Neuroscience M101B Notes

Lecture Notes

1/8 - Week 1

General Info on Nervous System Creation

- Course covers the development from an omnipotent cell to a brain and all the other types of cells and organs in our nervous system.
- The growth of the human brain depends on transcriptional regulators, signals, and adhesion/recognition molecules that determine what/where cells should form and how they should interact.
- Neural circuits continue to change after birth depending on the different activities that you do.
    - Causes different synaptic efficacy, gene expression, and neuronal growth.
- Capacity of the mature brain for repair is limited.
- There are 3 body axes
    - Anterior posterior: Mouth-anus
    - Dorsal ventral: Back-belly
    - Medial lateral: Midline-periphery.
- Hard to figure out how development in mammals occurs because everything is in the womb.

Gastrulation

- Gastrulation is the process of the creation of the 3 germ layers and it's the start of neural development. The whole nervous system gets created through inductive interactions between neighboring cells. Specifically, it is the conversion of the blastula to gastrula.
    - The embryo starts as a continuous epithelial sheet of cells and ends as embryo that has differentiated into different layers.
        - In terms of the development of those germ layers, there is first a splitting of ectoderm and mesendoderm, and then the mesendoderm splits into the mesoderm and the endoderm.
    - Gastrulation defines the locations of all the axes which determine the position of all the organ systems.
    - It is also the process of conversion from blastula (stage where embryo is single layer hollow sphere of cells called blastomeres) to gastrula (more differentiated embryo with 3 germ layers and basic axes of the body)
        - When the embryo is still a blastula, there is group of cells called involuting marginal zone that grow on the interior at the point called the blastopore. Those cells form the mesodermal tissues and then cause the cells in the ectoderm to become neural tissue. Without the mesoderm, that overlying ectoderm would stay as skin (aka before gastrulation, skin; after

gastrulation, neurons). That tissue ends up becoming the neural plate, which ends up generating the neurons and glia in the brain and spinal cord.

    - When embryo is gastrulating, first key event is the formation of the notochord (cylinder of mesodermal cells) at the midline. This notochord defines the embryonic midline and the axis of symmetry for the body.
        - The job of this notochord is to define where the midline is, determine where the position of the nervous system should be, and then it goes away. It is transient.
        - The notochord also sends signals to the ectoderm causes a subset of cells to become neuroectodermal precursor cells. This is called neurulation. Those cells thicken into a neural plate, which folds inwards and becomes a neural tube. The neural stem cells within the tube give rise to the brain and spinal cord.
- Each of the 3 germ layers gives rise to specific tissues and organs in the developing embryo.
    - Outer ectoderm - Creates epidermis (skin), nervous system
    - Middle mesoderm - Creates skeleton, muscle, bone, and connective tissue.
    - Inner endoderm - Creates epithelium of the digestive system and respiratory system and organs of digestive system (gut, liver, bladder, etc).
- Animals that have yolks will have cell divisions that result in cells of different sizes. Also differences in the RNA and proteins between the cells. This already helps to create differentiation between the new cells that get made because the cells each have different proteins and thus different capabilities.
- As time goes on, the fates of cells, specifically genes inside of cells get turned off and on depending on the function of that cell.
    - The transcriptomes (set of RNA molecules inside a particular cell) are different for each cell type even though the genome is the same in each cell.
- Interaction between neural plate and mesoderm determine the boundaries between nervous system and rest of body.
- Brain forms in a particular area because of the particular cells and the proteins they have.
    - Mesoderm induces the overlying ectoderm to become brain tissue and not skin. This is due to the expression of certain proteins in the dorsal blastopore lip. The signaling proteins that it has tells the ectoderm to be neural tissue. If you transplant it to the ventral part, then the nervous tissue will grow in that area. A lot of the host cells will become induced to be nervous tissue. Basically, the dorsal lip cells are the organizer that causes the host cells to form a new body axis.
    - Basically, there is some factor in the mesoderm that induces the ectoderm to become neural ectoderm. And that factor must induce neural tissue without inducing mesodermal tissue (because if it did, we wouldn't know if it was this

tissue or the newly created mesodermal tissue that induces the ectoderm's neural tissue).
    - Indirect induction of neural tissue is when both mesoderm and neural ectoderm tissue gets created.
    - Direct induction of neural tissue is when just neural ectoderm tissue gets created.
- The neural ectoderm is now called the neural plate, and that region is now restricted to only producing neural tissue. So basically, at this point in development, you just had the mesoderm induce neural ectoderm (now calling that region the neural plate). That neural plate is flanked on either side by normal ectoderm. Eventually, that neural plate is going to roll up into a neural tube which kinda connects to normal ectoderm regions back together. The neural crest is the top of the neural tube (basically the regions where the folded plate come together) and the cells that arise from that region migrate to areas between the tube and the somites. They are undifferentiated and according to their position and migration, they get exposed to different transcription factors causing them to change into different types of cells. At this point, the notochord is also forming underneath the neural plate/tube from mesodermal tissue.

Neural Induction

- Neural inducers - If you put them in the ventral region then you will get two nervous systems. These are factors that are expressed in the blastopore lip.
    - Noggin is an example of a protein that is a neural inducer
    - Chordin is another example
    - Follistatin is another example
- The first two are proteins expressed in the dorsal blastopore lip, or more generally from the cells in the IMZ.
    - These cells are the ones that form mesodermal tissue and I'm guessing that tissue also contains the neural inducer proteins that affect the overlying ectoderm.
    - All 3 of the neural inducers (noggin, chordin and follistatin) all promote the formation of anterior neural tissue.
- Default condition of the ectoderm is to make epidermis because BMP pathway is normally activated. The ectoderm is always exposed to BMP that is getting released from the somites and mesodermal tissue. When it is exposed, ectoderm -> epidermis.
- BMP signaling impairs neural induction. BMP signaling basically induces epithelial (skin) tissue. No BMP signaling gives nervous tissue.
    - The receptor for BMP is membrane bound and so therefore if cells are touching (and you get cell-cell signaling) or if you pour BMP over the cells, then you get BMP signaling and this means epidermis. But when the cells are separated, then BMP won't bind with its receptor, and there would be no signaling and Sox will not be inhibited and thus there would be no signaling.
- Noggin and Chordin and Follistatin bind to BMP which causes BMP to get blocked from attaching to its receptor. Sox is a transcription factor.

    - Let's say BMP signaling is on which it normally is. Then, Smad is a protein that turns on, and induces the transcription of the genes Gata and Msx. Those proteins inhibit Sox transcription, and that creates epithelial.
    - However, when the neural inducers are around, then BMP signaling turns off, the proteins are not turned on, causing the disinhibition of Sox and the creation of neural tissue.
    - Those 3 proteins (Noggin, Chordin, Follistatin) are the things getting released by the mesoderm, and that's what's getting in the way of BMP signaling.
- As a side note, Cerberus is also a BMP antagonist.
- In conclusion, the inhibitory effects of those 3 proteins on BMP signaling causes the neural induction of the overlying ectoderm (aka creation of the neural plate).
- The ectoderm is exposed to non-homogenous proteins as a result of the position, the overlying ectoderm is seeing different levels of proteins and that impacts the overall creation of the neural plate.
- Fgf is another protein that when it binds to the receptor on the cell, it will activate Sox and that has the effect of inducing neural tissue. It also turns off BMP expression. So this could be another pathway.

Neural Stem Cells and Creation of Neurons

- Need to make a whole bunch of stem cells so that we can create all the different types of cells rather than everything becoming neurons and the offspring being neurons.
    - Stem cells in the embryo are what generate the entire nervous system. Overall, they are what create the neurons, there are glial cells, and the neurons move from generation to their final positions.
    - One of the key points about neural stem cells is that they can divide into more precursor cells and they can give rise to the full range of cell classes, including neurons, astrocytes, oligodendroglial cells, etc.
- Neural stem cell division results in more neural stem cells.
    - Those stem cells will be either radial glial cells (which are a type of NSC) or they will differentiate into actual neurons.
    - Can also become oligodendrocytes form the myelin which is called the white matter, and the actual neurons are called the grey matter.
        - Those oligodendrocytes can also become astrocytes.
- Basically, all the neurons that make up the CNS come from the neural stem cells.
- Factors released by neurons will tell the other neural stem cells to become either oligodendrocytes, astrocytes, etc depending on what cell type is needed.
    - Cells are born into constantly changing environments.

Delta Notch Signaling

- Notch is a receptor protein and Delta is the corresponding ligand. Notch is a protein that is present on neural stem cells and they are key regulators of the decision for the cell to generate additional stem cells or different neurons.

    - Same sort of impact on our development from neural stem cells to either neurons or oligodendrocytes, astrocytes, etc.
- Signaling via Delta ligands binding to Notch receptors happens only between cells that are next to one another.
- When Delta ligands bind, this turns on suppressor of hairless, which then turns on enhancer split proteins, and then inhibits ASC, which has a positive relationship with Delta expression. Enhancer split, at the same time, has a positive relationship with Notch.
    - So Delta signaling to another cell will inhibit ASC and will cause that neighboring cell to express less delta.
    - Basically, one cell will express more Delta causing a higher level of Notch and lower Delta expression in the adjacent cell.
- When Delta ligands bind, this kicks off a process where HES genes are turned on, and this encourages the cell to become an actual neuron.
    - Cells with low levels of Delta remain neural stem cells.
    - To be specific, when the ligands bind, this induces proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to modify gene expression (HES).
- Local Delta-Notch signaling among neighboring cells leads to downregulation of Delta in several cells (thus diminishing signaling capacity), but to its upregulation in one or a few of the neighboring cells. This means that there will be cells with low levels of Delta surrounding a cell with high levels. That cell will high levels will end up being a neuron, and the others will remain progenitor cells.
    - The effect of local cell level interactions on neural differentiation is one of the key points of Delta Notch signaling.

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Creation of Different Neural Cells

- Ventricular zone is where neurons are born. It primarily contains neural stem cells at first and most importantly, radial glial cells. That birth process is neurogenesis and radial glial cells help with it by acting as these rods that migrating neurons can move on. This is where the neural stem cells are dividing and this is the origin point where all the neurons migrate from. Specifically the location of the VZ is the lumen of the neural tube. The VZ is the outer layer of the tube.
    - At the beginning, we have a lot of neural stem cells or progenitors in the VZ, but over time, more and more of the created cells are neurons.
    - There are distinct stages in making progenitor cells vs making neurons.
- The neural tube will start folding in on itself which creates the outer form of the brain.
- The cells in the different areas of the neural tube are different since the leading edge of the mesoderm is different from the trailing edge.

Why Neurons Before Others (Astrocytes, Glial, etc)

- HES is a homolog to E(s). HES is in mammals.
- Different genes end up turning on which causes the differentiation of cells
    - Ngn for motor neurons
- HES will suppress Ngn and thus the cell will remain in the VZ and stay as a stem cell.
- The first cells will be neurons, then oligo, then astro
- Turning on of Ngn gene leads cells down a fate of becoming a neuron. Wnt signals are the ones that bind to the open chromatin at the Ngn loci and activate the expression of Ngn on those neural progenitor cells. At the same time, we also inhibit astrocyte fate.
- In the case where the cell is becoming an astrocyte (in postnatal neural stem cells), the transcription factor (Wnt) can't bind to the open chromatin, since the Ngn loci have been methylated and bound by PcG. Thus the chromatin is closed and Ngn can't be turned on, meaning that the NPC will go toward an astrocyte fate.
    - The genes that encode for astrocytes are methylated and thus transcription factors can't bind.
- Once the Ngn neurons are made, those neurons express factors (which weren't there before the neurons were made) cause the binding to the chromatin and the astrocyte gene to get turned on.
    - Thus neurons have to be made before the astrocytes can be made.
- As the concentration of neurons build up, we start ramping up the production of oligodendrocytes and astrocytes. There is the suppression of neuron creation, I guess.
- The leading edge of the IMZ will pattern the head, trailing edge will be the tail.

Impact of SHH and BMP on Neuron Creation

- There are also changes from front to back in addition to top and bottom. The motor neurons are in the bottom (ventral) and the sensory neurons' axons are in the top (dorsal).
    - Sensory neurons will be more dorsal, and motor neurons will be more ventral.
    - This is caused by the underlying mesoderm. The notochord is a specialized form of the mesoderm. Somites are also created and that portion of the mesoderm is affecting the neural tube.
        - To clarify, somites are located to the sides of the neural tube and the notochord is located underneath.
- The mesoderm is also undergoing differentiation in the form of the creation of the somites and the notochord.
- Notochord (as well as the floorplate) will release SHH and that is a transcription factor and the gene expression of the neurons created in that ventral area of the VZ are affected. There is a lot of difference between dorsal and ventral neurons because of the difference in exposure to SHH.
    - Dorsal side gets exposed to Wnt and BMP
        - The reasoning is that before the neural tube was created, it was a neural plate that was flanked by parts of non-neural ectoderm which allowed for BMP signaling. In between, there was also a part that expressed Wnt as well. So when, the plate rolled up and became a tube the top/dorsal side

of the tube gets that exposure. It's also getting exposure because above the neural tube lies the non-neural ectoderm where BMP is expressed.
        - BMP originally inhibits neural tissue formation in early development, but once the neural tube has formed, the BMPs now act to induce dorsal neural structures.
        - Wnt will have high concentration posterior and low concentration anterior and will thus induce the posterior structures. All the head organizers will inhibit Wnt.
    - Ventral side gets exposed to SHH. SHH induces ventral differentiation.
    - Every cell along the dorsal ventral axis of the tube will be exposed to different levels of the transcription factors because SHH and BMP are acting antagonistically to each other.
        - Through this mutual antagonism they set up opposing gradients that control both the polarity of spinal cord differentiation and the amount of spinal cord tissue that differentiates into dorsal, ventral, and intermediate cell fates.
- In the absence of the notochord, you don't have SHH, then you don't get ventralized structures, which include the motor neurons and the flooplate. The presence of these are the characteristics of ventral differentiation.
- If we transplant the notochord to another embryo, then there are two ectopic floorplates, then we get motor neurons in a very dorsal area.
    - The neurons are the floor plate are affected the most by SHH.
- Basically, we can transform the fates of the neurons by exposing neurons to SHH, and that's enough to induce ventral neural tissue.
    - At different concentrations of SHH exposure, you get different types of neurons. Constantly affects the fate of those ventral areas.
    - We have precise boundaries between the different types of cells because of the different transcription factors that are turned on for each type (other factors also get suppressed as a result). Each layer is defined by a unique layer of transcription factors. There is also an effect caused by the concentration of SHH. Expression of those factors is influenced by the concentration of SHH. The cells that migrate at the beginning at first experience lower levels of SHH. As the concentration goes up, then the classification of the cell changes.
- Wnt and BMP are also doing similar things on the dorsal side.

