NEURAL DEVELOPMENT
Tuesday, November 13th, 2018

Chordates - recall dorsal hollow neural tube
Adjacent to lumen - **ventricular zone** - zone of proliferating neuronal progenitor cells
Mantle layer right outside ventricular zone
When cells become neurons - they are post-mitotic and migrate to mantle zone
Axons are sent out to marginal zone
Elsewhere in nervous system, arrangement is not maintained.
E.g. in cortex the cell bodies are on the outside
But throughout the nervous system the early arrangement is w cell bodies on the inside and processes extending outward.

(almost) All neurons you will ever have are generated during gestation.
Exceptions: 1) granule cells of cerebellum (recall they are the most abundant cell type of CNS - 3*10^10 neurons); for the first three months of your life there is a special germinal layer that produces granule cells
2) subgranular zone in dentate gyrus of hippocampus
3) subventricular zone of lateral ventricles
4) primary olfactory epithelium

Three Germ Layers
Ectoderm (skin (epidermis), neuroectoderm)
Mesoderm (skin (dermal layer), majority of bodily tissues e.g. skeletal muscles, connective tissue, blood, vasculature, kidneys, etc.)
Endoderm (gut)
Gastrulation - endoderm moving inside mesoderm, ectoderm spreading out over whole animal

Invertebrates:
Ectodermal cells **ingress** into animal to become invertebrate nervous system; those cells become **neuroblasts** that will give rise to neurons.
Rest of ventral ectoderm on surface will become skin

Cell Fate
Doe & Goodman - label neuroblasts and see how a specific neuroblast arose - 7-3;
can identify the ectodermal cell by sight (v large) which will become 7-3;
laser oblate prospective 7-3; yet 7-3 has still developed!
One of the other cells stepped up. By unknown mechanisms, a cell is destined to become neuroblast, but other cells can replace it if sth goes wrong. All these cells are potent at this stage - there are a group of cells - the **proneural cluster** - that can acquire the 7-3 neuroblast fate.
Maybe at this stage, the cell which will become 7-3 sends lateral inhibition to neighboring cells.
Proneural cluster signal each other via cell membrane molecules. All express a ligand Delta and the receptor Notch.

One cell expresses a little more Delta than surrounding cells. Its Delta signals via Notch to surrounding cells to express less Delta. Reinforcing signal. So original cell is the only one producing the most Delta, and eventually it switches to become a neuron.
By controlling transcription factors, this signaling pathway affects cell fate.
Delta-Notch signaling is found in both vertebrate and invertebrate animals. Perhaps all cell fates are determined at some stage by this type of signaling.

Amniotes - birds, mammals, reptiles
Create neural tube through **neurulation** process - not the same thing as inducing neuroectoderm. This is the morphogenetic process of forming the neural tube.
Neurulation is not a single process across vertebrates - there's a whole different process for amphibians and fish to form their dorsal neural tubes.

How is neuroectoderm induced?
The 'organizer' sets up patterning for the whole body structure, it itself is only a small part of embryo. It's a signaling center and sets up parts of ectoderm to become neural.
Whole bunch of stuff can act as neural inducers - including diet coke. But many of these are not endogenous molecules.
Early ectoderm in a dish will always only form skin. Skin was thought to be the default state. But this interpretation was wrong. A group dissociated ectoderm into many separate dishes. Many dishes of cells became neural tissue. Conclusion: default state is to become neuron. Associating the cells washes out the signal.
In signaling center are molecules that bind to BMP (bone morphogenetic protein) and inhibit it.
BMP says: make skin from default neuroectoderm.
Organizer says: BMP stop! And therefore makes neuroectoderm.

Neural crest
Derived from neuroectoderm. Certain cells in dorsal neural tube flee the developing CNS and become neural crest cells. These are no longer closely packed epithelium (where epithelium means closely spaced cells - epithelium is not equivalent to ectoderm; the alternative to being an epithelium is an organization of v diffuse cells and this is **mesenchyme** - mesenchyme is not equivalent to mesoderm!). So neural crest becomes mesenchymal cells.

The above is what neural crest does in the trunk. What it does in the head is quite different.
Neural crest does not become all of PNS in the head. Some of PNS in the head derive from placodes. Placodes are just ectodermal thickenings. Some placodes do not give rise to neurons - e.g. placodes form the lens in the eye - nonneuronal. Olfactory epithelium derive from placodes. The neural crest gives rise also to dermis, many of the bones (including teeth), connective tissue, etc.
The moving parts of the head arise primarily in **pharyngeal pouches** or **brancheal arches** - small outpouchings around the head. Ectoderm outside, endoderm inside, and the space in between has some mesoderm but mostly neural crest.
Key moving parts of head - jawbone, etc.
All of that is supplied by fifth cranial nerve.

