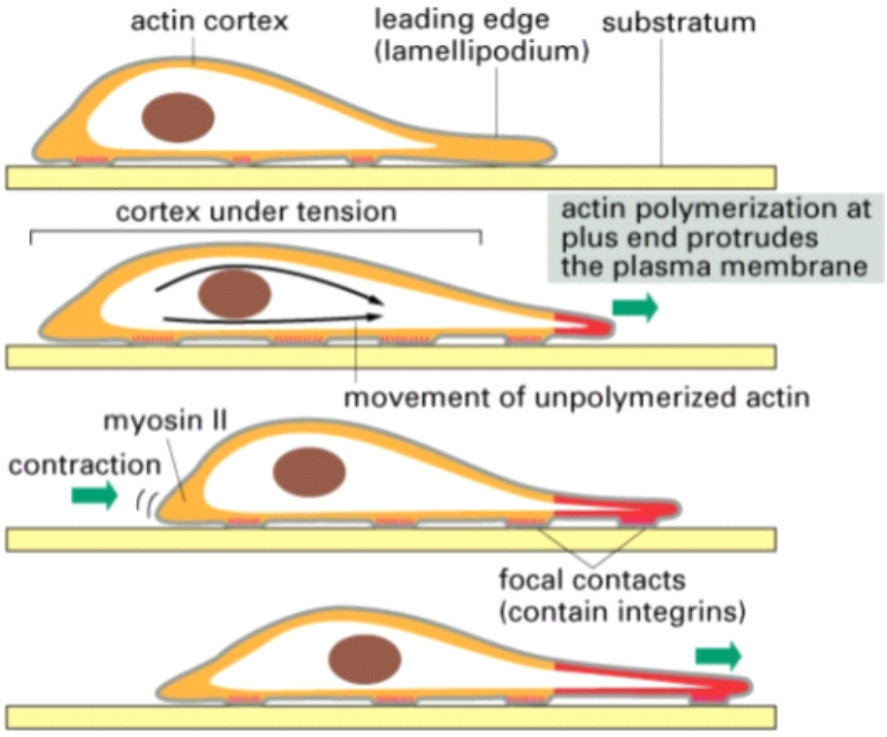
Built from monomers
Grow on the plus end



Actin stabilized by bound ATP - But there is no dynamic instability(once ATP leaves, the molecule does not break down)

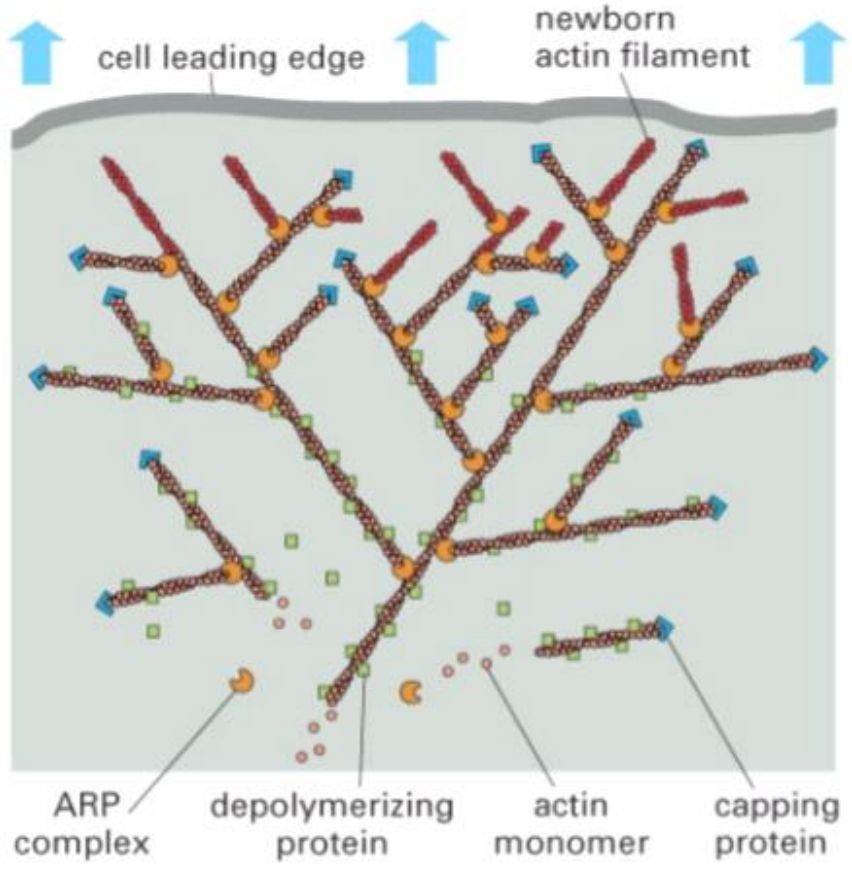
Many different proteins regulate the actin cytoskeleton
- Capping and nucleating protein
- Severing/bundling

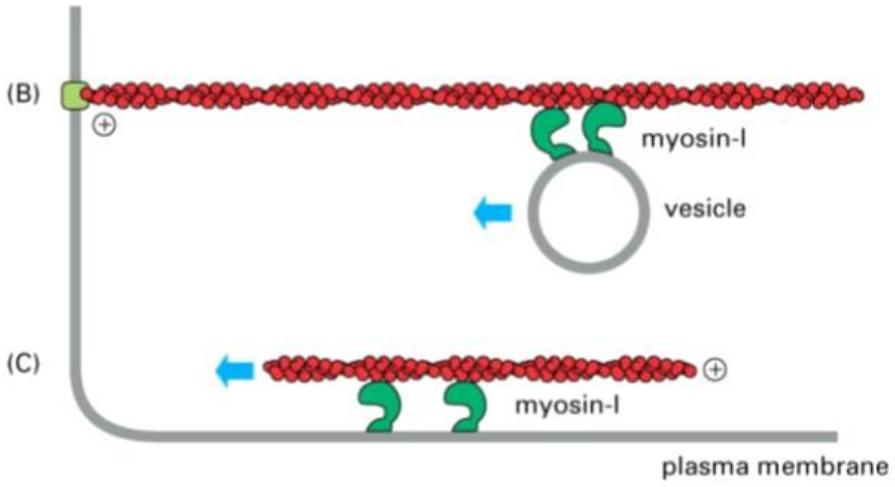
Important role in movement - Actin pushes cells to a particular direction due to tension



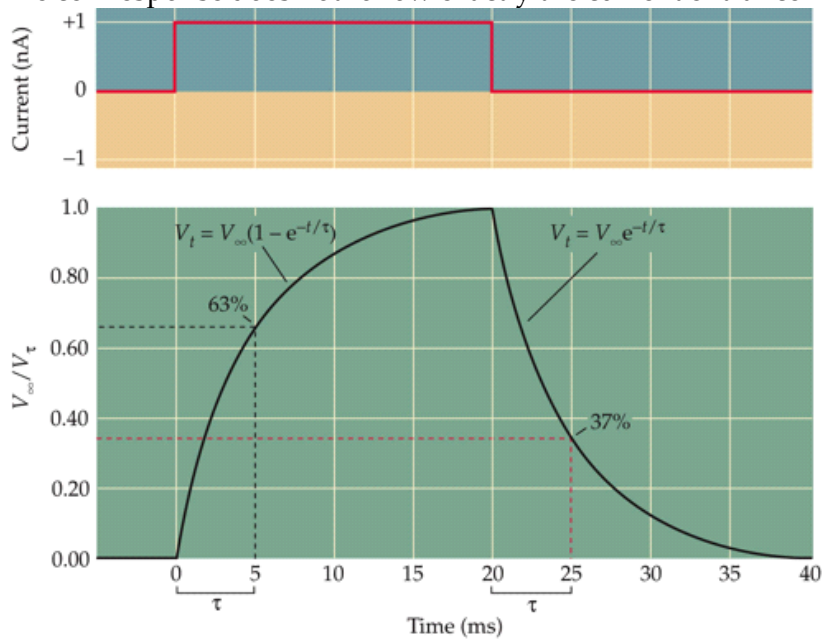
Motor proteins bind to the substrate (extracellular matrix)

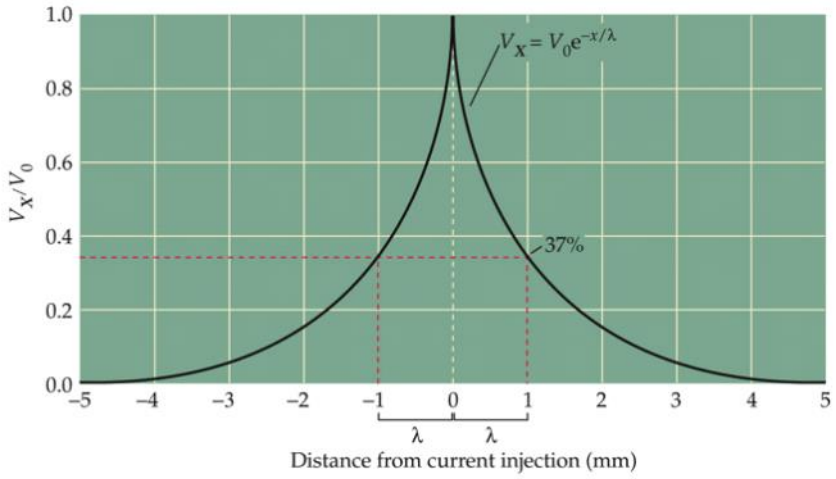
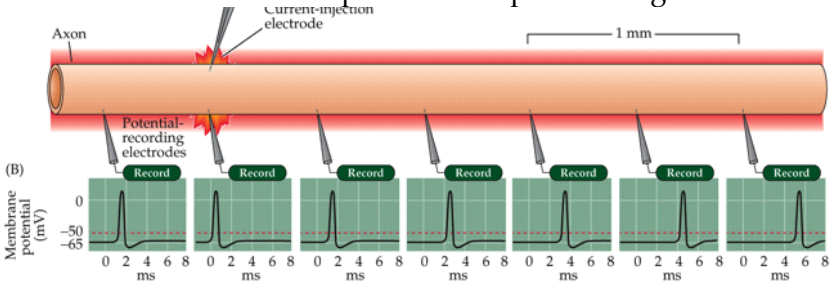
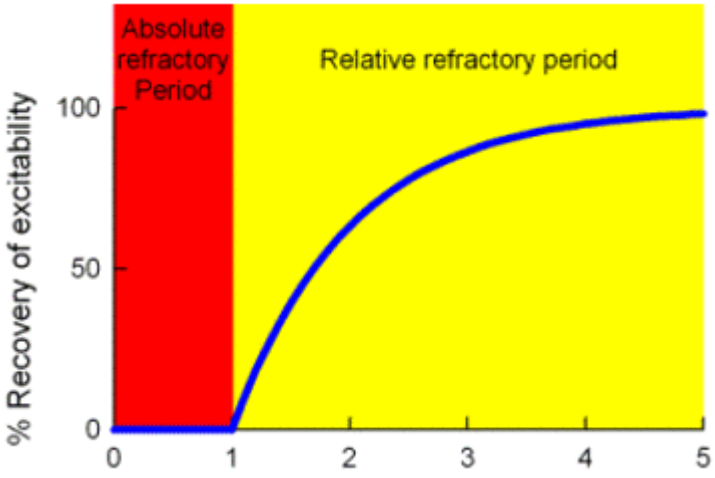
Cell growth - Essentially random until it finds the extracellular matrix of a neighbouring cell

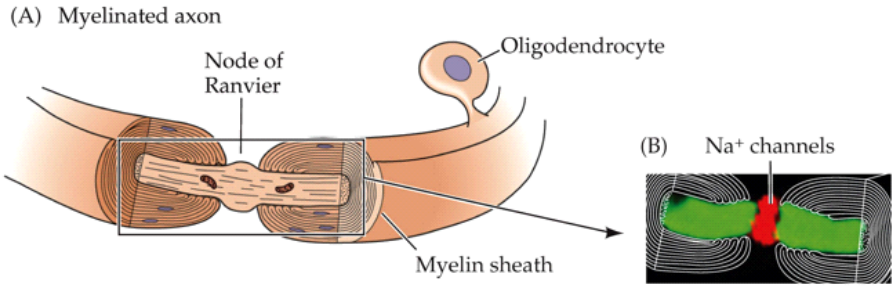
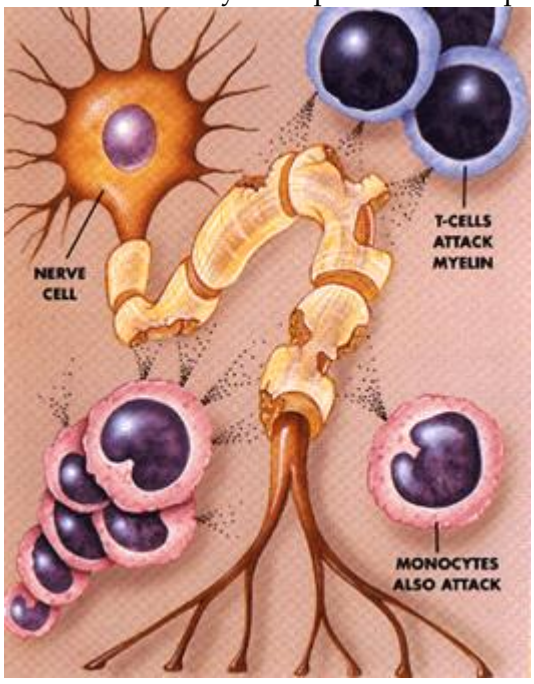


	Depolymerizing - Actin monomers will be reused at the new cell edge
	Myosin (monomeric dimer)- Motor protein involved in transport along actin filaments  Vesicle - Lipids that fuse with the cell membrane Transport requires two myosin molecules
Summary	• The 3 main components of the cystoskeleton and their function in cellular structure, transport and motility • Mechanisms of assembly and disassembly of the Cytoskeleton • Proteins involved in regulation of the cytoskeleton • The different types of motor proteins and their function

2b. Action potential propagation

Why doesn't the cell response follow exactly the current entrance? Which property of the cell could you modify to change this curve shape?	Passive properties: Input resistance The cell response does not follow exactly the current entrance  Ohm's law - Membrane potential = current injected * resistance
What does the time constant describe in the cell?	Lipid core - Behaves as a **capacitor** Some ion charge up the cell membrane - It becomes easier for other ions to go through ion channels than to the cell membrane (explains curve) Ion channel - Behaves as a **resistor** (only a few ions can go through at one time) If there were only ion channels, the cell response would be equal to the current Time constant (tau)- Time it takes the membrane potential to reach **63%** of the new membrane potential (positive or negative)
What happens to the time constant when you increase cell size? And when you increase the amount of ion channels?	Membrane capacitance **prolongs** time course $\tau = Rm * Cm$ Time constant = Membrane resistance (amount of ion channels) * Capacitance of the cell (bigger if the cell is larger)
What is the role of passive spread in action potentials? When do they occur?	**Passive spread** - Occurs when the membrane potential does not reach the threshold Fast decay of electrical signal Length constant (lambda) - length it takes for the membrane potential to reach 63% of the new value

	
	Active spread - Occurs when the membrane potential reaches the threshold Amplitude of the injection does not change It takes time for the action potential to spread along the axon  Main difference between active and passive spread: Openess of sodium channels Action potential spreading requires **passive and active properties**
What is the explanation at a molecular level of absolute refractory periods and relative refractory periods of sodium channel openings?	Direction of propagation - Moves in only one direction because there is a refractory period of sodium channels  **Absolute refractory period** - Cannot generate action potential **Relative refractory period** - Generates a small action potential (some sodium channels have recovered)
	Speed of action potentials can be regulated The larger the axon, the longer the length constant (reaches

	longer) The smaller the axon, the shorter the length constant
	In mammals, there is myelination - Changes the resistance of the membrane  Produced by glial cells - Oligodendrocytes/Schwann cells High concentration of sodium channels at Nodes of Ranvier **Saltatory action potential -** There are only influxes of Na at certain points of the neuron
	Multiple Sclerosis - Own immune system attacks myelin sheets, reduces efficiency and speed of action potentials 

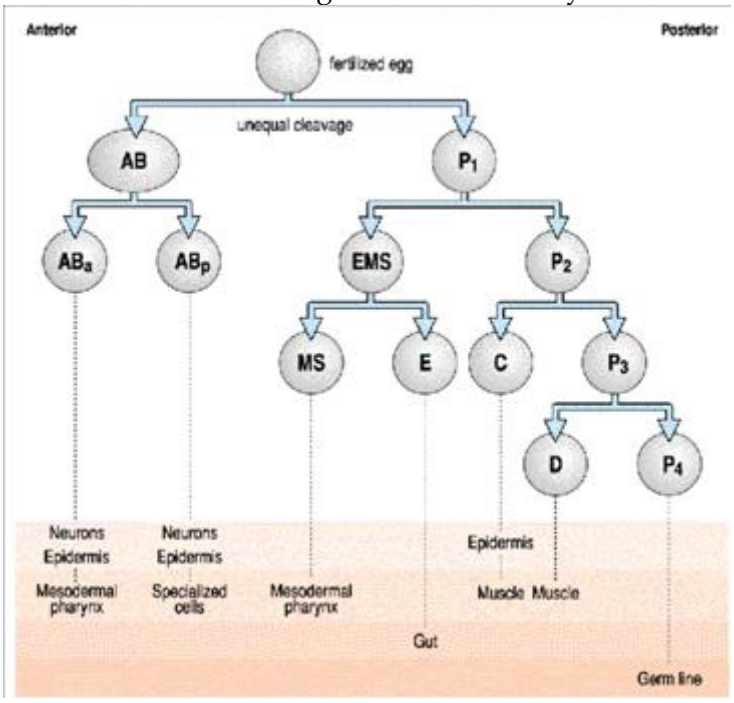
2c. Invertebrate model organisms

	Yeast model system Cell communication is the same as neurons Drosophila - Nervous system with enough complexity (e.g. it can fly) C. Elegans - Simply built nervous system There are many other animal models - Snails for synaptic plasticity, zebra fish for developmental neurobiology(transparent), finch for plasticity (learning new birdsongs), humans (non-invasive methods, tissue studies)
How many genes do C. elegans have? How does it compare to humans?	Homo sapiens - 3.2 Gb, estimated gene number of 27 thousand C. elegans - 100 Mb, 23 thousand genes
How many cells do C. elegans have? How many are nervous cells?	C. elegans - Nematode worm 1.5 mm in length - Lives in the soil, feeds on bacteria, no economic importance 959 cells - One third of that are neurons (302) All individuals have the same number of cells
Why is C. elegans a model organism?	Why is C. elegans a model organism? Small, easy to maintain Short generation time(3-days) and self-fertilizing Cell lineage is constant and mapped Transparent body Sequenced genome - wormbase.org
What percentage of C. elegans is male? What is the role of males in the population?	They are hermaphrodites, but can also reproduce sexually (only 1% of offspring made by cross-fertilization) Male offspring is really rare - 5% Females can reproduce by themselves
	C elegans life cycle Has an embryonic development of 12 hours (fertilized egg to larvae) Complete life cycle in three days (from egg to adult) Dauer larva - 'Hibernation', occurs when organism does not find food or when the temperature is too cold (lives four months) It does not make sense to complete development when the environment is not favorable Can be kept in a freezer in oil (avoid ice crystals)
How many chromosomes does C. elegans have?	C. elegans genome 6 chromosomes - 5 autosomes and 1 X chromosome

Around 100 million base pairs
20470 genes - 40% of which have human equivalents

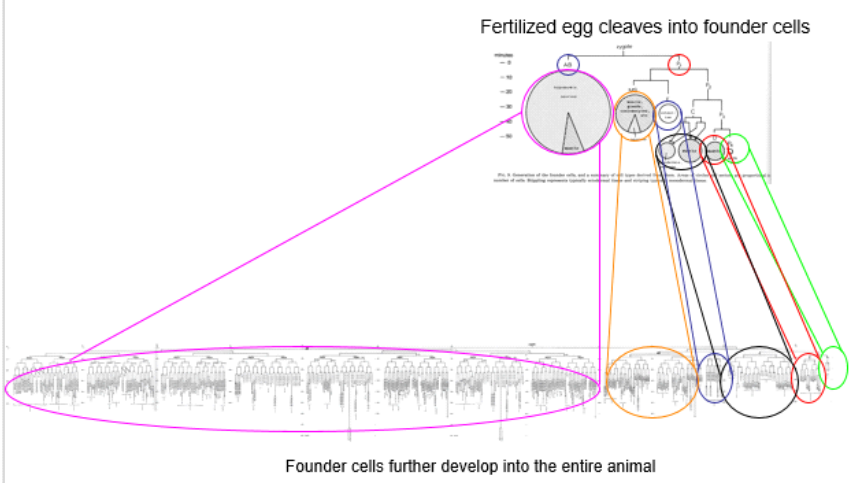
Embryonic lineage of C. elegans

You can see which cells give rise to which systems



Nervous system is formed really early on

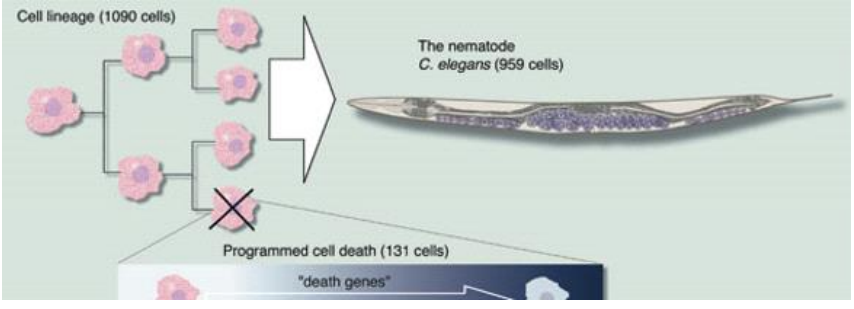
You can do this for the entire development

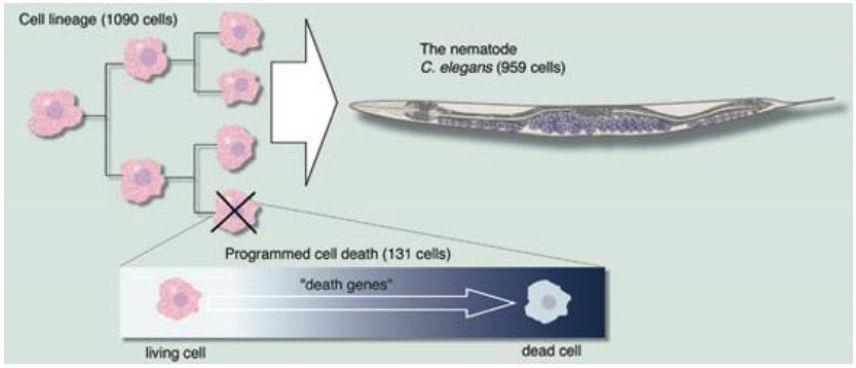


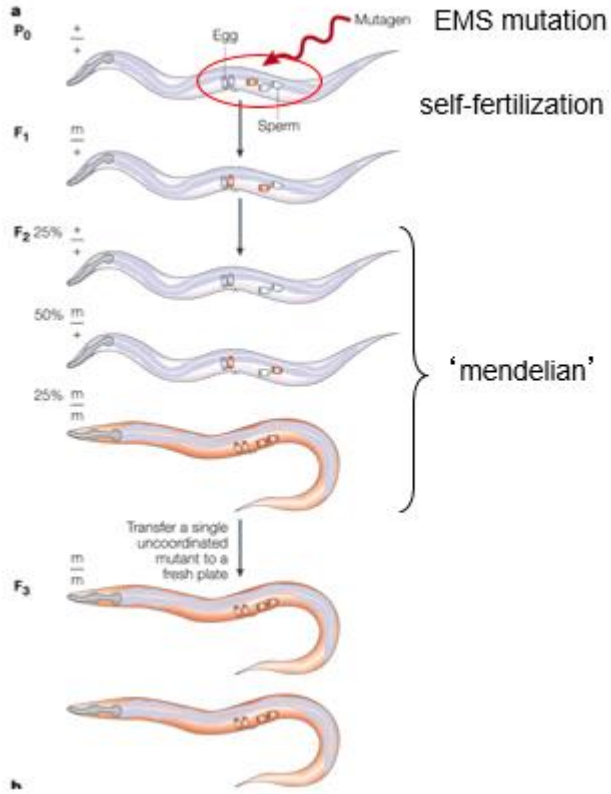
Some cells keep dividing a bit longer than others

Why does the organism have 959 cells (an uneven number)?

Some of the cells are programmed to die right after division



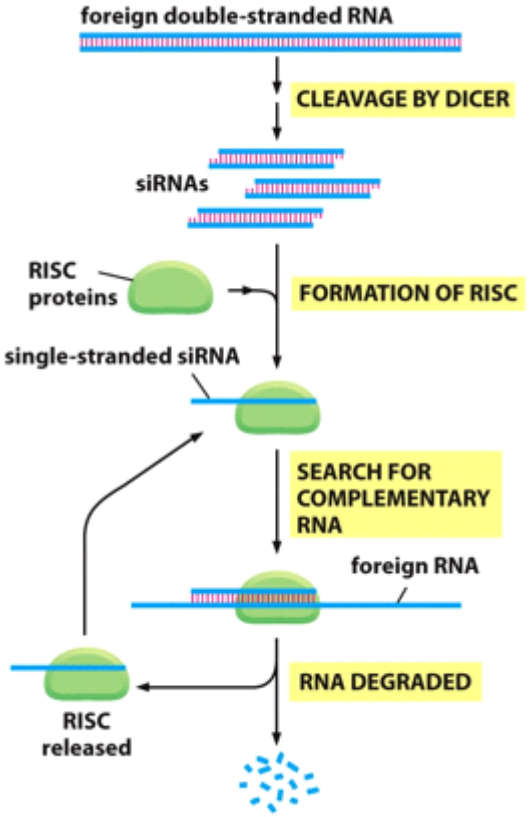
	 Nobel Prize in 2002
	Simple nervous system (302 neurons) Similar neurotransmitter as humans (Acetylcholine, GABA, dopamine, serotonin) Sophisticated behaviour - Moving backwards and forwards, respond to odors, chemicals, temperature differences, associative learning and habituation
What are the techniques related to forward genetics?	Genetic experiments **Forward genetics -** Mutations occur, breeding takes place, mutant offspring is isolated and the gene is mapped (observing phenotype first) 1) Random Mutagenesis - Generate small mutations (chemicals) 2) Transposons - DNA elements (Tc1) that jump around genome, may inactive a gene Induced mutation - Self-fertilization - full phenotype! (double mutation)

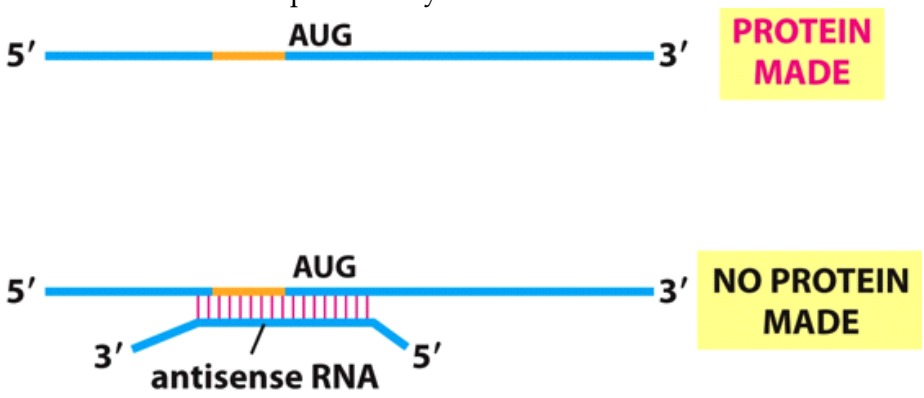
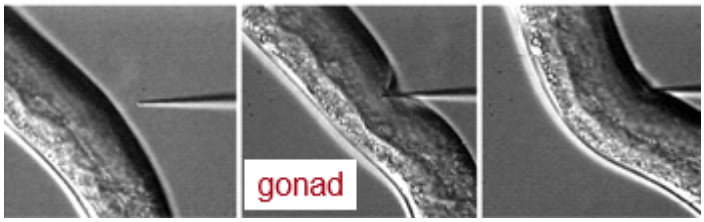


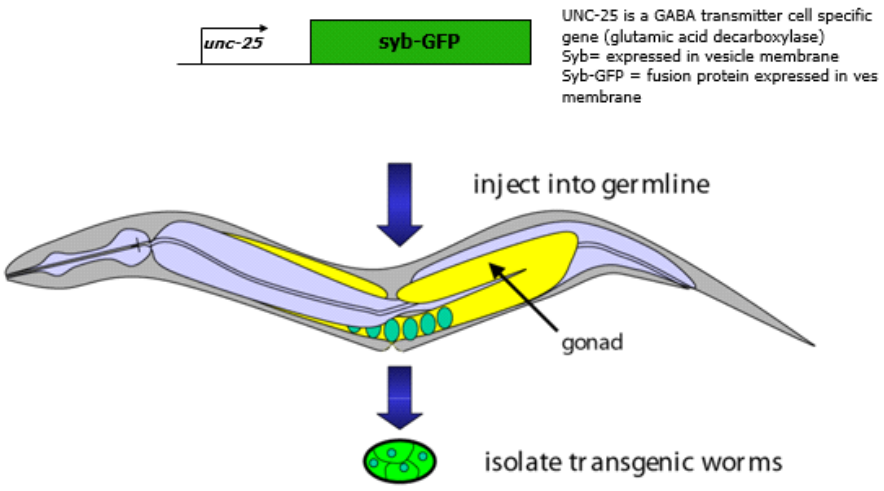
What are the techniques related to reverse genetics?

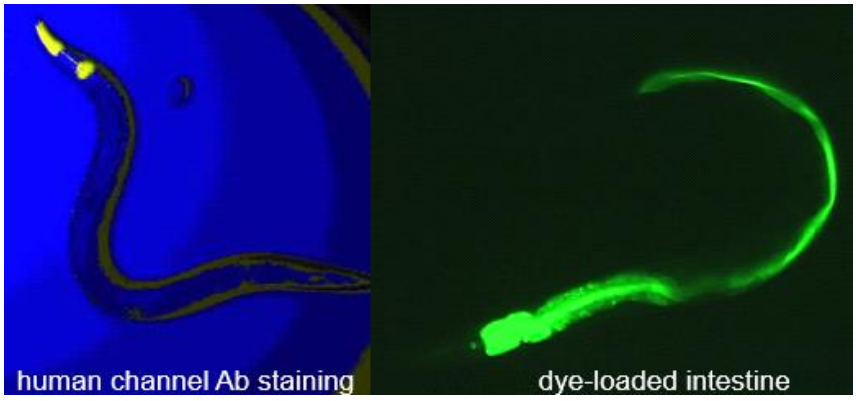
Reverse Genetics - Knockout of a particular gene and observe its effects on phenotypes

1) RNAi - Small RNA molecule (antisense RNA) makes a double strand with target RNA and prevent protein production



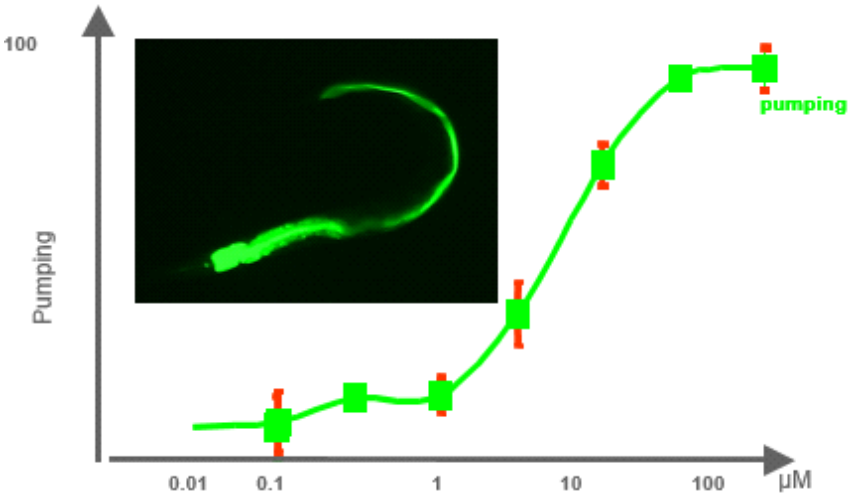
	RNAi can be feed to C. elegans in bacteria! Easier to do than inject RNA in the gonads of the animal Does not generate a full knockout, only a partial knockout This is advantage, because most full knockouts in C. elegans are lethal - Allows more interesting observations than 'This is an essential gene' RNAi has been done to knockdown all C. elegans genome 2) PCR identification of rearrangements
What are the two molecular functions that Antisense RNA has in a cell?	Antisense RNA - Complementary to the normal mRNA  Physically blocks translation Induces RNA degradation - RISC complex - Breaks down double stranded RNA in organisms (innate protection against virus) Small RNA molecules (siRNAs) - Also is broken down by RISC complex
What are the three ways to get dsRNA into C. elegans?	3 ways to interfere using double stranded RNA Injection of dsRNA in gonads Soaking animals in dsRNA Feeding animals with bacteria producing dsRNA Produces a transient knockdown - Gives interesting phenotypes when the full KO is lethal
What are possible research purposes of injecting extrachromosomal DNA into C. elegans gonads?	Getting DNA into c. Elegans DNA is injected into the cytoplasm of the gonads The DNA can pass through the germline in the form of extrachromosomal DNA  Purposes: a) Identification of genes by rescuing a mutant phenotype - Insert a copy of gene and see if it changes the phenotype b) Expression pattern using the gene of interest with reporter - GFP c) Interference of a biological process by overexpression of WT or

	mutated gene - RNA interference
How can a fluorescent marker like GFP be inserted specifically into specific types of neurons, such as GABAergic neurons?	 Syb - Synaptobrevin Every vesicle becomes a green vesicle Unc-25 - GABA transmitter cell specific gene (glutamic acid decarboxylase) Mutant offspring will express GFP in all GABAergic neurons  Fluorescence measurement - Screening for drug that inhibit a target promoter
How can C. elegans be useful in biomedical research for diseases such as Parkinson's or Alzheirmer's, if the nematodes do not have higher cognitive behaviour?	C. Elegans as a model in biomedical research CNS - Depression, psychosis, Parkinson's, Alzheimer's, pain Metabolic - Type II diabetes, obesity Other - Cardiovascular, oncology, muscle disease Many conserved molecular pathways Worms do not express the same higher cognitive behavior as humans - Research in this area is important for pharmacological/cell biology breakthroughs
How can human channels be used in C. elegans in a high-throughput essay?	Pharyngeal pumping depends on voltage-gated channels Express human ion channel after knocking down the endogenous channel present in the pharynx If human channels are able to replace them, the system would work again



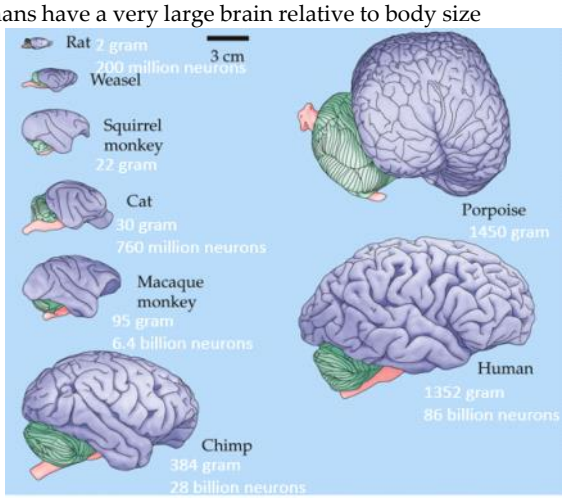
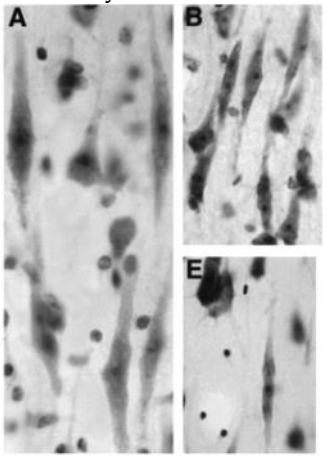
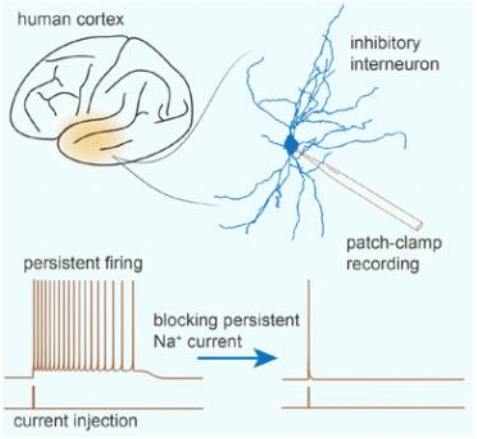
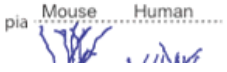
Feed the animals with dye - If the pharynx works, the work will become fluorescent

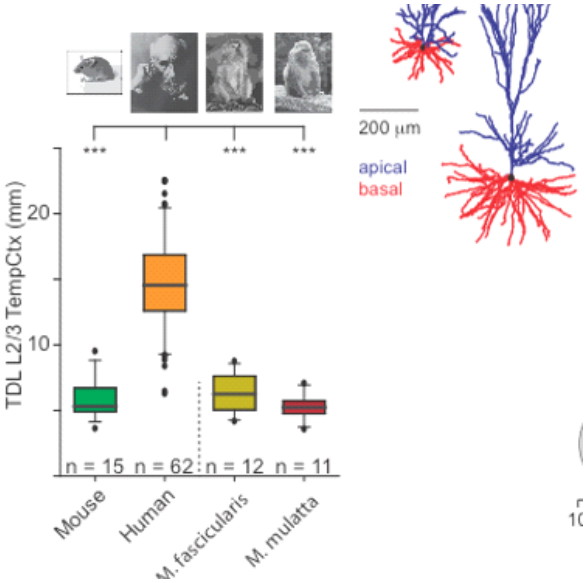
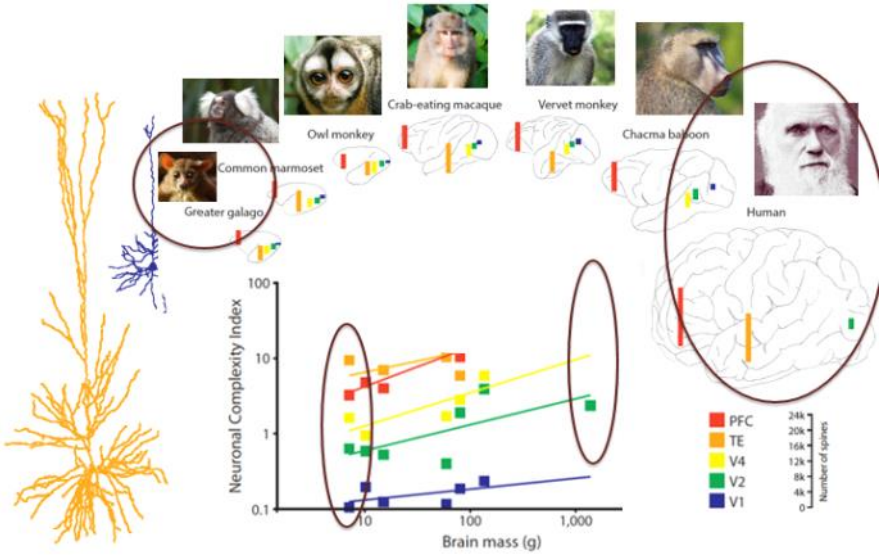
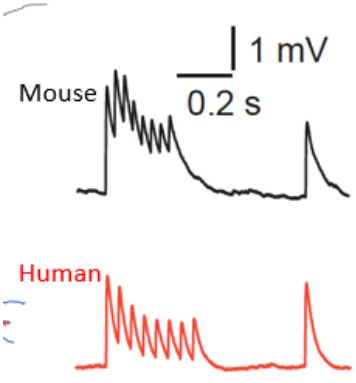
High-throughput essay - Test thousands of compounds for inhibition of the human channel

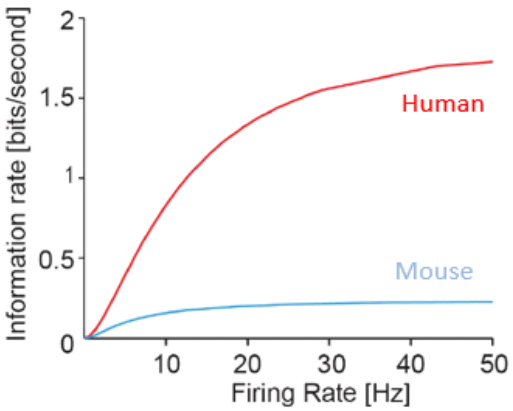
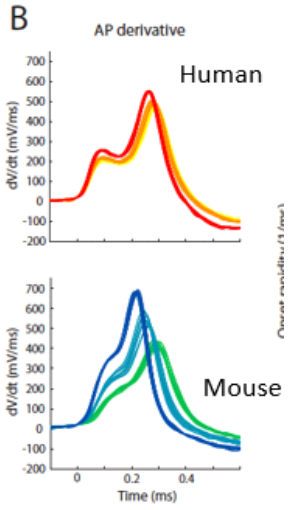


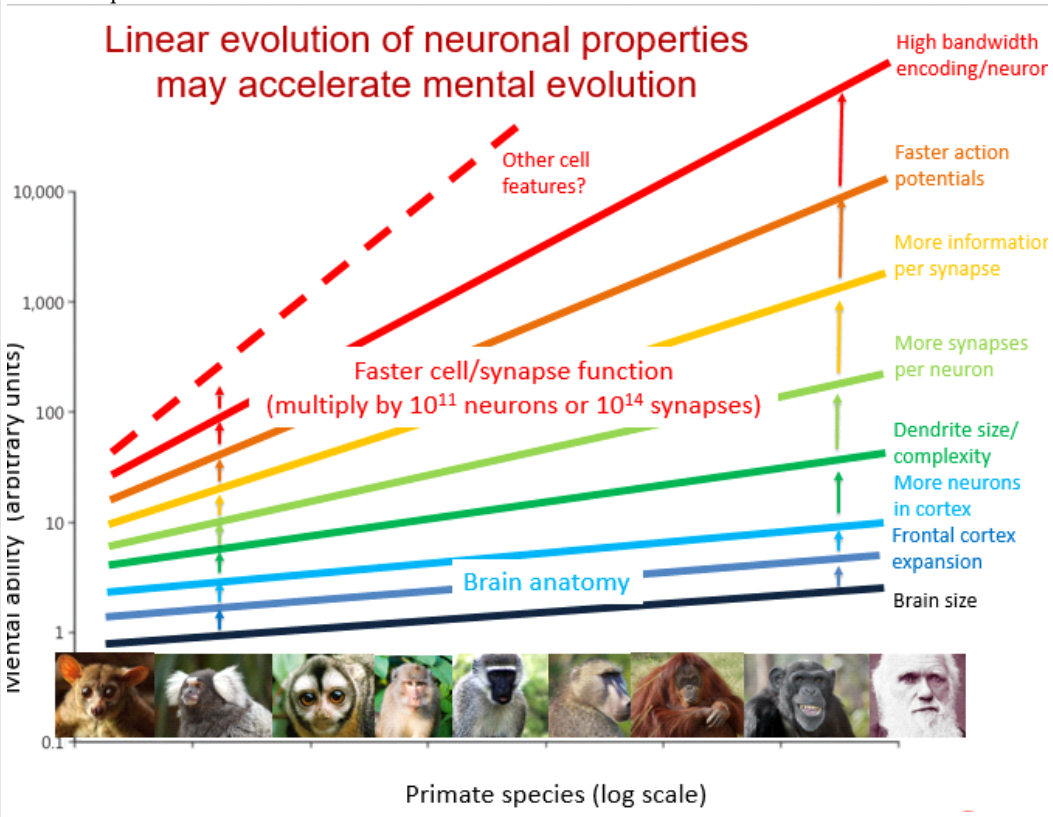
Potential gastric acid inhibitor

2d. Function of neurons and synapses in the human brain

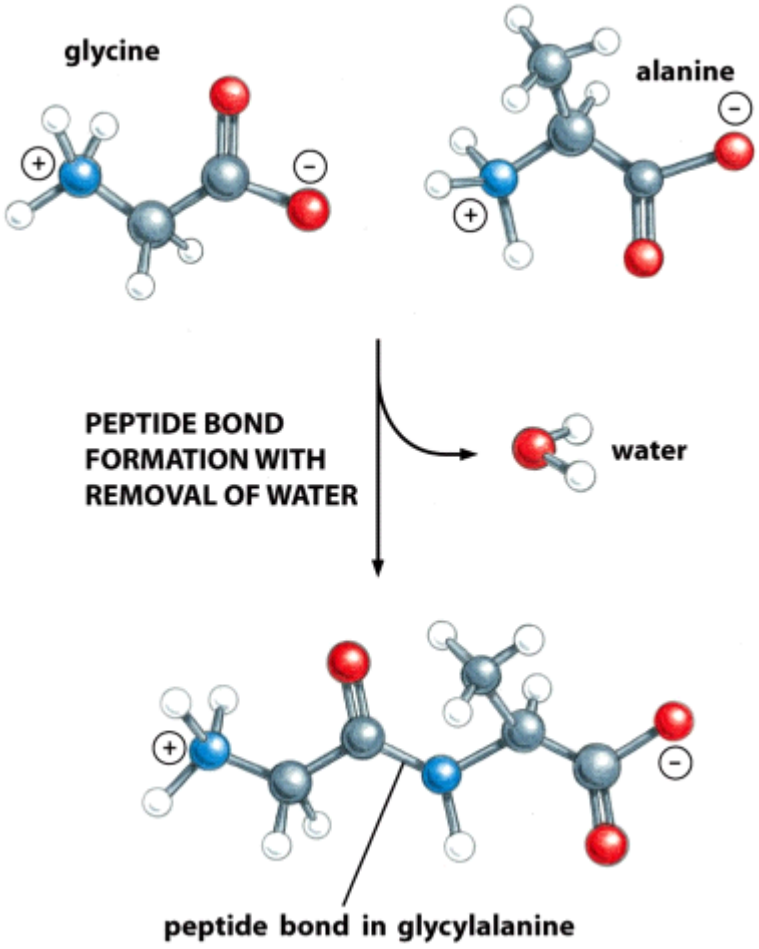
What is the weight of a human brain? How many neurons does it have?	Chimp brain - 384 gram, 28 billion neurons Human brain - **1352 grams, 86 billion neurons** Humans have a very large brain relative to body size  Humans do not have a unexpectedly large frontal cortex Number of neurons in the human cerebral cortex is also not unexpectedly large Are human neurons and synapses different? Cellular properties relate to mental ability?
Why there are very few papers on human neuronal function?	Very few papers have been published about human neuron function - The majority of them are from epileptic patients They already need to have implants to assess areas that initiate seizures
What are some unique cell types that only humans have?	Unique neocortical neurons in primates **Von Economo neurons** - Social behaviour **Persistently activated neurons**  Von Economo Neurons (VEN) Nimchinsky et al., PNAS 1999  Persistently Activated Neurons (PAN) Wang et al., Cell reports 2015
Why most studies conducted with human tissues have been done with epilepsy patients? Does that mean that the information obtained	Human brain tissue in the lab - From epileptic patients or tumor patients Centimeters of tissue Human neurons have much larger dendrites than other mammals Human neurons have more synapses 