Anterior/Posterior Look at Neural Tube and Cell Creation

- Leading edge of mesoderm (specifically IMZ) experience high levels of Frzb. Dickkopf and Cerberus and they inhibit Wnt receptor function.
- Somites are not found near the head and the neck, it's found more in the hindbrain and spinal cord. It's likely because it is a posteriorizing agent
- Cells along anterior posterior axis also have similar patterning.
- You can get a second head if you suppress Wnt and you also suppress BMP to get a neural fate. Suppressing Wnt will give you the head neural fate.

    - Overexpression of dikkopf will also suppress Wnt.
- tBR is a truncated or bad BMP receptor.
- Dikkopf, Cerberus, and Frizb inhibit Wnt which gives you more head structure (anterior). If you have things that inhibit those inhibitors, you get smaller head structures.
    - Dikkopf blocks Wnt receptor function, while Cerberus and Frizb block Wnt ligand binding.
- All of this is dependent on whether you can suppress BMP so that you get neural fates.
- Somites release RA and act as posteriorizing agent. Increasing RA will cause no head structures (aka the loss of anterior fate).
    - Retinoic acid binds to ligand receptor complexes that modulate the expression of several target genes.
    - RA signaling drives cellular differentiation, regulating the transitions between various classes of neural stem cells leading up to their terminal division for neurogenesis
- In review
    - SHH: Induce ventral structures
    - BMP: Induce dorsal structures
    - WNT: Induce dorsal + posterior structures
    - RA: Induce posterior structures (or more specifically loss of anterior fates)
    - FGF: Induce anterior structure

Cerebral Cortex

- The cortex is made up of 6 layers where Layer 1 is the outermost and Layer 6 is the innermost. Layer 6 will contain the oldest neurons and Layer 1 will contain the youngest neurons. This is because the neurons that are made later on in time will migrate further up the layers of the cortex.
    - More specifically, the cortex goes from ventricular zone -> subventricular zone -> Intermediate zone -> subplate -> cortical plate (contains 6 layers of the cortex) -> Cajal-Retzius cell layer -> marginal zone
- Before the majority of neurons migrate to the cortical plate, some cells withdraw from the cell cycle and establish the preplate, which has Cajal-Retzius cells and subplate cells. These cells end up being in between the cortical plate. After this stage where the preplate is filled up, then we see newly generated neurons migrate along the radial glial cells to go to the cortical plate.
- You can describe the movement of the neurons through somal translocation and glial guided.
    - Somal translocation is independent of glial cells and is what the early cells used to establish preplate and early cortical plate.
    - Glial guided is used for the later neurons and this movement uses the radial glial processes and establishes the pyramidal neurons in the six cortical layers.
    - The Cajal-Retzius cells release reelin which is important for the detachment of migrating neurons from radial glia. It inhibits locomotion, allowing neurons to detach and to switch to somal translocation once the leading process touches the

marginal zone. Basically, you go from glial guided to somal once the neuron has gotten far enough up in the cortex. These cells move tangentially instead of radially.

- No ventricular zone until you close the neural tube.
- At neurons are created in the VZ, neurons migrate along radial glial processes and they sorta jump off and start forming different layers in the cortex.
- As a side note, the offspring of radial glial cells, depending on if they are dividing symmetrically or asymmetrically, can be actual neurons that migrate using the RGC's processes. Those cells are considered neuroblasts before they go through the migration.
    - When the neuroblasts are created, they are both kicked out of the ventricular zone by repulsive signals and attracted to the radial glia by attractive signals.
    - More specifically, the glial tubes will secrete MIA (migration inducing activity), and there will be chemoattractive (netrin) and chemorepulsive (slit/robo) signals.
- Neural stem cells, which only divide symmetrically to produce more neural stem cells, transition gradually into radial glial cells. Radial glial cells also called radial glial progenitor cells, divide asymmetrically to produce a neuroblast and another radial glial cell that will re-enter the cell cycle.
- The first neurons that extend their own process out and pull their cell body out of the VZ instead of needing glial guided migration.
- Neurons in the VZ come from asymmetric division and can move to any layer of the cortex, while neurons in the SVZ come from symmetric division and can move to upper layers only.
- All the cells that are born in the ventricular zone, can they become cells in each of the layers in the cortex? Nah, it depends on when you are born. As time goes by, the fates of cells become more and more restricted.
    - Younger cells have more potency than older cells.
- If you put a cell that was going to by Layer 5 cell in a young host, into an older host, then that becomes a Layer 4 cell because of the different transcription factors in the ventricular zone.
    - But doesn't go the other way. Layer 4 cell in an old cell can't go up to layer 5 in a younger one. That cell doesn't really understand the things necessary for Layer 5?

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- GCaMP6 is a good fluorescent protein to use when you want to see whether neurons are working properly.
- Want to have a virus that targets neuron precursors rather
- The figure shows a general overview of the experiment by first going through the experimental timeline and then showing the main result of different age groups of ferrets showing differences in the responses from neurons. Between panels b and c,

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More Cortex and Inhibitory Neurons

- When neural stem division happens, a neuron gets created through Delta Notch signaling.
- Not all the neurons that are created are born in the VZ. In the cortex, we have inhibitory neurons that release GABA. They are not born in the VZ, but rather in the LGE (lateral ganglionic eminence), and they migrate into the cortex. They're not following a scaffold, but rather they are more free to go wherever into the different layers of the cortex. This is in contrast to the radial glial migration from the normal neurons where the neurons are just moving up and down.
    - In other words, the interneurons migrate perpendicularly through the radial glial cells to reach their final location
    - Some migrate into the cortical plate (place where the cortical layers are formed) and some go to the VZ.
    - All the cortical layers have been formed by the time the inhibitory neurons are born. The specific layer where the neurons go to is a function of their age. They need to check in with the VZ to get info on which layer they need to go to.
        - Ones that are born early innervate the lower cortical layers.
    - As a side note, if you remove the LGE, then you'll see a lower number of neurons that are GABA+ in the cortex
- Proteins that some neurons have will influence their direction of migration.

Axon Growth

- Once neurons are created and neurogenesis is over, the cell has initial sprouts, they become neurites, and one becomes an axon, and the others become dendrites.
    - Microtubule and actin components of the cytoskeleton are redistributed among the neurites so that a single process is identified as the axon.
- Axons have growth cones, which are specialized endings of growing axons. The behavior of these cones influence the direction the axons grow, and the cones themselves are affected by attractive and repulsive forces from the environment.
    - On a growth cone, the fan shaped sheets are the lamellipodia and the filopodia are the projections or the fingers. The filopodia contain actin filaments and they polymerize and depolymerize based on the growth cone direction. The actin filament slides along the microtubules that make up the long part of the axon body.
- Axon growth is influenced by signaling factors that tell the axon which direction to grow in. Sometimes there isn't even a target the axon is going to, the direction is influenced more locally.
    - Short-range (or contact) and long-range attractant and repellents guide an axon
    - Once the growth cone reaches its target, it is transformed into either a presynaptic ending for an axon or the terminal domain of a dendrite.
- As the axons enter different unfamiliar areas of the body, growth cones will change shape. The factors that affect the change of shape and the direction are cell surface

adhesion molecules on muscles, receptors for secreted signals, and specialized extracellular matrix molecules.

- Axon branches will want to spread out as much as possible, this is called self avoidance. The axons and dendrites don't want to be bunched up. This is in the same way a tree wants to get as much sunlight by branching out.
- PNS is created through the cells in the neural crest. Many neural ectodermal cells pinch off from the neural tube and migrate laterally to create the structures of the PNS, sensory neurons, connective tissue, etc. Depending on their direction of migration, they will differentiate into different types of cells.
    - The cranial ones will become neurons, glia, and cranial ganglia. They will also become cartilage, bone, and connective tissue. The ones in the trunk will become pigment cells and sensory neurons and glia.
        - Cranial and trunk refer to different anteroposterior levels. Depending on where those neurons are generated, they will differentiate into different types of neurons.
    - As those cells migrate out, they are differentiating into neurons. They release neuregulin (Nrg).
    - If there is Nrg present, then glial cells form. If not, then neurons form. The neural crest cells have receptors for Nrg and are thus affected by Nrg. If nothing binds to the receptors, the cells themselves become the source of the Nrg and they release them and they themselves turn into neurons. Now, the concentration of Nrg is increased, the crest cells have Nrg attached to their receptors, and the cell will become pro-glial.
- Growth of an axon comes through adding protein tubulin dimers to a microtubule, which is a protein structure. It basically is a controlled rearrangement of the cytoskeleton. Microtubule cytoskeleton causes elongation of the axon while actin cytoskeleton changes the structure of the “fingers”. The minus end of the microtubule is nearest the cell body and the plus end is where the dimers get added.
    - You find microtubules in the axon, but not in the dendrites.
    - The polymerization and depolymerization of actin and tubulin is what specifically causes the growth and change in structure.
- Even as neurons are migrating out of the VZ, the axons can start lengthening and start projecting to other places.
- Polarization of proteins inside the neuron is what causes one neurite to become the axon. Once one of the neurites is “chosen”, the other neurites stop production of their microtubules.
- The leading edge of the axon or growth cone have receptors that detect “smell” and “feel” the environment in order to guide the direction of their growth. The cones get cues from the environment in the form of molecules binding to receptors, turning on 2nd messenger pathways for signal amplification, and then attraction/repulsion based on the molecule.
    - All the sensing is done in the growth cone which is ahead of the end of the microtubule.

    - Actin is also growing and push out little fingers on the growth cone. Those fingers are the things that have the sensory receptors (ligand to the receptor is either attractive or repulsive) that investigate the local environment. Actin also has protein interactions with the microtubule (similar to muscle contraction interaction) which helps pull the microtubule in a particular direction.
    - The growth is all local (you can cut the axon from the cell body and you'd still get growth for a little bit).
    - Depending on attraction or repulsion, you have depolarization or repolarization of actin.
    - Attraction associated with exocytosis and cytoskeleton assembly. Repulsion is with endocytosis and cytoskeleton disassembly.
- There are different classes of axon guidance molecules (aka molecules that affect the direction of growth for axons).
    - The extracellular matrix molecules and their integrin receptors
    - Ca2+-independent cell adhesion molecules (CAMs)
    - Cadherins - Ca2+-dependent cell adhesion molecules
    - Ephrin ligands and Eph receptors: These ligands and receptors are used to recognize appropriate pathways for growth. Limiting axon growth is done through the cleaving of extracellular domain of the ephrin ligand and endocytosis of Eph receptors.
- Chemoattraction is the idea that cellular targets will release molecules that selectively attract the growth cones to move to particular locations.
    - Tropic molecules are the ones that affect axon direction, while trophic molecules are the ones that support the survival and growth of neurons.
- Netrins are one of the families of chemoattractants. Netrins are like molecules in the ECM space and once they bind to receptors, it causes a cascade of events that affects axon direction.
    - Netrin signaling can cause an axon to cross over the midline. However, slit and its receptor robo cause the axon to stay on that side once it has crossed over by terminating the growth cone's sensitivity to netrin.
- Semaphorins are molecules that bind to receptors, causing changes in calcium concentration that often cause growth cones to collapse and axon extensions to stop.
- Chemoattractants: Netrins, Ephrins,
- Chemorepellents: Slit (receptor is Robo), Semaphorins, Ephrins
- There are 4 families of guidance molecules that are found on the actin fingers. They are receptor ligand pairs.
    - Ephrins -> Eph
    - Slit -> Robo
    - Netrins -> Unc 40/DCC
    - Semaphorins -> Plexins
- ER is the hub of calcium. Other sources are TRPC and LVDCC channels that bring in calcium.

- Repulsion prevents release of calcium from ER. When cGMP is activated, it inhibits the release of calcium from the ER. Attractive cues cause cAMP to be activated which activates the release of internal calcium (There is also a positive feedback loop between Ca and cAMP). In other words, activation of cAMP creates high-amplitude attractive Ca2+ signals accompanied by Ca2+ release from the ER, while activation of cGMP creates low-amplitude repulsive Ca2+ influx that does not trigger Ca2+ release from the ER. cAMP and cGMP also have inhibitory connections with each other so if one concentration is high, the other would have to be low.
    - Basically, activation of 2nd messenger pathways cause release or inhibition of release of calcium from the ER.
    - Attractive Ca+ signals can also promote the transport of VAMP2-containing vesicles with exocytosis afterwards. On the other hand, repulsive signals cause formation of clathrin-coated pits and cause endocytosis.
- The attractive signals and the exocytosis lead to actin assembly and adhesion, while those things are inhibited when there are repulsive signals.