Spinal accessory nerve controls movement of clavicles - which are also derived from neural crest.
Ectoskeleton is a term applied to the outside bones of the head/shoulder that come from neural crest.
Neural crest only derives from caudal part of diencephalon, midbrain, and hindbrain - never telencephalon.

Hindbrain
Each segment - **rhombomere** - or hindbrain part, have very specific roles.
Each brancheal archs' derivatives are innervated by specific rhombomeres.
In other words, the cells that fill each brancheal arch and innervate derivatives of each brancheal arch come from v specific rhombomeres.

Motoneurons that innervate the derivatives of brancheal arches are called brancheal motoneurons. We have also somatic motoneurons that control the rest.
Somatic motoneurons send axons in p direct path.
Branchial motoneurons may have a loop before they exit the neural tube in a lateral position.
Positioning & exit points are different btwn the two groups.

How are rhombomeres set up?
Hox genes set up patterning throughout the neural tube from bottom to anterior part of hindbrain
Hox complex - found in flies and four separate genomic loci in amniotes
Contain anterior-posterior specification of hindbrain and spinal cord
All of them are transcription factors
Set up anterior borders of each rhombomere
Hindbrain and forebrain controlled by entirely different set of transcription factors

How are neuronal cell types specified in neural tube?
Examine specific case of epithelium patterning:
If I want 30 diff cell types, do I need 30 separate signals?
Wolpert suggested instead that position plays a role - the cell is told where it's positioned across the epithelium. They can then 'determine' what they 'should' become.
Could achieve this via a diffusible signal, w source at one end of epithelium; concentration drops off by distance. The cells know their position by detecting the concentration of the signal.
Thus it could be v few signals that set up complex patterns; i.e. only require two signals in many cases - one for anterior-posterior axis and one for dorsal-ventral axis.
Groups of signals: Hedgehog proteins like Shh (sonic hedgehog), morphogenetic proteins (BMPs), WNT genes, FGFs (fibroblast growth factors), EGFs (epidermal growth factors)

Two signaling centers right on midline - floor plate (& notochord) and roof plate.
They originate signals that diffuse out and set up a dorsal-ventral axis.
Shh is one that will diffuse from floor plate & notochord to pattern the neural tube.

In specifying cell types with concentrations, we are recruiting thousands of transcription factors!

Thursday, November 15th, 2018
Development of Nervous System Wiring
Early Mechanisms (Signaling Molecule Dependent)
Later Mechanisms (Activity Dependent)

How do we achieve the correct numbers of neurons in each population?
Overproduction of neurons, then trimmed back based on factors received from projection targets.

Hamburger experiments on chick embryos:
Removal of limb buds on one side of embryo; the sensory ganglia are smaller on the side which the bud was removed from.
In spinal cord - the motoneurons are lacking on the deprived side. Motoneurons die in absence of normal target.
Put in an additional limb - increase in motoneurons on that side, great expansion in size of DRG
Explanation - normally would've died w/o target, receive signals from extra target and more survive.
Bueker - add sarcoma to body of normal mouse - massive innervation of tumor including from DRG and sympathetic chain; sympathetic ganglia are much expanded on side of tumor. The tumor may make an endogenous neurotrophic factor.
Levi-Montalcini - exuberant axonal outgrowth in petri dishes with NGF added
How did they purify NGF? Didn't have enough factor in sarcoma cells to isolate.
In dish they introduced PDE, combined with the factor, led to more proliferation and not less.
Control: no sarcoma, put cells in dish with 'only' PDE. Yet PDE led to an NFG-like effect! Turns out the PDE came from snake salivary glands, which was rich with NGF. From this source they were able to purify NGF!

NGF was first of neurotrophic factors to be identified.
BDNF derived decades later. There's something in the brain / CNS itself - not in sympathetic or DRG cells - that lead to axonal outgrowth.
Cloning frenzy - identified NT-3 and NT-4.
Receptors on which these factors operate were also identified at the same time NT-3 was.
NGF - TRK-A
BDNF - TRK-B
NT-3 - TRK-C
NT-4 - TRK-B
Neurotrophic factors only or are they neurotropic?
Neurotropic factors lead to **directed** outgrowth to a target, **neurotrophic** means just eliciting **general** outgrowth responses.

Lamellae - 'broad sheets' - as wide as a cell - at the ends of growth processes, or **growth cones**. On the lamellae are small extensions called **filopodia**. These processes bind factors and 'sniff out' where they should go.
Spritzing a high concentration of NGF to the side of the process will make the growth cone turn towards it.