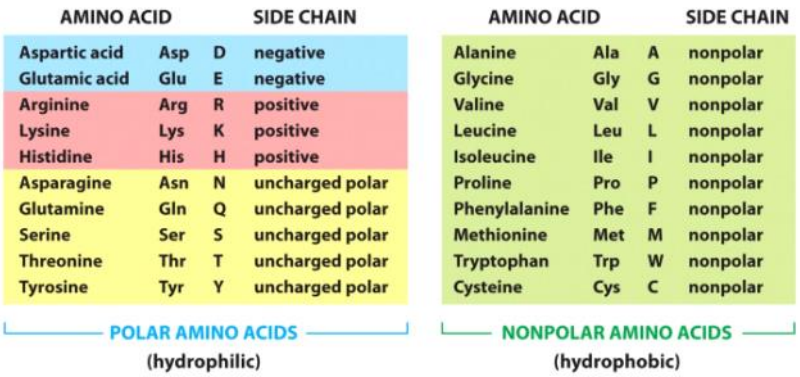
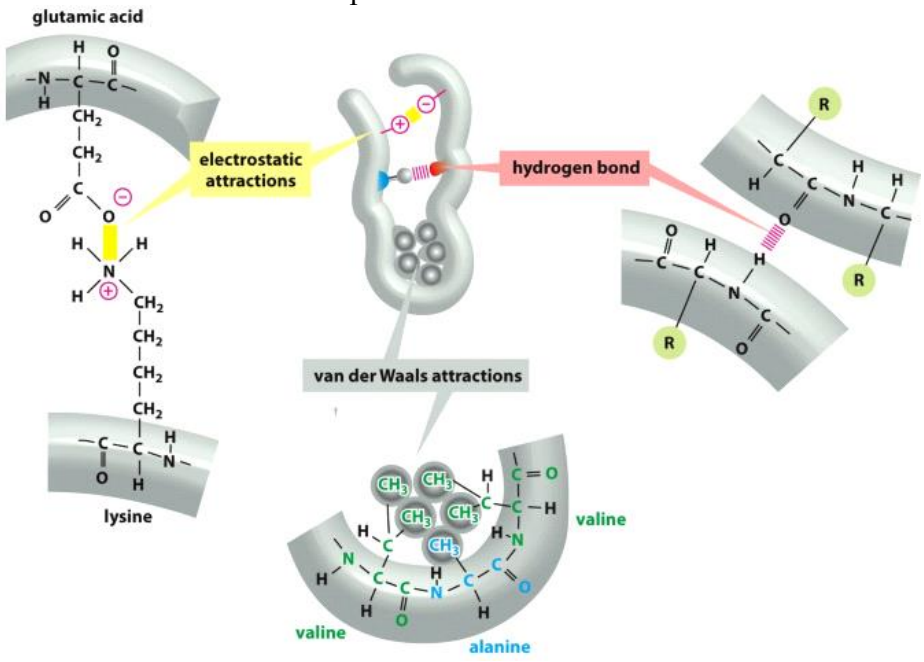
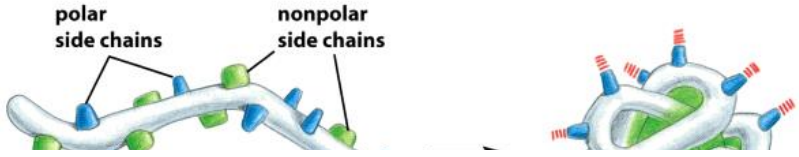
cannot be generalized among the whole population?	 If the patients are sick, how can the information be generalized to all humans? Non-pathological samples - Not present in the disease (tumor or epilepsy center) Correlations with disease history
Do humans have unexpectedly more complex neurons across different brain areas?	Humans have more complex neurons across brain areas Larger, more dendrites and receive more synapses Linear relationship with body size 
What does it mean that `human neurons recover faster from depression`?	Human synapses recover faster from depression  Time constant of recovery - Less than 200 ms This is **not age-dependent**
	Human synapsis transfer 10 times more information

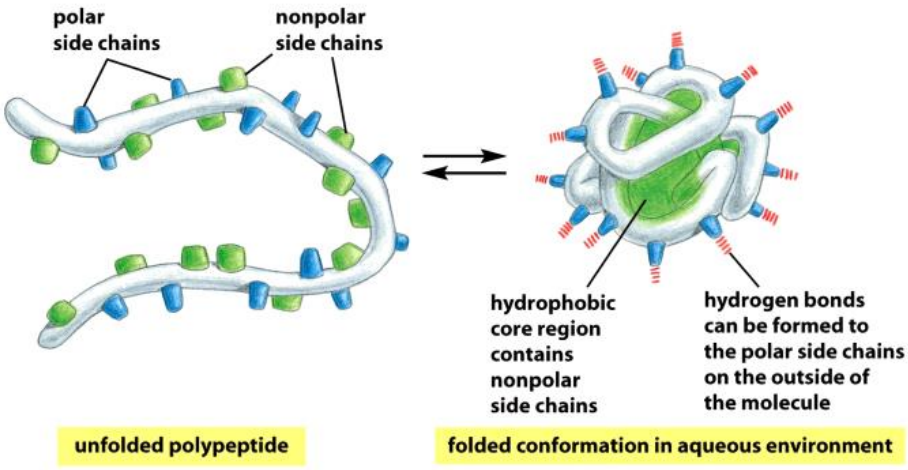
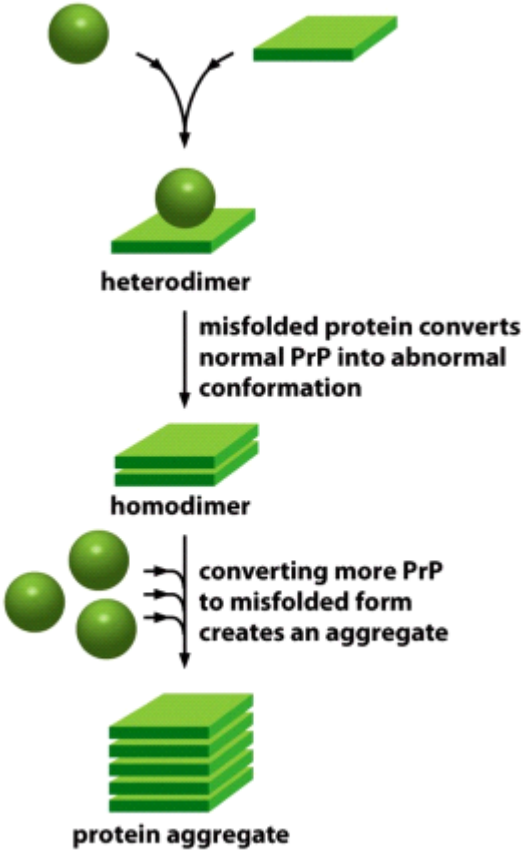
	Based on the post-synaptic response quality 
What is the underlying mechanism that allows human neurons to respond to repeated firing?	Human neurons reliability respond - Noise + sine wave Allows a known change in frequency Humans can distinguish 1000 Hz frequency Mechanism: Humans have fast action potential **even during repeated firing** 
How can mental ability be measure? What are some correlations between physiological and anatomical features of the human brain and mental ability?	How do neurons give rise to mental ability? Testing cognitive ability Attention Language Spatial orientation IQ(WAIS-III) - Average = 100 Short and long-term memory Correlations **Brain size and IQ**: The larger the brain, the larger the IQ Albert Einstein had a brian weight of 1.23 kg **Temporal cortex gray matter thickness and IQ:** The thicker, the larger the IQ Bigger pyramidal neurons - bigger dendrites and more information processing **Dendrite length** and **complexity** also correlates positevely with IQ
	Larger neurons have faster action potentials People with high IQ have faster action potentials Small differences become huge - The brain has 86 billion neurons and one thousand times the number of synapses
Why large scale neuroscience is	Publications on the brain - 100000 publications per year, **very little understanding** Large scale neuroscience - More than one billion euros/dollars project

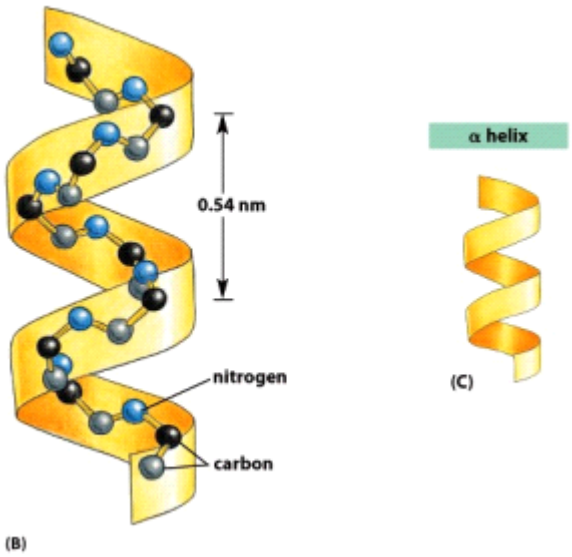
important?	- Allen Institute (2003) - Human connectome project (2009) - EU human brain project (2013) - ICT based brain research; Mining data from Hospitals Review: Neuron (2016 in November) - Global Neuroscience
	Summary - Small differences in many neuronal properties may reflect the big differences observed between humans and other primates 

3. Building the Basics - Protein structure and function (ECB 4)

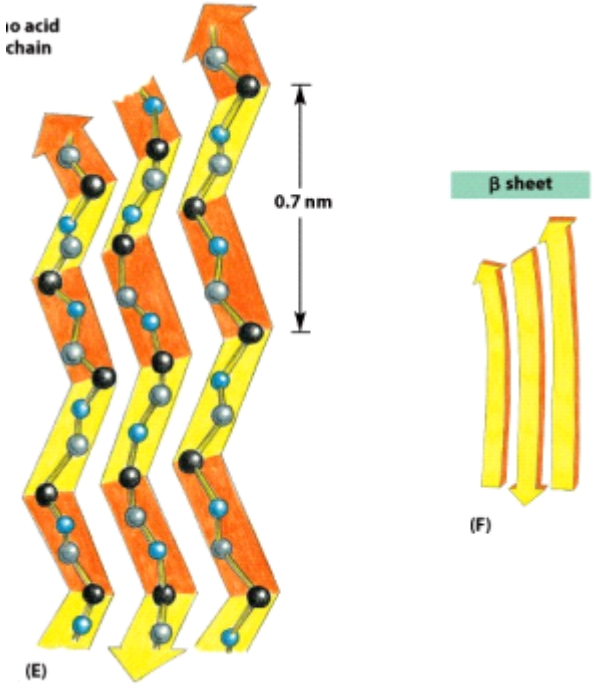
What does the water come from during the formation of a peptide bond?	Proteins - Amino acids connected by **peptide bonds** (catalysed by enzymes) Release a water molecule  Proteins are different because of their side chains - Dictated by the gene information in DNA
What does it mean to say that an amino acid is polar but uncharged?	The side chain dictates the behaviour of the protein - Nonpolar and polar side chains behave differently **Hydrophilic** (uneven electron distribution)/**Hydrophobic** (even electron distribution) **Uncharged polar** - Net charge equals 0, but the electron distribution is uneven

	 Mutations causes variations on amino acids - Proteins do not work the same way
Which are the four forces contribute to protein conformation?	Which forces contribute to protein conformation?  - **Electrostatic attractions** (between positive and negatively charges aminoacids) In solution, the protein interactions compete between other interactions in the solution (egg denatures in acid) - **Hydrogen bonds** - **Van der Waals** attractions - Small fluctuation of eletronic distribution - **Covalent bonds** - Protein backbones and between cysteins (disulfide bonds) These interactions occur between backbone to backbone, sidechain to backbone and sidechain to sidechain
Why mutations in nucleotides that code hydrophobic amino acids tend to be more severe than those in hydrophilic amino acids?	Hydrophobic side chains do not like water - They tend to be hidden in the middle 

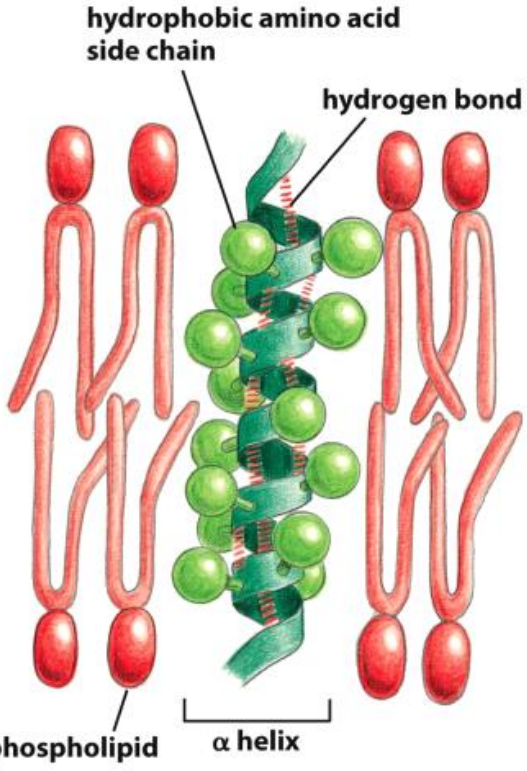
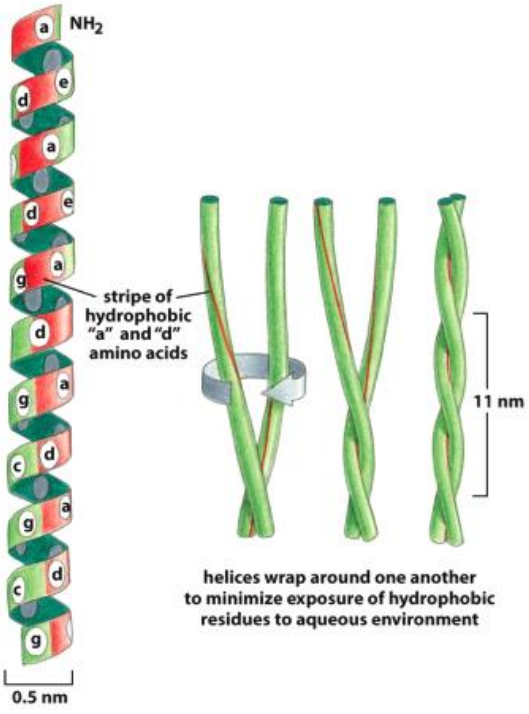
hydrophobic amino acids tend to be more severe than those in hydrophilic amino acids?	 Hydrophilic side chains stick out of the protein Mutations on hydrophobic side chains tend to be more serious - Changes the conformation of the **entire** protein
Why some proteins are not able to come back to their normal configuration after being denatured?	Protein aggregates - The proteins are no longer able to refold 
What are the most common tertiary structures?	**Backbone structures** **Alpha-helix** - Fixed distance of **0.54 nm** Only with a few side chains - Mutations can cause the alpha-helix not to be formed

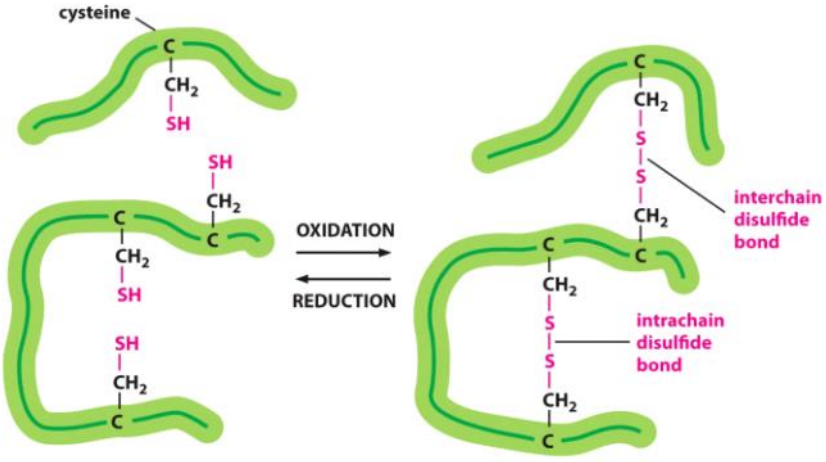
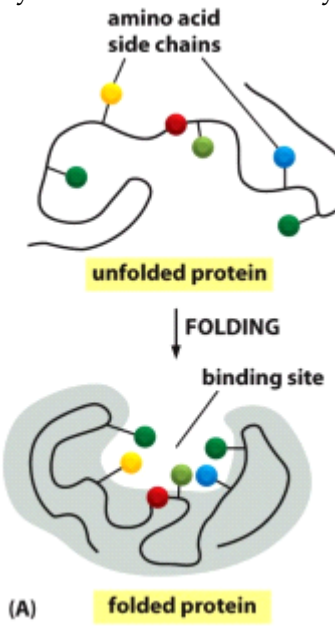


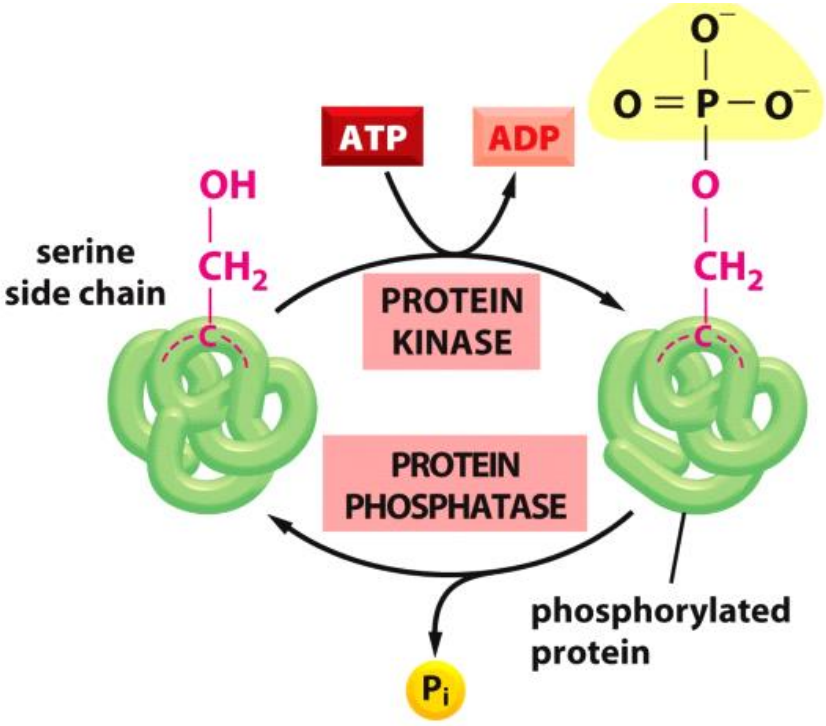
Beta-sheets - Fixed structure of **0.7 nm**



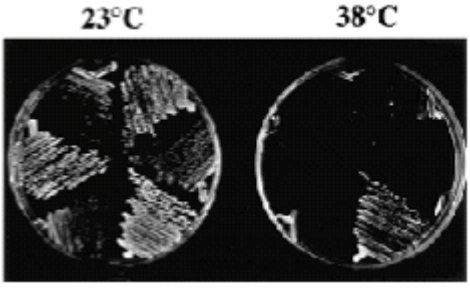
Alpha helix with hydrophobic side chains - Where a protein sits in the cell membrane

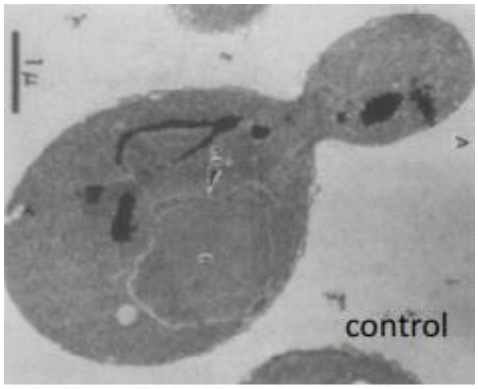
	 Alpha helix with polar and non-polar side chains - Helices wrap around one another to minimize exposure to the aqueous environment (**coiled coil**) 
Define primary, secondary, tertiary and quaternary protein structures.	**Primary structure** - Sequence of amino acids **Secondary structure** - Three-dimensional forms of local protein segments (alpha-helix and beta-sheets) **Tertiary structure** - Spatial distribution of the protein

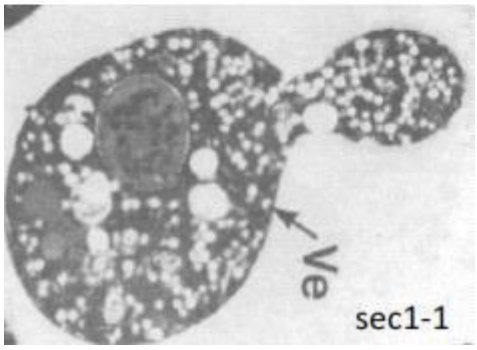
	Quaternary structure - Connections between two or more proteins (dimers, tetramers) E.g. Disulfide bonds between different proteins, cysteine mutations are usually very problematic 
	Aminoacids in very different positions in the primary structure, but may be close in the tertirary structure  Denatured protein does not work because it does not have its original tertirary structure
Phosphatases and kinase usually act upon which amino acid side chains?	Enzymes can be regulated with simpler compounds - Either activating or deactivating it Covalent interactions - Some side chains can be regulated with kinases and phosphatases **Serine chain** - Kinase puts a phosphate, phophatase removes a phosphate


O⁻
O = P − O⁻
O
CH2
C
ATP
ADP
OH
CH2
C
serine
side chain
PROTEIN
KINASE
PROTEIN
PHOSPHATASE
phosphorylated
protein
Pi

3b. Model Organism in Neurogenomics: Yeast

Why is yeast used as a model in the context of Neuroscience?	**6000 genes** - 25% essential, 75% non-essential Generation time: **20 minutes** **Eukaryotic cell** - Have organelles and vesicle secretion *Genes are not a predictor of complexity: Nematodes have the same amount of genes as humans* Yeast has many similar proteins - Vesicle proteins It is useful to study molecular processes In Science, you should answer your questions with **the simplest organism possible**
Explain Randy Schekman experiments with yeast.	Randy Schekman - Won a Nobel Prize studying yeast (check online lectures https://wn.com/randy_schekman_univ_calif_berkeley_part_1_biochemical_reconstitution_of_transport_vesicle_budding) **Random mutagenis** - Carcinogens inserted into yeast Mutations in proteins do not cause problems at 23 degrees, but cause problems at 38 degree - Temperature sensitive mutation  - You can use these yeast in the lab at lower temperatures - Sec-1 - Vacuoles filled with enzymes that are secreted in a normal yeast cell

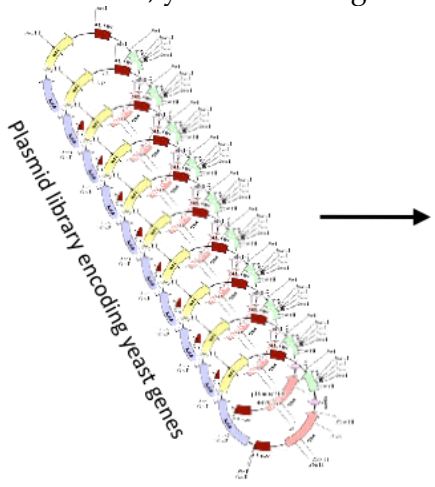


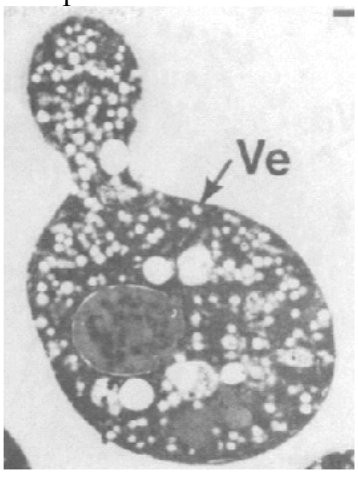


Buoyancy - Mutant yeast with vesicles are **more buoyant** than control cells (easy to collect with centrifuging)

Put human genes in plasmid - Also works in yeast cells!

How to find out which gene was mutated?
Plasmid library encoding yeast genes - Random collection of genes in plasmids and put it in the cell; if the vesicle function comes back, you know the gene is in the plasmid



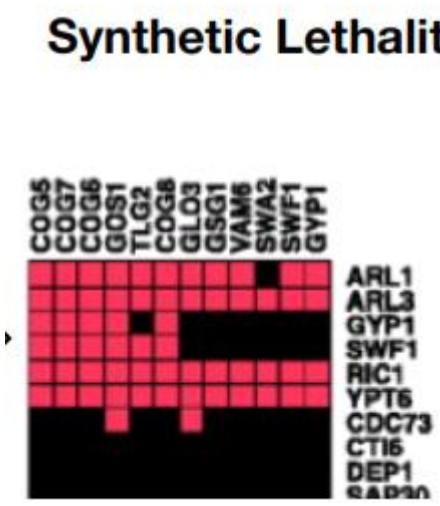
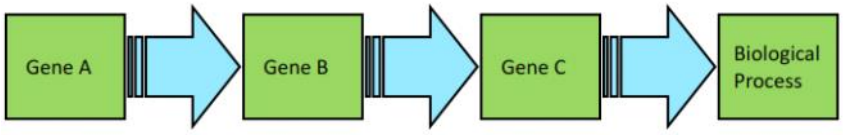


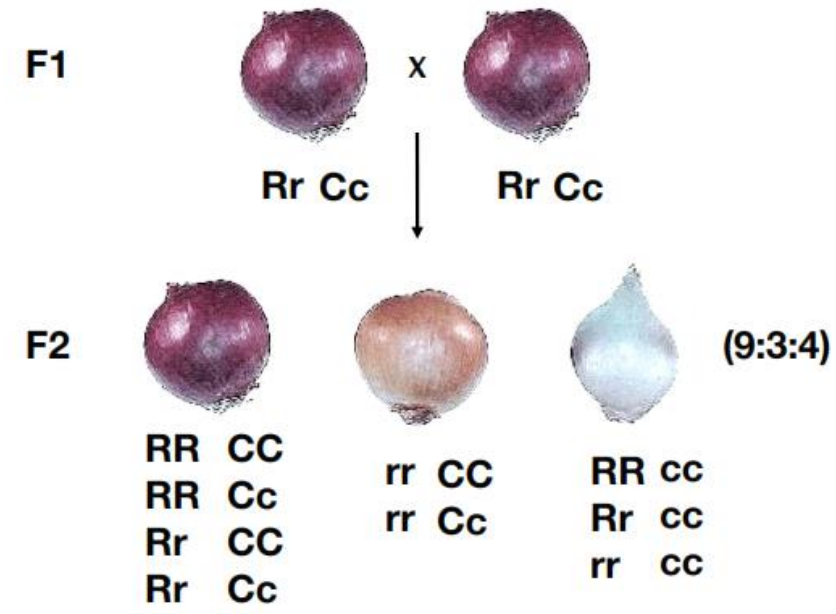
Sec1 mutant

Why the collection of sec wasn't complete for a long time?

Collection of sec genes is not complete

- For some genes, that are two proteins made by two different genes that make the same thing
    - **Robustness** - An organism tolerates mutations due to redundancy; this is present in complex organisms, since it prevents a single mutation to kill the organism

	○ E.g. Sec4 overexpression rescues Sec1 mutant - To determine two redundant genes, you need more advanced technology: Synthetic Lethality  *The middle squares, in which the combination of two mutations causes the cell to die, probably represents two redundant genes for the same function*
Why an overexpression of one gene may compensate for the absence of another gene?	**Epistasis** - The effect of one gene depends on one or more modifier genes All genes have a residual activity, even when they are not activated(stochastic nature)  If you overexpress gene B, even if it is gene A mutant, the process might still work
Where does the ratio 9:3:4 come from?	Onions - Red is dominant, white is recessive Epistasis - Ratio of 9:3:4 (C gene needs to produce something that the R gene can act upon)



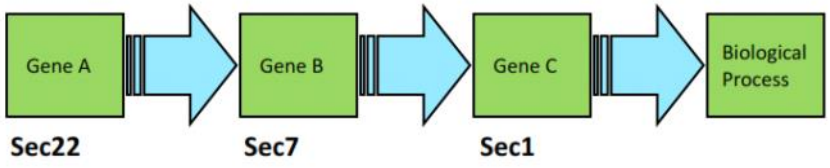
Colorless -> Yellow -> Red

"c genetically interacts with r"
"allele c enhances the phenotype of allele r"
"c is epistatic to r"

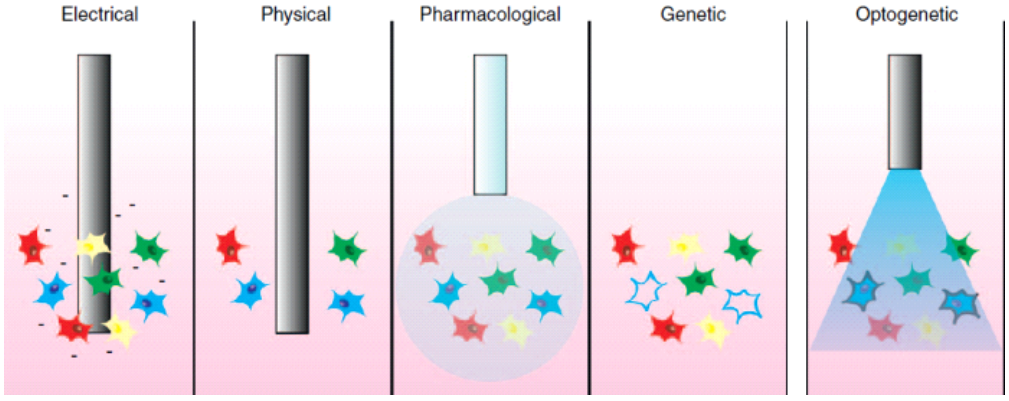
You cross a plant with a gene mutation A with another plant with a gene mutation B and the offspring only presents the mutant A phenotype. What is a possible explanation for this?

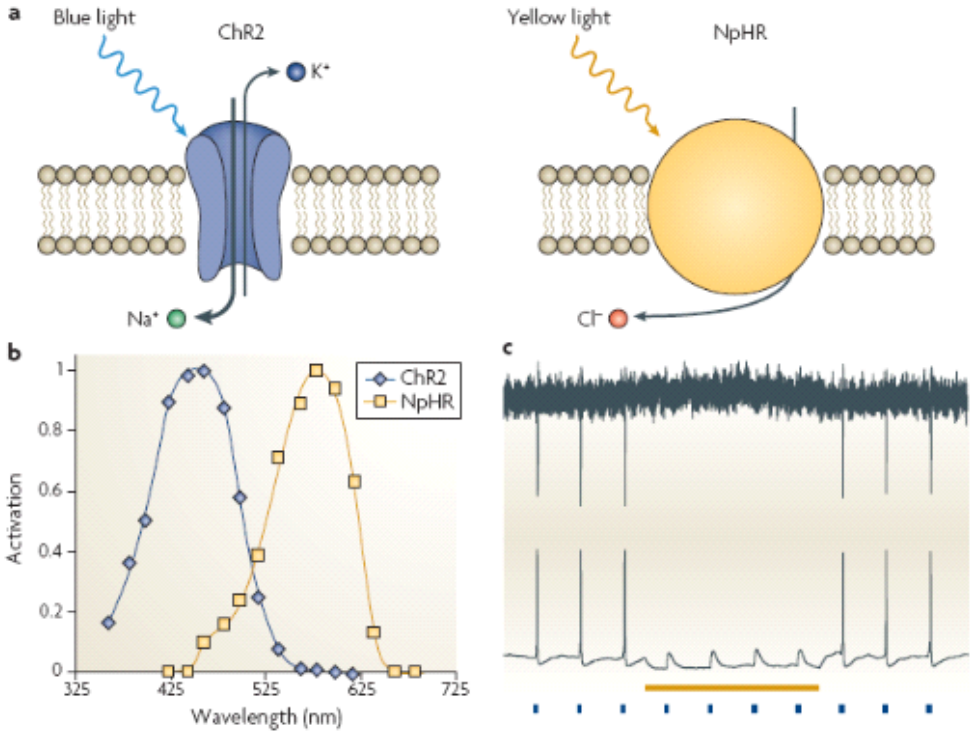
Upstream/Downstream epistasis - Determined by crossing mutants

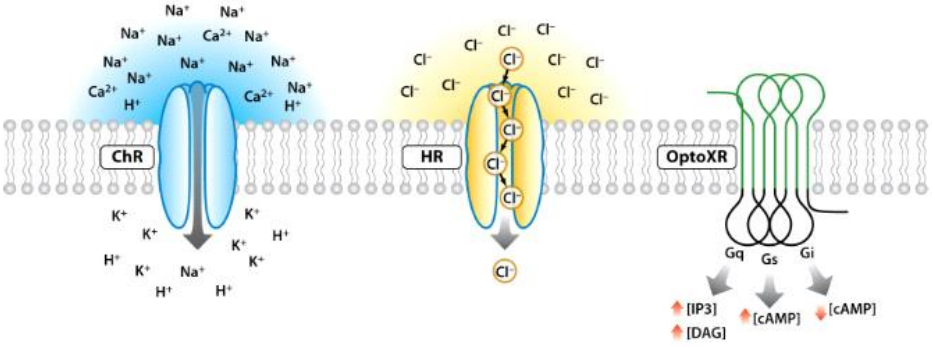
Sec22 + Sec7 = Sec22 (it means that Sec 22 is upstream)
Sec22 + Sec1 = Sec22 (it means that Sec 22 is upstream)
Sec7 + Sec1 = Sec7 (it means that Sec7 is upstream)



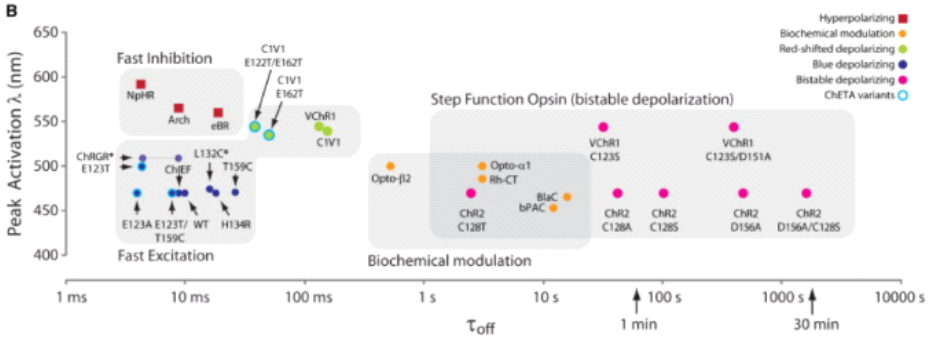
4. Optogenetics

Describe which approaches have been used to try to assess causal relationships between brain regions and behaviour. What are their main limitations?	Traditional approaches to have a causal understanding of brain areas - Lesions - Mechanical and chemical ○ **Irreversible** ○ HM - Both hippocampi lesioned - Short-term memory loss ○ Phineas Gage - Prefrontal cortex lesion - Personality change/ impulsivity - Pharmacological - Receptor agonists and antagonists ○ Reversible ○ **Not very spatially precise** - A drug acts on the entire brain at once; not selective to cell types, many receptors are expressed in many brain cell types - Deep brain stimulation ○ Fast - Electrical stimulation ○ **Not very precise as well** - Many neurons in the viscinity will be activated along with the desired area - Genetic intervention - Full/partial knock out of genes ○ **Bad temporal resolution** - Takes a long time 
What are the main advantages of optogenetics?	Optogenetics - Activation of cells using light Gene sensitivity to light can be **selectively expressed** **Very high temporal resolution** - Use of light! **Reversible** - Light can be turned off Can induce **any firing frequency**, even **silence neuronal activation** Integration of genetics, neurophysiology, neuroanatomy and behaviour
	Optogenetics tools Bacteria, fungi and animals express light-sensitive ion channels and pumps - E.g. Discovery and use of GFP (jellyfish)
What are the two most common opsin genes used in optogenetics? What is their effect in the neuron?	Opsin genes Channelrhodopsin (ChR2) - Sensitive to blue light Potassium and sodium flows through - Cells will be depolarized Halorhodopsin (eNpHR3.0) - Sensitive to yellow light Chloride flows through - Hyperpolarizes the cell

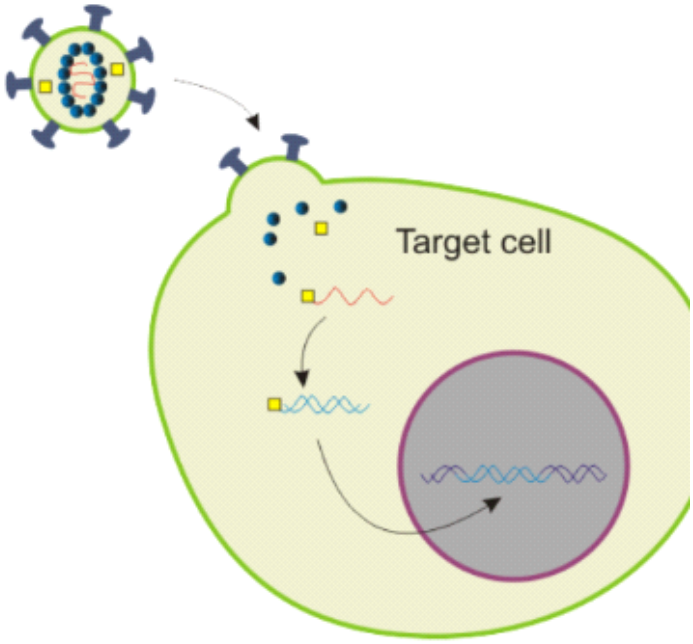
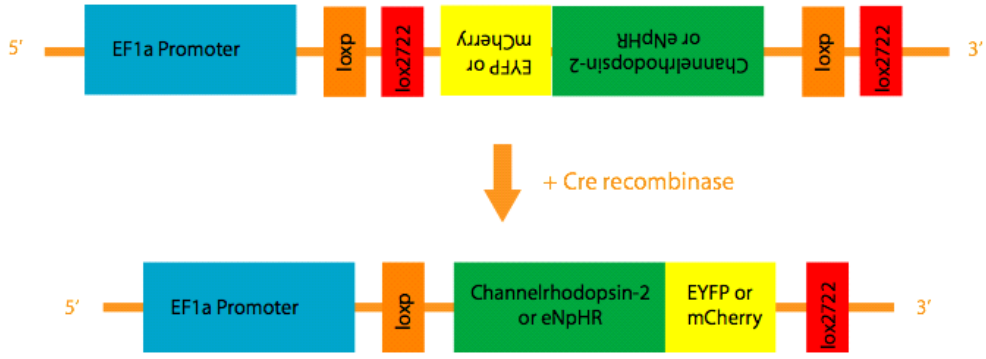




Many variations were created - Sensitivity to the whole visible light spectrum, many different types of channels (adrenaline, glutamate, adenylyl cyclases)



Why lentivirus are used in animal models to transfect cells with target genes?	Virus-mediated transfection - Virus are very efficient machine of injecting DNA into cells - AAV (adeno associated virus)/ Lentivirus - Can be used in living organisms

How can gene delivery be specific if virus infect cells indiscriminately?	 How to make sure the virus only affect target areas? Neuron subtype specific promoters Expression of Cre recombinase  E.g. CAMKII - Excitatory channel only expressed in the forebrain **LoxP site** - Recognized by Cre recombinase - Flips around DNA into the right order **eYFP** - Used to visualize in which cells this process was sucessful **ChR2 expression** is uncoupled from promoter expression By using special strains of transgenic mice that only express Cre recombinase under the control of specific promotors such as TH or ChAT, expression of the light sensitive channels and reporters are constrained to cells that express TH or ChAT. It is possible not only to get cell type specificity, but also projection specificity *Parvalbumin - Weak promoter, present in interneurons*
Why mice are most commonly used to assess in vivo modulation of behaviour?	In vivo modulation - Chronic implantation of optic fiber in brain region of interest Deisseroth paper - Activation of motor cortex

5B. Models and methodologies - Mutant mice

How humans compare with other organisms in terms of gene-rich areas, mRNA splicing and repeated sequences?

How humans compare with other organisms?

- Humans have seemingly random gene-rich areas, while the genome of other animals is more evenly distributed and predictable
- Humans have mRNA splicing occur more intensely than in other animals - multiple different proteins can be derived from the same gene
- Humans have most of the same protein families as in other model animals, but with a greater variety of family members
- Humans have more repeated sequences than other model animals

What does the forced swim test reveal about mice as model organisms?

Forced swim test (with pigment so that the mouse cannot see the platform)

Mice do not like to swim - Once they find the platform, they are happy

After a few trials, mice learn where the platform is, it goes straight to it (uses cues in the environment to navigate - complex behaviour, simpler organisms cannot do this)

Why are mice better than rats in terms of genetic manipulation?

Mice compared to other organisms

	man	monkey	rat	mouse	fly	worm	yeast
Behaviour	✓	✓	✓	✓	✓/x	✓/x	x
Electro-physiology	x	✓	✓	✓	✓	✓	x
Neuro-anatomy	✓	✓	✓	✓	✓	✓	x
Neuronal biochemistry	x	x	✓	✓	✓/x	✓/x	x
Neuronal Cell biology	x	x	✓	✓	✓	✓	x
Genetic manipulation	x	x	✓/x	✓	✓	✓	✓

Men and chimpanzees - Problematic for ethical reasons

What is syntheny?

What is the difference between construct validity and face validity?

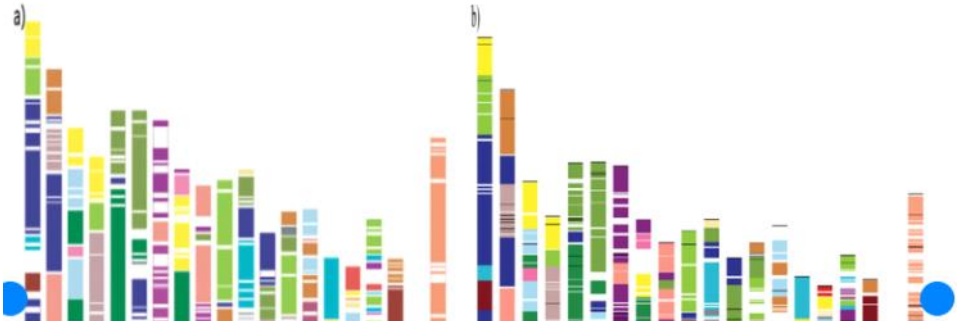
Mice

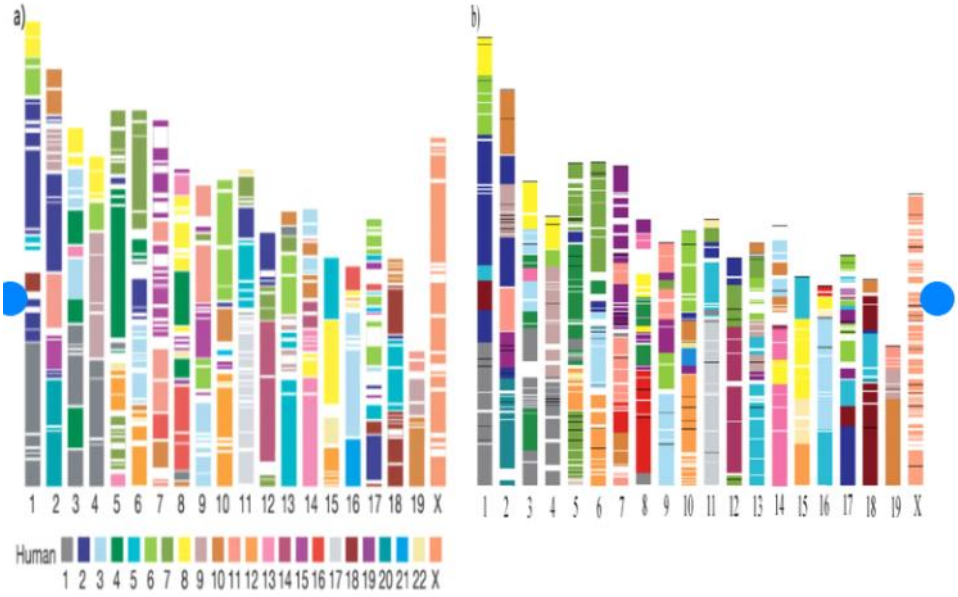
Similar chromosomes

Similar genes - Few exceptions of genes present in humans that are not present in mice

Syntheny - Physical colocation of genetic loci between different species

Color code - Same genes that mice have in common with humans





Means that we can have construct validity - Model human disease in mice

Trissomy of the 21 chromosome - mice present the same symptoms

Does not mean that it has **face validity** (you have not proved that there are no differences between mice and humans)

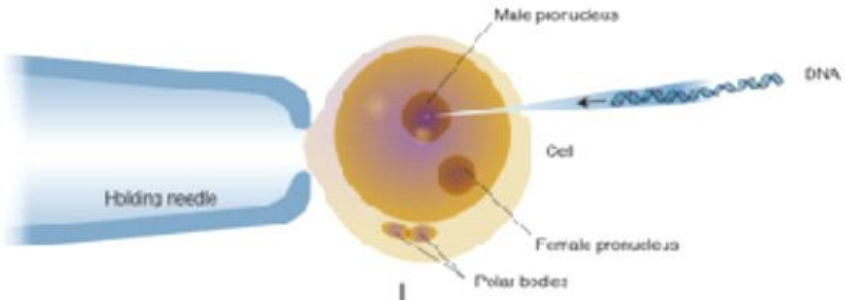
What is the process for transgenesis?