Semaphorins, Netrin, and Ephrins (Axon Guidance Protein Classes)

- Semaphorins are proteins that impact the growth direction of axons in relation to other neurons. They will primarily repel certain axons since they are a chemorepellent.
    - They are usually cues to deflect axons from inappropriate regions
    - Specifically, they are repulsive to pain fibers in the dorsal root ganglion.
- DCI neurons are innervated by dorsal ganglion neurons. Their axons are initially attracted to the netrins released by floorplate.
- Netrin is a protein that is a chemoattractant.
    - Dorsal commissural interneurons located in the dorsal column are attracted by a gradient of netrin
    - The receptors on growth cones will be attracted to netrin and will move toward floorplate where the concentration of netrin is high (b/c netrin is released by neurons in the floorplate). There is also receptor desensitization. However, the high levels of slit (also released by the floorplate) are what turn it around. Basically, the slit binds to robo and that activates a pathway that blocks Netrin signaling. The reason they don't immediately get repelled by the floorplate is because there is protein called commissureless that is expressed by axons before they cross the midline. It binds Robo and removes it from the growth cone surface, so that Slit cannot bind and thus axons are not repelled by Slit. Once the axons cross the midline, commissureless protein levels are reduced and Robo returns to the surface so the axons can respond to Slit.
- Canonical model for netrin is where the protein is expressed by the floorplate and the axons of the neurons in the dorsal area move toward the bottom. In the growth substrate model, netrin is produced by neural stem cells that have moved to the outer edge of the neural tube due to the radial glial processes to form a growth substrate and then axons adjacent to the substrate.
- Ephrins are another class of proteins involved in growth cone guidance.

- Ephrins are in between the LGN and MGN serving as a kind of a barrier, keeping the visual area in the visual region, and same with auditory.
    - LGN is the middle point between the retina and the visual cortex. MGN is the middle point between the hair cells in the ear and the auditory cortex.
    - In the situations with there is no ephrin, then retinal fibers invade into the MGN since there is no barrier. With ephrin, the fibers stayed on the LGN side even though the auditory fibers were cut and there was no input into the MGN.
- Cells in temporal part of retina avoided posterior tectum, while cells in the nasal part went to both. However, if PI-linked membrane proteins are released, then temporal axons grow on both types of membrane.
    - Thus posterior membranes normally have a PI-linked repulsive guidance molecule.
- There is a gradient of Ephrins in the tectum, high in the posterior pole and low in the anterior pole.
    - There are opposing gradients of active Eph receptor in the retina and in the tectum.
- Ephrin-A5 has a high concentration in layer 4 of the cortex. It enhances growth and branching of thalamic and Layer 6 neurons, but inhibit the growth of Layer 2/3 neurons.

1/17 - Week 2

Semaphorins, Netrin, and Ephrins (Axon Guidance Proteins)

- Ligands can act as attractive and repulsive based on the cell type of the neuron it is binding to,
- If you have multiple factors in the environment, how does the growth cone respond?
- Growth cone will encounter a lot of factors and those will influence the direction the axon will grow.
    - Combination of different factors can cause attraction or repulsion.
- The following process is for creating a retinotopic map. It's the path that the axon of one retinal ganglion cell will take to get to the tectum. Different transcription factors are basically leading the neuron.
    - For example, growth cone is happy on the yellow laminate.
    - Then, it will unbind when it reaches the green netrin, which attracts the RGC. However, the combination of laminin and netrin is repulsive so the axon leaves.
    - The blue ephrin-B will affect whether or not the axon will cross over. Depends on whether that cell is part of the nasal or temporal part of the retina (repulsive to RGCs from the temporal retina, but not to RGCs from the nasal retina).
    - Slit and SHH are repulsive.
    - Orange sema3a will repel and keeps the RGC near the side.
    - When the growth cone hits the boundary between the ephrin and the end of the FGF or when it hits the tectum, the axon will break up and start branching to multiple targets.

Cell Death and Survival

- Trophic molecules are ones that support the survival and growth of neurons. More broadly, in order to create mature neural circuits, you need to have a specific subset of neurons survive, connections between neurons need to be made and maintained, and there needs to be dendritic branches to support the connections.
    - In order to achieve these goals, neurons need to be signaled by factors that tell them how much to grow, if they are needed, etc. These factors come from target tissues.
    - Neurons can receive survival signals from the cells that they innervate (target-derived or retrograde), from their synaptic inputs (afferent-derived or anterograde), from neighboring neuron cell bodies (paracrine), from distant sources via the circulatory system (blood-born), and from nonneuronal cells (glia-derived).
- The nervous system doesn't really know about the size of the target tissues that the neurons are supposed to innervate since those things are still yet to be formed. At the beginning, we just have to make as many neurons as possible.
    - Cell to cell signaling is important in determining which neurons need to stay and which die.
    - There will be some proteins that a cell body releases, goes to other cells through the axon and synapse onto the dendrites, and that helps regulate the survival. It also forms some output that regulates its survival. Paracrine signaling also affects survival without direct contact.
- Somewhere between 20 and 70 percent of differentiated neurons die.
    - The reasoning is that as development happens, some of the target neurons don't have a job because the target limb didn't end up being big enough, we have too many motor neurons. This increases the amount of motor neuron death.
- Basically, the size of the neuronal pool is correlated with the size of the innervation site.
    - At the beginning the number of neurons in the dorsal root ganglia are relatively the same in all the areas. However, as the limbs start to grow, then the number of neurons in particular area in the dorsal root will increase proportionally to the size of the innervation site.
        - Specifically, there will be less cell death in the limb DRG because that limb is growing and it needs all the motor neurons it can get. More cell death in the regions that haven't had as much growth.
        - One thing to note is that there is always a decline of the number of neurons (aka cell death). The difference is that some areas will experience more/less cell death than others. The number of neurons never really increases.
    - Tl;dr the survival of motor neurons and neurons in general are regulated by the size of the target.
        - For example, When a limb bud is removed, the process of cell death is enhanced, and there are fewer motor neurons and DRG cells

        - If an animal has 3 limbs instead of 2, cell death is decreased and the animal will have more target tissue and thus you need more motor neurons.
- So what is being released by the target tissue? How does the body know to create more motor neurons.
    - Nerve growth factor is a protein that was the first neurotrophic factor. NGF is part of the neurotrophin family, which contains a lot of factors that help neurons survive. They each bind to particular receptors on cells. In a sentence, NGF basically supports the survival and neurite outgrowth of sympathetic neurons.
    - If you remove these growth factors, there is an increased amount of cell death in certain kinds of neurons in the body. NGF only affects certain classes of nerve cells.
- Cellular activity is what keeps cells alive. If you depolarize the cells and block BDNF (one of the trophic factors), then most of the cells die.
- In other words, electrical activity enhances the survival of neurons and acts like a neurotrophic factor.
    - If you add KCl to a cell, more calcium will enter and you'll get more activity, level of BDNF increases -> greater neuron survival.
    - But if you add a function blocking antibody of BDNF, then the trophic influence of the KCl is eliminated.
- Innervation regulates the survival of the target neurons, so if the cochlear nucleus dies, then it's not innervating the neurons in the cortex. For a while, there is a bit of a time period, those neurons are still unaffected (up to a week later). The very young neurons don't need a whole lot of input, mainly because the axons have barely started extending, and that's when you can have external components innervating them and receiving some sort of survival factors. Over time, they gain sensitivity to trophic support.
    - However, for older animals (posthatch 14), the effect is immediate. Most of the cells die.
    - When you're an adult, then the cochlear nucleus being removed doesn't really affect the neurons that region innervates.
- The survival of neurons in the gerbil cochlear nucleus depends on afferent innervation from the cochlea only through the first postnatal week.
    - As the removal happens on a later day, there is less neuron loss.
- Some groups of cells have a window where they need trophic support, and some don't need any support at all
    - The window occurs because at the beginning, they are not connected to anything.

Dendrites

- Dendrites are basically wires with resistance. They are tubes of cytoplasm. They can do logical operations, can do coincidence detection, and amplification.

- Regions of the dendritic tree may only receive inputs from one part of the brain (thalamus goes to lower order dendrites, higher cortex goes higher on the tree - apical dendrites).
    - The types of information are segregated on the tree.
- Dendrites grow up, axons grow down.
    - But high source of semaphorin will cause the dendrites to grow down. Evidence that semaphorin is attractive to dendrites, and they are repulsive to axons.
        - Different receptors in the dendrites than receptors in the growth cones.
- Neurons don't want their dendrites to cross over with each other. Branches from different neurons, however, can overlap freely with one another. This propriety demands that neurons are able to discriminate "self", which they avoid, from "non-self" branches, with which they coexist
- Glial cells tessellate the cortex while starburst amacrine cells have a lot of overlap. They have high coverage values.
- Protocadherins
    - Same complement of proteins all over the dendritic tree and thus cells can tell when the dendrites are in each other and so that covers self avoidance. Other neurons will have different proteins on their dendritic trees. In this case, you also see non-self adhesion.
        - If you have a tree with no protocadherins, then you lose the self avoidance characteristic.
        - If you have a tree with only 1 kind of protocadherin, you get the self avoidance but you don't get the non-self adhesion if the neuron it is next to has the same protocadherin.
    - Basically, combination of protocadherins gives you an indication of how much overlap there will be between SACs.
- Dendritic growth is regulated by cell activity. If a cell becomes more active then that stimulates the growth of dendrites.
    - For example, in the light, the neurons connected to the retina will be more active and thus net growth is positive. In the dark, it will be 0 or will be negative.
    - Also, it's not only the elongating of the dendrites, but it's also increasing the amount of branching.

1/18 - Week 2

- Ligands for axonal growth cones are netrins, semaphorins, and slit. The first two are more transcription factors that interact with the nucleus, while slit robo is a ligand receptor interaction.
    - Robos is a receptor, and slit is the ligand
- Lots of activity (aka high levels of calcium) in the axonal guidance cone means that you have an attractive pathway.
    - Repulsive cues involve low levels of calcium release.
- Younger donors have more potential as a stem cell, less confined in its cell fate.

    - On the contrary, the old donors are more confined so if they became a layer 3 cells in one organism and you transport it, it will still be a layer 3 cell even in a younger donor.
- Delta Notch pathway is one of the first pathways that starts to figure out what the precursors are and what the cells will become. After delta notch, you have the differentiation between the ones that become neurons and the astrocytes. Then, after the differentiated cells have been made, then you get into the growth of axons, dendrites, etc. They need to be able to communicate and find their place in the world.
- Used a retrovirus in the paper because it is able to integrate into the genome and for the mitotically dividing astrocyte, this is important for us to be able to detect the device in the subsequent copies of the astrocytes.
- Figure shows the procedure going to use to label the astrocytes (electroporation and then imaging), location of the astrocytes after a certain amount of time, their respective percentages in a pie chart (most of them are located in the subventricular zone), and then d) is looking at the location of locally proliferating cells instead of cells from the SVZ that they obtained through staining.

1/22 - Week 3

Continuation

- Neural circuits are intrinsically built, not based on sensory cues.
    - Experience isn't building the circuit itself but it is adjusting it in some ways.
- Information from the retina goes to the LGN and some part of the information goes to the superior colliculus.
- Neurons in the retina are connected by gap junctions, and thus you get small nodes of activity that go through the retina. This creates waves of activity that propagate further down the pathway to the colliculus and the cortex.
- Spontaneous neural activity plays a role in affecting dendrite growth.
- BDNF has some impact on promoting cell growth which means dendritic growth.
    - The dendrites that succeed in finding connections are the ones that stay.

1/29 - Week 4

Ligand Gated Ion Channels

- As a review, ionotropic receptors are the receptors that form a channel pore that can open and close letting ions through. Metabotropic receptors, though, are ones that use signal transduction mechanisms and are thus indirectly linked to the channels.
    - In other words, when an ionotropic receptor is activated, it opens a channel that allows ions such as Na^+, K^+, or Cl^- to flow. When a metabotropic receptor is activated, a series of intracellular events are triggered that can also result in ion channels opening or other intracellular events, but the key is that it involves a range of second messenger chemicals.
- Internal/endogenous chemical substances or neurotransmitters induce physiological effects.

