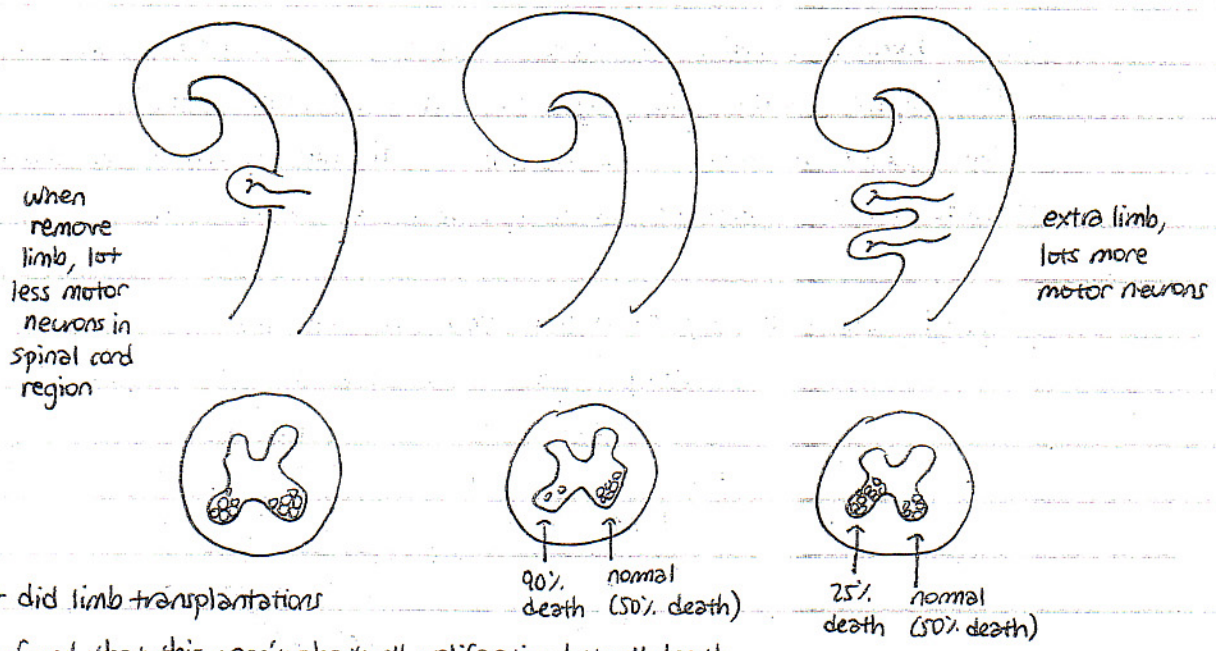
4/7/04

proneural genes - drive neuron cell fate (eg Achaete-scute)

neurogenic genes - limit neuronal cell fate (eg Notch-Delta)

growth factors: many neurons die in formation of neural system

- Victor Hamburger, Rita Montalcini (?), Stanley Cohen



- Victor did limb transplantations
- Rita found that this wasn't about cell proliferation but cell death

- purification of NGF (nerve growth factor): drives motor neuron cell survival
  - started looking also in sarcoma cell lines, found similar effects: when digest w/ snake venom, very robust outgrowth of neurons, used snake venom to purify factor
  - took submandibular glands of mice (like venom producing parts of snake), purified to single protein to sequence: lead to NGF

↳ whole family, includes BDNF
neurotrophin 3
neurotrophin 4/5
(look very similar to BMPs; lack gives less motor neurons)

if leave limb bud intact but paralyze muscles (nAChR antagonists), huge increase in number of neurons

⇓

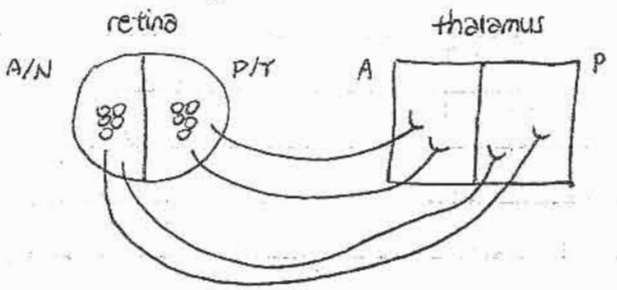
bind trk receptors: signal to cell death machinery (turn off): this inhibits caspases through signaling pathway

- as soon as muscle gets innervated, doesn't need to have more neurons innervating, turns off NGF (so too many neurons initially → proper wiring of brain)

axonal pathfinding:

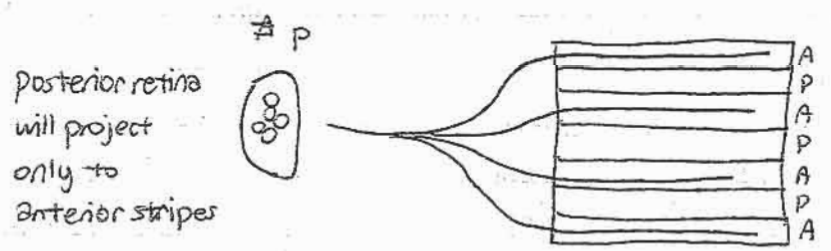
- Ramon y Cajal: identified growth cones, proposed idea that growth cones integrate information to find target
- R. G. Hamison: techniques for culturing neurons; saw living neurons migrating 1910
- Roger Sperry: chemoaffinity hypothesis (target secretes factor 1940-1960
- Corey Goodman: labeled track hypothesis (Abs label specific ~~tracts~~ tracts) 1980
- Mark Tessier-Lavigne: genetic manipulation (w/ Corey Goodman)

- Sperry's experiment: frog has retina → thalamus connections
  very specific regions of retina connect w/ specific regions of thalamus



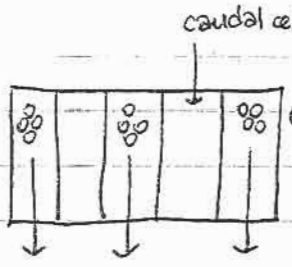
- can cut optic nerve of amphibians: will regrow (mammals won't): rotate eye 180°
- this frog will shoot 180° wrong way at flies
- R-A will always go to T-P, etc

- cut thalamus in A/P stripes, lay next to each other on collagen matrix



anterior

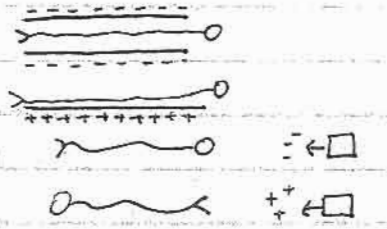
- if boil stripes, will kill factor that causes this? no, same pattern of migration
- if boil posterior stripes, axons go everywhere: make inhibitory factor



- this happens in neural crest cells
- also in axon trafficking (stripe experiment)

4 ways neuron can get to target: (cues)

1. inhibitory short-range
2. chemoattraction short-range
3. inhibitory long-range (eg ephrin stripes)
4. chemoattraction long-range

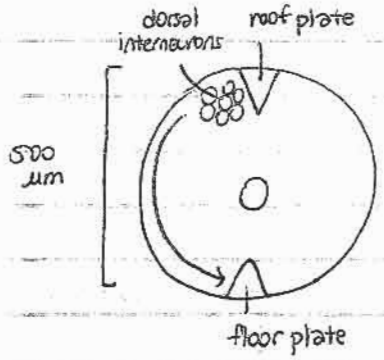


} must be type of cell adhesion molecule, usually

} must be secreted molecule: 50 μm → 250 μm

- advantage of negative cue: safety factor built in (if short-range positive cue & axon gets lost, you're screwed)

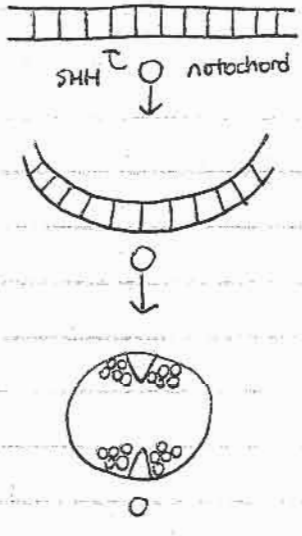
- all axonal migration events require influencing actin cytoskeleton



dorsal interneurons migrate through cord to get to floor plate

(could be neg. roof plate cues
pos. ~~roof~~ floor plate cues
local neg./pos. cues)

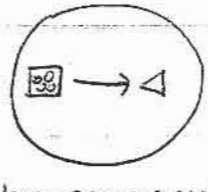
- test w/ cell culture: take floor plate, new dorsal interneurons



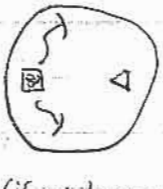
BMPs give dorsal fates, later repel those cells away: also, SHH (along w/ netrin) is attractant at floor plate

- redundancy in nervous system

this is true



(if long-range pos. cue)



(if not long-range pos. cue)

- same experiment w/ roof plate

this is true

roof

if neg. cue from floor plate

roof

if no neg. cue from floor plate

so: positive attractant from floor plate, negative repellant from roof plate : isolate factors?