Principles for producing mutant mice

Transgenesis (random insertion)

Happens naturally all the time - Virus insert DNA

- Occurs more frequently with open DNA (active being transcripted)
- You take a gene with its promoter (maybe a reporter gene such as GFP or channelrhodopsin), insert it in a fertilized egg



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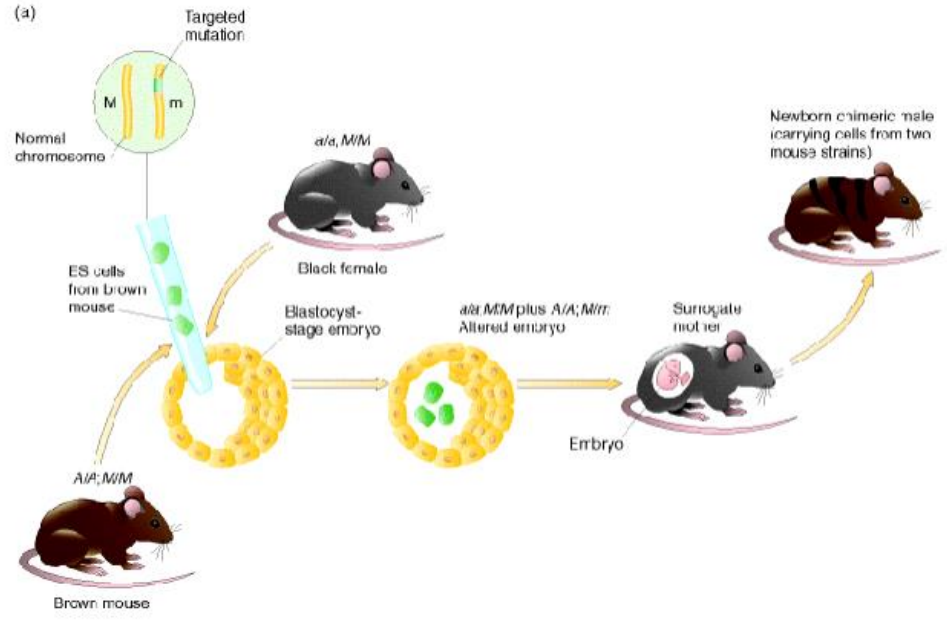
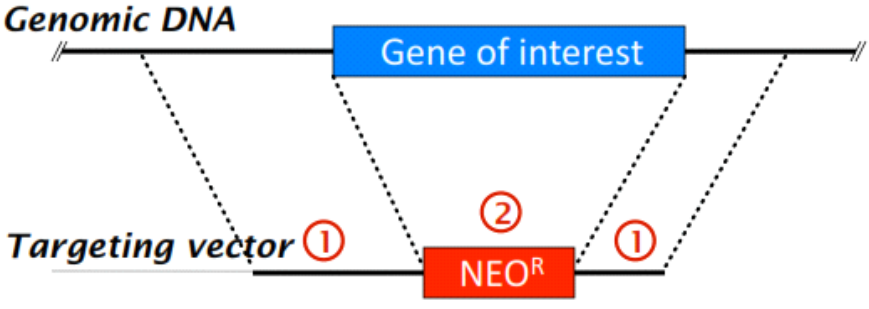
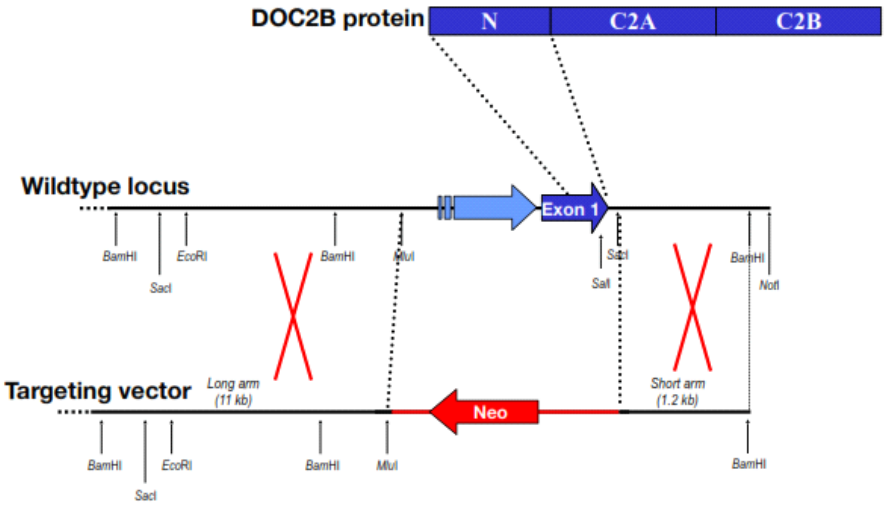
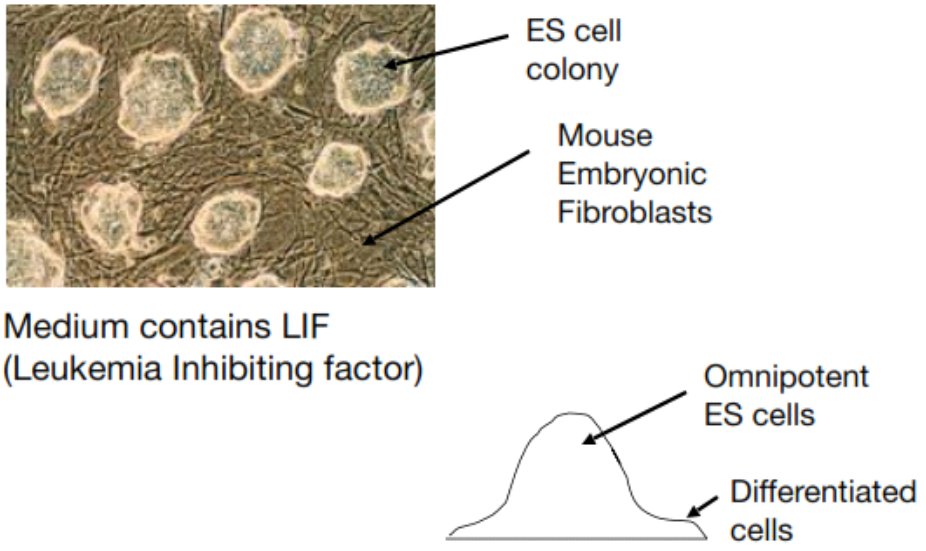
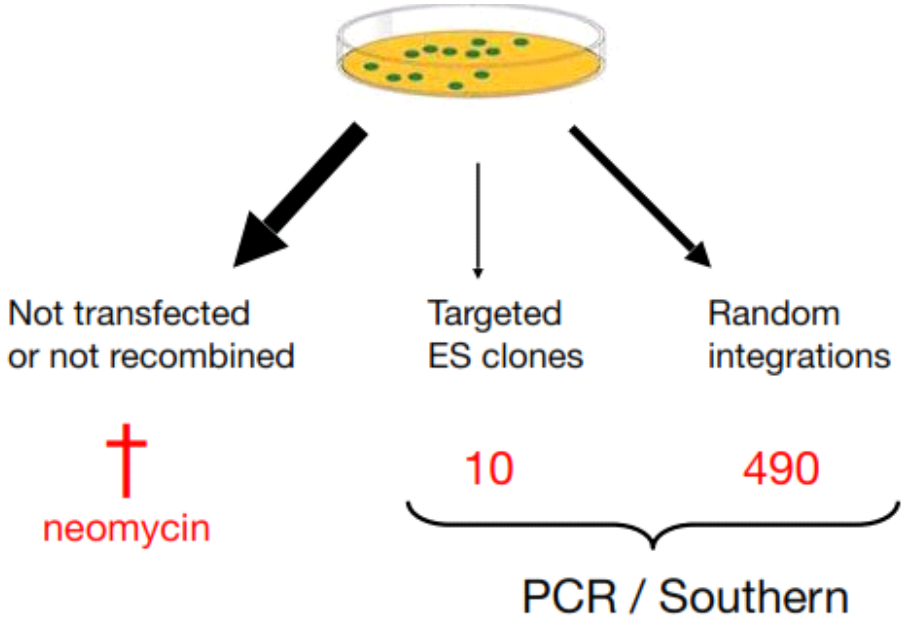
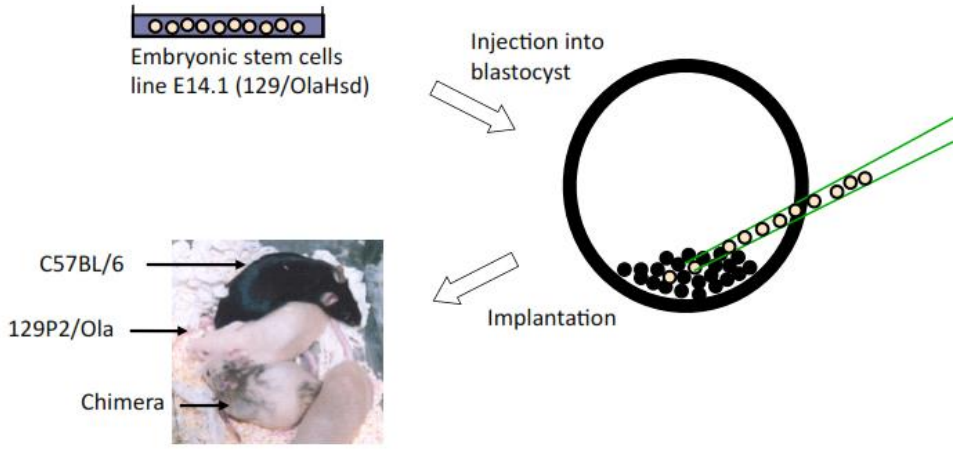
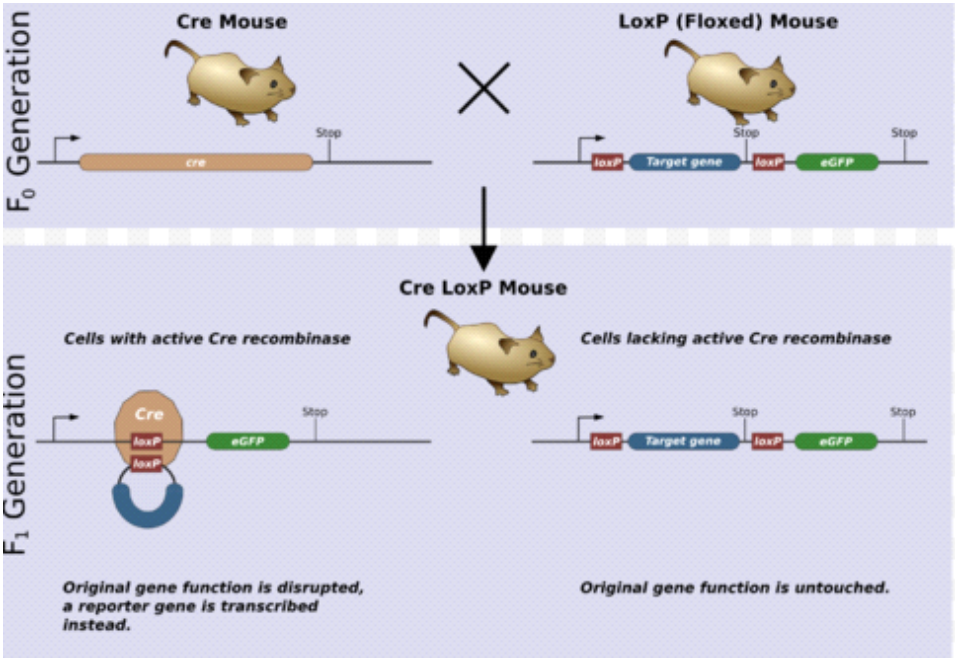
- Test of offspring - PCR/Western Blot
  - Measure protein or RNA expression to be sure that the transgenesis was sucessful

	- Cheaper and faster - There are many limitations ▪ You cannot be sure how much protein is expressed and where exactly
Describe the process of homologous recombination. What is its main advantage over transgenesis?	**Homologous recombination (targeted modification)** Two main goals: **Knockout a particular gene** (block gene expression - normally this does not occur in humans), **single nucleotide mutation** (normally occurs naturally) Done with stem cells: Inserted in blastocyst-stage embryo (all cells are omnipotent)  Omnipotent cells remain with their potency and divide (millions of cells are necessary) - Offspring become a mosaic - Fur colour is an indication of how much the green cells contributed to the organism (Aguti/Brown is dominant over black fur) - The green cells need to contribute to the reproductive system, so that the process can be repeated
Describe how homologous recombination occurs. What is the important of Neomycin resistance gene?	 Gene of interest (in the organism, you want to knockout) Black portion (arms for homologous recombination) - High affinity to the opposite strand 1 to 7 kb in size Targeting vector - NEOr (replaces gene of interest in dividing cells) Does not hybrize well

	Selectable marker - Neomycin (confers resistance against G418)
Why the neomycin gene is often reversed in the process of homologous recombination?	DOC2B protein  BamHI to MluI - Long arm MluI to SacI - Replaces gene of interest Reversed gene to make sure that it integrated with DNA SacI to Bam - Short arm
What is the importance of leukemia inhibiting factor in stem cell colonies?	Embryonic stem cell colony LIF - Leukemia inhibiting factor  Base - Differentiated cells Apex - Omnipotent ES cells

How can you differentiate homologous recombination and random insertion in terms of neomycin expression?	 Cells without neomycin - Die Random integrations (the knockout happens in the genome, but not in the locus of interest) - Express neomycin (do not die) Homologous recombination - Express neomycin (do not die) Need PCR/Southern blot to differentiate
Why can't you generate a double knockout mouse with homologous recombination?	**Chimeric mice** A lot of the fur derived from aguti mice, some black fur  Use the most chimeric mice to reproduce again The next generation, a complete mutant mice is available
	Nicotinic acetylcholine receptor B2 subunits in the medial prefrontal cortex control attention B-gal - Acetylcholine receptor DTA - Diphteria toxin-A (cells that express this will die) Random insertions will die Homologous recombination will live
How can LoxP and Cre be useful to create	Conditional Knock-out using Cre Insertion of a LoxP site - Insert a specific mutation

mutant mice?

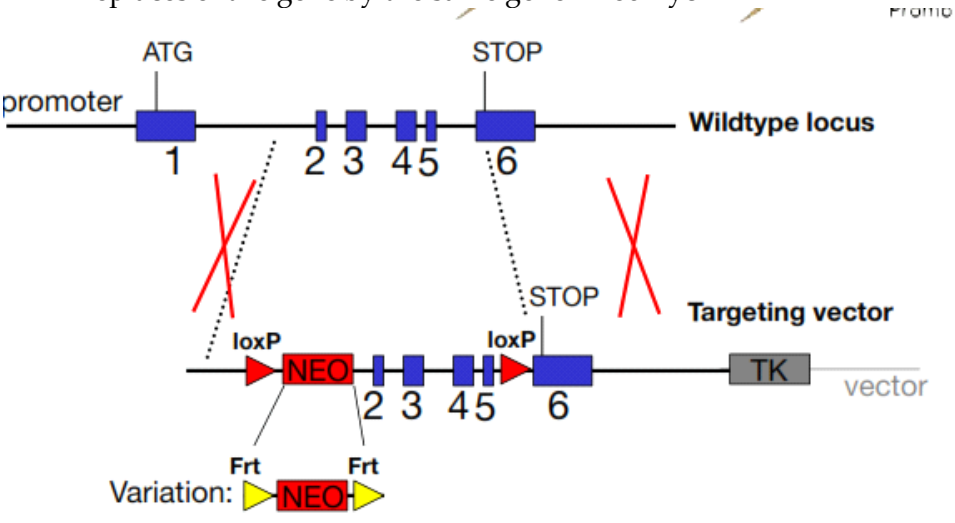


Produced by bacteriophages (virus) - Chops up its own genome to amplify it

Guarantee that the knockout happened in only a specific cell type (that expressed LoxP) - in all other cells, gene expression remains normal

Flank gene + Neomycin - Homologous receombination replaces it

Replaces entire gene by the same gene + neomycin



When you cross this organism with another mouse that expresses CRE - there will be a mutation in the offspring

CRE can be selectively expressed in tissues

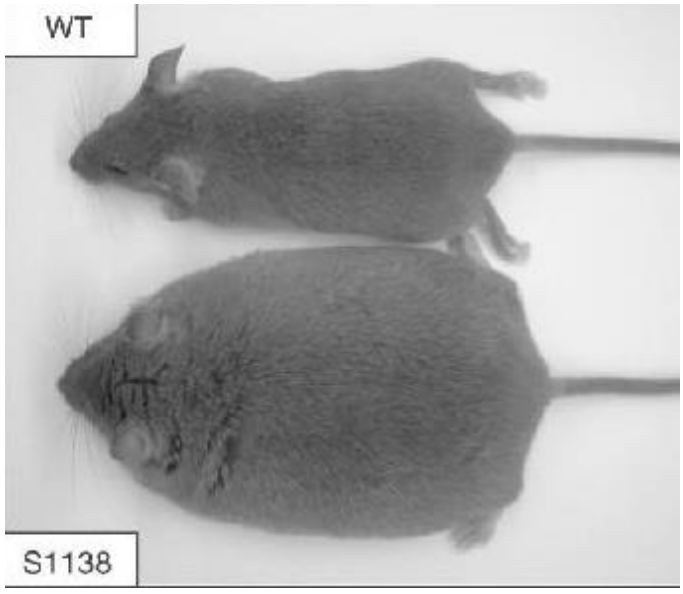
Variation with Frt - **LoxP site to remove Frt cassette** (remove neomycin)

Neomycin is used to select, but then **removed** for behavioural studies

Knock-in study to leptin signalling

ERK(P)- Leptin gene

STAT3(P) - Activation of transcription factor



STAT(3) - Important in leptin signalling

Review

Homologous recombination - Insertion of foreign DNA molecule into the genome

The inserted DNA is double helix - Each recombines with their respective DNA sisters

Why is the gene reversed?

So that the gene of interest is not combined with introns of the opposite strand

Neomycin is an antibiotic - All cells die, except the ones that express the gene

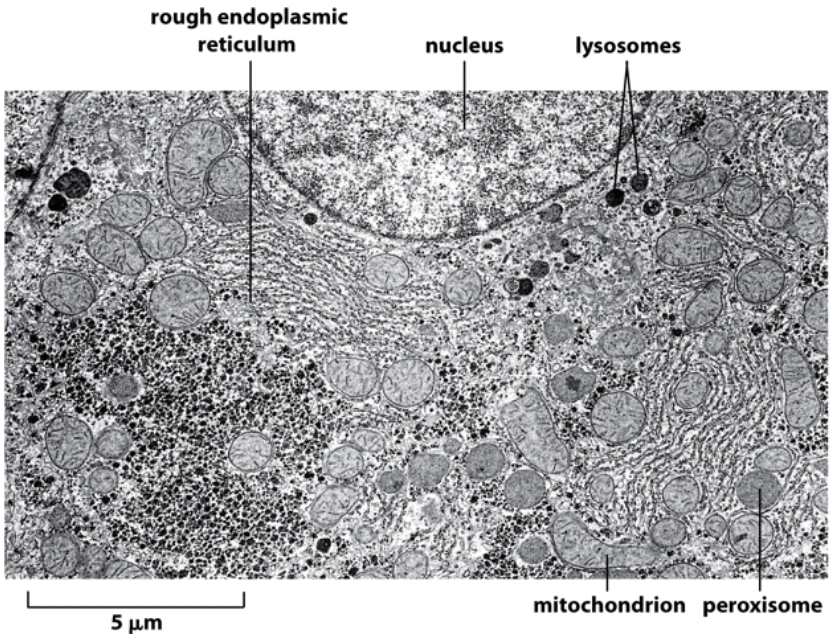
It can also be inserted into a random part of the genome

How to differentiate that? PCR that amplify a select segment of primer + gene of interest

6. Intracellular compartments and transport (chap 15)

How can an organelle visually be observed in an electronic microscope?

Organelles - Defined by membrane structure



Define the rough functionality of every organelles in the eukaryotic cell.

Function of organelles

Cytosol - Contains metabolic pathways
Nucleus - Contains genome, DNA and RNA synthesis
Endoplasmic reticulum - Synthesis of proteins and lipids
Golgi apparatus - Modification and secretion of proteins (railway station)
Lysossomes -Intracellular degradation
Endosomes - Sorting of endocytosed material (from outside)
Mitochondria - ATP synthesis by oxidative phosphorylation
Peroxisomes - Oxidation of toxic molecules

What are the four largest cell structures by percentage of cell volume? And the four largest by amount per cell?

Size of organelles in the cell

Structure	Percentage of cell volume	Number per cell
Cytosol	54	1
Mitochondria	22	1700
ER	12	1
Nucleus	6	1
Golgi	3	1
Peroxissomes	1	400
Lysosomes	1	300
Endosomes	1	200

What are the three types of transport that occur in an eukaryotic cell?

Types of transport

Through nuclear pores
Across membranes
Via vesicles

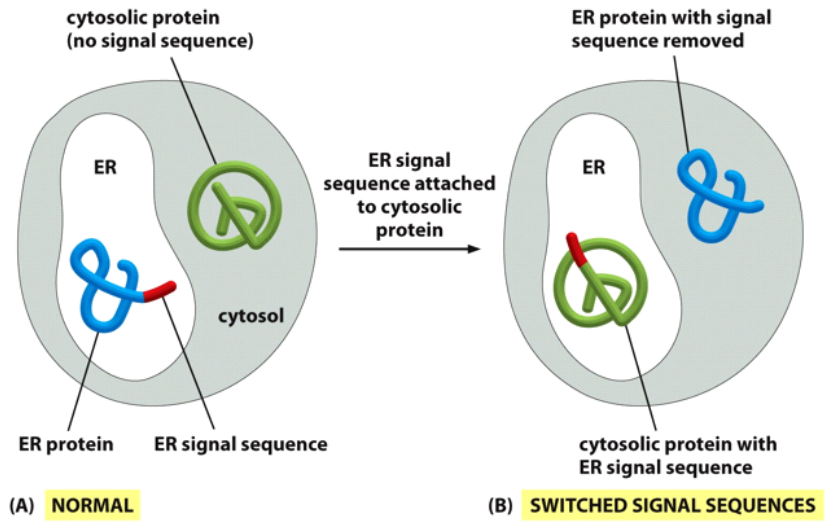
What are signal sequences? Describe the experiment that discovered their existence.

Chemical signaling - Some sequences of amino acids indicate where the proteins need to go

FUNCTION OF SIGNAL	EXAMPLE OF SIGNAL SEQUENCE
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids are shown in *red*, and negatively charged amino acids in *blue*. An extended block of hydrophobic amino acids is shown in *green*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus. The ER retention signal is commonly referred to by its single-letter amino acid abbreviation, KDEL.

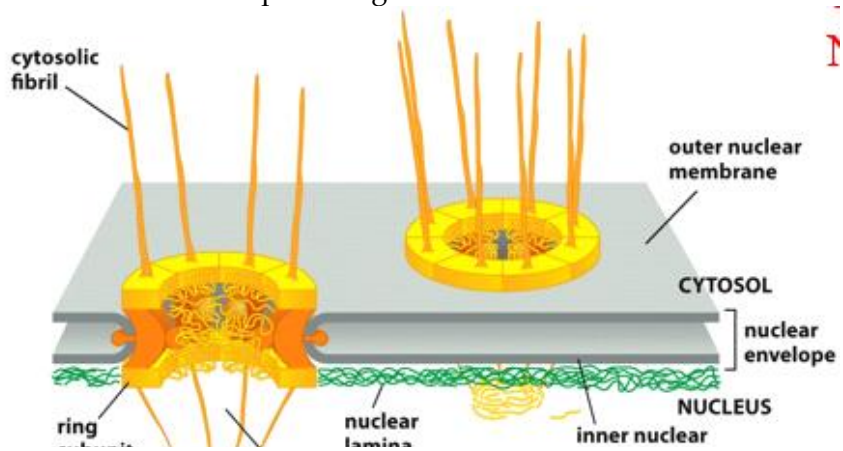
Signal sequences - If you add these sequences to other proteins, they switch places

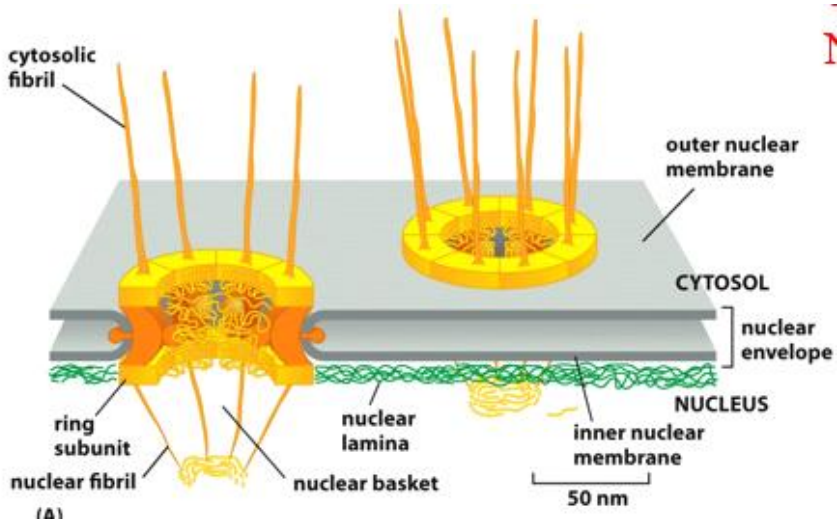
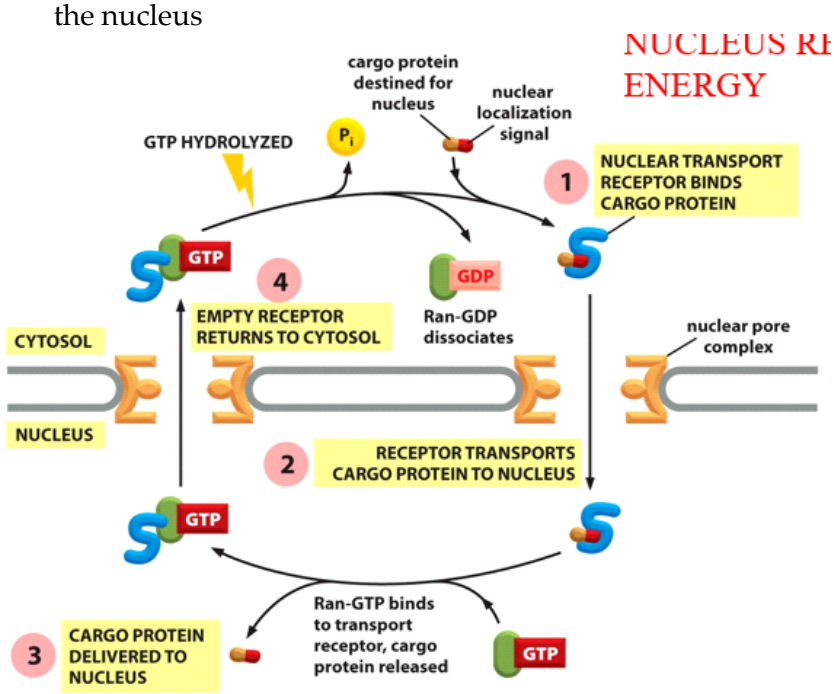


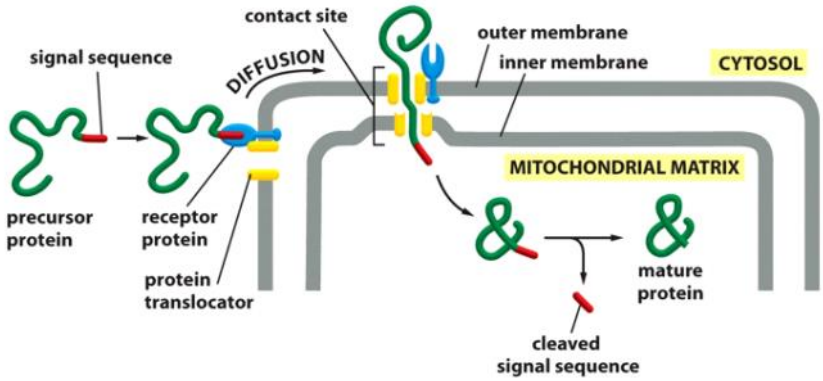
Describe how nuclear transport works. What is the role of GTP in the process?

Transport into the nucleus

Occur in the nuclear pore - Eight transmembrane sections



the role of GTP in the process?	 Nuclear transport receptors - Recognize nuclear localization signal Needs to release protein inside the nucleus and go into the cytosol again - Depends on energy (GTP) GTP can move in and out of the nucleus - It is a very small peptide and it is produced in the ER, which is very close to the nucleus 
How transport into the mitochondria differs from nuclear transport?	**Transport into mitochondria** Similar mechanism - The receptor protein is anchored by one transmembrane domain; pore opens in the inner and outer membrane; protein goes through The signal sequence is then cleaved - The mature protein is available inside the nucleus

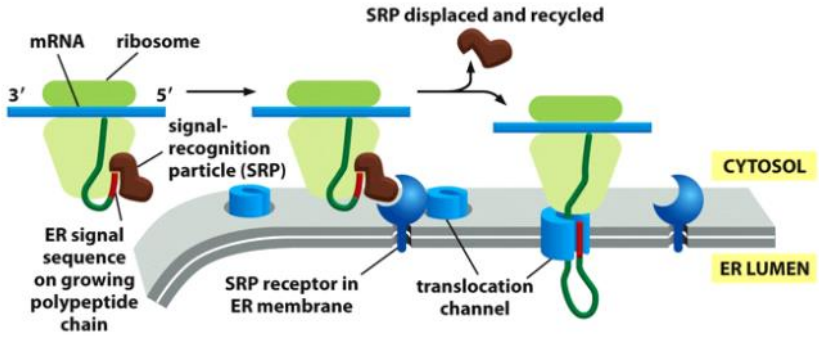


What is unusual about transport into the endoplasmatic reticulum?

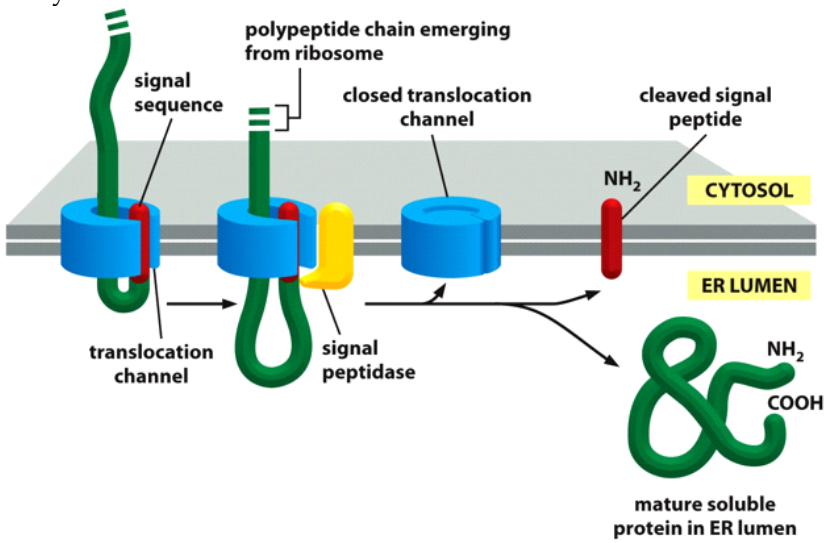
How does the production of cytosolic proteins and transmembrane proteins differ?

Transport into ER

Proteins produced in the ER are synthesided inside the ER
Ribosome + Protein is recognized the ER lumen
Protein is synthesized already docked in the ER membrane

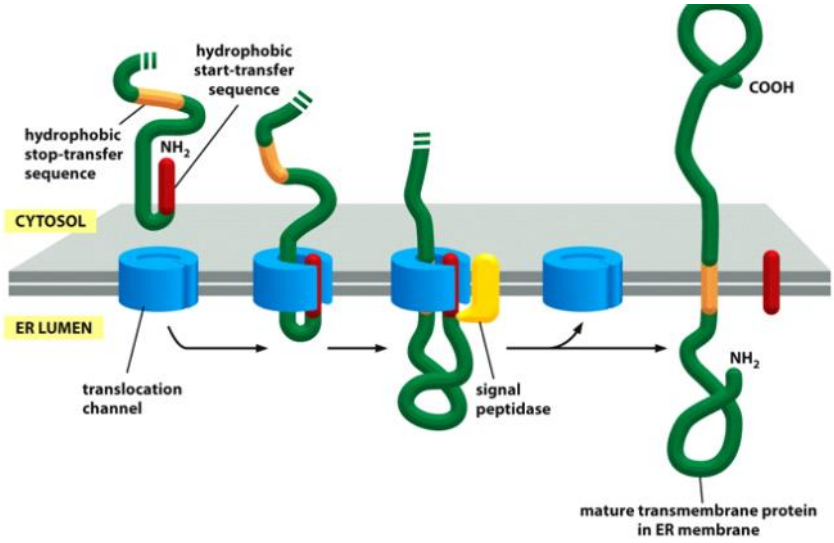


At the end, the signal sequence needs to be cleaved - there are enzymes inside the ER membrane



Transmembrane proteins have internal hydrophobic sequences that do not go through the membrane - High affinity

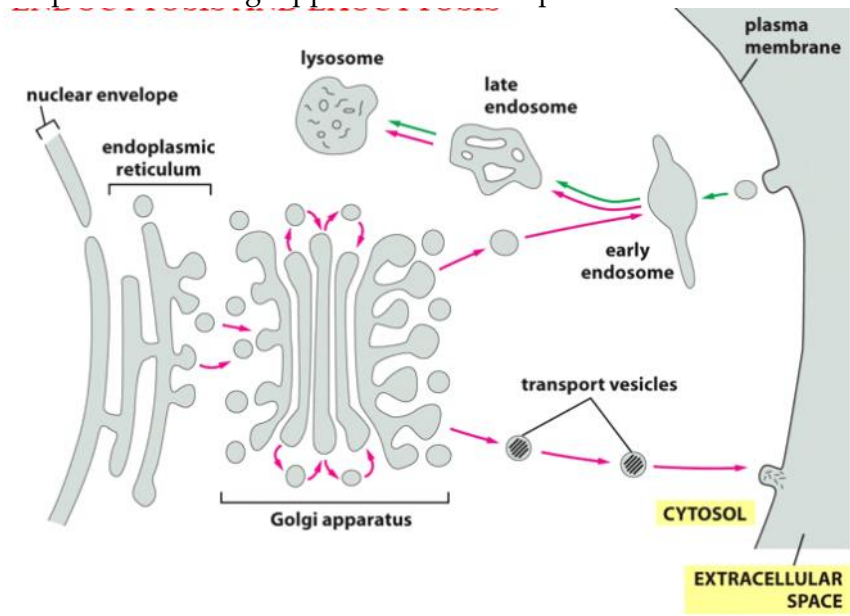
They are always attached to the membrane and are transported with the membrane (e.g. glutamate receptor in neurons)



What is the difference between cytosolic and transmembrane proteins in terms of transport between the ER and the extracellular space?

Endocytosis and exocytosis

All proteins that are secreted need to be produced in the ER, adapted in the Golgi apparatus and transported via vesicles

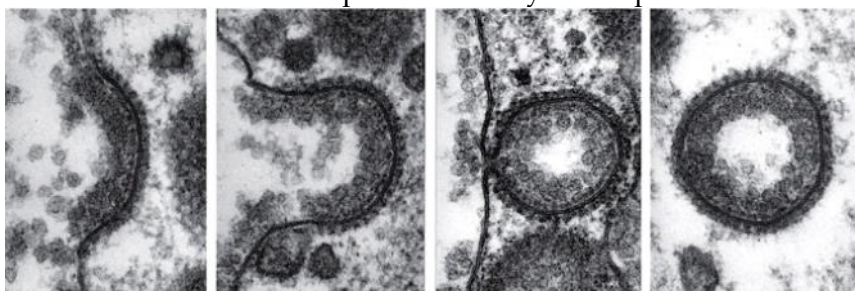


Vesicles that bud off and fuse again in the golgi apparatus - Small protein modifications
Entry of compounds via endocytosis

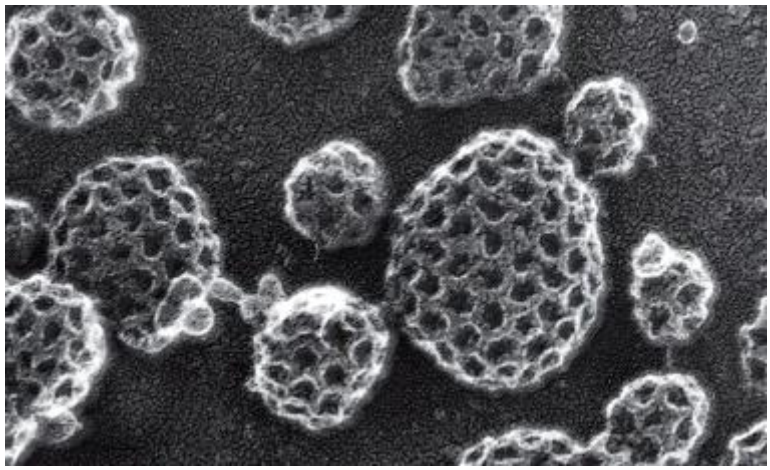
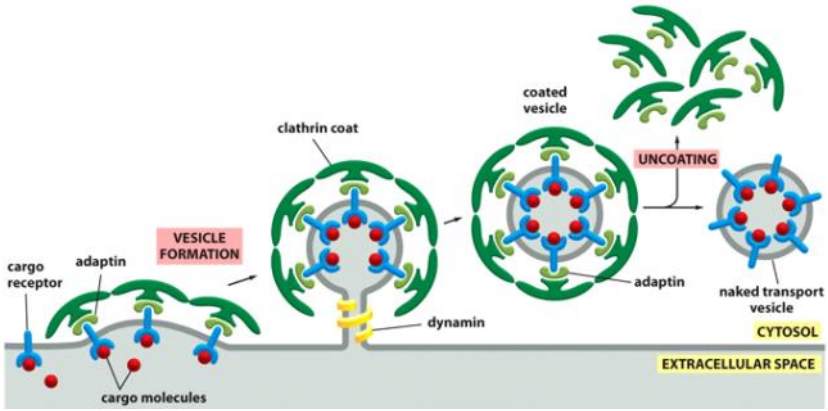
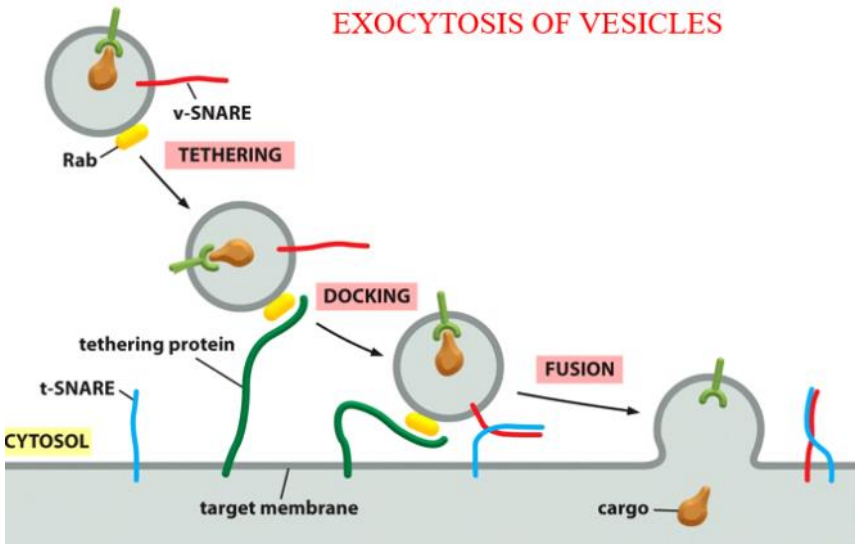
Describe the roles of clathrin, adaptin and dynamin in the process of endocytosis.

Endocytosis

Contains both membrane proteins and cytosolic proteins



Network structure on the outside - Comes from proteins that are

	sitting outside the membrane (clathrin) Vesicles that contain clathrin come from the outside  Dynamin - Helps the vesicle pinch off 
Describe the importance of SNARE proteins and RAB in the process of exocytosis.	Exocytosis of vesicles Guided by proteins - v-SNARE + t-SNARE (membrane coallesces) RAB recognizes tethering protein 
Where does glycosilation	**Glycosilation of proteins in ER**

of transmembrane proteins happen in the cell?

Modifications on the inside of the ER will be sticking outside of the cell

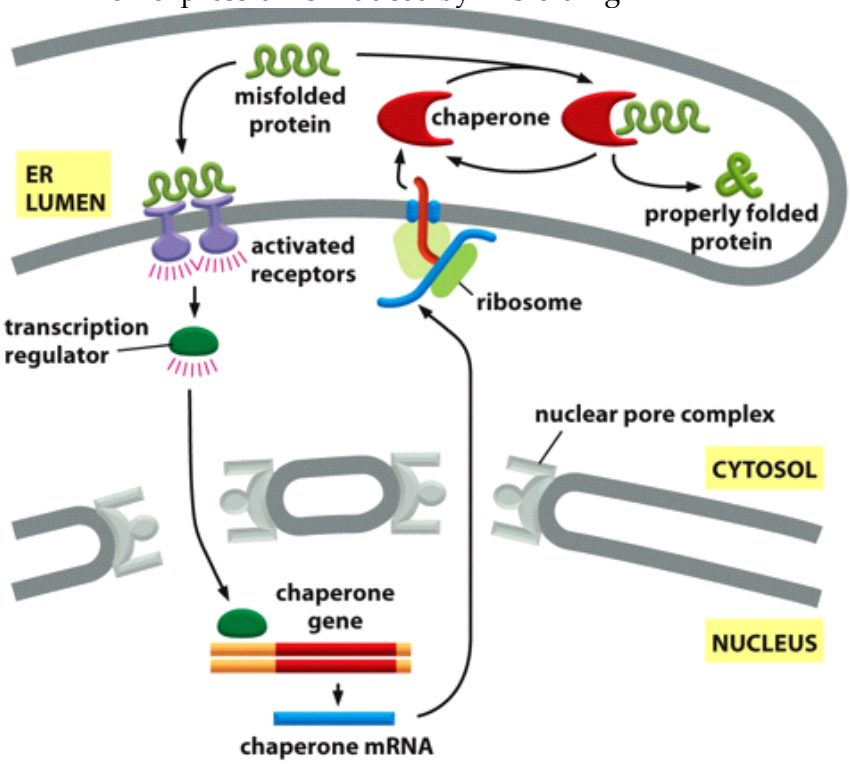
Chaperones govern folding of proteins

What is the importance of chaperone in protein folding?

Make sure that the protein folds in the right shape - Shields off binding site with neighbouring proteins

There is no one-to-one ratio between chaperone and proteins

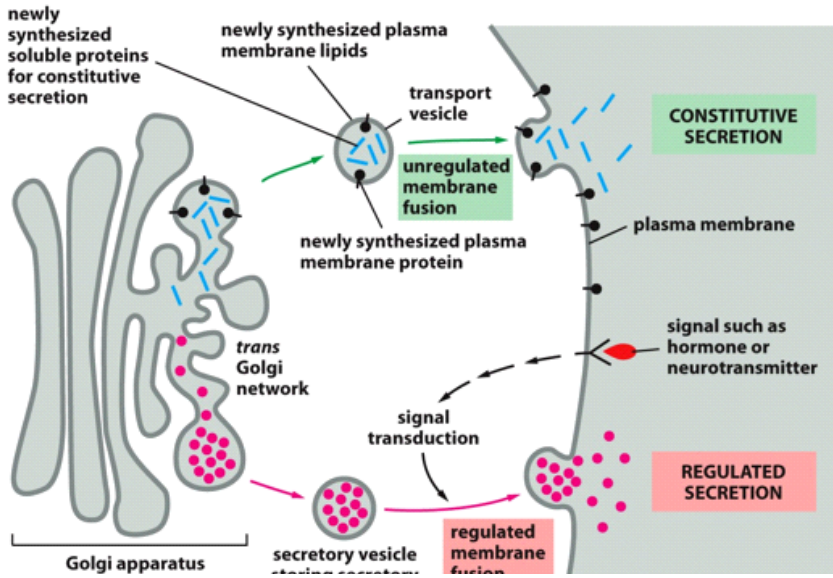
Chaperones are not being actively produced all the time - Their expression is induced by misfolding

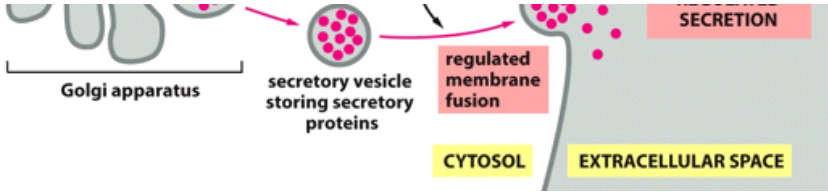


What is the difference between protein secretion and lipid secretion from the Golgi apparatus?

Golgi apparatus

Secretion of proteins and lipids in vesicles (transported in microtubules)





Protein - Regulated secretion (signaling required)
Lipid - Constitutive secretion (no-signaling required)

Protein sorting by the endosome

Some protein enter the cell and need to go out - E.g. LDL (lipid) cannot be transported in the aqueous environment of the cytosol

Why doesn't the proteins in the lysozome destroy the cell?

Lysozomes serve degradation

Creates building blocks for the cell - Nucleotides, amino acids

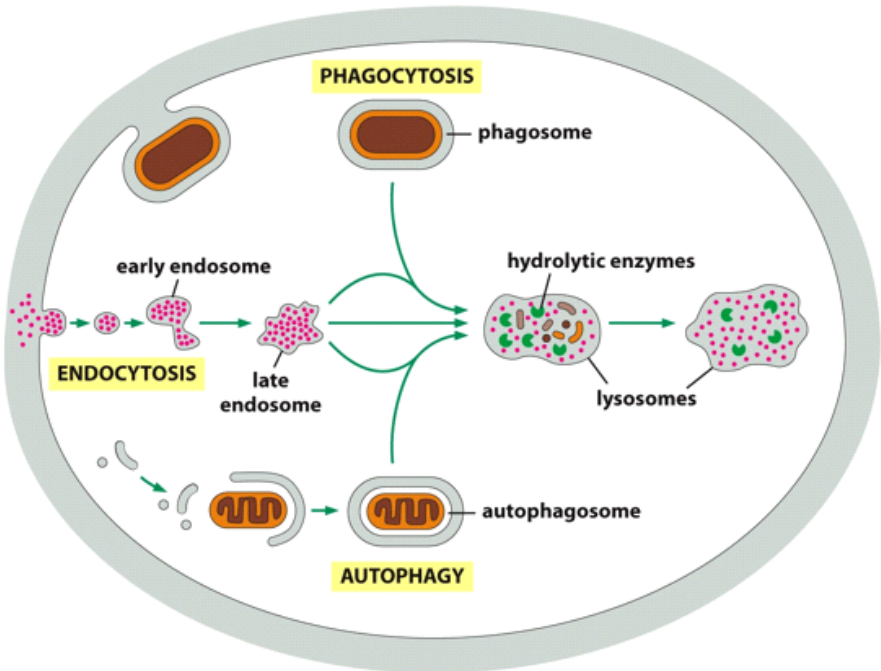
Enzymes - Active in pH 5.0 (hydrogen pumps)

- Nuclease
- Protease
- Glycosidase
- Lipase
- Phosphatase
- Sulfatase
- Phospholipase

These enzymes are *inactivated* in the cytosol - This is why the cell does not destroy itself from lysozome protein activity

Describe the difference between phagocytosis, endocytosis, pynocitosis and autophagy.

Different inputs

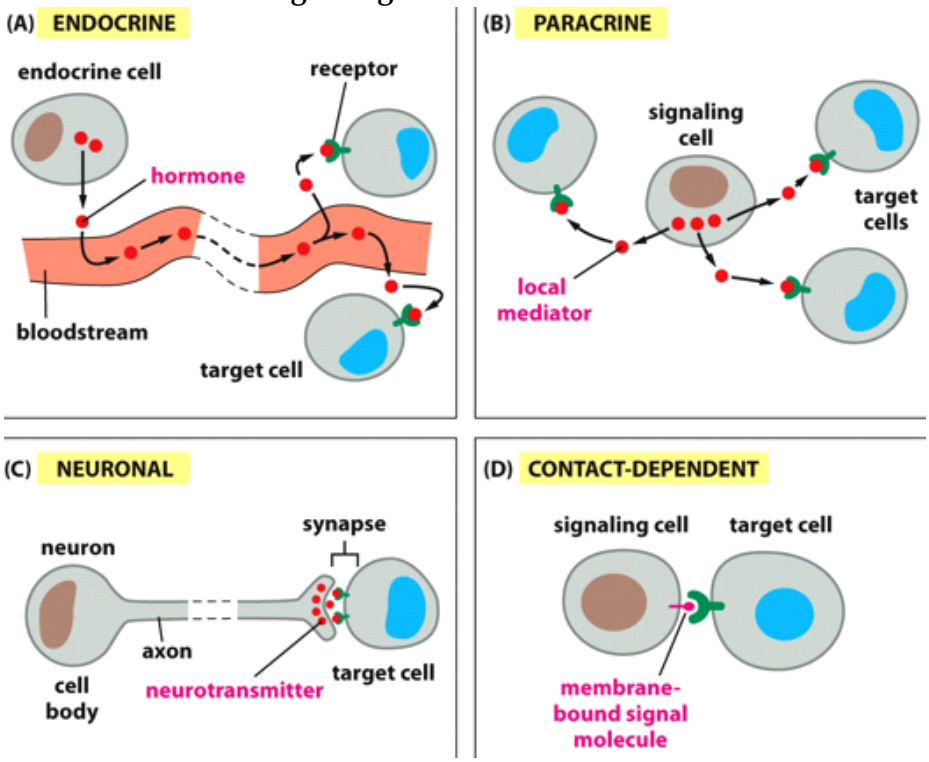


Phagocytosis - Solid structures
Pynocytosis - Liquid structures
Endocytosis - Absorbtion of extracelullar material from the cell
Autophagy - Breakdown of cells own organelles

6b. Cell communication (ECB chap 16 + Purves chap 7)

Describe four different types of cell signaling.

Different modes of signaling

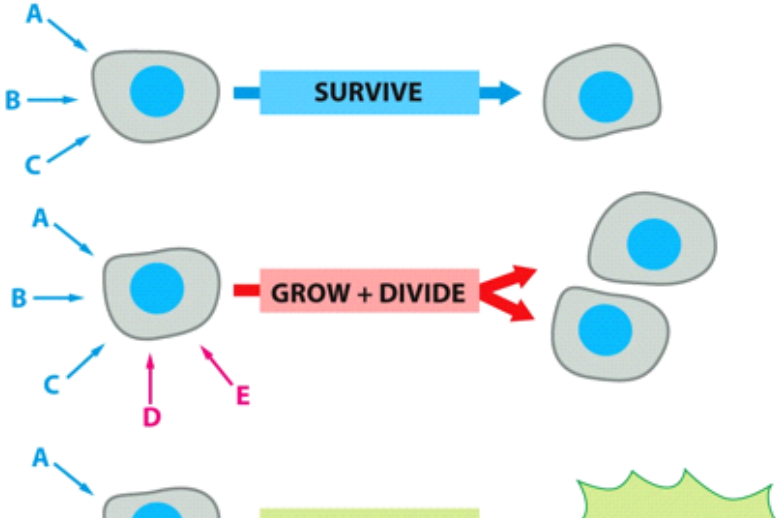


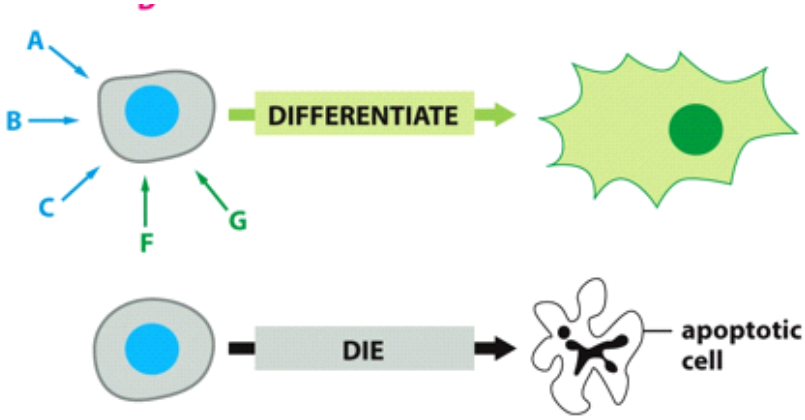
Endocrine - Target and release cells are far apart (transport via blood stream)
Paracrine - Target and release cells are close (transport in the extracellular medium)
Neuronal - Specific type of paracrine (cells are essentially adjacent)
Contact dependant - Via transmembrane proteins are receptors (molecule is not released)

Why does the lack of signal triggers apoptosis?

Signaling pathways integrate information into a unique output

May induce differentiation, grow, survive



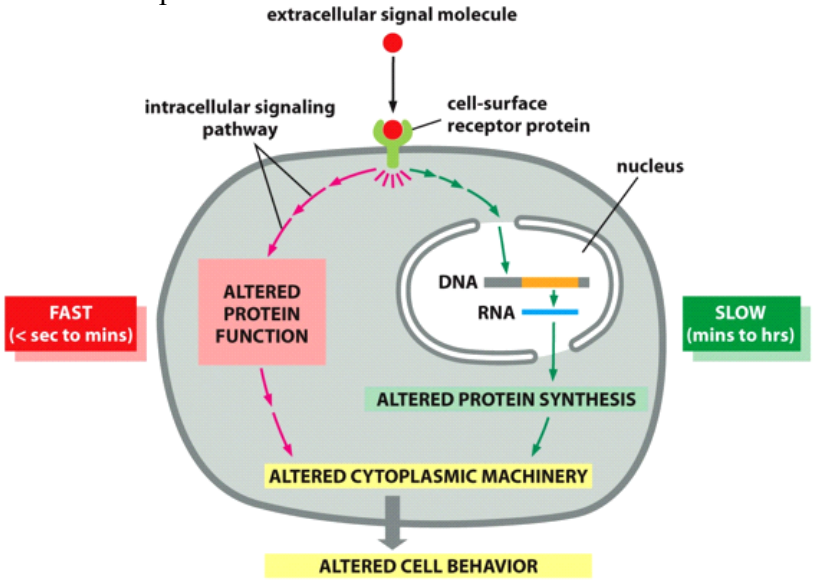


No signal = triggers apoptosis

Which signaling process takes longer - modifying protein function or gene function? Why?