- Acetylcholine and epinephrine are the first couple molecules discovered in inducing effects on biological tissue. Scientists figured this out by putting large concentrations of them on living tissue.
    - The substances were found to be secreted by nerves and used to communicate with target organs. In other words, these substances have a purpose in our bodies and are used for communication between different parts.
- In an experiment, a heart with vagus nerve attached was stimulated, thus causing a neurotransmitter to be released, causing the heart rate to slow down and the amplitude of contraction also decreased. Then put a tube between the solution with the first heart and another solution with another heart that didn't have the vagus nerve. The heart rate of the 2nd heart also slowed down. The inhibitory effect of the stimulation of the vagus nerve was transferred.
    - This was proof of chemical synaptic transmission from the vagus nerve. There is some chemical released and the heart had receptors for it, and the chemical was able to travel through the solution through the tube and affect the 2nd heart. It wasn't necessarily the direct stimulation of the nerve that caused the change in contraction rate, but rather the stimulation caused certain chemicals to be released which is then what caused the change.
- Way back when, muscle tissue was thought to contain receptive substances to receive the stimulus and transmit it to other places. We found out that the receptive substance is an ion channel. It's basically a protein with a pore and it is membrane bound and when it is activated, then it selectively passes certain types of ions through based on the ion concentration gradient and the electric gradient.
- These ion channels are classified by the way they are activated or opened. These channels can be:
    - Ligand gated: Some substance binding to the receptor, causing the opening.
    - Voltage gated: Change in membrane potential causes the actual opening.
    - Mechanosensitive / Thermosensitive: Directly interact with environmental cues.
- Agonist is any ligand (aka molecule) that activates the ion channel.
    - Endogenous agonists are those that the body produces itself.
        - Ex) Acetylcholine
    - The ones that aren't made by the body as just known as agonists.
- Antagonist are any molecule that interferes with the channel function (aka stops the channel from opening).
    - Competitive antagonists are the ones that compete with the agonist for binding to the receptor. One way to stop a channel from working is to stop the agonist from binding by binding themselves to the binding site. However, the key is that the binding does not cause activation of the channel.
    - Non-competitive antagonist is one that stops the activation of the channel by binding to a site on the receptor that is different from the one that the agonist binds to. So, once that non-competitive antagonist is on there, then the channel won't be activated regardless of whether agonist binds in its normal place.

    - Pore blockers: Enter the channel when the channel is activated and stops ion flow.
    - Allosteric Modulators: Change the channel function when the channel is activated. The ligand is still required to activate the channel, but this modulator comes in afterwards and turns the knobs that already exist in the channel (aka small changes instead of large scale ones).
- There are a bunch of ligands that activate the ion channels. The ligands can be neurotransmitters, acids (extracellular proteins), calcium, cyclic nucleotides (cAMP and cGMP), etc.
- There are 4 different families of ligand gated ion channels. There are named based on the number of subunits that come together to form the protein. While each of the families has a specific number of subunits that come together to form those receptors, within each family, there is variety in terms of the type of subunits that bind.
    - Pentameric Ligand Gated Ion Channel: 5 subunits coming together
    - Glutamatergic (Tetrameric): 4 subunits coming together
    - Purinergic (Trimeric): 3 subunits coming together
    - GluR0
- The first 3 are the ones that have been found in mammals. The families had convergent evolution.
- The individual types of receptors within each family are different based on the ligand that they have receptors for.
- Basically, different types of subunits come together in unique combinations which leads to different ligands that bind. The receptors can also be different in the speed at which ions can pass through.
- All acetylcholine activated ion channels are also activated by nicotine, therefore called nicotinic acetylcholine receptors. These are ligand gated channels. Specifically they are pentameric ligand gated ion channels.
    - The electric ray has a large amount of nicotinic acetylcholine receptors.
- There is a particular type of snake where in its venom, there is an alpha-bungarotoxin which is a molecule that binds irreversibly to nicotinic acetylcholine receptors thus not allowing acetylcholine to bind.
    - This allows us a way to isolate certain receptors.
- Patch clamp method allows you to look at a piece of membrane and then you can put agonists around it and see the effects.
- The channel response of one single receptor is different from the synaptic response from all the receptors as a whole.
    - Single channel wise, the graph of current as a function of time will show the times when the channel is closed and when the channel is opened. There is an immediate current response to the agonist being there and not being there, resulting in a blocky, not smooth, graph.
    - However, since there are lots of channels in one synapse and they open/close at similar but different times, the overall curves for current and membrane potential are relatively smooth over time.

- In order to channel to open, agonist and receptor have to bind and this creates a reaction. The length of time the channel is open is dependent on how long it takes for the agonist to go away from the receptor.
- Once the binding happens and the channel starts to open, the ion current through that channel is a function of
    - Ion concentration inside and outside the neuron. These differences create a diffusional force. Often times, this will be in opposition to the electrophoretic force.
    - Membrane potential: Electrophoretic force. Equilibrium or reversal potential is the voltage/current that has to be created/maintained so that no net flow of ions happens.
    - Ion Channel permeability
- The inward and outward current at a particular channel always depends on the above factors. There are no absolute statements about whether particular ions will flow particular directions in particular channels. It all depends.
- Determining ion channel permeability involves doing patch clamp and noticing the changes in current after changes in ion concentration in the bath of the pipette. Specifically, we look at the change in the reversal potential. If it changes as the concentration of the ion changes, then that means it is permeable and it is affecting the current.
- nAchr receptors are permeable to Na+, K+, and sometimes Ca2+. They are basically cation selective.
- For most ligand gated channels, they will pass more than one ion, but most often they will only pass positive ions or only pass negative ions.
    - ACh receptors are cation permeable. They aren't permeable to all of them, but they do pass more than one cation (sodium and potassium are examples).
- A channel can be inhibitory or excitatory based on the concentrations of ions on the outsides, the neurotransmitters, the thresholds, the potentials, etc.
    - Inhibitory is where activation resists firing.
        - You could have a receptor that binds glutamate and allows chloride to pass.
    - Excitatory brings the neurons towards firing.
- There are not any excitatory or inhibitory neurotransmitters because it depends on those channels.
- For ACh receptors in particular, in order to double the amount of response, you need 4 times as much concentration of choline.
    - The graph of response as a function of the concentration of choline plateaus. At high concentrations, it's hard to determine the impact that a change in concentration will have on the response.
- ACh receptors also exhibit desensitization. Glutamate receptors also do this. It's a property of most ligand gated receptors.
    - A 2:3 or 3:2 ratio of alpha4 and beta2. Both of those are types of subunits. The stoichiometry of the receptor refers to what types of subunits come together.

    - For situations where you have plenty of agonist around, the channel no longer responds despite the presence of the agonist. The reasoning is that once you've had the same amount of agonist around, the rate of binding steadies to a certain rate. The channel goes into a different conformation over time, which causes a different response when the agonist binds.
- These channels are protein structures that are being affected by thermal noise and thus there are possibilities that it can jump into different conformations.
- In the alpha4beta2 nicotinic acetylcholine receptor has 4 transmembrane domains per subunit (As a side note, the pentameric channels that we saw earlier have 5 of those subunits).
    - Nicotine binds at the interfaces of alpha(primary) and beta(complementary) subunits. No binding at the beta beta interface.
    - All depends on where there is a pocket of space for nicotine to bind.
    - There are two available binding pockets in total. A particular side of the beta unit has to come into contact with a particular side of the alpha unit in order to create the binding site for nicotine. At the other interfaces, nicotine isn't able to fit.
    - These are all structures in the extracellular domain, so that nicotine can bind there.
- Different combinations of subunits on the ligand gated ion channels give you different properties (different affinities for the ligand, different amounts of desensitization, different pore conductances affecting the speed of ions going through the pore).
    - We just saw how 3 alpha4 subunits and 2 beta2 subunits came together to form the channel, but we could also have 5 alpha7 subunits come together. Those will have different properties
- The M2 transmembrane domain for each of the subunits faces in on the pore and has a highly conserved set of amino acids.
- Desensitization and deactivation are different in that desentization has the agonist bound and there are no ions passing through while deactivation is when agonist is not bound and the channel is not open.
- Cation Selectivity is a Function of 3 Amino Acids
    - Insertion of Proline creates a bunch of changes.
    - P236' is essential to change selectivity from cation to anion and is considered "exclusive" for selectivity.
    - E237A and V261T are considered "permissive" in the sense that they have to be present for the proline substitution to work, but alone they don't affect ion selectivity
    - We only needed a few mutations to evolve anion or cation permeability
    - Keep the same receptor plan, but the change in current in or out is due to the changing of a few amino acids
    - The ionic selectivity filter is at the cytoplasmic end of the pore is formed by the specific combination of amino acids on the M2 transmembrane domains of the 5 LGIC subunits. They are the ones that will determine whether cations pass, anions pass, etc.

- Ion Conduction Depends Upon Continuous Pore Hydration
    - If we close the pores tighter, there is less permeability => less conductance
    - The closed state is not fully closed, but is closed enough where the ion doesn't pass through the pore
    - Amino acid sequences are important in keeping the water around the ion
    - Can't get water into the closed section even though it's wide enough to pass through, and thus if you can't get water, then the ions can't pass either.
        - Proper ion hydration is essential to ion flow through the channel pore.

1/29 - Week 4 (Based on Stephanie's Notes)

Metabotropic Receptors

- The 2 major types of NT receptors are ligand gated ion channels (where an NT binds causing a channel to open up) and G-protein coupled receptors (where an NT binds and there is a signal transduction pathway where messages are relayed and a channel is eventually opened).
- G-protein coupled receptors form 5 families
    - Glutamate, secretin, Frizzled, Adhesion, Rhodopsin
- Two Major Types of Neurotransmitter Receptors
    - Heterotrimeric G-proteins are just wandering in the intracellular area. When together and inactive, GDP is bounded to it. Then it sees a receptor that has undergone a conformational change (because a ligand binded to it) and the GDP turns into GTP and is active. Alpha subunit is the one that is activated and can affect other processes.
        - Hydrolysis is the process that converts the GTP back to GDP.
    - Many heterotrimeric g-proteins are activated for the activation of 1 receptor.
- The Endogenous Ligands For >140 GPCRs Remain Unknown
    - We don't know what activates the endogenous ligands even though we know its there
        - Ex. sense of smell is dependent on g-coupled receptors
- Heterotrimeric G-Protein-Coupled Receptors (GPCRs) Are Signaling-Cascade Triggers
    - These receptors are single proteins with 7 transmembrane domains. That + the interactions with the heterotrimeric G-Proteins is what characterizes the GPCRs.
- Effector Molecule:
    - G-protein is NOT part of the receptor
    - Ligand gated channels, binds ions, opens - very fast
    - This metabotropic receptor process is slow, but last long
- G-protein signaling can happen even if the receptor isn't there, but the receptor allows the signaling to happen as a result of some external influence like an NT binding.
- G-Protein Coupled Receptor Signaling Cycle
    - NT binds to the receptor changing the conformation, G-protein binds to the receptor, GDP -> GTP, alpha gets activated and interacts with effectors. Effectors activate second messengers, GTP hydrolysis to get the GTP that is on

the subunit back to GDP, phosphorylation of the receptor allowing arrestin to bind causing endocytosis of the receptor, brings it into the cell, recycles/removes the ligand, and then puts it back on the membrane.

- Ligand Bind Changes The Free Energy of GPCR States Making Activation More Or Less Likely
    - 1st graph: Higher climb to reach activation. We have an inverse agonist that doesn't want the protein to be more active.
    - 2nd graph: We can get stuck in the "valleys" with high slopes and thus large amount of energy to get into a more active state.
    - 3ds graph: Application of the agonist, there is a lower required energy than the inactive states. Ligand binding changing the confirmation so that the least energy state changes
        - Goal to achieve low energy
        - Agonist -> lowers the energy to active (lowers the activation)
    - 4th graph: When it bumps into a not activated G-protein
        - Positive feedback
        - Little activation leads to even more activation due to lowering of the activation state
        - The conformational change is lowering the required energy to active which leads to further activation
    - The real key here is that the ligand binding is changing the conformation of the receptor such that they are in low energy states and are active.
- "Turning Off" G-Protein Signaling In Two Parts
    - Part A: Turning Off The Receptor Through Internalization - Need to tell internalize the receptor by having the GPCR be phosphorylated by GPCR Kinase (GRK), which allows arrestin to bind to the receptor and initiate clathrin-mediated endocytosis to internalize the receptor.
    - Part B: Turning Off The G-Protein Through GTP Hydrolysis - Need to convert GTP back to GDP, which inactivates the g-protein subunits.
- GPCRs Have "Funky" Pharmacology
    - Agonists promote exchange activity above the basal level and therefore favor an active conformation.
    - Inverse agonists lower activity below that of the unliganded, basal state and thus favor an inactive conformation.
    - Neutral antagonists do not affect the basal activity (i.e., the inactive–active equilibrium) but compete with inverse agonists or agonists for the same ligand-binding site.
    - Partial agonists produce weaker maximal activity at saturation than do full agonists.
    - Biased agonists can stimulate both G-protein and arrestin pathways, or multiple G-proteins, and the greater efficacy toward one or the other is known as ligand bias
- Signaling Diversity Through G-Protein Subunit Diversity & Function

    - Some receptors only like the G-proteins that have specific types of alpha subunits on them. This creates signaling diversity.
    - The beta gamma does important functions. They are bound to the ends of the receptors of the K channels of the C-N terminus. G beta gamma subunit causes it to open.
- Examples Of Signaling Cascades Activated By G-Protein βγ Subunits
    - Gβγ Subunits Target Effectors Throughout The Neuron
    - G proteins can be activate without coming in contact with any receptor
- Examples Of Signaling Cascades Activated By G-Protein α Subunits
    - The process is actually very complicated
    - One transcription factor opens up not just 1 gene but a whole host of genes
- G-Protein Signaling Supports Amplification
    - While the receptor is in its active form, it is turning on many G-proteins, which bind to and activate the adenylyl cyclase which create cAMP, which activate protein kinases and phosphates are transferred to target proteins
        - This is called Amplification. This is an important distinction between ligand gated ion channels. For those, you need a bunch of receptors to get activated to get the appropriate ions flowing in and thus the appropriate voltage change. However, here, one receptor activation can affect hundreds of other molecules and longer lasting changes.
    - Neural Modulator: Cause a change that lasts and changes how the neuron responds
    - Ligand gated ion channels: This is about fast info flow
    - G-protein coupled receptors: Sensing everything around it and making the decision of how to be activated and what downstream effects to have.