Lumsden - collagen matrix cocultures - emulate extracellular space.
Place trigeminal ganglion into that space on its own or with its canonical target (maxillary process) or with a target it never innervates (hyoid process).
On its own or w hyoid process there was no outgrowth.
With maxillary process present, there is outgrowth from the trigeminal ganglion cells towards it.
Problem w this set-up is that there is a concentration gradient. The side with a higher gradient means that there may just be growth on that one side.
To test: placed a second trigeminal ganglion in series. They found that this second ganglion, even at a place of lower concentration, was still displaying axonal outgrowth.
Strong evidence that this is a neurotropic process.
Evidence that BDNF and NT-3 can act as neurotropic agents. 'Chemoattractive', 'chemotropic', 'neurotropic', etc.

Spinal cord - CNS neurotrophic factors?
Primary sensory neurons send processes. Secondary sensory neurons decussate and ascend to the brain.
Explants of dorsal and ventral spinal cord placed into collagen coculture. Do we see outgrowth from dorsal to ventral explants through collagen matrix?
No outgrowth.
Placed dorsal spinal cord in coculture with ventral explant - nothing happened. Then placed dorsal spinal cord with floor plate - exuberant outgrowth. Maybe there's a neurotrophic agent in floor plate.
Presented floor plate at an angle. The processes turned towards the floor plate explant.
Long-range chemoattractor - Netrin, Slits, HHs
We can also have long-range chemorepellants - Semaphorins, WNTs, BMPs
In short ranges, we have contact attraction and contact repulsion. Contact attraction relies on members of Cadherin family. They're adhesion molecules that cause cells to stick together. Dependent on calcium. Contact repulsion depends on Ephrins. In invertebrates, protocadherins / DSCAM (within cadherin superfamily) are responsible.
One consideration - you don't want processes from the same cell to seek each other. Self-avoidance strategies must be in place. Turns out protocadherins do this work.
Starburst amacrine cells do this hardcore.

Charron 2003; explant cells in coculture, abutted w tissue expressing Shh - find outgrowth towards the source. Shh is another chemoattractor.
Specific inhibitor of Shh - cyclopamine.
Cyclopamine + Shh leads to no directed outgrowth.

Canonical pathway - mechanism going through nucleus for these agents.
Also all have non-canonical pathways. They all act through the cytoplasm.

Later development
Experience from the environment affects organization of the brain
Case study: visual system
Visual target in the thalamus is the LGN (lateral geniculate nucleus)
Other visual targets include the superior colliculus
Many studies about how retinotopy is maintained relies upon the neurotrophic factors described

Hubel & Wiesel's Nobel lecture as reading for this week
How can we wire up to have binocular / stereoscopic vision? We need projections from both eyes to go in some patterned way to the LGN.
Radioactive labeling - ipsilateral labels to 5 3 2 layers of LGN, contralateral labels to 6 4 1 layers of LGN. Interdigitated ipsi/contra in each layer of the LGNs on both sides.
Hubel & Wiesel - use sharp electrode at oblique angle through V1
Ask of the cells - do they respond to stim from ipsi, contra, or both eyes?
Found that the preferred eye - ocular dominance - would shift in a systematic way
Postulated that if they moved the electrode straight down, all the cells in a column would respond to the same eye
Could we alter this somehow during development?
Cut one of the extraocular muscles of eye - two eyes are no longer in alignment (Happens in people - strabismus)
Used to be the case that in development, eyes may be patched to correct for lazy eye, etc.
They found that there was a dramatic shift in ocular dominance. Very few cells respond to both eyes. Most cells responded only to one or the other eye.
Experience impacted physiology.
Blakemore & VanSluyters - Suture shut ipsilateral eye - monocular deprivation - all units respond not at all to that eye; they respond exclusively to the eye that's open.
Reverse suture - suture one eye, open that eye, suture the other eye. Has the animal switched from one ocular dominance pattern to the other?
Found ocular dominance shift as expected. A complete reversal when performed at 5 weeks. If performed at 6 weeks, we have a large shift but it's not complete. At 8 weeks - balance between both eyes. At 14 weeks - the first eye is stable. There's a **critical period** during which neocortex is plastic. After that, no chance at affecting ocular dominance. The critical period varies from species to species and system to system.

Hubel - transsynaptic tracing - can literally view ocular dominance columns manifest in macaque V1 layer 4.
Will suturing affect this pattern? Yes, you change the structure by which input arrives in V1. Open eye has much more of the layer 4 surface.