Signaling occurs at different time scales

Direct altered protein functions = seconds to minutes



Gene expression modification = hours

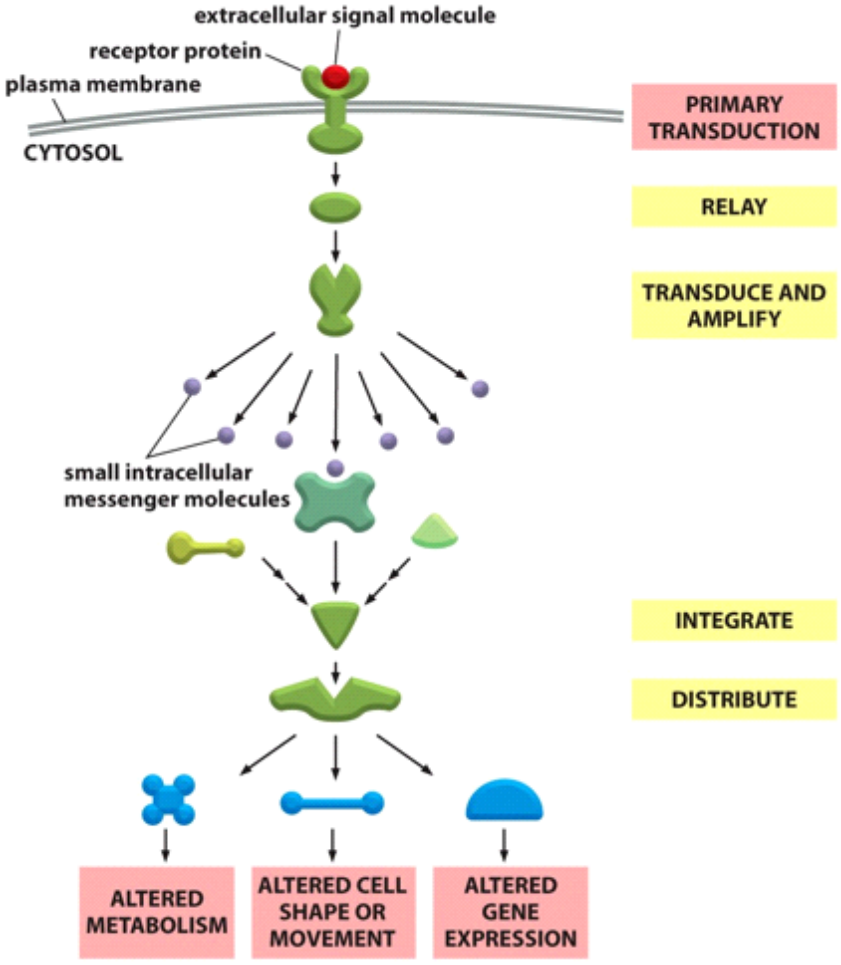
What is the overall structure of a signaling cascade. What is the main advantage of having many steps in the signaling?

Signaling cascade

Signaling cell
Signal
Receptor
Target molecule
Response

In neurons, signaling cell is presynaptic, rest is postsynaptic

This structure allows convergence and divergence - The signal is amplified to be perceived and integrated to trigger a specific signal

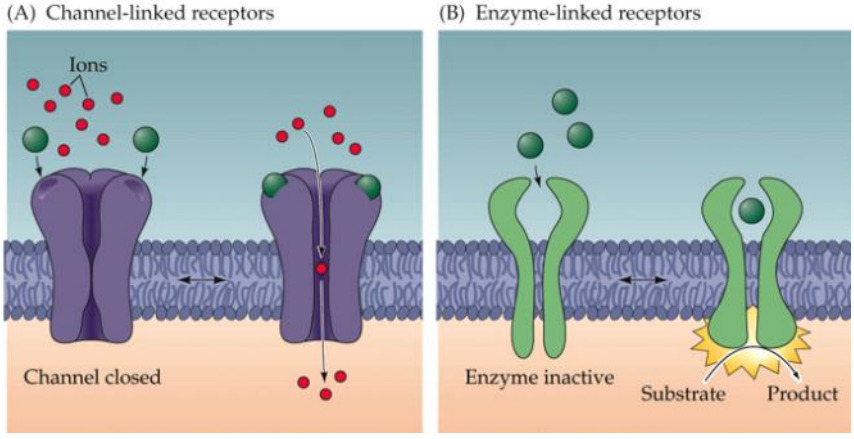


Describe the four different types of signaling receptors and how they differ at a molecular level.

Different receptors

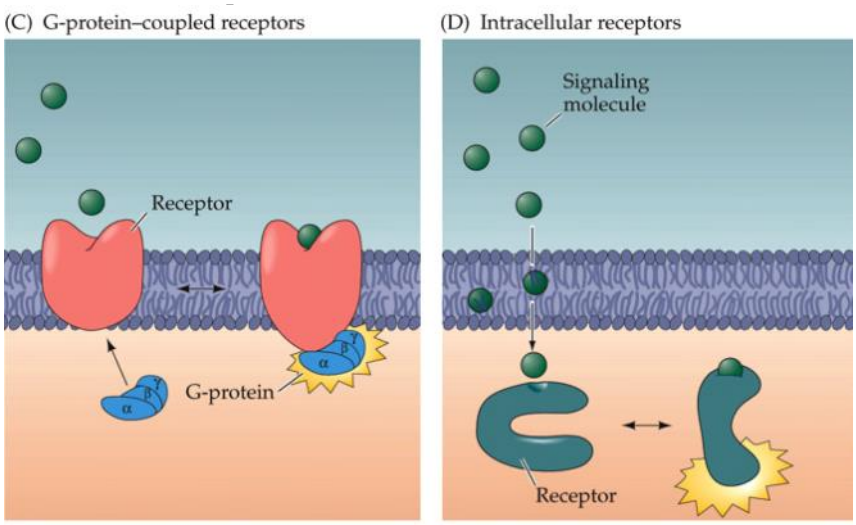
Channel-linked receptors - Link between inside and outside

Enzyme-linked receptors - Binding promote a change in the intracellular conformation of receptor (no link between inside and outside)



G-protein-coupled receptors

Intracellular receptors



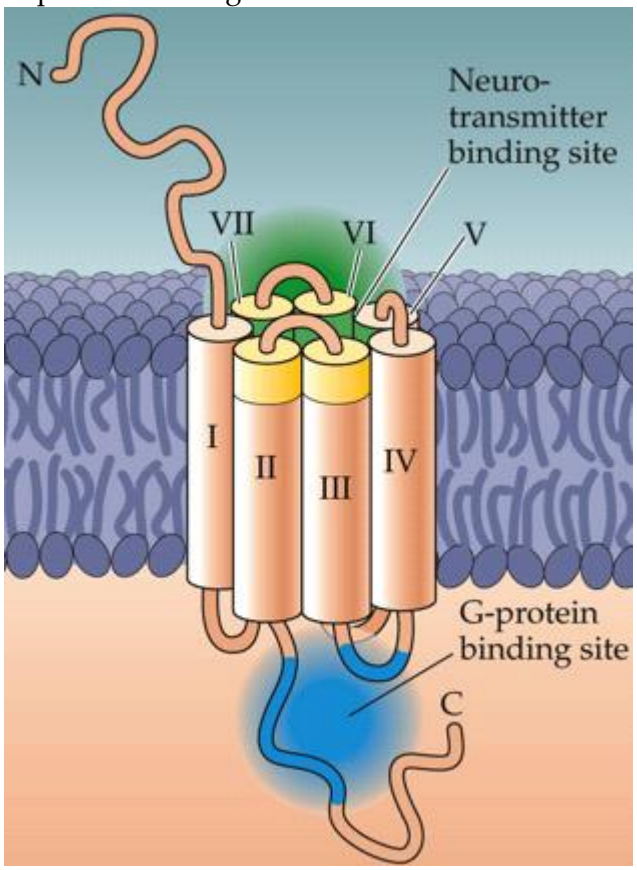
Different drugs act on different regions of receptors (allosteric modulation)

Describe the structure of a GPCR. Why is it a receptor, not a channel?

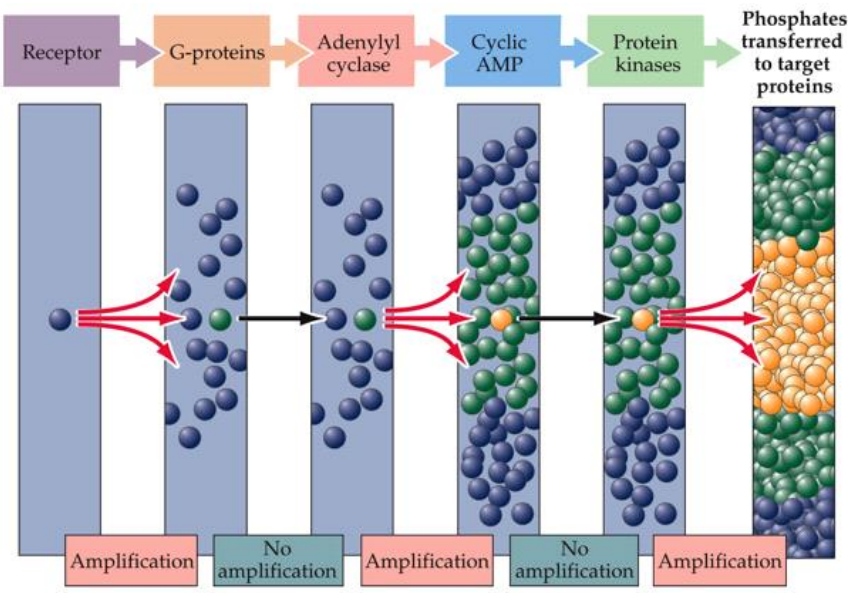
GPCR - Seven transmembranes

Do not form a pore, but change conformation when a ligand is bound

G-protein binding site is revealed



Amplification of signal occurs in many points in the GPCR signaling



Which sense is involved in almost half of all GPCR subtypes?

Types of GPCR

Receptor class	Glutamate	$GABA_B$	Dopamine	NE, Epi	Histamine	Serotonin	Purines	Muscarinic
Receptor subtype	Class I	$GABA_B$ R1	$D1_A$	α1	H1	5-HT 1	A type	M1
	mGlu R1	$GABA_B$ R2	$D1_B$	α2	H2	5-HT 2	A1	M2
	mGlu R5		D2	β1	H3	5-HT 3	A2a	M3
	Class II		D3	β2		5-HT 4	A2b	M4
	mGlu R2		D4	β3		5-HT 5	A3	M5
	mGlu R3					5-HT 6	P type	
	Class III					5-HT 7	P2x	
	mGlu R4						P2y	
	mGlu R6						P2z	
	mGlu R7						P2t	
	mGlu R8						P2u	

Half of GPCR (150 out of 300) - Olfactory

Describe the full cycle of GPCR signaling.

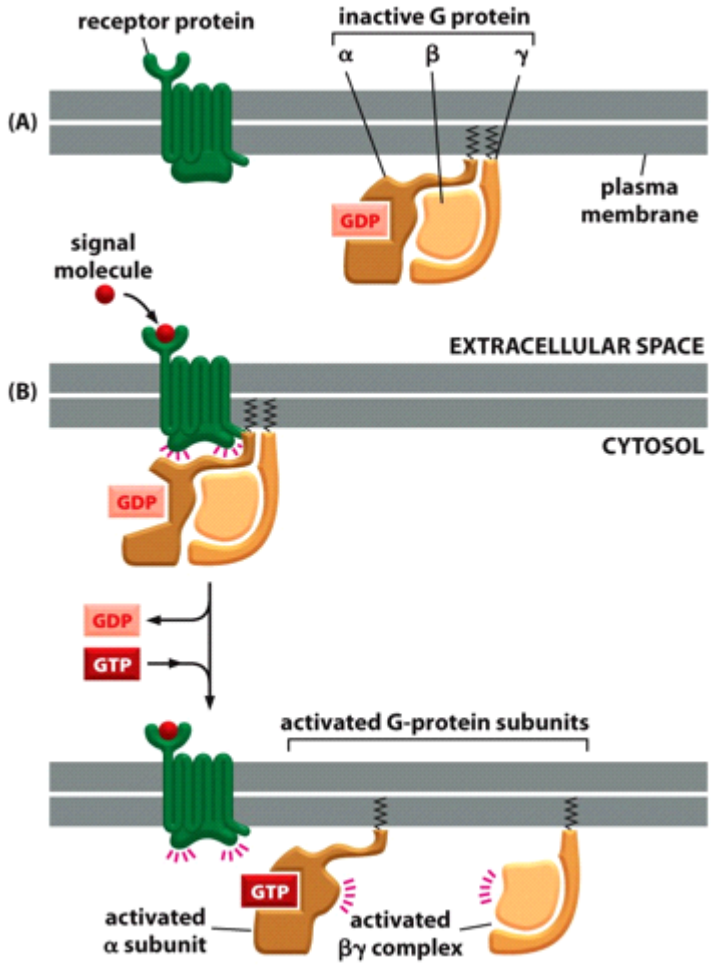
Steps in GPCR signaling

Signaling molecule - change protein conformation

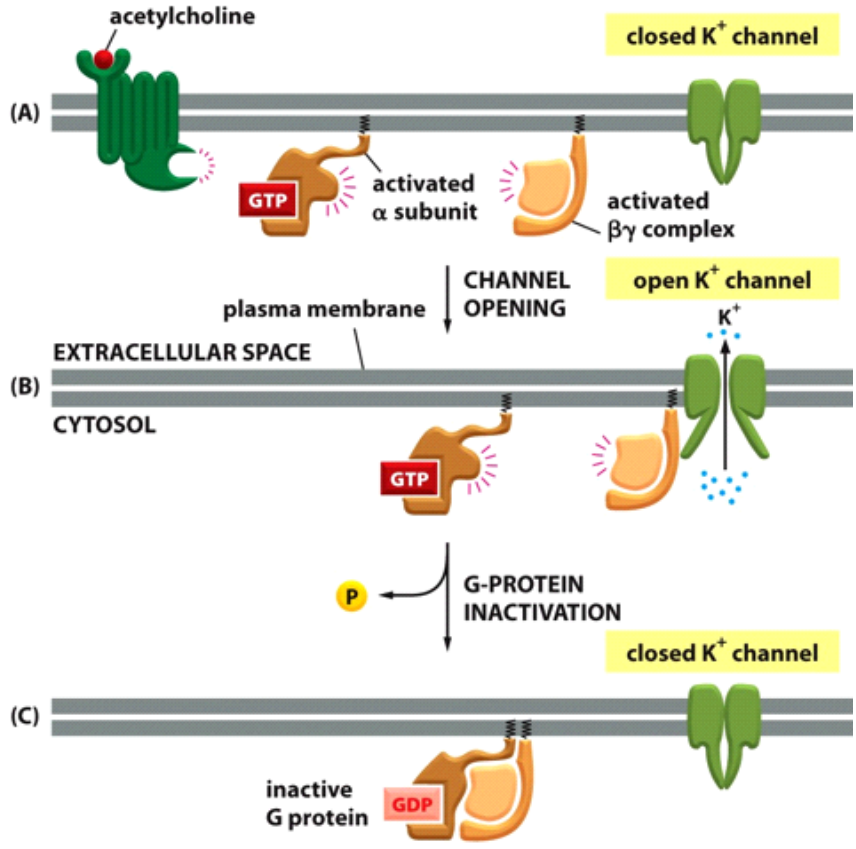
G-protein interact with the membrane - Increases likelihood of binding with the receptor

Alpha subunit of G-protein binds to GPCR - Releases GDP

Release an activated GTP-alpha and beta-gamma complex



Activated beta-gamma activates another channel
Activated GTP-alpha subunit is autophosphorylated after some time - GTP becomes GDP and unbinds from alpha subunit
Alpha subunit binds again to beta-gamma subunit



What are the three different GPCR effector pathways? What is their effect on the cell?

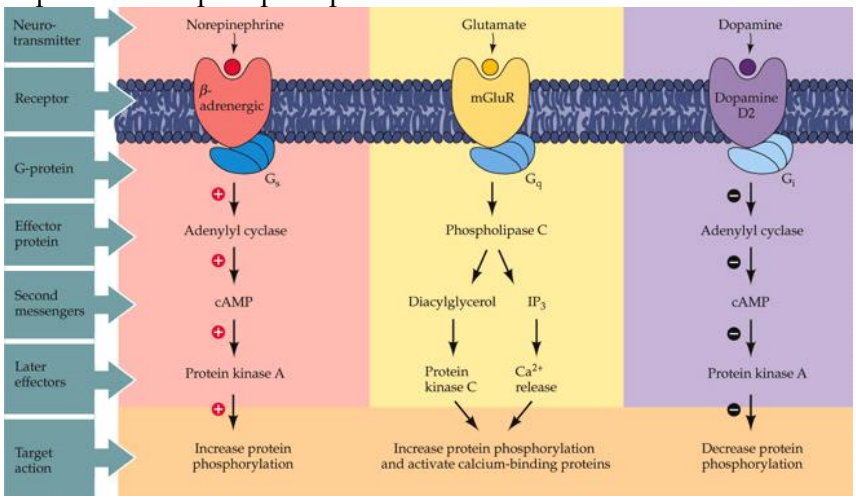
Effector pathways associated with GPCRs

Gs (stimulating) - Activates Adenylyl cyclase

Gi (inhibitor) - Inhibits adenyly cyclase

They usually act together in neurons

Gq - Activates phospholipase C

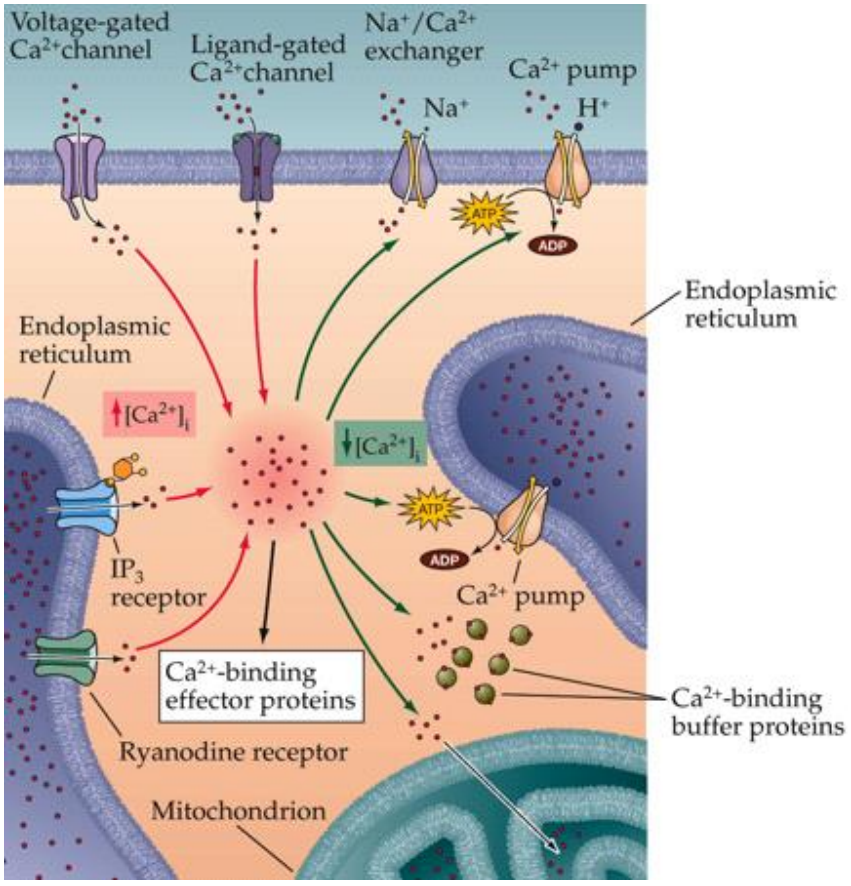


Describe how Ca+2 levels are regulated in the the cell (both from membrane channels and

Neuronal second messenger

Calcium - Has a double charge (2+), can bind to two proteins at the same time or at two regions of the same protein (changing its conformation)

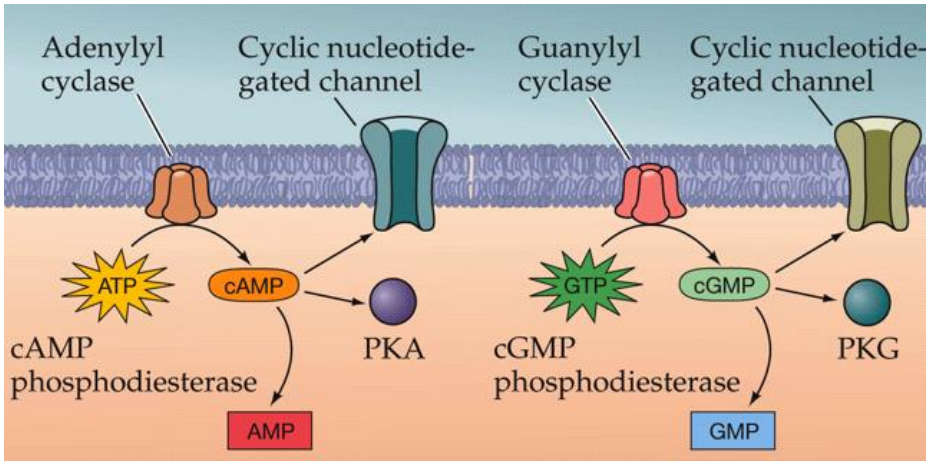
intracellular channels).



- Calcium is actively pumped into organelles (Endoplasmatic reticulum and mitochondrion)
- Calcium is actively pumped out of the cell (pumps and Na/Ca exchangers)
- Ligand-gated Ca channels let calcium passively come into the cell (NMDA)
- Voltage-gated Ca channels let calcium passively com into the cell
- IP3 receptor - Ligand-gated Ca channel in the ER

What is the role of phosphodiasterases in the cell?

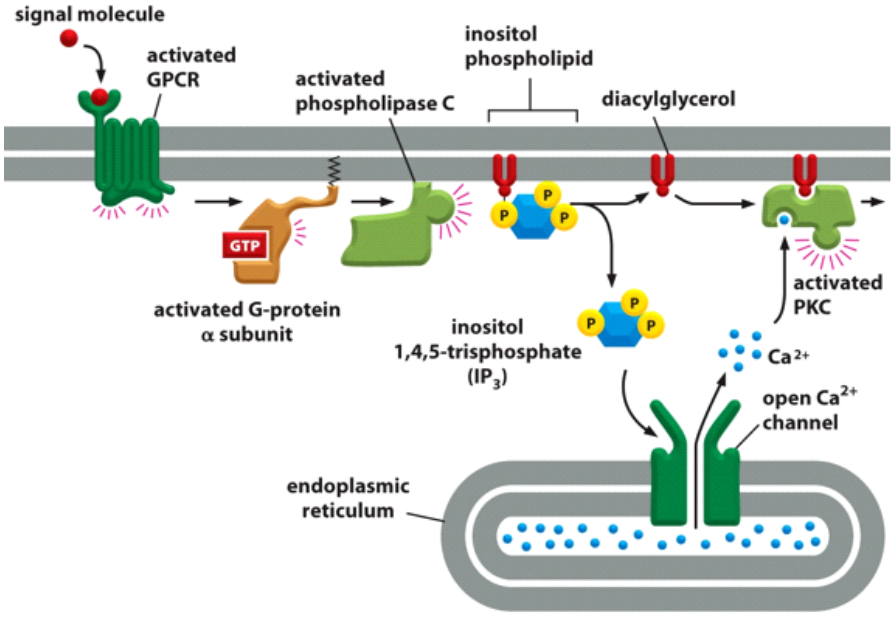
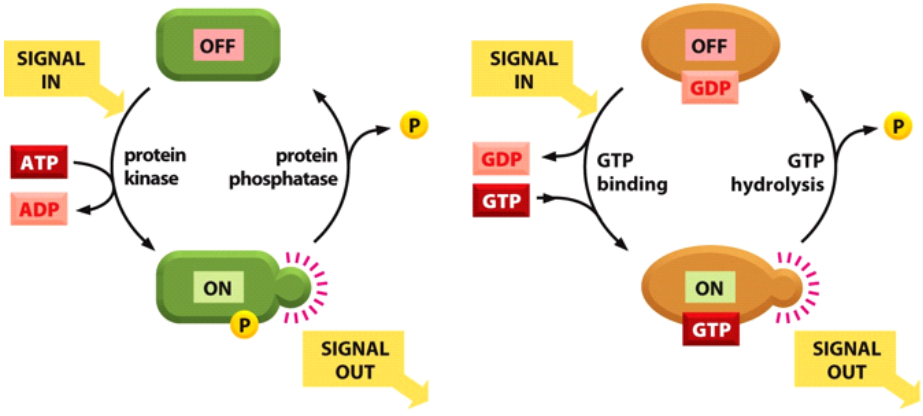
Formation of cAMP or cGMP

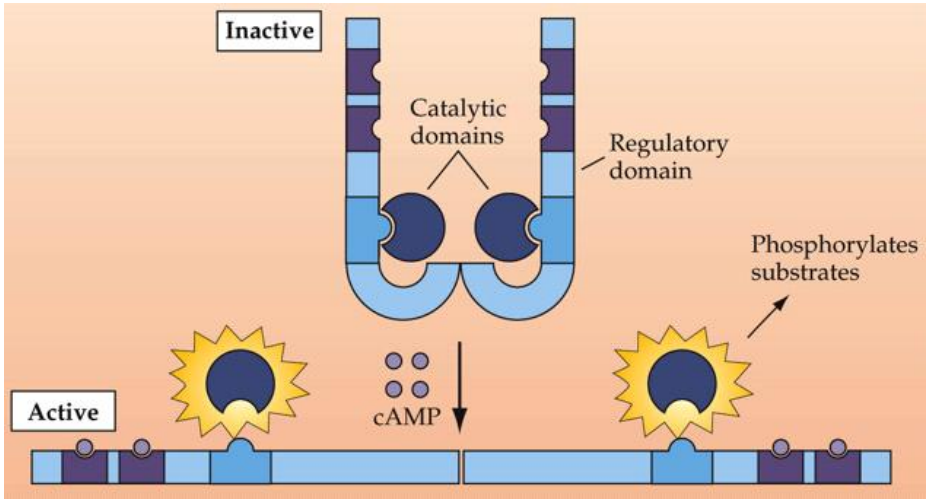
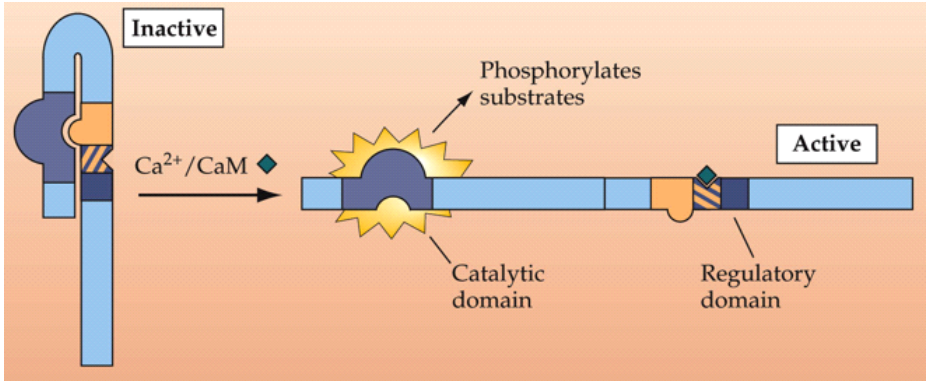
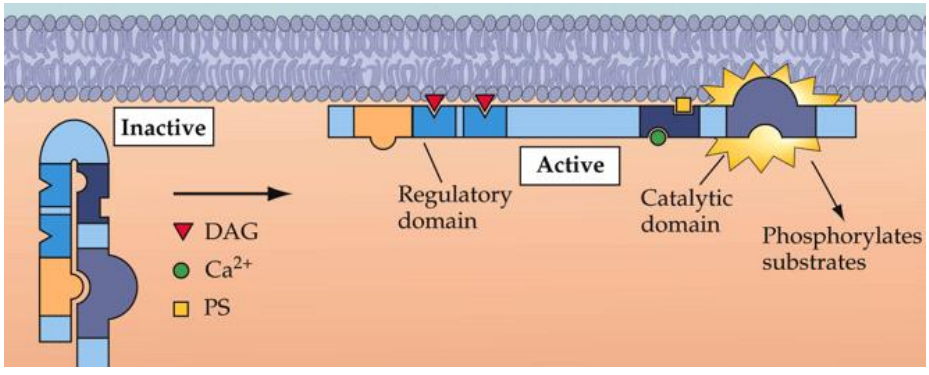


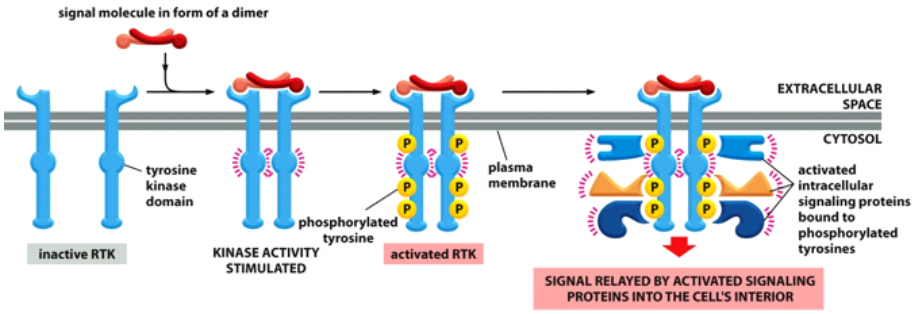
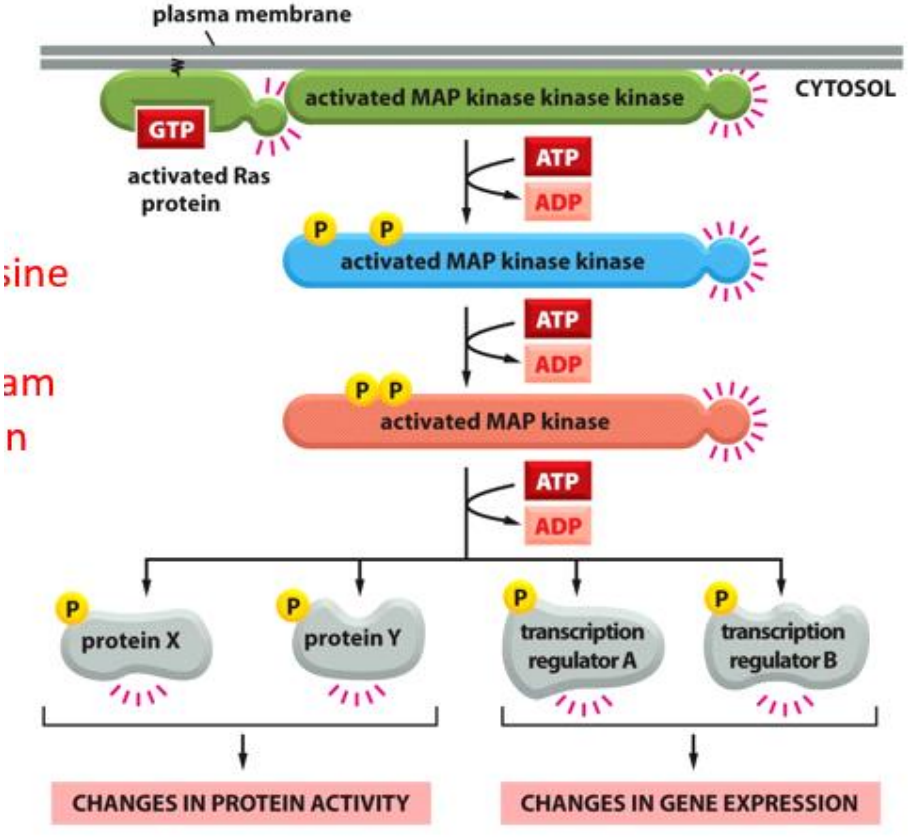
Adenylyl cyclase - breaks down ATP into cAMP, which can activate PKA

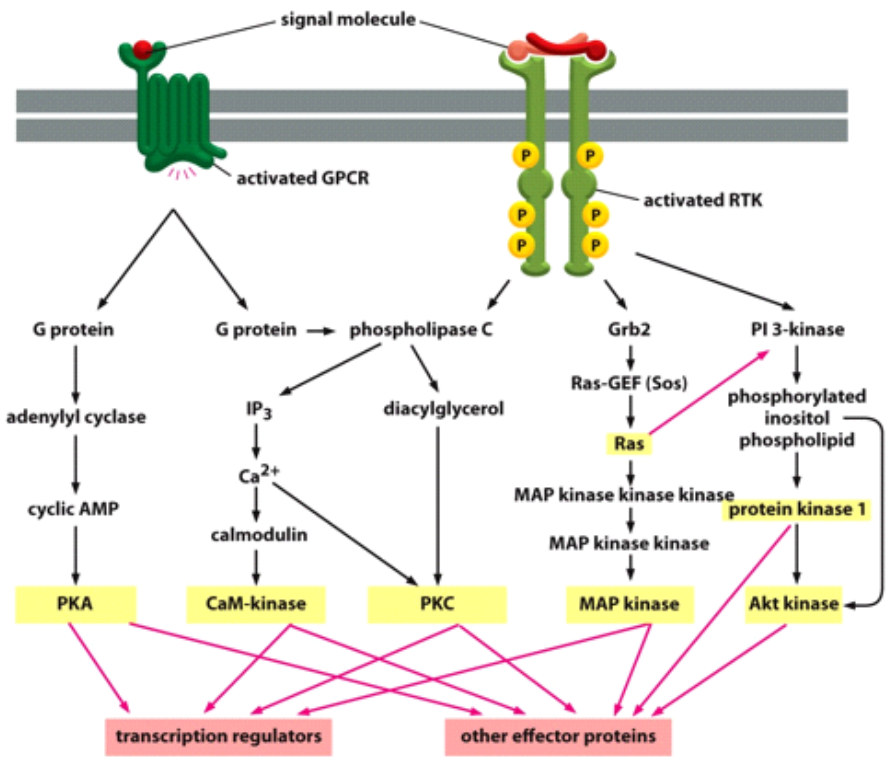
cAMP phosphodiesterase are readily available in the cytosol - Maintains cAMP concentration low

Guanylyl cyclase = breaks down GTP into cGMP, which can

	activate PKG cGMP phosphodiesterase - Maintains cGMP concentration low
How is phospholipase C activated upon the activation of a Gq GPCR?	**Activation of phospholipase C**  Alpha-GTP activates phospholipase C - Breaks down Inositol phospholip into inositol triphosphate (IP3, a sugar group) and diacylglycerol IP3 activates calcium channels in ER Calcium activates PKC
How ATP and GTP differ in their signaling roles in the cell?	**Phosphorylation is an important signal**  Phosphate group - Comes from ATP -> ADP breakdown Protein-protein interactions are facilitated by binding of phosphate group GTP is not broken down into GDP - The GDP group leaves the molecule, the GTP group attaches
Describe how protein	**Mechanism of protein kinase A activation**

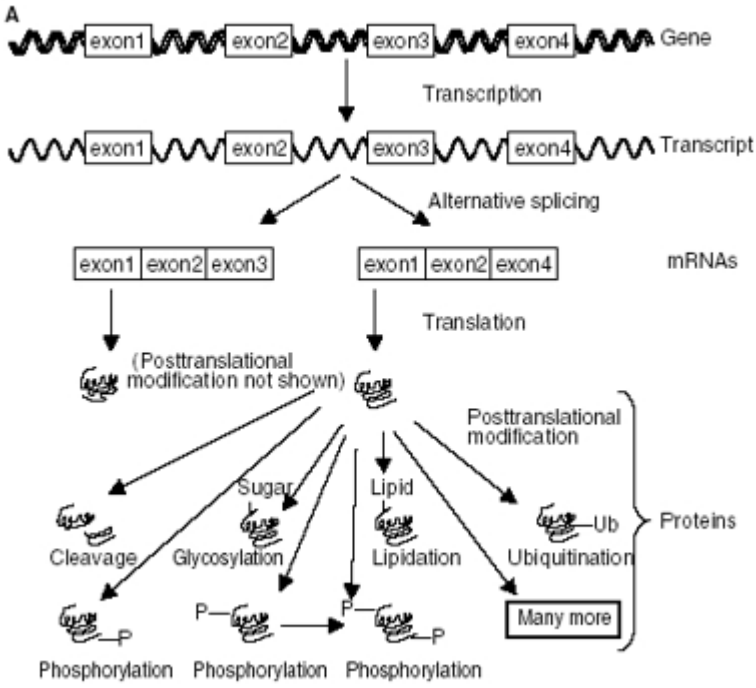
kinase A is activated.	 PKA inactive - Has two regulatory domains bound to catalytic domains PKA active - Bound to cAMP, the regulatory domain changes conformation, the catalytic domains is free to act in the cell Example of action - Regulate transcription factors(5% of transcription factors are activated by PKA) Involved in learning (activation of 'learning' genes in neurons)
Describe the process of CAM-kinase activation.	**Mechanism of CAM-kinase activation**  Inactive state - Catalytic domain is silenced by regulatory domain - Same protein Calmodulin, when activated by calcium, binds to regulatory domain - Changes protein conformation
Describe the process of PKC activation.	**Mechanism of PKC activation**  Combination of DAG (diacylglycerol), calcium and

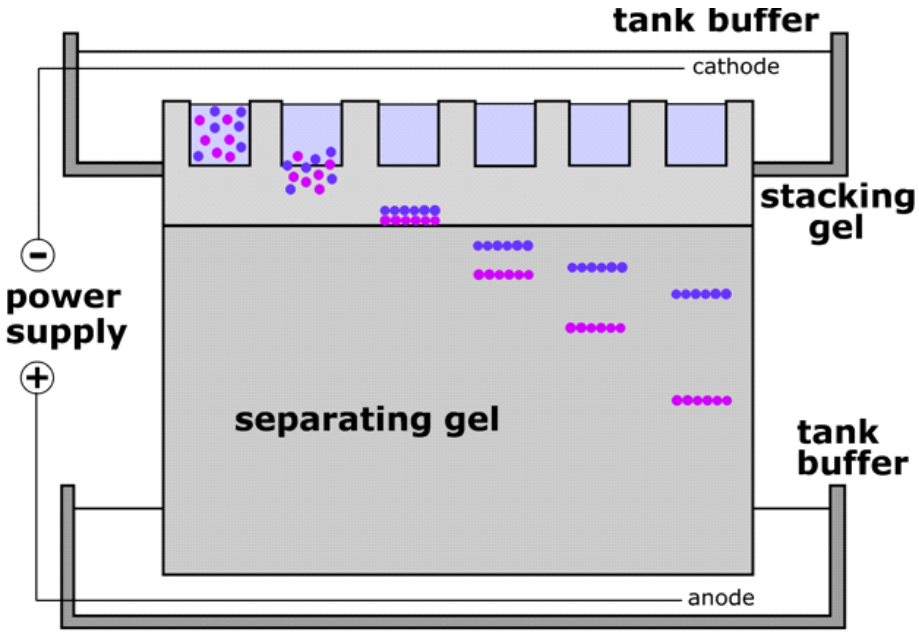
	PS(pseudosubstrate) change the conformation of the protein
Describe the process of RTK activation.	**Mechanism of tyrosine kinase receptor activation**  Transmembrane proteins - Separated when inactive Signal molecule (e.g. growth factors) - Couple protein, activate catalytic domains -> phosphorylation at tyrosine amino acids Attracts proteins and promotes a protein-protein interaction Adaptor protein -> Ras-activating protein Ras protein - Active when GTP is bound
Describe the general structure of the Ras pathway.	**Ras pathway**  MAPKKK MAPKK MAPK Activation of proteins and transcription factors

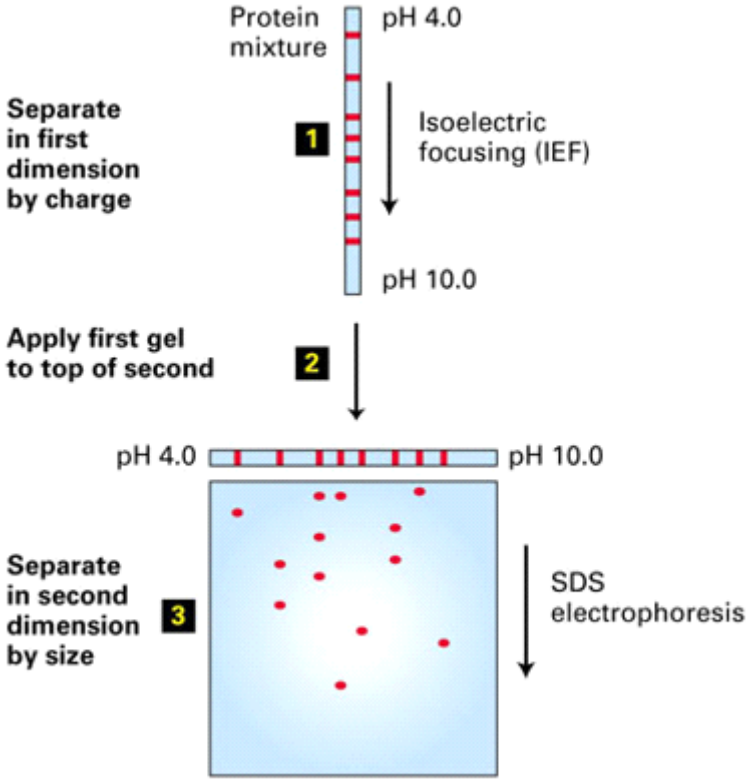
	Promotes high divergence of signaling Mutations in this pathway is associated with certain types of cancer
	Integration of information in intracellular signaling 

7. Proteomics - Methods to identify proteins and to understand their function

What is a proteome?	**What is a proteome?** Catalog of all proteins expressed throughout life and under all conditions Sub-proteomes - Of cells or organelles - Expressed at a given time and under specific conditions
What are the aims of proteomics and their practical applications?	**Aims of proteomics** Provide a catalog of all proteins present Quantitative data - How much protein is expressed To understand protein functions How proteins interact and what they interact with (interactome) **Practical applications** Variance in protein expression between healthy and disease cells Likely to reveal new drug targets - Clinical trials become more efficient
What is the main reason that proteomics is more difficult to study than transcriptomics?	**Transcriptomics vs Proteomics** Proteomics require 1000 - 3000 cells, transcriptomics require 1 cell Transcriptomics has amplification steps - PCR!
What are the challenges of proteomics?	**Challenges of proteomics** Transcript splice variants create many different proteins - 25000 genes create 200.000 proteins Protein expression varies with age, health, tissue and stimuli from the environment Proteomics requires a broader range of technologies than transcriptomics
Why is the proteome complex?	Complexity of proteome - Splicing, post-translational modifications, phosphorylation changes protein function

	 E.g. Calcitonin gene produces two different proteins through splicing; Insulin cleavage
What are the three most important types of protein chemical modifications?	**Types of chemical modifications** Phosphorylation - Activation Glycosylation - Cell-cell recognition, signaling Ubiquitination - Destruction signal
How can proteins be separated from one another?	**Separation of proteins** Proteins have different physical properties - Size, charge (average of basic and acidic amino acids) and hydrophobicity
Describe how a one-dimensional SDS-PAGE works.	**Polyacrylamide Gel Electrophoresis (PAGE)** Electrode - Proteins travel to the plus end (anode) of the electrode Porous gel - Small proteins travel faster than larger proteins Gravity plays almost no role - Proteins move according to thermodynamic forces Process: - Denaturation - Unfolding is necessary because some proteins are more folded than others - Sodium Dodecyl Sulfate (SDS) - Soap, binds and confers negative charge to proteins (charge becomes proportional to mass) - You always run the gel with a known reference

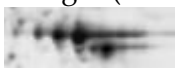
	 Staining is proportional to the amount of protein quantity
What is the difference between PAGE and protein electrophoresis?	**Protein electrophoresis (non-denaturing conditions)** No pretreatment of proteins before electrophoresis Proteins retain their shape and charge - Separation is dependant on charge, size and shape of natural proteins
Describe how a two dimensional SDS-PAGE works.	**Two-dimensional PAGE** Separates proteins from charge (number of protons changes according to pH) and mass Process: - Separate in first dimension by charge (pH difference) - Apply first gel on top of second (soaked in SDS) - Separate in second dimension by size (normal SDS electrophoresis)



Isoeletric focusing (IEF) - Proteins sit at the point of the gel in which the sum of its charge is zero

The more positive amino acids, the more negative in the gel it will go

Phosphorylation state - Proteins of same size but different charges (more phosphorylation, more to the right)



Left - Acidic
Right - Basic

Quantification by densitometry - Spot intensity (measured by pixel intensity)

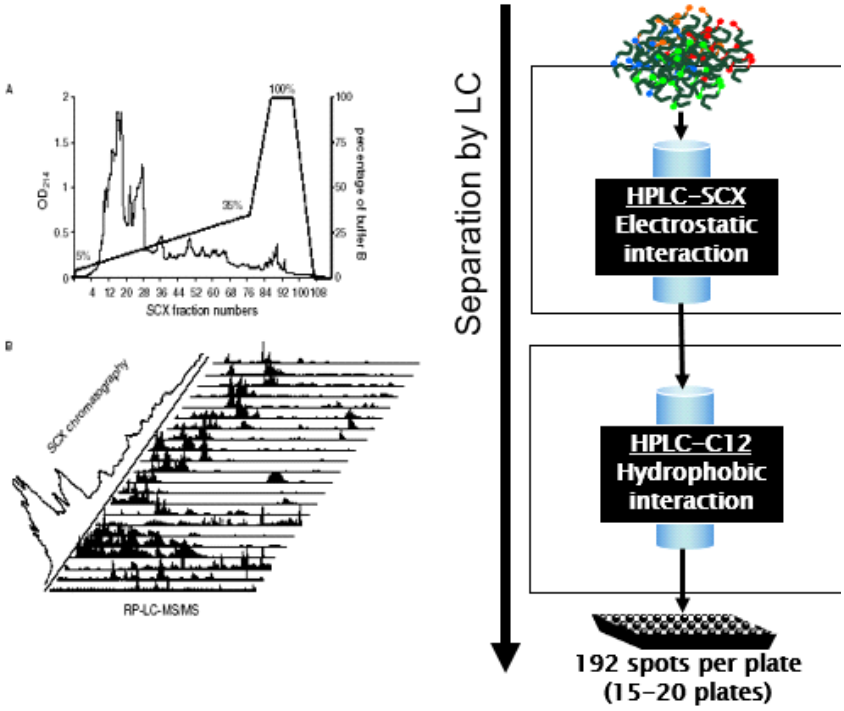
Most proteins do not change expression - House keeping gene

Problems with 2D gels - Poor performance to large proteins, very small proteins, less abundant proteins, membrane-bound proteins (stay at a lipid environment - they bind with each other in an aqueous environment)

Describe how 2D liquid chromatography works.

2D Liquid Chromotography

First phase - Charge
Second phase - Hydrophobicity



Matrix material in column - Proteins stick to the column at different rates, fluid change the environment
Fraction numbers - Different tubes that collect different peaks
Each tube is then separated by hydrophobicity

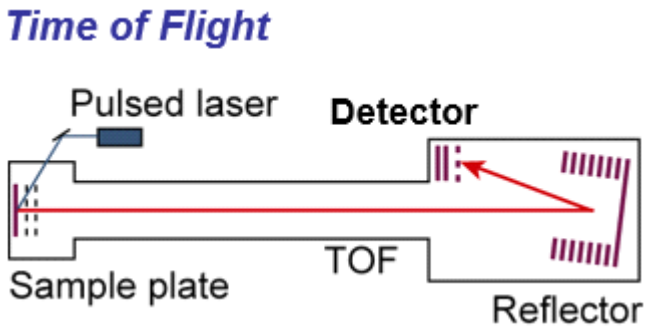
Describe how a mass spectrometer works.