2/5 - Week 5

Membrane Potential

- **Just honestly reread Chandler notes from m101A.**
- Neurons use electrical signaling to transmit info because it's fast, lossless, and metabolically efficient.
    - Time increases with the square of the distance when it comes to 1 dimensional diffusion and so diffusion takes a lot of time. However, conduction of a signal along a myelinated neuron is a lot faster.
    - The intensity of an electrical signal does not change as the signal is passing through the axon. No loss of amplitude of the signal. Thus, no loss of information.
        - Diffusion doesn't have this property since the intensity will decrease as the signal moves outward.
    - Don't have to expend much energy to pump ions to where they need to be.
- Membrane potential is the voltage difference across the lipid membrane.
- Resting membrane potential is the steady (unchanging) voltage across the membrane in the absence of other influences.

    - Some cells don't have a stable resting potential, but for most cells, it is -65 mV for neurons and -90 mV for the muscle and the heart.
- The resting membrane potential arises because of the membrane's selective permeability to different ions as well as the concentration gradient for those ions between the inside and outside of the neuron.
- The concentrations of the ions don't really change as the ions move from one side to another (since the number of those ions is so low).
    - As ions move, then this creates a charge separation and thus a voltage.
    - The two driving forces on ions are the concentration gradient (diffusion) and the electrical gradient (electric field)
- The Nernst equation predicts the equilibrium potential which is the voltage at which the net ion flux is 0.
    - At this point of equilibrium, the diffusion force has to balance with the electrical force. A constant membrane potential implied that net ionic current.
    - Each ion has its own equilibrium potential.
- The voltage exists across the membrane, and not within or outside of the neuron.
- Condition caused by ion channel mutation that destabilizes the resting potential.
- What enables a muscle fiber to contract is an internal release of calcium. This is done when it gets electrically excited by a motor neuron. The motor neuron produces an AP and the signal gets sent through the axon and to the muscle fiber.
- In some cases, you have an abnormally highly excitable set of muscle fibers. We find this by having an electrode placed outside the muscle.
    - Recording the electrical activity of muscle is an electromyogram.
- Myotonia (https://en.wikipedia.org/wiki/Myotonia) is inappropriate force occurring in a muscle. It is a disease that destabilizes the resting potential. Basically causes lots of action potentials in the muscle when the muscle should be relaxed.
    - It's unclear whether the problem is with the nerve which is sending a bunch of APs or with the muscle which is just contracting over and over again.
    - Proved the problem was with the muscle itself by directing injecting current into the muscle and seeing the effect. In the myotonic goats, the excitability of myotonic muscle is enhanced. We saw a more APs for the same current. Since the same current was applied, and the voltage increased by a greater amount, this must be because the resistance for the cell membrane of the cell is higher. The greater voltage change is what causes the higher number of APs and the general increase in excitability.
        - In the experiment, when they injected a small current, the normal goat got an increase in voltage but no AP, while the myotonic goat got an AP. Also, when they injected a larger current, the normal goat got an AP and then went back to normal, while the myotonic goat got repeated APs over and over again.
    - The increase of resistance of myotonic muscle is caused by a loss of chloride conductance. Equilibrium potential for chloride is very close to the resting potential, which means that in normal muscle the chloride conductance is the

largest of them all. This is very important for stabilizing the resting potential, since whenever it strays away from rest, a large chloride current will bring it back to normal.
        - The lower conductance meant that the resistance increased and that V_m is not able to reset back to a low value because it is getting affected more by E_Na and E_K.
        - The lower conductance was caused by a mutation in the chloride channel.
    - The action potentials keep going because the E_K increases with the increase in extracellular concentration of K+ due to the increase in APs. It's a positive feedback cycle.
- Myotonia congenita was the first ion channelopathy to be discovered in humans.
- Overall, turning down chloride conductance causes problems for muscles. This will lead to myotonia.
- The channel is also a dimer.
    - Dominant negative effects are when if you have a mutant and regular copy of the dimer, then the channel will be inactive.

2/7 - Week 5

Action Potentials

- **Reread Chandler notes on action potentials.**
- Voltage changing over time will depend on the capacitance changes.
- Passive responses are the voltage changes that are linear with the current applied. These responses don't get the neuron to send out an action potential.
- As the current increases (assuming we're already at the point where an AP will happen), the rate/frequency of the APs will increase rather than the amplitude of APs increasing.
- Action potentials always have all or none firing, they have a peak of some voltage above 0, and there is conduction of the AP without the loss of amplitude.
    - However, some variable characteristics are the action potential duration and the amplitude of after-hyperpolarization.
        - Undershoot is prominent in neurons, but no real undershoot in rat muscles because the resting potential is already pretty low. Also, the cat heart muscles takes a longer time to get back to resting potential.
- The biological basis of capacitance is the lipid bilayer. The capacitance determines how many charges what to move across the membrane in order to get a certain voltage change and influences the rate of change of voltage in response to current flow.
    - In other words, high capacitance means the capacitor (the membrane) can absorb a lot of charges before the voltage changes. Low capacitance means it can only absorb a few charges for the voltage to change.
- Capacitance is proportional to area. More area means that the amount of charge it can absorb goes up. However, it is inversely proportional to the thickness of the capacitor. Farther away plates means that the charges are further from each other and they don't

really cancel out (which happens for capacitors of small thickness) and therefore every additional charge added won't have as great of an effect.
    - Aka, low thickness means high capacitance
- Specific capacitance is capacitance per unit area. For the most part, the specific capacitance for a membrane is constant.
- Using Q = CV and if we know the specific capacitance for a membrane, then we can figure out the number of charges that need to move across to get a particular voltage change.
- By taking the derivative of the charge with respect to time, we can see a relationship between a large value for current flow and the rate at which the voltage is changing (dV/dt).
    - Large current flow + small capacitance means that the voltage will be changing rapidly.
- When you input a constant current, you'll see a gradual increase in voltage, The rate eventually plateaus.
    - The final voltage change is equal to the change in current multiplied by resistance.
- The fact that the voltage peak is above 0 means that we don't have a total breakdown of the membrane.
- The membrane permeability is selective for sodium at the peak of the action potential, but at the resting membrane potential, there is a very low permeability to sodium.
- The undershoot happens because the conductance of K is greater than it normally is at rest (since the K channels close pretty slowly), and therefore the membrane potential gets even closer to E_K.
- Voltage clamp experiments work by putting recording electrodes into the axon and into the isopotential bath, allowing us to measure voltage, and then having another component to detect when it's not 0, and apply the necessary current to move it to 0.
- Both sodium and potassium channels are activated and start to open more as the voltage rises, but the sodium one activates faster than the potassium one.
- Myotonia is a disorder of muscle membrane excitability. Another one is periodic paralysis.
    - In the individuals that have it, during the episodes of weakness, the muscles are sitting at -50 to -20 mV resting potential. One of the effects is that the sodium channels are stuck at inactive because the membrane potential is not going down, and the muscle is perpetually refractory.
- There is one gene that has the possibility of giving you myotonia (hyperexcitability) and periodic paralysis.
- Hyperkalemic periodic paralysis shows a much larger depolarization of the membrane in response to larger concentration of potassium. However, if you add TTX (blocker of sodium channels) then the behavior goes away, which indicates that something was causing those sodium channels to open when there is an increase in potassium. We found out that there is a mutation with the sodium channel (Nav1.4)

- All the mutations for these sodium channels are missense mutations that create functional channels with some things that are messed up about them.
- With the sodium channel myotonia, we see that the sodium inactivation is happening too slowly, which means that there are more APs.
- With the hyperkalemic periodic paralysis, the mutations cause openings of sodium channels very late even after the onset of the current. There is something going wrong with the inactivation. It activates quickly, but fails to inactivate. There is thus a persistent stable sodium current. You get extra spikes at the beginning, but then you just get this steady and stuck constant depolarization of the membrane. It turns off the normally functioning sodium channels.

2/12 - Week 6

- Slide 80 is showing when you clamp the voltage to a particular value. For some of the mutant channels, the inactivation is too slow (middle) or the inactivation fails to go to completion (right).
    - If it turns off too slowly, then you get myotonia since there is more likely to be more APs since there is a higher current as a result of the slow inactivation.
    - If it does not complete, then you have dysfunctioning channels leading to periodic paralysis because you can't send APs and can't contract your muscles.
- Inactivation gate in the channel is located on the loop between the 3rd and the 4th domain.
- In SCM, the voltage does not go all the way back down to original value, and this depolarizing shift continues. This could be affecting the continuous later firing of the neuron.
- In muscle fibers, APs travel longitudinally along the fiber but they also travel radially with the transverse tubules. This allows the components on the inner part of the fiber to get exposed to the calcium.
    - The fiber is susceptible to a buildup of potassium in the T tubules, which causes extracellular potassium to increase?
    - Tl;dr is that T tubules cause the depolarizing shift and the later APs.
- More stuff on different general ion channels
    - Voltage gated ion channels are selective about the particular ion(s) that can go through.
    - For some ligand gated channels, those ligands can bind intracellularly.
    - There are also stretch and heat activated channels.
- Voltage gated sodium channel was the first one discovered in 1984 (the gene for the channel was isolated). Found inside of the electric fish, which had a lot of those channels. Then, first potassium channel gene isolated in 1987 from the fruit fly.
- There are large gene superfamilies that code for different types of channels. Specifically, the voltage gated ion channel superfamily has many more members than expected.
    - These channels are different in terms of what they open to, the types of blockers, etc.

- The fundamental properties of the ion channels are:
    - The pore controls the ion permeation. It is a conduction pathway for the ions to flow through. There is also selectivity in the pore that allows certain ions to go through. The channel conductance affects how fast the ions will move.
    - The gates control the opening of the channel. The channels can be voltage or ligand.
- Single channel recording gives you an idea of those two functions above. How well the ions go through (the permeation) is reflected by the current and the amplitude along the y-axis. The recordings also show the all-or-nothing nature of the channels at any point in time, which reflects the gating characteristic of the channel.
- Some sodium channels are resistant to TTX and this affects pore function. In the P loop, amino acid substitutions affected the block of toxins.
    - Exchanging the P loops between channels change the characteristics of the pore. Kv 3.1 functionality inside of the Chimera. The permeation properties move along with the switch.
- The creation of a potassium selective pore is a function of the type of amino acids in the pore region.
- The pore is lined up with carbonyl oxygens and the oxygens are pointing to the center of the pore so that they can keep the potassium ions that are passing through happy.
- How do the pores prevent sodium ions from coming through if they are smaller than potassium?
    - Sodium being smaller means that it can't fully interact with all 4 oxygen atoms. They basically don’t fit in the binding site.
- The potassium and sodium voltage gated ion channels have 4 subunits and thus 4 P loops.
- Pronase was used to clear out the proteins in the axoplasm. Without those proteins, there is no inactivation of the potassium channels, which means that that machinery probably lives on the intracellular side where those proteins are.
    - The real mechanism of potassium channel inactivation comes when a blocking particle “the ball” can bind in a pocket on the intracellular end of the pore. The ball is connected by a peptide chain to the channel.
        - Channel each is connected to 4 balls are only one is needed to inactivate the channel.
        - Without the ball or any type of free ball peptide, there is no inactivation. Even if you delete the ball, you can replicate the same functionality by adding the free ball peptide.
    - The shorter the chain, the faster the inactivation because I think it takes less time for the ball to block the channel.
    - The channel being in the open conformation favors the ball to come in to block the region.
- In sodium channels, the inactivation function is created through that inactivation gate found in the loop between the 3rd and 4th domains.

    - Specifically, there is some IFM fast inactivation motif that binds to a hydrophobic pocket that pushes the S6 segments into the pore, closing it.
        - It's kind of like inactivation at a distance.
    - It is called a greasy interaction of the gate with the intracellular mouth of the pore to keep it occluded.
- There is a fixed positive charge in one of the proteins in the channel and that is the component that is sensing voltage changes and is changing its shape.
- Gating current is the current of the sensor moving through the gate as a result of the outside change in voltage. This can cause a very short lived outward current of sodium when the channel gates first start to open.
- Ion channels have been used as therapeutic targets for certain medicines. They can also be involved in human disease. There can be toxins that affect the function of channels.
- Identification of channel diseases need some delineation of familial inheritance, you can do genetic linkage analysis, and positional cloning.
- Particular channel defects can affect your tendency to get a certain disease/symptoms.

2/14 - Week 6

- There is a mapping between channel defects and the symptoms you see from patients.
- Epilepsy is functional disorder, characterized by temporary disruptions of brain activity called a seizure which is a situation where a large volume of neural tissue is firing synchronously (tl;dr abnormal excessive, hypersynchronous discharges). This seizure interferes with the normal function of the brain. The symptoms are variable because there are a large of different areas of the brain that could be affected (temporal lobe, motor cortex, etc)
- After a seizure happens, brain has to recover and there are side effects of headaches, tiredness, etc.
- Epilepsy is a condition where people have recurrent seizures that is due to some chronic underlying process.
    - One of the most common neurological disorders.
    - Some people will continue to have seizures even with medication.
    - Common disorder with high morbidity (interferes with daily activity) and mortality.
- Surface electrodes will attempt to find evidence of abnormal electrical activity.
    - However, the amplitude of the signals will be really small because you measure at a distance.
    - There is also poor localization of where the signal is coming from. There is also so much tissue in between the electrode and the synapses.
    - Time resolution is also compromised because you are measure way after the seizure.
- After doing an EEG, we see that there are interictal epileptiform discharges (IEDs). They are discharges between two seizures. Amplitude is larger because the neurons' activation add up.