(hard to do this b/c floor plate very small, hard to isolate factor)

Marc Tessier-Levigne found that axons also attracted to chick brains, used 20,000 chick brains to isolate attractant factor (hoped was same one in spinal cord)

- isolated netrin : secreted protein

  similarity to laminins (basal membrane components)

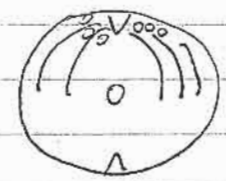
- tested by doing in situs, saw that floor plate making netrins

  knocked out, got axons going about halfway (driven away by roof plate), then stopped

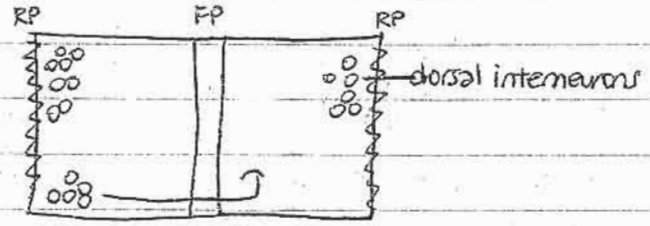
- DCC receptor on growth cones recognizes netrins (deleting this also gets axons halfway)

  ↳ plays role in colorectal cancer

(in mice?)



- open up spinal cord:

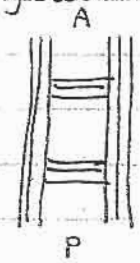


cross floor plate, head upwards towards brain

must turn off netrin signal, get another signal

maybe floor plate also secreting something negative

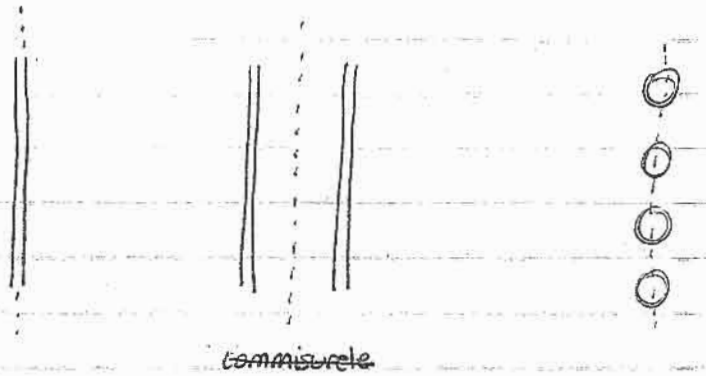
- Corey Goodman's lab dissected w/ genetics:



crossing of midline of axonal tracts

but can't test in cell culture (axons pass through floor plate, no longer attracted, but NOT repelled)

~~slit mutant~~
mutants:



~~commisurele~~

slit mutant (or comm GOF +++)

commissureless mutant (or robo GOF)

roundabout mutant (robo) (or comm GOF +)

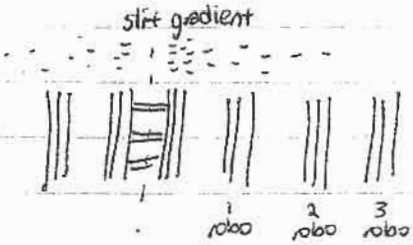
slit: may be enhancement of signal, lack of negative signal?
large secreted molecule w/ many extracellular motifs, secreted at midline + gradient outwards (inhibitory): w/o, axons never want to leave midline

commissureless: may be constitutive slit signaling, or positive always off? protein in vesicular trafficking (endocytosis)
neg. signal always on until midline crossed

roundabout: receptors for slit
↳ on growth cones

- in floor plate, slit always on: robo binds + is repelled
positive signal is netrin (bound by DCC)
- somehow can't get negative until after receive positive, must then switch pos. off
- robo expressed only after axon crosses midline, b/c comm inhibits robo by endocytosing + getting it degraded beforehand
- Some signal at midline turns comm off, so slit/robo can repel
robo binding slit lets it bind DCC, inactivating it; won't cross midline again
↳ robo

- axons follow more than one tract:



different tracts express different robo receptors: # of robo will drive you farther from midline: robo code

netrin & slit - secreted molecules w/ EGF repeats

- diffuse across ECM

- robo + DCC have extracellular repeats; have Ig & fibronectin repeats, TM domains

- semaphorins also guidance molecules
  - bind plexins