Detection and Identification of Proteins

Mass spectrometry - Nobel Prize in Chemistry in 2002
- Measures mass-to-charge ration

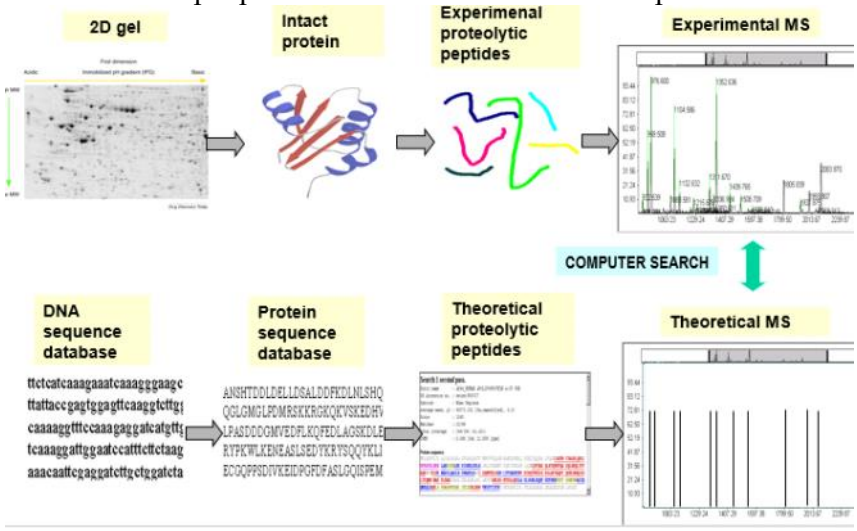
Components - Ion source, mass analyser, ion detector

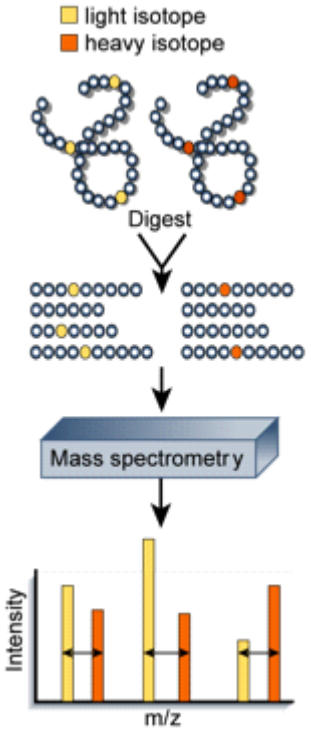
Time of flight (TOF)



- Time for accelerated ion to reach detector indicates mass-to-charge ratio (the larger the protein, the longer it takes to reach the detector)
- Proteins are ionized - They only get one electron (native protein charges does not matter in vacuum)
- Vacuum - Prevents colisions of proteins with air molecules
- At a second step - A little nitrogen can be inserted into the tube of the mass spectrometer, so that the protein is broken into its peptides

Peaks of different intensities

	The peptides are not charged in the same way Mass spectrometer is not useful for quantification
How can you identify a protein if you know its mass?	**How to identify proteins from their mass?** Protein size is proportional to their amino acid sequence  You can predict the amino acid sequence from the DNA sequence - Theoretical proteolytic peptides K or R - Cleavage of tripsin
Describe how stable isotope protein labeling works. What is its main shortcoming?	How to solve the problem of quantification? Labeling methods - Enzymatic, metabolic, via chemical reaction Detect the relative abundance of labeled and non-labeled proteins measures in mass spectrum Mass spectrometer does not detect light, only mass! Create a system with small variation in mass Stable isotope protein labeling E.g. Carbon 12, 14 - Creates a double peak in the reading



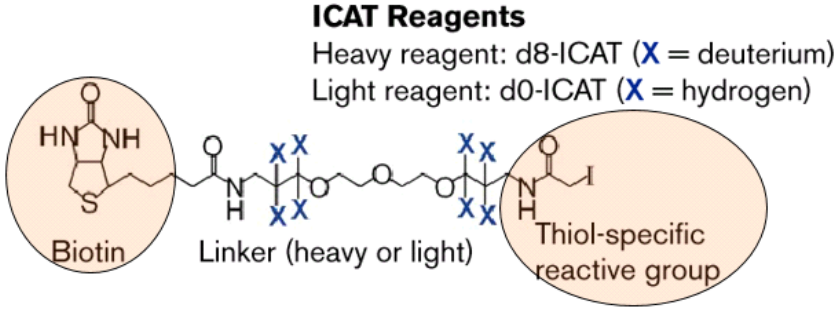
The peptide is identical, the only change is the weight of carbon -> The ionization will be the same -> Allows the quantification of the signal of the same peptide

This experiment is problematic because you need to feed cells/animals C14 for a long time

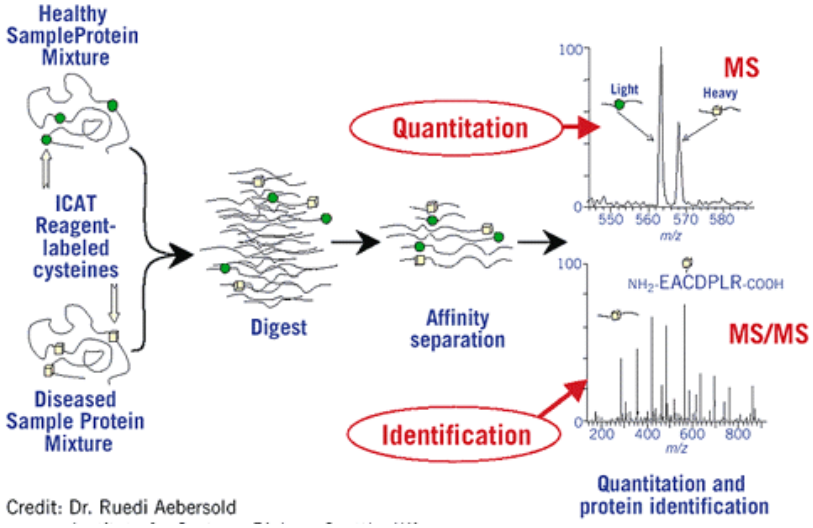
Describe how isotope coded affinity tag works.

Isotope Coded Affinity Tag (ICAT)

Label cysteine residues with light/heavy ICAT



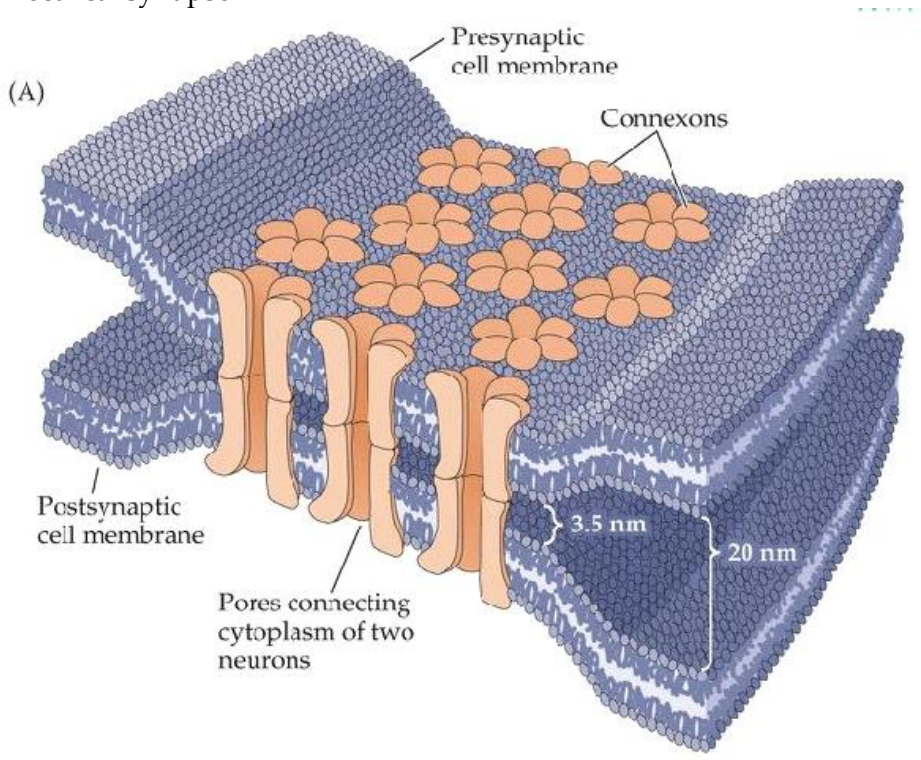
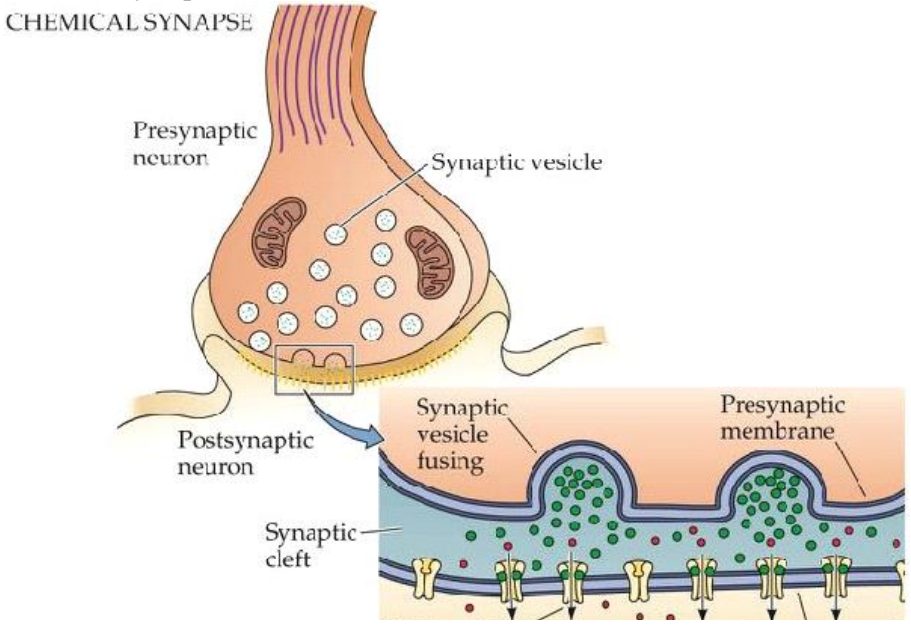
Biotin - Binds to avidin protein (used to separate which proteins have the ICAT construct)
Thiol-specific reactive group - Binds to cystein
Linking chain - Can be light or heavy

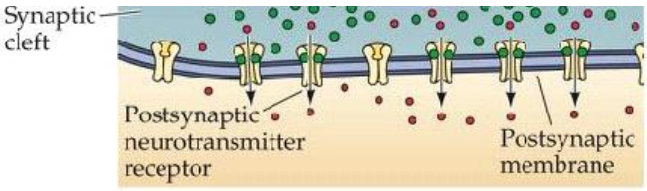


Digest proteins
Isolate ICAT containing peptides
Liquid chromatography separation
Mass spectrometry

Double peaks - Reagent with light heavy chain

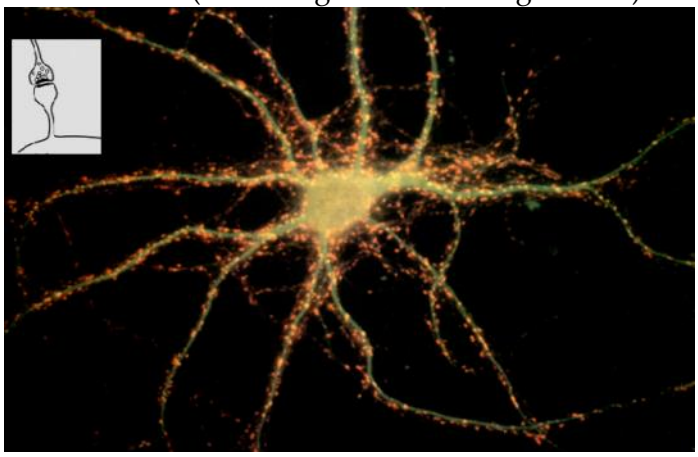
8. Synapses (chap 5 Purves)

Why are synapses important in the brain?	Synpases are involved in storing, retriving and processing information from the outside world
What are gap junctions? **Which protein is involved in gap junctions and how do they work?**	Electrical synapse  Gap junctions Connexons are channels, made by six connexins No modulation - A depolarization of presynaptic cells causes a depolarization of postsynaptic cells
What is the main difference between chemical and electrical synapses?	Chemical synapse 



Cells are not touching, they are separated by a space
Molecules are transmited from presynaptic (fusing vesicles), change conformation of recpetors, open sodium channels, new action potentials are made

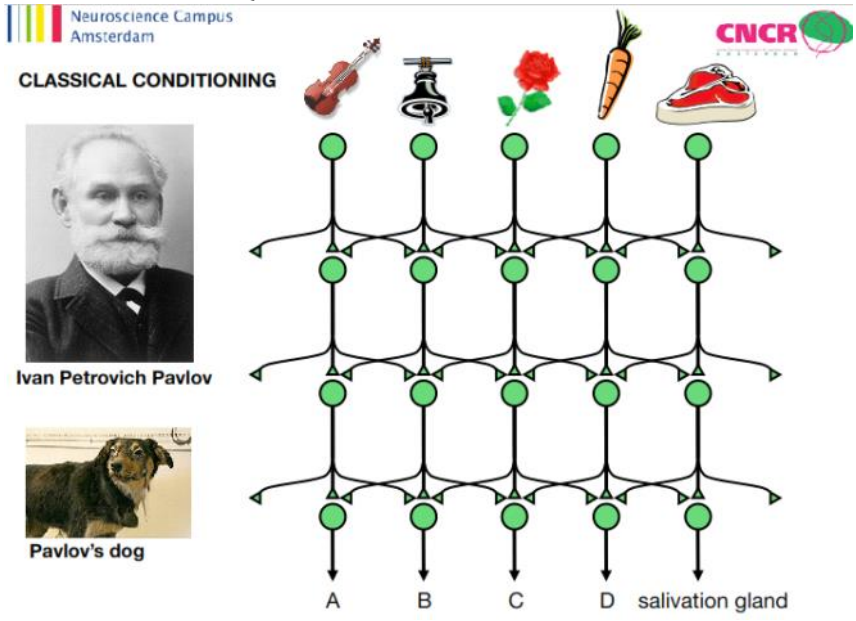
Activation of a neuron depends on thousands of synaptic transmissions (activating and deactiving the cell)



Describe how Pavlov's dog experiment can be described in a quantal system?

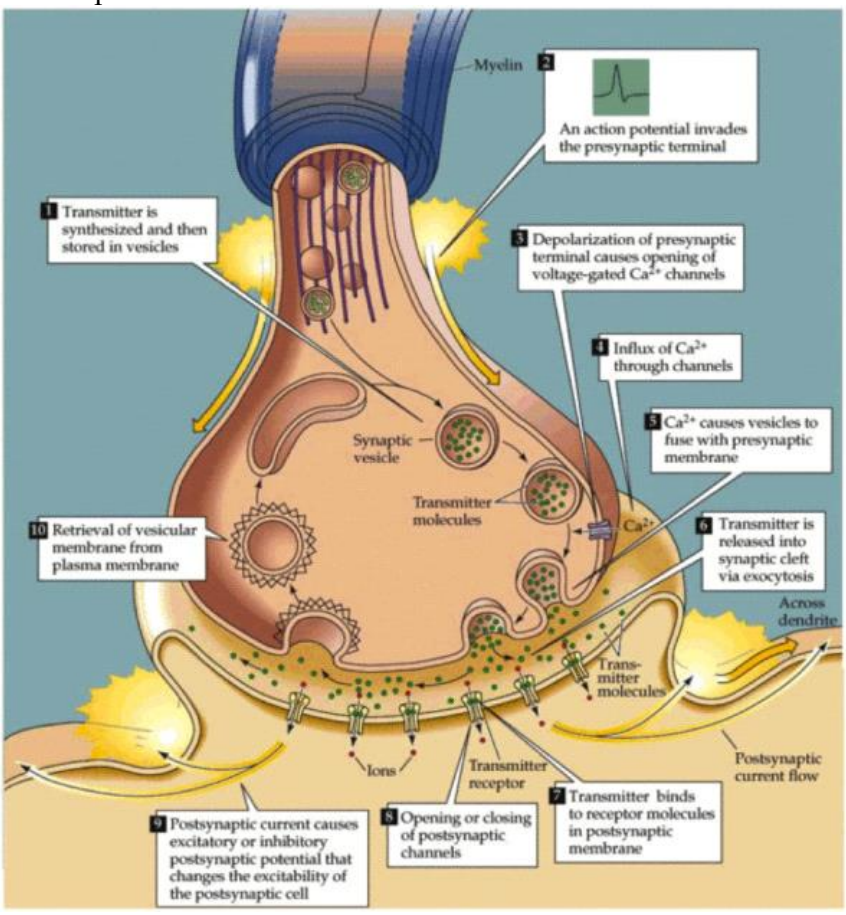
Synaptic computation

Turn on and off - Quantal difference

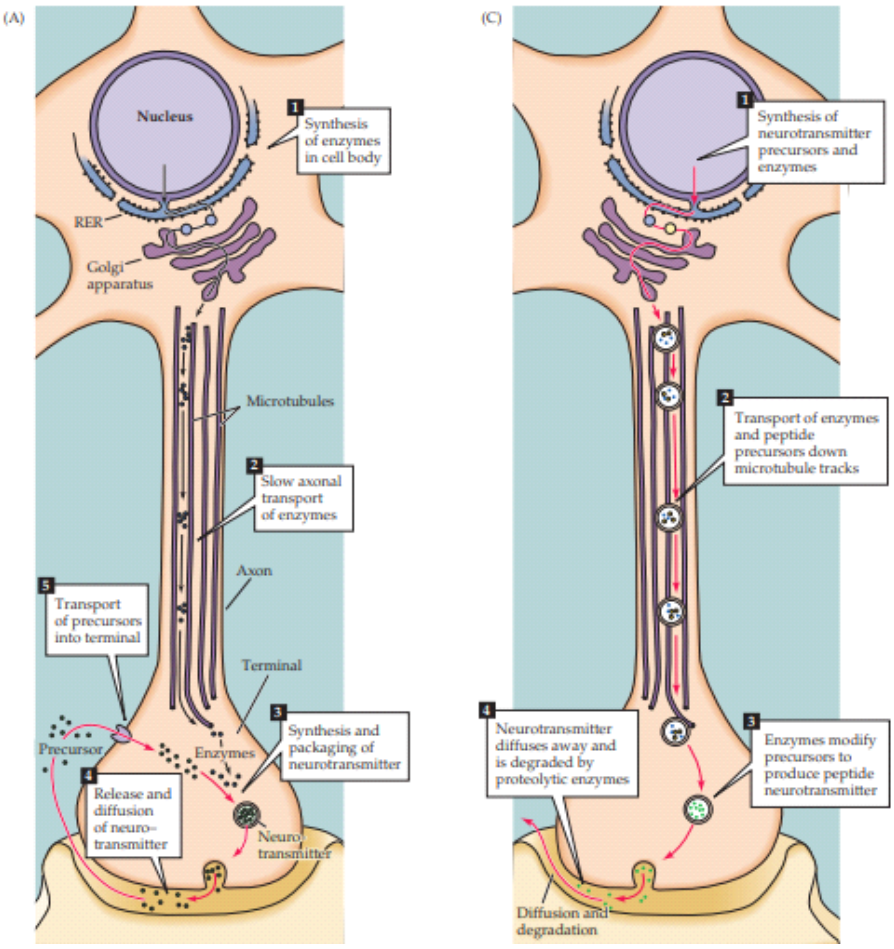


Example: Pavlov's dog - Classical conditioning

- Smells of food triggers salivation
- After conditioning, bell ring triggers salivation - Not in the genetic code
- Coincidence detection - Time is an important factor (a neuron needs to be active to associate memories)

	- Underlying memory strengthening (?) *Behaviour is overcoming inhibition*
Describe the ten steps of how chemical synapses work.	How do chemical synapses work? Neurotransmiter release Receptor binding Ion channels open or close Conductance change causes current flow (inhibition or excitation) Postsynaptic potential changes Postsynaptic cells excited or inhibitedd Action potential occurs or not  Memorize steps 1-10 for exam 4. Calcium is a universal signal for vesicle binding (influx of calcium occurs before cell depolarization) 10. Clathrin coats

What is the difference between small chemical transmitter and peptide transmitters in the process of transport?

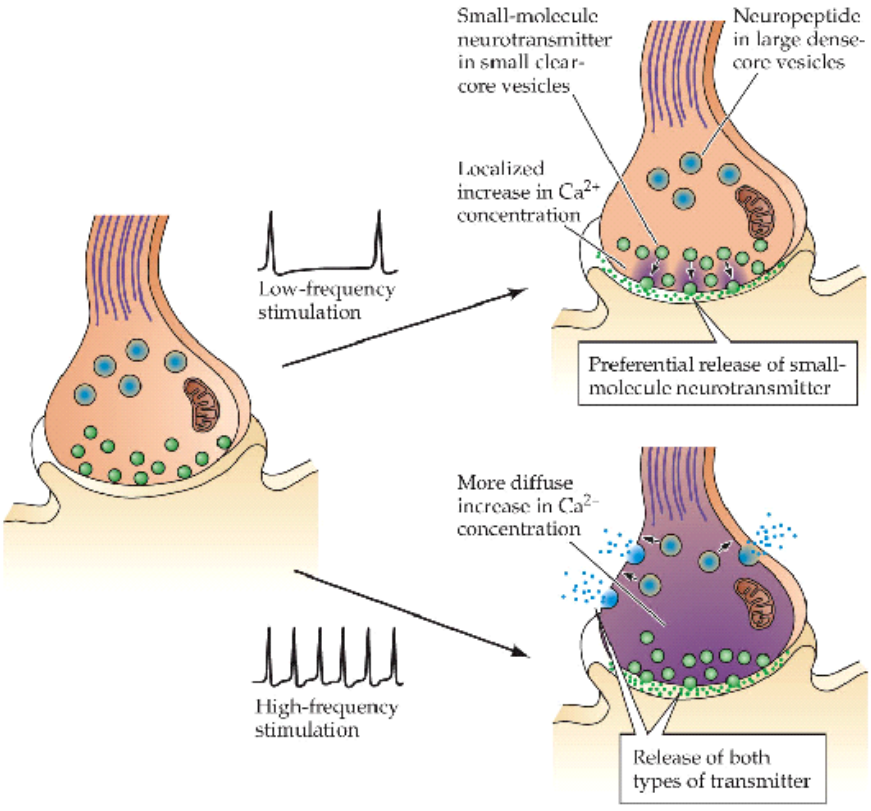


Small molecule transmitter - The axon is really far away from the soma; there is a local cycle of chemicals

Peptide transmitter - Does not have a local cycle, it is produced in the soma and transported to the axons

Dense core vesicles

What is the difference between small chemical transmitter and peptide transmitters in terms of calcium activation?



Low frequency stimulation -> Small molecule neurotransmitter release

- Increase in calcium remains localized

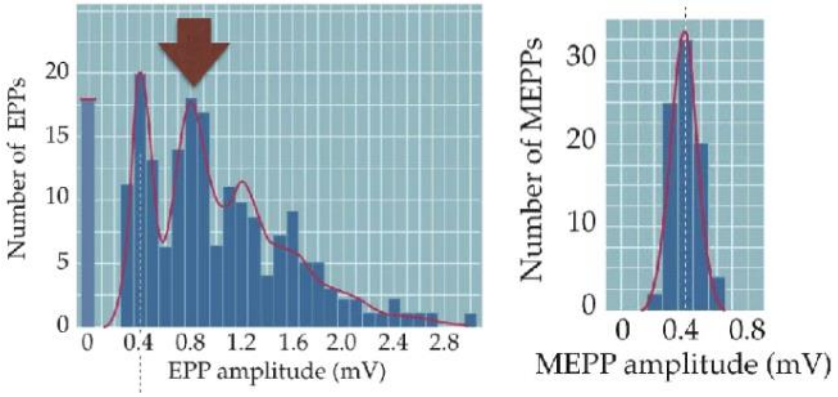
High frequency stimulation -> Small molecule and peptide neurotransmitter release

- The entire terminal fills with calcium
- This is the basis for LTP and LTD - The more often used, the more calcium a synapse will have

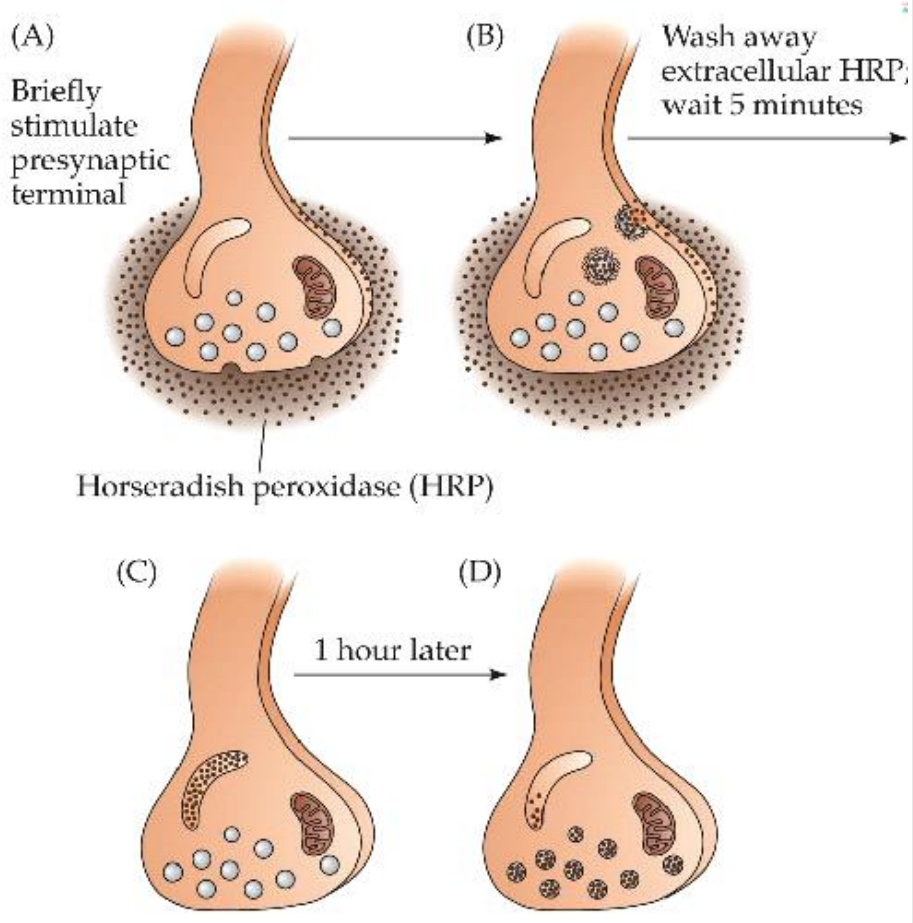
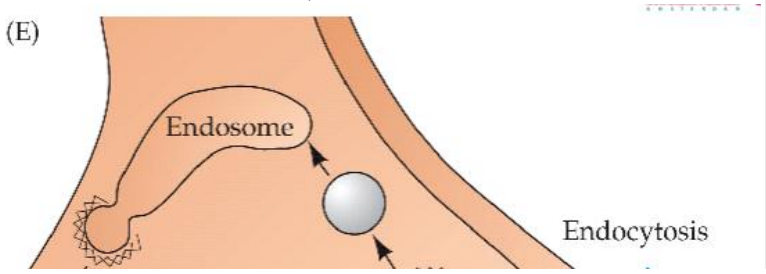
How was a vesicle transmition hypothesized even before the invention of microscopy?

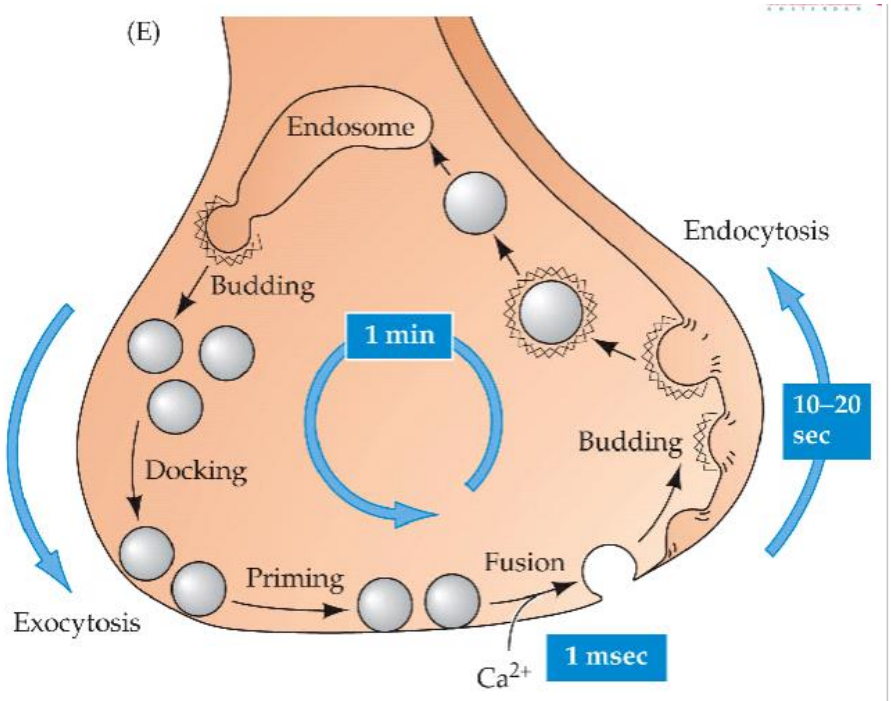
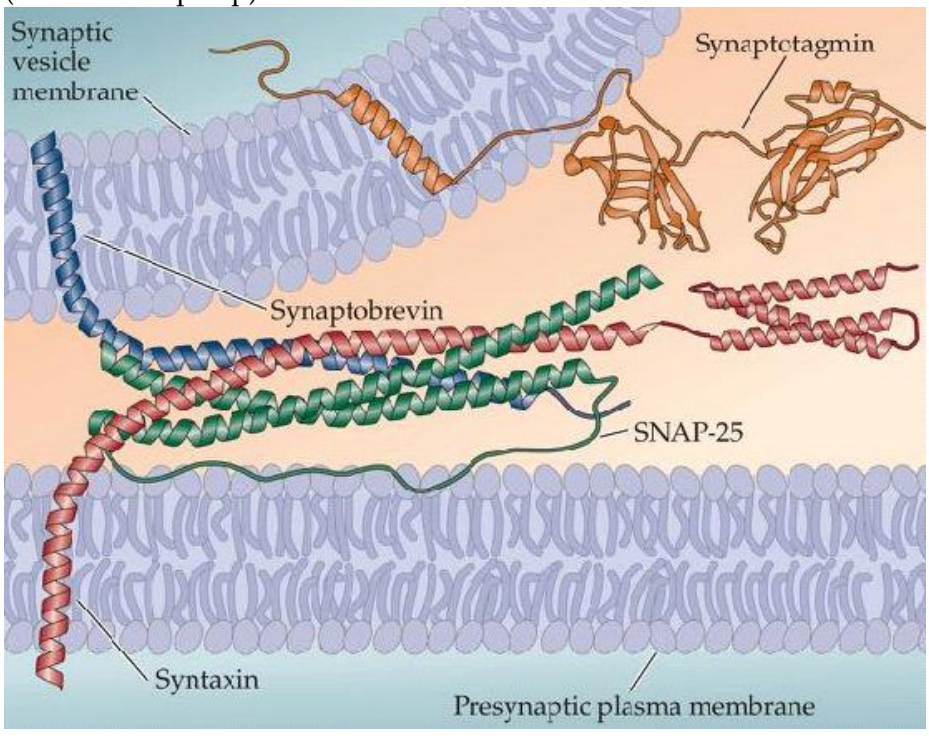
Electrode at a receiving cell (muscle fiber)

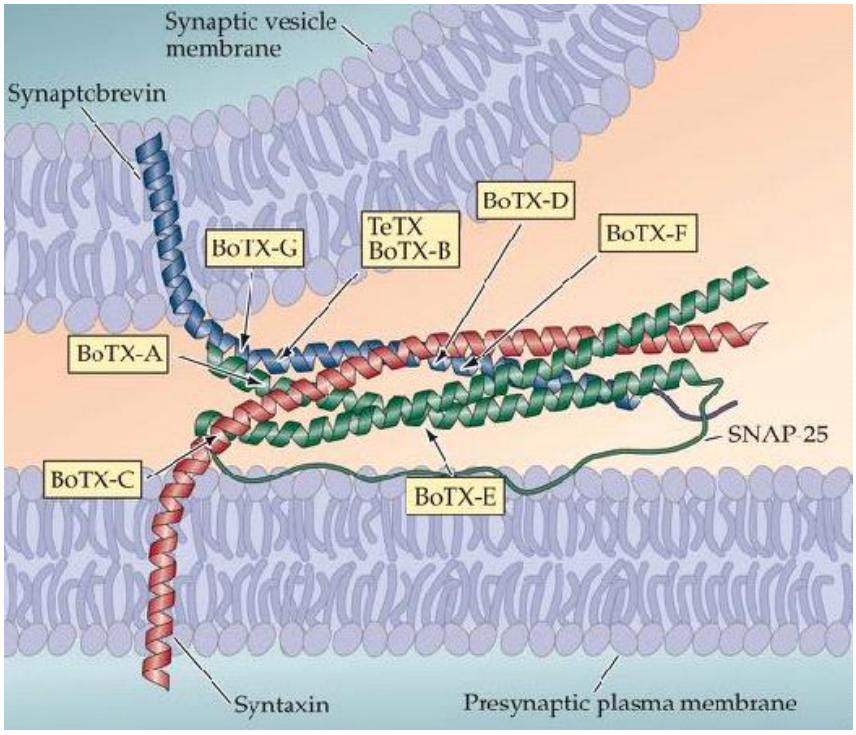
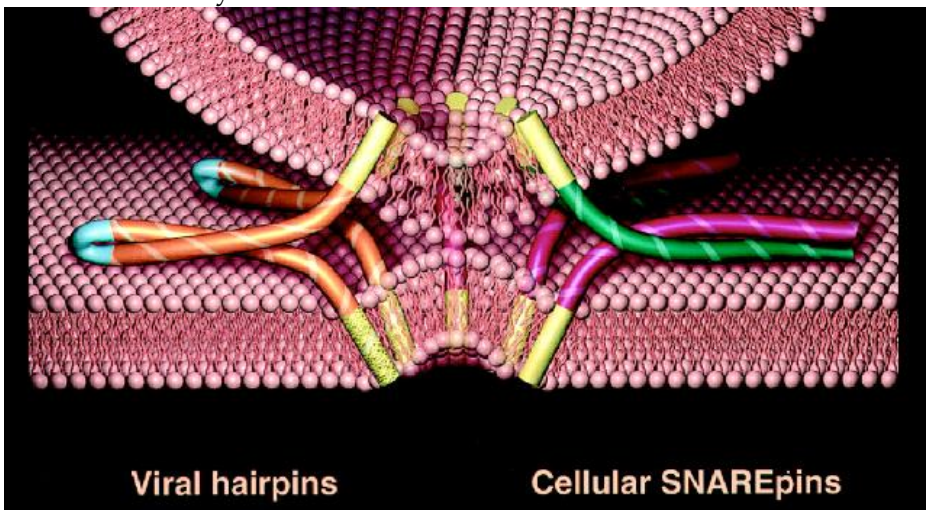
- There are sub-threshold events - Suggested quantal neurotransmission, even before people saw a vesicle



- Fourier transform - The amplitude of the signal suggests signal events over baseline
- Bar chart of amplitude frequencies -> Does not look like a normal distribution
  - Peaks at 0.4, 0.8, 1.2 - Suggests that there was a quantal release of chemical signals
  - How can there be a 0.6 signal? Mix of different vesicle

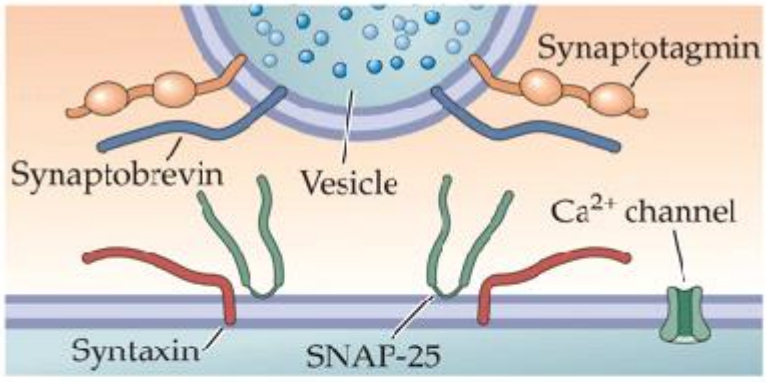
	release/the size of the vesicle is not the same all the time/Modulatory signals may alter the muscle excitation What happens to the curve when the receiving muscle cell becomes more sensitive? - Curve is maintained over a wider range of amplitude - There is still a skew to the right, since this is a property of the presynaptic cell What happen to the curve if the presynaptic side becomes more efficient? - 0 events decreases - Higher peakes in 0.4 multiples (because there are more vesicles being released)
What was the experiment that describe endocytosis?	Horseradish peroxidase (HRP) - extracelullar marker is taken into the cell and can be observed after washing 
In chemical synapses, which processes are faster and which are slower?	Different cycles - some take minutes, some take miliseconds 

which processes are faster and which are slower?	
Describe which main proteins are involved in the process of vesicle docking.	Synaptic fusion - Hydrophobic amino acids interact with each other (coiled coil zips up)  - Syntaxin (through the membrane) - Synaptobrevin (through the vesicle) - SNAP-25 (at the membrane) - Synaptotagmin - Calcium sensor that triggers fusion 4 helices are involved in the fusion How to we know it? Botulin toxin acts in different parts of the process (inhibits chemical transmittion)

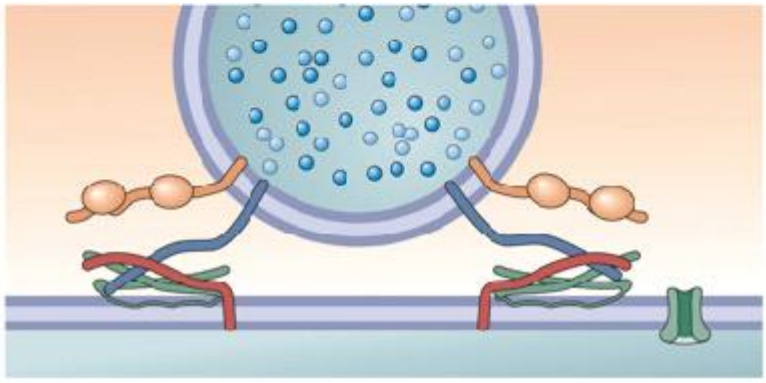
	 BoTX-C - Botox
What does the similarity between viral hairpins and mammalian SNARE pins imply?	Virus has a single molecule that binds and pulls it through the membrane - Very similar to the mammalian mechanism  Deletion probably happen in the mammalian DNA -> mammalian mechanism has two separate proteins
Describe the process by which vesicles bind to	1. Vesicle docks 2. SNARE complex - Zipper

the neuronal membrane.

(1) Vesicle docks

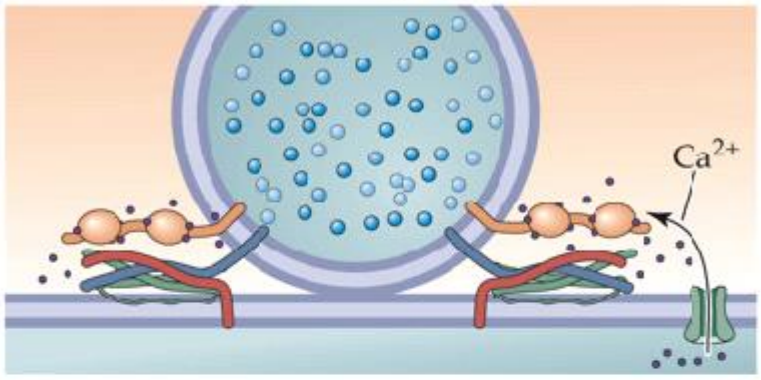


(2) SNARE complexes form to pull membranes together

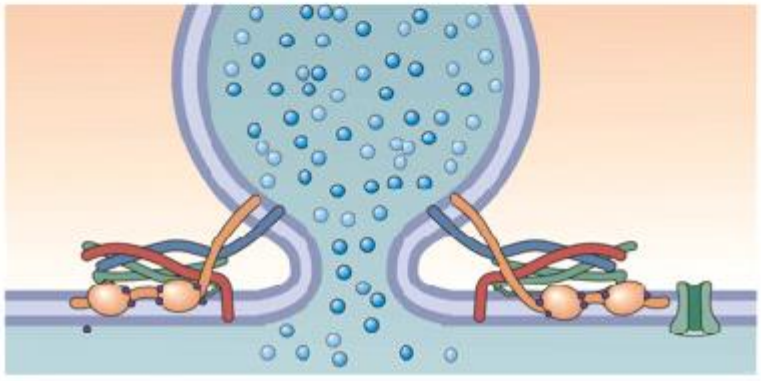


3. Calcium binds to synaptotagmin, which buries itself in the membrane (higher affinity for lipids -> hydrophobic amino acids are revelead)

(3) Entering Ca^{2+} binds to synaptotagmin



(4) Ca^{2+}-bound synaptotagmin catalyzes membrane fusion

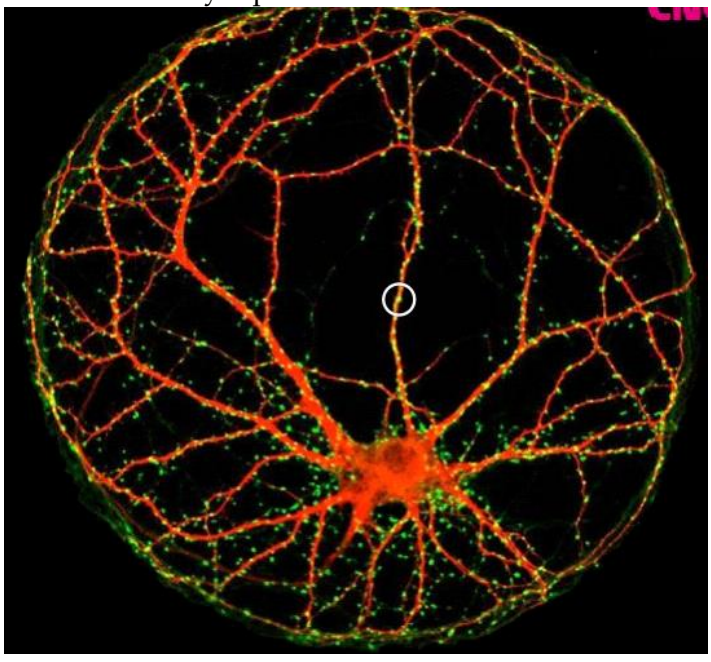


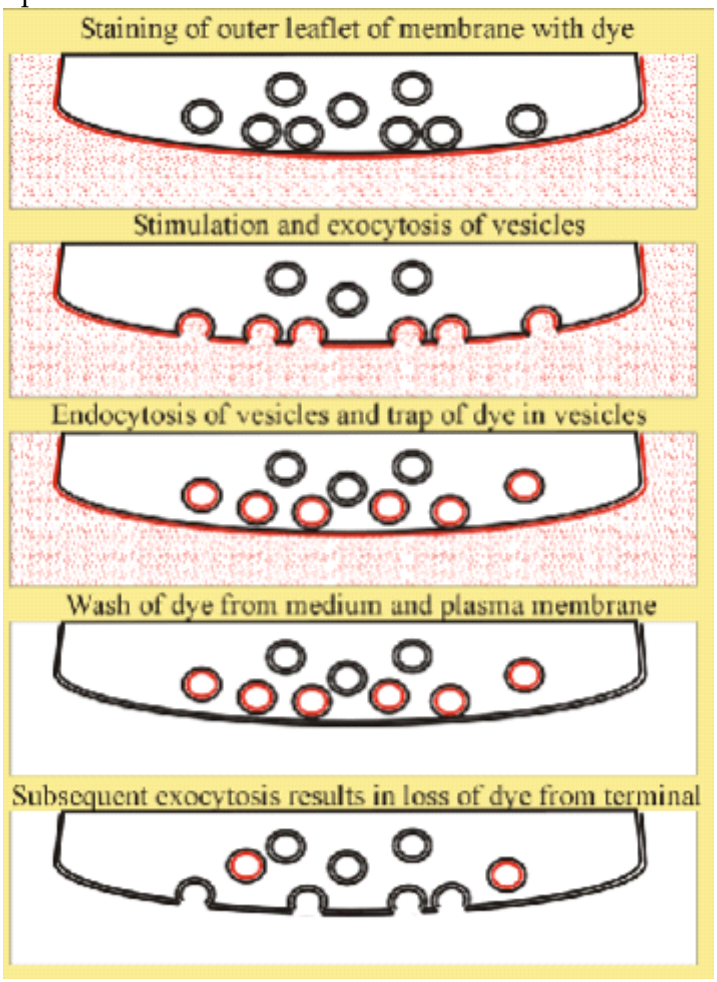
What is an autaptic neuron?