- Generalized seizure means that the seizure is spreading from one hemisphere to another.
- Some types of epilepsy are caused by mutations in ion channels leading to changes in behavior while makes someone susceptible to these discharges.
    - However, there are many routes that can lead to epilepsy symptoms.
- Benign familial neonatal convulsions are those shown by babies and they indicate some problem that is inherited. Only 10-15 percent develop epilepsy. There was a clear autosomal dominant inheritance.
    - Two papers studied this and they mapped to different chromosomes but both mutant genes were coding for potassium channel subunits.
- Next step is to induce a mutation in those channels in a frog and see what the impact is to the channels and the current that passes through them.
- The amplitude of the current going through each channel were small individually, but when expressed together, then the current was a lot bigger which means that you have both subunits coming together. They come together to make the channel that conducts the M current.
    - In the presence of a point mutation in either but not both subunit, you lose a bit of current, very modest reduction. Because it's modest, this makes sense since kids can grow out of it.
    - Less potassium conductance = channel is more sensitive and will fire more.
- Then, scientists looked at a cohort of 80 patients with this disease and they wanted to do genetic screening. 10% had mutations in KCNQ2 and none in KCNQ3.
- So severe epilepsy, on the other hand, had missense mutations in the voltage sensor section, the pore loop, and the C-terminal tail.
    - Voltage sensor mutations cause it to be much harder to open to channel, The mutations cause the channel to have a much lower probability of being open at -40 mV, which is the time at which neurons will inch closer to AP.
    - Dominant negative defects in KCNQ2 cause this.
- The solutions/drugs to this are ones that are M-channel openers, which increases the potassium current which reduces the firing of neurons.
- There are also multiple epilepsy syndromes associated with NaV1.1 mutations.
    - A lot of the mutations causing SMEI were nonsense and frameshift mutations which destroys the coding potential. So why would the excess activity characteristic of a seizure happen? Because there shouldn't be as many functional sodium channels. It's because of the cell type. Removing the channel won't affect the pyramidal excitatory neurons while removal of the channel will affect the inhibitory neurons.
        - Tl;dr Loss of excitability in inhibitory interneurons means greater excitability of neurons as a whole.
- Review
    - Mutations in channels can cause loss of function or pathological gain of function
        - Gain means that there's more current when there shouldn't be. More often these come through sodium.

        - Loss of function means that there is less current due to the number of functional channels. More often these come through potassium and chloride.
    - Most disease associated channel mutations affect gating (whether channels open or close).
- Like mentioned before, the action potential does not change along the fiber. If you reduce the current and that isn't enough to get to AP, then the lossless characteristic doesn't hold, the response at the other end of the axon will be decreased. The positive charges dissipate as it moves along the axon. The diameter of the axon affects the rate of dissipation. As the axon becomes larger, you can more effectively depolarize farther parts of the axon.
- Myelin will enhance the propagation of APs because
    - Increase in membrane resistance makes it harder for the charges to dissipate to the sides.
    - Capacitance decreases
- Depolarization is spread across a lot of nodes of Ranvier, not just at the spaces.
    - The density of current is higher at the nodes (b/c the density of ion channels is greater and you don't have to deal with the additional resistance added by the myelin), but the APs are spread out.
- Conduction velocity is also proportional to the fiber diameter in the myelinated axons.
- Losing the myelin will cause big sections of the nerve with low density of channels which can cause the failure of conduction.
- Proving that a symptom is a myelination problem instead of a nerve problem should involve measuring the conduction speed for the axon.
    - You can have decreased speed or a not uniform graph of speeds.

2/15 - Week 6

- As you increase the extracellular concentration of K, then Ek becomes more positive.
- Resistance inversely related to conductance.
- TTX blocks Na channel and TEA blocks K channel. Both are voltage gated channels.
    - TTX blocks the pore section of the channel.
- Hydrophobic interactions cause the pores to be closed.
- Practice Questions
    - 1. Inhibitory. When glycine activates receptor, it will depolarize the cell at v_rest but will hyperpolarize it for any membrane potential above -20 mV.
    - 2. Receptor becomes excitatory now because opening the channel will always generate an AP.
    - 3. Inside
    - 4. Right for K+ and Left for Cl-
    - 5. Figure out the slope.

- 6. Mutations in a channel such as nonsense mutations with the channels of inhibitory interneurons being rendered nonfunctional, meaning that there is greater excitability as a whole.
    - Sodium channel mutations in inhibitory neurons.
- 7. Ionotropic and metabotropic receptors both activate due to ligands binding to the receptors. They are different in that
    - Ionotropic receptors have channels that allow ions to pass through while metabotropic cause a cell change as a result of 2nd messenger pathways.
    - Ionotropic cause change in voltage faster than metabotropic.
- 8. Chloride channel is dysfunctional and thus chloride conductance is decreased, causing an increased membrane resistance, more APs since it's easier to hit the potential?
- 9. Refute. Shaker K is ball and chain, while sodium is Greasy
- 10.
- 11. Higher membrane resistance (so charges cannot leak) and increase in thickness leads to smaller capacitance and so the number of charges required to get to a particular voltage decreases.

2/21 - Week 7

- There was a debate in the beginning of neuroscience research between thinking about the electrical properties in the brain/neurons and the impact of chemicals
    - Soups believed in chemical transmission while Sparks believed in electrical transmission.
    - People differed on which one was the main method of transmission.
- The main reason people didn't think chemical transmission was the answer was that they thought it would be too slow to communicate info between neurons.
- The other reason is that of parsimony. It's just simpler. Nerves conduct electricity. So why doesn't electricity just turn into electricity. Neurons must communicate in this way.
    - The chemical transmission theory says that you have to have neurotransmitters, receptors that detect it, mechanisms to release NTs, and you have to stop the message.
- In order to show chemical transmission, you would need to determine what goes in between the action potential and the response of the postsynaptic neuron. We need to prove the following attributes.
    - The AP has to give rise to the chemical transmitting agent. So the AP should be the signal for the chemical to be released.
    - The NT has to work at specific regions on the target cell. We needed the NT to only be at that synapse.
    - The receptors on that postsynaptic cell need to be activated in response in the NT and needs to lead to general downstream effects like a muscle contraction.
        - There needs to be clearance or termination of that effect.

    - All the above needs to be fast.
- In the vagus nerve experiment, when the vagus nerve was stimulated, the heart was slowed down and the contractile force would decrease. It was hard to tell if electricity was the cause or if there was a chemical substance involved. When the second heart was connected through a bath, it slowed down after a delay. Since there was a delay, this means that it probably isn't electrical since that would happen immediately. This was evidence for some sort of chemical being released, moving through the tube, and affecting the 2nd heart.
    - People said that's cool and all but if this is going to be a central mechanism through the whole nervous system, then it has to be fast, and this experiment didn't show it. They didn't know about those metabotropic receptors which take a bit of time though.
    - They did another experiment where they looked at the times it took to slow the first heart down (basically time between the stimulation and the slow down), and saw that none of the responses occurred in shorter than 150 seconds, and thus people were like nah, central synapses can't be chemical transmission, they're so slow!
- The next experiment was to look at the neuromuscular junction specifically where you stimulate a motor neuron and you record the postsynaptic neuron (muscle cell) membrane potential. Eccles wanted to try this because it looked at a synapse that was much closer to what a central synapse should look like (compared to the vagus heart experiment).
    - Here the delay between the stimulation and the recording of the start of the change in voltage of the postsynaptic neuron was 1 mS which was 100x faster than the time for the heart experiment.
    - As you move away from the end plate the voltage change goes down.
    - The reason is that they put curare in since they didn't want the muscle to twitch and mess up the recording. The curare makes sure to reduce the amount of depolarization in the muscle cell so that it doesn't fire a muscle contraction or twitch.
        - The recording electrode then recorded subthreshold electrical responses (voltage changes) in the muscle that resulted from motor nerve activation.
    - This also provided evidence for there being a specific place where the NT is received (aka a special region for receptors). Showed this through an experiment where they puffed on acetylcholine (the normal NT for this type of synapse) at different locations on the NMJ and at different locations there were different levels of voltage change in the postsynaptic cell. The highest voltage change was at the motor end plate (where nerve meets muscle) and as you move farther away the voltage response decreases. This means that all the receptors for the acetylcholine must have been at the motor end plate and not really anywhere else on the NMJ.

- The two above experiments were focused on trying to determine if chemical or synaptic transmission as well as the delay between nerve stimulation and postsynaptic cell response.
- There was also another experiment that basically proved that central inhibition is chemical. Basically we're talking about the inhibitory interneurons in the dorsal root ganglia.
    - One of the key points to know is that the difference between the potential of the outside of a neuron and the potential at a bath electrode is not 0. So a situation that describes this is when an inhibitory neuron that is presynaptic to a given motor neuron is very active causing greater amounts of K+ to be in the area which, when those ions diffuse, make the area outside of the motor neuron to be more positive as well. At this point, the outside is relatively depolarized wrt the bath electrode. Now, when we think about the inside of the neuron wrt to the outside, the inside is already hyperpolarized compared to the outside, and now with the recent developments, the magnitude of that hyperpolarization grows.
    - Now, let's say that through leak channels, some amount of the positive ions flow in. Now, if we compare the inside of the neuron to the bath electrode, the inside seems a bit more depolarized because the inside of the cell increased the its number of positive ions and the bath electrode didn't change. However, if we compare the inside of the neuron to the outside, the inside seems little less hyperpolarized, but since only a small number of those ions are coming through leak channels, the net effect is that the inside of the neuron is still hyperpolarized as a result of the inhibitory presynaptic neuron.
- Chemical synaptic transmission is calcium dependent.
    - Action potential is not really necessary for the NT release, but rather just an increase in the calcium concentration in that axon terminal is what's necessary.
    - They did an experiment where they had the presynaptic cell undergo action potentials, and then removed extracellular calcium (which is the molecule that flows in once the depolarization reaches the axon terminal), which prevented a response in the postsynaptic cell.
- There was also another experiment where they looked at the effect of the timing of adding extracellular calcium wrt the AP. They found that the calcium is needed at the time of the AP and if you add the calcium after the AP then you don't get any postsynaptic response. This makes sense because the AP in the presynaptic neuron opens up those voltage gated gated calcium channels and the calcium needs to come inside the presynaptic cell terminal in order to bind with the vesicles and for the NTs to be released. If that calcium isn't there at the time of the AP, those channels will close down and the calcium won't come in, the vesicles won't get released, and there won't be a postsynaptic response.
- Also another experiment that shows when you increase the extracellular calcium levels, then you don't need as large of a presynaptic depolarization to obtain the same postsynaptic voltage change. Basically, the curve shifts to the left.

    - This implies that when extracellular calcium levels increase, the probability of release increases.
        - Important to note, though, that the maximum postsynaptic response does not change and the minimum depolarization required for the presynaptic doesn't change either (since you need a certain level of depolarization to even begin to open those calcium channels).
    - Also showed that postsynaptic cell potential dies down faster the presynaptic potential.
- Another experiment shows that based on the depolarization of the presynaptic cell, there is an upside U shape curve for the inward calcium current.
    - Basically the calcium starts to flow in because those voltage gated calcium channels start to open, and then the calcium current decreases because the driving force decreases as the membrane voltage gets larger and larger.
- Another experiment shows the presynaptic injection of calcium can generate a small postsynaptic response (since the calcium influx causes small amount of NT to be released).
    - They also showed that if you have a calcium chelator (something that gobbles up free calcium) presynaptically and you have your normal presynaptic action potential, the cell that has the chelator will not cause a postsynaptic response (since the chelator removes all the calcium from the presynaptic cell thus meaning that they can't bind to vesicles and the NTs are not released) while the normal cell will cause an AP in the postsynaptic cell.
        - Note that the chelator doesn't affect the presynaptic action potential, but rather affects whether or not there is a postsynaptic response.
- The hypothesis that we eventually came up with was that presynaptic cell depolarization -> calcium influx in the presynaptic cell -> neurotransmitter release

2/22 - Week 7

- For the paper, a-d show some characteristics of the fibre volley and the field EPSPs that result, and these are shown comparing the wild type and the Syt7 knockout and there weren't too many differences which was a sign that the probability of release does not change. In e-g, the graphs show how when you have multiple action potentials, or multiple stimuli, then the probability of release will change because of facilitation which is shown in graph f.

2/26 - Week 8

- Another important observation is that chemical synaptic transmission is quantal or is released in discrete chunks. The amount of NT released is released in particular multiples.
- The spontaneous activity in the NMJ (no action potential) are miniature endplate potentials. The different mEPP amplitudes end up being multiples of a single mEPP.

- Each quantum release is independent of another. At any point in time, there is a probability of a vesicle being released.
- In the chemical synapse there is a dark band, meaning that there are proteins presynaptic and postsynaptic.
- NTs rely on diffusion to get from pre to postsynaptic cell (aka crossing the cleft).
- In the tripartite synapse, you have astrocytes in addition to the pre/post neurons. Those surround the cleft and help clean things up. They will contain transporters and pumps that take up the remaining NTs that don't get to the post neuron.
- The NMJ is really large compared to a central synapse. In the NMJ you have many release sites. There are lots of folds in the sarcolemma (which is on the postsynaptic muscle cell) and the folds increase the surface area and increase the number of receptors there can be.
- Central synapses are faulty in terms of having a low probability of release, but with NMJ, we dump out more ACh than we need to make sure that it is reliable and fault tolerant. This is the reason for the increase in the number of release sites.
    - This is quantified by the safety factor which is a measure of excess released NTs.
- The NMJ for a human is much larger than the NMJ for a rat.
- Each muscle fiber controlled by one motor neuron, and one motor neuron can control multiple muscle fibers. Also, they don't innervate consecutive muscle fibers necessarily.
- Parts of the presynaptic terminal
    - Active zone: Disc-like structure that has the role of organizing and affecting synaptic release.
    - Perisynaptic zone: Site of vesicle endocytosis, basically the phase where the vesicles get pulled back in. Contains adhesion molecules. Also has presynaptic receptors that modulate release of NT.
- Active zone roles
    - Dock and prime synaptic vesicles: Basically make them ready to be released
    - Recruit voltage activated calcium channels so that once the excitation from the presynaptic AP comes, we can open up these channels, get calcium in, and release the vesicles containing the NTs.
    - Contribute to the location of pre and postsynaptic specializations opposite of each other via transsynaptic cell-adhesion molecules
    - Mediate much of the short- and long-term presynaptic plasticity in synapses.
- The tl;dr of the synaptic vesicle release process is docking -> priming -> fusion.
- Munc13, RIM, and RIM-BP are part of the RIM Coordinating Complex.
- Synaptotagmin-1 is a calcium sensor that is part of the synaptic vesicle membrane. The core release machinery is Snap-25, Munc18, Complexin, etc.
- Docking refers to the act of getting the vesicle to the active zone and holding it there. Docking is mediated by RIM (in the plasma membrane) binding with Rab 3/27 which is bound to the membrane of the vesicle and RIM grabs the vesicle into place. It moves the vesicle close to the membrane.

    - Know this because there was an experiment where they tried a cDKO (knockout of both the isoforms of RIM) in the Calyx of Held synapse (large synapse in mammalian auditory system), there are a lot less vesicles very close to the active zone. They are more spread out in the cDKO which means that RIM has a role in bringing the vesicles close to the membrane.
- Priming is the step after the vesicle is in position. Priming has the goal of making the vesicle ready to be triggered for calcium dependent fusion. It's basically when we hold te vesicle right at the membrane but we don't actually let it go. It is basically just waiting for calcium which is the signal for the vesicle to get released.
    - It involves the coming together of the vesicular SNARE synaptobrevin and target SNAREs SNAP-25 and Syntaxin. To be clear, these v-SNAREs are synaptic vesicle bound and the t-SNAREs are plasma membrane bound. They bind together to form a partial SNARE complex with the complexin protein that acts as a trigger/brake. In order for this complex to form, Munc13 has to interact with the t-SNARE syntaxin to put it into an open configuration.
- 4 alpha helices
    - 2 from SNAP-25
    - 1 from syntaxin
    - 1 from v-SNARE synaptobrevin
- Partial SNARE complex stops here because complexin stops the fusion and it is at an energetically stable position. It is energetically favorable for the snares to get together (at least partially).
- Munc13 makes sure that the syntaxin is open, and RIM makes sure that Munc13 is not homodimerized (dimerized with another Munc13) which if it was, would make Munc13 inactive, and thus syntaxin wouldn't be open and the complex couldn't be formed.
- Munc13-1 has more impact than Munc13-2 in excitatory EPSC. In IPSC, we need to knockout both of them out.
- Fusion is the step that requires calcium entry, a calcium sensor, and SNARE complex completion. It's also the step when the pore starts to open and the NTs can diffuse out.
- Calcium channels are located in the same places that the vesicles are in. They are in that location because of RIM and RIM-BP.
- Complexin provides a brake/clamp on the docking/priming/releasing. When you downregulate complexin, then you get lots of spontaneous release. The vesicles are being released without anything stopping it. There is more release, but it's not equivalent to the amount released by an AP.

2/28 - Week 8

- Fusion is when the vesicle becomes one with the plasma membrane.
- The calcium channels are really close to the vesicle.
- Because calcium concentration is very tightly controlled, increase in calcium can be a very local signal.

- RIM binds with Rab 3/27 (role in docking), it prevents Munc-13 from homodimerizing (role in priming), and it brings calcium channels to the active zone (role in fusion).
- Removing RIM and RIM Binding Protein means that the calcium channels won't localize to where the vesicles are.
- Synaptotagmin is the default calcium sensor. It is able to bind multiple molecules of calcium and it changes conformation so that it will interact with the SNARE complex (specifically complexin) to form the final fusion.
    - So basically when calcium comes into the cell and multiple calcium ions bind to one synaptotagmin, then it changes from a trans to a cis configuration, causes it to interact with complexin, and that interaction removes the complexin brake or triggering of release.
- Munc18 has to be around for docking, priming, and for fusion.
    - A KO of the protein will completely stop activity in the NMJ. No spontaneous release anywhere. It's a conserved effect in all synaptic situations.
- 3 Hypotheses for why Munc18 is important
    - Munc18 is mediating the process to make the two membranes into one.
    - Could be responsible for catalyzing SNARE complex formation in the active zone. Basically in charge of attracting the pieces that form the complex.
    - Spatially organizes the SNARE complex around the fusion site.
- We have to end the message in synaptic transmission in order to separate between APs.
    - Facilitation happens because we don’t have rapid enough cleanup in the synaptic cleft.
- Vesicles are recycled locally. Basically, after the vesicles fuses, releases NT, it becomes part of the plasma membrane. Endocytosis is what happens to create new vesicles. You don’t have to go to the Golgi and bud off a vesicle. The new vesicles come straight from the plasma membrane.
    - The evidence for this is that there was an experiment where you put a lot of HRP (horseradish peroxidase) in the cleft, stimulate the cell a lot so that there is a lot of vesicle release, which means that the number of vesicles recycled increases, and the amount of endocytosis increases and thus we can see if the vesicles are taking up the HRP in the cleft. They used an electron microscope to see where the HRP reaction products are. The experiment found that HRP were inside of coated pits and coated vesicles. They also found HRP in the local endosome, and the HRP migrate to the vesicles.
- Takes about a minute to go full life cycle of the vesicle. All the steps are local at the terminal. No need to go back to the nucleus to get a new vesicle. Vesicles are released and recycled locally.
- Clathrin are proteins that bind to other proteins in the membrane and you’re sucking membrane on the inside of its shell. Adaptor proteins (AP-2 and AP-180) are the ones that connect clathrin to the membrane. The membrane gets turned into a ball and is pulled up with the clathrin. There is another protein called dynamin and wraps around the neck of the ball (which is called a lipid stalk) that is getting created and snips it so

that the ball can go into the cytosol. Auxilin and Hsc70 are proteins that rip the clathrin off and the vesicle is free.
    - Clathrin self assembles into a ball. It's picking up and providing the shape to the vesicle.
- None of this is happening in the active zone, the recycling happens in the perisynaptic region.
- Once the NTs are released into the cleft, we need to clean it up after some time period.
    - There is enzymatic degradation by the Acetylcholinesterase. These are enzymes specifically in the cleft and there is a race between the acetylcholine diffusing across and going to the receptors and the Acetylcholinesterase turning the ACh into something else.
        - Inhibiting Acetylcholinesterase would mean that the acetylcholine stays around longer which would be good in the case of Myasthenia Gravis patients (who have condition where it is hard to get signal across).
    - There could also be astrocyte mediated reuptake. There are astrocytes that are surrounding the cleft and they suck up the NTs and recycle them back into the presynaptic cell.
        - There are 3 types of synapses
            - Glutamatergic - Astrocytes suck up the glutamate and it turns it into glutamine which goes back into the presynaptic cell where it can by synthesized back into glutamate and packaged into vesicles.
            - GABAergic - Astrocytes suck up GABA and returns it to the presynaptic cell.
            - Glycinergic - Astrocytes suck up glycine and returns it to the presynaptic cell.
        - They all rely on astrocytes in the reuptake process.
        - If you want to keep these NTs (glutamate, GABA, glycine) in the cleft for longer, you can inject substances that block the transporters on the astrocytes.
    - There is also direct reuptake which is where the presynaptic cell itself uses transporters on its own membrane to get the NTs directly back into the presynaptic cell. This prolongs the message.
        - There are direct reuptake transporters on the presynaptic cell for dopamine, norepinephrine, and serotonin
- Tldr Different neurotransmitters will have different ways that they are removed from the cleft and recycled.
    - Dopamine, norepinephrine, and serotonin are all largely removed from the cleft via reuptake through transporters. Acetylcholine is largely terminated through the activity of enzymes (i.e. acetylcholinesterase). Glutamate, GABA, and glycine are terminated by diffusion or reuptake into astrocytes or glial cells.

- Dale's principle (given by Eccles) said that one type of neurotransmitter gets released in all the axonal branches (which means that you're saying that the NT is excitatory/inhibitory) of a particular axon. Basically one type of NT per axon.
    - The problem is that NTs can have excitatory or inhibitory effects. This is caused by metabotropic signaling from GPCRs. The downstream effects from G-protein signaling can make the glutamatergic neurons inhibitory.
    - The synaptic actions of neurons vary with developmental age, postsynaptic membrane potential, and the biochemical state of the postsynaptic neuron.
    - Invertebrates have been shown to release more than one NT.
- The evidence for transmission of glycine and GABA was shown through experiment. In the experiment, the neuron we are measuring is getting input from one presynaptic neuron (which should in theory be releasing one type of NT).
    - First they spritz on GABA and glycine and they find the the postsynaptic cell responds to both, which is weird.
    - Now, they actually look at the spontaneous activity of that postsynaptic neuron (which is going to be caused by the activity of the presynaptic neuron), they block sodium, glutamate, NMDA, etc. and they record the response. They get a bunch of activity. Then, use strychnine to block glycine receptors, and you still get response. And then block GABA and then you get no response.
- Definition of corelease is the process by which 2 or more NTs are released by a single neuron in response to an AP.
    - Cotransmission is where you have a release of an NT and a peptide.
- For there to be corelease it has to be done through the normal synaptic transmission process.
    - It can result from packaging multiple types of NTs in the same vesicle. This can be done 2 ways.
        - 1 is where you have a vesicle where you have a common vesicular transporter for both NT 1 and NT 2.
        - Another is where you have a vesicle that has different transporters on the outside based on the types of NTs it lets in.
    - In the active zone, you could have a case where vesicles release only type of NT but there are different vesicles with different NTs being released in the same zone.
        - There is also a case where you can have multiple active zones, where each releases vesicles with only one type of NT.
    - Can also be coincident membrane fusion of different vesicles that have different NTs themselves.
- Corelease is a presynaptic phenomenon, but whether it means anything will depend on the postsynaptic cell.
- Examples of corelease
    - Glycine (inhibitory) - GABA: Spinal cord, brainstem, cerebellum
        - Provides tuning of the size and duration of the inhibition since there are two types of GABA receptors (ionotropic and metabotropic). When the

GABA is released, we can get longer lasting inhibition with the metabotropic GABA receptors. Release of glycine will cause short term inhibition.

- Glutamate (excitatory or inhibitory) - GABA: EP to LHb.
- Acetylcholine (excitatory) - GABA: Retina, globus pallidus
  - Provides a spatially gated signal
  - There are direction sensitive ganglion cells (DSGCs) and starburst amacrine cells (SACs). SACs are presynaptic to the DSGCs. If you block GABA signaling, then you eliminate direction selectivity. Depending on E_Rev for ACh and GABA, we will get different responses for different directions. Levels of corelease from the SAC affects the direction the DSGCs will respond to.
- Dopamine (excitatory or inhibitory) - GABA: Olfactory bulb, retina, substantia pars compacta
  - Dopamine receptors are slow.

3/1 - Week 8

- The tripartite synapse consists of presynapse neuron, postsynapse neuron, and the astrocyte.
- Main function of synaptotagmin is that it is a calcium sensor. It is the signal that releases the vesicle that has already been docked and primed.
- Priming stabilizes the vesicle and it is energetically favorable to be in that position. Something that messes with priming, even in the presence of calcium, will reduce the amount of vesicle release.
- Article tweet: GABA and glutamate corelease affects the strength of input to the LHb which is linked to depression.
- Figure 1:
  - A: Injection of AAV into EP and after 2-3 weeks, we see yellow fluorescent protein fluorescence in both EP and LHb .
  - B: Picrotoxin blocks GABA so the responses of light pulses are larger after picrotoxin.
  - C: Shows same thing except in terms of the number of spikes.
  - D: Shows effect of current when adding certain drugs (APV is blocking glutamate, Picrotoxin is blocking GABA, NBQX is blocking Ampa). Less outward current when picrotoxin and less inward current when you

3/5 - Week 9

- Co-release can be the release from the same vesicle with both NTs and can also be the release of several vesicles with different NTs in them.
- There is corelease of GABA so that we can create the same functionality as local inhibitory interneurons, which may not be present.
- Neuropeptides are signaling molecules. Hormones are examples.