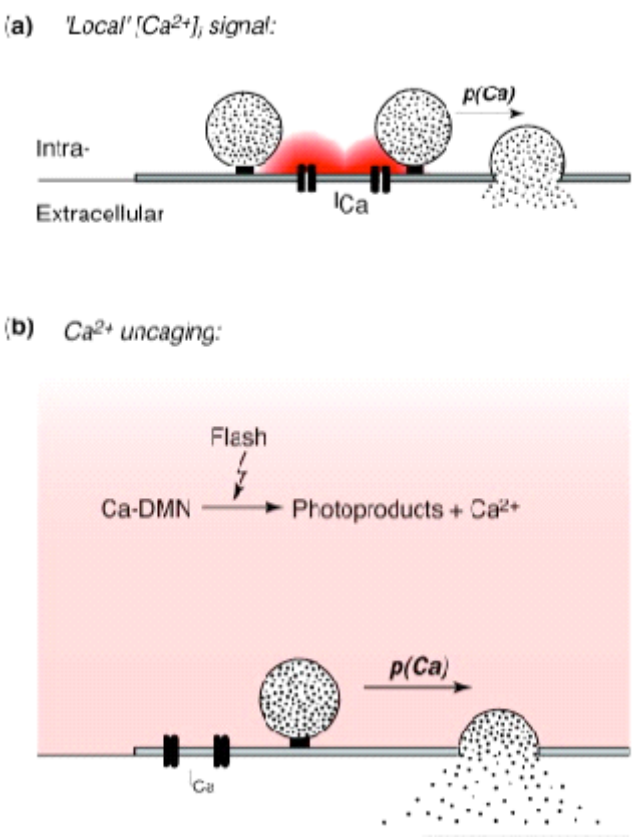
Main models to study secretion

Cultured neurons (mouse or humans) - Only dissection materials from a surgery can be used for culture

Autaptic neurons - Single neuron grown with glial cells, and that synapse with itself

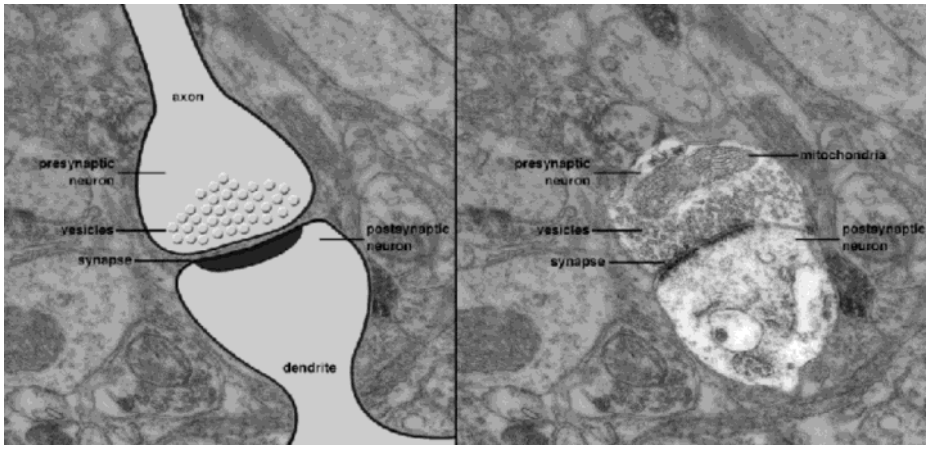
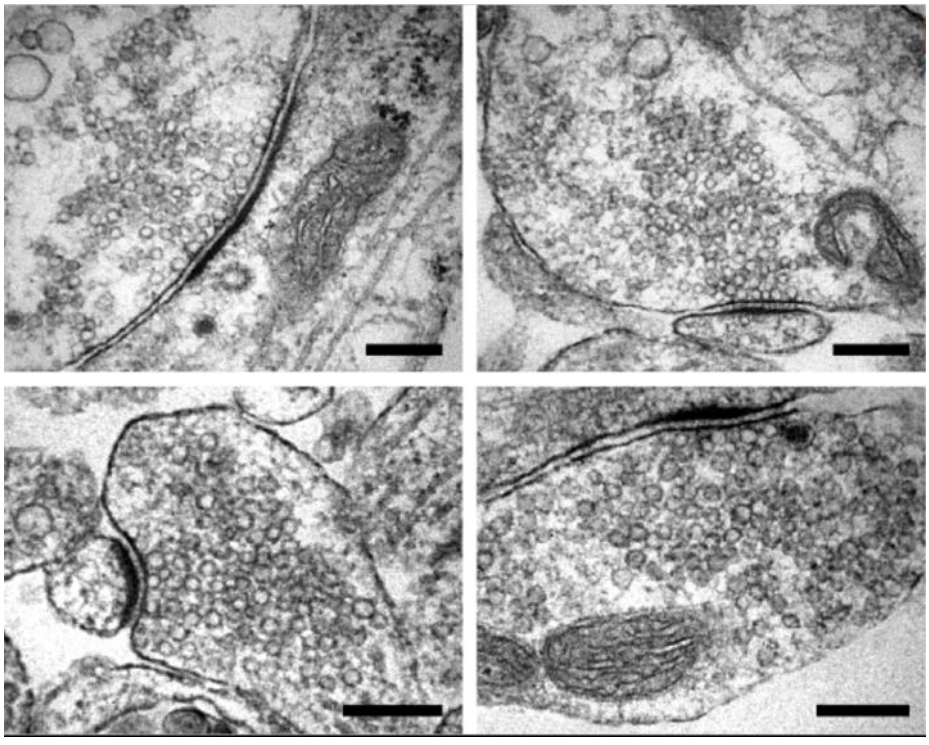


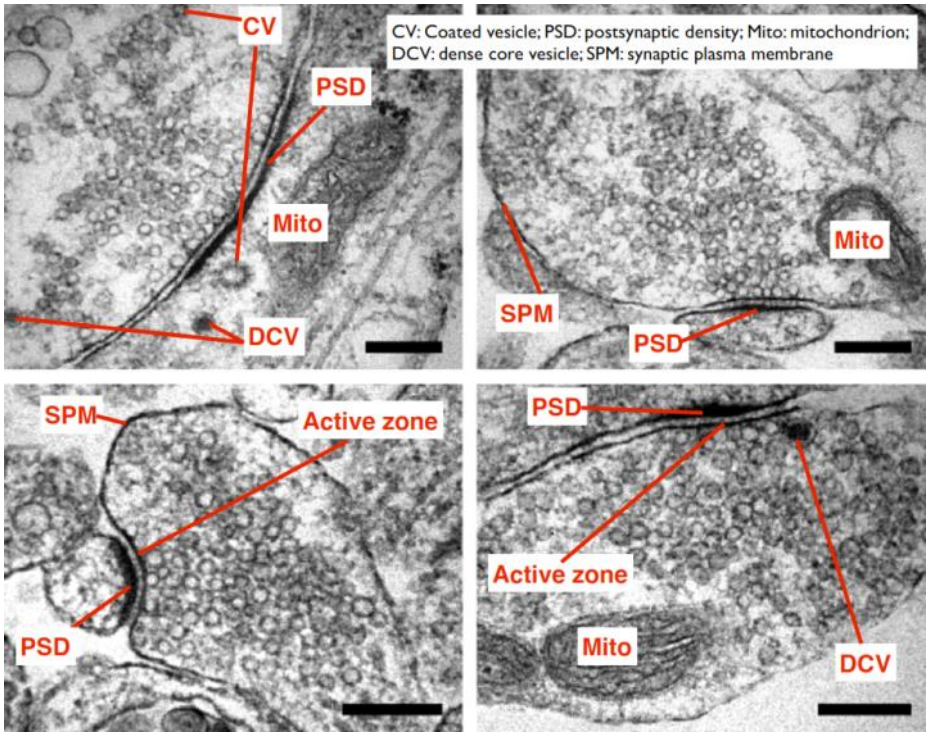
How does an FM dye work?	FM dies - Lipophilic molecules that become red when they are in lipids 
How does calcium caging work?	Calix of Held - Presynaptic neuron encapsulates the post synaptic neuron Allow many release sites to be made Calcium is bound to cage - Inactive When you flash a light, cage releases calcium -> Induces vesicle binding

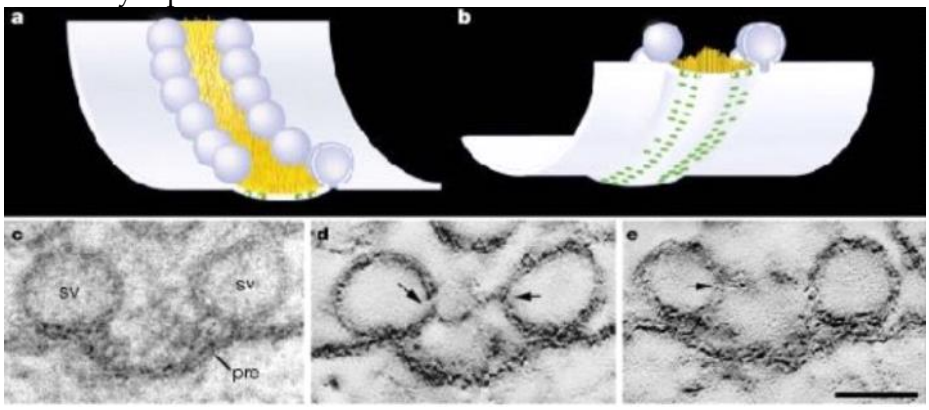


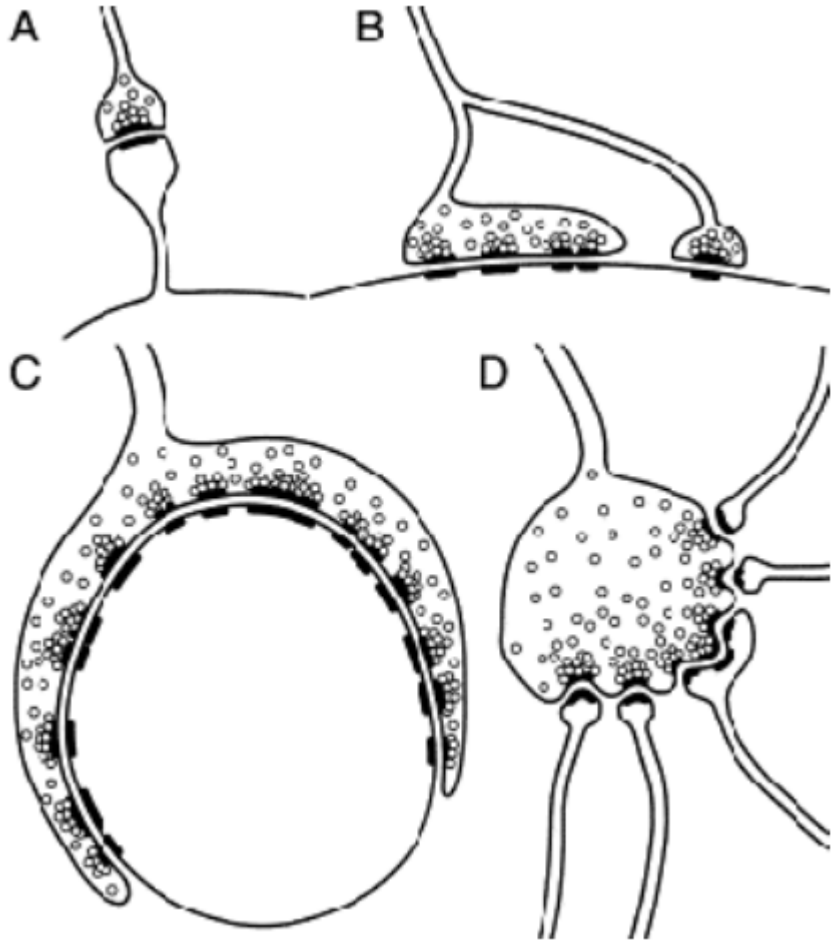
Bypasses a lot of the uncertainty of the process

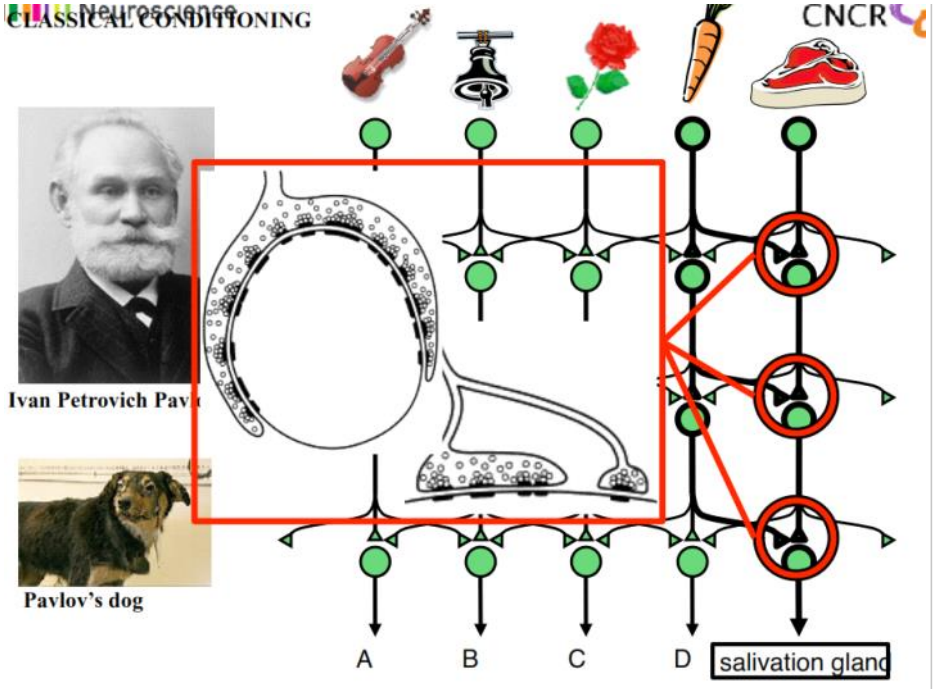
8b. SynBio2: Chemical synapses

Why is it often difficult to observe a synapse under the microscope?	Real reference  Normally this is more difficult to observe - Nerves are normally not oriented with the cut that was made
Test yourself with the following image. Try to identify • CV - coated vesicle • PSD - Post synaptic density • DCV - Dense core vesicle • SPM - Plasma membrane	



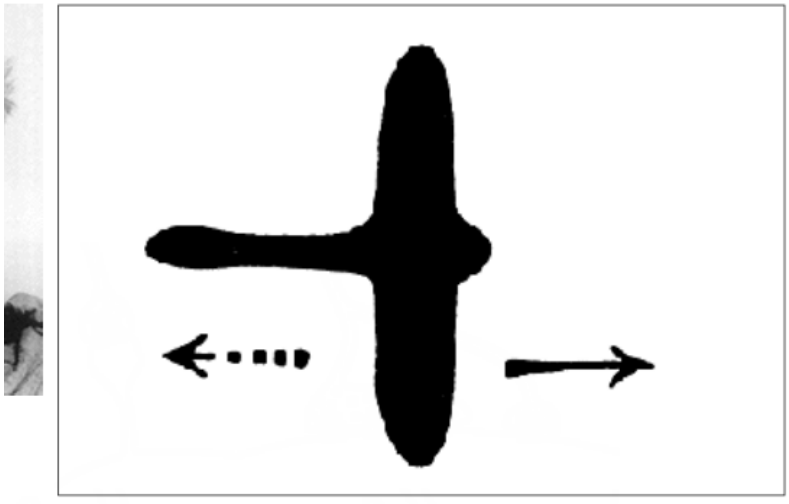
Why is a neuromuscular junction more reliable than interneurons in the CNS? Why is that important?	Neuromuscular junction - Organization is more complex than a normal synapse  Multiple release sites - Often more than 20 Element of robustness - If one or two vesicles do not fuse, the muscle is still able to contract This occurs because signals for muscle contraction need to be reliable 100% of the time - for the animal's survival! Endosome - Vesicles bud off from here There are six clathrin molecules per vesicle
Describe four different types of neurons and define how much they	Differences in release probability of different synapses play an important role of the brain's ability to process information and retrieve it

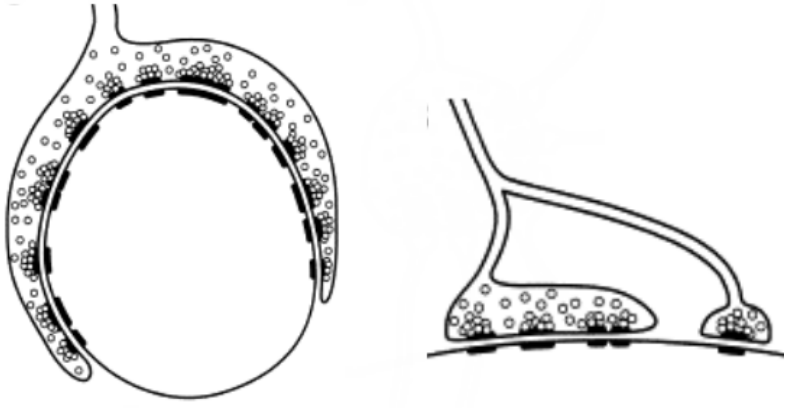
are reliable?	 More reliable - C>B>D>A - The more release sites, the more reliable - Often related to genetically sent and evolutionary important behaviour - The less reliable, the lesser probability of vesicle release after action potential
Which type of synapse would a genetically encoded signal have?	Certain connections are inherited -> Encoded by DNA A dog will salivate from the smell of food - Synapses are reliable ones (calix of Held and neuromuscular junctions)

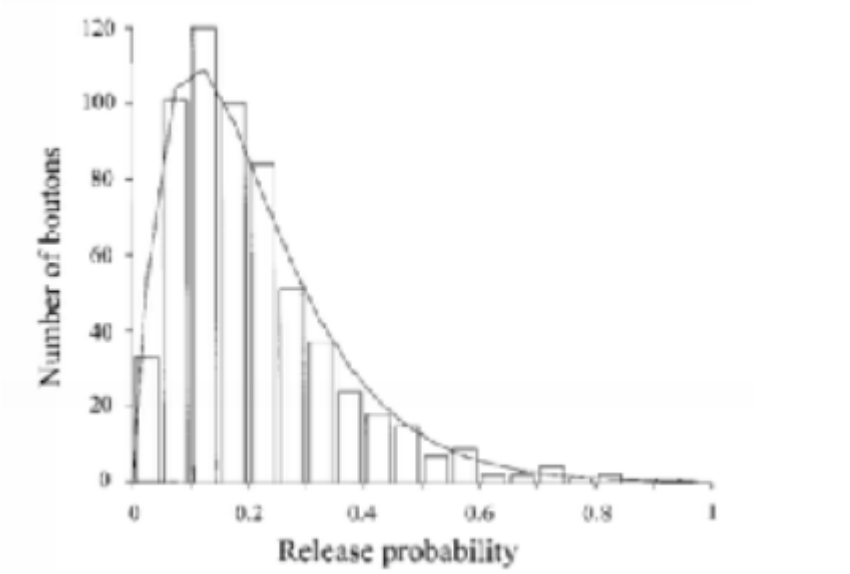
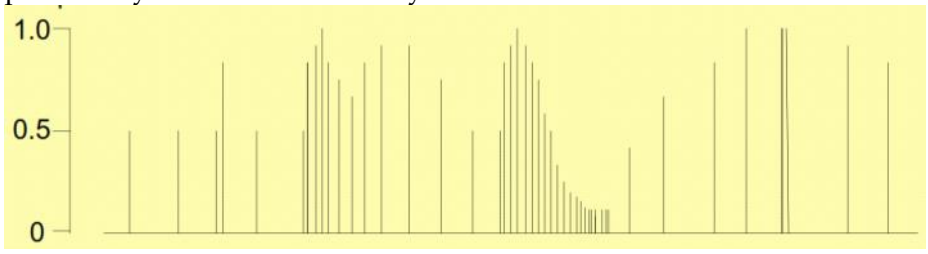


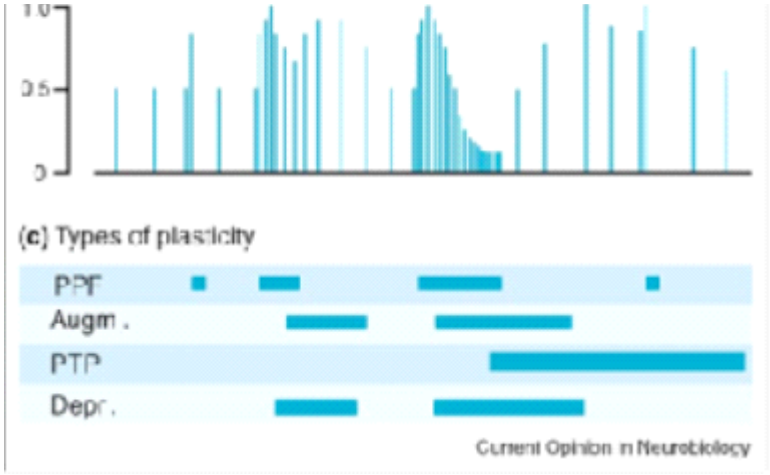
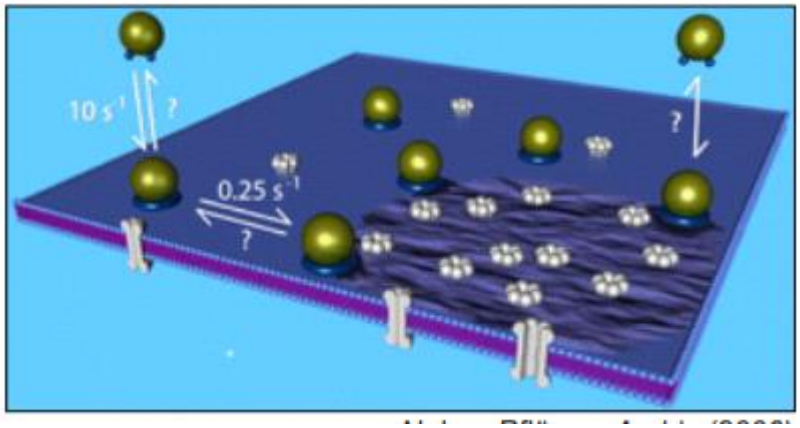
Dutch Nobel Prize - Baby geese behaviour in feeding and danger

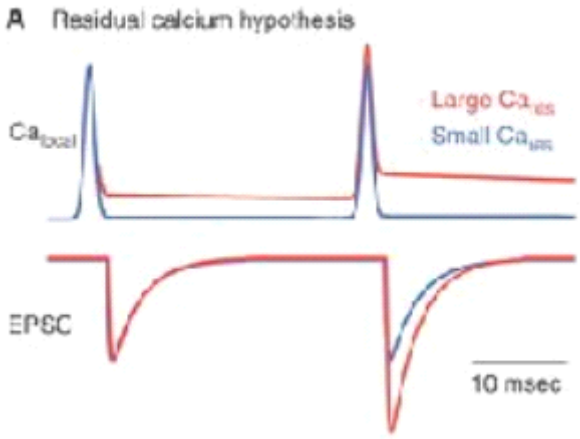
- Baby geese open their mouth when they see their mothers
- Drawings were enough to trigger a response
- Left to right -> Looks like mother (long neck) -> Feeding response
- Right to left -> Looks like predator (short neck) -> Fear response
    - Humans also have many hard wired behaviour



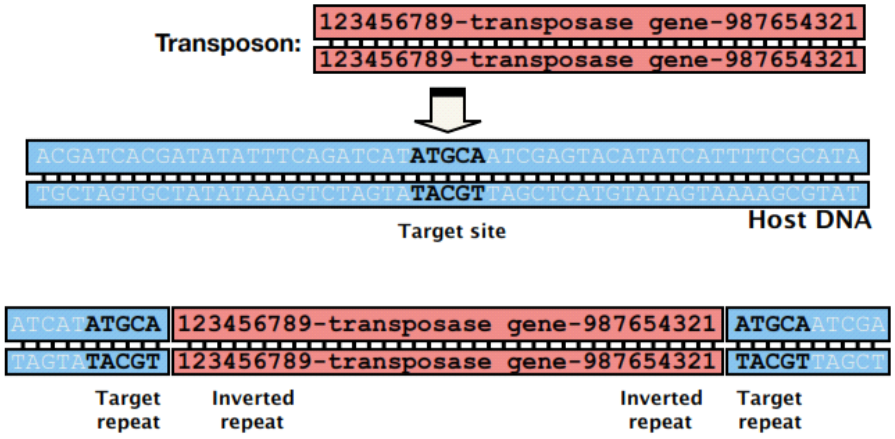
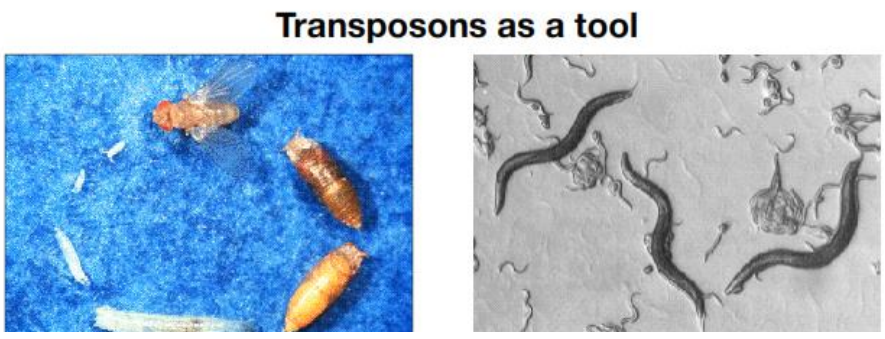


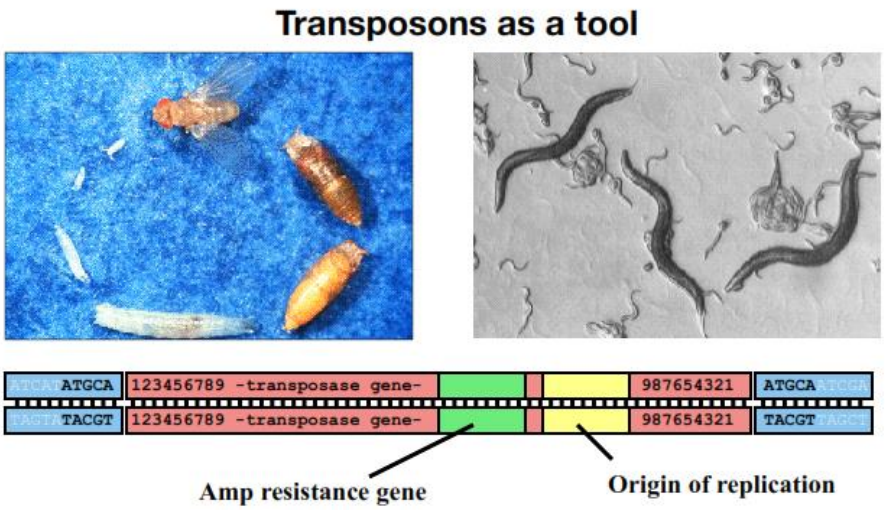
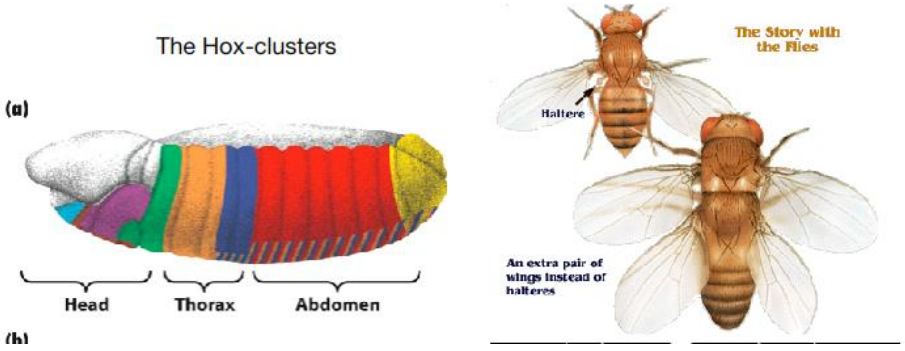
Why is the synapse 'unreliable'?	The unreliable synapse Synapses have a stochastic release probability - Only one in five (0.2) action potential will cause a release of neurotransmitters  Small synapses have very few vesicles docked and ready to be released In the absence of stimulation, there is also a basal level of activation  The neurons are always receiving excitatory and inhibitory inputs -> The postsynaptic neurons do not necessarily fire when the presynaptic neuron is activated! Muscle activation is more reliable than CNS interneurons!
Describe what happens to the presynaptic neurons if two action potentials occur in close sucession.	If two action potentials occurs in close sucession, the release probability increases immensely  If the constant stimulation persists, the release probability goes down - due to the limited supply of vesicles If you excite the neuron again after this overstimulation, the release probability increases again - vesicle recruitment Post-titanic potentiation - Short term potentiation, takes miliseconds
Describe **a) Paired pulse facilitation**	Types of plasticity (presynaptic) 

b) Augmentation **c) Post-titanic potentiation** **d) Depression**	 Paired pulse facilitation - Two concurrent stimuli cause a higher release probability Augmentation - Shorter lasting than PTP, the release probability goes down faster PTP - Post-titanic potentation - After intense activation, a long change in release probability happens Depression - Due to thelack of vesicle release
What is the molecular process that underlies paired pulse facilitation?	Paired pulse facilitation Vesicle with calcium ions are close to the membrane Calcium channels are not randomly distributed - they are localized in the presynaptic membrane  The calcium is diffused in the synapse when it is not activated When two pulses occurs at the same time - The local calcium concentration is higher for a longer period of time - increased chance of binding to synaptotagmin and vesicle release


A Residual calcium hypothesis
Large Ca_{ext}
Small Ca_{ext}
Ca_{local}
EPSC
10 msec

9b. Models and Methodologies - Drosophila

	Flybase: Genome of Drosophila is fully mapped and available online Two Nobel prizes: Principles of translocation (1925) - Certain traits are more likely to occur together (unit: morgan)
What are transposons? **How can transposons be used as a genetic tool?**	Transposons as a tool  Small piece of DNA that codes a single gene Can insert itself into host DNA Less commonly sucessful in eukaryotes - DNA is not acessible (histones) Transposase protein: Binds to transposon DNA Excises transposon from host genome (precise/imprecise) Can cause problems if it loses 1 or 2 nucleotides (changes reading frame) Induces integration into novel site
What is the important of transposons in evolution?	Transposons cause greater variability in the genetic material Explains the immense variability and our fast evolution (random mutations are too slow to explain it) A lot of our own genome can be recognized as transposons
How can transposon be used in genetic screening?	Transposons as tools - Insert transposons into Drosophila genome 

used in genetic screening?	 Behavioural screens - See which genes are important for particular behaviour A transposon will land itself in important genome regions - disrupting their functions Transposase gene can be inactived Origin of replication - Bacterial replication that express a specific part of fly genome Today it is cheaper to map the entire genome of the fly instead of using bacteria to produce enough DNA Ampiciline - Resistance marker
What are hox genes?	Hox-cluster - Division of head, thorax and abdomen 
Give three examples of traits discovered in Forward behavioural screenings in fruitflies.	Forward behavioural screens Period - Loss of sense of time The gene encodes a transcription factor that periodically transcribes other genes Fruitless - Loss of copulation impetus Gene only expressed in males and involved in sexual differentiation Dunce - Loss of odor discrimination General enzyme present in every cell of the body

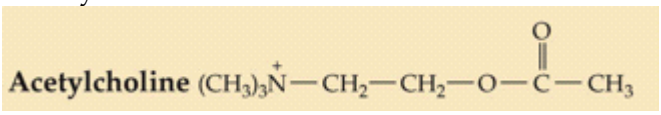
10. Neurotransmitters and their receptors (Chapter 6 Purves)

Why neurotransmitters tend to be small?

What are six examples of small molecule neurotransmitters?

Small molecule transmitters

Acethylcholine



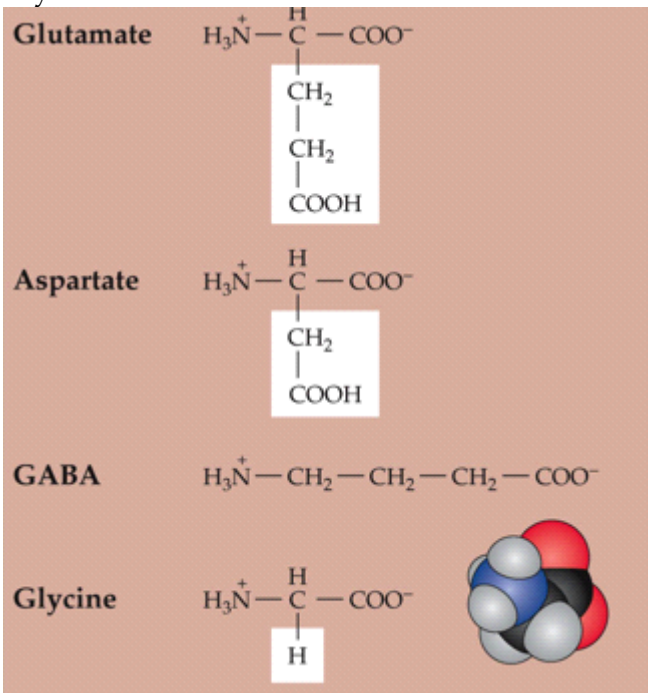
Amino acids:

Glutamate

Aspartate

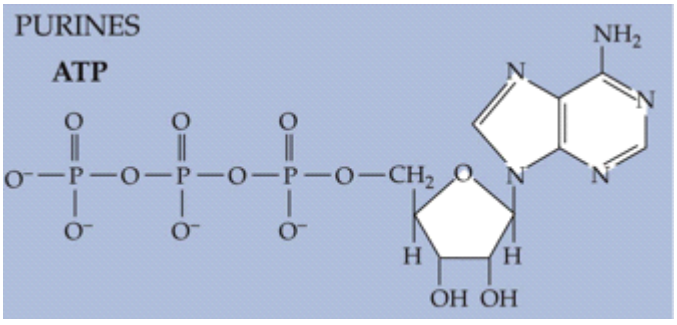
GABA (derivative of an amino acid)

Glycine



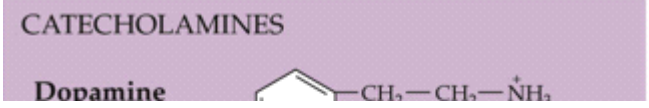
Nucleic acids:

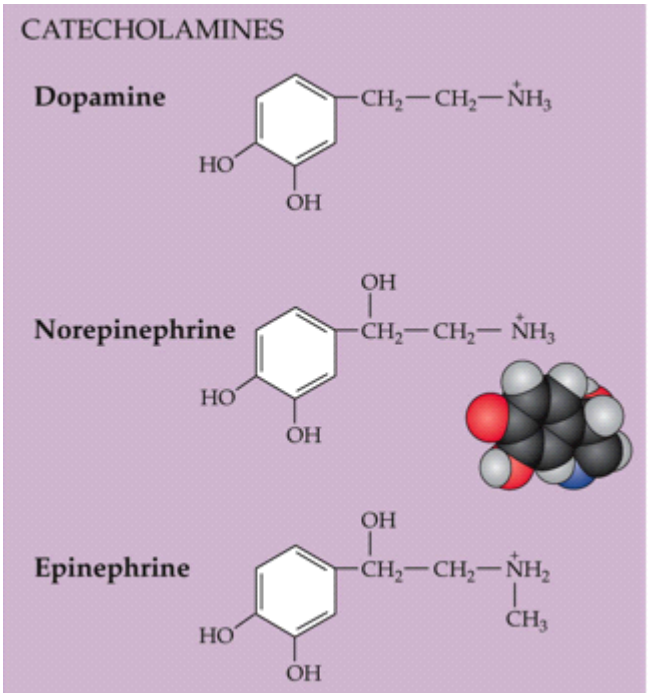
ATP



Catecholamine

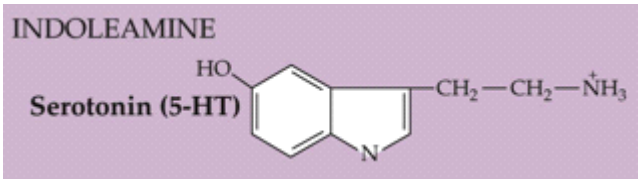
- Dopamine
- Norepinephrine
- Epinephrine





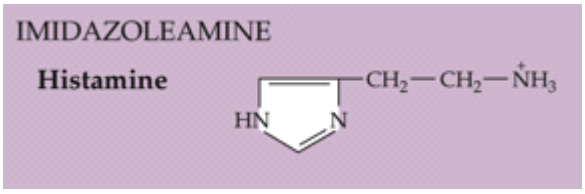
Indoleamine - Derived from tryptophan

Serotonin



Imidazoleamine - Derived from histidine

Histamine

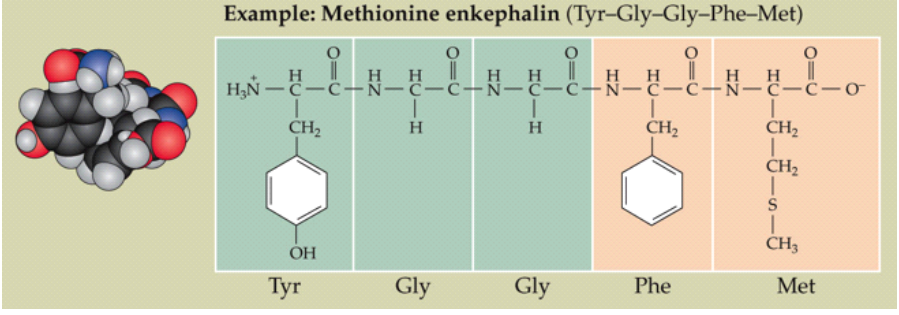


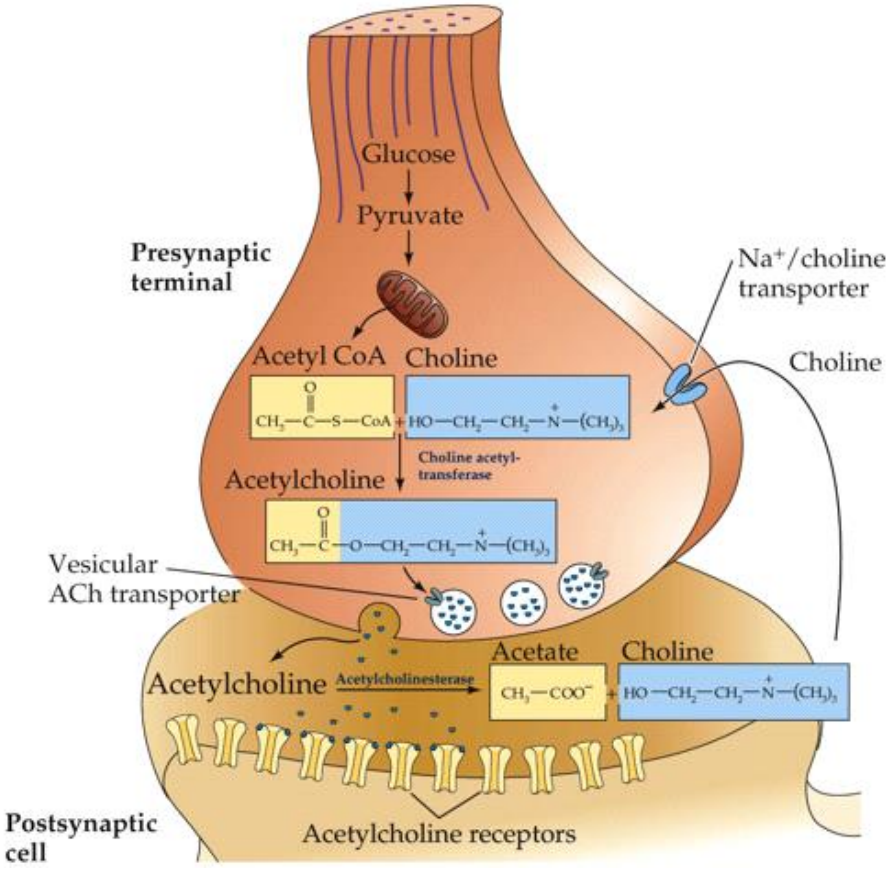
Nucleic acids and acethylcholine came later in evolutionary history - since amino acids are already a building block of an organism
Why small molecules?
Large molecules diffuse less; that is why neurotransmitters tend to be small - because they transmit information very fast!

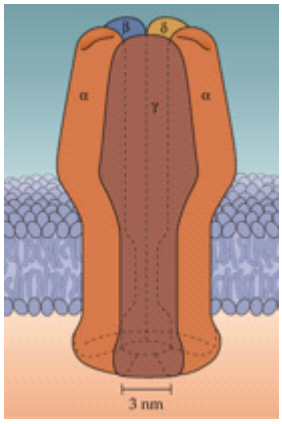
What is the relationship between peptide neurotransmitter and evolutionary history of an organism?

Peptide neurotransmitter - More than 100 peptides, usually 3-36 amino acids long

E.g. Methionine enkephalin



	The simpler the organism, the more peptide neurotransmitters they have!
Which molecule is used to produce acetylcholine? Which organelle does it come from? **Which enzyme breaks down Ach in the synaptic terminal?**	Acethylcholine metabolism in cholinergic nerve terminals  Acetyl-CoA - Derived from a metabolic byproduct in mitochondrias Choline -> Acethylcholine via choline acetyl-transferase Acethylcholine -> Acetate + choline in the synapse (breakdown is very fast, since you need to contract and relax your muscles very fast) Acethylcholine is inactive but choline can act upon certain types of Ach receptors Reuptake on choline by Na/choline transporter Organic phosphates - Block acethylcholinesterase -> muscle cells cannot relax Thousands of people die of organic phosphate poisoning **Small chemical transmitter are always produced locally**
How many subunits does a Ach receptor have?	**Structure of Ach receptor** 5 subunits - Extracellular portion binds to acethylcholine (2 molecules need to bind to alpha subunits) -> allows passive diffusion of ions when it is active 



What is the physiological advantages of having many different types of subunits for channels?

General architecture of ligand-gated receptors

Different combination of subunits have different kinetics and can allows different ions to go through

Different cell types express different receptor types

Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nACh	Serotonin	Purines
Subunits (combination of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5	α_{1-7}	α1	α_{2-9}	5-HT_3	P_{2X1}
	Glu R2	NR2A	Glu R6	β_{1-4}	α2	β_{1-4}		P_{2X2}
	Glu R3	NR2B	Glu R7	γ_{1-4}	α3	γ		P_{2X3}
	Glu R4	NR2C	KA1	δ	α4	δ		P_{2X4}
		NR2D	KA2	ε	β			P_{2X5}
				ρ_{1-3}				P_{2X6}
								P_{2X7}

AMPA, NMDA, Kainate - 4 subunits

GABA, glycine, acethylcholine, serotonin - 5 subunits

How many subunits does a metabotropic receptor have?

Metabotropic receptors - 7 transmembrane subunits

Great variety also - 5-HT 3 is a mistake in Purves

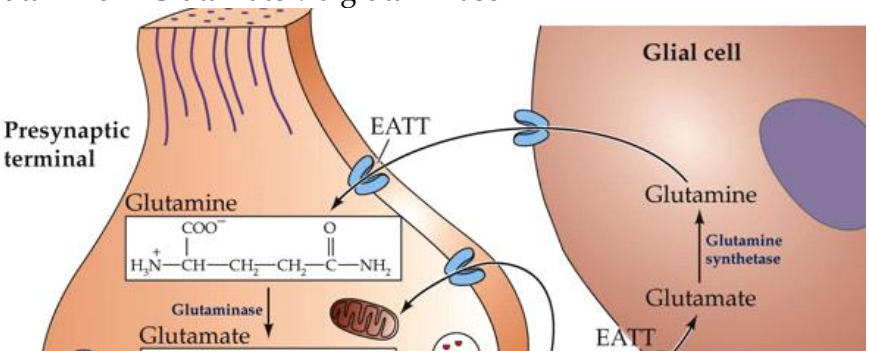
Receptor class	Glutamate	$GABA_B$	Dopamine	NE, Epi	Histamine	Serotonin	Purines	Muscarinic
Receptor subtype	Class I	$GABA_B$ R1	$D1_A$	α1	H1	5-HT 1	A type	M1
	mGlu R1	$GABA_B$ R2	$D1_B$	α2	H2	5-HT 2	A1	M2
	mGlu R5		D2	β1	H3	5-HT 3	A2a	M3
	Class II		D3	β2		5-HT 4	A2b	M4
	mGlu R2		D4	β3		5-HT 5	A3	M5
	mGlu R3					5-HT 6	P type	
	Class III					5-HT 7	P2x	
	mGlu R4						P2y	
	mGlu R6						P2z	
	mGlu R7						P2t	
	mGlu R8						P2u	

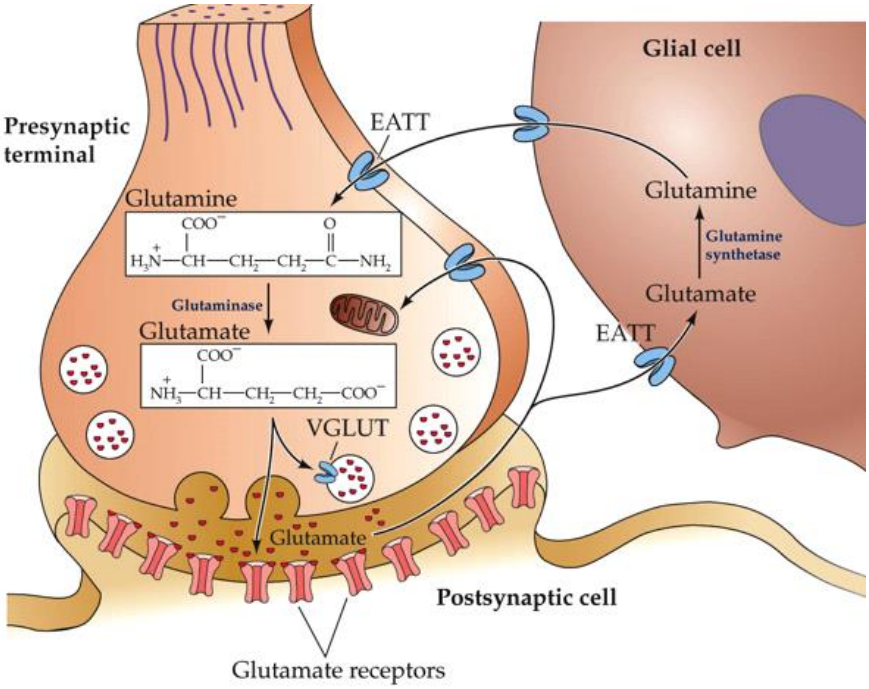
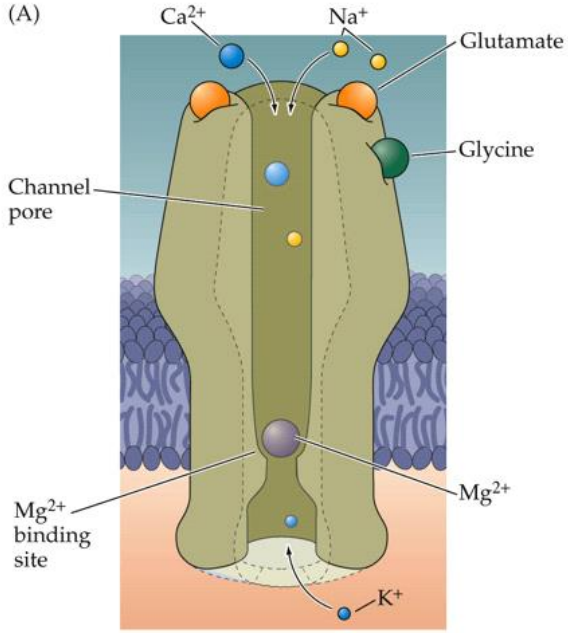
Dopamine has no ligand-gated ion channels in the mammalian brain

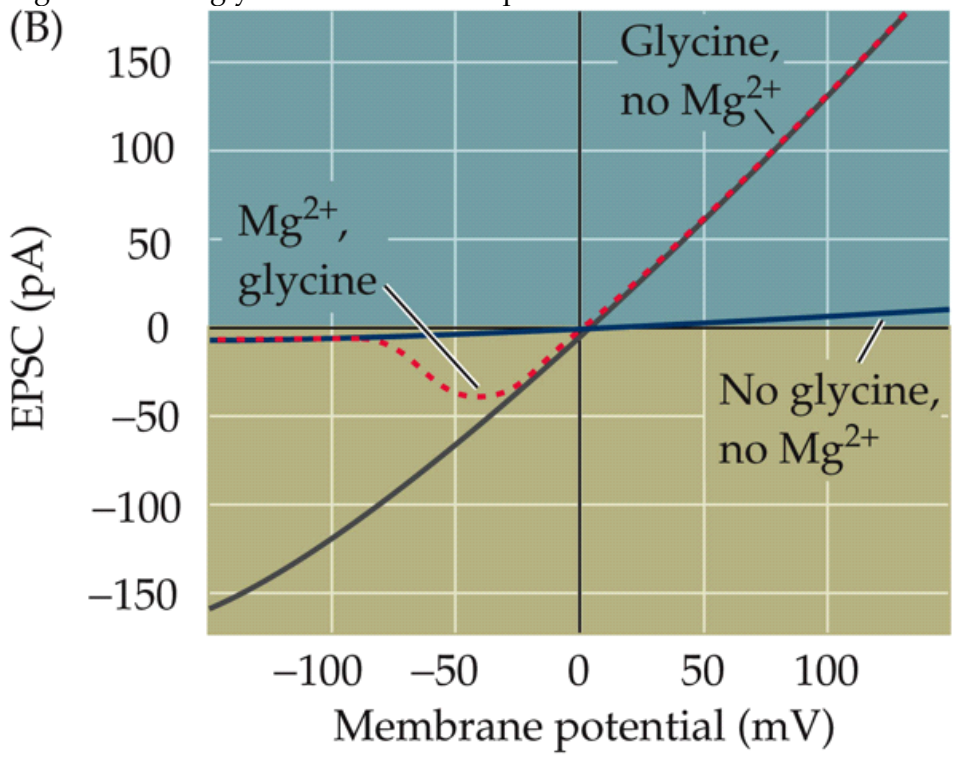
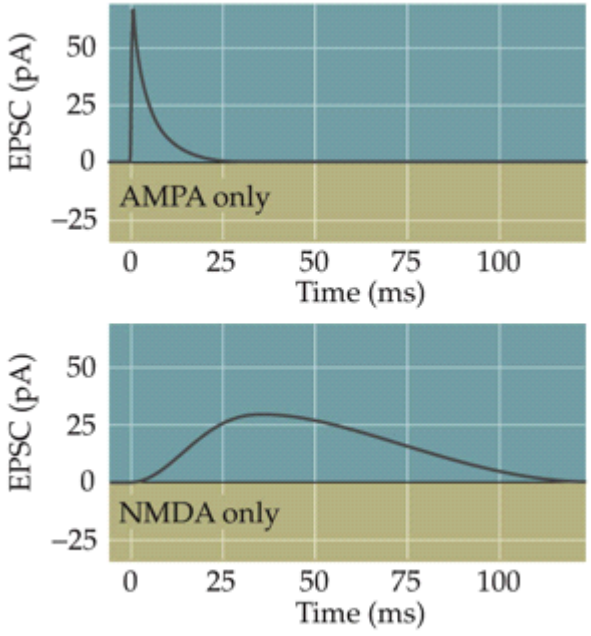
Which molecule is converted into glutamate? Which enzyme does that process?

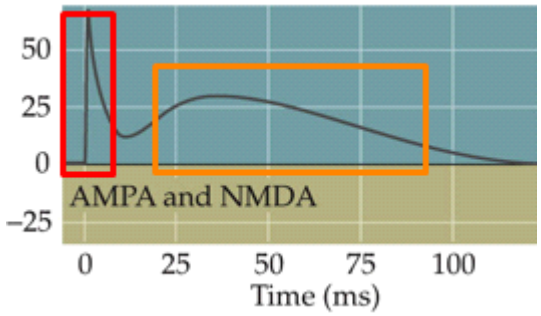
Glutamate synthesis and cycling between neurons and glia

Glutamine -> Glutamate via glutaminase



glutamate? Which enzyme does that process?	 VGLUT - vesicle binding of glutamate EATT (excitatory amino acid transporters) - reuptake of glutamate/glutamine in glial cells and neurons Astrocytes around synapses (trifactor: presynaptic neuron, postsynaptic neuron and glial cell)- Control the amount of neurotransmitter in the synapse Mutations in EATT - Hyperexcitation -> migraines/epilepsy
What is the main characteristics of NMDA and AMPA receptors?	NMDA/AMPA/Kainate receptors - Carry different ions depeding on the subunit compositions NMDA - Blocked by magnesium, usually allow calcium to go through AMPA - Usually allows sodium ions to go through  Allosteric binding - Glycine and Glutamate

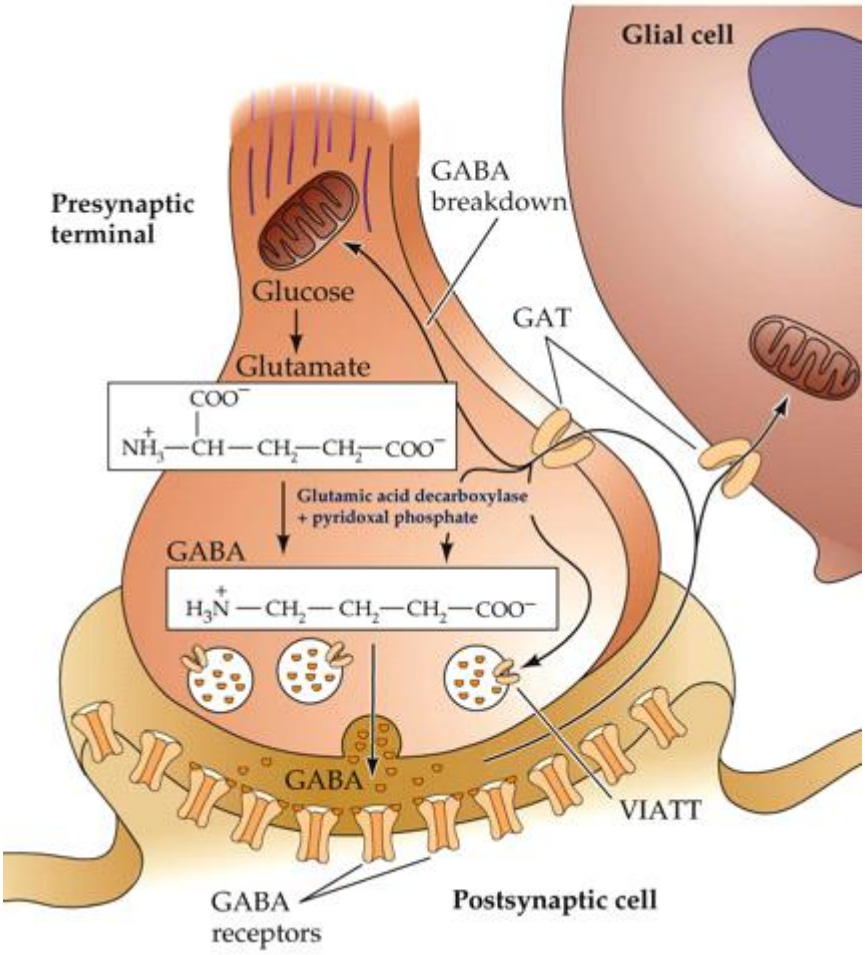
What is the relationship between magnesium and glycine in a NMDA receptor?	Magnesium and glycine in NMDA receptor  No glycine, no magnesium - No excitation Glycine, no magnesium - Straight line Glycine, magnesium - Negative membrane potential -> Positive magnesium ions are bound, therefore there is no conductance
Describe the EPSC of a neuron which expresses both AMPA and NMDA receptors.	AMPA and NMDA kinetics AMPA opens really quickly NMDA are slower  Compound EPSCs - Left curve in red = sodium only, right curve in orange = sodium and calcium



Calcium has more time to flow in the neuron

Which molecule is converted into GABA?

GABA



Derivative of glutamate - If the neuron is excitatory or inhibitory depends on the enzime present in the neuronal terminal

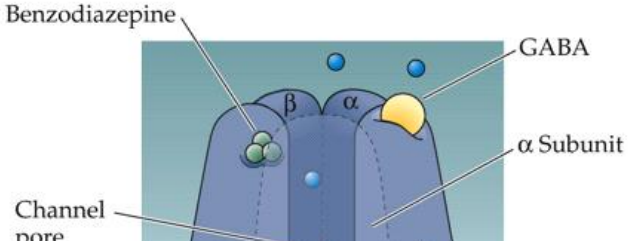
Transmitter phenotype is always kept - A GABAergic neuron does not change to glutamatergic neurons

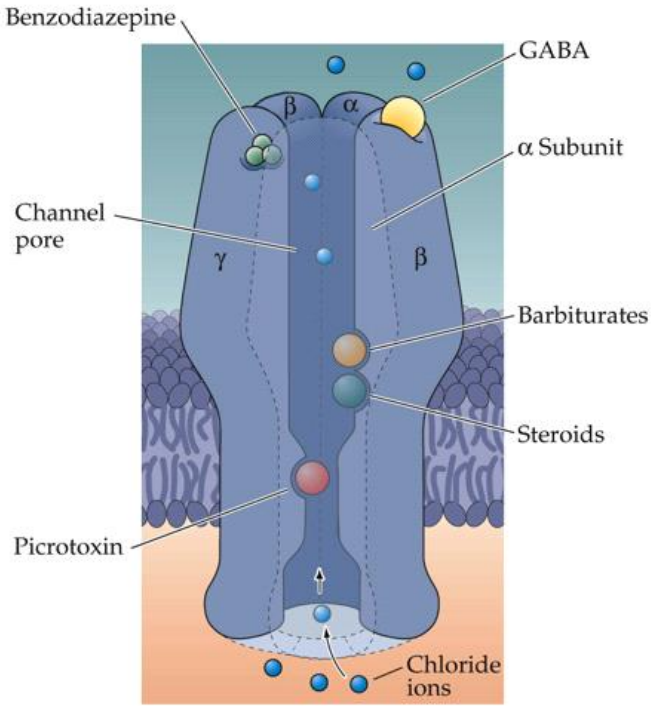
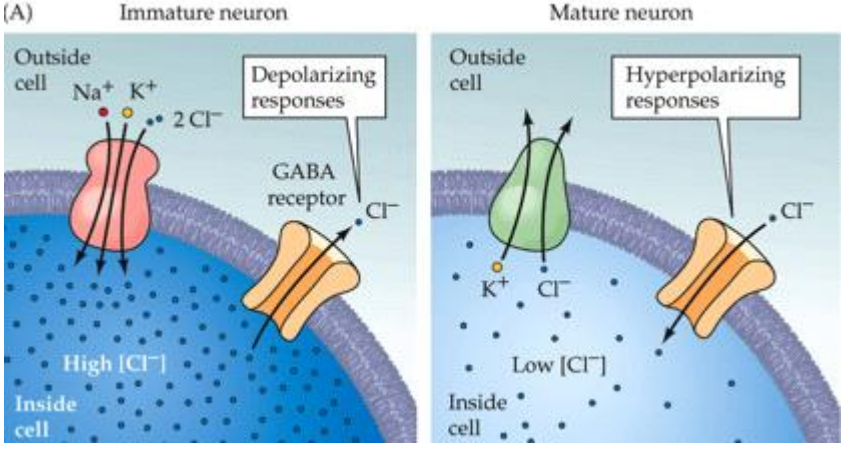
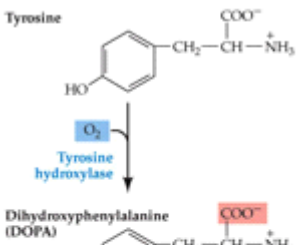
VIATT - vesicle binding of GABA

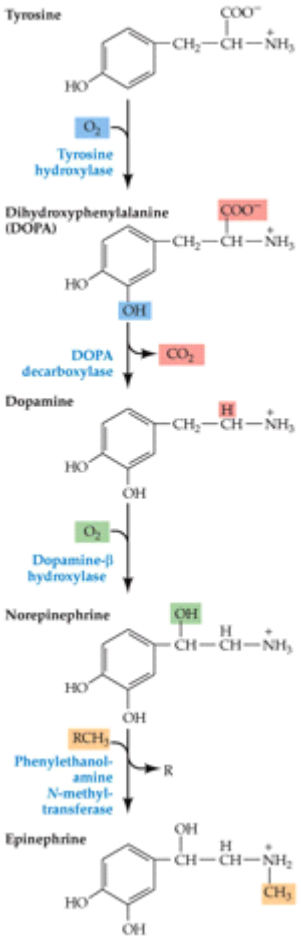
GAT (excitatory amino acid transporters) - reuptake of GABA

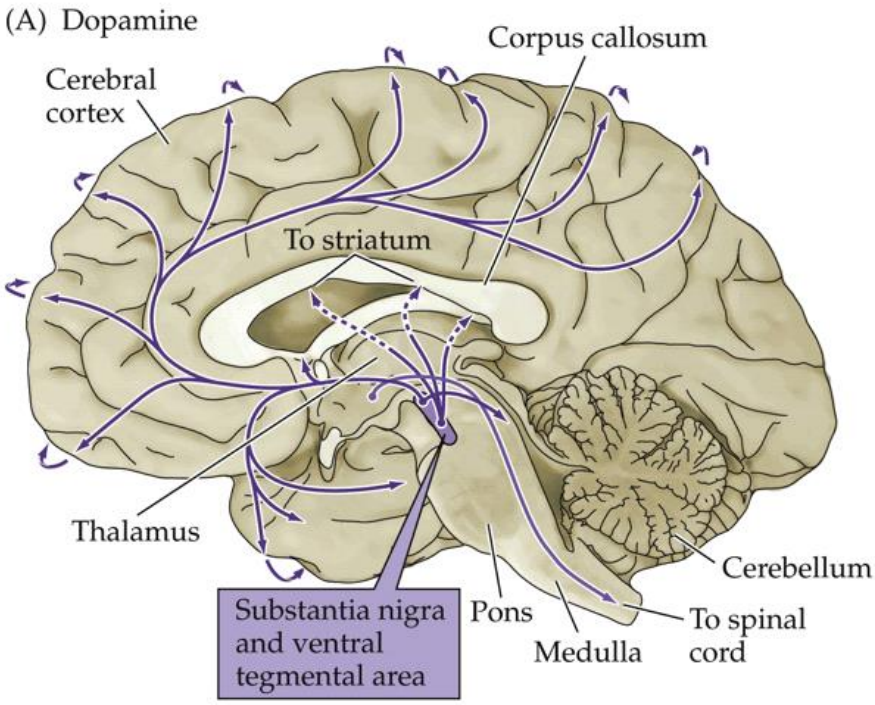
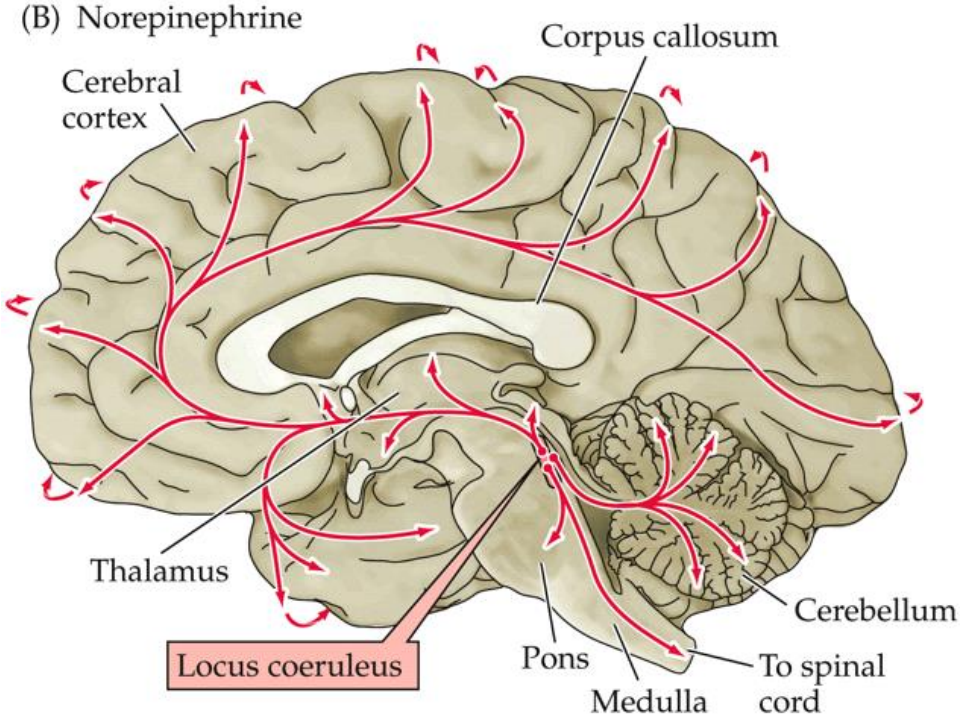
Which ions generally go through GABA channels?

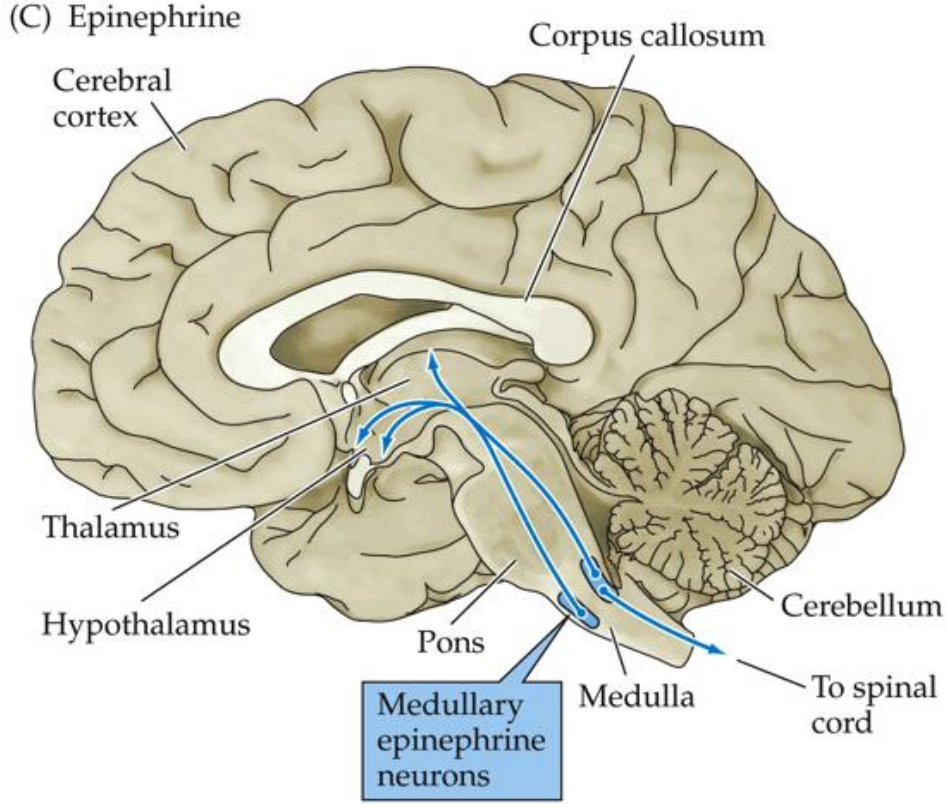
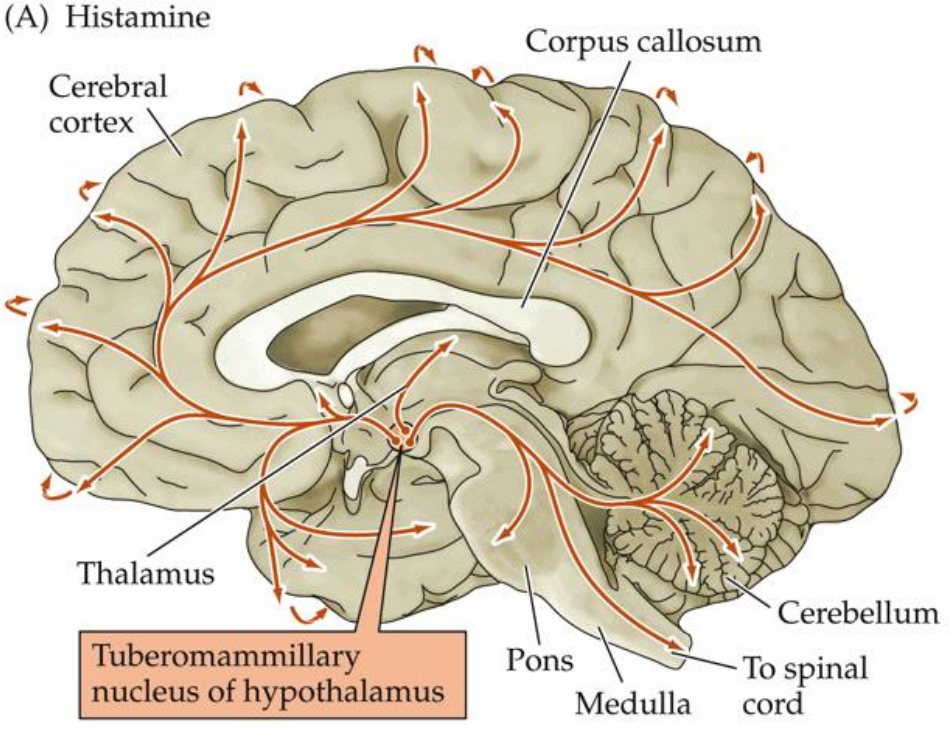
Ionotropic GABA channels

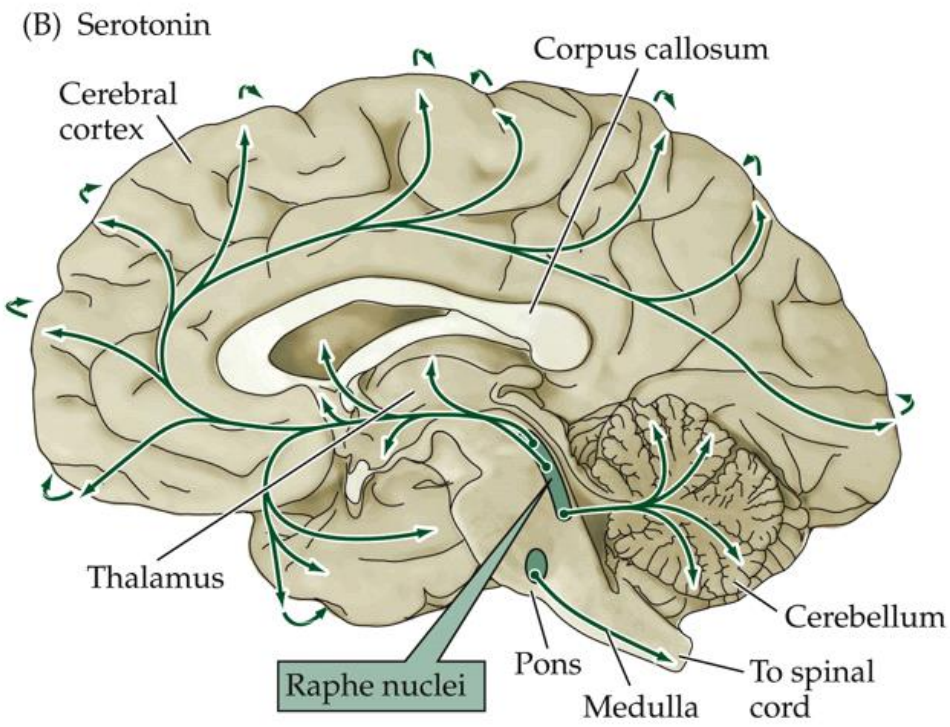
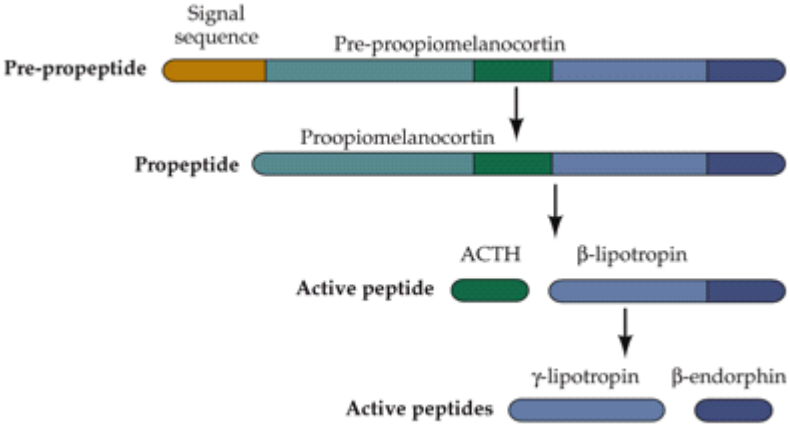


through GABA channels?	 Allosteric ligands - Benzodiazepine, barbiturates, steroids, picrotoxin Chloride ions go through
Why GABA is different in an immature neuron and a mature one?	GABA in the developing brain  In immature neurons - GABA is excitatory There are transporters that are only expressed in immature neurons -> Na+K+Cl- cotransportes High chloride inside, low outside -> Promotes depolarizing responses In mature neurons - GABA is inhibitory Low chloride inse, high outside -> Promotes hyperpolarization
In which regions are catecholamine neurotransmitters produced?	Biosynthetic pathway for catecholamine neurotransmitters 

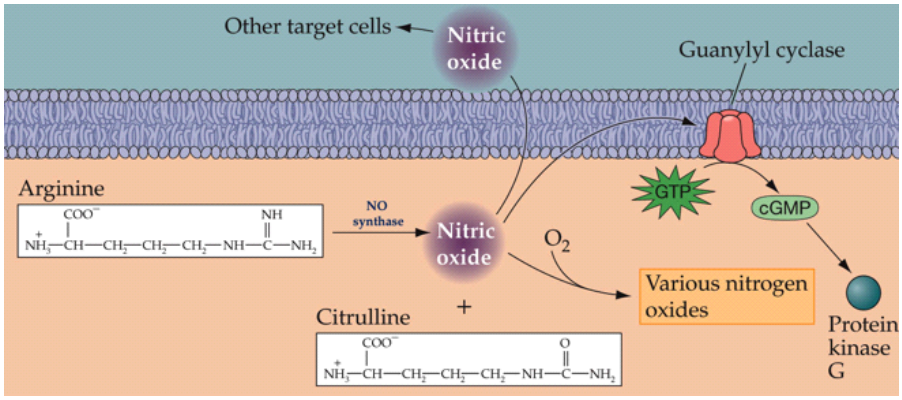
catecholamine neurotransmitters produced?	 Tyrosine (normal amino acid) DOPA Dopamine Norepinephrine Epinephrine Depending on the enzyme expression in this pathway, the neuron will produce different transmitters These are produced in basal brain areas - Indicates an old evolutionary history
Which brain regions produce dopamine?	Dopamine is produced by very localized neurons Substantia nigra Vental tegmental area

	 Dopamine is used as a modulator - Changes synaptic transmition by modulating GPCR, not ion channels
Which brain regions produce norepinephrine?	Norepinephrine  Locus coeruleus - 20000 cells produces all norepinephrine in the brain
Which brain regions	Epinephrine

produce epinephrine?	(C) Epinephrine  Medullary epinephrine neurons
Which brain regions produce histamine?	Histamine (A) Histamine  Tuberomammilary nucleus of hypothalamus
Which brain regions	Serotonin

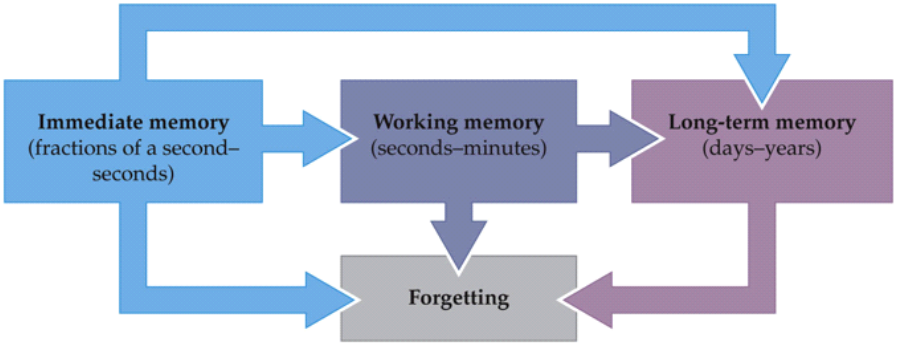
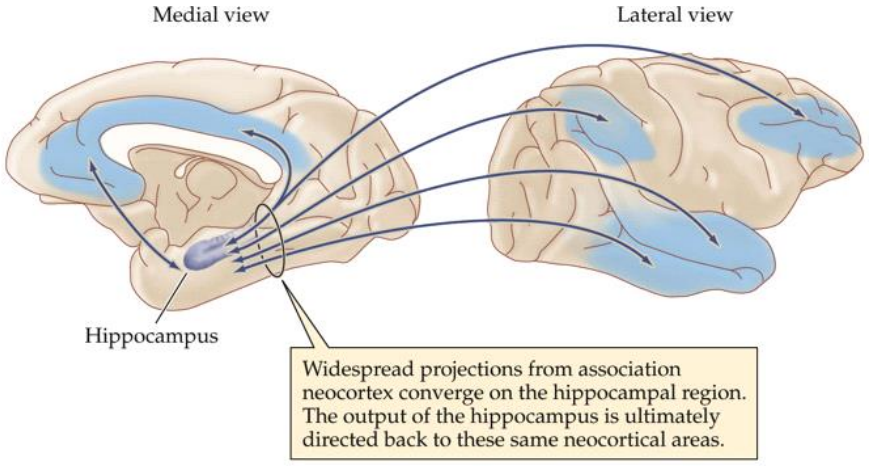
produce serotonin?	 Ralphe nuclei
What is the difference between pre-propeptide, propeptide, and an active peptide form?	Proteolytic processing of pre-propeptides Pre-propeptide form - Signal sequence Propeptide - Only one sequence Active peptide - Cleaved and small peptide 
Which are two endogenous ligands for cannabinoid receptors?	THC - Tetrahydrocannabinol Binds to putamen, substantia nigra, hippocampus and cerebellum Binds to cannabinoid receptors - Most commonly found GPCR in the brain Endogenous ligands - Anandamide + 2-AG (arachidonylglycerol) Not released in vesicles -> they are hydrophobic, can go through the membrane
Which molecule is the substrate to produce nitric oxide?	Synthesis, release and termination of NO Produced from arginine + NO synthase Binds to guanylyl cyclase -> Increase cGMP -> acts on protein kinase

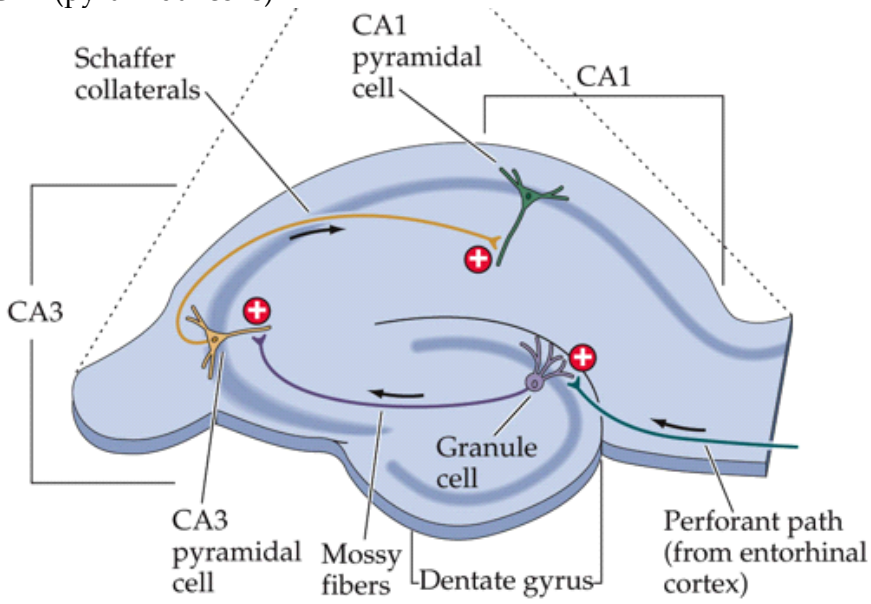
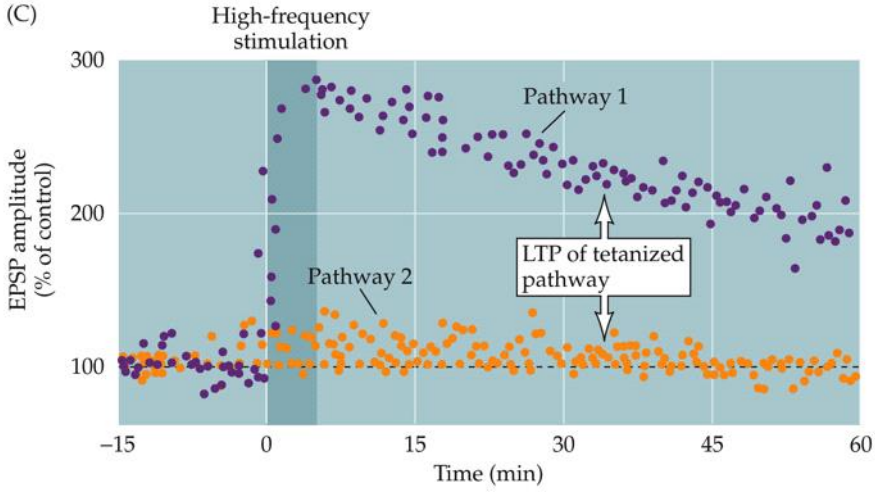
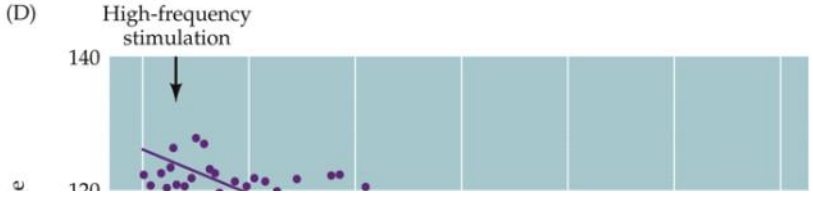
G

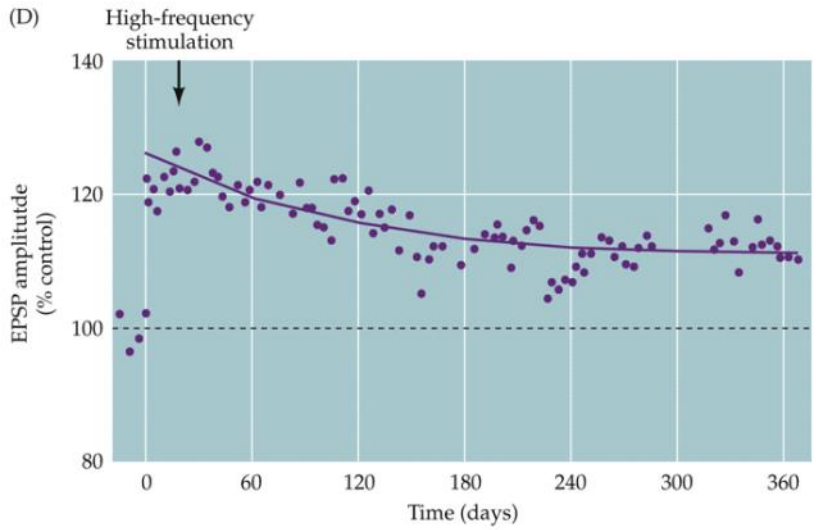


Very small molecule -> Can pass through the membrane, was used as an anesthetic

10b. Synaptic Plasticity (Chapter 8 Purves)

What are the three different types of memory?	Major temporal categories of human memory Immediate memory - Seconds Working memory - Seconds/minutes Long-term memory - days/years 
What is one of the current theories about information flow from the hippocampus to the cortex?	Areas related to memory formation Amygdala - Emotional memory Hippocampus - Spatial memory; Relay station to the neocortex Thalamus - Sensory input from all over the body The information from the hippocampus goes to different areas in the cortex  Associated with already existing information Cortical information is built after some time (requires hours) After some time, the information is not found in the hippocampus anymore The information goes from cortex also -> Memory retrieval goes back to the hippocampus -> Memory is actively reassembled (you build a new memory every time you recall it)

	- Treatment of PTSD - Memory is recalled and then stored in a different way
Describe the neuronal pathway that happens in the hippocampus.	Hippocampal data processing Incoming information (e.g. entorihnal cortex) Dentate gyrus (granule cells) CA3 (pyramidal cells) CA1 (pyramidal cells)  There are also other direct pathways
How is LTP observed in terms of EPSP? **How long can a LTP event be maintained in a neuronal circuitry?**	Schaffer collaterals - Connection between CA3 and CA1 CA3 - Glutamatergic neurons  Long-term potentiation of CA1 Activation of high frequency - Post synaptic response EPSP increases immensely over time (tetanus in pathway 1) Both pathway LTP occur independent of each other (pathway 2 was not stimulated at a high frequency) 

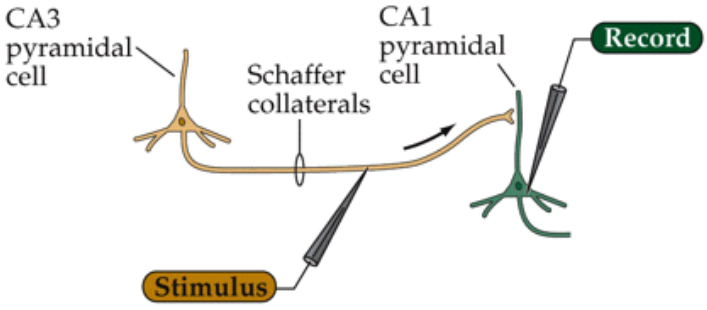


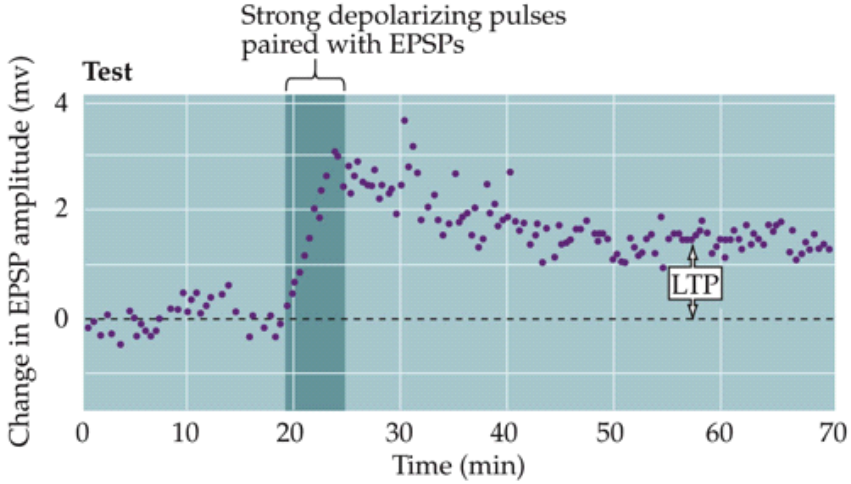
This increase in EPSP is maintained for many days/months over time

If LTP is maintained even with the post synaptic cell depolarized, what does that mean in terms of specificity?

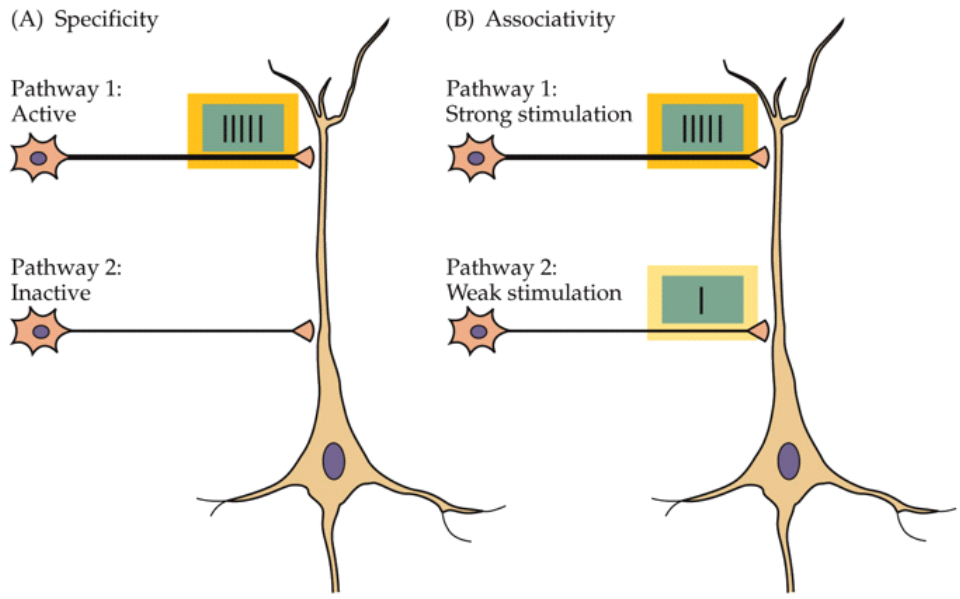
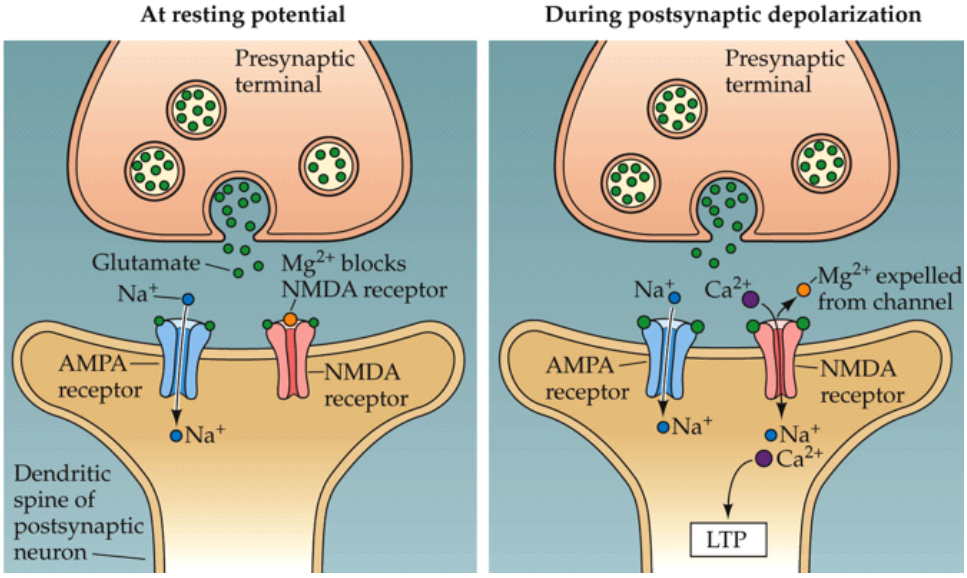
Post synaptic cell (CA1) is always depolarized

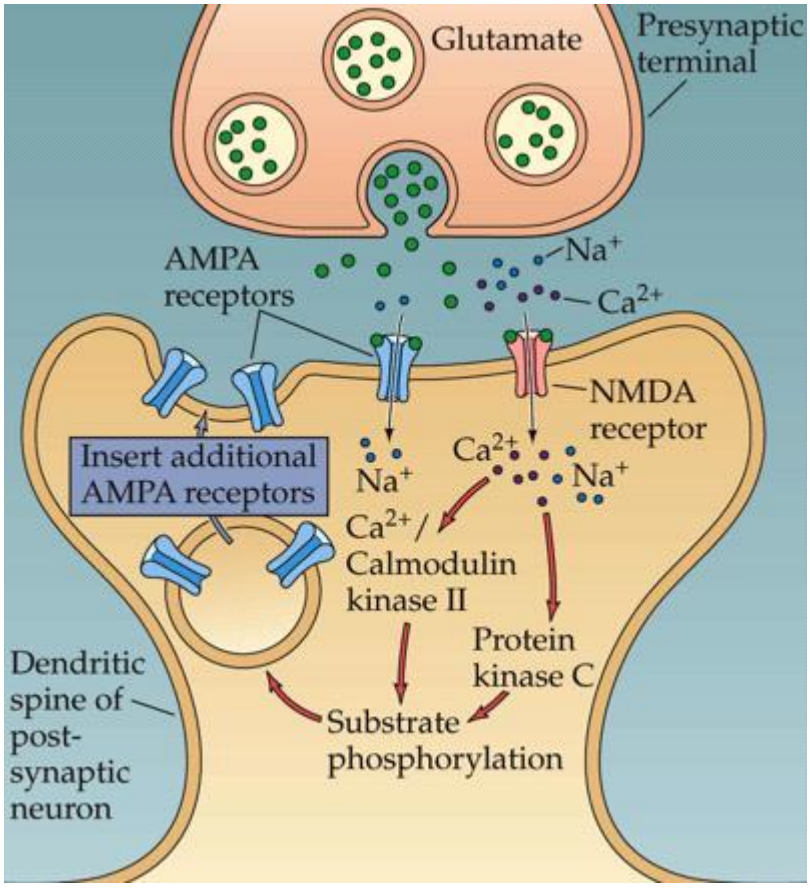
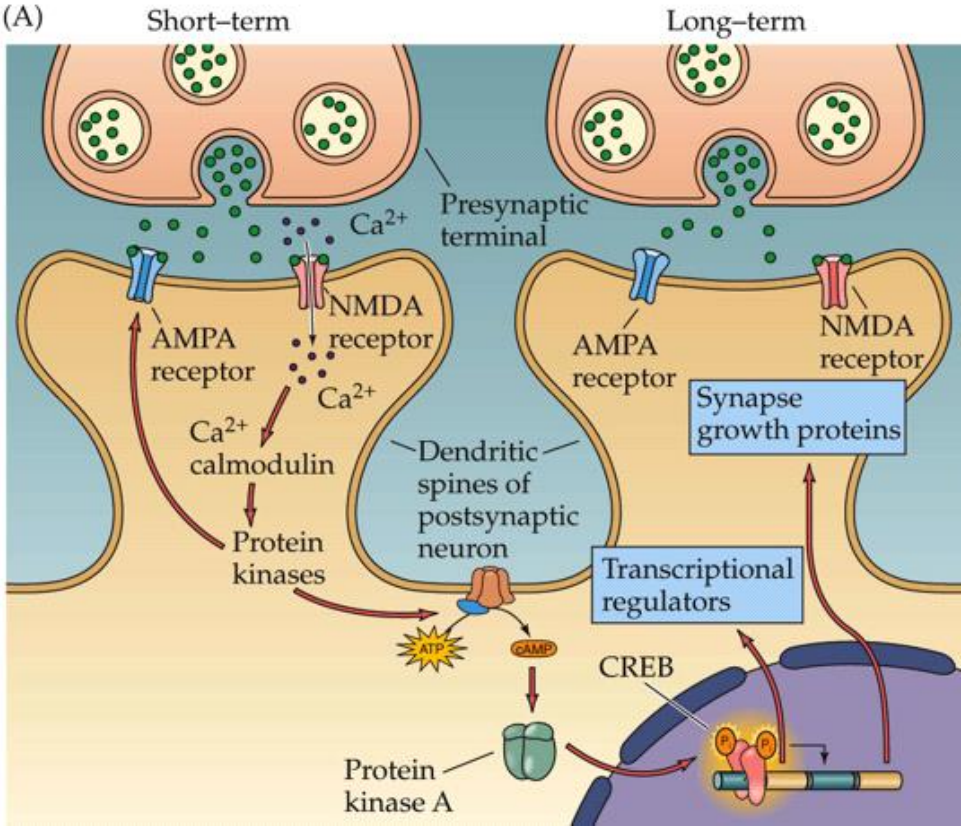
Strong depolarizing pulses with EPSPs

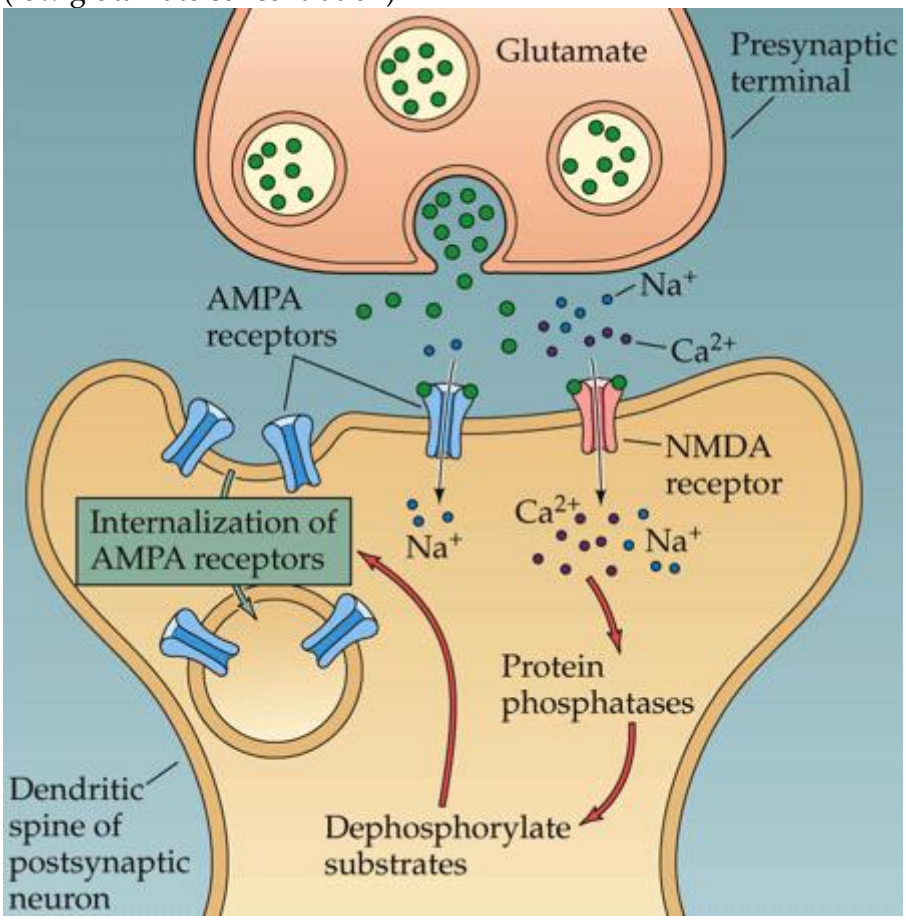
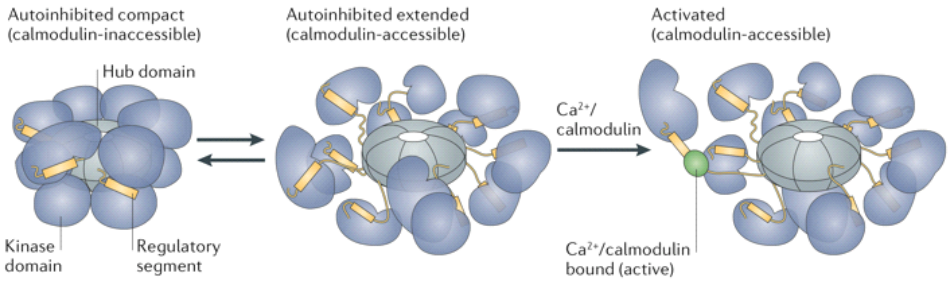


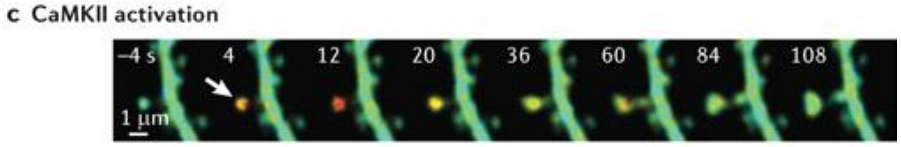
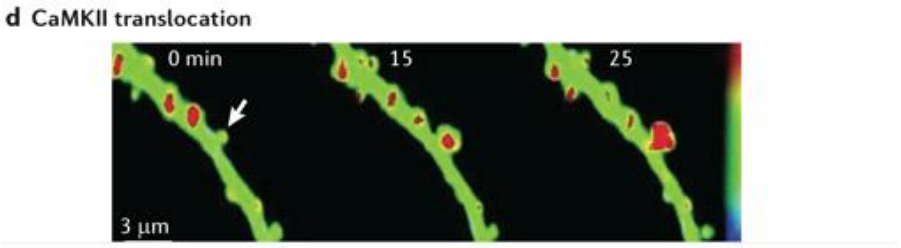
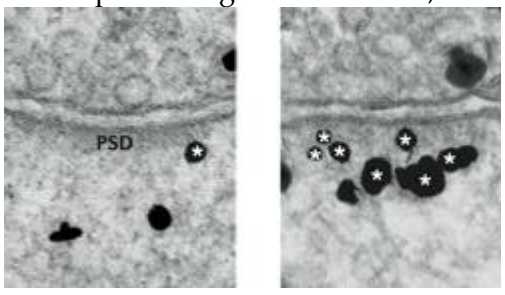
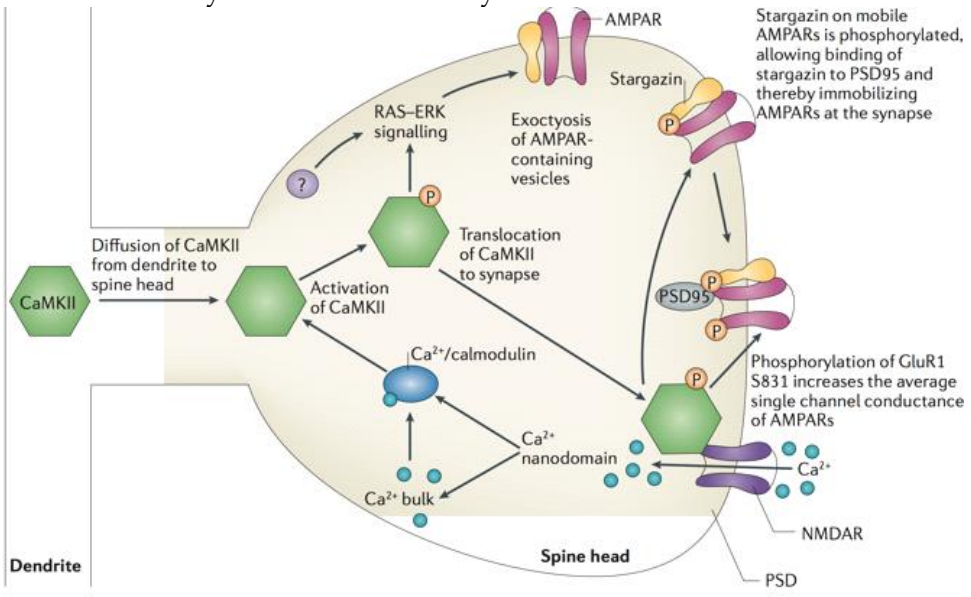