    - These peptide substances can be secreted endogenously by the cells in the nervous system.
- In the rat grid experiment, they showed that neurons release neuropeptides onto target cells.
- Neuropeptides are released by neurons as the result of its own AP and for the purpose of signaling to other neurons.
    - They are encoded in genes and synthesized in neurons (specifically inside the nucleus).
    - They are stored, and they have regulated release (aka they are released on demand as a result of a signal).
    - They have the ability to modulate firing of postsynaptic neurons and they do this by acting on receptors.
    - They can get released from areas outside of the active zone.
- Glia can release these type of substances, but they aren't called neuropeptides unless they are released by neurons.
- For neuropeptides, they have to go through a longer vesicle secretory process than the process for NTs.
    - The release is calcium dependent, very similar to the processes by which the small molecule vesicles are released.
    - However, neuropeptide release is slower because the large dense core vesicles (LDCVs) are not docked.
        - Thus, more calcium is required for release and that means it'll take more time in general to get to the threshold and this slows down the release.
        - Release is not only at the nerve endings.
        - Release can be independent of APs due to intracellular calcium sparks.
- A prepropeptide is the prior state of the neuropeptide. 2 neuropeptides are created from one prepropeptide.
- Neuropeptide synthesis involves a prepropeptide produced ribosomally in the ER and in some dendrites.
    - At the end terminus there is a signal peptide that identifies that the peptide will be secreted by the regulated secretory process.
- Then, the signal peptide is removed in the rough ER and this leaves a propeptide which goes through the Golgi and gets packaged into the LDCVs in the trans Golgi network. More still has to happen though to turn that propeptide into a neuropeptide.
- The LDCVs are 100:35 nm bigger than the normal vesicles, and they can also contain small NTs. They also have processing enzymes called convertases, and they convert the propeptides into several neuropeptides.
    - These convertases determine where they chop up the propeptides. Therefore different convertases will create different neuropeptides from the same propeptide.
- Tldr for Neuropeptide synthesis:
    - Propeptide created ribosomally -> signal peptide removed -> propeptides move across Golgi and placed inside of LDCVs -> LDCVs travel down microtubules to

the axon terminal -> convertases turning them into neuropeptides -> vesicles fused with membrane and peptides released.

- After the neuropeptides are released, they will bind to GPCRs on the postsynaptic cell. Since the neuropeptides are large, they have more recognition sites for the receptor which results in an increase in receptor binding affinity.
    - 20 percent of GPCRs are targets for neuropeptides.
    - There are over 70 types of neuropeptides.
- Once the neuropeptides have been released, they diffuse across and have actions several microns (large amount) from the release point (the neuropeptides can diffuse to and act on receptors that are far away from the presynaptic cell - basically they can go to other neurons, not just the neurons they are connected to in the cleft).
    - The onset (bc of the distance and because we are dealing with a GPCR) and the duration of the signaling is slow.
        - Acting on GPCR's means that the neuropeptides will be slower acting (compared to ionotropic receptors) but can often cause longer lasting responses because of the signal cascade that they begin.
    - There also isn't a lot of machinery to stop the signal. The signaling ends when the extracellular peptidases break down the neuropeptides. Very different from NTs which have a lot of mechanisms to control the signal at a very fine granularity. Neuropeptides thus have more of a modulatory and pervasive effect.
- Cotransmission is where you have a release of an NT and a neuropeptide (or multiple NTs) onto the same or different targets.
- Cotransmission provides signal diversity.
    - At different axon terminals and active sites where you have NTs and neuropeptides, there will be differences in the ratios of release (more NT release at some sites and more peptide release at some sites). There are also differences in what the postsynaptic neurons respond to (some won't even have neuropeptide receptors).
    - Because of the lack of docking and priming, the neuropeptides may need bursts of APs rather than a steady train of APs (which will only release small NTs).
        - As a result, whether or not a peptide gets transmitted will give the postsynaptic cell an idea of what the firing rate is like for the presynaptic cell.
    - There can also be axo-axonic synapses that affect the rate of release of small NTs and neuropeptides.
        - LDCVs need more calcium (and thus by effect, more excitation) to get released, so if you go from a cell that is releasing neuropeptides to one that isn't, there must have been some inhibition (in this case by the axo-axonic synapse)
        - Release of neuropeptides means bursts of APs (high frequency firing) while just release of NTs means there is a steady train of APs (low frequency).

    - There are also differences in that synapses can have different levels of peptidases which affects the amount of peptide that is in the cleft and that is able to diffuse out to other places.

3/7 - Week 9

- Difference between release of neuropeptides and small NTs: Vesicles are bigger, peptides are modified inside of the vesicle, it takes more excitation for release, neuropeptides can be released outside the active zone, diffusion is slower but there is a more long lasting effect since the only stopping mechanism is the extracellular peptidases.
- Evidence of corelease of serotonin and substance from the Raph-a to Pre-Botc.
    - Both serotonin and substance P is needed for respiratory function.
- RT (thalamoreticular) neuron has inhibitory synapse onto itself so that it doesn't overexcite itself and so it doesn't overly inhibit the downstream neuron (relay neuron).
    - Blocking the neuropeptide Y receptors abolishes the inhibitory input.
        - In the VB neuron, there is no effect. But if you block with the GABA_b receptor blockers, we uncover the excitatory input
    - There is cotransmission but neuropeptides are getting released to the self synapse while GABA is being released in the synapse with the relay neuron.
- In cases of high activity into the RT neuron, RT inhibits itself with the release of the large peptides and RT shuts down and this leads to not inhibiting the relay neuron.
- There is another experiment (unrelated to one above) that provided the first evidence for electrical synaptic transmission. It found direct hyperpolarization on the postsynaptic neuron with hyperpolarization of the presynaptic. How can this be chemical synaptic transmission if nothing gets released in hyperpolarization?
    - Other piece of evidence was that modifying the pre or post synaptic neuron (hyperpolarizing or depolarizing) caused a change in voltage in the other neuron.
        - Specifically, hyperpolarization of pre caused small hyperpolarization in post. Depolarization of pre caused significant depolarization in post.
        - Hyperpolarization of post caused significant hyperpolarization in pre. Depolarization of post caused small depolarization in pre.
    - Other evidence was that for this neuronal setup, the delay between AP in presynaptic and response in post was less than 100 microseconds, which was a lot faster than chemical synaptic transmission.
- Basically, the main issue is that we can depolarize one cell and get a response in another, but this could be because of chemical or electrical transmission. Main issue if what happens in hyperpolarization situations?
- Electrical synapse is not guaranteed to be directional.
- The distance between pre and post neurons in electrical synapses is extremely small. The synapses are formed by many gap junction channels.
- The junctions are made of two hemi-channels called connexons. One channel is on the pre neuron and one is on the post.

    - Connexon pore is larger than the pore of ion channels. Thus, molecules of .5-1 kD may pass through the pore in addition to ions.
- The gap junctions are held together with scaffolding that holds them in place in the same position.
- There are hundreds of connexons per gap junction. However, only 10 percent of those are electrically open at a time.
- Electrical synapses mediated by gap junctions mediated by connexons.
- 6 connexins make up a connexon hemi-channel.
    - 12 proteins come together to make one gap junction channel.
- There are many isoforms of connexins. Gap junctions occur in a lot of parts of the body and thus they must be made up of slightly different connexins.
    - For example, the junctions in cortical inhibitory neurons are composed of Cx36 connexins.
        - Could cause the rhythm of activity that you see in EEGs.
- When we KO connexin 36, this messed up a lot of functions (basically destroyed the electrical synapses) in the retina, neocortex, hippocampus, etc so we figured out that it is extremely important.
    - Gap junctions and electrical synapses are everywhere, even in the mammalian brain.
- Functional diversity of neurons that have different sensitivities to light is caused by having chemical and electrical synapses. In the KO of connexin 36, all the 4 different kinds of neurons become the same low sensitivity.
- Not all gap junctions are electrical synapses though. Not all of them will conduct electrically. Similarly, presence of connexins don't guarantee electrical synapses.
    - Also, not all neurons with gap junctions display dye coupling.
    - Also, not all gap junctions are unique to neurons (some astrocytes and glia have gap junctions).
- Coupling by gap junctions is modifiable through activity, pH, calcium, etc.
- Electrical synapses are very fast (microseconds instead of milliseconds in chemical synapses) and they are reliable and they are good for synchronization (you can put a lot of input into one neuron that is connected by gap junctions to a bunch of other neurons, and then have those other neurons fire immediately).
    - Good for escape behaviour in motion.

3/12 - Week 10

- Glia are very important in terminating a message by helping to collect the NTs in the cleft.
- The ionotropic channels with ligand gates are really what you're watching since they cause the major deflections of current.
- We don't get a rise in postsynaptic change because channels get opened more or more NT per channel gets released but rather it's that more active sites have channels that are opening.

- We will get an inhibitory or an excitatory postsynaptic potential.
    - Excitatory means that the postsynaptic will get pushed towards firing. Doesn't necessarily mean going toward depolarization.
    - Inhibitory means that the postsynaptic will get pushed towards not firing. Doesn't necessarily mean going toward hyperpolarization.
- Reversal potential of the channel is when there is no net current, considering all the ions. Reversal potential of a specific ion is when the net current of that ion is 0.
- E_rev > threshold means excitatory
- E_rev < threshold means inhibitory
- In B) when GABA binds, chloride comes in and that hyperpolarizes and V moves toward E_rev. When E_rev is originally greater than V_rest (in pic C), then there is a depolarization (chloride flows out) and as soon as you go north of E_rev, current direction is going to switch and chloride will start coming in and will fight against firing of the neuron. Basically, the channel depolarizes but it's not excitatory, since it's always going to go down once it goes over E_rev. If the E_rev was above threshold, then it would be excitatory because you'd be moving toward E_rev and then at a certain point you hit threshold, you fire, and you never get the chance to flip current.
- Synaptic summation (whether it be temporal or spatial) is required to generate an action potential.
- Dendritic conduction is passive and it decays over distance. Therefore if you stimulate the dendrite at any point along it, the signal amplitude will decrease as you get further and further away from the point of stimulation.
- The capacitance of the neuron will affect how much temporal and spatial summation affects a neuron.
- There is a bigger change in voltage at the dendrites than at the soma.
- Synaptic scaling is that you get a larger postsynaptic response at the dendrite so that at the soma the responses seem equivalent.
    - You get much more depolarization at places on the dendrites really far away from the soma, which makes it so that when the signals all travel to the soma, when they get there, the signals will all be relatively the same since the decay for the signal farther away is larger.
    - The above is accomplished by putting more receptors per synapse the farther the synapse is from the soma.
- The closer you are to the soma, the more synaptic scaling you get and the more active conductances there are.
- Shunting inhibition is different from normal hyperpolarization (summation) inhibition in that when you activate the excitatory and inhibitory synapses and E_rev for the inhibitory synapse is equal to V_Rest, normally you get an initial downward deflection because of the inhibitory synapse, but in shunting inhibition you don't get a hyperpolarization (since there is no voltage difference and thus no current) and the excitatory synapse doesn't have as much of an effect because the input resistance for the excitatory synapse is lower.

    - The reason is that you open a large hole in the membrane which decreases the resistance and thus decreases the change in resulting membrane potential.
    - This effect of shunting inhibition will occur when E_synapse is equal to E_rest.
- Hyperpolarizing inhibition will last longer than shunting even after the inhibitory synapse has completely deactivated. In the shunting inhibition case, the effect of inhibition disappears completely right at the moment of deactivation, while in the hyperpolarizing case, the effect will last longer, and we won't get peak excitation from the excitatory synapse.

3/14 - Week 10

- Neural circuits are composed of local circuits which mediate the interactions between neurons within a region.
    - We can express them through motifs which are the building blocks of neurons and their connections that perform certain functions.
- One type of local circuit motif is feedforward excitation. You have some input that has an excitatory input onto another neuron which excitedly influences another neuron.
    - Each of the synapses in the circuit is excitatory.
    - The reason we have multiple steps/synapses is that we want to allow for fine tuning of signal through convergence and divergence of information/signal at each synapse.
- Feedforward excitation can be series, convergent, divergent, and in parallel (basically divergence and then serial).
    - At each stage, you have opportunities to fine tune and modulate the signal. You get a chance to increase the richness because you have a composite representation of all the inputs.
- Feedback/recurrent excitation. This mainly is able to create oscillation like behavior. The axon for a neuron will have a synapse that has a terminal onto itself.
    - Can often lead to runaway excitation. Can often happen in places that are associated with epilepsy. This means that we need some control mechanisms for these types of circuits.
    - 2 reasons for the positive feedback in this circuit
        - Allows for small set of neurons to recruit a larger set of neurons.
        - Maintain persistent activity
- Feedforward inhibition is basically the excitation of inhibitory neurons causing it to do feedforward inhibition onto whatever downstream neuron it is connected to. If you have a divergent signal (one path being excitatory and one connected to an inhibitory neuron) then the IPSP will come a *little* bit later than the EPSP.
    - Reasons for this type of inhibition is that it controls excitation of downstream neurons and we enhance timing by shortening the postsynaptic EPSP response (better than having no inhibition and just having to rely on the decay of the excitatory signal).

- Feedback inhibition is where you decide whether you want to the cut the message off based on the response from the excitatory neuron. Basically you have a signal onto an excitatory neuron which is connected to an inhibitory one which goes and inhibits the excitatory neuron.
    - You can work through that inhibitory neuron or not. Depends on if that neuron is excitatory or inhibitory. If excitatory, need that interneuron. If inhibitory, then don't need it.
- Lateral inhibition is when you get input into one excitatory neuron and that then connects to two or more other inhibitory neurons when then inhibit all the other neurons that are around them.
    - The purpose is you can select inputs based on the strength of the surrounding responses.
- There are lots of types of synapses in terms of inputs to outputs and they can all either be electrical or chemical synapses.
    - Axodendritic, axoaxonic, axosomatic.
- Having multiple active sites onto one dendrite, then you increase the probability that the signal will get through.
- Rhythmic generation contains initial feedforward excitation pathways and then a feedback inhibition which causes the constant tonic input to turn into bursts of activity.
- Spatial contrast contains initial feedforward excitation pathways and then a lateral inhibition which causes an imbalanced input to be amplified.