- Results in the same result - Even with a depolarized post-synaptic cell, indicates that the LTP is synaptic specific
- If two inputs come at the same time - they are paired in the post-synaptic cell (memories become coupled)

What is specificity and associativity?	 Specificity - Inactived cell are not strengthed Associativity - Weakly active cells are strengthed by strongly active ones
What is the basic mechanism by which LTP is mediated by AMPA and NMDA receptors?	LTP depends on coaction of AMPA and NMDA receptors  AMPA receptors - Depolarized by sodium NMDA loses magnesium - Depolarized by calcium If the circuit is not used -> NMDA is blocked by magnesium
What is the short-term	Signaling mechanism underlying LTP

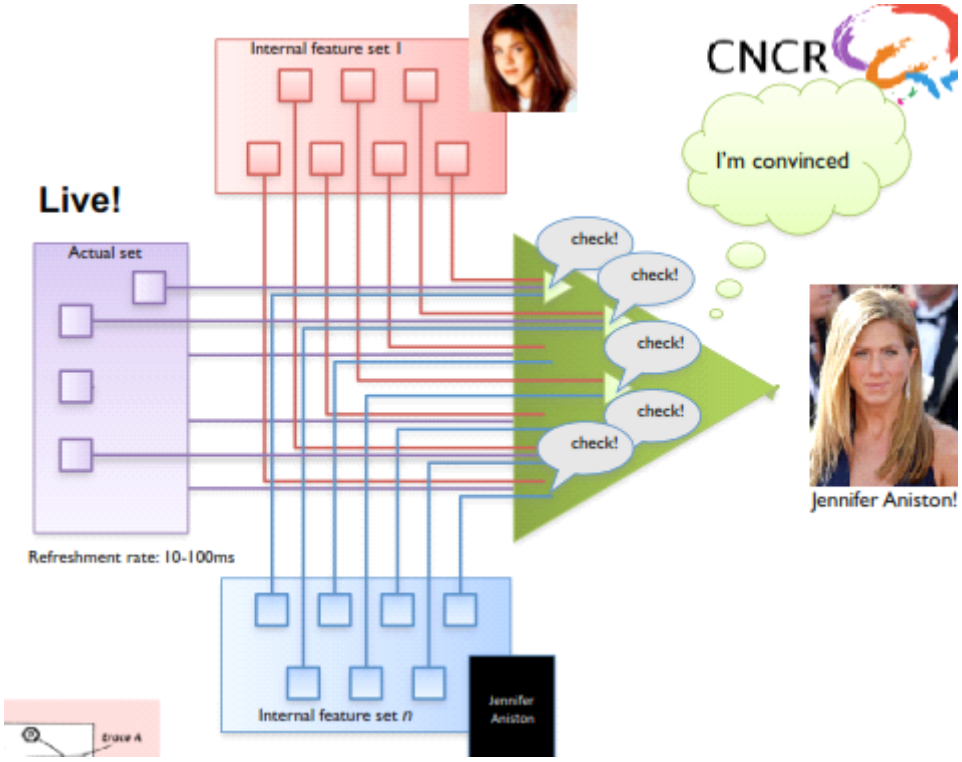
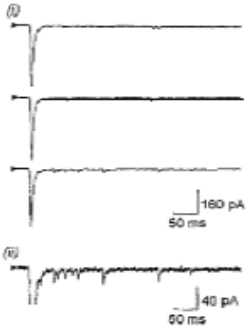
consequence of LTP?	 Calcium -> Activates CaMKII and Protein kinase C -> phosphorylate proteins -> Vesicles that contain AMPA receptors are added to the membrane (are already present in the cell)
What is the long-term change that LTP causes?	Long-term changes in synaptic transmission during LTP 

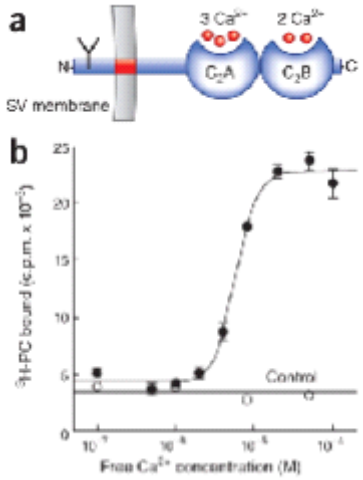
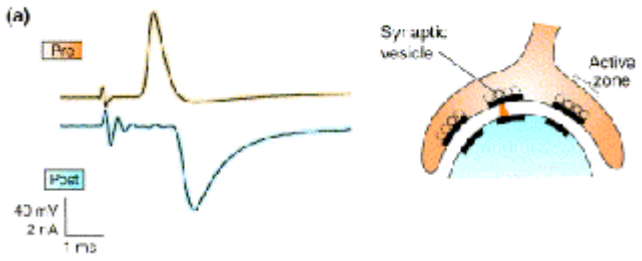
	Phosphorylation-> Protein kinase A -> Activates CREB and other transcription factors -> Active genes in the nucleus -> More AMPA/NMDA receptors -> Bigger synapses This takes a lot of time -> Memories become stronger (days/months) If you block gene expression hours after an event -> The memory is not stored
What is the difference between LTD and LTP?	Long term depression (LTD) - Happens due to low frequency stimulation (low glutamate concentration)  Calcium in a lower level Activation of protein phosphatasates Dephosphorylate substrates Internalization of AMPA receptors
How does calmodulin and calcium mediate the kinetics of CaMKII?	CaMKII activation by calmodulin  Kinase domain is inacessible in a state You need enough calcium to maintain the open the structure of CaMKII

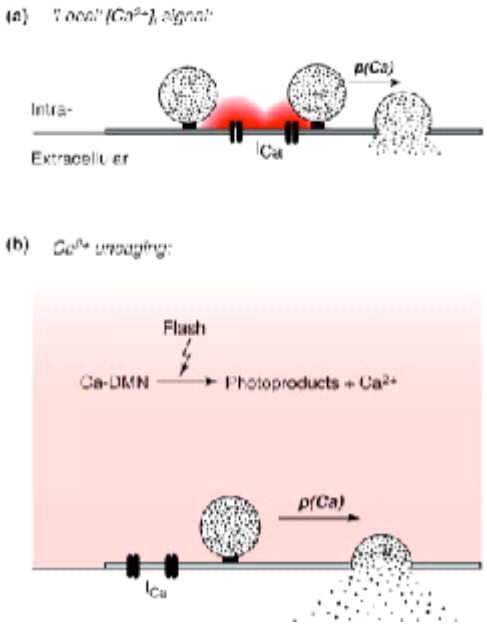
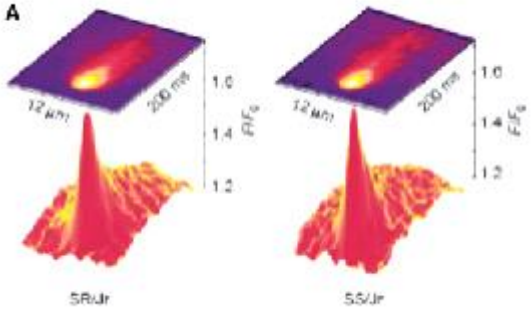
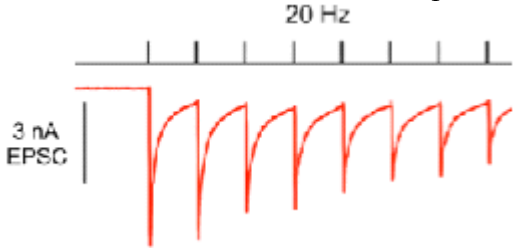
What happens to CaMKII in the neuron over time during the event of LTP?	Features of synaptic activation Calcium influx is localized in one synapse   CaMKII becomes more active with calcium CaMKII moves between different synapses
What experiments have been done to demonstrate the relationship between CaMKII and NMDA receptors?	CaMKII recruits to the postsynaptic neuron Black spots - Large ones CaMKII; small ones NMDA receptors  NMDA receptors without intracellular tail - No place where CaMKII can bind -> no LTP is observed Blocking the site where CaMKII is bound -> no LTP is observed also
How can the effect of LTP be sustained over a longer period of time, even if the high frequency stimulation stops?	CaMKII - After you activate the catalytic domain  Phosphorylation in CaMKII - It autophosphorylates itself (it stays active for a long time) Even if the input stops -> CaMKII still is active for quite some

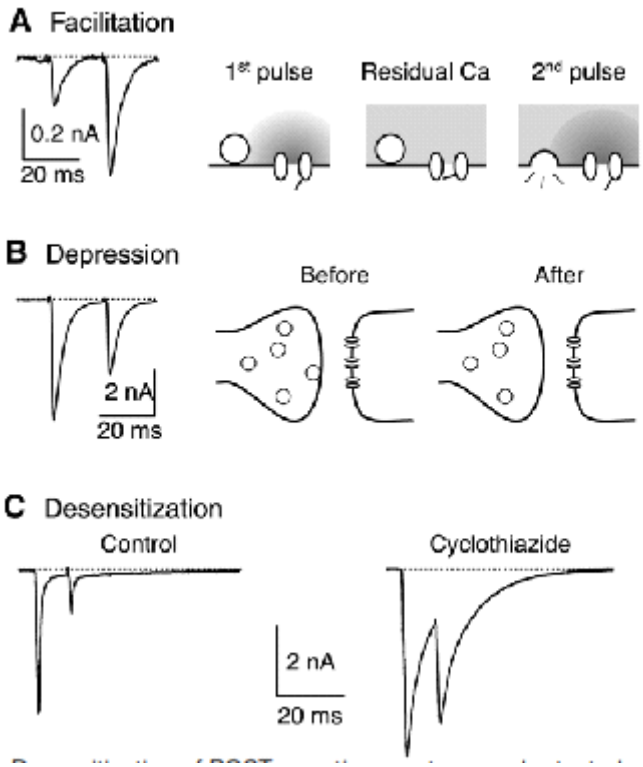
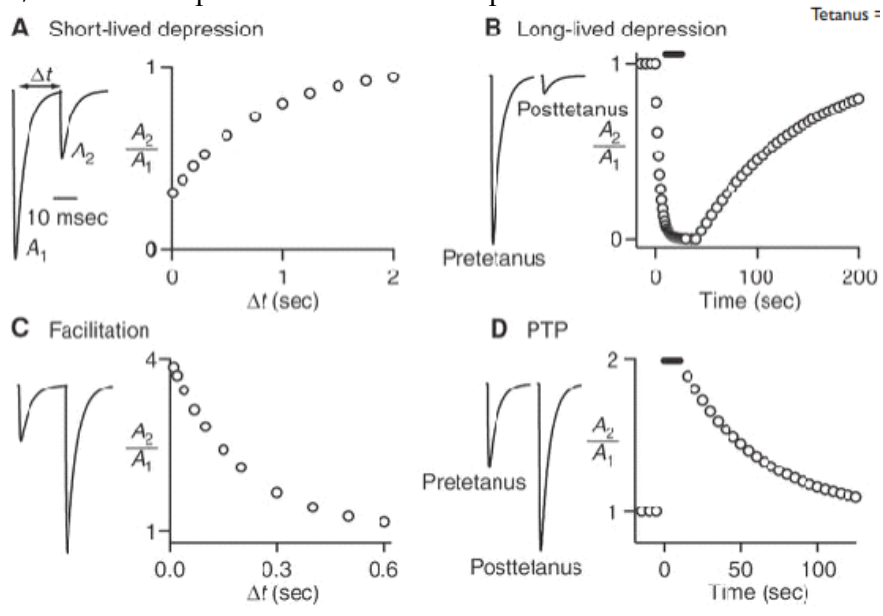
	time, increasing conductance of AMPA receptors

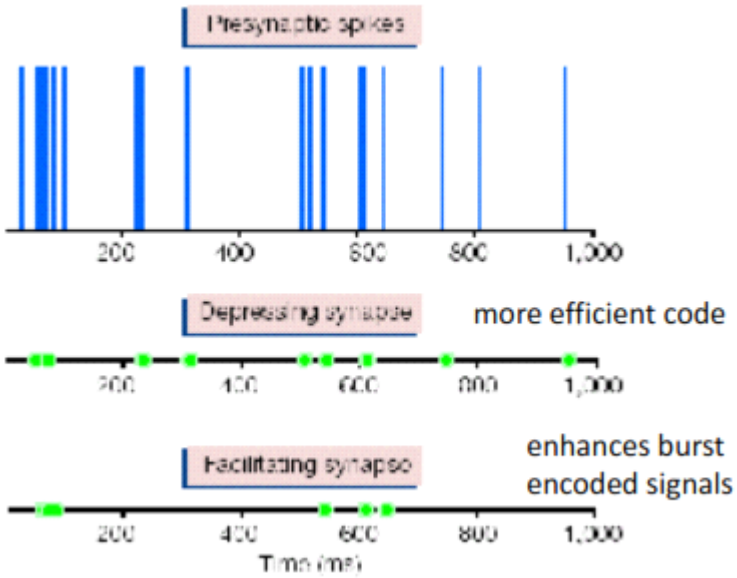
11. Synbio8: Short term plasticity

How can different sensory inputs - an image of Jennifer Aniston and the words 'Jennifer Aniston' elicit the the same response in the brain?	Live information received by sensory organs Comparisions to internal feature sets - Engrams are distributed in the brain  This comparision needs to happen in a very short time scale When these neurons fire together - more calcium available, higher probability of postsynaptic firing
If a synapse is unreliable, why does every stimulation produce a synaptic response?	Why does every stimulation produce a synaptic response? *200 synapses with a low release probability -> Still yields a very likely results of activation* 1 - 0.2^(synapses) - The more synapses you have, the more reliable the activation pattern is Voltage clamp - Electrode in the postsynaptic cell  Fixed voltage, measures current Net influx of positive ions (sodium) Downward deflection - The amount of current the amplifier

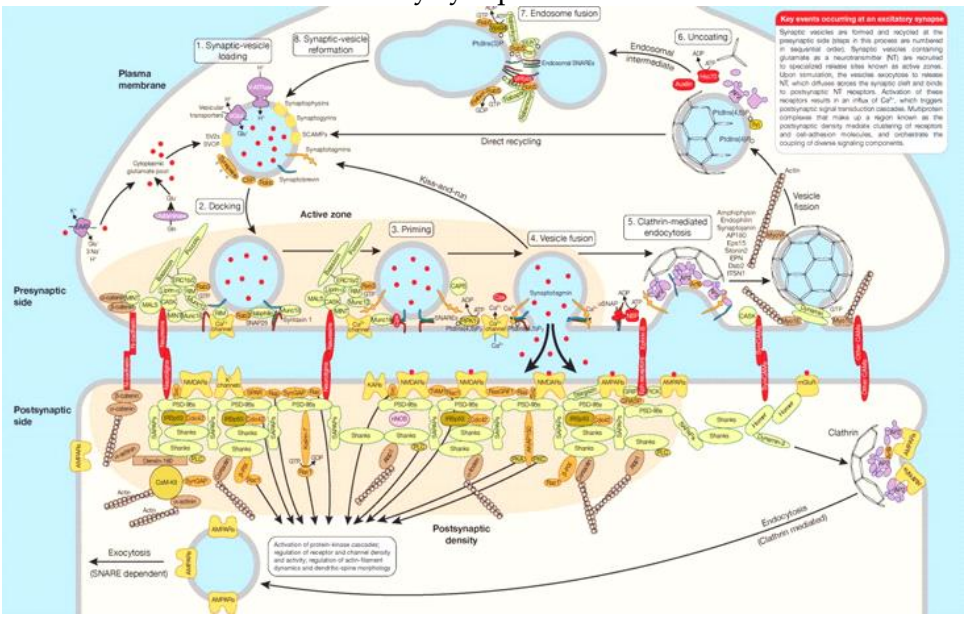
	needed to maintain the potential of the cell
Why is the affinity of synaptotagmin sensor not very high?	Synaptotagmin knockout Synchronous fusion is lost Asynchronous fusion increases Affinity of synaptotagmin sensor is not very high -> Otherwise, the offrate is very low, creates an irreversible system; The binding only happens when there is a lot of calcium -> synaptic transmission is really brief  Resting calcium concentration in synapses - 10e-5 is necessary to promote vesicle binding
Where is the calyx of Held present in the nervous system?	Calyx of Held - Thin layer of presynaptic structure, many release sites Unusual structure (incredibly reliable, but high energy cost) - Present in very localized and important systems. e.g. Auditory response (fight or flight)  Current clamp - Fixed current, measure voltage Voltage is proportional to the number of vesicles B - Voltage is reduced because there are not enough vesicles
Describe the advantage of using calcium gating.	Ca-DMN - Photoproducts + calcium - Reliable measure of calcium release throughout the entire cell - you can very the wavelength of light or the time of pulses Normally, increase in calcium is very localized

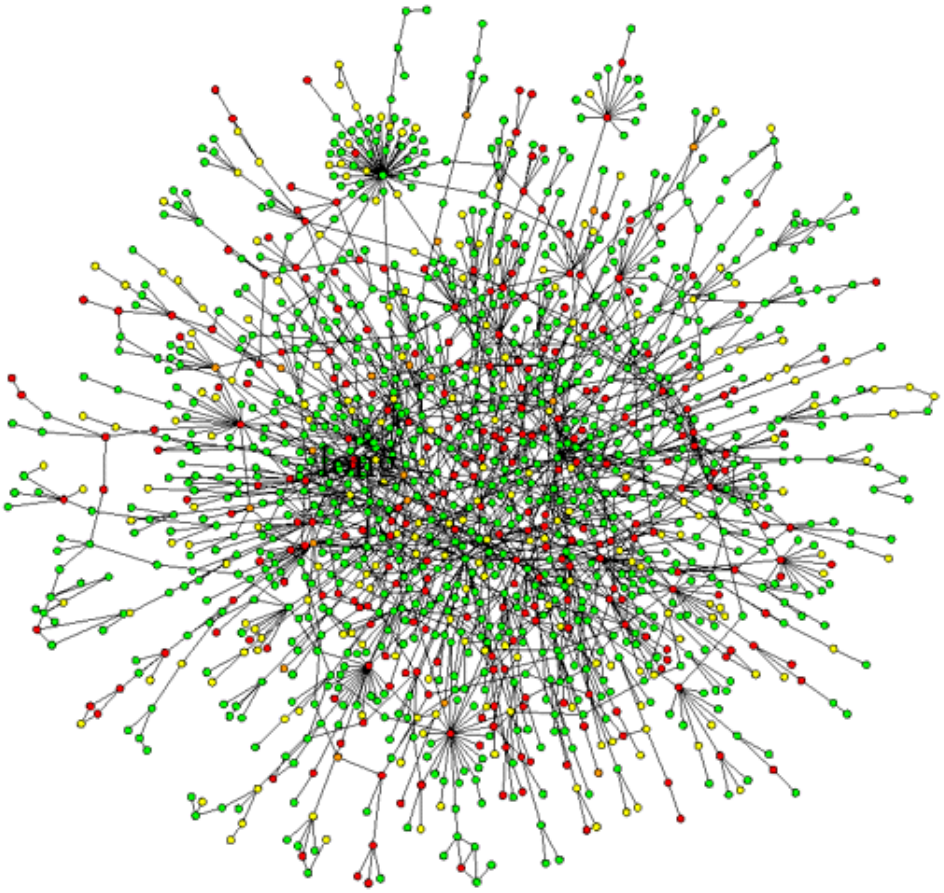
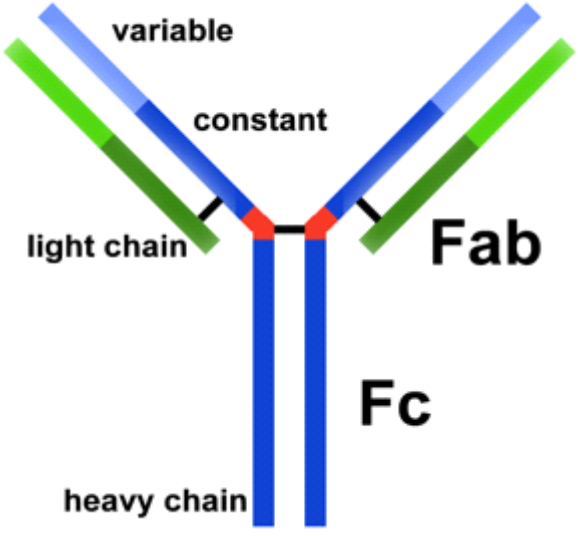
	 Relation between synaptic transmission and calcium A little more calcium gives a massive effect on the release rate
Why doesn't the increase of calcium always promove vesicle release?	Relation between calcium transients and release probability Calcium transients are local  If the vesicle is away from the channel, it does not bind -> Calcium channel coupling determines probability
What happens when you give a neuron a low excitation frequency (e.g. 20hz)? Why? **What happens when you give a neuron a high excitation frequency (e.g. 200 hz)? Why?**	Hard evidence - 20 hz - Decreases due to vesicle depletion - Depression  - 200 hz - More release initially (increased synaptotagmin binding to channels due to increased calcium concentration) - PTP (sufficient to overcome depression initially due to the immense calcium increase)
Describe the processes involved in short-term plasticity. **a) Facilitation** **b) Depression**	Short term plasticity: Facilitation & Depression Facilitation - Second pulse has an increased release probability (increased probability of vesicle binding) Depression - Second pulse has a decreased release probability (lack of vesicles)

c) Desensitization	 The same neurons have these two processes ocurring at the same time in different synapses/different extracellular calcium concentrations On the other synapse: Desensitization Depression due to diminished response of receptors (not the vesicles!)
What does A2/A1 graph measure?	Short-term, use-dependent plasticity on two timescales A2/A1 - Second pulse divide over first pulse  Measures the replacement of vesicles Takes two seconds to replace the vesicles to a naïve level Different synapses have different kinetics
In eletronic circuits,	Electronic circuits have similar behaviors

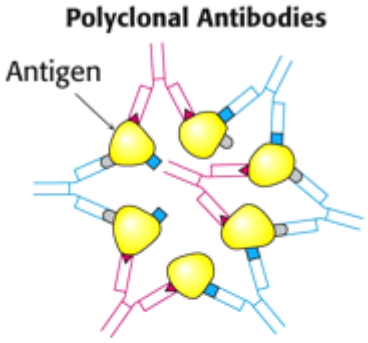
what is the equivalent of **a) Depressing synapses** **b) Facilitating synapses**	Low pass - Amount of total activity High pass - Counter  In synapses - Depressing synapses acts as a low pass filter (good at detecting events, not good at integrating information), facilitating synapses acts as high pass filter (not good at detecting events, good at integrating) Two different types of synapses -> Detect different types of information

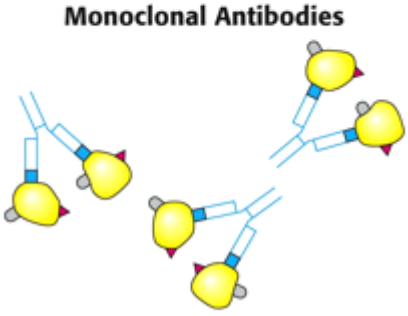
12. Dissecting protein interactions

How much information increases from the human genome all the way to protein interactions?	Dimensions of information complexity Genome: 25,000 genes Transcriptome: 100,000 mRNAs Proteome: 400,000 proteins -> millions of interactions
	The Architecture of an excitatory synapse  Alignment of presynaptic release sites with post synaptic receptors
Describe this figure.	Interactions in the yeast proteome

What does it mean?	 Some proteins only have one known interaction, others have dozens of known interactions
What are the four methods and tools for investigating protein interactions?	Methods and tools for investigating protein interactions Antibodies Immunoprecipitation Pull-down assay Yeast two-hybrid system
Describe the general structure of an antibody. **What is the difference between polyclonal and monoclonal antibodies?** **Which techniques to study protein interactions use antibodies?**	Antibodies  General structure - Heavy chain (blue), light chain (green), variable

region at the end (binds to amino acid, highly selective and high affinity)



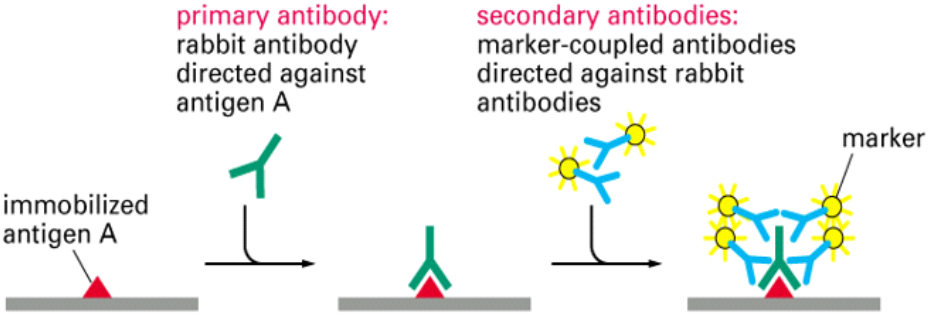


Polyclonal (different antibodies made by different cells) - Clump together -> can be separated by centrifuging
Monoclonal antibodies - More selective
Epitopes - Antigen part recognized by the antibody

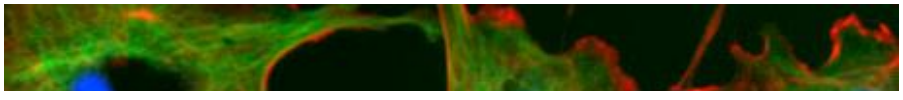
Analytical techniques that use antibodies:
Flow cytometry (sorting cells)
Gel electrophoresis (immunoprecipitation, immunobloting)
Microscopy - Immunofluorescence, electron microscopy
ELISA - Using antibodies to quantify proteins

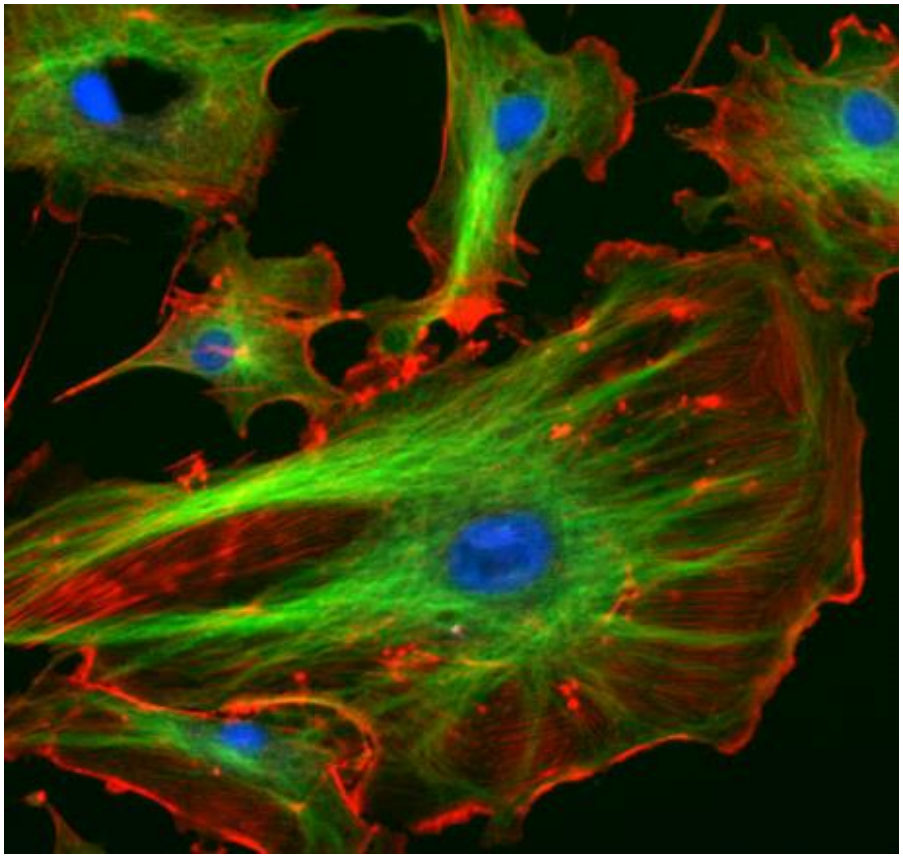
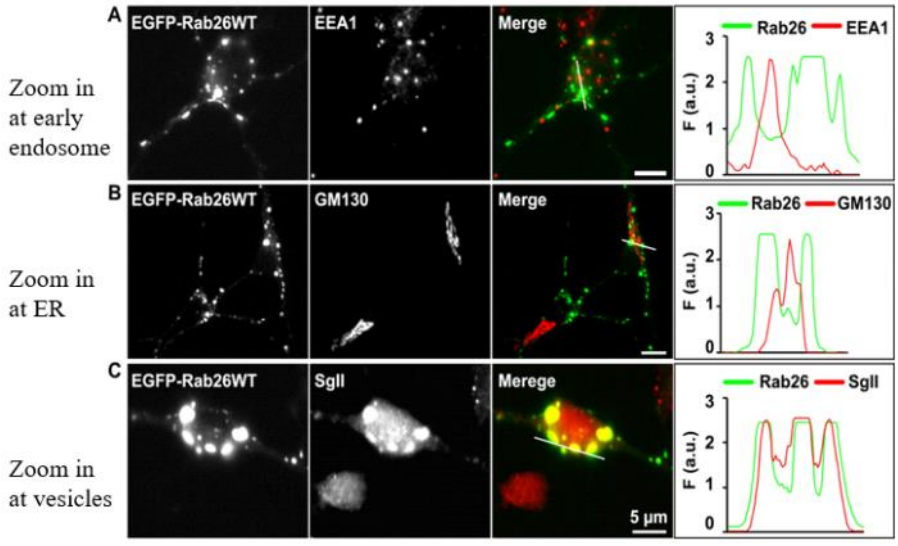
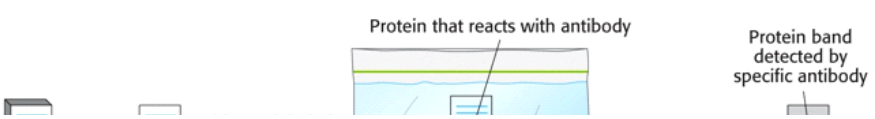
Describe how immunodetection works.

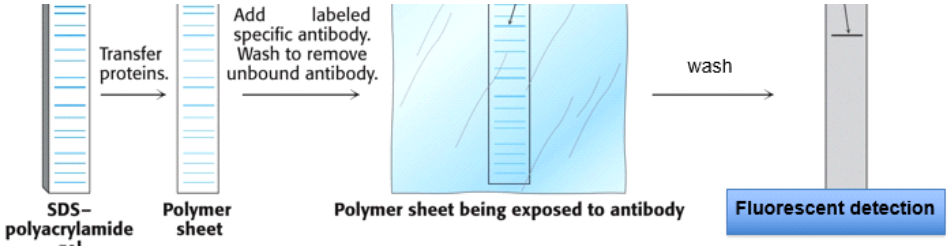
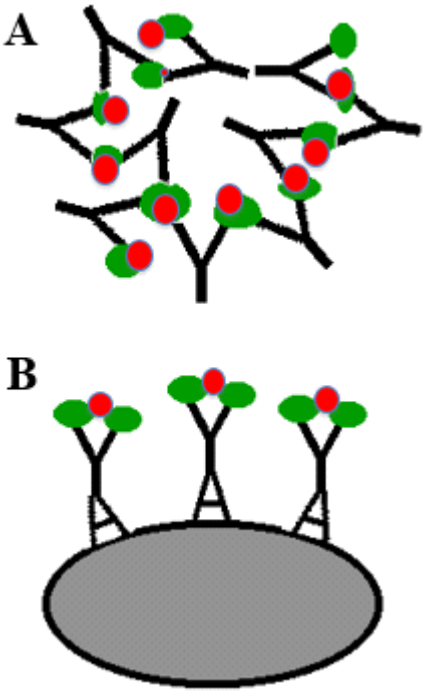
Immunodetection - The antibody detects the protein

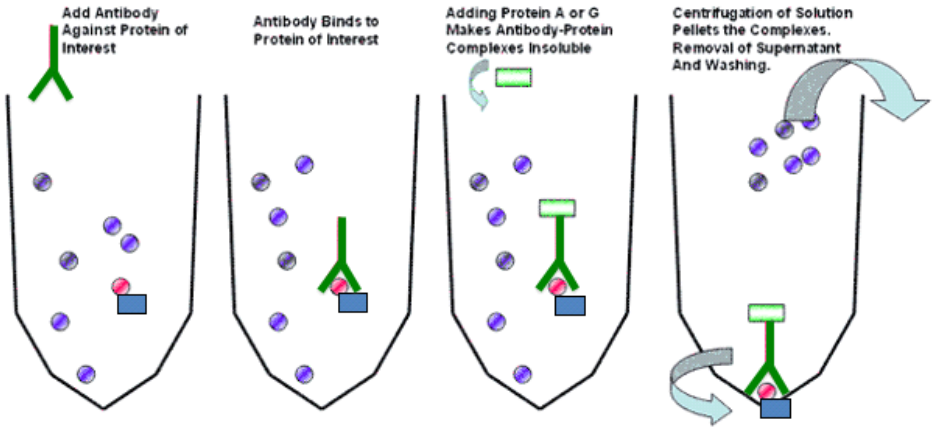


Primary antibody - Binds to antigen
Secondary antibody - Binds to primary antibody, has some marker (GFP)
Recognizes antibodies from particular species



	 Example: Detection of 3 cytoskeletal proteins (requires 3 different primary antibodies and 3 different secondary antibodies)
If you would like to investigate protein expression using immunodetection in three different organelles on the same cell, how many different primary antibodies and secondary antibodies would you need?	Colocalization of proteins to understanding their role in the cell Rab26 - Protein of interest (labeled in green) EEA1/GM130/SgII - Proteins specific to the organelle  Y-axis - Intensity of fluorescence X-axis - Distance (white line in the image)
Describe how immunobloting works. **What difference you would expect if you**	Immunoblotting (Western Blot) - Immunodetect specific proteins (e.g. separated by size via SDS-PAGE) 

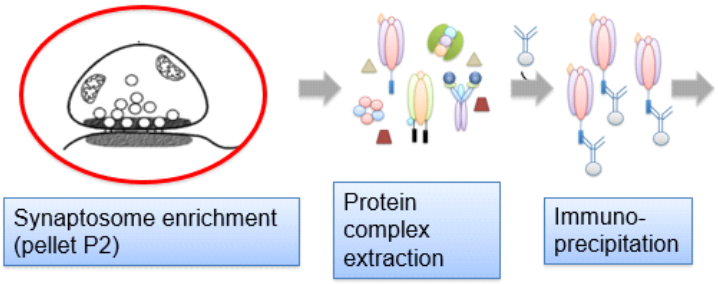
used polyclonal or monoclonal antibodies for a Western Blot?	 Polymer sheet exposed to antibody Fluorescence/Enzymatic detection -> Enzyme converts substrate into product (which is colored or fluorescent) Polyclonal antibodies -> often show multiple bands Monoclonal antibodies -> often show only one band
Describe how immunoprecipitation works. **What is the difference in using polyclonal or monoclonal antibodies in an immunoprecipitation essay?**	Immunoprecipitation - Affinity purification based on complexes of proteins A + proteins B  A - Polyclonal antibodies (separation by centrifuging) B - Monoclonal antibodies (binds to a bead via the heavy chain -> separation by centrifuging)
Describe a typical immunoprecipitation essay.	Typical immunoprecipitation protocol 1. Solubilize protein - Non-denaturing 2. Mix extract and antibody 3. Add bead-Ab 4. Wash 5. Elute with sample buffer (detergent) 6. SDS-PAGE 7. Detection - Protein staining (e.g. Comassi blue) or immuno-detection

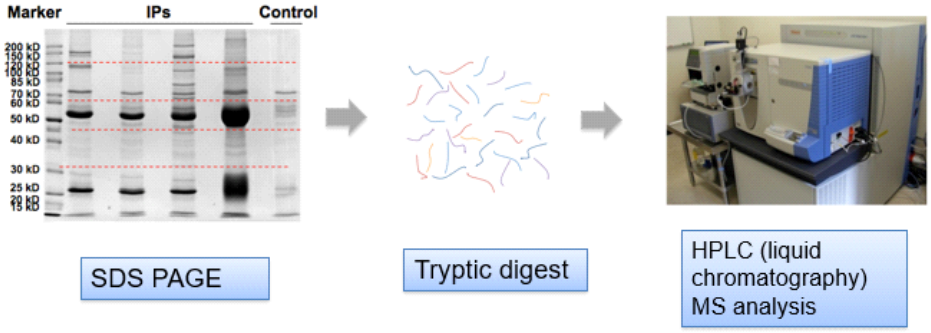


Validation requires controls - Only beads, antibodies and no beads

How could immunoprecipitation be used to study proteomics of an entire synapse?

IP interactome proteomics analysis



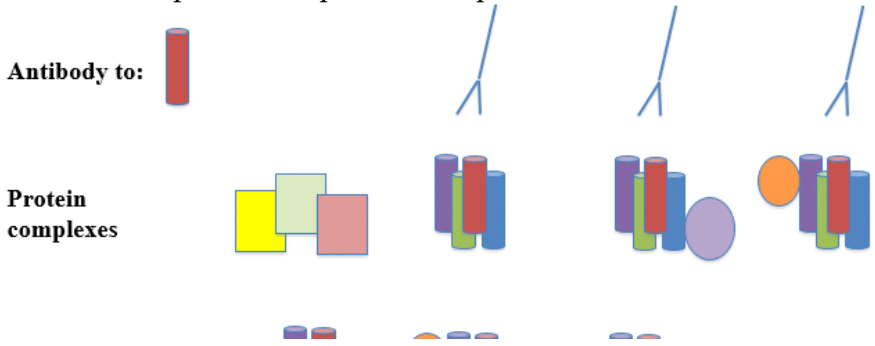


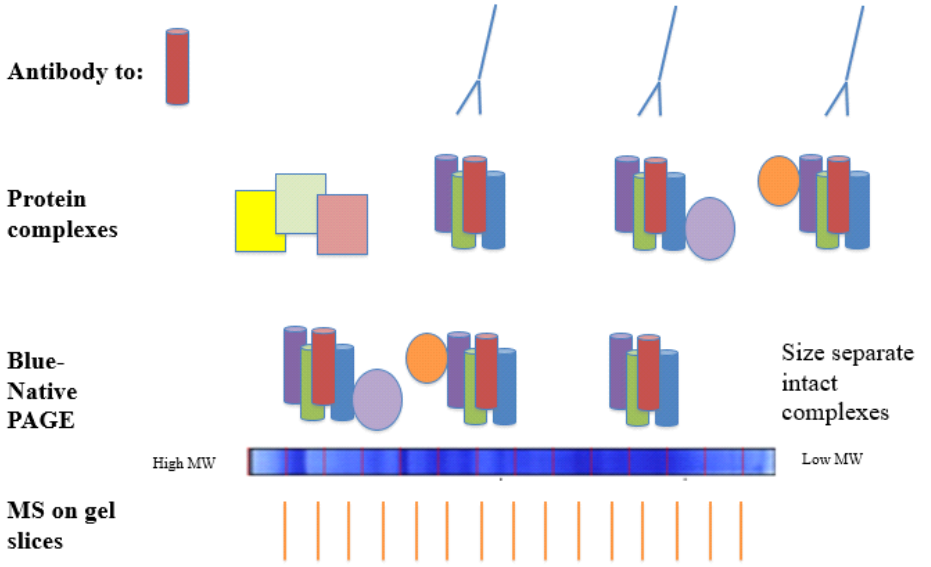
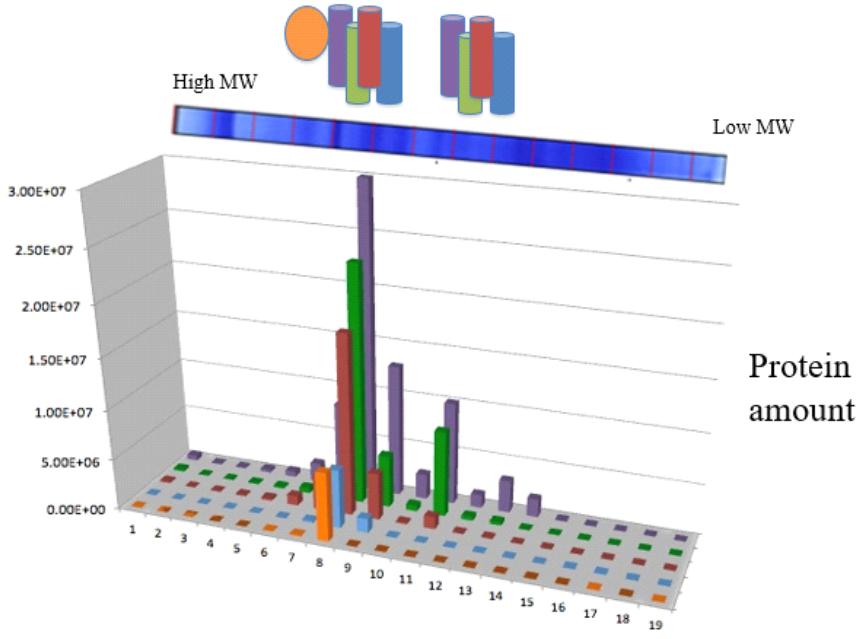
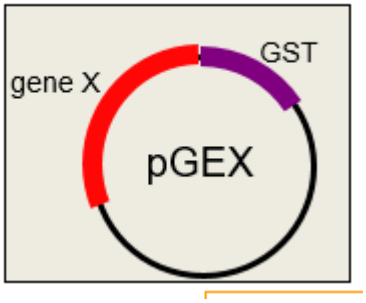
Proteins for an entire synapse
Immunoprecipitation
SDS-PAGE
Trypic digest
HPLC and Mass spectrometry -> List of all the components, but does not tell you the composition of individual protein complexes
Solution: BlueNative-PAGE

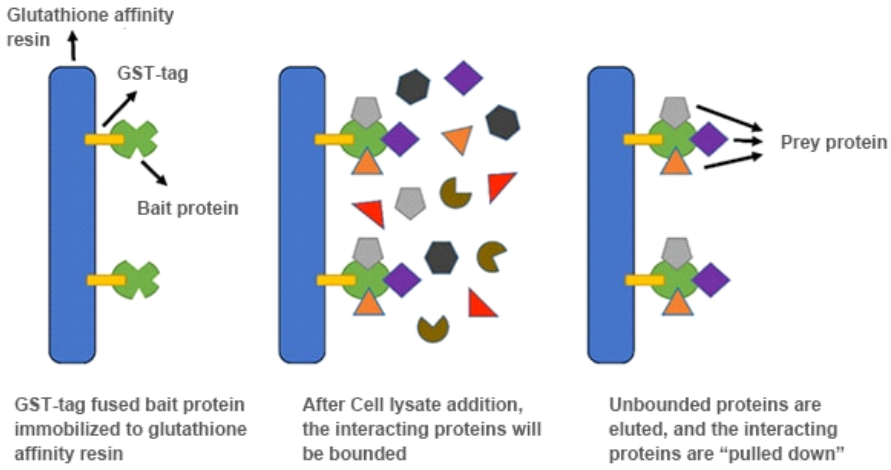
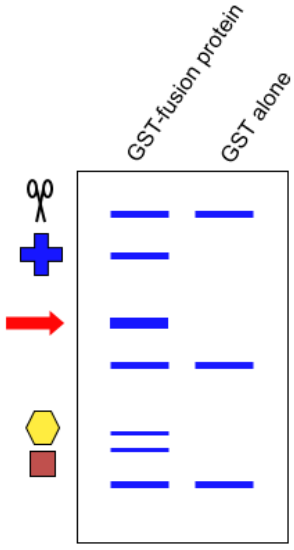
What is the difference between a BlueNative-PAGE and a SDS-PAGE?

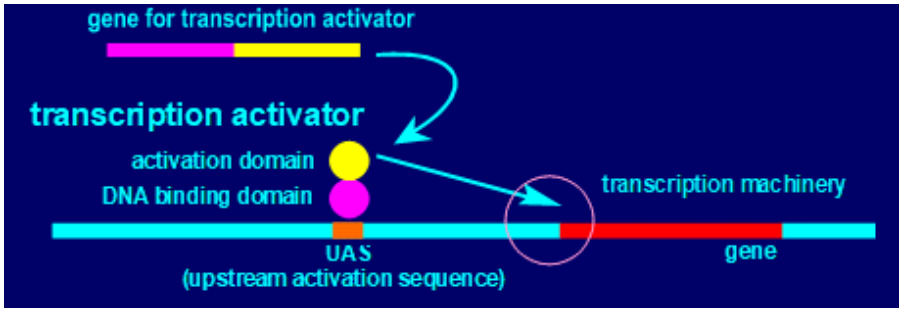
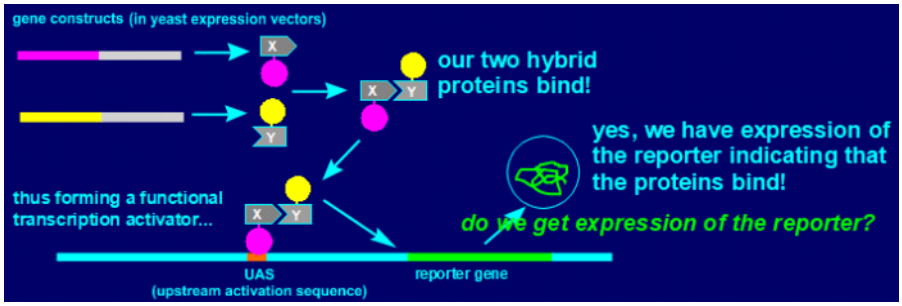
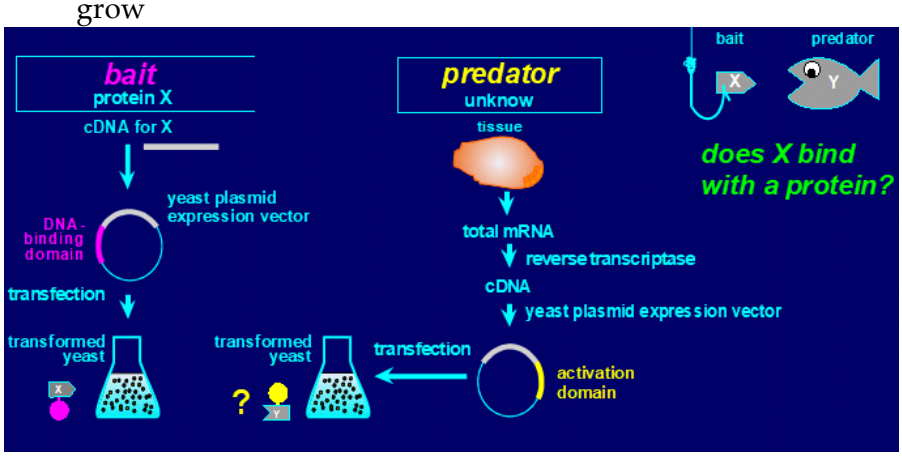
What do you do after the BlueNative-PAGE gel is complete?

Individual composition of protein complex - BlueNative-PAGE



between a BlueNative-PAGE and a SDS-PAGE? **What do you do after the BlueNative-PAGE gel is complete?**	 No SDS - Proteins are functional Separation of intact protein complexes Slice the gel for the different bands and perform mass spectrometry for each one 
Describe how a pull-down essay works.	Pull-down Plasmid - Express protein of interest fused with GST (binds to bead/known column protein) 

	Protein of interest binds to endogenous partner Pull down in a column - Only proteins with GST will bind  Then separate - pH or enzymatic reaction BlueNative-PAGE  Can be used to identify protein interactions in vivo - Allows identification of individual interactions
What are the advantages and disadvantages of immunoprecipitation and pulldown?	IP and pulldown - Identify protein complexes Disadvantage - Low-affinity interactions cannot be identified -> alternative is yeast two-hibrid
How the yeast two hybrid method takes advantage of the ways genes are expressed in	Yeast two hibrid - Can identify one-to-one interactions, including low affinity How gene expression works in yeast - Transcription factor has an activation binding domain + DNA binding domain

yeast? **Describe how the yeast two hybrid method works?**	 Separate DNA binding and transcription factor domains -> You can then add proteins of interest Formation of a hybrid protein complex that can activate the gene  Fuse protein X to DNA binding domain in a plasmid -> Perform transfection Unknown protein fused to activation domain -> Transfection in other yeast cell group Mate two yeast cells -> Retransformed yeast If reporter gene is active, then X and Y are present and are binding -> Yeast are able to grow Isolate plasmid from colonies and sequence it X-Y binding can be quantified by the rate of growth in the yeast colony - The lesser the affinity, the longer they take to grow 
What is the main disadvantge of the yeast two hybrid method?	Yeast two hybrid need validation - May provide false data (proteins that never interact in the endogenous cell environment) E.g. IP, immunocytochemistry -> X and Y should always be together! If they are separated, the result is a false positive